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(54) Title: ENZYMES WITH RUVC DOMAINS

(57) Abstract: The present disclosure provides for endonuclease enzymes having distinguishing domain features, as well as methods of using such enzymes or variants thereof.

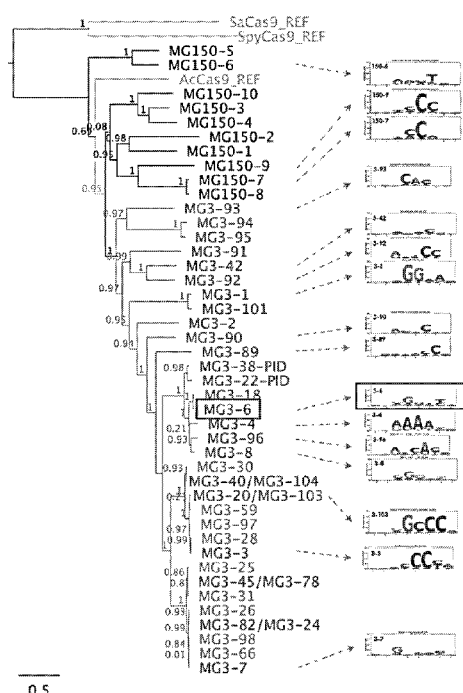


FIG. 24

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.

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ENZYMES WITH RUVB DOMAINS

RELATED APPLICATIONS

[0001] This application is related to PCT application no. PCT/US21/31136, which is incorporated by reference in its entirety herein.

CROSS-REFERENCE

[0002] This application claims the benefit of U.S. Provisional Application Nos: 63/237,791, filed on August 27, 2021; 63/245,629 filed on September 17, 2021; 63/252,956, filed on October 6, 2021; 63/282,909, filed on November 24, 2021; 63/316,895, filed on March 4, 2022; 63/319,681, filed on March 14, 2022; 63/322,944, filed on March 23, 2022; and 63/369,858, filed on July 29, 2022; each of which is incorporated by reference herein in its entirety.

BACKGROUND

[0003] Cas enzymes along with their associated Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) guide ribonucleic acids (RNAs) appear to be a pervasive (~45% of bacteria, ~84% of archaea) component of prokaryotic immune systems, serving to protect such microorganisms against non-self nucleic acids, such as infectious viruses and plasmids by CRISPR-RNA guided nucleic acid cleavage. While the deoxyribonucleic acid (DNA) elements encoding CRISPR RNA elements may be relatively conserved in structure and length, their CRISPR-associated (Cas) proteins are highly diverse, containing a wide variety of nucleic acid-interacting domains. While CRISPR DNA elements have been observed as early as 1987, the programmable endonuclease cleavage ability of CRISPR/Cas complexes has only been recognized relatively recently, leading to the use of recombinant CRISPR/Cas systems in diverse DNA manipulation and gene editing applications.

SEQUENCE LISTING

[0004] The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on August 26, 2022, is named 55921-731_601_SL.xml and is 23,191,225 bytes in size.

SUMMARY

[0005] In some aspects, the present disclosure provides for a method of disrupting a Beta-2-Microglobulin (B2M) locus in a cell, comprising contacting to the cell (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein the engineered guide RNA is

configured to form a complex with the endonuclease and the engineered guide RNA comprises a spacer sequence configured to hybridize to a region of the B2M locus, wherein the region of the B2M locus comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6387-6468. In some embodiments, the RNA-guided endonuclease is a class 2, type II Cas endonuclease. In some embodiments, the RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 2242 or SEQ ID NO: 2244. In some embodiments, the RNA-guided endonuclease further comprises an HNH domain. In some embodiments, the engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 6305-6386. In some embodiments, the region of the B2M locus comprises a sequence at least 75%, 80%, or 90% identical to at least 19 of the non-degenerate nucleotides of any one of SEQ ID NOs: 6388, 6399, 6401, 6403, 6410, 6413, 6421, 6446, and 6448.

[0006] In some aspects, the present disclosure provides for a method of editing a T Cell Receptor Alpha Constant (TRAC) locus in a cell, comprising contacting to the cell: (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein the engineered guide RNA is configured to form a complex with the endonuclease and the engineered guide RNA comprises a spacer sequence configured to hybridize to a region of the TRAC locus, wherein the region of the TRAC locus comprises a targeting sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6509-6548 or 6805. In some embodiments, the RNA-guided endonuclease is a class 2, type II Cas endonuclease. In some embodiments, the RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 2242 or SEQ ID NO: 2244. In some embodiments, the RNA-guided endonuclease further comprises an HNH domain. In some embodiments, the engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%,

at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 6469-6508 or 6804. In some embodiments, the region of the TRAC locus comprises a sequence at least 75%, 80%, or 90% identical to at least 19 of the non-degenerate nucleotides of any one of SEQ ID NOs: 6517, 6520, and 6523.

[0007] In some aspects, the present disclosure provides for a method of disrupting a Hypoxanthine Phosphoribosyltransferase 1 (HPRT) locus in a cell, comprising contacting to the cell: (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein the engineered guide RNA is configured to form a complex with the endonuclease and the engineered guide RNA comprises a spacer sequence configured to hybridize to a region of the HPRT locus, wherein the region of the HPRT locus comprises a targeting sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6616-6682. In some embodiments, the RNA-guided endonuclease is a class 2, type II Cas endonuclease. In some embodiments, the RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 2242 or SEQ ID NO: 2244. In some embodiments, the RNA-guided endonuclease further comprises an HNH domain. In some embodiments, the engineered guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 6549-6615. In some embodiments, the region of the HPRT locus comprises a sequence at least 75%, 80%, or 90% identical to at least 19 of the non-degenerate nucleotides of any one of SEQ ID NOs: 6619, 6634, 6673, 6675, and 6679.

[0008] In some aspects, the present disclosure provides for a method of editing a T Cell Receptor Beta Constant 1 or T Cell Receptor Beta Constant 2 (TRBC1/2) locus in a cell, comprising contacting to the cell: (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein the engineered guide RNA is configured to form a complex with the endonuclease and the engineered guide RNA comprises a spacer sequence configured to hybridize to a region of the TRBC1/2 locus, wherein the region of the TRBC1/2 locus comprises a targeting sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence

identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6722-6760 or 6782-6802. In some embodiments, the RNA-guided endonuclease is a class 2, type II Cas endonuclease. In some embodiments, the RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 2242 or SEQ ID NO: 2244. In some embodiments, the RNA-guided endonuclease further comprises an HNH domain. In some embodiments, the engineered guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 6683-6721 and 6761-6781. In some embodiments, the region of the TRBC1/2 locus comprises a sequence at least 75%, 80%, or 90% identical to at least 19 of the non-degenerate nucleotides of any one of SEQ ID NOs: 6734, 6753, 6790, and 6800.

[0009] In some aspects, the present disclosure provides for a method of editing an Hydroxyacid Oxidase 1 (HAO1) locus in a cell, comprising contacting to the cell: (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein the engineered guide RNA is configured to form a complex with the endonuclease and the engineered guide RNA comprises a spacer sequence configured to hybridize to a region of the HAO1 locus, wherein the region of the HAO1 locus comprises a targeting sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 11802-11820. In some embodiments, the RNA-guided endonuclease is a class 2, type II Cas endonuclease. In some embodiments, the RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 2242. In some embodiments, the RNA-guided endonuclease further comprises an HNH domain. In some embodiments, the region of the HAO1 locus comprises a sequence at least 75%, 80%, or 90% identical to at least 19 of the non-degenerate nucleotides of any one of SEQ ID NOs: 11806, 11813, 11816, and 11819.

[0010] In some aspects, the present disclosure provides for an engineered nuclease system comprising: (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein the engineered guide RNA comprises (i) a 2'-O-methyl nucleotide; (ii) a 2'-fluoro nucleotide; or (iii) a phosphorothioate bond; wherein the RNA-guided endonuclease has at least about 75%, at

least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 421-431 or a variant thereof. In some embodiments, the RNA-guided endonuclease comprises a sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 421.

[0011] In some aspects, the present disclosure provides for an engineered nuclease system comprising: (a) an endonuclease having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 421-431 or a variant thereof; and (b) an engineered guide RNA, wherein the engineered guide RNA is configured to form a complex with the endonuclease and the engineered guide RNA comprises a spacer sequence configured to hybridize to a target nucleic acid sequence, wherein the system has reduced immunogenicity when administered to a human subject compared to an equivalent system comprising a Cas9 enzyme. In some embodiments, the Cas9 enzyme is an SpCas9 enzyme. In some embodiments, the immunogenicity is antibody immunogenicity. In some embodiments, the engineered guide RNA comprises a sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of any one of SEQ ID NOs: 5466-5467 and 11160-11162. In some embodiments, the engineered nuclease has at least about 75% sequence identity at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 421 or 423 or a variant thereof.

[0012] In some aspects, the present disclosure provides for a method of editing a locus in a cell, comprising contacting to the cell: (a) an RNA-guided endonuclease or a nucleic acid encoding the RNA-guided endonuclease; and (b) an engineered guide RNA, wherein the engineered guide RNA is configured to form a complex with the RNA-guided endonuclease and the engineered guide RNA comprises a spacer sequence configured to hybridize to a region of the locus; wherein the cell is a peripheral blood mononuclear cell (PBMC), a hematopoietic stem cell

(HSC), or an induced pluripotent stem cell (iPSC). In some embodiments, the RNA-guided endonuclease is a class 2, type II Cas endonuclease. In some embodiments, the RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 2242, or a variant thereof. In some embodiments, the RNA-guided endonuclease further comprises an HNH domain. In some embodiments, the RNA-guided endonuclease has at least about 75% sequence identity at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 421 or a variant thereof. In some embodiments, the engineered guide RNA comprises a sequence at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 6804, 6806, and 6808. In some embodiments, the nucleic acid encoding the RNA-guided endonuclease comprises a sequence comprising at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 6803 or a variant thereof. In some embodiments, the region of the locus comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to at least 18 nucleotides of any one of SEQ ID NOs: 6805, 6807, and 6809.

[0013] In some aspects, the present disclosure provides for a method of editing a CD2 Molecule (CD2) locus in a cell, comprising contacting to the cell: (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein the engineered guide RNA is configured to form a complex with the endonuclease and the engineered guide RNA comprises a spacer sequence configured to hybridize to a region of the CD2 locus, wherein the engineered guide RNA comprises or is configured to hybridize to a sequence having at least 18-22 consecutive nucleotides complementary to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 6853-6894; or wherein the engineered

guide RNA comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to the non-degenerate nucleotides of any one of SEQ ID NOs: 6811-6852. In some embodiments, the RNA-guided endonuclease is a class 2, type II Cas endonuclease. In some embodiments, the RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 2242 or SEQ ID NO: 2244, or a variant thereof. In some embodiments, the RNA-guided endonuclease further comprises an HNH domain. In some embodiments, the RNA-guided endonuclease comprises a sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 421-431. In some embodiments, the RNA-guided endonuclease comprises a sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 421, or a variant thereof. In some embodiments, the engineered guide RNA comprises a sequence at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to the non-degenerate nucleotides of any one of SEQ ID NOs: 6813, 6841, 6843-6847, 6852, or 6852. In some embodiments, the engineered guide RNA further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 6A. In some embodiments, the engineered guide RNA comprises or is configured to hybridize to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6855, 6883, 6885-6889, 6892, or 6984.

[0014] In some aspects, the present disclosure provides for an isolated RNA molecule comprising a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at

least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 6811-6852. In some embodiments, the RNA molecule further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 6A.

[0015] In some aspects, the present disclosure provides for a method of editing a CD5 Molecule (CD5) locus in a cell comprising contacting to the cell: (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein the engineered guide RNA is configured to form a complex with the endonuclease and the engineered guide RNA comprises a spacer sequence configured to hybridize to a region of the CD5 locus, wherein the engineered guide RNA comprises or is configured to hybridize to a sequence having at least 18-22 consecutive nucleotide complementary to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID Nos: 6959-7022; or wherein the engineered guide RNA comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity the non-degenerate nucleotides of any one of SEQ ID NOs: 5466 or 6895-6958. In some embodiments, the RNA-guided endonuclease is a class 2, type II Cas endonuclease. In some embodiments, the RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 2242 or SEQ ID NO: 2244, or a variant thereof. In some embodiments, the RNA-guided endonuclease further comprises an HNH domain. In some embodiments, the RNA-guided endonuclease comprises an endonuclease comprising a sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 421-431 or a variant thereof. In some embodiments, the RNA-guided endonuclease comprises a sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 421. In some embodiments, the engineered guide RNA comprises a sequence

having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of SEQ ID NO: 5466. In some embodiments, the engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to the non-degenerate nucleotides of any one of SEQ ID NOs: 6897, 6904, 6906, 6911, 6928, 6930, 6932, 6934, 6938, 6945, 6950, 6952, and 6958. In some embodiments, the engineered guide RNA further comprises a pattern of nucleotide modification recited in any of the guide RNAs recited in Table 7A. In some embodiments, the engineered guide RNA is configured to hybridize to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6961, 6968, 6970, 6975, 6992, 6994, 6996, 6998, 7002, 7009, 7014, 7016, and 7022.

[0016] In some aspects, the present disclosure provides for an isolated RNA molecule comprising a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 6895-6958. In some embodiments, the RNA molecule further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 7A.

[0017] In some aspects, the present disclosure provides for a method of editing an RNA locus in a cell, comprising contacting to the cell: (a) an RNA-guided endonuclease comprising a sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 2242 or SEQ ID NO: 2244, or a variant thereof, and (b) an engineered guide RNA, wherein the engineered guide RNA is configured to form a complex with the endonuclease and the engineered guide RNA comprises a spacer sequence configured to hybridize to a region of the RNA locus, wherein the RNA locus does not comprise bacterial or microbial RNA. In some embodiments, the guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%,

at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of SEQ ID NO: 5466 or SEQ ID NO: 5539.

[0018] In some aspects, the present disclosure provides for a method of disrupting a Fas Cell Surface Death Receptor (FAS) locus in a cell, comprising introducing to the cell: (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein the engineered guide RNA is configured to form a complex with the endonuclease and the engineered guide RNA comprises a spacer sequence configured to hybridize to a region of the human FAS locus, wherein the engineered guide RNA comprises or is configured to hybridize to a sequence having at least 18-22 consecutive nucleotides complementary to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7057-7090; or wherein the engineered guide RNA comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7023-7056. In some embodiments, the RNA-guided endonuclease is a class 2, type II Cas endonuclease. In some embodiments, the RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 2242, or a variant thereof. In some embodiments, the RNA-guided endonuclease further comprises an HNH domain. In some embodiments, the engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of SEQ ID NO: 5466. In some embodiments, the engineered guide RNA comprises or is configured to hybridize to a sequence having at least 80% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 7059, 7061, 7069, 7070, 7076, 7080, 7083, 7084, 7085, or 7088. In some embodiments, the RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 421, or a variant thereof. In some embodiments, the guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 7025, 7027, 7035, 7036, 7042, 7046, 7049-7051, or 7054. In some embodiments, the guide RNA further

comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 8.

[0019] In some aspects, the present disclosure provides for an isolated RNA molecule comprising a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7023-7056. In some embodiments, the RNA molecule further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 8.

[0020] In some aspects, the present disclosure provides for a method of disrupting a Programmed Cell Death 1 (PD-1) locus in a cell, comprising introducing to the cell: (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein the engineered guide RNA is configured to form a complex with the endonuclease and the engineered guide RNA comprises a spacer sequence configured to hybridize to a region of the human PD-1 locus, wherein the engineered guide RNA comprises or is configured to hybridize to a sequence having at least 18-22 consecutive nucleotides complementary to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7129-7166; or wherein the engineered guide RNA comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7091-7128. In some embodiments, the RNA-guided endonuclease is a class 2, type II Cas endonuclease. In some embodiments, the RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 2242, or a variant thereof. In some embodiments, the RNA-guided endonuclease further comprises an HNH domain. In some embodiments, the engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of SEQ ID NO: 5466. In some embodiments, the engineered guide RNA comprises or is configured to

hybridize to a sequence having at least 80% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 7135, 7137, 7146, 7149, 7152, 7156, 7160, 7161, 7164, 7165, or 7166. In some embodiments, the RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 421, or a variant thereof. In some embodiments, the guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 7097, 7099, 7108, 7111, 7114, 7118, 7122, 7123, 7126, 7127, or 7128. In some embodiments, the guide RNA further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 9.

[0021] In some aspects, the present disclosure provides for an isolated RNA molecule comprising a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7091-7128. In some embodiments, the RNA molecule further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 9.

[0022] In some aspects, the present disclosure provides for a method of disrupting an human Rosa26 (hRosa26) locus in a cell, comprising introducing to the cell: (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein the engineered guide RNA is configured to form a complex with the endonuclease and the engineered guide RNA comprises a spacer sequence configured to hybridize to a region of the hRosa26 locus, wherein the engineered guide RNA comprises or is configured to hybridize to a sequence having at least 18-22 consecutive nucleotides complementary to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7199-7230; or wherein the engineered guide RNA comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7167-7198. In some embodiments, the RNA-guided endonuclease is a class 2, type II Cas endonuclease. In some embodiments, the RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 2242, or a variant thereof. In some

embodiments, the RNA-guided endonuclease further comprises an HNH domain. In some embodiments, the engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of SEQ ID NO: 5466. In some embodiments, the engineered guide RNA comprises or is configured to hybridize to a sequence having at least 80% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 7205-7206, 7215, 7220, 7223, or 7225. In some embodiments, the RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 421, or a variant thereof. In some embodiments, the guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 7173, 7174, 7183, 7188, 7191, or 7193. In some embodiments, the guide RNA further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 10.

[0023] In some aspects, the present disclosure provides for an isolated RNA molecule comprising a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7167-7198. In some embodiments, the RNA molecule further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 10.

[0024] In some aspects, the present disclosure provides for a method of disrupting an T Cell Receptor Alpha Constant (TRAC) locus in a cell, comprising introducing to the cell: (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein the engineered guide RNA is configured to form a complex with the endonuclease and the engineered guide RNA comprises a spacer sequence configured to hybridize to a region of the TRAC locus, wherein the engineered guide RNA comprises or is configured to hybridize to a sequence having at least 18-22 consecutive nucleotides complementary to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7235-7238, 7248-7256, 7270, or 7278-7284; or wherein the engineered guide RNA comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7231-7234, 7239-7247, 7269, or 7271-7277. In some embodiments,

the RNA-guided endonuclease is a class 2, type II Cas endonuclease. In some embodiments, the RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 1512, 1756, 11711-11713, or a variant thereof. In some embodiments, the engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of SEQ ID NO: 5473, 5475, 11145, 11714, or 11715. In some embodiments, the engineered guide RNA comprises or is configured to hybridize to a sequence having at least 80% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 7235-7238, 7248-7256, 7270, or 7278-7284. In some embodiments, the guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 7231-7234, 7239-7244, 7269, or 7271-7277. In some embodiments, the engineered guide RNA further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 11.

[0025] In some aspects, the present disclosure provides for an isolated RNA molecule comprising a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7231-7234, 7239-7247, 7269, or 7271-7277. In some embodiments, the RNA molecule further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 11.

[0026] In some aspects, the present disclosure provides for a method of disrupting an Adeno-Associated Virus Integration Site 1 (AAVS1) locus in a cell, comprising introducing to the cell: (a) a class 2, type II Cas endonuclease; and (b) an engineered guide RNA, wherein the engineered guide RNA is configured to form a complex with the endonuclease and the engineered guide RNA comprises a spacer sequence configured to hybridize to a region of the AAVS1 locus, wherein the engineered guide RNA comprises or is configured to hybridize to a sequence having at least 18-22 consecutive nucleotides complementary to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7261-7264 or 7267-7268; or wherein the engineered guide RNA comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to

any one of SEQ ID NOs: 7257-7260 or 7265-7266. In some embodiments, the RNA-guided endonuclease is a class 2, type II Cas endonuclease. In some embodiments, the RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 1756 or 11711, or a variant thereof. In some embodiments, the engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of SEQ ID NO: 5475 or 11715. In some embodiments, the engineered guide RNA comprises or is configured to hybridize to a sequence having at least 80% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 7261-7263 or 7267-7268. In some embodiments, the guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 7257-7260 or 7265-7266. In some embodiments, the engineered guide RNA further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 12.

[0027] In some aspects, the present disclosure provides for an isolated RNA molecule comprising a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7257-7260 or 7265-7266. In some embodiments, the RNA molecule further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 12.

[0028] In some aspects, the present disclosure provides for a method of disrupting an Hydroxyacid Oxidase 1 (HAO-1) locus in a cell, comprising introducing to the cell: (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein the engineered guide RNA is configured to form a complex with the endonuclease and the engineered guide RNA comprises a spacer sequence configured to hybridize to a region of the HAO-1 locus, wherein the engineered guide RNA comprises or is configured to hybridize to a sequence having at least 18-22 consecutive nucleotides complementary to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11773-11793. In some embodiments, the RNA-guided endonuclease is a class 2, type II Cas endonuclease. In some embodiments, the RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about

95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 2242, or a variant thereof. In some embodiments, the RNA-guided endonuclease further comprises an HNH domain. In some embodiments, the engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of SEQ ID NO: 5466. In some embodiments, the engineered guide RNA comprises or is configured to hybridize to a sequence having at least 80% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 11773, 11780, 11786, or 11787. In some embodiments, the RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 421, or a variant thereof.

[0029] In some aspects, the present disclosure provides for an isolated RNA molecule comprising a spacer sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11773-11793 and a scaffold sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 5466.

[0030] In some aspects, the present disclosure provides for a method of disrupting a human G Protein-Coupled Receptor 146 (GPR146) locus in a cell, comprising introducing to the cell: (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein the engineered guide RNA is configured to form a complex with the endonuclease and the engineered guide RNA comprises a spacer sequence configured to hybridize to a region of the GPR146 locus, wherein the engineered guide RNA comprises or is configured to hybridize to a sequence having at least 18-22 consecutive nucleotides complementary to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11406-11437; or wherein the engineered guide RNA comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11374-11405. In some embodiments, the RNA-guided endonuclease is a class 2, type II Cas

endonuclease. In some embodiments, the RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 2242, or a variant thereof. In some embodiments, the RNA-guided endonuclease further comprises an HNH domain. In some embodiments, the engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of SEQ ID NO: 5466. In some embodiments, the engineered guide RNA comprises or is configured to hybridize to a sequence having at least 80% identity to at least 18 consecutive nucleotides of SEQ ID NO: 11425. In some embodiments, the RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 421, or a variant thereof. In some embodiments, the guide RNA comprises a sequence having at least 80% identity to SEQ ID NO: 11393. In some embodiments, the engineered guide RNA further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 15.

[0031] In some aspects, the present disclosure provides for an isolated RNA molecule comprising a spacer sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11374-11405. In some embodiments, the RNA molecule further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 15.

[0032] In some aspects, the present disclosure provides for a method of disrupting a mouse G Protein-Coupled Receptor 146 (GPR146) locus in a cell, comprising introducing to the cell: (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein the engineered guide RNA is configured to form a complex with the endonuclease and the engineered guide RNA comprises a spacer sequence configured to hybridize to a region of the GPR146 locus, wherein the engineered guide RNA comprises or is configured to hybridize to a sequence having at least 18-22 consecutive nucleotides complementary to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11473-11507; or wherein the engineered guide RNA comprises a nucleotide sequence having at least about 80%,

at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11438-11472. In some embodiments, the RNA-guided endonuclease is a class 2, type II Cas endonuclease. In some embodiments, the RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 2242, or a variant thereof. In some embodiments, the RNA-guided endonuclease further comprises an HNH domain. In some embodiments, the engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of SEQ ID NO: 5466. In some embodiments, the engineered guide RNA comprises or is configured to hybridize to a sequence having at least 80% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 11482, 11488, or 11490. In some embodiments, the RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 421, or a variant thereof. In some embodiments, the guide RNA comprises a sequence having at least 80% identity to SEQ ID NO: 11447, 11453, or 11455. In some embodiments, the engineered guide RNA further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 16.

[0033] In some aspects, the present disclosure provides for an isolated RNA molecule comprising a spacer sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11438-11472. In some embodiments, the RNA molecule further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 16.

[0034] In some aspects, the present disclosure provides for a method of disrupting a T Cell Receptor Alpha Constant (TRAC) locus in a cell, comprising introducing to the cell: (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein the engineered guide RNA is configured to form a complex with the endonuclease and the engineered guide RNA comprises a spacer sequence configured to hybridize to a region of the TRAC locus, wherein the engineered guide RNA comprises or is configured to hybridize to a sequence having at least 18-

22 consecutive nucleotides complementary to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11516-11517; or wherein the engineered guide RNA comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11514-11515. In some embodiments, the RNA-guided endonuclease is a class 2, type II Cas endonuclease. In some embodiments, the engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of SEQ ID NO: 11153. In some embodiments, the engineered guide RNA comprises or is configured to hybridize to a sequence having at least 80% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 11516. In some embodiments, the RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 11716, or a variant thereof. In some embodiments, the guide RNA comprises a sequence having at least 80% identity to SEQ ID NO: 11514. In some embodiments, the engineered guide RNA further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 17.

[0035] In some aspects, the present disclosure provides for an isolated RNA molecule comprising a spacer sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11514-11515. In some embodiments, the RNA molecule further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 17.

[0036] In some aspects, the present disclosure provides for a method of disrupting an Adeno-Associated Virus Integration Site 1 (AAVS1) locus in a cell, comprising introducing to the cell: (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein the engineered guide RNA is configured to form a complex with the endonuclease and the engineered guide RNA comprises a spacer sequence configured to hybridize to a region of the AAVS1 locus, wherein the engineered guide RNA comprises or is configured to hybridize to a sequence having at least 18-22 consecutive nucleotides complementary to a sequence having at

least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11511-11513; or wherein the engineered guide RNA comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11508-11510. In some embodiments, the RNA-guided endonuclease is a class 2, type II Cas endonuclease. In some embodiments, the engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of SEQ ID NO: 11717. In some embodiments, the engineered guide RNA comprises or is configured to hybridize to a sequence having at least 80% identity to at least 18 consecutive nucleotides of SEQ ID NO: 11511. In some embodiments, the RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 914, or a variant thereof. In some embodiments, the guide RNA comprises a sequence having at least 80% identity to SEQ ID NO: 11508. In some embodiments, the engineered guide RNA further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 17.

[0037] In some aspects, the present disclosure provides for an isolated RNA molecule comprising a spacer sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11508-11510. In some embodiments, the RNA molecule further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 17.

[0038] In some aspects, the present disclosure provides for an engineered nuclease system comprising: (a) an endonuclease having at least at least about 80%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to a PI domain of any of the Cas effector protein sequences described herein, or a variant thereof; and (b) an engineered guide RNA, wherein the engineered guide RNA is configured to form a complex with the endonuclease and the engineered guide RNA comprises a

spacer sequence configured to hybridize to a target nucleic acid sequence, wherein the engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of any of the sgRNA sequences described herein. In some embodiments, the endonuclease further comprises a RuvCIII domain or a HNH domain having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to RuvCIII domains or HNH domains of any of the Cas effector nucleases described herein. In some embodiments, the endonuclease is configured to have selectivity for any of the PAM sequences described herein. In some embodiments, the endonuclease further comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any of the Cas effector sequences described herein.

[0039] In some aspects, the present disclosure provides for use of any of the methods described herein for disrupting a B2M locus in a cell.

[0040] In some aspects, the present disclosure provides for use of any of the methods described herein or any of the RNA molecules described herein for disrupting a TRAC locus in a cell.

[0041] In some aspects, the present disclosure provides for use of any of the methods described herein for disrupting an HPRT locus in a cell.

[0042] In some aspects, the present disclosure provides for use of any of the methods described herein for disrupting a TRBC1/2 locus in a cell.

[0043] In some aspects, the present disclosure provides for use of any of the methods described herein or any of the RNA molecules described herein for disrupting an HAO-1 locus in a cell.

[0044] In some aspects, the present disclosure provides for use of any of the methods described herein or any of the RNA molecules described herein for disrupting a CD2 locus in a cell.

[0045] In some aspects, the present disclosure provides for use of any of the methods described herein or any of the RNA molecules described herein for disrupting a CD5 locus in a cell.

[0046] In some aspects, the present disclosure provides for use of any of the methods described herein or any of the RNA molecules described herein for disrupting a FAS locus in a cell.

[0047] In some aspects, the present disclosure provides for use of any of the methods described herein or any of the RNA molecules described herein for disrupting a PD-1 locus in a cell.

[0048] In some aspects, the present disclosure provides for use of any of the methods described herein or any of the RNA molecules described herein for disrupting an hRosa26 locus in a cell.

[0049] In some aspects, the present disclosure provides for use of any of the methods described herein or any of the RNA molecules described herein for disrupting an AAVS1 locus in a cell.

[0050] In some aspects, the present disclosure provides for use of any of the methods described herein or any of the RNA molecules described herein for disrupting a GPR146 locus in a cell.

[0051] In some aspects, the present disclosure provides for an engineered nuclease system, comprising: (a) an endonuclease comprising a RuvC_III domain and an HNH domain, wherein the endonuclease is derived from an uncultivated microorganism, wherein the endonuclease is a class 2, type II Cas endonuclease; and (b) an engineered guide ribonucleic acid structure configured to form a complex with the endonuclease comprising: (i) a guide ribonucleic acid sequence configured to hybridize to a target deoxyribonucleic acid sequence; and (ii) a tracr ribonucleic acid sequence configured to bind to the endonuclease. In some embodiments, the RuvC_III domain comprises a sequence with at least 70%, at least 75%, at least 80%, at least 85%, at least 88%, at least 90%, at least 92%, at least 95%, or at least 98% sequence identity to any one of SEQ ID NOs: 1827-3637.

[0052] In some aspects, the present disclosure provides for an engineered nuclease system comprising: (a) an endonuclease comprising a RuvC_III domain having at least 70%, at least 75%, at least 80%, at least 85%, at least 88%, at least 90%, at least 92%, at least 95%, or at least 98% sequence identity to any one of SEQ ID NOs: 1827-3637; and (b) an engineered guide ribonucleic acid structure configured to form a complex with the endonuclease comprising: (i) a guide ribonucleic acid sequence configured to hybridize to a target deoxyribonucleic acid sequence; and (ii) a tracr ribonucleic acid sequence configured to bind to the endonuclease.

[0053] In some aspects, the present disclosure provides for an engineered nuclease system comprising: (a) an endonuclease configured to bind to a protospacer adjacent motif (PAM) sequence comprising SEQ ID NOs: 5512-5537, wherein the endonuclease is a class 2, type II Cas endonuclease; and (b) an engineered guide ribonucleic acid structure configured to form a complex with the endonuclease comprising: (i) a guide ribonucleic acid sequence configured to hybridize to a target deoxyribonucleic acid sequence; and (ii) a tracr ribonucleic acid sequence configured to bind to the endonuclease.

[0054] In some embodiments, the endonuclease is derived from an uncultivated microorganism. In some embodiments, the endonuclease has not been engineered to bind to a different PAM sequence. In some embodiments, the endonuclease is not a Cas9 endonuclease, a Cas14 endonuclease, a Cas12a endonuclease, a Cas12b endonuclease, a Cas 12c endonuclease, a Cas12d endonuclease, a Cas12e endonuclease, a Cas13a endonuclease, a Cas13b endonuclease,

a Cas13c endonuclease, or a Cas 13d endonuclease. In some embodiments, the endonuclease has less than 80% identity to a Cas9 endonuclease. In some embodiments, the endonuclease further comprises an HNH domain. In some embodiments, the tracr ribonucleic acid sequence comprises a sequence with at least 80% sequence identity to about 60 to 90 consecutive nucleotides selected from any one of SEQ ID NOs: 5476-5511 and SEQ ID NO: 5538.

[0055] In some aspects, the present disclosure provides for an engineered nuclease system comprising, (a) an engineered guide ribonucleic acid structure comprising: (i) a guide ribonucleic acid sequence configured to hybridize to a target deoxyribonucleic acid sequence; and (ii) a tracr ribonucleic acid sequence configured to bind to an endonuclease, wherein the tracr ribonucleic acid sequence comprises a sequence with at least 80% sequence identity to about 60 to 90 consecutive nucleotides selected from any one of SEQ ID NOs: 5476-5511 and SEQ ID NO: 5538; and (b) a class 2, type II Cas endonuclease configured to bind to the engineered guide ribonucleic acid. In some embodiments, the endonuclease is configured to bind to a protospacer adjacent motif (PAM) sequence selected from the group comprising SEQ ID NOs: 5512-5537.

[0056] In some embodiments, the engineered guide ribonucleic acid structure comprises at least two ribonucleic acid polynucleotides. In some embodiments, the engineered guide ribonucleic acid structure comprises one ribonucleic acid polynucleotide comprising the guide ribonucleic acid sequence and the tracr ribonucleic acid sequence.

[0057] In some embodiments, the guide ribonucleic acid sequence is complementary to a prokaryotic, bacterial, archaeal, eukaryotic, fungal, plant, mammalian, or human genomic sequence. In some embodiments, the guide ribonucleic acid sequence is 15-24 nucleotides in length. In some embodiments, the endonuclease comprises one or more nuclear localization sequences (NLSs) proximal to an N- or C-terminus of the endonuclease. In some embodiments, the NLS comprises a sequence selected from SEQ ID NOs: 5597-5612.

[0058] In some embodiments, the engineered nuclease system further comprises a single- or double-stranded DNA repair template comprising from 5' to 3': a first homology arm comprising a sequence of at least 20 nucleotides 5' to the target deoxyribonucleic acid sequence, a synthetic DNA sequence of at least 10 nucleotides, and a second homology arm comprising a sequence of at least 20 nucleotides 3' to the target sequence. In some embodiments, the first or second homology arm comprises a sequence of at least 40, 80, 120, 150, 200, 300, 500, or 1,000 nucleotides.

[0059] In some embodiments, the system further comprises a source of Mg²⁺.

[0060] In some embodiments, the endonuclease and the tracr ribonucleic acid sequence are derived from distinct bacterial species within a same phylum. In some embodiments, the

endonuclease is derived from a bacterium belonging to a genus *Dermabacter*. In some embodiments, the endonuclease is derived from a bacterium belonging to Phylum Verrucomicrobia, Phylum Candidatus Peregrinibacteria, or Phylum Candidatus Melainabacteria. In some embodiments, the endonuclease is derived from a bacterium comprising a 16S rRNA gene having at least 90% identity to any one of SEQ ID NOs: 5592-5595.

[0061] In some embodiments, the HNH domain comprises a sequence with at least 70% or at least 80% identity to any one of SEQ ID NOs: 5638-5460. In some embodiments, the endonuclease comprises SEQ ID NOs: 1-1826 or a variant thereof having at least 55% identity thereto. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 1827-1830 or SEQ ID NOs: 1827-2140.

[0062] In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 3638-3641 or SEQ ID NOs: 3638-3954. In some embodiments, the endonuclease comprises at least 1, at least 2, at least 3, at least 4, or at least 5 peptide motifs selected from the group consisting of SEQ ID NOs: 5615-5632. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 1-4 or SEQ ID NOs: 1-319.

[0063] In some embodiments, the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 5461-5464, SEQ ID NOs: 5476-5479, or SEQ ID NOs: 5476-5489. In some embodiments, the guide RNA structure comprises an RNA sequence predicted to comprise a hairpin consisting of a stem and a loop, wherein the stem comprises at least 10, at least 12 or at least 14 base-paired ribonucleotides, and an asymmetric bulge within 4 base pairs of the loop.

[0064] In some embodiments, the endonuclease is configured to bind to a PAM comprising a sequence selected from the group consisting of SEQ ID NOs: 5512-5515 or SEQ ID NOs: 5527-5530.

[0065] In some embodiments: (a) the endonuclease comprises a sequence at least 70%, at least 80%, or at least 90% identical to SEQ ID NO: 1827; (b) the guide RNA structure comprises a sequence at least 70%, at least 80%, or at least 90% identical to at least one of SEQ ID NO: 5461 or SEQ ID NO: 5476; and (c) the endonuclease is configured to bind to a PAM comprising SEQ ID NO: 5512 or SEQ ID NO: 5527. In some embodiments: (a) the endonuclease comprises a sequence at least 70%, at least 80%, or at least 90% identical to SEQ ID NO: 1828; (b) the guide RNA structure comprises a sequence at least 70%, at least 80%, or at least 90% identical to at least one of SEQ ID NO: 5462 or SEQ ID NO: 5477; and (c) the endonuclease is

configured to bind to a PAM comprising SEQ ID NO: 5513 or SEQ ID NO: 5528. In some embodiments: (a) the endonuclease comprises a sequence at least 70%, at least 80%, or at least 90% identical to SEQ ID NO: 1829; (b) the guide RNA structure comprises a sequence at least 70%, at least 80%, or at least 90% identical to at least one of SEQ ID NO: 5463 or SEQ ID NO: 5478; and (c) the endonuclease is configured to bind to a PAM comprising SEQ ID NO: 5514 or SEQ ID NO: 5529. In some embodiments: (a) the endonuclease comprises a sequence at least 70%, at least 80%, or at least 90% identical to SEQ ID NO: 1830; (b) the guide RNA structure comprises a sequence at least 70%, at least 80%, or at least 90% identical to at least one of SEQ ID NO: 5464 or SEQ ID NO: 5479; and (c) the endonuclease is configured to bind to a PAM comprising SEQ ID NO: 5515 or SEQ ID NO: 5530.

[0066] In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 2141-2142 or SEQ ID NOs: 2141-2241. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 3955-3956 or SEQ ID NOs: 3955-4055. In some embodiments, the endonuclease comprises at least 1, at least 2, at least 3, at least 4, or at least 5 peptide motifs selected from the group consisting of SEQ ID NOs: 5632-5638. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 320-321 or SEQ ID NOs: 320-420. In some embodiments, the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 5465, SEQ ID NOs: 5490-5491 or SEQ ID NOs: 5490-5494. In some embodiments, the guide RNA structure comprises a tracr ribonucleic acid sequence comprising a hairpin comprising at least 8, at least 10, or at least 12 base-paired ribonucleotides. In some embodiments, the endonuclease is configured to bind to a PAM comprising a sequence selected from the group consisting of SEQ ID NOs: 5516 and SEQ ID NOs: 5531. In some embodiments: (a) the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 2141; (b) the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 5490; and (c) the endonuclease is configured to binding to a PAM comprising SEQ ID NO: 5531. In some embodiments: (a) the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 2142; (b) the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 5465 or SEQ ID NO: 5491; and (c) the endonuclease is configured to binding to a PAM comprising SEQ ID NO: 5516.

[0067] In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 2245-2246. In

some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 4059-4060. In some embodiments, the endonuclease comprises at least 1, at least 2, at least 3, at least 4, or at least 5 peptide motifs selected from the group consisting of SEQ ID NOs: 5639-5648. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 424-425. In some embodiments, the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 5498-5499 and SEQ ID NO: 5539. In some embodiments, the guide RNA structure comprises a guide ribonucleic acid sequence predicted to comprise a hairpin with an uninterrupted base-paired region comprising at least 8 nucleotides of a guide ribonucleic acid sequence and at least 8 nucleotides of a tracr ribonucleic acid sequence, and wherein the tracr ribonucleic acid sequence comprises, from 5' to 3', a first hairpin and a second hairpin, wherein the first hairpin has a longer stem than the second hairpin.

[0068] In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 2242-2244 or SEQ ID NOs: 2247-2249. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 4056-4058 and SEQ ID NOs 4061-4063. In some embodiments, the endonuclease comprises at least 1, at least 2, at least 3, at least 4, or at least 5 peptide motifs selected from the group consisting of SEQ ID NOs: 5639-5648. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 421-423 or SEQ ID NOs: 426-428. In some embodiments, the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 5466-5467, SEQ ID NOs: 5495-5497, SEQ ID NO: 5500-5502, and SEQ ID NO: 5539. In some embodiments, the guide RNA structure comprises a guide ribonucleic acid sequence predicted to comprise a hairpin with an uninterrupted base-paired region comprising at least 8 nucleotides of a guide ribonucleic acid sequence and at least 8 nucleotides of a tracr ribonucleic acid sequence, and wherein the tracr ribonucleic acid sequence comprises, from 5' to 3', a first hairpin and a second hairpin, wherein the first hairpin has a longer stem than the second hairpin. In some embodiments, the endonuclease is configured to binding to a PAM comprising a sequence selected from the group consisting of SEQ ID NOs: 5517-5518 or SEQ ID NOs: 5532-5534. In some embodiments: (a) the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 2247; (b) the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 5500; and (c) the endonuclease is configured to binding to a PAM comprising SEQ ID NO: 5517 or SEQ

ID NO: 5532. In some embodiments: (a) the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 2248; (b) the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 5501; and (c) the endonuclease is configured to binding to a PAM comprising SEQ ID NO: 5518 or SEQ ID NOs: 5533. In some embodiments: (a) the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 2249; (b) the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 5502; and (c) the endonuclease is configured to binding to a PAM comprising SEQ ID NO: 5534.

[0069] In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 2253 or SEQ ID NOs: 2253-2481. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 4067 or SEQ ID NOs: 4067-4295. In some embodiments, the endonuclease comprises a peptide motif according to SEQ ID NO: 5649. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 432 or SEQ ID NOs: 432-660. In some embodiments, the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 5468 or SEQ ID NO: 5503. In some embodiments, the endonuclease is configured to binding to a PAM comprising a sequence selected from the group consisting of SEQ ID NOs: 5519. In some embodiments: (a) the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 2253; (b) the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 5468 or SEQ ID NO: 5503; and (c) the endonuclease is configured to binding to a PAM comprising SEQ ID NO: 5519.

[0070] In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 2482-2489. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 4296-4303. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of or SEQ ID NOs: 661-668. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of or SEQ ID NOs: 2490-2498. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 4304-4312. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 669-677. In some embodiments, the guide

RNA structure comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 5504.

[0071] In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 2499 or SEQ ID NOs: 2499-2750. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 4313 or SEQ ID NOs: 4313-4564. In some embodiments, the endonuclease comprises at least 1, at least 2, at least 3, at least 4, or at least 5 peptide motifs selected from the group consisting of SEQ ID NOs: 5650-5667. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 678 or SEQ ID NOs: 678-929. In some embodiments, the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 5469 or SEQ ID NO: 5505. In some embodiments, the endonuclease is configured to binding to a PAM comprising SEQ ID NOs: 5520 or SEQ ID NOs: 5535. In some embodiments: (a) the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 2499; (b) the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 5469 or SEQ ID NO: 5505; and (c) the endonuclease is configured to binding to a PAM comprising SEQ ID NO: 5520 or SEQ ID NO: 5535.

[0072] In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 2751 or SEQ ID NOs: 2751-2913. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 4565 or SEQ ID NOs: 4565-4727. In some embodiments, the endonuclease comprises at least 1, at least 2, at least 3, at least 4, or at least 5 peptide motifs selected from the group consisting of SEQ ID NOs: 5668-5678. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 930 or SEQ ID NOs: 930-1092. In some embodiments, the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 5470 or SEQ ID NOs: 5506. In some embodiments, the endonuclease is configured to binding to a PAM comprising a sequence selected from the group consisting of SEQ ID NOs: 5521 or SEQ ID NOs: 5536. In some embodiments: (a) the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 2751; (b) the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 5470 or SEQ ID NO: 5506; and (c) the endonuclease is configured to binding to a PAM comprising SEQ ID NO: 5521 or SEQ ID NO: 5536.

[0073] In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 2914 or SEQ ID NOs: 2914-3174. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 4728 or SEQ ID NOs: 4728-4988. In some embodiments, the endonuclease comprises at least 1, at least 2, or at least 3 peptide motifs selected from the group consisting of SEQ ID NOs: 5676-5678. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 1093 or SEQ ID NOs: 1093-1353. In some embodiments, the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 5471, SEQ ID NO: 5507, and SEQ ID NOs: 5540-5542. In some embodiments, the guide RNA structure comprises a tracr ribonucleic acid sequence predicted to comprise at least two hairpins comprising less than 5 base-paired ribonucleotides. In some embodiments, the endonuclease is configured to binding to a PAM comprising SEQ ID NO: 5522. In some embodiments: (a) the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 2914; (b) the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 5471 or SEQ ID NO: 5507; and (c) the endonuclease is configured to binding to a PAM comprising SEQ ID NO: 5522.

[0074] In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 3175 or SEQ ID NOs: 3175-3330. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 4989 or SEQ ID NOs: 4989-5146. In some embodiments, the endonuclease comprises at least 1, at least 2, at least 3, at least 4, or at least 5 peptide motifs selected from the group consisting of SEQ ID NOs: 5679-5686. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 1354 or SEQ ID NOs: 1354-1511. In some embodiments, the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 5472 or SEQ ID NOs: 5508. In some embodiments, the endonuclease is configured to binding to a PAM comprising a sequence selected from the group consisting of SEQ ID NO: 5523 or SEQ ID NO: 5537. In some embodiments: (a) the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 3175; (b) the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 5472 or SEQ ID NO: 5508; and (c) the endonuclease is configured to binding to a PAM comprising SEQ ID NO: 5523 or SEQ ID NO: 5537.

[0075] In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 3331 or SEQ ID NOs: 3331-3474. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 5147 or SEQ ID NOs: 5147-5290. In some embodiments, the endonuclease comprises at least 1, at least 2, at least 3, at least 4, or at least 5 peptide motifs selected from the group consisting of SEQ ID NOs: 5674-5675 and SEQ ID NOs: 5687-5693. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 1512 or SEQ ID NOs: 1512-1655. In some embodiments, the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 5473 or SEQ ID NO: 5509. In some embodiments, the endonuclease is configured to binding to a PAM comprising SEQ ID NO: 5524. In some embodiments: (a) the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 3331; (b) the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 5473 or SEQ ID NO: 5509; and (c) the endonuclease is configured to binding to a PAM comprising SEQ ID NO: 5524.

[0076] In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 3475 or SEQ ID NOs: 3475-3568. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 5291 or SEQ ID NOs: 5291-5389. In some embodiments, the endonuclease comprises at least 1, at least 2, at least 3, at least 4, or at least 5 peptide motifs selected from the group consisting of SEQ ID NOs: 5694-5699. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 1656 or SEQ ID NOs: 1656-1755. In some embodiments, the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 5474 or SEQ ID NO: 5510. In some embodiments, the endonuclease is configured to binding to a PAM comprising SEQ ID NOs: 5525. In some embodiments: (a) the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 3475; (b) the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 5474 or SEQ ID NO: 5510; and (c) the endonuclease is configured to binding to a PAM comprising SEQ ID NO: 5525.

[0077] In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 3569 or SEQ ID NOs: 3569-3637. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 5390 or

SEQ ID NOs: 5390-5460. In some embodiments, the endonuclease comprises at least 1, at least 2, at least 3, at least 4, or at least 5 peptide motifs selected from the group consisting of SEQ ID NOs: 5700-5717. In some embodiments, the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to a sequence selected from the group consisting of SEQ ID NO: 1756 or SEQ ID NOs: 1756-1826. In some embodiments, the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 5475 or SEQ ID NOs: 5511. In some embodiments, the endonuclease is configured to binding to a PAM comprising SEQ ID NO: 5526. In some embodiments: (a) the endonuclease comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 3569; (b) the guide RNA structure comprises a sequence at least 70%, 80%, or 90% identical to SEQ ID NO: 5475 or SEQ ID NO: 5511; and (c) the endonuclease is configured to binding to a PAM comprising SEQ ID NO: 5526. In some embodiments, the sequence identity is determined by a BLASTP, CLUSTALW, MUSCLE, MAFFT, or CLUSTALW with the parameters of the Smith-Waterman homology search algorithm. In some embodiments, the sequence identity is determined by the BLASTP homology search algorithm using parameters of a wordlength (W) of 3, an expectation (E) of 10, and a BLOSUM62 scoring matrix setting gap costs at existence of 11, extension of 1, and using a conditional compositional score matrix adjustment.

[0078] In some aspects, the present disclosure provides for an engineered guide ribonucleic acid polynucleotide comprising: (a) a DNA-targeting segment comprising a nucleotide sequence that is complementary to a target sequence in a target DNA molecule; and (b) a protein-binding segment comprising two complementary stretches of nucleotides that hybridize to form a double-stranded RNA (dsRNA) duplex, wherein the two complementary stretches of nucleotides are covalently linked to one another with intervening nucleotides, and wherein the engineered guide ribonucleic acid polynucleotide is configured to forming a complex with an endonuclease comprising a RuvC_III domain having at least 70%, at least 75%, at least 80%, at least 85%, at least 88%, at least 90%, at least 92%, at least 95%, or at least 98% sequence identity to any one of SEQ ID NOs: 1827-3637 and targeting the complex to the target sequence of the target DNA molecule. In some embodiments, the DNA-targeting segment is positioned 5' of both of the two complementary stretches of nucleotides.

[0079] In some embodiments: (a) the protein binding segment comprises a sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 88%, at least 90%, at least 92%, at least 95%, or at least 98% identity to a sequence selected from the group consisting of SEQ ID NOs: 5476-5479 or SEQ ID NOs: 5476-5489; (b) the protein binding segment comprises a sequence having at least 70%, at least 80%, or at least 90% identity to a sequence selected from the group consisting of (SEQ ID NOs: 5490-5491 or SEQ ID NOs: 5490-5494) and SEQ ID

NO: 5538; (c) the protein binding segment comprises a sequence having at least 70%, at least 80%, or at least 90% identity to a sequence selected from the group consisting of SEQ ID NOs: 5498-5499; (d) the protein binding segment comprises a sequence having at least 70%, at least 80%, or at least 90% identity to a sequence selected from the group consisting of SEQ ID NOs: 5495-5497 and SEQ ID NOs: 5500-5502; (e) the protein binding segment comprises a sequence having at least 70%, at least 80%, or at least 90% identity to SEQ ID NO: 5503; (f) the protein binding segment comprises a sequence having at least 70%, at least 80%, or at least 90% identity to SEQ ID NO: 5504; (g) the protein binding segment comprises a sequence having at least 70%, at least 80%, or at least 90% identity to SEQ ID NOs: 5505; (h) protein binding segment comprises a sequence having at least 70%, at least 80%, or at least 90% identity to SEQ ID NO: 5506; (i) protein binding segment comprises a sequence having at least 70%, at least 80%, or at least 90% identity to SEQ ID NO: 5507; (j) the protein binding segment comprises a sequence having at least 70%, at least 80%, or at least 90% identity to SEQ ID NO: 5508; (k) the protein binding segment comprises a sequence having at least 70%, at least 80%, or at least 90% identity to SEQ ID NO: 5509; (l) the protein binding segment comprises a sequence having at least 70%, at least 80%, or at least 90% identity to SEQ ID NO: 5510; or (m) the protein binding segment comprises a sequence having at least 70%, at least 80%, or at least 90% identity to SEQ ID NO: 5511.

[0080] In some embodiments: (a) the guide ribonucleic acid polynucleotide comprises an RNA sequence comprising a hairpin comprising a stem and a loop, wherein the stem comprises at least 10, at least 12, or at least 14 base-paired ribonucleotides, and an asymmetric bulge within 4 base pairs of the loop; (b) the guide ribonucleic acid polynucleotide comprises a tracr ribonucleic acid sequence predicted to comprise a hairpin comprising at least 8, at least 10, or at least 12 base-paired ribonucleotides; (c) the guide ribonucleic acid polynucleotide comprises a guide ribonucleic acid sequence predicted to comprise a hairpin with an uninterrupted base-paired region comprising at least 8 nucleotides of a guide ribonucleic acid sequence and at least 8 nucleotides of a tracr ribonucleic acid sequence, and wherein the tracr ribonucleic acid sequence comprises, from 5' to 3', a first hairpin and a second hairpin, wherein the first hairpin has a longer stem than the second hairpin; or (d) the guide ribonucleic acid polynucleotide comprises a tracr ribonucleic acid sequence predicted to comprise at least two hairpins comprising less than 5 base-paired ribonucleotides.

[0081] In some aspects, the present disclosure provides for a deoxyribonucleic acid polynucleotide encoding any of the engineered guide ribonucleic acid polynucleotides described herein.

[0082] In some aspects, the present disclosure provides for a nucleic acid comprising an engineered nucleic acid sequence optimized for expression in an organism, wherein the nucleic acid encodes a class 2, type II Cas endonuclease comprising a RuvC_III domain and an HNH domain, and wherein the endonuclease is derived from an uncultivated microorganism.

[0083] In some aspects, the present disclosure provides for a nucleic acid comprising an engineered nucleic acid sequence optimized for expression in an organism, wherein the nucleic acid encodes an endonuclease comprising a RuvC_III domain having at least 70% sequence identity to any one of SEQ ID NOs: 1827-3637. In some embodiments, the endonuclease comprises an HNH domain having at least 70% or at least 80% sequence identity to any one of SEQ ID NOs: 3638-5460. In some embodiments, the endonuclease comprises SEQ ID NOs: 5572-5591 or a variant thereof having at least 70% sequence identity thereto. In some embodiments, the endonuclease comprises a sequence encoding one or more nuclear localization sequences (NLSs) proximal to an N- or C-terminus of the endonuclease. In some embodiments, the NLS comprises a sequence selected from SEQ ID NOs: 5597-5612.

[0084] In some embodiments, the organism is prokaryotic, bacterial, eukaryotic, fungal, plant, mammalian, rodent, or human. In some embodiments, the organism is *E. coli*, and: (a) the nucleic acid sequence has at least 70%, 80%, or 90% identity to a sequence selected from the group consisting of SEQ ID NOs: 5572-5575; (b) the nucleic acid sequence has at least 70%, 80%, or 90% identity to a sequence selected from the group consisting of SEQ ID NOs: 5576-5577; (c) the nucleic acid sequence has at least 70%, 80%, or 90% identity to a sequence selected from the group consisting of SEQ ID NOs: 5578-5580; (d) the nucleic acid sequence has at least 70%, 80%, or 90% identity to SEQ ID NO: 5581; (e) the nucleic acid sequence has at least 70%, 80%, or 90% identity to SEQ ID NO: 5582; (f) the nucleic acid sequence has at least 70%, 80%, or 90% identity to SEQ ID NO: 5583; (g) the nucleic acid sequence has at least 70%, 80%, or 90% identity to SEQ ID NO: 5584; (h) the nucleic acid sequence has at least 70%, 80%, or 90% identity to SEQ ID NO: 5585; (i) the nucleic acid sequence has at least 70%, 80%, or 90% identity to SEQ ID NO: 5586; or (j) the nucleic acid sequence has at least 70%, 80%, or 90% identity to SEQ ID NO: 5587. In some embodiments, the organism is human, and: (a) the nucleic acid sequence has at least 70%, 80%, or 90% identity to SEQ ID NO: 5588 or SEQ ID NO: 5589; or (b) the nucleic acid sequence has at least 70%, 80%, or 90% identity to SEQ ID NO: 5590 or SEQ ID NO: 5591.

[0085] In some aspects, the present disclosure provides for a vector comprising a nucleic acid sequence encoding a class 2, type II Cas endonuclease comprising a RuvC_III domain and an HNH domain, wherein the endonuclease is derived from an uncultivated microorganism.

[0086] In some aspects, the present disclosure provides for a vector comprising the any of the nucleic acids described herein. In some embodiments, the vector further comprises a nucleic acid encoding an engineered guide ribonucleic acid structure configured to form a complex with the endonuclease comprising: (a) a guide ribonucleic acid sequence configured to hybridize to a target deoxyribonucleic acid sequence; and (b) a tracr ribonucleic acid sequence configured to binding to the endonuclease. In some embodiments, the vector is a plasmid, a minicircle, a CELiD, an adeno-associated virus (AAV) derived virion, or a lentivirus.

[0087] In some aspects, the present disclosure provides for a cell comprising any of the vectors described herein.

[0088] In some aspects, the present disclosure provides for a method of manufacturing an endonuclease, comprising cultivating any of the cells described herein.

[0089] In some aspects, the present disclosure provides for a method for binding, cleaving, marking, or modifying a double-stranded deoxyribonucleic acid polynucleotide, comprising: (a) contacting the double-stranded deoxyribonucleic acid polynucleotide with a class 2, type II Cas endonuclease in complex with an engineered guide ribonucleic acid structure configured to bind to the endonuclease and the double-stranded deoxyribonucleic acid polynucleotide; (b) wherein the double-stranded deoxyribonucleic acid polynucleotide comprises a protospacer adjacent motif (PAM); and (c) wherein the PAM comprises a sequence selected from the group consisting of SEQ ID NOs: 5512-5526 or SEQ ID NOs: 5527-5537. In some embodiments, the double-stranded deoxyribonucleic acid polynucleotide comprises a first strand comprising a sequence complementary to a sequence of the engineered guide ribonucleic acid structure and a second strand comprising the PAM. In some embodiments, the PAM is directly adjacent to the 3' end of the sequence complementary to the sequence of the engineered guide ribonucleic acid structure.

[0090] In some embodiments, the class 2, type II Cas endonuclease is not a Cas9 endonuclease, a Cas14 endonuclease, a Cas12a endonuclease, a Cas12b endonuclease, a Cas 12c endonuclease, a Cas12d endonuclease, a Cas12e endonuclease, a Cas13a endonuclease, a Cas13b endonuclease, a Cas13c endonuclease, or a Cas 13d endonuclease. In some embodiments, the class 2, type II Cas endonuclease is derived from an uncultivated microorganism. In some embodiments, the double-stranded deoxyribonucleic acid polynucleotide is a eukaryotic, plant, fungal, mammalian, rodent, or human double-stranded deoxyribonucleic acid polynucleotide.

[0091] In some embodiments: (a) the PAM comprises a sequence selected from the group consisting of SEQ ID NOs: 5512-5515 and SEQ ID NOs: 5527-5530; (b) the PAM comprises SEQ ID NO: 5516 or SEQ ID NO: 5531; (c) the PAM comprises SEQ ID NO: 5539; (d) the PAM comprises SEQ ID NO: 5517 or SEQ ID NO: 5518; (e) the PAM comprises SEQ ID NO:

5519; (f) the PAM comprises SEQ ID NO: 5520 or SEQ ID NO: 5535; (g) the PAM comprises SEQ ID NO: 5521 or SEQ ID NO: 5536; (h) the PAM comprises SEQ ID NO: 5522; (i) the PAM comprises SEQ ID NO: 5523 or SEQ ID NO: 5537; (j) the PAM comprises SEQ ID NO: 5524; (k) the PAM comprises SEQ ID NO: 5525; or (l) the PAM comprises SEQ ID NO: 5526.

[0092] In some aspects, the present disclosure provides for a method of modifying a target nucleic acid locus, the method comprising delivering to the target nucleic acid locus any of the engineered nuclease systems described herein, wherein the endonuclease is configured to form a complex with the engineered guide ribonucleic acid structure, and wherein the complex is configured such that upon binding of the complex to the target nucleic acid locus, the complex modifies the target nucleic locus. In some embodiments, modifying the target nucleic acid locus comprises binding, nicking, cleaving, or marking the target nucleic acid locus. In some embodiments, the target nucleic acid locus comprises deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). In some embodiments, the target nucleic acid comprises genomic DNA, viral DNA, viral RNA, or bacterial DNA. In some embodiments, the target nucleic acid locus is in vitro. In some embodiments, the target nucleic acid locus is within a cell. In some embodiments, the cell is a prokaryotic cell, a bacterial cell, a eukaryotic cell, a fungal cell, a plant cell, an animal cell, a mammalian cell, a rodent cell, a primate cell, or a human cell.

[0093] In some embodiments, delivering the engineered nuclease system to the target nucleic acid locus comprises delivering any of the nucleic acids described herein or any of the vectors described herein. In some embodiments, delivering the engineered nuclease system to the target nucleic acid locus comprises delivering a nucleic acid comprising an open reading frame encoding the endonuclease. In some embodiments, the nucleic acid comprises a promoter to which the open reading frame encoding the endonuclease is operably linked. In some embodiments, the engineered nuclease system to the target nucleic acid locus comprises delivering a capped mRNA containing the open reading frame encoding the endonuclease. In some embodiments, the engineered nuclease system to the target nucleic acid locus comprises delivering a translated polypeptide. In some embodiments, the engineered nuclease system to the target nucleic acid locus comprises delivering a deoxyribonucleic acid (DNA) encoding the engineered guide ribonucleic acid structure operably linked to a ribonucleic acid (RNA) pol III promoter. In some embodiments, the endonuclease induces a single-stranded break or a double-stranded break at or proximal to the target locus.

[0094] In some aspects, the present disclosure provides for an engineered nuclease system comprising: (a) an endonuclease comprising a sequence having at least 75% sequence identity to any one of SEQ ID NOs: 5718-5846 or 6257; and (b) an engineered guide ribonucleic acid structure configured to form a complex with said endonuclease comprising: (i) a ribonucleic acid

sequence configured to hybridize to a target deoxyribonucleic acid sequence; and (ii) a ribonucleic acid sequence configured to bind to said endonuclease. In some aspects, the present disclosure provides for an engineered nuclease system comprising: (a) an endonuclease configured to bind to a protospacer adjacent motif (PAM) sequence comprising SEQ ID NOs: 5847-5861 or 6258-6278, wherein said endonuclease is a class 2, type II Cas endonuclease; and (b) an engineered guide ribonucleic acid structure configured to form a complex with said endonuclease comprising: (i) a ribonucleic acid sequence configured to hybridize to a target deoxyribonucleic acid sequence; and (ii) a ribonucleic acid sequence configured to bind to said endonuclease. In some embodiments, said endonuclease is derived from an uncultivated microorganism. In some embodiments, said endonuclease has not been engineered to bind to a different PAM sequence. In some embodiments, said endonuclease is not a Cas9 endonuclease, a Cas14 endonuclease, a Cas12a endonuclease, a Cas12b endonuclease, a Cas12c endonuclease, a Cas12d endonuclease, a Cas12e endonuclease, a Cas13a endonuclease, a Cas13b endonuclease, a Cas13c endonuclease, or a Cas13d endonuclease. In some embodiments, said endonuclease has less than 80% identity to a Cas9 endonuclease. In some embodiments, said ribonucleic acid sequence comprises a sequence with at least 80% sequence identity to (a) any one of SEQ ID NOs: 5886-5887, 5891, 5893, or 5894; or (b) the non-degenerate nucleotides of any one of SEQ ID NOs: 5862-5885, 5888-5890, 5892, 5895-5896, or 6279-6301. In some aspects, the present disclosure provides for an engineered nuclease system comprising, (a) an engineered guide ribonucleic acid structure comprising: (i) a ribonucleic acid sequence configured to hybridize to a target deoxyribonucleic acid sequence; and (ii) a ribonucleic acid sequence configured to bind to an endonuclease, wherein said ribonucleic acid sequence comprises a sequence with at least 80% sequence identity (a) any one of SEQ ID NOs: 5886-5887, 5891, 5893, or 5894; or (b) the non-degenerate nucleotides of any one of SEQ ID NOs: 5862-5885, 5888-5890, 5892, 5895-5896, or 6279-6301; and a class 2, type II Cas endonuclease configured to bind to said engineered guide ribonucleic acid. In some embodiments, endonuclease is configured to bind to a protospacer adjacent motif (PAM) sequence selected from the group comprising SEQ ID NOs: 5847-5861 or 6258-6278. In some embodiments, said guide ribonucleic acid sequence is 15-24 nucleotides in length or 19-24 nucleotides in length. In some embodiments, said endonuclease comprises one or more nuclear localization sequences (NLSs) proximal to an N- or C-terminus of said endonuclease. In some embodiments, said NLS comprises a sequence selected from SEQ ID NOs: 5597-5612. In some embodiments, the system further comprises a single- or double-stranded DNA repair template comprising from 5' to 3': a first homology arm comprising a sequence of at least 20 nucleotides 5' to said target deoxyribonucleic acid sequence, a synthetic DNA sequence of at least 10 nucleotides, and a

second homology arm comprising a sequence of at least 20 nucleotides 3' to said target sequence. In some embodiments, said first or second homology arm comprises a sequence of at least 40, 80, 120, 150, 200, 300, 500, or 1,000 nucleotides. In some embodiments, said sequence identity is determined by a BLASTP, CLUSTALW, MUSCLE, MAFFT, or CLUSTALW with the parameters of the Smith-Waterman homology search algorithm. In some embodiments, said sequence identity is determined by said BLASTP homology search algorithm using parameters of a wordlength (W) of 3, an expectation (E) of 10, and a BLOSUM62 scoring matrix setting gap costs at existence of 11, extension of 1, and using a conditional compositional score matrix adjustment.

[0095] In some aspects, the present disclosure provides for an engineered guide ribonucleic acid polynucleotide comprising: (a) a DNA-targeting segment comprising a nucleotide sequence that is complementary to a target sequence in a target DNA molecule; and (b) a protein-binding segment comprising two complementary stretches of nucleotides that hybridize to form a double-stranded RNA (dsRNA) duplex, wherein said two complementary stretches of nucleotides are covalently linked to one another with intervening nucleotides, and wherein said engineered guide ribonucleic acid polynucleotide is configured to form a complex with an endonuclease comprising sequence having at least 75% sequence identity to any one of SEQ ID NOs: 5718-5846 or 6257 and target said complex to said target sequence of said target DNA molecule. In some embodiments, said DNA-targeting segment is positioned 5' of both of said two complementary stretches of nucleotides.

[0096] In some aspects, the present disclosure provides for a deoxyribonucleic acid polynucleotide encoding any of the engineered guide ribonucleic acid polynucleotides described herein.

[0097] In some aspects, the present disclosure provides for a nucleic acid comprising an engineered nucleic acid sequence optimized for expression in an organism, wherein said nucleic acid encodes an endonuclease comprising a sequence having at least 75% sequence identity to any one of SEQ ID NOs: 5718-5846 or 6257. In some embodiments, said endonuclease comprises a sequence encoding one or more nuclear localization sequences (NLSs) proximal to an N- or C-terminus of said endonuclease. In some embodiments, said NLS comprises a sequence selected from SEQ ID NOs: 5597-5612. In some embodiments, said organism is prokaryotic, bacterial, eukaryotic, fungal, plant, mammalian, rodent, or human.

[0098] In some aspects, the present disclosure provides for a vector comprising any of the nucleic acids described herein. In some embodiments, the vector further comprises a nucleic acid encoding an engineered guide ribonucleic acid structure configured to form a complex with said endonuclease comprising: (a) a ribonucleic acid sequence configured to hybridize to a target

deoxyribonucleic acid sequence; and (b) a ribonucleic acid sequence configured to bind to said endonuclease. In some embodiments, the vector is a plasmid, a minicircle, a CELiD, an adeno-associated virus (AAV) derived virion, or a lentivirus.

[0099] In some aspects, the present disclosure provides for a cell comprising any of the vectors described herein

[00100] In some aspects, the present disclosure provides for a method of manufacturing an endonuclease, comprising cultivating any of the cells described herein.

[00101] In some aspects, the present disclosure provides for a method for binding, cleaving, marking, or modifying a double-stranded deoxyribonucleic acid polynucleotide, comprising: contacting said double-stranded deoxyribonucleic acid polynucleotide with a class 2, type II Cas endonuclease in complex with an engineered guide ribonucleic acid structure configured to bind to said endonuclease and said double-stranded deoxyribonucleic acid polynucleotide; wherein said double-stranded deoxyribonucleic acid polynucleotide comprises a protospacer adjacent motif (PAM); and wherein said PAM comprises a sequence selected from the group consisting of SEQ ID NOs: 5847-5861 or 6258-6278. In some embodiments, said double-stranded deoxyribonucleic acid polynucleotide comprises a first strand comprising a sequence complementary to a sequence of said engineered guide ribonucleic acid structure and a second strand comprising said PAM. In some embodiments, said PAM is directly adjacent to the 3' end of said sequence complementary to said sequence of said engineered guide ribonucleic acid structure. In some embodiments, said class 2, type II Cas endonuclease is not a Cas9 endonuclease, a Cas14 endonuclease, a Cas12a endonuclease, a Cas12b endonuclease, a Cas 12c endonuclease, a Cas12d endonuclease, a Cas12e endonuclease, a Cas13a endonuclease, a Cas13b endonuclease, a Cas13c endonuclease, or a Cas 13d endonuclease. In some embodiments, said double-stranded deoxyribonucleic acid polynucleotide is a eukaryotic, plant, fungal, mammalian, rodent, or human double-stranded deoxyribonucleic acid polynucleotide.

[00102] In some aspects, the present disclosure provides for a method of modifying a target nucleic acid locus, said method comprising delivering to said target nucleic acid locus any of the engineered nuclease systems described herein, wherein said endonuclease is configured to form a complex with said engineered guide ribonucleic acid structure, and wherein said complex is configured such that upon binding of said complex to said target nucleic acid locus, said complex modifies said target nucleic locus. In some embodiments, said target nucleic acid locus comprises binding, nicking, cleaving, or marking said target nucleic acid locus. In some embodiments, said target nucleic acid locus comprises deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). In some embodiments, said target nucleic acid comprises genomic DNA, viral DNA, viral RNA, or bacterial DNA. In some embodiments, said target nucleic acid

locus is in vitro. In some embodiments, said target nucleic acid locus is within a cell. In some embodiments, said cell is a prokaryotic cell, a bacterial cell, a eukaryotic cell, a fungal cell, a plant cell, an animal cell, a mammalian cell, a rodent cell, a primate cell, or a human cell. In some embodiments, said engineered nuclease system to said target nucleic acid locus comprises delivering any of the nucleic acids described herein or any of the vectors described herein. In some embodiments, delivering said engineered nuclease system to said target nucleic acid locus comprises delivering a nucleic acid comprising an open reading frame encoding said endonuclease. In some embodiments, said nucleic acid comprises a promoter to which said open reading frame encoding said endonuclease is operably linked. In some embodiments, delivering said engineered nuclease system to said target nucleic acid locus comprises delivering a capped mRNA containing said open reading frame encoding said endonuclease. In some embodiments, delivering said engineered nuclease system to said target nucleic acid locus comprises delivering a translated polypeptide. In some embodiments, delivering said engineered nuclease system to said target nucleic acid locus comprises delivering a deoxyribonucleic acid (DNA) encoding said engineered guide ribonucleic acid structure operably linked to a ribonucleic acid (RNA) pol III promoter. In some embodiments, said endonuclease induces a single-stranded break or a double-stranded break at or proximal to said target locus.

[00103] In some aspects, the present disclosure provides for a method of editing a TRAC locus in a cell, comprising contacting to said cell (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said TRAC locus, wherein said engineered guide RNA comprises a targeting sequence having at least 80% identity, at least 82% identity, at least 84% identity, at least 86% identity, at least 88% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 100% identity to at least 19, at least 20, at least 21, at least 22, at least 23, or at least 24 consecutive nucleotides of any one of SEQ ID NOs: 5950-5958 or 5959-5965. In some embodiments, said RNA-guided endonuclease is a class II, type II Cas endonuclease. In some embodiments, said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% identity, at least 80% identity, at least 82% identity, at least 84% identity, at least 86% identity, at least 88% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 100% identity to SEQ ID NO: 2242 or SEQ ID NO: 2244. In some embodiments, said RNA-guided endonuclease further comprises an

HNH domain. In some embodiments, said RNA-guided endonuclease comprises a sequence having at least 75% identity, at least 80% identity, at least 82% identity, at least 84% identity, at least 86% identity, at least 88% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 100% identity to SEQ ID NO: 421 or SEQ ID NO: 423. In some embodiments, said engineered guide RNA comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 5950-5958 and said endonuclease comprises a sequence having at least 75% identity to SEQ ID NO: 421. In some embodiments, said engineered guide RNA comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 5959-5965 and said endonuclease comprises a sequence having at least 75% identity to SEQ ID NO: 423. In some embodiments, said engineered guide RNA comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 5953-5957. In some embodiments, said engineered guide RNA comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 5960-5961 or 5963-5964.

[00104] In some aspects, the present disclosure provides for a method of editing a TRBC locus in a cell, comprising contacting to said cell (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said TRBC locus, wherein said engineered guide RNA comprises a targeting sequence having at least 80% identity, at least 82% identity, at least 84% identity, at least 86% identity, at least 88% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 100% identity to at least 19, at least 20, at least 21, at least 22, at least 23, or at least 24 consecutive nucleotides of any one of SEQ ID NOs: 5966-6004 or 6005-6025. In some embodiments, said RNA-guided endonuclease is a class II, type II Cas endonuclease. In some embodiments, said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% identity, at least 80% identity, at least 82% identity, at least 84% identity, at least 86% identity, at least 88% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 100% identity to SEQ ID NO: 2242 or SEQ ID NO: 2244. In some embodiments, said RNA-guided endonuclease further comprises an HNH domain. In some embodiments, said RNA-guided endonuclease comprises a sequence

having at least 75% identity, at least 80% identity, at least 82% identity, at least 84% identity, at least 86% identity, at least 88% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 100% identity to SEQ ID NO: 421 or SEQ ID NO: 423. In some embodiments, said engineered guide RNA comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 5966-6004 and said endonuclease comprises a sequence having at least 75% identity to SEQ ID NO: 421. In some embodiments, said engineered guide RNA comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6005-6025 and said endonuclease comprises a sequence having at least 75% identity to SEQ ID NO: 423. In some embodiments, said engineered guide RNA comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 5970, 5971, 5983, or 5984. In some embodiments, said engineered guide RNA comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6006, 6010, 6011, or 6012.

[00105] In some aspects, the present disclosure provides for a method of editing a GR (NR3C1) locus in a cell, comprising contacting to said cell (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said GR (NR3C1) locus, wherein said engineered guide RNA comprises a targeting sequence having at least 80% identity, at least 82% identity, at least 84% identity, at least 86% identity, at least 88% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 100% identity to at least 19, at least 20, at least 21, at least 22, at least 23, or at least 24 consecutive nucleotides consecutive nucleotides of any one of SEQ ID NOs: 6026-6090 or 6091-6121. In some embodiments, said RNA-guided endonuclease is a class II, type II Cas endonuclease. In some embodiments, said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% identity, at least 80% identity, at least 82% identity, at least 84% identity, at least 86% identity, at least 88% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 100% identity to SEQ ID NO: 2242 or SEQ ID NO: 2244. In some embodiments, said RNA-guided endonuclease further comprises an HNH domain. In some embodiments, said RNA-guided endonuclease comprises a sequence having at least 75% identity to SEQ ID NO: 421 or SEQ ID

NO: 423. In some embodiments, said engineered guide RNA comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6026-6090 and said endonuclease comprises a sequence having at least 75% identity to SEQ ID NO: 421. In some embodiments, said engineered guide RNA comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6091-6121 and said endonuclease comprises a sequence having at least 75% identity to SEQ ID NO: 423. In some embodiments, said engineered guide RNA comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6027-6028, 6029, 6038, 6043, 6049, 6076, 6080, 6081, or 6086. In some embodiments, said engineered guide RNA comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6092, 6115, or 6119.

[00106] In some aspects, the present disclosure provides for a method of editing an AAVS1 locus in a cell, comprising contacting to said cell (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said AAVS1 locus, wherein said engineered guide RNA comprises a targeting sequence having at least 80% identity, at least 82% identity, at least 84% identity, at least 86% identity, at least 88% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 100% identity to at least 19, at least 20, at least 21, at least 22, at least 23, or at least 24 consecutive nucleotides of any one of SEQ ID NOs: 6122-6152. In some embodiments, said RNA-guided endonuclease is a class II, type II Cas endonuclease. In some embodiments, said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% identity, at least 80% identity, at least 82% identity, at least 84% identity, at least 86% identity, at least 88% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 100% identity to SEQ ID NO: 2242 or SEQ ID NO: 2244. In some embodiments, said RNA-guided endonuclease further comprises an HNH domain. In some embodiments, said RNA-guided endonuclease comprises a sequence having at least 75% identity, at least 80% identity, at least 82% identity, at least 84% identity, at least 86% identity, at least 88% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 100% identity to SEQ ID NO: 421 or SEQ ID NO: 423. In some embodiments, said engineered guide RNA comprises a

targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6122, 6125-6126, 6128, 6131, 6133, 6136, 6141, 6143, or 6148.

[00107] In some aspects, the present disclosure provides for a method of editing an TIGIT locus in a cell, comprising contacting to said cell (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said TIGIT locus, wherein said engineered guide RNA comprises a targeting sequence having at least 80% identity, at least 82% identity, at least 84% identity, at least 86% identity, at least 88% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 100% identity to at least 19, at least 20, at least 21, at least 22, at least 23, or at least 24 consecutive nucleotides of any one of SEQ ID NOs: 6153-6181. In some embodiments, said RNA-guided endonuclease is a class II, type II Cas endonuclease. In some embodiments, said RNA-guided endonuclease comprises a sequence having at least 75% identity, at least 80% identity, at least 82% identity, at least 84% identity, at least 86% identity, at least 88% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 100% identity to SEQ ID NO: 421 or SEQ ID NO: 423. In some embodiments, said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% identity, at least 80% identity, at least 82% identity, at least 84% identity, at least 86% identity, at least 88% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 100% identity to SEQ ID NO: 2242 or SEQ ID NO: 2244. In some embodiments, said RNA-guided endonuclease further comprises an HNH domain. In some embodiments, said engineered guide RNA comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 66155, 6159, 616, or 6172.

[00108] In some aspects, the present disclosure provides for a method of editing an CD38 locus in a cell, comprising contacting to said cell (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said CD38 locus, wherein said engineered guide RNA comprises a targeting sequence having at least 80% identity, at least 82% identity, at least 84% identity, at least 86% identity, at least 88% identity, at least 90% identity, at least 91% identity, at least 92%

identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 100% identity to at least 19, at least 20, at least 21, at least 22, at least 23, or at least 24 consecutive nucleotides of any one of SEQ ID NOs: 6182-6248 or 6249-6256. In some embodiments, said RNA-guided endonuclease is a class II, type II Cas endonuclease. In some embodiments, said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% identity, at least 80% identity, at least 82% identity, at least 84% identity, at least 86% identity, at least 88% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 100% identity to SEQ ID NO: 2242 or SEQ ID NO: 2244. In some embodiments, said RNA-guided endonuclease further comprises an HNH domain. In some embodiments, said RNA-guided endonuclease comprises a sequence having at least 75% identity, at least 80% identity, at least 82% identity, at least 84% identity, at least 86% identity, at least 88% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 100% identity to SEQ ID NO: 421 or SEQ ID NO: 423. In some embodiments, said engineered guide RNA comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6182-6248 and said endonuclease comprises a sequence having at least 75% identity to SEQ ID NO: 421. In some embodiments, said engineered guide RNA comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6249-6256 and said endonuclease comprises a sequence having at least 75% identity to SEQ ID NO: 423. In some embodiments, said engineered guide RNA comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6182-6183, 6189, 6191, 6208, 6210, 6211, or 6215. In some embodiments, said engineered guide RNA comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of SEQ ID NO: 6251.

[00109] In some embodiments of any of the methods for editing particular loci in cells above, said cell is a peripheral blood mononuclear cell, a T-cell, an NK cell, a hematopoietic stem cell (HSCT), or a B-cell, or any combination thereof.

[00110] In some aspects, the present disclosure provides for an engineered guide ribonucleic acid polynucleotide comprising: (a) a DNA-targeting segment comprising a nucleotide sequence that is complementary to a target sequence in a target DNA molecule; and (b) a protein-binding segment comprising two complementary stretches of nucleotides that hybridize to form a double-stranded RNA (dsRNA) duplex, wherein said two complementary stretches of

nucleotides are covalently linked to one another with intervening nucleotides, and wherein said engineered guide ribonucleic acid polynucleotide is configured to form a complex with a class 2, type II Cas endonuclease and target said complex to said target sequence of said target DNA molecule, wherein said DNA-targeting segment comprises a sequence having at least 80% identity, at least 82% identity, at least 84% identity, at least 86% identity, at least 88% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 100% identity to at least 19, at least 20, at least 21, at least 22, at least 23, or at least 24 consecutive nucleotides of any one of SEQ ID NOs: 5950-5965, 5966-6025, 6026-6121, 6122-6152, 6153-6181, or 6182-6256. In some embodiments, said protein-binding segment comprises a sequence having at least 85% identity to any one of SEQ ID NOs: 5466 or 6304.

[00111] In some aspects, the present disclosure provides for a system for generating an edited immune cell, comprising: (a) an RNA-guided endonuclease; (b) an engineered guide ribonucleic acid polynucleotide according to claim 97 configured to bind said RNA-guided endonuclease; and (c) a single- or double-stranded DNA repair template comprising first and second homology arms flanking a sequence encoding a chimeric antigen receptor (CAR). In some embodiments, said cell is a peripheral blood mononuclear cell, a T-cell, an NK cell, a hematopoietic stem cell (HSCT), or a B-cell, or any combination thereof. In some aspects, said RNA-guided endonuclease is a class II, type II Cas endonuclease. In some aspects, said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% identity, at least 80% identity, at least 82% identity, at least 84% identity, at least 86% identity, at least 88% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 100% identity to SEQ ID NO: 2242 or SEQ ID NO: 2244. In some aspects, said RNA-guided endonuclease further comprises an HNH domain. In some aspects, said RNA-guided endonuclease comprises a sequence having at least 75% identity, at least 80% identity, at least 82% identity, at least 84% identity, at least 86% identity, at least 88% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, or at least 100% identity to SEQ ID NO: 421 or SEQ ID NO: 423.

[00112] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized,

the present disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

In some aspects, the present disclosure provides for a method of editing a B2M locus in a cell, comprising contacting to said cell (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said B2M locus, wherein said region of said B2M locus comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6387-6468. In some embodiments, said RNA-guided endonuclease is a Cas endonuclease. In some embodiments, said Cas endonuclease is a class 2, type II Cas endonuclease. In some embodiments, said class 2, type II Cas endonuclease comprises an endonuclease having at least 75% sequence identity to any one of SEQ ID NOs: 421-431. In some embodiments, said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% sequence identity to SEQ ID NO: 2242 or SEQ ID NO: 2244. In some embodiments, said RNA-guided endonuclease further comprises an HNH domain. In some embodiments, said RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 421. In some embodiments, said engineered guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 6305-6386. In some embodiments, said region of said B2M locus comprises a sequence at least 75%, 80%, or 90% identical to at least 19 of the non-degenerate nucleotides of any one of SEQ ID NOs: 6388, 6399, 6401, 6403, 6410, 6413, 6421, 6446, and 6448. In some embodiments, said engineered guide RNA comprises a sequence at 80%, or at least 90% identical to any one of SEQ ID NOs: 6306, 6317, 6319, 6321, 6328, 6331, 6339, 6364, and 6366.

[00113] In some aspects, the present disclosure provides for a method of editing a TRAC locus in a cell, comprising contacting to said cell (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said TRAC locus, wherein said region of said TRAC locus comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6509-6548. In some embodiments, said RNA-guided endonuclease is a Cas endonuclease. In some embodiments, said Cas endonuclease is a class 2, type II Cas endonuclease. In some embodiments, said class 2, type II Cas endonuclease comprises an endonuclease having at least 75% sequence identity to any one of SEQ ID NOs: 421-431. In

some embodiments, said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% sequence identity to SEQ ID NO: 2242 or SEQ ID NO: 2244. In some embodiments, said RNA-guided endonuclease further comprises an HNH domain. In some embodiments, said RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 421. In some embodiments, said engineered guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 6469-6508. In some embodiments, said region of said TRAC locus comprises a sequence at least 75%, 80%, or 90% identical to at least 19 of the non-degenerate nucleotides of any one of SEQ ID NOs: 6517, 6520, and 6523. In some embodiments, said engineered guide RNA comprises a sequence at 80%, or at least 90% identical to any one of SEQ ID NOs: 6477, 6480, and 6483.

[00114] In some aspects, the present disclosure provides for a method of editing a HPRT locus in a cell, comprising contacting to said cell (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said HPRT locus, wherein said region of said HPRT locus comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6616-6682. In some embodiments, said RNA-guided endonuclease is a Cas endonuclease. In some embodiments, said Cas endonuclease is a class 2, type II Cas endonuclease. In some embodiments, said class 2, type II Cas endonuclease comprises an endonuclease having at least 75% sequence identity to any one of SEQ ID NOs: 421-431. In some embodiments, said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% sequence identity to SEQ ID NO: 2242 or SEQ ID NO: 2244. In some embodiments, said RNA-guided endonuclease further comprises an HNH domain. In some embodiments, said RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 421 or SEQ ID NO: 423. In some embodiments, said engineered guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 6549-6615. In some embodiments, said region of said HPRT locus comprises a sequence at least 75%, 80%, or 90% identical to at least 19 of the non-degenerate nucleotides of any one of SEQ ID NOs: 6619, 6634, 6673, 6675, and 6679. In some embodiments, said engineered guide RNA comprises a sequence at 80%, or at least 90% identical to any one of SEQ ID NOs: : 6552, 6567, 6606, 6608, and 6612.

[00115] In some aspects, the present disclosure provides for a method of editing a TRBC1/2 locus in a cell, comprising contacting to said cell (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured

to hybridize to a region of said TRBC1/2 locus, wherein said region of said TRBC1/2 locus comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6722-6760 or 6782-6802. In some embodiments, said RNA-guided endonuclease is a Cas endonuclease. In some embodiments, said Cas endonuclease is a class 2, type II Cas endonuclease. In some embodiments, said class 2, type II Cas endonuclease comprises an endonuclease having at least 75% sequence identity to any one of SEQ ID NOs: 421-431. In some embodiments, said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% sequence identity to SEQ ID NO: 2242 or SEQ ID NO: 2244. In some embodiments, said RNA-guided endonuclease further comprises an HNH domain. In some embodiments, said RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 421 or SEQ ID NO: 423. In some embodiments, said engineered guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 6683-6721 and 6761-6781. In some embodiments, said region of said TRBC1/2 locus comprises a sequence at least 75%, 80%, or 90% identical to at least 19 of the non-degenerate nucleotides of any one of SEQ ID NOs: 6734, 6753, 6790, and 6800. In some embodiments, said engineered guide RNA comprises a sequence at 80%, or at least 90% identical to any one of SEQ ID NOs: 6695, 6714, 6769, and 6779.

[00116] In some aspects, the present disclosure provides for a method of editing a HAO1 locus in a cell, comprising contacting to said cell (a) an RNA-guided endonuclease; and (b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said HAO1 locus, wherein said region of said HAO1 locus comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 11802-11820. In some embodiments, said RNA-guided endonuclease is a Cas endonuclease. In some embodiments, said Cas endonuclease is a class 2, type II Cas endonuclease. In some embodiments, said class 2, type II Cas endonuclease comprises an endonuclease having at least 75% sequence identity to any one of SEQ ID NOs: 421-431. In some embodiments, said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% sequence identity to SEQ ID NO: 2242. In some embodiments, said RNA-guided endonuclease further comprises an HNH domain. In some embodiments, said RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 421. In some embodiments, said region of said HAO1 locus comprises a sequence at least 75%, 80%, or 90% identical to at least 19 of the non-degenerate nucleotides of any one of SEQ ID NOs: 11806, 11813, 11816, and 11819. In some embodiments, said cell is a peripheral blood

mononuclear cell (PBMC). In some embodiments, said cell is a T-cell or a precursor thereof or a hematopoietic stem cell (HSC).

INCORPORATION BY REFERENCE

[00117] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[00118] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings (also “Figure” and “FIG.” herein), of which:

[00119] **FIG. 1** depicts the gene editing outcomes at the DNA level for B2M.

[00120] **FIG. 2A** and **FIG. 2B** depicts the gene editing outcomes at the DNA level for mouse TRAC.

[00121] **FIG. 3** depicts the gene editing outcomes at the DNA level for HPRT.

[00122] **FIG. 4** depicts the flow cytometry results for gene editing of human TRBC1/2.

[00123] **FIG. 5** depicts the results of a guide screen in Hepa1-6 cells; guides were delivered as mRNA and gRNA using lipofectamine Messenger Max.

[00124] **FIG. 6** depicts analysis of gene-editing outcomes by NGS for mRNA electroporation in T cells.

[00125] **FIG. 7** depicts ELISA results from a screen performed at a serum dilution of 1:50 to detect antibodies against MG-3-6 and MG3-8 (n = 50). Tetanus toxoid was used as the positive control due to wide-spread vaccination against this antigen. Serum samples above the dashed line were considered antibody-positive; the line represents the mean absorbance of the negative control (human albumin) plus two standard deviations from the mean. *P<0.05, **P<0.01, ****P<0.0001 as determined by an unpaired Student's *t*-test; ns, not significant.

[00126] **FIG. 8** depicts the gene editing outcomes at the DNA and cell-surface protein level for TRAC in human peripheral blood B cells.

[00127] **FIG. 9** depicts the gene editing outcomes at the DNA level for TRAC in hematopoietic stem cells.

[00128] **FIG. 10** depicts the gene editing outcomes at the DNA and cell-surface protein level for TRAC in induced pluripotent stem cells (iPSCs) for MG3-6 delivered as a ribonucleoprotein.

[00129] **FIG. 11** depicts the gene editing outcomes at the DNA level for TRAC in induced pluripotent stem cells (iPSCs) for MG3-6 delivered as mRNA.

[00130] **FIG. 12** depicts the gene editing outcomes at the DNA level for CD2 in primary T cells.

[00131] **FIG. 13** depicts the gene editing outcomes at the DNA level for CD5 in primary T cells.

[00132] **FIG. 14** depicts targeted RNA cleavage by MG3-6 and MG3-8.

[00133] **FIG. 15** depicts the gene editing outcomes at the DNA level for FAS in T cells.

[00134] **FIG. 16** depicts the gene editing outcomes at the DNA level for PD-1 in T cells.

[00135] **FIG. 17** depicts the gene editing outcomes at the DNA level for hRosa26 in T cells.

[00136] **FIG. 18** depicts the gene editing outcomes at the DNA level for TRAC and AAVS1 in K562 cells.

[00137] **FIG. 19** depicts the activity of chemically modified MG3-6 human HAO-1 guides in Hep3B cells when delivered as mRNA and gRNA using Lipofectamine Messenger Max.

[00138] **FIG. 20** depicts the gene editing outcomes at the DNA level for human GPR146 in Hep3B cells.

[00139] **FIG. 21** depicts the gene editing outcomes at the DNA level for mouse GPR146 in Hepa1-6 cells.

[00140] **FIG. 22** depicts the gene editing outcomes at the DNA level for mouse GPR146 in primary mouse hepatocytes.

[00141] **FIG. 23** depicts the gene editing outcomes at the DNA level for TRAC and AAVS1 in K562 cells.

[00142] **FIG. 24** depicts phylogenetic analysis of nucleases from the MG3 and MG150 families. PAM SeqLogo representations are shown for some active candidates. Reference SaCas9 and SpyCas9 sequences were included.

[00143] **FIG. 25** depicts phylogenetic analysis of nucleases from the MG15 family. Active candidates are highlighted with circles. Reference SaCas9, SpyCas9, and AcCas9 sequences were included as outgroup.

[00144] **FIG. 26** depicts SeqLogos of the PAMs for MG123-1, MG124-2, MG 125-1 and MG125-2.

[00145] **FIG. 27** depicts SeqLogos of the PAMs for MG125-3, MG125-4, MG125-5, and MG150-5.

[00146] **FIG. 28** depicts SeqLogos of the PAMs for MG150-6, MG150-7, MG150-8, and MG150-9.

[00147] **FIG. 29** depicts SeqLogos of the PAMs for MG3-18, MG3-89, MG3-90, and MG3-91.

[00148] **FIG. 30** depicts SeqLogos of the PAMs for MG3-92, MG3-93, MG3-95, and MG3-96.

[00149] **FIG. 31** depicts SeqLogos of the PAMs for MG3-103, MG15-130, MG15-146, and

MG15-164.

[00150] FIG. 32 depicts SeqLogos of the PAMs for MG15-166, MG15-171, MG15-172, and MG15-174.

[00151] FIG. 33 depicts SeqLogos of the PAMs for MG15-184, MG15-187, MG15-191, and MG15-193.

[00152] FIG. 34 depicts SeqLogos of the PAMs for MG15-195, MG15-217, MG15-218, and MG15-219.

[00153] FIG. 35 depicts a SeqLogo of the PAM for MG15-177.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

[00154] The Sequence Listing filed herewith provides exemplary polynucleotide and polypeptide sequences for use in methods, compositions and systems according to the disclosure. Below are exemplary descriptions of sequences therein.

MG1

[00155] SEQ ID NOs: 1-319 and 7285-7293 show the full-length peptide sequences of MG1 nucleases.

[00156] SEQ ID NOs: 1827-2140 show the peptide sequences of RuvC_III domains of MG1 nucleases above.

[00157] SEQ ID NOs: 3638-3955 show the peptide of HNH domains of MG1 nucleases above.

[00158] SEQ ID NOs: 5476-5479 show the nucleotide sequences of MG1 tracrRNAs derived from the same loci as MG1 nucleases above (e.g., same loci as SEQ ID NO:1-4, respectively).

[00159] SEQ ID NOs: 5461-5464 and 11130 show the nucleotide sequences of sgRNAs engineered to function with an MG1 nuclease (e.g., SEQ ID NO:1-4, respectively), where Ns denote nucleotides of a targeting sequence.

[00160] SEQ ID NOs: 5572-5575 show nucleotide sequences for E. coli codon-optimized coding sequences for MG1 family enzymes (SEQ ID NOs: 1-4).

[00161] SEQ ID NOs: 5588-5589 show nucleotide sequences for human codon-optimized coding sequences for MG1 family enzymes (SEQ ID NOs: 1 and 3).

[00162] SEQ ID NOs: 5616-5632 show peptide motifs characteristic of MG1 family enzymes.

[00163] SEQ ID NOs: 9192-9255 show the peptide sequences of PAM-interacting domains of MG1 nucleases.

[00164] SEQ ID NOs: 11229-11269 show the nucleotide sequences of target sites of MG1 nucleases.

MG2

[00165] SEQ ID NOs: 320-420 and 7294-7358 show the full-length peptide sequences of MG2 nucleases.

[00166] SEQ ID NOs: 2141-2241 show the peptide sequences of RuvC_III domains of MG2 nucleases above.

[00167] SEQ ID NOs: 3955-4055 show the peptide of HNH domains of MG2 nucleases above.

[00168] SEQ ID NOs: 5490-5494 and 11159 show the nucleotide sequences of MG2 tracrRNAs derived from the same loci as MG2 nucleases above (e.g., same loci as SEQ ID NOs: 320, 321, 323, 325, and 326, respectively).

[00169] SEQ ID NO: 5465 shows the nucleotide sequence of an sgRNA engineered to function with an MG2 nuclease (e.g., SEQ ID NO: 321 above).

[00170] SEQ ID NOs: 5572-5575 show nucleotide sequences for E. coli codon-optimized coding sequences for MG2 family enzymes.

[00171] SEQ ID NOs: 5631-5638 show peptide sequences characteristic of MG2 family enzymes.

[00172] SEQ ID NOs: 9256-9322 show the peptide sequences of PAM-interacting domains of MG2 nucleases.

[00173] SEQ ID NOs: 11270-11275 show the nucleotide sequences of target sites of MG2 nucleases.

MG3

[00174] SEQ ID NOs: 421-431 show the full-length peptide sequences of MG3 nucleases.

[00175] SEQ ID NO: 6803 shows the nucleotide sequence of an MG3-6 nuclease containing 5' UTR, NLS, CDS, NLS, 3' UTR, and polyA tail.

[00176] SEQ ID NOs: 2242-2252 show the peptide sequences of RuvC_III domains of MG3 nucleases above.

[00177] SEQ ID NOs: 4056-4066 show the peptide of HNH domains of MG3 nucleases above.

[00178] SEQ ID NOs: 5495-5502 and 11160-11162 show the nucleotide sequences of MG3 tracrRNAs derived from the same loci as MG3 nucleases above (e.g., same loci as SEQ ID NOs: 421-428, respectively).

[00179] SEQ ID NOs: 5466-5467, 11131, and 11567-11576 show the nucleotide sequences of sgRNAs engineered to function with MG3 nucleases (e.g., SEQ ID NOs: 421 — 423).

[00180] SEQ ID NOs: 5578-5580 show nucleotide sequences for E. coli codon-optimized coding sequences for MG3 family enzymes.

[00181] SEQ ID NOs: 5639-5648 show peptide sequences characteristic of MG3 family enzymes.

[00182] SEQ ID NOs: 9323-9329 show the peptide sequences of PAM-interacting domains of MG3 nucleases.

[00183] SEQ ID NOs: 11108 and 11530-11538 show the nucleotide sequences of single guide PAMs of MG3 nucleases.

[00184] SEQ ID NOs: 11276-11294 show the nucleotide sequences of target sites of MG1 nucleases.

[00185] SEQ ID NO: 11373 shows the nucleotide sequence of a DNA sequence encoding MG3-6 mRNA.

MG3a

[00186] SEQ ID NOs: 7369-7375 show the full-length peptide sequences of MG3a nucleases.

[00187] SEQ ID NOs: 11099 show the peptide sequences of PAM-interacting domains of MG3a nucleases.

MG3b

[00188] SEQ ID NOs: 7376-7390 show the full-length peptide sequences of MG3b nucleases.

[00189] SEQ ID NOs: 11100-11107 show the peptide sequences of PAM-interacting domains of MG3b nucleases.

MG4

[00190] SEQ ID NOs: 432-660 and 7391-7535 show the full-length peptide sequences of MG4 nucleases.

[00191] SEQ ID NOs: 2253-2481 show the peptide sequences of RuvC_III domains of MG4 nucleases above.

[00192] SEQ ID NOs: 4067-4295 show the peptide of HNH domains of MG4 nucleases above.

[00193] SEQ ID NO: 5503 shows the nucleotide sequences of an MG4 tracrRNA derived from the same loci as MG4 nucleases above.

[00194] SEQ ID NO: 5468 shows the nucleotide sequence of sgRNAs engineered to function with an MG4 nuclease.

[00195] SEQ ID NO: 5649 shows a peptide sequence characteristic of MG4 family enzymes.

[00196] SEQ ID NOs: 9330-9485 show the peptide sequences of PAM-interacting domains of MG4 nucleases.

[00197] SEQ ID NOs: 11295-11303 show the nucleotide sequences of target sites of MG4 nucleases.

MG5

[00198] SEQ ID NOs: 7536-7583 show the full-length peptide sequences of MG5 nucleases.

[00199] SEQ ID NOs: 9486-9526 show the peptide sequences of PAM-interacting domains of MG5 nucleases.

MG6

[00200] SEQ ID NOs: 661-668 and 7584-7587 show the full-length peptide sequences of MG6 nucleases.

[00201] SEQ ID NOs: 2482-2489 show the peptide sequences of RuvC_III domains of MG6 nucleases above.

[00202] SEQ ID NOs: 4296-4303 show the peptide of HNH domains of MG3 nucleases above.

[00203] SEQ ID NOs: 9527-9531 show the peptide sequences of PAM-interacting domains of MG6 nucleases.

MG7

[00204] SEQ ID NOs: 669-677 show the full-length peptide sequences of MG7 nucleases.

[00205] SEQ ID NOs: 2490-2498 show the peptide sequences of RuvC_III domains of MG7 nucleases above.

[00206] SEQ ID NOs: 4304-4312 show the peptide of HNH domains of MG3 nucleases above.

[00207] SEQ ID NO: 5504 shows the nucleotide sequence of an MG7 tracrRNA derived from the same loci as MG7 nucleases above.

[00208] SEQ ID NOs: 9532-9535 show the peptide sequences of PAM-interacting domains of MG7 nucleases.

MG14

[00209] SEQ ID NOs: 678-929 and 7588-7597 show the full-length peptide sequences of MG14 nucleases.

[00210] SEQ ID NOs: 2499-2750 show the peptide sequences of RuvC_III domains of MG14 nucleases above.

[00211] SEQ ID NOs: 4313-4564 show the peptide of HNH domains of MG14 nucleases above.

[00212] SEQ ID NOs: 5505 and 11163-11167 show nucleotide sequences of MG14 tracrRNAs derived from the same loci as MG14 nucleases above.

[00213] SEQ ID NO: 5581 shows a nucleotide sequence for an E. coli codon-optimized coding sequences for an MG14 family enzyme.

[00214] SEQ ID NOs: 5650-5667 show peptide sequences characteristic of MG14 family enzymes.

[00215] SEQ ID NOs: 9536-9611 show the peptide sequences of PAM-interacting domains of MG14 nucleases.

[00216] SEQ ID NOs: 11109-11113 show the nucleotide sequences of single guide PAMs of MG14 nucleases.

[00217] SEQ ID NOs: 11132-11136 shows the nucleotide sequence of sgRNAs engineered to function with an MG14 nuclease.

[00218] SEQ ID NOs: 11304-11312 show the nucleotide sequences of target sites of MG14 nucleases.

MG15

[00219] SEQ ID NOs: 930-1092, 7598-7622, and 11593-11616 show the full-length peptide sequences of MG15 nucleases.

[00220] SEQ ID NOs: 2751-2913 show the peptide sequences of RuvC_III domains of MG15 nucleases above.

[00221] SEQ ID NOs: 4565-4727 show the peptide of HNH domains of MG15 nucleases above.

[00222] SEQ ID NOs: 5506 and 11168-11172 show nucleotide sequences of MG15 tracrRNAs derived from the same loci as MG15 nucleases above.

[00223] SEQ ID NOs: 5470 and 11577-11592 show the nucleotide sequences of sgRNAs engineered to function with MG15 nucleases.

[00224] SEQ ID NO: 5582 shows a nucleotide sequence for an E. coli codon-optimized coding sequences for an MG15 family enzyme.

[00225] SEQ ID NOs: 5668-5675 show peptide sequences characteristic of MG15 family enzymes.

[00226] SEQ ID NOs: 9612-9671 show the peptide sequences of PAM-interacting domains of MG15 nucleases.

[00227] SEQ ID NOs: 11539-11554 show the nucleotide sequences of single guide PAMs of MG15 nucleases.

MG16

[00228] SEQ ID NOs: 1093-1353 and 7623-7698 show the full-length peptide sequences of MG16 nucleases.

[00229] SEQ ID NOs: 2914-3174 show the peptide sequences of RuvC_III domains of MG16 nucleases above.

[00230] SEQ ID NOs: 4728-4988 show the peptide of HNH domains of MG16 nucleases above.

[00231] SEQ ID NOs: 5507 and 11173-11174 show nucleotide sequences of MG16 tracrRNAs derived from the same loci as MG16 nucleases above.

[00232] SEQ ID NOs: 5471 and 11137 show nucleotide sequences of sgRNAs engineered to function with an MG16 nuclease.

[00233] SEQ ID NO: 5583 shows a nucleotide sequence for an E. coli codon-optimized coding sequences for an MG16 family enzyme.

[00234] SEQ ID NOs: 5676-5678 show peptide sequences characteristic of MG16 family enzymes.

[00235] SEQ ID NOs: 9672-9842 show the peptide sequences of PAM-interacting domains of MG16 nucleases.

[00236] SEQ ID NO: 11114 shows the nucleotide sequence of a single guide PAM of an MG16 nuclease.

[00237] SEQ ID NOs: 11313-11320 show the nucleotide sequences of target sites of MG16 nucleases.

MG17

[00238] SEQ ID NOs: 7699-7715 show the full-length peptide sequences of MG17 nucleases.

[00239] SEQ ID NOs: 9843-9856 show the peptide sequences of PAM-interacting domains of MG17 nucleases.

[00240] SEQ ID NO: 11115 shows the nucleotide sequence of a single guide PAM of an MG17 nuclease.

[00241] SEQ ID NO: 11138 shows the nucleotide sequence of an sgRNA engineered to function with an MG17 nuclease.

[00242] SEQ ID NO: 11175 shows the nucleotide sequence of an MG17 tracrRNA derived from the same loci as MG17 nucleases above.

MG18

[00243] SEQ ID NOs: 1354-1511 show the full-length peptide sequences of MG18 nucleases.

[00244] SEQ ID NOs: 3175-3330 show the peptide sequences of RuvC_III domains of MG18 nucleases above.

[00245] SEQ ID NOs: 4989-5146 show the peptide of HNH domains of MG18 nucleases above.

[00246] SEQ ID NO: 5508 shows the nucleotide sequences of MG18 tracrRNA derived from the same loci as MG18 nucleases above.

[00247] SEQ ID NOs: 5472 shows the nucleotide sequence of an sgRNA engineered to function with an MG18 nuclease.

[00248] SEQ ID NO: 5584 shows a nucleotide sequence for an E. coli codon-optimized coding sequences for an MG18 family enzyme.

[00249] SEQ ID NOs: 5679-5686 show peptide sequences characteristic of MG18 family enzymes.

[00250] SEQ ID NOs: 9857-9891 show the peptide sequences of PAM-interacting domains of MG18 nucleases.

[00251] SEQ ID NOs: 11321-11327 show the nucleotide sequences of target sites of MG18 nucleases.

MG21

[00252] SEQ ID NOs: 1512-1655 and 7716-7733 show the full-length peptide sequences of MG21 nucleases.

[00253] SEQ ID NOs: 3331-3474 show the peptide sequences of RuvC_III domains of MG21 nucleases above.

[00254] SEQ ID NOs: 5147-5290 show the peptide of HNH domains of MG21 nucleases above.

[00255] SEQ ID NOs: 5509 and 11176-11178 show nucleotide sequences of MG21 tracrRNAs derived from the same loci as MG21 nucleases above.

[00256] SEQ ID NOs: 5473 and 11139 show nucleotide sequences of sgRNAs engineered to function with an MG21 nuclease.

[00257] SEQ ID NO: 5585 shows a nucleotide sequence for an E. coli codon-optimized coding sequences for an MG21 family enzyme.

[00258] SEQ ID NOs: 5687-5692 and 5674-5675 show peptide sequences characteristic of MG21 family enzymes.

[00259] SEQ ID NOs: 9892-9951 show the peptide sequences of PAM-interacting domains of MG21 nucleases.

[00260] SEQ ID NO: 11116 shows the nucleotide sequence of a single guide PAM of an MG21 nuclease.

[00261] SEQ ID NOs: 11328-11336 show the nucleotide sequences of target sites of MG21 nucleases.

MG22

[00262] SEQ ID NOs: 1656-1755 show the full-length peptide sequences of MG22 nucleases.

[00263] SEQ ID NOs: 3475-3568 show the peptide sequences of RuvC_III domains of MG22 nucleases above.

[00264] SEQ ID NOs: 5291-5389 show the peptide of HNH domains of MG22 nucleases above.

[00265] SEQ ID NOs: 5510 and 11179-11180 show nucleotide sequences of MG22 tracrRNAs derived from the same loci as MG22 nucleases above.

[00266] SEQ ID NOs: 5474 shows the nucleotide sequence of an sgRNAs engineered to function with an MG22 nuclease.

[00267] SEQ ID NO: 5586 shows a nucleotide sequence for an E. coli codon-optimized coding sequences for an MG22 family enzyme.

[00268] SEQ ID NOs: 5694-5699 show peptide sequences characteristic of MG22 family enzymes.

[00269] SEQ ID NOs: 9952-9982 show the peptide sequences of PAM-interacting domains of MG22 nucleases.

[00270] SEQ ID NOs: 11337-11344 show the nucleotide sequences of target sites of MG22 nucleases.

MG23

[00271] SEQ ID NOs: 1756-1826 and 7734-7735 show the full-length peptide sequences of MG23 nucleases.

[00272] SEQ ID NOs: 3569-3637 show the peptide sequences of RuvC_III domains of MG23 nucleases above.

[00273] SEQ ID NOs: 5390-5460 show the peptide of HNH domains of MG23 nucleases above.

[00274] SEQ ID NOs: 5511 and 11181-11182 show nucleotide sequences of MG23 tracrRNAs derived from the same loci as MG23 nucleases above.

[00275] SEQ ID NOs: 5475 and 11140 show nucleotide sequences of sgRNAs engineered to function with an MG23 nuclease.

[00276] SEQ ID NO: 5587 shows a nucleotide sequence for an E. coli codon-optimized coding sequences for an MG23 family enzyme.

[00277] SEQ ID NOs: 5700-5717 show peptide sequences characteristic of MG23 family enzymes.

[00278] SEQ ID NOs: 9983-10004 show the peptide sequences of PAM-interacting domains of MG23 nucleases.

[00279] SEQ ID NOs: 11345-11351 show the nucleotide sequences of target sites of MG23 nucleases.

MG24

[00280] SEQ ID NOs: 7736-8027 show the full-length peptide sequences of MG24 nucleases.

[00281] SEQ ID NOs: 10005-10162 show the peptide sequences of PAM-interacting domains of MG24 nucleases.

MG25

[00282] SEQ ID NOs: 8028-8091 show the full-length peptide sequences of MG25 nucleases.

[00283] SEQ ID NOs: 10163-10211 show the peptide sequences of PAM-interacting domains of MG25 nucleases.

MG38

[00284] SEQ ID NOs: 8092-8095 show the full-length peptide sequences of MG38 nucleases.

[00285] SEQ ID NOs: 10212-10214 show the peptide sequences of PAM-interacting domains of MG38 nucleases.

MG40

[00286] SEQ ID NOs: 5718-5750 and 8096-8163 show the full-length peptide sequences of MG40 nucleases.

[00287] SEQ ID NOs: 5847-5852 show protospacer adjacent motifs associated with MG 40 nucleases.

[00288] SEQ ID NOs: 5862-5873 show the nucleotide sequence of an sgRNA engineered to function with an MG40 nuclease.

[00289] SEQ ID NOs: 10215-10263 show the peptide sequences of PAM-interacting domains of MG40 nucleases.

[00290] SEQ ID NOs: 11183-11188 show nucleotide sequences of MG40 tracrRNAs derived from the same loci as MG40 nucleases above.

MG41

[00291] SEQ ID NOs: 8164-8286 show the full-length peptide sequences of MG41 nucleases.

[00292] SEQ ID NOs: 10264-10304 show the peptide sequences of PAM-interacting domains of MG41 nucleases.

MG42

[00293] SEQ ID NOs: 8287-8356 show the full-length peptide sequences of MG42 nucleases.

[00294] SEQ ID NOs: 10305-10355 show the peptide sequences of PAM-interacting domains of MG42 nucleases.

MG43

[00295] SEQ ID NOs: 8357-8453 show the full-length peptide sequences of MG43 nucleases.

[00296] SEQ ID NOs: 10356-10412 show the peptide sequences of PAM-interacting domains of MG43 nucleases.

[00297] SEQ ID NO: 11117 shows the nucleotide sequence of a single guide PAM of an MG43 nuclease.

[00298] SEQ ID NO: 11141 shows the nucleotide sequence of an sgRNA engineered to function with an MG43 nuclease.

[00299] SEQ ID NO: 11189 shows the nucleotide sequence of an MG43 tracrRNA derived from the same loci as MG43 nucleases above.

MG44

[00300] SEQ ID NOs: 8454-8496 show the full-length peptide sequences of MG44 nucleases.

[00301] SEQ ID NOs: 10413-10555 show the peptide sequences of PAM-interacting domains of MG44 nucleases.

[00302] SEQ ID NO: 11190 shows the nucleotide sequence of an MG44 tracrRNA derived from the same loci as MG44 nucleases above.

MG46

[00303] SEQ ID NOs: 8497-8634 show the full-length peptide sequences of MG46 nucleases.

[00304] SEQ ID NOs: 10556-10633 show the peptide sequences of PAM-interacting domains of MG46 nucleases.

[00305] SEQ ID NO: 11191 shows the nucleotide sequence of an MG46 tracrRNA derived from the same loci as MG46 nucleases above.

MG47

[00306] SEQ ID NOs: 5751-5768 and 8635-8664 show the full-length peptide sequences of MG47 nucleases.

[00307] SEQ ID NOs: 5853-5854 show protospacer adjacent motifs associated with MG47 nucleases.

[00308] SEQ ID NOs: 5878-5881 show the nucleotide sequence of an sgRNA engineered to function with an MG47 nuclease.

[00309] SEQ ID NOs: 10634-10656 show the peptide sequences of PAM-interacting domains of MG47 nucleases.

[00310] SEQ ID NOs: 11192-11193 show nucleotide sequences of MG47 tracrRNAs derived from the same loci as MG47 nucleases above.

MG48

[00311] SEQ ID NOs: 5769-5804 and 8665 show the full-length peptide sequences of MG48 nucleases.

[00312] SEQ ID NOs: 5855-5856 show protospacer adjacent motifs associated with MG48 nucleases.

[00313] SEQ ID NOs: 5886, 5890, 5893, and 11194 show the nucleotide sequences of MG48 tracrRNA derived from the same loci as MG48 nucleases above

[00314] SEQ ID NOs: 5887, 5891 and 5894 show CRISPR repeats associated with MG48 nucleases described herein.

[00315] SEQ ID NOs: 5888-5889, 5892 and 5895-5896 show putative sgRNA designed to function with an MG48 nuclease.

[00316] SEQ ID NOs: 10657-10662 show the peptide sequences of PAM-interacting domains of MG48 nucleases.

[00317] SEQ ID NOs: 11142-11143 show nucleotide sequences of sgRNAs engineered to function with an MG48 nuclease.

MG49

[00318] SEQ ID NOs: 5805-5823 and 8666-8677 show the full-length peptide sequences of MG49 nucleases.

[00319] SEQ ID NOs: 5857-5858 show protospacer adjacent motifs associated with MG49 nucleases.

[00320] SEQ ID NOs: 5862-5873 show the nucleotide sequence of an sgRNA engineered to function with an MG40 nuclease.

[00321] SEQ ID NOs: 5876-5877 show the nucleotide sequence of an sgRNA engineered to function with an MG49 nuclease.

[00322] SEQ ID NOs: 10663-10675 show the peptide sequences of PAM-interacting domains of MG49 nucleases.

[00323] SEQ ID NOs: 11195-11196 show nucleotide sequences of MG49 tracrRNAs derived from the same loci as MG49 nucleases above.

MG50

[00324] SEQ ID NOs: 5824-5826 and 8678-8682 show the full-length peptide sequences of MG50 nucleases.

[00325] SEQ ID NO: 5859 shows a protospacer adjacent motif associated with MG50 nucleases.

[00326] SEQ ID NOs: 5884-5885 show the nucleotide sequence of an sgRNA engineered to function with an MG50 nuclease.

[00327] SEQ ID NOs: 10676-10682 show the peptide sequences of PAM-interacting domains of MG50 nucleases.

[00328] SEQ ID NO: 11197 shows the nucleotide sequence of an MG50 tracrRNA derived from the same loci as MG50 nucleases above.

MG51

[00329] SEQ ID NOs: 5827-5830 and 8683-8705 show the full-length peptide sequences of MG51 nucleases.

[00330] SEQ ID NO: 5860 shows a protospacer adjacent motif associated with MG51 nucleases.

[00331] SEQ ID NOs: 5882-5883 show the nucleotide sequence of an sgRNA engineered to function with an MG51 nuclease.

[00332] SEQ ID NOs: 10683-10704 show the peptide sequences of PAM-interacting domains of MG51 nucleases.

[00333] SEQ ID NO: 11198 shows the nucleotide sequence of an MG51 tracrRNA derived from the same loci as MG51 nucleases above.

MG52

[00334] SEQ ID NOs: 5831-5846 and 8706 show the full-length peptide sequences of MG52 nucleases.

[00335] SEQ ID NO: 5861 shows a protospacer adjacent motif associated with MG52 nucleases.

[00336] SEQ ID NOs: 5874-5875 show the nucleotide sequence of an sgRNA engineered to function with an MG52 nuclease.

[00337] SEQ ID NOs: 10705-10710 show the peptide sequences of PAM-interacting domains of MG52 nucleases.

[00338] SEQ ID NO: 11199 shows the nucleotide sequence of an MG52 tracrRNA derived from the same loci as MG52 nucleases above.

MG71

[00339] SEQ ID NOs: 10711-10712 show the peptide sequences of PAM-interacting domains of MG71 nucleases.

[00340] SEQ ID NOs: 11144-11145 show nucleotide sequences of sgRNAs engineered to function with an MG71 nuclease.

[00341] SEQ ID NOs: 11200-11201 show nucleotide sequences of MG71 tracrRNAs derived from the same loci as MG71 nucleases above.

MG72

[00342] SEQ ID NO: 11202 shows the nucleotide sequence of an MG72 tracrRNA derived from the same loci as MG72 nucleases above.

MG73

[00343] SEQ ID NOs: 10713-10718 show the peptide sequences of PAM-interacting domains of MG73 nucleases.

[00344] SEQ ID NOs: 11203-11204 show nucleotide sequences of MG73 tracrRNAs derived from the same loci as MG73 nucleases above.

MG74

[00345] SEQ ID NOs: 10719-10732 show the peptide sequences of PAM-interacting domains of MG74 nucleases.

[00346] SEQ ID NO: 11205 shows the nucleotide sequence of an MG74 tracrRNA derived from the same loci as MG74 nucleases above.

MG86

[00347] SEQ ID NOs: 8707-8737 show the full-length peptide sequences of MG86 nucleases.

[00348] SEQ ID NOs: 10733-10791 show the peptide sequences of PAM-interacting domains of MG86 nucleases.

[00349] SEQ ID NO: 11118 shows the nucleotide sequence of a single guide PAM of an MG86 nuclease.

[00350] SEQ ID NOs: 11206-11207 show nucleotide sequences of MG86 tracrRNAs derived from the same loci as MG86 nucleases above.

MG87

[00351] SEQ ID NOs: 8738-8747 show the full-length peptide sequences of MG87 nucleases.

[00352] SEQ ID NOs: 10792-10828 show the peptide sequences of PAM-interacting domains of MG87 nucleases.

[00353] SEQ ID NOs: 11208-11210 show nucleotide sequences of MG87 tracrRNAs derived from the same loci as MG87 nucleases above.

MG88

[00354] SEQ ID NOs: 10829-10841 show the peptide sequences of PAM-interacting domains of MG88 nucleases.

[00355] SEQ ID NOs: 11211-11213 show nucleotide sequences of MG88 tracrRNAs derived from the same loci as MG88 nucleases above.

MG89

[00356] SEQ ID NOs: 10842-10854 show the peptide sequences of PAM-interacting domains of MG89 nucleases.

[00357] SEQ ID NOs: 11214-11215 show nucleotide sequences of MG89 tracrRNAs derived from the same loci as MG89 nucleases above.

MG94

[00358] SEQ ID NOs: 8748-8781 show the full-length peptide sequences of MG94 nucleases.

[00359] SEQ ID NOs: 10855-10860 show the peptide sequences of PAM-interacting domains of MG94 nucleases.

[00360] SEQ ID NOs: 11119-11120 show the nucleotide sequences of single guide PAMs of MG94 nucleases.

[00361] SEQ ID NOs: 11146-11147 show nucleotide sequences of sgRNAs engineered to function with an MG94 nuclease.

[00362] SEQ ID NOs: 11216-11217 show nucleotide sequences of MG94 tracrRNAs derived from the same loci as MG94 nucleases above.

MG95

[00363] SEQ ID NOs: 8782-8785 show the full-length peptide sequences of MG95 nucleases.

[00364] SEQ ID NOs: 10861-10863 show the peptide sequences of PAM-interacting domains of MG95 nucleases.

[00365] SEQ ID NOs: 11121-11122 show the nucleotide sequences of single guide PAMs of MG95 nucleases.

[00366] SEQ ID NOs: 11148-11149 show nucleotide sequences of sgRNAs engineered to function with an MG95 nuclease.

[00367] SEQ ID NOs: 11218-11219 show nucleotide sequences of MG95 tracrRNAs derived from the same loci as MG95 nucleases above.

MG96

[00368] SEQ ID NOs: 8786-8814 show the full-length peptide sequences of MG96 nucleases.

[00369] SEQ ID NOs: 10864-10884 show the peptide sequences of PAM-interacting domains of MG96 nucleases.

[00370] SEQ ID NO: 11123 shows the nucleotide sequence of a single guide PAM of an MG96 nuclease.

[00371] SEQ ID NO: 11150 shows the nucleotide sequence of an sgRNA engineered to function with an MG96 nuclease.

[00372] SEQ ID NO: 11220 shows the nucleotide sequence of an MG96 tracrRNA derived from the same loci as MG96 nucleases above.

MG97

[00373] SEQ ID NOs: 8815-8818 show the full-length peptide sequences of MG97 nucleases.

[00374] SEQ ID NOs: 10885-10887 show the peptide sequences of PAM-interacting domains of MG97 nucleases.

MG98

[00375] SEQ ID NOs: 8819-8959 show the full-length peptide sequences of MG98 nucleases.

[00376] SEQ ID NOs: 10888-10936 show the peptide sequences of PAM-interacting domains of MG98 nucleases.

[00377] SEQ ID NOs: 11124-11125 show the nucleotide sequences of single guide PAMs of MG98 nucleases.

[00378] SEQ ID NOs: 11151-11152 show nucleotide sequences of sgRNAs engineered to function with an MG98 nuclease.

[00379] SEQ ID NOs: 11221-11222 show nucleotide sequences of MG98 tracrRNAs derived from the same loci as MG98 nucleases above.

MG99

[00380] SEQ ID NO: 11153 shows the nucleotide sequence of an sgRNA engineered to function with an MG99 nuclease.

[00381] SEQ ID NO: 11223 shows the nucleotide sequence of an MG99 tracrRNA derived from the same loci as MG99 nucleases above.

MG100

[00382] SEQ ID NOs: 8960-9036 show the full-length peptide sequences of MG100 nucleases.

[00383] SEQ ID NOs: 10937-10991 show the peptide sequences of PAM-interacting domains of MG100 nucleases.

[00384] SEQ ID NO: 11126 shows the nucleotide sequence of a single guide PAM of an MG100 nuclease.

[00385] SEQ ID NOs: 11154-11155 show nucleotide sequences of sgRNAs engineered to function with an MG100 nuclease.

[00386] SEQ ID NOs: 11224-11225 show nucleotide sequences of MG100 tracrRNAs derived from the same loci as MG100 nucleases above.

MG111

[00387] SEQ ID NOs: 9037-9126 show the full-length peptide sequences of MG111 nucleases.

[00388] SEQ ID NOs: 10992-11046 show the peptide sequences of PAM-interacting domains of MG111 nucleases.

[00389] SEQ ID NOs: 11127-11128 show the nucleotide sequences of single guide PAMs of MG111 nucleases.

[00390] SEQ ID NOs: 11156-11157 show nucleotide sequences of sgRNAs engineered to function with an MG111 nuclease.

[00391] SEQ ID NOs: 11226-11227 show nucleotide sequences of MG111 tracrRNAs derived from the same loci as MG111 nucleases above.

MG112

[00392] SEQ ID NOs: 9127-9149 show the full-length peptide sequences of MG112 nucleases.

[00393] SEQ ID NOs: 11047-11062 show the peptide sequences of PAM-interacting domains of MG112 nucleases.

MG116

[00394] SEQ ID NOs: 9150-9191 show the full-length peptide sequences of MG116 nucleases.

[00395] SEQ ID NOs: 11063-11098 show the peptide sequences of PAM-interacting domains of MG116 nucleases.

[00396] SEQ ID NO: 11129 shows the nucleotide sequence of a single guide PAM of an MG116 nuclease.

[00397] SEQ ID NO: 11158 shows the nucleotide sequence of an sgRNA engineered to function with an MG116 nuclease.

[00398] SEQ ID NO: 11228 shows the nucleotide sequence of an MG116 tracrRNA derived from the same loci as MG116 nucleases above.

MG123

[00399] SEQ ID NOs: 11617-11624 show the full-length peptide sequences of MG123 nucleases.

[00400] SEQ ID NO: 11518 shows the nucleotide sequence of a single guide PAM of an MG123 nuclease.

[00401] SEQ ID NO: 11555 shows the nucleotide sequence of an sgRNA engineered to function with an MG123 nuclease.

MG124

[00402] SEQ ID NOs: 11625-11626 show the full-length peptide sequences of MG124 nucleases.

[00403] SEQ ID NO: 11519 shows the nucleotide sequence of a single guide PAM of an MG124 nuclease.

[00404] SEQ ID NO: 11556 shows the nucleotide sequence of an sgRNA engineered to function with an MG124 nuclease.

MG125

[00405] SEQ ID NOs: 11627-11707 show the full-length peptide sequences of MG125 nucleases.

[00406] SEQ ID NOs: 11520-11524 show the nucleotide sequences of single guide PAMs of MG125 nucleases.

[00407] SEQ ID NOs: 11557-11561 show the nucleotide sequences of sgRNAs engineered to function with MG125 nucleases.

MG150

[00408] SEQ ID NOs: 7359-7368 and 11708-11710 show the full-length peptide sequences of MG150 nucleases.

[00409] SEQ ID NOs: 11525-11529 show the nucleotide sequences of single guide PAMs of MG150 nucleases.

[00410] SEQ ID NOs: 11562-11566 show the nucleotide sequences of sgRNAs engineered to function with MG150 nucleases.

B2M Targeting

[00411] SEQ ID NOs: 6305-6386 show the nucleotide sequences of sgRNAs engineered to function with an MG3-6 nuclease in order to target B2M.

[00412] SEQ ID NOs: 6387-6468 show the DNA sequences of B2M target sites.

TRAC Targeting

[00413] SEQ ID NOs: 6469-6508 and 6804 show the nucleotide sequences of sgRNAs engineered to function with an MG3-6 nuclease in order to target TRAC.

[00414] SEQ ID NOs: 6509-6548 and 6805 show the DNA sequences of TRAC target sites.

HPRT Targeting

[00415] SEQ ID NOs: 6549-6615 show the nucleotide sequences of sgRNAs engineered to function with an MG3-6 nuclease in order to target HPRT.

[00416] SEQ ID NOs: 6616-6682 show the DNA sequences of HPRT target sites.

MG3-6 TRBC1/2 Targeting

[00417] SEQ ID NOs: 6683-6721 show the nucleotide sequences of sgRNAs engineered to

function with an MG3-6 nuclease in order to target TRBC1/2.

[00418] SEQ ID NOs: 6722-6760 show the DNA sequences of TRBC1/2 target sites.

MG3-8 TRBC1/2 Targeting

[00419] SEQ ID NOs: 6761-6781 show the nucleotide sequences of sgRNAs engineered to function with an MG3-8 nuclease in order to target TRBC1/2.

[00420] SEQ ID NOs: 6782-6802 show the DNA sequences of TRBC1/2 target sites.

MG3-6 CD2 Targeting

[00421] SEQ ID NOs: 6811-6852 show the nucleotide sequences of sgRNAs engineered to function with an MG3-6 nuclease in order to target CD2.

[00422] SEQ ID NOs: 6853-6894 show the DNA sequences of CD2 target sites.

MG3-6 CD5 Targeting

[00423] SEQ ID NOs: 6895-6958 show the nucleotide sequences of sgRNAs engineered to function with an MG3-6 nuclease in order to target CD5.

[00424] SEQ ID NOs: 6959-7022 show the DNA sequences of CD5 target sites.

MG3-6 FAS Targeting

[00425] SEQ ID NOs: 7023-7056 show the nucleotide sequences of sgRNAs engineered to function with an MG3-6 nuclease in order to target FAS.

[00426] SEQ ID NOs: 7057-7090 show the DNA sequences of FAS target sites.

MG3-6 PD-1 Targeting

[00427] SEQ ID NOs: 7091-7128 show the nucleotide sequences of sgRNAs engineered to function with an MG3-6 nuclease in order to target PD-1.

[00428] SEQ ID NOs: 7129-7166 show the DNA sequences of PD-1 target sites.

MG3-6 hRosa26 Targeting

[00429] SEQ ID NOs: 7167-7198 show the nucleotide sequences of sgRNAs engineered to function with an MG3-6 nuclease in order to target hRosa26.

[00430] SEQ ID NOs: 7199-7230 show the DNA sequences of hRosa26 target sites.

MG21-1 TRAC Targeting

[00431] SEQ ID NOs: 7231-7234 show the nucleotide sequences of sgRNAs engineered to function with an MG21-1 nuclease in order to target TRAC.

[00432] SEQ ID NOs: 7235-7238 show the DNA sequences of TRAC target sites.

MG23-1 TRAC Targeting

[00433] SEQ ID NOs: 7239-7247 show the nucleotide sequences of sgRNAs engineered to function with an MG23-1 nuclease in order to target TRAC.

[00434] SEQ ID NOs: 7248-7256 show the DNA sequences of TRAC target sites.

MG14-241 AAVS1 Targeting

[00435] SEQ ID NOs: 11508-11510 show the nucleotide sequences of sgRNAs engineered to function with an MG14-241 nuclease in order to target AAVS1.

[00436] SEQ ID NOs: 11511-11513 show the DNA sequences of AAVS1 target sites.

MG23-1 AAVS1 Targeting

[00437] SEQ ID NOs: 7257-7260 show the nucleotide sequences of sgRNAs engineered to function with an MG23-1 nuclease in order to target AAVS1.

[00438] SEQ ID NOs: 7261-7264 show the DNA sequences of AAVS1 target sites.

MG71-2 AAVS1 Targeting

[00439] SEQ ID NOs: 7265-7266 show the nucleotide sequences of sgRNAs engineered to function with an MG71-2 nuclease in order to target AAVS1.

[00440] SEQ ID NOs: 7267-7268 show the DNA sequences of AAVS1 target sites.

MG73-1 TRAC Targeting

[00441] SEQ ID NO: 7269 shows the nucleotide sequence of an sgRNA engineered to function with an MG73-1 nuclease in order to target TRAC.

[00442] SEQ ID NO: 7270 shows the DNA sequence of a TRAC target site.

MG89-2 TRAC Targeting

[00443] SEQ ID NOs: 7271-7277 show the nucleotide sequences of sgRNAs engineered to function with an MG89-2 nuclease in order to target TRAC.

[00444] SEQ ID NOs: 7278-7284 show the DNA sequences of TRAC target sites.

MG99-1 TRAC Targeting

[00445] SEQ ID NOs: 11514-11515 show the nucleotide sequences of sgRNAs engineered to function with an MG99-1 nuclease in order to target TRAC.

[00446] SEQ ID NOs: 11516-11517 show the DNA sequences of TRAC target sites.

MG3-6 Human HAO-1 Targeting

[00447] SEQ ID NOs: 11352-11372 show the nucleotide sequences of sgRNAs engineered to function with an MG3-6 nuclease in order to target human HAO-1.

MG3-6 human GPR146 Targeting

[00448] SEQ ID NOs: 11374-11405 show the nucleotide sequences of sgRNAs engineered to function with an MG3-6 nuclease in order to target human GPR146.

[00449] SEQ ID NOs: 11406-11437 show the DNA sequences of human GPR146 target sites.

MG3-6 mouse GPR146 Targeting

[00450] SEQ ID NOs: 11438-11472 show the nucleotide sequences of sgRNAs engineered to function with an MG3-6 nuclease in order to target mouse GPR146.

[00451] SEQ ID NOs: 11473-11507 show the DNA sequences of mouse GPR146 target sites.

DETAILED DESCRIPTION

[00452] While various embodiments of the 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. Numerous variations, changes, and substitutions may occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed.

[00453] The practice of some methods disclosed herein employ, unless otherwise indicated, techniques of immunology, biochemistry, chemistry, molecular biology, microbiology, cell biology, genomics and recombinant DNA. See for example Sambrook and Green, *Molecular Cloning: A Laboratory Manual*, 4th Edition (2012); the series *Current Protocols in Molecular Biology* (F. M. Ausubel, et al. eds.); the series *Methods In Enzymology* (Academic Press, Inc.), *PCR 2: A Practical Approach* (M.J. MacPherson, B.D. Hames and G.R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) *Antibodies, A Laboratory Manual*, and *Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications*, 6th Edition (R.I. Freshney, ed. (2010)) (which is entirely incorporated by reference herein).

[00454] As used herein, the singular forms “a”, “an” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms “including”, “includes”, “having”, “has”, “with”, or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term “comprising”.

[00455] The term “about” or “approximately” means 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 one or more than one standard deviation, per the practice in the art. Alternatively, “about” can mean a range of up to 20%, up to 15%, up to 10%, up to 5%, or up to 1% of a given value.

[00456] As used herein, a “cell” generally refers to a biological cell. A cell may be the basic structural, functional or biological unit of a living organism. A cell may originate from any organism having one or more cells. Some non-limiting examples include: a prokaryotic cell, eukaryotic cell, a bacterial cell, an archaeal cell, a cell of a single-cell eukaryotic organism, a protozoa cell, a cell from a plant (e.g., cells from plant crops, fruits, vegetables, grains, soy bean, corn, maize, wheat, seeds, tomatoes, rice, cassava, sugarcane, pumpkin, hay, potatoes, cotton, cannabis, tobacco, flowering plants, conifers, gymnosperms, ferns, clubmosses, hornworts, liverworts, mosses), an algal cell, (e.g., *Botryococcus braunii*, *Chlamydomonas reinhardtii*, *Nannochloropsis gaditana*, *Chlorella pyrenoidosa*, *Sargassum patens* C. Agardh, and the like),

seaweeds (e.g., kelp), a fungal cell (e.g., a yeast cell, a cell from a mushroom), an animal cell, a cell from an invertebrate animal (e.g., fruit fly, cnidarian, echinoderm, nematode, etc.), a cell from a vertebrate animal (e.g., fish, amphibian, reptile, bird, mammal), a cell from a mammal (e.g., a pig, a cow, a goat, a sheep, a rodent, a rat, a mouse, a non-human primate, a human, etc.), and etcetera. Sometimes a cell is not originating from a natural organism (e.g., a cell can be a synthetically made, sometimes termed an artificial cell).

[00457] The term “nucleotide,” as used herein, generally refers to a base-sugar-phosphate combination. A nucleotide may comprise a synthetic nucleotide. A nucleotide may comprise a synthetic nucleotide analog. Nucleotides may be monomeric units of a nucleic acid sequence (e.g., deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)). The term nucleotide may include ribonucleoside triphosphates adenosine triphosphate (ATP), uridine triphosphate (UTP), cytosine triphosphate (CTP), guanosine triphosphate (GTP) and deoxyribonucleoside triphosphates such as dATP, dCTP, dITP, dUTP, dGTP, dTTP, or derivatives thereof. Such derivatives may include, for example, [α S]dATP, 7-deaza-dGTP and 7-deaza-dATP, and nucleotide derivatives that confer nuclease resistance on the nucleic acid molecule containing them. The term nucleotide as used herein may refer to dideoxyribonucleoside triphosphates (ddNTPs) and their derivatives. Illustrative examples of dideoxyribonucleoside triphosphates may include, but are not limited to, ddATP, ddCTP, ddGTP, ddITP, and ddTTP. A nucleotide may be unlabeled or detectably labeled, such as using moieties comprising optically detectable moieties (e.g., fluorophores). Labeling may also be carried out with quantum dots. Detectable labels may include, for example, radioactive isotopes, fluorescent labels, chemiluminescent labels, bioluminescent labels and enzyme labels. Fluorescent labels of nucleotides may include but are not limited fluorescein, 5-carboxyfluorescein (FAM), 2'7'-dimethoxy-4'5-dichloro-6-carboxyfluorescein (JOE), rhodamine, 6-carboxyrhodamine (R6G), N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA), 6-carboxy-X-rhodamine (ROX), 4-(4'dimethylaminophenylazo) benzoic acid (DABCYL), Cascade Blue, Oregon Green, Texas Red, Cyanine and 5-(2'-aminoethyl)aminonaphthalene-1-sulfonic acid (EDANS). Specific examples of fluorescently labeled nucleotides can include [R6G]dUTP, [TAMRA]dUTP, [R110]dCTP, [R6G]dCTP, [TAMRA]dCTP, [JOE]ddATP, [R6G]ddATP, [FAM]ddCTP, [R110]ddCTP, [TAMRA]ddGTP, [ROX]ddTTP, [dR6G]ddATP, [dR110]ddCTP, [dTAMRA]ddGTP, and [dROX]ddTTP available from Perkin Elmer, Foster City, Calif; FluoroLink DeoxyNucleotides, FluoroLink Cy3-dCTP, FluoroLink Cy5-dCTP, FluoroLink Fluor X-dCTP, FluoroLink Cy3-dUTP, and FluoroLink Cy5-dUTP available from Amersham, Arlington Heights, Ill.; Fluorescein-15-dATP, Fluorescein-12-dUTP, Tetramethyl-rhodamine-6-dUTP, IR770-9-dATP, Fluorescein-12-ddUTP, Fluorescein-12-UTP, and Fluorescein-15-2'-dATP available from Boehringer Mannheim,

Indianapolis, Ind.; and Chromosome Labeled Nucleotides, BODIPY-FL-14-UTP, BODIPY-FL-4-UTP, BODIPY-TMR-14-UTP, BODIPY-TMR-14-dUTP, BODIPY-TR-14-UTP, BODIPY-TR-14-dUTP, Cascade Blue-7-UTP, Cascade Blue-7-dUTP, fluorescein-12-UTP, fluorescein-12-dUTP, Oregon Green 488-5-dUTP, Rhodamine Green-5-UTP, Rhodamine Green-5-dUTP, tetramethylrhodamine-6-UTP, tetramethylrhodamine-6-dUTP, Texas Red-5-UTP, Texas Red-5-dUTP, and Texas Red-12-dUTP available from Molecular Probes, Eugene, Oreg. Nucleotides can also be labeled or marked by chemical modification. A chemically-modified single nucleotide can be biotin-dNTP. Some non-limiting examples of biotinylated dNTPs can include, biotin-dATP (e.g., bio-N6-ddATP, biotin-14-dATP), biotin-dCTP (e.g., biotin-11-dCTP, biotin-14-dCTP), and biotin-dUTP (e.g., biotin-11-dUTP, biotin-16-dUTP, biotin-20-dUTP).

[00458] The terms “polynucleotide,” “oligonucleotide,” and “nucleic acid” are used interchangeably to generally refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof, either in single-, double-, or multi-stranded form. A polynucleotide may be exogenous or endogenous to a cell. A polynucleotide may exist in a cell-free environment. A polynucleotide may be a gene or fragment thereof. A polynucleotide may be DNA. A polynucleotide may be RNA. A polynucleotide may have any three-dimensional structure and may perform any function. A polynucleotide may comprise one or more analogs (e.g., altered backbone, sugar, or nucleobase). If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. Some non-limiting examples of analogs include: 5-bromouracil, peptide nucleic acid, xeno nucleic acid, morpholinos, locked nucleic acids, glycol nucleic acids, threose nucleic acids, dideoxynucleotides, cordycepin, 7-deaza-GTP, fluorophores (e.g., rhodamine or fluorescein linked to the sugar), thiol containing nucleotides, biotin linked nucleotides, fluorescent base analogs, CpG islands, methyl-7-guanosine, methylated nucleotides, inosine, thiouridine, pseudouridine, dihydrouridine, queuosine, and wyosine. Non-limiting examples of polynucleotides include coding or non-coding regions of a gene or gene fragment, loci (locus) defined from linkage analysis, exons, introns, messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), short interfering RNA (siRNA), short-hairpin RNA (shRNA), micro-RNA (miRNA), ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, cell-free polynucleotides including cell-free DNA (cfDNA) and cell-free RNA (cfRNA), nucleic acid probes, and primers. The sequence of nucleotides may be interrupted by non-nucleotide components.

[00459] The terms “transfection” or “transfected” generally refer to introduction of a nucleic acid into a cell by non-viral or viral-based methods. The nucleic acid molecules may be gene

sequences encoding complete proteins or functional portions thereof. See, e.g., Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*, 18.1-18.88.

[00460] The terms “peptide,” “polypeptide,” and “protein” are used interchangeably herein to generally refer to a polymer of at least two amino acid residues joined by peptide bond(s). This term does not connote a specific length of polymer, nor is it intended to imply or distinguish whether the peptide is produced using recombinant techniques, chemical or enzymatic synthesis, or is naturally occurring. The terms apply to naturally occurring amino acid polymers as well as amino acid polymers comprising at least one modified amino acid. In some cases, the polymer may be interrupted by non-amino acids. The terms include amino acid chains of any length, including full length proteins, and proteins with or without secondary or tertiary structure (e.g., domains). The terms also encompass an amino acid polymer that has been modified, for example, by disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, oxidation, and any other manipulation such as conjugation with a labeling component. The terms “amino acid” and “amino acids,” as used herein, generally refer to natural and non-natural amino acids, including, but not limited to, modified amino acids and amino acid analogues. Modified amino acids may include natural amino acids and non-natural amino acids, which have been chemically modified to include a group or a chemical moiety not naturally present on the amino acid. Amino acid analogues may refer to amino acid derivatives. The term “amino acid” includes both D-amino acids and L-amino acids.

[00461] As used herein, the “non-native” can generally refer to a nucleic acid or polypeptide sequence that is not found in a native nucleic acid or protein. Non-native may refer to affinity tags. Non-native may refer to fusions. Non-native may refer to a naturally occurring nucleic acid or polypeptide sequence that comprises mutations, insertions or deletions. A non-native sequence may exhibit or encode for an activity (e.g., enzymatic activity, methyltransferase activity, acetyltransferase activity, kinase activity, ubiquitinating activity, etc.) that may also be exhibited by the nucleic acid or polypeptide sequence to which the non-native sequence is fused. A non-native nucleic acid or polypeptide sequence may be linked to a naturally-occurring nucleic acid or polypeptide sequence (or a variant thereof) by genetic engineering to generate a chimeric nucleic acid or polypeptide sequence encoding a chimeric nucleic acid or polypeptide.

[00462] The term “promoter”, as used herein, generally refers to the regulatory DNA region which controls transcription or expression of a gene, and which may be located adjacent to or overlapping a nucleotide or region of nucleotides at which RNA transcription is initiated. A promoter may contain specific DNA sequences which bind protein factors, often referred to as transcription factors, which facilitate binding of RNA polymerase to the DNA leading to gene transcription. A ‘basal promoter’, also referred to as a ‘core promoter’, may generally refer to a

promoter that contains all the basic elements to promote transcriptional expression of an operably linked polynucleotide. Eukaryotic basal promoters often contain a TATA-box or a CAAT box.

[00463] The term “expression”, as used herein, generally refers to the process by which a nucleic acid sequence or a polynucleotide is transcribed from a DNA template (such as into mRNA or other RNA transcript) or the process by which a transcribed mRNA is subsequently translated into peptides, polypeptides, or proteins. Transcripts and encoded polypeptides may be collectively referred to as “gene product.” If the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA in a eukaryotic cell.

[00464] As used herein, “operably linked”, “operable linkage”, “operatively linked”, or grammatical equivalents thereof generally refer to juxtaposition of genetic elements, e.g., a promoter, an enhancer, a polyadenylation sequence, etc., wherein the elements are in a relationship permitting them to operate in the expected manner. For instance, a regulatory element, which may comprise promoter or enhancer sequences, is operatively linked to a coding region if the regulatory element helps initiate transcription of the coding sequence. There may be intervening residues between the regulatory element and coding region so long as this functional relationship is maintained.

[00465] A “vector” as used herein, generally refers to a macromolecule or association of macromolecules that comprises or associates with a polynucleotide and which may be used to mediate delivery of the polynucleotide to a cell. Examples of vectors include plasmids, viral vectors, liposomes, and other gene delivery vehicles. The vector generally comprises genetic elements, e.g., regulatory elements, operatively linked to a gene to facilitate expression of the gene in a target.

[00466] As used herein, “an expression cassette” and “a nucleic acid cassette” are used interchangeably generally to refer to a combination of nucleic acid sequences or elements that are expressed together or are operably linked for expression. In some cases, an expression cassette refers to the combination of regulatory elements and a gene or genes to which they are operably linked for expression.

[00467] A “functional fragment” of a DNA or protein sequence generally refers to a fragment that retains a biological activity (either functional or structural) that is substantially similar to a biological activity of the full-length DNA or protein sequence. A biological activity of a DNA sequence may be its ability to influence expression in a manner attributed to the full-length sequence.

[00468] As used herein, an “engineered” object generally indicates that the object has been modified by human intervention. According to non-limiting examples: a nucleic acid may be

modified by changing its sequence to a sequence that does not occur in nature; a nucleic acid may be modified by ligating it to a nucleic acid that it does not associate with in nature such that the ligated product possesses a function not present in the original nucleic acid; an engineered nucleic acid may be synthesized in vitro with a sequence that does not exist in nature; a protein may be modified by changing its amino acid sequence to a sequence that does not exist in nature; an engineered protein may acquire a new function or property. An “engineered” system comprises at least one engineered component.

[00469] As used herein, “synthetic” and “artificial” are used interchangeably to refer to a protein or a domain thereof that has low sequence identity (e.g., less than 50% sequence identity, less than 25% sequence identity, less than 10% sequence identity, less than 5% sequence identity, less than 1% sequence identity) to a naturally occurring human protein. For example, VPR and VP64 domains are synthetic transactivation domains.

[00470] The term “tracrRNA” or “tracr sequence”, as used herein, can generally refer to a nucleic acid with at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% sequence identity or sequence similarity to a wild type exemplary tracrRNA sequence (e.g., a tracrRNA from *S. pyogenes* *S. aureus*, etc or SEQ ID NOs: 5476-5511). tracrRNA can refer to a nucleic acid with at most about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% sequence identity or sequence similarity to a wild type exemplary tracrRNA sequence (e.g., a tracrRNA from *S. pyogenes* *S. aureus*, etc). tracrRNA may refer to a modified form of a tracrRNA that can comprise a nucleotide change such as a deletion, insertion, or substitution, variant, mutation, or chimera. A tracrRNA may refer to a nucleic acid that can be at least about 60% identical to a wild type exemplary tracrRNA (e.g., a tracrRNA from *S. pyogenes* *S. aureus*, etc) sequence over a stretch of at least 6 contiguous nucleotides. For example, a tracrRNA sequence can be at least about 60% identical, at least about 65% identical, at least about 70% identical, at least about 75% identical, at least about 80% identical, at least about 85% identical, at least about 90% identical, at least about 95% identical, at least about 98% identical, at least about 99% identical, or 100 % identical to a wild type exemplary tracrRNA (e.g., a tracrRNA from *S. pyogenes* *S. aureus*, etc) sequence over a stretch of at least 6 contiguous nucleotides. Type II tracrRNA sequences can be predicted on a genome sequence by identifying regions with complementarity to part of the repeat sequence in an adjacent CRISPR array.

[00471] As used herein, a “guide nucleic acid” can generally refer to a nucleic acid that may hybridize to another nucleic acid. A guide nucleic acid may be RNA. A guide nucleic acid may be DNA. The guide nucleic acid may be programmed to bind to a sequence of nucleic acid site-specifically. The nucleic acid to be targeted, or the target nucleic acid, may comprise

nucleotides. The guide nucleic acid may comprise nucleotides. A portion of the target nucleic acid may be complementary to a portion of the guide nucleic acid. The strand of a double-stranded target polynucleotide that is complementary to and hybridizes with the guide nucleic acid may be called the complementary strand. The strand of the double-stranded target polynucleotide that is complementary to the complementary strand, and therefore may not be complementary to the guide nucleic acid may be called noncomplementary strand. A guide nucleic acid may comprise a polynucleotide chain and can be called a “single guide nucleic acid.” A guide nucleic acid may comprise two polynucleotide chains and may be called a “double guide nucleic acid.” If not otherwise specified, the term “guide nucleic acid” may be inclusive, referring to both single guide nucleic acids and double guide nucleic acids. A guide nucleic acid may comprise a segment that can be referred to as a “nucleic acid-targeting segment” or a “nucleic acid-targeting sequence.” A nucleic acid-targeting segment may comprise a sub-segment that may be referred to as a “protein binding segment” or “protein binding sequence” or “Cas protein binding segment”.

[00472] The term “sequence identity” or “percent identity” in the context of two or more nucleic acids or polypeptide sequences, generally refers to two (e.g., in a pairwise alignment) or more (e.g., in a multiple sequence alignment) sequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence over a local or global comparison window, as measured using a sequence comparison algorithm. Suitable sequence comparison algorithms for polypeptide sequences include, e.g., BLASTP using parameters of a wordlength (W) of 3, an expectation I of 10, and the BLOSUM62 scoring matrix setting gap costs at existence of 11, extension of 1, and using a conditional compositional score matrix adjustment for polypeptide sequences longer than 30 residues; BLASTP using parameters of a wordlength (W) of 2, an expectation(E) of 1000000, and the PAM30 scoring matrix setting gap costs at 9 to open gaps and 1 to extend gaps for sequences of less than 30 residues (these are the default parameters for BLASTP in the BLAST suite available at <https://blast.ncbi.nlm.nih.gov>); CLUSTALW with parameters of ; the Smith-Waterman homology search algorithm with parameters of a match of 2, a mismatch of -1, and a gap of -1; MUSCLE with default parameters; MAFFT with parameters retree of 2 and maxiterations of 1000; Novafold with default parameters; HMMER hmalign with default parameters.

[00473] Included in the current disclosure are variants of any of the enzymes described herein with one or more conservative amino acid substitutions. Such conservative substitutions can be made in the amino acid sequence of a polypeptide without disrupting the three-dimensional structure or function of the polypeptide. Conservative substitutions can be accomplished by

substituting amino acids with similar hydrophobicity, polarity, and R chain length for one another. Additionally or alternatively, by comparing aligned sequences of homologous proteins from different species, conservative substitutions can be identified by locating amino acid residues that have been mutated between species (e.g. non-conserved residues) without altering the basic functions of the encoded proteins. Such conservatively substituted variants may include variants with at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% identity to any one of the endonuclease protein sequences described herein (e.g. MG1, MG2, MG3, MG3a, MG3b, MG4, MG5, MG6, MG7, MG14, MG15, MG16, MG17, MG18, MG21, MG22, MG23, MG24, MG25, MG38, MG40, MG41, MG42, MG43, MG44, MG46, MG47, MG48, MG49, MG50, MG51, MG52, MG71, MG72, MG73, MG74, MG86, MG87, MG88, MG89, MG94, MG95, MG96, MG97, MG98, MG99, MG100, MG111, MG112, MG116, MG123, MG124, MG125, or MG150 family endonucleases described herein). In some embodiments, such conservatively substituted variants are functional variants. Such functional variants can encompass sequences with substitutions such that the activity of critical active site residues of the endonuclease are not disrupted. In some embodiments, a functional variant of any of the proteins described herein lacks substitution of at least one conserved or functional residue.

[00474] Conservative substitution tables providing functionally similar amino acids are available from a variety of references (see, for e.g., Creighton, *Proteins: Structures and Molecular Properties* (W H Freeman & Co.; 2nd edition (December 1993)). The following eight groups each contain amino acids that are conservative substitutions for one another:

- 1) Alanine (A), Glycine (G);
- 2) Aspartic acid (D), Glutamic acid (E);
- 3) Asparagine (N), Glutamine (Q);
- 4) Arginine (R), Lysine (K);
- 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
- 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);
- 7) Serine (S), Threonine (T); and
- 8) Cysteine (C), Methionine (M)

[00475] Also included in the current disclosure are variants of any of the nucleic acid sequences described herein with one or more substitutions. Such variants may include variants with at least

about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to any one of the nucleic acid sequences described herein.

[00476] As used herein, the term “RuvC_III domain” generally refers to a third discontinuous segment of a RuvC endonuclease domain (the RuvC nuclease domain being comprised of three discontinuous segments, RuvC_I, RuvC_II, and RuvC_III). A RuvC domain or segments thereof can generally be identified by alignment to documented domain sequences, structural alignment to proteins with annotated domains, or by comparison to Hidden Markov Models (HMMs) built based on documented domain sequences (e.g., Pfam HMM PF18541 for RuvC_III).

[00477] As used herein, the term “HNH domain” generally refers to an endonuclease domain having characteristic histidine and asparagine residues. An HNH domain can generally be identified by alignment to documented domain sequences, structural alignment to proteins with annotated domains, or by comparison to Hidden Markov Models (HMMs) built based on documented domain sequences (e.g., Pfam HMM PF01844 for domain HNH).

[00478] *Overview*

[00479] The discovery of new Cas enzymes with unique functionality and structure may offer the potential to further disrupt deoxyribonucleic acid (DNA) editing technologies, improving speed, specificity, functionality, and ease of use. Relative to the predicted prevalence of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) systems in microbes and the sheer diversity of microbial species, relatively few functionally characterized CRISPR/Cas enzymes exist in the literature. This is partly because a huge number of microbial species may not be readily cultivated in laboratory conditions. Metagenomic sequencing from natural environmental niches that represent large numbers of microbial species may offer the potential to drastically increase the number of new CRISPR/Cas systems documented and speed the discovery of new oligonucleotide editing functionalities. A recent example of the fruitfulness of such an approach is demonstrated by the 2016 discovery of CasX/CasY CRISPR systems from metagenomic analysis of natural microbial communities.

[00480] CRISPR/Cas systems are RNA-directed nuclease complexes that have been described to function as an adaptive immune system in microbes. In their natural context, CRISPR/Cas systems occur in CRISPR (clustered regularly interspaced short palindromic repeats) operons or loci, which generally comprise two parts: (i) an array of short repetitive sequences (30-40bp) separated by equally short spacer sequences, which encode the RNA-based targeting element; and (ii) ORFs encoding the Cas encoding the nuclease polypeptide directed by the RNA-based targeting element alongside accessory proteins/enzymes. Efficient nuclease targeting of a

particular target nucleic acid sequence generally requires both (i) complementary hybridization between the first 6-8 nucleic acids of the target (the target seed) and the crRNA guide; and (ii) the presence of a protospacer-adjacent motif (PAM) sequence within a defined vicinity of the target seed (the PAM usually being a sequence not commonly represented within the host genome). Depending on the exact function and organization of the system, CRISPR-Cas systems are commonly organized into 2 classes, 5 types and 16 subtypes based on shared functional characteristics and evolutionary similarity.

[00481] Class I CRISPR-Cas systems have large, multisubunit effector complexes, and comprise Types I, III, and IV.

[00482] Type I CRISPR-Cas systems are considered of moderate complexity in terms of components. In Type I CRISPR-Cas systems, the array of RNA-targeting elements is transcribed as a long precursor crRNA (pre-crRNA) that is processed at repeat elements to liberate short, mature crRNAs that direct the nuclease complex to nucleic acid targets when they are followed by a suitable short consensus sequence called a protospacer-adjacent motif (PAM). This processing occurs via an endoribonuclease subunit (Cas6) of a large endonuclease complex called Cascade, which also comprises a nuclease (Cas3) protein component of the crRNA-directed nuclease complex. Cas I nucleases function primarily as DNA nucleases.

[00483] Type III CRISPR systems may be characterized by the presence of a central nuclease, known as Cas10, alongside a repeat-associated mysterious protein (RAMP) that comprises Csm or Cmr protein subunits. Like in Type I systems, the mature crRNA is processed from a pre-crRNA using a Cas6-like enzyme. Unlike type I and II systems, type III systems appear to target and cleave DNA-RNA duplexes (such as DNA strands being used as templates for an RNA polymerase).

[00484] Type IV CRISPR-Cas systems possess an effector complex that consists of a highly reduced large subunit nuclease (csf1), two genes for RAMP proteins of the Cas5 (csf3) and Cas7 (csf2) groups, and, in some cases, a gene for a predicted small subunit; such systems are commonly found on endogenous plasmids.

[00485] Class II CRISPR-Cas systems generally have single-polypeptide multidomain nuclease effectors, and comprise Types II, V and VI.

[00486] Type II CRISPR-Cas systems are considered the simplest in terms of components. In Type II CRISPR-Cas systems, the processing of the CRISPR array into mature crRNAs does not require the presence of a special endonuclease subunit, but rather a small trans-encoded crRNA (tracrRNA) with a region complementary to the array repeat sequence; the tracrRNA interacts with both its corresponding effector nuclease (e.g. Cas9) and the repeat sequence to form a precursor dsRNA structure, which is cleaved by endogenous RNase III to generate a mature

effector enzyme loaded with both tracrRNA and crRNA. Cas II nucleases are known as DNA nucleases. Type 2 effectors generally exhibit a structure consisting of a RuvC-like endonuclease domain that adopts the RNase H fold with an unrelated HNH nuclease domain inserted within the folds of the RuvC-like nuclease domain. The RuvC-like domain is responsible for the cleavage of the target (e.g., crRNA complementary) DNA strand, while the HNH domain is responsible for cleavage of the displaced DNA strand.

[00487] Type V CRISPR-Cas systems are characterized by a nuclease effector (e.g. Cas12) structure similar to that of Type II effectors, comprising a RuvC-like domain. Similar to Type II, most (but not all) Type V CRISPR systems use a tracrRNA to process pre-crRNAs into mature crRNAs; however, unlike Type II systems which requires RNase III to cleave the pre-crRNA into multiple crRNAs, type V systems are capable of using the effector nuclease itself to cleave pre-crRNAs. Like Type-II CRISPR-Cas systems, Type V CRISPR-Cas systems are again known as DNA nucleases. Unlike Type II CRISPR-Cas systems, some Type V enzymes (e.g., Cas12a) appear to have a robust single-stranded nonspecific deoxyribonuclease activity that is activated by the first crRNA directed cleavage of a double-stranded target sequence.

[00488] Type VI CRISPR-Cas systems have RNA-guided RNA endonucleases. Instead of RuvC-like domains, the single polypeptide effector of Type VI systems (e.g. Cas13) comprises two HEPN ribonuclease domains. Differing from both Type II and V systems, Type VI systems also appear to not require a tracrRNA for processing of pre-crRNA into crRNA. Similar to type V systems, however, some Type VI systems (e.g., C2C2) appear to possess robust single-stranded nonspecific nuclease (ribonuclease) activity activated by the first crRNA directed cleavage of a target RNA.

[00489] Because of their simpler architecture, Class II CRISPR-Cas have been most widely adopted for engineering and development as designer nuclease/genome editing applications.

[00490] One of the early adaptations of such a system for in vitro use can be found in Jinek et al. (Science. 2012 Aug 17;337(6096):816-21, which is entirely incorporated herein by reference). The Jinek study first described a system that involved (i) recombinantly-expressed, purified full-length Cas9 (e.g., a Class II, Type II Cas enzyme) isolated from *S. pyogenes* SF370, (ii) purified mature ~42 nt crRNA bearing a ~20 nt 5' sequence complementary to the target DNA sequence desired to be cleaved followed by a 3' tracr-binding sequence (the whole crRNA being in vitro transcribed from a synthetic DNA template carrying a T7 promoter sequence); (iii) purified tracrRNA in vitro transcribed from a synthetic DNA template carrying a T7 promoter sequence, and (iv) Mg²⁺. Jinek later described an improved, engineered system wherein the crRNA of (ii) is joined to the 5' end of (iii) by a linker (e.g., GAAA) to form a single fused synthetic guide RNA (sgRNA) capable of directing Cas9 to a target by itself.

[00491] Mali et al. (Science. 2013 Feb 15; 339(6121): 823–826.), which is entirely incorporated herein by reference, later adapted this system for use in mammalian cells by providing DNA vectors encoding (i) an ORF encoding codon-optimized Cas9 (e.g., a Class II, Type II Cas enzyme) under a suitable mammalian promoter with a C-terminal nuclear localization sequence (e.g., SV40 NLS) and a suitable polyadenylation signal (e.g., TK pA signal); and (ii) an ORF encoding an sgRNA (having a 5' sequence beginning with G followed by 20 nt of a complementary targeting nucleic acid sequence joined to a 3' tracr-binding sequence, a linker, and the tracrRNA sequence) under a suitable Polymerase III promoter (e.g., the U6 promoter).

[00492] *MG Enzymes*

[00493] In one aspect, the present disclosure provides for an engineered nuclease system discovered through metagenomic sequencing. In some cases, the metagenomic sequencing is conducted on samples. In some cases, the samples may be collected by a variety of environments. Such environments may be a human microbiome, an animal microbiome, environments with high temperatures, environments with low temperatures. Such environments may include sediment.

[00494] *MG3 Enzymes*

[00495] In one aspect, the present disclosure provides for an engineered nuclease system comprising (a) an endonuclease. In some cases, the endonuclease is a Cas endonuclease. In some cases, the endonuclease is a Type II, Class II Cas endonuclease. The endonuclease may comprise a RuvC_III domain, wherein said RuvC_III domain has at least about 70% sequence identity to any one of SEQ ID NOs: 2242-2251. In some cases, the endonuclease may comprise a RuvC_III domain, wherein the RuvC_III domain has at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% identity to any one of SEQ ID NOs: 2242-2251. In some cases, the endonuclease may comprise a RuvC_III domain, wherein the substantially identical to any one of SEQ ID NOs: 2242-2251. The endonuclease may comprise a RuvC_III domain having at least about 70% sequence identity to any one of SEQ ID NOs: 2242-2244. In some cases, the endonuclease may comprise a RuvC_III domain having at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least

about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% identity to any one of SEQ ID NOs: 2242-2244. In some cases, the endonuclease may comprise a RuvC_III domain substantially identical to any one of SEQ ID NOs: 2242-2244.

[00496] The endonuclease may comprise an HNH domain having at least about 70% identity to any one of SEQ ID NOs: 4056-4066. In some cases, the endonuclease may comprise an HNH domain having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to any one of SEQ ID NOs: 4056-4066. The endonuclease may comprise an HNH domain substantially identical to any one of SEQ ID NOs: 4056-4066. The endonuclease may comprise an HNH domain having at least about 70% identity to any one of SEQ ID NOs: 4056-4058. In some cases, the endonuclease may comprise an HNH domain having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to any one of SEQ ID NOs: 4056-4058. The endonuclease may comprise an HNH domain substantially identical to any one of SEQ ID NOs: 4056-4058.

[00497] In some cases, the endonuclease may comprise a variant having at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to any one of SEQ ID NOs: 421-431. In some cases, the endonuclease may be substantially identical to any one of SEQ ID NOs: 421-431. In some cases, the endonuclease may comprise a variant having at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to any one of SEQ ID NOs: 421-423. In some cases, the endonuclease may be substantially identical to any one of SEQ ID NOs: 421-423.

[00498] In some cases, the endonuclease may comprise a variant having one or more nuclear localization sequences (NLSs). The NLS may be proximal to the N- or C-terminus of said endonuclease. The NLS may be appended N-terminal or C-terminal to any one of SEQ ID NOs: 421-431, or to a variant having at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%,

at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to any one of SEQ ID NOs: 421-431. The NLS may be an SV40 large T antigen NLS. The NLS may be a c-myc NLS. The NLS can comprise a sequence with at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99% identity to any one of SEQ ID NOs: 5593-5608. The NLS can comprise a sequence substantially identical to any one of SEQ ID NOs: 5593-5608.

[00499] In some cases, sequence identity may be determined by the BLASTP, CLUSTALW, MUSCLE, MAFFT, Novafold, or CLUSTALW with the parameters of the Smith-Waterman homology search algorithm. The sequence identity may be determined by the BLASTP algorithm using parameters of a wordlength (W) of 3, an expectation (E) of 10, and using a BLOSUM62 scoring matrix setting gap costs at existence of 11, extension of 1, and using a conditional compositional score matrix adjustment.

[00500] In some cases, the system above may comprise (b) at least one engineered synthetic guide ribonucleic acid (sgRNA) capable of forming a complex with the endonuclease bearing a 5' targeting region complementary to a desired cleavage sequence. In some cases, the 5' targeting region may comprises a PAM sequence compatible with the endonuclease. In some cases, the 5' most nucleotide of the targeting region may be G. In some cases, the 5' targeting region may be 15-23 nucleotides in length. The guide sequence and the tracr sequence may be supplied as separate ribonucleic acids (RNAs) or a single ribonucleic acid (RNA). The guide RNA may comprise a crRNA tracrRNA binding sequence 3' to the targeting region. The guide RNA may comprise a tracrRNA sequence preceded by a 4-nucleotide linker 3' to the crRNA tracrRNA binding region. The sgRNA may comprise, from 5' to 3': a non-natural guide nucleic acid sequence capable of hybridizing to a target sequence in a cell; and a tracr sequence. In some cases, the non-natural guide nucleic acid sequence and the tracr sequence are covalently linked.

[00501] In some cases, the tracr sequence may have a particular sequence. The tracr sequence may have at least about 80% to at least about 60-100 (e.g., at least about 60, at least about 65, at least about 70, at least about 75, at least about 80, at least about 85, or at least about 90) consecutive nucleotides of a natural tracrRNA sequence. The tracr sequence may have at least about 80% sequence identity to at least about 60-100 (e.g., at least about 60, at least about 65, at least about 70, at least about 75, at least about 80, at least about 85, or at least about 90) consecutive nucleotides of any one of SEQ ID NOs: 5495-5502. In some cases, the tracrRNA may have at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to at least about 60-90 (e.g.,

at least about 60, at least about 65, at least about 70, at least about 75, at least about 80, at least about 85, or at least about 90) consecutive nucleotides of any one of SEQ ID NOs: 5495-5502. In some cases, the tracrRNA may be substantially identical to at least about 60-100 (e.g., at least about 60, at least about 65, at least about 70, at least about 75, at least about 80, at least about 85, or at least about 90) consecutive nucleotides of any one of SEQ ID NOs: 5495-5502. The tracrRNA may comprise any of SEQ ID NOs: 5495-5502.

[00502] In some cases, the at least one engineered synthetic guide ribonucleic acid (sgRNA) capable of forming a complex with the endonuclease may comprise a sequence having at least about 80% identity to any one of SEQ ID NOs: 5466-5467. The sgRNA may comprise a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to any one of SEQ ID NOs: 5466-5467. The sgRNA may comprise a sequence substantially identical to any one of SEQ ID NOs: 5466-5467.

[00503] In some cases, the system above may comprise two different sgRNAs targeting a first region and a second region for cleavage in a target DNA locus, wherein the second region is 3' to the first region. In some cases, the system above may comprise a single- or double-stranded DNA repair template comprising from 5' to 3': a first homology arm comprising a sequence of at least about 20 (e.g., at least about 40, 80, 120, 150, 200, 300, 500, or 1kb) nucleotides 5' to the first region, a synthetic DNA sequence of at least about 10 nucleotides, and a second homology arm comprising a sequence of at least about 20 (e.g., at least about 40, 80, 120, 150, 200, 300, 500, or 1kb) nucleotides 3' to the second region.

[00504] In another aspect, the present disclosure provides a method for modifying a target nucleic acid locus of interest. The method may comprise delivering to the target nucleic acid locus any of the non-natural systems disclosed herein, including an enzyme and at least one synthetic guide RNA (sgRNA) disclosed herein. The enzyme may form a complex with the at least one sgRNA, and upon binding of the complex to the target nucleic acid locus of interest, may modify the target nucleic acid locus of interest. Delivering the enzyme to said locus may comprise transfecting a cell with the system or nucleic acids encoding the system. Delivering the nuclease to said locus may comprise electroporating a cell with the system or nucleic acids encoding the system. Delivering the nuclease to said locus may comprise incubating the system in a buffer with a nucleic acid comprising the locus of interest. In some cases, the target nucleic acid locus comprises deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). The target nucleic acid locus may comprise genomic DNA, viral DNA, viral RNA, or bacterial DNA. The target nucleic acid locus may be within a cell. The target nucleic acid locus may be in vitro. The

target nucleic acid locus may be within a eukaryotic cell or a prokaryotic cell. The cell may be an animal cell, a human cell, bacterial cell, archaeal cell, or a plant cell. The enzyme may induce a single or double-stranded break at or proximal to the target locus of interest.

[00505] In cases where the target nucleic acid locus may be within a cell, the enzyme may be supplied as a nucleic acid containing an open reading frame encoding the enzyme having a RuvC_III domain having at least about 75% (e.g., at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%) identity to any one of SEQ ID NOs: 2242-2251. The deoxyribonucleic acid (DNA) containing an open reading frame encoding said endonuclease may comprise a sequence substantially identical to any of SEQ ID NOs: 5578-5580 or at variant having at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to any one of SEQ ID NOs: 5578-5580. In some cases, the nucleic acid comprises a promoter to which the open reading frame encoding the endonuclease is operably linked. The promoter may be a CMV, EF1a, SV40, PGK1, Ubc, human beta actin, CAG, TRE, or CaMKIIa promoter. The endonuclease may be supplied as a capped mRNA containing said open reading frame encoding said endonuclease. The endonuclease may be supplied as a translated polypeptide. The at least one engineered sgRNA may be supplied as deoxyribonucleic acid (DNA) containing a gene sequence encoding said at least one engineered sgRNA operably linked to a ribonucleic acid (RNA) pol III promoter. In some cases, the organism may be eukaryotic. In some cases, the organism may be fungal. In some cases, the organism may be human.

[00506] Systems of the present disclosure may be used for various applications, such as, for example, nucleic acid editing (e.g., gene editing), binding to a nucleic acid molecule (e.g., sequence-specific binding). Such systems may be used, for example, for addressing (e.g., removing or replacing) a genetically inherited mutation that may cause a disease in a subject, inactivating a gene in order to ascertain its function in a cell, as a diagnostic tool to detect disease-causing genetic elements (e.g. via cleavage of reverse-transcribed viral RNA or an amplified DNA sequence encoding a disease-causing mutation), as deactivated enzymes in combination with a probe to target and detect a specific nucleotide sequence (e.g. sequence encoding antibiotic resistance in bacteria), to render viruses inactive or incapable of infecting host cells by targeting viral genomes, to add genes or amend metabolic pathways to engineer organisms to produce valuable small molecules, macromolecules, or secondary metabolites, to

establish a gene drive element for evolutionary selection, to detect cell perturbations by foreign small molecules and nucleotides as a biosensor.

EXAMPLES

Example 1 – Metagenomic analysis for new proteins

[00507] Metagenomic samples were collected from sediment, soil and animal. Deoxyribonucleic acid (DNA) was extracted with a Zymobiomics DNA mini-prep kit and sequenced on an Illumina HiSeq® 2500. Samples were collected with consent of property owners. Additional raw sequence data from public sources included animal microbiomes, sediment, soil, hot springs, hydrothermal vents, marine, peat bogs, permafrost, and sewage sequences. Metagenomic sequence data was searched using Hidden Markov Models generated based on documented Cas protein sequences including type II Cas effector proteins to identify new Cas effectors. Novel effector proteins identified by the search were aligned to documented proteins to identify potential active sites. This metagenomic workflow resulted in delineation of the families of class II, type II CRISPR endonucleases described herein.

Example 2 – (General protocol) PAM Sequence identification/confirmation for the endonucleases described herein

[00508] PAM sequences were determined by sequencing plasmids containing randomly-generated PAM sequences that can be cleaved by putative endonucleases expressed in an *E. coli* lysate-based expression system (myTXTL, Arbor Biosciences). In this system, an *E. coli* codon optimized nucleotide sequence was transcribed and translated from a PCR fragment under control of a T7 promoter. A second PCR fragment with a tracr sequence under a T7 promoter and a minimal CRISPR array composed of a T7 promoter followed by a repeat-spacer-repeat sequence was transcribed in the same reaction. Successful expression of the endonuclease and tracr sequence in the TXTL system followed by CRISPR array processing provided active *in vitro* CRISPR nuclease complexes.

[00509] A library of target plasmids containing a spacer sequence matching that in the minimal array followed by 8N mixed bases (putative PAM sequences) was incubated with the output of the TXTL reaction. After 1-3 hr, the reaction was stopped and the DNA was recovered via a DNA clean-up kit, e.g., Zymo DCC, AMPure XP beads, QiaQuick etc. Adapter sequences were blunt-end ligated to DNA with active PAM sequences that had been cleaved by the endonuclease, whereas DNA that had not been cleaved was inaccessible for ligation. DNA segments comprising active PAM sequences were then amplified by PCR with primers specific to the library and the adapter sequence. The PCR amplification products were resolved on a gel

to identify amplicons that corresponded to cleavage events. The amplified segments of the cleavage reaction were also used as template for preparation of an NGS library. Sequencing this resulting library, which was a subset of the starting 8N library, revealed the sequences which contain the correct PAM for the active CRISPR complex. For PAM testing with a single RNA construct, the same procedure was repeated except that an *in vitro* transcribed RNA was added along with the plasmid library and the tracr/minimal CRISPR array template was omitted. For endonucleases where NGS libraries were prepared, seqLogo (see e.g., Huber et al. Nat Methods. 2015 Feb;12(2):115-21) representations were constructed. The seqLogo module used to construct these representations takes the position weight matrix of a DNA sequence motif (e.g. a PAM sequence) and plots the corresponding sequence logo as introduced by Schneider and Stephens (see e.g. Schneider et al. Nucleic Acids Res. 1990 Oct 25;18(20):6097-100. The characters representing the sequence in the seqLogo representations have been stacked on top of each other for each position in the aligned sequences (e.g. PAM sequences). The height of each letter is proportional to its frequency, and the letters have been sorted so the most common one is on top.

Example 3 – (General protocol) RNA Folding of tracrRNA and sgRNA structures

[00510] Folded structures of guide RNA sequences at 37°C were computed using the method of Andronescu et al. Bioinformatics. 2007 Jul 1;23(13):i19-28, which is incorporated by reference herein in its entirety.

Example 4 – (General protocol) In vitro cleavage efficiency of MG CRISPR Complexes

[00511] Endonucleases were expressed as His-tagged fusion proteins from an inducible T7 promoter in a protease deficient *E. coli* B strain. Cells expressing the His-tagged proteins were lysed by sonication and the His-tagged proteins were purified by Ni-NTA affinity chromatography on a HisTrap FF column (GE Lifescience) on an AKTA Avant FPLC (GE Lifescience). The eluate was resolved by SDS-PAGE on acrylamide gels (Bio-Rad) and stained with InstantBlue Ultrafast coomassie (Sigma-Aldrich). Purity was determined using densitometry of the protein band with ImageLab software (Bio-Rad). Purified endonucleases were dialyzed into a storage buffer composed of 50 mM Tris-HCl, 300 mM NaCl, 1 mM TCEP, 5% glycerol; pH 7.5 and stored at -80°C.

[00512] Target DNAs containing spacer sequences and PAM sequences (determined e.g., as in Example 2) were constructed by DNA synthesis. A single representative PAM was chosen for testing when the PAM had degenerate bases. The target DNAs comprised 2200 bp of linear DNA derived from a plasmid via PCR amplification with a PAM and spacer located 700 bp

from one end. Successful cleavage resulted in fragments of 700 and 1500 bp. The target DNA, *in vitro* transcribed single RNA, and purified recombinant protein were combined in cleavage buffer (10 mM Tris, 100 mM NaCl, 10 mM MgCl₂) with an excess of protein and RNA and incubated for 5 minutes to 3 hours, usually 1 hr. The reaction was stopped via addition of RNase A and incubation at 60 minutes. The reaction was then resolved on a 1.2% TAE agarose gel and the fraction of cleaved target DNA is quantified in ImageLab software.

Example 5 – (General protocol) Testing of Genome Cleavage Activity of MG CRISPR Complexes in *E. coli*

[00513] *E. coli* lacks the capacity to efficiently repair double-stranded DNA breaks. Thus, cleavage of genomic DNA can be a lethal event. Exploiting this phenomenon, endonuclease activity was tested in *E. coli* by recombinantly expressing an endonuclease and a tracrRNA in a target strain with spacer/target and PAM sequences integrated into its genomic DNA.

[00514] In this assay, the PAM sequence is specific for the endonuclease being tested as determined by the methods described in Example 2. sgRNA sequences were determined based upon the sequence and predicted structure of the tracrRNA. Repeat-anti-repeat pairings of 8-12 bp (generally 10bp) were chosen, starting from the 5' end of the repeat. The remaining 3' end of the repeat and 5' end of the tracrRNA were replaced with a tetraloop. Generally, the tetraloop was GAAA, but other tetraloops can be used, particularly if the GAAA sequence is predicted to interfere with folding. In these cases, a TTCG tetraloop was used.

[00515] Engineered strains with PAM sequences integrated into their genomic DNA were transformed with DNA encoding the endonuclease. Transformants were then made chemocompetent and transformed with 50 ng of single guide RNAs either specific to the target sequence (“on target”), or non-specific to the target (“non target”). After heat shock, transformations were recovered in SOC for 2 hrs at 37°C. Nuclease efficiency was then determined by a 5-fold dilution series grown on induction media. Colonies were quantified from the dilution series in triplicate.

Example 6a – (General protocol) Testing of Genome Cleavage Activity of MG CRISPR Complexes in Mammalian Cells

[00516] To show targeting and cleavage activity in mammalian cells, the MG Cas effector protein sequences were tested in two mammalian expression vectors: (a) one with a C-terminal SV40 NLS and a 2A-GFP tag, and (b) one with no GFP tag and two SV40 NLS sequences, one on the N-terminus and one on the C-terminus. In some instances, nucleotide sequences encoding the endonucleases were codon-optimized for expression in mammalian cells.

[00517] The corresponding single guide RNA sequence (sgRNA) with targeting sequence attached is cloned into a second mammalian expression vector. The two plasmids are cotransfected into HEK293T cells. 72 hr after co-transfection of the expression plasmid and a sgRNA targeting plasmid into HEK293T cells, the DNA is extracted and used for the preparation of an NGS-library. Percent NHEJ is measured via indels in the sequencing of the target site to demonstrate the targeting efficiency of the enzyme in mammalian cells. At least 10 different target sites were chosen to test each protein's activity.

Example 6b – (General Protocol) Testing of Genome Cleavage Activity of MG CRISPR Complexes in Mammalian Cells

[00518] To show targeting and cleavage activity in mammalian cells, the MG Cas effector protein sequences were cloned into two mammalian expression vector: (a) one with flanking N and C-terminal SV40 NLS sequences, a C-terminal His tag, and a 2A-GFP tag at the C terminus after the His tag (Backbone 1), and (b) one with flanking NLS sequences and C-terminal His tag but no T2A GFP tag (Backbone 2). In some instances, nucleotide sequences encoding the endonucleases were the native sequence, codon-optimized for expression in *E. coli*, or codon-optimized for expression in mammalian cells.

[00519] The corresponding single guide RNA sequence (sgRNA) with targeting sequence attached was cloned into a second mammalian expression vector. The two plasmids were cotransfected into HEK293T cells. 72 hr after co-transfection of the expression plasmid and a sgRNA targeting plasmid into HEK293T cells, the DNA was extracted and used for the preparation of an NGS-library. Percent NHEJ was measured via indels in the sequencing of the target site to demonstrate the targeting efficiency of the enzyme in mammalian cells. About 7-12 different target sites were chosen for testing each protein's activity. An arbitrary threshold of 5% indels was used to identify active candidates.

Example 7 – Gene editing outcomes at the DNA level for B2M

[00520] Primary T cells were purified from PBMCs using a negative selection kit (Miltenyi) according to the manufacturer's recommendations. Nucleofection of MG3-6 RNPs (106 pmol protein/160 pmol guide) (SEQ ID NOs: 6305-6386) was performed into T cells (200,000) using the Lonza 4D electroporator. Cells were harvested and genomic DNA prepared five days post-transfection. PCR primers appropriate for use in NGS-based DNA sequencing were generated, optimized, and used to amplify the individual target sequences for each guide RNA (SEQ ID NOs: 6387-6468). The amplicons were sequenced on an Illumina MiSeq machine and analyzed with a proprietary Python script to measure gene editing (**FIG. 1**).

Table 1A: Guide sequences used in Example 7

SEQ ID NO:	Entity Name	Sequence
6305	MG3-6-B2M-sgRNA-A1	mC*mG*mC*rUrArCrUrCrUrCrUrCrUrUrCrUrGrGrCrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6306	MG3-6-B2M-sgRNA-B1	mA*mG*mA*rGrArCrUrCrArCrGrCrUrGrGrArUrArGrCrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6307	MG3-6-B2M-sgRNA-C1	mG*mA*mG*rArGrArGrUrArGrCrGrCrGrArGrCrArCrArGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6308	MG3-6-B2M-sgRNA-D1	mC*mC*mC*rGrArUrArUrUrCrCrUrCrArGrGrUrArCrUrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6309	MG3-6-B2M-sgRNA-E1	mA*mU*mU*rCrCrUrCrArGrGrUrArCrUrCrCrArArArGrArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6310	MG3-6-B2M-sgRNA-F1	mA*mA*mU*rUrUrCrCrUrGrArArUrUrGrCrUrArUrGrUrGrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6311	MG3-6-B2M-sgRNA-G1	mG*mA*mG*rArArUrUrGrArArArArArGrUrGrGrArGrCrArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6312	MG3-6-B2M-sgRNA-H1	mG*mC*mA*rUrUrCrArGrArCrUrUrGrUrCrUrUrUrCrArGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6313	MG3-6-B2M-sgRNA-A2	mA*mG*mA*rCrUrUrArCrCrCrArCrUrUrArArCrUrArUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6314	MG3-6-B2M-sgRNA-B2	mU*mU*mC*rArGrUrGrUrArGrUrArCrArArGrArGrArUrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6315	MG3-6-B2M-sgRNA-C2	mA*mG*mU*rUrCrUrCrCrUrUrGrGrUrGrGrCrCrCrGrCrCrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU

SEQ ID NO:	Entity Name	Sequence
6316	MG3-6-B2M-sgRNA-D2	mG*mU*mG*rGrCrCrGrCrCrGrUrGrGrGrGrCrUrArGrUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6317	MG3-6-B2M-sgRNA-E2	mC*mC*mG*rCrCrGrUrGrGrGrGrCrUrArGrUrCrCrArGrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6318	MG3-6-B2M-sgRNA-F2	mG*mC*mC*rCrCrUrUrUrCrGrGrCrGrGrGrArGrCrArGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6319	MG3-6-B2M-sgRNA-G2	mG*mA*mC*rCrUrUrUrGrGrCrCrUrArCrGrGrCrGrArCrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6320	MG3-6-B2M-sgRNA-H2	mG*mC*mG*rUrCrGrArUrArArGrCrGrUrCrArGrArGrCrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6321	MG3-6-B2M-sgRNA-A3	mC*mG*mU*rCrArGrArGrCrGrCrCrGrArGrGrUrUrGrGrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6322	MG3-6-B2M-sgRNA-B3	mG*mG*mG*rUrUrUrCrUrCrUrUrCrCrGrCrUrCrUrUrUrCrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6323	MG3-6-B2M-sgRNA-C3	mG*mC*mG*rCrArGrCrUrGrGrArGrUrGrGrGrGrGrArCrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6324	MG3-6-B2M-sgRNA-D3	mG*mC*mU*rCrGrUrCrCrCrArArArGrGrCrGrGrCrGrCrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6325	MG3-6-B2M-sgRNA-E3	mU*mG*mU*rGrArArCrGrCrGrUrGrGrArGrGrGrGrCrGrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6326	MG3-6-B2M-sgRNA-F3	mG*mU*mC*rUrGrCrUrGrCrGrGrCrUrCrUrGrCrUrUrCrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6327	MG3-6-B2M-sgRNA-G3	mG*mC*mU*rUrCrCrCrUrUrArGrArCrUrGrGrArGrArGrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr

SEQ ID NO:	Entity Name	Sequence
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6328	MG3-6-B2M-sgRNA-H3	mA*mA*mG*rUrUrCrGrCrArUrGrUrCrCrUrArGrCrArCrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6329	MG3-6-B2M-sgRNA-A4	mU*mC*mC*rUrArGrCrArCrCrUrCrUrGrGrGrUrCrUrArUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6330	MG3-6-B2M-sgRNA-B4	mC*mC*mU*rCrCrCrCrArCrGrGrUrGrGrCrCrCrCrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6331	MG3-6-B2M-sgRNA-C4	mA*mA*mG*rGrGrArArGrCrArGrArGrCrCrGrCrArGrCrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6332	MG3-6-B2M-sgRNA-D4	mG*mC*mU*rUrArCrCrCrGrGrGrCrGrArCrGrCrCrUrCrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6333	MG3-6-B2M-sgRNA-E4	mC*mU*mC*rCrArGrCrUrGrCrGrCrUrGrGrGrGrArGrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6334	MG3-6-B2M-sgRNA-F4	mU*mU*mG*rUrCrCrCrGrArCrCrCrUrCrCrCrGrUrCrGrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6335	MG3-6-B2M-sgRNA-G4	mG*mA*mC*rCrCrUrCrCrCrGrUrCrGrCrCrGrUrArGrGrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6336	MG3-6-B2M-sgRNA-H4	mG*mU*mG*rCrGrCrArCrCrCrCrUrUrCrCrCrCrArCrUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6337	MG3-6-B2M-sgRNA-A5	mC*mC*mA*rGrGrCrCrArCrCrCrCrGrCrCrGrCrUrUrCrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6338	MG3-6-B2M-sgRNA-B5	mG*mC*mC*rGrCrUrUrCrCrCrCrGrArGrArUrCrCrArGrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6339	MG3-6-B2M-sgRNA-C5	mC*mC*mA*rGrCrCrCrUrGrGrArCrUrArGrCrCrCrCrArCrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArAr

SEQ ID NO:	Entity Name	Sequence
		ArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGr GrGrCrGrGrUrArUrGrU*mU*mU*mU
6340	MG3-6-B2M-sgRNA-D5	mU*mC*mA*rCrGrGrArGrCrGrArGrArGrArGrCrArCrArGrCr GrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrAr ArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGr GrGrCrGrGrUrArUrGrU*mU*mU*mU
6341	MG3-6-B2M-sgRNA-E5	mA*mG*mA*rGrGrGrUrGrCrArGrArGrCrGrGrArGrArGrGr GrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrAr ArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGr GrGrCrGrGrUrArUrGrU*mU*mU*mU
6342	MG3-6-B2M-sgRNA-F5	mA*mG*mG*rArCrCrArGrArGrCrGrGrGrArGrGrGrUrArGrGr GrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrAr ArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGr GrGrCrGrGrUrArUrGrU*mU*mU*mU
6343	MG3-6-B2M-sgRNA-G5	mC*mG*mA*rGrArUrUrGrArArGrUrCrArArGrCrCrUrArArCr GrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrAr ArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGr GrGrCrGrGrUrArUrGrU*mU*mU*mU
6344	MG3-6-B2M-sgRNA-H5	mA*mG*mA*rArArArArCrGrCrCrUrGrCrCrUrUrCrUrGrCrGr GrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrAr ArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGr GrGrCrGrGrUrArUrGrU*mU*mU*mU
6345	MG3-6-B2M-sgRNA-A6	mU*mC*mU*rCrCrArGrArGrCrArArArCrUrGrGrGrCrGrCr GrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrAr ArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGr GrGrCrGrGrUrArUrGrU*mU*mU*mU
6346	MG3-6-B2M-sgRNA-B6	mG*mG*mC*rCrCrUrGrUrGrGrUrCrUrUrUrUrCrGrUrArCrAr GrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrAr ArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGr GrGrCrGrGrUrArUrGrU*mU*mU*mU
6347	MG3-6-B2M-sgRNA-C6	mA*mC*mU*rUrUrCrGrGrUrUrUrUrGrArArArArCrArUrGrAr GrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrAr ArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGr GrGrCrGrGrUrArUrGrU*mU*mU*mU
6348	MG3-6-B2M-sgRNA-D6	mA*mA*mA*rGrArGrGrArArGrCrCrCrUrCrUrGrUrArCrGrAr GrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrAr ArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGr GrGrCrGrGrUrArUrGrU*mU*mU*mU
6349	MG3-6-B2M-sgRNA-E6	mA*mG*mC*rCrCrUrCrUrGrUrArCrGrArArArArGrArCrCrAr GrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrAr ArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGr GrGrCrGrGrUrArUrGrU*mU*mU*mU
6350	MG3-6-B2M-sgRNA-F6	mU*mG*mC*rGrCrUrCrCrCrGrCrArArArArGrCrCrCrUrGrGr GrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrAr ArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGr GrGrCrGrGrUrArUrGrU*mU*mU*mU

SEQ ID NO:	Entity Name	Sequence
6351	MG3-6-B2M-sgRNA-G6	mA*mA*mA*rArGrArArArGrArArArGrArArArGrArArGrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6352	MG3-6-B2M-sgRNA-H6	mA*mA*mA*rGrArUrArArUrCrCrArArGrArUrGrGrUrUrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6353	MG3-6-B2M-sgRNA-A7	mU*mA*mC*rCrArArGrArCrUrGrUrUrGrArGrGrArCrGrCrCrGrUrUrGrArGrArArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6354	MG3-6-B2M-sgRNA-B7	mU*mC*mC*rArArArGrUrArArUrArCrArUrGrCrCrArUrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6355	MG3-6-B2M-sgRNA-C7	mA*mU*mU*rArCrUrUrUrGrGrArArArUrUrUrUrCrArArArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6356	MG3-6-B2M-sgRNA-D7	mA*mA*mA*rUrArArGrArUrUrUrUrUrUrUrUrArArArUrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6357	MG3-6-B2M-sgRNA-E7	mU*mG*mC*rCrArGrGrUrArCrUrUrArGrArArArGrUrGrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6358	MG3-6-B2M-sgRNA-F7	mC*mU*mC*rArArCrArGrUrCrUrUrGrGrUrArArCrCrArUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6359	MG3-6-B2M-sgRNA-G7	mU*mG*mA*rUrArCrUrUrGrUrCrCrUrCrUrUrCrUrUrArGrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6360	MG3-6-B2M-sgRNA-H7	mG*mC*mU*rUrUrUrArArUrGrUrUrArUrGrArArArArArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6361	MG3-6-B2M-sgRNA-A8	mU*mA*mU*rGrArArArArArArArUrCrArGrGrUrCrUrUrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6362	MG3-6-B2M-sgRNA-B8	mG*mA*mU*rUrCrCrCrCrArArUrCrCrArCrCrUrCrUrUrGrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr

SEQ ID NO:	Entity Name	Sequence
		CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6363	MG3-6-B2M-sgRNA-C8	mG*mG*mC*rArGrCrUrArCrUrCrCrUrCrCrUrUrGrUrCrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6364	MG3-6-B2M-sgRNA-D8	mG*mC*mU*rGrUrGrGrGrGrArGrArArGrGrArGrGrArGrUrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6365	MG3-6-B2M-sgRNA-E8	mU*mA*mG*rArArArCrArCrCrCrUrArUrCrArUrUrArArGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6366	MG3-6-B2M-sgRNA-F8	mA*mG*mG*rCrUrArCrUrArGrCrCrCrCrArUrCrArArGrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6367	MG3-6-B2M-sgRNA-G8	mG*mA*mG*rGrUrGrGrArUrUrGrGrGrGrArArUrCrUrArArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6368	MG3-6-B2M-sgRNA-H8	mA*mU*mA*rArGrArArCrArUrArUrUrArArArUrGrCrCrUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6369	MG3-6-B2M-sgRNA-A9	mU*mA*mA*rArUrGrCrCrUrCrArGrGrGrArUrCrArGrArGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6370	MG3-6-B2M-sgRNA-B9	mC*mU*mC*rUrCrUrGrUrUrUrGrArGrGrGrArArGrGrCrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6371	MG3-6-B2M-sgRNA-C9	mC*mU*mA*rArGrArArGrArGrGrArCrArArGrUrArUrCrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6372	MG3-6-B2M-sgRNA-D9	mC*mA*mC*rCrUrArUrCrCrCrUrGrUrUrGrUrArUrUrUrUrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6373	MG3-6-B2M-sgRNA-E9	mA*mU*mU*rUrGrCrCrArGrCrUrCrUrUrGrUrArUrGrCrArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6374	MG3-6-B2M-sgRNA-F9	mG*mA*mA*rArUrUrArGrGrUrArCrArArArGrUrCrArGrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArAr

SEQ ID NO:	Entity Name	Sequence
		ArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGr GrGrCrGrGrUrArUrGrU*mU*mU*mU
6375	MG3-6-B2M-sgRNA-G9	mU*mA*mU*rArArArArCrCrUrCrArGrCrArGrArArArUrArAr GrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrAr ArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGr GrGrCrGrGrUrArUrGrU*mU*mU*mU
6376	MG3-6-B2M-sgRNA-H9	mU*mG*mU*rUrGrUrUrUrGrGrUrArArGrArArCrArUrArCrCr GrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrAr ArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGr GrGrCrGrGrUrArUrGrU*mU*mU*mU
6377	MG3-6-B2M-sgRNA-A10	mA*mA*mC*rArArArArCrCrUrCrUrUrUrArUrUrUrCrUrGrCr GrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrAr ArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGr GrGrCrGrGrUrArUrGrU*mU*mU*mU
6378	MG3-6-B2M-sgRNA-B10	mA*mU*mU*rUrCrUrGrCrUrGrArGrGrUrUrUrUrArUrArUrGr GrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrAr ArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGr GrGrCrGrGrUrArUrGrU*mU*mU*mU
6379	MG3-6-B2M-sgRNA-C10	mG*mC*mA*rArArUrArCrCrUrUrArArArUrGrGrUrUrGrArGr GrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrAr ArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGr GrGrCrGrGrUrArUrGrU*mU*mU*mU
6380	MG3-6-B2M-sgRNA-D10	mA*mU*mA*rArArArUrArCrArArCrArGrGrGrArUrArGrGrUr GrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrAr ArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGr GrGrCrGrGrUrArUrGrU*mU*mU*mU
6381	MG3-6-B2M-sgRNA-E10	mA*mA*mU*rGrGrArGrUrArArUrGrCrArUrGrUrGrArCrArGr GrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrAr ArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGr GrGrCrGrGrUrArUrGrU*mU*mU*mU
6382	MG3-6-B2M-sgRNA-F10	mA*mC*mA*rGrGrUrGrArUrUrGrCrUrGrUrArArArCrUrArGr GrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrAr ArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGr GrGrCrGrGrUrArUrGrU*mU*mU*mU
6383	MG3-6-B2M-sgRNA-G10	mC*mU*mU*rUrCrCrArArArArUrGrArGrArGrGrCrArUrGrAr GrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrAr ArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGr GrGrCrGrGrUrArUrGrU*mU*mU*mU
6384	MG3-6-B2M-sgRNA-H10	mA*mA*mU*rArUrUrGrCrCrArGrGrGrUrArUrUrUrCrArCrUr GrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrAr ArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGr GrGrCrGrGrUrArUrGrU*mU*mU*mU
6385	MG3-6-B2M-sgRNA-A11	mU*mG*mC*rCrUrUrUrUrUrUrGrUrUrUrUrUrUrUrUrCrUrAr GrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrAr ArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr CrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGr GrGrCrGrGrUrArUrGrU*mU*mU*mU

SEQ ID NO:	Entity Name	Sequence
6386	MG3-6-B2M-sgRNA-B11	mU*mC*mU*rArGrCrArGrUrArUrCrUrUrCrUrGrUrCrArCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
Notations for chemical modifications: m = 2'-O-methyl ribonucleotide (e.g mC = cytosine ribonucleotide with 2'-O Methyl in place of 2' hydroxyl); f = 2'-fluoro ribonucleotide (e.g fC = cytosine ribonucleotide with 2' fluorine in place of 2' hydroxyl); * = phosphorothioate bond; r: native RNA linkage comprising the sugar ribose (for example the ribose or RNA form of the A base is written rA), d: deoxyribose sugar (DNA) linkage (for example a deoxyribose form of the A base is written dA)		

Table 1B: Sites targeted in Example 7

SEQ ID NO:	Entity Name	Sequence
6387	MG3-6-B2M-target site-A1	CGCTACTCTCTCTTTCTGGCCT
6388	MG3-6-B2M-target site-B1	AGAGACTCACGCTGGATAGCCT
6389	MG3-6-B2M-target site-C1	GAGAGAGTAGCGCGAGCACAGC
6390	MG3-6-B2M-target site-D1	CCCGATATTCCTCAGGTACTCC
6391	MG3-6-B2M-target site-E1	ATTCCTCAGGTACTCCAAAGAT
6392	MG3-6-B2M-target site-F1	AATTTCTGAATTGCTATGTGT
6393	MG3-6-B2M-target site-G1	GAGAATTGAAAAAGTGGAGCAT
6394	MG3-6-B2M-target site-H1	GCATTCAGACTTGTCTTTTCAGC
6395	MG3-6-B2M-target site-A2	AGACTTACCCCACTTAACATC
6396	MG3-6-B2M-target site-B2	TTCAGTGTAGTACAAGAGATAG
6397	MG3-6-B2M-target site-C2	AGTTCTCCTTGGTGGCCCGCCG
6398	MG3-6-B2M-target site-D2	GTGGCCCGCCGTGGGGCTAGTC
6399	MG3-6-B2M-target site-E2	CCGCCGTGGGGCTAGTCCAGGG
6400	MG3-6-B2M-target site-F2	GCCCCTTTCGGCGGGGAGCAGG
6401	MG3-6-B2M-target site-G2	GACCTTTGGCCTACGGCGACGG
6402	MG3-6-B2M-target site-H2	GCGTCGATAAGCGTCAGAGCGC
6403	MG3-6-B2M-target site-A3	CGTCAGAGCGCCGAGGTTGGGG
6404	MG3-6-B2M-target site-B3	GGGTTTCTCTTCCGCTCTTTTCG
6405	MG3-6-B2M-target site-C3	GCGCAGCTGGAGTGGGGGACGG
6406	MG3-6-B2M-target site-D3	GCTCGTCCCAAAGGCGCGGCGC
6407	MG3-6-B2M-target site-E3	TGTGAACGCGTGGAGGGGCGCT
6408	MG3-6-B2M-target site-F3	GTCTGCTGCGGCTCTGCTTCCC
6409	MG3-6-B2M-target site-G3	GCTTCCCTTAGACTGGAGAGCT
6410	MG3-6-B2M-target site-H3	AAGTTCGCATGTCCTAGCACCT
6411	MG3-6-B2M-target site-A4	TCCTAGCACCTCTGGGTCTATG
6412	MG3-6-B2M-target site-B4	CCTCCCCACGGTGTGGCCCCAC
6413	MG3-6-B2M-target site-C4	AAGGGAAGCAGAGCCGCAGCAG
6414	MG3-6-B2M-target site-D4	GCTTACCCGGGCGACGCCTCCC
6415	MG3-6-B2M-target site-E4	CTCCAGCTGCGCTGGGGGAGCC
6416	MG3-6-B2M-target site-F4	TTGTCCCGACCCTCCCGTCGCC
6417	MG3-6-B2M-target site-G4	GACCCCTCCCGTCGCCGTAGGCC
6418	MG3-6-B2M-target site-H4	GTGCGCACCCCCTTCCCCACTC

SEQ ID NO:	Entity Name	Sequence
6419	MG3-6-B2M-target site-A5	CCAGGCCACCCCGCCGCTTCCC
6420	MG3-6-B2M-target site-B5	GCCGCTTCCCCGAGATCCAGCC
6421	MG3-6-B2M-target site-C5	CCAGCCCTGGACTAGCCCCACG
6422	MG3-6-B2M-target site-D5	TCACGGAGCGAGAGAGCACAGC
6423	MG3-6-B2M-target site-E5	AGAGGGTGCAGAGCGGGAGAGG
6424	MG3-6-B2M-target site-F5	AGGACCAGAGCGGGAGGGTAGG
6425	MG3-6-B2M-target site-G5	CGAGATTGAAGTCAAGCCTAAC
6426	MG3-6-B2M-target site-H5	AGAAAAACGCCTGCCTTCTGCG
6427	MG3-6-B2M-target site-A6	TCTCCAGAGCAAACCTGGGCGGC
6428	MG3-6-B2M-target site-B6	GGCCCTGTGGTCTTTTCGTACA
6429	MG3-6-B2M-target site-C6	ACTTTCGGTTTTGAAAACATGA
6430	MG3-6-B2M-target site-D6	AAAGAGGAAGCCCTCTGTACGA
6431	MG3-6-B2M-target site-E6	AGCCCTCTGTACGAAAAGACCA
6432	MG3-6-B2M-target site-F6	TGCGCTCCCGCAAAAGCCCTGG
6433	MG3-6-B2M-target site-G6	AAAAGAAAAGAAAAGAAAGT
6434	MG3-6-B2M-target site-H6	AAAGATAATCCAAGATGGTTAC
6435	MG3-6-B2M-target site-A7	TACCAAGACTGTTGAGGACGCC
6436	MG3-6-B2M-target site-B7	TCCAAAGTAATACATGCCATGC
6437	MG3-6-B2M-target site-C7	ATTACTTTGGAAATTTTCAAAA
6438	MG3-6-B2M-target site-D7	AAATAAGATTTTTTTTTTAAATA
6439	MG3-6-B2M-target site-E7	TGCCAGGTACTTAGAAAGTGCT
6440	MG3-6-B2M-target site-F7	CTCAACAGTCTTGGAACCATC
6441	MG3-6-B2M-target site-G7	TGATACTTGTCTCTTCTTAGA
6442	MG3-6-B2M-target site-H7	GCTTTTAATGTTATGAAAAAAA
6443	MG3-6-B2M-target site-A8	TATGAAAAAAATCAGGTCTTCA
6444	MG3-6-B2M-target site-B8	GATTCCCCAATCCACCTCTTGA
6445	MG3-6-B2M-target site-C8	GGCAGCTACTCCTCCTTGCTG
6446	MG3-6-B2M-target site-D8	GCTGTGGGGAGAAGGAGGAGTA
6447	MG3-6-B2M-target site-E8	TAGAAACACCCTATCATTAAAGG
6448	MG3-6-B2M-target site-F8	AGGCTACTAGCCCCATCAAGAG
6449	MG3-6-B2M-target site-G8	GAGGTGGATTGGGGAATCTAAT
6450	MG3-6-B2M-target site-H8	ATAAGAACATATTAAATGCCTC
6451	MG3-6-B2M-target site-A9	TAAATGCCTCAGGGATCAGAGC
6452	MG3-6-B2M-target site-B9	CTCTCTGTTTGAGGGAAGGCGG
6453	MG3-6-B2M-target site-C9	CTAAGAAGAGGACAAGTATCAG
6454	MG3-6-B2M-target site-D9	CACCTATCCCTGTTGTATTTTA
6455	MG3-6-B2M-target site-E9	ATTTGCCAGCTCTTGTATGCAT
6456	MG3-6-B2M-target site-F9	GAAATTAGGTACAAAGTCAGAG
6457	MG3-6-B2M-target site-G9	TATAAAACCTCAGCAGAAATAA
6458	MG3-6-B2M-target site-H9	TGTTGTTTGGTAAGAACATACC
6459	MG3-6-B2M-target site-A10	AACAAAACCTCTTTATTTCTGC
6460	MG3-6-B2M-target site-B10	ATTTCTGCTGAGGTTTATATG
6461	MG3-6-B2M-target site-C10	GCAAATACCTTAAATGGTTGAG
6462	MG3-6-B2M-target site-D10	ATAAAATACAACAGGGATAGGT

SEQ ID NO:	Entity Name	Sequence
6463	MG3-6-B2M-target site-E10	AATGGAGTAATGCATGTGACAG
6464	MG3-6-B2M-target site-F10	ACAGGTGATTGCTGTAAACTAG
6465	MG3-6-B2M-target site-G10	CTTTCCAAAATGAGAGGCATGA
6466	MG3-6-B2M-target site-H10	AATATTGCCAGGGTATTTCACT
6467	MG3-6-B2M-target site-A11	TGCCTTTTTTTGTTTTTTTTCTA
6468	MG3-6-B2M-target site-B11	TCTAGCAGTATCTTCTGTCACT

Example 8 – Gene editing outcomes at the DNA level for mouse TRAC

[00521] Primary T cells were purified from C57BL/6 mouse spleens. Nucleofection of MG3-6 RNPs (126 pmol protein/160 pmol guide) (SEQ ID NOs: 6469-6508) was performed into T cells (200,000) using the Lonza 4D electroporator and 100 pmol transfection enhancer (IDT). Cells were harvested and genomic DNA prepared five days post-transfection. PCR primers appropriate for use in NGS-based DNA sequencing were generated, optimized, and used to amplify the individual target sequences for each guide RNA (SEQ ID NOs: 6509-6548). The amplicons were sequenced on an Illumina MiSeq machine and analyzed with a proprietary Python script to measure gene editing (**FIG. 2**). For analysis by flow cytometry, 3 days post-nucleofection, 100,000 mouse T cells were stained with anti-mouse CD3 antibody (Clone 17A2, Invitrogen 11-0032-82) for 30 minutes at 4 °C and analyzed on an Attune Nxt flow cytometer.

Table 2A: Guide sequences used in Example 8

SEQ ID NO:	Entity Name	Sequence
6469	MG3-6-mTRAC-sgRNA-A1	mA*mG*mA*rArCrCrUrGrCrUrGrUrGrUrArCrCrArGrUrUrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrArCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6470	MG3-6-mTRAC-sgRNA-B1	mA*mA*mC*rUrGrUrGrCrUrGrGrArCrArUrGrArArArGrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6471	MG3-6-mTRAC-sgRNA-C1	mA*mA*mG*rCrUrArUrGrGrArUrUrCrCrArArGrArGrCrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6472	MG3-6-mTRAC-sgRNA-D1	mU*mC*mA*rCrCrUrGrCrCrArArGrArUrArUrCrUrUrCrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6473	MG3-6-mTRAC-sgRNA-E1	mA*mG*mU*rUrUrUrGrUrCrArGrUrGrArUrGrArArCrGrUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCr

SEQ ID NO:	Entity Name	Sequence
		CrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6474	MG3-6-mTRAC-sgRNA-F1	mG*mA*mA*rCrArGrGrCrArGrArGrGrUrGrCrUrGrUrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6475	MG3-6-mTRAC-sgRNA-G1	mC*mA*mG*rArGrGrGrUrGrCrUrGrUrCrCrUrGrArGrArCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6476	MG3-6-mTRAC-sgRNA-H1	mA*mG*mG*rArUrCrUrUrUrUrArArCrUrGrGrUrArCrArCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6477	MG3-6-mTRAC-sgRNA-A2	mU*mU*mU*rArArCrUrGrGrUrArCrArCrArGrCrArGrGrUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6478	MG3-6-mTRAC-sgRNA-B2	mA*mG*mG*rUrUrCrUrGrGrGrUrUrCrUrGrGrArUrGrUrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6479	MG3-6-mTRAC-sgRNA-C2	mA*mA*mC*rUrUrUrCrArArArArCrCrUrGrUrCrArGrUrUrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6480	MG3-6-mTRAC-sgRNA-D2	mC*mG*mA*rArUrCrCrUrCrCrUrGrCrUrGrArArArGrUrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6481	MG3-6-mTRAC-sgRNA-E2	mG*mG*mA*rUrUrUrArArCrCrUrGrCrUrCrArUrGrArCrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6482	MG3-6-mTRAC-sgRNA-F2	mC*mU*mU*rUrCrArUrGrCrCrUrUrCrUrUrArCrCrUrCrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6483	MG3-6-mTRAC-sgRNA-G2	mG*mA*mC*rCrArCrArGrCrCrUrCrArGrCrGrUrCrArUrGrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6484	MG3-6-mTRAC-sgRNA-H2	mU*mA*mA*rArUrCrCrGrGrCrUrArCrUrUrUrCrArGrCrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6485	MG3-6-mTRAC-sgRNA-A3	mA*mG*mG*rArUrUrCrGrGrArGrUrCrCrCrArUrArArCrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGr

SEQ ID NO:	Entity Name	Sequence
		GrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6486	MG3-6-mTRAC-sgRNA-B3	mA*mU*mA*rArCrUrGrArCrArGrGrUrUrUrUrGrArArArGrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6487	MG3-6-mTRAC-sgRNA-C3	mG*mU*mA*rCrUrUrCrCrUrCrArCrUrCrCrArGrGrUrCrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6488	MG3-6-mTRAC-sgRNA-D3	mC*mC*mA*rCrCrUrCrGrUrCrArArGrArCrGrGrCrUrGrUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6489	MG3-6-mTRAC-sgRNA-E3	mU*mG*mG*rCrCrCrUrGrArUrUrCrArCrArArUrCrCrCrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6490	MG3-6-mTRAC-sgRNA-F3	mU*mU*mC*rArCrArArUrCrCrCrArCrCrUrGrGrArUrCrUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6491	MG3-6-mTRAC-sgRNA-G3	mC*mU*mG*rGrArUrCrUrCrCrArGrArUrUrUrGrUrGrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6492	MG3-6-mTRAC-sgRNA-H3	mC*mA*mU*rUrCrArCrArArArArArArCrGrGrCrArGrGrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6493	MG3-6-mTRAC-sgRNA-A4	mA*mA*mC*rGrGrCrArGrGrGrGrGrCrGrGrGrGrCrUrUrCrUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6494	MG3-6-mTRAC-sgRNA-B4	mG*mG*mG*rCrGrGrGrGrCrUrUrCrUrCrCrUrGrGrArUrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6495	MG3-6-mTRAC-sgRNA-C4	mA*mU*mC*rUrGrArArGrArCrCrCrCrUrCrCrCrCrCrArUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6496	MG3-6-mTRAC-sgRNA-D4	mG*mU*mU*rUrUrUrUrGrUrUrUrUrUrUrUrUrUrUrUrUrUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU

SEQ ID NO:	Entity Name	Sequence
6497	MG3-6-mTRAC-sgRNA-E4	mG*mG*mU*rGrUrArGrArArUrUrArUrCrUrCrArUrUrGrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6498	MG3-6-mTRAC-sgRNA-F4	mG*mG*mC*rUrCrArArUrArCrArCrArCrArGrUrArGrCrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6499	MG3-6-mTRAC-sgRNA-G4	mA*mU*mU*rUrUrUrUrUrArCrArArCrArUrUrCrUrCrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6500	MG3-6-mTRAC-sgRNA-H4	mA*mC*mA*rGrGrGrGrArGrUrCrUrGrCrCrArUrGrGrGrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6501	MG3-6-mTRAC-sgRNA-A5	mG*mU*mC*rUrGrCrCrArUrGrGrGrGrGrArGrGrGrGrUrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6502	MG3-6-mTRAC-sgRNA-B5	mA*mG*mC*rArArCrCrUrUrCrCrUrCrArCrArArArUrCrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6503	MG3-6-mTRAC-sgRNA-C5	mU*mC*mA*rCrArArArUrCrUrGrGrGrArGrArUrCrCrArGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6504	MG3-6-mTRAC-sgRNA-D5	mA*mG*mA*rUrCrCrArGrGrUrGrGrGrArUrUrGrUrGrArArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6505	MG3-6-mTRAC-sgRNA-E5	mU*mG*mG*rGrArUrUrGrUrGrArArUrCrArGrGrGrCrCrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6506	MG3-6-mTRAC-sgRNA-F5	mG*mA*mC*rArGrCrCrGrUrCrUrUrGrArCrGrArGrGrUrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6507	MG3-6-mTRAC-sgRNA-G5	mU*mG*mA*rGrGrArGrGrArUrGrGrArGrCrUrUrGrGrGrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6508	MG3-6-mTRAC-sgRNA-H5	mG*mG*mA*rGrUrCrArGrGrCrUrCrUrGrUrCrArGrUrCrUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCr

SEQ ID NO:	Entity Name	Sequence
		CrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
Notations for chemical modifications: m = 2'-O-methyl ribonucleotide (e.g mC = cytosine ribonucleotide with 2'-O Methyl in place of 2' hydroxyl); f = 2'-fluoro ribonucleotide (e.g fC = cytosine ribonucleotide with 2' fluorine in place of 2' hydroxyl); * = phosphorothioate bond; r: native RNA linkage comprising the sugar ribose (for example the ribose or RNA form of the A base is written rA), d: deoxyribose sugar (DNA) linkage (for example a deoxyribose form of the A base is written dA)		

Table 2B: List of sites targeted in Example 8

SEQ ID NO:	Entity Name	Sequence
6509	MG3-6-mTRAC-target site-A1	AGAACCTGCTGTGTACCAGTTA
6510	MG3-6-mTRAC-target site-B1	AACTGTGCTGGACATGAAAGCT
6511	MG3-6-mTRAC-target site-C1	AAGCTATGGATTCCAAGAGCAA
6512	MG3-6-mTRAC-target site-D1	TCACCTGCCAAGATATCTTCAA
6513	MG3-6-mTRAC-target site-E1	AGTTTTGTCAGTGATGAACGTT
6514	MG3-6-mTRAC-target site-F1	GAACAGGCAGAGGGTGCTGTCC
6515	MG3-6-mTRAC-target site-G1	CAGAGGGTGCTGTCCTGAGACC
6516	MG3-6-mTRAC-target site-H1	AGGATCTTTTAACTGGTACACA
6517	MG3-6-mTRAC-target site-A2	TTTAACTGGTACACAGCAGGTT
6518	MG3-6-mTRAC-target site-B2	AGGTTCTGGGTTCTGGATGTCT
6519	MG3-6-mTRAC-target site-C2	AACTTTCAAAACCTGTCAGTTA
6520	MG3-6-mTRAC-target site-D2	CGAATCCTCCTGCTGAAAGTAG
6521	MG3-6-mTRAC-target site-E2	GGATTTAACCTGCTCATGACGC
6522	MG3-6-mTRAC-target site-F2	CTTTCATGCCTTCTTACCTCAA
6523	MG3-6-mTRAC-target site-G2	GACCACAGCCTCAGCGTCATGA
6524	MG3-6-mTRAC-target site-H2	TAAATCCGGCTACTTTCAGCAG
6525	MG3-6-mTRAC-target site-A3	AGGATTCGGAGTCCCATAACTG
6526	MG3-6-mTRAC-target site-B3	ATAACTGACAGGTTTTGAAAGT
6527	MG3-6-mTRAC-target site-C3	GTACTTCCTCACTCCAGGTCTG
6528	MG3-6-mTRAC-target site-D3	CCACCTCGTCAAGACGGCTGTC
6529	MG3-6-mTRAC-target site-E3	TGGCCCTGATTCACAATCCCAC
6530	MG3-6-mTRAC-target site-F3	TTCACAATCCCACCTGGATCTC

SEQ ID NO:	Entity Name	Sequence
6531	MG3-6-mTRAC-target site-G3	CTGGATCTCCAGATTGTGAG
6532	MG3-6-mTRAC-target site-H3	CATTCAAAAAACGGCAGGGG
6533	MG3-6-mTRAC-target site-A4	AACGGCAGGGGCGGGGCTTCTC
6534	MG3-6-mTRAC-target site-B4	GGGCGGGGCTTCTCCTGGATCT
6535	MG3-6-mTRAC-target site-C4	ATCTGAAGACCCCTCCCCATG
6536	MG3-6-mTRAC-target site-D4	GTTTTTTGTTTTTTTTTTTTTT
6537	MG3-6-mTRAC-target site-E4	GGTGTAGAAATTATCTCATTGT
6538	MG3-6-mTRAC-target site-F4	GGCTCAATACACACAGTAGCAG
6539	MG3-6-mTRAC-target site-G4	ATTTTTTTTACAACATTCTCCA
6540	MG3-6-mTRAC-target site-H4	ACAGGGGAGTCTGCCATGGGGG
6541	MG3-6-mTRAC-target site-A5	GTCTGCCATGGGGGAGGGGTCT
6542	MG3-6-mTRAC-target site-B5	AGCAACCTTCCTCACAAATCTG
6543	MG3-6-mTRAC-target site-C5	TCACAAATCTGGGAGATCCAGG
6544	MG3-6-mTRAC-target site-D5	AGATCCAGGTGGGATTGTGAAT
6545	MG3-6-mTRAC-target site-E5	TGGGATTGTGAATCAGGGCCAA
6546	MG3-6-mTRAC-target site-F5	GACAGCCGTCTTGACGAGGTGG
6547	MG3-6-mTRAC-target site-G5	TGAGGAGGATGGAGCTTGGGAG
6548	MG3-6-mTRAC-target site-H5	GGAGTCAGGCTCTGTCAGTCTT

Example 9 – Gene editing outcomes at the DNA level for HPRT

[00522] Primary T cells were purified from PBMCs using a negative selection kit (Miltenyi) according to the manufacturer's recommendations. Nucleofection of MG3-6 RNPs (126 pmol protein/160 pmol guide) (SEQ ID NOs: 6549-6615) was performed into T cells (200,000) using the Lonza 4D electroporator. Cells were harvested and genomic DNA prepared five days post-transfection. PCR primers appropriate for use in NGS-based DNA sequencing were generated, optimized, and used to amplify the individual target sequences for each guide RNA (SEQ ID NOs: 6616-6682). The amplicons were sequenced on an Illumina MiSeq machine and analyzed with a proprietary Python script to measure gene editing (**FIG. 3**).

Table 3A: Guide sequences used in Example 9

SEQ ID NO:	Entity Name	Sequence
6549	MG3-6-HPRT-sgRNA-A1	mU*mU*mC*rCrUrCrUrGrCrArUrCrArGrUrUrUrArArUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6550	MG3-6-HPRT-sgRNA-B1	mG*mU*mG*rGrGrCrUrUrGrUrGrUrUrCrUrArArArGrGrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6551	MG3-6-HPRT-sgRNA-C1	mA*mG*mG*rArGrUrGrArGrArUrUrGrGrUrUrUrUrUrGrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6552	MG3-6-HPRT-sgRNA-D1	mU*mA*mA*rArArArArUrArArUrArUrUrArArUrUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6553	MG3-6-HPRT-sgRNA-E1	mG*mA*mG*rUrArUrUrUrUrUrArUrUrGrArArArArGrCrArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6554	MG3-6-HPRT-sgRNA-F1	mA*mA*mA*rArArUrArUrUrUrCrCrCrUrArArCrArArArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6555	MG3-6-HPRT-sgRNA-G1	mA*mU*mC*rUrCrArGrCrUrArUrUrUrArGrUrCrArArArArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6556	MG3-6-HPRT-sgRNA-H1	mG*mU*mC*rCrUrArCrUrUrUrUrGrArCrUrArArArUrArGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6557	MG3-6-HPRT-sgRNA-A2	mA*mA*mC*rUrCrUrCrCrArArUrArUrArGrGrUrGrGrCrUrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6558	MG3-6-HPRT-sgRNA-B2	mA*mU*mU*rUrUrUrCrCrCrArUrArArArUrUrCrArArGrArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6559	MG3-6-HPRT-sgRNA-C2	mC*mC*mA*rGrGrArCrUrGrGrArUrUrUrUrGrUrArGrGrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6560	MG3-6-HPRT-sgRNA-D2	mU*mG*mC*rArCrCrUrArCrArArArUrCrCrArGrUrCrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCr

SEQ ID NO:	Entity Name	Sequence
		rGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6561	MG3-6-HPRT-sgRNA-E2	mG*mU*mA*rArGrArArUrGrCrCrArGrCrCrCrCrArGrGrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6562	MG3-6-HPRT-sgRNA-F2	mA*mA*mG*rCrArGrUrArArGrArArUrGrCrCrArGrCrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6563	MG3-6-HPRT-sgRNA-G2	mG*mC*mU*rGrGrCrArUrCrUrUrArCrUrGrCrUrUrGrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6564	MG3-6-HPRT-sgRNA-H2	mU*mG*mC*rUrUrGrCrUrGrArGrGrGrCrCrArGrArUrGrArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6565	MG3-6-HPRT-sgRNA-A3	mA*mU*mA*rGrArUrUrCrCrArGrArArUrArUrCrUrCrCrArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6566	MG3-6-HPRT-sgRNA-B3	mU*mG*mA*rCrArGrUrArUrUrGrCrArGrUrUrArUrArCrArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6567	MG3-6-HPRT-sgRNA-C3	mC*mG*mA*rArArArGrUrArArUrGrUrArArUrCrUrCrArUrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6568	MG3-6-HPRT-sgRNA-D3	mG*mG*mA*rUrUrArUrArUrCrUrUrArArGrUrCrUrUrArUrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6569	MG3-6-HPRT-sgRNA-E3	mA*mA*mC*rArCrArUrGrArCrArArArArUrUrArUrUrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6570	MG3-6-HPRT-sgRNA-F3	mG*mU*mU*rUrGrUrCrCrUrGrArArUrArGrCrArUrGrGrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6571	MG3-6-HPRT-sgRNA-G3	mC*mU*mG*rArArUrArGrCrArUrGrGrCrArGrArGrArUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6572	MG3-6-HPRT-sgRNA-H3	mA*mU*mC*rCrUrUrArUrUrCrUrUrArArUrUrUrGrCrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGr

SEQ ID NO:	Entity Name	Sequence
		rCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6573	MG3-6-HPRT-sgRNA-A4	mG*mC*mC*rCrCrCrUrUrGrCrArArArUrUrArArGrArArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6574	MG3-6-HPRT-sgRNA-B4	mG*mG*mU*rGrArGrGrArArGrUrGrArUrArGrGrArArGrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6575	MG3-6-HPRT-sgRNA-C4	mG*mU*mG*rArUrArGrGrArArGrGrGrGrUrGrGrGrCrCrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6576	MG3-6-HPRT-sgRNA-D4	mG*mG*mA*rArGrGrGrGrUrGrGrGrCrCrCrUrGrArArGrArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6577	MG3-6-HPRT-sgRNA-E4	mA*mA*mU*rUrCrCrArGrGrArGrGrUrCrCrArGrArUrCrUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6578	MG3-6-HPRT-sgRNA-F4	mU*mC*mA*rUrCrArCrUrCrArArUrUrCrCrArGrGrArGrGrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6579	MG3-6-HPRT-sgRNA-G4	mC*mA*mG*rCrArUrUrCrArUrCrArCrUrCrArArUrUrCrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6580	MG3-6-HPRT-sgRNA-H4	mC*mA*mG*rCrArUrArGrGrUrArArGrGrUrGrArGrGrArGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6581	MG3-6-HPRT-sgRNA-A5	mA*mC*mA*rUrArArArArArCrUrGrCrArGrArCrUrGrArUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6582	MG3-6-HPRT-sgRNA-B5	mA*mC*mC*rUrGrArCrCrCrUrArCrArUrArArArArArCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6583	MG3-6-HPRT-sgRNA-C5	mG*mA*mU*rCrArGrUrCrUrGrCrArGrUrUrUrUrUrArUrGrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU

SEQ ID NO:	Entity Name	Sequence
6584	MG3-6-HPRT-sgRNA-D5	mU*mC*mU*rGrCrUrUrUrUrCrCrUrArArGrUrGrArUrUrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6585	MG3-6-HPRT-sgRNA-E5	mA*mC*mA*rGrArUrArCrCrGrUrGrArUrUrUrUrUrCrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6586	MG3-6-HPRT-sgRNA-F5	mA*mC*mU*rGrCrUrGrArCrArUrArUrGrArCrUrCrArCrUrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6587	MG3-6-HPRT-sgRNA-G5	mC*mA*mU*rArUrGrUrCrArGrCrArGrUrUrUrGrArCrUrGrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6588	MG3-6-HPRT-sgRNA-H5	mU*mA*mU*rCrArGrUrGrArGrUrUrUrUrUrCrUrUrUrUrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6589	MG3-6-HPRT-sgRNA-A6	mG*mC*mU*rUrArUrUrUrUrUrCrUrArCrArUrGrCrUrCrUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6590	MG3-6-HPRT-sgRNA-B6	mU*mU*mA*rArArUrGrUrCrArArCrCrUrArCrUrGrUrGrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6591	MG3-6-HPRT-sgRNA-C6	mG*mA*mG*rGrArUrUrArArArGrUrCrUrArUrGrCrCrArCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6592	MG3-6-HPRT-sgRNA-D6	mA*mA*mG*rArArCrArArCrArArArGrArArUrArCrCrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6593	MG3-6-HPRT-sgRNA-E6	mU*mG*mG*rUrArUrArUrGrCrUrGrUrGrGrArArUrUrGrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6594	MG3-6-HPRT-sgRNA-F6	mG*mA*mG*rArUrArGrArCrUrGrGrUrUrCrGrUrGrArGrCrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6595	MG3-6-HPRT-sgRNA-G6	mG*mU*mA*rGrGrArCrArUrGrCrUrCrArArArCrArArUrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCr

SEQ ID NO:	Entity Name	Sequence
		CrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6596	MG3-6-HPRT-sgRNA-H6	mA*mU*mU*rArArGrCrArGrCrUrGrCrUrCrArCrUrArCrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6597	MG3-6-HPRT-sgRNA-A7	mG*mA*mG*rArUrGrGrArGrCrUrUrUrArUrUrArArArCrArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6598	MG3-6-HPRT-sgRNA-B7	mG*mA*mA*rCrUrCrArGrCrArCrUrUrCrArUrArUrGrCrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6599	MG3-6-HPRT-sgRNA-C7	mU*mG*mA*rGrUrUrCrUrCrUrUrGrArArCrUrCrCrUrArArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6600	MG3-6-HPRT-sgRNA-D7	mA*mU*mU*rCrCrUrGrArGrUrUrCrArGrGrUrArGrGrGrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6601	MG3-6-HPRT-sgRNA-E7	mU*mA*mU*rArUrArUrGrUrUrUrArArArGrArGrCrUrGrGrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6602	MG3-6-HPRT-sgRNA-F7	mG*mG*mU*rArUrGrArArArGrCrArUrArArGrUrUrUrUrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6603	MG3-6-HPRT-sgRNA-G7	mU*mG*mA*rGrArCrUrGrCrCrUrUrUrArArCrArUrCrUrGrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6604	MG3-6-HPRT-sgRNA-H7	mA*mA*mU*rArUrUrUrUrUrCrArArCrArGrGrCrArGrCrArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6605	MG3-6-HPRT-sgRNA-A8	mC*mU*mC*rCrCrArCrArCrCrCrUrUrUrUrArUrArGrUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6606	MG3-6-HPRT-sgRNA-B8	mU*mA*mU*rArGrUrUrUrArGrGrGrArUrUrGrUrArUrUrUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6607	MG3-6-HPRT-sgRNA-C8	mA*mG*mG*rGrArUrUrGrUrArUrUrUrCrCrArArGrGrUrUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGr

SEQ ID NO:	Entity Name	Sequence
		GrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6608	MG3-6-HPRT-sgRNA-D8	mA*mG*mU*rGrUrCrArArUrGrArGrCrArArGrArUrGrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6609	MG3-6-HPRT-sgRNA-E8	mC*mC*mA*rUrUrGrArArGrGrGrGrArGrCrUrArArUrArArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6610	MG3-6-HPRT-sgRNA-F8	mU*mG*mG*rArCrArCrArUrGrGrGrUrArGrUrCrArGrGrGrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6611	MG3-6-HPRT-sgRNA-G8	mC*mC*mU*rGrGrArArCrCrUrGrArArGrGrArCrArGrUrUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6612	MG3-6-HPRT-sgRNA-H8	mG*mU*mG*rCrArGrGrUrCrUrCrArGrArArCrUrGrUrCrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6613	MG3-6-HPRT-sgRNA-A9	mA*mU*mG*rArArArUrGrGrArGrArGrCrUrArArArUrUrArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6614	MG3-6-HPRT-sgRNA-B9	mG*mU*mC*rArCrUrUrUrUrArArCrArCrArCrCrArArGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6615	MG3-6-HPRT-sgRNA-C9	mU*mA*mG*rArGrArGrGrCrArCrArUrUrUrGrCrCrArGrUrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU

Notations for chemical modifications: m = 2'-O-methyl ribonucleotide (e.g mC = cytosine ribonucleotide with 2'-O Methyl in place of 2' hydroxyl); f = 2'-fluoro ribonucleotide (e.g fC = cytosine ribonucleotide with 2' fluorine in place of 2' hydroxyl); * = phosphorothioate bond; r: native RNA linkage comprising the sugar ribose (for example the ribose or RNA form of the A base is written rA), d: deoxyribose sugar (DNA) linkage (for example a deoxyribose form of the A base is written dA)

Table 3B: Sites targeted in Example 9

SEQ ID NO:	Entity Name	Sequence
6616	MG3-6-HPRT-target site-A1	TTCCTCTGCATCAGTTTAAATG
6617	MG3-6-HPRT-target site-B1	GTGGGCTTGTGTTCTAAAGGAG

SEQ ID NO:	Entity Name	Sequence
6618	MG3-6-HPRT-target site-C1	AGGAGTGAGATTGGTTTTTTGT
6619	MG3-6-HPRT-target site-D1	TAAAAATAATATTTATAATTT
6620	MG3-6-HPRT-target site-E1	GAGTATTTTTATTGAAAAGCAT
6621	MG3-6-HPRT-target site-F1	AAAAATATTTTCCCTAACAAAG
6622	MG3-6-HPRT-target site-G1	ATCTCAGCTATTTAGTCAAAAG
6623	MG3-6-HPRT-target site-H1	GTCCTACTTTTGACTAAATAGC
6624	MG3-6-HPRT-target site-A2	AACTCTCCAATATAGGTGGCTA
6625	MG3-6-HPRT-target site-B2	ATTTTCCCATAAAATTCAAGAT
6626	MG3-6-HPRT-target site-C2	CCAGGACTGGATTTTGTAGGTG
6627	MG3-6-HPRT-target site-D2	TGCACCTACAAAATCCAGTCCT
6628	MG3-6-HPRT-target site-E2	GTAAGAATGCCAGCCCCCAGGA
6629	MG3-6-HPRT-target site-F2	AAGCAGTAAGAATGCCAGCCCC
6630	MG3-6-HPRT-target site-G2	GCTGGCATTCTTACTGCTTGCT
6631	MG3-6-HPRT-target site-H2	TGCTTGCTGAGGGCCAGATGAT
6632	MG3-6-HPRT-target site-A3	ATAGATTCCAGAATATCTCCAT
6633	MG3-6-HPRT-target site-B3	TGACAGTATTGCAGTTATACAT
6634	MG3-6-HPRT-target site-C3	CGAAAAGTAATGTAATCTCATA
6635	MG3-6-HPRT-target site-D3	GGATTATATCTTAAGTCTTATA
6636	MG3-6-HPRT-target site-E3	AACACATGACAAAATTATTTAA
6637	MG3-6-HPRT-target site-F3	GTTTGTCTGAATAGCATGGCA
6638	MG3-6-HPRT-target site-G3	CTGAATAGCATGGCAGAGGATT
6639	MG3-6-HPRT-target site-H3	ATCCTTATTCTTAATTTTGCAA
6640	MG3-6-HPRT-target site-A4	GCCCCCTTGCAAAATTAAGAAT
6641	MG3-6-HPRT-target site-B4	GGTGAGGAAGTGATAGGAAGGG
6642	MG3-6-HPRT-target site-C4	GTGATAGGAAGGGGTGGGCCCT
6643	MG3-6-HPRT-target site-D4	GGAAGGGGTGGGCCCTGAAGAT
6644	MG3-6-HPRT-target site-E4	AATTCCAGGAGGTCCAGATCTT
6645	MG3-6-HPRT-target site-F4	TCATCACTCAATTCCAGGAGGT
6646	MG3-6-HPRT-target site-G4	CAGCATTCATCACTCAATTCCA

SEQ ID NO:	Entity Name	Sequence
6647	MG3-6-HPRT-target site-H4	CAGCATAGGTAAGGTGAGGAGG
6648	MG3-6-HPRT-target site-A5	ACATAAAAACTGCAGACTGATC
6649	MG3-6-HPRT-target site-B5	ACCTGACCCCTACATAAAAACT
6650	MG3-6-HPRT-target site-C5	GATCAGTCTGCAGTTTTTATGT
6651	MG3-6-HPRT-target site-D5	TCTGCTTTTTCTTAAGTGATTA
6652	MG3-6-HPRT-target site-E5	ACAGATACCGTGATTTTTTCAA
6653	MG3-6-HPRT-target site-F5	ACTGCTGACATATGACTCACTA
6654	MG3-6-HPRT-target site-G5	CATATGTCAGCAGTTTGACTGT
6655	MG3-6-HPRT-target site-H5	TATCAGTGAGTTTTTCTTTTAA
6656	MG3-6-HPRT-target site-A6	GCTTATTTTTCTACATGCTCTT
6657	MG3-6-HPRT-target site-B6	TTAAATGTCAACCTACTGTGGC
6658	MG3-6-HPRT-target site-C6	GAGGATTAAAGTCTATGCCACA
6659	MG3-6-HPRT-target site-D6	AAGAACAACAAAAGAATACCCA
6660	MG3-6-HPRT-target site-E6	TGGTATATGCTGTGGAATTGAG
6661	MG3-6-HPRT-target site-F6	GAGATAGACTGGTTCGTGAGCG
6662	MG3-6-HPRT-target site-G6	GTAGGACATGCTCAAACAATAC
6663	MG3-6-HPRT-target site-H6	ATTAAGCAGCTGCTCACTACAA
6664	MG3-6-HPRT-target site-A7	GAGATGGAGCTTTATTAAACAT
6665	MG3-6-HPRT-target site-B7	GAACTCAGCACTTCATATGCCT
6666	MG3-6-HPRT-target site-C7	TGAGTTCTCTTGAACCTCCTAAT
6667	MG3-6-HPRT-target site-D7	ATTCCTGAGTTCAGGTAGGGAG
6668	MG3-6-HPRT-target site-E7	TATATATGTTTAAAGAGCTGGA
6669	MG3-6-HPRT-target site-F7	GGTATGAAAGCATAAGTTTTCT
6670	MG3-6-HPRT-target site-G7	TGAGACTGCCTTTAACATCTGT
6671	MG3-6-HPRT-target site-H7	AATATTTTTCAACAGGCAGCAT
6672	MG3-6-HPRT-target site-A8	CTCCCACACCCTTTTATAGTTT
6673	MG3-6-HPRT-target site-B8	TATAGTTTAGGGATTGTATTTC
6674	MG3-6-HPRT-target site-C8	AGGGATTGTATTTCCAAGGTTT
6675	MG3-6-HPRT-target site-D8	AGTGTCAATGAGCAAAGATGAA

SEQ ID NO:	Entity Name	Sequence
6676	MG3-6-HPRT-target site-E8	CCATTGAAGGGGAGCTAATAAG
6677	MG3-6-HPRT-target site-F8	TGGACACATGGGTAGTCAGGGT
6678	MG3-6-HPRT-target site-G8	CCTGGAACCTGAAGGACAGTTC
6679	MG3-6-HPRT-target site-H8	GTGCAGGTCTCAGAACTGTCCT
6680	MG3-6-HPRT-target site-A9	ATGAAATGGAGAGCTAAATTAT
6681	MG3-6-HPRT-target site-B9	GTCACCTTTTAACACACCCAAGG
6682	MG3-6-HPRT-target site-C9	TAGAGAGGCACATTTGCCAGTA

Example 10 – Gene editing outcomes at the DNA level for human TRBC1/2

[00523] Primary T cells were purified from PBMCs using a negative selection kit (Miltenyi) according to the manufacturer's recommendations. Nucleofection of MG3-6 or MG3-8 RNPs (106 pmol protein/160 pmol guide) (MG3-6: SEQ ID NOs: 6683-6721; MG3-8: SEQ ID NOs: 6761-6781) was performed into T cells (200,000) using the Lonza 4D electroporator. For analysis by flow cytometry, 3 days post-nucleofection, 100,000 T cells were stained with anti-CD3 antibody for 30 minutes at 4 °C and analyzed on an Attune Nxt flow cytometer (**FIG. 4**).

Table 4A: Guide sequences used in Example 10

SEQ ID NO:	Entity Name	Sequence
6683	MG3-6-TRBC1/2-sgRNA-A6	mA*mG*mG*rUrCrCrUrCrUrGrGrArArArGrGrGrArArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6684	MG3-6-TRBC1/2-sgRNA-B6	mC*mA*mG*rGrUrCrCrUrCrUrGrGrArArArGrGrGrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6685	MG3-6-TRBC1/2-sgRNA-C6	mC*mC*mA*rCrArCrUrGrGrUrGrUrGrCrCrUrGrGrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6686	MG3-6-TRBC1/2-sgRNA-D6	mG*mA*mA*rUrGrGrGrArArGrGrArGrGrUrGrCrArCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6687	MG3-6-TRBC1/2-sgRNA-E6	mU*mG*mA*rGrGrGrCrGrGrGrCrUrGrCrUrCrCrUrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU

SEQ ID NO:	Entity Name	Sequence
6688	MG3-6-TRBC1/2-sgRNA-F6	mA*mG*mU*rArUrCrUrGrGrArGrUrCrArUrUrGrArGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6689	MG3-6-TRBC1/2-sgRNA-G6	mA*mU*mA*rCrUrGrCrCrUrGrArGrCrArGrCrCrGrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6690	MG3-6-TRBC1/2-sgRNA-H6	mU*mU*mG*rArCrArGrCrGrGrArArGrUrGrGrUrUrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6691	MG3-6-TRBC1/2-sgRNA-A7	mC*mU*mU*rGrArCrArGrCrGrGrArArGrUrGrGrUrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6692	MG3-6-TRBC1/2-sgRNA-B7	mC*mC*mG*rCrUrGrUrCrArArGrUrCrCrArGrUrUrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6693	MG3-6-TRBC1/2-sgRNA-C7	mU*mC*mG*rGrArGrArArUrGrArCrGrArGrUrGrGrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6694	MG3-6-TRBC1/2-sgRNA-D7	mC*mA*mG*rArUrCrGrUrCrArGrCrGrCrGrArGrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6695	MG3-6-TRBC1/2-sgRNA-E7	mC*mG*mG*rCrGrCrUrGrArCrGrArUrCrUrGrGrGrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6696	MG3-6-TRBC1/2-sgRNA-F7	mG*mC*mC*rArArCrArGrUrGrUrCrCrUrArCrCrArGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6697	MG3-6-TRBC1/2-sgRNA-G7	mC*mU*mU*rCrCrCrUrArGrCrArGrGrArUrCrUrCrArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6698	MG3-6-TRBC1/2-sgRNA-H7	mA*mU*mA*rCrArGrGrGrUrGrGrCrCrUrUrCrCrCrUrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6699	MG3-6-TRBC1/2-sgRNA-A8	mG*mG*mC*rGrCrUrGrArCrCrArGrCrArCrArGrCrArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGr

SEQ ID NO:	Entity Name	Sequence
		UrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6700	MG3-6-TRBC1/2-sgRNA-B8	mU*mC*mU*rCrUrUrCrUrGrCrArGrGrUrCrArArGrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6701	MG3-6-TRBC1/2-sgRNA-C8	mC*mC*mU*rGrCrArGrArArGrArGrArArGrUrUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6702	MG3-6-TRBC1/2-sgRNA-D8	mA*mC*mC*rUrGrCrArGrArArGrArGrArArGrUrUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6703	MG3-6-TRBC1/2-sgRNA-E8	mC*mC*mA*rCrArCrUrGrGrUrGrUrGrCrCrUrGrGrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6704	MG3-6-TRBC1/2-sgRNA-F8	mA*mC*mC*rArGrCrUrCrArGrCrUrCrCrArCrGrUrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6705	MG3-6-TRBC1/2-sgRNA-G8	mG*mA*mA*rUrGrGrGrArArGrGrArGrGrUrGrCrArCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6706	MG3-6-TRBC1/2-sgRNA-H8	mU*mG*mA*rGrGrGrCrGrGrGrCrUrGrCrUrCrCrUrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6707	MG3-6-TRBC1/2-sgRNA-A9	mA*mG*mU*rArUrCrUrGrGrArGrUrCrArUrUrGrArGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6708	MG3-6-TRBC1/2-sgRNA-B9	mA*mU*mA*rCrUrGrCrCrUrGrArGrCrArGrCrCrGrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6709	MG3-6-TRBC1/2-sgRNA-C9	mU*mU*mG*rArCrArGrCrGrGrArArGrUrGrGrUrUrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6710	MG3-6-TRBC1/2-sgRNA-D9	mC*mU*mU*rGrArCrArGrCrGrGrArArGrUrGrGrUrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6711	MG3-6-TRBC1/2-sgRNA-E9	mC*mC*mG*rCrUrGrUrCrArArGrUrCrCrArGrUrUrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCr

SEQ ID NO:	Entity Name	Sequence
		ArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGr UrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUr ArUrGrU*mU*mU*mU
6712	MG3-6-TRBC1/2-sgRNA-F9	mU*mC*mG*rGrArGrArArUrGrArCrGrArGrUrGrGrArCrGrUrUr GrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCr ArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGr UrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUr ArUrGrU*mU*mU*mU
6713	MG3-6-TRBC1/2-sgRNA-G9	mC*mA*mG*rArUrCrGrUrCrArGrCrGrCrGrArGrGrCrGrUrUr GrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCr ArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGr UrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUr ArUrGrU*mU*mU*mU
6714	MG3-6-TRBC1/2-sgRNA-H9	mC*mG*mG*rCrGrCrUrGrArCrGrArUrCrUrGrGrGrUrGrGrUrUr GrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCr ArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGr UrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUr ArUrGrU*mU*mU*mU
6715	MG3-6-TRBC1/2-sgRNA-A10	mG*mU*mC*rArArCrArGrArGrUrCrUrUrArCrCrArGrCrGrUrUr GrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCr ArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGr UrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUr ArUrGrU*mU*mU*mU
6716	MG3-6-TRBC1/2-sgRNA-B10	mC*mU*mU*rCrCrCrUrArGrCrArArGrArUrCrUrCrArUrGrUrUr GrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCr ArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGr UrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUr ArUrGrU*mU*mU*mU
6717	MG3-6-TRBC1/2-sgRNA-C10	mG*mC*mU*rGrArUrGrGrCrCrArUrGrGrUrArArGrGrArGrUrUr GrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCr ArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGr UrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUr ArUrGrU*mU*mU*mU
6718	MG3-6-TRBC1/2-sgRNA-D10	mU*mG*mU*rGrGrArArGrArGrArGrArArCrArUrUrUrGrUrUr GrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCr ArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGr UrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUr ArUrGrU*mU*mU*mU
6719	MG3-6-TRBC1/2-sgRNA-E10	mC*mU*mG*rUrGrGrArArGrArGrArGrArArCrArUrUrUrGrUrUr GrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCr ArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGr UrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUr ArUrGrU*mU*mU*mU
6720	MG3-6-TRBC1/2-sgRNA-F10	mU*mC*mU*rCrUrUrCrCrArCrArGrGrUrCrArArGrArGrGrUrUr GrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCr ArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGr UrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUr ArUrGrU*mU*mU*mU
6721	MG3-6-TRBC1/2-sgRNA-G10	mA*mG*mG*rUrCrArArGrArGrArArArGrGrArUrUrCrCrGrUrUr GrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCr ArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGr UrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUr ArUrGrU*mU*mU*mU
6761	MG3-8-TRBC1/2-sgRNA-D3	mA*mG*mG*rUrCrCrUrCrUrGrGrArArArGrGrGrArArGrGrUrUr GrArGrArArUrCrUrUrUrCrGrArArArGrArArArGrArUrUrCrUrUr ArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUr UrCrUrCrArCrCrGrUrCrCrGrGrCrUrCrCrUrCrUrUrArGrGrArAr CrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU

SEQ ID NO:	Entity Name	Sequence
6762	MG3-8-TRBC1/2-sgRNA-E3	mC*mU*mG*rArArCrArArGrGrUrGrUrUrCrCrCrArCrCrGrUrUrGrArGrArArUrCrUrUrUrCrGrArArArGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrGrCrUrCrCrUrCrUrUrArGrGrArArCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6763	MG3-8-TRBC1/2-sgRNA-F3	mC*mU*mC*rGrGrGrUrGrGrGrArArCrArCrCrUrUrGrUrGrUrUrGrArGrArArUrCrUrUrUrCrGrArArArGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrGrCrUrCrCrUrCrUrUrArGrGrArArCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6764	MG3-8-TRBC1/2-sgRNA-G3	mA*mA*mU*rGrGrGrArArGrGrArGrGrUrGrCrArCrArGrGrUrUrGrArGrArArUrCrUrUrCrGrArArArGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrGrCrUrCrCrUrCrUrUrArGrGrArArCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6765	MG3-8-TRBC1/2-sgRNA-H3	mG*mG*mG*rCrUrGrCrUrCrCrUrUrGrArGrGrGrGrCrUrGrUrUrGrArGrArArUrCrUrUrUrCrGrArArArGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrGrCrUrCrCrUrCrUrUrArGrGrArArCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6766	MG3-8-TRBC1/2-sgRNA-A4	mU*mA*mC*rUrGrCrCrUrGrArGrCrArGrCrCrGrCrCrUrGrUrUrGrArGrArArUrCrUrUrUrCrGrArArArGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrGrCrUrCrCrUrCrUrUrArGrGrArArCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6767	MG3-8-TRBC1/2-sgRNA-B4	mU*mU*mG*rArCrArGrCrGrGrArArGrUrGrGrUrUrGrCrGrUrUrGrArGrArArUrCrUrUrUrCrGrArArArGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrGrCrUrCrCrUrCrUrUrArGrGrArArCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6768	MG3-8-TRBC1/2-sgRNA-C4	mG*mU*mG*rArCrGrGrGrUrUrUrGrGrCrCrCrUrArUrCrGrUrUrGrArGrArArUrCrUrUrUrCrGrArArArGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrGrCrUrCrCrUrCrUrUrArGrGrArArCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6769	MG3-8-TRBC1/2-sgRNA-D4	mC*mG*mG*rCrGrCrUrGrArCrGrArUrCrUrGrGrGrUrGrGrUrUrGrArGrArArUrCrUrUrUrCrGrArArArGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrGrCrUrCrCrUrCrUrUrArGrGrArArCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6770	MG3-8-TRBC1/2-sgRNA-E4	mC*mC*mA*rArCrArGrUrGrUrCrCrUrArCrCrArGrCrArGrUrUrGrArGrArArUrCrUrUrUrCrGrArArArGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrGrCrUrCrCrUrCrUrUrArGrGrArArCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6771	MG3-8-TRBC1/2-sgRNA-F4	mC*mC*mU*rGrCrArGrArArGrArGrArArArGrUrUrUrUrGrUrUrGrArGrArArUrCrUrUrUrCrGrArArArGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrGrCrUrCrCrUrCrUrUrArGrGrArArCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6772	MG3-8-TRBC1/2-sgRNA-G4	mC*mU*mG*rArArArArArCrGrUrGrUrUrCrCrCrArCrCrGrUrUrGrArGrArArUrCrUrUrUrCrGrArArArGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrGrCrUrCrCrUrCrUrUrArGrGrArArCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6773	MG3-8-TRBC1/2-sgRNA-H4	mC*mU*mU*rGrGrGrUrGrGrGrArArCrArCrGrUrUrUrUrGrUrUrGrArGrArArUrCrUrUrUrCrGrArArArGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUr

SEQ ID NO:	Entity Name	Sequence
		UrCrUrCrArCrCrGrUrCrCrGrGrCrUrCrCrUrCrUrUrArGrGrArArCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6774	MG3-8-TRBC1/2-sgRNA-A5	mA*mA*mU*rGrGrGrArArGrGrArGrGrUrGrCrArCrArGrGrUrUrGrArGrArArUrCrUrUrUrCrGrArArArGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrGrCrUrCrCrUrCrUrUrArGrGrArArCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6775	MG3-8-TRBC1/2-sgRNA-B5	mG*mG*mG*rCrUrGrCrUrCrCrUrUrGrArGrGrGrGrCrUrGrUrUrGrArGrArArUrCrUrUrUrCrGrArArArGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrGrCrUrCrCrUrCrUrUrArGrGrArArCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6776	MG3-8-TRBC1/2-sgRNA-C5	mU*mA*mC*rUrGrCrCrUrGrArGrCrCrCrCrUrGrUrUrGrArGrArArUrCrUrUrUrCrGrArArArGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrGrCrUrCrCrUrCrUrUrArGrGrArArCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6777	MG3-8-TRBC1/2-sgRNA-D5	mU*mU*mG*rArCrArGrCrGrGrArArGrUrGrGrUrUrGrCrGrUrUrGrArGrArArUrCrUrUrUrCrGrArArArGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrGrCrUrCrCrUrCrUrUrArGrGrArArCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6778	MG3-8-TRBC1/2-sgRNA-E5	mG*mU*mG*rArCrArGrGrUrUrUrGrGrCrCrCrUrArUrCrGrUrUrGrArGrArArUrCrUrUrUrCrGrArArArGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrGrCrUrCrCrUrCrUrUrArGrGrArArCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6779	MG3-8-TRBC1/2-sgRNA-F5	mC*mG*mG*rCrGrCrUrGrArCrGrArUrCrUrGrGrGrUrGrGrUrUrGrArGrArArUrCrUrUrUrCrGrArArArGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrGrCrUrCrCrUrCrUrUrArGrGrArArCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6780	MG3-8-TRBC1/2-sgRNA-G5	mU*mC*mA*rArCrArGrArGrUrCrUrUrArCrCrArGrCrArGrUrUrGrArGrArArUrCrUrUrUrCrGrArArArGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrGrCrUrCrCrUrCrUrUrArGrGrArArCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6781	MG3-8-TRBC1/2-sgRNA-H5	mU*mG*mU*rGrGrArArGrArGrArGrArArCrArUrUrUrUrGrUrUrGrArGrArArUrCrUrUrUrCrGrArArArGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrGrCrUrCrCrUrCrUrUrArGrGrArArCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU

Notations for chemical modifications: m = 2'-O-methyl ribonucleotide (e.g mC = cytosine ribonucleotide with 2'-O Methyl in place of 2' hydroxyl); f = 2'-fluoro ribonucleotide (e.g fC = cytosine ribonucleotide with 2' fluorine in place of 2' hydroxyl); * = phosphorothioate bond; r: native RNA linkage comprising the sugar ribose (for example the ribose or RNA form of the A base is written rA), d: deoxyribose sugar (DNA) linkage (for example a deoxyribose form of the A base is written dA)

Table 4B: Sites targeted in Example 10

SEQ ID NO:	Entity Name	Sequence
6722	MG3-6-TRBC1/2-target site-A6	AGGTCCTCTGGAAAGGGAAG
6723	MG3-6-TRBC1/2-target site-B6	CAGGTCCTCTGGAAAGGGAA
6724	MG3-6-TRBC1/2-target site-C6	CCACACTGGTGTGCCTGGCC

SEQ ID NO:	Entity Name	Sequence
6725	MG3-6-TRBC1/2-target site-D6	GAATGGGAAGGAGGTGCACA
6726	MG3-6-TRBC1/2-target site-E6	TGAGGGCGGGCTGCTCCTTG
6727	MG3-6-TRBC1/2-target site-F6	AGTATCTGGAGTCATTGAGG
6728	MG3-6-TRBC1/2-target site-G6	ATACTGCCTGAGCAGCCGCC
6729	MG3-6-TRBC1/2-target site-H6	TTGACAGCGGAAGTGGTTGC
6730	MG3-6-TRBC1/2-target site-A7	CTTGACAGCGGAAGTGGTTG
6731	MG3-6-TRBC1/2-target site-B7	CCGCTGTCAAGTCCAGTTCT
6732	MG3-6-TRBC1/2-target site-C7	TCGGAGAATGACGAGTGGAC
6733	MG3-6-TRBC1/2-target site-D7	CAGATCGTCAGCGCCGAGGC
6734	MG3-6-TRBC1/2-target site-E7	CGGCGCTGACGATCTGGGTG
6735	MG3-6-TRBC1/2-target site-F7	GCCAACAGTGTCTACCAGC
6736	MG3-6-TRBC1/2-target site-G7	CTTCCCTAGCAGGATCTCAT
6737	MG3-6-TRBC1/2-target site-H7	ATACAGGGTGGCCTTCCCTA
6738	MG3-6-TRBC1/2-target site-A8	GGCGCTGACCAGCACAGCAT
6739	MG3-6-TRBC1/2-target site-B8	TCTCTTCTGCAGGTCAAGAG
6740	MG3-6-TRBC1/2-target site-C8	CCTGCAGAAGAGAAAGTTT
6741	MG3-6-TRBC1/2-target site-D8	ACCTGCAGAAGAGAAAGTTT
6742	MG3-6-TRBC1/2-target site-E8	CCACACTGGTGTGCCTGGCC
6743	MG3-6-TRBC1/2-target site-F8	ACCAGCTCAGCTCCACGTGG
6744	MG3-6-TRBC1/2-target site-G8	GAATGGGAAGGAGGTGCACA
6745	MG3-6-TRBC1/2-target site-H8	TGAGGGCGGGCTGCTCCTTG
6746	MG3-6-TRBC1/2-target site-A9	AGTATCTGGAGTCATTGAGG
6747	MG3-6-TRBC1/2-target site-B9	ATACTGCCTGAGCAGCCGCC
6748	MG3-6-TRBC1/2-target site-C9	TTGACAGCGGAAGTGGTTGC
6749	MG3-6-TRBC1/2-target site-D9	CTTGACAGCGGAAGTGGTTG
6750	MG3-6-TRBC1/2-target site-E9	CCGCTGTCAAGTCCAGTTCT
6751	MG3-6-TRBC1/2-target site-F9	TCGGAGAATGACGAGTGGAC
6752	MG3-6-TRBC1/2-target site-G9	CAGATCGTCAGCGCCGAGGC
6753	MG3-6-TRBC1/2-target site-H9	CGGCGCTGACGATCTGGGTG

SEQ ID NO:	Entity Name	Sequence
6754	MG3-6-TRBC1/2-target site-A10	GTCAACAGAGTCTTACCAGC
6755	MG3-6-TRBC1/2-target site-B10	CTTCCCTAGCAAGATCTCAT
6756	MG3-6-TRBC1/2-target site-C10	GCTGATGGCCATGGTAAGGA
6757	MG3-6-TRBC1/2-target site-D10	TGTGGAAGAGAGAACATTTT
6758	MG3-6-TRBC1/2-target site-E10	CTGTGGAAGAGAGAACATT
6759	MG3-6-TRBC1/2-target site-F10	TCTCTTCCACAGGTCAAGAG
6760	MG3-6-TRBC1/2-target site-G10	AGGTCAAGAGAAAGGATTCC
6782	MG3-8-TRBC1/2-target site-D3	AGGTCCTCTGGAAAGGGAAG
6783	MG3-8-TRBC1/2-target site-E3	CTGAACAAGGTGTTCCCACC
6784	MG3-8-TRBC1/2-target site-F3	CTCGGGTGGGAACACCTTGT
6785	MG3-8-TRBC1/2-target site-G3	AATGGGAAGGAGGTGCACAG
6786	MG3-8-TRBC1/2-target site-H3	GGGCTGCTCCTTGAGGGGCT
6787	MG3-8-TRBC1/2-target site-A4	TACTGCCTGAGCAGCCGCCT
6788	MG3-8-TRBC1/2-target site-B4	TTGACAGCGGAAGTGGTTGC
6789	MG3-8-TRBC1/2-target site-C4	GTGACGGGTTTGGCCCTATC
6790	MG3-8-TRBC1/2-target site-D4	CGGCGCTGACGATCTGGGTG
6791	MG3-8-TRBC1/2-target site-E4	CCAACAGTGTCTTACCAGCA
6792	MG3-8-TRBC1/2-target site-F4	CCTGCAGAAGAGAAAGTTTT
6793	MG3-8-TRBC1/2-target site-G4	CTGAAAAACGTGTTCCCACC
6794	MG3-8-TRBC1/2-target site-H4	CTTGGGTGGGAACACGTTTT
6795	MG3-8-TRBC1/2-target site-A5	AATGGGAAGGAGGTGCACAG
6796	MG3-8-TRBC1/2-target site-B5	GGGCTGCTCCTTGAGGGGCT
6797	MG3-8-TRBC1/2-target site-C5	TACTGCCTGAGCAGCCGCCT
6798	MG3-8-TRBC1/2-target site-D5	TTGACAGCGGAAGTGGTTGC
6799	MG3-8-TRBC1/2-target site-E5	GTGACAGGTTTGGCCCTATC
6800	MG3-8-TRBC1/2-target site-F5	CGGCGCTGACGATCTGGGTG
6801	MG3-8-TRBC1/2-target site-G5	TCAACAGAGTCTTACCAGCA
6802	MG3-8-TRBC1/2-target site-H5	TGTGGAAGAGAGAACATTTT

Example 11 – MG3-6 guide screen for mouse HAO-1 gene using mRNA transfection

[00524] Guides for MG3-6 were identified in exons 1, 2, 3, and 4 of the human HAO1 gene using a guide-finding algorithm that searches for the appropriate PAM sequence. A total of 19 guides were selected for evaluation in mammalian cells. 300 ng mRNA and 120 ng single guide RNA were transfected into Hepa1-6 cells as follows. One day prior to transfection, Hepa1-6 cells that had been cultured for less than 10 days in DMEM, 10% FBS, 1xNEAA media, without Pen/Step, were seeded into a TC-treated 24 well plate. Cells were counted, and the equivalent volume to 60,000 viable cells were added to each well. Additional pre-equilibrated media was added to each well to bring the total volume to 500 μ L. On the day of transfection, 25 μ L of OptiMEM media and 1.25 μ L of Lipofectamine Messenger Max Solution (Thermo Fisher) were mixed in a mastermix solution, vortexed, and allowed to sit for at least 5 minutes at room temperature. In separate tubes, 300 ng of the MG3-6 mRNA and 120 ng of the sgRNA were mixed together with 25 μ L of OptiMEM media and vortexed briefly. The appropriate volume of MessengerMax solution was added to each RNA solution, mixed by flicking the tube, and briefly spun down at a low speed. The complete editing reagent solutions were allowed to incubate for 10 minutes at room temperature, then added directly to the Hepa1-6 cells. Two days post transfection, the media was aspirated off of each well of Hepa1-6 cells and genomic DNA was purified by automated magnetic bead purification, via the KingFisher Flex with the MagMAX™ DNA Multi-Sample Ultra 2.0 Kit. The activity of the guides is summarized in Table 5A and FIG. 5, while the primers used are summarized in Table 5B.

Table 5A: Average Activity of MG3-6 guides at mouse HAO1 delivered by mRNA Transfection

Guide Name	PAM	SEQ ID NO.	Spacer Sequence	Editing Activity (Average % INDELS)
mH36-1	GCAGACC	11802	CATGCTGTTTCATAATCACTGAT	0
mH36-2	ACAGGTC	11803	CAGAAGTCAGTGTATGACTATT	12.5
mH36-3	CCAGACC	11804	TAACGTCTCCTGATCATTTGCC	0
mH36-4	ACAGATC	11805	CTCTGTCCTAAAACAGAAGTTG	25
mH36-5	TGGGGCT	11806	GAGTCAGCATGCCAATATGTGT	33.5
mH36-6	TCAGACC	11807	TTCTCCATTTCATTACAGCCTG	0
mH36-7	ACAGGCT	11808	TCATGCCAGTTCCCATGGTCTG	0
mH36-8	TTGGGCT	11809	GAAGTGGCATGATGCTGAGTTC	5
mH36-9	CTGGGCC	11810	AGTTGCATCCAGCGAAGTGCCT	28
mH36-10	AAAGACC	11811	CTGGATGCAACTGTACATCTAC	0

Guide Name	PAM	SEQ ID NO.	Spacer Sequence	Editing Activity (Average % INDELs)
mH36-11	TGAGATC	11812	AACTGTACATCTACAAAGACCG	0
mH36-12	CAGGGTT	11813	GATAGTGAAGCGAGCTGAGAAG	45.5
mH36-13	CAAGGCC	11814	AGCGAGCTGAGAAGCAGGGTTA	27.5
mH36-14	ACAGGTT	11815	AACCGCATTGATGACGTGCGGA	0
mH36-15	TCAGGTC	11816	AGGTTCAAGCTGCCACCACAAC	41.5
mH36-16	GTGGACT	11817	AAGGGAAATTTTGGAGACAACA	0
mH36-17	ATAGACC	11818	TGCTGAATATGTGGCACAAGCT	0
mH36-18	ATGGGTC	11819	GTAATATCATCCCAGCTGAGAG	35
mH36-19	AGAGGTT	11820	TATTGTTGTAAAGGGCATTTTG	21

Table 5B: Primers designed for the mouse HAO1 gene, used for PCR at each of the first four exons, and for sanger sequencing

Target Exon	Use	Primer Name	SEQ ID NO.	Primer Sequence
Mouse HAO1 Exon 1	Fwd PCR	PCR_mHE1_F_+233	11821	GTGACCAACCCTACCCGTTT
	Rev PCR	PCR_mHE1_R_-553	11822	GCAAGCACCTACTGTCTCGT
	Sequencing	Seq_mHE1_F_+139	11823	GTCTAGGCATACAATGTTTGCTCA
Mouse HAO1 Exon 2	Fwd PCR	HAO1_E2_F5721	11824	CAACGAAGGTTCCCTCCAGG
	Rev PCR	HAO1_E2_R6271	11825	GGAAGGGTGTTCGAGAAGGA
	Sequencing	5938F Seq_HAO1_E2	11826	CTATGCAAGGAAAAGATTTGGCC
Mouse HAO1 Exon 3	Fwd PCR	HAO1_E3_F23198	11827	TGCCCTAGACAAGCTGACAC
	Rev PCR	HAO1_E3_R23879	11828	CAGATTCTGGAAGTGGCCCA
	Sequencing	HAO1_E3_F23198	11827	Same as Fwd PCR Primer
Mouse HAO1 Exon 4	Fwd PCR	PCR_mHE4_F_+300	11829	GGCTGGCTGAAAATAGCATCC
	Rev PCR	HAO1_E4_R31650	11830	AGGTTTGGTTCCCCTCACCT
	Sequencing	PCR_mHE4_R_-149	11831	TCTGCCATGAAGGCATATGGAC

Example 12 – Guide Chemistry Optimization for the MG3-6 Type II nuclease (Prophetic)

[00525] Various chemically modified guides are designed and tested for activity. The most active guide in a guide screen in mouse hepatocytes (Hepa1-6 cells) – targeting albumin intron 1 is chosen as the spacer sequence model to insert various chemical modifications. The gRNA

comprises the spacer located in the 5' followed by the CRISPR repeat and the trans-activating CRISPR RNA (tracr). The CRISPR repeat and the tracr are identical to MG3-6. The CRISPR repeat and tracr form a structured RNA comprising 3 stem loops. Different areas of the stem loops are modified by replacing the 2' hydroxyl in the ribose by 2'-O-methyl groups or replacing the phosphodiester backbone by a phosphorothioate (PS) bond. Moreover, the spacer in the 5' of the guide is modified by adding 2'-O-methyls, PS bonds, and 2'-fluoros. The editing activity of guides with the exact same base sequence but different chemical modifications is evaluated in Hepa1-6 cells by co-transfection of mRNA encoding MG3-6 and the guide. A guide with the same base sequence and a commercially available chemical modification called AltR1/AltR2 is used as a control. The spacer sequence in these guides targets a 22 nucleotide region in albumin intron1 of the mouse genome.

[00526] In order to test the stability of the chemically modified guides compared to the guide with no chemical modification (native RNA), a stability assay using crude cell extracts is used. Crude cell extracts from mammalian cells are selected because they contain the mixture of nucleases that a guide RNA will be exposed to when delivered to mammalian cells in vitro or in vivo. Hepa 1-6 cells are collected by adding 3ml of cold PBS per 15 cm dish of confluent cells and releasing the cells from the surface of the dish using a cell scraper. The cells are pelleted at 200 g for 10 min and frozen at -80 °C for future use. For the stability assays, cells are resuspended in 4 volumes of cold PBS (e.g., for a 100 mg pellet cells are resuspended in 400 µL of cold PBS). Triton X-100 is added to a concentration of 0.2% (v/v), cells are vortexed for 10 seconds, put on ice for 10 minutes, and vortexed again for 10 seconds. Triton X-100 is a mild non-ionic detergent that disrupts cell membranes but does not inactivate or denature proteins at the concentration used. Stability reactions are set up on ice and comprise 20 µL of cell crude extract with 2 pmoles of each guide (1 µL of a 2 µM stock). Six reactions are set up per guide comprising: input, 0.5 hour, 1 hour, 4 hours, 9 hours, and in some cases 21 hours (The time in hours referring to the length of time each sample is incubated). Samples are incubated at 37 °C from 0.5 hours up to 21 hours while the input control is left on ice for 5 minutes. After each incubation period, the reaction is stopped by adding 300 µL of a mixture of phenol and guanidine thiocyanate (Tri reagent, Zymo Research) which immediately denatures all proteins and efficiently inhibits ribonucleases and facilitates the subsequent recovery of RNA. After adding Tri Reagent, the samples are vortexed for 15 seconds and stored at -20 °C. RNA is extracted from the samples using Direct-zol RNA miniprep kit (Zymo Research) and eluted in 100 µL of nuclease-free water. Detection of the modified guide is performed using Taqman RT – qPCR using the Taqman miRNA Assay technology (Thermo Fisher). Data is plotted as a function of percentage of sgRNA remaining in relation to the input sample.

Example 13 – Efficiency of mRNA electroporation in T cells

[00527] Primary T cells were purified from PBMCs using a negative selection kit (Miltenyi) according to the manufacturer's recommendations. Nucleofection of mRNA was performed as follow: 200,000 cells were co-transfected with 500 ng of mRNA and the indicated amount of guide RNA using a Lonza 4D electroporator (DS-120). Cells were harvested and genomic DNA prepared three days post initial transfection. For conditions labeled "+gRNA": 15h post initial transfection, cells were nucleofected with indicated amount of additional guide. PCR primers appropriate for use in NGS-based DNA sequencing were generated, optimized, and used to amplify the individual target sequences for each guide RNA. The amplicons were sequenced on an Illumina MiSeq machine and analyzed with a proprietary Python script to measure gene editing (**FIG. 6**).

Example 14 – ELISA assay to assess pre-existing antibody response

[00528] MG3-6 and MG3-8 were expressed in and purified from human HEK293 cells using the Expi293™ Expression System Kit (ThermoFisher Scientific). Briefly, 293 cells were lipofected with plasmids encoding the nucleases driven by a strong viral promoter. Cells were grown in suspension culture with agitation and harvested two days post-transfection. The nuclease proteins were fused to a Six-His affinity tag and purified by metal-affinity chromatography to between 50-60% purity. Parallel lysates were made from mock-transfected cells and were subjected to an identical metal-affinity chromatography process. Cas9 was purchased from IDT and was >95% pure.

[00529] MaxiSorp® ELISA plates (Thermo Scientific) were coated with 0.5 µg of nucleases or control proteins diluted in 1X phosphate buffered saline (PBS) and incubated overnight at room temperature. Plates were then washed and incubated with a 1% (w/v) bovine serum albumin (BSA) (Sigma-Aldrich)/1X PBS solution (1% BSA-PBS) for an hour at room temperature. After another washing step, wells were incubated for 1 h at room temperature with more than 50 separate serum samples taken from randomly selected donors (1:50 dilution in 1% BSA-PBS). Plates were then washed and incubated for an hour at room temperature with a peroxidase-labeled goat anti-human (Fcγ fragment-specific) secondary antibody (Jackson Immuno Research), diluted 1:50,000 in 1% BSA-PBS. The assay was developed using a 3,3',5,5'-Tetramethylbenzidine (TMB) Liquid Substrate System kit (Sigma-Aldrich), according to the manufacturer's specifications. Antibody titers are reported as absorbance values measured at 450 nm (**FIG. 7**). Tetanus toxoid was used as the positive control due to wide-spread vaccination against this antigen and was purchased from Sigma Aldrich.

Example 15 – Gene editing outcomes at the DNA and cell-surface protein level for TRAC in human peripheral blood B cells

[00530] Human Peripheral Blood B cells were purchased from STEMCELL Technologies and expanded using ImmunoCult™ Human B Cell Expansion Kit for 2 days prior to nucleofection. Nucleofection of MG3-6 RNPs (106 pmol protein/160 pmol guide) was performed into B cells (200,000) using the Lonza 4D electroporator. Post-nucleofection cells were immediately recovered into media containing AAV-6 sourced from Virovek. Cells were harvested and genomic DNA prepared five days post-transfection. For NGS analysis, PCR primers appropriate for use in NGS-based DNA sequencing were used to amplify the target sequence for the TRAC 6 guide RNA (SEQ ID NO: 6804). The amplicon was sequenced on an Illumina MiSeq machine and analyzed with a proprietary Python script to measure gene editing. For analysis by flow cytometry, 100,000 cells were stained for viability, expression of B cell surface marker CD19 (CD19 Monoclonal Antibody (HIB19), APC, eBioscience™) and for transgene (SEQ ID NO: 6810) insertion as measured by expression of tLNGFR (CD271 (LNGFR) Antibody, anti-human, REAfinity™). Cells were stained for 30 min at 4°C and data was acquired on an Attune Nxt flow cytometer. Cells expressing tLNGFR were gated on single, live, CD19+ cells (**FIG. 8**).

Table 6: Guide sequences used in Example 15

SEQ ID NO:	Entity Name	Sequence
6804	MG3-6-TRAC-sgRNA-6	mC*mG*mA*rArUrCrCrUrCrCrUrCrCrUrGrArArArGrUrGrGrGrUrUrGrArGrArArUrCrGrArArGrArUrCrUrUrArArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrArUrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU

Example 16 – Gene editing outcomes at the DNA level for TRAC and AAVS1 in hematopoietic stem cells (HSCs)

[00531] Mobilized peripheral blood CD34+ cells were acquired from AllCells and cultured in STEMCELL StemSpan™ SFEM II media supplemented with StemSpan™ CC110 cytokine cocktail for 48 hours prior to nucleofection. Nucleofection of MG3-6 RNPs (106 pmol protein/120 pmol guide for standard dose, 52 pmol protein/60 pmol guide for half dose) was performed into HSCs (200,000) using the Lonza 4D electroporator. Cells were harvested and genomic DNA prepared three days post-transfection. PCR primers appropriate for use in Sanger and NGS-based DNA sequencing were generated, optimized, and used to amplify the individual target sequences for each guide RNA (SEQ ID NOs: 6804, 6806, and 6808). The NGS

amplicons were sequenced on an Illumina MiSeq machine and analyzed with a proprietary Python script to measure gene editing. The ICE amplicons were sent to Elim Biopharmaceuticals Inc. for Sanger sequencing and analyzed with a proprietary Python script to measure gene editing (**FIG. 9**).

Table 7: Guide sequences used in Example 16

SEQ ID NO:	Entity Name	Sequence
6804	MG3-6-TRAC-sgRNA-6	mC*mG*mA*rArUrCrCrUrCrCrUrCrCrUrGrArArArGrUrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6806	MG3-6-AAVS1-sgRNA-B2	mA*mG*mG*rArArUrCrUrGrCrCrUrArArCrArGrGrArGrGrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6808	MG3-6-AAVS1-sgRNA-D2	mU*mA*mG*rGrArArGrGrArGrGrArGrGrCrCrUrArArGrGrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU

Example 17 – Gene editing outcomes at the DNA and cell-surface protein level for TRAC in induced pluripotent stem cells (iPSCs) for MG3-6 delivered as ribonucleoprotein

[00532] ATCC-BXS0116 Human [Non-Hispanic Caucasian Female] Induced Pluripotent Stem (IPS) Cells are cultured on Corning Matrigel-coated plasticware in mTESR Plus media (STEMCELL Technologies) containing 10 μ M ROCK inhibitor Y-27632 for 24 hr prior to nucleofection. Nucleofection of MG3-6 RNPs (106 pmol protein/120 pmol guide) was performed into iPSCs (200,000) using the Lonza 4D electroporator. Cells were harvested with Accutase for flow cytometry and genomic DNA extraction five days post-transfection. PCR primers appropriate for use in NGS-based DNA sequencing were used to amplify the individual target sequences for the TRAC 6 gRNA (SEQ ID NO: 6804). The amplicons were sequenced on an Illumina MiSeq machine and analyzed with a proprietary Python script to measure gene editing. For analysis by flow cytometry, 5 days post-nucleofection 100,000 iPSCs per sample were stained with LIVE/DEAD™ Fixable Near-IR Dead Cell Stain Kit and CD271 (LNGFR) Antibody, anti-human, REAfinity™ to measure viability and transgene (SEQ ID NO: 6810) insertion, respectively. Cells were fixed and permeabilized (Inside Stain Kit, Miltenyi) and further stained for pluripotency transcription factors Oct4 and Sox2 (Anti-Oct3/4 Isoform A-APC, human and mouse REA338 1; Anti-Sox2-FITC, human and mouse REA320). Cells were

acquired on an Attune NxT flow cytometer, and analyzed for tLNGFR expression based on gating on single, live, Oct4+Sox2+ cells (**FIG. 10**).

Table 8: Guide sequences used in Example 17

SEQ ID NO:	Entity Name	Sequence
6804	MG3-6-TRAC-sgRNA-6	mC*mG*mA*rArUrCrCrUrCrCrUrCrCrUrGrArArArGrUrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU

Example 18 – Gene editing outcomes at the DNA protein level for TRAC in induced pluripotent stem cells (iPSCs) for MG3-6 delivered as mRNA

[00533] ATCC-BXS0116 Human [Non-Hispanic Caucasian Female] Induced Pluripotent Stem (IPS) Cells are cultured on Corning Matrigel-coated plasticware in mTESR Plus (STEMCELL Technologies) containing 10 μ M ROCK inhibitor Y-27632 for 24 hr prior to nucleofection. Nucleofection of MG3-6 RNPs (106 pmol protein/120 pmol guide) or mRNA (250 or 500 ng mRNA/12 pmol guide) was performed into iPSCs (200,000) using the Lonza 4D electroporator. Cells were harvested with Accutase for genomic DNA extraction five days post-transfection. PCR primers appropriate for use in NGS-based DNA sequencing were used to amplify the individual target sequences for the TRAC 6 gRNA (SEQ ID NO: 6804). The amplicons were sequenced on an Illumina MiSeq machine and analyzed with a proprietary Python script to measure gene editing (**FIG. 11**).

Table 9: Guide sequences used in Example 18

SEQ ID NO:	Entity Name	Sequence
6804	MG3-6-TRAC-sgRNA-6	mC*mG*mA*rArUrCrCrUrCrCrUrCrCrUrGrArArArGrUrUrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU

Example 19 – Gene editing outcomes at the DNA level for CD2

[00534] Primary T cells were purified from PBMCs using a negative selection kit (Miltenyi) according to the manufacturer's recommendations. Nucleofection of MG3-6 RNPs (106 pmol protein/160 pmol guide) was performed into T cells (200,000) using the Lonza 4D electroporator. Cells were harvested and genomic DNA prepared five days post-transfection. PCR primers appropriate for use in NGS-based DNA sequencing were generated, optimized,

and used to amplify the individual target sequences for each guide RNA. The amplicons were sequenced on an Illumina MiSeq machine and analyzed with a proprietary Python script to measure gene editing (FIG. 12).

Table 10A: Guide sequences used in Example 19

SEQ ID NO:	Entity Name	Sequence
6811	MG3-6-CD2-sgRNA-A1	mA*mU*mU*rUrArCrArUrGrGrArArGrCrUrCrArUrCrUrUrGrUrUrGrArGrArArUrCrGrArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6812	MG3-6-CD2-sgRNA-B1	mU*mU*mU*rUrUrArUrArGrGrUrGrCrArGrUrCrUrCrCrArArGrUrUrGrArGrArArUrCrGrArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6813	MG3-6-CD2-sgRNA-C1	mU*mG*mC*rCrUrUrGrGrArArArCrCrUrGrGrGrGrUrGrCrCrGrUrUrGrArGrArArUrCrGrArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6814	MG3-6-CD2-sgRNA-D1	mG*mG*mG*rArArArArArArCrUrUrCrArGrArCrArArGrArArGrUrUrGrArGrArArUrCrGrArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6815	MG3-6-CD2-sgRNA-E1	mU*mU*mG*rCrArCrArArUrUrCrArGrArArArGrArGrArArGrUrUrGrArGrArArUrCrGrArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6816	MG3-6-CD2-sgRNA-F1	mG*mA*mA*rCrUrCrUrGrArArArArUrUrArArGrCrArUrCrUrGrUrUrGrArGrArArUrCrGrArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6817	MG3-6-CD2-sgRNA-G1	mU*mG*mU*rUrGrGrArArArArArUrArUrUrGrArUrUrUrGrUrUrGrArGrArArUrCrGrArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6818	MG3-6-CD2-sgRNA-H1	mU*mG*mA*rUrGrUrCrCrUrGrArCrCrCrArArGrGrCrArCrCrGrUrUrGrArGrArArUrCrGrArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6819	MG3-6-CD2-sgRNA-A2	mC*mC*mA*rArGrGrCrArUrUrCrGrUrArArUrCrUrCrUrUrUrGrUrUrGrArGrArArUrCrGrArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6820	MG3-6-CD2-sgRNA-B2	mU*mU*mU*rUrArGrArGrArGrGrUrCrUrCrArArArArCrCrGrUrUrGrArGrArArUrCrGrArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU

SEQ ID NO:	Entity Name	Sequence
6821	MG3-6-CD2-sgRNA-C2	mG*mG*mG*rUrCrUrCrArArArArCrCrArArArGrArUrCrUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6822	MG3-6-CD2-sgRNA-D2	mG*mG*mA*rArArCrArUrCrUrArArArArCrUrUrUrCrUrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6823	MG3-6-CD2-sgRNA-E2	mC*mU*mC*rArGrArGrGrGrUrCrArUrCrArCrArCrArCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6824	MG3-6-CD2-sgRNA-F2	mC*mC*mG*rCrCrArCrGrCrArCrCrUrGrGrArCrArGrCrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6825	MG3-6-CD2-sgRNA-G2	mU*mG*mG*rArCrArGrCrUrGrArCrArGrGrCrUrCrGrArCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6826	MG3-6-CD2-sgRNA-H2	mG*mC*mU*rGrUrGrCrArCrUrUrGrArArUrUrUrGrCrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6827	MG3-6-CD2-sgRNA-A3	mU*mU*mU*rArGrArUrGrUrUrUrCrCrCrArUrCrUrUrGrArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6828	MG3-6-CD2-sgRNA-B3	mC*mC*mA*rUrCrUrUrGrArUrArCrArGrGrUrUrUrArArUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6829	MG3-6-CD2-sgRNA-C3	mG*mG*mU*rCrArGrUrUrCrCrArUrUrCrArUrUrArCrCrUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6830	MG3-6-CD2-sgRNA-D3	mU*mU*mC*rCrArUrUrCrArUrUrArCrCrUrCrArCrArGrGrUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6831	MG3-6-CD2-sgRNA-E3	mG*mG*mG*rUrUrGrUrGrUrUrGrArUrArCrArArGrUrCrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6832	MG3-6-CD2-sgRNA-F3	mA*mA*mG*rUrCrCrArGrGrArGrArUrCrUrUrUrGrGrUrUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCr

SEQ ID NO:	Entity Name	Sequence
		CrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6833	MG3-6-CD2-sgRNA-G3	mG*mG*mC*rArGrCrArUrCrCrUrUrGrGrCrCrArGrArGrUrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6834	MG3-6-CD2-sgRNA-H3	mG*mC*mC*rArGrArGrUrArArUrGrGrGrCrUrCrUrCrUrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6835	MG3-6-CD2-sgRNA-A4	mC*mA*mC*rUrUrCrUrCrUrUrCrCrUrUrUrGrCrArGrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6836	MG3-6-CD2-sgRNA-B4	mU*mA*mU*rArGrArArArArCrGrArGrCrArGrUrGrCrCrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6837	MG3-6-CD2-sgRNA-C4	mA*mG*mC*rArGrUrGrCrCrArCrArArArGrArCrCrArUrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6838	MG3-6-CD2-sgRNA-D4	mA*mU*mG*rCrCrArArUrGrArUrGrArGrArUrArGrArUrGrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6839	MG3-6-CD2-sgRNA-E4	mG*mA*mA*rGrArGrArArGrUrGrGrGrArUrGrGrCrUrGrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6840	MG3-6-CD2-sgRNA-F4	mC*mC*mA*rCrArGrArGrUrArGrCrUrArCrUrGrArArGrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6841	MG3-6-CD2-sgRNA-G4	mC*mG*mU*rGrUrUrCrArGrCrArCrCrArGrCrCrUrCrArGrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6842	MG3-6-CD2-sgRNA-H4	mG*mG*mG*rCrArCrArCrArArGrUrUrCrArCrCrArGrCrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6843	MG3-6-CD2-sgRNA-A5	mC*mA*mG*rCrArGrArArArGrGrCrCrCrGrCrCrCrUrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6844	MG3-6-CD2-sgRNA-B5	mU*mG*mA*rGrUrUrUrUrCrUrGrCrUrGrCrCrCrCrArUrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGr

SEQ ID NO:	Entity Name	Sequence
		GrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6845	MG3-6-CD2-sgRNA-C5	mA*mU*mG*rGrGrGrArGrGrUrUrUrUrGrGrCrUrGrArArCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6846	MG3-6-CD2-sgRNA-D5	mU*mG*mA*rArCrUrCrGrArGrGrUrCrUrGrGrGrGrArGrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6847	MG3-6-CD2-sgRNA-E5	mA*mA*mC*rUrUrGrUrGrUrGrCrCrCrGrArCrGrGrArGrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6848	MG3-6-CD2-sgRNA-F5	mC*mG*mA*rCrGrGrArGrCrArGrGrArGrGrCrCrUrCrUrUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6849	MG3-6-CD2-sgRNA-G5	mG*mG*mA*rGrGrArGrGrArUrGrUrUrGrGrGrArArGrUrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6850	MG3-6-CD2-sgRNA-H5	mG*mU*mU*rGrGrGrArArGrUrUrGrCrUrGrGrArUrUrCrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6851	MG3-6-CD2-sgRNA-A6	mA*mG*mG*rGrGrUrUrGrArArGrCrUrGrGrArArUrUrUrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6852	MG3-6-CD2-sgRNA-B6	mC*mC*mC*rUrUrUrCrUrUrCrArGrUrArGrCrUrArCrUrCrUrGrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
Notations for chemical modifications: m = 2'-O-methyl ribonucleotide (e.g mC = cytosine ribonucleotide with 2'-O Methyl in place of 2' hydroxyl); f = 2'-fluoro ribonucleotide (e.g fC = cytosine ribonucleotide with 2' fluorine in place of 2' hydroxyl); * = phosphorothioate bond; r: native RNA linkage comprising the sugar ribose (for example the ribose or RNA form of the A base is written rA), d: deoxyribose sugar (DNA) linkage (for example a deoxyribose form of the A base is written dA)		

Table 10B: Sites Targeted in Example 19

SEQ ID NO:	Entity Name	Sequence
6853	MG3-6-CD2-target site-A1	ATTTACATGGAAAGCTCATCTT
6854	MG3-6-CD2-target site-B1	TTTTTATAGGTGCAGTCTCCAA
6855	MG3-6-CD2-target site-C1	TGCCTTGGAAACCTGGGGTGCC

SEQ ID NO:	Entity Name	Sequence
6856	MG3-6-CD2-target site-D1	GGGAAAAAACTTCAGACAAGAA
6857	MG3-6-CD2-target site-E1	TTGCACAATTCAGAAAAGAGAA
6858	MG3-6-CD2-target site-F1	GAACTCTGAAAATTAAGCATCT
6859	MG3-6-CD2-target site-G1	TGTTGGAAAAAATATTTGATTT
6860	MG3-6-CD2-target site-H1	TGATGTCCTGACCCAAGGCACC
6861	MG3-6-CD2-target site-A2	CCAAGGCATTCGTAATCTCTTT
6862	MG3-6-CD2-target site-B2	TTTAGAGAGGGTCTCAAAACC
6863	MG3-6-CD2-target site-C2	GGGTCTCAAAACCAAAGATCTC
6864	MG3-6-CD2-target site-D2	GGAAACATCTAAAACTTTCTCA
6865	MG3-6-CD2-target site-E2	CTCAGAGGGTCATCACACACAA
6866	MG3-6-CD2-target site-F2	CCGCCACGCACCTGGACAGCTG
6867	MG3-6-CD2-target site-G2	TGGACAGCTGACAGGCTCGACA
6868	MG3-6-CD2-target site-H2	GCTGTGCACTTGAATTTTGCAC
6869	MG3-6-CD2-target site-A3	TTTAGATGTTTCCCATCTTGAT
6870	MG3-6-CD2-target site-B3	CCATCTTGATACAGGTTTAATT
6871	MG3-6-CD2-target site-C3	GGTCAGTTCCATTCATTACCTC
6872	MG3-6-CD2-target site-D3	TTCCATTCATTACCTCACAGGT
6873	MG3-6-CD2-target site-E3	GGGTTGTGTTGATACAAGTCCA
6874	MG3-6-CD2-target site-F3	AAGTCCAGGAGATCTTTGGTTT
6875	MG3-6-CD2-target site-G3	GGCAGCATCCTTGGCCAGAGTA
6876	MG3-6-CD2-target site-H3	GCCAGAGTAATGGGCTCTCTGC
6877	MG3-6-CD2-target site-A4	CACTTCTCTTCCTTTTGCAGAG
6878	MG3-6-CD2-target site-B4	TATAGAAAACGAGCAGTGCCAC
6879	MG3-6-CD2-target site-C4	AGCAGTGCCACAAAGACCATCA
6880	MG3-6-CD2-target site-D4	ATGCCAATGATGAGATAGATGT
6881	MG3-6-CD2-target site-E4	GAAGAGAAGTGGGATGGCTGGG
6882	MG3-6-CD2-target site-F4	CCACAGAGTAGCTACTGAAGAA
6883	MG3-6-CD2-target site-G4	CGTGTTTCAGCACCAGCCTCAGA
6884	MG3-6-CD2-target site-H4	GGGCACACAAGTTCACCAGCAG
6885	MG3-6-CD2-target site-A5	CAGCAGAAAGGCCCGCCCCTCC
6886	MG3-6-CD2-target site-B5	TGAGTTTTCTGCTGCCCCATGG
6887	MG3-6-CD2-target site-C5	ATGGGGAGGTTTTTGGCTGAACT
6888	MG3-6-CD2-target site-D5	TGAACTCGAGGTCTGGGGAGGG
6889	MG3-6-CD2-target site-E5	AACTTGTGTGCCCCGACGGAGCA
6890	MG3-6-CD2-target site-F5	CGACGGAGCAGGAGGCCTCTTC
6891	MG3-6-CD2-target site-G5	GGAGGAGGATGTTGGGAAGTTG
6892	MG3-6-CD2-target site-H5	GTTGGGAAGTTGCTGGATTCTG
6893	MG3-6-CD2-target site-A6	AGGGGTTGAAGCTGGAATTTGG
6894	MG3-6-CD2-target site-B6	CCCTTTCTTCAGTAGCTACTCT

Example 20 – Gene editing outcomes at the DNA level for CD5

[00535] Primary T cells were purified from PBMCs using a negative selection kit (Miltenyi) according to the manufacturer's recommendations. Nucleofection of MG3-6 RNPs (106 pmol

protein/160 pmol guide) was performed into T cells (200,000) using the Lonza 4D electroporator. Cells were harvested and genomic DNA prepared five days post-transfection. PCR primers appropriate for use in NGS-based DNA sequencing were generated, optimized, and used to amplify the individual target sequences for each guide RNA. The amplicons were sequenced on an Illumina MiSeq machine and analyzed with a proprietary Python script to measure gene editing (FIG. 13).

Table 11A: Guide sequences used in Example 20

SEQ ID NO:	Entity Name	Sequence
6895	MG3-6-CD5-sgRNA-A1	mA*mG*mA*rArGrGrCrCrArGrArArArCrCrArUrGrCrCrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6896	MG3-6-CD5-sgRNA-B1	mG*mU*mA*rCrArArGrGrUrGrGrCrCrArGrCrGrGrUrUrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6897	MG3-6-CD5-sgRNA-C1	mU*mU*mC*rUrGrArCrCrCrCrCrArGrArUrUrUrCrCrArGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6898	MG3-6-CD5-sgRNA-D1	mC*mA*mC*rCrCrGrUrUrCrCrArArCrUrCrGrArArGrUrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6899	MG3-6-CD5-sgRNA-E1	mA*mC*mU*rCrGrArArGrUrGrCrCrArGrGrGrCrCrArGrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6900	MG3-6-CD5-sgRNA-F1	mG*mC*mA*rCrArUrGrGrUrUrUrGrCrArGrCrCrArGrArGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6901	MG3-6-CD5-sgRNA-G1	mG*mG*mG*rCrCrGrGrArGrCrUrCrCrArArGrCrArGrUrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6902	MG3-6-CD5-sgRNA-H1	mC*mU*mC*rArArUrCrArUrCrUrGrCrUrArCrGrGrArCrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6903	MG3-6-CD5-sgRNA-A2	mC*mA*mG*rArArArUrGrArCrArUrGrUrGrUrCrArCrUrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU

SEQ ID NO:	Entity Name	Sequence
6904	MG3-6-CD5-sgRNA-B2	mG*mG*mC*rUrGrGrCrUrArGrUrUrArCrCrCrArCrCrUrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6905	MG3-6-CD5-sgRNA-C2	mG*mC*mU*rArGrUrUrArCrCrCrArCrCrUrArArGrCrArGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6906	MG3-6-CD5-sgRNA-D2	mU*mG*mA*rGrGrUrGrUrGrUrArGrGrUrGrArCrArArGrGrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6907	MG3-6-CD5-sgRNA-E2	mG*mU*mG*rUrArGrGrUrGrArCrArArGrGrArArGrGrGrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6908	MG3-6-CD5-sgRNA-F2	mG*mC*mA*rCrCrCrCrArCrArGrUrUrCrArGrCrCrGrCrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6909	MG3-6-CD5-sgRNA-G2	mU*mG*mG*rCrArGrArCrUrUrUrUrGrArCrGrCrUrUrGrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6910	MG3-6-CD5-sgRNA-H2	mC*mC*mA*rUrGrUrGrCrCrArUrCrCrGrUrCrCrUrUrGrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6911	MG3-6-CD5-sgRNA-A3	mG*mG*mU*rGrArGrCrCrUrUrGrCrCrUrGrGrArArArUrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6912	MG3-6-CD5-sgRNA-B3	mC*mA*mG*rArArGrArCrArArCrArCrUrCrCrArArCrGrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6913	MG3-6-CD5-sgRNA-C3	mC*mA*mA*rCrUrCrCrArGrArGrCrCrCrArCrArGrGrUrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6914	MG3-6-CD5-sgRNA-D3	mG*mG*mG*rCrUrCrUrGrGrArGrUrUrGrUrGrGrUrGrGrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6915	MG3-6-CD5-sgRNA-E3	mU*mG*mU*rCrGrUrUrGrGrArGrGrUrGrUrUrGrUrCrUrUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCr

SEQ ID NO:	Entity Name	Sequence
		CrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6916	MG3-6-CD5-sgRNA-F3	mC*mU*mC*rUrCrUrCrCrUrCrUrCrCrUrArGrCrUrCrCrUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6917	MG3-6-CD5-sgRNA-G3	mG*mC*mC*rUrGrGrGrGrGrGrUrArCrCrArUrCrArGrCrUrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6918	MG3-6-CD5-sgRNA-H3	mC*mC*mA*rUrCrArGrCrUrArUrGrArGrGrCrCrCrArGrGrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6919	MG3-6-CD5-sgRNA-A4	mC*mU*mA*rUrGrArGrGrCrCrCrArGrGrArCrArArGrArCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6920	MG3-6-CD5-sgRNA-B4	mG*mC*mU*rCrCrUrUrCrUrUrGrArArGrCrArUrCrUrGrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6921	MG3-6-CD5-sgRNA-C4	mA*mG*mA*rGrArCrUrGrArGrGrCrArGrGrCrArGrArGrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6922	MG3-6-CD5-sgRNA-D4	mA*mC*mC*rArGrCrCrCrUrUrGrCrCrArArUrCrCrArArUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6923	MG3-6-CD5-sgRNA-E4	mU*mG*mC*rCrCrUrCrCrUrUrUrGrCrUrCrArGrGrUrArArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6924	MG3-6-CD5-sgRNA-F4	mG*mG*mC*rArArGrArArCrUrCrGrGrCrCrArCrUrUrUrUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6925	MG3-6-CD5-sgRNA-G4	mC*mC*mA*rGrGrGrArGrGrUrArCrArGrCrUrUrGrArGrUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6926	MG3-6-CD5-sgRNA-H4	mA*mG*mC*rUrUrGrArGrUrUrCrUrGrGrArUrCrUrUrCrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6927	MG3-6-CD5-sgRNA-A5	mC*mU*mG*rGrArUrCrUrUrCrCrArUrUrGrGrArUrUrGrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGr

SEQ ID NO:	Entity Name	Sequence
		GrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6928	MG3-6-CD5-sgRNA-B5	mG*mG*mC*rUrGrGrUrGrUrUrCrCrCrGrUrGrGrCrUrCrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6929	MG3-6-CD5-sgRNA-C5	mG*mU*mU*rCrCrCrGrUrGrGrCrUrCrCrCrCrUrGrGrGrUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6930	MG3-6-CD5-sgRNA-D5	mU*mG*mC*rUrUrCrArArGrArArGrGrArGrCrCrArCrArCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6931	MG3-6-CD5-sgRNA-E5	mA*mG*mG*rUrUrGrUrUrGrCrArGrArGrGrArArGrUrUrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6932	MG3-6-CD5-sgRNA-F5	mU*mG*mC*rArGrArGrGrArArGrUrUrCrUrCrCrArGrGrUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6933	MG3-6-CD5-sgRNA-G5	mU*mC*mU*rCrCrArGrGrUrCrCrUrGrGrGrUrCrUrUrGrUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6934	MG3-6-CD5-sgRNA-H5	mG*mC*mC*rUrCrArUrArGrCrUrGrArUrGrGrUrArCrCrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6935	MG3-6-CD5-sgRNA-A6	mC*mA*mC*rGrCrCrGrGrCrArCrArGrUrGrCrUrGrGrCrCrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6936	MG3-6-CD5-sgRNA-B6	mA*mA*mG*rGrCrArCrCrGrUrGrGrArGrGrUrGrCrGrCrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6937	MG3-6-CD5-sgRNA-C6	mG*mA*mC*rGrCrUrGrGrUrGrArCrCrCrArArCrArUrCrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6938	MG3-6-CD5-sgRNA-D6	mG*mG*mA*rCrArGrArArGrArGrCrCrCrCrGrGrGrArUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU

SEQ ID NO:	Entity Name	Sequence
6939	MG3-6-CD5-sgRNA-E6	mU*mC*mC*rUrGrGrCrUrGrArArGrArGrCrUrGrUrCrArCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6940	MG3-6-CD5-sgRNA-F6	mC*mC*mC*rCrArCrCrArGrArCrGrGrCrUrCrUrGrCrArCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6941	MG3-6-CD5-sgRNA-G6	mC*mC*mC*rArGrGrCrCrArGrGrArUrCrCrArArArCrCrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6942	MG3-6-CD5-sgRNA-H6	mU*mU*mC*rArCrUrArGrCrUrUrCrUrUrGrUrArGrGrCrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6943	MG3-6-CD5-sgRNA-A7	mC*mC*mA*rGrCrArGrCrArCrCrArCrCrArGrGrArGrCrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6944	MG3-6-CD5-sgRNA-B7	mA*mU*mG*rCrUrUrGrCrCrArCrCrGrUrGrCrCrUrGrCrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6945	MG3-6-CD5-sgRNA-C7	mA*mC*mC*rGrUrGrCrCrUrGrCrGrGrCrCrArGrGrCrCrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6946	MG3-6-CD5-sgRNA-D7	mC*mC*mU*rGrCrGrGrCrCrArGrGrCrCrUrGrCrGrGrGrGrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6947	MG3-6-CD5-sgRNA-E7	mU*mC*mC*rGrCrCrArGrArArGrArArGrCrArGrCrGrCrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6948	MG3-6-CD5-sgRNA-F7	mU*mU*mA*rCrUrGrUrUrUrGrGrUrUrCrArUrUrCrCrCrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6949	MG3-6-CD5-sgRNA-G7	mU*mC*mC*rArCrUrGrGrCrGrCrUrGrCrUrUrCrUrUrCrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6950	MG3-6-CD5-sgRNA-H7	mA*mG*mC*rUrGrArCrArGrGrUrGrGrGrArGrUrUrCrCrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCr

SEQ ID NO:	Entity Name	Sequence
		CrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6951	MG3-6-CD5-sgRNA-A8	mG*mG*mC*rUrGrUrArUrUrCrGrUrUrArUrCrCrArCrGrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6952	MG3-6-CD5-sgRNA-B8	mU*mC*mG*rUrUrArUrCrCrArCrGrUrGrGrGrArGrGrCrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6953	MG3-6-CD5-sgRNA-C8	mG*mG*mA*rGrGrCrUrGrGrGrGrUrUrCrUrCrArGrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6954	MG3-6-CD5-sgRNA-D8	mC*mC*mU*rUrUrCrUrUrUrCrCrCrCrArGrCrUrCrUrGrGrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6955	MG3-6-CD5-sgRNA-E8	mC*mC*mG*rArCrArGrUrGrArCrUrArUrGrArUrCrUrGrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6956	MG3-6-CD5-sgRNA-F8	mG*mA*mC*rUrArUrGrArUrCrUrGrCrArUrGrGrGrGrCrUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6957	MG3-6-CD5-sgRNA-G8	mC*mU*mU*rUrArCrArGrCrCrUrCrUrGrArGrCrCrCrCrArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
6958	MG3-6-CD5-sgRNA-H8	mA*mU*mA*rGrUrCrArCrUrGrUrCrGrGrArGrGrArGrUrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
Notations for chemical modifications: m = 2'-O-methyl ribonucleotide (e.g mC = cytosine ribonucleotide with 2'-O Methyl in place of 2' hydroxyl); f = 2'-fluoro ribonucleotide (e.g fC = cytosine ribonucleotide with 2' fluorine in place of 2' hydroxyl); * = phosphorothioate bond; r: native RNA linkage comprising the sugar ribose (for example the ribose or RNA form of the A base is written rA), d: deoxyribose sugar (DNA) linkage (for example a deoxyribose form of the A base is written dA)		

Table 11B: Sites Targeted in Example 20

SEQ ID NO:	Entity Name	Sequence
6959	MG3-6-CD5-target site-A1	AGAAGGCCAGAAACCATGCCCA
6960	MG3-6-CD5-target site-B1	GTACAAGGTGGCCAGCGGTTGC
6961	MG3-6-CD5-target site-C1	TTCTGACCCCCAGATTTCCAGG
6962	MG3-6-CD5-target site-D1	CACCCGTTCCAACCTCGAAGTGC

SEQ ID NO:	Entity Name	Sequence
6963	MG3-6-CD5-target site-E1	ACTCGAAGTGCCAGGGCCAGCT
6964	MG3-6-CD5-target site-F1	GCACATGGTTTGCAGCCAGAGC
6965	MG3-6-CD5-target site-G1	GGGCCGGAGCTCCAAGCAGTGG
6966	MG3-6-CD5-target site-H1	CTCAATCATCTGCTACGGACAA
6967	MG3-6-CD5-target site-A2	CAGAAATGACATGTGTCACTCT
6968	MG3-6-CD5-target site-B2	GGCTGGCTAGTTACCCACCTAA
6969	MG3-6-CD5-target site-C2	GCTAGTTACCCACCTAAGCAGG
6970	MG3-6-CD5-target site-D2	TGAGGTGTGTAGGTGACAAGGA
6971	MG3-6-CD5-target site-E2	GTGTAGGTGACAAGGAAGGGGC
6972	MG3-6-CD5-target site-F2	GCACCCACAGTTCAGCCGCTG
6973	MG3-6-CD5-target site-G2	TGGCAGACTTTTGACGCTTGAC
6974	MG3-6-CD5-target site-H2	CCATGTGCCATCCGTCCTTGAG
6975	MG3-6-CD5-target site-A3	GGTGAGCCTTGCCCTGGAAATCT
6976	MG3-6-CD5-target site-B3	CAGAAGACAACACCTCCAACGA
6977	MG3-6-CD5-target site-C3	CAACTCCAGAGCCACAGGTAA
6978	MG3-6-CD5-target site-D3	GGGCTCTGGAGTTGTGGTGGGC
6979	MG3-6-CD5-target site-E3	TGTCGTTGGAGGTGTTGTCTTC
6980	MG3-6-CD5-target site-F3	CTCTCTCCTCTCCTAGCTCCTC
6981	MG3-6-CD5-target site-G3	GCCTGGGGGGTACCATCAGCTA
6982	MG3-6-CD5-target site-H3	CCATCAGCTATGAGGCCAGGA
6983	MG3-6-CD5-target site-A4	CTATGAGGCCAGGACAAGACC
6984	MG3-6-CD5-target site-B4	GCTCCTTCTTGAAGCATCTGCC
6985	MG3-6-CD5-target site-C4	AGAGACTGAGGCAGGCAGAGCC
6986	MG3-6-CD5-target site-D4	ACCAGCCCTTGCCAATCCAATG
6987	MG3-6-CD5-target site-E4	TGCCCTCCTTTGCTCAGGTAAG
6988	MG3-6-CD5-target site-F4	GGCAAGAAGTCCGGCCACTTTTC
6989	MG3-6-CD5-target site-G4	CCAGGGAGGTACAGCTTGAGTT
6990	MG3-6-CD5-target site-H4	AGCTTGAGTTCTGGATCTTCCA
6991	MG3-6-CD5-target site-A5	CTGGATCTTCCATTGGATTGGC
6992	MG3-6-CD5-target site-B5	GGCTGGTGTTCCTCGTGGCTCCC
6993	MG3-6-CD5-target site-C5	GTTCCCGTGGCTCCCCTGGGTC
6994	MG3-6-CD5-target site-D5	TGCTTCAAGAAGGAGCCACACT
6995	MG3-6-CD5-target site-E5	AGGTTGTTGCAGAGGAAGTTCT
6996	MG3-6-CD5-target site-F5	TGCAGAGGAAGTTCTCCAGGTC
6997	MG3-6-CD5-target site-G5	TCTCCAGGTCCTGGGTCTTGTC
6998	MG3-6-CD5-target site-H5	GCCTCATAGCTGATGGTACCCC
6999	MG3-6-CD5-target site-A6	CACGCCGGCACAGTGCTGGCCG
7000	MG3-6-CD5-target site-B6	AAGGCACCGTGGAGGTGCGCCA
7001	MG3-6-CD5-target site-C6	GACGCTGGTGACCCAACATCCC
7002	MG3-6-CD5-target site-D6	GGACAGAAGAGCCCCCGGGATG
7003	MG3-6-CD5-target site-E6	TCCTGGCTGAAGAGCTGTCACA
7004	MG3-6-CD5-target site-F6	CCCCACCAGACGGCTCTGCACC
7005	MG3-6-CD5-target site-G6	CCCAGGCCAGGATCCAAACCCC
7006	MG3-6-CD5-target site-H6	TTCACTAGCTTCTTGTAGGCAA

SEQ ID NO:	Entity Name	Sequence
7007	MG3-6-CD5-target site-A7	CCAGCAGCACCACCAGGAGCAC
7008	MG3-6-CD5-target site-B7	ATGCTTGCCACCGTGCCTGCGG
7009	MG3-6-CD5-target site-C7	ACCGTGCCTGCGGCCAGGCCTG
7010	MG3-6-CD5-target site-D7	CCTGCGGCCAGGCCTGCGGGGT
7011	MG3-6-CD5-target site-E7	TCCGCCAGAAGAAGCAGCGCCA
7012	MG3-6-CD5-target site-F7	TTACTGTTTTGGTTCATTCCTG
7013	MG3-6-CD5-target site-G7	TCCACTGGCGCTGCTTCTTCTG
7014	MG3-6-CD5-target site-H7	AGCTGACAGGTGGGAGTTCCTG
7015	MG3-6-CD5-target site-A8	GGCTGTATTCTGTTATCCACGTG
7016	MG3-6-CD5-target site-B8	TCGTTATCCACGTGGGAGGCTG
7017	MG3-6-CD5-target site-C8	GGAGGCTGTGGGGTTCTCAGCA
7018	MG3-6-CD5-target site-D8	CCTTTCTTTCCCCAGCTCTGGA
7019	MG3-6-CD5-target site-E8	CCGACAGTGACTATGATCTGCA
7020	MG3-6-CD5-target site-F8	GACTATGATCTGCATGGGGCTC
7021	MG3-6-CD5-target site-G8	CTTTACAGCCTCTGAGCCCCAT
7022	MG3-6-CD5-target site-H8	ATAGTCACTGTCGGAGGAGTTG

Example 21 – Targeted RNA cleavage by MG3-6 and MG3-8

[00536] A 101 nt RNA containing the spacer

(GGUCAGGGCGCGUCAGCGGGUGUUGGCGGGUGUCGGGGCUGGCUUAAAUUUUG GACCAGUCGAGGCUUGCGACGUGGUGGCUUUUCCAGUCGGGAAACCUG) with 5' adjacent sequence UUGGACCA were prepared via transcription of a T7 promoter-containing PCR product using the T7 Megascript kit (NEB) according to manufacturer instructions. The resulting RNA was purified using a Monarch RNA prep spin column (NEB) and then labeled with the 5' EndTag kit (Vector labs) using a FAM-maleimide dye per recommended instructions. The resulting RNA has one 5' label and an expected band size of 60 nt if cleaved at a single position in the spacer. For testing RNA cleavage, 2 pmol of protein and sgRNA were pre-incubated for 15 minutes before adding ssRNA target. The RNP complex was added to the labeled RNA at a 10:1 ratio (200 nM RNA: 2 μ M RNP) in cleavage buffer (10 mM Tris, 100 mM NaCl, and 10 mM MgCl₂) and incubated at 37 °C for 1 hr. Reactions were quenched with proteinase K and resolved on a 15% TBE Urea-PAGE gel (Bio-rad). The gel shows site-directed RNA cleavage by MG3-6 and MG3-8 as well as commercial positive control SauCas9 (NEB) (FIG. 14). The results indicated that MG3-6 and MG3-8 are capable of targeted RNA cleavage and are comparable in terms of RNA cleavage to SauCas9.

Example 22 – Gene editing outcomes at the DNA level for FAS

[00537] Primary T cells were purified from PBMCs using a negative selection kit (Miltenyi) according to the manufacturer's recommendations. Nucleofection of MG3-6 RNPs (104 pmol protein/120 pmol guide) (SEQ ID NOs: 7023-7056) was performed into T cells (200,000) using the Lonza 4D electroporator. Cells were harvested and genomic DNA prepared three days post-transfection. PCR primers appropriate for use in NGS-based DNA sequencing were generated, optimized, and used to amplify the individual target sequences for each guide RNA (SEQ ID NOs: 7057-7090). The amplicons were sequenced on an Illumina MiSeq machine and analyzed with a proprietary Python script to measure gene editing (FIG. 15).

Table 12: Guide RNAs and Sequences Targeted for Example 22

SEQ ID NO:	NAME	SEQUENCE
7023	MG3-6-FAS-sgRNA-A1	mC*mU*mG*rArUrGrArGrUrGrGrUrUrUrCrCrCrUrGrArGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7024	MG3-6-FAS-sgRNA-B1	mU*mU*mA*rGrArUrGrCrUrCrArGrArGrUrGrUrGrUrGrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7025	MG3-6-FAS-sgRNA-C1	mG*mU*mG*rUrGrCrArCrArArGrGrCrUrGrGrCrArCrGrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7026	MG3-6-FAS-sgRNA-D1	mG*mA*mG*rArCrArArGrCrCrUrArUrCrArArCrArCrCrUrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7027	MG3-6-FAS-sgRNA-E1	mA*mC*mC*rArCrCrArGrUrCrUrUrGrUrArGrGrUrGrUrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7028	MG3-6-FAS-sgRNA-F1	mC*mU*mU*rGrUrCrUrCrUrGrUrUrCrCrArCrCrUrUrUrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7029	MG3-6-FAS-sgRNA-G1	mA*mG*mU*rArGrArCrUrGrUrUrArGrUrGrCrCrArUrGrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7030	MG3-6-FAS-sgRNA-H1	mU*mU*mA*rCrArGrGrUrUrCrUrUrArCrGrUrCrUrGrUrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU

SEQ ID NO:	NAME	SEQUENCE
7031	MG3-6-FAS-sgRNA-A2	mC*mA*mA*rGrUrGrArCrUrGrArCrArUrCrArArCrUrCrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7032	MG3-6-FAS-sgRNA-B2	mA*mC*mU*rCrCrArArGrGrGrArUrUrGrGrArArUrUrGrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7033	MG3-6-FAS-sgRNA-C2	mU*mG*mA*rGrGrArArGrArCrUrGrUrUrArCrUrArCrArGrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7034	MG3-6-FAS-sgRNA-D2	mU*mA*mC*rArGrUrUrGrArGrArCrUrCrArGrArArCrUrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7035	MG3-6-FAS-sgRNA-E2	mU*mU*mU*rGrUrGrUrArArCrArUrArCrCrUrGrGrArGrGrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7036	MG3-6-FAS-sgRNA-F2	mU*mG*mG*rCrArGrArArUrUrGrGrCrCrArUrCrArUrGrArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7037	MG3-6-FAS-sgRNA-G2	mU*mU*mG*rGrGrCrArGrGrUrGrArArArGrGrArArArGrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7038	MG3-6-FAS-sgRNA-H2	mC*mU*mG*rCrArCrArGrUrCrArArUrGrGrGrGrArUrGrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7039	MG3-6-FAS-sgRNA-A3	mU*mU*mU*rUrCrUrUrCrCrArArArUrGrCrArGrArArGrArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7040	MG3-6-FAS-sgRNA-B3	mA*mU*mC*rUrUrCrUrGrCrArUrUrUrGrGrArArGrArArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7041	MG3-6-FAS-sgRNA-C3	mU*mA*mG*rArArGrUrGrGrArArArUrArArArCrUrGrCrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7042	MG3-6-FAS-sgRNA-D3	mA*mA*mG*rArCrUrArArArArCrUrUrArCrUrUrGrGrUrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrAr

SEQ ID NO:	NAME	SEQUENCE
		CrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7043	MG3-6-FAS-sgRNA-E3	mG*mU*mU*rUrArCrArUrCrUrGrCrArCrUrUrGrGrUrArUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7044	MG3-6-FAS-sgRNA-F3	mA*mA*mG*rArArGrArCrArArArGrCrCrArCrCrCrArArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7045	MG3-6-FAS-sgRNA-G3	mA*mC*mA*rArArGrCrCrArCrCrCrArArGrUrUrArGrArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7046	MG3-6-FAS-sgRNA-H3	mC*mC*mC*rCrArArGrUrUrArGrArUrCrUrGrGrArUrCrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7047	MG3-6-FAS-sgRNA-A4	mC*mA*mG*rArArArGrCrArCrArGrArArArGrGrArArArArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7048	MG3-6-FAS-sgRNA-B4	mA*mA*mU*rArCrCrUrArCrArGrGrArUrUrUrArArArGrUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7049	MG3-6-FAS-sgRNA-C4	mC*mA*mG*rUrGrGrCrArArUrArArArUrUrUrArUrCrUrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7050	MG3-6-FAS-sgRNA-D4	mA*mG*mU*rCrArUrGrArCrArCrUrArArGrUrCrArArGrUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7051	MG3-6-FAS-sgRNA-E4	mG*mU*mG*rUrCrArArUrGrArArGrCrCrArArArArUrArGrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7052	MG3-6-FAS-sgRNA-F4	mA*mG*mA*rArGrCrGrUrArUrGrArCrArCrArUrUrGrArUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7053	MG3-6-FAS-sgRNA-G4	mU*mU*mU*rGrUrArCrUrCrUrUrGrCrArGrArGrArArArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7054	MG3-6-FAS-sgRNA-H4	mG*mU*mU*rUrUrUrCrArCrUrCrUrArGrArCrCrArArGrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGr

SEQ ID NO:	NAME	SEQUENCE
		rGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7055	MG3-6-FAS-sgRNA-A5	mU*mG*mA*rArUrUrUrUrCrUrCrUrGrCrArArGrArGrUrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7056	MG3-6-FAS-sgRNA-B5	mA*mG*mA*rGrUrArCrArArArGrArUrUrGrGrCrUrUrUrUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7057	MG3-6-FAS-target site-A1	CTGATGAGTGGTTTCCCTGAGC
7058	MG3-6-FAS-target site-B1	TTAGATGCTCAGAGTGTGTGCA
7059	MG3-6-FAS-target site-C1	GTGTGCACAAGGCTGGCACGCC
7060	MG3-6-FAS-target site-D1	GAGACAAGCCTATCAACACCTA
7061	MG3-6-FAS-target site-E1	ACCACCAGTCTTGTAGGTGTTG
7062	MG3-6-FAS-target site-F1	CTTGTCTCTGTTCCACCTTTCA
7063	MG3-6-FAS-target site-G1	AGTAGACTGTTAGTGCCATGAG
7064	MG3-6-FAS-target site-H1	TTACAGGTTCTTACGTCTGTTG
7065	MG3-6-FAS-target site-A2	CAAGTGACTGACATCAACTCCA
7066	MG3-6-FAS-target site-B2	ACTCCAAGGGATTGGAATTGAG
7067	MG3-6-FAS-target site-C2	TGAGGAAGACTGTTACTACAGT
7068	MG3-6-FAS-target site-D2	TACAGTTGAGACTCAGAACTTG
7069	MG3-6-FAS-target site-E2	TTTGTGTAACATACCTGGAGGA
7070	MG3-6-FAS-target site-F2	TGGCAGAATTGGCCATCATGAT
7071	MG3-6-FAS-target site-G2	TTGGGCAGGTGAAAGGAAAGCT
7072	MG3-6-FAS-target site-H2	CTGCACAGTCAATGGGGATGAA
7073	MG3-6-FAS-target site-A3	TTTTCTTCCAAATGCAGAAGAT
7074	MG3-6-FAS-target site-B3	ATCTTCTGCATTTGGAAGAAAA
7075	MG3-6-FAS-target site-C3	TAGAAGTGGAATAAACTGCAC
7076	MG3-6-FAS-target site-D3	AAGACTAAAACCTTACTTGGTGTC
7077	MG3-6-FAS-target site-E3	GTTTACATCTGCACTTGGTATT
7078	MG3-6-FAS-target site-F3	AAGAAGACAAAGCCACCCCAAG

SEQ ID NO:	NAME	SEQUENCE
7079	MG3-6-FAS-target site-G3	ACAAAGCCACCCCAAGTTAGAT
7080	MG3-6-FAS-target site-H3	CCCCAAGTTAGATCTGGATCCT
7081	MG3-6-FAS-target site-A4	CAGAAAGCACAGAAAGGAAAAC
7082	MG3-6-FAS-target site-B4	AATACCTACAGGATTTAAAGTT
7083	MG3-6-FAS-target site-C4	CAGTGGCAATAAATTTATCTGG
7084	MG3-6-FAS-target site-D4	AGTCATGACACTAAGTCAAGTT
7085	MG3-6-FAS-target site-E4	GTGTCAATGAAGCCAAAATAGA
7086	MG3-6-FAS-target site-F4	AGAAGCGTATGACACATTGATT
7087	MG3-6-FAS-target site-G4	TTTGTACTCTTGCAGAGAAAAT
7088	MG3-6-FAS-target site-H4	GTTTTTCACTCTAGACCAAGCT
7089	MG3-6-FAS-target site-A5	TGAATTTTCTCTGCAAGAGTAC
7090	MG3-6-FAS-target site-B5	AGAGTACAAAGATTGGCTTTTT
r =native ribose base, m = 2'-O methyl modified base, F = 2' Fluro modified base, * = phosphorothioate bond		

Example 23 – Gene editing outcomes at the DNA level for PD-1

[00538] Primary T cells were purified from PBMCs using a negative selection kit (Miltenyi) according to the manufacturer's recommendations. Nucleofection of MG3-6 RNPs (104 pmol protein/120 pmol guide) (SEQ ID NOs: 7091-7128) was performed into T cells (200,000) using the Lonza 4D electroporator. Cells were harvested and genomic DNA prepared three days post-transfection. PCR primers appropriate for use in NGS-based DNA sequencing were generated, optimized, and used to amplify the individual target sequences for each guide RNA (SEQ ID NOs: 7129-7166). The amplicons were sequenced on an Illumina MiSeq machine and analyzed with a proprietary Python script to measure gene editing (**FIG. 16**).

Table 13: Guide RNAs and Sequences Targeted for Example 23

SEQ ID NO:	NAME	SEQUENCE
7091	MG3-6-PD-1-sgRNA-A1	mG*mG*mU*rGrGrCrCrArArGrGrArArGrCrCrGrGrUrCrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7092	MG3-6-PD-1-sgRNA-B1	mG*mG*mG*rCrCrArArGrArGrCrArGrUrGrUrCrCrArUrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrA

SEQ ID NO:	NAME	SEQUENCE
		rCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7093	MG3-6-PD-1-sgRNA-C1	mG*mG*mC*rCrCrUrCrGrGrArGrUrGrCrCrCrArGrCrCrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7094	MG3-6-PD-1-sgRNA-D1	mG*mG*mC*rCrUrCrArGrUrGrGrCrUrGrGrGrCrArCrUrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7095	MG3-6-PD-1-sgRNA-E1	mG*mG*mG*rCrArCrCrUrCrArUrCrCrCrCrGrCrCrCrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7096	MG3-6-PD-1-sgRNA-F1	mC*mU*mG*rCrUrCrArGrGrGrArCrArCrArGrGrGrCrArCrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7097	MG3-6-PD-1-sgRNA-G1	mG*mG*mA*rCrArCrArGrGrGrCrArCrGrGrGrGrGrGrCrUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7098	MG3-6-PD-1-sgRNA-H1	mA*mG*mC*rUrGrGrArUrUrUrCrCrArGrUrGrGrCrGrArGrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7099	MG3-6-PD-1-sgRNA-A2	mG*mU*mU*rCrUrCrUrGrUrGrGrArCrUrArUrGrGrGrGrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7100	MG3-6-PD-1-sgRNA-B2	mC*mU*mC*rArGrCrCrGrUrGrCrCrUrGrUrGrUrUrCrUrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7101	MG3-6-PD-1-sgRNA-C2	mA*mC*mA*rGrArGrArArCrArCrArGrGrCrArCrGrGrCrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7102	MG3-6-PD-1-sgRNA-D2	mG*mG*mG*rUrCrCrUrGrGrCrCrGrUrCrArUrCrUrGrCrUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7103	MG3-6-PD-1-sgRNA-E2	mC*mG*mG*rCrCrCrGrGrGrArGrCrArGrArUrGrArCrGrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7104	MG3-6-PD-1-sgRNA-F2	mG*mG*mG*rArGrCrArGrArUrGrArCrGrGrCrCrArGrGrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArAr

SEQ ID NO:	NAME	SEQUENCE
		GrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrAr rCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGr CrGrGrUrArUrGrU*mU*mU*mU
7105	MG3-6-PD-1-sgRNA-G2	mU*mG*mG*rGrCrArGrCrCrUrGrGrUrGrCrUrGrCrUrArGrUrG rUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArAr GrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrA rCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGr CrGrGrUrArUrGrU*mU*mU*mU
7106	MG3-6-PD-1-sgRNA-H2	mG*mC*mC*rArGrGrArCrCrCrArGrArCrUrArGrCrArGrCrArG rUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArAr GrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrA rCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGr CrGrGrUrArUrGrU*mU*mU*mU
7107	MG3-6-PD-1-sgRNA-A3	mA*mC*mU*rArGrCrArGrCrArCrCrArGrGrCrUrGrCrCrCrArGr UrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArG rGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrAr CrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGr CrGrGrUrArUrGrU*mU*mU*mU
7108	MG3-6-PD-1-sgRNA-B3	mG*mG*mC*rCrGrCrCrCrArCrGrArCrArCrCrArArCrCrArCrGr UrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArG rGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrAr CrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGr CrGrGrUrArUrGrU*mU*mU*mU
7109	MG3-6-PD-1-sgRNA-C3	mA*mA*mC*rUrGrGrCrCrGrGrCrUrGrGrCrCrUrGrGrGrUrGrG rUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArAr GrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrA rCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGr CrGrGrUrArUrGrU*mU*mU*mU
7110	MG3-6-PD-1-sgRNA-D3	mA*mC*mA*rGrCrCrCrArCrCrCrCrArGrCrCrCrCrUrCrArCrGr UrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArG rGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrAr CrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGr CrGrGrUrArUrGrU*mU*mU*mU
7111	MG3-6-PD-1-sgRNA-E3	mC*mU*mG*rGrCrCrUrGrGrGrUrGrArGrGrGrGrCrUrGrGrGrG rUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArAr GrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrA rCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGr CrGrGrUrArUrGrU*mU*mU*mU
7112	MG3-6-PD-1-sgRNA-F3	mC*mC*mU*rGrUrCrArCrCrCrUrGrArGrCrUrCrUrGrCrCrCrGr UrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArG rGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrAr CrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGr CrGrGrUrArUrGrU*mU*mU*mU
7113	MG3-6-PD-1-sgRNA-G3	mG*mG*mC*rUrCrUrCrUrUrUrGrArUrCrUrGrCrGrCrUrUrGr UrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArG rGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrAr CrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGr CrGrGrUrArUrGrU*mU*mU*mU
7114	MG3-6-PD-1-sgRNA-H3	mC*mC*mA*rUrCrUrCrCrCrUrGrGrCrCrCrCrArArGrGrCrGr UrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArG rGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrAr CrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGr CrGrGrUrArUrGrU*mU*mU*mU
7115	MG3-6-PD-1-sgRNA-A4	mA*mU*mG*rArCrArGrCrGrGrCrArCrCrUrArCrCrUrCrUrGrGr UrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArG rGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrAr CrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGr CrGrGrUrArUrGrU*mU*mU*mU

SEQ ID NO:	NAME	SEQUENCE
7116	MG3-6-PD-1-sgRNA-B4	mG*mG*mU*rArGrGrUrGrCrCrGrCrUrGrUrCrArUrUrGrCrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7117	MG3-6-PD-1-sgRNA-C4	mG*mU*mG*rArCrUrUrCrCrArCrArUrGrArGrCrGrUrGrGrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7118	MG3-6-PD-1-sgRNA-D4	mG*mA*mC*rArCrGrGrArArGrCrGrGrCrArGrUrCrCrUrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7119	MG3-6-PD-1-sgRNA-E4	mC*mG*mA*rGrGrArCrCrGrCrArGrCrCrArGrCrCrCrGrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7120	MG3-6-PD-1-sgRNA-F4	mG*mG*mA*rCrArArGrCrUrGrGrCrCrGrCrCrUrUrCrCrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7121	MG3-6-PD-1-sgRNA-G4	mG*mC*mC*rArGrCrUrUrGrUrCrCrGrUrCrUrGrGrUrUrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7122	MG3-6-PD-1-sgRNA-H4	mU*mG*mU*rCrCrCrCrUrUrCrGrGrUrCrArCrCrArCrGrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7123	MG3-6-PD-1-sgRNA-A5	mA*mG*mC*rArGrGrGrCrUrGrGrGrGrArGrArArGrGrUrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7124	MG3-6-PD-1-sgRNA-B5	mU*mG*mG*rGrGrArGrArArGrGrUrGrGrGrGrGrGrGrUrUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7125	MG3-6-PD-1-sgRNA-C5	mC*mU*mC*rCrArUrCrUrCrUrCrArGrArCrUrCrCrCrCrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7126	MG3-6-PD-1-sgRNA-D5	mG*mC*mC*rArGrGrArUrGrGrUrUrCrUrUrArGrGrUrArGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7127	MG3-6-PD-1-sgRNA-E5	mA*mG*mU*rCrGrUrCrUrGrGrGrCrGrGrUrGrCrUrArCrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrAr

SEQ ID NO:	NAME	SEQUENCE
		rCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7128	MG3-6-PD-1-sgRNA-F5	mA*mG*mA*rCrGrArCrUrGrGrCrCrArGrGrGrCrGrCrCrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7129	MG3-6-PD-1-target site-A1	GGTGGCCAAGGAAGCCGGTCAG
7130	MG3-6-PD-1-target site-B1	GGGCCAAGAGCAGTGTCCATCC
7131	MG3-6-PD-1-target site-C1	GGCCCTCGGAGTGCCCAGCCAC
7132	MG3-6-PD-1-target site-D1	GGCCTCAGTGGCTGGGCACTCC
7133	MG3-6-PD-1-target site-E1	GGGCACCTCATCCCCGCCCCG
7134	MG3-6-PD-1-target site-F1	GGACACAGGGCACGGGGGGCTC
7135	MG3-6-PD-1-target site-G1	GGACACAGGGCACGGGGGGCTC
7136	MG3-6-PD-1-target site-H1	AGCTGGATTTCCAGTGGCGAGA
7137	MG3-6-PD-1-target site-A2	GTTCTCTGTGGACTATGGGGAG
7138	MG3-6-PD-1-target site-B2	CTCAGCCGTGCCTGTGTCTCT
7139	MG3-6-PD-1-target site-C2	ACAGAGAACACAGGCACGGCTG
7140	MG3-6-PD-1-target site-D2	GGGTCCTGGCCGTCATCTGCTC
7141	MG3-6-PD-1-target site-E2	CGGCCCCGGGAGCAGATGACGGC
7142	MG3-6-PD-1-target site-F2	GGGAGCAGATGACGGCCAGGAC
7143	MG3-6-PD-1-target site-G2	TGGGCAGCCTGGTGCTGCTAGT
7144	MG3-6-PD-1-target site-H2	GCCAGGACCCAGACTAGCAGCA
7145	MG3-6-PD-1-target site-A3	ACTAGCAGCACCAGGCTGCCCA
7146	MG3-6-PD-1-target site-B3	GGCCGCCCACGACACCAACCAC
7147	MG3-6-PD-1-target site-C3	AACTGGCCGGCTGGCCTGGGTG
7148	MG3-6-PD-1-target site-D3	ACAGCCCACCCCAGCCCCTCAC
7149	MG3-6-PD-1-target site-E3	CTGGCCTGGGTGAGGGGCTGGG
7150	MG3-6-PD-1-target site-F3	CCTGTCACCCTGAGCTCTGCCC
7151	MG3-6-PD-1-target site-G3	GGCTCTCTTTGATCTGCGCCTT
7152	MG3-6-PD-1-target site-H3	CCATCTCCCTGGCCCCCAAGGC
7153	MG3-6-PD-1-target site-A4	ATGACAGCGGCACCTACCTCTG

SEQ ID NO:	NAME	SEQUENCE
7154	MG3-6-PD-1-target site-B4	GGTAGGTGCCGCTGTCATTGCG
7155	MG3-6-PD-1-target site-C4	GTGACTTCCACATGAGCGTGGT
7156	MG3-6-PD-1-target site-D4	GACACGGAAGCGGCAGTCCTGG
7157	MG3-6-PD-1-target site-E4	CGAGGACCGCAGCCAGCCCGGC
7158	MG3-6-PD-1-target site-F4	GGACAAGCTGGCCGCCTTCCCC
7159	MG3-6-PD-1-target site-G4	GCCAGCTTGTCCGTCTGGTTGC
7160	MG3-6-PD-1-target site-H4	TGTCCCCTTCGGTCACCACGAG
7161	MG3-6-PD-1-target site-A5	AGCAGGGCTGGGGAGAAGGTGG
7162	MG3-6-PD-1-target site-B5	TGGGGAGAAGGTGGGGGGGTTC
7163	MG3-6-PD-1-target site-C5	CTCCATCTCTCAGACTCCCCAG
7164	MG3-6-PD-1-target site-D5	GCCAGGATGGTTCTTAGGTAGG
7165	MG3-6-PD-1-target site-E5	AGTCGTCTGGGCGGTGCTACAA
7166	MG3-6-PD-1-target site-F5	AGACGACTGGCCAGGGCGCCTG
r = native ribose base, m = 2'-O methyl modified base, F = 2' Fluro modified base, * = phosphorothioate bond		

Example 24 – Gene editing outcomes at the DNA level for hRosa26

[00539] Primary T cells were purified from PBMCs using a negative selection kit (Miltenyi) according to the manufacturer's recommendations. Nucleofection of MG3-6 RNPs (104 pmol protein/120 pmol guide) (SEQ ID NOs: 7167-7198) was performed into T cells (200,000) using the Lonza 4D electroporator. Cells were harvested and genomic DNA prepared three days post-transfection. PCR primers appropriate for use in NGS-based DNA sequencing were generated, optimized, and used to amplify the individual target sequences for each guide RNA (SEQ ID NOs: 7199-7230). The amplicons were sequenced on an Illumina MiSeq machine and analyzed with a proprietary Python script to measure gene editing (**FIG. 17**).

Table 14: Guide RNAs and Sequences Targeted for Example 24

SEQ ID NO:	NAME	SEQUENCE
7167	MG3-6-hRosa26-sgRNA- A1	mA*mU*mC*rUrGrUrCrUrGrGrUrUrUrCrGrCrGrArGrArCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU

SEQ ID NO:	NAME	SEQUENCE
7168	MG3-6-hRosa26-sgRNA- B1	mU*mU*mU*rCrGrCrGrArGrArCrArCrArGrGrCrUrArCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7169	MG3-6-hRosa26-sgRNA- C1	mA*mG*mC*rArArGrUrArCrArArCrArArArUrGrGrArArArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7170	MG3-6-hRosa26-sgRNA- D1	mG*mC*mA*rArArArGrCrUrArArArCrArArArUrUrUrUrCrUrArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7171	MG3-6-hRosa26-sgRNA- E1	mU*mG*mC*rUrArCrArCrUrUrUrGrGrUrGrGrUrGrCrArGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7172	MG3-6-hRosa26-sgRNA- F1	mA*mC*mU*rCrCrCrCrUrGrCrArGrGrGrCrArArCrGrCrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7173	MG3-6-hRosa26-sgRNA- G1	mC*mG*mA*rCrUrCrGrArCrArUrGrGrArGrGrCrGrArUrGrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7174	MG3-6-hRosa26-sgRNA- H1	mA*mU*mC*rArCrGrCrGrArGrGrArGrGrArArArGrGrArGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7175	MG3-6-hRosa26-sgRNA- A2	mA*mG*mG*rArArArGrGrArGrGrGrArGrGrGrCrUrUrCrUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7176	MG3-6-hRosa26-sgRNA- B2	mA*mC*mC*rUrCrCrUrCrCrArCrCrGrCrArGrCrUrCrCrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7177	MG3-6-hRosa26-sgRNA- C2	mG*mC*mG*rCrCrUrCrCrCrArCrCrCrArCrArArArCrCrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7178	MG3-6-hRosa26-sgRNA- D2	mC*mC*mC*rArCrCrCrCrCrArCrGrArGrUrGrCrCrUrGrUrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7179	MG3-6-hRosa26-sgRNA- E2	mC*mU*mC*rGrUrGrGrGrGrUrGrGrGrGrGrArGrGrArGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCr

SEQ ID NO:	NAME	SEQUENCE
		ArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7180	MG3-6-hRosa26-sgRNA- F2	mG*mC*mU*rGrCrGrGrUrGrGrArGrGrArGrGrUrGrGrArGrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7181	MG3-6-hRosa26-sgRNA- G2	mU*mC*mU*rCrUrGrCrUrGrCrCrUrCrCrCrGrUrCrUrUrGrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7182	MG3-6-hRosa26-sgRNA- H2	mC*mU*mC*rCrCrGrUrCrUrGrUrArArGrGrArCrCrGrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7183	MG3-6-hRosa26-sgRNA- A3	mC*mG*mA*rGrUrCrGrCrUrUrCrUrCrGrArUrUrArUrGrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7184	MG3-6-hRosa26-sgRNA- B3	mA*mU*mU*rArUrGrGrGrCrGrGrGrArUrUrCrUrUrUrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7185	MG3-6-hRosa26-sgRNA- C3	mG*mG*mG*rArUrCrUrUrUrUrGrCrCrUrArGrGrCrUrUrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7186	MG3-6-hRosa26-sgRNA- D3	mC*mC*mU*rGrCrArGrGrGrGrArGrUrGrArGrCrArGrCrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7187	MG3-6-hRosa26-sgRNA- E3	mA*mC*mU*rCrCrGrArUrUrArGrUrUrUrArUrCrUrUrCrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7188	MG3-6-hRosa26-sgRNA- F3	mU*mC*mC*rCrArCrGrGrArCrUrArGrArGrUrUrGrGrUrUrGrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7189	MG3-6-hRosa26-sgRNA- G3	mA*mA*mA*rUrGrGrArGrCrUrUrArGrUrCrArUrUrCrArCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7190	MG3-6-hRosa26-sgRNA- H3	mA*mC*mC*rUrGrGrGrGrCrUrGrArUrUrUrUrArUrGrCrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7191	MG3-6-hRosa26-sgRNA- A4	mG*mC*mU*rGrArUrUrUrArUrGrCrArArCrGrArGrArCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArG

SEQ ID NO:	NAME	SEQUENCE
		rGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7192	MG3-6-hRosa26-sgRNA- B4	mA*mU*mC*rArCrCrUrGrArGrUrUrUrUrArUrArCrCrArUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7193	MG3-6-hRosa26-sgRNA- C4	mG*mC*mU*rGrCrArCrCrArCrCrArArArGrUrGrUrArGrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7194	MG3-6-hRosa26-sgRNA- D4	mU*mU*mC*rCrCrUrCrCrCrUrCrArCrCrCrUrCrUrCrUrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7195	MG3-6-hRosa26-sgRNA- E4	mG*mC*mC*rUrGrGrUrGrUrCrUrCrGrCrGrArArArCrCrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7196	MG3-6-hRosa26-sgRNA- F4	mA*mC*mA*rGrArUrUrGrGrUrUrCrCrArCrCrArCrArArArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7197	MG3-6-hRosa26-sgRNA- G4	mC*mA*mC*rCrArCrArArArUrUrArArGrGrCrUrUrGrArGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7198	MG3-6-hRosa26-sgRNA- H4	mC*mA*mU*rUrUrUrArUrCrCrUrUrUrUrCrCrUrUrArGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
7199	MG3-6-hRosa26-target site- A1	ATCTGTCTGGTTTCGCGAGACA
7200	MG3-6-hRosa26-target site- B1	TTTCGCGAGACACCAGGCTACC
7201	MG3-6-hRosa26-target site- C1	AGCAAGTACAACAAATGGAAAA
7202	MG3-6-hRosa26-target site- D1	GCAAAAGCTAAAATTTTCTAT
7203	MG3-6-hRosa26-target site- E1	TGCTACACTTTGGTGGTGCAGC
7204	MG3-6-hRosa26-target site- F1	ACTCCCCTGCAGGGCAACGCCC
7205	MG3-6-hRosa26-target site- G1	CGACTCGACATGGAGGCGATGA
7206	MG3-6-hRosa26-target site- H1	ATCACGCGAGGAGGAAAGGAGG
7207	MG3-6-hRosa26-target site- A2	AGGAAAGGAGGGAGGGCTTCTT
7208	MG3-6-hRosa26-target site- B2	ACCTCCTCCACCGCAGCTCCCT

SEQ ID NO:	NAME	SEQUENCE
7209	MG3-6-hRosa26-target site- C2	GCGCCTCCCACCCACAAACCAG
7210	MG3-6-hRosa26-target site- D2	CCCACCCCCACGAGTGCCTGTA
7211	MG3-6-hRosa26-target site- E2	CTCGTGGGGGTGGGGGAGGAGC
7212	MG3-6-hRosa26-target site- F2	GCTGCGGTGGAGGAGGTGGAGA
7213	MG3-6-hRosa26-target site- G2	TCTCTGCTGCCTCCCGTCTTGT
7214	MG3-6-hRosa26-target site- H2	CTCCCGTCTTGTAAGGACCGCC
7215	MG3-6-hRosa26-target site- A3	CGAGTCGCTTCTCGATTATGGG
7216	MG3-6-hRosa26-target site- B3	ATTATGGGCGGGATTCTTTTGC
7217	MG3-6-hRosa26-target site- C3	GGGATTCTTTTGCCTAGGCTTA
7218	MG3-6-hRosa26-target site- D3	CCTGCAGGGGAGTGAGCAGCTG
7219	MG3-6-hRosa26-target site- E3	ACTCCGATTAGTTTATCTTCCC
7220	MG3-6-hRosa26-target site- F3	TCCCACGGACTAGAGTTGGTGT
7221	MG3-6-hRosa26-target site- G3	AAATGGAGCTTAGTCATTACCC
7222	MG3-6-hRosa26-target site- H3	ACCTGGGGCTGATTTTATGCAA
7223	MG3-6-hRosa26-target site- A4	GCTGATTTTATGCAACGAGACT
7224	MG3-6-hRosa26-target site- B4	ATCACCTGAGTTTATACCATT
7225	MG3-6-hRosa26-target site- C4	GCTGCACCACCAAAGTGTAGCA
7226	MG3-6-hRosa26-target site- D4	TTCCCTCCCTCACCCCTCTCTCC
7227	MG3-6-hRosa26-target site- E4	GCCTGGTGTCTCGCGAAACCAG
7228	MG3-6-hRosa26-target site- F4	ACAGATTGGTTCCACCACAAAT
7229	MG3-6-hRosa26-target site- G4	CACCACAAATTAAGGCTTGAGC
7230	MG3-6-hRosa26-target site- H4	CATTTTATCCTTTTTCCTTAGC
r =native ribose base, m = 2'-O methyl modified base, F = 2' Fluro modified base, * = phosphorothioate bond		

Example 25 – Gene editing outcomes at the DNA level for TRAC and AAVS1 in K562 cells

[00540] Nucleofection of MG21-1, MG23-1, MG73-1, MG89-2, and MG71-2 mRNA along with the matching guide RNA (500 ng mRNA/150 pmol guide) was performed into K562 cells (200,000) using the Lonza 4D electroporator. Cells were harvested and genomic DNA prepared three days post-transfection. PCR primers appropriate for use in NGS-based DNA sequencing were generated, optimized, and used to amplify the individual target sequences for each guide

RNA. The amplicons were sequenced on an Illumina MiSeq machine and analyzed with a proprietary Python script to measure gene editing (FIG. 18).

Table 15: Guide RNAs and Sequences Targeted for Example 25 When Targeting TRAC

SEQ ID NO:	NAME	SEQUENCE
7231	MG21-1-TRAC-sgRNA-H9	mC*mA*mC*rCrUrUrCrUrUrCrCrCrCrArGrCrCrCrArGrGrUrGrUrUrGrUrArGrUrUrCrCrCrCrUrUrUrGrArArArArArArGrUrGrUrGrUrUrArCrUrGrCrArArUrArArGrGrUrArArArArCrArCrCrArCrGrArArGrCrUrCrUrGrCrCrCrUrArArCrUrGrCrCrUrUrArGrCrArGrUrUrArGrGrGrCrArUrC*mU*mU*mU
7232	MG21-1-TRAC-sgRNA-B7	mC*mA*mA*rGrArGrCrArArCrArGrUrGrCrUrGrUrGrGrCrCrGrUrUrGrUrArGrUrUrCrCrCrCrUrUrUrGrArArArArArArGrUrGrUrGrUrUrArCrUrGrCrArArUrArArGrGrUrArArArArCrArCrCrArCrGrArArGrCrUrCrUrGrCrCrCrUrArArCrUrGrCrCrUrUrArGrCrArGrUrUrArGrGrGrCrArUrC*mU*mU*mU
7233	MG21-1-TRAC-sgRNA-D6	mA*mG*mA*rCrArUrGrArGrGrUrCrUrArUrGrGrArCrUrUrCrGrUrUrGrUrArGrUrUrCrCrCrCrUrUrUrGrArArArArArArGrUrGrUrGrUrUrArCrUrGrCrArArUrArArGrGrUrArArArArCrArCrCrArCrGrArArGrCrUrCrUrGrCrCrCrUrArArCrUrGrCrCrUrUrArGrCrArGrUrUrArGrGrGrCrArUrC*mU*mU*mU
7234	MG21-1-TRAC-sgRNA-C10	mC*mC*mA*rArArGrCrUrGrCrCrCrUrUrArCrCrUrGrGrGrCrGrUrUrGrUrArGrUrUrCrCrCrCrUrUrUrGrArArArArArArGrUrGrUrGrUrUrArCrUrGrCrArArUrArArGrGrUrArArArArCrArCrCrArCrGrArArGrCrUrCrUrGrCrCrCrUrArArCrUrGrCrCrUrUrArGrCrArGrUrUrArGrGrGrCrArUrC*mU*mU*mU
7235	MG21-1-TRAC-target site-H9	CACCTTCTTCCCCAGCCAGGT
7236	MG21-1-TRAC-target site-B7	CAAGAGCAACAGTGCTGTGGCC
7237	MG21-1-TRAC-target site-D6	AGACATGAGGTCTATGGACTTC
7238	MG21-1-TRAC-target site-C10	CCAAAGCTGCCCTTACCTGGGC
7239	MG23-1-TRAC-sgRNA-E1	mC*mC*mG*rUrGrUrArCrCrArGrCrUrGrArGrArGrArCrUrCrGrUrUrUrGrArGrArArCrCrUrGrArArArArGrGrUrGrArGrUrGrCrArArArUrArArGrGrUrUrUrArArCrCrGrArArArUrUrGrUrUrUrArCrCrUrGrCrArUrUrGrUrGrCrArGrUrArArArGrArArArGrArCrCrGrCrGrArGrGrUrCrU*mU*mU*mU
7240	MG23-1-TRAC-sgRNA-H8	mU*mU*mG*rGrGrUrUrCrCrGrArArUrCrCrUrCrCrUrCrCrUrGrUrUrGrArGrArArCrCrUrGrArArArArGrGrUrGrArGrUrGrCrArArArUrArArGrGrUrUrUrArArCrCrGrArArArUrUrGrUrUrUrArCrCrUrGrCrArUrUrGrUrGrCrArGrUrArArArGrArArArGrArCrCrGrCrGrArGrGrUrCrU*mU*mU*mU
7241	MG23-1-TRAC-sgRNA-A3	mA*mC*mA*rGrUrGrCrUrGrGrCrCrUrGrGrArGrCrArArGrUrUrUrGrArArArCrCrUrGrArArArArGrGrUrGrArGrUrGrCrArArArUrArArGrGrUrUrUrArArCrCrGrArArArUrUrGrUrUrUrArCrCrUrGrCrArUrUrGrUrGrCrArGrUrArUrArArGrArArArGrArCrCrGrCrGrArGrGrUrCrU*mU*mU*mU
7242	MG23-1-TRAC-sgRNA-C10	mU*mG*mA*rArArGrUrUrUrArGrGrUrUrCrGrUrArUrCrUrGrGrUrUrUrGrArGrArArCrCrUrGrArArArArGrGrUrGrArGrUrGrCrArArArUrArArGrGrUrUrUrArArCrCrGrArArArUrUrGrUrUrUrArCrCrUrGrCrArUrUrGrUrGrCrArGrUrArUrArArGrArArArGrArCrCrGrCrGrArGrGrUrCrU*mU*mU*mU
7243	MG23-1-TRAC-sgRNA-H7	mG*mC*mU*rUrGrArCrArUrCrArCrArGrGrArArCrUrUrUrCrGrUrUrUrGrArGrArArCrCrUrGrArArArArGrGrUrGrArGrUrGrCrArArArUrArArGrGrUrUrUrArArCrCrGrArArArUrUrGrUrUrUrAr

SEQ ID NO:	NAME	SEQUENCE
		rCrCrUrGrCrArUrUrGrUrGrCrArGrUrArUrArArGrArArArGrArCrCrGrCrGrArGrGrUrCrU*mU*mU*mU
7244	MG23-1-TRAC-sgRNA-B10	mA*mA*mC*rCrCrArArUrCrArCrUrGrArCrArGrGrUrUrUrUrGrUrUrUrGrArGrArArCrCrUrGrArArArArGrGrUrGrArGrUrGrCrArArArUrArArGrGrUrUrUrArArCrCrGrArArArUrUrGrUrUrUrArCrCrUrGrCrArUrUrGrUrGrCrArGrUrArArArGrArArArGrArCrCrGrCrGrArGrGrUrCrU*mU*mU*mU
7245	MG23-1-TRAC-sgRNA-H6	mC*mC*mU*rGrUrGrArUrGrUrCrArArGrCrUrGrGrUrCrGrArGrUrUrUrGrArGrArArCrCrUrGrArArArArGrGrUrGrArGrUrGrCrArArArUrArArGrGrUrUrUrArArCrCrGrArArArUrUrGrUrUrUrArCrCrUrGrCrArUrUrGrUrGrCrArGrUrArArArGrArArArGrArCrCrGrCrGrArGrGrUrCrU*mU*mU*mU
7246	MG23-1-TRAC-sgRNA-E7	mU*mA*mG*rArCrCrCrCrUrGrUrCrUrUrArCrCrUrGrUrUrUrGrUrUrUrGrArGrArArCrCrUrGrArArArArGrGrUrGrArGrUrGrCrArArArUrArArGrGrUrUrUrArArCrCrGrArArArUrUrGrUrUrUrArCrCrUrGrCrArUrUrGrUrGrCrArGrUrArUrArArGrArArArGrArCrCrGrCrGrArGrGrUrCrU*mU*mU*mU
7247	MG23-1-TRAC-sgRNA-C9	mA*mG*mC*rCrGrCrArGrCrGrUrCrArUrGrArGrCrArGrArUrGrUrUrGrArGrArArCrCrUrGrArArArArGrGrUrGrArGrUrGrCrArArArUrArArGrGrUrUrUrArArCrCrGrArArArUrUrGrUrUrUrArCrCrUrGrCrArUrUrGrUrGrCrArGrUrArUrArArGrArArArGrArCrCrGrCrGrArGrGrUrCrU*mU*mU*mU
7248	MG23-1-TRAC-target site-E1	CCGTGTACCAGCTGAGAGACTC
7249	MG23-1-TRAC-target site-H8	TTGGGTTCCGAATCCTCCTCCT
7250	MG23-1-TRAC-target site-A3	ACAGTGCTGTGGCCTGGAGCAA
7251	MG23-1-TRAC-target site-C10	TGAAAGTTTAGGTTTCGTATCTG
7252	MG23-1-TRAC-target site-H7	GCTTGACATCACAGGAAC TTTC
7253	MG23-1-TRAC-target site-B10	AACCCAATCACTGACAGGTTTT
7254	MG23-1-TRAC-target site-H6	CCTGTGATGTCAAGCTGGTCTGA
7255	MG23-1-TRAC-target site-E7	TAGACCCCTGTCTTACCTGTTT
7256	MG23-1-TRAC-target site-C9	AGCCGCAGCGTCATGAGCAGAT
7269	MG73-1-TRAC-sgRNA-G3	mU*mC*mU*rUrGrGrUrUrUrUrArCrArGrArUrArCrGrArArCrCrUrGrUrUrArUrArGrUrGrGrGrArArArUrCrArCrUrArUrArUrArArGrUrGrArArArUrCrGrCrArArGrGrCrUrCrUrGrUrUrCrUrUrGrArArCrArUrCrCrUrUrUrArUrUrArArArCrUrCrCrUrGrCrCrArArUrCrCrGrArArCrCrGrCrCrCrUrCrArCrUrUrArGrUrGrArGrGrGrCrU*mU*mU*mU
7270	MG73-1-TRAC-target site-G3	TCTTGTTTTTACAGATACGAACCT
7271	MG89-2-TRAC-sgRNA-F1	mA*mU*mA*rUrCrCrArGrArArCrCrCrUrGrArCrCrCrUrGrCrCrGrGrUrUrGrUrArGrCrUrUrCrCrUrUrGrArArGrArArArUrUrCrArArCrGrUrUrGrUrUrArCrArArUrArArGrGrUrUrUrUrCrGrArArArGrArUrUrArCrCrGrArArCrCrGrCrCrCrUrCrArCrUrUrArGrUrGrArGrGrGrCrU*mU*mU*mU
7272	MG89-2-TRAC-sgRNA-G5	mG*mG*mC*rCrArCrUrUrUrCrArGrGrArGrGrArGrGrArUrUrCrGrGrUrUrGrUrArGrCrUrUrCrCrUrUrGrArArGrArArArUrUrCrArArCrGrUrUrGrUrUrArCrArArUrArArGrGrUrUrUrUrCrGrArArArGrArUrUrArCrCrGrArArCrCrGrCrCrCrUrCrArCrUrUrArGrGrUrGrArGrGrGrCrU*mU*mU*mU
7273	MG89-2-TRAC-sgRNA-E5	mC*mG*mC*rArGrCrGrUrCrArUrGrArGrCrArGrArUrUrArArArCrGrUrUrGrUrArGrCrUrUrCrCrUrUrGrArArGrArArArUrUrCrAr

SEQ ID NO:	NAME	SEQUENCE
		rArCrGrUrUrGrUrUrArCrArArUrArArGrGrUrUrUrUrCrGrArArArGrArUrUrArCrCrGrArArCrCrGrCrCrCrUrCrArCrUrUrArGrGrUrGrArGrGrGrCrU*mU*mU*mU
7274	MG89-2-TRAC-sgRNA-F5	mC*mG*mG*rCrCrArCrUrUrUrCrArGrGrArGrGrArGrUrUrCrGrUrUrGrUrArGrCrUrUrCrCrUrUrGrArArGrArArArUrUrCrArArCrGrUrUrGrUrUrArCrArArUrArArGrGrUrUrUrUrCrGrArArArGrArUrUrArCrCrGrArArCrCrGrCrCrCrUrCrArCrUrUrArGrGrUrGrArGrGrGrCrU*mU*mU*mU
7275	MG89-2-TRAC-sgRNA-G1	mG*mC*mC*rGrUrGrUrArCrCrArGrCrUrGrArGrArGrArCrUrCrUrGrUrUrGrUrArGrCrUrUrCrCrUrUrGrArArGrArArArUrUrCrArArCrGrUrUrGrUrUrArCrArArUrArArGrGrUrUrUrUrCrGrArArArGrArUrUrArCrCrGrArArCrCrGrCrCrCrUrCrArCrUrUrArGrGrUrGrArGrGrGrCrU*mU*mU*mU
7276	MG89-2-TRAC-sgRNA-E1	mC*mC*mC*rArCrArGrArUrArUrCrCrArGrArArCrCrCrUrGrArCrGrUrUrGrUrArGrCrUrUrCrCrUrUrGrArArGrArArArUrUrCrArArCrGrUrUrGrUrUrArCrArArUrArArGrGrUrUrUrUrCrGrArArArGrArUrUrArCrCrGrArArCrCrGrCrCrCrUrCrArCrUrUrArGrGrUrGrArGrGrGrCrU*mU*mU*mU
7277	MG89-2-TRAC-sgRNA-B1	mA*mU*mC*rCrUrCrUrUrGrUrCrCrArCrArGrArUrArUrCrCrArGrUrUrGrUrArGrCrUrUrCrCrUrUrGrArArGrArArArUrUrCrArArCrGrUrUrGrUrUrArCrArArUrArArGrGrUrUrUrUrCrGrArArArGrArUrUrArCrCrGrArArCrCrGrCrCrCrUrCrArCrUrUrArGrGrUrGrArGrGrGrCrU*mU*mU*mU
7278	MG89-2-TRAC-target site-F1	ATATCCAGAACCCTGACCCTGCCG
7279	MG89-2-TRAC-target site-G5	GGCCACTTTCAGGAGGAGGATTCG
7280	MG89-2-TRAC-target site-E5	CGCAGCGTCATGAGCAGATTAAAC
7281	MG89-2-TRAC-target site-F5	CGGCCACTTTCAGGAGGAGGATTC
7282	MG89-2-TRAC-target site-G1	GCCGTGTACCAGCTGAGAGACTCT
7283	MG89-2-TRAC-target site-E1	CCCACAGATATCCAGAACCCTGAC
7284	MG89-2-TRAC-target site-B1	ATCCTCTTGTCACACAGATATCCA
r = native ribose base, m = 2'-O methyl modified base, F = 2' Fluoro modified base, * = phosphorothioate bond		

Table 16: Guide RNAs and Sequences Targeted for Example 25 When Targeting AAVS1

SEQ ID NO:	NAME	SEQUENCE
7257	MG23-1-AAVS1-sgRNA-B1	mG*mC*mU*rArCrUrGrGrCrCrUrUrArUrCrUrCrArCrArGrGrUrUrUrGrArGrArArCrCrUrGrArArArGrGrUrGrArGrUrGrCrArArArUrArArGrGrUrUrUrArArCrCrGrArArArUrUrGrUrUrUrArCrCrUrGrCrArUrUrGrUrGrCrArGrUrArUrArArGrArArArGrArCrCrGrCrGrArGrGrUrCrU*mU*mU*mU
7258	MG23-1-AAVS1-sgRNA-C1	mC*mU*mA*rCrUrGrGrCrCrUrUrArUrCrUrCrArCrArGrGrUrGrUrUrGrArGrArArCrCrUrGrArArArGrGrUrGrArGrUrGrCrArArArUrArArGrGrUrUrUrArArCrCrGrArArArUrUrGrUrUrUrArCrCrUrGrCrArUrUrGrUrGrCrArGrUrArUrArArGrArArArGrArCrCrGrCrGrArGrGrUrCrU*mU*mU*mU
7259	MG23-1-AAVS1-sgRNA-G1	mA*mC*mU*rGrArCrGrCrArCrGrGrArGrGrArCrArArUrArGrUrUrUrGrArGrArArCrCrUrGrArArArGrGrUrGrArGrUrGrCr

SEQ ID NO:	NAME	SEQUENCE
		ArArArUrArArGrGrUrUrUrArArCrCrGrArArArUrUrGrUrUrUrArArCrCrUrGrCrArUrUrGrUrGrCrArGrUrArUrArArGrArArArGrArCrCrGrCrGrArGrGrUrCrU*mU*mU*mU
7260	MG23-1-AAVS1-sgRNA-B2	mG*mG*mA*rArCrArArUrArUrArArArUrUrGrGrGrGrArCrUrGrUrUrUrGrArGrArArCrCrUrGrArArArArGrGrUrGrArGrUrGrCrArArArUrArArGrGrUrUrUrArArCrCrGrArArArUrUrGrUrUrUrArArCrCrUrGrCrArUrUrGrUrGrCrArGrUrArArArGrArArArGrArCrCrGrCrGrArGrGrUrCrU*mU*mU*mU
7261	MG23-1-AAVS1-target site-B1	GCTACTGGCCTTATCTCACAGG
7262	MG23-1-AAVS1-target site-C1	CTACTGGCCTTATCTCACAGGT
7263	MG23-1-AAVS1-target site-G1	ACTGACGCACGGAGGAACAATA
7264	MG23-1-AAVS1-target site-B2	GGAACAATATAAATTGGGGACT
7265	MG71-2-AAVS1-sgRNA-C3	mG*mG*mA*rGrArGrGrGrUrArGrCrGrCrArGrGrGrUrGrGrUrUrGrArGrArGrUrGrArGrArArArUrCrArCrGrArGrUrUrCrArArArArArArCrArUrGrArUrUrUrArUrUrCrArArArCrCrGrUrCrUrUrCrUrUrCrGrGrArArGrGrCrCrCrCrArCrArGrUrGrUrGrUrGrGrArCrArGrUrArArArGrCrUrUrGrCrUrUrCrGrGrCrArArGrCrU*mU*mU*mU
7266	MG71-2-AAVS1-sgRNA-E2	mG*mC*mC*rCrUrGrCrCrArGrGrArCrGrGrGrGrCrUrGrGrUrUrGrArGrArGrUrGrArGrArArArUrCrArCrGrArGrUrUrCrArArArArArArCrArUrGrArUrUrUrArUrUrCrArArArCrCrGrUrCrUrUrCrUrUrCrGrGrArArGrGrCrCrCrCrArCrArGrUrGrUrGrUrGrGrArCrArGrUrArArArGrCrUrUrGrCrUrUrCrGrGrCrArArGrCrU*mU*mU*mU
7267	MG71-2-AAVS1-target site-C3	GGAGAGGGTAGCGCAGGGTG
7268	MG71-2-AAVS1-target site-E2	GCCCTGCCAGGACGGGGCTG
r = native ribose base, m = 2'-O methyl modified base, F = 2' Fluoro modified base, * = phosphorothioate bond		

Example 26 – MG3-6 nuclease guide screen for human HAO-1 gene using mRNA transfection of Hep3B cells

[00541] Guide RNAs for the MG3-6 nuclease targeting exons 1 to 4 of the human HAO-1 gene (encodes glycolate oxidase) were identified *in silico* by searching for the PAM sequence 5' NNRGRYY 3'. A total of 21 guides with the fewest predicted off-target sites in the human genome were chemically synthesized as single guide RNAs with AltR1/AltR2 end-modifications (IDT). The full sequences of the sgRNA are SEQ ID NOs: 11352-11372.

Table 17: Guide sequences used in Example 26

SEQ ID NO:	Entity Name	Sequence
11352	hH36-1	AAUUAGCCGGGGGAGCAUUUUCGUUGAGAAUCGAAAGAUUCUAAUAAGGCAUCCUCCGAUGCUGACUUCACCGUCCGUUUUCCAAUAGGAGCGGGCGGUAUGUUUU

SEQ ID NO:	Entity Name	Sequence
1135 3	hH36-2	CCCAGACCUGUAAUAGUCAUAUGUUGAGAAUCGAAAGAUUCUAAUAAGG CAUCCUUCCGAUGCUGACUUCUCACCGUCCGUUUUCCAAUAGGAGCGGGC GGUAUGUUUU
1135 4	hH36-3	CCAAAGUCUAUAUAUGACUAUUGUUGAGAAUCGAAAGAUUCUAAUAAGG CAUCCUUCCGAUGCUGACUUCUCACCGUCCGUUUUCCAAUAGGAGCGGGC GGUAUGUUUU
1135 5	hH36-4	CAAAGUUCUUCAUCAUUUGCCGUUGAGAAUCGAAAGAUUCUAAUAAGG CAUCCUUCCGAUGCUGACUUCUCACCGUCCGUUUUCCAAUAGGAGCGGGC GGUAUGUUUU
1135 6	hH36-5	GAUGCUCGCGAAUGUUGCUGAAGUUGAGAAUCGAAAGAUUCUAAUAAGG CAUCCUUCCGAUGCUGACUUCUCACCGUCCGUUUUCCAAUAGGAGCGGGC GGUAUGUUUU
1135 7	hH36-6	CUCUGUCCUAAAACAGAAGUCGGUUGAGAAUCGAAAGAUUCUAAUAAGG CAUCCUUCCGAUGCUGACUUCUCACCGUCCGUUUUCCAAUAGGAGCGGGC GGUAUGUUUU
1135 8	hH36-7	UGUCGACUUCUGUUUUAGGACAGUUGAGAAUCGAAAGAUUCUAAUAAGG CAUCCUUCCGAUGCUGACUUCUCACCGUCCGUUUUCCAAUAGGAGCGGGC GGUAUGUUUU
1135 9	hH36-8	GGGUCAGCAUGCCAAUAUGUGUGUUGAGAAUCGAAAGAUUCUAAUAAGG CAUCCUUCCGAUGCUGACUUCUCACCGUCCGUUUUCCAAUAGGAGCGGGC GGUAUGUUUU
1136 0	hH36-9	UCAUGCCCGUUC CAGGGACUGGUUGAGAAUCGAAAGAUUCUAAUAAGG CAUCCUUCCGAUGCUGACUUCUCACCGUCCGUUUUCCAAUAGGAGCGGGC GGUAUGUUUU
1136 1	hH36-10	ACUCAACAUC AUGCCCGUUC CCGUUGAGAAUCGAAAGAUUCUAAUAAGG CAUCCUUCCGAUGCUGACUUCUCACCGUCCGUUUUCCAAUAGGAGCGGGC GGUAUGUUUU
1136 2	hH36-11	GAACGGGCAUGAUGUUGAGUUCGUUGAGAAUCGAAAGAUUCUAAUAAG GCAUCCUUCCGAUGCUGACUUCUCACCGUCCGUUUUCCAAUAGGAGCGGG CGGUAUGUUUU
1136 3	hH36-12	AGUUGCAGCCAACGAAGUGCCUGUUGAGAAUCGAAAGAUUCUAAUAAGG CAUCCUUCCGAUGCUGACUUCUCACCGUCCGUUUUCCAAUAGGAGCGGGC GGUAUGUUUU
1136 4	hH36-13	UUGGCUGCAACUGUAUAUCUACGUUGAGAAUCGAAAGAUUCUAAUAAGG CAUCCUUCCGAUGCUGACUUCUCACCGUCCGUUUUCCAAUAGGAGCGGGC GGUAUGUUUU
1136 5	hH36-14	GCUAGUGCGGCAGGCAGAGAAGGUUGAGAAUCGAAAGAUUCUAAUAAG GCAUCCUUCCGAUGCUGACUUCUCACCGUCCGUUUUCCAAUAGGAGCGGG CGGUAUGUUUU
1136 6	hH36-15	GGCAGGCAGAGAAGAUGGGCUAGUUGAGAAUCGAAAGAUUCUAAUAAG GCAUCCUUCCGAUGCUGACUUCUCACCGUCCGUUUUCCAAUAGGAGCGGG CGGUAUGUUUU
1136 7	hH36-16	AACCGUCUGGAUGAUGUGCGUAGUUGAGAAUCGAAAGAUUCUAAUAAGG CAUCCUUCCGAUGCUGACUUCUCACCGUCCGUUUUCCAAUAGGAGCGGGC GGUAUGUUUU
1136 8	hH36-17	GAGGAAAAUUUUGGAGACGACAGUUGAGAAUCGAAAGAUUCUAAUAAGG CAUCCUUCCGAUGCUGACUUCUCACCGUCCGUUUUCCAAUAGGAGCGGGC GGUAUGUUUU
1136 9	hH36-18	UGCUGCAUAUGUGGCUAAAGCAGUUGAGAAUCGAAAGAUUCUAAUAAGG CAUCCUUCCGAUGCUGACUUCUCACCGUCCGUUUUCCAAUAGGAGCGGGC GGUAUGUUUU
1137 0	hH36-19	UUGAUUCUUC CAGCUGAUAGGUUGAGAAUCGAAAGAUUCUAAUAAGG CAUCCUUCCGAUGCUGACUUCUCACCGUCCGUUUUCCAAUAGGAGCGGGC GGUAUGUUUU
1137 1	hH36-20	UGGGAAGAUAUCAAUUGGCUGAGUUGAGAAUCGAAAGAUUCUAAUAAGG CAUCCUUCCGAUGCUGACUUCUCACCGUCCGUUUUCCAAUAGGAGCGGGC GGUAUGUUUU

SEQ ID NO:	Entity Name	Sequence
11372	hH36-21	AAUUGUUGCAAAGGGCAUUUUGGUUGAGAAUCGAAAGAUUCUAAUAAGGCAUCCUCCGAUGCUGACUUCACCGUCCGUUUUCCAAUAGGAGCGGGC GGUAUGUUUU

Hep3B Transfection Protocol

[00542] The mRNA encoding MG3-6 was generated by T7 polymerase in vitro transcription of a plasmid in which the coding sequence of MG3-6 had been cloned. The MG3-6 coding sequence was codon optimized using human codon usage tables and flanked by nuclear localization signals derived from SV40 (at the N-terminus) and from Nucleoplasmin (at the C-terminus). In addition, a 5' untranslated region (5' UTR) was included at the 5' end of the coding sequence to improve translation. A 3' UTR followed by an approximately 90 to 110 nucleotide poly A tract was included in the mRNA (encoded in the plasmid) at the 3' end of the coding sequence to improve mRNA stability in vivo. The DNA sequence that encodes the MG3-6 mRNA without the polyA tail is shown in SEQ ID 22. The in vitro transcription reaction included the Clean Cap® capping reagent (Trilink BioTechnologies) and the resulting RNA was purified using the MEGAClear™ Transcription Clean-Up kit (Invitrogen) and purity was evaluated using the TapeStation (Agilent) and found to be composed of >90% full length RNA.

[00543] 300 ng of MG3-6 mRNA and 120 ng of each single guide RNA were transfected into Hep3B cells as follows. One day prior to transfection, Hep3B cells that had been cultured for less than 10 days in EMEM-10% FBS-2 mM glutamine-1% NEAA media, without Pen/Step, were seeded into a TC-treated 24 well plate. Cells were counted, and the equivalent volume to 60,000 viable cells were added to each well. Additional pre-equilibrated media was added to each well to bring the total volume to 500 µL. On the day of transfection, 25 µL of OptiMEM media and 1.25 µL of Lipofectamine Messenger Max Solution (Thermo Fisher) were mixed in a master mix solution, vortexed, and allowed to sit for at least 5 minutes at room temperature. In separate tubes, 300 ng of the MG3-6/3-4 mRNA and 120 ng of the sgRNA were mixed with 25 µL of OptiMEM media and vortexed briefly. The appropriate volume of MessengerMax solution was added to each RNA solution, mixed by flicking the tube, and briefly spun down at a low speed. The complete editing reagent solutions were allowed to incubate for 10 minutes at room temperature, then added directly to the Hep3B cells. Two days post transfection, the media was aspirated from each well of Hep3B cells and genomic DNA was purified by automated magnetic bead purification on the KingFisher Flex robot with the MagMAX™ DNA Multi-Sample Ultra 2.0 Kit.

PCR amplification and editing analysis by Sanger sequencing

[00544] HAO-1 gene sequences targeted by the different sgRNA were amplified by PCR from purified genomic DNA using the exon-specific primers of Table 18 and Phusion Flash High-Fidelity PCR Master Mix (Thermo Fisher).

Table 18: Primers designed for the human HAO1 gene, used for PCR at each of the first four exons, and for Sanger sequencing.

Target Exon	Use	Primer Name	Primer Sequence
Human HAO1 Exon 1	Fwd PCR	PCR_hHe1_F_+490	TTTCATGGATGCCCCGTTCA
	Rev PCR	PCR_hHe1_R_-412	ACGAAAAGCCAGCAGGAAGA
	Sequencing	Seq_hHe1_R_-121	AGCCCCAAGAACTTTTCCCT
Human HAO1 Exon 2	Fwd PCR	PCR_hHe2_F_+391	TGCATCAGTGGTTGTCAGGG
	Rev PCR	PCR_hHe2_R_-387	CCTAGCTGTGACTTTGGGCA
	Sequencing	Seq_hHe2_R_-152	TGGAAAGAAGAGGAGCAGGAC
Human HAO1 Exon 3	Fwd PCR	PCR_hHe3_F_+238	AGGCTGGATGTTTCAGGTTCTT
	Rev PCR	PCR_hHe3_R_-212	TCCCAAAGCCAAAGCCCTTA
	Sequencing	Seq_hHe3_F_+186	AGCAGAAATAACTCCAGTAGCCA
Human HAO1 Exon 4	Fwd PCR	PCR_hHe4_F_+324	GCTGGCTGAAAATCGTGTCAA
	Rev PCR	PCR_hHe4_R_-348	TCCTTGGGGCTTCTCTTTGG
	Sequencing	Seq_hHe4_F_+174	ACTGATTAAGACCACTAGAGTATCACA

[00545] PCR products were purified and concentrated using DNA clean & concentrator 5 (Zymo Research) and 40 ng of PCR product subjected to Sanger sequencing (ELIM Biosciences).

[00546] The Sanger sequencing chromatograms were analyzed for insertions and deletions (INDELS) at the predicted target site for each sgRNA by an algorithm called Tracking of Indels by DEcomposition (TIDE) as described by Brinkman *et al.* (Nucleic Acids Res. 2014 Dec 16; 42(22): e168. Published online 2014 Oct 9. doi: 10.1093/nar/gku936). From this screen guides hH364-1, 14, and 15 were identified as having the highest editing activity in Hep3B cells (**FIG. 19** and Table 19).

Table 19: Editing activity of MG3-6 guides at human HAO1 gene delivered by mRNA transfection

Guide Name	PAM	Spacer Sequence	Editing Activity in Hep3B (Average % indels)	Spacer Sequence SEQ ID NO:
hH36-1	ACAGG TT	AATTAGCCGGGGGAGCA TTTTC	34.0	11773
hH36-2	ATAGA CT	CCCAGACCTGTAATAGT CATAT	0.0	11774
hH36-3	ACAGG TC	CCAAAGTCTATATATGA CTATT	4.0	11775
hH36-4	CCAGA CC	CAAAGTTTCTTCATCATT TGCC	0.0	11776
hH36-5	ACAGA TC	GATGCTCCGGAATGTTG CTGAA	0.0	11777
hH36-6	ACAGA TC	CTCTGTCCTAAAACAGA AGTCG	0.0	11778
hH36-7	GAGGG TC	TGTCGACTTCTGTTTTAG GACA	1.0	11779
hH36-8	GGGGG CT	GGGTCAGCATGCCAATA TGTGT	10.5	11780
hH36-9	ACAGG CT	TCATGCCCCGTTCCCAGG GACTG	0.0	11781
hH36-10	AGGGA CT	ACTCAACATCATGCCCG TTCCC	0.0	11782
hH36-11	CTGGG CC	GAACGGGCATGATGTTG AGTTC	0.0	11783
hH36-12	CAGGA CC	AGTTGCAGCCAACGAAG TGCCT	0.0	11784
hH36-13	AAGGA CC	TTGGCTGCAACTGTATAT CTAC	0.0	11785
hH36-14	ATGGG CT	GCTAGTGCGGCAGGCAG AGAAG	19.5	11786
hH36-15	CAAGG CC	GGCAGGCAGAGAAGATG GGCTA	14.5	11787
hH36-16	ACAGA TT	AACCGTCTGGATGATGT GCGTA	0.0	11788
hH36-17	GTGGA CT	GAGGAAAATTTTGGAGA CGACA	0.0	11789
hH36-18	ATAGA CC	TGCTGCATATGTGGCTA AAGCA	0.0	11790
hH36-19	ATGGG TC	TTGATATCTTCCCAGCTG ATAG	8.5	11791

Guide Name	PAM	Spacer Sequence	Editing Activity in Hep3B (Average % indels)	Spacer Sequence SEQ ID NO:
hH36-20	GAAGA CT	TGGGAAGATATCAAATG GCTGA	0.0	11792
hH36-21	AGAGG TT	AATTGTTGCAAAGGGCA TTTTG	5.0	11793

Example 27 – Gene editing outcomes at the DNA level for human GPR146

[00547] Nucleofection of MG3-6 RNPs (104 pmol protein/120 pmol guide) (SEQ ID NOs: 11374-11405) was performed into Hep3B cells (100,000) using the Lonza 4D electroporator. Cells were harvested and genomic DNA prepared three days post-transfection. PCR primers appropriate for use in NGS-based DNA sequencing were generated, optimized, and used to amplify the individual target sequences for each guide RNA (SEQ ID NOs: 11406-11437). The amplicons were sequenced on an Illumina MiSeq machine and analyzed with a proprietary Python script to measure gene editing (FIG. 20).

Table 20: Guide RNAs and Sequences Targeted for Example 25 When Targeting GPR146

SEQ ID NO:	NAME	SEQUENCE
11374	MG3-6-human GPR146-A1	mA*mG*mC*rUrGrCrArGrCrUrGrGrUrUrCrArArCrGrGrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11375	MG3-6-human GPR146-B1	mG*mG*mU*rGrGrArGrGrArGrCrUrGrCrCrUrGrCrCrUrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11376	MG3-6-human GPR146-C1	mC*mC*mU*rGrCrCrUrGrCrCrArGrGrArCrCrUrGrCrArGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11377	MG3-6-human GPR146-D1	mG*mG*mG*rGrCrUrGrUrCrArCrUrGrUrUrGrUrCrGrCrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11378	MG3-6-human GPR146-E1	mG*mG*mG*rCrCrUrGrGrUrGrGrUrGrGrGrCrGrUrGrCrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11379	MG3-6-human GPR146-F1	mU*mG*mG*rUrGrCrUrGrGrCrCrArArCrCrUrArCrArCrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCr

SEQ ID NO:	NAME	SEQUENCE
		ArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11380	MG3-6-human GPR146-G1	mG*mU*mA*rCrUrUrUrGrUrCrArArCrArUrGrGrCrArGrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11381	MG3-6-human GPR146-H1	mG*mC*mG*rGrCrGrArArGrUrCrCrArCrGrUrGrGrCrArCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11382	MG3-6-human GPR146-A2	mA*mC*mU*rArCrArUrCrGrArGrCrGrUrGrCrArCrUrGrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11383	MG3-6-human GPR146-B2	mC*mG*mU*rCrCrGrCrArGrGrGrArGrGrArCrArCrGrCrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11384	MG3-6-human GPR146-C2	mG*mG*mA*rCrArCrGrCrCrCrCrUrGrGrArCrCrGrGrGrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11385	MG3-6-human GPR146-D2	mG*mG*mC*rCrGrGrCrUrGrGrArGrCrCrCrUrCrGrGrCrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11386	MG3-6-human GPR146-E2	mG*mU*mG*rGrCrCrArCrCrGrUrGrUrGrCrArCrGrCrArGrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11387	MG3-6-human GPR146-F2	mA*mA*mG*rCrCrCrGrUrGrGrArCrGrCrArCrArCrUrArCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11388	MG3-6-human GPR146-G2	mC*mC*mU*rGrGrGrGrCrUrArCrUrGrCrArCrUrUrUrGrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11389	MG3-6-human GPR146-H2	mG*mC*mU*rGrArUrGrArArArArGrCrUrGrCrCrCrUrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11390	MG3-6-human GPR146-A3	mC*mU*mG*rCrGrGrGrGrArCrCrGrGrCrArCrUrGrCrUrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11391	MG3-6-human GPR146-B3	mG*mU*mG*rGrUrGrUrCrArCrArArArGrCrUrGrCrUrGrGrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr

SEQ ID NO:	NAME	SEQUENCE
		CrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11392	MG3-6-human GPR146-C3	mU*mA*mG*rCrCrCrCrArGrGrUrArGrUrGrUrGrCrGrUrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11393	MG3-6-human GPR146-D3	mA*mG*mA*rUrGrArUrGrArCrCrGrUrGrUrGrCrCrCrCrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11394	MG3-6-human GPR146-E3	mG*mG*mC*rCrArCrCrArGrCrArGrCrCrUrGrUrGrUrGrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11395	MG3-6-human GPR146-F3	mG*mC*mG*rUrGrUrUrGrUrArCrArCrGrCrUrGrGrCrCrArUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11396	MG3-6-human GPR146-G3	mA*mG*mU*rGrCrArCrGrCrUrCrGrArUrGrUrArGrUrGrGrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11397	MG3-6-human GPR146-H3	mC*mC*mA*rCrCrArGrUrGrArGrGrArCrArCrArUrUrGrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11398	MG3-6-human GPR146-A4	mU*mG*mA*rArGrGrGrGrArUrCrUrGrCrArGrUrGrCrCrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11399	MG3-6-human GPR146-B4	mC*mA*mC*rArGrCrGrCrCrArCrCrGrGrGrArGrCrUrCrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11400	MG3-6-human GPR146-C4	mU*mC*mG*rGrGrGrGrGrCrCrGrArGrCrArGrGrUrGrCrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11401	MG3-6-human GPR146-D4	mA*mC*mA*rGrGrGrGrCrCrArGrGrGrCrGrCrUrGrArGrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11402	MG3-6-human GPR146-E4	mA*mU*mG*rGrUrCrArUrGrCrUrGrGrCrCrUrUrGrCrUrGrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11403	MG3-6-human GPR146-F4	mA*mG*mC*rArCrCrArGrCrArGrGrGrCrGrUrUrGrUrArGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr

SEQ ID NO:	NAME	SEQUENCE
		CrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11404	MG3-6-human GPR146-G4	mA*mG*mG*rCrCrCrArCrUrGrGrCrArCrGrCrCrCrArCrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11405	MG3-6-human GPR146-H4	mG*mA*mC*rArArCrArGrUrGrArCrArGrCrCrCrCrArGrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11406	MG3-6-human GPR146-A1	AGCTGCAGCTGGTTCAACGGCA
11407	MG3-6-human GPR146-B1	GGTGGAGGAGCTGCCTGCCTGC
11408	MG3-6-human GPR146-C1	CCTGCCTGCCAGGACCTGCAGC
11409	MG3-6-human GPR146-D1	GGGGCTGTCACTGTTGTGCTG
11410	MG3-6-human GPR146-E1	GGGCCTGGTGGTGGGCGTGCCA
11411	MG3-6-human GPR146-F1	TGGTGCTGGCCAACCTACACAG
11412	MG3-6-human GPR146-G1	GTACTTTGTCAACATGGCAGTG
11413	MG3-6-human GPR146-H1	GCGGCGAAGTCCACGTGGCACT
11414	MG3-6-human GPR146-A2	ACTACATCGAGCGTGCACTGCC
11415	MG3-6-human GPR146-B2	CGTCCGCAGGGAGGACACGCCC
11416	MG3-6-human GPR146-C2	GGACACGCCCCTGGACCGGGAC
11417	MG3-6-human GPR146-D2	GGCCGGCTGGAGCCCTCGGCAC
11418	MG3-6-human GPR146-E2	GTGGCCACCGTGTGCACGCAGT
11419	MG3-6-human GPR146-F2	AAGCCCGTGGACGCACACTACC
11420	MG3-6-human GPR146-G2	CCTGGGGCTACTGCACTTTGTG
11421	MG3-6-human GPR146-H2	GCTGATGAAAAAGCTGCCCTGC
11422	MG3-6-human GPR146-A3	CTGCGGGGACCGGCACTGCTCC
11423	MG3-6-human GPR146-B3	GTGGTGTCAAAAGCTGCTGGA
11424	MG3-6-human GPR146-C3	TAGCCCCAGGTAGTGTGCGTCC
11425	MG3-6-human GPR146-D3	AGATGATGACCGTGTGCCCCAG
11426	MG3-6-human GPR146-E3	GGCCACCAGCAGCCTGTGTGCC
11427	MG3-6-human GPR146-F3	GCGTGTTGTACACGCTGGCCAT
11428	MG3-6-human GPR146-G3	AGTGCACGCTCGATGTAGTGGT

SEQ ID NO:	NAME	SEQUENCE
11429	MG3-6-human GPR146-H3	CCACCAGTGAGGACACATTGAA
11430	MG3-6-human GPR146-A4	TGAAGGGGATCTGCAGTGCCAC
11431	MG3-6-human GPR146-B4	CACAGCGCCCCACCGGGAGCTCG
11432	MG3-6-human GPR146-C4	TCGGGGGGCCGAGCAGGTGCAC
11433	MG3-6-human GPR146-D4	ACAGGGGCCAGGGCGCTGAGCA
11434	MG3-6-human GPR146-E4	ATGGTCATGCTGGCCTTGCTGT
11435	MG3-6-human GPR146-F4	AGCACCAGCAGGGCGTTGTAGC
11436	MG3-6-human GPR146-G4	AGGCCCACTGGCACGCCACCA
11437	MG3-6-human GPR146-H4	GACAACAGTGACAGCCCCAGCT
r = native ribose base, m = 2'-O methyl modified base, F = 2' Fluro modified base, * = phosphorothioate bond		

Example 28 – Gene editing outcomes at the DNA level for mouse GPR146 in Hepa1-6 cells [00548] Nucleofection of MG3-6 RNPs (104 pmol protein/120 pmol guide) (SEQ ID NOs: 11438-11472) was performed into Hepa1-6 cells (100,000) using the Lonza 4D electroporator. Cells were harvested and genomic DNA prepared five days post-transfection. PCR primers appropriate for use in NGS-based DNA sequencing were generated, optimized, and used to amplify the individual target sequences for each guide RNA (SEQ ID NOs: 11473-11507). The amplicons were sequenced on an Illumina MiSeq machine and analyzed with a proprietary Python script to measure gene editing (**FIG. 21**).

Table 21: Guide RNAs and Sequences Targeted for Example 25 When Targeting GPR146

SEQ ID NO:	NAME	SEQUENCE
11438	MG3-6-mouse GPR146-A1	mG*mU*mG*rGrCrCrCrArCrUrCrArArCrArGrCrArCrArGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11439	MG3-6-mouse GPR146-B1	mC*mC*mG*rCrUrGrUrGrCrCrGrGrArArCrCrUrGrCrGrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11440	MG3-6-mouse GPR146-C1	mG*mC*mC*rGrGrArArCrCrUrGrCrGrCrCrUrGrGrGrGrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11441	MG3-6-mouse GPR146-D1	mU*mC*mU*rCrGrCrUrGrCrUrCrUrArCrCrUrGrGrGrGrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr

SEQ ID NO:	NAME	SEQUENCE
		CrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11442	MG3-6-mouse GPR146-E1	mG*mG*mG*rGrGrCrArGrGrGrGrUrCrCrCrUrGrUrGrArGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11443	MG3-6-mouse GPR146-F1	mU*mA*mC*rUrUrCrGrUrGrArArCrArUrGrGrCrCrGrUrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11444	MG3-6-mouse GPR146-G1	mG*mG*mC*rArCrUrGrGrCrArCrCrUrGrCrGrUrArCrCrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11445	MG3-6-mouse GPR146-H1	mU*mG*mU*rUrGrGrGrCrCrCrUrGrCrCrCrArCrUrCrCrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11446	MG3-6-mouse GPR146-A2	mG*mG*mG*rCrCrCrUrGrUrGrGrArGrCrCrUrCrArGrCrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11447	MG3-6-mouse GPR146-B2	mU*mG*mU*rGrCrUrCrArUrCrGrGrCrUrArCrGrUrGrGrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11448	MG3-6-mouse GPR146-C2	mA*mA*mU*rCrGrGrGrArArGrGrArArGrArCrArCrArCrCrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11449	MG3-6-mouse GPR146-D2	mA*mC*mA*rCrCrCrUrGrGrArCrCrArGrGrArCrArCrCrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11450	MG3-6-mouse GPR146-E2	mC*mC*mU*rGrGrArCrCrArGrGrArCrArCrCrArGrCrArGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11451	MG3-6-mouse GPR146-F2	mA*mG*mC*rArGrGrCrUrGrGrArCrCrCrCrUrCrGrGrUrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11452	MG3-6-mouse GPR146-G2	mA*mC*mA*rCrArGrUrGrCrUrGrArCrGrUrCrArCrGrGrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11453	MG3-6-mouse GPR146-H2	mA*mG*mG*rGrGrCrArUrUrArUrCrUrGrGrGrCrArUrCrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUr

SEQ ID NO:	NAME	SEQUENCE
		CrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11454	MG3-6-mouse GPR146-A3	mU*mC*mU*rGrGrGrCrArUrCrCrUrArCrArGrGrUrUrGrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11455	MG3-6-mouse GPR146-B3	mG*mC*mC*rArUrCrArCrCrUrGrCrUrGrUrArUrCrCrCrCrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11456	MG3-6-mouse GPR146-C3	mU*mC*mA*rGrCrCrGrCrCrGrGrArGrCrUrUrGrCrCrGrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11457	MG3-6-mouse GPR146-D3	mG*mU*mG*rGrUrGrUrCrArCrArGrArArCrUrGrCrUrUrGrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11458	MG3-6-mouse GPR146-E3	mC*mU*mU*rGrArGrArArGrGrCrCrArGrGrArArCrUrUrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11459	MG3-6-mouse GPR146-F3	mC*mG*mU*rGrArCrGrUrCrArGrCrArCrUrGrUrGrUrGrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11460	MG3-6-mouse GPR146-G3	mA*mG*mG*rCrUrCrArArGrUrArGrUrArArGrGrUrGrUrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11461	MG3-6-mouse GPR146-H3	mG*mC*mC*rArCrCrArGrCrArGrCrCrUrGrUrGrCrArCrCrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11462	MG3-6-mouse GPR146-A4	mA*mG*mU*rGrCrCrArGrGrGrCrArUrArCrArArCrArCrArGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11463	MG3-6-mouse GPR146-B4	mA*mG*mG*rGrCrArCrGrCrUrCrGrArUrGrUrArGrUrArGrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11464	MG3-6-mouse GPR146-C4	mU*mG*mA*rArCrArGrGrArUrGrArGrCrArGrUrGrUrCrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11465	MG3-6-mouse GPR146-D4	mA*mG*mU*rGrUrCrArCrArUrGrGrGrCrCrUrCrArCrUrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCr

SEQ ID NO:	NAME	SEQUENCE
		ArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11466	MG3-6-mouse GPR146-E4	mG*mG*mG*rCrCrUrCrArCrUrGrCrUrGrArGrGrCrUrCrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11467	MG3-6-mouse GPR146-F4	mC*mA*mC*rArGrGrGrCrCrCrArCrCrUrGrGrArGrUrGrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11468	MG3-6-mouse GPR146-G4	mA*mU*mG*rGrUrCrArUrGrGrUrGrUrUrCrUrUrGrCrUrGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11469	MG3-6-mouse GPR146-H4	mG*mA*mG*rCrArUrUrArUrArGrCrCrUrArArGrCrUrCrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11470	MG3-6-mouse GPR146-A5	mC*mU*mG*rCrCrCrCrCrArGrGrUrArGrArGrCrArGrCrGrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11471	MG3-6-mouse GPR146-B5	mG*mA*mG*rArGrGrArCrCrCrArCrArGrCrCrCrCrArGrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11472	MG3-6-mouse GPR146-C5	mU*mC*mA*rGrCrCrCrArCrGrCrUrGrUrGrCrUrGrUrUrGrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
11473	MG3-6-mouse GPR146-A1	GTGGCCCACTCAACAGCACAGC
11474	MG3-6-mouse GPR146-B1	CCGCTGTGCCGGAACCTGCGCC
11475	MG3-6-mouse GPR146-C1	GCCGGAACCTGCGCCTGGGGCT
11476	MG3-6-mouse GPR146-D1	TCTCGCTGCTCTACCTGGGGGC
11477	MG3-6-mouse GPR146-E1	GGGGGCAGGGGTCCCTGTGAGC
11478	MG3-6-mouse GPR146-F1	TACTTCGTGAACATGGCCGTGG
11479	MG3-6-mouse GPR146-G1	GGCACTGGCACCTGCGTACCTG
11480	MG3-6-mouse GPR146-H1	TGTTGGGCCCCTGCCCCTCCAG
11481	MG3-6-mouse GPR146-A2	GGGCCCTGTGGAGCCTCAGCAG
11482	MG3-6-mouse GPR146-B2	TGTGCTCATCGGCTACGTGGTG
11483	MG3-6-mouse GPR146-C2	AATCGGGAAGGAAGACACACC

SEQ ID NO:	NAME	SEQUENCE
11484	MG3-6-mouse GPR146-D2	ACACCCCTGGACCAGGACACCA
11485	MG3-6-mouse GPR146-E2	CCTGGACCAGGACACCAGCAGG
11486	MG3-6-mouse GPR146-F2	AGCAGGCTGGACCCCTCGGTGC
11487	MG3-6-mouse GPR146-G2	ACACAGTGCTGACGTCACGGGG
11488	MG3-6-mouse GPR146-H2	AGGGGCATTATCTGGGCATCCT
11489	MG3-6-mouse GPR146-A3	TCTGGGCATCCTACAGGTTGCT
11490	MG3-6-mouse GPR146-B3	GCCATCACCTGCTGTATCCCCG
11491	MG3-6-mouse GPR146-C3	TCAGCCGCCGGAGCTTGCCGGG
11492	MG3-6-mouse GPR146-D3	GTGGTGTACAGAACTGCTTGA
11493	MG3-6-mouse GPR146-E3	CTTGAGAAGGCCAGGAACTTGG
11494	MG3-6-mouse GPR146-F3	CGTGACGTCAGCACTGTGTGCC
11495	MG3-6-mouse GPR146-G3	AGGCTCAAGTAGTAAGGTGTCC
11496	MG3-6-mouse GPR146-H3	GCCACCAGCAGCCTGTGCACCG
11497	MG3-6-mouse GPR146-A4	AGTGCCAGGGCATAACAACACAG
11498	MG3-6-mouse GPR146-B4	AGGGCACGCTCGATGTAGTAGT
11499	MG3-6-mouse GPR146-C4	TGAACAGGATGAGCAGTGTAC
11500	MG3-6-mouse GPR146-D4	AGTGTACATGGGCCTCACTGC
11501	MG3-6-mouse GPR146-E4	GGGCCTCACTGCTGAGGCTCCA
11502	MG3-6-mouse GPR146-F4	CACAGGGCCCACCTGGAGTGGG
11503	MG3-6-mouse GPR146-G4	ATGGTCATGGTGTCTTGCTGG
11504	MG3-6-mouse GPR146-H4	GAGCATTATAGCCTAAGCTCAC
11505	MG3-6-mouse GPR146-A5	CTGCCCCCAGGTAGAGCAGCGA
11506	MG3-6-mouse GPR146-B5	GAGAGGACCCACAGCCCCAGGC
11507	MG3-6-mouse GPR146-C5	TCAGCCCACGCTGTGCTGTTGA
r =native ribose base, m = 2'-O methyl modified base, F = 2' Fluro modified base, * = phosphorothioate bond		

Example 29 – Gene editing outcomes at the DNA level for mouse GPR146 in primary mouse hepatocytes

[00549] Lipofection with MessengerMax of MG3-6 mRNA and guide (0.42 ug mRNA, 1:20 nuclease:guide molar ratio) was performed in primary mouse hepatocytes (1E5 viable cells/guide)

using the guide RNAs described in Example 28 above. Cells were harvested and genomic DNA prepared three days post-transfection. PCR primers appropriate for use in NGS-based DNA sequencing were used to amplify the individual target sequences for each guide RNA. The amplicons were sequenced on an Illumina MiSeq machine and analyzed with a proprietary Python script to measure gene editing (**FIG. 22**). The results indicated that the GPR146-H2 sgRNA was highly effective for editing in mouse hepatocytes.

Example 30 – Gene editing outcomes at the DNA level for TRAC and AAVS1 in K562 cells
[00550] Nucleofection of MG14-241 and MG99-1 mRNA along with the matching guide RNA (500 ng mRNA/150 pmol guide) was performed into 200,000 human lymphoblasts (K562 cells) using the Lonza 4D electroporator. Cells were harvested and genomic DNA prepared three days post-transfection. PCR primers appropriate for use in NGS-based DNA sequencing were generated, optimized, and used to amplify the individual target sequences for each guide RNA. The amplicons were sequenced on an Illumina MiSeq machine and analyzed with a proprietary Python script to measure gene editing. (**FIG. 23**).

Table 22: Guide RNAs and Sequences Targeted for Example 30

SEQ ID NO:	NAME	SEQUENCE
11508	MG14-241-AAVS1-E2	mU*mG*mG*rCrArCrArGrGrCrCrCrCrArGrArArGrGrArGrUrCrUrUrGrCrCrGrGrArArArCrGrGrCrUrArGrArCrArArGrGrGrArArUrCrGrCrUrUrUrUrArCrGrCrGrArUrUrArCrCrGrCrArArGrGrUrArArGrCrCrCrGrUrCrArGrCrArCrCrUrUrGrGrUrGrUrCrGrGrCrGrGrGrCrGrArUrCrCrU*mU*mU*mU
11509	MG14-241-AAVS1-F2	mC*mC*mA*rCrUrArGrGrGrArCrArGrGrArUrUrGrGrUrGrUrCrUrUrGrCrCrGrGrArArArCrGrGrCrUrArGrArCrArArGrGrGrArArUrCrGrCrUrUrUrUrArCrGrCrGrArUrUrArCrCrGrCrArArGrGrUrArArGrCrCrCrGrUrCrArGrCrArCrCrUrUrGrGrUrGrUrCrGrGrCrGrGrGrCrGrArUrCrCrU*mU*mU*mU
11510	MG14-241-AAVS1-B2	mA*mG*mG*rArGrArArCrGrGrGrGrUrGrUrCrCrArGrGrGrUrCrUrUrGrCrCrGrGrArArArCrGrGrCrUrArGrArCrArArGrGrGrArArUrCrGrCrUrUrUrUrArCrGrCrGrArUrUrArCrCrCrGrCrArArGrGrUrArArGrCrCrCrGrUrCrArGrCrArCrCrUrUrGrGrUrGrUrCrGrGrCrGrGrGrCrGrArUrCrCrU*mU*mU*mU
11511	MG14-241-AAVS1-E2	TGGCACAGGCCCCAGAAGGA
11512	MG14-241-AAVS1-F2	CCACTAGGGACAGGATTGGT
11513	MG14-241-AAVS1-B2	AGGAGAACGGGGTGTCCAGG
11514	MG99-1-TRAC-G1	mG*mA*mC*rArCrCrUrUrCrUrUrCrCrCrArGrCrCrCrArGrGrUrGrUrUrUrUrArGrUrUrCrUrCrUrGrArUrGrArArArArUrCrArGrUrArArGrUrUrCrUrArArArArUrArArGrGrCrArUrUrArUrGrCrCrGrUrGrGrGrUrArUrGrGrUrGrGrUrArUrCrCrUrCrGrUrUrCrArArArUrArUrCrCrArCrCrGrUrUrCrUrArArArArArArUrCrGrCrGrCrGrCrCrGrCrCrGrCrGrUrGrCrU*mU*mU*mU
11515	MG99-1-TRAC-H6	mC*mC*mC*rGrGrCrCrArCrUrUrUrCrArGrGrArGrGrArGrGrArUrGrUrUrUrArGrUrUrCrUrCrUrGrArUrGrArArArArUrCrArGrUrArArGrUrUrCrUrArArArArUrArArGrGrCrArUrUrArUrGrCrCrGrUrGrGrGrGrUrArUrGrGrUrGrGrUrArUrCrCrUrCrGrUrUrCrArArArUrArUrCrCrArCr

SEQ ID NO:	NAME	SEQUENCE
		CrGrUrUrUrCrUrArArArArArArArUrCrGrCrGrCrGrCrCrGrCrCrGrCrCrGrCrGrCrCrGrCrU*mU*mU*mU
11516	MG99-1-TRAC-G1	GACACCTTCTTCCCCAGCCCAGGT
11517	MG99-1-TRAC-H6	CCCGGCCACTTTCAGGAGGAGGAT
r =native ribose base, m = 2'-O methyl modified base, F = 2' Fluro modified base, * = phosphorothioate bond		

Example 31 – Novel Type II CRISPR effectors are active nucleases with diverse PAM requirements

[00551] Novel nucleases of the MG3, MG15, MG150, MG123, MG124, and MG125 families were identified from phylogenetic analysis. The MG150 family of nucleases is more closely related to the MG3 family than to any other family identified (**FIG. 24**), and a new group of divergent effectors expanded the MG15 family of nucleases (**FIG. 25**). *In vitro* cleavage activity assays show that nucleases reported here generally have preference for cleavage at positions three or four from the PAM (**Table 23**). In addition, PAM sequence determination for Type II nucleases indicates diverse PAM requirements, as shown by the SeqLogo images from NGS data. (**FIGs. 26-35**)

Table 23: Cut sites of MG Family Variants

Candidate	Cut Site from NGS		Candidate	Cut Site from NGS
MG1-2 (SEQ ID NO: 6)	3		MG71-2	2 or 3
MG1-4 (SEQ ID NO: 1)	4		MG72-1	3
MG1-5 (SEQ ID NO: 2)	4		MG73-1 (SEQ ID NO: 11720)	3
MG1-6 (SEQ ID NO: 3)	4		MG73-2 (SEQ ID NO: 11721)	3
MG1-7 (SEQ ID NO: 4)	4		MG74-1 (SEQ ID NO: 11722)	3
MG14-1 (SEQ ID NO: 678)	1 or 3		MG86-1 (SEQ ID NO: 11723)	3
MG14-241 (SEQ ID NO: 914)	3		MG86-2 (SEQ ID NO: 11724)	3
MG14-244 (SEQ ID NO: 917)	3		MG87-1 (SEQ ID NO: 11725)	3
MG14-246 (SEQ ID NO: 919)	3		MG87-2 (SEQ ID NO: 11726)	3
MG14-248 (SEQ ID NO: 921)	3		MG87-3 (SEQ ID NO: 11727)	3
MG14-5 (SEQ ID NO: 681)	3		MG88-1 (SEQ ID NO: 11728)	3
MG15-1 (SEQ ID NO: 930)	1 or 3		MG88-2 (SEQ ID NO: 11729)	3
MG15-115 (SEQ ID NO: 1042)	3		MG88-3 (SEQ ID NO: 11730)	3
MG15-135 (SEQ ID NO: 1062)	3		MG89-2 (SEQ ID NO: 11731)	3
MG15-54 (SEQ ID NO: 981)	3		MG89-3 (SEQ ID NO: 11732)	3
MG15-66 (SEQ ID NO: 993)	3		MG94-1 (SEQ ID NO: 11733)	3

MG15-94 (SEQ ID NO: 1021)	3		MG94-2 (SEQ ID NO: 8748)	3
MG16-1 (SEQ ID NO: 11718)	1 or 3		MG95-1 (SEQ ID NO: 8782)	3
MG16-2 (SEQ ID NO: 1093)	3		MG95-2 (SEQ ID NO: 8783)	3
MG16-3 (SEQ ID NO: 11734)	3		MG96-1 (SEQ ID NO: 8786)	3
MG17-2 (SEQ ID NO: 7700)	3		MG98-1 (SEQ ID NO: 8819)	3
MG18-1 (SEQ ID NO: 1354)	1 or 3		MG98-2 (SEQ ID NO: 8820)	3
MG2-4 (SEQ ID NO: 11735)	1 or 3		MG99-1 (SEQ ID NO: 11748)	3
MG2-5 (SEQ ID NO: 323)	3		MG100-1 (SEQ ID NO: 8960)	3
MG2-55 (SEQ ID NO: 371)	3		MG100-2 (SEQ ID NO: 8961)	3
MG2-7 (SEQ ID NO: 321)	3		MG111-1 (SEQ ID NO: 9037)	3
MG21-1 (SEQ ID NO: 1512)	1 or 3		MG111-2 (SEQ ID NO: 9038)	3
MG21-2 (SEQ ID NO: 11736)	3		MG112-3 (SEQ ID NO: 11749)	3
MG21-3 (SEQ ID NO: 1513)	3		MG116-1 (SEQ ID NO: 9150)	3
MG21-97 (SEQ ID NO: 1607)	3		MG123-1 (SEQ ID NO: 11750)	3
MG22-1 (SEQ ID NO: 1656)	1 or 3		MG124-2 (SEQ ID NO: 11751)	3
MG22-2 (SEQ ID NO: 11737)	3		MG125-1 (SEQ ID NO: 11752)	3
MG22-3 (SEQ ID NO: 1657)	3		MG125-2 (SEQ ID NO: 11753)	3

MG23-1 (SEQ ID NO: 1756)	3	MG125-3 (SEQ ID NO: 11754)	3
MG23-2 (SEQ ID NO: 11738)	3	MG125-4 (SEQ ID NO: 11755)	3
MG23-3 (SEQ ID NO: 1757)	3	MG125-5 (SEQ ID NO: 11756)	3
MG3-1_long (SEQ ID NO: 424)	3	MG150-5 (SEQ ID NO: 7363)	3
MG3-3 (SEQ ID NO: 11739)	3	MG150-6 (SEQ ID NO: 7364)	3
MG3-4 (SEQ ID NO: 11740)	3	MG150-7 (SEQ ID NO: 7365)	3
MG3-42 (SEQ ID NO: 429)	3 or 4	MG150-8 (SEQ ID NO: 7366)	3
MG3-6 (SEQ ID NO: 426)	3	MG150-9 (SEQ ID NO: 7367)	3
MG3-7 (SEQ ID NO: 422)	3	MG3-18 (SEQ ID NO: 11757)	3
MG3-8 (SEQ ID NO: 428)	3	MG3-89 (SEQ ID NO: 11758)	3
MG4-2 (SEQ ID NO: 11741)	2 or 3	MG3-90 (SEQ ID NO: 11759)	3
MG4-5 (SEQ ID NO: 432)	1 or 3	MG3-91 (SEQ ID NO: 11760)	3
MG40-1 (SEQ ID NO: 5718)	3	MG3-92 (SEQ ID NO: 11761)	3
MG40-2 (SEQ ID NO: 5719)	3	MG3-93 (SEQ ID NO: 11762)	3
MG40-3 (SEQ ID NO: 5720)	3	MG3-95 (SEQ ID NO: 11763)	3
MG40-4 (SEQ ID NO: 5721)	3	MG3-96 (SEQ ID NO: 11764)	3
MG40-5 (SEQ ID NO: 5722)	3	MG3-103 (SEQ ID NO: 11765)	3

MG40-6 (SEQ ID NO: 5723)	3		MG15-130 (SEQ ID NO: 1057)	3
MG43-3 (SEQ ID NO: 8359)	3		MG15-146 (SEQ ID NO: 1073)	3
MG44-1 (SEQ ID NO: 11742)	3		MG15-164 (SEQ ID NO: 1091)	3
MG46-1 (SEQ ID NO: 11743)	3		MG15-166 (SEQ ID NO: 11766)	3
MG47-1 (SEQ ID NO: 5751)	3		MG15-171 (SEQ ID NO: 7605)	3
MG47-2 (SEQ ID NO: 5752)	3		MG15-172 (SEQ ID NO: 7606)	3
MG48-1 (SEQ ID NO: 5769)	3		MG15-174 (SEQ ID NO: 7608)	3
MG48-3 (SEQ ID NO: 5771)	3		MG15-184 (SEQ ID NO: 7618)	3
MG49-1 (SEQ ID NO: 5805)	3		MG15-187 (SEQ ID NO: 7621)	3
MG49-2 (SEQ ID NO: 5806)	3		MG15-191 (SEQ ID NO: 11767)	2
MG50-1 (SEQ ID NO: 5824)	3		MG15-193 (SEQ ID NO: 11768)	3
MG51-1 (SEQ ID NO: 5827)	3		MG15-195 (SEQ ID NO: 11769)	3
MG52-1 (SEQ ID NO: 5831)	2		MG15-217 (SEQ ID NO: 11770)	3
MG6-3 (SEQ ID NO: 11744)	1		MG15-218 (SEQ ID NO: 11771)	4
MG6-5 (SEQ ID NO: 11745)	3		MG15-219 (SEQ ID NO: 11772)	4
MG7-1 (SEQ ID NO: 11746)	3		MG15-177 (SEQ ID NO: 7611)	3
MG71-1 (SEQ ID NO: 11747)	3			

EMBODIMENTS

The following embodiments are illustrative in nature and are not intended to be limiting in any way:

1. A method of editing a B2M locus in a cell, comprising contacting to said cell
 - (a) an RNA-guided endonuclease; and
 - (b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said B2M locus,
wherein said region of said B2M locus comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6387-6468.
2. The method of embodiment 1, wherein said RNA-guided endonuclease is a class 2, type II Cas endonuclease.
3. The method of embodiment 1, wherein said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% sequence identity to SEQ ID NO: 2242 or SEQ ID NO: 2244.
4. The method of embodiment 3, wherein said RNA-guided endonuclease further comprises an HNH domain.
5. The method of embodiment 1, wherein said engineered guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 6305-6386.
6. The method of embodiment 1, wherein said region of said B2M locus comprises a sequence at least 75%, 80%, or 90% identical to at least 19 of the non-degenerate nucleotides of any one of SEQ ID NOs: 6388, 6399, 6401, 6403, 6410, 6413, 6421, 6446, and 6448.
7. A method of editing a TRAC locus in a cell, comprising contacting to said cell
 - (a) an RNA-guided endonuclease; and
 - (b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA

comprises a spacer sequence configured to hybridize to a region of said TRAC locus,

wherein said region of said TRAC locus comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6509-6548.

8. The method of embodiment 7, wherein said RNA-guided endonuclease is a class 2, type II Cas endonuclease.

9. The method of embodiment 7, wherein said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% sequence identity to SEQ ID NO: 2242 or SEQ ID NO: 2244.

10. The method of embodiment 9, wherein said RNA-guided endonuclease further comprises an HNH domain.

11. The method of embodiment 7, wherein said engineered guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 6469-6508.

12. The method of embodiment 7, wherein said region of said TRAC locus comprises a sequence at least 75%, 80%, or 90% identical to at least 19 of the non-degenerate nucleotides of any one of SEQ ID NOs: 6517, 6520, and 6523.

13. A method of editing a HPRT locus in a cell, comprising contacting to said cell

(a) an RNA-guided endonuclease; and

(b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said HPRT locus,

wherein said region of said HPRT locus comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6616-6682.

14. The method of embodiment 13, wherein said RNA-guided endonuclease is a class 2, type II Cas endonuclease.

15. The method of embodiment 13, wherein said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% sequence identity to SEQ ID NO: 2242 or SEQ ID NO: 2244.
16. The method of embodiment 15, wherein said RNA-guided endonuclease further comprises an HNH domain.
17. The method of embodiment 13, wherein said engineered guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 6549-6615.
18. The method of embodiment 13, wherein said region of said HPRT locus comprises a sequence at least 75%, 80%, or 90% identical to at least 19 of the non-degenerate nucleotides of any one of SEQ ID NOs: 6619, 6634, 6673, 6675, and 6679.
19. A method of editing a TRBC1/2 locus in a cell, comprising contacting to said cell
- (a) an RNA-guided endonuclease; and
 - (b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said TRBC1/2 locus,
- wherein said region of said TRBC1/2 locus comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6722-6760 or 6782-6802.
20. The method of embodiment 19, wherein said RNA-guided endonuclease is a class 2, type II Cas endonuclease.
21. The method of embodiment 19, wherein said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% sequence identity to SEQ ID NO: 2242 or SEQ ID NO: 2244.
22. The method of embodiment 21, wherein said RNA-guided endonuclease further comprises an HNH domain.

23. The method of embodiment 19, wherein said engineered guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 6683-6721 and 6761-6781.

24. The method of embodiment 19, wherein said region of said TRBC1/2 locus comprises a sequence at least 75%, 80%, or 90% identical to at least 19 of the non-degenerate nucleotides of any one of SEQ ID NOs: 6734, 6753, 6790, and 6800.

25. A method of editing a HAO1 locus in a cell, comprising contacting to said cell

- (a) an RNA-guided endonuclease; and
- (b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said HAO1 locus,

wherein said region of said HAO1 locus comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 11802-11820.

26. The method of embodiment 25, wherein said RNA-guided endonuclease is a class 2, type II Cas endonuclease.

27. The method of embodiment 25, wherein said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% sequence identity to SEQ ID NO: 2242.

28. The method of embodiment 27, wherein said RNA-guided endonuclease further comprises an HNH domain.

29. The method of embodiment 25, wherein said region of said HAO1 locus comprises a sequence at least 75%, 80%, or 90% identical to at least 19 of the non-degenerate nucleotides of any one of SEQ ID NOs: 11806, 11813, 11816, and 11819.

30. The method of embodiment 1 wherein said RNA-guided endonuclease is a Cas endonuclease.

31. The method of embodiment 2, wherein said class 2, type II Cas endonuclease comprises an endonuclease having at least 75% sequence identity to any one of SEQ ID NOs: 421-431.

32. The method of any one of embodiments 1-4, 30-31, wherein said RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 421.

33. The method of any one of embodiments 1-4, 30-32, wherein said engineered guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 6305-6386.

34. The method of any one of embodiments 1-4, 30-32, wherein said engineered guide RNA comprises a sequence at 80%, or at least 90% identical to any one of SEQ ID NOs: 6306, 6317, 6319, 6321, 6328, 6331, 6339, 6364, and 6366.

35. The method of embodiment 7, wherein said RNA-guided endonuclease is a Cas endonuclease.

36. The method of embodiment 8, wherein said class 2, type II Cas endonuclease comprises an endonuclease having at least 75% sequence identity to any one of SEQ ID NOs: 421-431.

37. The method of any one of embodiments 7-10, 35-36, wherein said RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 421.

38. The method of any one of embodiments 7-10, 35-37, wherein said engineered guide RNA comprises a sequence at 80%, or at least 90% identical to any one of SEQ ID NOs: 6477, 6480, and 6483.

39. The method of embodiment 13, wherein said RNA-guided endonuclease is a Cas endonuclease.

40. The method of embodiment 14, wherein said class 2, type II Cas endonuclease comprises an endonuclease having at least 75% sequence identity to any one of SEQ ID NOs: 421-431.

41. The method of any one of embodiments 13-16, 39-40, wherein said RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 421 or SEQ ID NO: 423.

42. The method of any one of embodiments 13-16, 39-40, wherein said engineered guide RNA comprises a sequence at 80%, or at least 90% identical to any one of SEQ ID NOs: : 6552, 6567, 6606, 6608, and 6612.

43. The method of embodiment 19, wherein said RNA-guided endonuclease is a Cas endonuclease.

44. The method of embodiment 20, wherein said class 2, type II Cas endonuclease comprises an endonuclease having at least 75% sequence identity to any one of SEQ ID NOs: 421-431.

45. The method of any one of embodiments 19-22, 43-44, wherein said RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 421 or SEQ ID NO: 423.

46. The method of any one of embodiments 19-22, 43-45, wherein said engineered guide RNA comprises a sequence at 80%, or at least 90% identical to any one of SEQ ID NOs: 6695, 6714, 6769, and 6779.

47. The method of embodiment 25, wherein said RNA-guided endonuclease is a Cas endonuclease.

48. The method of embodiment 26, wherein said class 2, type II Cas endonuclease comprises an endonuclease having at least 75% sequence identity to any one of SEQ ID NOs: 421-431.

49. The method of any one of embodiments 25-28, 47-48, wherein said RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 421.

50. The method of any one of embodiments 1-24, 30-46, wherein said cell is a peripheral blood mononuclear cell (PBMC).

51. The method of any one of embodiments 1-24, 30-46, wherein said cell is a T-cell or a precursor thereof or a hematopoietic stem cell (HSC).

[00553] 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. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

Table 24 – Listing of additional protein and nucleic acid sequences referred to herein not included in the sequence listing

Category	SEQ ID:	Description	Type	Organism	Sequence
MG123 active effectors PAM	11518	MG123-1 3' PAM	Nucleotide	artificial sequence	nnnnCAAA
MG124 active effectors PAM	11519	MG124-2 3' PAM	Nucleotide	artificial sequence	nnnnATAA
MG125 active effectors PAM	11520	MG125-1 3' PAM	Nucleotide	artificial sequence	nnnnATAA
MG125 active effectors PAM	11521	MG125-2 3' PAM	Nucleotide	artificial sequence	nnGTACAA
MG125 active effectors PAM	11522	MG125-3 3' PAM	Nucleotide	artificial sequence	nnnnRCAC
MG125 active effectors PAM	11523	MG125-4 3' PAM	Nucleotide	artificial sequence	nnnnGnnA
MG125 active effectors PAM	11524	MG125-5 3' PAM	Nucleotide	artificial sequence	nnnnCnnn
MG150 active effectors PAM	11525	MG150-5 3' PAM	Nucleotide	artificial sequence	nnnMCMnn
MG150 active effectors PAM	11526	MG150-6 3' PAM	Nucleotide	artificial sequence	nnRMYTnn
MG150 active effectors PAM	11527	MG150-7 3' PAM	Nucleotide	artificial sequence	nnwMCrnn
MG150 active effectors PAM	11528	MG150-8 3' PAM	Nucleotide	artificial sequence	nnwMCCnn
MG150 active effectors PAM	11529	MG150-9 3' PAM	Nucleotide	artificial sequence	nnnMCMnn
MG3 active effectors PAM	11530	MG3-18 3' PAM	Nucleotide	artificial sequence	nnRGGTY
MG3 active effectors PAM	11531	MG3-89 3' PAM	Nucleotide	artificial sequence	nnRwwYYn
MG3 active effectors PAM	11532	MG3-90 3' PAM	Nucleotide	artificial sequence	nnRmwYnn
MG3 active effectors PAM	11533	MG3-91 3' PAM	Nucleotide	artificial sequence	nnRwYCCn
MG3 active effectors PAM	11534	MG3-92 3' PAM	Nucleotide	artificial sequence	nnRGnCAAn
MG3 active effectors PAM	11535	MG3-93 3' PAM	Nucleotide	artificial sequence	nnCACnn

Category	SEQ ID:	Description	Type	Organism	Sequence
MG3 active effectors PAM	11536	MG3-95 3' PAM	Nucleotide	artificial sequence	nnnMYAY
MG3 active effectors PAM	11537	MG3-96 3' PAM	Nucleotide	artificial sequence	nRwYAYn
MG3 active effectors PAM	11538	MG3-103 3' PAM	Nucleotide	artificial sequence	nnRGCCcn
MG15 active effectors PAM	11539	MG15-130 3' PAM	Nucleotide	artificial sequence	nnnnCwAC
MG15 active effectors PAM	11540	MG15-146 3' PAM	Nucleotide	artificial sequence	nnnnGTRY
MG15 active effectors PAM	11541	MG15-164 3' PAM	Nucleotide	artificial sequence	nnRnRYAW
MG15 active effectors PAM	11542	MG15-166 3' PAM	Nucleotide	artificial sequence	nnnnGYTA
MG15 active effectors PAM	11543	MG15-171 3' PAM	Nucleotide	artificial sequence	nnnnCWAW
MG15 active effectors PAM	11544	MG15-172 3' PAM	Nucleotide	artificial sequence	nnCCRCAT
MG15 active effectors PAM	11545	MG15-174 3' PAM	Nucleotide	artificial sequence	nnRGAYA
MG15 active effectors PAM	11546	MG15-184 3' PAM	Nucleotide	artificial sequence	nnRnRYAA
MG15 active effectors PAM	11547	MG15-187 3' PAM	Nucleotide	artificial sequence	nnnnCAAn
MG15 active effectors PAM	11548	MG15-191 3' PAM	Nucleotide	artificial sequence	GnnnnCMA
MG15 active effectors PAM	11549	MG15-193 3' PAM	Nucleotide	artificial sequence	nnRnRYAY
MG15 active effectors PAM	11550	MG15-195 3' PAM	Nucleotide	artificial sequence	nnnnGAAA
MG15 active effectors PAM	11551	MG15-217 3' PAM	Nucleotide	artificial sequence	nnRnRTGA
MG15 active effectors PAM	11552	MG15-218 3' PAM	Nucleotide	artificial sequence	nnnCnAT
MG15 active effectors PAM	11553	MG15-219 3' PAM	Nucleotide	artificial sequence	nnnRTATW
MG15 active effectors PAM	11554	MG15-177 3' PAM	Nucleotide	artificial sequence	nnnSRYTA

Category	SEQ ID:	Description	Type	Organism	Sequence
MG71 effectors	11711	MG71-2 effector	protein	unknown	MFDNLDASKVNDNFNAVSEVENVYVRDEMGIEDWSCQNIDELAKVLCD VNLGRITTKQKAMQSLPAQTKQKQKAIQIFSGGKAKLAELFVDEELD ECEKKSVSFQDDDLNEFEFVLTAAALGERYEGLLRFKAIYDWLAKIL HLDTSKKDEDEQHLSCECKMQVYEDHKRDLAVLKSMLKGKPLYNKIF RQGDGDISYEKYAKGIKGRNQIDFKDLKKQLEGLAEYKKIMQETSIDD AKTEERLLFRITNGFAFPKQTTKDNNGIPIQVHLAELKCLDNAEGYLP FLGETDNNGLSVREKIEQIVKFRIPYVVGPLAGTRMSREQGRCWVVRK NEKYPWNFTIVNLEESAEEKFITNMTAKTYLVGEDVLPKESLLYSEF MVRNAINNITVDGERLPVDVLEKIFKQLAKTSKVTKTKTLERFFRQE NISFQNIIGIDDKINASMIKSYNDFRRIFGEDYIQLHRDEIENIIRWITLFC DEKKCWSLK
MG73 effectors	11712	MG73-1 effector	protein	unknown	MTKILGLDLGIASVGYAVVNLDEQKFDGGEILTAVGRIFEAAENPKDG ASLSAPRREARALRRILRRKTIRLQQRNLFIKYQILTTEELNHLVASPL PSVWEIRTLSEYEQPLQHIARALLHIAKRGRFRSMRKSAAEKNYETG QLLQGISLLQNLKQSGRQTIGEFYHLPQSEPKRNKAGSYNHSIARSM LEEEVRLILEKQRTYGTALSSSEFEQEFAIAFDQQLKPSPPGCKTFLP DEDRAPKQAYTAELFAALSKINHRIVSQGTSRALSADERQIALDLCLC KENNFAQLRKLELEQENEFNISYIIPRAKQNTDYQPEKKTAVYKMT GYHALRKALKDHHKPLWTTYMDNPNPGGLDQIAVLAATFKSDKEINAL EKLQFPSELIEAVKSLFSFGFMHLSLKAMRNINPFLLEGHTYDKACELA GYNFQAAKRNAGLTKLPPLTEENFSITSPVVKRSIAQTRKVVNALNRK YGPFDVHIELAREMGRNWAQRKELTQQQKENQEERDLIKAQIEGL FPKNSLDIKKIRLWKEQGGYCIYSNQYKPEQILEEGYCVQVDHIPIYSRS FDNTLSNQVLCLTKENQDKRNDIPDYFQRIQRDWDVSFVLVNASPTM RPNKKQKLLRTELSEEDLAGFKDRNLNDTRFISSFVRKYLQNLQLTN KYKQGVFCRNGKITADLRNMWGLSKIREQDDKHHDALDAIVLACCSNA MMQQISTQYTHINKETAALKIKPLFPWPWKEFRITDVENALLSIFVSRPP RKKITGAFHKETYYSAKHLARGFKTLTKTDINTLTAEKLAQRDLEIKY YGVERNKKLYDAIEQALLARTDAKQPLKVYLGPATPVKKIKLIMEG NKGVPVLKGTAVAENGAMPVDFYKNGTYFLVPVYTIDFTKEKLPLI SIPDNQPMVDVDRFSLYKDDYVQIKNKTGETFEGYFKQYNAQTGQIY LETHDRSDSYTVSGKPASEKKFSKSTFVDFTKYQIDILGNQHRVEKEY TGITRKNKGFGG
MG89 effectors	11713	MG89-2 effectors	protein	unknown	MSYVLGLDLGIASVGSIVPEPGRNIIDLGVRVFKAE/TDKEGDPLNLR RESRLSRRRLYRRAHRLSRLNLFNLSSGLIHSKDEVLKNVYNENPWALR TLGLNSVLTNNQLARVYHICKHRGFYWASSADDGQADNGKIKSLSS NQLVMKEKGKGYKTVGQMIFTEYPCQQRNKSGEYSKSLPRTDLDELRA IFKAQQSFSNPVITKDFINAVGCGDRKTGFLWEQRPALQGEDLLKMV GHCREFEKDELRAAANFYSEQLVWLTKNLNRVYDEDSQERPLTREER

Category	SEQ ID:	Description	Type	Organism	Sequence
					DLILNMPLEMKSDIKYSSLTSAFEKANLWKSQGFYKYSVDYEQKTQKK KNTAKSVDTIKSSKNPEDKVFYKSSHLHEIRKALGSSLSEWEKIRTE VLSGKYDRYNRIAYVLTVYKEDSDVIEQLSPYESRTLEALLPVRFSGFV ALSEKSLKKIIPHMVLGKRYDEACSEANYKHYKQNAEFKLYLPLPL FSGREPNGTLIFNEEIGDIPRNPVLRVINQTRKVVNAIVKKGSPKSVH IELARDLAKSRAERNEIEKNEEASRHIKERDEFKLFSGKCLNGTNL LKYRLYKEQDCKSMYSSEIDHKRLFEGYVQIDHILPYSRSDDSQSN KVLVLTNENQDKGNRIPEYFEAKQHGFQWVVKSCNKNQKK KRNLLRCSLSKDAKKDFLERNLNDTRYACRFVKNYIDSLCLSENDSNS GCVVAGQLTAYLRNCWGLNKVREENDRHHALDATTIACCTRKIVQ KVGAWSKSREMNSYSSVDPDDEKLLQKLYVNTTRKPDFFPKP WECFRSEVSRVFESALEKLEKLQCSYTESELKNVTLFVSRACE KIGKGAHGDVTVYRQTSERKENVAVKVSLLKLYARIEAIVDADT RNKNLCDALKKRYEYARKIGKIEDFVDKDIKIFADDNPLHMPNSD GQEDPCNPVKSVRVKEAFSGVPIRNGVAGNSTIIRVDLFKKDGKYCI PVYAWNKTLPNRAVVGKKTEDWALVDDSFECFVSIRQNELLLKIKLK GETIFGYNGFDRDRGSFNILLHNRQDGKDHKQGLIRKGIKTAISITKY DVDVLGNYVLSKPEKRLLEA
MG73-1 sgRNA	11714	MG73-1 sgRNA	Nucleotide (RNA)	unknown	(N24)GUUAUAGUGGGAUACACUAAUAAAGUGAAAAUCGCAAGGCU CUGUUCUUGAACAUCCUUUAUUAUAAACUCCUGCCCAAUCCGGUUG GGAGUUUU
MG89-2 sgRNA	11715	MG89-2 sgRNA	Nucleotide (RNA)	unknown	(N24)GUUGAGCUUCCUUGAAGAAAUUACACGUUGUUAUAAUAGG UUUUCGAAAGAUUACCGAACCCGCCUCCACUUAGGUGAGGGCUUU U
MG99 effectors	11716	MG99-1 effector	protein	unknown	MRDLSYRIGLDIGISIGWAVVVSSETEDHPARIENFGTRIFDSGEDPKTR ESLCQARRADRGVRRLERRRAFRKEMLKNHFQNIQNLNNTFNDDYES CRDDDVYLLKV/KGLDGKLEAAELFKCLAHTCNHRYKDFYEPEDDDDE NEESGVNEKAANLFEKEFAASGKRTVSEYLVKEYFNNGFVKFRNRSGS DAPYMLIRRSLLKDEAEKIKKQSEYYPCLGGINAEERTVSIIFSQRDFED GPGDPNDPHRRYHGFLETLGRCPYKDEKRGFRGTVISDVFAVTNLS QYVFFEKETGECRLDPKIANELVSYLLTNAGLTMTVEVKKLKSHGYEL KKSEKSDDKAISKAVKFLSIKKCVVEAGKSWAALISEDQFDAANLSTL HRIGELISKFTQPSRRVQEMKKAIGIDGLIKAFSGKKISGTSSVSYSYKM TDSINAFLSGDIYGNFQANFIKENAAVKEEERSYKLEPRHIDDPEVRDN RVVFKAINETRKVVNAIDYVGPEDIVIEVASELGKSVAEAREETKRQR ANEKENDRIKSEIAKLISIDVQSVKTTMIERYKLYNIQEGKCAYSLEPL GDLKDVVENVNKVVYEDHIVPFSLLDNTLNNKALVFTRENQIKGQRT PLMYLSEEEKAKEFLAFSNHLSKKTGGISKTKLEYLKLLETIVGEAAAEK LNAWKSRLNDRYITKYIAGLFDKQLIFAGDKKQHVFTVKGSVTQKF

Category	SEQ ID:	Description	Type	Organism	Sequence
					RRWFRGTWGWGKDEKDRITTYLNHALDALVAANLTKAYIEIGSDAIIKS QIYRAHRYQITEEYESYLDKCVKKMSKYGFSEGYTKKLLSKPERIPSF VPRLKEEVAVRFNDSDEAFDGVSKLYSAEAPFDPHPHITSHKQNKK FKGCADSNPIRVEEIDGEAHKIRRIDIKTLSAKKLDLYTGDVSLREEL AAMLGDKPESYTVGDSLKESGKEFFLSKSGAVIRKVSDDGIVSNYYR KEIKDGQYSTLGMILKYCYEVYKDAKGKTRIVGIRFVDVVKKNKKLY QKAESYPEDYASHVMYLFDTGDFVRITDKKGLKFEFGFYQAVKNINSSI LYFSPVNLANTVIKISLTDNIEKYVVDILGRIGGKIRKCEPLQSTAEEK SL
MG14 sgRNA	11717	MG14-241 sgRNA	Nucleotide (RNA)	unknown	N(20)GUUCUUGCCGGAAACGGCUAGACAAGGGAUUCGUUUUACGGC AUUACCCGCAAGGUAAGCCCCGUCAGCACCCUUGGUGUGCGGGGGC GAUCCUUUU
MG16 effectors	11718	MG16-1 effector	protein	unknown	MIKNILGLDLGVGSIGWALIQTEDDQPKQIHGMGSRIVPLTKDDSDQFT KGQAISKNAERTARRTRKGYDRYQLRRALLTQVLRQNGMLPECMD ENMIDLWKLRSDAATEGKQLTLQQIGRVLYHINQKRGYKHAKSDDNG DSKQTKYVEAVNLRYKEIQEKNVTVGQHFYAELLNSKVESGNGPYVTF RIKDKVFPRAAYIAEFDMQMGVQKEYYPNVLTDLEITLNRRIIFYQRPL KSCKHLVGLCEFEMRPYKKDGKIVYGGPKCAPRTSPLAQLCAMWET VNNITLNRNNELEISNEQRRQLVQLCTHETLTKLTDLYKILGITKKD GWYGGKAIGKGIKGNVTLNQLRKALDGKYSQWLEMPERIDVVDNRT AEAFWAVSPKVEETPLFQLWHAVYSLQNVVEELTKLQNRFSITDPQVI DALCKIDFVKPGYANKSHKFFIRLLPYLMEGMMYSEACACIQNHNS MTKAEREARPLAERIELLOKNALRQPVIEKILNQMINLVNRLQQEYGP DEARVELARELKQSRERKDAFDNRNKNKRNKEISALISEQGIRPSRS RIQKYKMWESESEHRCMYCGKVNLSEFLNGADVEIEHIIHPRISILFDDSF SNKVCACRDCNREKDNMTAMDYMASKPEGEFEAYKQRVDEAFNAHR ISKTKRDHLLWRRADIPQDFIDRLRLSQVIATKAVEILQQGIRQVWTS GGGVTDFLRHQWGYDEILHTLNLPRYRQVEDLTEMVHYEHAGQEHD EERIKNWSKRIDIRHHAIDALTVALTRQSYIQRNLTLASHEHMEKLV KEANTPYKEKKSLEKVVVALQPHFSVEEVTQVDGILVSFRAGKRVT PARRAVYHGGKRTIVQRGIQVPRGALTEDTYGLGDKFVVKYALDH PSMKPENIVDPTIRLLVENRITAGKKDAFKTPLYSAEGMEIKSVRCYT SLSEKGVVPIKYNEKGNAIGFAKKGNNHHVAYKDKQSGQYQEMVVSF WDAVERKLYGVPTVITNPKTVWDELLEKELPQDFLEKLPKDNWQYVL SMQENEMFVLGMEEDFNDAIDTQDYNLTNKKHLYRVQKLSHADYTFR FHTETKVDDKDYDGVENGRNTMSLKALVRIRSFNGLFTQFPHKVKIDI MGRITKA
MG16-1 sgRNA	11719	MG16-1 sgRNA	Nucleotide (RNA)	unknown	N(22)GUUGUGUAUGGAAACAACACAUAAGGAUUAUCCGUUGUG AAAACAUCAGGGUGGAGGACCAAGUCUCGCCUUUU

Category	SEQ ID:	Description	Type	Organism	Sequence
MG73 effectors	11720	MG73-1 effector	protein	unknown	MTKILGLDLGIASVGYAVVNLDEQKFDGGEILTAGVRIFEAAENPKDG ASLSAPRRERARRRRTIRLQIRNLFIKYQILTTEELNHLVASPL PSVWEIRTLSEYKQPLQHIARALLHIAKRRGFRSMRKSAAEKNYETG QLLQGISLLQNLKQSGRQTIGEFYHLPQSEPKRNKAGSYNHSIARSM LEEEVRLILEKQRTYNTALSSSEFEQEFRAIAFDQQPLKPSSPGKCTFLP DEDRAPKQAYTAELFAALSKNHRIIVSQGTSRALSADERQIALDLCLE KENNFAQLRKLELQENEFNISIYIIPRAKQNTDYQPEKKTAVYKMT GYHALRKALKDHHKPLWTTYYMDNPNNGGLDQJAVVLATFKSDKEIINAL EKLQFSELIEAVKSLFSFGFMHLSLKAMRNINPFLLEGHTYDKACELA GYNFQAAAKRNAGLTKPLPTEENFISITSPVVKRSIAQTRKVVNALNRK YGPFDVAVHIELAREMGRNWAQRKELTQQQKENQEERDLIKAQGIEGL FPKNSLDIKKIRLWKEQGGYCIYSNQYIKPEQILEEGYCQVDHIIPYSRS FDNTLSNQVLCITKENQDKRNDIPDYFQRIQRDWDVSFVTLVNASPTM RPNKKQKLLRTELSEEDLAGFKDRNLNDRFISFVRKYLLQNLQLTN KYYQGVFCRNGKITADLRNMWGLSKIREQDDKHIALDAIVLACCSNA MMQQISTQYTHNKETAALKIKPLFPWPWKEFRTDVENALLSIFVSRPP RKKITGAFHKETYYSAKHLARGFKTLTKTIDINTLTAEKLAQRDLEIKY YGVERNKKLYDAIEQALLARTDAKQPLKAYLGPATQTPVKKIKLIMEG NKGVPVLKGTAVAENGAMPVVDVYKNGTYFLVPVYTFIDTKEKPLI SIPDNQPMVDVDRFSLYKDDYVQIKNKTGETTEGYFKQYNAQTGGQIY LETHDRSDSYTVSGKPASEKKFSKSTFVFTKYQIDILGNQHRVEKEY TGITRKNKGFGG
MG73 effectors	11721	MG73-2 effector	protein	unknown	MNKLGDMDGIASLGYAVVNNIDENFVNGDILASGVRIFDVAESPDGSSL AAPRRAARSVRRILRRKVMRIKAQKQLFDLNLSPQELDLLSKQDFKT LYQATPEGQPIPSVWEIRARALDNPCLVDICRALLHIAKRRGFRSMRK SEKLTGEAGKLLKGVEEMQKKLQESNFRITIGELLFHLPAATEPKRNKD GSYSHSVARSLLEEVHLILQVQRAKGANALSQEFETQFCIAFLQNPL QPSDPGFCFTEPTEPRAPKNAYTAELFAALCKINHIYLEEDGGQSHALSA AQRALALEKCFSTQKTNYKQLRELFLNPNDIKFNISYTAAPAKKSKKK EEAQSPQAVQAPQEQYDAEKNTLYNMAGFHAKKALKSSPLWAEYQ TNPNGILDKIAEVLRSYKSDGEIRQHLTALGLPAEAVEKLQNVNFSGFM NLSLVAMNKIIPFLKEGFRYDVACKKAGYNFQAPQNNKGLSKLPLPTE EDNHTITSPVAKRSLAQARKVINALNKKYGPFDVAVHIEVAREIGKSFKE RKEIEAEQKAHAEERARLKEVGIDGIIPQTEITDLKKLRLWKEQDGRG MYSLOYIEPRKILEEGYCQVDHIIPYSLSFDNSLNNQVLCITSENQHKK NQIPYEFHSGKAQITWEDFEGYVNSLQNRIMAKKHLRLKQELTEDDL QGFERNLSDTKLISRFMKNYLLANRLTGKYGKQGVFCVNGKHTSTL RGFWRLQKIREDDGDKHHALDAIVIACTNRLMQYISTKYRQNRLEL QRKEVAVPWPFPHFKHAVENSLLSIFVSRPPRKVTGALHKNTEFSAK

Category	SEQ ID:	Description	Type	Organism	Sequence
MG74 effectors	11722	MG74-1 effector	protein	unknown	HIKKGIKTLRTDIQKLTLDLKKQRELEVYKFGVERNKPLYDLIETALN NRPNDKTIQVQMPTKNGGFVVRHIKLISETSGIPVGLGGTALAENDS MPRVDFLEEDGQYVVPVYTMDFAKGVLPVLAQPSGRLMKKENFVFS LYKDDYVNLVNKQGETWEGYFKQLNAQTQIYLESHDRSAQYTVSGK PSNQKKIASSTLKLIEKYQIDIFGKGKHLVKEKYIGIIRKNKGFGG MQVHDDVILGLDIGVSGSLGWALLEEDLQTEGEQRLLRQRTQPQGETTYA LGVRLFHVPENAKTKELNVKRRRTARMQRRRTARRAQRMRVRALL DSLGVPGVRDADAFHLGKGRGAQCDPWQLRREGLRERLEAREWAVV LLHIAKHRRGFRNSKTDNRSGDKEMGQVLQAVSNLQEQEASGLTVG ALLASRERRRRNRADHTGAPRYDLMLSLQENEDILFTRQRELGNPL AGEEVRCRYAELAFAPRPLAPVSHMVGPCAFLEGERRAPRAFTAEFL RYVQALCNMRLRQETGEEPTLSEEQQAACAVFGSVQSVTYKKLRQV LRLPAGCRFAGLSYGVTEKGNVQDPEKADVVMRTGKCCQGTACLRGI LGDAYEALHEQRLDEADAARLAATGALSAPAAALLARGMAGLRLTDA VARIVSELNDLDQIRAVLALLPLAAPRIAALSQAAGEGKLGFQGTARL SLRAMEAILPHMLACGEYAAACELAGFDPRAAQKTDVDIRNPVVLRV FREVRQFSAICREFGFLPGRVHVELLRELVGKSGEERNRISRGLERRTK EKEAARKAVAQLLGKSPETVSAGEVQRYELWRQDQKGCAYYMLWR HAGGERAYGDAMPQGSIPPDWLADGVNAVQVVDHILPRSRTFDNSFHN LCLCCHAAANQAKGGRTPWEWLGAAPQVAAWHDFEQVQSLPLKGLK KRNLYLLRDLNAEVQGRFHARNLTDGYYVARTLRLWLEEEYARHDVP MQDADGRTTRRRFFARPGQVTDLRRHWGVQALKKIDGQRSQDRHHA LDALLVAAACSEGMLQRMVMVRAFQREENGPERLHPCPWQDFSATVGRA LGSVFVSRVERGRTKGPLHEETLRAIREERQPDGETRRVLYERKAVAR LTAADLDKIKDAARCPDVVAALRRWLDAGKPGDALPRSAHGDIIRHVR VCAGEFSSGVVLQSGGQAQASNGGMVRTDIYSRDGKFMVVPVYAKD VADKRLPLRACVAAKAEKDWREMTADYRFLSLTPDCYVLTENRKGE VKEGYFAGANRNTSAISLSLAHDKQNVIQIGIGITTLKRFEKYRVDRFGR LSLVRRREADPRGRS MKKLSFDLGITSIGYSVLTDEAQKYSLLDYGVSMFDKPTDKDGNKSK KLLHAQALSTKKLYLKERKKNLALLFEKYALAKASKLLEQEKKNL YMYKWQLRAKKVFEERLSIGEITLYHIAKHRYGKSLDSGDLLEELC VELGIKIDVKKKEKDDKEGKIKQALSTIESLRKEYPKTVAAQIYEVEL QKERPVFRNHDNRYNVMIRREHINDEIATIRKQKEFGNFENDSEVFVD IIAADDDQKESTNDMSLFGEYYPKEHVAHQYSLSDIFKMYQAVANI TFNKEKIKITKEQIRLLTEDFLNKKKGVKSVKELKYKDVVRKILKLDES KIFNKEDSYQRAKKVEHTITKFFHVDNLKIDKSFIEDIFNADESYVL MREIFDVIHKEKSPKRIEQLKSKVSSLAVIDLIRYKKGSSLNISYAMA KFLPYFEEGMTLDAIKEKLDLGRKEDYSYKKGKIKYLHISTYEKDDDL
MG86 effectors	11723	MG86-1 effector	protein	unknown	

Category	SEQ ID:	Description	Type	Organism	Sequence
MG86 effectors	11724	MG86-2 effector	protein	unknown	EINHPVYVYVSAVLRVVVHLHAKHGTFDEIKVESTRELSLNDKVKKE IDKANAREKEIEKISNDEYQKIAKEYGKNIHKYARKILMWEAQERFD VYSGKSIGIDDFSNRVVDHIVPQSLGGLYVQHNLVLRDENLQKSN QLPMNYITDKEAYINRVEHLEFSEHKINWKKRKNLLASNLDIYKDTFES KDLRATSYEALTANILKRYYPFIDEKKSVDSAVRHIQGRATANIRKV LGVKTKSRSENIHGVDAALLIGVTNPWLQKLSNIFRENFQKIDDEARK NIKKALPYIDGVEVKDIEQKYNISYGEDSIFYKDIWKGAKTVNFW VSKKPMISKVHKDITIYADKNGGIFTVRESIAKFINLKITPTTFPEDFMK KEHKEILEKMYLYKTNSNDVICKIVQQRAEEIEKELLWSEFLDVKNKE EMQEAKANLESVHRELFNNGNVVRKVYQTNLTGFKVIRGGLAT KEKTFIGFRAFKDKKLEYKRIDVSNFEKIKKSDGSKVYKNDIVFFV FDEEKYKGGKIVSFLEDKKMAAFSPNPKYANIQAQPESEFLTFKGGKANS HKQVSVGKAKGHIKLVKVDILGNIESYQVLGNAKSKLLDEIKSIVSH MKKILSLDLGITSIGYSVIEEFGNDRYSLIDYGVSMFDKATDKDGNSSK LLHSASTSTSKLYDLRKKRKKDLAQLFHNFGLGDKNSLLSQEKQNIYK NKWYLRCHEKAFKEKLNINELFTIFYTLAKHRYGKSLDSSDLLEELCEK LNPLETKTKKDDERGGKIKKALKTIELKQNSTKTVAQHYEIELKKENP TFRNHDNRYNMIRREYIDQEIETKQKDFGLDFFDKFDIDNFIEKLKDI ITYQNPSNDMRLFGNCEYVEDEKAAHQYSVISDFKMYESVSNTFNT KPSIKITKEQINKIADDDFTKIKKGNIAIKYKDIRKILALSDDTKIFNK DDSYISKGGKVEHTIIFHFNNSKFDNSFIVENLSLENLKEFEVLQ FEKDPYAIYEKLDKIEDKQTIINLKHKSGNSLSAKAMVEFIPYFKD GLTTDKIKEKLELNRCEDEYSFYKGIKYLNRQFEQDDNLDINNHPVK YVVSATLRVYKYLHIVCGTFDEIRVESTRELSQNEETKKAIEKANKELE KQINDIVQNEQYQIAQHYGKNLQKYARKILLVEAQNRRIYTGEGIE FEDFTKQVDDHIVPQSVGGLSVKHNLVLFVFRDTNIQKSNQLPMNFVK DKQDFINRVEHLEFSEHKINWKKRKNLLATNLDIYKDTFESKSLRATSY IEALTAQILKRYYPFKDKEKQENGKAVSYIQGRATSNIRKILKVKTKTR DTNIHHAIDAILIGLTNQSWLQKLSNIFRENFQKIDDEARANIKKDMPIF EIHDDDEVKYLEPKELVELIEKNYNYDGENSYFYKDIWKGKSVNFWVSK KPMVSKIHKDTIYSKKDDGIYTVRENIINHFNLKITPKTSSKKEFEEN KKILNKMVLFKTNPKDAVCKAIIKRANDIKTLLDSFIDIDTKDKEAMNN AKTKLDELHKKDIPDNNNGKPIRKIKFYQTNLTGFDVIRGGLATKEKTFIG FKAQIINGKLNITRIDVANIDKIKKENDNSFKVYKNDLVFFIYTDGINK GGKIVSFLEDKRIAFAFSPNPFPSIGFQPHFFVTIFNGKANSKQHSNLK AIGHIKLNLDILGNISYQKIGSCSELEEFIKKVIKD MEKYIIGLDLGINNVGWSSVDAQTNNKIKYLGVKQFEASDSAKDRRTQR NTRRLKRRRETRKTDILKILSNINFPNNLTIDTMLIETRCCKGINEQISKQ DITNILCYMATHRGYIPFGDEEVSVFVLDLGGKYPCEYYYEFMKSSNNK
MG87 effectors	11725	MG87-1 effector	protein	unknown	

Category	SEQ ID:	Description	Type	Organism	Sequence
MG87 effectors	11726	MG87-2 effector	protein	unknown	YRALRNTVKNENINEVKKMLETQQRKYYPETNETIENIVTTLQRKRK FWEGPGSINALTDYGRFKTPEDVVEYLDKKKENPEYKYEFDLIGKCS VIPNEKCVSQINFYAEKFNLLNDFINISFKSIEELNKKDDFYETQVKT LRESGLNKVFDYCMSKDTLTIKGLFKDLFATTIDNISGYRQDGHDPNK PEMSTMTNFRSIRKTFKECNANMDFKPEPENTDLYNEINYMMLVPGQV ELINMLSTIMPLSENDEKALKKVFKSKKTNLKYHSLCEKILIRACNDML SLQKNYMQVYKLDYGKESKEFVKRYEESNKGKELMNPFTFDDIIS PQVKKTLRQSVKVIIEIKNEKSLPDVIVVESTKDTLNSEKMRKVYIDIN KKQKALHDKAIKTLSSIGYSEKDISKKIEKMLYEEDGGLCPYCINNQI TLKYLINGSDEIEHILPRSNFDDSDFNRTVSCANCNNKNNQTPLEFLK GNEKESFIERIKSNKNISEFKKENFLFAGDISKYRTRFFNRNLRDTAYAT KEMINQINFNLYLESKNKDERIKTLSTPGQTHSIRKRYDLEKDRDIDI PYHHAIDASILALLPTTKIGSKVVMFQNDNKFFLNENNKDKMTEIGLEL KYVDTSEGKIEYDDYADFKNINDTSNIFMYSPEVKKEPNKGLFNANMY KVIKIDDKYYKIDQINDIYNLSDSKKLLPKLFDDAKNETLLCKLQHKE FYEKLKNYIKYSDSKNSPFEDYQREINNLSKEDFDYKLGHLKMSENA PSVKRLRYTPISEPYLIDKKSNKKGDTYLAFLDSLAQAAGVYVYNETK NCEAFVPIPSVCYNLKTTRKVNRRKHKLKRYKELNLKDYKVYIVITLYN GNTIEVLKKDNTIHKGVVSSYHKTNDKIVLKNGSYFTKSDLEFSIIDYNPI GKSQKRLTKRIK MKKYTIGLDLGINNVGWAKYDLETKKVIDKGVVRFKESSTAQDRRIIR GSRRLRKRQHRVERLAIQLSNINFTSRSEPELLNKRKIKGLNESLSE QEITNIYWEFAHRYGYPFDEEKEPEREVHKADEYPCQYFIDVYKEYG VYRGQCDLISLKDNLKELKQILLTQQKYHSLKTDEVIDNLIYHQSKEF WEGPGASKENQSPYGRYRTLEDEKYEKADPTYHQYLYEMLIGKCEL SIDKDGFMIDQVAPKCNFYAEFNFNDFINMSVKEPSQIDEYRNKITL KGKFTEDTIEEIKKEIISTKKVSLDKLVKKILGLELKDQGYRIDKKYKP EISQFEFYKYLKSFKDEKLNPSWLENDKTIYNQIVYILTVAPSTYAE DMLKDRVKEVEFKKEIDVLIDIKKKKNPDLKYHLSERILKKALDDIK RHNCENFMQIMKRLYEKEMKEYQNNYSTKTQSPYTIEDQYIDHLI ANPQVKTLRKAIKINAIKEEKNYPETIVIESATSLNSKERKKQEEEQ KTFNQLNKEVKELEDNGYEATDKNMQLLNWKNETNESCIYCGESISL KEVLITEIEHILPKSKSMDSNNHNTTCSCLKCNKEKNRTPYQYLTSKN MYEGFKNRV/MNQYDKMSQDKKSNLLFEGDIDKYSIKFINRNLRTSYA SVLVQELKKYNEYLGAKEGYKINIITSPGQLTSKIRQYLKIKDKDRTY LYHHVVDAMILASIPDTEIGKVLIEAQNDQYWFKDKNKENYKEEVY NMLNNVWLSNRDQIQKFNQDCDNMPDNNKEGLIKRSYEVLPKPVRF SQYTEYAKYIKQNDVYKISQIDNIYNLLIRKKDGSADKDKLLDELF LSNKKNTLLCEKKDPKLYQKLKNIYEKNFSINPFVDESKYMYGLED

Category	SEQ ID:	Description	Type	Organism	Sequence
MG87 effectors	11727	MG87-3 effector	protein	unknown	GDKFDCLKQGIRKTDNSNSPLVIKRLYLEKVTPNPKNNITRRKNLYN EFTINKPKKDTLIGLDGLKQVSTRIFYSYEDKKFELPICAFSVNGKLN KKEKNYQTTYNRLIGNKNVKEMGNIYVGEWVGVIYKNGEYFEGRYK GYHKTSNVLEYYINGLDTLSCATIGSSDLRIIHYTTDILGNRHRLDTQKE I MTKNYSIGIDMGVNNIGWSILNNDTKKLENYGVRLFPTSNDAKERREV RNRRLKREKTRLDLDTLLKKGYNEDNTIEENLIEKRVKGLNEKL EKQDIVNLCYMIKHRGYIPFGDEEVLVDLNGKYPCEFYEMYNKNGG KYRNRKMTVRITDNEKEIKILETQSKVYKNINQDFINKVNLNLTTRRK YWEGPSINDLTPFRFKTQEDVENYLEEKRNKPSYEKYIYEDLIKKC DYELEERCCKLNIYYELFNMYQDFINVSFKNIEELENKDCFYETKNGL YKLNKKGLLMVKDYCMNNFKLYTDILKKLFTDKDNISGYRIDKDH KPEFSTLSYRKIMLEFTEKGFDTTWSDYCNEMEKMMLTPGGVE FIKEIENKLVPIYKFNEEEKSLFKELKEYFNKKTLLSYGSLSQKILQKAI NDMLDLEKNFMQVSRIDYGKEARENFIKQYKKTNSKLEINASFVDDII ASPQVKKSLRQAIIINAIEKEGCLPVSIAIESTKELNSDKKKKEIEKEQ KIQENLRKQASNYLSTVFGDSSVETNLIKVMLYNETNGHCAYCNKPL SINDIFADNIQVDHILPISKSFNDSFNKIIICKKCNDDKKNNTPYQFLKN KNYFEFEKRVLENKNISDYKKNLLYKDDLDKYKTRFFNRLRDTAY ATTELINQIEFNLYEILDKKRINTLSVPQITTSIRNRYVKNEDKTSLE KNRDAGVFHHAVDASIVSVSDTHIGQIMLKAQNDKEYWIKNKSNYDD IYKYLINLRIDDTINQIEKINNENIKVSKQSKNPQKLANSIYKMIKK DNEQYIINQIDNIYTFDFKDKDENKKLFEKLLNEENEFLLCYDNDKNT FNYIKIYNEYKNEKGNPFVNYLIDKGEIPDGNISFDYDITGIRMVTKKG NGPIIKRLRYYSKKNDLYILNKKNNKKNKDSNYLALDSLKQFCVKVYVD NDNKKFVFLPIYITISLDPKTKKVNENDEFYKLFVYKNYIGNKNVTFFADI YNGNKEITKKGDTIVSGYYSTFNKANNKLILNNGDTFTLSDKKKSIHT DILGNEKKG MKYKIGLDLSTSLGWAVVELNEADQITSLVDMGVRIFFPDGRDAKSH KPINVIRREHRQMRRRGDRVLLRKRVLQLHKYGLDFDISADIKLED PYVLRARAVSDKLSSAELGRVLFHLALRRGFKSNRKETRGDSGGKLLK KATLALHDAIGDKTLGEFQVDSKRYFADQFDGNKIKDKGALYPTRDM YLDEFNRICSVQNMSSDDMRQQFEVAIFHQRPVLPPEIGTCMFELDQPR AYKFEVPVQRFRVLQINQIRIINNNGEIKELTAEQREKLQDALLATFCG VKRDKSGRPKITFAEVKKLLGLSRNTKENLESEKRRKMDVDATAFAFA ECELADFWRACITDNVKSQVLAHLNDDLSDSLVDYLAHEYGISQDDK AEKLIQPPFEDGVANLSVCAMQKMLPFLEQQGHLYHIAAKEAGYDHAD RGIVHLDTLPYVYGDVMAIRPSLVQDKMGRYRTMNATVHIALNQLRAV VNDLIARFDGEPYAINIEMGRDVSAGADERAEIEKQQAANKRENDRIAT
MG88 effectors	11728	MG88-1 effector	protein	unknown	

Category	SEQ ID:	Description	Type	Organism	Sequence
					ELVAMGVRCRENIQKYKLWENLGKSPDRRCVYTGEISKEKLFSP FEIEHILPFSRTLDDSMANKTISAAVANRFKGNRSPDEAFSDPKSPWKY EDVVARAQNLPDATKWRFNRGAMDFVLQGEKECIARAMNDTRFMTRM AVTYLQHVCAKDNVNGMPGRLTASLDEWHLNWKNAEESKYR GNHIIHAIDAFVIACDENILRKADAKSDMHTTFFPGDFYDFDKMRCE NTIISYRQSQKNPKDAHSTVGCLHEDTAYNLECFEDGGCGVNAVMSHR EELPTTDDKDKAFADKFNVPKTLQMFNDAGVANEEDIAIKFLD WCAMRNIRKVRMYKTGVDVTYVPVFTKQORDVYRAAYLNWYVN TGVASGIVDKKLRAVQQEKEKHLLEQFQDAKQAYKWYVGGNNFCA EIEFIRDDDDTRYPKLRGRWQVEILSNYNAQLNAGAPLWRHKYATAKRI MSLRINDMVMAEFSKDDPKLPKGLVETVAHQCAIEKTDKVNNVVRVK KLNSSGTVYLRPHFIAKEDADTKSWIASATSLQEHKARKVCVSPSGKIL GLK
MG88 effectors	11729	MG88-2 effector	protein	unknown	MNYRLGLDMGATSIGWSIYDVETEKLDTGVRIFFDDGREDKSKASLCV KRRNARGARKLNRRRIHKTQELLKILTTLLGLFPQEONKREDLKNNDP YKLRKEALDRQLSTYELGRTLMLQAKRKGFSNRKDNREEGGKLLK GEAELKDIMQKENARTYGEFLYNCMQRPNDKPIRLKNTFDESGKYKG GLFPFREIYVNEFEQIWHKQKEYYPQILTDENKEKLKNIFFORPLKEA EEGECQFEKGEKIPRAHPLFQEFRIWQVNLNLTSAENEPDYKPLEK VQELIKLLMNPQEVKPNKQGHYANLKKALGLDKNGVNFENFERQNNRD TDLEKGLLVNTTQNAINESEFLAPYWNFFSDAQKGELINVIMRPHNYP FPKTRISIEEEDDLIINYVRKRNLPQEAEEELLFDIDLEDDFGSLSEKAI SKILPFMKQGTPTYHDACQSAGYHHSYKEYEYEHDKLPYYGELGQSCLG KKNPKCVVEEFGKINNATVHVVALNQIRHLINELIIMRYGKPYDIAVEYA RDLNASTQERLAMTDTRDKNELENNQRIIKELQSKLGNHPYSKNDIQKY KIWKKLFPYDKNPLIRECPFSGEQIPLSELNGQKFQIEHLIPFSRSLDD SLNNKVIAIVEANRYKGNRTPFQAFGQSKDGYNWKDIQHRAKKLSVE QQWRFAPDAMQRFQEKQEGPIVRSIDTRYMTRLQDYLYQPIVKEDGK QRVQAVVQGLTSLVRKAWGLNVYKEKADKDYREFHNHHAIDAIVIS AINRAQIKDVVGI LAHVRRDDIREEYKDELQFQSDNNVPEDKKREIKKEI RDVTAKREIAIAKKYFPLPKSFNIPDILQQVAAINISHKPNLKNKQKDS TIGQLHQDTAYGLQKFVDDKSLKAIFKTKKAGDEASDDKTTPKIDITQY IPMFRNKEDKKAYYDAFREWFKFNGKAASMDAKTKEDKKLKAELAQ KEQAAIQTLRQTALKAFFKWFVGGNNFCAEIEYNPNKNIEGVASKDRG EWKTEIVSNYNATVRNSRGEDIAVYWRKYKYPNAKRIMSLRGNDMMVMA FSREQAFDEKFPKGIQYVREKQKSDCQCDLDFRVKMKMSGNGICF TPHNIKENADTKSWIASAGAMQKYRVVRKIHISYMGRIQNA
MG88 effectors	11730	MG88-3 effector	protein	unknown	MKYKFAFDLGSTSCGWA VNTDEDDGNVVGGLADMGVRIFFPDGRNAKT KEPLQVARRNARGARVRNDRILQRRHIKIDILLKENGMIDCSDERENP

Category	SEQ ID:	Description	Type	Organism	Sequence
					YKLRSDAVDKETLTKQLGRIMYNLSLRRGFKSNRKPQTQKENDSDLKKA TEKLKQELKGQTLGQYLWGKMKKLNLDKEKENKGDKVKNKIGKVR SNLFDGNKIKDGSVPSREMYEDEFNRWISQAEEHDLTDELKGGK NAIFFQRPLKQQRGFCMFEDGELRIYKAHPLFQRFRALQTINQMEID DKPLTEQREALKKELEFEEKTKNTKAGTVSFSDIRTLGLKGVKINL EKNDDEENGDNTRADKTPEDKDNKKDDDDKEYDNIYADKTAYLLSRP ECFGEKWFEIPFDEQCSIVDILTDVYNNIEKKKKYDEQEQNGKHILE DDEIKQFLQEHYNLDDEQCNAINMNALEEGTGSLSQKAIEKILPYLEEG QLYNDAKKSAGYHHSFDDGDMELGELPYYGDLKKSCVQDDKDN RITNISVHVVALNQLRLVVELIKKYGNPDFVAVEIARDLKMGTIELKN LNKQNSNKKENDKITKAKEANGPNNAKDRKFKLWELCQKRCVY TGKQISATELFSRVRHVDHILPFSRTFNNGFFNKVVCFGDANEDKGNST PYEAFKNGYQGQSYEELDRVKKIVEAMKEKMKKTKTVKDKDGKK KKVDIDEFSWRFKEDAMEKFEQEGLIARQLNDTKYMSRLAVQYLKH ICKVEYTTDEEGKEHRKNKNCYGLPGTMTDFCRKGWGVNWLKDKSNK EGYRSSHAHHAVDADFVACMTIRGQLQKIASMANWIEHGGQCQDDK LYLSILFKKCKKPPDITFDREIRYELCDKMPISFKPKLKDQPKQENSTVGA LCEDTAYSLLEFDKGLNGVFKREDVGSVLKDLNIDTQADKLKEY VETEEAFNKFKEYCEKNGIKKIRCKSFADSYTPIFKTKEERDEYHKA YEDWFIFGRSPANETEEQKQERKEQELLKIVQQKALKAYKWFVG GNFCADVYQISPRDKVYTDKKEQGSWKVEVLSNYMATLNKGQALW RKKHPTARLVMRLKIDDMVMGENFTKEEAFQKLQEEIEKWEKSKEM KKYKDKLLEWETHEGKEPKKPKPKSINEHIEKCNKEKTSSTSLFR VKKISSDGSVYIRPDFITKEESDKSVKLSASSYQKYKIRKVFVSPAGKL VDNGFSDKWNDTKCN
MG89 effectors	11731	MG89-2 effector	protein	unknown	MSYVLGLDLGLIASVGWSIVEPGNRIIDLGVRFKKAETDKEGDPLNLIR RESRLSRRRLYRRAHRLSRLNLFSSGLIHSKDEVLKNVYNENPWALR TLGLNSVLTNNQLARVYHICKHRGFYWASSADDGQADNGKIKKSLSS NQLVMKEKGYKTVGMIFTEYPNCQRNKSGEYSKSLPRTDLDELRA IFKAQQSFSNPVTKDFINAVGCGDRKTGFLWEQRPALQGEDLLKMV GHCRFEKDELAPAAANFYSEQLVWLTINNLRVYDEDSQERPLTREER DLILNMPLEMKSDIKYSSLTSAFEKANLWKSQGFKYKSVDYEQKTQKK KNTAKSVDTKSSKNPEDKVFYKSSHLHEIRKALGSSLSSEWEKIRTE VLSGKYDRYNRIAYVLTVYKEDSDVIEQLSPYESRITLIEALLPVRSGFV ALSEKSLKIIPIHVMVLGKRYDEACSEANVYKHYKQNAEFKLYLPLPL FSGREPNGTLIFNEEIGDIPRNPVVRVNRVQTRKVNVAIVKKYGPSKSVH IELARDLAKSRAERNEIEKRNEEAASRHIKDEFEFKLFSGKCLNGTNL LKYRLYKEQDCKSMYSSEIDHKRLFEKGYVQIDHILPYRSYSDDSQSN KVLVLTNENQDKGNRIPEYFEAKQIHGFSWYEFQWVKSCKNLNQKK

Category	SEQ ID:	Description	Type	Organism	Sequence
					KRNLLRCSLSKDAKKDFLERNLNDTRYACRFVKNYIDSFCLSENSDNS GCVVAGQLTAYLRNCWGLNKVREENDRHHALDATTACCTRKIVQ KVGAWSKSREMNSYVDPDPSVDEDEKLLQKLYVNTKRPDPFKP WECFRSEVESRFESALEKLEKLLQCSYSELSKNVTLFVSRA KIGKALHGDVYRQTSEMRKENVAVKVSLLKLYARIEAIVDADT RNKNLADALKKRYEYARKIGKIEDFVDKIAKIFADDNPLHMPNSD GQEDPCNPIVKSVRVKEAFSGVPIRNGVAGNSTIIRVDLFKKDGKYYCI PVYAWNKTLNRAVYVSGKKTEDWALVDDSEWCFSSIRQNELLLKIKLK GETIFGYNGFDRDRGSFNILLHQRDQDGKHKQGLIRKGIKTAISITKY DVDVLGNYLSKPEKRELA
MG89 effectors	11732	MG89-3 effector	protein	unknown	MDLIFGLDLGASVGSVVDNENKRIVDLGVRAFKAETEDKGSNLN VRRSRLSRRRIYRRANRLNSLLNYLKSLGSLSSKDEILNNEHENPWNL RVKGLDGVLSNNQLARIYHICKHRGFYWSSEAETEDTEKGKIKKCL AQNSLALTNEHFTIGETILNKYPDAQRNKADEYTKSISRVLLNEELKQ ILTVQKEVFHNPLTDDFFKAILGTGDKKSGFLWKQKPLQGEQLLK MVGHCRFEKDELRSKPANYFAERHVWLTLLNLRYSDEDCEDRALTV EEISIVINKIYEQKSDIRYSSLTTFVKSIGWPKNHQYKYKGLNYDQLTS SKKKKVEDTASTSEDSTDTKTKAKTNPESKIFYKSSCYQNIKDAYMSN SLEQEWNLSTQISQGNVDYRNRIAYILSIYKDDDEVKKHLLDCGEKSE VAEALLKIRFNGFSALSEKALKIIVPIMEQKGRFVACSEAGYAHYKQ SQDSKEKRKYLPPLFSGREPNGTLIINSELDLPRNPVVMRVINQTRKV VNALVKKYGSPKSVHIELARDLSKTFEERIDIQKQKEIKERRQKEEE FDRIFGVGIRSGKNLEKYSLYKQDQCKSIYGETIDLGRLEQGYVEVD HVLPSYRSFNSQDNKVLVLTKENQNKGNMLPYEFMSHNLNDWNQFE ARVLSNKKLRKNKRCNLLKKSLLNRNSKKEFLDRNLNDTRYACRFVKN FDKYLRLSDKADKSGCVVVSQQLTAYLRKHWWGLNKNRSENDRHHAV DATVVAACCSRRMVQLIGYWSKHKEQYLLKDSQSDPDLEDELLKK SIGSQKLYFPYPWVKFKELNLRVSSDIEELKNELSLFESYTEEDISKV KTLFVSRAIQIGRGALHADTVRSQTEEMNKEKVAVSRVKLSSELSYDR ISKIVDSDTRNKNLCNALKRRFEAYCKSNNEISKLKAKDSAKIFTNN PMHMPNSNGEEDPLNPVTVRVKETFSGVPIRHGIAGNGDIFRVDIFF KEGYVLPVIYALAKELPNRACVAKKHESEWTVDDTYQWCFSVTQYD LIKIELKKETYFGYFNGFDRATGAVNIIHLDHRSSTEKVKKGLRSIGLKTA KSVTKYRVDVLGNYLAGAEKRELA
MG94 effectors	11733	MG94-1 effector	protein	unknown	MRKKIRYVIGLDIGLASVGWAALLDENDNVCGIVRAGVHTFDEAVVG QSKITGAAYRRGYRGRIRRKVNRIQRVKNLQRLNIISKKDLLEYF SGAVENIYYLRCAAIQNEPAILNNKELAQLLIYAKHRGYKSNTSYEQ KTDDSKKVLALSSENKKVMLEKGYQTAGEMLYRDEKFRKRYGSSEE CELLLVNSGDDYSHSISRELLVEEVHIVIFARQRELGNKLTTKLEDEDQF

Category	SEQ ID:	Description	Type	Organism	Sequence
					VEIMQSRNNYDEGSGEGSPYGGNLEKMGVGECTFEKGEKCRACKASYTS ERFVLEKLNHLRIQSKNGDVRLATEERDAIHLAYKNKDVKYKALR TILKLNPFERFGLTYSRGDIENSTEGKSVFVSEYWEYKVKVGLFYD DLDDNEETQQLDSIGTILTCYKSDDLRRRKFEQLHLEQEKIEHLLALNY TKFQNLSEKAMKNMPELEKGLSYTEACSNAGYGDKEETEGKNKYISK ELLNNTLDSIMNPTVKRAVRRTRILNELIKQYGSPPVEHVEMARDLTH SQVTNKMKKRQDENKAEKEAKRFICENFGKTEAQVSGKDILRYRL WKSQNQIDIYSNTMIPVSDILDYEKYEVDHIIPYSCSFNDSFNKMLVRK KDNQDKKNRTPYEYIGSDEKKWEAFATCANTYVMNYGKRKNMLTKV PASNTGEWMSRNLNDTRYTTKVVTDLRKHLEKFEAYVDQKRKKHIYPI NGGITAKLRYEWGLEKREKSKHHAQDAVVIACCTDGMQRLSRQY MLQEIGIVTWKNHKLVDRTTGEIVETNLPWECFEEVEMFMADSP DYIEKAKKNKYKGEAPKPIFVSRLPQKTTGKINEDTLRSVRIDSKGKA RFVNTKTLQDLKLVVDGKQKDYRPEDDKLLYDKLLERLVKNDD AKVVFAEPFYKPKKDGSDGPIVRSVKTYGKTVKNQVLVGDGVAERGG IYRCDVFKRKDEYAVVYVYRDLYIGNLPNNAHFDIEMKKGEFEFSL YKDDLIRFVKDGKEQYAYYKYINANNSQITYTEHDTSKETKCTTIRILD KFQKMNVDLLGNYSDDKEEREW*
MG16 effectors	11734	MG16-3 effector	protein	unknown	MATKKILGLDLGTNSIGWALIEDTSDNPKSILAMGSRIVPLSTDDSTQFA KGQAITKNADRTQKRTARKGLNRYQMRRAAMLTEELRRHGMPLPERTD ENIMDLWRLRSDAATDGKQLSLPQIGRVLHYHINQKRGYKSHKADNSA NTKQTKYVEAVNQRYRDIQACHQITIGQYFYEQLSSAVQTPSGSYTY RIKDKVLPREAYIAEFQIMKVQRVFPVDPVLTDELVDLNRNHIIFQRP LKSCKHLVSLCEFEKRPFKREDGQIVYSGPKCAPRTSPLAQFCTVWEA VNNITLNRQNETFEITQEQRVAMADFLNQHDKMGVKDLQKILGISPK DGWWAGKAIGKGLKGNITFTQLREALGNLPNAEHLKMKLSMVDAA VDTTGTGELRQVSPQVEEPLFRLWHLVYSLQNEDELKALRKQFGID DEEVLDKLCKIDFVKPGYANKSHKFKRLPYLMGYQYHEACAHIGV NHSDSLTAEQNAARPLLDKIPLLEKNELRQPVIEKILNQMINVVAALKA EYGDIDDVRIELARELKSSKDEREAAFKRNNENERQNKIYENRREYGI QPSRSRIQYKMWEESENHLCFYCGKPNVNTDLAGAEVEIEHIPQSVL FDDSYSNKVACACRACNQAQGNLTAREFMEKHSKEEYDYLRRVDDAF NAHRISKTGRDHLWWRKEDIPQDFDRQLQSQYIAKKAEEILRQGYR NVYATSGSVTDFLRHQWGYDEILHRLNLPYQQVEGLTEDVTYDHCG QEHQQRERIKGWTKRDLHRHHAIDALTIATLQQSVIQRNLTLNNSREQ MFDELGKRTDTPEYTEKRSLEKWWDAQPHFSVQEVTDKVDGILYSFR AGKRAATPAKRAVYQNGKRHIVQTGLQVPRGALSEETVYGKLGKNKY VVKYPLGHQSMKMDDIVDPTIREIVTRLNFAFGKAKDAFAEPLYSDA AHQMGIKTVRCYTGLODKAVVPVRFNAQGEFVGFVKMGNHHIAIYR

Category	SEQ ID:	Description	Type	Organism	Sequence
MG2 effectors	11735	MG2-4 effector	protein	unknown	DAKGYQESVVSFWQAVERKRYGIPVVIEQPHEVVDKLNSDNIPQDF LETLPHDDWQFVVSQQNEMFILGMDDADFEAAEMEOKDYRTLNKYL YRVQKISSKEYCFRYHTTETSVDDKYDGVINKSISMELQKLRLTSISAFF SQPHKVRVNNLLGEVSAL MNSTRSTPLVLSFDIGYASIGWSVAEVDPAANNLQAGVVTFPSDDVLNS ERAGHRRARRNIAARRNRVQRKLKLSVAGFVTAEEETLDRMERKS APEWRHCPWFLLAARVLGESPESTLTGLQHFHLRWYAHNRGYAPPSW GLFDEADGEQEEDEFKVRNAEKAMHEFGADTMAQTWCALLDADPTA GRWPEPRHWAKGENMAFRERVQAEVTRLLVAHVGLKPGVTSDFVR CLIDDWEHHPKIRSWLQAGADPRTGKLDYALPKRYEGGLFGQHPR FDNKIIPWCPFTLKEDTNSGKISGRNVPKKHSRAFLDFKVA MRLNDLA LSELGESGGRLSAGQRMTLFKRIEGYGDVTVRQFADLVAEVCAIPKPD LTTRIPEREGSDRPFELRPERKALIAILTGGRLSPWPLIHSIWDCCFEDA EAVLRPVFHGKPTSWADLIEQAKAPDVLLELEELFRLGKGKPSRSKK AQPPPDFEVLLRCKITITKAHGRARYCSDKLRAATDEALGGLDPRRAP TTETSDDAGCLYLNERQDLQDLPLSKLTNNHLVRHRLIFRRLIQD LVNTYANGDRERIDAIVVEVARDLNEYSGKNTKQRVALFQEKQRPEND AAEFAQAALIDDAQYTPAQALATYANVRFRMLREQNFECPTTGR HLSAEDIAKDRVDFEHIIPRSLKPTDSLDAVITYRAVNAQKRNRMTAMR FIKDMAGERLDADDAGWSYHTPQQFETAVNMRMPKGRNLTAAKRSQ ANRCDALLVEDYEPREADFLOQDLTQTGYLMKYAVGEARKFFRSAPR QPRFHLEGRVTTFFKKA WRLTETLAPISPLFLREYADPRTGRWQSTV RPKAE LRRFTQLHHALDALTLGLATALVPGERKELRRALSRLQAKGD DATLLRSDPKLGEALRWRTEDRFEAAPLSGKLESAAVRRALAEGRVVQ HVPKAKRQGMKVDNSNFFGFVEFDETGRLRVQRKMRSPTRRREIKTV KNGKNLHTLSLSDPKSWLGA PDHPLRRKQLEHGLRTENDLANPKL GNIRGMLPIRENWGIALITKDGSPRLDIVIPYINVHQWLEVLALENGSGS PVVLRKGHLVGFDAEKCEPEYCGAWMLLGVDKGRSGTTLELRPWW VAPRKGGTKESSAKQAIPASGYSEKEGKASGVFLQRSADVFLKLGLR PLDHDLTGIAAF HNTSGVYDLTVARTFIEEAHKLFAAQRQFGNAFATENIENEYCELLS QRHFSDDGPGGERTFKFDLRGNCTEFKEDELRAFKACYTFFFKLLQDIN HLRIUPEYRKGSNKQTRPLTPEERQKIIDLCLKSSSIDFSKRLKELAD DEIFNRVGYDVKKKKSKKDAEEQPEEQLTPEQRTKCEEATKFTQM QSYHEMRKALDKVAKGTISKFSHDKLDEIGEILSLYKADDKRRERLEQ IGLSNEEIEALLPLTFTKAGNLSLGA MRKLIPYLEQGLTYDKACEIVYG DIHRAQYKGERMPLLSFGKLEEGALDSVNNVVLRAIAQTFFKVVNAII RRYGSPQAIHIELARDMKRNFADRDQDIKSKQKDNWSENRRRREKVEEI KGSVATGQDIVKMKLYEDQNGVCLYSGKQLELHRLFEVGYAEVDHIV
MG21 effectors	11736	MG21-2 effector	protein	unknown	

Category	SEQ ID:	Description	Type	Organism	Sequence
					PYSKCFDDSYNNKVLVFSSENQRKGNRPLEYMLAEGDEDEKLDDYVTL VEANIKNTRKKQRLQLTEQDITDQWKNLTDQYITKAADILRN YLAFEEDSPFIKKPVRSGAVTDQVRKRLGLQKHREEDLHAMD AVIAVTTDGYNNRISRYTQRREFGKRIGCYKDKQTEGEVEIERQKQQA PLYIDPETGEKLTQVFDHKYAPTFAPWKEFTKELKARMAPNADEAI RQLYLPYSYSEEIKPIFVSQMPDRKISGQAHAEIIRSAIDVDSEGKERII AVAKTPLTSLKLDKDEIDGYMPSDDRLLYEELKNRLIKTKTSCKTY GNAEQAFKEPVYKPKKDGSGQPRVYKVKTWKPTTSNVKAVAGGIACK GDIVRVDFHITGKDKQGYVFPYVADTIKNTLPKHAVVTNFKSSKVE WKEMDDSNFISLYKGDLIHIELCACDRDKDSNNKIRKAKDMVYVYDG MKNILGLDLGTNSIGWAWIQSKVPQQTDDCFSSSEYLMPCATIRMAG SRVLPMDGKMLSGFESGLAVSKTKERTTYRMARRVNERFQLRRERLN RVLRILGFLPAHYEACLDYRGKIDEKNVTIPWVPTADGKRKFLFYAS FLEMKEFHEHPNLEKIPLDWTLYYLRTKALQQAITKEELAWVLHS FNQKRGYNQSRDEVKDEDAEQKEEYVVKVSVVSDGEKKKGKTSYI VTTESNLQFTTENAAAPSWLKDEREFIVTKLNPTGLPKMNQEGRIDC TVRIPKEEDWELKKKRTEALJADSHMTVGEYFHSLLDNDPDAKIKGAK VGTIDRHFYKEELAILKKQAAFPPELKDAAERYAECVSALYASNEMHR NILSAWDMPRLIIVNDIIFYQRPLKSKKSDIAECPFESRYFMDKEKKLQK QGKICPTSHPHFQYRIWQFLSNLRLRREVRNGRYMTDVEDVSAHY LTDKVVELYEWLAGKANVKQKELLSKLRMSEKEFRWNYVEDNIYP CGETRSLSTRKKAKPLSLDSSSDSGSHTFELWHILYVSDLAEL RKALRRFARKHDFTAEQEAFVETFKCPFKDYGAYSKALVKLL SLMRIGKYWNADRIDTNTRRRIA CLLDGEACDSISLRTREKVAERGLQ QSIEQFQGLPQSLACYVYVDRHAEASEVVRWESPADLQQTQRFKQYS LRNPIVEQVVLTLRVVHDLWEEIQKDGDTIDEIHLEMGRLKNTAEQ RARIMRRNRENEMTNFRIKLLQELHDCQPDIEGIRPYSPSQQLRLRY EETVWESESGKLGGEASKDIPDDIKKIRDLKSKPSDKPIPQSAIQRYRL WLDQKYLSPYTGRPIPLARLFTADYEIEHIIPRSIFFDDSVANKVICESAV NKLKNNRLGMQFIREMDGRVETVKLGEGETSILSVGYVDLVNDLF RNNPRKRNNLLAEETEDFCHRLQSDTRYIARYIKGILSNIVRQRAADG TLEQEATSKNLIVCTGQITDRVKQDWGLNDVWNHITPRFERLNRMTG SNDYGEWCCKEGKRYFQTRVPLHLQIGFNKKRIDRHHAAMDALAIAC VNRNVVNYLNNAHAHADRLMDLRMRICRPSGNGQTCKEIRSPWKNEFA HDAECALQAITVSFKQNVRIITKATNTYEGYDASGKKVRRCCQTSSDHY SIRKPLHKDITVYGEVVLPPVNVQVPLKALLRVNVRNGKLRKKIQEM QSSGLTDKQIVDFFMKT CADSPENWNSINFKKIEVRAYSNEEGQTRMAAI RTAIDSESEKVGISITDVSIQRILLNHLRECNCGDSEAFSPGIEIETMNRN IVRLNGGKPHLPIYKVRLGEAMGKFAVGQGRGNKGKGFVITAKNTNL
MG22 effectors	11737	MG22-2 effector	protein	unknown	

Category	SEQ ID:	Description	Type	Organism	Sequence
MG23 effectors	11738	MG23-2 effector	protein	unknown	FFAVYANDEGKRSFETIDLHCALQMKGQSSVAPPINENGDKLLFVLSP NDLVYVPSESELQHDIDSEDLDHVFVKVVSFSENRCFCVPHSMSSPIAA GFEFNSPNKIEVVSRLGLFEQDKETVSIKNICLPKMDRLGHLHLVKI MSEKIPYIIGLDMGTNSVGWAVTDENYKILRGKGKDMWGVRLFDEA QTAERRTNRVSRRRRQREVARGLVKEYFADALNAVDPGFMVRLLEE SKYWLEDRSEENQKQKALFNDKDFDKEYTVTYPTIFHLRKELIESTE QHDVRLVYLAILNLFKRRRGHFLNKSLESDGETMSMAEAYAAVDEAA ALEITLPMPIIDAKKLEEVLSQKGVSRKFVEQDTNEFFGFSKASEAREL VKLMCGLTGKMRNIYGEELIDDDNKKLALSFNSNDYEEKMNEVAELV GDEMRLLEAVKEVHDIALANILSGEQLSVARVKQYNKHKEDLQQ LKRVLRTYDKAAAYKKMFRVMGKDNYSAYVGSVNYKEHKERRNAGA GKDGESFRKAVEKVIDALPEEAQLDQDVIEIREKIKNEAFLPKQLTSAN GHPNQVHLRELKRILENASGYLPFLNEIDESGLTVKERIAQLYEFQIPY YVGPLSKQNSKNAWANRRPGEEKGRILPWNFQKIDVNAQAAEDFIKR MVRHCSYLDAAFTLPKQSLMYEKYMLNELNLRINGEKPTVLQKQKQ IYNELFGKGKRITQKALINYLKDEGIVEKDEPIISGIDGDFKASLSTFG KLRTVLKEEARKSSQEMMDQIVFWATVYGDDKRFIRARIEEHYSEIL DDHAIKQLLGMKFNWGNLSKAFLEMEGASKEDGVYRSVITQALWET NDNLMLLGRQVITYKEELEKRVQTEKEPLAEWTIDDDGMYLSPSVK RMVWQTLKIIREITEVRSAPSIFVEMARDDAQTAKNKGKRTKSRK DFLECYKDDKAWKDELTSVDDGELRAKLLYLQMGRCMYSGEA IDLASMSGNTMYDIDHIHPRHFVKDDSLNENLVVKDKNAHKSDNY PLESEIRNKQFGFWKSLLDKGLITKTKFTRLRSEDESPPELAGFINRQL VETRQGTAKITKILQQAFPDMDMEVFTKAGVAAKLHDFDLVKVRC VNDTHAHDAYLNIVAGNVYNNAKFTSNPLRFKNEVKKGNASYHMDKI FFRDVKGKGNKLAVWQAPNNEEKTPTGTAIVREQLARRTVLQTRRSYMA HGILSDATYISKDTAKTESYRPVKSSDERLSDVKKYGGMTSIKNTAYAL VEYTVKGKTIRSLGVPYLGNCSDKDDKLLQYLQELQRENKKNQVE NVSVMYPIRQSYLVKVDGYYYLGGATGSSVYLLDAMSVYLSKEDM GYVKKVEKAVAQQRYDECTKEGEFVLTREKNMDLYNKLVDKFSHG FIKRKASILKTLEEGIDVFSELNIEKQCGIIMQIFAWITTSQQNVNLTDIG GVAHAGTLLISKKLSTREALLIEQSLTGLWSKTDTLLTV MSADSLNYRIGVDVGDERSVGLAAIELDDGDFPLKLLAMVTFRHDGGK DPATGKTPKSRKETAGVARRTMRMRMRKRLKLDLKKLRDLGYFV PRDEEPQTYEAWSSRARLAESRFEDPHERGEHLVRAVRHMARHRGW RNPWWFSQSLEEAQQSEPTFGRILERAQHEWGERVSDNATLGMGLGA LAANNILLRPRRYEHNPKTGKNAEKLNRVGGQEPILLDKVRQEDVLAE LRRICKVQGEDQYPELAHAFTQVRPVPTERYVGDPLQPMKIRASR ASLEFQEFRIRDAVANLRIRVGGSERRPLTEEEYDRAVDYLMYSDTTP
MG3 effectors	11739	MG3-3 effector	protein	unknown	

Category	SEQ ID:	Description	Type	Organism	Sequence
MG3 effectors	11740	MG3-4 effector	protein	unknown	PTWGEVADLEIAENTLIAPVDDVRLNVAPYDRSSAIVEAKLKRKTQA RQWWDDDDANLDRSQLILLVSDATDDTARVAENSGLLLEVFESWDEE KQTLQDLKFDSGRAAYSIDTLNKLNAVMHEHRVGLHEARQNVFGVSD TWRPPRDLDEPTGQPTVDRVLTIVRRFLDCERAWGRPQKIVVEHAR TGLMGPSQRADVLEIARNRNANERIRQELREGGIEAPNADIRRNII QDQESQCLYCGKEIGVLTAELDHIVPRAGGGSSKRENLAACACNAS KGSRPFAVWAGPARLERTIQRLRELQAFKTKSKRITLNAIIRRLKQRE EDEPIDERSLASTSYAATSIRELERLEQHFENDDLPDGFAPVAVDVYGGSLT RESRRAGGIDKSIMLRGQSDKNRFDVRHHAIDAAVMTLLNPSVAVTLE QRRMLKQENDYSSPRGQHDNGWRDFIGRGEASQSKFLHWKKTAVVL ADLISEAIEQDTIPVNPRLRPQNGSVHKDTVEAVLERTVGDSTWTDK QVSRIVDPNTYIAFLSLGRKKELADHQRLVSVSAGVKLLADERVQIF PEEAASILTPRGVVKIGDSIHARLYGWKNQQRGDIQVGMRLRVFGAEFP WFMRESGVKDILRVPIPGSQSYRDLAATTRKFIENGQATEFGWITQN DEIEISAEYLATDKGDLSDFLGILPEIRWKVVTGIEDNRRIRLRPLLLSS EAPNMLNGRLLTQEEHDLIALVINKGVVVVSTFLALPSTKIIRNNL GIPRWGRNGHLPTSLDIQRAATQALEGRD MSTDPKNYRIGVDVGDVSVGLAAIEFDDAGFPIQKLALVTRHDDGGLD PTKNKTPVSRKKTGRDARRTMRMNRNRKQRLRDLDMMLTNLGYTVP EGPEPTYEAWTSRALLASIKLANVDENELVRAVRHMARHRGWAN PWWSIDRLENASREPSETFEIILARARELFGEKVPADPTLGMALGALAAN NEVLLRPRDGKKKTGYVRGTPLVAKVRQEDQLAELRRICEIQIEG QYDALRSAIFTHKMAYVPTERVGKDPNPSKNRITRASLEFEQFRILDS VANLRVRTDSRSKRELTEAEYDVAVEFLMSYTANEQPSWADVAEVIGV PGNRLIAPVLEDVQQKTAPFDRSSAAFEKAMGKKTARQWWESTDDD QLRSFLISFLVDATDDTEEAAGLPELYMSWPAEEREVLSDIDFEKG RVAYSQETLSKLSYMEYRVGLHEARKAVFGVDDTWRPPLDKLDEQ TGQPTVDRVLTILRRFVLDCEQWGRPRAITVEHARTGLVGPQRQEI LNEQKKNNRENNELIRGDLRKSGVENPSRAEVRRLHIVQEESQCLYCG AVIRTDTSeldHIVPRAGGGSSRRNMAAVCHYCNKKKRTLFYDWA GPVKLQETVDRVRQLEAFKDSKAKMFKNQIRRLKQTEADVPIDERSL ASTSYAAVAVRERLEQHFNEGLAPDDKSRVVDVYAGAVTRESRRAG GIDERILLRGERDKNRFDRHHAIDAAVMTLLNRSVALTLEQRSQRLRR AFYELELDKLDRLDQKPGEDWRNFTGLYEASQNKFSWKAAATVLDG LLAEAIEDDAIAVVSPRLRPQNGSVHDDTINAVKLTLSAWPADAV KRIVDPETIYLA MKDVLGKLKELPEDSARSLESDGRYIEADDEVLFPPK KAAASILTPRGAAEIGNSIHARLYSWLTKKGELKFGMLRVYGAFFPWL MRESGSRDVLHMPHPGQSFRGMQDGVKAVESGEAVEFGWITQDD ELEFPEDYIAHGGDDDELNRLLRVMPERRWRVDFGYNAGTLRIRPALL

Category	SEQ ID:	Description	Type	Organism	Sequence
MG4 effectors	11741	MG4-2 effector	protein	unknown	SAEQLPSELQKKVADKTLSDVELILRAVQQRGLFVAISSFLPLESLKVVIR RNNLGFPWRWNGNLPSTSEVRSSALRALGVEG MLREPGNSVSKIMGQQAARRSYVGLDIGTHSVGWALLKFRDGRPC GVERAGVRIFEGVEEVAFERGRAEPPGQKRGRQARALRRQTERRARR KAKLLHILQRAGLLPKGEADEILPALDRDILARHSAAWPGARDALPYW LRSGALDHRLEPHEFRALYHLGQRRGFSLNRRAPMRKNEEDGKVKA GISTLKEQMEKAGARTLGEFFAGLDPHQERIRQRYTSREMYEQEFVAV WSAQAAHHPAILTDDLKARVHHAVFHQRPLHNQSVLAGSCTLEPDRK RTPWACLIAQRFRMLQKLNDRVLPAASGPERPLSDEERQIVLTLEDRK KELKFDVRVKLLGLSADSNWESGGERLVGNNTTNAKLVFGKRW WSLSPDDRQVVEDVRSYKAEALARRGREHWGLDEKAAAGELSKLSL EDGYCRLSRQAIERLLPGMEKGTAYMELVRKLYPDRWAAAGKPVDDL ALAEITDLMRNPVVRCLTELKVVNAVVRHYGKPSAIRIELARDLRK SAKQREQTWRRNRNQDREAAAEKLLQEARIANPSRADVEKVLAE ECGWHCPTYTGHGFGMADLFGPHPHFDVEHVPFSRSLDNSFLNKTICE ARENDRKRNHPTPEAYGADAERWDQIARVQSFGRGTASREKLRFFQ QHEVEDLDGVAQRELNDRYASLLAVQVYGMLYGGAVDAGHVRVQ AAKGGTTGYLRDMYGLFVLGEGRKESDRHHAADVAIALTDPA ALKSISQAASDERRGGRVSGAVALPWVDFIGDVQAAIEAINVSHRPSR KVNAGALHEETFGPRGMDGDRPTGYVQRKPVVERLSAKEIPNIPDAV REAVQAKLDEVGGTPAQAFKDPANHPVRKRGIPVHKVRLRLNINPVQ VGSATERHVL TGSNHMEIEVRDAKGGKWTGRLVHRLEAKRRA LGRETIVDRAVQAGRQFQFSLSPGDMIELTGEDGERKLVHVRSEIGRI EYVDARDARKKADIRASGDWWRKPAVGSLLRLHCRKVVVTPFGEIRYA ND
MG44 effectors	11742	MG44-1 effector	protein	unknown	MEKFYLGADIGTNSVGIACTDENYELIRAKGKDCWAVRLFDESKTAET RRNFRTSRRRLERRKQRISWLQALFAPYINDETFFIRLNNSQFLPEDKD EILQADKNALFGDEGYTDKNYHVEFTIYHLREKLEGGKYDLKLYYL TIHHIVKYRGHFLFEGATMEERDIKRLFENLNAVWEATYAENVPHINL AKSDEAKEILLDTKKGLRDKQIALEKLFEGENTALMKESIKAMLGKKIS PETLFGEYKDEKSFKDMDEEAFDALQSTYGDNFECLNALSINNFV AFEKLLCGHKNISSAMIAVYNKHAADLSLLKSFIRSERPNYNKIFKSTT EKANYVNYIGYTKKGGEKKVAKCKSDDEFFAYMKKYLSSLLDDIKDG ATRDKILGEIENGSLPKILHSDKGLFPRQVNEAELKALASNMVVKYYPE TKELADKIIPLEFYRIPYFVGLAGVNSWAVRKSGETTPWNIGEKIDLAA SNEEFMRKMTSKCSYIFGEDVLPKCSHYQKDFDVLNQLNKLVRNDRPLT VDLKKGIFNELFLKYPKVSDDKKIDYLRNGHFSPTDGETITLSGKDGFE KAMSSSYIQLKRILGDFVDKDLENGGEVGENILWHTLNTDKKIVYDLI EKRYKNIPEIADSVKALKGLSFKNFCRLSKKFLVDLYSADNETGELVNI

Category	SEQ ID:	Description	Type	Organism	Sequence
					LDVLYETNENLEILNDEKYAFGKLVDEANGVADSKITYEDIEKLYVSP AVRRGIWQTVTMIDEYVEAIGRTPDKIFVEVTREDGVKGDAGRTPRRK RQLQEKYKNVSKTYADVISELGEKYSMDKMLRQERLFLYFRQLGKCM YSGQRIDLRLDLDYDVLHILPRTFIKDDSLDNKVLVLRSKNAEKADR YPLPQGFSDQDQFWKMLLDKNLIAKTTDYDRLTRTEPLGDNNDYKDFINR QKVITDQTVKAVAEMLMKRYPTAKIVYSKAKNVNDFKNKDFDKCRET NDLHHARDAYLNVVGVNVDVTFNSPLDMFRKDGDMWRVTYNLKKLF TRDVKGAWDCSRIARIKICSGSHTMAVTRYAYCNKGEFYNQTVYGKD DAGVSSPRKSNGLSDTKKYGGYKSQTTAYFAIVSSLDKKNRVKTIEA VPVLVAYRLKNNPKAVEEYFNSYLSKPEVLIPKIKNKQLVSYNGSLVYI AGVTGDRISVHNATQLFTDNKMDYVNGLLKLLDMDAKKMLVGDPE RYVIKTNRNKEELVIDKEKNVELYGYLKNKCLDKIYSGLSAFATAK NIENGKEKFIDL TIVEQAKVLIQILMMFKRKDILSDTLIGGSSHSKIL FNKKIDDVNFELHLSAFLRVIKNV
MG46 effectors	11743	MG46-1 effector	protein	unknown	MKENYYLGLDIGTNSVGYAVTDGGFNLLKYKGPEPMWGS HVFEEGKQ CSDRRMHRTARRRLDRRQQRVHLTQEIFA KAISEVDERFFVRLKESAL FREDTSGRDTVIFFDENYTDKEYHRDYPTIHLIKELMEDTTPHDVRL VYLAVALMAHRGHFLSEVNKDNI TELDFDSIYGNLMELFTTPPWIC SDKEEFKNILLHQTIKNKERKFWGLLYEGKKPKTDEEDYINKEGMIR LLSGGTVEAGKLFNQKEQEKISLKKSEDFQLLDEMDDEEDSEYLI RLRALYDWALLVDSLHGCSSEAKVQDYAQHEADLMLKNFVRKYC PNEYAAIFKNAEKENYASYVYNIPKGRKTKYKKITQEEFCDYLLKK LKDIQIEEEDQEIYQDMMFRLETYTFMPKQVTGDNRVIPYQLYYDELK KILENAENYLPFLKKVDEQGISNKTLLSIFEFRIPIYVVGPLCSASKYA WLKRKAEGKIYPWNFEKVDLDQSEKAFINRMTNNC SYLPGETVLPQ NSLLYCKFTVLNEINNIKINGIPISECKQEYIRLFEENKKVTVDKIKKY LISNNYMEKEDVLGGDITIKSSLPQHDFKRLHLSKILNEKEVEQIECI TYSEKSRVLRRLREFPKLSDERRRYLSKLYSGFGRLSREFFTGHHG ANKETGESFSIQALWDTNDNLMLQLLSDRYTFKDSIEEQRYVEEHP MTVESLLEEMVYVNAVKRPIRYTLDVIKDIQSVCKAAPKKIYEMARG QEEGSRNKRSRKDKILELYKNMDKGEVREL SKQLEDSCDRELSEVLF LYFMQLGRSMYSGKPIDIEKLKTNAYDVVDHIYPQCRVKDDSLSNKVLV LSEENRAKGD KYPISAVIRQNMGMWVRVYHEKGLISDEKVRRLTRVS AFNSAEKMEFINRQLVETRQSTKALTRIFRYIFPETEIVFVKAGLVSDFR NEVLKCAKSRIVNDLHHAKDAYLNI VAGEVYKHARTSKFFKIDQDEYS VKTAKIFGNKVWNGKELVWDGEKDIA RVKILTKNSVHYTRYAFERK GGLFDQQLRAASGLIERKAGLDEKYG YGYNKSTASYFLLVKYAEAG KKPKQDVMFVPIDLMESEQIUKSESYAKYVYVRNAIAHHIGKSREIVQEV FPLGMRKMKNVTLLTLDGFEAILASKSNGGKTLVFGSMMPFLVNNKK

Category	SEQ ID:	Description	Type	Organism	Sequence
MG6 effectors	11744	MG6-3 effector	protein	unknown	EIYIKRLESFSKKKQNNFLFVDEVYDKITKEENRELFLLTNKVVEEP YCLIFGSQLQVLHDKENEFENLNLEQQVETLLNLSIFKTGRITGCDL KLLGGAGQAGIFTESKLSNWKSYKDVRIVDISAAAGLHRKTSQNLLE LL MKKVLGIDLGVASIGWGIETDEKENENGRILKSGVRIFQNEQRADAA PGESSNADRRNKRVRQRDRRTKRKINLYVTLKKNGLAPNKSEWDK WVSINPYTIRAKALDEKVSLEHFGRALYHLNQRRGYKSNRKAGSDKE GAVKEGISKVRNHNMAKHNAARTIGEYFHEIYDNHLQNDTEHDDFDWRI RDKYTHRKMPKEEFDALWDAQSVFHKELTNDLGEVLKIIHFHQKRM KSQSHLIGKCELETDKKRIAKAHLFQEFRLKNINLSIDENGFPKIL TEGDRKLFKEIFDKKDKVSWSQLKTALNSGTIGNKNNAVFNLERGGRK NIEGRTNAALSHKKAFGEKWYELEDDFFKHHVVDVLIHVDPKPEIVKNL ALNKWDRTEEQAETHTKLTLQRYGGFSEKAIKKLLPYLKKGMES SAIKKAGYSLFEQNPCKMNQLPMPDQTIKNPVVYHALIELRKVNGIIR EYGMPPVIRVELARDLKAGYERRQKMTKKMRELEKKNDAKAYKALQ KEPFIQYPGYNDIWFNLWEEDKTCPTGKTIPAEAFNSGEFQIEHIL PFSRLDNSYANKTLCEDAFNRKKGNRTPWECVEAGIMEEDTMLQRIR NLPWNKRNFQKEIDEDKFLNRQLSDTRYISKEASSYLKHLSCERVE VVKGQTTSLRLHLWGLNGVLNKEGPMKNRDDHRAIDAVVAFTN RSTLKRSLDENKRIGTAEWMDADESGRATNDEIKRRLGGRIDLSEWP TFRNDVEVSINNTVSHRVNRKVSALHEETYYGPTDEPAPKNKEMMV LRKSVHQLSKKDLGLRDETIRQIVNDEVQKRMNNGESQANAIASLEA DPPFIISPKAQVPIRKVRLMKKDPQIMHYFENKNGEEDRAALYGNNH HIAIYETSDKNGVKKQIGIVIPMMEAARRVKDGDPIVMKDYRPDHTFL YSLAKNDMIFNHEDEQIYRVQKINSDBGTIMFRQNNVAMKGQSDPGVYF KSGSRLGASKIKISPIGEIIFAND MDSYTLGLDIGSNSIGWSLIKEDKNPTIIDIGVRVFPPEGIDRDTKGAEISK NKTRRNARSSRRMHQRRSYRKSCLKVKISREQGILPQEDKELDKLFLKD PYELRAKGIDEKISLFEFGRALFHLNQRRGFLSNRKSCKSKEDGVVTKS ASELQSTIKKTGCRTLGEYLNKLDSTEERRRSYYTFRSMYEEFEKLW EKQKEFYPKILNDDLLKKVIKDETTFQRPPIRWDRDTIRDCDLEPGEKVC PRSDWHARRFRILQDINNLEIYNTDSSDKLSDERKVLLEELLNKKD MTFGALRKKYGLFESQTFNLEEGSADKKAKLKGDEFAAQMRSAKIL GKKGWEKLNESQRIENDLIVDDIEDNELVKILDKYRFSQTQAEATL DISLPSKYSFSKVALQKLLIYMEKGLVHEAIAQAVYGKPAITNKGEI MDFLPMPEDLRNPANRGLFEVRKLVNAIREYGKPKKINIEMAREVK GSKREREIHLKQYKNERINEEARKTLDDFKIPNPSRDDIHKYKLWVE CNKVCPYTGKSISQHLFGPNPEFQIEHIIPYSRCLDDSYMNKTLCFVD ENKEKGNETPYEYSEKIPKQYEQILQRITLPPYKRRRRFSQEVKLDN
MG6 effectors	11745	MG6-5 effector	protein	unknown	

Category	SEQ ID:	Description	Type	Organism	Sequence
MG7 effectors	11746	MG7-1 effector	protein	unknown	FIERQLNDTRYISREVVVYLLKKLVIVKGTGRGQVTSCLRHHQWGLNNIL DLAGEGLKNRDDNRRHSIDAAVAVIGNEHLRELARTKFRKNKEFK QPWPDFREELEEKIKHINVSYRVQRKVSAGALHEETSYGPTGRKDEKGGQ DIFVYRKKLEDLTISMVNKIVDLVVRDIVKKRLVERGIDPEKKIPKE VVNEPLYMKTTSKDKKVQIRKVRIQDVFNMMMLDKDKGKPCRAVA PGNNHHIEFEYKDKKGGKKRDGRVITMFDVQRSQKRESVVKRDYG DGKEFVCSLATNEMFIMDNVDGNTELYRIQKITQSGNKNKTILRPHTYA GKLSDDKPLIQRKSPNTLKGHKVTVDMGLGRFHMAND MSNKTILGLDLGVSSIGWAIERNDENGRIVKSGVRVIPSSKSELSVFKD FDKGKPASFSEKTERTEKGRIRRSYFRKKLRRAKLIEHLKENNMFDPPELL GPKYSIDVWWEWEKATKEKITLAQLGRVLLHINQKRGYKSNRKAIVD EESDSNWLNAINDNSKLLREKGTVEYFYQEGKLLHERKPKVKFALHF RMKVRFNRKDYLDIEQIWKQSEFYPELTDELKESIDHTIFYQRPL KSAKHLSECRYEKMHKVIARSNPLQLFRVLEKVNNLRAEDAFGNR EITDEEKLKIEACTSAQSWKLLDKKKNLSKSKISILGLGKDYENLDS IEGSKTLHSIWEVLMKSWGEGADWDWDFDWSIQNDFSKQKSYQLWHA LYSIDEPQYLRKKLCEGFGFDLDTARLLMNIRLESYGAISARAIRIP ELLKFPKDATKAENAGYKFTDSETEEERSRELKDRIEHLKKGALRN PVVEKVLNQLVTLNAYIAHPPELNPDEIRVELARELKSAGAKERRAE LGMARAAKNDRIRELLQTEFGIPYPGRRDILRYRFWEEQDMRCVYS GDVIPRNKLFVGEYELDHIIPRARLNFNDSNSNLVLVKSENKDKSDMT AADYMKSKGEKAFEEYLVRVKNLYDKGAKKAGERGSGINKGKRN LIMKKEEIPQDFIERQLRESQYIVKEAVKLLKEVCRDVTITTKITDLL KHQWGANDVFRNIQVPKYRKWGMTETIVDRKTGEVIERIIDWSKRKD HRHHALDAIIVACTRQSYIQQLNRLNVLYENDYESLSYRKFPWPSPF HNDLSSLESLLVSFRNKRVRATMKNKRIKVGKKKYIVQKTLTPRDA FHELETTYGRRLVNNYKLVLNKKFSMELAEVDPDLKEKILNRLMEF GNDPQKAFANLKKNPFWKNENLEEVLIYDEVFTTRKKLDEKFNPNSE IIDPEVREIVTQRLKEFDNPKKAFADIENKPVWYNKDKQIRIKTVNTR AKASDLYPVRTKENGPKDFVTRNNHHITVYQKEDGKYDYDKVTSFW EAFELKKAKMPIYKENDDAKAAVLHLKINDMVLVDLNPEDLDQNDPE FFNTLSEHLYRVQKLASGDVTFRRHLETELSNKNTEVRVTNAESLYNR VVMYPLDLVLGLPK
MG7 effectors	11747	MG71-1 effector	protein	unknown	MDNLILRREKMLVTKIKNTYQISDDEIKAKKLYKDWGRISATLNL SSTIAYEDKAFGELVTIISALRHTNKNFMELSSYCSYDFIGKKEFNCS RQSSNGKLTYYKDVEELYVSPSAKRSIWQTLTILEIKKIMGCEPKRIFIE MARSKEESKRTDSRLKQLQDLYKKCREENIDFMPRKDEFNALKTQLSS KKEEDLRSKLYLYYTQMGRCMYTGRIELASLYDNLYDIDHIYPRS KTKDDSLSNRVLVKKQVNAAKTDIYPLDAAIRTKMHISFWKLLYDKGFI

Category	SEQ ID:	Description	Type	Organism	Sequence
					DERKYERLTRSTQLRDEELAGFISRQLVETRQSTKAVAAILKTAYQNSE VYVKAAGNSDFRQKFKVCRDNDLHHAQDAYLVNIVGNCYHVKF TANPLNFTTKNDNRRYSLKPEIFYKFSIKRSGEJAWLGGEDGTMATV ARTMHKNNILFTRQPLEGKGELFKQNPLKAKSGQLPLKAGLSVEKYG GYDSLTTAYFALVKSEKQGSVQLSIEGIPLVYAKQGEKAVQDYLTEV VQLCKPEIKPIKKYSLFKINGFPMHISGRGTGKQLVFGAGQLCIAADD VADYLKALKKHETDIAEKEKTLADQADDDIQKQKAGKGLDFYEQKW GISGAVNVQLYDMFIKSAANLYKNRPASQTITLKEKREHFVKLTLSK QIHIIKEILNLFKASASADFKLIDKGTSCGTLKISNNITKLKECILNQSP TGVFEQEVDLMKL
MG99 effectors	11748	MG99-1 effector	protein	unknown	Same as SEQ ID 11716 above
MG112 effectors	11749	MG112-3 effector	protein	unknown	MGYNKVVGLDVGVSIGWGLVQLDEEKYADEKQDGTVEEKYKID GKIIAAGVRRFQLPQDRQKKSLALIRGTARRSRRTIKRRARRLKRJEL GKEFNLLGNDFDRDKFLIPKKGDKKEKWDTWFRKEALERKLTDEEF FRVLYHIAKHARGAYFTQRAERLELEKDSKAAKQGEKGEKQDNEKK KEREKMKKGLKRIQELLKRSQYKTVGAMFYEMFKNGRKRNNAPDKYS NSIRRELLHDEINEIFKAQRALGNEKADPDLEKQYLRAVLMQEKGPDD EKMQMIGRCEIKELCSQAGKECTADCPDLNRCRCAPKESYTAERFV LFNRLNSLKIIGGQAIDLAHRDNEKLAAYTHDKIDFSQIRKELCLTDKP HLRFNLSYSEKNPEYEKTLKYEVSNGQLQFGPEHRVQMDNFDGET KVFDKEIRAIFQKRLATTPNYKINVRYSIDIRKELQGPQVDLAGFKFTA LKKEYTKSSAQLESEFTKPKNGKGNFNGDAAYIKQFEDDAIFVELKG YHKIRKVLNDRDGTWEKLSKSDGTRIDTIAEALTYCKKDETRTAYLKER GITDESVIDAALTLNMEKIATYSKEAMVLLHEHMEKGLLVNDKARC GYDKFEHKKQAYLAPYSGFFENNPPVVARVIAQTRKVVNAIVRKYGEQ YPIDQIHIEVATELANSEKTRIKDAQDKNKDEKSARSICEEFGINSD EGQNLTMVRLLEQGHFCPYTGKAVLRSTGAANEVHINDCEIDHIP MSRSFNDGMNNKILCCAKANQDKRNRIPEFEWFEETHGPNSSQWFEFT RRVEKMYDVVPYSKKKNLLRKSWTDEEMKFMMDRNLNDTRVATRHIA DYLRYFDFSNRRDDIKDVSRIKLRSGGVTAFLRYLCGLNKNRDNEL HHATDALITACATDGHVFLVSNLSKQIEEKGNWYKHFMEKFKPLR PWETVREDILEATQKIFVSRMPRHKVTSAAHEDEVWSFDEKRTTKA QKKKSSIPKDTARVMKINNGYAKIGEIVRADVFEDGKHKNVYVPIYA VDIFSCKPLPKYLLKNNTPYDEWPASAADNLTFFSLFKDDDLITINGT PYVYVDFVEGTQANIKVRNINGSKFESTNEKTRKFGYRNIELKKFSVDM LGNYKEVKEKRLGNEGKVTWKRPKK*
MG123 effectors	11750	MG123-1 effector	protein	unknown	MRILGLDLGIASCGWALIDQAKDGEGRILALGVRCFDAPEDSKDRTP NNQARRQHRLGLRRVLRRRRQRMQLRHLFLAHGLLASAGPDALALP GIDPWAMRAEALDRALAPCELAVALGHIARHGRFSNRNRQNEAED

Category	SEQ ID:	Description	Type	Organism	Sequence
MG124 effectors	11751	MG124-1 effector	protein	unknown	RTMLAAIAAARQERTGHTYRTIGEAFARDFEFARRKRNRDGDYGRSILRE EHREVRLLIFARQALGSQCATEALEQAFTDIAFFORPLAASEDRVGPC PFEPGERRSARFAPSFELFRFLARLTTLRTGTRREARLTAEEIARAEQGG FGTQQGMTTKRLKLLNLAAEAGFIGISPEDEGRDVNRRSPNGGCMR GSAALRQAIGEGAWLRLSTPERLDAIAFVLSFAAAKEFEPLAALGIE EDVIAAVLAGVEQGMFDHFAAGHISAKACRKIPGLRRGLVYSEACQ EAGYDHARRPETSLSDVANPVARKAIGETLKQVRAIVAEYGLPERIHIE LARDVGKSAEERAEIARGIEKRNRRDRRLRRIFETVGREPAGSEDML RFELWLEQAGRCLYTDHCIPDDAIVAADNRVQVDHILPWSRFGDDSDFA NKTLCFATANQEKGRTPFEWLGAQDQWRNRFVAVVEGCKGMKGR KKRIYLLKDAVSSEKFRTRNLNDTRYAARIVLEHLAHFYPEDGSRVVF ARPGALTDLRRGWGLQDLKKLEPDGCKRHHEDDRHHALDALIVAA TSESALQRLTRAFQEAETRGSHRDFSALTSPWPGFVDQAQAEFKTILVS RAERGRARGEAEATIRQVRKDEDEGPVVYERKSVEALTEKDLARVKD PERNAALIESLRAWIAAGKPKASPPSPKGDPIAKVRLRTDKKPAIEVR GGVAERGEMVRVDVFRARNRHGRWFEFYLVPYIPHQVADKVRWPTPP DRAVQGNTPTEQWPVMDAGYEFELSLHQRSFIEVEKRDRTVITGYFM GLDRHTGSAISTPHSTKALARGIGARTLMRFEKFRVDRGLRGTFAVRQ ETRTWHGVPT*
MG124 effectors	11751	MG124-1 effector	protein	unknown	MTLTGLDIGTNSIGHALVETDEQGNVISLKHIGVRIEFSRSDRTDKEKCP LNEARRTARQRRQRERKKSKMKAVLRVLRHGLDPQDSLLESYPYAA RAAALTGPLSRSQVGRAIWHIAKHGRPLVRKDDKEQGVKEGIRSLE TEMLAQKARTYGELLERIRLNGGSRVLRANSEGSYNRYPSRQVMEAEF NHLWESQVPHHPEVMTALRERLITAIFYQRPLKPVYPGRCTLEPEDEY RMPKAMPMAHEFRIRSEVANLTQKQGDDEVRTLTAENERQIVVDGLNS EKLTFATAIAKLLGFRAGVKFNLEGGDDGGGKARNYLGDLTSSKIRSV WPNFSKMPENMRLSLJLALLDDDELELKCKLRSDFGISPEVVDQLSSL MLPAGYNLSQKAVRAVLPYLQQGMGYAQACAAAGYHSHDRPEEL TPILPFYNELPGMKRYLGQEQKGKPGRISNPTVHVNLNQRKVYNTLV EVFGVPDCVNEVTRELKQTAKQKIAANKQNAANKKVRDAFKEKFE RANSDDQLVRWRLWNLPEERKVCVYSGKEITLNDLFSRVEIDHIPH SISFDNSPSNLVLCQAQGANRLKTNKTPHEAFAPGLHKEFNWSAIEQRVVF ELAADKCGTWAKKKLRFKPDYLDVGGDFATRQLNDNTAYLSKVVRIVYL GHSCPVLAVRGAVTAICRKEWGLDRLRLDITVSDHADLFCLSVVKSTG VFMAPHHVTGGSHDIREDCSVNKLKFGAEVVRVDPGRFQTPGEIRRI EPRGPKVNFQNLRTTTRKPLTSLTANSISQIKDDGLRTKTAHIKEVNP KLLTDTLNRKAKVELSRLLGEFGQKHSVGRVIRVANKSGVVRHGAAN QHTKVLIADTNHHGDVVVRDQKVKMLLTYYAELNHPPEVEAGWTLK MRLHKGDMIRVPGYVYVNYKPKPNYKDKDRSDHRHHHAADFVIACTIP

Category	SEQ ID:	Description	Type	Organism	Sequence
MG125 effector	11752	MG125-1 effector	protein	unknown	SLLRKISRSIALSKYVNLHFEPEPYERFKQELKTHLRKLVVSNKLDHSIS APFTETNYGFTA* MRPYGIGLDIGISSVGWAAIALDHQDSPCGILDMGARIFDAAENPKDGA SLAAPRRREKRSQRRRLRRHRHRNERIRRMILLKEGLLEAEALGLFDGA LEDIYALRTRALDEALTKEEFARVLLHLSQRRGRFSNRRTAAQEDGK LLDAVSENAKRMADCGYRTVGEMLYRDVAFKHKRNKGGEYLTTVS RAMIEDEVKLVFASQRRRLGSAFALEQGYLDILLSQRSFDEGPGGNS PYGGAQIERMIGKCTFYPEEPRAACYSFEYFSLQKVNHIRLQKDG ESTPLTSEQRLQLELAHKTENLDYARIRRALQIPDAYRENTVSYRIESD PAAAEKKEKFQYLRAYHTMRKAIDGASKGRFALLSQEQRDQIGTVLT LYKSQERISEKLTAEAGIEPCDIAALESVSGFSKTGHISLRACKELIPYLEQ GMNYNEACAAAGIEFHSGTERTVVLHPTPDDLADITSPVVRRAVAQ TVKVINAVIRRYGSPVFVNIELARELAKDFTERKKLEKDNKTNRAENE RLMRRIREEYGKMNPTGLDLVKRLYEQAGVCPYSQKQMSLQRLFE PNYAEVDHIIPYISISFDDSRNKNVLAENRNKGNRLPLQYLTGERRD NFIVWVNSSVRDYRKKQKLLKPTVTDEDKQKERNLQDTKTMRSFL MNYINDHLQFGVSAKERKKRVTAVNGIVTSYLRKRWGITKIRGDGDL HHAVDALVIACATDGMIRQITRYAQYRECRYMQTDTGSAIDEATGEV LRIFYPWEHFRKELEARLSSDPARAVNALPFLYLDGSEPLPKPLFVS RMPRRKVSGAAHKDVTKSPKAMAEKVIVRRALTLKLNKEIENYF DPGSDRLLYDALKARLAFFGGDGAKAFREFFYKPRHDGTGCPPLVKV KLCEPTTLNVAVHGGKGVADNDSMVIDVFEVGDGYFVPIYIADTL KPVLPNKACVAFKPYSEWRTMDDRDFSLYPNDLIRVTHKSALKLSR VSKESTLPESIESKTALLYVVSAGISGAASCRNHDNSYEIKSMGIKTL KLEKYTVDLGEYHKVEKERMPFTGKRS* MLSYAIGLDIGISSVGWATVALDGEDRPSGIIGMSRIFDAAEQPKTGD SLAAPRRREARSARRRLRRRRHRKERIRALILREGLLNETQLAALFDGQ LEDIYALRVRALDEAITAEALARIMLHLSQRRGFSLNRKTAASKEDGEL LAASANRARMQAHGYHTVGEMLLKDESIREHRRNKGGAIVISTVGR DMIVEEVRFIAAQRTFGNVAAEALAEANYLEILLSQRSFDAGPGEPS YAGSQIENMVGKCTLEPDESRAARATYSFEYFALLEAVNHIRLTGAGV SAPLTAEQREERLIALAHKTADLSVAKIRKELNPAEQRFNAVSYGKSDS PDEAEKTKLKQKAYHQMRGAFAFEKASKGSFVLLTKEQRNAIGQTLS IYKTGDNIRRSRLDAGLSEEQIAIVEGLSFGFGLSVKACDKLIPCLEQ GMNYNDACAAAGYAFRAHEGQEKKKLPPNAAEAKDITTSPPVVLRAV SQTIKVVAIIRERGGSPTFINIELAREMAKDFSERTQIKHEHDENRKQN ERLMERIKNEYGKSAPTGLDLVKLYEEQAGVCAYSLRQMSLEHLF DPNYAEIDHIIPYISISFDDGYKNKVLVLAENRDKGNRLPLEYLNKRR EDFIVWVNSAVRDWKKKQRLLEKHITQEDEAKFKERNLQDTKTASRF
MG125 effector	11753	MG125-2 effector	protein	unknown	

Category	SEQ ID:	Description	Type	Organism	Sequence
					LLNYADNLAFAPFQTERKKHVTAVNGSVTAYLKRKRWGITKTRANGD LHHAVDALVIACTTDGLIQKVSRYAQYQENRYSADGGGLVVDLHTGEV VAQFPEWAFHRQELDARLSDDPARAVRGLGLAHYATGEIPRPLFVS RMPRRKITGAHKEITKSPRALDEGLITKTPDLALKLDKDEIAGYYK PESDRLLYEALKERLRQFGDGKKAFAEPFRPKPHDGTGTPPLVTKVK LCEPTTSLVAVHGGGLGAANNDSMVRIDVFHVEGDGYFVPIYIADTLK PELPNKAQVAGKKPSEWKRMPNPNDFVSLFPNDLIVVSHRKGICLSLV NKESTLPASREEKCTFLYLKVGKSTASLECRNHDNTYHIKSLGIKITLE KIEKYTVDVLGVEVHKIEKEPRMPFTNMEG*
MG125 effector	11754	MG125-3 effector	protein	unknown	MYPYAIGFDIGITSVGWAVVALDGEDKPFGIINMGSRIFDAAEQSKTGA SLAAPRREARSMRRRLRRHRHLRERIRHLLVAENVISQAELDALFEGK LEDIYTLRVKALDTAVSHADFARILLHIAQRRGFKSNRKSSTSKEDGEL LAAVSANRALMAEKGYRTVGEMLLKDPQYSGSKRNKGGKYLATVGR DMVEEEVRAIFKAQREQQQAFAATELEEQYLEILLSQRSFDEGPGEGS PYRGSQIEKMIGKCTLEAGEPRAAKASYSFEYFTLLQNINHLRLICGGE SRPLSDAQREFLIALAHKTKDNLNFSRIKELDIPADTTFNNAVSYKSADGY EDAEKKAFCYLKAVYHQMAAFNKLKSGHFDLSLARQQKNELGRVLS TYKTSANIRPRLAAAGLSEMEIDIAETMSFKFGHISVKACDKLPFLEK GLKYNDACAAAGYDFKGYDSETRTLRHPTDFADVTVSPVVRRAISQ TAKV/LNAIIRERGNSTFINELAREMARDFTERSKMKKMDMDENHARN ERIMERIRTEYGKEHTGQDLVKFKLWEEQHGECAYSQKHLHLKHLF DPDYAEVDHIIPYISISFDDGYKNKVLVLAENRNKGNRLPLLVLQGERR ADFIWVWENSIDHYRKKQRLKETITAEDEKGFERNLQDTKMSRFL LNYISDHLEFSDSTGRKKHVTAVNGAITSYLRKRWGIKIRENGDLH HAVDALVVVCTTDGMIQQLSRYSTLRECEYVQTEAGSIAVSMHTGEVL KRFYPWPFEFRRLEARLGDDPRRAVISQRFVYANGDIPVRKLFVSR MPRRKVTGAAHKETIKSPKALNDGIVVVKRALTDLKLDPKTGEISNY MPQSDRLLYEALKEALKKHGGDAAKAFAPFHKPKSDGTPGPVVNKV KLCEPTTLNVAVLNGAGVADNDSMVRIDVFRVENDGYFVPIYIADTL KAELPNKACTRGKPYAEWREMDAEDFLSLYPNDLIRVTSQKGLTSLK AQKESTLPDTYETKQEMLYYTSASINTAAVACRTHDINSYEIKSMGIKTL EKLEKFTVDVLGEYHKVEKEPRMAFCRK*
MG125 effector	11755	MG125-4 effector	protein	unknown	MRSYAIGLDIGITSVGWATLALDGNENPCGHIIMGARIFDAAEQPKTGE SLAAPRRAARSMRRRLRRHRHNRERIKNLMVSKGVLSSDELETLFDGR LEDIYALRVKALDGKVSRSSEFARILLHLQRRGFRSNRKNPSSKEDGAL LKAVSENAERMEKHGYRTVGEMLLCDEAFKQKRNKGGNGLTIVTR DMVADAEARAFAAQRSFGSEYASEEFENEYLEILLSQRSFDEGPGGNSP YGGSQIERMIGRCTFFPEERRAARATYSFEYFSLLOKVNHIRVINGAA ERLTAEQRNTVIELAHTTKDLSYAKIRKALKLSDGQLENIRYSDKASAE

Category	SEQ ID:	Description	Type	Organism	Sequence
					DTEKKEKLGVMKAYHQMRSAFEKQSKGRFDFVTTTPQRNDIGTALSLY KTSDKIREYLKDSGFEIDMDAVESIGSFSGFHISVKACDMLPFLERG MNYNEACAAA GLNFKAHDTGEKTRFLHPTDEDDYEDITSVVRRAISQT VKVINAIIRKEGGSPTFINIELAREMAKDFTERNKLKKENDENRAKNEK LLERIRTEYGKSDPSGLDLVKLRLYEEQGGVCMYSLRQMSLEKLFSPN YAEVDHIVPYSKSFDDSRKNKVLVLTEENRNKGNRLPLQYLTGQRRDD FIVVNNNNVDRYKRRLLLKETLDEDELGFKERNLQDTKTMSRFL NYISDNLEFAESTCGRKKKVTAVNGAVTAYMRKRWGITKIREDDGLH HAVDAVVIACTTDDGMIQRVSKYARLRECRYMPTEEGSLVDDGTGEVL HQFPYPWRDRKELERIGTDPARTINDLRPLFYMSSGMPLPEPIFVSR MPKRKVTGA AHKDTVSPKELDKGCVVVKRPLTDLKLKDKGKIENYY NPQSDRLLYDALKKALIEHGGDANKAFAGFEHFKPSDGTGPIVSKVK LLEPTTLNVPVHGGAGVADNDSMVRVDVFLTKGKYNLVPIYVADTLK PELPNKAIAAHKPYSEWPEMSDDDDFFSLYPNDLVCVTHKKGIKLTVTN KNSTLPPTVEGKSFMLYYISTNISGGSIKGITHDNTYEIGGLGAKTLEKL EKYTVDVVLGEYHKVQKEVRQPFNIKRR*
MG125 effector	11756	MG125-5 effector	protein	unknown	MRPYAIGLDIGITSVGAALADADENPCGIIDLGSRIFYAAEHPQTGE SLAAPRREARGSRRLRRHRHNRERISLMLLEERLISQDELETLFDGRL EDIYALRVKALDEIVSRTDFARILLHISQRRGFKSNKKNPTTKEDGILLA AVNENKQRMSEHGVRTVGEMFLLDETFFKDHKRNKGNGYITTVARDM VADEVRAIFSAQRELGAFASEFEERYLEILLQSRSFDEGPGGNSPYGG SQIERMVGRCTFFPDEPRAAKATYSFEYFTLLQKVNHIRIVENGVSCKL TDEQRRRIELAHTTKDVSYTKIRKALKLSDKQLFNRYTDNLPAEDSEK KEKGLMKAYHQMRSAIDRISKGRFAMMPRAQRNAIGTALSLYKTSD KIRKYLTDAGLDEIDINSADSIGSFSGFHISVKACDMLPFLFLEQGMNIN EACAAA GLNFKGHDAGEKSKLLHPKEEDYEDITSVVRRAIAQTIKVIN AIIRKEGSSPTFINIELAREMAKDFRERNRIKKENDDNRAKNERLLEIR TEYGKNNPTGLDLVKLRLYEEQGGVCMYSLKQMSLEKLFEPNYAEVD HIVPYSISFDDSRKNKVLVLTEENRNKGNRLPLQYLTGRRRDFIVV NNVKDYRKRRLLLKEELTAEDESGFKERNLQDTKTMSRFLNLIADN LEFAESTRGRKKKVTAVNGAVTAYMRKRWGITKIREDDGCHHAVA VVIACCTDAMIRQVSRVYARFRECEYMQTESGSAVDGTGTGEVLRTPY PWPDFRKELEARLANDPAKVINDLHLPFYMSAGRPLPEPVFSRMPRR KVTGAAAHKDTIKSARELDNGYLIVKRPLTNLKNKEIENYNNPQSDK CLYDALKNALIEHGGDAKKAFADEFKPKSDGTGPIVNVKVKLLESAT MCVPVHGGKGAAYNDSMVRVDVFLSGGKYLVPIYVADTLKPELPNK AVTRGKKYSEWLEMADENFISLYPNDLICATSKNGITLSVCRKDSTLP PTVENKSFMLYYRGTDISTGSISCITHDNYAKRLGLGVKTLEKLEKYTV DVLGEYHKVQKEVRQPFNIKRRKACPSEML*

Category	SEQ ID:	Description	Type	Organism	Sequence
MG3 effector	11757	MG3-18 effector	protein	unknown	MSTDMKNYRIGVDVGDERSVGLAAIEFDDDDGLPIQKLALVTRHDGGGLD PTKNKTPMSRKETRGIAARRTMNREKRRLRLNDNVLENLGYSVPE GPEPETYEAWTSRALLASIKLASADELNEHLVRAVRHMAHRGWANP WWSLDQLEKASQEPSETFEILARARELFGEKVPANPTLGLMGLAAN NEVLLRPRDEKKRKTYGVVGTPLMFAQVRQGDQLAELRRICEVQIE DQYELRLGVFDHKKHPYVPKERVGKDPNPNSTNRTIRASLEFFQEFRLD SVANLRVRIGSRKRELTEAEYDAAVEFLMDYADKEQPSWADVAEKIG VPGNRLVAPVLEDVQKTAQYDRSSAAFEKAMGKKTEARQWWESTD DDQLRSLLIAFLVDAATNDTEAAEAGLSELYKSWPAEEREALSNIDFE KGRVAYSQETLSKLSEYMHEYRVGLHEARKAVFGVDDTWRPPLDKLE EPTGQPAVDRLVTILRRFVLDLCERQWGRPRATVEHTRTGLMGPTQR QKILNEQKKNRADNERIRDELRESGVDPNPSRAEVRRLHIVQECCQCL YCGTMITTTISELDHIVPRAGGSSRRRENLAACRACNAKKKRELFYA WAGPVKSQETIERVRLKAFKDKSKAKMFKNQIRRLNQTEADEPIDER SLASTSYAAVAVRERLEQHFNELGLADDDKSRVVLDVYAGAVTRESRA GGIDERILRGERDKNRFDVRRHHAADAAVMTLLNRSVALTLEQRSQLR RTFYEQGLDKLDRDQLHPGEDWRNFTGLYPASQKKFLEWKDAATAL GNVLAIEAIEDDSIAVVSPLRLPQNGSVHDETIDPVKKQTLGSDWPADA VKRIVDPEIYLAMKDALGKLKELPEDSARSELPGDGRFVEADDEVLFPP ENAAASILTPRGVAEIGSHHARLYGWLTKKELKVGMLRVYGAFFP WLMRESGSRNVLSMPIHRSQSFRDMQDTRKAVESEGAFAVITQ NDELEFDPDDYIAHGGKDELRLQFLGFMPECRWRVVDGFKKNYQIRIPA MLSREQLPSDIQRRLKESKTLTKNESLLKALDGLVVAIGGLPLETLK VIRRNGLGPRWRGNGNLPTSFEVRSSALRALGVEG
MG3 effector	11758	MG3-89 effector	protein	unknown	MSAPLNRYRLGFDVGERSVGFAAVEYDDQGYPLKFLAIGSYLHDGGMD PTTNKNPKSRKETRGVARRTMNRRQKIKRLKKTDKVLRRELGYQVS HYTDEPQTYEAWYSRRLATQKLSSEELNDHVMVRAVRHMAHRGWWR NPWWSLTQLESASAEPSSETVQMFKAQERWADELILPIEETTLGMLG ALSDDNKVLLRPRTYDSKKEHKEKLVKGEPPVFAKVRQEDILREL RIICVRQGVESQYELRKALFDQIRPHVPQENVGRDPLDPSQYRALRAS LEFQRYRILDALANLGVREGRGKPRSLTGERTQAWKFLSTYRDQKN APTWGDVAEAMGVPEALLVAPVIDEVRUNKAPYFSSLVAVEKKLKKK HQYKWWVEASVESRGLLRVLADATNATLDEASEAGLLELIEGLPEE EREVLDSLSEFETGRAAYASADTLTKLADYMEEHAEIGVGEARCAVF GVDDSWPPPKATLEATGQPTVDRVLTIVRRVLSAQQRQWGDPAEIM VEYARTGLMGPAQLAEVKREIAKNRKEIRDRQDLKGGVSEPKKRH IMAHRIVQDQNCQCMYCGAMITAAACELDHIVPRAAGGSSRRRENLAG VCRDCNASKGGRMFADWAADNPRGVSLKDTLRLRLSWEPFKKADKK RLCLKIERRLKQSVMSPEQIDERSLAPTAYATAIRERLRRHFEDDAKP

Category	SEQ ID:	Description	Type	Organism	Sequence
					IPRKDVVKAYAGGLTRESRRAGLIDEKLLRGRDKSRLDVRHHAIDA AVLTMLNVPVARTLEERRLMKRRERDLSARDNDWRDYTGTAEDKPKF VQWKQAAAGEMADLLIDAVDQDSIAVINPLRLRPQNGAVHDDTIRPLEE RALGAEWDPATIKRVVDPSIYALVDALGKSKSLPADSQRELVLDDGT LMSADDSIALFSTNAASILTTPRGAEEIGSVHVRVLYAWRDRKGEIEVG MQRVFGAEFPWLMRESGVKDVFKVPVHRGSQSYRDLQDGVKQIESG AAVEIGWITQGEIQNLDEVRDSMRSKELLTFLEIFFETRWVRVDGLPD NRRLMRPVLLASEGIEEFLKNAPEDIQTIVVNFKNAGALLSVSKVLAL TETKIIRYNHLGFRWRGVGRPVSLDIQRAAREALEGKK
MG3 effector	11759	MG3-90 effector	protein	unknown	MSQEATKYRIGIDVGDERSVGLAAFEFDDAGFPLRKLAMVYRHDGGL DPTQNKSPKSRKETAGVARRVRLRRRERLKKDLKLELGYPLP EGEEAQTYQAWKSRALLTSQKIEDKAEQAEHLVRLRHMARHRGWR NPWWQFGQLDSAPVPSETMVENLNHARLLWPGYITDQTTVGELGALA ASPDILLRPRTRDIKKKPNGLHHQEGVRAVLGSKVRQEDLLAEVKKIW QVQELPVSHYEELARALFEQVRPVPAQNVGRDPLPGRHHLPRAPRAS LEFTFEIRQAVANLRVREGREKVPLTSGQHIAAVNYLMNYADKQPPT WGDVAEQIGVEPTRLVAPVIDDVRLNKAPYNHSTSVFTRALPKKSEAM QWWSADVSLRSLLIHFLSDPTEETAADAEGLSAIFESWPEKEREKL EGLDFESGRAAYSIQSLIDLNQYMEEHQSDLHTARKEVFGVDDSWQPP RENLHEPTGQPAVDRLTVRRFVMACERKWKGPDRIVIEHARTALM GPTQRHEVLREIQNRDANERIREELRADGLTSPTRADVRRHRVVQNG DCKCLYCGTMITTATAELDHIVPRAGGSSKIDNLVAVCRGCNADKGR IPFAVWAEQTSREGVSLDDALNRLWSFDKVVYKGVAGRKLKAQIARR LRQAEDEPIDERSLESTAYSAAVIRHRLETYFNTSRGLNRGDDGFTFID VYAGALTREARRAGGIDEQILLRGQRDKNRFDVRHHAADAAMTVLD HSVARTLAQRNLIYREDRLKRRENQDDTRWREFTGLGGEAQEKFLVW KQKSYVVLADLLAEAAEDSIPVINPLRLTPRNGSVHKDTLSSLDKMYLG GSWSSKDIARVDPDIYALMELLGRAITLDEDPQRSITVKGKVLQAD QEIKLFPESAASILAGTGAVKIGDSLHHARLYAWPTKKGHEIGMLRVF GAFFPWLFKTYGTKNALTVPHPGSQSYRDMKDTLRKKIESGEAREIG WITQGEIEKIESYLQENDELGRFINLPENRWKIDGNDNGRLRLRPI LLSYEEIPEESYGEDVLGAKNHQLIRKIVLERGAHTAGKILGAEGTKVLR RNHLGAPVWQGGQAEARSLDISRAITEKLEG
MG3 effector	11760	MG3-91 effector	protein	unknown	MSMISTNDDDRVEGMMHMARYGERKYRVGIDVGDERSVGLAFIEFDEQDM PSELLRMFTVRHDGGIDPTTNKTPKSRKETAGVARRVRLRRRRTKR LQELDALLMASGFPIVDVSVGETYECWQARAAAVEGFTIDEQTRLETV SRAIRHMARHRGWRNPWLTVWQGFRELEVPTANHRKNIESANSKLHLD LEDTSTLGQIAASASASNNWMLRPNRNGQKRKEASNPLVAQVQQADQL AELYKILEVQRIDSTITEKIARA VFDQVRPVVPKGNIGLDELPGMGAYY

Category	SEQ ID:	Description	Type	Organism	Sequence
MG3 effector	11761	MG3-92 effector	protein	unknown	RASKASLAFQEFRRVRAAVANLRVKNSPRGQERLRLDPQDAQAAEYL LTWREDQPPQWGDVANALESIDENLLVIPVFEDAFKLAPYDRTSSDLE QRLSSANKKKLAGVREWWDSADTQMRELFIEFTSPNESVYEEADES GFSDFNGWSDEAKEVLLGMQFESGRSAYSVESLRLNLRLTGEVDL HEARLEFGVDDTWRPSLPSIDERTGQPAVDRLTVRRALMGAVDKW GVPEAVVVEHARSFGMGASARNLYLNEVSRRTATRAKLREIVKQQGV ERPSDGIHKWECINRQRCCTCAYCGNEITFTAEEMDHIVPRAGGGSNV RENLAVCRRRCNSEKRNIPFAFAESDAHPYTSLETTLTRVRNWDWSR DRSEQRLKNMLQRLKRRESDEIDERSLASTAYAAVEVRQIAEYLG RITGEDELNRVQVYTGGAATREARRAGKIDARIRGKDEKDFDVRHH AIDAAVMATLNHSAFTLRERAEMKRSATMNYLDPNEEWKDYSGRT SSAQKRFTVWRQQAHLADLIVDAVEADSVPAQQQLRLSASRGSVHK DTVSAVLRKQLGAPWTAQELQIVDPEIYVHLRLAAQNKGVLELDP RRIQLLSGVWISGSELVEVFPKAAASMKVSSGSVEIGEIQHHARVYAW RGTKEFQYGILRVFTAELPWLQRQAQSKDLFTMNIDDRSMYSYRDL QTVRKKIESDEARCGIWLQNDDELVLNVEVLRQGSQDKIAHFLSTYPESR WKLDGFPENRRFRIRPLYLSREGSDHLDPCAIELEKGAIVGTSNLLSNV HQIIRDSLGFERWRSQGGPASPWSVPDSTADTVETGLK MAQRRYRVGIDVGRDSVGAALVAFDDDGIPERVLHAVSYRHDGGIDPT TNKTPQSRKHTAGVARRVRRMRARRTKRLAALDRALIELGLPVRDVS DGETYEPWRMRARCVEGFIEDDAERDDAVSRALRHIAHRGWNRNPW HSVRRERLESVPTATHQKNVAAAQAVFPGEIADATVQQLGEVASRFN HMIRPTGKKNPKQKTAVLNERVMQADQMAEVVAIWTTQRMPEAEL NAVLELVFGQERPVVKAENIGRDELPGMGHLPAPKASFQEFIRIRA TAATIGVRARQGSKAERLDADAVDAVSHWLEWDADDDPTWADVA VEALNLDPRFIKAPVFDDVRRMTAPVNRRTARILRKALKKRHNAPVRT WWESASAESRAALVAFVDPDTEDELLDRTGLSDVVAAPVEVLDD LTNLNVEVGRAAAYSRESLSKMTIMAEQVRVGLHDARKIAFGVDDTWA PALPOLSEPTGQPTVDRVLPVRRIVMAAERYGVPEAVVIEHARSALL GPAARAEHQREVNANRREREKNRQILIEQGIDDPNRSIRRWVHQVQLQ NCLCLYCGQTISAAAGGAELDHVVPAGGGSNRRENLAVCRCQNSE KGKLPFAVFAARTQREGVSVEAALERVGRFQWRPADRAVKRGLVHR LKQTAADDPIDERSLESTAYSAVEVRRRLERFYSDDHASPEAPRPEFVFG GSITSEARKAGGIDAIIRLRGKDVKDRFDARHHAVDAAVMTLVDRSVA RTLQQRSDMRYAHRLTGSEPAWREHAGDSAAAQSRFAEWKKSRYRL AELLREISAADAIPVIEPLRLGVNRGSHKDTVRAAVPKRLHADWASAE LDVVSPAAMALSSVFDGAAELPHDDGRMLTVKRRLHADDTISLLP GHAAAIEVNGGVVEIGESIHHARLVAVWRDRKGVIOFGMVRVFTAEIPF MORLAGKKDVFSPVHPSTLSYRGVQLRVRKALDAGIAGVELGWITQGD

Category	SEQ ID:	Description	Type	Organism	Sequence
MG3 effector	11762	MG3-93 effector	protein	unknown	EIEIGLDDVASMTPFEFRQIAEIQWRVVDGFKDGGRLRVRPALLSAE GDAAVSDLVSKTLDKGSFVNAAGFIGAPSSVVRSSALGVPRWRSQAQG HLPVSFSPYQRAENLL* MNEGVIQYRIGDIVGQNGVGLAAIAFEAGNPSEVLAMVTHRHDAGLDDP AAAKQGYSRKKTSGVARRTRLRNRARRLKKLDELITSLGLEVPAAH EVPQTWEAWEARAELQEFIDNEQELHEKLVAVRHMARHARGWKNP WWSWNTLWEAPTPTSNMNEIRDNARKVFADLPPTATIGQIGFVAAAT NRLRPRKDTTKAKTKRPTPVNAKVMQEDQLAELRSYWDKQNLDP EWLEKIAKAVFYQTRPKVKPELVGHDDLPGMTKLPRASRSSLEFQEFR IRAAIANLKVVPGRSQENFLSVGDKNRIVDLMGWDDDEAPTWADV AEELNISVRDLKRPEFDDSPRVAPYDRSSNAIRNKLVSRLKDGKEALE WWDTADRDQRSFATWLGDSQHDAAFLTSGLSDIASWSEGVMEK IDSLSLEPGRAAAYSIESLKILNRHLAEGDNLHEARKHGFNVDDSWTPSL PFEDRTGQPVVDVIRIVHRFINGCVYKVGVPESIVIEHVRSGLLGP ALLDYKRETTTRHNEREQIARDLKDGISERPSSGDITRMIRIVQEQQG VCLYCGTAINIHCELDHIVPRAGGGSNFRENLAACRECNRLKGGKTPF ARWARETSQEGVSLGALDRVKNFVNGSTADLRNLKRRVSQRLRQT DEDQPIDERSMASTAYAAAEVDRVRREFTSRFTDTDIPVEVYRGSTISAA RKAGHIDKLILLRDKDIDRGRHHAIDAAMTVINRNSQVFAIREA MRSAHAMTGPSEPWNKDFRGNPRQEEKFEEFETRAAHLARIIRERIDA DRIPVISPLRLKPKETGVHADTIVPFDYKALESEWTDKDIVRVVDNELY LALVDALGGKKVLPTDKISVIDQDLAKDHRQVALYGTSPQIPVRGGS AAISDSLHFRVYAWKDKKGAIEFGQQRQVFGAEFRAMWGDPRQVDV FTAPVPRWAFAHREMPKVKAAIEAGNAKQIGWYTRNDELDLSEL MAQSNVLGEFLRELPEKSWRIRGSHLSRLSPLYSLESEGTDLSEQSRP VQDAVSKGIPVSASSILDSESMHRRGPIGPKWNGGNATSYFRQVAEE VLGTD MTNHSPAGSISDWDVVLGVDLGQRSVGLAAVALDPDGTPTTEILASNVV RIDGGLLPGSEESPVRKAAAGMARRVRRHRRRRARLALDRRLMD LGFVDDGPETYESWLDRLARLVEGRIANETERKTRATSRAVRIARHRG WRNPWLSWSAFAEELSTPSDNHRRNLAAAERFDRETEGWTVGQLGA AGTDPRTITRPTAKDSRRIVHGEAALEHRLVQEDQLAELRQIWTQ EWDPAELPELEKAVFTQAEFVPPGNVGRDALPGMQTHPRAPRASLEF QRFRIQSVVGNLRVPVPTERSGDLRPLTPEERERVTLGLERWHEREGHV PGERPTWRDVAAEAVGVSLNLRGRDTSYATATPTMETLRLKAGI AALKPAAVRRDVAAWFDEAEDDQISAFVSYLADTSASNEALDEAGLT DIITGWDEAAALEKLGLDPLEPGRAAAYSLESNRLTGRMQADAVDLQEA RKREFGVDDSWTPPTPTSETPTGMPAVDRNLVAVRRFVMSMVQFGM PQLVVIEHVRESFLGVTAVEELRRQRLDRRRRRETAAKEVAASGKEP
MG3 effector	11763	MG3-95 effector	protein	unknown	

Category	SEQ ID:	Description	Type	Organism	Sequence
MG3 effector	11764	MG3-96 effector	protein	unknown	RAADVRRYSLIQRQNGQCAYCGTAIGLRNSELDDHIVPRAVGGASTRAN LLAVCRACNNLKGKQPFVAVAAAADTRIDEDGEKLVSEAAALARTS WMAIPRSIRERNELREIRQLRQRESDPEDERSMESTAYAAATALVERV QNYLESQTPEGVQTPPVVYRGSIASARKSSGVKIVRLRGKSMKHR GDFRHHIDAIVCALITPSVARTLAIRESLRTAHRFTGDEHDWKSFTG DSPQARAETHAWTARMQTLAKLLRDADADQVPVSIPLRLGRRVGRV HEDKVRPFDSRPLGGTWSAKELEIRIVDRRLYTALHHVAAASATSGVEVT PELCTELGSSMGATVKLYGSPAAQIPVRGSGSARLGAHHARLYAWRGT RGIEFGMMRVFAGELTAMWPSATDVLTAPVPEWSMSYRRTAPAVM QQLRSDTAVQVGIAPGDEIVLPPREGARASSLDSLVSSEGDRWTV TGFERATTINVAPRLLSAEGISEETPKAVTSVLNRPGRLLAASSLLPRI RRDALGRERRRGGRLLPSSFVPFEVAEQRLQGD MSTDMKNYRIGVDVGDRSVGLAAIEFDDAGFPIQKLALVTFRHDGGLD PTDNPKSKEITRGEARRMRMTTRRKQRLCDLDKVLNGLGYTVPEGP EPETYEAWTSRALLASIKLASADELNEHLVRAVRHMARHGWANPW WSLDQLERASQEPSETFEIILARARELFGKVPANPTLGMGLAAN EVLPRPRAEKKKKTGYVRGTPPLAAQVVRIDQVAELRRICEVQDIEEQ YETLRNAIFAHKVAAYVPTERVGKDPPLAPSKNRTIRASLEFQEFRLDSV ANLRVTDTSRAKRELTEGEYDAAVEFLMGYTAKEQPSWADVAAEIGV PGNRLLIAPVLEQVQKTAPFDRSSAAFEKAMSKKTEARQWWSNDDDD QLRSLFMFLADATNDTEAAAVAGLPELYMSWPAEEREALSNIDFEK GRVAYSHEITLSKLSEYMHEYRVGLHEARKAVFGVDDTWRPPLAKLEE PTSQPTVDRVLTILRRFVLCERQWGRPQAITEHARIGLMGPVQRQK ILNEQKKNGENERIRNELRESGVENPNRAEVRRHLIVQECEQCCLYC GTMITTTTSELDDHIVPRAGGGSSRRLENAAVCRYCNGKKNRKLFEW AGPVKMQETIDRVVQLRAFKDKSKKAKMFKNQIRRLKQTEADEPIDERS LASTSYAAVAVRERLEQHFNEGLTLDKSRVVDVYAGAVTRESRRAG GIDERILLRGERDKNRFVRRHADVAAVMTLLNRSVALTLEQRSQLRR AFYEQGLDKLDRDQLKPEEDWRDFIGLYPASKEKFLEWKKTATVLGD VLAEAIEEDSIAVVSPLRLRPQNGSVHKTIAAVKKQTLGSSWSADAVK RIVDPEIYLAMKDALGKLKELPEDSARSLESDGRYIEADDEVLEFFPEN AASILTTPRGVAEIGGSIHHARLYSWLTKKGDVKVNVLRVYGAEFPWL MRESGSSDVLRMPHPGQSFRDVQEEETIEMEGGFAKEIAWITQNDDEL EFDPEYINLPGRSCLKTRFLIYMPETRWVRVDGFPESNLRIRPLMLSQ EDLPSEIKKHKEEKQLSDEEKLVEALEKGLIITSSKLLGLKSIKVIIRRN NLGFPWRGNGNLPSTSEVRSSALRALGVEG MSADSLNYRIGVDVGDRSVGLAAIEFDDGGFPIKKLAMVTFRHDGGM DPATGKTTPKSRKETAGVARRTMRMRRRKKRLKDLKDLKRLDLGYFV PRDEEPQTYEAWSSRARLAESRFEDPHERGEHLVRAVRHMARHRCGW
MG3 effector	11765	MG3-103 effector	protein	unknown	

Category	SEQ ID:	Description	Type	Organism	Sequence
					RNPWFSQLEEAQSEPSFTFGRILERAQHEWGERVSDNATLGMILGA LAANNILLRPRRYEHNPKTGKNAEKLNVRGQEPILLDKVRQEDVLAEL LRRICKVQGIEDQYPELAHAVFTQVRPVYPTERVGKDPQLQPMKIRASR ASLEFQEFRIQDAVANLRIRVGGSERRPLTEEEYDRAVDYLMYSDTTP PTWGEVADLEIAENTLIAPVDDVRLNVPYDRSSAIVEAKLKRKTQA RQWWDDDDANLRLSQILLVSDATDDTARVAENSGLLSEFESWDEE KQTLQDLKFDGSGRAAAYSIDTLNKLNAYMHEHRVGLHEARQNVFVSD TWRPRDRLEPTQPTVDRVLTIVRRFILDCEAWGRPQKIVVEHAR TGLMGPSQRADVLEKELARNANERIRQELREGGIEAPNADIRRNII QDQESQCLYCGKEIGVLTAELDHIVPRAGGGSSKRNLAAVCRACNAS KGSRPFAVWAGPARLERTIQRLRELQAFKTKSKKRTLNAIIRLLKQRE EDEPIDERSLASTSYAATSIRELEQHFENDDLDPGFAPVSDVYGGSLTR ESRRAGGIDKSIMLRGQRDKNRFVRRHHAIDAAVMTLLNPSVAVTLEQ RRMLKQENDYSSPRGQHDNGWRDFIGRGEASQSKFLHWKKTAVVLA DLISEAVEQDTIPVNPRLRPQNGSVHKDTVEAVLERTVGDSTWDKQ VSRIVDPNTYIAFLSLGKKELEADHQRLVSVSAGVKLLADERVQIFP EEAASILTPRGVVKIGDSIIHARLYGWKNQRGDIQVGMRLVFGEAEP WFMRESGVKDILRVPIPGSQSYRDLAATTTRKFIENGQATEFGWITQN DEIEISAEYLAATDKGDLSDFLTVPENRWKVVGIGDNRFRKIRPLLLS NEIPDTLNGRSIKSEERDLIVSVLDKGVVVASTLLTLPSTKIIRNNLG IPRWGRNSHLPTSLDIQRAATQALEGRD
MG15 effector	11766	MG15-166 effector	protein	unknown	MKYIIGLDMGITSVGFASMLMDNDNDEPCRIIHMGSRIFFAAENPKDGSS LAAPRRRENRRRRRLRRKRHRKERIKNLIIQNMMMTADEIDAIVNSGK ELPDYIKVRAEALDRKLDTEEFVRLIIHLSQRRRGFKSNRKVDAKEKGS EAGKLLSAVKSNKELMVERNYRTIGEMLYKDEKFAEFKRNKADDDYSN TFARSEYEEIREIFRAQQEYGNPYATEELKDSYLEIYLSQRSFDEGPGGG DSPYAGNQIEKMVGSCITLEPDEKRAAKATFSFEYFNLLTKVNSIKVVSS AGKRSLNEDERKRVIKLAFAKNAISYASIRKELNLGDGERFNISYSQSD KSIEIEKKTFTYLTAYHTFKKAYGSVFNWSAEKKNHLYALTAYK NDNKKKYLTEGFDVETDIALTLPSFSKWGNLSEKALNKIIPYLEQG MLYHDACTAAGYNFKADDTDKRMYLPAHEKEAPELGDITNPVVRRAI SQTIKVVNAIIREMGESPCFVNIELARELSKNAERSKIEKGQKENQAR NDRIMERLRNEFGLSPTGQDLIKLKLWEEQDQICPYSLKPIKIENLFD VGYTDIDHIIPYSLSFDDTYNNKVLVMSSENRQKGNRIPMQYLDGKRR DDFWLWVGSSNLSRRKKQNLKETLSDDDLSGFKKRNLDQTQYLSRF MLNYLKKYLTVPAPNATGRKNTIQAVNGAVTSYMRKRWGIQKVREDG DTHHAVDVAVISCVTAGMTKRISYAKYKETEYQNPETGEYFDVKNKT GEVINRFPMPYAWFRNELLMRCSDEPSRILHEMPLPNYATDEAVAPIFV SRMPKHKVRGSAHKETIROSFEDGKKFTVSKTPLTDLKLKNGEIEY

Category	SEQ ID:	Description	Type	Organism	Sequence
MG15 effector	11767	MG15-191 effector	protein	unknown	FNPESDVLLYNALKERLIAFGGDAKKAFAEPFHKPKSDGSEGLVKKV KLIKSTLTVPLKNTAVADNGSMVRVDVFFVEGEGYLVPIYVSDTV KKELPNKAIVAHKPYEEWKEMREENYVFSLYQNDLIGIKLKKEKMF LVQKNSTLPKNINVKDGLFYKGTNIGANISVINNDNTYTVESLGVKR IPVIEKYQVDVLGNVSKVGKEKRVRFQ* MKYIIGLDMGISSVGFATMMLNEKDEPCRMHMGSRIFEAAEHPKDG SLAAPRRNRSMRRRLRRKCHRIKLNIVSNNILTADEIDTYNSGK DLTDIYQIRAESLDRKLNTEEFVRLIHLISQRRRGFKSNRKVDAKEKGE AGKLSAVNSNKMTEKNYRTIGEMLYKDDKFAVFKRNKADDDYNT FAREEYEEIQIFSAQQEYGNQYATDELKEGYLEIYLSQRSFDEGPGG NSKYAGDQIEKMVGFCITLEPDEKRAAKATYSEFYFNLLTKVNSFKIL AEGKRSNENRQKIIKLAFNKNAISYASLRKELSVGYSERFNISYSQSD KSIDEIEKKTFTYLTAYHTFKKAYGSALJEWSTEKKNHILAYALTAYK NDNKIKNCLTEHGFNETECEIALTLPSFSKWGNLSEKALNKIPIYLEQG MLYHDACTAAGYNYKADDDTKRMYPAPAHEKEAPELENISNPVVRRAV SQTIKVINAIHREIGESPCFVNIELARELSKNKTERNKIEKGQKDNQARN DRIMERLNEEGLSPTGQDLIKLWEEQDGICPYSLQAISIERLFEAG YTDIDHIIPYSISFDDTYNNKVLVMSSENRQKGNRIPMQYLQGGRRDEF WLWVDSSNLSRRKKQNLKLTSLDSSDLSGFKKRNQLQDTQYLSRFMLN YLKKYLKLAPNATGRKNITQAVNGAVTSYMRKRWGQKQVRENGDTH HAVDAAVISCVTAGMTKRISYAKYKETEYQYPENGEYFDVDRKTGE VINRFPMPYPWFRNELLMRCSNPRLQEMPLPNYAADAADVDFVSR MPKHKAAGSAHKEITIRKAFEEEDGKKYTVSKVPLEDLKLNKEIENYF NPKSDTLNLYNALKSRLIEFCGDAKKAFAEPFYKPKSDGSKGPLVKKVKI VNKATLTVPLKNTAVADNGSMVRVDVFFVEGEGYLVPIYVADTVK KELPQKAIANKTYENWKEMKEENFVFSLYPNDLIRIKSKKEMKFNLV NKESTLAPHYQSKDAYVYKGSDDISTAATAITHDNTYKLRGLGVKTLL AIEKYQVDVLGNIIGIKREKRMFR* MNYRLGLDIGITSVGWAVLEHDSSEEPFRIADLGVRFDAEHPKDGSA LALPRREARSSRRRRHRRLERIKALLESQGIITIEKLQEVYHGSKEL TDIYELRCLGLDNLTLQEEWARVLIHLAQRRGFKSNRKKKEIKENKKEK NEDGKLLAAVRDNQALMQEKNYRTIGEMFYRDDKFSLNKRNSKSELYS HTVGREQILDEIAQLFTAQRRLGNSYAGEKVEQLYTDIVSRQRSFDEGP GTPSPVAGNLIERMRGKCTFEKEEPRAKACYSFELFNLLQKINSRLID DKNSASRPLNKEERQLLIQSAHEKADIKYTDLRKKQLSPEQRNLTLSY GRDDVEEIEKKKNFNLYKAYHDIRIALDKVSKGRKQLPVEHDMIGEI LTLYKNEEDRITSKLRKTGLTEYDIEELLSYSGFGRSLKAIKNILPYL QEGHIYSEASTLAGYNFRGHDNVVKQMYLPANNEQLQDITNPVVRRA VQOTIKVINAVIRKYGSPQLICIELSREMGKNFFDRKRIEKQIKENTDLN
MG15 effector	11768	MG15-193 effector	protein	unknown	

Category	SEQ ID:	Description	Type	Organism	Sequence
MG15 effector	11769	MG15-195 effector	protein	unknown	DAVRNKIIIEYGHLPNTGLDIVKMKLWQEQDGRCAYSGEPIQDLDLFA GIADVHIIPYSVSFDDSYANKVLVKSSNRQKGNLLPLEYMKNNPNKQ EKFIWVNTNVRNFRKQRLKLTITEEDRNKWKERQNLNDTKYISRF MLNYIRDYLEFAENIGKRKVISVNGNITAYMRKRWGLKDRLAGD LHHAQDAVVACVTEGMIQKITRYSQYCEAKNNRSHFVDYETGEVIDT LRNRFGADFVEPWNFKIEMVSRISDDPALRIDAYKLDNYLDLKDIPKPI FISRMNPNRKNKGAAHQETIRSSRLTGEGLVSVSKNITKLKLDADGEIAN YYNPKSDMRLYNALKNRLQQFNGNGEAAFEKPEVYKPAAPGKTISPVK KVKVIDKSNLNVAVGKGVAAANGDMLRIDVFKKGDDGYVWVPIYVADIV KETLPNKACVHSPYELWKEMDDNDFISLYPNDLIRFIPNNGEEAFM YYIKAGISTASITVESHDRSQSIPSLGVKTLKILEKWNVDVLGNRTLKVN EKRQYYPGQTTR*
MG15 effector	11770	MG15-217 effector	protein	unknown	MKYIGIGLDIGIASVGSIVLLDGSDEPYKIYRLSSRVFPKAEATDKGESLA SDRRNNRGMRRLRRRRHRKERIRNLIDYVDFVNEDEYITEIYAESGLK DIYQIRYEALDRKLDKDEFIRLLIHLSQLRRGFKSNRKSVDGKKDDGKLL DAVKKNTELQRQKMYRTIGEMLYCDDREADSKRNTGEGNYKNTFSRSEY GEEIKCFENQRAFGNEYATEDFEKEFGIIMSQRSGDEGPGGKDSKYS GNLERLVGKCTFEREMRAPKASYTFEYFNLLSKINAIVSANNTRC LTEEQRAIKNLAFSKNDLSYKSLRKALGLNEDELEFISYTDSDTNKKK AKNKKGTADKFSERDAVEDKTKFSYLKAVHTFKKAYGDEYDRWSTD KKNYLGVLTVFKTDKNVELKLRENFSDDEINIAQTIPSFKLGNLSV KAMNKMIPFLENGEINYKAAEMAGYNFKADDDKACAGMYLPANESKAPE LGDIANPVVRRSVSQTIKVINAIIVREMGESPVYVNIELARDLAKSHDER EKIEKNNANRQKNKLMLEELRKEFKLANPKGEDLIKLLWNEQNGR CMYSYEPIVRERLFEPGYAEDHIIPYSISFDDTMSNKKVLVKAENRDK GDRPLQYMTGKKADDFRVRVSNLSRKKNNLLKEQLTEEDRKSF KQNLQDTQYISRFMNFYKYLKFAKIVAVNGRATDYMRKRW GIRKIRADGDTHHADVACVACATHGMVQRSEYSEYKYKETEYIDDDGR IYDINKKTGELTDRFPMPYPMFRKELEMLTSDNDPQRILSQSKFPNYSGD EQLEPIFVSRMPQHVKVTGAHEDTLRKPVTENGQNYVVKVLTLLK LNDNGEINYMPQSDKLLYNALKERLAEYGGDGEKAFKNLTETPRK PKSDGTPGPVTKVKTIEKQTCGVPLADNTIADNGPMVRVDVYVAG EGYVLYVPIYVSDTVKKELPNRACVANKPYSEWKVMDDKNLFSLYNN DLVKITFKREKKFSLVNDKSTLDKEHRTKSELLYYKGTNIHTASITVIT HDNTYIFEGMGVKTLSIEKYEVDVLGRVRKVNKEKRMGF* MRYVVLGLDIGIASVGWAVLELDSFDEPFKIIDLNSRIFTKAENPDGSSL AKPRREARGNRRRLRRRRHRLDRIKHLIYVVGGLMSKEGYDKLYTSGF DKDVVYTLRAEGLDRSLAAEWTRVLIHIAKHGRFKSNRKSSTVAGEDG KVLQAVKENQEILSKYRTVGEFMFHNDDKFKSRKRNTTDSYILCVSRH

Category	SEQ ID:	Description	Type	Organism	Sequence
					MLKDEIKALFTAQRSFNNPFTDEKFEAKYIEFESQRAFDGPGSESYPYG GNQIEKMIGQCTTFEDGEKRAPKASYSMRFNLLQKVNHIRIKSSSATRA LSEERSHIALAYKSPNFTYGSIRKAIKLPYDMTTFSDVYKYKGLSEE ELIDKNEKSNKISLEPYHTIRKALDKVYKNRIEELSEDNINDIAYAFSV YKTNAKISQKLKECGIDNKDIEALINNLGTFKFGHLSVKACKKINKYL ETGMTYDKACEAAGYDFKGHCGETKFLSGAADEKEIPNPVVKRAALS QTIKVINAVVRKYGSPVEVHVELAREMARSKKDRDKINSIMKDNQAAN DRIRGILKNEFNINPTGIDILKYRLYQEQQGICVYSQKVMDLERVMMK DGKYAEIDHILPYSRSFDDSYNNKVLVKTEENRLKRNRTPEYMQDNE VKYKGCEIVKSHINPTKVSNNLLRENNVQVLVDWKARNINDTRYISK FVYNFLNDHLLADGMRKRRIIANGAVTGYIRKRLGINKIRANGDTH HAVDAVVIACVTQGVISKVTKYSQWQEVFYKNNNTGKLVDTYETGEIT KDNFEFIDSKFPEPWPLFRKELEARAGTNPKYEIECLRLDTYSPEEVT SLRPFVSRMPNRKVTGQAHQETIRSSRMANDGMTVSKVPLTSLKLS KDGQSIGEYFSPESDRLLYEALLNRLQGFGGKADKAFTPEFYKPKNDG SKGPIVKAVKITAPSTLNVRINNGKGLADNGSMVRIDVFHITAGKGAGY YLVPIYVADTKKDKLPSKAIVAHKKYDEWKLMDEKDFVFSLYPNDLIY VEKKGNIELSLDKKIEKDSLTPKKISSPQGYFYVVKAGIGDGSVQIKSH DGVYLLPSMGVKTLLKLRKCVVDELGNISFVGKEKRQHF
MG15 effector	11771	MG15-218 effector	protein	unknown	MNKSVMFGLGLDVGIASIGWATVALDEKGEPEYKLLNLGSRIFDKAENP KDGSSLAMQRRQFRSQRRLRRRRRLDRVIFLQKIGLCTNDELNKL FQTPAPKNVVELVDALDRKLDKQEWIRVLYSVLKHGRGFSNRKNKAK SEDGLLLKAIQSNEEIEFNNGYRTVGEMLFKDPFTNSKRNGGGSYKNC VSRISIQKELDLLFEKQREYGNFTSDYFKESFLNIFLAQRNFDEGPAP SCYGGNLEKMGYCALKKDKRAPKASLSFALFSFWSKINNIRYYDSN RCKEYSISTEQARKVLQKALIKSDLNYSDIRKIHGLDDGCVFKDITYTSK KSKTKKKQQAANNESYLSIDGLTLVEDVLTPEKDANEQSVDTILDIE KSKIKFFENSFISIKKAANGMLDHLNPFDPEDRKRKNRISYAFTVSKNDD GIAGYLNEIDISAEIKESLNNLDGFSQFGHISEEACEKLLPFLENGCDYT SACVEAGYVSSDDVVGKDLLPARSELDDVNPVRRSVSOLIKVVNSI IRENGKPEYNVEFARDLARSFADREITKEQEDRKAHNEKVRKIHET FGRARASSKDILYKLMWMEQESKCIYSGKHIDAHRLFEPGYVEVDHILP FSKSFDDSMITNKVLVFKENQNKANRTPLEYMRASKPMYVDSYLAMV KFLYKSNFKKLQNLTFECGNDREEWSTSNLNDTRYIAKFIHKYIKDH LYPAKTGIKRVRAVNGRITSLRHFWGIKKIRENGDLHHAVIDAAVISV TTDMIKKFSEASKRHEEYDEKIMVEKPWEMFVDEISARISENPADQVE RLGLSTYSDEEKSSSLKTPFVSRMPRHKVTGVSVDHSTLSPVLLKQGINA YVSKREINDKLLKVFDKCKCEFFNMQLDAKFYASLSKSYLEDKNNKG EFHKIKKDGSLGPVVRKVKVVENCSSGVFLNNGKAFKNGDMIRIDVF

Category	SEQ ID:	Description	Type	Organism	Sequence
MG15 effector	11772	MG15-219 effector	protein	unknown	QVKEGKDKGFYFVPIYVADKVKDKLPSRAVIQKDPKDWKEMKDEDF IYSLYPNDLIYFSSKKIVGFKNNNNKSEILGVDMSEGYLYYTIGADISTASI NVDFVDNSFSKIGLGVTKLKEFRKFTIDVLGNIHEVVKKEKRLVFGRK MNYILGLDIGIASVGAVALDANDEPCKILDNLARIFEAAEQPKTGAS LAAPRRARGSRRTTRRRHRMERLRLHFAREELISAENIAALFEAPA DVYRLRAEGLSRRLDEGEWARVLYHIAKRRGFKSNRKGAAADAEKG VLEAVKENEALLKNYKTVGEMMFREKFTAKRNKGGSYTFCVSRG MLAEEIGELFAAQREQGNPHASETFETAYSKIFADQRSFDDGPDANSRS PYAGNQIEKMIGTCSLETDPPEKRAAKASYSFMFSLQKINHLRLKD AKGEERPLTDEERAAVEALAWKSPSLTYGAIRKALPLDELFTDLYY RWDKKPEEIEKKKLPFAAPYHEIRKALDKREKGRIQSLTPDALDAVG AFTVFNDAKIEAALSAAGIDGEDAVALMAAGLTFRGFGHISVKACRK LIPHLEKGMTYDKACEAGYDLQKTGGEKTKLLSGNLDEIREIPNVV RRAIAQTVKVNAVIRRYGSPVAVNVELAREMGRTFQERRDMMKSM DNNAENEKREELKGYGVVHPSGLDIVKLKLYKEQGGVCAYSLAAMP IEKVLKDHVYAEVDHILPYSRSFDDSYANKVLVLSKENRDKGNRTPME YMANMPGRRHDFITWVKS AVRNPRKRDNLLEKFGEDKEAAWKERH LTDTKYIGSFIANLLRDHLEFAPWLNKKKQHVLA VNGAVTDYTRKR LGIRKIREDDGLHHA VDAVIAVTQGNIQKLT DYSKQIERAFVKNRD GRYVNPDTGEVLKKDEWIVQRSRHFPEPWPGRHELEARVSDHPKEM IESRLPTYTPEEIDGLKPPFVSRMPTRKVRGA AHLET VVSPRLKDEGM IVKKVSLDALKLTDKDAIENYYAPESDHLLEYALLHRLQAFGGGGEK AFAESFHKPKADGTPGPVVKVIAEKSTLSVPVHHGRGLAANGMV RVDVFFIPEGKDRGYLYVPVYTS DVVRGELPMRAV VQGKSYAEWKLM REEDFISLYPNDLVYIEHEKGVVKIQKQKLEISTLPREKMTSGLFY YRTMGIAVASHIYAPDGVYVQESLGVKTLKEFKKWTIDILGGEHPV QKEKRQDFASVKRDPHAAKSTSSG HGDVLTATIPASTFSFRNTPATLRKKLLAGEAVSVGWLTQNDIEIEVD EFACGNTSFAKFLTEIPEKRWVRVDGFYDNRRLRIRPAYLSAEGLTDNHS KVVHETLEKGGQFVNAGALLSASRTLIRRTALGAPRWKLDSSSGLPVSF SPLKLAEEAL (N22) GTTGGGAATCGTCACTGAAAAGTGACGATTCTCAACAAAAAGACTTT TGCTCTTGATTCTTATCCCCCGGCATTTGTGCGGGGGGATTGCTT ATT GCATCGTTTGAAGAAGTGACGATTCTCAACAAAAAGACTTTTGTCTTT GATTTCTTTATCCCCCGGCATTTGTGCGGGGGGATTGCTTATT MATKKILGLDLGNSIGWALIEATEDSNPKSILAMGSRIVPLSTDSTQFA KGQAITKNADRTQKRTARKGLNRYQMRRA MLTEELRRHGM LPERTD
MG3 effectors	11794	MG3-42 effectors P1 domain	protein	unknown	
MG3 effectors sgRNA	11795	MG3-42 effector sgRNA	Nucleotide (RNA)	unknown	
MG3 effectors tracr	11796	MG3-42 effector tracr	Nucleotide (RNA)	unknown	
MG16 effectors	11797	MG16-3 effector	protein	unknown	

Category	SEQ ID:	Description	Type	Organism	Sequence
					ENIMDLWRLRSDAAATDGKQLSLPQIGRVLVYHINQKRGYKHSKADNSA NTKQTKYVEAVNQRYRDIQAACHQTIGQYFYEQLLSSAVQTPSGSYTY RIKDKVLPREAVIAEFDQIMKVQRVFPDVLDELVDTLRNHIIFYQRP LKSCKHLVSLCEFEKRPFKREDGQIVYSGPKCAPRTSPLAQFCTVWEA VNNITLTNRQNETFEITQEQRVAMADFLNQHDKMGVKDLQKILGISPK DGWWAGKAIGKGLKGNFTFTQLREALGNLPNAEHLKMKLSMVDAA VDTTGTGELRQVSPQVEEPLFRLWHLVYSLQNEDELKALRKQFGID DEEVLDKICKIDFVKPGYANKSHKFIKRLPVLMEGYQYHEACAHIGV NHSDSLTAEQNAARPLDDKIPLLEKNELRQPVIEKILNQMINVNVNALK EYGDIDDVRIELARELKSSKDEREAAFKRNNEENERQNKIYENRREYGI QPSRSRIQKYKMWEESNHLCFYCGKPNVTDFLAGAEVEIEHIPQSVL FDDSYSNKVACACRACNQAKGNLTAREFMEKHSKEEYDYLRRVDDAF NAHRISKTTRDHLWRKEDIPQDFIDRLRLNLPYQQVEGLTEDVTYDHCG NVYATSGSVTDFLRHQWGYDEILHRLNLPYQQVEGLTEDVTYDHCG QEHQQUERIKGWTKRLDHRHHAIDALTIALTQQSVIQRNLNLNSREQ MFDELGKRTDPEYTEKRSLLLEKWDVAQPHFSVQEVTDKVDGILVSFR AGKRAATPAKRAVYQNGKRHIVQTGLQVPRGALSEETVYGKLGKNY VKYPLGHQSMKDDIDVPTIREIVTRNAFGGAKADAFAEPLYSDA AHQMIQITVRCYTGLODKAVVPVRNAAQGEVPGFVKMGNNHHIAYR DAKGQYQESVVSFWQAVERKRYGIPVVEQPHIEVVDKLNDSNPQDF LETLPHDDWQFVVSQQNEMFILGMDDDAFEAAMEQKDRTLNKYL YRVQKISSKEYCFRYHTETSVDDKYDGVINKSISMELQKLRKLTSSIAFF SQPHPKVRVNLGVSAL
MG86 effectors	11798	MG86-1 effector	protein	unknown	MKKILSFDLIGTISIGYSVLTEDEAQKYSLLDYGVSMFDKPTDKDGNK KLLHAQALSTKKLYKLRKERKKNLALLFEKYALAKASKLLEQEKKNL YMYKWQLRAKKVFEERLSIGEITILYHIAKHRYGYSKSDSGDLLEELC VELGIKIDVKKKDEKKGKIKQALSTIESLRKEYPKKTVAQIHYEVEL QKERPVFRNHDNRYNVMIRREHINDEIAIIRKQKEFGNFENIDSEVFVD IIAADDQKESTNDMSLFKCEYYPKYEHVAHQYSLSDIFKMYQAVANI TFNKEKITKEQIRLLTEDFLNKIKKKGKSVKELKYKDVVRKILKLDES KIFNKEDSYQRAGKKVEHTTKTFHFVDNLKIDKSFIEDIFNADESIVL MREIFDVIHKEKSPKRIYEQKSKVSSEAVIIDLRYKKGSSLNISYAMA KFLPYFEEGMITLDAIKEKLDLGRKEDYSVYKKGKIKYHISTYEKDDDL EINNHPVKYVVSAYLVRVVKHLHAKHGTFDEIKVESTRELSLNDKVKE IDKANKAREKEIEKISNDEYQKIAKEYGKNIHKEYARKILMWEAQERFD VYSGKSIGIDDIENRVDVHIYVQSLGGLYVQHNLVLRHNDENLQKSN QLPMNYITDKAEAYINRVEHLESEHKINWKKRKNLLASNLDIYKDTFES KDLRATSYIEALTANILKRYYPFIDEKKSVDGSAVRHIQGRATANIRKV LGVKTKSRESNIHGVDAALLIGVTNPWSVLQKLSNIFRENFCKIDDEARK

Category	SEQ ID:	Description	Type	Organism	Sequence
MG86 effector sgRNA	11799	MG86-1 effector sgRNA	Nucleotide (RNA)	unknown	NIKKALPYIDGVEVKDIVKEIEQKYN SYGEDSIFYKDIWGA KAKTVNFW VSKKPMISKVHKDTIYADKNGGIFTVRESIAKFINKITPTTFPEDFMK KFHEILEKMYLYKTNSNDVICKIVQQRAEEIEKELLWSFEFLDVKNKE EMQEA KANLES LVHRELF DNNGNVVRKV FYQTNL TGFKV RGGGLAT KEKTFIGFRAFKDKKLEYKRIDVSNEFEKIKSNDGSKFVYKNDIVFFV FDEEKYKGGKIVSFLEDKKMAAFSPKYPANIQAPESFLTIFK GKANS HKQVS VGGKAKGHKLKVDILGNIESYQVLGN AKSKLLDEIKSIVSH (N20)GUUUUAAUACCCGAAAGGUAUAAAACUAAAGGUCACUUUUA GUGGUGACUUUAGAGUAUGGCUUUGGCUAAUACUCCAUUUU
MG94 effector	11800	MG94-1 effector	protein	unknown	MRKKIRYVIGLDIGLASVGWAALLDENDNVCGIVRAGVHTFDEAVVG QSKITGAAYRRGYSGRRIRRKVNRIQRVKNLQRLNIISKKDL EYF SGAVENIYLRCAAIQNEPAYILNNKELAQLLIYYAKHRYGKYSNTSYEQ KTDDSKKVL SALS ENKKYMLEKGYQTAGEMLYRDEKFRKRKRYGSSEE CELLVRNSGDDYSHSISRELVEEVHVIFARQRELGNKLT TKELEDQF VEIMQSQRNYDESGSGEPYGGNLEKMGVGECTFEKGEKCRACKASYTS ERFVLLLEKLNHLRIQSKNGDVRALTEERDAIHKLAYKNKDVKYKALR TILKLNPERFGGLTYSRGDIENSTEGKSVFVSLEYWYIEKKVLGLFYD DLDNEETQQLDSIGTILTCYKSDDLRRRKFEQLHLEQEKIEHLALNY TKFQNL SFKAMKNIMPELEKGLSYTEACSNAGYGDKETIEGKNKYISK ELLNNTLDSIMNPTVKRAVRRRTIRILNELIKQYGSPVEVHVEMARDLTH SQTVTNKMKKRQDENKAEKEEAKRFICENFGKTEAQVSGKDILRYRL WKSQNQIDIYSNTMIPVSDILDYKYEVDHIIPYSCSFNDSFNKNMLVRK KDNQDKKNRTPYEYIGSDEKKWEAFATCANTYVMNYGKRKNMLTKV PASNTGEWMSRNLDTRYTKVVTDLIRKHLKFEAYVDQKRKKHIYPI NGGITAKLR YEWGLEKDRKSDKHHAQDAVVIACCTDGMQRLSRQY MLQEIGIVTWKNHKLVD RRTGEIV EETNLPWECFREEVEMFMADSPE DYIEKAKKNGYKGEAPKPIFVSRLPQKKTTGKINEDTLRSVRIDSKGKA RFVNKTKLQDLKLVEVDGKKQIKDYRPEDDKLLYDKLLERLVKNDD AKVVFAEPFYKPKKDGSDGPVRSVKTYGKTVKNQVLVGDGVAERGG IYRCDVFKRKDEYYA VVYYRDL YIGNLPNNA AHFDIEMKKGEFEFSL YKDDLIRFVKDGKEQYAYYKYINANN SQITYTEHDTSKETKCTTIRTL D KFQKMNVDLLGNIYSSDKEEREWN*
MG94 effector	11801	MG94-1 effector PI domain	protein	unknown	KTVKNQVLVGDGVAERGIYRCDVFKRKDEYYAVVYYRDL YIGNLP NNA AHFDIEMKKGEFEFSLYKDDLIRFVKDGKEQYAYYKYINANN SQI TYTEHDTSKETKCTTIRTL DKFQKMNVDLLGNIYSSDKEEREWN*

CLAIMS

WHAT IS CLAIMED IS:

1. A method of disrupting a Beta-2-Microglobulin (B2M) locus in a cell, comprising contacting to said cell
 - (a) an RNA-guided endonuclease; and
 - (b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said B2M locus,
 - wherein said region of said B2M locus comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6387-6468.
2. The method of claim 1, wherein said RNA-guided endonuclease is a class 2, type II Cas endonuclease.
3. The method of claim 1, wherein said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% sequence identity to SEQ ID NO: 2242 or SEQ ID NO: 2244.
4. The method of claim 3, wherein said RNA-guided endonuclease further comprises an HNH domain.
5. The method of claim 1, wherein said engineered guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 6305-6386.
6. The method of claim 1, wherein said region of said B2M locus comprises a sequence at least 75%, 80%, or 90% identical to at least 19 of the non-degenerate nucleotides of any one of SEQ ID NOs: 6388, 6399, 6401, 6403, 6410, 6413, 6421, 6446, and 6448.
7. A method of disrupting a T Cell Receptor Alpha Constant (TRAC) locus in a cell, comprising contacting to said cell
 - (a) an RNA-guided endonuclease; and

(b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said TRAC locus,

wherein said region of said TRAC locus comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6509-6548 or 6805.

8. The method of claim 7, wherein said RNA-guided endonuclease is a class 2, type II Cas endonuclease.

9. The method of claim 7, wherein said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% sequence identity to SEQ ID NO: 2242 or SEQ ID NO: 2244.

10. The method of claim 9, wherein said RNA-guided endonuclease further comprises an HNH domain.

11. The method of claim 7, wherein said engineered guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 6469-6508 or 6804.

12. The method of claim 7, wherein said region of said TRAC locus comprises a sequence at least 75%, 80%, or 90% identical to at least 19 of the non-degenerate nucleotides of any one of SEQ ID NOs: 6517, 6520, and 6523.

13. A method of disrupting a Hypoxanthine Phosphoribosyltransferase 1 (HPRT) locus in a cell, comprising contacting to said cell

(a) an RNA-guided endonuclease; and

(b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said HPRT locus,

wherein said region of said HPRT locus comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6616-6682.

14. The method of claim 13, wherein said RNA-guided endonuclease is a class 2, type II Cas endonuclease.

15. The method of claim 13, wherein said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% sequence identity to SEQ ID NO: 2242 or SEQ ID NO: 2244.

16. The method of claim 15, wherein said RNA-guided endonuclease further comprises an HNH domain.

17. The method of claim 13, wherein said engineered guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 6549-6615.

18. The method of any one of claim 13, wherein said region of said HPRT locus comprises a sequence at least 75%, 80%, or 90% identical to at least 19 of the non-degenerate nucleotides of any one of SEQ ID NOs: 6619, 6634, 6673, 6675, and 6679.

19. A method of disrupting a T Cell Receptor Beta Constant 1 or T Cell Receptor Beta Constant 2 (TRBC1/2) locus in a cell, comprising contacting to said cell

(a) an RNA-guided endonuclease; and

(b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said TRBC1/2 locus,

wherein said region of said TRBC1/2 locus comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6722-6760 or 6782-6802.

20. The method of claim 19, wherein said RNA-guided endonuclease is a class 2, type II Cas endonuclease.

21. The method of claim 19, wherein said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% sequence identity to SEQ ID NO: 2242 or SEQ ID NO: 2244.

22. The method of claim 21, wherein said RNA-guided endonuclease further comprises an HNH domain.

23. The method of claim 19, wherein said engineered guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 6683-6721 and 6761-6781.

24. The method of claim 19, wherein said region of said TRBC1/2 locus comprises a sequence at least 75%, 80%, or 90% identical to at least 19 of the non-degenerate nucleotides of any one of SEQ ID NOs: 6734, 6753, 6790, and 6800.

25. A method of disrupting a Hydroxyacid Oxidase 1 (HAO1) locus in a cell, comprising contacting to said cell

(a) an RNA-guided endonuclease; and

(b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said HAO1 locus,

wherein said region of said HAO1 locus comprises a targeting sequence having at least 85% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 11802-11820.

26. The method of claim 25, wherein said RNA-guided endonuclease is a class 2, type II Cas endonuclease.

27. The method of claim 25, wherein said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% sequence identity to SEQ ID NO: 2242.

28. The method of claim 27, wherein said RNA-guided endonuclease further comprises an HNH domain.

29. The method of claim 25, wherein said region of said HAO1 locus comprises a sequence at least 75%, 80%, or 90% identical to at least 19 of the non-degenerate nucleotides of any one of SEQ ID NOs: 11806, 11813, 11816, and 11819.

30. An engineered nuclease system comprising:

- (a) an RNA-guided endonuclease; and
- (b) an engineered guide RNA, wherein said engineered guide RNA comprises
 - (i) a 2'-O-methyl nucleotide;
 - (ii) a 2'-fluoro nucleotide; or
 - (iii) a phosphorothioate bond;

wherein said RNA-guided endonuclease has at least 75% sequence identity to any one of SEQ ID NOs: 421-431 or a variant thereof.

31. The engineered nuclease system of claim 30, wherein said RNA-guided endonuclease comprises a sequence having at least 75% sequence identity to SEQ ID NO: 421.

32. An engineered nuclease system comprising:

- (a) an endonuclease having at least 75% sequence identity to any one of SEQ ID NOs: 421-431 or a variant thereof;
- (b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a target nucleic acid sequence,
wherein said system has reduced immunogenicity when administered to a human subject compared to an equivalent system comprising a Cas9 enzyme.

33. The system of claim 32, wherein said Cas9 enzyme is an SpCas9 enzyme.

34. The system of claim 32 or 33, wherein said immunogenicity is antibody immunogenicity.

35. The system of any one of claims 32-34, wherein said engineered guide RNA comprises a sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of any one of SEQ ID NOs: 5466-5467 and 11160-11162.

36. The system of any one of claims 32-35, wherein said engineered nuclease has at least about 75% sequence identity at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 421 or 423 or a variant thereof.

37. A method of disrupting a locus in a cell, comprising contacting to said cell
(a) an RNA-guided endonuclease or a nucleic acid encoding said RNA-guided endonuclease; and
(b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said RNA-guided endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said locus;
wherein said cell is a peripheral blood mononuclear cell (PBMC), a hematopoietic stem cell (HSC), or an induced pluripotent stem cell (iPSC).

38. The method of claim 37, wherein said RNA-guided endonuclease is a class 2, type II Cas endonuclease.

39. The method of claim 37 or 38, wherein said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% sequence identity to SEQ ID NO: 2242, or a variant thereof.

40. The method of any one of claims 37-39, wherein said RNA-guided endonuclease further comprises an HNH domain.

41. The method of any one of claims 37-40, wherein said RNA-guided endonuclease has at least about 75% sequence identity at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 421 or a variant thereof.

42. The method of any one of claims 37-41, wherein said engineered guide RNA comprises a sequence at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at

least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 6804, 6806, and 6808.

43. The method of claim any one of claims 37-42, wherein said nucleic acid encoding said RNA-guided endonuclease comprises a sequence comprising at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 6803 or a variant thereof.

44. The method of any one of claims 37-43, wherein said region of said locus comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to at least 18 nucleotides of any one of SEQ ID NOs: 6805, 6807, and 6809.

45. A method of disrupting a CD2 Molecule (CD2) locus in a cell, comprising contacting to said cell

(a) an RNA-guided endonuclease; and

(b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said CD2 locus,

wherein said engineered guide RNA comprises or is configured to hybridize to a sequence having at least 18-22 consecutive nucleotides complementary to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 6853-6894; or

wherein said engineered guide RNA comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to the non-degenerate nucleotides of any one of SEQ ID NOs: 6811-6852.

46. The method of claim 45, wherein said RNA-guided endonuclease is a class 2, type II Cas endonuclease.

47. The method of claim 45 or 46, wherein said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least about 75% sequence identity to SEQ ID NO: 2242 or SEQ ID NO: 2244, or a variant thereof.

48. The method of any one of claims 45-47, wherein said RNA-guided endonuclease further comprises an HNH domain.

49. The method of any one of claims 45-48, wherein said RNA-guided endonuclease comprises a sequence having at least about 75% sequence identity to any one of SEQ ID NOs: 421-431.

50. The method of any one of claims 45-49, wherein said RNA-guided endonuclease comprises a sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 421, or a variant thereof.

51. The method of any one of claims 45-50, wherein said engineered guide RNA comprises a sequence at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to the non-degenerate nucleotides of any one of SEQ ID NOs: 6813, 6841, 6843-6847, 6852, or 6852.

52. The method of claim 51, wherein said engineered guide RNA further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 6A.

53. The method of any one of claims 45-52, wherein said engineered guide RNA comprises or is configured to hybridize to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6855, 6883, 6885-6889, 6892, or 6984.

54. An isolated RNA molecule comprising a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 6811-6852.

55. The isolated RNA molecule of claim 54, further comprising a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 6A.

56. A method of disrupting a CD5 Molecule (CD5) locus in a cell comprising contacting to said cell

(a) an RNA-guided endonuclease; and

(b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said CD5 locus,

wherein said engineered guide RNA comprises or is configured to hybridize to a sequence having at least 18-22 consecutive nucleotide complementary to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID Nos: 6959-7022; or

wherein said engineered guide RNA comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity the non-degenerate nucleotides of any one of SEQ ID NOs: 5466 or 6895-6958.

57. The method of claim 56, wherein said RNA-guided endonuclease is a class 2, type II Cas endonuclease.

58. The method of claim 56 or 57, wherein said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% sequence identity to SEQ ID NO: 2242 or SEQ ID NO: 2244, or a variant thereof.

59. The method of any one of claims 56-58, wherein said RNA-guided endonuclease further comprises an HNH domain.

60. The method of any one of claims 56-59, wherein said RNA-guided endonuclease comprises an endonuclease having at least 75% sequence identity to any one of SEQ ID NOs: 421-431 or a variant thereof.

61. The method of any one of claims 56-60, wherein said RNA-guided endonuclease comprises a sequence at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 421.

62. The method of any one of claims 56-61, wherein said engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of SEQ ID NO: 5466.

63. The method of any one of claims 56-62, wherein said engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to the non-degenerate nucleotides of any one of SEQ ID NOs: 6897, 6904, 6906, 6911, 6928, 6930, 6932, 6934, 6938, 6945, 6950, 6952, and 6958.

64. The method of claim 63, wherein said engineered guide RNA further comprises a pattern of nucleotide modification recited in any of the guide RNAs recited in Table 7A.

65. The method of any one of claims 56-64, wherein said engineered guide RNA is configured to hybridize to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 6961, 6968, 6970, 6975, 6992, 6994, 6996, 6998, 7002, 7009, 7014, 7016, and 7022.

66. An isolated RNA molecule comprising a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least

about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 6895-6958.

67. The isolated RNA molecule of claim 66, further comprising a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 7A.

68. A method of disrupting an RNA locus in a cell, comprising contacting to said cell

(a) an RNA-guided endonuclease comprising a sequence having at least 75% sequence identity to SEQ ID NO: 2242 or SEQ ID NO: 2244, or a variant thereof; and

(b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said RNA locus,

wherein said RNA locus does not comprise bacterial or microbial RNA.

69. The method of claim 68, wherein said guide RNA comprises a sequence having at least 80% sequence identity to non-degenerate nucleotides of SEQ ID NO: 5466 or SEQ ID NO: 5539.

70. A method of disrupting a Fas Cell Surface Death Receptor (FAS) locus in a cell, comprising introducing to said cell:

(a) an RNA-guided endonuclease; and

(b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said human FAS locus,

wherein said engineered guide RNA comprises or is configured to hybridize to a sequence having at least 18-22 consecutive nucleotides complementary to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7057-7090; or

wherein said engineered guide RNA comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7023-7056.

71. The method of claim 70, wherein said RNA-guided endonuclease is a class 2, type II Cas endonuclease.

72. The method of claim 70 or 71, wherein said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% sequence identity to SEQ ID NO: 2242, or a variant thereof.

73. The method of any one of claims 70-72, wherein said RNA-guided endonuclease further comprises an HNH domain.

74. The method of any one of claims 70-73, wherein said engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of SEQ ID NO: 5466.

75. The method of any one of claims 70-74, wherein said engineered guide RNA comprises or is configured to hybridize to a sequence having at least 80% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 7059, 7061, 7069, 7070, 7076, 7080, 7083, 7084, 7085, or 7088.

76. The method of any one of claims 70-75, wherein said RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 421, or a variant thereof.

77. The method of any one of claims 70-76, wherein said guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 7025, 7027, 7035, 7036, 7042, 7046, 7049-7051, or 7054.

78. The method of claim 77, wherein said guide RNA further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 8.

79. An isolated RNA molecule comprising a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7023-7056.

80. The isolated RNA molecule of claim 79, further comprising a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 8.

81. A method of disrupting a Programmed Cell Death 1 (PD-1) locus in a cell, comprising introducing to said cell:

(a) an RNA-guided endonuclease; and

(b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said human PD-1 locus,

wherein said engineered guide RNA comprises or is configured to hybridize to a sequence having at least 18-22 consecutive nucleotides complementary to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7129-7166; or

wherein said engineered guide RNA comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7091-7128.

82. The method of claim 81, wherein said RNA-guided endonuclease is a class 2, type II Cas endonuclease.

83. The method of claim 81 or 82, wherein said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% sequence identity to SEQ ID NO: 2242, or a variant thereof.

84. The method of any one of claims 81-83, wherein said RNA-guided endonuclease further comprises an HNH domain.

85. The method of any one of claims 81-84, wherein said engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of SEQ ID NO: 5466.

86. The method of any one of claims 81-85, wherein said engineered guide RNA comprises or is configured to hybridize to a sequence having at least 80% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 7135, 7137, 7146, 7149, 7152, 7156, 7160, 7161, 7164, 7165, or 7166.

87. The method of any one of claims 81-86, wherein said RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 421, or a variant thereof.

88. The method of any one of claims 81-87, wherein said guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 7097, 7099, 7108, 7111, 7114, 7118, 7122, 7123, 7126, 7127, or 7128.

89. The method of claim 88, wherein said guide RNA further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 9.

90. An isolated RNA molecule comprising a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7091-7128.

91. The isolated RNA molecule of claim 79, further comprising a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 9.

92. A method of disrupting an human Rosa26 (hRosa26) locus in a cell, comprising introducing to said cell:

(a) an RNA-guided endonuclease; and

(b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said hRosa26 locus,

wherein said engineered guide RNA comprises or is configured to hybridize to a sequence having at least 18-22 consecutive nucleotides complementary to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7199-7230; or

wherein said engineered guide RNA comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7167-7198.

93. The method of claim 92, wherein said RNA-guided endonuclease is a class 2, type II Cas endonuclease.

94. The method of claim 92 or 93, wherein said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% sequence identity to SEQ ID NO: 2242, or a variant thereof.

95. The method of any one of claims 92-94, wherein said RNA-guided endonuclease further comprises an HNH domain.

96. The method of any one of claims 92-95, wherein said engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of SEQ ID NO: 5466.

97. The method of any one of claims 92-96, wherein said engineered guide RNA comprises or is configured to hybridize to a sequence having at least 80% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 7205-7206, 7215, 7220, 7223, or 7225.

98. The method of any one of claims 92-97, wherein said RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 421, or a variant thereof.

99. The method of any one of claims 92-98, wherein said guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 7173, 7174, 7183, 7188, 7191, or 7193.

100. The method of claim 99, wherein said guide RNA further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 10.

101. An isolated RNA molecule comprising a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7167-7198.

102. The isolated RNA molecule of claim 101, further comprising a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 10.

103. A method of disrupting an T Cell Receptor Alpha Constant (TRAC) locus in a cell, comprising introducing to said cell:

(a) an RNA-guided endonuclease; and

(b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said TRAC locus,

wherein said engineered guide RNA comprises or is configured to hybridize to a sequence having at least 18-22 consecutive nucleotides complementary to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7235-7238, 7248-7256, 7270, or 7278-7284; or

wherein said engineered guide RNA comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7231-7234, 7239-7247, 7269, or 7271-7277.

104. The method of claim 103, wherein said RNA-guided endonuclease is a class 2, type II Cas endonuclease.

105. The method of any one of claims 103-104, wherein said RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 1512, 1756, 11711-11713, or a variant thereof.

106. The method of any one of claims 103-105, wherein said engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about

95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of SEQ ID NO: 5473, 5475, 11145, 11714, or 11715.

107. The method of any one of claims 103-106, wherein said engineered guide RNA comprises or is configured to hybridize to a sequence having at least 80% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 7235-7238, 7248-7256, 7270, or 7278-7284.

108. The method of any one of claims 103-107, wherein said guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 7231-7234, 7239-7244, 7269, or 7271-7277.

109. The method of claim 108, wherein the engineered guide RNA further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 11.

110. An isolated RNA molecule comprising a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7231-7234, 7239-7247, 7269, or 7271-7277.

111. The isolated RNA molecule of claim 110, further comprising a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 11.

112. A method of disrupting an Adeno-Associated Virus Integration Site 1 (AAVS1) locus in a cell, comprising introducing to said cell:

(a) a class 2, type II Cas endonuclease; and

(b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said AAVS1 locus,

wherein said engineered guide RNA comprises or is configured to hybridize to a sequence having at least 18-22 consecutive nucleotides complementary to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7261-7264 or 7267-7268; or

wherein said engineered guide RNA comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7257-7260 or 7265-7266.

113. The method of claim 112, wherein said RNA-guided endonuclease is a class 2, type II Cas endonuclease.

114. The method of any one of claims 112-113 wherein said RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 1756 or 11711, or a variant thereof.

115. The method of any one of claims 112-114, wherein said engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of SEQ ID NO: 5475 or 11715.

116. The method of any one of claims 112-115, wherein said engineered guide RNA comprises or is configured to hybridize to a sequence having at least 80% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 7261-7263 or 7267-7268.

117. The method of any one of claims 112-116, wherein said guide RNA comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 7257-7260 or 7265-7266.

118. The method of claim 117, wherein said engineered guide RNA further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 12.

119. An isolated RNA molecule comprising a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 7257-7260 or 7265-7266.

120. The isolated RNA molecule of claim 119, further comprising a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 12.

121. A method of disrupting an Hydroxyacid Oxidase 1 (HAO-1) locus in a cell, comprising introducing to said cell:

(a) an RNA-guided endonuclease; and

(b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said HAO-1 locus,

wherein said engineered guide RNA comprises or is configured to hybridize to a sequence having at least 18-22 consecutive nucleotides complementary to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11773-11793.

122. The method of claim 121, wherein said RNA-guided endonuclease is a class 2, type II Cas endonuclease.

123. The method of claim 121 or 122, wherein said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% sequence identity to SEQ ID NO: 2242, or a variant thereof.

124. The method of any one of claims 121-123, wherein said RNA-guided endonuclease further comprises an HNH domain.

125. The method of any one of claims 121-124, wherein said engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of SEQ ID NO: 5466.

126. The method of any one of claims 121-125, wherein said engineered guide RNA comprises or is configured to hybridize to a sequence having at least 80% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 11773, 11780, 11786, or 11787.

127. The method of any one of claims 121-126, wherein said RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 421, or a variant thereof.

128. An isolated RNA molecule comprising a spacer sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11773-11793 and

a scaffold sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 5466.

129. A method of disrupting a human G Protein-Coupled Receptor 146 (GPR146) locus in a cell, comprising introducing to said cell:

(a) an RNA-guided endonuclease; and

(b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said GPR146 locus,

wherein said engineered guide RNA comprises or is configured to hybridize to a sequence having at least 18-22 consecutive nucleotides complementary to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11406-11437; or

wherein said engineered guide RNA comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11374-11405.

130. The method of claim 129, wherein said RNA-guided endonuclease is a class 2, type II Cas endonuclease.

131. The method of claim 129 or 130, wherein said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% sequence identity to SEQ ID NO: 2242, or a variant thereof.

132. The method of any one of claims 129-131, wherein said RNA-guided endonuclease further comprises an HNH domain.

133. The method of any one of claims 129-132, wherein said engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of SEQ ID NO: 5466.

134. The method of any one of claims 129-133, wherein said engineered guide RNA comprises or is configured to hybridize to a sequence having at least 80% identity to at least 18 consecutive nucleotides of SEQ ID NO: 11425.

135. The method of any one of claims 129-134, wherein said RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 421, or a variant thereof.

136. The method of any one of claims 129-135, wherein said guide RNA comprises a sequence having at least 80% identity to SEQ ID NO: 11393.

137. The method of claim 129-136, wherein said engineered guide RNA further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 15.

138. An isolated RNA molecule comprising a spacer sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11374-11405.

139. The isolated RNA molecule of claim 138, further comprising a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 15.

140. A method of disrupting a mouse G Protein-Coupled Receptor 146 (GPR146) locus in a cell, comprising introducing to said cell:

(a) an RNA-guided endonuclease; and

(b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said GPR146 locus,

wherein said engineered guide RNA comprises or is configured to hybridize to a sequence having at least 18-22 consecutive nucleotides complementary to a sequence having

at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11473-11507; or

wherein said engineered guide RNA comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11438-11472.

141. The method of claim 140, wherein said RNA-guided endonuclease is a class 2, type II Cas endonuclease.

142. The method of claim 140 or 141, wherein said RNA-guided endonuclease comprises a RuvCIII domain comprising a sequence having at least 75% sequence identity to SEQ ID NO: 2242, or a variant thereof.

143. The method of any one of claims 140-142, wherein said RNA-guided endonuclease further comprises an HNH domain.

144. The method of any one of claims 140-143, wherein said engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of SEQ ID NO: 5466.

145. The method of any one of claims 140-144, wherein said engineered guide RNA comprises or is configured to hybridize to a sequence having at least 80% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 11482, 11488, or 11490.

146. The method of any one of claims 140-145, wherein said RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 421, or a variant thereof.

147. The method of any one of claims 140-146, wherein said guide RNA comprises a sequence having at least 80% identity to SEQ ID NO: 11447, 11453, or 11455.

148. The method of claim 140-147, wherein said engineered guide RNA further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 16.

149. An isolated RNA molecule comprising a spacer sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11438-11472.

150. The isolated RNA molecule of claim 149, further comprising a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 16.

151. A method of disrupting a T Cell Receptor Alpha Constant (TRAC) locus in a cell, comprising introducing to said cell:

(a) an RNA-guided endonuclease; and

(b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said TRAC locus,

wherein said engineered guide RNA comprises or is configured to hybridize to a sequence having at least 18-22 consecutive nucleotides complementary to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11516-11517; or

wherein said engineered guide RNA comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11514-11515.

152. The method of claim 151, wherein said RNA-guided endonuclease is a class 2, type II Cas endonuclease.

153. The method of any one of claims 151-152, wherein said engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about

95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of SEQ ID NO: 11153.

154. The method of any one of claims 151-153, wherein said engineered guide RNA comprises or is configured to hybridize to a sequence having at least 80% identity to at least 18 consecutive nucleotides of any one of SEQ ID NOs: 11516.

155. The method of any one of claims 151-154, wherein said RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 11716, or a variant thereof.

156. The method of any one of claims 151-155, wherein said guide RNA comprises a sequence having at least 80% identity to SEQ ID NO: 11514.

157. The method of claim 156, wherein said engineered guide RNA further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 17.

158. An isolated RNA molecule comprising a spacer sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11514-11515.

159. The isolated RNA molecule of claim 158, further comprising a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 17.

160. A method of disrupting an Adeno-Associated Virus Integration Site 1 (AAVS1) locus in a cell, comprising introducing to said cell:

(a) an RNA-guided endonuclease; and

(b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a region of said AAVS1 locus,

wherein said engineered guide RNA comprises or is configured to hybridize to a sequence having at least 18-22 consecutive nucleotides complementary to a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11511-11513; or

wherein said engineered guide RNA comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11508-11510.

161. The method of claim 160, wherein said RNA-guided endonuclease is a class 2, type II Cas endonuclease.

162. The method of any one of claims 160-161, wherein said engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of SEQ ID NO: 11717.

163. The method of any one of claims 160-162, wherein said engineered guide RNA comprises or is configured to hybridize to a sequence having at least 80% identity to at least 18 consecutive nucleotides of SEQ ID NO: 11511.

164. The method of any one of claims 160-163, wherein said RNA-guided endonuclease comprises a sequence at least 75%, 80%, or 90% identical to SEQ ID NO: 914, or a variant thereof.

165. The method of any one of claims 160-164, wherein said guide RNA comprises a sequence having at least 80% identity to SEQ ID NO: 11508.

166. The method of claim 165, wherein the engineered guide RNA further comprises a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 17.

167. An isolated RNA molecule comprising a spacer sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of SEQ ID NOs: 11508-11510.

168. The isolated RNA molecule of claim 167, further comprising a pattern of nucleotide modifications recited in any of the guide RNAs recited in Table 17.

169. An engineered nuclease system comprising:

(a) an endonuclease having at least at least about 80%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to a PI domain of any of the Cas effector protein sequences described herein, or a variant thereof; and

(b) an engineered guide RNA, wherein said engineered guide RNA is configured to form a complex with said endonuclease and said engineered guide RNA comprises a spacer sequence configured to hybridize to a target nucleic acid sequence,

wherein said engineered guide RNA comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to non-degenerate nucleotides of any of the sgRNA sequences described herein.

170. The engineered nuclease system of claim 169, further comprising a RuvCIII domain or a HNH domain having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to RuvCIII domains or HNH domains of any of the Cas effector nucleases described herein.

171. The engineered nuclease system of claim 169 or 170, wherein said endonuclease is configured to have selectivity for any of the PAM sequences described herein.

172. The engineered nuclease system of any one of claims 169-171, wherein the endonuclease further comprises a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any of the Cas effector sequences described herein.

173. Use of the methods of any one of claims 1-6 for disrupting a B2M locus in a cell.

174. Use of the methods of any one of claims 7-12, 103-109, or 151-157 or the RNA of any one of claims 110-111 or 158-159 for disrupting a TRAC locus in a cell.

175. Use of the methods of any one of claims 13-18 for disrupting an HPRT locus in a cell.
176. Use of the methods of any one of claims 19-24 for disrupting a TRBC1/2 locus in a cell.
177. Use of the methods of any one of claims 25-29 or 121-127 or the RNA of any one of claims 128-129 for disrupting an HAO-1 locus in a cell.
178. Use of the methods of any one of claims 45-53 or the RNA of any one of claims 54-55 for disrupting a CD2 locus in a cell.
179. Use of the methods of any one of claims 56-65 or the RNA of any one of claims 66-67 for disrupting a CD5 locus in a cell.
180. Use of the methods of any one of claims 70-78 or the RNA of any one of claims 79-80 for disrupting a FAS locus in a cell.
181. Use of the methods of any one of claims 81-89 or the RNA of any one of claims 90-91 for disrupting a PD-1 locus in a cell.
182. Use of the methods of any one of claims 92-100 or the RNA of any one of claims 101-102 for disrupting an hRosa26 locus in a cell.
183. Use of the methods of any one of claims 112-118 or 160-166 or the RNA of any one of claims 119-120 or 167-168 for disrupting an AAVS1 locus in a cell.
184. Use of the methods of any one of claims 129-137 or 140-148 or the RNA of any one of claims 138-139 or 149-150 for disrupting a GPR146 locus in a cell.

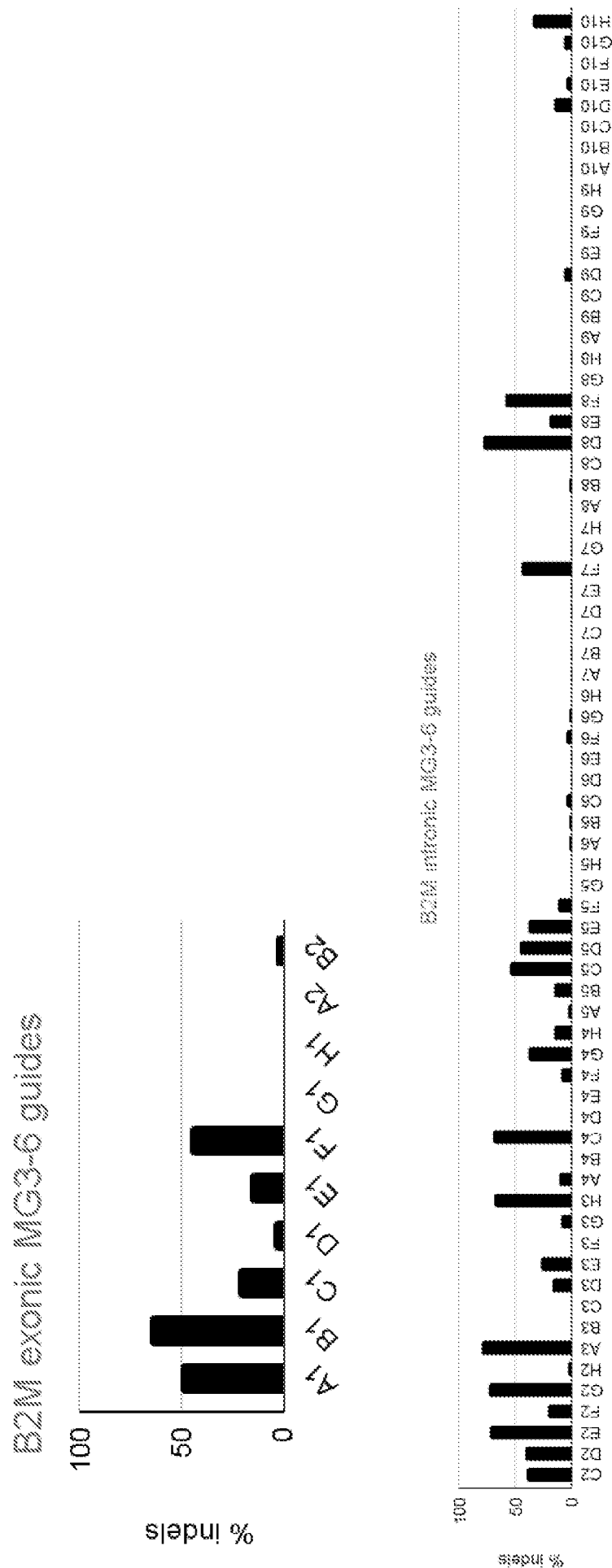


FIG. 1

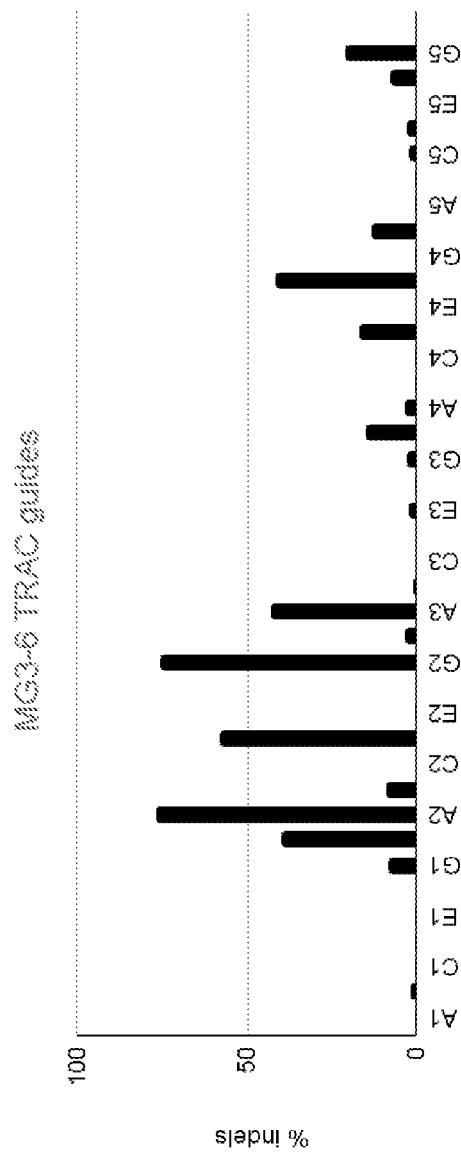


FIG. 2A

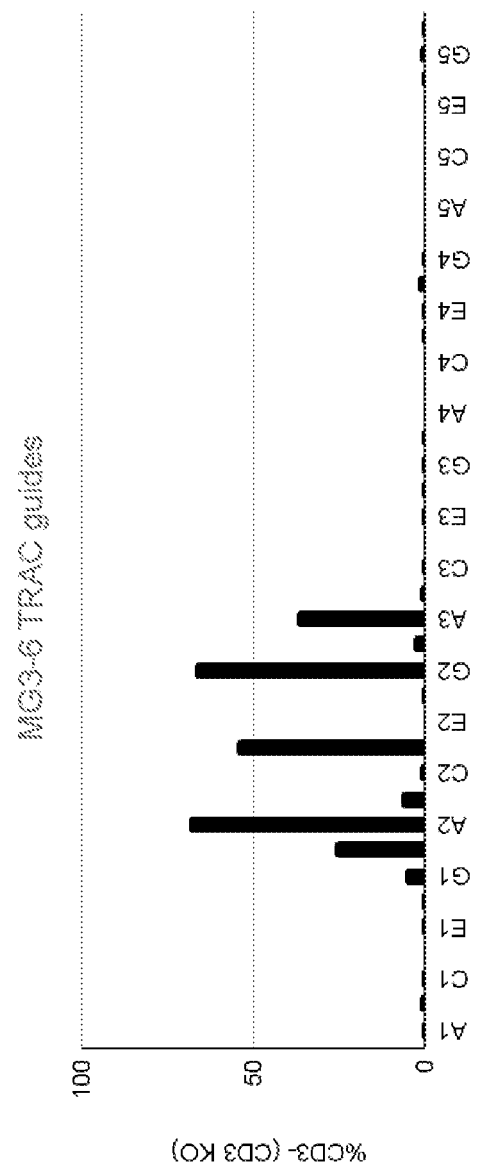


FIG. 2B

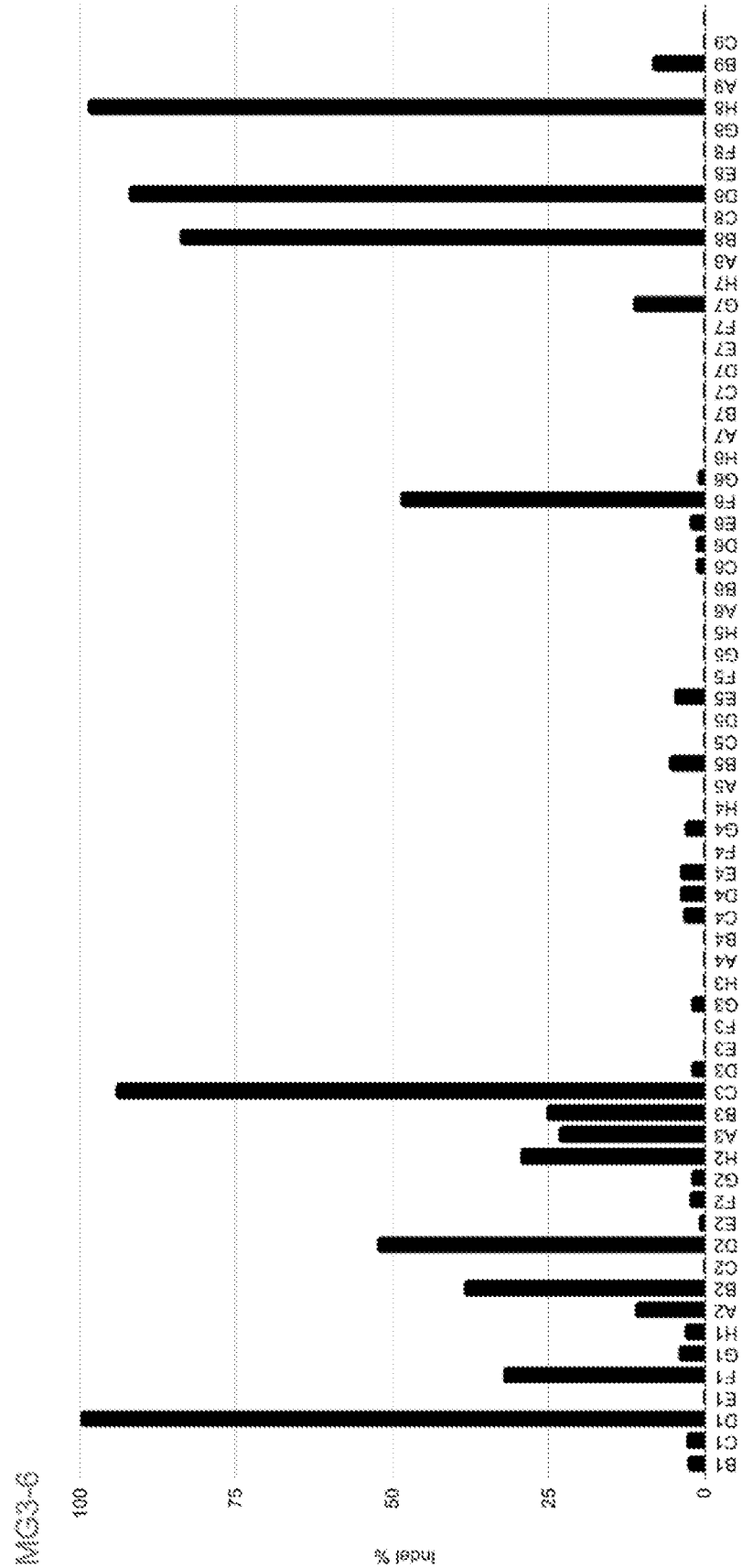


FIG. 3

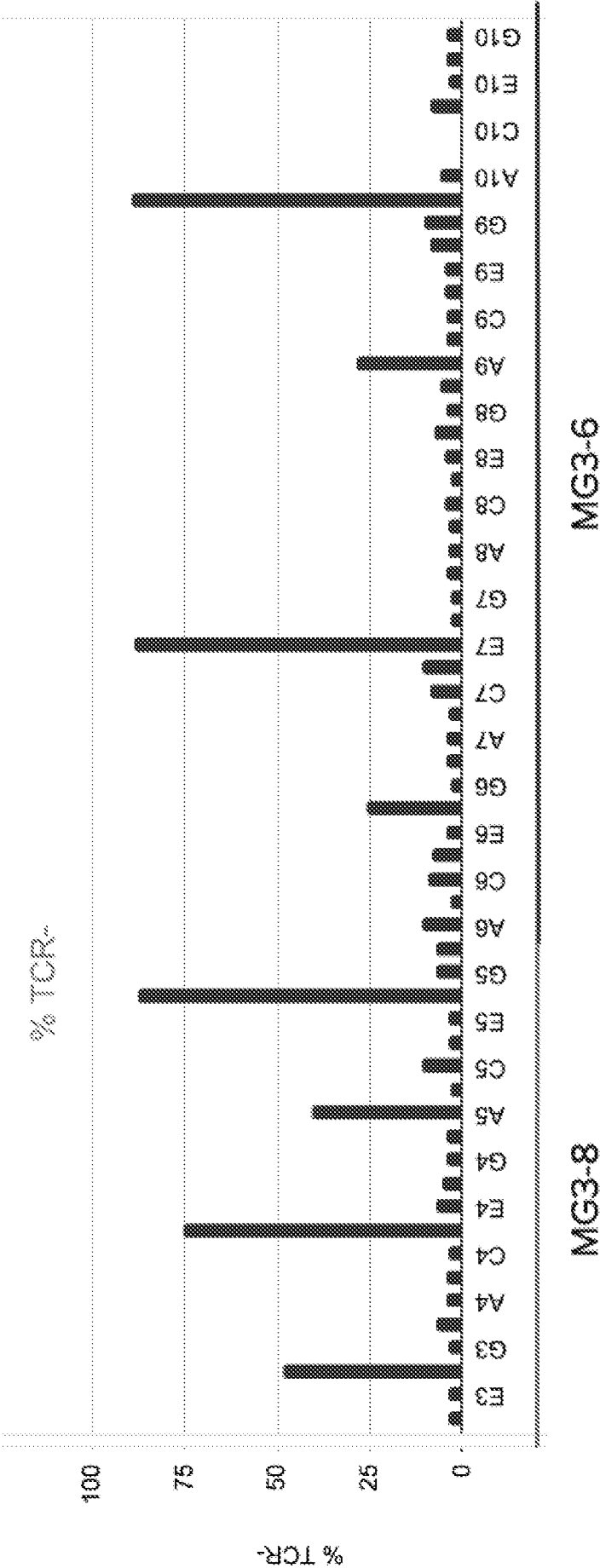


FIG. 4

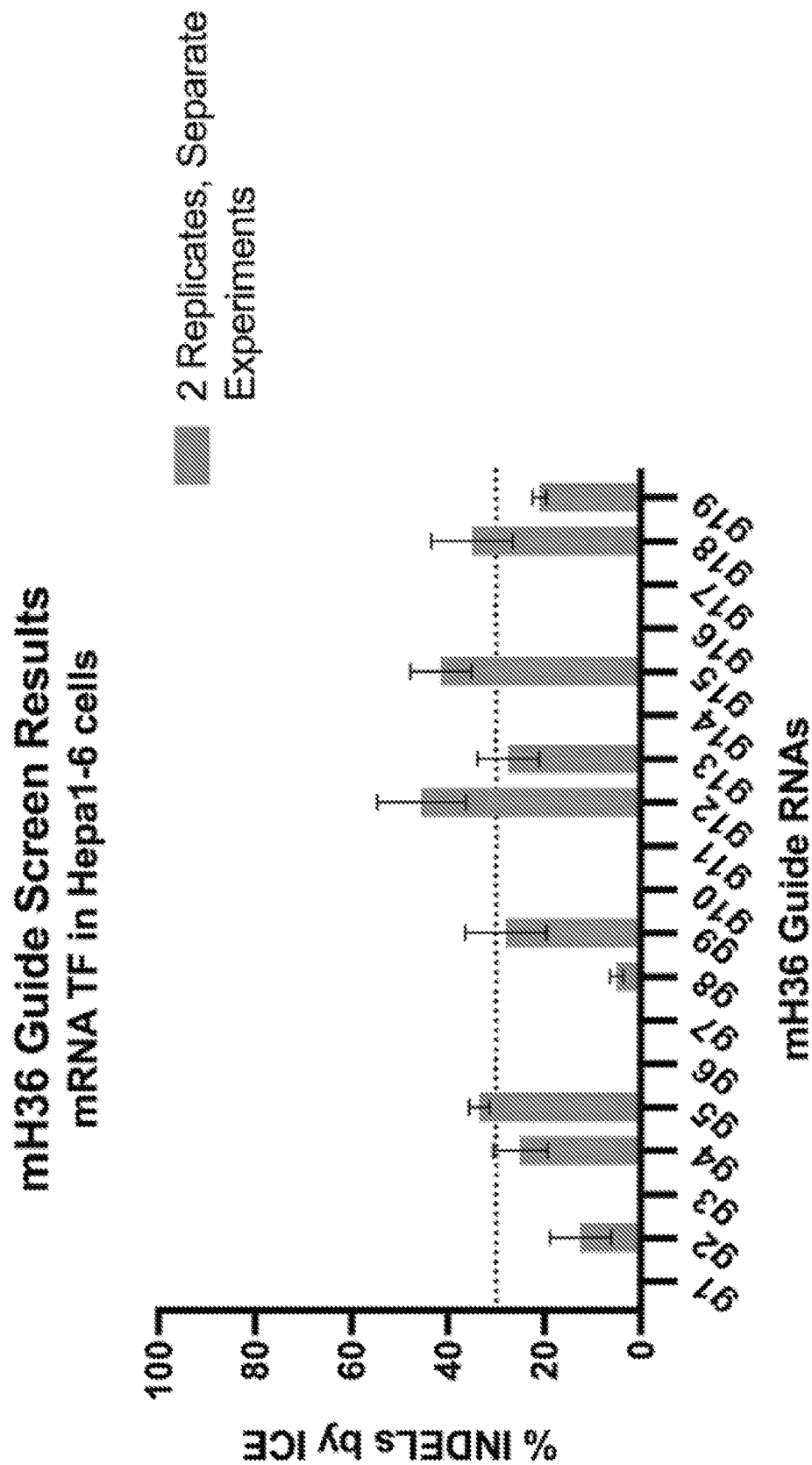


FIG. 5

Efficiency of mRNA electroporation in T cells

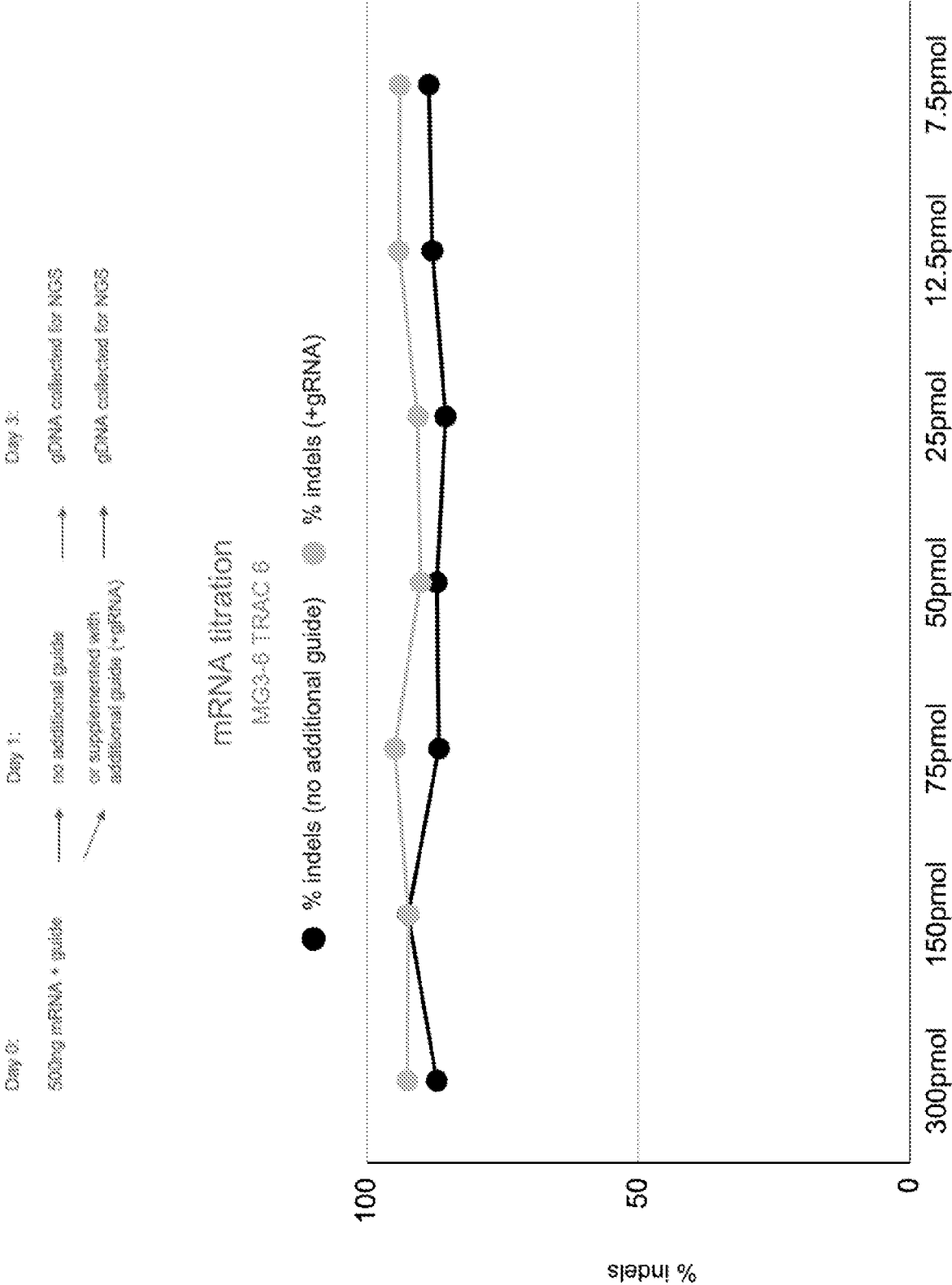


FIG. 6

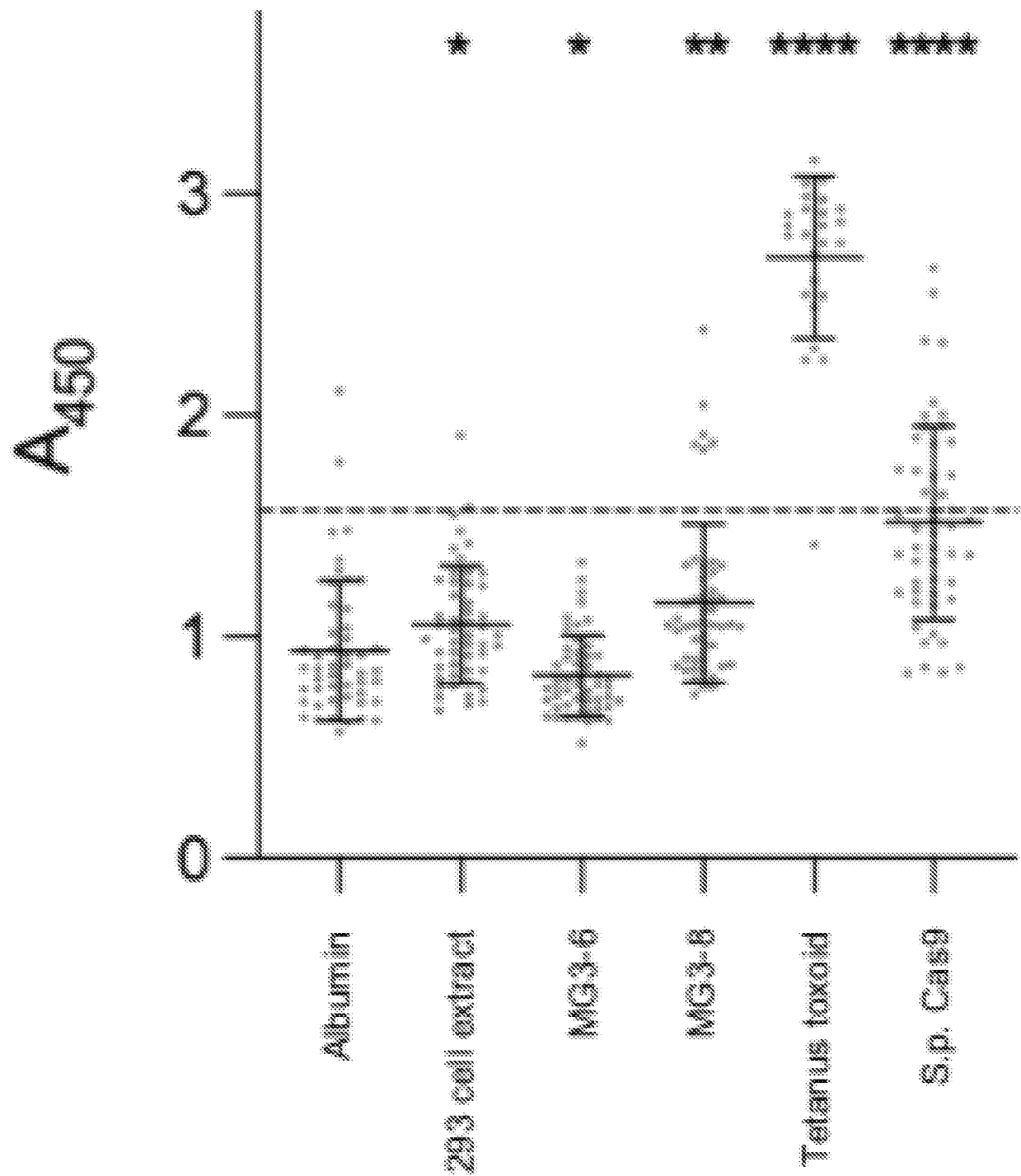


FIG. 7

B cell editing, MG3-6

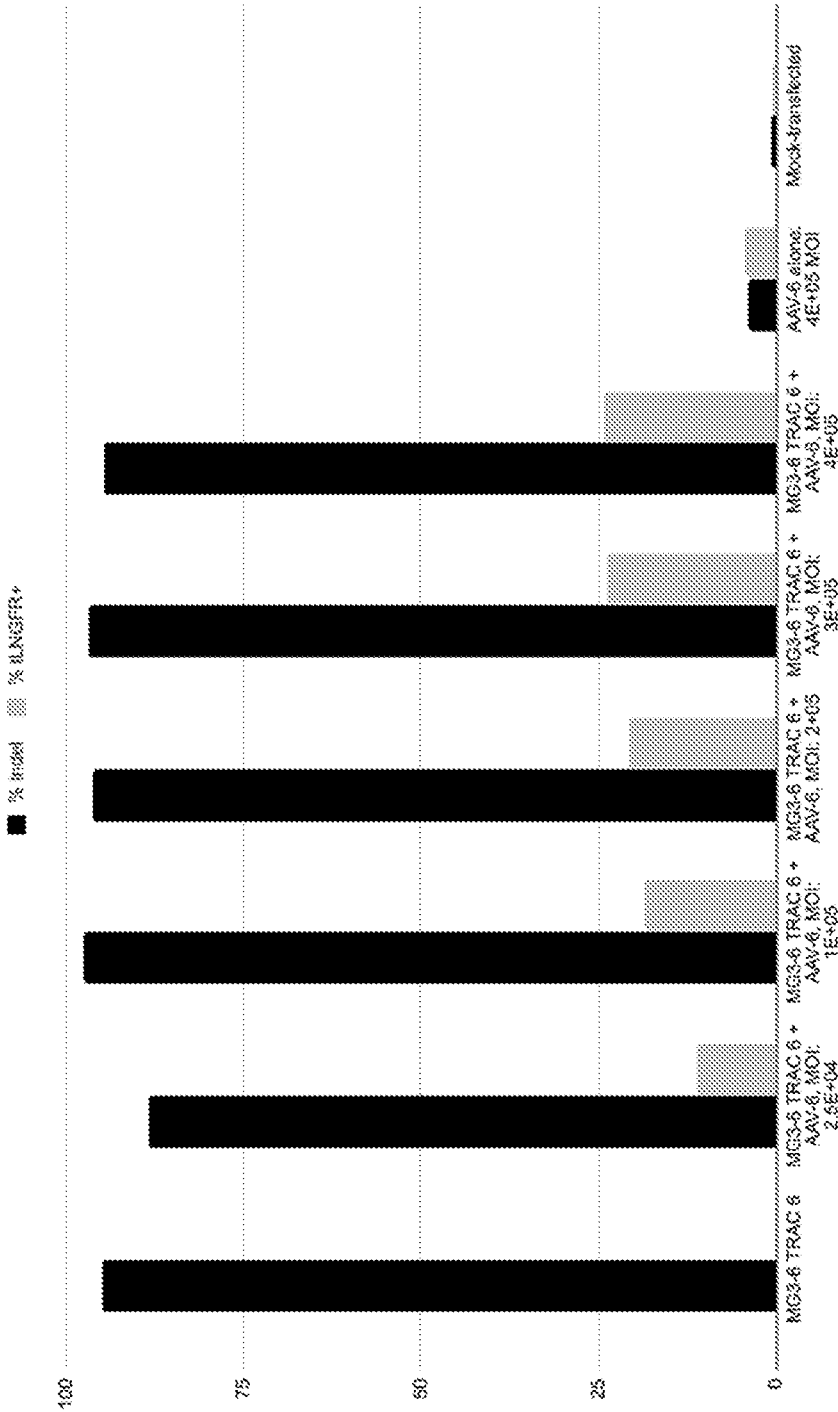


FIG. 8

HSC editing, MG3-6

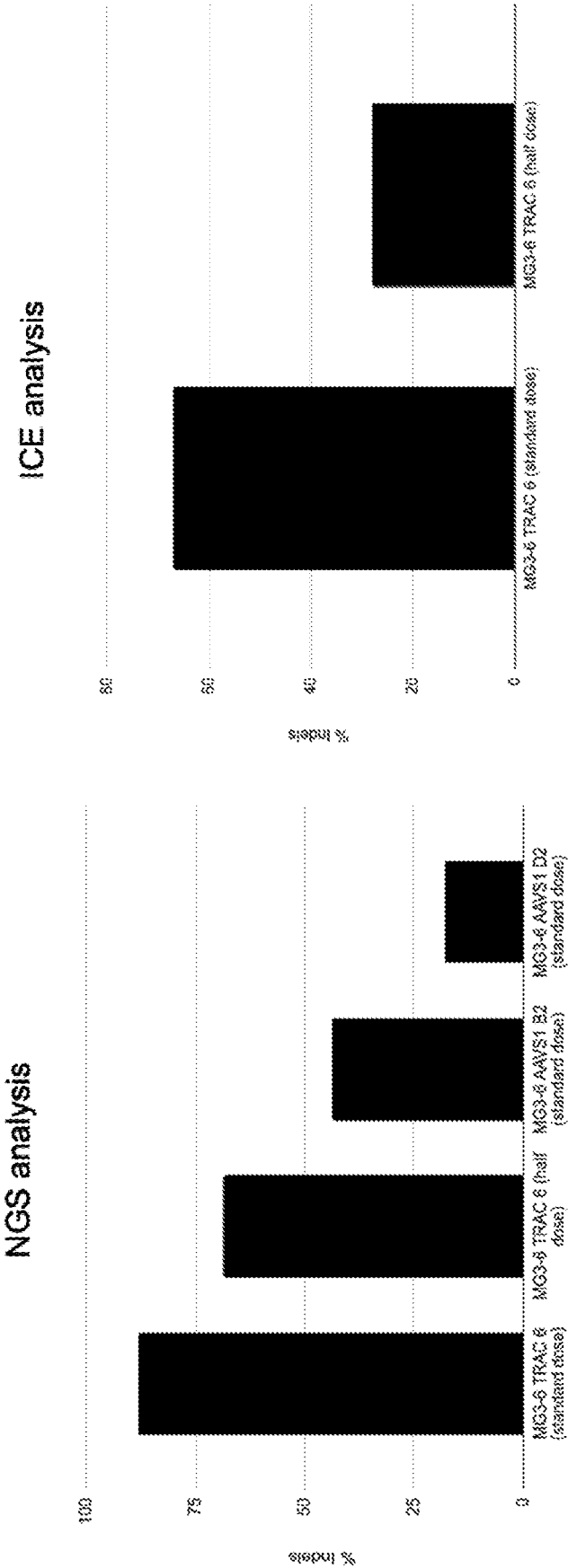


FIG. 9

ipSC editing MG3-6 RNP

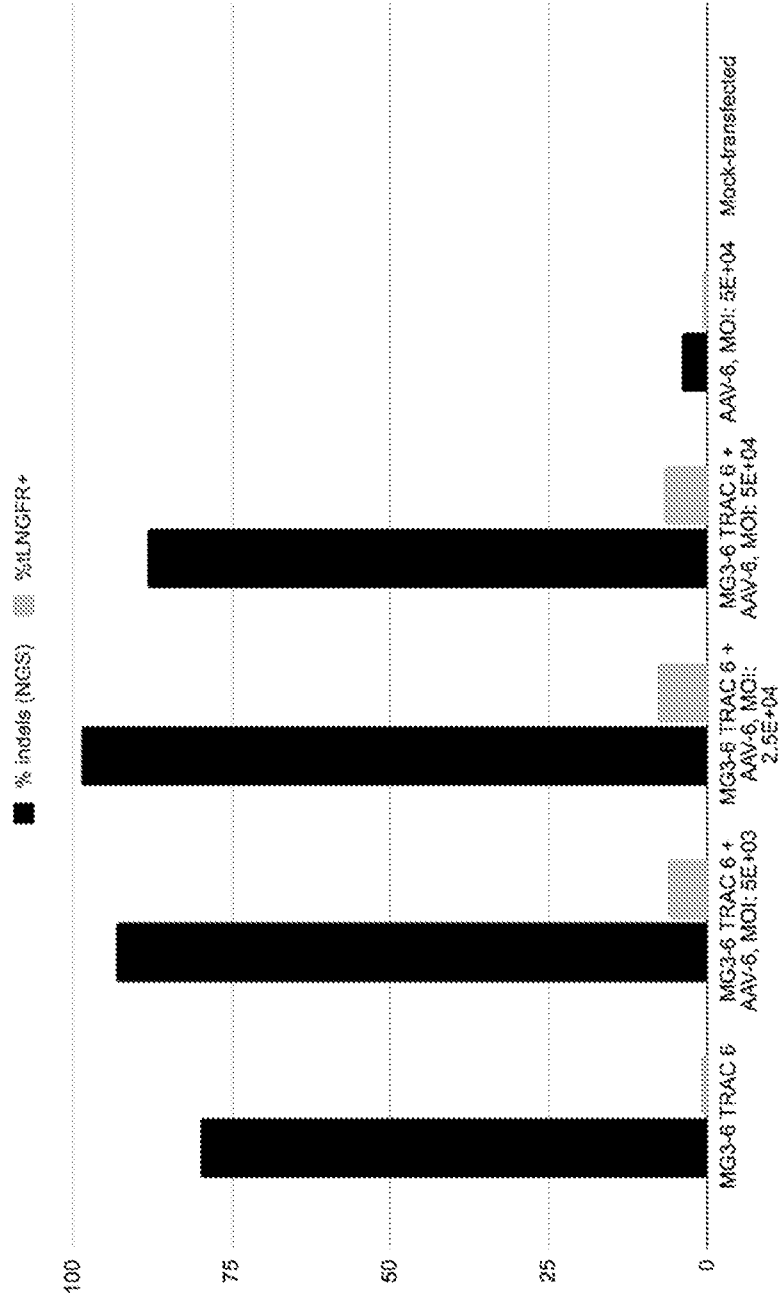


FIG. 10

iPSC editing MG3-6 mRNA

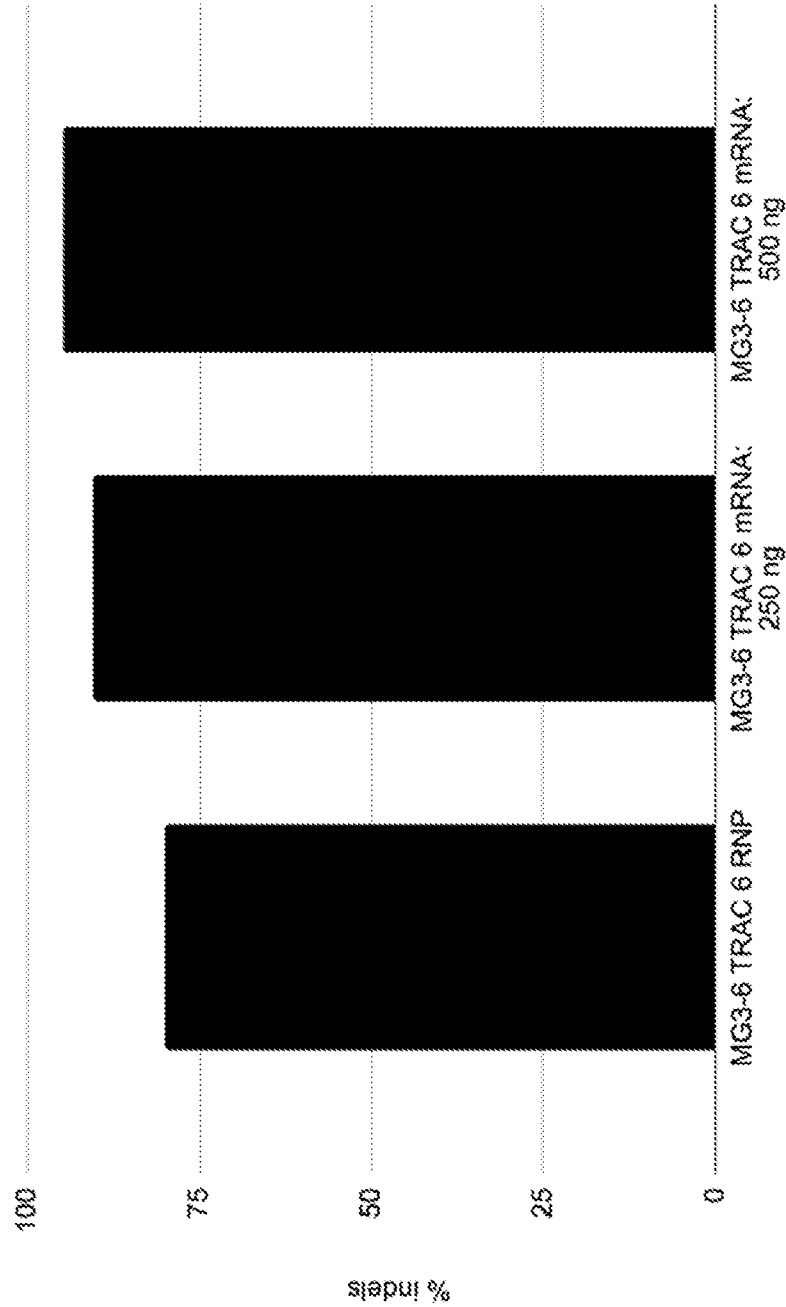
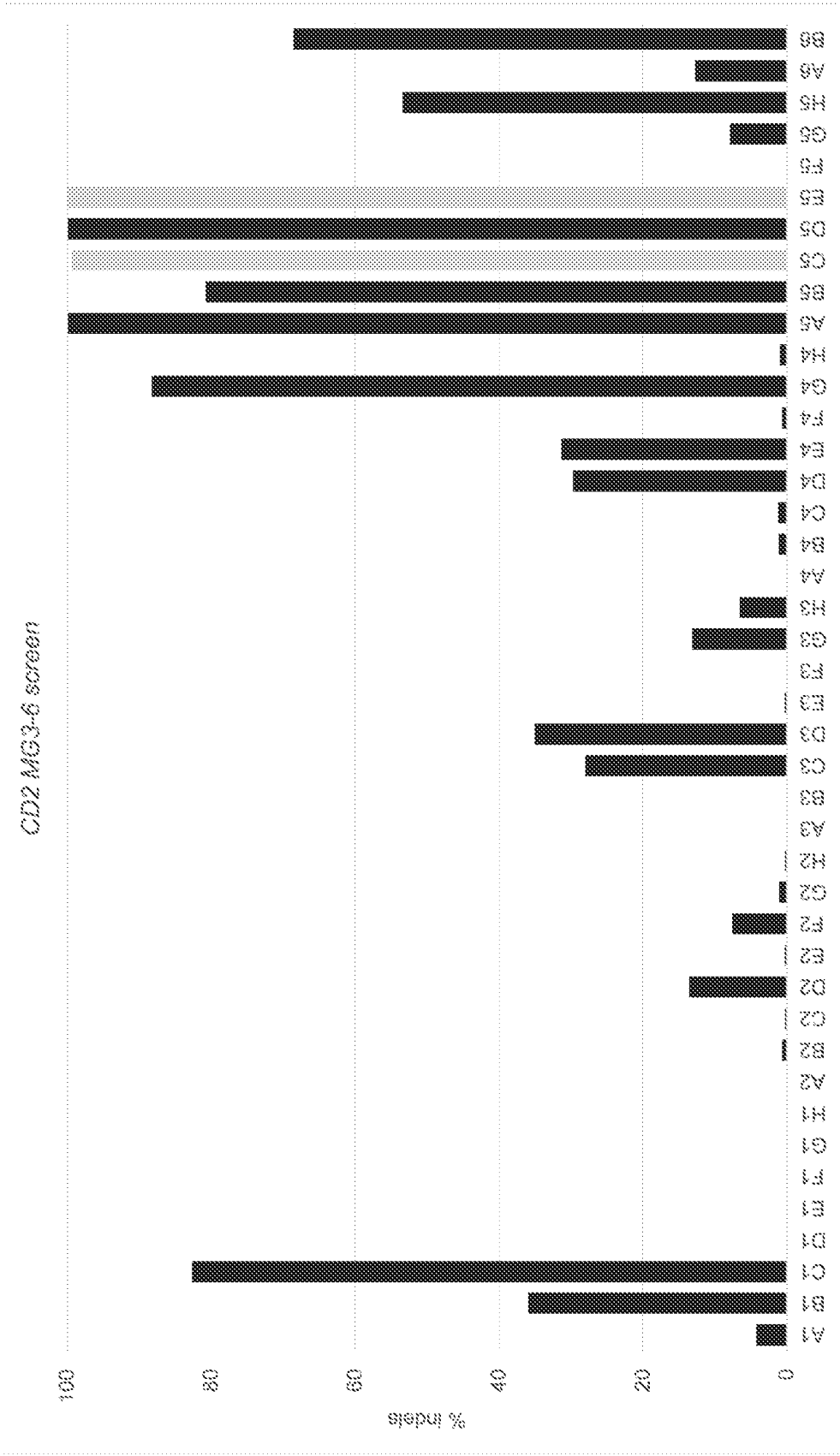


FIG. 11



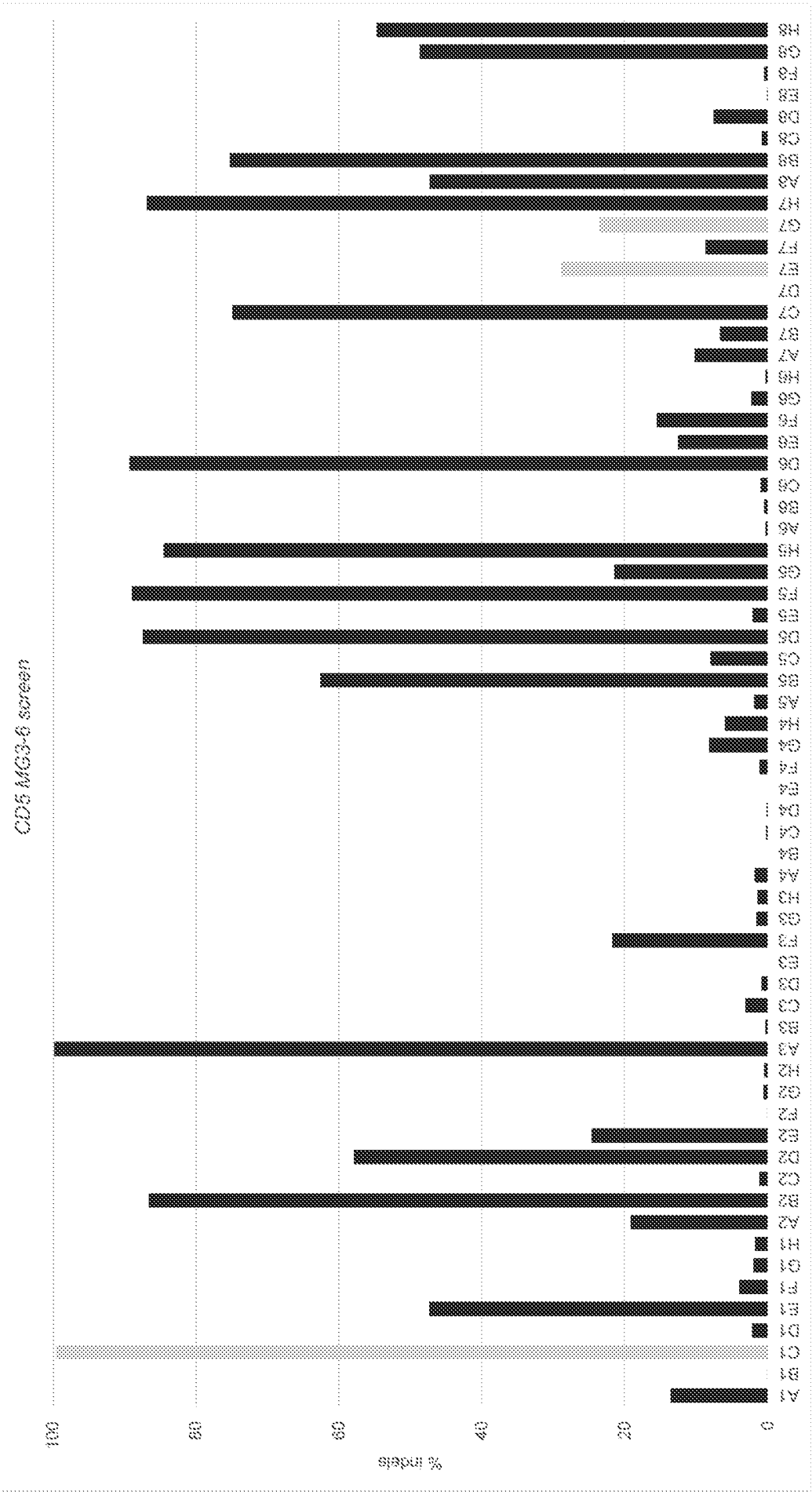


FIG. 13

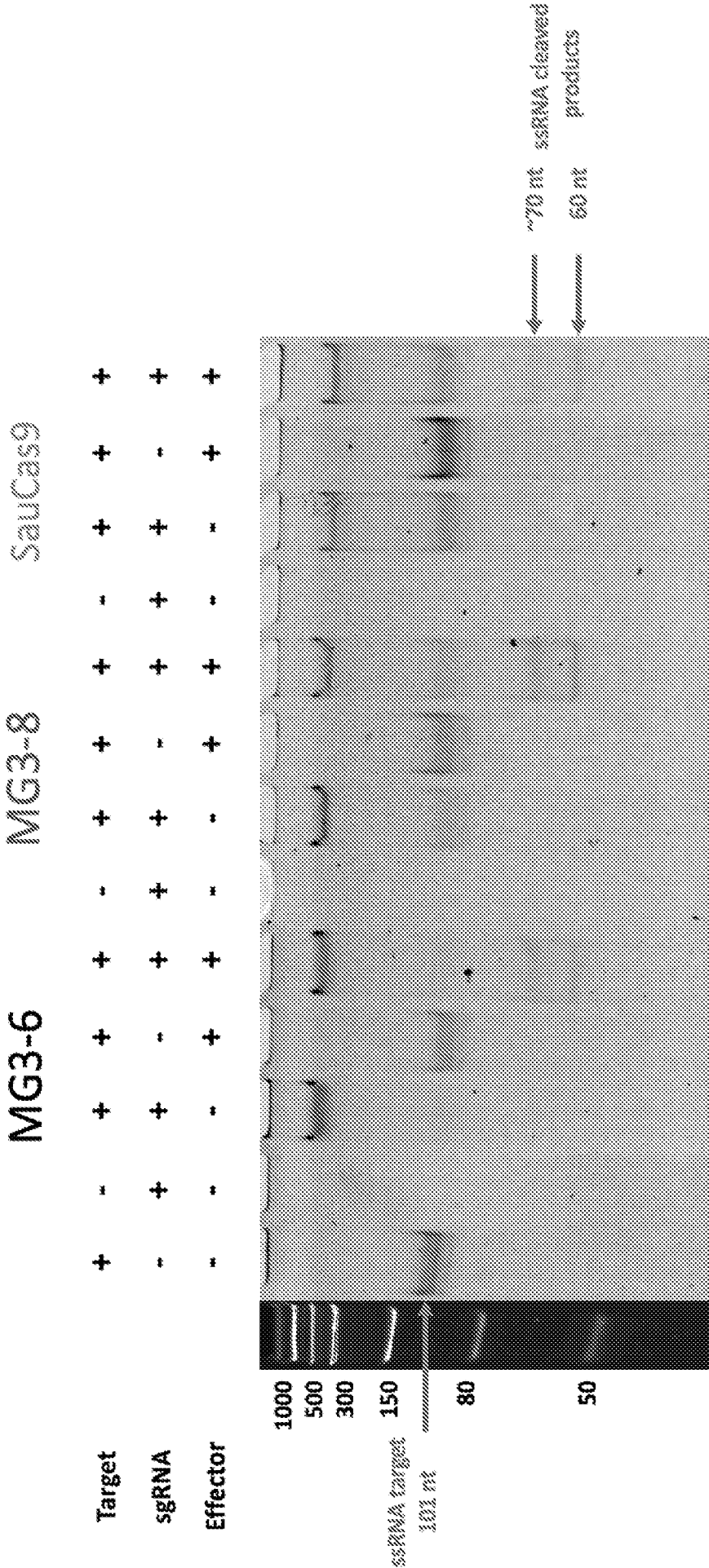


FIG. 14

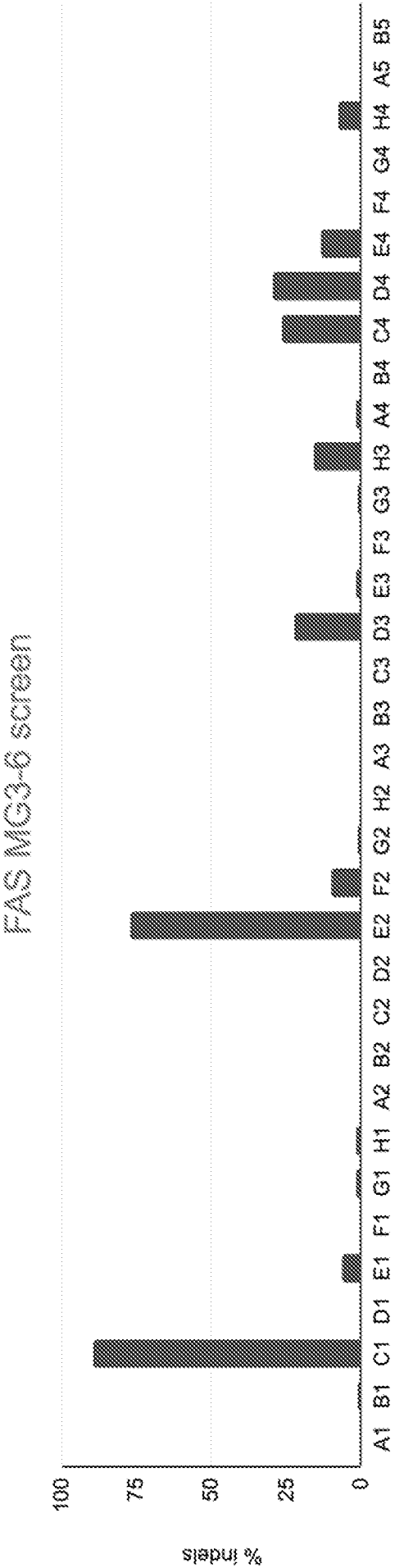


FIG. 15

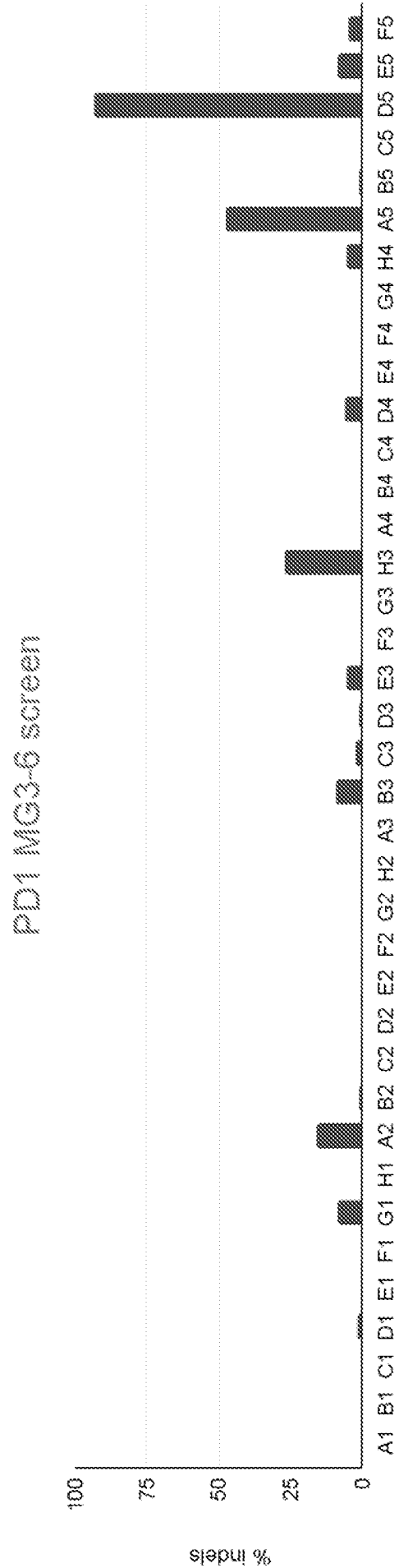


FIG. 16

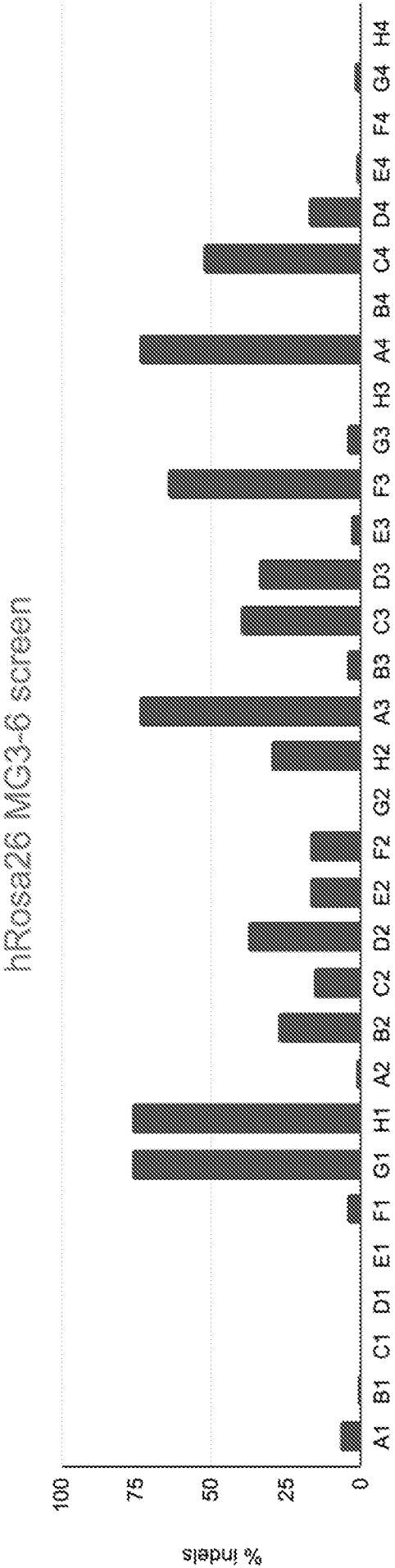


FIG. 17

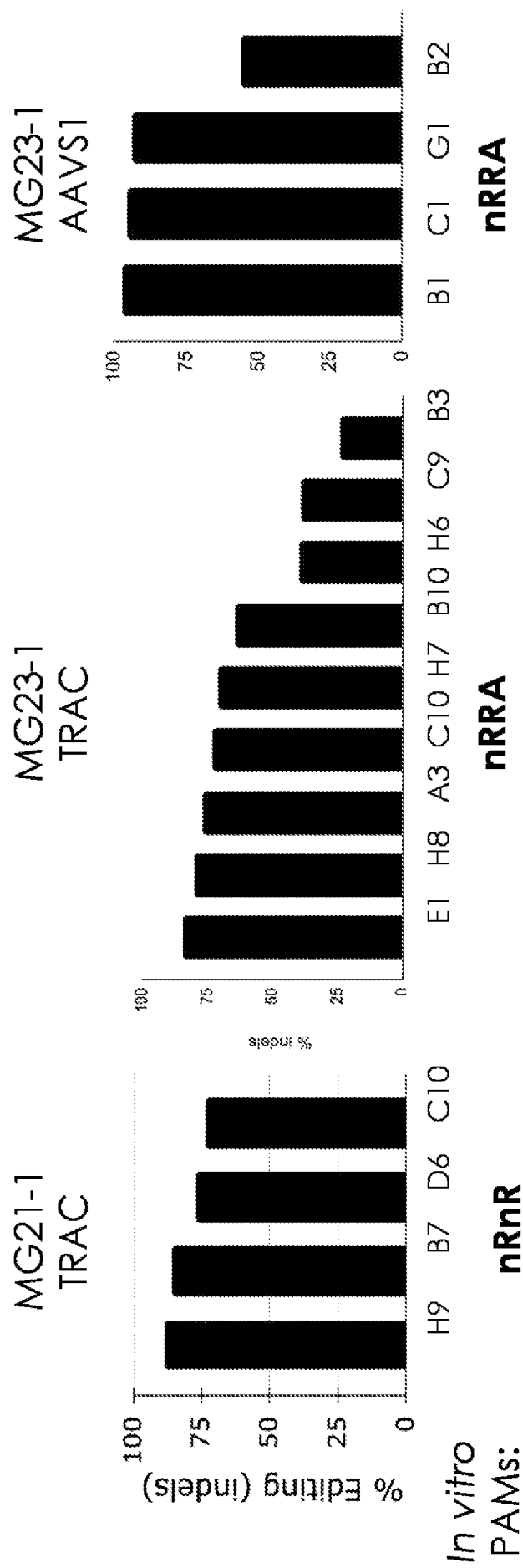


FIG. 18

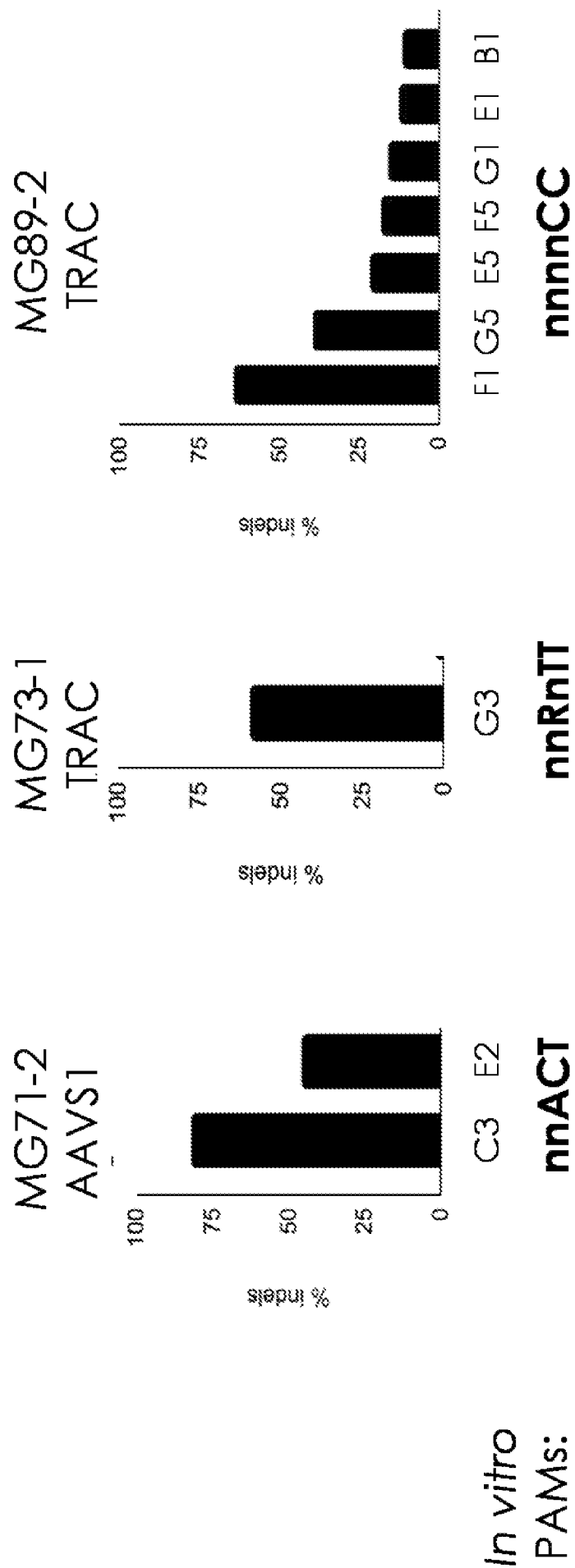


FIG. 18 Cont'd

Screen of MG3-6 human HAO-1 guides in Hep3B cells

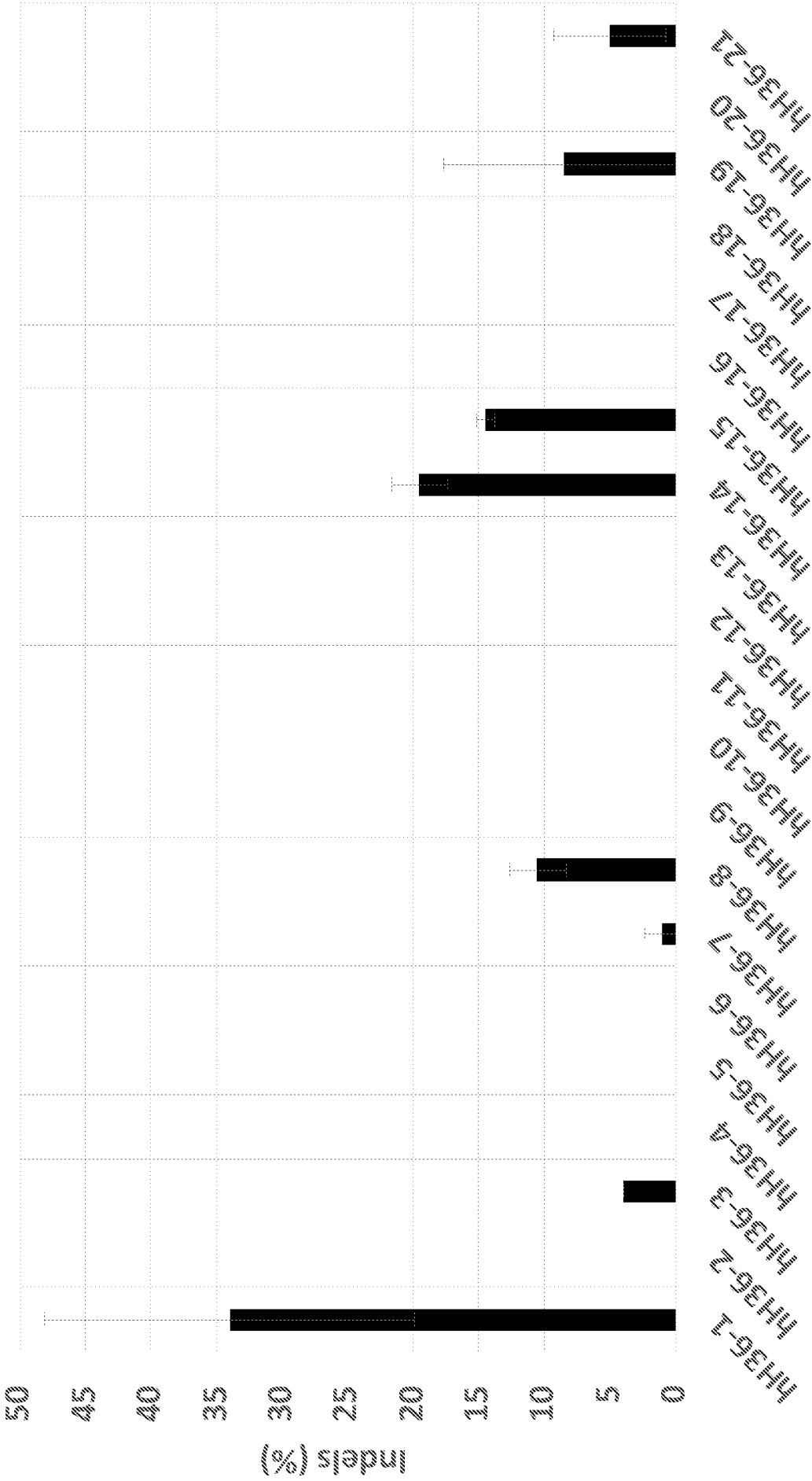


FIG. 19

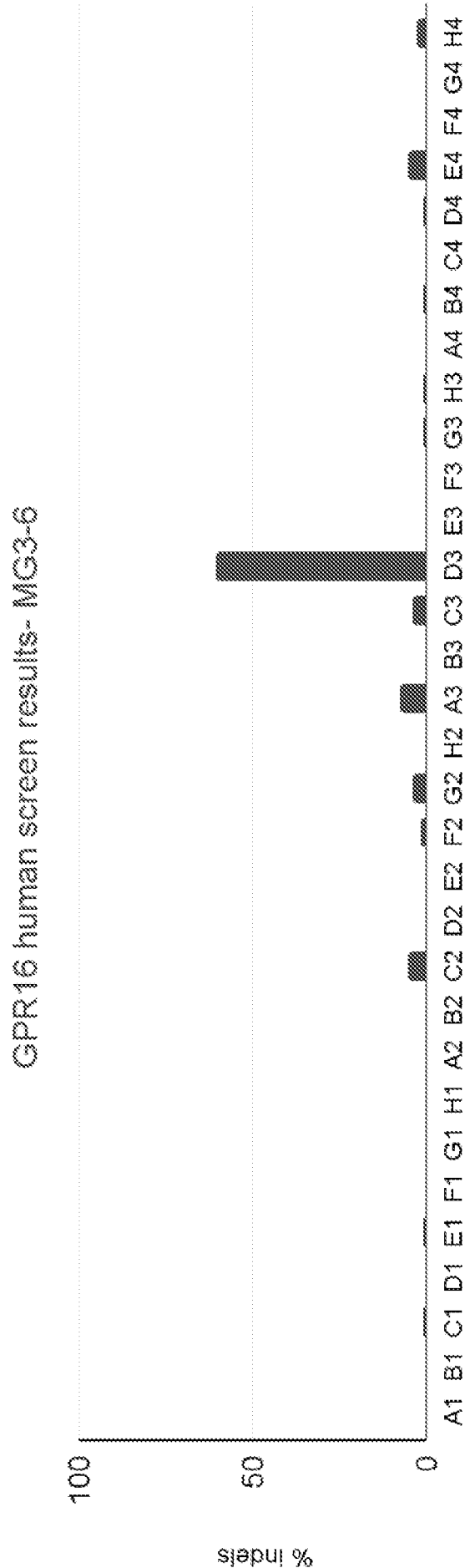


FIG. 20

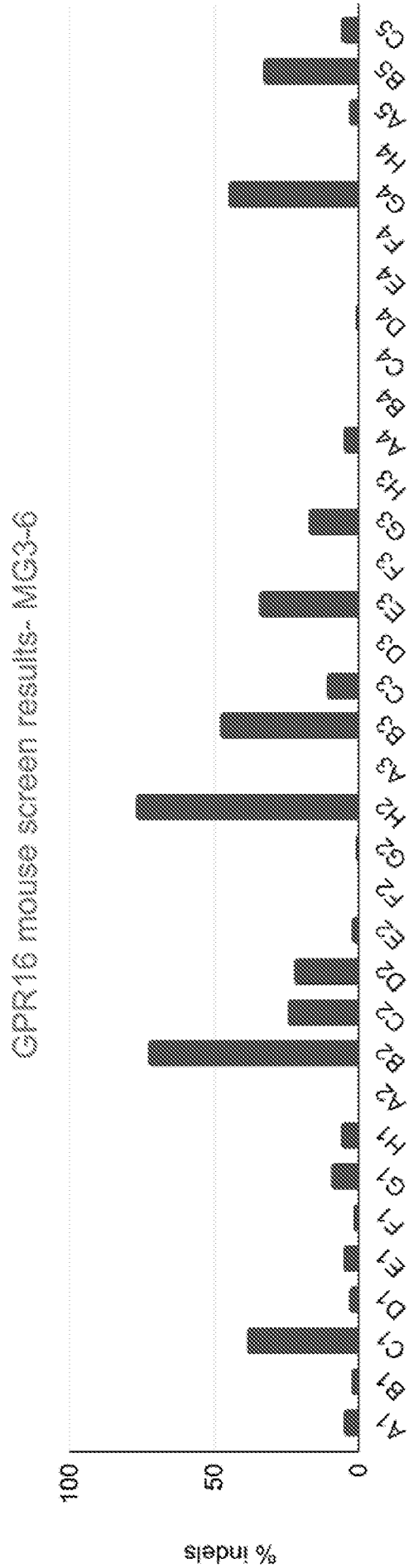


FIG. 21

Genome editing of GPR146 via mRNA
Primary Mouse Hepatocytes

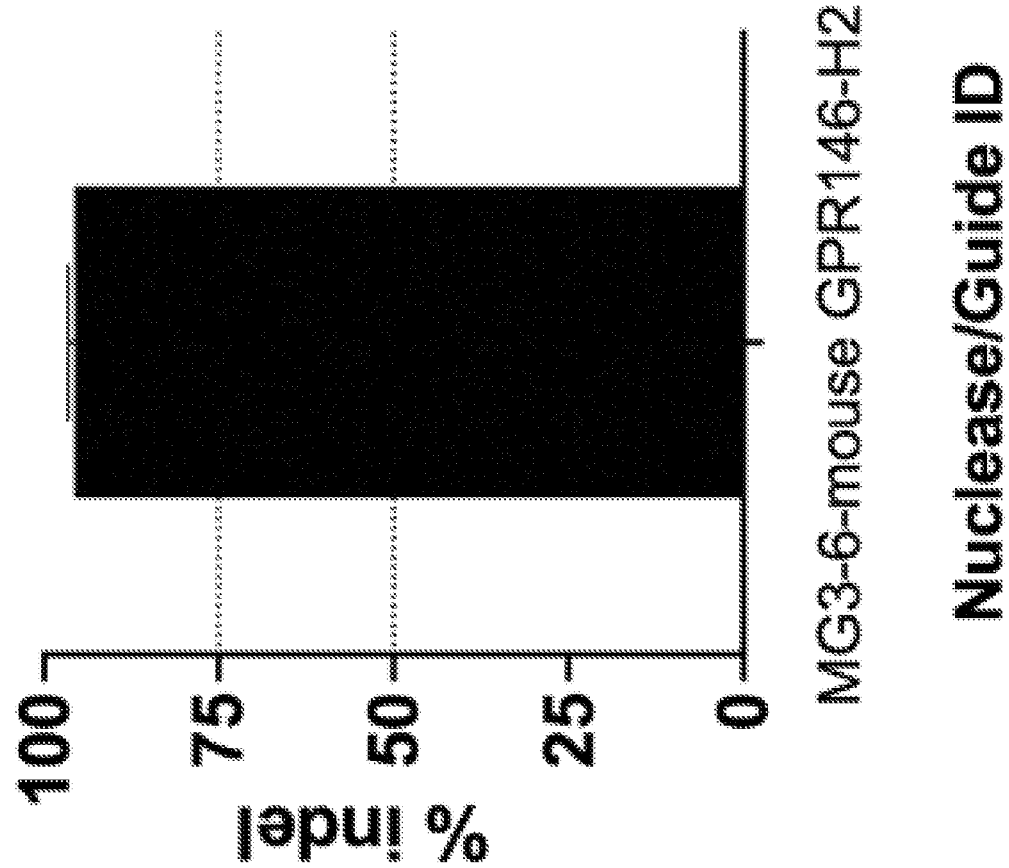


FIG. 22

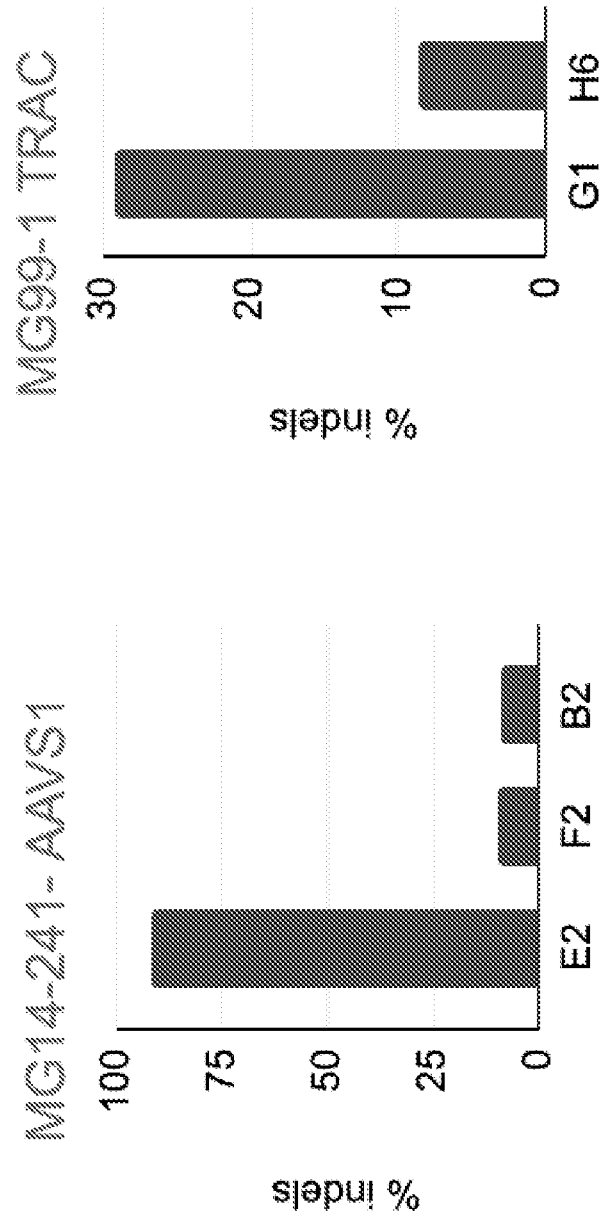


FIG. 23

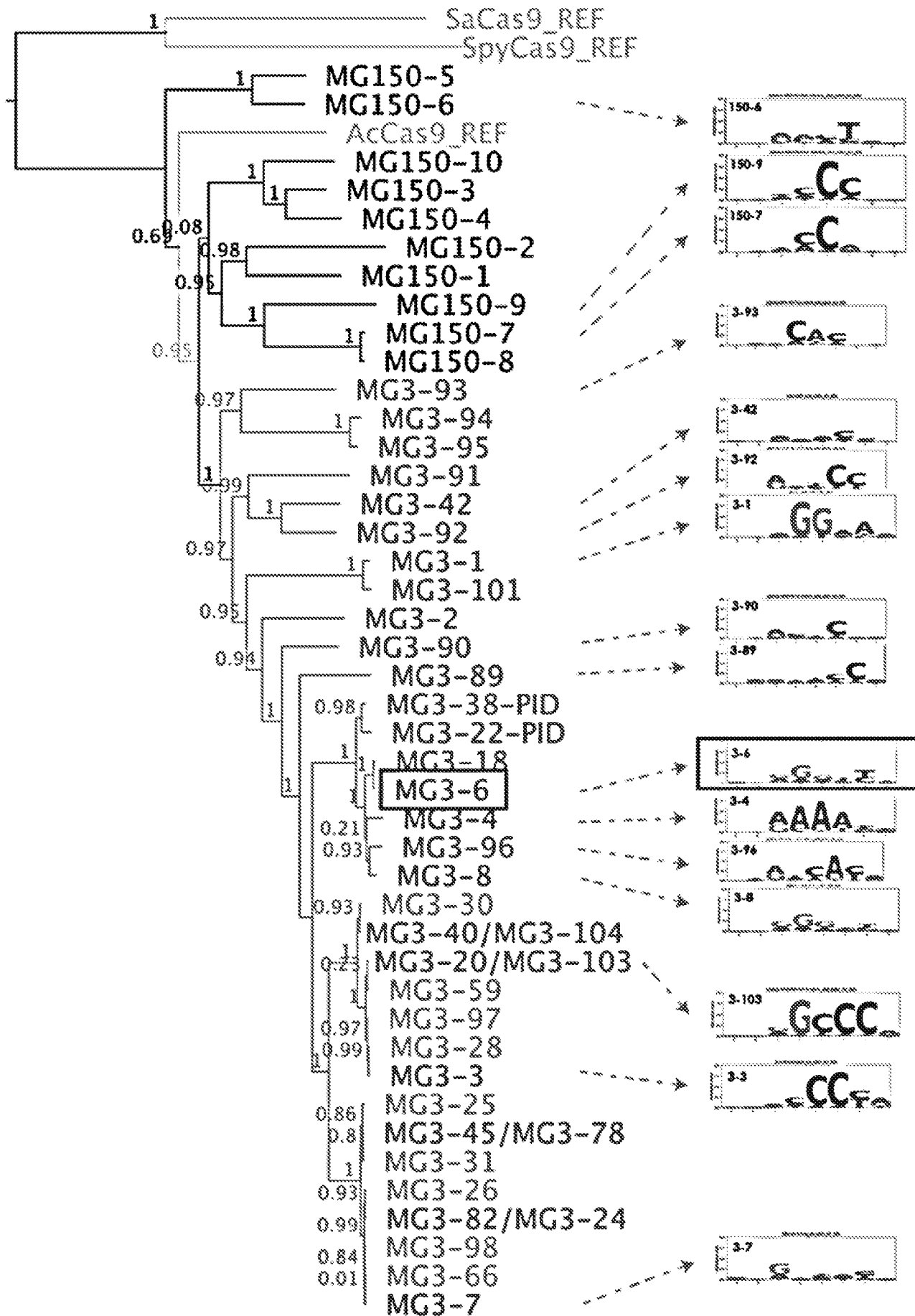
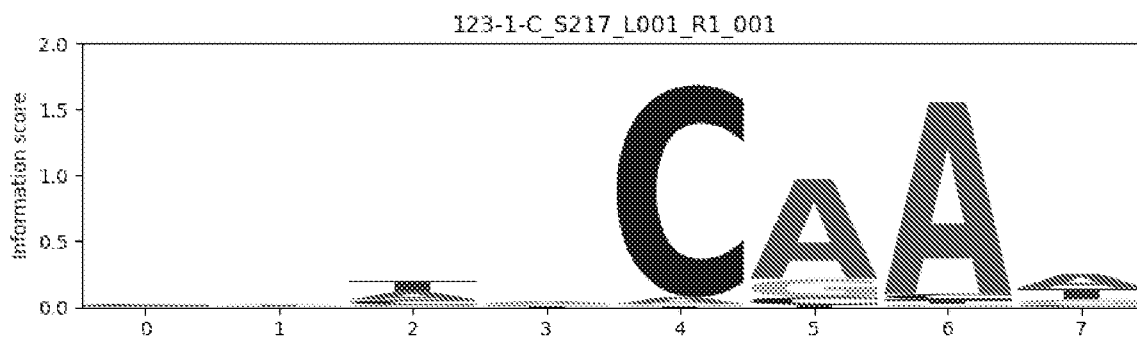


FIG. 24

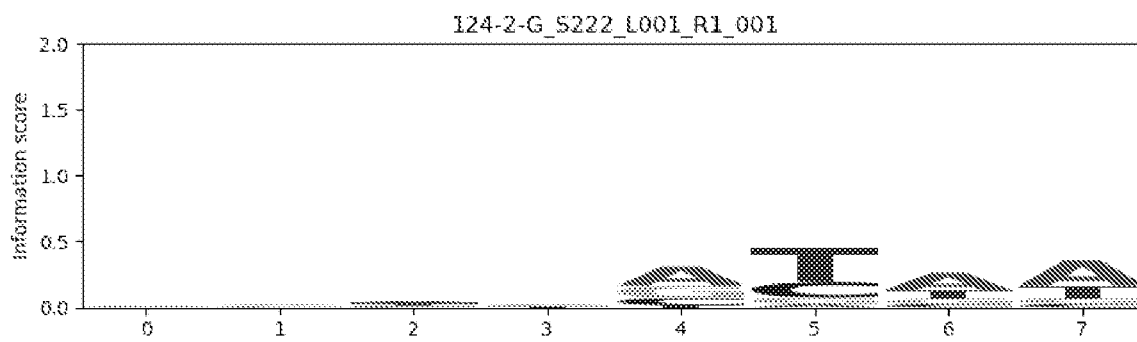


FIG. 25

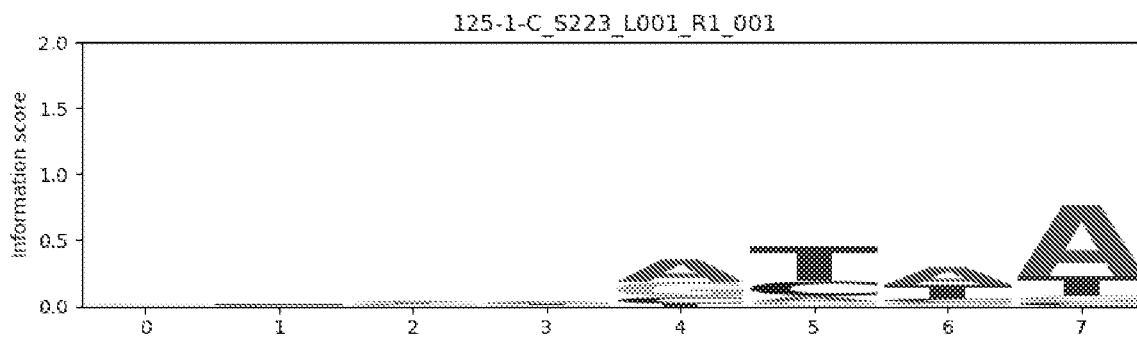
MG123-1



MG124-2



MG125-1



MG125-2

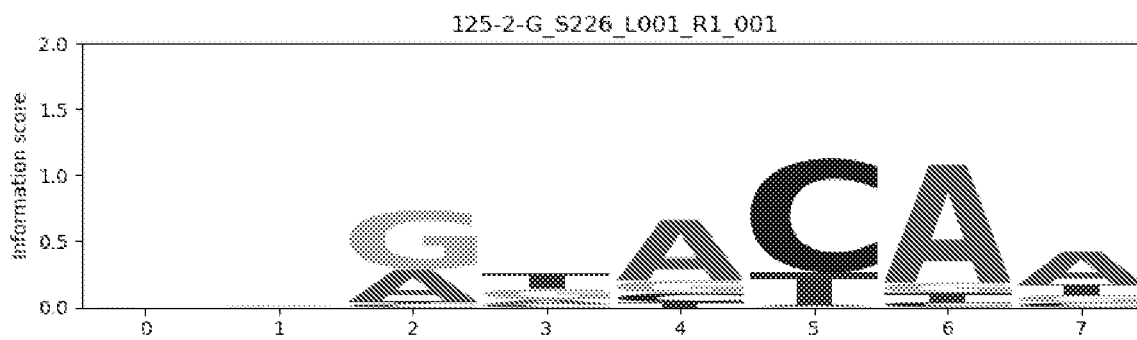
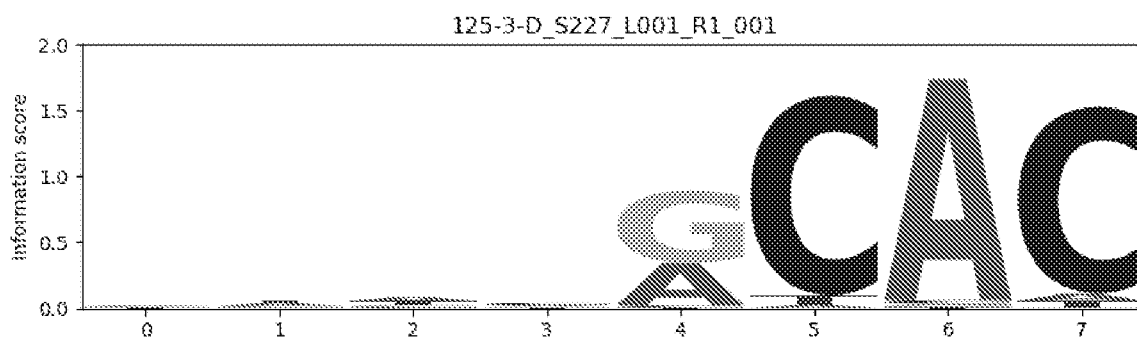
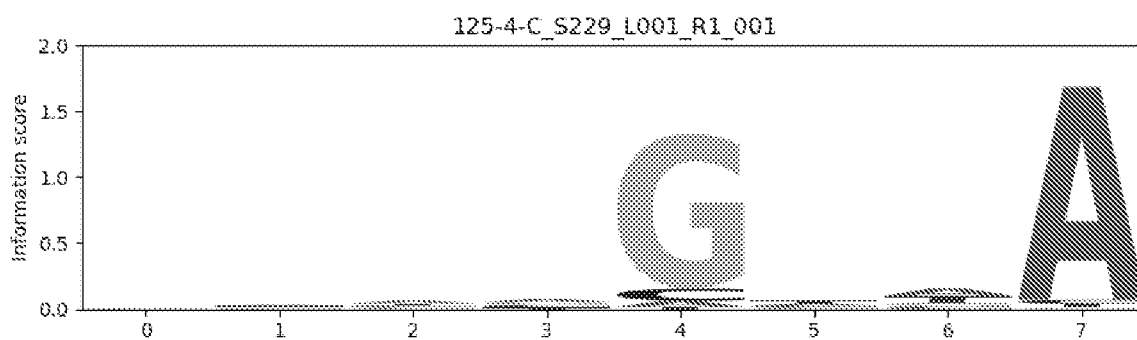


FIG. 26

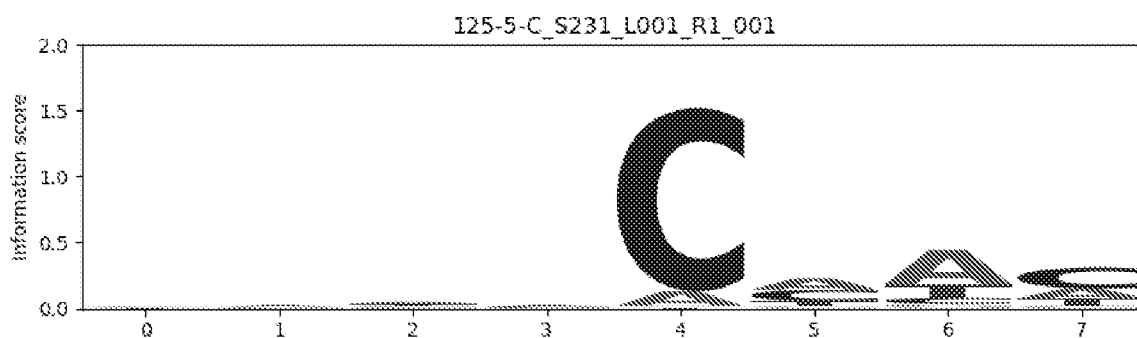
MG125-3



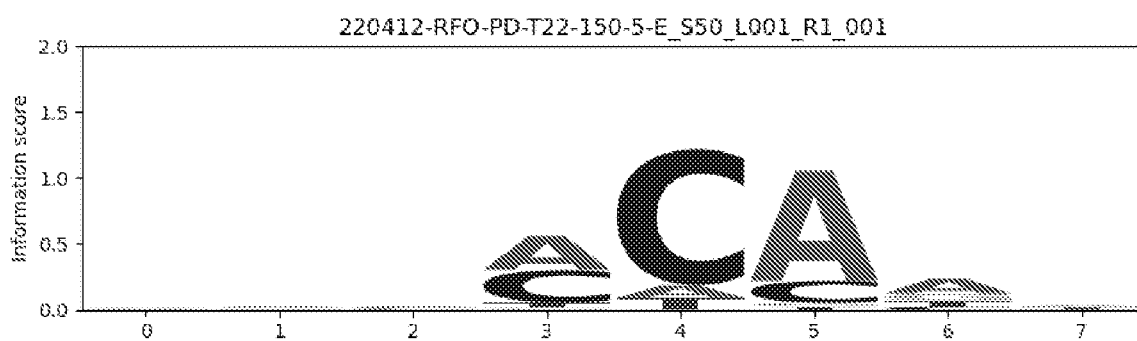
MG125-4



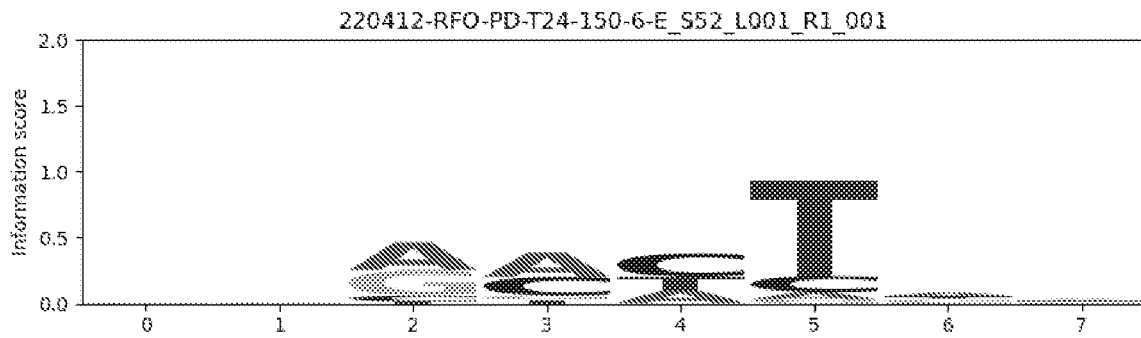
MG125-5



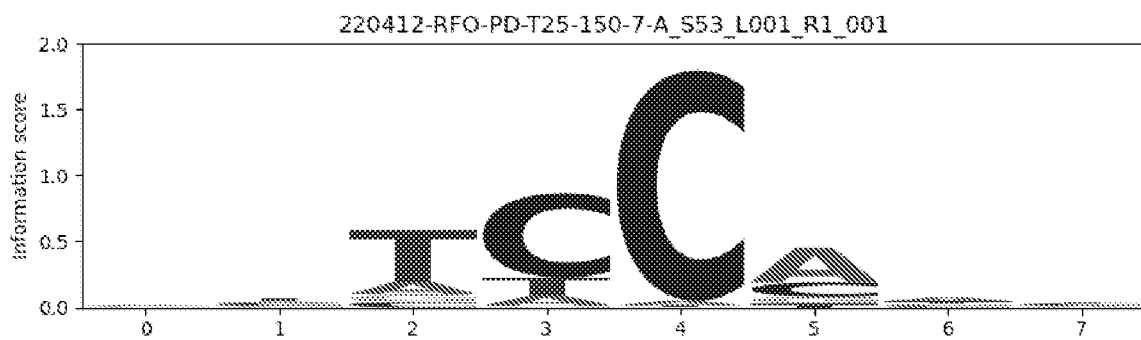
MG150-5

*FIG. 27*

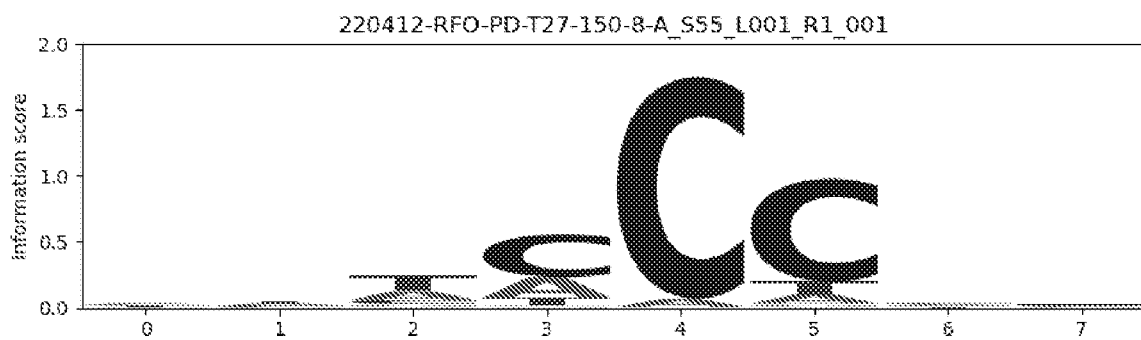
MG150-6



MG150-7



MG150-8



MG150-9

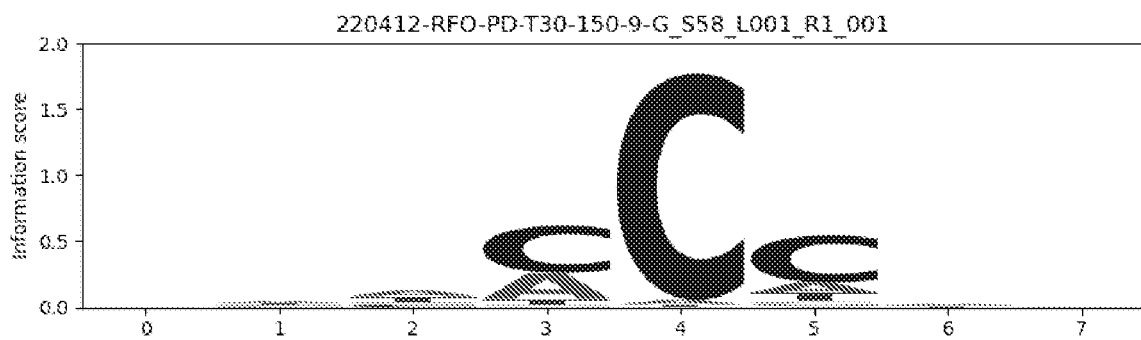
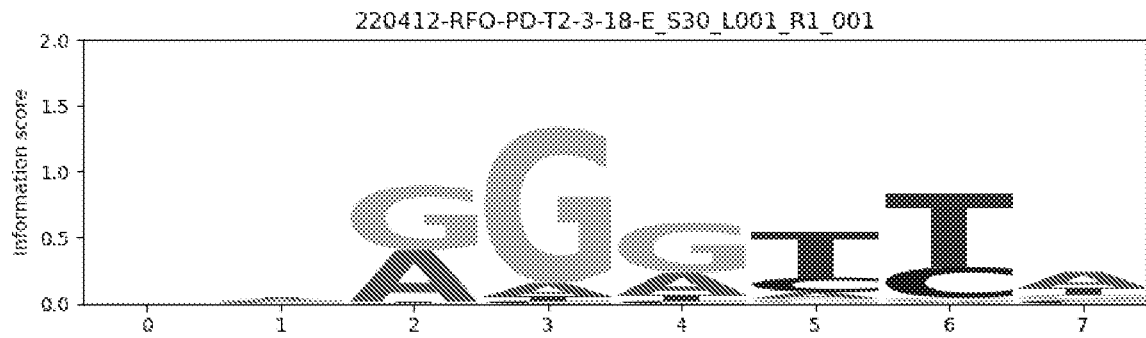
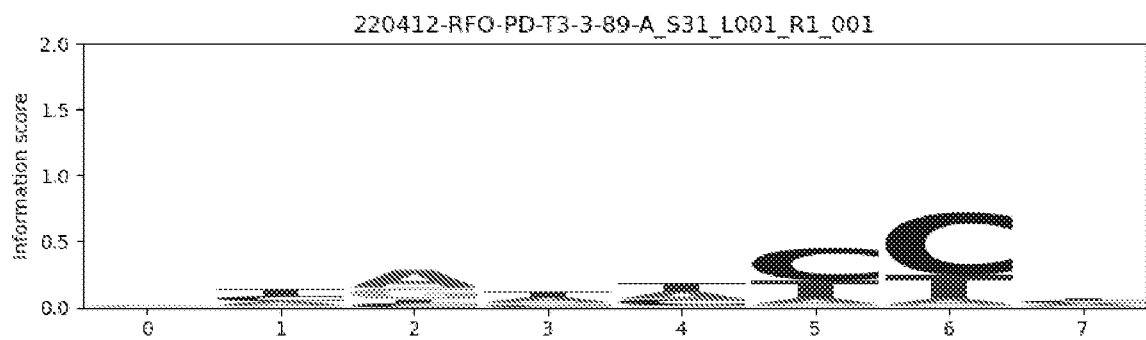


FIG. 28

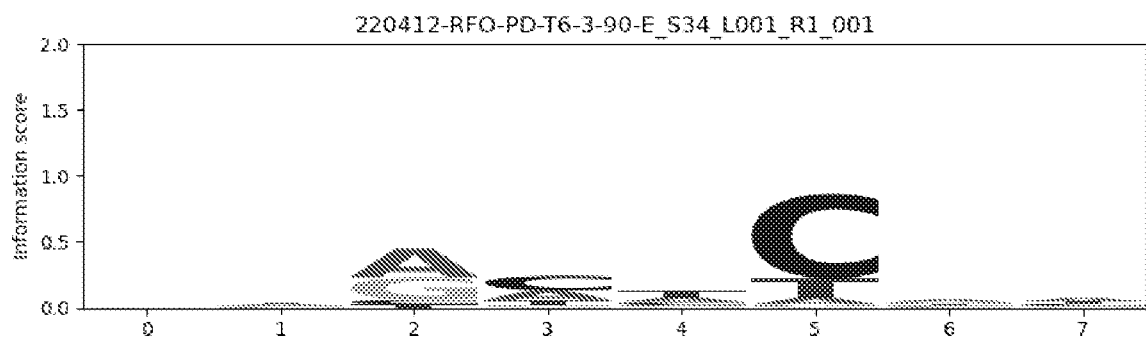
MG3-18



MG3-89



MG3-90



MG3-91

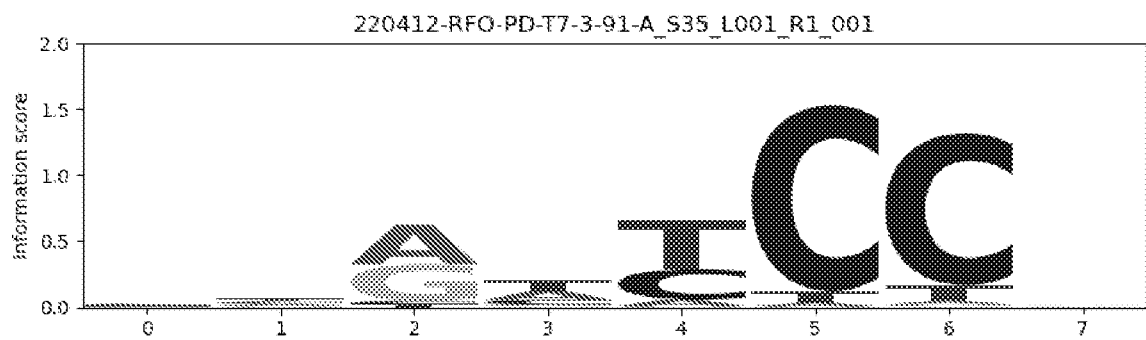
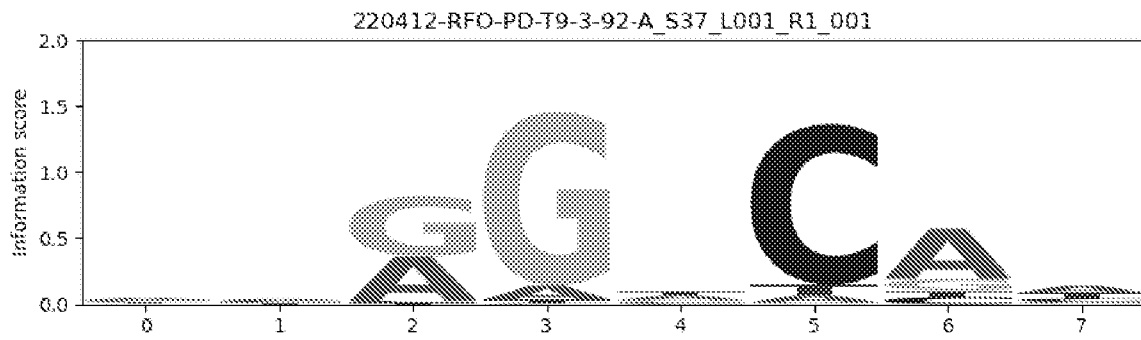
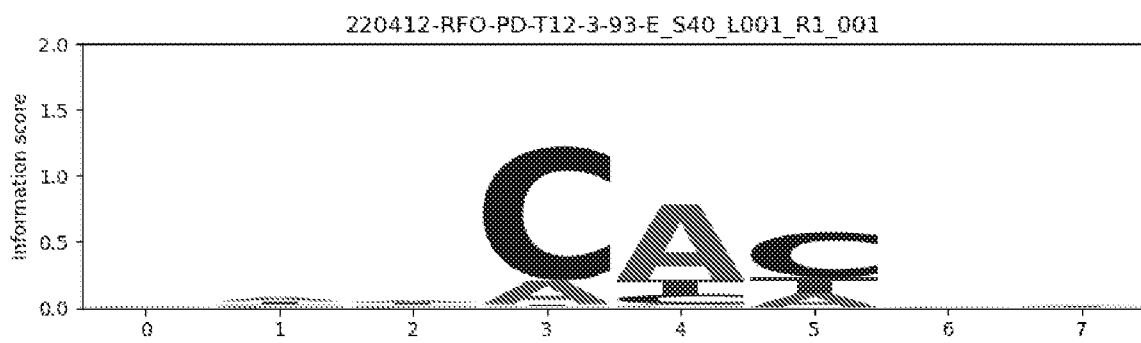


FIG. 29

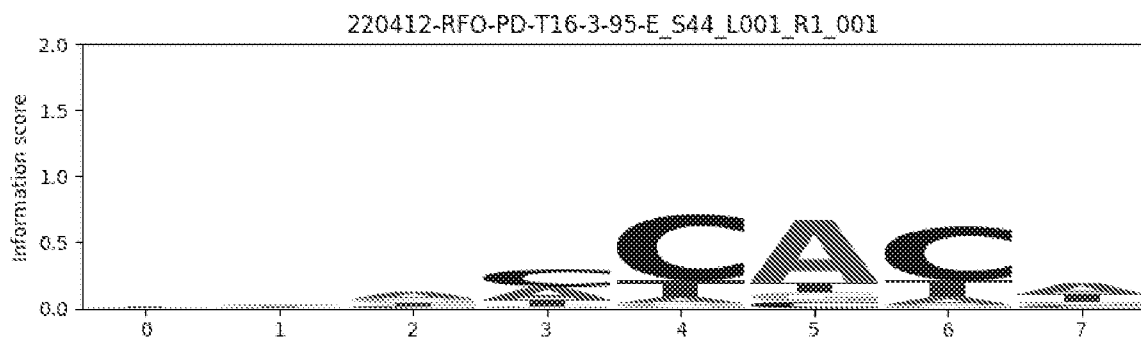
MG3-92



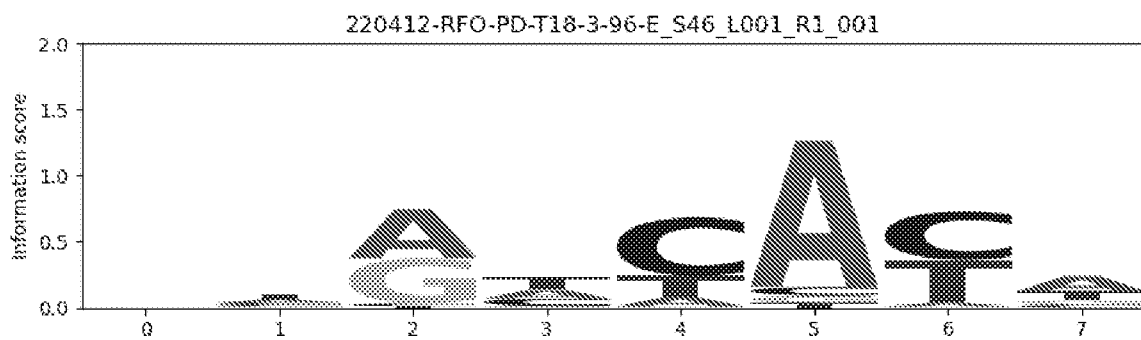
MG3-93



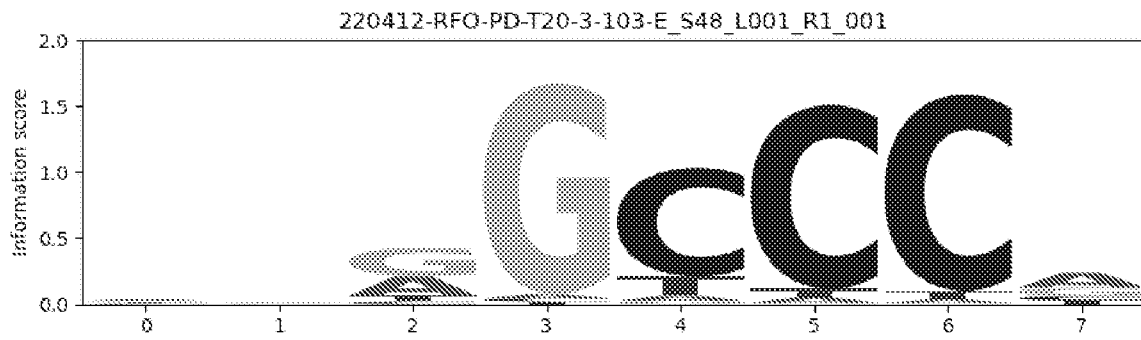
MG3-95



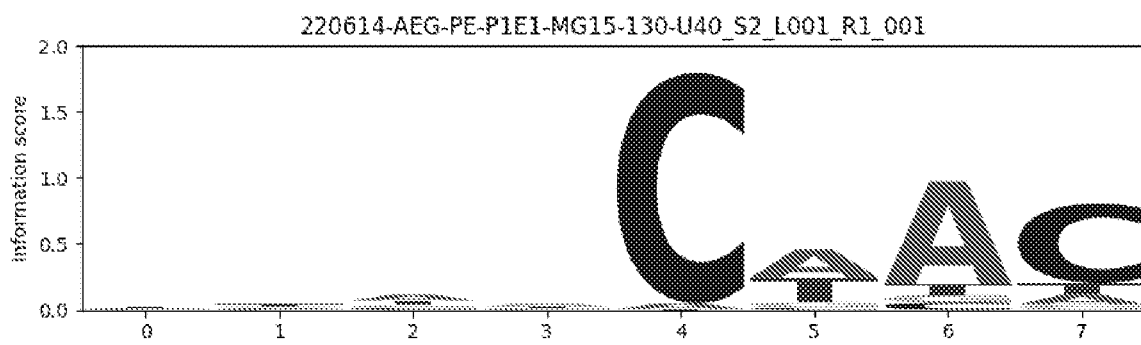
MG3-96

*FIG. 30*

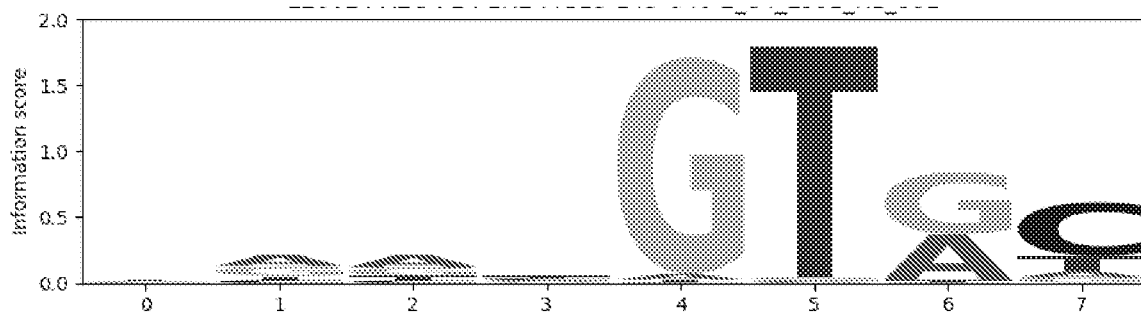
MG3-103



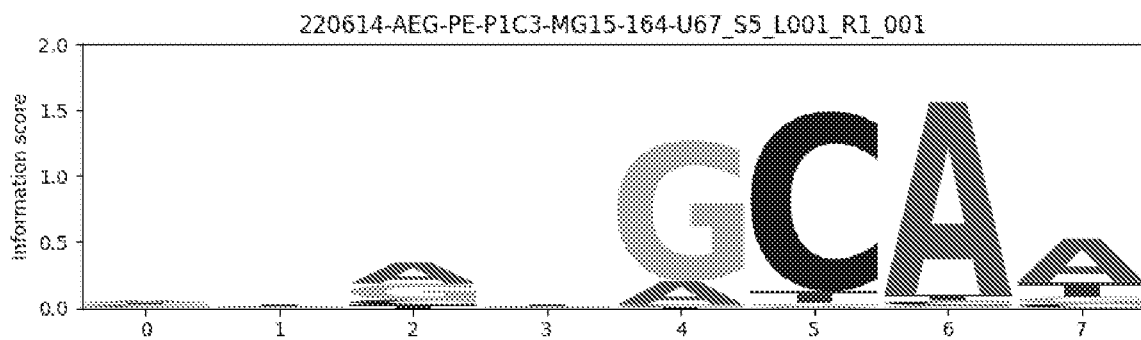
MG15-130



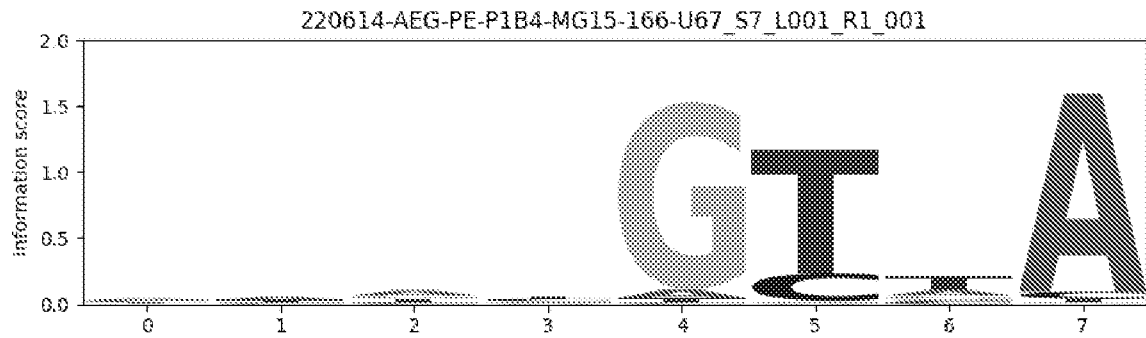
MG15-146



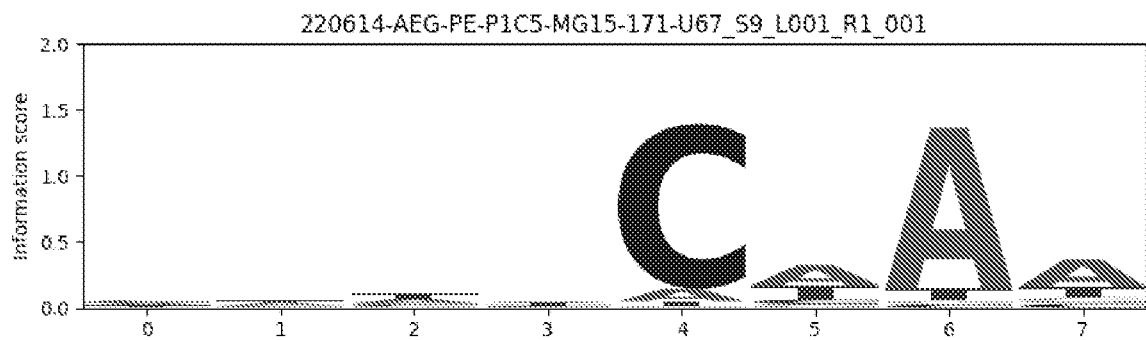
MG15-164

*FIG. 31*

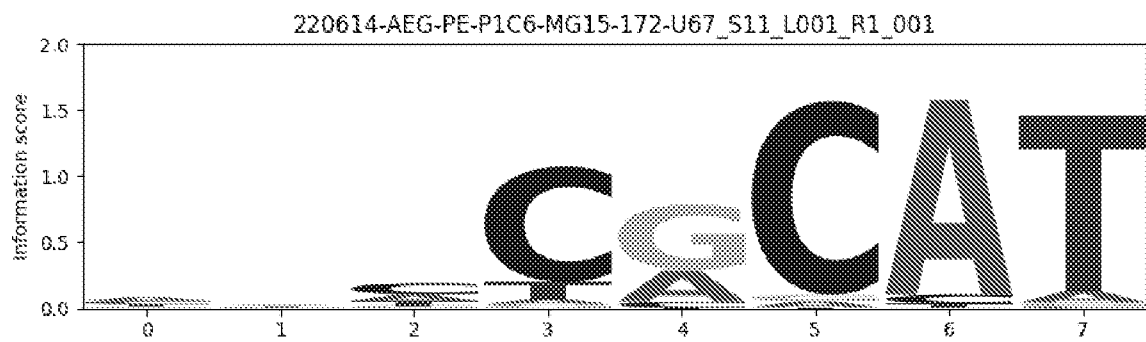
MG15-166



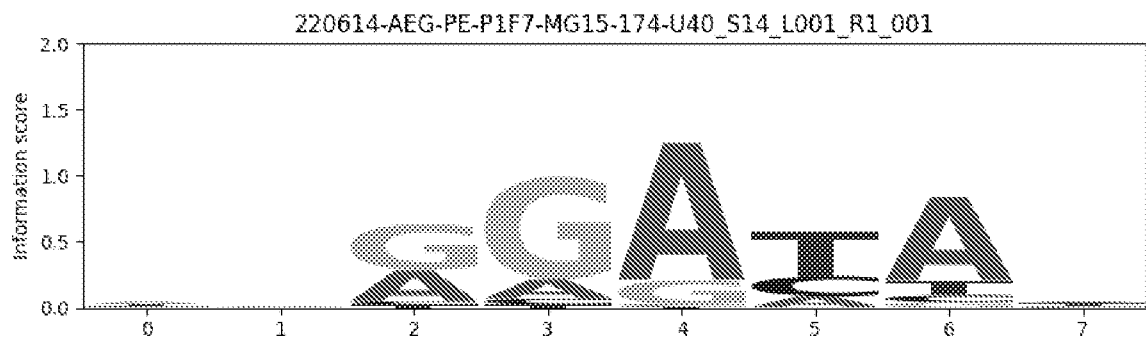
MG15-171



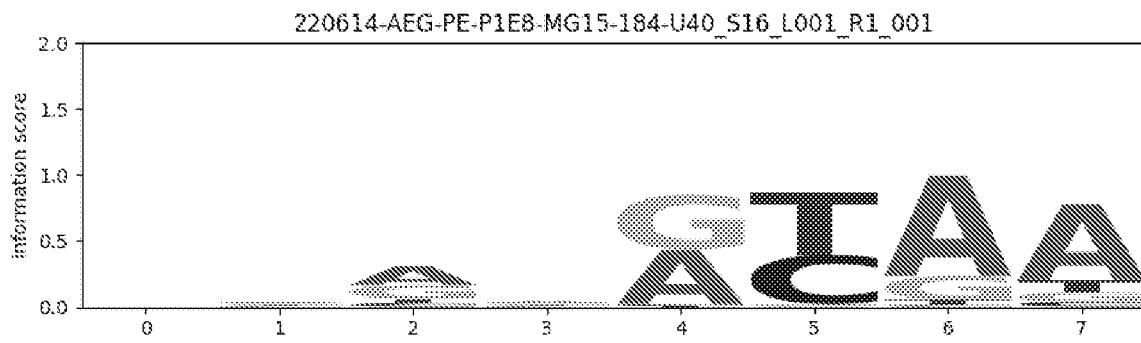
MG15-172



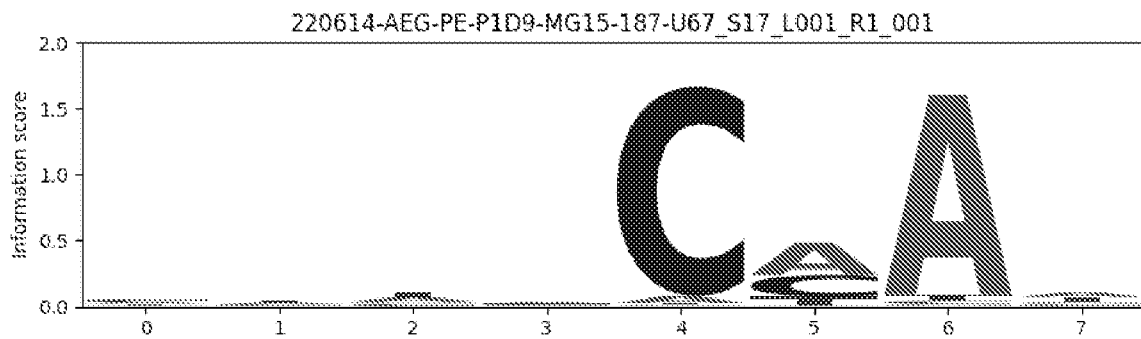
MG15-174

*FIG. 32*

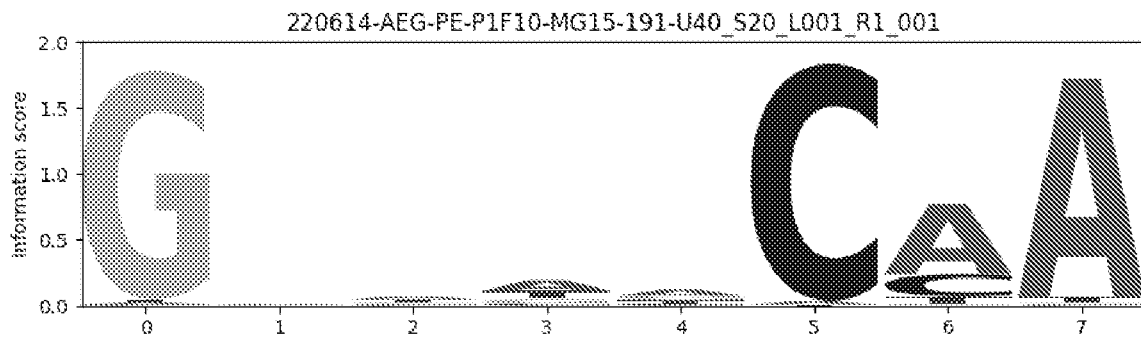
MG15-184



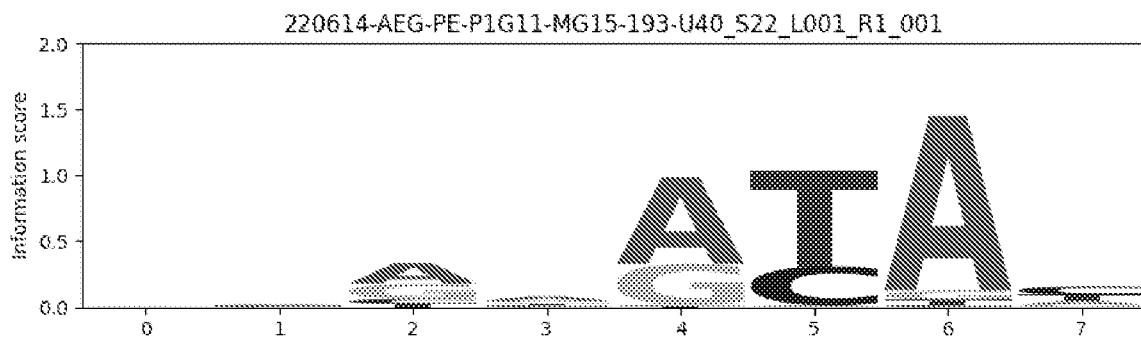
MG15-187



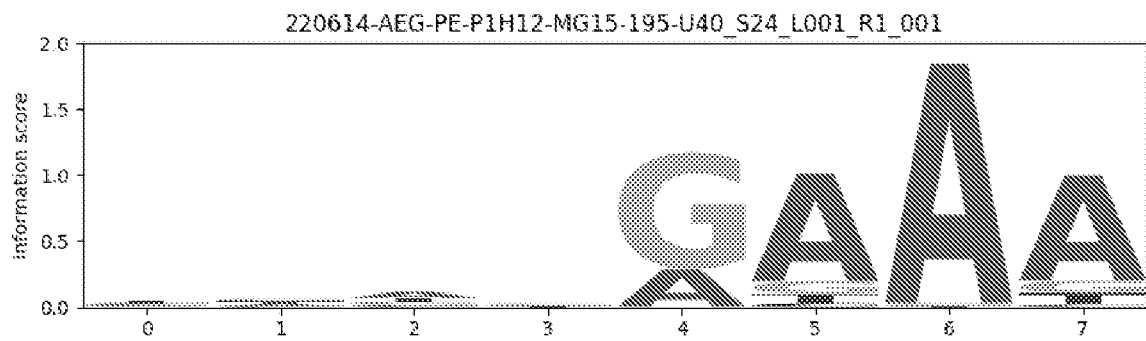
MG15-191



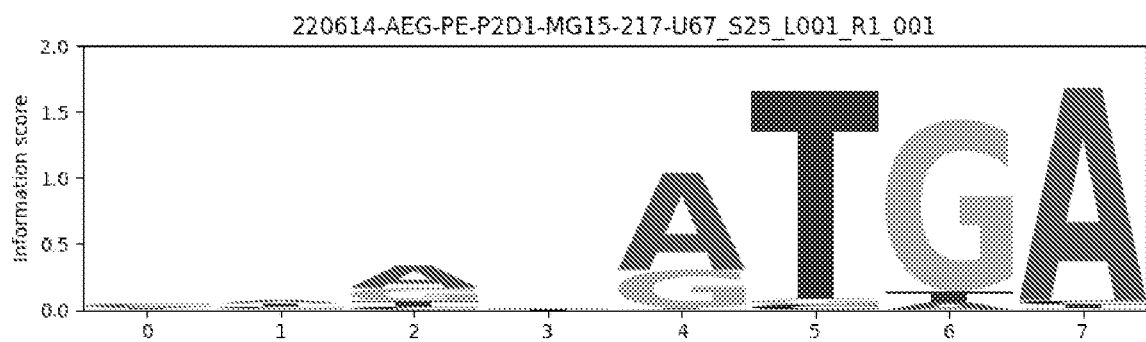
MG15-193

*FIG. 33*

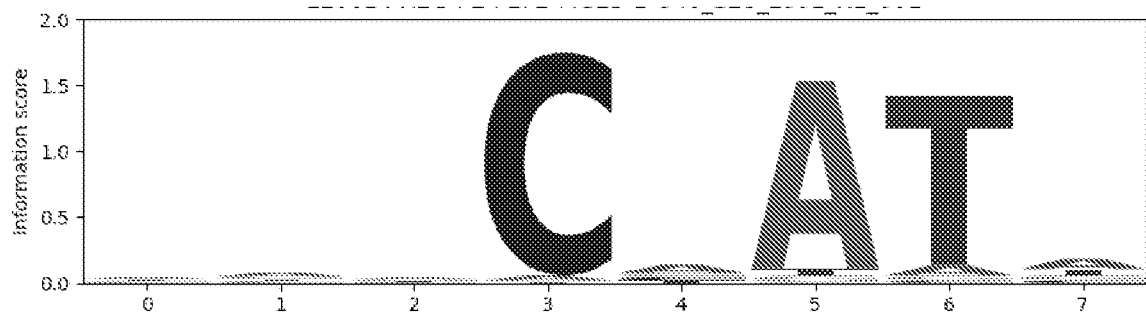
MG15-195



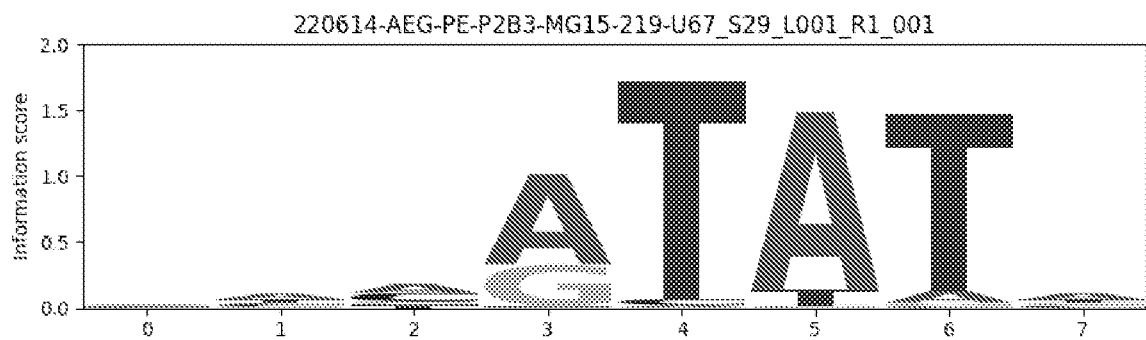
MG15-217



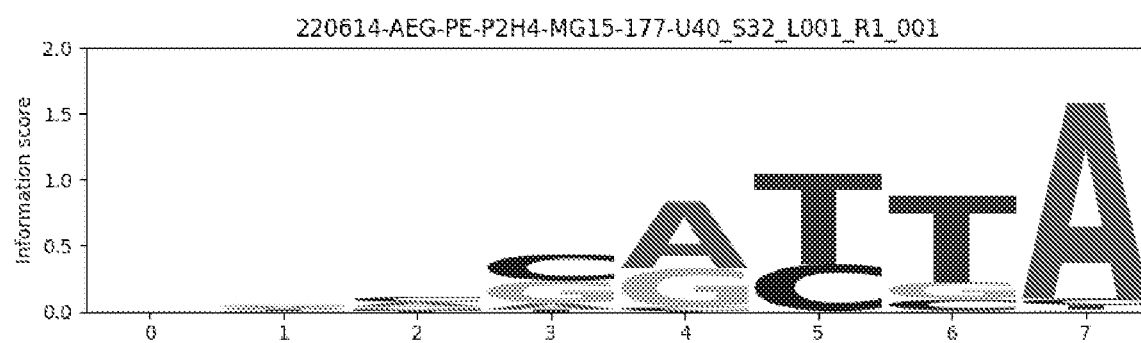
MG15-218



MG15-219

*FIG. 34*

MG15-177

*FIG. 35*