(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 October 2021 (14.10.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/207712 A2

(51) International Patent Classification:
C12N 15/11 (2006.01)

(21) International Application Number:
PCT/US2021/026732

(22) International Filing Date:
09 April 2021 (09.04.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

63/007,803	09 April 2020 (09.04.2020)	US
63/007,797	09 April 2020 (09.04.2020)	US
63/045,032	26 June 2020 (26.06.2020)	US
63/045,033	26 June 2020 (26.06.2020)	US
63/136,087	11 January 2021 (11.01.2021)	US

(71) Applicant: **VERVE THERAPEUTICS, INC.** [US/US]; 500 Technology Square, Suite 901, Cambridge, Massachusetts 02139 (US).

(72) Inventors: **CHADWICK, Alexandra**; 500 Technology Square, Suite 901, Cambridge, Massachusetts 02139 (US). **RAJEEV, Kallanthottathil G.**; 500 Technology Square, Suite 901, Cambridge, Massachusetts 02139 (US). **ROHDE, Ellen**; 500 Technology Square, Suite 901, Cambridge, Massachusetts 02139 (US). **CHENG, Christopher**; 500 Technology Square, Suite 901, Cambridge, Massachusetts 02139 (US). **REISS, Caroline**; 500 Technology Square, Suite 901, Cambridge, Massachusetts 02139 (US).

(74) Agent: **UM, Moonkyoung**; Wilson Sonsini Goodrich & Rosati, 650 Page Mill Road, Palo Alto, California 94304 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(54) **Title:** BASE EDITING OF PCSK9 AND METHODS OF USING SAME FOR TREATMENT OF DISEASE

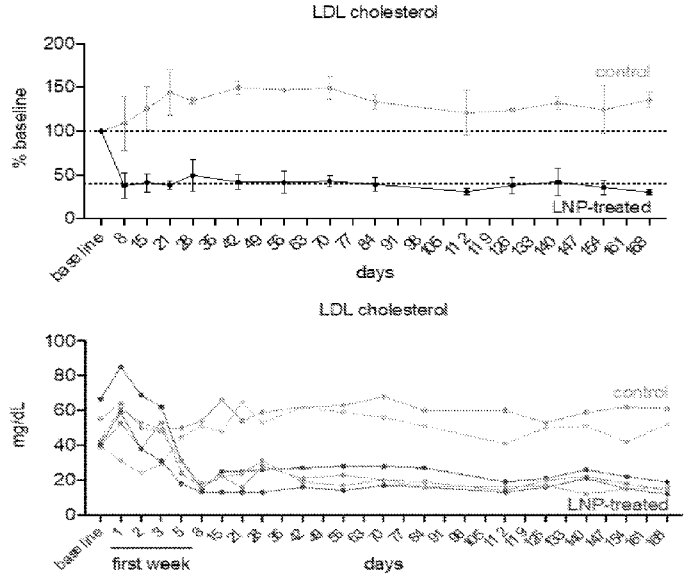


FIG. 34

(57) **Abstract:** Provided herein are compositions for gene modification or editing and methods of using same to treat or prevent certain conditions. Specific compositions and methods capable of safely and effectively editing gene targets expressed in the liver to durably lower LDL-C thereby treating a leading cause of cardiovascular disease are disclosed.

WO 2021/207712 A2

[Continued on next page]

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

— *with sequence listing part of description (Rule 5.2(a))*

BASE EDITING OF PCSK9 AND METHODS OF USING SAME FOR TREATMENT OF DISEASE

CROSS REFERENCE TO RELATED APPLICATIONS

[1] This application claims benefit under 35 U.S.C. §119 from Provisional Application Serial No. 63/007,803, filed April 9, 2020; Provisional Application Serial No. 63/007,797, filed April 9, 2020; Provisional Application Serial No. 63/136,087, filed January 11, 2021; Provisional Application Serial No.63/045,032, filed June 26, 2020; Provisional Application Serial No. 63/045,033, filed June 26, 2020, the disclosures of which are incorporated herein by reference in their entirety.

SEQUENCE LISTING

[2] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on April 8, 2021, is named 53989_707_601_SL.txt and is 1,105,762 bytes in size.

FIELD OF DISCLOSURE

[3] Provided herein are compositions for gene modification or editing, and methods of using same that are capable of treating or preventing certain conditions, such as cardiovascular disease and conditions or diseases associated therewith such as diabetes.

BACKGROUND

[4] All publications, patents, and patent applications mentioned in this specification are herein incorporated by references to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. It is not an admission that any publication or information specifically or implicitly referenced herein is prior art or necessarily relevant to the claimed subject matter. To the extent publications or patents or patent applications incorporated by reference contradict or are inconsistent with the disclosure contained in the specification, such cited or incorporated references should be considered supplementary to this disclosure with the understanding that the specification is intended to supersede and/or take precedence over any irreconcilable inconsistencies or contradictory material.

SUMMARY

[5] The inventive subject matter disclosed in this application is directed to compositions capable of editing a polynucleotide or target gene and methods of use of those compositions. The compositions and the constituent components thereof, individiualy and in combination, are considered separate aspects of the inventive subject matter, which may be defined and claimed by their respective physical and/or functional attributes described herein in any combination without limitation. The breadth, specificity, and variation of the inventive subject matter are further illustrated by specific aspects summarized here.

[6] One significant aspect of the inventive subject matter described herein is directed to the treatment of cardiovascular disease (CVD), which is the leading cause of death worldwide, responsible for nearly one in three deaths according to the World Health Organization. CVD is also a leading contributor to reductions in life expectancy and is one of the most expensive health conditions to care for. According to the Centers for Disease Control and Prevention (CDC), CVD is a significant economic burden, costing the U.S. healthcare system more than $320 billion per year in annual costs and lost productivity. CVD collectively refers to diseases of the heart and blood vessels, which are diagnosed as either atherosclerotic cardiovascular disease (ASCVD) or cardiomyopathy, among others. ASCVD is a large subset of CVD for which cholesterol drives the development of atherosclerotic plaque, a mixture of cholesterol, cells and cellular debris in the wall of a blood vessel that results in the hardening of the arteries. The current cornerstone of the treatment and prevention of ASCVD is to lower cumulative exposure to blood lipids aiming to maintain low-density lipoprotein cholesterol (LDL-C, commonly known as “bad” cholesterol) and/or triglycerides as low as possible for as long as possible. There is significant evidence, for example, demonstrating that individuals who maintain LDL-C at sufficiently low levels over a sufficiently long period of time are substantially less likely to develop ASCVD. The relationship between the lowering of LDL-C and reduction in ASCVD is amongst the best understood of all relationships in medicine. It has been shown that lowering LDL-C by 39 mg/dL for five years in a patient with established ASCVD reduces ASCVD risk by 21%, whereas that same 39 mg/dL degree of LDL-C reduction over a lifetime reduces risk of a first ASCVD event by 88%. The current standard of care is a chronic care model that typically requires numerous daily pills and/or intermittent injections and often insufficiently controls cumulative exposure to LDL-C. Despite the availability of such chronic care therapies, cumulative exposure to LDL-C is often insufficiently controlled in many patients with ASCVD, and a large fraction of individuals with established ASCVD have LDL-C levels

above the recommend goal. Higher cumulative LDL-C exposure leads to accelerated cholesterol plaque build-up in the heart or neck arteries and the rupture of which can result in heart attack, cardiac death, stroke and the need for invasive medical procedures, such as intracoronary stenting and coronary artery bypass surgery.

[7] One aspect, disclosed herein, are compositions and methods that are capable of safely and effectively editing gene targets expressed in the liver to durably lower LDL-C and/or triglycerides thereby treating CVD such as ASCVD.

[8] Another aspect, disclosed herein, is the efficacy and safety of the gene editing compositions described herein when administered as a single-course or dose (once-and-done) therapy, in repeat or successive doses, and combination gene editing therapy doses. The efficacy and safety of the compositions described herein are shown in *in vitro* and *in vivo* studies described herein involving various cell and animal experiments including mouse and non-human primate experiments, and the results or performance of the compositions disclosed herein represented in those studies each constitute aspects of the invention.

[9] Another aspect of the composition and methods disclosed herein relates to the location where the edit in the gene is made, including for example compositions and methods directed at editing a gene in the splice site.

[10] Another aspect of the compositions and methods disclosed herein are the constituent guide RNAs (gRNAs) and base editors that comprise the compositions that are capable of precisely editing a gene at a single base pair without imparting double-stranded breaks in the target gene. The compositions and method of use of those gRNAs and base editors including nucleotide or mRNA sequences that express and encode the base editor constitute yet another aspect.

[11] Another aspect, disclosed herein, are the lipid nanoparticle (LNPs) formulations that encapsulate the gRNA and base editor drug substances, the selection of an LNP, and the relative ratios between the various components of the drug substance compositions alone and as part of the LNP.

[12] Another aspect, disclosed herein, is directed at the dosing of gene editing compositions, such as those described herein, and the impact of dosing and repeat dosing on efficacy and safety profile indicia.

[13] Another aspect, disclosed herein, are that the compositions and methods of use include implementations that are designed to target or edit specific genes such PCSK9, ANGPTL3, APOC3, and/or Lp(a) and/or have an impact on the protein levels of proteins expressed by those genes.

[14] In one aspect, provided herein is a composition for editing a gene target comprising: (i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same, (ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a PCSK9 gene, wherein the guide RNA directs the base editor fusion protein to effect a nucleobase alteration in the PCSK9 gene *in vivo* when administered to a mammalian subject, wherein when the guide RNA and the mRNA is administered at a total amount of at least 0.05 mg/kg, the base alteration occurs in at least 35% of whole liver cells in the mammalian subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the mammalian subject is a cynomolgus monkey, wherein when the guide RNA and the mRNA is administered at a total amount of at least 0.5 mg/kg, the base alteration occurs in at least 40% of whole liver cells in the cynomolgus monkey as measured by next generation sequencing or Sanger sequencing. In some embodiments, the mammalian subject is a cynomolgus monkey, wherein when the guide RNA and the mRNA is administered at a total amount of at least 1 mg/kg, the base alteration occurs in at least 45% of whole liver cells in the cynomolgus monkey as measured by next generation sequencing or Sanger sequencing. In some embodiments, the mammalian subject is a cynomolgus monkey, wherein when the guide RNA and the mRNA is administered at a total amount of at least 1.5 mg/kg, the base alteration occurs in at least 50% of whole liver cells in the cynomolgus monkey as measured by next generation sequencing or Sanger sequencing. In some embodiments, the mammalian subject is a cynomolgus monkey, wherein when the guide RNA and the mRNA is administered at a total amount of at least 3 mg/kg, the base alteration occurs in at least 55% of whole liver cells in the cynomolgus monkey as measured by next generation sequencing or Sanger sequencing. In some embodiments, the mammalian subject is a mouse, wherein when the guide RNA and the mRNA is administered at a total amount of at least 0.125 mg/kg, the base alteration occurs in at least 40% of whole liver cells in the mouse as measured by next generation sequencing or Sanger sequencing. In some embodiments, the mammalian subject is a mouse, wherein when the guide RNA and the mRNA is administered at a total amount of at least 0.5 mg/kg, the base alteration occurs in at least 45% of whole liver cells in the mouse as measured by next generation sequencing or Sanger sequencing. In some embodiments, the mammalian subject is a mouse, wherein when the guide RNA and the mRNA is administered at a total amount of at least 2 mg/kg, the base alteration occurs in at least 50% of whole liver cells in the mouse as measured by next generation sequencing or Sanger sequencing. In some embodiments, the

nucleobase alteration results in a reduction of at least 50% in blood low-density lipoprotein cholesterol (LDL-C) level in the subject as compared to prior to the administration. In some embodiments, the protospacer is located in a splice site. In some embodiments, the protospacer complementary sequence is in the antisense strand of the PCSK9 gene. In some embodiments, the protospacer complementary sequence is in the sense strand of the PCSK9 gene. In some embodiments, the base alteration happens outside of the protospacer on the PCSK9 gene (off-target sites), wherein the editing percentages of off-target sites set forth in Table 11 are below or equal to the editing percentages set forth in Table 11, respectively. In some embodiments, the deaminase is an adenine deaminase and wherein the nucleobase alteration is a A•T to G•C alteration. In some embodiments, the programmable DNA binding domain comprises a nuclease inactive Cas9 or a Cas9 nickase. In some embodiments, the nucleobase alteration is at a splice site of the PCSK9 gene. In some embodiments, the nucleobase alteration is at a splice donor site of the PCSK9 gene. In some embodiments, the splice donor site is at 5' end of PCSK9 intron 1 as referenced in SEQ ID NO: 5. In some embodiments, the nucleobase alteration is at a splice acceptor site of the PCSK9 gene. In some embodiments, the nucleobase alteration results in a frame shift, a premature stop codon, an insertion or deletion in a transcript encoded by the PCSK9 gene. In some embodiments, the nucleobase alteration results in an aberrant transcript encoded by the PCSK9 gene. In some embodiments, the guide RNA is chemically modified. In some embodiments, the tracr sequence of the guide RNA is chemically modified following the scheme depicted in Fig. 7. In some embodiments, the spacer sequence comprises a PCSK9 ABE guide RNA spacer sequence set forth in Table 1. In some embodiments, the guide RNA comprises the PCSK9 ABE guide RNA sequence of GA096, GA097, GA343, GA346, GA375-377, GA380-389, GA391, GA439 or GA440 as set forth in Table 1. In some embodiments, the protospacer sequence comprises a PCSK9 ABE protospacer sequence set forth in Table 1. In some embodiments, the protospacer comprises the sequence 5'-CCCGCACCTTGGCGCAGCGG-3' (SEQ ID No: 13) or 5'-CCGCACCTTGGCGCAGCGG-3' (SEQ ID No: 247). In some embodiments, the base editor fusion protein comprises an amino acid sequence of SEQ ID No: 2137. In some embodiments, the GC% content of the mRNA sequence is greater than 50%. In some embodiments, the GC% content of the mRNA sequence is greater than 56%. In some embodiments, the GC% content of the mRNA sequence is greater than or equal to 63%. In some embodiments, the mRNA comprises an adenine tTNA deaminase (TadA) region, a Cas9 region and a nuclear localization sequence (NLS) region. In some embodiments, the mRNA further comprises a first linker region which connects the TadA region and the Cas9

region, and a second linker region which connects the Cas9 region and the NLS region. In some embodiments, the GC% content of the TadA region is greater than 60%. In some embodiments, the GC% content of the TadA region is greater than or equal to 70%. In some embodiments, the GC% content of the Cas9 region is greater than 56%. In some embodiments, the GC% content of the Cas9 region is greater than or equal to 62%. In some embodiments, the GC% content of the NLS region is greater than 54%. In some embodiments, the GC% content of the NLS region is greater than or equal to 63%. In some embodiments, the GC% content of the first linker region is greater than 65%. In some embodiments, the GC% content of the first linker region is greater than or equal to 79%. In some embodiments, the GC% content of the second linker region is greater than 67%. In some embodiments, the GC% content of the second linker region is greater than or equal to 83%. In some embodiments, the GC% content of the TadA region is greater than 60%, the GC% content of the Cas9 region is greater than 56%, the GC% content of the NLS region is greater than 54%, the GC% content of the first linker region is greater than 65%, and the GC% content of the second linker region is greater than 67%. In some embodiments, the mRNA comprises a mRNA sequence selected from Table 23. In some embodiments, the mRNA comprises a mRNA sequence of SEQ ID No: 2136. In some embodiments, the mRNA comprises a poly A tail. In some embodiments, the composition further comprises a lipid nanoparticle (LNP) enclosing (i). In some embodiments, the LNP further encloses (ii). In some embodiments, the composition further comprises a second LNP enclosing (ii). In some embodiments, the ratio of the guide RNA and the mRNA encoding the base editor fusion protein is about 1:10 to about 10:1 by weight. In some embodiments, the ratio of the guide RNA and the mRNA encoding the base editor fusion protein is about 1:1, 1.5:1, 2:1, 3:1, 4:1, 1:1.5, 1:2, 1:3, or 1:4 by weight. In some embodiments, the ratio of the guide RNA and the mRNA encoding the base editor fusion protein is about 1:1 by weight.

[15] In another aspect, provided herein is a pharmaceutical composition comprising the composition as provided herein and a pharmaceutically acceptable carrier or excipient.

[16] In another aspect, provided herein is a method for treating or preventing a condition in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the composition as provided herein. In some embodiments, the administration is via intravenous infusion. In some embodiments, the method comprises sequential administration of a LNP enclosing (i) and a LNP enclosing (ii). In some embodiments, the method comprises concurrent administration of the t LNP enclosing (i) and the LNP enclosing (ii). In some embodiments, the method comprises

administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 1 day. In some embodiments, the method comprises administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 2 days. In some embodiments, the method comprises administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 3 days. In some embodiments, the method comprises administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 4 days. In some embodiments, the method comprises administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 5 days. In some embodiments, the method comprises administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 6 days. In some embodiments, the method comprises administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 7 days. In some embodiments, the method comprises administering a single dose of the LNP enclosing (i) and (ii). In some embodiments, the single dose of the LNP is at about 0.3 to about 3mg/kg. In some embodiments, the method comprises administering a treatment course of one or more treatments to the subject, wherein each one of the one or more treatment comprises one or more of the single doses of the LNP. In some embodiments, the method comprises administering a treatment course of two to ten treatments. In some embodiments, the method comprises administering a treatment course of two to five treatments. In some embodiments, the method comprises administering a treatment course of two treatments. In some embodiments, the method comprises administering a treatment course of three treatments. In some embodiments, the method comprises administering a treatment course of four treatments. In some embodiments, the method comprises administering a treatment course of five treatments. In some embodiments, the condition is an atherosclerotic cardiovascular disease. In some embodiments, the condition is an atherosclerotic vascular disease. In some embodiments, the subject is a human.

[17] In another aspect, provided herein is a composition for editing a gene target comprising: (i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same, (ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a PCSK9 gene, wherein the guide RNA directs the base editor fusion protein to effect a nucleobase alteration in the PCSK9 gene *in vivo*

when administered to a mammalian subject, and wherein the guide RNA comprises the PCSK9 ABE guide RNA sequences as set forth in Table 1. In another aspect, provided herein is a composition for editing a gene target comprising: (i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same, (ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a PCSK9 gene, wherein the guide RNA directs the base editor fusion protein to effect a nucleobase alteration in the PCSK9 gene *in vivo* when administered to a mammalian subject, and wherein the mRNA comprises a sequence selected from Table 23.

[18] In another aspect, provided herein is a method for treating or preventing an atherosclerotic cardiovascular disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a first composition, comprising (i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same, (ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a PCSK9 gene, wherein the guide RNA directs the base editor fusion protein to effect a nucleobase alteration in the PCSK9 gene *in vivo* when administered to a mammalian subject, wherein when the guide RNA and the mRNA is administered at a total amount of at least 0.05 mg/kg, the base alteration occurs in at least 35% of whole liver cells in the mammalian subject as measured by next generation sequencing or Sanger sequencing; and a second composition, comprising (i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same, (ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a ANGPTL3 gene, wherein the guide RNA directs the base editor fusion protein to effect a nucleobase alteration in the ANGPTL3 gene *in vivo* when administered to a mammalian subject, wherein when the guide RNA and the mRNA is administered at a total amount of at least 0.5 mg/kg, the base alteration occurs in at least 35% of whole liver cells in the mammalian subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the method comprises sequential administration of the first composition and the second composition. In some embodiments, the method comprises administering one or more doses of the first composition followed by one or more dose of the second composition. In some embodiments, the method comprises administering one or more doses of the second composition followed by one or more dose of the first composition. In some

embodiments, the method comprises concurrent administration of the first composition and the second composition. In some embodiments, the method comprises one or more doses of the first composition and the second composition.

[19] In another aspect, provided herein is a composition for editing a gene target comprising: (i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same, (ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a APOC3 gene, wherein the guide RNA comprises the APOC3 ABE guide RNA sequence of GA300-303 as set forth in Table 24. In another aspect, provided herein is a composition for editing a gene target comprising: (i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same, (ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a PCSK9 gene, wherein the guide RNA directs the base editor fusion protein to effect a nucleobase alteration in the PCSK9 gene *in vitro*, wherein when the guide RNA and the mRNA is administered at a total amount of at least 2.5 mg/kg, the base alteration occurs in at least 35% of whole liver cells in the mammalian subject as measured by next generation sequencing or Sanger sequencing. In another aspect, provided herein is a composition for editing a PCSK9 gene comprising: (a) a mRNA encoding an adenine base editor protein having an editing window, and (b) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor protein and a spacer sequence that serves to guide the base editor protein to a protospacer sequence on the PCSK9 gene, wherein the spacer sequence is complimentary, at least in part, to a splice site or an exon region of the PCSK9 gene. In some embodiments, when the base editor protein is operatively bound to the guide RNA and the guide RNA is hybridized with the complementary strand to the protospacer sequence on the PCSK9 gene, the editing window encompasses the splice site of the PCSK9 gene. In some embodiments, when the base editor protein is operatively bound to the guide RNA and the guide RNA is hybridized with the complementary strand to the protospacer sequence on the PCSK9 gene, the editing window encompasses a region of an intron of the PCSK9 gene. In some embodiments, when the base editor protein is operatively bound to the guide RNA and the guide RNA is hybridized with the complementary strand to the protospacer sequence on the PCSK9 gene, the editing window encompasses a region of intron 1, intron 3 or intron 4 of the PCSK9 gene. In some embodiments, when the base editor protein is operatively bound to the guide RNA and the

guide RNA is hybridized with the complementary strand to the protospacer sequence on the PCSK9 gene, the editing window encompasses a region of intron 1 of the PCSK9 gene. In some embodiments, the spacer sequence has a 80-100 % nucleotide sequence identity to a spacer sequence selected from the group of guide RNA sequences identified as GA066, GA073 and GA074. In some embodiments, the tracr sequence has a 80-100% nucleotide sequence identity to a tracr sequence selected from the group of guide RNA sequences identified as GA066, GA095, GA096, GA097, GA343, GA346, GA375, GA376, GA377, GA380, GA381, GA382, GA383, GA384, GA385, GA386, GA387, GA388, GA389, GA439, and GA440. In some embodiments, the mRNA has an 80-100% sequence identity to the mRNA sequences identified as MA002, MA004, MA040, MA0041, or MA045. In some embodiments, the mRNA has one or more of the GC nucleotide region percentages set forth in the following table:

Nucleotide region	Average GC Nucleotide Content
27-213	67-73%
389-661	67-71%
735-829	63-74%
4207-4286	67-70%
4537-4569	65-73%
4683-4741	62- 67%

[20] In some embodiments, the mRNA has one or more of the GC nucleotide region percentages set forth in the following table:

Nucleotide region	Average GC Nucleotide Content
27-213	At least 73%
389-661	At least 71%
735-829	At least 74%
4207-4286	At least 70%
4537-4569	At least 73%
4683-4741	At least 67%

[21] In some embodiments, the mRNA and gRNA are encapsulated within a lipid nanoparticle. In some embodiments, the mRNA and gRNA are encapsulated within a lipid nanoparticle having the following: LNP composition (mol%):

40-65% iLipid

2-20% DSPC

1-5% PEG

Remaining mol% balance is cholesterol;

LNP Particle size: 55-120 nm Z average hydrodynamic diameter; and

Polydispersity index of <0.2 as determined by dynamic light scattering.

[22] In some embodiments, the mRNA and gRNA are encapsulated within the lipid nanoparticle having an LNP particle size between 50-70 nm Z average hydrodynamic diameter. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the *PCSK9* target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 40 percent. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 50 percent. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 30 percent. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 40 percent. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the

cynomolgus monkeys with an average editing percentage of greater than 50 percent. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 60 percent. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 70 percent. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 40 percent. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 50 percent. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 60 percent. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 70 percent. In some embodiments, the composition when at administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 80 percent. In some

embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 40 percent. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 50 percent. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 60 percent. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 70 percent. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 80 percent. In some embodiments, the percent editing is determined at 15 days after dosing through analysis of dosed cynomolgus monkey liver either via liver biopsy or necropsy of the monkey. In some embodiments, the percent editing is determined to be durably maintained by periodic liver biopsy testing of the dosed cynomolgus monkeys over a span of at least 168 days after dosing. In some embodiments, the percent editing is determined to be durably maintained by periodic liver biopsy testing of the dosed cynomolgus monkeys over a span of at least 300 days after dosing. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline. In

some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed monkeys on average of at least 60 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 70 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 80 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline. In some embodiments, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at

least 50 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 60 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 70 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 80 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 60 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9

protein in the plasma of the dosed cynomolgus monkeys on average of at least 70 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 80 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 60 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 70 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 80 percent as compared to baseline. In some embodiments, the reduction in plasma protein is determined at 15 days after dosing via blood sampling and analysis of the dosed cynomolgus monkey. In some embodiments, the composition when administered to a

group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of monkey weight is capable of reducing LDL-C in the plasma of the dosed monkeys on average of at least 20 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 25 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 30 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 45 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 20 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 25 percent as compared to baseline. In some embodiments, the composition when

administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 30 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 45 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 55 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 60 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 20 percent as

compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 25 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 30 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 45 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 55 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed

cynomolgus monkeys on average of at least 60 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 65 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 20 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 25 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 30 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 45 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is

capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 55 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 60 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 65 percent as compared to baseline. In some embodiments, the reduction in LDL-C is determined at 15 days after dosing via blood sampling and analysis of the dosed cynomolgus monkey. In some embodiments, the reduction in LDL-C is determined to be durably maintained over a span of at least 168 days by periodic blood sampling and analysis of the dosed cynomolgus monkey. In some embodiments, the reduction in LDL-C is determined to be durably maintained over a span of at least 300 days by periodic blood sampling and analysis of the dosed cynomolgus monkey. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing lipoprotein(a) in the plasma of the dosed cynomolgus monkeys on average of at least 10 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing lipoprotein(a) level in the plasma of the dosed cynomolgus monkeys on average of at least 15 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing lipoprotein(a) level in the plasma of the dosed cynomolgus monkeys on average of at least 20 percent as compared to baseline. In some embodiments, the

composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing lipoprotein(a) level in the plasma of the dosed cynomolgus monkeys on average of at least 25 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing lipoprotein(a) level in the plasma of the dosed cynomolgus monkeys on average of at least 30 percent as compared to baseline. In some embodiments, the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing lipoprotein(a) level in the plasma of the dosed cynomolgus monkeys on average of approximately 35 percent as compared to baseline. In some embodiments, the reduction in lipoprotein(a) is determined at 15 days after dosing via blood sampling and analysis of the dosed cynomolgus monkey. In some embodiments, the reduction in lipoprotein(a) is determined to be durably maintained over a span of at least 224 days by periodic blood sampling and analysis of the dosed cynomolgus monkey. In some embodiments, the reduction in lipoprotein(a) is determined to be durably maintained over a span of at least 300 days by periodic blood sampling and analysis of the dosed cynomolgus monkey. In some embodiments, to the extent that the dosing of the cynomolgus monkeys results in elevation of AST, ALT, or Cytokines, the elevations resulting from the dosing of the composition are transient and resolved back to approximately baseline levels within 3-15 days after dosing. In some embodiments, the percent editing of PCSK9 is negligible outside of the liver, spleen and adrenal glands tissues as illustrated in FIG. 27. In some embodiments, repeat dosing results is additive with respect to the editing percentage of PCSK9 editing percentage. In some embodiments, the repeat dosing does not elicit cytokine activation nor an immune response. In some embodiments, the spacer sequence has at least 80% nucleotide correlation with the nucleotide sequence of a targeted protospacer on the PCSK9 gene, wherein an RNA nucleotide on the spacer sequence is in correlation with a DNA nucleotide of the protospacer if it has the same nucleotide as the DNA nucleotide in the same order and wherein uracil and thymine bases are considered the same nucleotide for purposes of determining correlation. In some embodiments, the spacer sequence has at least 85% nucleotide correlation with the nucleotide sequence of a targeted protospacer on the PCSK9 gene. In some embodiments, the spacer sequence has at least 90% nucleotide correlation with

the nucleotide sequence of a targeted protospacer on the PCSK9 gene. In some embodiments, the spacer sequence has at least 95% nucleotide correlation with the nucleotide sequence of a targeted protospacer on the PCSK9 gene. In some embodiments, the spacer sequence has at least 99% nucleotide correlation with the nucleotide sequence of a targeted protospacer on the PCSK9 gene. In some embodiments, the spacer sequence has at least 100% nucleotide correlation with the nucleotide sequence of a targeted protospacer on the PCSK9 gene.

[23] In another aspect, provided herein is a method for treating or preventing an atherosclerotic cardiovascular disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of (a) a mRNA encoding an adenine base editor protein having an editing window, (b) a first guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor protein and a spacer sequence that serves to guide the base editor protein to a protospacer sequence on a PCSK9 gene, wherein the spacer sequence is complimentary, at least in part, to a splice site or an exon region of the PCSK9 gene; and (c) a second guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor protein and a spacer sequence that serves to guide the base editor protein to a protospacer sequence on a ANGPTL3 gene, wherein the spacer sequence is complimentary, at least in part, to a splice site or an exon region of the ANGPTL3 gene. In some embodiments, the method further comprises a first LNP enclosing (a). In some embodiments, the first LNP encloses (b) and (c). In some embodiments, the first LNP was administered repeatedly. In some embodiments, the first LNP was administered repeatedly at an interval of one to sixty days. In some embodiments, the first LNP was administered repeatedly at an interval of seven days. In some embodiments, the first LNP further encloses (b). In some embodiments, the method further comprises a second LNP enclosing (a) and (c). In some embodiments, the first LNP and the second LNP are administered sequentially. In some embodiments, the first LNP and the second LNP are administered sequentially at an interval of one day to 12 months. In some embodiments, the interval is one day. In some embodiments, the interval is five days. In some embodiments, the interval is ten days. In some embodiments, the interval is fifteen days. In some embodiments, the interval is twenty days. In some embodiments, the interval is twenty-five days. In some embodiments, the interval is one month. In some embodiments, the interval is two months. In some embodiments, the interval is three months. In some embodiments, the interval is five months. In some embodiments, the interval is eight months. In some embodiments, the interval is ten months. In some embodiments, the interval is twelve months.

BRIEF DESCRIPTION OF THE DRAWINGS

[24] The features, principles, advantages, and illustrative embodiments, implementations, analysis, and examples of the subject matter of this application are set forth herein including in the appended claims with aspects of which being illustrated in the accompanying drawings of which:

[25] **FIGs. 1A-1C** illustrate the modes of operation of Cas9, cytidine base editors (CBE), and adenine base editors (ABE) respectively, along with relevant terminology used in this application including "protospacer", "PAM", "spacer" (Mali, P. et al 2013 *Nat Methods* 10, 957-963; Anzalone, AV. 2020 *Nat Biotechnol* 38, 824-844). **FIG. 1D** is a schematic representation of how mcPCSK9 guide RNA (gRNA)–Tracr disclosed herein were designed. Shown is a ribonucleoprotein (RNP)–single guide RNA (sgRNA) alignment with stem-loop guide intramolecular interactions (W-C base pairing at the stem). The tracrRNA sequence of the gRNA serves as a binding scaffold for the cas protein. When designing a gRNA, loop nucleotides can be aligned with a protein and the interaction of base (H-bond), 2'-hydroxyl (2'-OH), 4'-oxygen (ring-oxygen) of the sugar moiety, phosphate linkage of each nucleotide to amino acid side chains of the protein can be considered in the design of a gRNA-Tracr. Spatial arrangement of nucleotide within RNP-steric interaction, room to accommodate bulky substitution like 2'-O-Methyl (2'-OMe) and phosphorothioate (PS) can be also taken into consideration. FIG. 1D discloses SEQ ID NOS 70-71, respectively, in order of appearance.

[26] **FIG. 2** are two tables that describe how SpCas9 crystal structures were chosen in connection with designing a gRNAs disclosed herein based on structure. The top panel is a table summarizing different components of CRISPR/Cas system with Protein Data Bank (PDB) IDs and the state. The bottom panel shows the root mean square deviation (RMSD) values after alignment between the protein chains of each structure. The RNP state (4ZT0) and pre-catalytic ternary complex (5F9R) are the most relevant to determine contacts related to RNP formation and contacts related to catalysis. Some rearrangement of the protein occurs between these two states. Massive rearrangements may occur in the protein between the apo state and the RNP.

[27] **FIG. 3** depicts a gRNA secondary structure of a sgRNA based on the 4ZT0 and 5F9R crystal structures, in which the sgRNA is bound to SpCas9 either as an RNP alone or as part of a ternary complex with DNA. Secondary structure relationships are shown in the Leontis and Westhof nomenclature (RNA, 2001, 7, 499–512) (SEQ ID NO: 72).

[28] **FIG. 4** depicts a gRNA secondary structure with a PCSK9 spacer showing predicted contacts based on RNP PDB 4ZT0 (sgRNA + SpCas9 RNP). Positions 1-10 and positions 83-

100 were not modeled in this crystal structure due to a lack of clear electron density (SEQ ID NO: 73). Black circle with white letter labels: protein contact, light gray with black letter: steric clash if 2'-O-Me incorporated or distant contact, dark grey with black letter: RNA contact. As a person of skill in the art would understand, the term "clash" as used herein refers to physically-unlikely overlapping atomic volumes in a structure. Incorporation of a 2'O-Me modification in these situations would potentially result in structural rearrangement(s) that could be detrimental to RNP function. As a person of skill in the art would understand, the term "contact" as used herein means a stable, non-covalent interaction between two functional groups, e.g. a hydrogen bond.

[29] **FIG. 5** depicts a gRNA secondary structure with a PCSK9 spacer showing predicted contacts based on PDB 5F9R (precatalytic ternary complex) (SEQ ID NO: 74). Black circle with white letter labels: protein contact, light gray with black letter: steric clash if 2'-O-Me incorporated or distant contact, dark grey with black letter: RNA contact.

[30] **FIG. 6** depicts plausible positions of 2'-OMe substitutions determined by structure-based designs (SEQ ID NO: 73). Black circle with white letter labels: 2'-OMe and phosphorothioate substitution, light gray circle with black letter labels: 2'-OMe substitution only, white circle with black letter labels: unmodified nucleotide.

[31] **FIG. 7A** depicts three patterns of 2'-OMe position identified in the tracr region of the single guide RNA from structure-guided incorporation of 2'-O-methylribosugar modification that produced robust editing in vivo: (1) mice: FIG. 16, GA054 (tracr1) and GA055 (tracr2) and (2) NHP: FIG. 28, GA096 (tracr1), GA097 (tracr2) and GA346 (tracr3). Black circle with white letter labels: 2'-OMe and phosphorothioate substitution, light gray circle with black letter labels: 2'-OMe substitution only, white circle with black letter labels: unmodified nucleotide. (SEQ ID NO: 73) **FIG. 7B** depicts the sequence alignment of unmodified SEQ ID NO: 61, a reference tracrRNA sequence ("Lit Tracr"), tracr1, tracr2, and tracr3. (SEQ ID NO: 73)

[32] **FIG. 8** shows base editing of the target splice site by an adenosine base editor system in modifying *PCSK9* in primary human hepatocytes. The dark line represents the percent splice site editing obtained using gRNA identified a GA066.

[33] **FIG. 9** depicts a Sanger sequencing chromatogram demonstrating editing of adenine base in the antisense strand at the splice donor at the end of *PCSK9* exon 1 (PCR amplification from the genomic DNA of the cells transfected with the 2500-ng/mL dose), portraying how splice-site disruption results in an in-frame stop codon. Heterozygosity for a naturally occurring single nucleotide polymorphism (SNP) is evident downstream of the

editing site. The scheme shows A-to-G base editing by an adenosine base editor system to knock-out *PCSK9* in primary human hepatocytes. FIG. 9 discloses SEQ ID NO: 75.

[34] **FIG. 10** depicts a Sanger sequencing chromatogram demonstrating editing of adenine base in the antisense strand at the splice donor at the end of *ANGPTL3* exon 6 (PCR amplification from the genomic DNA of the cells transfected with the 2500-ng/mL dose), portraying how splice-site disruption results in an in-frame stop codon. A-to-G base editing effected by an adenosine base editor system in modifying *ANGPTL3* in primary human hepatocytes. FIG. 10 discloses SEQ ID NO: 76.

[35] **FIG. 11** shows three sanger sequencing chromatograms from PCR amplified genomic DNA isolated from: 1) untreated primary hepatocytes (top panel); 2) SpCas9 mRNA/PCSK9 gRNA GA097 treated primary hepatocytes (middle panel); 3) ABE8.8 mRNA/PCSK9 gRNA treated primary hepatocytes (bottom panel). The PCSK9-gRNA GA097 protospacer sequence is highlighted in grey. The arrow points to the position 6 of the protospacer that is targeted for A-to-G base editing by the ABE8.8 editor. The scissors depict the general site of double stranded break that occurs upon cutting by the SpCas9 nuclease at this target site. SpCas9 mRNA and GA097 delivery in cells resulted in significant gene editing at the site of the double strand break (as denoted with the scissors) but this was not seen in the ABE8.8 and GA097 delivery in cells. Instead, the same gRNA GA097 in combination with ABE8.8 mRNA resulted in robust A-to-G base editing. FIG. 11 discloses SEQ ID NO 77-79, respectively in order of appearance.

[36] **FIG. 12** depicts a schematic showing potential splicing outcomes with disruption of splice donor or splice acceptor sequences. Other outcomes are possible, such as inclusion of part of intron 1 in the splicing product. Alteration of the splice donor or splice acceptor sites are shown (top panel). Alternative splice donor sites within *PCSK9* intron 1 resulting from editing of *PCSK9* exon 1 splice-donor adenine base in primary human hepatocytes is shown in the bottom panel. *Four different primer pairs (Table 9) were used for RT-PCR of the control/treated samples.

[37] **FIG. 13** shows lack of guide RNA-dependent DNA off-target editing in primary human hepatocytes. Human primary hepatocytes were incubated with gRNA (GA097 or GA346) and ABE8.8 mRNA as described in Example 4. The off-target reading was calculated as net adenine editing (proportion of sequencing reads with alteration of one or more adenine bases in LNP-treated cells versus untreated cells) at the on-target *PCSK9* site and more than 50 candidate off-target *PCSK9* sites in primary human hepatocytes from four individual donors.

[38] **FIG. 14** depicts guide RNA-independent RNA editing, assessed in SpCas9-treated or ABE8.8-treated hepatocytes after 2 days (n = 4 biological replicates). Each replicate was compared against each of four untreated hepatocyte samples to eliminate any positions with editing that were common to both conditions. The jitter plots portray transcriptomic loci with editing in the treated sample (number indicates total edited loci identified in the treated sample, boxplot indicates median ± interquartile range of proportion of edited reads across all edited loci in the sample). gRNA GA097, SpCas9 mRNA MS010, and ABE8.8 mRNA MA004 were used in this study as described in example 4.

[39] **FIG. 15** shows *PCSK9* and *ANGPTL3* base editing with lipid nanoparticles (LNPs) formulated with an embodiment of base editor system, ABE mRNA and a guide RNA, in primary human hepatocytes and primary non-human primates (NHP) hepatocytes. The human-specific ANGPTL3 guide RNA showed low editing efficiency in NHP hepatocytes within the concentration evaluated, whereas the PCSK9 guide RNA cross-reactive to both human and NHP showed high editing efficiency in both cell lines.

[40] **FIG. 16** shows gene editing of *Pcsk9* in mice (n=2-5 mice) via LNPs containing SpCas9 mRNA MS002 (TriLink Biotechnologies) and mouse *Pcsk9*-targeting gRNA with different tracr designs (GA052, GA053, GA054, and GA055) at a 1:1 weight ratio. Wild-type C57BL/6 mice were dosed with 2 mg/kg of the LNP test article. The mg/kg dose was calculated based on total RNA and the total RNA is the quantified sum of mRNA and gRNA present in the LNP after formulation. Seven days after dosing, the mice were euthanized and genomic DNA was harvested from mouse liver, and then assessed for base editing of the target site with next-generation sequencing. All four guide RNAs evaluated carry same spacer and same pattern of chemical modification within the first 20 nucleotides from the 5'-end, but all four differ in chemical modification pattern within the tracr between nucleotides at position 21 and at position 100 of the 100-mer guide RNA.

[41] **FIG. 17** shows base editing of *PCSK9* in mice (n=5) via LNPs with an embodiment of base editor system, ABE mRNA MA004 and PCSK9 guide RNA GA256 (targeting mouse intron 1 splice donor). Wild-type C57BL/6 mice were dosed with 2 mg/kg total RNA of the LNP test article. Seven days after dosing, the mice were euthanized and genomic DNA was harvested from mouse liver, and then assessed for base editing of the target site with next-generation sequencing.

[42] **FIG. 18** depicts editing of the *Pcsk9* exon 1 splice-donor adenine base in wild-type mouse liver, assessed 1 week following treatment with different doses of same LNP

formulation with ABE8.8 mRNA MA004 and Pcsk9 gRNA GA256 (n = 4 to 5 mice per dosing group, bar indicates mean editing in group).

[43] **FIG. 19** depicts editing of the *Pcsk9* exon 1 splice-donor adenine base in wild-type mouse liver, after dosed with LNPs at 0.05mg/kg total RNA dose containing different ratios of gRNA GA256 and mRNA MA002. Additional guides GA255 and GA257 with different chemical modifications were also assessed for base editing efficiency, at mRNA to gRNA 1:1 wt ratio.

[44] **FIG. 20** shows the results from dosing LNPs containing ABE8.8 mRNA and mouse *Angptl3*-targeting gRNAs (GA258, GA259, GA260, GA349, GA353) at 0.05 mg/kg total RNA dose at a 1:1 weight ratio into mice. GA258, GA259 and GA260 contain three different structure-guided tracr design. GA349 and GA353 tracr designs were from published literature (Cell Reports, 2018 22, 2227–2235). The mice were later euthanized and genomic DNA was harvested from mouse liver, and then assessed for base editing of the target splice site with next-generation sequencing.

[45] **FIG. 21** is a schematic showing the general dosing strategy for introducing adenine base editing of a target gene in NHP via LNPs formulated with an embodiment of base editor system, ABE mRNA and a guide RNA, and subsequent analysis after 2 weeks.

[46] **FIG. 22** shows that administration of LNPs formulated with an embodiment of base editor system, ABE mRNA MA002 and PCSK9 guide RNA GA066, at 1mg/kg and 3mg/kg total RNA dose to cynomolgus monkeys via intravenous infusion, induced adenine base editing at the *PCSK9* target splice site in the liver of cynomolgus monkeys.

[47] **FIG. 23** shows that administration of LNPs formulated with an embodiment of base editor system, ABE mRNA MA004 and PCSK9 guide RNA GA066, to cynomolgus monkeys via intravenous infusion resulted in reduction in the blood PCSK9 protein level compared to pre-dosing levels at 2 weeks after dosing.

[48] **FIG. 24** shows that administration of LNPs at 3mg/kg formulated with an embodiment of base editor system, ABE mRNA MA002 and PCSK9 guide RNA GA066, to cynomolgus monkeys via intravenous infusion resulted in reduction of the blood low-density lipoprotein cholesterol (LDL-C) level at 1 and 2 weeks after dosing compared to pre-dosing levels.

[49] **FIGs. 25A-25C** show short-term adenine base editing of *PCSK9* in non-human primates. FIG. 25A depicts editing of the *PCSK9* exon 1 splice-donor adenine base in the livers of cynomolgus monkeys receiving an intravenous infusion of a 1 mg/kg dose of an LNP formulation with ABE8.8 mRNA MA004 and PCSK9 gRNA GA097 with necropsy at

either 2 weeks (three animals) or 24 hours (two animals) following treatment. For each animal, editing was assessed in samples collected from sites distributed throughout the liver (n = 8 samples; bar indicates mean editing in animal). Reduction of the blood PCSK9 protein level (FIG. 25B) or blood LDL-C level (FIG. 25C) in the three animals that underwent necropsy at 2 weeks following treatment are shown, comparing the level at 2 weeks versus the baseline pre-treatment level (n = 1 blood sample per animal).

[50] **FIGs. 26A-26C** shows adenine base editing of *PCSK9* in non-human primates. FIG. 26A depicts editing of the *PCSK9* exon 1 splice-donor adenine base in the livers of cynomolgus monkeys receiving an intravenous infusion of 0.5, 1.0, or 1.5 mg/kg dose of an LNP formulation with ABE8.8 mRNA MA004 and PCSK9 gRNA GA346. Reduction of the blood PCSK9 protein level (FIG. 26B) or blood LDL-C level (FIG. 26C) for the animals are shown.

[51] **FIG. 27** depicts tissue distribution of editing of the *PCSK9* exon 1 splice donor adenine base in the three animals that underwent necropsy at 2 weeks following treatment (n = 1 sample per animal for each indicated organ except liver; the liver data represent the means shown in a calculated from eight liver samples each). The LNP constituted with ABE mRNA MA004 and guide RNA GA346 was used in this study, and the dose administered was 0.5 mg/kg.

[52] **FIG. 28** illustrates editing of the *PCSK9* exon 1 splice-donor adenine base editing in the livers of cynomolgus monkeys following intravenous infusions of individual lipid nanoparticles (LNPs) constituted with ABE mRNA MA004 and different guide RNAs with same spacer but with different tracer modifications. The guide RNAs used in this study were GA066, GA096, GA097 and GA346. The gRNA GA097 from two different sources were used in the same study and the sources are identified as (1) and (2). For tracr comparison studies the LNPS were dosed at 1 mg/kg total RNA dose where the guide RNA and mRNA were mixed at 1:1 weight ratio. The GA066 with published tracr design (Cell Reports, 2018 22, 2227–2235) produced lower base editing in monkey compared to all other tracr designs (FIG. 7) - GA095, GA097, and GA346 - under same experimental conditions.

[53] **FIG. 29** shows SpCas9 nuclease versus adenine base editing of *PCSK9* in non-human primates. LNPs containing either SpCas9 mRNA and PCSK9 gRNA, (MS010/GA097) or ABE8.8 mRNA and PCSK9 gRNA (MA004/GA097) were infused intravenously in cynomolgus monkeys. The MS010/GA097 LNP was dosed at 0.75 and 1.5 mg/kg, and the MA004/GA097 LNP was dosed at 1 mg/kg total RNA dose. The MS010/GA097 LNP test article at 1.5 mg/kg produced low single digit gene editing in NHP whereas the ABE/GA097

test article produced about 40% adenine base editing at 1 mg/kg, showing the robustness of ABE base editor over SpCas9 system. All LNPs used in this study were prepared using same excipients and compositions.

[54] **FIG. 30** shows adenine base editing of *PCSK9* in non-human primates. This graph depicts editing of the *PCSK9* exon 1 splice-donor adenine base in the livers of cynomolgus monkeys (each bar is an individual animal, with editing recorded for multiple sampling areas) receiving an intravenous infusion of 0.5, 1.0, 1.5, or 3 mg/kg total RNA dose of LNP#1. LNP#2 at 3mg/kg total RNA was dosed as a benchmark from a previous study. Both LNP formulations contain ABE8.8 mRNA MA004 and PCSK9 gRNA GA346.

[55] **FIG. 31** shows the reduction of PCSK9 protein levels from basal on Day 15, from the same experiment described in FIG. 30.

[56] **FIG. 32** depicts editing of the *PCSK9* exon 1 splice-donor adenine base in the livers of four cynomolgus monkeys received an intravenous infusion of a 3 mg/kg total RNA dose of an LNP formulation with ABE8.8 mRNA MA004 and PCSK9 gRNA GA066. For each animal, editing was assessed in a liver biopsy sample at 2 weeks following treatment (n = 1 sample per animal).

[57] **FIG. 33** depicts reduction of the blood PCSK9 protein levels in the four animals from FIG. 32 (animals that received 3mg/kg total RNA dose of an LNP formulation with ABE8.8 mRNA MA004 and PCSK9 gRNA GA066) and in two contemporaneous control animals that received phosphate-buffered saline, comparing levels at various time points following treatment versus the baseline pre-treatment level (mean ± standard deviation for each group, n = 4 or n = 2, at each time point). The dotted lines indicate 100% and 10% of baseline levels, respectively.

[58] **FIG. 34** depicts reduction of the blood LDL-C level in the four animals from FIG. 32 (animals that received 3mg/kg total RNA dose of an LNP formulation with ABE8.8 mRNA MA004 and PCSK9 gRNA GA066) and in two contemporaneous control animals that received phosphate-buffered saline, comparing levels at various time points following treatment versus the baseline pre-treatment level (mean ± standard deviation for each group, n = 4 or n = 2, at each time point). The dotted lines indicate 100% and 40% of baseline levels, respectively (top panel). The absolute values of individual animals are shown in the bottom panel.

[59] **FIG. 35** depicts reduction of Lipoprotein(a) in the four animals from FIG. 32 and in two contemporaneous control animals that received phosphate-buffered saline, comparing

levels at various time points following treatment versus the baseline pre-treatment level (mean ± standard deviation for each group, n = 4 or n = 2, at each time point).

[60] **FIG. 36** shows long-term phenotypic effects of liver *PCSK9* base editing in non-human primates. Absolute values of aspartate aminotransferase (AST) (top panel), and alanine aminotransferase (ALT) (bottom panel) in the individual animals portrayed in FIG. 32 (n = 4 animals treated with 3 mg/kg dose of an LNP formulation with ABE8.8 mRNA and PCSK9-gRNA, and n = 2 animals treated with phosphate-buffered saline) at various time points following treatment.

[61] **FIGs. 37A-37G** show liver function markers of individual animals. AST (FIG. 37A, FIG. 37B), ALT (FIG. 37A, FIG. 37C), Alkaline phosphate (FIG. 37A, FIG. 37D), gamma-glutamyltransferase (FIG. 37A, FIG. 37E), total bilirubin (FIG. 37A, FIG. 37F), and albumin (FIG. 37A, FIG. 37G), up to 15 days post dose, from cynomolgus monkeys that received an intravenous infusion of a 0.5, 1.0, or 1.5 mg/kg dose of an LNP formulation with ABE8.8 mRNA and PCSK9 gRNA.

[62] **FIG. 38** is a schematic of the representative candidate ONE-seq sites using a specific library designed against the cynomolgus genome. The PCSK9 protospacer is depicted at the top with a ONE-seq score of 1.00. All sites listed are ranked by decreasing ONE-seq score, with mismatches to the protospacer sequence identified. FIG. 38 discloses SEQ ID NO: 80, 2193-2196, 566, 2197-2205, 675, 2206-2220, 564, 2221-224, 638, 644, 506, 621, 2225-2226, 681, and 2227-2230, respectively, in order of appearance.

[63] **FIG. 39** shows gRNA-dependent, DNA off-target analysis of the sites identified in FIG. 38. Samples from cynomolgus primary hepatocytes (top panel) or cynomolgus monkey livers (bottom panel) were assessed for net A>G base editing (n=3 treated, n=3 untreated samples).

[64] **FIG. 40** shows net A>G % base editing at one off-target site (C5), identified in FIG. 39, from livers of NHPs that received either 0.5, 1.0, or 1.5 mg/kg LNP.

[65] **FIG. 41** shows base editing of *ANGPTL3* in NHPs via LNPs formulated with an embodiment of base editor system, ABE mRNA MA004 and ANGPTL3 guide RNA GA067. Liver editing (left panel), ANGPTL3 protein levels (middle panel), and triglyceride levels (right panel) are shown for three NHPs.

[66] **FIG. 42** shows simultaneous ANGPTL3 and PCSK9 base editing with lipid nanoparticles (LNPs) formulated with an embodiment of base editor system, ABE mRNA MA002 and dual guide RNAs GA095 (hcPCSK9) and GA098 (hANGPTL3), in human primary hepatocytes at concentrations ranging from 0-2500 ng/test article/mL.

[67] **FIG. 43** shows Day 15 and Day 44 liver biopsy adenosine base editing results. NHPs were dosed with LNPs formulated with ABE8.8 mRNA MA004 and either a gRNA targeting *PCSK9* (GA346) or a gRNA targeting *ANGPTL3* (GA347) via intravenous infusion at a total RNA doses ranging from 0.5-2mg/kg. Two weeks after administration of test article, biopsies were performed to assess base editing. After 30 days from the initiation of the study, the opposite LNP was administered. Following a second biopsy after an additional 2 weeks, the gDNA was extracted, and base editing was assessed using next generation sequencing. Results for *PCSK9* base editing (top panel) and *ANGPTL3* base editing (bottom panel) are shown. 2 mg/kg total RNA dose of LNP encapsulating ABE8.8 mRNA, PCSK9 gRNA GA346 and ANGPTL3 gRNA GA347 at 1:0.5:0.5 weight ratio produced robust synchronized PCSK9 and ANGPTL3 gene editing (Example 10).

[68] **FIG. 44** illustrates the corresponding % change in PCSK9 (top panel) and ANGPTL3 (bottom panel) protein levels from NHPs described in FIG. 43.

[69] **FIG. 45** illustrates that repeat LNP dosing in NHPs causes additive adenosine base editing in the liver, post Day 14, Day 46, and Day75 liver biopsies. NHPs were dosed with either LNP#1 or LNP#2 formulated with ABE8.8 mRNA MA004 and a gRNA targeting PCSK9 (GA097) via intravenous infusion at a total RNA doses of 0.5mg/kg (see Example 10 for details on dosing intervals and related details).

[70] **FIG. 46** illustrates that repeat LNP dosing in NHPs causes additive base editing in the liver and translates to dose-dependent additive decrease in plasma PCSK9 protein levels over 90 days. As described in FIG. 45, NHPs were repeat dosed with LNPs formulated with ABE8.8 mRNA MA004 and a gRNA targeting PCSK9 (GA097). NHPs were dosed via intravenous infusion at a total RNA doses of 0.5mg/kg at days 0, 30, and 60 (arrow is illustrated on graph to depict dosing). For description of analysis of PCSK9 protein levels, see detailed methods section.

[71] **FIG. 47** illustrates that repeat LNP dosing in NHPs causes additive base editing in the liver with only transient liver marker increase, and the transient liver marker increase correlates well with the day of each dose administered. The data shows 71 days of the liver marker levels of ALT, AST, total bilirubin, and creatine kinase post first dose (see Example 10, FIG. 45 for details).

[72] **FIG. 48** illustrates that repeat LNP dosing in NHPs causes additive base editing in the liver, with only transient liver marker increase showing up to 71 days post dose of the liver enzyme levels of LDH, GLDH, GGT, and ALP, in NHPs (See Example 10, FIG. 45 for details).

[73] **FIGs. 49A and 49B** illustrate that base editing of ANGPTL3 results in long-term decreased ANGPTL3 protein and triglyceride levels after a single dose of LNP constituted with ABE8.8 mRNA MA004 and ANGPTL3 gRNA GA067. The results illustrates the effect of long-term adenine base editing of ANGPTL3 on ANGPTL3 protein **(FIG. 49A)** and triglycerides **(FIG. 49B)** in non-human primates over 6 months**.** ANGPTL3 protein (96% reduction) and triglyceride levels were substantially decreased upon single dose administration of the LNP, and remain stably reduced for more than 170 days (see Example 10 for details).

[74] **FIGs. 50A-50E** illustrate the effect of LNP dosing in NHPs on cytokine activation and immune response. Cynomolgus monkeys received intravenous infusions of 0.5 mg/kg doses at specified time points (**FIG. 50A** and **FIG. 50B**) of an LNP formulation with ABE8.8 mRNA MA004 and PCSK9 gRNA GA346. Blood was collected at timepoints specified and the graph, and IP-10 and MCP-1 were analyzed. In additional studies, IL-6, MCP-1, and SC5b-9 (**FIG. 50C**, **FIG. 50D**, and **FIG. 50E**, respectively) were analyzed at different time points from blood collected from NHPs that received an intravenous infusion of 1.0 mg/kg total RNA dose of LNP formulated with MA004 and PCSK9 gRNA GA346.

[75] **FIG. 51** illustrates liver editing in NHPs at 15 days after treatment with an LNP containing SpCas9 mRNA/gRNA. **FIG. 51** shows the results from gene editing of ANGPTL3 or PCSK9 in non-human primates. Cynomolgus monkeys received an intravenous infusion of a 1.5 mg/kg dose of an LNP formulation with SpCas9 mRNA MS004 and one gRNA targeting either ANGPTL3 (GA261-GA263) or PCSK9 (GA266-GA271). Upon necropsy after 2 weeks, two pieces from each liver lobe (8 pieces total) were isolated and gDNA was extracted. Samples were processed as described in the detailed methods section. Indel % was analyzed for each separate piece and are graphed as individual points. High editing efficiency was observed in most NHP livers.

[76] **FIG. 52** illustrates LDL-C levels in NHPs at 15 days after treatment with SpCas9/gRNA. **FIG. 52** shows the reduction of LDL-C from gene editing of ANGPTL3 or PCSK9 in non-human primates. Cynomolgus monkeys received an intravenous infusion of a 1.5 mg/kg dose of an LNP formulation with SpCas9 mRNA MS004 and one gRNA targeting either ANGPTL3 (GA261-GA263) or PCSK9 (GA266-GA271. Samples were processed as described in the detailed methods section. All NHPs that received LNPs with SpCas9 mRNA/PCSK9 gRNA had at least 35% reduction in circulating LDL-C levels. Although more modest, LNPs with SpCas9 mRNA/ANGPTL3 gRNA had 10-25% reduction in circulating LDL-C levels.

[77] **FIG. 53** illustrates triglyceride levels in NHPs at 15 days after treatment with SpCas9/gRNA. **FIG. 53** shows the triglyceride levels from gene editing of ANGPTL3 or PCSK9 in non-human primates. Cynomolgus monkeys received an intravenous infusion of a 1.5 mg/kg dose of an LNP formulation with SpCas9 mRNA MS004 and one gRNA targeting either ANGPTL3 (GA261-GA263) or PCSK9 (GA266-GA271. Samples were processed as described in the detailed methods section. NHPs that received LNPs with SpCas9 mRNA/ANGPTL3 gRNA had around 10-50% reduction in triglyceride levels. NHPs that received LNPs with SpCas9 mRNA/PCSK9 gRNA did not show a significant reduction in triglyceride levels.

[78] **FIGs. 54A and 54B** illustrate that PACE-modifications to the gRNA decrease off-target editing efficiency. Human primary hepatocytes were transfected at 2500, 1250, 500, and 250 ng/test article/mL with SpCas9 mRNA (commercially purchased from Trilink) and a gRNA targeting PCSK9 with modifications to the tracr. Genomic DNA was processed, sequenced, and analyzed as described in the detailed methods section. GA156 was transfected to serve as a positive control. GA248 and GA249 contain PACE-modifications to the gRNA that have previously been demonstrated to decrease off-target editing efficiency. Indeed, although GA248 and GA249 had lower on-target editing compared to the unmodified gRNA, GA156 (**FIG. 54A**), GA248 and GA249 showed decreased off-target editing at an identified off-target site (**FIG. 54B**).

[79] **FIG. 55A** illustrates the GC comparison of ABE-encoding nucleotides, MA004, MA019, MA020, MA021, and ABE8.8m (Table 23), as well as a more detailed look at MA004 (FIG 55A, bottom panel). **FIG. 55B** illustrates the editing % obtained using ABE-encoding nucleotides, MA004, MA019, MA020, and MA021 (Table 23).

DETAILED DESCRIPTION

[80] Certain specific details of this description are set forth in order to provide a thorough understanding of various embodiments. However, one skilled in the art will understand that the present disclosure may be practiced without these details. In other instances, well–known structures have not been shown or described in detail to avoid unnecessarily obscuring descriptions of the embodiments.

[81] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods, and materials are

described below. Further, headings provided herein are for convenience only and do not interpret the scope or meaning of the claimed disclosure. It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the present disclosure, and vice versa. Furthermore, compositions of the present disclosure can be used to achieve methods of the present disclosure.

Definitions

[82] To facilitate an understanding of the present disclosure, a number of terms and phrases are defined below.

[83] As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the content clearly dictates otherwise.

[84] It should also be noted that the term "or" is generally employed in its sense including "and/or" unless the content clearly dictates otherwise. The terms "and/or" and "any combination thereof" and their grammatical equivalents as used herein, can be used interchangeably. These terms can convey that any combination is specifically contemplated. Solely for illustrative purposes, the following phrases "A, B, and/or C" or "A, B, C, or any combination thereof" can mean "A individually; B individually; C individually; A and B; B and C; A and C; and A, B, and C." The term "or" can be used conjunctively or disjunctively, unless the context specifically refers to a disjunctive use.

[85] The term "about" or "approximately" can mean within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, up to 10%, up to 5%, or up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, within 5-fold, and more preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated the term "about" meaning within an acceptable error range for the particular value should be assumed.

[86] As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

[87] As used herein, "some embodiments," "an embodiment," "one embodiment," "embodiments" or "other embodiments" means that a particular feature, structure, or characteristic described in connection with the embodiments is included in at least some embodiments, but not necessarily all embodiments, of the present disclosures.

[88] The term "nucleic acid" as used herein refers to a polymer containing at least two nucleotides (*i.e.*, deoxyribonucleotides or ribonucleotides) in either single- or double-stranded form and includes DNA and RNA. "Nucleotides" contain a sugar deoxyribose (DNA) or ribose (RNA), a base, and a phosphate group. Nucleotides are linked together through the phosphate groups. "Bases" include purines and pyrimidines, which further include natural compounds adenine, thymine, guanine, cytosine, uracil, inosine, and natural analogs, and synthetic derivatives of purines and pyrimidines, which include, but are not limited to, modifications which place new reactive groups such as, but not limited to, amines, alcohols, thiols, carboxylates, and alkylhalides. A nucleic acid includes any oligonucleotide or polynucleotide, with fragments containing up to 60 nucleotides generally termed oligonucleotides, and longer fragments termed polynucleotides. A deoxyribooligonucleotide consists of a 5-carbon sugar called deoxyribose joined covalently to phosphate at the 5' and 3' carbons of this sugar to form an alternating, unbranched polymer. DNA may be in the form of, *e.g.*, antisense molecules, plasmid DNA, pre-condensed DNA, a PCR product, vectors, expression cassettes, chimeric sequences, chromosomal DNA, or derivatives and combinations of these groups. A ribooligonucleotide consists of a similar repeating structure where the 5-carbon sugar is ribose. Accordingly, the terms "polynucleotide" and "oligonucleotide" can refer to a polymer or oligomer of nucleotide or nucleoside monomers consisting of naturally-occurring bases, sugars and intersugar (backbone) linkages. Additionally, nucleic acids include nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, nonstandard, and/or non-naturally occurring, and which have similar binding properties as the reference nucleic acid. The nucleic acid may be modified at the base moiety (*e.g.*, at one or more atoms that typically are available to form a hydrogen bond with a complementary nucleotide and/or at one or more atoms that are not typically capable of forming a hydrogen bond with a complementary nucleotide), sugar moiety, or phosphate backbone. Backbone modifications can include, but are not limited to, a phosphorothioate, a phosphorodithioate, a phosphoroselenoate, a phosphorodiselenoate, a phosphoroanilothioate, a phosphoraniladate, a phosphoramidate, and a phosphorodiamidate linkage. A phosphorothioate linkage substitutes a sulfur atom for a non-bridging oxygen in the phosphate backbone and delays nuclease degradation of

oligonucleotides. A phosphorodiamidate linkage (N3'→P5') allows preventing nuclease recognition and degradation. Backbone modifications can also include having peptide bonds instead of phosphorous in the backbone structure (*e.g.*, N-(2-aminoethyl)-glycine units linked by peptide bonds in a peptide nucleic acid), or linking groups including carbamate, amides, and linear and cyclic hydrocarbon groups. Oligonucleotides with modified backbones are reviewed in Micklefield, Backbone modification of nucleic acids: synthesis, structure and therapeutic applications, Curr. Med. Chem., 8 (10): 1157-79, 2001 and Lyer et al., Modified oligonucleotides-synthesis, properties and applications, Curr. Opin. Mol. Ther., 1 (3): 344-358, 1999. Nucleic acid molecules described herein may contain a sugar moiety that comprises ribose or deoxyribose, as present in naturally occurring nucleotides, or a modified sugar moiety or sugar analog. The examples of modified sugar moieties include, but are not limited to, 2'-O-methyl, 2'-O-methoxyethyl, 2'-O-aminoethyl, 2'-Flouro, N3'→P5' phosphoramidate, 2'dimethylaminooxyethoxy, 2' 2′dimethylaminoethoxyethoxy, 2′-guanidinidium, 2′-O-guanidinium ethyl, carbamate modified sugars, and bicyclic modified sugars. 2'-O-methyl or 2'-O-methoxyethyl modifications promote the A-form or RNA-like conformation in oligonucleotides, increase binding affinity to RNA, and have enhanced nuclease resistance. Modified sugar moieties can also include having an extra bridge bond (*e.g.*, a methylene bridge joining the 2'-O and 4'-C atoms of the ribose in a locked nucleic acid) or sugar analog such as a morpholine ring (*e.g.*, as in a phosphorodiamidate morpholino). Examples of such analogs and/or modified residues include, but are not limited to diaminopurine, 5–fluorouracil, 5–bromouracil, 5–chlorouracil, 5–iodouracil, hypoxanthine, xantine, 4–acetylcytosine, 5–(carboxyhydroxylmethyl)uracil, 5–carboxymethylaminomethyl–2–thiouridine, 5–carboxymethylaminomethyluracil, dihydrouracil, beta–D–galactosylqueosine, inosine, N6–isopentenyladenine, 1–methylguanine, 1–methylinosine, 2,2–dimethylguanine, 2–methyladenine, 2–methylguanine, 3–methylcytosine, 5–methylcytosine, N6–adenine, 7–methylguanine, 5–methylaminomethyluracil, 5–methoxyaminomethyl–2–thiouracil, beta–D– mannosylqueosine, 5'–methoxycarboxymethyluracil, 5–methoxyuracil, 2–methylthio–N6– isopentenyladenine, uracil–5–oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2–thiocytosine, 5–methyl–2–thiouracil, 2–thiouracil, 4–thiouracil, 5–methyluracil, uracil–5– oxyacetic acid methylester, 5–methyl–2–thiouracil, 3–(3–amino– 3– N–2–carboxypropyl) uracil, (acp3)w, 2,6–diaminopurine, methyl phosphonates, chiral-methyl phosphonates, 2'-O-methyl ribonucleotides, peptide-nucleic acids (PNAs), and the like. In some cases, nucleotides may include modifications in their phosphate moieties, including modifications to a triphosphate

moiety. Non–limiting examples of such modifications include phosphate chains of greater length (e.g., a phosphate chain having, 4, 5, 6, 7, 8, 9, 10 or more phosphate moieties) and modifications with thiol moieties (e.g., alpha–thiotriphosphate and beta–thiotriphosphates). Such modified or substituted oligonucleotides are often preferred over native forms because of properties such as, for example, enhanced cellular uptake, reduced immunogenicity, and increased stability in the presence of nucleases. Thus, the terms "polynucleotide" and "oligonucleotide" can also include polymers or oligomers comprising non-naturally occurring monomers, or portions thereof, which function similarly.

[89] Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (*e.g.*, degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res., 19:5081 (1991); Ohtsuka et al., J. Biol. Chem., 260:2605-2608 (1985); Rossolini et al., Mol. Cell. Probes, 8:91-98 (1994)).

[90] The present disclosure encompasses isolated or substantially purified nucleic acid molecules and compositions containing those molecules. As used herein, an "isolated" or "purified" DNA molecule or RNA molecule is a DNA molecule or RNA molecule that exists apart from its native environment. An isolated DNA molecule or RNA molecule may exist in a purified form or may exist in a non-native environment such as, for example, a transgenic host cell. For example, an "isolated" or "purified" nucleic acid molecule or biologically active portion thereof, is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. In one embodiment, an "isolated" nucleic acid is free of sequences that naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in some embodiments, the isolated nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, or 0.1 kb of nucleotide sequences that naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived.

[91] The term "vector," as used herein, refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. In some examples, a vector is an expression vector that is capable of directing the expression of nucleic acids to which they

are operatively linked. The term "operably linked," as used herein, means that the nucleotide sequence of interest is linked to regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence. The term "regulatory sequence," as used herein, includes, but is not limited to promoters, enhancers and other expression control elements. Such regulatory sequences are well known in the art and are described, for example, in Goeddel; Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). Examples of expression vectors include, but are not limited to, plasmid vectors, viral vectors based on vaccinia virus, poliovirus, adenovirus, adeno-associated virus, SV40, herpes simplex virus, human immunodeficiency virus, retrovirus (e.g., Murine Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, a lentivirus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus) and other recombinant vectors.

[92] As used herein, the terms "protein," "polypeptide," and "peptide" are used interchangeably and refer to a polymer of amino acid residues linked via peptide bonds and which may be composed of two or more polypeptide chains. The terms "polypeptide," "protein," and "peptide" refer to a polymer of at least two amino acid monomers joined together through amide bonds. An amino acid may be the L–optical isomer or the D–optical isomer. More specifically, the terms "polypeptide," "protein," and "peptide" refer to a molecule composed of two or more amino acids in a specific order; for example, the order as determined by the base sequence of nucleotides in the gene or RNA coding for the protein. Proteins are essential for the structure, function, and regulation of the body's cells, tissues, and organs, and each protein has unique functions. Examples are hormones, enzymes, antibodies, and any fragments thereof. In some cases, a protein can be a portion of the protein, for example, a domain, a subdomain, or a motif of the protein. In some cases, a protein can be a variant (or mutation) of the protein, wherein one or more amino acid residues are inserted into, deleted from, and/or substituted into the naturally occurring (or at least a known) amino acid sequence of the protein. A protein or a variant thereof can be naturally occurring or recombinant. Methods for detection and/or measurement of polypeptides in biological material are well known in the art and include, but are not limited to, Western–blotting, flow cytometry, ELISAs, RIAs, and various proteomics techniques. An exemplary method to measure or detect a polypeptide is an immunoassay, such as an ELISA. This type of protein quantitation can be based on an antibody capable of capturing a specific antigen, and a second antibody capable of detecting the captured antigen. Exemplary assays for

detection and/or measurement of polypeptides are described in Harlow, E. and Lane, D. Antibodies: A Laboratory Manual, (1988), Cold Spring Harbor Laboratory Press.

[93] The term "sequence identity," as used herein, refers to the amount of nucleotide which match exactly between two different sequences. When comparing RNA and DNA sequences Uracil and Thymine bases are considered to be the same base. Gaps are not counted and the measurement is typically in relation to the shorter of the two sequences. For example:

A: AAGGCTT

B: AAGGC

C: AAGGCAT

Here identity (A,B)=100% (5 identical nucleotides / min(length(A),length(B))). Identity(B,C)=100%, but identity(A,C)=85% ((6 identical nucleotides / 7). So 100% identity does not mean two sequences are the same.

[94] The term "sequence similarity," as used herein, can be described as an optimal matching problem that finds the minimal number of edit operations (inserts, deletes, and substitutions) in order to transform the one sequence into an exact copy of the other sequence being aligned (edit distance). Using this, the percentage sequence similarity of the examples above are sim(A,B)=60%, sim(B,C)=60%, sim(A,C)=86% (semi-global, sim=1-(edit distance/unaligned length of the shorter sequence)).

[95] A "subject" in need thereof, refers to an individual who has a disease, a symptom of the disease, or a predisposition toward the disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disease, the symptom of the disease, or the predisposition toward the disease. In some embodiments, the subject has hypercholesterolemia. In some embodiments, the subject has atherosclerotic vascular disease. In some embodiments, the subject has hypertriglyceridemia. In some embodiments, the subject has diabetes. The term "subject" or "patient" encompasses mammals. Examples of mammals include, but are not limited to, any member of the mammalian class: humans, non-human primates such as chimpanzees, and other apes and monkey species; farm animals such as cattle, horses, sheep, goats, swine; domestic animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice and guinea pigs, and the like.

[96] The term "condition," as used herein, includes diseases, disorders, and susceptibilities. In some embodiments, the condition is an atherosclerotic vascular disease. In some embodiments, the condition is a hypertriglyceridemia. In some embodiments, the condition is a diabetes.

[97] The term "atherosclerosis" or "atherosclerotic vascular disease," as used herein, refers to a disease in which the inside of an artery narrows due to the buildup of plaque. In some embodiments, it may result in coronary artery disease, stroke, peripheral artery disease, or kidney problems.

[98] The term "hypertriglyceridemia," as used herein, refers to high (hyper-) blood levels (-emia) of triglycerides, the most abundant fatty molecule in most organisms. In some embodiments, elevated levels of triglycerides are associated with atherosclerosis, even in the absence of hypercholesterolemia (high cholesterol levels), and predispose to cardiovascular disease. In some embodiments, very high triglyceride levels increase the risk of acute pancreatitis. In some embodiments, hypertriglyceridemia is associated with overeating, obesity, diabetes mellitus and insulin resistance, excess alcohol consumption, kidney failure, nephrotic syndrome, genetic predisposition (e.g., familial combined hyperlipidemia, i.e., Type II hyperlipidemia), lipoprotein lipase deficiency, lysosomal acid lipase deficiency, cholesteryl ester storage disease, certain medications (e.g., isotretinoin, hydrochlorothiazide diuretics, beta blockers, protease inhibitors), hypothyroidism (underactive thyroid), systemic lupus erythematosus and associated autoimmune responses, glycogen storage disease type 1, propofol, or HIV medications.

[99] The term "diabetes," as used herein, refers to a group of metabolic disorders characterized by a high blood sugar level over a prolonged period of time. In some embodiments, diabetes is type 1 diabetes that results from the pancreas's failure to produce enough insulin due to loss of beta cells. In some embodiments, diabetes is type 2 diabetes characterized by insulin resistance, a condition in which cells fail to respond to insulin properly. In some embodiments, diabetes is gestational diabetes that occurs when pregnant women without a previous history of diabetes develop high blood sugar levels.

[100] The term "low-density lipoprotein (LDL)," as used herein, refers to a microscopic blob made up of an outer rim of lipoprotein and a cholesterol center. In some embodiments, LDL has a highly hydrophobic core composed of a polyunsaturated fatty acid known as linoleate and hundreds to thousands esterified and unesterified cholesterol molecules. In some embodiments, the core of LDL also carries triglycerides and other fats and is surrounded by a shell of phospholipids and unesterified cholesterol.

[101] The term "high-density lipoprotein (HDL)," as used herein, refers to the smallest lipoprotein particles. In embodiments, plasma enzyme lecithin-cholesterol acyltransferase (LCAT) converts the free cholesterol into cholesteryl, which is then sequestered into the core of the lipoprotein particle, eventually causing the newly synthesized HDL to assume a

spherical shape. In embodiments, HDL particles increase in size as they circulate through the bloodstream and incorporate more cholesterol and phospholipid molecules from cells and other lipoproteins.

[102] The term "cholesterol," as used herein, refers to a lipid with a unique structure composed of four linked hydrocarbon rings forming the bulky steroid structure. The term "triglyceride," as used herein, refers to a tri-ester composed of a glycerol bound to three fatty acid molecules. In some embodiments, the fatty acids are saturated or unsaturated fatty acids.

[103] The terms "treat," "treating," or "treatment," and its grammatical equivalents as used herein, can include alleviating, abating, or ameliorating at least one symptom of a disease or a condition, preventing additional symptoms, inhibiting the disease or the condition, *e.g.*, delaying, decreasing, suppressing, attenuating, diminishing, arresting, or stabilizing the development or progression of a disease or the condition, relieving the disease or the condition, causing regression of the disease or the condition, relieving a condition caused by the disease or the condition, reducing disease severity, or stopping the symptoms of the disease or the condition either prophylactically and/or therapeutically. "Treating" also includes lessening the frequency of occurrence or recurrence, or the severity, of any symptoms or other ill effects related to a disease or condition and/or the side effects associated with the disease or condition. "Treating" does not necessarily require curative results. It is appreciated that, although not precluded, treating a disorder or condition also does not require that the disorder, condition, or symptoms associated therewith be completely eliminated. The term "treating" encompasses the concept of "managing" which refers to reducing the severity of a particular disease or disorder in a patient or delaying its recurrence, *e.g.*, lengthening the period of remission in a patient who had suffered from the disease. "Treating" may refer to the application or administration or a composition to a subject after the onset, or suspected onset, of a disease or condition.

[104] The term "treating" further encompasses the concept of "prevent," "preventing," and "prevention." The terms "prevent," "preventing," and "prevention," as used herein, refer to a decrease in the occurrence of pathology of a condition in a subject, who does not have, but is at risk of or susceptible to developing a disease or condition. The prevention may be complete, e.g., the total absence of pathology of a condition in a subject. The prevention may also be partial, such that the occurrence of pathology of a condition in a subject is less than that which would have occurred without the present disclosure.

[105] By "treating or preventing a condition," for example, as compared with an equivalent untreated control, alleviating a symptom of a disorder may involve reduction or degree of

prevention at least 3%, 5%, 10%, 20%, 40%, 50%, 60%, 80%, 90%, 95%, 98%, 99%, 99.5%, 99.9%, or 100% as measured by any standard technique. In some embodiments, alleviating a symptom of a disorder may involve reduction or degree of prevention by at least 2, 3, 4, 5, 10, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, or 10000 fold as compared with an equivalent untreated control.

[106] As used therein, "delaying" the development of a disease means to defer, hinder, slow, retard, stabilize, and/or postpone progression of the disease. This delay can be of varying lengths of time, depending on the history of the disease and/or individuals being treated. A method that "delays" or alleviates the development of a disease, or delays the onset of the disease, is a method that reduces probability of developing one or more symptoms of the disease in a given time frame and/or reduces extent of the symptoms in a given time frame, when compared to not using the method. Such comparisons are typically based on clinical studies, using a number of subjects sufficient to give a statistically significant result.

[107] "Development" or "progression" of a disease means initial manifestations and/or ensuing progression of the disease. Development of the disease can be detectable and assessed using standard clinical techniques as well known in the art. However, development also refers to progression that may be undetectable. For purpose of this disclosure, development or progression refers to the biological course of the symptoms. "Development" includes occurrence, recurrence, and onset.

[108] As used herein "onset" or "occurrence" of a disease includes initial onset and/or recurrence.

[109] "Administering" and its grammatical equivalents as used herein can refer to providing pharmaceutical compositions described herein to a subject or a patient. Conventional methods, known to those of ordinary skill in the art of medicine, can be used to administer the composition to the subject, depending upon the type of disease to be treated or the site of the disease. For example, the composition can be administered, *e.g.*, orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally, via an implanted reservoir, or via infusion. One or more such routes can be employed.

[110] The term "parenteral" as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intraperitoneal, intradermal, intraarterial, intrasynovial, intrastemal, intrathecal, intravascular, intralesional, and intracranial injection or infusion techniques. In addition, it can be administered to the subject via injectable depot routes of

administration such as using 1-, 3-, or 6-month depot injectable or biodegradable materials and methods.

[111] By "co-administering" is meant administering one or more additional therapeutic regimens or agents or treatments and the composition of the disclosure sufficiently close in time to enhance the effect of one or more additional therapeutic agents, or vice versa. In this regard, the composition of the disclosure described herein can be administered simultaneously with one or more additional therapeutic regimens or agents or treatments, at a different time, or on an entirely different therapeutic schedule (e.g., the first treatment can be daily, while the additional treatment is weekly). For example, in embodiments, the secondary therapeutic regimens or agents or treatments are administered simultaneously, prior to, or subsequent to the composition of the disclosure.

[112] The terms "pharmaceutical composition" and its grammatical equivalents as used herein can refer to a mixture or solution comprising a therapeutically effective amount of an active pharmaceutical ingredient together with one or more pharmaceutically acceptable excipients, carriers, and/or a therapeutic agent to be administered to a subject, *e.g.*, a human in need thereof.

[113] The term "pharmaceutically acceptable" and its grammatical equivalents as used herein can refer to an attribute of a material which is useful in preparing a pharmaceutical composition that is generally safe, non–toxic, and neither biologically nor otherwise undesirable and is acceptable for veterinary as well as human pharmaceutical use. "Pharmaceutically acceptable" can refer a material, such as a carrier or diluent, which does not abrogate the biological activity or properties of the compound, and is relatively nontoxic, *i.e.*, the material may be administered to a subject without causing undesirable biological effects or interacting in a deleterious manner with any of the components of the pharmaceutical composition in which it is contained.

[114] A "pharmaceutically acceptable excipient, carrier, or diluent" refers to an excipient, carrier, or diluent that can be administered to a subject, together with an agent, and which does not destroy the pharmacological activity thereof and is nontoxic when administered in doses sufficient to deliver a therapeutic amount of the agent.

[115] A "pharmaceutically acceptable salt" may be an acid or base salt that is generally considered in the art to be suitable for use in contact with the tissues of human beings or animals without excessive toxicity, irritation, allergic response, or other problem or complication. Such salts include mineral and organic acid salts of basic residues such as amines, as well as alkali or organic salts of acidic residues such as carboxylic acids. Specific

pharmaceutical salts include, but are not limited to, salts of acids such as hydrochloric, phosphoric, hydrobromic, malic, glycolic, fumaric, sulfuric, sulfamic, sulfanilic, formic, toluenesulfonic, methanesulfonic, benzene sulfonic, ethane disulfonic, 2-hydroxyethyl sulfonic, nitric, benzoic, 2-acetoxybenzoic, citric, tartaric, lactic, stearic, salicylic, glutamic, ascorbic, pamoic, succinic, fumaric, maleic, propionic, hydroxymaleic, hydroiodic, phenylacetic, alkanoic such as acetic, HOOC-(CH_2)n-COOH where n is 0-4, and the like. Similarly, pharmaceutically acceptable cations include, but are not limited to sodium, potassium, calcium, aluminum, lithium and ammonium. Those of ordinary skill in the art will recognize from this disclosure and the knowledge in the art that further pharmaceutically acceptable salts include those listed by Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, PA, p. 1418 (1985). In general, a pharmaceutically acceptable acid or base salt can be synthesized from a parent compound that contains a basic or acidic moiety by any conventional chemical method. Briefly, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in an appropriate solvent.

[116] The term "therapeutic agent" can refer to any agent that, when administered to a subject, has a therapeutic, diagnostic, and/or prophylactic effect and/or elicits a desired biological and/or pharmacological effect. Therapeutic agents can also be referred to as "actives" or "active agents." Such agents include, but are not limited to, cytotoxins, radioactive ions, chemotherapeutic agents, small molecule drugs, proteins, and nucleic acids.

[117] "A therapeutically effective amount" as used herein refers to the amount of each composition of the present disclosure required to confer therapeutic effect on the subject, either alone or in combination with one or more other therapeutic agents. Hence, as used herein, the term "therapeutically effective amount" means an amount of an agent to be delivered (*e.g.*, nucleic acid, composition, therapeutic agent, prophylactic agent, etc.) that is sufficient, when administered to a subject suffering from or susceptible to a disease, disorder, and/or condition, to treat, improve symptoms of, diagnose, prevent, and/or delay the onset of the disease, disorder, and/or condition. In terms of treatment, a "therapeutically effective amount" is an amount that is sufficient to palliate, ameliorate, stabilize, reverse or slow the progression of a disease or a condition, e.g., an atherosclerotic vascular disease, hypertriglyceridemia, or diabetes. A "therapeutically effective amount" varies, as recognized by those skilled in the art, depending on the particular condition being treated, the severity of the condition, the individual subject parameters including age, physical condition, size, gender and weight, the duration of the treatment, the nature of concurrent therapy (if any), the

specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is generally preferred that a maximum dose of the individual components or combinations thereof be used, that is, the highest safe dose according to sound medical judgment. It will be understood by those of ordinary skill in the art, however, that a subject may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reasons. Additionally, other medication the patient may be receiving will affect the determination of the therapeutically effective amount of the therapeutic agent to administer. Empirical considerations, such as the half-life, generally will contribute to the determination of the dosage. A "therapeutically effective amount" may be of any of the compositions of the disclosure used alone or in conjunction with one or more agents used to treat a condition. A therapeutically effective amount can be administered in one or more administrations.

[118] An effective initial method to determine a "therapeutically effective amount" may be by carrying out cell culture assays (for example, using neuronal cells) or using animal models (for example, mice, rats, rabbits, dogs or pigs). A dose may be formulated in animal models to achieve a concentration range that includes the IC50 (i.e., the concentration of the composition which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. In addition to determining the appropriate concentration range for an disclosure composition to be therapeutically effective, animal models may also yield other relevant information such as preferable routes of administration that will give maximum effectiveness. Adjusting the dose to achieve maximal efficacy in humans based on the methods described above and other methods is well within the capabilities of the ordinarily skilled artisan.

[119] Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50, as well as all intervening decimal values between the aforementioned integers such as, for example, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, and 1.9. With respect to sub-ranges, "nested sub-ranges" that extend from either end point of the range are specifically contemplated. For example, a nested sub-range of an exemplary range of 1 to 50 may comprise 1 to 10, 1 to 20, 1 to 30, and 1 to 40 in one direction, or 50 to 40, 50 to 30, 50 to 20, and 50 to 10 in the other direction.

[120] The term "protospacer," or "target sequence" and their grammatical equivalents as used herein can refer to a DNA sequence of a target gene. In the native state, a protospacer is adjacent to a PAM (protospacer adjacent motif). The term "spacer" can be the RNA version of the protospacer that binds to the complementary strand of the protospacer. A spacer can be within a guide RNA (gRNA). The site of cleavage by an RNA-guided nuclease is within a protospacer sequence. Please see Fig. 1A for an illustration.

[121] The term "base editing," "gene editing," or "gene modification" and its grammatical equivalents as used herein can refer to genetic engineering in which one or more nucleotides are inserted, replaced, or removed from a genome. Gene editing can be performed using a nuclease (*e.g.*, a natural-existing nuclease or an artificially engineered nuclease). Gene modification can include introducing a double stranded break, a non-sense mutation, a frameshift mutation, a splice site alteration, or an inversion in a polynucleotide sequence, *e.g.*, a target polynucleotide sequence.

[122] The term "base editor (BE)" or "nucleobase editor (NBE)" as used herein can refer to an agent that binds a polynucleotide and has nucleobase modifying activity. In various embodiments, the base editor comprises a nucleobase modifying polypeptide (*e.g.*, a deaminase) and a nucleic acid programmable nucleotide binding domain in conjunction with a guide polynucleotide (*e.g.*, guide RNA), or nucleic acids encoding the programmable nucleotide binding domain and the deaminase. In various embodiments, the agent is a biomolecular complex comprising a protein domain having base editing activity, *i.e.*, a domain capable of modifying a base (*e.g.*, A, T, C, G, or U) within a nucleic acid molecule (*e.g.*, DNA), or a nucleic acid encoding the same. In some embodiments, the polynucleotide programmable DNA binding domain is fused or linked to a deaminase domain, resulting in a base editor fusion protein. In some embodiments, the base editor comprises a nucleic acid encoding the base editor fusion protein, e.g., a mRNA encoding the base editor fusion protein. The base editor fusion protein may comprise one or more linkers, for example, peptide linkers. In one embodiment, the agent is a fusion protein comprising a domain having base editing activity. In another embodiment, the protein domain having base editing activity is linked to the guide RNA (*e.g.*, via an RNA binding motif on the guide RNA and an RNA binding domain fused to the deaminase). In some embodiments, the domain having base editing activity is capable of deaminating a base within a nucleic acid molecule. In some embodiments, the base editor is capable of deaminating one or more bases within a DNA molecule. In some embodiments, the base editor is capable of deaminating an adenosine (A) within DNA. In some embodiments, the base editor is an adenosine base editor (ABE). In

some embodiments, the base editor is capable of deaminating an cytosine (C) within DNA. In some embodiments, the base editor is a cytosine base editor (CBE).

[123] The term "base editor system" refers to a system for editing a nucleobase of a target nucleotide sequence. In various embodiments, the base editor system comprises (1) a polynucleotide programmable nucleotide binding domain (*e.g.*, Cas9); (2) a deaminase domain (*e.g.*, an adenosine deaminase or a cytidine deaminase) for deaminating said nucleobase; and (3) one or more guide polynucleotide (*e.g.*, guide RNA). In some embodiments, the base editor system comprises a base editor fusion protein comprising (1) and (2). In some embodiments, the polynucleotide programmable nucleotide binding domain is a polynucleotide programmable DNA binding domain. In some embodiments, the base editor is an adenine or adenosine base editor (ABE). In some embodiments, the base editor is a cytosine base editor (CBE).

Nucleobase Editor Systems

[124] In some aspects, provided herein are base editor systems capable of nucleobase modifications. In some embodiments, the base editor system comprises (i) a guide polynucleotide or a nucleic acid encoding same, and (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same. In some embodiments, the base editor system comprises a guide polynucleotide. In some embodiments, the base editor system comprises a nucleic acid encoding a guide polynucleotide. In some embodiments, the base editor system comprises a base editor fusion protein comprising a programmable DNA binding domain and a deaminase. In some embodiments, the base editor system comprises a nucleic acid encoding a base editor fusion protein comprising a programmable DNA binding domain and a deaminase.

[125] In some embodiments, the guide polynucleotide directs the base editor system to effect a nucleobase alteration in a PCSK9 or ANGPTL3 gene *in vivo* when administered to a subject.

[126] In some embodiments, the base alteration occurs in at least 35% of whole liver cells in the subject as measured by next generation sequencing or Sanger sequencing.

[127] In some embodiments, the base alteration occurs in at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.3%, 99.5%, 99.7%, 99.8%, or 99.9% of whole liver cells in the subject as measured by next generation sequencing or Sanger

sequencing. In some embodiments, the base alteration occurs in at most 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.3%, 99.5%, 99.7%, 99.8%, or 99.9% of whole liver cells in the subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the base alteration occurs in 1%-99.9%, 2%-99.9%, 3%-99.9%, 4%-99.9%, 5%-99.9%, 6%-99.9%, 7%-99.9%, 8%-99.9%, 9%-99.9%, 10%-99.9%, 15%-99.9%, 20%-99.9%, 25%-99.9%, 30%-99.9%, 35%-99.9%, 40%-99.9%, 45%-99.9%, 50%-99.9%, 55%-99.9%, 60%-99.9%, 65%-99.9%, 70%-99.9%, 75%-99.9%, 80%-99.9%, 85%-99.9%, 90%-99.9%, or 95-99.9% of whole liver cells in the subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the base alteration occurs in 1%-99.5%, 1%-99%, 1%-98%, 1%-97%, 1%-96%, 1%-95%, 1%-90%, 1%-85%, 1%-80%, 1%-75%, 1%-70%, 1%-65%, 1%-60%, 1%-55%, 1%-50%, 1%-45%, 1%-40%, 1%-35%, 1%-30%, 1%-25%, 1%-20%, 1%-15%, 1%-10%, 1%-9%, 1%-8%, 1%-7%, 1%-6%, 1%-5%, 1%-4%, 1%-3%, or 1%-2% of whole liver cells in the subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the base alteration occurs in 1%-90%, 5%-85%, 10%-80%, 15%-75%, 20%-70%, 25%-65%, 30%-60%, 35%-55%, or 40%-50% of whole liver cells in the subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the base alteration occurs in 100% of whole liver cells in the subject as measured by next generation sequencing or Sanger sequencing.

[128] In some embodiments, the base alteration occurs in hepatocytes in the subject. In some embodiments, the base alteration occurs in at least 30% of hepatocytes in the subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the base alteration occurs in hepatocytes in the subject. In some embodiments, the base alteration occurs in at least % of hepatocytes in the subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the base alteration occurs in at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.3%, 99.5%, 99.7%, 99.8%, or 99.9% of hepatocytes in the subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the base alteration occurs in at most 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.3%, 99.5%, 99.7%, 99.8%, or 99.9% of hepatocytes in the subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the base alteration occurs in 1%-99.9%, 2%-99.9%, 3%-99.9%, 4%-

99.9%, 5%-99.9%, 6%-99.9%, 7%-99.9%, 8%-99.9%, 9%-99.9%, 10%-99.9%, 15%-99.9%, 20%-99.9%, 25%-99.9%, 30%-99.9%, 35%-99.9%, 40%-99.9%, 45%-99.9%, 50%-99.9%, 55%-99.9%, 60%-99.9%, 65%-99.9%, 70%-99.9%, 75%-99.9%, 80%-99.9%, 85%-99.9%, 90%-99.9%, or 95-99.9% of hepatocytes in the subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the base alteration occurs in 1%-99.5%, 1%-99%, 1%-98%, 1%-97%, 1%-96%, 1%-95%, 1%-90%, 1%-85%, 1%-80%, 1%-75%, 1%-70%, 1%-65%, 1%-60%, 1%-55%, 1%-50%, 1%-45%, 1%-40%, 1%-35%, 1%-30%, 1%-25%, 1%-20%, 1%-15%, 1%-10%, 1%-9%, 1%-8%, 1%-7%, 1%-6%, 1%-5%, 1%-4%, 1%-3%, or 1%-2% hepatocytes in the subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the base alteration occurs in 1%-90%, 5%-85%, 10%-80%, 15%-75%, 20%-70%, 25%-65%, 30%-60%, 35%-55%, or 40%-50% of hepatocytes in the subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the base alteration occurs in 100% of hepatocytes in the subject as measured by next generation sequencing or Sanger sequencing.

[129] In some embodiments, the base alteration occurred in whole liver cells in the subject is measured by next generation sequencing. In some embodiments, the base alteration occurred in whole liver cells in the subject is measured by Sanger sequencing. In some embodiments, the base alteration occurred in hepatocytes in the subject is measured by next generation sequencing. In some embodiments, the base alteration occurred in hepatocytes in the subject is measured by Sanger sequencing.

[130] In some embodiments, the nucleobase alteration results in a reduction of at least 35% in blood PCSK9 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in a reduction of at least 35% in blood ANGPTL3 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS

[131] In some embodiments, the nucleobase alteration results in a reduction of at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% in blood PCSK9 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in a reduction of at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 85%, 90%, 95%, 97%, 98%, 90%, 95%, 97%, 98%, 99%, 99.3%, 99.5%, 99.7%, 99.8%, or 99.9% in

blood PCSK9 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in a reduction of at most 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 85%, 90%, 95%, 97%, 98%, 90%, 95%, 97%, 98%, 99%, 99.3%, 99.5%, 99.7%, 99.8%, or 99.9% in blood PCSK9 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in a reduction of 1%-99.9%, 2%-99.9%, 3%-99.9%, 4%-99.9%, 5%-99.9%, 6%-99.9%, 7%-99.9%, 8%-99.9%, 9%-99.9%, 10%-99.9%, 15%-99.9%, 20%-99.9%, 25%-99.9%, 30%-99.9%, 31%-99.9%, 32%-99.9%, 33%-99.9%, 34%-99.9%, 35%-99.9%, 36%-99.9%, 37%-99.9%, 38%-99.9%, 39%-99.9%, 40%-99.9%, 45%-99.9%, 50%-99.9%, 55%-99.9%, 60%-99.9%, 65%-99.9%, 70%-99.9%, 75%-99.9%, 80%-99.9%, 85%-99.9%, 90%-99.9%, or 95-99.9% in blood PCSK9 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in a reduction of 1%-99.5%, 1%-99%, 1%-98%, 1%-97%, 1%-96%, 1%-95%, 1%-90%, 1%-85%, 1%-80%, 1%-79%, 1%-78%, 1%-77%, 1%-76%, 1%-75%, 1%-74%, 1%-73%, 1%-72%, 1%-71%, 1%-70%, 1%-65%, 1%-60%, 1%-55%, 1%-50%, 1%-45%, 1%-40%, 1%-39%, 1%-38%, 1%-37%, 1%-36%, 1%-35%, 1%-34%, 1%-33%, 1%-32%, 1%-31%, 1%-30%, 1%-25%, 1%-20%, 1%-15%, 1%-10%, 1%-9%, 1%-8%, 1%-7%, 1%-6%, 1%-5%, 1%-4%, 1%-3%, or 1%-2% in blood PCSK9 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in a reduction of 1%-99.9%, 5%-99.5%, 10%-99%, 15%-97%, 20%-95%, 25%-90%, 30%-85%, 31%-80%, 32%-79%, 33%-78%, 34%-77%, 35%-76%, 36%-76%, 37%-75%, 38%-74%, 39%-73%, 40%-72%, 45%-71%, 50%-70%, or 55%-65% in blood PCSK9 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in a reduction of 100% in blood PCSK9 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS.

[132] In some embodiments, the nucleobase alteration results in at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 75%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 400% 500%, 600%,

700%, 800%, 900%, 1000% less blood PCSK9 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in at least 1.1–fold, 1.2–fold, 1.3–fold, 1.4–fold, 1.5–fold, 1.6–fold, 1.7–fold, 1.8–fold, 1.9–fold, 2–fold, 2.5–fold, 3–fold, 3.5–fold, 4–fold, 4.5–fold, 5–fold, 6–fold, 7–fold, 8–fold, 9–fold, 10–fold, or more than 10-fold less blood PCSK9 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS.

[133] In some embodiments, the reduction of blood PCSK9 protein level or the blood PCSK9 protein level in the subject as compared to prior to the administration is measured by ELISA (enzyme-linked immunosorbent assay). In some embodiments, the reduction of blood PCSK9 protein level or the blood PCSK9 protein level in the subject as compared to prior to the administration is measured by Western blot analysis. In some embodiments, the reduction of blood PCSK9 protein level or the blood PCSK9 protein level in the subject as compared to prior to the administration is measured by LC-MS/MS (liquid chromatography -tandem mass spectrometry).

[134] In some embodiments, the nucleobase alteration results in a reduction of at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% in blood ANGPTL3 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in a reduction of at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 85%, 90%, 95%, 97%, 98%, 90%, 95%, 97%, 98%, 99%, 99.3%, 99.5%, 99.7%, 99.8%, or 99.9% in blood ANGPTL3 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in a reduction of at most 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 85%, 90%, 95%, 97%, 98%, 90%, 95%, 97%, 98%, 99%, 99.3%, 99.5%, 99.7%, 99.8%, or 99.9% in blood ANGPTL3 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in a reduction of 1%-99.9%, 2%-99.9%, 3%-99.9%, 4%-99.9%, 5%-99.9%, 6%-99.9%, 7%-99.9%, 8%-99.9%, 9%-99.9%, 10%-99.9%, 15%-99.9%, 20%-99.9%, 25%-99.9%, 30%-99.9%, 31%-99.9%, 32%-99.9%, 33%-99.9%, 34%-99.9%,

35%-99.9%, 36%-99.9%, 37%-99.9%, 38%-99.9%, 39%-99.9%, 40%-99.9%, 45%-99.9%, 50%-99.9%, 55%-99.9%, 60%-99.9%, 65%-99.9%, 70%-99.9%, 75%-99.9%, 80%-99.9%, 85%-99.9%, 90%-99.9%, or 95-99.9% in blood ANGPTL3 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in a reduction of 1%-99.5%, 1%-99%, 1%-98%, 1%-97%, 1%-96%, 1%-95%, 1%-90%, 1%-85%, 1%-80%, 1%-79%, 1%-78%, 1%-77%, 1%-76%, 1%-75%, 1%-74%, 1%-73%, 1%-72%, 1%-71%, 1%-70%, 1%-65%, 1%-60%, 1%-55%, 1%-50%, 1%-45%, 1%-40%, 1%-39%, 1%-38%, 1%-37%, 1%-36%, 1%-35%, 1%-34%, 1%-33%, 1%-32%, 1%-31%, 1%-30%, 1%-25%, 1%-20%, 1%-15%, 1%-10%, 1%-9%, 1%-8%, 1%-7%, 1%-6%, 1%-5%, 1%-4%, 1%-3%, or 1%-2% in blood ANGPTL3 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in a reduction of 1%-99.9%, 5%-99.5%, 10%-99%, 15%-97%, 20%-95%, 25%-90%, 30%-85%, 31%-80%, 32%-79%, 33%-78%, 34%-77%, 35%-76%, 36%-76%, 37%-75%, 38%-74%, 39%-73%, 40%-72%, 45%-71%, 50%-70%, or 55%-65% in blood ANGPTL3 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in a reduction of 100% in blood ANGPTL3 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS.

[135] In some embodiments, the nucleobase alteration results in at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 75%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 400% 500%, 600%, 700%, 800%, 900%, 1000% less blood ANGPTL3 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in at least 1.1–fold, 1.2–fold, 1.3–fold, 1.4–fold, 1.5–fold, 1.6–fold, 1.7–fold, 1.8–fold, 1.9–fold, 2–fold, 2.5–fold, 3–fold, 3.5–fold, 4–fold, 4.5–fold, 5–fold, 6–fold, 7–fold, 8–fold, 9–fold, 10–fold, or more than 10-fold less blood ANGPTL3 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS.

[136] In some embodiments, the reduction of blood ANGPTL3 protein level or the blood ANGPTL3 protein level in the subject as compared to prior to the administration is measured by ELISA (enzyme-linked immunosorbent assay). In some embodiments, the reduction of blood ANGPTL3 protein level or the blood ANGPTL3 protein level in the subject as

compared to prior to the administration is measured by Western blot analysis. In some embodiments, the reduction of blood ANGPTL3 protein level or the blood ANGPTL3 protein level in the subject as compared to prior to the administration is measured by LC-MS/MS (liquid chromatography -tandem mass spectrometry).

[137] In some embodiments, the nucleobase alteration results in a reduction of at least 35% in blood or low-density lipoprotein cholesterol (LDL-C) levels in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of at least 35%, 40%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% in blood low-density lipoprotein cholesterol (LDL-C) level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.3%, 99.5%, 99.7%, 99.8%, or 99.9% in blood low-density lipoprotein cholesterol (LDL-C) level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of at most 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.3%, 99.5%, 99.7%, 99.8%, or 99.9% in blood low-density lipoprotein cholesterol (LDL-C) level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of 1%-99.9%, 2%-99.9%, 3%-99.9%, 4%-99.9%, 5%-99.9%, 6%-99.9%, 7%-99.9%, 8%-99.9%, 9%-99.9%, 10%-99.9%, 15%-99.9%, 20%-99.9%, 25%-99.9%, 30%-99.9%, 35%-99.9%, 40%-99.9%, 45%-99.9%, 50%-99.9%, 55%-99.9%, 60%-99.9%, 65%-99.9%, 70%-99.9%, 75%-99.9%, 80%-99.9%, 85%-99.9%, 90%-99.9%, or 95%-99.9% in blood low-density lipoprotein cholesterol (LDL-C) level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of 1%-99.5%, 1%-99%, 1%-98%, 1%-97%, 1%-96%, 1%-95%, 1%-90%, 1%-85%, 1%-80%, 1%-75%, 1%-70%, 1%-65%, 1%-60%, 1%-55%, 1%-50%, 1%-45%, 1%-40%, 1%-35%, 1%-30%, 1%-25%, 1%-20%, 1%-15%, 1%-10%, 1%-9%, 1%-8%, 1%-7%, 1%-6%, 1%-5%, 1%-4%, 1%-3%, or 1%-2% in blood low-density lipoprotein cholesterol (LDL-C) level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of 1%-99.9%, 5%-99.5%, 10%-99%, 15%-97%, 20%-95%, 25%-90%, 30%-85%, 35%-80%, 40%-75%, 45%-70%, 50%-65%, or 55%-60% in

blood low-density lipoprotein cholesterol (LDL-C) level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of 100% in blood low-density lipoprotein cholesterol (LDL-C) level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 75%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 400% 500%, 600%, 700%, 800%, 900%, 1000% less blood low-density lipoprotein cholesterol (LDL-C) level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in at least 1.1–fold, 1.2–fold, 1.3–fold, 1.4–fold, 1.5–fold, 1.6–fold, 1.7–fold, 1.8–fold, 1.9–fold, 2–fold, 2.5–fold, 3–fold, 3.5–fold, 4–fold, 4.5–fold, 5–fold, 6–fold, 7–fold, 8–fold, 9–fold, 10–fold, or more than 10-fold less blood low-density lipoprotein cholesterol (LDL-C) level in the subject as compared to prior to the administration.

[138] In some embodiments, the nucleobase alteration results in a reduction of at least 35% in blood triglyceride levels in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of at least 30%, 35%, 40%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% in blood triglyceride level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.3%, 99.5%, 99.7%, 99.8%, or 99.9% in blood triglyceride level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of at most 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.3%, 99.5%, 99.7%, 99.8%, or 99.9% in blood triglyceride level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of 1%-99.9%, 2%-99.9%, 3%-99.9%, 4%-99.9%, 5%-99.9%, 6%-99.9%, 7%-99.9%, 8%-99.9%, 9%-99.9%, 10%-99.9%, 15%-99.9%, 20%-99.9%, 25%-99.9%, 30%-99.9%, 35%-99.9%, 40%-99.9%, 45%-99.9%, 50%-99.9%, 55%-99.9%, 60%-99.9%, 65%-99.9%, 70%-99.9%, 75%-99.9%, 80%-99.9%, 85%-99.9%, 90%-99.9%, or 95-99.9% in blood triglyceride level in the subject as compared

to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of 1%-99.5%, 1%-99%, 1%-98%, 1%-97%, 1%-96%, 1%-95%, 1%-90%, 1%-85%, 1%-80%, 1%-75%, 1%-70%, 1%-65%, 1%-60%, 1%-55%, 1%-50%, 1%-45%, 1%-40%, 1%-35%, 1%-30%, 1%-25%, 1%-20%, 1%-15%, 1%-10%, 1%-9%, 1%-8%, 1%-7%, 1%-6%, 1%-5%, 1%-4%, 1%-3%, or 1%-2% in blood triglyceride level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of 1%-99.9%, 5%-99.5%, 10%-99%, 15%-97%, 20%-95%, 25%-90%, 30%-85%, 35%-80%, 40%-75%, 45%-70%, 50%-65%, or 55%-60% in blood triglyceride level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of 100% in blood triglyceride level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 75%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 400% 500%, 600%, 700%, 800%, 900%, 1000% less blood triglyceride level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in at least 1.1–fold, 1.2–fold, 1.3–fold, 1.4–fold, 1.5–fold, 1.6–fold, 1.7–fold, 1.8–fold, 1.9–fold, 2–fold, 2.5–fold, 3–fold, 3.5–fold, 4–fold, 4.5–fold, 5–fold, 6–fold, 7–fold, 8–fold, 9–fold, 10–fold, or more than 10-fold less blood triglyceride level in the subject as compared to prior to the administration.

[139] In some embodiments, the blood triglyceride level or the reduction of blood triglyceride level in the subject as compared to prior to the administration is measured by any standard technique. In some embodiments, the blood low-density lipoprotein cholesterol (LDL-C) level or the reduction of blood low-density lipoprotein cholesterol (LDL-C) level in the subject as compared to prior to the administration is measured by any standard technique. For example, a clinical analyzer instrument may be used to measure a ‘lipid panel’ in serum samples which entails the direct measurement of cholesterol (total C), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) enzymatically. Reagent kits specific for each analyte contain buffers, calibrators, blanks and controls. As used in the present disclosure, cholesterol, triglycerides and HDL-C may be quantified using absorbance measurements of specific enzymatic reaction products. LDL-C may be determined indirectly. In some instances, most of circulating cholesterol can be found in three major lipoprotein fractions: very low-density lipoproteins (VLDL), LDL and HDL. In some embodiments, total circulating cholesterol may be estimated with the formula [Total C] = [VLDL-C] + [LDL-C]

+ [HDL-C]. Thus the LDL-C can be calculated from measured values of total cholesterol, triglycerides and HDL-C according to the relationship: [LDL-C] = [total C] - [HDL-C] - [TG]/5, where [TG]/5 is an estimate of VLDL-cholesterol. A reagent kit specific for triglycerides containing buffers, calibrators, blanks and controls may be used. As used herein, serum samples from the study may be analyzed and triglycerides may be measured using a series of coupled enzymatic reactions. In some embodiments, H_2O_2 may be used to quantify the analyte.as the end product of the last one and its absorbance at 500nm, and the color intensity is proportional to triglyceride concentrations.

[140] In some embodiments, the guide polynucleotide is a guide RNA, wherein the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the PCSK9 gene with 0, 1, or 2 mismatches. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the PCSK9 gene with no mismatches. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the PCSK9 gene with 1 mismatch. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the PCSK9 gene with 2 mismatches. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the PCSK9 gene with 3 mismatches. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the PCSK9 gene with 4 mismatches. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the PCSK9 gene with 5 mismatches.

[141] In some embodiments, the guide polynucleotide is a guide RNA, wherein the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the ANGPTL3 gene with 0, 1, or 2 mismatches. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the ANGPTL3 gene with no mismatches. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the ANGPTL3 gene with 1 mismatch. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the ANGPTL3 gene with 2 mismatches. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the ANGPTL3 gene with 3 mismatches. In some embodiments, the guide RNA

comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the ANGPTL3 gene with 4 mismatches. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the ANGPTL3 gene with 5 mismatches.

[142] In some embodiments, the nucleobase alteration is outside of the protospacer sequence in less than 1% of whole liver cells in the subject as measured by net nucleobase editing. In some embodiments, the nucleobase alteration is outside of the protospacer sequence in less than 1% of hepatocytes in the subject as measured by net nucleobase editing. In some embodiments, the nucleobase alteration is only within the protospacer sequence as measured by net nucleobase editing.

[143] In some embodiments, the nucleobase alteration is outside of the protospacer sequence in less than 0.01%. 0.02%, 0.03% 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 2.0%, 3.0% 4.0%, 5.0%, 6.0%, 7.0%, 8.0%, 9.0%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 65%, 80%, 85%, 90% of whole liver cells in the subject as measured by net nucleobase editing. In some embodiments, the nucleobase alteration is outside of the protospacer sequence in less than 0.01%. 0.02%, 0.03% 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 2.0%, 3.0% 4.0%, 5.0%, 6.0%, 7.0%, 8.0%, 9.0%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 65%, 80%, 85%, 90% of hepatocytes in the subject as measured by net nucleobase editing. In some embodiments, the nucleobase alteration is outside of the protospacer sequence in less than 0.01%. 0.02%, 0.03% 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 2.0%, 3.0% 4.0%, 5.0%, 6.0%, 7.0%, 8.0%, 9.0%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 65%, 80%, 85%, 90% of cells in the subject as measured by net nucleobase editing.

[144] In some embodiments, the deaminase is an adenine deaminase. In some embodiments, the nucleobase alteration is a A•T to G•C alteration. In some embodiments, the deaminase is an adenine deaminase and the nucleobase alteration is a A•T to G•C alteration. In some embodiments, the programmable DNA binding domain comprises a nuclease inactive Cas9 or a Cas9 nickase. In some embodiments, the programmable DNA binding domain comprises a Cas9.

[145] In some embodiments, the nucleobase alteration is at a splice site of the PCSK9 gene. In some embodiments, the nucleobase alteration is at a splice donor site of the PCSK9 gene.

In some embodiments, the splice donor site is at 5’ end of PCSK9 intron 1 as referenced in SEQ ID NO: 5. In some embodiments, the nucleobase alteration is at a splice acceptor site of the PCSK9 gene. In some embodiments, the nucleobase alteration results in a frame shift, a premature stop codon, a insertion or deletion in a transcript encoded by the PCSK9 gene. In some embodiments, the nucleobase alteration results in an aberrant transcript encoded by the PCSK9 gene. In some embodiments, the guide polynucleotide is a guide RNA. In some embodiments, the guide RNA is chemically modified. In some embodiments, the guide RNA comprises a tracrRNA sequence. In some embodiments, the guide RNA comprises a chemical modification as set forth in Table 1 or Table 24.

[146] In some embodiments, the nucleobase alteration is at a splice site of the ANGPTL3 gene. In some embodiments, the nucleobase alteration is at a splice donor site of the ANGPTL3 gene. In some embodiments, the splice donor site is at 5’ end of ANGPTL3 intron 6 as referenced in SEQ ID NO: 7. In some embodiments, the nucleobase alteration is at a splice acceptor site of the ANGPTL3 gene. In some embodiments, the nucleobase alteration results in a frame shift, a premature stop codon, a insertion or deletion in a transcript encoded by the ANGPTL3 gene. In some embodiments, the nucleobase alteration results in an aberrant transcript encoded by the ANGPTL3 gene. In some embodiments, the guide polynucleotide is a guide RNA. In some embodiments, the guide RNA is chemically modified. In some embodiments, the guide RNA comprises a tracrRNA sequence. In some embodiments, the guide RNA comprises a chemical modification as set forth in Table 1 or Table 24.

[147] In some embodiments, the guide RNA comprises a guide RNA sequence set forth in Table 1 or Table 24. In some embodiments, the guide RNA comprises the sequence 5'-5'-cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcG UUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususu-3' (SEQ ID NO: 9), 5'-cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGU UAucAAcuugaaaaagugGcaccgagucggugcusususu-3' (SEQ ID NO: 9), 5'-cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu-3' (SEQ ID NO: 9) (GA346), 5’-cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAacAAcuugaaaaagugGcaccgagucggugcusususu-3’ (SEQ ID NO: 10) (GA374), 5’-cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuuuu-3’ (SEQ ID NO: 11) (GA385), 5’-cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUcc

GUUAucAAcuugaaaaagugGcaccgagucggugcusususuuUu-3' (SEQ ID NO: 11) (GA386) or 5'-
cscscsGCACCUUGGCGCAGCGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccG
UUAucAAcuuGaaaaagugGcaccgagucggugcuusususuuuu-3' (SEQ ID NO: 12) (GA387).

[148] In some embodiments, the protospacer sequence comprises a protospacer sequence set forth in Table 1 or Table 24. In some embodiments, the protospacer comprises the sequence 5'-CCCGCACCTTGGCGCAGCGG-3' (SEQ ID NO: 13), AAGATACCTGAATAACTCTC-3' (SEQ ID NO: 14), and 5'-AAGATACCTGAATAACCCTC-3' (SEQ ID NO: 15).

[149] In some embodiments, the base editor fusion protein comprises the sequence of SEQ ID NO: 3. In some embodiments, the adenosine deaminase comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to the amino acid sequence set forth in SEQ ID NO: 3 or to any of the adenosine deaminases provided herein. It should be appreciated that adenosine deaminases provided herein may include one or more mutations (e.g., any of the mutations provided herein). The disclosure provides any deaminase domains with a certain percent identity plus any of the mutations or combinations thereof described herein. In some embodiments, the adenosine deaminase comprises an amino acid sequence that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more mutations compared to the amino acid sequence set forth in SEQ ID NO: 3 or any of the adenosine deaminases provided herein. In some embodiments, the adenosine deaminase comprises an amino acid sequence that has at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, or at least 170 identical contiguous amino acid residues as compared to any one of the amino acid sequences set forth in SEQ ID NO: 3 or any of the adenosine deaminases provided herein.

[150] In some embodiments, the nucleic acid encoding the base editor fusion protein is a mRNA. The mRNA may comprise modifications, for example, modifications at 3' or 5' end of the mRNA. In some embodiments, the mRNA comprises a cap analog.

[151] In some embodiments, the mRNA comprises at least 1, 2, or 3 nucleotides at the 5' end that comprises 2'-hydroxyl group, 2'-O-methyl group, or additional 2' chemical modification or a combination thereof. In some embodiments, the mRNA comprises at least

1, 2, or 3 nucleotides at the 5' end that comprises 2'-hydroxyl group, 2'-O-methyl group, or additional 2' chemical modification or a combination thereof. In some embodiments, the mRNA comprises at least 1 nucleotide at the 5' end that comprises 2'-hydroxyl group, 2'-O-methyl group, or additional 2' chemical modification or a combination thereof. In some embodiments, the mRNA comprises at least 2 nucleotides at the 5' end that comprises 2'-hydroxyl group, 2'-O-methyl group, or additional 2' chemical modification or a combination thereof. In some embodiments, the mRNA comprises at least 3 nucleotides at the 5' end that comprises 2'-hydroxyl group, 2'-O-methyl group, or additional 2' chemical modification or a combination thereof. In some embodiments, the mRNA comprises at least 4 nucleotides at the 5' end that comprises 2'-hydroxyl group, 2'-O-methyl group, or additional 2' chemical modification or a combination thereof. In some embodiments, the mRNA comprises at least 5 nucleotides at the 5' end that comprises 2'-hydroxyl group, 2'-O-methyl group, or additional 2' chemical modification or a combination thereof. In some embodiments, the mRNA comprises at least 6 nucleotides at the 5' end that comprises 2'-hydroxyl group, 2'-O-methyl group, or additional 2' chemical modification or a combination thereof. In some embodiments, the mRNA comprises at least 7 nucleotides at the 5' end that comprises 2'-hydroxyl group, 2'-O-methyl group, or additional 2' chemical modification or a combination thereof. In some embodiments, the mRNA comprises at least 8 nucleotides at the 5' end that comprises 2'-hydroxyl group, 2'-O-methyl group, or additional 2' chemical modification or a combination thereof. In some embodiments, the mRNA comprises at least 9 nucleotides at the 5' end that comprises 2'-hydroxyl group, 2'-O-methyl group, or additional 2' chemical modification or a combination thereof. In some embodiments, the mRNA comprises at least 10 nucleotides at the 5' end that comprises 2'-hydroxyl group, 2'-O-methyl group, or additional 2' chemical modification or a combination thereof.

[152] In some embodiments, the mRNA comprises a poly A tail. The poly A tail may be at the 3' end of the mRNA.

[153] In some embodiments, the GC% content of the mRNA sequence is greater than or equal to 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90%. In some embodiments, the GC% content of the mRNA sequence is greater than or equal to 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75% or 80%.

[154] In some embodiments, the mRNA sequence comprises an adenine tTNA deaminase (TadA) region. In some embodiments, the GC% of the TadA region is greater than or equal to

40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90%. In some embodiments, the GC% content of the TadA region is greater than or equal to 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75% or 80%.

[155] In some embodiments, the mRNA sequence comprises a Cas9 region. In some embodiments, the GC% of the Cas9 region is greater than or equal to 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90%. In some embodiments, the GC% content of the Cas9 region is greater than or equal to 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75% or 80%.

[156] In some embodiments, the mRNA sequence comprises a NLS region. In some embodiments, the GC% of the NLS region is greater than or equal to 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90%. In some embodiments, the GC% content of the NLS region is greater than or equal to 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75% or 80%.

[157] In some embodiments, the mRNA sequence comprises a first linker region that connects the TadA region and the Cas9 region. In some embodiments, the GC% of the first linker region is greater than or equal to 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90%. In some embodiments, the GC% content of the first linker region is greater than or equal to 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75% or 80%.

[158] In some embodiments, the mRNA sequence comprises a second linker region that connects the Cas9 region and the NLS region. In some embodiments, the GC% of the second linker region is greater than or equal to 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90%. In some embodiments, the GC%

content of the second linker region is greater than or equal to 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75% or 80%.

[159] In some embodiments, the base editor system as provided herein further comprises a lipid nanoparticle (LNP) enclosing a guide polynucleotide or a nucleic acid encoding the guide polynucleotide (i). In some embodiments, the LNP further encloses a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same (ii). In some embodiments, the base editor system further comprises a second LNP enclosing a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same (ii).

[160] A base editor system as provided herein can include one or more LNPs. For example, a base editor system may comprise a LNP enclosing both a guide polynucleotide and a nucleic acid encoding the base editor fusion protein, e.g. an mRNA encoding the base editor fusion protein. In another example, a base editor system may comprise a LNP enclosing a guide polynucleotide, e.g. a guide RNA, and a LNP enclosing a nucleic acid, e.g. an mRNA, encoding the base editor fusion protein. LNPs separately enclosing the guide polynucleotide and the base editor fusion protein or mRNA encoding the base editor fusion protein may allow for flexible dosing and administration of the base editor system. For example, a LNP enclosing a guide RNA can be administered first, followed by administration of a LNP enclosing a mRNA encoding the base editor fusion protein. In some embodiments, a LNP enclosing a guide RNA and a second LNP enclosing a mRNA encoding the base editor fusion protein are administered to a subject at the same time. In some embodiments, a LNP enclosing a guide RNA and a LNP enclosing a mRNA encoding the base editor protein are administered to a subject sequentially. In some embodiments, a LNP enclosing mRNA encoding the base editor fusion protein is administered to a subject, followed by multiple administration or doses of a second LNP enclosing a guide RNA after 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, or 12 weeks or more. The multiple doses of the second LNP may be administered with intervals of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 days or more.

[161] In some embodiments, the ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 1:10 to about 10: 1 by weight. In some embodiments, the ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 1:1, 1.5:1, 2:1, 3:1, 4:1, 1:1.5, 1:2, 1:3, 1:4 or any ratio between 4:1 or 1:4 by weight. In some embodiments, the ratio of the guide polynucleotide and the

nucleic acid encoding the base editor fusion protein can be determined by titration of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein.

[162] In some embodiments, the molar ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 500:1 to about 1:500.

[163] In some embodiments, the ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 1000:1 to about 1:1000 by weight. In some embodiments, the ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 1000:1, 950:1, 900:1, 850:1, 800:1, 750:1, 700:1, 650:1, 600:1, 550:1, 500:1, 450:1, 400:1, 350:1, 300:1, 250:1, 200:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 65:1, 60:1, 55:1, 50:1, 45:1, 40:1, 35:1, 30:1, 25:1, 20:1, 19:1, 18:1, 17:1, 17:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2;1, 1.9:1, 1.8:1, 1.7:1, 1.6:1, 1.5:1, 1.4:1, 1.3:1, 1.2:1, 1.1:1, 1.0:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, 0.5:1, 0.4:1, 0.3:1, 0.2:1, or 0.1 by weight. In some embodiments, the ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 1:0.1, 1:0.2, 1:0.3, 1:0.4, 1:0.5, 1:0.6, 1:0.7, 1:0.8, 1:0.9, 1:1.0, 1:1.1, 1:1.2, 1:1.3, 1:1.4, 1:1.5, 1:1.6, 1:1.7, 1:1.8, 1:1.9, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, 1:100, 1:150, 1:200, 1:250, 1:300, 1:350, 1:400, 1:450, 1:500, 1:550, 1:600, 1:650, 1:700, 1:750, 1:800, 1:850, 1:900, 1:950, or 1:1000 by weight. In some embodiments, the ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at least about 1000:1, 950:1, 900:1, 850:1, 800:1, 750:1, 700:1, 650:1, 600:1, 550:1, 500:1, 450:1, 400:1, 350:1, 300:1, 250:1, 200:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 65:1, 60:1, 55:1, 50:1, 45:1, 40:1, 35:1, 30:1, 25:1, 20:1, 19:1, 18:1, 17:1, 17:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2;1, 1.9:1, 1.8:1, 1.7:1, 1.6:1, 1.5:1, 1.4:1, 1.3:1, 1.2:1, 1.1:1, 1.0:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, 0.5:1, 0.4:1, 0.3:1, 0.2:1, or 0.1 by weight. In some embodiments, the ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at least about 1:0.1, 1:0.2, 1:0.3, 1:0.4, 1:0.5, 1:0.6, 1:0.7, 1:0.8, 1:0.9, 1:1.0, 1:1.1, 1:1.2, 1:1.3, 1:1.4, 1:1.5, 1:1.6, 1:1.7, 1:1.8, 1:1.9, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, 1:100, 1:150, 1:200, 1:250, 1:300, 1:350, 1:400, 1:450, 1:500, 1:550, 1:600, 1:650, 1:700, 1:750, 1:800, 1:850, 1:900, 1:950, or 1:1000 by weight. In some embodiments, the ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at most about 1000:1, 950:1, 900:1, 850:1, 800:1, 750:1, 700:1, 650:1, 600:1, 550:1, 500:1, 450:1, 400:1, 350:1, 300:1, 250:1,

200:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 65:1, 60:1, 55:1, 50:1, 45:1, 40:1, 35:1, 30:1, 25:1, 20:1, 19:1, 18:1, 17:1, 17:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2;1, 1.9:1, 1.8:1, 1.7:1, 1.6:1, 1.5:1, 1.4:1, 1.3:1, 1.2:1, 1.1:1, 1.0:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, 0.5:1, 0.4:1, 0.3:1, 0.2:1, or 0.1 by weight. In some embodiments, the ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at most about 1:0.1, 1:0.2, 1:0.3, 1:0.4, 1:0.5, 1:0.6, 1:0.7, 1:0.8, 1:0.9, 1:1.0, 1:1.1, 1:1.2, 1:1.3, 1:1.4, 1:1.5, 1:1.6, 1:1.7, 1:1.8, 1:1.9, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, 1:100, 1:150, 1:200, 1:250, 1:300, 1:350, 1:400, 1:450, 1:500, 1:550, 1:600, 1:650, 1:700, 1:750, 1:800, 1:850, 1:900, 1:950, or 1:1000 by weight.

[164] In some embodiments, the molar ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 10000:1 to about 1:10000. In some embodiments, the molar ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 10000:1, 9500:1, 9000:1, 8500:1, 8000:1, 7500:1, 7000:1, 6500:1, 6000:1, 5500:1, 5000:1, 4500:1, 4000:1, 3500:1, 3000:1, 2500:1, 2000:1, 1500:1, 1000:1, 950:1, 900:1, 850:1, 800:1, 750:1, 700:1, 650:1, 600:1, 550:1, 500:1, 450:1, 400:1, 350:1, 300:1, 250:1, 200:1, 190:1, 180:1, 170:1, 160:1, 150:1, 140:1, 130:1, 120:1, 110:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 69:1, 68:1, 67:1, 66:1, 65:1, 64:1, 63:1, 62:1, 61:1, 60:1, 59:1, 58:1, 57:1, 56:1, 55:1, 54:1, 53:1, 52:1, 51:1, 50:1, 49:1, 48:1, 47:1, 46:1, 45:1, 44:1, 43:1, 42:1, 41:1, 40:1, 39:1, 38:1, 37:1, 36:1, 35:1, 34:1, 33:1, 32:1, 31:1, 30:1, 29:1, 28:1, 27:1, 26:1, 25:1, 24:1, 23:1, 22:1, 21:1, 20:1, 19:1, 18:1, 17:1, 16:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, 0.5:1, 0.4:1, 0.3:1, 0.2:1, or 0.1:1. In some embodiments, the molar ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at least about 1:10000, 1:9500, 1:9000, 1:8500, 1:8000, 1:7500, 1:7000, 1:6500, 1:6000, 1:5500, 1:5000, 1:4500, 1:4000, 1:3500, 1:3000, 1:2500, 1:2000, 1:1500, 1:1000, 1:950, 1:900, 1:850, 1:800, 1:750, 1:700, 1:650, 1:600, 1:550, 1:500, 1:450, 1:400, 1:350, 1:300, 1:250, 1:200, 1:190, 1:180, 1:170, 1:160, 1:150, 1:140, 1:130, 1:120, 1:110, 1:100, 1:95, 1:90, 1:85, 1:80, 1:75, 1:70, 1:69, 1:68, 1:67, 1:66, 1:65, 1:64, 1:63, 1:62, 1:61, 1:60, 1:59, 1:58, 1:57, 1:56, 1:55, 1:54, 1:53, 1:52, 1:51, 1:50, 1:49, 1:48, 1:47, 1:46, 1:45, 1:44, 1:43, 1:42, 1:41, 1:40, 1:39, 1:38, 1:37, 1:36, 1:35, 1:34, 1:33, 1:32, 1:31, 1:30, 1:29, 1:28, 1:27, 1:26, 1:25, 1:24, 1:23, 1:22, 1:21, 1:20, 1:19, 1:18, 1:17, 1:16, 1:15, 1:14, 1:13, 1:12, 1:11, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 1:0.9, 1:0.8, 1:0.7, 1:0.6, 1:0.5, 1:0.4, 1:0.3, 1:0.2, or 1:0.1. In some

embodiments, the molar ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at least about 10000:1, 9500:1, 9000:1, 8500:1, 8000:1, 7500:1, 7000:1, 6500:1, 6000:1, 5500:1, 5000:1, 4500:1, 4000:1, 3500:1, 3000:1, 2500:1, 2000:1, 1500:1, 1000:1, 950:1, 900:1, 850:1, 800:1, 750:1, 700:1, 650:1, 600:1, 550:1, 500:1, 450:1, 400:1, 350:1, 300:1, 250:1, 200:1, 190:1, 180:1, 170:1, 160:1, 150:1, 140:1, 130:1, 120:1, 110:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 69:1, 68:1, 67:1, 66:1, 65:1, 64:1, 63:1, 62:1, 61:1, 60:1, 59:1, 58:1, 57:1, 56:1, 55:1, 54:1, 53:1, 52:1, 51:1, 50:1, 49:1, 48:1, 47:1, 46:1, 45:1, 44:1, 43:1, 42:1, 41:1, 40:1, 39:1, 38:1, 37:1, 36:1, 35:1, 34:1, 33:1, 32:1, 31:1, 30:1, 29:1, 28:1, 27:1, 26:1, 25:1, 24:1, 23:1, 22:1, 21:1, 20:1, 19:1, 18:1, 17:1, 16:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, 0.5:1, 0.4:1, 0.3:1, 0.2:1, or 0.1:1. In some embodiments, the molar ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at least about 1:10000, 1:9500, 1:9000, 1:8500, 1:8000, 1:7500, 1:7000, 1:6500, 1:6000, 1:5500, 1:5000, 1:4500, 1:4000, 1:3500, 1:3000, 1:2500, 1:2000, 1:1500, 1:1000, 1:950, 1:900, 1:850, 1:800, 1:750, 1:700, 1:650, 1:600, 1:550, 1:500, 1:450, 1:400, 1:350, 1:300, 1:250, 1:200, 1:190, 1:180, 1:170, 1:160, 1:150, 1:140, 1:130, 1:120, 1:110, 1:100, 1:95, 1:90, 1:85, 1:80, 1:75, 1:70, 1:69, 1:68, 1:67, 1:66, 1:65, 1:64, 1:63, 1:62, 1:61, 1:60, 1:59, 1:58, 1:57, 1:56, 1:55, 1:54, 1:53, 1:52, 1:51, 1:50, 1:49, 1:48, 1:47, 1:46, 1:45, 1:44, 1:43, 1:42, 1:41, 1:40, 1:39, 1:38, 1:37, 1:36, 1:35, 1:34, 1:33, 1:32, 1:31, 1:30, 1:29, 1:28, 1:27, 1:26, 1:25, 1:24, 1:23, 1:22, 1:21, 1:20, 1:19, 1:18, 1:17, 1:16, 1:15, 1:14, 1:13, 1:12, 1:11, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 1:0.9, 1:0.8, 1:0.7, 1:0.6, 1:0.5, 1:0.4, 1:0.3, 1:0.2, or 1:0.1. In some embodiments, the molar ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at most about 10000:1, 9500:1, 9000:1, 8500:1, 8000:1, 7500:1, 7000:1, 6500:1, 6000:1, 5500:1, 5000:1, 4500:1, 4000:1, 3500:1, 3000:1, 2500:1, 2000:1, 1500:1, 1000:1, 950:1, 900:1, 850:1, 800:1, 750:1, 700:1, 650:1, 600:1, 550:1, 500:1, 450:1, 400:1, 350:1, 300:1, 250:1, 200:1, 190:1, 180:1, 170:1, 160:1, 150:1, 140:1, 130:1, 120:1, 110:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 69:1, 68:1, 67:1, 66:1, 65:1, 64:1, 63:1, 62:1, 61:1, 60:1, 59:1, 58:1, 57:1, 56:1, 55:1, 54:1, 53:1, 52:1, 51:1, 50:1, 49:1, 48:1, 47:1, 46:1, 45:1, 44:1, 43:1, 42:1, 41:1, 40:1, 39:1, 38:1, 37:1, 36:1, 35:1, 34:1, 33:1, 32:1, 31:1, 30:1, 29:1, 28:1, 27:1, 26:1, 25:1, 24:1, 23:1, 22:1, 21:1, 20:1, 19:1, 18:1, 17:1, 16:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, 0.5:1, 0.4:1, 0.3:1, 0.2:1, or 0.1:1. In some embodiments, the molar ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at most about 1:10000, 1:9500, 1:9000, 1:8500, 1:8000, 1:7500, 1:7000, 1:6500, 1:6000, 1:5500, 1:5000,

1:4500, 1:4000, 1:3500, 1:3000, 1:2500, 1:2000, 1:1500, 1:1000, 1:950, 1:900, 1:850, 1:800, 1:750, 1:700, 1:650, 1:600, 1:550, 1:500, 1:450, 1:400, 1:350, 1:300, 1:250, 1:200, 1:190, 1:180, 1:170, 1:160, 1:150, 1:140, 1:130, 1:120, 1:110, 1:100, 1:95, 1:90, 1:85, 1:80, 1:75, 1:70, 1:69, 1:68, 1:67, 1:66, 1:65, 1:64, 1:63, 1:62, 1:61, 1:60, 1:59, 1:58, 1:57, 1:56, 1:55, 1:54, 1:53, 1:52, 1:51, 1:50, 1:49, 1:48, 1:47, 1:46, 1:45, 1:44, 1:43, 1:42, 1:41, 1:40, 1:39, 1:38, 1:37, 1:36, 1:35, 1:34, 1:33, 1:32, 1:31, 1:30, 1:29, 1:28, 1:27, 1:26, 1:25, 1:24, 1:23, 1:22, 1:21, 1:20, 1:19, 1:18, 1:17, 1:16, 1:15, 1:14, 1:13, 1:12, 1:11, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 1:0.9, 1:0.8, 1:0.7, 1:0.6, 1:0.5, 1:0.4, 1:0.3, 1:0.2, or 1:0.1.

[165] In some embodiments, the ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 10:1 to about 1:10 by weight. In some embodiments, the ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 4:1, 3;1, 2:1, 1.5:1, 1:1, 1:1.5, 1:2, 1:3, or 1:4 by weight. In some embodiments, the molar ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 500:1 to about 1:500.

[166] In some embodiments, the ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 1000:1 to about 1:1000 by weight. In some embodiments, the ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 1000:1, 950:1, 900:1, 850:1, 800:1, 750:1, 700:1, 650:1, 600:1, 550:1, 500:1, 450:1, 400:1, 350:1, 300:1, 250:1, 200:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 65:1, 60:1, 55:1, 50:1, 45:1, 40:1, 35:1, 30:1, 25:1, 20:1, 19:1, 18:1, 17:1, 17:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2;1, 1.9:1, 1.8:1, 1.7:1, 1.6:1, 1.5:1, 1.4:1, 1.3:1, 1.2:1, 1.1:1, 1.0:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, 0.5:1, 0.4:1, 0.3:1, 0.2:1, or 0.1 by weight. In some embodiments, the ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 1:0.1, 1:0.2, 1:0.3, 1:0.4, 1:0.5, 1:0.6, 1:0.7, 1:0.8, 1:0.9, 1:1.0, 1:1.1, 1:1.2, 1:1.3, 1:1.4, 1:1.5, 1:1.6, 1:1.7, 1:1.8, 1:1.9, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, 1:100, 1:150, 1:200, 1:250, 1:300, 1:350, 1:400, 1:450, 1:500, 1:550, 1:600, 1:650, 1:700, 1:750, 1:800, 1:850, 1:900, 1:950, or 1:1000 by weight. In some embodiments, the ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at least about 1000:1, 950:1, 900:1, 850:1, 800:1, 750:1, 700:1, 650:1, 600:1, 550:1, 500:1, 450:1, 400:1, 350:1, 300:1, 250:1, 200:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 65:1, 60:1,

55:1, 50:1, 45:1, 40:1, 35:1, 30:1, 25:1, 20:1, 19:1, 18:1, 17:1, 17:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2;1, 1.9:1, 1.8:1, 1.7:1, 1.6:1, 1.5:1, 1.4:1, 1.3:1, 1.2:1, 1.1:1, 1.0:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, 0.5:1, 0.4:1, 0.3:1, 0.2:1, or 0.1 by weight. In some embodiments, the ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at least about 1:0.1, 1:0.2, 1:0.3, 1:0.4, 1:0.5, 1:0.6, 1:0.7, 1:0.8, 1:0.9, 1:1.0, 1:1.1, 1:1.2, 1:1.3, 1:1.4, 1:1.5, 1:1.6, 1:1.7, 1:1.8, 1:1.9, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, 1:100, 1:150, 1:200, 1:250, 1:300, 1:350, 1:400, 1:450, 1:500, 1:550, 1:600, 1:650, 1:700, 1:750, 1:800, 1:850, 1:900, 1:950, or 1:1000 by weight. In some embodiments, the ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at most about 1000:1, 950:1, 900:1, 850:1, 800:1, 750:1, 700:1, 650:1, 600:1, 550:1, 500:1, 450:1, 400:1, 350:1, 300:1, 250:1, 200:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 65:1, 60:1, 55:1, 50:1, 45:1, 40:1, 35:1, 30:1, 25:1, 20:1, 19:1, 18:1, 17:1, 17:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2;1, 1.9:1, 1.8:1, 1.7:1, 1.6:1, 1.5:1, 1.4:1, 1.3:1, 1.2:1, 1.1:1, 1.0:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, 0.5:1, 0.4:1, 0.3:1, 0.2:1, or 0.1 by weight. In some embodiments, the ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at most about 1:0.1, 1:0.2, 1:0.3, 1:0.4, 1:0.5, 1:0.6, 1:0.7, 1:0.8, 1:0.9, 1:1.0, 1:1.1, 1:1.2, 1:1.3, 1:1.4, 1:1.5, 1:1.6, 1:1.7, 1:1.8, 1:1.9, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, 1:100, 1:150, 1:200, 1:250, 1:300, 1:350, 1:400, 1:450, 1:500, 1:550, 1:600, 1:650, 1:700, 1:750, 1:800, 1:850, 1:900, 1:950, or 1:1000 by weight.

[167] In some embodiments, the molar ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 10000:1 to about 1:10000. In some embodiments, the molar ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 10000:1, 9500:1, 9000:1, 8500:1, 8000:1, 7500:1, 7000:1, 6500:1, 6000:1, 5500:1, 5000:1, 4500:1, 4000:1, 3500:1, 3000:1, 2500:1, 2000:1, 1500:1, 1000:1, 950:1, 900:1, 850:1, 800:1, 750:1, 700:1, 650:1, 600:1, 550:1, 500:1, 450:1, 400:1, 350:1, 300:1, 250:1, 200:1, 190:1, 180:1, 170:1, 160:1, 150:1, 140:1, 130:1, 120:1, 110:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 69:1, 68:1, 67:1, 66:1, 65:1, 64:1, 63:1, 62:1, 61:1, 60:1, 59:1, 58:1, 57:1, 56:1, 55:1, 54:1, 53:1, 52:1, 51:1, 50:1, 49:1, 48:1, 47:1, 46:1, 45:1, 44:1, 43:1, 42:1, 41:1, 40:1, 39:1, 38:1, 37:1, 36:1, 35:1, 34:1, 33:1, 32:1, 31:1, 30:1, 29:1, 28:1, 27:1, 26:1, 25:1, 24:1, 23:1, 22:1,

21:1, 20:1, 19:1, 18:1, 17:1, 16:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, 0.5:1, 0.4:1, 0.3:1, 0.2:1, or 0.1:1. In some embodiments, the molar ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at least about 1:10000, 1:9500, 1:9000, 1:8500, 1:8000, 1:7500, 1:7000, 1:6500, 1:6000, 1:5500, 1:5000, 1:4500, 1:4000, 1:3500, 1:3000, 1:2500, 1:2000, 1:1500, 1:1000, 1:950, 1:900, 1:850, 1:800, 1:750, 1:700, 1:650, 1:600, 1:550, 1:500, 1:450, 1:400, 1:350, 1:300, 1:250, 1:200, 1:190, 1:180, 1:170, 1:160, 1:150, 1:140, 1:130, 1:120, 1:110, 1:100, 1:95, 1:90, 1:85, 1:80, 1:75, 1:70, 1:69, 1:68, 1:67, 1:66, 1:65, 1:64, 1:63, 1:62, 1:61, 1:60, 1:59, 1:58, 1:57, 1:56, 1:55, 1:54, 1:53, 1:52, 1:51, 1:50, 1:49, 1:48, 1:47, 1:46, 1:45, 1:44, 1:43, 1:42, 1:41, 1:40, 1:39, 1:38, 1:37, 1:36, 1:35, 1:34, 1:33, 1:32, 1:31, 1:30, 1:29, 1:28, 1:27, 1:26, 1:25, 1:24, 1:23, 1:22, 1:21, 1:20, 1:19, 1:18, 1:17, 1:16, 1:15, 1:14, 1:13, 1:12, 1:11, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 1:0.9, 1:0.8, 1:0.7, 1:0.6, 1:0.5, 1:0.4, 1:0.3, 1:0.2, or 1:0.1. In some embodiments, the molar ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at least about 10000:1, 9500:1, 9000:1, 8500:1, 8000:1, 7500:1, 7000:1, 6500:1, 6000:1, 5500:1, 5000:1, 4500:1, 4000:1, 3500:1, 3000:1, 2500:1, 2000:1, 1500:1, 1000:1, 950:1, 900:1, 850:1, 800:1, 750:1, 700:1, 650:1, 600:1, 550:1, 500:1, 450:1, 400:1, 350:1, 300:1, 250:1, 200:1, 190:1, 180:1, 170:1, 160:1, 150:1, 140:1, 130:1, 120:1, 110:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 69:1, 68:1, 67:1, 66:1, 65:1, 64:1, 63:1, 62:1, 61:1, 60:1, 59:1, 58:1, 57:1, 56:1, 55:1, 54:1, 53:1, 52:1, 51:1, 50:1, 49:1, 48:1, 47:1, 46:1, 45:1, 44:1, 43:1, 42:1, 41:1, 40:1, 39:1, 38:1, 37:1, 36:1, 35:1, 34:1, 33:1, 32:1, 31:1, 30:1, 29:1, 28:1, 27:1, 26:1, 25:1, 24:1, 23:1, 22:1, 21:1, 20:1, 19:1, 18:1, 17:1, 16:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, 0.5:1, 0.4:1, 0.3:1, 0.2:1, or 0.1:1. In some embodiments, the molar ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at least about 1:10000, 1:9500, 1:9000, 1:8500, 1:8000, 1:7500, 1:7000, 1:6500, 1:6000, 1:5500, 1:5000, 1:4500, 1:4000, 1:3500, 1:3000, 1:2500, 1:2000, 1:1500, 1:1000, 1:950, 1:900, 1:850, 1:800, 1:750, 1:700, 1:650, 1:600, 1:550, 1:500, 1:450, 1:400, 1:350, 1:300, 1:250, 1:200, 1:190, 1:180, 1:170, 1:160, 1:150, 1:140, 1:130, 1:120, 1:110, 1:100, 1:95, 1:90, 1:85, 1:80, 1:75, 1:70, 1:69, 1:68, 1:67, 1:66, 1:65, 1:64, 1:63, 1:62, 1:61, 1:60, 1:59, 1:58, 1:57, 1:56, 1:55, 1:54, 1:53, 1:52, 1:51, 1:50, 1:49, 1:48, 1:47, 1:46, 1:45, 1:44, 1:43, 1:42, 1:41, 1:40, 1:39, 1:38, 1:37, 1:36, 1:35, 1:34, 1:33, 1:32, 1:31, 1:30, 1:29, 1:28, 1:27, 1:26, 1:25, 1:24, 1:23, 1:22, 1:21, 1:20, 1:19, 1:18, 1:17, 1:16, 1:15, 1:14, 1:13, 1:12, 1:11, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 1:0.9, 1:0.8, 1:0.7, 1:0.6, 1:0.5, 1:0.4, 1:0.3, 1:0.2, or 1:0.1. In

some embodiments, the molar ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at most about 10000:1, 9500:1, 9000:1, 8500:1, 8000:1, 7500:1, 7000:1, 6500:1, 6000:1, 5500:1, 5000:1, 4500:1, 4000:1, 3500:1, 3000:1, 2500:1, 2000:1, 1500:1, 1000:1, 950:1, 900:1, 850:1, 800:1, 750:1, 700:1, 650:1, 600:1, 550:1, 500:1, 450:1, 400:1, 350:1, 300:1, 250:1, 200:1, 190:1, 180:1, 170:1, 160:1, 150:1, 140:1, 130:1, 120:1, 110:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 69:1, 68:1, 67:1, 66:1, 65:1, 64:1, 63:1, 62:1, 61:1, 60:1, 59:1, 58:1, 57:1, 56:1, 55:1, 54:1, 53:1, 52:1, 51:1, 50:1, 49:1, 48:1, 47:1, 46:1, 45:1, 44:1, 43:1, 42:1, 41:1, 40:1, 39:1, 38:1, 37:1, 36:1, 35:1, 34:1, 33:1, 32:1, 31:1, 30:1, 29:1, 28:1, 27:1, 26:1, 25:1, 24:1, 23:1, 22:1, 21:1, 20:1, 19:1, 18:1, 17:1, 16:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, 0.5:1, 0.4:1, 0.3:1, 0.2:1, or 0.1:1. In some embodiments, the molar ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at most about 1:10000, 1:9500, 1:9000, 1:8500, 1:8000, 1:7500, 1:7000, 1:6500, 1:6000, 1:5500, 1:5000, 1:4500, 1:4000, 1:3500, 1:3000, 1:2500, 1:2000, 1:1500, 1:1000, 1:950, 1:900, 1:850, 1:800, 1:750, 1:700, 1:650, 1:600, 1:550, 1:500, 1:450, 1:400, 1:350, 1:300, 1:250, 1:200, 1:190, 1:180, 1:170, 1:160, 1:150, 1:140, 1:130, 1:120, 1:110, 1:100, 1:95, 1:90, 1:85, 1:80, 1:75, 1:70, 1:69, 1:68, 1:67, 1:66, 1:65, 1:64, 1:63, 1:62, 1:61, 1:60, 1:59, 1:58, 1:57, 1:56, 1:55, 1:54, 1:53, 1:52, 1:51, 1:50, 1:49, 1:48, 1:47, 1:46, 1:45, 1:44, 1:43, 1:42, 1:41, 1:40, 1:39, 1:38, 1:37, 1:36, 1:35, 1:34, 1:33, 1:32, 1:31, 1:30, 1:29, 1:28, 1:27, 1:26, 1:25, 1:24, 1:23, 1:22, 1:21, 1:20, 1:19, 1:18, 1:17, 1:16, 1:15, 1:14, 1:13, 1:12, 1:11, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 1:0.9, 1:0.8, 1:0.7, 1:0.6, 1:0.5, 1:0.4, 1:0.3, 1:0.2, or 1:0.1.

[168] Precision genome editing is a growing field with industrial, agricultural, and biomedical applications. One of the dominant genome-editing systems available today is clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR associated 9 (Cas9). Through the use of a guide RNA (gRNA) with a sequence homologous to that of a sequence of DNA in the target genome (known as the protospacer) adjacent to a specific protospacer-adjacent motif (PAM) comprising the sequence NGG (N is any standard base) in the DNA, Cas9 can be used to create a double-strand break (DSB) at the targeted sequence. Non-homologous end joining (NHEJ) at DSBs can be used to create indels and knock out genes at genetic loci; likewise, homology-directed repair (HDR) can be used, with an introduced template DNA, to insert genes or modify the targeted sequence. A variety of Cas9-based tools have been developed in recent years, including tools that methylate DNA, recognize broader sequence space, or create single-strand nicks. In 2016, Komor et al.

described the use of CRISPR-Cas9 to convert a cytosine base to a thymine base without the introduction of a template DNA strand and without the need for DSBs (Komor AC, Kim YB, Packer MS, et al. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature,* 2016, 533: 420–4, incorporated herein by reference in its entirety). After the cytidine deaminase domain of rat APOBEC1 was fused to the N-terminus of catalytically-dead Cas9 (dCas9) using the linker XTEN (resulting in a fusion protein called base editor 1, or BE1), conversion of cytosine to uracil was observed between position 4 and position 8 within the 20-nt protospacer region of DNA (or, to express it a different way, 13 to 17 nucleotides upstream of the PAM). Of note, any cytosine base within this "window" was amenable to editing, resulting in varied outcomes depending on how many and which cytosines were edited. After DNA replication or repair, each uracil was replaced by a thymine, completing the C to T base editing.3 The next version of base editor (BE2) incorporated a uracil glycosylase inhibitor fused to the C-terminus of dCas9 to help inhibit base excision repair of the uracil bases resulting from the cytidine deaminase activity (which otherwise would act to restore the original cytosine bases); this improved the efficiency of C to T base editing. The final version, BE3, used a Cas9 nickase rather than dCas9; the nickase cut the unedited strand opposite the edited C to T bases, stimulating the removal of the opposing guanidine through eukaryotic mismatch repair. BE2 and BE3 base editing was observed in both human and murine cell lines. The specificity of base editing has been further improved through the addition of mutations to the Cas9 nickase; in similar fashion, Cas9 has been mutated to narrow the width of the editing window from approximately 5 nucleotides to as little as 1-2 nucleotides (Rees HA, Komor AC, Yeh WH, et al. Improving the DNA specificity and applicability of base editing through protein engineering and protein delivery. Nat Commun, 2017, 8: 15790, Kim YB, Komor AC, Levy JM, et al. Increasing the genome-targeting scope and precision of base editing with engineered Cas9-cytidine deaminase fusions. Nat Biotechnol, 2017, 35: 371–6, each of which is incorporated herein by reference in its entirety).

[169] An alternative cytosine base editing platform is by linking the activation-induced cytosine deaminase domain PmCDA1 to dCas9 (Target-AID), they were able to demonstrate targeted C to T base editing in yeast. Furthermore, an alternative C to T editing strategy was also demonstrated without fusing a deaminase domain to Cas9; instead, a SH3 (Src 3 homology) domain was added to the C-terminus of dCas9 while a SHL (SH3 interaction ligand) was added to PmCDA1.6 Optimization of efficiency was achieved through the use of a Cas9 nickase rather than dCas9. Further, an uracil DNA glycosylase inhibitor was added to

enhance base editing in the mammalian CHO cell line. The resulting platform was able to consistently edit bases within 3 to 5 bases of the 18th nucleotide upstream of the PAM sequence (Nishida K, Arazoe T, Yachie N, et al. Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems. Science, 2016, 353: aaf8729, incorporated herein by reference in its entirety).

[170] A distinct cytosine base editing platform used Cpf1 (also known as Cas12a) instead of Cas9 as the RNA-guided endonuclease. Catalytically-inactive Cpf1 was fused to APOBEC1 (dLbCpf1-BE0), leading to C to T conversion in a human cell line. While the Cas9 base editor variants BE3 and Target-AID recognize the PAM sequence NGG, dLbCpf1-BE0 recognizes the T-rich PAM sequence TTTV. Although base editing was observed between positions 8 and 13 of the protospacer sequence with dLbCpf1-BE0, the introduction of additional mutations into Cpf1 was able to reduce the window to positions 10 to 12. However, narrowing of the base editing window correlated with a decrease in editing efficiency (Li X, Wang Y, Liu Y, et al. Base editing with a Cpf1-cytidine deaminase fusion. Nat Biotechnol, 2018, 36: 324–7, incorporated herein by reference in its entirety).

[171] Cytosine base editing is not wholly predictable; indels can occur at the target site, albeit at lower frequencies that those observed for C to T editing editors. Furthermore, cytosine base editors can occasionally cause C to A or C to G edits rather than the expected C to T edits. Adding linker lengths between Cas9 nickase and the rat APOBEC1 cytosine deaminase domain from 16 amino acids to 32 amino acids, the linker between the Cas9 nickase and the uracil glycosylase inhibitor from 4 amino acids to 9 amino acids, and using a second uracil glycosylase inhibitor was appended to the C-terminus of the new cytosine base editor using another 9 amino acid linker improved cytosine base editor termed “BE4” (Komor AC, Zhao KT, Packer MS, et al. Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity. Sci Adv, 2017, 3: eaao4774. Incorporated herein by reference in its entirety).

[172] By fusing Escherichia coli adenine tTNA deaminase TadA (ecTadA) to dCas9 and mutagenesis of the ecTadA domain in conjunction with selection for editing activity revealed that A106V and D108N mutations yielded a base editor capable of editing adenine to guanine in DNA, termed ABE7.10 (Gaudelli NM, Komor AC, Rees HA, et al. Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. Nature, 2017, 551: 464–71. Incorporated herein by reference in its entirety). Koblan et al. improved the efficiency of ABE7.10 through modification of nuclear localization signals and codon optimization, yielding a version called ABEmax; a similar approach improved the efficiency of the

cytosine base editor BE4.10 Huang et al. performed further development of both adenine and cytosine base editors to use alternative PAMs and to expand their editing windows, thereby increasing their targeting range (Improving cytidine and adenine base editors by expression optimization and ancestral reconstruction. Nat Biotechnol, 2018, 36: 843–6, Incorporated herein by reference in its entirety).

[173] The same has proven to be true of base editors,12–14 although comparisons of Cas9, cytosine base editors, and adenine base editors using the same gRNAs have shown distinct off-target profiles.

[174] A variety of studies have raised concern about gRNA-independent off-target base editing, caused by the deaminase domain acting in isolation (without the need for engagement of DNA by the Cas9-gRNA complex). Additional studies showed that the gRNA-independent off-target effects of base editors are not limited to DNA. RNA sequencing of cells treated with either cytosine base editors or adenine base editors revealed transcriptome-wide off-target editing of RNA, and that introduction of amino acid substitution in the deaminase domain of adenine base (e.g. R106W) editors reduced off-target editing of RNA without substantially reducing on-target DNA base-editing efficiency. Zuo E, Sun Y, Wei W, et al. Cytosine base editor generates substantial off-target single-nucleotide variants in mouse embryos. Science, 2019, 364: 289–92; Jin S, Zong Y, Gao Q, et al. Cytosine, but not adenine, base editors induce genome-wide off-target mutations in rice. Science, 2019, 364: 292–5; Grünewald J, Zhou R, Garcia SP, et al. Transcriptome-wide off-target RNA editing induced by CRISPR-guided DNA base editors. Nature, 2019, 569: 433–7; Grünewald J, Zhou R, Iyer S, et al. CRISPR DNA base editors with reduced RNA off-target and self-editing activities. Nat Biotechnol, 2019, 37: 1041–8; Zhou C, Sun Y, Yan R, et al. Off-target RNA mutation induced by DNA base editing and its elimination by mutagenesis. Nature, 2019, 571: 275–8; Rees HA, Wilson C, Doman JL, et al. Analysis and minimization of cellular RNA editing by DNA adenine base editors. Sci Adv, 2019, 5: eaax5717, each of which is incorporated herein by reference in its entirety).

[175] Correction of disease-causing mutations via precision editing with standard Cas9 genome editing has largely required HDR. Since HDR is limited to cells in S or G2 phase of mitosis, precision editing of non-mitotic cells is difficult. However, base editors are not reliant on HDR; the editing of postmitotic cochlear cells in mice is feasible with cytosine base editor BE3. By injecting BE3 and a gRNA in the form of a preassembled ribonucleoprotein via cationic liposomes, serine-33 in beta-catenin was edited to phenylalanine (TCT codon edited to TTT), allowing for the transdifferentiation of supporting

cells into hair cells. Base editing of cochlear tissue was confirmed via sequencing, showing an editing rate between 0.7% and 3.0% depending on the region of the cochlea. In contrast, standard Cas9 editing via HDR showed negligible signs of efficacy in cochlear cells. A variant of SaBE3, delivered into the liver via adeno-associated viral (AAV) vectors, was reported to directly correct a pathogenic T to C mutation in the Pah gene with an editing rate as high as 29% and thereby treat the disease phenylketonuria in adult mice. Adenine base editing has also been demonstrated to generate mutations in mice. Yeh WH, Chiang H, Rees HA, et al. In vivo base editing of post-mitotic sensory cells. Nat Commun, 2018, 9: 2184; Villiger L, Grisch-Chan HM, Lindsay H, et al. Treatment of a metabolic liver disease by in vivo genome base editing in adult mice. Nat Med, 2018, 24: 1519–25; Ryu SM, Koo T, Kim K, et al. Adenine base editing in mouse embryos and an adult mouse model of Duchenne muscular dystrophy. Nat Biotechnol, 2018, 36: 536–9; Song CQ, Jiang T, Richter M, et al. Adenine base editing in an adult mouse model of tyrosinaemia. Nat Biomed Eng, 2020, 4: 125–30; Pisciotta L, Favari E, Magnolo L, et al. Characterization of three kindreds with familial combined hypolipidemia caused by loss-of-function mutations of ANGPTL3. Circ Cardiovasc Genet, 2012, 5: 42–50. each of which is incorporated herein by reference in its entirety.

[176] Provided herein are compositions of nucleobase editor systems that comprises nucleobase editor proteins, complexes, or compounds that is capable of making a modification or conversion to a nucleobase (e.g., A, T, C, G, or U) within a target nucleotide sequence.

[177] A nucleobase editor or a base editor (BE) refers to an agent comprising a polypeptide that is capable of making a modification to a base (e.g., A, T, C, G, or U) within a nucleic acid sequence (e.g., DNA or RNA). In some embodiments, the base editor is capable of deaminating a base within a nucleic acid. In some embodiments, the base editor is capable of deaminating a base within a DNA molecule. In some embodiments, the base editor is capable of deaminating an adenine (A) in DNA.

[178] In some embodiments, the base editor comprises a fusion protein comprising a programmable DNA binding protein fused to an adenosine deaminase. In some embodiments, the base editor comprises a fusion protein comprising a Cas9 protein and an adenosine deaminase. In some embodiments, the base editor is a Cas9 nickase (nCas9) fused to an adenosine deaminase. In some embodiments, the base editor is a nuclease-inactive Cas9 (dCas9) fused to an adenosine deaminase. In some embodiments, the base editor comprises a fusion protein comprising a programmable DNA binding protein fused to an cytidine

deaminase. In some embodiments, the base editor comprises a fusion protein comprising a Cas9 protein and an cytidine deaminase. In some embodiments, the base editor is a Cas9 nickase (nCas9) fused to an cytidine deaminase. In some embodiments, the base editor is a nuclease-inactive Cas9 (dCas9) fused to an cytidine deaminase. In some embodiments, the base editor further comprises, an inhibitor of base excision repair, for example, a UGI domain. In some embodiments, the fusion protein comprises a Cas9 nickase fused to a deaminase and an inhibitor of base excision repair, such as a UGI or dISN domain. In some embodiments, the dCas9 domain of the fusion protein comprises a D10A and a H840A mutation as numbered in the wild type SpCas9 amino acid sequence. In some embodiments, the UGI comprises the following amino acid sequence:

[179] >splP14739IUNGI_BPPB2 Uracil-DNA glycosylase inhibitor MTNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLT S D APE YKPW ALVIQDS NGENKIKML (SEQ ID NO: 16)

[180] In some embodiments, a base editor system provided herein comprises a base editor fusion protein. For example, a base editor fusion protein may comprise a programmable DNA binding protein and a deaminase, e.g. an adenosine deaminase. In some embodiments, any of the fusion proteins provided herein are base editors. In some embodiments, the programmable DNA binding protein is a Cas9 domain, a Cpf1 domain, a CasX domain, a CasY domain, a Cas12b domain, a C2c2 domain, aC2c3 domain, or an Argonaute domain. In some embodiments, the programmable DNA binding protein is a Cas9 domain. The Cas9 domain may be any of the Cas9 domains or Cas9 proteins (e.g., nuclease inactive Cas9 or Cas9 nickase, or a Cas9 variant from any species) provided herein. In some embodiments, any of the Cas9 domains or Cas9 proteins provided herein may be fused with any of the deaminases provided herein. In some embodiments, the base editor comprises a deaminase, e.g., an adenosine deaminase and a programmable DNA binding protein, e.g., a Cas9 domain joined via a linker. In some embodiments, the base editor comprises a fusion protein comprising a deaminase, e.g., an adenosine deaminase and a programmable DNA binding protein, e.g., a Cas9 domain joined via a linker. In some embodiments, the linker is a peptide linker. In some embodiments, a linker is present between the deaminase domain and the Cas9 domain. In some embodiments, an deaminase and a programmable DNA binding domain are fused via any of the peptide linkers provided herein. For example, an adenosine deaminase and a Cas9 domain may be fused via a linker that comprises between 1 and 200 amino acids. In some embodiments, the adenosine deaminase and the programmable DNA binding protein are fused via a linker that comprises from 1 to 5, 1 to 10, 1 to 20, 1 to 30, 1 to 40, 1 to 50, 1 to 60,

1 to 80, 1 to 100, 1 to 150, 1 to 200, 5 to 10, 5 to 20, 5 to 30, 5 to 40, 5 to 60, 5 to 80, 5 to 100, 5 to 150, 5 to 200, 10 to 20, 10 to 30, 10 to 40, 10 to 50, 10 to 60, 10 to 80, 10 to 100, 10 to 150, 10 to 200, 20 to 30, 20 to 40, 20 to 50, 20 to 60, 20 to 80, 20 to 100, 20 to 150, 20 to 200, 30 to 40, 30 to 50, 30 to 60, 30 to 80, 30 to 100, 30 to 150, 30 to 200, 40 to 50, 40 to 60, 40 to 80, 40 to 100, 40 to 150, 40 to 200, 50 to 60 50 to 80, 50 to 100, 50 to 150, 50 to 200, 60 to 80, 60 to 100, 60 to 150, 60 to 200, 80 to 100, 80 to 150, 80 to 200, 100 to 150, 100 to 200, or 150 to 200 amino acids in length. In some embodiments, the adenosine deaminase and the programmable DNA binding protein are fused via a linker that comprises 4, 16, 32, or 104 amino acids in length. In some embodiments, the adenosine deaminase and the programmable DNA binding protein are fused via a linker that comprises the amino acid sequence of SGSETPGTSESATPES (SEQ ID NO: 17), SGGS (SEQ ID NO: 18), SGGSSGSETPGTSESATPESSGGS (SEQ ID NO: 19), SGGSSGGSSGSETPGTSESATPESSGGSSGGS (SEQ ID NO: 20), or GGSGGSPGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGSEPATSGGSGGS (SEQ ID NO: 21). In some embodiments, the adenosine deaminase and the programmable DNA binding protein are fused via a linker comprising the amino acid sequence SGSETPGTSESATPES (SEQ ID NO: 17), which may also be referred to as the XTEN linker. In some embodiments, the linker is 24 amino acids in length. In some embodiments, the linker comprises the amino acid sequence SGGSSGGSSGSETPGTSESATPES (SEQ ID NO: 23). In some embodiments, the linker is 40 amino acids in length. In some embodiments, the linker comprises the amino acid sequence SGGSSGGSSGSETPGTSESATPESSGGSSGGSSGGSSGGS (SEQ ID NO: 24). In some embodiments, the linker is 64 amino acids in length. In some embodiments, the linker comprises the amino acid sequence SGGSSGGSSGSETPGTSESATPESSGGSSGGSSGGSSGGSSGSETPGTSESATPESSGGSSGGS (SEQ ID NO: 25). In some embodiments, the linker is 92 amino acids in length. In some embodiments, the linker comprises the amino acid sequence PGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGSEPATS (SEQ ID NO: 26).

[181] In some embodiments, a base editor system provided herein comprises a base editor comprising a fusion protein comprising an inhibitor of base repair. In some embodiments, a base editor comprises a fusion protein comprising a cytidine deaminase and a programmable DNA binding domain, e.g. a Cas9 domain. In some embodiments, a base editor comprises a fusion protein comprising an adenosine deaminase and a programmable DNA binding

domain, e.g. a Cas9 domain. In some embodiments, the base editor or the fusion protein further comprises an inhibitor of base repair (IBR). In some embodiments, the IBR comprises an inhibitor of inosine base repair. In some embodiments, the IBR is an inhibitor of inosine base excision repair. In some embodiments, the inhibitor of inosine base excision repair is a catalytically inactive inosine specific nuclease (dISN). In some embodiments, a dISN may inhibit (e.g., by steric hindrance) inosine removing enzymes from excising the inosine residue from DNA. For example, catalytically dead inosine glycosylases (e.g., alkyl adenine glycosylase [AAG]) will bind inosine but will not create an abasic site or remove the inosine, thereby sterically blocking the newly-formed inosine moiety from potential DNA damage/repair mechanisms. Thus, this disclosure contemplates a fusion protein comprising a programmable DNA binding protein and an adenosine deaminase further fused to a dISN. This disclosure contemplates a fusion protein comprising any Cas9 domain, for example, a Cas9 nickase (nCas9) domain, a catalytically inactive Cas9 (dCas9) domain, a high fidelity Cas9 domain, or a Cas9 domain with reduced PAM exclusivity. It should be understood that the use of a dISN may increase the editing efficiency of a adenosine deaminase that is capable of catalyzing a A to I change. For example, fusion proteins comprising a dISN domain may be more efficient in deaminating A residues.

[182] In some embodiments, the base editors provided herein comprise fusion proteins that further comprise one or more nuclear targeting sequences, for example, a nuclear localization sequence (NLS). In some embodiments, the fusion protein comprises multiple NLSs. In some embodiments, the fusion protein comprises a NLS at the N-terminus and the C-terminus of the fusion protein. In some embodiments, a NLS comprises an amino acid sequence that facilitates the importation of a protein, that comprises an NLS, into the cell nucleus. In some embodiments, the NLS is fused to the N-terminus of the fusion protein. In some embodiments, the NLS is fused to the C- terminus of the fusion protein. In some embodiments, the NLS is fused to the N-terminus of the programmable DNA binding protein, e.g. the Cas9. In some embodiments, the NLS is fused to the C-terminus of the programmable DNA binding protein. In some embodiments, the NLS is fused to the N-terminus of the adenosine deaminase. In some embodiments, the NLS is fused to the C-terminus of the adenosine deaminase. In some embodiments, the NLS is fused to the fusion protein via one or more linkers. In some embodiments, the NLS is fused to the fusion protein without a linker. In some embodiments, the NLS comprises an amino acid sequence of any one of the NLS sequences provided or referenced herein. In some embodiments, a NLS comprises the amino acid sequence PKKKRKV (SEQ ID NO: 27) or

MDSLLMNRRKFLYQFKNVRWAKGRRETYLC (SEQ ID NO: 28). Additional nuclear localization sequences are known in the art and would be apparent to the skilled artisan. For example, NLS sequences are described in Plank et ah , PCT/EP2000/011690, the contents of which are incorporated herein by reference for their disclosure of exemplary nuclear localization sequences.

[183] In some embodiments, the fusion proteins provided herein do not comprise a linker. In some embodiments, a linker is present between one or more of the domains or proteins (e.g., adenosine deaminase, napDNAbp, NLS, and/or IBR). In some embodiments, the "-" used in the general architecture above indicates the presence of an optional linker.

[184] Some aspects of the disclosure provide base editors or fusion proteins that comprise a programmable DNA binding protein and at least two adenosine deaminase domains. Without wishing to be bound by any particular theory, dimerization of adenosine deaminases (e.g., in cis or in trans) may improve the ability (e.g., efficiency) of the fusion protein to modify a nucleic acid base, for example to deaminate adenine. In some embodiments, any of the fusion proteins may comprise 2, 3, 4 or 5 adenosine deaminase domains. In some embodiments, any of the fusion proteins provided herein comprise two adenosine deaminases. In some embodiments, any of the fusion proteins provided herein contain only two adenosine deaminases. In some embodiments, the adenosine deaminases are the same. In some embodiments, the adenosine deaminases are any of the adenosine deaminases provided herein. In some embodiments, the adenosine deaminases are different. In some embodiments, the first adenosine deaminase is any of the adenosine deaminases provided herein, and the second adenosine is any of the adenosine deaminases provided herein, but is not identical to the first adenosine deaminase. In some embodiments, the first adenosine deaminase comprises any one of the mutations provided herein as numbered in SEQ ID NO: 1. In some embodiments, the second adenosine deaminase comprises any one of the mutations provided herein as numbered in SEQ ID NO: 1. In some embodiments, the first adenosine deaminase comprises any one of the mutations provided herein as numbered in SEQ ID NO: 1, and the second adenosine deaminase comprises a wild type adenosine deaminase sequence. In some embodiments, the second adenosine deaminase comprises any one of the mutations provided herein as numbered in SEQ ID NO: 1, and the first adenosine deaminase comprises a wild type adenosine deaminase sequence. As one example, the fusion protein may comprise a first adenosine deaminase and a second adenosine deaminase that both comprise a A106V, D108N, D147Y, and E155V mutation from ecTadA (SEQ ID NO: 1). As another example, the fusion protein may comprise a first adenosine deaminase domain that comprises a

A106V, D108N, D147Y, and E155V mutation from ecTadA (SEQ ID NO: 1), and a second adenosine deaminase that comprises a L84F, A106V, D108N, H123Y, D147Y, E155V, and I156F mutation from ecTadA (SEQ ID NO: 1).

[185] In some embodiments, the adenosine deaminase comprises the amino acid sequence: MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPT AHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKT GAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTD (SEQ ID NO: 1)

[186] In some embodiments, the fusion protein comprises two adenosine deaminases (e.g., a first adenosine deaminase and a second adenosine deaminase). In some embodiments, the first adenosine deaminase is N-terminal to the second adenosine deaminase in the fusion protein. In some embodiments, the first adenosine deaminase is C-terminal to the second adenosine deaminase in the fusion protein. In some embodiments, the first adenosine deaminase and the second deaminase are fused directly or via a linker. In some embodiments, the linker is any of the linkers provided herein, for example, any of the linkers described in the "Linkers" section. In some embodiments, the first adenosine deaminase is the same as the second adenosine deaminase. In some embodiments, the first adenosine deaminase and the second adenosine deaminase are any of the adenosine deaminases described herein. In some embodiments, the first adenosine deaminase and the second adenosine deaminase are different. In some embodiments, the first adenosine deaminase is any of the adenosine deaminases provided herein. In some embodiments, the second adenosine deaminase is any of the adenosine deaminases provided herein but is not identical to the first adenosine deaminase. In some embodiments, the first adenosine deaminase is an ecTadA adenosine deaminase. In some embodiments, the first adenosine deaminase comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to the amino acid sequence set forth SEQ ID NO: 1 or to any of the adenosine deaminases provided herein. In some embodiments, the second adenosine deaminase comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to the amino acid sequence set forth SEQ ID NO: 1 or to any of the adenosine deaminases provided herein. In some embodiments, the second adenosine deaminase comprises the amino acid sequence of SEQ ID NO: 1.

[187] In some embodiments, the fusion proteins provided herein do not comprise a linker. In some embodiments, a linker is present between one or more of the domains or proteins (e.g., first adenosine deaminase, second adenosine deaminase, programmable DNA binding protein, and/or NLS).

[188] It should be appreciated that the fusion proteins of the present disclosure may comprise one or more additional features. For example, in some embodiments, the fusion protein may comprise cytoplasmic localization sequences, export sequences, such as nuclear export sequences, or other localization sequences, as well as sequence tags that are useful for solubilization, purification, or detection of the fusion proteins. Suitable protein tags provided herein include, but are not limited to, biotin carboxylase carrier protein (BCCP) tags, myc-tags, calmodulin-tags, FLAG-tags, hemagglutinin (HA)-tags, polyhistidine tags, also referred to as histidine tags or His-tags, maltose binding protein (MBP)-tags, nus-tags, glutathione-S-transferase (GST)-tags, green fluorescent protein (GFP)-tags, thioredoxin-tags, S-tags, Softags (e.g. , Softag 1, Softag 3), strep-tags , biotin ligase tags, FlAsH tags, V5 tags, and SBP-tags. Additional suitable sequences will be apparent to those of skill in the art. In some embodiments, the fusion protein comprises one or more His tags.

[189] In some embodiments, the fusion protein comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the amino acid sequences listed in Table 23. In some embodiments, the fusion protein comprises any one of the amino acid sequences listed in Table 23. In some embodiments, the sequence of the fusion protein is any one of the amino acid sequences listed in Table 23. In some embodiments, the fusion protein comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the amino acid sequences of SEQ ID NOs: 2137, 2149, 2154, 2158, 2188, 2140, 40, 2146, 2152, 2156, and 2160. In some embodiments, the fusion protein comprises any one of the amino acid sequences of SEQ ID NOs: 2137, 2149, 2154, 2158, 2188, 2140, 40, 2146, 2152, 2156, and 2160. In some embodiments, the sequence of the fusion protein is any one of the amino acid sequences of SEQ ID NOs: 2137, 2149, 2154, 2158, and 2188.

[190] In some embodiments, the fusion protein is encoded by the polynucleotide sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%,

at least 99.7%, or at least 99.9% identical to any one of the polynucleotide sequences listed in Table 23. In some embodiments, the fusion protein is encoded by any one of the polynucleotide sequences listed in Table 23. In some embodiments, the fusion protein is expressed by the polynucleotide sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the polynucleotide sequences listed in Table 23. In some embodiments, the fusion protein is expressed by any one of the polynucleotide sequences listed in Table 23. In some embodiments, the fusion protein is encoded by the polynucleotide sequence that comprises at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the polynucleotide sequences of SEQ ID NOs: 2192, 2148, 2153, 2157, 2161, 2168, 2174, 2180, 2186, 2189, 2139, 2142, 2145, 2151, 2155, 2159, 2162, 2164, 2167, 2169, 2171, 2173, 2175, 2177, 2179, 2181, 2183, 2185, 2187, and 2190. In some embodiments, the fusion protein is encoded by the polynucleotide sequence that comprises any one of the polynucleotide sequences of SEQ ID NOs: 2192, 2148, 2153, 2157, 2161, 2168, 2174, 2180, 2186, 2189, 2139, 2142, 2145, 2151, 2155, 2159, 2162, 2164, 2167, 2169, 2171, 2173, 2175, 2177, 2179, 2181, 2183, 2185, 2187, and 2190. In some embodiments, the fusion protein is encoded by any one of the polynucleotide sequences of SEQ ID NOs: 2192, 2148, 2153, 2157, 2161, 2168, 2174, 2180, 2186, and 2189. In some embodiments, the fusion protein is expressed by the polynucleotide sequence that comprises at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the polynucleotide sequences of SEQ ID NOs: 2192, 2148, 2153, 2157, 2161, 2168, 2174, 2180, 2186, 2189, 2139, 2142, 2145, 2151, 2155, 2159, 2162, 2164, 2167, 2169, 2171, 2173, 2175, 2177, 2179, 2181, 2183, 2185, 2187, 2190, 2138, 2147, 2158, or a combination thereof: In some embodiments, the fusion protein is encoded by the polynucleotide sequence that comprises any one of the polynucleotide sequences of SEQ ID NOs: 2192, 2148, 2153, 2157, 2161, 2168, 2174, 2180, 2186, 2189, 2139, 2142, 2145, 2151, 2155, 2159, 2162, 2164, 2167, 2169, 2171, 2173, 2175, 2177, 2179, 2181, 2183, 2185, 2187, 2190, 2138, 2147, 2158, or a combination thereof. In some embodiments, the fusion protein is expressed by any one of the polynucleotide sequences of SEQ ID NOs: 2192, 2148, 2153, 2157, 2161, 2168, 2174, 2180, 2186, and 2189. In some embodiments, the polynucleotide sequence further comprises at least 60%, at least 65%, at

least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the polynucleotide sequences of SEQ ID NOs: 2138, 2147, 2158, or a combination thereof. In some embodiments, the polynucleotide sequence further comprises any one of the polynucleotide sequences of SEQ ID NOs: 2138, 2147, 2158, or a combination thereof.

[191] In some embodiments, the nucleobase editor ABE8.8 comprises a fusion protein comprising the sequence as provided below:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPT
AHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKT
GAAGSLMDVLHHPGMNHRVEITEGILADECAALLCRFFRMPRRVFNAQKKAQSSTD
SGGSSGGSSGSETPGTSESATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVP
SKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIF
SNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLV
DSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPI
NASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLA
EDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPL
SASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYK
FIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFL
KDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSF
IERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAI
VDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDF
LDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS
RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLH
EHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSR
ERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSD
YDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAK
LITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEND
KLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKL
ESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPL
IETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLI
ARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEK
NPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYV
NFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVL

SAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIH QSITGLYETRIDLSQLGGD (SEQ ID NO: 3)

[192] In some embodiments, the nucleobase editor comprises a fusion protein comprising a polypeptide encoded by the polynucleotide (herein also referenced as MA002) sequence as provided below:

ATGAGCGAGGTCGAGTTCTCTCACGAATATTGGATGAGACACGCTCTCACCCTGG
CTAAGAGAGCCAGGGACGAAAGAGAGGTGCCAGTTGGCGCTGTCCTGGTGTTGA
ACAATCGCGTCATCGGAGAAGGATGGAATCGCGCCATTGGCCTGCACGATCCAA
CCGCACATGCCGAAATTATGGCTCTGCGGCAAGGCGGCCTCGTGATGCAAAATT
ACAGACTGATCGATGCTACCCTCTACGTCACCTTCGAGCCCTGTGTCATGTGTGC
TGGGGCAATGATTCACTCCCGGATTGGCCGCGTGGTGTTTGGAGTGCGGAATGCC
AAGACTGGCGCCGCTGGATCTCTGATGGACGTCCTGCACcatCCTGGGATGAACCA
CCGGGTCGAGATCACAGAGGGAATTCTGGCTGACGAGTGCGCTGCCCTGCTGTG
CaggTTCTTTAGAATGCCtAGAaggGTGTTCAACGCCCAGAAAAAAGCTCAGAGCAG
CACCGATTCCGGCGGAAGCAGCGGAGGATCTTCTGGAAGCGAAACCCCAGGCAC
CAGCGAGTCTGCCACACCAGAATCATCTGGCGGTAGCTCCGGCGGCAGCGACAA
GAAGTATTCTATCGGACTGGCCATCGGCACCAACTCTGTTGGATGGGCCGTGATC
ACCGACGAGTACAAGGTGCCCAGCAAGAAATTCAAGGTGCTGGGCAACACCGAC
AGGCACAGCATCAAGAAGAACCTGATCGGCGCACTGCTGTTCGACTCTGGCGAA
ACAGCCGAGGCCACCAGACTGAAGAGAACAGCCCGCAGACGGTACACCAGAAG
AAAGAACCGGATCTGCTACCTCCAAGAGATCTTCAGCAACGAGATGGCCAAGGT
GGACGACAGCTTCTTCCACAGACTGGAAGAGTCCTTCCTGGTGGAAGAGGACAA
GAAGCACGAGAGACACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCA
CGAGAAGTACCCCACCATCTACCACCTGAGAAAGAAACTGGTGGACAGCACCGA
CAAGGCCGACCTGAGACTGATCTATCTGGCCCTGGCTCACATGATCAAGTTCCGG
GGCCACTTCCTGATCGAGGGCGACCTGAATCCTGACAACAGCGACGTGGACAAG
CTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAAAACCCCATCA
ACGCCAGCGGAGTGGATGCCAAGGCCATCCTGTCTGCCAGACTGAGCAAGAGCA
GACGGCTGGAAAATCTGATCGCCCAGCTGCCTGGCGAGAAGAAGAATGGCCTGT
TCGGCAACCTGATTGCCCTGAGCCTGGGCCTGACACCTAACTTCAAGAGCAACTT
CGACCTGGCCGAGGACGCCAAACTGCAGCTGAGCAAGGACACCTACGACGACGA
CCTGGACAATCTGCTGGCCCAGATCGGCGATCAGTACGCCGACTTGTTTCTGGCC
GCCAAGAATCTGAGCGACGCCATCCTGCTGTCCGACATCCTGAGAGTGAACACC
GAGATCACCAAGGCACCTCTGAGCGCCTCTATGATCAAGAGATACGACGAGCAC

CACCAGGATCTGACCCTGCTGAAGGCCCTCGTTAGACAGCAGCTGCCAGAGAAG
TACAAAGAGATTTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATTGAT
GGCGGAGCCAGCCAAGAGGAATTCTACAAGTTCATCAAGCCCATCCTCGAGAAG
ATGGACGGCACCGAGGAACTGCTGGTCAAGCTGAACAGAGAGGACCTGCTGAGA
AAGCAGAGAACCTTCGACAACGGCAGCATCCCTCACCAGATCCACCTGGGAGAA
CTGCACGCCATTCTGCGGAGACAAGAGGACTTTTACCCATTCCTGAAGGACAACC
GGGAAAAGATCGAGAAAATCCTGACCTTCAGGATCCCCTACTACGTGGGACCAC
TGGCCAGAGGCAATAGCAGATTCGCCTGGATGACCAGAAAGAGCGAGGAAACC
ATCACTCCCTGGAACTTCGAGGAAGTGGTGGACAAGGGCGCCAGCGCTCAGTCC
TTCATCGAGCGGATGACCAACTTCGATAAGAACCTGCCTAACGAGAAGGTGCTG
CCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAA
GTGAAATACGTGACCGAGGGAATGAGAAAGCCCGCCTTTCTGAGCGGCGAGCAG
AAAAAGGCCATCGTGGATCTGCTGTTCAAGACCAACCGGAAAGTGACCGTGAAG
CAGCTGAAAGAGGACTACTTCAAGAAAATCGAGTGCTTCGACAGCGTCGAGATC
TCCGGCGTGGAAGATCGGTTCAATGCCAGCCTGGGCACATACCACGATCTGCTG
AAAATTATCAAGGACAAGGACTTCCTGGACAACGAAGAGAACGAGGACATCCTT
GAGGACATCGTGCTGACACTGACCCTGTTTGAGGACAGAGAGATGATCGAGGAA
CGGCTGAAAACATACGCCCACCTGTTCGACGACAAAGTGATGAAGCAACTGAAG
CGGCGGAGATACACCGGCTGGGGCAGACTGTCTCGGAAGCTGATCAACGGCATC
CGGGATAAGCAGTCCGGCAAGACCATCCTGGACTTTCTGAAGTCCGACGGCTTC
GCCAACAGAAACTTCATGCAGCTGATTCACGACGACAGCCTCACCTTCAAAGAG
GATATCCAGAAAGCCCAGGTGTCCGGCCAGGGCGATTCTCTGCATGAGCACATT
GCCAACCTGGCCGGCTCTCCCGCCATTAAGAAAGGCATCCTGCAGACAGTGAAG
GTGGTGGACGAGCTTGTGAAAGTGATGGGCAGACACAAGCCCGAGAACATCGTG
ATCGAAATGGCCAGAGAGAACCAGACCACACAGAAGGGACAGAAGAACAGCCG
CGAGAGAATGAAGCGGATCGAAGAGGGCATCAAAGAGCTGGGCAGCCAGATCC
TGAAAGAACACCCCGTGGAAAACACCCAGCTGCAGAACGAGAAGCTGTACCTGT
ACTACCTGCAGAATGGACGGGATATGTACGTGGACCAAGAGCTGGACATCAACA
GACTGTCCGACTACGATGTGGACCATATCGTGCCCCAGTCTTTTCTGAAGGACGA
CTCCATCGACAACAAGGTCCTGACCAGATCCGACAAGAATCGGGGCAAGAGCGA
CAACGTGCCCTCCGAAGAGGTGGTCAAGAAGATGAAGAACTACTGGCGACAGCT
GCTGAACGCCAAGCTGATTACCCAGCGGAAGTTCGACAATCTGACCAAGGCCGA
AAGAGGCGGCCTGAGCGAACTGGATAAGGCCGGCTTCATCAAGAGACAGCTGGT
GGAAACCCGGCAGATCACAAAGCACGTGGCACAGATTCTGGACTCTCGGATGAA

CACTAAGTACGACGAGAACGACAAACTGATCCGCGAAGTGAAAGTCATCACCCT
GAAGTCCAAGCTGGTGTCCGATTTCCGGAAGGATTTCCAGTTCTACAAAGTGCGC
GAGATCAACAACTACCATCACGCCCACGACGCCTACCTGAATGCCGTTGTTGGA
ACAGCCCTGATCAAAAAGTACCCTAAGCTGGAAAGCGAGTTCGTGTACGGCGAC
TACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAAGAGATTGGC
AAGGCAACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTTTTCAAGACAG
AGATCACCCTCGCCAACGGCGAGATCAGAAAGCGGCCTCTGATCGAGACAAACG
GCGAAACCGGCGAGATTGTGTGGGATAAGGGCAGAGACTTTGCCACAGTGCGGA
AAGTGCTGAGCATGCCCCAAGTGAATATCGTGAAGAAAACCGAGGTGCAGACAG
GCGGCTTCAGCAAAGAGTCTATCCTGCCTAAGCGGAACTCCGACAAGCTGATCG
CCAGAAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGATTCTCCTACCG
TGGCCTATAGCGTGCTGGTGGTGGCCAAAGTGGAAAAGGGCAAGTCCAAGAAAC
TCAAGAGCGTGAAAGAGCTGCTGGGGATCACCATCATGGAAAGAAGCAGCTTCG
AGAAGAATCCGATCGATTTCCTCGAGGCCAAGGGCTACAAAGAAGTGAAAAAGG
ACCTGATCATCAAGCTCCCCAAGTACTCCCTGTTCGAGCTGGAAAACGGCCGGA
AGAGAATGCTGGCCTCTGCTGGCGAACTGCAGAAGGGAAACGAACTGGCCCTGC
CTAGCAAATATGTGAACTTCCTGTACCTGGCCAGCCACTATGAGAAGCTGAAGG
GCAGCCCCGAGGACAATGAGCAAAAGCAGCTGTTTGTGGAACAGCACAAGCACT
ACCTGGACGAGATCATCGAGCAGATCAGCGAGTTTAGCAAGAGAGTGATTCTGG
CCGACGCCAATCTGGACAAAGTGCTGTCCGCCTACAACAAGCACCGGGACAAGC
CTATCAGAGAGCAGGCCGAGAATATCATCCACCTGTTTACCCTGACCAACCTGGG
AGCCCCTGCCGCCTTCAAGTACTTTGACACCACCATCGACCGGAAGCGGTACACC
TCCACCAAAGAGGTGCTGGACGCCACTCTGATCCACCAGTCTATCACCGGCCTGT
ACGAGACACGGATCGACCTGTCTCAACTCGGAGGCGACGAAGGCGCCGATAAGA
GAACCGCCGATGGCTCTGAGTTCGAGAGCCCCAAGAAAAAGCGCAAAGTG (SEQ
ID NO: 4)

[193] In an aspect, a nucleobase editor system provided herein comprises a guide polynucleotide. In some embodiments, the guide polynucleotide binds and forms a complex with the base editor fusion protein. In some embodiments, the guide polynucleotide directs the base editor fusion protein to effect a modification at a target sequence. The guide polynucleotide may comprise a single nucleic acid sequence or two separate nucleic acid sequences. In some embodiments, the guide polynucleotide is a single guide, e.g. a single guide RNA. In some embodiments, the single guide RNA comprises a spacer sequence that is capable of hybridizing with a target sequence. In some embodiments, the single guide RNA

comprises a tracrRNA sequence that binds the programmable DNA binding protein, e.g. the Cas9 protein of the base editor fusion protein. In some embodiments, the single guide RNA comprises a stem loop structure, a tracrRNA sequence, a crRNA sequence, a direct repeat, and/or an anti-repeat. In some embodiments, the single guide RNA comprises a chemical modification. In some embodiments, the guide polynucleotide is any one of the guide polynucleotides provided herein, as described in the "Guide polynucleotide" section.

Deaminase Domains

[194] Disclosed herein are base editor systems for editing, modifying or altering a target nucleotide sequence of a polynucleotide.

[195] A base editor system provided herein may comprise a programmable DNA binding protein and a deaminase. As used herein, a deaminase may refer to an enzyme that catalyzes the removal of an amine group from a molecule, or deamination, for example through hydrolysis. In some embodiments, the deaminase is a cytidine deaminase, catalyzing the deamination of cytidine (C) to uridine (U), deoxycytidine (dC) to deoxyuridine (dU), or 5-methyl-cytidine to thymidine (T, 5-methyl-U), respectively. Subsequent DNA repair mechanisms ensure that a dU is replaced by T, as described in Komor et al, Nature, Programmable editing of a target base in genomic DNA without double- stranded DNA cleavage, 533, 420-424 (2016), which is incorporated herein by reference in its entirety. In some embodiments, the deaminase is a cytosine deaminase, catalyzing and promoting the conversion of cytosine to uracil (e.g., in RNA) or thymine (e.g., in DNA). In some embodiments, the deaminase is an adenosine deaminase, catalyzing and promoting the conversion of adenine to guanine. In some embodiments, the deaminase is a naturally-occurring deaminase from an organism, such as a human, chimpanzee, gorilla, monkey, cow, dog, rat, or mouse. In some embodiments, the deaminase is a variant of a naturally-occurring deaminase from an organism, and the variants do not occur in nature. For example, in some embodiments, the deaminase or deaminase domain is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75% at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to a naturally-occurring deaminase from an organism

[196] A cytidine deaminase (or cytosine deaminase) comprises an enzyme that catalyzes the chemical reaction "cytosine + H20 -> uracil + NH3" or "5-methyl-cytosine + H20 -> thymine + NH3." In the context of a gene, such nucleotide change, or mutation, may in turn lead to an amino acid change in the protein, which may affect the protein's function, e.g., loss-of-

function or gain-of-function. Subsequent DNA repair mechanisms ensure that uracil bases in DNA are replaced by T, as described in Komor et al. (Nature, Programmable editing of a target base in genomic DNA without double- stranded DNA cleavage, 533, 420-424 (2016), which is incorporated herein by reference in its entirety).

[197] One exemplary suitable class of cytosine deaminases is the apolipoprotein B mRNA-editing complex (APOBEC) family of cytosine deaminases encompassing eleven proteins that serve to initiate mutagenesis in a controlled and beneficial manner. The apolipoprotein B editing complex 3 (APOBEC3) enzyme provides protection to human cells against a certain HIV-1 strain via the deamination of cytosines in reverse-transcribed viral ssDNA. These cytosine deaminases all require a Zn -coordinating motif (His-X-Glu-X23_26-Pro-Cys-X2_4-Cys (SEQ ID NO: 29)) and bound water molecule for catalytic activity. The glutamic acid residue acts to activate the water molecule to a zinc hydroxide for nucleophilic attack in the deamination reaction. Each family member preferentially deaminates at its own particular "hotspot," for example, WRC (W is A or T, R is A or G) for hAID, or TTC for hAPOBEC3F. A recent crystal structure of the catalytic domain of APOBEC3G revealed a secondary structure comprising a five-stranded β-sheet core flanked by six α-helices, which is believed to be conserved across the entire family. The active center loops have been shown to be responsible for both ssDNA binding and in determining "hotspot" identity. Overexpression of these enzymes has been linked to genomic instability and cancer, thus highlighting the importance of sequence- specific targeting. Another suitable cytosine deaminase is the activation-induced cytidine deaminase (AID), which is responsible for the maturation of antibodies by converting cytosines in ssDNA to uracils in a transcription-dependent, strand-biased fashion.

[198] An adenosine deaminase (or adenine deaminase) comprises an enzyme that catalyzes the hydrolytic deamination of adenosine or deoxy adenosine to inosine or deoxyinosine, respectively. In some embodiments, the adenosine deaminase catalyzes the hydrolytic deamination of adenine or adenosine in deoxyribonucleic acid (DNA). The adenosine deaminases (e.g. engineered adenosine deaminases, evolved adenosine deaminases) provided herein may be from any organism, such as a bacterium. In some embodiments, the deaminase or deaminase domain is a variant of a naturally-occurring deaminase from an organism. In some embodiments, the deaminase or deaminase domain does not occur in nature. For example, in some embodiments, the deaminase or deaminase domain is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75% at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5%

identical to a naturally-occurring deaminase. In some embodiments, the adenosine deaminase is from a bacterium, such as, E.coli, S. aureus, S. typhi, S. putrefaciens, H. influenzae, or C. crescentus. In some embodiments, the adenosine deaminase is a TadA deaminase. In some embodiments, the TadA deaminase is an E. coli TadA deaminase. In some embodiments, the TadA deaminase is a truncated E. coli TadA deaminase. For example, the truncated ecTadA may be missing one or more N-terminal amino acids relative to a full-length ecTadA. In some embodiments, the truncated ecTadA may be missing 1, 2, 3, 4, 5 ,6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 6, 17, 18, 19, or 20 N-terminal amino acid residues relative to the full length ecTadA. In some embodiments, the truncated ecTadA may be missing 1, 2, 3, 4, 5 ,6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 6, 17, 18, 19, or 20 C-terminal amino acid residues relative to the full length ecTadA. In some embodiments, the ecTadA deaminase does not comprise an N-terminal methionine.

[199] In some embodiments, the adenosine deaminase comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to the amino acid sequence set forth in SEQ ID NO:1 or to any of the adenosine deaminases provided herein. It should be appreciated that adenosine deaminases provided herein may include one or more mutations (e.g., any of the mutations provided herein). The disclosure provides any deaminase domains with a certain percent identity plus any of the mutations or combinations thereof described herein. In some embodiments, the adenosine deaminase comprises an amino acid sequence that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more mutations compared to the amino acid sequence set forth in SEQ ID NO:1 or any of the adenosine deaminases provided herein. In some embodiments, the adenosine deaminase comprises an amino acid sequence that has at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, or at least 170 identical contiguous amino acid residues as compared to any one of the amino acid sequences set forth in SEQ ID NO:1 or any of the adenosine deaminases provided herein.

[200] In some embodiments, the adenosine deaminase comprises a D108X mutation in ecTadA SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a D108G,

D108N, D108V, D108A, or D108Y mutation in SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase. It should be appreciated, however, that additional deaminases may similarly be aligned to identify homologous amino acid residues that can be mutated as provided herein.

[201] In some embodiments, the adenosine deaminase comprises an A106X mutation in ecTadA SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an A106V mutation in SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase.

[202] In some embodiments, the adenosine deaminase comprises a E155X mutation in SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase, where the presence of X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a E155D, E155G, or E155V mutation in SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase.

[203] In some embodiments, the adenosine deaminase comprises a D147X mutation in SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase, where the presence of X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a D147Y, mutation in SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase.

[204] It should be appreciated that any of the mutations provided herein (e.g., based on the ecTadA amino acid sequence of SEQ ID NO: 1) may be introduced into other adenosine deaminases, such as S. aureus TadA (saTadA), or other adenosine deaminases (e.g., bacterial adenosine deaminases). It would be apparent to the skilled artisan how to are homologous to the mutated residues in ecTadA. Thus, any of the mutations identified in ecTadA may be made in other adenosine deaminases that have homologous amino acid residues. It should also be appreciated that any of the mutations provided herein may be made individually or in any combination in ecTadA or another adenosine deaminase. For example, an adenosine deaminase may contain a D108N, a A106V, a E155V, and/or a D147Y mutation in ecTadA SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase. In some embodiments, an adenosine deaminase comprises the following group of mutations (groups of mutations are separated by a ";") in ecTadA SEQ ID NO: 1, or corresponding mutations in another adenosine deaminase: D108N and A106V; D108N and E155V; D108N and D147Y; A106V and E155V; A106V and D147Y; E155V and D147Y; D108N, A106V, and E55V;

D108N, A106V, and D147Y; D108N, E55V, and D147Y; A106V, E55V, and D 147Y; and D108N, A106V, E55V, and D147Y. It should be appreciated, however, that any combination of corresponding mutations provided herein may be made in an adenosine deaminase (e.g., ecTadA)

[205] In some embodiments, the adenosine deaminase comprises one or more of a H8X, T17X, L18X, W23X, L34X, W45X, R51X, A56X, E59X, E85X, M94X, I95X, V102X, F104X, A106X, R107X, D108X, K110X, M118X, N127X, A138X, F149X, M151X, R153X, Q154X, I156X, and/or K157X mutation in SEQ ID NO: 1, or one or more corresponding mutations in another adenosine deaminase, where the presence of X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one or more of H8Y, T17S, L18E, W23L, L34S, W45L, R51H, A56E, or A56S, E59G, E85K, or E85G, M94L, 1951, V102A, F104L, A106V, R107C, or R107H, or R107P, D108G, or D108N, or D108V, or D108A, or D108Y, K110l, M118K, N127S, A138V, F149Y, M151V, R153C, Q154L, I156D, and/or K157R mutation in SEQ ID NO: 1, or one or more corresponding mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises one or more of the mutations corresponding to SEQ ID NO: 1, or one or more corresponding mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises the mutation or mutations of in any constructs shown in Table 23 corresponding to SEQ ID NO: 1, or a corresponding mutation or mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises one or more of a H8X, D108X, and/or N127X mutation in SEQ ID NO: 1, or one or more corresponding mutations in another adenosine deaminase, where X indicates the presence of any amino acid. In some embodiments, the adenosine deaminase comprises one or more of a H8Y, D108N, and/or N127S mutation in SEQ ID NO: 1, or one or more corresponding mutations in another adenosine deaminase.

[206] In some embodiments, the adenosine deaminase comprises one or more of H8X, R26X, M61X, L68X, M70X, A106X, D108X, A109X, N127X, D147X, R152X, Q154X, E155X, K161X, Q163X, and/or T166X mutation in SEQ ID NO: 1, or one or more corresponding mutations in another adenosine deaminase, where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one or more of H8Y, R26W, M61I, L68Q, M70V, A106T, D108N, A109T, N127S, D147Y, R152C, Q154H or

Q154R, E155G or E155V or E155D, K161Q, Q163H, and/or T166P mutation in SEQ ID NO: 1, or one or more corresponding mutations in another adenosine deaminase.

[207] In some embodiments, the adenosine deaminase comprises one, two, three, four, five, or six mutations selected from the group consisting of H8X, D108X, N127X, D147X, R152X, and Q154X in SEQ ID NO: 1, or a corresponding mutation or mutations in another adenosine deaminase, where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, five, six, seven, or eight mutations selected from the group consisting of H8X, M61X, M70X, D108X, N127X, Q154X, E155X, and Q163X in SEQ ID NO: 1, or a corresponding mutation or mutations in another adenosine deaminase, where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, or five mutations selected from the group consisting of H8X, D108X, N127X, E155X, and T166X in SEQ ID NO: 1, or a corresponding mutation or mutations in another adenosine deaminase, where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, five, or six mutations selected from the group consisting of H8X, A106X, D108X, mutation or mutations in another adenosine deaminase, where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, five, six, seven, or eight mutations selected from the group consisting of H8X, R126X, L68X, D108X, N127X, D147X, and E155X in SEQ ID NO: 1, or a corresponding mutation or mutations in another adenosine deaminase, where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, or five mutations selected from the group consisting of H8X, D108X, A109X, N127X, and E155X in SEQ ID NO: 1, or a corresponding mutation or mutations in another adenosine deaminase, where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase.

[208] In some embodiments, the adenosine deaminase comprises one, two, three, four, five, or six mutations selected from the group consisting of H8Y, D108N, N127S, D147Y, R152C, and Q154H in SEQ ID NO: 1, or a corresponding mutation or mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four,

five, six, seven, or eight mutations selected from the group consisting of H8Y, M61I, M70V, D108N, N127S, Q154R, E155G and Q163H in SEQ ID NO: 1, or a corresponding mutation or mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, or five mutations selected from the group consisting of H8Y, D108N, N127S, E155V, and T166P in SEQ ID NO: 1, or a corresponding mutation or mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, five, or six mutations selected from the group consisting of H8Y, A106T, D108N, N127S, E155D, and K161Q in SEQ ID NO: 1, or a corresponding mutation or mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, five, six, seven, or eight mutations selected from the group consisting of H8Y, R126W, L68Q, D108N, N127S, D147Y, and E155V in SEQ ID NO: 1, or a corresponding mutation or mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, or five mutations selected from the group consisting of H8Y, D108N, A109T, N127S, and E155G in SEQ ID NO: 1, or a corresponding mutation or mutations in another adenosine deaminase.

[209] In some embodiments, the adenosine deaminase comprises a D108N, D108G, or D108V mutation in SEQ ID NO: 1, or corresponding mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises a A106V and D108N mutation in SEQ ID NO: 1, or corresponding mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises R107C and D108N mutations in SEQ ID NO: 1, or corresponding mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises a H8Y, D108N, N127S, D147Y, and Q154H mutation in SEQ ID NO: 1, or corresponding mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises a H8Y, R24W, D108N, N127S, D147Y, and E155V mutation in SEQ ID NO: 1, or corresponding mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises a D108N, D147Y, and E155V mutation in SEQ ID NO: 1, or corresponding mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises a H8Y, D108N, and S 127S mutation in SEQ ID NO: 1, or corresponding mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises a A106V, D108N, D147Y and E155V mutation in SEQ ID NO: 1, or corresponding mutations in another adenosine deaminase.

[210] In some embodiments, the adenosine deaminase comprises one or more of a S2X, H8X, I49X, L84X, H123X, N127X, I156X and/or K160X mutation in SEQ ID NO: 1, or one or more corresponding mutations in another adenosine deaminase, where the presence of X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one or more of S2A, H8Y, I49F, L84F, H123Y, N127S, I156F and/or K160S mutation in SEQ ID NO: 1, or one or more corresponding mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises one or more of the mutations in SEQ ID NO: 1, or one or more corresponding mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises the mutation or mutations of any one of clones corresponding to SEQ ID NO: 1, or a corresponding mutation or mutations in another adenosine deaminase.

[211] In some embodiments, the adenosine deaminase comprises an L84X mutation adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an L84F mutation in SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase.

[212] In some embodiments, the adenosine deaminase comprises an H123X mutation in ecTadA SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an H123Y mutation in SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase.

[213] In some embodiments, the adenosine deaminase comprises an I157X mutation in ecTadA SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an I157F mutation in SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase.

[214] In some embodiments, the adenosine deaminase comprises one, two, three, four, five, six, or seven mutations selected from the group consisting of L84X, A106X, D108X, H123X, D147X, E155X, and I156X in SEQ ID NO: 1, or a corresponding mutation or mutations in another adenosine deaminase, where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, five, or six mutations selected from the group consisting of S2X, I49X, A106X, D108X, D147X, and E155X in SEQ ID NO: 1,

or a corresponding mutation or mutations in another adenosine deaminase, where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, or five mutations selected from the group consisting of H8X, A106X, D108X, N127X, and K160X in SEQ ID NO: 1, or a corresponding mutation or mutations in another adenosine deaminase, where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase.

[215] In some embodiments, the adenosine deaminase comprises one, two, three, four, five, six, or seven mutations selected from the group consisting of L84F, A106V, D108N, H123Y, D147Y, E155V, and I156F in SEQ ID NO: 1, or a corresponding mutation or mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, five, or six mutations selected from the group consisting of S2A, I49F, A106V, D108N, D147Y, and E155V in SEQ ID NO: 1, or a corresponding amino acid in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, or five mutations selected from the group consisting of H8Y, A106T, D108N, N127S, and K160S in SEQ ID NO: 1, or a corresponding mutation or mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises one or more of a E25X, R26X, R107X, A142X, and/or A143X mutation in SEQ ID NO: 1, or one or more corresponding mutations in another adenosine deaminase, where the presence of X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one or more of E25M, E25D, E25A, E25R, E25V, E25S, E25Y, R26G, R26N, R26Q, R26C, R26L, R26K, R107P, R07K, R107A, R107N, R107W, R107H, R107S, A142N, A142D, A142G, A143D, A143G, A143E, A143L, A143W, A143M, A143S, A143Q and/or A143R mutation in SEQ ID NO: 1, or one or more corresponding mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises one or more of the mutations provided in Table 23 corresponding to SEQ ID NO: 1, or one or more corresponding mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises the mutation or mutations of any one shown in Table 23 corresponding to SEQ ID NO: 1, or a corresponding mutation or mutations in another adenosine deaminase.

[216] In some embodiments, the adenosine deaminase comprises an E25X mutation in ecTadA SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an E25M,

E25D, E25A, E25R, E25V, E25S, or E25Y mutation in SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase.

[217] In some embodiments, the adenosine deaminase comprises an R26X mutation in ecTadA SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an , R26G, R26N, R26Q, R26C, R26L, or R26K mutation in SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase.

[218] In some embodiments, the adenosine deaminase comprises an R107X mutation in ecTadA SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an R107P, R07K, R107A, R107N, R107W, R107H, or R107S mutation in SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase.

[219] In some embodiments, the adenosine deaminase comprises an A142X mutation in ecTadA SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an A142N, A142D, A142G, mutation in SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase.

[220] In some embodiments, the adenosine deaminase comprises an A143X mutation in ecTadA SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an A143D, A143G, A143E, A143L, A143W, A143M, A143S, A143Q and/or A143R mutation in SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase.

[221] In some embodiments, the adenosine deaminase comprises one or more of a H36X, N37X, P48X, I49X, R51X, M70X, N72X, D77X, E134X, S 146X, Q154X, K157X, and/or K161X mutation in SEQ ID NO: 1, or one or more corresponding mutations in another adenosine deaminase, where the presence of X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one or more of H36L, N37T, N37S, P48T, P48L, I49V, R51H, R51L, M70L, N72S, D77G, E134G, S 146R, S 146C, Q154H, K157N, and/or K161T

mutation in SEQ ID NO: 1, or one or more corresponding mutations in another adenosine deaminase.

[222] In some embodiments, the adenosine deaminase comprises an H36X mutation in ecTadA SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an H36L mutation in SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises an N37X mutation in ecTadA SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an N37T, or N37S mutation in SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase.

[223] In some embodiments, the adenosine deaminase comprises an P48X mutation in ecTadA SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an P48T, or P48L mutation in SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase.

[224] In some embodiments, the adenosine deaminase comprises an R51X mutation in ecTadA SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an R51H, or R51L mutation in SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase.

[225] In some embodiments, the adenosine deaminase comprises an S 146X mutation in ecTadA SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an S 146R, or S 146C mutation in SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase.

[226] In some embodiments, the adenosine deaminase comprises an K157X mutation in ecTadA SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type

adenosine deaminase. In some embodiments, the adenosine deaminase comprises a K157N mutation in SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase.

[227] In some embodiments, the adenosine deaminase comprises an P48X mutation in ecTadA SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a P48S, P48T, or P48A mutation in SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase.

[228] In some embodiments, the adenosine deaminase comprises an A142X mutation in ecTadA SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a A142N mutation in SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase.

[229] In some embodiments, the adenosine deaminase comprises an W23X mutation in ecTadA SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a W23R, or W23L mutation in SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase.

[230] In some embodiments, the adenosine deaminase comprises an R152X mutation in ecTadA SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a R152P, or R52H mutation in SEQ ID NO: 1, or a corresponding mutation in another adenosine deaminase.

[231] Additional adenosine deaminase mutations and variants are described in Patent Application WO2018119354, which is incorporated herein by reference in its entirety.

[232] Additional adenosine deaminases useful in the present application would be apparent to the skilled artisan and are within the scope of this disclosure. For example, the adenosine deaminase may be a homolog of an AD AT. Exemplary AD AT homologs include, without limitation:

[233] Staphylococcus aureus TadA:
MGSHMTNDIYFMTLAIEEAKKAAQLGEVPIGAIITKDDEVIARAHNLRETLQQPTAH
AEHIAIERAAKVLGSWRLEGCTLYVTLEPCVMCAGTIVMSRIPRVVYGADDPKGGCS

GS LMNLLQQS NFNHRAIVDKG VLKE AC S TLLTTFFKNLRANKKS TN (SEQ ID NO: 30)

[234] Bacillus subtilis TadA: MTQDELYMKEAIKEAKKAEEKGEVPIGAVLVINGEIIARAHNLRETEQRSIAHAEML VIDE AC KALGT WRLEG ATLY VTLEPCPMC AG A V VLS R VEK V VFG AFDPKGGC S GTLMN LLQEERFNHQ AE V VS G VLEEEC GGMLS AFFRELRKKKK A ARKNLS E (SEQ ID NO: 31)

[235] Salmonella typhimurium (S. typhimurium) TadA: MPPAFITGVTSLSDVELDHEYWMRHALTLAKRAWDEREVPVGAVLVHNHRVIGEG WNRPIGRHDPTAHAEIMALRQGGLVLQNYRLLDTTLYVTLEPCVMCAGAMVHSRIG RVVFGARDAKTGAAGSLIDVLHHPGMNHRVEIIEGVLRDECATLLSDFFRMRRQEIK ALKKADRAEGAGPAV (SEQ ID NO: 32)

[236] Shewanella putrefaciens (S. putrefaciens) TadA: MDEYWMQVAMQMAEKAEAAGEVPVGAVLVKDGQQIATGYNLSISQHDPTAHAEI LCLRSAGKKLENYRLLDATLYITLEPCAMCAGAMVHSRIARVVYGARDEKTGAAGT VVNLLQHPAFNHQVEVTSGVLAEACSAQLSRFFKRRRDEKKALKLAQRAQQGIE (SEQ ID NO: 33)

[237] Haemophilus influenzae F3031 (H. influenzae) TadA: MDAAKVRSEFDEKMMRYALELADKAEALGEIPVGAVLVDDARNIIGEGWNLSIVQS DPTAHAEIIALRNGAKNIQNYRLLNSTLYVTLEPCTMCAGAILHSRIKRLVFGASDYK TGAIGSRFHFFDDYKMNHTLEITSGVLAEECSQKLSTFFQKRREEKKIEKALLKSLSD K (SEQ ID NO: 34)

[238] Caulobacter crescentus (C. crescentus) TadA: MRTDESEDQDHRMMRLALDAARAAAEAGETPVGAVILDPSTGEVIATAGNGPIAAH DPTAHAEIAAMRAAAAKLGNYRLTDLTLVVTLEPCAMCAGAISHARIGRVVFGADD PKGGAVVHGPKFFAQPTCHWRPEVTGGVLADESADLLRGFFRARRKAKI (SEQ ID NO: 35)

[239] Geobacter sulfurreducens (G. sulfurreducens) TadA: MSSLKKTPIRDDAYWMGKAIREAAKAAARDEVPIGAVIVRDGAVIGRGHNLREGSN DPSAHAEMIAIRQAARRSANWRLTGATLYVTLEPCLMCMGAIILARLERVVFGCYDP KGGAAGSLYDLSADPRLNHQVRLSPGVCQEECGTMLSDFFRDLRRRKKAKATPALF IDERKVPPEP (SEQ ID NO: 36)

[240] In some embodiments, the adenosine deaminase comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at

least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the amino acid sequences listed in Table 23. In some embodiments, the adenosine deaminase comprises any one of the amino acid sequences listed in Table 23. In some embodiments, the sequence of the adenosine deaminase is any one of the amino acid sequences listed in Table 23. In some embodiments, the adenosine deaminase comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the amino acid sequences of SEQ ID NOs: SEQ ID NOs: 2137, 2149, 2154, 2158, 2188, 2140, 40, 2146, 2152, 2156, and 2160. In some embodiments, the adenosine deaminase comprises any one of the amino acid sequences of SEQ ID NOs: 2137, 2149, 2154, 2158, 2188, 2140, 40, 2146, 2152, 2156, and 2160. In some embodiments, the sequence of the adenosine deaminase is any one of the amino acid sequences of SEQ ID NOs: 2137, 2149, 2154, 2158, and 2188.

[241] In some embodiments, the adenosine deaminase is encoded by the polynucleotide sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the polynucleotide sequences listed in Table 23. In some embodiments, the adenosine deaminase is encoded by any one of the polynucleotide sequences listed in Table 23. In some embodiments, the adenosine deaminase is expressed by the polynucleotide sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the polynucleotide sequences listed in Table 23. In some embodiments, the adenosine deaminase is expressed by any one of the polynucleotide sequences listed in Table 23. In some embodiments, the adenosine deaminase is encoded by the polynucleotide sequence that comprises at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the polynucleotide sequences of SEQ ID NOs: 2192, 2148, 2153, 2157, 2161, 2168, 2174, 2180, 2186, 2189, 2139, 2142, 2145, 2151, 2155, 2159, 2162, 2164, 2167, 2169, 2171, 2173, 2175, 2177, 2179, 2181, 2183, 2185, 2187, and 2190. In some embodiments, the adenosine deaminase is encoded by the polynucleotide sequence that comprises any one of the polynucleotide sequences of SEQ ID NOs: 2192, 2148, 2153, 2157, 2161, 2168, 2174, 2180,

2186, 2189, 2139, 2142, 2145, 2151, 2155, 2159, 2162, 2164, 2167, 2169, 2171, 2173, 2175, 2177, 2179, 2181, 2183, 2185, 2187, and 2190. In some embodiments, the adenosine deaminase is encoded by any one of the polynucleotide sequences of SEQ ID NOs: 2192, 2148, 2153, 2157, 2161, 2168, 2174, 2180, 2186, and 2189. In some embodiments, the adenosine deaminase is expressed by the polynucleotide sequence that comprises at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the polynucleotide sequences of SEQ ID NOs: 2192, 2148, 2153, 2157, 2161, 2168, 2174, 2180, 2186, 2189, 2139, 2142, 2145, 2151, 2155, 2159, 2162, 2164, 2167, 2169, 2171, 2173, 2175, 2177, 2179, 2181, 2183, 2185, 2187, 2190, 2138, 2147, 2158, or a combination thereof: In some embodiments, the adenosine deaminase is encoded by the polynucleotide sequence that comprises any one of the polynucleotide sequences of SEQ ID NOs: 2192, 2148, 2153, 2157, 2161, 2168, 2174, 2180, 2186, 2189, 2139, 2142, 2145, 2151, 2155, 2159, 2162, 2164, 2167, 2169, 2171, 2173, 2175, 2177, 2179, 2181, 2183, 2185, 2187, 2190, 2138, 2147, 2158, or a combination thereof. In some embodiments, the adenosine deaminase is expressed by any one of the polynucleotide sequences of SEQ ID NOs: 2192, 2148, 2153, 2157, 2161, 2168, 2174, 2180, 2186, and 2189. In some embodiments, the polynucleotide sequence further comprises at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the polynucleotide sequences of SEQ ID NOs: 2138, 2147, 2158, or a combination thereof. In some embodiments, the polynucleotide sequence further comprises any one of the polynucleotide sequences of SEQ ID NOs: 2138, 2147, 2158, or a combination thereof.

Programmable DNA binding Proteins

[242] Provided herein are programmable DNA-binding proteins that can be programmed to target to bind DNA sequences in any desired nucleotide sequence within a genome. To program the DNA-binding protein to bind a desired nucleotide sequence, the DNA binding protein may be modified to change its binding specificity, e.g., zinc finger DNA-binding domain, zinc finger nuclease (ZFN), a CRISPR-Cas9 protein, or a transcription activator- like effector proteins (TALE). ZFNs are artificial restriction enzymes generated by fusing a zinc finger DNA-binding domain to a DNA-cleavage domain. Zinc finger domains can be engineered to target specific desired DNA sequences and this enables zinc-fingers to

bind unique sequences within complex genomes. Transcription activator-like effector nucleases (TALEN) are engineered restriction enzymes that can be engineered to cut specific sequences of DNA. They are made by fusing a TAL effector DNA-binding domain to a nuclease domain (e.g. Fokl). Transcription activator-like effectors (TALEs) can be engineered to bind practically any desired DNA sequence. Methods for programming ZFNs and TALEs are familiar to one skilled in the art. For example, such methods are described in Maeder, et al, Mol. Cell 31 (2): 294-301, 2008; Carroll et al, Genetics Society of America, 188 (4): 773-782, 2011; Miller et al., Nature Biotechnology 25 (7): 778-785, 2007; Christian et al, Genetics 186 (2): 757-61, 2008; Li et al, Nucleic Acids Res. 39 (1): 359-372, 2010; and Moscou et al, Science 326 (5959): 1501, 2009, each of which are incorporated herein by reference.

[243] A CRISPR/Cas system or a Cas protein in a base editor system provided herein may comprise Class 1 or Class 2 system components, including ribonucleic acid protein complexes. The Class 2 Cas nuclease families of proteins are enzymes with DNA endonuclease activity, and they can be directed to cleave a desired nucleic acid target by designing an appropriate guide RNA, as described further herein. A Class 2 CRISPR/Cas system component may be from a Type II, Type IIA, Type IIB, Type IIC, Type V, or Type VI system. Class 2 Cas nucleases include, for example, Cas9 (also known as Csn1 or Csx12), Csn2, Cas4, Cas12a (Cpf1), Cas12b (C2c1), Cas12c (C2c3), Cas13a (C2c2), Cas13b, Cas13c, and Cas13d proteins. In some embodiments, the Cas protein is from a Type II CRISPR/Cas system, i.e., a Cas9 protein from a CRISPR/Cas9 system, or a Type V CRISPR/Cas system, e.g., a Cas12a protein. In some embodiments, the Cas protein is from a Class 2 CRISPR/Cas system, i.e., a single-protein Cas nuclease such as a Cas9 protein or a Cas12a protein.

[244] Other non-limiting examples of Cas proteins can include Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas10, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx1S, Csf1, Csf2, CsO, Csf4, Cpf1, Cas9HiFi, homologues thereof, or modified versions thereof.

[245] In some embodiments, provided herein are guide nucleotide sequence-programmable DNA-binding protein or RNA guided programable DNA binding proteins that are able to bind DNA, and the binding to its target DNA sequence is mediated by a guide nucleotide sequence. Thus, it is appreciated that the guide nucleotide sequence-programmable DNA-binding protein binds to a guide nucleotide sequence. The guide nucleotide may be an RNA or DNA molecule (e.g., a single-stranded DNA or ssDNA molecule) that is

complementary to the target sequence and can guide the DNA binding protein to the target sequence. As such, a guide nucleotide sequence-programmable DNA-binding protein may be a RNA-programmable DNA-binding protein (e.g., a Cas9 protein), or an ssDNA-programmable DNA-binding protein (e.g., an Argonaute protein). "Programmable" means the DNA-binding protein may be programmed to bind any DNA sequence that the guide nucleotide targets. Exemplary guide nucleotide sequence-programmable DNA-binding proteins include, but are not limited to, Cas9 (e.g., dCas9 and nCas9), saCas9 (e.g., saCas9d, saCas9d, saKKH Cas9) CasX, CasY, Cpf1, C2c1, C2c2, C2c3, Argonaute, and any other suitable protein described herein, or variants thereof.

[246] In some embodiments, the guide nucleotide sequence exists as a single nucleotide molecule and comprises two domains: (1) a domain that shares homology to a target nucleic acid (e.g., and directs binding of a guide nucleotide sequence-programmable DNA-binding protein to the target); and (2) a domain that binds a guide nucleotide sequence-programmable DNA-binding protein. In some embodiments, domain (1) comprises a spacer sequence. In some embodiments, domain (2) is referred to as a tracrRNA sequence. In some embodiments, domain (2) corresponds to a sequence known as a tracrRNA, and comprises a stem-loop structure. For example, in some embodiments, domain (2) is identical or homologous to a tracrRNA as provided in Jinek et al., Science 337:816-821(2012), which is incorporated herein by reference. Other examples of gRNAs (e.g., those including domain 2) can be found in U.S. Patent Application Publication US20160208288 and U.S. Patent Application Publication US20160200779 each of which is herein incorporated by reference in their entirety.

[247] Methods of using guide nucleotide sequence-programmable DNA-binding protein, such as Cas9, for site-specific cleavage (e.g., to modify a genome) are known in the art (see e.g., Cong, L. et al. Multiplex genome engineering using CRISPR/Cas systems. Science 339, 819-823 (2013); Mali, P. et al. RNA-guided human genome engineering via Cas9. Science 339, 823-826 (2013); Hwang, W.Y. et al. Efficient genome editing in zebrafish using a CRISPR-Cas system. Nature biotechnology 31, 227-229 (2013); Jinek, M. et al. RNA-programmed genome editing in human cells. eLife 2, e00471 (2013); Dicarlo, J.E. et al. Genome engineering in Saccharomyces cerevisiae using CRISPR-Cas systems. Nucleic acids research (2013); Jiang, W. et al. RNA-guided editing of bacterial genomes using CRISPR-Cas systems. Nature biotechnology 31, 233-239 (2013); each of which are incorporated herein by reference).

[248] CRISPR is an adaptive immune system that provides protection against mobile genetic elements (viruses, transposable elements and conjugative plasmids). CRISPR clusters contain spacers, sequences complementary to antecedent mobile elements, and target invading nucleic acids. A CRISPR/Cas system comprises a non-coding RNA molecule (*e.g.*, guide RNA) that binds to DNA (*e.g.*, target DNA sequence) and Cas proteins (*e.g.*, Cas9) with nuclease functionality (*e.g.*, two nuclease domains). See, *e.g.*, Sander, et al., Nature Biotechnology, 32:347–355 (2014); see also *e.g.*, Hsu, et al., Cell 157(6):1262-1278 (2014). The general mechanism and recent advances of CRISPR system is discussed in Cong, et al., Science, 339(6121): 819-823 (2013); Fu, et al., Nature Biotechnology, 31, 822–826 (2013); Chu, et al., Nature Biotechnology 33, 543–548 (2015); Shmakov, et al., Molecular Cell, 60, 1-13 (2015); Makarova, et al., Nature Reviews Microbiology, 13, 1-15 (2015). CRISPR/Cas systems can be used to introduce site-specific cleavage of a target DNA. The locations for site-specific cleavage are determined by both 1) base-pairing complementarity between the guide RNA (gRNA) and the target DNA (a protospacer) and 2) a short motif in the target DNA referred to as the protospacer adjacent motif (PAM). CRISPR/Cas systems (*e.g.*, Type II CRISPR/Cas system) can be used to generate, *e.g.*, an engineered cell in which a target gene is disrupted or mutated. A Cas enzyme (*e.g.*, Cas9) can be used to catalyze DNA cleavage. A Cas9 protein (*e.g.*, a *Streptococcus pyogenes* Cas9 or any closely related Cas9) can derive an enzymatic action to generate double stranded breaks at target site sequences which hybridize to about 20 nucleotides of a guide sequence (*e.g.*, gRNA) and that have a protospacer-adjacent motif (PAM) following the target sequence.

[249] CRISPR/Cas system comprises Class 1 or Class 2 system components, including ribonucleic acid protein complexes. The Class 2 Cas nuclease families of proteins are enzymes with DNA endonuclease activity, and they can be directed to cleave a desired nucleic acid target by designing an appropriate guide RNA, as described further herein. A Class 2 CRISPR/Cas system component may be from a Type II, Type IIA, Type IIB, Type IIC, Type V, or Type VI system. Class 2 Cas nucleases include, for example, Cas9 (also known as Csn1 or Csx12), Csn2, Cas4, Cas12a (Cpf1), Cas12b (C2c1), Cas12c (C2c3), Cas13a (C2c2), Cas13b, Cas13c, and Cas13d proteins. In some embodiments, the Cas protein is from a Type II CRISPR/Cas system, *i.e.*, a Cas9 protein from a CRISPR/Cas9 system, or a Type V CRISPR/Cas system, *e.g.*, a Cas12a protein. In some embodiments, the Cas protein is from a Class 2 CRISPR/Cas system, *i.e.*, a single-protein Cas nuclease such as a Cas9 protein or a Cas12a protein.

[250] Other non-limiting examples of Cas proteins can include Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas10, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx1S, Csf1, Csf2, CsO, Csf4, Cpf1, Cas9HiFi, homologues thereof, or modified versions thereof.

[251] A base editor system provided herein may comprise a Cas9 or a Cas9 nuclease. A Cas9 protein or a Cas9 nuclease refers to an RNA-guided nuclease comprising a Cas9 protein, a fragment, or a variant thereof. A Cas9 nuclease is also referred to sometimes as a casnl nuclease or a CRISPR (clustered regularly interspaced short palindromic repeat)-associated nuclease. CRISPR is an adaptive immune system that provides protection against mobile genetic elements (viruses, transposable elements and conjugative plasmids). CRISPR clusters contain spacers, sequences complementary to antecedent mobile elements, and target invading nucleic acids. CRISPR clusters are transcribed and processed into CRISPR RNA (crRNA). In type II CRISPR systems correct processing of pre-crRNA requires a trans-encoded small RNA (tracrRNA), endogenous ribonuclease 3 (rnc) and a Cas9 protein. The tracrRNA serves as a guide for ribonuclease 3-aided processing of pre-crRNA. Subsequently, Cas9/crRNA/tracrRNA endonucleolytically cleaves linear or circular dsDNA target complementary to the spacer. The target strand not complementary to crRNA is first cut endonucleolytically, then trimmed 3 '-5' exonucleolytically. In nature, DNA-binding and cleavage typically requires protein and both RNAs. However, single guide RNAs ("sgRNA", or simply "gRNA") can be engineered so as to incorporate aspects of both the crRNA and tracrRNA into a single RNA species. See, e.g., Jinek et al., Science 337:816-821(2012), which is incorporated herein by reference in its entirety.

[252] The term “Cas9” refers to an RNA guided nuclease comprising a Cas9 protein, or a fragment thereof (e.g., a protein comprising an active, inactive, or partially active DNA cleavage domain of Cas9, and/or the gRNA binding domain of Cas9). A Cas9 nuclease is also referred to as a Casnl nuclease or a CRISPR (clustered regularly interspaced short palindromic repeat) associated nuclease. Cas9 can refer to a polypeptide with at least or at least about 50%, 60%, 70%, 80%, 90%, 100% sequence identity and/or sequence similarity to a wild type exemplary Cas9 polypeptide (e.g., Cas9 from S. pyogenes). Cas9 can refer to a polypeptide with at most or at most about 50%, 60%, 70%, 80%, 90%, 100% sequence identity and/or sequence similarity to a wild type exemplary Cas9 polypeptide (e.g., from S. pyogenes). Cas9 can refer to the wild type or a modified form of the Cas9 protein that can comprise an amino acid change such as a deletion, insertion, substitution, variant, mutation,

fusion, chimera, or any combination thereof.

[253] Cas9 nuclease sequences and structures of variant Cas9 orthologs have been described in various species. Exemplary species that the Cas9 protein or other components can be from include, but are not limited to, Streptococcus pyogenes, Streptococcus thermophilus, Streptococcus sp., Staphylococcus aureus, Listeria innocua, Lactobacillus gasseri, Francisella novicida, Wolinella succinogenes, Sutterella wadsworthensis, Gamma proteobacterium, Neisseria meningitidis, Campylobacter jejuni, Pasteurella multocida, Fibrobacter succinogene, Rhodospirillum rubrum, Nocardiopsis dassonvillei, Streptomyces pristinaespiralis, Streptomyces viridochromogenes, Streptomyces viridochromogenes, Streptosporangium roseum, Alicyclobacillus acidocaldarius, Bacillus pseudomycoides, Bacillus selenitireducens, Exiguobacterium sibiricum, Lactobacillus delbrueckii, Lactobacillus salivarius, Lactobacillus buchneri, Treponema denticola, Microscilla marina, Burkholderiales bacterium, Polar omonas naphthalenivorans, Polar omonas sp., Crocosphaera watsonii, Cyanothece sp., Microcystis aeruginosa, Synechococcus sp., Acetohalobium arabaticum, Ammonifex degensii, Caldicelulosiruptor becscii, Candidatus Desulforudis, Clostridium botulinum, Clostridium difficile, Finegoldia magna, Natranaerobius thermophilus, Pelotomaculum thermopropionium, Acidithiobacillus caldus, Acidithiobacillus ferrooxidans, Allochromatium vinosum, Marinobacter sp., Nitrosococcus halophilus, Nitrosococcus watsoni, Pseudoalteromonas haloplanktis, Ktedonobacter racemifer, Methanohalobium evestigatum, Anabaena variabilis, Nodularia spumigena, Nostoc sp., Arthrospira maxima, Arthrospira platensis, Arthrospira sp., Lyngbya sp., Microcoleus chthonoplastes, Oscillator ia sp., Petrotoga mobilis, Thermosipho africanus, Streptococcus pasteurianus, Neisseria cinerea, Campylobacter lari, Parvibaculum lavamentivorans, Coryne bacterium diphtheria, or Acaryochloris marina. In some embodiments, the Cas9 protein is from Streptococcus pyogenes. In some embodiments, the Cas9 protein may be from Streptococcus thermophilus. In some embodiments, the Cas9 protein is from Staphylococcus aureus.

[254] Additional suitable Cas9 nucleases and sequences will be apparent to those of skill in the art based on this disclosure, and such Cas9 nucleases and sequences include Cas9 sequences from the organisms and loci disclosed in Chylinski et al., (2013) RNA Biology 10:5, 726-737; which are incorporated herein by reference.

[255] In some embodiments, wild-type Cas9 corresponds to Cas9 from *Streptococcus pyogenes* (NCBI Reference Sequence: NC_002737.2 (nucleotide sequence as follows); and Uniprot Reference Sequence: Q99ZW2 (amino acid sequence as follows)

ATGGATAAGAAATACTCAATAGGCTTAGATATCGGCACAAATAGCGTCGGATGG
GCGGTGATCACTGATGAATATAAGGTTCCGTCTAAAAAGTTCAAGGTTCTGGGAA
ATACAGACCGCCACAGTATCAAAAAAAATCTTATAGGGGCTCTTTTATTTGACAG
TGGAGAGACAGCGGAAGCGACTCGTCTCAAACGGACAGCTCGTAGAAGGTATAC
ACGTCGGAAGAATCGTATTTGTTATCTACAGGAGATTTTTTCAAATGAGATGGCG
AAAGTAGATGATAGTTTCTTTCATCGACTTGAAGAGTCTTTTTTGGTGGAAGAAG
ACAAGAAGCATGAACGTCATCCTATTTTTGGAAATATAGTAGATGAAGTTGCTTA
TCATGAGAAATATCCAACTATCTATCATCTGCGAAAAAAATTGGTAGATTCTACT
GATAAAGCGGATTTGCGCTTAATCTATTTGGCCTTAGCGCATATGATTAAGTTTC
GTGGTCATTTTTTGATTGAGGGAGATTTAAATCCTGATAATAGTGATGTGGACAA
ACTATTTATCCAGTTGGTACAAACCTACAATCAATTATTTGAAGAAAACCCTATT
AACGCAAGTGGAGTAGATGCTAAAGCGATTCTTTCTGCACGATTGAGTAAATCA
AGACGATTAGAAAATCTCATTGCTCAGCTCCCCGGTGAGAAGAAAAATGGCTTA
TTTGGGAATCTCATTGCTTTGTCATTGGGTTTGACCCCTAATTTTAAATCAAATTT
TGATTTGGCAGAAGATGCTAAATTACAGCTTTCAAAAGATACTTACGATGATGAT
TTAGATAATTTATTGGCGCAAATTGGAGATCAATATGCTGATTTGTTTTTGGCAG
CTAAGAATTTATCAGATGCTATTTTACTTTCAGATATCCTAAGAGTAAATACTGA
AATAACTAAGGCTCCCCTATCAGCTTCAATGATTAAACGCTACGATGAACATCAT
CAAGACTTGACTCTTTTAAAAGCTTTAGTTCGACAACAACTTCCAGAAAAGTATA
AAGAAATCTTTTTTGATCAATCAAAAAACGGATATGCAGGTTATATTGATGGGGG
AGCTAGCCAAGAAGAATTTTATAAATTTATCAAACCAATTTTAGAAAAAATGGAT
GGTACTGAGGAATTATTGGTGAAACTAAATCGTGAAGATTTGCTGCGCAAGCAA
CGGACCTTTGACAACGGCTCTATTCCCCATCAAATTCACTTGGGTGAGCTGCATG
CTATTTTGAGAAGACAAGAAGACTTTTATCCATTTTTAAAAGACAATCGTGAGAA
GATTGAAAAAATCTTGACTTTTCGAATTCCTTATTATGTTGGTCCATTGGCGCGTG
GCAATAGTCGTTTTGCATGGATGACTCGGAAGTCTGAAGAAACAATTACCCCATG
GAATTTTGAAGAAGTTGTCGATAAAGGTGCTTCAGCTCAATCATTTATTGAACGC
ATGACAAACTTTGATAAAAATCTTCCAAATGAAAAAGTACTACCAAAACATAGT
TTGCTTTATGAGTATTTTACGGTTTATAACGAATTGACAAAGGTCAAATATGTTA
CTGAAGGAATGCGAAAACCAGCATTTCTTTCAGGTGAACAGAAGAAAGCCATTG
TTGATTTACTCTTCAAAACAAATCGAAAAGTAACCGTTAAGCAATTAAAAGAAG
ATTATTTCAAAAAAATAGAATGTTTTGATAGTGTTGAAATTTCAGGAGTTGAAGA
TAGATTTAATGCTTCATTAGGTACCTACCATGATTTGCTAAAAATTATTAAAGAT
AAAGATTTTTTGGATAATGAAGAAAATGAAGATATCTTAGAGGATATTGTTTTAA

CATTGACCTTATTTGAAGATAGGGAGATGATTGAGGAAAGACTTAAAACATATG
CTCACCTCTTTGATGATAAGGTGATGAAACAGCTTAAACGTCGCCGTTATACTGG
TTGGGGACGTTTGTCTCGAAAATTGATTAATGGTATTAGGGATAAGCAATCTGGC
AAAACAATATTAGATTTTTTGAAATCAGATGGTTTTGCCAATCGCAATTTTATGC
AGCTGATCCATGATGATAGTTTGACATTTAAAGAAGACATTCAAAAAGCACAAG
TGTCTGGACAAGGCGATAGTTTACATGAACATATTGCAAATTTAGCTGGTAGCCC
TGCTATTAAAAAAGGTATTTTACAGACTGTAAAAGTTGTTGATGAATTGGTCAAA
GTAATGGGGCGGCATAAGCCAGAAAATATCGTTATTGAAATGGCACGTGAAAAT
CAGACAACTCAAAAGGGCCAGAAAAATTCGCGAGAGCGTATGAAACGAATCGA
AGAAGGTATCAAAGAATTAGGAAGTCAGATTCTTAAAGAGCATCCTGTTGAAAA
TACTCAATTGCAAAATGAAAAGCTCTATCTCTATTATCTCCAAAATGGAAGAGAC
ATGTATGTGGACCAAGAATTAGATATTAATCGTTTAAGTGATTATGATGTCGATC
ACATTGTTCCACAAAGTTTCCTTAAAGACGATTCAATAGACAATAAGGTCTTAAC
GCGTTCTGATAAAAATCGTGGTAAATCGGATAACGTTCCAAGTGAAGAAGTAGT
CAAAAAGATGAAAAACTATTGGAGACAACTTCTAAACGCCAAGTTAATCACTCA
ACGTAAGTTTGATAATTTAACGAAAGCTGAACGTGGAGGTTTGAGTGAACTTGAT
AAAGCTGGTTTTATCAAACGCCAATTGGTTGAAACTCGCCAAATCACTAAGCATG
TGGCACAAATTTTGGATAGTCGCATGAATACTAAATACGATGAAAATGATAAAC
TTATTCGAGAGGTTAAAGTGATTACCTTAAAATCTAAATTAGTTTCTGACTTCCG
AAAAGATTTCCAATTCTATAAAGTACGTGAGATTAACAATTACCATCATGCCCAT
GATGCGTATCTAAATGCCGTCGTTGGAACTGCTTTGATTAAGAAATATCCAAAAC
TTGAATCGGAGTTTGTCTATGGTGATTATAAAGTTTATGATGTTCGTAAAATGATT
GCTAAGTCTGAGCAAGAAATAGGCAAAGCAACCGCAAAATATTTCTTTTACTCTA
ATATCATGAACTTCTTCAAAACAGAAATTACACTTGCAAATGGAGAGATTCGCA
AACGCCCTCTAATCGAAACTAATGGGGAAACTGGAGAAATTGTCTGGGATAAAG
GGCGAGATTTTGCCACAGTGCGCAAAGTATTGTCCATGCCCCAAGTCAATATTGT
CAAGAAAACAGAAGTACAGACAGGCGGATTCTCCAAGGAGTCAATTTTACCAAA
AAGAAATTCGGACAAGCTTATTGCTCGTAAAAAAGACTGGGATCCAAAAAAATA
TGGTGGTTTTGATAGTCCAACGGTAGCTTATTCAGTCCTAGTGGTTGCTAAGGTG
GAAAAAGGGAAATCGAAGAAGTTAAAATCCGTTAAAGAGTTACTAGGGATCACA
ATTATGGAAAGAAGTTCCTTTGAAAAAAATCCGATTGACTTTTTAGAAGCTAAAG
GATATAAGGAAGTTAAAAAAGACTTAATCATTAAACTACCTAAATATAGTCTTTT
TGAGTTAGAAAACGGTCGTAAACGGATGCTGGCTAGTGCCGGAGAATTACAAAA
AGGAAATGAGCTGGCTCTGCCAAGCAAATATGTGAATTTTTTATATTTAGCTAGT

CATTATGAAAAGTTGAAGGGTAGTCCAGAAGATAACGAACAAAAACAATTGTTT
GTGGAGCAGCATAAGCATTATTTAGATGAGATTATTGAGCAAATCAGTGAATTTT
CTAAGCGTGTTATTTTAGCAGATGCCAATTTAGATAAAGTTCTTAGTGCATATAA
CAAACATAGAGACAAACCAATACGTGAACAAGCAGAAAATATTATTCATTTATT
TACGTTGACGAATCTTGGAGCTCCCGCTGCTTTTAAATATTTTGATACAACAATTG
ATCGTAAACGATATACGTCTACAAAAGAAGTTTTAGATGCCACTCTTATCCATCA
ATCCATCACTGGTCTTTATGAAACACGCATTGATTTGAGTCAGCTAGGAGGTGAC
TGA (SEQ ID NO: 37)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGE
TAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHE
RHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG
DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLP
GEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYA
DLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPE
KYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQ
RTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRF
AWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFT
VYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECF
DSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE
RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFAN
RNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDEL
VKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENT
QLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRS
DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAG
FIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFY
KVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEI
GKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL
SMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVL
VVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYS
LFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLF
VEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTN
LGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD (SEQ ID
NO: 38)

[256] In some embodiments, Cas9 is a Cas9 protein from species Corynebacterium ulcerans (NCBI Refs: NC_015683.1, NC_017317.1); Corynebacterium diphtheria (NCBI Refs. NC 016782.1, NC_016786.1); Spiroplasma syrphidicola (NCBI Ref: NC_021284.1); Prevotella intermedia (NCBI Ref: NC_017861.1); Spiroplasma taiwanense (NCBI Ref: NC_021846.1); Streptococcus iniae (NCBI Ref: NC_021314.1); Belliella baltica (NCBI Ref: NC_018010.1); Psychroflexus torquisl (NCBI Ref: NC_018721.1); Listeria innocua (NCBI Ref: NP_472073.1), Campylobacter jejuni (NCBI Ref: YP_002344900.1) or Neisseria, meningitidis (NCBI Ref: YP_002342100.1).

[257] In some embodiments, a base editor provided herein comprises a programmable DNA binding protein, e.g. a Cas nuclease, with reduced or abolished nuclease activity. For example a Ca9 protein may be nuclease inactive or may be a Cas9 nickase. Methods for generating a Cas9 protein (or a fragment thereof) having an inactive DNA cleavage domain are known (See, e.g., Jinek et al., Science. 337:816-821(2012); Qi et al, (2013) Cell. 28; 152(5): 1173-83, each of which are incorporated herein by reference in its entirety. For example, the DNA cleavage domain of Cas9 is known to include two subdomains, the HNH nuclease subdomain and the RuvC1 subdomain. The HNH subdomain cleaves the strand complementary to the gRNA, whereas the RuvC1 subdomain cleaves the non-complementary strand. Mutations within these subdomains can silence the nuclease activity of Cas9. For example, the mutations D10A and H840A completely inactivate the nuclease activity of S. pyogenes Cas9 (Jinek et al., Science. 337:816-821(2012); Qi et al, Cell. 28;152(5): 1173-83 (2013)). The Cas9 nickase suitable for use in accordance with the present disclosure has an active HNH domain and an inactive RuvC domain and is able to cleave only the strand of the target DNA that is bound by the sgRNA (which is the opposite strand of the strand that is being edited via cytidine deamination). The Cas9 nickase of the present disclosure may comprise mutations that inactivate the RuvC domain, e.g., a D10A mutation. It is to be understood that any mutation that inactivates the RuvC domain may be included in a Cas9 nickase, e.g., insertion, deletion, or single or multiple amino acid substitution in the RuvC domain. In a Cas9 nickase described herein, while the RuvC domain is inactivated, the HNH domain remains activate. Thus, while the Cas9 nickase may comprise mutations other than those that inactivate the RuvC domain (e.g., D10A), those mutations do not affect the activity of the HNH domain. In a non-limiting Cas9 nickase example, the histidine at position 840 remains unchanged.

[258] In some embodiments, a nuclease inactive Cas9 comprises the amino acid sequence of dCas9 (D10A and H840A) provided below:

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGE

TAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHE
RHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG
DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLP
GEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYA
DLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPE
KYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQ
RTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRF
AWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFT
VYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECF
DSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE
RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFAN
RNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDEL
VKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENT
QLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRS
DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAG
FIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFY
KVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEI
GKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL
SMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVL
VVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYS
LFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLF
VEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTN
LGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD (SEQ ID
NO: 39)

[259] In some embodiments, a Cas9 nickase comprises the amino acid sequence of an exemplary catalytically Cas9 nickase (nCas9) is as follows:

DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERH
PIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL
NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGE
KKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADL
FLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKY
KEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTF
DNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAW

MTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVY
NELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDS
VEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERL
KTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRN
FMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVK
VMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDK
NRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK
RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGK
ATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSM
PQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVV
AKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFE
LENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQ
HKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGA
PAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD (SEQ ID NO:
40)

[260] Additional suitable mutations that inactivate Cas9 will be apparent to those of skill in the art based on this disclosure and knowledge in the field, and are within the scope of this disclosure. Such additional exemplary suitable nuclease-inactive Cas9 domains include, but are not limited to, D839A and/or N863A (See, e.g., Prashant et al, Nature Biotechnology. 2013; 31(9): 833-838, which are incorporated herein by reference), or), or K603R {See, e.g., Chavez et al., Nature Methods 12, 326-328, 2015, which is incorporated herein by reference). Cas9, dCas9, or Cas9 variant also encompasses Cas9, dCas9, or Cas9 variants from any organism. Also appreciated is that dCas9, Cas9 nickase, or other appropriate Cas9 variants from any organisms may be used in accordance with the present disclosure.

[261] In some embodiments, Cas9 refers to a Cas9 from archaea (e.g. nanoarchaea), which constitute a domain and kingdom of single-celled prokaryotic microbes.

[262] In some embodiments, the programmable DNA binding protein comprises a CasX or CasY, or a variant thereof, which have been described in, for example, Burstein et al., "New CRISPR-Cas systems from uncultivated microbes." Cell Res. 2017 Feb 21. doi: 10.1038/cr.2017.21, the entire contents of which is hereby incorporated by reference. Using genome-resolved metagenomics, a number of CRISPR-Cas systems were identified, including the first reported Cas9 in the archaeal domain of life. This divergent Cas9 protein

was found in little- studied nanoarchaea as part of an active CRISPR-Cas system. In bacteria, two previously unknown systems were discovered, CRISPR-CasX and CRISPR-CasY, which are among the most compact systems yet discovered.

[263] Some aspects of the disclosure provide high fidelity Cas9 domains of the nucleobase editors provided herein. In some embodiments, high fidelity Cas9 domains are engineered Cas9 domains comprising one or more mutations that decrease electrostatic interactions between the Cas9 domain and the sugar-phosphate backbone of DNA, as compared to a corresponding wild-type Cas9 domain. Without wishing to be bound by any particular theory, high fidelity Cas9 domains that have decreased electrostatic interactions with the sugar-phosphate backbone of DNA may have less off-target effects. In some embodiments, the Cas9 domain comprises one or more mutations that decreases the association between the Cas9 domain and the sugar-phosphate backbone of DNA. In some embodiments, a Cas9 domain comprises one or more mutations that decreases the association between the Cas9 domain and the sugar-phosphate backbone of DNA by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at leastl0%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, or more. In some embodiments, any of the Cas9 fusion proteins provided herein comprise one or more of N497X, R661X, Q695X, and/or Q926X mutation as numbered in the wild type Cas9 amino acid sequence or a corresponding amino acid in another Cas9, wherein X is any amino acid. In some embodiments, any of the Cas9 or Cas9 fusion proteins provided herein comprise one or more of N497A, R661A, Q695A, and/or Q926A mutation of the amino acid sequence provided in the wild type Cas9 sequence, or a corresponding mutation as numbered in the wild type Cas9 amino acid sequence or a corresponding amino acid in another Cas9. Cas9 domains with high fidelity have been described in Kleinstiver, B.P., et al. "High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects." Nature 529, 490-495 (2016); and Slaymaker, I.M., et al. "Rationally engineered Cas9 nucleases with improved specificity." Science 351, 84-88 (2015); the entire contents of each are incorporated herein by reference.

[264] It should be appreciated that any of the base editors provided herein, for example, any of the adenosine deaminase base editors provided herein, may be converted into high fidelity base editors by modifying the Cas9 domain as described herein to generate high fidelity base editors, for example, a high fidelity adenosine base editor. In some embodiments, the high fidelity Cas9 domain is a nuclease inactive Cas9 domain. In some embodiments, the high fidelity Cas9 domain is a Cas9 nickase domain.

[265] In some embodiments, a Cas protein comprises a CasX or CasY, or a variant thereof, which have been described in, for example, Burstein et al., "New CRISPR-Cas systems from uncultivated microbes." Cell Res. 2017 Feb 21. doi: 10.1038/cr.2017.21, the entire contents of which is hereby incorporated by reference. Using genome-resolved metagenomics, a number of CRISPR-Cas systems were identified, including the first reported Cas9 in the archaeal domain of life. This divergent Cas9 protein was found in little- studied nanoarchaea as part of an active CRISPR-Cas system. In bacteria, two previously unknown systems were discovered, CRISPR-CasX and CRISPR-CasY, which are among the most compact systems yet discovered.

[266] Alternatives to *S. pyogenes* Cas9 may include RNA-guided endonucleases from the Cpf1 family that exhibit cleavage activity in mammalian cells. Cpf1-mediated DNA cleavage is a double-strand break with a short 3′ overhang, which is different from Cas9-mediated DNA cleavage. The staggered cleavage pattern of Cpf1 may open up the possibility of directional gene transfer, analogous to traditional restriction enzyme cloning, which may increase the efficiency of gene editing. Like the Cas9 variants and orthologues described above, Cpf1 may also expand the number of sites that can be targeted by CRISPR to AT-rich regions or AT-rich genomes that lack the NGG PAM sites favored by SpCas9.

[267] In some embodiments, the Cas9 protein comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the amino acid sequences listed in Table 23. In some embodiments, the Cas9 protein comprises any one of the amino acid sequences listed in Table 23. In some embodiments, the sequence of the Cas9 protein is any one of the amino acid sequences listed in Table 23. In some embodiments, the Cas9 protein comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the amino acid sequences of SEQ ID NOs: 2137, 2149, 2154, 2158, 2188, 2140, 40, 2146, 2152, 2156, and 2160. In some embodiments, the Cas9 protein comprises any one of the amino acid sequences of SEQ ID NOs: 2137, 2149, 2154, 2158, 2188, 2140, 40, 2146, 2152, 2156, and 2160. In some embodiments, the sequence of the Cas9 protein is any one of the amino acid sequences of SEQ ID NOs: 2137, 2149, 2154, 2158, and 2188.

[268] In some embodiments, the Cas9 protein is encoded by the polynucleotide sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at

least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the polynucleotide sequences listed in Table 23. In some embodiments, the Cas9 protein is encoded by any one of the polynucleotide sequences listed in Table 23. In some embodiments, the Cas9 protein is expressed by the polynucleotide sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the polynucleotide sequences listed in Table 23. In some embodiments, the Cas9 protein is expressed by any one of the polynucleotide sequences listed in Table 23. In some embodiments, the Cas9 protein is encoded by the polynucleotide sequence that is at In some embodiments, the Cas9 protein is encoded by the polynucleotide sequence that comprises at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the polynucleotide sequences of SEQ ID NOs: 2192, 2148, 2153, 2157, 2161, 2168, 2174, 2180, 2186, 2189, 2139, 2142, 2145, 2151, 2155, 2159, 2162, 2164, 2167, 2169, 2171, 2173, 2175, 2177, 2179, 2181, 2183, 2185, 2187, and 2190. In some embodiments, the Cas9 protein is encoded by the polynucleotide sequence that comprises any one of the polynucleotide sequences of SEQ ID NOs: 2192, 2148, 2153, 2157, 2161, 2168, 2174, 2180, 2186, 2189, 2139, 2142, 2145, 2151, 2155, 2159, 2162, 2164, 2167, 2169, 2171, 2173, 2175, 2177, 2179, 2181, 2183, 2185, 2187, and 2190. In some embodiments, the Cas9 protein is encoded by any one of the polynucleotide sequences of SEQ ID NOs: 2192, 2148, 2153, 2157, 2161, 2168, 2174, 2180, 2186, and 2189. In some embodiments, the Cas9 protein is expressed by the polynucleotide sequence that comprises at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the polynucleotide sequences of SEQ ID NOs: 2192, 2148, 2153, 2157, 2161, 2168, 2174, 2180, 2186, 2189, 2139, 2142, 2145, 2151, 2155, 2159, 2162, 2164, 2167, 2169, 2171, 2173, 2175, 2177, 2179, 2181, 2183, 2185, 2187, 2190, 2138, 2147, 2158, or a combination thereof: In some embodiments, the Cas9 protein is encoded by the polynucleotide sequence that comprises any one of the polynucleotide sequences of SEQ ID NOs: 2192, 2148, 2153, 2157, 2161, 2168, 2174, 2180, 2186, 2189, 2139, 2142, 2145, 2151, 2155, 2159, 2162, 2164, 2167, 2169, 2171, 2173, 2175, 2177, 2179, 2181, 2183, 2185, 2187, 2190, 2138, 2147, 2158, or a combination thereof. In some embodiments, the Cas9 protein is expressed by any one of the polynucleotide sequences of

SEQ ID NOs: 2192, 2148, 2153, 2157, 2161, 2168, 2174, 2180, 2186, and 2189. In some embodiments, the polynucleotide sequence further comprises at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the polynucleotide sequences of SEQ ID NOs: 2138, 2147, 2158, or a combination thereof. In some embodiments, the polynucleotide sequence further comprises any one of the polynucleotide sequences of SEQ ID NOs: 2138, 2147, 2158, or a combination thereof.

Linkers

[269] Base editors provided herein may comprises linkers that connect one or more components of the base editors. In certain embodiments, linkers may be used to link any of the protein or protein domains described herein. The linker may be as simple as a covalent bond, or it may be a polymeric linker many atoms in length. In certain embodiments, the linker is a polypeptide or based on amino acids. In other embodiments, the linker is not peptide-like. In some embodiments, the linker is carbon bond, disulfide bond, carbon-heteroatom bond, etc. In certain embodiments, the linker is a carbon-nitrogen bond of an amide linkage. In certain embodiments, the linker is a cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic or heteroaliphatic linker. In certain embodiments, the linker is polymeric (e.g. , polyethylene, polyethylene glycol, polyamide, polyester, etc.). In certain embodiments, the linker comprises a monomer, dimer, or polymer of aminoalkanoic acid. In certain embodiments, the linker comprises an aminoalkanoic acid (e.g. , glycine, ethanoic acid, alanine, beta-alanine, 3-aminopropanoic acid, 4-aminobutanoic acid, 5-pentanoic acid, etc.). In certain embodiments, the linker comprises a monomer, dimer, or polymer of aminohexanoic acid (Ahx). In certain embodiments, the linker is based on a carbocyclic moiety (e.g. , cyclopentane, cyclohexane). In other embodiments, the linker comprises a polyethylene glycol moiety (PEG). In other embodiments, the linker comprises amino acids. In certain embodiments, the linker comprises a peptide. In certain embodiments, the linker comprises an aryl or heteroaryl moiety. In certain embodiments, the linker is based on a phenyl ring. The linker may include functionalized moieties to facilitate attachment of a nucleophile (e.g. , thiol, amino) from the peptide to the linker. Any electrophile may be used as part of the linker. Exemplary electrophiles include, but are not limited to, activated esters, activated amides, alkyl halides, aryl halides, acyl halides, and isothiocyanates.

[270] In some embodiments, the linker is an amino acid or a plurality of amino acids (e.g. , a peptide or protein). In some embodiments, the linker is a bond (e.g. , a covalent bond), an organic molecule, group, polymer, or chemical moiety. In some embodiments, the linker is 5-100 amino acids in length, for example, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 30-35, 35-40, 40-45, 45-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-110, 110- 120, 120-130, 130-140, 140- 150, or 150-200 amino acids in length. Longer or shorter linkers are also contemplated. In some embodiments, a linker comprises the amino acid sequence SGSETPGTSESATPES (SEQ ID NO: 17), which may also be referred to as the XTEN linker. In some embodiments, a linker comprises the amino acid sequence SGGS. In some embodiments, a linker comprises (SGGS)n (SEQ ID NO: 41) , (GGGS)n (SEQ ID NO: 42), (GGGGS)n (SEQ ID NO: 43), (G)n (SEQ ID NO: 44), (EAAAK)n (SEQ ID NO: 45), (GGS)n (SEQ ID NO: 46), SGSETPGTSESATPES (SEQ ID NO: 17), or (XP)n (SEQ ID NO: 47) motif, or a combination of any of these, wherein n is independently an integer between 1 and 30, and wherein X is any amino acid. In some embodiments, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15. In some embodiments, a linker comprises SGSETPGTSESATPES (SEQ ID NO: 17), SGGSSGSETPGTSESATPESSGGS (SEQ ID NO: 19). In some embodiments, a linker comprises SGGSSGGSSGSETPGTSESATPESSGGSSGGS (SEQ ID NO: 20). In some embodiments, a linker comprises GGSGGSPGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGSEPATSGGSGGS (SEQ ID NO: 21). In some embodiments, the linker is 24 amino acids in length. In some embodiments, the linker comprises the amino acid sequence SGGSSGGSSGSETPGTSESATPES (SEQ ID NO: 23). In some embodiments, the linker is 40 amino acids in length. In some embodiments, the linker comprises the amino acid sequence SGGSSGGSSGSETPGTSESATPESSGGSSGGSSGGSSGGS (SEQ ID NO: 24). In some embodiments, the linker is 64 amino acids in length. In some embodiments, the linker comprises the amino acid sequence SGGSSGGSSGSETPGTSESATPESSGGSSGGSSGGSSGGSSGSETPGTSESATPESSGGSSGGS (SEQ ID NO: 25). In some embodiments, the linker is 92 amino acids in length. In some embodiments, the linker comprises the amino acid sequence PGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGSEPATS (SEQ ID NO: 26). It should be appreciated that any of the linkers provided herein may be used to link a first adenosine deaminase and a

second adenosine deaminase; a deaminase (e.g. , a first or a second adenosine deaminase) and a RNA guided programmable DNA binding protein; a RNA guided programmable DNA binding protein and an NLS; or a deaminase (e.g. , a first or a second adenosine deaminase) and an NLS.

[271] Various linker lengths and flexibilities between a deaminase (e.g. , an engineered ecTadA) and a RNA guided programmable DNA binding protein (e.g., a Cas9 domain), and/or between a first adenosine deaminase and a second adenosine deaminase can be employed (e.g., ranging from very flexible linkers of the form (GGGGS)n (SEQ ID NO: 22), (GGGGS)n (SEQ ID NO: 22), and (G)n to more rigid linkers of the form (EAAAK)n (SEQ ID NO: 48), (SGGS)n (SEQ ID NO: 49), and (XP)n) in order to achieve the optimal length for deaminase activity for the specific application. In some embodiments, n is any integer between 3 and 30. In some embodiments, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15. In some embodiments, the linker comprises a (GGS)n (SEQ ID NO: 51) motif, wherein n is 1, 3, or 7.

Protospacer Adjacent Motifs

[272] CRISPR clusters contain spacers, sequences complementary to antecedent mobile elements, and target invading nucleic acids. CRISPR clusters are transcribed and processed into CRISPR RNA (crRNA). For example, in type II CRISPR systems correct processing of pre-crRNA requires a trans-encoded small RNA (tracrRNA), endogenous ribonuclease 3 (rnc) and a Cas9 protein. The tracrRNA serves as a guide for ribonuclease 3-aided processing of pre-crRNA. Cas9 recognizes a short motif in the CRISPR repeat sequences (the PAM or protospacer adjacent motif) (see, e.g. , "Complete genome sequence of an Ml strain of Streptococcus pyogenes." Ferretti et ah , J.J., McShan W.M., Ajdic D.J., Savic D.J., Savic G., Lyon K., Primeaux C, Sezate S., Suvorov A.N., Kenton S., Lai H.S., Lin S.P., Qian Y., Jia H.G., Najar F.Z., Ren Q., Zhu H., Song L., Natl. Acad. Sci. U.S.A. 98:4658-4663(2001); "CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III." Deltcheva E., Chylinski K., Sharma CM., Gonzales K., Chao Y., Pirzada Z.A., Eckert M.R., Vogel J., Charpentier E., Nature 471:602-607(2011); and "A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity." Jinek M., Chylinski K., Fonfara I., Hauer M., Doudna J.A., Charpentier E. Science 337:816-821(2012), the entire contents of each of which are incorporated herein by reference). Cas9 orthologs have been described in various species, including, but not limited to, S. pyogenes and S. thermophilus.

[273] Some aspects of the disclosure provide programmed DNA binding protein domains, e.g. Cas9 domains that have altered PAM specificities. In some embodiments, a Cas9 derived from S. pyogenes (spCas9) recognizes a canonical NGG PAM sequence where the "N" in "NGG" is adenine (A), thymine (T), guanine (G), or cytosine (C), and the G is guanine. In some embodiments, the base editing fusion proteins provided herein function by deaminating a target nucleobase within a 4 base region (e.g., a "deamination window"), which is approximately 15 bases upstream of the PAM. In some embodiments, the deamination window is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 bases upstream of the PAM. Accordingly, in some embodiments, any of the fusion proteins provided herein may contain a Cas9 domain that is capable of binding a nucleotide sequence that does not contain a canonical (e.g., NGG) PAM sequence. Cas9 domains that bind to non-canonical PAM sequences have been described in the art and would be apparent to the skilled artisan. For example, Cas9 domains that bind non-canonical PAM sequences have been described in Kleinstiver, B. P., et al., "Engineered CRISPR-Cas9 nucleases with altered PAM specificities" Nature 523, 481-485 (2015); and Kleinstiver, B. P., et ah, "Broadening the targeting range of Staphylococcus aureus CRISPR-Cas9 by modifying PAM recognition" Nature Biotechnology 33, 1293-1298 (2015); the entire contents of each are hereby incorporated by reference. In some embodiments, the Cas9 domain is a Cas9 domain from Staphylococcus aureus (SaCas9). In some embodiments, the SaCas9 domain is a nuclease active SaCas9, a nuclease inactive SaCas9 (SaCas9d), or a SaCas9 nickase (SaCas9n). A wild type SaCas9 amino acid sequence is provided below:

KRNYILGLDIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSKRGARRLKRR
RRHRIQRVKKLLFDYNLLTDHSELSGINPYEARVKGLSQKLSEEEFSAALLHLAKRR
GVHNVNEVEEDTGNELSTKEQISRNSKALEEKYVAELQLERLKKDGEVRGSINRFKT
SDYVKEAKQLLKVQKAYHQLDQSFIDTYIDLLETRRTYYEGPGEGSPFGWKDIKEW
YEMLMGHCTYFPEELRSVKYAYNADLYNALNDLNNLVITRDENEKLEYYEKFQIIEN
VFKQKKKPTLKQIAKEILVNEEDIKGYRVTSTGKPEFTNLKVYHDIKDITARKEIIENA
ELLDQIAKILTIYQSSEDIQEELTNLNSELTQEEIEQISNLKGYTGTHNLSLKAINLILDE
LWHTNDNQIAIFNRLKLVPKKVDLSQQKEIPTTLVDDFILSPVVKRSFIQSIKVINAIIK
KYGLPNDIIIELAREKNSKDAQKMINEMQKRNRQTNERIEEIIRTTGKENAKYLIEKIK
LHDMQEGKCLYSLEAIPLEDLLNNPFNYEVDHIIPRSVSFDNSFNNKVLVKQEENSKK
GNRTPFQYLSSSDSKISYETFKKHILNLAKGKGRISKTKKEYLLEERDINRFSVQKDFI
NRNLVDTRYATRGLMNLLRSYFRVNNLDVKVKSINGGFTSFLRRKWKFKKERNKG
YKHHAEDALIIANADFIFKEWKKLDKAKKVMENQMFEEKQAESMPEIETEQEYKEIF

ITPHQIKHIKDFKDYKYSHRVDKKPNRELINDTLYSTRKDDKGNTLIVNNLNGLYDK
DNDKLKKLINKSPEKLLMYHHDPQTYQKLKLIMEQYGDEKNPLYKYYEETGNYLTK
YSKKDNGPVIKKIKYYGNKLNAHLDITDDYPNSRNKVVKLSLKPYRFDVYLDNGVY
KFVTVKNLDVIKKENYYEVNSKCYEEAKKLKKISNQAEFIASFYNNDLIKINGELYR
VIGVNNDLLNRIEVNMIDITYREYLENMNDKRPPRIIKTIASKTQSIKKYSTDILGNLY
EVKSKKHPQIIKKG (SEQ ID NO: 52)

[274] In some embodiments, the SaCas9 comprises a N579X mutation, or a corresponding mutation in any of the amino acid sequences as numbered in the wild type SaCas9 sequence, wherein X is any amino acid except for N. In some embodiments, the SaCas9 comprises a N579A mutation as numbered in the wild type SaCas9 sequence or a corresponding mutation in another SaCas9 protein.

[275] In some embodiments, the SaCas9 domain, the nuclease inactive SaCas9 domain, or the SaCas9 nickase domain can bind to a nucleic acid sequence having a non-canonical PAM. In some embodiments, the SaCas9 domain, the SaCas9d domain, or the SaCas9n domain can bind to a nucleic acid sequence having a NNGRRT PAM sequence, where N = A, T, C, or G, and R = A or G. In some embodiments, the SaCas9 domain comprises one or more of E781X, N967X, and R1014X as numbered in the wild type SaCas9 sequence or a corresponding mutation in another SaCas9 protein wherein X is any amino acid. In some embodiments, the SaCas9 domain comprises one or more of a E781K, a N967K, and a R1014H mutation as numbered in the wild type SaCas9 sequence or a corresponding mutation in another SaCas9 protein. In some embodiments, the SaCas9 domain comprises a E781K, a N967K, or a R1014H mutation as numbered in the wild type SaCas9 sequence or a corresponding mutation in another SaCas9 protein.

[276] In some embodiments, the Cas9 domain is a Cas9 domain from Streptococcus pyogenes (SpCas9). In some embodiments, the SpCas9 domain is a nuclease active SpCas9, a nuclease inactive SpCas9 (SpCas9d), or a SpCas9 nickase (SpCas9n). In some embodiments, the SpCas9 comprises a D10X mutation as numbered in the wild type SpCas9 amino acid sequence or a corresponding mutation in another SapCas9 protein, wherein X is any amino acid except for D. In some embodiments, the SpCas9 comprises a D10A mutation as numbered in the wild type SpCas9 amino acid sequence or a corresponding mutation in another SpCas9 protein. In some embodiments, the SpCas9 domain, the nuclease in active SpCas9 domain, or the SpCas9 nickase domain can bind to a nucleic acid sequence having a non-NGG PAM. In some embodiments, the SpCas9 domain, the SpCas9 domain, the nuclease in active SpCas9 domain, or the SpCas9 nickase domain can bind to a nucleic acid

sequence having a NGG, a NGA, or a NGCG PAM sequence. In some embodiments, the SpCas9 domain comprises one or more of a D1135X, a R1335X, and a T1337X mutation as numbered in the wild type SpCas9 amino acid sequence or a corresponding mutation in another SpCas9 protein, wherein X is any amino acid. In some embodiments, the SpCas9 domain comprises one or more of a D1135E, R1335Q, and T1337R mutation as numbered in the wild type SpCas9 amino acid sequence or a corresponding mutation in another SpCas9 protein. In some embodiments, the SpCas9 domain comprises one or more of a Dl 134V, a R1334Q, and a T1336R mutation as numbered in the wild type Cas9 amino acid sequence, or a corresponding mutation thereof. In some embodiments, the SpCas9 domain comprises a D1135V, a R1335Q, and a T1337R mutation as numbered in the wild type SpCas9 amino acid sequence or a corresponding mutation in another SpCas9 protein. In some embodiments, the SpCas9 domain comprises one or more of a D1135X, a G1218X, a R1335X, and a T1337X mutation as numbered in the wild type SpCas9 amino acid sequence or a corresponding mutation in another SpCas9 protein, wherein X is any amino acid. In some embodiments, the SpCas9 domain comprises one or more of a D1135V, a G1218R, a R1335Q, and a T1337R mutation as numbered in the wild type SpCas9 amino acid sequence or a corresponding mutation in another SpCas9 protein. In some embodiments, the SpCas9 domain comprises a D1135V, a G1218R, a R1335Q, and a T1337R mutation as numbered in the wild type SpCas9 amino acid sequence or a corresponding mutation in another SpCas9 protein.

[277] In some embodiments, the Cas9 domain of any of the fusion proteins provided herein comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any one of the Cas9 sequences provided herein.

[278] In some embodiments, a base editor provided herein comprises a RNA guide programmable DNA binding protein that may be used to target and modify a specific nucleic acid (e.g., DNA or RNA) sequence. Nucleic acid programmable DNA binding proteins include, without limitation, Cas9 (e.g., nuclease inactive Cas9 and Cas9 nickase), CasX, CasY, Cas12a (Cpf1), Cas12b, C2c1, C2c2, C2C3, and Argonaute. One example of an nucleic acid programmable DNA-binding protein that has different PAM specificity than Cas9 is Clustered Regularly Interspaced Short Palindromic Repeats from Prevotella and Francisella 1 (Cpf1). Similar to Cas9, Cpf1 is also a class 2 CRISPR effector. It has been shown that Cpf1 mediates robust DNA interference with features distinct from Cas9. Cpf1 is

a single RNA-guided endonuclease lacking tracrRNA, and it utilizes a T-rich protospacer-adjacent motif (TTN, TTTN, or YTN). Cpf1 proteins described in Yamano et ah, "Crystal structure of Cpf1 in complex with guide RNA and target DNA." Cell (165) 2016, p. 949-962; the entire contents of which is hereby incorporated by reference.

[279] In some embodiments, a base editor provided herein comprises a nuclease inactive Cpf1 protein or a variant thereof. The Cpf1 protein has a RuvC-like endonuclease domain that is similar to the RuvC domain of Cas9 but does not have a HNH endonuclease domain, and the N-terminal of Cpf1 does not have the alfa-helical recognition lobe of Cas9. In some embodiments, the Cpf1 nickase comprises one or more mutations corresponding to D917A, E1006A, or D1255A as numbered in the Francisella novicida Cpf1 protein.

[280] Wild type Francisella novicida Cpf1 amino acid sequence is provided below:

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYH
QFFIEEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSE
KFKNLFNQNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWT
TYFKGFHENRKNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIK
KDLAEELTFDIDYKTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGEN
TKRKGINEYINLYSQQINDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTM
QSFYEQIAAFKTVEEKSIKETLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDY
SVIGTAVLEYITQQIAPKNLDNPSKKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDI
DKQCRFEEILANFAAIPMIFDEIAQNKDNLAQISIKYQNQGKKDLLQASAEDDVKAIK
DLLDQTNNLLHKLKIFHISQSEDKANILDKDEHFYLVFEECYFELANIVPLYNKIRNYI
TQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAILFIKDDKYYLGVMNKKNNKIFD
DKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFYNPSEDILRIRNHSTHTKN
GSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDTQRYNSIDEFYREVE
NQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTLYWKALFDER
NLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLIKDKR
FTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSIDRGERHLAYYTLVDG
KGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQV
VHEIAKLVIEYNAIVVFEDLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEF
DKTGGVLRAYQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESV
SKSQEFFSKFDKICYNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKN
HNWDTREVYPTKELEKLLKDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQM
RNSKTGTELDYLISPVADVNGNFFDSRQAPKNMPQDADANGAYHIGLKGLMLLGRI
KNNQEGKKLNLVIKNEEYFEFVQNRNN (SEQ ID NO: 53)

[281] In some embodiments, the base editor comprises a Cpf1 protein. In some embodiments, the Cpf1 protein is a Cpf1 nickase. In some embodiments, the Cpf1 protein is a nuclease inactive Cpf1. In some embodiments, the Cpf1 protein comprises an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at ease 99.5% identical to the FnCpf1 sequence provided herein. In some embodiments, the Cpf1 protein comprises mutations corresponding to D917A, E1006A, D1255A, D917A/E1006A, D917A/D1255A, E1006A/D1255A, or D917A/ E1006A/D1255A as numbered in the wild type FnCpf1 sequence provided herein. It should be appreciated that Cpf1 from other bacterial species may also be used in accordance with the present disclosure.

[282] In some embodiments, the nucleic acid programmable DNA binding protein comprises a Cas12b (C2c1), C2c2, or C2c3 protein or a variant thereof. Additional features of Cas proteins in Class 2 CRISPR-Cas systems described in Shmakov et al., "Discovery and Functional Characterization of Diverse Class 2 CRISPR Cas Systems", Mol. Cell, 2015 Nov 5; 60(3): 385-397, the entire contents of which is hereby incorporated by reference. Effectors of two of the systems, C2c1 and C2c3, contain RuvC-like endonuclease domains related to Cpf1. A third system, C2c2 contains an effector with two predicated HEPN RNase domains. Production of mature CRISPR RNA is tracrRNA-independent, unlike production of CRISPR RNA by C2c1. C2c1 depends on both CRISPR RNA and tracrRNA for DNA cleavage. The crystal structure of Alicyclobaccillus acidoterrastris C2c1 (AacC2c1) in complex with a chimeric single-molecule guide RNA (sgRNA) is described in Liu et al., "C2c1-sgRNA Complex Structure Reveals RNA-Guided DNA Cleavage Mechanism", Mol. Cell, 2017 Jan 19;65(2):310-322; Yang et al., "PAM-dependent Target DNA Recognition and Cleavage by C2C1 CRISPR-Cas, the entire contents of which are hereby incorporated by reference.

[283] An exemplary Cas12b amino acid sequence (Bacillus hisashii Cas12b) is provided below:

BhCas12b (*Bacillus hisashii*) NCBI Reference Sequence: WP_095142515

MAPKKKRKVGIHGVPAAATRSFILKIEPNEEVKKGLWKTHEVLNHGIAYYMNILKLI
RQEAIYEHHEQDPKNPKKVSKAEIQAELWDFVLKMQKCNSFTHEVDKDEVFNILRE
LYEELVPSSVEKKGEANQLSNKFLYPLVDPNSQSGKGTASSGRKPRWYNLKIAGDPS
WEEEKKKWEEDKKKDPLAKILGKLAEYGLIPLFIPYTDSNEPIVKEIKWMEKSRNQS
VRRLDKDMFIQALERFLSWESWNLKVKEEYEKVEKEYKTLEERIKEDIQALKALEQY
EKERQEQLLRDTLNTNEYRLSKRGLRGWREIIQKWLKMDENEPSEKYLEVFKDYQR
KHPREAGDYSVYEFLSKKENHFIWRNHPEYPYLYATFCEIDKKKKDAKQQATFTLA

DPINHPLWVRFEERSGSNLNKYRILTEQLHTEKLKKKLTVQLDRLIYPTESGGWEEK
GKVDIVLLPSRQFYNQIFLDIEEKGKHAFTYKDESIKFPLKGTLGGARVQFDRDHLRR
YPHKVESGNVGRIYFNMTVNIEPTESPVSKSLKIHRDDFPKVVNFKPKELTEWIKDSK
GKKLKSGIESLEIGLRVMSIDLGQRQAAAASIFEVVDQKPDIEGKLFFPIKGTELYAVH
RASFNIKLPGETLVKSREVLRKAREDNLKLMNQKLNFLRNVLHFQQFEDITEREKRV
TKWISRQENSDVPLVYQDELIQIRELMYKPYKDWVAFLKQLHKRLEVEIGKEVKHW
RKSLSDGRKGLYGISLKNIDEIDRTRKFLLRWSLRPTEPGEVRRLEPGQRFAIDQLNH
LNALKEDRLKKMANTIIMHALGYCYDVRKKKWQAKNPACQIILFEDLSNYNPYEER
SRFENSKLMKWSRREIPRQVALQGEIYGLQVGEVGAQFSSRFHAKTGSPGIRCSVVT
KEKLQDNRFFKNLQREGRLTLDKIAVLKEGDLYPDKGGEKFISLSKDRKCVTTHADI
NAAQNLQKRFWTRTHGFYKVYCKAYQVDGQTVYIPESKDQKQKIIEEFGEGYFILK
DGVYEWVNAGKLKIKKGSSKQSSSELVDSDILKDSFDLASELKGEKLMLYRDPSGN
VFPSDKWMAAGVFFGKLERILISKLTNQYSISTIEDDSSKQSMKRPAATKKAGQAKK
KK (SEQ ID NO: 54)

[284] In some embodiments, the programmable DNA binding protein comprises an argonaute protein. One example of such a nucleic acid programmable DNA binding protein is an Argonaute protein from Natronobacterium gregoryi (NgAgo). NgAgo is a ssDNA-guided endonuclease. NgAgo binds 5' phosphorylated ssDNA of ~24 nucleotides (gDNA) to guide it to its target site and will make DNA double-strand breaks at the gDNA site. In contrast to Cas9, the NgAgo-gDNA system does not require a protospacer-adjacent motif (PAM). Using a nuclease inactive NgAgo (dNgAgo) can greatly expand the bases that may be targeted. The characterization and use of NgAgo have been described in Gao et al., Nat Biotechnol., 2016 Jul;34(7):768-73. PubMed PMID: 27136078; Swarts et al., Nature. 507(7491) (2014):258-61; and Swarts et al., Nucleic Acids Res. 43(10) (2015):5120-9, each of which is incorporated herein by reference.

[285] In some embodiments, the protospacer sequence comprises a nucleotide sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the protospacer sequences listed in Table 1 or Table 24. In some embodiments, the protospacer sequence comprises any one of the protospacer sequences listed in Table 1 or Table 24. In some embodiments, the protospacer sequence is any one of the protospacer sequences listed in Table 1 or Table 24. In some embodiments, the protospacer sequence comprises a nucleotide sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least

95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the sequences of SEQ ID NOs: 13-15, 50, 66-69, 81-252, and 1594-1617. In some embodiments, the protospacer sequence comprises any one of the sequences of SEQ ID NOs: 13-15, 50, 66-69, 81-252, and 1594-1617. In some embodiments, the protospacer sequence is any one of the sequences of SEQ ID NOs: 13-15, 50, 66-69, 81-252, and 1594-1617.

Guide Polynucleotides

[286] Some aspects of this disclosure provide complexes comprising any of the fusion proteins provided herein, and a guide polynucleotide bound to a programmable DNA binding protein, e.g. a Cas9 domain of the base editor or fusion protein.

[287] In some embodiments, a guide polynucleotide is a guide polynucleotide, a guide RNA (gRNA), or a nucleic acid encoding the same.

[288] In some embodiments, the guide polynucleotide comprises a single nucleic acid sequence. In some embodiments, the guide polynucleotide comprises two nucleic acid sequences. In some embodiments, the guide nucleic acid (e.g., guide RNA) is from 15- 100 nucleotides long and comprises a sequence of at least 10 contiguous nucleotides that is complementary to a target sequence. In some embodiments, the guide RNA is 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides long. In some embodiments, the guide RNA comprises a sequence of 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 contiguous nucleotides that is complementary to a target sequence. In some embodiments, the target sequence is a DNA sequence. In some embodiments, the target sequence is a sequence in the genome of a mammal. In some embodiments, the target sequence is a sequence in the genome of a human. In some embodiments, the 3' end of the target sequence is immediately adjacent to a canonical PAM sequence (NGG). In some embodiments, the guide nucleic acid (e.g., guide RNA) is complementary to a sequence associated with a disease or disorder. In some embodiments, the guide nucleic acid (e.g., guide RNA) is complementary to a sequence associated with a disease or disorder having a mutation in a PCSK9 gene, a ANGPTL3 gene, or a APOC3 gene.

[289] Some aspects of this disclosure provide methods of using the fusion proteins, or complexes comprising a guide nucleic acid (e.g., gRNA) and a nucleobase editor provided herein. For example, some aspects of this disclosure provide methods comprising contacting

a DNA, or RNA molecule with any of the fusion proteins provided herein, and with at least one guide nucleic acid (e.g., guide RNA), wherein the guide nucleic acid, (e.g., guide RNA) is about 15-100 nucleotides long and comprises a sequence of at least 10 contiguous nucleotides that is complementary to a target sequence. In some embodiments, the 3' end of the target sequence is immediately adjacent to a canonical PAM sequence (NGG). In some embodiments, the 3' end of the target sequence is not immediately adjacent to a canonical PAM sequence (NGG). In some embodiments, the 3' end of the target sequence is immediately adjacent to an AGC, GAG, TTT, GTG, or CAA sequence.

[290] In some embodiments, a guide polynucleotide is a DNA. In some embodiments, a guide polynucleotide is an RNA. In some embodiments, a guide polynucleotide is a modified, artificial polynucleotides. In some embodiments, a guide polynucleotide is a single or single molecule polynucleotide. In some embodiments, a guide polynucleotide comprises dual polynucleotides. In some embodiments, a guide polynucleotide is a dual polynucleotides connected by a linker. In some embodiments, a guide polynucleotide is a dual polynucleotides connected by a non-nucleic acid linker. In some embodiments, a guide polynucleotide is a dual polynucleotides connected by a peptide linker or a chemical linker. In some embodiments, the guide polynucleotide is a single guide RNA. The guide RNA (gRNA) may guide the programmable DNA binding protein, e.g., a Class 2 Cas nuclease, e.g. a Cas9 to a target sequence on a target nucleic acid molecule, where the gRNA hybridizes with and the programmable DNA binding protein and modifies the target sequence. In some embodiments, the gRNA and the base editor fusion protein may form a ribonucleoprotein (RNP), e.g., a CRISPR/Cas complex. In some embodiments, the CRISPR complex may be a Type II CRISPR/Cas9 complex. In some embodiments, the CRISPR/Cas complex may be a Type V CRISPR/Cas complex, such as a Cpf1/guide RNA complex.

[291] A gRNA can comprise at least three regions: a first region at the 5’ end that can bind to the complementary strand of a target site in a chromosomal sequence (spacer region), a second internal region that can form a stem loop structure, and a third 3’ region that can be single-stranded. A first region of each guide RNA can also be different such that each gRNA guides a protein, e.g., Cas9, to a specific target site. Further, second and third regions of each gRNA can be identical in all gRNAs. A second region of a gRNA may form a secondary structure. In some embodiments, a secondary structure formed by a gRNA can comprise a stem (or hairpin) and a loop. A length of a loop and a stem can vary. In some embodiments, a loop can range from about 3 to about 10 nucleotides in length. In some embodiments, a stem can range from about 6 to about 20 nucleotides in length. A stem can comprise one or more

bulges of 1 to 10 nucleotides or about 10 nucleotides. In some embodiments, the overall length of a second region can range from about 16 to 60 nucleotides in length. In some embodiments, a loop can be about 4 nucleotides in length. In some embodiments, a stem can be about 12 in length. A third region of gRNA at the 3' end can be essentially single-stranded. In some embodiments, a third region is sometimes not complementary to any chromosomal sequence in a cell of interest and is sometimes not complementary to the rest of a gRNA. In addition, the length of a third region can vary. In some embodiments, a third region can be more than 3 or more than 4 nucleotides in length. For example, the length of a third region can range from about 5 to 60 nucleotides in length.

[292] A gRNA for a base editor system can comprise a CRISPR RNA (crRNA) and a trans-activating crRNA (tracrRNA). In some embodiments, the crRNA may comprise a targeting sequence (or spacer sequence) that hybridizes with the complementary strand of the target sequence on the target nucleic acid molecule. The crRNA may also comprise a flagpole that is complementary to and hybridizes with a portion of the tracrRNA. In some embodiments, the crRNA may parallel the structure of a naturally occurring crRNA transcribed from a CRISPR locus of a bacteria, where the targeting sequence acts as the protospacer of the Cas9 in the base editor system. The gRNA may target any sequence of interest via the targeting sequence of the crRNA. In some embodiments, the degree of complementarity between the targeting sequence of the gRNA and the target sequence on the target nucleic acid molecule is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100%. In some embodiments, the targeting sequence of the gRNA and the target sequence on the target nucleic acid molecule may be 100% complementary. In other embodiments, the targeting sequence of the gRNA and the target sequence on the target nucleic acid molecule may contain at least one mismatch. For example, the targeting sequence of the gRNA and the target sequence on the target nucleic acid molecule may contain 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mismatches.

[293] In some embodiments, the length of the targeting sequence depends on the CRISPR/Cas component of the base editor system and components used. For example, different Cas proteins from different bacterial species have varying optimal targeting sequence lengths. Accordingly, the targeting sequence may comprise 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more than 50 nucleotides in length. In some embodiments, the targeting sequence comprised 18-24 nucleotides in length. In some

embodiments, the targeting sequence comprises 19-21 nucleotides in length. In some embodiments, the targeting sequence comprises 20 nucleotides in length.

[294] In some embodiments, the guide RNA is a "dual guide RNA" or "dgRNA". In some embodiments, the dgRNA comprises a first RNA molecule comprising a crRNA, and a second RNA molecule comprising a tracrRNA. The first and second RNA molecules may form a RNA duplex via the base pairing between the crRNA and the tracrRNA (e.g. the repeat and anti repeat). In some embodiments, the guide RNA is a "single guide RNA" or "sgRNA." In some embodiments, the sgRNA may comprise a crRNA covalently linked to a tracrRNA. In some embodiments, the crRNA and the tracrRNA may be covalently linked via a linker. In some embodiments, the single-molecule guide RNA may comprise a stem-loop structure via the base pairing between the crRNA and the tracrRNA. In some embodiments, the sgRNA is a "Cas9 sgRNA" capable of directing a Cas9 protein. In certain embodiments, the guide RNA comprises a crRNA and tracrRNA sufficient for forming an active complex with a Cas9 protein and mediating RNA-guided DNA modification. The term "gRNA" and "sgRNA" are used interchangeably throughout this application. In some embodiments, more than one guide RNAs can be used; each guide RNA contains a different targeting sequence, such that the base editor system modifies more than one target sequence. In some embodiments, one or more guide RNAs may have the same or differing properties such as activity or stability within a base editor complex. Where more than one guide RNA is used, each guide RNA can be encoded on the same or on different expression cassettes. The promoters used to drive expression of the more than one guide RNA may be the same or different.

[295] In some embodiments, the gRNA or mRNA encoding Cas protein is modified. The modifications can comprise chemical alterations, synthetic modifications, nucleotide additions, and/or nucleotide subtractions and modified nucleosides or nucleotides can be present in a gRNA. A gRNA or Cas protein-encoding mRNA comprising one or more modified nucleosides or nucleotides is called a "modified" RNA to describe the presence of one or more non-naturally and/or naturally occurring components or configurations that are used instead of or in addition to the canonical A, G, C, and U residues. In some embodiments, a modified RNA is synthesized with a non-canonical nucleoside or nucleotide. Modified nucleosides and nucleotides can include one or more of: (i) alteration, e.g., replacement, of one or both of the non-linking phosphate oxygens and/or of one or more of the linking phosphate oxygens in the phosphodiester backbone linkage (an exemplary backbone modification); (ii) alteration, e.g., replacement, of a constituent of the ribose sugar, e.g., of

the 2' hydroxyl on the ribose sugar (an exemplary sugar modification); (iii) wholesale replacement of the phosphate moiety with "dephospho" linkers (an exemplary backbone modification); (iv) modification or replacement of a naturally occurring nucleobase, including with a non-canonical nucleobase (an exemplary base modification); (v) replacement or modification of the ribose-phosphate backbone (an exemplary backbone modification); (vi) modification of the 3' end or 5' end of the oligonucleotide, e.g., removal, addition, modification, or replacement of a terminal phosphate group or conjugation of a moiety, cap, or linker (such 3' or 5' cap modifications may comprise a sugar and/or backbone modification); and (vii) modification or replacement of the sugar (an exemplary sugar modification). The modifications can enhance genome editing by CRISPR/Cas. A modification can alter chirality of a gRNA. In some cases, chirality may be uniform or stereopure after a modification. A guide RNA can also be truncated. Truncation can be used to reduce undesired off-target mutagenesis. The truncation can comprise any number of nucleotide deletions. For example, the truncation can comprise 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 50 or more nucleotides. In some embodiments, the term "cap," as used herein, may be used to refer to one or more specially altered or modified nucleotides, e.g., specially altered or modified nucleotides on the 5' end of mRNA. In some embodiments, the cap comprises to a modified guanine (G) nucleotide. In some embodiments, the cap refers to the 5' end modification of mRNAs by addition of 7-Methylguanosine (N7-methyl guanosine or m7G). In some embodiments, "capping" of the mRNA structure plays a crucial role in a variety of cellular processes which include translation initiation, splicing, intracellular transport, and turnover. In some embodiment, the 5' cap enhances mRNA stability as well as translation efficiency. Exemplary cap analogs include, but are not limited to, standard cap analog m7G(5′)ppp(5′)G, anti-reverse cap analog (ARCA) m7,3'-OGpppG (or 3′-O-Me-m7G(5′) ppp(5′)G), unmethylated cap analog G (5′)ppp(5′)G, methylated cap analog for A+1 sites m7G(5′)ppp(5′)A, and unmethylated cap analog for A+1 sites G(5′)ppp(5′)A. Additional methods of obtaining mRNA cap analogs are described in Kowalska et al., RNA 2008 14(6): 1119-1131, which is incorporated herein in its entirety.

[296] In some embodiments, the guide RNA sequence comprises a nucleotide sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the guide sequences listed in Table 1 or Table 24. In some embodiments, the guide RNA sequence comprises any one of the guide RNA sequences listed in Table 1 or Table 24. In some embodiments, the guide RNA

sequence is any one of the guide RNA sequences listed in Table 1 or Table 24. In some embodiments, the guide RNA sequence comprises a nucleotide sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the sequences of SEQ ID NOs: 9-11, 55, 59, 253-452, 1618-1635, 1637-1800, 1802-2135, and 2191. In some embodiments, the guide RNA sequence comprises any one of the sequences of SEQ ID NOs: 9-11, 55, 59, 253-452, 1618-1635, 1637-1800, 1802-2135, and 2191. In some embodiments, the guide RNA sequence is any one of the sequences of SEQ ID NOs: 9-11, 55, 59, 253-452, 1618-1635, 1637-1800, 1802-2135, and 2191.

Off-target Effects

[297] With standard CRISPR-Cas9 genome editing, off-target mutagenesis—i.e., edits at genomic sites other than the intended target site—can occur at sites that share a high degree of sequence similarity with the protospacer sequence encoded in the gRNA.

[298] As used herein, off-target effect may be used refer to modifications, including indels, at genomic sites other than intended target site, for example, a protospacer sequence recognized by the guide RNA sequence. In the context of base editing, off-target editing may occur at genomic sites remote from an intended target site, or in close proximity to the intended target site. For example, one or more bases besides the target nucleobase in the base editing window may be edited ("bystander editing"). In certain embodiments, off-target base editing, e.g. bystander editing does not impact expression or function of the target gene.

[299] Potential off-target sequence candidates in the genome may be predicted by bioinformatic analyses of the human genome using, e.g., the MIT Specificity Score (calculated by http://crispor.tefor.net/; minimum score of 50) or by *in vitro* biochemical assays, e.g., ONE-seq.

[300] Off-target editing may be determined by sequencing analysis. In some embodiments, off-target editing is calculated using net nucleobase editing at potential off-target sites in the genome. For example, net nucleobase editing efficiency may be determined using no-base editor or no-guide RNA as a control, where net nucleobase editing efficiency is obtained by editing rate observed in cells contacted with a LNP enclosing a base editor mRNA and a guide RNA subjected by editing rate observed in control cells at predicted off-target sites. Additional methods for estimating and reducing off-target editing are described in Rees et al.,

Nat. Rev. Genet. 2018 19(12): 770-788, which is incorporated herein by reference in its entirety.

[301] Methods for selecting, designing, and validating gRNAs and targeting sequences (or spacer sequences) are described herein and known to those skilled in the art. Software tools can be used to optimize the gRNAs corresponding to a target nucleic acid sequence, e.g., to minimize total off-target activity across the genome. For example, for each possible targeting domain choice using S. pyogenes Cas9, all off-target sequences (preceding selected PAMs, e.g., NAG or NGG) may be identified across the genome that contain up to certain number (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) of mismatched base-pairs. First regions of gRNAs complementary to a target site can be identified, and all first regions (e.g., crRNAs) can be ranked according to its total predicted off-target score; the top-ranked targeting domains represent those that are likely to have the greatest on-target and the least off-target activity. In some embodiments, a DNA sequence searching algorithm can be used to identify a target sequence in crRNAs of a gRNA for use with Cas9. A custom gRNA design software based on the public tool cas-offinder, which scores guides after calculating their genome-wide off-target propensity, can be also used to design a gRNA (Bae, et al., Cas-OFFinder: A fast and versatile algorithm that searches for potential off-target sites of Cas9 RNA-guided endonucleases. Bioinformatics 30, 1473-1475 (2014)). In some embodiments, RepeatMasker program can be used to screen repeat elements and regions of low complexity in the input DNA sequences. In addition, the number of residues that could unintentionally be targeted (e.g., off-target residues that could potentially reside on ssDNA within the target nucleic acid locus) may be minimized to reduce the impact of potential substrate promiscuity of a deaminase domain in the nucleobase editor system. Candidate gRNAs can be functionally evaluated by using methods known in the art and/or as set forth herein.

[302] In some embodiments, target sequences for a Cas9 protein in a base editor system can be selected based on the presence of target sequences of a gene of interest in one or more species. For example, a sequence may be selected as a target sequence if the sequence match target sequences in both human and cynomolgus monkey orthologs of a gene of interest. In some embodiments, target sequences for a Cas9 nuclease can be selected based on predicted off-target profiles, as judged by the MIT Specificity Score (calculated by http://crispor.tefor.net/). For example, a sequence may be selected as a target sequence if the sequence has a favorable predicted off-target profiles (e.g., minimum score of 50 when judged by the MIT Specificity Score). In some embodiments, target sequences for an adenine base editor (ABE) can be selected based on the ability of editing an adenine base within a

splice donor site or a splice acceptor site in a target gene. In some embodiments, target sequences for an ABE can be selected based on the position of an adenine, e.g., if the adenine lies within the editing window of the ABE.

[303] The gRNAs described herein can be synthesized chemically, enzymatically, or a combination thereof. For example, the gRNA can be synthesized using standard phosphoramidite-based solid-phase synthesis methods. Alternatively, the gRNA can be synthesized in vitro by operably linking DNA encoding the gRNA to a promoter control sequence that is recognized by a phage RNA polymerase. Examples of suitable phage promoter sequences include, but are not limited to, T7, T3, SP6 promoter sequences, or variations thereof. In some embodiments, gRNA comprises two separate molecules (e.g., crRNA and tracrRNA) and one molecule (e.g., crRNA) can be chemically synthesized and the other molecule (e.g., tracrRNA) can be enzymatically synthesized.

[304] In some aspects, provided herein, is a composition for gene modification comprising a single guide RNA (sgRNA) described herein and the base editor fusion protein or a nucleic acid sequence encoding the base editor fusion protein. In some embodiments, the composition further comprises a vector that comprises the nucleic acid sequence encoding the base editor fusion protein. In some embodiments, base editor fusion protein comprises a Cas9 protein. In some embodiments, the Cas9 protein is a the Cas9 protein is a Streptococcus pyogenes Cas9. In some embodiments, the composition further comprises a pharmaceutically acceptable carrier. Non-limiting examples of pharmaceutically acceptable carriers are listed in "Pharmaceutical composition and methods of treatment" section of this application. In some embodiments, the composition described herein can be provided in a lipid nanoparticle.

Chemically modified guide RNAs (gRNAs)

[305] The present disclosure, provided herein, relates to a chemically modified CRISPR guide RNA (gRNA) that may be used in association with any general CRISPR/Cas system, for example, a base editor system as described herein. gRNAs (e.g., sgRNAs, short-sgRNAs, crRNAs, tracrRNAs, or dgRNAs) may comprise modifications at various nucleotide positions. In some aspects, provided herein, is a guide RNA (gRNA) or a single guide RNA (sgRNA) that comprises (i) a chemical modification at a nucleotide and (ii) an unmodified nucleotide. In some embodiments, the gRNA or the sgRNA may comprise a modification at a specific nucleotide position. In some embodiments, the gRNA or the sgRNA may comprise one or more modifications at one or more specific positions.

[306] In an aspect, chemically modified CRISPR guide RNAs provided herein comprise modifications based on crystal structures. Not intended to be bound by a particular theory, X-ray crystal structure based guide design may be used to optimize gRNA modification in complex with the Cas protein, e.g. Cas9. In some embodiments, a nucleotide where a 2'-OH is in close proximity with a Cas9 protein in the Cas9-gRNA complex is unmodified. In some embodiments, a nucleotide where a 2'-OH is in contact with a Cas9 protein in the Cas9-gRNA complex is unmodified. In some embodiments, a nucleotide where a 2'-OH is in contact with a Cas9 protein with a hydrogen bond in the Cas9-gRNA complex is unmodified. In some embodiments, the Cas9-gRNA complex is a pre-catalytic ternary complex. In some embodiments, a nucleotide where a 2'-OH is in close proximity with another part of the gRNA in the Cas9-gRNA complex is unmodified. In some embodiments, a nucleotide where a 2'-OH is in contact with another part of the gRNA in the Cas9-gRNA complex is unmodified. In some embodiments, a nucleotide where a 2'-OH is in contact with another part of the gRNA with a hydrogen bond in the Cas9-gRNA complex is unmodified. In some embodiments, the Cas9-gRNA complex is a pre-catalytic ternary complex. As a person of skill in the art would understand, the term "clash" as used herein refers to physically-unlikely overlapping atomic volumes in a structure. Incorporation of a 2'O-Me modification in these situations would potentially result in structural rearrangement(s) that could be detrimental to RNP function. As a person of skill in the art would understand, the term "contact" as used herein means a stable, non-covalent interaction between two functional groups, e.g. a hydrogen bond. In some embodiments, a nucleotide where a clash with another part of the gRNA is predicted if a 2'-OH is replaced with a 2'-OMe in the Cas9-gRNA complex is unmodified. In some embodiments, a nucleotide where a clash with another 2'-OH in the gRNA is predicted if a 2'-OH is replaced with a 2'-OMe in the Cas9-gRNA complex is unmodified. In some embodiments, the Cas9-gRNA complex is a pre-catalytic ternary complex. In some embodiments, a nucleotide that is solvent-exposed or otherwise distant from Cas protein residues in the Cas9-gRNA complex comprises a chemical modification. In some embodiments, a nucleotide that is solvent-exposed or otherwise distant from another nucleotide in the gRNA in the Cas9-gRNA complex comprises a chemical modification. In some embodiments, the chemical modification is a 2'-OMe modification. Additional description of Cas9-gRNA crystal structure is included in Jiang F, Taylor DW, Chen JS, Kornfeld JE, Zhou K, Thompson AJ, Nogales E, Doudna JA. Structures of a CRISPR-Cas9 R-loop complex primed for DNA cleavage. Science. 2016 Feb 19;351(6275):867-71, incorporated herein by reference in its entirety.

[307] Modifications to guide RNA nucleotides can include, but are not limited to, 2'-O-methyl modifications, 2'-O-(2-methoxyethyl) modifications, 2'-fluoro modifications, phosphorothioate modifications, inverted abasic modifications, deoxyribonucleotides, bicylic ribose analog (e.g., locked nucleic acid (LNA), C-ethylene-bridged nucleic acid (ENA), bridged nucleic acid (BNA), unlocked nucleic acid (UNA)), base or nucleobase modifications, internucleoside linkage modifications, ribonebularine, 2'-O-methylnebularine, or 2'-deoxynebularine. Other examples of modifications include, but are not limited to, 5'adenylate, 5' guanosine-triphosphate cap, 5'N7-Methylguanosine-triphosphate cap, 5'triphosphate cap, 3'phosphate, 3'thiophosphate, 5'phosphate, 5'thiophosphate, Cis-Syn thymidine dimer, trimers, C12 spacer, C3 spacer, C6 spacer, dSpacer, PC spacer, rSpacer, Spacer 18, Spacer 9,3'-3' modifications, 5'-5' modifications, abasic, acridine, azobenzene, biotin, biotin BB, biotin TEG, cholesteryl TEG, desthiobiotin TEG, DNP TEG, DNP-X, DOTA, dT-Biotin, dual biotin, PC biotin, psoralen C2, psoralen C6, TINA, 3'DABCYL, black hole quencher 1, black hole quencer 2, DABCYL SE, dT-DABCYL, IRDye QC-1, QSY-21, QSY-35, QSY-7, QSY-9, carboxyl linker, thiol linkers, 2'deoxyribonucleoside analog purine, 2'deoxyribonucleoside analog pyrimidine, ribonucleoside analog, 2'-0-methyl ribonucleoside analog, sugar modified analogs, wobble/universal bases, fluorescent dye label, 2'fluoro RNA, 2'O-methyl RNA, methylphosphonate, phosphodiester DNA, phosphodiester RNA, phosphothioate DNA, phosphorothioate RNA, UNA, pseudouridine-5'-triphosphate, 5-methylcytidine-5'-triphosphate, 2-O-methyl 3phosphorothioate or any combinations thereof.

[308] In some embodiments, the ribose group (or sugar) may be modified. In some embodiments, modified ribose group may control oligonucleotide binding affinity for complementary strands, duplex formation, or interaction with nucleases. Examples of chemical modifications to the ribose group include, but are not limited to, 2'-O-methyl (2'-OMe), 2'-fluoro (2'-F), 2'-deoxy, 2'-O-(2-methoxyethyl) (2'-MOE), 2'-NH2, 2'-O-Allyl, 2'-O-Ethylamine, 2'-O-Cyanoethyl, 2'-O-Acetalester, or a bicyclic nucleotide such as locked nucleic acid (LNA), 2'-(5-constrained ethyl (S-cEt)), constrained MOE, or 2'-0,4'-C-aminomethylene bridged nucleic acid (2',4'-BNANC). In some embodiments, 2'-O-methyl modification can increase binding affinity of oligonucleotides. In some embodiments, 2'-O-methyl modification can enhance nuclease stability of oligonucleotides. In some embodiments, 2'-fluoro modification can increase oligonucleotide binding affinity and nuclease stability. In this application, a ribonucleotide in lowercase in gRNA sequence depicts 2'-OMe modification (e.g., Table 1 or Table 24) (lowercase letter s indicates a phosphorothioate linkage).

[309] In some embodiments, the phosphate group may be chemically modified. Examples of chemical modifications to the phosphate group includes, but are not limited to, a phosphorothioate (PS), phosphonoacetate (PACE), thiophosphonoacetate (thioPACE), amide, triazole, phosphonate, or phosphotriester modification. In some embodiments, PS linkage can refer to a bond where a sulfur is substituted for one nonbridging phosphate oxygen in a phosphodiester linkage, e.g., between nucleotides. An "s" may be used to depict a PS modification in gRNA sequences in this application (e.g., Table 1 or Table 24). In some embodiments, a gRNA or an sgRNA may comprise a phosphorothioate (PS) linkage at a 5' end or at a 3' end. In some embodiments, a gRNA or an sgRNA may comprise a phosphorothioate (PS) linkage at a 5' end. In some embodiments, a gRNA or an sgRNA may comprise a phosphorothioate (PS) linkage at a 3' end. In some embodiments, a gRNA or an sgRNA may comprise a phosphorothioate (PS) linkage at a 5' end and at a 3' end. In some embodiments, a gRNA or an sgRNA may comprise one, two, or three, or more than three phosphorothioate linkages at the 5' end or at the 3' end. In some embodiments, a gRNA or an sgRNA may comprise three phosphorothioate (PS) linkages at the 5' end or at the 3' end. In some embodiments, a gRNA or an sgRNA may comprise three phosphorothioate linkages at the 3' end. In some embodiments, a gRNA or an sgRNA may comprise two and no more than two (i.e., only two) contiguous phosphorothioate (PS) linkages at the 5' end or at the 3' end. In some embodiments, a gRNA or an sgRNA may comprise three contiguous phosphorothioate (PS) linkages at the 5' end or at the 3' end. In some embodiments, a gRNA or an sgRNA may comprise the sequence 5'- UsUsU-3' at the 3'end or at the 5' end, wherein U indicates a uridine and wherein s indicates a phosphorothioate (PS) linkage. In some embodiments, a gRNA or an sgRNA may comprise the sequence 5'- ususu-3' at the 3'end or at the 5' end, wherein u indicates a 2'-O-methyluridine and wherein s indicates a phosphorothioate (PS) linkage. In some embodiments, a gRNA or an sgRNA may comprise the sequence 5'- ususuUUU-3' at the 3'end or at the 5' end, wherein U and u indicate uridine and 2'-O-methyluridine respectively, and wherein s indicates a phosphorothioate (PS) linkage. In some embodiments, a gRNA or an sgRNA may comprise the sequence 5'- ususuUuU-3' at the 3'end or at the 5' end, wherein U and u indicate uridine and 2'-O-methyluridine respectively, and wherein s indicates a phosphorothioate (PS) linkage. In some embodiments, a gRNA or an sgRNA may comprise the sequence 5'- ususususUuU-3' at the 3'end, wherein U and u indicate uridine and 2'-O-methyluridine respectively, and wherein s indicates a phosphorothioate (PS) linkage. In some embodiments, a gRNA or an sgRNA may

comprise the sequence 5'- ususuuuu-3' at the 3'end, wherein u indicates 2'-O-methyluridine, and wherein s indicates a phosphorothioate (PS) linkage.

[310] In some embodiments, the nucleobase may be chemically modified. Examples of chemical modifications to the nucleobase include, but are not limited to, 2-thiouridine, 4-thiouridine, N6-methyladenosine, pseudouridine, 2,6-diaminopurine, inosine, thymidine, 5-methylcytosine, 5-substituted pyrimidine, isoguanine, isocytosine, or halogenated aromatic groups.

[311] In some embodiments the chemically modified gRNAs comprise nebularine. Nebularine is a purine ribonucleoside that is derived from a beta-D-ribose and is 9H-purine attached to a beta-D-ribofuranosyl residue at position 9 via a glycosidic (N-glycosyl) linkage. Nebularine is a purine ribonucleoside with no exocyclic functional moiety or substitution. In some embodiments, it is a purine ribonucleoside. In some embodiments, it is a purine D-ribonucleoside. In some embodiments, nebularine is further modified chemically. In this application, "X," "x," and "dX" may be used to depict a ribonebularine modification, a 2'-O-methylnebularine modification, and a 2'-deoxynebularine modification, respectively, in gRNA sequences (e.g., Table 1 or Table 24). In some embodiments, substitution of a nucleotide (e.g., A) of a gRNA sequence (e.g., spacer region or tracr region) with nebularine may reduce off-target effects without affecting the gRNA activity. In some embodiments, the nebularine, the deoxynebularine, or 2'-O-methylnebularine replaces an adenine in an unmodified gRNA or sgRNA. In some embodiments, the nebularine, the deoxynebularine, or 2'-O-methylnebularine is in the spacer sequence. In some embodiments, the nebularine, the deoxynebularine, or 2'-O-methylnebularine is in the tracrRNA sequence. In some embodiments, the nebularine, the deoxynebularine, or 2'-O-methylnebularine is in a tracrRNA sequence in the tracrRNA sequence. In some embodiments, the nebularine, the deoxynebularine, or 2'-O-methylnebularine is in a crRNA sequence in the tracrRNA sequence. In some embodiments, the nebularine, the deoxynebularine, or 2'-O-methylnebularine is in a stem loop structure in the tracrRNA sequence.

[312] In some embodiments, the chemically modified gRNAs may comprise a total of 50-150 base pairs in length. In some embodiments, the chemically modified gRNAs may comprise a total of 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, or 150

base pairs in length. In some embodiments, the chemically modified gRNAs may comprise a total of about 50 to about 140 base pairs in length. In some embodiments, the chemically modified gRNAs may comprise a total of about 50 to about 60, about 50 to about 70, about 50 to about 80, about 50 to about 90, about 50 to about 100, about 50 to about 110, about 50 to about 120, about 50 to about 130, about 50 to about 140, about 60 to about 70, about 60 to about 80, about 60 to about 90, about 60 to about 100, about 60 to about 110, about 60 to about 120, about 60 to about 130, about 60 to about 140, about 70 to about 80, about 70 to about 90, about 70 to about 100, about 70 to about 110, about 70 to about 120, about 70 to about 130, about 70 to about 140, about 80 to about 90, about 80 to about 100, about 80 to about 110, about 80 to about 120, about 80 to about 130, about 80 to about 140, about 90 to about 100, about 90 to about 110, about 90 to about 120, about 90 to about 130, about 90 to about 140, about 100 to about 110, about 100 to about 120, about 100 to about 130, about 100 to about 140, about 110 to about 120, about 110 to about 130, about 110 to about 140, about 120 to about 130, about 120 to about 140, or about 130 to about 140 base pairs in length. In some embodiments, the chemically modified gRNAs may comprise a total of about 50, about 60, about 70, about 80, about 90, about 100, about 110, about 120, about 130, or about 140 base pairs in length. In some embodiments, the chemically modified gRNAs may comprise a total of at least about 50, about 60, about 70, about 80, about 90, about 100, about 110, about 120, or about 130 base pairs in length. In some embodiments, the chemically modified gRNAs may comprise a total of at most about 60, about 70, about 80, about 90, about 100, about 110, about 120, about 130, or about 140 base pairs in length. In one embodiment, the chemically modified gRNAs may comprise a total of 100 base pairs in length. In another embodiment, the chemically modified gRNAs may comprise a total of 103 base pairs in length.

[313] In some embodiments, the chemically modified gRNAs may comprise from 1 to 150 chemically modified nucleotides. In some embodiments, the chemically modified gRNAs may comprise a total of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, or 150 chemically modified nucleotides. In some embodiments, the chemically modified gRNAs

may comprise a total of about 1 to about 45 chemically modified nucleotides. In some embodiments, the chemically modified gRNAs may comprise a total of about 1 to about 3, about 1 to about 5, about 1 to about 10, about 1 to about 15, about 1 to about 20, about 1 to about 25, about 1 to about 30, about 1 to about 35, about 1 to about 40, about 1 to about 45, about 3 to about 5, about 3 to about 10, about 3 to about 15, about 3 to about 20, about 3 to about 25, about 3 to about 30, about 3 to about 35, about 3 to about 40, about 3 to about 45, about 5 to about 10, about 5 to about 15, about 5 to about 20, about 5 to about 25, about 5 to about 30, about 5 to about 35, about 5 to about 40, about 5 to about 45, about 10 to about 15, about 10 to about 20, about 10 to about 25, about 10 to about 30, about 10 to about 35, about 10 to about 40, about 10 to about 45, about 15 to about 20, about 15 to about 25, about 15 to about 30, about 15 to about 35, about 15 to about 40, about 15 to about 45, about 20 to about 25, about 20 to about 30, about 20 to about 35, about 20 to about 40, about 20 to about 45, about 25 to about 30, about 25 to about 35, about 25 to about 40, about 25 to about 45, about 30 to about 35, about 30 to about 40, about 30 to about 45, about 35 to about 40, about 35 to about 45, about 40 to about 45, or about 45 to about 50 chemically modified nucleotides. In some embodiments, the chemically modified gRNAs may comprise a total of about 1, about 3, about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, or about 50 chemically modified nucleotides. In some embodiments, the chemically modified gRNAs may comprise a total of at least about 1, about 3, about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, or about 50 chemically modified nucleotides. In some embodiments, the chemically modified gRNAs may comprise a total of at most about 3, about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, or about 50 chemically modified nucleotides. In some embodiments, the chemically modified gRNAs may comprise a total of about 50 to about 140 chemically modified nucleotides. In some embodiments, the chemically modified gRNAs may comprise a total of about 50 to about 60, about 50 to about 70, about 50 to about 80, about 50 to about 90, about 50 to about 100, about 50 to about 110, about 50 to about 120, about 50 to about 130, about 50 to about 140, about 60 to about 70, about 60 to about 80, about 60 to about 90, about 60 to about 100, about 60 to about 110, about 60 to about 120, about 60 to about 130, about 60 to about 140, about 70 to about 80, about 70 to about 90, about 70 to about 100, about 70 to about 110, about 70 to about 120, about 70 to about 130, about 70 to about 140, about 80 to about 90, about 80 to about 100, about 80 to about 110, about 80 to about 120, about 80 to about 130, about 80 to about 140, about 90 to about 100, about 90 to about 110, about 90 to about 120, about 90 to about 130, about 90 to about 140, about 100 to about 110,

about 100 to about 120, about 100 to about 130, about 100 to about 140, about 110 to about 120, about 110 to about 130, about 110 to about 140, about 120 to about 130, about 120 to about 140, or about 130 to about 140 chemically modified nucleotides. In some embodiments, the chemically modified gRNAs may comprise a total of about 50, about 60, about 70, about 80, about 90, about 100, about 110, about 120, about 130, or about 140 chemically modified nucleotides. In some embodiments, the chemically modified gRNAs may comprise a total of at least about 50, about 60, about 70, about 80, about 90, about 100, about 110, about 120, or about 130 chemically modified nucleotides. In some embodiments, the chemically modified gRNAs may comprise a total of at most about 60, about 70, about 80, about 90, about 100, about 110, about 120, about 130, or about 140 chemically modified nucleotides. In one embodiment, the chemically modified gRNAs may comprise a total of 54 chemically modified nucleotides. In another embodiment, the chemically modified gRNAs may comprise a total of 62 chemically modified nucleotides.

[314] In some embodiments, the chemically modified gRNAs may comprise about 1 % to about 100 % chemically modified nucleotides. In some embodiments, the chemically modified gRNAs may comprise about 1 % to about 10 %, about 1 % to about 20 %, about 1 % to about 30 %, about 1 % to about 40 %, about 1 % to about 50 %, about 1 % to about 60 %, about 1 % to about 70 %, about 1 % to about 80 %, about 1 % to about 90 %, about 1 % to about 95 %, about 1 % to about 100 %, about 10 % to about 20 %, about 10 % to about 30 %, about 10 % to about 40 %, about 10 % to about 50 %, about 10 % to about 60 %, about 10 % to about 70 %, about 10 % to about 80 %, about 10 % to about 90 %, about 10 % to about 95 %, about 10 % to about 100 %, about 20 % to about 30 %, about 20 % to about 40 %, about 20 % to about 50 %, about 20 % to about 60 %, about 20 % to about 70 %, about 20 % to about 80 %, about 20 % to about 90 %, about 20 % to about 95 %, about 20 % to about 100 %, about 30 % to about 40 %, about 30 % to about 50 %, about 30 % to about 60 %, about 30 % to about 70 %, about 30 % to about 80 %, about 30 % to about 90 %, about 30 % to about 95 %, about 30 % to about 100 %, about 40 % to about 50 %, about 40 % to about 60 %, about 40 % to about 70 %, about 40 % to about 80 %, about 40 % to about 90 %, about 40 % to about 95 %, about 40 % to about 100 %, about 50 % to about 60 %, about 50 % to about 70 %, about 50 % to about 80 %, about 50 % to about 90 %, about 50 % to about 95 %, about 50 % to about 100 %, about 60 % to about 70 %, about 60 % to about 80 %, about 60 % to about 90 %, about 60 % to about 95 %, about 60 % to about 100 %, about 70 % to about 80 %, about 70 % to about 90 %, about 70 % to about 95 %, about 70 % to about 100 %, about 80 % to about 90 %, about 80 % to about 95 %, about 80 % to about 100 %, about 90 % to

about 95 %, about 90 % to about 100 %, or about 95 % to about 100 % chemically modified nucleotides. In some embodiments, the chemically modified gRNAs may comprise about 1 %, about 10 %, about 20 %, about 30 %, about 40 %, about 50 %, about 60 %, about 70 %, about 80 %, about 90 %, about 95 %, or about 100 % chemically modified nucleotides. In some embodiments, the chemically modified gRNAs may comprise at least about 1 %, about 10 %, about 20 %, about 30 %, about 40 %, about 50 %, about 60 %, about 70 %, about 80 %, about 90 %, or about 95 % chemically modified nucleotides. In some embodiments, the chemically modified gRNAs may comprise at most about 10 %, about 20 %, about 30 %, about 40 %, about 50 %, about 60 %, about 70 %, about 80 %, about 90 %, about 95 %, or about 100 % chemically modified nucleotides.

[315] In some embodiments, the chemically modified gRNAs may have chemically modified nucleotide on one or more of the positions 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, or 150 in the 5'– 3' direction in the gRNA sequence.

[316] In some embodiments, the chemically modified gRNAs may have chemically modified nucleotide on one or more of the positions which are 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, or 149 base pairs from the 5' end of the gRNA sequence.

[317] In some embodiments, the chemically modified gRNAs may have chemically modified nucleotide on one or more the positions where are 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108,

109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, or 149 base pairs from the 3' end of the gRNA sequence.

[318] In some embodiments, the chemically modified gRNA may be a sgRNA. The term "gRNA" and "sgRNA" are used interchangeably in this application. In some embodiments, the sgRNA may comprise one or more chemically modified nucleotides. In some embodiments, the chemically modified sgRNA may comprise a total of 50-150 base pairs in length. In some embodiments, the chemically modified sgRNA may comprise a total of 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, or 150 base pairs in length. In some embodiments, the chemically modified sgRNA may comprise a total of about 50 to about 140 base pairs in length. In some embodiments, the chemically modified sgRNA may comprise a total of about 50 to about 60, about 50 to about 70, about 50 to about 80, about 50 to about 90, about 50 to about 100, about 50 to about 110, about 50 to about 120, about 50 to about 130, about 50 to about 140, about 60 to about 70, about 60 to about 80, about 60 to about 90, about 60 to about 100, about 60 to about 110, about 60 to about 120, about 60 to about 130, about 60 to about 140, about 70 to about 80, about 70 to about 90, about 70 to about 100, about 70 to about 110, about 70 to about 120, about 70 to about 130, about 70 to about 140, about 80 to about 90, about 80 to about 100, about 80 to about 110, about 80 to about 120, about 80 to about 130, about 80 to about 140, about 90 to about 100, about 90 to about 110, about 90 to about 120, about 90 to about 130, about 90 to about 140, about 100 to about 110, about 100 to about 120, about 100 to about 130, about 100 to about 140, about 110 to about 120, about 110 to about 130, about 110 to about 140, about 120 to about 130, about 120 to about 140, or about 130 to about 140 base pairs in length. In some embodiments, the chemically modified sgRNAs may comprise a total of about 50, about 60, about 70, about 80, about 90, about 100, about 110, about 120, about 130, or about 140 base pairs in length. In some embodiments, the chemically modified sgRNAs may comprise a total of at least about 50, about 60, about 70, about 80, about 90, about 100, about 110, about 120, or about 130 base pairs in length. In some embodiments, the chemically modified sgRNA may comprise a total of at most about 60, about 70, about 80, about 90, about 100, about 110, about 120, about 130, or about 140 base pairs in length. In one embodiment, the chemically

modified sgRNA may comprise a total of 100 base pairs in length. In another embodiment, the chemically modified sgRNA may comprise a total of 103 base pairs in length.

[319] In some embodiments, the chemically modified sgRNAs may comprise a total of from 1 to 150 chemically modified nucleotides. In some embodiments, the chemically modified sgRNAs may comprise a total of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, or 150 chemically modified nucleotides. In some embodiments, the chemically modified sgRNAs may comprise a total of about 1 to about 45 chemically modified nucleotides. In some embodiments, the chemically modified sgRNAs may comprise a total of about 1 to about 3, about 1 to about 5, about 1 to about 10, about 1 to about 15, about 1 to about 20, about 1 to about 25, about 1 to about 30, about 1 to about 35, about 1 to about 40, about 1 to about 45, about 3 to about 5, about 3 to about 10, about 3 to about 15, about 3 to about 20, about 3 to about 25, about 3 to about 30, about 3 to about 35, about 3 to about 40, about 3 to about 45, about 5 to about 10, about 5 to about 15, about 5 to about 20, about 5 to about 25, about 5 to about 30, about 5 to about 35, about 5 to about 40, about 5 to about 45, about 10 to about 15, about 10 to about 20, about 10 to about 25, about 10 to about 30, about 10 to about 35, about 10 to about 40, about 10 to about 45, about 15 to about 20, about 15 to about 25, about 15 to about 30, about 15 to about 35, about 15 to about 40, about 15 to about 45, about 20 to about 25, about 20 to about 30, about 20 to about 35, about 20 to about 40, about 20 to about 45, about 25 to about 30, about 25 to about 35, about 25 to about 40, about 25 to about 45, about 30 to about 35, about 30 to about 40, about 30 to about 45, about 35 to about 40, about 35 to about 45, about 40 to about 45, or about 45 to about 50 chemically modified nucleotides. In some embodiments, the chemically modified sgRNAs may comprise a total of about 1, about 3, about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, or about 50 chemically modified nucleotides. In some embodiments, the chemically modified sgRNAs may comprise a total of at least about 1, about 3, about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, or about 50 chemically modified nucleotides. In some embodiments, the chemically modified sgRNAs may comprise a total of at most about 3, about 5, about 10, about 15, about 20, about 25, about 30, about 35, about

40, about 45, or about 50 chemically modified nucleotides. In some embodiments, the chemically modified sgRNAs may comprise a total of about 50 to about 140 chemically modified nucleotides. In some embodiments, the chemically modified sgRNAs may comprise a total of about 50 to about 60, about 50 to about 70, about 50 to about 80, about 50 to about 90, about 50 to about 100, about 50 to about 110, about 50 to about 120, about 50 to about 130, about 50 to about 140, about 60 to about 70, about 60 to about 80, about 60 to about 90, about 60 to about 100, about 60 to about 110, about 60 to about 120, about 60 to about 130, about 60 to about 140, about 70 to about 80, about 70 to about 90, about 70 to about 100, about 70 to about 110, about 70 to about 120, about 70 to about 130, about 70 to about 140, about 80 to about 90, about 80 to about 100, about 80 to about 110, about 80 to about 120, about 80 to about 130, about 80 to about 140, about 90 to about 100, about 90 to about 110, about 90 to about 120, about 90 to about 130, about 90 to about 140, about 100 to about 110, about 100 to about 120, about 100 to about 130, about 100 to about 140, about 110 to about 120, about 110 to about 130, about 110 to about 140, about 120 to about 130, about 120 to about 140, or about 130 to about 140 chemically modified nucleotides. In some embodiments, the chemically modified sgRNAs may comprise a total of about 50, about 60, about 70, about 80, about 90, about 100, about 110, about 120, about 130, or about 140 chemically modified nucleotides. In some embodiments, the chemically modified sgRNAs may comprise a total of at least about 50, about 60, about 70, about 80, about 90, about 100, about 110, about 120, or about 130 chemically modified nucleotides. In some embodiments, the chemically modified sgRNAs may comprise a total of at most about 60, about 70, about 80, about 90, about 100, about 110, about 120, about 130, or about 140 chemically modified nucleotides. In one embodiment, the chemically modified sgRNAs may comprise a total of 54 chemically modified nucleotides. In another embodiment, the chemically modified sgRNAs may comprise a total of 62 chemically modified nucleotides.

[320] In some embodiments, the chemically modified sgRNAs may comprise about 1 % to about 100 % chemically modified nucleotides. In some embodiments, the chemically modified sgRNAs may comprise about 1 % to about 10 %, about 1 % to about 20 %, about 1 % to about 30 %, about 1 % to about 40 %, about 1 % to about 50 %, about 1 % to about 60 %, about 1 % to about 70 %, about 1 % to about 80 %, about 1 % to about 90 %, about 1 % to about 95 %, about 1 % to about 100 %, about 10 % to about 20 %, about 10 % to about 30 %, about 10 % to about 40 %, about 10 % to about 50 %, about 10 % to about 60 %, about 10 % to about 70 %, about 10 % to about 80 %, about 10 % to about 90 %, about 10 % to about 95 %, about 10 % to about 100 %, about 20 % to about 30 %, about 20 % to about 40 %, about

20 % to about 50 %, about 20 % to about 60 %, about 20 % to about 70 %, about 20 % to about 80 %, about 20 % to about 90 %, about 20 % to about 95 %, about 20 % to about 100 %, about 30 % to about 40 %, about 30 % to about 50 %, about 30 % to about 60 %, about 30 % to about 70 %, about 30 % to about 80 %, about 30 % to about 90 %, about 30 % to about 95 %, about 30 % to about 100 %, about 40 % to about 50 %, about 40 % to about 60 %, about 40 % to about 70 %, about 40 % to about 80 %, about 40 % to about 90 %, about 40 % to about 95 %, about 40 % to about 100 %, about 50 % to about 60 %, about 50 % to about 70 %, about 50 % to about 80 %, about 50 % to about 90 %, about 50 % to about 95 %, about 50 % to about 100 %, about 60 % to about 70 %, about 60 % to about 80 %, about 60 % to about 90 %, about 60 % to about 95 %, about 60 % to about 100 %, about 70 % to about 80 %, about 70 % to about 90 %, about 70 % to about 95 %, about 70 % to about 100 %, about 80 % to about 90 %, about 80 % to about 95 %, about 80 % to about 100 %, about 90 % to about 95 %, about 90 % to about 100 %, or about 95 % to about 100 % chemically modified nucleotides. In some embodiments, the chemically modified sgRNAs may comprise about 1 %, about 10 %, about 20 %, about 30 %, about 40 %, about 50 %, about 60 %, about 70 %, about 80 %, about 90 %, about 95 %, or about 100 % chemically modified nucleotides. In some embodiments, the chemically modified sgRNAs may comprise at least about 1 %, about 10 %, about 20 %, about 30 %, about 40 %, about 50 %, about 60 %, about 70 %, about 80 %, about 90 %, or about 95 % chemically modified nucleotides. In some embodiments, the chemically modified sgRNAs may comprise at most about 10 %, about 20 %, about 30 %, about 40 %, about 50 %, about 60 %, about 70 %, about 80 %, about 90 %, about 95 %, or about 100 % chemically modified nucleotides.

[321] In some embodiments, the chemically modified sgRNAs may have chemically modified nucleotide on one or more of the positions 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, or 150 in the 5’ – 3’ direction in the sgRNA sequence.

[322] In some embodiments, the chemically modified sgRNAs may have chemically modified nucleotide on one or more of the positions 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39,

40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, or 149 base pairs from the 5' end of the sgRNA sequence.

[323] In some embodiments, the chemically modified sgRNAs may have chemically modified nucleotide on one or more of the positions 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, or 149 base pairs from the 3' end of the sgRNA sequence.

[324] In some embodiments, the chemically modified gRNAs or sgRNAs may have one or more chemical modifications in the spacer or protospacer region, i.e., the target sequence. In some embodiments, the chemically modified gRNAs or sgRNAs may comprise a spacer sequence of from 10 to 30 base pairs in length. In some embodiments, the chemically modified gRNAs or sgRNAs may comprise a spacer sequence of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 base pairs in length. In some embodiments, the chemically modified gRNAs or sgRNAs may comprise a spacer sequence of about 10 to about 30 base pairs in length. In some embodiments, the chemically modified gRNAs or sgRNAs may comprise a spacer sequence of about 10 to about 15, about 10 to about 20, about 10 to about 25, about 10 to about 30, about 15 to about 20, about 15 to about 25, about 15 to about 30, about 20 to about 25, about 20 to about 30, or about 25 to about 30 base pairs in length. In some embodiments, the chemically modified gRNAs or sgRNAs may comprise a spacer sequence of about 10, about 15, about 20, about 25, or about 30 base pairs in length. In some embodiments, the chemically modified gRNAs or sgRNAs may comprise a spacer sequence of at least about 10, about 15, about 20, or about 25 base pairs in length. In some embodiments, the chemically modified gRNAs or sgRNAs may comprise a spacer sequence of at most about 15, about 20, about 25, or about 30 base pairs in length. In one embodiment, the chemically modified gRNAs or sgRNAs may comprise a spacer sequence of 18 base pairs in length. In another embodiment, the chemically modified gRNAs or sgRNAs

may comprise a spacer sequence of 22 base pairs in length. In a preferred embodiment, the chemically modified gRNAs or sgRNAs may comprise a spacer sequence of 20 base pairs in length.

[325] In some embodiments, the spacer sequence may comprise a total of from 1 to 30 chemically modified nucleotides. In some embodiments, the spacer region may comprise a total of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 chemically modified nucleotides. In some embodiments, the spacer sequence may comprise a total of about 1 to about 10 chemically modified nucleotides. In some embodiments, the spacer sequence may comprise a total of about 1 to about 2, about 1 to about 3, about 1 to about 4, about 1 to about 5, about 1 to about 6, about 1 to about 7, about 1 to about 8, about 1 to about 9, about 1 to about 10, about 2 to about 3, about 2 to about 4, about 2 to about 5, about 2 to about 6, about 2 to about 7, about 2 to about 8, about 2 to about 9, about 2 to about 10, about 3 to about 4, about 3 to about 5, about 3 to about 6, about 3 to about 7, about 3 to about 8, about 3 to about 9, about 3 to about 10, about 4 to about 5, about 4 to about 6, about 4 to about 7, about 4 to about 8, about 4 to about 9, about 4 to about 10, about 5 to about 6, about 5 to about 7, about 5 to about 8, about 5 to about 9, about 5 to about 10, about 6 to about 7, about 6 to about 8, about 6 to about 9, about 6 to about 10, about 7 to about 8, about 7 to about 9, about 7 to about 10, about 8 to about 9, about 8 to about 10, or about 9 to about 10 chemically modified nucleotides. In some embodiments, the spacer sequence may comprise a total of about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, or about 10 chemically modified nucleotides. In some embodiments, the spacer sequence may comprise a total of at least about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, or about 9 chemically modified nucleotides. In some embodiments, the spacer sequence may comprise a total of at most about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, or about 10 chemically modified nucleotides. In some embodiments, the spacer sequence may comprise a total of about 10 to about 30 chemically modified nucleotides. In some embodiments, the spacer sequence may comprise a total of about 10 to about 15, about 10 to about 20, about 10 to about 25, about 10 to about 30, about 15 to about 20, about 15 to about 25, about 15 to about 30, about 20 to about 25, about 20 to about 30, or about 25 to about 30 chemically modified nucleotides. In some embodiments, the spacer sequence may comprise a total of about 10, about 15, about 20, about 25, or about 30 chemically modified nucleotides. In some embodiments, the spacer sequence may comprise a total of at least about 10, about 15, about 20, or about 25 chemically modified nucleotides. In some embodiments, the spacer sequence may comprise a total of at most

about 15, about 20, about 25, or about 30 chemically modified nucleotides. In one embodiment, the spacer sequence may comprise a total of 3 chemically modified nucleotides. In another embodiment, the spacer sequence may comprise a total of 5 chemically modified nucleotides.

[326] In some embodiments, the spacer sequence may comprise from 1% to 100% chemically modified nucleotides. In some embodiments, the spacer sequence may comprise about 1 % to about 100 % chemically modified nucleotides. In some embodiments, the spacer sequence may comprise about 1 % to about 10 %, about 1 % to about 20 %, about 1 % to about 30 %, about 1 % to about 40 %, about 1 % to about 50 %, about 1 % to about 60 %, about 1 % to about 70 %, about 1 % to about 80 %, about 1 % to about 90 %, about 1 % to about 95 %, about 1 % to about 100 %, about 10 % to about 20 %, about 10 % to about 30 %, about 10 % to about 40 %, about 10 % to about 50 %, about 10 % to about 60 %, about 10 % to about 70 %, about 10 % to about 80 %, about 10 % to about 90 %, about 10 % to about 95 %, about 10 % to about 100 %, about 20 % to about 30 %, about 20 % to about 40 %, about 20 % to about 50 %, about 20 % to about 60 %, about 20 % to about 70 %, about 20 % to about 80 %, about 20 % to about 90 %, about 20 % to about 95 %, about 20 % to about 100 %, about 30 % to about 40 %, about 30 % to about 50 %, about 30 % to about 60 %, about 30 % to about 70 %, about 30 % to about 80 %, about 30 % to about 90 %, about 30 % to about 95 %, about 30 % to about 100 %, about 40 % to about 50 %, about 40 % to about 60 %, about 40 % to about 70 %, about 40 % to about 80 %, about 40 % to about 90 %, about 40 % to about 95 %, about 40 % to about 100 %, about 50 % to about 60 %, about 50 % to about 70 %, about 50 % to about 80 %, about 50 % to about 90 %, about 50 % to about 95 %, about 50 % to about 100 %, about 60 % to about 70 %, about 60 % to about 80 %, about 60 % to about 90 %, about 60 % to about 95 %, about 60 % to about 100 %, about 70 % to about 80 %, about 70 % to about 90 %, about 70 % to about 95 %, about 70 % to about 100 %, about 80 % to about 90 %, about 80 % to about 95 %, about 80 % to about 100 %, about 90 % to about 95 %, about 90 % to about 100 %, or about 95 % to about 100 % chemically modified nucleotides. In some embodiments, the spacer sequence may comprise about 1 %, about 10 %, about 20 %, about 30 %, about 40 %, about 50 %, about 60 %, about 70 %, about 80 %, about 90 %, about 95 %, or about 100 % chemically modified nucleotides. In some embodiments, the spacer sequence may comprise at least about 1 %, about 10 %, about 20 %, about 30 %, about 40 %, about 50 %, about 60 %, about 70 %, about 80 %, about 90 %, or about 95 % chemically modified nucleotides. In some embodiments, the spacer sequence may comprise at most about 10 %, about 20 %, about 30 %, about 40 %, about 50 %, about 60 %,

about 70 %, about 80 %, about 90 %, about 95 %, or about 100 % chemically modified nucleotides.

[327] In some embodiments, the chemically modified spacer sequence may have chemically modified nucleotide on one or more of the positions 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 in the 5' – 3' direction in the spacer sequence.

[328] In some embodiments, the chemically modified spacer region may have chemically modified nucleotide on one or more of the positions which are 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29 base pairs from the 5' end of the spacer sequence.

[329] In some embodiments, the chemically modified spacer region may have chemically modified nucleotide on one or more the positions which are 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29 base pairs from the 3' end of the spacer sequence.

[330] In some embodiments, the chemically modified gRNAs or sgRNAs may have one or more chemical modifications in the tracrRNA sequence. In some embodiments, the chemically modified gRNAs or sgRNAs may comprise a tracrRNA sequence of from 30 to 100 base pairs in length. In some embodiments, the chemically modified gRNAs or sgRNAs may comprise a tracrRNA sequence of 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, or 130 base pairs in length. In some embodiments, the chemically modified gRNAs or sgRNAs may comprise a tracrRNA sequence of from about 30 to about 130 base pairs in length. In some embodiments, the chemically modified gRNAs or sgRNAs may comprise a tracrRNA sequence of from about 30 to about 40, about 30 to about 50, about 30 to about 60, about 30 to about 70, about 30 to about 75, about 30 to about 80, about 30 to about 90, about 30 to about 100, about 30 to about 110, about 30 to about 120, about 30 to about 130, about 40 to about 50, about 40 to about 60, about 40 to about 70, about 40 to about 75, about 40 to about 80, about 40 to about 90, about 40 to about 100, about 40 to about 110, about 40 to about 120, about 40 to about 130, about 50 to about 60, about 50 to about 70, about 50 to about 75, about 50 to about 80, about 50 to about 90, about 50 to about 100, about 50 to about 110, about 50 to about 120, about 50 to about 130, about 60 to about 70, about 60 to about 75,

about 60 to about 80, about 60 to about 90, about 60 to about 100, about 60 to about 110, about 60 to about 120, about 60 to about 130, about 70 to about 75, about 70 to about 80, about 70 to about 90, about 70 to about 100, about 70 to about 110, about 70 to about 120, about 70 to about 130, about 75 to about 80, about 75 to about 90, about 75 to about 100, about 75 to about 110, about 75 to about 120, about 75 to about 130, about 80 to about 90, about 80 to about 100, about 80 to about 110, about 80 to about 120, about 80 to about 130, about 90 to about 100, about 90 to about 110, about 90 to about 120, about 90 to about 130, about 100 to about 110, about 100 to about 120, about 100 to about 130, about 110 to about 120, about 110 to about 130, or about 120 to about 130 base pairs in length. In some embodiments, the chemically modified gRNAs or sgRNAs may comprise a tracrRNA sequence of from about 30, about 40, about 50, about 60, about 70, about 75, about 80, about 90, about 100, about 110, about 120, or about 130 base pairs in length. In some embodiments, the chemically modified gRNAs or sgRNAs may comprise a tracrRNA sequence of from at least about 30, about 40, about 50, about 60, about 70, about 75, about 80, about 90, about 100, about 110, or about 120 base pairs in length. In some embodiments, the chemically modified gRNAs or sgRNAs may comprise a tracrRNA sequence of from at most about 40, about 50, about 60, about 70, about 75, about 80, about 90, about 100, about 110, about 120, or about 130 base pairs in length. In some embodiments, the chemically modified gRNAs or sgRNAs may comprise a tracrRNA sequence of 70 base pairs in length. In some embodiments, the chemically modified gRNAs or sgRNAs may comprise a tracrRNA sequence of 73 base pairs in length. In some embodiments, the chemically modified gRNAs or sgRNAs may comprise a tracrRNA sequence of 68 base pairs in length. In some embodiments, the chemically modified gRNAs or sgRNAs may comprise a tracrRNA sequence of 71 base pairs in length.

[331] In some embodiments, the tracrRNA sequence may comprise a total of from 1 to 130 chemically modified nucleotides. In some embodiments, the chemically modified gRNAs or sgRNAs may comprise a tracrRNA sequence of from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, or 130 chemically modified nucleotides. In some embodiments, the tracrRNA sequence may comprise a total of from about 1 to about 10 chemically modified nucleotides.

In some embodiments, the tracrRNA sequence may comprise a total of from about 1 to about 2, about 1 to about 3, about 1 to about 4, about 1 to about 5, about 1 to about 6, about 1 to about 7, about 1 to about 8, about 1 to about 9, about 1 to about 10, about 2 to about 3, about 2 to about 4, about 2 to about 5, about 2 to about 6, about 2 to about 7, about 2 to about 8, about 2 to about 9, about 2 to about 10, about 3 to about 4, about 3 to about 5, about 3 to about 6, about 3 to about 7, about 3 to about 8, about 3 to about 9, about 3 to about 10, about 4 to about 5, about 4 to about 6, about 4 to about 7, about 4 to about 8, about 4 to about 9, about 4 to about 10, about 5 to about 6, about 5 to about 7, about 5 to about 8, about 5 to about 9, about 5 to about 10, about 6 to about 7, about 6 to about 8, about 6 to about 9, about 6 to about 10, about 7 to about 8, about 7 to about 9, about 7 to about 10, about 8 to about 9, about 8 to about 10, or about 9 to about 10 chemically modified nucleotides. In some embodiments, the tracrRNA sequence may comprise a total of from about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, or about 10 chemically modified nucleotides. In some embodiments, the tracrRNA sequence may comprise a total of from at least about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, or about 9 chemically modified nucleotides. In some embodiments, the tracrRNA sequence may comprise a total of from at most about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, or about 10 chemically modified nucleotides. In some embodiments, the tracrRNA sequence may comprise a total of from about 10 to about 30 chemically modified nucleotides. In some embodiments, the tracrRNA sequence may comprise a total of from about 10 to about 15, about 10 to about 20, about 10 to about 25, about 10 to about 30, about 15 to about 20, about 15 to about 25, about 15 to about 30, about 20 to about 25, about 20 to about 30, or about 25 to about 30 chemically modified nucleotides. In some embodiments, the tracrRNA sequence may comprise a total of from about 10, about 15, about 20, about 25, or about 30 chemically modified nucleotides. In some embodiments, the tracrRNA sequence may comprise a total of from at least about 10, about 15, about 20, or about 25 chemically modified nucleotides. In some embodiments, the spacer region may comprise a total of at most about 15, about 20, about 25, or about 30 chemically modified nucleotides. In some embodiments, the tracrRNA sequence may comprise a total of from about 30 to about 130 chemically modified nucleotides. In some embodiments, the tracrRNA sequence may comprise a total of from about 30 to about 40, about 30 to about 50, about 30 to about 60, about 30 to about 70, about 30 to about 75, about 30 to about 80, about 30 to about 90, about 30 to about 100, about 30 to about 110, about 30 to about 120, about 30 to about 130, about 40 to about 50, about 40 to about 60, about 40 to about 70, about 40 to about 75, about 40 to

about 80, about 40 to about 90, about 40 to about 100, about 40 to about 110, about 40 to about 120, about 40 to about 130, about 50 to about 60, about 50 to about 70, about 50 to about 75, about 50 to about 80, about 50 to about 90, about 50 to about 100, about 50 to about 110, about 50 to about 120, about 50 to about 130, about 60 to about 70, about 60 to about 75, about 60 to about 80, about 60 to about 90, about 60 to about 100, about 60 to about 110, about 60 to about 120, about 60 to about 130, about 70 to about 75, about 70 to about 80, about 70 to about 90, about 70 to about 100, about 70 to about 110, about 70 to about 120, about 70 to about 130, about 75 to about 80, about 75 to about 90, about 75 to about 100, about 75 to about 110, about 75 to about 120, about 75 to about 130, about 80 to about 90, about 80 to about 100, about 80 to about 110, about 80 to about 120, about 80 to about 130, about 90 to about 100, about 90 to about 110, about 90 to about 120, about 90 to about 130, about 100 to about 110, about 100 to about 120, about 100 to about 130, about 110 to about 120, about 110 to about 130, or about 120 to about 130 chemically modified nucleotides. In some embodiments, the tracrRNA sequence may comprise a total of from about 30, about 40, about 50, about 60, about 70, about 75, about 80, about 90, about 100, about 110, about 120, or about 130 chemically modified nucleotides. In some embodiments, the tracrRNA sequence may comprise a total of from at least about 30, about 40, about 50, about 60, about 70, about 75, about 80, about 90, about 100, about 110, or about 120 chemically modified nucleotides. In some embodiments, the tracrRNA sequence may comprise a total of from at most about 40, about 50, about 60, about 70, about 75, about 80, about 90, about 100, about 110, about 120, or about 130 chemically modified nucleotides. In one embodiment, the tracrRNA sequence may comprise a total of 51 chemically modified nucleotides. In another embodiment, the tracrRNA sequence may comprise a total of 59 chemically modified nucleotides.

[332] In some embodiments, the tracrRNA sequence may comprise from 1% to 100% chemically modified nucleotides. In some embodiments, the tracrRNA sequence may comprise about 1 % to about 100 % chemically modified nucleotides. In some embodiments, the tracrRNA sequence may comprise about 1 % to about 10 %, about 1 % to about 20 %, about 1 % to about 30 %, about 1 % to about 40 %, about 1 % to about 50 %, about 1 % to about 60 %, about 1 % to about 70 %, about 1 % to about 80 %, about 1 % to about 90 %, about 1 % to about 95 %, about 1 % to about 100 %, about 10 % to about 20 %, about 10 % to about 30 %, about 10 % to about 40 %, about 10 % to about 50 %, about 10 % to about 60 %, about 10 % to about 70 %, about 10 % to about 80 %, about 10 % to about 90 %, about 10 % to about 95 %, about 10 % to about 100 %, about 20 % to about 30 %, about 20 % to about

40 %, about 20 % to about 50 %, about 20 % to about 60 %, about 20 % to about 70 %, about 20 % to about 80 %, about 20 % to about 90 %, about 20 % to about 95 %, about 20 % to about 100 %, about 30 % to about 40 %, about 30 % to about 50 %, about 30 % to about 60 %, about 30 % to about 70 %, about 30 % to about 80 %, about 30 % to about 90 %, about 30 % to about 95 %, about 30 % to about 100 %, about 40 % to about 50 %, about 40 % to about 60 %, about 40 % to about 70 %, about 40 % to about 80 %, about 40 % to about 90 %, about 40 % to about 95 %, about 40 % to about 100 %, about 50 % to about 60 %, about 50 % to about 70 %, about 50 % to about 80 %, about 50 % to about 90 %, about 50 % to about 95 %, about 50 % to about 100 %, about 60 % to about 70 %, about 60 % to about 80 %, about 60 % to about 90 %, about 60 % to about 95 %, about 60 % to about 100 %, about 70 % to about 80 %, about 70 % to about 90 %, about 70 % to about 95 %, about 70 % to about 100 %, about 80 % to about 90 %, about 80 % to about 95 %, about 80 % to about 100 %, about 90 % to about 95 %, about 90 % to about 100 %, or about 95 % to about 100 % chemically modified nucleotides. In some embodiments, the tracrRNA sequence may comprise about 1 %, about 10 %, about 20 %, about 30 %, about 40 %, about 50 %, about 60 %, about 70 %, about 80 %, about 90 %, about 95 %, or about 100 % chemically modified nucleotides. In some embodiments, the tracrRNA sequence may comprise at least about 1 %, about 10 %, about 20 %, about 30 %, about 40 %, about 50 %, about 60 %, about 70 %, about 80 %, about 90 %, or about 95 % chemically modified nucleotides. In some embodiments, the tracrRNA sequence may comprise at most about 10 %, about 20 %, about 30 %, about 40 %, about 50 %, about 60 %, about 70 %, about 80 %, about 90 %, about 95 %, or about 100 % chemically modified nucleotides.

[333] In some embodiments, the chemically modified tracrRNA sequence may have chemically modified nucleotide on one or more of the positions 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, or 130 in the 5’ – 3’ direction in the tracrRNA sequence.

[334] In some embodiments, the chemically modified tracr region may have chemically modified nucleotide on one or more of the positions which are 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61,

62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, or 129 base pairs from the 5' end of the tracrRNA sequence.

[335] In some embodiments, the chemically modified tracr region may have chemically modified nucleotide on one or more the positions which are 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, or 129 base pairs from the 3' end of the tracrRNA sequence.

[336] In some embodiments, the chemically modified gRNAs may have one or more chemical modifications in the 5' end of the gRNA sequence. In some embodiments, the chemically modified gRNAs may comprise one or more chemically modified nucleotides in the 5' end of the gRNA sequence. In some embodiments, the chemically modified gRNAs may have one or more chemical modifications in the 3' end of the gRNA sequence. In some embodiments, the chemically modified gRNAs may comprise one or more chemically modified nucleotides in the 3' end of the gRNA sequence.

[337] In some embodiments, the chemically modified gRNAs may comprise a crRNA. In some embodiments, the crRNA may comprise one or more chemically modified nucleotides. In some embodiments, the chemically modified crRNA may be of from 30 to 50 base pairs in length. In some embodiments, the chemically modified crRNA may be of 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 base pairs in length. In some embodiments, the chemically modified crRNA may be of from about 30 to about 50 base pairs in length. In some embodiments, the chemically modified crRNA may be of from about 30 to about 32, about 30 to about 34, about 30 to about 36, about 30 to about 38, about 30 to about 40, about 30 to about 42, about 30 to about 44, about 30 to about 46, about 30 to about 48, about 30 to about 50, about 32 to about 34, about 32 to about 36, about 32 to about 38, about 32 to about 40, about 32 to about 42, about 32 to about 44, about 32 to about 46, about 32 to about 48, about 32 to about 50, about 34 to about 36, about 34 to about 38, about 34 to about 40, about 34 to about 42, about 34 to about 44, about 34 to about 46, about 34 to about 48, about 34 to about 50, about 36 to about 38, about 36 to about 40, about 36 to about 42, about 36 to about 44, about 36 to about 46, about 36 to about 48, about 36 to about 50, about

38 to about 40, about 38 to about 42, about 38 to about 44, about 38 to about 46, about 38 to about 48, about 38 to about 50, about 40 to about 42, about 40 to about 44, about 40 to about 46, about 40 to about 48, about 40 to about 50, about 42 to about 44, about 42 to about 46, about 42 to about 48, about 42 to about 50, about 44 to about 46, about 44 to about 48, about 44 to about 50, about 46 to about 48, about 46 to about 50, or about 48 to about 50 base pairs in length. In some embodiments, the chemically modified crRNA may be of from about 30, about 32, about 34, about 36, about 38, about 40, about 42, about 44, about 46, about 48, or about 50 base pairs in length. In some embodiments, the chemically modified crRNA may be of from at least about 30, about 32, about 34, about 36, about 38, about 40, about 42, about 44, about 46, or about 48 base pairs in length. In some embodiments, the chemically modified crRNA may be of from at most about 32, about 34, about 36, about 38, about 40, about 42, about 44, about 46, about 48, or about 50 base pairs in length.

[338] In some embodiments, the crRNA may comprise a total of from 1 to 50 chemically modified nucleotides. In some embodiments, the chemically modified crRNA may comprise a total of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 chemically modified nucleotides. In some embodiments, the chemically modified crRNA may comprise a total of about 1 to about 10 chemically modified nucleotides. In some embodiments, the chemically modified crRNA may comprise a total of about 1 to about 2, about 1 to about 3, about 1 to about 4, about 1 to about 5, about 1 to about 6, about 1 to about 7, about 1 to about 8, about 1 to about 9, about 1 to about 10, about 2 to about 3, about 2 to about 4, about 2 to about 5, about 2 to about 6, about 2 to about 7, about 2 to about 8, about 2 to about 9, about 2 to about 10, about 3 to about 4, about 3 to about 5, about 3 to about 6, about 3 to about 7, about 3 to about 8, about 3 to about 9, about 3 to about 10, about 4 to about 5, about 4 to about 6, about 4 to about 7, about 4 to about 8, about 4 to about 9, about 4 to about 10, about 5 to about 6, about 5 to about 7, about 5 to about 8, about 5 to about 9, about 5 to about 10, about 6 to about 7, about 6 to about 8, about 6 to about 9, about 6 to about 10, about 7 to about 8, about 7 to about 9, about 7 to about 10, about 8 to about 9, about 8 to about 10, or about 9 to about 10 chemically modified nucleotides. In some embodiments, the chemically modified crRNA may comprise a total of about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, or about 10 chemically modified nucleotides. In some embodiments, the chemically modified crRNA may comprise a total of at least about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, or about 9 chemically modified nucleotides. In some embodiments, the chemically modified crRNA may comprise a total of

at most about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, or about 10 chemically modified nucleotides. In some embodiments, the chemically modified crRNA may comprise a total of about 10 to about 30 chemically modified nucleotides. In some embodiments, the chemically modified crRNA may comprise a total of about 10 to about 15, about 10 to about 20, about 10 to about 25, about 10 to about 30, about 15 to about 20, about 15 to about 25, about 15 to about 30, about 20 to about 25, about 20 to about 30, or about 25 to about 30 chemically modified nucleotides. In some embodiments, the chemically modified crRNA may comprise a total of about 10, about 15, about 20, about 25, or about 30 chemically modified nucleotides. In some embodiments, the chemically modified crRNA may comprise a total of at least about 10, about 15, about 20, or about 25 chemically modified nucleotides. In some embodiments, the chemically modified crRNA may comprise a total of at most about 15, about 20, about 25, or about 30 chemically modified nucleotides. In some embodiments, the chemically modified crRNA may comprise a total of from about 30 to about 50 chemically modified nucleotides. In some embodiments, the chemically modified crRNA may comprise a total of from about 30 to about 32, about 30 to about 34, about 30 to about 36, about 30 to about 38, about 30 to about 40, about 30 to about 42, about 30 to about 44, about 30 to about 46, about 30 to about 48, about 30 to about 50, about 32 to about 34, about 32 to about 36, about 32 to about 38, about 32 to about 40, about 32 to about 42, about 32 to about 44, about 32 to about 46, about 32 to about 48, about 32 to about 50, about 34 to about 36, about 34 to about 38, about 34 to about 40, about 34 to about 42, about 34 to about 44, about 34 to about 46, about 34 to about 48, about 34 to about 50, about 36 to about 38, about 36 to about 40, about 36 to about 42, about 36 to about 44, about 36 to about 46, about 36 to about 48, about 36 to about 50, about 38 to about 40, about 38 to about 42, about 38 to about 44, about 38 to about 46, about 38 to about 48, about 38 to about 50, about 40 to about 42, about 40 to about 44, about 40 to about 46, about 40 to about 48, about 40 to about 50, about 42 to about 44, about 42 to about 46, about 42 to about 48, about 42 to about 50, about 44 to about 46, about 44 to about 48, about 44 to about 50, about 46 to about 48, about 46 to about 50, or about 48 to about 50 chemically modified nucleotides. In some embodiments, the chemically modified crRNA may comprise a total of from about 30, about 32, about 34, about 36, about 38, about 40, about 42, about 44, about 46, about 48, or about 50 chemically modified nucleotides. In some embodiments, the chemically modified crRNA may comprise a total of from at least about 30, about 32, about 34, about 36, about 38, about 40, about 42, about 44, about 46, or about 48 chemically modified nucleotides. In some embodiments, the chemically modified crRNA may comprise a total of from at most about

32, about 34, about 36, about 38, about 40, about 42, about 44, about 46, about 48, or about 50 chemically modified nucleotides.

[339] In some embodiments, the crRNA may comprise from 1% to 100% chemically modified nucleotides. In some embodiments, the crRNA may comprise about 1 % to about 100 % chemically modified nucleotides. In some embodiments, the crRNA may comprise about 1 % to about 10 %, about 1 % to about 20 %, about 1 % to about 30 %, about 1 % to about 40 %, about 1 % to about 50 %, about 1 % to about 60 %, about 1 % to about 70 %, about 1 % to about 80 %, about 1 % to about 90 %, about 1 % to about 95 %, about 1 % to about 100 %, about 10 % to about 20 %, about 10 % to about 30 %, about 10 % to about 40 %, about 10 % to about 50 %, about 10 % to about 60 %, about 10 % to about 70 %, about 10 % to about 80 %, about 10 % to about 90 %, about 10 % to about 95 %, about 10 % to about 100 %, about 20 % to about 30 %, about 20 % to about 40 %, about 20 % to about 50 %, about 20 % to about 60 %, about 20 % to about 70 %, about 20 % to about 80 %, about 20 % to about 90 %, about 20 % to about 95 %, about 20 % to about 100 %, about 30 % to about 40 %, about 30 % to about 50 %, about 30 % to about 60 %, about 30 % to about 70 %, about 30 % to about 80 %, about 30 % to about 90 %, about 30 % to about 95 %, about 30 % to about 100 %, about 40 % to about 50 %, about 40 % to about 60 %, about 40 % to about 70 %, about 40 % to about 80 %, about 40 % to about 90 %, about 40 % to about 95 %, about 40 % to about 100 %, about 50 % to about 60 %, about 50 % to about 70 %, about 50 % to about 80 %, about 50 % to about 90 %, about 50 % to about 95 %, about 50 % to about 100 %, about 60 % to about 70 %, about 60 % to about 80 %, about 60 % to about 90 %, about 60 % to about 95 %, about 60 % to about 100 %, about 70 % to about 80 %, about 70 % to about 90 %, about 70 % to about 95 %, about 70 % to about 100 %, about 80 % to about 90 %, about 80 % to about 95 %, about 80 % to about 100 %, about 90 % to about 95 %, about 90 % to about 100 %, or about 95 % to about 100 % chemically modified nucleotides. In some embodiments, the crRNA may comprise about 1 %, about 10 %, about 20 %, about 30 %, about 40 %, about 50 %, about 60 %, about 70 %, about 80 %, about 90 %, about 95 %, or about 100 % chemically modified nucleotides. In some embodiments, the crRNA may comprise at least about 1 %, about 10 %, about 20 %, about 30 %, about 40 %, about 50 %, about 60 %, about 70 %, about 80 %, about 90 %, or about 95 % chemically modified nucleotides. In some embodiments, the crRNA may comprise at most about 10 %, about 20 %, about 30 %, about 40 %, about 50 %, about 60 %, about 70 %, about 80 %, about 90 %, about 95 %, or about 100 % chemically modified nucleotides.

[340] In some embodiments, the chemically modified crRNA may have chemically modified nucleotide on one or more of the positions 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 in the 5' – 3' direction in the crRNA sequence.

[341] In some embodiments, the chemically modified crRNA may have chemically modified nucleotide on one or more of the positions which are 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, or 49 base pairs from the 5' end of the crRNA sequence.

[342] In some embodiments, the chemically modified crRNA may have chemically modified nucleotide on one or more of the positions which are 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, or 49 base pairs from the 3' end of the crRNA sequence.

[343] In some embodiments, the chemically modified gRNAs may comprise a tracrRNA. In some embodiments, the tracrRNA may comprise one or more chemically modified nucleotides. In some embodiments, the chemically modified tracrRNA may comprise a total of from 50 to 130 base pairs in length. In some embodiments, the chemically modified tracrRNA may comprise a total of 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, or 130 base pairs in length. In some embodiments, the chemically modified tracrRNA may comprise a total of about 30 to about 130 base pairs in length. In some embodiments, the chemically modified tracrRNA may comprise a total of about 30 to about 40, about 30 to about 50, about 30 to about 60, about 30 to about 70, about 30 to about 75, about 30 to about 80, about 30 to about 90, about 30 to about 100, about 30 to about 110, about 30 to about 120, about 30 to about 130, about 40 to about 50, about 40 to about 60, about 40 to about 70, about 40 to about 75, about 40 to about 80, about 40 to about 90, about 40 to about 100, about 40 to about 110, about 40 to about 120, about 40 to about 130, about 50 to about 60, about 50 to about 70, about 50 to about 75, about 50 to about 80, about 50 to about 90, about 50 to about 100, about 50 to about 110, about 50 to about 120, about 50 to about 130, about 60 to about 70, about 60 to about 75, about 60 to about 80, about 60 to about 90, about 60 to about 100, about 60 to

about 110, about 60 to about 120, about 60 to about 130, about 70 to about 75, about 70 to about 80, about 70 to about 90, about 70 to about 100, about 70 to about 110, about 70 to about 120, about 70 to about 130, about 75 to about 80, about 75 to about 90, about 75 to about 100, about 75 to about 110, about 75 to about 120, about 75 to about 130, about 80 to about 90, about 80 to about 100, about 80 to about 110, about 80 to about 120, about 80 to about 130, about 90 to about 100, about 90 to about 110, about 90 to about 120, about 90 to about 130, about 100 to about 110, about 100 to about 120, about 100 to about 130, about 110 to about 120, about 110 to about 130, or about 120 to about 130 base pairs in length. In some embodiments, the chemically modified tracrRNA may comprise a total of about 30, about 40, about 50, about 60, about 70, about 75, about 80, about 90, about 100, about 110, about 120, or about 130 base pairs in length. In some embodiments, the chemically modified tracrRNA may comprise a total of at least about 30, about 40, about 50, about 60, about 70, about 75, about 80, about 90, about 100, about 110, or about 120 base pairs in length. In some embodiments, the chemically modified tracrRNA may comprise a total of at most about 40, about 50, about 60, about 70, about 75, about 80, about 90, about 100, about 110, about 120, or about 130 base pairs in length.

[344] In some embodiments, the chemically modified tracrRNA may comprise a total of from 1 to 130 chemically modified nucleotides. In some embodiments, the chemically modified tracrRNA may comprise a total of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, or 130 chemically modified nucleotides. In some embodiments, the chemically modified tracrRNA may comprise a total of about 1 to about 10 chemically modified nucleotides. In some embodiments, the chemically modified tracrRNA may comprise a total of about 1 to about 2, about 1 to about 3, about 1 to about 4, about 1 to about 5, about 1 to about 6, about 1 to about 7, about 1 to about 8, about 1 to about 9, about 1 to about 10, about 2 to about 3, about 2 to about 4, about 2 to about 5, about 2 to about 6, about 2 to about 7, about 2 to about 8, about 2 to about 9, about 2 to about 10, about 3 to about 4, about 3 to about 5, about 3 to about 6, about 3 to about 7, about 3 to about 8, about 3 to about 9, about 3 to about 10, about 4 to about 5, about 4 to about 6, about 4 to about 7, about 4 to about 8, about 4 to about 9, about 4 to about 10, about 5 to about 6, about 5 to about 7, about 5 to about 8, about 5 to

about 9, about 5 to about 10, about 6 to about 7, about 6 to about 8, about 6 to about 9, about 6 to about 10, about 7 to about 8, about 7 to about 9, about 7 to about 10, about 8 to about 9, about 8 to about 10, or about 9 to about 10 chemically modified nucleotides. In some embodiments, the chemically modified tracrRNA may comprise a total of about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, or about 10 chemically modified nucleotides. In some embodiments, the chemically modified tracrRNA may comprise a total of at least about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, or about 9 chemically modified nucleotides. In some embodiments, the chemically modified tracrRNA may comprise a total of at most about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, or about 10 chemically modified nucleotides. In some embodiments, the chemically modified tracrRNA may comprise a total of about 10 to about 30 chemically modified nucleotides. In some embodiments, the chemically modified tracrRNA may comprise a total of about 10 to about 15, about 10 to about 20, about 10 to about 25, about 10 to about 30, about 15 to about 20, about 15 to about 25, about 15 to about 30, about 20 to about 25, about 20 to about 30, or about 25 to about 30 chemically modified nucleotides. In some embodiments, the chemically modified tracrRNA may comprise a total of about 10, about 15, about 20, about 25, or about 30 chemically modified nucleotides. In some embodiments, the chemically modified tracrRNA may comprise a total of at least about 10, about 15, about 20, or about 25 chemically modified nucleotides. In some embodiments, the spacer region may comprise a total of at most about 15, about 20, about 25, or about 30 chemically modified nucleotides. In some embodiments, the chemically modified tracrRNA may comprise a total of about 30 to about 130 chemically modified nucleotides. In some embodiments, the chemically modified tracrRNA may comprise a total of about 30 to about 40, about 30 to about 50, about 30 to about 60, about 30 to about 70, about 30 to about 75, about 30 to about 80, about 30 to about 90, about 30 to about 100, about 30 to about 110, about 30 to about 120, about 30 to about 130, about 40 to about 50, about 40 to about 60, about 40 to about 70, about 40 to about 75, about 40 to about 80, about 40 to about 90, about 40 to about 100, about 40 to about 110, about 40 to about 120, about 40 to about 130, about 50 to about 60, about 50 to about 70, about 50 to about 75, about 50 to about 80, about 50 to about 90, about 50 to about 100, about 50 to about 110, about 50 to about 120, about 50 to about 130, about 60 to about 70, about 60 to about 75, about 60 to about 80, about 60 to about 90, about 60 to about 100, about 60 to about 110, about 60 to about 120, about 60 to about 130, about 70 to about 75, about 70 to about 80, about 70 to about 90, about 70 to about 100, about 70 to about 110, about 70 to about 120, about 70 to about 130, about 75 to about 80, about 75 to about 90,

about 75 to about 100, about 75 to about 110, about 75 to about 120, about 75 to about 130, about 80 to about 90, about 80 to about 100, about 80 to about 110, about 80 to about 120, about 80 to about 130, about 90 to about 100, about 90 to about 110, about 90 to about 120, about 90 to about 130, about 100 to about 110, about 100 to about 120, about 100 to about 130, about 110 to about 120, about 110 to about 130, or about 120 to about 130 chemically modified nucleotides. In some embodiments, the chemically modified tracrRNA may comprise a total of about 30, about 40, about 50, about 60, about 70, about 75, about 80, about 90, about 100, about 110, about 120, or about 130 chemically modified nucleotides. In some embodiments, the chemically modified tracrRNA may comprise a total of at least about 30, about 40, about 50, about 60, about 70, about 75, about 80, about 90, about 100, about 110, or about 120 chemically modified nucleotides. In some embodiments, the chemically modified tracrRNA may comprise a total of at most about 40, about 50, about 60, about 70, about 75, about 80, about 90, about 100, about 110, about 120, or about 130 chemically modified nucleotides.

[345] In some embodiments, the chemically modified tracrRNA may comprise about 1 % to about 100 % chemically modified nucleotides. In some embodiments, the chemically modified tracrRNA may comprise about 1 % to about 10 %, about 1 % to about 20 %, about 1 % to about 30 %, about 1 % to about 40 %, about 1 % to about 50 %, about 1 % to about 60 %, about 1 % to about 70 %, about 1 % to about 80 %, about 1 % to about 90 %, about 1 % to about 95 %, about 1 % to about 100 %, about 10 % to about 20 %, about 10 % to about 30 %, about 10 % to about 40 %, about 10 % to about 50 %, about 10 % to about 60 %, about 10 % to about 70 %, about 10 % to about 80 %, about 10 % to about 90 %, about 10 % to about 95 %, about 10 % to about 100 %, about 20 % to about 30 %, about 20 % to about 40 %, about 20 % to about 50 %, about 20 % to about 60 %, about 20 % to about 70 %, about 20 % to about 80 %, about 20 % to about 90 %, about 20 % to about 95 %, about 20 % to about 100 %, about 30 % to about 40 %, about 30 % to about 50 %, about 30 % to about 60 %, about 30 % to about 70 %, about 30 % to about 80 %, about 30 % to about 90 %, about 30 % to about 95 %, about 30 % to about 100 %, about 40 % to about 50 %, about 40 % to about 60 %, about 40 % to about 70 %, about 40 % to about 80 %, about 40 % to about 90 %, about 40 % to about 95 %, about 40 % to about 100 %, about 50 % to about 60 %, about 50 % to about 70 %, about 50 % to about 80 %, about 50 % to about 90 %, about 50 % to about 95 %, about 50 % to about 100 %, about 60 % to about 70 %, about 60 % to about 80 %, about 60 % to about 90 %, about 60 % to about 95 %, about 60 % to about 100 %, about 70 % to about 80 %, about 70 % to about 90 %, about 70 % to about 95 %, about 70 % to about 100 %, about

80 % to about 90 %, about 80 % to about 95 %, about 80 % to about 100 %, about 90 % to about 95 %, about 90 % to about 100 %, or about 95 % to about 100 % chemically modified nucleotides. In some embodiments, the chemically modified tracrRNA may comprise about 1 %, about 10 %, about 20 %, about 30 %, about 40 %, about 50 %, about 60 %, about 70 %, about 80 %, about 90 %, about 95 %, or about 100 % chemically modified nucleotides. In some embodiments, the chemically modified tracrRNA may comprise at least about 1 %, about 10 %, about 20 %, about 30 %, about 40 %, about 50 %, about 60 %, about 70 %, about 80 %, about 90 %, or about 95 % chemically modified nucleotides. In some embodiments, the chemically modified tracrRNA may comprise at most about 10 %, about 20 %, about 30 %, about 40 %, about 50 %, about 60 %, about 70 %, about 80 %, about 90 %, about 95 %, or about 100 % chemically modified nucleotides.

[346] In some embodiments, the chemically modified tracrRNA may have chemically modified nucleotide on one or more of the positions 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, or 130 in the 5’ – 3’ direction in the tracrRNA sequence.

[347] In some embodiments, the chemically modified tracrRNA may have chemically modified nucleotide on one or more of the positions 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, or 129 base pairs from the 5’ end of the tracrRNA sequence.

[348] In some embodiments, the chemically modified tracrRNA may have chemically modified nucleotide on one or more of the positions 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110,

111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, or 129 base pairs from the 3' end of the tracrRNA sequence.

[349] In some embodiments, the chemically modified gRNA or sgRNA comprises phosphorothioate (PS) linkages at the 5' or 3' end of the gRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 0, 1, 2, 3, 4, or 5 PS linkages at the 5' end of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 0, 1, 2, 3, 4, or 5 PS linkages at the 3' end of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 0, 1, 2, 3, 4, or 5 PS linkages, or any combinations thereof at each of 5' and 3' end of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 0, 1, 2 or 3 PS linkages, or any combinations thereof at each of 5' and 3' end of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 1 PS linkage at each of 5' and 3' end of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 2 PS linkages at each of 5' and 3' end of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 PS linkages at each of 5' and 3' end of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 4 PS linkages at each of 5' and 3' end of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 5 PS linkages at each of 5' and 3' end of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 PS linkages at the 5' end and 0 PS linkage (i.e., no modification) at the 3' end of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 PS linkages at the 5' end and 1 PS linkage at the 3' end of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 PS linkages at the 5' end and 2 PS linkages at the 3' end of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 PS linkages at the 5' end and 4 PS linkages at the 3' end of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 PS linkages at the 5' end and 5 PS linkages at the 3' end of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 2 PS linkages at the 5' end and 0 PS linkage (i.e., no modification) at the 3' end of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 2 PS linkages at the 5' end and 1 PS linkage at the 3' end of the gRNA or sgRNA sequence. In some embodiments,

the chemically modified gRNA or sgRNA comprises 2 PS linkages at the 5' end and 3 PS linkages at the 3' end of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 2 PS linkages at the 5' end and 4 PS linkages at the 3' end of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 2 PS linkages at the 5' end and 5 PS linkages at the 3' end of the gRNA or sgRNA sequence.

[350] In some embodiments, the chemically modified gRNA or sgRNA comprises 2 and no more than 2 contiguous phosphorothioate (PS) linkages at the 5' end. In some embodiments, the chemically modified gRNA or sgRNA comprises 2 and no more than 2 contiguous phosphorothioate (PS) linkages at the 3' end. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 contiguous phosphorothioate (PS) linkages at the 5' end. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 contiguous phosphorothioate (PS) linkages at the 3' end. In some embodiments, the chemically modified gRNA or sgRNA comprises the sequence 5'- UsUsU-3' at the 3'end or at the 5' end, wherein U indicates a uridine and wherein s indicates a phosphorothioate (PS) linkage. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 phosphorothioate (PS) linkages at the 5' end. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 phosphorothioate (PS) linkages at the 3' end. In some embodiments, the chemically modified gRNA or sgRNA comprises the two phosphorothioate linkages at the 5' end, wherein the two phosphorothioate (PS) linkages are two contiguous phosphorothioate (PS) linkages at the first two nucleotide positions of the 5' end. In some embodiments, the chemically modified gRNA or sgRNA comprises the two phosphorothioate linkages at the 5' end, wherein the two phosphorothioate (PS) linkages are within the first 3-10 nucleotides of the 5' end. In some embodiments, the chemically modified gRNA or sgRNA comprises the two phosphorothioate (PS) linkages at the 3' end, wherein the two phosphorothioate (PS) linkages are two contiguous phosphorothioate (PS) linkages at the first two nucleotide positions of the 3' end. In some embodiments, the chemically modified gRNA or sgRNA comprises the two phosphorothioate (PS) linkages at the 3' end, wherein the two phosphorothioate (PS) linkages are within the first 3-10 nucleotides of the 3' end. In some embodiments, the chemically modified gRNA or sgRNA comprises the sequence 5'-UsUsUs-3' at the 3' end, wherein U indicates a uridine and s indicates a phosphorothioate (PS) linkage. In some embodiments, the chemically modified gRNA or sgRNA comprises the sequence 5'-UsUsU-3' at the 3'end, wherein U indicates a uridine and s indicates a phosphorothioate (PS) linkage.

[351] In some embodiments, the chemically modified gRNA or sgRNA comprises PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 1 PS linkage at the internal position of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 2 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 4 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 5 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 6 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 7 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 8 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 9 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 10 PS linkages at the internal positions of the gRNA or sgRNA sequence.

[352] In some embodiments, the chemically modified gRNA or sgRNA comprises PS linkages at the 5’ end, the 3’ end, or at the internal positions, or any combination thereof, of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 PS linkages at the 5’end, the 3’ end, or at the internal positions, or any combination thereof, of the gRNA or sgRNA sequence.

[353] In some embodiments, the chemically modified gRNA or sgRNA comprises 2 PS linkages at the 5’ end, 3 PS linkages at the 3’ end, and 0 PS linkage (i.e., no modification) at the internal position of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 2 PS linkages at the 5’ end, 3 PS linkages at the 3’ end, and 1 PS linkage at the internal position of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 2 PS linkages at the 5’ end, 3 PS linkages at the 3’ end, and 2 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA

comprises 2 PS linkages at the 5' end, 3 PS linkages at the 3' end, and 3 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 2 PS linkages at the 5' end, 3 PS linkages at the 3' end, and 4 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 2 PS linkages at the 5' end, 3 PS linkages at the 3' end, and 5 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 2 PS linkages at the 5' end, 3 PS linkages at the 3' end, and 6 PS linkages at the internal positions of the gRNA or sgRNA sequence.

[354] In some embodiments, the chemically modified gRNA or sgRNA comprises 3 PS linkages at the 5' end, 2 PS linkages at the 3' end, and 0 PS linkage (i.e., no modification) at the internal position of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 PS linkages at the 5' end, 2 PS linkages at the 3' end, and 1 PS linkage at the internal position of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 PS linkages at the 5' end, 2 PS linkages at the 3' end, and 2 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 PS linkages at the 5' end, 2 PS linkages at the 3' end, and 3 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 PS linkages at the 5' end, 2 PS linkages at the 3' end, and 4 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 PS linkages at the 5' end, 2 PS linkages at the 3' end, and 5 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 PS linkages at the 5' end, 2 PS linkages at the 3' end, and 6 PS linkages at the internal positions of the gRNA or sgRNA sequence.

[355] In some embodiments, the chemically modified gRNA or sgRNA comprises 2 PS linkages at the 5' end, 2 PS linkages at the 3' end, and 0 PS linkage (i.e., no modification) at the internal position of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 2 PS linkages at the 5' end and 2 PS linkages at the 3' end and 1 PS linkage at the internal position of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 2 PS linkages at the 5' end and 2 PS linkages at the 3' end and 2 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA

comprises 2 PS linkages at the 5' end and 2 PS linkages at the 3' end and 3 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 2 PS linkages at the 5' end and 2 PS linkages at the 3' end and 4 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 2 PS linkages at the 5' end and 2 PS linkages at the 3' end and 5 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 2 PS linkages at the 5' end and 2 PS linkages at the 3' end and 6 PS linkages at the internal positions of the gRNA or sgRNA sequence.

[356] In some embodiments, the chemically modified gRNA or sgRNA comprises 3 PS linkages at the 5' end, 3 PS linkages at the 3' end, and 0 PS linkage (i.e., no modification) at the internal position of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 PS linkages at the 5' end and 3 PS linkages at the 3' end and 1 PS linkage at the internal position of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 PS linkages at the 5' end and 3 PS linkages at the 3' end and 2 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 PS linkages at the 5' end and 3 PS linkages at the 3' end and 3 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 PS linkages at the 5' end and 3 PS linkages at the 3' end and 4 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 PS linkages at the 5' end and 3 PS linkages at the 3' end and 5 PS linkages at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 PS linkages at the 5' end and 3 PS linkages at the 3' end and 6 PS linkages at the internal positions of the gRNA or sgRNA sequence.

[357] In some embodiments, the chemically modified gRNA or sgRNA comprises additional modified or unmodified nucleotide (N) with phosphodiester linkage, wherein N is an A, C, G, U, dA (deoxyA), dC (deoxyC), dG (deoxyG), or T, and any combinations thereof. In some embodiments, the chemically modified gRNA or sgRNA comprises 1, 2, 3, 4, or 5 additional N with phosphodiester linkage, wherein N is an A, G, U, dA, dG, dC, or T, and any combinations thereof. In some embodiments, the chemically modified gRNA or sgRNA comprises 1, 2, 3, 4, or 5 additional N with phosphodiester linkage at the 5' or 3' end. In one embodiment, the chemically modified gRNA or sgRNA comprises 1, 2, 3, 4, or 5

additional N with phosphodiester linkage at the 5' end. In a preferred embodiment, the chemically modified gRNA or sgRNA comprises 1, 2, 3, 4, or 5 additional N with phosphodiester linkage at the 3' end. In some embodiments, the chemically modified gRNA or sgRNA comprises 1, 2, 3, 4, or 5 additional N with phosphodiester linkage and each N is the same modified or unmodified nucleotide. For example, the chemically modified gRNA or sgRNA may comprise 4 additional N with phosphodiester linkage and each N of the 4 additional N is A, C, G, U, dA, dC, dG, or T. For example, the chemically modified gRNA or sgRNA may comprise 4 additional N with phosphodiester linkage and each N of the 4 additional N is A. For example, the chemically modified gRNA or sgRNA may comprise 3 additional N with phosphodiester linkage and each N of the 3 additional N is A, C, G, U, dA, dC, dG, or T. For example, the chemically modified gRNA or sgRNA may comprise 3 additional N with phosphodiester linkage and each N of the 3 additional N is A. For example, the chemically modified gRNA or sgRNA may comprise 3 additional N with phosphodiester linkage and each N of the 3 additional N is C. For example, the chemically modified gRNA or sgRNA may comprise 3 additional N with phosphodiester linkage and each N of the 3 additional N is G. For example, the chemically modified gRNA or sgRNA may comprise 3 additional N with phosphodiester linkage and each N of the 3 additional N is U. For example, the chemically modified gRNA or sgRNA may comprise 3 additional N with phosphodiester linkage and each N of the 3 additional N is dA. For example, the chemically modified gRNA or sgRNA may comprise 3 additional N with phosphodiester linkage and each N of the 3 additional N is dC. For example, the chemically modified gRNA or sgRNA may comprise 3 additional N with phosphodiester linkage and each N of the 3 additional N is dG. For example, the chemically modified gRNA or sgRNA may comprise 3 additional N with phosphodiester linkage and each N of the 3 additional N is T.

[358] In some embodiments, the chemically modified gRNA or sgRNA comprises additional Uracil (U) with phosphodiester linkage at the 5' or 3' end. In one embodiment, the chemically modified gRNA or sgRNA comprises additional U with phosphodiester linkage at the 5' end. In a preferred embodiment, the chemically modified gRNA or sgRNA comprises additional U with phosphodiester linkage at the 3' end. In some embodiments, the chemically modified gRNA or sgRNA comprises 1, 2, 3, 4, or 5 additional U with phosphodiester linkage at the 5' or 3' end. In some embodiments, the chemically modified gRNA or sgRNA comprises 1 additional U with phosphodiester linkage at the 5' end. In some embodiments, the chemically modified gRNA or sgRNA comprises 1 additional U with phosphodiester linkage at the 3' end. In some embodiments, the chemically modified gRNA or sgRNA

comprises 2 additional U with phosphodiester linkage at the 5' end. In some embodiments, the chemically modified gRNA or sgRNA comprises 2 additional U with phosphodiester linkage at the 3' end. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 additional U with phosphodiester linkage at the 5' end. In a preferred embodiment, the chemically modified gRNA or sgRNA comprises 3 additional U with phosphodiester linkage at the 3' end. In some embodiments, the chemically modified gRNA or sgRNA comprises 4 additional U with phosphodiester linkage at the 5' end. In some embodiments, the chemically modified gRNA or sgRNA comprises 4 additional U with phosphodiester linkage at the 3' end. In some embodiments, the chemically modified gRNA or sgRNA comprises 5 additional U with phosphodiester linkage at the 5' end. In some embodiments, the chemically modified gRNA or sgRNA comprises 5 additional U with phosphodiester linkage at the 3' end.

[359] In some embodiments, the chemically modified gRNA or sgRNA comprises a ribonebularine (depicted as "X" in this application, e.g., in Table 1 or Table 24). In some embodiments, the chemically modified gRNA or sgRNA comprises 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 ribonebularines. In some embodiments, the chemically modified gRNA or sgRNA does not comprise ribonebularine. In some embodiments, the chemically modified gRNA or sgRNA comprises 1 ribonebularine. In some embodiments, the chemically modified gRNA or sgRNA comprises 2 ribonebularines. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 ribonebularines. In some embodiments, the chemically modified gRNA or sgRNA comprises 4 ribonebularines. In some embodiments, the chemically modified gRNA or sgRNA comprises 5 ribonebularines. In some embodiments, the chemically modified gRNA or sgRNA comprises 6 ribonebularines. In some embodiments, the chemically modified gRNA or sgRNA comprises 7 ribonebularines. In some embodiments, the chemically modified gRNA or sgRNA comprises 8 ribonebularines. In some embodiments, the chemically modified gRNA or sgRNA comprises 9 ribonebularines. In some embodiments, the chemically modified gRNA or sgRNA comprises 10 ribonebularines. In some embodiments, the nebularine replaces an adenine in an unmodified gRNA or sgRNA or sgRNA or sgRNA. In some embodiments, the nebularine is in the spacer sequence. In some embodiments, the nebularine is in a tracrRNA sequence. In some embodiments, the nebularine is in a crRNA sequence in the tracrRNA sequence. In some embodiments, the nebularine is in a stem loop structure in the tracrRNA sequence.

[360] In some embodiments, the chemically modified gRNA or sgRNA comprises a 2'-O-methylnebularine (depicted as "x" in this application, e.g., in Table 1 or Table 24). In some

embodiments, the chemically modified gRNA or sgRNA comprises 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 2'-O-methylnebularines. In some embodiments, the chemically modified gRNA or sgRNA does not comprise 2'-O-methylnebularine. In some embodiments, the chemically modified gRNA or sgRNA comprises 1 2'-O-methylnebularine. In some embodiments, the chemically modified gRNA or sgRNA comprises 2 2'-O-methylnebularines. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 2'-O-methylnebularines. In some embodiments, the chemically modified gRNA or sgRNA comprises 4 2'-O-methylnebularines. In some embodiments, the chemically modified gRNA or sgRNA comprises 5 2'-O-methylnebularines. In some embodiments, the chemically modified gRNA or sgRNA comprises 6 2'-O-methylnebularines. In some embodiments, the chemically modified gRNA or sgRNA comprises 7 2'-O-methylnebularines. In some embodiments, the chemically modified gRNA or sgRNA comprises 8 2'-O-methylnebularines. In some embodiments, the chemically modified gRNA or sgRNA comprises 9 2'-O-methylnebularines. In some embodiments, the chemically modified gRNA or sgRNA comprises 10 2'-O-methylnebularines. In some embodiments, the 2'-O-methylnebularine replaces an adenine in an unmodified gRNA or sgRNA or sgRNA or sgRNA. In some embodiments, the 2'-O-methylnebularine is in the spacer sequence. In some embodiments, the 2'-O-methylnebularine is in a tracrRNA sequence. In some embodiments, the 2'-O-methylnebularine is in a crRNA sequence in the tracrRNA sequence. In some embodiments, the 2'-O-methylnebularine is in a stem loop structure in the tracrRNA sequence.

[361] In some embodiments, the chemically modified gRNA or sgRNA comprises a 2'-deoxynebularine (depicted as "dX" in this application, e.g., in Table 1 or Table 24). In some embodiments, the chemically modified gRNA or sgRNA comprises 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 2'-deoxynebularine. In some embodiments, the chemically modified gRNA or sgRNA does not comprise 2'-deoxynebularine. In some embodiments, the chemically modified gRNA or sgRNA comprises 1 2'-deoxynebularine. In some embodiments, the chemically modified gRNA or sgRNA comprises 2 2'-deoxynebularine. In some embodiments, the chemically modified gRNA or sgRNA comprises 3 2'-deoxynebularines. In some embodiments, the chemically modified gRNA or sgRNA comprises 4 2'-deoxynebularines. In some embodiments, the chemically modified gRNA or sgRNA comprises 5 2'-deoxynebularines. In some embodiments, the chemically modified gRNA or sgRNA comprises 6 2'-deoxynebularines. In some embodiments, the chemically modified gRNA or sgRNA comprises 7 2'-deoxynebularines. In some embodiments, the chemically modified

gRNA or sgRNA comprises 8 2'-deoxynebularines. In some embodiments, the chemically modified gRNA or sgRNA comprises 9 2'-deoxynebularines. In some embodiments, the chemically modified gRNA or sgRNA comprises 10 2'-deoxynebularines. In some embodiments, the 2'-deoxynebularine replaces an adenine in an unmodified gRNA or sgRNA or sgRNA or sgRNA. In some embodiments, the 2'-deoxynebularine is in the spacer sequence. In some embodiments, the 2'-deoxynebularine is in a tracrRNA sequence. In some embodiments, the 2'-deoxynebularine is in a crRNA sequence in the tracrRNA sequence. In some embodiments, the 2'-deoxynebularine is in a stem loop structure in the tracrRNA sequence.

[362] In some embodiments, the chemically modified gRNA or sgRNA comprises a 2'-O-methylribonucleotide (2'-OMe). In some embodiments, the chemically modified gRNA or sgRNA comprises from about 0 to about 70 2'-OMe. In some embodiments, the chemically modified gRNA or sgRNA comprises 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, or 70 2'-OMe. In some embodiments, the chemically modified gRNA or sgRNAs may comprise from about 0 to about 70 2'-OMe. In some embodiments, the chemically modified gRNA or sgRNAs may comprise from about 0 to about 10, about 0 to about 20, about 0 to about 30, about 0 to about 35, about 0 to about 40, about 0 to about 45, about 0 to about 50, about 0 to about 55, about 0 to about 60, about 0 to about 65, about 0 to about 70, about 10 to about 20, about 10 to about 30, about 10 to about 35, about 10 to about 40, about 10 to about 45, about 10 to about 50, about 10 to about 55, about 10 to about 60, about 10 to about 65, about 10 to about 70, about 20 to about 30, about 20 to about 35, about 20 to about 40, about 20 to about 45, about 20 to about 50, about 20 to about 55, about 20 to about 60, about 20 to about 65, about 20 to about 70, about 30 to about 35, about 30 to about 40, about 30 to about 45, about 30 to about 50, about 30 to about 55, about 30 to about 60, about 30 to about 65, about 30 to about 70, about 35 to about 40, about 35 to about 45, about 35 to about 50, about 35 to about 55, about 35 to about 60, about 35 to about 65, about 35 to about 70, about 40 to about 45, about 40 to about 50, about 40 to about 55, about 40 to about 60, about 40 to about 65, about 40 to about 70, about 45 to about 50, about 45 to about 55, about 45 to about 60, about 45 to about 65, about 45 to about 70, about 50 to about 55, about 50 to about 60, about 50 to about 65, about 50 to about 70, about 55 to about 60, about 55 to about 65, about 55 to about 70, about 60 to about 65, about 60 to about 70, or about 65 to about 70 2'-OMe. In some embodiments, the chemically modified gRNA or sgRNAs may comprise from

about 0, about 10, about 20, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, or about 70 2'-OMe. In some embodiments, the chemically modified gRNA or sgRNAs may comprise from at least about 0, about 10, about 20, about 30, about 35, about 40, about 45, about 50, about 55, about 60, or about 65 2'-OMe. In some embodiments, the chemically modified gRNA or sgRNAs may comprise from at most about 10, about 20, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, or about 70 2'-OMe. In some embodiments, the chemically modified gRNA or sgRNA does not comprise 2'-OMe. In some embodiments, the chemically modified gRNA or sgRNA comprises 62 2'-OMe. In some embodiments, the chemically modified gRNA or sgRNA comprises 54 2'-OMe.

[363] In some embodiments, the chemically modified gRNA or sgRNA comprises 2'-OMe at the 5' end of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 2'-OMe at the 3' end of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 2'-OMe at the internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 2'-OMe at the 5' end, 3' end, or internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA comprises 2'-OMe at the 5' end, 3' end, and internal positions of the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA does not comprise 2'-OMe at the positions 2, 3, 4, 23, 24, 25, 27, 31, or 42, or any combinations thereof, in the 5' – 3' direction in the gRNA or sgRNA sequence. In some embodiments, the chemically modified gRNA or sgRNA does not comprise 2'-OMe at the positions 2, 3, 4, 23, 24, 25, 27, 31, and 42 in the 5' – 3' direction in the gRNA or sgRNA sequence.

[364] An exemplary chemically modified gRNA or sgRNA sequence is shown below:

5' –

csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGU CcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususu – 3 (SEQ ID NO: 55)

wherein the uppercase A, U, G, or C denote a ribonucleotide adenosine, uridine, guanine, or cytidine, the lowercase a, u, g, and c indicate 2'-O-Methyl- modified adenine, uridine, guanine, and cytidine, and "s" denotes phosphorothioate (PS) linkage.

[365] An exemplary chemically modified spacer sequence is shown below:

5' – csasgsGUUCCAUGGGAUGCUCU – 3' (SEQ ID NO: 56)

wherein the uppercase A, U, G, or C denote a ribonucleotide adenosine, uridine, guanine, or cytidine, the lowercase a, u, g, and c indicate 2'-O-Methyl- modified adenine, uridine, guanine, and cytidine, and "s" denotes phosphorothioate (PS) linkage.

[366] An exemplary chemically modified tracrRNA sequence is shown below:
5' –
gUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcacc
gAgUCggugcusususu – 3' (SEQ ID NO: 57)
wherein the uppercase A, U, G, or C denote a ribonucleotide adenosine, uridine, guanine, or cytidine, the lowercase a, u, g, and c indicate 2'-O-Methyl- modified adenine, uridine, guanine, and cytidine, and "s" denotes phosphorothioate (PS) linkage.

[367] Another exemplary chemically modified gRNA or sgRNA sequence is shown below:
5' –
csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUcc
GUUAucAAcuugaaaaagugGcaccgagucggugcusususu – 3' (SEQ ID NO: 55)
wherein the uppercase A, U, G, or C denote a ribonucleotide adenosine, uridine, guanine, or cytidine, the lowercase a, u, g, and c indicate 2'-O-Methyl- modified adenine, uridine, guanine, and cytidine, and "s" denotes phosphorothioate (PS) linkage.

[368] Another exemplary chemically modified tracrRNA sequence is shown below:
: 5'-
gUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgag
ucggugcusususu-3' (SEQ ID NO: 57)

[369] Another exemplary chemically modified guide RNA sequence is shown below:
5' –
cscscsGCACCTTGGCGCAGCGGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAA
GGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu -3'
(SEQ ID NO: 58), wherein the uppercase A, U, G, or C denote a ribonucleotide adenosine, uridine, guanine, or cytidine, the lowercase a, u, g, and c indicate 2'-O-Methyl- modified adenine, uridine, guanine, and cytidine, and "s" denotes phosphorothioate (PS) linkage.

[370] Another exemplary chemically modified guide RNA sequence is shown below:
5' –
asasgsAUACCUGAAUAACCCUCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAA
GGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu-3'
(SEQ ID NO: 59) (GA091)

[371] Another exemplary chemically modified guide RNA sequence is shown below:
5' –
asasgsAUACCUGAAUAACCCUCGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCU
AGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu 3' (SEQ ID NO: 60) (GA098)

wherein the uppercase A, U, G, or C denote a ribonucleotide adenosine, uridine, guanine, or cytidine, the lowercase a, u, g, and c indicate 2'-O-Methyl- modified adenine, uridine, guanine, and cytidine, and "s" denotes phosphorothioate (PS) linkage.

[372] Another exemplary chemically modified guide RNA sequence is shown below:

5' –

asasgsAUACCUGAAUAACCCUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUC cGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususu-3' (SEQ ID NO: 59) (GA099)

wherein the uppercase A, U, G, or C denote a ribonucleotide adenosine, uridine, guanine, or cytidine, the lowercase a, u, g, and c indicate 2'-O-Methyl- modified adenine, uridine, guanine, and cytidine, and "s" denotes phosphorothioate (PS) linkage.

[373] Another exemplary chemically modified guide RNA sequence is shown below:

[374] 5' –

asasgsAUACCUGAAUAACCCUCgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUcc GUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3' (SEQ ID NO: 59) (GA100)

wherein the uppercase A, U, G, or C denote a ribonucleotide adenosine, uridine, guanine, or cytidine, the lowercase a, u, g, and c indicate 2'-O-Methyl- modified adenine, uridine, guanine, and cytidine, and "s" denotes phosphorothioate (PS) linkage.

[375] Another exemplary chemically modified guide RNA sequence is shown below:

5'–

cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUcc GUUAucAAcuugaaaaagugGcaccgagucggugcusususuuuu-3' (SEQ ID NO: 11) (GA385)

wherein the uppercase A, U, G, or C denote a ribonucleotide adenosine, uridine, guanine, or cytidine, the lowercase a, u, g, and c indicate 2'-O-Methyl- modified adenine, uridine, guanine, and cytidine, and "s" denotes phosphorothioate (PS) linkage.

[376] Another exemplary chemically modified guide RNA sequence is shown below:

5'–

cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUcc GUUAucAAcuugaaaaagugGcaccgagucggugcusususuUu-3' (SEQ ID NO: 11) (GA386),

wherein the uppercase A, U, G, or C denote a ribonucleotide adenosine, uridine, guanine, or cytidine, the lowercase a, u, g, and c indicate 2'-O-Methyl- modified adenine, uridine, guanine, and cytidine, and "s" denotes phosphorothioate (PS) linkage.

[377] Another exemplary chemically modified guide RNA sequence is shown below:

5'–

cscscsGCACCUUGGCGCAGCGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAuc

AAcuuGaaaaagugGcaccgagucggugcuusususuuuu-3' (SEQ ID NO: 12), (GA387)
wherein the uppercase A, U, G, or C denote a ribonucleotide adenosine, uridine, guanine, or cytidine, the lowercase a, u, g, and c indicate 2'-O-Methyl- modified adenine, uridine, guanine, and cytidine, and "s" denotes phosphorothioate (PS) linkage.

[378] Additional chemically modified guide RNA sequences and target gene information are provided in Table 1 or Table 24.

[379] In some embodiments, the chemical modification comprises a phosphorothioate linkage (PS). In some embodiments, the sgRNA comprises a phosphorothioate linkage (PS) at a 5' end or at a 3' end. In some embodiments, the sgRNA comprises two and no more than two contiguous phosphorothioate linkages (PS) at the 5' end or at the 3' end. In some embodiments, the sgRNA comprises three contiguous phosphorothioate linkages (PS) at the 5' end or at the 3' end. In some embodiments, the sgRNA comprises the sequence 5'-UsUsU-3' at the 3'end or at the 5' end, wherein U indicates a uridine and wherein s indicates a phosphorothioate linkage (PS).

[380] In some aspects, provided herein, is an sgRNA that comprises (i) a spacer sequence and (ii) a tracrRNA sequence, wherein the spacer sequence hybridizes with a target polynucleotide sequence in a PCSK9 gene or an ANGPTL3 gene when contacted with the target polynucleotide sequence, wherein the tracrRNA sequence binds a Cas protein in a base editor system when contacted with the base editor system, and wherein the sgRNA comprises a nebularine or a deoxynebularine. In some embodiments, the nebularine or the deoxynebularine replaces an adenine in an unmodified sgRNA. In some embodiments, the nebularine or the deoxynebularine is in the spacer sequence. In some embodiments, wherein the nebularine or the deoxynebularine is in the tracrRNA sequence. In some embodiments, the nebularine or the deoxynebularine is in a tracrRNA sequence. In some embodiments, the nebularine or the deoxynebularine is in a crRNA sequence in the tracrRNA sequence. In some embodiments, the nebularine or the deoxynebularine is in a stem loop structure in the tracrRNA sequence.

[381] In some aspects, provided herein, is a single guide RNA that comprises a sequence selected from Table 1 or Table 24, wherein a, u, g, and c indicate 2'-OMe modified adenine, uridine, guanine, and cytidine, wherein s indicates a phosphorothioate (PS) linkage.

[382] In some embodiments, the chemically modified guide RNA comprises an unmodified tracrRNA sequence GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUU GAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 61). In some embodiments,

the chemically modified guide RNA comprises a tracrRNA sequence GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUU GAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 61), which comprises 1 or more modification.

[383] In some embodiments, the chemically modified guide RNA comprises an oligonucleotide that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the chemically modified guide RNAs listed in Table 1 or Table 24. In some embodiments, the chemically modified guide RNA comprises any one of the chemically modified guide RNAs listed in Table 1 or Table 24. In some embodiments, the chemically modified guide RNA is any one of the chemically modified guide RNAs listed in Table 1 or Table 24. In some embodiments, the chemically modified guide RNA comprises an oligonucleotide that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or at least 99.9% identical to any one of the chemically modified guide RNAs of SEQ ID NOs: 9-11, 55, 59, 253-452, 1618-1635, 1637-1800, 1802-2135, and 2191. In some embodiments, the chemically modified guide RNA comprises any one of the chemically modified guide RNAs of SEQ ID NOs: 9-11, 55, 59, 253-452, 1618-1635, 1637-1800, 1802-2135, and 2191. In some embodiments, the chemically modified guide RNA is any one of the chemically modified guide RNAs of SEQ ID NOs: 9-11, 55, 59, 253-452, 1618-1635, 1637-1800, 1802-2135, and 2191.

Base Editing at Splice Sites

[384] In an aspect, provided herein are base editor systems and methods of using same for modifying target genes by generating a genetic alteration in the DNA sequence that occurs at the boundary of an exon and an intron (splice site) at a splice site of a target gene. In some embodiments, the base editor system modifies a nucleobase at a splice acceptor site of a target gene. In some embodiments, the base editor system modifies a nucleobase at a splice donor site of a target gene. Modification at a splice donor or splice acceptor site may result in alternated transcripts. In some embodiments, modification at a splice donor or splice acceptor site results in aberrant transcripts. In some embodiments, modification at a splice donor or splice acceptor site results in unstable transcripts that are subject to degradation upon transcription. In some embodiments, modification at a splice donor or splice acceptor site

results in a premature stop codon in the transcript. In certain embodiments, the methods comprise knocking out or knocking down genes by targeting splice acceptor-splice donor (SA-SD) sites or premature STOP (pmSTOP) sites. For such methods, guide polynucleotides are designed to disrupt one or more slice acceptor/donor sites within the target nucleotide sequence. In some embodiments, a guide polynucleotide, e.g. a single guide RNA comprises a sequence of at least 10 contiguous nucleotides or a sequence of 17-23 contiguous nucleotides, that is complementary to a target sequence in the genome of an organism and comprises a target base pair.

[385] Disruptions at SA-SD sites are particularly advantageous because one may knock out coding sequence and non-coding RNAs (ncRNAs) without stop codon read through. Efficiency of base editing can be determined on the genomic level by EditR analysis of Sanger sequencing traces or by next generation sequencing (NGS), and also on the protein level by flow cytometry. Splice acceptor-splice donor base editing gRNAs that target the splice donor regions and the splice acceptor region exhibit base conversion efficiency of at least 5% and, in some cases, at least 80% or greater (e.g., 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%). In some cases, SA-SD gRNAs are significantly more efficient at C-to-T conversions than gRNAs that introduce premature stop codons disrupting.

[386] Guide RNAs for targeting SA-SD sites can be designed using an R based program that identifies gRNAs targeting all ncRNAs and protein coding gene SA-SD sites. In some cases, the user supplies the reference genome, Ensembl transcript ID of the reference sequence, protospacer adjacent motif (PAM) site, and distance to subset upstream and downstream of exon-intron boundary. The program extracts sequences of 20 base pairs + the PAM length upstream and 15 base pairs downstream of an exon-intron boundary, as well as the splice site motif. In some embodiments, a guide molecule can be from 20 to 120 bases in length, or more. In certain embodiments, a guide molecule can be from 20 to 60 bases in length, or 20 to 50 bases, or 30 to 50 bases, or 39 to 46 bases.

[387] In some cases, it is advantageous to use chemically modified gRNAs having increased stability when transfected into mammalian cells. For example, gRNAs can be chemically modified to comprise 2'-0-methyl phosphorthioate modifications on at least one 5' nucleotide and at least one 3' nucleotide of each gRNA. In some cases, the three terminal 5' nucleotides and three terminal 3' nucleotides are chemically modified to comprise 2'-O-methyl phosphorthioate modifications.

[388] In an aspect, provided herein are base editing systems and methods for targeting diseases for base editing disruption. The target sequence can be any disease-associated polynucleotide or gene, as have been established in the art.

[389] Without being bound by a particular theory, where the goal is to disrupt a gene in vivo for a therapeutic purpose, cytosine base editors may be used to directly introduce stop codons into the coding sequence of the gene (nonsense mutations) by altering specific codons for glutamine (CAG-TAG, CAA-TAA), arginine (CGA-TGA), and tryptophan (TGG-TAG/TAA/TGA, with editing of cytosines on the antisense strand). In certain embodiments, adenine base editors may be used to disrupt gene function and/or expression by modifying nucleobases at splice sites of target genes. The more favorable off-target profile of adenine base editors, particularly with respect to gRNA-independent off-target DNA base editing, recommend the use of adenine base editors over cytosine base editors for therapeutic purposes. In some embodiments, a base editor, e.g. an adenosine nucleobase editor as describe herein may be used to disrupt a splice donors at the 5' ends of introns or splice acceptors at the 3' ends of introns. In some embodiments, splice site disruption results in the inclusion of intronic sequences in messenger RNA (mRNA)—potentially introducing nonsense, frameshift, or in-frame indel mutations that result in premature stop codons or in insertion/deletion of amino acids that disrupt protein activity—or in the exclusion of exonic sequences, which can also introduce nonsense, frameshift, or in-frame indel mutations.

[390] Canonical splice donors comprise the DNA sequence GT on the sense strand, whereas canonical splice acceptors comprise the DNA sequence AG. In some embodiments, a base editor, e.g. an adenosine nucleobase editor as described herein can be used to generate alteration of the sequence disrupts normal splicing. In some embodiments, the adenosine base editor disrupts a complementary base in the second position in the antisense strand (GT-GC). In some embodiments, the adenosine base editor disrupts the first position in the sense strand (AG-GG).

[391] Examples of useful applications of mutation or disruption of an endogenous gene sequence that reduces or abolishes expression of the target gene related to the disease, with significantly reduced off-target effect, indels frequency, and/or other unintended genetic interruptions related to double stranded breaks that result from traditional CRISPR-Cas9 nuclease modification. In some embodiments, the target gene is a PCSK9 gene. In some embodiments, the target gene is an ANGPTL3 gene.

[392] The chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the binding affinity between a programmable DNA binding component of a base

editor fusion protein, such as a Cas protein (e.g., a Cas9 protein, a nuclease inactive Cas9 protein, a Cas9 nickase) and guide RNAs (e.g., gRNAs or sgRNAs). In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the binding affinity between a Cas protein and gRNAs or sgRNAs by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 75%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 400% 500%, 600%, 700%, 800%, 900%, or 1000% compared to unmodified gRNAs or sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the binding affinity between a Cas protein, e.g. a Cas9 protein, a nuclease inactive Cas9 protein, a Cas9 nickase, and gRNAs or sgRNAs by about 20% to about 300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about 100%, about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to about 250%, about 200% to about 250%, at least about 20%, at least about 50%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% compared to unmodified gRNAs or sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the binding affinity between a base editor fusion or Cas protein and gRNAs or sgRNAs by about 20% to about 300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about 100%, about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to about 250%, about 200% to about 250%, at least about 20%, at least about 50%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% compared unmodified gRNAs or sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the binding affinity between a Cas protein or component of a base editor fusion protein and gRNAs or sgRNAs by at least 1.1–fold, 1.2–fold, 1.3–fold, 1.4–fold, 1.5–fold, 1.6–fold, 1.7–fold, 1.8–fold, 1.9–fold, 2–fold, 2.5–fold, 3–fold, 3.5–fold, 4–fold, 4.5–fold, 5–fold, 6–fold,

7–fold, 8–fold, 9–fold, 10–fold, or more than 10-fold compared to unmodified gRNAs or sgRNAs.

[393] The chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the binding affinity between a programmable DNA binding component of a base editor fusion protein, such as a Cas protein (e.g., a Cas9 protein, a nuclease inactive Cas9 protein, a Cas9 nickase) and the tracrRNA sequence of the guide RNAs (e.g., gRNAs or sgRNAs). In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the binding affinity between a base editor fusion or Cas protein and the tracrRNA sequence of the gRNAs or sgRNAs by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 75%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 400% 500%, 600%, 700%, 800%, 900%, or 1000% compared to unmodified gRNAs or sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the binding affinity between a base editor fusion or Cas protein and the tracrRNA sequence of the gRNAs or sgRNAs by about 20% to about 300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about 100%, about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to about 250%, about 200% to about 250%, at least about 20%, at least about 50%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% compared to unmodified gRNAs or sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the binding affinity between a base editor fusion or Cas protein and the tracrRNA sequence of the gRNAs or sgRNAs by about 20% to about 300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about 100%, about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to about 250%, about 200% to about 250%, at least about 20%, at least about 50%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% compared unmodified gRNAs or sgRNAs. In some embodiments, the

chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the binding affinity between a base editor fusion or Cas protein and the tracrRNA sequence of the gRNAs or sgRNAs by at least 1.1–fold, 1.2–fold, 1.3–fold, 1.4–fold, 1.5–fold, 1.6–fold, 1.7–fold, 1.8–fold, 1.9–fold, 2–fold, 2.5–fold, 3–fold, 3.5–fold, 4–fold, 4.5–fold, 5–fold, 6–fold, 7–fold, 8–fold, 9–fold, 10–fold, or more than 10-fold compared to unmodified gRNAs or sgRNAs.

[394] In some embodiments, the tracrRNA sequence of the gRNAs or sgRNAs may bind a programmable DNA binding component of a base editor fusion protein, such as a Cas protein (,) with increased binding affinity compared to a tracrRNA sequence in an unmodified sgRNA. The type II Cas protein may be used as a system or base editor as described herein. In some embodiments, the tracrRNA sequence of the gRNAs or sgRNAs may bind a Type II Cas protein with at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 75%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 400% 500%, 600%, 700%, 800%, 900%, or 1000% increased binding affinity compared to a tracrRNA sequence in an unmodified sgRNA. In some embodiments, the tracrRNA sequence of the gRNAs or sgRNAs may bind a base editor comprising a Type II Cas protein with at least 1.1–fold, 1.2–fold, 1.3–fold, 1.4–fold, 1.5–fold, 1.6–fold, 1.7–fold, 1.8–fold, 1.9–fold, 2–fold, 2.5–fold, 3–fold, 3.5–fold, 4–fold, 4.5–fold, 5–fold, 6–fold, 7–fold, 8–fold, 9–fold, 10–fold, or more than 10-fold increased binding affinity compared to a tracrRNA sequence in an unmodified sgRNA. In some embodiments, the tracrRNA sequence of the gRNAs or sgRNAs may bind a base editor fusion protein comprising a Cas9 protein with increased binding affinity compared to a tracrRNA sequence in an unmodified sgRNA. In some embodiments, the tracrRNA sequence of the gRNAs or sgRNAs may bind a Cas9 protein with at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 75%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, or 300% increased binding affinity compared to a tracrRNA sequence in an unmodified sgRNA. In some embodiments, the tracrRNA sequence of the gRNAs or sgRNAs may bind a base editor fusion protein comprising a Cas9 protein with at least 1.1–fold, 1.2–fold, 1.3–fold, 1.4–fold, 1.5–fold, 1.6–fold, 1.7–fold, 1.8–fold, 1.9–fold, 2–fold, 2.5–fold, 3–fold, 3.5–fold, 4–fold, 4.5–fold, 5–fold, 6–fold, 7–fold, 8–fold, 9–fold, 10–fold, or more than 10-fold increased binding affinity compared to a tracrRNA sequence in an unmodified sgRNA.

[395] The chemically modified gRNAs or sgRNAs of the present disclosure can reduce off-target effect in gene modification (e.g., gene or genome editing) of the target sequence. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can reduce off-target effect in gene modification of the target sequence by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 75%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 400% 500%, 600%, 700%, 800%, 900%, or 1000% compared to unmodified gRNAs or sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can reduce off-target effect in gene modification of the target sequence by about 20% to about 300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about 100%, about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to about 250%, about 200% to about 250%, at least about 20%, at least about 50%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% compared to unmodified gRNAs or sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can reduce off-target effect in gene modification of the target sequence by about 20% to about 300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about 100%, about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to about 250%, about 200% to about 250%, at least about 20%, at least about 50%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% compared unmodified gRNAs or sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can reduce off-target effect in gene modification of the target sequence by at least 1.1–fold, 1.2–fold, 1.3–fold, 1.4–fold, 1.5–fold, 1.6–fold, 1.7–fold, 1.8–fold, 1.9–fold, 2–fold, 2.5–fold, 3–fold, 3.5–fold, 4–fold, 4.5–fold, 5–fold, 6–fold, 7–fold, 8–fold, 9–fold, 10–fold, or more than 10-fold compared to unmodified gRNAs or sgRNAs.

[396] In some embodiments, the target polynucleotide sequence is in a genome, wherein the gRNA or sgRNA is capable of directing a component (e.g. Cas9) of a base editor fusion protein to effect a modification in the target polynucleotide sequence, and wherein the modification results in less off-target effect in the genome as compared to an unmodified gRNA or sgRNA. In some embodiments, the gRNA or sgRNA is capable of directing a Type II Cas protein to effect a modification in the target polynucleotide sequence wherein the modification results in at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 75%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 400% 500%, 600%, 700%, 800%, 900%, or 1000% less off-target effect in the genome as compared to an unmodified gRNA or sgRNA. In some embodiments, the gRNA or sgRNA is capable of directing a Type II Cas protein of a base editor fusion protein to effect a modification in the target polynucleotide sequence wherein the modification results in at least 1.1–fold, 1.2–fold, 1.3–fold, 1.4–fold, 1.5–fold, 1.6–fold, 1.7–fold, 1.8–fold, 1.9–fold, 2–fold, 2.5–fold, 3–fold, 3.5–fold, 4–fold, 4.5–fold, 5–fold, 6–fold, 7–fold, 8–fold, 9–fold, 10–fold, or more than 10-fold less off-target effect in the genome as compared to an unmodified gRNA or sgRNA. In some embodiments, the target polynucleotide sequence is in a genome, wherein the gRNA or sgRNA is capable of directing a Cas9 protein to effect a modification in the target polynucleotide sequence, and wherein the modification results in less off-target effect in the genome as compared to an unmodified gRNA or sgRNA. In some embodiments, the gRNA or sgRNA is capable of directing a Cas9 protein to effect a modification in the target polynucleotide sequence wherein the modification results in at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 75%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 400% 500%, 600%, 700%, 800%, 900%, or 1000% less off-target effect in the genome as compared to an unmodified gRNA or sgRNA. In some embodiments, the gRNA or sgRNA is capable of directing a Cas9. In some embodiments, the target polynucleotide sequence is in a genome, wherein the gRNA or sgRNA is capable of directing a base editor fusion protein to effect a modification in the target polynucleotide sequence, and wherein the modification results in less off-target effect in the genome as compared to an unmodified gRNA or sgRNA. In some embodiments, the gRNA or sgRNA is capable of directing a base editor fusion protein to effect a modification in the target polynucleotide sequence wherein the modification results in at least 1%, 2%, 3%, 4%, 5%,

6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 75%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 400% 500%, 600%, 700%, 800%, 900%, or 1000% less off-target effect in the genome as compared to an unmodified gRNA or sgRNA. In some embodiments, the gRNA or sgRNA is capable of directing a base editor fusion protein to effect a modification in the target polynucleotide sequence wherein the modification results in at least 1.1–fold, 1.2–fold, 1.3–fold, 1.4–fold, 1.5–fold, 1.6–fold, 1.7–fold, 1.8–fold, 1.9–fold, 2–fold, 2.5–fold, 3–fold, 3.5–fold, 4–fold, 4.5–fold, 5–fold, 6–fold, 7–fold, 8–fold, 9–fold, 10–fold, or more than 10-fold less off-target effect in the genome as compared to an unmodified gRNA or sgRNA. In some embodiments, the modification using the chemically modified gRNAs or sgRNAs results in less off-target effect in a cell as compared to an unmodified gRNA or sgRNA. In some embodiments, the modification using the chemically modified gRNAs or sgRNAs results in at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 75%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 400% 500%, 600%, 700%, 800%, 900%, or 1000% less off-target effect in a cell as compared to an unmodified gRNA or sgRNA. In some embodiments, the modification using the chemically modified gRNAs or sgRNAs results in at least 1.1–fold, 1.2–fold, 1.3–fold, 1.4–fold, 1.5–fold, 1.6–fold, 1.7–fold, 1.8–fold, 1.9–fold, 2–fold, 2.5–fold, 3–fold, 3.5–fold, 4–fold, 4.5–fold, 5–fold, 6–fold, 7–fold, 8–fold, 9–fold, 10–fold, or more than 10-fold less off-target effect in a cell as compared to an unmodified gRNA or sgRNA.

[397] The chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the gene modification (e.g., gene or genome editing) efficiency. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the gene modification (e.g., gene or genome editing) efficiency by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 75%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 400% 500%, 600%, 700%, 800%, 900%, or 1000% compared to unmodified gRNAs or sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the gene modification (e.g., gene or genome editing) efficiency by about 20% to about 300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about 100%,

about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to about 250%, about 200% to about 250%, at least about 20%, at least about 50%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% compared to unmodified gRNAs or sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the gene modification (e.g., gene or genome editing) efficiency by about 20% to about 300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about 100%, about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to about 250%, about 200% to about 250%, at least about 20%, at least about 50%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% compared unmodified gRNAs or sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the gene modification (e.g., gene or genome editing) efficiency by at least 1.1–fold, 1.2–fold, 1.3–fold, 1.4–fold, 1.5–fold, 1.6–fold, 1.7–fold, 1.8–fold, 1.9–fold, 2–fold, 2.5–fold, 3–fold, 3.5–fold, 4–fold, 4.5–fold, 5–fold, 6–fold, 7–fold, 8–fold, 9–fold, 10–fold, or more than 10-fold compared to unmodified gRNAs or sgRNAs.

[398] The chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the stability of the complex of a base editor protein or component (e.g., Cas9 protein) and the guide RNAs (e.g., gRNAs or sgRNAs), for example, the stability of base editor complex. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the stability of the Cas protein-gRNA complex by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 75%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 400% 500%, 600%, 700%, 800%, 900%, or 1000% compared to unmodified gRNAs or sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the stability of the base editor fusion or Cas protein-gRNA complex by about 20% to about 300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about

100%, about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to about 250%, about 200% to about 250%, at least about 20%, at least about 50%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% compared to unmodified gRNAs or sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the stability of the base editor fusion or Cas protein-gRNA complex by about 20% to about 300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about 100%, about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to about 250%, about 200% to about 250%, at least about 20%, at least about 50%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% compared unmodified gRNAs or sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the stability of the base editor fusion or Cas protein-gRNA complex by at least 1.1–fold, 1.2–fold, 1.3–fold, 1.4–fold, 1.5–fold, 1.6–fold, 1.7–fold, 1.8–fold, 1.9–fold, 2–fold, 2.5–fold, 3–fold, 3.5–fold, 4–fold, 4.5–fold, 5–fold, 6–fold, 7–fold, 8–fold, 9–fold, 10–fold, or more than 10-fold compared to unmodified gRNAs or sgRNAs.

[399] In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the stability of the base editor complex by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 75%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 400% 500%, 600%, 700%, 800%, 900%, or 1000% compared to unmodified sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the stability of the base editor complex by about 20% to about 300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about 100%, about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to

about 250%, about 200% to about 250%, at least about 20%, at least about 50%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% compared to unmodified sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the stability of the base editor complex by about 20% to about 300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about 100%, about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to about 250%, about 200% to about 250%, at least about 20%, at least about 50%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% compared unmodified sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the stability of the base editor-sgRNA or Cas9-sgRNA complex by at least 1.1–fold, 1.2–fold, 1.3–fold, 1.4–fold, 1.5–fold, 1.6–fold, 1.7–fold, 1.8–fold, 1.9–fold, 2–fold, 2.5–fold, 3–fold, 3.5–fold, 4–fold, 4.5–fold, 5–fold, 6–fold, 7–fold, 8–fold, 9–fold, 10–fold, or more than 10-fold compared to unmodified sgRNAs.

[400] The chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the stability of the guide RNAs (e.g., gRNAs or sgRNAs) in vitro, in vivo, and/or ex vivo. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the stability of the gRNAs or sgRNAs in vitro, in vivo, and/or ex vivo by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 75%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 400% 500%, 600%, 700%, 800%, 900%, or 1000% compared to unmodified gRNAs or sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the stability of the gRNAs or sgRNAs in vitro, in vivo, and/or ex vivo by about 20% to about 300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about 100%, about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to about 250%, about 200% to about 250%, at least about 20%, at least about 50%, at least about 100%, at least

about 150%, at least about 200%, at least about 250%, or at least about 300% compared to unmodified gRNAs or sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the stability of the gRNAs or sgRNAs in vitro, in vivo, and/or ex vivo by about 20% to about 300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about 100%, about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to about 250%, about 200% to about 250%, at least about 20%, at least about 50%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% compared unmodified gRNAs or sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can enhance or increase the stability of the gRNAs or sgRNAs in vitro, in vivo, and/or ex vivo by at least 1.1–fold, 1.2–fold, 1.3–fold, 1.4–fold, 1.5–fold, 1.6–fold, 1.7–fold, 1.8–fold, 1.9–fold, 2–fold, 2.5–fold, 3–fold, 3.5–fold, 4–fold, 4.5–fold, 5–fold, 6–fold, 7–fold, 8–fold, 9–fold, 10–fold, or more than 10-fold compared to unmodified gRNAs or sgRNAs.

[401] In some embodiments, the chemically modified gRNAs or sgRNAs exhibit increased stability in a cell as compared to unmodified gRNAs or sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs exhibit at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 75%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 400% 500%, 600%, 700%, 800%, 900%, or 1000% higher or increased stability in a cell as compared to unmodified gRNAs or sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs exhibit be at least 1.1–fold, 1.2–fold, 1.3–fold, 1.4–fold, 1.5–fold, 1.6–fold, 1.7–fold, 1.8–fold, 1.9–fold, 2–fold, 2.5–fold, 3–fold, 3.5–fold, 4–fold, 4.5–fold, 5–fold, 6–fold, 7–fold, 8–fold, 9–fold, 10–fold, or more than 10-fold higher or increased stability in a cell as compared to unmodified gRNAs or sgRNAs.

[402] In some embodiments, the stability of the chemically modified gRNAs or sgRNAs is measured by half-life of the gRNAs or sgRNAs in the cell. In some embodiments, the chemically modified gRNAs or sgRNAs exhibit increased half-life in the cell compared to unmodified gRNAs or sgRNAs. In some embodiments, the gRNAs or sgRNAs exhibit increased half-life in the cell by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%,

12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 75%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 400% 500%, 600%, 700%, 800%, 900%, or 1000% as compared to unmodified gRNAs or sgRNAs. In some embodiments, the gRNAs or sgRNAs exhibit increased half-life in the cell by about 20% to about 300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about 100%, about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to about 250%, about 200% to about 250%, at least about 20%, at least about 50%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% as compared to unmodified gRNAs or sgRNAs. In some embodiments, the gRNAs or sgRNAs exhibit increased half-life in the cell by at least 1.1–fold, 1.2–fold, 1.3–fold, 1.4–fold, 1.5–fold, 1.6–fold, 1.7–fold, 1.8–fold, 1.9–fold, 2–fold, 2.5–fold, 3–fold, 3.5–fold, 4–fold, 4.5–fold, 5–fold, 6–fold, 7–fold, 8–fold, 9–fold, 10–fold, or more than 10-fold as compared to unmodified gRNAs or sgRNAs.

[403] The chemically modified gRNAs or sgRNAs of the present disclosure can be more resistant to degradation as compared to an unmodified gRNA or sgRNA. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can be at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 75%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 400% 500%, 600%, 700%, 800%, 900%, or 1000% more resistant to degradation compared to unmodified gRNAs or sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can be about 20% to about 300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about 100%, about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to about 250%, about 200% to about 250%, at least about 20%, at least about 50%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% more resistant to degradation compared to unmodified gRNAs or sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can be about 20% to about

300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about 100%, about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to about 250%, about 200% to about 250%, at least about 20%, at least about 50%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% more resistant to degradation compared unmodified gRNAs or sgRNAs. In some embodiments, the chemically modified gRNAs or sgRNAs of the present disclosure can be at least 1.1–fold, 1.2–fold, 1.3–fold, 1.4–fold, 1.5–fold, 1.6–fold, 1.7–fold, 1.8–fold, 1.9–fold, 2–fold, 2.5–fold, 3–fold, 3.5–fold, 4–fold, 4.5–fold, 5–fold, 6–fold, 7–fold, 8–fold, 9–fold, 10–fold, or more than 10-fold more resistant to degradation compared to unmodified gRNAs or sgRNAs.

Target gene modification

[404] In some aspects, provided herein, is a method for treating or preventing a condition in a subject in need thereof, the method comprising administering to the subject the composition or lipid nanoparticle comprising chemically modified gRNAs or sgRNAs described herein, wherein the gRNAs or sgRNAs direct a base editor protein to effect a modification in a target polynucleotide sequence in a cell of the subject, thereby treating or preventing the condition. In some embodiments, the target polynucleotide is in a PCSK9 gene. In some embodiments, the target polynucleotide is in an ANGPTL3 gene. In some embodiments, the modification is at a splice site of the target polynucleotide. In some embodiments, the modification is at a splice donor site of the target polynucleotide. In some embodiments, the modification is at a splice acceptor site of the target polynucleotide. In some embodiments, the modification reduces expression of functional PCSK9 protein encoded by the PCSK9 gene in the subject. In some embodiments, the modification reduces expression of functional ANGPTL3 protein encoded by the ANGPTL3 gene in the subject. In some embodiments, the condition is atherosclerotic vascular disease. In some embodiments, the condition is an atherosclerotic vascular disease, hypertriglyceridemia, or diabetes. In some embodiments, the subject exhibits a reduced blood LDL cholesterol level and/or a reduced blood triglycerides level as compared to before the administration.

[405] In some embodiments, the disclosure provides base-editing systems, compositions and methods for editing a polynucleotide encoding an Apolipoprotein C3 (APOC3) protein

and variants thereof. In some embodiments, provided herein are genome/base-editing systems, compositions and methods for editing a polynucleotide encoding Proprotein convertase subtilisin/kexin type 9 (PCSK9) and variants thereof. In some embodiments, provided herein are genome/base-editing systems, compositions and methods for editing a polynucleotide encoding Angiopoietin-like 3 (ANGPTL3) and variants thereof. To edit a target gene, a target gene polynucleotide may contact the compositions disclosed herein comprising a sgRNA and a adenosine base editor protein, wherein the sgRNA comprises a spacer sequence and a tracrRNA sequence, wherein the spacer sequence hybridizes with a target polynucleotide sequence in a PCSK9 or ANGPTL3 gene and the tracrRNA sequence binds the adenosine base editor protein (e.g. a Cas9 component of the adenosine base editor). In some embodiments, the sgRNA therefore directs the base editor protein to the target polynucleotide sequence to result in a to G modification in the target gene. In some embodiments, the target gene or target polynucleotide is selected from a gene encoding PCSK9, APOC3, LPA, and ANGPTL3. In some embodiments, the target polynucleotide sequence is in a PCSK9 gene. In some embodiments, the target polynucleotide sequence is in an ANGPTL3 gene. In some embodiments, the modification reduces or abolishes expression of functional PCSK9 protein encoded by the PCSK9 gene in the cell. In some embodiments, the modification reduces or abolishes expression of functional ANGPTL3 protein encoded by the ANGPTL3 gene in the cell. In some embodiments, the introduction is performed via a lipid nanoparticle that comprises the composition.

[406] For example, the sgRNA and the Adenosine base editor protein may be expressed in a cell where a target gene editing is desired (e.g., a liver cell), to thereby allowing contact of the target gene with the composition disclosed herein (e.g., sgRNA and the Adenosine base editor protein). In some embodiments, the binding of the Adenosine base editor protein to its target polynucleotide sequence in the target gene is directed by a single guide RNA disclosed herein, e.g., a single guide RNA comprising (i) a spacer sequence and (ii) a tracrRNA sequence, wherein the spacer sequence hybridizes with a target polynucleotide sequence in a target gene. Thus, by designing the guide RNA sequence, the Adenosine base editor protein can be directed to edit any target polynucleotide sequence in the target gene (e.g., target gene encoding PCSK9, APOC3 and ANGPTL3). In some embodiments, the guide RNA sequence is co-expressed with the Adenosine base editor protein in a cell where editing is desired.

[407] In some embodiments, a target gene comprising more than one mutations described herein are contemplated. For example, a target gene encoding a variant protein can be produced using the methods described herein that includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more

mutations. To make multiple mutations in the target gene, a plurality of guide RNA sequences can be used, each guide RNA sequence targeting one target polynucleotide sequence in the target gene. The Adenosine base editor protein is capable of editing each and every target polynucleotide sequence dictated by the guide RNA sequence. For example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more guide RNA sequences can be used in a gene editing reaction. In some embodiments, the guide RNA sequences as used (e.g., gRNA). In some embodiments, DNA molecule encoding the guide RNA sequences can also be used.

[408] In some embodiments, simultaneous modifications into more than one target genes (e.g., more than one target gene in the LDL-mediated cholesterol clearance pathway) are also contemplated herein. For example, in some embodiments, a modification may be simultaneously introduced into PCSK9 and APOC3 gene. In some embodiments, a modification may be simultaneously introduced into PCSK9 and LDL-R gene. In some embodiments, a modification may be simultaneously introduced into PCSK9 and IODL gene. In some embodiments, a modification may be simultaneously introduced into PCSK9 and LPA gene. In some embodiments, a modification may be simultaneously introduced into APOC3 and IODL gene. In some embodiments, a modification may be simultaneously introduced into LDL-R and APOC3 gene. In some embodiments, a modification may be simultaneously introduced into LDL-R and IDOL gene. In some embodiments, a modification may be simultaneously introduced into PCSK9, APOC3, LDL-R and IDOL gene. In some embodiments, a modification can be simultaneously introduced into PCSK9 and ANGPTL3 gene. To simultaneously introduce of modifications into more than one target genes, multiple guide nucleotide sequences are used.

[409] To edit a gene encoding the PCSK9 or ANGPTL3 protein, the gene is contacted with the composition described herein. In some embodiments, a target polynucleotide sequence in a target gene is contacted with the single guide RNA disclosed herein and a Adenosine base editor protein or a nucleic acid sequence encoding the Adenosine base editor protein, wherein the single guide RNA directs the Adenosine base editor protein to effect a modification in the target gene (e.g., target gene encoding PCSK9, APOC3 and ANGPTL3). In some embodiments, the target polynucleotide sequence is the gene locus in the genomic DNA of a cell. In some embodiments, the cell is a cultured cell. In some embodiments, the cell is in vivo. In some embodiments, the cell is in vitro. In some embodiments, the cell is ex vivo.

[410] In some embodiments, the cell is from a mammal. In some embodiments, the mammal is a human. In some embodiments, the mammal is a rodent. In some embodiments, the rodent is a mouse. In some embodiments, the rodent is a rat. As would be understood be those

skilled in the art, a target polynucleotide sequence may be a DNA molecule comprising a coding strand and a complementary strand, e.g., the PCSK9 or ANGPTL3 gene locus in a genome. As such, the target polynucleotide sequence may also include coding regions (e.g., exons) and non-coding regions (e.g., introns or splicing sites). In some embodiments, the target polynucleotide sequence is located in the coding region (e.g., an exon) of the target gene (e.g., the PCSK9 or ANGPTL3 gene locus). As such, the modification in the coding region may result in an amino acid change in the protein encoded by the target gene, i.e., a mutation. In some embodiments, the mutation is a loss of function mutation. In some embodiments, the loss-of-function mutation is a naturally occurring loss-of-function mutation. In some embodiments, the target polynucleotide sequence is located in a non-coding region of the target gene, e.g., in an intron or a splicing site. In some embodiments, the base editor system provided herein results in a A•T to G•C alteration at a splice site in a PCSK9 gene or a ANGPTL3 gene. In some embodiments, the A•T to G•C alteration is at a splice acceptor site of the PCSK9 gene. In some embodiments, the A•T to G•C alteration results in an aberrant PCSK9 transcript encoded by the PCSK9 gene. In some embodiments, the A•T to G•C alteration results in a non-functional PCSK9 polypeptide encoded by the PCSK9 gene when expressed in a cell. In some embodiments, the A•T to G•C alteration is at a 5' end of a splice donor site of an intron 1 of the PCSK9 gene. In some embodiments, the A•T to G•C alteration is at a 5' end of a splice donor site of an intron 4 of the PCSK9 gene. In some embodiments, the base editor system provided herein results in a A•T to G•C alteration at a splice site ANGPTL3 gene, or the second A•T to G•C alteration is at a splice donor site of the ANGPTL3 gene. In some embodiments, the A•T to G•C alteration or the second A•T to G•C alteration is at a splice acceptor site of the ANTPTL3 gene. In some embodiments, the A•T to G•C alteration or the second A•T to G•C alteration results in an aberrant ANGPTL3 transcript encoded by the ANGPTL3 gene. In some embodiments, the A•T to G•C alteration or the second A•T to G•C alteration results in a non-functional ANGPTL3 polypeptide encoded by the ANGPTL3 gene. In some embodiments, the A•T to G•C alteration or the second A•T to G•C alteration is at a 5' end of a splice donor site of an intron 6 of the ANGPLT3 gene.

[411] In some embodiments, a target polynucleotide sequence is located in a splicing site and the editing of such sequence causes alternative splicing of the mRNA of a target gene. In some embodiments, the alternative splicing leads to leading to loss-of-function mutants. In some embodiments, the alternative splicing leads to the introduction of a premature stop codon in a mRNA encoded by the target gene, resulting in truncated and unstable proteins. In

some embodiments, mutants that are defective in folding are produced. A loss-of-function variant generated by a gene that is modified using the compositions and methods disclosed herein, may have reduced activity compared to a wild type protein encoded by an unmodified target gene. Activity refers to any known biological activity of the wild-type protein in the art.

[412] In some embodiments, the activity of a loss-of-function variant may be reduced by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 99%, or more. In some embodiments, the loss-of-function variant has no more than 50%, no more than 40%, no more than 30%, no more than 20%, no more than 10%, no more than 5%, no more than 1% or less activity compared to a wild type protein.

[413] In some embodiments, cellular activity of a protein encoded by a target gene may be reduced by reducing the level of properly folded and active protein. Introducing destabilizing mutations into the wild type protein may cause misfolding or deactivation of the protein. A variant generated by modifying a target gene using the compositions and methods disclosed herein comprises one or more destabilizing mutations may have reduced activity compared to the wild type protein encoded by an unmodified target gene. For example, the activity of a variant comprising one or more destabilizing mutations may be reduced by at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 99%, or more.

[414] In some embodiments, the methods and composition disclosed herein reduces or abolishes expression and/or function of protein encoded by a target gene. For example, the methods and composition disclosed herein reduces expression and/or function of protein encoded by the target gene by at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, or at least 10-fold relative to a control.

[415] In some embodiments, the methods and composition disclosed herein reduces or abolishes expression and/or function of the protein encoded by a target gene by at least 2-fold relative to a control. For example, the methods and composition disclosed herein reduces or abolishes expression and/or function of the protein encoded by a target gene by at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, or at least 10-fold relative to a control.

[416] Some aspects of the present disclosure provide strategies of editing target gene to reduce the amount of full-length, functional protein being produced. In some embodiments, stop codons may be introduced into the coding sequence of target gene upstream of the

normal stop codon (referred to as a "premature stop codon"). Premature stop codons cause premature translation termination, in turn resulting in truncated and nonfunctional proteins and induces rapid degradation of the mRNA via the non- sense mediated mRNA decay pathway. See, e.g., Baker et al., Current Opinion in Cell Biology 16 (3): 293-299, 2004; Chang et al, Annual Review of Biochemistry 76: 51-74, 2007; and Behm-Ansmant et ah, Genes & Development 20 (4): 391-398, 2006, each of which is incorporated herein by reference.

Proprotein convertase subtilisin-kexin type 9 (PCSK9)

[417] In some embodiments, the target gene for modification using the compositions and methods disclosed herein is gene encoding PCSK9. Proprotein convertase subtilisin-kexin type 9 (PCSK9), also known as neural apoptosis- regulated convertase 1 (NARC-I), is a proteinase K-like subtilase identified as the 9th member of the secretory subtilase family. "Proprotein convertase subtilisin/kexin type 9 (PCSK9)" refers to an enzyme encoded by the PCSK9 gene. PCSK9 binds to the receptor for low-density lipoprotein (LDL) particles. In the liver, the LDL receptor removes LDL particles from the blood through the endocytosis pathway. When PCSK9 binds to the LDL receptor, the receptor is channeled towards the lysosomal pathway and broken down by proteolytic enzymes, limiting the number of times that a given LDL receptor is able to uptake LDL particles from the blood. Thus, blocking PCSK9 activity may lead to more LDL receptors being recycled and present on the surface of the liver cells, and will remove more LDL cholesterol from the blood.

[418] Therefore, blocking PCSK9 can lower blood cholesterol levels. PCSK9 orthologs are found across many species. PCSK9 is inactive when first synthesized, a pre-pro enzyme, because a section of the peptide chain blocks its activity; proprotein convertases remove that section to activate the enzyme. Pro-PCSK9 is a secreted, globular, serine protease capable of proteolytic auto-processing of its N-terminal pro-domain into a potent endogenous inhibitor of PCSK9, which blocks its catalytic site. PCSK9's role in cholesterol homeostasis has been exploited medically. Drugs that block PCSK9 can lower the blood level of low-density lipoprotein cholesterol (LDL-C). The first two PCSK9 inhibitors, alirocumab and evolocumab, were approved by the U.S. Food and Drug Administration in 2015 for lowering cholesterol where statins and other drugs were insufficient.

[419] The human gene for PCSK9 localizes to human chromosome Ip33-p34.3. PCSK9 is expressed in cells capable of proliferation and differentiation including, for example, hepatocytes, kidney mesenchymal cells, intestinal ileum, and colon epithelia as well as

embryonic brain telencephalon neurons. See, e.g., Seidah et al., 2003 PNAS 100:928-933, which is incorporated herein by reference.

[420] Original synthesis of PCSK9 is in the form of an inactive enzyme precursor, or zymogen, of 72-kDa, which undergoes autocatalytic, intramolecular processing in the endoplasmic reticulum (ER) to activate its functionality. This internal processing event has been reported to occur at the SSVFAQ jSIP motif, and has been reported as a requirement of exit from the ER. "j" indicates cleavage site. See, Benjannet et al., 2004 J. Biol. Chem. 279:48865-48875, and Seidah et al, 2003 PNAS 100:928-933, each of which are incorporated herein by reference. The cleaved protein is then secreted. The cleaved peptide remains associated with the activated and secreted enzyme.

[421] The gene sequence for human PCSK9 is ~22-kb long with 12 exons encoding a 692 amino acid protein. The protein sequence of human PCSK9 can be found, for example, at Deposit No. NP_777596.2, which sequence is incorporated herein in its entirety. Human, mouse and rat PCSK9 nucleic acid sequences have been deposited; see, e.g., GenBank Accession Nos.: AX127530 (also AX207686), AX207688, and AX207690, respectively, each of which sequence is incorporated herein in its entirety. The gene sequence of Macaca fascicularis can be found publically, for example, NCBI Gene ID: 102142788, which sequence is incorporated herein in their entirety. Macaca fascicularis proprotein convertase subtilisin/kexin type 9 isoform X2 sequence can be found publically, for example, at NCBI Reference Sequence: XP_005543317.1, which sequence is incorporated herein in its entirety.

[422] The translated protein contains a signal peptide in the NH2-terminus, and in cells and tissues an about 74 kDa zymogen (precursor) form of the full-length protein is found in the endoplasmic reticulum. During initial processing in the cell, the about 14 kDa prodomain peptide is autocatalytically cleaved to yield a mature about 60 kDa protein containing the catalytic domain and a C-terminal domain often referred to as the cysteine-histidine rich domain (CHRD). This about 60 kDa form of PCSK9 is secreted from liver cells. The secreted form of PCSK9 appears to be the physiologically active species, although an intracellular functional role of the about 60 kDa form has not been ruled out.

[423] Numerous PCSK9 variants are disclosed and/or claimed in several patent publications including, but not limited to the following: PCT Publication Nos. WO2001031007, WO2001057081, WO2002014358, WO2001098468, WO2002102993, WO2002102994, WO2002046383, WO2002090526, WO2001077137, and WO2001034768; US Publication Nos. US 2004/0009553 and US 2003/0119038, and European Publication Nos. EP 1 440 981, EP 1 067 182, and EP 1 471 152, each of which are incorporated herein by reference.

[424] Several mutant forms of PCSK9 are well characterized, including S 127R, N157K, F216L, R218S, and D374Y, with S 127R, F216L, and D374Y being linked to autosomal dominant hypercholesterolemia (ADH). Benjannet et al. (J. Biol. Chem., 279(47):48865-48875 (2004)) demonstrated that the S 127R and D374Y mutations result in a significant decrease in the level of pro-PCSK9 processed in the ER to form the active secreted zymogen. As a consequence, it is believed that wild-type PCSK9 increases the turnover rate of the LDL receptor causing inhibition of LDL clearance (Maxwell et al, PNAS, 102(6):2069-2074 (2005); Benjannet et al, and Lalanne et al), while PCSK9 autosomal dominant mutations result in increased levels of LDLR, increased clearance of circulating LDL, and a corresponding decrease in plasma cholesterol levels. See, Rashid et al, PNAS, 102(15):5374-5379 (2005); Abifadel et al, 2003 Nature Genetics 34: 154-156; Timms et al, 2004 Hum. Genet. 114:349-353; and Leren, 2004 Clin. Genet. 65:419-422, each of which are incorporated herein by reference.

[425] A later-published study on the S127R mutation of Abifadel et al, reported that patients carrying such a mutation exhibited higher total cholesterol and apoB 100 in the plasma attributed to (1) an overproduction of apoB 100-containing lipoproteins, such as low density lipoprotein (LDL), very low density lipoprotein (VLDL) and intermediate density lipoprotein (IDL), and (2) an associated reduction in clearance or conversion of said lipoproteins. Together, the studies referenced above evidence the fact that PCSK9 plays a role in the regulation of LDL production. Expression or upregulation of PCSK9 is associated with increased plasma levels of LDL cholesterol, and inhibition or the lack of expression of PCSK9 is associated with low LDL cholesterol plasma levels. Significantly, lower levels of LDL cholesterol associated with sequence variations in PCSK9 have conferred protection against coronary heart disease; Cohen et al, 2006 N. Engl. J. Med. 354: 1264-1272.

[426] Lalanne et al. demonstrated that LDL catabolism was impaired and apolipoprotein B-containing lipoprotein synthesis was enhanced in two patients harboring S 127R mutations in PCSK9 (J. Lipid Research, 46: 1312-1319 (2005)). Sun et al. also provided evidence that mutant forms of PCSK9 are also the cause of unusually severe dominant hypercholesterolaemia as a consequence of its effect of increasing apolipoprotein B secretion (Sun et al, Hum. Mol. Genet, 14(9): 1161-1169 (2005)). These results were consistent with earlier results which demonstrated adenovirus -mediated overexpression of PCSK9 in mice results in severe hypercholesteromia due to drastic decreases in the amount of LDL receptor Dubuc et al., Thromb. Vase. Biol., 24: 1454-1459 (2004), in addition to results demonstrating mutant forms of PCSK9 also reduce the level of LDL receptor (Park et al., J. Biol. Chem.,

279:50630-50638 (2004). The overexpression of PCSK9 in cell lines, including liver-derived cells, and in livers of mice in vivo, results in a pronounced reduction in LDLR protein levels and LDLR functional activity without changes in LDLR mRNA level (Maxwell et al., Proc. Nat. Amer. Set, 101:7100-7105 (2004); Benjannet S. et al, J. Bio. Chem. 279: 48865-48875 (2004)).

[427] Various therapeutic approaches to the inhibition of PSCK9 have been proposed, including: inhibition of PSCK9 synthesis by gene silencing agents, e.g., RNAi; inhibition of PCSK9 binding to LDLR by monoclonal antibodies, small peptides or adnectins; and inhibition of PCSK9 autocatalytic processing by small molecule inhibitors. These strategies have been described in Hedrick et al., Curr Opin Investig Drugs 2009;10:938-46; Hooper et al, Expert Opin Biol Ther, 2013;13:429-35; Rhainds et al, Clin Lipid, 2012;7:621-40; Seidah et al;, Expert Opin Ther Targets 2009;13:19-28; and Seidah et al, Nat Rev Drug Discov 2012; 11:367-83, each of which are incorporated herein by reference.

[428] In some embodiments, the loss of function mutation induced in PCSK9 e.g., G106R, L253F, A443T, R93C, etc. In some embodiments, the loss-of-function mutation is engineered (i.e., not naturally occurring), e.g., G24D, S47F, R46H, S 153N, H193Y, etc.

[429] PCSK9 variants that can be useful in the present disclosure are loss-of-function variants that may boost LDL receptor-mediated clearance of LDL cholesterol, alone or in combination with other genes involved in the pathway, e.g., APOC3, LDL-R, or Idol. In some embodiments, the PCSK9 loss-of-function variants produced using the methods of the present disclosure express efficiently in a cell. In some embodiments, the PCKS9 loss-of-function variants produced using the methods of the present disclosure is activated and exported to engage the clathrin-coated pits from unmodified cells in a paracrine mechanism, thus competing with the wild-type PCSK9 protein. In some embodiments, the PCSK9 loss-of-function variant comprises mutations in residues in the LDL-R bonding region that make direct contact with the LDL-R protein. In some embodiments, the residues in the LDL-R bonding region that make direct contact with the LDL-R protein are selected from the group consisting of R194, R237, F379, S372, D374, D375, D378, R46, R237, and A443.

[430] As described herein, a loss-of-function PCSK9 variant, may have reduced activity compared to a wild type PCSK9 protein. PCSK9 activity refers to any known biological activity of the PCSK9 protein in the art. For example, in some embodiments, PCSK9 activity refers to its protease activity. In some embodiments, PCSK9 activity refers to its ability to be secreted through the cellular secretory pathway. In some embodiments, PCSK9 activity refers to its ability to act as a protein-binding adaptor in clathrin-coated vesicles. In some

embodiments, PCSK9 activity refers to its ability to interact with LDL receptor. In some embodiments, PCSK9 activity refers to its ability to prevent LDL receptor recycling. These examples are not meant to be limiting.

[431] In some embodiments, the activity of a loss-of-function PCSK9 variant may be reduced by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 99%, or more. In some embodiments, the loss-of-function PCSK9 variant has no more than 50%, no more than 40%, no more than 30%, no more than 20%, no more than 10%, no more than 5%, no more than 1% or less activity compared to a wild type PCSK9 protein. Non-limiting, exemplary assays for determining PCSK9 activity have been described in the art, e.g., in US Patent Application Publication US20120082680, which are incorporated herein by reference.

[432] In some embodiments, cellular PCSK9 activity may be reduced by reducing the level of properly folded and active PCSK9 protein. Introducing destabilizing mutations into the wild type PCSK9 protein may cause misfolding or deactivation of the protein. A PCSK9 variant comprising one or more destabilizing mutations described herein may have reduced activity compared to the wild type PCSK9 protein. For example, the activity of a PCSK9 variant comprising one or more destabilizing mutations described herein may be reduced by at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 99%, or more.

[433] In some embodiments, the methods and composition disclosed herein reduce or abolish expression of protein encoded by a target gene and/or function thereof. For example, the methods and composition disclosed herein reduces expression and/or function of PCSK9 protein encoded by the PCSK9 gene by at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, or at least 10-fold relative to a control. For example, the methods and composition disclosed herein reduces expression and/or function of APOC3 protein encoded by the APOC3 gene by at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, or at least 10-fold relative to a control. For example, the methods and composition disclosed herein reduces expression and/or function of ANGPTL3 protein encoded by the ANGPTL3 gene by at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, or at least 10-fold relative to a control.

[434] In some embodiments, the gene modification methods and compositions disclosed herein reduces expression of functional PCSK9 protein encoded by the PCSK9 gene in the

cell by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or 100%. In some embodiments, the modification reduces expression of functional PCSK9 protein encoded by the PCSK9 gene in the cell by at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 10 fold, at least 20 fold, at least 25 fold, at least 30 fold, at least 40 fold, at least 50 fold, at least 60 fold, at least 70 fold, at least 80 fold, at least 90 fold, at least 100 fold, at least 200 fold, at least 300 fold, at least 400 fold, at least 500 fold, at least 600 fold, at least 700 fold, at least 800 fold, at least 900 fold, at least 1000 fold, at least 2000 fold, at least 3000 fold, at least 4000 fold, at least 5000 fold, at least 6000 fold, at least 7000 fold, at least 8000 fold, at least 9000 fold, or at least 10000 fold. In some embodiments, the modification abolishes expression of functional PCSK9 protein encoded by the PCSK9 gene in the cell.

[435] Some aspects of the present disclosure provide strategies of reducing cellular PCSK9 activity via preventing PCSK9 mRNA maturation and production. In some embodiments, such strategies involve alterations of splicing sites in the PCSK9 gene. Altered splicing site may lead to altered splicing and maturation of the PCSK9 mRNA. For example, in some embodiments, an altered splicing site may lead to the skipping of an exon, in turn leading to a truncated protein product or an altered reading frame. In some embodiments, an altered splicing site may lead to translation of an intron sequence and premature translation termination when an in frame stop codon is encountered by the translating ribosome in the intron. In some embodiments, a start codon is edited and protein translation initiates at the next ATG codon, which may not be in the correct coding frame.

[436] The splicing sites typically comprises an intron donor site, a Lariat branch point, and an intron acceptor site. The mechanism of splicing is familiar to those skilled in the art. As a non-limiting example, the intron donor site has a consensus sequence of GGGTRAGT, and the C bases paired with the G bases in the intron donor site consensus sequence may be targeted by the methods and compositions described herein, thereby altering the intron donor site. The Lariat branch point also has consensus sequences, e.g., YTRAC, wherein Y is a pyrimidine and R is a purine. The C base in the Lariat branch point consensus sequence may be targeted by the nucleobase editors described herein, leading to the skipping of the following exon. The intron acceptor site has a consensus sequence of YNCAGG, wherein Y is a pyrimidine and N is any nucleotide. The C base of the consensus sequence of the intron acceptor site, and the C base paired with the G bases in the consensus sequence of the intron

acceptor site may be targeted by the nucleobase editors described herein, thereby altering the intron acceptor site, in turn leading the skipping of an exon. As described herein, gene sequence for human PCSK9 is -22- kb long and contains 12 exons and 11 introns. Each of the exon-intron junction may be altered to disrupt the processing and maturation of the PCSK9 mRNA.

[437] In some embodiments, a splice site disruption generated by a base editor system disclosed herein can result in the inclusion of intronic sequences in messenger RNA (mRNA) encoded by the PCSK9 gene. In some embodiments, the splice site disruption generates a nonsense, frameshift, or an in-frame indel mutation that result in premature stop codons or in insertion/deletion of amino acids that disrupt protein activity. In some embodiments, the splice site disruption generates exclusion of exonic sequences. In some embodiments, the splice site disruption generates exclusion of exonic sequences that results in nonsense, frameshift, or in-frame indel mutations in the PCSK9 transcript. Canonical splice donors comprise the DNA sequence GT on the sense strand, whereas canonical splice acceptors comprise the DNA sequence AG. Alteration of the sequence disrupts normal splicing. Splice donors can be disrupted by adenine base editing of the complementary base in the second position in the antisense strand (GT to GC), and splice acceptors can be disrupted by adenine base editing of the first position in the sense strand (AG to GG).

[438] Further, the present disclosure also contemplates the use of destabilizing mutations to counteract the effect of gain-of-function PCSK9 variant. Gain-of-function PCSK9 variants (e.g., the gain-of-function variants have been described in the art and are found to be associated with hypercholesterolemia (e.g., in Peterson et al., J Lipid Res. 2008 Jun; 49(6): 1152-1156; Benjannet et al., J Biol Chem. 2012 Sep 28;287(40):33745-55; Abifadel et al, Atherosclerosis. 2012 Aug;223(2):394-400; and Cameron et al, Hum. Mol. Genet. (1 May 2006) 15(9): 1551-1558, each of which is incorporated herein by reference). Introducing destabilizing mutations into these gain-of-function PCSK9 variants may cause misfolding and deactivation of these gain-of-function variants, thereby counteracting the hyper- activity caused by the gain-of-function mutation. Further, gain-of-function mutations in several other key factors in the LDL-R mediated cholesterol clearance pathway, e.g., LDL- R, APOB, or APOC, have also been described in the art. Thus, making destabilizing mutations in these factors to counteract the deleterious effect of the gain-of-function mutation using the compositions and methods described herein, is also within the scope of the present disclosure. As such, the present disclosure further provides mutations that cause misfolding of PCSK9 protein or structurally destabilization of PCSK9 protein.

[439] The polypeptide and coding nucleic acid sequences of PCSK9 and of other members of the family of human origin and those of a number of animals are publicly available, *e.g.*, from the NCBI website or ENSEMBL website. Examples include, but are not limited to the following sequences, each of which sequences are incorporated herein in their entireties; Wild Type PCSK9 Gene (NG_009061.1), Homo sapiens proprotein convertase subtilisin/kexin type 9 (PCSK9), RefSeqGene (LRG_275) on chromosome 1

GTCCGATGGGGCTCTGGTGGCGTGATCTGCGCGCCCCAGGCGTCAAGCACCCAC
ACCCTAGAAGGTTTCCGCAGCGACGTCGAGGCGCTCATGGTTGCAGGCGGGCGC
CGCCGTTCAGTTCAGGGTCTGAGCCTGGAGGAGTGAGCCAGGCAGTGAGACTGG
CTCGGGCGGGCCGGGACGCGTCGTTGCAGCAGCGGCTCCCAGCTCCCAGCCAGG
ATTCCGCGCGCCCCTTCACGCGCCCTGCTCCTGAACTTCAGCTCCTGCACAGTCCT
CCCCACCGCAAGGCTCAAGGCGCCGCCGGCGTGGACCGCGCACGGCCTCTAGGT
CTCCTCGCCAGGACAGCAACCTCTCCCCTGGCCCTCATGGGCACCGTCAGCTCCA
GGCGGTCCTGGTGGCCGCTGCCACTGCTGCTGCTGCTGCTGCTCCTGGGTCC
CGCGGGCGCCCGTGCGCAGGAGGACGAGGACGGCGACTACGAGGAGCTGGTGC
TAGCCTTGCGTTCCGAGGAGGACGGCCTGGCCGAAGCACCCGAGCACGGAACCA
CAGCCACCTTCCACCGCTGCGCCAAGGTGCGGGTGTAGGGATGGGAGGCCGGGG
CGAACCCGCAGCCGGGACGGTGCGGTGCTGTTTCCTCTCGGGCCTCAGTTTCCCC
CCATGTAAGAGAGGAAGTGGAGTGCAGGTCGCCGAGGGCTCTTCGCTTGGCACG
ATCTTGGGGACTGCAGGCAAGGCGGCGGGGGAGGACGGGTAGTGGGGAGCACG
GTGGAGAGCGGGGACGGCCGGCTCTTTGGGGACTTGCTGGGGCGTGCGGCTGCG
CTATTCAGTGGGAAGGTTCGCGGGGTTGGGAGACCCGGAGGCCGAGGAAGGGCG
AGCAGAGCACTGCCAGGATATCCTGCCCAGATTTCCCAGTTTCTGCCTCGCCGCG
GCACAGGTGGGTGAAGGAGTGAATGCCTGGAACGTACTGGGAACTGCACCAGGC
ACAGAGAAAGCGGGCTTGCCATTATAGTGGGTTCCGATTTGGTTTGGAAAACATG
GGCAGCGGAGGGTGGAGGGCCTGGAGAGAAGGCCCTACCCGAGACAGGGGCGG
GGTGGGAAGGACGGCAGATGCTGGGAGCACGAGGCAATTTCTTTATGACACAGA
ACTCATGCTCTAGTATTCCATCTGTTTCAGCCGAAGAAAAGAACCAGCTGAAGGG
GCAGGGGAGAAGGGGCGGAGGTATTCTCGAGGCCCATTGGCGTCCTTTAGGACT
CAGGCAGGGAAGGGCCCTTGGTGCTCTGGAGCCGGAGGTGGTGCGCCTGGTACT
GGGACCCCGGAGCTGAGCCCGGCGCCTCAGCCCACCTGGCTGTCTGCCGACCGT
GTGCGGGGCGAGTTTGCTCAACAACTCTGCCAGCTTCTGGCCCTCAGGCTGTGGG
AAGCTTCTTCCCGGGGCGAGACCACTAGCTTTTTCTAAGTATTACCAGCCCAGGA
CTTGGCTGAGGTTCTGTGTCCCCCAGCTTGGAGTCAGATGTGGGGTTGAATCTTG

GCTTCCTCTCACTAGCTGTGGTGCTTGACAAGTCACTTATCCTTGAGCCTCCATTG
CCTAATCTTTAAAAGGGAGGTGACAATCGTCCCTACGGCTCAGTGGCAGCAGAT
GGGGAGATGAAGGGAAAGTTCTGTTGACCATGAGTGAACTTACAATGCAAGCCC
CGGGGGGATCACTTGCAGTTTTGTCCCTGTCTGCAGTGTGACCTGTTGGTGACAT
TGTCTTTGCTCCAAACCACAGCTCCTGGGGCAGAGGGGAAAATTCTGCCACTCAC
AGCTGCCTGCCCACGCTTCTGTCTGAGTGTGCTGGGTGGCAGGATGGCAAGTCCT
TACTCAGCTCAGTATAGCCCTCTTCCTTGTTCCCTGAGCCTTTGACTTTCTCGAGG
GATGTTGTGGGGTTGTGGCCAGGATAAGAAAGGGCATTTCAAGTTACCACTGCTC
CAAAACAACTGTTCTGGAAATAGTGAGTACCCCATCCTGAGAGGTGAGTAAGCA
GAGGCTGTATGACCACCTGAACCAAGCCCTTGAGGATGTTTCTTCTCTGGTGGAA
GTTTGGAACAGGAGCCTCCTCAAGTTCATTTATTCATTCATTCAATGGTTATTTTG
TGGGAATCGAATTTAGAATGAAAATATTTTTTGGCAAGCAGAAAATAATTTTTAG
ACCAATCCTTTTCTTTTAGTCATGAGAAACTGAGGCCCAGAGAGAGGAGGTCACC
CCAGGTGCATTAGAACTGGGTTTCCAGAACTGACACTCCACTGCACAGAGTACTC
TCCCAATTCATTCAATTTTTATTTAGCGGAAGGCATTTTCAGATGGGTCTTTGAAG
CATTAGTAGGAGTTCAGCGATGATGGTGTCATGAGAATTTTATTCTAGGATTAGG
AGGTACCATGAACAAAGATACAGAGCTGGGAAAACCAGAGGTGGAAGATAAGG
AGCACATGTCCACAGTTCTTTTTCTTTTTTTTTTGAGATGGAGTTTCGCTCTTGTTG
CCCAGGCTGGAGTGCAATGGTGCAGTCTCAGCTCACTGCAACATCTGTCTCCCGG
GTTCAAGTGGTTCTCCTGCCTCAGCCTCCCAAGAAGCTGGGATTACAGGTACCTG
CCACCACGCCCGGCTAATTTTTGTATTTTTAGTAGAGAAGGGGTTTCACCACGTT
GGCCAGGCTAGTCGCAAACTCCTGACCTCCTCAGTGGATCCGAGGAGGTGATCCT
CCCGCCTCAGCCTCCCAAAGTGCTCGAATTACAGGTGTGAGCCACCACGCCTGGC
CTCCACAGTTCTTTATCCACCGTCTGAAATGTAAAATGTTACGAAAACCAAAAGT
TTTTTTTGTGATTTATTTGATGGTAGCACCTGACGTGAACTGACATGAGATTATTT
TTAATTTAGTTGTGTGAATATGCATATTCATATATTTTGCTGCATAGATTACAGTA
TGCAGCTCCAGATTCTTCCAAGCAGACTCTGATTGCCCATTACTGCCTTTCTAAA
ATCCAAACAAGTTCTGAGGTTCAAAACCGTTTTGGCCCTAAGGCTTTGGGTAAAG
GGGGTGGACTCTGTTCTACTCTGACTGGAGTCCAAGATGCATATATACAGAGATA
TGGGTGATGGGGCTGCAAGGTAGGTTGAGGTAGGGGCCAAGGAGGAGCATGGA
GTTTGGACTTGATTCATGAGGCTGTGGGGAGCCAGTGAAGGTTCTTAAGCAGGTA
TGTCTGCCTGAGAGCAGTTGGAGCAGACAAGAGCTAAAAACCAAACAAATCACC
ATAGATAGTGGCTGCTATAATTTGTTTGTCCCCTCCAAATCTCATGTGGAAATTTG
GTCCTCAGTGTTGGAAGTGGGGCCTAATGGGAGGTGTTTGGGTCATGGGGGAGG

AACCCCTGTGAAAGGCTTGGTGCCGTCCTTGTGATAATGAGTAAGTTCTCCCGCT
ATGATTTCCCTTGAAGGCTGATTATTAAAAAGAGCTTGGCACCTCCCTCTCTTCTC
TCTTGCTTCTTCTCTTGCCATGTGATTGATCTCTGCACATGTAGGCTCCCCTTCAC
CTTCTGCCATCAGTGAAAGCAGCTTAAGGCCCTCACCAGAAGCAGATGCTGGTG
CCATGCTTCCTGGAGAGCTTGCAGAATCATGAGCTGAATAAATCCCTTTTCCTTG
TAAATTACTCACCTTCAGGTATTCCTTTATATAGCAACACAAAAGGACTAAGACA
GTGGCCTTGACTTTTCTCTCTCTTTAAGAAGTGTTGCCTTTGCTCACTTAGTCATC
CCTTCTGCCTGCATTTGTAGAGCATCTGGATGGGAGATTTATATAACCGTCACTC
TTGACTTTCCCAGCAGGCCTATGTCATAGGTACTGTGGTCTCTACAATACAGCAG
AGGTATCTGAGGCTCCGAGAGGTTGAGTGACTTGCTCATGGCTGCACAACCAGT
AAATATTGGAGCTGGAATTCAGGTCCACGGTTTCCTGGCTCCAAAGCCCATGATT
TTTTCCCTCAATTTATTCTGACTGGGGCATGGGGGAGGGGGTGGCCTTTGGGCAG
GGCCACCAGGAGCGACCAGGCCCGTAGAGAGCTGGGTGCAGGTACAGAGGAAA
ACCTGTTGTCGAGTGTGGCCCGTAGTTCCCATTTTTGCCTGAATGGCACATTTGA
AAGTGTTATATAACCATGTGAATAATAATAGTTGGCCTATATGAGTTCTTTAATTT
GCTTTTTGGTCCGCATTTGGTAACTTCTTTATCATCTACTATACTCTGTTGTGTCTC
TTTTGTTGTAATTTGTAAGTAGGGGTGAGATAAAGTACACCTAGGGTTTGCTGGG
TTTCTTCCATGTCATCATGTTCCTCCTTGCATGGGGCCAGGATCCGTGGAGGTTGC
CTGGCACCTACGTGGTGGTGCTGAAGGAGGAGACCCACCTCTCGCAGTCAGAGC
GCACTGCCCGCCGCCTGCAGGCCCAGGCTGCCCGCCGGGGATACCTCACCAAGA
TCCTGCATGTCTTCCATGGCCTTCTTCCTGGCTTCCTGGTGAAGATGAGTGGCGAC
CTGCTGGAGCTGGTGAGCCACCCTTTTTGGGAATGGCACTTCCTGATAGGGCTGG
GCCACTGCATATACACTGGGGACTGTGCTTAGTAGGCCCATTGCTGAAAATCAGA
AGGGGACAGCAAGTATGTATTGAGCACTTATCGGGTACCAAGCACAGTAACTAC
TGGCTTTCTGTATAGAATTCCCTTTAAGCCTGGCCATGCCCCAGTGGTACGTCTAT
CTTCATTTGAAAGACGAGGAGACTGAAGTTCAGAGGGGACCACACAGACAGCTA
GGGGTAGAGCCTGGATCAAACCCATTGGTCTGCCTGCCAGCCATTCTTGTGCCAA
TGCATCTGCTGCCTACGGAAACCTGTAGGGACAAGGCCCTGGGATGTTCAGTGG
AGCCTGAGTCATTTTATAAAAAAGCATGACTCTAGGGTCCAAAATTCCTTTGAAG
CTGTTGCTATCCAGAGTGAAGTCCCTTCTTTAGGACAGGGTGGCCCTCCTCCCTC
CTGGATGTCACATCTTCGGTGGAGGGGCAGAAAGGGGACTGGGTATTCTCCTCA
CCCTGGCCCTAGTGCTTCAAATCTTAAAAAAACGTTTTTATTTGTGCTTCTGCACC
ACCTTCTAGCCCACCTCGTTTCCTGGCCTCTAACTTGATGAGAGCGTGTGTCATTT
TCACACTGATTCTCCACATGGCAGGCGGTGCTTCTTAGCCTCCTGCAGACAGTGA

GGCCCCACGGTCTTGTCCAAGGTCACACAGCGTGTAATGGGCAGGGTCAGAGTC
TGGAGTCTGGACCTGGGTCTCCTAGCTGCACTGCACTGCTGCCCCATGGGTTAAT
CAGCTCAGCATACCGTGGCTGAACAGCTACCTCATACCAAGGCCTGTGGCGCCAT
GACAGGGATTGACAGGGTCCCTGCCTTGGAAACCCGTAGTCTAAGTAGAGGAGA
CTGACAAGTCAATGCCTTCCATCAGTCTGCTCAACACACGTTTACCAAGTGCCTA
CTGTGTGCTGCAGAGGCGAAGATGACACAGCTCAGGCCTTTCCCTTGAGCTTACA
GTTCAGGAGGAGAGACTGACCAGTGACTGCCAGTACAGTTGACTATGGGACAAT
GTGCTCAGCCTTGGGGAGAGACGAAGAAGGTACCCGTATAGCACCAGATGACAG
GCACGAGCCCCACAGGCCAGGGCAGCTGCTCAGAGGAGAGTAGGCCAAGCAGA
AGGCAAACAGAAGGCTGCAGGCATTTGCCATCGAGAGCTGGACTTCAAACTGGG
CATCATACCAGCCTGGGTTCGAGTCCTGCCCAGCCCCTTATTGGCTGTCTAACCC
TGAGCAAATCCCTTCACCTCTCTGAGCCTCATTCCTCTATCTGTAAACCAGTTATA
ATAATTGGAACATTCATTTAAGGACTAAATGAGGTCGTGAAGCATTCAGCAGAT
GCTAGGTACGGAAACTCGCTGAAGTGGGGGCAGGTTAAGAAGCCTCTGGGGATA
CGAAGGCATCCAGGGACTAGTTGTGGCAGGAGGCTGTTACCACTTAGGTCTGAA
GGGTAAGGAGAGGGAATAGCTTTCCCTCTGCCCAGTTGGAGCCGGTGGCATGGA
GGAGAGGCTGCCTGTGGGGAATCACCCGAGGGTTCACCGCTGCCATGCGCAGGG
AGTCAGGAGGTAGGGAGGGAGTGGGGCAGATGCACACCATTTTTTTTTTTTTTG
AGACTCTGTTGCCCAGACTGGAGTGCAGTGGTGCCATATCTGCACCTCTGCCTCC
CGGGTTCAAGCTCACTGCAACCTCTGCCTCCCGGGTTCAAGCGATTCTCCTGCCT
CAGCCTCCCGAGTAGCTGGGACTACAGGTGTGTGCCACCATGCCTGGCTAATTTT
TGTATTTTTAATAGAGATGGGGTTTCACCATGTTGGCCAGGCTGGTCTCGAACTC
TCGACCTCAGGTGATCCCCCACCTCGGCCTCCCAAAGTGCTGGGATTACAGGCGT
GAGTCACCGCTCCCAGCTGCTGATGCACTCTTGTCCTTCTAACTCCTGCTAGTGCC
TCCCATTGGCTGAGCCCAACTGGAAGCTTTGCAAGGGAGCTGGTGCTGCAGTTTG
CACTGAGCAGGCTGGAGAAGGCTGGAGAATAGACTAGGGGACAAACCGAATTG
CCAGTGCTGTTATGTCATGATTTAGGCATGGAGTCCAGGGCCTGAGCTTCACTCC
ATGTCCATCCTGCCCAGAGCCTTGGCACAGCCTGGCTCCCAGACAAGATGTCAAG
TTCAGAATCCTTCCTAAAAGGAATCCTCTATGCCAGACCGTGTTGCAGGGATATG
GGAGTGCTGGGCTCCCAGCCTGATCAAGGAGCGAGAAAACTCAGGCTCCTAGTC
TGTCCTCCGGGGCACTAGCAGGGACAAGGTGGGAGGCTGCTGGGCTGGGATGTG
GGGACAGGTTTGATCAGGTAAGGCCAGGCTGTGGCTGTGTTTGCTGCTGTCCAAA
TGGCTTAAGCAGAGTCCCCCGGCCTCTCTGGCTTCTGCAGGCCTTGAAGTTGCCC
CATGTCGACTACATCGAGGAGGACTCCTCTGTCTTTGCCCAGAGCATCCCGTGGA

ACCTGGAGCGGATTACCCCTCCACGGTACCGGGCGGATGAATACCAGCCCCCCG
GTAAGACCCCCATCTGTGCCCTGCCCCACCCCATCTGAGCTGAATCCATTTGCTC
TGCCCTGGCCTGGCCTCCCTGCTGGTGGTTTCCACTTCTCGGGGGGCTTTGGGACT
CAGCACCTCCACTGACCCCTTTTTTTCTGTCCCATCCCCATCCCCTGCAGCCCCCA
CTGCCTGCCTTCCTGTTGCCCCACAAATGCAAAAGTCTTGCCTTAAATGATCCTCT
TTTCCTTCTTTTCTCTTGTTTTCCTTTTCTCACCATTTGGAATGGCCCAGCAGGCTG
CACTTACCTTGGAAGGAGGGTTCATCTGATGGTGACTCTACCTAGGGCCCCCAGG
CCTCTATAACTCCCAGTGCCCTGCAGACTGGACCAGATCCTTTAATGGGATAGAC
ACAACCCTGTCTGGGATGCCTCTGCCTACCTTCCTGTTTTGCTGCTCCACCTGCCT
CCAGCTCCGTTTGGCTTCCTGGGGCTCCCTGCCTGGGCCACTTTGTGTCTTCCCTC
TAGGCCTTTCTTTCCACTGTTCCCTCTGCCTGGTGTGGCCTGGCTATGGAAGGGA
GGGAGGAGGAGCGGCCATGGAAAACGGTCTGCATTCTAGCAGGGACTTGCAGGT
GGCAATTCAGTCGGGGAAGACTCTAGATGCACCTGGCCTGAGGAGAGAATGAAG
GGTTCTAGTTGGACTGTGTTAAGTTTGAGGTGCCCATGGTGTGAGGTCTGGAGCT
CAGCGCAGAGATGATGCAATGTGGTGGGTCCATGCAACATGGTGCCAGGACGCA
GAGCTTGGGGTGAACTCAGCTTTCACCCCTTACCGGTTCTCGTGGGATCTTGGGA
AGCCACTTTCTTCTATGAGCTTTGTCGTTCTTGTCTGTAAAATGGGCACATAACCC
TGTCCCTGTCCTTCTCACAGGTTGCTGTGAGACTCCAATGAGTTGAAGGATGTGC
AGATGCTTTTGGAAGTGAAAAGTTGGGGGGCTACTGTGTGACTTTGCATACACCC
AAACTGTGTGACCTTGCATATGTCTGAGTTGCTGCCATTGCAACAGATCAGAGCT
GGTGGGCTGGGTGTGGAGAAAGGGTTTGTGTGGGGGACATCCTCTGGCAAGGGT
GGCAGCAGCAGAAGTGAGGGGCCTGGTCGGTCATGTGTGCTGACCCGGCCTGGG
CAGCCTGTGGCCAGGGAGAGGACAGCTCCTCTGTAGGAAGAGCCTGTTCCTTTCC
AACCAGGTGAGACCTCTTCAGTGGAGCCCTGGAGCCCCCTGTACTCCACATCAGT
GCCTCAGGGACCTCCCGGAGCAGGCTAATATCAGAGACCAAGAGGGACACTGGC
AGAGGATCACAGAGACCCCAGTCCAGGCAGGGACTGAGAAGATCTTGCCCCCTA
AGTTAGTTTCCTAGCACTGCTGTGACAAATTACCACCCCCTCGGTTGGAACAAGT
TGATTCTCTGCAGTCCTGGAGGCCAGAAGCCTGAATCAGTGTCGGCAGGACCACT
TTCTCCCGGGGGGCTCCAGGGAGAAGCTTCTCTTGCCTCTTCCGTGTCCCAACAG
CGGCAGCACACCAATCCCAGCCTCTGTCTTCACACAGCCTTCTCTGTGTCTCTCTC
CTCTTCATTGTCTCATAAGGACACTTGTCATTGGATTTAGGGCCCACTGGATCCTC
CAGGATGATCTCATGTGGGGAACCTTAACCACATCTGCAAGGACCCTTTTTCCAA
ATAAGGTCACAGCCACAGGTTGTGGGGGTTAGGATGTGAGTGTATCTCTTTGGCA
GCCACTGTTCCCTCCTCTCCCTTGGGCCAGAAGCAGACGTGGGGCCCTTTCTTCC

CCATAGGATGCCCATGGATTGCCCCCCTTCCCGCTTCCCCCGAGTGTCTGTGGGA
GGTGGCAGGAATGGCAGGCAGGGGTGTGGAACCCCTTCTGGAGTCATATCAAGG
GCTTGGCTGGAGGAAGTCCTCCTGGAGCTGTTGGGCTGGCATGGGGCAGGCTGG
CTGGGCCCAGCAGCAGCTTCTTCATTCATGGGGAGGCCACAAGCATGGGCCCTA
GAGCTGGCTGCCGCCCTCAAACCCAGACCCTGCACTCTTAACTGTGTGACCTTGC
ATACGTCACTCACCCTCTCTGATCTTCAGGTTCCTCTGCAAAAGGGAGGTAATGA
TAACCCTCACTCTGGGGGGCTGTTTGGAGGGTTAAATCAGTTATTGCTGTAGCAT
GCATTTCTCTGTCAGGTATTGAGTGAGGTGCTGTGATTTTAGCCCTGCATTTTTCT
TTTCTTACCATTCAATAATAACGTTTTGAGCACCCACTGTGCGCCAGGCACCATA
TTAGGTGCTGGGGATACAAATGTGAATGAAATGAATGTGGTCTCTTCCCCCAACA
GTGTATCCAGAAGATTAATCCATTCCTTAAACAAATGCTACTTGACACAGATTAG
TTCTGGATAGGCTGAGAGCTCTGAAGGAGTGCAGGCAGCTGCGAGCCTGTGTAT
CCAGCAGAAGGATCAGGAAAGGATTCCTGGAGGAAGCGCTGTTCTAGCCAAGAC
CTACGGGGGCATTATTAACCAGGCAAAGGGGACGGTGTCCAAGCAGTGGAATGA
ACGTGGATTGAAGCTGTGAGGCAGGAGGGAGTGTGGCCTGTGCAGAAGGGACCG
AGGCTGGTGAGACCAGGAGGGCCTGGGTGGCCTCCAGGTCAGATGTGAAAGGAA
GAACTTGGCCACAGTCTGAGCTTCTCAGGCGTATGGCAGGGCTGCCTGGTGAGA
GGGAATGAGCTCCCTGCTCTGGAGGTATGCAAGCAGGACTGGGCTCTCACCTGC
CAGAGGCCACAGAGCTTTCCAGAGGCTGGAAGAGGCCACTCCAAGGCCTCTTTG
CCCCTGAGAGTGGTGGCTCTTCTTGAGGCCACCTTGCCACGCTGTCACAGGGAAC
TAGCAGCCCCTGCCTCACCCGGGGGTTTGGAAGATAGAGGGAGGCCTAGGAAGG
GCCCTGTGTCTCATCCGAGCTGGGCCCCTTTCCAGCCTCTCACTGGAAGGAAGCC
CAAGGATGTTCCTGTGGGGGCTTTTACCAGGCCCACCTGCCCTCTGCTGGCCATG
CTTGCAGCCTCCTGACCCTGTCCCAGCAGGACAGTGGGCTGGTGTGAGCGGGCA
GGAACCGCCTGCACTTAGAAGGTGTGGGGCTGCCTCCCCGAGCTTCCATCTGCCG
CTGGGGCCACACCCCAGGCCCAGGGATGGGACCCCACAGTGGTCACATCATCTT
GCAGCAGAACCCAGGTACAGCTCCTGGAGCAGATGGTGGTCCCAAGCACGGGTG
GGACCAGAAAGGACTCTCACCTGGGCTAACTCAGCTGCAGCCTCAGTTCCCTCCT
CACACACGACGAGGAACATGGACTGGAAGCCTGCCCAGCAGGCCTTCTGCTCGA
TGTGCGTTGTGTGGCTTACGTCCAGGGAGGGAAGCAGCCTCTGTGCTGTCTTCTA
GATAAGCCTGTATTCCCCGGGCTGTCTGCCAATGTATCCAGTTGTCCCGTCAGCC
TGGAAGCTCTGAGGGAAAACCTTGGGCTGCTTCCTGAGCACCTGTATCCCCTGCA
GCCAGCCCGGGGCCTCTGCTAGGAGCAGACTGAGCATGGCTTATGGGCCTGGCA
CCATCTGGCCTCTGCCCACCTTGCTGGCCTTGTCTTGTGTCTGCCCCTTCGACATT

CCATAGCCCAGCTCAATATCTAGTGGTTCCTCTAGGGTGGCGAGCACTGTTTGGT
CTCCAGATGTCTTCAGGTCGGAGCTCACAGCGCTCTCAGCCACCCCTTCCCAGTG
TAGCACCGGGCACATGGTAGATGCCTATTGATGAGTGAAAGCTCCTAACACACT
CAGAGAGCAAGGACTCCGCCTCATCCCACAGCCTGGGAGGAGAGGCAGACTGCC
AAGGACCTGCTCAGCATGCTACAGAAGAAACCAAAGTGCCCACGGGACTGATCA
GTGGAGCTTCCTGCCGAGACTGGAGGCCTTAGGGCAGGGTAGACAGTGTGTGTG
CAGGCTGGGGACTCACAGTTCGGACTGTGCCCAGACCTACTAGCATAGTGGGTG
GGTGGGAGGATGCGGGACTGGGGGCCGACCTTGCCTGAAATTCATGTGGGATCT
CAGAGCAGCCACTGAATTGCTCTGTAGGGGGCTAAATAGTGGCCCCCACAGATA
CACACACCCAGACAGAGCCTGTGAGCCAGACCTTATTTGGAGAAAAGGTCTTTG
TAGATGTAATTAAGCATCTCAAGATGGCATCATCTGGATTATGCGGTGGGCTGTA
AGTCCTGTGATGTGTCTTTATGAGAGAAAGGCAGAGGGAGATTTGACACACACA
GGAGGGGCCACGTGGAGACAGAGGTGGAGATTGGAGAAATGTGGCCACAAGCC
AGGGAACACCAGCAGCCACCAGAAGCCGGAAGACGTGAGGCAGGGTTCTTCCCA
GAGCCTTCGCTGCTGAGTCTGGGAATTTGTGACCGAAGCCATAAGAAGTGGGTA
CACGCCCTGAGCCTCCCACACTTGCTCACCTGTCCTGAGATGAGAATCTCTACTC
TGCAGCATATTTGGAGGATCACTGCGGGGGCCACAGAGGTGCTGTTCAGATGGC
ACTTCAGAAGACTCAGGAGACCCTGGGGCAGGAGCAGTTTGACTGACAGCCCAG
AGGGCTGCCCTCTGATTCCACCTGAGGCCCTGCTTTTCCTGGCTGCAGGGGTTCC
AGGGCCAGGCCATTTCCGCTGGCGCAGGACTCTGCTAGCAGCAACCTGCCTGAA
GTCTTCCTTTGGCCTGGCTGAGAGTTTCTGAGACCTGCGCTGGAGCGGAGGTGCT
TCCTTCCTTGCTTCCTTTCTTCCTCTCTCCCTTCTCCATCCAGCAGGCTGGACCTGC
CTGGCATCTGTGAGCTCTCCCTACTTTCTCCTATACCCTAACCTTTGTCCTGCATG
GGCGACTCCCCCAGTGAGTCTCTTGCAGCTTTTACCCCAGTGCCTGCTTCTTGGA
GAATCCAAACTGATCCAGTTAGGGATGATAAAGTGTAGGGTAGGCGCTCGGTGA
CTGTTTTCTCTGAGGTTGTGACTCGTGTGAGGCAGAAGCAGTCCCCGTGAGCCCT
CCTGGTATCTTGTGGAGTGGAGAACGCTTGGACCTGGAGCCAGGAGGCCCAGAC
ATACATCCTGTCCGAGCTGCAGCTTCCTGTCTCTAAAATGAGCCGGCCAGCGCAG
GTGGCCAGACATCACTGTTATTCTCCTTTGAGTCTTTAAATCTTGTTGTCTTTCTTG
CAGACTCGGTGAGCTGTGAAAGGCTATAATAGGGGCTTTATTTTACACTTTGATA
CTATTTTTTGAACATTCATATTATTGTTAGATATTGATATTCATATGAAGGAGCAG
GATGACTTGGGTCCTTCTTGGCAGTAGCATTGCCAGCTGATGGCCTTGGACAGTT
ACCTGCCCTCTCTAGGCCTCCCTTTCCTTGTCTATGAAATACATTATAGAATAGGA
TGTAGTGTGTGAGGATTTTTTGGAGGTTAAACGAGTGAATATATTTAAGGCGCTT

TCACCAGTGCCTGGGATGTGCTCTGTAGTTTCTGTGTGTTAACTATAAGGTTGACT
TTATGCTCATTCCCTCCTCTCCCACAAATGTCGCCTTGGAAAGACGGAGGCAGCC
TGGTGGAGGTGTATCTCCTAGACACCAGCATACAGAGTGACCACCGGGAAATCG
AGGGCAGGGTCATGGTCACCGACTTCGAGAATGTGCCCGAGGAGGACGGGACCC
GCTTCCACAGACAGGTAAGCACGGCCGTCTGATGGGAGGGCTGCCTCTGCCCAT
ATCCCCATCCTGGAGGTGGGTGGGGACTGCCACCCCAGAGCGTTGCAGCTGTACT
CCTGGGTTGCACCCCCCCCAGCTGTCACTGTCCCCTCCCTGCCATCAGTTGTGGG
AAGGGCGTTCATCCATCCAGCCACCTGCTGATTTGTTATAGGGTGGAGGGGGGGT
CTTTCTCATGTGGTCCTTGTGTTCGTCGAGCAGGCCAGCAAGTGTGACAGTCATG
GCACCCACCTGGCAGGGGTGGTCAGCGGCCGGGATGCCGGCGTGGCCAAGGGTG
CCAGCATGCGCAGCCTGCGCGTGCTCAACTGCCAAGGGAAGGGCACGGTTAGCG
GCACCCTCATAGGTAAGTGATGGCCCCAGACGCTGGTCTCTCTCCATCTGGACCT
GGCCTGGGAGGTGGCTTGGGCTGGGCCCAGGGAGAGCTAATGTCTCCTAACCAA
GAATGCTGTGGCAGCCTCTGCCGCAGAGCCAGAGAACCAGAGTGCCAAGGCTGG
CAGGGTTCCCAGTGGCCACGAGTGCAGATGAAGAAACCCAGGCCCCAAGAGGGT
CATGCAGGTAGCCCAGGGAGTTCAGCCTTGACCCTGGGTCAATGACCTTTCCACA
GTTCCACACTGCTCCCCTTTTAAAATCCGGTGATGTCTTTATGTCTTTTGTTATGTT
ATCTTCAATGTGGAGGGACTCGAGGTGATCTAAGCAAACTTTTTCTATCTTCTGCT
TGCATACCTCTGAGACCAGGGGACTCACTCACTTGCATGACTGGGCCCTGCAGGT
CACACTGGCCAGGCAGATGTGGTGGAGGAACTGGCAGAGGACTTTTTCTAGACT
GTGACTACATTTAGTCCACCCAGCGGCCCCCCTATGAAGTCCAGTTGAGAACTAG
GACTCTGGGGGCCGGTGGACAGAGAAGAGGGAGGGTTCTCTCCCTTACTGACTT
CCTTCTGTGGCCAGACATTGAGCAAGGCCTCTGTACAGCATGTCCTGGGGCTGGC
CTTGCCGTAGCTGCTAAATAGTTGACGAAACCAGTCCAGAGAGGGGAGGTGACT
GCCAGGGTCGCACAGCTCAAGCTGGGGAACTCGCTGGGAAAACTGTCAGCTCTG
GGCAGCAGCTTGACTTCCACTGTAAGCCCCAGCCCCCAGGGTCAAACACTGGCTC
TGGTGCTGGCAGAGGCAGCCCACTAGCCTGTTTCAAAGGCTGAGAAGGCCCAGG
AGTCTGCCCTGTGCTCCACCAGTTCTGCCCTGAGACTTTCCTACAGAGTACAGGT
TTTGATGTTCAGTTTTAAAGGCAAGAATCAATAACCTTCTGCCCCATCAGGTGAC
CCCTTGTGCCTGTCCCACCCCTTTATTGACTGACCTCGGCTCAGTCAGGTCAGTTC
CTGAAGGTCAGTGTGTGGAGGGGAGGCTGTTCTTTCCCAGAAAGGCCTTCCCCAG
GCCTGGTGCTCTGGCCTCTGGAGGACTTCCTGGAGAAGTCCCTTCTTTGGGGTCC
CAGTCAGTGTATGGGAAGCCCTTATTGCATGACCTGGCACGGGGCAGGGGCTCA
ACAGTCACTATTGCCTTCCTTGCCACTGCCATTTCCTCCTCTGTAAGCAGGTGATT

GTGTGTCCAGTCTGAGCACAGAGATAAGCACACAGCAGGTGCTTAATAACTAGC
AGCTGTAGGCTGGGCGCGGTGGCTCATGCCTGTAATCCCAGCACTTTGGGAGGCC
GAGGTGGGCAGATCACCTGAGGTCAGGAGTTCGAGACCAGCCTGTTCAACATGG
TGAAACCCCGTCTCTACTAAAAATACAAAAATTAGCCAGGCATGGTGGTGGGTG
TCTGTATCCCAGCTACTTGGGAGGCTAAGGCAGGAGAATCGCTTGAACCCAGGA
GGTGGAGGTTGCAGTGAGCTGAGATCGTGCCACTGCAATCCAGCCTGAGTGATA
GAGCGAGATTCCATCTCAAAAATAAATAAGTAAATAACTAGCAGCTGTAAATGT
GGCTGTTGTTCTTCACCTCCACACTCAGTGCCACTCCACTCCCTCCCTCCGTGGTG
TGAGGGGCCTCACTAGCTGTCTCCTAGGAGGAGCATGGCTGTGAGATTCCAGCTC
CATCCTTGGCCACGGCTCCTGGAGACATCTTAGAGGCCAGGATCCAGAAGGCTC
CCACACCTCATTTGACAGGGGAGAAGCTGTCAGTTCCAGGTCCCCTTGCACATCA
GGGCCAGAGCTGCGTTAGGCCTCCAGTCTCCAGGCCACTGGGCCAGAGCTCACA
GGCTGGCAGAGGGTTAGAACTGTTACTGGTGGCTGGGTGCAGTGGCTCACGCCT
GTAATCTTAGCACTTTGGGAGGGCAAGGCGGGAGGATCATGAGGTCAGGACATC
GAGACCATCCTTGCTAACACGGTGAAGCCCCGTCTCTACTAAAACTACAAAAAA
TTAGCCGGGCGTGGTGGCAGGCGCCTGTAGTCCCAGCTACTCAGGAGGCTGAGG
CAGGAGAATGGCGTGAACCCGGGAGGCGGAGCTTGCAGTGAGCCGAGATTGCGC
CACTGCACTCCAGCCTGGGCAATAGAGCGAGACTCCGTCTGGAAAGAAAAAAAA
AAAAAAGAGCTGTTACTGTTGACAGTAGCATGAGGTAGACCATGGCCTGCACCA
AAATGGGGGAGTGGAGTGCCACTGAGGCCAGAAGGAACCACACCCTCAAGGGT
GGGGAGTTATGGTATGGGGGGTCCTAGGCATGGAGTCTTTTAATTCTTTAGACAA
TCCTGGGAGCAACTGTCCCTGTTTCACAGAGGGCGGGGCCACACAGCTGGTGAG
TGGGCAGCCAAGACTCTGTTCAAGTTTGTGTGGGTCCAACACTTGCGGCCACGGT
GGAGGGGCATCTGAGCCAGGCCTCAGAGAGTGGCGGGGGAAGTTGGGTGGGGA
AGTGTGCCCTTCTCATTCCTCTGAGGCTCATCCTCTTGGTGCCTCTCTTTCATGGA
AAGGGATAATAAGGTTATTGTGAGGATCCCCTGAGTTCGTATATTCAGACGCTTA
GACAGAGCCAGGCACAGAGAAGGGCCCGGGGTTGGCTAGTTTGATTGCTGGTGT
AATTGCTAATATCTTCCAGTTTGTATTGGTCAAGGTTCTGCAGAGAAGCAGAACC
AGTAGGATGTATATATTAAGAGTTTCAAGCTCATGTGACCGTGCGGGCTGGCAAG
TCTGAAATCCGCAGGGCAGGCCAGGCAGGCTGGCAATTCCTGCAGAATTTGATG
TTGCAATACTGAGTCCTAAGGCAGTCCTGGGGCAGAATTCCTTCTTCCCTGGGAG
GCCTCAGTCTGTTCTCTTAAGGCCTTCAACTGATTAAATGAGGCCTGCCCAAGTT
ATAGAGAGTAACCTGCCTTACTCCGTCTTCTGATTTAAATGTTAGTCACATCTAA
AAAATATTTTCGCAGCAGCATTTCCACTGGCTTTTGACCAAACATCAGGCCACAA

AGTTGATCCCCAAAATTAACCATCACTCTGTGCCTGTAAGGGAGGGGCTGGGAA
AGGGGAGCAGGTCTCCCCAAGGGGTGACCTTGGCTTTGTTCCTCCCAGGCCTGGA
GTTTATTCGGAAAAGCCAGCTGGTCCAGCCTGTGGGGCCACTGGTGGTGCTGCTG
CCCCTGGCGGGTGGGTACAGCCGCGTCCTCAACGCCGCCTGCCAGCGCCTGGCG
AGGGCTGGGGTCGTGCTGGTCACCGCTGCCGGCAACTTCCGGGACGATGCCTGC
CTCTACTCCCCAGCCTCAGCTCCCGAGGTAGGTGCTGGGGCTGCTGCCCCAAGGC
GCGGGTAGGGGGCGGAGGGCGGAGGGCGGAGGGAGGGCGGGCGGGCAGGCGG
GCTTCTTGTGGCACGTGGGCTTCTTGTGGCACGTTCCTGGAGGCCGAACCCTTCT
GGCTTTGGAAGGAGTCGTCAGAGACCCCCGCCATGCGGGAGGCTGGGGAGGAAG
GGGCTCGAAACCTCCATCATCGCAGAGTCTGAATAGCAGTGGCCCCGCCATGCG
CCCACGTAGCGGCGCCTACGTAGCCACGCCCCCACACCCCGTCCTGGCCACTCTC
CCTCCTGAAGGTCTTCTGGTACCCGCCCCCTCCCCATCTCCATCCCCAGGCCCTGC
GTCCTCTGCCCAATACTCTTTGGGCCTCCCTGTTGTCCAGCTCTCTCCGCGGCTCC
ATGACTGACAACTTGAGCAAGGCTAATGTGAATGGGAGCGGTTGAGGGCTCAGA
CCTCTCACCCGAGGAACATCCACAGAGTGTGCCGCATGCCCGGTGCAGTGTGGCT
GCGGGGACACAGACACGGAGCCTCGGCCCTGAGGAGCTGGGGGGCAGTGACCG
TCCCTCCTCTGACCCACCACTCCTCCAGTGTCAGGACACTGCGGGTATCTAGGGG
AAGGAATCTTGTTCCACTTCAAGTCTGGAACTTCAAGTCTGTGTGTGTGCGTGCG
CGCGCGCGCGTTGGGGGTGGGGGTTGCAGAGCAGATGCGTACCTGACAGCGGTA
ACCTAGGTCCCCCCTGGCCTATCAAGGCTTCCCTGGCGGCCGAATTTAAAGGCAT
CAAGCAAACAAAGCCCAACACATCTCTGCCTTGTCCTCTCAGTTTCCCCCCGTGG
CACTTAGAACCACTTGATACACCGAATAGTTTCCTATCTCCCCCACTAGGATGTA
AACTCCACAGGGGCATTGGGAATGCTGCCTGGCTATGGTAGGGACAGAGGGGAG
CACCAGGGCGGGGCAGGGGTGCCAGAGTTCTGCCTGGGCAGTCAGATTTTCCTT
AGGAGGGGACATTTGAGTGGGACCCAAACAGGTGTATAGCAGTTGTCCAGCCCA
GCTGGCAAGGCCTGAGTCTGCCTCTGCAACCCCTCTCTTGGGCTCCTTTCTCTGCC
ACCCACCTCCTCACCTTTCCAGGTCATCACAGTTGGGGCCACCAATGCCCAAGAC
CAGCCGGTGACCCTGGGGACTTTGGGGACCAACTTTGGCCGCTGTGTGGACCTCT
TTGCCCCAGGGGAGGACATCATTGGTGCCTCCAGCGACTGCAGCACCTGCTTTGT
GTCACAGAGTGGGACATCACAGGCTGCTGCCCACGTGGCTGGTAAGTCACCACC
CCACTGCCTCGGCCACCGTGATGCTAACAGCCCCTTTGGCAGTCAGGGTCTGTGC
CGGGACCTCCAGTGCCAGGCTCTGTGCAGGGGGACCAGAGATGAAGTAGGCCTG
ATGGTGCCTTCAAGGACACTCAGTCTGATGAGGGAGGCGAGTGCACAGAGGAAA
CACGAGGTCAGGGCTGTATTAGAGGGAGCCCAGAGGAGGCACCTGCCCAGCCCG

AGGGTCAGAGAAGGCATCTTGGAGGAGGGACATTTGATCGGGAGCTTGATGGAT
GAATAGGAGTTCACCTGGCCGATAAGACAGCAACTACCAAGGCTTAGAGGTGTG
AGAGGAGGCTGTCTTACCTCACTGAGTAAGGACTGCAGGCGGCTTACCTTCGAG
AAGAGAGCTTAGTGTCTGTGTGCACGTGTGTTTGTGTGTATGTGTGTGCGTGTGT
GCACTGGCAGGAGTCCCCTGCTGGGGCAGGAGGGCCGGGCCATCACCATCTTTC
ACCATTCACCCCTGCACCAGGCATTGCAGCCATGATGCTGTCTGCCGAGCCGGAG
CTCACCCTGGCCGAGTTGAGGCAGAGACTGATCCACTTCTCTGCCAAAGATGTCA
TCAATGAGGCCTGGTTCCCTGAGGACCAGCGGGTACTGACCCCCAACCTGGTGG
CCGCCCTGCCCCCCAGCACCCATGGGGCAGGTAAGCAGGATGGCAGGGTGGGCA
AGTCCAGGCTGGGGCTTGGGAGGTCTGTGTGACCTTGACAGTCTCTCCCTTCTCC
CTTGTCTGTGTAAGGAGGATGACGCCACCTTAAATAGGATTAAATGAGAATGGG
GCTCTGAAAGGGCTGTGCAATATTTTCATAACGTGTTTTTATAGAGACAGTTGAG
TATGTTCTTTAAGCCCTCCTCTCTCCTACCATGAACTAAAGATTTCTGTGGAGGTC
CCCTCACTCCCAGCACCCCCTCCTCATCCCAGGCCCTTTTTGCAGGTTGGCAGCTG
TTTTGCAGGACTGTATGGTCAGCACACTCGGGGCCTACACGGATGGCCACAGCC
GTCGCCCGCTGCGCCCCAGATGAGGAGCTGCTGAGCTGCTCCAGTTTCTCCAGGA
GTGGGAAGCGGCGGGGCGAGCGCATGGAGGTGACTGTACCCCTCCTTCGTGTGT
GTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTCAGTGCTGGG
CCCTCAGGGACCCCCAGCAAGCCCCTCCATCCTCCAGACTCCAGCTCTTCTGTAA
GCTTACAGGGCTGGCCAGACCAGGAGTGGGGCACTCCTCACTTCACGCGGCTGG
GGGCTGCTGGAGAGAGCCACAGCGGGAAGGGTTTCCTAGAGGCTGCAGGACAGT
GCTGGATGGATTTTCAATGCTCACCTGGGTGTGAGCGTGCGGCAGGGCCGCGTG
AGGGTCAGCGATCTGCTACTCTGGACTCAGCCATCTCTAGGCCCCTCTCACTCAG
GTGCTCCATGGTTCTGGGAGCTGAGAAATCTCAAACCAGCAAAAAAGTGGAATT
GATGTTGATGCTACAGGATAGTGCACAGATGCCATCTGGTTGCAGCATTTTGGTG
GAAGGGCAGTGCCCAGCTAGGAGAGTGAGGAGGGGCAGGCATTTCTGGCTTGAG
GAGATGGGGTCTTAATGCTCGTGTGAGAGGCAGAGTGGGTGGAGTGGAGCTGGC
TGGATCCTTGCTTTGGCCTCCTGGATTTCTCTCTATCTCCATTTTGAAACCACTCT
GTGTTTGGAAGAACTTTTGAGTATTCAGAGCTGCCCACTGGCAGAACAGTCTTCC
TTGGGCAGGAGTGAGCTCCTTGTCCCCAGAAGGCTGGGTCTGGCTGGCCCCTGGC
AGGGACACTGATGAGGGTGCTTGAGTTGATCCTGTCTAGTCCCTTTCTGTGTTTTC
AAAGCCCATTCTAAAGCAGATTCCCATTTCCGTCTTTGACTCTAAGGCCCAAGGG
GGCAAGCTGGTCTGCCGGGCCCACAACGCTTTTGGGGGTGAGGGTGTCTACGCC
ATTGCCAGGTGCTGCCTGCTACCCCAGGCCAACTGCAGCGTCCACACAGCTCCAC

CAGCTGAGGCCAGCATGGGGACCCGTGTCCACTGCCACCAACAGGGCCACGTCC
TCACAGGTAGGAGGCTGGGCTTGCCCTGGGGTGAGGAGGGGTCTCTTTCTCCTTA
TGCACCCACTGCCCGCGAGGCTTGGTCCTCACAAGTGTGATCCATGAGACTCAAG
CCTGACTTGCAGTTCCATACTCTGGTTCTGCCACTTCCATGCCCTTTGAGCCTGGG
CAGGTGACCTTACTTCTCCTCATCTCAGCTTCCTCCTCCATAAGAGGGAAAAAGG
TATTACCTGCCTCATTGTGTTGCAAGGAGATGGGCAGCATCTAGGGCACTGGCCT
GGAGTATCGCAGGTGCTTTGCCTAAGGTGGTGCAGTCCAGGAGAGGCAGCTCCA
GAGAGAGGCCCCCGGCTGGGGCTGAAAGGAGGGCAGACCTCGGTTTGAATTTCA
CCCTGCCGCTCTATAGCTGTGTGACTTGGGCAAATTACTTAACATCTCTGTATGA
GGAAATGATGAGTGCTAAGCACTTAGCTTAGTGCCGGGACAATATAAATTCTAG
CTATCGTTACTATTGTTTTCATCACCCGTTGCTTTAAAATCCAGCCTCTGGTATAG
GCAACTATTGACGGGCTACCCTGTGTCGAAAACATGCCCAGGCAGGTAGCAGGA
AGTCACAGATGGGGACCTCTTGGGGCATCAAGGGATGGTGCCCTGAGGCTGAGC
TGTTCTGGTTGGGTGGAGCATGAGAGGTCTGGGAAGACAGTGGGACTCCAGCCT
GGAATAAGAGGCTCAGAGTTGATTCTCGTCTGAGCACGTCCAGGGGAACCACTG
AGGGTTTGGGAACAGGAGAGTGAGGGTGAGAACCTGGTTCTGGGCACAGCAGGC
TGGCATGTAGGATGGATGTTCAGGAAAGATGAGCATAGTCAGGTGGCTGGTGCC
CTTGTCCAGGGGAGAGGCTCCGTCAGGTTCAGGGGTCCTGGCTTGGAGGGAAGT
CCGCCATGCTCTAATCACGCTCCCCTTTGGAAGTGCTCAGCCGATGAGCTCACAG
GCACATGTCAGTTTGAAGTCATGGAATCTGACTCCATGAAGCGCACCTCAAAGA
GCACCATTTTGCAGCTAAGGGAACTGCAGGCTGGACATGCTGAGTGGCTGCCCC
GAGCCCTTGCAGCTAGGACATAGAGAATGCTAGTAACCACAACCCTACCATGTT
CAGAGCACATGCCAGGCTCCATGCTGGGGCTTCGCACGTGTCATCTTCACAGTGT
CCCTGTGAGTAGGTGTGGTTTCTCTTTCCATCTTACAAATGAGTAAACAGAGCCT
CAGTGTAGCTAAGTAACCACTATTTTAGGTTTCTTAGCCAATGGGTGTGTCTGAC
TCCTAAGCCCATGGAGGGCATTCTGAGGTGGTTCAGACAGACCCCGGCTTACCCT
TGAACTTCTGCCTGCTGGCTGCATAGGGAGGGGCTGGGGGGAGTTTGAGCATCTC
AGGCCATAGAGCCCCTGCCTCACTGTCTCCATCTCTGGGTGGAAAGATGGTGTTT
TCCCTGAGAAACTAAGGCTCAGAGAGGTTGAATGGCTCTCCCAAGGTCACACAG
CTGGTCAGCTGCAGAGTTGAGAACACAGGAGTCCTGGTGCTCAGGCCAGCATCT
CTTTTTTTCTTTGAGTTGTTTCTAGGTTTCCTAGCTCTTGCCTCAGACCTTAAAGAG
AGAGGGTCTGATGGGGATGGGCACTGGAGACGGAGCATCCCAGCATTTCACATC
TGAGCTGGCTTTCCTCTGCCCCAGGCTGCAGCTCCCACTGGGAGGTGGAGGACCT
TGGCACCCACAAGCCGCCTGTGCTGAGGCCACGAGGTCAGCCCAACCAGTGCGT

GGGCCACAGGGAGGCCAGCATCCACGCTTCCTGCTGCCATGCCCCAGGTCTGGA
ATGCAAAGTCAAGGAGCATGGAATCCCGGCCCCTCAGGAGCAGGTGAAGAGGCC
CGTGAGGCCGGGTGGGTGGGGTGCTGCGTGTCTCTCCTGCACAGCTTTTCTGTGT
CAGTTTGTGCCACCACCATACCGCCATGCATCAGGGTGGCGGTTTGCCAGGTAGA
TGCTGTGGGCAGCTTCCGCCATTGTGTGGACAGCATGTATATGTGTCTCTGTGTG
GCTGGGTCTGTTTTTGCTTTTGTCCAGATCAGTAAGGTTTGCTACCTGGGTACCCC
ACTCCACTTGGAGTAGAATGTGCATAAATATGGCATAAAGAAATGCAATATGCA
TGCATTTATTGATTGATCTATTTTTTTCTGAGATGGGGTCTTGCTGTGTTGCCCAG
GCTGGTCTCAAATTCCTGGGCTCAAGCAATCCTCTGGTCTCAGCCTCCCCAAGTG
TTGGGATTATAGGCATGAGCCGCTGCACCTGGCCTCTCTGATCTATTTAACAAAC
CTGCTGGGAGGGTCTCAGGGTCAGGAGCAGCACTGGGCTCTGAGGACACAGAGC
TCACTCAGCCGTGACCCAGAGGGGGTGCCTGAGCTGCATGCTGAAGGTTGTTAG
CATGACCAGCAAGGCAAGAAAAGGCCCTGCCGAGATTAGCAAGGCATGTGCCAA
GCCCTGGAATGTGACAGCCGGGCCTTCTAGAAACCTGAGTGTATAACTCTCCTTA
AAAGCCAGTAGGAGCTCCTCAAAAGGCAGCCCTAAGGAGTCCACTCTTAAATGA
ACTCAGAGTCAGTTTTAAAATGCAAGTCTGTGTTGATTCTGGTCTGGATGGTGCA
TTCCTCGAGAGCAAAAGACAGTCTTGGTCTTGGATCCACTTGCCCTGGGTACACT
GAGGGCTGCTAGGTTCCAGGTGCTCTTCCTGGCACTGGGGAGGGATACAGGCCC
AAGAGACATGCTGTTCTCCCTCCTGGAGCATCTATTTTAGTGGAGGAAGACAGAA
AACAAACCATTAATATAGAGTACTGAAAAGATGCGATGGAGAAAACTATAGCAA
GGAAGGGAATGGGGTGGGAGAGAGGTCAGGAGAGGTCTCGCTGACAAGGTGGA
CGAAACAGGCCATGAGGCAGAGAACATGTTCCAGGCAAAGCAAAGGCCCCCAG
GTGGGGATGTGCAGGGAGTACCAGGAAACCAGAGAGGTGGGAATAGTTATGAG
ATGGGGGGTGCCTCAGAGGGGACAGGGCCAAGTCAGGTGAGACCTGAGGGTCA
CAGTCAGCAGTGAGCTGGGGCCATGCAGGGGTCTGGCCTCAGAGGAGTGTGGTC
TGGCCTGGATCTGAACCTCTCACTGTGGCCTAGCTGCTGAGCTGAGAAGAGATGA
CAAGGACCTTGGGCAGAAGCAGGGAGACTGGAGGGAGGCGGTGGAGGGTCCAG
GCGTTGGGGCGGGGCTCAGGCTGGAGTCTGAAGGGAGCCTGCAGGCCTGGTGGG
TGGATGTGGGTGGGAGAGGGGGAGGATGGCACCAAGGCTCGGGCCCCTGGACA
GATGGAGTTGCCATTAAGTGGGATGGGGCAGGCTATGGGGCCATCAGTTTCAGA
GGGATGAGTTTGGCACTGGCATGGTAGGCATCTGTCTATCTCCACGGCCCTCAAA
CCAGGCATGAAGCAGGAGCTCACGTGTTTGGTCAGCCATGGTGCAGAACCGCCT
GGGTGGGAGGTGCGGGGTGGGAGATACACGGTTGTGTCCCAAATGGGCTCTGAG
CCAGCGAGGGCCGTCTGCACTTTGGCCTCACAGAAGGATGTCGGAGGGAGAAAT

GAAGTGTGGGTGGGGGTCCCGGGCCACGCTAGACATGTGCTTTCTTTTCCTCGGG
CTCTGGCAGGTGACCGTGGCCTGCGAGGAGGGCTGGACCCTGACTGGCTGCAGT
GCCCTCCCTGGGACCTCCCACGTCCTGGGGGCCTACGCCGTAGACAACACGTGTG
TAGTCAGGAGCCGGGACGTCAGCACTACAGGCAGCACCAGCGAAGGGGCCGTG
ACAGCCGTTGCCATCTGCTGCCGGAGCCGGCACCTGGCGCAGGCCTCCCAGGAG
CTCCAGTGACAGCCCCATCCCAGGATGGGTGTCTGGGGAGGGTCAAGGGCTGGG
GCTGAGCTTTAAAATGGTTCCGACTTGTCCCTCTCTCAGCCCTCCATGGCCTGGC
ACGAGGGGATGGGGATGCTTCCGCCTTTCCGGGGCTGCTGGCCTGGCCCTTGAGT
GGGGCAGCCTCCTTGCCTGGAACTCACTCACTCTGGGTGCCTCCTCCCCAGGTGG
AGGTGCCAGGAAGCTCCCTCCCTCACTGTGGGGCATTTCACCATTCAAACAGGTC
GAGCTGTGCTCGGGTGCTGCCAGCTGCTCCCAATGTGCCGATGTCCGTGGGCAGA
ATGACTTTTATTGAGCTCTTGTTCCGTGCCAGGCATTCAATCCTCAGGTCTCCACC
AAGGAGGCAGGATTCTTCCCATGGATAGGGGAGGGGGCGGTAGGGGCTGCAGG
GACAAACATCGTTGGGGGGTGAGTGTGAAAGGTGCTGATGGCCCTCATCTCCAG
CTAACTGTGGAGAAGCCCCTGGGGGCTCCCTGATTAATGGAGGCTTAGCTTTCTG
GATGGCATCTAGCCAGAGGCTGGAGACAGGTGCGCCCCTGGTGGTCACAGGCTG
TGCCTTGGTTTCCTGAGCCACCTTTACTCTGCTCTATGCCAGGCTGTGCTAGCAAC
ACCCAAAGGTGGCCTGCGGGGAGCCATCACCTAGGACTGACTCGGCAGTGTGCA
GTGGTGCATGCACTGTCTCAGCCAACCCGCTCCACTACCCGGCAGGGTACACATT
CGCACCCCTACTTCACAGAGGAAGAAACCTGGAACCAGAGGGGGCGTGCCTGCC
AAGCTCACACAGCAGGAACTGAGCCAGAAACGCAGATTGGGCTGGCTCTGAAGC
CAAGCCTCTTCTTACTTCACCCGGCTGGGCTCCTCATTTTTACGGGTAACAGTGA
GGCTGGGAAGGGGAACACAGACCAGGAAGCTCGGTGAGTGATGGCAGAACGAT
GCCTGCAGGCATGGAACTTTTTCCGTTATCACCCAGGCCTGATTCACTGGCCTGG
CGGAGATGCTTCTAAGGCATGGTCGGGGGAGAGGGCCAACAACTGTCCCTCCTT
GAGCACCAGCCCCACCCAAGCAAGCAGACATTTATCTTTTGGGTCTGTCCTCTCT
GTTGCCTTTTTACAGCCAACTTTTCTAGACCTGTTTTGCTTTTGTAACTTGAAGAT
ATTTATTCTGGGTTTTGTAGCATTTTTATTAATATGGTGACTTTTTAAAATAAAAA
CAAACAAACGTTGTCCTAAC (SEQ ID NO: 5)

[440] Human PCSK9 Amino Acid Sequence (NP_777596.2)

MGTVSSRRSWWPLPLLLLLLLLLGPAGARAQEDEDGDYEELVLALRSEEDGLAEAP
EHGTTATFHRCAKDPWRLPGTYVVVLKEETHLSQSERTARRLQAQAARRGYLTKIL
HVFHGLLPGFLVKMSGDLLELALKLPHVDYIEEDSSVFAQSIPWNLERITPPRYRADE
YQPPDGGSLVEVYLLDTSIQSDHREIEGRVMVTDFENVPEEDGTRFHRQASKCDSHG

THLAGVVSGRDAGVAKGASMRSLRVLNCQGKGTVSGTLIGLEFIRKSQLVQPVGPL
VVLLPLAGGYSRVLNAACQRLARAGVVLVTAAGNFRDDACLYSPASAPEVITVGAT
NAQDQPVTLGTLGTNFGRCVDLFAPGEDIIGASSDCSTCFVSQSGTSQAAAHVAGIA
AMMLSAEPELTLAELRQRLIHFSAKDVINEAWFPEDQRVLTPNLVAALPPSTHGAGW
QLFCRTVWSAHSGPTRMATAVARCAPDEELLSCSSFSRSGKRRGERMEAQGGKLVC
RAHNAFGGEGVYAIARCCLLPQANCSVHTAPPAEASMGTRVHCHQQGHVLTGCSSH
WEVEDLGTHKPPVLRPRGQPNQCVGHREASIHASCCHAPGLECKVKEHGIPAPQEQ
VTVACEEGWTLTGCSALPGTSHVLGAYAVDNTCVVRSRDVSTTGSTSEGAVTAVAI
CCRSRHLAQASQELQ (SEQ ID NO: 6)

Apolipoprotein C3 (APOC3)

[441] In some embodiments, the target gene for modification using the compositions and methods disclosed herein is gene encoding APOC3. The LDL-R mediated cholesterol clearance pathway involves multiple players. Non- limiting examples of protein factors involved in this pathway include: Apolipoprotein C3 (APOC3), LDL receptor (LDL-R), and Increased Degradation of LDL Receptor Protein (IDOL). These protein factors and their respective function are described in the art. Further, loss-of-function variants of these factors have been identified and characterized, and are determined to have cardio protective functions. See, e.g., Jorgensen et al., N Engl J Med 2014; 371:32-41July 3, 2014; Scholtzl et al, Hum. Mol. Genet. (1999) 8 (11): 2025-2030; De Castro-Oros et al., BMC Medical Genomics, 20147: 17; and Gu et al., J Lipid Res. 2013, 54(12):3345-57, each of which are incorporated herein by reference. Thus, some aspects of the present disclosure provide the generation of loss-of- function variants of APOC3 (e.g., A43T and R19X), LDL-R, and IDOL (e.g., R266X) using the methods and compositions disclosed herein.

[442] Apolipoprotein C-III (APOC3) is a protein that in humans is encoded by the APOC3 gene. APOC3 is a component of very low density lipoproteins (VLDL). APOC3 inhibits lipoprotein lipase and hepatic lipase. It is also thought to inhibit hepatic uptake of triglyceride-rich particles. An increase in APOC3 levels induces the development of hypertriglyceridemia. Recent evidence suggests an intracellular role for APOC3 in promoting the assembly and secretion of triglyceride-rich VLDL particles from hepatic cells under lipid-rich conditions. However, two naturally occurring point mutations in human apoC3 coding sequence, A23T and K58E have been shown to abolish the intracellular assembly and secretion of triglyceride-rich VLDL particles from hepatic cells.

[443] Loss-of-function mutations that may be made in APOC3 gene using the methods and compositions described herein are also provided. The strategies to generate loss-of-function mutation are similar to that used for PCSK9 or ANGPTL3 (e.g., premature stop codons, destabilizing mutations, altering splicing, etc.).

[444] In some embodiments, the gene modification methods and compositions described herein reduces expression of functional APOC3 protein encoded by the APOC3 gene in the cell. In some embodiments, the modification reduces expression of functional APOC3 protein encoded by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or 100%. In some embodiments, the modification reduces expression of functional ANGPTL3 protein encoded by the APOC3 or ANGPTL3 gene in the cell by at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 10 fold, at least 20 fold, at least 25 fold, at least 30 fold, at least 40 fold, at least 50 fold, at least 60 fold, at least 70 fold, at least 80 fold, at least 90 fold, at least 100 fold, at least 200 fold, at least 300 fold, at least 400 fold, at least 500 fold, at least 600 fold, at least 700 fold, at least 800 fold, at least 900 fold, at least 1000 fold, at least 2000 fold, at least 3000 fold, at least 4000 fold, at least 5000 fold, at least 6000 fold, at least 7000 fold, at least 8000 fold, at least 9000 fold, or at least 10000 fold. In some embodiments, the modification abolishes expression of functional APOC3 protein encoded by the APOC3 gene in the cell.

[445] The protein sequence of human APOC3 can be found, for example, at Deposit No. NP_000031.1, which reference is incorporated herein in its entirety. Human nucleic acid sequences can be found at e.g., GenBank Accession Nos.: NG_008949.1, which sequence is incorporated herein in its entirety. Mouse, rat and monkey APOC3 nucleic acid sequences have been deposited; see, e.g., Ensembl accession number ENSMUSG00000032081, ENSRNOG00000047503, and ENSMFAG00000001837 respectively, each of which sequences is incorporated herein in its entirety.

[446] The polypeptide and coding nucleic acid sequences of APOC3 and of other members of the family of human origin and those of a number of animals are publicly available, e.g., from the NCBI website or ENSEMBL website. Examples include, but are not limited to the following sequences, each of which sequences are incorporated herein in their entireties;

[447] NG_008949.1:5000-8165 Homo sapiens apolipoprotein C3 (APOC3), RefSeqGene on chromosome 11

CTGCTCAGTTCATCCCTAGAGGCAGCTGCTCCAGGTAATGCCCTCTGGGGAGGGG

AAAGAGGAGGGGAGGAGGATGAAGAGGGGCAAGAGGAGCTCCCTGCCCAGCCC
AGCCAGCAAGCCTGGAGAAGCACTTGCTAGAGCTAAGGAAGCCTCGGAGCTGGA
CGGGTGCCCCCCACCCCTCATCATAACCTGAAGAACATGGAGGCCCGGGAGGGG
TGTCACTTGCCCAAAGCTACACAGGGGGTGGGGCTGGAAGTGGCTCCAAGTGCA
GGTTCCCCCCTCATTCTTCAGGCTTAGGGCTGGAGGAAGCCTTAGACAGCCCAGT
CCTACCCCAGACAGGGAAACTGAGGCCTGGAGAGGGCCAGAAATCACCCAAAG
ACACACAGCATGTTGGCTGGACTGGACGGAGATCAGTCCAGACCGCAGGTGCCT
TGATGTTCAGTCTGGTGGGTTTTCTGCTCCATCCCACCCACCTCCCTTTGGGCCTC
GATCCCTCGCCCCTCACCAGTCCCCCTTCTGAGAGCCCGTATTAGCAGGGAGCCG
GCCCCTACTCCTTCTGGCAGACCCAGCTAAGGTTCTACCTTAGGGGCCACGCCAC
CTCCCCAGGGAGGGGTCCAGAGGCATGGGGACCTGGGGTGCCCCTCACAGGACA
CTTCCTTGCAGGAACAGAGGTGCCATGCAGCCCCGGGTACTCCTTGTTGTTGCCC
TCCTGGCGCTCCTGGCCTCTGCCCGTAAGCACTTGGTGGGACTGGGCTGGGGGCA
GGGTGGAGGCAACTTGGGGATCCCAGTCCCAATGGGTGGTCAAGCAGGAGCCCA
GGGCTCGTCCAGAGGCCGATCCACCCCACTCAGCCCTGCTCTTTCCTCAGGAGCT
TCAGAGGCCGAGGATGCCTCCCTTCTCAGCTTCATGCAGGGTTACATGAAGCACG
CCACCAAGACCGCCAAGGATGCACTGAGCAGCGTGCAGGAGTCCCAGGTGGCCC
AGCAGGCCAGGTACACCCGCTGGCCTCCCTCCCCATCCCCCCTGCCAGCTGCCTC
CATTCCCACCCGCCCCTGCCCTGGTGAGATCCCAACAATGGAATGGAGGTGCTCC
AGCCTCCCCTGGGCCTGTGCCTCTTCAGCCTCCTCTTTCCTCACAGGGCCTTTGTC
AGGCTGCTGCGGGAGAGATGACAGAGTTGAGACTGCATTCCTCCCAGGTCCCTC
CTTTCTCCCCGGAGCAGTCCTAGGGCGTGCCGTTTTAGCCCTCATTTCCATTTTCC
TTTCCTTTCCCTTTCTTTCTCTTTCTATTTCTTTCTTTCTTTCTTTCTTTCTTTC
TTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCCTTTCTTTCTTTCCTTTCTTTCTT
TCCTTTCTTTCTTTCTTTCCTTTCTTTCTCTTTCTTTCTTTCTTTCCTTTTTCTTTCTTT
CCCTCTCTTCCTTTCTCTCTTTCTTTCTTCTTCTTTTTTTTTTTAATGGAGTCTCCCTC
TGTCACCTAGGCTGGAGTGCAGTGGTGCCATCTCGGCTCACTGCAACCTCCGTCT
CCCGGGTTCAACCCATTCTCCTGCCTCAGCCTCCCAAGTAGCTGGGATTACAGGC
ACGCGCCACCACACCCAGCTAATTTTTGTATTTTTAGCAGAGATGGGGTTTCACC
ATGTTGGCCAGGTTGGTCTTGAATTCCTGACCTCAGGGGATCCTCCTGCCTCGGC
CTCCCAAAGTGCTGGGATTACAGGCATGAGCCACTGCGCCTGGCCCCATTTTCCT
TTTCTGAAGGTCTGGCTAGAGCAGTGGTCCTCAGCCTTTTTGGCACCAGGGACCA
GTTTTGTGGTGGACAATTTTTCCATGGGCCAGCGGGGATGGTTTTGGGATGAAGC
TGTTCCACCTCAGATCATCAGGCATTAGATTCTCATAAGGAGCCCTCCACCTAGA

TCCCTGGCATGTGCAGTTCACAATAGGGTTCACACTCCTATGAGAATGTAAGGCC
ACTTGATCTGACAGGAGGCGGAGCTCAGGCGGTATTGCTCACTCACCCACCACTC
ACTTCGTGCTGTGCAGCCCGGCTCCTAACAGTCCATGGACCAGTACCTATCTATG
ACTTGGGGGTTGGGGACCCCTGGGCTAGGGGTTTGCCTTGGGAGGCCCCACCTG
ACCCAATTCAAGCCCGTGAGTGCTTCTGCTTTGTTCTAAGACCTGGGGCCAGTGT
GAGCAGAAGTGTGTCCTTCCTCTCCCATCCTGCCCCTGCCCATCAGTACTCTCCTC
TCCCCTACTCCCTTCTCCACCTCACCCTGACTGGCATTAGCTGGCATAGCAGAGG
TGTTCATAAACATTCTTAGTCCCCAGAACCGGCTTTGGGGTAGGTGTTATTTTCTC
ACTTTGCAGATGAGAAAATTGAGGCTCAGAGCGATTAGGTGACCTGCCCCAGAT
CACACAACTAATCAATCCTCCAATGACTTTCCAAATGAGAGGCTGCCTCCCTCTG
TCCTACCCTGCTCAGAGCCACCAGGTTGTGCAACTCCAGGCGGTGCTGTTTGCAC
AGAAAACAATGACAGCCTTGACCTTTCACATCTCCCCACCCTGTCACTTTGTGCC
TCAGGCCCAGGGGCATAAACATCTGAGGTGACCTGGAGATGGCAGGGTTTGACT
TGTGCTGGGGTTCCTGCAAGGATATCTCTTCTCCCAGGGTGGCAGCTGTGGGGGA
TTCCTGCCTGAGGTCTCAGGGCTGTCGTCCAGTGAAGTTGAGAGGGTGGTGTGGT
CCTGACTGGTGTCGTCCAGTGGGGACATGGGTGTGGGTCCCATGGTTGCCTACAG
AGGAGTTCTCATGCCCTGCTCTGTTGCTTCCCCTGACTGATTTAGGGGCTGGGTG
ACCGATGGCTTCAGTTCCCTGAAAGACTACTGGAGCACCGTTAAGGACAAGTTCT
CTGAGTTCTGGGATTTGGACCCTGAGGTCAGACCAACTTCAGCCGTGGCTGCCTG
AGACCTCAATACCCCAAGTCCACCTGCCTATCCATCCTGCGAGCTCCTTGGGTCC
TGCAATCTCCAGGGCTGCCCCTGTAGGTTGCTTAAAAGGGACAGTATTCTCAGTG
CTCTCCTACCCCACCTCATGCCTGGCCCCCCTCCAGGCATGCTGGCCTCCCAATA
AAGCTGGACAAGAAGCTGCTATGA (SEQ ID NO: 62)

NP_000031.1 Human apolipoprotein C-III precursor

MQPRVLLVVALLALLASARASEAEDASLLSFMQGYMKHATKTAKDALSSVQESQV
AQQARGWVTDGFSSLKDYWSTVKDKFSEFWDLDPEVRPTSAVAA (SEQ ID NO: 63)

Angiopoietin-Like 3 (ANGPTL3)

[448] In some embodiments, the target gene for modification using the compositions and methods disclosed herein is gene encoding ANGPTL3. ANGPTL3 has been associated with diseases and disorders such as, but not limited to, Arteriosclerosis, Atherosclerosis, Cardiovascular Diseases, Coronary heart disease, Diabetes, Diabetes Mellitus, Non-Insulin-Dependent Diabetes Mellitus, Fatty Liver, Hyperinsulinism, Hyperlipidemia, Hypertriglyceridemia, Hypobetalipoproteinemias, Inflammation, Insulin Resistance,

Metabolic Diseases, Obesity, Malignant neoplasm of mouth, Lipid Metabolism Disorders, Lip and Oral Cavity Carcinoma, Dyslipidemias, Metabolic Syndrome X, Hypotriglyceridemia, Opitz trigonocephaly syndrome, Ischemic stroke, Hypertriglyceridemia result, Hypobetalipoproteinemia Familial 2, Familial hypobetalipoproteinemia, and Ischemic Cerebrovascular Accident. Editing the ANGPTL3 gene using any of the methods described herein may be used to treat, prevent and/or mitigate the symptoms of the diseases and disorders described herein.

[449] The ANGPTL3 gene encodes the Angiopoietin-Like 3 protein, which is a determinant factor of high density lipoprotein (HDL) level in human. It positively correlates with plasma triglyceride and HDL cholesterol. The activity of ANGPTL3 is expressed predominantly in the liver. ANGPTL3 is associated with Dyslipidemias. Dyslipidemias is a genetic disease characterized by elevated level of lipids in the blood that contributes to the development of clogged arteries (atherosclerosis). These lipids include plasma cholesterol, triglycerides, or high-density lipoprotein. Dyslipidemia increases the risk of heart attacks, stroke, or other circulatory concerns. Current management includes lifestyle changes such as exercise and dietary modifications as well as use of lipid-lowering drugs such as statins. Non-statin lipid-lowering drugs include bile acid sequestrants, cholesterol absorption inhibitors, drugs for homozygous familial hypercholesteremia, fibrates, nicotinic acid, omega-3 fatty acids and/or combination products. Treatment options usually depend on the specific lipid abnormality, although different lipid abnormalities often coexist. Treatment of children is more challenging as dietary changes may be difficult to implement and lipid-lowering therapies have not been proven effective.

[450] ANGPTL3 is also known to cause hypobetalipoproteinemia. Hypobetalipoproteinemia is an inherited disease (autosomal recessive) that affects between 1 in 1000 and 1 in 3000 people worldwide. Common symptoms of hypobetalipoproteinemia include plasma levels of LDL cholesterol or apolipoprotein B below the 5th percentile which impairs the body's ability to absorb and transport fats and can lead to retinal degeneration, neuropathy, coagulopathy, or abnormal buildup of fats in the liver called hepatic steatosis. In severely affected patients, hepatic steatosis may progress to chronic liver disease (cirrhosis). Current treatment of hypobetalipoproteinemia includes severe restriction of long-chain fatty acids to 15 grams per day to improve fat absorption. In infants with hypobetalipoproteinemia, brief supplementation with medium-chain triglycerides may be effective but amount must be closely monitored to avoid liver toxicity. Another option for treating hypobetalipoproteinemia is administration high doses of vitamin E to prevent neurologic

complications. Alternatively, vitamin A (10,000-25,000 IU/d) supplementation may be effective if an elevated prothrombin time suggests vitamin K depletion.

[451] In one example, the target tissue for the compositions and methods described herein is liver tissue. In one example, the gene is ANGPTL3 which may also be referred to as Angiopoietin 5, ANGPT5, ANG-5, Angiopoietin-Like Protein 3, Angiopoietin- 5, FHBL2, and ANL3. ANGPTL3 has a cytogenetic location of lp31.3 and the genomic coordinate are on Chromosome 1 on the forward strand at position 62,597,487-62,606,159.

[452] Loss-of-function mutations that may be made in ANGPTL3 gene using the methods and compositions described herein are also provided. The strategies to generate loss-of-function mutation are similar to that used for PCSK9 (e.g., include, but are not limited to premature stop codons, destabilizing mutations, altering splicing, etc.).

[453] In some embodiments, the modification reduces expression of functional ANGPTL3 protein encoded by the ANGPTL3 gene in the cell. In some embodiments, the modification reduces expression of functional ANGPTL3 protein encoded by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or 100%. In some embodiments, the modification reduces expression of functional ANGPTL3 protein encoded by the ANGPTL3 gene in the cell by at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 10 fold, at least 20 fold, at least 25 fold, at least 30 fold, at least 40 fold, at least 50 fold, at least 60 fold, at least 70 fold, at least 80 fold, at least 90 fold, at least 100 fold, at least 200 fold, at least 300 fold, at least 400 fold, at least 500 fold, at least 600 fold, at least 700 fold, at least 800 fold, at least 900 fold, at least 1000 fold, at least 2000 fold, at least 3000 fold, at least 4000 fold, at least 5000 fold, at least 6000 fold, at least 7000 fold, at least 8000 fold, at least 9000 fold, or at least 10000 fold. In some embodiments, the modification abolishes expression of functional ANGPTL3 protein encoded by the ANGPTL3 gene in the cell.

[454] In some embodiments, a splice site disruption generated by a base editor system disclosed herein can result in the inclusion of intronic sequences in messenger RNA (mRNA) encoded by the ANGPTL3 gene. In some embodiments, the splice site disruption generates a nonsense, frameshift, or an in-frame indel mutation that result in premature stop codons or in insertion/deletion of amino acids that disrupt protein activity. In some embodiments, the splice site disruption generates exclusion of exonic sequences. In some embodiments, the splice site disruption generates exclusion of exonic sequences that results in nonsense,

frameshift, or in-frame indel mutations in the ANGPTL3 transcript. Canonical splice donors comprise the DNA sequence GT on the sense strand, whereas canonical splice acceptors comprise the DNA sequence AG. Alteration of the sequence disrupts normal splicing. Splice donors can be disrupted by adenine base editing of the complementary base in the second position in the antisense strand (GT to GC), and splice acceptors can be disrupted by adenine base editing of the first position in the sense strand (AG to GG).

[455] In some embodiments, a base editor system provided herein effects an A•T to G•C alteration in a ANGPTL3 gene when contacted with the ANGPTL3 gene. In some embodiments, the A•T to G•C alteration is at a splice donor site of the ANGPTL3 gene. In some embodiments, the A•T to G•C alteration is at a splice acceptor site of the ANTPTL3 gene. In some embodiments, the A•T to G•C alteration results in an aberrant ANGPTL3 transcript encoded by the ANGPTL3 gene. In some embodiments, the A•T to G•C alteration results in a non-functional ANGPTL3 polypeptide encoded by the ANGPTL3 gene. In some embodiments, the A•T to G•C alteration is at a 5' end of a splice donor site of an intron 6 of the ANGPLT3 gene.

[456] The nucleotide sequence of human ANGPTL3 is provided, for example, in NG_028169.1, which is incorporated herein in its entirety. The protein sequence of human ANGPTL3 is provided, for example, AAD34156.1, which is incorporated herein in its entirety.

[457] Mouse, rat, and monkey ANGPTL3 nucleic acid sequences have been deposited; see, e.g., Ensembl accession number ENSMUSG00000028553, ENSRNOG00000008638, and ENSMFAG00000007083 respectively., each of which sequences are incorporated herein its entirety.

[458] The polypeptide and coding nucleic acid sequences of ANGPTL3 and of other members of the family of human origin and those of a number of animals are publicly available, e.g., from the NCBI website or ENSEMBL website. Examples include, but are not limited to the following sequences, each of which sequences are incorporated herein in their entireties;

NG_028169.1 Human angiopoietin like 3 (ANGPTL3), RefSeqGene on chromosome 1

AATGACAAACTGAAAAAATCTATTGTTTGTTATATATATAACAAAGAATTAGTAT
CCACAATATGTAAATAATTCCTAAAATTAGTCAGAAAGAGACAAACTTAAAAAG
AGGGTAACAAGGAGGGGAGCAAATTATGTACATAACCAGATGATTCGCAAAGAC
GGCAACAGAGATGGCCAGCAAAACAAACTAGATATATACTTGTCTATTAGATTT
ATCAACATTTTTTGCCTTTTTCATTAAAAGCATTTGTAAAAGGATATAGGAAAAG

AGGAACTCTCATATACTCCTGGCAGGGATGTAAATTGGTACAACCCTTTTGAAGG
ACAATCTGACAAAAGCAATCGTAAGTTACAAGTCAACATCTATGAATGTATATG
AAAATATTTATATACATACATCACCACCATAAAAGCATTTTCTATACATACTGTTT
ATAATTAGAAAATTGGAAACAAATGATTAAAAGGGGGCTGATTAAATTAAGGTT
CATCTATATAACAGGATTATGCAGCTATTAAAAAGGACGTGGTAACTCTATAGAC
ATTCATAGGAAAATAAATTTTAAAATACTAAGATCCTGAATGATATATATATCAT
GAGCTATTATACATAACAAGATCCCACTTGTGTTATAAAAAATTATGTTTAGTCA
TTCAAAGGGTCTGGTATGATAGACCCAAAATGTTAATAGAGTCGAGATTTTTATT
TTTTATAGGTTTTTGAAATACCTGAATTTTCACAATAAGTACTTTGCACATTAAAA
ATCTTAGCTGGGCATGGTGGCTCACGCTTGTAATCCCAGCACTCTGGGAGGCCAA
GGTAGGCAGATCACCTGAGGTCAGGAGTTCGAGACCAGTCTGGCCAACATGGTG
AAACCCCGTCTCTACTAAAAATACAAAAATTAGCCAGGCTTGGTTGGGGGTGCCT
GTAATTCCAGATACTCGGGAAGCTGAGACAGGAGAATCGCTTGAACCCAGGAGG
GGGAAGTTGCAGTGAGCTGAGATCACGACGCTGCACTCCAGCCTGGGCAACAAA
GAGCAAAACTCCGTCTCAAAAATAAATAAAGAAAAAATCTTTACATGTCCAAAG
ATACGGCTGTTCAACTAAAAAATATATATGTATAAAACTTAACATGTTAATAGTG
AACACACAAAACAGTAAGATAGATAAAATTATTCCTTCAAAGCTCACTTAACCTC
TGGATCTACACTGTCCAAAAAGACGGTCTAATGAGACAATTGAGCACTTGATAG
GTGAGTGGTTCTAACTGAGATATGTTCTCAGTATAAAACATACAATAGGATCTTC
CTATACAACATTAATTAAAAAACAAACTATTGTAGTTAAAAAGGAAAAAATTAG
AGATACTATGTAAAAAAGAGCCAAAATACCTTGTATTTTATTTGAAAGACATATC
TCCATAAGATTACACAACCTCGTGTAGGATAAAGGACTTTGCTTTGCTTGGAATT
TAAACAATTTAGGCTCTTAAATGTCCTAAAATTCTCTGTAGCTAAGAAATTTTTAT
ATTGGTTCCTAGGAACTAGGAATCCTTAAATTAGGCCCTACATTTGCTTACAAGT
TTATTTTCCTTGGCATAAAATTTTTTAGTTTTTACATTACTGGTTATATTTGATCAG
GGTTCTATTTAAATAGGCACAAGTTCAAGCAAAGATCAGATTCTGCTTTTAGCAG
TGTGTACTCAGACAGGAAGTATTAAAAGGCAGGCAGAAAATCCTTTATAAAATT
ACTACTTTCAATGCATTTTCCCACGTTGAAATGCTTCTGCAGTTTATAATTAGGCA
AATTACTTTAATTATAATCAATAATGCTGTTCAAATTACTATAAGAATTATACAG
ATATTTATACCAAGAGACAATATACTAGAAACCAAGACTACGTGACCATTACCTC
TACTCTGTCAGTGTTATTTGTGAGAAATTGCACAAATTTTGCAAAAAGTGTTAGT
ATCCTACTACAGTAGGATATAATATAGAAGGAAATAATTTCATAAAGCCTGTCTT
TGGTACTAGTGCTCAGTTACTTTCATTAACTAAAAAAGGGGCTACTCTTCAAATT
CCCTTCTCTAAAAAGAATGTACTATATCAAAAGGGGGTAAACACTACTACGTATA

CATTCTGCACTTAGAAATCCCTATATGTTGATTTTATCATTCTCTTATTCAATAAA
TATTGTTTCTACAATGTGTAAGGCATTACTGTACTAAAGCATTATAAGGAATATA
AGTTAAAAACACATACAAATCTTGCCAATCAACAGCTTATAGTGTAATAGGGGA
GAGAAGCTGGCCCATCTATATTCTCCCTCAACTAGCAAGTGGATGAAATATCAGG
GTCAATAGTTATAAGCCACAAAAGCTGACAGCTTAATTAAGAGAAGTTTTGAAA
TATGTATTTCATGACCAATAATTACAACTGTAACTTTTCTATTTAAAGAAGGAGA
AAATTTGAATTTCTTCTCTAGCTCAACATACACTTCTATAATTCCATTACATGAAC
CAGAGTAAAGGGTAAGATGGAAATGAAGAATATTTTCTTACCCTTTTGTGGTTCT
ATATTGGACACTTAAAAATCATACACAACCTAATCAAAAGATGTAATTCTTTAAA
AAGGTACGAGACCAAAATTCAGAAAATCTAGACTATAACAAAATTTCTCAATTT
ACATTATCTTAATATGCAATTAATTTTCACCAGTAAAATACTATAGTATGGGTAC
AAATGCATTGATTAGTTCTAATTACAAAAATGGCTAATATATAATACTGTGTAGT
GTTTATGATACATCAGATAATGTTCTAAGTGCTCTGAAAATATAAACTTTTAATCT
TTTATACGACCCTATAAAATAGGTGTTATTCTCACTGGAGAGATGAGAAAACAGG
GGTTCAGAGATGTGAAGTAATTTGACCAAAGGTCACAAAGCTGAAGAATATGAA
ATCCGGGATTCTGATTCAGGCAGTCTTATTCCAGAATCATGCTCTTAACCACTAT
GGAATACTGCCTCTACTGTAACTATTATACCCAAAACCCTTAATCCTAAGTCATC
AAAAGGAAGAGCCTCTATTTTACACAATGAAGAGGCATTTCTAAGAATAGAAAT
TTAGGGACGAGCACAGTGGCTTACTCCTTTAATATCAGCACTTTGAGAGGCTGAT
ATGGGAGGTTCACTTGAAGTCAGGAGTTCAAGGTCAGCTTGGGCAACATAGTGA
AACACAGTCTCTACAAAATATTTAAAAATTAGCTGGGTGTGGTGGCATGCATCTA
TAGTCTCAGCTACTTGGGAGACAGAGGGAGGAGGCTTGCTCGAGCCCAGGAGTT
CGTGGCTATAGTGAGCTATGATCATGCCACTGCACTCCAGCCTGGACAACAGAG
CAAGACCCTGTCTCTAAAAAAGAAAAGAAATTTGGAAATGGTTTATTTTGTATTA
ACAATTTATAATTTACACTGAAATTTATTATGATAAAACTTTTCCCTGTGTTAAAA
AGCTATTAACTTTATGAAAAATTTCTTTTAGGTAAGGTTGATTATATATACCCACA
CACATACACAGGTTAAAAGTTAGTTTCATGTGACATAATAACTAGCATTTTGAGC
ACTACCTGTTTGCCCAGCACTGTTCTAAGTGCTCTACATGTATTATTGTTAAATTA
TCATAACACTATGAATTATGTACTATAATTACCCCAGCTTTACAGATGAGGAGAC
TAATCCATGGGGAGGTTAAGTAACTTGTCCAAGGCCAGACAGCTAGAGCCGGCT
TTTGGACCCACACCACAGTCTGACTCCAGCACCCATATTCTTAACAATTTCACCA
TATTAATATGTCAAGATTAAGCAGTTTTAAAGGATGCTATTTTCTCACAAATTTCT
TAATATGAACACTCAATAAGAATAATCACTAATATAAGCATTTAGTATTTTTTTA
ACACTAAGTTGGAAGCATAGTGGAACATTTATTTTTAGAAATATTATTAATTGGC

TGGGCTCACGCTTGTAATCGGCTGGGCTCATGCCTGTAAATTTTGGGAGGCCAAG
GTAAAAGAATTGCTTGAGCCCAGTATTTCCAGACCAGCATGGGCAATACATTAA
GACATCATCTTTAAAAAAAAAATGTTATTAATCTCCTCTTTTTGTTAAATGTATAT
TATCAAAATTGTTACTAAGCTAACAAACTTCAGAAAAACTTATGATGGGCAAGCT
GCTTGTGACATTGAAGGTATTTAAGATTCAATTCTAGTTTGGTCCTAGATGACCA
CATATCCATTGTTCCTTCAACGAGCACATGGTAAAGAGCCTAGAACACAGAGAC
ACAGAACACAGTGGAGAAAAGGGAGTGAAATGTCTTTAATGACACTTACTATAT
ATGGGATTTTGTGACAATATACAAGGATGGTTAAGACATATAAGGTGATGCAAA
AAAACATATTAACAATTATAGTGACAAAAAATGAGGAGCATATAATTATACATT
GATTTATACAGAGTACCAGAGGAACACAGCATTGAGAGCCGTAACACCACCTGA
GGGAGTGGAGAAAGGCTTCAGAGAGAAAGTGTTTTTTGGAATGGATCACTGTTT
CCAAAAGAACTAAAGTACAGTTTGAGAAATGCATACTTAATTCATTACTTTTTTC
CCCTCAACTTTAATAATAAATTTACCCAACAAAAAAGTTTATTTTTGACTTGTAA
ATCTCTTAAAATCATAAAAAAGTAAAATTAGCTTTTAAAAACAGGTAGTCACCAT
AGCATTGAATGTGTAGTTTATAATACAGCAAAGTTAAATACAATTTCAAATTACC
TATTAAGTTAGTTGCTCATTTCTTTGATTTCATTTAGCATTGATCTAACTCAATGT
GGAAGAAGGTTACATTCGTGCAAGTTAACACGGCTTAATGATTAACTATGTTCAC
CTACCAACCTTACCTTTTCTGGGCAAATATTGGTATATATAGAGTTAAGAAGTCT
AGGTCTGCTTCCAGAAGAAAACAGTTCCACGTTGCTTGAAATTGAAAATCAAGA
TAAAAATGTTCACAATTAAGCTCCTTCTTTTTATTGTTCCTCTAGTTATTTCCTCCA
GAATTGATCAAGACAATTCATCATTTGATTCTCTATCTCCAGAGCCAAAATCAAG
ATTTGCTATGTTAGACGATGTAAAAATTTTAGCCAATGGCCTCCTTCAGTTGGGA
CATGGTCTTAAAGACTTTGTCCATAAGACGAAGGGCCAAATTAATGACATATTTC
AAAAACTCAACATATTTGATCAGTCTTTTTATGATCTATCGCTGCAAACCAGTGA
AATCAAAGAAGAAGAAAAGGAACTGAGAAGAACTACATATAAACTACAAGTCA
AAAATGAAGAGGTAAAGAATATGTCACTTGAACTCAACTCAAAACTTGAAAGCC
TCCTAGAAGAAAAAATTCTACTTCAACAAAAAGTGAAATATTTAGAAGAGCAAC
TAACTAACTTAATTCAAAATCAACCTGAAACTCCAGAACACCCAGAAGTAACTTC
ACTTAAAGTAAGTAGAAAATAAAGAGGGTTCATGTTTATGTTTTCAATGTGGATC
TTTTAAAAAAAATATTTCTAAGGCATGCCATTTGAAATACTTTGTTGCATTGTTGA
AATACTTTTTTTTCCAAGAAAAATAATCTCCAGAAAATAAAATTTCCTATTATAA
TTTCAAGTTAGTTTTTTGTTTCCCTAATGTTATATATGAAAACACTGAAAATTTGC
ATTTTATATGAAAATTACAAATCGGTTAAATTATACAATCTAGAACACTATGTCA
TTACACTATTGTAAATTACTGAAGGTAAGTAAAAAGTTAAAAAAAATTTAAAACT

ATTCTCCAGTGTTTAAAACAGATTAAATAATACAGTAAATGGAAAAGATTTATTC
ATATGAAAATATGCTGGGCTTTTTCTTTTAATTGAAGTTCAGAAAATCAAATTTTA
GAGATAGTACAATTTAAATAAAATGTTAAGGACAAAAATATGTGCTATTTGAAA
GAAGCATACAAGGGGAAGGAATTGCCAATATTCATTTTTCAAATCCATTATTAGT
TTAAAAATTTAGATTATGATAGTGTTACAGGAAATTAATAGAAAAGAAAGAGGA
AAGCAACTTATAACCAACCTACTCTCTATATCCAGACTTTTGTAGAAAAACAAGA
TAATAGCATCAAAGACCTTCTCCAGACCGTGGAAGACCAATATAAACAATTAAA
CCAACAGCATAGTCAAATAAAAGAAATAGAAAATCAGGTAAGTCAGTATTTTAA
TGGTATGTCCCATCTTTCACACAGGTCTGTAAAAACACTGAATCCTAAAATTATT
TACAAGCTTTAACTGGATCATGAGTAAAATTATCACATCAGCATAACTGTTAAAA
TTGCAGGCTCTGAAGCTAATAAACTACCTGCATTTAAACCATGGCTCTAAAACTT
TGTGTGACCTTGAATAAATTACTTCACCCCTTTATCTCTCAGTTTCCTCACATATA
CTACAAAGATAATAACAGAACTTATAGGATTATTGTAAGAAAAAAAATTAATTC
ATAGCAGCCAATGTCATCTTACTAAAATTCAAATTAGATCATGTTTCTCTTTGCTC
AAAACCACACAATAGCTTTCCATTTCACTCATATTGGCTCTTTAGACCAAGATTA
CCCAACCCTTCGTCATCTCACTGACTTCACCTCCTCTACTCTAGTTATTCTGACCG
CTTTACCAGTATTCAAACACATCAAACATACTGCCACCTCAAAGCCTTTGCCCTT
GTTGTTTCCTCTAACTGGAACGCTCTTCTGCCCTGGTATCTACGTGGCCCACTCTC
TGATTTCCCTTAGGGTCGTTATCAAACAAAAAATTCCCAATGAAGACTTACAAGG
TCACTTAACCAAAAATCACAACCGCCTGGTCCCATCCCTGAAAACTTCTACTTCC
TTAGCTACTTTTCTCCTGCACACTCACCTTTATTTAACATAACATAAATTTTAGTT
ATTTATCTCTTCTATTCCTGCACTAAAATGTAAGCTCTGTGAATACAGGGATTTTT
TCCATTATCTTCATATTTTCCATTATTTGTATATACTCCAGAATATAGAATACTGT
ATGGCACACAGTAGGCATTTCTGTTGAATTAATAAATGTAATGTCATATTCACAC
AGAAGCGTGTGCTATGATTATTATTACTTGGATTACTAGAAATAGTGTGCCTCAT
AATTAAAGGTCAACATTCAACAATGTAATTAATCTACAATGTAAACATCTGGTGA
AGTGACAGAGGGAAGCACTTGTTTAGAAAAAAGCTATGTCAGAATCCATGTATT
CTAATATGCAGTACAATAGTTTAAAAATATTAATAATACTCTCAAACAGCTATTC
AAGAGGATTCAAAAAACATAATATAAACTCAGAGAAACTGGTAAACAAAATCAT
TTTCAAGAGATATAAAACAAATATTATTACCAATTTCCACTAAACAAACATAATG
TTAGTAGTGCTGCTAAAAGGTTTTTTATCAACTACTTTTGGTTTCCATACTTTCCTT
CTTATGATGTTATTATTCTAAATTCTTTTCAATTATATCTTTTACTATGATTAAATG
AACCTGCTCCCCAAAGCAAAATGTTACTATAGTAATATACATTGTGTCTAAAAAT
AAAAATGTGTGAAGAAACCAAAACAATGAATTTCTGAGTTGGAAGAAGAGTTAG

ATCATTTAACTTTCTCATATTTAAATTAAAAAAACAAAACTCTAAAAATTTAAGT
AACTTTAAGATCACATAGTTACTTAGTAGAAAAGAGTAATACCCAGCAAGCAAA
CTTTACAATAGATCCTTTTAAATAAGGTCCTAGGAAATATCATTCATGCCAGCAT
CAAAAAACTAACACTAATAATGCAAGATATTATATATTCTGCTTTTCTTACTGTC
AATGAGAAAAACTATCATTCAATAAATTGCAAACCCAACACACTTAAATAAAAA
TAAAATGTTACTGCTAAACTAACGATAAACTACTGAATATATAGAAAGTAAGCA
AACAAACTTGCCAACCTGCCAACATCTACAGATATGTTTACAGGTCAAAAATTAT
CAAATTATCAAGAAAGCCTGGTTCAAATTATGTATTATGTCTTTATCACAGGTCT
GAAGATCAGTAAGACCTAAAACTGAAAATTATTAAACTTAAAATCTGAACAGAA
TATCAAATATATTTTATTCATATAAATAAAAGAATACATTACAATATTCTAAGCA
AAGCAGTCTCTACTTTTGGCCTTGCTCTGTTTTCCGACCAATGTCTGCTTTTTTGC
CTTGCTTTATTTTTTTATCTTATTAAATAATGTCCCTGATTAAATATTTTGAGAAC
AGGTAATCTGTACAATCTGAATAACACTGTTTATCTAAATATCAAACACCGTTAT
AACATTATGAACTGAAAGACAAACTGTACTTCTGACATCCTTACTCAGATTTCCC
CTAATTGTATATTCAGTATCATTTTAAAAAACAGATTTATATTCTTTTATCAGCTC
AGAAGGACTAGTATTCAAGAACCCACAGAAATTTCTCTATCTTCCAAGCCAAGA
GCACCAAGAACTACTCCCTTTCTTCAGTTGAATGAAATAAGAAATGTAAAACATG
ATGGTAAGACACTTTGGTGGGTTTCCTTCTTGAAGCTATTATTATCAAATTCCCTA
TTCTTAGGACTTGTTCTAGACTAAAAGATAGTTAAGAGATATCCATCAAATACAA
TGTATCAACCTAAACTGGATGCTGGGGTTCTTTTTACACCCTATAAAAGACATAC
CTAAGACAATCAGAGAAATACAAATATGGACTTGATTATTAGATAATATAGAAG
GTTTATTAATTTTCTTAGATGTGATCATGGTATTGCAGTTTTAAAGGAGAACAATC
TCCTGTTTAAGAGATACATGCTGAAATATTTACGGAGTTAAAGGTCACTGGACTC
CAGACTGGTGATAGAACAAGACTCTGTCTCTAAAAAATAATTAATTTTTTAAAAG
AAAATAGTTTGGTAAGATGATTCTTACATTCTTAAATAACACGCCATCTAAGAAA
AATGCTTTAACATAAACATTACTGAAAAAATGCTACATTTGCCACAACTTCATAA
AATGTCAAGTGAAATCTCAAGCTCCAAAGATATTATTCCTATTACTAAATCTGAT
GTAATAACATTTTATTGATTCTAGGCATTCCTGCTGAATGTACCACCATTTATAAC
AGAGGTGAACATACAAGTGGCATGTATGCCATCAGACCCAGCAACTCTCAAGTT
TTTCATGTCTACTGTGATGTTATATCAGGTAAAACCTGTCTAAGGAGAATAGACA
GTAGTTAGTTCAACTTACTCATTACGTATTAGGAAGATTAACCTGGTTATCATTGT
TTTATACATATATATATGAAATATATATGAGTATTCGTATAAATATAATACTTTTA
CCTTGTTTATGTATTTACTCAATATTCTCCTTTTCCTCTAAAATAATCTGAAGTGA
CTATTATCAATAAGTTTACTATGCCAAAATTCATTAATTGCCTTTCACTTAACTTT

TGGGACCATAATAAATAATAAAATGTATTGCCATAACATTAATAAACTACCTTAC
AAAACCACCAATTAAAATCAAACAAACAAAAAAGTGTTATTTACATCTGTCAAC
ATAAATCTACTAAAAATACATGATTTCATTCATTATATTCAGGTAGTCCATGGAC
ATTAATTCAACATCGAATAGATGGATCACAAAACTTCAATGAAACGTGGGAGAA
CTACAAATATGGTTTTGGGAGGCTTGATGGTAAGGGGACTACATTCAATCATTCA
TTCACTTGCTAATCTACAAATATTTACTGAGAACCTCTTATGGACCAGGTATTAG
GAAAAGTAGTAACGAACGAGAAGCAGTCTCAGCCTTCATATAATTTATTATCAA
ACAATTACACATTTGTTAGTAAATTACACTTATTACAACTGTTATTATTTGAATTA
TATTTATCACAATTACATGTCTGTTCTTAAATATACTTATCACAATTTAATTCCAC
GGCTTACAATGATCATAACTATAATTATTAAAGACAATTTTGATTAAATGTTATG
TCATAAGTAGTAACTGTTACAAATAAGCTGTGAAAAGAACCACTCCTAGCATTA
GTCACTCTATTCTCTCATTAACGTTTTACATATCAATTAATTGGAAGTTAAAAGGA
CCAGGAAACTCAGACATACAGTATACATTTTAAAATTTCAATTATTTAAATATAA
TATATAGAATGTATGGCTTATAATGAATTAGTTAACTCAATGCAAATTATTCTATT
TTGATTACAAATAGTAAAATAAGCAAGATAAAATAACAGATGTTTAAAATCCAA
AAAGCACATACAAAAATCCATGAATGATGTCTAAGTACTCACTTATAAAGTAGA
AGACATTCATTATTATATCAAATTTTTAAATGCTCAGTACTATTTGACCATTTAAA
AATTTTGTATTCAAACTACCAGTGAAAGCCCTACCTAGAAGGTATACTCAGTGAT
AAGTTTTGTAGCTCCAAATCTTCTAATAGTGAGTGTAACCCCAAAATAAAAGGCT
GACAGGTAAGTCGAGAATACTCACTTAATTCTGGTAAGAAAGCAACCCATTTGT
ACTTGTATTTACCAGCAATCCTTAAAATGAAGCTTCCTACTAACTCAATAGCAAT
AAGACAATAGTGAATGTTTAATGAAAACAGTATTTTATAAATACTTTAATAAAAA
GGATTGTGATGAAGAACAATCTATTTATATTTGTTATTTGTTTTTAATTCCAATAA
AAATAATTTTTAAAATTACAGAAAAAAGTTATTAAGAACCATGCTTTTAAATTTA
AAATGATTTTTTAAATTTATTCCTGTCTTTTTCTACAAAGAAAGCATACATTAAGC
AAATACCAAAGGCCAGGTTTACATTTGAAGAAAGTGACATTATTATTACTCAAGT
CTCTAGGAATACTTAACACATCTCTTGACTGTATATGGATGTTAATAAATAGCTG
ACAGTAAAGTTTATCCATATAAAGACTTGCAAATATTCCTCTACCAATGACGAGA
CTTTAAAATATCTATAATAATGTAACACATTTCACTGGTGAAACATGTCTTGTCAT
ATGCATTATAGAAAGGATAATCAGACTTTCAGTTATATTAATATTTTTAACATTTT
TGTGCACATAGCTATCTTCAATAAAATTGTTTTAAAAGGTATTATTTTAAGATAC
ACTAAAATGATCAAGGGATTCAAGACTAAACAACTCAATTAGTTGCACCAATAA
AAAACACTTAAAAAAACTGTCAGTGTCCAACCTGTACTTAATAACTCACAGATTT
TTAAAACTTTTCTTTTCAGGAGAATTTTGGTTGGGCCTAGAGAAGATATACTCCA

TAGTGAAGCAATCTAATTATGTTTTACGAATTGAGTTGGAAGACTGGAAAGACA
ACAAACATTATATTGAATATTCTTTTTACTTGGGAAATCACGAAACCAACTATAC
GCTACATCTAGTTGCGATTACTGGCAATGTCCCCAATGCAATCCCGGAAAACAAA
GATTTGGTGTTTTCTACTTGGGATCACAAAGCAAAAGGACACTTCAACTGTCCAG
AGGGTTATTCAGGTATCTTTTTCTGATACCAATACTTTATTTTCATATCTTCAAAG
TATCTTCCCACATTATTAGCTATTATCTGCAATGACAACTTTTAAAAATCCGAATC
CCAAATAAGCGTTTTCTCTCTAGACGAAAACCTCTTAACTATAATGAAAGTGTTC
ATTCTAGTTCAATCAGGTATTTTACCTCTAATCTTCCTCAGATTTTCTATTTTTTGG
TAGTGTATAGATTATTTATACAGATTATTTAAAATTGGGACTTATACAGATTATTT
AAAACTGGGATACATGCATCTAAAACACTGTAATATTTATAAGAAAGGAAGATA
AACTTACGGGGAAATACAGTAACAGTAACTACATACGAGTCTGTACCCATTAAA
TTGCATATCTATCTCCTTTAGGAGGCTGGTGGTGGCATGATGAGTGTGGAGAAAA
CAACCTAAATGGTAAATATAACAAACCAAGAGCAAAATCTAAGCCAGAGAGGA
GAAGAGGATTATCTTGGAAGTCTCAAAATGGAAGGTTATACTCTATAAAATCAA
CCAAAATGTTGATCCATCCAACAGATTCAGAAAGCTTTGAATGAACTGAGGCAA
ATTTAAAAGGCAATAATTTAAACATTAACCTCATTCCAAGTTAATGTGGTCTAAT
AATCTGGTATTAAATCCTTAAGAGAAAGCTTGAGAAATAGATTTTTTTTATCTTA
AAGTCACTGTCTATTTAAGATTAAACATACAATCACATAACCTTAAAGAATACCG
TTTACATTTCTCAATCAAAATTCTTATAATACTATTTGTTTTAAATTTTGTGATGTG
GGAATCAATTTTAGATGGTCACAATCTAGATTATAATCAATAGGTGAACTTATTA
AATAACTTTTCTAAATAAAAAATTTAGAGACTTTTATTTTAAAAGGCATCATATG
AGCTAATATCACAACTTTCCCAGTTTAAAAAACTAGTACTCTTGTTAAAACTCTA
AACTTGACTAAATACAGAGGACTGGTAATTGTACAGTTCTTAAATGTTGTAGTAT
TAATTTCAAAACTAAAAATCGTCAGCACAGAGTATGTGTAAAAATCTGTAATACA
AATTTTTAAACTGATGCTTCATTTTGCTACAAAATAATTTGGAGTAAATGTTTGAT
ATGATTTATTTATGAAACCTAATGAAGCAGAATTAAATACTGTATTAAAATAAGT
TCGCTGTCTTTAAACAAATGGAGATGACTACTAAGTCACATTGACTTTAACATGA
GGTATCACTATACCTTATTTGTTAAAATATATACTGTATACATTTTATATATTTTA
ACACTTAATACTATGAAAACAAATAATTGTAAAGGAATCTTGTCAGATTACAGTA
AGAATGAACATATTTGTGGCATCGAGTTAAAGTTTATATTTCCCCTAAATATGCT
GTGATTCTAATACATTCGTGTAGGTTTTCAAGTAGAAATAAACCTCGTAACAAGT
TACTGAACGTTTAAACAGCCTGACAAGCATGTATATATGTTTAAAATTCAATAAA
CAAAGACCCAGTCCCTAAATTATAGAAATTTAAATTATTCTTGCATGTTTATCGA
CATCACAACAGATCCCTAAATCCCTAAATCCCTAAAGATTAGATACAAATTTTTT

ACCACAGTATCACTTGTCAGAATTTATTTTTAAATATGATTTTTTAAAACTGCCAG
TAAGAAATTTTAAATTAAACCCATTTGTTAAAGGATATAGTGCCCAAGTTATATG
GTGACCTACCTTTGTCAATACTTAGCATTATGTATTTCAAATTATCCAATATACAT
GTCATATATATTTTTATATGTCACATATATAAAAGATATGTATGATCTATGTGAAT
CCTAAGTAAATATTTTGTTCCAGAAAAGTACAAAATAATAAAGGTAAAAATAAT
CTATAATTTTCAGGACCACAGACTAAGCTGTCGAAATTAACGCTGATTTTTTTAG
GGCCAGAATACCAAAATGGCTCCTCTCTTCCCCCAAAATTGGACAATTTCAAATG
CAAAATAATTCATTATTTAATATATGAGTTGCTTCCTCTATTTGGTTTCCTTAAAA
AAAAAAAAAACTCTCATAGGACATGTTTCATTTTGTTCCTTTCAGGAGTAGTAAA
TTAGACGTTTTCCCCATATAAAGCTTTTTTCTACCAGAAAGATACTTCTGGTAGAA
GAAGAGAAAGGAGCTCTTTATGGTTCACACGACTGTCTCCTGTCCTAACTACTTT
GCTTAAAGTGCTCAAATTCCATCACTACTCACAGTTGTCTAATCTAAGTCTAATC
CCCTTTGATCTCTCAGACTACCTTCCCTTTTATCTCTCTACTACTTAATAATAAGA
ATATCTTTTTTTCAAACTTGACCTTCATTTTGCTTTCACAATACTATACTCTCCATG
GATTATCCCTTATCTGAATCCATCTTTATAACCCTATTCCTTTCTCATATTTAGTAC
TGTGGGCCAATGGACAACCTTCAATCATCTTTTCTACACTGACCCTCAGACATTC
TATCTGCTCTCACGGACTCCTTTATTTACCATGAATAAAGTTCCAAAATCTACATA
TTCATCCCAAGTCTCTTTCCAGTTCCCCTTCTTACATTGCCTATTTGCCATTTCTCC
CTTCAATACCCTATACTTCACTCAAATTCAACATACCAAAAATAAAAGGCCAGGC
ACGGTGGCTCACACCTGTAATCCCAGGACTTTGGGAGGCTGAGGCAGGTGGATC
ACCTGAGGTCAGGAGTCTGACCAGCCTGACCAATATGGTGAAACCCCGTCTCTAC
CTAAAATACAAAAATTAGCCAGGCGTGGTGGCATGTGCCTACAGTCCCAGCTAC
TCAAGAGGCTGAGACAGGAGAATCGCTTGAACCCAGGAGGCGGAGGTTGCAGTG
AGCTGAGATCACACCAATGCACTGGGTGACAGAACAAGACTGACTCAAAAAAAA
ATAAATAACAAATTCCCCAGCCCCTTACTGCTACTGCTATCCCTTTCTACCCACCT
TTCCCTCCTTTATACTCTTTCACACCATCTTCCTCACTTCTTTATATCCATTAATAT
GACCAGCATGTTCCCAGTCACAGAAGCCTGGAACCCGGAAGACATCTCTGGCTTT
TCACTCAACTTTGTAAACTACCTCTTTTGTATCATAAGCCACCAAGTTCAATACA
ATCTTCTCTTGAAACGTCTCTTAATCTTATAAGCTTTCTTCCCCAAAGACTGTCTT
TAACTTCAGTGCTAGATTATATAAGT (SEQ ID NO: 7)

[459] AAD34156.1 human angiopoietin-related protein 3

MFTIKLLLFIVPLVISSRIDQDNSSFDSLSPEPKSRFAMLDDVKILANGLLQLGHGLKD
FVHKTKGQINDIFQKLNIFDQSFYDLSLQTSEIKEEEKELRRTTYKLQVKNEEVKNMS
LELNSKLESLLEEKILLQQKVKYLEEQLTNLIQNQPETPEHPEVTSLKTFVEKQDNSIK

DLLQTVEDQYKQLNQQHSQIKEIENQLRRTSIQEPTEISLSSKPRAPRTTPFLQLNEIRN
VKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQN
FNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFYL
GNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWHD
ECGENNLNGKYNKPRAKSKPERRRGLSWKSQNGRLYSIKSTKMLIHPTDSESFE
(SEQ ID NO: 8)

Lipoprotein (a) (LPA)

[460] In some embodiments, the target gene for modification using the compositions and methods disclosed herein is gene encoding lipoprotein a (LPA). LPA is a low-density lipoprotein variant. Genetic and epidemiologic studies have identified LPA as a risk factor for atherosclerosis and related diseases, such as coronary heart disease and stroke. LPA concentrations vary more than one thousand times between individuals: from <0.2 to >200 mg/dL. This range of concentrations is observed in all populations studied by scientists so far. The mean and median concentrations between different world populations show distinct particularities, the main being the two to threefold higher LPA plasma concentration of populations of African descent compared to Asian, Oceanic, or European populations. High LPA in blood correlates with coronary heart disease (CHD), cardiovascular disease (CVD), atherosclerosis, thrombosis, and stroke. Individuals without LPA or with very low LPA levels seem to be healthy. Thus, plasma LPA is not vital, at least under normal environmental conditions. Since apo(a)/LPA appeared rather recently in mammalian evolution - only old world monkeys and humans have been shown to harbor LPA - its function might not be vital, but just evolutionarily advantageous under certain environmental conditions, e.g. in case of exposure to certain infectious diseases.

[461] An exemplary LPA amino acid sequence encoded by Human reference sequence NG_016147.1 is provided below:

>sp|P08519|APOA_HUMAN Apolipoprotein(a) OS=Homo sapiens OX=9606 GN=LPA PE=1 SV=1

MEHKEVVLLLLLFLKSAAPEQSHVVQDCYHGDGQSYRGTYSTTVTGRTCQAWSSM
TPHQHNRTTENYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEG
TAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWS
SMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAE
GTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQA
WSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCS

DAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTC
QAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQ
CSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGR
TCQAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNL
TQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVT
GRTCQAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYC
NLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTT
VTGRTCQAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWE
YCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYS
TTVTGRTCQAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVR
WEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRG
TYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPG
VRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSY
RGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRD
PGVRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQ
SYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYT
RDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGN
GQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYC
YTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYH
GNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAP
YCYTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQEC
YHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAV
AAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGV
QECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPD
AVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRP
GVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNAGLIMNYCR
NPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTE
QRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNAGLIMNY
CRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAP
TEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNAGLIM
NYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQ
APTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNAGL
IMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPS
EQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNA

GLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEA
PSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYP
NAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVPSL
EAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEY
YPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVP
SLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPE
YYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTP
VPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSR
TPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPPT
VTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHS
HSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVA
PPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTP
HSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTA
VAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSS
MTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAE
GTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQA
WSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCS
DAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTC
QAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQ
CSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGR
TCQAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDPVAAPYCYTRDPSVRWEYCNLT
QCSDAEGTAVAPPTITPIPSLEAPSEQAPTEQRPGVQECYHGNGQSYQGTYFITVTGR
TCQAWSSMTPHSHSRTPAYYPNAGLIKNYCRNPDPVAAPWCYTTDPSVRWEYCNLT
RCSDAEWTAFVPPNVILAPSLEAFFEQALTEETPGVQDCYYHYGQSYRGTYSTTVTG
RTCQAWSSMTPHQHSRTPENYPNAGLTRNYCRNPDAEIRPWCYTMDPSVRWEYCN
LTQCLVTESSVLATLTVVPDPSTEASSEEAPTEQSPGVQDCYHGDGQSYRGSFSTTVT
GRTCQSWSSMTPHWHQRTTEYYPNGGLTRNYCRNPDAEISPWCYTMDPNVRWEYC
NLTQCPVTESSVLATSTAVSEQAPTEQSPTVQDCYHGDGQSYRGSFSTTVTGRTCQS
WSSMTPHWHQRTTEYYPNGGLTRNYCRNPDAEIRPWCYTMDPSVRWEYCNLTQCP
VMESTLLTTPTVVPVPSTELPSEEAPTENSTGVQDCYRGDGQSYRGTLSTTITGRTCQ
SWSSMTPHWHRRIPLYYPNAGLTRNYCRNPDAEIRPWCYTMDPSVRWEYCNLTRCP
VTESSVLTTPTVAPVPSTEAPSEQAPPEKSPVVQDCYHGDGRSYRGISSTTVTGRTCQ
SWSSMIPHWHQRTPENYPNAGLTENYCRNPDSGKQPWCYTTDPCVRWEYCNLTQC
SETESGVLETPTVVPVPSMEAHSEAAPTEQTPVVRQCYHGNGQSYRGTFSTTVTGRT

CQSWSSMTPHRHQRTPENYPNDGLTMNYCRNPDADTGPWCFTMDPSIRWEYCNLT
RCSDTEGTVVAPPTVIQVPSLGPPSEQDCMFGNGKGYRGKKATTVTGTPCQEWAAQ
EPHRHSTFIPGTNKWAGLEKNYCRNPDGDINGPWCYTMNPRKLFDYCDIPLCASSSF
DCGKPQVEPKKCPGSIVGGCVAHPHSWPWQVSLRTRFGKHFCGGTLISPEWVLTAA
HCLKKSSRPSSYKVILGAHQEVNLESHVQEIEVSRLFLEPTQADIALLKLSRPAVITDK
VMPACLPSPDYMVTARTECYITGWGETQGTFGTGLLKEAQLLVIENEVCNHYKYIC
AEHLARGTDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCARPNKPGVYARVSRFVT
WIEGMMRNN (SEQ ID NO: 64)

Base editor protein-gRNA complexes

[462] In another aspect, provided herein is a complex comprising the single guide RNA as provided herein in complex with the base editor fusion protein, e.g. the adenosine base editor fusion protein, wherein the complex comprises increased stability as compared to a complex with an unmodified single guide RNA and a base editor protein, wherein the stability is measured by half life of the complex ex vivo or in vitro.

[463] In some embodiments, the complex comprises increased stability as compared to a complex with an unmodified single guide RNA and a Cas9 protein. In some embodiments, the complex comprises increased stability by at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 200%, at least 300%, at least 400%, at least 500%, at least 600%, at least 700%, at least 800%, at least 900%, at least 1000%, at least 2000%, at least 3000%, at least 4000%, at least 5000%, at least 6000%, at least 7000%, at least 8000%, at least 9000%, at least 10000%, at least 20000%, at least 30000%, at least 40000%, at least 50000%, at least 60000%, at least 70000%, at least 80000%, at least 90000%, or at least 100000% as compared to a complex with an unmodified single guide RNA and a Cas9 protein. In some embodiments, the complex comprises increased stability by at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 10 fold, at least 20 fold, at least 25 fold, at least 30 fold, at least 40 fold, at least 50 fold, at least 60 fold, at least 70 fold, at least 80 fold, at least 90 fold, at least 100 fold, at least 200 fold, at least 300 fold, at least 400 fold, at least 500 fold, at least 600 fold, at least 700 fold, at least 800 fold, at least 900 fold, at least 1000 fold, at least 2000 fold, at least 3000 fold, at least 4000 fold, at least 5000 fold, at least 6000 fold, at least 7000 fold, at least 8000 fold, at least 9000 fold, or at least 10000 fold as compared to a complex with an unmodified single guide RNA and a Cas9 protein.

[464] In some embodiments, wherein the stability of the complex is measured by half life of the complex. In some embodiments, wherein the stability of the complex is measured by half life of the complex ex vivo. In some embodiments, wherein the stability of the complex is measured by half life of the complex in vitro.

[465] In some embodiments, the complex comprises increased half life by at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 200%, at least 300%, at least 400%, at least 500%, at least 600%, at least 700%, at least 800%, at least 900%, at least 1000%, at least 2000%, at least 3000%, at least 4000%, at least 5000%, at least 6000%, at least 7000%, at least 8000%, at least 9000%, at least 10000%, at least 20000%, at least 30000%, at least 40000%, at least 50000%, at least 60000%, at least 70000%, at least 80000%, at least 90000%, or at least 100000% as compared to a complex with an unmodified single guide RNA and a Cas9 protein wherein half life of the complex is measured ex vivo. In some embodiments, the single guide RNA exhibits increased half life of the complex by at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 10 fold, at least 20 fold, at least 25 fold, at least 30 fold, at least 40 fold, at least 50 fold, at least 60 fold, at least 70 fold, at least 80 fold, at least 90 fold, at least 100 fold, at least 200 fold, at least 300 fold, at least 400 fold, at least 500 fold, at least 600 fold, at least 700 fold, at least 800 fold, at least 900 fold, at least 1000 fold, at least 2000 fold, at least 3000 fold, at least 4000 fold, at least 5000 fold, at least 6000 fold, at least 7000 fold, at least 8000 fold, at least 9000 fold, or at least 10000 fold as compared to a complex with an unmodified single guide RNA and a Cas9 protein, wherein half life of the complex is measured ex vivo.

[466] In some embodiments, the complex comprises increased half life when measured in vitro by at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 200%, at least 300%, at least 400%, at least 500%, at least 600%, at least 700%, at least 800%, at least 900%, at least 1000%, at least 2000%, at least 3000%, at least 4000%, at least 5000%, at least 6000%, at least 7000%, at least 8000%, at least 9000%, at least 10000%, at least 20000%, at least 30000%, at least 40000%, at least 50000%, at least 60000%, at least 70000%, at least 80000%, at least 90000%, or at least 100000% as compared to a complex with an unmodified single guide RNA and a Cas9 protein, wherein half life of the complex is measured in vitro. In some embodiments, the single guide RNA exhibits increased half life of the complex by at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 10 fold, at least 20 fold, at least 25 fold, at least 30 fold, at least 40 fold, at least 50 fold, at least 60 fold, at least 70 fold,

at least 80 fold, at least 90 fold, at least 100 fold, at least 200 fold, at least 300 fold, at least 400 fold, at least 500 fold, at least 600 fold, at least 700 fold, at least 800 fold, at least 900 fold, at least 1000 fold, at least 2000 fold, at least 3000 fold, at least 4000 fold, at least 5000 fold, at least 6000 fold, at least 7000 fold, at least 8000 fold, at least 9000 fold, or at least 10000 fold as compared to a complex with an unmodified single guide RNA and a Cas9 protein, wherein half life of the complex is measured in vitro.

[467] In another aspect, provided herein is a cell comprising the complex as provided herein. In some embodiments, the cell may be an in vitro cell. In some embodiments, the cell may be an ex vivo cell. In some embodiments, the cell may be an in vivo cell. In some embodiments, the cell may be an isolated cell.

Gene Modification Compositions

[468] In another aspect, provided herein is a composition for gene modification comprising the single guide RNA as provided herein and a base editor protein or a nucleic acid sequence encoding the base editor protein. In some embodiments, the composition further comprises a vector that comprises the nucleic acid sequence encoding the base editor protein.

[469] In some embodiments, the nucleic acid sequence may be a DNA, an RNA or mRNA, or a modified nucleic acid sequence. In some embodiments, the vector may be an expression vector. In some embodiments, the nucleic acid is operatively linked to a promoter of the vector. In some embodiments, the vector is a plasmid or a viral vector.

Therapeutics and methods of treatment

[470] In some aspects, provided herein is a method for treating or preventing a condition in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of (i) a guide polynucleotide or a nucleic acid encoding same, and (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same. In some embodiments, the base editor system comprises a guide polynucleotide. In some embodiments, the base editor system comprises a nucleic acid encoding a guide polynucleotide. In some embodiments, the base editor system comprises a base editor fusion protein comprising a programmable DNA binding domain and a deaminase. In some embodiments, the base editor system comprises a nucleic acid encoding a base editor fusion protein comprising a programmable DNA binding domain and a deaminase.

[471] In some embodiments, the guide polynucleotide directs the base editor system to effect a nucleobase alteration in a ANGPTL3 gene in the subject. In some embodiments, the guide polynucleotide directs the base editor system to effect a nucleobase alteration in a PCSK9gene in the subject.

[472] In some embodiments, the base alteration occurs in at least 35% of whole liver cells in the subject as measured by next generation sequencing or Sanger sequencing, thereby treating or preventing the condition in the subject.

[473] In some aspects, provided herein is a method for treating or preventing a condition in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a first composition, comprising (i) a guide polynucleotide or a nucleic acid encoding same, and (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same, wherein the guide polynucleotide directs the base editor system to effect a nucleobase alteration in a PCSK9 gene in the subject, wherein the base alteration occurs in at least 35% of whole liver cells in the subject as measured by next generation sequencing or Sanger sequencing; and a second composition, comprising (i) a guide polynucleotide or a nucleic acid encoding same, and (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same, wherein the guide polynucleotide directs the base editor system to effect a nucleobase alteration in a ANGPTL3 gene in the subject, wherein the base alteration occurs in at least 35% of whole liver cells in the subject as measured by next generation sequencing or Sanger sequencing.

[474] In some embodiments, the first composition and the second composition are administered sequentially. In some embodiment, the first composition is administered for one or more doses, then the second composition is administered for one or more doses. In some embodiments, the first composition and the second composition interspersed. In some embodiments, the first composition and the second composition are administered concurrently. In some embodiments, the first composition and the second composition are administered for one or more doses. In some embodiments, the first composition and the second composition are administered over and interval of over an interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 hours. In some embodiments, the first composition and the second composition are administered over and interval of over an interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 days. In some embodiments, the first composition and the second composition are administered over and interval of over an

interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 weeks.

[475] In some embodiments, the base alteration occurs in at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.3%, 99.5%, 99.7%, 99.8%, or 99.9% of whole liver cells in the subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the base alteration occurs in at most 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.3%, 99.5%, 99.7%, 99.8%, or 99.9% of whole liver cells in the subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the base alteration occurs in 1%-99.9%, 2%-99.9%, 3%-99.9%, 4%-99.9%, 5%-99.9%, 6%-99.9%, 7%-99.9%, 8%-99.9%, 9%-99.9%, 10%-99.9%, 15%-99.9%, 20%-99.9%, 25%-99.9%, 30%-99.9%, 35%-99.9%, 40%-99.9%, 45%-99.9%, 50%-99.9%, 55%-99.9%, 60%-99.9%, 65%-99.9%, 70%-99.9%, 75%-99.9%, 80%-99.9%, 85%-99.9%, 90%-99.9%, or 95-99.9% of whole liver cells in the subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the base alteration occurs in 1%-99.5%, 1%-99%, 1%-98%, 1%-97%, 1%-96%, 1%-95%, 1%-90%, 1%-85%, 1%-80%, 1%-75%, 1%-70%, 1%-65%, 1%-60%, 1%-55%, 1%-50%, 1%-45%, 1%-40%, 1%-35%, 1%-30%, 1%-25%, 1%-20%, 1%-15%, 1%-10%, 1%-9%, 1%-8%, 1%-7%, 1%-6%, 1%-5%, 1%-4%, 1%-3%, or 1%-2% of whole liver cells in the subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the base alteration occurs in 1%-90%, 5%-85%, 10%-80%, 15%-75%, 20%-70%, 25%-65%, 30%-60%, 35%-55%, or 40%-50% of whole liver cells in the subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the base alteration occurs in 100% of whole liver cells in the subject as measured by next generation sequencing or Sanger sequencing.

[476] In some embodiments, the base alteration occurs in hepatocytes in the subject. In some embodiments, the base alteration occurs in at least 30% of hepatocytes in the subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the base alteration occurs in hepatocytes in the subject. In some embodiments, the base alteration occurs in at least 35% of hepatocytes in the subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the base alteration occurs in at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.3%, 99.5%, 99.7%,

99.8%, or 99.9% of hepatocytes in the subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the base alteration occurs in at most 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.3%, 99.5%, 99.7%, 99.8%, or 99.9% of hepatocytes in the subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the base alteration occurs in 1%-99.9%, 2%-99.9%, 3%-99.9%, 4%-99.9%, 5%-99.9%, 6%-99.9%, 7%-99.9%, 8%-99.9%, 9%-99.9%, 10%-99.9%, 15%-99.9%, 20%-99.9%, 25%-99.9%, 30%-99.9%, 35%-99.9%, 40%-99.9%, 45%-99.9%, 50%-99.9%, 55%-99.9%, 60%-99.9%, 65%-99.9%, 70%-99.9%, 75%-99.9%, 80%-99.9%, 85%-99.9%, 90%-99.9%, or 95-99.9% of hepatocytes in the subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the base alteration occurs in 1%-99.5%, 1%-99%, 1%-98%, 1%-97%, 1%-96%, 1%-95%, 1%-90%, 1%-85%, 1%-80%, 1%-75%, 1%-70%, 1%-65%, 1%-60%, 1%-55%, 1%-50%, 1%-45%, 1%-40%, 1%-35%, 1%-30%, 1%-25%, 1%-20%, 1%-15%, 1%-10%, 1%-9%, 1%-8%, 1%-7%, 1%-6%, 1%-5%, 1%-4%, 1%-3%, or 1%-2% hepatocytes in the subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the base alteration occurs in 1%-90%, 5%-85%, 10%-80%, 15%-75%, 20%-70%, 25%-65%, 30%-60%, 35%-55%, or 40%-50% of hepatocytes in the subject as measured by next generation sequencing or Sanger sequencing. In some embodiments, the base alteration occurs in 100% of hepatocytes in the subject as measured by next generation sequencing or Sanger sequencing.

[477] In some embodiments, the base alteration occurred in whole liver cells in the subject is measured by next generation sequencing. In some embodiments, the base alteration occurred in whole liver cells in the subject is measured by Sanger sequencing. In some embodiments, the base alteration occurred in hepatocytes in the subject is measured by next generation sequencing. In some embodiments, the base alteration occurred in hepatocytes in the subject is measured by Sanger sequencing.

[478] In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering (i) the guide polynucleotide and (ii) the nucleic acid encoding the base editor fusion protein to the subject. In some embodiments, the base editor system comprises a guide polynucleotide. In some embodiments, the base editor system comprises a nucleic acid encoding a guide polynucleotide.

[479] In some embodiments, the nucleic acid encoding the base editor fusion protein is a mRNA. In some embodiments, the mRNA generates the base editor fusion protein upon

translation in the subject after the administration. In some embodiments, the base editor fusion protein forms a RNP complex in the subject.

[480] In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein further comprises administering a lipid nanoparticle (LNP) enclosing a guide polynucleotide or a nucleic acid encoding the guide polynucleotide (i). In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein further comprises administering a second LNP enclosing a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same (ii). In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein further comprises administering a LNP enclosing a guide polynucleotide or a nucleic acid encoding the guide polynucleotide (i) and a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same (ii). In some embodiments, the RNP complex is formed upon uptake of the LNP or the second LNP by a liver cell in the subject.

[481] In some embodiments, the ratio of a guide polynucleotide or a nucleic acid encoding the guide polynucleotide (i) and a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same (ii) is about 1:10 to about 10:1 by weight. In some embodiments, the ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 1:1, 1.5:1, 2:1, 3:1, 4:1, 1:1.5, 1:2, 1:3, or 1:4 by weight. In some embodiments, the molar ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 500:1 to about 1:500.

[482] In some embodiments, the ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 1000:1 to about 1:1000 by weight. In some embodiments, the ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 1000:1, 950:1, 900:1, 850:1, 800:1, 750:1, 700:1, 650:1, 600:1, 550:1, 500:1, 450:1, 400:1, 350:1, 300:1, 250:1, 200:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 65:1, 60:1, 55:1, 50:1, 45:1, 40:1, 35:1, 30:1, 25:1, 20:1, 19:1, 18:1, 17:1, 17:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2;1, 1.9:1, 1.8:1, 1.7:1, 1.6:1, 1.5:1, 1.4:1, 1.3:1, 1.2:1, 1.1:1, 1.0:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, 0.5:1, 0.4:1, 0.3:1, 0.2:1, or 0.1 by weight. In some embodiments, the ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 1:0.1, 1:0.2, 1:0.3, 1:0.4, 1:0.5, 1:0.6, 1:0.7, 1:0.8, 1:0.9, 1:1.0, 1:1.1, 1:1.2, 1:1.3, 1:1.4, 1:1.5, 1:1.6, 1:1.7, 1:1.8, 1:1.9, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, 1:20,

1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, 1:100, 1:150, 1:200, 1:250, 1:300, 1:350, 1:400, 1:450, 1:500, 1:550, 1:600, 1:650, 1:700, 1:750, 1:800, 1:850, 1:900, 1:950, or 1:1000 by weight. In some embodiments, the ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at least about 1000:1, 950:1, 900:1, 850:1, 800:1, 750:1, 700:1, 650:1, 600:1, 550:1, 500:1, 450:1, 400:1, 350:1, 300:1, 250:1, 200:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 65:1, 60:1, 55:1, 50:1, 45:1, 40:1, 35:1, 30:1, 25:1, 20:1, 19:1, 18:1, 17:1, 17:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2;1, 1.9:1, 1.8:1, 1.7:1, 1.6:1, 1.5:1, 1.4:1, 1.3:1, 1.2:1, 1.1:1, 1.0:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, 0.5:1, 0.4:1, 0.3:1, 0.2:1, or 0.1 by weight. In some embodiments, the ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at least about 1:0.1, 1:0.2, 1:0.3, 1:0.4, 1:0.5, 1:0.6, 1:0.7, 1:0.8, 1:0.9, 1:1.0, 1:1.1, 1:1.2, 1:1.3, 1:1.4, 1:1.5, 1:1.6, 1:1.7, 1:1.8, 1:1.9, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, 1:100, 1:150, 1:200, 1:250, 1:300, 1:350, 1:400, 1:450, 1:500, 1:550, 1:600, 1:650, 1:700, 1:750, 1:800, 1:850, 1:900, 1:950, or 1:1000 by weight. In some embodiments, the ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at most about 1000:1, 950:1, 900:1, 850:1, 800:1, 750:1, 700:1, 650:1, 600:1, 550:1, 500:1, 450:1, 400:1, 350:1, 300:1, 250:1, 200:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 65:1, 60:1, 55:1, 50:1, 45:1, 40:1, 35:1, 30:1, 25:1, 20:1, 19:1, 18:1, 17:1, 17:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2;1, 1.9:1, 1.8:1, 1.7:1, 1.6:1, 1.5:1, 1.4:1, 1.3:1, 1.2:1, 1.1:1, 1.0:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, 0.5:1, 0.4:1, 0.3:1, 0.2:1, or 0.1 by weight. In some embodiments, the ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at most about 1:0.1, 1:0.2, 1:0.3, 1:0.4, 1:0.5, 1:0.6, 1:0.7, 1:0.8, 1:0.9, 1:1.0, 1:1.1, 1:1.2, 1:1.3, 1:1.4, 1:1.5, 1:1.6, 1:1.7, 1:1.8, 1:1.9, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, 1:100, 1:150, 1:200, 1:250, 1:300, 1:350, 1:400, 1:450, 1:500, 1:550, 1:600, 1:650, 1:700, 1:750, 1:800, 1:850, 1:900, 1:950, or 1:1000 by weight.

[483] In some embodiments, the molar ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 10000:1 to about 1:10000. In some embodiments, the molar ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 10000:1, 9500:1, 9000:1, 8500:1, 8000:1, 7500:1, 7000:1, 6500:1, 6000:1, 5500:1, 5000:1, 4500:1, 4000:1, 3500:1, 3000:1, 2500:1, 2000:1, 1500:1,

1000:1, 950:1, 900:1, 850:1, 800:1, 750:1, 700:1, 650:1, 600:1, 550:1, 500:1, 450:1, 400:1, 350:1, 300:1, 250:1, 200:1, 190:1, 180:1, 170:1, 160:1, 150:1, 140:1, 130:1, 120:1, 110:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 69:1, 68:1, 67:1, 66:1, 65:1, 64:1, 63:1, 62:1, 61:1, 60:1, 59:1, 58:1, 57:1, 56:1, 55:1, 54:1, 53:1, 52:1, 51:1, 50:1, 49:1, 48:1, 47:1, 46:1, 45:1, 44:1, 43:1, 42:1, 41:1, 40:1, 39:1, 38:1, 37:1, 36:1, 35:1, 34:1, 33:1, 32:1, 31:1, 30:1, 29:1, 28:1, 27:1, 26:1, 25:1, 24:1, 23:1, 22:1, 21:1, 20:1, 19:1, 18:1, 17:1, 16:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, 0.5:1, 0.4:1, 0.3:1, 0.2:1, or 0.1:1. In some embodiments, the molar ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at least about 1:10000, 1:9500, 1:9000, 1:8500, 1:8000, 1:7500, 1:7000, 1:6500, 1:6000, 1:5500, 1:5000, 1:4500, 1:4000, 1:3500, 1:3000, 1:2500, 1:2000, 1:1500, 1:1000, 1:950, 1:900, 1:850, 1:800, 1:750, 1:700, 1:650, 1:600, 1:550, 1:500, 1:450, 1:400, 1:350, 1:300, 1:250, 1:200, 1:190, 1:180, 1:170, 1:160, 1:150, 1:140, 1:130, 1:120, 1:110, 1:100, 1:95, 1:90, 1:85, 1:80, 1:75, 1:70, 1:69, 1:68, 1:67, 1:66, 1:65, 1:64, 1:63, 1:62, 1:61, 1:60, 1:59, 1:58, 1:57, 1:56, 1:55, 1:54, 1:53, 1:52, 1:51, 1:50, 1:49, 1:48, 1:47, 1:46, 1:45, 1:44, 1:43, 1:42, 1:41, 1:40, 1:39, 1:38, 1:37, 1:36, 1:35, 1:34, 1:33, 1:32, 1:31, 1:30, 1:29, 1:28, 1:27, 1:26, 1:25, 1:24, 1:23, 1:22, 1:21, 1:20, 1:19, 1:18, 1:17, 1:16, 1:15, 1:14, 1:13, 1:12, 1:11, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 1:0.9, 1:0.8, 1:0.7, 1:0.6, 1:0.5, 1:0.4, 1:0.3, 1:0.2, or 1:0.1. In some embodiments, the molar ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at least about 10000:1, 9500:1, 9000:1, 8500:1, 8000:1, 7500:1, 7000:1, 6500:1, 6000:1, 5500:1, 5000:1, 4500:1, 4000:1, 3500:1, 3000:1, 2500:1, 2000:1, 1500:1, 1000:1, 950:1, 900:1, 850:1, 800:1, 750:1, 700:1, 650:1, 600:1, 550:1, 500:1, 450:1, 400:1, 350:1, 300:1, 250:1, 200:1, 190:1, 180:1, 170:1, 160:1, 150:1, 140:1, 130:1, 120:1, 110:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 69:1, 68:1, 67:1, 66:1, 65:1, 64:1, 63:1, 62:1, 61:1, 60:1, 59:1, 58:1, 57:1, 56:1, 55:1, 54:1, 53:1, 52:1, 51:1, 50:1, 49:1, 48:1, 47:1, 46:1, 45:1, 44:1, 43:1, 42:1, 41:1, 40:1, 39:1, 38:1, 37:1, 36:1, 35:1, 34:1, 33:1, 32:1, 31:1, 30:1, 29:1, 28:1, 27:1, 26:1, 25:1, 24:1, 23:1, 22:1, 21:1, 20:1, 19:1, 18:1, 17:1, 16:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, 0.5:1, 0.4:1, 0.3:1, 0.2:1, or 0.1:1. In some embodiments, the molar ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at least about 1:10000, 1:9500, 1:9000, 1:8500, 1:8000, 1:7500, 1:7000, 1:6500, 1:6000, 1:5500, 1:5000, 1:4500, 1:4000, 1:3500, 1:3000, 1:2500, 1:2000, 1:1500, 1:1000, 1:950, 1:900, 1:850, 1:800, 1:750, 1:700, 1:650, 1:600, 1:550, 1:500, 1:450, 1:400, 1:350, 1:300, 1:250, 1:200, 1:190, 1:180, 1:170, 1:160, 1:150, 1:140, 1:130, 1:120, 1:110, 1:100, 1:95, 1:90, 1:85, 1:80, 1:75,

1:70, 1:69, 1:68, 1:67, 1:66, 1:65, 1:64, 1:63, 1:62, 1:61, 1:60, 1:59, 1:58, 1:57, 1:56, 1:55, 1:54, 1:53, 1:52, 1:51, 1:50, 1:49, 1:48, 1:47, 1:46, 1:45, 1:44, 1:43, 1:42, 1:41, 1:40, 1:39, 1:38, 1:37, 1:36, 1:35, 1:34, 1:33, 1:32, 1:31, 1:30, 1:29, 1:28, 1:27, 1:26, 1:25, 1:24, 1:23, 1:22, 1:21, 1:20, 1:19, 1:18, 1:17, 1:16, 1:15, 1:14, 1:13, 1:12, 1:11, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 1:0.9, 1:0.8, 1:0.7, 1:0.6, 1:0.5, 1:0.4, 1:0.3, 1:0.2, or 1:0.1. In some embodiments, the molar ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at most about 10000:1, 9500:1, 9000:1, 8500:1, 8000:1, 7500:1, 7000:1, 6500:1, 6000:1, 5500:1, 5000:1, 4500:1, 4000:1, 3500:1, 3000:1, 2500:1, 2000:1, 1500:1, 1000:1, 950:1, 900:1, 850:1, 800:1, 750:1, 700:1, 650:1, 600:1, 550:1, 500:1, 450:1, 400:1, 350:1, 300:1, 250:1, 200:1, 190:1, 180:1, 170:1, 160:1, 150:1, 140:1, 130:1, 120:1, 110:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 69:1, 68:1, 67:1, 66:1, 65:1, 64:1, 63:1, 62:1, 61:1, 60:1, 59:1, 58:1, 57:1, 56:1, 55:1, 54:1, 53:1, 52:1, 51:1, 50:1, 49:1, 48:1, 47:1, 46:1, 45:1, 44:1, 43:1, 42:1, 41:1, 40:1, 39:1, 38:1, 37:1, 36:1, 35:1, 34:1, 33:1, 32:1, 31:1, 30:1, 29:1, 28:1, 27:1, 26:1, 25:1, 24:1, 23:1, 22:1, 21:1, 20:1, 19:1, 18:1, 17:1, 16:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, 0.5:1, 0.4:1, 0.3:1, 0.2:1, or 0.1:1. In some embodiments, the molar ratio of the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at most about 1:10000, 1:9500, 1:9000, 1:8500, 1:8000, 1:7500, 1:7000, 1:6500, 1:6000, 1:5500, 1:5000, 1:4500, 1:4000, 1:3500, 1:3000, 1:2500, 1:2000, 1:1500, 1:1000, 1:950, 1:900, 1:850, 1:800, 1:750, 1:700, 1:650, 1:600, 1:550, 1:500, 1:450, 1:400, 1:350, 1:300, 1:250, 1:200, 1:190, 1:180, 1:170, 1:160, 1:150, 1:140, 1:130, 1:120, 1:110, 1:100, 1:95, 1:90, 1:85, 1:80, 1:75, 1:70, 1:69, 1:68, 1:67, 1:66, 1:65, 1:64, 1:63, 1:62, 1:61, 1:60, 1:59, 1:58, 1:57, 1:56, 1:55, 1:54, 1:53, 1:52, 1:51, 1:50, 1:49, 1:48, 1:47, 1:46, 1:45, 1:44, 1:43, 1:42, 1:41, 1:40, 1:39, 1:38, 1:37, 1:36, 1:35, 1:34, 1:33, 1:32, 1:31, 1:30, 1:29, 1:28, 1:27, 1:26, 1:25, 1:24, 1:23, 1:22, 1:21, 1:20, 1:19, 1:18, 1:17, 1:16, 1:15, 1:14, 1:13, 1:12, 1:11, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 1:0.9, 1:0.8, 1:0.7, 1:0.6, 1:0.5, 1:0.4, 1:0.3, 1:0.2, or 1:0.1.

[484] In some embodiments, the ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 10:1 to about 1:10 by weight. In some embodiments, the ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 4:1, 3;1, 2:1, 1.5:1, 1:1, 1:1.5, 1:2, 1:3, or 1:4 by weight. In some embodiments, the molar ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 500:1 to about 1:500.

[485] In some embodiments, the ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 1000:1 to about 1:1000 by weight. In some embodiments, the ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 1000:1, 950:1, 900:1, 850:1, 800:1, 750:1, 700:1, 650:1, 600:1, 550:1, 500:1, 450:1, 400:1, 350:1, 300:1, 250:1, 200:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 65:1, 60:1, 55:1, 50:1, 45:1, 40:1, 35:1, 30:1, 25:1, 20:1, 19:1, 18:1, 17:1, 17:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2;1, 1.9:1, 1.8:1, 1.7:1, 1.6:1, 1.5:1, 1.4:1, 1.3:1, 1.2:1, 1.1:1, 1.0:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, 0.5:1, 0.4:1, 0.3:1, 0.2:1, or 0.1 by weight. In some embodiments, the ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 1:0.1, 1:0.2, 1:0.3, 1:0.4, 1:0.5, 1:0.6, 1:0.7, 1:0.8, 1:0.9, 1:1.0, 1:1.1, 1:1.2, 1:1.3, 1:1.4, 1:1.5, 1:1.6, 1:1.7, 1:1.8, 1:1.9, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, 1:100, 1:150, 1:200, 1:250, 1:300, 1:350, 1:400, 1:450, 1:500, 1:550, 1:600, 1:650, 1:700, 1:750, 1:800, 1:850, 1:900, 1:950, or 1:1000 by weight. In some embodiments, the ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at least about 1000:1, 950:1, 900:1, 850:1, 800:1, 750:1, 700:1, 650:1, 600:1, 550:1, 500:1, 450:1, 400:1, 350:1, 300:1, 250:1, 200:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 65:1, 60:1, 55:1, 50:1, 45:1, 40:1, 35:1, 30:1, 25:1, 20:1, 19:1, 18:1, 17:1, 17:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2;1, 1.9:1, 1.8:1, 1.7:1, 1.6:1, 1.5:1, 1.4:1, 1.3:1, 1.2:1, 1.1:1, 1.0:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, 0.5:1, 0.4:1, 0.3:1, 0.2:1, or 0.1 by weight. In some embodiments, the ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at least about 1:0.1, 1:0.2, 1:0.3, 1:0.4, 1:0.5, 1:0.6, 1:0.7, 1:0.8, 1:0.9, 1:1.0, 1:1.1, 1:1.2, 1:1.3, 1:1.4, 1:1.5, 1:1.6, 1:1.7, 1:1.8, 1:1.9, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, 1:100, 1:150, 1:200, 1:250, 1:300, 1:350, 1:400, 1:450, 1:500, 1:550, 1:600, 1:650, 1:700, 1:750, 1:800, 1:850, 1:900, 1:950, or 1:1000 by weight. In some embodiments, the ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at most about 1000:1, 950:1, 900:1, 850:1, 800:1, 750:1, 700:1, 650:1, 600:1, 550:1, 500:1, 450:1, 400:1, 350:1, 300:1, 250:1, 200:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 65:1, 60:1, 55:1, 50:1, 45:1, 40:1, 35:1, 30:1, 25:1, 20:1, 19:1, 18:1, 17:1, 17:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2;1, 1.9:1, 1.8:1, 1.7:1,

1.6:1, 1.5:1, 1.4:1, 1.3:1, 1.2:1, 1.1:1, 1.0:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, 0.5:1, 0.4:1, 0.3:1, 0.2:1, or 0.1 by weight. In some embodiments, the ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at most about 1:0.1, 1:0.2, 1:0.3, 1:0.4, 1:0.5, 1:0.6, 1:0.7, 1:0.8, 1:0.9, 1:1.0, 1:1.1, 1:1.2, 1:1.3, 1:1.4, 1:1.5, 1:1.6, 1:1.7, 1:1.8, 1:1.9, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, 1:100, 1:150, 1:200, 1:250, 1:300, 1:350, 1:400, 1:450, 1:500, 1:550, 1:600, 1:650, 1:700, 1:750, 1:800, 1:850, 1:900, 1:950, or 1:1000 by weight.

[486] In some embodiments, the molar ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 10000:1 to about 1:10000. In some embodiments, the molar ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is about 10000:1, 9500:1, 9000:1, 8500:1, 8000:1, 7500:1, 7000:1, 6500:1, 6000:1, 5500:1, 5000:1, 4500:1, 4000:1, 3500:1, 3000:1, 2500:1, 2000:1, 1500:1, 1000:1, 950:1, 900:1, 850:1, 800:1, 750:1, 700:1, 650:1, 600:1, 550:1, 500:1, 450:1, 400:1, 350:1, 300:1, 250:1, 200:1, 190:1, 180:1, 170:1, 160:1, 150:1, 140:1, 130:1, 120:1, 110:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 69:1, 68:1, 67:1, 66:1, 65:1, 64:1, 63:1, 62:1, 61:1, 60:1, 59:1, 58:1, 57:1, 56:1, 55:1, 54:1, 53:1, 52:1, 51:1, 50:1, 49:1, 48:1, 47:1, 46:1, 45:1, 44:1, 43:1, 42:1, 41:1, 40:1, 39:1, 38:1, 37:1, 36:1, 35:1, 34:1, 33:1, 32:1, 31:1, 30:1, 29:1, 28:1, 27:1, 26:1, 25:1, 24:1, 23:1, 22:1, 21:1, 20:1, 19:1, 18:1, 17:1, 16:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, 0.5:1, 0.4:1, 0.3:1, 0.2:1, or 0.1:1. In some embodiments, the molar ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at least about 1:10000, 1:9500, 1:9000, 1:8500, 1:8000, 1:7500, 1:7000, 1:6500, 1:6000, 1:5500, 1:5000, 1:4500, 1:4000, 1:3500, 1:3000, 1:2500, 1:2000, 1:1500, 1:1000, 1:950, 1:900, 1:850, 1:800, 1:750, 1:700, 1:650, 1:600, 1:550, 1:500, 1:450, 1:400, 1:350, 1:300, 1:250, 1:200, 1:190, 1:180, 1:170, 1:160, 1:150, 1:140, 1:130, 1:120, 1:110, 1:100, 1:95, 1:90, 1:85, 1:80, 1:75, 1:70, 1:69, 1:68, 1:67, 1:66, 1:65, 1:64, 1:63, 1:62, 1:61, 1:60, 1:59, 1:58, 1:57, 1:56, 1:55, 1:54, 1:53, 1:52, 1:51, 1:50, 1:49, 1:48, 1:47, 1:46, 1:45, 1:44, 1:43, 1:42, 1:41, 1:40, 1:39, 1:38, 1:37, 1:36, 1:35, 1:34, 1:33, 1:32, 1:31, 1:30, 1:29, 1:28, 1:27, 1:26, 1:25, 1:24, 1:23, 1:22, 1:21, 1:20, 1:19, 1:18, 1:17, 1:16, 1:15, 1:14, 1:13, 1:12, 1:11, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 1:0.9, 1:0.8, 1:0.7, 1:0.6, 1:0.5, 1:0.4, 1:0.3, 1:0.2, or 1:0.1. In some embodiments, the molar ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at least about 10000:1, 9500:1, 9000:1, 8500:1, 8000:1, 7500:1,

7000:1, 6500:1, 6000:1, 5500:1, 5000:1, 4500:1, 4000:1, 3500:1, 3000:1, 2500:1, 2000:1, 1500:1, 1000:1, 950:1, 900:1, 850:1, 800:1, 750:1, 700:1, 650:1, 600:1, 550:1, 500:1, 450:1, 400:1, 350:1, 300:1, 250:1, 200:1, 190:1, 180:1, 170:1, 160:1, 150:1, 140:1, 130:1, 120:1, 110:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 69:1, 68:1, 67:1, 66:1, 65:1, 64:1, 63:1, 62:1, 61:1, 60:1, 59:1, 58:1, 57:1, 56:1, 55:1, 54:1, 53:1, 52:1, 51:1, 50:1, 49:1, 48:1, 47:1, 46:1, 45:1, 44:1, 43:1, 42:1, 41:1, 40:1, 39:1, 38:1, 37:1, 36:1, 35:1, 34:1, 33:1, 32:1, 31:1, 30:1, 29:1, 28:1, 27:1, 26:1, 25:1, 24:1, 23:1, 22:1, 21:1, 20:1, 19:1, 18:1, 17:1, 16:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, 0.5:1, 0.4:1, 0.3:1, 0.2:1, or 0.1:1. In some embodiments, the molar ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at least about 1:10000, 1:9500, 1:9000, 1:8500, 1:8000, 1:7500, 1:7000, 1:6500, 1:6000, 1:5500, 1:5000, 1:4500, 1:4000, 1:3500, 1:3000, 1:2500, 1:2000, 1:1500, 1:1000, 1:950, 1:900, 1:850, 1:800, 1:750, 1:700, 1:650, 1:600, 1:550, 1:500, 1:450, 1:400, 1:350, 1:300, 1:250, 1:200, 1:190, 1:180, 1:170, 1:160, 1:150, 1:140, 1:130, 1:120, 1:110, 1:100, 1:95, 1:90, 1:85, 1:80, 1:75, 1:70, 1:69, 1:68, 1:67, 1:66, 1:65, 1:64, 1:63, 1:62, 1:61, 1:60, 1:59, 1:58, 1:57, 1:56, 1:55, 1:54, 1:53, 1:52, 1:51, 1:50, 1:49, 1:48, 1:47, 1:46, 1:45, 1:44, 1:43, 1:42, 1:41, 1:40, 1:39, 1:38, 1:37, 1:36, 1:35, 1:34, 1:33, 1:32, 1:31, 1:30, 1:29, 1:28, 1:27, 1:26, 1:25, 1:24, 1:23, 1:22, 1:21, 1:20, 1:19, 1:18, 1:17, 1:16, 1:15, 1:14, 1:13, 1:12, 1:11, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 1:0.9, 1:0.8, 1:0.7, 1:0.6, 1:0.5, 1:0.4, 1:0.3, 1:0.2, or 1:0.1. In some embodiments, the molar ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at most about 10000:1, 9500:1, 9000:1, 8500:1, 8000:1, 7500:1, 7000:1, 6500:1, 6000:1, 5500:1, 5000:1, 4500:1, 4000:1, 3500:1, 3000:1, 2500:1, 2000:1, 1500:1, 1000:1, 950:1, 900:1, 850:1, 800:1, 750:1, 700:1, 650:1, 600:1, 550:1, 500:1, 450:1, 400:1, 350:1, 300:1, 250:1, 200:1, 190:1, 180:1, 170:1, 160:1, 150:1, 140:1, 130:1, 120:1, 110:1, 100:1, 95:1, 90:1, 85:1, 80:1, 75:1, 70:1, 69:1, 68:1, 67:1, 66:1, 65:1, 64:1, 63:1, 62:1, 61:1, 60:1, 59:1, 58:1, 57:1, 56:1, 55:1, 54:1, 53:1, 52:1, 51:1, 50:1, 49:1, 48:1, 47:1, 46:1, 45:1, 44:1, 43:1, 42:1, 41:1, 40:1, 39:1, 38:1, 37:1, 36:1, 35:1, 34:1, 33:1, 32:1, 31:1, 30:1, 29:1, 28:1, 27:1, 26:1, 25:1, 24:1, 23:1, 22:1, 21:1, 20:1, 19:1, 18:1, 17:1, 16:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, 0.5:1, 0.4:1, 0.3:1, 0.2:1, or 0.1:1. In some embodiments, the molar ratio of a nucleic acid encoding the guide polynucleotide and the nucleic acid encoding the base editor fusion protein is at most about 1:10000, 1:9500, 1:9000, 1:8500, 1:8000, 1:7500, 1:7000, 1:6500, 1:6000, 1:5500, 1:5000, 1:4500, 1:4000, 1:3500, 1:3000, 1:2500, 1:2000, 1:1500, 1:1000, 1:950, 1:900, 1:850, 1:800, 1:750, 1:700, 1:650, 1:600, 1:550, 1:500,

1:450, 1:400, 1:350, 1:300, 1:250, 1:200, 1:190, 1:180, 1:170, 1:160, 1:150, 1:140, 1:130, 1:120, 1:110, 1:100, 1:95, 1:90, 1:85, 1:80, 1:75, 1:70, 1:69, 1:68, 1:67, 1:66, 1:65, 1:64, 1:63, 1:62, 1:61, 1:60, 1:59, 1:58, 1:57, 1:56, 1:55, 1:54, 1:53, 1:52, 1:51, 1:50, 1:49, 1:48, 1:47, 1:46, 1:45, 1:44, 1:43, 1:42, 1:41, 1:40, 1:39, 1:38, 1:37, 1:36, 1:35, 1:34, 1:33, 1:32, 1:31, 1:30, 1:29, 1:28, 1:27, 1:26, 1:25, 1:24, 1:23, 1:22, 1:21, 1:20, 1:19, 1:18, 1:17, 1:16, 1:15, 1:14, 1:13, 1:12, 1:11, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 1:0.9, 1:0.8, 1:0.7, 1:0.6, 1:0.5, 1:0.4, 1:0.3, 1:0.2, or 1:0.1.

[487] In some embodiments, the nucleobase alteration results in a reduction of at least 35% in blood PCSK9 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS.

[488] In some embodiments, the nucleobase alteration results in a reduction of at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% in blood PCSK9 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS.

[489] In some embodiments, the nucleobase alteration results in a reduction of at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 85%, 90%, 95%, 97%, 98%, 90%, 95%, 97%, 98%, 99%, 99.3%, 99.5%, 99.7%, 99.8%, or 99.9% in blood PCSK9 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in a reduction of at most 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 85%, 90%, 95%, 97%, 98%, 90%, 95%, 97%, 98%, 99%, 99.3%, 99.5%, 99.7%, 99.8%, or 99.9% in blood PCSK9 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in a reduction of 1%-99.9%, 2%-99.9%, 3%-99.9%, 4%-99.9%, 5%-99.9%, 6%-99.9%, 7%-99.9%, 8%-99.9%, 9%-99.9%, 10%-99.9%, 15%-99.9%, 20%-99.9%, 25%-99.9%, 30%-99.9%, 31%-99.9%, 32%-99.9%, 33%-99.9%, 34%-99.9%, 35%-99.9%, 36%-99.9%, 37%-99.9%, 38%-99.9%, 39%-99.9%, 40%-99.9%, 45%-99.9%, 50%-99.9%, 55%-99.9%, 60%-99.9%, 65%-99.9%, 70%-99.9%, 75%-99.9%, 80%-99.9%, 85%-99.9%, 90%-99.9%, or 95-99.9% in blood PCSK9 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in a

reduction of 1%-99.5%, 1%-99%, 1%-98%, 1%-97%, 1%-96%, 1%-95%, 1%-90%, 1%-85%, 1%-80%, 1%-79%, 1%-78%, 1%-77%, 1%-76%, 1%-75%, 1%-74%, 1%-73%, 1%-72%, 1%-71%, 1%-70%, 1%-65%, 1%-60%, 1%-55%, 1%-50%, 1%-45%, 1%-40%, 1%-39%, 1%-38%, 1%-37%, 1%-36%, 1%-35%, 1%-34%, 1%-33%, 1%-32%, 1%-31%, 1%-30%, 1%-25%, 1%-20%, 1%-15%, 1%-10%, 1%-9%, 1%-8%, 1%-7%, 1%-6%, 1%-5%, 1%-4%, 1%-3%, or 1%-2% in blood PCSK9 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in a reduction of 1%-99.9%, 5%-99.5%, 10%-99%, 15%-97%, 20%-95%, 25%-90%, 30%-85%, 31%-80%, 32%-79%, 33%-78%, 34%-77%, 35%-76%, 36%-76%, 37%-75%, 38%-74%, 39%-73%, 40%-72%, 45%-71%, 50%-70%, or 55%-65% in blood PCSK9 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in a reduction of 100% in blood PCSK9 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS.

[490] In some embodiments, the nucleobase alteration results in at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 75%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 400% 500%, 600%, 700%, 800%, 900%, 1000% less blood PCSK9 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in at least 1.1–fold, 1.2–fold, 1.3–fold, 1.4–fold, 1.5–fold, 1.6–fold, 1.7–fold, 1.8–fold, 1.9–fold, 2–fold, 2.5–fold, 3–fold, 3.5–fold, 4–fold, 4.5–fold, 5–fold, 6–fold, 7–fold, 8–fold, 9–fold, 10–fold, or more than 10-fold less blood PCSK9 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS.

[491] In some embodiments, the reduction of blood PCSK9 protein level or the blood PCSK9 protein level in the subject as compared to prior to the administration is measured by ELISA (enzyme-linked immunosorbent assay). In some embodiments, the reduction of blood PCSK9 protein level or the blood PCSK9 protein level in the subject as compared to prior to the administration is measured by Western blot analysis. In some embodiments, the reduction of blood PCSK9 protein level or the blood PCSK9 protein level in the subject as compared to prior to the administration is measured by LC-MS/MS (liquid chromatography -tandem mass spectrometry).

[492] In some embodiments, the nucleobase alteration results in a reduction of at least 35% in blood ANGPTL3 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS.

[493] In some embodiments, the nucleobase alteration results in a reduction of at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% in blood ANGPTL3 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS.

[494] In some embodiments, the nucleobase alteration results in a reduction of at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 85%, 90%, 95%, 97%, 98%, 90%, 95%, 97%, 98%, 99%, 99.3%, 99.5%, 99.7%, 99.8%, or 99.9% in blood ANGPTL3 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in a reduction of at most 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 85%, 90%, 95%, 97%, 98%, 90%, 95%, 97%, 98%, 99%, 99.3%, 99.5%, 99.7%, 99.8%, or 99.9% in blood ANGPTL3 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in a reduction of 1%-99.9%, 2%-99.9%, 3%-99.9%, 4%-99.9%, 5%-99.9%, 6%-99.9%, 7%-99.9%, 8%-99.9%, 9%-99.9%, 10%-99.9%, 15%-99.9%, 20%-99.9%, 25%-99.9%, 30%-99.9%, 31%-99.9%, 32%-99.9%, 33%-99.9%, 34%-99.9%, 35%-99.9%, 36%-99.9%, 37%-99.9%, 38%-99.9%, 39%-99.9%, 40%-99.9%, 45%-99.9%, 50%-99.9%, 55%-99.9%, 60%-99.9%, 65%-99.9%, 70%-99.9%, 75%-99.9%, 80%-99.9%, 85%-99.9%, 90%-99.9%, or 95-99.9% in blood ANGPTL3 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in a reduction of 1%-99.5%, 1%-99%, 1%-98%, 1%-97%, 1%-96%, 1%-95%, 1%-90%, 1%-85%, 1%-80%, 1%-79%, 1%-78%, 1%-77%, 1%-76%, 1%-75%, 1%-74%, 1%-73%, 1%-72%, 1%-71%, 1%-70%, 1%-65%, 1%-60%, 1%-55%, 1%-50%, 1%-45%, 1%-40%, 1%-39%, 1%-38%, 1%-37%, 1%-36%, 1%-35%, 1%-34%, 1%-33%, 1%-32%, 1%-31%, 1%-30%, 1%-25%, 1%-20%, 1%-15%, 1%-10%, 1%-9%, 1%-8%, 1%-7%, 1%-6%, 1%-5%, 1%-4%, 1%-3%, or 1%-2% in blood ANGPTL3 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some

embodiments, the nucleobase alteration results in a reduction of 1%-99.9%, 5%-99.5%, 10%-99%, 15%-97%, 20%-95%, 25%-90%, 30%-85%, 31%-80%, 32%-79%, 33%-78%, 34%-77%, 35%-76%, 36%-76%, 37%-75%, 38%-74%, 39%-73%, 40%-72%, 45%-71%, 50%-70%, or 55%-65% in blood ANGPTL3 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in a reduction of 100% in blood ANGPTL3 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS.

[495] In some embodiments, the nucleobase alteration results in at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 75%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 400% 500%, 600%, 700%, 800%, 900%, 1000% less blood ANGPTL3 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS. In some embodiments, the nucleobase alteration results in at least 1.1–fold, 1.2–fold, 1.3–fold, 1.4–fold, 1.5–fold, 1.6–fold, 1.7–fold, 1.8–fold, 1.9–fold, 2–fold, 2.5–fold, 3–fold, 3.5–fold, 4–fold, 4.5–fold, 5–fold, 6–fold, 7–fold, 8–fold, 9–fold, 10–fold, or more than 10-fold less blood ANGPTL3 protein level in the subject as compared to prior to the administration as measured by ELISA, Western blots, or LC-MS/MS.

[496] In some embodiments, the reduction of blood ANGPTL3 protein level or the blood ANGPTL3 protein level in the subject as compared to prior to the administration is measured by ELISA (enzyme-linked immunosorbent assay). In some embodiments, the reduction of blood ANGPTL3 protein level or the blood ANGPTL3 protein level in the subject as compared to prior to the administration is measured by Western blot analysis. In some embodiments, the reduction of blood ANGPTL3 protein level or the blood ANGPTL3 protein level in the subject as compared to prior to the administration is measured by LC-MS/MS (liquid chromatography -tandem mass spectrometry).

[497] In some embodiments, the nucleobase alteration results in a reduction of at least 35% in blood low-density lipoprotein cholesterol (LDL-C) level in the subject as compared to prior to the administration.

[498] In some embodiments, the nucleobase alteration results in a reduction of at least 30%, 35%, 40%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% in blood low-density lipoprotein cholesterol (LDL-C) level in the subject as compared to

prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.3%, 99.5%, 99.7%, 99.8%, or 99.9% in blood low-density lipoprotein cholesterol (LDL-C) level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of at most 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.3%, 99.5%, 99.7%, 99.8%, or 99.9% in blood low-density lipoprotein cholesterol (LDL-C) level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of 1%-99.9%, 2%-99.9%, 3%-99.9%, 4%-99.9%, 5%-99.9%, 6%-99.9%, 7%-99.9%, 8%-99.9%, 9%-99.9%, 10%-99.9%, 15%-99.9%, 20%-99.9%, 25%-99.9%, 30%-99.9%, 35%-99.9%, 40%-99.9%, 45%-99.9%, 50%-99.9%, 55%-99.9%, 60%-99.9%, 65%-99.9%, 70%-99.9%, 75%-99.9%, 80%-99.9%, 85%-99.9%, 90%-99.9%, or 95-99.9% in blood low-density lipoprotein cholesterol (LDL-C) level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of 1%-99.5%, 1%-99%, 1%-98%, 1%-97%, 1%-96%, 1%-95%, 1%-90%, 1%-85%, 1%-80%, 1%-75%, 1%-70%, 1%-65%, 1%-60%, 1%-55%, 1%-50%, 1%-45%, 1%-40%, 1%-35%, 1%-30%, 1%-25%, 1%-20%, 1%-15%, 1%-10%, 1%-9%, 1%-8%, 1%-7%, 1%-6%, 1%-5%, 1%-4%, 1%-3%, or 1%-2% in blood low-density lipoprotein cholesterol (LDL-C) level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of 1%-99.9%, 5%-99.5%, 10%-99%, 15%-97%, 20%-95%, 25%-90%, 30%-85%, 35%-80%, 40%-75%, 45%-70%, 50%-65%, or 55%-60% in blood low-density lipoprotein cholesterol (LDL-C) level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of 100% in blood low-density lipoprotein cholesterol (LDL-C) level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 75%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 400% 500%, 600%, 700%, 800%, 900%, 1000% less blood low-density lipoprotein cholesterol (LDL-C) level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in at least 1.1–fold, 1.2–fold, 1.3–fold, 1.4–

fold, 1.5–fold, 1.6–fold, 1.7–fold, 1.8–fold, 1.9–fold, 2–fold, 2.5–fold, 3–fold, 3.5–fold, 4–fold, 4.5–fold, 5–fold, 6–fold, 7–fold, 8–fold, 9–fold, 10–fold, or more than 10-fold less blood low-density lipoprotein cholesterol (LDL-C) level in the subject as compared to prior to the administration.

[499] In some embodiments, the nucleobase alteration results in a reduction of at least 35% in blood triglyceride level in the subject as compared to prior to the administration.

[500] In some embodiments, the nucleobase alteration results in a reduction of at least 30%, 35%, 40%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% in blood triglyceride level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.3%, 99.5%, 99.7%, 99.8%, or 99.9% in blood triglyceride level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of at most 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.3%, 99.5%, 99.7%, 99.8%, or 99.9% in blood triglyceride level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of 1%-99.9%, 2%-99.9%, 3%-99.9%, 4%-99.9%, 5%-99.9%, 6%-99.9%, 7%-99.9%, 8%-99.9%, 9%-99.9%, 10%-99.9%, 15%-99.9%, 20%-99.9%, 25%-99.9%, 30%-99.9%, 35%-99.9%, 40%-99.9%, 45%-99.9%, 50%-99.9%, 55%-99.9%, 60%-99.9%, 65%-99.9%, 70%-99.9%, 75%-99.9%, 80%-99.9%, 85%-99.9%, 90%-99.9%, or 95-99.9% in blood triglyceride level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of 1%-99.5%, 1%-99%, 1%-98%, 1%-97%, 1%-96%, 1%-95%, 1%-90%, 1%-85%, 1%-80%, 1%-75%, 1%-70%, 1%-65%, 1%-60%, 1%-55%, 1%-50%, 1%-45%, 1%-40%, 1%-35%, 1%-30%, 1%-25%, 1%-20%, 1%-15%, 1%-10%, 1%-9%, 1%-8%, 1%-7%, 1%-6%, 1%-5%, 1%-4%, 1%-3%, or 1%-2% in blood triglyceride level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of 1%-99.9%, 5%-99.5%, 10%-99%, 15%-97%, 20%-95%, 25%-90%, 30%-85%, 35%-80%, 40%-75%, 45%-70%, 50%-65%, or 55%-60% in blood triglyceride level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in a reduction of 100% in blood triglyceride

level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 75%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, 400% 500%, 600%, 700%, 800%, 900%, 1000% less blood triglyceride level in the subject as compared to prior to the administration. In some embodiments, the nucleobase alteration results in at least 1.1–fold, 1.2–fold, 1.3–fold, 1.4–fold, 1.5–fold, 1.6–fold, 1.7–fold, 1.8–fold, 1.9–fold, 2–fold, 2.5–fold, 3–fold, 3.5–fold, 4–fold, 4.5–fold, 5–fold, 6–fold, 7–fold, 8–fold, 9–fold, 10–fold, or more than 10-fold less blood triglyceride level in the subject as compared to prior to the administration.

[501] In some embodiments, the blood triglyceride level or the reduction of blood triglyceride level in the subject as compared to prior to the administration is measured by any standard technique. In some embodiments, the blood low-density lipoprotein cholesterol (LDL-C) level or the reduction of blood low-density lipoprotein cholesterol (LDL-C) level in the subject as compared to prior to the administration is measured by any standard technique. For example, a clinical analyzer instrument may be used to measure a 'lipid panel' in serum samples which entails the direct measurement of cholesterol (total C), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) enzymatically. Reagent kits specific for each analyte contain buffers, calibrators, blanks and controls. As used in the present disclosure, cholesterol, triglycerides and HDL-C may be quantified using absorbance measurements of specific enzymatic reaction products. LDL-C may be determined indirectly. In some instances, most of circulating cholesterol can be found in three major lipoprotein fractions: very low-density lipoproteins (VLDL), LDL and HDL. In some embodiments, total circulating cholesterol may be estimated with the formula [Total C] = [VLDL-C] + [LDL-C] + [HDL-C]. Thus the LDL-C can be calculated from measured values of total cholesterol, triglycerides and HDL-C according to the relationship: [LDL-C] = [total C] - [HDL-C] - [TG]/5, where [TG]/5 is an estimate of VLDL-cholesterol. A reagent kit specific for triglycerides containing buffers, calibrators, blanks and controls . As used herein, serum samples from the study may be analyzed and triglycerides may be measured using a series of coupled enzymatic reactions. In some embodiments, H2O2 may be used to quantify the analyte.as the end product of the last one and its absorbance at 500nm, and the color intensity is proportional to triglyceride concentrations.

[502] In some embodiments, the guide polynucleotide is a guide RNA. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary

strand of a protospacer sequence of the ANGPTL3 gene with 0, 1, or 2 mismatches. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the ANGPTL3 gene with no mismatches. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the ANGPTL3 gene with 1 mismatch. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the ANGPTL3 gene with 2 mismatches. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the ANGPTL3 gene with 3 mismatches. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the ANGPTL3 gene with 4 mismatches. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the ANGPTL3 gene with 5 mismatches.

[503] In some embodiments, the guide polynucleotide is a guide RNA. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the PCSK9 gene with 0, 1, or 2 mismatches. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the PCSK9 gene with no mismatches. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the PCSK9 gene with 1 mismatch. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the PCSK9 gene with 2 mismatches. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the PCSK9 gene with 3 mismatches. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the PCSK9 gene with 4 mismatches. In some embodiments, the guide RNA comprises a spacer sequence that binds to the complementary strand of a protospacer sequence of the PCSK9 gene with 5 mismatches.

[504] In some embodiments, the nucleobase alteration is outside of the protospacer sequence in less than 1% of whole liver cells in the subject as measured by net nucleobase editing. In some embodiments, the nucleobase alteration is outside of the protospacer sequence in less than 1% of hepatocytes in the subject as measured by net nucleobase editing. In some embodiments, the nucleobase alteration is only within the protospacer sequence as measured by net nucleobase editing.

[505] In some embodiments, the nucleobase alteration is outside of the protospacer sequence in less than 0.01%. 0.02%, 0.03% 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 2.0%, 3.0% 4.0%, 5.0%, 6.0%, 7.0%, 8.0%, 9.0%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 65%, 80%, 85%, 90% of whole liver cells in the subject as measured by net nucleobase editing. In some embodiments, the nucleobase alteration is outside of the protospacer sequence in less than 0.01%. 0.02%, 0.03% 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 2.0%, 3.0% 4.0%, 5.0%, 6.0%, 7.0%, 8.0%, 9.0%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 65%, 80%, 85%, 90% of hepatocytes in the subject as measured by net nucleobase editing. In some embodiments, the nucleobase alteration is outside of the protospacer sequence in less than 0.01%. 0.02%, 0.03% 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 2.0%, 3.0% 4.0%, 5.0%, 6.0%, 7.0%, 8.0%, 9.0%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 65%, 80%, 85%, 90% of cells in the subject as measured by net nucleobase editing.

[506] In some embodiments, the administration is via intravenous infusion. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises sequential administration of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding the guide polynucleotide and the second LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises concurrent administration of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding the guide polynucleotide and the second LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same followed by staggered doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding the guide polynucleotide over an interval of 1, 2, 3, 4, 5, 6, or 7 days.

[507] In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP

enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same followed by staggered doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding the guide polynucleotide over an interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 hours. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same followed by staggered doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding the guide polynucleotide over an interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 days. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same followed by staggered doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding the guide polynucleotide over an interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 months.

[508] The method as described herein may be used for treating or preventing a condition in a subject in need thereof. In some embodiments, the method comprises administering a single dose of a LNP enclosing (i) a guide polynucleotide, e.g., a guide RNA, or a nucleic acid encoding the guide polynucleotide and (ii) a base editor fusion protein or a nucleic acid encoding the base editor fusion protein, e.g., an mRNA encoding the base editor fusion protein. For example, the method may comprise administering an LNP enclosing (i) a guide RNA and (i) an mRNA encoding the base editor fusion protein. The LNP may be administered at a single dose or multiple doses. In other embodiments, the method comprises administering a LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding the guide polynucleotide, and a second LNP enclosing (ii) the base editor fusion protein or a nucleic acid encoding the base editor fusion protein. The LNP may be administered at a single dose or multiple doses.

[509] In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a

programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 1 day, and the multiple doses are given at 1 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 1 day, and the multiple doses are given at 2 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 1 day, and the multiple doses are given at 3 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 1 day, and the multiple doses are given at 4 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 1 day, and the multiple doses are given at 5 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 1 day, and the multiple doses are given at 6 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain

and a deaminase, or a nucleic acid encoding same after 1 day, and the multiple doses are given at 7 day intervals.

[510] In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 2 days, and the multiple doses are given at 1 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 2 days, and the multiple doses are given at 2 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 2 days, and the multiple doses are given at 3 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 2 days, and the multiple doses are given at 4 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 2 days, and the multiple doses are given at 5 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid

encoding same after 2 days, and the multiple doses are given at 6 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 2 days, and the multiple doses are given at 7 day intervals.

[511] In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 3 days, and the multiple doses are given at 1 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 3 days, and the multiple doses are given at 2 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 3 days, and the multiple doses are given at 3 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 3 days, and the multiple doses are given at 4 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 3 days, and the multiple doses are

given at 5 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 3 days, and the multiple doses are given at 6 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 3 days, and the multiple doses are given at 7 day intervals.

[512] In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 4 days, and the multiple doses are given at 1 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 4 days, and the multiple doses are given at 2 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 4 days, and the multiple doses are given at 3 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 4 days, and the multiple doses are given at 4 day intervals. In some

embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 4 days, and the multiple doses are given at 5 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 4 days, and the multiple doses are given at 6 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 4 days, and the multiple doses are given at 7 day intervals.

[513] In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 5 days, and the multiple doses are given at 1 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 5 days, and the multiple doses are given at 2 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 5 days, and the multiple doses are given at 3 day intervals. In some embodiments, the method for treating or preventing a

condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 5 days, and the multiple doses are given at 4 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 5 days, and the multiple doses are given at 5 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 5 days, and the multiple doses are given at 6 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 5 days, and the multiple doses are given at 7 day intervals.

[514] In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 6 days, and the multiple doses are given at 1 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 6 days, and the multiple doses are given at 2 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need

thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 6 days, and the multiple doses are given at 3 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 6 days, and the multiple doses are given at 4 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 6 days, and the multiple doses are given at 5 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 6 days, and the multiple doses are given at 6 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 6 days, and the multiple doses are given at 7 day intervals.

[515] In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 7 days, and the multiple doses are given at 1 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein

comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 7 days, and the multiple doses are given at 2 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 7 days, and the multiple doses are given at 3 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 7 days, and the multiple doses are given at 4 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 7 days, and the multiple doses are given at 5 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 7 days, and the multiple doses are given at 6 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same after 7 days, and the multiple doses are given at 7 day intervals.

[516] In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP

enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 1 day, and the multiple doses are given at 1 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 1 day, and the multiple doses are given at 2 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 1 day, and the multiple doses are given at 3 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 1 day, and the multiple doses are given at 4 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 1 day, and the multiple doses are given at 5 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 1 day, and the multiple doses are given at 6 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and

administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 1 day, and the multiple doses are given at 7 day intervals.

[517] In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 2 days, and the multiple doses are given at 1 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 2 days, and the multiple doses are given at 2 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 2 days, and the multiple doses are given at 3 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 2 days, and the multiple doses are given at 4 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 2 days, and the multiple doses are given at 5 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 2 days,

and the multiple doses are given at 6 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 2 days, and the multiple doses are given at 7 day intervals.

[518] In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 3 days, and the multiple doses are given at 1 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 3 days, and the multiple doses are given at 2 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 3 days, and the multiple doses are given at 3 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 3 days, and the multiple doses are given at 4 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 3 days, and the multiple doses are given at 5 day intervals. In some embodiments, the method for treating or preventing a condition in

a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 3 days, and the multiple doses are given at 6 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 3 days, and the multiple doses are given at 7 day intervals.

[519] In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 4 days, and the multiple doses are given at 1 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 4 days, and the multiple doses are given at 2 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 4 days, and the multiple doses are given at 3 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 4 days, and the multiple doses are given at 4 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein

comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 4 days, and the multiple doses are given at 5 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 4 days, and the multiple doses are given at 6 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 4 days, and the multiple doses are given at 7 day intervals.

[520] In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 5 days, and the multiple doses are given at 1 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 5 days, and the multiple doses are given at 2 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 5 days, and the multiple doses are given at 3 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple

doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 5 days, and the multiple doses are given at 4 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 5 days, and the multiple doses are given at 5 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 5 days, and the multiple doses are given at 6 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 5 days, and the multiple doses are given at 7 day intervals.

[521] In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 6 days, and the multiple doses are given at 1 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 6 days, and the multiple doses are given at 2 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 6 days, and the multiple doses are

given at 3 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 6 days, and the multiple doses are given at 4 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 6 days, and the multiple doses are given at 5 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 6 days, and the multiple doses are given at 6 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 6 days, and the multiple doses are given at 7 day intervals.

[522] In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 7 days, and the multiple doses are given at 1 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 7 days, and the multiple doses are given at 2 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as

described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 7 days, and the multiple doses are given at 3 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 7 days, and the multiple doses are given at 4 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 7 days, and the multiple doses are given at 5 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 7 days, and the multiple doses are given at 6 day intervals. In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same and administering multiple doses of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding same after 7 days, and the multiple doses are given at 7 day intervals.

[523] In some embodiments, the method for treating or preventing a condition in a subject in need thereof as described herein comprises administering a single dose of the LNP enclosing (i) a guide polynucleotide or a nucleic acid encoding the guide polynucleotide and (ii) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a nucleic acid encoding same. In some embodiments, the single dose of the LNP is at 0.3 mg/kg. In some embodiments, the single dose of the LNP is at 0.5 mg/kg. In some embodiments, the single dose of the LNP is at 1 mg/kg. In some embodiments, the

single dose of the LNP is at 1 mg/kg. In some embodiments, the single dose of the LNP is at about 0.3 to about 3 mg/kg.

[524] Methods for treatment of a condition as provided herein may comprise a treatment course of one or more treatments, with each treatment comprising a single dose or multiple doses of a base editor system, or a LNP enclosing one or more components of the base editor system. For example, a subject in need thereof may be administered a single dose of a LNP enclosing a mRNA encoding a base editor fusion protein and a guide RNA for a treatment. In some embodiments, a subject may be administered a treatment course of one or more treatments, wherein each treatment comprises one or more of the single doses of the LNP, for example, a subject may also be administered 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more doses of a LNP enclosing a mRNA encoding a base editor fusion protein and a guide RNA for a treatment. The subject may receive a treatment course of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more treatments, where each dose may be administered 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months, 24 months, 48 months or apart. A single dose for the treatment as described herein may comprise a LNP enclosing a guide RNA or a mRNA encoding the base editor fusion protein, or both.

[525] In some embodiments, the single dose of the LNP comprises 0.1 mg/kg, 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, 0.9 mg/kg, 1.0 mg/kg, 1.1 mg/kg, 1.2 mg/kg, 1.3 mg/kg, 1.4 mg/kg, 1.5 mg/kg, 1.6 mg/kg, 1.7 mg/kg, 1.8 mg/kg, 1.9 mg/kg, 2.0 mg/kg, 2.1 mg/kg, 2.2 mg/kg, 2.3 mg/kg, 2.4 mg/kg, 2.5 mg/kg, 2.6 mg/kg, 2.7 mg/kg, 2.8 mg/kg, 2.9 mg/kg, 3.0 mg/kg, 3.1 mg/kg, 3.2 mg/kg, 3.3 mg/kg, 3.4 mg/kg, 3.5 mg/kg, 3.6 mg/kg, 3.7 mg/kg, 3.8 mg/kg, 3.9 mg/kg, 4.0 mg/kg, 4.1 mg/kg, 4.2 mg/kg, 4.3 mg/kg, 4.4 mg/kg, 4.5 mg/kg, 4.6 mg/kg, 4.7 mg/kg, 4.8 mg/kg, 4.9 mg/kg, 5.0 mg/kg, 5.1 mg/kg, 5.2 mg/kg, 5.3 mg/kg, 5.4 mg/kg, 5.5 mg/kg, 5.6 mg/kg, 5.7 mg/kg, 5.8 mg/kg, 5.9 mg/kg, 6.0 mg/kg, 6.1 mg/kg, 6.2 mg/kg, 6.3 mg/kg, 6.4 mg/kg, 6.5 mg/kg, 6.6 mg/kg, 6.7 mg/kg, 6.8 mg/kg, 6.9 mg/kg, 7.0 mg/kg, 7.1 mg/kg, 7.2 mg/kg, 7.3 mg/kg, 7.4 mg/kg, 7.5 mg/kg, 7.6 mg/kg, 7.7 mg/kg, 7.8 mg/kg, 7.9 mg/kg, 8.0 mg/kg, 8.1 mg/kg, 8.2 mg/kg, 8.3 mg/kg, 8.4 mg/kg, 8.5 mg/kg, 8.6 mg/kg, 8.7 mg/kg, 8.8 mg/kg, 8.9 mg/kg, 9.0 mg/kg, 9.1 mg/kg, 9.2 mg/kg, 9.3 mg/kg, 9.4 mg/kg, 9.5 mg/kg, 9.6 mg/kg, 9.7 mg/kg, 9.8 mg/kg, 9.9 mg/kg, 10 mg/kg, 11 mg/kg, 12 mg/kg, 13 mg/kg, 14 mg/kg, 15 mg/kg, 16 mg/kg, 17

mg/kg, 18 mg/kg, 19 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 45 mg/kg, 50 mg/kg, 55 mg/kg, 60 mg/kg, 65 mg/kg, 70 mg/kg, 75 mg/kg, 80 mg/kg, 85 mg/kg, 90 mg/kg, 95 mg/kg, 100 mg/kg, 105 mg/kg, 110 mg/kg, 115 mg/kg, 120 mg/kg, 125 mg/kg, 130 mg/kg, 135 mg/kg, 140 mg/kg, 145 mg/kg, 150 mg/kg, 155 mg/kg, 160 mg/kg, 165 mg/kg, 170 mg/kg, 175 mg/kg, 180 mg/kg, 185 mg/kg, 190 mg/kg, 195 mg/kg, 200 mg/kg, 250 mg/kg, 300 mg/kg, 350 mg/kg, 400 mg/kg, 450 mg/kg, or 500 mg/kg.

[526] In some embodiments, the condition is a atherosclerotic cardiovascular disease. In some embodiments, the condition is a cardiovascular disease, or diabetes. In some embodiments, the condition is a atherosclerotic vascular disease.

[527] In some embodiments, the subject is a non-human primate. In some embodiments, the subject is a monkey. In some embodiments, the subject is a human. In some embodiments, the deaminase is an adenine deaminase. In some embodiments, the nucleobase alteration is a A•T to G•C alteration. In some embodiments, the deaminase is an adenine deaminase and the nucleobase alteration is a A•T to G•C alteration. In some embodiments, the programmable DNA binding domain comprises a nuclease inactive Cas9. In some embodiments, the programmable DNA binding domain comprises a Cas9 nickase. In some embodiments, the programmable DNA binding domain comprises a Cas9.

[528] In some embodiments, the nucleobase alteration is at a splice site of the PCSK9 gene. In some embodiments, the nucleobase alteration is at a splice donor site of the PCSK9 gene. In some embodiments, the splice donor site is at 5' end of PCSK9 intron 1 as referenced in SEQ ID NO: 5. In some embodiments, the nucleobase alteration is at a splice acceptor site of the PCSK9 gene. In some embodiments, the nucleobase alteration results in a frame shift, a premature stop codon, a insertion or deletion in a transcript encoded by the PCSK9 gene. In some embodiments, the nucleobase alteration results in an aberrant transcript encoded by the PCSK9 gene. In some embodiments, the guide polynucleotide is a guide RNA. In some embodiments, the guide RNA is chemically modified. In some embodiments, the guide RNA comprises a tracrRNA sequence. In some embodiments, the guide RNA comprises a chemical modification as set forth in Table 1 or Table 24.

[529] In some embodiments, the nucleobase alteration is at a splice site of the ANGPTL3 gene. In some embodiments, the nucleobase alteration is at a splice donor site of the ANGPTL3 gene. In some embodiments, the splice donor site is at 5' end of ANGPTL3 intron 6 as referenced in SEQ ID NO: 7. In some embodiments, the nucleobase alteration is at a splice acceptor site of the ANGPTL3 gene. In some embodiments, the nucleobase alteration results in a frame shift, a premature stop codon, a insertion or deletion in a transcript encoded

by the ANGPTL3 gene. In some embodiments, the nucleobase alteration results in an aberrant transcript encoded by the ANGPTL3 gene. In some embodiments, the guide polynucleotide is a guide RNA. In some embodiments, the guide RNA is chemically modified. In some embodiments, the guide RNA comprises a tracrRNA sequence. In some embodiments, the guide RNA comprises a chemical modification as set forth in Table 1 or Table 24.

[530] In some embodiments, the guide RNA comprises a guide RNA sequence set forth in Table 1 or Table 24. In some embodiments, the guide RNA comprises the sequence 5'-5'-cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcG UUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususu-3' (SEQ ID NO: 9), 5'-cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGU UAucAAcuugaaaaagugGcaccgagucggugcusususu-3' (SEQ ID NO: 9), 5'-cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu-3' (SEQ ID NO: 9) (GA346), 5'-cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAacAAcuugaaaaagugGcaccgagucggugcusususu-3 (SEQ ID NO: 65) (GA374), 5'-cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuuuu-3' (SEQ ID NO: 11) (GA385), 5'-cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuUu-3' (SEQ ID NO: 11) (GA386) or 5'-cscscsGCACCUUGGCGCAGCGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuusususuuu-3' (SEQ ID NO: 12) (GA387).

[531] In some embodiments, the protospacer sequence comprises a protospacer sequence set forth in Table 1 or Table 24. In some embodiments, the protospacer comprises the sequence 5'-CCCGCACCTTGGCGCAGCGG-3' (SEQ ID NO: 13), AAGATACCTGAATAACTCTC-3' (SEQ ID NO: 14) or 5'-AAGATACCTGAATAACCCTC-3' (SEQ ID NO: 15).

[532] In some embodiments, the base editor fusion protein comprises the sequence of SEQ ID NO: 3. In some embodiments, the adenosine deaminase comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to the amino acid sequence set forth in SEQ ID NO: 3 or to any of the adenosine deaminases provided herein. It should be appreciated that adenosine deaminases

provided herein may include one or more mutations (e.g., any of the mutations provided herein). The disclosure provides any deaminase domains with a certain percent identity plus any of the mutations or combinations thereof described herein. In some embodiments, the adenosine deaminase comprises an amino acid sequence that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more mutations compared to the amino acid sequence set forth in SEQ ID NO: 3 or any of the adenosine deaminases provided herein. In some embodiments, the adenosine deaminase comprises an amino acid sequence that has at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, or at least 170 identical contiguous amino acid residues as compared to any one of the amino acid sequences set forth in SEQ ID NO: 3 or any of the adenosine deaminases provided herein.

[533] In some embodiments, the mRNA comprises a cap analog.

[534] In some embodiments, the mRNA comprises at least 1, 2, or 3 nucleotides at the 5' end that comprises 2'-hydroxyl group, 2'-O-methyl group, or additional 2' chemical modification or a combination thereof. In some embodiments, the mRNA comprises at least 1, 2, or 3 nucleotides at the 5' end that comprises 2'-hydroxyl group, 2'-O-methyl group, or additional 2' chemical modification or a combination thereof. In some embodiments, the mRNA comprises at least 1 nucleotide at the 5' end that comprises 2'-hydroxyl group, 2'-O-methyl group, or additional 2' chemical modification or a combination thereof. In some embodiments, the mRNA comprises at least 2 nucleotides at the 5' end that comprises 2'-hydroxyl group, 2'-O-methyl group, or additional 2' chemical modification or a combination thereof. In some embodiments, the mRNA comprises at least 3 nucleotides at the 5' end that comprises 2'-hydroxyl group, 2'-O-methyl group, or additional 2' chemical modification or a combination thereof. In some embodiments, the mRNA comprises at least 4 nucleotides at the 5' end that comprises 2'-hydroxyl group, 2'-O-methyl group, or additional 2' chemical modification or a combination thereof. In some embodiments, the mRNA comprises at least 5 nucleotides at the 5' end that comprises 2'-hydroxyl group, 2'-O-methyl group, or additional 2' chemical modification or a combination thereof. In some embodiments, the mRNA comprises at least 6 nucleotides at the 5' end that comprises 2'-hydroxyl group, 2'-O-methyl group, or additional 2' chemical modification or a combination thereof. In some embodiments, the mRNA comprises at least 7 nucleotides at the 5' end that comprises 2'-hydroxyl group, 2'-O-methyl group, or additional 2' chemical modification or a combination

thereof. In some embodiments, the mRNA comprises at least 8 nucleotides at the 5' end that comprises 2'-hydroxyl group, 2'-O-methyl group, or additional 2' chemical modification or a combination thereof. In some embodiments, the mRNA comprises at least 9 nucleotides at the 5' end that comprises 2'-hydroxyl group, 2'-O-methyl group, or additional 2' chemical modification or a combination thereof. In some embodiments, the mRNA comprises at least 10 nucleotides at the 5' end that comprises 2'-hydroxyl group, 2'-O-methyl group, or additional 2' chemical modification or a combination thereof.

[535] In some embodiments, the mRNA comprises a poly A tail.

[536] The compositions described herein, may be administered to a subject in need thereof, in a therapeutically effective amount, to treat conditions related to high circulating cholesterol levels. Conditions related to high circulating cholesterol level that may be treated using the compositions and methods described herein include, without limitation: hypercholesterolemia, elevated total cholesterol levels, elevated low-density lipoprotein (LDL) levels, elevated blood LDL-cholesterol levels, reduced blood high-density lipoprotein cholesterol level, liver steatosis, coronary heart disease, vascular disease, ischemia, stroke, peripheral vascular disease, thrombosis, type 2 diabetes, hypertriglyceridemia, high elevated blood pressure, atherosclerosis, obesity, Alzheimer's disease, neurodegeneration, and combinations thereof. The compositions and methods disclosed herein are effective in reducing the circulating cholesterol level in the subject, thus treating the conditions. The compositions and methods disclosed herein are effective in reducing blood LDL cholesterol level and/or a reducing blood triglycerides level as compared to before the administration.

[537] In another aspect, provided herein is a method for treating or preventing a condition in a subject in need thereof, the method comprising administering to the subject the complex as provided herein, the composition as provided herein, or the lipid nanoparticle as provided herein, wherein the sgRNA directs the adenosine base editor protein to effect a modification in a target polynucleotide sequence in a cell of the subject, thereby treating or preventing the condition.

[538] In some embodiments, the target polynucleotide sequence is in a PCSK9 gene. In some embodiments, the modification reduces expression of functional PCSK9 protein encoded by the PCSK9 gene in the subject. In some embodiments, the condition is atherosclerotic vascular disease. In some embodiments, the target polynucleotide sequence is in an ANGPTL3 gene. In some embodiments, the modification reduces expression of functional ANGPTL3 protein encoded by the ANGPTL3 gene in the subject. In some

embodiments, the condition is an atherosclerotic vascular disease, hypertriglyceridemia, or diabetes.

[539] A patient who is being treated for a condition, a disease or a disorder is one who a medical practitioner has diagnosed as having such a condition. Diagnosis may be by any suitable means. Diagnosis and monitoring may involve, for example, detecting the presence of diseased, dying or dead cells in a biological sample (e.g., tissue biopsy, blood test, or urine test), detecting the presence of plaques, detecting the level of a surrogate marker in a biological sample, or detecting symptoms associated with a condition. A patient in whom the development of a condition is being prevented may or may not have received such a diagnosis. One in the art will understand that these patients may have been subjected to the same standard tests as described above or may have been identified, without examination, as one at high risk due to the presence of one or more risk factors (e.g., family history or genetic predisposition).

[540] The therapeutic methods of the disclosure may be carried out on subjects displaying pathology resulting from a disease or a condition, subjects suspected of displaying pathology resulting from a disease or a condition, and subjects at risk of displaying pathology resulting from a disease or a condition. For example, subjects that have a genetic predisposition to a disease or a condition can be treated prophylactically. Subjects exhibiting symptoms associated with a condition, a disease or a disorder may be treated to decrease the symptoms or to slow down or prevent further progression of the symptoms. The physical changes associated with the increasing severity of a disease or a condition are shown herein to be progressive. Thus, in embodiments of the disclosure, subjects exhibiting mild signs of the pathology associated with a condition or a disease may be treated to improve the symptoms and/or prevent further progression of the symptoms.

[541] In some embodiments, the subject exhibits a reduced blood LDL cholesterol level and/or a reduced blood triglycerides level as compared to before the administration.

[542] In some embodiments, after the administration, the subject exhibits a reduced blood low-density lipoprotein (LDL) cholesterol level by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or 100% as compared to before the administration. In some embodiments, after the administration, the subject exhibits a reduced blood low-density lipoprotein (LDL) cholesterol level by at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 10

fold, at least 20 fold, at least 25 fold, at least 30 fold, at least 40 fold, at least 50 fold, at least 60 fold, at least 70 fold, at least 80 fold, at least 90 fold, at least 100 fold, at least 200 fold, at least 300 fold, at least 400 fold, at least 500 fold, at least 600 fold, at least 700 fold, at least 800 fold, at least 900 fold, at least 1000 fold, at least 2000 fold, at least 3000 fold, at least 4000 fold, at least 5000 fold, at least 6000 fold, at least 7000 fold, at least 8000 fold, at least 9000 fold, or at least 10000 fold as compared to before the administration.

[543] In some embodiments, after the administration, the subject exhibits a reduced blood triglycerides level by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or 100% as compared to before the administration.

[544] In some embodiments, after the administration, the subject exhibits a reduced blood triglycerides level by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or 100% as compared to before the administration. In some embodiments, after the administration, the subject exhibits a reduced blood triglycerides level by at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 10 fold, at least 20 fold, at least 25 fold, at least 30 fold, at least 40 fold, at least 50 fold, at least 60 fold, at least 70 fold, at least 80 fold, at least 90 fold, at least 100 fold, at least 200 fold, at least 300 fold, at least 400 fold, at least 500 fold, at least 600 fold, at least 700 fold, at least 800 fold, at least 900 fold, at least 1000 fold, at least 2000 fold, at least 3000 fold, at least 4000 fold, at least 5000 fold, at least 6000 fold, at least 7000 fold, at least 8000 fold, at least 9000 fold, or at least 10000 fold as compared to before the administration.

[545] The dosage and frequency (single or multiple doses) administered to a mammal can vary depending upon a variety of factors, for example, whether the mammal suffers from another disease, and its route of administration; size, age, sex, health, body weight, body mass index, and diet of the recipient; nature and extent of symptoms of the disease being treated, kind of concurrent treatment, complications from the disease being treated or other health-related problems. Adjustment and manipulation of established dosages (e.g., frequency and duration) are well within the ability of those skilled in the art. The treatment, such as those disclosed herein, can be administered to the subject on a daily, twice daily, biweekly, monthly or any applicable basis that is therapeutically effective. In embodiments, the

treatment is only on an as-needed basis, e.g., upon appearance of signs or symptoms of a condition or a disease, e.g., an atherosclerotic vascular disease, hypertriglyceridemia, or diabetes.

[546] The compositions described herein, may be administered to a subject in need thereof, in a therapeutically effective amount, to treat conditions related to high circulating cholesterol levels. Conditions related to high circulating cholesterol level that may be treated using the compositions and methods described herein include, without limitation: hypercholesterolemia, elevated total cholesterol levels, elevated low-density lipoprotein (LDL) levels, elevated blood LDL-cholesterol levels, reduced blood high-density lipoprotein cholesterol level, liver steatosis, coronary heart disease, vascular disease, ischemia, stroke, peripheral vascular disease, thrombosis, type 2 diabetes, hypertriglyceridemia, high elevated blood pressure, atherosclerosis, obesity, Alzheimer's disease, neurodegeneration, and combinations thereof. The compositions and methods disclosed herein are effective in reducing the circulating cholesterol level in the subject, thus treating the conditions. The compositions and methods disclosed herein are effective in reducing blood LDL cholesterol level and/or a reducing blood triglycerides level as compared to before the administration.

[547] Toxicity and therapeutic efficacy of the compositions of the disclosure can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects (the ratio LD50/ED50) is the therapeutic index. Agents that exhibit high therapeutic indices are preferred. The dosage of agents lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. While agents that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such agents to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[548] The skilled artisan will appreciate that certain factors may influence the dosage and frequency of administration required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general characteristics of the subject including health, sex, weight and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of the compositions can include a single treatment or, preferably, can include a series of treatments. It will also be appreciated that the effective dosage of the composition of the disclosure used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may

result and become apparent from the results of diagnostic assays as described herein. The therapeutically-effective dosage will generally be dependent on the patient's status at the time of administration. The precise amount can be determined by routine experimentation but may ultimately lie with the judgment of the clinician, for example, by monitoring the patient for signs of disease and adjusting the treatment accordingly.

[549] Frequency of administration may be determined and adjusted over the course of therapy, and is generally, but not necessarily, based on treatment and/or suppression and/or amelioration and/or delay of a disease. Alternatively, sustained continuous release formulations of a polypeptide or a polynucleotide may be appropriate. Various formulations and devices for achieving sustained release are known in the art. In some embodiments, dosage is daily, every other day, every three days, every four days, every five days, or every six days. In some embodiments, dosing frequency is once every week, every 2 weeks, every 4 weeks, every 5 weeks, every 6 weeks, every 7 weeks, every 8 weeks, every 9 weeks, or every 10 weeks; or once every month, every 2 months, or every 3 months, or longer. The progress of this therapy is easily monitored by conventional techniques and assays.

[550] The dosing regimen (including a composition disclosed herein) can vary over time. In some embodiments, for an adult subject of normal weight, doses ranging from about 0.01 to 1000 mg/kg may be administered. In some embodiments, the dose is between 1 to 200 mg. The particular dosage regimen, i.e., dose, timing and repetition, will depend on the particular subject and that subject's medical history, as well as the properties of the polypeptide or the polynucleotide (such as the half-life of the polypeptide or the polynucleotide, and other considerations well known in the art).

[551] For the purpose of the present disclosure, the appropriate therapeutic dosage of a composition as described herein will depend on the specific agent (or compositions thereof) employed, the formulation and route of administration, the type and severity of the disease, whether the polypeptide or the polynucleotide is administered for preventive or therapeutic purposes, previous therapy, the subject's clinical history and response to the antagonist, and the discretion of the attending physician. Typically, the clinician will administer a polypeptide until a dosage is reached that achieves the desired result.

[552] Administration of one or more compositions can be continuous or intermittent, depending, for example, upon the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of a composition may be essentially continuous over a

preselected period of time or may be in a series of spaced dose, e.g., either before, during, or after developing a disease.

[553] The methods and compositions of the disclosure described herein including embodiments thereof can be administered with one or more additional therapeutic regimens or agents or treatments, which can be co-administered to the mammal.

Metabolic Syndrome and Cardiovascular Disease

[554] The ability of gene modification, especially to edit bases directly allows for the precise edit, modification, and/or disruption of genes in vivo without the need to create DSBs and improved methods of treating human disease. For example, Chadwick et. al used cytosine base editing to introduce nonsense mutations into the Pcsk9 gene in adult mice. Delivery of BE3 and a gRNA via adenoviral vector into the liver caused a reduction in plasma PCSK9 levels by 56%. Furthermore, cholesterol levels in the base-edited mice were reduced by roughly 30%. In subsequent work, cytosine base editing to disrupt the Angptl3 gene in adult mice resulted in substantially reduced blood cholesterol and triglyceride levels, and cytosine base editing to disrupt the Pcsk9 gene or the Hpd gene in fetal mice resulted in postnatal reduction of cholesterol levels or cure of the disease hereditary tyrosinemia type 1.

[555] Cardiovascular disease, obesity, type 2 diabetes, metabolic syndrome may share one or more similar underlying etiologies. The human PCSK9 gene encodes a protein that helps regulate the amount of cholesterol in the bloodstream. The liver protein Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) in human is a secreted, globular, auto-activating serine protease that acts as a protein-binding adaptor within endosomal vesicles to bridge a pH-dependent interaction with the low-density lipoprotein receptor (LDL-R) during endocytosis of LDL particles, preventing recycling of the LDL-R to the cell surface and leading to reduction of LDL-cholesterol clearance. PCSK9 orthologs are found across many species. The PCSK9 protein breaks down low-density lipoprotein receptors before they reach the cell surface, so more cholesterol can remain in the bloodstream. As a result, the PCSK9 gene and the encoded PCSK9 protein are attractive targets for regulating cholesterol metabolism, especially in lowering blood cholesterol levels.

[556] Other targets have also been shown that are associated with decreased plasma levels of triglycerides (TGs), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C), which leads to a significant reduction in cardiovascular risk. For example, human ANGPTL3 is considered an important new pharmacological target for the treatment of cardiovascular diseases. Experimental evidence demonstrates that anti-

ANGPTL3 therapies have an important anti-atherosclerotic effect. Results from phase I clinical trials with a monoclonal anti-ANGPTL3 antibody (evinacumab) and anti-sense oligonucleotide (ASO) clearly show a significant lipid lowering effect.

[557] Human ANGPTL3 gene is located on chromosome 1p31. Genetic variants of human ANGPTL3 gene have been linked to different plasma lipid profiles. For example, homozygous loss of function (LOF) variants of ANGPTL3 gene cause the levels of all plasma lipoproteins to be greatly reduced. ANGPTL3 is exclusively expressed in the liver and is secreted into circulation where it goes through cleavage by hepatic proprotein convertases. The ANGPTL3 protein is a 460-amino-acid (aa) polypeptide with a distinctive signal peptide sequence, a N-terminal helical domain and a C-terminal globular fibrinogen homology domain. The N-terminal coiled-coil region (17-207aa) affects plasma TG levels via reversibly inhibiting catalytic activity of LPL while the fibrinogen-like domain (207-460aa) binds to integrin αvβ3 receptor and affects angiogenesis, which is similar to the function of angiopoietins. A short linker region (at 221–222 and 224–225) between N- and C-terminal domains is a special zone, which has been verified to be split by furin. Existed results have shown that the truncated form of cleavage ANGPTL3 could reinforce the inhibitory activity of LPL and endothelial lipase (EL), suggesting that the cleavage type of ANGPTL3 may function more effectively.

[558] Moreover, human apolipoprotein C-III (APOC3) could potentially be another therapeutic target. APOC3 is a protein that in humans is encoded by the APOC3 gene. APOC3 is a component of VLDL. APOC3 inhibits lipoprotein lipase and hepatic lipase. It is also thought to inhibit hepatic uptake of triglyceride-rich particles. An increase in APOC3 levels induces the development of hypertriglyceridemia. Recent evidence suggests an intracellular role for APOC3 in promoting the assembly and secretion of triglyceride-rich VLDL particles from hepatic cells under lipid- rich conditions. However, two naturally occurring point mutations in human apoC3 coding sequence, A23T and K58E have been shown to abolish the intracellular assembly and secretion of triglyceride-rich VLDL particles from hepatic cells.

[559] Targeted base editing with target genes involved in vascular disease and diabetes as described in Chadwick AC, Wang X, Musunuru K. In vivo base editing of PCSK9 (proprotein convertase subtilisin/kexin type 9) as a therapeutic alternative to genome editing. Arterioscler Thromb Vasc Biol, 2017, 37: 1741–7; Chadwick AC, Evitt NH, Lv W, et al. Reduced blood lipid levels with in vivo CRISPR-Cas9 base editing of ANGPTL3. Circulation, 2018, 137: 975–7; Rossidis AC, Stratigis JD, Chadwick AC, et al. In utero

CRISPR-mediated therapeutic editing of metabolic genes. Nat Med, 2018, 24: 1513–8 is incorporated herein by reference in its entirety. While genes involved in lipid metabolism, including PCSK9, have been edited by targeted by current gene editing technologies, there remains a need for precise base editing targeting these genes for improved editing outcomes.

Pharmaceutical composition

[560] In some aspects, provided herein is a pharmaceutical composition comprising the base editor system as provided herein and a pharmaceutically acceptable carrier or excipient.

[561] In some aspects, provided herein, is a pharmaceutical composition for gene modification comprising a gRNA or sgRNA described herein and a Base editor fusion protein or a nucleic acid sequence encoding the Base editor fusion protein and a pharmaceutically acceptable carrier. The composition for gene modification comprising a gRNA or sgRNA described herein and a Base editor fusion protein or a nucleic acid sequence encoding the Base editor fusion protein can be formulated into pharmaceutical compositions. Pharmaceutical compositions are formulated in a conventional manner using one or more pharmaceutically acceptable inactive ingredients that facilitate processing of the active compounds into preparations that can be used pharmaceutically. Suitable formulations for use in the present disclosure and methods of delivery are generally well known in the art. Proper formulation is dependent upon the route of administration chosen. A summary of pharmaceutical compositions described herein can be found, for example, in Remington: The Science and Practice of Pharmacy, Nineteenth Ed (Easton, Pa.: Mack Publishing Company, 1995); Hoover, John E., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pennsylvania 1975; Liberman, H.A. and Lachman, L., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, N.Y., 1980; and Pharmaceutical Dosage Forms and Drug Delivery Systems, Seventh Ed. (Lippincott Williams & Wilkins1999), herein incorporated by reference for such disclosure.

[562] A pharmaceutical composition can be a mixture of a gRNA or sgRNA described herein and a Base editor fusion protein or a nucleic acid sequence encoding the Base editor fusion protein with one or more of other chemical components (i.e., pharmaceutically acceptable ingredients), such as carriers, excipients, binders, filling agents, suspending agents, flavoring agents, sweetening agents, disintegrating agents, dispersing agents, surfactants, lubricants, colorants, diluents, solubilizers, moistening agents, plasticizers, stabilizers, penetration enhancers, wetting agents, anti–foaming agents, antioxidants, preservatives, or one or more combination thereof. The pharmaceutical composition

facilitates administration of the gRNA or sgRNA described herein and the Base editor fusion protein or a nucleic acid sequence encoding the Base editor fusion protein to an organism or a subject in need thereof.

[563] The pharmaceutical compositions of the present disclosure can be administered to a subject using any suitable methods known in the art. The pharmaceutical compositions described herein can be administered to the subject in a variety of ways, including parenterally, intravenously, intradermally, intramuscularly, colonically, rectally, or intraperitoneally. In some embodiments, the pharmaceutical compositions can be administered by intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection of the subject. In some embodiments, the pharmaceutical compositions can be administered parenterally, intravenously, intramuscularly, or orally.

[564] For administration by inhalation, the adenovirus described herein can be formulated for use as an aerosol, a mist, or a powder. For buccal or sublingual administration, the pharmaceutical compositions may be formulated in the form of tablets, lozenges, or gels formulated in a conventional manner. In some embodiments, the adenovirus described herein can be prepared as transdermal dosage forms. In some embodiments, the adenovirus described herein can be formulated into a pharmaceutical composition suitable for intramuscular, subcutaneous, or intravenous injection. In some embodiments, the adenovirus described herein can be administered topically and can be formulated into a variety of topically administrable compositions, such as solutions, suspensions, lotions, gels, pastes, medicated sticks, balms, creams, or ointments. In some embodiments, the adenovirus described herein can be formulated in rectal compositions such as enemas, rectal gels, rectal foams, rectal aerosols, suppositories, jelly suppositories, or retention enemas. In some embodiments, the adenovirus described herein can be formulated for oral administration such as a tablet, a capsule, or liquid in the form of aqueous suspensions or solutions selected from the group including, but not limited to, aqueous oral dispersions, emulsions, solutions, elixirs, gels, and syrups.

[565] In some embodiments, the pharmaceutical composition for gene modification comprising a gRNA or sgRNA described herein and a Type II Cas protein or a nucleic acid sequence encoding the Type II Cas protein further comprises a therapeutic agent. The additional therapeutic agent may modulate different aspects of the disease, disorder, or condition being treated and provide a greater overall benefit than administration of either the replication competent recombinant adenovirus or the therapeutic agent alone. Therapeutic agents include, but are not limited to, a chemotherapeutic agent, a radiotherapeutic agent, a

hormonal therapeutic agent, and/or an immunotherapeutic agent. In some embodiments, the therapeutic agent may be a radiotherapeutic agent. In some embodiments, the therapeutic agent may be a hormonal therapeutic agent. In some embodiments, the therapeutic agent may be an immunotherapeutic agent. In some embodiments, the therapeutic agent is a chemotherapeutic agent. Preparation and dosing schedules for additional therapeutic agents can be used according to manufacturers' instructions or as determined empirically by a skilled practitioner. For example, preparation and dosing schedules for chemotherapy are also described in The Chemotherapy Source Book, 4th Edition, 2008, M. C. Perry, Editor, Lippincott, Williams & Wilkins, Philadelphia, PA.

[566] The subjects that can be treated with gene modification compositions of chemically modified gRNAs or sgRNAs described herein and a Type II Cas protein or a nucleic acid sequence encoding the Type II Cas protein and methods described herein can be any subject with a disease or a condition. For example, the subject may be a eukaryotic subject, such as an animal. In some embodiments, the subject is a mammal, e.g., human. In some embodiments, the subject is a human. In some embodiments, the subject is a non–human animal. In some embodiments, the subject is a fetus, an embryo, or a child. In some embodiments, the subject is a non–human primate such as chimpanzee, and other apes and monkey species; farm animals such as cattle, horses, sheep, goats, pigs; domestic animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice, and guinea pigs, and the like.

[567] In some embodiments, the subject is prenatal (e.g., a fetus), a child (e.g., a neonate, an infant, a toddler, a preadolescent), an adolescent, a pubescent, or an adult (e.g., an early adult, a middle-aged adult, a senior citizen). The human subject can be between about 0 month and about 120 years old, or older. The human subject can be between about 0 and about 12 months old; for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months old. The human subject can be between about 0 and 12 years old; for example, between about 0 and 30 days old; between about 1 month and 12 months old; between about 1 year and 3 years old; between about 4 years and 5 years old; between about 4 years and 12 years old; about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 years old. The human subject can be between about 13 years and 19 years old; for example, about 13, 14, 15, 16, 17, 18, or 19 years old. The human subject can be between about 20 and about 39 years old; for example, about 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, or 39 years old. The human subject can be between about 40 to about 59 years old; for example, about 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, or 59 years old. The human subject can be greater than

59 years old; for example, about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, or 120 years old. The human subjects can include male subjects and/or female subjects.

Lipid Nanoparticle (LNP) Composition

[568] In some embodiments, LNPs are prepared in accordance with the methods described in Conway, A. et al. 2019 *Mol. Ther.* **27**, 866–877, and Villiger, L. et al 2021 *Nat. Biomed. Eng.* 5, 179-18, which are incorporated by reference. In some embodiments, LNPs are composed of an amino lipid, a monomethoxypolyethylene glycol (or methoxypoluyethyle glycol) of average molecular weight 2000 Da conjugated to a lipid called PEG-Lipid, cholesterol and 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC). In some embodiments, LNPs are composed of proprietary ionizable cationic lipid, 1,2-distearoyl-sn-glycero-3-phosphocholine, cholesterol, and a PEG-lipid.

[569] In some embodiments, LNPs have an average hydrodynamic diameter of about 30 – about 160, about 35 – about 160, about 40 - about 160, about 45 - about 160, about 50 - about 160, about 55 - about 160, about 60 - about 160, about 65 - about 160, about 70 - about 160, about 75 - about 160, about 80 - about 160, about 85 - about 160, about 90 - about 160, about 95 - about 160, about 100 - about 160, about 105 - about 160, about 110 - about 160, about 115 - about 160, about 120 - about 160, about 125 - about 160, about 130 - about 160, about 135 - about 160, about 140 - about 160, about 145 - about 160, about 150 - about 160, about 30 - about 155, about 30 - about 150, about 30 - about 145, about 30 - about 140, about 30 - about 135, about 30 - about 130, about 30 - about 125, about 30 - about 120, about 30 - about 115, about 30 - about 110, about 30 - about 105, about 30 - about 100, about 30 - about 95, about 30 - about 90, about 30 - about 85, about 30 - about 80, about 30 - about 75, about 30 - about 70, about 30 - about 65, about 30 - about 60, about 30 - about 55, about 30 - about 50, about 30 - about 45, about 30 - about 40, about 30 - about 45, about 35 - about 45, about 35 - about 50, about 40 - about 50, about 40 - about 55, about 45 - about 55, about 45 - about 60, about 50 - about 60, about 50 - about 65, about 55 - about 60, about 55 - about 65, about 55 - about 70, about 60 - about 70, about 60 - about 75, about 65 - about 75, about 65 - about 80, about 70 - about 80, about 70 - about 85, about 75 - about 85, about 75 - about 90, about 80 - about 90, about 80 - about 95, about 85 - about 95, about 85 - about 100, about 90 - about 100, about 90 - about 105, about 95 - about 105, about 95 - about 110, about 100 - about 110,

about 100 - about 115, about 105 - about 115, about 105 - about 120, about 110 - about 120, about 110 - about 125, about 115 - about 125, about 115 - about 130, about 120 - about 130, about 120 - about 135, about 125 - about 135, about 125 - about 140, about 130 - about 140, about 130 - about 145, about 35 - about 140, about 45 - about 130, about 55 - about 120, about 65 - about 110, about 75 - about 100, or about 85 - about 90 nm.

[570] In some embodiments, LNPs comprise about 1 - about 97, about 5 - about 97, about 10 - about 97, about 15 - about 97, about 20 - about 97, about 25 - about 97, about 30 - about 97, about 35 - about 97, about 40 - about 97, about 45 - about 97, about 50 - about 97, about 55 - about 97, about 60 - about 97, about 65 - about 97, about 70 - about 97, about 75 - about 97, about 80 - about 97, about 1 - about 95, about 1 - about 90, about 1 - about 85, about 1 - about 80, about 1 - about 75, about 1 - about 70, about 1 - about 65, about 1 - about 60, about 1 - about 55, about 1 - about 50, about 1 - about 45, about 1 - about 40, about 1 - about 35, about 1 - about 30, about 1 - about 25, about 1 - about 20, about 1 - about 15, about 1 - about 10, about 10 - about 30, about 10 - about 35, about 15 - about 35, about 15 - about 40, about 20 - about 40, about 20 - about 45, about 25 - about 45, about 25 - about 50, about 30 - about 50, about 30 - about 55, about 35 - about 55, about 35 - about 60, about 40 - about 60, about 40 - about 65, about 45 - about 65, about 45 - about 70, about 50 - about 70, about 50 - about 75, about 55 - about 75, about 55 - about 80, or about 60 - about 80% of amino lipids (in mol%).

[571] In some embodiments, LNPs comprise about 1 - about 40, about 1 - about 38, about 1 - about 36, about 1 - about 34, about 1 - about 32, about 1 - about 30, about 1 - about 28, about 1 - about 26, about 1 - about 24, about 1 - about 22, about 1 - about 20, about 1 - about 18, about 1 - about 16, about 1 - about 14, about 1 - about 12, about 1 - about 10, about 1 - about 8, about 1 - about 6, about 1 - about 4, about 1 - about 2, about 2 - about 40, about 4 - about 40, about 6 - about 40, about 8 - about 40, about 10 - about 40, about 12 - about 40, about 14 - about 40, about 16 - about 40, about 18 - about 40, about 20 - about 40, about 22 - about 40, about 24 - about 40, about 26 - about 40, about 28 - about 40, about 30 - about 40, about 32 - about 40, about 34 - about 40, about 36 - about 40, about 38 - about 40, about 2 - about 35, about 2 - about 30, about 2 - about 25, about 2 - about 20, about 2 - about 15, about 2 - about 10, about 2 - about 8, about 2 - about 6, or about 2 - about 4% DSPC (in mol%).

[572] In some embodiments, LNPs comprise about 1 - about 20, about 1 - about 19, about 1 - about 18, about 1 - about 17, about 1 - about 16, about 1 - about 15, about 1 - about 14, about 1 - about 13, about 1 - about 12, about 1 - about 11, about 1 - about 10, about 1 - about 9, about 1 - about 8, about 1 - about 7, about 1 - about 6, about 1 - about 5, about 1 - about 4,

about 1 - about 3, about 2 - about 6, about 3 - about 7, about 4 - about 8, about 5 - about 9, about 6 - about 10, about 7 - about 11, about 8 - about 12, about 9 - about 13, about 10 - about 14, about 11 - about 15, about 12 - about 16, about 13 - about 17, about 14 - about 18, about 15 - about 19, or about 16 - about 20% PEG-Lipid (in mol%),

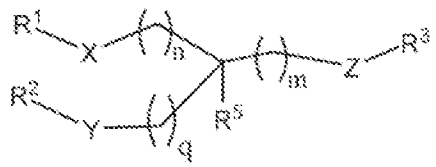
[573] In some embodiments, LNPs comprise about 1 - about 97, about 5 - about 97, about 10 - about 97, about 15 - about 97, about 20 - about 97, about 25 - about 97, about 30 - about 97, about 35 - about 97, about 40 - about 97, about 45 - about 97, about 50 - about 97, about 55 - about 97, about 60 - about 97, about 65 - about 97, about 70 - about 97, about 75 - about 97, about 80 - about 97, about 1 - about 95, about 1 - about 90, about 1 - about 85, about 1 - about 80, about 1 - about 75, about 1 - about 70, about 1 - about 65, about 1 - about 60, about 1 - about 55, about 1 - about 50, about 1 - about 45, about 1 - about 40, about 1 - about 35, about 1 - about 30, about 1 - about 25, about 1 - about 20, about 1 - about 15, about 1 - about 10, about 10 - about 30, about 10 - about 35, about 15 - about 35, about 15 - about 40, about 20 - about 40, about 20 - about 45, about 25 - about 45, about 25 - about 50, about 30 - about 50, about 30 - about 55, about 35 - about 55, about 35 - about 60, about 40 - about 60, about 40 - about 65, about 45 - about 65, about 45 - about 70, about 50 - about 70, about 50 - about 75, about 55 - about 75, about 55 - about 80, or about 60 - about 80% of cholesterol (in mol%).

[574] In some embodiments, LNPs comprise about 1 - about 97, 5 - about 97, 10 - about 97, 15 - about 97, 20 - about 97, 25 - about 97, 30 - about 97, 35 - about 97, 40 - about 97, 45 - about 97, 50 - about 97, 55 - about 97, 60 - about 97, 65 - about 97, 70 - about 97, 75 - about 97, 80 - about 97, 1 - about 95, 1 - about 90, 1 - about 85, 1 - about 80, 1 - about 75, 1 - about 70, 1 - about 65, 1 - about 60, 1 - about 55, 1 - about 50, 1 - about 45, 1 - about 40, 1 - about 35, 1 - about 30, 1 - about 25, 1 - about 20, 1 - about 15, 1 - about 10, 10 - about 30, 10 - about 35, 15 - about 35, 15 - about 40, 20 - about 40, 20 - about 45, 25 - about 45, 25 - about 50, 30 - about 50, 30 - about 55, 35 - about 55, 35 - about 60, 40 - about 60, 40 - about 65, 45 - about 65, 45 - about 70, 50 - about 70, 50 - about 75, 55 - about 75, 55 - about 80, or 60 - about 80% of amino lipids; 1 - about 40, 1 - about 38, 1 - about 36, 1 - about 34, 1 - about 32, 1 - about 30, 1 - about 28, 1 - about 26, 1 - about 24, 1 - about 22, 1 - about 20, 1 - about 18, 1 - about 16, 1 - about 14, 1 - about 12, 1 - about 10, 1 - about 8, 1 - about 6, 1 - about 4, 1 - about 2, 2 - about 40, 4 - about 40, 6 - about 40, 8 - about 40, 10 - about 40, 12 - about 40, 14 - about 40, 16 - about 40, 18 - about 40, 20 - about 40, 22 - about 40, 24 - about 40, 26 - about 40, 28 - about 40, 30 - about 40, 32 - about 40, 34 - about 40, 36 - about 40, 38 - about 40, 2 - about 35, 2 - about 30, 2 - about 25, 2 - about 20, 2 - about 15, 2 - about 10, 2 - about

8, 2 - about 6, or 2 - about 4% DSPC; 1 - about 20, 1 - about 19, 1 - about 18, 1 - about 17, 1 - about 16, 1 - about 15, 1 - about 14, 1 - about 13, 1 - about 12, 1 - about 11, 1 - about 10, 1 - about 9, 1 - about 8, 1 - about 7, 1 - about 6, 1 - about 5, 1 - about 4, 1 - about 3, 2 - about 6, 3 - about 7, 4 - about 8, 5 - about 9, 6 - about 10, 7 - about 11, 8 - about 12, 9 - about 13, 10 - about 14, 11 - about 15, 12 - about 16, 13 - about 17, 14 - about 18, 15 - about 19, and about 16 - about 20% PEG-Lipid, with the balance being cholesterol (all in mol%).

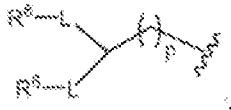
Amino lipid

[575] Described herein are LNP compositions comprising an amino lipid, a phospholipid, a PEGlipid, a cholesterol or a derivative thereof, a payload, or any combination thereof. In some embodiments, the LNP composition comprises an amino lipid. In one aspect, disclosed herein is an amino lipid having the structure of. Formula (I), or a pharmaceutically acceptable salt or solvate thereof,



Formula (I)

wherein

each of R^1 and R^2 is independently C_7-C_{22} alkyl, C_7-C_{22} alkenyl, C_3-C_8 cycloalkyl,- C_2-C_{10} allkylene-L-R^6, or  , wherein each of the alkyl, alkylene, alkenyl, and cycloalkyl is independently substituted or unsubstituted;

each of X, Y, and Z is independently -C(=O)NR^4-, -NR^4C(=O)-, -C(=O)O-, -OC(=O)-, -OC(=O)O-, -NR^4C(=O)O-, -OC(=O)NR^4-, -NR^4C(=O)NR^4-, -NR^4C(=NR^4)NR^4-, -C(=S)NR^4-, -NR^4C(=S)- , -C(=O)O-, -OC(=S)-, OC(=S)O-, -NR^4C(=S)O-, -OC(=S)NR^4-, -NR^4C(=S)NR^4-, -C(=O)S-, -SC(=O)-, -OC(=O)S-, -NR^4C(=O)S-. -SC(=O)NR^4- , -C(=S)S-, -SC(=S)-, -SC(=S)O-, -NR^4C(=S)S-, -SC(=S)NR^4-, -C(=S)S-, -SC(=S)-, -SC(=O)S-, -SC(=S)S-, -NR^4C(=S)S-, - SC(=S)NR^4- O, S, or a bond;

each of L is independently -C(=O)NR^4-, -NR^4C(=O)-, -C(=O)O-. -OC(=O)O-, -NR^4C(=O)O-, -OC(=O)NR^4-, -NR^4C(=O)NR^4-, -NR^4C(=NR^4)NR^4-, -C(=S)NR^4-, -NR^4C(=S)- , -C(=O)O-, -OC(=S)-, OC(=S)O-, -NR^4C(=S)O-, -OC(=S)NR^4-, -NR^4C(=S)NR^4-, -C(=O)S-, SC(=O)-, -OC(=O)S-, -NR^4C(=O)S-, -SC(=O)NR^4- , -C(=S)S-, -SC(=S)-, -SC(=S)O-, - NR^4C(=S)S-, -SC(=S)NR^4-, -C(=S)S-, -SC(=S)-, -SC(=O)S-, -SC(=S)S-, -NR^4C(=S)S-, - SC(=S)NR^4-, O,

S, -C_1-C_{10} alkylene-O-, -C_1-C_{10} alkylene-C(=O)O-, -C_1-C_{10} alkylene-OC(=O)-, or a bond, wherein the alkylene is substituted or unsubstituted;

R^3 is -C_0-C_{10} alkylene-NR^7R^8, -C_0-C_{10} alkylene-heterocycloalkyl, or -C_0-C_{10} alkylene-heterocycloaryl,

wherein the alkylene, heterocycloalkyl and heterocycloaiyl is independently substituted or unsubstituted; each of R^4 is independently hydrogen or substituted or unsubstituted C_1-C_6 alkyl;

R^5 is hydrogen or substituted or unsubstituted C_1-C_6 alkyl;

each of R^6 is independently substituted or unsubstituted C_3-C_{22} alkyl or substituted or unsubstituted C_3-C_{22} alkenyl;

each of R^7 and R^8 is independently hydrogen or substituted or unsubstituted C_1-C_6 alkyl, or R^7 and R^8 taken together with the nitrogen to which they are attached form a substituted or unsubstituted C_2-C_6 heterocyclyl;

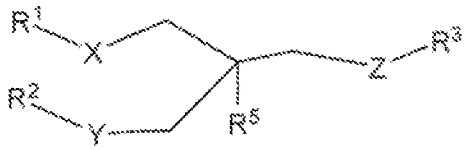
p is an integer selected from 1 to 10; and

each of n, m, and q is independently 0, 1, 2, 3, 4, or 5.

[576] In some embodiments of Formula (I), if the structure carries more than one asymmetric C- atom, each asymmetric C-atom independently represents racemic, chirally pure R and/or chirally pure *S* isomer, or a combination thereof.

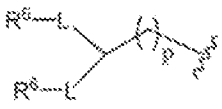
[577] In some embodiments, each of n, in, and q in Formula (I) is independently 0, 1, 2, or 3. In some embodiments, each of n, m, and q in Formula (I) is 1.

[578] In some embodiments, the compound of Formula (1) has a structure of Formula (Ia), or a pharmaceutically acceptable salt or pharmaceutically acceptable solvate thereof:



Formula (Ia)

wherein

each of R^1 and R^2 is independently C_7-C_{22} alkyl, C_7-C_{22} alkenyl, C_3-C_8 cycloalkyl, -C_2-C_{10} alkylene-L-R^6, or  , wherein each of the alkyl, alkylene, alkenyl, and cycloalkyl is independently substituted or unsubstituted;

each of X, Y, and Z is independently C(=O)NR^4-, -NR^4C(D)-, -C(=O)O-, -OC(=O)-, -OC(=O)O-, - NR^4C(=O)O-, -OC(=O)NR^4-, -NR^4C=O)NR^4-, -NR^4C(=NR^4)NR^4-. -C(=S)NR^4-

, -$NR^4C(=S)$- , -C(E)O-, -OC(=S)-, OC(=S)O-, -$NR^4C(=S)O$-, -$OC(=S)NR^4$-, -$NR^4C(=S)NR^4$-, -C(=O)S-, -SC(=O)-, -OC(=O)S-, -$NR^4C(=O)S$-, -$SC(=O)NR^4$- -C(=S)S-, -SC(=S)-, -SC(=S)O-, - $NR^4C(=S)S$-, -$SC(=S)NR^4$-, -C(=S)S-. -SC(=S)-, -SC(=O)S-, -SC(=S)S-. -$NR^4C(=S)S$-, - $SC(=S)NR^4$-, O, S, -C_1-C_{10} alkylene-O-, or a bond, wherein the alkylene is substituted or unsubstituted;

each of L is independently -$C(=O)NR^4$-, -$NR^4C(=O)$-, -C(=O)O-, -OC(=O)-, -OC(=O)O-, -$NR^4C(=O)O$-, -$OC(=O)NR^4$-, -$NR^4C(=O)NR^4$-, -$NR^4C(=NR^4)NR^4$-. -$C(=S)NR^4$-, -$NR^4C(=S)$- , -C(=O)O-, -OC(=S)-, OC(=S)O-, -$NR^4C(=S)O$-, -$OC(=S)NR^4$-, -$NR^4C(=S)NR^4$-, -C(=O)S-, -SC(=O)-, -OC(=O)S-, -$NR^4C(=O)S$-, -$SC(=O)NR^4$- -C(=S)S-, -SC(=S)-, -SC(=S)O-, -$NR^4C(=S)S$-, -$SC(=S)NR^4$-, -C(=S)S-, -SC(=S)-, -SC(=O)S-, -SC(=S)S-, -$NR^4C(=S)S$-, - $SC(=S)NR^4$-, O, S. -C_1-C_{10} alkylene-O-, -C_1-C_{10} alkylene-C(=O)O-, -C_1-C_{10} alkylene- OC(=O)-, or a bond, wherein the alkylene is substituted or unsubstituted;

R^3 is - C_0-C_{10} alkylene-NR^7R^8, - C_0-C_{10} alkylene-heterocycloalkyl, or - C_0-C_{10} alkylene-heterocyclowyl, wherein the alkylene, heterocycloalkyl and heterocycloaryl is independently substituted or unsubstituted;

each of R^4 is independently hydrogen or substituted or unsubstituted CI-C6 alkyl;

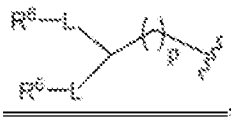
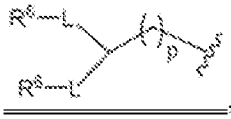
R^5 is hydrogen or substituted or unsubstituted C_1-C_6 alkyl;

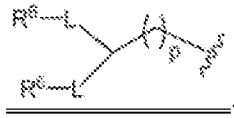
each of R^6 is independently substituted or unsubstituted C_3-C_{22} alkyl or substituted or unsubstituted C_3-C_{22} alkenyl;

each of R^7 and R^8 is independently hydrogen or substituted or unsubstituted C_1-C_6 alkyl, or R^7 and R^8 taken together with the nitrogen to which they are attached form a substituted or unsubstituted C_2-C_6 heterocyclyl; and

p is an integer selected from 1 to 10.

[579] In some embodiments of Formula (la), if the structure carries more than one asymmetric Catom, each asymmetric C-atom independently represents *racemic*, chirally pure *R* and/or chirally pure *S* isomer, or a combination thereof.

[580] In some embodiments, R^1 and R^2 in Formula (I) and Formula (Ia) is independently C_7-C_{22} alkyl, C_7-C_{22} alkenyl, -C_2-C_{10} alkylene-L- R^6, or  , wherein each of the alkyl, alkylene, alkenyl, and cycloalkyl is independently substituted or unsubstituted. In some embodiments, R^1 and R^2 in Formula (I) and Formula (la) is independently C_{10}-C_{20} alkyl, C_{10}-C_{20} alkenyl. - C_8-C_7 alkylene-L- R^6, or  , wherein each of the

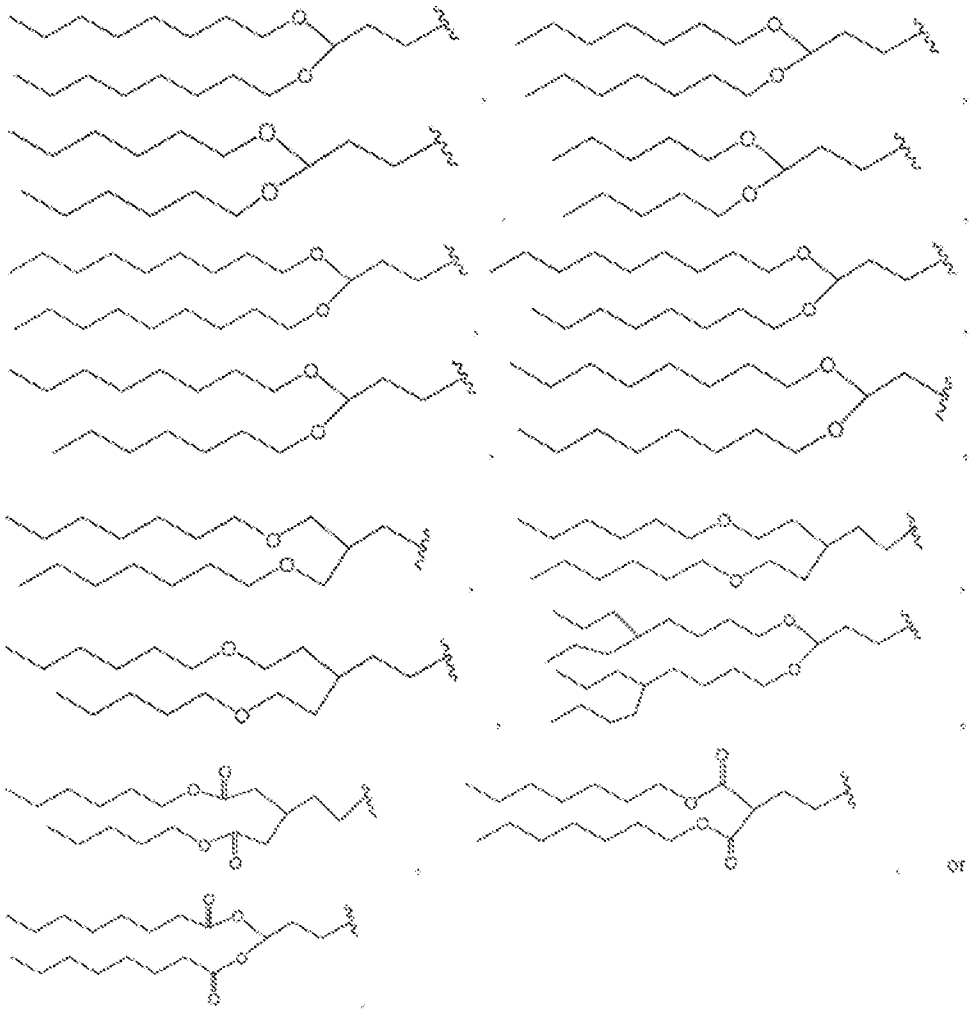
alkyl, alkylene, alkenyl, and cycloalkyl is independently substituted or unsubstituted. In some embodiments, R^1 in Formula (I) and Formula (la) is  .

[581] In some embodiments, each of L in Formula (I) and Formula (Ia) is independently O, S, -C_1-C_{10} alkylene-O-, - C_1-C_{10} alkylene-C(=O)O-, - C_1-C_{10} alkylene-OC(=O)-, or a bond, wherein the alkylene is substituted or unsubstituted. In some embodiments, each of L in Formula (I) and Formula (Ia) is independently O, S, - C_1-C_3 alkylene-O-, - C_1-C_3 alkylene-C(=O)O-, - C_1-C_3 alkylene-OC(=O)-, or a bond, wherein the alkylene is substituted or unsubstituted. In some embodiments, each of L in Formula (I) and Formula (la) is independently O, S, - C_1-C_3 alkylene-O-, - C_1-C_3 alkylene-C(=O)O-, -C_1-C_3 alkylene-OC(=O)-, or a bond, wherein the alkylene is linear or branched unsubstituted alkylene.

[582] In some embodiments, each of R^6 in Formula (I) and Formula (la) is independently substituted or unsubstituted linear C_3-C_{22} alkyl or substituted or unsubstituted linear C_3-C_{22} alkenyl. In some embodiments, each of R^6 in Formula (I) and Formula (Ia) is independently substituted or unsubstituted C_3-C_{20} alkyl or substituted or unsubstituted C_3-C_{20} alkenyl. In some embodiments, each of R^6 in Formula (I) and Formula (Ia) is independently substituted or unsubstituted C_3-C_{10} alkyl or substituted or unsubstituted C_3-C_{10} alkenyl. In some embodiments, each of R^6 in Formula (I) and Formula (Ia) is independently substituted or unsubstituted C_3-C_{10} alkyl. In some embodiments, each of R^6 in Formula (I) and Formula (la) is independently substituted or unsubstituted linear C_3-C_{10} alkyl. In some embodiments, each of R^6 in Formula (I) and Formula (la) is independently substituted or unsubstituted n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, n-decyl, n-undecyl, or n-dodecyl. In some embodiments, each of R^6 in Formula (I) and Formula (Ia) is independently substituted or unsubstituted n-octyl. In some embodiments, each of R^6 in Formula (I) and Formula (Ia) is n-octyl.

[583] In some embodiments, each of L in Formula (I) and Formula (Ia) is independently -C(=O)O-, -OC(=O)-, -C_1-C_{10} alkylene-O-, or O. In some embodiments, each of. L in Formula (I) and Formula (Ia) is O. In some embodiments, each of L in Formula (I) and Formula (Ia) is -C_1-C_3 alkylene-O-. In some embodiments, p in Formula (I) and Formula (la) is 1, 2, 3, 4, or 5. In some embodiments, p in Formula (I) and Formula (Ia) is 2.

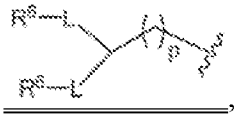
[584] In some embodiments, R^1 in Formula (I) and Formula (Ia) is



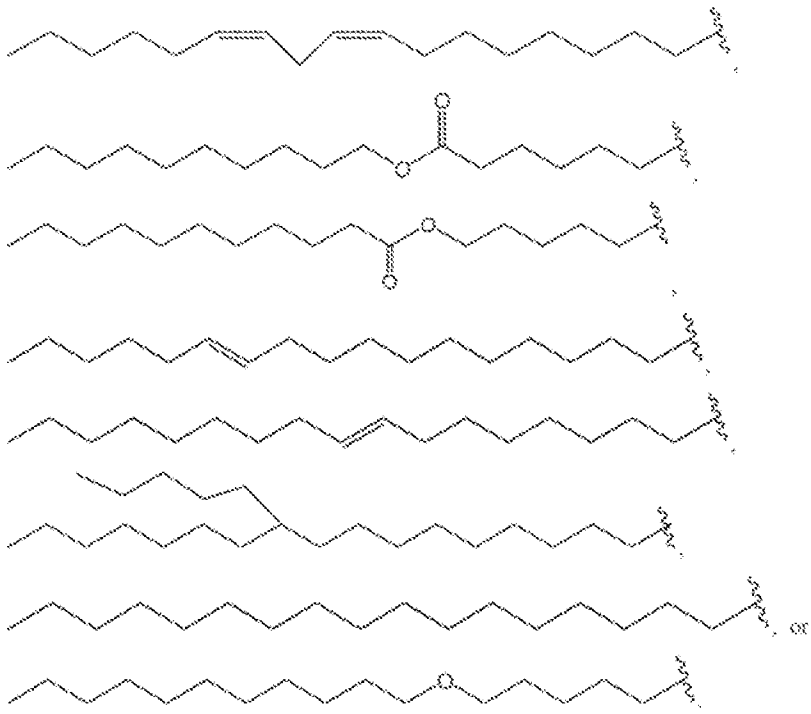
[585] In some embodiments, each of R^4 in Formula (I) and Formula (Ia) is independently H or substituted or unsubstituted C_1-C_4 alkyl. In some embodiments, each of. R^4 in Formula (I) and Formula (la) is independently substituted or unsubstituted linear C_1-C_4 alkyl. In some embodiments, each of R^4 in Formula (1) and Formula (la) is H. In some embodiments, each of R^4 in Formula (I) and Formula (Ia) is independently H, -CH_3, -CH_2CH_3, -$CH_2CH_2CH_3$, or -$CH(CH_3)_2$. In some embodiments, each of R^4 in Formula (I) and Formula (Ia) is independently H or -CH3. In some embodiments, each of R^4 in Formula (1) and Formula (Ia) is -CH_3.

[586] In some embodiments, X in Formula (I) and Formula (Ia) is -C(=O)O- or -OC(=O))-. In some embodiments, X in Formula (I) and Formula (Ia) is -C(=O)NR^4- or -NR^4C(=O)-. In some embodiments, X in Formula (I) and Formula (Ia) is -C(=O)N(CH_3)-, -N(CH_3)C(=O)-, -C(=O)NH-, or -NHC(=O)-. In some embodiments, X in Formula (I) and Formula (Ia) is -C(=O))NH-, -C(=O)N(CH_3)-. -OC(=O))-, -NHC(=O)-, -N(CH_3)C(=O))-, -C(=O)O-, -OC(=O)O-, -NHC(=O)O-, -N(CH_3)C(=O)O-, - OC(=O))NH-, -OC(=O)N(CH_3)-, -NHC(=O)NH-, -N(CH_3)C(=O))NH-, -NHC(=O)N(CH_3)-, - N(CH_3)C(=O)N(CH_3)-,

NHC(=NH)NH-, -N(CH_3)C(=NH)NH-, -NHC(=NH)N(CH_3)-, - N(CH_3)C(=NH)N(CH_3)-, NHC(=NMe)NH-, -N(CH_3)C(=NMe)NH-, -NHC(=NMe)N(CH_3)-, or - N(CH_3)C(=NMe)N(CH_3)-.

[587] In some embodiments. R^2 in Formula (I) and Formula (Ia) is C_7-C_{22} alkyl, C_7-C_{22} alkenyl, - C_2-C_{10} alkylene-L- R^6, or  , wherein each of the alkyl, alkylene, alkenyl, and cycloalkyl is independently substituted or unsubstituted. In some embodiments, R^2 in Formula (I) and Formula (Ia) is substituted or unsubstituted C_7-C_{22} alkyl or substituted or unsubstituted C_7-C_{22} alkenyl. In some embodiments, R^2 in Formula (I) and Formula (la) is substituted or unsubstituted linear C_7-C_{22} alkyl or substituted or unsubstituted linear C_7-C_{22} alkenyl. In some embodiments, R^2 in Formula (I) and Formula (la) is substituted or unsubstituted C_{10}-C_{20} alkyl or substituted or unsubstituted C_{10}-C_{20} alkenyl. In some embodiments, R^2 in Formula (I) and Formula (Ia) is unsubstituted C_{10}-C_{20} alkyl. In some embodiments, R^2 in Formula (I) and Formula (Ia) is unsubstituted C_{10}-C_{20} alkenyl. In some embodiments, R^2 in Formula (I) and Formula (la) is -C_2-C_{10} alkylene-L- R^6. In some embodiments, R^2 in Formula (I) and Formula (Ia) is -C_2-C_{10} alkylene- C(=O)O- R^6 or -C_2-C_{10} alkylene-OC(=O)- R^6.

[588] In some embodiments, R^2 in Formula (I) and Formula (Ia) is



[589] In some embodiments, Y in Formula (I) and Formula (Ia) is -C(=O)O- or -OC(=O)-. In some embodiments, Y in Formula (I) and Formula (Ia) is -C(=O)NR^4- or -NR^4C(=O)-. In

some embodiments, Y in Formula (I) and Formula (Ia) is -C(=O)N(CH_3)-, -N(CH_3)C(=O)-, -C(=O)NH-, or -NHC(=O)-. In some embodiments, Y in Formula (I) and Formula (Ia) is -OC(=O)O-, -NR^4C(=O)O-, -OC(=O)NR^4-, or -NR^4C(=O)NR^4-. In some embodiments. Y in Formula (I) and Formula (la) is - OC(=O)O-, -NHC(=O)O-, -OC(=O)NH-, -NHC(=O)NH-, -N(CH_3)C(=O)O-. -OC(=O)N(CH_3)-, - N(CH_3)C(=O)N(CH_3)- or -N(CH_3)C(=O)NH-. In some embodiments, Y in Formula (I) and Formula (Ia) is -OC(=O)O-, -NHC(=O)0-, -OC(=O)NH-, or -NHC(=O)NH-.

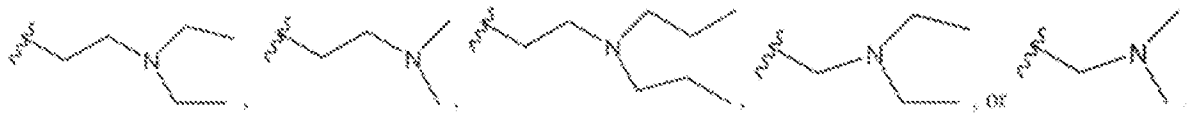
[590] In some embodiments, R^3 in Formula (I) and Formula (Ia) is -C_0-C_{10} alkylene-NR^7R^8 or -C_0-C_{10} alkylene-heterocycloalkyl, wherein the alkylene and heterocycloalkyl is independently substituted or unsubstituted. In some embodiments, R^3 in Formula (I) and Formula (Ta) is -C_0-C_{10} alkylene-NR^7R^8. Tn some embodiments, R^3 in Formula (I) and Formula (Ia) is -C_1-C_6 alkylene-NR^7R^8. In some embodiments, R^3 in Formula (I) and Formula (Ia) is - C_1-C_4 alkylene-NR^7R^8. In some embodiments, R^3 in Formula (I) and Formula (Ia) is -C_1- alkylene-NR^7R^8. In some embodiments, R^3 in Formula (I) and Formula (Ia) is -C_2-- alkylene-NR^7R^8. Tn some embodiments, R^3 in Formula (I) and Formula (Ia) is -C_3- alkylene-NR^7R^8. In some embodiments, R^3 in Formula (I) and Formula (Ia) is -C_4-alkylene- NR^7R^8. In some embodiments, R^3 in Formula (I) and Formula (Ia) is -C_5- alkylene-NR^7R^8. In some embodiments, R^3 in Formula (I) and Formula (Ia) is - C_0-C_{10} alkylene-heterocycloalkyl. In some embodiments, R^3 in Formula (I) and Formula (Ia) is - C_1-C_6 alkylene-heterocycloalkyl, wherein the heterocycloalkyl comprises 1 to 3 nitrogen and 0-2 oxygen. In some embodiments, R^3 in Formula (I) and Formula (la) is -C_1-C_6 alkylene-heterocycloaryl.

[591] In some embodiments, each of R^7 and R^8 in Formula (I) and Formula (Ia) is independently hydrogen or substituted or unsubstituted C_1-C_6 alkyl. Tn some embodiments, each of R^7 and R^8 is independently hydrogen or substituted or unsubstituted C_1-C_3 alkyl. In some embodiments, each of R^7 and R^8 is independently substituted or unsubstituted C_1-C_3 alkyl. In some embodiments, each of R^7 and R^8 is independently -CH_3, -CH_2CH_3, -$CH_2CH_2CH_3$, or -$CH(CH_3)_2$. In some embodiments, each of R and R^8 is CH_3. In some embodiments, each of R^7 and R^8 is -CH_2CH_3.

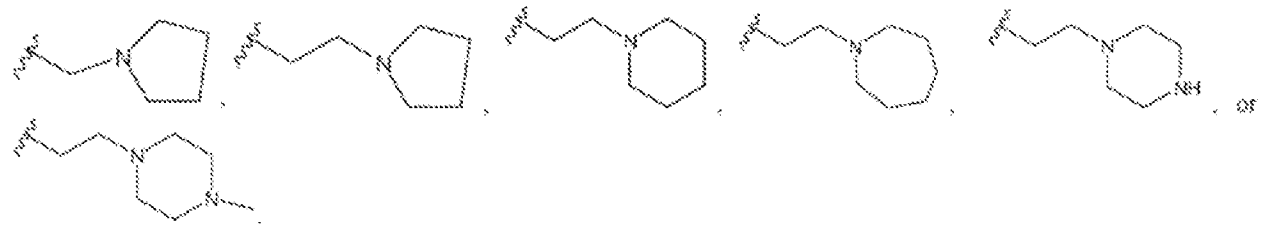
[592] In some embodiments, R^7 and R^8 in Formula (I) and Formula (Ia) taken together with the nitrogen to which they are attached form a substituted or unsubstituted C2-C6 heterocyclyl. In some embodiments, R^7 and R^8 taken together with the nitrogen to which they are attached form a substituted or unsubstituted C_2-C_6 heterocycloalkyl. In some

embodiments, R^7 and R^8 taken together with the nitrogen to which they are attached form a substituted or unsubstituted 3-7 membered heterocycloalkyl.

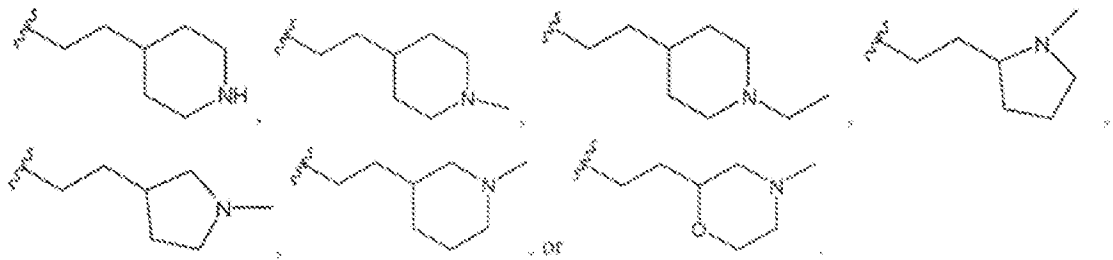
[593] In some embodiments, R^3 in Formula (I) and Formula (la) is



[594] In some embodiments. R^3 in Formula (I) and Formula (Ia) is



[595] In some embodiments. R^3 in Formula (1) and Formula (la) is



[596] In some embodiments, Z in Formula (I) and Formula (Ia) is -C(=O)O- or -OC(=O)-. In some embodiments, Z in Formula (1) and Formula (Ia) is -C(=O)NR^4- or -NR^4C(=O)-. In some embodiments, Z in Formula (I) and Formula (Ia) is -C(=O)N(CH_3)-, -N(CH_3)C(=O)-, -C(=O)NH-, or -NHC(=O)-. In some embodiments, Z in Formula (I) and Formula (Ia) is -OC(=O)O-, -NR^4C(=O)O-, -OC(O)NR^4-, or -NR^4C(=O)NR^4-. In some embodiments. Z in Formula (I) and Formula (Ia) is -OC(=O)O-, - NHC(=O)O-, -OC(=O)NH-, -NHC(=O)NH-, -N(CH_3)C(=O)O-, -OC(=O)N(CH_3)-, - N(CH_3)C(=O)N(CH_3)-, -NHC(=O)N(CH_3)- or -N(CH_3)C(=O)NH-. In some embodiments, Y in Formula (I) and Formula (Ia) is -OC(=O)O-, -NHC(=O)O-, -OC(=O)NH-, or -NHC(=O)NH-.

[597] In some embodiments, R^5 in Formula (I) and Formula (Ia) is hydrogen or substituted or unsubstituted C_1-C_3 alkyl. In some embodiments, R^5 in Formula (I) and Formula (Ia) is H, -CH_3, - CH-)CH_3, -$CH_2CH_2CH_3$, or -$CH(CH_3)_2$. In some embodiments, R^5 in Formula (I) and Formula (Ia) is H.

[598] In some embodiments, the LNP comprises a plurality of amino lipids. For example, the LNP composition can comprise 2, 3, 4, 5, 6.7, 8, 9. 10, or more amino lipids. For another

example, the LNP composition can comprise at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 9, at least 10, or at least 20 amino lipids. For yet another example, the LNP composition can comprise at most 2, at most 3, at most 4, at most 5, at most 6, at most 7, at most 9, at most 10, at most 20, or at most 30 amino lipids.

[599] In some embodiments, the LNP composition comprises a first amino lipid. In some embodiments, the LNP composition comprises a first amino lipid and a second amino lipid. In some embodiments, the LNP composition comprises a first amino lipid, a second amino lipid, and a third amino lipid. In some embodiments, the LNP composition comprises a first amino lipid, a second amino lipid, a third amino lipid, and a fourth amino lipid. In some embodiments, the LNP composition does not comprise a fourth amino lipid. In some embodiments, the LNP composition does not comprise a third amino lipid. In some embodiments, a molar ratio of the first amino lipid to the second amino lipid is from about 0.1 to about 10. In some embodiments, a molar ratio of the first amino lipid to the second amino lipid is from about 0.20 to about 5. In some embodiments, a molar ratio of the first amino lipid to the second amino lipid is from about 0.25 to about 4. In some embodiments, a molar ratio of the first amino lipid to the second amino lipid is about 0.25, about 0.33, about 0.5, about 1, about 2, about 3, or about 4.

[600] In some embodiments, a molar ratio of the first amino lipid: the second amino lipid: the third amino lipid is about 4:1:1. In some embodiments, a molar ratio of the first amino lipid: the second amino lipid: the third amino lipid is about 1:1:1. In some embodiments, a molar ratio of the first amino lipid: the second amino lipid: the third amino lipid is about 2:1:1. In some embodiments, a molar ratio of the first amino lipid: the second amino lipid: the third amino lipid is about 2:2:1. In some embodiments, a molar ratio of the first amino lipid: the second amino lipid: the third amino lipid is about 3:2:1. In some embodiments, a molar ratio of the first amino lipid: the second amino lipid: the third amino lipid is about 3:1:1. In some embodiments, a molar ratio of the first amino lipid: the second amino lipid: the third amino lipid is about 5:1:1. In some embodiments, a molar ratio of the first amino lipid: the second amino lipid: the third amino lipid is about 3:3:1. In some embodiments, a molar ratio of the first amino lipid: the second amino lipid: the third amino lipid is about 4:4:1.

[601] In some embodiments, the LNP composition comprises one or more amino lipids. In some embodiments, the one or more amino lipids comprise from about 40 mol% to about 65 mol% of the total lipid present in the particle. In some embodiments, the one or more amino lipids comprise about 40 mol%, about 41 mol%, about 42 mol%, about 43 mol%, about 44 mol%, about 45 mol%, about 46 mol%, about 47 mol%, about 48 mol%, about 49 mol%,

about 50 mol%, about 51 mol%, about 52 mol%, about 53 mol%, about 54 mol%, about 55 mol%, about 56 mol%, about 57 mol%, about 58 mol%, about 59 mol%, about 60 mol%, about 61 mol%, about 62 mol%, about 63 mol%, about 64 mol%, or about 65 mol% of the total lipid present in the particle. In some embodiments, the first amino lipid comprises from about 1 mol% to about 99 mol% of the total amino lipids present in the particle. In some embodiments, the first amino lipid comprises from about 16.7 mol% to about 66.7 mol% of the total amino lipids present in the particle. In some embodiments, the first amino lipid comprises from about 20 mol% to about 60 mol% of the total amino lipids present in the particle.

[602] In some embodiments, the amino lipid is an ionizable lipid. An ionizable lipid can comprise one or more ionizable nitrogen atoms. In some embodiments, at least one of the one or more ionizable nitrogen atoms is positively charged. Tn some embodiments, at least 10 mol%, 20 mol%, 30 mol%, 40 mol%, 50 mol%, 60 mol%, 70 mol%, 80 mol%. 90 mol%, 95 mol%, or 99 mol% of the ionizable nitrogen atoms in the LNP composition are positively charged. In some embodiments, the amino lipid comprises a primary amine, a secondary amine, a tertiary amine, an imine, an amide, a guanidine moiety, a histidine residue, a lysine residue, an arginine residue, or any combination thereof. In some embodiments, the amino lipid comprises a primary amine, a secondary amine, a tertiary amine, a guanidine moiety, or any combination thereof. In some embodiments, the amino lipid comprises a tertiary amine.

[603] In some embodiments, the amino lipid is a cationic lipid. Tn some embodiments, the amino lipid is an ionizable lipid. In some embodiments, the amino lipid comprises one or more nitrogen atoms. In some embodiments, the amino lipid comprises one or more ionizable nitrogen atoms. Exemplary cationic and/or ionizable lipids include, but are not limited to, 3-(didodecylamino)- N1,N1,4-tri dodecyl-l-piperazineethan amine (KL10), N142-(didodecylamino)ethy1]-N1,N4,N4- tridodecyl-1,4-piperazinediethanamine (KL22), 14,25-ditridecy1-15,18,21 ,24-tetraaza-octatriacontane (KL25), 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLin-DMA), 2,2-dilinoley1-4- dimethylaminomethy1-[1,3]-dioxolane (DLin-K-DMA), heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (DLin-MC 3-DMA), 2,2-dilinoley1-4-(2-dimethylaminoethy1)-[1,3]- dioxolane (DLin-KC2-DMA), 1,2-dioleyloxy-N,N-dimethylaminopropane (DODMA), 2-({8-[(3β)- cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethy1-3-[(9Z,12Z)-octadeca-9,12-dien-l-yloxy]propan-l-amine (Octyl-CLinDMA), (2R)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethy1-3- [(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine

(Octyl-CLinDMA (2R)), and (2S)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethy1-3-[(9Z,12Z)-octadeca-9,12-dien-l-yloxy]propan-l-amine (Octyl-CLinDMA (2S)).

[604] In some embodiments, an amino lipid described herein can take the form of a salt, such as a pharmaceutically acceptable salt. All pharmaceutically acceptable salts of the amino lipid are encompassed by this disclosure. As used herein, amino lipid also includes its pharmaceutically acceptable salts, and its diastereomeric, enantiomeric, and epimeric forms.

[605] In some embodiments, an amino lipid described herein, possesses one or more stereocenters and each stereocenter exists independently in either the R or S configuration. The lipids presented herein include all diastereomeric, enantiomeric, and epimeric forms as well as the appropriate mixtures thereof. The lipids provided herein include all cis. trans, syn, anti, entgegen (E), and zusammen (Z) isomers as well as the appropriate mixtures thereof. Tn certain embodiments, lipids described herein are prepared as their individual stereoisomers by reacting a racemic mixture of the compound with an optically active resolving agent to form a pair of diastereoisomeric compounds/salts, separating the diastereomers and recovering the optically pure enantiomers. In some embodiments, resolution of enantiomers is carried out using covalent diastereomeric derivatives of the compounds described herein. In another embodiment, diastereomers are separated by separation/resolution techniques based upon differences in solubility. In other embodiments, separation of stereoisomers is performed by chromatography or by the forming diastereomeric salts and separation by recrystallization, or chromatography, or any combination thereof. Jean Jacques, Andre Collet, Samuel H. Wilen, "Enantiomers, Racemates and Resolutions", John Wiley and Sons, Inc., 1981. In one aspect, stereoisomers are obtained by stereoselective synthesis.

[606] In some embodiments, the lipids such as the amino lipids are substituted based on the structures disclosed herein. In some embodiments, the lipids such as the amino lipids are unsubstituted. In another embodiment, the lipids described herein are labeled isotopically (e.g. with a radioisotope) or by another other means, including, but not limited to, the use of chromophores or fluorescent moieties, bioluminescent labels, or chemiluminescent labels.

[607] Lipids described herein include isotopically-labeled compounds, which are identical to those recited in the various formulae and structures presented herein, but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into the present lipids include isotopes of hydrogen, carbon, nitrogen, oxygen, sulfur, fluorine and chlorine, such as, for example, ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, ^{35}S, ^{18}F, ^{36}Cl. In one aspect, isotopically-labeled lipids described herein, for example those

into which radioactive isotopes such as ^{3}H and ^{14}C are incorporated, are useful in drug and/or substrate tissue distribution assays. In one aspect, substitution with isotopes such as deuterium affords certain therapeutic advantages resulting from greater metabolic stability, such as, for example, increased in vivo half-life or reduced dosage requirements.

[608] In some embodiments, the asymmetric carbon atom of the amino lipid is present in enantiomerically enriched form. In certain embodiments, the asymmetric carbon atom of the amino lipid has at least 50% enantiomeric excess, at least 60 % enantiomeric excess, at least 70 % enantiomeric excess, at least 80 % enantiomeric excess, at least 90 % enantiomeric excess, at least 95 % enantiomeric excess, or at least 99 % enantiomeric excess in the (S)- or (R)-configuration.

[609] In some embodiments, the disclosed amino lipids can be converted to N-oxides. In some embodiments, N-oxides are formed by a treatment with an oxidizing agent (e.g. , 3-chloroperoxybenzoic acid and/or hydrogen peroxides). Accordingly, disclosed herein are N-oxide compounds of the described amino lipids, when allowed by valency and structure, which can be designated as N O or N^{+}-O^{-}. In some embodiments, the nitrogen in the compounds of the disclosure can be converted to N-hydroxy or N-alkoxy. For example, N-hydroxy compounds can be prepared by oxidation of the parent amine by an oxidizing agent such as ra-CPBA. All shown and claimed nitrogencontaining compounds are also considered. Accordingly, also disclosed herein are N-hydroxy and Nal koxy (e.g., N-OR, wherein R is substituted or unsubstituted C_1-C_6 alkyl, C_1-C_6 alkenyl, C_1-C_6 alkynyl, 3-14-membered carbocycle or 3-14-membered heterocycle) derivatives of the described amino lipids.

PEG-Lipid

[610] As used herein, a “PEG lipid” or “PEG-lipid” refers to a lipid comprising a polyethylene glycol component.

[611] In some embodiments, the described LNP composition comprises a PEG-lipid. In some embodiments, the described LNP composition comprises two or more PEG-lipids. Exemplary PEG- lipids include, but are not limited to, the lipids. Exemplary PEG-lipids also include, but are not limited to, PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG- modified ceramides, PEG-modified dialkylamines, PEG-modified diacylglycerols, PEG-modified dialkylglycerols, and mixtures thereof. For example, the one or more PEG-lipids can comprise PEG-c- DOMG, PEG-DMG, PEG-DLPE, PEG-DMPE, PEG-DPPC, a PEG-DSPE lipid, or a combination thereof. In some embodiments, PEG moiety is an optionally substituted linear or branched polymer of ethylene glycol or ethylene

oxide. In some embodiments, the PEG moiety is substituted, e.g., by one or more alkyl, alkoxy, acyl, hydroxy, or aryl groups. In some embodiments, the PEG moiety includes PEG copolymer such as PEG-polyurethane or PEG-polypropylene (see, e.g., j. Milton Harris, Poly(ethylene glycol) chemistry: biotechnical and biomedical applications (1992)). In some embodiments, the PEG moiety does not include PEG copolymers, e.g., it may be a PEG monopolymer. Exemplary PEG-lipids include, but are not limited to, PEG-dilauroylglycerol, PEG- dimyristoylglycerol (PEG-DMG), PEG-dipalmitoylglycerol, PEG-distearoylgiycerol (PEG-DSPE), PEG-dipalmitoylglycerol, PEG-disteiylglycerol, PEG-dilawylglycamide, PEG-dimyristylglycamide, PEG-dipalmitoylglycamide, PEG-disterylglycamide, PEG-cholesterol, and PEG-DMB (3,4- Ditetradecoxylbenzyl- [omega]-methyl-poly(ethylene glycol) ether), 1,2-dimyristoyl-sn-glycero-3- phosphoethanolamine-N-[methoxy(polyethylene glycol) - 2000]).

[612] In some embodiments, a PEG-lipid is a PEG-lipid conjugate, for example, PEG coupled to dialkyloxypropyls (e.g., PEG-DAA conjugates), PEG coupled to diacylglycerols (e.g., PEG-DAG conjugates), PEG coupled to cholesterol, PEG coupled to phosphatidylethanolamines, and PEG conjugated to ceramides (see, e.g., U.S. Pat. No. 5,885,613), cationic PEG lipids, polyoxazoline (POZ)-lipid conjugates (e.g. POZ-DAA conjugates; see, e.g., WO 2010/006282), polyamide oligomers (e.g., ATTA-lipid conjugates), and mixtures thereof.

[613] A PEG-lipid can comprise one or more ethylene glycol units, for example, at least 1, at least 2, at least 5, at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 120, or at least 150 ethylene glycol units. In some embodiments, a number average molecular weight of the PEG-lipids is from about 200 Da to about 5000 Da. In some embodiments, a number average molecular weight of the PEG-lipids is from about 500Da to about 3000 Da. In some embodiments, a number average molecular weight of the PEG-lipids is from about 750 Da to about 2500 Da. In some embodiments, a number average molecular weight of the PEG- lipids is from about 750 Da to about 2500 Da. In some embodiments, a number average molecular weight of the PEG-lipids is about 500 Da, about 750 Da, about 1000 Da, about 1250 Da, about 1500 Da, about 1750 Da, or about 2000 Da. In some embodiments, a polydispersity index (PD1) of the one or more PEG-lipids is smaller than 2. In some embodiments, a PDI of the one or more PEG-lipids is at most 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3. 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, or 3.0. In some embodiments, a PDI of the one or more PEG-lipids is at least 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, or 3.0.

[614] In some embodiments, the PEG-lipid comprises from about 0.1 mol% to about 10 mol% of the total lipid present in the particle. In some embodiments, the PEG-lipid comprises from about 0.1 mol% to about 6 mol% of the total lipid present in the particle. In some embodiments, the PEG-lipid comprises from about 0.5 mol% to about 5 mol% of the total lipid present in the particle. In some embodiments, the PEG-lipid comprises from about 1 mol% to about 3 mol% of the total lipid present in the particle. In some embodiments, the PEG-lipid comprises about 2.0 mol% to about 2.5 mol% of the total lipid present in the particle. In some embodiments, the PEG-lipid comprises about 1 mol%, about 1.1 mol%, about 1.2 mol%, about 1.3 mol%, about 1.4 mol%, about 1.5 mol%, about 1.6 mol%, about 1.7 mol%, about 1.8 mol%, about 1.9 mol%, about 2.0 mol%, about 2.1 mol%, about 2.2 mol%, about 2.3 mol%, about 2.4 mol%, about 2.5 mol%, about 2.6 mol%, about 2.7 mol%, about 2.8 mol%, about 2.9 mol%, or about 3.0 mol% of the total lipid present in the particle.

[615] In some embodiments, the LNP composition comprises a plurality of PEG-lipids, for example, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, or more distinct PEG-lipids.

Phospholipid

[616] As used herein, a "phospholipid" refers to a lipid that includes a phosphate moiety and one or more carbon chains, such as unsaturated fatty acid chains. A phospholipid may include one or more multiple (e.g., double or triple) bonds. In some embodiments, a phospholipid may facilitate fusion to a membrane. For example, a cationic phospholipid may interact with one or more negatively charged phospholipids of a membrane (e.g., a cellular or intracellular membrane). Fusion of a phospholipid to a membrane may allow one or more elements of an LNP to pass through the membrane, i.e., delivery of the one or more elements to a cell.

[617] In some embodiments, the described LNP composition comprises a phospholipid. In some embodiments, the phospholipid comprises a lipid selected from the group consisting of: phosphatidylcholine (PC), phosphatidylethanolamine amine, glycerophospholipid, sphingophospholipids, Guriserohosuhono, sphingolipids phosphono lipids, natural lecithins, and hydrogenated phospholipid. In some embodiments, the phospholipid comprises a phosphatidylcholine. Exemplary phosphatidylcholines include, but are not limited to, soybean phosphatidylcholine, egg yolk phosphatidylcholine (EPC), distearoylphosphatidylcholine, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), dipalmitoyl phosphatidylcholine, dipalmitoyl -sn-glycero-3-phosphocholine (DPPC), 2-Oleoy1-1-palmitoyl-sn-glycero-3-

phosphocholine (POPC), dimyristoyl phosphatidylcholine (DMPC), and dioleoyl phosphatidylcholine (DOPC). In certain specific embodiments, the phospholipid is DSPC.

[618] In some embodiments, the phospholipid comprises a phosphatidylethanolamine amine. In some embodiments, the phosphatidylethanolamine amine is distearoyl phosphatidylethanolamine (DSPE), dipalmitoyl phosphatidyl ethanolamine (DPPE), 1,2-dioleoyl-sn-glycero-3- phosphoethanolamine (DOPE), dimyristoyl phosphoethanolamine (DMPE), 16-0-Monome Le PE, 16-0-dimethyl PE, 18-1-trans PE, palmitoyl oleoyl-phosphatidylethanolamine (POPE), or 1-stearoy1-2- oleoyl-phosphatidyl ethanolamine (SOPE). In some embodiments, the phospholipid comprises a glycerophospholipid. In some embodiments, the glycerophospholipid is plasmalogen, phosphatidate, or phosphatidylcholine. In some embodiments, the glycerophospholipid is phosphatidylserine, phosphatidic acid, phosphatidylglycerol, phosphatidylinositol, palmitoyl oleoyl phosphatidylglycerol (POPG), or lysophosphatidylcholine. In some embodiments, the phospholipid comprises a sphingophospholipid. In some embodiments, the sphingophospholipid is sphingomyelin, ceramide phosphoethanolamine, ceramide phosphoglycerol, or ceramide phosphoglycerophosphoric acid. In some embodiments, the phospholipid comprises a natural lecithin. In some embodiments, the natural lecithin is egg yolk lecithin or soybean lecithin. Tn some embodiments, the phospholipid comprises a hydrogenated phospholipid. In some embodiments, the hydrogenated phospholipid is hydrogenated soybean phosphatidylcholine. In some embodiments, the phospholipid is selected from the group consisting of: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, palmitoyloleoyl phosphatidylcholine, lysophosphatidylcholine, lysophosphatidylethanolamine, dipalmitoylphosphatidylcholine, dioleoylphosphatidylcholine, distearoylphosphatidylcholine, and dilinoleoylphosphatidylcholine.

[619] In some embodiments, the phospholipid comprises a lipid selected from: 1,2-distearoyl-sn- glycero-3-phosphocholine (DSPC). 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2- dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3- phosphocholine (DPPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 2-Oleoy1-1- pahnitoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3- phosphocholine,

1,2-di arachidonoyl-sn-glycero-3-phosphocholine, 1 ,2-di docosahexaenoyl-sn- glycero-3-phosphocholine, 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diaracliidonoyl-sn-gly cero-3- phosphoethanol amine, 1,2-di docosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diol eoy I -sn- glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), and sphingomyelin.

[620] A phospholipid can comprise a phospholipid moiety and one or more fatty acid moieties. A phospholipid moiety can comprise phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl serine, phosphatidic acid, 2-lysophosphatidyl choline, or a sphingomyelin. A fatty acid moiety can comprise lauric acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, erucic acid, phytanoic acid, arachidic acid, arachidonic acid, eicosapentaenoic acid, behenic acid, docosapentaenoic acid, or docosahexaenoic acid. In some specific embodiments, a phospholipid can be functionalized with or cross-linked to one or more alkynes, which may undergo a copper-catalyzed cycloaddition upon exposure to an azide.

[621] In some embodiments, the LNP composition comprises a plurality of phospholipids, for example, at least 2, 3, 4, 5, or more distinct phospholipids. In some embodiments, the phospholipid comprises from 1 mol% to 20 mol% of the total lipid present in the particle. In some embodiments, the phospholipid comprises from about 5 mol% to about 15 mol% of the total lipid present in the particle. In some embodiments, the phospholipid comprises from about 8 mol% to about 12 mol% of the total lipid present in the particle. In some embodiments, the phospholipid comprises from about 9 mol%, 10 mol%, or 11 mol% of the total lipid present in the particle.

Cholesterol

[622] In some embodiments, the LNP composition comprises a cholesterol or a derivative thereof. In some embodiments, the LNP composition comprises a structural lipid. The structural lipid can be selected from steroid, sterol, alkyl resoreinol, cholesterol or derivative thereof, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, tomatine, ursolic acid, alphatocopherol, and a combination thereof. In some embodiments, the structural lipid is a corticosteroid such as prednisolone, dexamethasone, prednisone, and hydrocortisone. In some embodiments, the cholesterol or derivative thereof is

cholesterol, 5- heptadecylresorcinol, or cholesterol hemisuccinate. Tn some embodiments, the cholesterol or derivative thereof is cholesterol.

[623] In some embodiments, the cholesterol or derivative thereof is a cholesterol derivative. In some embodiments, the cholesterol derivative is a polar cholesterol analogue. In some embodiments, the polar cholesterol analogue is 5a-cholestanol, 513-coprostanol, cholesteryl-(2'-hydroxy)-ethyl ether, cholestely1-(4'-hydroxy)-butyl ether, or 6-ketocholestanol. In some embodiments, the polar cholesterol analogue is cholestery1-(4'-hydroxy)-butyl ether. In some embodiments, the cholesterol derivative is a non-polar cholesterol analogue. In some embodiments, the non-polar cholesterol analogue is 5acholestane, cholestenone, 5α-cholestanone, 5β-cholestanone, or cholestetyl decanoate.

[624] In some embodiments, the cholesterol or the derivative thereof comprises from 20 mol% to 50 mol% of the total lipid present in the particle. In some embodiments, the cholesterol or the derivative thereof comprises about 20 mol%, about 21 mol%, about 22 mol%, about 23 mol%, about 24 mol%, about 25 mol%, about 26 mol%, about 27 mol%, about 28 mol%, about 29 mol%, about 30 mol%, about 31 mol%, about 32 mol%, about 33 mol%, about 34 mol%, about 35 mol%, about 36 mol%, about 37 mol%, about 38 mol%, about 39 mol%, about 40 mol%, about 41 mol%, about 42 mol%, about 43 mol%, about 44 mol%. about 45 mol%. about 46 mol%, about 47 mol%, about 48 mol%, or about 50 mol% of the total lipid present in the particle.

Phosphate charge neutralizer

[625] In some embodiments, the LNP described herein comprises a phosphate charge neutralizer. In some embodiments, the phosphate charge neutralizer comprises arginine, asparagine, glutamine, lysine, histidine, cationic dendrimers, polyamines, or a combination thereof. In some embodiments, the phosphate charge neutralizer comprises one or more nitrogen atoms. In some embodiments, the phosphate charge neutralizer comprises a polyamine. In some embodiments, the polyamine is 1,3- propanediamine, spermine, spermidine, Norspermidine, Tris(2-aminoethyDamine, Cyclen, 1,4,7- Triazacyclononane, 1,1,1-Tris(aminomethyl)ethane, Diethylenetriamine, Triethylenetetramine, or a combination thereof. In some embodiments, the polyamine is 1,3-propanediamine, 1,4-butanediamine, spermine, spermidine, or a combination thereof. In some embodiments, the N/P ratio for the phosphate charge neutralizer is from 0.01 to 10. In some embodiments, the N/P ratio for the phosphate charge neutralizer is from about 0.05 to about 2. In some embodiments, the N/P ratio for the phosphate charge neutralizer is from about 0.1 to about 1. In some embodiments,

the N/P ratio for the phosphate charge neutralizer is about 0.1, about 0.2, about 0.3, about 0.4, about 0.5, about 0.6, about 0.7, about 0.8, about 0.9, or about 1. In some embodiments, the NIP ratio for the phosphate charge neutralizer is about 0.25, 0.5, or 0.75.

Antioxidants

[626] In some embodiments, the LNP described herein comprises one or more antioxidants. In some embodiments, the one or more antioxidants function to reduce a degradation of the cationic lipids, the payload, or both. In some embodiments, the one or more antioxidants comprise a hydrophilic antioxidant. In some embodiments, the one or more antioxidants is a chelating agent such as ethylenediaminetetraacefic acid (EDTA) and citrate. In some embodiments, the one or more antioxidants is EDTA. In some embodiments, the one or more antioxidants comprise a lipophilic antioxidant. In some embodiments, the lipophilic antioxidant comprises a vitamin E isomer or a polyphenol. In some embodiments, the one or more antioxidants are present in the LNP composition at a concentration of at least 1 mM, at least 10 mM, at least 20 mM, at least 50 mM, or at least 100 mM. In some embodiments, the one or more antioxidants are present in the particle at a concentration of about 20 mM.

Payload

[627] The LNPs described herein can be designed to deliver a payload, such as a therapeutic agent, or a target of interest. In some embodiments, an LNP described herein encloses one or more components of a base editor system as described herein. For example, a LNP may enclose one or more of a guide RNA, a nucleic acid encoding the guide RNA, a vector encoding the guide RNA, a base editor fusion protein, a nucleic acid encoding the base editor fusion protein, a programmable DNA binding domain, a nucleic acid encoding the programmable DNA binding domain, a deaminase, a nucleic acid encoding the deaminase, or all or any combination thereof. In some embodiments, the nucleic acid is a DNA. In some embodiments, the nucleic acid is a RNA, for example, a mRNA.

[628] Additional exemplary therapeutic agents include, but are not limited to, antibodies (e.g., monoclonal, chimeric, humanized, nanobodies, and fragments thereof etc.), cholesterol, hormones, peptides, proteins, chemotherapeutics and other types of antineoplastic agents, low molecular weight drugs, vitamins, co-factors, nucleosides, nucleotides, oligonucleotides, enzymatic nucleic acids, antisense nucleic acids, triplex forming oligonucleotides, antisense DNA or RNA compositions, chimeric DNA:RNA compositions, allozymes, aptamers, ribozyme, decoys and analogs thereof, plasmids and other types of expression vectors, and

small nucleic acid molecules, RNAi agents, short interfering nucleic acid (siNA), messenger ribonucleic acid (messenger RNA, mRNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules, peptide nucleic acid (PNA), a locked nucleic acid ribonucleotide (LNA), morpholino nucleotide, threose nucleic acid (TNA), glycol nucleic acid (GNA), sisiRNA (small internally segmented interfering RNA), aiRNA (asymetrical interfering RNA), and siRNA with 1 , 2 or more mismatches between the sense and anti-sense strand to relevant cells and/or tissues, such as in a cell culture, subject or organism. Therapeutic agents can be purified or partially purified, and can be naturally occurring or synthetic, or chemically modified. In some embodiments, the therapeutic agent is an RNAi agent, short interfering nucleic acid (siNA), short interfering RNA (siRNA), double- stranded RNA (dsRNA), micro-RNA (miRNA), or a short hairpin RNA (shRNA) molecule. In some embodiments, the therapeutic agent is an mRNA.

[629] In some embodiments, the payload comprises one or more nucleic acid(s) (i.e., one or more nucleic acid molecular entities). In some embodiments, the nucleic acid is a single-stranded nucleic acid. In some embodiments, single-stranded nucleic acid is a DNA. In some embodiments, single- stranded nucleic acid is an RNA. In some embodiments, the nucleic acid is a double-stranded nucleic acid. In some embodiments, the double-stranded nucleic acid is a DNA. In some embodiments, the double-stranded nucleic acid is an RNA. In some embodiments, the double-stranded nucleic acid is a DNA-RNA hybrid. In some embodiments, the nucleic acid is a messenger RNA (mRNA), a microRNA, an asymmetrical interfering RNA (aiRNA), a small hairpin RNA (shRNA), or a Dicer- Substrate dsRNA.

Other lipids

[630] In some embodiments, the disclosed LNP compositions comprise a helper lipid. In some embodiments, the disclosed LNP compositions comprise a neutral lipid. In some embodiments, the disclosed LNP compositions comprise a stealth lipid. In some embodiments, the disclosed LNP compositions comprises additional lipids.

[631] As used herein, neutral lipids suitable for use in a lipid composition of the disclosure include, for example, a variety of neutral, uncharged or zwitterionic lipids. Examples of neutral phospholipids suitable for use in the present disclosure include, but are not limited to, 5- heptadecylbenzene-1,3-diol (resorcinol), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn- glycero-3- phosphocholine (DSPC), phosphocholine (DOPC), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), phosphatidylcholine (PLPC), 1,2-distearoyl- sn-glycero-3-phosphocholine (DAPC), phosphatidylethanolamine (PE), egg

phosphatidylcholine (EPC), dilauryloylphosphatidylcholine (DLPC), 1-myristoy1-2-palmitoyl phosphatidylcholine (MPPC), 1-palmitoy1-2-myristoyl phosphatidylcholine (PMPC), 1-palmitoy1-2-stearoyl phosphatidylcholine (PSPC), 1,2-diarachidoylsn- glycero-3-phosphocholine (DBPC), 1- stearoy1-2-palmitoyl phosphatidylcholine (SPPC), 1,2-dieicosenoyl-sn-glycero-3- phosphocholine (DEPC), 2-Oleoy1-1-palmitoyl-sn-glycero-3-phosphocholine (POPC), lysophosphatidyl choline, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), dilinoleoylphosphatidylcholine distearoylphosphatidylethanolamine (DSPE), dimyristoyl phosphatidylethanolamine (DMPE), dipalmitoyl phosphatidylethanolamine (DPPE), palmitoyloleoyl phosphatidylethanolamine (POPE), lysophosphatidylethanolamine and combinations thereof. In some embodiments, the neutral phospholipid is selected from the group consisting of DSPC and dimyristoyl phosphatidyl ethanolamine (DMPE). In some embodiments, the neutral phospholipid is DSPC. Neutral lipids can function to stabilize and improve processing of the LNPs.

[632] Helper lipids can refer to lipids that enhance transfection (e.g. transfection of the nanoparticle (LNP) comprising the composition as provided herein, including the biologically active agent). The mechanism by which the helper lipid enhances transfection includes enhancing particle stability. In some embodiments, the helper lipid enhances membrane fusogenicity. Helper lipids can include steroids, sterols, and alkyl resorcinols. Helper lipids suitable for use in the present disclosure can include, but are not limited to, cholesterol, 5-heptadecylresorcinol, and cholesterol hemisuccinate. In some embodiments, the helper lipid is cholesterol. In some embodiments, the helper lipid is be cholesterol hemisuccinate.

[633] Stealth lipids can refer to lipids that alter the length of time the nanoparticles can exist in vivo (e.g., in the blood). Stealth lipids can assist in the formulation process by, for example, reducing particle aggregation and controlling particle size. Stealth lipids used herein may modulate pharmacokinetic properties of the LNP. Stealth lipids suitable for use in a lipid composition of the disclosure can include, but are not limited to, stealth lipids having a hydrophilic head group linked to a lipid moiety. Stealth lipids suitable for use in a lipid composition of the present disclosure and information about the biochemistry of such lipids can be found in Romberg et al, Pharmaceutical Research, Vol. 25, No. 1, 2008, pg. 55-71 and I-Toekstra et al, Biochimica et Biophysica Acta 1660 (2004) 41-52. Additional suitable PEG lipids are disclosed, e.g., in WO 2006/007712.

[634] In some embodiments, the stealth lipid is a PEG-lipid. In one embodiment, the hydrophilic head group of stealth lipid comprises a polymer moiety selected from polymers

based on PEG (sometimes referred to as poly(ethylene oxide)), poly(oxazoline), poly(vinyl alcohol), poly(glycerol), poly(N- vinylpyrrolidone), polyaminoacids and poly N-(2-hydroxypropyl)methacrylamide]. Stealth lipids can comprise a lipid moiety. In some embodiments, the lipid moiety of the stealth lipid may be derived from diacylglycerol or diacylglycamide, including those comprising a dialkylglycerol or dialkylglycamide group having alkyl chain length independently comprising from about C4 to about C40 saturated or unsaturated carbon atoms, wherein the chain may comprise one or more functional groups such as, for example, an amide or ester. The dialkylglycerol or dialkylglycamide group can further comprise one or more substituted alkyl groups.

[635] The structures and properties of helper lipids, neutral lipids, stealth lipids, and/or other lipids are further described in W02017173054A1, W02019067999A1, US20180290965A1, US20180147298A1, US20160375134A1, US8236770, US8021686, US8236770B2, US7371404B2, US7780983B2, US7858117B2, US20180200186A1, US20070087045A1, W02018119514A1, and W02019067992A1, all of which are hereby incorporated by reference in their entirety.

LNP Formulations

[636] The LNPs described herein can be designed for one or more specific applications or targets. The elements of a nanoparticle (LNP) composition or a composition can be selected based on a particular application or target, and/or based on the efficacy, toxicity, expense, ease of use, and availability. Similarly, the particular formulation of a nanoparticle composition is a composition comprising one or more described lipids. may be selected for the particular application or target. Suitable phosphate charge neutralizers to be used in formulations include, but are not limited to, Spermidine and 1,3-propanediamine.

[637] The described LNP formulations can be designed for one or more specific applications or targets. For example, a nanoparticle composition may be designed to deliver a therapeutic agent such as an RNA to a particular cell, tissue, organ, or system or group thereof in a mammal's body. Physiochemical properties of nanoparticle compositions may be altered in order to increase selectivity for particular bodily targets. For instance, particle sizes may be adjusted based on the fenestration sizes of different organs. The therapeutic agent included in a nanoparticle composition may also be selected based on the desired delivery target or targets. For example, a therapeutic agent may be selected for a particular indication, condition, disease, or disorder and/or for delivery to a particular cell, tissue, organ, or system or group thereof (e.g., localized or specific delivery). In certain embodiments, a nanoparticle

composition may include an mRNA encoding a polypeptide of interest capable of being translated within a cell to produce the polypeptide of interest. Such a composition may be designed to be specifically delivered to a particular organ.

[638] The amount of a therapeutic agent in an LNP composition may depend on the size, composition, desired target and/or application, or other properties of the nanoparticle composition. For example, the amount of an RNA comprised in a nanoparticle composition may depend on the size, sequence, and other characteristics of the RNA. The relative amounts of a therapeutic agent and other elements (e.g., lipids) in a nanoparticle composition may also vary. In some embodiments, the wt/wt ratio of the lipid component to a therapeutic agent in a nanoparticle composition may be from about 5:1 to about 60:1, such as about 5:1. 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, 13:1, 14:1, 15:1, 16:1, 17:1, 18:1, 19:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, 50:1, and 60:1. For example, the wt/wt ratio of the lipid component to a therapeutic agent may be from about 10:1 to about 40:1. In certain embodiments, the wt/wt ratio is about 20:1. The amount of a therapeutic agent in a nanoparticle composition can be measured using absorption spectroscopy (e.g., ultraviolet-visible spectroscopy).

[639] In some embodiments, an LNP composition comprises one or more nucleic acids such as RNAs. In some embodiments, the one or more RNAs, lipids, and amounts thereof may be selected to provide a specific NIP ratio. The NIP ratio can be selected from about 1 to about 30. The N/P ratio can be selected from about 2 to about 10. In some embodiments, the NIP ratio is from about 0.1 to about 50. In some embodiments, the N/P ratio is from about 2 to about 8. hi some embodiments, the NIP ratio is from about 2 to about 15, from about 2 to about 10, from about 2 to about 8, from about 2 to about 6, from about 3 to about 15, from about 3 to about 10, from about 3 to about 8, from about 3 to about 6, from about 4 to about 15, from about 4 to about 10, from about 4 to about 8, or from about 4 to about 6. hi some embodiments, the N/P ratio is about 2, about 2.5, about 3, about 3.5, about 4, about 4.5, about 5, about 5.5, about 6, or about 6.5. In some embodiments, the NIP ratio is from about 4 to about 6. In some embodiments, the NIP ratio is about 4, about 4.5, about 5, about 5.5, or about 6.

[640] In some embodiments, the LNPs are formed with an average encapsulation efficiency ranging from about 50% to about 70%, from about 70% to about 90%, or from about 90% to about 100%. In some embodiments, the LNPs are formed with an average encapsulation efficiency ranging from about 75% to about 95%.

[641] In another aspect, provided herein is a lipid nanoparticle (LNP) comprising the composition as provided herein. As used herein, a lipid nanoparticle (LNP) composition or a

nanoparticle composition is a composition comprising one or more described lipids. LNP compositions are typically sized on the order of micrometers or smaller and may include a lipid bilayer. Nanoparticle compositions encompass lipid nanoparticles (LNPs), liposomes (e.g., lipid vesicles), and lipoplexes. In some embodiments, a LNP refers to any particle that has a diameter of less than 1000 nm, 500 nm, 250 nm, 200 nm, 150 nm, 100 nm, 75 nm, 50 nm, or 25 nm. In some embodiments, a nanoparticle may range in size from 1-1000 nm, 1-500 nm, 1-250 nm, 25-200 nm, 25-100 nm, 35-75 nm, or 25-60 nm. In some embodiments, a liposome having a lipid bilayer with a diameter of 500 rim or less. In some embodiments, the LNPs described herein can have a mean diameter of from about 1 nm to about 2500 nm, from about 10 nm to about 1500 nm, from about 20 nm to about 1000 nm, from about 30 nm to about 150 nm, from about 40 nm to about 150 nm, from about 50 rim to about 150 nm, from about 60 nm to about 130 nm, from about 70 nm to about 110 nm, from about 70 nm to about 100 nm, from about 80 nm to about 100 nm, from about 90 nm to about 100 nm, from about 70 to about 90 nm, from about 80 nm to about 90 nm- or from about 70 nm to about 80 nm. The LNPs described herein can have a mean diameter of about 30 nm, 35 nm, 40 nm, 45 nm, 50 nm, 55 nm, 60 nm, 65 nm, 70 nm, 75 nm, 80 nm, 85 nm, 90 nm, 95 nm, 100 nm, 105 nm, 110 nm, 115 nm, 120 nm, 125 nm, 130 nm, 135 nm, 140 nm, 145 nm, 150 nm, or greater. The LNPs described herein can be substantially non-toxic.

[642] In some embodiments, an LNP may be made from cationic, anionic, or neutral lipids. In some embodiments, an LNP may comprise neutral lipids, such as the fusogenic phospholipid 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) or the membrane component cholesterol, as helper lipids to enhance transfection activity and nanoparticle stability. In some embodiments, an LNP may comprise hydrophobic lipids, hydrophilic lipids, or both hydrophobic and hydrophilic lipids. Any lipid or combination of lipids that are known in the art can be used to produce an LNP. Examples of lipids used to produce LNPs include, but are not limited to DOTMA (N[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride), DOSPA (N,N-dimethyl-N-([2-sperminecarboxamido]ethyl)-2,3-bis(dioleyloxy)-1-propaniminium pentahydrochloride), DOTAP (1,2-Dioleoyl-3-trimethylammonium propane), DMRIE (N-(2-hydroxyethyl)- N,N-dimethyl-2,3-bis(tetradecyloxy-1-propanaminiumbromide), DC-cholesterol (3β-[N-(N',N'-dimethylaminoethane)-carbamoyl]cholesterol), DOTAP-cholesterol, GAP-DMORIE-DPyPE, and GL67A-DOPE-DMPE (,2-Bis(dimethylphosphino)ethane)-polyethylene glycol (PEG). Examples of cationic lipids include, but are not limited to, 98N12-5, C12-200, DLin-KC2-DMA (KC2), DLin-MC3 -DMA (MC3), XTC, MD1, and 7C1. Examples of neutral lipids

include, but are not limited to, DPSC, DPPC (Dipalmitoylphosphatidylcholine), POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine), DOPE, and SM (sphingomyelin). Examples of PEG-modified lipids include, but are not limited to, PEG-DMG (Dimyristoyl glycerol), PEG-CerC14, and PEG-CerC20. In some embodiments, the lipids may be combined in any number of molar ratios to produce a LNP. In some embodiments, the polynucleotide may be combined with lipid(s) in a wide range of molar ratios to produce an LNP.

[643] The term "substituted", unless otherwise indicated, refers to the replacement of one or more hydrogen radicals in a given structure with the radical of a specified substituent including, but not limited to: halo, alkyl, alkenyl, alkynyl, aryl, heterocyclyl, thiol, alkylthio, oxo, thioxy, arylthio, alkylthioalkyl, arylthioallcyl, alkylsulfonyl, alkylsulfonylalkyl, arylsulfonylalkyl, alkoxy, aryloxy, aralkoxy, aminocarbonyl, alkylaminocarbonyl, aiylaminocarbonyl, alkoxycarbonyl, aryloxycarbonyl, haloalkyl, amino, trifluoromethyl, cyano, nitro, alkylamino, arylamino, alkylaminoalkyl, aiylaminoalkyl, aminoalkylamino, hydroxy, alkoxyalkyl, carboxyalkyl, alkoxycarbonylalkyl, aminocarbonylalkyl, acyl, aralkoxycarbonyl, carboxylic acid, sulfonic acid, sulfonyl, phosphonic acid, aryl, heteroaryl, heterocyclic, and an aliphatic group. It is understood that the substituent may be further substituted. Exemplary• substituents include amino, alkylamino, and the like.

[644] As used herein, the term "substituent" means positional variables on the atoms of a core molecule that are substituted at a designated atom position, replacing one or more hydrogens on the designated atom, provided that the designated atom's normal valency is not exceeded, and that the substitution results in a stable compound. Combinations of substituents and/or variables are permissible only if such combinations result in stable compounds. A person of ordinal), skill in the art should note that any carbon as well as heteroatom with valences that appear to be unsatisfied as described or shown herein is assumed to have a sufficient number of hydrogen atom(s) to satisfy the valences described or shown. In certain instances one or more substituents having a double bond (e.g., "oxo" or "=O") as the point of attachment may be described, shown or listed herein within a substituent group, wherein the structure may only show a single bond as the point of attachment to the core structure of Formula (I). A person of ordinary skill in the art would understand that, while only a single bond is shown, a double bond is intended for those substituents.

[645] The term "alkyl" refers to a straight or branched hydrocarbon chain radical, having from one to twenty carbon atoms, and which is attached to the rest of the molecule by a single bond. An alkyl comprising up to 10 carbon atoms is referred to as a C_1-C_{10} alkyl,

likewise, for example, an alkyl comprising up to 6 carbon atoms is a C_1-C_6 alkyl. Alkyls (and other moieties defined herein) comprising other numbers of carbon atoms are represented similarly. Alkyl groups include, but are not limited to, C_1-C_{10} alkyl, C_1-C_9 alkyl, C_1-C_8 alkyl. C_1-C_7 alkyl, C_1-C_6 alkyl, C_1-C_5 alkyl, C_1-C_4 alkyl, C_1-C_3 alkyl, C_1-C_2 alkyl, C_2-C_8 alkyl, C_3-C_8 alkyl and C_4-C_8 alkyl. Representative alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, 1-methylethyl (i-propyl), n-butyl, i-butyl, s-butyl, n- pentyl, 1,1-dimethylethyl (t-butyl), 3-methylhexyl, 2-methylhexyl, 1-ethyl-propyl, and the like. In some embodiments, the alkyl is methyl or ethyl. In some embodiments, the alkyl is -$CH(CH_3)_2$ or -$C(CH_3)_3$. Unless stated otherwise specifically in the specification, an alkyl group may be optionally substituted as described below. "Alkylene" or "alkylene chain" refers to a straight or branched divalent hydrocarbon chain linking the rest of the molecule to a radical group. In some embodiments, the alkylene is -CI-12-, -CH_2CH_2-, or -$CH_2CH_2CH_2$-. In some embodiments, the alkylene is -CH_2-. In some embodiments, the alkylene is -CH_2CH_2-. In some embodiments, the alkylene is -$CH_2CH_2CH_2$-.

[646] The term "alkenyl" refers to a type of alkyl group in which at least one carbon-carbon double bond is present. In one embodiment, an alkenyl group has the formula -$C(R)=CR^2$, wherein R refers to the remaining portions of the alkenyl group, which may be the same or different. In some embodiments, R is H or an alkyl. In some embodiments, an alkenyl is selected from ethenyl (i.e., vinyl), propenyl (i.e., allyl), butenyl, pentenyl, pentadienyl, and the like. Non-limiting examples of an alkenyl group include -$CH=CH_2$, -$C(CH_3)=CH_2$, -$CH=CHCH_3$, -$C(CH_3)=CHCH_3$, and -$CH_2CH=CH_2$.

[647] The term "cycloalkyl" refers to a monocyclic or polycyclic non-aromatic radical, wherein each of the atoms forming the ring (i.e. skeletal atoms) is a carbon atom. In some embodiments, cycloalkyls are saturated or partially unsaturated. In some embodiments, cycloalkyls are spirocyclic or bridged compounds. In some embodiments, cycloalkyls are fused with an aromatic ring (in which case the cycloalkyl is bonded through a non-aromatic ring carbon atom). Cycloalkyl groups include groups having from 3 to 10 ring atoms. Representative cycloalkyls include, but are not limited to, cycloalkyls having from three to ten carbon atoms, from three to eight carbon atoms, from three to six carbon atoms, or from three to five carbon atoms. Monocyclic cycloalkyl radicals include, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. In some embodiments, the monocyclic cycloalkyl is cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl. In some embodiments, the monocyclic cycloalkyl is cyclopentenyl or cyclohexenyl. In some embodiments, the monocyclic cycloalkyl is cyclopenteny 1. Polycyclic radicals include, for

example, adamantyl, 1,2- dihydronaphthalenyl, 1,4-dihydronaphthalenyl, tetrainyl, decalinyl, 3,4-dihydronaphthalenyl-.l (2H)- one. spiro[2.2]pentyl, norbomyl and bicycle[1.1.1]pentyl. Unless otherwise stated specifically in the specification, a cycloalkyl group may be optionally substituted. Depending on the structure, a cycloalkyl group can be monovalent or divalent (i.e., a cycloalkylene group).

[648] The term "heterocycle" or "heterocyclic" refers to heteroaromatic rings (also known as heteroaryls) and heterocycloallcyl rings (also known as heteroalicyclic groups) that includes at least one heteroatom selected from nitrogen, oxygen and sulfur, wherein each heterocyclic group has from 3 to 12 atoms in its ring system, and with the proviso that any ring does not contain two adjacent O or S atoms. A "heterocyclyl" is a univalent group formed by removing a hydrogen atom from any ring atoms of a heterocyclic compound. In some embodiments, heterocycles are monocyclic, bicyclic, polycyclic, spirocyclic or bridged compounds. Non-aromatic heterocyclic groups (also known as heterocycloalkyls) include rings having 3 to 12 atoms in its ring system and aromatic heterocyclic groups include rings having 5 to 12 atoms in its ring system. The heterocyclic groups include benzofused ring systems. Examples of non-aromatic heterocyclic groups are pyrrolidinyl, tetrahydrofuranyl, dihydrofuranyl, tetrahydrothienyl, oxazolidinonyl, tetrahydropyranyl, dihydropyranyl, tetrahydrothiopyranyl, piperidinyl, morpholinyl. thiomorpholinyl, thioxanyl, piperazinyl, aziridinyl, azetidinyl, oxetanyl, thietanyl, homopiperidinyl, oxepanyl, thiepanyl, oxazepinyl, diazepinyl, thiazepinyl, 1,2,3,6-tetrahydropyridinyl, pyrrolin-2-yl, pyrrolin-3-yl, indolinyl, 2H-pyranyl, 4Hpyranyl, dioxanyl, 1,3-dioxolanyl, pyrazolinyl, dithianyl, dithiolanyl, dihydropyranyl, dihydrothienyl, dihydrofuranyl, pyrazolidinyl, imidazolinyl, imidazoli diny I , 3-az.abicy cl o[3. 1.0]hexany 1,3- azabicyclo[4.1.0]heptanyl, 3 h-indolyl, indolin-2-onyl, isoindolin-l-onyl, isoindoline-1,3-dionyl, 3,4- dihydroisoquinolin-1(2H)-onyl, 3,4-dihydroquinolin-2(1H)-onyl, isoindoline-1,3-dithionyl, benzo[d]oxazol-2(3H)-onyl, 1H-benzo[d]imidazol-2(3H)-onyl, benzo[d]thiazol-2(3H)-onyl, and quinolizinyl. Examples of aromatic heterocyclic groups are pyridinyl, imidazolyl, pyrimidinyl, pyrazolyl, triazolyl, pyrazinyl, tetrazolyl, futyl, thienyl, isoxazolyl, thiazolyl, oxazolyl, isothiazolyl, pyrrolyl, quinolinyl, isoquinolinyl, indolyl, benzimidazolyl, benzofuranyl, cinnolinyl, indazolyl, indolizinyl, phthalazinyl, pyridazinyl, triazinyl, isoindolyl, pteridinyl, purinyl, oxadiazolyl, thiadiazolyl, furaz.anyl, benzofuraz.anyl, benzothiophenyl, benzothiazolyl, benzoxazolyl, quinazolinyl, quinoxalinyl, naphthyridinyl, and furopyridinyl. The foregoing groups are either C-attached (or Clinked) or N-attached where such is possible. For instance, a group derived from pyrrole includes both pyrrol-1-yl (N-attached) or pyrrol-3-yl (C-attached).

Further, a group derived from imidazole includes imidazol-1-y1 or imidazol-3-yl (both N-attached) or imidazol-2-yl, imidazol-4-yl or imidazol-5-yl (all C-attached). The heterocyclic groups include benzo-fused ring systems. Non-aromatic heterocycles are optionally substituted with one or two oxo (= O) moieties, such as pyrrolidin-2-one. In some embodiments, at least one of the two rings of a bicyclic heterocycle is aromatic. In some embodiments, both rings of a bicyclic heterocycle are aromatic.

[649] The term "heterocycloalkyl" refers to a cycloalkyl group that includes at least one heteroatom selected from nitrogen, oxygen, and sulfur. Unless stated otherwise specifically in the specification, the heterocycloalkyl radical may be a monocyclic, or bicyclic ring system, which may include fused (when fused with an aryl or a heterowyl ring, the heterocycloalkyl is bonded through a non-aromatic ring atom) or bridged ring systems. The nitrogen, carbon or sulfur atoms in the heterocyclyl radical may be optionally oxidized. The nitrogen atom may be optionally quaternized. The heterocycloalkyl radical is partially or fully saturated. Examples of heterocycloalkyl radicals include, but are not limited to, dioxolanyl, thienyl[1,3]dithianyl, tetrahydroquinolyl, tetrahydroisoquinolyl, decahydroquinolyl, decahydroisoquinolyl, imidazolinyl, imidazolidinyl, isothiazolidinyl, isoxazolidinyl, morpholinyl, octahydroindolyl, octahydroisoindolyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolidinyl, oxazolidinyl, piperidinyl, piperazinyl, 4-piperidonyl, pyrrolidinyl, pyrazolidinyl, quinuclidinyl, thiazolidinyl, tetrahydrofuryl, trithianyl, tetrahydropyranyl, thiomorpholinyl, thiamorpholinyl, 1-oxothiomorpholinyl, 1,1-dioxo-thiomorpholinyl. The term heterocycloalkyl also includes all ring forms of carbohydrates, including but not limited to monosaccharides, disaccharides and oligosaccharides. Unless otherwise noted, heterocycloalkyls have from 2 to 12 carbons in the ring. In some embodiments, heterocycloalkyls have from 2 to 10 carbons in the ring. In some embodiments, heterocycloalkyls have from 2 to 10 carbons in the ring and 1 or 2 N atoms. In some embodiments, heterocycloalkyls have from 2 to 10 carbons in the ring and 3 or 4 N atoms. In some embodiments, heterocycloalkyls have from 2 to 12 carbons, 0-2 N atoms, 0-2 O atoms, 0-2 P atoms, and 0-1 S atoms in the ring. In some embodiments, heterocycloalkyls have from 2 to 12 carbons, 1-3 N atoms, 0-1 O atoms, and 0-1 S atoms in the ring. It is understood that when referring to the number of carbon atoms in a heterocycloalkyl, the number of carbon atoms in the heterocycloalkyl is not the same as the total number of atoms (including the heteroatoms) that make up the heterocycloalkyl (i.e. skeletal atoms of the heterocycloalkyl ring). Unless stated otherwise specifically in the specification, a heterocycloalkyl group may

be optionally substituted. As used herein, the term "teterocycloalkylene" can refer to a divalent heterocycloalkyl group.

[650] The term "heteroaryl" refers to an aryl group that includes one or more ring heteroatoms selected from nitrogen, oxygen and sulfur. The heteroaryl is monocyclic or bicyclic. Illustrative examples of monocyclic heteroaryls include pyridinyl, imidazolyl, pyrimidinyl, pyrazolyl, triazolyl, pyrazinyl, tetrazolyl, furyl, thienyl, isoxazolyl, thiazolyl, oxazolyl, isothiazolyl, pyrrolyl, pyridazinyl, triazinyl, oxadiazolyl, thiadiazolyl, furazanyl, indolizine, indole, benzofuran, benzothiophene, indazole, benzimidazole, purine, quinolizine, quinoline, isoquinoline, cinnoline, phthalazine, quinazoline, quinoxaline, 1,8-naphthyridine, and pteridine. Illustrative examples of monocyclic heteroaryls include pyridinyl, imidazolyl, pyrimidinyl, pyrazolyl, triazolyl, pyrazinyl, tetrazolyl, furyl, thienyl, isoxazolyl, thiazolyl, oxazolyl, isothiazolyl, pyrrolyl, pyridazinyl, triazinyl, oxadiazolyl, thiadiazolyl, and furazanyl. Illustrative examples of bicyclic heteroaryls include indolizine, indole, benzofuran, benzothiophene, indazole, benzimidazole, purine, quinolizine, quinoline, isoquinoline, cinnoline, phthalazine, quinazoline, quinoxaline, 1,8-naphthyridine, and pteridine. In some embodiments, heteroaryl is pyridinyl, pyrazinyl, pyrimidinyl, thiazolyl, thienyl, thiadiazolyl or furyl. In some embodiments, a heteroaryl contains 0-6 N atoms in the ring. In some embodiments, a heteroaryl contains 1-4 N atoms in the ring. In some embodiments, a heteroaryl contains 4-6 N atoms in the ring. In some embodiments, a heteroaryl contains 0-4 N atoms, 0-1 O atoms, 0-1 P atoms, and 0-1 S atoms in the ring. In some embodiments, a heteroaryl contains 1-4 N atoms, 0-1 O atoms, and 0-1 S atoms in the ring. In some embodiments, heteroaryl is a C_1-C_9 heteroaryl. In some embodiments, monocyclic heteroaryl is a C_1-C_5 heteroaryl. In some embodiments, monocyclic heteroaryl is a 5- membered or 6-membered heteroaryl. In some embodiments, a bicyclic heteroaryl is a C_6-C_9 heteroaryl. In some embodiments, a heteroaryl group is partially reduced to form a heterocycloalkyl group defined herein. In some embodiments, a heteroaryl group is fully reduced to form a heterocycloalkyl group defined herein.

[651] As used herein, the "N/P ratio" is the molar ratio of ionizable (e.g., in the physiological pH range) nitrogen atoms in a lipid (or lipids) to phosphate groups in a nucleic acid molecular entity (or nucleic acid molecular entities), e.g., in a nanoparticle composition comprising a lipid component and an RNA. Ionizable nitrogen atoms can include, for example, nitrogen atoms that can be protonated at about pH 1, about pH 2, about pH 3, about pH 4, about pH5, about pH 6, about pH 7, about pH 7.5, or about pH 8 or higher. The physiological pH range can include, for example, the pH range of different cellular

compartments (such as organs, tissues, and cells) and bodily fluids (such as blood, CSF, gastric juice, milk, bile, saliva, tears, and urine). In certain specific embodiments, the physiological pH range refers to the pH range of blood in a mammal, for example, from about 7.35 to about 7.45. Similarly, for phosphate charge neutralizers that have one or more ionizable nitrogen atoms, the N/P ratio can refer to a molar ratio of ionizable nitrogen atoms in the phosphate charge neutralizer to the phosphate groups in a nucleic acid. In some embodiments, ionizable nitrogen atoms refer to those nitrogen atoms that are ionizable within a pH range between 5 and 14.

[652] It is further contemplated that the LNP formulations that encapsulate the gRNAs and mRNAs drug substances described herein may be modified to include GalNac lipid formulations such as those disclosed in co-owned U.S. Patent application Serial Number 17/192,709 filed on March 4, 2021 with a parallel PCT application being filed on same date having International Application Number PCT/US21/20955. Employment of such GalNac lipid formulations are understood to be capable of enhancing LNP uptake in LDL-R deficient cells, such as those associated with heterozygous and homozygous familial hypercholesterolemia patient populations.

[653] For the payload that does not contain a phosphate group, the N/P ratio can refer to a molar ratio of ionizable nitrogen atoms in a lipid to the total negative charge in the payload. For example, the N/P ratio of an LNP composition can refer to a molar ratio of the total ionizable nitrogen atoms in the LNP composition to the total negative charge in the payload that is present in the composition.

[654] As used herein, amino lipids can contain at least one primary, secondary or tertiary amine moiety that is protonatable (or ionizable) between pH range 4 and 14. In some embodiments, the amine moiety/moieties function as the hydrophilic headgroup of the amino lipids. When most of the amine moiety(ies) of an amino lipid (or amino lipids) in a nucleic acid- lipid nanoparticle formulation is protonated at physiological pH, then the nanoparticles can be termed as cationic lipid nanoparticle (cLNP). When most of the amine moiety(ies) of an amino lipid (or amino lipids) in a nucleic acid-lipid nanoparticle formulation is not protonated at physiological pH but can be protonated at acidic pH, endosomal pH for example, can be termed as ionizable lipid nanoparticle (iLNP). The amino lipids that constitute cLNPs can be generally called cationic amino lipids (cLipids). The amino lipids that constitute iLNPs can be called ionizable amino lipids (iLipids). The amino lipid can be an iLipid or a cLipid at physiological pH.

Kits

[655] One aspect of the disclosure relates to kits including the compositions comprising a single guide RNA as provided herein, the base editor system and complex as provided herein, the composition as provided herein, or the lipid nanoparticle as provided herein for treating or preventing a condition. The kits can further include one or more additional therapeutic regimens or agents for treating or preventing a condition.

[656] Also disclosed herein, in certain embodiments, are kits and articles of manufacture for use with one or more methods described herein. Such kits include a carrier, package, or container that is compartmentalized to receive one or more containers such as vials, tubes, and the like, each of the container(s) comprising one of the separate elements to be used in a method described herein. Suitable containers include, for example, bottles, vials, syringes, and test tubes. In one embodiment, the containers are formed from a variety of materials such as glass or plastic.

[657] The articles of manufacture provided herein contain packaging materials. Examples of pharmaceutical packaging materials include, but are not limited to, blister packs, bottles, tubes, bags, containers, bottles, and any packaging material suitable for a selected formulation and intended mode of administration and treatment.

[658] For example, the container(s) include the composition of the disclosure, and optionally in addition with therapeutic regimens or agents disclosed herein. Such kits optionally include an identifying description or label or instructions relating to its use in the methods described herein.

[659] A kit typically includes labels listing contents and/or instructions for use, and package inserts with instructions for use. A set of instructions will also typically be included.

[660] In embodiments, a label is on or associated with the container. In one embodiment, a label is on a container when letters, numbers or other characters forming the label are attached, molded or etched into the container itself; a label is associated with a container when it is present within a receptacle or carrier that also holds the container, e.g., as a package insert. In one embodiment, a label is used to indicate that the contents are to be used for a specific therapeutic application. The label also indicates directions for use of the contents, such as in the methods described herein.

EXAMPLES

Introduction

[661] The present disclosure will be described in greater detail by way of specific examples. These examples are provided for illustrative purposes only and not to limit the scope of the claims provided herein. Those of skill in the art will readily recognize a variety of non-critical parameters that can be changed or modified to yield alternative embodiments according to the present disclosure.

[662] Gene-editing technologies, including CRISPR-Cas9 nucleases and CRISPR base editors, have the potential to precisely and permanently modify disease-causing genes in human patients. The demonstration of durable editing in target organs of non-human primates (NHPs) is an important and necessary step prior to *in vivo* administration of gene editors to patients in clinical trials. Herein provided is the first demonstration that CRISPR base editors delivered *in vivo* using lipid nanoparticles (LNP) can efficiently modify disease-related genes in living NHPs. In addition to providing proof-of-concept for a once-and-done approach to reduce cholesterol and treat coronary heart disease, the leading cause of death worldwide, these results demonstrate how CRISPR base editors can be productively applied to a variety of therapeutic target genes in the liver and potentially other organs.

[663] *In vivo* gene editing is an emerging new therapeutic approach to make DNA modifications in a patient's own body in organs such as the liver. Gene-editing methods include CRISPR-Cas9 and -Cas12 nucleases, CRISPR cytosine base editors, CRISPR adenine base editors, and CRISPR prime editors. The human *PCSK9* and *ANGPTL3* gene are particularly attractive potential target(s) for *in vivo* gene editing. In principle, editing of *PCSK9* could produce durable reductions in blood LDL-C levels and thereby dramatically lower one's cumulative exposure to LDL-C in contrast to all existing therapies (e.g., statins, ezetimibe, or PCSK9 inhibitors), which must be taken daily or every few weeks to months and can suffer from lack of patient adherence.

[664] *In vivo* delivery of a gene editor that can introduce a *PCSK9* or *ANGPTL3* loss-of-function mutation in the liver has the potential to offer a new kind of once-and-done therapy that offers lifelong treatment of coronary heart disease. CRISPR base editors make an attractive gene-editing modality for this purpose because they function efficiently for introducing precise targeted alterations and, in contrast to CRISPR-Cas9 and other gene-editing nucleases, minimize any deleterious consequences of introducing double-strand DNA breaks.

[665] CRISPR adenine base editors can induce targeted A→G edits in DNA (T→C on the opposing strand) and can be used to inactivate genes by disrupting start codons, splice donors (canonical GT sequence on the sense strand) or splice acceptors (canonical AG sequence on

the sense strand) at exon-intron or intron-exon boundaries, or introducing missense mutations. Adenine 8.8-m (hereafter referred to as ABE8.8) uses its core *Streptococcus pyogenes* nickase Cas9 (nSpCas9) protein with a guide RNA (gRNA) to engage a double-strand protospacer DNA sequence, flanked by an NGG protospacer-adjacent motif (PAM) sequence on its 3' end. The protospacer sequence is specified via hybridization of the first 20 bases of the gRNA with a complementary sequence on the "target" DNA strand, leaving part of the other ("non-target") strand in exposed single-strand form structure called the R-loop. The ABE base editor uses an evolved deoxyadenosine deaminase domain—fused to nSpCas9—to chemically modify an adenosine nucleoside, contained in the single-stranded DNA portion of the R-loop, into inosine and nicks the target DNA strand within the DNA:RNA heteroduplex of the R-loop. This nick biases DNA repair machinery to use the freshly deaminated strand as a template, enabling highly efficient transition mutation at the targeted site. The activity window of ABE8.8 typically ranges from positions 3 to 9 in the protospacer DNA sequence specified by the gRNA, 12 to 18 base pairs 5' of the NGG PAM (positions 21 to 23), with peak editing observed at position 6 of the protospacer. Although the mechanism of action of ABE8.8 does not involve double-strand DNA breaks, indel mutagenesis can occur low frequency.

[666] The advantages of base editing compared to CRISPR-Cas9 genome editing for gene inactivation or other types of gene alteration is noted. Standard CRISPR-Cas9 editing carries higher risks of unwanted on-target effects and off-target effects emanating from double-strand breaks (large deletions, integration of vector DNA sequences, chromosomal rearrangements, induction of p53 activity, etc.). Even the intended on-target effects—small indel mutations—have an element of unpredictability, as they can result in frameshift mutations that add varied strings of anomalous amino acids at the end of the truncated protein product, or in-frame mutations that add or remove amino acids from the protein without knocking out its function. In contrast, base editing offers a means to efficiently make precise, stereotyped changes in the genome, allowing for more reproducible alteration of gene function. Base editing mitigates unintended on-target effects by virtue of not requiring double-strand breaks but instead acting through enzymatic modification of DNA bases via the deaminase domain.

[667] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. It is not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described

with reference to the aforementioned specification, the descriptions and illustrations of the embodiments herein are not meant to be construed in a limiting sense. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is therefore contemplated that the invention shall also cover any such alternatives, modifications, variations or equivalents.

Protospacer Selection and Editing

Example 1. gRNA design for targeting PCSK9 and ANGPTL3

[668] All protospacers shown in **Table 1** and **Table 24** were chosen by two criteria. First, they matched (or very closely matched) sequences in the human, cynomolgus monkey, and/or mouse orthologs of the genes. Second, they had favorable predicted off-target profiles, as judged by the MIT Specificity Score (calculated by http://crispor.tefor.net/; minimum score of 50).

[669] Where the goal is to disrupt a gene *in vivo* for a therapeutic purpose, cytosine base editors have the advantage of being able to directly introduce stop codons into the coding sequence of the gene (nonsense mutations) by altering specific codons for glutamine (CAG→TAG, CAA→TAA), arginine (CGA→TGA), and tryptophan (TGG→TAG/TAA/TGA, with editing of cytosines on the antisense strand). In contrast, adenine base editors cannot directly introduce stop codons, as there are no A→G changes that result in nonsense mutations. Nonetheless, the more favorable off-target profile of adenine base editors, particularly with respect to gRNA-independent off-target DNA base editing, recommend the use of adenine base editors over cytosine base editors for therapeutic purposes. Similar approaches were followed to identify and select mouse/rodent specific protospacers listed in Tables 1 and 24.

[670] One strategy by which adenine base editors might be used to disrupt gene function is to edit the start codon, such as from ATG→GTG or ATG→ACG. Therefore, the resultant translation into protein will not initiate at the canonical ATG site. A second strategy by which adenine base editors might be used to disrupt gene function is to edit splice sites, whether splice donors at the 5' ends of introns or splice acceptors at the 3' ends of introns. Splice site disruption can result in the inclusion of intronic sequences in messenger RNA (mRNA)—

potentially introducing nonsense, frameshift, or in-frame indel mutations that result in premature stop codons or in insertion/deletion of amino acids that disrupt protein activity—or in the exclusion of exonic sequences, which can also introduce nonsense, frameshift, or in-frame indel mutations. Canonical splice donors comprise the DNA sequence GT on the sense strand, whereas canonical splice acceptors comprise the DNA sequence AG. Alteration of the sequence disrupts normal splicing. Splice donors can be disrupted by adenine base editing of the complementary base in the second position in the antisense strand (GT→GC), and splice acceptors can be disrupted by adenine base editing of the first position in the sense strand (AG→GG). A third strategy by which adenine base editors might be used to disrupt gene function is to introduce a missense mutation(s) into the coding region of the gene that results in production of a less functional, or non-functional protein.

[671] All gRNA spacer sequences that would permit ABE8.8 (and other ABE variants containing *Streptococcus pyogenes* Cas9, such as ABE7.10, or another Cas protein that can use the NGG PAM) to disrupt splice sites were identified, whether donors or acceptors, via A→G editing within its editing window (roughly positions 3 to 9 in the 20-nt protospacer region of DNA) were identified. Guide RNAs matching each of the protospacer sequences and otherwise conforming to the standard 100-nt *Streptococcus pyogenes* CRISPR gRNA sequence were synthesized, with each gRNA molecule having a modest degree of chemical modifications (e.g., in **Table 1**).

Table 1. Guide RNAs (SgRNA/gRNA) for Cas9 nuclease and ABE editors

Gene Target	gRNA ID	Protospacer (5'-3')	SEQ ID NO	Sequence (5'-3')	SEQ ID NO	Editor associated data
hcPCSK9	GA001	GGTGCTAGCCTTGCGTTCCG	66	GsGsUsGCUAGCCUUGCGUUCCGGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu	253	SpCas9
hcPCSK9	GA002	GGTGCTAGCCTTGCGTTCCG	66	gsgsusGCUAGCCUUGCGUUCCGGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu	253	SpCas9
hcPCSK9	GA003	GGTGCTAGCCTTGCGTTCCG	66	gsgsusGCUAGCCUUGCGUUCCGGUUUUsAGAgcuagaaauagcAAGUUsAAAAUsAAGGCUsAGUCCGUUsAUCsAacuugaaaaaguggcaccgagucggugcusususu	254	SpCas9

hcPCSK9	GA004	GGTGCTAGCCTTGCGTTCCG	66	gsgsusGCUAGCCUUGCGUUCCGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuuuuu	255	SpCas9
hcPCSK9	GA005	GGTGCTAGCCTTGCGTTCCG	66	GsGsUsGCUAGCCUUGCGUUCCGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuuuuu	255	SpCas9
hcPCSK9	GA006	GGTGCTAGCCTTGCGTTCCG	66	GsGsUsGCUAGCCUUGCGUUCCGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu	253	SpCas9
hcPCSK9	GA007	GGTGCTAGCCTTGCGTTCCG	66	gsgsusGCUAGCCUUGCGUUCCGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu	253	SpCas9
hcPCSK9	GA007/GA252	GGTGCTAGCCTTGCGTTCCG	66	gsgsusGCUAGCCUUGCGUUCCGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu	253	SpCas9
hcPCSK9	GA008	GGTGCTAGCCTTGCGTTCCG	66	gsgsusGCUAGCCUUGCGUUCCGgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcusususu	256	SpCas9
hcPCSK9	GA009	GGTGCTAGCCTTGCGTTCCG	66	gsgsusGCUAGCCUUGCGUUCCGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	253	SpCas9
mcPCSK9	GA052	CAGGTTCCATGGGATGCTCT	81	csasgsGUUCCAUGGGAUGCUCUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	55	SpCas9
mcPCK9	GA053	CAGGTTCCATGGGATGCTCT	81	csasgsGUUCCAUGGGAUGCUCUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu	55	SpCas9
mcPCSK9	GA054	CAGGTTCCATGGGATGCTCT	81	csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususu	55	SpCas9
mcPCSK9	GA055	CAGGTTCCATGGGATGCTCT	81	csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu	55	SpCas9
hcPCSK9	GA066/GA095	CCCGCACCTTGGCGCAGCGG	13	cscscsGCACCUUGGCGCAGCGGGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu	9	ABE/SpCas9

cANGPTL3	GA067/GA101	AAGATACCTGAATAACTCTC	14	asasgsAUACCUGAAUAACUCUCGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcususu	257	ABE/SpCas9
hcPCSK9	GA072	GGTGGCTCACCAGCTCCAGC	82	gsgsusGGCUCACCAGCUCCAGCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	258	ABE
hcPCSK9	GA073	GCTTACCTGTCTGTGGAAGC	67	gscsusUACCUGUCUGUGGAAGCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	259	ABE
hcPCSK9	GA074	TGCTTACCTGTCTGTGGAAG	68	usgscsUUACCUGUCUGUGGAAGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	260	ABE
hPCSK9	GA075	TTGGAAAGACGGAGGCAGCC	83	ususgsGAAAGACGGAGGCAGCCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	261	ABE
hPCSK9	GA076	GAAAGACGGAGGCAGCCTGG	84	gsasasAGACGGAGGCAGCCUGGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	262	ABE
hcPCSK9	GA077	TCCCAGGCCTGGAGTTTATT	85	uscscsCAGGCCUGGAGUUUAUUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	263	ABE
hPCSK9	GA078	AGCACCTACCTCGGGAGCTG	86	asgscsACCUACCUCGGGAGCTGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	264	ABE
hcPCSK9	GA079	CTTTCCAGGTCATCACAGTT	87	csususUCCAGGUCAUCACAGUUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	265	ABE
hcPCSK9	GA080	CCTTTCCAGGTCATCACAGT	88	cscsusUUCCAGGUCAUCACAGUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	266	ABE
hcPCSK9	GA081	TTTCCAGGTCATCACAGTTG	89	usususCCAGGUCAUCACAGUUGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCG	267	ABE

				UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu		
hPCSK9	GA082	CTTACCTG CCCCATGG GTGC	90	csususACCUGCCCCAUGGGUGCG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	268	ABE
hPCSK9	GA083	TAAGGCCC AAGGGGG CAAGC	91	usasasGGCCCAAGGGGGCAAGCG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	269	ABE
hPCSK9	GA084	CCTCTTCA CCTGCTCC TGAG	92	cscsusCUUCACCUGCUCCUGAGG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	270	ABE
hPCSK9	GA085	GCCTCTTC ACCTGCTC CTGA	93	gscscsUCUUCACCUGCUCCUGAG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	271	ABE
hPCSK9	GA086	TTCACCTG CTCCTGAG GGGC	94	ususcsACCUGCUCCUGAGGGGCG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	272	ABE
hcPCSK9	GA087	TCACCTGC TCCTGAGG GGCC	95	uscsasCCUGCUCCUGAGGGGCCG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	273	ABE
hcPCSK9	GA088	CCCAGGCT GCAGCTCC CACT	96	cscscsAGGCUGCAGCUCCCACUG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	274	ABE
hPCSK9	GA089	CCCCAGGC TGCAGCTC CCAC	97	cscscsCAGGCUGCAGCUCCCACG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	275	ABE
hPCSK9	GA090	GCAGGTG ACCGTGGC CTGCG	98	gscsasGGUGACCGUGGCCUGCGG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	276	ABE
hANGPTL3	GA091	AAGATACC TGAATAAC CCTC	15	asasgsAUACCUGAAUAACCCUCG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	59	ABE

hcANGPTL3	GA092	CTCCTTTAGGAGGCTGGTGG	99	csuscsCUUUAGGAGGCUGGUGGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	277	ABE
hANGPTL3	GA093	TTTTCAGGAGAATTTTGGTT	100	usususUCAGGAGAAUUUUGGUUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	278	ABE
hANGPTL3	GA094/GA153	CTTTTCAGGAGAATTTTGGT	101	csususUUCAGGAGAAUUUUGGUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	279	ABE
hcPCSK9	GA095 / GA066	CCCGCACCTTGGCGCAGCGG	13	cscscsGCACCUUGGCGCAGCGGGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu	9	ABE
hcPCSK9	GA096	CCCGCACCTTGGCGCAGCGG	13	cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususu	9	ABE
hcPCSK9	GA097	CCCGCACCTTGGCGCAGCGG	13	cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu	9	ABE
hANGPTL3	GA098	AAGATACCTGAATAACCCTC	15	asasgsAUACCUGAAUAACCCUCGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu	59	ABE
hANGPTL3	GA099	AAGATACCTGAATAACCCTC	15	asasgsAUACCUGAAUAACCCUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususu	59	ABE
hANGPTL3	GA100	AAGATACCTGAATAACCCTC	15	asasgsAUACCUGAAUAACCCUCgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu	59	ABE
cANGPTL3	GA101/GA067	AAGATACCTGAATAACTCTC	14	asasgsAUACCUGAAUAACUCUCGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu	257	ABE
cANGPTL3	GA102	AAGATACCTGAATAACTCTC	14	asasgsAUACCUGAAUAACUCUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususu	257	ABE
cANGPTL3	GA103	AAGATACCTGAATAACTCTC	14	asasgsAUACCUGAAUAACUCUCgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu	257	ABE

hANGPTL3	GA104	TAATTTGGCCCTTCGTCTTA	102	usasasUUUGGCCCUUCGUCUUAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	280	ABE
hANGPTL3	GA105	AGACTTTGTCCATAAGACGA	103	asgsasCUUUGUCCAUAAGACGAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	281	ABE
hANGPTL3	GA106	GACTTTGTCCATAAGACGAA	104	gsascsUUUGUCCAUAAGACGAAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	282	ABE
hcANGPTL3	GA107	AGCCAATGGCCTCCTTCAGT	105	asgscsCAAUGGCCUCCUUCAGUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	283	SpCas9
hcANGPTL3	GA108	TCCCAACTGAAGGAGGCCAT	106	uscscsCAACUGAAGGAGGCCAUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	284	SpCas9
hcANGPTL3	GA109	GGCCTCCTTCAGTTGGGACA	107	gsgscsCUCCUUCAGUUGGGACAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	285	SpCas9
hcANGPTL3	GA110	GACCATGTCCCAACTGAAGG	108	gsascsCAUGUCCCAACUGAAGGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	286	SpCas9
hcANGPTL3	GA111	GCCAATGGCCTCCTTCAGTT	109	gscscsAAUGGCCUCCUUCAGUUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	287	SpCas9
hANGPTL3	GA112	ATTGTCTTGATCAATTCTGG	110	asususGUCUUGAUCAAUUCUGGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	288	ABE/SpCas9
hcANGPTL3	GA113	ATTCTGGAGGAAATAACTAG	111	asususCUGGAGGAAAUAACUAGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	289	ABE/SpCas9
hANGPTL3	GA114	TCTGGGTGTTCTGGAGTTTC	112	uscsusGGGUGUUCUGGAGUUUCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCG	290	SpCas9

				UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu		
hcAN GPTL3	GA115	AACATAGC AAATCTTG ATTT	113	asascsAUAGCAAAUCUUGAUUU GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	291	ABE/ SpCas 9
hcAN GPTL3	GA116	GTAGAATT TTTTCTTC TAGG	114	gsusasGAAUUUUUUCUUCUAGG GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	292	ABE/ SpCas 9
hcAN GPTL3	GA117	ACTACAAG TCAAAAAT GAAG	115	ascsusACAAGUCAAAAAUGAAG GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	293	ABE/ SpCas 9
hANG PTL3	GA118	TATATTGG TCTTCCAC GGTC	116	usasusAUUGGUCUUCCACGGUCG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	294	ABE/ SpCas 9
hANG PTL3	GA119	CAAAGAC CTTCTCCA GACCG	117	csasasAGACCUUCUCCAGACCGG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	295	ABE/ SpCas 9
hANG PTL3	GA120	GGTCTTCC ACGGTCTG GAGA	118	gsgsusCUUCCACGGUCUGGAGA GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	296	ABE/ SpCas 9
hANG PTL3	GA121	TTGTTTAT ATTGGTCT TCCA	119	ususgsUUUAUAUUGGUCUUCCA GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	297	ABE/ SpCas 9
hcAN GPTL3	GA122	CTTTTATT TGACTATG CTGT	120	csususUUAUUUGACUAUGCUGU GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	298	ABE/ SpCas 9
hANG PTL3	GA123	AAAGTCTG GATATAGA GAGT	121	asasasGUCUGGAUAUAGAGAGU GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	299	ABE/ SpCas 9
hANG PTL3	GA124	GTTGGTTT AATTGTTT ATAT	122	gsususGGUUUAAUUGUUUAUAU GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	300	ABE/ SpCas 9

hcAN GPTL3	GA125	TGATGGTA AGACACTT TGGT	123	usgsasUGGUAAGACACUUUGGU GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	301	ABE/ SpCas 9
hcAN GPTL3	GA126	GGAGTAGT TCTTGGTG CTCT	124	gsgsasGUAGUUCUUGGUGCUCU GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	302	ABE/ SpCas 9
hcAN GPTL3	GA127	AACATGAT GGTAAGA CACTT	125	asascsAUGAUGGUAAGACACUU GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	303	ABE/ SpCas 9
hcAN GPTL3	GA128	TGAAGAA AGGGAGT AGTTCT	126	usgsasAGAAAGGGAGUAGUUCU GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	304	ABE/ SpCas 9
hcAN GPTL3	GA129	AGTTCTTG GTGCTCTT GGCT	127	asgsusUCUUGGUGCUCUUGGCU GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	305	ABE/ SpCas 9
hcAN GPTL3	GA130	ATGATGGT AAGACACT TTGG	128	asusgsAUGGUAAGACACUUUGG GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	306	ABE/ SpCas 9
hcAN GPTL3	GA131	GAAGATA GAGAAATT TCTGT	129	gsasasGAUAGAGAAAUUUCUGU GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	307	ABE/ SpCas 9
hcAN GPTL3	GA132	GGAAGAT AGAGAAA TTTCTG	130	gsgsasAGAUAGAGAAAUUUCUG GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	308	ABE/ SpCas 9
hANG PTL3	GA133	TATTTCAT TCAACTGA AGAA	131	usasusUUCAUUCAACUGAAGAA GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	309	ABE/ SpCas 9
hANG PTL3	GA134	ATTTCATT CAACTGAA GAAA	132	asususUCAUUCAACUGAAGAAA GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	310	ABE/ SpCas 9
hANG PTL3	GA135	GTCTACTG TGATGTTA TATC	133	gsuscsUACUGUGAUGUUAUAUC GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG	311	ABE/ SpCas 9

				UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu		
hANGPTL3	GA136	TAAATGGTGGTACATTCAGC	134	usasasAUGGUGGUACAUUCAGC GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	312	ABE/SpCas9
hcANGPTL3	GA137	TATCAGGTAAAACCTGTCTA	135	usasusCAGGUAAAACCUGUCUA GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	313	ABE/SpCas9
hANGPTL3	GA138	TGTACCACCATTTATAACAG	136	usgsusACCACCAUUUAUAACAG GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	314	ABE/SpCas9
hANGPTL3	GA139	TTCACCTCTGTTATAAATGG	137	ususcsACCUCUGUUAUAAAUGG GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	315	ABE/SpCas9
hANGPTL3	GA140	AACAGAGGTGAACATACAAG	138	asascsAGAGGUGAACAUACAAG GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	316	ABE/SpCas9
hcANGPTL3	GA141	TTGAGAGTTGCTGGGTCTGA	139	ususgsAGAGUUGCUGGGUCUGA GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	317	ABE/SpCas9
hcANGPTL3	GA142	TGAAAAACTTGAGAGTTGCT	140	usgsasAAAACUUGAGAGUUGCU GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	318	SpCas9
hcANGPTL3	GA143	TTAATTCAACATCGAATAGA	141	ususasAUUCAACAUCGAAUAGA GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	319	ABE/SpCas9
hANGPTL3	GA144	TTTGGGAGGCTTGATGGTAA	142	usususGGGAGGCUUGAUGGUAA GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	320	ABE/SpCas9
hcANGPTL3	GA145	CATTATATTCAGGTAGTCCA	143	csasusUAUAUUCAGGUAGUCCA GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	321	ABE/SpCas9

hANGPTL3	GA146	TTGGGAGGCTTGATGGTAAG	144	ususgsGGAGGCUUGAUGGUAAGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	322	ABE/SpCas9
hANGPTL3	GA147	TTTTGGGAGGCTTGATGGTA	145	usususUGGGAGGCUUGAUGGUAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	323	ABE/SpCas9
hcANGPTL3	GA148	ACAAAACTTCAATGAAACGT	146	ascsasAAACUUCAAUGAAACGUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	324	ABE/SpCas9
hANGPTL3	GA149	TATGGTTTTGGGAGGCTTGA	147	usasusGGUUUUGGGAGGCUUGAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	325	ABE/SpCas9
hANGPTL3	GA150	ACTACAAATATGGTTTTGGG	148	ascsusACAAAUAUGGUUUUGGGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	326	ABE/SpCas9
hcANGPTL3	GA151	GAGAACTACAAATATGGTTT	149	gsasgsAACUACAAAUAUGGUUUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	327	ABE/SpCas9
hANGPTL3	GA152	AGGACACTTCAACTGTCCAG	150	asgsgsACACUUCAACUGUCCAGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	328	ABE/SpCas9
hANGPTL3	GA094/GA153	CTTTTCAGGAGAATTTTGGT	101	csususUUCAGGAGAAUUUUGGUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	279	ABE/SpCas9
hcPCSK9	GA156	GGTGCTAGCCTTGCGTTCCG	66	gsgsusGCUAGCCUUGCGUUCCGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	253	SpCas9
hcPCSK9	GA157	GCCGTCCTCCTCGGAACGCA	151	gscscsGUCCUCCUCGGAACGCAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	329	SpCas9
hcPCSK9	GA158	GCTAGCCTTGCGTTCCGAGG	152	gscsusAGCCUUGCGUUCCGAGGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGU	330	SpCas9

				UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu		
hcPCSK9	GA159	GCGTTCCGAGGAGGACGGCC	153	gscsgsUUCCGAGGAGGACGGCCG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	331	SpCas9
hcPCSK9	GA160	GCCTTGCGTTCCGAGGAGGA	154	gscscsUUGCGUUCCGAGGAGGA GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	332	SpCas9
hcPCSK9	GA161	GGACGAGGACGGCGACTACG	155	gsgsasCGAGGACGGCGACUACGG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	333	SpCas9
hcPCSK9	GA162	GGACGGCGACTACGAGGAGC	156	gsgsasCGGCGACUACGAGGAGCG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	334	SpCas9
hcPCSK9	GA163	CGTCCTCGTCCTCCTGCGCA	157	csgsusCCUCGUCCUCCUGCGCAG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	335	SpCas9
hcPCSK9	GA164	GTCCTCGTCCTCCTGCGCAC	158	gsuscsCUCGUCCUCCUGCGCACG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	336	SpCas9
hcPCSK9	GA165	CCGTCAGCTCCAGGCGGTCC	159	cscsgsUCAGCUCCAGGCGGUCCG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	337	SpCas9
hcPCSK9	GA166	CGCCCGTGCGCAGGAGGACG	160	csgscsCCGUGCGCAGGAGGACGG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	338	SpCas9
hcPCSK9	GA167	TCAGCTCCAGGCGGTCCTGG	161	uscsasGCUCCAGGCGGUCCUGGG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	339	SpCas9
hcPCSK9	GA168	CTTGGCGCAGCGGTGGAAGG	162	csususGGCGCAGCGGUGGAAGG GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	340	SpCas9

hcPCSK9	GA169	CGTGCGCAGGAGGACGAGGA	163	csgsusGCGCAGGAGGACGAGGAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	341	SpCas9
hcPCSK9	GA170	CCAGGACCGCCTGGAGCTGA	164	cscsasGGACCGCCUGGAGCUGAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	342	SpCas9
hcPCSK9	GA171	TCTTGGTGAGGTATCCCCGG	165	uscsusUGGUGAGGUAUCCCCGGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	343	SpCas9
hcPCSK9	GA172	CAGTGCGCTCTGACTGCGAG	166	csasgsUGCGCUCUGACUGCGAGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	344	SpCas9
hcPCSK9	GA173	CTTGGTGAGGTATCCCCGGC	167	csususGGUGAGGUAUCCCCGGCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	345	SpCas9
hcPCSK9	GA174	GGATCTTGGTGAGGTATCCC	168	gsgsasUCUUGGUGAGGUAUCCCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	346	SpCas9
hcPCSK9	GA175	GAAGATGAGTGGCGACCTGC	169	gsasasGAUGAGUGGCGACCUGCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	347	SpCas9
hcPCSK9	GA176	AGCACCACCACGTAGGTGCC	170	asgscsACCACCACGUAGGUGCCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	348	SpCas9
hcPCSK9	GA177	GGTCGCCACTCATCTTCACC	171	gsgsusCGCCACUCAUCUUCACCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	349	SpCas9
hcPCSK9	GA178	CTCCTTCAGCACCACCACGT	172	csuscsCUUCAGCACCACCACGUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	350	SpCas9
hcPCSK9	GA179	GAGTGGCGACCTGCTGGAGC	173	gsasgsUGGCGACCUGCUGGAGCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGU	351	SpCas9

				UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu		
hcPCSK9	GA180	GCGCACTGCCCGCCGCCCTGC	174	gscsgsCACUGCCCGCCGCCUGCG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	352	SpCas9
hcPCSK9	GA181	GGCTTCCTGGTGAAGATGAG	175	gsgscsUUCCUGGUGAAGAUGAG GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	353	SpCas9
hcPCSK9	GA182	CACCTACGTGGTGGTGCTGA	176	csascsCUACGUGGUGGUGCUGAG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	354	SpCas9
hcPCSK9	GA183	CTACGTGGTGGTGCTGAAGG	177	csusasCGUGGUGGUGCUGAAGG GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	355	SpCas9
hcPCSK9	GA184	ATGGAAGACATGCAGGATCT	178	asusgsGAAGACAUGCAGGAUCU GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	356	SpCas9
hcPCSK9	GA185	AGACATGCAGGATCTTGGTG	179	asgsasCAUGCAGGAUCUUGGUG GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	357	SpCas9
hcPCSK9	GA186	CTCATCTTCACCAGGAAGCC	180	csuscsAUCUUCACCAGGAAGCCG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	358	SpCas9
hcPCSK9	GA187	CTCCTCGATGTAGTCGACAT	181	csuscsCUCGAUGUAGUCGACAUG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	359	SpCas9
hcPCSK9	GA188	TATTCATCCGCCCGGTACCG	182	usasusUCAUCCGCCCGGUACCGG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	360	SpCas9
hcPCSK9	GA189	TCCTCGATGTAGTCGACATG	183	uscscsUCGAUGUAGUCGACAUG GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	361	SpCas9

hcPCSK9	GA190	CCTCCTCGATGTAGTCGACA	184	cscsusCCUCGAUGUAGUCGACAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	362	SpCas9
hcPCSK9	GA191	GCCCCATGTCGACTACATCG	185	gscscsCCAUGUCGACUACAUCGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	363	SpCas9
hcPCSK9	GA192	CCATGTCGACTACATCGAGG	186	cscsasUGUCGACUACAUCGAGGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	364	SpCas9
hcPCSK9	GA193	GGGGCTGGTATTCATCCGCC	187	gsgsgsGCUGGUAUUCAUCCGCCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	365	SpCas9
hcPCSK9	GA194	GTCGACATGGGGCAACTTCA	188	gsuscsGACAUGGGGCAACUUCAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	366	SpCas9
hcPCSK9	GA195	ACCACCGGGAAATCGAGGGC	189	ascscsACCGGGAAAUCGAGGGCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	367	SpCas9
hcPCSK9	GA196	GAGTGACCACCGGGAAATCG	190	gsasgsUGACCACCGGGAAAUCGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	368	SpCas9
hcPCSK9	GA197	AGTGACCACCGGGAAATCGA	191	asgsusGACCACCGGGAAAUCGAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	369	SpCas9
hcPCSK9	GA198	CCACCGGGAAATCGAGGGCA	192	cscsasCCGGGAAAUCGAGGGCAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	370	SpCas9
hcPCSK9	GA199	GAAGCGGGTCCCGTCCTCCT	193	gsasasGCGGGUCCCGUCCUCCUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	371	SpCas9
hcPCSK9	GA200	CTAGGAGATACACCTCCACC	194	csusasGGAGAUACACCUCCACCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGU	372	SpCas9

				UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu		
hcPCSK9	GA201	CAGCATACAGAGTGACCACC	195	csasgsCAUACAGAGUGACCACCG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	373	SpCas9
hcPCSK9	GA202	AAGCGGGTCCCGTCCTCCTC	196	asasgsCGGGUCCCGUCCUCCUCG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	374	SpCas9
hcPCSK9	GA203	TGACCCTGCCCTCGATTTCC	197	usgsasCCCUGCCCUCGAUUUCCG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	375	SpCas9
hcPCSK9	GA204	CACTCTGTATGCTGGTGTCT	198	csascsUCUGUAUGCUGGUGUCUG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	376	SpCas9
hcPCSK9	GA205	CCCTGCCCTCGATTTCCCGG	199	cscscsUGCCCUCGAUUUCCCGGG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	377	SpCas9
hcPCSK9	GA206	CCGGTGGTCACTCTGTATGC	200	cscsgsGUGGUCACUCUGUAUGCG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	378	SpCas9
hcPCSK9	GA207	GGAAATCGAGGGCAGGGTCA	201	gsgsasAAUCGAGGGCAGGGUCA GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	379	SpCas9
hcPCSK9	GA208	CCAGCATACAGAGTGACCAC	202	cscsasGCAUACAGAGUGACCACG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	380	SpCas9
hcPCSK9	GA209	CTTGGCAGTTGAGCACGCGC	203	csususGGCAGUUGAGCACGCGCG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	381	SpCas9
hcPCSK9	GA210	CTGCGCGTGCTCAACTGCCA	204	csusgsCGCGUGCUCAACUGCCAG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	382	SpCas9

hcPCSK9	GA211	TGCGCGTGCTCAACTGCCAA	205	usgscsGCGUGCUCAACUGCCAAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	383	SpCas9
hcPCSK9	GA212	CGGGATGCCGGCGTGGCCAA	206	csgsgsGAUGCCGGCGUGGCCAAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	384	SpCas9
hcPCSK9	GA213	CGTGCTCAACTGCCAAGGGA	207	csgsusGCUCAACUGCCAAGGGAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	385	SpCas9
hcPCSK9	GA214	CCTTGGCCACGCCGGCATCC	208	cscsusUGGCCACGCCGGCAUCCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	386	SpCas9
hcPCSK9	GA215	CAGCGGCCGGGATGCCGGCG	209	csasgsCGGCCGGGAUGCCGGCGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	387	SpCas9
hcPCSK9	GA216	CCGGGATGCCGGCGTGGCCA	210	cscsgsGGAUGCCGGCGUGGCCAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	388	SpCas9
hcPCSK9	GA217	GTGGTCAGCGGCCGGGATGC	211	gsusgsGUCAGCGGCCGGGAUGCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	389	SpCas9
hcPCSK9	GA218	CGCTGACCACCCCTGCCAGG	212	csgscsUGACCACCCCUGCCAGGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	390	SpCas9
hcPCSK9	GA219	GGCAGGGGTGGTCAGCGGCC	213	gsgscsAGGGGUGGUCAGCGGCCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	391	SpCas9
hcPCSK9	GA220	GTGCTCAACTGCCAAGGGAA	214	gsusgsCUCAACUGCCAAGGGAAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	392	SpCas9
hPCSK9	GA221	TCATGGCACCCACCTGGCAG	215	uscsasUGGCACCCACCUGGCAGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGU	393	SpCas9

				UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu		
hcPCSK9	GA222	TGGCAGGGGTGGTCAGCGGC	216	usgsgsCAGGGGUGGUCAGCGGC GUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu	394	SpCas9
hcPCSK9	GA223	GCTGACCACCCCTGCCAGGT	217	gscsusGACCACCCCUGCCAGGUG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	395	SpCas9
hcPCSK9	GA224	GGCCGCTGACCACCCCTGCC	218	gsgscsCGCUGACCACCCCUGCCG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	396	SpCas9
hcPCSK9	GA225	GGCATCGTCCCGGAAGTTGC	219	gsgscsAUCGUCCCGGAAGUUGCG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	397	SpCas9
hcPCSK9	GA226	GGCTTTTCCGAATAAACTCC	220	gsgscsUUUUCCGAAUAAACUCCG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	398	SpCas9
hcPCSK9	GA227	GTCCCGGAAGTTGCCGGCAG	221	gsuscsCCGGAAGUUGCCGGCAGG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	399	SpCas9
hcPCSK9	GA228	CGGCTGTACCCACCCGCCAG	222	csgsgsCUGUACCCACCCGCCAGG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	400	SpCas9
hcPCSK9	GA229	GTCGTGCTGGTCACCGCTGC	223	gsuscsGUGCUGGUCACCGCUGCG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	401	SpCas9
hcPCSK9	GA230	AGTAGAGGCAGGCATCGTCC	224	asgsusAGAGGCAGGCAUCGUCCG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	402	SpCas9
hcPCSK9	GA231	TCCGAATAAACTCCAGGCCT	225	uscscsGAAUAAACUCCAGGCCUG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	403	SpCas9

hcPCSK9	GA232	GTTTATTCGGAAAAGCCAGC	226	gsususUAUUCGGAAAAGCCAGCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	404	SpCas9
hPCSK9	GA233	GCGGCTGTACCCACCCGCCA	227	gscsgsGCUGUACCCACCCGCCAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	405	SpCas9
hcPCSK9	GA235	CACCGCTGCCGGCAACTTCC	228	csascsCGCUGCCGGCAACUUCCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	406	SpCas9
hcPCSK9	GA236	AGCCCTCGCCAGGCGCTGGC	229	asgscsCCUCGCCAGGCGCUGGCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	407	SpCas9
hcPCSK9	GA237	CAACGCCGCCTGCCAGCGCC	230	csasasCGCCGCCUGCCAGCGCCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	408	SpCas9
hcPCSK9	GA238	GCACGACCCCAGCCCTCGCC	231	gscsasCGACCCCAGCCCUCGCCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	409	SpCas9
hcPCSK9	GA239	CCGCCTGCCAGCGCCTGGCG	232	cscsgsCCUGCCAGCGCCUGGCGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	410	SpCas9
hcPCSK9	GA240	TGCCAGCGCCTGGCGAGGGC	233	usgscsCAGCGCCUGGCGAGGGCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	411	SpCas9
hcPCSK9	GA241	TGCTGCTGCCCCTGGCGGGT	234	usgscsUGCUGCCCCUGGCGGGUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	412	SpCas9
hcPCSK9	GA242	TCACCGCTGCCGGCAACTTC	235	uscsasCCGCUGCCGGCAACUUCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	413	SpCas9
hcPCSK9	GA243	GGCGAGGGCTGGGGTCGTGC	236	gsgscsGAGGGCUGGGGUCGUGCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCG	414	SpCas9

				UUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUsususu		
hcPCSK9	GA244	TTCCGAATAAACTCCAGGCC	237	ususcsCGAAUAAACUCCAGGCCG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	415	SpCas9
hcPCSK9	GA245	GTGCTGCTGCCCCTGGCGGG	238	gsusgsCUGCUGCCCCUGGCGGGG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	416	SpCas9
hcPCSK9	GA246	GCCAGCGCCTGGCGAGGGCT	239	gscscsAGCGCCUGGCGAGGGCUG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	417	SpCas9
hcPCSK9	GA247	CCTCGCCAGGCGCTGGCAGG	240	cscsusCGCCAGGCGCUGGCAGGG UUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCA CCGAGUCGGUGCUsususu	418	SpCas9
hcPCSK9	GA248	GGTGCTAGCCTTGCGTTCCG	66	gsgsusG(cPACE)UAGCCUUGCGU UCCGGUUUUAGAgcuagaaauagcA AGUUAAAAUAAGGCUAGUCCG UUAUCAacuugaaaaaguggcaccgaguc ggugcusususu	419	SpCas9
hcPCSK9	GA249	GGTGCTAGCCTTGCGTTCCG	66	gsgsusGCUAGCC(uPACE)UGCGU UCCGGUUUUAGAgcuagaaauagcA AGUUAAAAUAAGGCUAGUCCG UUAUCAacuugaaaaaguggcaccgaguc ggugcusususu	420	SpCas9
hcPCSK9	GA250	GGTGCTAGCCTTGCGTTCCG	66	gsgsusGcUAGCCUUGCGUUCCGG UUUUAGAgcuagaaauagcAAGUUA AAAUAAGGCUAGUCCGUUAUC Aacuugaaaaaguggcaccgagucggugcusu susu	253	SpCas9
hcPCSK9	GA251	GGTGCTAGCCTTGCGTTCCG	66	gsgsusGCUAGCCuUGCGUUCCGG UUUUAGAgcuagaaauagcAAGUUA AAAUAAGGCUAGUCCGUUAUC Aacuugaaaaaguggcaccgagucggugcusu susu	253	SpCas9
hcPCSK9	GA253	GGTGCTAGCCTTGCGTTCCG	66	gsgsusG(cPACE)UAGCCUUGCGU UCCGgUUUUAGagcuagaaauagcaaG UUaAaAuAaggcuaGUccGUUAucA AcuugaaaaagugGcaccgagucggugcusu susu	419	SpCas9
hcPCSK9	GA254	GGTGCTAGCCTTGCGTTCCG	66	gsgsusGCUAGCC(uPACE)UGCGU UCCGgUUUUAGagcuagaaauagcaaG UUaAaAuAaggcuaGUccGUUAucA AcuugaaaaagugGcaccgagucggugcusu susu	420	SpCas9

mPCSK9	GA255	CCCATACCTTGGAGCAACGG	69	cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususu	421	ABE
mPCSK9	GA256	CCCATACCTTGGAGCAACGG	69	cscscsAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu	421	ABE
mPCSK9	GA257	CCCATACCTTGGAGCAACGG	69	cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu	421	ABE
mANGPTL3	GA258	GAGATACCTGAGTAACTTTC	241	gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususu	422	ABE
mANGPTL3	GA259	GAGATACCTGAGTAACTTTC	241	gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu	422	ABE
mANGPTL3	GA260	GAGATACCTGAGTAACTTTC	241	gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu	422	ABE
hcANGPTL3	GA276	ACGTGGGAGAACTACAAATA	242	ascsgsUGGGAGAACUACAAAUAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	423	ABE
hcANGPTL3	GA277	CGATGTTGAATTAATGTCCA	243	csgsasUGUUGAAUUAAUGUCCAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	424	ABE
hcANGPTL3	GA278	CACAAAACTTCAATGAAACG	244	csascsAAAACUUCAAUGAAACGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	425	ABE
hcANGPTL3	GA279	TACGAATTGAGTTGGAAGAC	245	usascsGAAUUGAGUUGGAAGACGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	426	ABE
hcANGPTL3	GA284	CTATGGAGTATATCTTCTCT	246	csusasUGGAGUAUAUCUUCUCUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	427	ABE
hcPCSK9	GA343	CCCGCACCTTGGCGCAGCGG	13	cscscsGCACCUUGGCGCAGCGGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	9	ABE

cANGPTL3	GA344	AAGATACCTGAATAACTCTC	14	asasgsAUACCUGAAUAACUCUCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	257	ABE
hcPCSK9	GA346	CCCGCACCTTGGCGCAGCGG	13	cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu	9	ABE
cANGPTL3	GA347	AAGATACCTGAATAACTCTC	14	asasgsAUACCUGAAUAACUCUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu	257	ABE
mANGPTL3	GA349	GAGATACCTGAGTAACTTTC	241	gsasgsAUACCUGAGUAACUUUCGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu	422	ABE
mANGPTL3	GA353	GAGATACCTGAGTAACTTTC	241	gsasgsAUACCUGAGUAACUUUCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	422	ABE
hcPCSK9	GA375	CCCGCACCTTGGCGCAGCGG	13	cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAacAAcgggaaccgugGcaccgagucggugcusususu	428	ABE
hcPCSK9	GA376	CCCGCACCTTGGCGCAGCGG	13	cscscsdGCdACdCUdUGdGCdGCdAGdCGdGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu	429	ABE
hcPCSK9	GA377	CCCGCACCTTGGCGCAGCGG	13	cscscsGdCAdCCdUUdGGdCGdCAdGCdGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu	430	ABE
hcPCSK9	GA380	CCCGCACCTTGGCGCAGCGG	13	cscscsGdCACCUUGGCGdCAGCGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu	431	ABE
hcPCSK9	GA381	CCCGCACCTTGGCGCAGCGG	13	cscscsGCdACCUUGGCGCdAGCGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu	432	ABE
hcPCSK9	GA382	CCCGCACCTTGGCGCAGCGG	13	cscscsGCACCUdUGGdCGCAGdCGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu	433	ABE
hcPCSK9	GA383	CCCGCACCTTGGCGCAGCGG	13	cscscsGCACCUUdGGCdGCAGCdGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu	434	ABE

hcPCSK9	GA384	CCCGCACCTTGGCGCAGCGG	13	cscscsdGdCdACCdUdUGGdCGCAdGCGdGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu	435	ABE
hcPCSK9	GA385	CCCGCACCTTGGCGCAGCGG	13	cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuuuu	11	ABE
hcPCSK9	GA386	CCCGCACCTTGGCGCAGCGG	13	cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuuUu	11	ABE
hcPCSK9	GA387	CCCGCACCTTGGCGCAGCGG	13	cscscsGCACCUUGGCGCAGCGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuusususuuu	436	ABE
hcPCSK9	GA388	CCCGCACCTTGGCGCAGCGG	13	cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUCcGUUAucAAcuugaaaaaguggcaccgagucggugcusususu	9	ABE
hcPCSK9	GA389	CCCGCACCTTGGCGCAGCGG	13	cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGuCcGUUaucAAcuugaaaaaguggcaccgagucggugcusususu	9	ABE
hcPCSK9	GA391	CCGCACCTTGGCGCAGCGG	247	cscsGCACCUUGGCGCAGCGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu	437	ABE
hANGPTL3	GA441	AAGATACCTGAATAACCCTC	15	asasgsAUACCUGAAUAACCCUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu	59	ABE
hANGPTL3	GA442	AAGATACCTGAATAACCCTC	15	asasgsAUACCUGAAUAACCCUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUUu	438	ABE
hANGPTL3	GA472	AAGATACCTGAATAACCCTC	15	asasisAUACCUGAAUAACCCUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu	439	ABE
hANGPTL3	GA473	AAGATACCTGAATAACCCTC	15	asasisAUACCUIAAUAACCCUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu	440	ABE
hANGPTL3	GA474	AAGATACCTGAATAACCCTC	15	asasgsAUACCUIAAUAACCCUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu	441	ABE
hANGPTL3	GA475	AGATACCTGAATAACCCTC	248	asgsasUACCUGAAUAACCCUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu	442	ABE

hANGPTL3	GA476	GATACCTGAATAACCCTC	249	gsasUACCUGAAUAACCCUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu	443	ABE
hANGPTL3	GA477	ATACCTGAATAACCCTC	250	asusACCUGAAUAACCCUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu	444	ABE
hANGPTL3	GA547	AAGATACCTGAATAACTCTC	14	asasgsAUACCUGAAUAACUCUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguggcaccgagucggugcusususuUUu	445	ABE
mPCSK9	GA010	GGCTGATGAGGCCGCACATG	251	5’gsgscsUGAUGAGGCCGCACAUGGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3’	446	SpCas9
mPCSK9	GA011	GGCTGATGAGGCCGCACATG	251	5’gsgscsUGAUGAGGCCGCACAUGGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3’	446	SpCas9
hcANGPTL3	GA016	GGCCTCCTTCAGTTGGGACA	107	5’gsgscsCUCCUUCAGUUGGGACAGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3’	285	SpCas9
hcANGPTL3	GA017	GCCAATGGCCTCCTTCAGTT	109	5’gscscsAAUGGCCUCCUUCAGUUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3’	287	SpCas9
hcPCSK9	GA395	TCAGCTCCAGGCGGTCCTGG	161	5’uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuuuu-3’	447	SpCas9
hcPCSK9	GA396	TCAGCTCCAGGCGGTCCTGG	161	5’uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuuuu-3’	448	SpCas9
hcPCSK9	GA397	TCAGCTCCAGGCGGTCCTGG	161	5’uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuuuu-3’	449	SpCas9
hcPCSK9	GA398	TCAGCTCCAGGCGGTCCTGG	161	5’uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUCcGUUAucAAcuugaaaaaguggcaccgagucggugcusususuuu-3’	447	SpCas9

hcPCSK9	GA399	TCAGCTCCAGGCGGTCCTGG	161	5'uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGuCcGUUaucAAcuugaaaaaguggcaccgagucggugcusususuuuu-3'	447	SpCas9
hcPCSK9	GA400	TCAGCTCCAGGCGGTCCTGG	161	5'uscsasGCUCCAGGCGGUCCUGGgUUUuagagcuagaaauagcaaGUUaAaAuaaggcuaGuccgUUaucaacuugaaaaaguggcaccgagucggugcusususuuuu-3'	447	SpCas9
hcPCSK9	GA401	TCAGCTCCAGGCGGTCCTGG	161	5'uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuuuu-3'	447	SpCas9
hcPCSK9	GA402	TCAGCTCCAGGCGGTCCTGG	161	5'uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUu-3'	447	SpCas9
hcPCSK9	GA403	TCAGCTCCAGGCGGTCCTGG	161	5'uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUu-3'	448	SpCas9
hcPCSK9	GA404	TCAGCTCCAGGCGGTCCTGG	161	5'uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuUu-3'	449	SpCas9
hcPCSK9	GA405	TCAGCTCCAGGCGGTCCTGG	161	5'uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUCcGUUAucAAcuugaaaaaguggcaccgagucggugcusususuUu-3'	447	SpCas9
hcPCSK9	GA406	TCAGCTCCAGGCGGTCCTGG	161	5'uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGuCcGUUaucAAcuugaaaaaguggcaccgagucggugcusususuUu-3'	447	SpCas9
hcPCSK9	GA407	TCAGCTCCAGGCGGTCCTGG	161	5'uscsasGCUCCAGGCGGUCCUGGgUUUuagagcuagaaauagcaaGUUaAaAuaaggcuaGuccgUUaucaacuugaaaaaguggcaccgagucggugcusususuUu-3'	447	SpCas9
hcPCSK9	GA408	TCAGCTCCAGGCGGTCCTGG	161	5'uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuUu-3'	447	SpCas9
hcPCSK9	GA409	TCAGCTCCAGGCGGTCCTGG	161	5'uscsasG(C-PACE)UCCAGGCGGUCCUGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	450	SpCas9

hcPCSK9	GA410	TCAGCTCCAGGCGGTCCTGG	161	5'uscsasGCUCCAG(G-PACE)CGGUCCUGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	451	SpCas9
hcPCSK9	GA439	CCCGCACCTTGGCGCAGCGG	13	5'cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUUu-3'	11	ABE
hcPCSK9	GA440	CCCGCACCTTGGCGCAGCGG	13	5'cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuUUu-3'	11	ABE
hcPCSK9	GA234	CGCCTGCCAGCGCCTGGCGA	252	csgscsCUGCCAGCGCCUGGCGAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	452	SpCas9
hcANGPTL3	GA261	TGAAGAAAGGGAGTAGTTCT	126	5'usgsasAGAAAGGGAGUAGUUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	304	SpCas9
hcANGPTL3	GA262	CATTATATTCAGGTAGTCCA	143	5'csasusUAUAUUCAGGUAGUCCAgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	321	SpCas9
hcANGPTL3	GA263	ACAAAACTTCAATGAAACGT	146	5'ascsasAAACUUCAAUGAAACGUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	324	SpCas9
hcANGPTL3	GA264	GCCAATGGCCTCCTTCAGTT	109	5'gscscsAAUGGCCUCCUUCAGUUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	287	SpCas9
hcANGPTL3	GA265	GCCAATGGCCTCCTTCAGTT	109	5'gscscsAAUGGCCUCCUUCAGUUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuGaaaaaguGgcaccgAgUCggugcusususu-3'	287	SpCas9
hcPCSK9	GA266	TCAGCTCCAGGCGGTCCTGG	161	5'uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	339	SpCas9
hcPCSK9	GA267	GCCCCATGTCGACTACATCG	185	5'gscscsCCAUGUCGACUACAUCGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	363	SpCas9

hcPCSK9	GA268	GGGGCTGGTATTCATCCGCC	187	5'gsgsgsGCUGGUAUUCAUCCGCCgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	365	SpCas9
hcPCSK9	GA269	CTAGGAGATACACCTCCACC	194	5'csusasGGAGAUACACCUCCACCgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	372	SpCas9
hcPCSK9	GA270	CTGCGCGTGCTCAACTGCCA	204	5'csusgsCGCGUGCUCAACUGCCAgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	382	SpCas9
hcPCSK9	GA271	CGGGATGCCGGCGTGGCCAA	206	5'csgsgsGAUGCCGGCGUGGCCAAgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	384	SpCas9
hcPCSK9	GA272	CGCTGACCACCCCTGCCAGG	212	5'csgscsUGACCACCCCUGCCAGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	390	SpCas9
mPCSK9	GA292	cccatacCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu-3'	421	ABE

[672] As used in Table 1 above, Table 23, and Table 24, in the Protospacer: uppercase nucleotides (A, G, C, I and T) indicate 2'-deoxyribonucleotides, adenine, guanine, cytosine, inosine, and thymine, respectively; in Guide RNA sequence: uppercase nucleotides (A, C, G, I and U) indicate ribonucleotides, adenine, guanine, cytosine, inosine, and uracil, respectively and lowercase nucleotides (a, g, c, i and u) indicate 2'-O-methylribonucleotide (2'-OMe) unless otherwise specified. It is understood that the DNA protospacer is converted to RNA or RNA-equivalent in the guide RNA design except when other modifications including 2'-deoxyribonucleotides are introduced into the spacer section of the single guide RNA; s: phosphorothioate (PS), X = ribonebularine; x= 2'-O-methylnebularine; dX = 2'-deoxynebularine; 5'-NNN-3' indicates uniform A, C, G, I, U, dA, dG, dC, dI, T, X, x, dX, and combinations thereof. 5'-nnn-3' indicates uniform a, c, g, i, u, dA, dG, dC or T, x, dX, and combinations thereof. mANGPTL3: mouse ANGPTL3; hANGPTL3: human ANGPTL3; cANGPTL3: cynomolgus ANGPTL3; mcANGPTL3: mouse, cynomolgus cross-reactive ANGPTL3; hcANGPTL3: human, cynomolgus cross-reactive ANGPTL3; mPCSK9: mouse PCSK9; hPCSK9: human PCSK9; cPCSK9: cynomolgus PCSK9; mcPCSK9: mouse,

cynomolgus cross-reactive PCSK9; hcPCSK9: human, cynomolgus cross-reactive PCSK9; hcAPOC3: human, cynomolgus cross-reactive APOC3. As disclosed herein, the nucleotide sequences and modification patterns encompass all length, structure, and type of RNAs or fragments thereof, CRISPR guide RNAs, e.g. sgRNAs, dual guide RNAs, or mRNAs. For example, nucleotide sequences and modification patterns as described in the Table above may indicate RNA sequences and modification patterns in a single guide RNA, a dual guide RNA, a nuclease mRNA, or any fragment or segment thereof. In Table 23, u' indicates N^1-methylpseudouridine.

Example 2. PCSK9 and ANGPTL3 Cas9 editing in vitro

[673] In set of experiments, PCSK9 gRNAs were co-transfected with an equivalent amount (1:1 ratio by weight) of in vitro transcribed commercially available SpCas9 mRNA MS002 purchased from TriLink Biotechnologies into primary human hepatocytes at 2500, 500, or 100ng/RNA/mL and processed as described in detailed methods. Extracted genomic DNA was analyzed for gene editing at the target site with next-generation sequencing of PCR amplicons generated around the target sites. Samples were prepared using the Nextera XT DNA library preparation kit (Illumina) according to the manufacturer's protocol. Briefly, two rounds of PCR were performed first to amplify the region of interest and second to add DNA sequences required for next generation sequencing and sample identification to the initial product. The final amplicon was sequenced on the Illumina MiSeq instrument according to the manufacturer's protocol. A wide range of editing activities (**Table 2**) were observed. In a second set of experiments, ANGPTL3 gRNAs were co-transfected with an equivalent amount of in vitro transcribed SpCas9 mRNA (1:1 ratio by weight) into primary human hepatocytes and processed and analyzed similarly (**Table 3**).

Table 2. PCSK9 gRNA/SpCas9 editing in primary hepatocytes

gRNA	Protospacer (5'-3')	SEQ ID NO:	Human Primary Hepatocyte Editing %			Cyno Primary Hepatocyte Editing %		
			2500	500	100	2500	500	100
GA156	GGTGCTAGCCTTGCGTTCCG	66	26.34	6.22	1.48	33.94	14.31	2.34
GA157	GCCGTCCTCCTCGGAACGCA	151	10.03	2.82	0.62	13.78	2.93	0.78
GA158	GCTAGCCTTGCGTTCCGAGG	152	37.94	6.55	2	18.27	4.42	1.04
GA159	GCGTTCCGAGGAGGACGGCC	153	14.68	3.74	1.35	12.21	3.69	1.19

GA160	GCCTTGCGTTCCGAGGAGGA	154	23.3	5.58	1.19	23.15	6.60	1.38
GA161	GGACGAGGACGGCGACTACG	155	35.08	8.74	3.65	48.24	14.98	4.52
GA162	GGACGGCGACTACGAGGAGC	156	42.8	12.97	2.14	28.26	11.18	1.62
GA163	CGTCCTCGTCCTCCTGCGCA	157	29.32	9.2	2.31	49.50	17.69	5.99
GA164	GTCCTCGTCCTCCTGCGCAC	158	25.77	11.12	2.68	34.89	13.96	2.75
GA165	CCGTCAGCTCCAGGCGGTCC	159	40.27	22.3	4.99	35.57	18.00	5.43
GA166	CGCCCGTGCGCAGGAGGACG	160	36.89	10.99	2.89	44.77	14.39	3.93
GA167	TCAGCTCCAGGCGGTCCTGG	161	53.71	25.87	8.2	66.07	35.98	11.98
GA168	CTTGGCGCAGCGGTGGAAGG	162	54.99	25.77	ND	41.49	15.90	4.75
GA169	CGTGCGCAGGAGGACGAGGA	163	46.65	15.15	5.49	42.38	18.28	3.45
GA170	CCAGGACCGCCTGGAGCTGA	164	31.35	15.84	2.9	23.17	11.48	4.03
GA171	TCTTGGTGAGGTATCCCCGG	165	31.75	9.38	2.37	27.79	9.06	1.29
GA172	CAGTGCGCTCTGACTGCGAG	166	14.01	3.52	0.32	15.58	2.11	0.51
GA173	CTTGGTGAGGTATCCCCGGC	167	23.38	3.94	0.69	35.03	8.70	2.06
GA174	GGATCTTGGTGAGGTATCCC	168	14.74	5.24	1.18	18.65	4.88	1.60
GA175	GAAGATGAGTGGCGACCTGC	169	24.81	7.53	1.55	44.77	12.37	1.45
GA176	AGCACCACCACGTAGGTGCC	170	4.98	1.26	0.36	22.65	8.11	2.11
GA177	GGTCGCCACTCATCTTCACC	171	12.57	2.67	0.65	15.91	4.68	0.38
GA178	CTCCTTCAGCACCACCACGT	172	18.29	5.43	1.26	25.22	5.78	2.21
GA179	GAGTGGCGACCTGCTGGAGC	173	21.87	6	1.29	12.94	4.21	1.06
GA180	GCGCACTGCCCGCCGCCTGC	174	17.16	2.22	0.22	14.12	2.45	0.91
GA181	GGCTTCCTGGTGAAGATGAG	175	ND	ND	28.87	45.12	15.43	3.66
GA182	CACCTACGTGGTGGTGCTGA	176	17.44	4.49	0.95	22.89	6.14	1.55
GA183	CTACGTGGTGGTGCTGAAGG	177	32.35	9.89	2.21	39.56	15.99	2.87
GA184	ATGGAAGACATGCAGGATCT	178	29.55	8.29	2.65	31.87	10.54	2.90
GA185	AGACATGCAGGATCTTGGTG	179	27.98	10.91	1.6	20.29	7.75	1.14
GA186	CTCATCTTCACCAGGAAGCC	180	12.01	3.57	0.79	33.48	7.59	1.96

GA187	CTCCTCGATGTAGTCGACAT	181	ND	6.47	2.44	29.24	12.30	3.77
GA188	TATTCATCCGCCCGGTACCG	182	41.02	15.38	4.21	0.06	0.01	0.01
GA189	TCCTCGATGTAGTCGACATG	183	31.23	8.94	1.76	41.70	15.58	3.30
GA190	CCTCCTCGATGTAGTCGACA	184	25.39	9.73	2.78	26.78	5.53	1.17
GA191	GCCCCATGTCGACTACATCG	185	34.6	12.2	3	51.79	26.79	5.46
GA192	CCATGTCGACTACATCGAGG	186	16.7	6.02	1.14	10.26	1.50	0.60
GA193	GGGGCTGGTATTCATCCGCC	187	35.77	15.68	3.92	52.89	32.02	8.70
GA194	GTCGACATGGGGCAACTTCA	188	32.97	12.39	2.32	30.91	9.49	2.38
GA195	ACCACCGGGAAATCGAGGGC	189	12.57	13.3	3.58	18.78	4.77	0.69
GA196	GAGTGACCACCGGGAAATCG	190	17.19	2.9	0.75	27.79	3.4	1.09
GA197	AGTGACCACCGGGAAATCGA	191	15.03	3.29	1.22	18.21	3.89	0.9
GA198	CCACCGGGAAATCGAGGGCA	192	30.93	10.58	0.04	49	16.15	3.53
GA199	GAAGCGGGTCCCGTCCTCCT	193	29.95	6.89	1.17	45.32	15.11	3.07
GA200	CTAGGAGATACACCTCCACC	194	5.31	8.68	3.8	54.36	20.51	5.15
GA201	CAGCATACAGAGTGACCACC	195	31.51	12.92	3.29	44.01	14.37	4.33
GA202	AAGCGGGTCCCGTCCTCCTC	196	34.75	10.75	2.54	31.13	8.91	1.54
GA203	TGACCCTGCCCTCGATTTCC	197	37.02	12.76	2.57	32.85	10.49	3.66
GA204	CACTCTGTATGCTGGTGTCT	198	24.98	8.98	1.27	19.37	5.27	0.91
GA205	CCCTGCCCTCGATTTCCCGG	199	38.04	12.42	3.74	36.43	12.04	3.01
GA206	CCGGTGGTCACTCTGTATGC	200	20.73	2.7	0.85	16.9	2.73	0.84
GA207	GGAAATCGAGGGCAGGGTCA	201	20.79	3.46	0.84	19.16	2.72	1.05
GA208	CCAGCATACAGAGTGACCAC	202	25.76	5.72	1.38	25.07	6.01	1.15
GA209	CTTGGCAGTTGAGCACGCGC	203	33.23	8.76	0.02	40.78	13.96	3.59
GA210	CTGCGCGTGCTCAACTGCCA	204	14.3	5.86	0.04	54.88	19.72	4.5
GA211	TGCGCGTGCTCAACTGCCAA	205	38.07	10.29	1.31	28.63	8.3	1.01
GA212	CGGGATGCCGGCGTGGCCAA	206	12.45	5.59	1.17	54.89	16.53	1.96
GA213	CGTGCTCAACTGCCAAGGGA	207	24.28	8.77	2.17	45.22	16.35	2.73

GA214	CCTTGGCCACGCCGGCATCC	208	22.92	6.28	1.94	50.35	14.84	3.06
GA215	CAGCGGCCGGGATGCCGGCG	209	4.94	1.01	0.13	7.52	1.07	
GA216	CCGGGATGCCGGCGTGGCCA	210	14.65	3.7	0.95	37.79	4.97	1.77
GA217	GTGGTCAGCGGCCGGGATGC	211	15.31	2.75	0.77	39.21	10.2	2.6
GA218	CGCTGACCACCCCTGCCAGG	212	31.71	8.96	3.81	58.66	19.95	5.42
GA219	GGCAGGGGTGGTCAGCGGCC	213	15.97	4.57	1.28	48.67	11.89	0.35
GA220	GTGCTCAACTGCCAAGGGAA	214	21.87	9.12	2.17	45.17	12.39	3.13
GA221	TCATGGCACCCACCTGGCAG	215	29.35	7.48	1.11	57.4	20.27	2.82
GA222	TGGCAGGGGTGGTCAGCGGC	216	2.04	0.66	0.5	16.7	3.32	0.88
GA223	GCTGACCACCCCTGCCAGGT	217	17.5	7.28	1.44	57.58	21.4	3.46
GA224	GGCCGCTGACCACCCCTGCC	218	13.02	2.19	1.01	47.5	12.34	2.61
GA225	GGCATCGTCCCGGAAGTTGC	219	18.82	6.39	0.89	8.57	3.11	2.03
GA226	GGCTTTTCCGAATAAACTCC	220	7.61	2.99	0.4	ND	ND	ND
GA227	GTCCCGGAAGTTGCCGGCAG	221	32.09	8.29	0.02	32.41	4.58	0.56
GA228	CGGCTGTACCCACCCGCCAG	222	37.1	10.96	5.9	31.36	7.89	1.95
GA229	GTCGTGCTGGTCACCGCTGC	223	30.41	6.85	0.47	34.28	5.39	0.75
GA230	AGTAGAGGCAGGCATCGTCC	224	29.31	12.83	ND	35.86	ND	ND
GA231	TCCGAATAAACTCCAGGCCT	225	ND	ND	ND	ND	ND	ND
GA232	GTTTATTCGGAAAAGCCAGC	226	ND	ND	ND	ND	ND	ND
GA233	GCGGCTGTACCCACCCGCCA	227	19.97	5.39	3.08	37.8	10.84	2.38
GA234	CGCCTGCCAGCGCCTGGCGA	252	5.45	2.29	0.06	6.44	1.53	0.21
GA235	CACCGCTGCCGGCAACTTCC	228	ND	ND	ND	50.03	17.51	2.51
GA236	AGCCCTCGCCAGGCGCTGGC	229	22.41	3.13	6.92	37.44	11.62	2.31
GA237	CAACGCCGCCTGCCAGCGCC	230	36.78	15.83	4.53	46.01	13.46	2.17
GA238	GCACGACCCCAGCCCTCGCC	231	ND	ND	ND	17.92	4.01	0.94
GA239	CCGCCTGCCAGCGCCTGGCG	232	3.49	1.73	0.06	4.55	0.55	0.02
GA240	TGCCAGCGCCTGGCGAGGGC	233	7.94	0.24	1.05	10.62	1.9	0.52

GA241	TGCTGCTGCCCCTGGCGGGT	234	5.39	1.49	0.61	16.11	2.94	0.97
GA242	TCACCGCTGCCGGCAACTTC	235	ND	ND	ND	31.71	7.13	1.79
GA243	GGCGAGGGCTGGGGTCGTGC	236	ND	ND	ND	10.18	1.84	0.51
GA244	TTCCGAATAAACTCCAGGCC	237	ND	ND	ND	28.16	7.89	1.63
GA245	GTGCTGCTGCCCCTGGCGGG	238	2.9	2.97	1.68	7.07	0.88	0.19
GA246	GCCAGCGCCTGGCGAGGGCT	239	17.95	2.17	0.08	24.63	5.78	1.11
GA247	CCTCGCCAGGCGCTGGCAGG	240	6.76	0.17	0.25	11.48	2.62	0.71

Table 3. ANGPTL3 gRNA/SpCas9 editing in primary hepatocytes

gRNA	Protospacer (5'-3')	SEQ ID NO:	Human Primary Hepatocyte Editing %		Cyno Primary Hepatocyte Editing %	
			500 ng/mL	100 ng/mL	500 ng/mL	100 ng/mL
GA107	AGCCAATGGCCTCCTTCAGT	105	5.09	3.72	ND	32.58
GA108	TCCCAACTGAAGGAGGCCAT	106	19.43	4.95	15.49	4.11
GA109	GGCCTCCTTCAGTTGGGACA	107	18.07	6.60	15.11	4.15
GA110	GACCATGTCCCAACTGAAGG	108	13.63	5.37	15.99	8.59
GA111	GCCAATGGCCTCCTTCAGTT	109	24.08	10.72	28.98	22.61
GA113	ATTCTGGAGGAAATAACTAG	111	19.97	6.00	26.67	12.07
GA115	AACATAGCAAATCTTGATTT	113	ND	ND	25.51	12.58
GA116	GTAGAATTTTTTCTTCTAGG	114	16.33	3.78	19.83	10.84
GA117	ACTACAAGTCAAAAATGAAG	115	16.55	7.08	26.11	10.01
GA122	CTTTTATTTGACTATGCTGT	120	13.82	4.45	16.76	7.28
GA125	TGATGGTAAGACACTTTGGT	123	16.27	5.04	19.72	12.91
GA126	GGAGTAGTTCTTGGTGCTCT	124	15.21	6.73	17.57	8.29
GA127	AACATGATGGTAAGACACTT	125	18.74	5.77	33.67	19.89
GA128	TGAAGAAAGGGAGTAGTTCT	126	25.39	10.29	32.55	0.19
GA129	AGTTCTTGGTGCTCTTGGCT	127	ND	ND	20.06	5.36
GA130	ATGATGGTAAGACACTTTGG	128	17.09	7.51	25.90	14.60

GA131	GAAGATAGAGA AATTTCTGT	129	18.64	ND	27.57	10.10
GA132	GGAAGATAGAG AAATTTCTG	130	28.39	15.25	26	15
GA137	TATCAGGTAAA ACCTGTCTA	135	29.70	11.44	30.57	28.01
GA141	TTGAGAGTTGC TGGGTCTGA	139	14.87	5.80	10.32	5.49
GA142	TGAAAAACTTG AGAGTTGCT	140	24.90	10.50	27.59	20.09
GA143	TTAATTCAACAT CGAATAGA	141	19.36	9.30	21.16	14.84
GA145	CATTATATTCAG GTAGTCCA	143	26.33	12.55	30.34	18.77
GA148	ACAAAACTTCA ATGAAACGT	146	31.67	12.01	69.75	28.57
GA151	GAGAACTACAA ATATGGTTT	149	31.88	10.56	38.91	11.32

[674] The modes of operation of Cas9, cytidine base editors (CBE), and adenine base editors (ABE) respectively, along with relevant terminology used in this application including "protospacer", "PAM", "spacer" are illustrated (**FIGs. 1A-1C**). For one of the guides that performed well, GA156, with spacer sequence 5'-GGTGCTAGCCTTGCGTTCCG-3' (SEQ ID NO: 66), additional guides were synthesized with modifications to the tracrRNA sequence. Additionally, an X-ray crystal structure-guided approach was used (**FIG. 1D**). Structure-guided spacer and tracr designs were informed by the crystal structures of *S. pyogenes* Cas9 in complex with sgRNA (Jiang et al., 2015, PDB ID 4ZT0) and *S. pyogenes* Cas9 in a pre-catalytic ternary complex (Jiang et al., 2016, PDB ID 5F9R) (**FIG. 2**). Any positions in the crystal structures where there appeared to be a hydrogen bond between the 2'-OH of a given nucleotide and the Cas9 protein (or another part of the sgRNA) were left unmodified. Additionally, any positions where a steric clash was predicted to occur between a 2'-OMe and the protein were left unmodified. At sites where the 2'-OH was solvent-exposed or otherwise distant from protein residues, a 2'-OMe substitution was made. In cases where the two crystal structures disagreed, no modification was made. This strategy was applied to the entire sgRNA or just the tracr region. Exemplary structures are shown in **FIGs 3-7**.

[675] PCSK9 gRNAs were co-transfected with an equivalent amount of in vitro transcribed SpCas9 mRNA MS002 (1:1 ratio by weight) into primary human hepatocytes and processed as described in detailed methods. A wide range of editing activities were observed (**Table 4** ; ND=Not determined). The guides with the highest editing activity at five days post

transfection were GA001, GA002, GA007, and GA008. The structure-guided 2'-OMe heavy guide RNA (gRNA) designs GA007 and GA008 showed improved editing over minimally modified control gRNA GA009.

Table 4. In vitro evaluation of modified gRNA via SpCas9 editing in primary human hepatocytes

gRNA	% Editing, day 3	% Editing, day 5
GA001	16	27
GA002	28	23
GA003	ND	7
GA004	7	12
GA005	1	15
GA006	ND	12
GA007	30	27
GA008	20	28
GA009	4	19

Example 3. PCSK9 and ANGPTL3 base editing in vitro

[676] Editing of *PCSK9* by ABE8.8 was observed in primary hepatocytes. PCSK9 gRNAs were co-transfected with an equivalent amount of in vitro transcribed ABE8.8 mRNA MA002 (1:1 ratio by weight) into primary hepatocytes and processed as described in detailed methods. In one set of experiments, extracted genomic DNA was analyzed for base editing of the target splice site with next-generation sequencing of PCR amplicons generated around the target sites. Samples were prepared using the Nextera XT DNA library preparation kit (Illumina) according to the manufacturer's protocol. Briefly, two rounds of PCR were performed first to amplify the region of interest and second to add DNA sequences required for next generation sequencing and sample identification to the initial product. The final amplicon was sequenced on the Illumina MiSeq instrument according to the manufacturer's protocol. A wide range of editing activities (**Table 5**; **FIG. 8**) were observed, with guides GA066, GA073, and GA074 performing the best.

Table 5. ABE8.8/PCSK9 gRNA editing in primary hepatocytes

		Human Primary Hepatocytes-splice site editing %			**Cynomolgous Primary Hepatocytes-splice site editing %**		
gRNA	**Species**	5000 ng/mL	2500 ng/mL	1250 ng/mL	5000 ng/mL	2500 ng/mL	1250 ng/mL
GA066	Human/Cyno	25.9	18.7	13.6	24.3	29.1	21.4
GA072	Human/Cyno	2.5	2.2	1.5	2.2	1.7	2.3
GA073	Human/Cyno	25.4	17.5	11.2	29.2	23.2	24.7

GA074	Human/Cyno	23.8	18.8	11.6	18.7	20.1	17.5
GA075	Human/Cyno	5.5	3.1	1.8	ND	ND	ND
GA076	Human/Cyno	4.9	3.9	1.6	ND	ND	ND
GA077	Human/Cyno	6.0	5.7	2.8	5.4	5.7	4.1
GA078	Human/Cyno	12.6	10.0	6.5	ND	ND	ND
GA079	Human/Cyno	6.5	4.9	2.2	0.5	0.5	0.6
GA080	Human/Cyno	0.6	0.5	0.4	7.0	8.8	7.1
GA081	Human/Cyno	8.3	6.1	4.3	0.3	0.3	0.3
GA082	Human/Cyno	9.8	9.6	5.6	ND	ND	ND
GA083	Human/Cyno	0.2	0.2	0.2	ND	ND	ND
GA084	Human/Cyno	8.6	7.6	4.9	ND	ND	ND
GA085	Human/Cyno	2.4	1.8	1.2	ND	ND	ND
GA086	Human/Cyno	5.7	4.9	2.9	ND	ND	ND
GA087	Human/Cyno	1.9	1.4	1.0	0.2	0.2	0.2
GA088	Human/Cyno	7.2	6.2	3.6	24.8	23.0	17.0
GA089	Human/Cyno	18.1	14.1	5.4	ND	ND	ND
GA090	Human/Cyno	0.4	0.5	0.3	ND	ND	ND

[677] When considering the data from primary human hepatocytes, *PCSK9*-targeting gRNAs were identified that matched the following three protospacer sequences as having the best cross-species activity between both primary human hepatocytes and primary cynomolgous hepatocytes: 5'-CCCGCACCTTGGCGCAGCGG-3' (SEQ ID NO: 13) (GA066), 5'-GCTTACCTGTCTGTGGAAGC-3' (SEQ ID NO: 67) (GA073), and 5'-TGCTTACCTGTCTGTGGAAG-3' (SEQ ID NO: 68) (GA074). The GA066 sequence targets the splice donor at the 5' end of human *PCSK9* intron 1 and is predicted to result in an aberrant PCSK9 protein translated from exon 1, followed by several amino acids translated from the beginning of intron 1 (read through from exon 1 into intron 1), followed by a premature stop codon (**FIG. 9**). The GA073 and GA074 sequences each target the splice donor at the 5' end of human PCSK9 intron 4 and is predicted to result in an aberrant PCSK9 protein translated from exons 1, 2, 3, and 4, followed by several amino acids translated from the beginning of intron 4 (readthrough from exon 4 into intron 4), followed by a premature stop codon. The predicted prematurely truncated protein in each of these cases is likely to have complete loss of function as well as having minimal immunogenicity due to the near-identical match to part of the naturally occurring wild-type human PCSK9 amino acid sequence. The same protospacer sequence, 5'-CCCGCACCTTGGCGCAGCGG-3' (SEQ ID NO: 13) (GA066), is found in the cynomolgus monkey genome, similarly targeting the splice donor at the 5' end of cyno *PCSK9* intron 1.

[678] Editing of *ANGPTL3* by ABE8.8 was observed in primary hepatocytes. ANGPTL3 gRNAs were co-transfected with an equivalent amount of in vitro transcribed ABE8.8 mRNA MA004 (1:1 ratio by weight) into primary hepatocytes and processed as described in detailed

methods. A human ANGPTL3-targeting gRNA matching the following protospacer sequence 5'- AAGATACCTGAATAACCCTC-3' (SEQ ID NO: 15) (GA091) was identified. The GA091 sequence targets the splice donor at the 5' end of human ANGPTL3 intron 6 and is predicted to result in an aberrant ANGPTL3 protein translated from exons 1, 2, 3, 4, 5, and 6, followed by several amino acids translated from the beginning of intron 6 (readthrough from exon 6 into intron 6), followed by a premature stop codon (**FIG. 10**). Notably, there is a naturally occurring rare human DNA variant that disrupts the same splice donor and bears the designation rs398122985. A gRNA was synthesized with the orthologous cynomolgous spacer sequence, differing by 1 nucleotide: 5'-AAGATACCTGAATAACTCTC-3' (SEQ ID NO: 14) (GA067) and bearing modest chemical modifications (**Table 1**). Both GA091 in human primary hepatocytes, and GA067 in cynomolgus primary hepatocytes, lead to substantial splice editing when co-transfected with ABE8.8 mRNA (protocol described in the detailed methods section) (**Table 6**). RNA was transfected at 2500, 1250, 625, and 312.5 ng/test article/mL, with two replicates reported (rep 1 and rep 2).

Table 6. ABE8.8/ANGPTL3 gRNA editing in primary hepatocytes

		Human Primary Hepatocytes- splice site editing % (Dose, Replicate #)							
gRNA	**Species**	**2500, rep 1**	**2500, rep 2**	**1250, rep 1**	**1250, rep 2**	**625, rep 1**	**625, rep 2**	**312.5, rep 1**	**312.5, rep 2**
GA091	Human	72.23	72.04	62.01	65.15	47.85	47.3	30.71	29.62
GA092	Human/ Cyno	38.39	41.71	33.38	29.83	20.73	19.94	8.54	10.92
GA093	Human	63.53	66.9	55.54	56.2	39.22	39.8	20.7	17.32
GA094	Human	44.84	46.65	39.62	46.8	28.75	14.74	14.12	26.7
		Cyno Primary Hepatocytes- splice site editing (Dose, Replicate #)							
		2500, rep 1	**2500, rep 2**	**1250, rep 1**	**1250, rep 2**	**625, rep 1**	**625, rep 2**	**312.5, rep 1**	**312.5, rep 2**
GA067	Cyno	66.06	64.84	64.92	63.78	53.56	53.66	37.05	39.36
GA092	Human/ Cyno	45.76	43.27	43	44.28	32.42	32.56	25.72	19.87

[679] Another strategy by which adenine base editors might be used to disrupt gene function is to introduce a missense mutation(s) into the coding region of the gene that results in production of a less functional, or non-functional protein. Guide RNAs targeting protospacers in *ANGPTL3* exons were assessed for base editing activity to introduce missense mutation(s). Each of the gRNAs with an equivalent amount of in vitro transcribed ABE8.8 mRNA MA004 (1:1 ratio by weight) were co- transfected in two replicates (rep 1 and rep 2) at 2500, 1250, 625, and 312.5 ng/RNA/mL into primary human hepatocytes and processed as

described in detailed methods. The resulting base editing efficiency, as well as one potential amino acid substitution, is listed in **Table 7**.

[680] In some embodiments, adenine base editors disrupts gene function by replacing any one amino acid selected from A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, and V with a non-identical amin acid A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, and V. In some embodiments, adenine base editors disrupt gene function by substituting I with T or V. In some embodiments, adenine base editors disrupt gene function by substituting D with G. In some embodiments, adenine base editors disrupt gene function by substituting E with G. In some embodiments, adenine base editors disrupt gene function by substituting F with L or P. In some embodiments, adenine base editors disrupt gene function by substituting H with R. In some embodiments, adenine base editors disrupt gene function by substituting L with P. In some embodiments, adenine base editors disrupt gene function by substituting S with P. In some embodiments, adenine base editors disrupt gene function by substituting M with T. In some embodiments, adenine base editors disrupt gene function by substituting N with G. In some embodiments, adenine base editors disrupt gene function by substituting Q with R. In some embodiments, adenine base editors disrupt gene function by substituting R with G. In some embodiments, adenine base editors disrupt gene function by substituting T with A. In some embodiments, adenine base editors disrupt gene function by substituting V with A. In some embodiments, adenine base editors disrupt gene function by substituting Y with C or H. In some embodiments, adenine base editors disrupt gene function by substituting K with G. In some embodiments, adenine base editors disrupt gene function by introducing an amino acid substitute as listed in Table 7.

Table 7. ANGPTL3 gRNA ABE editing can introduce missense mutations

gRNA	Protospacer (5'-3')	SEQ ID NO	Example Amino Acid Substitution	Human Primary Hepatocytes- Editing % (Dose, Replicate #)							
				2500, rep 1	2500, rep 2	1250, rep 1	1250, rep 2	625, rep 1	625, rep 2	312.5, rep 1	312.5, rep 2
GA104	TAATTTGGCCCTTCGTCTTA	102	I68T	0.27	0.31	0.17	0.29	0.27	0.31	0.21	0.27
GA105	AGACTTTGTCCATAAGACGA	103	D59G	1.12	1.06	0.98	1	0.95	1.28	1.07	1.01
GA	GACTTT	104	N/A (D59G)	1.24	1.08	1.15	1.13	1.2	1.2	1.07	1.34

106	GTCCATAAGACGAA							8	4		
GA112	ATTGTCTTGATCAATTCTGG	110	N/A	0.53	0.44	0.46	0.54	0.49	0.35	0.39	0.5
GA113	ATTCTGGAGGAAATAACTAG	111	S17P	1.22	1.31	1.17	1.26	1.23	1.19	1.21	1.13
GA115	AACATAGCAAATCTTGATTT	113	M39T	58.3	ND	50.19	ND	29.1	ND	16.55	ND
GA116	GTAGAATTTTTTCTTCTAGG	114	I132T	54.78	ND	65.37	ND	33.93	ND	19.8	ND
GA117	ACTACAAGTCAAAAATGAAG	115	Q107R	56.95	57.99	42.71	48.24	25.63	27.9	13.13	12.8
GA118	TATATTGGTCTTCCACGGTC	116	Y186C	37.26	37.2	30.38	30.76	20.85	20.44	9.23	11.01
GA119	CAAAGACCTTCTCCAGACCG	117	D177G	54.92	55.55	14.06	42.4	25.96	25.9	10.84	12.43
GA120	GGTCTTCCACGGTCTGGAGA	118	V182A	1.79	2.04	1.41	1.59	1.21	0.89	0.73	0.76
GA121	TTGTTTATATTGGTCTTCCA	119	Y186H	57.04	65.91	45.78	45.61	30.15	33.83	15.68	13.37
GA122	CTTTTATTTGACTATGCTGT	120	I196T	40.76	37.69	37.03	34.27	21.08	18.83	11.25	7.49
GA123	AAAGTCTGGATATAGAGAGT	121	F167L	12.51	13.68	8.79	12.05	8.1	5.76	3.63	2.83
GA124	GTTGGTTTAATTGTTTATAT	122	L189P	4.43	4.46	4.13	4.34	2.5	2.4	1.2	0.71
GA125	TGATGGTAAGACACTTTG	123	N/A	23.46	26.03	20.98	24.59	13.15	15.36	6.44	5.84

	GT										
GA 126	GGAGTA GTTCTT GGTGCT CT	124	N/A	30.9 3	32.1 9	18.1 1	28.5 7	15. 14	14. 56	7.37	5.22
GA 127	AACATG ATGGTA AGACAC TT	125	H239R	43.8	45.0 3	35.0 9	34.9 8	23. 07	24. 46	12.1 2	12.5
GA 128	TGAAGA AAGGGA GTAGTT CT	126	F228P, L229P	57.4 1	74.8 2	54.6 7	60.9	34. 35	48. 55	22.7 3	17.5 5
GA 129	AGTTCT TGGTGC TCTTGG CT	127	N/A	0.19	0.07	0.07	0.13	0.0 5	0.1 1	0.1	0.08
GA 130	ATGATG GTAAGA CACTTT GG	128	D240G	22.1 6	22.7 3	18.5 7	20.9 3	10. 79	10. 52	5.93	5.09
GA 131	GAAGAT AGAGAA ATTTCT GT	129	L216P, S217P	73.6 3	82.2 4	65.4	74.1 9	42. 42	47. 99	25.2 4	27.1 4
GA 132	GGAAGA TAGAGA AATTTC TG	130	S217P	76.4 3	88.7 7	69.6 5	70.5 5	49. 49	46. 32	22.3 6	23.8 3
GA 133	TATTTC ATTCAA CTGAAG AA	131	I234T	33.9	31.4 9	26.9 5	30.4 8	14. 09	21. 88	12.1	11.3 1
GA 134	ATTTCA TTCAAC TGAAGA AA	132	N/A	41.0 7	47.0 4	38.0 9	38.7 8	24. 71	21	15.4 8	15.6 8
GA 135	GTCTAC TGTGAT GTTATA TC	133	Y273C	34.5	36.7 3	31.3 3	31.8 8	19. 77	19. 85	12.1 2	10.6 1
GA 136	TAAATG GTGGTA CATTCA GC	134	I249T	31.4 5	31.4 6	22.0 8	22.4 2	12. 96	13. 18	6.23	5
GA 137	TATCAG GTAAAA CCTGTC TA	135	N/A	39.2 3	45.5	35.5 4	35.2 4	20. 93	15. 07	10.3	9.13
GA 138	TGTACC ACCATT TATAAC AG	136	T247A	38.6 5	38.2 4	29.1 6	29.4 2	19. 7	17. 09	10.3 2	8.59
GA	TTCACC	137	N/A	13	11.9	10.4	9.45	6.8	4.9	3.51	3.78

139	TCTGTTATAAATGG				6	7		6	5		
GA140	AACAGAGGTGAACATACAAG	138	R252G	30.27	41.96	32.49	33.65	18.78	17.9	9.48	7.7
GA141	TTGAGAGTTGCTGGGTCTGA	139	S267P	0.4	0.41	0.37	0.51	0.4	0.67	0.44	0.45
GA143	TTAATTCAACATCGAATAGA	141	I285V	12.33	10.68	9.23	8.81	5.47	4.63	3.05	2.94
GA144	TTTGGGAGGCTTGATGGTAA	142	R308G	15.05	15.17	10.82	12.69	7.52	8.04	4.16	4.56
GA145	CATTATATTCAGGTAGTCCA	143	N/A (intron)	66.32	62.98	55.96	58.5	37.77	37.26	20.55	17.46
GA146	TTGGGAGGCTTGATGGTAAG	144	R308G	22.26	23.4	16.22	18.81	9.91	10.61	4.57	4.62
GA147	TTTTGGGAGGCTTGATGGTA	145	R308G	3.99	3.67	3.46	2.72	1.96	2.41	1.03	1.2
GA148	ACAAAACTTCAATGAAACGT	146	N294G	25.08	21.83	22.57	21.33	12.96	16.13	8.62	7.59
GA149	TATGGTTTTGGGAGGCTTGA	147	Y304C	0.47	0.43	0.42	0.48	0.52	0.47	0.45	0.47
GA150	ACTACAAATATGGTTTTGGG	148	Y302C, K303G	51.08	51.83	40.09	43.73	23.76	22.55	10.14	9.52
GA151	GAGAACTACAAATATGGTTT	149	N301G	45.3	44.63	31.68	35.7	22.72	20.85	10.83	12.4
GA152	AGGACACTTCAACTGTCCAG	150	H391R	15.32	14.78	9.37	11.5	6.33	6.89	4.95	4.38
GA153	CTTTTCAGGAGAATTTTG	101	N/A (intron)	22.82	23.57	18.25	16.17	8.41	7.17	4.79	ND

	GT										
GA 276	ACGTGG GAGAAC TACAAA TA	242	E300G	4.85	5.62	3.78	4	2.9 8	2.7 5	2.38	1.96
GA 277	CGATGT TGAATT AATGTC CA	243	N/A	2.36	2.43	1.69	1.87	1.5 3	1.7 2	1.16	1.17
GA 278	CACAAA ACTTCA ATGAAA CG	244	Q293R	4.09	4.48	2.43	2.81	2.2	2.0 1	1.68	1.74
GA 279	TACGAA TTGAGT TGGAAG AC	245	I333V	22.6 9	29.4 8	19.3 5	21.1 9	12. 41	15. 31	7.23	7.37
GA 284	CTATGG AGTATA TCTTCT CT	246	S322P	22.7 8	30.9 5	22.2 7	19.1 2	16. 01	16. 29	11.2 9	5.95

[681] The gRNAs GA066/GA095, GA096, G097, GA346, matching the 5'-CCCGCACCTTGGCGCAGCGG-3' (SEQ ID NO: 13) (GA066) protospacer sequence but with more extensive chemical modifications of various kinds (**Table 1**) were synthesized. Similarly, three gRNAs (GA098, GA099, GA100) were synthesized matching the 5'-AAGATACCTGAATAACCCTC-3' (SEQ ID NO: 15) (GA091) human protospacer sequence, as well as four gRNAs (GA067/GA101, GA102, GA103, GA347) matching the 5'-AAGATACCTGAATAACTCTC-3' (SEQ ID NO: 14) (GA067) cynomolgus protospacer sequence, but with more extensive chemical modifications of various kinds (**Table 1**). GA066 and GA095 contain the same chemical composition and modification pattern, as do GA067 and GA101 with one another. It is contemplated that such gRNAs may improve stability against nucleases and gRNA-base editor complex, and reduce or suppress gRNA-triggered immune reaction. Each of the gRNAs described in Table 8, with an equivalent amount of in vitro transcribed ABE8.8 mRNA MA002 (1:1 ratio by weight), were co-transfected into primary human hepatocytes and primary cynomolgus hepatocytes via MessengerMax reagent, using various dilutions to assess for editing activity at different concentrations of test article. Three days after transfection, genomic DNA was harvested from the hepatocytes, and then assessed for base editing of the target splice site with next-generation sequencing. In both human and cynomolgus hepatocytes, as high as 60%–70%

editing of the target splice site (*PCSK9* intron 1 splice donor; *ANGPTL3* intron 6 splice donor) were observed (**Table 8**).

Table 8. Chemically modified gRNAs have high editing efficiency in human and cynomolgus primary hepatocytes

		Human Primary Hepatocytes- splice site editing %				Cynomolgus Primary Hepatocytes- splice site editing %			
gRNA	**Species**	**2500 ng/mL**	**500 ng/mL**	**100 ng/mL**	**20 ng/mL**	**2500 ng/mL**	**500 ng/mL**	**100 ng/mL**	**20 ng/mL**
GA066	Human /Cyno	67.7	55.1	38.9	13.3	48.2	55.3	43.8	19.0
GA096	Human /Cyno	62.8	49.9	41.2	15.6	65.9	51.4	48.0	19.6
GA097	Human /Cyno	67.3	68.1	54.1	22.6	62.2	58.1	56.8	28.0
GA098	Human	70.7	51.3	36.3	10.9	ND	ND	ND	ND
GA099	Human	70.3	47.0	34.4	11.5	ND	ND	ND	ND
GA100	Human	69.2	48.6	38.5	12.4	ND	ND	ND	ND
GA066 /GA101	Cyno	ND	ND	ND	ND	61.4	49.0	45.9	16.0
GA102	Cyno	ND	ND	ND	ND	62.5	51.7	58.1	25.9
GA103	Cyno	ND	ND	ND	ND	62.7	44.2	44.6	17.2

[682] The method by which classical CRISPR/Cas9 disrupts a gene by ultimately introducing an indel, is distinctly and significantly different than base editing. Specifically, base editing is used to introduce a base mutation(s) within a target window closer to the 5' region of the protospacer, as opposed to 3-4 bp away from the PAM, as routinely seen with CRISPR/Cas9. Further, a target region that is highly amenable to CRISPR/Cas9 editing does not necessarily mean base editing at that location will occur, and vice versa. LNPs containing either: (1) Cas9 mRNA MS010 and a gRNA matching 5'-CCCGCACCTTGGCGCAGCGG-3' (SEQ ID NO: 13) (GA097) protospacer sequence; or (2) ABE8.8 mRNA MA004 and gRNA matching 5'-CCCGCACCTTGGCGCAGCGG-3' (SEQ ID NO: 13) (GA097) protospacer sequence, were transfected in human primary hepatocytes. Sanger sequencing of the edited genomic DNA was performed and illustrated the difference in gene editing that occurred from CRISPR/Cas9 compared to base editing (**FIG. 11**). The arrow highlights the main base editing position, while the scissors highlight the general region where Cas9 cuts.

[683] To demonstrate that base editing of a splice site disrupts splicing, reverse transcription-PCR of mRNA from treated primary human hepatocytes, using primers in exon 1 and exon 2, was performed. Results confirmed that splice site disruption resulted in the use

of alternative splice donor sites within intron 1, well downstream of the in-frame TAG stop codon (**FIG. 12**, **Table 9**). The table reports the number of mapped reads for each splice site donor as determined from next generation sequencing.

Table 9. Alternative splice donor sites within *PCSK9* intron 1 resulting from editing of *PCSK9* exon 1 splice-donor adenine base in primary human hepatocytes.

Sample*	Mapped reads	Number of aligned reads corresponding to:					
		Intron length 0 (GT donor)	Intron length 83 (CC donor)	Intron length 87 (GT donor)	Intron length 135 (CG donor)	Intron length 153 (GC donor)	Intron length 154 (CA donor)
control (1)	7827	3187	0	0	0	0	0
control (2)	10584	5956	0	0	0	0	0
control (3)	8575	2307	0	0	0	0	0
control (4)	6721	5153	0	0	0	0	0
treated (1)	8278	2368	0	243	184	984	0
treated (2)	4127	1851	19	235	0	785	0
treated (3)	5694	1096	21	563	0	392	130
treated (4)	4214	2036	0	70	0	1203	0

*Four different primer pairs were used for RT-PCR of the control/treated samples.

Example 4. PCSK9 and ANGPTL3 off-target validation in vitro

[684] With a view towards establishing the safety of a base-editing therapy knocking down *PCSK9* in the human liver *in vivo*, off-target mutagenesis analysis was assessed. A list of candidate sites in the human genome for off-target mutagenesis was assembled using two different methods. The first method used bioinformatic analysis of the human genome, identifying all sites with a PAM sequence compatible with *Streptococcus pyogenes* Cas9 (and therefore ABE 8.8) and a protospacer sequence with up to 4 single-nucleotide mismatches with the GA066 spacer sequence 5'-CCCGCACCTTGGCGCAGCGG-3' (SEQ ID NO: 13). The second method to generate candidate sites used an *in vitro* biochemical assay, ONE-seq, that determined the propensity of a ribonucleoprotein comprising the ABE8.8 base editor protein and PCSK9 gRNA (5'-CCCGCACCTTGGCGCAGCGG-3'(SEQ ID NO: 13)) to cleave oligonucleotides in a library. The reference human genome (GRCh38) was searched for sites with up to 6 mismatches to the protospacer sequence specified by the PCSK9 gRNA

(5'-CCCGCACCTTGGCGCAGCGG-3' (SEQ ID NO: 13)), and sites with up to 4 mismatches plus up to 2 DNA or RNA bulges using Cas-Designer were identified. More specifics on ONE-seq library preparation, experimental protocol, and bioinformatic analysis are described in the additional detailed methods section.

[685] Any cleaved oligonucleotides are PCR-amplified and undergo next-generation sequencing. Oligonucleotides with higher sequence counts reflect a higher propensity for Cas9/gRNA cleavage *in vitro* and represent the sites most likely to suffer off-target mutagenesis in cells. The top site identified by the ONE-seq assay was the on-target *PCSK9* site. The list of candidate off-target sites comprises more than 250 sites, and warranted further investigation (**Table 10**).

[686] Primary human hepatocytes were treated with lipid nanoparticle (LNP) encapasulating ABE8.8 mRNA MA004 and PCSK9 gRNA (GA097 or GA346) at 1:1 weight ratio (see Lipid Nanoparticle Formulation and Analysis for details of LNP preparation) Upon next-generation sequencing using the Agilent SureSelect technology (**Table 11**), when the observed base editing rates in control cells were subtracted from the observed base editing rates in LNP-treated cells across the on-target site and candidate off-target sites (to account for background sequencing errors inherent in next-generation sequencing), appreciable base editing was observed at the on-target *PCSK9* target site. These results were replicated in three different primary hepatocyte lots (STL, HLY, and JLP) from two male and one female patients.

[687] Additionally, using hepatocytes from four individual donors (including lot TLY from a female patient, in addition to the three lots listed above), more than 50 off-target sites were assessed by performing next-generation sequencing of targeted PCR amplicons from LNP-treated versus untreated hepatocytes. Editing at none of these potential off-target sites, and only on-target editing at the *PCSK9* target site, was observed (**FIG. 13**).

Table 10. PCSK9 gRNA (Protospacer 5'-CCCGCACCTTGGCGCAGCGG-3'(SEQ ID NO: 13)) candidate off-target sites as determined by ONE-seq

Chromosome	Location	Potential Off-target sequence (5'-3')	SEQ ID NO:	Alignment
1	55040029-55040051	CCCGCACCTTGGCGCAGCGGTGG	80	X00
1	6888039-6888061	CTAGCACC-TGCCCCAGCGGTGG	453	RNA41
1	10640222-10640244	TCCCTACCCTGGCACAGCAGGGG	454	X60
1	15101074-15101096	CCCACACTCAGACGCAGCGAGGG	455	X60

1	20712184-20712206	CCCGCAC--TGGAGCAGCAGGGA	456	RNA32
1	22256342-22256365	CTTGCACCTTGGTAGCAGCTGGGG	457	DNA41
1	25103342-25103364	CTGGCACCATGGCCCAGCAGTGG	458	X50
1	29882027-29882049	ACTGCATCTTAGTGCAGAGGTGG	459	X60
1	40866872-40866894	TCCACACCTTGGCGCACTGGAAG	460	X50
1	43692630-43692652	CATTCACCTTGTCACAGCTGGGG	461	X60
1	44163492-44163514	ACCGCACCATTGCACAGCCTAGG	462	X60
1	54256943-54256966	GCCGCACCATGGGCACAGCGGGGA	463	DNA41
1	60335313-60335335	CCCATACC-TGGCACAGCGGTGG	464	RNA31
1	64198527-64198549	CAAGCACC-TGGCTCAGAGGTGG	465	RNA41
1	64266524-64266546	ACCGCACC-TGGCACAGTGGAAG	466	RNA41
1	110096163-110096185	CCCCACCCTTGGCACAGAGCAGG	467	X60
1	121262583-121262605	CCAGCACC-TAGCGCAGAGCTGG	468	RNA41
1	145017699-145017721	CCAGCACC-TAGCGCAGAGCTGG	468	RNA41
1	155615561-155615583	CCCGCACC-TACCTCAGCAGGGG	469	RNA41
1	155751756-155751778	CCCGCACC-TACCTCAGCAGGGG	469	RNA41
1	157194781-157194803	CCCGCACCCCACCGCAGCGGGGG	470	X40
1	163263975-163263997	TCTTTACCTTGGAGCAGCTGAGG	471	X60
1	175575740-175575762	CCCGCACCACAGTGCACCGGGGG	472	X50
1	204314754-204314776	CCTCCACAATGGCGCAGGGGCGG	473	X50
1	206281058-206281080	CCAGCACC-TAGCGCAGAGCTGG	468	RNA41
1	208574946-208574968	CCAGCACC-TGGCACAGAAGAGG	474	RNA41
1	228337504-228337526	ACCGCACCAAGGTGCATAGGAGG	475	X60
1	240808285-240808307	CCAGCA-CTTGCCCCAGCAGAGG	476	RNA41
2	9138986-9139008	CTCCCACCCTGGCCCAGCGGGGG	477	X40
2	11670027-11670049	ACAGCACCTTGGCCCAGAGGCGA	478	X50
2	15561025-15561047	ACGGCACCTTGGGCTAGCGGGGG	479	X50
2	42969964-	CCCGCACCCAGCTGCAGCCGAGG	480	X50

	42969986			
2	69112187-69112209	CCAGCACC-TGGTGCAGAGTAGG	481	RNA41
2	71667395-71667417	CCCACACCTGGGCCCAGTGGAGG	482	X40
2	72985325-72985347	CCAGCACC-TGGTGCAGCTTAGG	483	RNA41
2	85317392-85317414	TCCACACAATGGCACAGCAGAGG	484	X60
2	90356123-90356145	CCCGCACC-TGGCTCAGAGGGTC	485	RNA41
2	91447288-91447310	CCCGCACC-TGGCTCAGAGGGTC	485	RNA41
2	107914896-107914919	CGGGCACCATGGCAGCAGCGGAGG	486	DNA31
2	110633933-110633956	CGGGCACCATGGCAGCAGCGGAGG	486	DNA31
2	120922089-120922111	CCCACACCATGGCCCAGTGAGAG	487	X60
2	127571237-127571259	CCAGCACC-TGGCACAGTGAAGG	488	RNA41
2	132524147-132524169	CCCACACATGGCCACAGCAGTGG	489	X60
2	179801496-179801518	CTGGCACCTTAGCACAGAGGAGG	490	X50
2	182608809-182608831	CCCGCACCAGAGCGCAGGGGGAG	491	X50
2	197360047-197360069	GCCACACC-TGGCCCAGCAGGGG	492	RNA41
2	203642814-203642836	CCCGCACCAGGGCGCAGATGGAG	493	X50
2	203866756-203866778	TCCACACCTCAGCACAGCAGAGG	494	X60
2	219451287-219451309	ACCGCACCTTGGCCCAGTGCTGG	495	X40
2	231686140-231686162	ACCGCAGC-TGGCGCAGCATGGG	496	RNA41
3	3719723-3719745	CCCGCACC-TGGCTCAGAGGGTC	485	RNA41
3	10287991-10288013	CCCGCA-TTTGCCTCAGCGGTGG	497	RNA31
3	13178217-13178239	CCAGCA-CTTGGCACAGCAGTGG	498	RNA31
3	14242673-14242696	CCCACACCTTGGTGTCAGCGGAGG	499	DNA21
3	14819436-14819458	CCTGCACC-CGGAGCAGCAGGGG	500	RNA41
3	38369509-38369531	CCTCCACCTTGGCCCAGTGGAGG	501	X40
3	52005791-52005813	CTCCCACCCTGGCGCAGAGGAGG	502	X40
3	54016522-54016544	TCAGCACC-TGGTGCAGAGGAGG	503	RNA41
3	118171416-118171438	CGAGCACCATGGCGCAGCCCCGG	504	X50

3	118171480-118171502	CCCGCA-CTTGGAGCAGCCGGCG	505	RNA31
3	139288546-139288568	GGAGCACCTTGACCCAGCAGAGG	506	X60
3	139677330-139677352	CCAGCA-CTTAGCCCAGCAGCGG	507	RNA41
3	139731008-139731030	CCCGCACCTCGGCACAGCTAGGG	508	X40
3	150408362-150408384	GCCCCATCTTGGCCCAGCGGAGG	509	X40
3	182806878-182806900	CTAGAACCATGGTGCAGAGGGGG	510	X60
4	1221653-1221675	ATCCCACCTCGGCACAGCTGGGG	511	X60
4	1308120-1308142	CCCGCACCGTGGTACAGCCTGTG	512	X60
4	6008382-6008404	CCCGCACC-AGGCCCAGCTGGCG	513	RNA41
4	6747615-6747637	CCCACACCTTGGTGCAGCTCTGT	514	X50
4	7054192-7054214	ACCACACC-TGTCCCAGCGGAGG	515	RNA41
4	37229056-37229078	CACACTACTTGGCACAGAGGAGG	516	X60
4	84244376-84244398	ACCGCA-CTCAGAGCAGCGGAGG	517	RNA41
4	114598629-114598651	TCCGCA-CTTGGCTCAGCGGGGC	518	RNA31
4	115179695-115179717	CCCCGCACTCGGAGCAGCGGCGG	519	X60
4	139511785-139511808	TCCGCACCCTGAGAGCAGTGGAGG	520	DNA41
4	141615591-141615613	CCCTGCACTTGGTGCAGCTGGGG	521	X60
4	158623208-158623230	ACCGCACC-TGACCCAGTGGTGG	522	RNA41
4	182110224-182110246	CTCCTACCATGGCACAGCTGAGG	523	X60
5	767330-767352	CCCACACCAGGGCGCAGCTGAAG	524	X50
5	1516550-1516572	CCAGTACCTTGGCCCAGCTTTGG	525	X50
5	77203454-77203476	ACAGCACC-TGGCACAGTGGTGG	526	RNA41
5	126364896-126364918	CCCACACC-TGGAGCACCAGGGG	527	RNA41
5	126628420-126628443	CAAGCACCTTGGCAGCAGCTGAGG	528	DNA31
5	173667321-173667343	CCCATGCCTTAGCACAGCGATGG	529	X60
5	178526685-178526707	CTCTATCCTTGGCCCAGCAGTGG	530	X60
6	3751269-3751291	CCCGCACCTTGCCGCAGCGGCCC	531	X30
6	15092621-15092643	ATGGCACCTTGGCACATCAGAGG	532	X60
6	16346963-16346985	CCCCGCACTCGGAGCAGCGGCGG	519	X60
6	29016770-29016792	TCCCCACACTGTCGCAGAGGAGG	533	X60
6	36310604-	CCAGCACCATGGCACAGAGAGGT	534	X60

	36310626			
6	44047493-44047515	ACAGCACC-TGGCACAGAGGAGG	535	RNA41
6	47477658-47477680	ACCGCACCTGGCAGCAGCCGTGG	536	X50
6	73315113-73315135	CCAGCACC-TGGAGCAGCCGAGG	537	RNA31
6	138138382-138138404	CCAGCACC-TGGCACAAAGGAGG	538	RNA41
6	139537177-139537199	GAGGCACCTTGGACCAGCAGCGG	539	X60
6	148698322-148698344	CCCGCA-CTTGGAGCAGCCGGTG	540	RNA31
6	153756217-153756239	CCAGCA-CTTAGTGCAGCGCAGG	541	RNA41
6	165843913-165843935	CCCACACC-TGGAGCAGCAGGAG	542	RNA41
7	93102-93124	CCCACACC-AGGCACGGCGGGGG	543	RNA41
7	5360719-5360741	CCCTCACC-AGCCGCAGCAGGGG	544	RNA41
7	24849083-24849106	CACCCGACTTGGCGACAGCGGGGG	545	DNA41
7	74043103-74043125	ACCGCACC-TGGCACAGAAGGGG	546	RNA41
7	84150566-84150588	CCCGCACC-TGGCTCAGAGGGTC	485	RNA41
7	96041009-96041031	ACCACACC-TGGCCCAGCAGTGG	547	RNA41
7	98765214-98765236	CCCTCACC-GGGCACAGAGGAGG	548	RNA41
7	106882973-106882995	CCAGTACC-TGGCCCAGAGGAGG	549	RNA41
7	123763164-123763186	CCCGCACC-AGGCGCAGGTGGAG	550	RNA41
7	129225099-129225122	CAGGCACCTTGGCGGCAGCCGCGG	551	DNA31
7	139596895-139596917	TGTGCACCATGGCACAGCGGGGA	552	X60
7	147737806-147737828	CCCGCACC-TGGCTCAGAGGGTC	485	RNA41
7	150737589-150737611	CCCGCACACAGACGCAGAGCAGG	553	X60
7	153256176-153256198	TCCACACC-TGGCCCAGCTGTGG	554	RNA41
7	155527865-155527887	CCTGCAC--TGGCCCAGCAGCGG	555	RNA32
8	2485153-2485175	CCTGCA-CTAGCCGCAACGGGGG	556	RNA41
8	26191893-26191915	TGAGCACCTTGGGGCAGCTGGGG	557	X50
8	100957388-100957410	CCCGCA-CTTGGCGCAGCTGGCC	558	RNA31
8	139813559-139813582	CCAGCACCTTGGCAGCAGCACCGG	559	DNA31
8	142499068-	TCCCCCACTTGGCACAGCTGGGG	560	X60

	142499090			
8	143383198-143383220	CCAGCA-CAGGGCGCAGAGGTGG	561	RNA41
9	33873295-33873317	CCCACCCCTTAGCACAGCAATGG	562	X60
9	34958979-34959001	CCCACACCGTGGCCCAGAGGTGG	563	X40
9	109123571-109123593	CCAGCACC-TGGCACAGCATAGG	564	RNA41
9	137012994-137013016	ACCACTACCTGGCCCAGCGGTGG	565	X60
9	137167999-137168021	TCCGCACCTTGGTCCAGCAGGGG	566	X40
9	137477162-137477184	TACGCACC-TGGCTCAGCAGAGG	567	RNA41
10	1572578-1572600	AGCCCAGCATGGCGCAGAGGTGG	568	X60
10	5824416-5824438	CCAGCACC-TGGCACAGAGGAGA	569	RNA41
10	16285410-16285432	ACCGCACC-TGGCCCAGATGGGG	570	RNA41
10	20297215-20297237	CCCGCACC-TGGCTCAGAGGGGT	571	RNA31
10	21126683-21126705	ACCGCACC-TGGCCCAGTGGAGG	572	RNA31
10	28839554-28839576	CCCACATCCTGGCCCAGCAGGGG	573	X50
10	47223533-47223555	CCTGCACC-AGGCTCAGTGGGGG	574	RNA41
10	59089166-59089189	CGAGCACCTTGGCAGCAGCTGAGG	575	DNA31
10	60958888-60958910	ACCGCACC-TGGCCCAGAGTAGG	576	RNA41
10	88036335-88036357	ACAGCACC-TGGCGCAGAGCGGG	577	RNA41
10	97031490-97031512	ACTCCACCATGGCACAGCGTGGG	578	X60
10	111259337-111259359	ACCACACC-TGGCCCAGAGGAGG	579	RNA41
10	122572126-122572148	GCGGCACCTTGGCCCAGCGTGGG	580	X40
10	130560773-130560795	CCTGCACCTGAGCACAGAGGAGG	581	X50
11	2916587-2916609	CCCGCACCCT-GTGCAGCCGAGG	582	RNA31
11	10630862-10630884	CCCAC-TCTTGGCACAGGGGAGG	583	RNA41
11	18729719-18729741	CCCGCACC-AGGTGCAGGAGTGG	584	RNA41
11	47350413-47350435	ACCGCACC-TGGCCCAGCAAAGG	585	RNA41
11	47941203-47941225	GCTGCACCTTAGCACAGTGAAGG	586	X60
11	57761981-57762003	TCAGCACCTTGGCGAAGCCTTGG	587	X50
11	72870610-	ACCGCACC-TGGCCCAAAGGGGG	588	RNA41

	72870632			
11	76659109-76659131	CCCTCACCCTGGCACAGCCGGGG	589	X40
11	117692097-117692119	TTCACACCTTGGTGCAGAGGTGA	590	X60
11	121325368-121325390	CCAGCACC-TAGCGCAGCGGCTG	591	RNA31
11	129981605-129981627	ACCGCACC-TGGCCCATCAGGGG	592	RNA41
12	3851459-3851481	CCCGCACC-TGGCTCAGCGGGTC	593	RNA31
12	32850805-32850827	TCAGCACC-TGGAGCAGCCGAGG	594	RNA41
12	48000120-48000142	CCCCACCCTTGGTGCAGAGGAGG	595	X50
12	53328526-53328548	CATACACCTTGCCGCAGCCAGGG	596	X60
12	54190321-54190343	CACACACCTTAGCACAGCCAAGG	597	X60
12	65971780-65971802	CTAGCACC-TGGCACAGTGGTGG	598	RNA41
12	95590363-95590385	ACCGTACC-TGGCCCAGAGGAGG	599	RNA41
12	106690762-106690784	GCCCCACCTTAATGCAGCGGGGG	600	X50
12	114491014-114491036	CCAGCTACATGGCCCAGAGGAGG	601	X60
12	127431260-127431282	CCCACACCAT-GCGCAGAGTGGG	602	RNA41
13	25788073-25788095	CCCGCACCCAGGC-CAGCTGTGG	603	RNA31
13	69424400-69424422	CCAGCACC-TGCTGCAGCGATGG	604	RNA41
13	85917572-85917594	CCCGCACC-TGGCTCAGAGGGTC	485	RNA41
13	98388403-98388425	TCCGCACCTTGGCCCAGTTGAAG	605	X50
14	20813343-20813366	ACAGCACCTTGGCAGCAGAGGTGG	606	DNA31
14	24408232-24408254	CCCGCACC-AGGCTCAGCAGCAG	607	RNA41
14	38663021-38663043	CCAGCACC-TGGCACAGCCAGGG	608	RNA41
14	65250553-65250575	GCTGCACCCTGCCACAGAGGAGG	609	X60
14	89310097-89310119	CCAGCACCCTAGTGCAGAGTGGG	610	X60
14	90554536-90554558	CCCGCACC-TGGCACAGCACCAG	611	RNA41
14	100213915-100213937	CCCG-ACCCTGACGCAGCCTGGG	612	RNA41
14	101153383-101153405	CCCGCACCCTATCACAGCAGAGG	613	X50
14	102929418-102929440	CTCGCACC-AGGCGCAGCCGTGG	614	RNA31

15	42252041-42252063	CCCGCACC-TGGCTCAGCGGGTC	593	RNA31
15	47827093-47827115	CATGTACCTTGCCACAGCAGGGG	615	X60
15	69452825-69452848	CGAGCACCTTGGCGGCAGCTGAGG	616	DNA31
15	86059010-86059032	TCCACACCTAGGCACAGCCTAGG	617	X60
15	94089230-94089252	CCAGCACCAAGGCTCAGCTGAGG	618	X50
15	99993270-99993292	ACCGCACC-TGGCACATTGGAGG	619	RNA41
16	1003178-1003200	CCCACACCTTGGCCCAGCCCTGG	620	X40
16	2092489-2092511	GCCGCACC-TGCCGCAGCCGTGG	621	RNA31
16	3089598-3089620	CCCGCA-CTGGGTGCAGTGGTGG	622	RNA31
16	14882693-14882715	CCCACACCCAGGTGCAGCGGCAG	623	X50
16	16097875-16097897	CCAGCACC-TGGCACAGCAGGTG	624	RNA41
16	16281338-16281360	CCCACACCCAGGTGCAGCGGCAG	623	X50
16	18430777-18430799	CCCACACCCAGGTGCAGCGGCAG	623	X50
16	18513312-18513334	CCCACACCCAGGTGCAGCGGCAG	623	X50
16	21358246-21358268	CCCGCATCTTGGTGCAGCTGCTT	625	X50
16	28383449-28383471	ACCGCACCATGGTGCAGCTGGGC	626	X50
16	28731839-28731861	ACCGCACCATGGTGCAGCTGGGC	626	X50
16	29792483-29792505	CCAACACC-TGGCGCAGAGTAGG	627	RNA41
16	29863301-29863323	CTCGTACCAAAGCGCAGAGGAGG	628	X60
16	30783356-30783378	CCCGCA-CTCGGTGCAGCGGTAT	629	RNA41
16	31081352-31081374	CCCGCA-CTGGGTGCAGCGGAAG	630	RNA31
16	31436901-31436923	GCCGCA-CTTGGCGCAGCTGTGG	631	RNA21
16	67748649-67748671	CTAACACCTTGGTGCAGAGGTGG	632	X50
16	78768901-78768923	CCTACACCATGGCGCAGCGTCAG	633	X50
16	81278358-81278380	CCAGCACC-TGGCACATCGGTGG	634	RNA31
16	88044071-88044093	CCCGCATCTTGGTGCAGCTGCTT	625	X50
16	88839686-88839708	CCCGCA-CATGGTGCAGTGGCTG	635	RNA41
17	7223899-7223921	CCCAC-CCCTGGCCCAGCCGAGG	636	RNA41
17	7462659-7462681	TGCACACCTTGGCGCAGTGGGGG	637	X40

17	7511796-7511818	CCAGCACC-TGGCACAGGGGAGG	638	RNA31
17	19391599-19391621	CCCACACC-AGGCACAGAGGAGG	639	RNA41
17	26736056-26736078	CCCGCACC-TGGCTCAGAGGGTC	485	RNA41
17	28662198-28662220	GTAGCACGTTGACGCAGCAGCGG	640	X60
17	38870195-38870217	GCCGCACC-GGGCGCAGTTGGGG	641	RNA41
17	45753295-45753317	CCCACACC-TGCCCCAGAGGTGG	642	RNA41
17	45993823-45993845	CCCACAGC-TGGCCCAGCAGGGG	643	RNA41
17	63929863-63929885	TCAGCACCTTGGCACAGCTGAAG	644	X50
17	79154410-79154432	ACAGCACCTCGGTGCAGCAGAGA	645	X60
17	81131740-81131762	CCCTC-CCATGGCGCAGCTTCGG	646	RNA41
18	3448410-3448432	CCCGCACC-GGGCGCAGCAGCTG	647	RNA31
18	39380982-39381004	CATGCTACATGGCGCAGCGGTAG	648	X60
18	58247634-58247656	CTCGCACCCGAACGCAGCAGAGG	649	X60
18	59917417-59917439	TCCCCACCCCAGCGCAGCAGAGG	650	X60
18	78993257-78993279	CGAAGACCTTGGCGCAGAAGCGG	651	X60
19	1104611-1104633	CCCGCACCTTGGCCTAGCGCGGT	652	X40
19	1274125-1274147	TCTGCACCTTGGCGCAGCTGGAG	653	X40
19	2209581-2209603	CTCCCACCTTAAAGCAGCGGTGG	654	X50
19	5543277-5543299	ACCGCACC-TGGCCCAGAGTGGG	655	RNA41
19	6431799-6431821	CCCCCACCTTGGCCCAGCGTTGG	656	X30
19	10872441-10872463	GCCACACC-TCGCACAGCGGTGG	657	RNA41
19	11391283-11391305	ACCACACCTTGCTGCAGAGGAGG	658	X50
19	11406443-11406465	CCCGCA-GAGGGCGCAGCGGCTG	659	RNA41
19	12747607-12747629	CCAGCAC--TGGCTCAGCAGAGG	660	RNA32
19	36110180-36110202	ACCGCACC-TGGCCCAAAGGGGG	588	RNA41
19	46709648-46709670	ACCGCACC-CGGCCCAGTGGAGG	661	RNA41
19	49720095-49720117	CCTGCACTCAGGCGCAGACGGGG	662	X60
19	50490618-50490640	GCTGCACCTTGGCACAGTGGAGG	663	X40
19	51068476-51068498	CCCACACCCAGGCCCAGAGGAGG	664	X50
19	51606728-51606750	CCTGCACCT--GCACAGCAGGGG	665	RNA32

19	53775475-53775498	CGAGCACCTTGGCAGCAGCTGAGG	575	DNA31
19	56088833-56088855	CTCGCACC-TGGTGCAGCACCGG	666	RNA41
20	13900645-13900667	GCCGCAC--AGGCACAGCGGCGG	667	RNA32
20	23109029-23109051	TCAGCACCTTGGCACATCCAGGG	668	X60
20	31859435-31859457	CCTGCACCTTTGCACAGCACTGG	669	X50
20	45967752-45967774	CCCGCA-TGTGGCGCAGCAGTGT	670	RNA41
20	49558961-49558983	CCCAGCACTTGGTGAAGCGGAGG	671	X60
21	37176555-37176577	CCCACAC--TGGTGCAGAGGTGG	672	RNA32
22	22912001-22912023	CCTGCACCATGGTGCACCGGCAG	673	X50
22	25849024-25849046	CCCGCAC--AGCCGCAGAGGAGG	674	RNA32
22	27735501-27735523	ACCGCACC-TGGCACAGCTTGGG	675	RNA41
22	49971621-49971643	CCCACACCCTGGCCCAGCCTCGG	676	X50
X	1842130-1842152	CCAGCACTTTGGTACAGCATAGG	677	X60
X	23566446-23566468	CCCGCACC-TGGCTCAGAGGGGT	571	RNA31
X	50814798-50814820	CCCGCACCCTGGCTCAGTGTTGG	678	X40
X	76172984-76173006	CCCGCAC--AGGCGCAGAGGTGG	679	RNA22
X	83385071-83385093	CCAGCACC-TGGTCCAGTGGAGG	680	RNA41
X	107536875-107536897	CCAGCACC-TGGCACAGAGTAGG	681	RNA41

Table 11. PCSK9 gRNA GA346 off-target site validation in human primary hepatocytes

	Lot #:	STL	HLY	JLP	STL	HLY	JLP			
Chromosome	**Location**	**Treated**	**Treated**	**Treated**	**Control**	**Control**	**Control**	**Average treated**	**Average untreated**	**Net Editing**
1	55040029-55040051	42.18	49.76	78.54	0.13	0.19	0.03	56.83	0.12	56.71
1	54256943-54256966	0	0.07	0.04	0.1	0	0.03	0.04	0.04	-0.01
1	22256342-22256365	0	0	0.02	0	0.07	0.02	0.01	0.03	-0.02
1	10640222-10640244	0	0	0	0	0.04	0	0.00	0.01	-0.01
1	6888039-6888061	0	0	0.03	0.03	0.05	0.03	0.01	0.04	-0.03
1	60335313-	0	0.02	0.01	0.03	0	0.02	0.01	0.02	-0.01

	60335335									
1	64198527-64198549	0	0.07	0	0.02	0.06	0	0.02	0.03	0.00
1	64266524-64266546	0	0.04	0.05	0.02	0	0.05	0.03	0.02	0.01
1	121262583-121262605	0.02	0.04	0.05	0.02	0	0.04	0.04	0.02	0.02
1	20712184-20712206	0.05	0.05	0.03	0.03	0.06	0.06	0.04	0.05	-0.01
1	240808285-240808307	0.06	0.03	0.01	0	0.06	0.02	0.03	0.03	0.01
1	145017699-145017721	0.05	0.05	0.08	0.07	0.14	0.05	0.06	0.09	-0.03
1	155615561-155615583	0	0.04	0.01	0	0.05	0.03	0.02	0.03	-0.01
1	155751756-155751778	0.02	0	0.06	0.01	0.09	0.07	0.03	0.06	-0.03
1	206281058-206281080	0	0.08	0.08	0.02	0.07	0	0.05	0.03	0.02
1	208574946-208574968	0	0	0	0	0	0.05	0.00	0.02	-0.02
1	15101074-15101096	0	0	0.05	0.02	0.06	0	0.02	0.03	-0.01
1	25103342-25103364	0.06	0	0.03	0.03	0.03	0	0.03	0.02	0.01
1	29882027-29882049	0.03	0	0.04	0.05	0	0	0.02	0.02	0.01
1	40866872-40866894	0	0	0.05	0.02	0	0	0.02	0.01	0.01
1	43692630-43692652	0	0	0	0.02	0.04	0.01	0.00	0.02	-0.02
1	44163492-44163514	0	0	0	0	0	0.03	0.00	0.01	-0.01
1	110096163-110096185	0	0	0.02	0.03	0	0.03	0.01	0.02	-0.01
1	157194781-157194803	0	0	0.03	0	0	0	0.01	0.00	0.01
1	163263975-163263997	0	0	0	0	0	0	0.00	0.00	0.00
1	175575740-175575762	0	0.05	0.06	0.07	0	0	0.04	0.02	0.01
1	204314754-204314776	0	0.08	0.04	0.04	0.02	0.06	0.04	0.04	0.00
1	228337504-228337526	0.02	0	0.01	0.05	0.05	0.01	0.01	0.04	-0.03
2	107914896-107914919	0.04	0	0	0.11	0	0	0.01	0.04	-0.02
2	110633933-110633956	0	0	0.02	0.03	0	0.06	0.01	0.03	-0.02
2	69112187-69112209	0	0	0	0	0.07	0	0.00	0.02	-0.02
2	72985325-72985347	0.03	0	0	0.01	0	0.05	0.01	0.02	-0.01
2	90356123-	0.05	0	0	0.01	0	0	0.02	0.00	0.01

	90356145									
2	127571237-127571259	0.03	0.04	0.01	0	0.04	0.02	0.03	0.02	0.01
2	197360047-197360069	0	0.04	0	0	0	0.05	0.01	0.02	0.00
2	231686140-231686162	0.05	0.12	0	0.02	0	0	0.06	0.01	0.05
2	9138986-9139008	96.7	99.46	96.44	97.34	99.52	96.8	97.53	97.89	-0.35
2	11670027-11670049	0.03	0	0.02	0	0	0.11	0.02	0.04	-0.02
2	15561025-15561047	0	0	0	0.06	0.06	0.06	0.00	0.06	-0.06
2	42969964-42969986	0.03	0	0.04	0.05	0.08	0.03	0.02	0.05	-0.03
2	71667395-71667417	0	0.04	0.02	0.05	0.08	0	0.02	0.04	-0.02
2	85317392-85317414	0.02	0.06	0	0.01	0	0.03	0.03	0.01	0.01
2	120922089-120922111	0.03	0.03	0.03	0.01	0	0.05	0.03	0.02	0.01
2	132524147-132524169	0	0.05	0	0.06	0.08	0.08	0.02	0.07	-0.06
2	179801496-179801518	0.03	0.04	0	0.01	0	0.01	0.02	0.01	0.02
2	182608809-182608831	0	0	0.09	0.14	0	0	0.03	0.05	-0.02
2	203866756-203866778	0.02	0.11	0.04	0	0.04	0.01	0.06	0.02	0.04
2	219451287-219451309	0	0	0.02	0	0.08	0.02	0.01	0.03	-0.03
3	14242673-14242696	0.03	0	0	0.04	0	0.01	0.01	0.02	-0.01
3	10287991-10288013	0	0	0	0	0	0.06	0.00	0.02	-0.02
3	13178217-13178239	0.03	0	0.02	0.03	0.03	0.01	0.02	0.02	-0.01
3	118171480-118171502	0	0	0	0.05	0	0	0.00	0.02	-0.02
3	14819436-14819458	0.02	0	0	0.01	0	0.03	0.01	0.01	-0.01
3	54016522-54016544	0.06	0	0	0.01	0.05	0	0.02	0.02	0.00
3	139677330-139677352	0	0	0	0.06	0	0.03	0.00	0.03	-0.03
3	38369509-38369531	0.03	0	0	0	0	0	0.01	0.00	0.01
3	52005791-52005813	0.03	0	0.04	0	0	0.02	0.02	0.01	0.02
3	118171416-118171438	0.15	0.06	0	0	0	0.03	0.07	0.01	0.06
3	139288546-139288568	0	0.02	0.04	0.03	0	0	0.02	0.01	0.01
3	139731008-	0	0.02	0.07	0.03	0	0	0.03	0.01	0.02

	139731030									
3	150408362-150408384	0	0	0.04	0	0	0.04	0.01	0.01	0.00
3	182806878-182806900	0.09	0	0.04	0	0	0.05	0.04	0.02	0.03
4	139511785-139511808	0	0	0	0.03	0	0.03	0.00	0.02	-0.02
4	84244376-84244398	0.03	0	0.05	0.06	0	0.01	0.03	0.02	0.00
4	114598629-114598651	0	0	0.02	0	0	0	0.01	0.00	0.01
4	6008382-6008404	0	0	0.04	0	0	0.12	0.01	0.04	-0.03
4	7054192-7054214	0.14	0.02	0	0.02	0.07	0.01	0.05	0.03	0.02
4	158623208-158623230	0	0	0.12	0	0	0	0.04	0.00	0.04
4	1221653-1221675	0.05	0	0.01	0.03	0	0.05	0.02	0.03	-0.01
4	1308120-1308142	0.03	0	0.02	0	0	0	0.02	0.00	0.02
4	6747615-6747637	0.03	0	0.05	0.02	0	0.04	0.03	0.02	0.01
4	37229056-37229078	0.03	0.02	0	0.05	0	0.05	0.02	0.03	-0.02
4	115179695-115179717	0	0	0	0	0	0	0.00	0.00	0.00
4	141615591-141615613	0	0	0.05	0	0	0	0.02	0.00	0.02
4	182110224-182110246	0.04	0	0.05	0.05	0.06	0.02	0.03	0.04	-0.01
5	126628420-126628443	0.03	0.07	0	0.05	0	0	0.03	0.02	0.02
5	77203454-77203476	0.06	0	0.07	0.1	0	0.05	0.04	0.05	-0.01
5	126364896-126364918	0.03	0	0	0	0	0	0.01	0.00	0.01
5	767330-767352	0.09	0.04	0.05	0.03	0.06	0.04	0.06	0.04	0.02
5	1516550-1516572	0	0.06	0	0	0	0.03	0.02	0.01	0.01
5	173667321-173667343	0.02	0	0.01	0.03	0.04	0.02	0.01	0.03	-0.02
5	178526685-178526707	0	0	0	0	0.03	0.03	0.00	0.02	-0.02
6	44047493-44047515	0.06	0.02	0.04	0.09	0	0.05	0.04	0.05	-0.01
6	73315113-73315135	0	0	0	0.07	0.04	0	0.00	0.04	-0.04
6	148698322-148698344	0	0	0.13	0.07	0	0.11	0.04	0.06	-0.02
6	153756217-153756239	0	0	0.01	0.07	0.11	0	0.00	0.06	-0.06
6	138138382-	0.06	0	0	0.04	0	0.03	0.02	0.02	0.00

	138138404									
6	165843913-165843935	0.03	0.07	0	0.05	0	0.03	0.03	0.03	0.01
6	3751269-3751291	0	0	0	0	0	0	0.00	0.00	0.00
6	15092621-15092643	0	0.03	0.02	0.01	0	0	0.02	0.00	0.01
6	16346963-16346985	0.07	0	0	0	0	0	0.02	0.00	0.02
6	29016770-29016792	0	0	0	0.02	0.08	0.02	0.00	0.04	-0.04
6	36310604-36310626	0.06	0	0.06	0.01	0.1	0.09	0.04	0.07	-0.03
6	47477658-47477680	0.2	0	0	0.07	0	0.03	0.07	0.03	0.03
6	139537177-139537199	0	0	0	0.03	0	0	0.00	0.01	-0.01
7	24849083-24849106	0	0.05	0.06	0.03	0	0	0.04	0.01	0.03
7	129225099-129225122	0	0	0	0	0	0	0.00	0.00	0.00
7	93102-93124	0.37	0	0.28	0.04	0	0.05	0.22	0.03	0.19
7	5360719-5360741	0	0.03	0.07	0.02	0	0.1	0.03	0.04	-0.01
7	74043103-74043125	0.1	0	0.07	0.04	0	0.04	0.06	0.03	0.03
7	96041009-96041031	0.17	0.09	0.03	0.03	0.11	0.11	0.10	0.08	0.01
7	98765214-98765236	0	0	0	0	0.05	0.03	0.00	0.03	-0.03
7	106882973-106882995	0	0.12	0.02	0.09	0.05	0.02	0.05	0.05	-0.01
7	123763164-123763186	0.08	0.29	0	0	0	0.13	0.12	0.04	0.08
7	147737806-147737828	0	44.29	0.19	0.11	43.85	0	14.83	14.65	0.17
7	153256176-153256198	0	0.05	0.04	0	0	0.03	0.03	0.01	0.02
7	155527865-155527887	0	0	0	0	0	0	0.00	0.00	0.00
7	139596895-139596917	0	0.03	0.03	0.03	0.03	0	0.02	0.02	0.00
7	150737589-150737611	0.05	0.13	0.05	0.01	0.05	0.18	0.08	0.08	0.00
8	139813559-139813582	0	0.06	0	0	0.06	0	0.02	0.02	0.00
8	2485153-2485175	0.05	0	0.03	0.03	0	0.06	0.03	0.03	0.00
8	100957388-100957410	0	0	0	0.03	0	0	0.00	0.01	-0.01
8	143383198-143383220	0.02	0.11	0.04	0.11	0.09	0	0.06	0.07	-0.01
8	26191893-	0	0.06	0	0.02	0.13	0.02	0.02	0.06	-0.04

	26191915									
8	142499068-142499090	0.03	0	0	0	0.08	0	0.01	0.03	-0.02
9	137012994-137013016	0.05	0	0.07	0.02	0	0.03	0.04	0.02	0.02
9	137167999-137168021	0.03	0	0	0	0	0.03	0.01	0.01	0.00
9	109123571-109123593	0.03	0	0	0.02	0	0	0.01	0.01	0.00
9	137477162-137477184	0.05	0.06	0.02	0.02	0.03	0.05	0.04	0.03	0.01
9	33873295-33873317	0.05	0	0	0	0	0	0.02	0.00	0.02
9	34958979-34959001	0.1	0.03	0	0.01	0	0.01	0.04	0.01	0.04
10	28839554-28839576	0	0.18	0.08	0.11	0	0	0.09	0.04	0.05
10	59089166-59089189	0	0	0	0	0.06	0	0.00	0.02	-0.02
10	97031490-97031512	0.1	0.12	0	0.01	0.03	0.09	0.07	0.04	0.03
10	122572126-122572148	0	0.05	0.03	0	0	0	0.03	0.00	0.03
10	130560773-130560795	0.03	0.02	0.03	0.02	0	0	0.03	0.01	0.02
10	5824416-5824438	0	0.09	0.01	0	0	0	0.03	0.00	0.03
10	16285410-16285432	0.08	0.09	0	0.05	0	0.03	0.06	0.03	0.03
10	20297215-20297237	0	0	0	0	0	0	0.00	0.00	0.00
10	21126683-21126705	0.08	0.09	0	0.05	0	0	0.06	0.02	0.04
10	47223533-47223555	0	0	0.02	0.03	0	0.03	0.01	0.02	-0.01
10	60958888-60958910	0.1	0	0.12	0	0.14	0.03	0.07	0.06	0.02
10	88036335-88036357	0.03	0	0.02	0.02	0	0.02	0.02	0.01	0.00
10	111259337-111259359	0.08	0	0	0.09	0	0	0.03	0.03	0.00
10	1572578-1572600	0.03	0	0.03	0	0.05	0.05	0.02	0.03	-0.01
11	47941203-47941225	0	0.05	0.02	0	0	0.03	0.02	0.01	0.01
11	57761981-57762003	0	0	0.02	0.01	0	0.08	0.01	0.03	-0.02
11	76659109-76659131	0	0	0	0	0	0.01	0.00	0.00	0.00
11	117692097-117692119	0	0.04	0	0.01	0	0.02	0.01	0.01	0.00
11	10630862-10630884	0.03	0	0	0.02	0	0.06	0.01	0.03	-0.02
11	18729719-	0	0	0.06	0.06	0.1	0.08	0.02	0.08	-0.06

	18729741									
11	47350413-47350435	0.05	0	0	0.06	0.07	0	0.02	0.04	-0.03
11	72870610-72870632	0	0	0	0	0	0.24	0.00	0.08	-0.08
11	2916587-2916609	0	0	0.01	0	0	0	0.00	0.00	0.00
11	121325368-121325390	0	0.04	0.09	0.07	0.06	0.05	0.04	0.06	-0.02
11	129981605-129981627	0	0	0.04	0.06	0.13	0.04	0.01	0.08	-0.06
12	3851459-3851481	0	0	0	0	0	0	0.00	0.00	0.00
12	32850805-32850827	0.06	0	0.07	0.05	0.07	0	0.04	0.04	0.00
12	65971780-65971802	0.06	0.04	0.03	0	0	0	0.04	0.00	0.04
12	95590363-95590385	0	0.18	0	0.08	0	0.08	0.06	0.05	0.01
12	127431260-127431282	0.05	0.04	0.11	0.06	0.12	0.04	0.07	0.07	-0.01
12	48000120-48000142	0	0	0.02	0	0.06	0.02	0.01	0.03	-0.02
12	53328526-53328548	0	0	0	0.06	0.09	0.02	0.00	0.06	-0.06
12	54190321-54190343	0.06	0.02	0.03	0.01	0	0.05	0.04	0.02	0.02
12	106690762-106690784	0	0	0	0.01	0	0.01	0.00	0.01	-0.01
12	114491014-114491036	0.11	0	0	0.05	0.1	0.05	0.04	0.07	-0.03
13	69424400-69424422	0	0.04	0.03	0.01	0.13	0	0.02	0.05	-0.02
13	25788073-25788095	0.05	0.04	0.04	0.03	0.05	0	0.04	0.03	0.02
13	98388403-98388425	0	0.06	0.02	0	0	0	0.03	0.00	0.03
14	20813343-20813366	0.05	0.02	0.01	0.02	0	0.05	0.03	0.02	0.00
14	24408232-24408254	0	0.03	0.07	0.06	0	0.07	0.03	0.04	-0.01
14	38663021-38663043	0.08	0	0.01	0.06	0	0	0.03	0.02	0.01
14	100213915-100213937	0	0	0	0.03	0	0	0.00	0.01	-0.01
14	90554536-90554558	0	0	0.01	0.06	0	0.03	0.00	0.03	-0.03
14	102929418-102929440	0	0	0.03	0.03	0	0	0.01	0.01	0.00
14	65250553-65250575	0.04	0	0	0.04	0	0	0.01	0.01	0.00
14	89310097-89310119	0.06	0	0.06	0.03	0.06	0.04	0.04	0.04	0.00
14	101153383-	0	0.04	0	0	0	0	0.01	0.00	0.01

	101153405									
15	69452825-69452848	0	0	0.03	0.04	0	0.03	0.01	0.02	-0.01
15	42252041-42252063	0	0	0	0	0	0	0.00	0.00	0.00
15	99993270-99993292	0.03	0	0.02	0	0	0	0.02	0.00	0.02
15	47827093-47827115	0.08	0.04	0.02	0.04	0	0	0.05	0.01	0.03
15	86059010-86059032	0.05	0.02	0.07	0.05	0.05	0.01	0.05	0.04	0.01
15	94089230-94089252	0.11	0.04	0.04	0.07	0.02	0.02	0.06	0.04	0.03
16	3089598-3089620	0	0.03	0.01	0	0.07	0	0.01	0.02	-0.01
16	30783356-30783378	0	0	0	0.04	0	0.02	0.00	0.02	-0.02
16	31081352-31081374	0.03	0	0.04	0	0	0	0.02	0.00	0.02
16	31436901-31436923	0.02	0.02	0.01	0.01	0.05	0.03	0.02	0.03	-0.01
16	2092489-2092511	0.05	0	0	0	0.03	0	0.02	0.01	0.01
16	16097875-16097897	0.03	0.04	0.02	0.04	0.04	0.14	0.03	0.07	-0.04
16	29792483-29792505	0.06	0.03	0.11	0.11	0	0.03	0.07	0.05	0.02
16	88839686-88839708	0.03	0	0	0	0	0.04	0.01	0.01	0.00
16	81278358-81278380	0	0	0	0	0	0.04	0.00	0.01	-0.01
16	1003178-1003200	0.09	0	0.04	0.02	0	0	0.04	0.01	0.04
16	14882693-14882715	0.06	0.03	0.07	0.09	0	0.01	0.05	0.03	0.02
16	16281338-16281360	0.02	0.04	0.06	0.06	0	0.06	0.04	0.04	0.00
16	18430777-18430799	0.08	0	0.09	0.07	0.05	0.02	0.06	0.05	0.01
16	18513312-18513334	0.04	0	0	0.02	0	0.07	0.01	0.03	-0.02
16	21358246-21358268	0	0	0	0.02	0	0.02	0.00	0.01	-0.01
16	28383449-28383471	0.02	0.1	0.03	0.03	0.04	0.01	0.05	0.03	0.02
16	28731839-28731861	0.05	0.02	0.05	0.02	0	0.04	0.04	0.02	0.02
16	29863301-29863323	0	0	0	0.03	0	0	0.00	0.01	-0.01
16	67748649-67748671	0	0	0.03	0	0	0	0.01	0.00	0.01
16	78768901-78768923	0.04	0	0.01	0	0.02	0.03	0.02	0.02	0.00
16	88044071-	0	0	0	0	0	0	0.00	0.00	0.00

	88044093									
17	7223899-7223921	0	0	0	0.02	0	0.06	0.00	0.03	-0.03
17	7511796-7511818	0	0.02	0.05	0.04	0.09	0	0.02	0.04	-0.02
17	19391599-19391621	0.03	0.03	0.1	0.05	0.06	0.06	0.05	0.06	0.00
17	26736056-26736078	0.03	0.08	0	0.04	0	0	0.04	0.01	0.02
17	38870195-38870217	0	0.03	0.05	0.03	0.07	0	0.03	0.03	-0.01
17	45753295-45753317	0.03	0	0.02	0.04	0	0.04	0.02	0.03	-0.01
17	45993823-45993845	0	0.06	0.02	0.05	0.07	0	0.03	0.04	-0.01
17	81131740-81131762	0	0	0	0.01	0	0.03	0.00	0.01	-0.01
17	7462659-7462681	0.05	0	0.04	0.01	0.06	0	0.03	0.02	0.01
17	28662198-28662220	0	0	0	0.07	0	0.03	0.00	0.03	-0.03
17	63929863-63929885	0.07	0.02	0.03	0.01	0.02	0.03	0.04	0.02	0.02
17	79154410-79154432	0	0	0.03	0.02	0	0	0.01	0.01	0.00
18	3448410-3448432	0.03	0	0	0	0	0	0.01	0.00	0.01
18	39380982-39381004	0	0.04	0.03	0.01	0	0.05	0.02	0.02	0.00
18	58247634-58247656	0.08	0.02	0	0	0.05	0.03	0.03	0.03	0.01
18	59917417-59917439	0.06	0	0	0.02	0	0	0.02	0.01	0.01
18	78993257-78993279	0	0	0.04	0	0.11	0.02	0.01	0.04	-0.03
19	53775475-53775498	0.03	0	0.02	0.02	0	0	0.02	0.01	0.01
19	11406443-11406465	0.02	0	0.08	0.02	0	0.1	0.03	0.04	-0.01
19	10872441-10872463	0.06	0	0	0.03	0	0.03	0.02	0.02	0.00
19	36110180-36110202	0	0	0	0.13	0	0	0.00	0.04	-0.04
19	46709648-46709670	0	0	0.09	0.03	0	0.12	0.03	0.05	-0.02
19	12747607-12747629	0.03	0	0.06	0.04	0.05	0.02	0.03	0.04	-0.01
19	56088833-56088855	0	0.07	0	0.05	0.14	0	0.02	0.06	-0.04
19	51606728-51606750	0.02	0	0.01	0.01	0	0.06	0.01	0.02	-0.01
19	1104611-1104633	0	0	0	0	0	0	0.00	0.00	0.00
19	1274125-	0	0.05	0.02	0.05	0	0	0.02	0.02	0.01

	1274147									
19	2209581-2209603	0	0	0	0.02	0.07	0	0.00	0.03	-0.03
19	6431799-6431821	0	0	0	0	0	0.03	0.00	0.01	-0.01
19	11391283-11391305	0.03	0.04	0.03	0.07	0.1	0.03	0.03	0.07	-0.03
19	49720095-49720117	0.04	0	0	0	0	0.1	0.01	0.03	-0.02
19	50490618-50490640	0	0.05	0.02	0	0.03	0	0.02	0.01	0.01
19	51068476-51068498	0.07	0.06	0.1	0.08	0	0.02	0.08	0.03	0.04
20	45967752-45967774	0	0	0	0	0	0.03	0.00	0.01	-0.01
20	13900645-13900667	0.03	0	0.03	0.04	0.05	0.06	0.02	0.05	-0.03
20	23109029-23109051	0	0	0.01	0	0	0.01	0.00	0.00	0.00
20	31859435-31859457	0	0.02	0	0	0	0.03	0.01	0.01	0.00
20	49558961-49558983	0.04	0.09	0	0	0.07	0	0.04	0.02	0.02
21	37176555-37176577	0	0	0.03	0.07	0.05	0	0.01	0.04	-0.03
22	27735501-27735523	0	0.08	0	0	0.04	0.05	0.03	0.03	0.00
22	25849024-25849046	0.03	0.04	0.01	0	0	0.02	0.03	0.01	0.02
22	22912001-22912023	0	0	0.01	0	0.05	0	0.00	0.02	-0.01
22	49971621-49971643	0	0.15	0.06	0	0.11	0.03	0.07	0.05	0.02
X	1842130-1842152	0	0	0	0	0	0	0.00	0.00	0.00
X	50814798-50814820	0	0	0	0.06	0	0	0.00	0.02	-0.02
X	23566446-23566468	0	0	0	0	0	0	0.00	0.00	0.00
X	83385071-83385093	0	0.08	0	0	0	0	0.03	0.00	0.03
X	76172984-76173006	0	0	0.03	0	0	0.05	0.01	0.02	-0.01
X	107536875-107536897	0	0	0	0.03	0	0	0.00	0.01	-0.01

[688] Adenine base editors have been reported to induce gRNA-independent RNA editing via the deoxyadenosine deaminase domain. To assess for gRNA-independent RNA editing, primary human hepatocytes were treated with mRNA and PCSK9 gRNA (n = 4 biological replicates), SpCas9 mRNA and gRNA (n = 4), or were untreated (n = 4). RNA was extracted after 2 days, and processed as described in the additional detailed methods section.

Comparing the RNA profiles of the ABE8.8- and SpCas9-treated hepatocytes with untreated hepatocytes, no substantial RNA edits in the ABE8.8-treated hepatocytes was observed (**FIG. 14**). Each replicate was compared against each of four untreated hepatocyte samples to eliminate any positions with editing that were common to both conditions. The jitter plots portray transcriptomic loci with editing in the treated sample (number indicates total edited loci identified in the treated sample, boxplot indicates median ± interquartile range of proportion of edited reads across all edited loci in the sample).

[689] Modifications and/or truncations to either the spacer or tracr portion of the gRNA was assessed by alteration of the GA066 spacer (5'-CCCGCACCTTGGCGCAGCGG-3'(SEQ ID NO: 13)) (**Table 12).** The modifications to the guide can serve to improve on-target editing efficiency and/or improve off-target editing efficiency. Each of the gRNAs with an equivalent amount of in vitro transcribed ABE8.8 mRNA (1:1 ratio by weight) were co-transfected into primary human hepatocytes and primary cynomolgus hepatocytes and processed as described in detailed methods.

Table 12. Modifications and/or truncations of the PCSK9 guide GA346 results in high-level editing in human primary hepatocytes

	Human Primary Hepatocytes— Editing % at Position 6 (Dose, Replicate #)							
gRNA	**2500 ng/mL rep1**	**2500 ng/mL rep2**	**1250 ng/mL rep1**	**1250 ng/mL rep2**	**625 ng/mL rep1**	**625 ng/mL rep2**	**312.5 ng/mL rep1**	**312.5 ng/mL rep2**
GA346	30.69	35.58	19.99	34.67	27.13	16.93	12.56	9.92
GA376	38.15	33.98	31.43	25.28	24.02	22.03	14.81	12.02
GA377	35.11	37.31	26.39	31.48	21.39	23.19	15.34	14.9
GA380	38.5	35.38	32.41	32.43	25.28	20.36	12.85	ND
GA381	29.7	31.2	24.12	29.23	17.32	18.43	11.44	11
GA382	34.56	30.02	22.5	24.45	18.48	15.64	8.45	8.86
GA383	42.2	34.95	32.76	31.79	22.24	24.17	13.27	13.26
GA384	8.49	8.5	7.07	6.51	5.03	4.25	2.61	2.7
GA385	40.2	29.05	26.39	21.43	18.88	16.66	11.76	7.63
GA386	43.73	30.67	31.57	26.5	27.51	19.19	16.94	13.41
GA387	22.61	16.64	17.25	12.78	11.55	8.26	5.28	4.24
GA388	28.77	23.84	20.46	20.33	13.73	ND	7.26	ND
GA389	28.45	23.46	21.98	17.88	12.62	12.78	8.06	8.54
GA391	25.58	30.06	25.43	27.3	27.12	18.67	16.86	15.78
	Cyno Primary Hepatocytes— Editing % at Position 6 (Dose, Replicate #)							
gRNA	**2500 ng/mL rep1**	**2500 ng/mL rep2**	**1250 ng/mL rep1**	**1250 ng/mL rep2**	**625 ng/mL rep1**	**625 ng/mL rep2**	**312.5 ng/mL rep1**	**312.5 ng/mL rep2**
GA346	40.1	37.48	36.97	34.08	25.91	25.59	14.83	15.25
GA376	29.57	29.57	27.62	31.98	23.08	22.29	15.12	15.75

GA377	35.36	42.67	35.66	43.35	30.68	34.73	21.24	21.79
GA380	43.27	44.85	39.03	38.76	33.31	34.1	21.83	21.51
GA381	39.45	47.85	35.82	41.03	28.42	31.87	15.51	18.36
GA382	36.34	39.97	32.74	37.41	26.15	25.68	14.08	12.74
GA383	39.02	53.37	35.44	44.23	28	33.42	16	20.03
GA384	26.71	22.52	20.97	21.53	14.38	16.21	8.31	8.03
GA385	38.44	37.36	38.31	31.84	31.97	28.48	18.23	17.68
GA386	48.11	43.61	40.09	40.44	37.59	37.42	23.07	25.27
GA387	27.3	21.08	23.71	21.53	16.82	13.62	7.34	6.03
GA388	41.95	36.03	32.87	29.9	23.65	25.43	12.13	13.19
GA389	34.62	33.47	32.15	29.86	25.87	23.29	11.17	12.33

[690] With a view towards establishing the safety of a base-editing therapy knocking down *ANGPTL3* in the human liver *in vivo*, a list of candidate sites in the human genome for off-target mutagenesis was assembled using two different methods. The first method used bioinformatic analysis of the human genome, identifying all sites with a PAM sequence compatible with *Streptococcus pyogenes* Cas9 (and therefore ABE 8.8) and a protospacer sequence with up to 4 single-nucleotide mismatches with the GA100 spacer sequence 5'-AAGATACCTGAATAACTCTC-3' (SEQ ID NO: 14).

[691] The second method to generate candidate sites used an *in vitro* biochemical assay, ONE-seq, that determined the propensity of a ribonucleoprotein comprising the ABE8.8 base editor protein and gRNA GA441 to cleave oligonucleotides in a library. The reference human genome (GRCh38) was searched for sites with up to 6 mismatches to the protospacer sequence specified by the ANGPTL3 gRNA (5'-AAGATACCTGAATAACTCTC-3' (SEQ ID NO: 14)), and sites with up to 4 mismatches plus up to 2 DNA or RNA bulges using Cas-Designer were identified. More specifics on ONE-seq library preparation, experimental protocol, and bioinformatic analysis are described in the additional detailed methods section.

[692] Oligonucleotides with higher sequence counts reflect a higher propensity for Cas9/gRNA cleavage *in vitro* and represent the sites most likely to suffer off-target mutagenesis in cells. The top candidate sites are identified in Table 13. Validation of candidate sites was performed using *ANGPTL3* edited, and untreated human primary hepatocytes. Upon next-generation sequencing (Table 14), when the observed base editing rates in control cells were subtracted from the observed base editing rates in LNP-treated cells across the on-target site and candidate off-target sites (to account for background sequencing errors inherent in next-generation sequencing), appreciable base editing were observed at the on-target *ANGPTL3* target site.

Table 13. ANGPTL3 gRNA (Protospacer 5'-AAGATACCTGAATAACCCTC-3' (SEQ ID NO: 15)) candidate off-target sites identified by ONE-seq

chromosome	location	Potential Off-target Sequence (5'-3')	SEQ ID NO:	alignment
1	4546642	CAGATACCATAAAAATCATCTGG	682	X60
1	6955991	AAGATACTGAAAAACCCTGCAA	683	RNA41
1	16544549	CAGAGAGCAGAGTAAACCTCTGG	684	X60
1	23996891	CAGAAAACTATAACCCTCAGG	685	RNA32
1	26088616	AGAATGGCTGCATAACCCTTGGG	686	X60
1	27386898	AAGGTTACTTAATAAACATCTGG	687	X60
1	28115884	AAGATACCTGAATTAAATCTCAAA	688	DNA41
1	30795252	AAGTATCTTGAATAACCCTCCTG	689	X50
1	31542787	CAAATAACTAAATGACCCTCTTG	690	X60
1	32826604	AAGATACTGAAAAACTCTCTTC	691	RNA41
1	33418548	TAGATAACCTGAATAGCCCTATAG	692	DNA41
1	45361452	TAGATACTTCAATACCTCTCAGT	693	X60
1	57486127	AGCAATCCTGAAAAATCCTCTGG	694	X60
1	59422147	ACGATACTTGGATAACCCTCTGG	695	X30
1	59572755	GAGATACCTAATAAGCCTCTAT	696	RNA41
1	62604219	AAGATACCTGAATAACCCTCTGG	697	X00
1	66687653	AAGTTACTGAATATCTCTCAGC	698	RNA41
1	76436411	AGTATACCTCAATAACCACCTGG	699	X50
1	77254313	AAGATCCAGAAGAATCCCCTGG	700	RNA41
1	78181978	AGGATACCTGGAAACCCTACTGG	701	X60
1	83689742	AAGATAACTAGCTAGCTCTCTGG	702	X60
1	89670639	GAGATACTTGAATAAACTTCTAT	703	X60
1	93001069	AAGATACCTGACTAACCTGTTTT	704	X60
1	97782174	TAGATACATGATTAAACCACTGC	705	X60
1	110981606	TATATACATGAATAACTCCGG	706	RNA32
1	114341782	ATTATTCTTGAATAACCCCAGGG	707	X60
1	114403243	AAGATACCTGAGAAACTCTGCAA	708	X60
1	114800896	ATGACTGCTGAGAAACCCTCTGG	709	X60
1	114851709	AAGAATCCTAGATATCCCTCAGT	710	X60
1	116440140	AAAATACCAAAAAAACCCTATAG	711	X60
1	118751265	AAGAATTTGAATAACCCTCAAT	712	RNA41
1	119001156	AGGCTAACAGAATCACCCTCGGG	713	X50
1	150002706	GTGATCCTGAGTAAACCTCAGG	714	RNA41
1	156332931	TAGATACCTATAAGCCTCTGG	715	RNA22
1	162745410	AAGATACATGAAAAAAACATTGG	716	X60
1	164962899	AAGATACCTTAAAACCCTTCAGC	717	X50
1	165289582	AATTTACCTGAATAGTCATTAGG	718	X60
1	167577809	AAGATACACAAATAGCCATCAAG	719	X60
1	170883188	AACAAACCTGAATAGTCCTACTG	720	X60
1	175232700	CAGATATCTGAATCACTCTTCTG	721	X60
1	176788495	AAAATATGAATAACCCTTTGG	722	RNA22
1	177848832	CAGATACTGAGTCACCCTCAGC	723	RNA41
1	178104014	AAGTACCTAAATACCCTTGGGG	724	RNA41
1	179507899	AAGATACCATTCTAACCCTCTGA	725	X50
1	180840385	AAGGTAACTAAGAAACCCTCAGA	726	X60
1	183484429	CTCATACCACAATAACCCTCATG	727	X60
1	183743474	AAGATAATGAATATCACCCAGG	728	RNA41

1	185824500	AAGATACTAATAAACACTCAGG	729	RNA41
1	188687384	CAGATACCTGAATAAGCAGAAGC	730	X60
1	194817380	ATAATACCTGAATACTCTCAGA	731	RNA41
1	199542263	TCTATACCTGCATAACCCTAAGG	732	X50
1	201739039	AAGTTACCAGGAGGACCCTCAGG	733	X50
1	203127991	AAGAGACCGGAATGCCCCCTTGG	734	X60
1	209196372	AACAGGCCATAAGAACCCTCTGG	735	X60
1	209428278	TAAATGCCAGGATAAGCCTCTGG	736	X60
1	217518556	TACATAACTGAATAACTCTCCAA	737	X60
1	222014072	AAGAAAGCTGAGTAAGCCCGGG	738	RNA41
1	228023082	GGGATACCCGAATAACCCCACAG	739	X60
1	228546593	GAGACACCTGGGTAACCCCAGG	740	RNA41
1	228724320	GTTATACCTGAATAAATCTGGGG	741	X60
1	230479071	AAAATACCTGCATAACCCTGAGA	742	X40
1	230750818	CAGTTCCCTGAGTCACCCTCAGT	743	X60
1	231757688	AAGATACATGAACTTCCCACAGG	744	X50
1	232190288	AAAATAAGTGAATAACCCACTAA	745	X60
1	233265568	TAGGTACCTGAGTAACTCTGCGG	746	X50
1	235139684	AAGAAACCAGATAACCCACAGC	747	RNA41
1	238895801	AAGATACCTGCATAATCTAGGA	748	RNA41
1	244048003	AGGATACCTGCGTAACTCGCAGC	749	X60
1	245228289	AAGATACCTGGAGATGCCCCTTG	750	X60
2	2541015	AAGATATCAGAATAACTCTGGCT	751	X60
2	5866267	CCTGTACCTGCATAACCTTCTGG	752	X60
2	11192249	AAGATACCTAAAAAGCCCCCAGC	753	X50
2	14318310	TAGATACCCAAATAGCCCCTCAGG	754	DNA41
2	17555591	TCTATACCTAAATAGTCCTCAGG	755	X60
2	18083124	AAAATACCAGAATAACCCATTTA	756	X60
2	18570047	AAGATATCTGAATAAACTCCAAA	757	X60
2	23315873	ATGATTATTAAATAACCCTCAAG	758	X60
2	24850333	AAGATCCCTGATTACCATTCCAG	759	X60
2	28691746	AAGATACCTGGCTCAACCTCATC	760	X60
2	29326137	AATGTGGCTGAAAAAGCCTCTGG	761	X60
2	30453369	AAGATACCTAAACATTCCAGTGG	762	X60
2	31504056	AAGAGAGCTGGACAACTCTAAGG	763	X60
2	33271364	AAGATAGGTCAATAACCACCCGG	764	X50
2	33829479	TAGATAACTGAATAAACTCTATGG	765	DNA41
2	35540632	AAGATGTATTATTGACCCTCTGG	766	X60
2	38119450	TAGAAAACAGAATGACTCTCAGG	767	X60
2	42166900	AATAACCAAAATAACCCTCCAG	768	RNA41
2	46398173	TATGTACCTAATAAGCCTCAGG	769	RNA41
2	49841412	CATATATCTGAATAGCCTTCTGG	770	X50
2	56159084	AAGATACAGAATTCCCCTCTGA	771	RNA41
2	56257553	AAGTTACATGAAAAACATCTGG	772	RNA41
2	57130463	AAAATACCTGAATAATCCTCGAA	773	X40
2	59482506	AAGATACCAGAGAAAGTCTCAGA	774	X60
2	60290320	CAAATACCAGAATACCCTACAGG	775	X60
2	64169496	ATGATCCTAAATAACCTTCCAG	776	RNA41
2	68986545	AACAACATGAATGACTCTCTGG	777	RNA41
2	71741885	AAGATAAAGGTATCTCCCTCTGG	778	X60
2	72415642	AAGCCAACTGAATAACACTGTTG	779	X60
2	72619690	ATGATACCTGAATACCTCTAATG	780	X50
2	75266724	AAGCCAACTGAAGAGCCCTTGGG	781	X60

2	80321182	AAGAACTTGATAAACCCTCAAG	782	RNA41
2	87443936	TTGACACCTAAATAACCCTTTGG	783	X50
2	98349660	AAGAGACCAGAAGCAACCTCTGT	784	X60
2	100696913	AAGAAACATGAACAGAGCCTCAGG	785	DNA41
2	104408132	GAGATACCTGTATAAGTCACTAG	786	X60
2	108664444	AAAATGCCTTAAGAACCCTGGGG	787	X50
2	111506561	TTGACACCTAAATAACCCTTTGG	783	X50
2	112735695	TAGATACTCAGTAACCCTCTTG	788	RNA41
2	115162717	AAGAGACAGGAATAACCCTCAGC	789	X40
2	121516112	AAAATACCATGTAAAACACTCTGG	790	DNA41
2	122492850	GAGATACAGAATAGCCCTGGGG	791	RNA41
2	124401576	AAGAACCTAAATCACCATTGGG	792	RNA41
2	128856709	AAGATATCTGGATAATCCACCAA	793	X60
2	141714040	AAGATACTGGAATAAACATAAAG	794	X60
2	143719680	AAAATGCCTCAATATCTCTGTGG	795	X60
2	144191771	AAAATACCAAAATAACCCTAAAG	796	X50
2	149423627	TAGATACCTGAAATACTCTATGC	797	X60
2	153591411	AAAATACCATAATTTCCCTCAGA	798	X60
2	154142624	AAGATACCAAATAAACCTCTGA	799	RNA31
2	155791011	AAGATACCTAAAATACCTATTGG	800	X60
2	155965426	CAGATACCTGAAAAACTCTCTCT	801	X50
2	157734996	AATAAACACTGAATAACCCTTTGT	802	DNA41
2	163699397	CAGCTACCTAATTACCCTCTGG	803	RNA31
2	163782922	TAGGTACCTGATTTACCCTGATG	804	X60
2	166944919	AAGATACCTCAGGAACCAGCAGC	805	X60
2	168821195	AAAAGACCTAAAAAGCCCTCTGG	806	X50
2	169944031	TCAATTACTGAATAACCCTCTTG	807	X60
2	170941666	AATATACATGAATAAATCTCACA	808	X60
2	173215359	AAAAAACGTGAAAAACCTTAGGG	809	X60
2	174371138	AAGATACCTGTTAGCCCTCACC	810	RNA41
2	176698280	AAGTTTCATGCATAAAACTCTGG	811	X60
2	180424100	AAGGTACCTGAAGAACTCAACTG	812	X60
2	181934742	AAGATGTATGAATAACTCTCGGG	813	X40
2	183685747	AAGCAACCTAAATGTCCATCAGG	814	X60
2	183737398	GAGATACCTGGATAACCACTTGG	815	X50
2	184251994	AAGACACCTGGAGAACTCTCGGG	816	X40
2	188364288	AAGTAACCCAATAACCCTGAGG	817	RNA41
2	188883471	ACAATACGTGAATGAATCCTCTGG	818	DNA41
2	191075654	AAGATTCCAGACATACACTCTGG	819	X60
2	191371557	TAAGTAGCTGTATAACCCTGGGG	820	X60
2	195665474	TAAATACTTGAAAAACCCACAGA	821	X60
2	196276026	AAGTTATCTGACTAACAATCTGT	822	X60
2	196963744	AATATACATGAATTTCTCTTAGG	823	X60
2	197268832	AAGATGCCTCCCTAACACTGGGG	824	X60
2	197475333	TACATACCAACAACCCTCTGG	825	RNA32
2	198342120	AATAGAGCTGAAAAACCCTCACT	826	X60
2	198435701	AAATTAGATGAATAACCCTTAGT	827	X60
2	202129138	AAAATACCTGATACCCCCTGG	828	RNA32
2	202797188	AGAGTACCTGAATACCTCTGG	829	RNA32
2	203165696	AGAATATCTGAATTACCCTCAAG	830	X50
2	204928058	AAGATACATGAGTAAACCCAGC	831	RNA41
2	206764957	AGGAGACCTGCACAGGCCTCTGG	832	X60
2	216172775	AATTTATATGAAAAACCCACTGG	833	X60

2	217541307	AAGATACCTGATCAATCCTCTTC	834	X50
2	217737466	AAGATACCAGACCAAAGCTCAGG	835	X50
2	220751309	TAGAGACATGAAAAACCCTCCAA	836	X60
2	221762944	AATATTCCTGAATCCCTCGGG	837	RNA22
2	226128392	CTCATACCTGAATAACCTTCTCA	838	X60
2	226932531	CAGATACCGAGAAAAACCCTCTGG	839	DNA31
2	227560446	AAGATATATGAAAAAGCCTATGC	840	X60
2	228586979	CAGATACCTCACCAACACTCCAG	841	X60
2	232730167	CAGAGACCTGAAAAGCCCTGCCG	842	X60
2	240208622	AAAAGGCCTGCAGAAACCTCTGG	843	X60
2	241728292	CAAATCCTTGAATCACCCCCAGG	844	X60
2	241778506	AAGATACCTGGCCACACCCCAGG	845	X60
3	539550	GAGATAATGAATAGCCCTCCGG	846	RNA31
3	7611430	AAGATACCTGTCATCCCTCCGT	847	RNA41
3	8738135	GGGATACCTGCATAACTCTCAGG	848	X40
3	11156153	GAAATGCCTGAATCACACTCGAG	849	X60
3	11683514	AAGATACTGAATCAACTTCAGT	850	RNA41
3	16474484	AAGATACCAGTACACCCCCAGG	851	RNA41
3	19871463	ATGATACCTCAATAACCCTATTT	852	X50
3	25070750	AAAATACCTGAACATACCTTGAG	853	X60
3	35461847	TACATACCTAACAACCCCTCAGG	854	X60
3	35699317	AAGAAACAAGGGAAACCCTCAGG	855	X60
3	36482333	AAGATACCAGGATGGCTCTCTGC	856	X60
3	37570248	CAACAACCAGAATAACCCACAGG	857	X60
3	37680767	AAGATTAAAAGAATAAACCTCAGG	858	DNA41
3	38875399	AACTTACCTGCAAAACCATCTGG	859	X50
3	56933123	AAGATCTGAATTACCATCAGT	860	RNA32
3	61401423	GAGATCCTTAACAACCCTCAAG	861	RNA41
3	64532843	AAGATATCTGAGCCACACTCTCG	862	X60
3	68532737	AAGCTACCTGAAAACCTTCAGG	863	RNA21
3	70442317	AAGATGACTGAAGAAACCTTGGG	864	X50
3	70620477	AAGATCCCTGCAAACCCTTTTGG	865	X60
3	71429771	AAGACAGCTAAATCACCCCACGG	866	X60
3	71714408	AAGATCACAGAATCACCCTCCTC	867	X60
3	74054464	AATAGACATGAATAACCCTAAGA	868	X50
3	76238395	CAGACACCTTACTAACTCTCCTG	869	X60
3	82942470	AAGATACACAAATAAACTTCTAG	870	X60
3	83311768	AAGATATCTGAAAAACTCTCAAA	871	X50
3	84371314	AAGACACTGAATAACCCACAGA	872	RNA31
3	86668690	TAGATAGCAAATAACCCTAGGG	873	RNA41
3	98032158	AATAGACCTAAATAACCTCAAG	874	RNA41
3	99319950	AAGTTACCTGACTAACCCTGAGG	875	X30
3	100809940	AAGATATCTGAATAACTACAAGG	876	X50
3	107069791	AAGATACTTGGATAACTCTGCTA	877	X60
3	107509328	AAGAGGCTGGAATAAGCCACGGG	878	X60
3	111016912	AAGATACATAAATATCACTGCAG	879	X60
3	118950239	CACTAACCTGAATAGCCCTAAGG	880	X60
3	120988045	AATATACCTGAAAAACCATCAGG	881	X30
3	121667776	AAAATACCTAAAGAAATTTCAGG	882	X60
3	122037269	CGTGTACCTGAAAAAGCCTCAGG	883	X60
3	126759212	GTGAGGCCTGAAGAACACTCTGG	884	X60
3	133985020	AAGTTACTGAATATCCCTCCAG	885	RNA31
3	140863741	AAGAAACCGGAATGAGCCCCCAG	886	X60

3	141050688	CAGATACCAGAAGCACCCTCGAG	887	X50
3	142685343	TAGATAACTGAAATATCCCTCTGG	888	DNA31
3	143875802	GAGATACCCAAATAAACCTCAAA	889	X60
3	151175376	AAGATACTGAATAAAGCTCTTA	890	RNA41
3	151226712	AAGATAACTGAAGAAACTGAAGG	891	X60
3	151395707	AAGATACCAGGGTAACTCTATGA	892	X60
3	155083487	AAGATACCTGAATATCCAACCTA	893	X50
3	161319971	AAGCTGACTGAAGAGCCCTTGGG	894	X60
3	168443226	AGTATTTCTGACAAACCCTCAGG	895	X60
3	169534358	GAGATAGCTAATAACCCCCTGG	896	RNA31
3	171933625	AAGACAACTGAAAAACGCTGTGG	897	X50
3	173995167	AAGACACCTGAATAATCTCTGGA	898	X60
3	176890998	ATGATAAGTGAATAAGTCTCATG	899	X60
3	177768689	AGGATATATGAAAAAGCCTGCGG	900	X60
3	187379619	AAGATACCCTAAAAGCCTTTGG	901	RNA41
3	188484179	AAGATGCATGAATAACCTCTGGA	902	X60
3	190699564	ATGACACCAGAAAGACCCTAAGG	903	X60
3	192710228	AAAATACTAAATAACCCCATGG	904	RNA41
4	723653	CAGATTCCTGGAAGACCCTCAAG	905	X60
4	11334933	AAGAGACCTCAAGGTCTCTCTGG	906	X60
4	12971711	AAGACTAAAGAATAACCCTCTGG	907	X50
4	15327544	AATATGCCTACATTACACTCAGG	908	X60
4	16411507	AAGACACTGAATAACCCTTGGC	909	RNA31
4	20157051	AAGATAGTGAGTAAGTCTCAGG	910	RNA41
4	23244775	TAGATAAGAATAGCCCTCAGG	911	RNA32
4	26200297	TCTATACCTCAATAACCTCCTGG	912	X60
4	28742386	AAGATACCTAAGTGCTCCACAGG	913	X60
4	29008961	AAGAAACCTACAATAACCTTCATG	914	DNA41
4	33579022	CCGATATCAGAATATCCCTTAGG	915	X60
4	44748785	AAGATGCATGAAAAAGCTTCAGT	916	X60
4	44812393	TAGATATCTGCAGAACTCTCCGC	917	X60
4	45258647	AACATACATCAATAACTTTCTTG	918	X60
4	46495164	AAGATACTTGAATACCAATCAAG	919	X50
4	47192935	TAAAGACCTGAATAACCTCAAG	920	RNA41
4	47840228	AAGATATTGAATATACCTCAGG	921	RNA31
4	51965380	TAGTTACCTGAATACACCTCTGG	922	X40
4	66291743	TATATACCTGGAAAACCCTAAAG	923	X60
4	74170265	TAGATACATAATAACCAACTGG	924	RNA41
4	84374124	GAGATACTTGAATCCCTCAGG	925	RNA22
4	87976264	AAGATACCTGTATGACACTGTGT	926	X50
4	88117912	AAGATACCTAAATAACAAGCTGG	927	X40
4	90779595	AGGATACATGCATAACCCATGAG	928	X60
4	91244542	AAGATCCAGAATAATTCTCTGG	929	RNA31
4	91936467	AAGATACATGAAAAATTCTCTCA	930	X60
4	93373949	AATATACCCTAATAAGCCTTCTG	931	X60
4	93711948	AAGATGGCTGAATAGAACCTTCCAG	932	DNA42
4	96659665	AATATACCTTATTATCCTTCAGA	933	X60
4	100450684	CAGATACAAGAGTACTCCTCAGG	934	X60
4	101268626	TCTATACCTAAATAAACCTAAGG	935	X60
4	104738004	TAGATACCTCAATAATTCTCTCT	936	X60
4	115433239	AAATAACATAAATAACCCTCAGA	937	X60
4	115953135	CAGAAGCTGGATAGCCCTCGGG	938	RNA41
4	136101368	AAGAACATGAGTAACCCTAAGG	939	RNA31

4	136644335	AAGATACTTGAAAAACACCAGGT	940	X60
4	136853658	AAGAGAACTGAAAAGCCTCAAG	941	RNA41
4	144234750	AAGATACCAAATAACTCTCTGG	942	RNA21
4	146835606	AATATGCCTGTAACCCTCAAG	943	RNA32
4	149064984	AAGCTGACTGAAGAGCCCTTAGG	944	X60
4	155932126	AAAATTCATGAAGAACCCTGGGT	945	X60
4	157312624	AAGATACGTGAATAACTCAGGAT	946	X60
4	159332385	AAGATATCTGAACAATTCTAAGG	947	X50
4	160192361	AAGATACTTGAATCACTGTCAGT	948	X50
4	168412887	AAGATCCTGAATAATTCTCTGG	949	RNA21
4	170886954	AAGAAGCCTGGATAGAACTCTGG	950	X60
4	172543878	TTGATACCTGAATAACACTCCAG	951	X40
4	172763007	AAAAAAAATGAATTACCCTCATG	952	X60
4	174561024	GAGATCCAGAATTACCCTTGGG	953	RNA41
4	181671436	AACATACCTAAATAACTCCTGGC	954	X60
4	184625030	AATCTACCTGATGACCCTCGGC	955	RNA41
4	186075007	AAGTTAACTGTAAAACAGCCTCAGG	956	DNA42
5	6252615	AAGAAATCTGAAAGCCCTGGGG	957	RNA41
5	7102627	GAGATACCTGCATAACACTTAAT	958	X60
5	7274614	CAGATAACTGAACAACCAGATGG	959	X60
5	7657678	AACTTAGGTGATTAACCCTCACG	960	X60
5	8704833	ATGATACTGAATAAGTCTCAGG	961	RNA31
5	9774717	AAGATCCAGAATAATTCTCTGG	929	RNA31
5	13575913	AGAATACCTAGTGACCCTCAGG	962	RNA41
5	16123391	AACAGACCTTCATAGCCCTCAGA	963	X60
5	16404802	TATATACCTAAATAACCTAGGG	964	RNA41
5	20733472	AAGATCTCTGAAATGCCCTGAGG	965	X60
5	23248793	AACTTAACATAATAACCTTCAGG	966	X60
5	34351741	ATGATAGTGAATAATCCTCATG	967	RNA41
5	35773444	AAGATACGAGAATAACCTCAAGA	968	X60
5	35946321	AAACTACCTGGATGACCCTCTGA	969	X50
5	36662538	AAGAAGATGAATTACTCTCTGG	970	RNA41
5	42904833	AAGATACAGAGTAACTCTTGGG	971	RNA41
5	43693099	GAGATAGCTGAATAATCCCTCAG	972	X60
5	45541710	AAGATACATCAGTAAGCCACAGT	973	X60
5	55453226	AAAATACATTAATAAAACTAAGG	974	X60
5	58609559	AGTAGAGGTGAATAACCCTCAGC	975	X60
5	60295257	CAGATACTTAAATAACCATAAGG	976	X50
5	61555769	AAGAACCTGACAAGCCCTCAGA	977	RNA41
5	66999062	AAGATCCATCAATAAACCACGTG	978	X60
5	69412657	AAGATACTTTTAAAACCCAGTGG	979	X60
5	73013090	AAGATCCTGAATTACTCTCAAG	980	RNA31
5	73504738	AAGATACCTGAACAACTCCACAC	981	X60
5	75225969	AAGCTGACTGAAGAGCCCTAGGG	982	X60
5	78444765	TTGATACCTGTATTACACTCATG	983	X60
5	82799696	AAGATACAGAGAATAACCCTCACA	984	DNA41
5	83300276	AAAATACCTCACTAGCTCTCCAG	985	X60
5	85150663	AAAATAATTGAAAAACTCTCTGT	986	X60
5	87098398	ATAAGAACAGAATAACCTTCTGG	987	X60
5	91842929	AAGAGTCATGCATAGCCCTCCAG	988	X60
5	105774459	AAGATACTGAATAAACTCCTGC	989	RNA41
5	106733933	AAGAAACACAAATAACCATCAGA	990	X60
5	108874504	GAGATGCTGAATAACTCTCTGG	991	RNA31

5	112311434	AAGATACTGAGTATCTCACAGG	992	RNA41
5	112422645	AAGACACCAAGATAGCACCCTCGGG	993	DNA42
5	113833519	AGTATACCTTAATACCCCCTGG	994	RNA41
5	114129926	ATTATACCTAATAACCCTATGG	995	RNA31
5	117372593	AATATAGCCCAATATCCCTCATG	996	X60
5	118452920	CAGATACCTGAATGATCCTTTTC	997	X60
5	119760952	AAGAAAGCTGAGTAGTCCTCAGA	998	X60
5	132792147	AAGGGACAGGAAGAACCCTGAGG	999	X60
5	132904762	AAGATAGCTGTATAACCTTTATC	1000	X60
5	137163670	AAGTTAGCGGAACTCACCCTCAGG	1001	DNA41
5	137764269	AAGAGAGCTAGCTAGCCCTCTGG	1002	X60
5	142640990	AGGATTTCTGGATAACACTTTGG	1003	X60
5	143734240	GAAGTACCTGAAAAACCCTCTGC	1004	X50
5	148112086	GAGGTACTTGAATCACCATCAAG	1005	X60
5	151722432	TAGCTTACTGAATAACCCTAAGG	1006	X50
5	152708508	AAGAGGCATGAATAACCCTGTTC	1007	X60
5	153529703	CAGTTACGAATAACCCTCAGA	1008	RNA32
5	156251323	AAGATACTGAAAAACCCTCTGA	1009	RNA21
5	160330029	AACAGACATGAAGAAGGCTCAGG	1010	X60
5	163829739	ACCATACCTGAAGGACCATCTTG	1011	X60
5	164401431	AAGAGACCTTAAAACTCTCTGT	1012	RNA41
5	165782539	ACAAGACCAAAATAACCCACAGG	1013	X60
5	172047699	ACGATGGCAGAACAAACCTCAGG	1014	X60
5	173042104	AAGATACCTCAAACACCAGCCGT	1015	X60
5	175751428	AAGACACCTAAATATCCCTGGGA	1016	X50
5	177550455	AAGATACTGACTAATCATCTGC	1017	RNA41
6	2810238	TAGACACCTGAGTCACCCTCTTA	1018	X60
6	3633347	GTGATACCAGATTAACCTCTGG	1019	RNA41
6	3993855	AGAGTAACTGAATAACCCTACAG	1020	X60
6	6543792	TTTGTACCAGAATAACCCTCCTG	1021	X60
6	8845584	AAATTAACTGCATAACCCTGGGT	1022	X60
6	12146644	AAAAAACTTGAATTACCTTCTAG	1023	X60
6	13274573	CTGAAACCTAAATAATCCCCCGG	1024	X60
6	15494007	AAGACCCAGGAATCACACTCTGG	1025	X60
6	16159742	AGAAGACCTGAATACCCCCAGG	1026	RNA41
6	17837365	CAAATACCTAAAGAACTCTCCTG	1027	X60
6	18241159	CCTATACCTAATAACCTTCAGG	1028	RNA41
6	20501819	AAGATACCTGGAAAACTCCCAAA	1029	X60
6	22811364	TAGACACCTAATAATCCCCAGG	1030	RNA41
6	24130572	GTGATACCTGTCAGACCCTCGGG	1031	X60
6	25968782	AAGATACCAGAATTCCCCTCTTC	1032	X50
6	26726581	AAGATGGCTGAGAAAAACTCAGG	1033	X60
6	26775081	AAGATGGCTGAGAAAAACTCAGG	1033	X60
6	28642665	CGTGTACCAGAATCACCCTCAGG	1034	X60
6	28976992	AAGAACTTGTAGAACCATCAGG	1035	RNA41
6	31728324	AAGATACCCCATCACCCCTCCCG	1036	X60
6	31925117	AAGATCAGCAGAAGAACCTTCTGG	1037	DNA41
6	32569518	AGTATACCTAAACAAACCTCAGA	1038	X60
6	35414348	AAGATCCTGCATACCCTTCTGT	1039	RNA41
6	39979448	AAGATCCAGATTAACCCTCTAG	1040	RNA31
6	40805097	AACATCCCTGAAGACCCTTGAGG	1041	X60
6	41733389	AAGATACCTGGGTCCCCATTTGG	1042	X60
6	42264231	AAGATCCTTAAATACCCTCTGG	1043	RNA31

6	42676866	AAGATAACTAACTCACCTTCCCG	1044	X60
6	45506487	ATGATATATGAACAACCCTCTGG	1045	X40
6	45565147	AACATACTCGAATACCCCTCAAA	1046	X60
6	45812353	AGCTTACCTGCTTAACCCTCAGG	1047	X50
6	45989719	AAGATACCAAAATTCCCCAATGG	1048	X60
6	46206185	ACGATACTGGAATAAGCCTTTGA	1049	X60
6	51499203	CAGTACCTCAACAACCCCCTGG	1050	RNA41
6	51857715	AATATCCTGAATAAACCTCTAG	1051	RNA31
6	57915493	AAGACACCTGATAAAAACTCTGT	1052	X60
6	62452292	AAGATACAGAATAAACCTAAGG	1053	RNA31
6	66308858	GAGATACTTGAACAACTCACTGG	1054	X50
6	66899926	AAGAAACCAGAGAAACCTTCGAG	1055	X60
6	71004311	AAAGTACCTAAATAACCAACCAG	1056	X60
6	76467643	AAGAAGGTGAATAACACTTAGG	1057	RNA41
6	78402401	AAGATACCTGGCTAGCCATATGC	1058	X60
6	79368236	AAGCTACCAGAGGAACAATCAGG	1059	X60
6	84651006	CATTTACTGAATAACCCTTTGG	1060	RNA41
6	84877021	GATAAACATGAAGGACCCTCAGG	1061	X60
6	89636030	AACGTACCTGAACAACCCTCAGG	1062	X30
6	90026983	ACACTACCTGAATGACCCTCAAG	1063	X50
6	91316610	AATATACATGAAACACACTTAGG	1064	X60
6	100261928	AAGATACCTAAATGTCCATCAAC	1065	X60
6	101477606	CAGATATGAATAAACCTCTAG	1066	RNA32
6	104427293	AATAGTACTCAATAAACCTCTGG	1067	X60
6	105452361	AGTATACATGAATAGCCCTCAGG	1068	X40
6	113288211	TTGCTACCTGAATAACCCTCTTG	1069	X40
6	125169983	AAGATATCTGAGGAAGCGTCTTG	1070	X60
6	125494646	AAGATACCTGAAACCCTCTGG	1071	RNA02
6	126401280	CAGATATTGAAAAACCCTCTGG	1072	RNA31
6	127951944	AAGGTATCCAAATAACCCTTTGG	1073	X50
6	133766716	TAAATACCTGAACAACTCTAGGG	1074	X50
6	134020048	AAGATTACATGAGTACCCCTCCTG	1075	DNA41
6	141080346	ATGATAGTGAATAACTCTCTTG	1076	RNA41
6	141657228	AAGATATCTGAAAAATCCACAAA	1077	X60
6	142866995	AAGATTTCTTATAACCCTCTGG	1078	RNA31
6	146355335	AAGATACTCTGAATAACTTTCTGG	1079	DNA21
6	147553748	AAGTCATCTGAATAACCCTCAAG	1080	X40
6	152311384	AAGATACGTGCAATAACTATCAAG	1081	DNA41
6	158194025	AAGATACATTAGTAACATTTTGG	1082	X60
6	159249441	AAGAAACACTTAAAAACCATCTGG	1083	DNA41
6	159877092	TAGATACCTGTATAACCACCACC	1084	X60
6	163714704	AACTTACCTGAACATGCCCCTGG	1085	X60
6	166082374	TAGATACATGGATTAACTTCTGG	1086	X60
6	168015970	AAGACAGCAGTAAAACACTCTGG	1087	X60
6	168145686	AAGATCTGGAAGAACTCTCTGG	1088	RNA41
7	5143278	AAGAAAGGTGAGCAACACTCGGG	1089	X60
7	5180429	AAGATACTTTAAAAAACCCCTGC	1090	X60
7	12098480	AATATACCTAGCTAAGCCTCAGA	1091	X60
7	16189181	AAGATAGCCTGAGTCACACTCTTG	1092	DNA41
7	17175165	AAGAAAACAGCGTAAGCCTCAGG	1093	X60
7	18301478	ATGAAACCTAAATAAGTCTCTGG	1094	X50
7	18326887	TGTTTACCTGAATAACCTTCAGG	1095	X50
7	20063992	AAGATATCTGAAAAGCCCACTTT	1096	X60

7	21393775	TGTAAACCTGGATAACCCTCTAG	1097	X60
7	22673635	AAGTATCATGAATACCCTCAGG	1098	RNA41
7	25237966	AAGATCCTATATAACCCTCTGA	1099	RNA31
7	28451369	AAAATACCTGAATAACCTGGGCC	1100	X60
7	36309411	AAGTTACCAGAACAACCCTTAGG	1101	X40
7	43099959	AAGAATTTGAATAACTCTCTAG	1102	RNA41
7	45063943	AGGATACCTGAATCCCTCCAG	1103	RNA22
7	46108303	GAGACACCTGAATAAGTGTCTGG	1104	X50
7	46457470	AAGCTAGTGAAAAACCATCAGG	1105	RNA41
7	47819173	AGGATACTTGAATATCCCTGCCT	1106	X60
7	49066063	CAGATACTGAAAAACACTCTGG	1107	RNA31
7	49306954	AAGAAATCTGGATAACCCCCAAT	1108	X60
7	60892285	AACATACCCAAAAAAACCTCTGA	1109	X60
7	61597579	AACATACCCAAAAAAACCTCTGA	1109	X60
7	62026418	AACATACCCAAAAAAACCTCTGA	1109	X60
7	78658625	AAAATATCCAAATAACCCCCTGG	1110	X50
7	81886792	AAGATATCTGAATAATCATCAGG	1111	X30
7	88664778	AAGATACTGAGTAACACTGAAG	1112	RNA41
7	89093321	AAGTTACCTAAATAACTTTGG	1113	RNA32
7	89117479	ACCATAATTGATTAACTCTCTGG	1114	X60
7	94742185	AAGAAACCTAAGTAAACCTCTAA	1115	X60
7	96159184	AAGATACATCATCACCCTCCTG	1116	RNA41
7	96499495	AAGACACATAAGGAACCCTCATG	1117	X60
7	101395824	GAGATACCTGAGCTGCCCTCAGC	1118	X60
7	104765414	GAGATACCTAAATAACCTTCCAG	1119	X40
7	107527319	AAGATACGAATAATCCTCCGA	1120	RNA22
7	114126788	ATTAAACCAAAATAACCCTCGGG	1121	X50
7	117090821	CACAAACCTTAATAACCCTCAAG	1122	X50
7	117393513	AAGATAAAAAAATACCCCTAGGG	1123	X60
7	123985680	AAGATATCTGAAAAAGTCTTGAG	1124	X60
7	124105262	GAGATAACTGAATAACCATTTGG	1125	X40
7	125205185	AAGATATCTGTAAAACCCTTCAG	1126	X50
7	125899502	AACATACATGAAAAATCCTCAAC	1127	X60
7	127268120	AAGGTAACTGGATAGCCATCTGC	1128	X60
7	132695120	AAGAGACCTGAGTCACCCTGTCT	1129	X60
7	134060777	AAGATAAATGAATTTCCTTCTGT	1130	X60
7	140628498	AAGATACGGGAAATCCCCTTTGG	1131	X60
7	146763269	CAGATATCAGATAACCCTATGG	1132	RNA41
7	153436410	CAGATGCTTGTAAAACCATCAGG	1133	X60
7	153479243	ACAGTAGTTGCATAACCCTCTGG	1134	X60
7	155749430	AAGATTCAGCACTAACCCACAGG	1135	X60
7	157980250	GAGATACTGAATTACCCTCTGG	1136	RNA21
8	4038549	AAGAGACATGCATAATCTTCAGG	1137	X50
8	5643554	AAGTAAACTGAATACCTTCTGG	1138	RNA41
8	7420667	AAGAAACCACTATAACCATCAGT	1139	X60
8	7890852	AAGAAACCACTATAACCATCAGT	1139	X60
8	19687879	CAGATACTGTATAACCCTCAGA	1140	RNA31
8	21016005	AATGTAAATGTATAACCCTCTGT	1141	X60
8	29936242	AAGTTAACTGTAAAACAGCCTCAGG	956	DNA42
8	36495615	AGGATACCATAATAACCAGCATG	1142	X60
8	43072291	GCAATACCTGAATAACCCATTGT	1143	X60
8	47579895	AAGATACCAGACCATCTCCCAGG	1144	X60
8	49597827	AAGATCTGAATAGCACCCTGG	1145	RNA32

8	55071567	AAGATCACAGGACAACCCTCCGC	1146	X60
8	55401326	AAGATTCATGATAACCATCATG	1147	RNA41
8	60813307	CAGGTACCTGGAGAACCCTAGAG	1148	X60
8	61779630	AAGATACCTGAACACCCTACTGG	1149	X40
8	64267393	TAGCTAGCTGATAACACTCTGG	1150	RNA41
8	65828227	ATTATACTGAATAACCTTCCAG	1151	RNA41
8	66202960	AAGTTACCTGCATCAAACTCATG	1152	X60
8	66947451	AAGATACAGAATAACCCTCTAG	1153	RNA21
8	67331610	GATATACTCTAATAACCCTCAAG	1154	X60
8	67691390	AAGATACATAAATCACTTTCCAG	1155	X60
8	67694023	AAAATGCCTAATAGACCTCTGG	1156	RNA41
8	68072298	AAAACACCTGAAAAATCCTCCAC	1157	X60
8	73416526	AAGAAACACTGAATGTCCCTCTTG	1158	DNA41
8	74109927	GTGAAACCTAAATAACCCTCAGA	1159	X50
8	75900398	AATATACCAGAATAGTTCTCAGT	1160	X60
8	76479079	AAGATACTGAATATTCCTCAGG	1161	RNA21
8	78222169	GAGATACTTGAATAACCATCTCA	1162	X50
8	80098340	AAAGTACATTAAAAACCTTCTGG	1163	X60
8	80176030	AAGATAATTGAATAGCCATCTTA	1164	X60
8	80682169	AACAAACCCGTAAAACCCTCACG	1165	X60
8	83187784	AAGATAGTTAAATATCCCCCTGT	1166	X60
8	88225407	AAGATAATAAATAACCCTCAAG	1167	RNA31
8	91354172	AAGCTACTTGATAACCCTATGG	1168	RNA31
8	102629148	ATTATACCTGAATCCCTCAGG	1169	RNA22
8	106844104	AAGTTATCCTAATAACCCTATGG	1170	X50
8	106983770	ATGTTTACTGAATACCCCTCTGG	1171	X50
8	108954887	AAGGGACCTGAAATTCCCTGAGG	1172	X60
8	115458181	TAGATAACAGAATGACTCACAGG	1173	X60
8	119894846	AAGATTCTGAATAGACCCCAGG	1174	RNA41
8	120688940	AACATACCCAAATACTCTCAGG	1175	RNA41
8	121544316	AAGATACCTCAATAAGACTCGGG	1176	X30
8	124209633	TAGATACTGAAAATCTCTCAGG	1177	RNA41
8	124951793	AGAATACAAGAATAACCCTGGGG	1178	X50
8	125028680	AAGATACCTGAAATTCCTGCAGA	1179	X60
8	125768967	CTGGTACTTAATAACCCTCAGG	1180	RNA41
8	130261595	GAGATACCTAACACACACTCTGG	1181	X60
8	139484036	ACAATACCTGAAAAACTCCCTTG	1182	X60
8	140142948	CAGATACATTAAAACCCTCTGG	1183	RNA31
8	141381027	CAGAACCCACAATAAGCCTCTGG	1184	X60
9	1548217	AATAACATGAATAACACTCAAG	1185	RNA41
9	2362898	AAGTAACCCAAATAACCCCAGG	1186	RNA41
9	3085581	AAGATACAGAATTTCCCTTTGG	1187	RNA41
9	7499375	AAGGTCCCTGAGCCACCCTCCAG	1188	X60
9	7701793	AAGATCTGGAACAACTCTCTGG	1189	RNA41
9	9668247	AACACTATTGAATAACCATCTGG	1190	X60
9	13025629	GGGATACCTGAATATTCTCTGG	1191	RNA41
9	13825298	AAGATGCCTGCACATCCCTCCTT	1192	X60
9	15566128	AAGATACATGAATTACCAAAAAG	1193	X60
9	18157881	AAGATGCTTGAAGTAGCTTCAGG	1194	X60
9	21582692	TAGATACCTCTTATAACCCTCCAG	1195	DNA41
9	22799849	AAGATACATCAATAAACCTGATT	1196	X60
9	22914887	AAGATAGTCCTATAACTCTCTGG	1197	X60
9	27875283	AAAATTAGTGGATAAACCTCTGG	1198	X60

9	28235239	AAGATCCTGAAGAATTCTCTGA	1199	RNA41
9	29142022	AGAATACCTGAACAACACTCTAG	1200	X50
9	33293321	ATTAAACCTGAAACCCTCAGG	1201	RNA32
9	33907178	AAGATAGCTAAATAACCTCTGA	1202	RNA31
9	36483605	GAGACAACTGATTATCCCTCAAG	1203	X60
9	39480043	AATTTACCAGAATAGCCCTCTGA	1204	X50
9	66885197	AATTTACCAGAATAGCCCTCTGA	1204	X50
9	74963383	AAGATACTGAAATACCCCTATGG	1205	X50
9	81338482	AAGGTAAATGAGTAATCCTTTGG	1206	X60
9	82995745	AAGATTACTTGAAGAAGCCTCTGA	1207	DNA41
9	85121255	AAAGTAGCTGAATAAACCTCAAG	1208	X50
9	88846233	AAGATAACTGAAATGCCTTCGAG	1209	X60
9	89929285	ACTATTCCTGAATAACCCTTTGG	1210	X40
9	94468528	AACATCCTGAATCAACCTCTGC	1211	RNA41
9	96771534	GAAATACTGAGTACCCCTCAGG	1212	RNA41
9	97299066	TAGATCTAGAATAAGCCTCTGG	1213	RNA41
9	100767516	AAGATACCCAAATAACCATCAGA	1214	X40
9	107058386	AAGACAGCTAACTAACTCTCGGG	1215	X50
9	107981569	AAAGTAGCTGAACAGCCCTCAGG	1216	X50
9	109732394	AAGATACCTCATTAATCATCACA	1217	X60
9	110545175	AAGATATGGAATCAACCTCAGG	1218	RNA41
9	112078020	AAGATACCTAATAACCACAGGG	1219	RNA31
9	114633862	ACAGCACCTGAATCACACTCTGG	1220	X60
9	114864429	AAGATACAAAAACAACCGACTGG	1221	X60
9	114970717	AAGATACTCAATAAACCTCAAG	1222	RNA31
9	116496855	ATGATAGTGAATAATTCTCAGG	1223	RNA41
9	123598985	AAGACACATAAACCACTCTCAGG	1224	X60
9	125297490	AAGATACTGGATAACCTTCCAG	1225	RNA31
9	125378327	ATGATAATGAATAACCCTTGGG	1226	RNA31
9	125911093	AAGACACTGAAAAACCCTCAAG	1227	RNA31
9	127691320	AAGATAGCAGATTAGTCCTCAGT	1228	X60
9	127974450	AAGAGATCGGGATAAGCCTTTGG	1229	X60
10	7147046	TAAGTACCCAAATAAGCCTCTGG	1230	X60
10	7993040	AAGATATCTGAATATGGACCAGG	1231	X60
10	11705444	AAGATAGCTAAGAATCCCTCCAG	1232	X60
10	13360457	TAGTTACAAGAATAACCCCAGG	1233	RNA41
10	14735911	TAGATACATAATAGCCCTTTGG	1234	RNA41
10	15941531	AAGAGCCCTGAATAAGCTTTAGG	1235	X50
10	16541599	AAGATACTGAATAAACCCGAAG	1236	RNA41
10	21114219	AAGATATCTGGATAATCCCCAAA	1237	X60
10	27972145	AATATACCTTGAATACCCCTCCCT	1238	DNA41
10	32570562	GGGATACCTTAACAACCCTGTGG	1239	X50
10	35982871	AAAATACCTGACAGAACCACTGG	1240	X60
10	38157649	AAAATACCTGAGTGACCCTAGAA	1241	X60
10	47125548	AAGATTTGTGAATGACCCTAATG	1242	X60
10	50305665	AAGATACCAGTATACTCTCTGG	1243	RNA31
10	51383143	AAGATCCCTGAACAACACCAAAG	1244	X60
10	54571271	AAGATACCAGTCCCACCCTCTTG	1245	X60
10	55277105	TAGATACTCTCAATGACCCTCTGA	1246	DNA41
10	59699528	AAGTTACTTAAATAGCTCTCTGC	1247	X60
10	62667353	AAGATGCTTGAATTTCCCTAATG	1248	X60
10	82421522	AAGAGATCTGGATCACCCCCAGC	1249	X60
10	83225274	AAGATATTTGAATATTCCCTAGG	1250	X60

10	84218985	TAATTACCAGAATAACCCTATGA	1251	X60
10	90306612	AAGATACCTGAAATACCTGGGT	1252	RNA41
10	94280573	AAGGTACCTGAGTAAACATTCTG	1253	X60
10	102517636	AAGATACATGAATGAATTCAGG	1254	RNA41
10	102734271	CAGATACCTAAGAACCCTGACG	1255	RNA41
10	106248382	AGTATAACAGAATAACCCTATGT	1256	X60
10	118171466	AACATATCTGAATATCTCTCTAA	1257	X60
10	119789592	AAGATCCTGCCTCAACCTCTGG	1258	RNA41
10	120632845	AAGATACCAAGCTAATCCTCTGC	1259	X60
10	121862898	GAGATTTCTGAATAACCCACCCT	1260	X60
10	121868681	GAGAGGCCTGAGTAGCCCACGGG	1261	X60
10	122274421	AAGGAAACTGGAGAACTCTCCGG	1262	X60
10	130321175	AAGATACTTGAATAACCATTTCA	1263	X50
10	131549720	TGCTTACATGAATAACCCTCCAG	1264	X60
11	2376222	TCTGTACCTGGATAACCCCCTGG	1265	X60
11	3277015	AAGGTAACTGACAAACCATCTGA	1266	X60
11	3322831	AAGGTAACTGACAAACCATCTGA	1266	X60
11	3502236	AAAATAAATGAATAAAACTGTGG	1267	X60
11	4864723	AAGTCACCAGTTTAACACTCAGG	1268	X60
11	7440757	AAGATACCAGAATAAATCATTCTAG	1269	DNA42
11	12052022	TAAATACATGAATAACCATTAGG	1270	X50
11	12138824	AAAAGCCCTGATCAAACCTCAGG	1271	X60
11	12410906	CACTTACCTGTATAACCTCAGG	1272	RNA41
11	14162519	AAGATACTTGCAGAACCATCTGA	1273	X50
11	15458757	AAGATCTGAATTTCCATCAGG	1274	RNA32
11	22689538	AAGATATACATACTAACCCTCTGG	1275	DNA41
11	22781693	GAGTACCATAATAAACCTCAGG	1276	RNA41
11	22831675	AAAGAACCTAAATAAGCCTCTTG	1277	X60
11	25615424	AAGACATTAAAATAACCCACAGG	1278	X60
11	25752459	AATATACCTGGAAAACCCACACC	1279	X60
11	26574272	AAGACACATGAATATCACTAATG	1280	X60
11	26949218	AAGATATTGAATAACCCACATT	1281	RNA41
11	26997914	AACATCCCAGAATGACCCACAGC	1282	X60
11	28826023	AAGAAAGCTGGCTAGCTCTCTGG	1283	X60
11	32956605	AAGATAACTGACTTGACCCTGTGG	1284	DNA41
11	36609914	AAGATATGAATAAAACTCTGG	1285	RNA22
11	50574461	AAGCTATCTGAGAAACCCTTTTG	1286	X60
11	58705310	CAGATACCTAATAACCACACGG	1287	RNA41
11	63223967	AAGATACCTGAAATTATTTCAGG	1288	X60
11	79064018	TTGATACCTAAATACCCTGTGG	1289	RNA41
11	80186841	TAAATACATGAATAATCCTCGGG	1290	X40
11	81449300	AATTAACCTACATAACCCTCAGT	1291	X60
11	83977145	AAGATTCTTAAATAACCCACTTT	1292	X60
11	87592156	TAGAGACATGAAAAACCCTCCAA	836	X60
11	88339518	AAGATTTAAGAATGAACCTCAGG	1293	X60
11	88892935	AAGATACAGAATTTCCCTTTGG	1187	RNA41
11	92568878	TGGATACCTGTCAACCCTCTGG	1294	RNA41
11	101689415	AAGATCCGTGAATAAACCACAGC	1295	X50
11	104364397	AACATACACGAATACCTCACAGG	1296	X60
11	106039497	AAGATGATAAACAACCCTCTGG	1297	RNA41
11	109887670	TAGATCCTAATTAACCCTCAGA	1298	RNA41
11	114155888	AAGATACAGGTGTAGCCCTCCTG	1299	X60
11	117119305	CTGATATGTGAAAAACCCTAAGG	1300	X60

12	3649402	AACATACTGAACCAGCCTCTGG	1301	RNA41
12	3696467	AAGACACGAGAAGAACCTTCTGA	1302	X60
12	3830100	AAGACACTTGAATACCACTCTTT	1303	X60
12	4704350	TACATACCTTGATAACCTTCAGG	1304	X50
12	8120901	AAGTTACCTGAACAAACCTGCAA	1305	X60
12	9334202	AAAAAACTAGAAGAACCCTTTGG	1306	X60
12	10687002	AAGATCCCTGAATTTCCATCAGA	1307	X50
12	14689862	AAAATACCTGCATACCCTCAGG	1308	RNA21
12	20320965	AACTTACATGAATTACTCTTAGG	1309	X60
12	22372837	AACATTTCTCAATATCCCTTTGG	1310	X60
12	26607410	CAGATAACTGAATAACCCTCTGT	1311	X30
12	28330833	TAAATACCTATAACCCACTGG	1312	RNA32
12	28550781	GAGATACCTGAATATTCTTCCAA	1313	X60
12	32209572	ATTATATCTGAATATCCTCTGG	1314	RNA41
12	45545836	ATGATACCCAATACCCCTCCAG	1315	RNA41
12	47016633	CAGATACCTGAATCTCCTTCTTT	1316	X60
12	49068780	CAGTTACCTGGATAACTCCCTGG	1317	X50
12	54882194	AAAATACTAGAATAACCAGCTTG	1318	X60
12	61361291	AAGACTCAGGAATATCACTCAGG	1319	X60
12	66409389	AAGATCCAGAAGAATCATCTGG	1320	RNA41
12	72361250	AAAACACCTGAATAAATCCCATG	1321	X60
12	73122159	AACATCACTGAAGAACCCTGAAG	1322	X60
12	74390201	AAAATACCTCATAGCCCTCTGC	1323	RNA41
12	77975069	AAAATAGCTTAATAATGCTCAGT	1324	X60
12	78463857	AAGTTCCCTGAACACACCTCTGG	1325	X50
12	79756510	ACTATACTGACTAACCCTCTAG	1326	RNA41
12	79913270	AGAATATCTTCATAACCTTCGGG	1327	X60
12	81976639	AGAAAACCTGAATAACCTATGG	1328	RNA41
12	87296671	AAGATATTCAAATAACCCTGCTG	1329	X60
12	98856708	GAGATACTGAATAACCTCTGGG	1330	RNA41
12	105221258	AAGATAGCTGCTAACCCTGAGT	1331	RNA41
12	106415644	AAAATAAATGAATAACCATCAGA	1332	X50
12	109568847	AAGGTACTAGAAAACCCTGTGG	1333	RNA41
12	124304548	AAGACACTGCCAAACCCTCCGG	1334	RNA41
12	128916634	AAGATACATGAATGGCCAACGCG	1335	X60
12	131173514	CAGAGACCTGCATAGCACTGAGG	1336	X60
12	131502294	AAGATATCTGAAAATCCCTGCGC	1337	X50
13	20297900	AAGAACACTGAGAAAACCCTCTGG	1338	DNA41
13	22964102	AAGAAACCTCCATATCCATCGTG	1339	X60
13	24389118	AAGAAACCTCCATATCCATCGTG	1339	X60
13	25266108	AAGGTACCTTAACAACATTTTGG	1340	X60
13	26448879	AAGGTGACTGAAGGACCCTTGGG	1341	X60
13	26553083	ACGATAACTGAACAGCTCTCAAG	1342	X60
13	34458374	AAGAGGGCTTAATAACCTTCAAG	1343	X60
13	39980150	AAGATAACTGGATAGCTCTATGA	1344	X60
13	43747762	AAGATTCAGCAATACCCTCTGG	1345	RNA41
13	50325631	CAAAAACCAGAAAACCCTCAGG	1346	RNA41
13	60126783	CATATACCAAAATAACCCATGGG	1347	X60
13	61481200	AAAATACAAATAACCCTCAGA	1348	RNA32
13	66207288	AAAATACCTGAATAAACCTGAAG	1349	X40
13	68049114	AAGATAACTTAATAGGCCTCCAC	1350	X60
13	68754577	AAGATGGCTGAAAAAGCCTTTCG	1351	X60
13	74802880	AAGCTGACTGAAGAGCCCTTGGG	894	X60

13	77274559	AAGTTACCAGATAACCCACAGG	1352	RNA31
13	84432474	CAGATACCTGGTTAAACATCACG	1353	X60
13	86737129	AAGATACATGAATGGCCAACAGG	1354	X50
13	92119936	AAGCTACCAGCATAGCCCACTAG	1355	X60
13	109759900	AAGAAAGCTGGAAAACACTCCAG	1356	X60
13	111418659	AAGATGCCTGAGTCTCCCCCGTG	1357	X60
13	112083112	AAGGAAACTGAATAACCCTCAGA	1358	X40
13	113351476	CTGAGAACTGAATAAACCTCGAG	1359	X60
14	18485266	AAGGTTACAGAATAAAACTCTGG	1360	X60
14	20357994	CAGGATACTGAATAACCCTCAGA	1361	X60
14	21233079	CCATTACATGAATAACCTTCAGG	1362	X60
14	22348176	AAGACACCTGGATAACCATCAGG	1363	X30
14	26263145	AAATTATGAAAAACCCTCAGG	1364	RNA32
14	30878091	AAGATACTTGACAACTCTCTGA	1365	RNA41
14	31153973	AAGATAGATGAATGACCCACTAC	1366	X60
14	35231283	AAGATACTTAAATGATCCTTGGC	1367	X60
14	37392538	AATATACCTAAATAACTTACAGC	1368	X60
14	40710593	AAGATAACTGGATAGCCATATGC	1369	X60
14	40860782	AACAAACATGAAAAACACTCAGC	1370	X60
14	41931495	AAGATACCTAAATATATATCTGA	1371	X60
14	42141012	AGGATGACTGAAAAATCCCCAGG	1372	X60
14	49513652	AAGATGACTGCTTAACCCCCAGT	1373	X60
14	51112581	AAGATACCAAAATGTAACTCAGG	1374	X60
14	52885591	AAGATAACTTAAAAACTCTAAGG	1375	X50
14	52900831	ACAGAACCTGAATAGCCCTAGGG	1376	X60
14	53020328	AAGATGCCTGAACTAGTCCTTGGG	1377	DNA41
14	56099999	AAAATAACTGAAAACCCTCAAC	1378	RNA41
14	59879739	AACATACATCCACAACCATCAGG	1379	X60
14	64921531	AAGATACATGAATAAGCACCATG	1380	X50
14	66348379	CATATACTTGAATAACCAACTGG	1381	X50
14	68160233	AAGATCTTAATAATCATCTGG	1382	RNA32
14	72604917	AAGAAACCCGAGTCCCTCTGG	1383	RNA32
14	74660460	AAGATATCTCATATCACTCAGG	1384	RNA41
14	74867740	ACAATACCTTGAATAACCCACAAG	1385	DNA41
14	77627074	AAGATACATAAATAACCTCACAG	1386	X60
14	80214772	AAGTAACAGAATAATCCTAAGG	1387	RNA41
14	81206628	GAATAACCTGAATAACCCCCAGA	1388	X60
14	82199597	AAATTACCTAAATAACCCAAGGC	1389	X60
14	82935226	AAGGTACCTGAATATTGTTCTGT	1390	X60
14	84727246	AAGACAGCTGGGTATCTCTCTGG	1391	X60
14	87353900	CTGATAGTGAATAAGCCTCAGG	1392	RNA41
14	87564479	ATGATAGTGAATAAGTCTCAGG	1393	RNA41
14	90316482	TAGATATTTGAATAACCATTTAG	1394	X60
14	94330884	AAGATGCCCTGAGCAACCCTCCTG	1395	DNA41
14	100626641	ATGATCTCTGAATGGCCTTCAGG	1396	X60
14	104781000	CGTGTACCTGAATGACCCACGGG	1397	X60
14	106194663	TAGATACTGAGAAAGCCTCTGG	1398	RNA41
15	26338242	AAGTTATCTAAAAACCCTCTAG	1399	RNA41
15	26400480	AAGATATCTGAAAAATTCTCAAA	1400	X60
15	36010542	AAGTAAACTGAAAACCTTCTGG	1401	RNA41
15	37986317	AATATAACTGAATAAAACTCTTT	1402	X60
15	38076935	TAGCTACATGAAAACCCTGGGG	1403	RNA41
15	40609022	ATAATACTGAATAACCCTTTGA	1404	RNA41

15	43079813	CTTTTACCTGAATACCCTCAGG	1405	RNA41
15	44348352	AAGATGACTGAAGAGCCCTTGGG	1406	X50
15	49430075	ATGAGCCCAGAAGAACCCTCTTG	1407	X60
15	57756788	GAGATACCTGAAAAATCCTGGCT	1408	X60
15	58607272	TGTATACCAGAATCACCCTAGGG	1409	X60
15	60876750	CAGATACCTGGTAACTCTCAGG	1410	RNA31
15	61064287	AAATTACCTGGACAACCTTCGCG	1411	X60
15	63004747	AAGATACATGAATAACCTTGTCA	1412	X50
15	63156318	AAGATACATTAGAACCCTCTGT	1413	RNA41
15	69510497	AAGATATATGAATGGCCCTGAAG	1414	X60
15	71127689	AAGATACACAAACAGCCCACAGG	1415	X60
15	73446678	AACATACCTGAAAAAACCTCAGC	1416	X40
15	74253079	AGGATTCCAGAAGAAGCCTGTGG	1417	X60
15	75470662	AAGAAACCTGGATTTACCTCTGT	1418	X60
15	77101670	AGAATAGCTGAAGGACCCTGGGG	1419	X60
15	84875080	AGGATGCCAGGGTAACCCACTGG	1420	X60
15	85440610	TAGGTACCTGAATGACCTCAGG	1421	RNA31
15	85452623	AAGATACCTGACATACCCTCCCC	1422	X50
15	90002805	GAGATACATGAATGACCCTTTCA	1423	X60
15	94867496	GTGATAGTGAATAACTCTCAGG	1424	RNA41
15	96462615	AGGAAAGCTGAATAACTCTCTTA	1425	X60
15	96700894	AAGAAACGCTAATAAACCTCAGG	1426	X50
15	99174006	AACATTCCACACTAACCATCAGG	1427	X60
16	2950487	AAGATACGAGAGAAACGCCCGGG	1428	X60
16	7926633	CAATTACCAGAATAACCCACAGA	1429	X60
16	11910562	AAGAGATTTGATAACCTTCTGG	1430	RNA41
16	14200710	AAGATTCCTGATTCAGCCTCTGA	1431	X50
16	15502916	AAGAAGCCAGACTAACACTGGGG	1432	X60
16	15969961	CAAATAAATGAATAATCATCAGG	1433	X60
16	16048075	AAGAGGCCTGACTCTCCCTCCAG	1434	X60
16	17024068	AAGATACTTGTATAACCTCAAGA	1435	X60
16	17094952	AACATACCTGAACAACCCTCAGG	1436	X20
16	22614186	AAGATATTTATATACCCCTCCAG	1437	X60
16	24555817	TAGTTACATGAAGAAACCTCTAG	1438	X60
16	25905845	AAGATACATGCGTGAAGCCTCTGG	1439	DNA41
16	26382131	ATGGTACCTGTACAAACTTCAGG	1440	X60
16	27966927	AAGGCACCTGAAAGCTCTCTGG	1441	RNA41
16	35693983	GAGATACCTGGAAAACCCCAGGC	1442	X60
16	46657396	ATGTTAACAGAAGACCCCTCTGG	1443	X60
16	47691992	TAGATAACTGGATACCCCACTGC	1444	X60
16	49245974	AAAATTCCTGTCCAGCCCTCTGG	1445	X60
16	49984669	AGGATGCCTGAAAAAACCTCAAA	1446	X60
16	51935176	AAGATACATGAGAACCCTTTGC	1447	RNA41
16	52525199	AAGATACATGAATAGCCCTCCAC	1448	X40
17	5340518	TAAATACCTGAATAACCCATAGT	1449	X50
17	5857462	TATATACATGTATAACCCACAGG	1450	X50
17	6194882	AAGATACATTCTTAGCCCTCAAG	1451	X60
17	7932420	AAGAAACCTTGTGAACCCTCAGT	1452	X60
17	14407307	AAGAAATGGATAGCCCTCTGG	1453	RNA32
17	21332996	AAAATAGCTTAAAAAGCCTGTGG	1454	X60
17	27457685	AGGGTGCCCCAACAACCCTCGGG	1455	X60
17	28706042	AAGAAATGTGGGTAAGCCTCAGG	1456	X60
17	29645114	ATCATATCTGAATAACTCTCAAC	1457	X60

17	32458400	AACAAGCCTGTATAACCCTCATG	1458	X50
17	34969762	GTGATACTGAATAAGTCTCAGG	1459	RNA41
17	37872545	AACATACATGAATAATCTTCAAA	1460	X60
17	39300993	ATTATACCTCAATATCCCTCTGG	1461	X40
17	44931191	AAGATACATGAAAAACACTGGCT	1462	X60
17	47455028	AACATACATGAATAATCTTCAAA	1460	X60
17	47541148	AAGATACCTGTACAACACTATAC	1463	X60
17	54308512	CAGATACCTCATTTACCCTGATG	1464	X60
17	65806019	CAGATACTTATAACCCTCTGG	1465	RNA22
17	67990176	AAAATACTTGAAAAATCCCTCAGT	1466	DNA41
17	70972434	AAGATCAAGAATAACTGTCAGG	1467	RNA41
17	81310828	AAGACACTTGTCTAGTCCTCTGG	1468	X60
18	8119646	AAGAATCCGGAAAAACCCTATGC	1469	X60
18	10478571	AAGACAGCTGGAGAACTCTCAGG	1470	X50
18	11443150	AAGAAAAATGAACAAACCTCAGA	1471	X60
18	11651294	AAGTTACGTGAATAGGACTCAGT	1472	X60
18	13336259	AAGATAAAGAACAACTCTCTGG	1473	RNA41
18	21942917	CAGATACACAGATAACCCCCAGG	1474	X60
18	24883127	AAGAAACTTGAAAAATCCCAGG	1475	RNA41
18	35027674	CAGATAACAGAAAAACCCTGTTG	1476	X60
18	36792668	AAGATACCCAGATCCCCCTCCAG	1477	X60
18	36915438	TAGATATTCTGAATATACCTCTGG	1478	DNA41
18	39891882	CAGATACATGAAATAATCCTCCAG	1479	DNA41
18	40348238	AGTCATCCTGAATAACCCTCATG	1480	X60
18	47612935	GCCATACCAAATAACCCTCTGG	1481	RNA41
18	49237013	AAGATACCTGGATAAGCAACTGC	1482	X50
18	49759577	AAGAAACCTATAGAACCCTGAGT	1483	X60
18	52354807	AAGAAAACAGAATAAACCTCTAA	1484	X60
18	53136651	AAGATACCTTATCAACCCTAAAG	1485	X50
18	54113085	CAGAAATATAAAAAACCCTCAGG	1486	X60
18	55667190	AAGATTCAAGAAAGACCCTCTGC	1487	X60
18	56622476	TAAATACTGAATAACCCTGTGA	1488	RNA41
18	58463120	GAGATCTCAGCATAACTCTCTGG	1489	X60
18	66313025	AAGATACCTTATTTACCTATGG	1490	RNA41
18	68281088	TTCATACCTAAATAACCCTTAGA	1491	X60
18	69322487	AAGATACCTGAATAACCTAAGAC	1492	X50
18	74469327	AAGATACATAATAATCATCTGT	1493	RNA41
18	77464690	AAGAGCCCTTGCTAACCATCTGG	1494	X60
19	11998269	AAAGTATCTGAATAACACTTCTG	1495	X60
19	12455264	AGCCTACCTAAATAGCCCTAGGG	1496	X60
19	27693648	CAGACACCTGAATGACCCCAGGC	1497	X60
19	28546228	CACTGACCTGAATCACCCCCAGG	1498	X60
19	37641018	GAAATACTGAATAATCCTCAGG	1499	RNA31
19	38143521	AAGACACAGAAAGATCCCTCTGG	1500	X60
19	42798163	AAGGTACCAGATGAGCCCTTGGG	1501	X60
19	45644796	AGGATACCTCCCAAATCCTCTGG	1502	X60
19	46147476	CAAATACTTGAAAAGCCCTCGAG	1503	X60
19	52963761	TAAATATGAATGACCCTCAGG	1504	RNA32
19	53485175	CAGATATCTGAAGATCCCTCCAG	1505	X50
20	1471262	ACAGAACCTGTATAACCCTCCAG	1506	X60
20	2545286	AAGAAATCTAGAATACCCCTAGGG	1507	DNA41
20	6093655	AAGTTAAATGAATGACCCTGACCGG	1508	DNA42
20	7232430	AAGATATTTGATCAACCCTTGGA	1509	X60

20	10350390	AGGTAACATCAATATCCCTCCGG	1510	X60
20	12411793	AACGTACCTGAATAACTCATAAG	1511	X60
20	13449167	AAGATACCAGCATAGTCCTCCTG	1512	X50
20	13559179	AAGATATCTGAAGAGCCTTCCCA	1513	X60
20	18449496	AAGATACCTGAATTTACCATGAG	1514	X60
20	19443923	AAGATACTTTATTAACTCTCAAG	1515	X50
20	22704701	TAGATATGTGAATTAACTTCTGG	1516	X60
20	23658111	AAGATACCTGATCACACATCAGA	1517	X60
20	37569891	CAGATACCTCAATAACCCTGATG	1518	X40
20	44858917	AAGATACTTGGATAATGCTTGGT	1519	X60
20	45794486	AAGGTATCTGAATTACCCTCAAG	1520	X40
20	52287275	CTGAGACCTGAGTAACTCTCATG	1521	X60
20	56292197	AAGAAAATGAATAACCCTACAG	1522	RNA41
20	60198378	TAGATACATGAAAAACCCTTCAG	1523	X50
20	60509505	TAGATGCTTAAATGACCCTCTGC	1524	X60
21	17021098	TAGGATCCTGGATAACCCTCCAG	1525	X60
21	17566032	TTGATACTTGAATAACCATCTGA	1526	X50
21	17907925	AAGAAACCAATAACCCTCAGG	1527	RNA12
21	19274949	AAGGAACCAGAATTACTCTCAGT	1528	X60
21	24464330	CAGAAACCAGAATAACCTTCAGG	1529	X40
21	24537041	AAAATTCCTGAATAGCTCTCTGG	1530	X40
21	26666529	TAGAGACATGAAAAACCCTCCAA	836	X60
21	30710727	ATGATAGTGAATAACACTCATG	1531	RNA41
21	30757521	AGGATACAGAATATCCATCTGG	1532	RNA41
21	31767749	AATCTACCTGAAAAGCCCTCTGG	1533	X40
21	33249604	CTGATACCAGATAACCCTGAGG	1534	RNA41
21	45566319	CAGATATCTGAATAACCCACCAG	1535	X40
21	46274280	AAGATACATGGATAATGTTCAGG	1536	X50
21	46379614	AAGAAACATGAAGAAAACTCAGC	1537	X60
22	15415612	AAGGTTACAGAATAAAACTCTGG	1360	X60
22	16210764	AAAATACATGGAAAATCCTCATG	1538	X60
22	18016369	ATTATACCTGAATAAACCTGACT	1539	X60
22	22356999	GATTTACCAGAAAAACCCTCTGG	1540	X50
22	22409053	GATTGACCAGAATAACCCTCTGG	1541	X50
22	26261319	AAGATACATCGAATAACCCTTCTA	1542	DNA41
22	31956781	AAGGCAACTGAAGATCCTTCAGG	1543	X60
22	48095647	AAGATATCTGAATTCAGCTCTTG	1544	X60
X	3121703	AAGAAAACAGAATAAACCTCTCA	1545	X60
X	3288787	AAGATAGCTGATGAAATATCAGG	1546	X60
X	6286346	ATGATAGTGAATAAGCCTCAGG	1547	RNA31
X	15276107	AAGATTCATGTACAAACCTCATG	1548	X60
X	18754961	AAGACACATGAAGAAAACTGAGG	1549	X60
X	19389428	AAGTTGACTGAAACCCTCTGG	1550	RNA32
X	25123524	GAGATACCCTAGATAACCCTCAGA	1551	DNA41
X	33500389	GTGATAACTGAATAACCCTGCTC	1552	X60
X	57574816	GAGATAACTGCATAGCCCTAGGT	1553	X60
X	58085053	GAGACACCTGAACGACCTCAGG	1554	RNA41
X	58098997	AAGACACCTGACTAACCCCAGGC	1555	X50
X	58100889	AAGACACCTGACTAACCCCAGGC	1555	X50
X	58123415	TAGACACCTGAACAACCCCAGGC	1556	X60
X	58198267	AAGACACCTGGCTGACCCCAGG	1557	RNA41
X	58199277	AAGACACCTGAGCAACCTCAGG	1558	RNA31
X	58401983	AAGATATCTGAGCAACACTCTGT	1559	X50

X	63325513	GAGATATGTGAATAAAACTCTGA	1560	X60
X	63786600	CAGAAGCTGAATGACCCTTTGG	1561	RNA41
X	63945361	AAAATAACTGATAAATCCTCTGA	1562	X60
X	65679966	AAGCTCCTGCATAGCCCACAGG	1563	RNA41
X	70140322	AACATACCTAAATATACCCTGG	1564	RNA41
X	70754250	AAGCTGACTGAAGAGCCCTTGGG	894	X60
X	72198434	TAGATACCTGACTTTCCCACTTG	1565	X60
X	73418101	AAGATTCCTGAAGCCTCCTCAGA	1566	X60
X	73819519	CATATACTGAATAACCATCTGG	1567	RNA31
X	84823869	AAGATATCTGAAATGCCCTAGAG	1568	X60
X	89194879	AAATAGCCTGAATAACCCTAGTG	1569	X60
X	89316606	AACAAACATGAATAACACTAAGC	1570	X60
X	91030898	AAGAAATCTGACAAACCTTTAGG	1571	X60
X	91177495	GTGATACTGAATAAGTCTCAGG	1459	RNA41
X	99969578	AAGATCCAGAAGAACTCTCTGA	1572	RNA41
X	107292941	TAGATACCTAAATAGCCCATGGG	1573	X50
X	108681302	AAGAAACCTCTATAAGCCTCTTA	1574	X60
X	109943949	AATCTACTGAATAACTCTCAGG	1575	RNA31
X	111810669	ATGAATACTGAATAACATTCAGG	1576	X60
X	113640910	AAGATACCAATAACCCTCAAA	1577	RNA22
X	117399450	AATGTACCCGAACAACCCTCAGG	1578	X40
X	120228774	CAGATAGCTGAAAGAACACTCAGG	1579	DNA41
X	125113165	AACATAACTGAATAACCATAAGG	1580	X40
X	126715614	AATCAACCTGAATATCCATCAGT	1581	X60
X	130340267	AAGATACCCATTCACCCTCTGG	1582	RNA31
X	130463899	AAAGAACCTAAAAAACCCTCTTG	1583	X60
X	132229997	AAGATAGGGGAAAGCCCTCTGG	1584	RNA41
X	138021160	AAGGCAAATGAATAACCCACAGC	1585	X60
X	139830410	AAAATACAAATAACCCTCAGT	1586	RNA32
X	144953782	AAGATCCAGAAGAATTCTCTGG	1587	RNA41
X	150328565	AGAATACCTGAATACCCCCAGA	1588	RNA41
X	154034869	CAAATACCTTAATCACCATGAGG	1589	X60
Y	4605941	AAGAAATCTGACAAACCTTTAGG	1571	X60
Y	4641256	GTGATACTGAATAAGTCTCAGG	1459	RNA41
Y	6194908	AAGATGCCAACATAAAGCTCAGG	1590	X60
Y	13359292	TAAATACCTATAACCCTGAGG	1591	RNA32
Y	16565563	AGCACACATGAATAACCCTAAGG	1592	X50
Y	22230617	AAGATCCCAGAATAACTACTGG	1593	RNA41

Table 14. ANGPTL3 gRNA off-target site validation in human primary hepatocytes

		Editing %		
Chromosome	**Location**	**treated**	**control**	**Net Editing %**
1	62604219	61.93	0.39	61.54
1	2.28E+08	0.75	0.81	-0.06
1	2E+08	0.1	0.03	0.07
1	59422147	0.32	0.4	-0.08
1	76436411	0.3	0.26	0.04
1	19936662	0.26	0.2	0.06
1	1.8E+08	0.47	0.55	-0.08
2	1.7E+08	0.18	0.1	0.08
2	17555591	0.09	0.12	-0.03

2	87443936	0.27	0.26	0.01
2	2.26E+08	0.13	0.19	-0.06
2	1.22E+08	0.48	0.62	-0.14
2	1.84E+08	0.26	0.36	-0.1
2	1.88E+08	0.26	0.22	0.04
3	8738135	0.28	0.23	0.05
3	539550	1.24	0.44	0.8
3	1.21E+08	0.26	0.27	-0.01
3	99319950	0.21	0.22	-0.01
4	1.73E+08	0.22	0.22	0
4	1.44E+08	0.48	0.54	-0.06
4	51965380	0.09	0.08	0.01
4	1.01E+08	0.09	0.08	0.01
4	26200297	0.14	0.11	0.03
4	74170265	0.54	0.3	0.24
5	1.14E+08	0.16	0.13	0.03
5	1.56E+08	0.47	0.59	-0.12
5	8704833	0	0	0
5	16404802	0.15	0.19	-0.04
6	1.13E+08	0.09	0.11	-0.02
6	45506487	0.3	0.36	-0.06
6	89636030	0.28	0.3	-0.02
6	18241159	0.08	0.1	-0.02
6	1.05E+08	0.45	0.39	0.06
6	84651006	0.15	0.11	0.04
6	1.26E+08	0.17	0.14	0.03
6	1.34E+08	0.23	0.29	-0.06
6	22811364	0.3	0.31	-0.01
6	6543792	0.08	0.15	-0.07
6	3993855	0.39	0.45	-0.06
6	51857715	0.19	0.29	-0.1
6	90026983	0.28	0.31	-0.03
6	1.48E+08	0.15	0.17	-0.02
6	1.46E+08	0.32	0.35	-0.03
7	1.58E+08	0.5	0.45	0.05
7	1.05E+08	0.4	0.61	-0.21
7	18326887	0.03	0	0.03
7	81886792	0.23	0.47	-0.24
7	1.24E+08	0.62	0.44	0.18
7	1.14E+08	0.41	0	0.41
8	66947451	17.71	0.67	17.04
8	19687879	0.19	0.26	-0.07
8	76479079	0.44	0.44	0
8	1.09E+08	0.14	0.23	-0.09
8	88225407	0.44	0.57	-0.13
8	1.4E+08	0.29	0.34	-0.05
8	1.22E+08	0.42	0.57	-0.15
9	89929285	0.15	0.21	-0.06
9	1.01E+08	0.41	0.49	-0.08
10	32570562	0.17	0.23	-0.06
11	2376222	0.05	0.11	-0.06
11	58705310	0.36	0.39	-0.03
11	80186841	0.24	0.24	0

11	12052022	0.35	0.32	0.03
12	26607410	0.41	0.46	-0.05
12	64120945	0.26	0.27	-0.01
12	98856708	0.34	0.42	-0.08
12	71164415	0.42	0.37	0.05
13	60126783	0.23	0.22	0.01
13	1.12E+08	0.59	0.76	-0.17
14	20357994	2.53	0.43	2.1
14	66348379	0.21	0.22	-0.01
14	81206628	0.28	0.28	0
14	22348176	0.74	0.68	0.06
14	76759328	0.07	0.04	0.03
15	94867496	0.32	0.42	-0.1
15	60876750	0.3	0.27	0.03
15	43079813	100	99.93	0.07
16	17094952	7.87	1.57	6.3
16	52525199	0.83	0.69	0.14
17	39300993	0.08	0.11	-0.03
18	21942917	0.48	0.65	-0.17
18	47612935	0.24	0.3	-0.06
19	37641018	0.4	0.34	0.06
20	60198378	0.4	0.35	0.05
21	45566319	0.26	0.35	-0.09
21	17566032	0.15	0.18	-0.03
21	17021098	0.19	0.17	0.02
22	18016369	0.08	0.21	-0.13
X	1.07E+08	0.25	0.29	-0.04
X	1.17E+08	0.24	0.2	0.04
X	21471705	0.42	0.4	0.02
X	73819519	0.25	0.3	-0.05
X	6286346	0.58	0.83	-0.25
X	1.14E+08	0.38	0.38	0

[693] Modifications and/or truncations to either the spacer or tracr portion of the gRNA was assessed by alteration of the gRNA GA100 spacer (5'-AAGATACCTGAATAACCCTC-3' (SEQ ID NO: 15)) (**Table 15**). The modifications to the guide can serve to improve on-target editing efficiency and/or improve off-target editing efficiency. Additionally, four different ABE8.8 mRNAs were assessed (MA004, MA040, MA041, MA045; Table 23), in two different experiments (separated in the table below). Each of the gRNAs with an equivalent amount of in vitro transcribed ABE8.8 mRNA (1:1 ratio by weight) were co-transfected into primary human hepatocytes and primary cynomolgus hepatocytes at 5000, 2500, and 1250 ng/RNA/mL and processed as described.

Table 15. gRNA and/or mRNA modifications improve gRNA specificity

gRNA	Protospacer (5'-3')	SEQ		Human Primary Hepatocytes— Editing % at Position 6 (Dose, Replicate #)

		ID NO:	mRNA	5000, rep 1	5000, rep 2	2500, rep 1	2500, rep 2	1250, rep 1	1250, rep 2
GA441	AAGATACCTG AATAACCCTC	15	MA004	ND	47.29	52.04	49.69	41.45	42.72
GA442	AAGATACCTG AATAACCCTC	15	MA004	35.34	32.48	35.2	35.78	31.54	27.9
GA472	AAGATACCTG AATAACCCTC	15	MA004	37.65	36.08	37.98	32.46	28.85	32.1
GA473	AAGATACCTG AATAACCCTC	15	MA004	37.9	31.23	37.55	35.45	28.07	26.92
GA474	AAGATACCTG AATAACCCTC	15	MA004	30.59	39.55	37.46	37.26	28.79	25.37
GA475	AGATACCTGA ATAACCCTC	248	MA004	36.82	37.79	37.96	40.49	28.11	28.81
GA476	GATACCTGAAT AACCCTC	249	MA004	37.2	37.7	42.27	40.86	31.97	31.48
GA477	ATACCTGAATA ACCCTC	250	MA004	25.59	24.9	27.3	25.25	19.23	19.19
GA441	AAGATACCTG AATAACCCTC	15	MA040	43.09	45.18	29.68	47.56	41.53	35.98
GA442	AAGATACCTG AATAACCCTC	15	MA040	41.76	43.74	41.43	41.19	31.04	32.85
GA472	AAGATACCTG AATAACCCTC	15	MA040	41.15	42.31	40.66	40.5	31.38	28.23
GA473	AAGATACCTG AATAACCCTC	15	MA040	39.8	39	40.85	34.58	29.19	28.19
GA474	AAGATACCTG AATAACCCTC	15	MA040	38.99	33.48	30.43	34.63	24.83	22.76
GA475	AGATACCTGA ATAACCCTC	248	MA040	37.64	39.98	37.03	39.31	28.67	26.83
GA476	GATACCTGAAT AACCCTC	249	MA040	41.08	39.14	37.69	38.01	28.8	27.26
GA477	ATACCTGAATA ACCCTC	250	MA040	20.51	19.43	22.64	22.01	15.69	16.61
GA441	AAGATACCTG AATAACCCTC	15	MA041	28.97	20.87	31.35	37.98	27.9	27.48
GA442	AAGATACCTG AATAACCCTC	15	MA041	26.39	25.73	29.01	27.38	20.18	20.53
GA472	AAGATACCTG AATAACCCTC	15	MA041	27.32	29.91	28.54	28.29	23.4	23.15
GA473	AAGATACCTG AATAACCCTC	15	MA041	19.75	19.89	19.6	17.62	14.97	14.04
GA474	AAGATACCTG AATAACCCTC	15	MA041	22.56	20.87	18.86	17.38	15.4	14.14
GA475	AGATACCTGA ATAACCCTC	248	MA041	31.47	29.18	27.34	23.98	17.67	18.89
GA476	GATACCTGAAT AACCCTC	249	MA041	31.01	33.84	29.37	28.62	19.77	21.75
GA477	ATACCTGAATA ACCCTC	250	MA041	3.7	2.53	3.92	3.47	2.96	3.24
				Human Primary Hepatocytes— Editing % at Position 6 (Dose, Replicate #)					

Verve gRNA ID	Protospacer (5'-3')	SEQ ID NO:	Verve mRNA ID	5000, rep 1	5000, rep 2	2500, rep 1	2500, rep 2	1250, rep 1	1250, rep 2
GA441	AAGATACCTG AATAACCCTC	15	MA004	33.26	34.23	42	36.89	34.57	31.5
GA442	AAGATACCTG AATAACCCTC	15	MA004	27.1	35.52	36.03	32.59	28.21	32.14
GA472	AAGATACCTG AATAACCCTC	15	MA004	32.58	22.71	27.15	38.51	29.44	32.3
GA473	AAGATACCTG AATAACCCTC	15	MA004	28.63	27.84	18.2	36.76	31.84	29.58
GA474	AAGATACCTG AATAACCCTC	15	MA004	25.26	22.74	26.59	35.61	28.96	26.24
GA475	AGATACCTGA ATAACCCTC	248	MA004	28.61	34.79	30.52	35.29	29.14	30.03
GA476	GATACCTGAAT AACCCTC	249	MA004	31.45	27.75	31.88	32.11	26.99	30.15
GA477	ATACCTGAATA ACCCTC	250	MA004	15.07	13.4	15.01	18.73	16.78	16.96
GA441	AAGATACCTG AATAACCCTC	15	MA045	20.49	21.6	26.32	26.13	24.37	25.48
GA442	AAGATACCTG AATAACCCTC	15	MA045	25.65	17.51	25.36	18.73	18.54	20.34
GA472	AAGATACCTG AATAACCCTC	15	MA045	20.87	15.13	19.67	19.28	19.07	ND
GA473	AAGATACCTG AATAACCCTC	15	MA045	14.84	14.05	20.14	15.95	15.95	16.71
GA474	AAGATACCTG AATAACCCTC	15	MA045	15.81	16.04	17.08	20.15	15.02	14.33
GA475	AGATACCTGA ATAACCCTC	248	MA045	24.33	31.08	25.52	28.55	20.61	21.1
GA476	GATACCTGAAT AACCCTC	249	MA045	25.43	24.26	23.45	26.05	21.47	23.35
GA477	ATACCTGAATA ACCCTC	250	MA045	5.06	2.84	3.07	4.65	3.75	3.01

[694] Off-target analysis for altered gRNA/mRNA combinations at the highest dose of 5,000ng/RNA/mL was performed for two sites previously identified that showed off-target editing (**Table 16**). The calculated editing percentage across the protospacer was totaled, and the negative control editing % was subtracted. This data shows that modifications to the guide and/or to the ABE mRNA improve off-target editing efficiency.

Table 16. Modifications to the guide and/or to the ABE mRNA improve off-target editing efficiency.

		Human Primary Hepatocytes—SUM total editing % (all positions) minus control, AVG of ~2 replicates

gRNA	mRNA	OT Site A	OT Site B
GA441	MA004	9.785	1.605
	MA040	7.17	0.85
	MA041	-0.1	-0.27
GA442	MA004	7.65	0.77
	MA040	6.97	0.64
	MA041	-0.005	-0.17
GA472	MA004	0.36	-0.13
	MA040	0.78	-0.185
	MA041	-0.035	-0.205
GA473	MA004	-0.145	-0.135
	MA040	-0.1	-0.215
	MA041	-0.085	-0.19
GA474	MA004	1.075	0.03
	MA040	1.545	0.39
	MA041	-0.095	-0.14
GA475	MA004	2.79	0.39
	MA040	1.68	ND
	MA041	-0.13	-0.205
GA476	MA004	0.48	0.385
	MA040	0.785	0.295
	MA041	-0.17	ND
GA477	MA004	-0.13	-0.215
	MA040	-0.48	-0.23
	MA041	-0.225	-0.14
		Human Primary Hepatocytes—SUM total editing % (all positions) AVG of ~2 replicates	
GA441	MA004	6.575	2.345
	MA045	1.36	0.865
GA442	MA004	7.315	1.75
	MA045	1.355	0.895
GA472	MA004	2.195	1.005
	MA045	1.19	0.87
GA473	MA004	1.47	1.025
	MA045	1.495	0.98
GA474	MA004	2.145	1.055
	MA045	1.405	0.9
GA475	MA004	3.67	1.825
	MA045	1.39	0.955
GA476	MA004	1.775	1.675
	MA045	1.505	0.84
GA477	MA004	1.31	0.975
	MA045	1.135	0.83

[695] Lipid nanoparticles containing ABE8.8 mRNA and a gRNA matching the human/cynomolgus 5'-CCCGCACCTTGGCGCAGCGG-3' (SEQ ID NO: 13) sequence (GA066) that targets the splice donor at the 5' end of *PCSK9* intron 1 were formulated at a 1:1 ratio by weight. The LNPs were administered to primary human hepatocytes and primary cynomolgus hepatocytes, using various dilutions to assess for editing activity at different

concentrations of test article. LNP containing Cas9 mRNA and a gRNA matching a protospacer sequence in another gene, *ANGPTL3*, as a positive control was used for these experiments. This Cas9 mRNA/gRNA combination were chosen because it had been previously observed to produce high levels of genome-editing activity in primary human hepatocytes, primary cynomolgus hepatocytes, and cynomolgus liver *in vivo*. It was observed the ABE8.8/GA066 LNP substantially outperformed the control LNP with respect to editing, displaying much higher potency in both human and cynomolgus hepatocytes (**FIG. 15**).

Example 5. Pcsk9 gene editing in mice

[696] LNPs containing SpCas9 mRNA and mouse *Pcsk9*-targeting gRNA with different tracr designs (GA052, GA053, GA054, and GA055) at a 1:1 weight ratio were formulated. Wild-type C57BL/6 mice were dosed with 2 mg/kg of the LNP test article. Seven days after dosing, the mice were euthanized and genomic DNA was harvested from mouse liver, and then assessed for editing of the target site with next-generation sequencing. GA052, GA054, and GA055 all outperformed GA053 that had a previously disclosed tracr design (**FIG. 16**).

Example 6. Pcsk9 base editing in mice

[697] LNPs containing ABE8.8 mRNA and a mouse *Pcsk9*-targeting gRNA at a 1:1 weight ratio were formulated. This gRNA matches the 5'-CCCATACCTTGGAGCAACGG-3' (SEQ ID NO: 69) protospacer sequence, which is the mouse ortholog of the human 5'-CCCGCACCTTGGCGCAGCGG-3' (SEQ ID NO: 13) sequence (matched by GA066, GA095, GA096, GA097 and GA346) that targets the splice donor at the 5' end of PCSK9 intron 1. Wild-type C57BL/6 mice were dosed with 2 mg/kg of the LNP test article via the lateral tail vein or retro-orbital in a total volume of 10 ml/kg. Seven days after dosing, the mice were euthanized and genomic DNA was harvested from mouse liver, and then assessed for base editing of the target splice site with next-generation sequencing. Approximately 60% editing of the target splice site was observed (mouse Pcsk9 intron 1 splice donor) (**FIG. 17**), providing a preclinical proof of concept of a base-editing therapy knocking down PCSK9 in the liver in vivo.

[698] In a subsequent study, LNPs containing ABE8.8 mRNA and a mouse *Pcsk9*-targeting gRNA at a 1:1 weight ratio were formulated, and dosed in wild-type C57BL/6 mice at doses ranging from 0-2.0 mg total RNA/kg. The low dose of 0.05mg RNA/kg showed high editing (>45%), while saturation of base editing occurred around 0.25mg RNA/kg dose (**FIG. 18**).

[699] In an additional study, LNPs containing ABE8.8 mRNA and a mouse *Pcsk9*-targeting gRNA were formulated at different weight ratios of mRNA and gRNA ranging from 1:1-1:6 and 2:1-6:1. This gRNA matches the 5'-CCCATACCTTGGAGCAACGG-3' (SEQ ID NO: 69) protospacer sequence, which is the mouse ortholog of the human 5'-CCCGCACCTTGGCGCAGCGG-3' (SEQ ID NO: 13) sequence (matched by GA066, GA095, GA096, GA097 and GA346) that targets the splice donor at the 5' end of PCSK9 intron 1. Wild-type mice were dosed with the LNP at 0.05 mg/kg total RNA dose. Additionally, two individual LNPs constituted with the same mRNA and two guides, GA255 and GA257 , which matches the 5'-CCCATACCTTGGAGCAACGG-3' (SEQ ID NO: 69) protospacer sequence but has modifications to the tracr to improve stability, were also injected into mice. Seven days after dosing, the mice were euthanized and genomic DNA was harvested from mouse liver, and then assessed for base editing of the target splice site with next-generation sequencing (**FIG. 19**). Base editing was comparable between many of the ratios, with 1:6 and 6:1 (gRNA:mRNA) performing the poorest. The guides with modifications to the tracr had increased editing efficiency.

Example 7. Angptl3 base editing in mice

[700] LNPs containing ABE8.8 mRNA and mouse *Angptl3*-targeting gRNAs (GA258, GA259, GA260, GA349, GA353) at a 1:1 weight ratio were formulated. Wild-type C57BL/6 mice were dosed with LNP test article at 0.05 mg/kg total RNA dose. The mice were later euthanized and genomic DNA was harvested from mouse liver, and then assessed for base editing of the target splice site with next-generation sequencing (**FIG. 20**).

In Vivo evaluation in non-human primates (NHP)

Example 8. Evaluation of PCSK9/ABE

[701] First, a 2-week study was performed to evaluate editing of the PCSK9 gene in cynomolgus monkeys using specific gRNA and base editor nuclease ABE 8.8 through administration of LNPs (**FIG. 21**). The ABE8.8/GA066 LNP was administered to cynomolgus monkeys via intravenous infusion at two doses, 3 mg/kg (n = 3 animals) and 1 mg/kg (n = 2 animals) with the intent of producing high-level base editing of *PCSK9* gene in the liver. Two weeks after administration of test article, blood samples were collected for clinical chemistry assays and PCSK9 ELISA assay, and the animals underwent necropsy for collection of liver samples, 2 samples each from each of the 4 lobes of the liver (8 in total from each animal).

[702] Editing analysis of the liver specimens was performed by next generation sequencing. At the 3 mg/kg dose, a mean of 55% editing of the adenine base at the target splice site in the liver samples was observed; at the 1 mg/kg dose, a mean of 24% editing of the adenine base at the target splice site in the liver samples was observed (**FIG. 22**). For PCSK9 ELISA analysis, blood samples were collected from the animals on days D-10, D-7 and D-5 pre-dose (average shown) as well as on D8 and D15 post-dose. At 2 weeks after dosing there was a mean 76% reduction in the blood PCSK9 protein level compared to pre-dosing levels in the 3 mg/kg group (**FIG. 23**), and a mean 32% reduction in the blood PCSK9 protein level in the 1 mg/kg group (**Table 17**). Low density lipoprotein cholesterol (LDL-C) was determined in serum samples taken pre-dose and on D8 and D15 using a standard clinical analyzer (**Table 18**). There was a mean 57% reduction in the blood low-density lipoprotein cholesterol (LDL-C) level in the 3 mg/kg group (**FIG. 24**), and a mean 25% reduction in the blood LDL-C level in the 1 mg/kg group.

Table 17. Circulating PCSK9 protein levels decrease after PCSK9 gRNA/ABE editing

PCSK9 Group	Dose (total RNA, mg/kg)	Animal ID	Concentration (ng/ml)			Reduction from basal on D15 (%)
			Basal	D8	D15	
1	0	1001	432	387	366	15
2	1	2001	324	285	227	30
2	1	2002	144	116	95	34
3	3	3001	254	16	28	89
3	3	3002	170	34	31	82
3	3	3003	253	65	109	57

Table 18. LDL-C reduction after editing of the PCSK9 gene

Group	Dose (total RNA, mg/kg)	Animal ID	LDL-C concentration (mg/dl)			Reduction from basal on D15 (%)
			Basal	D8	D15	
1	0	1001	76.7	70	57	26
2	1	2001	72.3	55	54	25
2	1	2002	69.0	53	52	25
3	3	3001	57.0	23	17	70
3	3	3002	60.0	25	22	63
3	3	3003	107.7	88	66	39

[703] In a subsequent experiment, LNPs were delivered in monkeys via intravenous infusion at a 1.0 mg/kg dose. For three monkeys that underwent necropsy at 2 weeks after LNP infusion, there was a mean 63% base editing frequency of the *PCSK9* splice site adenine in the liver, with no bystander base editing observed elsewhere in the protospacer (**FIG. 25**);

there was a mean indel frequency of 0.5%. The editing was accompanied by a mean 81% reduction in blood PCSK9 levels and a mean 65% reduction in blood LDL-C levels. For two monkeys that underwent necropsy at 24 hours after LNP infusion, there was a mean 48% editing frequency.

[704] In a subsequent short-term dose-response study (0.5, 1.0, and 1.5 mg/kg doses, three monkeys each, with necropsy at 2 weeks), all doses achieved >50% mean base editing rates; *PCSK9* editing and reductions in PCSK9 protein and LDL-C appeared to saturate at doses ≥1.0 mg/kg (**FIG. 26**).

[705] In these studies we assessed liver function tests and in some groups noted moderate rises in AST and ALT that largely resolved by the end of the first week and entirely resolved by 2 weeks after LNP infusion, with no adverse health events observed in any of the animals. In assaying base editing in a wide variety of tissues, we found that the liver was the predominant site of editing, with much lower editing observed in the spleen and adrenal glands and minimal editing observed elsewhere (**FIG. 27**).

Table 25. Numeric formats of tissue distribution of editing of the *PCSK9* exon 1 splice donor adenine base in three animals, as shown in FIG. 27.

Tissue	control NHP	NHP #1	NHP #2	NHP #3
Liver	0.12	59.49	73.83	55.10
Spleen	0.02	5.25	7.99	5.67
Adrenal gland (left)	0.02	1.81	7.49	1.79
Adrenal gland (right)	0.10	1.54	0.22	2.18
Kidney (left)	0.10	0.63	0.89	0.24
Kidney (right)	0.06	0.64	0.27	0.32
Skin (injection site)	0.13	0.08	1.47	1.56
Mandibular lymph nodes (left)	0.06	0.15	0.48	0.43
Mandibular lymph nodes (right)	0.06	0.18	0.17	0.96
Mesenteric lymph nodes	0.04	0.12	0.15	0.12
Testis (left)	0.13	0.11	0.06	0.38
Testis (right)	0.07	0.16	0.09	0.38
Epididymis (left)	0.09	0.12	0.58	0.83
Epididymis (right)	0.80	0.25	0.79	0.52
Skeletal muscle	0.16	0.40	0.33	0.07
Duodenum	0.07	0.30	0.83	0.26
Jejunum	0.06	0.19	0.28	0.58
Colon	0.08	0.07	0.08	0.13
Lung (left)	0.01	0.16	0.22	0.18
Lung (right)	0.10	0.54	0.24	0.16
Brain	0.12	0.17	0.05	0.15

[706] In an additional study, different tracr designs were assessed. LNPs were delivered in NHPs via intravenous infusion at a 1.0 mg/kg total RNA dose, unless otherwise specified. Base editing of the liver was assessed, and several guides outperformed GA066 with the literature tracr (**FIG. 28**).

[707] In the same NHP study, LNPs containing either: 1) Cas9 mRNA and a gRNA matching 5'-CCCGCACCTTGGCGCAGCGG-3' (SEQ ID NO: 13) (GA097) protospacer sequence; or 2) ABE8.8 mRNA and gRNA matching 5'-CCCGCACCTTGGCGCAGCGG-3' (SEQ ID NO: 13) (GA097) protospacer sequence, were delivered intravenously into NHPs. Importantly, the method by which classical CRISPR/Cas9 disrupts a gene by ultimately introducing an indel, is different than base editing where a base mutation has occurred. Further, a target region that is highly amenable to CRISPR/Cas9 editing does not necessarily mean base editing at that location will occur, and vice versa. This is highlighted in the NHP study, where the LNP containing SpCas9/gRNA resulted in low liver editing in the NHPs (<5%), while the LNP containing ABE8.8/gRNA had significantly higher editing close to 40% (**FIG. 29**) with a dose (1 mg/kg) lower than the SpCas9/gRNA dose (1.5 mg/kg).

[708] In another study, two LNPs were compared for base editing activity after delivery in NHPs via intravenous infusion at doses ranging from 0.5mg/kg-3.0 mg/kg. Both LNPs showed high efficacy, with the first LNP (LNP#1) outperforming the second LNP (LNP#2), at doses as low as 0.5mg/kg having greater than 50% average editing (**FIG. 30**). Similar to past experiments, the PCSK9 protein levels decreased significantly upon high level of base editing at the splice site, decreasing circulating PCSK9 protein by 80-90% (**FIG. 31**).

[709] In a long-term study (four animals, with liver biopsy at 2 weeks) LNPs were introduced via intravenous infusion at a higher dose of 3.0 mg/kg to assess drug tolerability and the durability of PCSK9 protein and LDL-C reductions resulting from *PCSK9* editing. The liver biopsy samples showed a mean 66% base editing frequency (**FIG. 32**). Blood PCSK9 protein levels reached a trough by 1 week and have remained stable thereafter out to at least 6 months, settling at approximately 90% reductions (**FIG. 33**). Blood LDL-C and lipoprotein(a) [Lp(a)] levels have similarly achieved stable troughs persisting to 8 months, settling at ≈60% and ≈35% reductions, respectively (**FIG. 34**, **FIG. 35**).

[710] There were transient, moderate rises in AST and ALT that entirely resolved by 2 weeks following LNP infusion (**FIG. 36**), with no changes in any other liver function tests and no adverse health events observed to date (**FIG. 37**). Importantly, the persistence of PCSK9 and LDL-C reductions for 8 months with no late AST and ALT elevations

demonstrates that such a response, whatever its scale, does not adversely impact the treatment's efficacy.

[711] To evaluate ABE8.8/gRNA (protospacer matching GA346) LNP-mediated off-target editing in primary cyno hepatocytes and monkey liver samples, ONE-seq was performed with a synthetic cynomolgus genomic library selected by homology to the gRNA spacer sequence. This library was treated with ABE8.8 protein and PCSK9 gRNA, and the top 48 ONE-seq-nominated sites (**FIG. 38**)—of which the *PCSK9* target site was the very top site— was assessed with next-generation sequencing of targeted PCR amplicons from LNP-treated versus untreated NHP samples (**FIG. 39**).

[712] In LNP-treated primary cynomolgus hepatocytes, besides editing at the *PCSK9* target site, there was off-target editing (mean <1%) evident at only one site, designated C5, which has poor homology to the human genome (**FIG. 39**). Assessing the same 48 sites in liver samples from monkeys that were treated with a 1.0 mg/kg LNP dose (from the aforementioned dose-response study), low-level off-target editing (mean <1%) only at the C5 site (**FIG. 40**) was observed. No off-target editing with a 0.5 mg/kg LNP dose and only low-level off-target editing with a 1.5 mg/kg LNP dose (mean <1%) were detected.

Example 9. Evaluation of ANGPTL3/ABE

[713] Similar to the NHP studies performed with the ABE/PCSK9 guide(s), LNPs formulated with ABE8.8 mRNA and a gRNA with the 5'-AAGATACCTGAATAACTCTC-3' (SEQ ID NO: 14) (GA067) cynomolgus spacer was administered to cynomolgus monkeys via intravenous infusion at two doses, 3 mg/kg (n = 3 animals) and 1 mg/kg (n = 2 animals) with the intent of producing high-level base editing of *ANGPTL3* in the liver. Two weeks after administration of test article, blood samples were collected for clinical chemistry assays and ANGPTL3 ELISA assay, and the animals underwent necropsy for collection of liver samples. Small segments from the center and periphery of each lobe of the liver were excised and flash frozen in liquid nitrogen and stored at -86 to -60 °C. Splice-site editing was analyzed by next generation sequencing, confirming that base change occurred. At the highest dose (3 mg/kg), a mean of 61% editing of the adenine base was observed at the target splice site in the liver; at the lowest dose (1 mg/kg), the editing observed was 28% at the target splice site in the liver (**Table 19**).

[714] In addition, blood samples were collected pre-dose, on D7 and D15 post-dose to determine serum ANGPTL3, and triglycerides. At two weeks after dosing there was a mean 90% and 31% reduction in the blood ANGPTL3 protein level compared to pre-dosing levels

in the higher (3 mg/kg) and lower (1 mg/kg) dosing groups (**Table 20**).

Table 19. Liver Editing in cynomolgus monkeys

Group	Dose (total RNA, mg/kg)	Animal ID	Mean ± standard deviation
1	0	1001	0.25 ± 0.06
4	1	4001	39.6 ± 2.7
4	1	4002	15.1 ± 3.9
5	3	5001	61.2 ± 3.2
5	3	5002	56.4 ± 5.2
5	3	5003	64.3 ± 2.6

Table 20. Knockdown of circulating cynomolgus monkey ANGPTL3 following gene editing using ABE

Group	Dose (total RNA, mg/kg)	Animal ID	Concentration (ng/ml)			Reduction from basal on D15 (%)
			Basal	D8	D15	
1	0	1001	39	47	41	-7
4	1	4001	56	15	31	45
4	1	4002	38	22	31	17
5	3	5001	31	1	1	98
5	3	5002	33	11	9	72
5	3	5003	70	1	1	99

[715] Triglycerides were determined in serum samples taken pre-dose and on D8 and D15 using a standard clinical analyzer. Serum triglyceride reduction in response to LNP-mediated ANGPTL3 base editing with ABE8.8 and GA067 are summarized in **Table 21**. The levels of triglycerides were reduced by 24% and 59% in response to doses of 1 mg/kg and 3 mg/kg base editor ABE8.8 and GA067 targeting ANGPTL3.

Table 21. Triglyceride reduction in response to ANGPTL3 gene in liver

Group	Dose (total RNA, mg/kg)	Animal ID	Triglyceride concentration (mg/dl)			Reduction from basal on D15 (%)
			Basal	D8	D15	
1	0	1001	31.3	43	41	-31
4	1	4001	71.0	46	49	31
4	1	4002	67.7	52	56	17
5	3	5001	59.3	23	21	65
5	3	5002	41.3	33	25	40
5	3	5003	55.0	27	15	73

[716] In another independent study, the same ABE8.8/GA067 LNP was administered to cynomolgus monkeys via intravenous infusion at a dose of 3 mg/kg (n = 3 animals) with the

intent of producing high-level base editing of *ANGPTL3* in the liver. Two weeks after administration of test article, blood samples were collected for clinical chemistry assays, and the animals underwent a liver biopsy for collection of liver samples. At the 3 mg/kg dose, a mean of 60% editing of the adenine base at the target splice site was achieved in the liver biopsy samples. Concordantly, at 2 weeks after dosing there was a mean 95% reduction in the blood ANGPTL3 protein level compared to pre-dosing levels and there was a mean 64% reduction in the blood triglyceride level (**FIG. 41**). These results provide an additional preclinical proof of concept of a base-editing therapy knocking down *ANGPTL3* in the liver *in vivo* and effecting reductions in blood ANGPTL3 protein and triglyceride levels.

Example 10. Evaluation of dual PCSK9 and ANGPTL3 base editing

[717] To assess whether it was possible to affect simultaneous base editing of PCSK9 and ANGPTL3 with a single test article, LNPs were formulated containing a mix of three components: ABE8.8 mRNA, PCSK9-targeting gRNA (GA095), and ANGPTL3-targeting gRNA (GA098) at a 2:1:1 weight ratio. Primary human hepatocytes were incubated with various dilutions of the LNPs. Three days after incubation, genomic DNA was harvested from the hepatocytes, and then assessed for base editing of the target splice site with next-generation sequencing. At the highest LNP concentrations, ≈40% editing of the PCSK9 splice site (intron 1 splice donor) and ≈40% editing of the ANGPTL3 splice site (intron 6 splice donor) was observed (**FIG. 42**), demonstrating the feasibility of dual gene disruption in human hepatocytes with a single test article and providing a preclinical proof of concept of a single base-editing therapy simultaneously knocking down PCSK9 and ANGPTL3, which would be predicted to substantially reduce both blood LDL cholesterol levels and blood triglyceride levels in human recipients. An advantage of this approach is that because the base editors do not require double strand breaks (DSBs) for editing, in contrast to standard CRISPR-Cas9, there is substantially lower risk of chromosomal rearrangements or other structural changes inherent in the simultaneous targeting of two different sites in the genome.

[718] A follow-up NHP study was performed to address: 1) if a second dose of administered LNPs administered can cause editing of the target gene; 2) if a different target can be base edited. LNPs formulated with ABE8.8 mRNA and either a gRNA targeting *PCSK9* (GA346) or a gRNA targeting *ANGPTL3* (GA347) was administered to cynomolgus monkeys via intravenous infusion at doses ranging from 0.5-2mg/kg. Two weeks after administration of test article, biopsies were performed to assess for base editing. After 30 days from the initiation of the study, the opposite LNP was administered. Following a second biopsy after

an additional 2 weeks, the gDNA was extracted, and base editing was assessed using next generation sequencing. The findings from this study show that high level editing of both the *PCSK9* and *ANGPTL3* targets are achieved after the first and second doses of LNPs (**FIG. 43**). Blood samples were collected on both biopsy timepoints and show significant decrease of around 90% in circulating PCSK9 and ANGPTL3 after subsequent dosing with 1mg/kg LNPs (**FIG. 44**). In the same study an LNP encpasualting ABE8.8 mRNA, PCSK9 gRNA GA346 and ANGPTL3 gRNA GA347 at 1:0.5:0.5 weight ratio was admnistred at 2 mg/kg total RNA dose, which resulted in rubust synchronized PCSK9 and ANGPTL3 gene editing (**FIG. 44**, top and bottom data labelled GA346+GA347 (D1) – top and bottom panels show PSCK9 and ANGPTL3 editing). The data demonstrate that robust multi-gene editing is possible with single 1-dose administration of ABE base editor mRNA and two or more gRNAs targeting two or more genes of interest in mammals (and/or mammalian cell). **FIG. 44** mirrors corresponding knowckdown of ANGPTL3 (bottom) and PCSK9 (top) proteins.

[719] In another study, NHPs were repeat dosed with LNPs (**FIG. 45**). NHPs were dosed with LNP#1 and LNP#2 formulated with ABE8.8 mRNA MA004 and a gRNA targeting *PCSK9* (GA097) via intravenous infusion at a total RNA doses of 0.5mg/kg. NHPs received an additional LNP dose on day 30 and on day 60. Liver biopsies from Day 14, Day 46, and Day75 were extracted for adenosine base editing analysis. All gDNA was extracted, and base editing was assessed using next generation sequencing. These results demonstrate that repeat dosing of LNP containing ABE8.8 mRNA and PCSK9 gRNA causes additive base editing in the liver, as editing efficiency was near 30% after the first dose of LNP, while over 50% after the third dose LNP. It was also observed that the magnitude of additive editing was LNP-dependent.

[720] Further, repeat dosing of LNPs reduced PCSK9 protein levels in NHPs (**FIG. 46**). The PCSK9 protein levels were monitored over 90 days in NHPs that were repeat dosed with LNPs formulated with ABE8.8 mRNA MA004 and a gRNA targeting *PCSK9* (GA097). NHPs were dosed via intravenous infusion at a total RNA dose of 0.5mg/kg at days 0, 30, and 60 (arrow is illustrated on graph to depict dosing). For description of analysis of PCSK9 protein levels, see detailed methods section. Compared to basal levels, PCSK9 protein dropped by nearly 40% after the initial dose of LNP, but upon additional doses, decreased even further.

[721] Liver markers were assessed following repeat dosing of LNPs (**FIG. 47**). ALT, AST, total bilirubin, and creatine kinase levels were assessed up to 71 days in NHPs that were repeat dosed with LNPs formulated with ABE8.8 mRNA MA004 and a gRNA targeting

PCSK9 (GA097). NHPs were dosed via intravenous infusion at a total RNA dose of 0.5mg/kg at days 0, 30, and 60, which is shown as D1, D2 and D3 on graph, respectively. Liver biopsy was performed at days 14 and 46 which is shown as B1 and B2 on graph, respectively. Blood was collected at multiple timepoints, as described on the graph. Although ALT and AST minimally rose upon dosing, this was a transient response that returned to normal after a period of several days. Similarly, Creatine Kinase levels increased upon dosing, with the largest effect seen after the first dose, and returned to basal levels after a period of several days.

[722] The liver enzymes, LDH, GLDH, GGT, and ALP, were assessed in NHPs that were repeat dosed with LNPs formulated with ABE8.8 mRNA MA004 and a gRNA targeting *PCSK9* (GA097) (**FIG. 48**). NHPs were dosed via intravenous infusion at a total RNA doses of 0.5mg/kg at days 0, 30, and 60. Blood was collected at multiple timepoints, as described on the graph. Although LDH and GLDH rose upon dosing, this was a transient response that returned to normal after a period of several days.

[723] Further, long-term adenine base editing of *ANGPTL3* was assessed in non-human primates (**FIG. 49**). Cynomolgus monkeys received an intravenous infusion of a 3 mg/kg dose of an LNP formulation with ABE8.8 mRNA MA004 and ANGPTL3 gRNA GA067. Blood was collected at timepoints specified and the graph, and ANGPTL3 protein levels (**FIG. 49A**) and triglyceride levels (**FIG. 49B**) were analyzed. As compared to controls, both ANGPTL3 protein levels (96% reduction) and triglyceride levels substantially decreased upon base editing of the gene, and remain stably reduced for more than 170 days.

[724] Cytokine activation and immune response were assessed in NHPs receiving LNPs. In one set of studies, cynomolgus monkeys received intravenous infusions of 0.5 mg/kg doses at three specified time points (**FIG. 50A** and **FIG. 50B**) of an LNP formulation with ABE8.8 mRNA MA004 and PCSK9 gRNA GA346. Blood was collected at timepoints specified and the graph, and IP-10 and MCP-1 were analyzed. These results demonstrated: 1) that NHPs receiving LNPs had no evidence of cytokine activation nor immune response, as compared to control; and 2) that repeat dosing of the same LNP does not elicit cytokine activation nor an immune response.

[725] In additional studies, IL-6, MCP-1, and SC5b-9 (**FIG. 50C**, **FIG. 50D**, and **FIG. 50E**, respectively) were analyzed at different time points from blood collected from NHPs that received an intravenous infusion of 1.0 mg/kg total RNA dose of LNP formulated with MA004 and PCSK9 gRNA GA346. This caused minimal cytokine/complement activation

that returned to baseline by/before 336 hours, as compared to control.

Example 11. Evaluation of SpCa9-mediated on-target editing efficiency

[726] Gene editing of *ANGPTL3* or *PCSK9* in non-human primates was assessed. Cynomolgus monkeys received an intravenous infusion of a 1.5 mg/kg dose of an LNP formulation with SpCas9 mRNA MS004 and one gRNA targeting either *ANGPTL3* (GA261-GA263) or *PCSK9* (GA266-GA271). Upon necropsy after 2 weeks, two pieces from each liver lobe (8 pieces total) were isolated and gDNA was extracted. Samples were processed as described in the detailed methods section. Indel % was analyzed for each separate piece and are graphed as individual points (**FIG. 51**). High editing efficiency was observed in most NHP livers. LDL-C levels were measured (**FIG. 52**). All NHPs that received LNPs with SpCas9 mRNA/PCSK9 gRNA had at least 35% reduction in circulating LDL-C levels. Although more modest, LNPs with SpCas9 mRNA/ANGPTL3 gRNA had 10-25% reduction in circulating LDL-C levels. Additionally, NHPs that received LNPs with SpCas9 mRNA/ANGPTL3 gRNA had around 10-50% reduction in triglyceride levels (**FIG. 53**). NHPs that received LNPs with SpCas9 mRNA/PCSK9 gRNA did not show a significant reduction in triglyceride levels.

[727] Cynomolgus primary hepatocytes were transfected at 2500, 1250, and 625 ng/test article/mL with SpCas9 mRNA MS002 and a gRNA targeting PCSK9 with modifications to the tracr. Genomic DNA was processed, sequenced, and analyzed as described in the detailed methods section. GA266 was transfected independently twice to serve as two positive controls. Almost all transfections resulted in high editing efficiency compared to positive control, while GA405 had slightly lower editing efficiency.

Table 22. Modifications to guides retain on-target editing efficiency in cynomolgus primary hepatocytes.

	Cynomolgus Primary Hepatocytes- % Editing (dose, replicate #)					
gRNA	**2500, rep1**	**2500, rep2**	**1250, rep1**	**1250, rep2**	**625, rep1**	**625, rep2**
GA266, #1	56.51	49.95	51.81	55.2	44.53	42.53
GA266, #2	50.34	48.3	43.56	46.2	39.75	36.59
GA395	43.91	49.53	41.48	44.35	33.87	34.57
GA396	53.98	56.84	56.02	52.02	44.44	41.53
GA397	56.65	58.59	56.47	58.68	40.72	46.28
GA398	59.85	63.97	56.45	55.21	39.09	44
GA399	58.97	62.75	55.55	53.02	37.75	41.8
GA401	53.74	52.79	49.12	48.83	38.33	38.77
GA402	45.58	55.34	44.46	47.93	35.05	40.31
GA403	52.62	55.84	50.5	47.13	38.28	35.58

GA404	48.3	50.59	41.68	35.04	31.63	30.16
GA405	41.85	47.33	35.84	35.88	26.2	26.36
GA406	57.17	51.45	45.11	43.68	37.58	40.31
GA408	50.4	46.92	47.21	49.8	36.7	37.14

[728] PACE-modifications to the gRNA have been previously demonstrated to reduce off-target editing efficiency. Human primary hepatocytes were transfected at 2500, 1250, 500, and 250 ng/test article/mL with SpCas9 mRNA MS002 and a gRNA targeting PCSK9 with modifications to the tracr. Genomic DNA was processed, sequenced, and analyzed as described in the detailed methods section. GA156 was transfected to serve as a positive control. GA248 and GA249 contain PACE-modifications to the gRNA that have previously been demonstrated to decrease off-target editing efficiency. Indeed, although GA248 and GA249 had lower on-target editing compared to the unmodified gRNA, GA156 (**FIG. 54A**), GA248 and GA249 showed decreased off-target editing at an identified off-target site (**FIG. 54B**).

[729] ABE mRNA sequences - MA004, MA019, MA020, and MA021- were evaluated for their GC content **(FIG. 55A).** In general, MA004 sequence has a higher GC content than MA019, MA020, MA021, and ABE8.8m sequences. GC levels in sequence MA004 are also elevated (above 60%) in certain regions throughout the ABE sequence such as the TadA domain, N-terminal linker, and C-terminal linker, as well as various sub-regions. For all sequences, GC levels below 60% are shaded in gray to provide contrast to the elevated GC regions. GC content is calculated by determining the relative amount of G and C within a given 25 nucleotide stretch across the entire mRNA sequence, i.e. for every 25 nucleotides, the sum of G's and C's is divided by the total number of nucleotides. This comparison shows that mRNA MA004 sequence has a distinct distribution and enrichment of G and C nucleotides relative to other mRNA sequences.

[730] The region-specific GC characterization of sequence MA004 is further illustrated (**FIG. 55**). The graph in each row shows the GC content in every 1,000-nucleotide stretch comprising the full ABE coding sequence (4,767 nt). The GC content is particularly high in the 5' end of the sequence, which includes the TadA region and the N-terminal linker. The GC content is also high various parts of the Cas9 nickase and in the 3' end of the ABE sequence; there the high GC% is centered around the linker region instead of the NLS. Sub-regions with high GC content are delineated by where GC reaches a threshold level of 60%. GC content in each sub-region is calculated by determining the number of G and C nucleotides divided by the total number of nucleotides for each sub-region.

Table 26. GC comparison of ABE-encoding nucleotides, MA004, MA019, MA020, MA021, and ABE8.8m

ABE sequence	MA004	MA019	MA020	MA021	ABE8.8m	ΔGC*
Region-specific GC %						
Total sequence	63	54	47	54	55	11
TadA (nt: 1-501)	70	58	54	60	54	14
N-terminal linker (nt: 502-597)	79	64	65	54	59	19
Cas9 nickase (nt: 598-4698)	62	54	45	53	56	10
C-terminal linker (nt: 4699-4710)	83	58	67	58	60	22
NLS (nt: 4711-4767)	63	53	49	60	48	11
Sub-region GC%						
nt: 27-109	75	59	55	63	59	16
nt: 111-213	73	56	55	66	57	15
nt: 250-385	71	60	57	62	57	12
nt: 389-475	70	59	56	54	53	15
nt: 479-601	76	62	61	65	55	15
nt: 615-661	72	64	51	66	68	10
nt: 699-714	75	63	63	56	69	12
nt: 735-829	74	62	54	61	61	15
nt: 1192-1345	67	58	51	55	61	11
nt: 1363-1627	66	58	52	55	61	10
nt: 1661-1706	70	57	57	65	63	10
nt: 1735-1880	66	54	52	57	60	10
nt: 1938-1999	71	60	55	63	60	12
nt: 2023-2073	63	55	45	53	63	9
nt: 2184-2231	67	60	54	63	60	8
nt: 2542-2600	71	63	51	53	64	13
nt: 2719-2798	69	56	54	65	64	9
nt: 3294-3334	73	61	54	61	66	13
nt: 3542-3584	72	67	58	72	67	6
nt: 3745-3864	67	54	53	57	62	11
nt: 3977-4045	65	57	48	59	59	9
nt: 4207-4286	70	56	54	66	59	11
nt: 4427-4517	65	60	51	53	55	10
nt: 4537-4569	73	58	48	64	61	15
nt: 4583-4741	67	58	49	57	61	11

*ΔGC is the difference between the GC content of sequence MA004 CDS (cDNA sequence) and the average of sequences MA019 CDS, MA020 CDS, MA021 CDS, and ABE8.8m.

[731] The editing efficiency of these mRNA constructs were assessed in mice (**FIG. 55B**). These mRNAs, MA004, MA019, MA020, and MA021 code for the same ABE protein sequence but have different nucleotide sequence optimizations. The only difference between these groups is the mRNA sequence; the mRNAs have the same base modifications, and each group used the same LNP formulation and gRNA. Each group was administered intravenously to mouse, and a low dose of 0.05 mg/kg was used in this study to achieve sub-saturating levels of liver editing to resolve efficacy differences. At day 5 post-dose, gDNA

was isolated and base editing was assessed by next generation sequencing. These results demonstrate that mRNA sequence changes can affect adenosine base editing performance *in vivo*.

Additional details of methods described

[732] Plating, culturing, and transfection of primary hepatocytes. Primary human liver hepatocytes (PHH) and primary cynomolgus liver hepatocytes (PCH) from BioIVT were cultured per the manufacturer's protocol. Briefly, primary human hepatocytes and primary cyno hepatocytes were obtained as frozen aliquots from BioIVT. Four lots of primary human hepatocytes, each derived from a de-identified individual donor, were used for the experiments: STL (main donor) was used for all experiments, including screening experiments and off-target experiments; HLY, JLP, and TLY were used for off-target experiments. The HFG lot of primary cyno hepatocytes were used for experiments. Following the manufacturer's instructions, cells were thawed and rinsed prior to plating in 24-well plates that had been coated with bovine collagen overnight, with a density of approximate 350,000 cells/well in INVITROGRO hepatocyte medium supplemented with TORPEDO antibiotic mix (BioIVT). The cells were thawed and resuspended in hepatocyte thawing medium followed by centrifugation at 100 g for 10 min at 4 °C. The supernatant was discarded, and the pelleted cells resuspended in hepatocyte plating medium. Each vial contains approximately 5 million cells that were used for plating one 24-well plate. Plated cells were allowed to settle and adhere for 4-6 h in a tissue culture incubator at 37 °C under 5% CO_2 atmosphere. After incubation, cells were checked for monolayer formation. The incubating media were then replaced with fresh hepatocyte maintenance media (complete INVITROGRO medium obtained from BioIVT, the cell line provider). The cells thus became ready for transfection. Each of the gRNAs were co-transfected with an equivalent amount of in vitro transcribed ABE8.8 mRNA (1:1 ratio by molecular weight) into primary human hepatocytes via MessengerMax reagent (Lipofectamine), using various dilutions to assess for editing activity at different concentrations of test article. MessengerMAX from Thermo Fisher is used for transfection. Solution A: desired amount of guide RNA is mixed with 1:1 wt ratio of mRNA in OptiMEM. Solution B: MessengerMAX in OptiMEM. After mixing solutions A and B, the mixture was incubated at room temperature for 20 min. 60 µL of the incubated solution was added dropwise to each cell wells. For protospacer sequences that were a perfect match to the corresponding cynomolgous monkey *PCSK9* or *ANGPTL3* gene sequence, each gRNA was also co-transfected with an equivalent amount of ABE8.8 mRNA

(1:1 ratio by molecular weight) into primary cynomolgous hepatocytes, and followed the same transfection protocol. The cells were then allowed to remain at 37 °C for 3 days. Cells were harvested and prepared for genomic DNA extraction using either a Thermo Kingfisher, or Qiagen DNEasy blood and Tissue Kit, per manufacturer's instructions.

[733] Bioinformatic analysis. Targeted amplicon sequencing data were analyzed with CRISPResso2 v2.0.31 in batch mode (CRISPRessoBatch). For Cas9 experiments, the following parameters were set: "--quantification_window_center -3 --quantification_window_size 5 --min_frequency_alleles_around_cut_to_plot 0.1 --max_rows_alleles_around_cut_to_plot 100". For ABE experiments, the following parameters were set: "--default_min_aln_score 95 --quantification_window_center -10 --quantification_window_size 10 --base_editor_output --conversion_nuc_from A --conversion_nuc_to G --min_frequency_alleles_around_cut_to_plot 0.1 --max_rows_alleles_around_cut_to_plot 100". For NHP experiments, an additional parameter was set to exclude low quality reads: "--min_single_bp_quality 30". Moreover, in all cases, the parameter "--max_paired_end_reads_overlap" was set to 2R-F+0.25*F, following FLASH recommendations (http://ccb.jhu.edu/software/FLASH/), where R was the read length and F was the amplicon length.

[734] Editing was quantified from the "Quantification_window_nucleotide_percentage_table.txt" output table as the percentage of reads that supported any A-to-G/C/T substitution in the main edited position (position 6 of the protospacer DNA sequence). Indels were quantified from the "Alleles_frequency_table_around_sgRNA_*.txt" output table as the percentage of reads that supported insertions or deletions over a 5-bp window on either side of the nick site (at position –3 upstream of the PAM sequence), having excluded reads that supported deletions larger than 30 bp. For candidate off-target sites, editing was quantified from the "Alleles_frequency_table_around_sgRNA_*.txt" output table as the percentage of reads from alleles with an A->G substitution in the editing window (positions 1-10 in the PAM distal side of the protospacer), having excluded reads from alleles with deletions larger than 30 bp.

[735] Reverse transcription. The collected cells were processed with the miRNeasy Mini Kit (QIAGEN) according to the manufacturer's instructions to isolate both large and small RNA species, with the other part harvested for genomic DNA to establish *PCSK9* editing and thereby confirm base editor activity in the cells. Reverse transcription was performed using the iScript Reverse Transcription Supermix reagent according to the manufacturer's instructions, with four different primer pairs used for PCR amplification of transcripts

spanning exon 1 and exon 2, with or without any portions of intron 1. Paired-end reads of 250-bp length generated using an Illumina MiSeq System, as described above, were trimmed for adapters using trimmomatic v0.39 with parameters "ILLUMINACLIP:NexteraPE-PE.fa:2:30:10:1:true LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36". Reads were then merged with FLASH v1.2.11[34] and aligned to the *PCSK9* gene body with Bowtie2 v2.4.1 with parameters "--local --very-sensitive-local -k 1 --np 0". Gene annotations were obtained from Ensembl v98 (ftp://ftp.ensembl.org/pub/release-98/gtf/homo_sapiens/Homo_sapiens.GRCh38.98.gtf.gz). Alignments were filtered with samtools v1.10 and converted to BED format with the bedtools v2.25.0 bamtobed function. A minimum of 1000 mapped reads per sample were required and the end positions of mapped reads were tallied. Positions throughout *PCSK9* intron 1 supported by a minimum of 10 reads in at least one treated sample are report.

[736] ONE-seq analysis to predict candidate off-target sites. The design of a ONE-seq library starts with the computational identification of sites in a reference genome that have sequence homology to the on-target. For human ONE-seq libraries, the reference human genome (GRCh38, Ensembl v98, chromosomes ftp://ftp.ensembl.org/pub/release-98/fasta/homo_sapiens/dna/Homo_sapiens.GRCh38.dna.chromosome.{1-22,X,Y,MT}.fa and ftp://ftp.ensembl.org/pub/release-98/fasta/homo_sapiens/dna/Homo_sapiens.GRCh38.dna.nonchromosomal.fa), was searched for potential off-target sites with up to 6 mismatches to the protospacer sequence above, and sites with up to 4 mismatches plus up to 2 DNA or RNA bulges, using Cas-Designer v1.2 (http://www.rgenome.net/cas-designer/).

[737] For the ONE-seq library pertaining to the cynomolgus monkey, the reference cynomolgus monkey genome (macFas5, Ensembl 98, chromosomes ftp://ftp.ensembl.org/pub/release-98/fasta/macaca_fascicularis/dna/Macaca_fascicularis.Macaca_fascicularis_5.0.dna.chromosome.{1-20,X,MT}.fa.gz and ftp://ftp.ensembl.org/pub/release-98/fasta/macaca_fascicularis/dna/Macaca_fascicularis.Macaca_fascicularis_5.0.dna.nonchromosomal.fa.gz) was searched using similar parameters.

[738] Sites with up to 6 mismatches and no bulges are referred to using a *X<number of mismatches><number of bulges>* code. As such, the on-target site is labelled as X00; a site with 1 mismatch to the on-target and no bulges is labelled as X10, and so on. Sites with DNA bulges are referred to with a similar nomenclature, *DNA<number of mismatches><number of bulges>*. As such, a site with 4 mismatches to the on-target and 2 DNA bulges is labelled as

DNA42. The same nomenclature is used for RNA bulges, but these are coded as *RNA<number of mismatches><number of bulges>*.

[739] The protospacer sequences identified were extended by 10 nucleotides (nt) on both sides with adjacent sequence from the respective reference genome (these regions are herein referred to as the genomic context). These extended sequences were then padded by additional sequences up to a final length of approximately 200 nt, including 6 predefined constant regions of different nucleotide composition and sequence length; 2 copies of a 14-nt site-specific barcode, one on each side of the central protospacer sequence; and 2 distinct 11-nt unique molecular identifiers (UMIs), one on each side of the central protospacer sequence. The UMIs are used to correct for bias from PCR amplification, and the barcodes allow for the unambiguous identification of each site during analysis. The barcodes are selected from an initial list of 668,420 barcodes, which contain neither a CC nor a GG in their sequences, and each barcode has a Hamming distance of 2 from any other barcode. A custom Python script was used for designing the final library.

[740] The final oligonucleotide libraries are synthesized by a commercial vendor (Agilent Technologies). Each library is PCR-amplified and subjected to 1.25× AMPure XP bead purification (Beckman Coulter). After incubation at 25°C for 10 minutes in CutSmart buffer (New England Biolabs), RNP comprising 769 nM recombinant ABE8.8-m protein and 1.54 μM gRNA is mixed with 100 ng of the purified library and incubated at 37°C for 8 hours. The RNP dose is derived from an analysis documenting that it is a super-saturating dose, ie, above the dose that achieves the maximum amount of on-target editing in the biochemical assay.

[741] Proteinase K (New England Biolabs) is added to quench the reaction at 37°C for 45 minutes, followed by 2× AMPure XP bead purification. The reaction is then serially incubated with EndoV (New England Biolabs) at 37°C for 30 minutes, Klenow Fragment (New England Biolabs) at 37°C for 30 minutes, and NEBNext Ultra II End Prep Enzyme Mix (New England Biolabs) at 20°C for 30 minutes followed by 65°C for 30 minutes, with 2× AMPure XP bead purification after each incubation. The reaction is ligated with an annealed adaptor oligonucleotide duplex at 20°C for 1 hour to facilitate PCR amplification of the cleaved library products, followed by 2× AMPure XP bead purification. Size selection of the ligated reaction is performed on a PippinHT system (Sage Sciences) to isolate DNA of 150 to 200 bp on a 3% agarose gel cassette, followed by 2 rounds of PCR amplification to generate a barcoded library, which undergoes paired-end sequencing on an Illumina MiSeq System as described above.

[742] Two cleavage products are obtained in a ONE-seq experiment. The PROTO side includes the part of the oligonucleotide upstream of the cleavage position, whereas the PAM side includes part of the oligonucleotide downstream of the cleavage position. In an ABE experiment, only the PROTO side is informative of editing activity (an A→G substitution); therefore, only this side is sequenced.

[743] Paired-end reads were trimmed for sequencing adapters using trimmomatic v0.39 (Bolger et al., 2014) with custom Nextera adapters (PrefixPE/1: ACACTCTTTCCCTACACGACGCTCTTCCGATCT; PrefixPE/2: GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT; as specified in file) and parameters "ILLUMINACLIP:NEB_custom.fa:2:30:10:1:true LEADING:0 TRAILING:0 SLIDINGWINDOW:4:30 MINLEN:36". For experiments with lower sequencing quality (VOL014), these parameters were set to "ILLUMINACLIP NEB_custom.fa:2:30:10:1:true LEADING:2 TRAILING:0 SLIDINGWINDOW:30:30 MINLEN:36". Reads were then merged using FLASH v1.2.11 (Magoc and Salzberg, 2011) with parameters "--max-mismatch-density=0.25 --max-overlap=160". Merged reads were scanned for the constant sequences, barcodes and protospacer sequences unique to each site, and filtered to those with evidence of an A→G substitution in the editing window (defined as the 1-10 most PAM-distal positions of the protospacer). Duplicated reads were discarded.

[744] For each site, the total number of edited reads was normalized to the total number of edited reads assigned to the on-target site, and this ratio defines the ONE-seq score for the site. Sites were ranked by ONE-seq score, and those with a score equal to or larger than 0.001, were selected for validation. This implies the sponsor follows up on sites that have down to 1000-fold less editing activity in the biochemical assay compared to editing of the on-target site. This threshold is based on the premise that in cells, if there is 100% on-target editing, 1/1000-fold less editing activity would translate to < 0.1% off-target editing, which falls below the lower limit of detection of editing by NGS.

[745] **SureSelect.** SureSelect panels were designed and purchased from Agilent Technologies. Bases in raw FASTQ files with quality lower than 30 were masked to Ns using seqtk v1.3-r106 (https://github.com/lh3/seqtk). Adapters were trimmed using the "Trimmer" script in Agilent's AGeNT v2.0.5 tool. Reads were aligned to the GRCh38 reference human genome (ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/001/405/GCA_000001405.15_GRCh38/seq

s_for_alignment_pipelines.ucsc_ids/GCA_000001405.15_GRCh38_no_alt_analysis_set.fna) using BWA MEM v 0.7.17-r1188 with parameter "-C". Aligned reads were processed and duplicates were removed using the "LocatIt" script in Agilent's AGeNT v2.0.5 tool, with parameters "-i -R -IB -OB -U -l < Covered.bed >". Nucleotide distributions at each position in the editing window (positions 1-10 in the PAM distal side of the protospacer) were determined using perbase v0.6.3 (https://github.com/sstadick/perbase), with parameters "base-depth -F 3848". Editing was quantified by summing the percentage of reads supporting an A→G substitution in the editing window.

[746] RNA-seq for guide-independent off-target analysis. The RNA samples were processed and sequenced by GENEWIZ; following rRNA depletion, libraries were prepared and underwent 2×150-bp paired-end sequencing on an Illumina HiSeq System, with approximate 50 million reads per sample. RNA-seq variant calling for all samples was executed using GATK Best Practices. In short, reads were aligned using STAR to the GRCh38 reference genome (ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/001/405/GCA_000001405.15_GRCh38/seqs_for_alignment_pipelines.ucsc_ids/GCA_000001405.15_GRCh38_no_alt_analysis_set.fna.gz) with gencode v34 (ftp://ftp.ebi.ac.uk/pub/databases/gencode/Gencode_human/release_34/gencode.v34.primary_assembly.annotation.gtf.gz). PCR duplicates were removed using GATK MarkDuplicates, followed by variant identification using GATK HaplotypeCaller. Variants were then filtered by excluding those with QD (Quality of Depth) < 2.0 and FS (Fisher strand – evidence of strand bias) > 30. All GATK analyses were performed with gatk4 v4.1.8.1.

[747] Variants obtained as above were further filtered by comparison with untreated control samples as follows. (1) Nucleotide distributions at each identified variant in treated cells each untreated control sample and each treated sample using perbase v0.5.1 (https://github.com/sstadick/perbase). (2) For all variants covered by at least 20 reads in both treated and untreated conditions, RNA edits were identified as those that had the reference allele (A or T) in at least 95% of reads in all untreated control samples and the alternate allele (G or C) in at least one read in the treated sample. The above steps were executed with each of the ABE8.8-treated and SpCas9-treated samples.

[748] Guide RNA synthesis. The guide RNAs shown in **Table 1 w**ere/are synthesized under solid phase oligonucleotide synthesis and deprotection conditions using controlled pore glass support and commercially available phosphoramidite monomers and oligonucleotide

synthesis reagents (Methods in Molecular Biology, **1993**, *20*, 81-114; ACS Chem. Biol. **2015**, *10*, 1181−1187, incorporated herein by reference in its entirety). The spacer section of the guide RNAs were converted to the corresponding ribonucleotides except the first 1-3 nucleotides from the 5'-end. The first 1-3 nucleotides from the 5'-end was/were converted to the corresponding 2'-O-methylribonucleotide as outlined in Table 1. The deprotected guide RNAs were purified by HPLC and the integrity of each guide RNA was confirmed by mass spectrometric analysis. The observed mass of each guide RNA was conformed to calculated mass.

[749] mRNA production by *in vitro* transcription (IVT) The mRNA described herein are produced by different methods well known in the art. One of such methods is *in vitro* transcription (IVT) using T7 polymerase or additional RNA polymerase variants. Typically, IVT of mRNA uses a linearized DNA template that comprises a T7 polymerase promoter and associated regulatory sequences, mRNA coding sequence (CDS), 3' and 5' untranslated regions (UTRs), poly A tail, and additional sequence elements to enhance mRNA stability and *in vivo* performance. Prior to IVT, the DNA template is in the form of a plasmid, PCR product, synthetic DNA product, or any other double-stranded DNA construct; linearization of the DNA template, typically with a restriction digestion enzyme, is performed to promote run-off transcription. A typical IVT reaction includes T7 polymerase, DNA template, RNase inhibitor, cap analog, inorganic pyrophosphatase, and naturally occurring ribonucleotide triphosphates (NTPs) such as GTP, ATP, CTP, UTP, or substitutions of natural NTPs with modified NTPs such as pseudouridine, N1-methylpseudouridine, 5'methylcytidine, 5-methoxyuridine, N6-methyladenosine, and N4-acetylcytidine. The cap analog can be added during transcription or supplemented after the IVT reaction using a capping enzyme; in both instances a 2'-O-methyl group, or additional 2' chemical modification, is added to first initiating nucleotide to produce a cap-1 form of mRNA. In some instances, poly A tail is added to the mRNA after the IVT reaction using an RNA ligase enzyme. After IVT, in some cases DNase is added to the transcription mixture to remove DNA template; alternatively, residual DNA is removed with chromatography, precipitation, or tangential flow filtration. Purification and concentration of mRNA is performed with methods such as ion exchange chromatography, affinity chromatography, precipitation, ion-pairing reversed-phase chromatography, hydrogen bond chromatography, cellulose chromatography, reversed-phase chromatography, enzymatic reactions, size exclusion chromatography, and tangential flow filtration. Similar IVT and purification process are used to produce mRNA encoding

luciferase, eGFP, SpCas9, Cas12b, CBE, and ABE; in all cases the DNA template, reaction conditions, and purification parameters are optimized for the specific gene of interest.

[750] Lipid nanoparticle formulation and analysis. LNPs used were formulated as previously described (Conway, A. et al. 2019 *Mol. Ther.* 27, 866–877; Villiger, L. et al 2021 *Nat. Biomed. Eng.* 5, 179-189) and generated either by (1) microfluidic mixing using the Precision Nanosystems NanoAssemblr system according to the manufacturer's protocol, with some optimization for individual payloads or by (2) rapid inline mixing of a solution of lipid excipients in an organic solvent and an aqueous solution of gRNA and mRNA. The lipid solution generally comprises of a mixture of four formulation excipients namely: an amino lipid, a monomethoxypolyethylene glycol (or methoxypoluyethyle glycol) of average molecular weight 2000 Da conjugated to a lipid called PEG-Lipid, cholesterol and 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), mixed in a predetermined molar ratio in ethanol. LNP composition comprised 40-65% of amino lipid, 2-20% DSPC, 1-5% PEG-Lipid, with the balance being cholesterol (all in mol%). The RNA aqueous solution contains a 1:1 by weight mixture of desired mRNA and guide RNA (gRNA) unless otherwise specified. For evaluating impact of mRNA to gRNA ratio, aqueous solution containing desired weight ratio of mRNA and gRNA were prepared prior to the preparation of corresponding LNPs, for evaluation; for example, the preparation of LNP test article to evaluate 6:1, 3:1, 2:1, 1:1, 1:2, 1:3 and 1:6 mRNA to gRNA ratio in mice (Example 6, FIG. 19). In some other instances, the mRNA and two gRNAs were mixed in 1:0.5:0.5 (mRNA:gRNA1:gRNA2) weight ratio to prepare LNPs containing desired mRNA and two gRNAs in a single test article (Example 10, FIG. 43). The aqueous solution of desired mRNA to gRNA was then mixed with the lipid excipients in ethanol by microfluidic or by rapid inline mixing. All LNPs for reported NHP studies were prepared by following the inline mixing protocols as described WO 2019/036028 Al, WO 2015/199952 A1, WO 2017/004143, WO 2020/081938 Al and WO 2020/061426 A2. The PEG-Lipid used for the preparation of the NHP LNP test articles was selected from WO 2019/036028 Al, WO 2015/199952 A1. The LNP compositions and ionizable amino lipids were selected from the from Patent Publications WO/2017/004143A1, WO/2017/075531A1 and WO/2018/191719 A1. The ionizable amino lipids used for the preparation of LNP#1 and LNP#2 (Example 8, FIG. 30 & FIG. 45) were selected from the publication WO 2018/191719 Al. The resulting LNP formulations were subsequently dialyzed against test article buffer and filtered using a 0.2 μm sterile filter.

[751] As an example, the LNPs used for cellular and NHP studies had an average hydrodynamic diameter range of 55 to 65 nm, with a polydispersity index of <0.2 as

determined by dynamic light scattering and 85-98% total RNA encapsulation as measured by the Quant-iT Ribogreen Assay. The LNP particle size (Z-Ave, hydrodynamic diameter), polydispersity index and total RNA encapsulation were measured as described in the literature prior to administration.

[752] As an example, the LNPs used for cellular and mouse studies had a particle size of 55-120 nm (Z-Ave, hydrodynamic diameter), with a polydispersity index of <0.2 as determined by dynamic light scattering (Malvern NanoZS Zetasizer) and 85-100% total RNA encapsulation as measured by the Quant-iT Ribogreen Assay (Thermo Fisher).

[753] **Genomic DNA extraction**. The whole mouse liver or 100-200 mg of monkey liver was loaded into 2 mL lysing matrix tubes (MP Bio). Livers were lysed with 0.5 ml PBS for the mouse liver or 0.25mL PBS for the monkey liver, using the FastPrep-24 system (MP Bio) according to the manufacturer's protocol. Genomic DNA was isolated from approximately 20 µL of mouse or monkey liver lysate using a bead-based extraction kit, MagMAX-96 DNA Multi-Sample Kit (Thermo-Fisher Scientific) on the KingFisher Flex automated extraction instrument (Thermo-Fisher Scientific) according to the manufacturer's protocols. For monkey liver biopsy samples, Qiagen DNEasy Blood & Tissue kit extraction was used to extract genomic DNA according to the manufacturer's instructions. Extracted genomic DNA was stored at 4 °C until further use or at -80 °C for long term storage.

[754] **PCSK9 protein levels quantified by ELISA**. Blood samples were collected and processed to plasma following blood draw. The plasma cynomolgus PCSK9 levels were determined by ELISA using the described ELISA; briefly, test samples or standards of purified cynomolgus monkey PCSK9 diluted in assay diluent D (Biolegend, part# 76384) were incubated with assay buffer A (Biolegend, part# 78232) in a 96-well microplate coated with a monoclonal antibody specific for human PCSK9 (Biolegend, part# 76157). After four washes with wash buffer (Biolegend, part# 78233), a polyclonal antibody specific for human PCSK9 (Biolegend, part# 76158) was incubated in individual wells. After four washes, avidin-HRP (Biolegend, part# 77897) were next incubated in individual wells. After six washes, substrate solution F (Biolegend, part# 79132), which contains TMB, was used to develop the plate. Optical density was determined using on a microplate reader set to 450 nm. Readings at 570 nm were subtracted from the readings at 450 nm to correct for optical imperfections in the plate. Blood samples were collected and processed to plasma following blood draw and stored at -86 to -60 °C until analysis.

[755] **ANGPTL3 protein levels quantified by ELISA**. The plasma cynomolgus ANGPTL3 levels were determined by ELISA; briefly, test samples or standards of purified

cynomolgus monkey ANGPTL3 diluted in calibrator diluent RD6Q (R&D, part# 895128) were incubated with assay diluent RD1-76 (R&D, part#895812) in a 96-well microplate coated with a monoclonal antibody specific for human ANGPTL3 (R&D, part#893734). After four washes with wash buffer (R&D, part# 895003), human ANGPTL3 conjugate (R&D, part#893735) which contains a polyclonal antibody specific for human ANGPTL3 conjugated to horseradish peroxidase (HRP) were next incubated in individual wells. After four washes, TMB substrate solution (R&D, part# 895000 and 895001) was used to develop the plate. Optical density was determined using on a microplate reader set to 450 nm. Readings at 540 nm were subtracted from the readings at 450 nm to correct for optical imperfections in the plate.

[756] Quantifying lipid levels. Reagent kits each analyte contain reagent, cholesterol, triglycerides and HDL-C are quantified using absorbance measurements of specific enzymatic reaction products. LDL-C is determined indirectly. Most of the circulating cholesterol is found in three major lipoprotein fractions: very low-density lipoproteins (VLDL), LDL and HDL. [Total C] = [VLDL-C] + [LDL-C] + [HDL-C]. Thus the LDL-C can be calculated from measured values of total cholesterol, triglycerides and HDL-C according to the relationship: [LDL-C] = [total C] - [HDL-C] - [TG]/5, where [TG]/5 is an estimate of VLDL-cholesterol expressed. These results provide a preclinical proof of concept of a base-editing therapy knocking down *PCSK9* in the human liver *in vivo* and effecting reductions in blood PCSK9 protein and LDL-C levels.

[757] Direct measurement of triglyceride levels. A clinical analyzer instrument is used to measure a 'lipid panel' in serum samples. This entails the direct measurement of cholesterol (total C), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C). A reagent kit specific for triglycerides contains buffers, calibrators, blanks and controls. Using the provided reagents, serum samples from the study are analyzed. Triglycerides are measured using a series of coupled enzymatic reactions. H_2O_2 is the end product of the last one and its absorbance at 500nm is used to quantify the analyte. The color intensity is proportional to triglyceride concentrations. All values are reported in mg/dL.

[758] LNP treatment of mice. The mouse studies were approved by the Institutional Animal Care and Use Committee of the Charles River Accelerator and Development Lab (CRADL), where the studies were performed. Female C57BL/6J mice were obtained from The Jackson Laboratory and used for experiments at 8-10 weeks of age, with random assignment of animals to various experimental groups. LNP were administered to the mice via injection into the lateral tail vein and/or retro-orbital injection of the of the venous sinus

(*Lab Anim* 2011; 40(5): 155–160) at mg/kg doses that correspond to mg of total RNA per animal weight (kilogram). The dose is calculated based on total RNA that constitute the amount of mRNA and gRNA, after formulating the LNP. One week following treatment, the mice were euthanized unless otherwise stated, and liver samples were obtained on necropsy and processed with the KingFisher Flex Purification System according to the manufacturer's instructions to isolate genomic DNA.

[759] LNP treatment of NHPs. The NHP studies were approved by the Institutional Animal Care and Use Committees of Envol Biomedical and Altasciences, respectively. NHPs studies were performed at Envol Biomedical (study #VTP2001) and Altasciences (study #1388.02, 04, 05, 09, and 11), with both studies using *Macaca fascicularis*) of Cambodian origin. The animals were 2-3 years of age and 2-3 kilograms in weight at the time of study initiation. All the animals were genotyped at the PCSK9 and/or ANGPTL3 editing site(s) to ensure that any animals receiving LNPs were homozygous for the protospacer DNA sequences perfectly matching the gRNA sequence, and animals were randomly assigned to various experimental groups. The animals were premedicated with 1 mg/kg dexamethasone, 0.5 mg/kg famotidine, and 5 mg/kg diphenhydramine prior to LNP administration unless otherwise stated. The LNP were administered using a temporary catheter inserted into a peripheral vein connected to a primed infusion line, over the course of 1 hour (+/- 5 minutes). Dose formulations were administered at a volume of 6 ml/kg, unless otherwise specified, and dosed at mg/kg corresponding to mg of total RNA per animal weight (kilogram). The dose is calculated based on total RNA that constitute the amount of mRNA and gRNA, after formulating the LNP. The appropriate volume based on the weight of the animal was delivered using an infusion pump. Control animals received phosphate-buffered saline instead of LNP under the same infusion conditions. When there are two LNPs constituted from amino lipid 1 and amino lipid 2 used in the same NHP study, the test articles are identified as LNP#1 and LNP#2 where mRNA and gRNA used for preparing these LNPs are the same. If there is only one LNP composition used in a study, the test article is identified as LNP

[760] For blood chemistry samples, animals were fasted for at least 4 hours before collection via peripheral venipuncture. NHP studies generally followed collection on the following schedule: day –10, day –7, day –5, day 1 (6 hours after LNP infusion), day 2, day 3, day 5, day 8, and day 15. In the long-term study, samples were also collected at day 21 and day 28 and have generally been collected every 2 weeks thereafter and analyzed by the study site for LDL cholesterol, HDL cholesterol, total cholesterol, triglycerides, AST, and ALT.

For each analyte, the mean of the values at day –10, day –7, and day –5 were regarded as the baseline value. A portion of each blood sample was sent to the investigators for PCSK9 or ANGPTL3 protein measurement.

[761] Analysis of cytokine levels. The serum cynomolgus IL-6, MCP-1 and IP-10 levels were determined by U-PLEX Biomarker Group 1 (NHP) Assays (Meso Scale Discovery, #K15068L-2) according to manufacturer's instruction. Briefly, U-PLEX (Meso Scale Discovery, #N05230) plate was incubated overnight at 4 °C with linker-coupled capture antibodies (MCP-1 antibody; Meso Scale Discovery, #C26UG-3, IL-6 antibody; Meso Scale Discovery, #C21TX-3, IP-10 antibody; Meso Scale Discovery, #C21UF-3). After 3 washes with PBS-T (PBS containing 0.05% Tween 20), test samples or Calibrator standards (Calibrator 1; Meso Scale Discovery, #C0060-2, Calibrator 2: Meso Scale Discovery, #C0061-2) were incubated with assay diluent 43 (Meso Scale Discovery, #R50AG-2), which contains serum, blockers and preservatives, at room temperature for an hour. After 3 washes, SULFO-TAG conjugated detection antibodies for IL-6 (Meso Scale Discovery, #D26TX-3), MCP-1 (Meso Scale Discovery, #D26UG-3) and IP-10 (Meso Scale Discovery, #D21UF-3) were incubated in individual wells at at room temperature for an hour. After 3 washes and adding Gold™ read buffer B (Meso Scale Discovery, #R60AM-2) to each well, the plate was analyzed by MSD instrument (Meso Scale Discovery, #R31QQ-3.

Table 23. ABE variant sequences

MA004 mRNA and protein sequences			
Region	**Sequence**		**SEQ ID NO**
Full sequence	mRNA	AGGAAAu'AAGAGAGAAAAGAAGAGu'AAGAAGAAAu'Au'AAGAGCCACCAu'GAGCGAGGu'GGAGu'u'CAGCCACGAGu'ACu'GGAu'GCGGCACGCCCu'GACCCu'GGCCAAGCGGGCCCGGGACGAGCGGGAGGu'GCCCGu'GGGCGCCGu'GCu'GGu'GCu'GAACAACCGGGu'GAu'CGGCGAGGGCu'GGAACCGGGCCAu'CGGCCu'GCACGACCCCACCGCCCACGCCGAGAu'CAu'GGCCCu'GCGGCAGGGCGGCCu'GGu'GAu'GCAGAACu'ACCGGCu'GAu'CGACGCCACCCu'Gu'ACGu'GACCu'u'CGAGCCCu'GCGu'GAu'Gu'GCGCCGGCGCCAu'GAu'CCACAGCCGGAu'CGGCCGGGu'GGu'Gu'u'CGGCGu'GCGGAACGCCAAGACCGGCGCCGCCGGCAGCCu'GAu'GGACGu'GCu'GCACCACCCCGGCAu'GAACCACCGGGu'GGAGAu'CACCGAGGGCAu'CCu'GGCCGACGAGu'GCGCCGCCCu'GCu'Gu'GCCGGu'u'Cu'u'CCGGAu'GCCCCGGCGGGu'Gu'u'CAACGCCCAGAAGAAGGCCCAGAGCAGCACCGACAGCGGCGGCAGCAGCGGCGGCAGCAGCGGCAGCGAGACACCCGGCACCAGCGAGAGCGCCACCCCCGAGAGCAGCGGCGGCAGCAGCGGCGGCAGCGACAAGAAGu'ACAGCAu'CGGCCu'GGCCAu'CGGCACCAAC	2192

		AGCGu'GGGCu'GGGCCGu'GAu'CACCGACGAGu'ACAA GGu'GCCCAGCAAGAAGu'u'CAAGGu'GCu'GGGCAACA CCGACCGGCACAGCAu'CAAGAAGAACCu'GAu'CGGCG CCCu'GCu'Gu'u'CGACAGCGGCGAGACAGCCGAGGCCA CCCGGCu'GAAGCGGACCGCCCGGCGGCGGu'ACACCC GGCGGAAGAACCGGAu'Cu'GCu'ACCu'GCAGGAGAu'Cu 'u'CAGCAACGAGAu'GGCCAAGGu'GGACGACAGCu'u'C u'u'CCACCGGCu'GGAGGAGAGCu'u'CCu'GGu'GGAGGA GGACAAGAAGCACGAGCGGCACCCCAu'Cu'u'CGGCAA CAu'CGu'GGACGAGGu'GGCCu'ACCACGAGAAGu'ACCC CACCAu'Cu'ACCACCu'GCGGAAGAAGCu'GGu'GGACAG CACCGACAAGGCCGACCu'GCGGCu'GAu'Cu'ACCu'GGC CCu'GGCCCACAu'GAu'CAAGu'u'CCGGGGCCACu'u'CCu 'GAu'CGAGGGCGACCu'GAACCCCGACAACAGCGACGu' GGACAAGCu'Gu'u'CAu'CCAGCu'GGu'GCAGACCu'ACA ACCAGCu'Gu'u'CGAGGAGAACCCCAu'CAACGCCAGCG GCGu'GGACGCCAAGGCCAu'CCu'GAGCGCCCGGCu'GA GCAAGAGCCGGCGGCu'GGAGAACCu'GAu'CGCCCAGC u'GCCCGGCGAGAAGAAGAACGGCCu'Gu'u'CGGCAACC u'GAu'CGCCCu'GAGCCu'GGGCCu'GACCCCCAACu'u'CA AGAGCAACu'u'CGACCu'GGCCGAGGACGCCAAGCu'GC AGCu'GAGCAAGGACACCu'ACGACGACGACCu'GGACA ACCu'GCu'GGCCCAGAu'CGGCGACCAGu'ACGCCGACC u'Gu'u'CCu'GGCCGCCAAGAACCu'GAGCGACGCCAu'CC u'GCu'GAGCGACAu'CCu'GCGGGu'GAACACCGAGAu'C ACCAAGGCCCCCCu'GAGCGCCAGCAu'GAu'CAAGCGG u'ACGACGAGCACCACCAGGACCu'GACCCu'GCu'GAAG GCCCu'GGu'GCGGCAGCAGCu'GCCCGAGAAGu'ACAAG GAGAu'Cu'u'Cu'u'CGACCAGAGCAAGAACGGCu'ACGC CGGCu'ACAu'CGACGGCGGCGCCAGCCAGGAGGAGu'u' Cu'ACAAGu'u'CAu'CAAGCCCAu'CCu'GGAGAAGAu'GG ACGGCACCGAGGAGCu'GCu'GGu'GAAGCu'GAACCGGG AGGACCu'GCu'GCGGAAGCAGCGGACCu'u'CGACAACG GCAGCAu'CCCCCACCAGAu'CCACCu'GGGCGAGCu'GC ACGCCAu'CCu'GCGGCGGCAGGAGGACu'u'Cu'ACCCCu' u'CCu'GAAGGACAACCGGGAGAAGAu'CGAGAAGAu'CC u'GACCu'u'CCGGAu'CCCCu'ACu'ACGu'GGGCCCCCu'G GCCCGGGGCAACAGCCGGu'u'CGCCu'GGAu'GACCCGC AAGAGCGAGGAGACAAu'CACCCCCu'GGAACu'u'CGAG GAGGu'GGu'GGACAAGGGCGCCAGCGCCCAGAGCu'u'C Au'CGAGCGGAu'GACCAACu'u'CGACAAGAACCu'GCCC AACGAGAAGGu'GCu'GCCCAAGCACAGCCu'GCu'Gu'AC GAGu'ACu'u'CACCGu'Gu'ACAACGAGCu'GACCAAGGu' GAAGu'ACGu'GACCGAGGGCAu'GCGGAAGCCCGCCu'u' CCu'GAGCGGCGAGCAGAAGAAGGCCAu'CGu'GGACCu' GCu'Gu'u'CAAGACCAACCGGAAGGu'GACCGu'GAAGC AGCu'GAAGGAGGACu'ACu'u'CAAGAAGAu'CGAGu'GC u'u'CGACAGCGu'GGAGAu'CAGCGGCGu'GGAGGACCG Gu'u'CAACGCCAGCCu'GGGCACCu'ACCACGACCu'GCu' GAAGAu'CAu'CAAGGACAAGGACu'u'CCu'GGACAACG AGGAGAACGAGGACAu'CCu'GGAGGACAu'CGu'GCu'G ACCCu'GACCCu'Gu'u'CGAGGACCGGGAGAu'GAu'CGA GGAGCGGCu'GAAGACCu'ACGCCCACCu'Gu'u'CGACGA CAAGGu'GAu'GAAGCAGCu'GAAGCGGCGGCGGu'ACAC CGGCu'GGGGCCGGCu'GAGCCGGAAGCu'GAu'CAACGG	

		CAu'CCGGGACAAGCAGAGCGGCAAGACCAu'CCu'GGA Cu'u'CCu'CAAGAGCGACGGCu'u'CGCCAACCGGAACu'u 'CAu'GCAGCu'GAu'CCACGACGACAGCCu'GACCu'u'CA AGGAGGACAu'CCAGAAGGCCCAGGu'GAGCGGCCAGG GCGACAGCCu'GCACGAGCACAu'CGCCAACCu'GGCCG GCAGCCCCGCCAu'CAAGAAGGGCAu'CCu'GCAGACCG u'GAAGGu'GGu'GGACGAGCu'GGu'GAAGGu'GAu'GGGC CGGCACAAGCCCGAGAACAu'CGu'GAu'CGAGAu'GGCC CGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAAC AGCCGGGAGCGGAu'GAAGCGGAu'CGAGGAGGGCAu'C AAGGAGCu'GGGCAGCCAGAu'CCu'GAAGGAGCACCCC Gu'GGAGAACACCCAGCu'GCAGAACGAGAAGCu'Gu'AC Cu'Gu'ACu'ACCu'GCAGAACGGCCGGGACAu'Gu'ACGu' GGACCAGGAGCu'GGACAu'CAACCGGCu'GAGCGACu'A CGACGu'GGACCACAu'CGu'GCCCCAGAGCu'u'CCu'GAA GGACGACAGCAu'CGACAACAAGGu'GCu'GACCCGGAG CGACAAGAACCGGGGCAAGAGCGACAACGu'GCCCAG CGAGGAGGu'GGu'GAAGAAGAu'GAAGAACu'ACu'GGC GGCAGCu'GCu'GAACGCCAAGCu'GAu'CACCCAGCGGA AGu'u'CGACAACCu'GACCAAGGCCGAGCGGGGCGGCC u'GAGCGAGCu'GGACAAGGCCGGCu'u'CAu'CAAGCGGC AGCu'GGu'GGAGACACGGCAGAu'CACCAAGCACGu'GG CCCAGAu'CCu'GGACAGCCGGAu'GAACACCAAGu'ACG ACGAGAACGACAAGCu'GAu'CCGGGAGGu'GAAGGu'G Au'CACCCu'CAAGAGCAAGCu'GGu'GAGCGACu'u'CCG GAAGGACu'u'CCAGu'u'Cu'ACAAGGu'GCGGGAGAu'CA ACAACu'ACCACCACGCCCACGACGCCu'ACCu'GAACG CCGu'GGu'GGGCACCGCCCu'GAu'CAAGAAGu'ACCCCA AGCu'GGAGAGCGAGu'u'CGu'Gu'ACGGCGACu'ACAAG Gu'Gu'ACGACGu'GCGGAAGAu'GAu'CGCCAAGAGCGA GCAGGAGAu'CGGCAAGGCCACCGCCAAGu'ACu'u'Cu'u 'Cu'ACAGCAACAu'CAu'GAACu'u'Cu'u'CAAGACCGAGA u'CACCCu'GGCCAACGGCGAGAu'CCGGAAGCGGCCCCu 'GAu'CGAGACAAACGGCGAGACAGGCGAGAu'CGu'Gu' GGGACAAGGGCCGGGACu'u'CGCCACCGu'GCGGAAGG u'GCu'GAGCAu'GCCCCAGGu'GAACAu'CGu'GAAGAAG ACCGAGGu'GCAGACCGGCGGCu'u'CAGCAAGGAGAGC Au'CCu'GCCCAAGCGGAACAGCGACAAGCu'GAu'CGCC CGGAAGAAGGACu'GGGACCCCAAGAAGu'ACGGCGGC u'u'CGACAGCCCCACCGu'GGCCu'ACAGCGu'GCu'GGu' GGu'GGCCAAGGu'GGAGAAGGGCAAGAGCAAGAAGCu 'CAAGAGCGu'GAAGGAGCu'GCu'GGGCAu'CACCAu'CA u'GGAGCGGAGCAGCu'u'CGAGAAGAACCCCAu'CGACu' u'CCu'GGAGGCCAAGGGCu'ACAAGGAGGu'GAAGAAG GACCu'GAu'CAu'CAAGCu'GCCCAAGu'ACAGCCu'Gu'u' CGAGCu'GGAGAACGGCCGGAAGCGGAu'GCu'GGCCAG CGCCGGCGAGCu'GCAGAAGGGCAACGAGCu'GGCCCu' GCCCAGCAAGu'ACGu'GAACu'u'CCu'Gu'ACCu'GGCCA GCCACu'ACGAGAAGCu'GAAGGGCAGCCCCGAGGACA ACGAGCAGAAGCAGCu'Gu'u'CGu'GGAGCAGCACAAGC ACu'ACCu'GGACGAGAu'CAu'CGAGCAGAu'CAGCGAGu 'u'CAGCAAGCGGGu'GAu'CCu'GGCCGACGCCAACCu'G GACAAGGu'GCu'GAGCGCCu'ACAACAAGCACCGGGAC AAGCCCAu'CCGGGAGCAGGCCGAGAACAu'CAu'CCAC Cu'Gu'u'CACCCu'GACCAACCu'GGGCGCCCCCGCCGCCu	

		'u'CAAGu'ACu'u'CGACACCACCAu'CGACCGGAAGCGGu 'ACACCAGCACCAAGGAGGu'GCu'GGACGCCACCCu'GA u'CCACCAGAGCAu'CACCGGCCu'Gu'ACGAGACACGGA u'CGACCu'GAGCCAGCu'GGGCGGCGACGAGGGCGCCG ACAAGCGGACCGCCGACGGCAGCGAGu'u'CGAGAGCC CCAAGAAGAAGCGGAAGGu'Gu'GAGCGGCCGCu'u'AAu 'u'AAGCu'GCCu'u'Cu'GCGGGGCu'u'GCCu'u'Cu'GGCCAu 'GCCCu'u'Cu'u'Cu'Cu'CCCu'u'GCACCu'Gu'ACCu'Cu'u'G Gu'Cu'u'u'GAAu'AAAGCCu'GAGu'AGGAAGu'Cu'AGA	
	protein	MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNN RVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLID ATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHHPGMNHRVEITEGILADECAALLCRFFRMPRR VFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGG SSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVL GNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRR KNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHER HPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIY LALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKN GLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDL DNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKA PLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQS KNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLN REDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITP WNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLL YEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLF KTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD KQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM GRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKEL GSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDI NRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDN VPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAH DAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIA KSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT GGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAY SVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDF LEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGEL QKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFV EQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRD KPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTK EVLDATLIHQSITGLYETRIDLSQLGGDEGADKRTADGSE FESPKKKRKV	2137
5′ UTR	mRNA	AGGAAAu'AAGAGAGAAAAGAAGAGu'AAGAAGAAAu' Au'AAGAGCCACC	2138
TadA	mRNA	Au'GAGCGAGGu'GGAGu'u'CAGCCACGAGu'ACu'GGAu' GCGGCACGCCCu'GACCCu'GGCCAAGCGGGCCCGGGA	2139

		CGAGCGGGAGGu'GCCCGu'GGGCGCCGu'GCu'GGu'GCu'GAACAACCGGGu'GAu'CGGCGAGGGCu'GGAACCGGGCCAu'CGGCCu'GCACGACCCCACCGCCCACGCCGAGAu'CAu'GGCCCu'GCGGCAGGGCGGCCu'GGu'GAu'GCAGAACu'ACCGGCu'GAu'CGACGCCACCCu'Gu'ACGu'GACCu'u'CGAGCCCu'GCGu'GAu'Gu'GCGCCGGCGCCAu'GAu'CCACAGCCGGAu'CGGCCGGGu'GGu'Gu'u'CGGCGu'GCGGAACGCCAAGACCGGCGCCGCCGGCAGCCu'GAu'GGACGu'GCu'GCACCACCCCGGCAu'GAACCACCGGGu'GGAGAu'CACCGAGGGCAu'CCu'GGCCGACGAGu'GCGCCGCCCu'GCu'Gu'GCCGGu'u'Cu'u'CCGGAu'GCCCCGGCGGGu'Gu'u'CAACGCCCAGAAGAAGGCCCAGAGCAGCACCGAC	
	protein	MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLCRFFRMPRRVFNAQKKAQSSTD	2140
Linker between TadA and Cas9 nickase	mRNA	AGCGGCGGCAGCAGCGGCGGCAGCAGCGGCAGCGAGACACCCGGCACCAGCGAGAGCGCCACCCCCGAGAGCAGCGGCGGCAGCAGCGGCGGCAGC	2141
	protein	SGGSSGGSSGSETPGTSESATPESSGGSSGGS	20
Cas9 nickase	mRNA	GACAAGAAGu'ACAGCAu'CGGCCu'GGCCAu'CGGCACCAACAGCGu'GGGCu'GGGCCGu'GAu'CACCGACGAGu'ACAAGGu'GCCCAGCAAGAAGu'u'CAAGGu'GCu'GGGCAACACCGACCGGCACAGCAu'CAAGAAGAACCu'GAu'CGGCGCCCu'GCu'Gu'u'CGACAGCGGCGAGACAGCCGAGGCCACCCGGCu'GAAGCGGACCGCCCGGCGGCGGu'ACACCCGGCGGAAGAACCGGAu'Cu'GCu'ACCu'GCAGGAGAu'Cu'u'CAGCAACGAGAu'GGCCAAGGu'GGACGACAGCu'u'Cu'u'CCACCGGCu'GGAGGAGAGCu'u'CCu'GGu'GGAGGAGGACAAGAAGCACGAGCGGCACCCCAu'Cu'u'CGGCAACAu'CGu'GGACGAGGu'GGCCu'ACCACGAGAAGu'ACCCCACCAu'Cu'ACCACCu'GCGGAAGAAGCu'GGu'GGACAGCACCGACAAGGCCGACCu'GCGGCu'GAu'Cu'ACCu'GGCCCu'GGCCCACAu'GAu'CAAGu'u'CCGGGGCCACu'u'CCu'GAu'CGAGGGCGACCu'GAACCCCGACAACAGCGACGu'GGACAAGCu'Gu'u'CAu'CCAGCu'GGu'GCAGACCu'ACAACCAGCu'Gu'u'CGAGGAGAACCCCAu'CAACGCCAGCGGCGu'GGACGCCAAGGCCAu'CCu'GAGCGCCCGGCu'GAGCAAGAGCCGGCGGCu'GGAGAACCu'GAu'CGCCCAGCu'GCCCGGCGAGAAGAAGAACGGCCu'Gu'u'CGGCAACCu'GAu'CGCCCu'GAGCCu'GGGCCu'GACCCCCAACu'u'CAAGAGCAACu'u'CGACCu'GGCCGAGGACGCCAAGCu'GCAGCu'GAGCAAGGACACCu'ACGACGACGACCu'GGACAACCu'GCu'GGCCCAGAu'CGGCGACCAGu'ACGCCGACCu'Gu'u'CCu'GGCCGCCAAGAACCu'GAGCGACGCCAu'CCu'GCu'GAGCGACAu'CCu'GCGGGu'GAACACCGAGAu'CACCAAGGCCCCCCu'GAGCGCCAGCAu'GAu'CAAGCGGu'ACGACGAGCACCACCAGGACCu'GACCCu'GCu'GAAGGCCCu'GGu'GCGGCAGCAGCu'GCCCGAGAAGu'ACAAGGAGAu'Cu'u'Cu'u'CGACCAGAGCAAGAACGGCu'ACG	2142

		CCGGCu'ACAu'CGACGGCGGCGCCAGCCAGGAGGAGu' u'Cu'ACAAGu'u'CAu'CAAGCCCAu'CCu'GGAGAAGAu'G GACGGCACCGAGGAGCu'GCu'GGu'GAAGCu'GAACCGG GAGGACCu'GCu'GCGGAAGCAGCGGACCu'u'CGACAAC GGCAGCAu'CCCCCACCAGAu'CCACCu'GGGCGAGCu'G CACGCCAu'CCu'GCGGCGGCAGGAGGACu'u'Cu'ACCCC u'u'CCu'GAAGGACAACCGGGAGAAGAu'CGAGAAGAu' CCu'GACCu'u'CCGGAu'CCCCu'ACu'ACGu'GGGCCCCCu' GGCCCGGGGCAACAGCCGGu'u'CGCCu'GGAu'GACCCG CAAGAGCGAGGAGACAAu'CACCCCCu'GGAACu'u'CGA GGAGGu'GGu'GGACAAGGGCGCCAGCGCCCAGAGCu'u' CAu'CGAGCGGAu'GACCAACu'u'CGACAAGAACCu'GCC CAACGAGAAGGu'GCu'GCCCAAGCACAGCCu'GCu'Gu'A CGAGu'ACu'u'CACCGu'Gu'ACAACGAGCu'GACCAAGGu 'GAAGu'ACGu'GACCGAGGGCAu'GCGGAAGCCCGCCu'u 'CCu'GAGCGGCGAGCAGAAGAAGGCCAu'CGu'GGACCu 'GCu'Gu'u'CAAGACCAACCGGAAGGu'GACCGu'GAAGC AGCu'GAAGGAGGACu'ACu'u'CAAGAAGAu'CGAGu'GC u'u'CGACAGCGu'GGAGAu'CAGCGGCGu'GGAGGACCG Gu'u'CAACGCCAGCCu'GGGCACCu'ACCACGACCu'GCu' GAAGAu'CAu'CAAGGACAAGGACu'u'CCu'GGACAACG AGGAGAACGAGGACAu'CCu'GGAGGACAu'CGu'GCu'G ACCCu'GACCCu'Gu'u'CGAGGACCGGGAGAu'GAu'CGA GGAGCGGCu'GAAGACCu'ACGCCCACCu'Gu'u'CGACGA CAAGGu'GAu'GAAGCAGCu'GAAGCGGCGGCGGu'ACAC CGGCu'GGGGCCGGCu'GAGCCGGAAGCu'GAu'CAACGG CAu'CCGGGACAAGCAGAGCGGCAAGACCAu'CCu'GGA Cu'u'CCu'CAAGAGCGACGGCu'u'CGCCAAC**CGG**AACu'u 'CAu'GCAGCu'GAu'CCACGACGACAGCCu'GACCu'u'CA AGGAGGACAu'CCAGAAGGCCCAGGu'GAGCGGCCAGG GCGACAGCCu'GCACGAGCACAu'CGCCAACCu'GGCCG GCAGCCCCGCCAu'CAAGAAGGGCAu'CCu'GCAGACCG u'GAAGGu'GGu'GGACGAGCu'GGu'GAAGGu'GAu'GGGC CGGCACAAGCCCGAGAACAu'CGu'GAu'CGAGAu'GGCC CGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAAC AGCCGGGAGCGGAu'GAAGCGGAu'CGAGGAGGGCAu'C AAGGAGCu'GGGCAGCCAGAu'CCu'GAAGGAGCACCCC Gu'GGAGAACACCCAGCu'GCAGAACGAGAAGCu'Gu'AC Cu'Gu'ACu'ACCu'GCAGAACGGCCGGGACAu'Gu'ACGu' GGACCAGGAGCu'GGACAu'CAACCGGCu'GAGCGACu'A CGACGu'GGACCACAu'CGu'GCCCCAGAGCu'u'CCu'GAA GGACGACAGCAu'CGACAACAAGGu'GCu'GACCCGGAG CGACAAGAACCGGGGCAAGAGCGACAACGu'GCCCAG CGAGGAGGu'GGu'GAAGAAGAu'GAAGAACu'ACu'GGC GGCAGCu'GCu'GAACGCCAAGCu'GAu'CACCCAGCGGA AGu'u'CGACAACCu'GACCAAGGCCGAGCGGGGCGGCC u'GAGCGAGCu'GGACAAGGCCGGCu'u'CAu'CAAGCGGC AGCu'GGu'GGAGACACGGCAGAu'CACCAAGCACGu'GG CCCAGAu'CCu'GGACAGCCGGAu'GAACACCAAGu'ACG ACGAGAACGACAAGCu'GAu'CCGGGAGGu'GAAGGu'G Au'CACCCu'CAAGAGCAAGCu'GGu'GAGCGACu'u'CCG GAAGGACu'u'CCAGu'u'Cu'ACAAGGu'GCGGGAGAu'CA ACAACu'ACCACCACGCCCACGACGCCu'ACCu'GAACG CCGu'GGu'GGGCACCGCCCu'GAu'CAAGAAGu'ACCCCA AGCu'GGAGAGCGAGu'u'CGu'Gu'ACGGCGACu'ACAAG	

		Gu'Gu'ACGACGu'GCGGAAGAu'GAu'CGCCAAGAGCGA GCAGGAGAu'CGGCAAGGCCACCGCCAAGu'ACu'u'Cu'u 'Cu'ACAGCAACAu'CAu'GAACu'u'Cu'u'CAAGACCGAGA u'CACCCu'GGCCAACGGCGAGAu'CCGGAAGCGGCCCCu 'GAu'CGAGACAAACGGCGAGACAGGCGAGAu'CGu'Gu' GGGACAAGGGCCGGGACu'u'CGCCACCGu'GCGGAAGG u'GCu'GAGCAu'GCCCCAGGu'GAACAu'CGu'GAAGAAG ACCGAGGu'GCAGACCGGCGGCu'u'CAGCAAGGAGAGC Au'CCu'GCCCAAGCGGAACAGCGACAAGCu'GAu'CGCC CGGAAGAAGGACu'GGGACCCCAAGAAGu'ACGGCGGC u'u'C**GAC**AGCCCCACCGu'GGCCu'ACAGCGu'GCu'GGu' GGu'GGCCAAGGu'GGAGAAGGGCAAGAGCAAGAAGCu 'CAAGAGCGu'GAAGGAGCu'GCu'GGGCAu'CACCAu'CA u'GGAGCGGAGCAGCu'u'CGAGAAGAACCCCAu'CGACu' u'CCu'GGAGGCCAAGGGCu'ACAAGGAGGu'GAAGAAG GACCu'GAu'CAu'CAAGCu'GCCCAAGu'ACAGCCu'Gu'u' CGAGCu'GGAGAACGGCCGGAAGCGGAu'GCu'GGCCAG CGCCGGCGAGCu'GCAGAAGGGCAACGAGCu'GGCCCu' GCCCAGCAAGu'ACGu'GAACu'u'CCu'Gu'ACCu'GGCCA GCCACu'ACGAGAAGCu'GAAGGGCAGCCCCGAGGACA ACGAGCAGAAGCAGCu'Gu'u'CGu'GGAGCAGCACAAGC ACu'ACCu'GGACGAGAu'CAu'CGAGCAGAu'CAGCGAGu 'u'CAGCAAGCGGGu'GAu'CCu'GGCCGACGCCAACCu'G GACAAGGu'GCu'GAGCGCCu'ACAACAAGCACCGGGAC AAGCCCAu'CCGGGAGCAGGCCGAGAACAu'CAu'CCAC Cu'Gu'u'CACCCu'GACCAACCu'GGGCGCCCCCGCCGCCu 'u'CAAGu'ACu'u'CGACACCACCAu'CGACCGGAAGCGGu 'ACACCAGCACCAAGGAGGu'GCu'GGACGCCACCCu'GA u'CCACCAGAGCAu'CACCGGCCu'Gu'ACGAGACACGGA u'CGACCu'GAGCCAGCu'GGGCGGCGAC	
	protein	DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDR HSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRIC YLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFG NIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALA HMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEEN PINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFG NLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNL LAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSA SMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNG YAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDL LRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNRE KIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFE EVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYF TVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNR KVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDL LKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKT YAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSG KTILDFLKSDGFAN**R**NFMQLIHDDSLTFKEDIQKAQVSGQ GDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHK PENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQI LKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRL SDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPS EEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYL	40

		NAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQ EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGE TGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFS KESILPKRNSDKLIARKKDWDPKKYGGF**D**SPTVAYSVLV VAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAK GYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHK HYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIRE QAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLD ATLIHQSITGLYETRIDLSQLGGD	
Linker between Cas9 nickase and NLS	mRNA	GAGGGCGCCGAC	2143
	Protein	EGAD	2144
Nuclear localization sequence (NLS)	mRNA	AAGCGGACCGCCGACGGCAGCGAGu'u'CGAGAGCCCC AAGAAGAAGCGGAAGGu'Gu'GA	2145
	Protein	KRTADGSEFESPKKKRKV	2146
3′ UTR	mRNA	GCGGCCGCu'u'AAu'u'AAGCu'GCCu'u'Cu'GCGGGGCu'u' GCCu'u'Cu'GGCCAu'GCCCu'u'Cu'u'Cu'Cu'CCCu'u'GCAC Cu'Gu'ACCu'Cu'u'GGu'Cu'u'u'GAAu'AAAGCCu'GAGu'A GGAAGu'Cu'AGA	2147
MA040 mRNA and protein sequences			
Region	**Sequence**		**SEQ ID NO**
Full sequence	mRNA	AGGAAAu'AAGAGAGAAAAGAAGAGu'AAGAAGAAAu' Au'AAGAGCCACCAu'GAGCGAGGu'GGAGu'u'CAGCCA CGAGu'ACu'GGAu'GCGGCACGCCCu'GACCCu'GGCCAA GCGGGCCCGGGACGAGCGGGAGGu'GCCCGu'GGGCGC CGu'GCu'GGu'GCu'GAACAACCGGGu'GAu'CGGCGAGG GCu'GGAACCGGGCCAu'CGGCCu'GCACGACCCCACCG CCCACGCCGAGAu'CAu'GGCCCu'GCGGCAGGGCGGCCu 'GGu'GAu'GCAGAACu'ACCGGCu'GAu'CGACGCCACCCu 'Gu'ACGu'GACCu'u'CGAGCCCu'GCGu'GAu'Gu'GCGCCG GCGCCAu'GAu'CCACAGCCGGAu'CGGCCGGGu'GGu'Gu 'u'CGGCGu'GCGGAACGCCAAGACCGGCGCCGCCGGCA GCCu'GAu'GGACGu'GCu'GCACCACCCCGGCAu'GAACC ACCGGGu'GGAGAu'CACCGAGGGCAu'CCu'GGCCGACG AGu'GCGCCGCCCu'GCu'Gu'GCCGGu'u'Cu'u'CCGGAu'G CCCCGGCGGGu'Gu'u'CAACGCCCAGAAGAAGGCCCAG AGCAGCACCGACAGCGGCGGCAGCAGCGGCGGCAGCA GCGGCAGCGAGACACCCGGCACCAGCGAGAGCGCCAC CCCCGAGAGCAGCGGCGGCAGCAGCGGCGGCAGCGAC AAGAAGu'ACAGCAu'CGGCCu'GGCCAu'CGGCACCAAC AGCGu'GGGCu'GGGCCGu'GAu'CACCGACGAGu'ACAA GGu'GCCCAGCAAGAAGu'u'CAAGGu'GCu'GGGCAACA CCGACCGGCACAGCAu'CAAGAAGAACCu'GAu'CGGCG CCCu'GCu'Gu'u'CGACAGCGGCGAGACAGCCGAGGCCA CCCGGCu'GAAGCGGACCGCCCGGCGGCGGu'ACACCC GGCGGAAGAACCGGAu'Cu'GCu'ACCu'GCAGGAGAu'Cu 'u'CAGCAACGAGAu'GGCCAAGGu'GGACGACAGCu'u'C	2148

		u'u'CCACCGGCu'GGAGGAGAGCu'u'CCu'GGu'GGAGGA GGACAAGAAGCACGAGCGGCACCCCAu'Cu'u'CGGCAA CAu'CGu'GGACGAGGu'GGCCu'ACCACGAGAAGu'ACCC CACCAu'Cu'ACCACCu'GCGGAAGAAGCu'GGu'GGACAG CACCGACAAGGCCGACCu'GCGGCu'GAu'Cu'ACCu'GGC CCu'GGCCCACAu'GAu'CAAGu'u'CCGGGGCCACu'u'CCu 'GAu'CGAGGGCGACCu'GAACCCCGACAACAGCGACGu' GGACAAGCu'Gu'u'CAu'CCAGCu'GGu'GCAGACCu'ACA ACCAGCu'Gu'u'CGAGGAGAACCCCAu'CAACGCCAGCG GCGu'GGACGCCAAGGCCAu'CCu'GAGCGCCCGGCu'GA GCAAGAGCCGGCGGCu'GGAGAACCu'GAu'CGCCCAGC u'GCCCGGCGAGAAGAAGAACGGCCu'Gu'u'CGGCAACC u'GAu'CGCCCu'GAGCCu'GGGCCu'GACCCCCAACu'u'CA AGAGCAACu'u'CGACCu'GGCCGAGGACGCCAAGCu'GC AGCu'GAGCAAGGACACCu'ACGACGACGACCu'GGACA ACCu'GCu'GGCCCAGAu'CGGCGACCAGu'ACGCCGACC u'Gu'u'CCu'GGCCGCCAAGAACCu'GAGCGACGCCAu'CC u'GCu'GAGCGACAu'CCu'GCGGGu'GAACACCGAGAu'C ACCAAGGCCCCCCu'GAGCGCCAGCAu'GAu'CAAGCGG u'ACGACGAGCACCACCAGGACCu'GACCCu'GCu'GAAG GCCCu'GGu'GCGGCAGCAGCu'GCCCGAGAAGu'ACAAG GAGAu'Cu'u'Cu'u'CGACCAGAGCAAGAACGGCu'ACGC CGGCu'ACAu'CGACGGCGGCGCCAGCCAGGAGGAGu'u' Cu'ACAAGu'u'CAu'CAAGCCCAu'CCu'GGAGAAGAu'GG ACGGCACCGAGGAGCu'GCu'GGu'GAAGCu'GAACCGGG AGGACCu'GCu'GCGGAAGCAGCGGACCu'u'CGACAACG GCAGCAu'CCCCCACCAGAu'CCACCu'GGGCGAGCu'GC ACGCCAu'CCu'GCGGCGGCAGGAGGACu'u'Cu'ACCCCu' u'CCu'GAAGGACAACCGGGAGAAGAu'CGAGAAGAu'CC u'GACCu'u'CCGGAu'CCCCu'ACu'ACGu'GGGCCCCCu'G GCCCGGGGCAACAGCCGGu'u'CGCCu'GGAu'GACCCGC AAGAGCGAGGAGACAAu'CACCCCCu'GGAACu'u'CGAG GAGGu'GGu'GGACAAGGGCGCCAGCGCCCAGAGCu'u'C Au'CGAGCGGAu'GACCAACu'u'CGACAAGAACCu'GCCC AACGAGAAGGu'GCu'GCCCAAGCACAGCCu'GCu'Gu'AC GAGu'ACu'u'CACCGu'Gu'ACAACGAGCu'GACCAAGGu' GAAGu'ACGu'GACCGAGGGCAu'GCGGAAGCCCGCCu'u' CCu'GAGCGGCGAGCAGAAGAAGGCCAu'CGu'GGACCu' GCu'Gu'u'CAAGACCAACCGGAAGGu'GACCGu'GAAGC AGCu'GAAGGAGGACu'ACu'u'CAAGAAGAu'CGAGu'GC u'u'CGACAGCGu'GGAGAu'CAGCGGCGu'GGAGGACCG Gu'u'CAACGCCAGCCu'GGGCACCu'ACCACGACCu'GCu' GAAGAu'CAu'CAAGGACAAGGACu'u'CCu'GGACAACG AGGAGAACGAGGACAu'CCu'GGAGGACAu'CGu'GCu'G ACCCu'GACCCu'Gu'u'CGAGGACCGGGAGAu'GAu'CGA GGAGCGGCu'GAAGACCu'ACGCCCACCu'Gu'u'CGACGA CAAGGu'GAu'GAAGCAGCu'GAAGCGGCGGCGGu'ACAC CGGCu'GGGGCCGGCu'GAGCCGGAAGCu'GAu'CAACGG CAu'CCGGGACAAGCAGAGCGGCAAGACCAu'CCu'GGA Cu'u'CCu'CAAGAGCGACGGCu'u'CGCCAAC**<u>CGG</u>**AACu'u 'CAu'GCAGCu'GAu'CCACGACGACAGCCu'GACCu'u'CA AGGAGGACAu'CCAGAAGGCCCAGGu'GAGCGGCCAGG GCGACAGCCu'GCACGAGCACAu'CGCCAACCu'GGCCG GCAGCCCCGCCAu'CAAGAAGGGCAu'CCu'GCAGACCG u'GAAGGu'GGu'GGACGAGCu'GGu'GAAGGu'GAu'GGGC	

		CGGCACAAGCCCGAGAACAu'CGu'GAu'CGAGAu'GGCC CGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAAC AGCCGGGAGCGGAu'GAAGCGGAu'CGAGGAGGGCAu'C AAGGAGCu'GGGCAGCCAGAu'CCu'GAAGGAGCACCCC Gu'GGAGAACACCCAGCu'GCAGAACGAGAAGCu'Gu'AC Cu'Gu'ACu'ACCu'GCAGAACGGCCGGGACAu'Gu'ACGu' GGACCAGGAGCu'GGACAu'CAACCGGCu'GAGCGACu'A CGACGu'GGACCACAu'CGu'GCCCCAGAGCu'u'CCu'GAA GGACGACAGCAu'CGACAACAAGGu'GCu'GACCCGGAG CGACAAGAACCGGGGCAAGAGCGACAACGu'GCCCAG CGAGGAGGu'GGu'GAAGAAGAu'GAAGAACu'ACu'GGC GGCAGCu'GCu'GAACGCCAAGCu'GAu'CACCCAGCGGA AGu'u'CGACAACCu'GACCAAGGCCGAGCGGGGCGGCC u'GAGCGAGCu'GGACAAGGCCGGCu'u'CAu'CAAGCGGC AGCu'GGu'GGAGACACGGCAGAu'CACCAAGCACGu'GG CCCAGAu'CCu'GGACAGCCGGAu'GAACACCAAGu'ACG ACGAGAACGACAAGCu'GAu'CCGGGAGGu'GAAGGu'G Au'CACCCu'CAAGAGCAAGCu'GGu'GAGCGACu'u'CCG GAAGGACu'u'CCAGu'u'Cu'ACAAGGu'GCGGGAGAu'CA ACAACu'ACCACCACGCCCACGACGCCu'ACCu'GAACG CCGu'GGu'GGGCACCGCCCu'GAu'CAAGAAGu'ACCCCA AGCu'GGAGAGCGAGu'u'CGu'Gu'ACGGCGACu'ACAAG Gu'Gu'ACGACGu'GCGGAAGAu'GAu'CGCCAAGAGCGA GCAGGAGAu'CGGCAAGGCCACCGCCAAGu'ACu'u'Cu'u 'Cu'ACAGCAACAu'CAu'GAACu'u'Cu'u'CAAGACCGAGA u'CACCCu'GGCCAACGGCGAGAu'CCGGAAGCGGCCCCu 'GAu'CGAGACAAACGGCGAGACAGGCGAGAu'CGu'Gu' GGGACAAGGGCCGGGACu'u'CGCCACCGu'GCGGAAGG u'GCu'GAGCAu'GCCCCAGGu'GAACAu'CGu'GAAGAAG ACCGAGGu'GCAGACCGGCGGCu'u'CAGCAAGGAGAGC Au'CCu'GCCCAAGCGGAACAGCGACAAGCu'GAu'CGCC CGGAAGAAGGACu'GGGACCCCAAGAAGu'ACGGCGGC u'u'C**GAG**AGCCCCACCGu'GGCCu'ACAGCGu'GCu'GGu' GGu'GGCCAAGGu'GGAGAAGGGCAAGAGCAAGAAGCu 'CAAGAGCGu'GAAGGAGCu'GCu'GGGCAu'CACCAu'CA u'GGAGCGGAGCAGCu'u'CGAGAAGAACCCCAu'CGACu' u'CCu'GGAGGCCAAGGGCu'ACAAGGAGGu'GAAGAAG GACCu'GAu'CAu'CAAGCu'GCCCAAGu'ACAGCCu'Gu'u' CGAGCu'GGAGAACGGCCGGAAGCGGAu'GCu'GGCCAG CGCCGGCGAGCu'GCAGAAGGGCAACGAGCu'GGCCCu' GCCCAGCAAGu'ACGu'GAACu'u'CCu'Gu'ACCu'GGCCA GCCACu'ACGAGAAGCu'GAAGGGCAGCCCCGAGGACA ACGAGCAGAAGCAGCu'Gu'u'CGu'GGAGCAGCACAAGC ACu'ACCu'GGACGAGAu'CAu'CGAGCAGAu'CAGCGAGu 'u'CAGCAAGCGGGu'GAu'CCu'GGCCGACGCCAACCu'G GACAAGGu'GCu'GAGCGCCu'ACAACAAGCACCGGGAC AAGCCCAu'CCGGGAGCAGGCCGAGAACAu'CAu'CCAC Cu'Gu'u'CACCCu'GACCAACCu'GGGCGCCCCCGCCGCCu 'u'CAAGu'ACu'u'CGACACCACCAu'CGACCGGAAGCGGu 'ACACCAGCACCAAGGAGGu'GCu'GGACGCCACCCu'GA u'CCACCAGAGCAu'CACCGGCCu'Gu'ACGAGACACGGA u'CGACCu'GAGCCAGCu'GGGCGGCGACGAGGGCGCCG ACAAGCGGACCGCCGACGGCAGCGAGu'u'CGAGAGCC CCAAGAAGAAGCGGAAGGu'Gu'GAGCGGCCGCu'u'AAu 'u'AAGCu'GCCu'u'Cu'GCGGGGCu'u'GCCu'u'Cu'GGCCAu	

		'GCCCu'u'Cu'u'Cu'Cu'CCCu'u'GCACCu'Gu'ACCu'Cu'u'G Gu'Cu'u'u'GAAu'AAAGCCu'GAGu'AGGAAGu'Cu'AGA	
	Protein	MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNN RVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLID ATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHHPGMNHRVEITEGILADECAALLCRFFRMPRR VFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGG SSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVL GNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRR KNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHER HPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIY LALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKN GLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDL DNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKA PLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQS KNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLN REDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITP WNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLL YEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLF KTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD KQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM GRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKEL GSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDI NRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDN VPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAH DAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIA KSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT GGFSKESILPKRNSDKLIARKKDWDPKKYGGFESPTVAY SVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDF LEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGEL QKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFV EQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRD KPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTK EVLDATLIHQSITGLYETRIDLSQLGGDEGADKRTADGSE FESPKKKRKV	2149
5′ UTR	mRNA	AGGAAAu'AAGAGAGAAAAGAAGAGu'AAGAAGAAAu' Au'AAGAGCCACC	2138
TadA	mRNA	Au'GAGCGAGGu'GGAGu'u'CAGCCACGAGu'ACu'GGAu' GCGGCACGCCCu'GACCCu'GGCCAAGCGGGCCCGGGA CGAGCGGGAGGu'GCCCGu'GGGCGCCGu'GCu'GGu'GCu 'GAACAACCGGGu'GAu'CGGCGAGGGCu'GGAACCGGG CCAu'CGGCCu'GCACGACCCCACCGCCCACGCCGAGAu' CAu'GGCCCu'GCGGCAGGGCGGCCu'GGu'GAu'GCAGAA Cu'ACCGGCu'GAu'CGACGCCACCCu'Gu'ACGu'GACCu'u 'CGAGCCCu'GCGu'GAu'Gu'GCGCCGGCGCCAu'GAu'CC ACAGCCGGAu'CGGCCGGGu'GGu'Gu'u'CGGCGu'GCGG	2139

		AACGCCAAGACCGGCGCCGCCGGCAGCCu'GAu'GGAC Gu'GCu'GCACCACCCCGGCAu'GAACCACCGGGu'GGAG Au'CACCGAGGGCAu'CCu'GGCCGACGAGu'GCGCCGCC Cu'GCu'Gu'GCCGGu'u'Cu'u'CCGGAu'GCCCCGGCGGGu' Gu'u'CAACGCCCAGAAGAAGGCCCAGAGCAGCACCGA C	
	Protein	MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNN RVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLID ATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHHPGMNHRVEITEGILADECAALLCRFFRMPRR VFNAQKKAQSSTD	2140
Linker between TadA and Cas9 nickase	mRNA	AGCGGCGGCAGCAGCGGCGGCAGCAGCGGCAGCGAG ACACCCGGCACCAGCGAGAGCGCCACCCCCGAGAGCA GCGGCGGCAGCAGCGGCGGCAGC	2141
	protein	SGGSSGGSSGSETPGTSESATPESSGGSSGGS	20
Cas9 nickase	mRNA	GACAAGAAGu'ACAGCAu'CGGCCu'GGCCAu'CGGCACC AACAGCGu'GGGCu'GGGCCGu'GAu'CACCGACGAGu'AC AAGGu'GCCCAGCAAGAAGu'u'CAAGGu'GCu'GGGCAA CACCGACCGGCACAGCAu'CAAGAAGAACCu'GAu'CGG CGCCCu'GCu'Gu'u'CGACAGCGGCGAGACAGCCGAGGC CACCCGGCu'GAAGCGGACCGCCCGGCGGCGGu'ACAC CCGGCGGAAGAACCGGAu'Cu'GCu'ACCu'GCAGGAGAu' Cu'u'CAGCAACGAGAu'GGCCAAGGu'GGACGACAGCu'u 'Cu'u'CCACCGGCu'GGAGGAGAGCu'u'CCu'GGu'GGAGG AGGACAAGAAGCACGAGCGGCACCCCAu'Cu'u'CGGCA ACAu'CGu'GGACGAGGu'GGCCu'ACCACGAGAAGu'ACC CCACCAu'Cu'ACCACCu'GCGGAAGAAGCu'GGu'GGACA GCACCGACAAGGCCGACCu'GCGGCu'GAu'Cu'ACCu'GG CCCu'GGCCCACAu'GAu'CAAGu'u'CCGGGGCCACu'u'CC u'GAu'CGAGGGCGACCu'GAACCCCGACAACAGCGACG u'GGACAAGCu'Gu'u'CAu'CCAGCu'GGu'GCAGACCu'AC AACCAGCu'Gu'u'CGAGGAGAACCCCAu'CAACGCCAGC GGCGu'GGACGCCAAGGCCAu'CCu'GAGCGCCCGGCu'G AGCAAGAGCCGGCGGCu'GGAGAACCu'GAu'CGCCCAG Cu'GCCCGGCGAGAAGAAGAACGGCCu'Gu'u'CGGCAAC Cu'GAu'CGCCCu'GAGCCu'GGGCCu'GACCCCCAACu'u'C AAGAGCAACu'u'CGACCu'GGCCGAGGACGCCAAGCu'G CAGCu'GAGCAAGGACACCu'ACGACGACGACCu'GGAC AACCu'GCu'GGCCCAGAu'CGGCGACCAGu'ACGCCGAC Cu'Gu'u'CCu'GGCCGCCAAGAACCu'GAGCGACGCCAu'C Cu'GCu'GAGCGACAu'CCu'GCGGGu'GAACACCGAGAu' CACCAAGGCCCCCCu'GAGCGCCAGCAu'GAu'CAAGCG Gu'ACGACGAGCACCACCAGGACCu'GACCCu'GCu'GAA GGCCCu'GGu'GCGGCAGCAGCu'GCCCGAGAAGu'ACAA GGAGAu'Cu'u'Cu'u'CGACCAGAGCAAGAACGGCu'ACG CCGGCu'ACAu'CGACGGCGGCGCCAGCCAGGAGGAGu' u'Cu'ACAAGu'u'CAu'CAAGCCCAu'CCu'GGAGAAGAu'G GACGGCACCGAGGAGCu'GCu'GGu'GAAGCu'GAACCGG GAGGACCu'GCu'GCGGAAGCAGCGGACCu'u'CGACAAC GGCAGCAu'CCCCCACCAGAu'CCACCu'GGGCGAGCu'G CACGCCAu'CCu'GCGGCGGCAGGAGGACu'u'Cu'ACCCC u'u'CCu'GAAGGACAACCGGGAGAAGAu'CGAGAAGAu'	2151

		CCu'GACCu'u'CCGGAu'CCCCu'ACu'ACGu'GGGCCCCCu' GGCCCGGGGCAACAGCCGGu'u'CGCCu'GGAu'GACCCG CAAGAGCGAGGAGACAAu'CACCCCCu'GGAACu'u'CGA GGAGGu'GGu'GGACAAGGGCGCCAGCGCCCAGAGCu'u' CAu'CGAGCGGAu'GACCAACu'u'CGACAAGAACCu'GCC CAACGAGAAGGu'GCu'GCCCAAGCACAGCCu'GCu'Gu'A CGAGu'ACu'u'CACCGu'Gu'ACAACGAGCu'GACCAAGGu 'GAAGu'ACGu'GACCGAGGGCAu'GCGGAAGCCCGCCu'u 'CCu'GAGCGGCGAGCAGAAGAAGGCCAu'CGu'GGACCu 'GCu'Gu'u'CAAGACCAACCGGAAGGu'GACCGu'GAAGC AGCu'GAAGGAGGACu'ACu'u'CAAGAAGAu'CGAGu'GC u'u'CGACAGCGu'GGAGAu'CAGCGGCGu'GGAGGACCG Gu'u'CAACGCCAGCCu'GGGCACCu'ACCACGACCu'GCu' GAAGAu'CAu'CAAGGACAAGGACu'u'CCu'GGACAACG AGGAGAACGAGGACAu'CCu'GGAGGACAu'CGu'GCu'G ACCCu'GACCCu'Gu'u'CGAGGACCGGGAGAu'GAu'CGA GGAGCGGCu'GAAGACCu'ACGCCCACCu'Gu'u'CGACGA CAAGGu'GAu'GAAGCAGCu'GAAGCGGCGGCGGu'ACAC CGGCu'GGGGCCGGCu'GAGCCGGAAGCu'GAu'CAACGG CAu'CCGGGACAAGCAGAGCGGCAAGACCAu'CCu'GGA Cu'u'CCu'CAAGAGCGACGGCu'u'CGCCAAC**CGG**AACu'u 'CAu'GCAGCu'GAu'CCACGACGACAGCCu'GACCu'u'CA AGGAGGACAu'CCAGAAGGCCCAGGu'GAGCGGCCAGG GCGACAGCCu'GCACGAGCACAu'CGCCAACCu'GGCCG GCAGCCCCGCCAu'CAAGAAGGGCAu'CCu'GCAGACCG u'GAAGGu'GGu'GGACGAGCu'GGu'GAAGGu'GAu'GGGC CGGCACAAGCCCGAGAACAu'CGu'GAu'CGAGAu'GGCC CGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAAC AGCCGGGAGCGGAu'GAAGCGGAu'CGAGGAGGGCAu'C AAGGAGCu'GGGCAGCCAGAu'CCu'GAAGGAGCACCCC Gu'GGAGAACACCCAGCu'GCAGAACGAGAAGCu'Gu'AC Cu'Gu'ACu'ACCu'GCAGAACGGCCGGGACAu'Gu'ACGu' GGACCAGGAGCu'GGACAu'CAACCGGCu'GAGCGACu'A CGACGu'GGACCACAu'CGu'GCCCCAGAGCu'u'CCu'GAA GGACGACAGCAu'CGACAACAAGGu'GCu'GACCCGGAG CGACAAGAACCGGGGCAAGAGCGACAACGu'GCCCAG CGAGGAGGu'GGu'GAAGAAGAu'GAAGAACu'ACu'GGC GGCAGCu'GCu'GAACGCCAAGCu'GAu'CACCCAGCGGA AGu'u'CGACAACCu'GACCAAGGCCGAGCGGGGCGGCC u'GAGCGAGCu'GGACAAGGCCGGCu'u'CAu'CAAGCGGC AGCu'GGu'GGAGACACGGCAGAu'CACCAAGCACGu'GG CCCAGAu'CCu'GGACAGCCGGAu'GAACACCAAGu'ACG ACGAGAACGACAAGCu'GAu'CCGGGAGGu'GAAGGu'G Au'CACCCu'CAAGAGCAAGCu'GGu'GAGCGACu'u'CCG GAAGGACu'u'CCAGu'u'Cu'ACAAGGu'GCGGGAGAu'CA ACAACu'ACCACCACGCCCACGACGCCu'ACCu'GAACG CCGu'GGu'GGGCACCGCCCu'GAu'CAAGAAGu'ACCCCA AGCu'GGAGAGCGAGu'u'CGu'Gu'ACGGCGACu'ACAAG Gu'Gu'ACGACGu'GCGGAAGAu'GAu'CGCCAAGAGCGA GCAGGAGAu'CGGCAAGGCCACCGCCAAGu'ACu'u'Cu'u 'Cu'ACAGCAACAu'CAu'GAACu'u'Cu'u'CAAGACCGAGA u'CACCCu'GGCCAACGGCGAGAu'CCGGAAGCGGCCCCu 'GAu'CGAGACAAACGGCGAGACAGGCGAGAu'CGu'Gu' GGGACAAGGGCCGGGACu'u'CGCCACCGu'GCGGAAGG u'GCu'GAGCAu'GCCCCAGGu'GAACAu'CGu'GAAGAAG	

		ACCGAGGu'GCAGACCGGCGGCu'u'CAGCAAGGAGAGC Au'CCu'GCCCAAGCGGAACAGCGACAAGCu'GAu'CGCC CGGAAGAAGGACu'GGGACCCCAAGAAGu'ACGGCGGC u'u'C**GAG**AGCCCCACCGu'GGCCu'ACAGCGu'GCu'GGu' GGu'GGCCAAGGu'GGAGAAGGGCAAGAGCAAGAAGCu 'CAAGAGCGu'GAAGGAGCu'GCu'GGGCAu'CACCAu'CA u'GGAGCGGAGCAGCu'u'CGAGAAGAACCCCAu'CGACu' u'CCu'GGAGGCCAAGGGCu'ACAAGGAGGu'GAAGAAG GACCu'GAu'CAu'CAAGCu'GCCCAAGu'ACAGCCu'Gu'u' CGAGCu'GGAGAACGGCCGGAAGCGGAu'GCu'GGCCAG CGCCGGCGAGCu'GCAGAAGGGCAACGAGCu'GGCCCu' GCCCAGCAAGu'ACGu'GAACu'u'CCu'Gu'ACCu'GGCCA GCCACu'ACGAGAAGCu'GAAGGGCAGCCCCGAGGACA ACGAGCAGAAGCAGCu'Gu'u'CGu'GGAGCAGCACAAGC ACu'ACCu'GGACGAGAu'CAu'CGAGCAGAu'CAGCGAGu 'u'CAGCAAGCGGGu'GAu'CCu'GGCCGACGCCAACCu'G GACAAGGu'GCu'GAGCGCCu'ACAACAAGCACCGGGAC AAGCCCAu'CCGGGAGCAGGCCGAGAACAu'CAu'CCAC Cu'Gu'u'CACCCu'GACCAACCu'GGGCGCCCCCGCCGCCu 'u'CAAGu'ACu'u'CGACACCACCAu'CGACCGGAAGCGGu 'ACACCAGCACCAAGGAGGu'GCu'GGACGCCACCCu'GA u'CCACCAGAGCAu'CACCGGCCu'Gu'ACGAGACACGGA u'CGACCu'GAGCCAGCu'GGGCGGCGAC	
	Protein	DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDR HSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRIC YLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFG NIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALA HMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEEN PINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFG NLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNL LAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSA SMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNG YAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDL LRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNRE KIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFE EVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYF TVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNR KVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDL LKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKT YAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSG KTILDFLKSDGFAN**R**NFMQLIHDDSLTFKEDIQKAQVSGQ GDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHK PENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQI LKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRL SDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPS EEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYL NAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQ EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGE TGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFS KESILPKRNSDKLIARKKDWDPKKYGGF**E**SPTVAYSVLV VAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAK GYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHK	2152

		HYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIRE QAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLD ATLIHQSITGLYETRIDLSQLGGD	
Linker between Cas9 nickase and NLS	mRNA	GAGGGCGCCGAC	2143
	Protein	EGAD	2144
Nuclear localization sequence (NLS)	mRNA	AAGCGGACCGCCGACGGCAGCGAGu'u'CGAGAGCCCC AAGAAGAAGCGGAAGGu'Gu'GA	2145
	Protein	KRTADGSEFESPKKKRKV	2146
3′ UTR	mRNA	GCGGCCGCu'u'AAu'u'AAGCu'GCCu'u'Cu'GCGGGGCu'u' GCCu'u'Cu'GGCCAu'GCCCu'u'Cu'u'Cu'Cu'CCCu'u'GCAC Cu'Gu'ACCu'Cu'u'GGu'Cu'u'u'GAAu'AAAGCCu'GAGu'A GGAAGu'Cu'AGA	2147
MA041 mRNA and protein sequences			
Region	**Sequence**		**SEQ ID NO**
Full sequence	mRNA	AGGAAAu'AAGAGAGAAAAGAAGAGu'AAGAAGAAAu' Au'AAGAGCCACCAu'GAGCGAGGu'GGAGu'u'CAGCCA CGAGu'ACu'GGAu'GCGGCACGCCCu'GACCCu'GGCCAA GCGGGCCCGGGACGAGCGGGAGGu'GCCCGu'GGGCGC CGu'GCu'GGu'GCu'GAACAACCGGGu'GAu'CGGCGAGG GCu'GGAACCGGGCCAu'CGGCCu'GCACGACCCCACCG CCCACGCCGAGAu'CAu'GGCCCu'GCGGCAGGGCGGCCu 'GGu'GAu'GCAGAACu'ACCGGCu'GAu'CGACGCCACCCu 'Gu'ACGu'GACCu'u'CGAGCCCu'GCGu'GAu'Gu'GCGCCG GCGCCAu'GAu'CCACAGCCGGAu'CGGCCGGGu'GGu'Gu 'u'CGGCGu'GCGGAACGCCAAGACCGGCGCCGCCGGCA GCCu'GAu'GGACGu'GCu'GCACCACCCCGGCAu'GAACC ACCGGGu'GGAGAu'CACCGAGGGCAu'CCu'GGCCGACG AGu'GCGCCGCCCu'GCu'Gu'GCCGGu'u'Cu'u'CCGGAu'G CCCCGGCGGGu'Gu'u'CAACGCCCAGAAGAAGGCCCAG AGCAGCACCGACAGCGGCGGCAGCAGCGGCGGCAGCA GCGGCAGCGAGACACCCGGCACCAGCGAGAGCGCCAC CCCCGAGAGCAGCGGCGGCAGCAGCGGCGGCAGCGAC AAGAAGu'ACAGCAu'CGGCCu'GGCCAu'CGGCACCAAC AGCGu'GGGCu'GGGCCGu'GAu'CACCGACGAGu'ACAA GGu'GCCCAGCAAGAAGu'u'CAAGGu'GCu'GGGCAACA CCGACCGGCACAGCAu'CAAGAAGAACCu'GAu'CGGCG CCCu'GCu'Gu'u'CGACAGCGGCGAGACAGCCGAGGCCA CCCGGCu'GAAGCGGACCGCCCGGCGGCGGu'ACACCC GGCGGAAGAACCGGAu'Cu'GCu'ACCu'GCAGGAGAu'Cu 'u'CAGCAACGAGAu'GGCCAAGGu'GGACGACAGCu'u'C u'u'CCACCGGCu'GGAGGAGAGCu'u'CCu'GGu'GGAGGA GGACAAGAAGCACGAGCGGCACCCCAu'Cu'u'CGGCAA CAu'CGu'GGACGAGGu'GGCCu'ACCACGAGAAGu'ACCC CACCAu'Cu'ACCACCu'GCGGAAGAAGCu'GGu'GGACAG CACCGACAAGGCCGACCu'GCGGCu'GAu'Cu'ACCu'GGC CCu'GGCCCACAu'GAu'CAAGu'u'CCGGGGCCACu'u'CCu 'GAu'CGAGGGCGACCu'GAACCCCGACAACAGCGACGu'	2153

		GGACAAGCu'Gu'u'CAu'CCAGCu'GGu'GCAGACCu'ACA ACCAGCu'Gu'u'CGAGGAGAACCCCAu'CAACGCCAGCG GCGu'GGACGCCAAGGCCAu'CCu'GAGCGCCCGGCu'GA GCAAGAGCCGGCGGCu'GGAGAACCu'GAu'CGCCCAGC u'GCCCGGCGAGAAGAAGAACGGCCu'Gu'u'CGGCAACC u'GAu'CGCCCu'GAGCCu'GGGCCu'GACCCCCAACu'u'CA AGAGCAACu'u'CGACCu'GGCCGAGGACGCCAAGCu'GC AGCu'GAGCAAGGACACCu'ACGACGACGACCu'GGACA ACCu'GCu'GGCCCAGAu'CGGCGACCAGu'ACGCCGACC u'Gu'u'CCu'GGCCGCCAAGAACCu'GAGCGACGCCAu'CC u'GCu'GAGCGACAu'CCu'GCGGGu'GAACACCGAGAu'C ACCAAGGCCCCCCu'GAGCGCCAGCAu'GAu'CAAGCGG u'ACGACGAGCACCACCAGGACCu'GACCCu'GCu'GAAG GCCCu'GGu'GCGGCAGCAGCu'GCCCGAGAAGu'ACAAG GAGAu'Cu'u'Cu'u'CGACCAGAGCAAGAACGGCu'ACGC CGGCu'ACAu'CGACGGCGGCGCCAGCCAGGAGGAGu'u' Cu'ACAAGu'u'CAu'CAAGCCCAu'CCu'GGAGAAGAu'GG ACGGCACCGAGGAGCu'GCu'GGu'GAAGCu'GAACCGGG AGGACCu'GCu'GCGGAAGCAGCGGACCu'u'CGACAACG GCAGCAu'CCCCCACCAGAu'CCACCu'GGGCGAGCu'GC ACGCCAu'CCu'GCGGCGGCAGGAGGACu'u'Cu'ACCCCu' u'CCu'GAAGGACAACCGGGAGAAGAu'CGAGAAGAu'CC u'GACCu'u'CCGGAu'CCCCu'ACu'ACGu'GGGCCCCCu'G GCCCGGGGCAACAGCCGGu'u'CGCCu'GGAu'GACCCGC AAGAGCGAGGAGACAAu'CACCCCCu'GGAACu'u'CGAG GAGGu'GGu'GGACAAGGGCGCCAGCGCCCAGAGCu'u'C Au'CGAGCGGAu'GACCAACu'u'CGACAAGAACCu'GCCC AACGAGAAGGu'GCu'GCCCAAGCACAGCCu'GCu'Gu'AC GAGu'ACu'u'CACCGu'Gu'ACAACGAGCu'GACCAAGGu' GAAGu'ACGu'GACCGAGGGCAu'GCGGAAGCCCGCCu'u' CCu'GAGCGGCGAGCAGAAGAAGGCCAu'CGu'GGACCu' GCu'Gu'u'CAAGACCAACCGGAAGGu'GACCGu'GAAGC AGCu'GAAGGAGGACu'ACu'u'CAAGAAGAu'CGAGu'GC u'u'CGACAGCGu'GGAGAu'CAGCGGCGu'GGAGGACCG Gu'u'CAACGCCAGCCu'GGGCACCu'ACCACGACCu'GCu' GAAGAu'CAu'CAAGGACAAGGACu'u'CCu'GGACAACG AGGAGAACGAGGACAu'CCu'GGAGGACAu'CGu'GCu'G ACCCu'GACCCu'Gu'u'CGAGGACCGGGAGAu'GAu'CGA GGAGCGGCu'GAAGACCu'ACGCCCACCu'Gu'u'CGACGA CAAGGu'GAu'GAAGCAGCu'GAAGCGGCGGCGGu'ACAC CGGCu'GGGGCCGGCu'GAGCCGGAAGCu'GAu'CAACGG CAu'CCGGGACAAGCAGAGCGGCAAGACCAu'CCu'GGA Cu'u'CCu'CAAGAGCGACGGCu'u'CGCCAAC**GCC**AACu'u 'CAu'GCAGCu'GAu'CCACGACGACAGCCu'GACCu'u'CA AGGAGGACAu'CCAGAAGGCCCAGGu'GAGCGGCCAGG GCGACAGCCu'GCACGAGCACAu'CGCCAACCu'GGCCG GCAGCCCCGCCAu'CAAGAAGGGCAu'CCu'GCAGACCG u'GAAGGu'GGu'GGACGAGCu'GGu'GAAGGu'GAu'GGGC CGGCACAAGCCCGAGAACAu'CGu'GAu'CGAGAu'GGCC CGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAAC AGCCGGGAGCGGAu'GAAGCGGAu'CGAGGAGGGCAu'C AAGGAGCu'GGGCAGCCAGAu'CCu'GAAGGAGCACCCC Gu'GGAGAACACCCAGCu'GCAGAACGAGAAGCu'Gu'AC Cu'Gu'ACu'ACCu'GCAGAACGGCCGGGACAu'Gu'ACGu' GGACCAGGAGCu'GGACAu'CAACCGGCu'GAGCGACu'A	

		CGACGu'GGACCACAu'CGu'GCCCCAGAGCu'u'CCu'GAA GGACGACAGCAu'CGACAACAAGGu'GCu'GACCCGGAG CGACAAGAACCGGGGCAAGAGCGACAACGu'GCCCAG CGAGGAGGu'GGu'GAAGAAGAu'GAAGAACu'ACu'GGC GGCAGCu'GCu'GAACGCCAAGCu'GAu'CACCCAGCGGA AGu'u'CGACAACCu'GACCAAGGCCGAGCGGGGCGGCC u'GAGCGAGCu'GGACAAGGCCGGCu'u'CAu'CAAGCGGC AGCu'GGu'GGAGACACGGCAGAu'CACCAAGCACGu'GG CCCAGAu'CCu'GGACAGCCGGAu'GAACACCAAGu'ACG ACGAGAACGACAAGCu'GAu'CCGGGAGGu'GAAGGu'G Au'CACCCu'CAAGAGCAAGCu'GGu'GAGCGACu'u'CCG GAAGGACu'u'CCAGu'u'Cu'ACAAGGu'GCGGGAGAu'CA ACAACu'ACCACCACGCCCACGACGCCu'ACCu'GAACG CCGu'GGu'GGGCACCGCCCu'GAu'CAAGAAGu'ACCCCA AGCu'GGAGAGCGAGu'u'CGu'Gu'ACGGCGACu'ACAAG Gu'Gu'ACGACGu'GCGGAAGAu'GAu'CGCCAAGAGCGA GCAGGAGAu'CGGCAAGGCCACCGCCAAGu'ACu'u'Cu'u 'Cu'ACAGCAACAu'CAu'GAACu'u'Cu'u'CAAGACCGAGA u'CACCCu'GGCCAACGGCGAGAu'CCGGAAGCGGCCCCu 'GAu'CGAGACAAACGGCGAGACAGGCGAGAu'CGu'Gu' GGGACAAGGGCCGGGACu'u'CGCCACCGu'GCGGAAGG u'GCu'GAGCAu'GCCCCAGGu'GAACAu'CGu'GAAGAAG ACCGAGGu'GCAGACCGGCGGCu'u'CAGCAAGGAGAGC Au'CCu'GCCCAAGCGGAACAGCGACAAGCu'GAu'CGCC CGGAAGAAGGACu'GGGACCCCAAGAAGu'ACGGCGGC u'u'C**GAG**AGCCCCACCGu'GGCCu'ACAGCGu'GCu'GGu' GGu'GGCCAAGGu'GGAGAAGGGCAAGAGCAAGAAGCu 'CAAGAGCGu'GAAGGAGCu'GCu'GGGCAu'CACCAu'CA u'GGAGCGGAGCAGCu'u'CGAGAAGAACCCCAu'CGACu' u'CCu'GGAGGCCAAGGGCu'ACAAGGAGGu'GAAGAAG GACCu'GAu'CAu'CAAGCu'GCCCAAGu'ACAGCCu'Gu'u' CGAGCu'GGAGAACGGCCGGAAGCGGAu'GCu'GGCCAG CGCCGGCGAGCu'GCAGAAGGGCAACGAGCu'GGCCCu' GCCCAGCAAGu'ACGu'GAACu'u'CCu'Gu'ACCu'GGCCA GCCACu'ACGAGAAGCu'GAAGGGCAGCCCCGAGGACA ACGAGCAGAAGCAGCu'Gu'u'CGu'GGAGCAGCACAAGC ACu'ACCu'GGACGAGAu'CAu'CGAGCAGAu'CAGCGAGu 'u'CAGCAAGCGGGu'GAu'CCu'GGCCGACGCCAACCu'G GACAAGGu'GCu'GAGCGCCu'ACAACAAGCACCGGGAC AAGCCCAu'CCGGGAGCAGGCCGAGAACAu'CAu'CCAC Cu'Gu'u'CACCCu'GACCAACCu'GGGCGCCCCCGCCGCCu 'u'CAAGu'ACu'u'CGACACCACCAu'CGACCGGAAGCGGu 'ACACCAGCACCAAGGAGGu'GCu'GGACGCCACCCu'GA u'CCACCAGAGCAu'CACCGGCCu'Gu'ACGAGACACGGA u'CGACCu'GAGCCAGCu'GGGCGGCGACGAGGGCGCCG ACAAGCGGACCGCCGACGGCAGCGAGu'u'CGAGAGCC CCAAGAAGAAGCGGAAGGu'Gu'GAGCGGCCGCu'u'AAu 'u'AAGCu'GCCu'u'Cu'GCGGGGCu'u'GCCu'u'Cu'GGCCAu 'GCCCu'u'Cu'u'Cu'Cu'CCCu'u'GCACCu'Gu'ACCu'Cu'u'G Gu'Cu'u'u'GAAu'AAAGCCu'GAGu'AGGAAGu'Cu'AGA	
	Protein	MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNN RVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLID ATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHHPGMNHRVEITEGILADECAALLCRFFRMPRR VFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGG	2154

		SSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVL GNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRR KNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHER HPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIY LALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKN GLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDL DNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKA PLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQS KNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLN REDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITP WNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLL YEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLF KTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD KQSGKTILDFLKSDGFANANFMQLIHDDSLTFKEDIQKAQ VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM GRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKEL GSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDI NRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDN VPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAH DAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIA KSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT GGFSKESILPKRNSDKLIARKKDWDPKKYGGFESPTVAY SVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDF LEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGEL QKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFV EQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRD KPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTK EVLDATLIHQSITGLYETRIDLSQLGGDEGADKRTADGSE FESPKKKRKV	
5′ UTR	mRNA	AGGAAAu’AAGAGAGAAAAGAAGAGu’AAGAAGAAAu’ Au’AAGAGCCACC	2138
TadA	mRNA	Au’GAGCGAGGu’GGAGu’u’CAGCCACGAGu’ACu’GGAu’ GCGGCACGCCCu’GACCCu’GGCCAAGCGGGCCCGGGA CGAGCGGGAGGu’GCCCGu’GGGCGCCGu’GCu’GGu’GCu ’GAACAACCGGGu’GAu’CGGCGAGGGCu’GGAACCGGG CCAu’CGGCCu’GCACGACCCCACCGCCCACGCCGAGAu’ CAu’GGCCCu’GCGGCAGGGCGGCCu’GGu’GAu’GCAGAA Cu’ACCGGCu’GAu’CGACGCCACCCu’Gu’ACGu’GACCu’u ’CGAGCCCu’GCGu’GAu’Gu’GCGCCGGCGCCAu’GAu’CC ACAGCCGGAu’CGGCCGGGu’GGu’Gu’u’CGGCGu’GCGG AACGCCAAGACCGGCGCCGCCGGCAGCCu’GAu’GGAC Gu’GCu’GCACCACCCCGGCAu’GAACCACCGGGu’GGAG Au’CACCGAGGGCAu’CCu’GGCCGACGAGu’GCGCCGCC Cu’GCu’Gu’GCCGGu’u’Cu’u’CCGGAu’GCCCCGGCGGGu’ Gu’u’CAACGCCCAGAAGAAGGCCCAGAGCAGCACCGA C	2139
	Protein	MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNN	2140

		RVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLID ATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHHPGMNHRVEITEGILADECAALLCRFFRMPRR VFNAQKKAQSSTD	
Linker between TadA and Cas9 nickase	mRNA	AGCGGCGGCAGCAGCGGCGGCAGCAGCGGCAGCGAG ACACCCGGCACCAGCGAGAGCGCCACCCCCGAGAGCA GCGGCGGCAGCAGCGGCGGCAGC	2141
	Protein	SGGSSGGSSGSETPGTSESATPESSGGSSGGS	20
Cas9 nickase	mRNA	GACAAGAAGu'ACAGCAu'CGGCCu'GGCCAu'CGGCACC AACAGCGu'GGGCu'GGGCCGu'GAu'CACCGACGAGu'AC AAGGu'GCCCAGCAAGAAGu'u'CAAGGu'GCu'GGGCAA CACCGACCGGCACAGCAu'CAAGAAGAACCu'GAu'CGG CGCCCu'GCu'Gu'u'CGACAGCGGCGAGACAGCCGAGGC CACCCGGCu'GAAGCGGACCGCCCGGCGGCGGu'ACAC CCGGCGGAAGAACCGGAu'Cu'GCu'ACCu'GCAGGAGAu' Cu'u'CAGCAACGAGAu'GGCCAAGGu'GGACGACAGCu'u 'Cu'u'CCACCGGCu'GGAGGAGAGCu'u'CCu'GGu'GGAGG AGGACAAGAAGCACGAGCGGCACCCCAu'Cu'u'CGGCA ACAu'CGu'GGACGAGGu'GGCCu'ACCACGAGAAGu'ACC CCACCAu'Cu'ACCACCu'GCGGAAGAAGCu'GGu'GGACA GCACCGACAAGGCCGACCu'GCGGCu'GAu'Cu'ACCu'GG CCCu'GGCCCACAu'GAu'CAAGu'u'CCGGGGCCACu'u'CC u'GAu'CGAGGGCGACCu'GAACCCCGACAACAGCGACG u'GGACAAGCu'Gu'u'CAu'CCAGCu'GGu'GCAGACCu'AC AACCAGCu'Gu'u'CGAGGAGAACCCCAu'CAACGCCAGC GGCGu'GGACGCCAAGGCCAu'CCu'GAGCGCCCGGCu'G AGCAAGAGCCGGCGGCu'GGAGAACCu'GAu'CGCCCAG Cu'GCCCGGCGAGAAGAAGAACGGCCu'Gu'u'CGGCAAC Cu'GAu'CGCCCu'GAGCCu'GGGCCu'GACCCCCAACu'u'C AAGAGCAACu'u'CGACCu'GGCCGAGGACGCCAAGCu'G CAGCu'GAGCAAGGACACCu'ACGACGACGACCu'GGAC AACCu'GCu'GGCCCAGAu'CGGCGACCAGu'ACGCCGAC Cu'Gu'u'CCu'GGCCGCCAAGAACCu'GAGCGACGCCAu'C Cu'GCu'GAGCGACAu'CCu'GCGGGu'GAACACCGAGAu' CACCAAGGCCCCCCu'GAGCGCCAGCAu'GAu'CAAGCG Gu'ACGACGAGCACCACCAGGACCu'GACCCu'GCu'GAA GGCCCu'GGu'GCGGCAGCAGCu'GCCCGAGAAGu'ACAA GGAGAu'Cu'u'Cu'u'CGACCAGAGCAAGAACGGCu'ACG CCGGCu'ACAu'CGACGGCGGCGCCAGCCAGGAGGAGu' u'Cu'ACAAGu'u'CAu'CAAGCCCAu'CCu'GGAGAAGAu'G GACGGCACCGAGGAGCu'GCu'GGu'GAAGCu'GAACCGG GAGGACCu'GCu'GCGGAAGCAGCGGACCu'u'CGACAAC GGCAGCAu'CCCCCACCAGAu'CCACCu'GGGCGAGCu'G CACGCCAu'CCu'GCGGCGGCAGGAGGACu'u'Cu'ACCCC u'u'CCu'GAAGGACAACCGGGAGAAGAu'CGAGAAGAu' CCu'GACCu'u'CCGGAu'CCCCu'ACu'ACGu'GGGCCCCCu' GGCCCGGGGCAACAGCCGGu'u'CGCCu'GGAu'GACCCG CAAGAGCGAGGAGACAAu'CACCCCCu'GGAACu'u'CGA GGAGGu'GGu'GGACAAGGGCGCCAGCGCCCAGAGCu'u' CAu'CGAGCGGAu'GACCAACu'u'CGACAAGAACCu'GCC CAACGAGAAGGu'GCu'GCCCAAGCACAGCCu'GCu'Gu'A CGAGu'ACu'u'CACCGu'Gu'ACAACGAGCu'GACCAAGGu 'GAAGu'ACGu'GACCGAGGGCAu'GCGGAAGCCCGCCu'u	2155

		'CCu'GAGCGGCGAGCAGAAGAAGGCCAu'CGu'GGACCu 'GCu'Gu'u'CAAGACCAACCGGAAGGu'GACCGu'GAAGC AGCu'GAAGGAGGACu'ACu'u'CAAGAAGAu'CGAGu'GC u'u'CGACAGCGu'GGAGAu'CAGCGGCGu'GGAGGACCG Gu'u'CAACGCCAGCCu'GGGCACCu'ACCACGACCu'GCu' GAAGAu'CAu'CAAGGACAAGGACu'u'CCu'GGACAACG AGGAGAACGAGGACAu'CCu'GGAGGACAu'CGu'GCu'G ACCCu'GACCCu'Gu'u'CGAGGACCGGGAGAu'GAu'CGA GGAGCGGCu'GAAGACCu'ACGCCCACCu'Gu'u'CGACGA CAAGGu'GAu'GAAGCAGCu'GAAGCGGCGGCGGu'ACAC CGGCu'GGGGCCGGCu'GAGCCGGAAGCu'GAu'CAACGG CAu'CCGGGACAAGCAGAGCGGCAAGACCAu'CCu'GGA Cu'u'CCu'CAAGAGCGACGGCu'u'CGCCAAC**GCC**AACu'u 'CAu'GCAGCu'GAu'CCACGACGACAGCCu'GACCu'u'CA AGGAGGACAu'CCAGAAGGCCCAGGu'GAGCGGCCAGG GCGACAGCCu'GCACGAGCACAu'CGCCAACCu'GGCCG GCAGCCCCGCCAu'CAAGAAGGGCAu'CCu'GCAGACCG u'GAAGGu'GGu'GGACGAGCu'GGu'GAAGGu'GAu'GGGC CGGCACAAGCCCGAGAACAu'CGu'GAu'CGAGAu'GGCC CGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAAC AGCCGGGAGCGGAu'GAAGCGGAu'CGAGGAGGGCAu'C AAGGAGCu'GGGCAGCCAGAu'CCu'GAAGGAGCACCCC Gu'GGAGAACACCCAGCu'GCAGAACGAGAAGCu'Gu'AC Cu'Gu'ACu'ACCu'GCAGAACGGCCGGGACAu'Gu'ACGu' GGACCAGGAGCu'GGACAu'CAACCGGCu'GAGCGACu'A CGACGu'GGACCACAu'CGu'GCCCCAGAGCu'u'CCu'GAA GGACGACAGCAu'CGACAACAAGGu'GCu'GACCCGGAG CGACAAGAACCGGGGCAAGAGCGACAACGu'GCCCAG CGAGGAGGu'GGu'GAAGAAGAu'GAAGAACu'ACu'GGC GGCAGCu'GCu'GAACGCCAAGCu'GAu'CACCCAGCGGA AGu'u'CGACAACCu'GACCAAGGCCGAGCGGGGCGGCC u'GAGCGAGCu'GGACAAGGCCGGCu'u'CAu'CAAGCGGC AGCu'GGu'GGAGACACGGCAGAu'CACCAAGCACGu'GG CCCAGAu'CCu'GGACAGCCGGAu'GAACACCAAGu'ACG ACGAGAACGACAAGCu'GAu'CCGGGAGGu'GAAGGu'G Au'CACCCu'CAAGAGCAAGCu'GGu'GAGCGACu'u'CCG GAAGGACu'u'CCAGu'u'Cu'ACAAGGu'GCGGGAGAu'CA ACAACu'ACCACCACGCCCACGACGCCu'ACCu'GAACG CCGu'GGu'GGGCACCGCCCu'GAu'CAAGAAGu'ACCCCA AGCu'GGAGAGCGAGu'u'CGu'Gu'ACGGCGACu'ACAAG Gu'Gu'ACGACGu'GCGGAAGAu'GAu'CGCCAAGAGCGA GCAGGAGAu'CGGCAAGGCCACCGCCAAGu'ACu'u'Cu'u 'Cu'ACAGCAACAu'CAu'GAACu'u'Cu'u'CAAGACCGAGA u'CACCCu'GGCCAACGGCGAGAu'CCGGAAGCGGCCCCu 'GAu'CGAGACAAACGGCGAGACAGGCGAGAu'CGu'Gu' GGGACAAGGGCCGGGACu'u'CGCCACCGu'GCGGAAGG u'GCu'GAGCAu'GCCCCAGGu'GAACAu'CGu'GAAGAAG ACCGAGGu'GCAGACCGGCGGCu'u'CAGCAAGGAGAGC Au'CCu'GCCCAAGCGGAACAGCGACAAGCu'GAu'CGCC CGGAAGAAGGACu'GGGACCCCAAGAAGu'ACGGCGGC u'u'C**GAG**AGCCCCACCGu'GGCCu'ACAGCGu'GCu'GGu' GGu'GGCCAAGGu'GGAGAAGGGCAAGAGCAAGAAGCu 'CAAGAGCGu'GAAGGAGCu'GCu'GGGCAu'CACCAu'CA u'GGAGCGGAGCAGCu'u'CGAGAAGAACCCCAu'CGACu' u'CCu'GGAGGCCAAGGGCu'ACAAGGAGGu'GAAGAAG	

		GACCu'GAu'CAu'CAAGCu'GCCCAAGu'ACAGCCu'Gu'u' CGAGCu'GGAGAACGGCCGGAAGCGGAu'GCu'GGCCAG CGCCGGCGAGCu'GCAGAAGGGCAACGAGCu'GGCCCu' GCCCAGCAAGu'ACGu'GAACu'u'CCu'Gu'ACCu'GGCCA GCCACu'ACGAGAAGCu'GAAGGGCAGCCCCGAGGACA ACGAGCAGAAGCAGCu'Gu'u'CGu'GGAGCAGCACAAGC ACu'ACCu'GGACGAGAu'CAu'CGAGCAGAu'CAGCGAGu 'u'CAGCAAGCGGGu'GAu'CCu'GGCCGACGCCAACCu'G GACAAGGu'GCu'GAGCGCCu'ACAACAAGCACCGGGAC AAGCCCAu'CCGGGAGCAGGCCGAGAACAu'CAu'CCAC Cu'Gu'u'CACCCu'GACCAACCu'GGGCGCCCCCGCCGCCu 'u'CAAGu'ACu'u'CGACACCACCAu'CGACCGGAAGCGGu 'ACACCAGCACCAAGGAGGu'GCu'GGACGCCACCCu'GA u'CCACCAGAGCAu'CACCGGCCu'Gu'ACGAGACACGGA u'CGACCu'GAGCCAGCu'GGGCGGCGAC	
	Protein	DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDR HSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRIC YLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFG NIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALA HMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEEN PINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFG NLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNL LAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSA SMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNG YAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDL LRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNRE KIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFE EVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYF TVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNR KVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDL LKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKT YAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSG KTILDFLKSDGFANANFMQLIHDDSLTFKEDIQKAQVSGQ GDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHK PENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQI LKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRL SDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPS EEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYL NAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQ EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGE TGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFS KESILPKRNSDKLIARKKDWDPKKYGGFESPTVAYSVLV VAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAK GYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHK HYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIRE QAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLD ATLIHQSITGLYETRIDLSQLGGD	2156
Linker between Cas9 nickase and	mRNA	GAGGGCGCCGAC	2143
	Protein	EGAD	2144

NLS			
Nuclear localization sequence (NLS)	mRNA	AAGCGGACCGCCGACGGCAGCGAGu'u'CGAGAGCCCC AAGAAGAAGCGGAAGGu'Gu'GA	2145
	Protein	KRTADGSEFESPKKKRKV	2146
3′ UTR	mRNA	GCGGCCGCu'u'AAu'u'AAGCu'GCCu'u'Cu'GCGGGGCu'u' GCCu'u'Cu'GGCCAu'GCCCu'u'Cu'u'Cu'Cu'CCCu'u'GCAC Cu'Gu'ACCu'Cu'u'GGu'Cu'u'u'GAAu'AAAGCCu'GAGu'A GGAAGu'Cu'AGA	2147
MA045 mRNA and protein sequences			
Region	**Sequence**		**SEQ ID NO**
Full sequence	mRNA	AGGAAAu'AAGAGAGAAAAGAAGAGu'AAGAAGAAAu' Au'AAGAGCCACCAu'GAGCGAGGu'GGAGu'u'CAGCCA CGAGu'ACu'GGAu'GCGGCACGCCCu'GACCCu'GGCCAA GCGGGCCCGGGACGAGCGGGAGGu'GCCCGu'GGGCGC CGu'GCu'GGu'GCu'GAACAACCGGGu'GAu'CGGCGAGG GCu'GGAACCGGGCCAu'CGGCCu'GCACGACCCCACCG CCCACGCCGAGAu'CAu'GGCCCu'GCGGCAGGGCGGCCu 'GGu'GAu'GCAGAACu'ACCGGCu'GAu'CGACGCCACCCu 'Gu'ACGu'GACCu'u'CGAGCCCu'GCGu'GAu'Gu'GCGCCG GCGCCAu'GAu'CCACAGCCGGAu'CGGCCGGGu'GGu'Gu 'u'CGGCGu'GCGGAACGCCAAGACCGGCGCCGCCGGCA GCCu'GAu'GGACGu'GCu'GCACCACCCCGGCAu'GAACC ACCGGGu'GGAGAu'CACCGAGGGCAu'CCu'GGCCGACG AGu'GCGCCGCCCu'GCu'Gu'GCCGGu'u'Cu'u'CCGGAu'G CCCCGGCGGGu'Gu'u'CAACGCCCAGAAGAAGGCCCAG AGCAGCACCGACAGCGGCGGCAGCAGCGGCGGCAGCA GCGGCAGCGAGACACCCGGCACCAGCGAGAGCGCCAC CCCCGAGAGCAGCGGCGGCAGCAGCGGCGGCAGCGAC AAGAAGu'ACAGCAu'CGGCCu'GGCCAu'CGGCACCAAC AGCGu'GGGCu'GGGCCGu'GAu'CACCGACGAGu'ACAA GGu'GCCCAGCAAGAAGu'u'CAAGGu'GCu'GGGCAACA CCGACCGGCACAGCAu'CAAGAAGAACCu'GAu'CGGCG CCCu'GCu'Gu'u'CGACAGCGGCGAGACAGCCGAGGCCA CCCGGCu'GAAGCGGACCGCCCGGCGGCGGu'ACACCC GGCGGAAGAACCGGAu'Cu'GCu'ACCu'GCAGGAGAu'Cu 'u'CAGCAACGAGAu'GGCCAAGGu'GGACGACAGCu'u'C u'u'CCACCGGCu'GGAGGAGAGCu'u'CCu'GGu'GGAGGA GGACAAGAAGCACGAGCGGCACCCCAu'Cu'u'CGGCAA CAu'CGu'GGACGAGGu'GGCCu'ACCACGAGAAGu'ACCC CACCAu'Cu'ACCACCu'GCGGAAGAAGCu'GGu'GGACAG CACCGACAAGGCCGACCu'GCGGCu'GAu'Cu'ACCu'GGC CCu'GGCCCACAu'GAu'CAAGu'u'CCGGGGCCACu'u'CCu 'GAu'CGAGGGCGACCu'GAACCCCGACAACAGCGACGu' GGACAAGCu'Gu'u'CAu'CCAGCu'GGu'GCAGACCu'ACA ACCAGCu'Gu'u'CGAGGAGAACCCCAu'CAACGCCAGCG GCGu'GGACGCCAAGGCCAu'CCu'GAGCGCCCGGCu'GA GCAAGAGCCGGCGGCu'GGAGAACCu'GAu'CGCCCAGC u'GCCCGGCGAGAAGAAGAACGGCCu'Gu'u'CGGCAACC u'GAu'CGCCCu'GAGCCu'GGGCCu'GACCCCCAACu'u'CA AGAGCAACu'u'CGACCu'GGCCGAGGACGCCAAGCu'GC AGCu'GAGCAAGGACACCu'ACGACGACGACCu'GGACA	2157

		ACCu'GCu'GGCCCAGAu'CGGCGACCAGu'ACGCCGACC u'Gu'u'CCu'GGCCGCCAAGAACCu'GAGCGACGCCAu'CC u'GCu'GAGCGACAu'CCu'GCGGGu'GAACACCGAGAu'C ACCAAGGCCCCCCu'GAGCGCCAGCAu'GAu'CAAGCGG u'ACGACGAGCACCACCAGGACCu'GACCCu'GCu'GAAG GCCCu'GGu'GCGGCAGCAGCu'GCCCGAGAAGu'ACAAG GAGAu'Cu'u'Cu'u'CGACCAGAGCAAGAACGGCu'ACGC CGGCu'ACAu'CGACGGCGGCGCCAGCCAGGAGGAGu'u' Cu'ACAAGu'u'CAu'CAAGCCCAu'CCu'GGAGAAGAu'GG ACGGCACCGAGGAGCu'GCu'GGu'GAAGCu'GAACCGGG AGGACCu'GCu'GCGGAAGCAGCGGACCu'u'CGACAACG GCAGCAu'CCCCCACCAGAu'CCACCu'GGGCGAGCu'GC ACGCCAu'CCu'GCGGCGGCAGGAGGACu'u'Cu'ACCCCu' u'CCu'GAAGGACAACCGGGAGAAGAu'CGAGAAGAu'CC u'GACCu'u'CCGGAu'CCCCu'ACu'ACGu'GGGCCCCCu'G GCCCGGGGCAACAGCCGGu'u'CGCCu'GGAu'GACCCGC AAGAGCGAGGAGACAAu'CACCCCCu'GGAACu'u'CGAG GAGGu'GGu'GGACAAGGGCGCCAGCGCCCAGAGCu'u'C Au'CGAGCGGAu'GACCAACu'u'CGACAAGAACCu'GCCC AACGAGAAGGu'GCu'GCCCAAGCACAGCCu'GCu'Gu'AC GAGu'ACu'u'CACCGu'Gu'ACAACGAGCu'GACCAAGGu' GAAGu'ACGu'GACCGAGGGCAu'GCGGAAGCCCGCCu'u' CCu'GAGCGGCGAGCAGAAGAAGGCCAu'CGu'GGACCu' GCu'Gu'u'CAAGACCAACCGGAAGGu'GACCGu'GAAGC AGCu'GAAGGAGGACu'ACu'u'CAAGAAGAu'CGAGu'GC u'u'CGACAGCGu'GGAGAu'CAGCGGCGu'GGAGGACCG Gu'u'CAACGCCAGCCu'GGGCACCu'ACCACGACCu'GCu' GAAGAu'CAu'CAAGGACAAGGACu'u'CCu'GGACAACG AGGAGAACGAGGACAu'CCu'GGAGGACAu'CGu'GCu'G ACCCu'GACCCu'Gu'u'CGAGGACCGGGAGAu'GAu'CGA GGAGCGGCu'GAAGACCu'ACGCCCACCu'Gu'u'CGACGA CAAGGu'GAu'GAAGCAGCu'GAAGCGGCGGCGGu'ACAC CGGCu'GGGGCCGGCu'GAGCCGGAAGCu'GAu'CAACGG CAu'CCGGGACAAGCAGAGCGGCAAGACCAu'CCu'GGA Cu'u'CCu'CAAGAGCGACGGCu'u'CGCCAAC**GCC**AACu'u 'CAu'GCAGCu'GAu'CCACGACGACAGCCu'GACCu'u'CA AGGAGGACAu'CCAGAAGGCCCAGGu'GAGCGGCCAGG GCGACAGCCu'GCACGAGCACAu'CGCCAACCu'GGCCG GCAGCCCCGCCAu'CAAGAAGGGCAu'CCu'GCAGACCG u'GAAGGu'GGu'GGACGAGCu'GGu'GAAGGu'GAu'GGGC CGGCACAAGCCCGAGAACAu'CGu'GAu'CGAGAu'GGCC CGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAAC AGCCGGGAGCGGAu'GAAGCGGAu'CGAGGAGGGCAu'C AAGGAGCu'GGGCAGCCAGAu'CCu'GAAGGAGCACCCC Gu'GGAGAACACCCAGCu'GCAGAACGAGAAGCu'Gu'AC Cu'Gu'ACu'ACCu'GCAGAACGGCCGGGACAu'Gu'ACGu' GGACCAGGAGCu'GGACAu'CAACCGGCu'GAGCGACu'A CGACGu'GGACCACAu'CGu'GCCCCAGAGCu'u'CCu'GAA GGACGACAGCAu'CGACAACAAGGu'GCu'GACCCGGAG CGACAAGAACCGGGGCAAGAGCGACAACGu'GCCCAG CGAGGAGGu'GGu'GAAGAAGAu'GAAGAACu'ACu'GGC GGCAGCu'GCu'GAACGCCAAGCu'GAu'CACCCAGCGGA AGu'u'CGACAACCu'GACCAAGGCCGAGCGGGGCGGCC u'GAGCGAGCu'GGACAAGGCCGGCu'u'CAu'CAAGCGGC AGCu'GGu'GGAGACACGGCAGAu'CACCAAGCACGu'GG	

		CCCAGAu'CCu'GGACAGCCGGAu'GAACACCAAGu'ACG ACGAGAACGACAAGCu'GAu'CCGGGAGGu'GAAGGu'G Au'CACCCu'CAAGAGCAAGCu'GGu'GAGCGACu'u'CCG GAAGGACu'u'CCAGu'u'Cu'ACAAGGu'GCGGGAGAu'CA ACAACu'ACCACCACGCCCACGACGCCu'ACCu'GAACG CCGu'GGu'GGGCACCGCCCu'GAu'CAAGAAGu'ACCCCA AGCu'GGAGAGCGAGu'u'CGu'Gu'ACGGCGACu'ACAAG Gu'Gu'ACGACGu'GCGGAAGAu'GAu'CGCCAAGAGCGA GCAGGAGAu'CGGCAAGGCCACCGCCAAGu'ACu'u'Cu'u 'Cu'ACAGCAACAu'CAu'GAACu'u'Cu'u'CAAGACCGAGA u'CACCCu'GGCCAACGGCGAGAu'CCGGAAGCGGCCCCu 'GAu'CGAGACAAACGGCGAGACAGGCGAGAu'CGu'Gu' GGGACAAGGGCCGGGACu'u'CGCCACCGu'GCGGAAGG u'GCu'GAGCAu'GCCCCAGGu'GAACAu'CGu'GAAGAAG ACCGAGGu'GCAGACCGGCGGCu'u'CAGCAAGGAGAGC Au'CCu'GCCCAAGCGGAACAGCGACAAGCu'GAu'CGCC CGGAAGAAGGACu'GGGACCCCAAGAAGu'ACGGCGGC u'u'C**GAC**AGCCCCACCGu'GGCCu'ACAGCGu'GCu'GGu' GGu'GGCCAAGGu'GGAGAAGGGCAAGAGCAAGAAGCu 'CAAGAGCGu'GAAGGAGCu'GCu'GGGCAu'CACCAu'CA u'GGAGCGGAGCAGCu'u'CGAGAAGAACCCCAu'CGACu' u'CCu'GGAGGCCAAGGGCu'ACAAGGAGGu'GAAGAAG GACCu'GAu'CAu'CAAGCu'GCCCAAGu'ACAGCCu'Gu'u' CGAGCu'GGAGAACGGCCGGAAGCGGAu'GCu'GGCCAG CGCCGGCGAGCu'GCAGAAGGGCAACGAGCu'GGCCCu' GCCCAGCAAGu'ACGu'GAACu'u'CCu'Gu'ACCu'GGCCA GCCACu'ACGAGAAGCu'GAAGGGCAGCCCCGAGGACA ACGAGCAGAAGCAGCu'Gu'u'CGu'GGAGCAGCACAAGC ACu'ACCu'GGACGAGAu'CAu'CGAGCAGAu'CAGCGAGu 'u'CAGCAAGCGGGu'GAu'CCu'GGCCGACGCCAACCu'G GACAAGGu'GCu'GAGCGCCu'ACAACAAGCACCGGGAC AAGCCCAu'CCGGGAGCAGGCCGAGAACAu'CAu'CCAC Cu'Gu'u'CACCCu'GACCAACCu'GGGCGCCCCCGCCGCCu 'u'CAAGu'ACu'u'CGACACCACCAu'CGACCGGAAGCGGu 'ACACCAGCACCAAGGAGGu'GCu'GGACGCCACCCu'GA u'CCACCAGAGCAu'CACCGGCCu'Gu'ACGAGACACGGA u'CGACCu'GAGCCAGCu'GGGCGGCGACGAGGGCGCCG ACAAGCGGACCGCCGACGGCAGCGAGu'u'CGAGAGCC CCAAGAAGAAGCGGAAGGu'Gu'GAGCGGCCGCu'u'AAu 'u'AAGCu'GCCu'u'Cu'GCGGGGCu'u'GCCu'u'Cu'GGCCAu 'GCCCu'u'Cu'u'Cu'Cu'CCCu'u'GCACCu'Gu'ACCu'Cu'u'G Gu'Cu'u'u'GAAu'AAAGCCu'GAGu'AGGAAGu'Cu'AGA	
	Protein	MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNN RVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLID ATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHHPGMNHRVEITEGILADECAALLCRFFRMPRR VFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGG SSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVL GNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRYTRR KNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHER HPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIY LALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKN GLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDL DNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKA	2158

		PLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQS KNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLN REDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITP WNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLL YEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLF KTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD KQSGKTILDFLKSDGFAN**A**NFMQLIHDDSLTFKEDIQKAQ VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM GRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKEL GSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDI NRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDN VPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAH DAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIA KSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT GGFSKESILPKRNSDKLIARKKDWDPKKYGGF**D**SPTVAY SVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDF LEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGEL QKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFV EQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRD KPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTK EVLDATLIHQSITGLYETRIDLSQLGGDEGADKRTADGSE FESPKKKRKV	
5′ UTR	mRNA	AGGAAAu'AAGAGAGAAAAGAAGAGu'AAGAAGAAAu' Au'AAGAGCCACC	2138
TadA	mRNA	Au'GAGCGAGGu'GGAGu'u'CAGCCACGAGu'ACu'GGAu' GCGGCACGCCCu'GACCCu'GGCCAAGCGGGCCCGGGA CGAGCGGGAGGu'GCCCGu'GGGCGCCGu'GCu'GGu'GCu 'GAACAACCGGGu'GAu'CGGCGAGGGCu'GGAACCGGG CCAu'CGGCCu'GCACGACCCCACCGCCCACGCCGAGAu' CAu'GGCCCu'GCGGCAGGGCGGCCu'GGu'GAu'GCAGAA Cu'ACCGGCu'GAu'CGACGCCACCCu'Gu'ACGu'GACCu'u 'CGAGCCCu'GCGu'GAu'Gu'GCGCCGGCGCCAu'GAu'CC ACAGCCGGAu'CGGCCGGGu'GGu'Gu'u'CGGCGu'GCGG AACGCCAAGACCGGCGCCGCCGGCAGCCu'GAu'GGAC Gu'GCu'GCACCACCCCGGCAu'GAACCACCGGGu'GGAG Au'CACCGAGGGCAu'CCu'GGCCGACGAGu'GCGCCGCC Cu'GCu'Gu'GCCGGu'u'Cu'u'CCGGAu'GCCCCGGCGGGu' Gu'u'CAACGCCCAGAAGAAGGCCCAGAGCAGCACCGA C	2139
	Protein	MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNN RVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLID ATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHHPGMNHRVEITEGILADECAALLCRFFRMPRR VFNAQKKAQSSTD	2140
Linker between TadA and	mRNA	AGCGGCGGCAGCAGCGGCGGCAGCAGCGGCAGCGAG ACACCCGGCACCAGCGAGAGCGCCACCCCCGAGAGCA GCGGCGGCAGCAGCGGCGGCAGC	2141
	Protein	SGGSSGGSSGSETPGTSESATPESSGGSSGGS	20

Cas9 nickase			
Cas9 nickase	mRNA	GACAAGAAGu'ACAGCAu'CGGCCu'GGCCAu'CGGCACC AACAGCGu'GGGCu'GGGCCGu'GAu'CACCGACGAGu'AC AAGGu'GCCCAGCAAGAAGu'u'CAAGGu'GCu'GGGCAA CACCGACCGGCACAGCAu'CAAGAAGAACCu'GAu'CGG CGCCCu'GCu'Gu'u'CGACAGCGGCGAGACAGCCGAGGC CACCCGGCu'GAAGCGGACCGCCCGGCGGCGGu'ACAC CCGGCGGAAGAACCGGAu'Cu'GCu'ACCu'GCAGGAGAu' Cu'u'CAGCAACGAGAu'GGCCAAGGu'GGACGACAGCu'u 'Cu'u'CCACCGGCu'GGAGGAGAGCu'u'CCu'GGu'GGAGG AGGACAAGAAGCACGAGCGGCACCCCAu'Cu'u'CGGCA ACAu'CGu'GGACGAGGu'GGCCu'ACCACGAGAAGu'ACC CCACCAu'Cu'ACCACCu'GCGGAAGAAGCu'GGu'GGACA GCACCGACAAGGCCGACCu'GCGGCu'GAu'Cu'ACCu'GG CCCu'GGCCCACAu'GAu'CAAGu'u'CCGGGGCCACu'u'CC u'GAu'CGAGGGCGACCu'GAACCCCGACAACAGCGACG u'GGACAAGCu'Gu'u'CAu'CCAGCu'GGu'GCAGACCu'AC AACCAGCu'Gu'u'CGAGGAGAACCCCAu'CAACGCCAGC GGCGu'GGACGCCAAGGCCAu'CCu'GAGCGCCCGGCu'G AGCAAGAGCCGGCGGCu'GGAGAACCu'GAu'CGCCCAG Cu'GCCCGGCGAGAAGAAGAACGGCCu'Gu'u'CGGCAAC Cu'GAu'CGCCCu'GAGCCu'GGGCCu'GACCCCCAACu'u'C AAGAGCAACu'u'CGACCu'GGCCGAGGACGCCAAGCu'G CAGCu'GAGCAAGGACACCu'ACGACGACGACCu'GGAC AACCu'GCu'GGCCCAGAu'CGGCGACCAGu'ACGCCGAC Cu'Gu'u'CCu'GGCCGCCAAGAACCu'GAGCGACGCCAu'C Cu'GCu'GAGCGACAu'CCu'GCGGGu'GAACACCGAGAu' CACCAAGGCCCCCCu'GAGCGCCAGCAu'GAu'CAAGCG Gu'ACGACGAGCACCACCAGGACCu'GACCCu'GCu'GAA GGCCCu'GGu'GCGGCAGCAGCu'GCCCGAGAAGu'ACAA GGAGAu'Cu'u'Cu'u'CGACCAGAGCAAGAACGGCu'ACG CCGGCu'ACAu'CGACGGCGGCGCCAGCCAGGAGGAGu' u'Cu'ACAAGu'u'CAu'CAAGCCCAu'CCu'GGAGAAGAu'G GACGGCACCGAGGAGCu'GCu'GGu'GAAGCu'GAACCGG GAGGACCu'GCu'GCGGAAGCAGCGGACCu'u'CGACAAC GGCAGCAu'CCCCCACCAGAu'CCACCu'GGGCGAGCu'G CACGCCAu'CCu'GCGGCGGCAGGAGGACu'u'Cu'ACCCC u'u'CCu'GAAGGACAACCGGGAGAAGAu'CGAGAAGAu' CCu'GACCu'u'CCGGAu'CCCCu'ACu'ACGu'GGGCCCCCu' GGCCCGGGGCAACAGCCGGu'u'CGCCu'GGAu'GACCCG CAAGAGCGAGGAGACAAu'CACCCCCu'GGAACu'u'CGA GGAGGu'GGu'GGACAAGGGCGCCAGCGCCCAGAGCu'u' CAu'CGAGCGGAu'GACCAACu'u'CGACAAGAACCu'GCC CAACGAGAAGGu'GCu'GCCCAAGCACAGCCu'GCu'Gu'A CGAGu'ACu'u'CACCGu'Gu'ACAACGAGCu'GACCAAGGu 'GAAGu'ACGu'GACCGAGGGCAu'GCGGAAGCCCGCCu'u 'CCu'GAGCGGCGAGCAGAAGAAGGCCAu'CGu'GGACCu 'GCu'Gu'u'CAAGACCAACCGGAAGGu'GACCGu'GAAGC AGCu'GAAGGAGGACu'ACu'u'CAAGAAGAu'CGAGu'GC u'u'CGACAGCGu'GGAGAu'CAGCGGCGu'GGAGGACCG Gu'u'CAACGCCAGCCu'GGGCACCu'ACCACGACCu'GCu' GAAGAu'CAu'CAAGGACAAGGACu'u'CCu'GGACAACG AGGAGAACGAGGACAu'CCu'GGAGGACAu'CGu'GCu'G ACCCu'GACCCu'Gu'u'CGAGGACCGGGAGAu'GAu'CGA	2159

		GGAGCGGCu'GAAGACCu'ACGCCCACCu'Gu'u'CGACGA CAAGGu'GAu'GAAGCAGCu'GAAGCGGCGGCGGu'ACAC CGGCu'GGGGCCGGCu'GAGCCGGAAGCu'GAu'CAACGG CAu'CCGGGACAAGCAGAGCGGCAAGACCAu'CCu'GGA Cu'u'CCu'CAAGAGCGACGGCu'u'CGCCAAC**GCC**AACu'u 'CAu'GCAGCu'GAu'CCACGACGACAGCCu'GACCu'u'CA AGGAGGACAu'CCAGAAGGCCCAGGu'GAGCGGCCAGG GCGACAGCCu'GCACGAGCACAu'CGCCAACCu'GGCCG GCAGCCCCGCCAu'CAAGAAGGGCAu'CCu'GCAGACCG u'GAAGGu'GGu'GGACGAGCu'GGu'GAAGGu'GAu'GGGC CGGCACAAGCCCGAGAACAu'CGu'GAu'CGAGAu'GGCC CGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAAC AGCCGGGAGCGGAu'GAAGCGGAu'CGAGGAGGGCAu'C AAGGAGCu'GGGCAGCCAGAu'CCu'GAAGGAGCACCCC Gu'GGAGAACACCCAGCu'GCAGAACGAGAAGCu'Gu'AC Cu'Gu'ACu'ACCu'GCAGAACGGCCGGGACAu'Gu'ACGu' GGACCAGGAGCu'GGACAu'CAACCGGCu'GAGCGACu'A CGACGu'GGACCACAu'CGu'GCCCCAGAGCu'u'CCu'GAA GGACGACAGCAu'CGACAACAAGGu'GCu'GACCCGGAG CGACAAGAACCGGGGCAAGAGCGACAACGu'GCCCAG CGAGGAGGu'GGu'GAAGAAGAu'GAAGAACu'ACu'GGC GGCAGCu'GCu'GAACGCCAAGCu'GAu'CACCCAGCGGA AGu'u'CGACAACCu'GACCAAGGCCGAGCGGGGCGGCC u'GAGCGAGCu'GGACAAGGCCGGCu'u'CAu'CAAGCGGC AGCu'GGu'GGAGACACGGCAGAu'CACCAAGCACGu'GG CCCAGAu'CCu'GGACAGCCGGAu'GAACACCAAGu'ACG ACGAGAACGACAAGCu'GAu'CCGGGAGGu'GAAGGu'G Au'CACCCu'CAAGAGCAAGCu'GGu'GAGCGACu'u'CCG GAAGGACu'u'CCAGu'u'Cu'ACAAGGu'GCGGGAGAu'CA ACAACu'ACCACCACGCCCACGACGCCu'ACCu'GAACG CCGu'GGu'GGGCACCGCCCu'GAu'CAAGAAGu'ACCCCA AGCu'GGAGAGCGAGu'u'CGu'Gu'ACGGCGACu'ACAAG Gu'Gu'ACGACGu'GCGGAAGAu'GAu'CGCCAAGAGCGA GCAGGAGAu'CGGCAAGGCCACCGCCAAGu'ACu'u'Cu'u 'Cu'ACAGCAACAu'CAu'GAACu'u'Cu'u'CAAGACCGAGA u'CACCCu'GGCCAACGGCGAGAu'CCGGAAGCGGCCCCu 'GAu'CGAGACAAACGGCGAGACAGGCGAGAu'CGu'Gu' GGGACAAGGGCCGGGACu'u'CGCCACCGu'GCGGAAGG u'GCu'GAGCAu'GCCCCAGGu'GAACAu'CGu'GAAGAAG ACCGAGGu'GCAGACCGGCGGCu'u'CAGCAAGGAGAGC Au'CCu'GCCCAAGCGGAACAGCGACAAGCu'GAu'CGCC CGGAAGAAGGACu'GGGACCCCAAGAAGu'ACGGCGGC u'u'C**GAC**AGCCCCACCGu'GGCCu'ACAGCGu'GCu'GGu' GGu'GGCCAAGGu'GGAGAAGGGCAAGAGCAAGAAGCu 'CAAGAGCGu'GAAGGAGCu'GCu'GGGCAu'CACCAu'CA u'GGAGCGGAGCAGCu'u'CGAGAAGAACCCCAu'CGACu' u'CCu'GGAGGCCAAGGGCu'ACAAGGAGGu'GAAGAAG GACCu'GAu'CAu'CAAGCu'GCCCAAGu'ACAGCCu'Gu'u' CGAGCu'GGAGAACGGCCGGAAGCGGAu'GCu'GGCCAG CGCCGGCGAGCu'GCAGAAGGGCAACGAGCu'GGCCCu' GCCCAGCAAGu'ACGu'GAACu'u'CCu'Gu'ACCu'GGCCA GCCACu'ACGAGAAGCu'GAAGGGCAGCCCCGAGGACA ACGAGCAGAAGCAGCu'Gu'u'CGu'GGAGCAGCACAAGC ACu'ACCu'GGACGAGAu'CAu'CGAGCAGAu'CAGCGAGu 'u'CAGCAAGCGGGu'GAu'CCu'GGCCGACGCCAACCu'G	

		GACAAGGu'GCu'GAGCGCCu'ACAACAAGCACCGGGAC AAGCCCAu'CCGGGAGCAGGCCGAGAACAu'CAu'CCAC Cu'Gu'u'CACCCu'GACCAACCu'GGGCGCCCCCGCCGCCu 'u'CAAGu'ACu'u'CGACACCACCAu'CGACCGGAAGCGGu 'ACACCAGCACCAAGGAGGu'GCu'GGACGCCACCCu'GA u'CCACCAGAGCAu'CACCGGCCu'Gu'ACGAGACACGGA u'CGACCu'GAGCCAGCu'GGGCGGCGAC	
	Protein	DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDR HSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRIC YLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFG NIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALA HMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEEN PINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFG NLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNL LAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSA SMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNG YAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDL LRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNRE KIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFE EVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYF TVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNR KVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDL LKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKT YAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSG KTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQ GDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHK PENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQI LKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRL SDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPS EEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYL NAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQ EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGE TGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFS KESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLV VAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAK GYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHK HYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIRE QAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLD ATLIHQSITGLYETRIDLSQLGGD	
Linker between Cas9 nickase and NLS	mRNA	GAGGGCGCCGAC	2143
	Protein	EGAD	2144
Nuclear localization sequence (NLS)	mRNA	AAGCGGACCGCCGACGGCAGCGAGu'u'CGAGAGCCCC AAGAAGAAGCGGAAGGu'Gu'GA	2145
	Protein	KRTADGSEFESPKKKRKV	2146
3′ UTR	mRNA	GCGGCCGCu'u'AAu'u'AAGCu'GCCu'u'Cu'GCGGGGCu'u'	2147

		GCCu'u'Cu'GGCCAu'GCCCu'u'Cu'u'Cu'Cu'CCCu'u'GCAC Cu'Gu'ACCu'Cu'u'GGu'Cu'u'u'GAAu'AAAGCCu'GAGu'A GGAAGu'Cu'AGA	
MA019 mRNA and protein sequence			
Region	**Sequence**		**SEQ ID NO**
Full sequence	mRNA	AGGAAAu'AAGAGAGAAAAGAAGAGu'AAGAAGAAAu' Au'AAGAGCCACCAu'Gu'CCGAAGu'CGAAu'u'CAGCCA CGAGu'ACu'GGAu'GAGACAu'GCACu'GACCCu'CGCGA AGAGAGCu'CGGGAu'GAGAGGGAAGu'GCCAGu'GGGA GCu'Gu'GCu'GGu'GCu'CAACAACAGGGu'CAu'u'GGGGA AGGAu'GGAACAGGGCCAu'u'GGACu'u'CACGAu'CCGA CGGCu'CAu'GCGGAAAu'CAu'GGCACu'GAGACAGGGC GGu'Cu'u'Gu'GAu'GCAGAACu'Au'CGCCu'GAu'u'GAu'G CCACu'Cu'Gu'ACGu'GACCu'u'CGAACCu'u'GCGu'GAu'G u'GCGCCGGu'GCCAu'GAu'CCACu'CACGCAu'CGGu'CG GGu'GGu'Gu'u'CGGCGu'GCGAAACGCu'AAGACu'GGAG CCGCCGGAu'CACu'GAu'GGAu'Gu'GCu'CCAu'CACCCCG GGAu'GAACCACAGGGu'GGAGAu'CACCGAAGGCAu'u' Cu'GGCCGACGAAu'GCGCAGCCCu'Gu'u'Gu'Gu'CGCu'u' Cu'u'CCGGAu'GCCu'CGAAGAGu'Gu'u'CAACGCGCAGA AGAAGGCGCAGAGCu'CCACu'GACu'Cu'GGAGGu'u'CCu 'CGGGCGGAu'CGu'CAGGGu'CCGAAACu'CCu'GGAACC u'CGGAGu'CAGCAACu'CCGGAAu'CCu'CAGGAGGCu'CC AGCGGAGGCu'CCGAu'AAGAAGu'ACAGCAu'CGGGCu'u 'GCCAu'CGGAACCAACu'CGGu'CGGCu'GGGCu'Gu'GAu' CACu'GACGAGu'ACAAGGu'CCCu'u'CCAAAAAGu'u'CA AAGu'GCu'CGGCAACACu'GAu'CGGCACu'CGAu'u'AAG AAGAACCu'GAu'CGGu'GCACu'GCu'Gu'u'CGACu'Cu'GG GGAGACu'GCGGAAGCAACu'CGCCu'GAAGAGGACu'GC GCGCAGACGGu'ACACCAGGCGCAAGAACCGGAu'Cu'G Cu'ACu'u'GCAAGAGAu'u'u'u'CAGCAACGAGAu'GGCCA AGGu'CGACGACu'CGu'u'u'u'CCACCGGCu'CGAGGAA AGCu'u'CCu'GGu'GGAGGAAGAu'AAGAAGCAu'GAGCG CCAu'CCCAu'Cu'u'CGGAAACAu'CGu'GGACGAAGu'GG CCu'ACCAu'GAGAAGu'ACCCGACCAu'Cu'ACCAu'Cu'GC GCAAGAAGCu'CGu'GGACAGCACu'GACAAAGCCGACu' u'GCGCCu'GAu'Cu'ACCu'u'GCCCu'GGCCCACAu'GAu'C AAGu'u'CAGAGGGCACu'u'CCu'GAu'CGAGGGCGACCu' GAACCCGGACAAu'u'CCGACGu'GGAu'AAGCu'Gu'u'CA u'CCAACu'GGu'ACAAACu'u'ACAACCAGCu'Gu'u'u'GAG GAGAACCCGAu'CAACGCu'u'CCGGCGu'GGACGCGAAG GCCAu'u'Cu'GAGCGCACGGCu'Gu'CCAAGu'CCCGGAGA u'u'AGAAAACCu'GAu'u'GCCCAACu'GCCu'GGAGAAAA GAAGAAu'GGCCu'Gu'u'CGGAAACCu'GAu'CGCCCu'Cu' CGCu'CGGu'Cu'GACCCCCAACu'u'u'AAGAGCAACu'u'C GACCu'GGCu'GAGGACGCGAAGCu'CCAGCu'Gu'CCAAG GACACCu'ACGAu'GAu'GACCu'CGAu'AAu'Cu'CCu'CGC CCAAAu'CGGCGACCAAu'Au'GCGGACCu'Gu'u'u'Cu'GG CCGCCAAGAAu'Cu'Gu'CCGACGCCAu'CCu'CCu'GAGCG Au'Au'CCu'GCGCGu'GAACACu'GAAAu'CACCAAGGCCC CACu'GAGCGCu'AGCAu'GAu'u'AAGCGCu'ACGACGAA CACCAu'CAGGACCu'CACCCu'GCu'GAAGGCu'Cu'u'Gu' GCGGCAGCAGCu'CCCAGAGAAGu'ACAAGGAGAu'Cu'u	2161

		'Cu'u'CGACCAAu'CGAAGAAu'GGu'u'ACGCCGGCu'ACA u'u'GACGGAGGAGCGu'CACAGGAGGAGu'u'Cu'ACAAG u'u'CAu'u'AAGCCAAu'CCu'GGAGAAGAu'GGACGGAAC CGAGGAACu'GCu'CGu'GAAGCu'GAACAGAGAAGAu'Cu 'ACu'GCGCAAGCAGCGCACu'u'u'CGACAACGGAu'CGA u'CCCACAu'CAGAu'u'CACCu'GGGAGAGCu'GCACGCGA u'CCu'GCGGAGACAGGAAGAu'u'u'Cu'ACCCGu'u'CCu'G AAGGACAACCGCGAGAAGAu'CGAAAAGAu'CCu'GACC u'u'CCGCAu'u'CCGu'ACu'ACGu'GGGACCCCu'CGCAAG AGGAAACu'CGCGGu'u'CGCCu'GGAu'GACCAGGAAGu' CGGAAGAAACCAu'u'ACu'CCGu'GGAACu'u'CGAGGAA Gu'GGu'GGACAAGGGCGCCu'CAGCGCAAu'CCu'u'CAu' CGAACGGAu'GACCAACu'u'CGACAAGAACu'u'GCCu'A ACGAGAAAGu'GCu'u'CCu'AAGCAu'u'CCCu'CCu'u'u'AC GAAu'ACu'u'CACu'Gu'Gu'ACAACGAGCu'u'ACCAAGGu' CAAAu'Au'Gu'GACCGAGGGCAu'GCGCAAGCCGGCCu'u 'u'Cu'u'u'CCGGGGAGCAAAAGAAGGCCAu'CGu'GGACC u'Gu'u'Gu'u'CAAGACCAACCGGAAGGu'CACCGu'CAAG CAGCu'GAAGGAGGACu'ACu'u'u'AAGAAAAu'CGAGu'G Cu'u'u'GAu'u'CCGu'CGAAAu'Cu'CCGGAGu'GGAAGAu'A GAu'u'CAACGCCAGCCu'AGGCACCu'ACCACGAu'Cu'GC u'CAAGAu'CAu'u'AAGGAu'AAGGAu'u'u'CCu'GGACAAC GAGGAGAACGAGGAu'Au'CCu'GGAGGACAu'u'Gu'GCu' GACCCu'GACACu'Gu'u'CGAAGAu'CGCGAAAu'GAu'CG AGGAGCGGCu'GAAAACAu'ACGCCCACCu'Gu'u'CGACG ACAAGGu'CAu'GAAGCAGCu'CAAGCGGAGGAGGu'AC ACGGGAu'GGGGAAGGCu'Gu'CCCGGAAGCu'GAu'CAA CGGGAu'u'AGAGACAAGCAGu'CCGGAAAGACCAu'u'Cu 'GGACu'u'CCu'CAAGu'CGGACGGCu'u'CGCGAAC**CGG**A ACu'u'CAu'GCAACu'GAu'CCACGACGACu'CCCu'GACGu 'u'CAAGGAGGACAu'u'CAGAAGGCCCAAGu'Gu'CCGGA CAGGGAGAu'u'CACu'GCACGAACACAu'CGCCAAu'Cu' GGCu'GGAAGu'CCGGCCAu'CAAGAAAGGCAu'CCu'GCA AACCGu'GAAGGu'CGu'GGACGAGCu'CGu'GAAGGu'CA u'GGGCCGCCACAAGCCu'GAGAACAu'CGu'GAu'CGAAA u'GGCCCGGGAGAACCAGACCACGCAGAAAGGGCAGA AGAACAGCCGCGAGAGGAu'GAAGAGAAu'CGAGGAAG GAAu'CAAGGAACu'u'GGAAGCCAGAu'CCu'GAAGGAA CACCCCGu'GGAAAACACu'CAGCu'u'CAAAACGAAAAG Cu'Gu'ACCu'Cu'ACu'Au'Cu'GCAAAACGGACGGGAu'Au' Gu'ACGu'GGACCAGGAAu'u'GGACAu'CAAu'CGCCu'Gu' CCGACu'ACGAu'Gu'GGAu'CACAu'u'Gu'GCCGCAGAGCu 'u'u'Cu'GAAGGACGAu'u'CAAu'u'GAu'AACAAGGu'GCu' CACCCGGu'CCGACAAGAACCGGGGCAAAAGCGAu'AA CGu'GCCGAGCGAAGAGGu'GGu'CAAGAAGAu'GAAGA Au'u'ACu'GGCGGCAGCu'Cu'u'GAACGCCAAACu'GAu'C ACCCAGCGGAAGu'u'CGACAACCu'GACCAAGGCCGAA AGGGGCGGu'Cu'u'u'CCGAACu'GGACAAGGCCGGGu'u' u'Au'CAAGCGCCAACu'GGu'GGAAACCCGGCAGAu'CAC u'AAGCACGu'GGCCCAGAu'ACu'GGACu'Cu'AGGAu'GA Au'ACCAAAu'ACGACGAGAACGACAAGCu'GAu'u'CGC GAAGu'CAAAGu'CAu'u'ACCCu'GAAAu'CCAAGCu'CGu' Gu'CCGACu'u'CCGGAAGGACu'u'CCAGu'u'Cu'Au'AAGG u'u'CGCGAAAu'CAACAACu'ACCACCACGCCCAu'GACG CGu'ACCu'GAACGCCGu'CGu'GGGu'ACCGCCCu'GAu'u'	

		AAGAAGu'ACCCu'AAGCu'CGAGu'CCGAGu'u'CGu'Cu'A CGGAGACu'ACAAAGu'Gu'ACGACGu'GCGGAAGAu'GAu 'CGCCAAGu'CCGAGCAGGAAAu'AGGGAAGGCCACCGC CAAGu'ACu'u'Cu'u'Cu'ACu'CCAACAu'CAu'GAACu'u'Cu' u'CAAGACCGAGAu'CACCCu'u'GCCAACGGGGAGAu'u' CGGAAGCGCCCGu'u'GAu'u'GAGACu'AACGGAGAAAC CGGCGAAAu'u'Gu'Gu'GGGAu'AAGGGCAGAGACu'u'u'G CGACu'Gu'GCGCAAAGu'Cu'u'Gu'CAAu'GCCCCAAGu'C AACAu'u'Gu'CAAAAAGACCGAAGu'GCAAACCGGCGGC u'u'CAGCAAGGAAAGCAu'CCu'GCCCAAGCGCAAu'u'CC GACAAGCu'CAu'CGCCCGGAAGAAGGACu'GGGACCCC AAGAAAu'Au'GGAGGCu'u'C**GAC**u'CCCCCACu'Gu'GGC Cu'ACu'CCGu'CCu'GGu'GGu'CGCGAAAGu'GGAAAAGG GAAAGu'CGAAGAAGCu'CAAGu'CCGu'GAAGGAGCu'C Cu'GGGGAu'CACCAu'u'Au'GGAACGGu'CCAGCu'u'CGA GAAGAACCCGAu'u'GACu'u'CCu'GGAAGCCAAGGGu'u' ACAAGGAGGu'CAAGAAGGAu'Cu'GAu'u'Au'u'AAGCu'G CCGAAGu'Au'u'CCCu'Cu'u'CGAACu'CGAAAAu'GGCCG CAAGCGAAu'Gu'u'GGCAu'CGGCGGGAGAGCu'CCAGA AGGGAAACGAACu'GGCCu'u'GCCu'u'CCAAAu'ACGu'G AACu'u'u'Cu'Gu'ACCu'GGCCAGCCACu'ACGAGAAGCu' GAAGGGu'u'CCCCCGAGGACAACGAGCAGAAGCAGCu' Gu'u'CGu'GGAGCAGCACAAGCACu'ACCu'GGAu'GAGAu 'CAu'CGAACAGAu'Cu'CGGAAu'u'Cu'CCAAGAGAGu'GA u'CCu'GGCCGACGCGAACCu'GGAu'AAGGu'CCu'Gu'CC GCGu'ACAACAAGCACCGGGACAAGCCCAu'CCGGGAG CAGGCu'GAGAACAu'u'Au'CCACCu'Gu'u'CACCCu'CACu 'AACCu'u'GGAGCGCCu'GCCGCAu'u'CAAGu'ACu'u'CGA CACAACCAu'CGACAGAAAGCGCu'ACACu'u'CCACCAA GGAGGu'GCu'GGACGCCACCCu'GAu'CCACCAGu'CCAu' CACu'GGGCu'Cu'Au'GAGACu'CGCAu'u'GACCu'Gu'CCC AGu'u'GGGAGGGGACGAAGGAGCCGAu'AAGAGAACC GCCGAu'GGGu'CCGAAu'u'CGAGu'CGCCCAAGAAGAA GCGGAAAGu'Gu'AAGCGGCCGCu'u'AAu'u'AAGCu'GCC u'u'Cu'GCGGGGCu'u'GCCu'u'Cu'GGCCAu'GCCCu'u'Cu'u 'Cu'Cu'CCCu'u'GCACCu'Gu'ACCu'Cu'u'GGu'Cu'u'u'GAA u'AAAGCCu'GAGu'AGGAAGu'Cu'AGA	
	Protein	MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNN RVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLID ATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHHPGMNHRVEITEGILADECAALLCRFFRMPRR VFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGG SSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVL GNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRYTRR KNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHER HPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIY LALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKN GLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDL DNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKA PLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQS KNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLN REDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITP WNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLL	2137

		YEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLF KTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD KQSGKTILDFLKSDGFAN**R**NFMQLIHDDSLTFKEDIQKAQ VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM GRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKEL GSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDI NRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDN VPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAH DAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIA KSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT GGFSKESILPKRNSDKLIARKKDWDPKKYGGF**D**SPTVAY SVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDF LEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGEL QKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFV EQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRD KPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTK EVLDATLIHQSITGLYETRIDLSQLGGDEGADKRTADGSE FESPKKKRKV	
5′ UTR	mRNA	AGGAAAu'AAGAGAGAAAAGAAGAGu'AAGAAGAAAu' Au'AAGAGCCACC	2138
TadA	mRNA	Au'Gu'CCGAAGu'CGAAu'u'CAGCCACGAGu'ACu'GGAu' GAGACAu'GCACu'GACCCu'CGCGAAGAGAGCu'CGGGA u'GAGAGGGAAGu'GCCAGu'GGGAGCu'Gu'GCu'GGu'GC u'CAACAACAGGGu'CAu'u'GGGGAAGGAu'GGAACAGG GCCAu'u'GGACu'u'CACGAu'CCGACGGCu'CAu'GCGGA AAu'CAu'GGCACu'GAGACAGGGCGGu'Cu'u'Gu'GAu'GC AGAACu'Au'CGCCu'GAu'u'GAu'GCCACu'Cu'Gu'ACGu'G ACCu'u'CGAACCu'u'GCGu'GAu'Gu'GCGCCGGu'GCCAu' GAu'CCACu'CACGCAu'CGGu'CGGGu'GGu'Gu'u'CGGCG u'GCGAAACGCu'AAGACu'GGAGCCGCCGGAu'CACu'GA u'GGAu'Gu'GCu'CCAu'CACCCCGGGAu'GAACCACAGG Gu'GGAGAu'CACCGAAGGCAu'u'Cu'GGCCGACGAAu'G CGCAGCCCu'Gu'u'Gu'Gu'CGCu'u'Cu'u'CCGGAu'GCCu'C GAAGAGu'Gu'u'CAACGCGCAGAAGAAGGCGCAGAGCu 'CCACu'GAC	2162
	Protein	MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNN RVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLID ATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHHPGMNHRVEITEGILADECAALLCRFFRMPRR VFNAQKKAQSSTD	2140
N-terminal linker	mRNA	u'Cu'GGAGGu'u'CCu'CGGGCGGAu'CGu'CAGGGu'CCGA AACu'CCu'GGAACCu'CGGAGu'CAGCAACu'CCGGAAu'C Cu'CAGGAGGCu'CCAGCGGAGGCu'CC	2163
	Protein	SGGSSGGSSGSETPGTSESATPESSGGSSGGS	20
Cas9 nickase	mRNA	GAu'AAGAAGu'ACAGCAu'CGGGCu'u'GCCAu'CGGAAC CAACu'CGGu'CGGCu'GGGCu'Gu'GAu'CACu'GACGAGu' ACAAGGu'CCCu'u'CCAAAAAGu'u'CAAAGu'GCu'CGGC AACACu'GAu'CGGCACu'CGAu'u'AAGAAGAACCu'GAu' CGGu'GCACu'GCu'Gu'u'CGACu'Cu'GGGGAGACu'GCGG	2164

		AAGCAACu'CGCCu'GAAGAGGACu'GCGCGCAGACGGu' ACACCAGGCGCAAGAACCGGAu'Cu'GCu'ACu'u'GCAAG AGAu'u'u'u'CAGCAACGAGAu'GGCCAAGGu'CGACGACu 'CGu'u'u'u'u'CCACCGGCu'CGAGGAAAGCu'u'CCu'GGu' GGAGGAAGAu'AAGAAGCAu'GAGCGCCAu'CCCAu'Cu'u 'CGGAAACAu'CGu'GGACGAAGu'GGCCu'ACCAu'GAGA AGu'ACCCGACCAu'Cu'ACCAu'Cu'GCGCAAGAAGCu'CG u'GGACAGCACu'GACAAAGCCGACu'u'GCGCCu'GAu'Cu 'ACCu'u'GCCCu'GGCCCACAu'GAu'CAAGu'u'CAGAGGG CACu'u'CCu'GAu'CGAGGGCGACCu'GAACCCGGACAAu' u'CCGACGu'GGAu'AAGCu'Gu'u'CAu'CCAACu'GGu'ACA AACu'u'ACAACCAGCu'Gu'u'u'GAGGAGAACCCGAu'CA ACGCu'u'CCGGCGu'GGACGCGAAGGCCAu'u'Cu'GAGC GCACGGCu'Gu'CCAAGu'CCCGGAGAu'u'AGAAAACCu' GAu'u'GCCCAACu'GCCu'GGAGAAAAGAAGAAu'GGCCu 'Gu'u'CGGAAACCu'GAu'CGCCCu'Cu'CGCu'CGGu'Cu'GA CCCCCAACu'u'u'AAGAGCAACu'u'CGACCu'GGCu'GAG GACGCGAAGCu'CCAGCu'Gu'CCAAGGACACCu'ACGAu' GAu'GACCu'CGAu'AAu'Cu'CCu'CGCCCAAAu'CGGCGA CCAAu'Au'GCGGACCu'Gu'u'u'Cu'GGCCGCCAAGAAu'C u'Gu'CCGACGCCAu'CCu'CCu'GAGCGAu'Au'CCu'GCGC Gu'GAACACu'GAAAu'CACCAAGGCCCCACu'GAGCGCu' AGCAu'GAu'u'AAGCGCu'ACGACGAACACCAu'CAGGAC Cu'CACCCu'GCu'GAAGGCu'Cu'u'Gu'GCGGCAGCAGCu' CCCAGAGAAGu'ACAAGGAGAu'Cu'u'Cu'u'CGACCAAu' CGAAGAAu'GGu'u'ACGCCGGCu'ACAu'u'GACGGAGGA GCGu'CACAGGAGGAGu'u'Cu'ACAAGu'u'CAu'u'AAGCC AAu'CCu'GGAGAAGAu'GGACGGAACCGAGGAACu'GCu 'CGu'GAAGCu'GAACAGAGAAGAu'Cu'ACu'GCGCAAGC AGCGCACu'u'u'CGACAACGGAu'CGAu'CCCACAu'CAGA u'u'CACCu'GGGAGAGCu'GCACGCGAu'CCu'GCGGAGA CAGGAAGAu'u'u'Cu'ACCCGu'u'CCu'GAAGGACAACCG CGAGAAGAu'CGAAAAGAu'CCu'GACCu'u'CCGCAu'u'C CGu'ACu'ACGu'GGGACCCCu'CGCAAGAGGAAACu'CGC GGu'u'CGCCu'GGAu'GACCAGGAAGu'CGGAAGAAACC Au'u'ACu'CCGu'GGAACu'u'CGAGGAAGu'GGu'GGACAA GGGCGCCu'CAGCGCAAu'CCu'u'CAu'CGAACGGAu'GAC CAACu'u'CGACAAGAACu'u'GCCu'AACGAGAAAGu'GCu 'u'CCu'AAGCAu'u'CCCu'CCu'u'u'ACGAAu'ACu'u'CACu' Gu'Gu'ACAACGAGCu'u'ACCAAGGu'CAAAu'Au'Gu'GAC CGAGGGCAu'GCGCAAGCCGGCCu'u'u'Cu'u'u'CCGGGG AGCAAAAGAAGGCCAu'CGu'GGACCu'Gu'u'Gu'u'CAAG ACCAACCGGAAGGu'CACCGu'CAAGCAGCu'GAAGGAG GACu'ACu'u'u'AAGAAAAu'CGAGu'GCu'u'u'GAu'u'CCGu 'CGAAAu'Cu'CCGGAGu'GGAAGAu'AGAu'u'CAACGCCA GCCu'AGGCACCu'ACCACGAu'Cu'GCu'CAAGAu'CAu'u' AAGGAu'AAGGAu'u'u'CCu'GGACAACGAGGAGAACGA GGAu'Au'CCu'GGAGGACAu'u'Gu'GCu'GACCCu'GACAC u'Gu'u'CGAAGAu'CGCGAAAu'GAu'CGAGGAGCGGCu'G AAAACAu'ACGCCCACCu'Gu'u'CGACGACAAGGu'CAu' GAAGCAGCu'CAAGCGGAGGAGGu'ACACGGGAu'GGGG AAGGCu'Gu'CCCGGAAGCu'GAu'CAACGGGAu'u'AGAG ACAAGCAGu'CCGGAAAGACCAu'u'Cu'GGACu'u'CCu'C AAGu'CGGACGGCu'u'CGCGAAC**CGG**AACu'u'CAu'GCA ACu'GAu'CCACGACGACu'CCCu'GACGu'u'CAAGGAGG	

		ACAu'u'CAGAAGGCCCAAGu'Gu'CCGGACAGGGAGAu'u 'CACu'GCACGAACACAu'CGCCAAu'Cu'GGCu'GGAAGu' CCGGCCAu'CAAGAAAGGCAu'CCu'GCAAACCGu'GAAG Gu'CGu'GGACGAGCu'CGu'GAAGGu'CAu'GGGCCGCCA CAAGCCu'GAGAACAu'CGu'GAu'CGAAAu'GGCCCGGG AGAACCAGACCACGCAGAAAGGGCAGAAGAACAGCC GCGAGAGGAu'GAAGAGAAu'CGAGGAAGGAAu'CAAG GAACu'u'GGAAGCCAGAu'CCu'GAAGGAACACCCCGu'G GAAAACACu'CAGCu'u'CAAAACGAAAAGCu'Gu'ACCu' Cu'ACu'Au'Cu'GCAAAACGGACGGGAu'Au'Gu'ACGu'GG ACCAGGAAu'u'GGACAu'CAAu'CGCCu'Gu'CCGACu'AC GAu'Gu'GGAu'CACAu'u'Gu'GCCGCAGAGCu'u'u'Cu'GAA GGACGAu'u'CAAu'u'GAu'AACAAGGu'GCu'CACCCGGu' CCGACAAGAACCGGGGCAAAAGCGAu'AACGu'GCCGA GCGAAGAGGu'GGu'CAAGAAGAu'GAAGAAu'u'ACu'GG CGGCAGCu'Cu'u'GAACGCCAAACu'GAu'CACCCAGCGG AAGu'u'CGACAACCu'GACCAAGGCCGAAAGGGGCGGu' Cu'u'u'CCGAACu'GGACAAGGCCGGGu'u'u'Au'CAAGCG CCAACu'GGu'GGAAACCCGGCAGAu'CACu'AAGCACGu' GGCCCAGAu'ACu'GGACu'Cu'AGGAu'GAAu'ACCAAAu' ACGACGAGAACGACAAGCu'GAu'u'CGCGAAGu'CAAAG u'CAu'u'ACCCu'GAAAu'CCAAGCu'CGu'Gu'CCGACu'u'C CGGAAGGACu'u'CCAGu'u'Cu'Au'AAGGu'u'CGCGAAAu' CAACAACu'ACCACCACGCCCAu'GACGCGu'ACCu'GAA CGCCGu'CGu'GGGu'ACCGCCCu'GAu'u'AAGAAGu'ACC Cu'AAGCu'CGAGu'CCGAGu'u'CGu'Cu'ACGGAGACu'AC AAAGu'Gu'ACGACGu'GCGGAAGAu'GAu'CGCCAAGu'C CGAGCAGGAAAu'AGGGAAGGCCACCGCCAAGu'ACu'u' Cu'u'Cu'ACu'CCAACAu'CAu'GAACu'u'Cu'u'CAAGACCG AGAu'CACCCu'u'GCCAACGGGGAGAu'u'CGGAAGCGCC CGu'u'GAu'u'GAGACu'AACGGAGAAACCGGCGAAAu'u' Gu'Gu'GGGAu'AAGGGCAGAGACu'u'u'GCGACu'Gu'GCG CAAAGu'Cu'u'Gu'CAAu'GCCCCAAGu'CAACAu'u'Gu'CA AAAAGACCGAAGu'GCAAACCGGCGGCu'u'CAGCAAGG AAAGCAu'CCu'GCCCAAGCGCAAu'u'CCGACAAGCu'CA u'CGCCCGGAAGAAGGACu'GGGACCCCAAGAAAu'Au'G GAGGCu'u'C**GAC**u'CCCCCACu'Gu'GGCCu'ACu'CCGu'C Cu'GGu'GGu'CGCGAAAGu'GGAAAAGGGAAAGu'CGAA GAAGCu'CAAGu'CCGu'GAAGGAGCu'CCu'GGGGAu'CA CCAu'u'Au'GGAACGGu'CCAGCu'u'CGAGAAGAACCCG Au'u'GACu'u'CCu'GGAAGCCAAGGGu'u'ACAAGGAGGu' CAAGAAGGAu'Cu'GAu'u'Au'u'AAGCu'GCCGAAGu'Au'u 'CCCu'Cu'u'CGAACu'CGAAAAu'GGCCGCAAGCGAAu'G u'u'GGCAu'CGGCGGGAGAGCu'CCAGAAGGGAAACGA ACu'GGCCu'u'GCCu'u'CCAAAu'ACGu'GAACu'u'u'Cu'Gu 'ACCu'GGCCAGCCACu'ACGAGAAGCu'GAAGGGu'u'CC CCCGAGGACAACGAGCAGAAGCAGCu'Gu'u'CGu'GGAG CAGCACAAGCACu'ACCu'GGAu'GAGAu'CAu'CGAACAG Au'Cu'CGGAAu'u'Cu'CCAAGAGAGu'GAu'CCu'GGCCGA CGCGAACCu'GGAu'AAGGu'CCu'Gu'CCGCGu'ACAACA AGCACCGGGACAAGCCCAu'CCGGGAGCAGGCu'GAGA ACAu'u'Au'CCACCu'Gu'u'CACCCu'CACu'AACCu'u'GGA GCGCCu'GCCGCAu'u'CAAGu'ACu'u'CGACACAACCAu'C GACAGAAAGCGCu'ACACu'u'CCACCAAGGAGGu'GCu'G GACGCCACCCu'GAu'CCACCAGu'CCAu'CACu'GGGCu'C	

		u'Au'GAGACu'CGCAu'u'GACCu'Gu'CCCAGu'u'GGGAGGGGAC	
	Protein	DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD	40
C-terminal linker	mRNA	GAAGGAGCCGAu'	2166
	Protein	EGAD	2144
Nuclear localization sequence (NLS)	mRNA	AAGAGAACCGCCGAu'GGGu'CCGAAu'u'CGAGu'CGCCCAAGAAGAAGCGGAAAGu'Gu'AA	2167
	Protein	KRTADGSEFESPKKKRKV	2146
3′ UTR	mRNA	GCGGCCGCu'u'AAu'u'AAGCu'GCCu'u'Cu'GCGGGGCu'u'GCCu'u'Cu'GGCCAu'GCCCu'u'Cu'u'Cu'Cu'CCCu'u'GCACCu'Gu'ACCu'Cu'u'GGu'Cu'u'u'GAAu'AAAGCCu'GAGu'AGGAAGu'Cu'AGA	2147
MA020 mRNA and protein sequences			
Region	**Sequence**		**SEQ ID NO**
Full sequenc	mRNA	AGGAAAu'AAGAGAGAAAAGAAGAGu'AAGAAGAAAu'Au'AAGAGCCACCAu'Gu'CAGAAGu'AGAAu'u'Cu'CCCA	2168

e		CGAGu'Au'u'GGAu'GAGACACGCu'Cu'CACACu'CGCCA AGCGAGCu'AGGGAu'GAAAGGGAAGu'ACCAGu'GGGG GCCGu'ACu'GGu'ACu'CAACAAu'AGGGu'CAu'AGGAGA GGGGu'GGAAu'CGAGCAAu'u'GGGCu'GCAu'GAu'CCCA Cu'GCACACGCGGAGAu'u'Au'GGCu'Cu'u'AGACAAGGG GGCCu'GGu'GAu'GCAGAAu'u'ACCGCCu'CAu'u'GACGC AACCCu'u'u'ACGu'GACAu'u'u'GAGCCAu'GCGu'u'Au'Gu 'GCGCGGGCGCAAu'GAu'ACACu'Cu'AGGAu'AGGCCGC Gu'AGu'u'u'u'CGGAGu'CCGAAAu'GCGAAGACu'GGGGC GGCCGGAu'Cu'Cu'GAu'GGACGu'GCu'CCACCAu'CCAG GCAu'GAACCACCGAGu'GGAGAu'AACGGAAGGCAu'Cu 'u'GGCGGACGAAu'Gu'GCu'GCGCu'CCu'u'u'GCAGGu'u' Cu'u'CCGGAu'GCCAAGACGGGu'Au'u'CAACGCGCAGA AGAAGGCACAGu'CAAGCACu'GAu'AGu'GGCGGCAGu'u 'CCGGAGGAu'Cu'AGu'GGGu'CCGAAACGCCAGGGACCu 'CCGAAu'CCGCAACu'CCCGAAAGu'u'CAGGAGGCu'CCu 'CCGGCGGCAGCGACAAGAAGu'ACu'CCAu'u'GGu'Cu'G GCGAu'CGGu'ACGAAu'AGu'Gu'u'GGGu'GGGCu'Gu'AAu 'AACu'GACGAGu'Au'AAGGu'GCCCAGu'AAGAAGu'u'C AAAGu'CCu'GGGGAACACAGACCGACAu'AGCAu'CAAG AAGAACCu'GAu'AGGu'GCGCu'Cu'u'Gu'u'u'GACu'CCGG AGAGACAGCAGAAGCCACACGACu'u'AAACGAACCGC u'CGAAGGCGGu'ACACACGGCGAAAGAAu'AGGAu'u'u' Gu'u'ACCu'GCAGGAGAu'Au'u'u'AGCAACGAGAu'GGCA AAGGu'u'GACGAu'u'CCu'u'Cu'u'CCACCGGCu'u'GAAGA Gu'Cu'u'u'CCu'GGu'GGAGGAGGACAAGAAACAu'GAAC GCCACCCCAu'Au'u'u'GGCAAu'Au'AGu'GGACGAAGu'A GCAu'ACCAu'GAGAAAu'ACCCu'ACCAu'Au'Au'CAu'Cu' u'CGAAAGAAGCu'u'Gu'AGAu'u'CAACGGAu'AAAGCAG Au'Cu'CAGGu'u'GAu'Au'Au'Cu'GGCACu'GGCACACAu'G Au'CAAGu'u'u'CGAGGu'CACu'u'u'Cu'u'Au'CGAGGGCG Au'Cu'u'AAu'CCGGACAACu'CCGACGu'GGAu'AAGCu'G u'u'CAu'CCAGCu'CGu'CCAAACGu'Au'AACCAACu'Cu'u' CGAGGAGAAu'CCGAu'CAAu'GCCu'Cu'GGAGu'GGACG CGAAAGCGAu'ACu'Cu'CAGCAAGGCu'u'AGu'AAGu'Cu' CGCCGGCu'u'GAGAACCu'CAu'CGCACAGu'u'GCCAGGA GAGAAGAAGAAu'GGCu'u'Gu'u'u'GGu'AACCu'u'Au'u'G CACu'CAGu'Cu'CGGCCu'u'ACACCu'AACu'u'CAAGu'CC AACu'u'CGAu'Cu'u'GCCGAGGACGCCAAGCu'CCAGCu'u 'AGCAAGGAu'ACCu'ACGAu'GACGAu'Cu'u'GAu'AACCu 'GCu'GGCCCAGAu'AGGCGACCAGu'ACGCGGACCu'u'u' u'Cu'u'GGCAGCAAAGAAu'u'u'Gu'CCGACGCCAu'CCu'C u'u'GAGCGACAu'CCu'CCGGGu'AAACACu'GAAAu'u'AC u'AAGGCGCCACu'u'u'CCGCCAGCAu'GAu'CAAGAGAu' ACGAu'GAGCAu'CAu'CAGGAu'Cu'GACCCu'GCu'u'AAA GCGCu'u'Gu'ACGCCAGCAAu'u'GCCu'GAGAAGu'Au'AA AGAAAu'Cu'u'u'u'u'CGACCAAAGCAAGAACGGu'u'Au'G Cu'GGCu'ACAu'AGACGGCGGGGCu'u'Cu'CAGGAGGAAu 'u'Cu'ACAAGu'u'u'Au'CAAGCCGAu'u'Cu'CGAGAAGAu' GGAu'GGAACAGAGGAGCu'Gu'u'GGu'GAAACu'GAACC GGGAGGACCu'GCu'CAGAAAGCAGCGGACCu'u'CGAu' AAu'GGGAGCAu'CCCGCACCAGAu'u'CAu'u'u'GGGu'GA GCu'GCAu'GCAAu'Cu'u'GCGCAGGCAAGAAGAu'u'u'u'u' ACCCCu'u'CCu'CAAGGACAAu'AGAGAGAAGAu'u'GAG AAGAu'CCu'u'ACGu'u'u'AGGAu'u'CCGu'Au'u'ACGu'AG	

		GCCCGCu'CGCACGCGGu'AAu'u'Cu'CGCu'u'u'GCGu'GG Au'GACACGAAAGAGCGAGGAAACCAu'AACACCCu'GG AACu'u'u'GAAGAGGu'CGu'CGAu'AAGGGu'GCCAGCGC ACAAu'CAu'u'u'Au'CGAACGAAu'GACu'AAu'u'u'CGAu' AAGAAu'Cu'CCCAAAu'GAGAAGGu'Gu'u'GCCAAAGCA u'AGCCu'u'u'u'Gu'Au'GAGu'Au'u'u'u'ACu'Gu'Au'ACAAC GAGCu'CACCAAGGu'GAAAu'Au'Gu'CACu'GAAGGGAu' GCGCAAGCCGGCCu'u'u'u'u'GAGCGGAGAACAGAAGA AAGCu'Au'u'Gu'AGACu'u'GCu'u'u'u'u'AAGACGAAu'AG AAAGGu'u'ACGGu'CAAGCAGCu'CAAAGAAGACu'ACu' u'u'AAGAAGAu'AGAAu'GCu'u'u'GACu'Cu'Gu'CGAGAu' u'AGu'GGGGu'AGAGGAu'CGAu'u'CAAu'GCAAGCCu'GG GGACAu'ACCACGAu'Cu'Cu'u'GAAGAu'u'Au'CAAGGAu' AAGGAu'u'u'u'Cu'GGACAACGAAGAGAAu'GAAGACAu' Cu'u'GGAAGAu'Au'u'Gu'u'u'u'GACu'u'u'GACu'Cu'u'u'u' CGAAGAu'CGCGAGAu'GAu'AGAGGAAAGGu'u'GAAGA Cu'u'Au'GCCCAu'Cu'u'u'u'u'GAu'GACAAGGu'u'Au'GAA ACAACu'GAAGAGACGGCGAu'Au'ACu'GGu'u'GGGGGA GACu'Cu'CACGAAAGCu'CAu'u'AACGGCAu'CCGGGAC AAACAGAGu'GGu'AAGACGAu'u'u'u'GGACu'u'CCu'u'A AGAGCGACGGGu'u'u'GCGAAu'**AGG**AACu'u'CAu'GCAG u'u'GAu'CCAu'GAu'GAu'u'Cu'u'u'GACCu'u'u'AAGGAGG Au'Au'ACAGAAGGCACAAGu'CAGu'GGACAAGGAGAu' AGu'Cu'GCAu'GAGCACAu'u'GCCAACCu'u'GCGGGCu'C CCCAGCCAu'CAAGAAGGGu'Au'CCu'GCAAACCGu'CAA AGu'AGu'AGAu'GAACu'u'Gu'GAAAGu'CAu'GGGACGAC Au'AAGCCu'GAGAAu'Au'u'Gu'GAu'CGAAAu'GGCu'AG GGAGAAu'CAGACGACu'CAGAAGGGu'CAGAAGAAu'u' CCAGGGAACGGAu'GAAGAGAAu'AGAGGAGGGAAu'u' AAGGAACu'CGGGu'CACAAAu'Au'u'GAAGGAGCAu'CC u'Gu'AGAGAACACACAGCu'u'CAGAACGAGAAGCu'Gu' ACCu'Gu'Au'u'Au'Cu'CCAGAAu'GGCAGAGACAu'Gu'Au' Gu'CGACCAAGAACu'GGAu'Au'CAAu'CGCu'u'Gu'CCGA u'u'Au'GAu'Gu'AGAu'CACAu'AGu'CCCACAAu'CCu'u'Cu 'u'GAAAGACGAu'AGu'Au'u'GACAAu'AAGGu'ACu'u'AC GAGAu'CCGACAAGAACCGCGGAAAGu'CCGAu'AACGu' ACCGAGu'GAAGAAGu'GGu'AAAGAAGAu'GAAGAAu'u' Au'u'GGAGGCAGCu'CCu'CAACGCCAAACu'u'Au'u'ACu' CAGAGAAAGu'u'CGAu'AACCu'u'ACGAAAGCu'GAGCG AGGCGGGu'u'Gu'Cu'GAGCu'CGAu'AAAGCu'GGAu'u'CA u'AAAGCGGCAGCu'u'Gu'GGAAACu'AGACAGAu'CACA AAGCACGu'u'GCACAGAu'CCu'u'GAu'u'CCAGAAu'GAA CACu'AAAu'Au'GAu'GAGAACGAu'AAGCu'GAu'ACGCG AGGu'GAAAGu'AAu'AACACu'u'AAGu'CAAAGCu'GGu'A AGCGAu'u'u'u'CGCAAAGACu'u'CCAAu'u'u'u'Au'AAGGu 'CAGAGAGAu'AAACAAu'u'Au'CAu'CACGCGCAu'GACG Cu'u'ACCu'GAACGCAGu'u'Gu'GGGGACAGCACu'u'Au'A AAGAAAu'ACCCAAAGu'u'GGAAAGu'GAAu'u'u'Gu'Cu' Au'GGAGACu'Au'AAGGu'Au'ACGAu'Gu'ACGGAAGAu'G Au'u'GCGAAGu'CAGAGCAAGAAAu'CGGu'AAGGCAACu 'GCu'AAGu'ACu'u'Cu'u'u'u'ACu'CAAAu'Au'u'Au'GAACu 'u'u'u'u'CAAGACGGAAAu'CACu'Cu'GGCu'AACGGAGA AAu'u'CGGAAGAGGCCCu'u'GAu'AGAGACGAAu'GGGG AGACu'GGu'GAGAu'CGu'u'u'GGGACAAAGGGCGAGAu' u'u'CGCAACGGu'CCGAAAGGu'GCu'Cu'CAAu'GCCACA	

		AGu'CAAu'Au'AGu'CAAGAAGACAGAGGu'ACAAACAG GAGGu'u'u'Cu'CCAAGGAAu'CAAu'ACu'CCCAAAGCGG AAu'u'CCGACAAACu'GAu'AGCu'AGAAAGAAGGACu'G GGAu'CCGAAGAAGu'Au'GGAGGGu'u'u'**GAu'**u'CCCCAA CAGu'AGCGu'Au'u'CAGu'ACu'GGu'GGu'AGCu'AAGGu' GGAGAAAGGCAAGu'Cu'AAGAAGCu'u'AAGu'Cu'Gu'CA AAGAGCu'GCu'CGGCAu'CACCAu'u'Au'GGAGCGCAGu' AGCu'u'CGAGAAGAACCCu'Au'AGAu'u'u'u'Cu'GGAAGC CAAAGGAu'Au'AAGGAGGu'u'AAGAAGGAu'Cu'CAu'CA u'CAAACu'CCCGAAGu'Au'AGu'Cu'u'u'u'u'GAGu'u'GGA GAACGGACGAAAGCGCAu'GCu'u'GCGu'Cu'GCGGGu'G AAu'u'GCAGAAAGGAAAu'GAGCu'CGCCu'u'GCCGu'CA AAGu'Au'Gu'CAAu'u'u'Cu'u'Gu'ACCu'u'GCGAGu'CACu' Au'GAGAAGCu'GAAAGGAAGCCCAGAAGAu'AAu'GAA CAGAAGCAAu'u'Gu'u'CGu'u'GAACAACAu'AAGCAu'u'A u'u'u'GGACGAGAu'CAu'CGAGCAAAu'CAGCGAGu'u'CA GCAAGCGCGu'CAu'ACu'u'GCGGACGCCAAu'Cu'GGAC AAAGu'GCu'Gu'CCGCCu'Au'AACAAACAu'AGGGACAA ACCu'Au'ACGAGAGCAGGCAGAGAAu'Au'AAu'ACAu'C u'Cu'u'CACACu'u'ACu'AAu'Cu'u'GGAGCu'CCAGCCGCA u'u'CAAGu'Au'u'u'u'GACACGACu'Au'AGAu'CGCAAGCG Au'Au'ACCu'Cu'ACGAAAGAAGu'u'Cu'GGACGCu'ACu'C u'u'Au'ACAu'CAAu'CCAu'u'ACGGGCCu'Gu'ACGAGACG AGGAu'u'GACu'u'Gu'CACAGCu'u'GGu'GGu'GACGAGGG u'GCAGACAAGCGAACAGCGGACGGGu'CAGAAu'u'CGA AAGCCCCAAGAAGAAGCGGAAAGu'u'u'AAGCGGCCGC u'u'AAu'u'AAGCu'GCCu'u'Cu'GCGGGGCu'u'GCCu'u'Cu' GGCCAu'GCCCu'u'Cu'u'Cu'Cu'CCCu'u'GCACCu'Gu'ACC u'Cu'u'GGu'Cu'u'u'GAAu'AAAGCCu'GAGu'AGGAAGu'Cu 'AGA	
	Protein	MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNN RVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLID ATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHHPGMNHRVEITEGILADECAALLCRFFRMPRR VFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGG SSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVL GNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRR KNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHER HPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIY LALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKN GLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDL DNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKA PLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQS KNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLN REDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITP WNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLL YEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLF KTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD KQSGKTILDFLKSDGFAN**R**NFMQLIHDDSLTFKEDIQKAQ VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM GRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKEL	2137

		GSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDI NRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDN VPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAH DAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIA KSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT GGFSKESILPKRNSDKLIARKKDWDPKKYGGF**D**SPTVAY SVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDF LEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGEL QKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFV EQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRD KPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTK EVLDATLIHQSITGLYETRIDLSQLGGDEGADKRTADGSE FESPKKKRKV	
5′ UTR	mRNA	AGGAAAu'AAGAGAGAAAAGAAGAGu'AAGAAGAAAu' Au'AAGAGCCACC	2138
TadA	mRNA	Au'Gu'CAGAAGu'AGAAu'u'Cu'CCCACGAGu'Au'u'GGAu 'GAGACACGCu'Cu'CACACu'CGCCAAGCGAGCu'AGGG Au'GAAAGGGAAGu'ACCAGu'GGGGGCCGu'ACu'GGu'A Cu'CAACAAu'AGGGu'CAu'AGGAGAGGGGu'GGAAu'CG AGCAAu'u'GGGCu'GCAu'GAu'CCCACu'GCACACGCGG AGAu'u'Au'GGCu'Cu'u'AGACAAGGGGGCCu'GGu'GAu'G CAGAAu'u'ACCGCCu'CAu'u'GACGCAACCCu'u'u'ACGu' GACAu'u'u'GAGCCAu'GCGu'u'Au'Gu'GCGCGGGCGCAA u'GAu'ACACu'Cu'AGGAu'AGGCCGCGu'AGu'u'u'u'CGGA Gu'CCGAAAu'GCGAAGACu'GGGGCGGCCGGAu'Cu'Cu' GAu'GGACGu'GCu'CCACCAu'CCAGGCAu'GAACCACCG AGu'GGAGAu'AACGGAAGGCAu'Cu'u'GGCGGACGAAu' Gu'GCu'GCGCu'CCu'u'u'GCAGGu'u'Cu'u'CCGGAu'GCCA AGACGGGu'Au'u'CAACGCGCAGAAGAAGGCACAGu'CA AGCACu'GAu'	2169
	Protein	MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNN RVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLID ATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHHPGMNHRVEITEGILADECAALLCRFFRMPRR VFNAQKKAQSSTD	2140
N-terminal linker	mRNA	AGu'GGCGGCAGu'u'CCGGAGGAu'Cu'AGu'GGGu'CCGA AACGCCAGGGACCu'CCGAAu'CCGCAACu'CCCGAAAG u'u'CAGGAGGCu'CCu'CCGGCGGCAGC	2170
	Protein	SGGSSGGSSGSETPGTSESATPESSGGSSGGS	20
Cas9 nickase	mRNA	GACAAGAAGu'ACu'CCAu'u'GGu'Cu'GGCGAu'CGGu'AC GAAu'AGu'Gu'u'GGGu'GGGCu'Gu'AAu'AACu'GACGAGu 'Au'AAGGu'GCCCAGu'AAGAAGu'u'CAAAGu'CCu'GGG GAACACAGACCGACAu'AGCAu'CAAGAAGAACCu'GAu' AGGu'GCGCu'Cu'u'Gu'u'u'GACu'CCGGAGAGACAGCAG AAGCCACACGACu'u'AAACGAACCGCu'CGAAGGCGGu' ACACACGGCGAAAGAAu'AGGAu'u'u'Gu'u'ACCu'GCAG GAGAu'Au'u'u'AGCAACGAGAu'GGCAAAGGu'u'GACGA u'u'CCu'u'Cu'u'CCACCGGCu'u'GAAGAGu'Cu'u'u'CCu'G Gu'GGAGGAGGACAAGAAACAu'GAACGCCACCCCAu'A u'u'u'GGCAAu'Au'AGu'GGACGAAGu'AGCAu'ACCAu'GA GAAAu'ACCCu'ACCAu'Au'Au'CAu'Cu'u'CGAAAGAAGC	2171

		u'u'Gu'AGAu'u'CAACGGAu'AAAGCAGAu'Cu'CAGGu'u' GAu'Au'Au'Cu'GGCACu'GGCACACAu'GAu'CAAGu'u'u'C GAGGu'CACu'u'u'Cu'u'Au'CGAGGGCGAu'Cu'u'AAu'CC GGACAACu'CCGACGu'GGAu'AAGCu'Gu'u'CAu'CCAGCu 'CGu'CCAAACGu'Au'AACCAACu'Cu'u'CGAGGAGAAu'C CGAu'CAAu'GCCu'Cu'GGAGu'GGACGCGAAAGCGAu'A Cu'Cu'CAGCAAGGCu'u'AGu'AAGu'Cu'CGCCGGCu'u'GA GAACCu'CAu'CGCACAGu'u'GCCAGGAGAGAAGAAGAA u'GGCu'u'Gu'u'u'GGu'AACCu'u'Au'u'GCACu'CAGu'Cu'C GGCCu'u'ACACCu'AACu'u'CAAGu'CCAACu'u'CGAu'Cu' u'GCCGAGGACGCCAAGCu'CCAGCu'u'AGCAAGGAu'AC Cu'ACGAu'GACGAu'Cu'u'GAu'AACCu'GCu'GGCCCAGA u'AGGCGACCAGu'ACGCGGACCu'u'u'u'Cu'u'GGCAGCA AAGAAu'u'u'Gu'CCGACGCCAu'CCu'Cu'u'GAGCGACAu' CCu'CCGGGu'AAACACu'GAAAu'u'ACu'AAGGCGCCACu 'u'u'CCGCCAGCAu'GAu'CAAGAGAu'ACGAu'GAGCAu'C Au'CAGGAu'Cu'GACCCu'GCu'u'AAAGCGCu'u'Gu'ACGC CAGCAAu'u'GCCu'GAGAAGu'Au'AAAGAAAu'Cu'u'u'u'u 'CGACCAAAGCAAGAACGGu'u'Au'GCu'GGCu'ACAu'AG ACGGCGGGGCu'u'Cu'CAGGAGGAAu'u'Cu'ACAAGu'u'u' Au'CAAGCCGAu'u'Cu'CGAGAAGAu'GGAu'GGAACAGA GGAGCu'Gu'u'GGu'GAAACu'GAACCGGGAGGACCu'GC u'CAGAAAGCAGCGGACCu'u'CGAu'AAu'GGGAGCAu'C CCGCACCAGAu'u'CAu'u'u'GGGu'GAGCu'GCAu'GCAAu' Cu'u'GCGCAGGCAAGAAGAu'u'u'u'u'ACCCCu'u'CCu'CA AGGACAAu'AGAGAGAAGAu'u'GAGAAGAu'CCu'u'ACG u'u'u'AGGAu'u'CCGu'Au'u'ACGu'AGGCCCGCu'CGCACG CGGu'AAu'u'Cu'CGCu'u'u'GCGu'GGAu'GACACGAAAGA GCGAGGAAACCAu'AACACCCu'GGAACu'u'u'GAAGAG Gu'CGu'CGAu'AAGGGu'GCCAGCGCACAAu'CAu'u'u'Au' CGAACGAAu'GACu'AAu'u'u'CGAu'AAGAAu'Cu'CCCAA Au'GAGAAGGu'Gu'u'GCCAAAGCAu'AGCCu'u'u'u'Gu'Au 'GAGu'Au'u'u'u'ACu'Gu'Au'ACAACGAGCu'CACCAAGGu 'GAAAu'Au'Gu'CACu'GAAGGGAu'GCGCAAGCCGGCCu' u'u'u'u'GAGCGGAGAACAGAAGAAAGCu'Au'u'Gu'AGAC u'u'GCu'u'u'u'u'AAGACGAAu'AGAAAGGu'u'ACGGu'CA AGCAGCu'CAAAGAAGACu'ACu'u'u'AAGAAGAu'AGAA u'GCu'u'u'GACu'Cu'Gu'CGAGAu'u'AGu'GGGGu'AGAGG Au'CGAu'u'CAAu'GCAAGCCu'GGGGACAu'ACCACGAu' Cu'Cu'u'GAAGAu'u'Au'CAAGGAu'AAGGAu'u'u'u'Cu'GG ACAACGAAGAGAAu'GAAGACAu'Cu'u'GGAAGAu'Au'u' Gu'u'u'u'GACu'u'u'GACu'Cu'u'u'u'CGAAGAu'CGCGAGA u'GAu'AGAGGAAAGGu'u'GAAGACu'u'Au'GCCCAu'Cu'u 'u'u'u'GAu'GACAAGGu'u'Au'GAAACAACu'GAAGAGAC GGCGAu'Au'ACu'GGu'u'GGGGGAGACu'Cu'CACGAAAG Cu'CAu'u'AACGGCAu'CCGGGACAAACAGAGu'GGu'AA GACGAu'u'u'u'GGACu'u'CCu'u'AAGAGCGACGGGu'u'u' GCGAAu'**AGG**AACu'u'CAu'GCAGu'u'GAu'CCAu'GAu'G Au'u'Cu'u'u'GACCu'u'u'AAGGAGGAu'Au'ACAGAAGGC ACAAGu'CAGu'GGACAAGGAGAu'AGu'Cu'GCAu'GAGC ACAu'u'GCCAACCu'u'GCGGGCu'CCCCAGCCAu'CAAGA AGGGu'Au'CCu'GCAAACCGu'CAAAGu'AGu'AGAu'GAA Cu'u'Gu'GAAAGu'CAu'GGGACGACAu'AAGCCu'GAGAA u'Au'u'Gu'GAu'CGAAAu'GGCu'AGGGAGAAu'CAGACGA Cu'CAGAAGGGu'CAGAAGAAu'u'CCAGGGAACGGAu'G	

		AAGAGAAu'AGAGGAGGGAAu'u'AAGGAACu'CGGGu'C ACAAAu'Au'u'GAAGGAGCAu'CCu'Gu'AGAGAACACAC AGCu'u'CAGAACGAGAAGCu'Gu'ACCu'Gu'Au'u'Au'Cu'C CAGAAu'GGCAGAGACAu'Gu'Au'Gu'CGACCAAGAACu' GGAu'Au'CAAu'CGCu'u'Gu'CCGAu'u'Au'GAu'Gu'AGAu' CACAu'AGu'CCCACAAu'CCu'u'Cu'u'GAAAGACGAu'AG u'Au'u'GACAAu'AAGGu'ACu'u'ACGAGAu'CCGACAAGA ACCGCGGAAAGu'CCGAu'AACGu'ACCGAGu'GAAGAAG u'GGu'AAAGAAGAu'GAAGAAu'u'Au'u'GGAGGCAGCu'C Cu'CAACGCCAAACu'u'Au'u'ACu'CAGAGAAAGu'u'CGA u'AACCu'u'ACGAAAGCu'GAGCGAGGCGGGu'u'Gu'Cu'G AGCu'CGAu'AAAGCu'GGAu'u'CAu'AAAGCGGCAGCu'u' Gu'GGAAACu'AGACAGAu'CACAAAGCACGu'u'GCACA GAu'CCu'u'GAu'u'CCAGAAu'GAACACu'AAAu'Au'GAu' GAGAACGAu'AAGCu'GAu'ACGCGAGGu'GAAAGu'AAu' AACACu'u'AAGu'CAAAGCu'GGu'AAGCGAu'u'u'u'CGCA AAGACu'u'CCAAu'u'u'u'Au'AAGGu'CAGAGAGAu'AAAC AAu'u'Au'CAu'CACGCGCAu'GACGCu'u'ACCu'GAACGC AGu'u'Gu'GGGGACAGCACu'u'Au'AAAGAAAu'ACCCAA AGu'u'GGAAAGu'GAAu'u'u'Gu'Cu'Au'GGAGACu'Au'AA GGu'Au'ACGAu'Gu'ACGGAAGAu'GAu'u'GCGAAGu'CAG AGCAAGAAAu'CGGu'AAGGCAACu'GCu'AAGu'ACu'u'C u'u'u'u'ACu'CAAAu'Au'u'Au'GAACu'u'u'u'u'CAAGACGG AAAu'CACu'Cu'GGCu'AACGGAGAAAu'u'CGGAAGAGG CCCu'u'GAu'AGAGACGAAu'GGGGAGACu'GGu'GAGAu' CGu'u'u'GGGACAAAGGGCGAGAu'u'u'CGCAACGGu'CC GAAAGGu'GCu'Cu'CAAu'GCCACAAGu'CAAu'Au'AGu'C AAGAAGACAGAGGu'ACAAACAGGAGGu'u'u'Cu'CCAA GGAAu'CAAu'ACu'CCCAAAGCGGAAu'u'CCGACAAACu 'GAu'AGCu'AGAAAGAAGGACu'GGGAu'CCGAAGAAGu' Au'GGAGGGu'u'u'**GAu'**u'CCCCAACAGu'AGCGu'Au'u'C AGu'ACu'GGu'GGu'AGCu'AAGGu'GGAGAAAGGCAAGu' Cu'AAGAAGCu'u'AAGu'Cu'Gu'CAAAGAGCu'GCu'CGGC Au'CACCAu'u'Au'GGAGCGCAGu'AGCu'u'CGAGAAGAA CCCu'Au'AGAu'u'u'u'Cu'GGAAGCCAAAGGAu'Au'AAGG AGGu'u'AAGAAGGAu'Cu'CAu'CAu'CAAACu'CCCGAAG u'Au'AGu'Cu'u'u'u'u'GAGu'u'GGAGAACGGACGAAAGC GCAu'GCu'u'GCGu'Cu'GCGGGu'GAAu'u'GCAGAAAGGA AAu'GAGCu'CGCCu'u'GCCGu'CAAAGu'Au'Gu'CAAu'u'u 'Cu'u'Gu'ACCu'u'GCGAGu'CACu'Au'GAGAAGCu'GAAA GGAAGCCCAGAAGAu'AAu'GAACAGAAGCAAu'u'Gu'u' CGu'u'GAACAACAu'AAGCAu'u'Au'u'u'GGACGAGAu'CA u'CGAGCAAAu'CAGCGAGu'u'CAGCAAGCGCGu'CAu'A Cu'u'GCGGACGCCAAu'Cu'GGACAAAGu'GCu'Gu'CCGC Cu'Au'AACAAACAu'AGGGACAAACCu'Au'ACGAGAGC AGGCAGAGAAu'Au'AAu'ACAu'Cu'Cu'u'CACACu'u'ACu' AAu'Cu'u'GGAGCu'CCAGCCGCAu'u'CAAGu'Au'u'u'u'G ACACGACu'Au'AGAu'CGCAAGCGAu'Au'ACCu'Cu'ACG AAAGAAGu'u'Cu'GGACGCu'ACu'Cu'u'Au'ACAu'CAAu'C CAu'u'ACGGGCCu'Gu'ACGAGACGAGGAu'u'GACu'u'Gu' CACAGCu'u'GGu'GGu'GAC	
	Protein	DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDR HSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRIC YLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFG NIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALA	40

		HMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEEN PINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFG NLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNL LAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSA SMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNG YAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDL LRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNRE KIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFE EVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYF TVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNR KVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDL LKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKT YAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSG KTILDFLKSDGFAN**R**NFMQLIHDDSLTFKEDIQKAQVSGQ GDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHK PENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQI LKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRL SDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPS EEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYL NAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQ EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGE TGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFS KESILPKRNSDKLIARKKDWDPKKYGGF**D**SPTVAYSVLV VAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAK GYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHK HYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIRE QAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLD ATLIHQSITGLYETRIDLSQLGGD	
C-terminal linker	mRNA	GAGGGu'GCAGAC	2172
	Protein	EGAD	2144
Nuclear localization sequence (NLS)	mRNA	AAGCGAACAGCGGACGGGu'CAGAAu'u'CGAAAGCCCC AAGAAGAAGCGGAAAGu'u'u'AA	2173
	Protein	KRTADGSEFESPKKKRKV	2146
3′ UTR	mRNA	GCGGCCGCu'u'AAu'u'AAGCu'GCCu'u'Cu'GCGGGGCu'u' GCCu'u'Cu'GGCCAu'GCCCu'u'Cu'u'Cu'Cu'CCCu'u'GCAC Cu'Gu'ACCu'Cu'u'GGu'Cu'u'u'GAAu'AAAGCCu'GAGu'A GGAAGu'Cu'AGA	2147
MA021 mRNA and protein sequences			
Region	**Sequence**		**SEQ ID NO**
Full sequence	mRNA	AGGAAAu'AAGAGAGAAAAGAAGAGu'AAGAAGAAAu' Au'AAGAGCCACCAu'Gu'Cu'GAGGu'GGAGu'u'Cu'CCCA CGAGu'ACu'GGAu'GCGGCAu'GCu'Cu'GACu'u'u'GGCCA AGAGAGCCCGAGAu'GAGCGGGAGGu'CCCu'Gu'GGGCG CAGu'GCu'GGu'GCu'GAACAACAGGGu'GAu'CGGCGAG GGCu'GGAACCGGGCCAu'CGGCCu'GCACGACCCu'ACA GCu'CACGCCGAGAu'CAu'GGCCCu'u'AGACAGGGCGGC Cu'GGu'GAu'GCAGAACu'ACAGACu'GAu'CGACGCCAC	2174

		ACu'Gu'ACGu'CACCu'u'u'GAGCCu'u'GCGu'GAu'Gu'GCG CCGGAGCCAu'GAu'CCAu'AGCCGGAu'AGGACGGGu'G Gu'Cu'u'u'GGCGu'GCGGAACGCGAAGACCGGCGCCGCC GGCAGCCu'u'Au'GGAu'Gu'GCu'GCACCAu'CCu'GGCAu' GAACCACAGGGu'GGAGAu'CACCGAAGGCAu'CCu'GGC u'GAu'GAAu'Gu'GCCGCu'Cu'GCu'Gu'GCAGAu'u'Cu'u'u' CGGAu'GCCu'AGAAGAGu'Gu'u'CAACGCCCAGAAGAA AGCCCAGAGu'AGCACCGAu'AGCGGCGGCAGCAGCGG CGGCAGCAGCGGAAGCGAGACACCu'GGCACCAGCGA Au'CCGCCACACCu'GAGAGCu'Cu'GGAGGAAGCu'CAGG CGGCu'Cu'GACAAGAAAu'ACu'CAAu'CGGACu'GGCCAu 'CGGCACCAAu'AGCGu'GGGCu'GGGCCGu'GAu'CACAG Au'GAAu'Au'AAGGu'GCCu'AGCAAGAAGu'u'CAAGGu'G Cu'GGGCAACACCGAu'AGACACAGCAu'CAAGAAGAAC Cu'GAu'CGGAGCCCu'GCu'Gu'u'CGACAGCGGCGAAACC GCCGAAGCCACCAGACu'GAAGAGAACCGCCAGAAGA CGGu'ACACCCGGCGu'AAGAACAGGAu'Cu'Gu'u'ACCu' GCAGGAGAu'Cu'u'CAGCAACGAAAu'GGCCAAGGu'GG ACGACu'Cu'u'u'Cu'u'CCACAGACu'GGAGGAGAGCu'u'u' Cu'GGu'GGAAGAAGACAAGAAGCAu'GAGCGGCACCCC Au'Cu'u'u'GGCAACAu'CGu'GGACGAAGu'GGCCu'ACCA CGAGAAAu'ACCCAACAAu'Cu'Au'CACCu'GAGAAAGA AGCu'GGu'GGACAGCACCGAu'AAGGCCGACCu'GCGGC u'GAu'Cu'ACCu'GGCCCu'GGCACACAu'GAu'CAAGu'u'C AGAGGCCACu'u'CCu'GAu'CGAGGGCGACCu'GAACCCC GAu'AAu'AGCGAu'Gu'GGAu'AAGCu'Gu'u'CAu'CCAGCu 'GGu'GCAGACCu'ACAACCAGCu'Gu'u'CGAGGAGAACC CGAu'CAACGCCu'CGGGAGu'u'GAu'GCCAAGGCAAu'C Cu'Gu'Cu'GCu'AGACu'GAGu'AAGAGCAGAAGACu'GGA GAACCu'GAu'CGCCCAGCu'GCCCGGAGAGAAGAAGAA u'GGGCu'Gu'u'u'GGCAACCu'GAu'u'GCCCu'Gu'Cu'Cu'G GGCCu'u'ACACCu'AACu'u'CAAGAGCAACu'u'u'GACCu' GGCCGAAGACGCCAAGCu'GCAACu'Gu'Cu'AAGGAu'AC Cu'ACGACGACGAu'Cu'GGACAACCu'GCu'GGCCCAAAu' CGGGGAu'CAAu'ACGCCGACCu'Gu'u'CCu'GGCu'GCCA AGAAu'Cu'Gu'Cu'GACGCCAu'CCu'u'Cu'Gu'CCGAu'Au'C Cu'GCGGGu'GAACACAGAGAu'AACCAAAGCu'CCu'Cu'u 'AGu'GCGAGCAu'GAu'CAAGAGAu'Au'GAu'GAGCACCA CCAAGAu'Cu'GACGCu'CCu'GAAGGCCCu'GGu'CAGACA GCAGCu'CCCu'GAGAAAu'ACAAGGAAAu'u'u'u'Cu'u'CG ACCAGAGCAAGAAu'GGCu'ACGCCGGCu'ACAu'CGAu'G GCGGCGCCAGCCAGGAGGAAu'u'u'u'ACAAGu'u'u'Au'C AAACCAAu'CCu'CGAGAAGAu'GGACGGCACCGAGGAA Cu'GCu'CGu'GAAGCu'GAACAGAGAGGACCu'ACu'GCG GAAGCAGCGGACGu'u'CGACAACGGCAGCAu'CCCu'CA CCAGAu'CCACCu'GGGu'GAGCu'GCACGCAAu'CCu'GAG AAGACAGGAAGAu'u'u'Cu'ACCCCu'u'CCu'GAAAGACA ACCGGGAGAAGAu'CGAGAAGAu'CCu'GACAu'u'u'AGA Au'u'CCCu'ACu'ACGu'GGGCCCu'Cu'GGCu'AGAGGCAA Cu'CCCGGu'u'CGCCu'GGAu'GACCCGGAAGu'CCGAAGA AACCAu'CACCCCCu'GGAACu'u'u'GAGGAGGu'AGu'GG ACAAGGGCGCCAGCGCu'CAGAGCu'u'u'Au'AGAGAGA Au'GACCAACu'u'CGACAAGAAu'Cu'GCCAAACGAGAA AGu'CCu'GCCu'AAGCACu'Cu'Cu'GCu'Gu'Au'GAGu'ACu' u'CACCGu'u'u'ACAACGAGCu'GACAAAGGu'GAAAu'AC	

		Gu'GACGGAGGGGAu'GAGGAAGCCCGCCu'u'CCu'GAG CGGCGAACAGAAGAAGGCu'Au'CGu'u'GACCu'GCu'Gu' u'CAAGACCAAu'AGGAAGGu'GACCGu'GAAGCAGCu'C AAGGAGGACu'ACu'u'CAAGAAGAu'u'GAGu'GCu'u'CGA Cu'Cu'Gu'GGAAAu'Cu'Cu'GGAGu'u'GAGGACCGGu'u'CA ACGCAu'CCCu'GGGAACAu'ACCACGAu'Cu'GCu'GAAG Au'CAu'u'AAGGACAAGGACu'u'u'Cu'GGACAAu'GAGGA GAACGAAGAu'Au'CCu'GGAGGACAu'u'Gu'GCu'GACCC u'GACACu'Gu'u'u'GAAGAu'CGu'GAAAu'GAu'CGAAGA ACGGCu'GAAGACAu'ACGCCCAu'Cu'Gu'u'CGACGACA AGGu'u'Au'GAAGCAGCu'GAAGAGAAGAAGAu'Au'ACA GGGu'GGGGCAGGCu'Gu'CCAGGAAACu'GAu'CAACGG CAu'CCGGGACAAGCAGAGCGGCAAGACAAu'u'Cu'GGA Cu'u'CCu'CAAGAGCGACGGCu'u'CGCCAAC**AGA**AAu'u'u 'u'Au'GCAGCu'GAu'CCAu'GACGAu'AGCCu'GACAu'u'C AAGGAAGACAu'CCAGAAGGCCCAGGu'GAGCGGGCAA GGCGACAGCCu'GCACGAGCACAu'CGCCAACCu'GGCC GGCu'Cu'CCCGCu'Au'CAAGAAGGGAAu'CCu'GCAGAC AGu'GAAGGu'GGu'u'GAu'GAGu'u'AGu'GAAAGu'GAu'G GGCCGGCACAAGCCu'GAGAAu'Au'CGu'GAu'u'GAAAu' GGCCAGAGAGAACCAGACAACu'CAGAAGGGCCAGAA GAACu'CCCGAGAGAGAAu'GAAGAGAAu'CGAAGAGG GAAu'CAAAGAGCu'AGGCAGCCAAAu'CCu'CAAAGAAC ACCCCGu'GGAGAAu'ACCCAGCu'GCAGAACGAGAAGC u'Gu'ACCu'Gu'ACu'ACCu'GCAGAACGGCCGAGAu'Au'G u'ACGu'GGAu'CAGGAGCu'GGACAu'CAAu'AGGCu'Gu'C AGACu'ACGACGu'GGACCACAu'CGu'GCCu'CAGAGCu'u 'u'Cu'GAAGGAu'GAu'u'Cu'Au'CGACAACAAGGu'Gu'u'A ACCAGAu'CCGACAAGAACAGAGGCAAGAGCGAu'AAC Gu'CCCAu'CCGAAGAGGu'CGu'GAAGAAGAu'GAAGAA Cu'ACu'GGAGACAGCu'GCu'GAACGCGAAACu'GAu'u'A CCCAGAGAAAGu'u'u'GACAACCu'CACCAAAGCu'GAGA GAGGAGGCCu'GAGCGAGCu'GGACAAGGCu'GGu'u'u'C Au'CAAGCGGCAGCu'AGu'GGAAACACGGCAAAu'CACC AAGCACGu'GGCu'CAAAu'CCu'AGAu'AGCAGAAu'GAA u'ACCAAGu'ACGAu'GAGAACGACAAACu'GAu'CAGAG AAGu'GAAGGu'GAu'CACAu'u'AAAGu'Cu'AAACu'GGu'C AGCGACu'u'CAGAAAGGACu'u'CCAGu'u'Cu'ACAAAGu' CAGAGAGAu'CAACAACu'ACCACCACGCCCACGACGC Cu'ACCu'GAACGCCGu'GGu'GGGCACCGCCCu'GAu'CAA GAAGu'Au'CCCAAGCu'GGAGu'CCGAAu'u'CGu'Gu'ACG GCGACu'ACAAGGu'Gu'ACGACGu'GCGGAAGAu'GAu'C GCCAAGu'Cu'GAACAGGAGAu'CGGCAAGGCCACCGCC AAGu'Au'u'u'Cu'u'Cu'ACAGCAACAu'u'Au'GAACu'u'Cu' u'CAAGACCGAGAu'CACCCu'GGCu'AAu'GGAGAGAu'C AGAAAGCGCCCCCu'GAu'CGAAACCAACGGAGAAACC GGCGAGAu'CGu'Gu'GGGACAAGGGCAGAGACu'u'CGC CACCGu'GAGAAAGGu'Gu'u'Gu'CCAu'GCCu'CAGGu'GA ACAu'CGu'GAAGAAGACAGAGGu'GCAGACAGGAGGGu 'u'u'AGCAAGGAGAGCAu'CCu'GCCu'AAACGGAACAGC GACAAGCu'GAu'CGCCAGAAAGAAGGACu'GGGAu'CCu 'AAGAAAu'ACGGCGGCu'u'C**GAu'**u'CCCCu'ACCGu'GGC Cu'ACAGCGu'GCu'GGu'GGu'GGCCAAGGu'CGAGAAGG GCAAGAGCAAGAAACu'GAAGu'CCGu'GAAGGAACu'G Cu'GGGAAu'CACCAu'CAu'GGAAAGAu'Cu'AGCu'u'CGA	

		GAAGAAu'CCu'Au'CGACu'u'CCu'GGAAGCCAAGGGCu' Au'AAAGAGGu'GAAGAAAGACCu'GAu'CAu'CAAGCu'C CCGAAGu'ACAGCCu'Gu'u'CGAGCu'GGAGAACGGCCGC AAGCGGAu'GCu'GGCCu'CCGCCGGCGAGCu'GCAGAAG GGCAAu'GAGCu'CGCCCu'GCCu'u'Cu'AAAu'ACGu'GAA Cu'u'CCu'Gu'ACCu'GGCCu'CGCACu'ACGAGAAGCu'CA AGGGCAGu'CCAGAGGACAACGAGCAGAAACAGCu'Gu' u'CGu'GGAACAACACAAGCACu'ACCu'GGAu'GAGAu'u' Au'CGAGCAGAu'CAGCGAGu'u'Cu'CCAAACGGGu'GAu' CCu'GGCGGAu'GCu'AACCu'AGAu'AAGGu'u'Cu'GAGCG CCu'ACAACAAGCACCGCGACAAGCCu'Au'AAGAGAAC AGGCCGAGAAu'Au'CAu'CCACCu'Gu'u'CACCCu'GACC AACCu'GGGCGCACCu'GCu'GCCu'u'CAAGu'ACu'u'CGA CACCACCAu'CGACAGAAAGAGAu'ACACAAGCACCAA GGAGGu'u'Cu'GGACGCCACCCu'GAu'ACACCAGAGCAu 'CACAGGu'Cu'Gu'Au'GAAACCCGGAu'CGACCu'Gu'Cu'C AGCu'GGGCGGu'GACGAAGGu'GCu'GACAAGAGAACC GCCGACGGCAGCGAGu'u'CGAGAGCCCCAAGAAGAAG CGCAAGGu'Gu'GAGCGGCCGCu'u'AAu'u'AAGCu'GCCu' u'Cu'GCGGGGCu'u'GCCu'u'Cu'GGCCAu'GCCCu'u'Cu'u'C u'Cu'CCCu'u'GCACCu'Gu'ACCu'Cu'u'GGu'Cu'u'u'GAAu' AAAGCCu'GAGu'AGGAAGu'Cu'AGA	
	Protein	MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNN RVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLID ATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHHPGMNHRVEITEGILADECAALLCRFFRMPRR VFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGG SSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVL GNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRR KNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHER HPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIY LALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKN GLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDL DNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKA PLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQS KNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLN REDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITP WNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLL YEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLF KTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD KQSGKTILDFLKSDGFAN**R**NFMQLIHDDSLTFKEDIQKAQ VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM GRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKEL GSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDI NRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDN VPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAH DAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIA KSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT	2137

		GGFSKESILPKRNSDKLIARKKDWDPKKYGGF**D**SPTVAY SVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDF LEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGEL QKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFV EQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRD KPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTK EVLDATLIHQSITGLYETRIDLSQLGGDEGADKRTADGSE FESPKKKRKV	
5′ UTR	mRNA	AGGAAAu'AAGAGAGAAAAGAAGAGu'AAGAAGAAAu' Au'AAGAGCCACC	2138
TadA	mRNA	Au'Gu'Cu'GAGGu'GGAGu'u'Cu'CCCACGAGu'ACu'GGAu' GCGGCAu'GCu'Cu'GACu'u'u'GGCCAAGAGAGCCCGAG Au'GAGCGGGAGGu'CCCu'Gu'GGGCGCAGu'GCu'GGu'G Cu'GAACAACAGGGu'GAu'CGGCGAGGGCu'GGAACCGG GCCAu'CGGCCu'GCACGACCCu'ACAGCu'CACGCCGAG Au'CAu'GGCCCu'u'AGACAGGGCGGCCu'GGu'GAu'GCA GAACu'ACAGACu'GAu'CGACGCCACACu'Gu'ACGu'CAC Cu'u'u'GAGCCu'u'GCGu'GAu'Gu'GCGCCGGAGCCAu'GA u'CCAu'AGCCGGAu'AGGACGGGu'GGu'Cu'u'u'GGCGu'G CGGAACGCGAAGACCGGCGCCGCCGGCAGCCu'u'Au'G GAu'Gu'GCu'GCACCAu'CCu'GGCAu'GAACCACAGGGu' GGAGAu'CACCGAAGGCAu'CCu'GGCu'GAu'GAAu'Gu'G CCGCu'Cu'GCu'Gu'GCAGAu'u'Cu'u'u'CGGAu'GCCu'AGA AGAGu'Gu'u'CAACGCCCAGAAGAAAGCCCAGAGu'AGC ACCGAu'	2175
	Protein	MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNN RVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLID ATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHHPGMNHRVEITEGILADECAALLCRFFRMPRR VFNAQKKAQSSTD	2140
N-terminal linker	mRNA	AGCGGCGGCAGCAGCGGCGGCAGCAGCGGAAGCGAG ACACCu'GGCACCAGCGAAu'CCGCCACACCu'GAGAGCu 'Cu'GGAGGAAGCu'CAGGCGGCu'Cu'	2176
	Protein	SGGSSGGSSGSETPGTSESATPESSGGSSGGS	20
Cas9 nickase	mRNA	GACAAGAAAu'ACu'CAAu'CGGACu'GGCCAu'CGGCACC AAu'AGCGu'GGGCu'GGGCCGu'GAu'CACAGAu'GAAu'A u'AAGGu'GCCu'AGCAAGAAGu'u'CAAGGu'GCu'GGGCA ACACCGAu'AGACACAGCAu'CAAGAAGAACCu'GAu'CG GAGCCCu'GCu'Gu'u'CGACAGCGGCGAAACCGCCGAAG CCACCAGACu'GAAGAGAACCGCCAGAAGACGGu'ACA CCCGGCGu'AAGAACAGGAu'Cu'Gu'u'ACCu'GCAGGAG Au'Cu'u'CAGCAACGAAAu'GGCCAAGGu'GGACGACu'Cu 'u'u'Cu'u'CCACAGACu'GGAGGAGAGCu'u'u'Cu'GGu'GG AAGAAGACAAGAAGCAu'GAGCGGCACCCCAu'Cu'u'u' GGCAACAu'CGu'GGACGAAGu'GGCCu'ACCACGAGAAA u'ACCCAACAAu'Cu'Au'CACCu'GAGAAAGAAGCu'GGu' GGACAGCACCGAu'AAGGCCGACCu'GCGGCu'GAu'Cu'A CCu'GGCCCu'GGCACACAu'GAu'CAAGu'u'CAGAGGCCA Cu'u'CCu'GAu'CGAGGGCGACCu'GAACCCCGAu'AAu'A GCGAu'Gu'GGAu'AAGCu'Gu'u'CAu'CCAGCu'GGu'GCAG ACCu'ACAACCAGCu'Gu'u'CGAGGAGAACCCGAu'CAAC GCCu'CGGGAGu'u'GAu'GCCAAGGCAAu'CCu'Gu'Cu'GC u'AGACu'GAGu'AAGAGCAGAAGACu'GGAGAACCu'GA u'CGCCCAGCu'GCCCGGAGAGAAGAAGAAu'GGGCu'Gu	2177

		'u'u'GGCAACCu'GAu'u'GCCCu'Gu'Cu'Cu'GGGCCu'u'AC ACCu'AACu'u'CAAGAGCAACu'u'u'GACCu'GGCCGAAG ACGCCAAGCu'GCAACu'Gu'Cu'AAGGAu'ACCu'ACGAC GACGAu'Cu'GGACAACCu'GCu'GGCCCAAAu'CGGGGAu 'CAAu'ACGCCGACCu'Gu'u'CCu'GGCu'GCCAAGAAu'Cu' Gu'Cu'GACGCCAu'CCu'u'Cu'Gu'CCGAu'Au'CCu'GCGGG u'GAACACAGAGAu'AACCAAAGCu'CCu'Cu'u'AGu'GCG AGCAu'GAu'CAAGAGAu'Au'GAu'GAGCACCACCAAGAu 'Cu'GACGCu'CCu'GAAGGCCCu'GGu'CAGACAGCAGCu' CCCu'GAGAAAu'ACAAGGAAAu'u'u'u'Cu'u'CGACCAGA GCAAGAAu'GGCu'ACGCCGGCu'ACAu'CGAu'GGCGGCG CCAGCCAGGAGGAAu'u'u'u'ACAAGu'u'u'Au'CAAACCA Au'CCu'CGAGAAGAu'GGACGGCACCGAGGAACu'GCu'C Gu'GAAGCu'GAACAGAGAGGACCu'ACu'GCGGAAGCA GCGGACGu'u'CGACAACGGCAGCAu'CCCu'CACCAGAu' CCACCu'GGGu'GAGCu'GCACGCAAu'CCu'GAGAAGACA GGAAGAu'u'u'Cu'ACCCCu'u'CCu'GAAAGACAACCGGG AGAAGAu'CGAGAAGAu'CCu'GACAu'u'u'AGAAu'u'CCC u'ACu'ACGu'GGGCCCu'Cu'GGCu'AGAGGCAACu'CCCG Gu'u'CGCCu'GGAu'GACCCGGAAGu'CCGAAGAAACCAu 'CACCCCCu'GGAACu'u'u'GAGGAGGu'AGu'GGACAAGG GCGCCAGCGCu'CAGAGCu'u'u'Au'AGAGAGAAu'GACC AACu'u'CGACAAGAAu'Cu'GCCAAACGAGAAAGu'CCu' GCCu'AAGCACu'Cu'Cu'GCu'Gu'Au'GAGu'ACu'u'CACCG u'u'u'ACAACGAGCu'GACAAAGGu'GAAAu'ACGu'GACG GAGGGGAu'GAGGAAGCCCGCCu'u'CCu'GAGCGGCGAA CAGAAGAAGGCu'Au'CGu'u'GACCu'GCu'Gu'u'CAAGAC CAAu'AGGAAGGu'GACCGu'GAAGCAGCu'CAAGGAGG ACu'ACu'u'CAAGAAGAu'u'GAGu'GCu'u'CGACu'Cu'Gu' GGAAAu'Cu'Cu'GGAGu'u'GAGGACCGGu'u'CAACGCAu' CCCu'GGGAACAu'ACCACGAu'Cu'GCu'GAAGAu'CAu'u' AAGGACAAGGACu'u'u'Cu'GGACAAu'GAGGAGAACGA AGAu'Au'CCu'GGAGGACAu'u'Gu'GCu'GACCCu'GACAC u'Gu'u'u'GAAGAu'CGu'GAAAu'GAu'CGAAGAACGGCu' GAAGACAu'ACGCCCAu'Cu'Gu'u'CGACGACAAGGu'u'A u'GAAGCAGCu'GAAGAGAAGAAGAu'Au'ACAGGGu'GG GGCAGGCu'Gu'CCAGGAAACu'GAu'CAACGGCAu'CCGG GACAAGCAGAGCGGCAAGACAAu'u'Cu'GGACu'u'CCu' CAAGAGCGACGGCu'u'CGCCAAC**AGA**AAu'u'u'u'Au'GC AGCu'GAu'CCAu'GACGAu'AGCCu'GACAu'u'CAAGGAA GACAu'CCAGAAGGCCCAGGu'GAGCGGGCAAGGCGAC AGCCu'GCACGAGCACAu'CGCCAACCu'GGCCGGCu'Cu' CCCGCu'Au'CAAGAAGGGAAu'CCu'GCAGACAGu'GAA GGu'GGu'u'GAu'GAGu'u'AGu'GAAAGu'GAu'GGGCCGG CACAAGCCu'GAGAAu'Au'CGu'GAu'u'GAAAu'GGCCAG AGAGAACCAGACAACu'CAGAAGGGCCAGAAGAACu'C CCGAGAGAGAAu'GAAGAGAAu'CGAAGAGGGAAu'CA AAGAGCu'AGGCAGCCAAAu'CCu'CAAAGAACACCCCG u'GGAGAAu'ACCCAGCu'GCAGAACGAGAAGCu'Gu'AC Cu'Gu'ACu'ACCu'GCAGAACGGCCGAGAu'Au'Gu'ACGu' GGAu'CAGGAGCu'GGACAu'CAAu'AGGCu'Gu'CAGACu' ACGACGu'GGACCACAu'CGu'GCCu'CAGAGCu'u'u'Cu'G AAGGAu'GAu'u'Cu'Au'CGACAACAAGGu'Gu'u'AACCAG Au'CCGACAAGAACAGAGGCAAGAGCGAu'AACGu'CCC Au'CCGAAGAGGu'CGu'GAAGAAGAu'GAAGAACu'ACu'	

		GGAGACAGCu'GCu'GAACGCGAAACu'GAu'u'ACCCAG AGAAAGu'u'u'GACAACCu'CACCAAAGCu'GAGAGAGG AGGCCu'GAGCGAGCu'GGACAAGGCu'GGu'u'u'CAu'CA AGCGGCAGCu'AGu'GGAAACACGGCAAAu'CACCAAGC ACGu'GGCu'CAAAu'CCu'AGAu'AGCAGAAu'GAAu'ACC AAGu'ACGAu'GAGAACGACAAACu'GAu'CAGAGAAGu' GAAGGu'GAu'CACAu'u'AAAGu'Cu'AAACu'GGu'CAGCG ACu'u'CAGAAAGGACu'u'CCAGu'u'Cu'ACAAAGu'CAGA GAGAu'CAACAACu'ACCACCACGCCCACGACGCCu'AC Cu'GAACGCCGu'GGu'GGGCACCGCCCu'GAu'CAAGAAG u'Au'CCCAAGCu'GGAGu'CCGAAu'u'CGu'Gu'ACGGCGA Cu'ACAAGGu'Gu'ACGACGu'GCGGAAGAu'GAu'CGCCA AGu'Cu'GAACAGGAGAu'CGGCAAGGCCACCGCCAAGu' Au'u'u'Cu'u'Cu'ACAGCAACAu'u'Au'GAACu'u'Cu'u'CAA GACCGAGAu'CACCCu'GGCu'AAu'GGAGAGAu'CAGAA AGCGCCCCCu'GAu'CGAAACCAACGGAGAAACCGGCG AGAu'CGu'Gu'GGGACAAGGGCAGAGACu'u'CGCCACC Gu'GAGAAAGGu'Gu'u'Gu'CCAu'GCCu'CAGGu'GAACAu' CGu'GAAGAAGACAGAGGu'GCAGACAGGAGGGu'u'u'A GCAAGGAGAGCAu'CCu'GCCu'AAACGGAACAGCGACA AGCu'GAu'CGCCAGAAAGAAGGACu'GGGAu'CCu'AAG AAAu'ACGGCGGCu'u'C**GAu'**u'CCCCu'ACCGu'GGCCu'A CAGCGu'GCu'GGu'GGu'GGCCAAGGu'CGAGAAGGGCA AGAGCAAGAAACu'GAAGu'CCGu'GAAGGAACu'GCu'G GGAAu'CACCAu'CAu'GGAAAGAu'Cu'AGCu'u'CGAGAA GAAu'CCu'Au'CGACu'u'CCu'GGAAGCCAAGGGCu'Au'A AAGAGGu'GAAGAAAGACCu'GAu'CAu'CAAGCu'CCCG AAGu'ACAGCCu'Gu'u'CGAGCu'GGAGAACGGCCGCAA GCGGAu'GCu'GGCCu'CCGCCGGCGAGCu'GCAGAAGGG CAAu'GAGCu'CGCCCu'GCCu'u'Cu'AAAu'ACGu'GAACu' u'CCu'Gu'ACCu'GGCCu'CGCACu'ACGAGAAGCu'CAAG GGCAGu'CCAGAGGACAACGAGCAGAAACAGCu'Gu'u'C Gu'GGAACAACACAAGCACu'ACCu'GGAu'GAGAu'u'Au' CGAGCAGAu'CAGCGAGu'u'Cu'CCAAACGGGu'GAu'CCu 'GGCGGAu'GCu'AACCu'AGAu'AAGGu'u'Cu'GAGCGCCu 'ACAACAAGCACCGCGACAAGCCu'Au'AAGAGAACAGG CCGAGAAu'Au'CAu'CCACCu'Gu'u'CACCCu'GACCAACC u'GGGCGCACCu'GCu'GCCu'u'CAAGu'ACu'u'CGACACC ACCAu'CGACAGAAAGAGAu'ACACAAGCACCAAGGAG Gu'u'Cu'GGACGCCACCCu'GAu'ACACCAGAGCAu'CACA GGu'Cu'Gu'Au'GAAACCCGGAu'CGACCu'Gu'Cu'CAGCu' GGGCGGu'GAC	
	Protein	DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDR HSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRIC YLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFG NIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALA HMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEEN PINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFG NLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNL LAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSA SMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNG YAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDL LRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNRE KIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFE EVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYF	40

		TVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNR KVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDL LKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKT YAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSG KTILDFLKSDGFAN**R**NFMQLIHDDSLTFKEDIQKAQVSGQ GDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHK PENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQI LKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRL SDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPS EEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYL NAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQ EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGE TGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFS KESILPKRNSDKLIARKKDWDPKKYGGF**D**SPTVAYSVLV VAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAK GYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHK HYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIRE QAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLD ATLIHQSITGLYETRIDLSQLGGD	
C-terminal linker	mRNA	GAAGGu'GCu'GAC	2178
	Protein	EGAD	2144
Nuclear localization sequence (NLS)	mRNA	AAGAGAACCGCCGACGGCAGCGAGu'u'CGAGAGCCCC AAGAAGAAGCGCAAGGu'Gu'GA	2179
	Protein	KRTADGSEFESPKKKRKV	2146
3′ UTR	mRNA	GCGGCCGCu'u'AAu'u'AAGCu'GCCu'u'Cu'GCGGGGCu'u' GCCu'u'Cu'GGCCAu'GCCCu'u'Cu'u'Cu'Cu'CCCu'u'GCAC Cu'Gu'ACCu'Cu'u'GGu'Cu'u'u'GAAu'AAAGCCu'GAGu'A GGAAGu'Cu'AGA	2147
ABE8.8m DNA and protein sequences			
Region	**Sequence**		**SEQ ID NO**
Full sequence	DNA	ATGTCCGAAGTCGAGTTTTCCCATGAGTACTGGATGAG ACACGCATTGACTCTCGCAAAGAGGGCTCGAGATGAA CGCGAGGTGCCCGTGGGGGCAGTACTCGTGCTCAACA ATCGCGTAATCGGCGAAGGTTGGAATAGGGCAATCGG ACTCCACGACCCCACTGCACATGCGGAAATCATGGCCC TTCGACAGGGAGGGCTTGTGATGCAGAATTATCGACTT ATCGATGCGACGCTGTACGTCACGTTTGAACCTTGCGT AATGTGCGCGGGAGCTATGATTCACTCCCGCATTGGAC GAGTTGTATTCGGTGTTCGCAACGCCAAGACGGGTGCC GCAGGTTCACTGATGGACGTGCTGCATCATCCAGGCAT GAACCACCGGGTAGAAATCACAGAAGGCATATTGGCG GACGAATGTGCGGCGCTGTTGTGTCGTTTTTTTCGCAT GCCCAGGCGGGTCTTTAACGCCCAGAAAAAAGCACAA TCCTCTACTGACTCTGGTGGTTCTTCTGGTGGTTCTAGC GGCAGCGAGACTCCCGGGACCTCAGAGTCCGCCACAC CCGAAAGTTCTGGTGGTTCTTCTGGTGGTTCTGACAAG AAGTACAGCATCGGCCTGGCCATCGGCACCAACTCTGT	2180

		GGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCC AGCAAGAAATTCAAGGTGCTGGGCAACACCGACCGGC ACAGCATCAAGAAGAACCTGATCGGAGCCCTGCTGTT CGACAGCGGCGAAACAGCCGAGGCCACCCGGCTGAAG AGAACCGCCAGAAGAAGATACACCAGACGGAAGAAC CGGATCTGCTATCTGCAAGAGATCTTCAGCAACGAGAT GGCCAAGGTGGACGACAGCTTCTTCCACAGACTGGAA GAGTCCTTCCTGGTGGAAGAGGATAAGAAGCACGAGC GGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGC CTACCACGAGAAGTACCCCACCATCTACCACCTGAGA AAGAAACTGGTGGACAGCACCGACAAGGCCGACCTGC GGCTGATCTATCTGGCCCTGGCCCACATGATCAAGTTC CGGGGCCACTTCCTGATCGAGGGCGACCTGAACCCCG ACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGT GCAGACCTACAACCAGCTGTTCGAGGAAAACCCCATC AACGCCAGCGGCGTGGACGCCAAGGCCATCCTGTCTG CCAGACTGAGCAAGAGCAGACGGCTGGAAAATCTGAT CGCCCAGCTGCCCGGCGAGAAGAAGAATGGCCTGTTC GGAAACCTGATTGCCCTGAGCCTGGGCCTGACCCCCAA CTTCAAGAGCAACTTCGACCTGGCCGAGGATGCCAAA CTGCAGCTGAGCAAGGACACCTACGACGACGACCTGG ACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGA CCTGTTTCTGGCCGCCAAGAACCTGTCCGACGCCATCC TGCTGAGCGACATCCTGAGAGTGAACACCGAGATCAC CAAGGCCCCCCTGAGCGCCTCTATGATCAAGAGATAC GACGAGCACCACCAGGACCTGACCCTGCTGAAAGCTC TCGTGCGGCAGCAGCTGCCTGAGAAGTACAAAGAGAT TTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACA TTGACGGCGGAGCCAGCCAGGAAGAGTTCTACAAGTT CATCAAGCCCATCCTGGAAAAGATGGACGGCACCGAG GAACTGCTCGTGAAGCTGAACAGAGAGGACCTGCTGC GGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCA CCAGATCCACCTGGGAGAGCTGCACGCCATTCTGCGGC GGCAGGAAGATTTTTACCCATTCCTGAAGGACAACCG GGAAAAGATCGAGAAGATCCTGACCTTCCGCATCCCCT ACTACGTGGGCCCTCTGGCCAGGGGAAACAGCAGATT CGCCTGGATGACCAGAAAGAGCGAGGAAACCATCACC CCCTGGAACTTCGAGGAAGTGGTGGACAAGGGCGCTT CCGCCCAGAGCTTCATCGAGCGGATGACCAACTTCGAT AAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACA GCCTGCTGTACGAGTACTTCACCGTGTATAACGAGCTG ACCAAAGTGAAATACGTGACCGAGGGAATGAGAAAGC CCGCCTTCCTGAGCGGCGAGCAGAAAAAGGCCATCGT GGACCTGCTGTTCAAGACCAACCGGAAAGTGACCGTG AAGCAGCTGAAAGAGGACTACTTCAAGAAAATCGAGT GCTTCGACTCCGTGGAAATCTCCGGCGTGGAAGATCGG TTCAACGCCTCCCTGGGCACATACCACGATCTGCTGAA AATTATCAAGGACAAGGACTTCCTGGACAATGAGGAA AACGAGGACATTCTGGAAGATATCGTGCTGACCCTGA CACTGTTTGAGGACAGAGAGATGATCGAGGAACGGCT GAAAACCTATGCCCACCTGTTCGACGACAAAGTGATG AAGCAGCTGAAGCGGCGGAGATACACCGGCTGGGGCA GGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAA GCAGTCCGGCAAGACAATCCTGGATTTCCTGAAGTCCG ACGGCTTCGCCAAC**AGA**AACTTCATGCAGCTGATCCAC	

		GACGACAGCCTGACCTTTAAAGAGGACATCCAGAAAG CCCAGGTGTCCGGCCAGGGCGATAGCCTGCACGAGCA CATTGCCAATCTGGCCGGCAGCCCCGCCATTAAGAAG GGCATCCTGCAGACAGTGAAGGTGGTGGACGAGCTCG TGAAAGTGATGGGCCGGCACAAGCCCGAGAACATCGT GATCGAAATGGCCAGAGAGAACCAGACCACCCAGAAG GGACAGAAGAACAGCCGCGAGAGAATGAAGCGGATC GAAGAGGGCATCAAAGAGCTGGGCAGCCAGATCCTGA AAGAACACCCCGTGGAAAACACCCAGCTGCAGAACGA GAAGCTGTACCTGTACTACCTGCAGAATGGGCGGGAT ATGTACGTGGACCAGGAACTGGACATCAACCGGCTGT CCGACTACGATGTGGACCATATCGTGCCTCAGAGCTTT CTGAAGGACGACTCCATCGACAACAAGGTGCTGACCA GAAGCGACAAGAACCGGGGCAAGAGCGACAACGTGC CCTCCGAAGAGGTCGTGAAGAAGATGAAGAACTACTG GCGGCAGCTGCTGAACGCCAAGCTGATTACCCAGAGA AAGTTCGACAATCTGACCAAGGCCGAGAGAGGCGGCC TGAGCGAACTGGATAAGGCCGGCTTCATCAAGAGACA GCTGGTGGAAACCCGGCAGATCACAAAGCACGTGGCA CAGATCCTGGACTCCCGGATGAACACTAAGTACGACG AGAATGACAAGCTGATCCGGGAAGTGAAAGTGATCAC CCTGAAGTCCAAGCTGGTGTCCGATTTCCGGAAGGATT TCCAGTTTTACAAAGTGCGCGAGATCAACAACTACCAC CACGCCCACGACGCCTACCTGAACGCCGTCGTGGGAA CCGCCCTGATCAAAAAGTACCCTAAGCTGGAAAGCGA GTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGG AAGATGATCGCCAAGAGCGAGCAGGAAATCGGCAAGG CTACCGCCAAGTACTTCTTCTACAGCAACATCATGAAC TTTTTCAAGACCGAGATTACCCTGGCCAACGGCGAGAT CCGGAAGCGGCCTCTGATCGAGACAAACGGCGAAACC GGGGAGATCGTGTGGGATAAGGGCCGGGATTTTGCCA CCGTGCGGAAAGTGCTGAGCATGCCCCAAGTGAATAT CGTGAAAAAGACCGAGGTGCAGACAGGCGGCTTCAGC AAAGAGTCTATCCTGCCCAAGAGGAACAGCGATAAGC TGATCGCCAGAAAGAAGGACTGGGACCCTAAGAAGTA CGGCGGCTTC**GAC**AGCCCCACCGTGGCCTATTCTGTGC TGGTGGTGGCCAAAGTGGAAAAGGGCAAGTCCAAGAA ACTGAAGAGTGTGAAAGAGCTGCTGGGGATCACCATC ATGGAAAGAAGCAGCTTCGAGAAGAATCCCATCGACT TTCTGGAAGCCAAGGGCTACAAAGAAGTGAAAAAGGA CCTGATCATCAAGCTGCCTAAGTACTCCCTGTTCGAGC TGGAAAACGGCCGGAAGAGAATGCTGGCCTCTGCCGG CGAACTGCAGAAGGGAAACGAACTGGCCCTGCCCTCC AAATATGTGAACTTCCTGTACCTGGCCAGCCACTATGA GAAGCTGAAGGGCTCCCCCGAGGATAATGAGCAGAAA CAGCTGTTTGTGGAACAGCACAAGCACTACCTGGACG AGATCATCGAGCAGATCAGCGAGTTCTCCAAGAGAGT GATCCTGGCCGACGCTAATCTGGACAAAGTGCTGTCCG CCTACAACAAGCACCGGGATAAGCCCATCAGAGAGCA GGCCGAGAATATCATCCACCTGTTTACCCTGACCAATC TGGGAGCCCCTGCCGCCTTCAAGTACTTTGACACCACC ATCGACCGGAAGAGGTACACCAGCACCAAAGAGGTGC TGGACGCCACCCTGATCCACCAGAGCATCACCGGCCTG TACGAGACACGGATCGACCTGTCTCAGCTGGGAGGTG ACGAGGGAGCTGATAAGCGCACCGCCGATGGTTCCGA	

		GTTCGAAAGCCCCAAGAAGAAGAGGAAAGTCTAA	
	Protein	MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNN RVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLID ATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHHPGMNHRVEITEGILADECAALLCRFFRMPRR VFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGG SSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVL GNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRR KNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHER HPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIY LALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKN GLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDL DNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKA PLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQS KNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLN REDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITP WNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLL YEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLF KTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD KQSGKTILDFLKSDGFAN**R**NFMQLIHDDSLTFKEDIQKAQ VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM GRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKEL GSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDI NRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDN VPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAH DAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIA KSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT GGFSKESILPKRNSDKLIARKKDWDPKKYGGF**D**SPTVAY SVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDF LEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGEL QKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFV EQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRD KPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTK EVLDATLIHQSITGLYETRIDLSQLGGDEGADKRTADGSE FESPKKKRKV	2137
TadA	DNA	ATGTCCGAAGTCGAGTTTTCCCATGAGTACTGGATGAG ACACGCATTGACTCTCGCAAAGAGGGCTCGAGATGAA CGCGAGGTGCCCGTGGGGGCAGTACTCGTGCTCAACA ATCGCGTAATCGGCGAAGGTTGGAATAGGGCAATCGG ACTCCACGACCCCACTGCACATGCGGAAATCATGGCCC TTCGACAGGGAGGGCTTGTGATGCAGAATTATCGACTT ATCGATGCGACGCTGTACGTCACGTTTGAACCTTGCGT AATGTGCGCGGGAGCTATGATTCACTCCCGCATTGGAC GAGTTGTATTCGGTGTTCGCAACGCCAAGACGGGTGCC GCAGGTTCACTGATGGACGTGCTGCATCATCCAGGCAT GAACCACCGGGTAGAAATCACAGAAGGCATATTGGCG GACGAATGTGCGGCGCTGTTGTGTCGTTTTTTTCGCAT GCCCAGGCGGGTCTTTAACGCCCAGAAAAAAGCACAA	2181

		TCCTCTACTGAC	
	Protein	MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNN RVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLID ATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHHPGMNHRVEITEGILADECAALLCRFFRMPRR VFNAQKKAQSSTD	2140
Linker between TadA and Cas9 nickase	DNA	TCTGGTGGTTCTTCTGGTGGTTCTAGCGGCAGCGAGAC TCCCGGGACCTCAGAGTCCGCCACACCCGAAAGTTCTG GTGGTTCTTCTGGTGGTTCT	2182
	Protein	SGGSSGGSSGSETPGTSESATPESSGGSSGGS	20
Cas9 nickase	DNA	GACAAGAAGTACAGCATCGGCCTGGCCATCGGCACCA ACTCTGTGGGCTGGGCCGTGATCACCGACGAGTACAA GGTGCCCAGCAAGAAATTCAAGGTGCTGGGCAACACC GACCGGCACAGCATCAAGAAGAACCTGATCGGAGCCC TGCTGTTCGACAGCGGCGAAACAGCCGAGGCCACCCG GCTGAAGAGAACCGCCAGAAGAAGATACACCAGACGG AAGAACCGGATCTGCTATCTGCAAGAGATCTTCAGCA ACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACAG ACTGGAAGAGTCCTTCCTGGTGGAAGAGGATAAGAAG CACGAGCGGCACCCCATCTTCGGCAACATCGTGGACG AGGTGGCCTACCACGAGAAGTACCCCACCATCTACCA CCTGAGAAAGAAACTGGTGGACAGCACCGACAAGGCC GACCTGCGGCTGATCTATCTGGCCCTGGCCCACATGAT CAAGTTCCGGGGCCACTTCCTGATCGAGGGCGACCTGA ACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCA GCTGGTGCAGACCTACAACCAGCTGTTCGAGGAAAAC CCCATCAACGCCAGCGGCGTGGACGCCAAGGCCATCC TGTCTGCCAGACTGAGCAAGAGCAGACGGCTGGAAAA TCTGATCGCCCAGCTGCCCGGCGAGAAGAAGAATGGC CTGTTCGGAAACCTGATTGCCCTGAGCCTGGGCCTGAC CCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGAT GCCAAACTGCAGCTGAGCAAGGACACCTACGACGACG ACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTA CGCCGACCTGTTTCTGGCCGCCAAGAACCTGTCCGACG CCATCCTGCTGAGCGACATCCTGAGAGTGAACACCGA GATCACCAAGGCCCCCCTGAGCGCCTCTATGATCAAGA GATACGACGAGCACCACCAGGACCTGACCCTGCTGAA AGCTCTCGTGCGGCAGCAGCTGCCTGAGAAGTACAAA GAGATTTTCTTCGACCAGAGCAAGAACGGCTACGCCG GCTACATTGACGGCGGAGCCAGCCAGGAAGAGTTCTA CAAGTTCATCAAGCCCATCCTGGAAAAGATGGACGGC ACCGAGGAACTGCTCGTGAAGCTGAACAGAGAGGACC TGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCAT CCCCCACCAGATCCACCTGGGAGAGCTGCACGCCATTC TGCGGCGGCAGGAAGATTTTTACCCATTCCTGAAGGAC AACCGGGAAAAGATCGAGAAGATCCTGACCTTCCGCA TCCCCTACTACGTGGGCCCTCTGGCCAGGGGAAACAGC AGATTCGCCTGGATGACCAGAAAGAGCGAGGAAACCA TCACCCCCTGGAACTTCGAGGAAGTGGTGGACAAGGG CGCTTCCGCCCAGAGCTTCATCGAGCGGATGACCAACT TCGATAAGAACCTGCCCAACGAGAAGGTGCTGCCCAA GCACAGCCTGCTGTACGAGTACTTCACCGTGTATAACG	2183

		AGCTGACCAAAGTGAAATACGTGACCGAGGGAATGAG AAAGCCCGCCTTCCTGAGCGGCGAGCAGAAAAAGGCC ATCGTGGACCTGCTGTTCAAGACCAACCGGAAAGTGA CCGTGAAGCAGCTGAAAGAGGACTACTTCAAGAAAAT CGAGTGCTTCGACTCCGTGGAAATCTCCGGCGTGGAAG ATCGGTTCAACGCCTCCCTGGGCACATACCACGATCTG CTGAAAATTATCAAGGACAAGGACTTCCTGGACAATG AGGAAAACGAGGACATTCTGGAAGATATCGTGCTGAC CCTGACACTGTTTGAGGACAGAGAGATGATCGAGGAA CGGCTGAAAACCTATGCCCACCTGTTCGACGACAAAGT GATGAAGCAGCTGAAGCGGCGGAGATACACCGGCTGG GGCAGGCTGAGCCGGAAGCTGATCAACGGCATCCGGG ACAAGCAGTCCGGCAAGACAATCCTGGATTTCCTGAA GTCCGACGGCTTCGCCAAC**AGA**AACTTCATGCAGCTG ATCCACGACGACAGCCTGACCTTTAAAGAGGACATCC AGAAAGCCCAGGTGTCCGGCCAGGGCGATAGCCTGCA CGAGCACATTGCCAATCTGGCCGGCAGCCCCGCCATTA AGAAGGGCATCCTGCAGACAGTGAAGGTGGTGGACGA GCTCGTGAAAGTGATGGGCCGGCACAAGCCCGAGAAC ATCGTGATCGAAATGGCCAGAGAGAACCAGACCACCC AGAAGGGACAGAAGAACAGCCGCGAGAGAATGAAGC GGATCGAAGAGGGCATCAAAGAGCTGGGCAGCCAGAT CCTGAAAGAACACCCCGTGGAAAACACCCAGCTGCAG AACGAGAAGCTGTACCTGTACTACCTGCAGAATGGGC GGGATATGTACGTGGACCAGGAACTGGACATCAACCG GCTGTCCGACTACGATGTGGACCATATCGTGCCTCAGA GCTTTCTGAAGGACGACTCCATCGACAACAAGGTGCTG ACCAGAAGCGACAAGAACCGGGGCAAGAGCGACAAC GTGCCCTCCGAAGAGGTCGTGAAGAAGATGAAGAACT ACTGGCGGCAGCTGCTGAACGCCAAGCTGATTACCCA GAGAAAGTTCGACAATCTGACCAAGGCCGAGAGAGGC GGCCTGAGCGAACTGGATAAGGCCGGCTTCATCAAGA GACAGCTGGTGGAAACCCGGCAGATCACAAAGCACGT GGCACAGATCCTGGACTCCCGGATGAACACTAAGTAC GACGAGAATGACAAGCTGATCCGGGAAGTGAAAGTGA TCACCCTGAAGTCCAAGCTGGTGTCCGATTTCCGGAAG GATTTCCAGTTTTACAAAGTGCGCGAGATCAACAACTA CCACCACGCCCACGACGCCTACCTGAACGCCGTCGTGG GAACCGCCCTGATCAAAAAGTACCCTAAGCTGGAAAG CGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTG CGGAAGATGATCGCCAAGAGCGAGCAGGAAATCGGCA AGGCTACCGCCAAGTACTTCTTCTACAGCAACATCATG AACTTTTTCAAGACCGAGATTACCCTGGCCAACGGCGA GATCCGGAAGCGGCCTCTGATCGAGACAAACGGCGAA ACCGGGGAGATCGTGTGGGATAAGGGCCGGGATTTTG CCACCGTGCGGAAAGTGCTGAGCATGCCCCAAGTGAA TATCGTGAAAAAGACCGAGGTGCAGACAGGCGGCTTC AGCAAAGAGTCTATCCTGCCCAAGAGGAACAGCGATA AGCTGATCGCCAGAAAGAAGGACTGGGACCCTAAGAA GTACGGCGGCTTC**GAC**AGCCCCACCGTGGCCTATTCTG TGCTGGTGGTGGCCAAAGTGGAAAAGGGCAAGTCCAA GAAACTGAAGAGTGTGAAAGAGCTGCTGGGGATCACC ATCATGGAAAGAAGCAGCTTCGAGAAGAATCCCATCG ACTTTCTGGAAGCCAAGGGCTACAAAGAAGTGAAAAA GGACCTGATCATCAAGCTGCCTAAGTACTCCCTGTTCG	

		AGCTGGAAAACGGCCGGAAGAGAATGCTGGCCTCTGC CGGCGAACTGCAGAAGGGAAACGAACTGGCCCTGCCC TCCAAATATGTGAACTTCCTGTACCTGGCCAGCCACTA TGAGAAGCTGAAGGGCTCCCCCGAGGATAATGAGCAG AAACAGCTGTTTGTGGAACAGCACAAGCACTACCTGG ACGAGATCATCGAGCAGATCAGCGAGTTCTCCAAGAG AGTGATCCTGGCCGACGCTAATCTGGACAAAGTGCTGT CCGCCTACAACAAGCACCGGGATAAGCCCATCAGAGA GCAGGCCGAGAATATCATCCACCTGTTTACCCTGACCA ATCTGGGAGCCCCTGCCGCCTTCAAGTACTTTGACACC ACCATCGACCGGAAGAGGTACACCAGCACCAAAGAGG TGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGC CTGTACGAGACACGGATCGACCTGTCTCAGCTGGGAG GTGAC	
	Protein	DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDR HSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRIC YLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFG NIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALA HMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEEN PINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFG NLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNL LAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSA SMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNG YAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDL LRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNRE KIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFE EVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYF TVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNR KVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDL LKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKT YAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSG KTILDFLKSDGFAN**R**NFMQLIHDDSLTFKEDIQKAQVSGQ GDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHK PENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQI LKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRL SDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPS EEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYL NAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQ EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGE TGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFS KESILPKRNSDKLIARKKDWDPKKYGGF**D**SPTVAYSVLV VAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAK GYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHK HYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIRE QAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLD ATLIHQSITGLYETRIDLSQLGGD	40
Linker between Cas9 nickase and NLS	DNA	GAGGGAGCTGAT	2184
	Protein	EGAD	2144

Nuclear localization sequence (NLS)	DNA	AAGCGCACCGCCGATGGTTCCGAGTTCGAAAGCCCCA AGAAGAAGAGGAAAGTCTAA	2185
	Protein	KRTADGSEFESPKKKRKV	2146
MS004 mRNA and protein sequences			
Region	**Sequence**		**SEQ ID NO**
Full Sequence	mRNA	AGGAAAu'AAGAGAGAAAAGAAGAGu'AAGAAGAAAu' Au'AAGAGCCACCAu'GGCCCCCAAGAAGAAGCGGAAG Gu'GGGCAu'CCACGGCGu'GCCCGCCGCCGACAAGAAG u'ACAGCAu'CGGCCu'GGACAu'CGGCACCAACAGCGu'G GGCu'GGGCCGu'GAu'CACCGACGAGu'ACAAGGu'GCCC AGCAAGAAGu'u'CAAGGu'GCu'GGGCAACACCGACCGG CACAGCAu'CAAGAAGAACCu'GAu'CGGCGCCCu'GCu'G u'u'CGACAGCGGCGAGACGGCCGAGGCCACCCGGCu'G AAGCGGACCGCCCGGCGGCGGu'ACACCCGGCGGAAG AACCGGAu'Cu'GCu'ACCu'GCAGGAGAu'Cu'u'CAGCAA CGAGAu'GGCCAAGGu'GGACGACAGCu'u'Cu'u'CCACC GGCu'GGAGGAGAGCu'u'CCu'GGu'GGAGGAGGACAAG AAGCACGAGCGGCACCCCAu'Cu'u'CGGCAACAu'CGu'G GACGAGGu'GGCCu'ACCACGAGAAGu'ACCCCACCAu'C u'ACCACCu'GCGGAAGAAGCu'GGu'GGACAGCACCGAC AAGGCCGACCu'GCGGCu'GAu'Cu'ACCu'GGCCCu'GGCC CACAu'GAu'CAAGu'u'CCGGGGCCACu'u'CCu'GAu'CGA GGGCGACCu'GAACCCCGACAACAGCGACGu'GGACAA GCu'Gu'u'CAu'CCAGCu'GGu'GCAGACCu'ACAACCAGCu 'Gu'u'CGAGGAGAACCCCAu'CAACGCCAGCGGCGu'GG ACGCCAAGGCCAu'CCu'GAGCGCCCGGCu'GAGCAAGA GCCGGCGGCu'GGAGAACCu'GAu'CGCCCAGCu'GCCCG GCGAGAAGAAGAACGGCCu'Gu'u'CGGCAACCu'GAu'C GCCCu'GAGCCu'GGGCCu'GACCCCCAACu'u'CAAGAGC AACu'u'CGACCu'GGCCGAGGACGCCAAGCu'GCAGCu'G AGCAAGGACACCu'ACGACGACGACCu'GGACAACCu'G Cu'GGCCCAGAu'CGGCGACCAGu'ACGCCGACCu'Gu'u'C Cu'GGCCGCCAAGAACCu'GAGCGACGCCAu'CCu'GCu'G AGCGACAu'CCu'GCGGGu'GAACACCGAGAu'CACCAAG GCCCCCCu'GAGCGCCAGCAu'GAu'CAAGCGGu'ACGAC GAGCACCACCAGGACCu'GACCCu'GCu'GAAGGCCCu'G Gu'GCGGCAGCAGCu'GCCCGAGAAGu'ACAAGGAGAu'C u'u'Cu'u'CGACCAGAGCAAGAACGGCu'ACGCCGGCu'AC Au'CGACGGCGGCGCCAGCCAGGAGGAGu'u'Cu'ACAAG u'u'CAu'CAAGCCCAu'CCu'GGAGAAGAu'GGACGGCAC CGAGGAGCu'GCu'GGu'GAAGCu'GAACCGGGAGGACCu 'GCu'GCGGAAGCAGCGGACCu'u'CGACAACGGCAGCAu 'CCCCCACCAGAu'CCACCu'GGGCGAGCu'GCACGCCAu' CCu'GCGGCGGCAGGAGGACu'u'Cu'ACCCCu'u'CCu'GA AGGACAACCGGGAGAAGAu'CGAGAAGAu'CCu'GACCu' u'CCGGAu'CCCCu'ACu'ACGu'GGGCCCCCu'GGCCCGGG GCAACAGCCGGu'u'CGCCu'GGAu'GACCCGCAAGAGCG AGGAGACGAu'CACCCCCu'GGAACu'u'CGAGGAGGu'G Gu'GGACAAGGGCGCCAGCGCCCAGAGCu'u'CAu'CGAG CGGAu'GACCAACu'u'CGACAAGAACCu'GCCCAACGAG AAGGu'GCu'GCCCAAGCACAGCCu'GCu'Gu'ACGAGu'A	2186

		Cu'u'CACCGu'Gu'ACAACGAGCu'GACCAAGGu'GAAGu' ACGu'GACCGAGGGCAu'GCGGAAGCCCGCCu'u'CCu'GA GCGGCGAGCAGAAGAAGGCCAu'CGu'GGACCu'GCu'Gu 'u'CAAGACCAACCGGAAGGu'GACCGu'GAAGCAGCu'G AAGGAGGACu'ACu'u'CAAGAAGAu'CGAGu'GCu'u'CGA CAGCGu'GGAGAu'CAGCGGCGu'GGAGGACCGGu'u'CA ACGCCAGCCu'GGGCACCu'ACCACGACCu'GCu'GAAGA u'CAu'CAAGGACAAGGACu'u'CCu'GGACAACGAGGAG AACGAGGACAu'CCu'GGAGGACAu'CGu'GCu'GACCCu' GACCCu'Gu'u'CGAGGACCGGGAGAu'GAu'CGAGGAGC GGCu'GAAGACCu'ACGCCCACCu'Gu'u'CGACGACAAGG u'GAu'GAAGCAGCu'GAAGCGGCGGCGGu'ACACCGGCu 'GGGGCCGGCu'GAGCCGGAAGCu'GAu'CAACGGCAu'C CGGGACAAGCAGAGCGGCAAGACCAu'CCu'GGACu'u'C Cu'CAAGAGCGACGGCu'u'CGCCAACCGGAACu'u'CAu'G CAGCu'GAu'CCACGACGACAGCCu'GACCu'u'CAAGGAG GACAu'CCAGAAGGCCCAGGu'GAGCGGCCAGGGCGAC AGCCu'GCACGAGCACAu'CGCCAACCu'GGCCGGCAGC CCCGCCAu'CAAGAAGGGCAu'CCu'GCAGACCGu'GAAG Gu'GGu'GGACGAGCu'GGu'GAAGGu'GAu'GGGCCGGCA CAAGCCCGAGAACAu'CGu'GAu'CGAGAu'GGCCCGGGA GAACCAGACCACCCAGAAGGGCCAGAAGAACAGCCG GGAGCGGAu'GAAGCGGAu'CGAGGAGGGCAu'CAAGG AGCu'GGGCAGCCAGAu'CCu'GAAGGAGCACCCCGu'GG AGAACACCCAGCu'GCAGAACGAGAAGCu'Gu'ACCu'Gu' ACu'ACCu'GCAGAACGGCCGGGACAu'Gu'ACGu'GGACC AGGAGCu'GGACAu'CAACCGGCu'GAGCGACu'ACGACG u'GGACCACAu'CGu'GCCCCAGAGCu'u'CCu'GAAGGACG ACAGCAu'CGACAACAAGGu'GCu'GACCCGGAGCGACA AGAACCGGGGCAAGAGCGACAACGu'GCCCAGCGAGG AGGu'GGu'GAAGAAGAu'GAAGAACu'ACu'GGCGGCAG Cu'GCu'GAACGCCAAGCu'GAu'CACCCAGCGGAAGu'u'C GACAACCu'GACCAAGGCCGAGCGGGGCGGCCu'GAGC GAGCu'GGACAAGGCCGGCu'u'CAu'CAAGCGGCAGCu'G Gu'GGAGACGCGGCAGAu'CACCAAGCACGu'GGCCCAG Au'CCu'GGACAGCCGGAu'GAACACCAAGu'ACGACGAG AACGACAAGCu'GAu'CCGGGAGGu'GAAGGu'GAu'CAC CCu'CAAGAGCAAGCu'GGu'GAGCGACu'u'CCGGAAGG ACu'u'CCAGu'u'Cu'ACAAGGu'GCGGGAGAu'CAACAAC u'ACCACCACGCCCACGACGCCu'ACCu'GAACGCCGu'G Gu'GGGCACCGCCCu'GAu'CAAGAAGu'ACCCCAAGCu'G GAGAGCGAGu'u'CGu'Gu'ACGGCGACu'ACAAGGu'Gu'A CGACGu'GCGGAAGAu'GAu'CGCCAAGAGCGAGCAGGA GAu'CGGCAAGGCCACCGCCAAGu'ACu'u'Cu'u'Cu'ACA GCAACAu'CAu'GAACu'u'Cu'u'CAAGACCGAGAu'CACC Cu'GGCCAACGGCGAGAu'CCGGAAGCGGCCCCu'GAu'C GAGACGAACGGCGAGACGGGCGAGAu'CGu'Gu'GGGAC AAGGGCCGGGACu'u'CGCCACCGu'GCGGAAGGu'GCu'G AGCAu'GCCCCAGGu'GAACAu'CGu'GAAGAAGACCGAG Gu'GCAGACCGGCGGCu'u'CAGCAAGGAGAGCAu'CCu'G CCCAAGCGGAACAGCGACAAGCu'GAu'CGCCCGGAAG AAGGACu'GGGACCCCAAGAAGu'ACGGCGGCu'u'CGAC AGCCCCACCGu'GGCCu'ACAGCGu'GCu'GGu'GGu'GGCC AAGGu'GGAGAAGGGCAAGAGCAAGAAGCu'CAAGAGC Gu'GAAGGAGCu'GCu'GGGCAu'CACCAu'CAu'GGAGCG	

		GAGCAGCu'u'CGAGAAGAACCCCAu'CGACu'u'CCu'GG AGGCCAAGGGCu'ACAAGGAGGu'GAAGAAGGACCu'GA u'CAu'CAAGCu'GCCCAAGu'ACAGCCu'Gu'u'CGAGCu'G GAGAACGGCCGGAAGCGGAu'GCu'GGCCAGCGCCGGC GAGCu'GCAGAAGGGCAACGAGCu'GGCCCu'GCCCAGC AAGu'ACGu'GAACu'u'CCu'Gu'ACCu'GGCCAGCCACu'A CGAGAAGCu'GAAGGGCAGCCCCGAGGACAACGAGCA GAAGCAGCu'Gu'u'CGu'GGAGCAGCACAAGCACu'ACCu 'GGACGAGAu'CAu'CGAGCAGAu'CAGCGAGu'u'CAGCA AGCGGGu'GAu'CCu'GGCCGACGCCAACCu'GGACAAGG u'GCu'GAGCGCCu'ACAACAAGCACCGGGACAAGCCCA u'CCGGGAGCAGGCCGAGAACAu'CAu'CCACCu'Gu'u'CA CCCu'GACCAACCu'GGGCGCCCCCGCCGCCu'u'CAAGu' ACu'u'CGACACCACCAu'CGACCGGAAGCGGu'ACACCA GCACCAAGGAGGu'GCu'GGACGCCACCCu'GAu'CCACC AGAGCAu'CACCGGCCu'Gu'ACGAGACGCGGAu'CGACC u'GAGCCAGCu'GGGCGGCGACAGCGGCGGCAAGCGGC CCGCCGCCACCAAGAAGGCCGGCCAGGCCAAGAAGAA GAAGu'AAu'GAu'AGGCGGCCGCu'u'AAu'u'AAGCu'GCC u'u'Cu'GCGGGGCu'u'GCCu'u'Cu'GGCCAu'GCCCu'u'Cu'u 'Cu'Cu'CCCu'u'GCACCu'Gu'ACCu'Cu'u'GGu'Cu'u'u'GAA u'AAAGCCu'GAGu'AGGAAGu'Cu'AGA	
5′ UTR	mRNA	AGGAAAu'AAGAGAGAAAAGAAGAGu'AAGAAGAAAu' Au'AAGAGCCACC	2138
CDS	mRNA	Au'GGCCCCCAAGAAGAAGCGGAAGGu'GGGCAu'CCAC GGCGu'GCCCGCCGCCGACAAGAAGu'ACAGCAu'CGGC Cu'GGACAu'CGGCACCAACAGCGu'GGGCu'GGGCCGu'G Au'CACCGACGAGu'ACAAGGu'GCCCAGCAAGAAGu'u'C AAGGu'GCu'GGGCAACACCGACCGGCACAGCAu'CAAG AAGAACCu'GAu'CGGCGCCCu'GCu'Gu'u'CGACAGCGGC GAGACGGCCGAGGCCACCCGGCu'GAAGCGGACCGCCC GGCGGCGGu'ACACCCGGCGGAAGAACCGGAu'Cu'GCu' ACCu'GCAGGAGAu'Cu'u'CAGCAACGAGAu'GGCCAAG Gu'GGACGACAGCu'u'Cu'u'CCACCGGCu'GGAGGAGAG Cu'u'CCu'GGu'GGAGGAGGACAAGAAGCACGAGCGGCA CCCCAu'Cu'u'CGGCAACAu'CGu'GGACGAGGu'GGCCu' ACCACGAGAAGu'ACCCCACCAu'Cu'ACCACCu'GCGGA AGAAGCu'GGu'GGACAGCACCGACAAGGCCGACCu'GC GGCu'GAu'Cu'ACCu'GGCCCu'GGCCCACAu'GAu'CAAGu 'u'CCGGGGCCACu'u'CCu'GAu'CGAGGGCGACCu'GAAC CCCGACAACAGCGACGu'GGACAAGCu'Gu'u'CAu'CCAG Cu'GGu'GCAGACCu'ACAACCAGCu'Gu'u'CGAGGAGAA CCCCAu'CAACGCCAGCGGCGu'GGACGCCAAGGCCAu' CCu'GAGCGCCCGGCu'GAGCAAGAGCCGGCGGCu'GGA GAACCu'GAu'CGCCCAGCu'GCCCGGCGAGAAGAAGAA CGGCCu'Gu'u'CGGCAACCu'GAu'CGCCCu'GAGCCu'GG GCCu'GACCCCCAACu'u'CAAGAGCAACu'u'CGACCu'GG CCGAGGACGCCAAGCu'GCAGCu'GAGCAAGGACACCu' ACGACGACGACCu'GGACAACCu'GCu'GGCCCAGAu'CG GCGACCAGu'ACGCCGACCu'Gu'u'CCu'GGCCGCCAAGA ACCu'GAGCGACGCCAu'CCu'GCu'GAGCGACAu'CCu'GC GGGu'GAACACCGAGAu'CACCAAGGCCCCCCu'GAGCG CCAGCAu'GAu'CAAGCGGu'ACGACGAGCACCACCAGG ACCu'GACCCu'GCu'GAAGGCCCu'GGu'GCGGCAGCAGC u'GCCCGAGAAGu'ACAAGGAGAu'Cu'u'Cu'u'CGACCAG	2187

		AGCAAGAACGGCu'ACGCCGGCu'ACAu'CGACGGCGGC GCCAGCCAGGAGGAGu'u'Cu'ACAAGu'u'CAu'CAAGCC CAu'CCu'GGAGAAGAu'GGACGGCACCGAGGAGCu'GCu 'GGu'GAAGCu'GAACCGGGAGGACCu'GCu'GCGGAAGC AGCGGACCu'u'CGACAACGGCAGCAu'CCCCCACCAGAu 'CCACCu'GGGCGAGCu'GCACGCCAu'CCu'GCGGCGGCA GGAGGACu'u'Cu'ACCCCu'u'CCu'GAAGGACAACCGGG AGAAGAu'CGAGAAGAu'CCu'GACCu'u'CCGGAu'CCCCu 'ACu'ACGu'GGGCCCCCu'GGCCCGGGGCAACAGCCGGu' u'CGCCu'GGAu'GACCCGCAAGAGCGAGGAGACGAu'CA CCCCCu'GGAACu'u'CGAGGAGGu'GGu'GGACAAGGGC GCCAGCGCCCAGAGCu'u'CAu'CGAGCGGAu'GACCAAC u'u'CGACAAGAACCu'GCCCAACGAGAAGGu'GCu'GCCC AAGCACAGCCu'GCu'Gu'ACGAGu'ACu'u'CACCGu'Gu'A CAACGAGCu'GACCAAGGu'GAAGu'ACGu'GACCGAGGG CAu'GCGGAAGCCCGCCu'u'CCu'GAGCGGCGAGCAGAA GAAGGCCAu'CGu'GGACCu'GCu'Gu'u'CAAGACCAACC GGAAGGu'GACCGu'GAAGCAGCu'GAAGGAGGACu'ACu 'u'CAAGAAGAu'CGAGu'GCu'u'CGACAGCGu'GGAGAu'C AGCGGCGu'GGAGGACCGGu'u'CAACGCCAGCCu'GGGC ACCu'ACCACGACCu'GCu'GAAGAu'CAu'CAAGGACAAG GACu'u'CCu'GGACAACGAGGAGAACGAGGACAu'CCu' GGAGGACAu'CGu'GCu'GACCCu'GACCCu'Gu'u'CGAGG ACCGGGAGAu'GAu'CGAGGAGCGGCu'GAAGACCu'ACG CCCACCu'Gu'u'CGACGACAAGGu'GAu'GAAGCAGCu'G AAGCGGCGGCGGu'ACACCGGCu'GGGGCCGGCu'GAGC CGGAAGCu'GAu'CAACGGCAu'CCGGGACAAGCAGAGC GGCAAGACCAu'CCu'GGACu'u'CCu'CAAGAGCGACGGC u'u'CGCCAACCGGAACu'u'CAu'GCAGCu'GAu'CCACGA CGACAGCCu'GACCu'u'CAAGGAGGACAu'CCAGAAGGC CCAGGu'GAGCGGCCAGGGCGACAGCCu'GCACGAGCA CAu'CGCCAACCu'GGCCGGCAGCCCCGCCAu'CAAGAA GGGCAu'CCu'GCAGACCGu'GAAGGu'GGu'GGACGAGCu 'GGu'GAAGGu'GAu'GGGCCGGCACAAGCCCGAGAACAu 'CGu'GAu'CGAGAu'GGCCCGGGAGAACCAGACCACCCA GAAGGGCCAGAAGAACAGCCGGGAGCGGAu'GAAGCG GAu'CGAGGAGGGCAu'CAAGGAGCu'GGGCAGCCAGAu 'CCu'GAAGGAGCACCCCGu'GGAGAACACCCAGCu'GCA GAACGAGAAGCu'Gu'ACCu'Gu'ACu'ACCu'GCAGAACG GCCGGGACAu'Gu'ACGu'GGACCAGGAGCu'GGACAu'C AACCGGCu'GAGCGACu'ACGACGu'GGACCACAu'CGu'G CCCCAGAGCu'u'CCu'GAAGGACGACAGCAu'CGACAAC AAGGu'GCu'GACCCGGAGCGACAAGAACCGGGGCAAG AGCGACAACGu'GCCCAGCGAGGAGGu'GGu'GAAGAAG Au'GAAGAACu'ACu'GGCGGCAGCu'GCu'GAACGCCAA GCu'GAu'CACCCAGCGGAAGu'u'CGACAACCu'GACCAA GGCCGAGCGGGGCGGCCu'GAGCGAGCu'GGACAAGGC CGGCu'u'CAu'CAAGCGGCAGCu'GGu'GGAGACGCGGCA GAu'CACCAAGCACGu'GGCCCAGAu'CCu'GGACAGCCG GAu'GAACACCAAGu'ACGACGAGAACGACAAGCu'GAu' CCGGGAGGu'GAAGGu'GAu'CACCCu'CAAGAGCAAGCu 'GGu'GAGCGACu'u'CCGGAAGGACu'u'CCAGu'u'Cu'ACA AGGu'GCGGGAGAu'CAACAACu'ACCACCACGCCCACG ACGCCu'ACCu'GAACGCCGu'GGu'GGGCACCGCCCu'GA u'CAAGAAGu'ACCCCAAGCu'GGAGAGCGAGu'u'CGu'Gu	

		'ACGGCGACu'ACAAGGu'Gu'ACGACGu'GCGGAAGAu'G Au'CGCCAAGAGCGAGCAGGAGAu'CGGCAAGGCCACC GCCAAGu'ACu'u'Cu'u'Cu'ACAGCAACAu'CAu'GAACu'u' Cu'u'CAAGACCGAGAu'CACCCu'GGCCAACGGCGAGAu' CCGGAAGCGGCCCCu'GAu'CGAGACGAACGGCGAGAC GGGCGAGAu'CGu'Gu'GGGACAAGGGCCGGGACu'u'CG CCACCGu'GCGGAAGGu'GCu'GAGCAu'GCCCCAGGu'GA ACAu'CGu'GAAGAAGACCGAGGu'GCAGACCGGCGGCu' u'CAGCAAGGAGAGCAu'CCu'GCCCAAGCGGAACAGCG ACAAGCu'GAu'CGCCCGGAAGAAGGACu'GGGACCCCA AGAAGu'ACGGCGGCu'u'CGACAGCCCCACCGu'GGCCu' ACAGCGu'GCu'GGu'GGu'GGCCAAGGu'GGAGAAGGGC AAGAGCAAGAAGCu'CAAGAGCGu'GAAGGAGCu'GCu' GGGCAu'CACCAu'CAu'GGAGCGGAGCAGCu'u'CGAGA AGAACCCCAu'CGACu'u'CCu'GGAGGCCAAGGGCu'ACA AGGAGGu'GAAGAAGGACCu'GAu'CAu'CAAGCu'GCCC AAGu'ACAGCCu'Gu'u'CGAGCu'GGAGAACGGCCGGAA GCGGAu'GCu'GGCCAGCGCCGGCGAGCu'GCAGAAGGG CAACGAGCu'GGCCCu'GCCCAGCAAGu'ACGu'GAACu'u' CCu'Gu'ACCu'GGCCAGCCACu'ACGAGAAGCu'GAAGGG CAGCCCCGAGGACAACGAGCAGAAGCAGCu'Gu'u'CGu' GGAGCAGCACAAGCACu'ACCu'GGACGAGAu'CAu'CGA GCAGAu'CAGCGAGu'u'CAGCAAGCGGGu'GAu'CCu'GG CCGACGCCAACCu'GGACAAGGu'GCu'GAGCGCCu'ACA ACAAGCACCGGGACAAGCCCAu'CCGGGAGCAGGCCG AGAACAu'CAu'CCACCu'Gu'u'CACCCu'GACCAACCu'GG GCGCCCCCGCCGCCu'u'CAAGu'ACu'u'CGACACCACCA u'CGACCGGAAGCGGu'ACACCAGCACCAAGGAGGu'GC u'GGACGCCACCCu'GAu'CCACCAGAGCAu'CACCGGCC u'Gu'ACGAGACGCGGAu'CGACCu'GAGCCAGCu'GGGCG GCGACAGCGGCGGCAAGCGGCCCGCCGCCACCAAGAA GGCCGGCCAGGCCAAGAAGAAGAAGu'AAu'GAu'AG	
	Protein	MAPKKKRKVGIHGVPAADKKYSIGLDIGTNSVGWAVITD EYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATR LKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEE SFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLV DSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVD KLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLE NLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAK LQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILL SDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQ LPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEK MDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAI LRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFA WMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNL PNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLS GEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEIS GVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVL TLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGW GRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHD DSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQ TVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQ NGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKV LTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQ	2188

		RKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQI LDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFY KVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYG DYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEIT LANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMP QVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPK KYGGF**D**SPTVAYSVLVVAKVEKGKSKKLKSVKELLGITI MERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENG RKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKG SPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLD KVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFD TTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDS GGKRPAATKKAGQAKKKK	
3′ UTR	mRNA	GCGGCCGCu’u’AAu’u’AAGCu’GCCu’u’Cu’GCGGGGCu’u’ GCCu’u’Cu’GGCCAu’GCCCu’u’Cu’u’Cu’Cu’CCCu’u’GCAC Cu’Gu’ACCu’Cu’u’GGu’Cu’u’u’GAAu’AAAGCCu’GAGu’A GGAAGu’Cu’AGA	2147
MS010 mRNA and protein sequences			
Region	**Sequence**		**SEQ ID NO**
Full Sequence	mRNA	AGGAAAu’AAGAGAGAAAAGAAGAGu’AAGAAGAAAu’ Au’AAGAGCCACCAu’GGCCCCCAAGAAGAAGCGCAAG Gu’GGGCAu’CCACGGCGu’GCCCGCCGCCGACAAGAAG u’ACAGCAu’CGGCCu’GGACAu’CGGCACCAACAGCGu’G GGCu’GGGCCGu’GAu’CACCGACGAGu’ACAAGGu’GCCC AGCAAGAAGu’u’CAAGGu’GCu’GGGCAACACCGACCGC CACAGCAu’CAAGAAGAACCu’GAu’CGGCGCCCu’GCu’G u’u’CGACAGCGGCGAGACAGCCGAGGCCACCCGCCu’G AAGCGCACCGCCCGCCGCCGCu’ACACCCGCCGCAAGA ACCGCAu’Cu’GCu’ACCu’GCAGGAGAu’Cu’u’CAGCAAC GAGAu’GGCCAAGGu’GGACGACAGCu’u’Cu’u’CCACCG CCu’GGAGGAGAGCu’u’CCu’GGu’GGAGGAGGACAAGA AGCACGAGCGCCACCCCAu’Cu’u’CGGCAACAu’CGu’GG ACGAGGu’GGCCu’ACCACGAGAAGu’ACCCCACCAu’Cu’ ACCACCu’GCGCAAGAAGCu’GGu’GGACAGCACCGACA AGGCCGACCu’GCGCCu’GAu’Cu’ACCu’GGCCCu’GGCCC ACAu’GAu’CAAGu’u’CCGCGGCCACu’u’CCu’GAu’CGAG GGCGACCu’GAACCCCGACAACAGCGACGu’GGACAAG Cu’Gu’u’CAu’CCAGCu’GGu’GCAGACCu’ACAACCAGCu’ Gu’u’CGAGGAGAACCCCAu’CAACGCCAGCGGCGu’GGA CGCCAAGGCCAu’CCu’GAGCGCCCGCCu’GAGCAAGAG CCGCCGCCu’GGAGAACCu’GAu’CGCCCAGCu’GCCCGG CGAGAAGAAGAACGGCCu’Gu’u’CGGCAACCu’GAu’CGC CCu’GAGCCu’GGGCCu’GACCCCCAACu’u’CAAGAGCAA Cu’u’CGACCu’GGCCGAGGACGCCAAGCu’GCAGCu’GAG CAAGGACACCu’ACGACGACGACCu’GGACAACCu’GCu’ GGCCCAGAu’CGGCGACCAGu’ACGCCGACCu’Gu’u’CCu’ GGCCGCCAAGAACCu’GAGCGACGCCAu’CCu’GCu’GAG CGACAu’CCu’GCGCGu’GAACACCGAGAu’CACCAAGGC CCCCCu’GAGCGCCAGCAu’GAu’CAAGCGCu’ACGACGA GCACCACCAGGACCu’GACCCu’GCu’GAAGGCCCu’GGu’ GCGCCAGCAGCu’GCCCGAGAAGu’ACAAGGAGAu’Cu’u’ Cu’u’CGACCAGAGCAAGAACGGCu’ACGCCGGCu’ACAu’ CGACGGCGGCGCCAGCCAGGAGGAGu’u’Cu’ACAAGu’u’	2189

		CAu'CAAGCCCAu'CCu'GGAGAAGAu'GGACGGCACCGA GGAGCu'GCu'GGu'GAAGCu'GAACCGCGAGGACCu'GCu 'GCGCAAGCAGCGCACCu'u'CGACAACGGCAGCAu'CCC CCACCAGAu'CCACCu'GGGCGAGCu'GCACGCCAu'CCu' GCGCCGCCAGGAGGACu'u'Cu'ACCCCu'u'CCu'GAAGGA CAACCGCGAGAAGAu'CGAGAAGAu'CCu'GACCu'u'CCG CAu'CCCCu'ACu'ACGu'GGGCCCCCu'GGCCCGCGGCAA CAGCCGCu'u'CGCCu'GGAu'GACCCGCAAGAGCGAGGA GACAAu'CACCCCCu'GGAACu'u'CGAGGAGGu'GGu'GG ACAAGGGCGCCAGCGCCCAGAGCu'u'CAu'CGAGCGCA u'GACCAACu'u'CGACAAGAACCu'GCCCAACGAGAAGG u'GCu'GCCCAAGCACAGCCu'GCu'Gu'ACGAGu'ACu'u'C ACCGu'Gu'ACAACGAGCu'GACCAAGGu'GAAGu'ACGu' GACCGAGGGCAu'GCGCAAGCCCGCCu'u'CCu'GAGCGG CGAGCAGAAGAAGGCCAu'CGu'GGACCu'GCu'Gu'u'CA AGACCAACCGCAAGGu'GACCGu'GAAGCAGCu'GAAGG AGGACu'ACu'u'CAAGAAGAu'CGAGu'GCu'u'CGACAGC Gu'GGAGAu'CAGCGGCGu'GGAGGACCGCu'u'CAACGCC AGCCu'GGGCACCu'ACCACGACCu'GCu'GAAGAu'CAu'C AAGGACAAGGACu'u'CCu'GGACAACGAGGAGAACGAG GACAu'CCu'GGAGGACAu'CGu'GCu'GACCCu'GACCCu' Gu'u'CGAGGACCGCGAGAu'GAu'CGAGGAGCGCCu'GA AGACCu'ACGCCCACCu'Gu'u'CGACGACAAGGu'GAu'G AAGCAGCu'GAAGCGCCGCCGCu'ACACCGGCu'GGGGC CGCCu'GAGCCGCAAGCu'GAu'CAACGGCAu'CCGCGAC AAGCAGAGCGGCAAGACCAu'CCu'GGACu'u'CCu'CAAG AGCGACGGCu'u'CGCCAACCGCAACu'u'CAu'GCAGCu'G Au'CCACGACGACAGCCu'GACCu'u'CAAGGAGGACAu'C CAGAAGGCCCAGGu'GAGCGGCCAGGGCGACAGCCu'G CACGAGCACAu'CGCCAACCu'GGCCGGCAGCCCCGCCA u'CAAGAAGGGCAu'CCu'GCAGACCGu'GAAGGu'GGu'G GACGAGCu'GGu'GAAGGu'GAu'GGGCCGCCACAAGCCC GAGAACAu'CGu'GAu'CGAGAu'GGCCCGCGAGAACCAG ACCACCCAGAAGGGCCAGAAGAACAGCCGCGAGCGCA u'GAAGCGCAu'CGAGGAGGGCAu'CAAGGAGCu'GGGC AGCCAGAu'CCu'GAAGGAGCACCCCGu'GGAGAACACC CAGCu'GCAGAACGAGAAGCu'Gu'ACCu'Gu'ACu'ACCu' GCAGAACGGCCGCGACAu'Gu'ACGu'GGACCAGGAGCu' GGACAu'CAACCGCCu'GAGCGACu'ACGACGu'GGACCA CAu'CGu'GCCCCAGAGCu'u'CCu'GAAGGACGACAGCAu' CGACAACAAGGu'GCu'GACCCGCAGCGACAAGAACCG CGGCAAGAGCGACAACGu'GCCCAGCGAGGAGGu'GGu' GAAGAAGAu'GAAGAACu'ACu'GGCGCCAGCu'GCu'GA ACGCCAAGCu'GAu'CACCCAGCGCAAGu'u'CGACAACC u'GACCAAGGCCGAGCGCGGCGGCCu'GAGCGAGCu'GG ACAAGGCCGGCu'u'CAu'CAAGCGCCAGCu'GGu'GGAGA CACGCCAGAu'CACCAAGCACGu'GGCCCAGAu'CCu'GG ACAGCCGCAu'GAACACCAAGu'ACGACGAGAACGACA AGCu'GAu'CCGCGAGGu'GAAGGu'GAu'CACCCu'CAAG AGCAAGCu'GGu'GAGCGACu'u'CCGCAAGGACu'u'CCA Gu'u'Cu'ACAAGGu'GCGCGAGAu'CAACAACu'ACCACC ACGCCCACGACGCCu'ACCu'GAACGCCGu'GGu'GGGCA CCGCCCu'GAu'CAAGAAGu'ACCCCAAGCu'GGAGAGCG AGu'u'CGu'Gu'ACGGCGACu'ACAAGGu'Gu'ACGACGu'G CGCAAGAu'GAu'CGCCAAGAGCGAGCAGGAGAu'CGGC	

		AAGGCCACCGCCAAGu'ACu'u'Cu'u'Cu'ACAGCAACAu' CAu'GAACu'u'Cu'u'CAAGACCGAGAu'CACCCu'GGCCA ACGGCGAGAu'CCGCAAGCGCCCCCu'GAu'CGAGACAA ACGGCGAGACAGGCGAGAu'CGu'Gu'GGGACAAGGGCC GCGACu'u'CGCCACCGu'GCGCAAGGu'GCu'GAGCAu'GC CCCAGGu'GAACAu'CGu'GAAGAAGACCGAGGu'GCAGA CCGGCGGCu'u'CAGCAAGGAGAGCAu'CCu'GCCCAAGC GCAACAGCGACAAGCu'GAu'CGCCCGCAAGAAGGACu' GGGACCCCAAGAAGu'ACGGCGGCu'u'CGACAGCCCCA CCGu'GGCCu'ACAGCGu'GCu'GGu'GGu'GGCCAAGGu'G GAGAAGGGCAAGAGCAAGAAGCu'CAAGAGCGu'GAAG GAGCu'GCu'GGGCAu'CACCAu'CAu'GGAGCGCAGCAGC u'u'CGAGAAGAACCCCAu'CGACu'u'CCu'GGAGGCCAA GGGCu'ACAAGGAGGu'GAAGAAGGACCu'GAu'CAu'CA AGCu'GCCCAAGu'ACAGCCu'Gu'u'CGAGCu'GGAGAAC GGCCGCAAGCGCAu'GCu'GGCCAGCGCCGGCGAGCu'G CAGAAGGGCAACGAGCu'GGCCCu'GCCCAGCAAGu'AC Gu'GAACu'u'CCu'Gu'ACCu'GGCCAGCCACu'ACGAGAA GCu'GAAGGGCAGCCCCGAGGACAACGAGCAGAAGCA GCu'Gu'u'CGu'GGAGCAGCACAAGCACu'ACCu'GGACG AGAu'CAu'CGAGCAGAu'CAGCGAGu'u'CAGCAAGCGC Gu'GAu'CCu'GGCCGACGCCAACCu'GGACAAGGu'GCu' GAGCGCCu'ACAACAAGCACCGCGACAAGCCCAu'CCG CGAGCAGGCCGAGAACAu'CAu'CCACCu'Gu'u'CACCCu' GACCAACCu'GGGCGCCCCCGCCGCCu'u'CAAGu'ACu'u' CGACACCACCAu'CGACCGCAAGCGCu'ACACCAGCACC AAGGAGGu'GCu'GGACGCCACCCu'GAu'CCACCAGAGC Au'CACCGGCCu'Gu'ACGAGACACGCAu'CGACCu'GAGC CAGCu'GGGCGGCGACAGCGGCGGCAAGCGCCCCGCCG CCACCAAGAAGGCCGGCCAGGCCAAGAAGAAGAAGu' AAu'AAu'AAGCGGCCGCu'u'AAu'u'AAGCu'GCCu'u'Cu'G CGGGGCu'u'GCCu'u'Cu'GGCCAu'GCCCu'u'Cu'u'Cu'Cu'C CCu'u'GCACCu'Gu'ACCu'Cu'u'GGu'Cu'u'u'GAAu'AAAG CCu'GAGu'AGGAAGu'Cu'AGA	
5′ UTR	mRNA	AGGAAAu'AAGAGAGAAAAGAAGAGu'AAGAAGAAAu' Au'AAGAGCCACC	2138
CDS	mRNA	Au'GGCCCCCAAGAAGAAGCGCAAGGu'GGGCAu'CCAC GGCGu'GCCCGCCGCCGACAAGAAGu'ACAGCAu'CGGC Cu'GGACAu'CGGCACCAACAGCGu'GGGCu'GGGCCGu'G Au'CACCGACGAGu'ACAAGGu'GCCCAGCAAGAAGu'u'C AAGGu'GCu'GGGCAACACCGACCGCCACAGCAu'CAAG AAGAACCu'GAu'CGGCGCCCu'GCu'Gu'u'CGACAGCGGC GAGACAGCCGAGGCCACCCGCCu'GAAGCGCACCGCCC GCCGCCGCu'ACACCCGCCGCAAGAACCGCAu'Cu'GCu' ACCu'GCAGGAGAu'Cu'u'CAGCAACGAGAu'GGCCAAG Gu'GGACGACAGCu'u'Cu'u'CCACCGCCu'GGAGGAGAG Cu'u'CCu'GGu'GGAGGAGGACAAGAAGCACGAGCGCCA CCCCAu'Cu'u'CGGCAACAu'CGu'GGACGAGGu'GGCCu' ACCACGAGAAGu'ACCCCACCAu'Cu'ACCACCu'GCGCA AGAAGCu'GGu'GGACAGCACCGACAAGGCCGACCu'GC GCCu'GAu'Cu'ACCu'GGCCCu'GGCCCACAu'GAu'CAAGu 'u'CCGCGGCCACu'u'CCu'GAu'CGAGGGCGACCu'GAAC CCCGACAACAGCGACGu'GGACAAGCu'Gu'u'CAu'CCAG Cu'GGu'GCAGACCu'ACAACCAGCu'Gu'u'CGAGGAGAA CCCCAu'CAACGCCAGCGGCGu'GGACGCCAAGGCCAu'	2190

		CCu'GAGCGCCCGCCu'GAGCAAGAGCCGCCGCCu'GGA GAACCu'GAu'CGCCCAGCu'GCCCGGCGAGAAGAAGAA CGGCCu'Gu'u'CGGCAACCu'GAu'CGCCCu'GAGCCu'GG GCCu'GACCCCCAACu'u'CAAGAGCAACu'u'CGACCu'GG CCGAGGACGCCAAGCu'GCAGCu'GAGCAAGGACACCu' ACGACGACGACCu'GGACAACCu'GCu'GGCCCAGAu'CG GCGACCAGu'ACGCCGACCu'Gu'u'CCu'GGCCGCCAAGA ACCu'GAGCGACGCCAu'CCu'GCu'GAGCGACAu'CCu'GC GCGu'GAACACCGAGAu'CACCAAGGCCCCCCu'GAGCG CCAGCAu'GAu'CAAGCGCu'ACGACGAGCACCACCAGG ACCu'GACCCu'GCu'GAAGGCCCu'GGu'GCGCCAGCAGC u'GCCCGAGAAGu'ACAAGGAGAu'Cu'u'Cu'u'CGACCAG AGCAAGAACGGCu'ACGCCGGCu'ACAu'CGACGGCGGC GCCAGCCAGGAGGAGu'u'Cu'ACAAGu'u'CAu'CAAGCC CAu'CCu'GGAGAAGAu'GGACGGCACCGAGGAGCu'GCu 'GGu'GAAGCu'GAACCGCGAGGACCu'GCu'GCGCAAGC AGCGCACCu'u'CGACAACGGCAGCAu'CCCCCACCAGAu 'CCACCu'GGGCGAGCu'GCACGCCAu'CCu'GCGCCGCCA GGAGGACu'u'Cu'ACCCCu'u'CCu'GAAGGACAACCGCGA GAAGAu'CGAGAAGAu'CCu'GACCu'u'CCGCAu'CCCCu' ACu'ACGu'GGGCCCCCu'GGCCCGCGGCAACAGCCGCu' u'CGCCu'GGAu'GACCCGCAAGAGCGAGGAGACAAu'CA CCCCCu'GGAACu'u'CGAGGAGGu'GGu'GGACAAGGGC GCCAGCGCCCAGAGCu'u'CAu'CGAGCGCAu'GACCAAC u'u'CGACAAGAACCu'GCCCAACGAGAAGGu'GCu'GCCC AAGCACAGCCu'GCu'Gu'ACGAGu'ACu'u'CACCGu'Gu'A CAACGAGCu'GACCAAGGu'GAAGu'ACGu'GACCGAGGG CAu'GCGCAAGCCCGCCu'u'CCu'GAGCGGCGAGCAGAA GAAGGCCAu'CGu'GGACCu'GCu'Gu'u'CAAGACCAACC GCAAGGu'GACCGu'GAAGCAGCu'GAAGGAGGACu'ACu 'u'CAAGAAGAu'CGAGu'GCu'u'CGACAGCGu'GGAGAu'C AGCGGCGu'GGAGGACCGCu'u'CAACGCCAGCCu'GGGC ACCu'ACCACGACCu'GCu'GAAGAu'CAu'CAAGGACAAG GACu'u'CCu'GGACAACGAGGAGAACGAGGACAu'CCu' GGAGGACAu'CGu'GCu'GACCCu'GACCCu'Gu'u'CGAGG ACCGCGAGAu'GAu'CGAGGAGCGCCu'GAAGACCu'ACG CCCACCu'Gu'u'CGACGACAAGGu'GAu'GAAGCAGCu'G AAGCGCCGCCGCu'ACACCGGCu'GGGGCCGCCu'GAGC CGCAAGCu'GAu'CAACGGCAu'CCGCGACAAGCAGAGC GGCAAGACCAu'CCu'GGACu'u'CCu'CAAGAGCGACGGC u'u'CGCCAACCGCAACu'u'CAu'GCAGCu'GAu'CCACGAC GACAGCCu'GACCu'u'CAAGGAGGACAu'CCAGAAGGCC CAGGu'GAGCGGCCAGGGCGACAGCCu'GCACGAGCAC Au'CGCCAACCu'GGCCGGCAGCCCCGCCAu'CAAGAAG GGCAu'CCu'GCAGACCGu'GAAGGu'GGu'GGACGAGCu' GGu'GAAGGu'GAu'GGGCCGCCACAAGCCCGAGAACAu' CGu'GAu'CGAGAu'GGCCCGCGAGAACCAGACCACCCA GAAGGGCCAGAAGAACAGCCGCGAGCGCAu'GAAGCG CAu'CGAGGAGGGCAu'CAAGGAGCu'GGGCAGCCAGAu' CCu'GAAGGAGCACCCCGu'GGAGAACACCCAGCu'GCA GAACGAGAAGCu'Gu'ACCu'Gu'ACu'ACCu'GCAGAACG GCCGCGACAu'Gu'ACGu'GGACCAGGAGCu'GGACAu'CA ACCGCCu'GAGCGACu'ACGACGu'GGACCACAu'CGu'GC CCCAGAGCu'u'CCu'GAAGGACGACAGCAu'CGACAACA AGGu'GCu'GACCCGCAGCGACAAGAACCGCGGCAAGA	

		GCGACAACGu'GCCCAGCGAGGAGGu'GGu'GAAGAAGA u'GAAGAACu'ACu'GGCGCCAGCu'GCu'GAACGCCAAGC u'GAu'CACCCAGCGCAAGu'u'CGACAACCu'GACCAAGG CCGAGCGCGGCGGCCu'GAGCGAGCu'GGACAAGGCCG GCu'u'CAu'CAAGCGCCAGCu'GGu'GGAGACACGCCAGA u'CACCAAGCACGu'GGCCCAGAu'CCu'GGACAGCCGCA u'GAACACCAAGu'ACGACGAGAACGACAAGCu'GAu'CC GCGAGGu'GAAGGu'GAu'CACCCu'CAAGAGCAAGCu'G Gu'GAGCGACu'u'CCGCAAGGACu'u'CCAGu'u'Cu'ACAA GGu'GCGCGAGAu'CAACAACu'ACCACCACGCCCACGA CGCCu'ACCu'GAACGCCGu'GGu'GGGCACCGCCCu'GAu' CAAGAAGu'ACCCCAAGCu'GGAGAGCGAGu'u'CGu'Gu' ACGGCGACu'ACAAGGu'Gu'ACGACGu'GCGCAAGAu'G Au'CGCCAAGAGCGAGCAGGAGAu'CGGCAAGGCCACC GCCAAGu'ACu'u'Cu'u'Cu'ACAGCAACAu'CAu'GAACu'u' Cu'u'CAAGACCGAGAu'CACCCu'GGCCAACGGCGAGAu' CCGCAAGCGCCCCCu'GAu'CGAGACAAACGGCGAGAC AGGCGAGAu'CGu'Gu'GGGACAAGGGCCGCGACu'u'CG CCACCGu'GCGCAAGGu'GCu'GAGCAu'GCCCCAGGu'GA ACAu'CGu'GAAGAAGACCGAGGu'GCAGACCGGCGGCu' u'CAGCAAGGAGAGCAu'CCu'GCCCAAGCGCAACAGCG ACAAGCu'GAu'CGCCCGCAAGAAGGACu'GGGACCCCA AGAAGu'ACGGCGGCu'u'CGACAGCCCCACCGu'GGCCu' ACAGCGu'GCu'GGu'GGu'GGCCAAGGu'GGAGAAGGGC AAGAGCAAGAAGCu'CAAGAGCGu'GAAGGAGCu'GCu' GGGCAu'CACCAu'CAu'GGAGCGCAGCAGCu'u'CGAGAA GAACCCCAu'CGACu'u'CCu'GGAGGCCAAGGGCu'ACAA GGAGGu'GAAGAAGGACCu'GAu'CAu'CAAGCu'GCCCA AGu'ACAGCCu'Gu'u'CGAGCu'GGAGAACGGCCGCAAGC GCAu'GCu'GGCCAGCGCCGGCGAGCu'GCAGAAGGGCA ACGAGCu'GGCCCu'GCCCAGCAAGu'ACGu'GAACu'u'CC u'Gu'ACCu'GGCCAGCCACu'ACGAGAAGCu'GAAGGGCA GCCCCGAGGACAACGAGCAGAAGCAGCu'Gu'u'CGu'GG AGCAGCACAAGCACu'ACCu'GGACGAGAu'CAu'CGAGC AGAu'CAGCGAGu'u'CAGCAAGCGCGu'GAu'CCu'GGCC GACGCCAACCu'GGACAAGGu'GCu'GAGCGCCu'ACAAC AAGCACCGCGACAAGCCCAu'CCGCGAGCAGGCCGAG AACAu'CAu'CCACCu'Gu'u'CACCCu'GACCAACCu'GGGC GCCCCCGCCGCCu'u'CAAGu'ACu'u'CGACACCACCAu'C GACCGCAAGCGCu'ACACCAGCACCAAGGAGGu'GCu'G GACGCCACCCu'GAu'CCACCAGAGCAu'CACCGGCCu'G u'ACGAGACACGCAu'CGACCu'GAGCCAGCu'GGGCGGC GACAGCGGCGGCAAGCGCCCCGCCGCCACCAAGAAGG CCGGCCAGGCCAAGAAGAAGAAGu'AAu'AAu'AA	
	Protein	MAPKKKRKVGIHGVPAADKKYSIGLDIGTNSVGWAVITD EYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATR LKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEE SFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLV DSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVD KLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLE NLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAK LQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILL SDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQ LPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEK MDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAI	2188

		LRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFA WMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNL PNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLS GEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEIS GVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVL TLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGW GRLSRKLINGIRDKQSGKTILDFLKSDGFAN**R**NFMQLIHD DSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQ TVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQ NGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKV LTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQ RKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQI LDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFY KVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYG DYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEIT LANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMP QVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPK KYGGF**D**SPTVAYSVLVVAKVEKGKSKKLKSVKELLGITI MERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENG RKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKG SPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLD KVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFD TTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDS GGKRPAATKKAGQAKKKK	
3′ UTR	mRNA	GCGGCCGCu’u’AAu’u’AAGCu’GCCu’u’Cu’GCGGGGCu’u’ GCCu’u’Cu’GGCCAu’GCCCu’u’Cu’u’Cu’Cu’CCCu’u’GCAC Cu’Gu’ACCu’Cu’u’GGu’Cu’u’u’GAAu’AAAGCCu’GAGu’A GGAAGu’Cu’AGA	2147
The mutations at amino acid positions 691 and 1135 of the nCas9 component and their corresponding nucleotide sequences are indicated as bold and underlined. u’: N^1-methylpseudouridine The first nucleotide in the 5′ UTR has a 2′-O-methyl modification.			

Table 24. Guide RNA (SgRNA/gRNA) sequences

Gene Target	gRNA ID	Protospacer (5'-3')	SEQ ID NO:	Sequence (5'-3')	SEQ ID NO:	Editor
hcPCSK9	GA012	CGCCTGCCAGCGCCTGGCGA	252	csgscsCUGCCAGCGCCUGGCGAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	452	CBE
hcPCSK9	GA014	CGTGCGCAGGAGGACGAGGA	163	csgsusGCGCAGGAGGACGAGGAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	341	CBE
hPCSK9	GA018	GGTGCTAGCCTTGCGTTCCG	66	gsgsusGCUAGCCUUGCGUUCCGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuusususu	1618	SpCas9
hPCS	GA01	GGTGCT	66	gsgsuGCUAGCCUUGCGUUCCGgU	1619	SpCas

K9	9	AGCCTTG CGTTCCG		UUUAGagcuagaaauagcaaGUUaAaAu AaggcuaGUccGUUAucAAcuugaaaaa gugGcaccgagucggugcusususu		9
hPCS K9	GA02 0	GGTGCT AGCCTTG CGTTCCG	66	gsgsuGCUAGCCUUGCGUUCCGgU UUUAGagcuagaaauagcaaGUUaAaAu AaggcuaGUccGUUAucAAcuugaaaaa gugGcaccgagucggugcuususu	1620	SpCas 9
hPCS K9	GA02 1	GGTGCT AGCCTTG CGTTCCG	66	gsgsuGCUAGCCUUGCGUUCCGgU UUUAGagcuagaaauagcaaGUUaAaAu AaggcuaGUccGUUAucAAcuugaaaaa gugGcaccgagucggugcuuusu	1621	SpCas 9
hPCS K9	GA02 2	GGTGCT AGCCTTG CGTTCCG	66	gsgsusGCUAGCCUUGCGUUCCGg UUUuAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUUAucAAcuugaaaa agugGcaccgagucggugcusususu	253	SpCas 9
hPCS K9	GA02 3	GGTGCT AGCCTTG CGTTCCG	66	gsgsusGCUAGCCUUGCGUUCCGg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUuAucAAcuugaaaaa gugGcaccgagucggugcusususu	253	SpCas 9
hPCS K9	GA02 4	GGTGCT AGCCTTG CGTTCCG	66	gsgsusGCUAGCCUUGCGUUCCGg UUUuAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUuAucAAcuugaaaaa gugGcaccgagucggugcusususu	253	SpCas 9
hPCS K9	GA02 5	GGTGCT AGCCTTG CGTTCCG	66	gsgsusGCUAGCCUUGCGUUCCGg UUUuAGagcuagaaauagcaaGUuaAaA uAaggcuaGUccGUUAucAAcuugaaaa agugGcaccgagucggugcusususu	253	SpCas 9
hPCS K9	GA02 6	GGTGCT AGCCTTG CGTTCCG	66	gsgsusGCUAGCCUUGCGUUCCGg UUUUAGagcuagaaauagcaaGUuaAaA uAaggcuaGUccGUuAucAAcuugaaaaa gugGcaccgagucggugcusususu	253	SpCas 9
hPCS K9	GA02 7	GGTGCT AGCCTTG CGTTCCG	66	gsgsusGCUAGCCUUGCGUUCCGg UUUuAGagcuagaaauagcaaGUuaAaA uAaggcuaGUccGUuAucAAcuugaaaaa gugGcaccgagucggugcusususu	253	SpCas 9
hPCS K9	GA02 8	GGTGCT AGCCTTG CGTTCCG	66	gsgsusGCUAGCCUUGCGUUCCGg UUUuAGagcuagaaauagcaaGUusaAaA uAaggcuaGUccGUusAucAAcuugaaaa agugGcaccgagucggugcusususu	256	SpCas 9
hPCS K9	GA02 9	GGTGCT AGCCTTG CGTTCCG	66	gsgsusGCuAGCCUuGCGUUCcGgU UUUAGagcuagaaauagcaaGUUaAaAu AaggcuaGUccGUUAucAAcuugaaaaa gugGcaccgagucggugcusususu	253	SpCas 9
hPCS K9	GA03 9	GGTGCT AGCCTTG CGTTCCG	66	gsgsusGCuAGCCUuGCGUUCcGgU UUUAGagcuagaaauagcaaGUUsaAaA uAaggcuaGUccGUUsAucAAcuugaaaa agugGcaccgagucggugcusususu	256	SpCas 9
hANG PTL3	GA05 8	GCCAAT GGCCTCC TTCAGTT	109	iscscsAAUGGCCUCCUUCAGUUGU UUUAGAgcuagaaauagcAAGUUAAA AUAAGGCUAGUCCGUUAUCAacu ugaaaaaguggcaccgagucggugcusususu	1622	SpCas 9
hANG PTL3	GA05 9	CCAATG GCCTCCT TCAGTT	1594	cscsAAUGGCCUCCUUCAGUUGU UUUAGAgcuagaaauagcAAGUUAAA AUAAGGCUAGUCCGUUAUCAacu	1623	SpCas 9

				ugaaaaaguggcaccgagucggugcusususu		
hANGPTL3	GA060	CAATGGCCTCCTTCAGTT	1595	csAAUGGCCUCCUUCAGUUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu	1624	SpCas9
hANGPTL3	GA061	CAATGGCCTCCTTCAGTT	1595	csasAUGGCCUCCUUCAGUUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu	1625	SpCas9
hANGPTL3	GA062	AATGGCCTCCTTCAGTT	1596	AAUGGCCUCCUUCAGUUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu	1626	SpCas9
hANGPTL3	GA063	AATGGCCTCCTTCAGTT	1596	asasUGGCCUCCUUCAGUUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu	1627	SpCas9
hANGPTL3	GA064	GCCAATGGCCTCCTTCAGTT	109	gscscsAAUGGCCUCCUUCAIUUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu	1628	SpCas9
hANGPTL3	GA065	TCCAATGGCCTCCTTCAGTT	1597	iscscsAAUGGCCUCCUUCAIUUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu	1629	SpCas9
hANGPTL3	GA068	GCCAATGGCCTCCTTCAGTT	109	gscscsAAUIICCUCCUUCAIUUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu	1630	SpCas9
hANGPTL3	GA069	GCCAATGGCCTCCTTCAGTT	109	ascscsAAUGGCCUCCUUCAGUUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu	1631	SpCas9
hANGPTL3	GA070	GCCAATGGCCTCCTTCAGTT	50	iscscsAAUiGCCUCCUUCAGUUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu	1632	SpCas9
hANGPTL3	GA071	GCCAATGGCCTCCTTCAGTT	109	gscscsAAUiGCCUCCUUCAGUUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu	1633	SpCas9
mANGPTL3	GA154	GCGAATGGCCTCCTGCAGCT	1598	gscsgsAAUGGCCUCCUGCAGCUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	1634	SpCas9
mANGPTL3	GA155	AGCCCTTCAACACAAGGTCA	1599	asgscsCCUUCAACACAAGGUCAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	1635	SpCas9
hcANGPTL	GA275	TAAGACCATGTCC	1600	usasasGACCAUGUCCCAACUGAGUUUUAGAGCUAGAAAUAGCAAG	2191	SpCas9

3		CAACTGA		UUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu		
hcANGPTL3	GA280	CAATGTCCCCAATGCAATCC	1601	csasasUGUCCCCAAUGCAAUCCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	1637	SpCas9
hcANGPTL3	GA281	TTGTTTTCCGGGATTGCATT	1602	ususgsUUUUCCGGGAUUGCAUUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	1638	SpCas9
hcANGPTL3	GA282	TTTGTTTTCCGGGATTGCAT	1603	usususGUUUUCCGGGAUUGCAUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	1639	SpCas9
hcANGPTL3	GA283	AATCCCGGAAAACAAAGATT	1604	asasusCCCGGAAAACAAAGAUUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	1640	SpCas9
hcANGPTL3	GA285	TGTTTTCCGGGATTGCATTG	1605	usgsusUUUCCGGGAUUGCAUUGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	1641	SpCas9
mPCSK9	GA292	cccatacCTTGGAGCAACGG	69	cscscsAUACCUUGGAGCAACGGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	421	ABE
hcPCSK9	GA307	GCTTACCTGTCTGTGGAAGC	67	gscsusUACCUGUCUGUGGAAGCgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu	259	ABE
hcPCSK9	GA308	TGCTTACCTGTCTGTGGAAG	68	usgscsUUACCUGUCUGUGGAAGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu	260	ABE
mPCSK9	GA348	CCCATACCTTGGAGCAACGG	69	cscscsAUACCUUGGAGCAACGGGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu	421	ABE
mPCSK9	GA354	CCCATACCTTGGAGCAACGG	69	cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususu	1642	ABE
mPCSK9	GA355	CCCATACCTTGGAGCAACGG	69	cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuuuu	1643	ABE

mPCSK9	GA356	CCCATACCTTGGAGCAACGG	69	cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuusususuuu	1644	ABE
mPCSK9	GA357	CCCATACCTTGGAGCAACGG	69	cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuusususuuu	1645	ABE
mPCSK9	GA358	CCCATACCTTGGAGCAACGG	69	cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuusususuuu	1646	ABE
mPCSK9	GA359	CCCATACCTTGGAGCAACGG	69	cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuusususuuu	1647	ABE
mPCSK9	GA360	CCCATACCTTGGAGCAACGG	69	cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuusususuUu	1645	ABE
mPCSK9	GA361	CCCATACCTTGGAGCAACGG	69	cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuusususuUu	1646	ABE
mPCSK9	GA362	CCCATACCTTGGAGCAACGG	69	cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuusususuUu	1647	ABE
mPCSK9	GA363	CCCATACCTTGGAGCAACGG	69	cscscsAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUCcGUUAucAAcuugaaaaaguggcaccgagucggugcusususu	421	ABE
mPCSK9	GA364	CCCATACCTTGGAGCAACGG	69	cscscsAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGuCcGUUaucAAcuugaaaaaguggcaccgagucggugcusususu	421	ABE
mPCSK9	GA365	CCCATACCTTGGAGCAACGG	69	cscscsAUACCUUGGAGCAACGGgUUuagagcuagaaauagcaaGUUaAaAuaaggcuaGuccgUUaucaacuugaaaaaguggcaccgagucggugcusususu	421	ABE
hPCSK9	GA374	CCCGCACCTTGGCGCAGCGG	13	cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAacAAcuugaaaaagugGcaccgagucggugcusususu	10	ABE
hPCSK9	GA378	CCCGCACCTTGGCGCAGCGG	13	cscscsGdCACCUdUGGdCGdCAGdCGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu	1648	ABE
hPCSK9	GA379	CCCGCACCTTGGCGCAGCGG	13	cscscsGCdACCUUdGGCdGCdAGCdGGGUUUUAGAgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu	1649	ABE
hPCS	GA39	CCCGCA	13	cscscsGCACCUUGGCGCAGCGGgU	9	ABE

K9	0	CCTTGGCGCAGCGG		UUuagagcuagaaauagcaaGUUaAaAuaaggcuaGuccgUUaucaacuugaaaaaguggcaccgagucggugcusususu		
hANGPTL3	GA392	AAGATACCTGAATAACCCTC	15	asgsAUACCUGAAUAACCCUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu	1650	ABE
hcPCSK9	GA439	CCCGCACCTTGGCGCAGCGG	13	cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUUu	11	ABE
hcPCSK9	GA440	CCCGCACCTTGGCGCAGCGG	13	cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuUUu	11	ABE
hANGPTL3	GA462	AGATACCTGAATAACCCTC	248	asgsasUACCUGAAUAACCCUCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	442	ABE
hANGPTL3	GA463	GATACCTGAATAACCCTC	249	gsasUACCUGAAUAACCCUCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	443	ABE
hANGPTL3	GA464	ATACCTGAATAACCCTC	250	asusACCUGAAUAACCCUCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	444	ABE
hANGPTL3	GA465	AGATACCTGAATAACCCTC	248	asgsAUACCUGAAUAACCCUCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	1650	ABE
hANGPTL3	GA466	GATACCTGAATAACCCTC	249	gsasUsACCUGAAUAACCCUCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	1651	ABE
hANGPTL3	GA467	ATACCTGAATAACCCTC	250	asUsACCUGAAUAACCCUCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	444	ABE
cANGPTL3	GA517	GATACCTGAATAACTCTC	1606	gsasUsACCUGAAUAACUCUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu	1652	ABE
cANGPTL3	GA518	ATACCTGAATAACTCTC	1607	asusAsCCUGAAUAACUCUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu	1653	ABE
hANG	GA53	AAGATA	15	asasgsAUXCCUGAAUAACCCUCgU	1654	ABE

PTL3	9	CCTGAAT AACCCTC		UUUAGagcuaGaaauagcaaGUUaAaAu AaggcuaGUccGUUAucAAcuuGaaaaa gugGcaccgagucggugcusususu		
hANG PTL3	GA54 0	AAGATA CCTGAAT AACCCTC	15	asasgsAUXCCUGXAUAACCCUCgU UUUAGagcuaGaaauagcaaGUUaAaAu AaggcuaGUccGUUAucAAcuuGaaaaa gugGcaccgagucggugcusususu	1655	ABE
hANG PTL3	GA54 1	AAGATA CCTGAAT AACCCTC	15	asasgsAUXCCUGAAUAXCCCUCgU UUUAGagcuaGaaauagcaaGUUaAaAu AaggcuaGUccGUUAucAAcuuGaaaaa gugGcaccgagucggugcusususu	1656	ABE
hANG PTL3	GA54 2	AAGATA CCTGAAT AACCCTC	15	asasgsAUXCCUGXAUXACCCUCgU UUUAGagcuaGaaauagcaaGUUaAaAu AaggcuaGUccGUUAucAAcuuGaaaaa gugGcaccgagucggugcusususu	1657	ABE
hANG PTL3	GA54 3	AAGATA CCTGAAT AACCCTC	15	asasgsXUXCCUGAAUAACCCUCgU UUUAGagcuaGaaauagcaaGUUaAaAu AaggcuaGUccGUUAucAAcuuGaaaaa gugGcaccgagucggugcusususu	1658	ABE
hANG PTL3	GA54 4	AAGATA CCTGAAT AACCCTC	15	asasgsXUXCCUGAAUAXCCCUCgU UUUAGagcuaGaaauagcaaGUUaAaAu AaggcuaGUccGUUAucAAcuuGaaaaa gugGcaccgagucggugcusususu	1659	ABE
hANG PTL3	GA54 5	AAGATA CCTGAAT AACCCTC	15	asasgsXUACCUGAAUXACCCUCgU UUUAGagcuaGaaauagcaaGUUaAaAu AaggcuaGUccGUUAucAAcuuGaaaaa gugGcaccgagucggugcusususu	1660	ABE
hANG PTL3	GA54 6	AAGATA CCTGAAT AACCCTC	15	asasgsAUACCUGXAUAACCCUCgU UUUAGagcuaGaaauagcaaGUUaAaAu AaggcuaGUccGUUAucAAcuuGaaaaa gugGcaccgagucggugcusususu	1661	ABE
hANG PTL3	GA54 7	AAGATA CCTGAAT AACCCTC	15	asasgsAUACCUGAAUAACUCUCgU UUUAGagcuaGaaauagcaaGUUaAaAu AaggcuaGUccGUUAucAAcuuGaaaaa guggcaccgagucggugcusususuUUu	445	ABE
hANG PTL3	GA54 8	AAGATA CCTGAAT AACCCTC	15	asasgsAUACCUGXAUAXCCCUCgU UUUAGagcuaGaaauagcaaGUUaAaAu AaggcuaGUccGUUAucAAcuuGaaaaa gugGcaccgagucggugcusususu	1662	ABE
hcAN GPTL 3	GA54 9	CCGCAC CTTGGCG CAGCGG	247	asasgsAUXCCUGAAUAXCCCUCgU UUUAGagcuaGaaauagcaaGUUaAaAu AaggcuaGUccGUUAucAAcuuGaaaaa gugGcaccgagucggugcusususu	1656	ABE
mcPC SK9	GA00 394	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUaA aAuAaggCUaGUCcGUUAucAAcuug aaaaaguGgcaccgAgUcggugcuususu-3'	1663	SpCas 9
mcPC SK9	GA00 395	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUaA aAuAaggcuaGUccGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcuususu-3'	1663	SpCas 9
mcPC SK9	GA00 396	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUaA aAuAaggCUaGUCcGUUAucAAcuug	1664	SpCas 9

				aaaaaguGgcaccgAgUCggugcusususu-3'		
mcPCSK9	GA00397	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcuususu-3'	1663	SpCas9
mcPCSK9	GA00398	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1663	SpCas9
mPCSK9	GA00399	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1665	ABE
mPCSK9	GA00400	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	1665	ABE
mPCSK9	GA00401	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususu-3'	1665	ABE
mANGPTL3	GA00402	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1666	ABE
mANGPTL3	GA00403	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	1666	ABE
mANGPTL3	GA00404	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususu-3'	1666	ABE
mcPCSK9	GA00405	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususu-3'	1667	SpCas9
mcPCSK9	GA00406	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1667	SpCas9
mcPCSK9	GA00407	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1667	SpCas9
mcPCSK9	GA00408	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususu-3'	1667	SpCas9
mcPC	GA00	CAGGTTC	81	5'csasgsGUUCCAUGGGAUGCUCU	1668	SpCas

SK9	409	CATGGG ATGCTCT		gUUUUAGagcuagaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUcggugcusususu-3'		9
mcPC SK9	GA00 410	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcuususu-3'	1667	SpCas 9
mcPC SK9	GA00 411	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUCggugcuususu-3'	1667	SpCas 9
mcPC SK9	GA00 412	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUcggugcuususu-3'	1667	SpCas 9
mcPC SK9	GA00 413	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcuususu-3'	1667	SpCas 9
mPCS K9	GA00 414	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUAucAAcuuG aaaaaguGgcaccgAgUCggugcuususu-3'	1669	ABE
mPCS K9	GA00 415	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUAucAAcuugaa aaagugGcaccgagucggugcuususu-3'	1669	ABE
mPCS K9	GA00 416	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUAucAAcuuGa aaaagugGcaccgagucggugcuususu-3'	1669	ABE
mAN GPTL 3	GA00 417	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggCUaGUCcGUUAucAAcuu GaaaaaguGgcaccgAgUCggugcuususu-3'	1670	ABE
mAN GPTL 3	GA00 418	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUsAGagcuagaaauagcaaGUUsa AaAuAaggcuaGUccGUUAucAAcuug aaaaagugGcaccgagucggugcuususu-3'	1670	ABE
mAN GPTL 3	GA00 419	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggcuaGUccGUUAucAAcuuG aaaaagugGcaccgagucggugcuususu-3'	1670	ABE
mcPC SK9	GA00 420	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggcuaGUccGUUAucAAcuuG aaaaagugGcaccgagucggugcuususu-3'	1671	SpCas 9
mcPC SK9	GA00 421	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUsa AaAuAaggCUaGUCcGUUAucAAcuu	1671	SpCas 9

				GaaaaaguGgcaccgAgUCggugcuususu-3'		
mcPCSK9	GA00422	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1671	SpCas9
mcPCSK9	GA00423	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcuususu-3'	1671	SpCas9
mcPCSK9	GA00424	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcuususu-3'	1671	SpCas9
mcPCSK9	GA00425	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususu-3'	1672	SpCas9
mcPCSK9	GA00426	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcuususu-3'	1671	SpCas9
mcPCSK9	GA00427	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcuususu-3'	1671	SpCas9
mcPCSK9	GA00428	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1671	SpCas9
mPCSK9	GA00429	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1673	ABE
mPCSK9	GA00430	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	1673	ABE
mPCSK9	GA00431	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususu-3'	1673	ABE
mPCSK9	GA00432	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1674	ABE
mANGPTL	GA00433	GAGATACCTGAGT	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUaA	1674	ABE

3		AACTTTC		aAuAaggcuaGUccGUUsAucAAcuuga aaaagugGcaccgagucggugcuususu-3'		
mAN GPTL 3	GA00 434	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUsAGagcuaGaaauagcaaGUUaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaagugGcaccgagucggugcuususu-3'	1674	ABE
mAN GPTL 3	GA00 435	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaagugGcaccgagucggugcuususu-3'	1675	SpCas 9
mcPC SK9	GA00 436	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUaA aAuAaggCUaGUCcGUUsAucAAcuu GaaaaaguGgcaccgAgUCggugcuususu-3'	1675	SpCas 9
mcPC SK9	GA00 437	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcuususu-3'	1675	SpCas 9
mcPC SK9	GA00 438	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUaA aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUCggugcuususu-3'	1675	SpCas 9
mcPC SK9	GA00 443	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcuususu-3'	1675	SpCas 9
mPCS K9	GA00 444	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu GaaaaaguGgcaccgAgUCggugcuususu-3'	1676	ABE
mPCS K9	GA00 445	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuga aaaagugGcaccgagucggugcuususu-3'	1676	ABE
mPCS K9	GA00 446	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaagugGcaccgagucggugcuususu-3'	1676	ABE
mAN GPTL 3	GA00 447	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggCUaGUCcGUUsAucAAcu uGaaaaaguGgcaccgAgUCggugcuususu -3'	1677	ABE
mAN GPTL 3	GA00 448	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUsAGagcuagaaauagcaaGUUsa AaAuAaggcuaGUccGUUsAucAAcuu gaaaaagugGcaccgagucggugcuususu-3'	1677	ABE
mAN GPTL 3	GA00 449	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggcuaGUccGUUsAucAAcuu GaaaaagugGcaccgagucggugcuususu-3'	1677	ABE

mcPCSK9	GA00450	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususu-3'	1678	SpCas9
mcPCSK9	GA00451	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1678	SpCas9
mcPCSK9	GA00452	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1678	SpCas9
mcPCSK9	GA00453	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususu-3'	1678	SpCas9
mcPCSK9	GA00454	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcuususu-3'	1678	SpCas9
mcPCSK9	GA00455	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1678	SpCas9
mcPCSK9	GA00456	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususu-3'	1678	SpCas9
mcPCSK9	GA00457	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcuususu-3'	1678	SpCas9
mcPCSK9	GA00458	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1678	SpCas9
mcPCSK9	GA00459	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3	1679	SpCas9
mcPCSK9	GA00460	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	1680	SpCas9
mPCSK9	GA00461	CCCATACCTTGGA	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAa	1681	ABE

		GCAACGG		AuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'		
mPCSK9	GA00478	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususu-3'	1682	ABE
mANGPTL3	GA00479	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1683	ABE
mANGPTL3	GA00480	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	1683	ABE
mANGPTL3	GA00481	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususu-3'	1683	ABE
mcPCSK9	GA00482	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususu-3'	1684	SpCas9
mcPCSK9	GA00483	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1684	SpCas9
mcPCSK9	GA00484	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1684	SpCas9
mcPCSK9	GA00485	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususu-3'	1684	SpCas9
mcPCSK9	GA00486	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcuususu-3'	1684	SpCas9
mcPCSK9	GA00487	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1684	SpCas9
mcPCSK9	GA00488	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususu-3'	1684	SpCas9
mcPCSK9	GA00489	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcuususu-3'	1684	SpCas9
mcPCSK9	GA00490	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1684	SpCas9

mcPCSK9	GA00491	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1685	ABE
mcPCSK9	GA00492	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	1685	ABE
mcPCSK9	GA00493	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususu-3'	1685	ABE
mcPCSK9	GA00494	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1686	ABE
mcPCSK9	GA00495	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	1686	ABE
mcPCSK9	GA00496	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususu-3'	1686	ABE
mcPCSK9	GA00497	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususu-3'	1687	SpCas9
mcPCSK9	GA00498	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1687	SpCas9
mcPCSK9	GA00499	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1687	SpCas9
mcPCSK9	GA00500	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcuususu-3'	1688	SpCas9
mcPCSK9	GA00501	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcuususu-3'	1687	SpCas9
mcPCSK9	GA00502	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1687	SpCas9
mcPCSK9	GA00503	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcuususu-3'	1687	SpCas9
mcPCSK9	GA00504	CAGGTTCCATGGG	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAa	1687	SpCas9

		ATGCTCT		AuAaggCUaGUCcGUUAucAAcuuga aaaaguGgcaccgAgUcggugcuususu-3'		
mcPCSK9	GA00505	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1687	SpCas9
mPCSK9	GA00506	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1689	ABE
mPCSK9	GA00507	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	1689	ABE
mPCSK9	GA00508	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususu-3'	1689	ABE
mANGPTL3	GA00509	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1690	ABE
mANGPTL3	GA00510	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	1690	ABE
mANGPTL3	GA00511	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususu-3'	1690	ABE
mcPCSK9	GA00512	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususu-3'	1691	SpCas9
mcPCSK9	GA00513	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1691	SpCas9
mcPCSK9	GA00514	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1691	SpCas9
mcPCSK9	GA00515	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcuususu-3'	1691	SpCas9
mcPCSK9	GA00516	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcuususu-3'	1691	SpCas9
mcPCSK9	GA00550	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1692	SpCas9

mPCSK9	GA00551	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1693	ABE
mPCSK9	GA00552	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	1693	ABE
mPCSK9	GA00553	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususu-3'	1693	ABE
mANGPTL3	GA00554	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1694	ABE
mANGPTL3	GA00555	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	1694	ABE
mANGPTL3	GA00556	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususu-3'	1694	ABE
mcPCSK9	GA00557	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususu-3'	1695	SpCas9
mcPCSK9	GA00558	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1695	SpCas9
mcPCSK9	GA00559	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1695	SpCas9
mcPCSK9	GA00560	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususu-3'	1695	SpCas9
mcPCSK9	GA00561	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcuususu-3'	1695	SpCas9
mcPCSK9	GA00562	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1695	SpCas9
mcPCSK9	GA00563	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususu-3'	1695	SpCas9
mcPCSK9	GA00564	CAGGTTCCATGGG	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAa	1695	SpCas9

		ATGCTCT		AuAaggCUaGUCcGUUsAucAAcuug aaaaaguGgcaccgAgUcggugcuususu-3'		
mcPCSK9	GA00565	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1695	SpCas9
mPCSK9	GA00566	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1696	ABE
mPCSK9	GA00567	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	1696	ABE
mPCSK9	GA00568	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususu-3'	1696	ABE
mANGPTL3	GA00569	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1697	ABE
mANGPTL3	GA00570	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	1697	ABE
mANGPTL3	GA00571	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususu-3'	1697	ABE
mcPCSK9	GA00572	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususu-3'	1698	SpCas9
mcPCSK9	GA00573	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1698	SpCas9
mcPCSK9	GA00574	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1698	SpCas9
mcPCSK9	GA00575	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususu-3'	1698	SpCas9
mcPCSK9	GA00576	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcuususu-	1698	SpCas9

				3'		
mcPCSK9	GA00577	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1698	SpCas9
mcPCSK9	GA00578	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususu-3'	1698	SpCas9
mcPCSK9	GA00579	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcuususu-3'	1698	SpCas9
mcPCSK9	GA00580	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu-3'	1698	SpCas9
mPCSK9	GA00581	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1643	ABE
mPCSK9	GA00582	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuUUU-3'	1643	ABE
mPCSK9	GA00583	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1643	ABE
mANGPTL3	GA00584	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1699	ABE
mANGPTL3	GA00585	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuUUU-3'	1699	ABE
mANGPTL3	GA00586	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1699	ABE
mcPCSK9	GA00587	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1700	SpCas9
mcPC	GA00	CAGGTTC	81	5'csasgsGUUCCAUGGGAUGCUCU	1700	SpCas

SK9	588	CATGGG ATGCTCT		gUUUUAGagcuagaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcusususuU UU-3'		9
mcPC SK9	GA00 589	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaaguGgcaccgAgUCggugcusususuUU U-3'	1700	SpCas 9
mcPC SK9	GA00 590	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuga aaaaguGgcaccgAgUCggugcusususuU UU-3'	1700	SpCas 9
mcPC SK9	GA00 591	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuga aaaaguGgcaccgAgUcggugcusususuUU U-3'	1700	SpCas 9
mcPC SK9	GA00 592	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaaguGgcaccgAgUCggugcusususuUU U-3'	1700	SpCas 9
mcPC SK9	GA00 593	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuga aaaaguGgcaccgAgUCggugcusususuU UU-3'	1700	SpCas 9
mcPC SK9	GA00 594	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuga aaaaguGgcaccgAgUcggugcusususuUU U-3'	1700	SpCas 9
mcPC SK9	GA00 595	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaaguGgcaccgAgUCggugcusususuUU U-3'	1700	SpCas 9
mPCS K9	GA00 596	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuU UU-3'	1701	ABE
mPCS K9	GA00 597	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUUsAucAAcuugaaaa agugGcaccgagucggugcusususuUUU-3'	1701	ABE
mPCS K9	GA00 598	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaagugGcaccgagucggugcusususuUU U-3'	1701	ABE
mAN	GA00	GAGATA	241	5'gsasgsAUACCUGAGUAACUUUC	1702	ABE

GPTL 3	599	CCTGAGT AACTTTC		gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuU UU-3'		
mAN GPTL 3	GA00 600	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuugaa aaagugGcaccgagucggugcusususuUUU -3'	1702	ABE
mAN GPTL 3	GA00 601	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaagugGcaccgagucggugcusususuUU U-3'	1702	ABE
mcPC SK9	GA00 602	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaagugGcaccgagucggugcusususuUU U-3'	1703	SpCas 9
mcPC SK9	GA00 603	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggCUaGUCcGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuU UU-3'	1703	SpCas 9
mcPC SK9	GA00 604	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaaguGgcaccgAgUCggugcusususuU UU-3'	1703	SpCas 9
mcPC SK9	GA00 605	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggCUaGUCcGUUsAucAAcuug aaaaaguGgcaccgAgUCggugcusususuU UU-3'	1703	SpCas 9
mcPC SK9	GA00 606	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggCUaGUCcGUUsAucAAcuug aaaaaguGgcaccgAgUcggugcusususuU UU-3'	1703	SpCas 9
mcPC SK9	GA00 607	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaaguGgcaccgAgUCggugcusususuU UU-3'	1703	SpCas 9
mcPC SK9	GA00 608	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUsAucAAcuug aaaaaguGgcaccgAgUCggugcusususuU UU-3'	1703	SpCas 9
mcPC SK9	GA00 609	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUsAucAAcuug aaaaaguGgcaccgAgUcggugcusususuU UU-3'	1703	SpCas 9

mcPCSK9	GA00610	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1703	SpCas9
mPCSK9	GA00611	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1704	ABE
mPCSK9	GA00612	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuUUU-3'	1704	ABE
mPCSK9	GA00613	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1704	ABE
mANGPTL3	GA00614	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1705	ABE
mANGPTL3	GA00615	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuUUU-3'	1705	ABE
mANGPTL3	GA00616	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1705	ABE
mcPCSK9	GA00617	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1706	SpCas9
mcPCSK9	GA00618	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1706	SpCas9
mcPCSK9	GA00619	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaguGgcaccgAgUCggugcusususuUUU-3'	1706	SpCas9
mcPCSK9	GA00620	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcusususuU	1706	SpCas9

				UU-3'		
mcPCSK9	GA00621	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcusususuUUU-3'	1706	SpCas9
mcPCSK9	GA00622	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1706	SpCas9
mcPCSK9	GA00623	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1706	SpCas9
mcPCSK9	GA00624	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcusususuUUU-3'	1706	SpCas9
mcPCSK9	GA00625	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1706	SpCas9
mPCSK9	GA00626	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1707	ABE
mPCSK9	GA00627	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuUUU-3'	1707	ABE
mPCSK9	GA00628	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1707	ABE
mANGPTL3	GA00629	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1708	ABE
mANGPTL3	GA00630	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuUUU-3'	1708	ABE
mANGPTL3	GA00631	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGa	1708	ABE

				aaaagugGcaccgagucggugcusususuUU U-3'		
mcPC SK9	GA00 632	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUaA aAuAaggcuaGUccGUUAucAAcuuGa aaaagugGcaccgagucggugcusususuUU U-3'	1709	SpCas 9
mcPC SK9	GA00 633	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUaA aAuAaggCUaGUCcGUUAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuU UU-3'	1709	SpCas 9
mcPC SK9	GA00 634	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUaA aAuAaggcuaGUccGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcusususuU UU-3'	1709	SpCas 9
mcPC SK9	GA00 635	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUaA aAuAaggCUaGUCcGUUAucAAcuug aaaaaguGgcaccgAgUCggugcusususuU UU-3'	1709	SpCas 9
mcPC SK9	GA00 636	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUaA aAuAaggCUaGUCcGUUAucAAcuug aaaaaguGgcaccgAgUcggugcusususuU UU-3'	1709	SpCas 9
mcPC SK9	GA00 637	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUaA aAuAaggcuaGUccGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcusususuU UU-3'	1709	SpCas 9
mcPC SK9	GA00 638	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUaA aAuAaggCUaGUCcGUUAucAAcuug aaaaaguGgcaccgAgUCggugcusususuU UU-3'	1709	SpCas 9
mcPC SK9	GA00 639	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUaA aAuAaggCUaGUCcGUUAucAAcuug aaaaaguGgcaccgAgUcggugcusususuU UU-3'	1709	SpCas 9
mcPC SK9	GA00 640	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUaA aAuAaggcuaGUccGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcusususuU UU-3'	1709	SpCas 9
mPCS K9	GA00 641	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUAGagcuaGaaauagcaaGUUsaAa AuAaggCUaGUCcGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuU UU-3'	1710	ABE
mPCS K9	GA00 642	CCCATAC CTTGGA	69	5'cscscsAUACCUUGGAGCAACGGg UUUUAGagcuagaaauagcaaGUUsaAa	1710	ABE

		GCAACG G		AuAaggcuaGUccGUUsAucAAcuugaa aaagugGcaccgagucggugcusususuUUU -3'		
mPCS K9	GA00 643	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUAGagcuaGaaauagcaaGUUsaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaagugGcaccgagucggugcusususuUU U-3'	1710	ABE
mAN GPTL 3	GA00 644	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu GaaaaaguGgcaccgAgUCggugcusususu UUU-3'	1711	ABE
mAN GPTL 3	GA00 645	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuga aaaagugGcaccgagucggugcusususuUU U-3'	1711	ABE
mAN GPTL 3	GA00 646	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaagugGcaccgagucggugcusususuUU U-3'	1711	ABE
mcPC SK9	GA00 647	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaagugGcaccgagucggugcusususuUU U-3'	1712	SpCas 9
mcPC SK9	GA00 648	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu GaaaaaguGgcaccgAgUCggugcusususu UUU-3'	1712	SpCas 9
mcPC SK9	GA00 649	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuU UU-3'	1712	SpCas 9
mcPC SK9	GA00 650	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUCggugcusususu UUU-3'	1712	SpCas 9
mcPC SK9	GA00 651	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUcggugcusususu UUU-3'	1712	SpCas 9
mcPC SK9	GA00 652	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuU UU-3'	1712	SpCas 9
mcPC	GA00	CAGGTTC	81	5'csasgsGUUCCAUGGGAUGCUCU	1712	SpCas

SK9	653	CATGGG ATGCTCT		gUUUUAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUCggugcusususu UUU-3'		9
mcPC SK9	GA00 654	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUcggugcusususu UUU-3'	1712	SpCas 9
mcPC SK9	GA00 655	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuU UU-3'	1712	SpCas 9
mPCS K9	GA00 656	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuU UU-3'	1713	ABE
mPCS K9	GA00 657	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUAucAAcuugaa aaagugGcaccgagucggugcusususuUUU -3'	1713	ABE
mPCS K9	GA00 658	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUAucAAcuuGa aaaagugGcaccgagucggugcusususuUU U-3'	1713	ABE
mAN GPTL 3	GA00 659	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggCUaGUCcGUUAucAAcuu GaaaaaguGgcaccgAgUCggugcusususu UUU-3'	1714	ABE
mAN GPTL 3	GA00 660	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUsAGagcuagaaauagcaaGUUsa AaAuAaggcuaGUccGUUAucAAcuug aaaaagugGcaccgagucggugcusususuUU U-3'	1714	ABE
mAN GPTL 3	GA00 661	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggcuaGUccGUUAucAAcuuG aaaaagugGcaccgagucggugcusususuUU U-3'	1714	ABE
mcPC SK9	GA00 662	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggcuaGUccGUUAucAAcuuG aaaaagugGcaccgagucggugcusususuUU U-3'	1715	SpCas 9
mcPC SK9	GA00 663	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUsa AaAuAaggCUaGUCcGUUAucAAcuu GaaaaaguGgcaccgAgUCggugcusususu UUU-3'	1715	SpCas 9

mcPCSK9	GA00664	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1715	SpCas9
mcPCSK9	GA00665	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1715	SpCas9
mcPCSK9	GA00666	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcusususuUUU-3'	1715	SpCas9
mcPCSK9	GA00667	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1715	SpCas9
mcPCSK9	GA00668	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1715	SpCas9
mcPCSK9	GA00669	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcusususuUUU-3'	1715	SpCas9
mcPCSK9	GA00670	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1715	SpCas9
mPCSK9	GA00671	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1716	ABE
mPCSK9	GA00672	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcusususuUUU-3'	1716	ABE
mPCSK9	GA00673	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1716	ABE
mANGPTL3	GA00674	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususu	1717	ABE

				UUU-3'		
mANGPTL3	GA00675	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcusususuUUU-3'	1717	ABE
mANGPTL3	GA00676	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1717	ABE
mcPCSK9	GA00677	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1718	SpCas9
mcPCSK9	GA00678	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1718	SpCas9
mcPCSK9	GA00679	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1718	SpCas9
mcPCSK9	GA00680	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1718	SpCas9
mcPCSK9	GA00681	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcusususuUUU-3'	1718	SpCas9
mcPCSK9	GA00682	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1718	SpCas9
mcPCSK9	GA00683	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1718	SpCas9
mcPCSK9	GA00684	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcusususuUUU-3'	1718	SpCas9
mcPCSK9	GA00685	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuG	1718	SpCas9

				aaaaaguGgcaccgAgUCggugcusususuUUU-3'		
mPCSK9	GA00686	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1719	ABE
mPCSK9	GA00687	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcusususuUUU-3'	1719	ABE
mPCSK9	GA00688	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1719	ABE
mANGPTL3	GA00689	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1720	ABE
mANGPTL3	GA00690	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcusususuUUU-3'	1720	ABE
mANGPTL3	GA00691	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1720	ABE
mcPCSK9	GA00692	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1721	SpCas9
mcPCSK9	GA00693	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1721	SpCas9
mcPCSK9	GA00694	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1721	SpCas9
mcPCSK9	GA00695	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1721	SpCas9
mcPCSK9	GA00696	CAGGTTCCATGGG	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsa	1721	SpCas9

		ATGCTCT		AaAuAaggCUaGUCcGUUsAucAAcu ugaaaaaguGgcaccgAgUcggugcusususu UUU-3'		
mcPC SK9	GA00 697	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUsa AaAuAaggcuaGUccGUUsAucAAcuu GaaaaaguGgcaccgAgUCggugcusususu UUU-3'	1721	SpCas 9
mcPC SK9	GA00 698	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggCUaGUCcGUUsAucAAcu ugaaaaaguGgcaccgAgUCggugcusususu UUU-3'	1721	SpCas 9
mcPC SK9	GA00 699	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggCUaGUCcGUUsAucAAcu ugaaaaaguGgcaccgAgUcggugcusususu UUU-3'	1721	SpCas 9
mcPC SK9	GA00 700	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggcuaGUccGUUsAucAAcuu GaaaaaguGgcaccgAgUCggugcusususu UUU-3'	1721	SpCas 9
mcPC SK9	GA00 701	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcusususuU UU-3'	1722	SpCas 9
mcPC SK9	GA00 702	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUUAucAAcuugaaaa agugGcaccgagucggugcusususuUUU-3'	1722	SpCas 9
mPCS K9	GA00 703	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcusususuU UU-3'	1723	ABE
mPCS K9	GA00 704	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUUAucAAcuugaaaa agugGcaccgagucggugcusususuUUU-3'	1723	ABE
mPCS K9	GA00 705	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcusususuUUU -3'	1723	ABE
mAN GPTL 3	GA00 706	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcusususuU UU-3'	1724	ABE
mAN GPTL 3	GA00 707	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUUAucAAcuugaaaa	1724	ABE

				agugGcaccgagucggugcusususuUUU-3'		
mANGPTL3	GA00708	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1724	ABE
mcPCSK9	GA00709	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1722	SpCas9
mcPCSK9	GA00710	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1722	SpCas9
mcPCSK9	GA00711	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1722	SpCas9
mcPCSK9	GA00712	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1722	SpCas9
mcPCSK9	GA00713	CAGGTTCCATGGGATGCTCT	81	5csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcusususuUUU-3'	1725	SpCas9
mcPCSK9	GA00714	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1722	SpCas9
mcPCSK9	GA00715	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1722	SpCas9
mcPCSK9	GA00716	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcusususuUUU-3'	1722	SpCas9
mcPCSK9	GA00717	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1722	SpCas9
mPCSK9	GA00718	CCCATACCTTGGAGCAACG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuG	1726	ABE

		G		aaaaaguGgcaccgAgUCggugcusususuUUU-3'		
mPCSK9	GA00719	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcusususuUUU-3'	1726	ABE
mPCSK9	GA00720	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1726	ABE
mANGPTL3	GA00721	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1727	ABE
mANGPTL3	GA00722	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcusususuUUU-3'	1727	ABE
mANGPTL3	GA00723	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1727	ABE
mcPCSK9	GA00724	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1728	SpCas9
mcPCSK9	GA00725	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1728	SpCas9
mcPCSK9	GA00726	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1728	SpCas9
mcPCSK9	GA00727	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1728	SpCas9
mcPCSK9	GA00728	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcusususuUUU-3'	1728	SpCas9
mcPCSK9	GA00729	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU	1728	SpCas9

				-3'		
mcPCSK9	GA00730	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1728	SpCas9
mcPCSK9	GA00731	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcusususuUUU-3'	1728	SpCas9
mcPCSK9	GA00732	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1728	SpCas9
mPCSK9	GA00733	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1729	ABE
mPCSK9	GA00734	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuUUU-3'	1729	ABE
mPCSK9	GA00735	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1729	ABE
mANGPTL3	GA00736	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1730	ABE
mANGPTL3	GA00737	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuUUU-3'	1730	ABE
mANGPTL3	GA00738	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1730	ABE
mcPCSK9	GA00739	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1731	SpCas9
mcPCSK9	GA00740	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGa	1731	SpCas9

				aaaaguGgcaccgAgUCggugcusususuUUU-3'		
mcPCSK9	GA00741	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1731	SpCas9
mcPCSK9	GA00742	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1731	SpCas9
mcPCSK9	GA00743	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcusususuUUU-3'	1731	SpCas9
mcPCSK9	GA00744	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1731	SpCas9
mcPCSK9	GA00745	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1731	SpCas9
mcPCSK9	GA00746	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcusususuUUU-3'	1731	SpCas9
mcPCSK9	GA00747	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1731	SpCas9
mPCSK9	GA00748	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1732	ABE
mPCSK9	GA00749	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuUUU-3'	1732	ABE
mPCSK9	GA00750	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1732	ABE
mANGPTL	GA00751	GAGATACCTGAGT	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUaAa	1733	ABE

3		AACTTTC		AuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'		
mANGPTL3	GA00752	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuUUU-3'	1733	ABE
mANGPTL3	GA00753	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1733	ABE
mcPCSK9	GA00754	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1734	SpCas9
mcPCSK9	GA00755	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1734	SpCas9
mcPCSK9	GA00756	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1734	SpCas9
mcPCSK9	GA00757	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1734	SpCas9
mcPCSK9	GA00758	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcusususuUUU-3'	1734	SpCas9
mcPCSK9	GA00759	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1734	SpCas9
mcPCSK9	GA00760	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1734	SpCas9
mcPCSK9	GA00761	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1734	SpCas9
mcPC	GA00	CAGGTTC	81	5'csasgGUUCCAUGGGAUGCUCUg	1734	SpCas

SK9	762	CATGGG ATGCTCT		UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaaguGgcaccgAgUCggugcusususuUU U-3'		9
mPCS K9	GA00 763	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUAGagcuaGaaauagcaaGUUsaAa AuAaggCUaGUCcGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuU UU-3'	1735	ABE
mPCS K9	GA00 764	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUAGagcuagaaauagcaaGUUsaAa AuAaggcuaGUccGUUsAucAAcuugaa aaagugGcaccgagucggugcusususuUUU -3'	1735	ABE
mPCS K9	GA00 765	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUAGagcuaGaaauagcaaGUUsaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaagugGcaccgagucggugcusususuUU U-3'	1735	ABE
mAN GPTL 3	GA00 766	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUAGagcuaGaaauagcaaGUUsaAa AuAaggCUaGUCcGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuU UU-3'	1736	ABE
mAN GPTL 3	GA00 767	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUAGagcuagaaauagcaaGUUsaAa AuAaggcuaGUccGUUsAucAAcuugaa aaagugGcaccgagucggugcusususuUUU -3'	1736	ABE
mAN GPTL 3	GA00 768	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUAGagcuaGaaauagcaaGUUsaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaagugGcaccgagucggugcusususuUU U-3'	1736	ABE
mcPC SK9	GA00 769	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuaGaaauagcaaGUUsaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaagugGcaccgagucggugcusususuUU U-3'	1737	SpCas 9
mcPC SK9	GA00 770	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUsaAa AuAaggCUaGUCcGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuU UU-3'	1737	SpCas 9
mcPC SK9	GA00 771	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUsaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaaguGgcaccgAgUCggugcusususuU UU-3'	1737	SpCas 9
mcPC SK9	GA00 772	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUsaAa AuAaggCUaGUCcGUUsAucAAcuug aaaaaguGgcaccgAgUCggugcusususuU UU-3'	1737	SpCas 9

mcPCSK9	GA00773	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcusususuUUU-3'	1737	SpCas9
mcPCSK9	GA00774	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1737	SpCas9
mcPCSK9	GA00775	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1737	SpCas9
mcPCSK9	GA00776	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcusususuUUU-3'	1737	SpCas9
mcPCSK9	GA00777	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1737	SpCas9
mPCSK9	GA00778	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1738	ABE
mPCSK9	GA00779	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuUUU-3'	1738	ABE
mPCSK9	GA00780	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1738	ABE
mANGPTL3	GA00781	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1739	ABE
mANGPTL3	GA00782	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuUUU-3'	1739	ABE
mANGPTL3	GA00783	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUU	1739	ABE

				U-3'		
mcPCSK9	GA00784	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1740	SpCas9
mcPCSK9	GA00785	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1740	SpCas9
mcPCSK9	GA00786	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1740	SpCas9
mcPCSK9	GA00787	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1740	SpCas9
mcPCSK9	GA00788	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcusususuUUU-3'	1740	SpCas9
mcPCSK9	GA00789	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1740	SpCas9
mcPCSK9	GA00790	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1740	SpCas9
mcPCSK9	GA00791	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcusususuUUU-3'	1740	SpCas9
mcPCSK9	GA00792	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1740	SpCas9
mPCSK9	GA00793	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1741	ABE
mPCSK9	GA00794	CCCATACCTTGGAGCAACG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuugaa	1741	ABE

		G		aaagugGcaccgagucggugcusususuUUU-3'		
mPCSK9	GA00795	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1741	ABE
mANGPTL3	GA00796	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1742	ABE
mANGPTL3	GA00797	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcusususuUUU-3'	1742	ABE
mANGPTL3	GA00798	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1742	ABE
mcPCSK9	GA00799	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuUUU-3'	1743	SpCas9
mcPCSK9	GA00800	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1743	SpCas9
mcPCSK9	GA00801	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1743	SpCas9
mcPCSK9	GA00802	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1743	SpCas9
mcPCSK9	GA00803	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcusususuUUU-3'	1743	SpCas9
mcPCSK9	GA00804	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1743	SpCas9
mcPCSK9	GA00805	CAGGTTCCATGGG	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAa	1743	SpCas9

		ATGCTCT		AuAaggCUaGUCcGUUsAucAAcuug aaaaaguGgcaccgAgUCggugcusususuU UU-3'		
mcPC SK9	GA00 806	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUsAucAAcuug aaaaaguGgcaccgAgUcggugcusususuU UU-3'	1743	SpCas 9
mcPC SK9	GA00 807	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaaguGgcaccgAgUCggugcusususuU UU-3'	1743	SpCas 9
mPCS K9	GA00 808	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu GaaaaaguGgcaccgAgUCggugcusususu UUU-3'	1744	ABE
mPCS K9	GA00 809	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUsAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuga aaaagugGcaccgagucggugcusususuUU U-3'	1744	ABE
mPCS K9	GA00 810	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaagugGcaccgagucggugcusususuUU U-3'	1744	ABE
mAN GPTL 3	GA00 811	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu GaaaaaguGgcaccgAgUCggugcusususu UUU-3'	1745	ABE
mAN GPTL 3	GA00 812	GAGATA CCTGAGT AACTTTC	241	5'gsasAUACCUGAGUAACUUUCgU UUUsAGagcuagaaauagcaaGUUsaAa AuAaggcuaGUccGUUsAucAAcuugaa aaagugGcaccgagucggugcusususuUUU -3'	1746	ABE
mAN GPTL 3	GA00 813	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaagugGcaccgagucggugcusususuUU U-3'	1745	ABE
mcPC SK9	GA00 814	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaagugGcaccgagucggugcusususuUU U-3'	1747	SpCas 9
mcPC SK9	GA00 815	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuagaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu GaaaaaguGgcaccgAgUCggugcusususu UUU-3'	1747	SpCas 9
mcPC	GA00	CAGGTTC	81	5'csasgGUUCCAUGGGAUGCUCUg	1747	SpCas

SK9	816	CATGGG ATGCTCT		UUUUsAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuU UU-3'		9
mcPC SK9	GA00 817	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuagaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUCggugcusususu UUU-3'	1747	SpCas 9
mcPC SK9	GA00 818	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuagaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUcggugcusususu UUU-3'	1747	SpCas 9
mcPC SK9	GA00 819	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuU UU-3'	1747	SpCas 9
mcPC SK9	GA00 820	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUCggugcusususu UUU-3'	1747	SpCas 9
mcPC SK9	GA00 821	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUcggugcusususu UUU-3'	1747	SpCas 9
mcPC SK9	GA00 822	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuU UU-3'	1747	SpCas 9
mcPC SK9	GA00 823	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuUU U-3	1748	SpCas 9
mcPC SK9	GA00 824	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususuUUU-3'	1749	SpCas 9
mPCS K9	GA00 825	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuUU U-3'	1644	ABE
mPCS K9	GA00 826	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUUAucAAcuugaaaa agugGcaccgagucggugcuususuUUU-3'	1644	ABE
mPCS K9	GA00 827	CCCATAC CTTGGA	69	5'cscscsAUACCUUGGAGCAACGGg UUUUAGagcuaGaaauagcaaGUUaAa	1644	ABE

		GCAACGG		AuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuUUU-3'		
mANGPTL3	GA00828	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1750	ABE
mANGPTL3	GA00829	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususuUUU-3'	1750	ABE
mANGPTL3	GA00830	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuUUU-3'	1750	ABE
mcPCSK9	GA00831	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuUUU-3'	1749	SpCas9
mcPCSK9	GA00832	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1749	SpCas9
mcPCSK9	GA00833	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1749	SpCas9
mcPCSK9	GA00834	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1749	SpCas9
mcPCSK9	GA00835	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcuususuUUU-3'	1749	SpCas9
mcPCSK9	GA00836	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgAgUCggugcuususuUUU-3'	1749	SpCas9
mcPCSK9	GA00837	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1749	SpCas9
mcPCSK9	GA00838	CAGGTTCCATGGG	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAa	1749	SpCas9

		ATGCTCT		AuAaggCUaGUCcGUUAucAAcuuga aaaaguGgcaccgAgUcggugcuususuUU U-3'		
mcPC SK9	GA00 839	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaaguGgcaccgAgUCggugcuususuUU U-3'	1749	SpCas 9
mPCS K9	GA00 840	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcuususuU UU-3'	1647	ABE
mPCS K9	GA00 841	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUUsAucAAcuugaaaa agugGcaccgagucggugcuususuUUU-3'	1647	ABE
mPCS K9	GA00 842	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaagugGcaccgagucggugcuususuUUU -3'	1647	ABE
mAN GPTL 3	GA00 843	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcuususuU UU-3'	1751	ABE
mAN GPTL 3	GA00 844	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuugaa aaagugGcaccgagucggugcuususuUUU-3'	1751	ABE
mAN GPTL 3	GA00 845	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaagugGcaccgagucggugcuususuUUU -3'	1751	ABE
mcPC SK9	GA00 846	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaagugGcaccgagucggugcuususuUUU -3'	1752	SpCas 9
mcPC SK9	GA00 847	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggCUaGUCcGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcuususuU UU-3'	1752	SpCas 9
mcPC SK9	GA00 848	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuUU U-3'	1752	SpCas 9
mcPC SK9	GA00 849	CAGGTTC CATGGG	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa	1752	SpCas 9

		ATGCTCT		AuAaggCUaGUCcGUUsAucAAcuug aaaaaguGgcaccgAgUCggugcuususuU UU-3'		
mcPCSK9	GA00850	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuug aaaaaguGgcaccgAgUcggugcuususuUU U-3'	1752	SpCas9
mcPCSK9	GA00851	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuUU U-3'	1752	SpCas9
mcPCSK9	GA00852	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuug aaaaaguGgcaccgAgUCggugcuususuU UU-3'	1752	SpCas9
mcPCSK9	GA00853	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuug aaaaaguGgcaccgAgUcggugcuususuUU U-3'	1752	SpCas9
mcPCSK9	GA00854	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuUU U-3'	1752	SpCas9
mPCSK9	GA00855	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuUU U-3'	1646	ABE
mPCSK9	GA00856	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususuUUU-3'	1646	ABE
mPCSK9	GA00857	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcuususuUUU-3'	1646	ABE
mANGPTL3	GA00858	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuG aaaaaguGgcaccgAgUCggugcuususuUUU-3'	1753	ABE
mANGPTL3	GA00859	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaa aaagugGcaccgagucggugcuususuUUU-3'	1753	ABE
mANGPTL	GA00860	GAGATACCTGAGT	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaA	1753	ABE

3		AACTTTC		aAuAaggcuaGUccGUUAucAAcuuGa aaaagugGcaccgagucggugcuususuUUU -3'		
mcPC SK9	GA00 861	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUAucAAcuuGa aaaagugGcaccgagucggugcuususuUUU -3'	1754	SpCas 9
mcPC SK9	GA00 862	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUsaA aAuAaggCUaGUCcGUUAucAAcuuG aaaaaguGgcaccgAgUCggugcuususuU UU-3'	1754	SpCas 9
mcPC SK9	GA00 863	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuUU U-3'	1754	SpCas 9
mcPC SK9	GA00 864	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUsaA aAuAaggCUaGUCcGUUAucAAcuug aaaaaguGgcaccgAgUCggugcusususuU UU-3'	1706	SpCas 9
mcPC SK9	GA00 865	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUsaA aAuAaggCUaGUCcGUUAucAAcuug aaaaaguGgcaccgAgUcggugcuususuUU U-3'	1754	SpCas 9
mcPC SK9	GA00 866	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuUU U-3'	1754	SpCas 9
mcPC SK9	GA00 867	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUAucAAcuug aaaaaguGgcaccgAgUCggugcuususuU UU-3'	1754	SpCas 9
mcPC SK9	GA00 868	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUAucAAcuug aaaaaguGgcaccgAgUcggugcuususuUU U-3'	1754	SpCas 9
mcPC SK9	GA00 869	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuUU U-3'	1754	SpCas 9
mPCS K9	GA00 870	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuUU U-3'	1755	ABE
mPCS	GA00	CCCATAC	69	5'cscscsAUACCUUGGAGCAACGGg	1755	ABE

K9	871	CTTGGAGCAACGG		UUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususuUUU-3'		
mPCSK9	GA00872	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuUUU-3'	1755	ABE
mANGPTL3	GA00873	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1756	ABE
mANGPTL3	GA00874	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususuUUU-3'	1756	ABE
mANGPTL3	GA00875	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuUUU-3'	1756	ABE
mcPCSK9	GA00876	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuUUU-3'	1757	SpCas9
mcPCSK9	GA00877	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1757	SpCas9
mcPCSK9	GA00878	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1757	SpCas9
mcPCSK9	GA00879	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1757	SpCas9
mcPCSK9	GA00880	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcuususuUUU-3'	1757	SpCas9
mcPCSK9	GA00881	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1757	SpCas9
mcPC	GA00	CAGGTTC	81	5'csasgsGUUCCAUGGGAUGCUCU	1709	SpCas

SK9	882	CATGGG ATGCTCT		gUUUUsAGagcuaGaaauagcaaGUUaA aAuAaggCUaGUCcGUUAucAAcuug aaaaaguGgcaccgAgUCggugcusususuU UU-3'		9
mcPC SK9	GA00 883	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUaA aAuAaggCUaGUCcGUUAucAAcuug aaaaaguGgcaccgAgUcggugcuususuUU U-3'	1757	SpCas 9
mcPC SK9	GA00 884	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUaA aAuAaggcuaGUccGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuUU U-3'	1757	SpCas 9
mPCS K9	GA00 885	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUAGagcuaGaaauagcaaGUUsaAa AuAaggCUaGUCcGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcuususuU UU-3'	1645	ABE
mPCS K9	GA00 886	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUAGagcuagaaauagcaaGUUsaAa AuAaggcuaGUccGUUsAucAAcuugaa aaagugGcaccgagucggugcuususuUUU-3'	1645	ABE
mPCS K9	GA00 887	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUAGagcuaGaaauagcaaGUUsaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaagugGcaccgagucggugcuususuUUU -3'	1645	ABE
mAN GPTL 3	GA00 888	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu GaaaaaguGgcaccgAgUCggugcuususu UUU-3'	1758	ABE
mAN GPTL 3	GA00 889	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuga aaaagugGcaccgagucggugcuususuUUU -3'	1758	ABE
mAN GPTL 3	GA00 890	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaagugGcaccgagucggugcuususuUU U-3'	1758	ABE
mcPC SK9	GA00 891	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaagugGcaccgagucggugcuususuUU U-3'	1759	SpCas 9
mcPC SK9	GA00 892	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu GaaaaaguGgcaccgAgUCggugcuususu UUU-3'	1759	SpCas 9

mcPCSK9	GA00893	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1759	SpCas9
mcPCSK9	GA00894	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1759	SpCas9
mcPCSK9	GA00895	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcusususuUUU-3'	1712	SpCas9
mcPCSK9	GA00896	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1759	SpCas9
mcPCSK9	GA00897	CAGGTTCCATGGGATGCTCT	81	5csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1760	SpCas9
mcPCSK9	GA00898	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcuususuUUU-3'	1759	SpCas9
mcPCSK9	GA00899	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1759	SpCas9
mPCSK9	GA00900	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1761	ABE
mPCSK9	GA00901	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususuUUU-3'	1761	ABE
mPCSK9	GA00902	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuUUU-3'	1761	ABE
mANGPTL3	GA00903	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususu	1762	ABE

				UUU-3'		
mANGPTL3	GA00904	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususuUUU-3'	1762	ABE
mANGPTL3	GA00905	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuUUU-3'	1762	ABE
mcPCSK9	GA00906	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuUUU-3'	1763	SpCas9
mcPCSK9	GA00907	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1763	SpCas9
mcPCSK9	GA00908	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1763	SpCas9
mcPCSK9	GA00909	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1763	SpCas9
mcPCSK9	GA00910	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcuususuUUU-3'	1763	SpCas9
mcPCSK9	GA00911	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1715	SpCas9
mcPCSK9	GA00912	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1763	SpCas9
mcPCSK9	GA00913	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcuususuUUU-3'	1763	SpCas9
mcPCSK9	GA00914	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuG	1763	SpCas9

				aaaaaguGgcaccgAgUCggugcuususuUUU-3'		
mPCSK9	GA00915	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1764	ABE
mPCSK9	GA00916	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususuUUU-3'	1764	ABE
mPCSK9	GA00917	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuUUU-3'	1764	ABE
mANGPTL3	GA00918	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1765	ABE
mANGPTL3	GA00919	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususuUUU-3'	1765	ABE
mANGPTL3	GA00920	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuUUU-3'	1765	ABE
mcPCSK9	GA00921	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuUUU-3'	1766	SpCas9
mcPCSK9	GA00922	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1766	SpCas9
mcPCSK9	GA00923	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1766	SpCas9
mcPCSK9	GA00924	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1766	SpCas9
mcPCSK9	GA00925	CAGGTTCCATGGG	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaA	1766	SpCas9

		ATGCTCT		aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUcggugcuususuU UU-3'		
mcPC SK9	GA00 926	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcuususuU UU-3'	1766	SpCas 9
mcPC SK9	GA00 927	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUaA aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUCggugcuususuU UU-3'	1766	SpCas 9
mcPC SK9	GA00 928	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUaA aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUcggugcuususuU UU-3'	1766	SpCas 9
mcPC SK9	GA00 929	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcuususuU UU-3'	1766	SpCas 9
mPCS K9	GA00 930	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu GaaaaaguGgcaccgAgUCggugcuususu UUU-3'	1767	ABE
mPCS K9	GA00 931	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuga aaaagugGcaccgagucggugcuususuUUU -3'	1767	ABE
mPCS K9	GA00 932	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaagugGcaccgagucggugcuususuUU U-3'	1767	ABE
mAN GPTL 3	GA00 933	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggCUaGUCcGUUsAucAAcu uGaaaaaguGgcaccgAgUCggugcuususu UUU-3'	1768	ABE
mAN GPTL 3	GA00 934	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUsAGagcuagaaauagcaaGUUsa AaAuAaggcuaGUccGUUsAucAAcuu gaaaaagugGcaccgagucggugcuususuUU U-3'	1768	ABE
mAN GPTL 3	GA00 935	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggcuaGUccGUUsAucAAcuu GaaaaagugGcaccgagucggugcuususuU UU-3'	1768	ABE
mcPC	GA00	CAGGTTC	81	5'csasgsGUUCCAUGGGAUGCUCU	1769	SpCas

SK9	936	CATGGG ATGCTCT		gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggcuaGUccGUUsAucAAcuu GaaaaagugGcaccgagucggugcuususuU UU-3'		9
mcPC SK9	GA00 937	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUsa AaAuAaggCUaGUCcGUUsAucAAcu uGaaaaaguGgcaccgAgUCggugcuususu UUU-3'	1769	SpCas 9
mcPC SK9	GA00 938	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUsa AaAuAaggcuaGUccGUUsAucAAcuu GaaaaaguGgcaccgAgUCggugcuususu UUU-3'	1769	SpCas 9
mcPC SK9	GA00 939	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUsa AaAuAaggCUaGUCcGUUsAucAAcu ugaaaaaguGgcaccgAgUCggugcuususu UUU-3'	1769	SpCas 9
mcPC SK9	GA00 940	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUsa AaAuAaggCUaGUCcGUUsAucAAcu ugaaaaaguGgcaccgAgUcggugcuususu UUU-3'	1769	SpCas 9
mcPC SK9	GA00 941	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUsa AaAuAaggcuaGUccGUUsAucAAcuu GaaaaaguGgcaccgAgUCggugcuususu UUU-3'	1769	SpCas 9
mcPC SK9	GA00 942	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggCUaGUCcGUUsAucAAcu ugaaaaaguGgcaccgAgUCggugcuususu UUU-3'	1769	SpCas 9
mcPC SK9	GA00 943	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggCUaGUCcGUUsAucAAcu ugaaaaaguGgcaccgAgUcggugcuususu UUU-3'	1769	SpCas 9
mcPC SK9	GA00 944	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggcuaGUccGUUsAucAAcuu GaaaaaguGgcaccgAgUCggugcuususu UUU-3'	1769	SpCas 9
mcPC SK9	GA00 945	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuUU U-3	1770	SpCas 9
mcPC SK9	GA00 946	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUUAucAAcuugaaaa agugGcaccgagucggugcuususuUUU-3'	1771	SpCas 9
mPCS	GA00	CCCATAC	69	5'cscscAUACCUUGGAGCAACGGg	1772	ABE

K9	947	CTTGGAGCAACGG		UUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'		
mPCSK9	GA00948	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususuUUU-3'	1772	ABE
mPCSK9	GA00949	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuUUU-3'	1772	ABE
mANGPTL3	GA00950	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUU-3'	1773	ABE
mANGPTL3	GA00951	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususuUUU-3'	1773	ABE
mANGPTL3	GA00952	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuUUU-3'	1773	ABE
mcPCSK9	GA00953	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuUUU-3'	1771	SpCas9
mcPCSK9	GA00954	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1771	SpCas9
mcPCSK9	GA00955	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1771	SpCas9
mcPCSK9	GA00956	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1771	SpCas9
mcPCSK9	GA00957	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcuususuUUU-3'	1771	SpCas9
mcPCSK9	GA00958	CAGGTTCCATGGG	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaA	1771	SpCas9

		ATGCTCT		uAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'		
mcPCSK9	GA00959	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1771	SpCas9
mcPCSK9	GA00960	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcuususuUUU-3'	1771	SpCas9
mcPCSK9	GA00961	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1771	SpCas9
mPCSK9	GA00962	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1774	ABE
mPCSK9	GA00963	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususuUUU-3'	1774	ABE
mPCSK9	GA00964	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuUUU-3'	1774	ABE
mANGPTL3	GA00965	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1775	ABE
mANGPTL3	GA00966	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususuUUU-3'	1775	ABE
mANGPTL3	GA00967	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuUUU-3'	1775	ABE
mcPCSK9	GA00968	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuUUU-3'	1776	SpCas9
mcPCSK9	GA00969	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaa	1776	SpCas9

				aaaguGgcaccgAgUCggugcuususuUU U-3'		
mcPC SK9	GA00 970	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUUsAucAAcuuGaaa aaguGgcaccgAgUCggugcuususuUUU-3'	1776	SpCas 9
mcPC SK9	GA00 971	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUaAaA uAaggCUaGUCcGUUsAucAAcuugaa aaaguGgcaccgAgUCggugcuususuUU U-3'	1776	SpCas 9
mcPC SK9	GA00 972	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUaAaA uAaggCUaGUCcGUUsAucAAcuugaa aaaguGgcaccgAgUcggugcuususuUUU -3'	1776	SpCas 9
mcPC SK9	GA00 973	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUUsAucAAcuuGaaa aaguGgcaccgAgUCggugcuususuUUU-3'	1776	SpCas 9
mcPC SK9	GA00 974	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUsAucAAcuug aaaaaguGgcaccgAgUCggugcuususuU UU-3'	1776	SpCas 9
mcPC SK9	GA00 975	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUsAucAAcuug aaaaaguGgcaccgAgUcggugcuususuUU U-3'	1776	SpCas 9
mcPC SK9	GA00 976	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuUU U-3'	1776	SpCas 9
mPCS K9	GA00 977	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUAGagcuaGaaauagcaaGUUsaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuUU U-3'	1777	ABE
mPCS K9	GA00 978	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUAGagcuagaaauagcaaGUUsaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususuUUU-3'	1777	ABE
mPCS K9	GA00 979	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUAGagcuaGaaauagcaaGUUsaAa AuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcuususuUUU-3'	1777	ABE
mAN GPTL 3	GA00 980	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUAGagcuaGaaauagcaaGUUsaAa AuAaggCUaGUCcGUUAucAAcuuGa	1778	ABE

				aaaaguGgcaccgAgUCggugcuususuUU U-3'		
mAN GPTL 3	GA00 981	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUAGagcuagaaauagcaaGUUsaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususuUUU-3'	1778	ABE
mAN GPTL 3	GA00 982	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUAGagcuaGaaauagcaaGUUsaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuUU U-3'	1778	ABE
mcPC SK9	GA00 983	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuaGaaauagcaaGUUsaAa AuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcuususuUUU-3'	1779	SpCas 9
mcPC SK9	GA00 984	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUsaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuUU U-3'	1779	SpCas 9
mcPC SK9	GA00 985	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUsaAa AuAaggcuaGUccGUUAucAAcuuGaa aaaguGgcaccgAgUCggugcuususuUU U-3'	1779	SpCas 9
mcPC SK9	GA00 986	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUsaAa AuAaggCUaGUCcGUUAucAAcuuga aaaaguGgcaccgAgUCggugcususususU UU-3'	1731	SpCas 9
mcPC SK9	GA00 987	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUsaAa AuAaggCUaGUCcGUUAucAAcuuga aaaaguGgcaccgAgUcggugcuususuUU U-3'	1779	SpCas 9
mcPC SK9	GA00 988	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUsaAa AuAaggcuaGUccGUUAucAAcuuGaa aaaguGgcaccgAgUCggugcuususuUU U-3'	1779	SpCas 9
mcPC SK9	GA00 989	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuaGaaauagcaaGUUsaAa AuAaggCUaGUCcGUUAucAAcuuga aaaaguGgcaccgAgUCggugcuususuUU U-3'	1779	SpCas 9
mcPC SK9	GA00 990	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuaGaaauagcaaGUUsaAa AuAaggCUaGUCcGUUAucAAcuuga aaaaguGgcaccgAgUcggugcuususuUU U-3'	1779	SpCas 9
mcPC SK9	GA00 991	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuaGaaauagcaaGUUsaAa AuAaggcuaGUccGUUAucAAcuuGaa	1779	SpCas 9

				aaaguGgcaccgAgUCggugcuususuUU U-3'		
mPCS K9	GA00 992	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuUU U-3'	1780	ABE
mPCS K9	GA00 993	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUsAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususuUUU-3'	1780	ABE
mPCS K9	GA00 994	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcuususuUUU-3'	1780	ABE
mAN GPTL 3	GA00 995	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuUU U-3'	1781	ABE
mAN GPTL 3	GA00 996	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUsAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususuUUU-3'	1781	ABE
mAN GPTL 3	GA00 997	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcuususuUUU-3'	1781	ABE
mcPC SK9	GA00 998	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcuususuUUU-3'	1782	SpCas 9
mcPC SK9	GA00 999	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuagaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuUU U-3'	1782	SpCas 9
mcPC SK9	GA01 000	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaaguGgcaccgAgUCggugcuususuUU U-3'	1782	SpCas 9
mcPC SK9	GA01 001	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuagaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuga aaaaguGgcaccgAgUCggugcuususuUU U-3'	1782	SpCas 9
mcPC SK9	GA01 002	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuagaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuga aaaaguGgcaccgAgUcggugcuususuUU	1782	SpCas 9

				U-3'		
mcPCSK9	GA01003	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1782	SpCas9
mcPCSK9	GA01004	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcusususuUUU-3'	1734	SpCas9
mcPCSK9	GA01005	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcuususuUUU-3'	1782	SpCas9
mcPCSK9	GA01006	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1782	SpCas9
mPCSK9	GA01007	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1783	ABE
mPCSK9	GA01008	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususuUUU-3'	1783	ABE
mPCSK9	GA01009	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuUUU-3'	1783	ABE
mANGPTL3	GA01010	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1784	ABE
mANGPTL3	GA01011	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususuUUU-3'	1784	ABE
mANGPTL3	GA01012	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuUUU-3'	1784	ABE
mcPCSK9	GA01013	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGa	1785	SpCas9

				aaaagugGcaccgagucggugcuususuUUU-3'		
mcPCSK9	GA01014	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1785	SpCas9
mcPCSK9	GA01015	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1785	SpCas9
mcPCSK9	GA01016	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1785	SpCas9
mcPCSK9	GA01017	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcusususuUUU-3'	1737	SpCas9
mcPCSK9	GA01018	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1785	SpCas9
mcPCSK9	GA01019	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1785	SpCas9
mcPCSK9	GA01020	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcuususuUUU-3'	1785	SpCas9
mcPCSK9	GA01021	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1785	SpCas9
mPCSK9	GA01022	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1786	ABE
mPCSK9	GA01023	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususuUUU-3'	1786	ABE
mPCSK9	GA01024	CCCATACCTTGGA	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUsaA	1786	ABE

		GCAACG G		aAuAaggcuaGUccGUUAucAAcuuGa aaaagugGcaccgagucggugcuususuUUU -3'		
mAN GPTL 3	GA01 025	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUAucAAcuuG aaaaaguGgcaccgAgUCggugcuususuU UU-3'	1787	ABE
mAN GPTL 3	GA01 026	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUsAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUAucAAcuugaa aaagugGcaccgagucggugcuususuUUU- 3'	1787	ABE
mAN GPTL 3	GA01 027	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUAucAAcuuGa aaaagugGcaccgagucggugcuususuUUU -3'	1787	ABE
mcPC SK9	GA01 028	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUAucAAcuuGa aaaagugGcaccgagucggugcuususuUUU -3'	1788	SpCas 9
mcPC SK9	GA01 029	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuagaaauagcaaGUUsaA aAuAaggCUaGUCcGUUAucAAcuuG aaaaaguGgcaccgAgUCggugcuususuU UU-3'	1788	SpCas 9
mcPC SK9	GA01 030	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuUU U-3'	1788	SpCas 9
mcPC SK9	GA01 031	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuagaaauagcaaGUUsaA aAuAaggCUaGUCcGUUAucAAcuug aaaaaguGgcaccgAgUCggugcuususuU UU-3'	1788	SpCas 9
mcPC SK9	GA01 032	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuagaaauagcaaGUUsaA aAuAaggCUaGUCcGUUAucAAcuug aaaaaguGgcaccgAgUcggugcuususuUU U-3'	1788	SpCas 9
mcPC SK9	GA01 033	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcusususuU UU-3'	1740	SpCas 9
mcPC SK9	GA01 034	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUAucAAcuug aaaaaguGgcaccgAgUCggugcuususuU UU-3'	1788	SpCas 9
mcPC	GA01	CAGGTTC	81	5'csasgGUUCCAUGGGAUGCUCUg	1788	SpCas

SK9	035	CATGGG ATGCTCT		UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUAucAAcuug aaaaaguGgcaccgAgUcggugcuususuUU U-3'		9
mcPC SK9	GA01 036	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuUU U-3'	1788	SpCas 9
mPCS K9	GA01 037	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcuususuU UU-3'	1789	ABE
mPCS K9	GA01 038	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUsAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuugaa aaagugGcaccgagucggugcuususuUUU-3'	1789	ABE
mPCS K9	GA01 039	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaagugGcaccgagucggugcuususuUUU -3'	1789	ABE
mAN GPTL 3	GA01 040	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcuususuU UU-3'	1790	ABE
mAN GPTL 3	GA01 041	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUsAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuugaa aaagugGcaccgagucggugcuususuUUU-3'	1790	ABE
mAN GPTL 3	GA01 042	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaagugGcaccgagucggugcuususuUUU -3'	1790	ABE
mcPC SK9	GA01 043	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaagugGcaccgagucggugcuususuUUU -3'	1791	SpCas 9
mcPC SK9	GA01 044	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuagaaauagcaaGUUaAa AuAaggCUaGUCcGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcuususuU UU-3'	1791	SpCas 9
mcPC SK9	GA01 045	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuUU U-3'	1791	SpCas 9

mcPCSK9	GA01046	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1791	SpCas9
mcPCSK9	GA01047	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcuususuUUU-3'	1791	SpCas9
mcPCSK9	GA01048	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1791	SpCas9
mcPCSK9	GA01049	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1791	SpCas9
mcPCSK9	GA01050	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcuususuUUU-3'	1791	SpCas9
mcPCSK9	GA01051	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1791	SpCas9
mPCSK9	GA01052	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1792	ABE
mPCSK9	GA01053	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususuUUU-3'	1792	ABE
mPCSK9	GA01054	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuUUU-3'	1792	ABE
mANGPTL3	GA01055	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1793	ABE
mANGPTL3	GA01056	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususuUUU	1793	ABE

				-3'		
mANGPTL3	GA01057	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuUUU-3'	1793	ABE
mcPCSK9	GA01058	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuUUU-3'	1794	SpCas9
mcPCSK9	GA01059	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1794	SpCas9
mcPCSK9	GA01060	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1794	SpCas9
mcPCSK9	GA01061	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1794	SpCas9
mcPCSK9	GA01062	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcuususuUUU-3'	1794	SpCas9
mcPCSK9	GA01063	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1794	SpCas9
mcPCSK9	GA01064	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1794	SpCas9
mcPCSK9	GA01065	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcuususuUUU-3'	1794	SpCas9
mcPCSK9	GA01066	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuUUU-3'	1794	SpCas9
mPCSK9	GA01067	CCCATACCTTGGAGCAACG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGa	1795	ABE

		G		aaaaguGgcaccgAgUCggugcusususuNNN-3'		
mPCSK9	GA01068	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuNNN-3'	1795	ABE
mPCSK9	GA01069	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1795	ABE
mANGPTL3	GA01070	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1796	ABE
mANGPTL3	GA01071	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuNNN-3'	1796	ABE
mANGPTL3	GA01072	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1796	ABE
mcPCSK9	GA01073	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1797	SpCas9
mcPCSK9	GA01074	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1797	SpCas9
mcPCSK9	GA01075	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1797	SpCas9
mcPCSK9	GA01076	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1797	SpCas9
mcPCSK9	GA01077	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcusususuNNN-3'	1797	SpCas9
mcPCSK9	GA01078	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaa	1797	SpCas9

				aaaguGgcaccgAgUCggugcusususuNNN-3'		
mcPCSK9	GA01079	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1797	SpCas9
mcPCSK9	GA01080	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcusususuNNN-3'	1797	SpCas9
mcPCSK9	GA01081	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1797	SpCas9
mPCSK9	GA01082	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1798	ABE
mPCSK9	GA01083	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcusususuNNN-3'	1798	ABE
mPCSK9	GA01084	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1798	ABE
mANGPTL3	GA01085	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1799	ABE
mANGPTL3	GA01086	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcusususuNNN-3'	1799	ABE
mANGPTL3	GA01087	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1799	ABE
mcPCSK9	GA01088	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1800	SpCas9
mcPCSK9	GA01089	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuG	1800	SpCas9

				aaaaaguGgcaccgAgUCggugcusususuNNN-3'		
mcPCSK9	GA01090	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1800	SpCas9
mcPCSK9	GA01091	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1800	SpCas9
mcPCSK9	GA01092	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcusususuNNN-3'	1800	SpCas9
mcPCSK9	GA01093	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1800	SpCas9
mcPCSK9	GA01094	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1800	SpCas9
mcPCSK9	GA01095	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcusususuNNN-3'	1800	SpCas9
mcPCSK9	GA01096	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1800	SpCas9
mPCSK9	GA01097	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1802	ABE
mPCSK9	GA01098	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuNNN-3'	1802	ABE
mPCSK9	GA01099	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1802	ABE
mANGPTL	GA01100	GAGATACCTGAGT	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaA	1803	ABE

3		AACTTTC		aAuAaggCUaGUCcGUUAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuN NN-3'		
mAN GPTL 3	GA01 101	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUAucAAcuugaa aaagugGcaccgagucggugcusususuNNN -3'	1803	ABE
mAN GPTL 3	GA01 102	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUAucAAcuuGa aaaagugGcaccgagucggugcusususuNN N-3'	1803	ABE
mcPC SK9	GA01 103	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUAucAAcuuGa aaaagugGcaccgagucggugcusususuNN N-3'	1804	SpCas 9
mcPC SK9	GA01 104	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUsaA aAuAaggCUaGUCcGUUAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuN NN-3'	1804	SpCas 9
mcPC SK9	GA01 105	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcusususuN NN-3'	1804	SpCas 9
mcPC SK9	GA01 106	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUsaA aAuAaggCUaGUCcGUUAucAAcuug aaaaaguGgcaccgAgUCggugcusususuN NN-3'	1804	SpCas 9
mcPC SK9	GA01 107	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUsaA aAuAaggCUaGUCcGUUAucAAcuug aaaaaguGgcaccgAgUcggugcusususuN NN-3'	1804	SpCas 9
mcPC SK9	GA01 108	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcusususuN NN-3'	1804	SpCas 9
mcPC SK9	GA01 109	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUAucAAcuug aaaaaguGgcaccgAgUCggugcusususuN NN-3'	1804	SpCas 9
mcPC SK9	GA01 110	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUAucAAcuug aaaaaguGgcaccgAgUcggugcusususuN NN-3'	1804	SpCas 9
mcPC	GA01	CAGGTTC	81	5'csasgsGUUCCAUGGGAUGCUCU	1804	SpCas

SK9	111	CATGGG ATGCTCT		gUUUUAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcusususuN NN-3'		9
mPCS K9	GA01 112	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcusususuN NN-3'	1805	ABE
mPCS K9	GA01 113	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususuNNN-3'	1805	ABE
mPCS K9	GA01 114	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcusususuNNN-3'	1805	ABE
mAN GPTL 3	GA01 115	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUsAGagcuaGaaauagcaaGUUaA aAuAaggCUaGUCcGUUAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuN NN-3'	1806	ABE
mAN GPTL 3	GA01 116	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUsAGagcuagaaauagcaaGUUaA aAuAaggcuaGUccGUUAucAAcuugaa aaagugGcaccgagucggugcusususuNNN-3'	1806	ABE
mAN GPTL 3	GA01 117	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUsAGagcuaGaaauagcaaGUUaA aAuAaggcuaGUccGUUAucAAcuuGa aaaagugGcaccgagucggugcusususuNN N-3'	1806	ABE
mcPC SK9	GA01 118	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUaA aAuAaggcuaGUccGUUAucAAcuuGa aaaagugGcaccgagucggugcusususuNN N-3'	1807	SpCas 9
mcPC SK9	GA01 119	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUaA aAuAaggCUaGUCcGUUAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuN NN-3'	1807	SpCas 9
mcPC SK9	GA01 120	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUaA aAuAaggcuaGUccGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcusususuN NN-3'	1807	SpCas 9
mcPC SK9	GA01 121	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUaA aAuAaggCUaGUCcGUUAucAAcuug aaaaaguGgcaccgAgUCggugcusususuN NN-3'	1807	SpCas 9

mcPCSK9	GA01122	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcusususuNNN-3'	1807	SpCas9
mcPCSK9	GA01123	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1807	SpCas9
mcPCSK9	GA01124	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1807	SpCas9
mcPCSK9	GA01125	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1807	SpCas9
mcPCSK9	GA01126	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1807	SpCas9
mPCSK9	GA01127	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1808	ABE
mPCSK9	GA01128	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcusususuNNN-3'	1808	ABE
mPCSK9	GA01129	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1808	ABE
mANGPTL3	GA01130	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1809	ABE
mANGPTL3	GA01131	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcusususuNNN-3'	1809	ABE
mANGPTL3	GA01132	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuNN	1809	ABE

				N-3'		
mcPCSK9	GA01133	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1810	SpCas9
mcPCSK9	GA01134	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1810	SpCas9
mcPCSK9	GA01135	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1810	SpCas9
mcPCSK9	GA01136	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1810	SpCas9
mcPCSK9	GA01137	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcusususuNNN-3'	1810	SpCas9
mcPCSK9	GA01138	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1810	SpCas9
mcPCSK9	GA01139	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1810	SpCas9
mcPCSK9	GA01140	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcusususuNNN-3'	1810	SpCas9
mcPCSK9	GA01141	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1810	SpCas9
mPCSK9	GA01142	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1811	ABE
mPCSK9	GA01143	CCCATACCTTGGAGCAACG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaa	1811	ABE

		G		aaagugGcaccgagucggugcusususuNNN-3'		
mPCSK9	GA01144	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1811	ABE
mANGPTL3	GA01145	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1812	ABE
mANGPTL3	GA01146	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuNNN-3'	1812	ABE
mANGPTL3	GA01147	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1812	ABE
mcPCSK9	GA01148	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1813	SpCas9
mcPCSK9	GA01149	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1813	SpCas9
mcPCSK9	GA01150	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1813	SpCas9
mcPCSK9	GA01151	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1813	SpCas9
mcPCSK9	GA01152	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcusususuNNN-3'	1813	SpCas9
mcPCSK9	GA01153	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususu-NNN3'	1814	SpCas9
mcPCSK9	GA01154	CAGGTTCCATGGG	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsa	1813	SpCas9

		ATGCTCT		AaAuAaggCUaGUCcGUUAucAAcuu gaaaaaguGgcaccgAgUCggugcusususu NNN-3'		
mcPCSK9	GA01155	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggCUaGUCcGUUAucAAcuu gaaaaaguGgcaccgAgUcggugcusususu NNN-3'	1813	SpCas9
mcPCSK9	GA01156	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggcuaGUccGUUAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuN NN-3'	1813	SpCas9
mPCSK9	GA01157	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuN NN-3'	1815	ABE
mPCSK9	GA01158	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuugaa aaagugGcaccgagucggugcusususuNNN -3'	1815	ABE
mPCSK9	GA01159	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaagugGcaccgagucggugcusususuNN N-3'	1815	ABE
mANGPTL3	GA01160	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUsAGagcuaGaaauagcaaGUUaA aAuAaggCUaGUCcGUUsAucAAcuu GaaaaaguGgcaccgAgUCggugcusususu NNN-3'	1816	ABE
mANGPTL3	GA01161	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUsAGagcuagaaauagcaaGUUaA aAuAaggcuaGUccGUUsAucAAcuuga aaaagugGcaccgagucggugcusususuNN N-3'	1816	ABE
mANGPTL3	GA01162	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUsAGagcuaGaaauagcaaGUUaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaguGgcaccgagucggugcusususuNN N-3'	1816	ABE
mcPCSK9	GA01163	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaguGgcaccgagucggugcusususuNN N-3'	1817	SpCas9
mcPCSK9	GA01164	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUaA aAuAaggCUaGUCcGUUsAucAAcuu GaaaaaguGgcaccgAgUCggugcusususu NNN-3'	1817	SpCas9
mcPC	GA01	CAGGTTC	81	5'csasgsGUUCCAUGGGAUGCUCU	1817	SpCas

SK9	165	CATGGG ATGCTCT		gUUUUsAGagcuagaaauagcaaGUUaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuN NN-3'		9
mcPC SK9	GA01 166	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUaA aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUCggugcusususu NNN-3'	1817	SpCas 9
mcPC SK9	GA01 167	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUaA aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUcggugcusususu NNN-3'	1817	SpCas 9
mcPC SK9	GA01 168	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuN NN-3'	1817	SpCas 9
mcPC SK9	GA01 169	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUaA aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUCggugcusususu NNN-3'	1817	SpCas 9
mcPC SK9	GA01 170	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUaA aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUcggugcusususu NNN-3'	1817	SpCas 9
mcPC SK9	GA01 171	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuN NN-3'	1817	SpCas 9
mPCS K9	GA01 172	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu GaaaaaguGgcaccgAgUCggugcusususu NNN-3'	1818	ABE
mPCS K9	GA01 173	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuga aaaagugGcaccgagucggugcusususuNN N-3'	1818	ABE
mPCS K9	GA01 174	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaagugGcaccgagucggugcusususuNN N-3'	1818	ABE
mAN GPTL 3	GA01 175	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggCUaGUCcGUUsAucAAcu uGaaaaaguGgcaccgAgUCggugcususus uNNN-3'	1819	ABE

mANGPTL3	GA01176	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcusususuNNN-3'	1819	ABE
mANGPTL3	GA01177	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1819	ABE
mcPCSK9	GA01178	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1820	SpCas9
mcPCSK9	GA01179	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1820	SpCas9
mcPCSK9	GA01180	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1820	SpCas9
mcPCSK9	GA01181	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1820	SpCas9
mcPCSK9	GA01182	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcusususuNNN-3'	1820	SpCas9
mcPCSK9	GA01183	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1820	SpCas9
mcPCSK9	GA01184	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1820	SpCas9
mcPCSK9	GA01185	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcusususuNNN-3'	1820	SpCas9
mcPCSK9	GA01186	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususu	1820	SpCas9

				NNN-3'		
mcPCSK9	GA01187	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1821	SpCas9
mcPCSK9	GA01188	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuNNN-3'	1821	SpCas9
mPCSK9	GA01189	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1822	ABE
mPCSK9	GA01190	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuNNN-3'	1822	ABE
mPCSK9	GA01191	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1822	ABE
mANGPTL3	GA01192	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1823	ABE
mANGPTL3	GA01193	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuNNN-3'	1823	ABE
mANGPTL3	GA01194	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1823	ABE
mcPCSK9	GA01195	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1821	SpCas9
mcPCSK9	GA01196	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1821	SpCas9
mcPCSK9	GA01197	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1821	SpCas9
mcPC	GA01	CAGGTTC	81	5'csasgGUUCCAUGGGAUGCUCUg	1821	SpCas

SK9	198	CATGGG ATGCTCT		UUUUAGagcuagaaauagcaaGUUaAaA uAaggCUaGUCcGUUAucAAcuugaaa aaguGgcaccgAgUCggugcusususuNNN -3'		9
mcPC SK9	GA01 199	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUaAaA uAaggCUaGUCcGUUAucAAcuugaaa aaguGgcaccgAgUcggugcusususuNNN -3'	1821	SpCas 9
mcPC SK9	GA01 200	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUUAucAAcuuGaaaa aguGgcaccgAgUCggugcusususuNNN-3'	1821	SpCas 9
mcPC SK9	GA01 201	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuga aaaaguGgcaccgAgUCggugcusususuN NN-3'	1821	SpCas 9
mcPC SK9	GA01 202	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuga aaaaguGgcaccgAgUcggugcusususuNN N-3'	1821	SpCas 9
mcPC SK9	GA01 203	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaaguGgcaccgAgUCggugcusususuNN N-3'	1821	SpCas 9
mPCS K9	GA01 204	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuN NN-3'	1824	ABE
mPCS K9	GA01 205	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUUsAucAAcuugaaaa agugGcaccgagucggugcusususuNNN-3'	1824	ABE
mPCS K9	GA01 206	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaagugGcaccgagucggugcusususuNN N-3'	1824	ABE
mAN GPTL 3	GA01 207	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuN NN-3'	1825	ABE
mAN GPTL 3	GA01 208	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUUsAucAAcuugaaaa agugGcaccgagucggugcusususuNNN-3'	1825	ABE
mAN GPTL	GA01 209	GAGATA CCTGAGT	241	5'gsasgAUACCUGAGUAACUUUCg UUUUAGagcuaGaaauagcaaGUUaAa	1825	ABE

3		AACTTTC		AuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'		
mcPCSK9	GA01210	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1826	SpCas9
mcPCSK9	GA01211	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1826	SpCas9
mcPCSK9	GA01212	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNNN-3'	1826	SpCas9
mcPCSK9	GA01213	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1826	SpCas9
mcPCSK9	GA01214	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcusususuNNN-3'	1826	SpCas9
mcPCSK9	GA01215	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNNN-3'	1826	SpCas9
mcPCSK9	GA01216	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcusususuNNNN-3'	1826	SpCas9
mcPCSK9	GA01217	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcusususuNNNN-3'	1826	SpCas9
mcPCSK9	GA01218	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNNN-3'	1826	SpCas9
mPCSK9	GA01219	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNNN-3'	1827	ABE
mPCS	GA01	CCCATAC	69	5'cscscAUACCUUGGAGCAACGGg	1827	ABE

K9	220	CTTGGAGCAACGG		UUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuNNN-3'		
mPCSK9	GA01221	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1827	ABE
mANGPTL3	GA01222	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1828	ABE
mANGPTL3	GA01223	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuNNN-3'	1828	ABE
mANGPTL3	GA01224	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1828	ABE
mcPCSK9	GA01225	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1829	SpCas9
mcPCSK9	GA01226	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1829	SpCas9
mcPCSK9	GA01227	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1829	SpCas9
mcPCSK9	GA01228	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1829	SpCas9
mcPCSK9	GA01229	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcusususuNNN-3'	1829	SpCas9
mcPCSK9	GA01230	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1829	SpCas9

mcPCSK9	GA01231	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1829	SpCas9
mcPCSK9	GA01232	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcusususuNNN-3'	1829	SpCas9
mcPCSK9	GA01233	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1829	SpCas9
mPCSK9	GA01234	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1830	ABE
mPCSK9	GA01235	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuNNN-3'	1830	ABE
mPCSK9	GA01236	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1830	ABE
mANGPTL3	GA01237	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1831	ABE
mANGPTL3	GA01238	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuNNN-3'	1831	ABE
mANGPTL3	GA01239	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1831	ABE
mcPCSK9	GA01240	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1832	SpCas9
mcPCSK9	GA01241	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuN	1832	SpCas9

				NN-3'		
mcPCSK9	GA01242	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1832	SpCas9
mcPCSK9	GA01243	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1832	SpCas9
mcPCSK9	GA01244	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcusususuNNN-3'	1832	SpCas9
mcPCSK9	GA01245	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1832	SpCas9
mcPCSK9	GA01246	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1832	SpCas9
mcPCSK9	GA01247	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcusususuNNN-3'	1832	SpCas9
mcPCSK9	GA01248	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1832	SpCas9
mPCSK9	GA01249	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1833	ABE
mPCSK9	GA01250	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcusususuNNN-3'	1833	ABE
mPCSK9	GA01251	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1833	ABE
mANGPTL3	GA01252	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuG	1834	ABE

				aaaaaguGgcaccgAgUCggugcusususuNNN-3'		
mANGPTL3	GA01253	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcusususuNNN-3'	1834	ABE
mANGPTL3	GA01254	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1834	ABE
mcPCSK9	GA01255	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1835	SpCas9
mcPCSK9	GA01256	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1835	SpCas9
mcPCSK9	GA01257	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1835	SpCas9
mcPCSK9	GA01258	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1835	SpCas9
mcPCSK9	GA01259	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcusususuNNN-3'	1835	SpCas9
mcPCSK9	GA01260	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1835	SpCas9
mcPCSK9	GA01261	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1835	SpCas9
mcPCSK9	GA01262	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcusususuNNN-3'	1835	SpCas9
mcPCSK9	GA01263	CAGGTTCCATGGG	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAa	1835	SpCas9

		ATGCTCT		AuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'		
mPCSK9	GA01264	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1836	ABE
mPCSK9	GA01265	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuNNN-3'	1836	ABE
mPCSK9	GA01266	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1836	ABE
mANGPTL3	GA01267	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1837	ABE
mANGPTL3	GA01268	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuNNN-3'	1837	ABE
mANGPTL3	GA01269	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1837	ABE
mcPCSK9	GA01270	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1838	SpCas9
mcPCSK9	GA01271	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1838	SpCas9
mcPCSK9	GA01272	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1838	SpCas9
mcPCSK9	GA01273	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1838	SpCas9
mcPC	GA01	CAGGTTC	81	5'csasgGUUCCAUGGGAUGCUCUg	1838	SpCas

SK9	274	CATGGG ATGCTCT		UUUUsAGagcuagaaauagcaaGUUsaA aAuAaggCUaGUCcGUUAucAAcuug aaaaaguGgcaccgAgUcggugcusususuN NN-3'		9
mcPC SK9	GA01 275	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcusususuN NN-3'	1838	SpCas 9
mcPC SK9	GA01 276	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUAucAAcuug aaaaaguGgcaccgAgUCggugcusususuN NN-3'	1838	SpCas 9
mcPC SK9	GA01 277	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUAucAAcuug aaaaaguGgcaccgAgUcggugcusususuN NN-3'	1838	SpCas 9
mcPC SK9	GA01 278	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcusususuN NN-3'	1838	SpCas 9
mPCS K9	GA01 279	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuN NN-3'	1839	ABE
mPCS K9	GA01 280	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUsAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuugaa aaagugGcaccgagucggugcusususuNNN -3'	1839	ABE
mPCS K9	GA01 281	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaagugGcaccgagucggugcusususuNN N-3'	1839	ABE
mAN GPTL 3	GA01 282	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuN NN-3'	1840	ABE
mAN GPTL 3	GA01 283	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUsAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuugaa aaagugGcaccgagucggugcusususuNNN -3'	1840	ABE
mAN GPTL 3	GA01 284	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaagugGcaccgagucggugcusususuNN N-3'	1840	ABE

mcPCSK9	GA01285	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1841	SpCas9
mcPCSK9	GA01286	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1841	SpCas9
mcPCSK9	GA01287	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1841	SpCas9
mcPCSK9	GA01288	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1841	SpCas9
mcPCSK9	GA01289	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcusususuNNN-3'	1841	SpCas9
mcPCSK9	GA01290	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1841	SpCas9
mcPCSK9	GA01291	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1841	SpCas9
mcPCSK9	GA01292	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcusususuNNN-3'	1841	SpCas9
mcPCSK9	GA01293	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1841	SpCas9
mPCSK9	GA01294	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1842	ABE
mPCSK9	GA01295	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcusususuNN	1842	ABE

				N-3'		
mPCSK9	GA01296	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1842	ABE
mANGPTL3	GA01297	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1843	ABE
mANGPTL3	GA01298	GAGATACCTGAGTAACTTTC	241	5'gsasAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcusususuNNN-3'	1844	ABE
mANGPTL3	GA01299	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1843	ABE
mcPCSK9	GA01300	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuNNN-3'	1845	SpCas9
mcPCSK9	GA01301	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1845	SpCas9
mcPCSK9	GA01302	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1845	SpCas9
mcPCSK9	GA01303	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1845	SpCas9
mcPCSK9	GA01304	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcusususuNNN-3'	1845	SpCas9
mcPCSK9	GA01305	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1845	SpCas9
mcPCSK9	GA01306	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuu	1845	SpCas9

				gaaaaaguGgcaccgAgUCggugcusususu NNN-3'		
mcPCSK9	GA01307	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcusususu NNN-3'	1845	SpCas9
mcPCSK9	GA01308	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1845	SpCas9
mcPCSK9	GA01309	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3	1846	SpCas9
mcPCSK9	GA01310	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususuNNN-3'	1847	SpCas9
mPCSK9	GA01311	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1848	ABE
mPCSK9	GA01312	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususuNNN-3'	1848	ABE
mPCSK9	GA01313	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1848	ABE
mANGPTL3	GA01314	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1849	ABE
mANGPTL3	GA01315	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususuNNN-3'	1849	ABE
mANGPTL3	GA01316	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1849	ABE
mcPCSK9	GA01317	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1847	SpCas9

mcPCSK9	GA01318	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1847	SpCas9
mcPCSK9	GA01319	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1847	SpCas9
mcPCSK9	GA01320	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1847	SpCas9
mcPCSK9	GA01321	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcuususuNNN-3'	1847	SpCas9
mcPCSK9	GA01322	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1847	SpCas9
mcPCSK9	GA01323	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1847	SpCas9
mcPCSK9	GA01324	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcuususuNNN-3'	1847	SpCas9
mcPCSK9	GA01325	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1847	SpCas9
mPCSK9	GA01326	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1850	ABE
mPCSK9	GA01327	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususuNNN-3'	1850	ABE
mPCSK9	GA01328	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1850	ABE

mANGPTL3	GA01329	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1851	ABE
mANGPTL3	GA01330	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususuNNN-3'	1851	ABE
mANGPTL3	GA01331	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1851	ABE
mcPCSK9	GA01332	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1852	SpCas9
mcPCSK9	GA01333	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1852	SpCas9
mcPCSK9	GA01334	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1852	SpCas9
mcPCSK9	GA01335	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1852	SpCas9
mcPCSK9	GA01336	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcuususuNNN-3'	1852	SpCas9
mcPCSK9	GA01337	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1852	SpCas9
mcPCSK9	GA01338	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1852	SpCas9
mcPCSK9	GA01339	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcuususuNN	1852	SpCas9

				N-3'		
mcPCSK9	GA01340	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1852	SpCas9
mPCSK9	GA01341	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1853	ABE
mPCSK9	GA01342	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususuNNN-3'	1853	ABE
mPCSK9	GA01343	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1853	ABE
mANGPTL3	GA01344	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1854	ABE
mANGPTL3	GA01345	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususuNNN-3'	1854	ABE
mANGPTL3	GA01346	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1854	ABE
mcPCSK9	GA01347	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1855	SpCas9
mcPCSK9	GA01348	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1855	SpCas9
mcPCSK9	GA01349	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1855	SpCas9
mcPCSK9	GA01350	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcususuuN	1804	SpCas9

				NN-3'		
mcPCSK9	GA01351	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcuususuNNN-3'	1855	SpCas9
mcPCSK9	GA01352	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1855	SpCas9
mcPCSK9	GA01353	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1855	SpCas9
mcPCSK9	GA01354	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcuususuNNN-3'	1855	SpCas9
mcPCSK9	GA01355	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1855	SpCas9
mPCSK9	GA01356	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1856	ABE
mPCSK9	GA01357	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususuNNN-3'	1856	ABE
mPCSK9	GA01358	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1856	ABE
mANGPTL3	GA01359	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1857	ABE
mANGPTL3	GA01360	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususuNNN-3'	1857	ABE
mANGPTL3	GA01361	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN	1857	ABE

				-3'		
mcPCSK9	GA01362	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1858	SpCas9
mcPCSK9	GA01363	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1858	SpCas9
mcPCSK9	GA01364	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1858	SpCas9
mcPCSK9	GA01365	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1858	SpCas9
mcPCSK9	GA01366	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcuususuNNN-3'	1858	SpCas9
mcPCSK9	GA01367	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1858	SpCas9
mcPCSK9	GA01368	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcususususuNNN-3'	1807	SpCas9
mcPCSK9	GA01369	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcuususuNNN-3'	1858	SpCas9
mcPCSK9	GA01370	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1858	SpCas9
mPCSK9	GA01371	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1859	ABE
mPCSK9	GA01372	CCCATACCTTGGAGCAACG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaa	1859	ABE

		G		aaagugGcaccgagucggugcuusususuNNN-3'		
mPCSK9	GA01373	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuusususuNNN-3'	1859	ABE
mANGPTL3	GA01374	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuusususuNNN-3'	1860	ABE
mANGPTL3	GA01375	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuusususuNNN-3'	1860	ABE
mANGPTL3	GA01376	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuusususuNNN-3'	1860	ABE
mcPCSK9	GA01377	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuusususuNNN-3'	1861	SpCas9
mcPCSK9	GA01378	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuusususuNNN-3'	1861	SpCas9
mcPCSK9	GA01379	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuusususuNNN-3'	1861	SpCas9
mcPCSK9	GA01380	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuusususuNNN-3'	1861	SpCas9
mcPCSK9	GA01381	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcuusususuNNN-3'	1810	SpCas9
mcPCSK9	GA01382	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuusususuNNN-3'	1861	SpCas9
mcPCSK9	GA01383	CAGGTTCCATGGG	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaA	1861	SpCas9

		ATGCTCT		aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUCggugcuususuN NN-3'		
mcPC SK9	GA01 384	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUcggugcuususuN NN-3'	1861	SpCas 9
mcPC SK9	GA01 385	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcuususuN NN-3'	1861	SpCas 9
mPCS K9	GA01 386	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUAucAAcuuG aaaaaguGgcaccgAgUCggugcuususuN NN-3'	1862	ABE
mPCS K9	GA01 387	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUAucAAcuugaa aaagugGcaccgagucggugcuususuNNN-3'	1862	ABE
mPCS K9	GA01 388	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUAucAAcuuGa aaaagugGcaccgagucggugcuususuNNN -3'	1862	ABE
mAN GPTL 3	GA01 389	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggCUaGUCcGUUAucAAcuu GaaaaaguGgcaccgAgUCggugcuususu NNN-3'	1863	ABE
mAN GPTL 3	GA01 390	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUsAGagcuagaaauagcaaGUUsa AaAuAaggcuaGUccGUUAucAAcuug aaaaagugGcaccgagucggugcuususuNN N-3'	1863	ABE
mAN GPTL 3	GA01 391	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggcuaGUccGUUAucAAcuuG aaaaagugGcaccgagucggugcuususuNN N-3'	1863	ABE
mcPC SK9	GA01 392	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggcuaGUccGUUAucAAcuuG aaaaagugGcaccgagucggugcuususuNN N-3'	1864	SpCas 9
mcPC SK9	GA01 393	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUsa AaAuAaggCUaGUCcGUUAucAAcuu GaaaaaguGgcaccgAgUCggugcuususu NNN-3'	1864	SpCas 9
mcPC	GA01	CAGGTTC	81	5'csasgsGUUCCAUGGGAUGCUCU	1864	SpCas

SK9	394	CATGGG ATGCTCT		gUUUUsAGagcuagaaauagcaaGUUsa AaAuAaggcuaGUccGUUAucAAcuuG aaaaaguGgcaccgAgUCggugcuususuN NN-3'		9
mcPC SK9	GA01 395	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUsa AaAuAaggCUaGUCcGUUAucAAcuu gaaaaaguGgcaccgAgUCggugcuususuN NN-3'	1864	SpCas 9
mcPC SK9	GA01 396	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUsa AaAuAaggCUaGUCcGUUAucAAcuu gaaaaaguGgcaccgAgUcggugcuususuN NN-3'	1864	SpCas 9
mcPC SK9	GA01 397	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUsa AaAuAaggcuaGUccGUUAucAAcuuG aaaaaguGgcaccgAgUCggugcusususuN NN-3'	1813	SpCas 9
mcPC SK9	GA01 398	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggCUaGUCcGUUAucAAcuu gaaaaaguGgcaccgAgUCggugcuususuN NN-3'	1864	SpCas 9
mcPC SK9	GA01 399	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggCUaGUCcGUUAucAAcuu gaaaaaguGgcaccgAgUcggugcuususuN NN-3'	1864	SpCas 9
mcPC SK9	GA01 400	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggcuaGUccGUUAucAAcuuG aaaaaguGgcaccgAgUCggugcuususuN NN-3'	1864	SpCas 9
mPCS K9	GA01 401	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcuususuN NN-3'	1865	ABE
mPCS K9	GA01 402	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuugaa aaagugGcaccgagucggugcuususuNNN-3'	1865	ABE
mPCS K9	GA01 403	CCCATAC CTTGGA GCAACG G	69	5'cscscsAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUsAucAAcuuGa aaaagugGcaccgagucggugcuususuNNN-3'	1865	ABE
mAN GPTL 3	GA01 404	GAGATA CCTGAGT AACTTTC	241	5'gsasgsAUACCUGAGUAACUUUC gUUUUsAGagcuaGaaauagcaaGUUaA aAuAaggCUaGUCcGUUsAucAAcuu GaaaaaguGgcaccgAgUCggugcuususu NNN-3'	1866	ABE

mANGPTL3	GA01405	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuusususNNN-3'	1866	ABE
mANGPTL3	GA01406	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuusususNNN-3'	1866	ABE
mcPCSK9	GA01407	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuusususNNN-3'	1867	SpCas9
mcPCSK9	GA01408	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1867	SpCas9
mcPCSK9	GA01409	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1867	SpCas9
mcPCSK9	GA01410	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1867	SpCas9
mcPCSK9	GA01411	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcuususuNNN-3'	1867	SpCas9
mcPCSK9	GA01412	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1867	SpCas9
mcPCSK9	GA01413	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1867	SpCas9
mcPCSK9	GA01414	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcuususuNNN-3'	1867	SpCas9
mcPCSK9	GA01415	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuN	1867	SpCas9

				NN-3'		
mPCSK9	GA01416	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1868	ABE
mPCSK9	GA01417	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususuNNN-3'	1868	ABE
mPCSK9	GA01418	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1868	ABE
mANGPTL3	GA01419	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1869	ABE
mANGPTL3	GA01420	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususuNNN-3'	1869	ABE
mANGPTL3	GA01421	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1869	ABE
mcPCSK9	GA01422	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1870	SpCas9
mcPCSK9	GA01423	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1870	SpCas9
mcPCSK9	GA01424	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1870	SpCas9
mcPCSK9	GA01425	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1870	SpCas9
mcPCSK9	GA01426	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcu	1870	SpCas9

				ugaaaaaguGgcaccgAgUcggugcuususu NNN-3'		
mcPC SK9	GA01 427	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuagaaauagcaaGUUsa AaAuAaggcuaGUccGUUsAucAAcuu GaaaaaguGgcaccgAgUCggugcuususu NNN-3'	1870	SpCas 9
mcPC SK9	GA01 428	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggCUaGUCcGUUsAucAAcu ugaaaaaguGgcaccgAgUCggugcuususu NNN-3'	1870	SpCas 9
mcPC SK9	GA01 429	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggCUaGUCcGUUsAucAAcu ugaaaaaguGgcaccgAgUcggugcuususu NNN-3'	1870	SpCas 9
mcPC SK9	GA01 430	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUsAGagcuaGaaauagcaaGUUsa AaAuAaggcuaGUccGUUsAucAAcuu GaaaaaguGgcaccgAgUCggugcuususu NNN-3'	1870	SpCas 9
mcPC SK9	GA01 431	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuNN N-3	1871	SpCas 9
mcPC SK9	GA01 432	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUUAucAAcuugaaaa agugGcaccgagucggugcuususuNNN-3'	1872	SpCas 9
mPCS K9	GA01 433	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuNN N-3'	1873	ABE
mPCS K9	GA01 434	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUUAucAAcuugaaaa agugGcaccgagucggugcuususuNNN-3'	1873	ABE
mPCS K9	GA01 435	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcuususuNNN-3'	1873	ABE
mAN GPTL 3	GA01 436	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuNN N-3'	1874	ABE
mAN GPTL 3	GA01 437	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUUAucAAcuugaaaa agugGcaccgagucggugcuususuNNN-3'	1874	ABE

mANGPTL3	GA01438	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1874	ABE
mcPCSK9	GA01439	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1872	SpCas9
mcPCSK9	GA01440	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1872	SpCas9
mcPCSK9	GA01441	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1872	SpCas9
mcPCSK9	GA01442	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1872	SpCas9
mcPCSK9	GA01443	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcuususuNNN-3'	1872	SpCas9
mcPCSK9	GA01444	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1872	SpCas9
mcPCSK9	GA01445	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1872	SpCas9
mcPCSK9	GA01446	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcuususuNNN-3'	1872	SpCas9
mcPCSK9	GA01447	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1872	SpCas9
mPCSK9	GA01448	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuN	1875	ABE

				NN-3'		
mPCSK9	GA01449	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususuNNN-3'	1875	ABE
mPCSK9	GA01450	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1875	ABE
mANGPTL3	GA01451	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1876	ABE
mANGPTL3	GA01452	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususuNNN-3'	1876	ABE
mANGPTL3	GA01453	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1876	ABE
mcPCSK9	GA01454	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1877	SpCas9
mcPCSK9	GA01455	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1877	SpCas9
mcPCSK9	GA01456	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1877	SpCas9
mcPCSK9	GA01457	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1877	SpCas9
mcPCSK9	GA01458	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcuususuNNN-3'	1877	SpCas9
mcPCSK9	GA01459	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1877	SpCas9

mcPCSK9	GA01460	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1877	SpCas9
mcPCSK9	GA01461	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcuususuNNN-3'	1877	SpCas9
mcPCSK9	GA01462	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1877	SpCas9
mPCSK9	GA01463	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1878	ABE
mPCSK9	GA01464	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususuNNN-3'	1878	ABE
mPCSK9	GA01465	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1878	ABE
mANGPTL3	GA01466	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1879	ABE
mANGPTL3	GA01467	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususuNNN-3'	1879	ABE
mANGPTL3	GA01468	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1879	ABE
mcPCSK9	GA01469	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1880	SpCas9
mcPCSK9	GA01470	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1880	SpCas9
mcPC	GA01	CAGGTTC	81	5'csasgGUUCCAUGGGAUGCUCUg	1880	SpCas

SK9	471	CATGGG ATGCTCT		UUUUAGagcuagaaauagcaaGUUsaAa AuAaggcuaGUccGUUAucAAcuuGaa aaaguGgcaccgAgUCggugcuususuNN N-3'		9
mcPC SK9	GA01 472	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUsaAa AuAaggCUaGUCcGUUAucAAcuuga aaaaguGgcaccgAgUCggugcusususuN NN-3'	1829	SpCas 9
mcPC SK9	GA01 473	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUsaAa AuAaggCUaGUCcGUUAucAAcuuga aaaaguGgcaccgAgUcggugcuususuNN N-3'	1880	SpCas 9
mcPC SK9	GA01 474	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUsaAa AuAaggcuaGUccGUUAucAAcuuGaa aaaguGgcaccgAgUCggugcuususuNN N-3'	1880	SpCas 9
mcPC SK9	GA01 475	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuaGaaauagcaaGUUsaAa AuAaggCUaGUCcGUUAucAAcuuga aaaaguGgcaccgAgUCggugcuususuNN N-3'	1880	SpCas 9
mcPC SK9	GA01 476	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuaGaaauagcaaGUUsaAa AuAaggCUaGUCcGUUAucAAcuuga aaaaguGgcaccgAgUcggugcuususuNN N-3'	1880	SpCas 9
mcPC SK9	GA01 477	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuaGaaauagcaaGUUsaAa AuAaggcuaGUccGUUAucAAcuuGaa aaaguGgcaccgAgUCggugcuususuNN N-3'	1880	SpCas 9
mPCS K9	GA01 478	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuNN N-3'	1881	ABE
mPCS K9	GA01 479	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUsAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususuNNN-3'	1881	ABE
mPCS K9	GA01 480	CCCATAC CTTGGA GCAACG G	69	5'cscscAUACCUUGGAGCAACGGg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcuususuNNN-3'	1881	ABE
mAN GPTL 3	GA01 481	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuNN N-3'	1882	ABE
mAN	GA01	GAGATA	241	5'gsasgAUACCUGAGUAACUUUCg	1882	ABE

GPTL 3	482	CCTGAGT AACTTTC		UUUUsAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususuNNN-3'		
mAN GPTL 3	GA01 483	GAGATA CCTGAGT AACTTTC	241	5'gsasgAUACCUGAGUAACUUUCg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcuususuNNN-3'	1882	ABE
mcPC SK9	GA01 484	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcuususuNNN-3'	1883	SpCas 9
mcPC SK9	GA01 485	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuagaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcuususuNNN-3'	1883	SpCas 9
mcPC SK9	GA01 486	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaaguGgcaccgAgUCggugcuususuNNN-3'	1883	SpCas 9
mcPC SK9	GA01 487	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuagaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuga aaaaguGgcaccgAgUCggugcuususuNNN-3'	1883	SpCas 9
mcPC SK9	GA01 488	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuagaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuga aaaaguGgcaccgAgUcggugcuususuNNN-3'	1883	SpCas 9
mcPC SK9	GA01 489	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaaguGgcaccgAgUCggugcuususuNNN-3'	1883	SpCas 9
mcPC SK9	GA01 490	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuga aaaaguGgcaccgAgUCggugcusususuNNN-3'	1832	SpCas 9
mcPC SK9	GA01 491	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuga aaaaguGgcaccgAgUcggugcuususuNNN-3'	1883	SpCas 9
mcPC SK9	GA01 492	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaaguGgcaccgAgUCggugcuususuNNN-3'	1883	SpCas 9
mPCS	GA01	CCCATAC	69	5'cscscAUACCUUGGAGCAACGGg	1884	ABE

K9	493	CTTGGAGCAACGG		UUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'		
mPCSK9	GA01494	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususuNNN-3'	1884	ABE
mPCSK9	GA01495	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1884	ABE
mANGPTL3	GA01496	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1885	ABE
mANGPTL3	GA01497	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususuNNN-3'	1885	ABE
mANGPTL3	GA01498	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1885	ABE
mcPCSK9	GA01499	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1886	SpCas9
mcPCSK9	GA01500	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1886	SpCas9
mcPCSK9	GA01501	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1886	SpCas9
mcPCSK9	GA01502	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1886	SpCas9
mcPCSK9	GA01503	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcusususuNNN-3'	1835	SpCas9

mcPCSK9	GA01504	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1886	SpCas9
mcPCSK9	GA01505	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1886	SpCas9
mcPCSK9	GA01506	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcuususuNNN-3'	1886	SpCas9
mcPCSK9	GA01507	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1886	SpCas9
mPCSK9	GA01508	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1887	ABE
mPCSK9	GA01509	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususuNNN-3'	1887	ABE
mPCSK9	GA01510	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1887	ABE
mANGPTL3	GA01511	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1888	ABE
mANGPTL3	GA01512	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususuNNN-3'	1888	ABE
mANGPTL3	GA01513	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1888	ABE
mcPCSK9	GA01514	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN	1889	SpCas9

				-3'		
mcPCSK9	GA01515	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1889	SpCas9
mcPCSK9	GA01516	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1889	SpCas9
mcPCSK9	GA01517	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1889	SpCas9
mcPCSK9	GA01518	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcuususuNNN-3'	1889	SpCas9
mcPCSK9	GA01519	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1838	SpCas9
mcPCSK9	GA01520	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1889	SpCas9
mcPCSK9	GA01521	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUAucAAcuugaaaaaguGgcaccgAgUcggugcuususuNNN-3'	1889	SpCas9
mcPCSK9	GA01522	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1889	SpCas9
mPCSK9	GA01523	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1890	ABE
mPCSK9	GA01524	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususuNNN-3'	1890	ABE
mPCSK9	GA01525	CCCATACCTTGGAGCAACG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGa	1890	ABE

		G		aaaagugGcaccgagucggugcuususuNNN-3'		
mANGPTL3	GA01526	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1891	ABE
mANGPTL3	GA01527	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususuNNN-3'	1891	ABE
mANGPTL3	GA01528	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1891	ABE
mcPCSK9	GA01529	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1892	SpCas9
mcPCSK9	GA01530	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1892	SpCas9
mcPCSK9	GA01531	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1892	SpCas9
mcPCSK9	GA01532	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1892	SpCas9
mcPCSK9	GA01533	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUcggugcuususuNNN-3'	1892	SpCas9
mcPCSK9	GA01534	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1892	SpCas9
mcPCSK9	GA01535	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUsAucAAcuugaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1892	SpCas9
mcPCSK9	GA01536	CAGGTTCCATGGG	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAa	1892	SpCas9

		ATGCTCT		AuAaggCUaGUCcGUUsAucAAcuug aaaaaguGgcaccgAgUcggugcuususuNNN-3'		
mcPCSK9	GA01537	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1892	SpCas9
mPCSK9	GA01538	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1893	ABE
mPCSK9	GA01539	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususuNNN-3'	1893	ABE
mPCSK9	GA01540	CCCATACCTTGGAGCAACGG	69	5'cscscAUACCUUGGAGCAACGGgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1893	ABE
mANGPTL3	GA01541	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1894	ABE
mANGPTL3	GA01542	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuugaaaaagugGcaccgagucggugcuususuNNN-3'	1894	ABE
mANGPTL3	GA01543	GAGATACCTGAGTAACTTTC	241	5'gsasgAUACCUGAGUAACUUUCgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1894	ABE
mcPCSK9	GA01544	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuususuNNN-3'	1895	SpCas9
mcPCSK9	GA01545	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggCUaGUCcGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1895	SpCas9
mcPCSK9	GA01546	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUsAGagcuagaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaaguGgcaccgAgUCggugcuususuNNN-3'	1895	SpCas9
mcPC	GA01	CAGGTTC	81	5'csasgGUUCCAUGGGAUGCUCUg	1895	SpCas

SK9	547	CATGGG ATGCTCT		UUUUsAGagcuagaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUCggugcuususuN NN-3'		9
mcPC SK9	GA01 548	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuagaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUcggugcuususuN NN-3'	1895	SpCas 9
mcPC SK9	GA01 549	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcuususuN NN-3'	1895	SpCas 9
mcPC SK9	GA01 550	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUCggugcuususuN NN-3'	1895	SpCas 9
mcPC SK9	GA01 551	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggCUaGUCcGUUsAucAAcuu gaaaaaguGgcaccgAgUcggugcuususuN NN-3'	1895	SpCas 9
mcPC SK9	GA01 552	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUsAGagcuaGaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuG aaaaaguGgcaccgAgUCggugcuususuN NN-3'	1895	SpCas 9
mcPC SK9	GA01 553	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1896	SpCas 9
mcPC SK9	GA01 554	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1897	SpCas 9
mcPC SK9	GA01 555	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1898	SpCas 9
mcPC SK9	GA01 556	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagXaauagcaaGUUaA aAuAaggcuaGUccGUUAucAAcuugaa aaagugGcaccgagucggugcusususu-3'	1899	SpCas 9
mcPC SK9	GA01 557	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagXaauagcaaGUUaA aAuAaggcuXGUccGUUAucAAcuuga aaaagugGcaccgagucggugcusususu-3'	1900	SpCas 9
mcPC SK9	GA01 558	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuXGUccGUUAucAAcuugaa aaagugGcaccgagucggugcusususu-3'	1901	SpCas 9
mcPC	GA01	CAGGTTC	81	5'csasgsGUUCCAUGGGAUGCUCU	1902	SpCas

SK9	559	CATGGG ATGCTCT		gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUXucAAcuugaaa aagugGcaccgagucggugcusususu-3'		9
mcPC SK9	GA01 560	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagXaauagcaaGUUaA aAuAaggcuXGUccGUUXucAAcuuga aaaagugGcaccgagucggugcusususu-3'	1903	SpCas 9
mcPC SK9	GA01 561	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGXgcuagXaauagcaXGUUa AaAuAaggcuXGUccGUUXucAAcuug aaaaagugGcaccgagucggugcusususu-3'	1904	SpCas 9
mcPC SK9	GA01 562	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCU gUUUUAGagcuagXaauagcaaGUUaA aAuAaggcuaGUccGUUAucAAcuugaa aaagugGcaccgagucggugcusususu-3'	1905	SpCas 9
mcPC SK9	GA01 563	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCU gUUUUAGagcuagXaauagcaaGUUaA aAuAaggcuXGUccGUUAucAAcuuga aaaagugGcaccgagucggugcusususu-3'	1906	SpCas 9
mcPC SK9	GA01 564	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUXucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1907	SpCas 9
mcPC SK9	GA01 565	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCU gUUUUAGagcuagXaauagcaaGUUaA aAuAaggcuXGUccGUUXucAAcuuga aaaagugGcaccgagucggugcusususu-3'	1908	SpCas 9
mcPC SK9	GA01 566	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCU gUUUUAGXgcuagXaauagcaXGUUa AaAuAaggcuXGUccGUUXucAAcuug aaaaagugGcaccgagucggugcusususu-3'	1909	SpCas 9
mcPC SK9	GA01 567	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagXaauagcaaGUUaA aAuAaggcuaGUccGUUAucAAcuugaa aaagugGcaccgagucggugcusususu-3'	1899	SpCas 9
mcPC SK9	GA01 568	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCU gUUUUAGagcuagXaauagcaaGUUaA aAuAaggcuXGUccGUUAucAAcuuga aaaagugGcaccgagucggugcusususu-3'	1910	SpCas 9
mcPC SK9	GA01 569	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuXGUccGUUAucAAcuugaa aaagugGcaccgagucggugcusususu-3'	1911	SpCas 9
mcPC SK9	GA01 570	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUXucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1912	SpCas 9
mcPC SK9	GA01 571	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCU gUUUUAGagcuagXaauagcaaGUUaA aAuAaggcuXGUccGUUXucAAcuuga aaaagugGcaccgagucggugcusususu-3'	1913	SpCas 9
mcPC SK9	GA01 572	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCU gUUUUAGXgcuagXaauagcaXGUUa AaAuAaggcuXGUccGUUXucAAcuug	1914	SpCas 9

				aaaaagugGcaccgagucggugcusususu-3'		
mcPCSK9	GA01573	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCUgUUUUAGagcuagXaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1915	SpCas9
mcPCSK9	GA01574	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCUgUUUUAGagcuagXaauagcaaGUUaAaAuAaggcuXGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1916	SpCas9
mcPCSK9	GA01575	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuXGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1917	SpCas9
mcPCSK9	GA01576	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUXucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1918	SpCas9
mcPCSK9	GA01577	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCUgUUUUAGagcuagXaauagcaaGUUaAaAuAaggcuXGUccGUUXucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1919	SpCas9
mcPCSK9	GA01578	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCUgUUUUAGXgcuagXaauagcaXGUUaAaAuAaggcuXGUccGUUXucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1920	SpCas9
mcPCSK9	GA01579	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCUgUUUUAGxgcuagxaauagcaxGUUaAaAuAaggcuxGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1921	SpCas9
mcPCSK9	GA01580	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCUgUUUUAGagcuagxaauagcaxGUUaAaAuAaggcuxGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1922	SpCas9
mcPCSK9	GA01581	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCUgUUUUAGagcuagaaauagcaxGUUaAaAuAaggcuxGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1923	SpCas9
mcPCSK9	GA01582	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuxGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1924	SpCas9
mcPCSK9	GA01583	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCUgUUUUAGxgcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1925	SpCas9
mcPCSK9	GA01584	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCUgUUUUAGagcuagaaauagcaxGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1926	SpCas9
mcPCSK9	GA01585	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCUgUUUUAGagcuagxaauagcaxGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1927	SpCas9
mcPC	GA01	CAGGTTC	81	5'csasgsGUUCCXUGGGXUGCUCU	1905	SpCas

SK9	586	CATGGG ATGCTCT		gUUUUAGagcuagxaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'		9
mcPC SK9	GA01 587	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCU gUUUUAGxgcuagxaauagcaxGUUaAa AuAaggcuxGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1928	SpCas 9
mcPC SK9	GA01 588	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCU gUUUUAGagcuagxaauagcaxGUUaAa AuAaggcuxGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1929	SpCas 9
mcPC SK9	GA01 589	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCU gUUUUAGagcuagaaauagcaxGUUaAa AuAaggcuxGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1930	SpCas 9
mcPC SK9	GA01 590	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuxGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1917	SpCas 9
mcPC SK9	GA01 591	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCU gUUUUAGxgcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1931	SpCas 9
mcPC SK9	GA01 592	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCU gUUUUAGagcuagaaauagcaxGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1932	SpCas 9
mcPC SK9	GA01 593	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCU gUUUUAGagcuagxaauagcaxGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1933	SpCas 9
mcPC SK9	GA01 594	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCU gUUUUAGagcuagxaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1915	SpCas 9
mcPC SK9	GA01 595	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCU gUUUUAGxgcuagxaauagcaxGUUaAa AuAaggcuxGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1934	SpCas 9
mcPC SK9	GA01 596	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCU gUUUUAGagcuagxaauagcaxGUUaAa AuAaggcuxGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1935	SpCas 9
mcPC SK9	GA01 597	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCU gUUUUAGagcuagaaauagcaxGUUaAa AuAaggcuxGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1936	SpCas 9
mcPC SK9	GA01 598	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuxGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1911	SpCas 9
mcPC SK9	GA01 599	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCU gUUUUAGxgcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa	1937	SpCas 9

				aagugGcaccgagucggugcusususu-3'		
mcPCSK9	GA01600	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCUgUUUUAGagcuagaaauagcaxGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1938	SpCas9
mcPCSK9	GA01601	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCUgUUUUAGagcuagxaauagcaxGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1939	SpCas9
mcPCSK9	GA01602	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCUgUUUUAGagcuagxaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1940	SpCas9
mcPCSK9	GA01603	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCdXUGGGdXUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaaagugGcaccgagucggugcusususu-3'	1941	SpCas9
mcPCSK9	GA01604	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCdXUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaaagugGcaccgagucggugcusususu-3'	1942	SpCas9
mcPCSK9	GA01605	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGdXUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaaagugGcaccgagucggugcusususu-3'	1943	SpCas9
mcPCSK9	GA01606	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagdXaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaaagugGcaccgagucggugcusususu-3'	1944	SpCas9
mcPCSK9	GA01607	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagdXaauagcaaGUUaAaAuAaggcudXGUccGUUAucAAcuugaaaaaagugGcaccgagucggugcusususu-3'	1945	SpCas9
mcPCSK9	GA01608	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcudXGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1946	SpCas9
mcPCSK9	GA01609	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUdXucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1947	SpCas9
mcPCSK9	GA01610	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagdXaauagcaaGUUaAaAuAaggcudXGUccGUUdXucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1948	SpCas9
mcPCSK9	GA01611	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGdXgcuagdXaauagcadXGUUaAaAuAaggcudXGUccGUUdXucAAcuugaaaaagugGcaccgagucggugcususususu-3'	1949	SpCas9
mcPCSK9	GA01612	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaa	1950	SpCas9

				agugGcaccgagucggugcusususu-3'		
mcPCSK9	GA01613	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1951	SpCas9
mcPCSK9	GA01614	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1952	SpCas9
mcPCSK9	GA01615	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagXaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1953	SpCas9
mcPCSK9	GA01616	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagXaauagcaaGUUaAaAuAaggcuXGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1954	SpCas9
mcPCSK9	GA01617	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuXGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1955	SpCas9
mcPCSK9	GA01618	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUXucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1956	SpCas9
mcPCSK9	GA01619	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagXaauagcaaGUUaAaAuAaggcuXGUccGUUXucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1957	SpCas9
mcPCSK9	GA01620	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGXgcuagXaauagcaXGUUaAaAuAaggcuXGUccGUUXucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1958	SpCas9
mcPCSK9	GA01621	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUgUUUUAGagcuagXaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1959	SpCas9
mcPCSK9	GA01622	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUgUUUUAGagcuagXaauagcaaGUUaAaAuAaggcuXGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1960	SpCas9
mcPCSK9	GA01623	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUXucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1961	SpCas9
mcPCSK9	GA01624	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUgUUUUAGagcuagXaauagcaaGUUaAaAuAaggcuXGUccGUUXucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1962	SpCas9
mcPCSK9	GA01625	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUgUUUUAGXgcuagXaauagcaXGUUaAaAuAaggcuXGUccGUUXucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1963	SpCas9
mcPC	GA01	CAGGTTC	81	5'csasgGUUCCAUGGGAUGCUCUg	1953	SpCas

SK9	626	CATGGG ATGCTCT		UUUUAGagcuagXaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'		9
mcPC SK9	GA01 627	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUg UUUUAGagcuagXaauagcaaGUUaAa AuAaggcuXGUccGUUAucAAcuugaa aaagugGcaccgagucggugcusususu-3'	1964	SpCas 9
mcPC SK9	GA01 628	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuXGUccGUUAucAAcuugaaaa agugGcaccgagucggugcusususu-3'	1965	SpCas 9
mcPC SK9	GA01 629	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUUXucAAcuugaaaa agugGcaccgagucggugcusususu-3'	1966	SpCas 9
mcPC SK9	GA01 630	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUg UUUUAGagcuagXaauagcaaGUUaAa AuAaggcuXGUccGUUXucAAcuugaa aaagugGcaccgagucggugcusususu-3'	1967	SpCas 9
mcPC SK9	GA01 631	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUg UUUUAGXgcuagXaauagcaXGUUaA aAuAaggcuXGUccGUUXucAAcuuga aaaagugGcaccgagucggugcusususu-3'	1968	SpCas 9
mcPC SK9	GA01 632	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUg UUUUAGagcuagXaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1969	SpCas 9
mcPC SK9	GA01 633	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUg UUUUAGagcuagXaauagcaaGUUaAa AuAaggcuXGUccGUUAucAAcuugaa aaagugGcaccgagucggugcusususu-3'	1970	SpCas 9
mcPC SK9	GA01 634	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuXGUccGUUAucAAcuugaaaa agugGcaccgagucggugcusususu-3'	1971	SpCas 9
mcPC SK9	GA01 635	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUUXucAAcuugaaaa agugGcaccgagucggugcusususu-3'	1972	SpCas 9
mcPC SK9	GA01 636	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUg UUUUAGagcuagXaauagcaaGUUaAa AuAaggcuXGUccGUUXucAAcuugaa aaagugGcaccgagucggugcusususu-3'	1973	SpCas 9
mcPC SK9	GA01 637	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUg UUUUAGXgcuagXaauagcaXGUUaA aAuAaggcuXGUccGUUXucAAcuuga aaaagugGcaccgagucggugcusususu-3'	1974	SpCas 9
mcPC SK9	GA01 638	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUg UUUUAGxgcuagxaauagcaxGUUaAa AuAaggcuxGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1975	SpCas 9
mcPC SK9	GA01 639	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUg UUUUAGagcuagxaauagcaxGUUaAa AuAaggcuxGUccGUUAucAAcuugaaa	1976	SpCas 9

				aagugGcaccgagucggugcusususu-3'		
mcPCSK9	GA01640	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUgUUUUAGagcuagaaauagcaxGUUaAaAuAaggcuxGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1977	SpCas9
mcPCSK9	GA01641	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuxGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1978	SpCas9
mcPCSK9	GA01642	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUgUUUUAGxgcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1979	SpCas9
mcPCSK9	GA01643	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUgUUUUAGagcuagaaauagcaxGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1980	SpCas9
mcPCSK9	GA01644	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUgUUUUAGagcuagxaauagcaxGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1981	SpCas9
mcPCSK9	GA01645	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUgUUUUAGagcuagxaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1959	SpCas9
mcPCSK9	GA01646	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUgUUUUAGxgcuagxaauagcaxGUUaAaAuAaggcuxGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1982	SpCas9
mcPCSK9	GA01647	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCUgUUUUAGagcuagxaauagcaxGUUaAaAuAaggcuxGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1929	SpCas9
mcPCSK9	GA01648	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUgUUUUAGagcuagaaauagcaxGUUaAaAuAaggcuxGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1983	SpCas9
mcPCSK9	GA01649	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuxGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1971	SpCas9
mcPCSK9	GA01650	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUgUUUUAGxgcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1984	SpCas9
mcPCSK9	GA01651	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUgUUUUAGagcuagaaauagcaxGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1985	SpCas9
mcPCSK9	GA01652	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUgUUUUAGagcuagxaauagcaxGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1986	SpCas9
mcPC	GA01	CAGGTTC	81	5'csasgGUUCCAUGGGXUGCUCUg	1969	SpCas

SK9	653	CATGGG ATGCTCT		UUUUAGagcuagxaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'		9
mcPC SK9	GA01 654	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUg UUUUAGxgcuagxaauagcaxGUUaAa AuAaggcuxGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1987	SpCas 9
mcPC SK9	GA01 655	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUg UUUUAGagcuagxaauagcaxGUUaAa AuAaggcuxGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1988	SpCas 9
mcPC SK9	GA01 656	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUg UUUUAGagcuagaaauagcaxGUUaAa AuAaggcuxGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1989	SpCas 9
mcPC SK9	GA01 657	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuxGUccGUUAucAAcuugaaaa agugGcaccgagucggugcusususu-3'	1965	SpCas 9
mcPC SK9	GA01 658	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUg UUUUAGxgcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1990	SpCas 9
mcPC SK9	GA01 659	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUg UUUUAGagcuagaaauagcaxGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1991	SpCas 9
mcPC SK9	GA01 660	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUg UUUUAGagcuagxaauagcaxGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1992	SpCas 9
mcPC SK9	GA01 661	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUg UUUUAGagcuagxaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1993	SpCas 9
mcPC SK9	GA01 662	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCdXUGGGdXUGCUC UgUUUUAGagcuagaaauagcaaGUUa AaAuAaggcuaGUccGUUAucAAcuug aaaaagugGcaccgagucggugcusususu-3'	1994	SpCas 9
mcPC SK9	GA01 663	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCdXUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1995	SpCas 9
mcPC SK9	GA01 664	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGdXUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1996	SpCas 9
mcPC SK9	GA01 665	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagdXaauagcaaGUUaA aAuAaggcuaGUccGUUAucAAcuugaa aaagugGcaccgagucggugcusususu-3'	1997	SpCas 9
mcPC SK9	GA01 666	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagdXaauagcaaGUUaA aAuAaggcudXGUccGUUAucAAcuug	1998	SpCas 9

				aaaaagugGcaccgagucggugcusususu-3'		
mcPCSK9	GA01667	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcudXGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1999	SpCas9
mcPCSK9	GA01668	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUdXucAAcuugaaaaagugGcaccgagucggugcusususu-3'	2000	SpCas9
mcPCSK9	GA01669	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagdXaauagcaaGUUaAaAuAaggcudXGUccGUUdXucAAcuugaaaaagugGcaccgagucggugcusususu-3'	2001	SpCas9
mcPCSK9	GA01670	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGdXgcuagdXaauagcadXGUUaAaAuAaggcudXGUccGUUdXucAAcuugaaaaagugGcaccgagucggugcusususu-3'	2002	SpCas9
mcPCSK9	GA01671	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2003	SpCas9
mcPCSK9	GA01672	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2004	SpCas9
mcPCSK9	GA01673	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2005	SpCas9
mcPCSK9	GA01674	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagXaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2006	SpCas9
mcPCSK9	GA01675	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagXaauagcaaGUUaAaAuAaggcuXGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2007	SpCas9
mcPCSK9	GA01676	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuXGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2008	SpCas9
mcPCSK9	GA01677	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUXucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2009	SpCas9
mcPCSK9	GA01678	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagXaauagcaaGUUaAaAuAaggcuXGUccGUUXucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2010	SpCas9
mcPCSK9	GA01679	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGXgcuagXaauagcaXGUUaAaAuAaggcuXGUccGUUXucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2011	SpCas9

mcPCSK9	GA01680	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCUgUUUUAGagcuagXaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2012	SpCas9
mcPCSK9	GA01681	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCUgUUUUAGagcuagXaauagcaaGUUaAaAuAaggcuXGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2013	SpCas9
mcPCSK9	GA01682	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUXucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2014	SpCas9
mcPCSK9	GA01683	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCUgUUUUAGagcuagXaauagcaaGUUaAaAuAaggcuXGUccGUUXucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2015	SpCas9
mcPCSK9	GA01684	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCUgUUUUAGXgcuagXaauagcaXGUUaAaAuAaggcuXGUccGUUXucAAcuugaaaaaagugGcaccgagucggugcuususu-3'	2016	SpCas9
mcPCSK9	GA01685	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagXaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2006	SpCas9
mcPCSK9	GA01686	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCUgUUUUAGagcuagXaauagcaaGUUaAaAuAaggcuXGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2017	SpCas9
mcPCSK9	GA01687	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuXGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2018	SpCas9
mcPCSK9	GA01688	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUXucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2019	SpCas9
mcPCSK9	GA01689	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCUgUUUUAGagcuagXaauagcaaGUUaAaAuAaggcuXGUccGUUXucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2020	SpCas9
mcPCSK9	GA01690	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCUgUUUUAGXgcuagXaauagcaXGUUaAaAuAaggcuXGUccGUUXucAAcuugaaaaaagugGcaccgagucggugcuususu-3'	2021	SpCas9
mcPCSK9	GA01691	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCUgUUUUAGagcuagXaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2022	SpCas9
mcPCSK9	GA01692	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCUgUUUUAGagcuagXaauagcaaGUUaAaAuAaggcuXGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2023	SpCas9
mcPCSK9	GA01693	CAGGTTCCATGGG	81	5'csasgsGUUCCAUGGGXUGCUCUgUUUUAGagcuagaaauagcaaGUUaAa	2024	SpCas9

		ATGCTCT		AuAaggcuXGUccGUUAucAAcuugaa aaagugGcaccgagucggugcuususu-3'		
mcPC SK9	GA01 694	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUXucAAcuugaaa aagugGcaccgagucggugcuususu-3'	2025	SpCas 9
mcPC SK9	GA01 695	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCU gUUUUAGagcuagXaauagcaaGUUaA aAuAaggcuXGUccGUUXucAAcuuga aaaagugGcaccgagucggugcuususu-3'	2026	SpCas 9
mcPC SK9	GA01 696	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCU gUUUUAGXgcuagXaauagcaXGUUa AaAuAaggcuXGUccGUUXucAAcuug aaaaagugGcaccgagucggugcuususu-3'	2027	SpCas 9
mcPC SK9	GA01 697	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCU gUUUUAGxgcuagxaauagcaxGUUaAa AuAaggcuxGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'	2028	SpCas 9
mcPC SK9	GA01 698	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCU gUUUUAGagcuagxaauagcaxGUUaAa AuAaggcuxGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'	2029	SpCas 9
mcPC SK9	GA01 699	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCU gUUUUAGagcuagaaauagcaxGUUaAa AuAaggcuxGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'	2030	SpCas 9
mcPC SK9	GA01 700	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuxGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'	2031	SpCas 9
mcPC SK9	GA01 701	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCU gUUUUAGxgcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'	2032	SpCas 9
mcPC SK9	GA01 702	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCU gUUUUAGagcuagaaauagcaxGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'	2033	SpCas 9
mcPC SK9	GA01 703	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCU gUUUUAGagcuagxaauagcaxGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'	2034	SpCas 9
mcPC SK9	GA01 704	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCXUGGGXUGCUCU gUUUUAGagcuagxaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'	2012	SpCas 9
mcPC SK9	GA01 705	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCU gUUUUAGxgcuagxaauagcaxGUUaAa AuAaggcuxGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'	2035	SpCas 9
mcPC SK9	GA01 706	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCU gUUUUAGagcuagxaauagcaxGUUaAa AuAaggcuxGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'	2036	SpCas 9

mcPCSK9	GA01707	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCUgUUUUAGagcuagaaauagcaxGUUaAaAuAaggcuxGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2037	SpCas9
mcPCSK9	GA01708	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuxGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2024	SpCas9
mcPCSK9	GA01709	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCUgUUUUAGxgcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2038	SpCas9
mcPCSK9	GA01710	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCUgUUUUAGagcuagaaauagcaxGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2039	SpCas9
mcPCSK9	GA01711	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCUgUUUUAGagcuagxaauagcaxGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2040	SpCas9
mcPCSK9	GA01712	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCUgUUUUAGagcuagxaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2022	SpCas9
mcPCSK9	GA01713	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCUgUUUUAGxgcuagxaauagcaxGUUaAaAuAaggcuxGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2041	SpCas9
mcPCSK9	GA01714	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCUgUUUUAGagcuagxaauagcaxGUUaAaAuAaggcuxGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2042	SpCas9
mcPCSK9	GA01715	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCUgUUUUAGagcuagaaauagcaxGUUaAaAuAaggcuxGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2043	SpCas9
mcPCSK9	GA01716	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuxGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2018	SpCas9
mcPCSK9	GA01717	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCUgUUUUAGxgcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2044	SpCas9
mcPCSK9	GA01718	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCUgUUUUAGagcuagaaauagcaxGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2045	SpCas9
mcPCSK9	GA01719	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCXUGGGAUGCUCUgUUUUAGagcuagxaauagcaxGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2046	SpCas9
mcPCSK9	GA01720	CAGGTTCCATGGG	81	5'csasgsGUUCCXUGGGAUGCUCUgUUUUAGagcuagxaauagcaaGUUaAa	2047	SpCas9

		ATGCTCT		AuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'		
mcPCSK9	GA01721	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCdXUGGGdXUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2048	SpCas9
mcPCSK9	GA01722	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCdXUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2049	SpCas9
mcPCSK9	GA01723	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGdXUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2050	SpCas9
mcPCSK9	GA01724	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagdXaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2051	SpCas9
mcPCSK9	GA01725	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagdXaauagcaaGUUaAaAuAaggcudXGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2052	SpCas9
mcPCSK9	GA01726	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcudXGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2053	SpCas9
mcPCSK9	GA01727	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUdXucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2054	SpCas9
mcPCSK9	GA01728	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagdXaauagcaaGUUaAaAuAaggcudXGUccGUUdXucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2055	SpCas9
mcPCSK9	GA01729	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGdXgcuagdXaauagcadXGUUaAaAuAaggcudXGUccGUUdXucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2056	SpCas9
mcPCSK9	GA01730	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2057	SpCas9
mcPCSK9	GA01731	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2058	SpCas9
mcPCSK9	GA01732	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2059	SpCas9
mcPCSK9	GA01733	CAGGTTCCATGGG	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagXaauagcaaGUUaAa	2060	SpCas9

		ATGCTCT		AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'		
mcPC SK9	GA01 734	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagXaauagcaaGUUaAa AuAaggcuXGUccGUUAucAAcuugaa aaagugGcaccgagucggugcuususu-3'	2061	SpCas 9
mcPC SK9	GA01 735	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuXGUccGUUAucAAcuugaaaa agugGcaccgagucggugcuususu-3'	2062	SpCas 9
mcPC SK9	GA01 736	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUUXucAAcuugaaaa agugGcaccgagucggugcuususu-3'	2063	SpCas 9
mcPC SK9	GA01 737	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagXaauagcaaGUUaAa AuAaggcuXGUccGUUXucAAcuugaa aaagugGcaccgagucggugcuususu-3'	2064	SpCas 9
mcPC SK9	GA01 738	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGXgcuagXaauagcaXGUUaA aAuAaggcuXGUccGUUXucAAcuuga aaaagugGcaccgagucggugcuususu-3'	2065	SpCas 9
mcPC SK9	GA01 739	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUg UUUUAGagcuagXaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'	2066	SpCas 9
mcPC SK9	GA01 740	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUg UUUUAGagcuagXaauagcaaGUUaAa AuAaggcuXGUccGUUAucAAcuugaa aaagugGcaccgagucggugcuususu-3'	2067	SpCas 9
mcPC SK9	GA01 741	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUUXucAAcuugaaaa agugGcaccgagucggugcuususu-3'	2068	SpCas 9
mcPC SK9	GA01 742	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUg UUUUAGagcuagXaauagcaaGUUaAa AuAaggcuXGUccGUUXucAAcuugaa aaagugGcaccgagucggugcuususu-3'	2069	SpCas 9
mcPC SK9	GA01 743	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUg UUUUAGXgcuagXaauagcaXGUUaA aAuAaggcuXGUccGUUXucAAcuuga aaaagugGcaccgagucggugcuususu-3'	2070	SpCas 9
mcPC SK9	GA01 744	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUg UUUUAGagcuagXaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'	2060	SpCas 9
mcPC SK9	GA01 745	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUg UUUUAGagcuagXaauagcaaGUUaAa AuAaggcuXGUccGUUAucAAcuugaa aaagugGcaccgagucggugcuususu-3'	2071	SpCas 9
mcPC SK9	GA01 746	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuXGUccGUUAucAAcuugaaaa agugGcaccgagucggugcuususu-3'	2072	SpCas 9

mcPCSK9	GA01747	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUXucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2073	SpCas9
mcPCSK9	GA01748	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUgUUUUAGagcuagXaauagcaaGUUaAaAuAaggcuXGUccGUUXucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2074	SpCas9
mcPCSK9	GA01749	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUgUUUUAGXgcuagXaauagcaXGUUaAaAuAaggcuXGUccGUUXucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2075	SpCas9
mcPCSK9	GA01750	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUgUUUUAGagcuagXaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2076	SpCas9
mcPCSK9	GA01751	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUgUUUUAGagcuagXaauagcaaGUUaAaAuAaggcuXGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2077	SpCas9
mcPCSK9	GA01752	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuXGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2078	SpCas9
mcPCSK9	GA01753	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUXucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2079	SpCas9
mcPCSK9	GA01754	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUgUUUUAGagcuagXaauagcaaGUUaAaAuAaggcuXGUccGUUXucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2080	SpCas9
mcPCSK9	GA01755	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUgUUUUAGXgcuagXaauagcaXGUUaAaAuAaggcuXGUccGUUXucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2081	SpCas9
mcPCSK9	GA01756	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUgUUUUAGxgcuagxaauagcaxGUUaAaAuAaggcuxGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2082	SpCas9
mcPCSK9	GA01757	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUgUUUUAGagcuagxaauagcaxGUUaAaAuAaggcuxGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2083	SpCas9
mcPCSK9	GA01758	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUgUUUUAGagcuagaaauagcaxGUUaAaAuAaggcuxGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2084	SpCas9
mcPCSK9	GA01759	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuxGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2085	SpCas9
mcPCSK9	GA01760	CAGGTTCCATGGG	81	5'csasgGUUCCXUGGGXUGCUCUgUUUUAGxgcuagaaauagcaaGUUaAa	2086	SpCas9

		ATGCTCT		AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'		
mcPC SK9	GA01 761	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUg UUUUAGagcuagaaauagcaxGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	1980	SpCas 9
mcPC SK9	GA01 762	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUg UUUUAGagcuagxaauagcaxGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'	2087	SpCas 9
mcPC SK9	GA01 763	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGXUGCUCUg UUUUAGagcuagxaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'	2066	SpCas 9
mcPC SK9	GA01 764	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUg UUUUAGxgcuagxaauagcaxGUUaAa AuAaggcuxGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'	2088	SpCas 9
mcPC SK9	GA01 765	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGXUGCUCU gUUUUAGagcuagxaauagcaxGUUaAa AuAaggcuxGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'	2036	SpCas 9
mcPC SK9	GA01 766	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUg UUUUAGagcuagaaauagcaxGUUaAa AuAaggcuxGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'	2089	SpCas 9
mcPC SK9	GA01 767	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuxGUccGUUAucAAcuugaaaa agugGcaccgagucggugcuususu-3'	2078	SpCas 9
mcPC SK9	GA01 768	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUg UUUUAGxgcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'	2090	SpCas 9
mcPC SK9	GA01 769	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUg UUUUAGagcuagaaauagcaxGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'	2091	SpCas 9
mcPC SK9	GA01 770	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUg UUUUAGagcuagxaauagcaxGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'	2092	SpCas 9
mcPC SK9	GA01 771	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCAUGGGXUGCUCUg UUUUAGagcuagxaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'	2076	SpCas 9
mcPC SK9	GA01 772	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUg UUUUAGxgcuagxaauagcaxGUUaAa AuAaggcuxGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'	2093	SpCas 9
mcPC SK9	GA01 773	CAGGTTC CATGGG ATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUg UUUUAGagcuagxaauagcaxGUUaAa AuAaggcuxGUccGUUAucAAcuugaaa aagugGcaccgagucggugcuususu-3'	2094	SpCas 9

mcPCSK9	GA01774	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUgUUUUAGagcuagaaauagcaxGUUaAaAuAaggcuxGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2095	SpCas9
mcPCSK9	GA01775	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuxGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2072	SpCas9
mcPCSK9	GA01776	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUgUUUUAGxgcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2096	SpCas9
mcPCSK9	GA01777	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUgUUUUAGagcuagaaauagcaxGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2097	SpCas9
mcPCSK9	GA01778	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUgUUUUAGagcuagxaauagcaxGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2098	SpCas9
mcPCSK9	GA01779	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCXUGGGAUGCUCUgUUUUAGagcuagxaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2099	SpCas9
mcPCSK9	GA01780	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCdXUGGGdXUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaaugGcaccgagucggugcuususu-3'	2100	SpCas9
mcPCSK9	GA01781	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCdXUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2101	SpCas9
mcPCSK9	GA01782	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGdXUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2102	SpCas9
mcPCSK9	GA01783	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagdXaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2103	SpCas9
mcPCSK9	GA01784	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagdXaauagcaaGUUaAaAuAaggcudXGUccGUUAucAAcuugaaaaaugGcaccgagucggugcuususu-3'	2104	SpCas9
mcPCSK9	GA01785	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcudXGUccGUUAucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2105	SpCas9
mcPCSK9	GA01786	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUdXucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2106	SpCas9
mcPCSK9	GA01787	CAGGTTCCATGGG	81	5'csasgGUUCCAUGGGAUGCUCUgUUUUAGagcuagdXaauagcaaGUUaA	2107	SpCas9

		ATGCTCT		aAuAaggcudXGUccGUUdXucAAcuugaaaaagugGcaccgagucggugcuususu-3'		
mcPCSK9	GA01788	CAGGTTCCATGGGATGCTCT	81	5'csasgGUUCCAUGGGAUGCUCgUUUUAGdXgcuagdXaauagcadXGUUaAaAuAaggcudXGUccGUUdXucAAcuugaaaaagugGcaccgagucggugcuususu-3'	2108	SpCas9
mcPCSK9	GA01789	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusxsxsx-3'	2109	SpCas9
mcPCSK9	GA01790	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusxsxsx--3'	2110	SpCas9
mcPCSK9	GA01791	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusxsxsx--3'	2110	SpCas9
mcPCSK9	GA01792	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcususxsx-3'	2111	SpCas9
mcPCSK9	GA01793	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcususxsx--3'	2112	SpCas9
mcPCSK9	GA01794	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcususxsx--3'	2112	SpCas9
mcPCSK9	GA01795	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususx-3'	2113	SpCas9
mcPCSK9	GA01796	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcususususx--3'	2114	SpCas9
mcPCSK9	GA01797	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcususususx--3'	2114	SpCas9
mcPCSK9	GA01798	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusXsXsXsX-3'	2109	SpCas9

mcPCSK9	GA01799	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusXsXsX--3'	2110	SpCas9
mcPCSK9	GA01800	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusXsXsX--3'	2110	SpCas9
mcPCSK9	GA01801	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcususXsX-3'	2111	SpCas9
mcPCSK9	GA01802	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcususXsX--3'	2112	SpCas9
mcPCSK9	GA01803	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcususXsX--3'	2112	SpCas9
mcPCSK9	GA01804	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususX-3'	2113	SpCas9
mcPCSK9	GA01805	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcususususX--3'	2114	SpCas9
mcPCSK9	GA01806	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcususususX--3'	2114	SpCas9
mcPCSK9	GA01807	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcususususuNNN-3'	1797	SpCas9
mcPCSK9	GA01808	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcusususuNNN-3'	1797	SpCas9
mcPCSK9	GA01809	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcususususuNNN-3'	1797	SpCas9
mcPCSK10	GA01810	CAGGTTCCATGGG	81	5'csasgsGUUCCAUGGGAUGCUCUGUUUUAGAgcuagaaauagcAAGUUA	1797	SpCas9

		ATGCTCT		AAAUAAGGCUAGUCCGUUAUCA acuugaaaaaguggcaccgagucggugcususu sunnn-3'		
mcPC SK11	GA01 811	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcusususunn n-3'	1797	SpCas 9
mcPC SK12	GA01 812	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususunnn-3'	1797	SpCas 9
mcPC SK9	GA01 813	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU GUUUUAGAgcuagaaauagcAAGUUA AAAUAAGGCUAGUCCGUUAUCA acuugaaaaaguggcaccgagucggugcusdXs dXsdX-3'	2115	SpCas 9
mcPC SK9	GA01 814	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcusdXsdXs dX--3'	2116	SpCas 9
mcPC SK9	GA01 815	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusdXsdXsdX-- 3'	2116	SpCas 9
mcPC SK9	GA01 816	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU GUUUUAGAgcuagaaauagcAAGUUA AAAUAAGGCUAGUCCGUUAUCA acuugaaaaaguggcaccgagucggugcususd XsdX-3'	2117	SpCas 9
mcPC SK9	GA01 817	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcususdXsd X--3'	2118	SpCas 9
mcPC SK9	GA01 818	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcususdXsdX--3'	2118	SpCas 9
mcPC SK9	GA01 819	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU GUUUUAGAgcuagaaauagcAAGUUA AAAUAAGGCUAGUCCGUUAUCA acuugaaaaaguggcaccgagucggugcususu sdX-3'	2119	SpCas 9
mcPC SK9	GA01 820	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggCUaGUCcGUUAucAAcuuGa aaaaguGgcaccgAgUCggugcususdX- -3'	2120	SpCas 9
mcPC SK9	GA01 821	CAGGTTC CATGGG ATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa	2120	SpCas 9

				aagugGcaccgagucggugcusususdX--3'		
mcPCSK9	GA053	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcususususdXdXdX-3'	2121	SpCas9
mcPCSK9	GA01823	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcususususdXdXdX-3'	2121	SpCas9
mcPCSK9	GA01824	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcususususdXdXdX-3'	2121	SpCas9
mcPCSK99	GA01825	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGGcaccgAgUCggugcusususu-3'	55	SpCas9
mcPCSK99	GA01826	CAGGTTCCATGGGATGCTCT	81	5'csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaaguGGcaccgagucggugcusususu-3'	55	SpCas9
mPCSK9	GA010	GGCTGATGAGGCCGCACATG	251	5’gsgscsUGAUGAGGCCGCACAUGGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3’	446	SpCas9
mPCSK9	GA011	GGCTGATGAGGCCGCACATG	251	5’gsgscsUGAUGAGGCCGCACAUGGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3’	446	SpCas9
hPCSK9	GA013	CGCCTGCCAGCGCCTGGCGA	252	5’csgscsCUGCCAGCGCCUGGCGAGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3’	452	CBE
hPCSK9	GA015	CGCCTGCCAGCGCCTGGCGA	252	5’csgsusGCGCAGGAGGACGAGGAGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3’	341	CBE
hcANGPTL3	GA016	GGCCTCCTTCAGTTGGGACA	107	5’gsgscsCUCCUUCAGUUGGGACAGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3’	285	SpCas9
hcANGPTL3	GA017	GCCAATGGCCTCCTTCAGTT	109	5’gscscsAAUGGCCUCCUUCAGUUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3’	287	SpCas9

hcAN GPTL 3	GA03 1	GCCAAT GGCCTCC TTCAGTT	109	5'gscscsAAUGGCCUCCUUCAGUU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	287	SpCas 9
hcAN GPTL 3	GA03 2	GCCAAT GGCCTCC TTCAGTT	109	5'gscscsAAUGGCCUCCUUCAGUU gUUUUAGagcuagaaauagcaaGUUsaA aAuAaggcuaGUccGUUsAucAAcuuga aaaagugGcaccgagucggugcusususu-3'	2122	SpCas 9
hcAN GPTL 3	GA03 3	GCCAAT GGCCTCC TTCAGTT	109	5'gscscsAAUGGCCUCCUUCAGUU gUUUuAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	287	SpCas 9
hcAN GPTL 3	GA03 4	GCCAAT GGCCTCC TTCAGTT	109	5'gscscsAAUGGCCUCCUUCAGUU gUUUUAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUuAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	287	SpCas 9
hcAN GPTL 3	GA03 5	GCCAAT GGCCTCC TTCAGTT	109	5'gscscsAAUGGCCUCCUUCAGUU gUUUuAGagcuagaaauagcaaGUUaAa AuAaggcuaGUccGUuAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	287	SpCas 9
hcAN GPTL 3	GA03 6	GCCAAT GGCCTCC TTCAGTT	109	5'gscscsAAUGGCCUCCUUCAGUU gUUUuAGagcuagaaauagcaaGUuaAa AuAaggcuaGUccGUUAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	287	SpCas 9
hcAN GPTL 3	GA03 7	GCCAAT GGCCTCC TTCAGTT	109	5'gscscsAAUGGCCUCCUUCAGUU gUUUUAGagcuagaaauagcaaGUuaAa AuAaggcuaGUccGUuAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	287	SpCas 9
hcAN GPTL 3	GA03 8	GCCAAT GGCCTCC TTCAGTT	109	5'gscscsAAUGGCCUCCUUCAGUU gUUUuAGagcuagaaauagcaaGUuaAa AuAaggcuaGUccGUuAucAAcuugaaa aagugGcaccgagucggugcusususu-3'	287	SpCas 9
hcAN GPTL 3	GA04 0	GCCAAT GGCCTCC TTCAGTT	109	5'gscscsAAuGGCCUCCUUcAGUUg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUUAucAAcuugaaaa agugGcaccgagucggugcusususu-3'	287	SpCas 9
hcAN GPTL 3	GA04 1	GCCAAT GGCCTCC TTCAGTT	109	5'gscscsAAuGGCCUCCUUcAGUUg UUUUAGagcuagaaauagcaaGUUsaAa AuAaggcuaGUccGUUsAucAAcuugaa aaagugGcaccgagucggugcusususu-3'	2122	SpCas 9
hcAN GPTL 3	GA04 2	TCCCAAC TGAAGG AGGCCA T	106	5'uscscsCAACUGAAGGAGGCCAU GUUUUAGAgcuagaaauagcAAGUUA AAAUAAGGCUAGUCCGUUAUCA acuugaaaaaguggcaccgagucggugcususu su-3'	284	SpCas 9
hcAN GPTL 3	GA04 3	ATTCTGG AGGAAA TAACTA G	111	5'asususCUGGAGGAAAUAACUAG GUUUUAGAgcuagaaauagcAAGUUA AAAUAAGGCUAGUCCGUUAUCA acuugaaaaaguggcaccgagucggugcususu su-3'	289	SpCas 9
hcAN GPTL 3	GA04 4	ACTACA AGTCAA AAATGA AG	115	5'ascsusACAAGUCAAAAAUGAAG GUUUUAGAgcuagaaauagcAAGUUA AAAUAAGGCUAGUCCGUUAUCA acuugaaaaaguggcaccgagucggugcususu	293	SpCas 9

				su-3’		
hcANGPTL3	GA045	CTTTTATTTGACTATGCTGT	120	5’csususUUAUUUGACUAUGCUGUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcususu su-3’	298	SpCas9
hcANGPTL3	GA046	GGAGTAGTTCTTGGTGCTCT	124	5’gsgsasGUAGUUCUUGGUGCUCUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcususu su-3’	302	SpCas9
hcANGPTL3	GA047	AGTTCTTGGTGCTCTTGGCT	127	5’asgsusUCUUGGUGCUCUUGGCUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcususu su-3’	305	SpCas9
hcANGPTL3	GA048	GGAAGATAGAGAAATTTCTG	130	5’gsgsasAGAUAGAGAAAUUUCUGGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcususu su-3’	308	SpCas9
hcANGPTL3	GA049	CATTATATTCAGGTAGTCCA	143	5’csasusUAUAUUCAGGUAGUCCAGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcususu su-3’	321	SpCas9
hcANGPTL3	GA050	ACAAAACTTCAATGAAACGT	146	5’ascsasAAACUUCAAUGAAACGUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcususu su-3’	324	SpCas9
hcANGPTL3	GA051	GAGAACTACAAATATGGTTT	149	5’gsasgsAACUACAAAUAUGGUUUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcususu su-3’	327	SpCas9
mcPCSK9	GA052	CAGGTTCCATGGGATGCTCT	81	5’csasgsGUUCCAUGGGAUGCUCUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu-3’	55	SpCas9
mcPCSK9	GA053	CAGGTTCCATGGGATGCTCT	81	5’csasgsGUUCCAUGGGAUGCUCUGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcususu su-3’	55	SpCas9
hANGPTL3	GA098	AAGATACCTGAATAACCCTC	15	5’asasgsAUACCUGAAUAACCCUCGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcususu su-3’	59	ABE
cANGPTL3	GA101/GA06	AAGATACCTGAATAACTCTC	14	5’asasgsAUACCUGAAUAACUCUCGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCA	257	ABE

	6			acuugaaaaaguggcaccgagucggugcususu su-3’		
hcPCSK9	GA234	CGCCTGCCAGCGCCTGGCGA	252	5’csgscsCUGCCAGCGCCUGGCGAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu-3’	452	CBE
hcANGPTL3	GA261	TGAAGAAAGGGAGTAGTTCT	126	5’usgsasAGAAAGGGAGUAGUUCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcususu-3’	304	SpCas9
hcANGPTL3	GA262	CATTATATTCAGGTAGTCCA	143	5’csasusUAUAUUCAGGUAGUCCAgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcususu-3’	321	SpCas9
hcANGPTL3	GA263	ACAAAACTTCAATGAAACGT	146	5’ascsasAAACUUCAAUGAAACGUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcususu-3’	324	SpCas9
hcANGPTL3	GA264	GCCAATGGCCTCCTTCAGTT	109	5’gscscsAAUGGCCUCCUUCAGUUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcususu-3’	287	SpCas9
hcANGPTL3	GA265	GCCAATGGCCTCCTTCAGTT	109	5’gscscsAAUGGCCUCCUUCAGUUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggCUaGUCcGUUAucAAcuuGaaaaaguGgcaccgAgUCggugcususu-3’	287	SpCas9
hcPCSK9	GA266	TCAGCTCCAGGCGGTCCTGG	161	5’uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcususu-3’	339	SpCas9
hcPCSK9	GA267	GCCCCATGTCGACTACATCG	185	5’gscscsCCAUGUCGACUACAUCGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcususu-3’	363	SpCas9
hcPCSK9	GA268	GGGGCTGGTATTCATCCGCC	187	5’gsgsgsGCUGGUAUUCAUCCGCCgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcususu-3’	365	SpCas9
hcPCSK9	GA269	CTAGGAGATACACCTCCACC	194	5’csusasGGAGAUACACCUCCACCgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcususu-3’	372	SpCas9
hcPCSK9	GA270	CTGCGCGTGCTCAACTGCCA	204	5’csusgsCGCGUGCUCAACUGCCAgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcususu-3’	382	SpCas9
hcPCSK9	GA271	CGGGATGCCGGCGTGGCCAA	206	5’csgsgsGAUGCCGGCGUGGCCAAgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcususu-3’	384	SpCas9
hcPCSK9	GA272	CGCTGACCACCCCTGCCAG	212	5’csgscsUGACCACCCCUGCCAGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaa	390	SpCas9

		G		agugGcaccgagucggugcusususu-3’		
hcPCSK9	GA273	TCATGGCACCCACCTGGCAG	215	5’uscsasUGGCACCCACCUGGCAGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3’	393	SpCas9
hcPCSK9	GA274	GCTGACCACCCCTGCCAGGT	217	5’gscsusGACCACCCCUGCCAGGUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3’	395	SpCas9
hcANGPTL3	GA295	GCAAATCTTGATTTTGGCTC	1608	5’gscsasAAUCUUGAUUUUGGCUCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu-3’	2123	SpCas9
hcANGPTL3	GA296	GATGTAAAAATTTTAGCCAA	1609	5’gsasusGUAAAAAUUUUAGCCAAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu-3’	2124	SpCas9
hcANGPTL3	GA297	ATGAAAAACTTGAGAGTTGC	1610	5’asusgsAAAAACUUGAGAGUUGCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu-3’	2125	SpCas9
hcANGPTL3	GA298	ACTTGGGATCACAAAGCAAA	1611	5’ascsusUGGGAUCACAAAGCAAAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu-3’	2126	SpCa9
hcANGPTL3	GA299	AAGATTTGGTGTTTTCTACT	1612	5’asasgsAUUUGGUGUUUUCUACUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu-3’	2127	SpCas9
hcAPOC3	GA300	AAGGAGTACCCGGGGCTGCA	1613	5’asasgsGAGUACCCGGGGCUGCAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu-3’	2128	SpCas9
hcAPOC3	GA301	CAGAGGCCAGGAGCGCCAGG	1614	5’csasgsAGGCCAGGAGCGCCAGGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu-3’	2129	SpCas9
hcAPOC3	GA302	AGAGGCCAGGAGCGCCAGGA	1615	5’asgsasGGCCAGGAGCGCCAGGAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu	2130	SpCas9
hcAPOC3	GA303	CTCGGCCTCTGAAGCTCCTG	1616	5’csuscsGGCCUCUGAAGCUCCUGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu-3’	2131	SpCas9

hcANGPTL3	GA304	CCAATGGCCTCCTTCAGTT	1594	5'iscscsAAUGGCCUCCUUCAGUUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1622	SpCas9
hcANGPTL3	GA305	CCAATGGCCTCCTTCAGTT	1594	5'cscsAAUGGCCUCCUUCAGUUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	1623	SpCas9
hcPCSK9	GA306	CCACCGGGAAATCGAGGGCA	192	5'cscsasCCGGGAAAUCGAGGGCAgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	370	SpCas9
hcPCSK9	GA307	GCTTACCTGTCTGTGGAAGC	67	5'gscsusUACCUGUCUGUGGAAGCgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	259	ABE
hcPCSK9	GA309	CTTGGCAGTTGAGCACGCGC	203	5'csususGGCAGUUGAGCACGCGCgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3'	381	SpCas9
hcPCSK9	GA343	CCCGCACCTTGGCGCAGCGG	13	5'cscscsGCACCUUGGCGCAGCGGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu-3'	9	ABE
cANGPTL3	GA344	aagatacCTGAATAACTCTC	14	5'asasgsAUACCUGAAUAACUCUCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu-3'	257	ABEe
PCSK9	GA348	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'	421	ABE
mANGPTL3	GA349	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCGUUUUAGAgcuagaaauagcAAGUUAAAAUAAGGCUAGUCCGUUAUCAacuugaaaaaguggcaccgagucggugcusususu-3'	422	ABE
mANGPTL3	GA353	GAGATACCTGAGTAACTTTC	241	5'gsasgsAUACCUGAGUAACUUUCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu-3'	422	ABE
mPCSK9	GA355	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuuuu-3'	1643	ABE
mPCSK9	GA356	CCCATACCTTGGAGCAACGG	69	5'cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususuuuu-3'	1644	ABE

mPCSK9	GA357	CCCATACCTTGGAGCAACGG	69	5’cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuusususuuuu-3’	1645	ABE
mPCSK9	GA358	CCCATACCTTGGAGCAACGG	69	5’cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuusususuuuu-3’	1646	ABE
mPCSK9	GA359	CCCATACCTTGGAGCAACGG	69	5’cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuusususuuuu-3’	1647	ABE
mPCSK9	GA360	CCCATACCTTGGAGCAACGG	69	5’cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuusususuUu-3’	1645	ABE
mPCSK9	GA361	CCCATACCTTGGAGCAACGG	69	5’cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuusususuUu-3’	1646	ABE
mPCSK9	GA362	CCCATACCTTGGAGCAACGG	69	5’cscscsAUACCUUGGAGCAACGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuusususuUu-3’	1647	ABE
mPCSK9	GA363	CCCATACCTTGGAGCAACGG	69	5’cscscsAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUCcGUUAucAAcuugaaaaaguggcaccgagucggugcusususu-3’	421	ABE
mPCSK9	GA364	CCCATACCTTGGAGCAACGG	69	5’cscscsAUACCUUGGAGCAACGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGuCcGUUaucAAcuugaaaaaguggcaccgagucggugcusususu-3’	421	ABE
mPCSK9	GA365	CCCATACCTTGGAGCAACGG	69	5’cscscsAUACCUUGGAGCAACGGgUUUuagagcuagaaauagcaaGUUaAaAuaaggcuaGuccgUUaucaacuugaaaaaggcgcaccgagucggugcusususu-3’	421	ABE
mcPCSK9	GA366	CAGGTTCCATGGGATGCTCT	81	5’csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuusususuuuu-3’	1759	SpCas9
mcPCSK9	GA367	CAGGTTCCATGGGATGCTCT	81	5’csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuusususuuuu-3’	1754	SpCas9
mcPCSK9	GA368	CAGGTTCCATGGG	81	5’csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAa	1752	SpCas9

		ATGCTCT		AuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuusususuuu-3’		
mcPCSK9	GA369	CAGGTTCCATGGGATGCTCT	81	5’csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuusususuUu-3’	1759	SpCas9
mcPCSK9	GA370	CAGGTTCCATGGGATGCTCT	81	5’csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuusususuUu-3’	1754	SpCas9
mcPCSK9	GA371	CAGGTTCCATGGGATGCTCT	81	5’csasgsGUUCCAUGGGAUGCUCUgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcuusususuUu-3’	1752	SpCas9
mcPCSK9	GA372	CAGGTTCCATGGGATGCTCT	81	5’csasgsGdUUdCCdAUdGGdGAdUGdCUdCUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3’	2132	SpCas9
mcPCSK9	GA373	CAGGTTCCATGGGATGCTCT	81	5’csasgsdGUdUCdCAdUGdGGdAUdGCdUCdUgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcususususu-3’	2133	SpCas9
hPCSK9	GA374	CCCGCACCTTGGCGCAGCGG	13	5’cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAacAAcuugaaaaagugGcaccgagucggugcususususu-3’	10	ABE
hPCSK9	GA375	CCCGCACCTTGGCGCAGCGG	13	5’cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAacAAcgggaaaccgugGcaccgagucggugcususususu-3’	428	ABE
hcPCSK9	GA376	CCCGCACCTTGGCGCAGCGG	13	5’cscscsdGCdACdCUdUGdGCdGCdAGdCGdGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcususususu-3’	429	ABE
hcPCSK9	GA377	CCCGCACCTTGGCGCAGCGG	13	5’cscscsGdCAdCCdUUdGGdCGdCAdGCdGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcususususu-3’	430	ABE
hcPCSK9	GA378	CCCGCACCTTGGCGCAGCGG	13	5’cscscsGdCACCUdUGGdCGdCAGdCGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuGaaaaagugGcaccgagucggugcususususu-3’	1648	ABE
hcPCSK9	GA379	CCCGCACCTTGGCGCAGCG	13	5’cscscsGCdACCUUdGGCdGCdAGCdGGGUUUUAGAgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUcc	1649	ABE

		G		GUUAucAAcuuGaaaaagugGcaccgagu cggugcusususu-3’		
hcPCS K9	GA38 0	CCCGCA CCTTGGC GCAGCG G	13	5’cscscsGdCACCUUGGCGdCAGCG GgUUUUAGagcuaGaaauagcaaGUUa AaAuAaggcuaGUccGUUAucAAcuuG aaaaagugGcaccgagucggugcusususu-3’	431	ABE
hcPCS K9	GA38 1	CCCGCA CCTTGGC GCAGCG G	13	5’cscscsGCdACCUUGGCGCdAGCG GgUUUUAGagcuaGaaauagcaaGUUa AaAuAaggcuaGUccGUUAucAAcuuG aaaaagugGcaccgagucggugcusususu-3’	432	ABE
hcPCS K9	GA38 2	CCCGCA CCTTGGC GCAGCG G	13	5’cscscsGCACCUdUGGdCGCAGdC GGgUUUUAGagcuaGaaauagcaaGUU aAaAuAaggcuaGUccGUUAucAAcuu GaaaaagugGcaccgagucggugcusususu-3’	433	ABE
hcPCS K9	GA38 3	CCCGCA CCTTGGC GCAGCG G	13	5’cscscsGCACCUUdGGCdGCAGCd GGgUUUUAGagcuaGaaauagcaaGUU aAaAuAaggcuaGUccGUUAucAAcuu GaaaaagugGcaccgagucggugcusususu-3’	434	ABE
hcPCS K9	GA38 4	CCCGCA CCTTGGC GCAGCG G	13	5’cscscsdGdCdACCdUdUGGdCGCA dGCGdGgUUUUAGagcuaGaaauagca aGUUaAaAuAaggcuaGUccGUUAuc AAcuuGaaaaagugGcaccgagucggugcus ususu-3’	435	ABE
hcPCS K9	GA38 5	CCCGCA CCTTGGC GCAGCG G	13	5’cscscsGCACCUUGGCGCAGCGGg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUUAucAAcuugaaaa agugGcaccgagucggugcusususuuuu-3’	11	ABE
hcPCS K9	GA38 6	CCCGCA CCTTGGC GCAGCG G	13	5’cscscsGCACCUUGGCGCAGCGGg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGUccGUUAucAAcuugaaaa agugGcaccgagucggugcusususuUu-3’	11	ABE
hcPCS K9	GA38 7	CCCGCA CCTTGGC GCAGCG G	13	5’cscscsGCACCUUGGCGCAGCGgU UUUAGagcuaGaaauagcaaGUUaAaAu AaggcuaGUccGUUAucAAcuuGaaaaa gugGcaccgagucggugcuususuuuu-3’	436	ABE
hcPCS K9	GA38 8	CCCGCA CCTTGGC GCAGCG G	13	5’cscscsGCACCUUGGCGCAGCGGg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGUCcGUUAucAAcuugaaaa aguggcaccgagucggugcusususu-3’	9	ABE
hcPCS K9	GA38 9	CCCGCA CCTTGGC GCAGCG G	13	5’cscscsGCACCUUGGCGCAGCGGg UUUUAGagcuagaaauagcaaGUUaAaA uAaggcuaGuCcGUUaucAAcuugaaaaa guggcaccgagucggugcusususu	9	ABE
hcPCS K9	GA39 0	CCCGCA CCTTGGC GCAGCG G	13	5’cscscsGCACCUUGGCGCAGCGGg UUUuagagcuagaaauagcaaGUUaAaAu aaggcuaGuccgUUaucaacuugaaaaagugg caccgagucggugcusususu-3’	9	ABE
hcPCS K9	GA39 1	CCCGCA CCTTGGC GCAGCG G	13	5’cscsGCACCUUGGCGCAGCGGgU UUUAGagcuaGaaauagcaaGUUaAaAu AaggcuaGUccGUUAucAAcuuGaaaaa gugGcaccgagucggugcusususu-3’	437	ABE
hANG	GA39	AAGATA	15	5’asgsAUACCUGAAUAACCCUCgU	1650	ABE

PTL3	2	CCTGAATAACCCTC		UUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu-3’		
hcPCSK9	GA393	CAGCTCCAGGCGGTCCTGG	1617	5’csasGCUCCAGGCGGUCCUGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususu-3’	2134	
hcPCSK9	GA394	TCAGCTCCAGGCGGTCCTGG	161	5’uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcuususu-3’	2135	SpCas9
hcPCSK9	GA395	TCAGCTCCAGGCGGTCCTGG	161	5’uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuuuu-3’	447	SpCas9
hcPCSK9	GA396	TCAGCTCCAGGCGGTCCTGG	161	5’uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuuuu-3’	448	SpCas9
hcPCSK9	GA397	TCAGCTCCAGGCGGTCCTGG	161	5’uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuuuu-3’	449	SpCas9
hcPCSK9	GA398	TCAGCTCCAGGCGGTCCTGG	161	5’uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUCcGUUAucAAcuugaaaaaguggcaccgagucggugcusususuuuu-3’	447	SpCas9
hcPCSK9	GA399	TCAGCTCCAGGCGGTCCTGG	161	5’uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGuCcGUUaucAAcuugaaaaaguggcaccgagucggugcusususuuuu-3’	447	SpCas9
hcPCSK9	GA400	TCAGCTCCAGGCGGTCCTGG	161	5’uscsasGCUCCAGGCGGUCCUGGgUUUuagagcuagaaauagcaaGUUaAaAuaaggcuaGuccgUUaucaacuugaaaaagugcaccgagucggugcusususuuuu-3’	447	SpCas9
hcPCSK9	GA401	TCAGCTCCAGGCGGTCCTGG	161	5’uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuuuu-3’	447	SpCas9
hcPCSK9	GA402	TCAGCTCCAGGCGGTCCTGG	161	5’uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUu-3’	447	SpCas9
hcPCSK9	GA403	TCAGCTCCAGGCGGTCCTGG	161	5’uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuaGaaauagcaaGUUsaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUu-3’	448	SpCas9
hcPCSK9	GA404	TCAGCTCCAGGCG	161	5’uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuaGaaauagcaaGUUaAa	449	SpCas9

		GTCCTGG		AuAaggcuaGUccGUUsAucAAcuuGaaaaagugGcaccgagucggugcusususuuUu-3’		
hcPCSK9	GA405	TCAGCTCCAGGCGGTCCTGG	161	5’uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUCcGUUAucAAcuugaaaaaguggcaccgagucggugcusususuUu-3’	447	SpCas9
hcPCSK9	GA406	TCAGCTCCAGGCGGTCCTGG	161	5’uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGuCcGUUaucAAcuugaaaaaguggcaccgagucggugcusususuUu-3’	447	SpCas9
hcPCSK9	GA407	TCAGCTCCAGGCGGTCCTGG	161	5’uscsasGCUCCAGGCGGUCCUGGgUUUuagagcuagaaauagcaaGUUaAaAuaaggcuaGuccgUUaucaacuugaaaaaggcaccgagucggugcusususuUu-3’	447	SpCas9
hcPCSK9	GA408	TCAGCTCCAGGCGGTCCTGG	161	5’uscsasGCUCCAGGCGGUCCUGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuUu-3’	447	SpCas9
hcPCSK9	GA409	TCAGCTCCAGGCGGTCCTGG	161	5’uscsasG(C-PACE)UCCAGGCGGUCCUGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaaguGcaccgagucggugcusususu-3’	450	SpCas9
hcPCSK9	GA410	TCAGCTCCAGGCGGTCCTGG	161	5’uscsasGCUCCAG(G-PACE)CGGUCCUGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususu-3’	451	SpCas9
hcPCSK9	GA439	CCCGCACCTTGGCGCAGCGG	13	5’cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUUu-3’	11	ABE
hcPCSK9	GA440	CCCGCACCTTGGCGCAGCGG	13	5’cscscsGCACCUUGGCGCAGCGGgUUUUAGagcuagaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuugaaaaagugGcaccgagucggugcusususuUUu-3’	11	ABE
hANGPTL3	GA441	AAGATACCTGAATAACCCTC	15	5’asasgsAUACCUGAAUAACCCUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususu-3’	59	ABE
hANGPTL3	GA442	AAGATACCTGAATAACCCTC	15	5’asasgsAUACCUGAAUAACCCUCgUUUUAGagcuaGaaauagcaaGUUaAaAuAaggcuaGUccGUUAucAAcuuGaaaaagugGcaccgagucggugcusususuUUu-3’	438	ABE
hANGPTL3	GA462	AGATACCTGAATAACCCTC	248	5’asgsasUACCUGAAUAACCCUCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUsususu-3’	442	ABE
hANG	GA46	GATACCT	249	5’gsasUACCUGAAUAACCCUCGU	443	ABE

PTL3	3	GAATAA CCCTC		UUUAGAGCUAGAAAUAGCAAGU UAAAAUAAGGCUAGUCCGUUAU CAACUUGAAAAAGUGGCACCGA GUCGGUGCUsususu-3’		
hANG PTL3	GA46 4	ATACCTG AATAAC CCTC	250	5’asusACCUGAAUAACCCUCGUU UUAGAGCUAGAAAUAGCAAGUU AAAAUAAGGCUAGUCCGUUAUC AACUUGAAAAAGUGGCACCGAG UCGGUGCUsususu-3’	444	ABE
hANG PTL3	GA46 5	AGATAC CTGAAT AACCCTC	248	5’asgsAUACCUGAAUAACCCUCG UUUUAGAGCUAGAAAUAGCAAG UUAAAAUAAGGCUAGUCCGUUA UCAACUUGAAAAAGUGGCACCG AGUCGGUGCUsususu-3’	1650	ABE
hANG PTL3	GA46 6	GATACCT GAATAA CCCTC	249	5’gsasUsACCUGAAUAACCCUCGU UUUAGAGCUAGAAAUAGCAAGU UAAAAUAAGGCUAGUCCGUUAU CAACUUGAAAAAGUGGCACCGA GUCGGUGCUsususu-3’	1651	ABE
hANG PTL3	GA46 7	ATACCTG AATAAC CCTC	250	5’asUsACCUGAAUAACCCUCGUU UUAGAGCUAGAAAUAGCAAGUU AAAAUAAGGCUAGUCCGUUAUC AACUUGAAAAAGUGGCACCGAG UCGGUGCUsususu-3’	444	ABE
hANG PTL3	GA47 2	AAGATA CCTGAAT AACCCTC	15	5’asasisAUACCUGAAUAACCCUCg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcusususu-3’	439	ABE
hANG PTL3	GA47 3	AAGATA CCTGAAT AACCCTC	15	5’asasisAUACCUIAAUAACCCUCg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcusususu-3’	440	ABE
hANG PTL3	GA47 4	AAGATA CCTGAAT AACCCTC	15	5’asasgsAUACCUIAAUAACCCUCg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcusususu-3’	441	ABE
hANG PTL3	GA47 5	AGATAC CTGAAT AACCCTC	248	5’asgsasUACCUGAAUAACCCUCg UUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcusususu-3’	442	ABE
hANG PTL3	GA47 6	GATACCT GAATAA CCCTC	249	5’gsasUACCUGAAUAACCCUCgUU UUAGagcuaGaaauagcaaGUUaAaAuA aggcuaGUccGUUAucAAcuuGaaaaag ugGcaccgagucggugcusususu-3’	443	ABE
hANG PTL3	GA47 7	ATACCTG AATAAC CCTC	250	5’asusACCUGAAUAACCCUCgUUU UAGagcuaGaaauagcaaGUUaAaAuAa ggcuaGUccGUUAucAAcuuGaaaaagu gGcaccgagucggugcusususu-3’	444	ABE
cANG PTL3	GA51 7	GATACCT GAATAA CTCTC	1606	5’gsasUsACCUGAAUAACUCUCgU UUUAGagcuaGaaauagcaaGUUaAaAu AaggcuaGUccGUUAucAAcuuGaaaaa gugGcaccgagucggugcusususu-3’	1652	ABE
cANG PTL3	GA51 8	ATACCTG AATAAC	1607	5’asusAsCCUGAAUAACUCUCgUU UUAGagcuaGaaauagcaaGUUaAaAuA	1653	ABE

		TCTC		aggcuaGUccGUUAucAAcuuGaaaaag ugGcaccgagucggugcusususu-3’		
hANG PTL3	GA539	AAGATA CCTGAAT AACCCTC	15	5’asasgsAUXCCUGAAUAACCCUC gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcusususu-3’	1654	ABE
hANG PTL3	GA540	AAGATA CCTGAAT AACCCTC	15	5’asasgsAUXCCUGXAUAACCCUC gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcusususu-3’	1655	ABE
hANG PTL3	GA541	AAGATA CCTGAAT AACCCTC	15	5’asasgsAUXCCUGAAUAXCCCUC gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcusususu-3’	1656	ABE
hANG PTL3	GA542	AAGATA CCTGAAT AACCCTC	15	5’asasgsAUXCCUGXAUXACCCUC gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcusususu-3’	1657	ABE
hANG PTL3	GA543	AAGATA CCTGAAT AACCCTC	15	5’asasgsXUXCCUGAAUAACCCUC gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcusususu-3’	1658	ABE
hANG PTL3	GA544	AAGATA CCTGAAT AACCCTC	15	5’asasgsXUXCCUGAAUAXCCCUC gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcusususu-3’	1659	ABE
hANG PTL3	GA545	AAGATA CCTGAAT AACCCTC	15	5’asasgsXUACCUGAAUXACCCUC gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcusususu-3’	1660	ABE
hANG PTL3	GA546	AAGATA CCTGAAT AACCCTC	15	5’asasgsAUACCUGXAUAACCCUC gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcusususu-3’	1661	ABE
hANG PTL3	GA547	AAGATA CCTGAAT AACCCTC	15	5’asasgsAUACCUGAAUAACUCUC gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaaguggcaccgagucggugcusususuUUu-3’	445	ABE
hANG PTL3	GA548	AAGATA CCTGAAT AACCCTC	15	5’asasgsAUACCUGXAUAXCCCUC gUUUUAGagcuaGaaauagcaaGUUaAa AuAaggcuaGUccGUUAucAAcuuGaa aaagugGcaccgagucggugcusususu-3’	1662	ABE

[762] All patents and publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent and publication was specifically and individually indicated to be incorporated by reference.

Other Embodiments

[763] From the foregoing description, it will be apparent that variations and modifications may be made to the disclosure described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

[764] The recitation of a listing of elements in any definition of a variable herein includes definitions of that variable as any single element or combination (or subcombination) of listed elements. The recitation of an embodiment herein includes that embodiment as any single embodiment, any portion of the embodiment, or in combination with any other embodiments or any portion thereof.

[765] As is set forth herein, it will be appreciated that the disclosure comprises specific embodiments and examples of base editing systems to effect a nucleobase alteration in a gene and methods of using same for treatment of disease including compositions that comprise such base editing systems, designs and modifications thereto; and specific examples and embodiments describing the synthesis, manufacture, use, and efficacy of the foregoing individually and in combination including as pharmaceutical compositions for treating disease and for *in vivo* and *in vitro* delivery of active agents to mammalian cells under described conditions.

[766] While specific examples and numerous embodiments have been provided to illustrate aspects and combinations of aspects of the foregoing, it should be appreciated and understood that any aspect, or combination thereof, of an exemplary or disclosed embodiment may be excluded therefrom to constitute another embodiment without limitation and that it is contemplated that any such embodiment can constitute a separate and independent claim. Similarly, it should be appreciated and understood that any aspect or combination of aspects of one or more embodiments may also be included or combined with any aspect or combination of aspects of one or more embodiments and that it is contemplated herein that all such combinations thereof fall within the scope of this disclosure and can be presented as separate and independent claims without limitation. Accordingly, it should be appreciated that any feature presented in one claim may be included in another claim; any feature presented in one claim may be removed from the claim to constitute a claim without that feature; and any feature presented in one claim may be combined with any feature in another claim, each of which is contemplated herein. The following enumerated clauses are further illustrative examples of aspects and combination of aspects of the foregoing embodiments and examples:

[767] Following is the first example of enumerated clauses:

1. A composition for editing a gene target comprising:

(i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same,

(ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a PCSK9 gene,

wherein the guide RNA directs the base editor fusion protein to effect a nucleobase alteration in the PCSK9 gene *in vivo* when administered to a mammalian subject,

wherein when the guide RNA and the mRNA is administered at a total amount of at least 0.05 mg/kg, the base alteration occurs in at least 35% of whole liver cells in the mammalian subject as measured by next generation sequencing or Sanger sequencing.

2. The composition of clause 1, wherein the mammalian subject is a cynomolgus monkey,

wherein when the guide RNA and the mRNA is administered at a total amount of at least 0.5 mg/kg, the base alteration occurs in at least 40% of whole liver cells in the cynomolgus monkey as measured by next generation sequencing or Sanger sequencing.

3. The composition of clause 1, wherein the mammalian subject is a cynomolgus monkey,

wherein when the guide RNA and the mRNA is administered at a total amount of at least 1 mg/kg, the base alteration occurs in at least 45% of whole liver cells in the cynomolgus monkey as measured by next generation sequencing or Sanger sequencing.

4. The composition of clause 1, wherein the mammalian subject is a cynomolgus monkey,

wherein when the guide RNA and the mRNA is administered at a total amount of at least 1.5 mg/kg, the base alteration occurs in at least 50% of whole liver cells in the cynomolgus monkey as measured by next generation sequencing or Sanger sequencing.

5. The composition of clause 1, wherein the mammalian subject is a cynomolgus monkey,

wherein when the guide RNA and the mRNA is administered at a total amount of at least 3 mg/kg, the base alteration occurs in at least 55% of whole liver cells in the cynomolgus monkey as measured by next generation sequencing or Sanger

sequencing.

6. The composition of clause 1, wherein the mammalian subject is a mouse, wherein when the guide RNA and the mRNA is administered at a total amount of at least 0.125 mg/kg, the base alteration occurs in at least 40% of whole liver cells in the mouse as measured by next generation sequencing or Sanger sequencing.

7. The composition of clause 1, wherein the mammalian subject is a mouse, wherein when the guide RNA and the mRNA is administered at a total amount of at least 0.5 mg/kg, the base alteration occurs in at least 45% of whole liver cells in the mouse as measured by next generation sequencing or Sanger sequencing.

8. The composition of clause 1, wherein the mammalian subject is a mouse, wherein when the guide RNA and the mRNA is administered at a total amount of at least 2 mg/kg, the base alteration occurs in at least 50% of whole liver cells in the mouse as measured by next generation sequencing or Sanger sequencing.

9. The composition of clause 2, wherein the nucleobase alteration results in a reduction of at least 50% in blood low-density lipoprotein cholesterol (LDL-C) level in the subject as compared to prior to the administration.

10. The composition of any one of clauses 1-9, wherein the protospacer is located in a splice site.

11. The composition of any one of clauses 1-9, wherein the protospacer complementary sequence is in the antisense strand of the PCSK9 gene.

12. The composition of any one of clauses 1-9, wherein the protospacer complementary sequence is in the sense strand of the PCSK9 gene.

13. The composition of any one of clauses 1-12, wherein the base alteration happens outside of the protospacer on the PCSK9 gene (off-target sites), wherein the editing percentages of off-target sites set forth in Table 11 are below or equal to the editing percentages set forth in Table 11, respectively.

14. The composition of any one of clauses 1-13, wherein the deaminase is an adenine deaminase and wherein the nucleobase alteration is a A•T to G•C alteration.

15. The composition of any one of clauses 1-14, wherein the programmable DNA binding domain comprises a nuclease inactive Cas9 or a Cas9 nickase.

16. The composition of any one of clauses 1-15, wherein the nucleobase alteration is at a splice site of the PCSK9 gene.

17. The composition of clause 16, wherein the nucleobase alteration is at a splice donor site of the PCSK9 gene.

18. The composition of clause 17, wherein the splice donor site is at 5' end of PCSK9 intron 1 as referenced in SEQ ID NO: 5.
19. The composition of clause 16, wherein the nucleobase alteration is at a splice acceptor site of the PCSK9 gene.
20. The composition of any one of clauses 1-19, wherein the nucleobase alteration results in a frame shift, a premature stop codon, an insertion or deletion in a transcript encoded by the PCSK9 gene.
21. The composition of any one of clauses 1-20, wherein the nucleobase alteration results in an aberrant transcript encoded by the PCSK9 gene.
22. The composition of any one of clauses 1-21, wherein the guide RNA is chemically modified.
23. The composition of clause 16, wherein the tracr sequence of the guide RNA is chemically modified following the scheme depicted in Fig. 7.
24. The composition of any one of clauses 1-22, wherein the spacer sequence comprises a PCSK9 ABE guide RNA spacer sequence set forth in Table 1.
25. The composition of clause 24, wherein the guide RNA comprises the PCSK9 ABE guide RNA sequence of GA096, GA097, GA343, GA346, GA375-377, GA380-389, GA391, GA439 or GA440 as set forth in Table 1.
26. The composition of any one of clauses 1-23, wherein the protospacer sequence comprises a PCSK9 ABE protospacer sequence set forth in Table 1.
27. The composition of clause 26, wherein the protospacer comprises the sequence 5'-CCCGCACCTTGGCGCAGCGG-3' (SEQ ID No: 13) or 5'-CCGCACCTTGGCGCAGCGG-3' (SEQ ID No: 247).
28. The composition of any one of clauses 1-27, wherein the base editor fusion protein comprises an amino acid sequence of SEQ ID No: 2137.
29. The composition of any one of clauses 1-28, wherein the GC% content of the mRNA sequence is greater than 50%.
30. The composition of clause 29, wherein the GC% content of the mRNA sequence is greater than 56%.
31. The composition of clause 30, wherein the GC% content of the mRNA sequence is greater than or equal to 63%.
32. The composition of clause 29, wherein the mRNA comprises an adenine tTNA deaminase (TadA) region, a Cas9 region and a nuclear localization sequence (NLS) region.

33. The composition of clause 32, wherein the mRNA further comprises a first linker region which connects the TadA region and the Cas9 region, and a second linker region which connects the Cas9 region and the NLS region.
34. The composition of clause 32 or 33, wherein the GC% content of the TadA region is greater than 60%.
35. The composition of clause 32 or 33, wherein the GC% content of the TadA region is greater than or equal to 70%.
36. The composition of clause 32 or 33, wherein the GC% content of the Cas9 region is greater than 56%.
37. The composition of clause 32 or 33, wherein the GC% content of the Cas9 region is greater than or equal to 62%.
38. The composition of clause 32 or 33, wherein the GC% content of the NLS region is greater than 54%.
39. The composition of clause 32 or 33, wherein the GC% content of the NLS region is greater than or equal to 63%.
40. The composition of clause 33, wherein the GC% content of the first linker region is greater than 65%.
41. The composition of clause 33, wherein the GC% content of the first linker region is greater than or equal to 79%.
42. The composition of clause 33, wherein the GC% content of the second linker region is greater than 67%.
43. The composition of clause 33, wherein the GC% content of the second linker region is greater than or equal to 83%.
44. The composition of clause 33, wherein the GC% content of the TadA region is greater than 60%, the GC% content of the Cas9 region is greater than 56%, the GC% content of the NLS region is greater than 54%, the GC% content of the first linker region is greater than 65%, and the GC% content of the second linker region is greater than 67%.
45. The composition of clause 29, wherein the mRNA comprises a mRNA sequence selected from Table 23.
46. The composition of clause 45, wherein the mRNA comprises a mRNA sequence of SEQ ID No: 2136.
47. The composition of any one of clauses 29-46, wherein the mRNA comprises a poly A tail.

48. The composition of any one of clauses 1-47, further comprising a lipid nanoparticle (LNP) enclosing (i).
49. The composition of clause 48, wherein the LNP further encloses (ii).
50. The composition of clause 48, further comprising a second LNP enclosing (ii).
51. The composition of any one of clauses 1-44, wherein the ratio of the guide RNA and the mRNA encoding the base editor fusion protein is about 1:10 to about 10:1 by weight.
52. The composition of clause 51, wherein the ratio of the guide RNA and the mRNA encoding the base editor fusion protein is about 1:1, 1.5:1, 2:1, 3:1, 4:1, 1:1.5, 1:2, 1:3, or 1:4 by weight.
53. The composition of clause 51, wherein the ratio of the guide RNA and the mRNA encoding the base editor fusion protein is about 1:1 by weight.
54. A pharmaceutical composition comprising the composition of any one of the preceding clauses and a pharmaceutically acceptable carrier or excipient.
55. A method for treating or preventing a condition in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the composition of clause 1.
56. The method of clause 55, wherein the administration is via intravenous infusion.
57. The method of clause 55 or 56, comprising sequential administration of a LNP enclosing (i) and a LNP enclosing (ii).
58. The method of clause 55 or 56, comprising concurrent administration of the t LNP enclosing (i) and the LNP enclosing (ii).
59. The method of clause 57, comprising administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 1 day.
60. The method of clause 57, comprising administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 2 days.
61. The method of clause 57, comprising administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 3 days.
62. The method of clause 57, comprising administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 4 days.

63. The method of clause 57, comprising administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 5 days.
64. The method of clause 57, comprising administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 6 days.
65. The method of clause 57, comprising administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 7 days.
66. The method of clause 55 or 56, comprising administering a single dose of the LNP enclosing (i) and (ii).
67. The method of clause 66, wherein the single dose of the LNP is at about 0.3 to about 3mg/kg.
68. The method of clause 66 or 67, comprising administering a treatment course of one or more treatments to the subject, wherein each one of the one or more treatment comprises one or more of the single doses of the LNP.
69. The method of clause 68, comprising administering a treatment course of two to ten treatments.
70. The method of clause 68, comprising administering a treatment course of two to five treatments.
71. The method of clause 68, comprising administering a treatment course of two treatments.
72. The method of clause 68, comprising administering a treatment course of three treatments.
73. The method of clause 68, comprising administering a treatment course of four treatments.
74. The method of clause 68, comprising administering a treatment course of five treatments.
75. The method of any one of clauses 55-74, wherein the condition is an atherosclerotic cardiovascular disease.
76. The method of any one of clauses 55-74, wherein the condition is an atherosclerotic vascular disease.
77. The method of any one of clauses 55-74, wherein the subject is a human.
78. A composition for editing a gene target comprising:

(i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same,

(ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a PCSK9 gene,

wherein the guide RNA directs the base editor fusion protein to effect a nucleobase alteration in the PCSK9 gene *in vivo* when administered to a mammalian subject, and

wherein the guide RNA comprises the PCSK9 ABE guide RNA sequences as set forth in Table 1.

79. A composition for editing a gene target comprising:

(i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same,

(ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a PCSK9 gene,

wherein the guide RNA directs the base editor fusion protein to effect a nucleobase alteration in the PCSK9 gene *in vivo* when administered to a mammalian subject, and

wherein the mRNA comprises a sequence selected from Table 23.

80. A method for treating or preventing an atherosclerotic cardiovascular disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a first composition, comprising

(i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same,

(ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a PCSK9 gene,

wherein the guide RNA directs the base editor fusion protein to effect a nucleobase alteration in the PCSK9 gene *in vivo* when administered to a mammalian subject,

wherein when the guide RNA and the mRNA is administered at a total amount of at least 0.05 mg/kg, the base alteration occurs in at least 35% of whole liver cells in the mammalian subject as measured by next generation sequencing or Sanger sequencing; and

a second composition, comprising

(i) a base editor fusion protein comprising a programmable DNA binding domain and a

deaminase, or a mRNA encoding the same,

(ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a ANGPTL3 gene,

wherein the guide RNA directs the base editor fusion protein to effect a nucleobase alteration in the ANGPTL3 gene *in vivo* when administered to a mammalian subject,

wherein when the guide RNA and the mRNA is administered at a total amount of at least 0.5 mg/kg, the base alteration occurs in at least 35% of whole liver cells in the mammalian subject as measured by next generation sequencing or Sanger sequencing.

81. The method of clause 80, comprising sequential administration of the first composition and the second composition.

82. The method of clause 81, comprising administering one or more doses of the first composition followed by one or more dose of the second composition.

83. The method of clause 82, comprising administering one or more doses of the second composition followed by one or more dose of the first composition.

84. The method of clause 80, comprising concurrent administration of the first composition and the second composition.

85. The method of clause 84, comprising one or more doses of the first composition and the second composition.

86. A composition for editing a gene target comprising:

(i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same,

(ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a APOC3 gene,

wherein the guide RNA comprises the APOC3 ABE guide RNA sequence of GA300-303 as set forth in Table 24.

87. A composition for editing a gene target comprising:

(i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same,

(ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a PCSK9 gene,

wherein the guide RNA directs the base editor fusion protein to effect a nucleobase alteration

in the PCSK9 gene *in vitro*,

wherein when the guide RNA and the mRNA is administered at a total amount of at least 2.5 mg/kg, the base alteration occurs in at least 35% of whole liver cells in the mammalian subject as measured by next generation sequencing or Sanger sequencing.

88. A composition for editing a PCSK9 gene comprising:

(a) a mRNA encoding an adenine base editor protein having an editing window, and

(b) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor protein and a spacer sequence that serves to guide the base editor protein to a protospacer sequence on the PCSK9 gene,

wherein the spacer sequence is complimentary, at least in part, to a splice site or an exon region of the PCSK9 gene.

89. The composition for editing the PCSK9 gene of clause 88, wherein when the base editor protein is operatively bound to the guide RNA and the guide RNA is hybridized with the complementary strand to the protospacer sequence on the PCSK9 gene, the editing window encompasses the splice site of the PCSK9 gene.

90. The composition for editing the PCSK9 gene of clause 88, wherein when the base editor protein is operatively bound to the guide RNA and the guide RNA is hybridized with the complementary strand to the protospacer sequence on the PCSK9 gene, the editing window encompasses a region of an intron of the PCSK9 gene.

91. The composition for editing the PCSK9 gene of clause 88, wherein when the base editor protein is operatively bound to the guide RNA and the guide RNA is hybridized with the complementary strand to the protospacer sequence on the PCSK9 gene, the editing window encompasses a region of intron 1, intron 3 or intron 4 of the PCSK9 gene.

92. The composition for editing the PCSK9 gene of clause 88, wherein when the base editor protein is operatively bound to the guide RNA and the guide RNA is hybridized with the complementary strand to the protospacer sequence on the PCSK9 gene, the editing window encompasses a region of intron 1 of the PCSK9 gene.

93. The composition for editing the PCSK9 gene of clause 88, wherein the spacer sequence has a 80-100 % nucleotide sequence identity to a spacer sequence selected from the group of guide RNA sequences identified as GA066, GA073 and GA074.

94. The composition for editing the PCSK9 gene of clause 88, wherein the tracr sequence has a 80-100% nucleotide sequence identity to a tracr sequence selected from the group of guide RNA sequences identified as GA066, GA095, GA096, GA097,

GA343, GA346, GA375, GA376, GA377, GA380, GA381, GA382, GA383, GA384, GA385, GA386, GA387, GA388, GA389, GA439, and GA440.

95. The composition for editing the PCSK9 gene of clause 88, wherein the mRNA has an 80-100% sequence identity to the mRNA sequences identified as MA002, MA004, MA040, MA0041, or MA045.

96. The composition for editing the PCSK9 gene of clause 88, wherein the mRNA has one or more of the GC nucleotide region percentages set forth in the following table:

Nucleotide region	Average GC Nucleotide Content
27-213	67-73%
389-661	67-71%
735-829	63-74%
4207-4286	67-70%
4537-4569	65-73%
4683-4741	62- 67%

97. The composition for editing the PCSK9 gene of clause 88, wherein the mRNA has one or more of the GC nucleotide region percentages set forth in the following table:

Nucleotide region	Average GC Nucleotide Content
27-213	At least 73%
389-661	At least 71%
735-829	At least 74%
4207-4286	At least 70%
4537-4569	At least 73%
4683-4741	At least 67%

98. The composition for editing the PCSK9 gene of clause 88, wherein the mRNA and gRNA are encapsulated within a lipid nanoparticle.

99. The composition for editing the PCSK9 gene of clause 88, wherein the mRNA and gRNA are encapsulated within a lipid nanoparticle having the following:
LNP composition (mol%):
40-65% iLipid
2-20% DSPC
1-5% PEG

Remaining mol% balance is cholesterol;

LNP Particle size: 55-120 nm Z average hydrodynamic diameter; and

Polydispersity index of <0.2 as determined by dynamic light scattering.

100. The composition for editing the PCSK9 gene of clause 88, wherein the mRNA and gRNA are encapsulated within the lipid nanoparticle having an LNP particle size between 50-70 nm Z average hydrodynamic diameter.

101. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the *PCSK9* target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 40 percent.

102. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 50 percent.

103. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 30 percent.

104. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 40 percent.

105. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at

the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 50 percent.

106. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 60 percent.

107. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 70 percent.

108. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 40 percent.

109. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 50 percent.

110. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 60 percent.

111. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a

dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 70 percent.

112. The composition for editing the PCSK9 gene of clause 88, wherein the composition when at administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 80 percent.

113. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 40 percent.

114. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 50 percent.

115. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 60 percent.

116. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 70 percent.

117. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 80 percent.

118. The compositions for editing the PCSK9 gene of clauses 87-117, wherein the percent editing is determined at 15 days after dosing through analysis of dosed cynomolgus monkey liver either via liver biopsy or necropsy of the monkey.

119. The compositions for editing of the PCSK9 gene of clauses 87-117, wherein the percent editing is determined to be durably maintained by periodic liver biopsy testing of the dosed cynomolgus monkeys over a span of at least 168 days after dosing.

120. The compositions for editing of the PCSK9 gene of clauses 87-117, wherein the percent editing is determined to be durably maintained by periodic liver biopsy testing of the dosed cynomolgus monkeys over a span of at least 300 days after dosing.

121. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline.

122. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline.

123. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline.

124. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a

dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed monkeys on average of at least 60 percent as compared to baseline.

125. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 70 percent as compared to baseline.

126. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 80 percent as compared to baseline.

127. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline.

128. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline.

129. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline.

130. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 60 percent as compared to baseline.

131. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 70 percent as compared to baseline.

132. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 80 percent as compared to baseline.

133. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline.

134. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline.

135. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the

plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline.

136. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 60 percent as compared to baseline.

137. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 70 percent as compared to baseline.

138. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 80 percent as compared to baseline.

139. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline.

140. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline.

141. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a

dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline.

142. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 60 percent as compared to baseline.

143. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 70 percent as compared to baseline.

144. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 80 percent as compared to baseline.

145. The compositions for editing of the PCSK9 gene of clauses 121-144, wherein the reduction in plasma protein is determined at 15 days after dosing via blood sampling and analysis of the dosed cynomolgus monkey.

146. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of monkey weight is capable of reducing LDL-C in the plasma of the dosed monkeys on average of at least 20 percent as compared to baseline.

147. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of

the dosed cynomolgus monkeys on average of at least 25 percent as compared to baseline.

148. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 30 percent as compared to baseline.

149. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline.

150. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline.

151. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 45 percent as compared to baseline.

152. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 20 percent as compared to baseline.

153. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per

kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 25 percent as compared to baseline.

154. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 30 percent as compared to baseline.

155. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline.

156. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline.

157. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 45 percent as compared to baseline.

158. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline.

159. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 55 percent as compared to baseline.

160. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a

dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 60 percent as compared to baseline.

161. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 20 percent as compared to baseline.

162. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 25 percent as compared to baseline.

163. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 30 percent as compared to baseline.

164. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline.

165. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline.

166. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 45 percent as compared to baseline.

167. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline.

168. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 55 percent as compared to baseline.

169. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 60 percent as compared to baseline.

170. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 65 percent as compared to baseline.

171. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per

kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 20 percent as compared to baseline.

172. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 25 percent as compared to baseline.

173. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 30 percent as compared to baseline.

174. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline.

175. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline.

176. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 45 percent as compared to baseline.

177. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline.

178. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a

dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 55 percent as compared to baseline.

179. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 60 percent as compared to baseline.

180. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 65 percent as compared to baseline.

181. The compositions for editing the PCSK9 gene of clauses 148-182, wherein the reduction in LDL-C is determined at 15 days after dosing via blood sampling and analysis of the dosed cynomolgus monkey.

182. The compositions for editing the PCSK9 gene of clauses 148-182, wherein the reduction in LDL-C is determined to be durably maintained over a span of at least 168 days by periodic blood sampling and analysis of the dosed cynomolgus monkey.

183. The compositions for editing the PCSK9 gene of clauses 148-182, wherein the reduction in LDL-C is determined to be durably maintained over a span of at least 300 days by periodic blood sampling and analysis of the dosed cynomolgus monkey.

184. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing lipoprotein(a) in the plasma of the dosed cynomolgus monkeys on average of at least 10 percent as compared to baseline.

185. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing lipoprotein(a) level in the plasma of the dosed cynomolgus monkeys on average of at least 15 percent as compared to baseline.

186. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing lipoprotein(a) level in the plasma of the dosed cynomolgus monkeys on average of at least 20 percent as compared to baseline.

187. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing lipoprotein(a) level in the plasma of the dosed cynomolgus monkeys on average of at least 25 percent as compared to baseline.

188. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing lipoprotein(a) level in the plasma of the dosed cynomolgus monkeys on average of at least 30 percent as compared to baseline.

189. The composition for editing the PCSK9 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing lipoprotein(a) level in the plasma of the dosed cynomolgus monkeys on average of approximately 35 percent as compared to baseline.

190. The compositions for editing the PCSK9 gene of clauses 186-191, wherein the reduction in lipoprotein(a) is determined at 15 days after dosing via blood sampling and analysis of the dosed cynomolgus monkey.

191. The compositions for editing the PCSK9 gene of clauses 186-191, wherein the reduction in lipoprotein(a) is determined to be durably maintained over a span of at least 224 days by periodic blood sampling and analysis of the dosed cynomolgus monkey.

192. The compositions for editing the PCSK9 gene of clauses 186-191, wherein the reduction in lipoprotein(a) is determined to be durably maintained over a span of at

least 300 days by periodic blood sampling and analysis of the dosed cynomolgus monkey.

193. The compositions for editing the PCSK9 gene of clauses 101-194, wherein to the extent that the dosing of the cynomolgus monkeys results in elevation of AST, ALT, or Cytokines, the elevations resulting from the dosing of the composition are transient and resolved back to approximately baseline levels within 3-15 days after dosing.

194. The composition for editing the PCSK9 gene of clauses 101-103, 121-126, 148-153, wherein the percent editing of PCSK9 is negligible outside of the liver, spleen and adrenal glands tissues as illustrated in FIG. 27.

195. The compositions for editing the PCSK9 gene of clauses 101-107, 121-132, 148-162, wherein repeat dosing results is additive with respect to the editing percentage of PCSK9 editing percentage.

196. The compositions for editing the PCSK9 gene of clause 195, wherein the repeat dosing does not elicit cytokine activation nor an immune response.

197. The composition for editing the PCSK9 gene of clause 88, wherein the spacer sequence has at least 80% nucleotide correlation with the nucleotide sequence of a targeted protospacer on the PCSK9 gene, wherein an RNA nucleotide on the spacer sequence is in correlation with a DNA nucleotide of the protospacer if it has the same nucleotide as the DNA nucleotide in the same order and wherein uracil and thymine bases are considered the same nucleotide for purposes of determining correlation.

198. The composition for editing the PCSK9 gene of clause 197, wherein the spacer sequence has at least 85% nucleotide correlation with the nucleotide sequence of a targeted protospacer on the PCSK9 gene.

199. The composition for editing the PCSK9 gene of clause 197, wherein the spacer sequence has at least 90% nucleotide correlation with the nucleotide sequence of a targeted protospacer on the PCSK9 gene.

200. The composition for editing the PCSK9 gene of clause 197, wherein the spacer sequence has at least 95% nucleotide correlation with the nucleotide sequence of a targeted protospacer on the PCSK9 gene.

201. The composition for editing the PCSK9 gene of clause 197, wherein the spacer sequence has at least 99% nucleotide correlation with the nucleotide sequence of a targeted protospacer on the PCSK9 gene.

202. The composition for editing the PCSK9 gene of clause 197, wherein the spacer sequence has at least 100% nucleotide correlation with the nucleotide sequence of a targeted protospacer on the PCSK9 gene.

203. A method for treating or preventing an atherosclerotic cardiovascular disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of

(a) a mRNA encoding an adenine base editor protein having an editing window,

(b) a first guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor protein and a spacer sequence that serves to guide the base editor protein to a protospacer sequence on a PCSK9 gene, wherein the spacer sequence is complimentary, at least in part, to a splice site or an exon region of the PCSK9 gene; and

(c) a second guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor protein and a spacer sequence that serves to guide the base editor protein to a protospacer sequence on a ANGPTL3 gene, wherein the spacer sequence is complimentary, at least in part, to a splice site or an exon region of the ANGPTL3 gene.

204. The method of clause 203, further comprising a first LNP enclosing (a).

205. The method of clause 204, wherein the first LNP encloses (b) and (c).

206. The method of clause 204, wherein the first LNP was administered repeatedly.

207. The method of clause 204, wherein the first LNP was administered repeatedly at an interval of one to sixty days.

208. The method of clause 204, wherein the first LNP was administered repeatedly at an interval of seven days.

209. The method of clause 204, wherein the first LNP further encloses (b).

210. The method of clause 209, further comprising a second LNP enclosing (a) and (c).

211. The method of clause 210, wherein the first LNP and the second LNP are administered sequentially.

212. The method of clause 211, wherein the first LNP and the second LNP are administered sequentially at an interval of one day to 12 months.

213. The method of clause 212, wherein the interval is one day.

214. The method of clause 212, wherein the interval is five days.

215. The method of clause 212, wherein the interval is ten days.

216. The method of clause 212, wherein the interval is fifteen days.

217. The method of clause 212, wherein the interval is twenty days.
218. The method of clause 212, wherein the interval is twenty-five days.
219. The method of clause 212, wherein the interval is one month.
220. The method of clause 212, wherein the interval is two months.
221. The method of clause 212, wherein the interval is three months.
222. The method of clause 212, wherein the interval is five months.
223. The method of clause 212, wherein the interval is eight months.
224. The method of clause 212, wherein the interval is ten months.
225. The method of clause 212, wherein the interval is twelve months.

[768] Following is the second example of enumerated clauses:

1. A single guide RNA that comprises: (a) a spacer sequence, wherein the spacer sequence comprises (i) one or more chemical modification(s) and (ii) one or more unmodified nucleotide(s) at select position(s), and (b) a tracr sequence having at least 70% identity to SEQ ID NO: 61, wherein the tracr sequence serve as a binding scaffold for a Type II Cas protein, and wherein the tracr sequence comprises (i) one or more chemical modification(s) and (ii) one or more unmodified nucleotide(s) at select position(s).
2. The single guide RNA of clause 1, wherein the spacer sequence hybridizes with a target polynucleotide sequence in a gene of interest when contacted with the target polynucleotide sequence, wherein the single guide RNA directs the Cas protein to effect alteration in the gene when administered to a mammalian subject.
3. The single guide RNA of clause 1 or 2, wherein the tracr sequence comprises unmodified nucleotides at positions 2 to 7, 23 to 25, 27, 29, 31, 38, 39, 42 to 45 48, 49 and 62 as numbered in SEQ ID NO: 61 or a corresponding position thereof.
4. The single guide RNA of clause 1 or 2, wherein the tracr sequence comprises unmodified nucleotides at positions 2 to 7, 13, 23 to 25, 27, 29, 31, 38, 39, 42 to 45, 48, 49, 53 and 62 as numbered in SEQ ID NO: 61 or a corresponding position thereof.
5. The single guide RNA of clause 1 or 2, wherein the tracr sequence comprises unmodified nucleotides at positions 2 to 7, 13, 23 to 25, 27, 29, 31, 38, 39, 42 to 45, 48, 49, 53, 61, 68, 70 and 11 as numbered in SEQ ID NO: 61 or a corresponding position thereof.
6. The single guide RNA of any one of clauses 1 to 3, wherein the tracr sequence comprises modified nucleotides at positions 1, 8 to 22, 26, 28, 30, 32 to 37, 40, 41,

46, 47, 50 to 61, and 63 to 80 as numbered in SEQ ID NO: 61 or a corresponding position thereof.

7. The single guide RNA of any one of clauses 1 to 3, wherein the tracr sequence comprises modified nucleotides at positions 1, 8 to 12, 14 to 22, 26, 28, 30, 32 to 37, 40, 41, 46, 47, 50 to 52, 54 to 61, and 63 to 80 as numbered in SEQ ID NO: 61 or a corresponding position thereof.

8. The single guide RNA of any one of clauses 1 to 7, wherein the tracr sequence comprises modified nucleotides at positions 1, 8 to 12, 14 to 22, 26, 28, 30, 32 to 34, 37, 41, 46, 47, 50 to 52, 54 to 60, 62 to 67, 69 and 72 to 80 as numbered in SEQ ID NO: 61 or a corresponding position thereof.

9. The single guide RNA of any one of clauses 3 to 8, wherein more than 60% of the nucleotides in the tracr sequence with SEQ ID NO: 61 are modified.

10. The single guide RNA of clauses 9, wherein more than 70% of the nucleotides in the tracr sequence with SEQ ID NO: 61 are modified.

11. The single guide RNA of any one of clauses 1 to 10, wherein the tracr sequence comprises unmodified nucleotides at positions 13, 49, 53 and 62 as numbered in SEQ ID NO: 61 or a corresponding position thereof.

12. The single guide RNA of any one of clauses 11, wherein the tracr sequence comprises unmodified nucleotides at positions 49 and 62 as numbered in SEQ ID NO: 61 or a corresponding position thereof.

13. The single guide RNA of any one of clauses 1 to 10, wherein the tracr sequence comprises unmodified nucleotides at positions 13, 40, 49, 53, 61, 68, 70 and 71 as numbered in SEQ ID NO: 61 or a corresponding position thereof.

14. The single guide RNA of any one of clause 13, wherein the tracr sequence comprises unmodified nucleotides at positions 13, 49 and 53 as numbered in SEQ ID NO: 61 or a corresponding position thereof.

15. The single guide RNA of any one of clauses 1 to 10, wherein the tracr sequence comprises modified nucleotides at positions 1, 8, 21, 22, 26, 28, 30, 32 to 37, 40, 41, 46 and 47 as numbered in SEQ ID NO: 61 or a corresponding position thereof.

16. The single guide RNA of any one of clauses 1-15, wherein the tracr sequence comprises at least 80% identity to SEQ ID NO: 61.

17. The single guide RNA of any one of clauses 1-15, wherein the tracr sequence comprises at least 90% identity to SEQ ID NO: 61.

18. The single guide RNA of any one of clauses 1-17, wherein the chemical modification comprises a 2'-OMe modification.
19. The single guide RNA of any one of clauses 1-17, wherein the chemical modification comprises a nebularin or a deoxynebularin.
20. The single guide RNA of any one of clauses 1-17, wherein the chemical modification comprises a phosphorothioate linkage.
21. The single guide RNA of any one of clauses 1-20, wherein the tracr sequence comprises SEQ ID No. 61, and wherein the single guide RNA further comprises one or more phosphorothioate linkage at a 5' end, at a 3' end, at select internal positions or any combinations thereof.
22. The single guide RNA of clause 21, wherein the single guide RNA further comprises two and no more than two contiguous phosphorothioate linkages at the 5' end, at the 3' end or both.
23. The single guide RNA of clause 21, wherein the single guide RNA further comprises three contiguous phosphorothioate linkages at the 5' end, at the 3' end or both.
24. The single guide RNA of any one of clauses 1-21, wherein the single guide RNA comprises the sequence 5'- ususuNNN-3' at the 3'end, wherein N independently indicates a unmodified ribonucleotide, and wherein each u indicates 2'-O-methyluridine and each s indicates phosphorothioate linkage.
25. The single guide RNA of clauses 24, wherein each N is uridine.
26. The single guide RNA of any one of clauses 1-21, wherein the single guide RNA comprises the sequence 5'- ususuNNn-3' at the 3'end, wherein each N independently indicates a unmodified ribonucleotide, wherein the n indicates a modified nucleotide, wherein each u indicates 2'-O-methyluridine and wherein each s indicates phosphorothioate linkage.
27. The single guide RNA of clauses 26, wherein each N is uridine and the n is 2'-O-methyluridine.
28. A single guide RNA that comprises (i) a spacer sequence and (ii) a tracr sequence, wherein the spacer sequence hybridizes with a target polynucleotide sequence in a PCSK9 gene or an ANGPTL3 gene when contacted with the target polynucleotide sequence, wherein the tracr sequence binds a Type II Cas protein when contacted with the Type II Cas protein, and wherein the single guide RNA comprises a nebularine, a deoxynebularine, or a 2'-O-methylnebularine.

29. A single guide RNA that comprises (i) a spacer sequence and (ii) a tracr sequence, wherein the spacer sequence hybridizes with a target polynucleotide sequence in a PCSK9 gene or an ANGPTL3 gene when contacted with the target polynucleotide sequence, wherein the tracr sequence binds a Type II Cas protein when contacted with the Type II Cas protein, and wherein the single guide RNA comprises two and no more than two phosphorothioate linkages at a 5' end or at a 3' end.
30. The single guide RNA of clause 29, wherein the single guide RNA comprises two and no more than two phosphorothioate linkages at the 5' end and at the 3' end.
31. The single guide RNA of clause 29, wherein the single guide RNA comprises three phosphorothioate linkages at the 5' end.
32. The single guide RNA of clause 29, wherein the single guide RNA comprises three phosphorothioate linkages at the 3' end.
33. The single guide RNA of clause 29, wherein the two phosphorothioate linkages at the 5' end are two contiguous phosphorothioate linkages at the first two nucleotide positions of the 5' end.
34. The single guide RNA of clause 29, wherein the two phosphorothioate linkages at the 5' end are within the first 3-10 nucleotides of the 5' end.
35. The single guide RNA of clause 29, wherein the two phosphorothioate linkages at the 3' end are two contiguous phosphorothioate linkages at the last two nucleotide positions of the 3' end.
36. The single guide RNA of clause 29, wherein the two phosphorothioate linkages at the 3' end are within the last 3-10 nucleotides of the 3' end.
37. The single guide RNA of clause 29 comprising the sequence 5'-UsUsUs-3' at the 3' end, wherein U indicates a uridine and s indicates a phosphorothioate linkage.
38. The single guide RNA of clause 29 comprising the sequence 5'-UUU-3' at the 3'end.
39. The single guide RNA of any one of clauses 1-27, wherein the tracr sequence binds the Type II Cas protein with increased binding affinity compared to a tracr sequence in an unmodified single guide RNA.
40. The single guide RNA of any one of clauses 28-38, wherein the Type II Cas protein is a Cas9 protein.
41. The single guide RNA of clause 40, wherein the Cas9 protein is a Streptococcus pyogenes Cas9.
42. A single guide RNA that comprises a guide RNA sequence selected from Table 1, wherein a, u, g, and c indicate 2'-OMe modified adenine, uridine, guanine, and

cytidine, wherein s indicates a phosphorothioate linkage, wherein X indicates a nebularine, wherein x indicates a 2'-O-methylnebularine and wherein dX indicates a 2'-deoxynebularine.

43. A pharmaceutical composition for gene modification comprising the single guide RNA of any one of clauses 1-42 and a Type II Cas protein or a nucleic acid sequence encoding the Type II Cas protein.

44. The pharmaceutical composition of clause 43 further comprising a vector that comprises the nucleic acid sequence encoding the Type II Cas protein.

45. The pharmaceutical composition of clause 43 or 44, wherein the Type II Cas protein is a Cas9.

46. The pharmaceutical composition of any one of clauses 43-45 further comprising a pharmaceutically acceptable carrier.

47. A lipid nanoparticle comprising the pharmaceutical composition of any one of clauses 43-46.

48. A method for modifying a target polynucleotide sequence in a cell comprising introducing into the cell the pharmaceutical composition of any one of clauses 43-46, wherein the single guide RNA directs the Type II Cas protein to effect a modification in the target polynucleotide sequence in the cell.

49. The method of clause 48, wherein the target polynucleotide sequence is in a PCSK9 gene.

50. The method of clause 49, wherein the modification reduces expression of functional PCSK9 protein encoded by the PCSK9 gene in the cell.

51. The method of clause 50, wherein the target polynucleotide sequence is in an ANGPTL3 gene.

52. The method of clause 51 wherein the modification reduces expression of functional ANGPTL3 protein encoded by the ANGPTL3 gene in the cell.

53. The method of any one of clauses 48-52, wherein the introduction is performed via a lipid nanoparticle that comprises the composition.

54. A method for treating or preventing a condition in a subject in need thereof, the method comprising administering to the subject the pharmaceutical composition of any one of clauses 43-46 or the lipid nanoparticle of clause 47, wherein the single guide RNA directs the Type II Cas protein to effect a modification in a target polynucleotide sequence in a cell of the subject, thereby treating or preventing the condition.

55. The method of clause 54, wherein the target polynucleotide sequence is in a PCSK9 gene.
56. The method of clause 54, wherein the target polynucleotide sequence is in an ANGPTL3 gene.
57. The method of clause 55, wherein the modification reduces expression of functional PCSK9 protein encoded by the PCSK9 gene in the subject.
58. The method of clause 56, wherein the modification reduces expression of functional ANGPTL3 protein encoded by the ANGPTL3 gene in the subject.
59. The method of any one of clauses 48-58 wherein the condition is an atherosclerotic vascular disease.
60. The method of any one of clauses 48-58, wherein the condition is an atherosclerotic vascular disease, hypertriglyceridemia, or diabetes.
61. The method of any one of clauses 48-60, wherein the subject exhibits a reduced blood LDL cholesterol level, and/or a reduced blood triglycerides level as compared to before the administration.

[769] Following is the third example of enumerated clauses:

1. A composition for editing a gene target comprising:

(i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same,

(ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a ANGPTL3 gene,

wherein the guide RNA directs the base editor fusion protein to effect a nucleobase alteration in the ANGPTL3 gene *in vivo* when administered to a mammalian subject,

wherein when the guide RNA and the mRNA is administered at a total amount of at least 0.5 mg/kg, the base alteration occurs in at least 35% of whole liver cells in the mammalian subject as measured by next generation sequencing or Sanger sequencing.

2. The composition of clause 1, wherein the mammalian subject is a cynomolgus monkey,

wherein when the guide RNA and the mRNA is administered at a total amount of about 1 mg/kg, the base alteration occurs in at least 35% of whole liver cells in the cynomolgus monkey as measured by next generation sequencing or Sanger sequencing.

3. The composition of clause 1, wherein the mammalian subject is a cynomolgus monkey,

wherein when the guide RNA and the mRNA is administered at a total amount of about 3 mg/kg, the base alteration occurs in at least 50% of whole liver cells in the cynomolgus monkey as measured by next generation sequencing or Sanger sequencing.

4. The composition of clause 2, wherein the nucleobase alteration results in a reduction of at least 20% in blood triglyceride level in the cynomolgus monkey as compared to prior to the administration.

5. The composition of clause 3, wherein the nucleobase alteration results in a reduction of at least 50% in blood triglyceride level in the cynomolgus monkey as compared to prior to the administration.

6. The composition of any one of clauses 1-5, wherein the protospacer is located in a splice site.

7. The composition of any one of clauses 1-5, wherein the protospacer complementary sequence is in the antisense strand of the ANGPTL3 gene.

8. The composition of any one of clauses 1-5, wherein the protospacer complementary sequence is in the sense strand of the ANGPTL3 gene.

9. The composition of any one of clauses 1-8, wherein the base alteration happens outside of the protospacer on the ANGPTL3 gene (off-target sites),

wherein the editing percentages of off-target sites set forth in Table 14 are below or equal to the editing percentages set forth in Table 14, respectively.

10. The composition of any one of clauses 1-9, wherein the deaminase is an adenine deaminase and wherein the nucleobase alteration is a A•T to G•C alteration.

11. The composition of any one of clauses 1-10, wherein the programmable DNA binding domain comprises a nuclease inactive Cas9 or a Cas9 nickase.

12. The composition of any one of clauses 1-11, wherein the nucleobase alteration is at a splice site of the ANGPTL3 gene.

13. The composition of clause 12, wherein the nucleobase alteration is at a splice donor site of the ANGPTL3 gene.

14. The composition of clause 13, wherein the splice donor site is at 5' end of ANGPTL3 intron 6 as referenced in SEQ ID NO: 7.

15. The composition of clause 12, wherein the nucleobase alteration is at a splice acceptor site of the ANGPTL3 gene.

16. The composition of any one of clauses 1-15, wherein the nucleobase alteration results in a frame shift, a premature stop codon, an insertion or deletion in a transcript encoded by the ANGPTL3 gene.
17. The composition of any one of clauses 1-16, wherein the nucleobase alteration results in an aberrant transcript encoded by the ANGPTL3 gene.
18. The composition of any one of clauses 1-17, wherein the guide RNA is chemically modified.
19. The composition of clause 16, wherein the tracr sequence of the guide RNA is chemically modified following the scheme depicted in Fig. 7.
20. The composition of any one of clauses 1-19, wherein the spacer sequence comprises an ANGPTL3 ABE guide RNA spacer sequence set forth in Table 1.
21. The composition of clause 20, wherein the guide RNA comprises the ANGPTL3 ABE guide RNA sequence of GA067, GA091, GA098, GA099, GA100, GA101, GA102, GA103, GA347, GA441, GA442, GA472, GA473, GA474, GA475, GA476, GA517 or GA547 as set forth in Table 1.
22. The composition of any one of clauses 1-19, wherein the protospacer sequence comprises an ANGPTL3 ABE protospacer sequence set forth in Table 1.
23. The composition of clause 22, wherein the protospacer comprises the sequence 5'-AAGATACCTGAATAACTCTC-3' (SEQ ID No: 14), 5'-AAGATACCTGAATAACCCTC-3' (SEQ ID No: 15), 5'-GATACCTGAATAACTCTC-3' (SEQ ID No: 1606), 5'-AGATACCTGAATAACCCTC-3' (SEQ ID No: 248), or 5'-GATACCTGAATAACCCTC-3' (SEQ ID No: 249).
24. The composition of any one of clauses 1-23, wherein the base editor fusion protein comprises an amino acid sequence of SEQ ID No: 2137.
25. The composition of any one of clauses 1-24, wherein the GC% content of the mRNA sequence is greater than 50%.
26. The composition of clause 25, wherein the GC% content of the mRNA sequence is greater than 56%.
27. The composition of clause 26, wherein the GC% content of the mRNA sequence is greater than or equal to 63%.
28. The composition of clause 25, wherein the mRNA comprises an adenine tTNA deaminase (TadA) region, a Cas9 region and a nuclear localization sequence (NLS) region.

29. The composition of clause 28, wherein the mRNA further comprises a first linker region which connects the TadA region and the Cas9 region, and a second linker region which connects the Cas9 region and the NLS region.
30. The composition of clause 28 or 29, wherein the GC% content of the TadA region is greater than 60%.
31. The composition of clause 28 or 29, wherein the GC% content of the TadA region is greater than or equal to 70%.
32. The composition of clause 28 or 29, wherein the GC% content of the Cas9 region is greater than 56%.
33. The composition of clause 28 or 29, wherein the GC% content of the Cas9 region is greater than or equal to 62%.
34. The composition of clause 28 or 29, wherein the GC% content of the NLS region is greater than 54%.
35. The composition of clause 28 or 29, wherein the GC% content of the NLS region is greater than or equal to 63%.
36. The composition of clause 29, wherein the GC% content of the first linker region is greater than 65%.
37. The composition of clause 29, wherein the GC% content of the first linker region is greater than or equal to 79%.
38. The composition of clause 29, wherein the GC% content of the second linker region is greater than 67%.
39. The composition of clause 29, wherein the GC% content of the second linker region is greater than or equal to 83%.
40. The composition of clause 29, wherein the GC% content of the TadA region is greater than 60%, the GC% content of the Cas9 region is greater than 56%, the GC% content of the NLS region is greater than 54%, the GC% content of the first linker region is greater than 65%, and the GC% content of the second linker region is greater than 67%.
41. The composition of clause 25, wherein the mRNA comprises a mRNA sequence selected from Table 23.
42. The composition of clause 41, wherein the mRNA comprises a mRNA sequence of SEQ ID No: 2136.
43. The composition of any one of clauses 25-42, wherein the mRNA comprises a poly A tail.

44. The composition of any one of clauses 1-43, further comprising a lipid nanoparticle (LNP) enclosing (i).
45. The composition of clause 44, wherein the LNP further encloses (ii).
46. The composition of clause 44, further comprising a second LNP enclosing (ii).
47. The composition of any one of clauses 1-44, wherein the ratio of the guide RNA and the mRNA encoding the base editor fusion protein is about 1:10 to about 10:1 by weight.
48. The composition of clause 47, wherein the ratio of the guide RNA and the mRNA encoding the base editor fusion protein is about 1:1, 1.5:1, 2:1, 3:1, 4:1, 1:1.5, 1:2, 1:3, or 1:4 by weight.
49. The composition of clause 48, wherein the ratio of the guide RNA and the mRNA encoding the base editor fusion protein is about 1:1 by weight.
50. A pharmaceutical composition comprising the composition of any one of the preceding clauses and a pharmaceutically acceptable carrier or excipient.
51. A method for treating or preventing a condition in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the composition of clause 1.
52. The method of clause 51, wherein the administration is via intravenous infusion.
53. The method of clause 51 or 52, comprising sequential administration of a LNP enclosing (i) and a LNP enclosing (ii).
54. The method of clause 51 or 52, comprising concurrent administration of the LNP enclosing (i) and the LNP enclosing (ii).
55. The method of clause 53, comprising administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 1 day.
56. The method of clause 53, comprising administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 2 days.
57. The method of clause 53, comprising administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 3 days.
58. The method of clause 53, comprising administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 4 days.

59. The method of clause 53, comprising administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 5 days.
60. The method of clause 53, comprising administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 6 days.
61. The method of clause 53, comprising administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 7 days.
62. The method of clause 51 or 52, comprising administering a single dose of the LNP enclosing (i) and (ii).
63. The method of clause 62, wherein the single dose of the LNP is at about 0.3 to about 3mg/kg.
64. The method of clause 62 or 63, comprising administering a treatment course of one or more treatments to the subject, wherein each one of the one or more treatment comprises one or more of the single doses of the LNP.
65. The method of clause 64, comprising administering a treatment course of two to ten treatments.
66. The method of clause 64, comprising administering a treatment course of two to five treatments.
67. The method of clause 64, comprising administering a treatment course of two treatments.
68. The method of clause 64, comprising administering a treatment course of three treatments.
69. The method of clause 64, comprising administering a treatment course of four treatments.
70. The method of clause 62, comprising administering a treatment course of five treatments.
71. The method of any one of clauses 51-70, wherein the condition is an atherosclerotic cardiovascular disease.
72. The method of any one of clauses 51-70, wherein the condition is an atherosclerotic vascular disease.
73. The method of any one of clauses 51-72, wherein the subject is a human.
74. A composition for editing a gene target comprising:

(i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same,

(ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a ANGPTL3 gene,

wherein the guide RNA directs the base editor fusion protein to effect a nucleobase alteration in the ANGPTL3 gene *in vivo* when administered to a mammalian subject, and

wherein the guide RNA comprises the ANGPTL3 ABE guide RNA sequences as set forth in Table 1.

75. A composition for editing a gene target comprising:

(i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same,

(ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a ANGPTL3 gene,

wherein the guide RNA directs the base editor fusion protein to effect a nucleobase alteration in the ANGPTL3 gene *in vivo* when administered to a mammalian subject, and

wherein the mRNA comprises a sequence selected from Table 23.

76. A method for treating or preventing an atherosclerotic cardiovascular disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a first composition, comprising

(i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same,

(ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a PCSK9 gene,

wherein the guide RNA directs the base editor fusion protein to effect a nucleobase alteration in the PCSK9 gene *in vivo* when administered to a mammalian subject,

wherein when the guide RNA and the mRNA is administered at a total amount of at least 0.5 mg/kg, the base alteration occurs in at least 35% of whole liver cells in the mammalian subject as measured by next generation sequencing or Sanger sequencing; and

a second composition, comprising

(i) a base editor fusion protein comprising a programmable DNA binding domain and a

deaminase, or a mRNA encoding the same,

(ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a ANGPTL3 gene,

wherein the guide RNA directs the base editor fusion protein to effect a nucleobase alteration in the ANGPTL3 gene *in vivo* when administered to a mammalian subject,

wherein when the guide RNA and the mRNA is administered at a total amount of at least 1 mg/kg, the base alteration occurs in at least 35% of whole liver cells in the mammalian subject as measured by next generation sequencing or Sanger sequencing.

77. The method of clause 76, comprising sequential administration of the first composition and the second composition.

78. The method of clause 77, comprising administering one or more doses of the first composition followed by one or more dose of the second composition.

79. The method of clause 78, comprising administering one or more doses of the second composition followed by one or more dose of the first composition.

80. The method of clause 76, comprising concurrent administration of the first composition and the second composition.

81. The method of clause 80, comprising one or more doses of the first composition and the second composition.

82. A composition for editing a gene target comprising:

(i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same,

(ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a ANGPTL3 gene,

wherein the guide RNA directs the base editor fusion protein to effect a nucleobase alteration in the ANGPTL3 gene *in vitro*,

wherein when the guide RNA and the mRNA is administered at a total amount of at least 0.5 mg/kg, the base alteration occurs in at least 35% of whole liver cells in the mammalian subject as measured by next generation sequencing or Sanger sequencing.

83. The composition of clause 82, wherein when the guide RNA and the mRNA is administered at a total amount of at least 1 mg/kg, the base alteration occurs in at least 40% of whole liver cells in the mammalian subject as measured by next generation sequencing or Sanger sequencing.

84. The composition of clause 82, wherein when the guide RNA and the mRNA is administered at a total amount of at least 1.5 mg/kg, the base alteration occurs in at least 45% of whole liver cells in the mammalian subject as measured by next generation sequencing or Sanger sequencing.

85. The composition of clause 82, wherein when the guide RNA and the mRNA is administered at a total amount of at least 2 mg/kg, the base alteration occurs in at least 50% of whole liver cells in the mammalian subject as measured by next generation sequencing or Sanger sequencing.

86. The composition of clause 82, wherein when the guide RNA and the mRNA is administered at a total amount of at least 2.5 mg/kg, the base alteration occurs in at least 55% of whole liver cells in the mammalian subject as measured by next generation sequencing or Sanger sequencing.

87. The composition of clause 82, wherein when the guide RNA and the mRNA is administered at a total amount of at least 3 mg/kg, the base alteration occurs in at least 60% of whole liver cells in the mammalian subject as measured by next generation sequencing or Sanger sequencing.

88. A composition for editing an ANGPTL3 gene comprising:
(a) a mRNA encoding an adenine base editor protein having an editing window, and
(b) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor protein and a spacer sequence that serves to guide the base editor protein to a protospacer sequence on the ANGPTL3 gene,
wherein the spacer sequence is complimentary, at least in part, to a splice site or an exon region of the ANGPTL3 gene.

89. The composition for editing the ANGPTL3 gene of clause 83, wherein when the base editor protein is operatively bound to the guide RNA and the guide RNA is hybridized with the complementary strand to the protospacer sequence on the ANGPTL3 gene, the editing window encompasses the splice site of the ANGPTL3 gene.

90. The composition for editing the ANGPTL3 gene of clause 88, wherein when the base editor protein is operatively bound to the guide RNA and the guide RNA is hybridized with the complementary strand to the protospacer sequence on the ANGPTL3 gene, the editing window encompasses a region of an intron of the ANGPTL3 gene.

91. The composition for editing the ANGPTL3 gene of clause 88, wherein when the base editor protein is operatively bound to the guide RNA and the guide RNA is hybridized with the complementary strand to the protospacer sequence on the ANGPTL3 gene,

the editing window encompasses a region of intron 1, intron 3 or intron 4 of the ANGPTL3 gene.

92. The composition for editing the ANGPTL3 gene of clause 88, wherein when the base editor protein is operatively bound to the guide RNA and the guide RNA is hybridized with the complementary strand to the protospacer sequence on the ANGPTL3 gene, the editing window encompasses a region of intron 1 of the ANGPTL3 gene.

93. The composition for editing the ANGPTL3 gene of clause 88, wherein the spacer sequence has a 80-100 % nucleotide sequence identity to a spacer sequence selected from the group of guide RNA sequences identified as GA067, GA100 and GA574.

94. The composition for editing the ANGPTL3 gene of clause 88, wherein the tracr sequence has a 80-100% nucleotide sequence identity to a tracr sequence selected from the group of guide RNA sequences identified as GA067, GA091, GA098, GA099, GA100, GA101, GA102, GA103, GA347, GA441, GA442, GA472, GA473, GA474, GA475, GA476, GA517 and GA547.

95. The composition for editing the ANGPTL3 gene of clause 88, wherein the mRNA has an 80-100% sequence identity to the mRNA sequences identified as MA002, MA004, MA040, MA0041, or MA045.

96. The composition for editing the ANGPTL3 gene of clause 88, wherein the mRNA has one or more of the GC nucleotide region percentages set forth in the following table:

Nucleotide region	**Average GC Nucleotide Content**
27-213	67-73%
389-661	67-71%
735-829	63-74%
4207-4286	67-70%
4537-4569	65-73%
4683-4741	62- 67%

97. The composition for editing the ANGPTL3 gene of clause 88, wherein the mRNA has one or more of the GC nucleotide region percentages set forth in the following table:

Nucleotide region	**Average GC Nucleotide Content**
27-213	At least 73%
389-661	At least 71%
735-829	At least 74%

4207-4286	At least 70%
4537-4569	At least 73%
4683-4741	At least 67%

98. The composition for editing the ANGPTL3 gene of clause 88, wherein the mRNA and gRNA are encapsulated within a lipid nanoparticle.

99. The composition for editing the ANGPTL3 gene of clause 88, wherein the mRNA and gRNA are encapsulated within a lipid nanoparticle having the following:

LNP composition (mol%):

40-65% iLipid

2-20% DSPC

1-5% PEG

Remaining mol% balance is cholesterol;

LNP Particle size: 55-120 nm Z average hydrodynamic diameter; and

Polydispersity index of <0.2 as determined by dynamic light scattering.

100. The composition for editing the ANGPTL3 gene of clause 88, wherein the mRNA and gRNA are encapsulated within the lipid nanoparticle having an LNP particle size between 50-70 nm Z average hydrodynamic diameter.

101. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the ANGPTL3 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 40 percent.

102. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the ANGPTL3 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 50 percent.

103. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined

total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the ANGPTL3 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 30 percent.

104. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the ANGPTL3 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 40 percent.

105. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the ANGPTL3 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 50 percent.

106. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the ANGPTL3 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 60 percent.

107. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the ANGPTL3 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 70 percent.

108. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the ANGPTL3 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 40 percent.

109. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the ANGPTL3 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 50 percent.

110. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the ANGPTL3 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 60 percent.

111. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the ANGPTL3 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 70 percent.

112. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when at administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the ANGPTL3 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 80 percent.

113. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the ANGPTL3 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 40 percent.

114. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine

base editing at the ANGPTL3 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 50 percent.

115. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the ANGPTL3 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 60 percent.

116. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the ANGPTL3 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 70 percent.

117. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the ANGPTL3 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 80 percent.

118. The compositions for editing the ANGPTL3 gene of clauses 87-117, wherein the percent editing is determined at 15 days after dosing through analysis of dosed cynomolgus monkey liver either via liver biopsy or necropsy of the monkey.

119. The compositions for editing of the ANGPTL3 gene of clauses 87-117, wherein the percent editing is determined to be durably maintained by periodic liver biopsy testing of the dosed cynomolgus monkeys over a span of at least 168 days after dosing.

120. The compositions for editing of the ANGPTL3 gene of clauses 87-117, wherein the percent editing is determined to be durably maintained by periodic liver biopsy testing of the dosed cynomolgus monkeys over a span of at least 300 days after dosing.

121. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing ANGPTL3

protein in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline.

122. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing ANGPTL3 protein in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline.

123. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of monkey weight is capable of reducing ANGPTL3 protein in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline.

124. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing ANGPTL3 protein in the plasma of the dosed monkeys on average of at least 60 percent as compared to baseline.

125. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing ANGPTL3 protein in the plasma of the dosed cynomolgus monkeys on average of at least 70 percent as compared to baseline.

126. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing ANGPTL3 protein in the plasma of the dosed cynomolgus monkeys on average of at least 80 percent as compared to baseline.

127. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous

infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing ANGPTL3 protein in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline.

128. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing ANGPTL3 protein in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline.

129. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of monkey weight is capable of reducing ANGPTL3 protein in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline.

130. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing ANGPTL3 protein in the plasma of the dosed cynomolgus monkeys on average of at least 60 percent as compared to baseline.

131. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing ANGPTL3 protein in the plasma of the dosed cynomolgus monkeys on average of at least 70 percent as compared to baseline.

132. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing ANGPTL3 protein in the plasma of the dosed cynomolgus monkeys on average of at least 80 percent as compared to baseline.

133. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing ANGPTL3 protein in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline.

134. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing ANGPTL3 protein in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline.

135. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing ANGPTL3 protein in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline.

136. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing ANGPTL3 protein in the plasma of the dosed cynomolgus monkeys on average of at least 60 percent as compared to baseline.

137. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing ANGPTL3 protein in the plasma of the dosed cynomolgus monkeys on average of at least 70 percent as compared to baseline.

138. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing ANGPTL3

protein in the plasma of the dosed cynomolgus monkeys on average of at least 80 percent as compared to baseline.

139. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing ANGPTL3 protein in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline.

140. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing ANGPTL3 protein in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline.

141. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing ANGPTL3 protein in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline.

142. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing ANGPTL3 protein in the plasma of the dosed cynomolgus monkeys on average of at least 60 percent as compared to baseline.

143. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing ANGPTL3 protein in the plasma of the dosed cynomolgus monkeys on average of at least 70 percent as compared to baseline.

144. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous

infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing ANGPTL3 protein in the plasma of the dosed cynomolgus monkeys on average of at least 80 percent as compared to baseline.

145. The compositions for editing of the ANGPTL3 gene of clauses 121-144, wherein the reduction in plasma protein is determined at 15 days after dosing via blood sampling and analysis of the dosed cynomolgus monkey.

146. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of monkey weight is capable of reducing triglyceride level in the plasma of the dosed monkeys on average of at least 20 percent as compared to baseline.

147. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 25 percent as compared to baseline.

148. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 30 percent as compared to baseline.

149. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline.

150. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined

total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline.

151. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 45 percent as compared to baseline.

152. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 20 percent as compared to baseline.

153. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 25 percent as compared to baseline.

154. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 30 percent as compared to baseline.

155. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline.

156. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline.

157. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 45 percent as compared to baseline.

158. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline.

159. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 55 percent as compared to baseline.

160. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 60 percent as compared to baseline.

161. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride

level in the plasma of the dosed cynomolgus monkeys on average of at least 20 percent as compared to baseline.

162. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 25 percent as compared to baseline.

163. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 30 percent as compared to baseline.

164. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline.

165. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline.

166. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 45 percent as compared to baseline.

167. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous

infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline.

168. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 55 percent as compared to baseline.

169. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 60 percent as compared to baseline.

170. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 65 percent as compared to baseline.

171. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 20 percent as compared to baseline.

172. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 25 percent as compared to baseline.

173. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 30 percent as compared to baseline.

174. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline.

175. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline.

176. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 45 percent as compared to baseline.

177. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline.

178. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride

level in the plasma of the dosed cynomolgus monkeys on average of at least 55 percent as compared to baseline.

179. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 60 percent as compared to baseline.

180. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing triglyceride level in the plasma of the dosed cynomolgus monkeys on average of at least 65 percent as compared to baseline.

181. The compositions for editing the ANGPTL3 gene of clauses 148-182, wherein the reduction in triglyceride level is determined at 15 days after dosing via blood sampling and analysis of the dosed cynomolgus monkey.

182. The compositions for editing the ANGPTL3 gene of clauses 148-182, wherein the reduction in triglyceride level is determined to be durably maintained over a span of at least 168 days by periodic blood sampling and analysis of the dosed cynomolgus monkey.

183. The compositions for editing the ANGPTL3 gene of clauses 148-182, wherein the reduction in triglyceride level is determined to be durably maintained over a span of at least 300 days by periodic blood sampling and analysis of the dosed cynomolgus monkey.

184. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing lipoprotein(a) in the plasma of the dosed cynomolgus monkeys on average of at least 10 percent as compared to baseline.

185. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined

total weight per kg of cynomolgus monkey weight is capable of reducing lipoprotein(a) level in the plasma of the dosed cynomolgus monkeys on average of at least 15 percent as compared to baseline.

186. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing lipoprotein(a) level in the plasma of the dosed cynomolgus monkeys on average of at least 20 percent as compared to baseline.

187. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing lipoprotein(a) level in the plasma of the dosed cynomolgus monkeys on average of at least 25 percent as compared to baseline.

188. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing lipoprotein(a) level in the plasma of the dosed cynomolgus monkeys on average of at least 30 percent as compared to baseline.

189. The composition for editing the ANGPTL3 gene of clause 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing lipoprotein(a) level in the plasma of the dosed cynomolgus monkeys on average of approximately 35 percent as compared to baseline.

190. The compositions for editing the ANGPTL3 gene of clauses 186-191, wherein the reduction in lipoprotein(a) is determined at 15 days after dosing via blood sampling and analysis of the dosed cynomolgus monkey.

191. The compositions for editing the ANGPTL3 gene of clauses 186-191, wherein the reduction in lipoprotein(a) is determined to be durably maintained over a span of at least 224 days by periodic blood sampling and analysis of the dosed cynomolgus monkey.

192. The compositions for editing the ANGPTL3 gene of clauses 186-191, wherein the reduction in lipoprotein(a) is determined to be durably maintained over a span of at least 300 days by periodic blood sampling and analysis of the dosed cynomolgus monkey.

193. The compositions for editing the ANGPTL3 gene of clauses 101-194, wherein to the extent that the dosing of the cynomolgus monkeys results in elevation of AST, ALT, or Cytokines, the elevations resulting from the dosing of the composition are transient and resolved back to approximately baseline levels within 3-15 days after dosing.

194. The composition for editing the ANGPTL3 gene of clauses 101-103, 121-126, 148-153, wherein the percent editing of ANGPTL3 is negligible outside of the liver, spleen and adrenal glands tissues as illustrated in FIG. 27.

195. The compositions for editing the ANGPTL3 gene of clauses 101-107, 121-132, 148-162, wherein repeat dosing results is additive with respect to the editing percentage of ANGPTL3 editing percentage.

196. The compositions for editing the ANGPTL3 gene of clause 195, wherein the repeat dosing does not elicit cytokine activation nor an immune response.

197. The composition for editing the ANGPTL3 gene of clause 88, wherein the spacer sequence has at least 80% nucleotide correlation with the nucleotide sequence of a targeted protospacer on the ANGPTL3 gene, wherein an RNA nucleotide on the spacer sequence is in correlation with a DNA nucleotide of the protospacer if it has the same nucleotide as the DNA nucleotide in the same order and wherein uracil and thymine bases are considered the same nucleotide for purposes of determining correlation.

198. The composition for editing the ANGPTL3 gene of clause 197, wherein the spacer sequence has at least 85% nucleotide correlation with the nucleotide sequence of a targeted protospacer on the ANGPTL3 gene.

199. The composition for editing the ANGPTL3 gene of clause 197, wherein the spacer sequence has at least 90% nucleotide correlation with the nucleotide sequence of a targeted protospacer on the ANGPTL3 gene.

200. The composition for editing the ANGPTL3 gene of clause 197, wherein the spacer sequence has at least 95% nucleotide correlation with the nucleotide sequence of a targeted protospacer on the ANGPTL3 gene.

201. The composition for editing the ANGPTL3 gene of clause 197, wherein the spacer sequence has at least 99% nucleotide correlation with the nucleotide sequence of a targeted protospacer on the ANGPTL3 gene.

202. The composition for editing the ANGPTL3 gene of clause 197, wherein the spacer sequence has at least 100% nucleotide correlation with the nucleotide sequence of a targeted protospacer on the ANGPTL3 gene.

203. A method for treating or preventing an atherosclerotic cardiovascular disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of

(a) a mRNA encoding an adenine base editor protein having an editing window,

(b) a first guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor protein and a spacer sequence that serves to guide the base editor protein to a protospacer sequence on a PCSK9 gene, wherein the spacer sequence is complimentary, at least in part, to a splice site or an exon region of the PCSK9 gene; and

(c) a second guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor protein and a spacer sequence that serves to guide the base editor protein to a protospacer sequence on a ANGPTL3 gene, wherein the spacer sequence is complimentary, at least in part, to a splice site or an exon region of the ANGPTL3 gene.

204. The method of clause 203, further comprising a first LNP enclosing (a).

205. The method of clause 204, wherein the first LNP encloses (b) and (c).

206. The method of clause 204, wherein the first LNP was administered repeatedly.

207. The method of clause 204, wherein the first LNP was administered repeatedly at an interval of one to sixty days.

208. The method of clause 204, wherein the first LNP was administered repeatedly at an interval of seven days.

209. The method of clause 204, wherein the first LNP further encloses (b).

210. The method of clause 209, further comprising a second LNP enclosing (a) and (c).

211. The method of clause 210, wherein the first LNP and the second LNP are administered sequentially.

212. The method of clause 211, wherein the first LNP and the second LNP are administered sequentially at an interval of one day to 12 months.

213. The method of clause 212, wherein the interval is one day.

214. The method of clause 212, wherein the interval is five days.
215. The method of clause 212, wherein the interval is ten days.
216. The method of clause 212, wherein the interval is fifteen days.
217. The method of clause 212, wherein the interval is twenty days.
218. The method of clause 212, wherein the interval is twenty-five days.
219. The method of clause 212, wherein the interval is one month.
220. The method of clause 212, wherein the interval is two months.
221. The method of clause 212, wherein the interval is three months.
222. The method of clause 212, wherein the interval is five months.
223. The method of clause 212, wherein the interval is eight months.
224. The method of clause 212, wherein the interval is ten months.
225. The method of clause 212, wherein the interval is twelve months.

[770] It will also be appreciated from reviewing the present disclosure, that it is contemplated that the one or more aspects or features presented in one of or a group of related clauses may also be included in other clauses or in combination with the one or more aspects or features in other clauses.:

CLAIMS

What is claimed is:

1. A composition for editing a gene target comprising:

(i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same,

(ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a PCSK9 gene,

wherein the guide RNA directs the base editor fusion protein to effect a nucleobase alteration in the PCSK9 gene *in vivo* when administered to a mammalian subject,

wherein when the guide RNA and the mRNA is administered at a total amount of at least 0.05 mg/kg, the base alteration occurs in at least 35% of whole liver cells in the mammalian subject as measured by next generation sequencing or Sanger sequencing.

2. The composition of claim 1, wherein the mammalian subject is a cynomolgus monkey,

wherein when the guide RNA and the mRNA is administered at a total amount of at least 0.5 mg/kg, the base alteration occurs in at least 40% of whole liver cells in the cynomolgus monkey as measured by next generation sequencing or Sanger sequencing.

3. The composition of claim 1, wherein the mammalian subject is a cynomolgus monkey,

wherein when the guide RNA and the mRNA is administered at a total amount of at least 1 mg/kg, the base alteration occurs in at least 45% of whole liver cells in the cynomolgus monkey as measured by next generation sequencing or Sanger sequencing.

4. The composition of claim 1, wherein the mammalian subject is a cynomolgus monkey,

wherein when the guide RNA and the mRNA is administered at a total amount of at least 1.5 mg/kg, the base alteration occurs in at least 50% of whole liver cells in the cynomolgus monkey as measured by next generation sequencing or Sanger sequencing.

5. The composition of claim 1, wherein the mammalian subject is a cynomolgus monkey,

wherein when the guide RNA and the mRNA is administered at a total amount of at

least 3 mg/kg, the base alteration occurs in at least 55% of whole liver cells in the cynomolgus monkey as measured by next generation sequencing or Sanger sequencing.

6. The composition of claim 1, wherein the mammalian subject is a mouse, wherein when the guide RNA and the mRNA is administered at a total amount of at least 0.125 mg/kg, the base alteration occurs in at least 40% of whole liver cells in the mouse as measured by next generation sequencing or Sanger sequencing.

7. The composition of claim 1, wherein the mammalian subject is a mouse, wherein when the guide RNA and the mRNA is administered at a total amount of at least 0.5 mg/kg, the base alteration occurs in at least 45% of whole liver cells in the mouse as measured by next generation sequencing or Sanger sequencing.

8. The composition of claim 1, wherein the mammalian subject is a mouse, wherein when the guide RNA and the mRNA is administered at a total amount of at least 2 mg/kg, the base alteration occurs in at least 50% of whole liver cells in the mouse as measured by next generation sequencing or Sanger sequencing.

9. The composition of claim 2, wherein the nucleobase alteration results in a reduction of at least 50% in blood low-density lipoprotein cholesterol (LDL-C) level in the subject as compared to prior to the administration.

10. The composition of any one of claims 1-9, wherein the protospacer is located in a splice site.

11. The composition of any one of claims 1-9, wherein the protospacer complementary sequence is in the antisense strand of the PCSK9 gene.

12. The composition of any one of claims 1-9, wherein the protospacer complementary sequence is in the sense strand of the PCSK9 gene.

13. The composition of any one of claims 1-12, wherein the base alteration happens outside of the protospacer on the PCSK9 gene (off-target sites), wherein the editing percentages of off-target sites set forth in Table 11 are below or equal to the editing percentages set forth in Table 11, respectively.

14. The composition of any one of claims 1-13, wherein the deaminase is an adenine deaminase and wherein the nucleobase alteration is a A•T to G•C alteration.

15. The composition of any one of claims 1-14, wherein the programmable DNA binding domain comprises a nuclease inactive Cas9 or a Cas9 nickase.

16. The composition of any one of claims 1-15, wherein the nucleobase alteration is at a splice site of the PCSK9 gene.

17. The composition of claim 16, wherein the nucleobase alteration is at a splice donor site of the PCSK9 gene.
18. The composition of claim 17, wherein the splice donor site is at 5' end of PCSK9 intron 1 as referenced in SEQ ID NO: 5.
19. The composition of claim 16, wherein the nucleobase alteration is at a splice acceptor site of the PCSK9 gene.
20. The composition of any one of claims 1-19, wherein the nucleobase alteration results in a frame shift, a premature stop codon, an insertion or deletion in a transcript encoded by the PCSK9 gene.
21. The composition of any one of claims 1-20, wherein the nucleobase alteration results in an aberrant transcript encoded by the PCSK9 gene.
22. The composition of any one of claims 1-21, wherein the guide RNA is chemically modified.
23. The composition of claim 16, wherein the tracr sequence of the guide RNA is chemically modified following the scheme depicted in Fig. 7.
24. The composition of any one of claims 1-22, wherein the spacer sequence comprises a PCSK9 ABE guide RNA spacer sequence set forth in Table 1.
25. The composition of claim 24, wherein the guide RNA comprises the PCSK9 ABE guide RNA sequence of GA096, GA097, GA343, GA346, GA375-377, GA380-389, GA391, GA439 or GA440 as set forth in Table 1.
26. The composition of any one of claims 1-23, wherein the protospacer sequence comprises a PCSK9 ABE protospacer sequence set forth in Table 1.
27. The composition of claim 26, wherein the protospacer comprises the sequence 5'-CCCGCACCTTGGCGCAGCGG-3' (SEQ ID No: 13) or 5'-CCGCACCTTGGCGCAGCGG-3' (SEQ ID No: 247).
28. The composition of any one of claims 1-27, wherein the base editor fusion protein comprises an amino acid sequence of SEQ ID No: 2137.
29. The composition of any one of claims 1-28, wherein the GC% content of the mRNA sequence is greater than 50%.
30. The composition of claim 29, wherein the GC% content of the mRNA sequence is greater than 56%.
31. The composition of claim 30, wherein the GC% content of the mRNA sequence is greater than or equal to 63%.

32. The composition of claim 29, wherein the mRNA comprises an adenine tTNA deaminase (TadA) region, a Cas9 region and a nuclear localization sequence (NLS) region.
33. The composition of claim 32, wherein the mRNA further comprises a first linker region which connects the TadA region and the Cas9 region, and a second linker region which connects the Cas9 region and the NLS region.
34. The composition of claim 32 or 33, wherein the GC% content of the TadA region is greater than 60%.
35. The composition of claim 32 or 33, wherein the GC% content of the TadA region is greater than or equal to 70%.
36. The composition of claim 32 or 33, wherein the GC% content of the Cas9 region is greater than 56%.
37. The composition of claim 32 or 33, wherein the GC% content of the Cas9 region is greater than or equal to 62%.
38. The composition of claim 32 or 33, wherein the GC% content of the NLS region is greater than 54%.
39. The composition of claim 32 or 33, wherein the GC% content of the NLS region is greater than or equal to 63%.
40. The composition of claim 33, wherein the GC% content of the first linker region is greater than 65%.
41. The composition of claim 33, wherein the GC% content of the first linker region is greater than or equal to 79%.
42. The composition of claim 33, wherein the GC% content of the second linker region is greater than 67%.
43. The composition of claim 33, wherein the GC% content of the second linker region is greater than or equal to 83%.
44. The composition of claim 33, wherein the GC% content of the TadA region is greater than 60%, the GC% content of the Cas9 region is greater than 56%, the GC% content of the NLS region is greater than 54%, the GC% content of the first linker region is greater than 65%, and the GC% content of the second linker region is greater than 67%.
45. The composition of claim 29, wherein the mRNA comprises a mRNA sequence selected from Table 23.

46. The composition of claim 45, wherein the mRNA comprises a mRNA sequence of SEQ ID No: 2136.
47. The composition of any one of claims 29-46, wherein the mRNA comprises a poly A tail.
48. The composition of any one of claims 1-47, further comprising a lipid nanoparticle (LNP) enclosing (i).
49. The composition of claim 48, wherein the LNP further encloses (ii).
50. The composition of claim 48, further comprising a second LNP enclosing (ii).
51. The composition of any one of claims 1-44, wherein the ratio of the guide RNA and the mRNA encoding the base editor fusion protein is about 1:10 to about 10:1 by weight.
52. The composition of claim 51, wherein the ratio of the guide RNA and the mRNA encoding the base editor fusion protein is about 1:1, 1.5:1, 2:1, 3:1, 4:1, 1:1.5, 1:2, 1:3, or 1:4 by weight.
53. The composition of claim 51, wherein the ratio of the guide RNA and the mRNA encoding the base editor fusion protein is about 1:1 by weight.
54. A pharmaceutical composition comprising the composition of any one of the preceding claims and a pharmaceutically acceptable carrier or excipient.
55. A method for treating or preventing a condition in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the composition of claim 1.
56. The method of claim 55, wherein the administration is via intravenous infusion.
57. The method of claim 55 or 56, comprising sequential administration of a LNP enclosing (i) and a LNP enclosing (ii).
58. The method of claim 55 or 56, comprising concurrent administration of the t LNP enclosing (i) and the LNP enclosing (ii).
59. The method of claim 57, comprising administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 1 day.
60. The method of claim 57, comprising administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 2 days.
61. The method of claim 57, comprising administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 3 days.
62. The method of claim 57, comprising administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 4 days.

63. The method of claim 57, comprising administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 5 days.
64. The method of claim 57, comprising administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 6 days.
65. The method of claim 57, comprising administering a single dose of the LNP enclosing (ii) followed by staggered doses of the LNP enclosing (i) over an interval of 7 days.
66. The method of claim 55 or 56, comprising administering a single dose of the LNP enclosing (i) and (ii).
67. The method of claim 66, wherein the single dose of the LNP is at about 0.3 to about 3mg/kg.
68. The method of claim 66 or 67, comprising administering a treatment course of one or more treatments to the subject, wherein each one of the one or more treatment comprises one or more of the single doses of the LNP.
69. The method of claim 68, comprising administering a treatment course of two to ten treatments.
70. The method of claim 68, comprising administering a treatment course of two to five treatments.
71. The method of claim 68, comprising administering a treatment course of two treatments.
72. The method of claim 68, comprising administering a treatment course of three treatments.
73. The method of claim 68, comprising administering a treatment course of four treatments.
74. The method of claim 68, comprising administering a treatment course of five treatments.
75. The method of any one of claims 55-74, wherein the condition is an atherosclerotic cardiovascular disease.
76. The method of any one of claims 55-74, wherein the condition is an atherosclerotic vascular disease.
77. The method of any one of claims 55-74, wherein the subject is a human.
78. A composition for editing a gene target comprising:
(i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same,
(ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the

base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a PCSK9 gene,

wherein the guide RNA directs the base editor fusion protein to effect a nucleobase alteration in the PCSK9 gene *in vivo* when administered to a mammalian subject, and wherein the guide RNA comprises the PCSK9 ABE guide RNA sequences as set forth in Table 1.

79. A composition for editing a gene target comprising:

(i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same,

(ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a PCSK9 gene,

wherein the guide RNA directs the base editor fusion protein to effect a nucleobase alteration in the PCSK9 gene *in vivo* when administered to a mammalian subject, and wherein the mRNA comprises a sequence selected from Table 23.d

80. A method for treating or preventing an atherosclerotic cardiovascular disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a first composition, comprising

(i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same,

(ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a PCSK9 gene,

wherein the guide RNA directs the base editor fusion protein to effect a nucleobase alteration in the PCSK9 gene *in vivo* when administered to a mammalian subject, wherein when the guide RNA and the mRNA is administered at a total amount of at least 0.05 mg/kg, the base alteration occurs in at least 35% of whole liver cells in the mammalian subject as measured by next generation sequencing or Sanger sequencing; and

a second composition, comprising

(i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same,

(ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on

a ANGPTL3 gene,

wherein the guide RNA directs the base editor fusion protein to effect a nucleobase alteration in the ANGPTL3 gene *in vivo* when administered to a mammalian subject, wherein when the guide RNA and the mRNA is administered at a total amount of at least 0.5 mg/kg, the base alteration occurs in at least 35% of whole liver cells in the mammalian subject as measured by next generation sequencing or Sanger sequencing.

81. The method of claim 80, comprising sequential administration of the first composition and the second composition.

82. The method of claim 81, comprising administering one or more doses of the first composition followed by one or more dose of the second composition.

83. The method of claim 82, comprising administering one or more doses of the second composition followed by one or more dose of the first composition.

84. The method of claim 80, comprising concurrent administration of the first composition and the second composition.

85. The method of claim 84, comprising one or more doses of the first composition and the second composition.

86. A composition for editing a gene target comprising:

(i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same,

(ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a APOC3 gene,

wherein the guide RNA comprises the APOC3 ABE guide RNA sequence of GA300-303 as set forth in Table 24.

87. A composition for editing a gene target comprising:

(i) a base editor fusion protein comprising a programmable DNA binding domain and a deaminase, or a mRNA encoding the same,

(ii) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor fusion protein, and a spacer sequence that corresponds to a protospacer on a PCSK9 gene,

wherein the guide RNA directs the base editor fusion protein to effect a nucleobase alteration in the PCSK9 gene *in vitro*,

wherein when the guide RNA and the mRNA is administered at a total amount of at least 2.5 mg/kg, the base alteration occurs in at least 35% of whole liver cells in the

mammalian subject as measured by next generation sequencing or Sanger sequencing.

88. A composition for editing a PCSK9 gene comprising:
(a) a mRNA encoding an adenine base editor protein having an editing window, and
(b) a guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor protein and a spacer sequence that serves to guide the base editor protein to a protospacer sequence on the PCSK9 gene,
wherein the spacer sequence is complimentary, at least in part, to a splice site or an exon region of the PCSK9 gene.

89. The composition for editing the PCSK9 gene of claim 88, wherein when the base editor protein is operatively bound to the guide RNA and the guide RNA is hybridized with the complementary strand to the protospacer sequence on the PCSK9 gene, the editing window encompasses the splice site of the PCSK9 gene.

90. The composition for editing the PCSK9 gene of claim 88, wherein when the base editor protein is operatively bound to the guide RNA and the guide RNA is hybridized with the complementary strand to the protospacer sequence on the PCSK9 gene, the editing window encompasses a region of an intron of the PCSK9 gene.

91. The composition for editing the PCSK9 gene of claim 88, wherein when the base editor protein is operatively bound to the guide RNA and the guide RNA is hybridized with the complementary strand to the protospacer sequence on the PCSK9 gene, the editing window encompasses a region of intron 1, intron 3 or intron 4 of the PCSK9 gene.

92. The composition for editing the PCSK9 gene of claim 88, wherein when the base editor protein is operatively bound to the guide RNA and the guide RNA is hybridized with the complementary strand to the protospacer sequence on the PCSK9 gene, the editing window encompasses a region of intron 1 of the PCSK9 gene.

93. The composition for editing the PCSK9 gene of claim 88, wherein the spacer sequence has a 80-100 % nucleotide sequence identity to a spacer sequence selected from the group of guide RNA sequences identified as GA066, GA073 and GA074.

94. The composition for editing the PCSK9 gene of claim 88, wherein the tracr sequence has a 80-100% nucleotide sequence identity to a tracr sequence selected from the group of guide RNA sequences identified as GA066, GA095, GA096, GA097, GA343, GA346, GA375, GA376, GA377, GA380, GA381, GA382, GA383, GA384, GA385, GA386, GA387, GA388, GA389, GA439, and GA440.

95. The composition for editing the PCSK9 gene of claim 88, wherein the mRNA has an 80-100% sequence identity to the mRNA sequences identified as MA002, MA004, MA040, MA0041, or MA045.

96. The composition for editing the PCSK9 gene of claim 88, wherein the mRNA has one or more of the GC nucleotide region percentages set forth in the following table:

Nucleotide region	Average GC Nucleotide Content
27-213	67-73%
389-661	67-71%
735-829	63-74%
4207-4286	67-70%
4537-4569	65-73%
4683-4741	62- 67%

97. The composition for editing the PCSK9 gene of claim 88, wherein the mRNA has one or more of the GC nucleotide region percentages set forth in the following table:

Nucleotide region	Average GC Nucleotide Content
27-213	At least 73%
389-661	At least 71%
735-829	At least 74%
4207-4286	At least 70%
4537-4569	At least 73%
4683-4741	At least 67%

98. The composition for editing the PCSK9 gene of claim 88, wherein the mRNA and gRNA are encapsulated within a lipid nanoparticle.

99. The composition for editing the PCSK9 gene of claim 88, wherein the mRNA and gRNA are encapsulated within a lipid nanoparticle having the following:

LNP composition (mol%):

40-65% iLipid

2-20% DSPC

1-5% PEG

Remaining mol% balance is cholesterol;

LNP Particle size: 55-120 nm Z average hydrodynamic diameter; and

Polydispersity index of <0.2 as determined by dynamic light scattering.

100. The composition for editing the PCSK9 gene of claim 88, wherein the mRNA and gRNA are encapsulated within the lipid nanoparticle having an LNP particle size between 50-70 nm Z average hydrodynamic diameter.

101. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the *PCSK9* target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 40 percent.

102. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 50 percent.

103. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 30 percent.

104. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 40 percent.

105. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 50 percent.

106. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 60 percent.

107. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 70 percent.

108. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 40 percent.

109. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 50 percent.

110. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 60 percent.

111. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing

at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 70 percent.

112. The composition for editing the PCSK9 gene of claim 88, wherein the composition when at administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 80 percent.

113. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 40 percent.

114. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 50 percent.

115. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 60 percent.

116. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 70 percent.

117. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a

dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of the cynomolgus monkey weight is capable of inducing adenine base editing at the PCSK9 target splice site in the liver of the cynomolgus monkeys with an average editing percentage of greater than 80 percent.

118. The compositions for editing the PCSK9 gene of claims 87-117, wherein the percent editing is determined at 15 days after dosing through analysis of dosed cynomolgus monkey liver either via liver biopsy or necropsy of the monkey.

119. The compositions for editing of the PCSK9 gene of claims 87-117, wherein the percent editing is determined to be durably maintained by periodic liver biopsy testing of the dosed cynomolgus monkeys over a span of at least 168 days after dosing.

120. The compositions for editing of the PCSK9 gene of claims 87-117, wherein the percent editing is determined to be durably maintained by periodic liver biopsy testing of the dosed cynomolgus monkeys over a span of at least 300 days after dosing.

121. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline.

122. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline.

123. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline.

124. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the

plasma of the dosed monkeys on average of at least 60 percent as compared to baseline.

125. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 70 percent as compared to baseline.

126. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 80 percent as compared to baseline.

127. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline.

128. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline.

129. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline.

130. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per

kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 60 percent as compared to baseline.

131. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 70 percent as compared to baseline.

132. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 80 percent as compared to baseline.

133. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline.

134. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline.

135. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline.

136. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 60 percent as compared to baseline.

137. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 70 percent as compared to baseline.

138. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 80 percent as compared to baseline.

139. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline.

140. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline.

141. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the

plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline.

142. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 60 percent as compared to baseline.

143. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 70 percent as compared to baseline.

144. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing PCSK9 protein in the plasma of the dosed cynomolgus monkeys on average of at least 80 percent as compared to baseline.

145. The compositions for editing of the PCSK9 gene of claims 121-144, wherein the reduction in plasma protein is determined at 15 days after dosing via blood sampling and analysis of the dosed cynomolgus monkey.

146. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of monkey weight is capable of reducing LDL-C in the plasma of the dosed monkeys on average of at least 20 percent as compared to baseline.

147. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 25 percent as compared to baseline.

148. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 30 percent as compared to baseline.

149. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline.

150. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline.

151. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 0.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 45 percent as compared to baseline.

152. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 20 percent as compared to baseline.

153. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 25 percent as compared to baseline.

154. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 30 percent as compared to baseline.

155. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline.

156. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline.

157. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 45 percent as compared to baseline.

158. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline.

159. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 55 percent as compared to baseline.

160. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1 mg of the guide RNA and mRNA combined total weight per

kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 60 percent as compared to baseline.

161. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 20 percent as compared to baseline.

162. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 25 percent as compared to baseline.

163. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 30 percent as compared to baseline.

164. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline.

165. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline.

166. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a

dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 45 percent as compared to baseline.

167. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline.

168. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 55 percent as compared to baseline.

169. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 60 percent as compared to baseline.

170. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 1.5 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 65 percent as compared to baseline.

171. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 20 percent as compared to baseline.

172. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 25 percent as compared to baseline.

173. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 30 percent as compared to baseline.

174. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 35 percent as compared to baseline.

175. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 40 percent as compared to baseline.

176. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 45 percent as compared to baseline.

177. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 50 percent as compared to baseline.

178. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per

kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 55 percent as compared to baseline.

179. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 60 percent as compared to baseline.

180. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing LDL-C in the plasma of the dosed cynomolgus monkeys on average of at least 65 percent as compared to baseline.

181. The compositions for editing the PCSK9 gene of claims 148-180, wherein the reduction in LDL-C is determined at 15 days after dosing via blood sampling and analysis of the dosed cynomolgus monkey.

182. The compositions for editing the PCSK9 gene of claims 148-180, wherein the reduction in LDL-C is determined to be durably maintained over a span of at least 168 days by periodic blood sampling and analysis of the dosed cynomolgus monkey.

183. The compositions for editing the PCSK9 gene of claims 148-182, wherein the reduction in LDL-C is determined to be durably maintained over a span of at least 300 days by periodic blood sampling and analysis of the dosed cynomolgus monkey.

184. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing lipoprotein(a) in the plasma of the dosed cynomolgus monkeys on average of at least 10 percent as compared to baseline.

185. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing lipoprotein(a) level in the plasma of the dosed cynomolgus monkeys on average of at least 15 percent as compared to baseline.

186. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing lipoprotein(a) level in the plasma of the dosed cynomolgus monkeys on average of at least 20 percent as compared to baseline.

187. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing lipoprotein(a) level in the plasma of the dosed cynomolgus monkeys on average of at least 25 percent as compared to baseline.

188. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing lipoprotein(a) level in the plasma of the dosed cynomolgus monkeys on average of at least 30 percent as compared to baseline.

189. The composition for editing the PCSK9 gene of claim 88, wherein the composition when administered to a group of cynomolgus monkeys via intravenous infusion at a dose of approximately 3 mg of the guide RNA and mRNA combined total weight per kg of cynomolgus monkey weight is capable of reducing lipoprotein(a) level in the plasma of the dosed cynomolgus monkeys on average of approximately 35 percent as compared to baseline.

190. The compositions for editing the PCSK9 gene of claims 186-189, wherein the reduction in lipoprotein(a) is determined at 15 days after dosing via blood sampling and analysis of the dosed cynomolgus monkey.

191. The compositions for editing the PCSK9 gene of claims 186-189, wherein the reduction in lipoprotein(a) is determined to be durably maintained over a span of at least 224 days by periodic blood sampling and analysis of the dosed cynomolgus monkey.

192. The compositions for editing the PCSK9 gene of claims 186-189, wherein the reduction in lipoprotein(a) is determined to be durably maintained over a span of at

least 300 days by periodic blood sampling and analysis of the dosed cynomolgus monkey.

193. The compositions for editing the PCSK9 gene of claims 101-192, wherein to the extent that the dosing of the cynomolgus monkeys results in elevation of AST, ALT, or Cytokines, the elevations resulting from the dosing of the composition are transient and resolved back to approximately baseline levels within 3-15 days after dosing.

194. The composition for editing the PCSK9 gene of claims 101-103, 121-126, 148-153, wherein the percent editing of PCSK9 is negligible outside of the liver, spleen and adrenal glands tissues as illustrated in FIG. 27.

195. The compositions for editing the PCSK9 gene of claims 101-107, 121-132, 148-162, wherein repeat dosing results is additive with respect to the editing percentage of PCSK9 editing percentage.

196. The compositions for editing the PCSK9 gene of claim 195, wherein the repeat dosing does not elicit cytokine activation nor an immune response.

197. The composition for editing the PCSK9 gene of claim 88, wherein the spacer sequence has at least 80% nucleotide correlation with the nucleotide sequence of a targeted protospacer on the PCSK9 gene, wherein an RNA nucleotide on the spacer sequence is in correlation with a DNA nucleotide of the protospacer if it has the same nucleotide as the DNA nucleotide in the same order and wherein uracil and thymine bases are considered the same nucleotide for purposes of determining correlation.

198. The composition for editing the PCSK9 gene of claim 197, wherein the spacer sequence has at least 85% nucleotide correlation with the nucleotide sequence of a targeted protospacer on the PCSK9 gene.

199. The composition for editing the PCSK9 gene of claim 197, wherein the spacer sequence has at least 90% nucleotide correlation with the nucleotide sequence of a targeted protospacer on the PCSK9 gene.

200. The composition for editing the PCSK9 gene of claim 197, wherein the spacer sequence has at least 95% nucleotide correlation with the nucleotide sequence of a targeted protospacer on the PCSK9 gene.

201. The composition for editing the PCSK9 gene of claim 197, wherein the spacer sequence has at least 99% nucleotide correlation with the nucleotide sequence of a targeted protospacer on the PCSK9 gene.

202. The composition for editing the PCSK9 gene of claim 197, wherein the spacer sequence has at least 100% nucleotide correlation with the nucleotide sequence of a targeted protospacer on the PCSK9 gene.

203. A method for treating or preventing an atherosclerotic cardiovascular disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of

(a) a mRNA encoding an adenine base editor protein having an editing window,

(b) a first guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor protein and a spacer sequence that serves to guide the base editor protein to a protospacer sequence on a PCSK9 gene, wherein the spacer sequence is complimentary, at least in part, to a splice site or an exon region of the PCSK9 gene; and

(c) a second guide RNA comprising a tracr sequence that serves as a binding scaffold for the base editor protein and a spacer sequence that serves to guide the base editor protein to a protospacer sequence on a ANGPTL3 gene, wherein the spacer sequence is complimentary, at least in part, to a splice site or an exon region of the ANGPTL3 gene.

204. The method of claim 203, further comprising a first LNP enclosing (a).

205. The method of claim 204, wherein the first LNP encloses (b) and (c).

206. The method of claim 204, wherein the first LNP was administered repeatedly.

207. The method of claim 204, wherein the first LNP was administered repeatedly at an interval of one to sixty days.

208. The method of claim 204, wherein the first LNP was administered repeatedly at an interval of seven days.

209. The method of claim 204, wherein the first LNP further encloses (b).

210. The method of claim 209, further comprising a second LNP enclosing (a) and (c).

211. The method of claim 210, wherein the first LNP and the second LNP are administered sequentially.

212. The method of claim 211, wherein the first LNP and the second LNP are administered sequentially at an interval of one day to 12 months.

213. The method of claim 212, wherein the interval is one day.

214. The method of claim 212, wherein the interval is five days.

215. The method of claim 212, wherein the interval is ten days.

216. The method of claim 212, wherein the interval is fifteen days.

217. The method of claim 212, wherein the interval is twenty days.

218. The method of claim 212, wherein the interval is twenty-five days.

219. The method of claim 212, wherein the interval is one month.

220. The method of claim 212, wherein the interval is two months.

221. The method of claim 212, wherein the interval is three months.

222. The method of claim 212, wherein the interval is five months.

223. The method of claim 212, wherein the interval is eight months.

224. The method of claim 212, wherein the interval is ten months.

225. The method of claim 212, wherein the interval is twelve months.

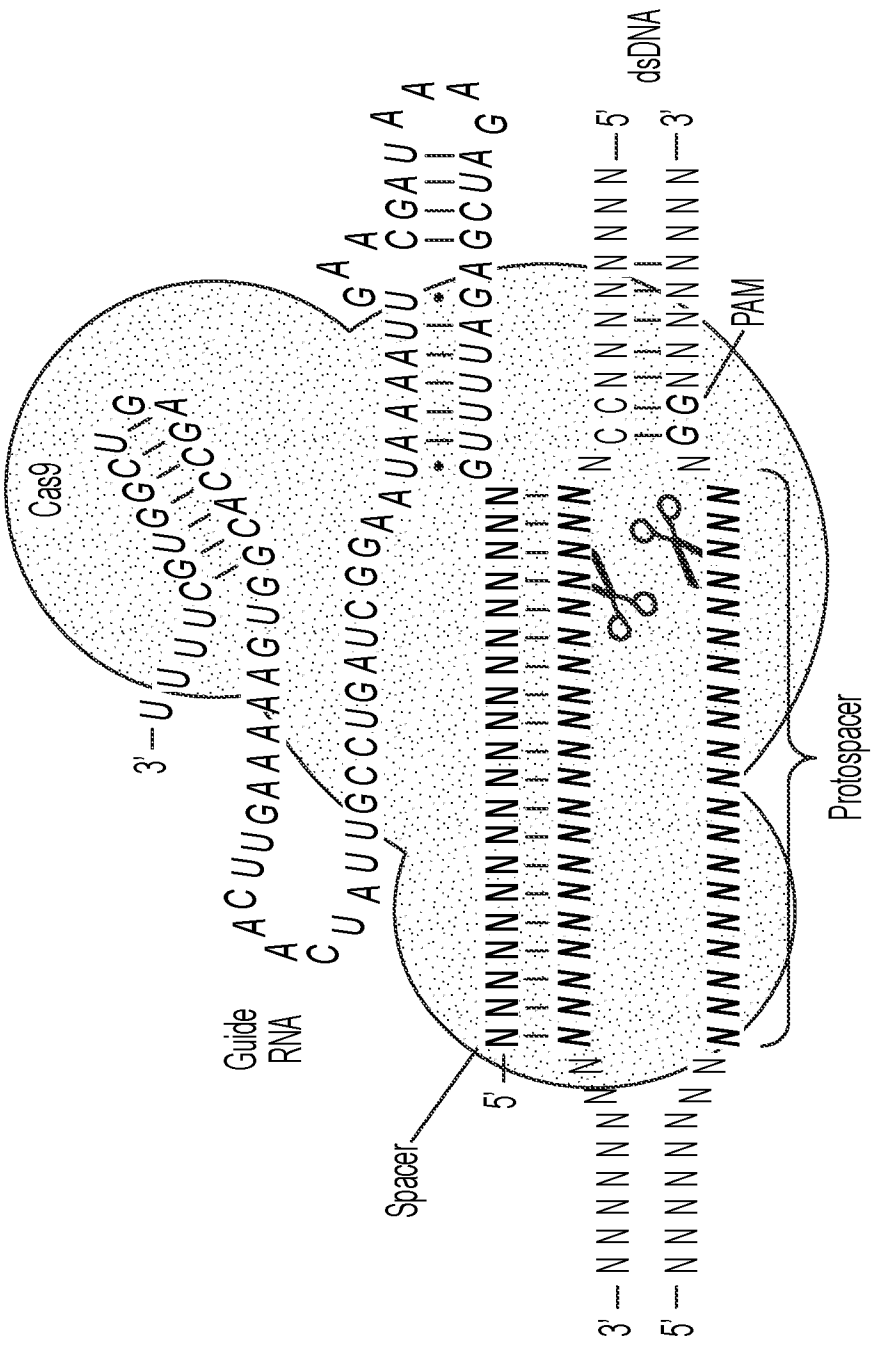


FIG. 1A

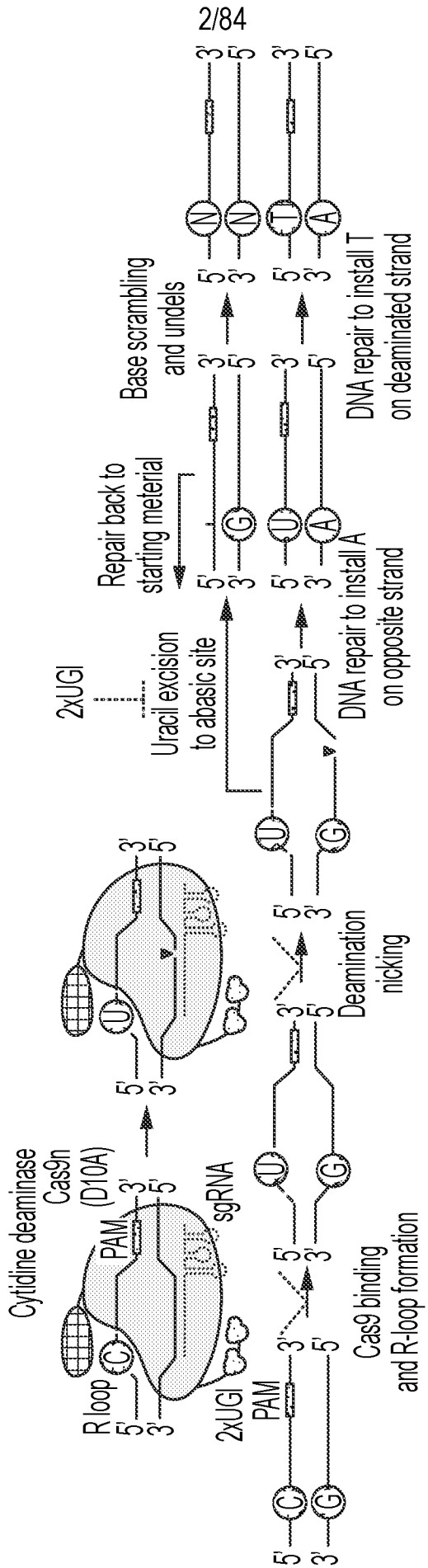

Cytidine deaminase
Cas9n
(D10A)
PAM
sgRNA
R loop
2xUGI
PAM
Cas9 binding
and R-loop formation
Deamination
nicking
2xUGI
Uracil excision
to abasic site
DNA repair to install A
on opposite strand
Repair back to
starting meterial
Base scrambling
and undels
DNA repair to install T
on deaminated strand

FIG. 1B

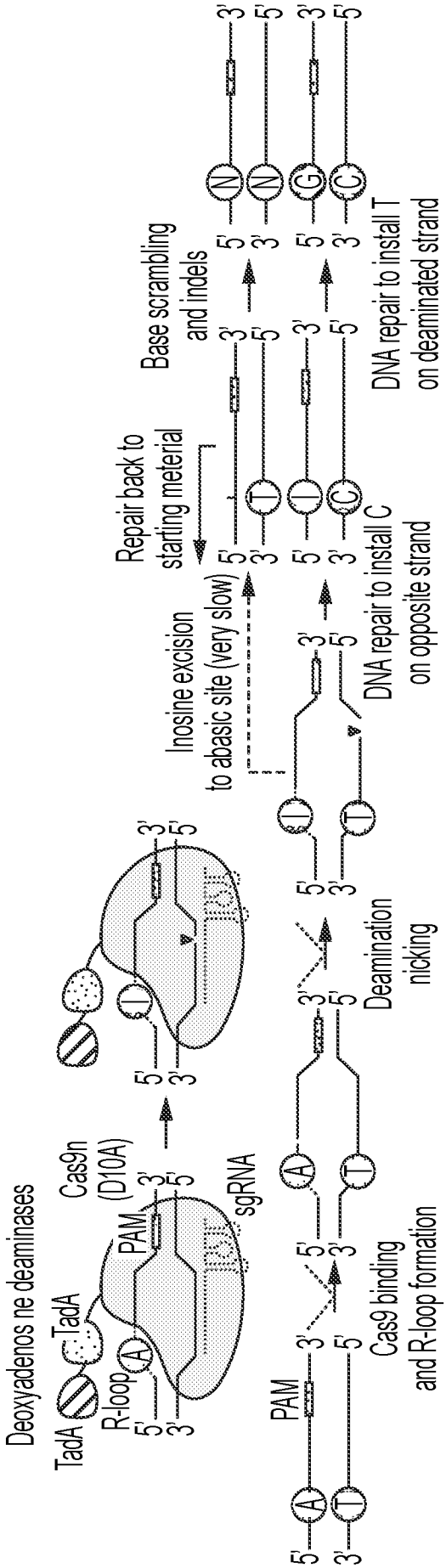

Deoxyadenos ne deaminases
TadA
TadA
R-loop
PAM
Cas9n
(D10A)
sgRNA
Cas9 binding
and R-loop formation
Deamination
nicking
Inosine excision
to abasic site (very slow)
DNA repair to install C
on opposite strand
Repair back to
starting meterial
Base scrambling
and indels
DNA repair to install T
on deaminated strand

FIG. 1C

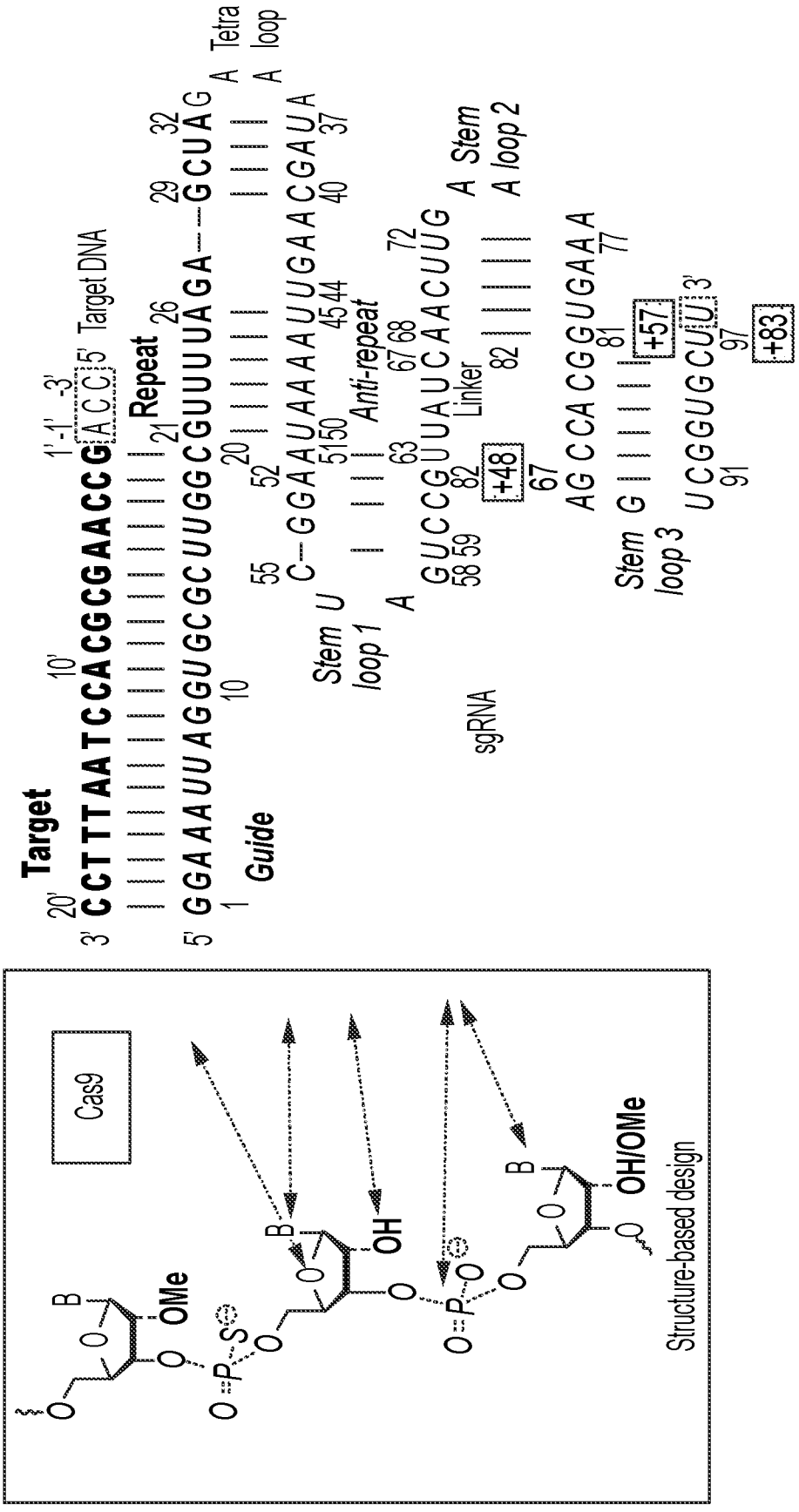

Target
Guide
Repeat
Target DNA
Tetra loop
Stem
loop 2
Stem
loop 3
Anti-repeat
Stem
loop 1
Linker
sgRNA
+48
+57
+83
Cas9
OMe
OH
OH/OMe
Structure-based design

FIG. 1D

PDB ID	Components	State
4CMP	SpCas9 protein	Apo (pre-RNP formation)
4ZT0	SpCas9 protein + gRNA	RNP (forms in cytosol)
5F9R	SpCas9 protein + gRNA + target/non-target DNA	Pre-catalytic ternary complex
4UN3	SpCas9 protein + gRNA + target/non-target DNA	Post-catalytic ternary complex

	4CMP	4ZT0	5F9R	4UN3
4CMP		15	20	20
4ZT0	15		4	3
5F9R	20	4		2
4UN3	20	3	2	

FIG. 2

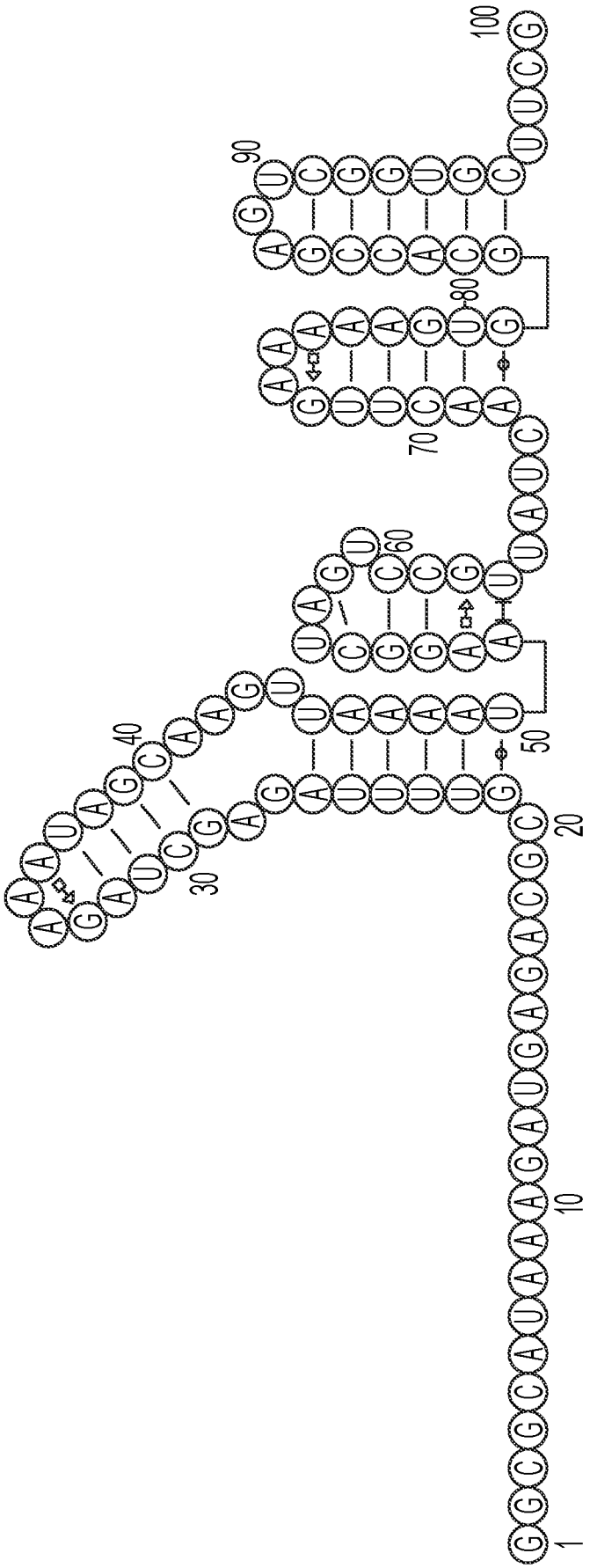


FIG. 3

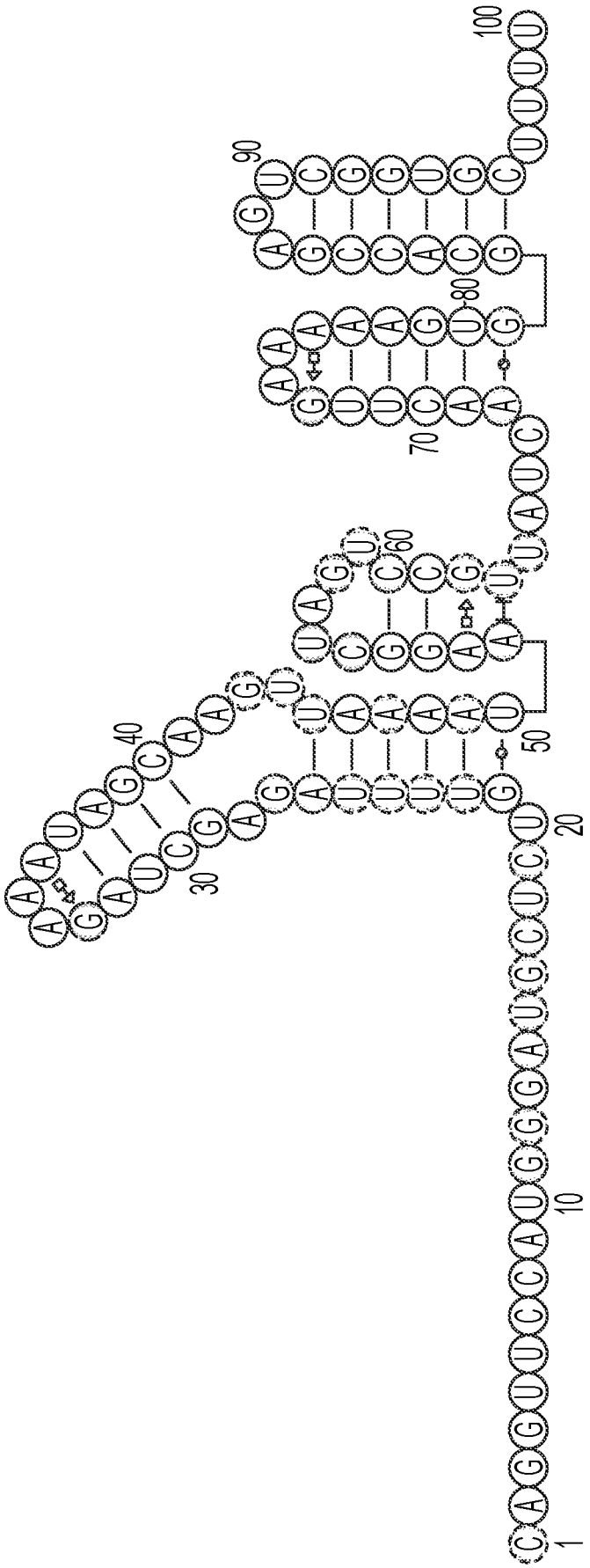


FIG. 4

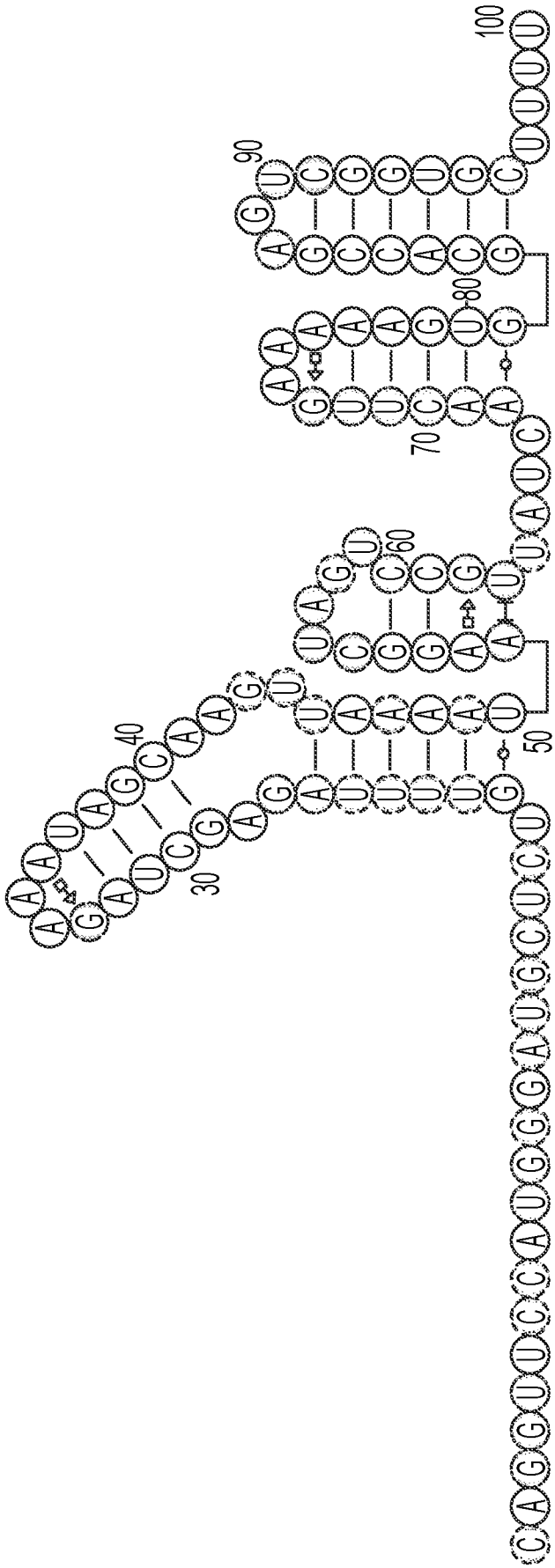


FIG. 5

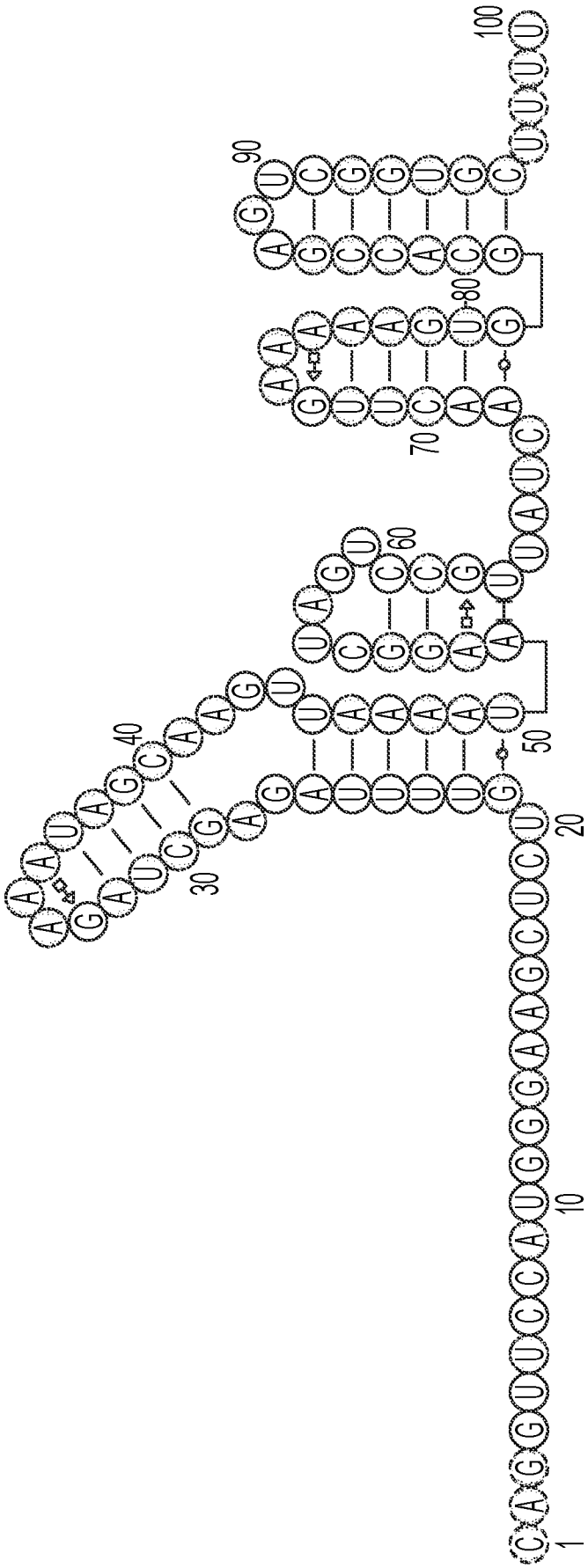


FIG. 6

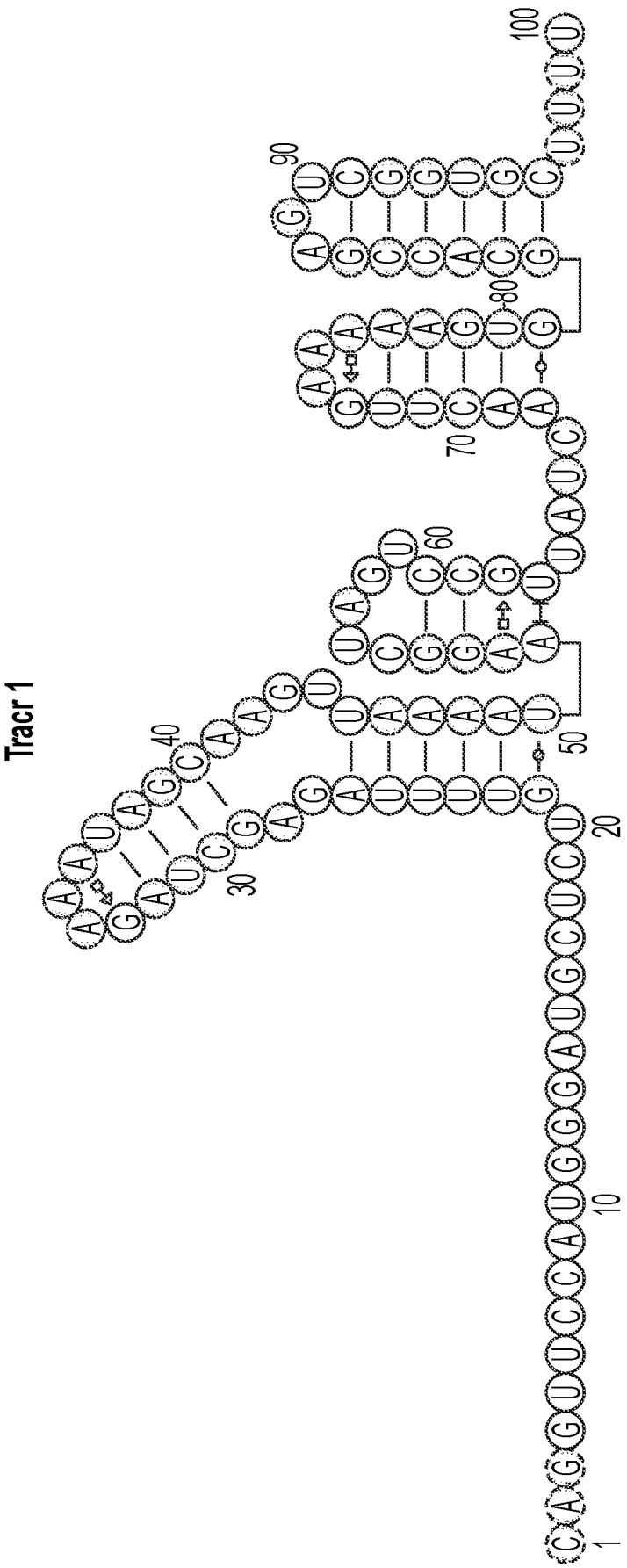


FIG. 7A

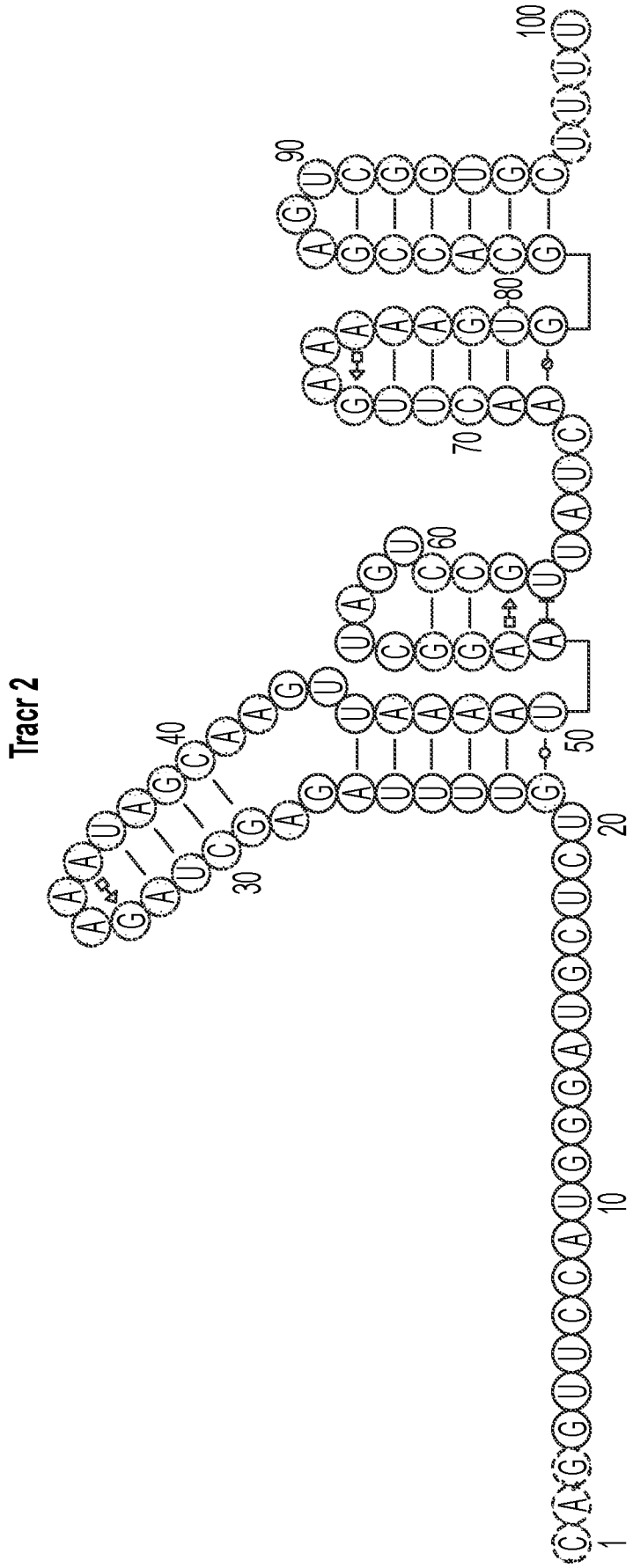


FIG. 7A
(Continued)

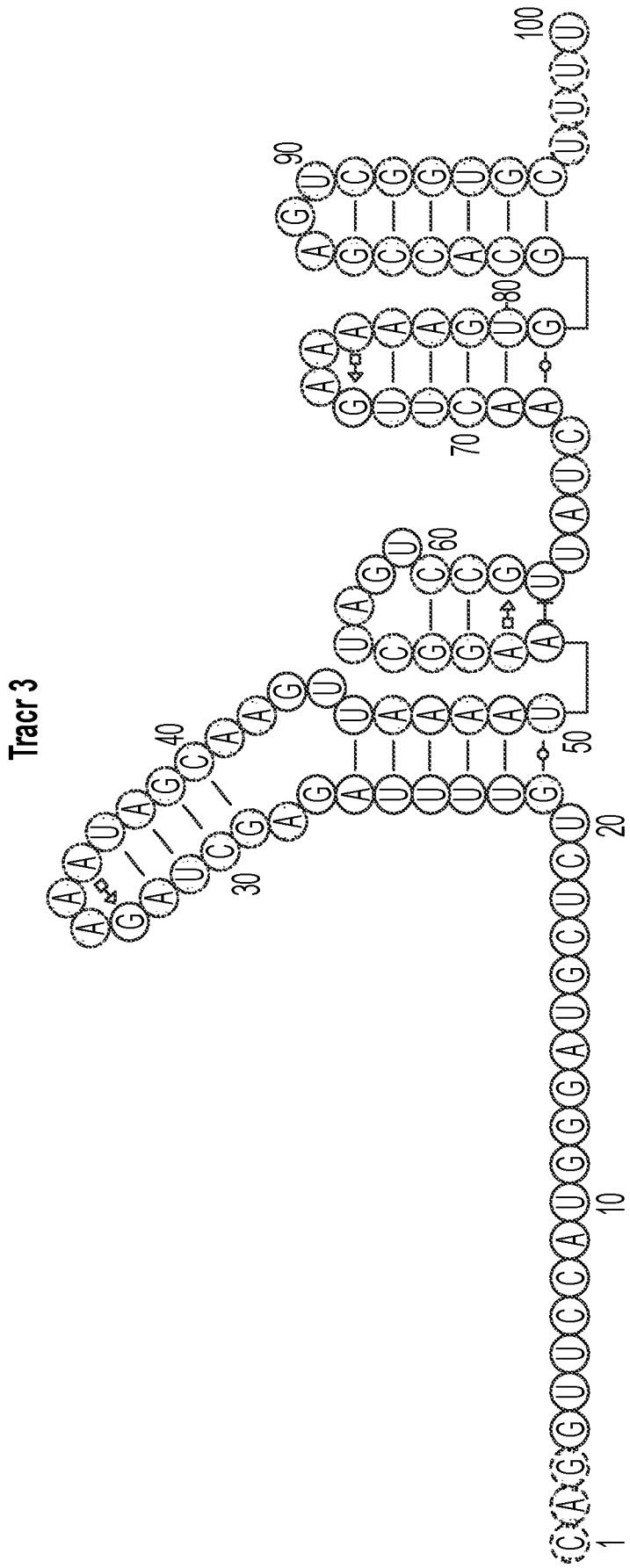


FIG. 7A
(Continued)

```
                                10        20        30        40        50        60        70        80
                       123456789X 123456789X 123456789X 123456789X 123456789X 123456789X 123456789X 123456789X
SEQ ID NO: 61 GUUUUAGAGC UAGAAAUAGC AAGUUAAAAU AAGGCUAGUC CGUUAUCAAC UUGAAAAAGU GGCACCGAGU CGGUGCUUUU
Lit Tracr     GUUUUAGAgc uagaaauagc AAGUUAAAAU AAGGCUAGUC CGUUAUCAac uugaaaaagu ggcaccgagu cggugcusususu
Tracr1        gUUUUAGagc uaGaaauagc aaGUUaAaAu AaggCUaGUC cGUUAucAAc uuGaaaaagu GgcaccgAgU Cggugcusususu
Tracr2        gUUUUAGagc uagaaauagc aaGUUaAaAu AaggcuaGUc cGUUAucAAc uugaaaaagu gGcaccgagu cggugcusususu
Tracr3        gUUUUAGagc uaGaaauagc aaGUUaAaAu AaggcuaGUc cGUUAucAAc uuGaaaaagu gGcaccgagu cggugcusususu
```

FIG. 7B

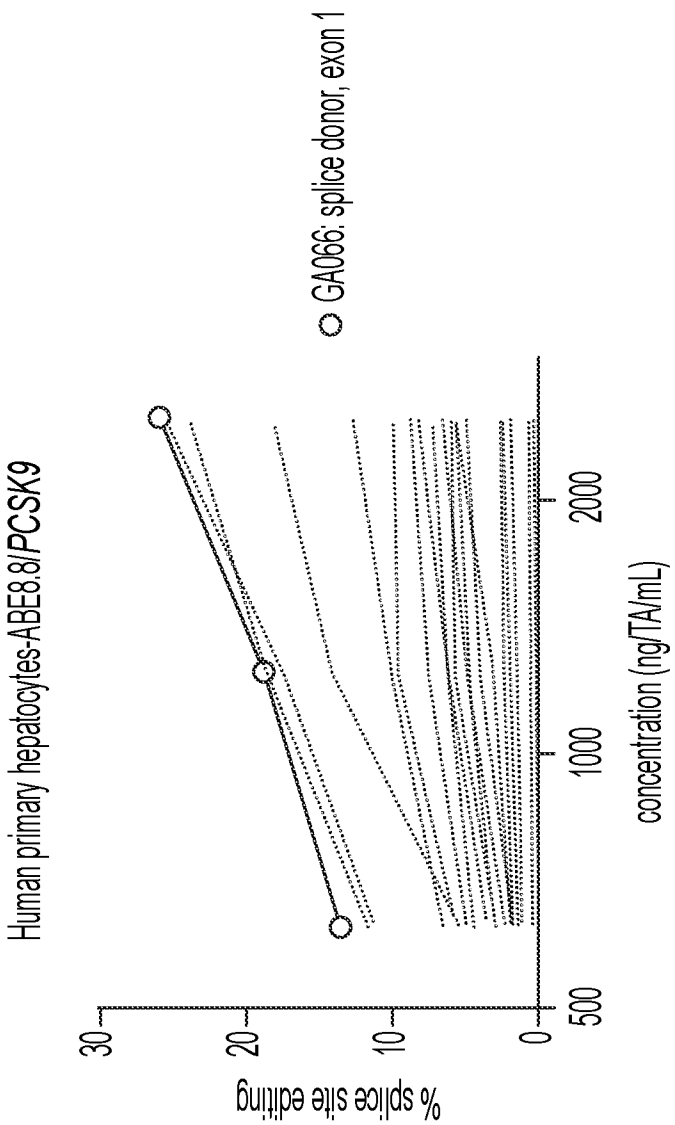

Human primary hepatocytes-ABE8.8/PCSK9
GA066: splice donor, exon 1
% splice site editing
30
20
10
0
500
1000
2000
concentration (ng/TA/mL)

FIG. 8

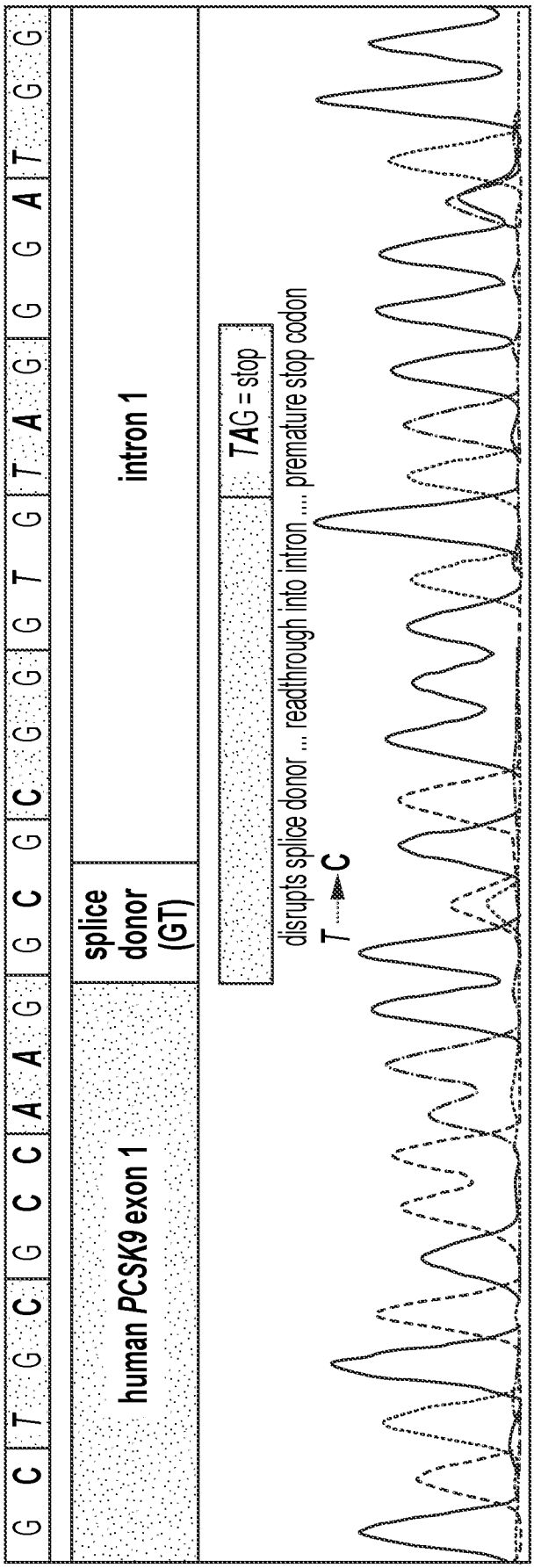

G C T G C G C C A A G G C G G C G G T G T A G G G A T G G
human PCSK9 exon 1
splice donor (GT)
intron 1
TAG = stop
disrupts splice donor ... readthrough into intron .... premature stop codon
T → C
natural SNP
≈ 60% adenine base editing
(editing of A in opposite strand)

FIG. 9

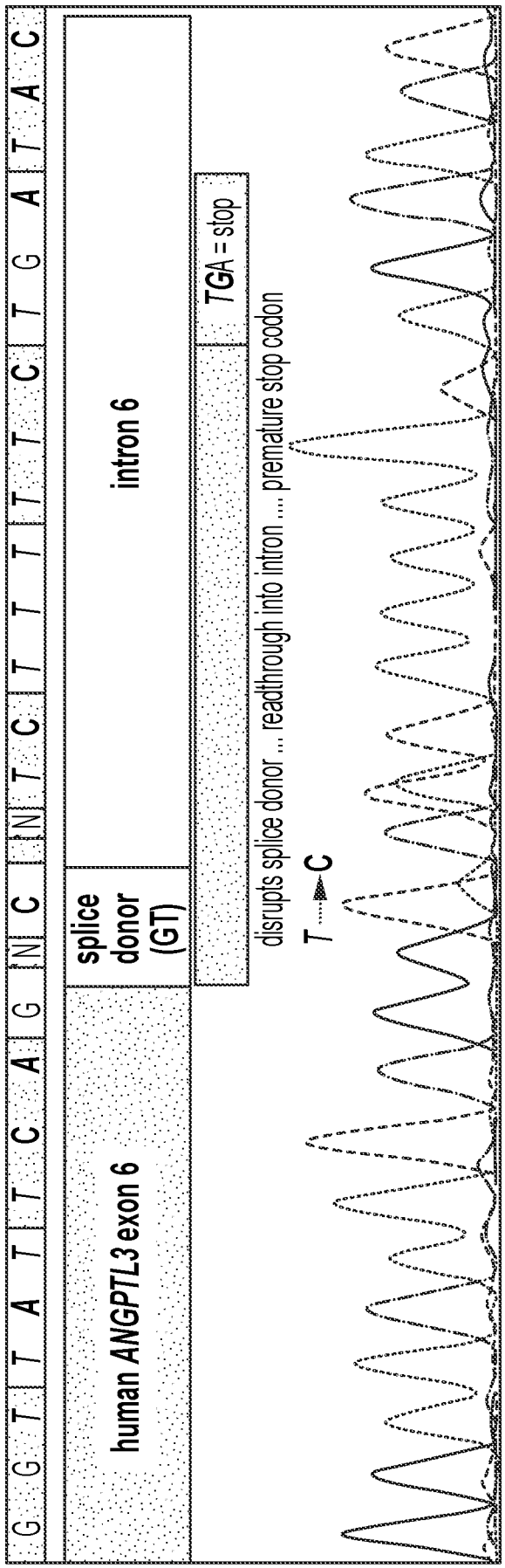

G G T T A T T C A G N C
human ANGPTL3 exon 6
splice donor (GT)
N T C T T T T T C T G A T A C
intron 6
TGA = stop
disrupts splice donor ... readthrough into intron .... premature stop codon
T ---> C
site of natural rare variant (rs398122985, G ---> T) that disrupts splice donor and in homozygous state causes combined hypolipidemia (TG 33, LDL-C 42, HDL-C 24)
T ---> C (additional base edit of no consequence)
≈ 70% adenine base editing (editing of A in opposite strand)

FIG. 10

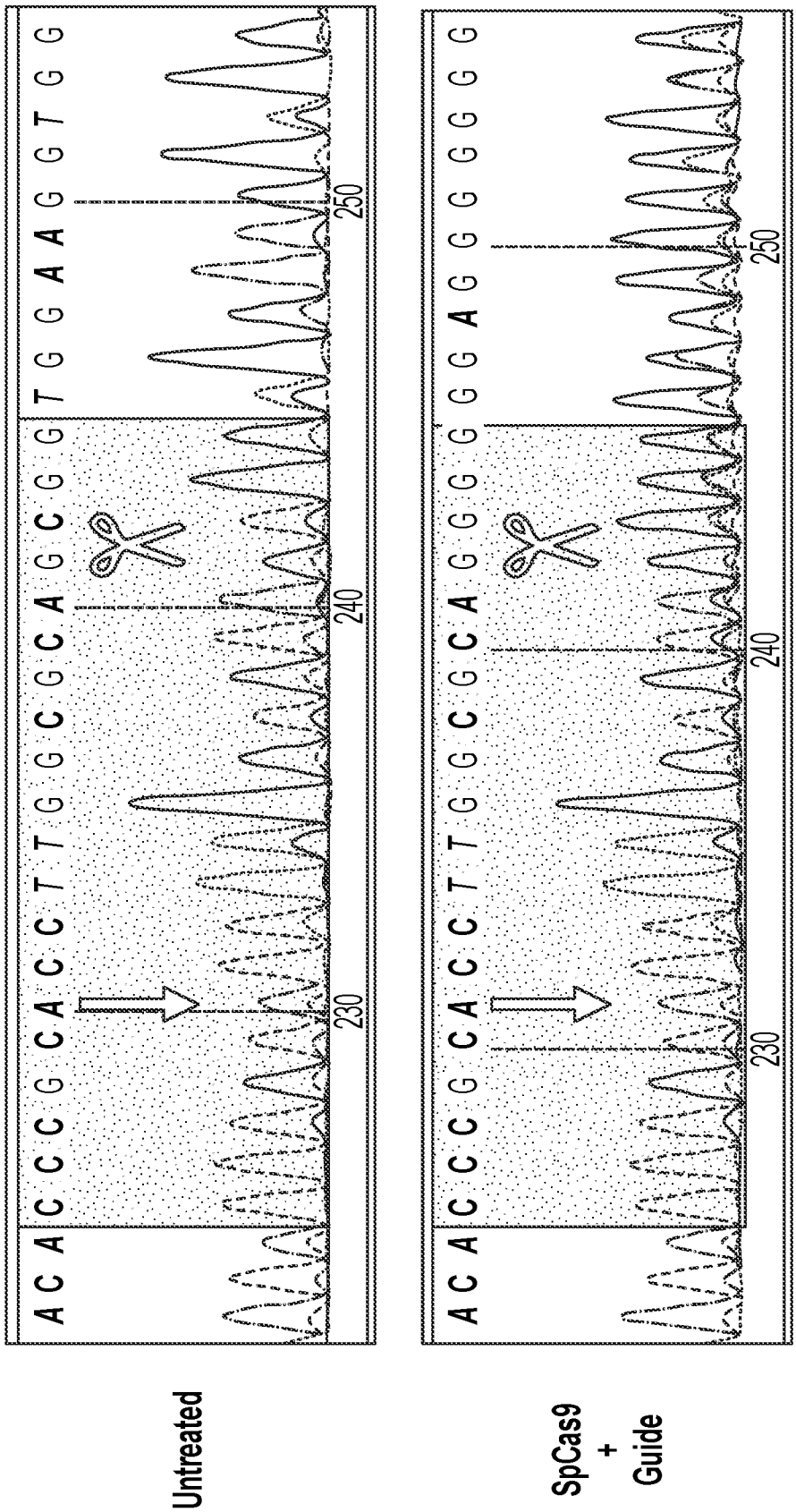

A C A C C C C A C C T T G G C C A G G G T G G
230
240
250
Untreated
A C A C C C C A C C T T G G C C A G G G G G G G
230
240
250
SpCas9
+
Guide

FIG. 11

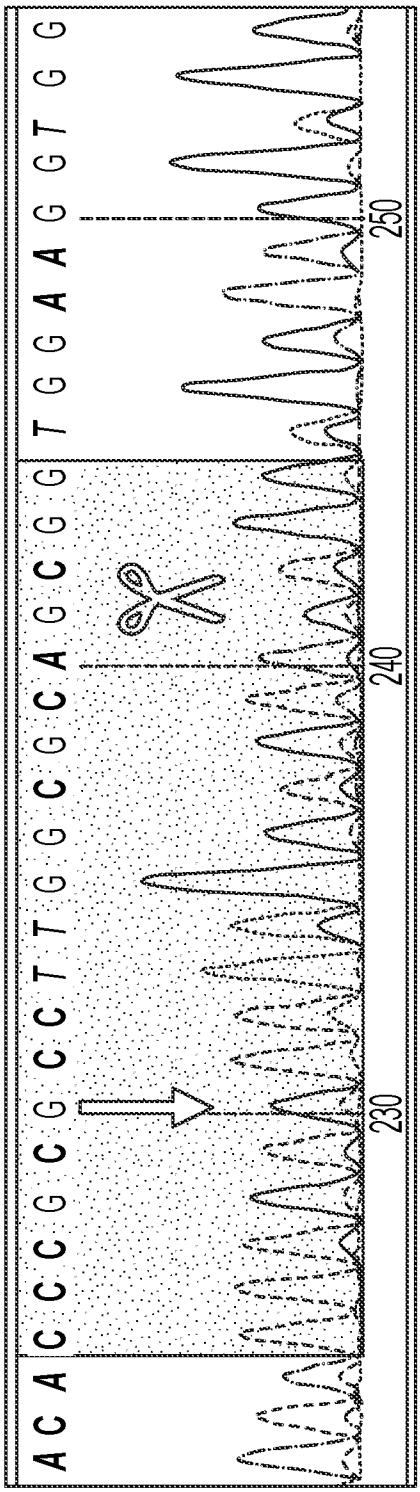

A C A C C C C G C C C C T T G G C G C A G C C G G T G G G A A G G T G G
230
240
250
ABE + Guide

FIG. 11 CONT.

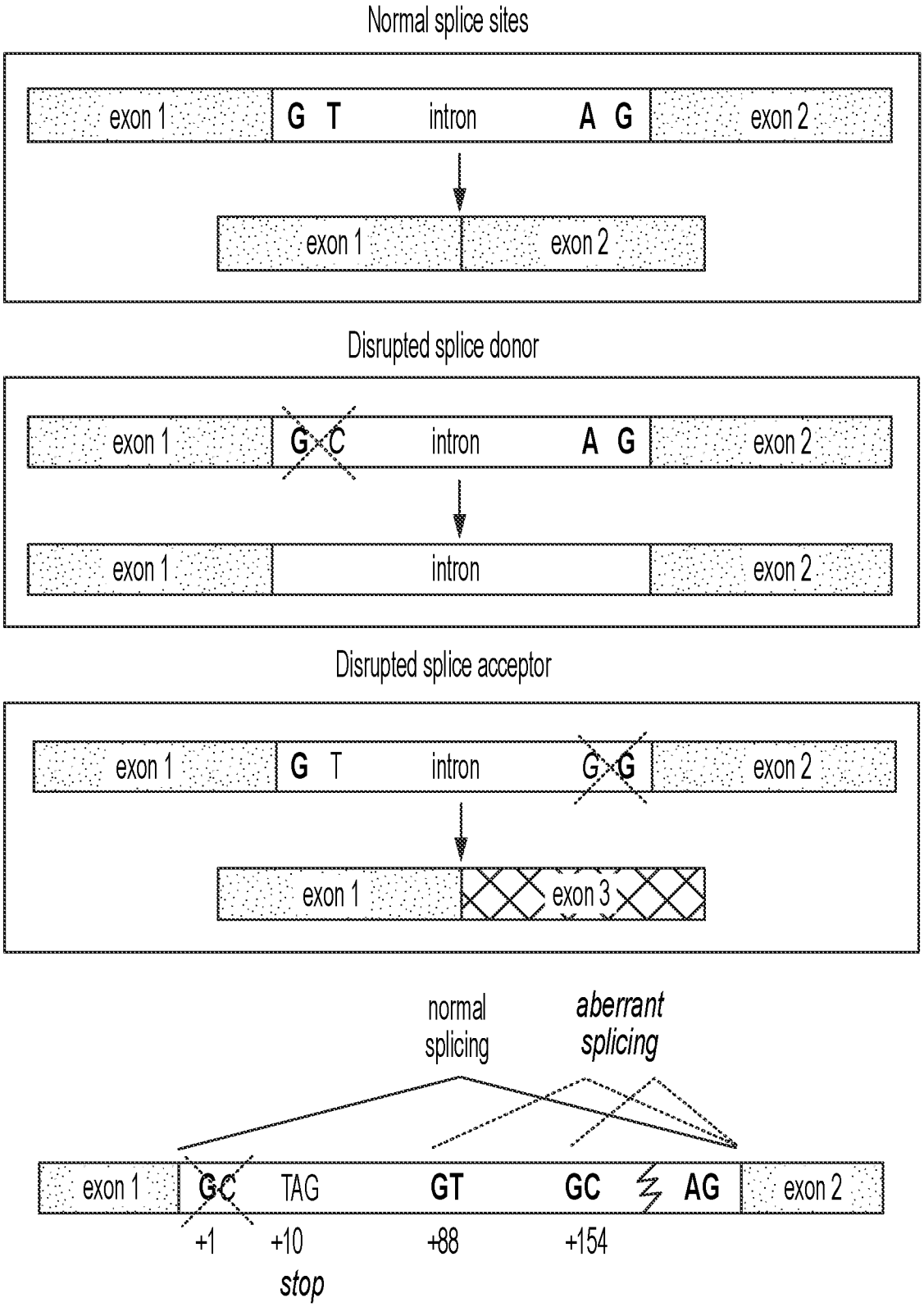

Normal splice sites
exon 1
G T
intron
A G
exon 2
exon 1
exon 2
Disrupted splice donor
exon 1
G C
intron
A G
exon 2
exon 1
intron
exon 2
Disrupted splice acceptor
exon 1
G T
intron
G G
exon 2
exon 1
exon 3
normal splicing
aberrant splicing
exon 1
GC
TAG
GT
GC
AG
exon 2
+1
+10
+88
+154
stop

FIG. 12

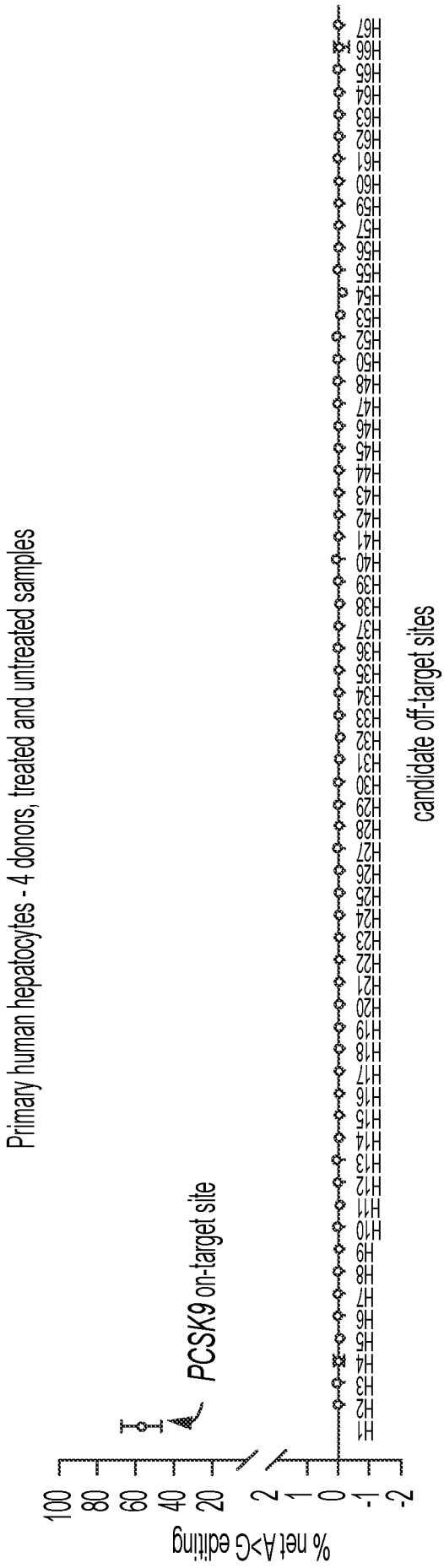

Primary human hepatocytes - 4 donors, treated and untreated samples
% net A>G editing
100
80
60
40
20
2
1
0
-1
-2
PCSK9 on-target site
candidate off-target sites

FIG. 13

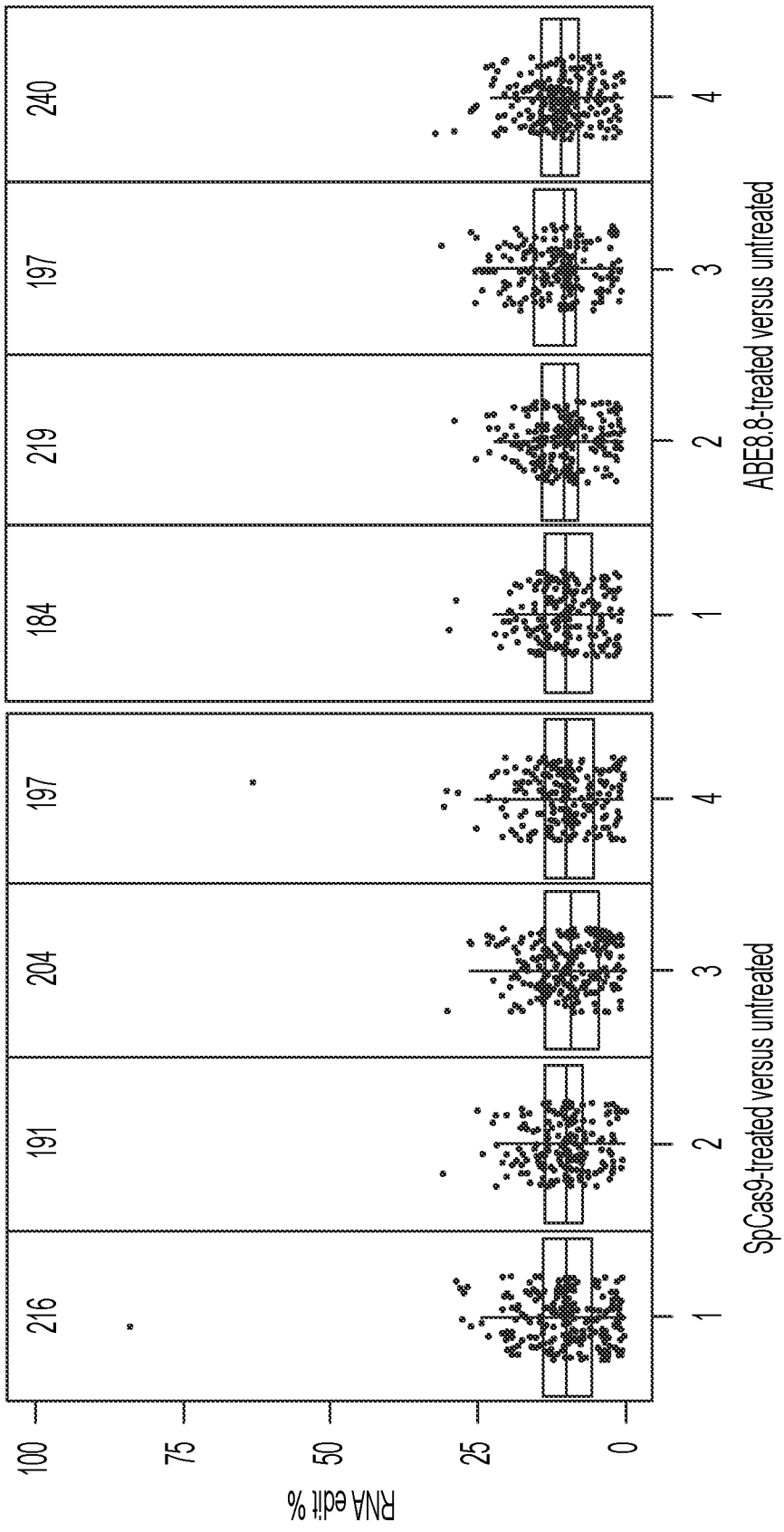

RNA edit %
100
75
50
25
0
216
191
204
197
184
219
197
240
1
2
3
4
1
2
3
4
SpCas9-treated versus untreated
ABE8.8-treated versus untreated

FIG. 14

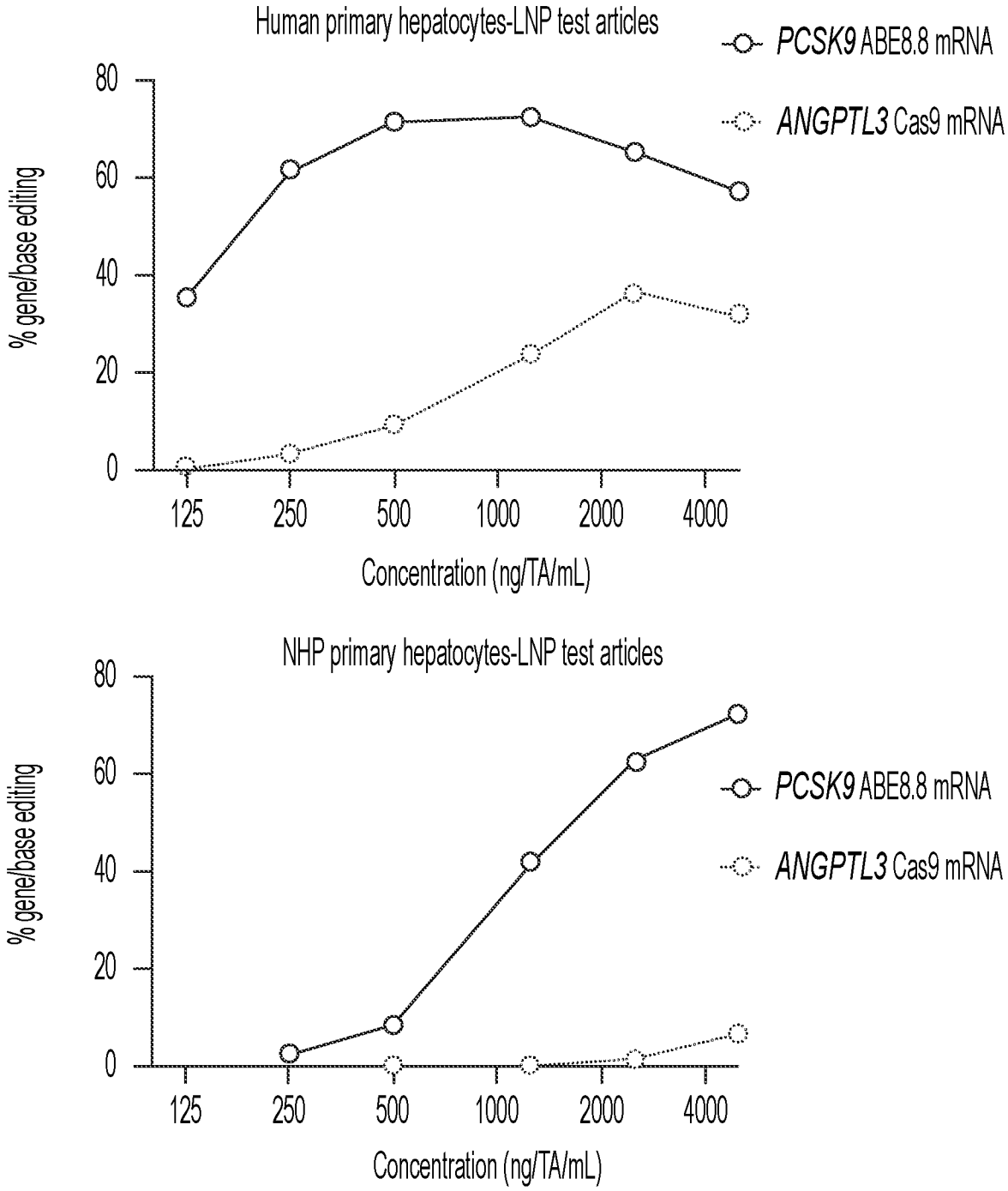

Human primary hepatocytes-LNP test articles
PCSK9 ABE8.8 mRNA
ANGPTL3 Cas9 mRNA
% gene/base editing
0
20
40
60
80
125
250
500
1000
2000
4000
Concentration (ng/TA/mL)
NHP primary hepatocytes-LNP test articles
PCSK9 ABE8.8 mRNA
ANGPTL3 Cas9 mRNA
% gene/base editing
0
20
40
60
80
125
250
500
1000
2000
4000
Concentration (ng/TA/mL)

FIG. 15

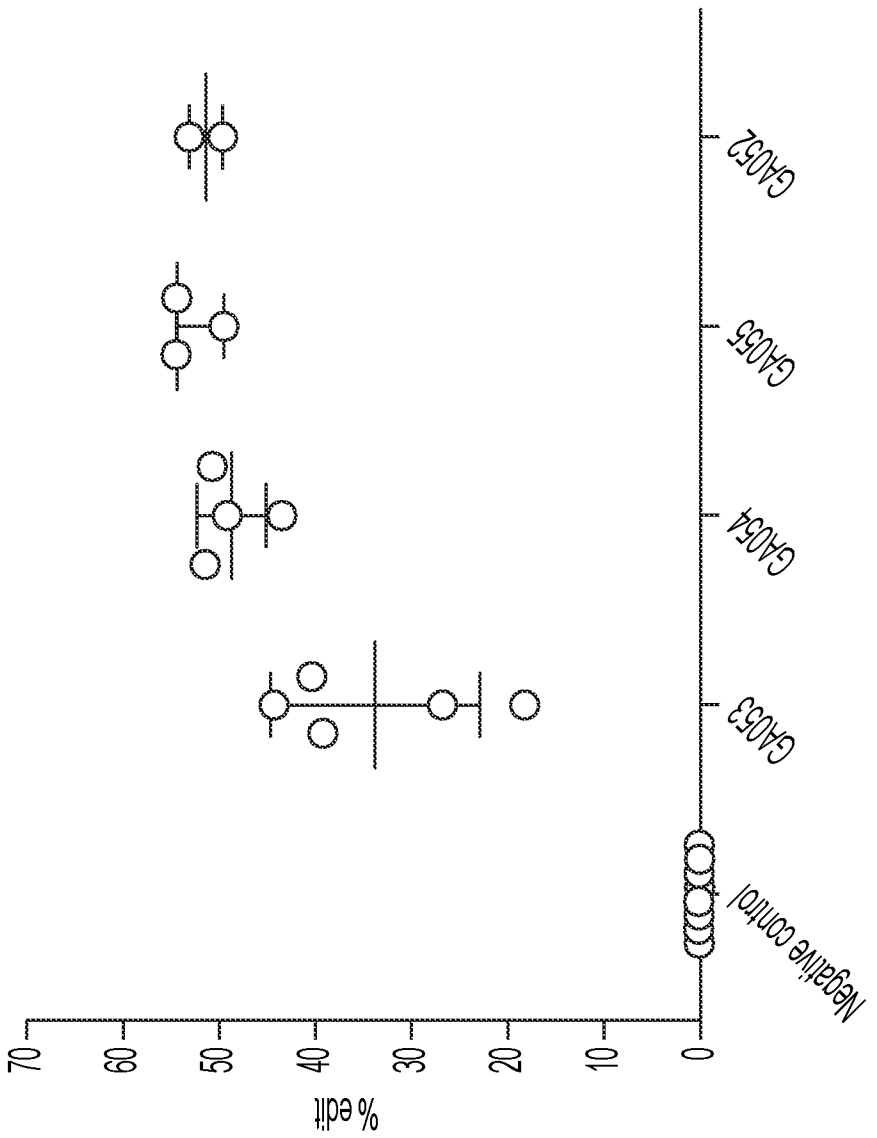

% edit
70
60
50
40
30
20
10
0
Negative control
GA053
GA054
GA055
GA052

FIG. 16

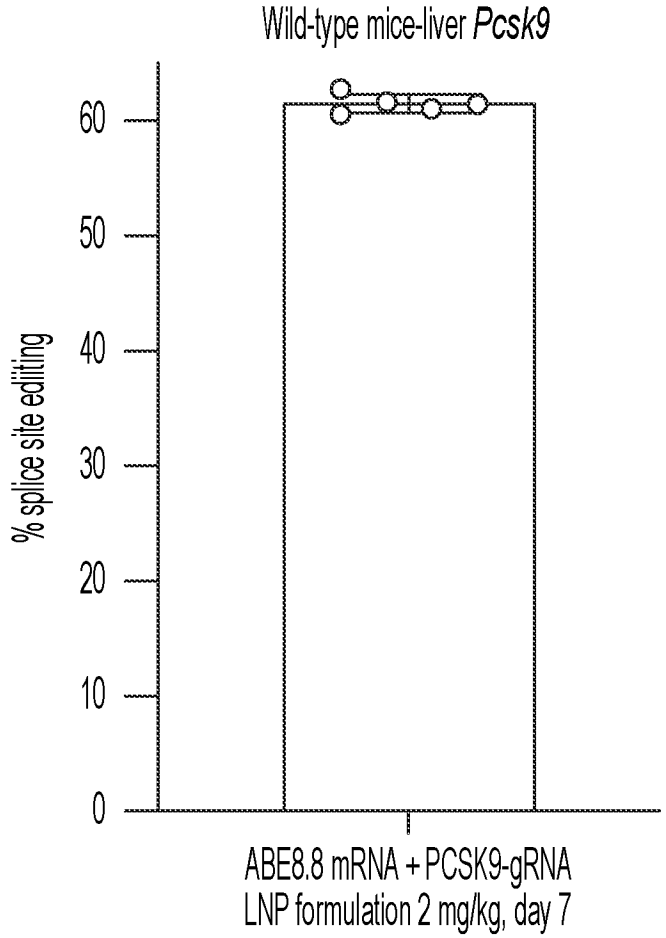

Wild-type mice-liver Pcsk9
% splice site editing
60
50
40
30
20
10
0
ABE8.8 mRNA + PCSK9-gRNA
LNP formulation 2 mg/kg, day 7

FIG. 17

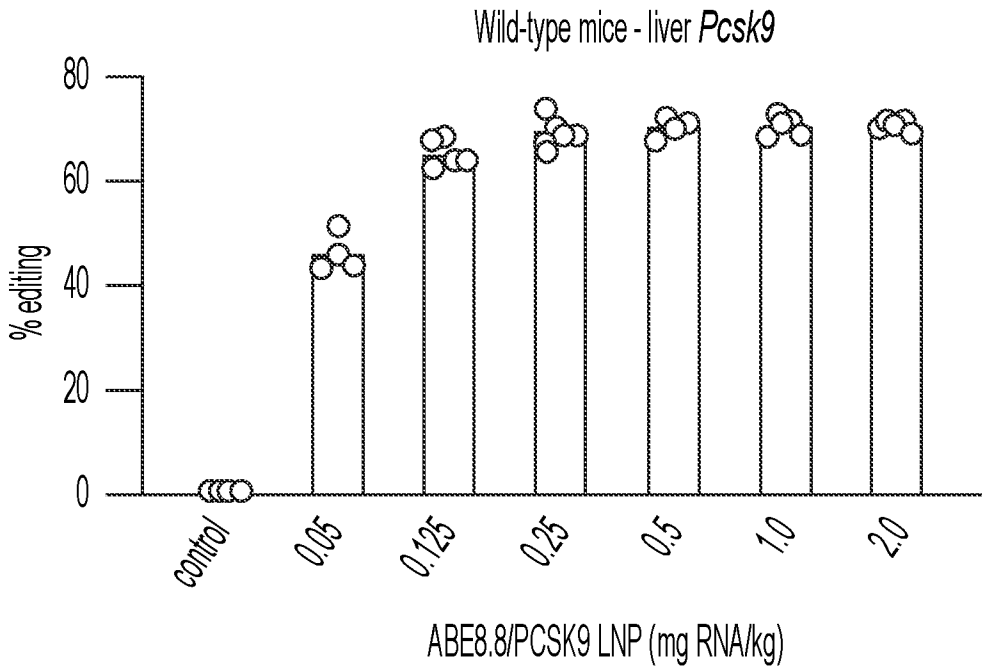

Wild-type mice - liver Pcsk9
% editing
80
60
40
20
0
control
0.05
0.125
0.25
0.5
1.0
2.0
ABE8.8/PCSK9 LNP (mg RNA/kg)

FIG. 18

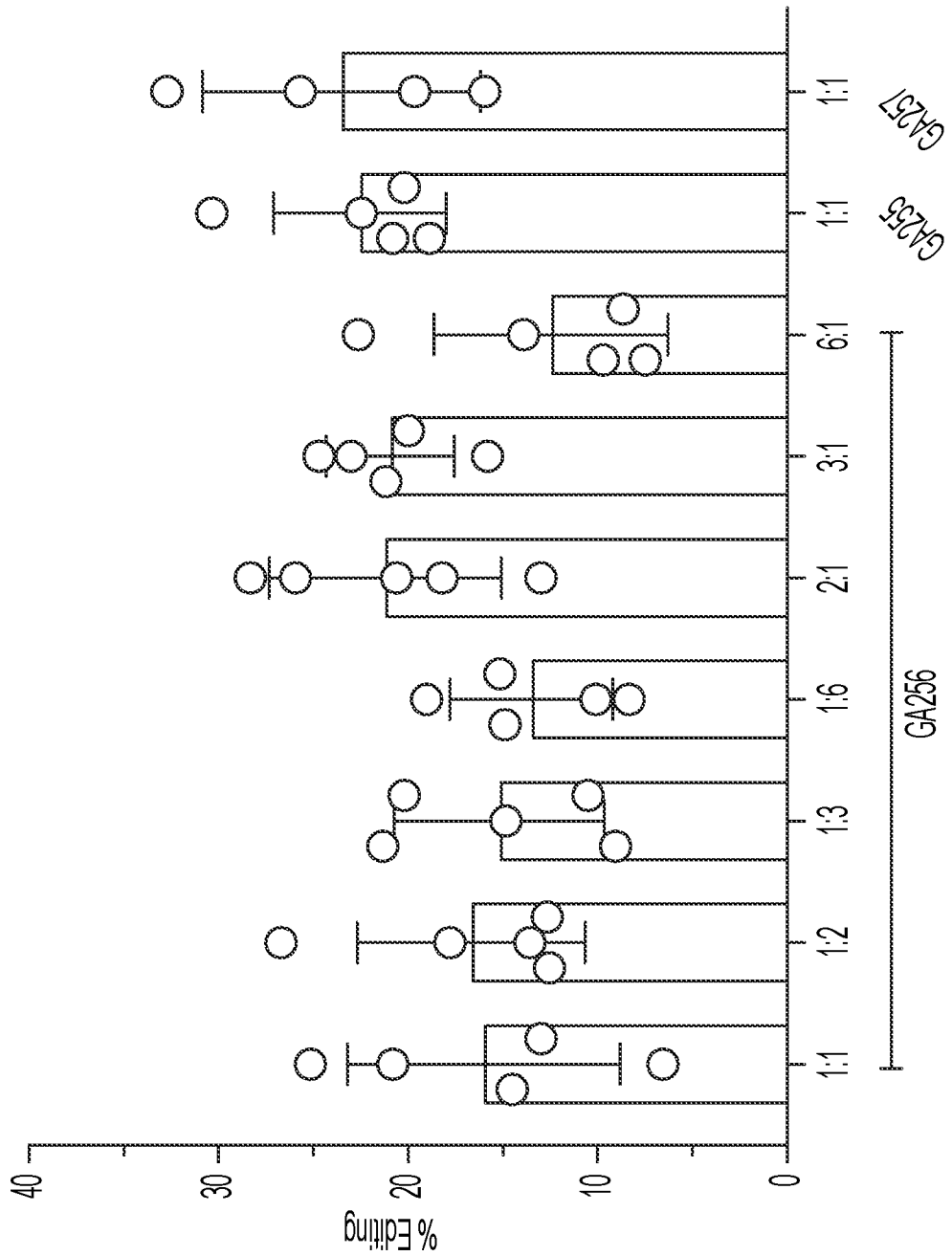

% Editing
0
10
20
30
40
1:1
1:2
1:3
1:6
2:1
3:1
6:1
1:1
1:1
GA256
GA255
GA257
Ratio of gRNA:mRNA

FIG. 19

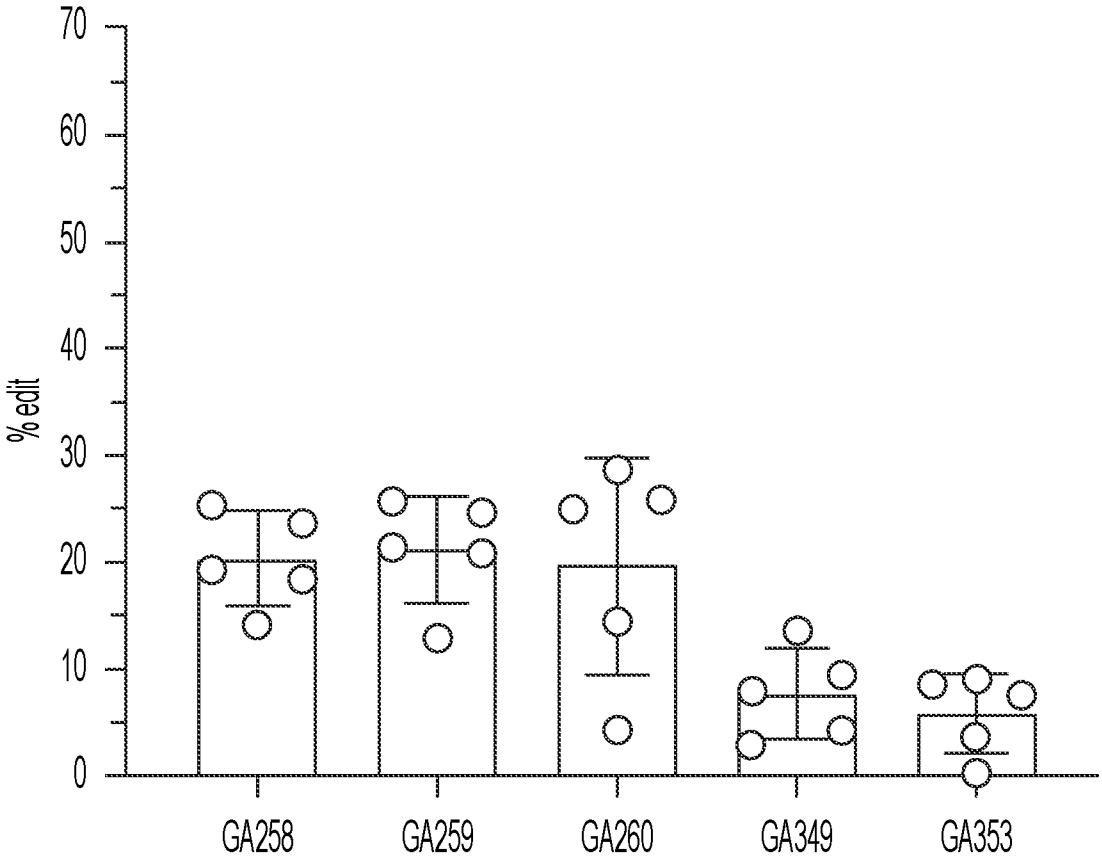


FIG. 20

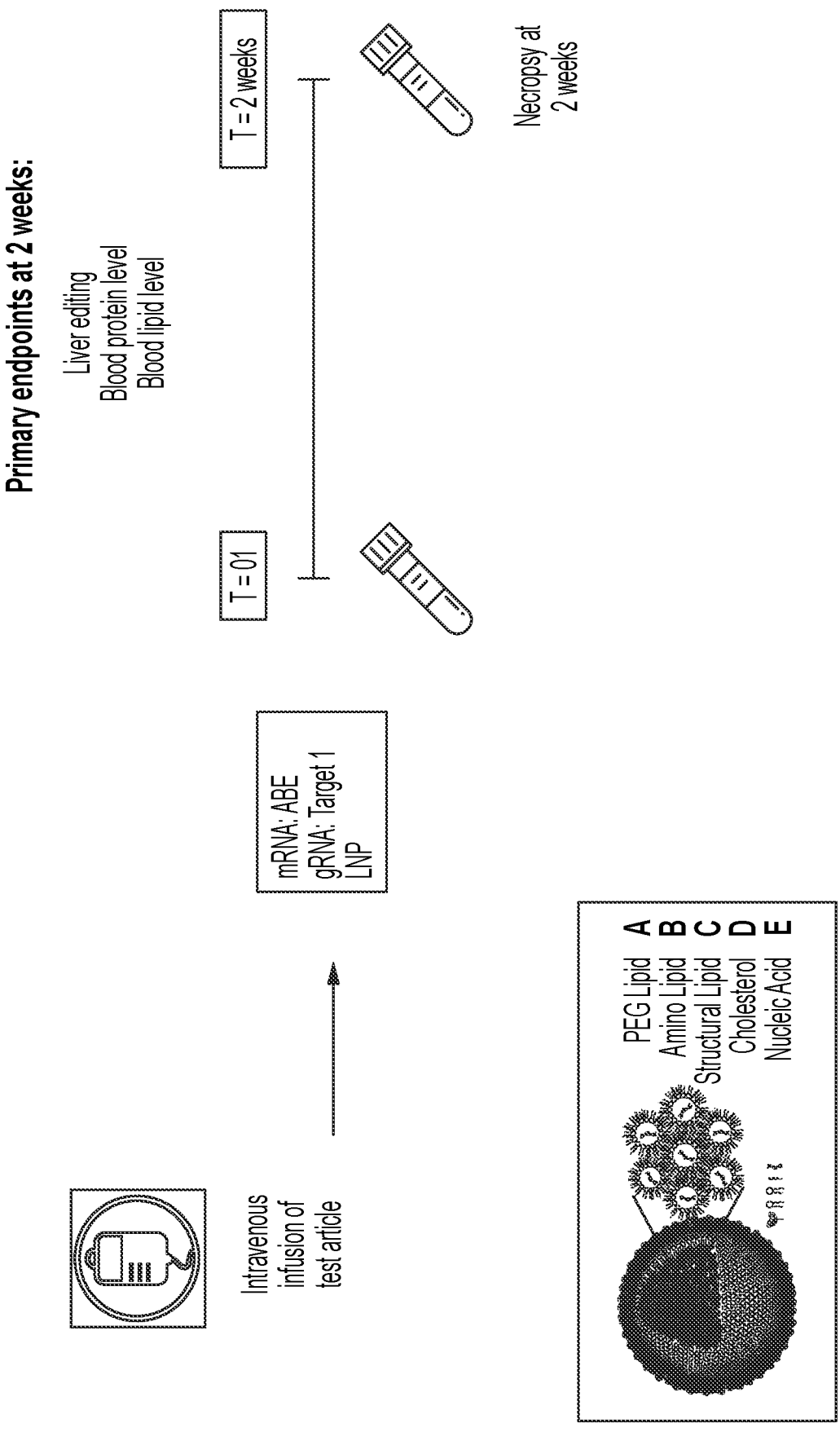

Primary endpoints at 2 weeks:
Liver editing
Blood protein level
Blood lipid level
T = 01
T = 2 weeks
Necropsy at 2 weeks
mRNA: ABE
gRNA: Target 1
LNP
Intravenous infusion of test article
A PEG Lipid
B Amino Lipid
C Structural Lipid
D Cholesterol
E Nucleic Acid

FIG. 21

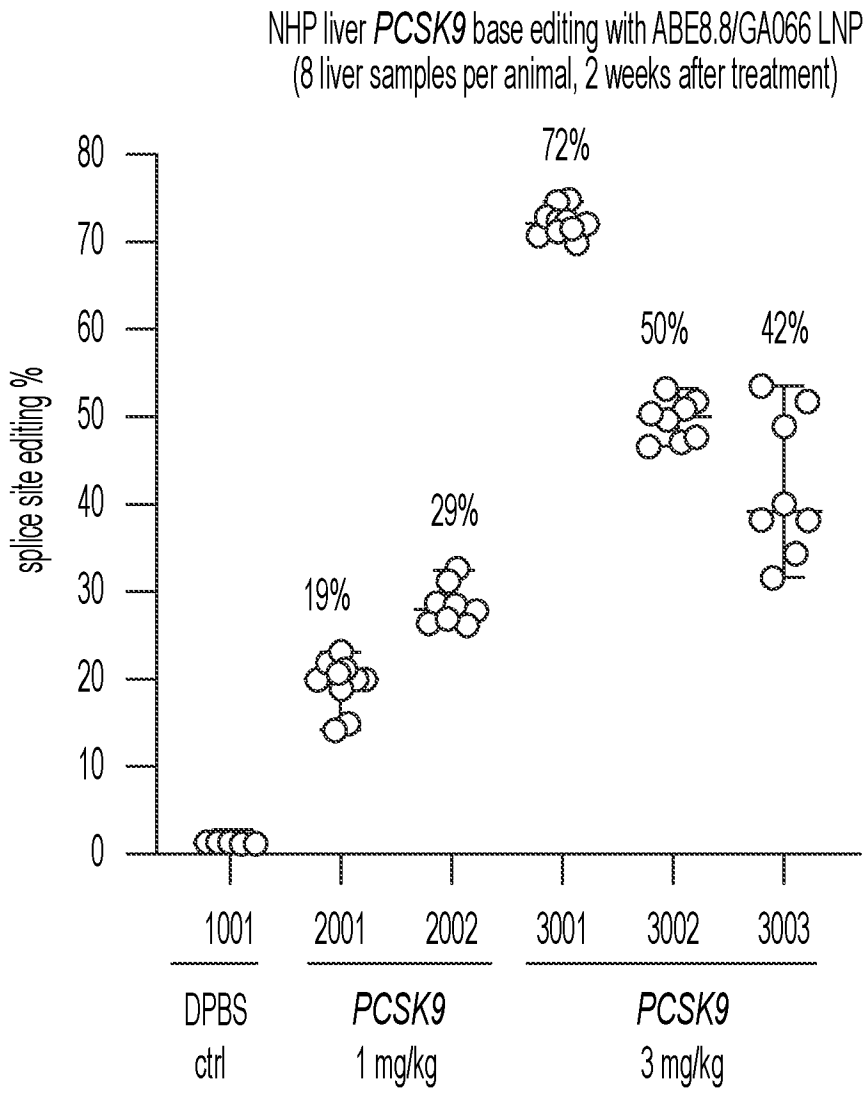

NHP liver *PCSK9* base editing with ABE8.8/GA066 LNP
(8 liver samples per animal, 2 weeks after treatment)
splice site editing %
80
70
60
50
40
30
20
10
0
72%
50%
42%
29%
19%
1001
2001
2002
3001
3002
3003
DPBS
ctrl
PCSK9
1 mg/kg
PCSK9
3 mg/kg

FIG. 22

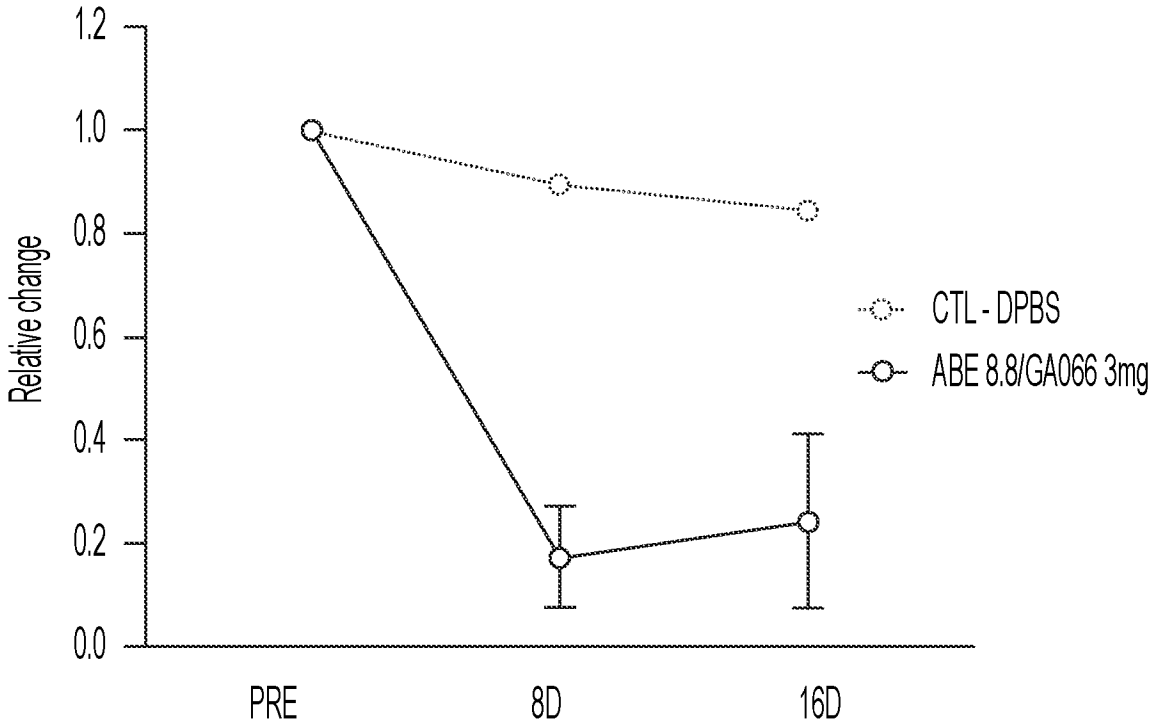

1.2
1.0
0.8
0.6
0.4
0.2
0.0
Relative change
PRE
8D
16D
CTL - DPBS
ABE 8.8/GA066 3mg

FIG. 23

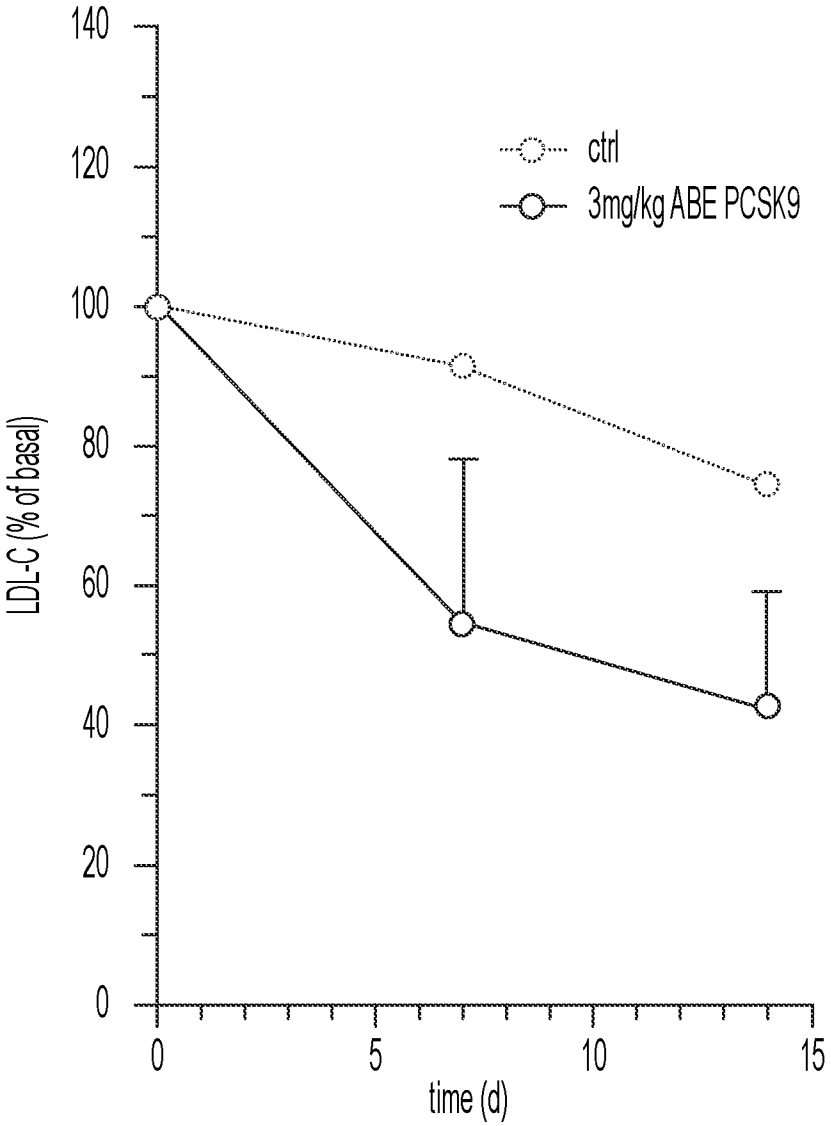

140
120
100
80
60
40
20
0
LDL-C (% of basal)
0
5
10
15
time (d)
ctrl
3mg/kg ABE PCSK9

FIG. 24

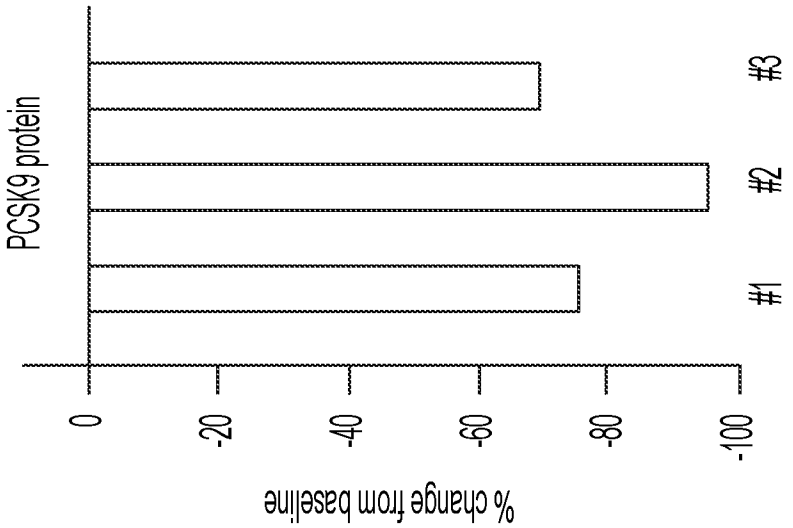


FIG. 25B

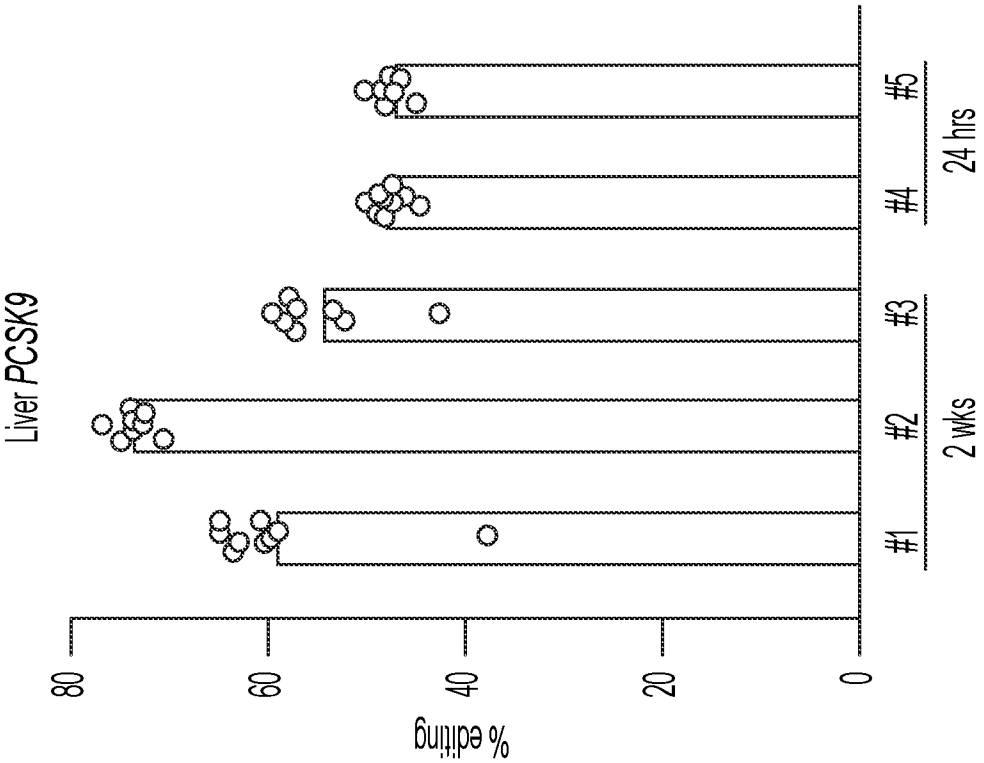


FIG. 25A

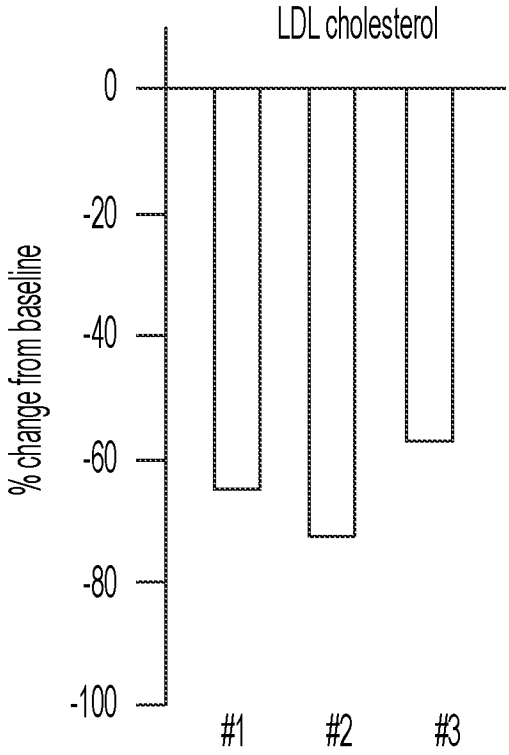


FIG. 25C

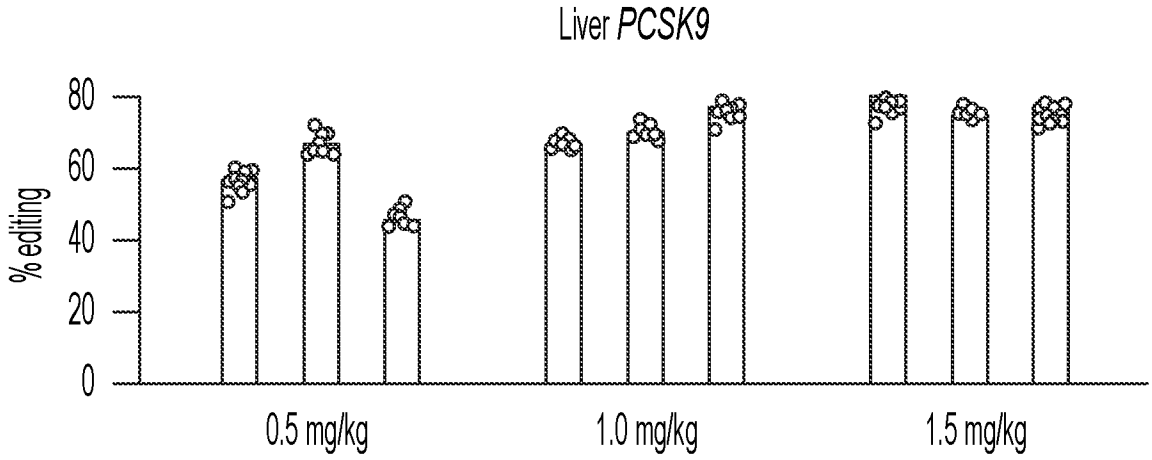


FIG. 26A

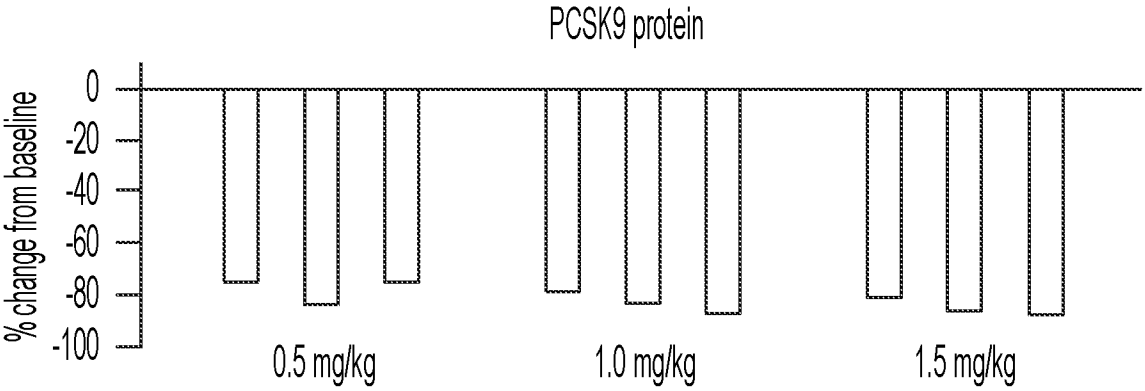


FIG. 26B

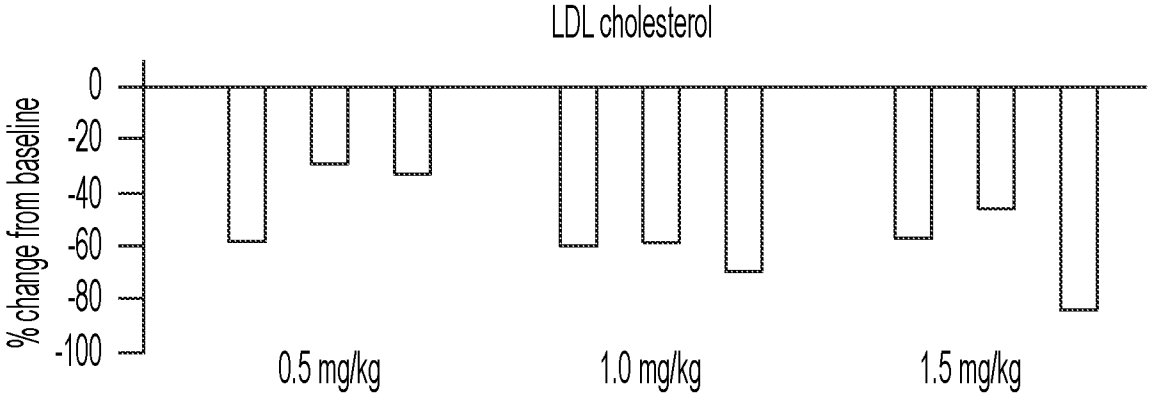


FIG. 26C

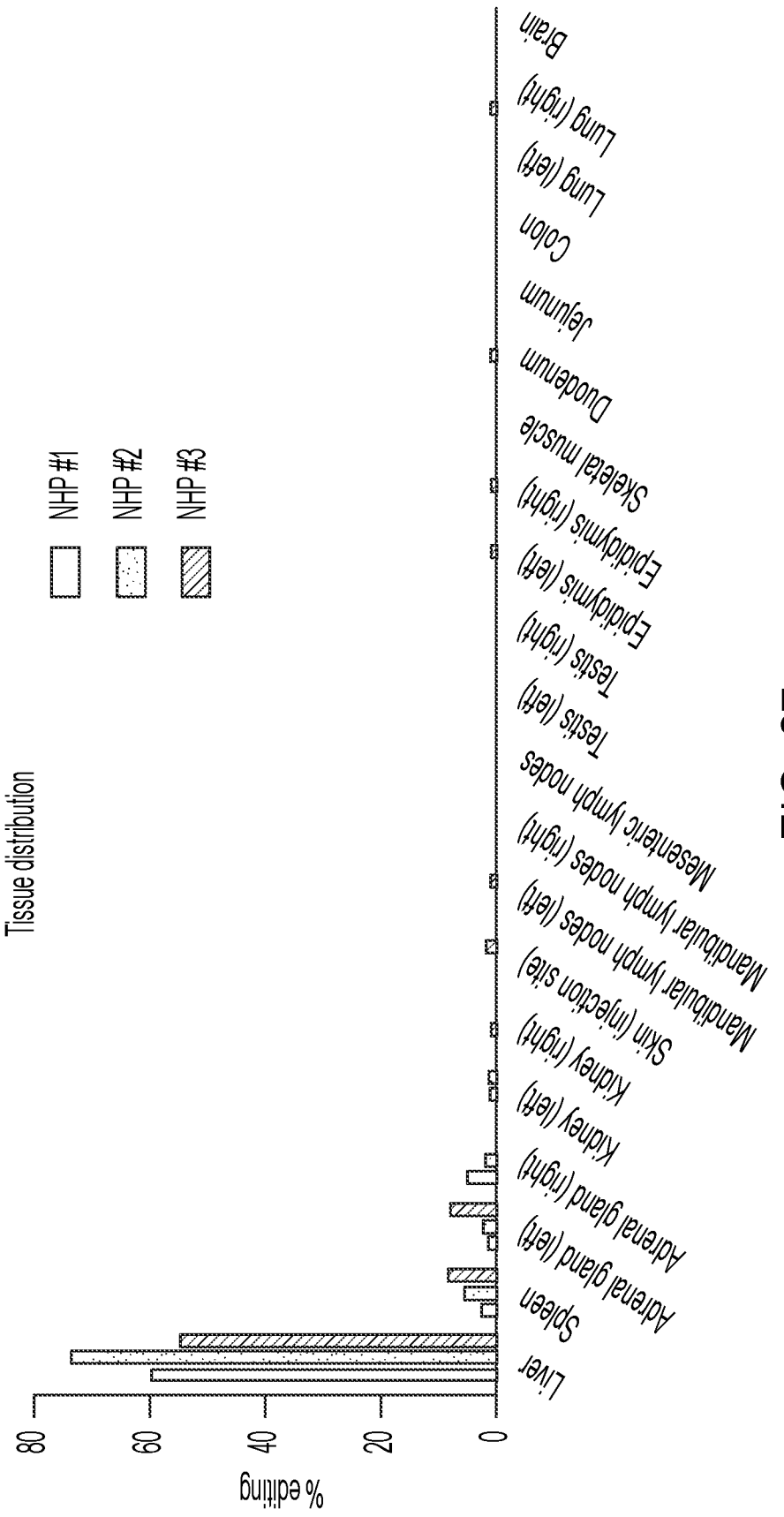

Tissue distribution
NHP #1
NHP #2
NHP #3
% editing
0
20
40
60
80
Liver
Spleen
Adrenal gland (left)
Adrenal gland (right)
Kidney (left)
Kidney (right)
Skin (injection site)
Mandibular lymph nodes (left)
Mandibular lymph nodes (right)
Mesenteric lymph nodes
Testis (left)
Testis (right)
Epididymis (left)
Epididymis (right)
Skeletal muscle
Duodenum
Jejunum
Colon
Lung (left)
Lung (right)
Brain

FIG. 27

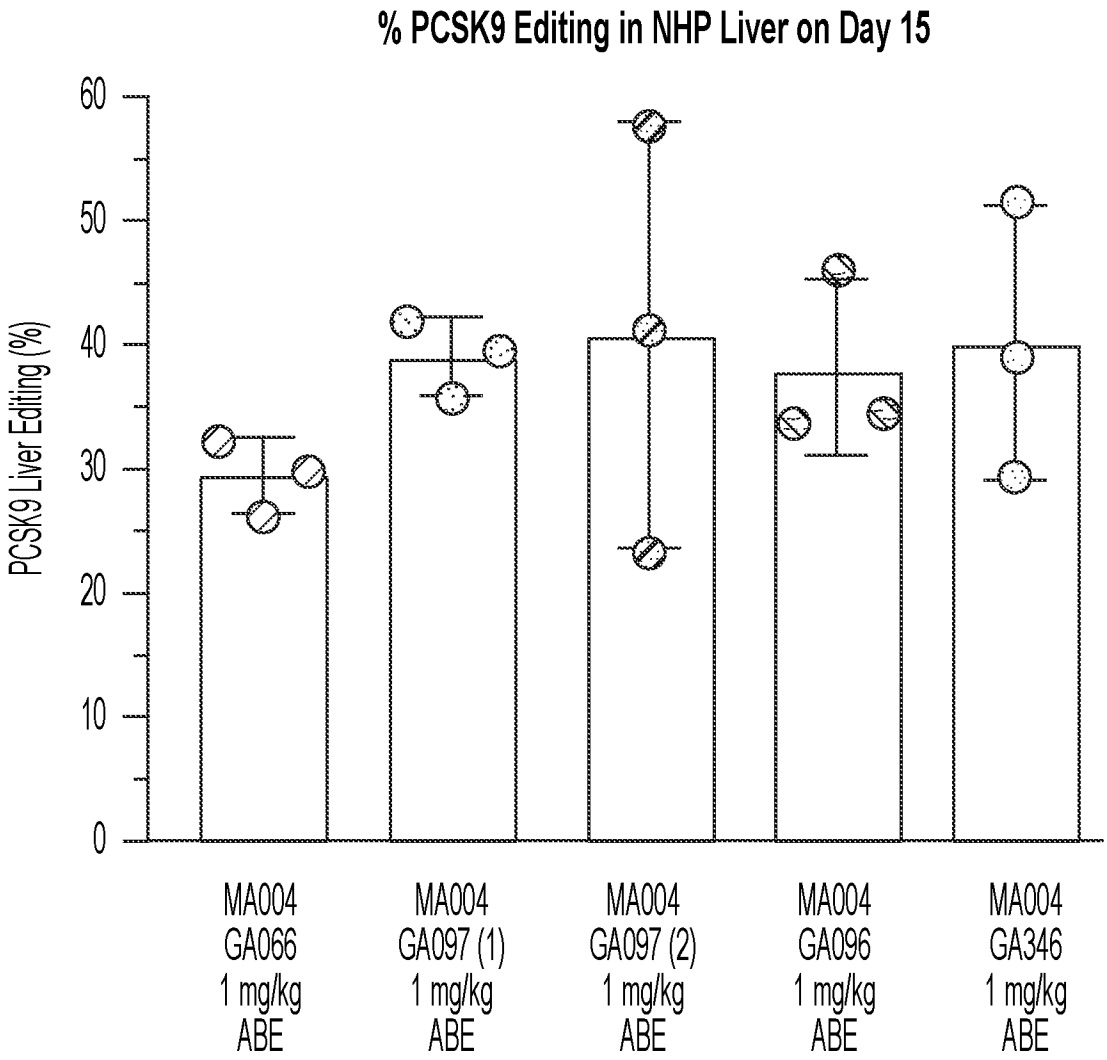

% PCSK9 Editing in NHP Liver on Day 15
PCSK9 Liver Editing (%)
60
50
40
30
20
10
0
MA004 GA066 1 mg/kg ABE
MA004 GA097 (1) 1 mg/kg ABE
MA004 GA097 (2) 1 mg/kg ABE
MA004 GA096 1 mg/kg ABE
MA004 GA346 1 mg/kg ABE

FIG. 28

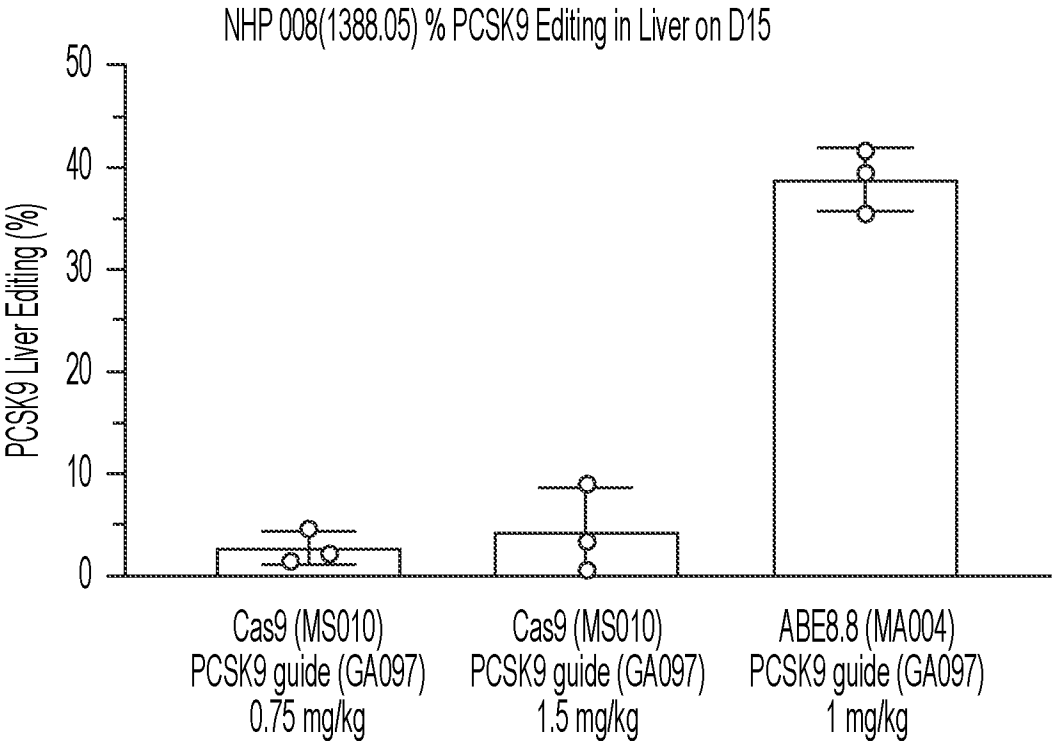

NHP 008(1388.05) % PCSK9 Editing in Liver on D15
PCSK9 Liver Editing (%)
50
40
30
20
10
0
Cas9 (MS010)
PCSK9 guide (GA097)
0.75 mg/kg
Cas9 (MS010)
PCSK9 guide (GA097)
1.5 mg/kg
ABE8.8 (MA004)
PCSK9 guide (GA097)
1 mg/kg

FIG. 29

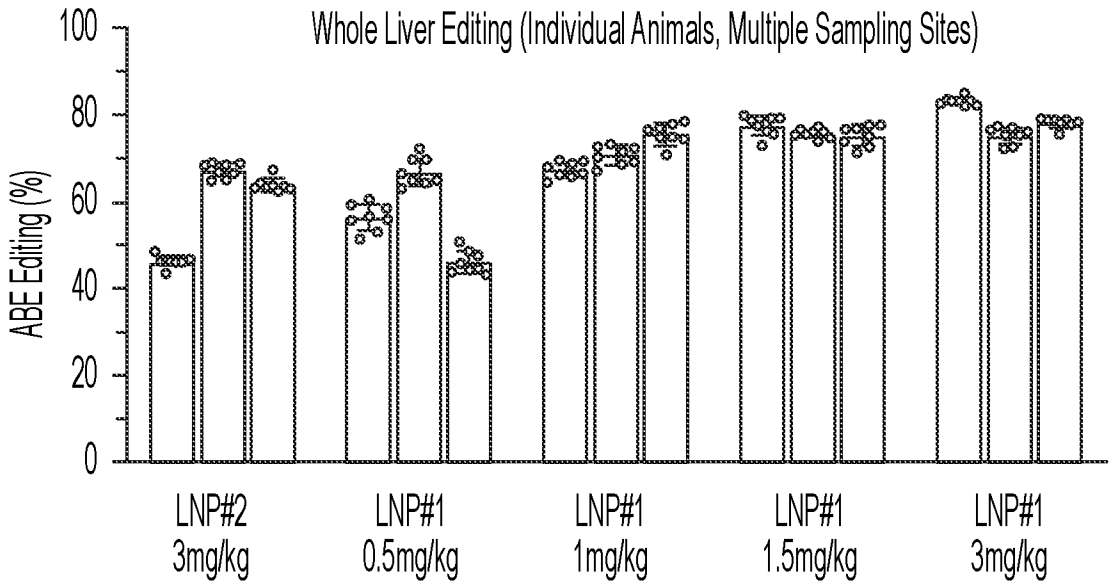

Whole Liver Editing (Individual Animals, Multiple Sampling Sites)
ABE Editing (%)
100
80
60
40
20
0
LNP#2
3mg/kg
LNP#1
0.5mg/kg
LNP#1
1mg/kg
LNP#1
1.5mg/kg
LNP#1
3mg/kg

FIG. 30

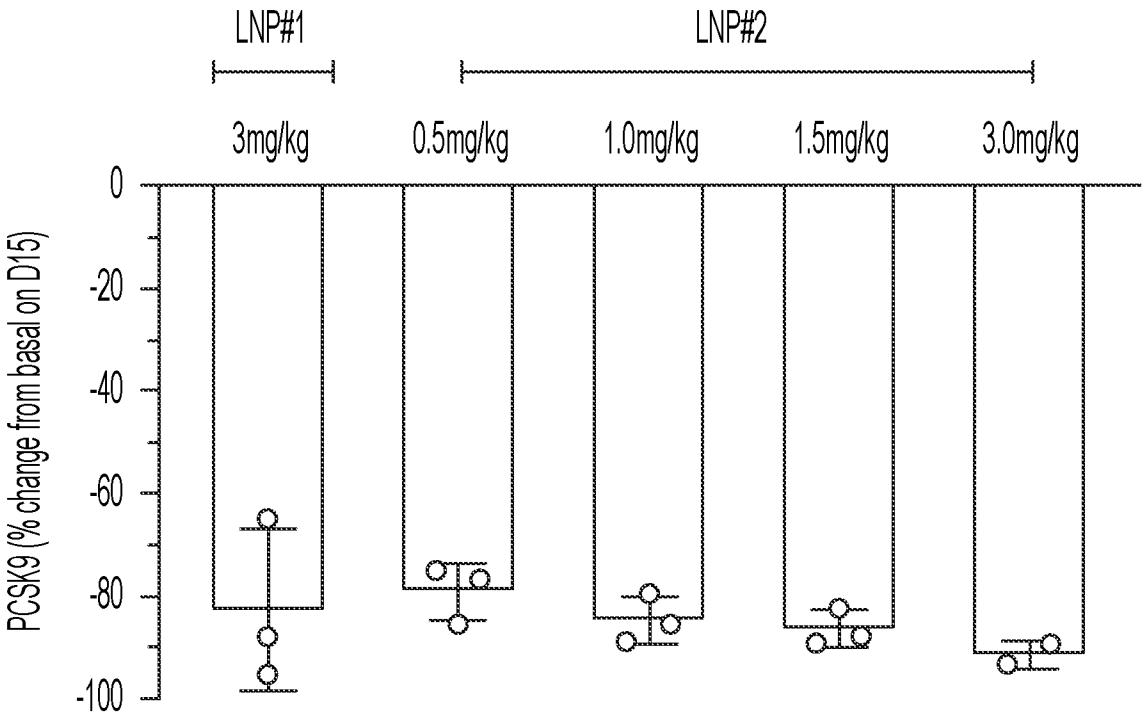

LNP#1
LNP#2
3mg/kg
0.5mg/kg
1.0mg/kg
1.5mg/kg
3.0mg/kg
PCSK9 (% change from basal on D15)
0
-20
-40
-60
-80
-100

FIG. 31

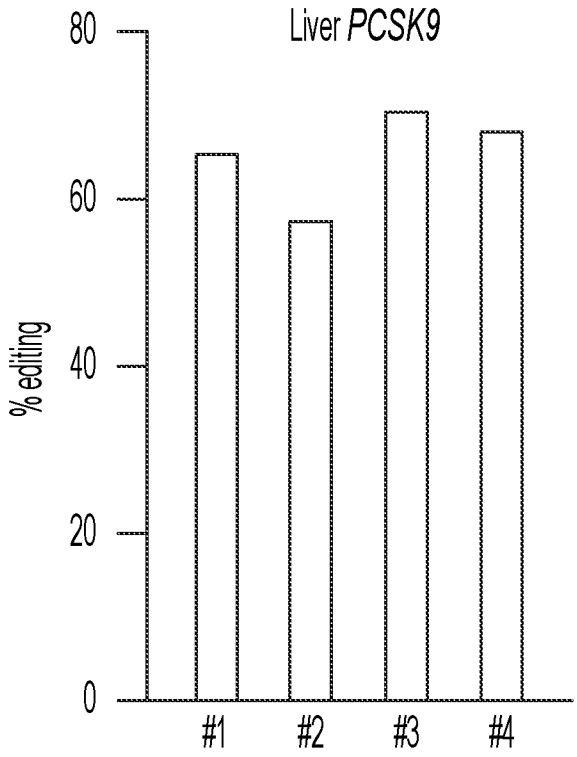

Liver PCSK9
% editing
80
60
40
20
0
#1
#2
#3
#4

FIG. 32

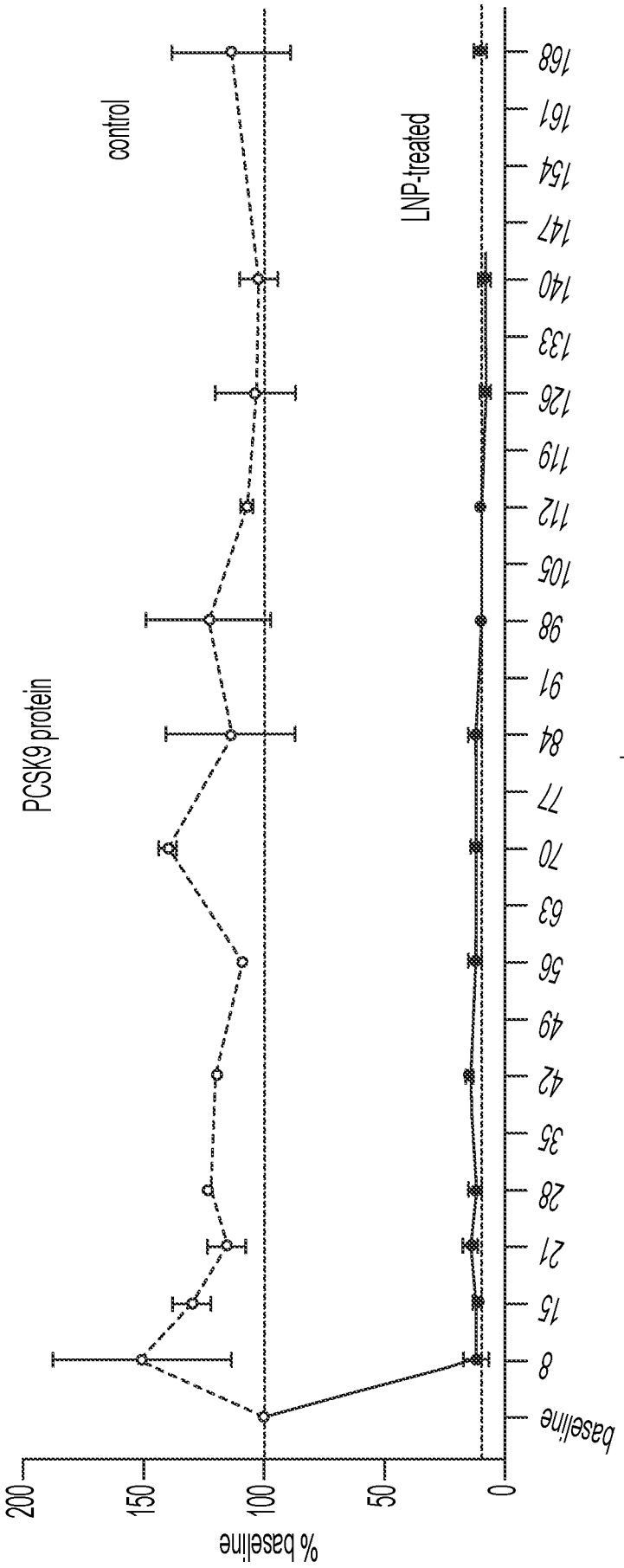

PCSK9 protein
% baseline
200
150
100
50
0
control
LNP-treated
baseline
8
15
21
28
35
42
49
56
63
70
77
84
91
98
105
112
119
126
133
140
147
154
161
168
days

FIG. 33

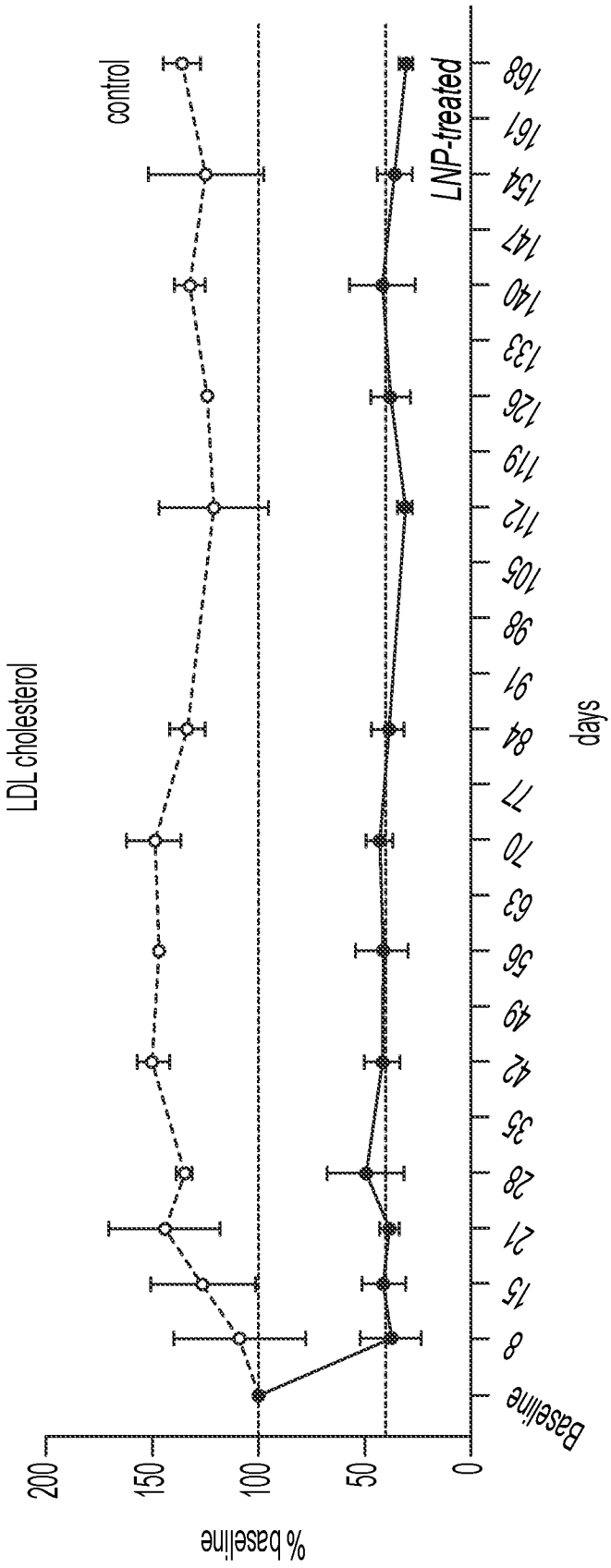

LDL cholesterol
% baseline
200
150
100
50
0
control
LNP-treated
Baseline
8
15
21
28
35
42
49
56
63
70
77
84
91
98
105
112
119
126
133
140
147
154
161
168
days

FIG. 34

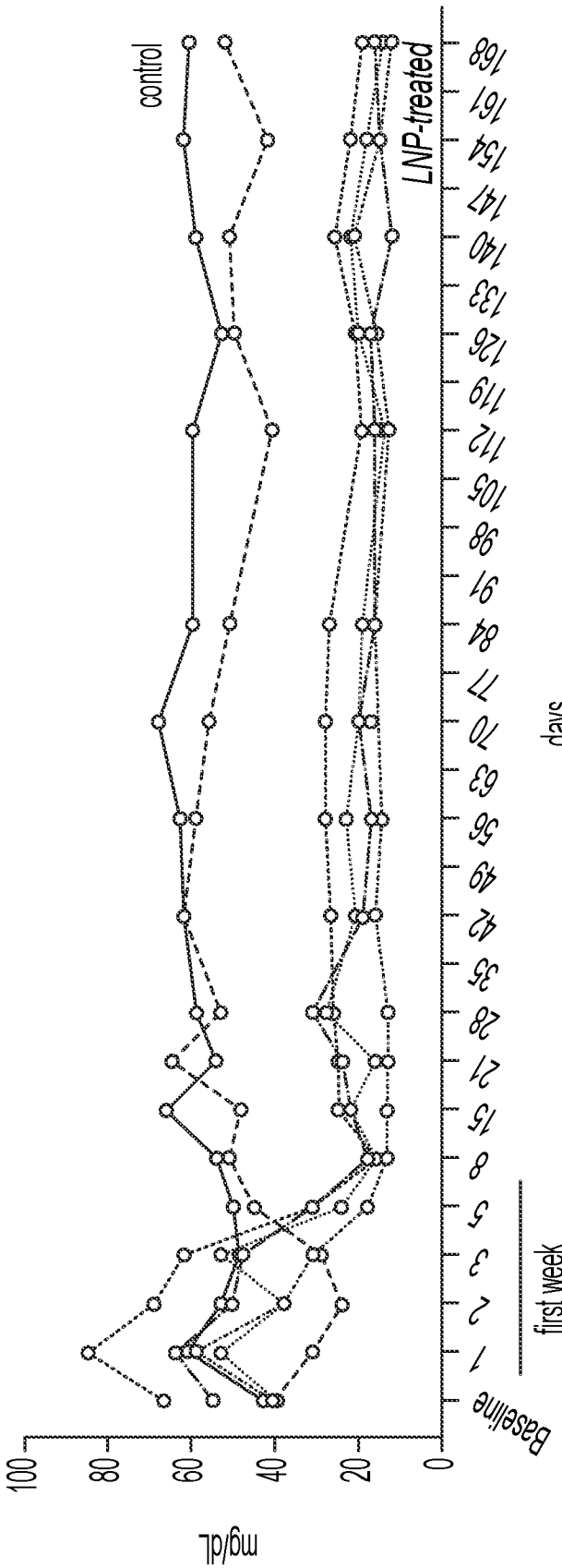


FIG. 34
(Continued)

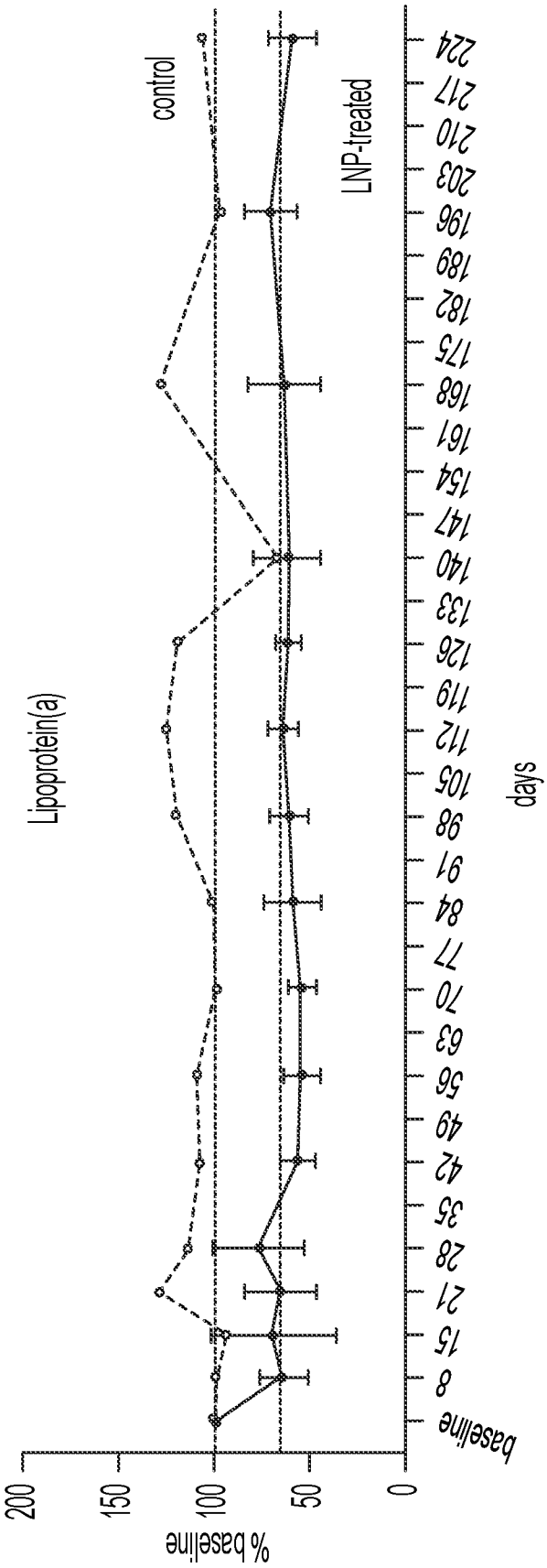

Lipoprotein(a)
% baseline
200
150
100
50
0
control
LNP-treated
baseline
8
15
21
28
35
42
49
56
63
70
77
84
91
98
105
112
119
126
133
140
147
154
161
168
175
182
189
196
203
210
217
224
days

FIG. 35

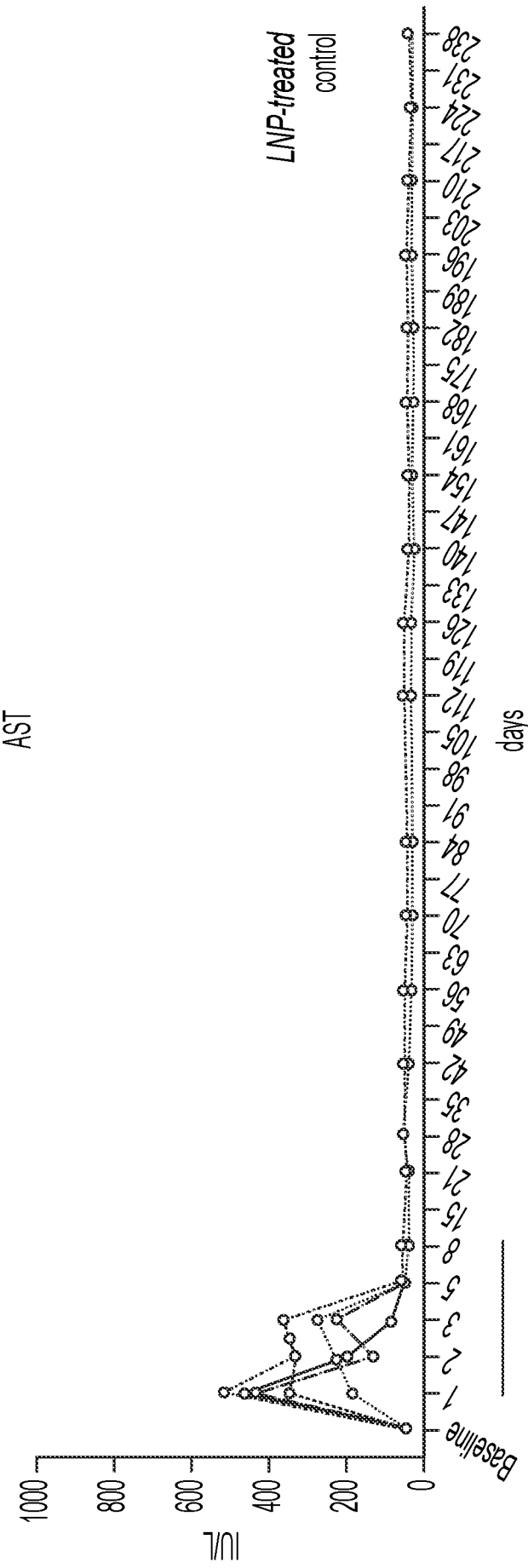

AST
LNP-treated
control
IU/L
1000
800
600
400
200
0
Baseline
1
2
3
5
8
15
21
28
35
42
49
56
63
70
77
84
91
98
105
112
119
126
133
140
147
154
161
168
175
182
189
196
203
210
217
224
231
238
days
first week

FIG. 36

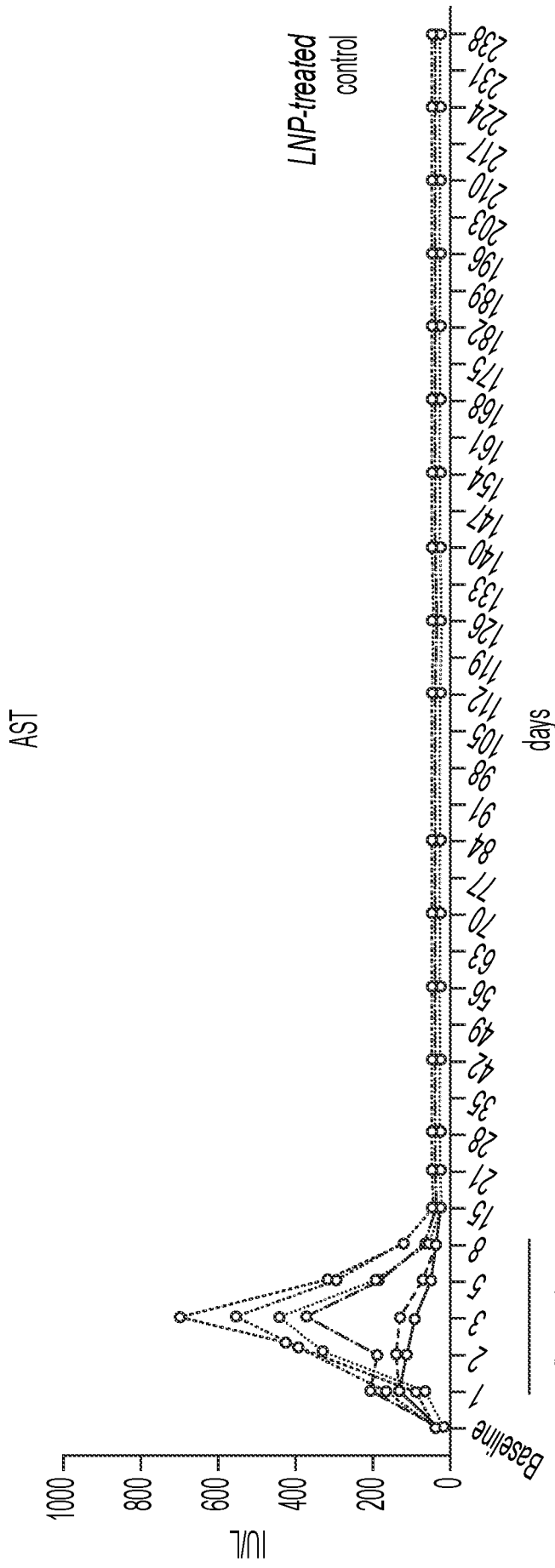


FIG. 36
(Continued)

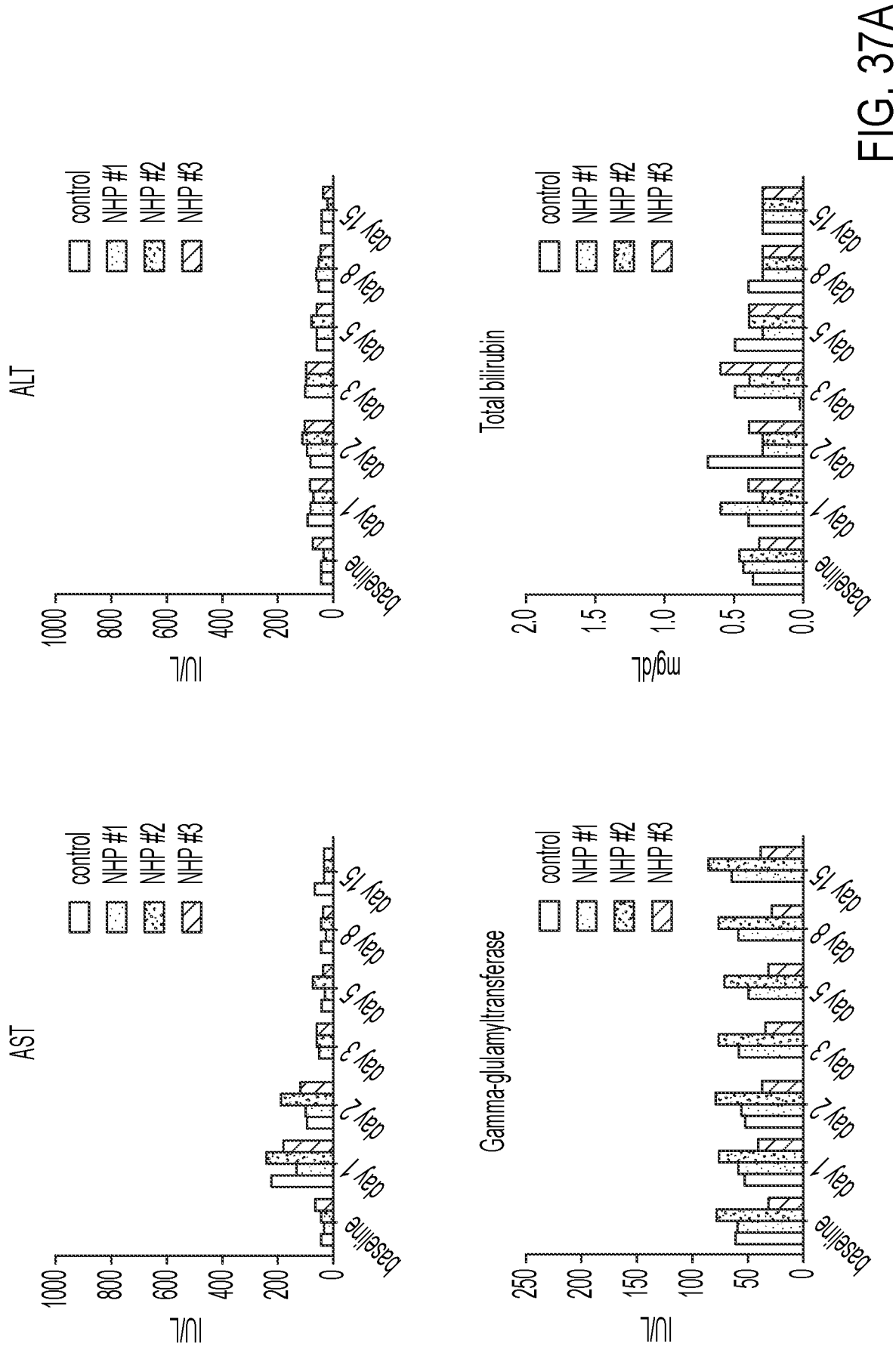

ALT
control
NHP #1
NHP #2
NHP #3
IU/L
1000
800
600
400
200
0
baseline
day 1
day 2
day 3
day 5
day 8
day 15
Total bilirubin
mg/dL
2.0
1.5
1.0
0.5
0.0
AST
Gamma-glulamyltransferase
250
200
150
100
50

FIG. 37A

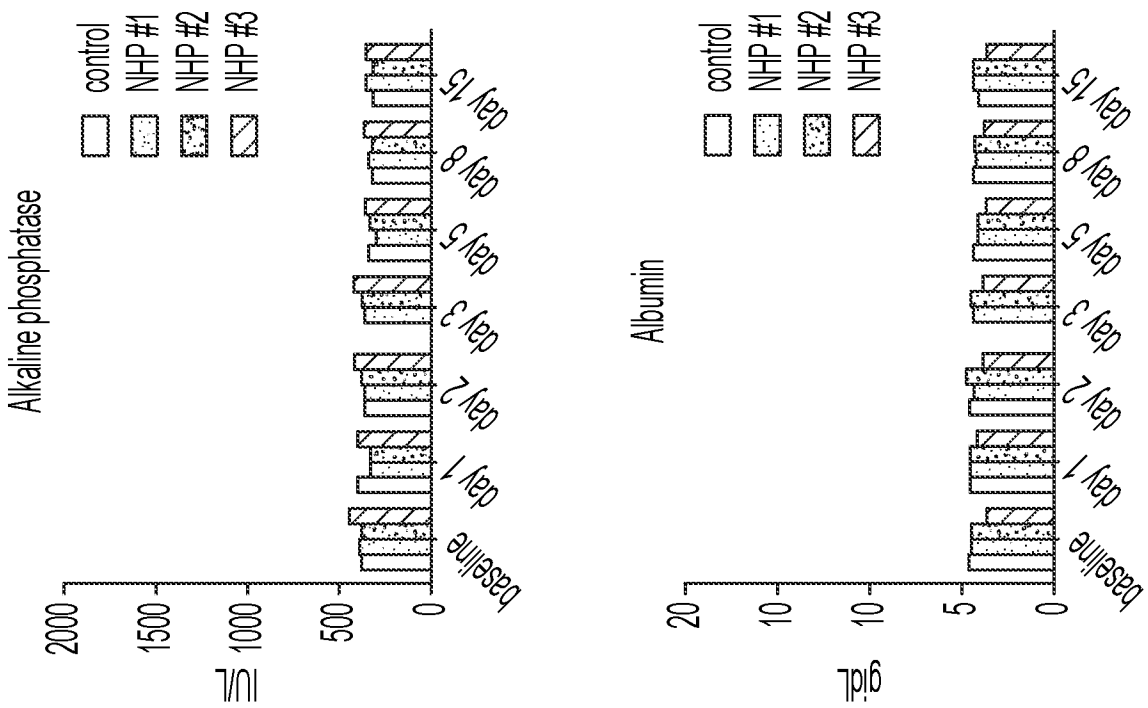


FIG. 37A
(Continued)

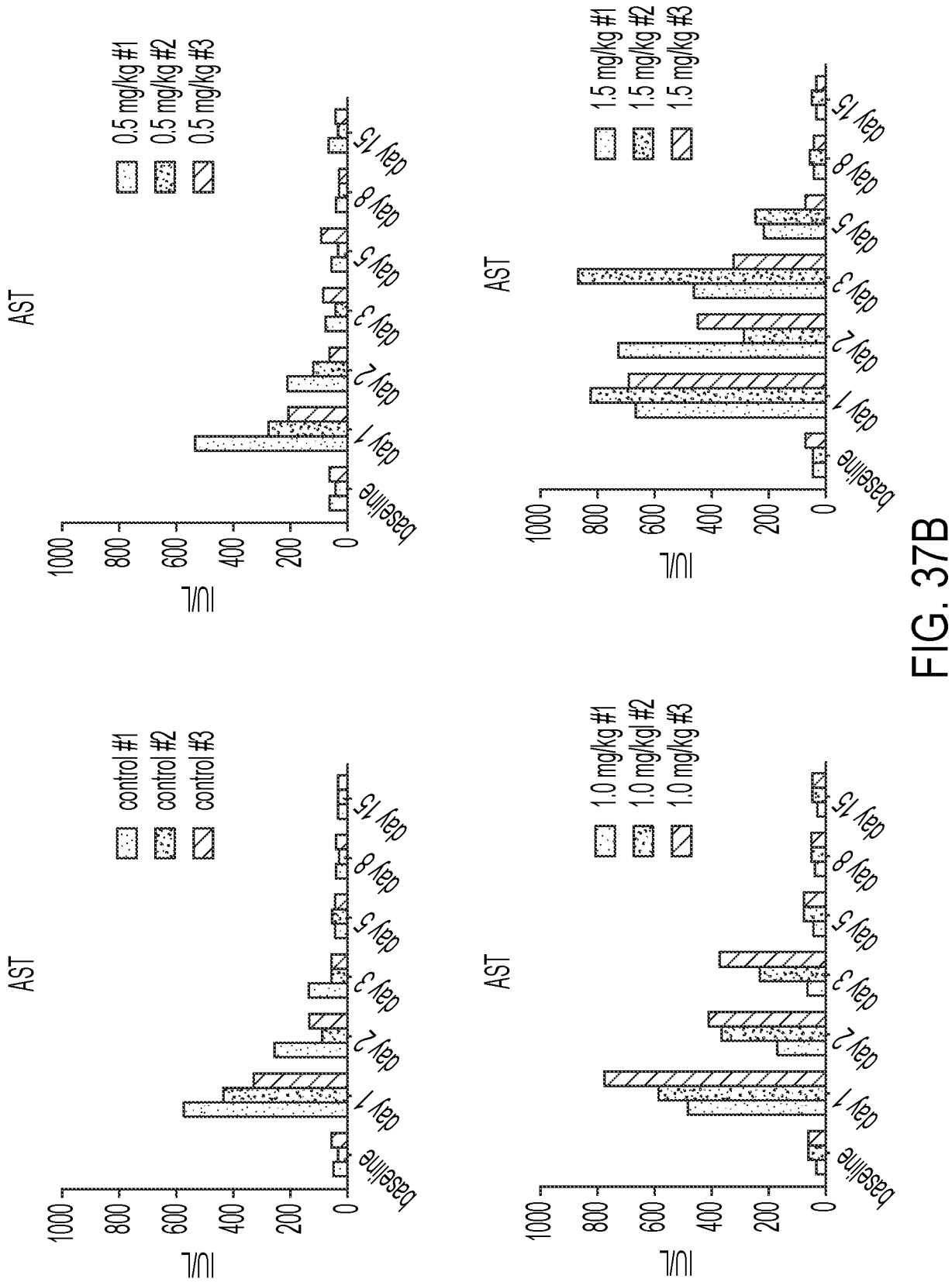

AST
IU/L
1000
800
600
400
200
0
baseline
day 1
day 2
day 3
day 5
day 8
day 15
control #1
control #2
control #3
0.5 mg/kg #1
0.5 mg/kg #2
0.5 mg/kg #3
1.0 mg/kg #1
1.0 mg/kg #2
1.0 mg/kg #3
1.5 mg/kg #1
1.5 mg/kg #2
1.5 mg/kg #3

FIG. 37B

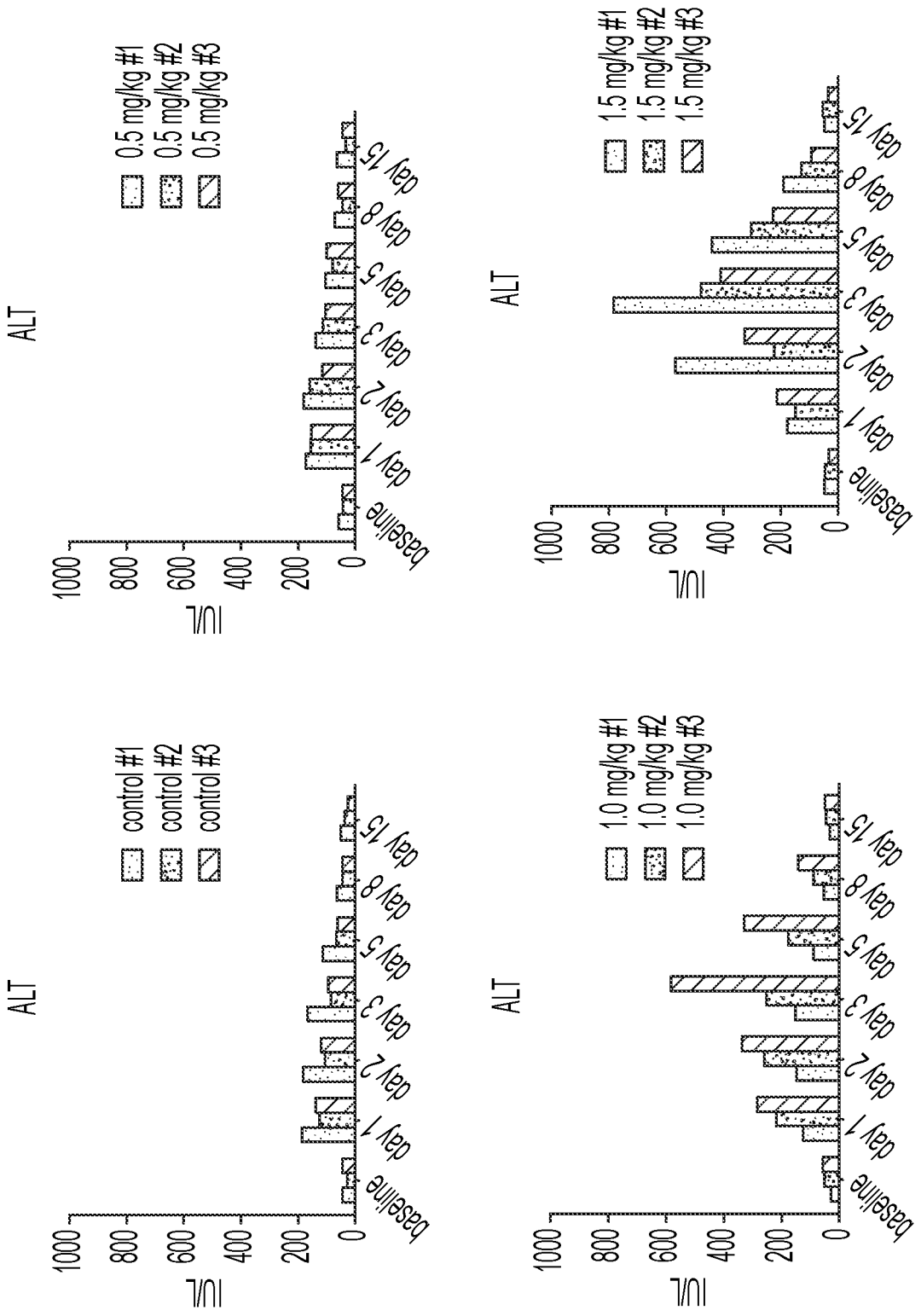

ALT
IU/L
1000
800
600
400
200
0
baseline
day 1
day 2
day 3
day 5
day 8
day 15
control #1
control #2
control #3
0.5 mg/kg #1
0.5 mg/kg #2
0.5 mg/kg #3
1.0 mg/kg #1
1.0 mg/kg #2
1.0 mg/kg #3
1.5 mg/kg #1
1.5 mg/kg #2
1.5 mg/kg #3

FIG. 37C

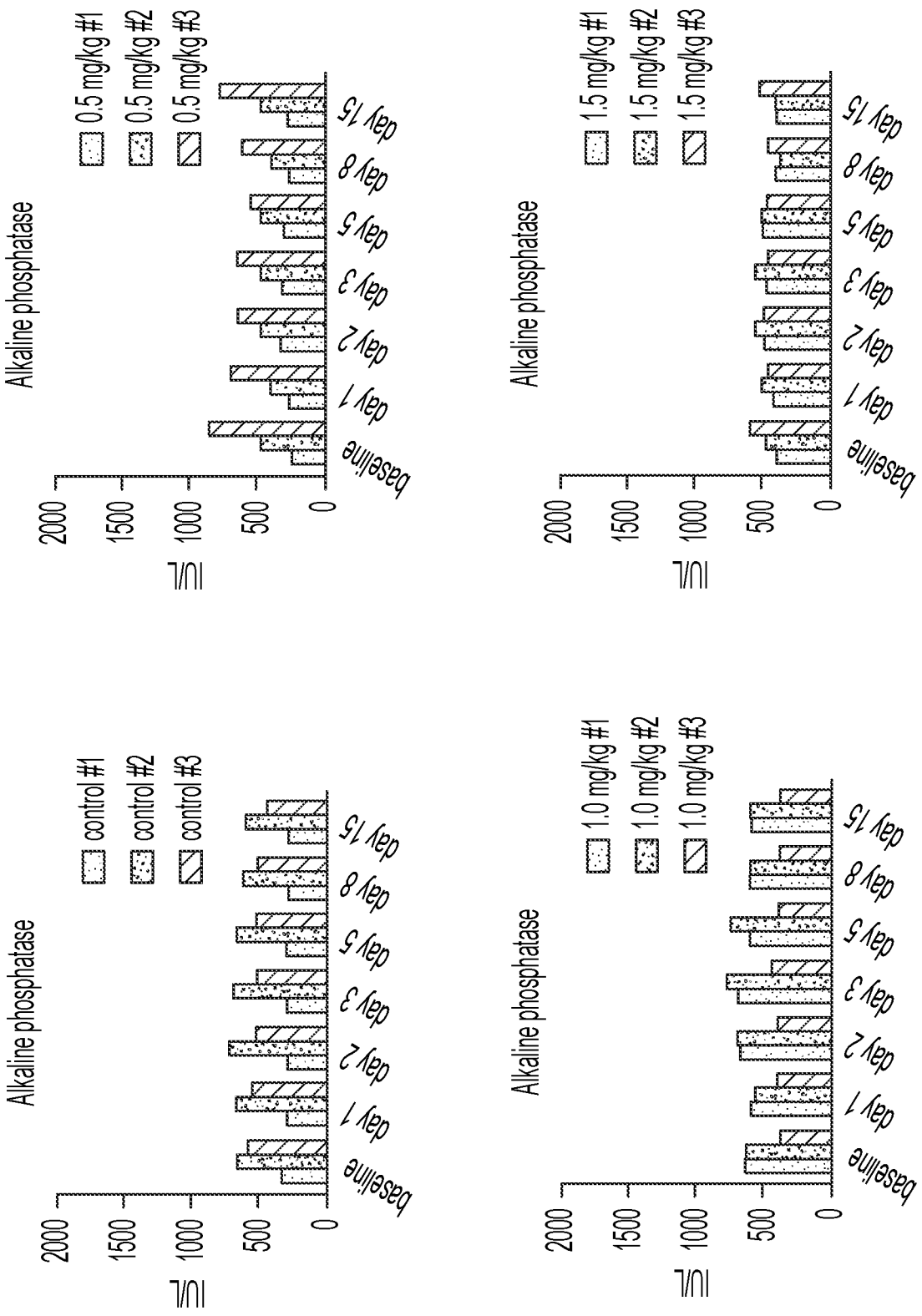

Alkaline phosphatase
IU/L
2000
1500
1000
500
0
baseline
day 1
day 2
day 3
day 5
day 8
day 15
control #1
control #2
control #3
0.5 mg/kg #1
0.5 mg/kg #2
0.5 mg/kg #3
1.5 mg/kg #1
1.5 mg/kg #2
1.5 mg/kg #3
1.0 mg/kg #1
1.0 mg/kg #2
1.0 mg/kg #3

FIG. 37D

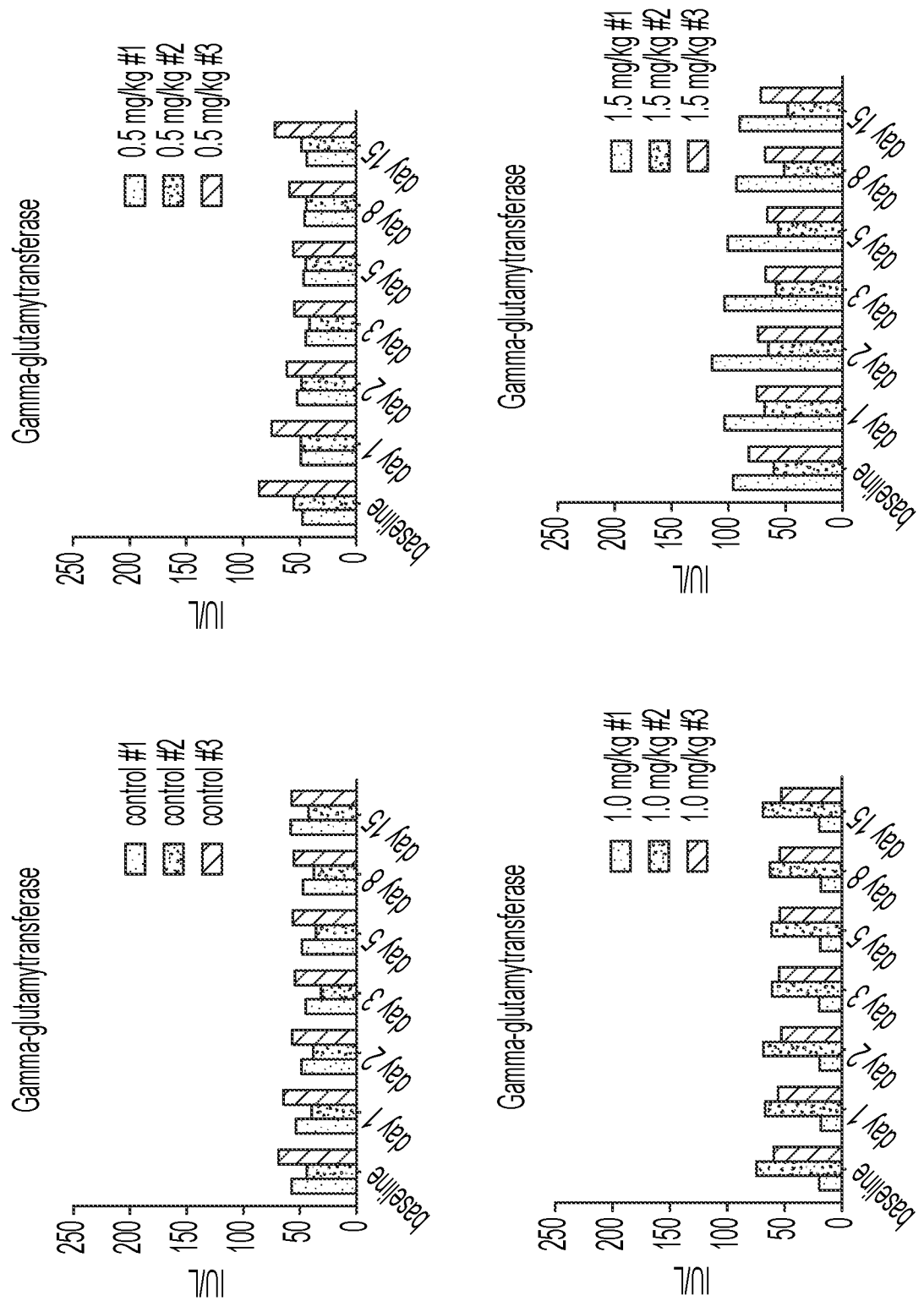

Gamma-glutamytransferase
IU/L
250
200
150
100
50
0
baseline
day 1
day 2
day 3
day 5
day 8
day 15
control #1
control #2
control #3
0.5 mg/kg #1
0.5 mg/kg #2
0.5 mg/kg #3
1.0 mg/kg #1
1.0 mg/kg #2
1.0 mg/kg #3
1.5 mg/kg #1
1.5 mg/kg #2
1.5 mg/kg #3

FIG. 37E

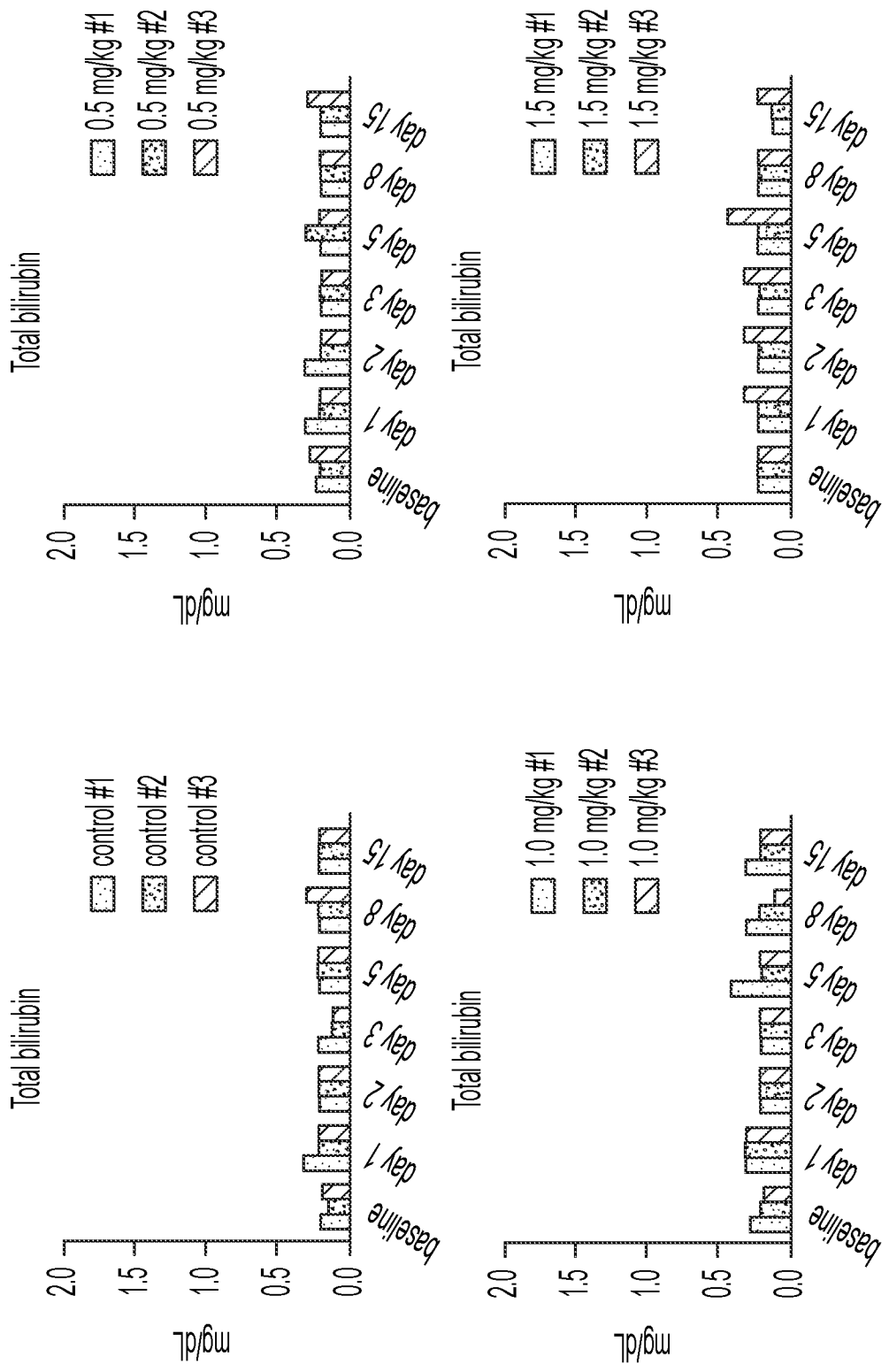

Total bilirubin
mg/dL
2.0
1.5
1.0
0.5
0.0
baseline
day 1
day 2
day 3
day 5
day 8
day 15
0.5 mg/kg #1
0.5 mg/kg #2
0.5 mg/kg #3
1.5 mg/kg #1
1.5 mg/kg #2
1.5 mg/kg #3
control #1
control #2
control #3
1.0 mg/kg #1
1.0 mg/kg #2
1.0 mg/kg #3

FIG. 37F

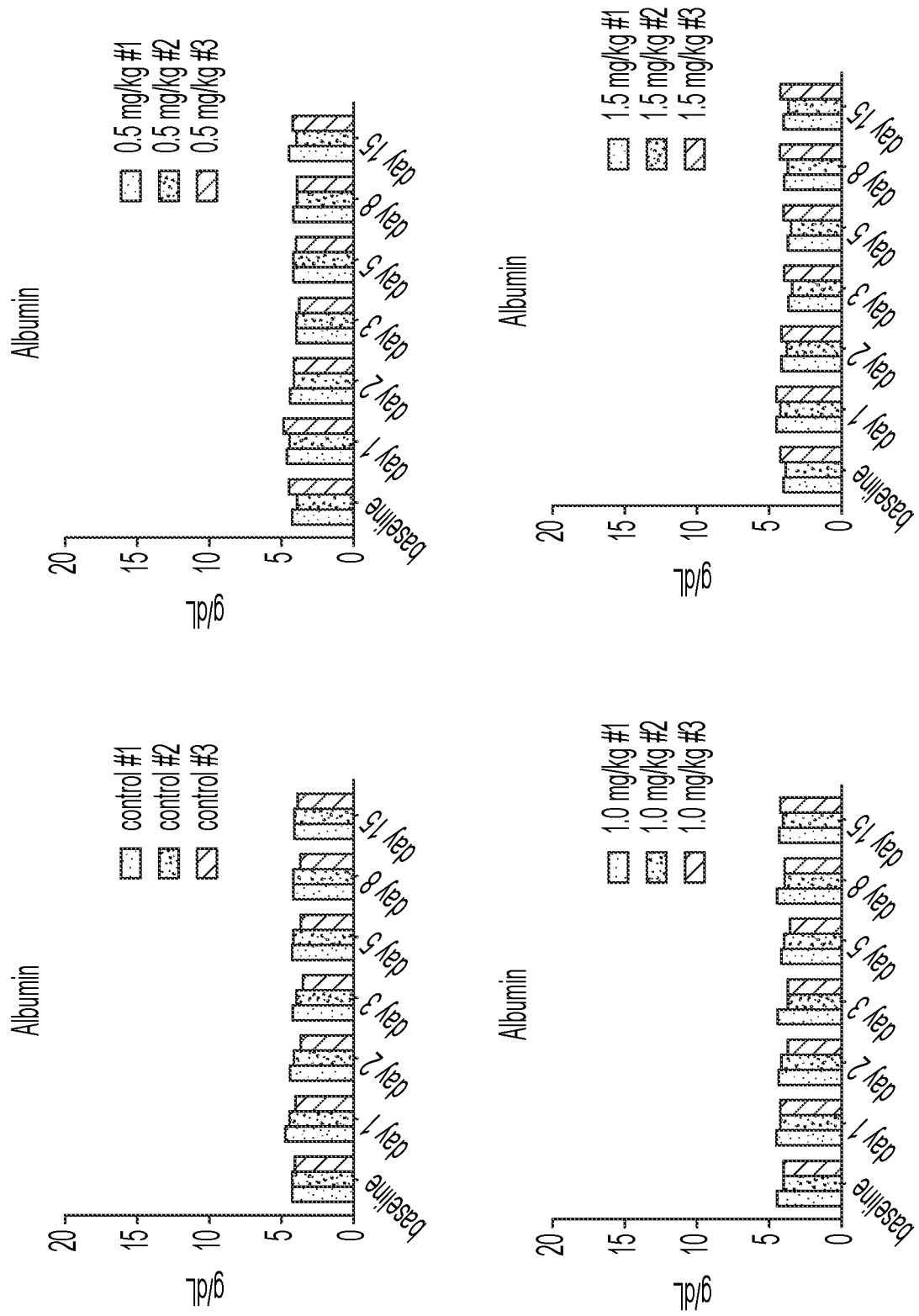

Albumin
g/dL
0
5
10
15
20
baseline
day 1
day 2
day 3
day 5
day 8
day 15
0.5 mg/kg #1
0.5 mg/kg #2
0.5 mg/kg #3
1.5 mg/kg #1
1.5 mg/kg #2
1.5 mg/kg #3
control #1
control #2
control #3
1.0 mg/kg #1
1.0 mg/kg #2
1.0 mg/kg #3

FIG. 37G

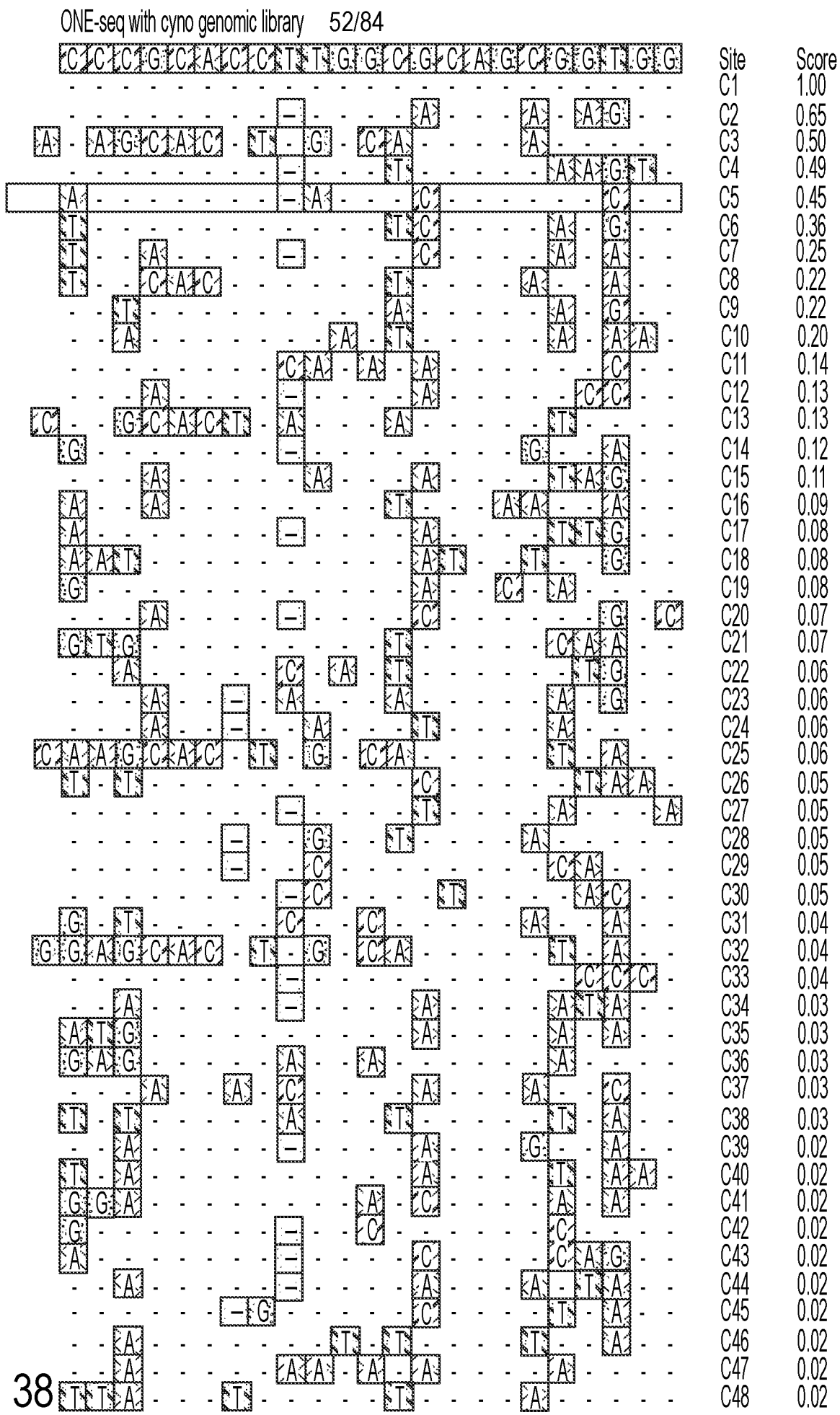

ONE-seq with cyno genomic library 52/84
Site
Score
C1 1.00
C2 0.65
C3 0.50
C4 0.49
C5 0.45
C6 0.36
C7 0.25
C8 0.22
C9 0.22
C10 0.20
C11 0.14
C12 0.13
C13 0.13
C14 0.12
C15 0.11
C16 0.09
C17 0.08
C18 0.08
C19 0.08
C20 0.07
C21 0.07
C22 0.06
C23 0.06
C24 0.06
C25 0.06
C26 0.05
C27 0.05
C28 0.05
C29 0.05
C30 0.05
C31 0.04
C32 0.04
C33 0.04
C34 0.03
C35 0.03
C36 0.03
C37 0.03
C38 0.03
C39 0.02
C40 0.02
C41 0.02
C42 0.02
C43 0.02
C44 0.02
C45 0.02
C46 0.02
C47 0.02
C48 0.02

FIG. 38

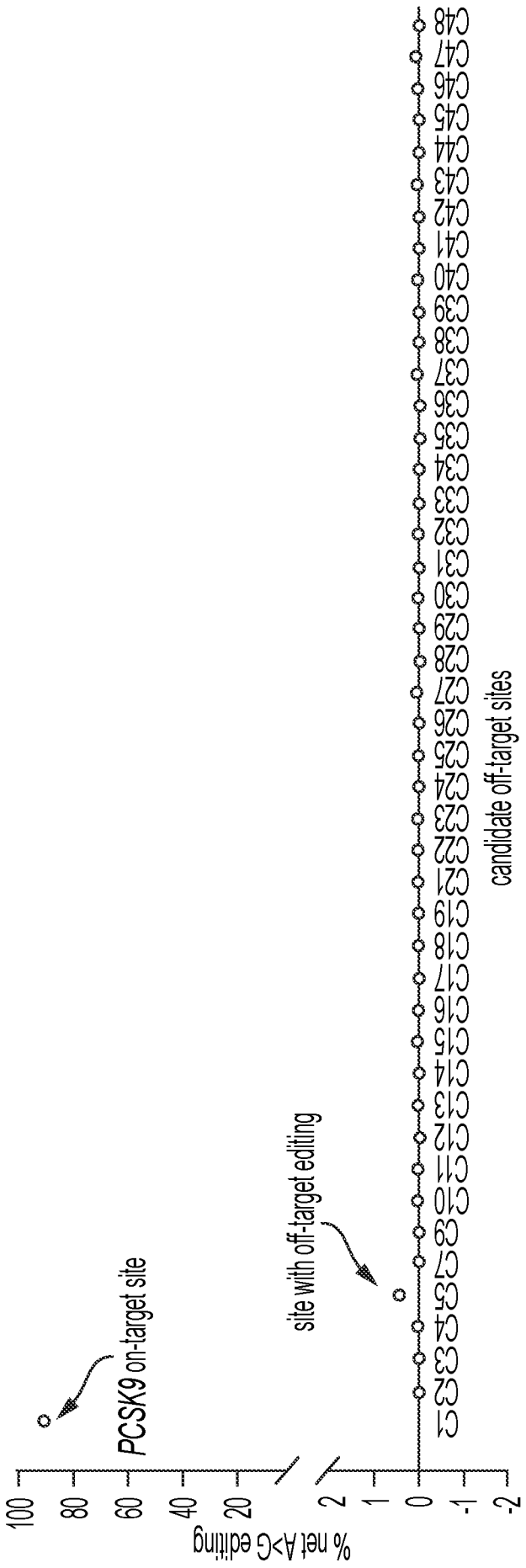

Primary cyno hepatocytes - 3 treated samples, 3 untreated samples
PCSK9 on-target site
site with off-target editing
% net A>G editing
100
80
60
40
20
2
1
0
-1
-2
candidate off-target sites

FIG. 39

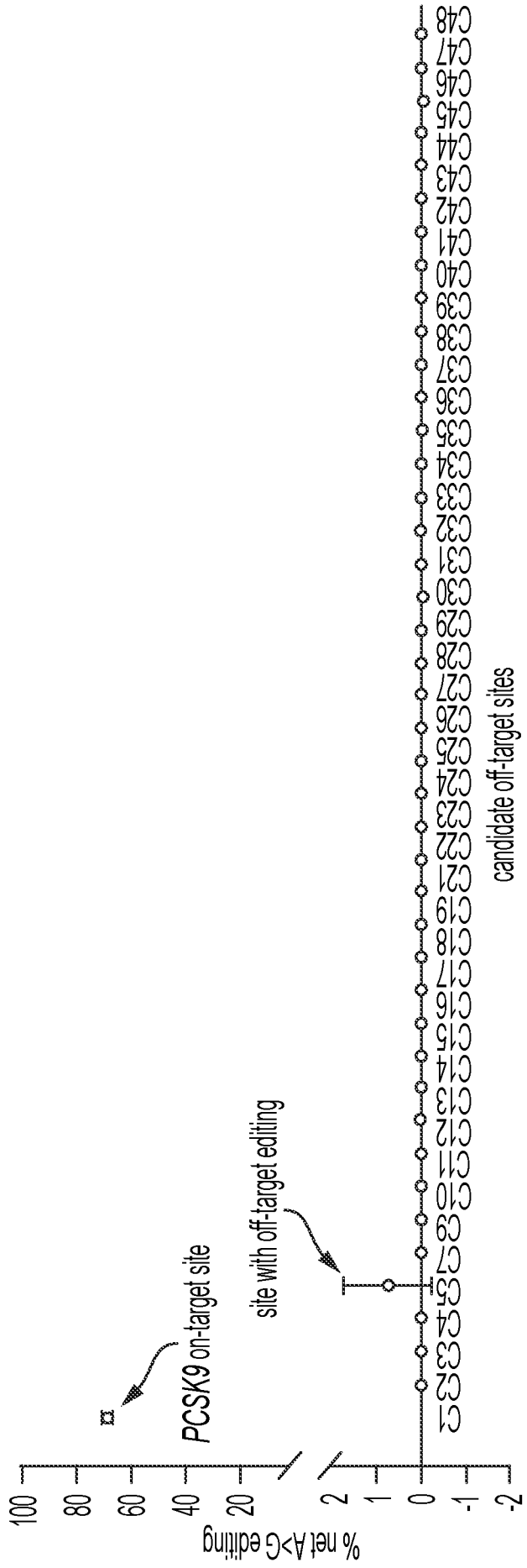


FIG. 39
(Continued)

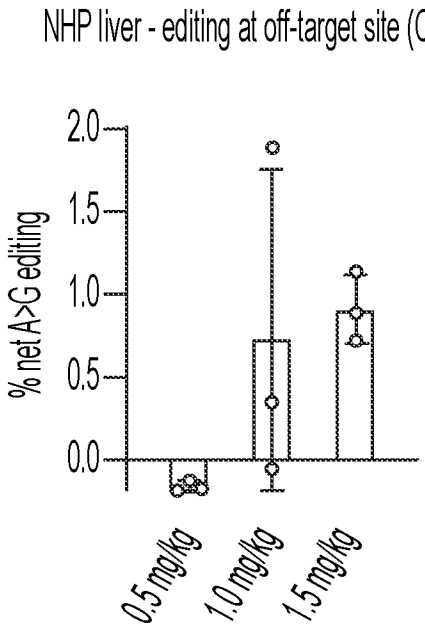

NHP liver - editing at off-target site (C5)
% net A>G editing
2.0
1.5
1.0
0.5
0.0
0.5 mg/kg
1.0 mg/kg
1.5 mg/kg

FIG. 40

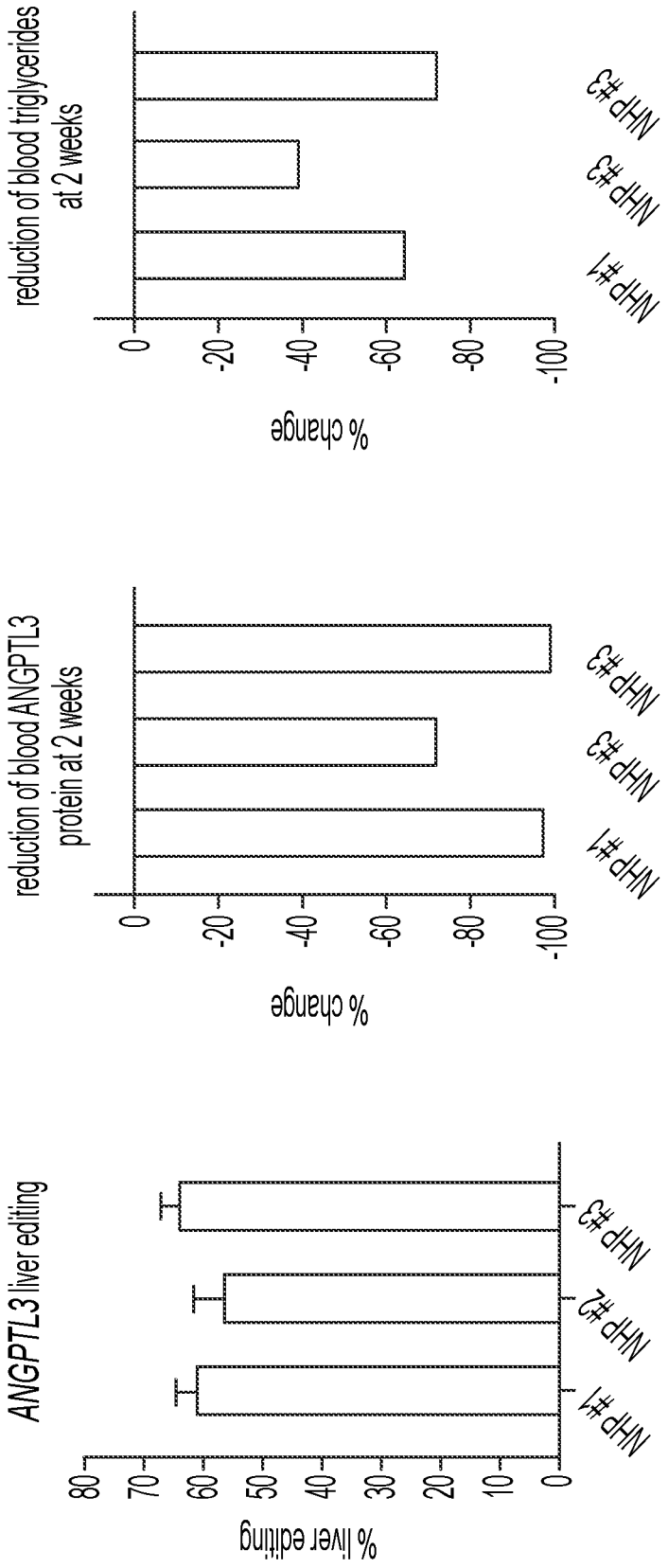

reduction of blood triglycerides at 2 weeks
% change
0
-20
-40
-60
-80
-100
NHP #1
NHP #3
NHP #3
reduction of blood ANGPTL3 protein at 2 weeks
% change
0
-20
-40
-60
-80
-100
NHP #1
NHP #3
NHP #3
ANGPTL3 liver editing
% liver editing
0
10
20
30
40
50
60
70
80
NHP #1
NHP #2
NHP #3

FIG. 41

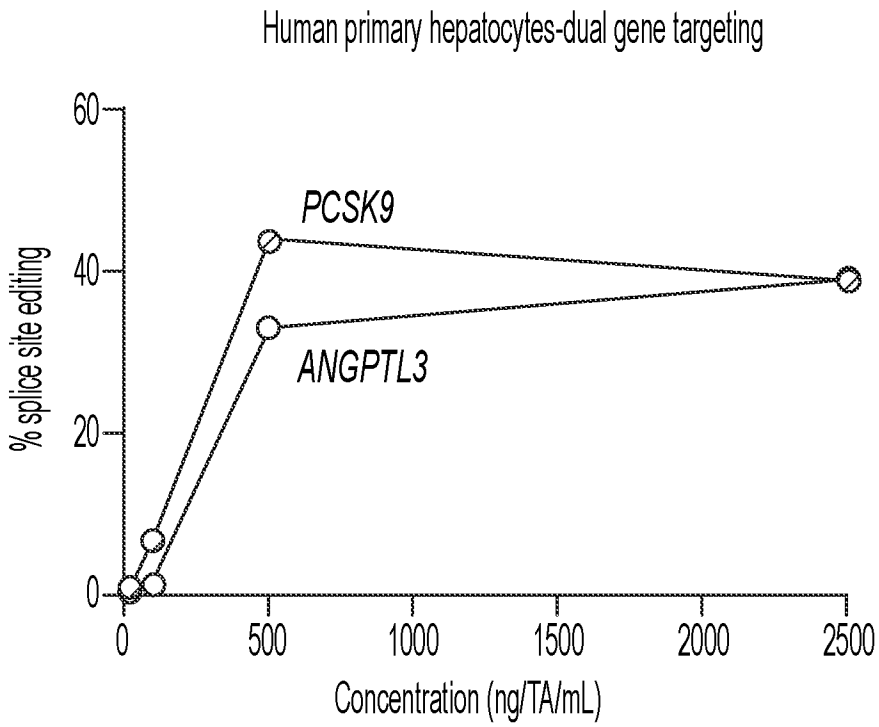

Human primary hepatocytes-dual gene targeting
% splice site editing
60
40
20
0
PCSK9
ANGPTL3
0
500
1000
1500
2000
2500
Concentration (ng/TA/mL)

FIG. 42

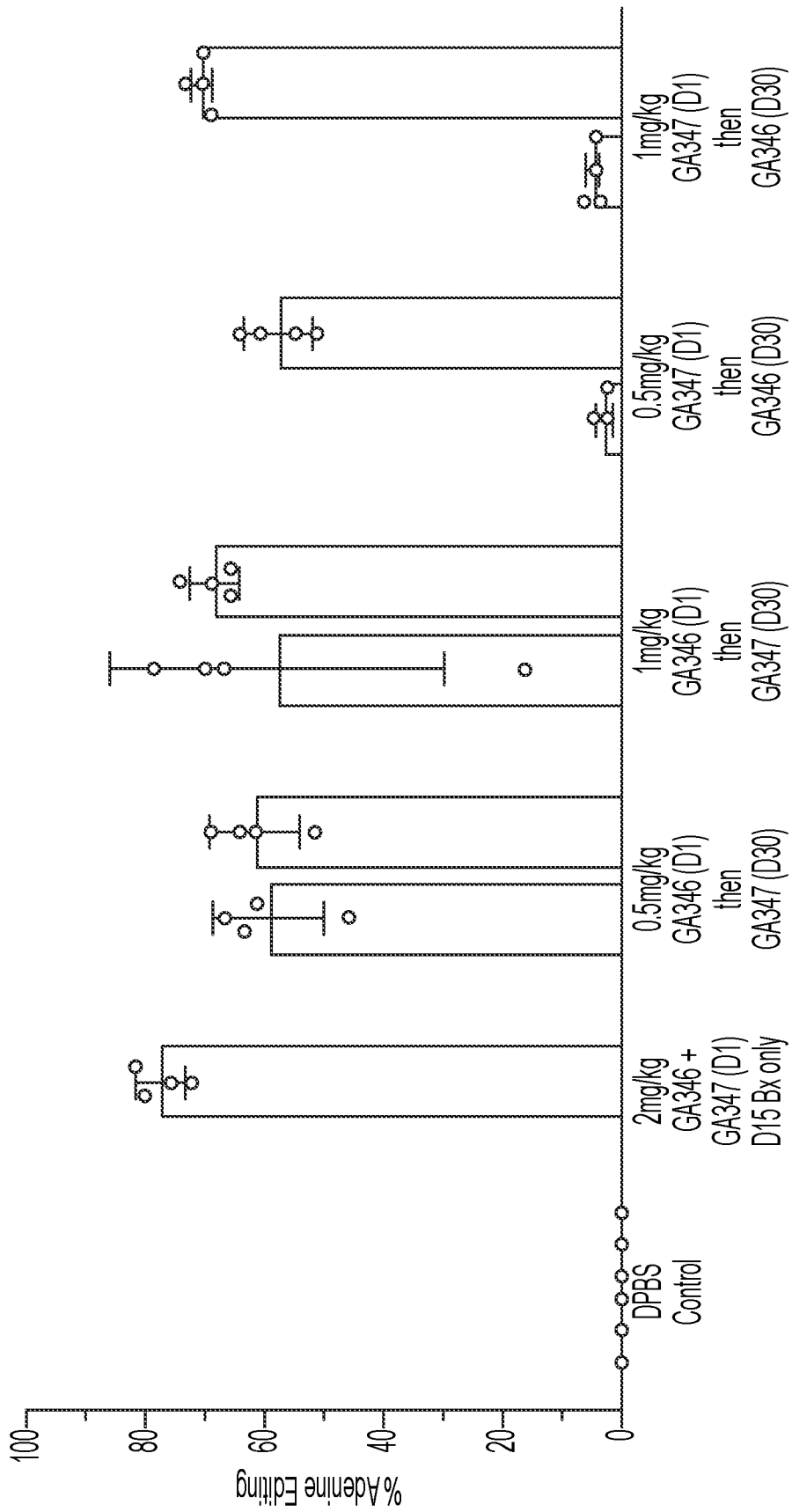

PCSK9 (GA346)
Day 15 & D44 Liver Biopsy
% Adenine Editing
100
80
60
40
20
0
DPBS
Control
2mg/kg
GA346 +
GA347 (D1)
D15 Bx only
0.5mg/kg
GA346 (D1)
then
GA347 (D30)
1mg/kg
GA346 (D1)
then
GA347 (D30)
0.5mg/kg
GA347 (D1)
then
GA346 (D30)
1mg/kg
GA347 (D1)
then
GA346 (D30)

FIG. 43

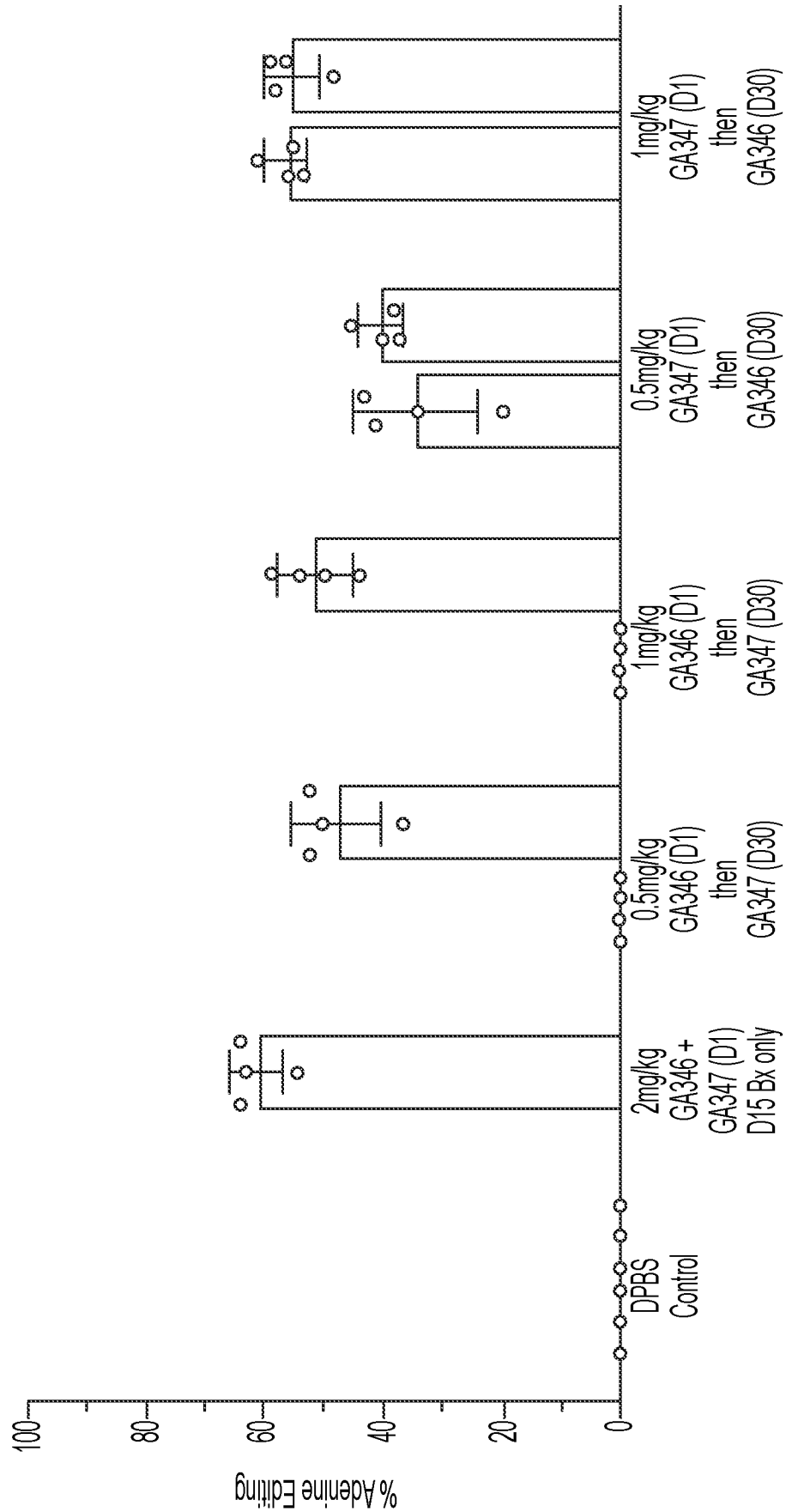


FIG. 43
(Continued)

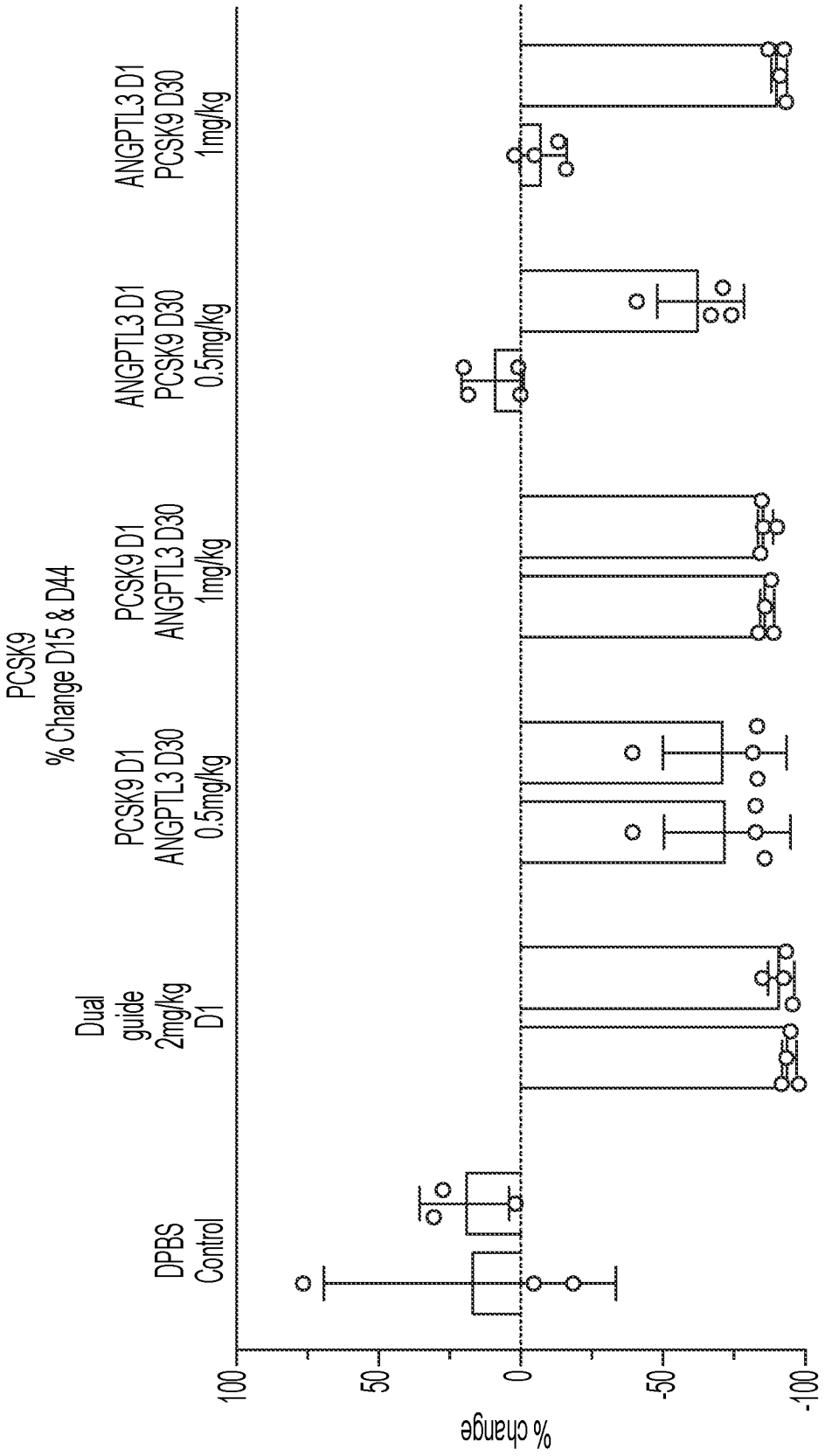

PCSK9
% Change D15 & D44
% change
100
50
0
-50
-100
DPBS Control
Dual guide 2mg/kg D1
PCSK9 D1 ANGPTL3 D30 0.5mg/kg
PCSK9 D1 ANGPTL3 D30 1mg/kg
ANGPTL3 D1 PCSK9 D30 0.5mg/kg
ANGPTL3 D1 PCSK9 D30 1mg/kg

FIG. 44

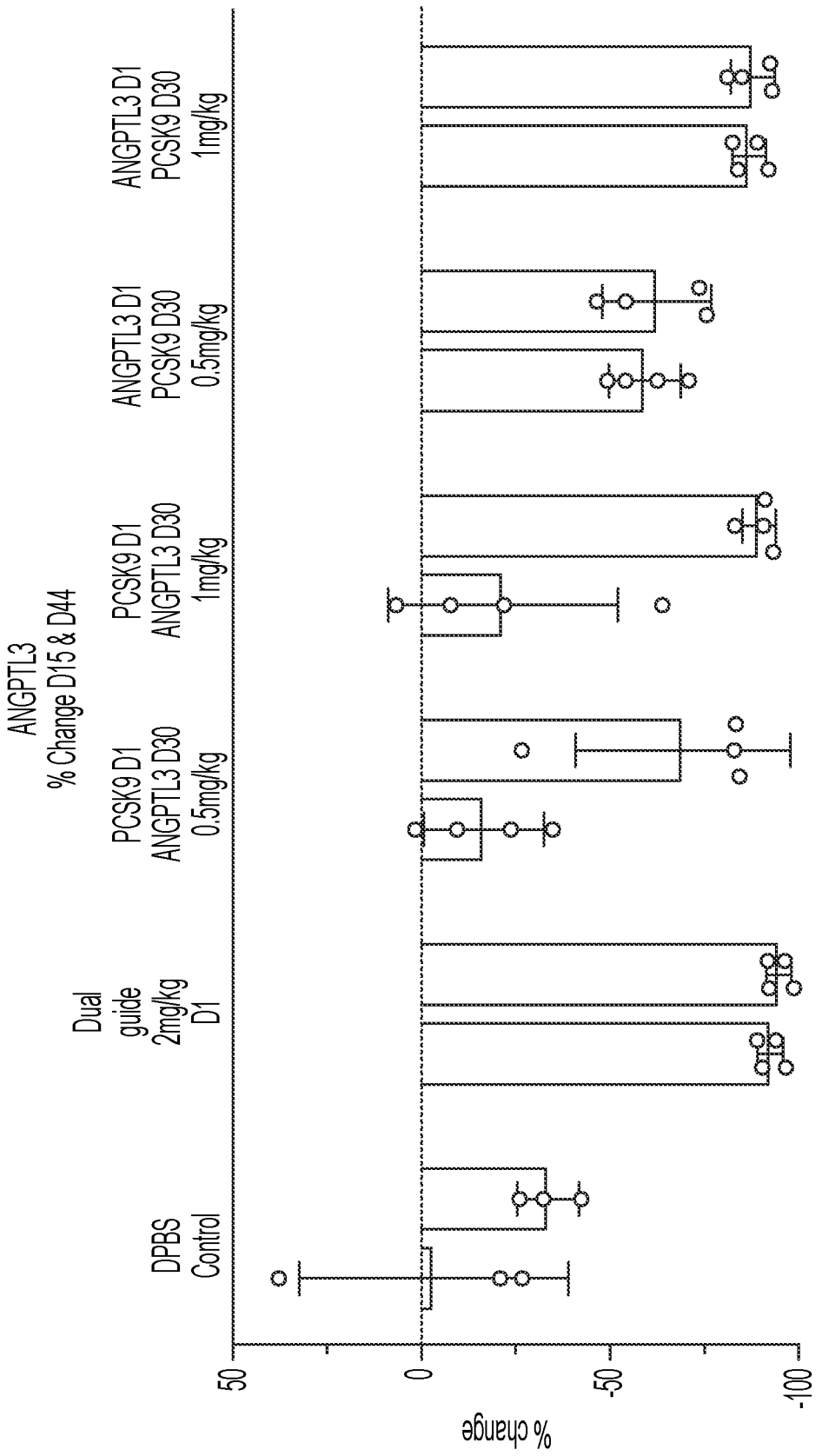


FIG. 44
(Continued)

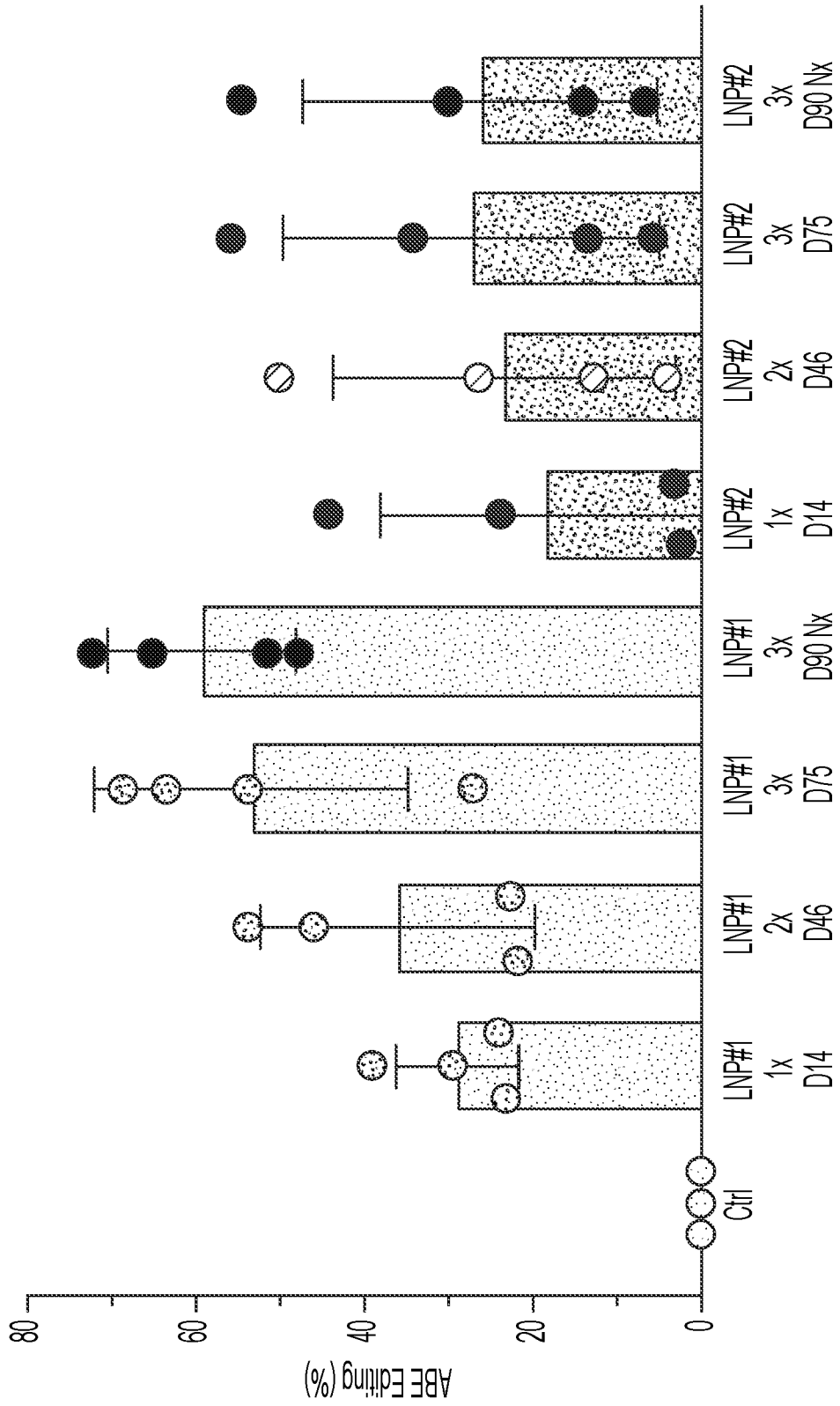

ABE Editing (%)
0
20
40
60
80
Ctrl
LNP#1 1x D14
LNP#1 2x D46
LNP#1 3x D75
LNP#1 3x D90 Nx
LNP#2 1x D14
LNP#2 2x D46
LNP#2 3x D75
LNP#2 3x D90 Nx

FIG. 45

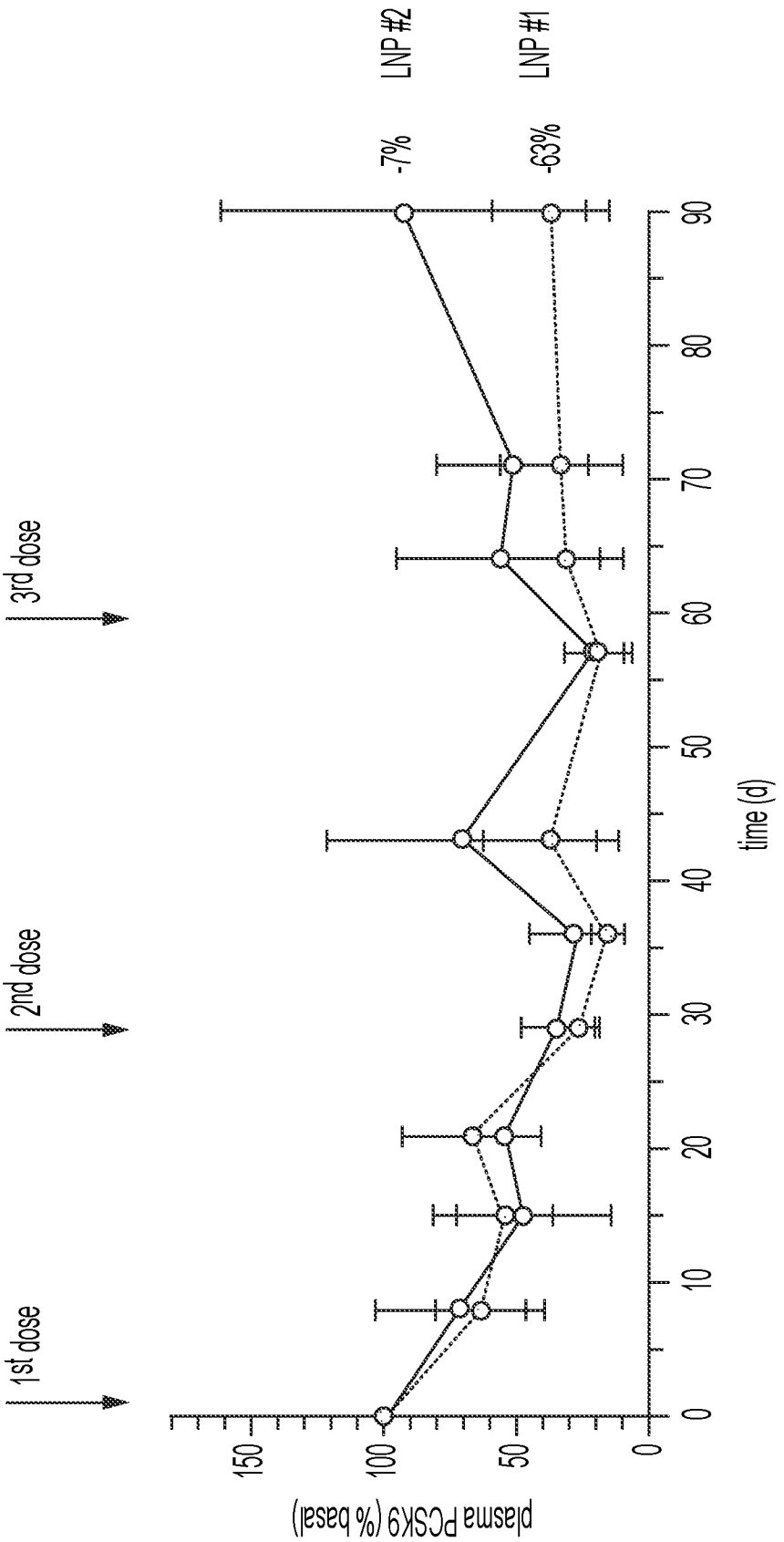

LNP #2
LNP #1
-7%
-63%
1st dose
2nd dose
3rd dose
plasma PCSK9 (% basal)
time (d)
0
50
100
150
0
10
20
30
40
50
60
70
80
90

FIG. 46

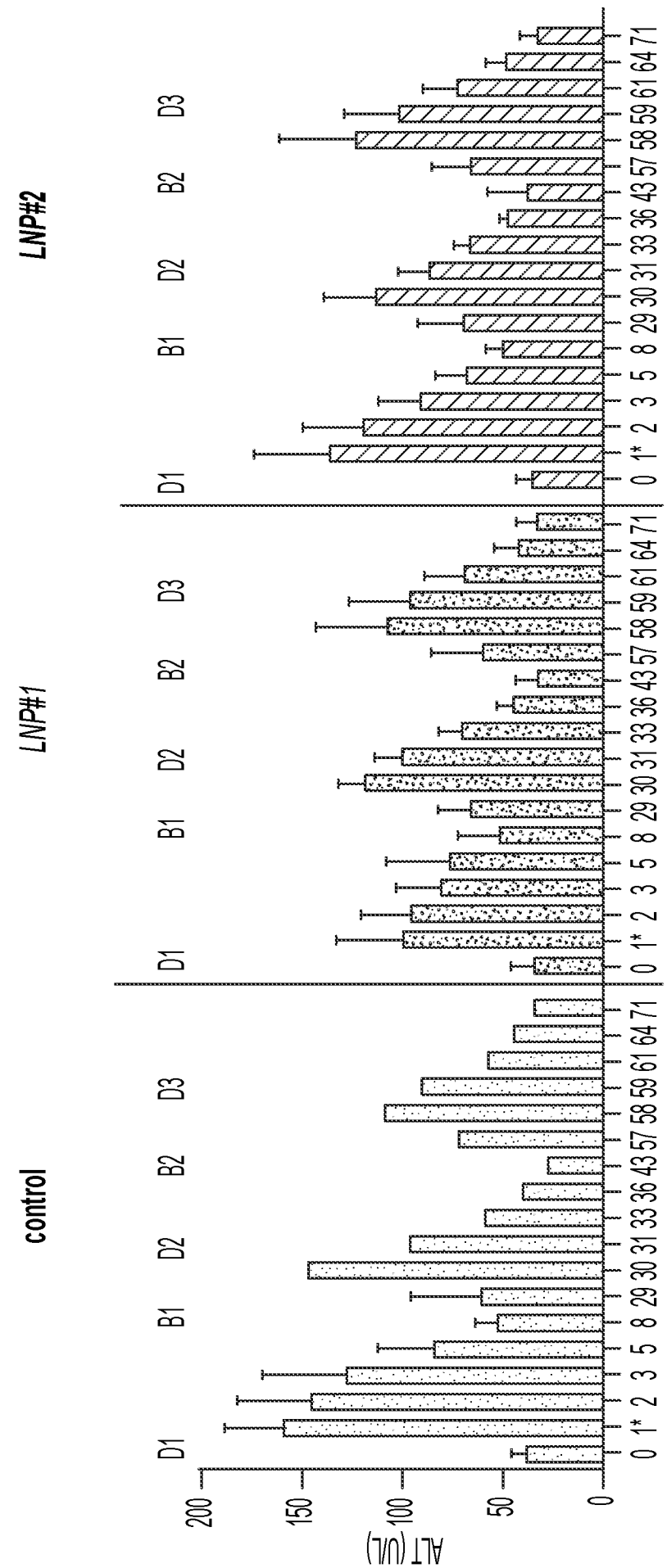

control
LNP#1
LNP#2
ALT (U/L)
200
150
100
50
0
time (d)
D1
B1
D2
B2
D3
0 1* 2 3 5 8 29 30 31 33 36 43 57 58 59 61 64 71

FIG. 47



FIG. 47
(Continued)

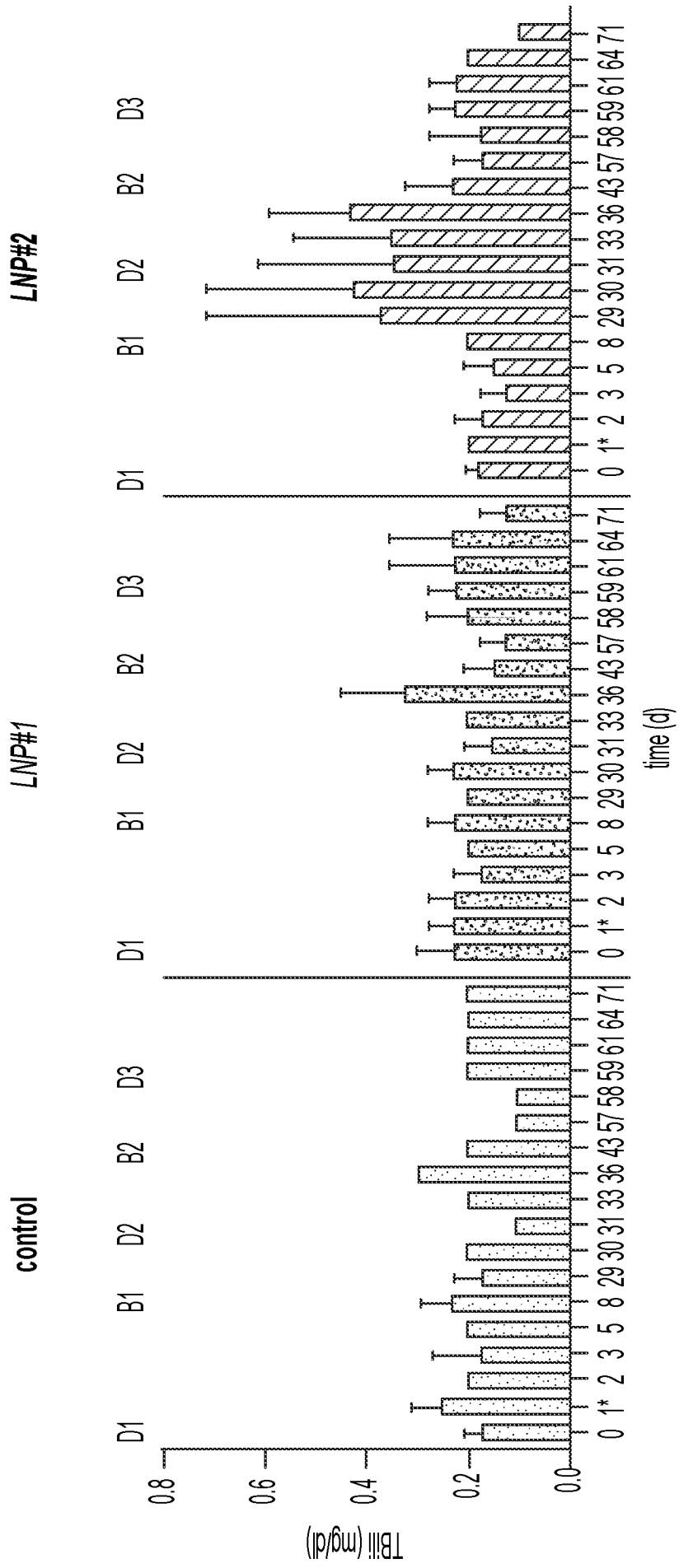


FIG. 47
(Continued)

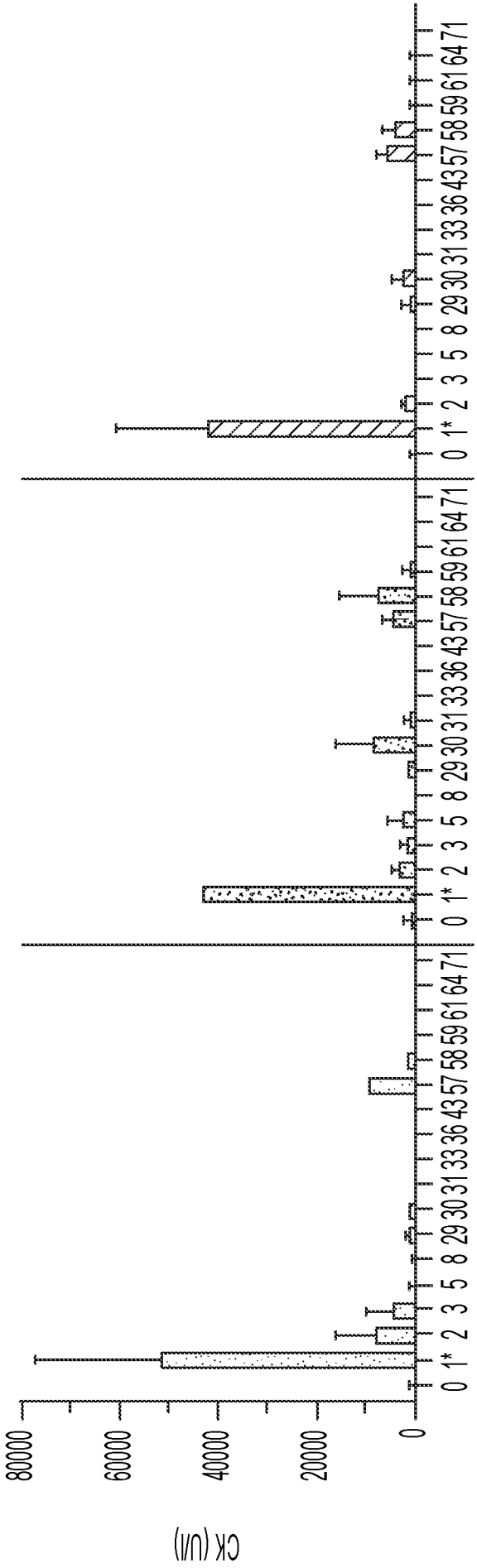


FIG. 47
(Continued)

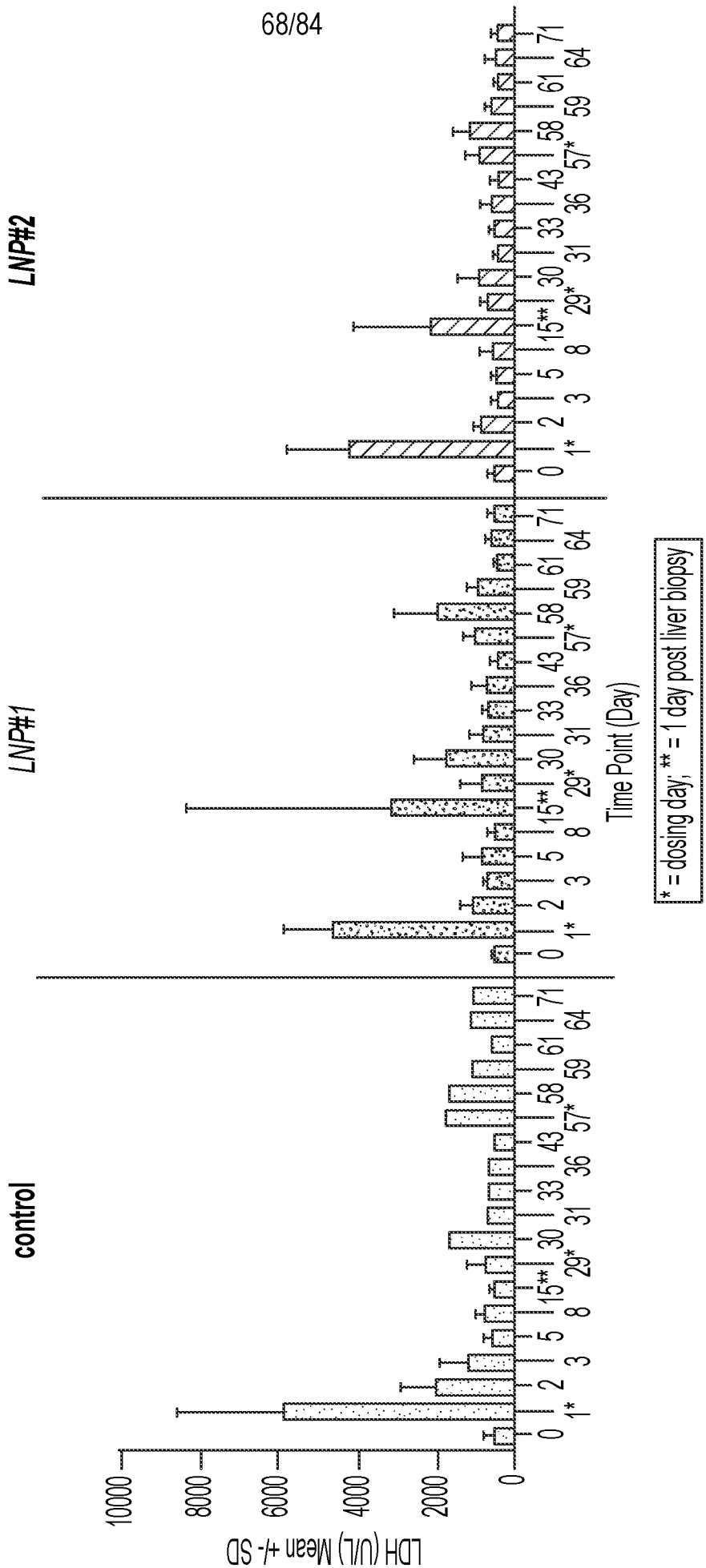

control
LNP#1
LNP#2
LDH (U/L) Mean +/- SD
10000
8000
6000
4000
2000
0
Time Point (Day)
* = dosing day; ** = 1 day post liver biopsy

FIG. 48

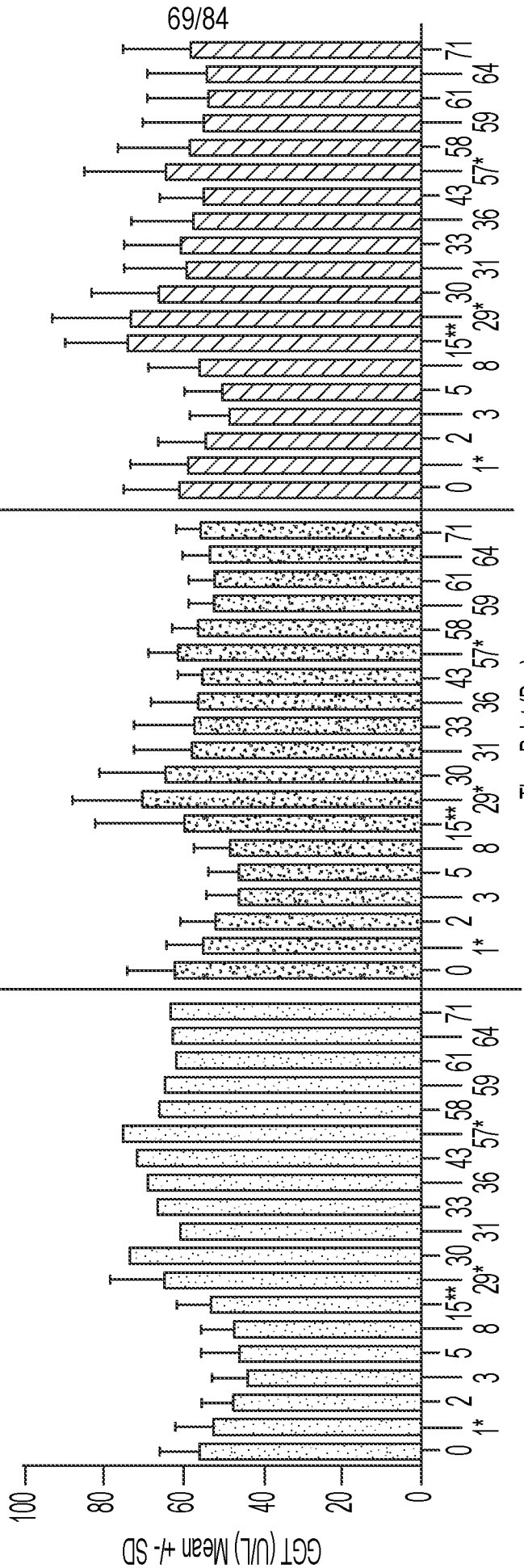


FIG. 48
(Continued)

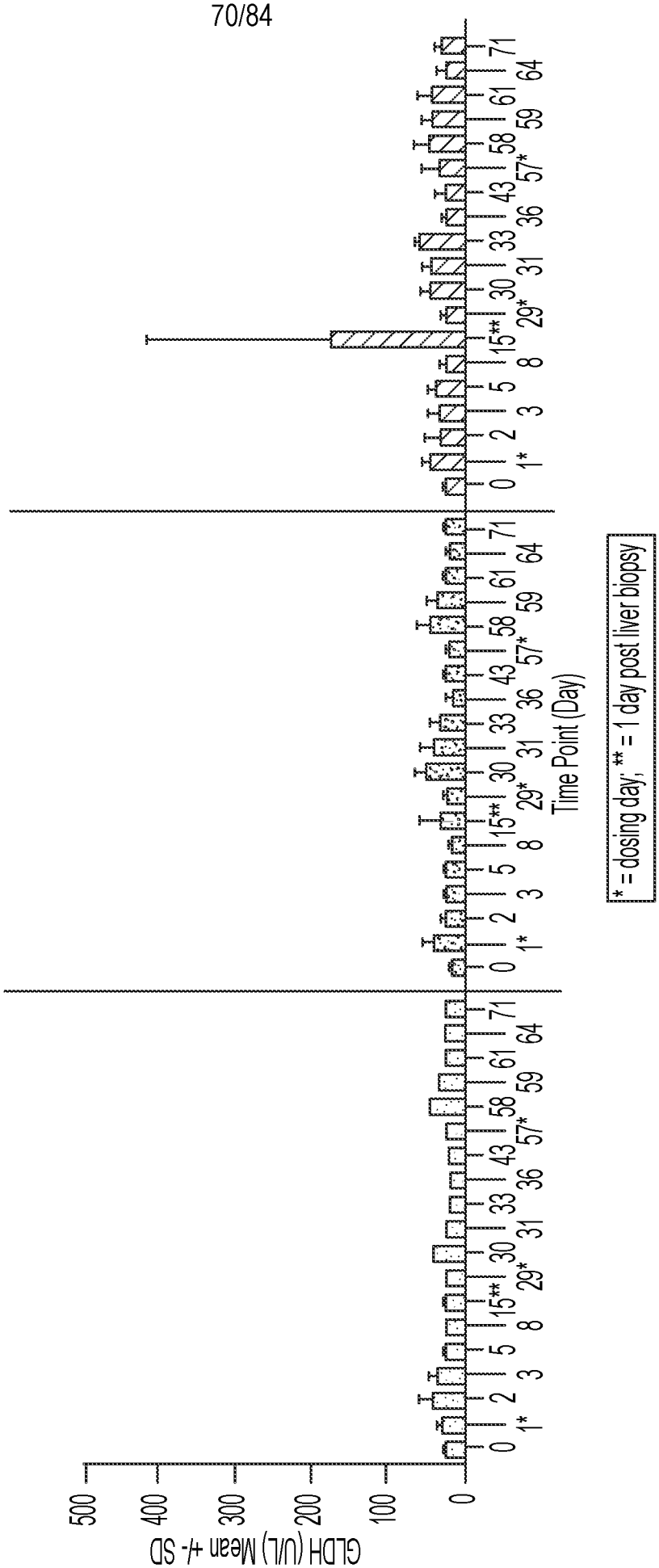


FIG. 48
(Continued)

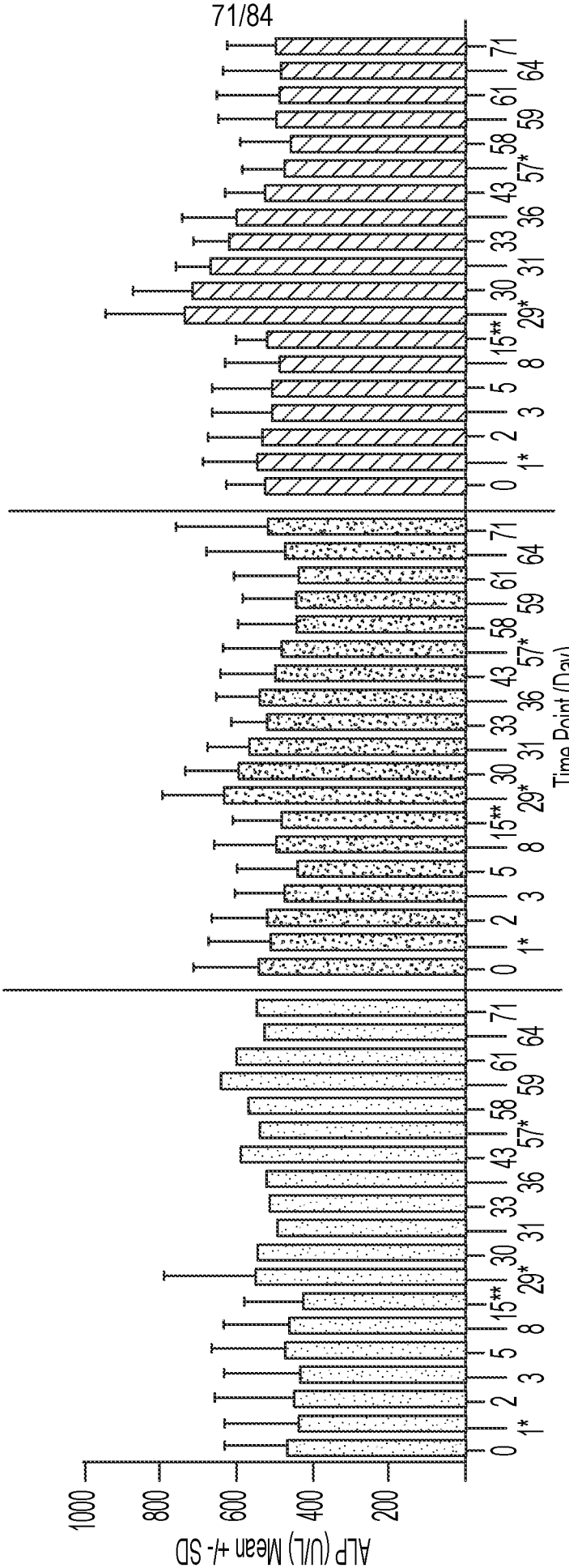


FIG. 48
(Continued)

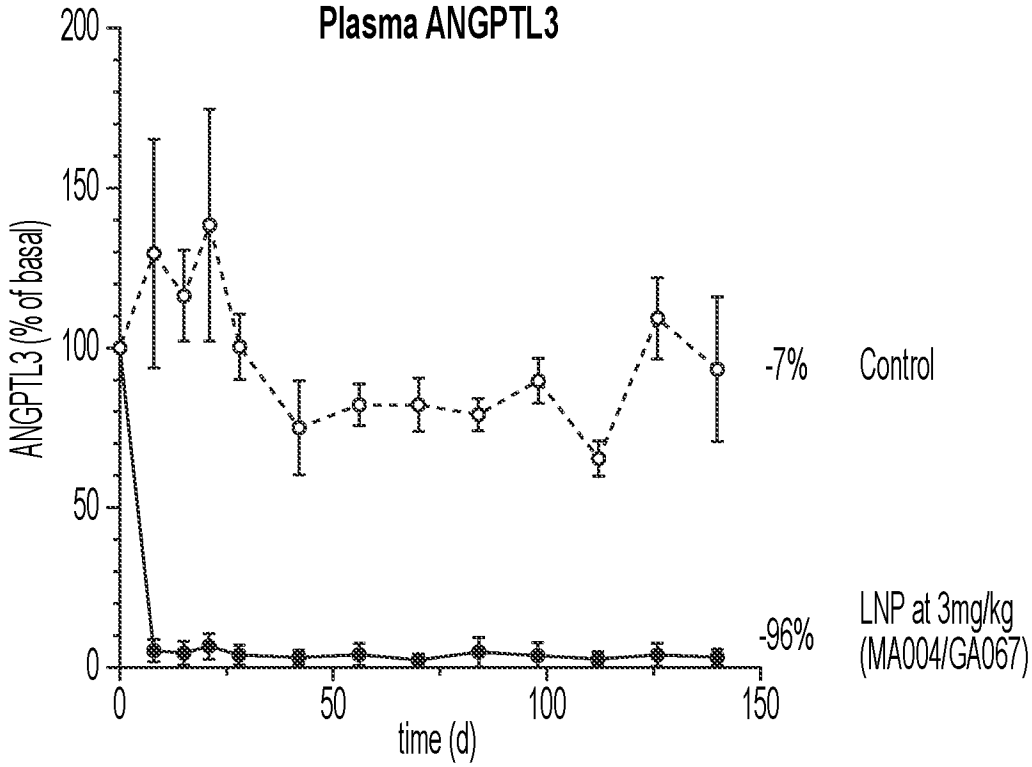


FIG. 49A

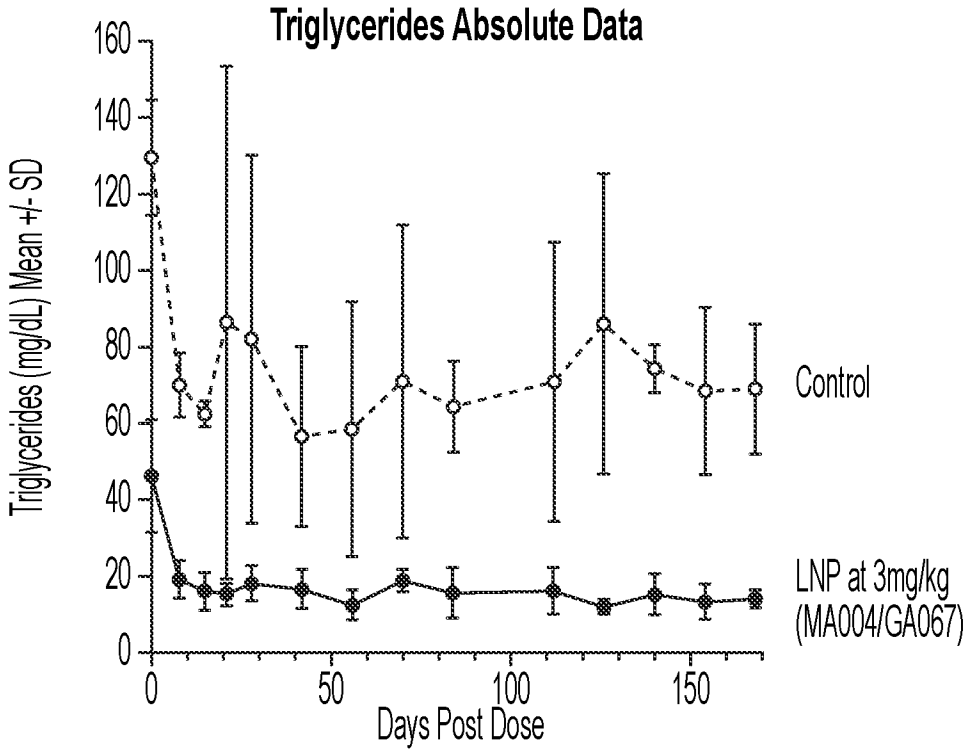


FIG. 49B

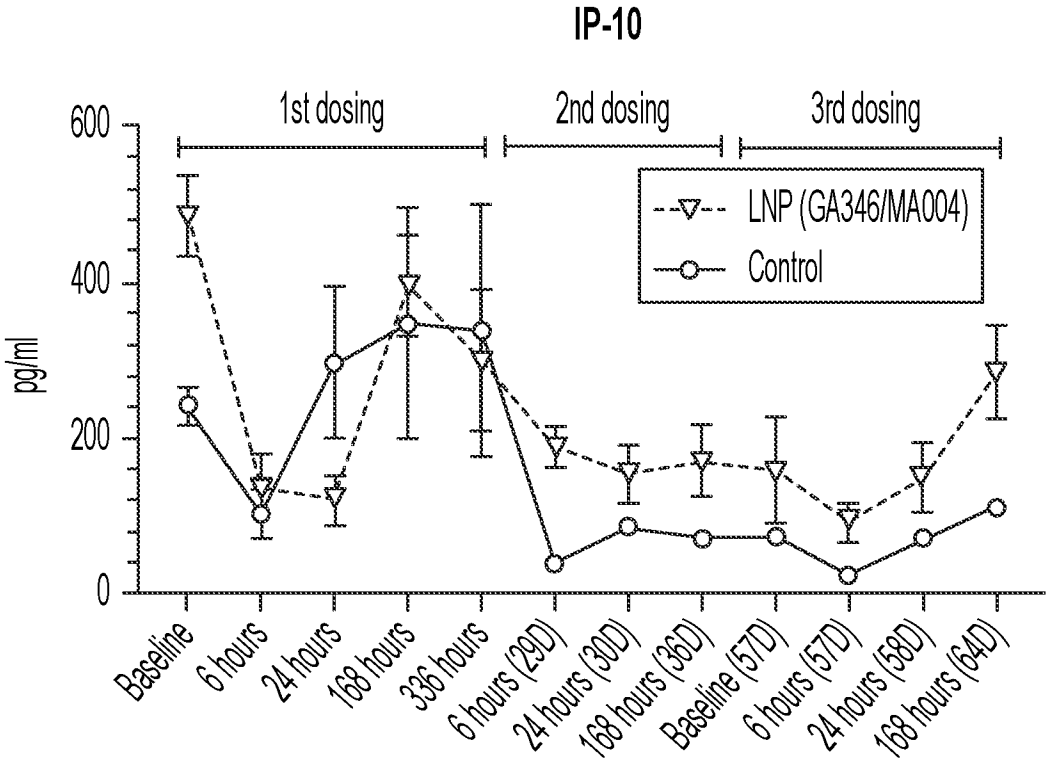


FIG. 50A

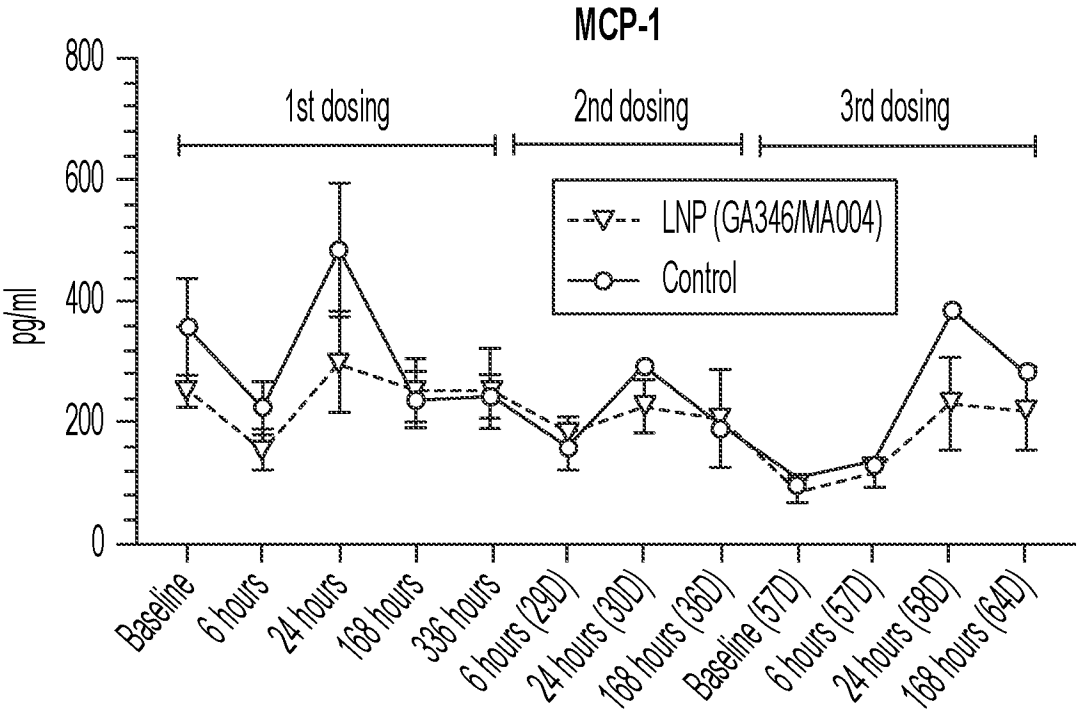


FIG. 50B

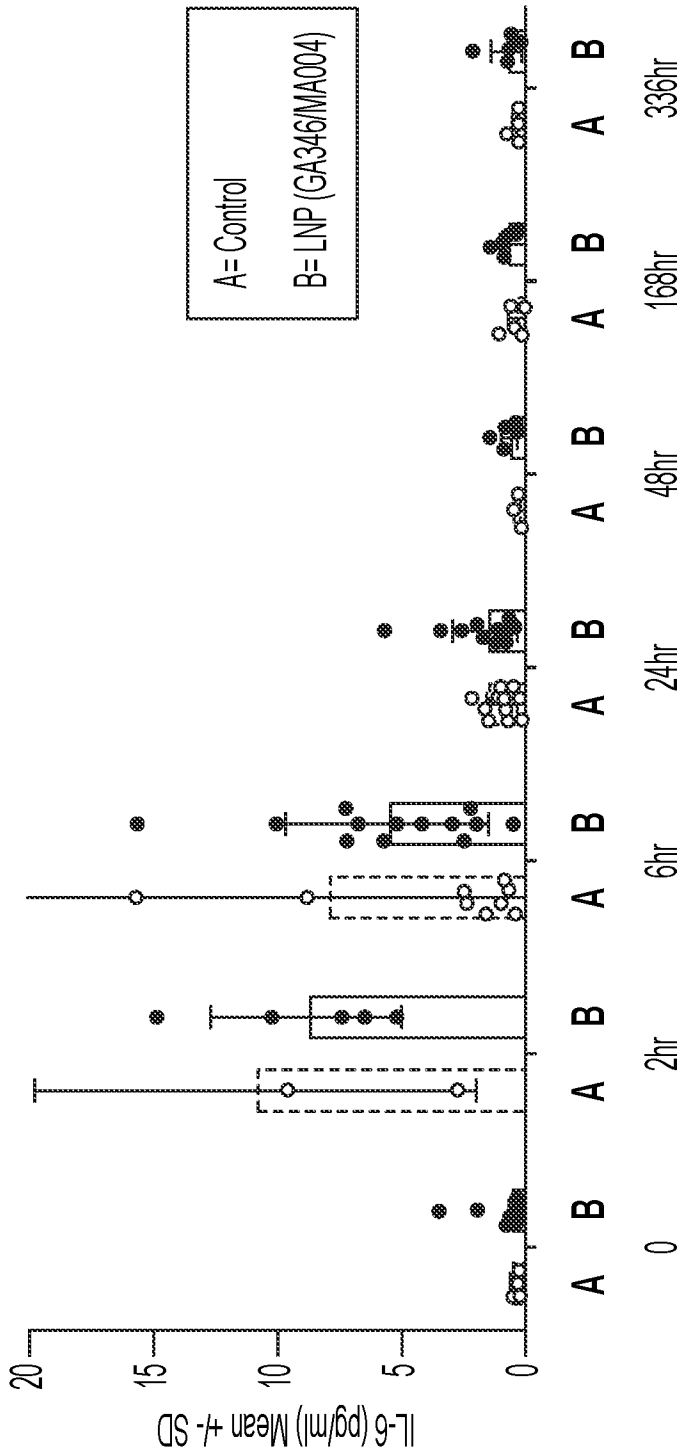

A= Control
B= LNP (GA346/MA004)
IL-6 (pg/ml) Mean +/- SD
20
15
10
5
0
A B A B A B A B A B A B
0
2hr
6hr
24hr
48hr
168hr
336hr
Time Point

FIG. 50C

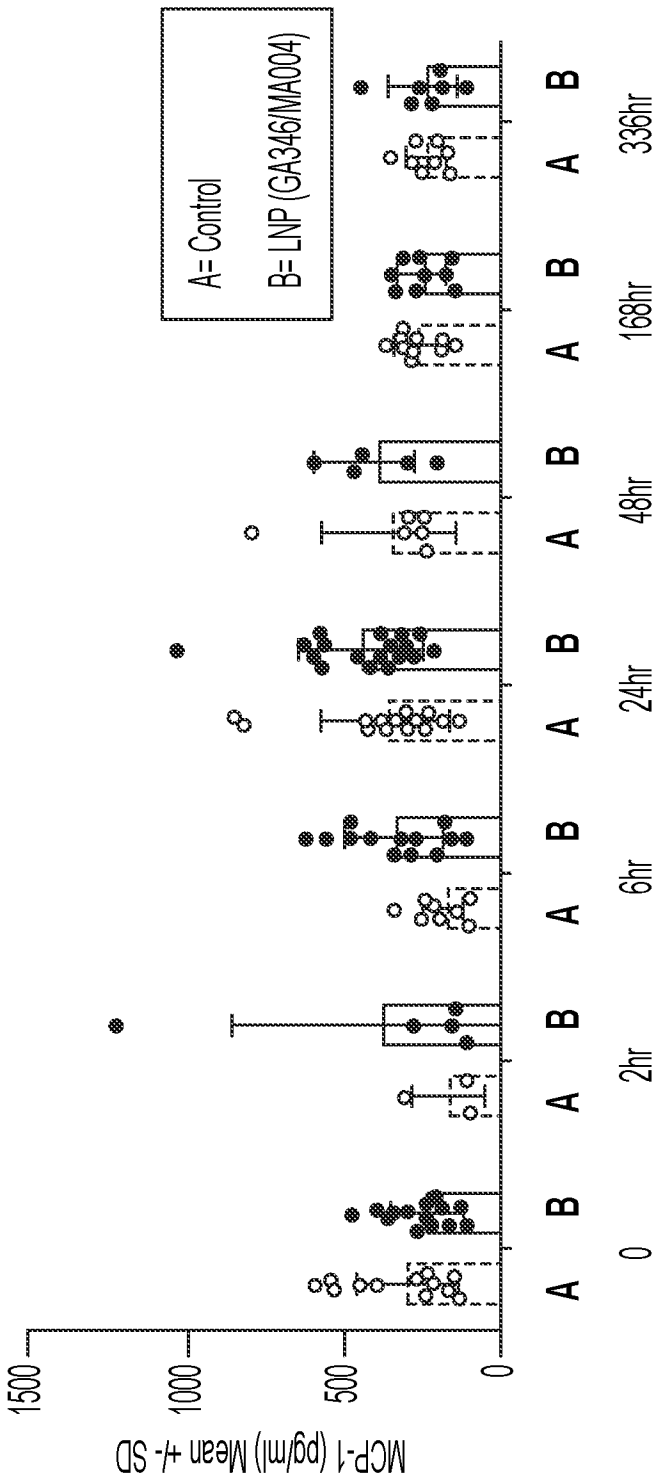

A= Control
B= LNP (GA346/MA004)
MCP-1 (pg/ml) Mean +/- SD
1500
1000
500
0
A B A B A B A B A B A B
0
2hr
6hr
24hr
48hr
168hr
336hr
Time Point

FIG. 50D

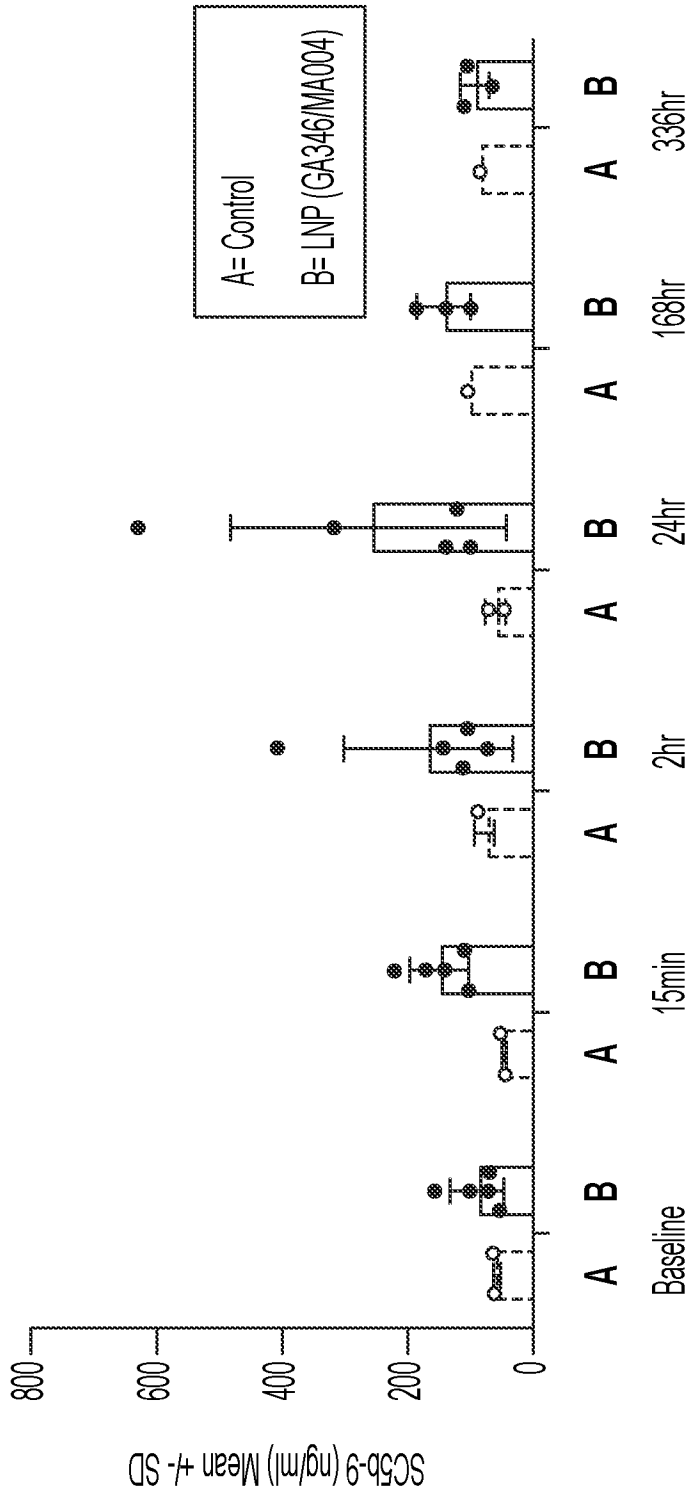

A= Control
B= LNP (GA346/MA004)
SC5b-9 (ng/ml) Mean +/- SD
800
600
400
200
0
A B A B A B A B A B
Baseline
15min
2hr
24hr
168hr
336hr
Time Point

FIG. 50E

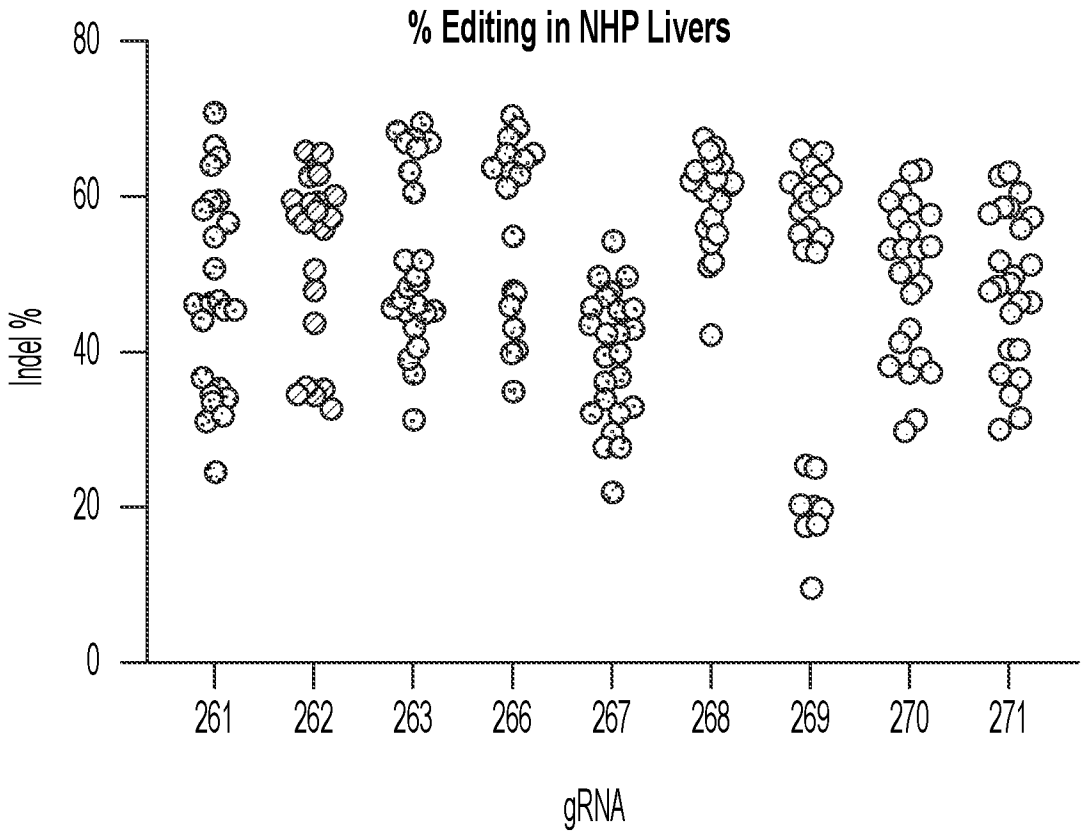

% Editing in NHP Livers
Indel %
80
60
40
20
0
261
262
263
266
267
268
269
270
271
gRNA

FIG. 51

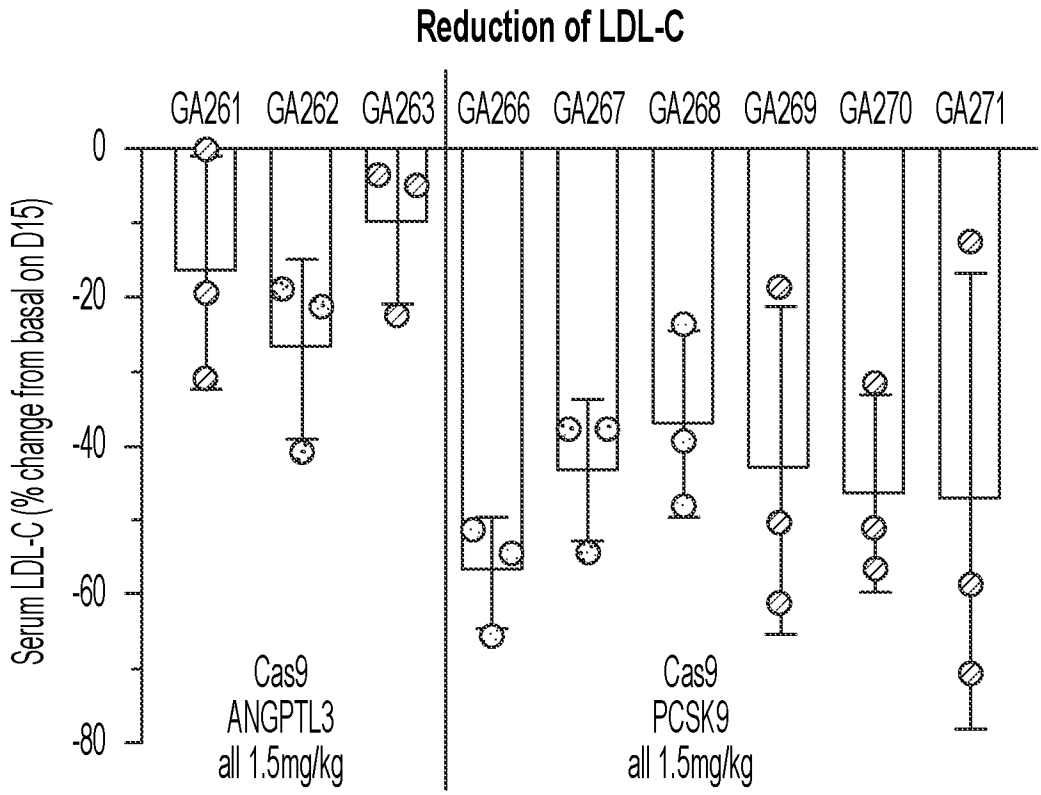

Reduction of LDL-C
GA261
GA262
GA263
GA266
GA267
GA268
GA269
GA270
GA271
0
-20
-40
-60
-80
Serum LDL-C (% change from basal on D15)
Cas9
ANGPTL3
all 1.5mg/kg
Cas9
PCSK9
all 1.5mg/kg

FIG. 52

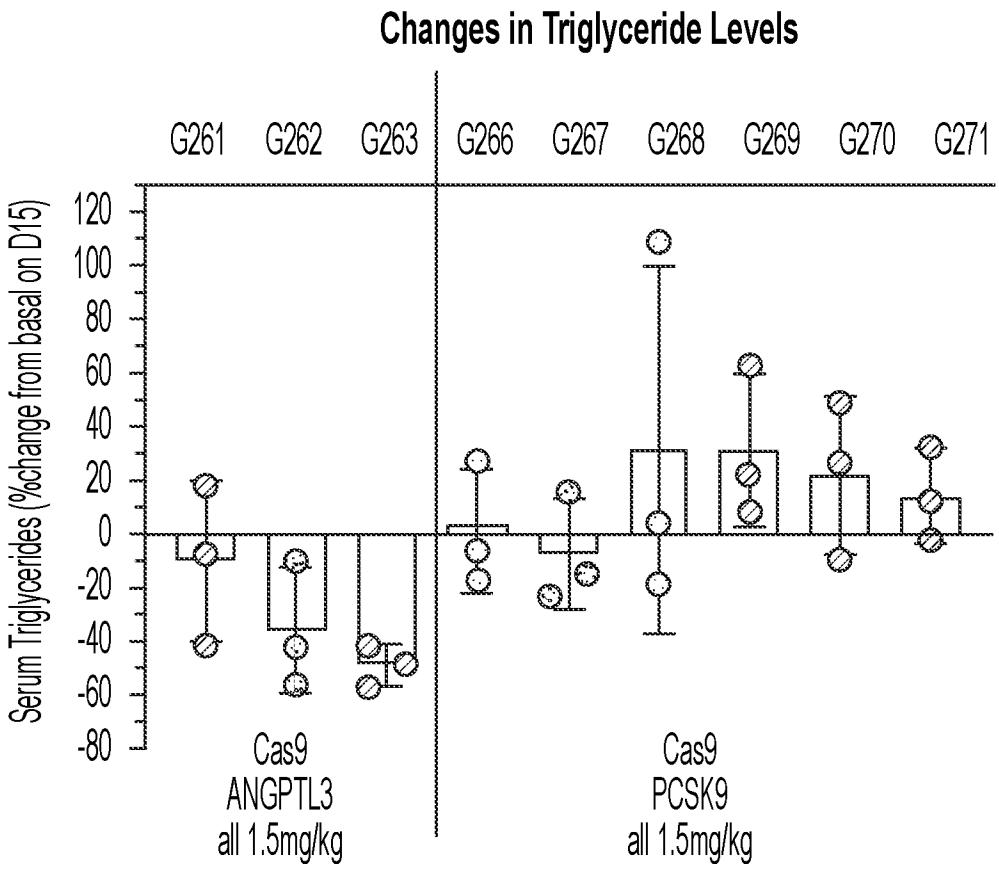

Changes in Triglyceride Levels
G261
G262
G263
G266
G267
G268
G269
G270
G271
Serum Triglycerides (%change from basal on D15)
120
100
80
60
40
20
0
-20
-40
-60
-80
Cas9
ANGPTL3
all 1.5mg/kg
Cas9
PCSK9
all 1.5mg/kg

FIG. 53

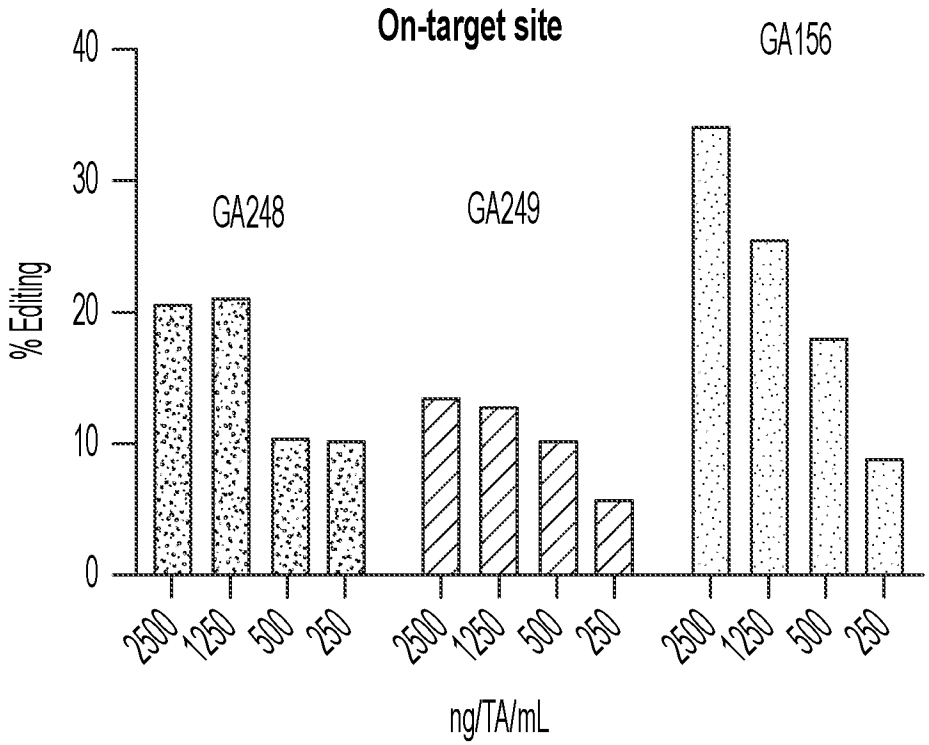


FIG. 54A

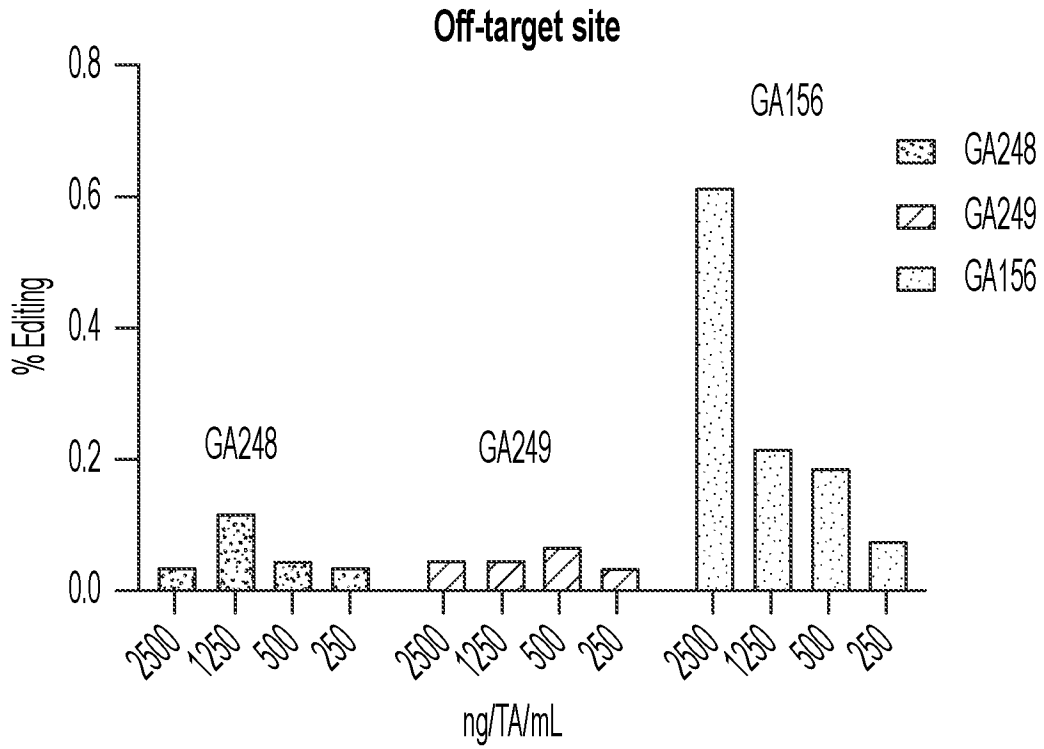


FIG. 54B

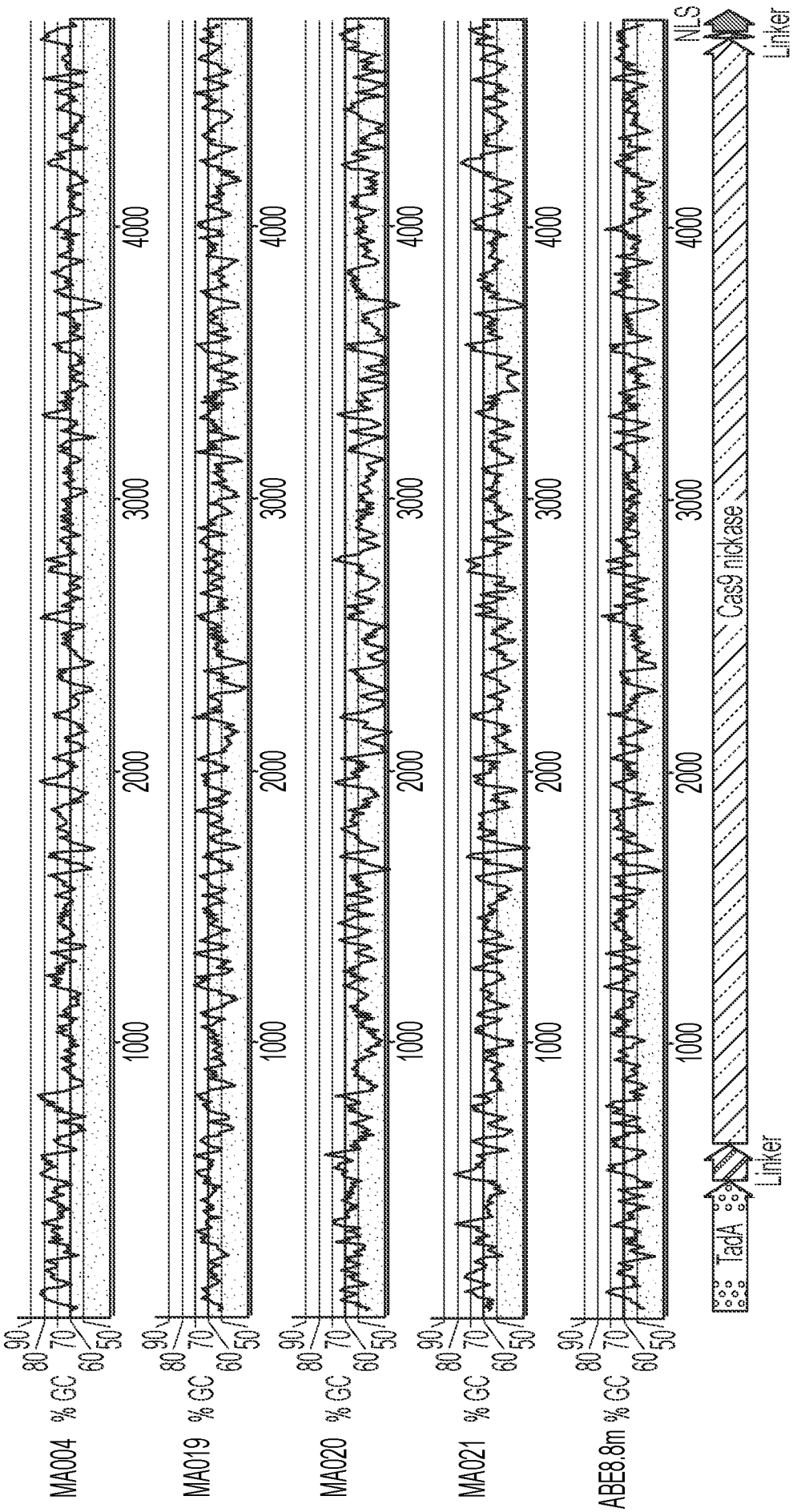

MA004 % GC
MA019 % GC
MA020 % GC
MA021 % GC
ABE8.8m % GC
90
80
70
60
50
1000
2000
3000
4000
TadA
Linker
Cas9 nickase
NLS
Linker

FIG. 55A

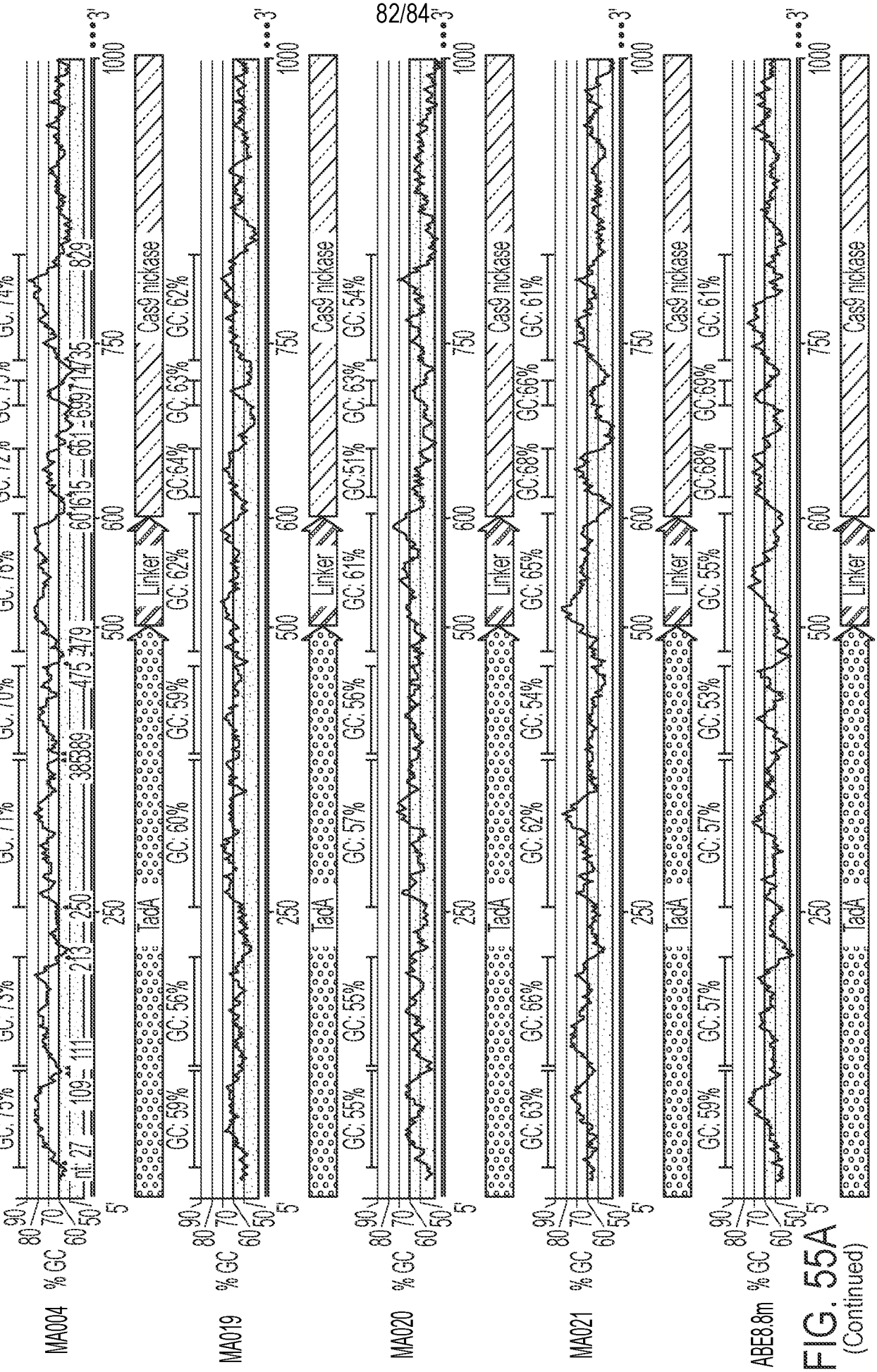


FIG. 55A
(Continued)

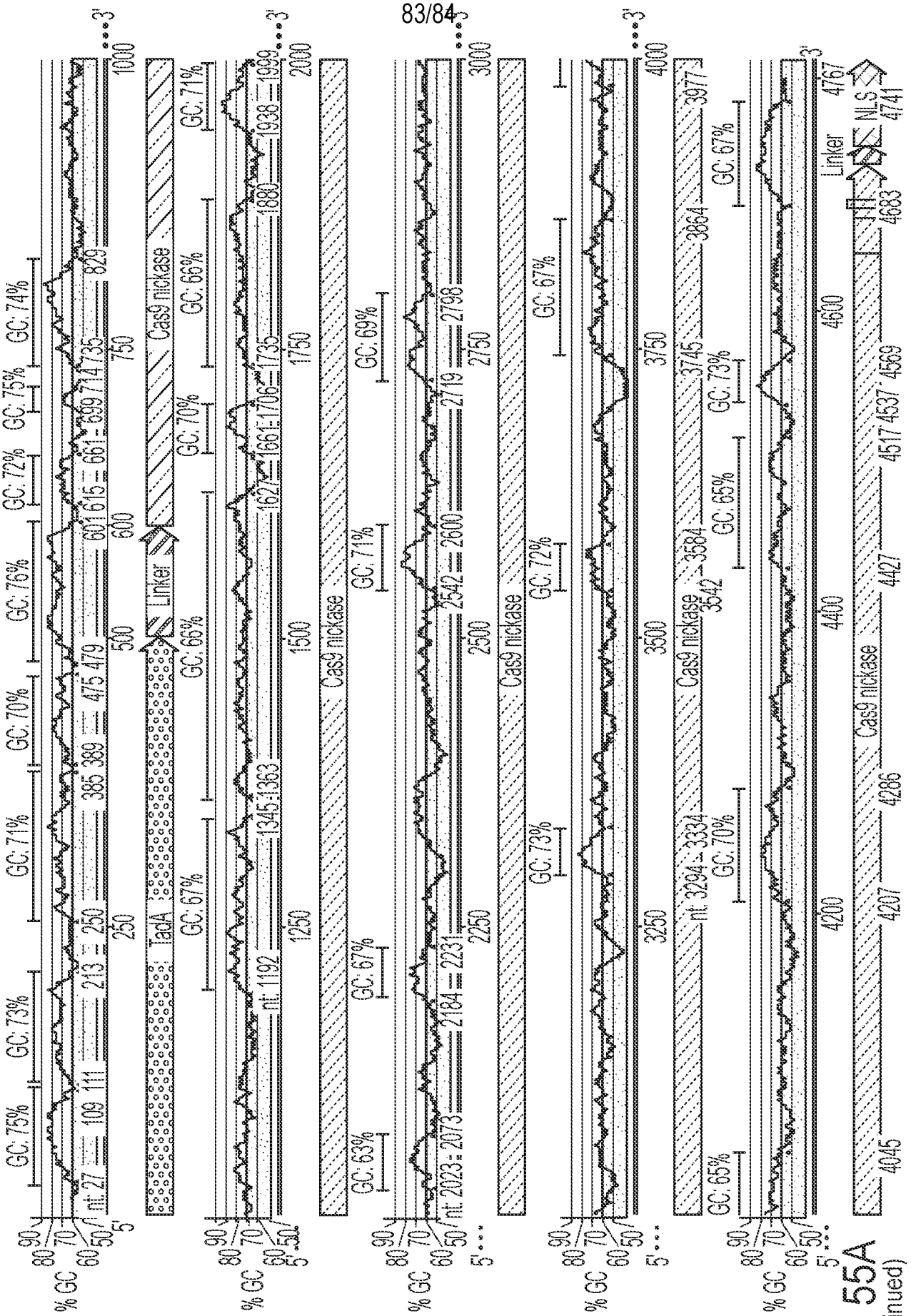


FIG. 55A
(Continued)

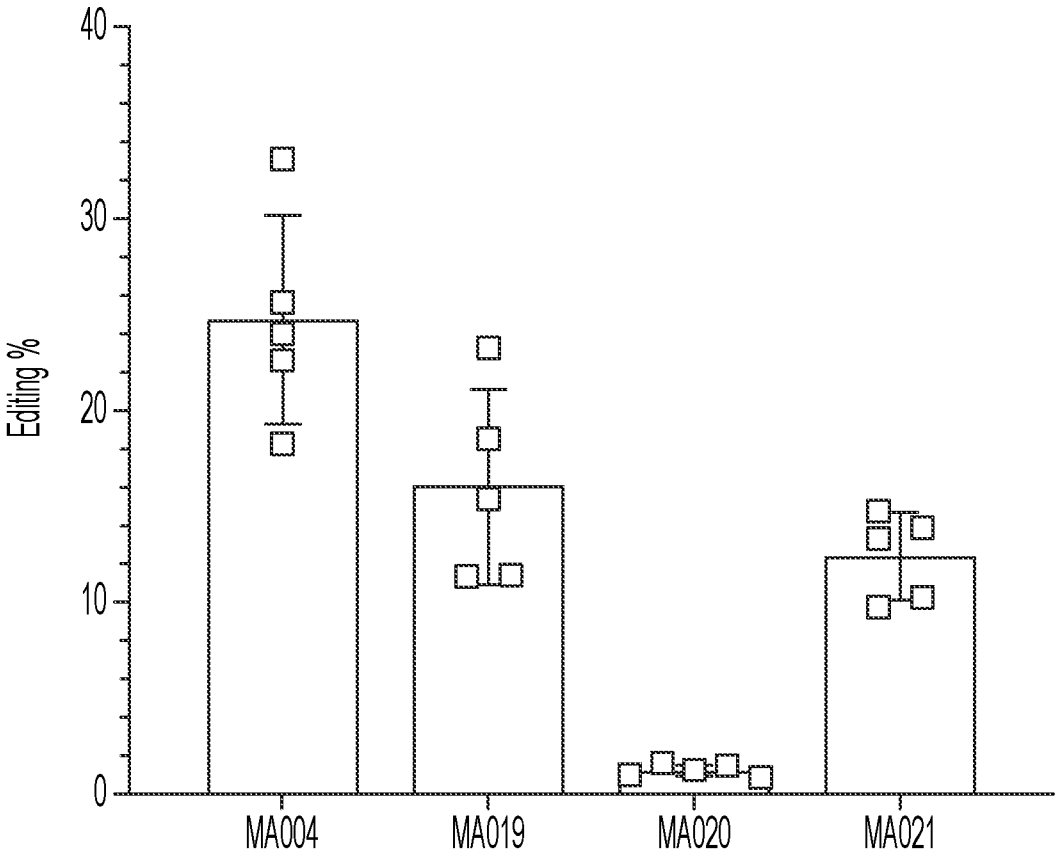


FIG. 55B