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(58) Field of Search:
As for published application 2610711 A viz:
INT CL **C12N**
Other: **eKOMPASS (KIPO internal)**
updated as appropriate

Additional Fields
Other: **BlastP**

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GB 2610711 B

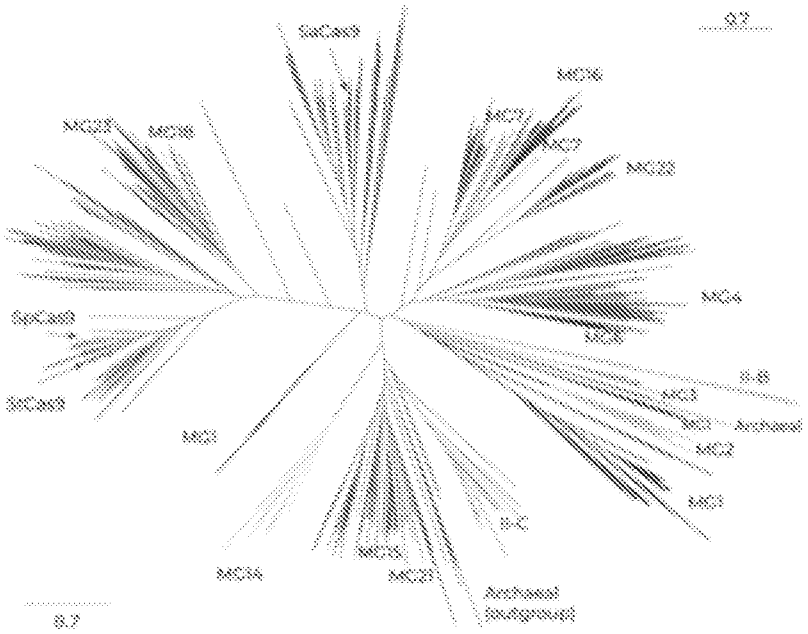


FIG. 1A

Gene	PAM
MG1-4	nRRR
MG1-5	nnnnYY
MG1-6	nnRRAY
MG1-7	nRRRAAG
MG2-4	nAGG
MG2-7	nnnRTA
MG3-1	Not applicable
MG3-2	Not applicable
MG3-3	nnnCCCYR
MG3-4	nnAAAABn
MG3-6	nnRGGnT
MG3-7	nnRnYAY
MG3-8	nnRGGTY
MG4-2	YRnMCC
MG4-5	nRCCV
MG6-3	nRRTA
MG7-1	nRRnCG
MG14-1	nRnnGRKA
MG15-1	CNNNCNAA
MG16-1	nRRnAC
MG18-1	nRWART
SpCas9	nGG
SaCas9	nGGRRT

FIG. 1B

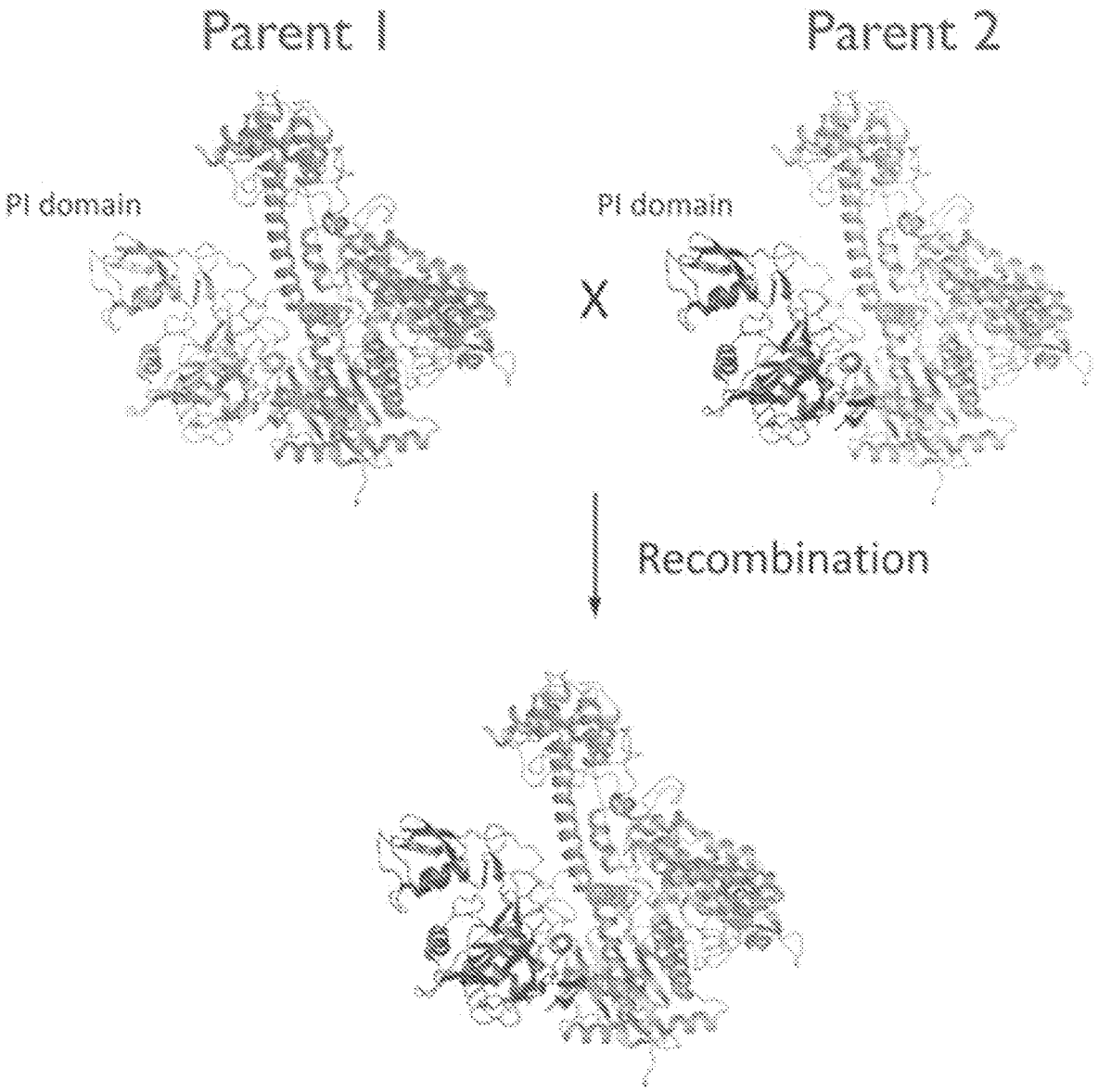


FIG. 2

FIG. 3A

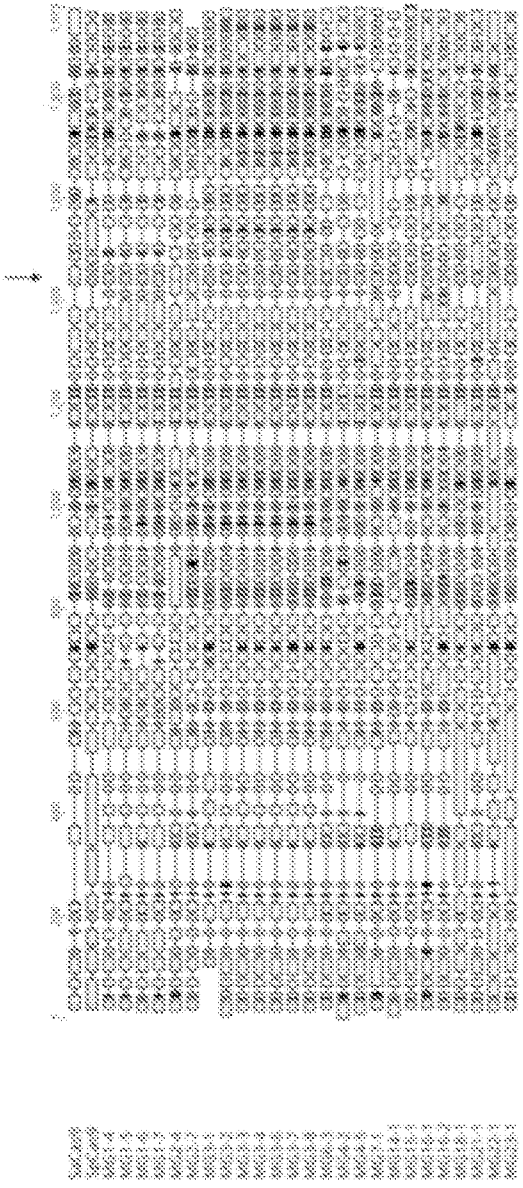
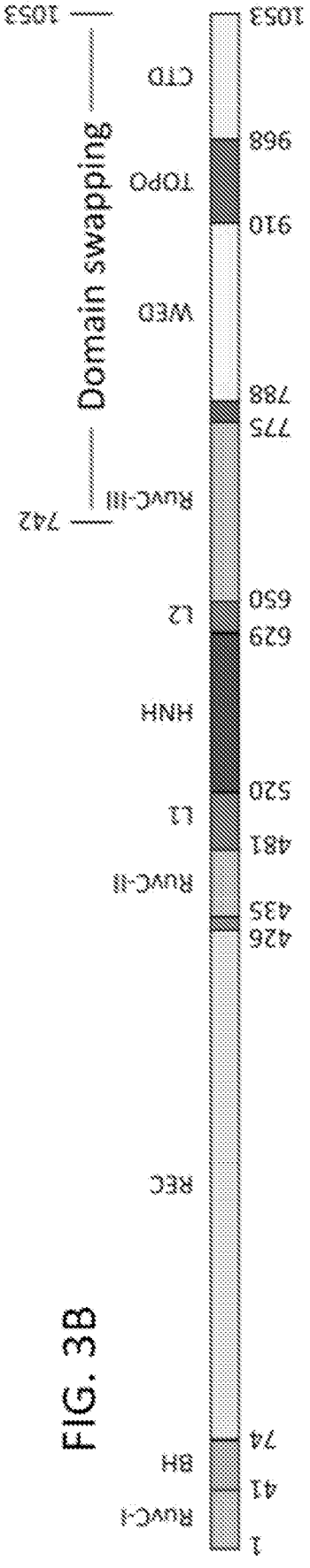


FIG. 3B


1053
CTD
968
TOPO
910
WED
788
775
RuvC-III
742
Domain swapping
650
L2
629
HNH
520
L1
481
RuvC-II
435
426
REC
74
BH
41
RuvC-I
1

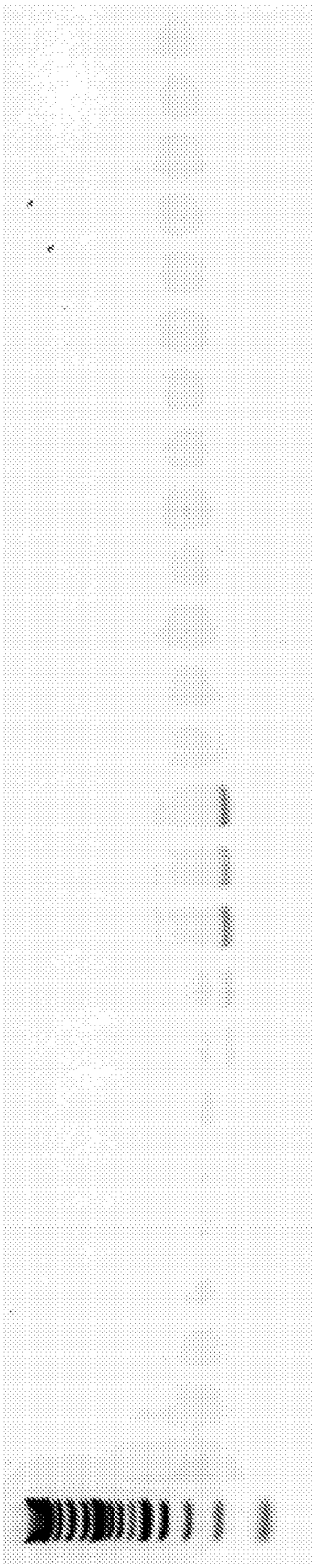

MG3-6+MG1-4
MG3-6+MG1-5
MG3-6+MG1-6
MG3-6+MG1-7
MG3-6+MG2-4
MG3-6+MG2-7
MG3-6+MG3-1
MG3-6+MG3-2
MG3-6+MG3-3
MG3-6+MG3-4
MG3-6+MG3-7
MG3-6+MB3-8
MG3-6+MG4-2
MG3-6+MG4-5
MG3-6+MG6-3
MG3-6+MG14-1
MG3-6+MG15-1
MG3-6+MG16-1
MG3-6+MG16-2
MG3-6+MG18-1
MG3-6+MG21-1
MG3-6+MG22-1
MG3-6+MG23-1
MG3-6+SaCas9
MG3-6+SpCas9

FIG. 4

FIG. 5A

Parents	Chimeras
MG3-3 (nnnCCCYR)	MG3-6+MG3-2 (nnRMYYMW)
MG3-4 (nnAAAAnn)	MG3-6+MG3-3 (nnnCCCYR)
MG3-6 (nnRGGnT)	MG3-6+MG3-4 (nnAAAAnn)
MG3-7 (nnRnYAY)	MG3-6+MG3-7 (nnRnYAY)
MG3-8 (nnRGGTY)	MG3-6+MG3-8 (nnRGGTY)

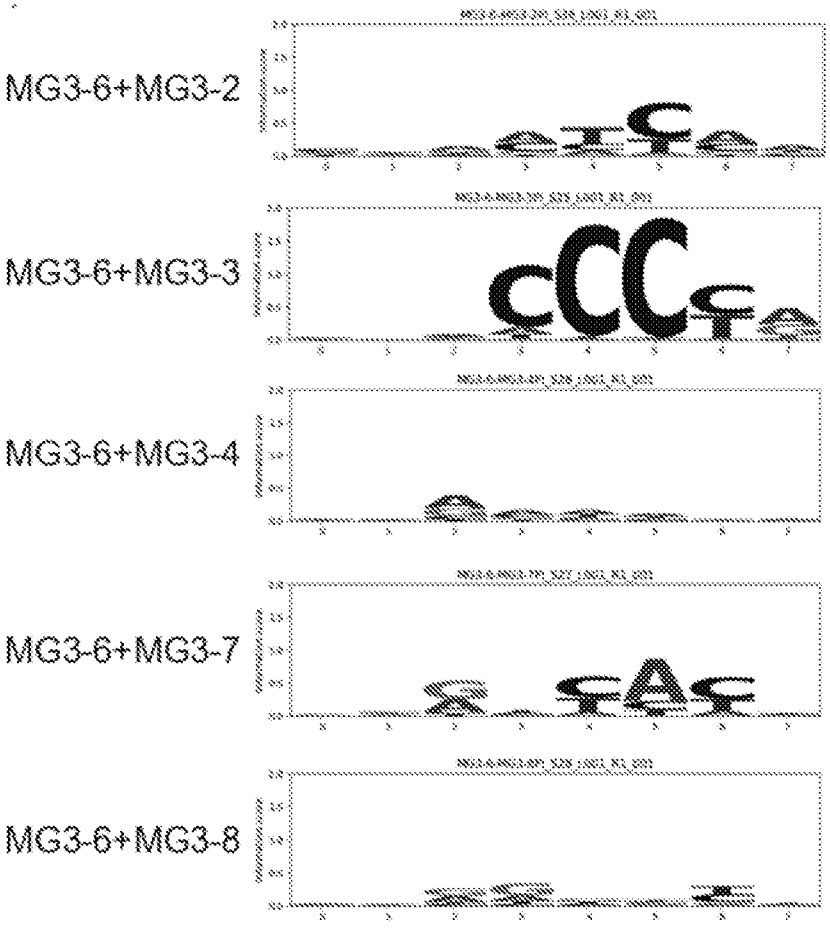


FIG. 5B

MG3-6 (3-6)
MG3-6+MG14-4 (14-4)
MG3-6+MG14-5 (14-5)
MG3-6+MG16-6 (16-6)
MG3-6+MG21-7 (21-7)
MG3-6+MG24 (24)
MG3-6+MG27 (27)
MG3-6+MG42 (42)
MG3-6+MG45 (45)
MG3-6+MG66-3 (66-3)
MG3-6+MG314-1 (314-1)
MG3-6+MG315-1 (315-1)
MG3-6+MG316-1 (316-1)
MG3-6+MG316-2 (316-1)
MG3-6+MG321-1 (321-1)
MG3-6+MG322-1 (322-1)
MG3-6+MG323-1 (323-1)
MG3-6+SpCas9 (SpCas9)

FIG. 6

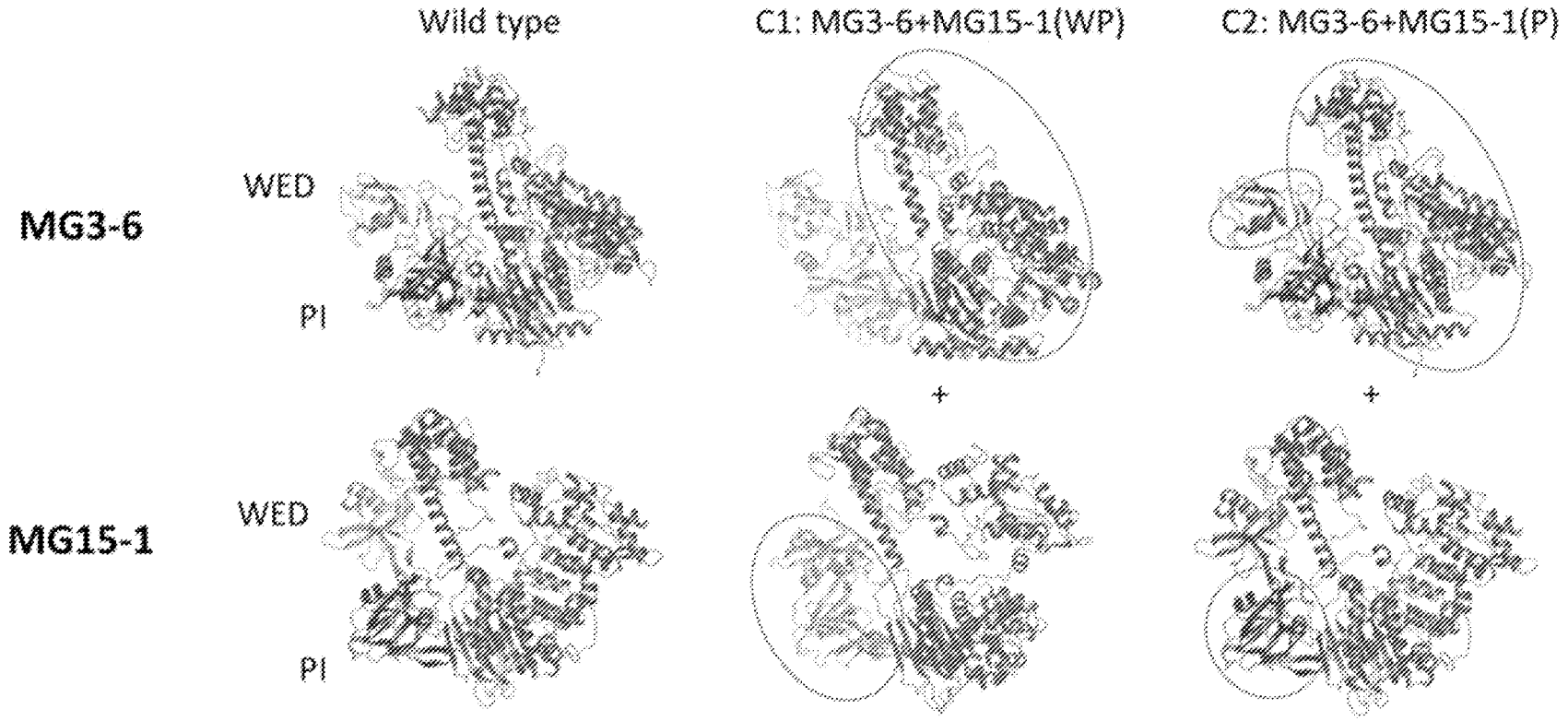

Wild type
C1: MG3-6+MG15-1(WP)
C2: MG3-6+MG15-1(P)
MG3-6
WED
PI
+
MG15-1
WED
PI
+

FIG. 7

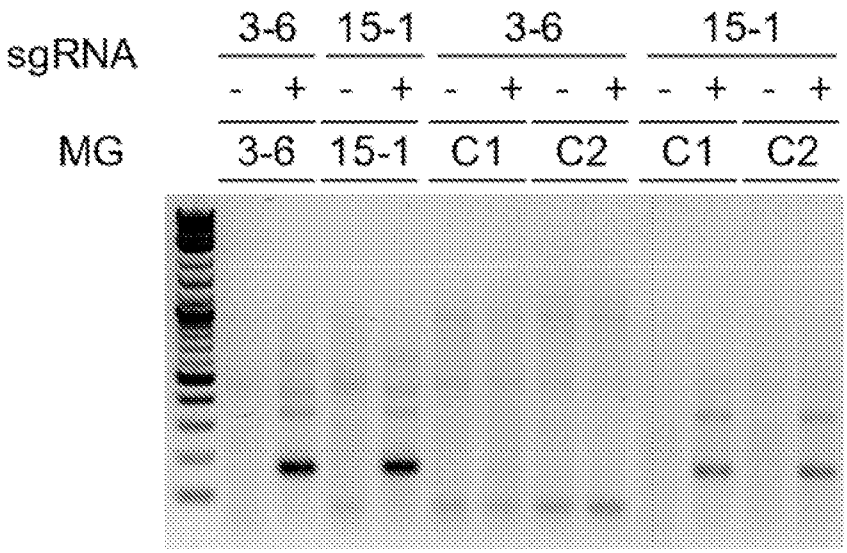


FIG. 8A

Sanger sequencing result

Gene	PAM		
	1	2	3
MG3-6	NNRGGTYA	NNRGGTYN	NNRGGTYN
MG15-1	CNNNCNAA	CNNNCCAA	CNNNCCAA
MG3-6_MG15-1(WP)	CNNNCNAA	CNANCWAA	CNANCWAA
MG3-6_MG15-1(P)		NNNNCWAA	CNNNCWAA

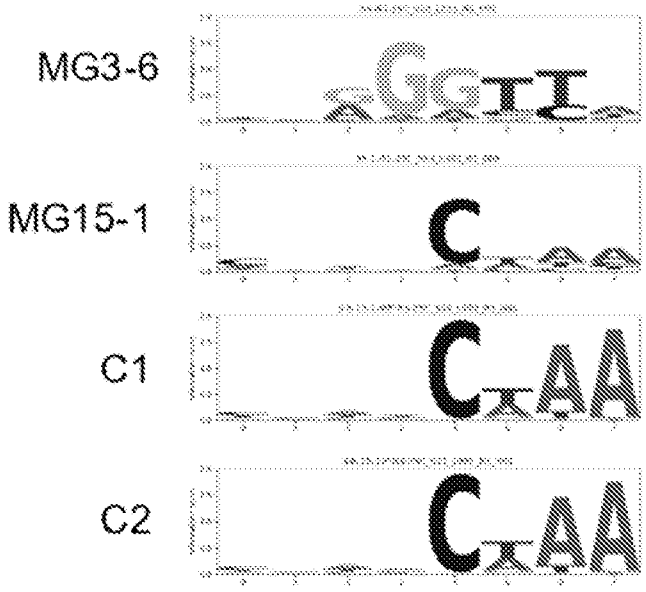


FIG. 8B

FIG. 9A

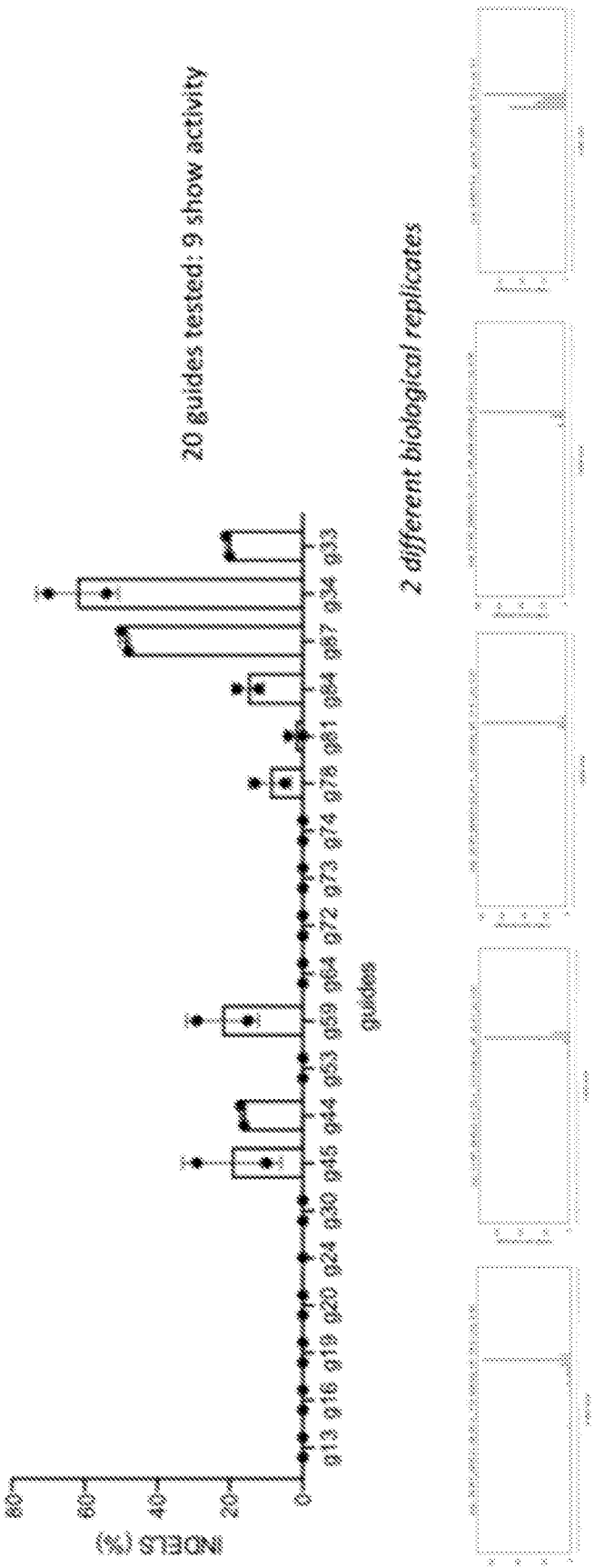
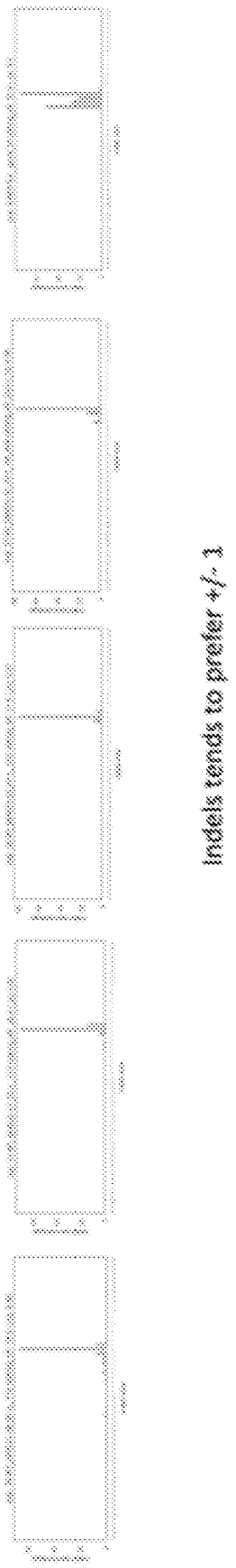

20 guides tested; 9 show activity
2 different biological replicates
INDELS (%)
0
20
40
60
80
g13 g16 g19 g20 g24 g30 g45 g44 g53 g59 g64 g72 g73 g74 g78 g81 g84 g87 g34 g33
guides

FIG. 9B


Indels tends to prefer +/- 1

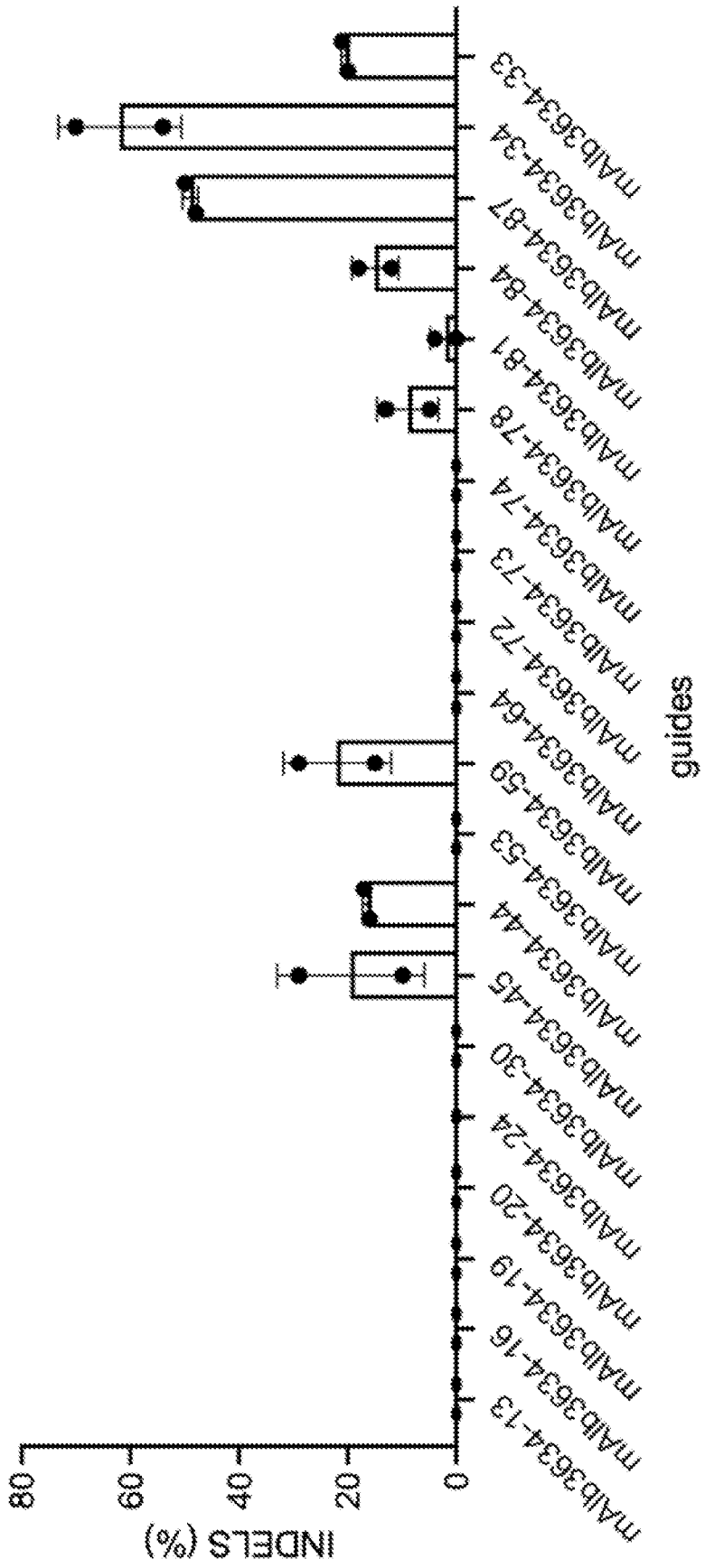

INDELS (%)
0
20
40
60
80
guides
mAlb3634-13
mAlb3634-16
mAlb3634-19
mAlb3634-20
mAlb3634-24
mAlb3634-30
mAlb3634-45
mAlb3634-44
mAlb3634-53
mAlb3634-59
mAlb3634-64
mAlb3634-72
mAlb3634-73
mAlb3634-74
mAlb3634-78
mAlb3634-81
mAlb3634-84
mAlb3634-87
mAlb3634-34
mAlb3634-33

FIG. 10

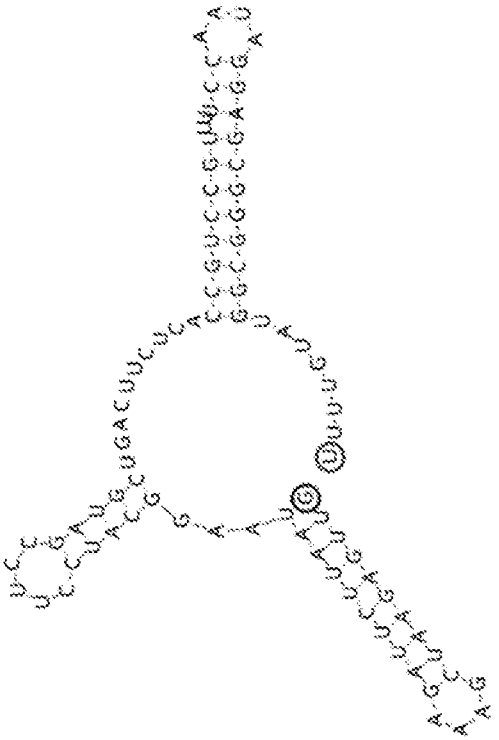


FIG. 11B

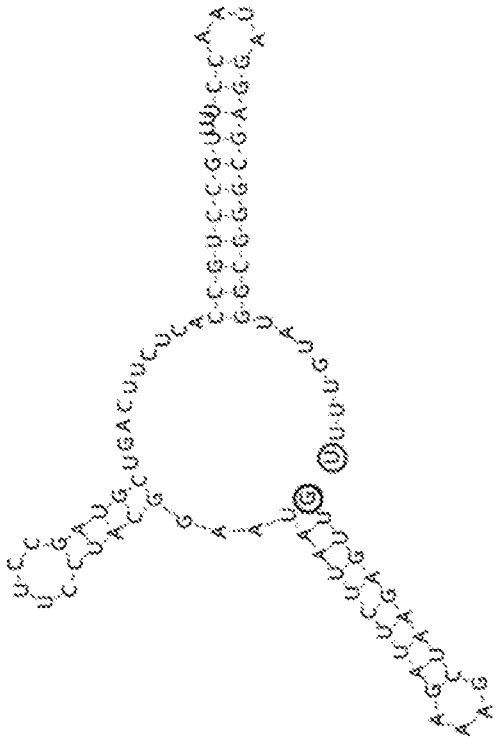


FIG. 11A

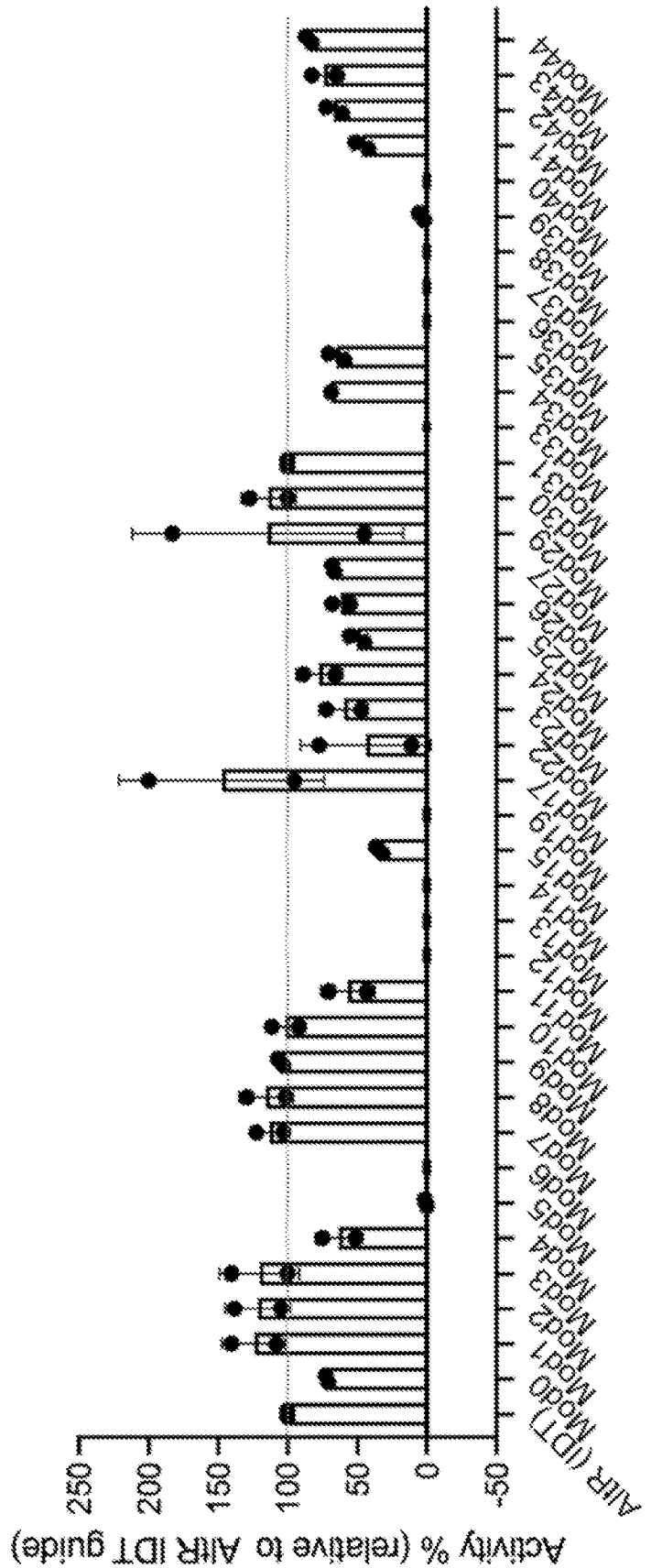

Activity % (relative to AIIR IDT guide)
250
200
150
100
50
0
-50
AIIR (IDT)
Mod0
Mod1
Mod2
Mod3
Mod4
Mod5
Mod6
Mod7
Mod8
Mod9
Mod10
Mod11
Mod12
Mod13
Mod14
Mod15
Mod16
Mod17
Mod18
Mod19
Mod20
Mod21
Mod22
Mod23
Mod24
Mod25
Mod26
Mod27
Mod28
Mod29
Mod30
Mod31
Mod32
Mod33
Mod34
Mod35
Mod36
Mod37
Mod38
Mod39
Mod40
Mod41
Mod42
Mod43
Mod44

FIG. 12

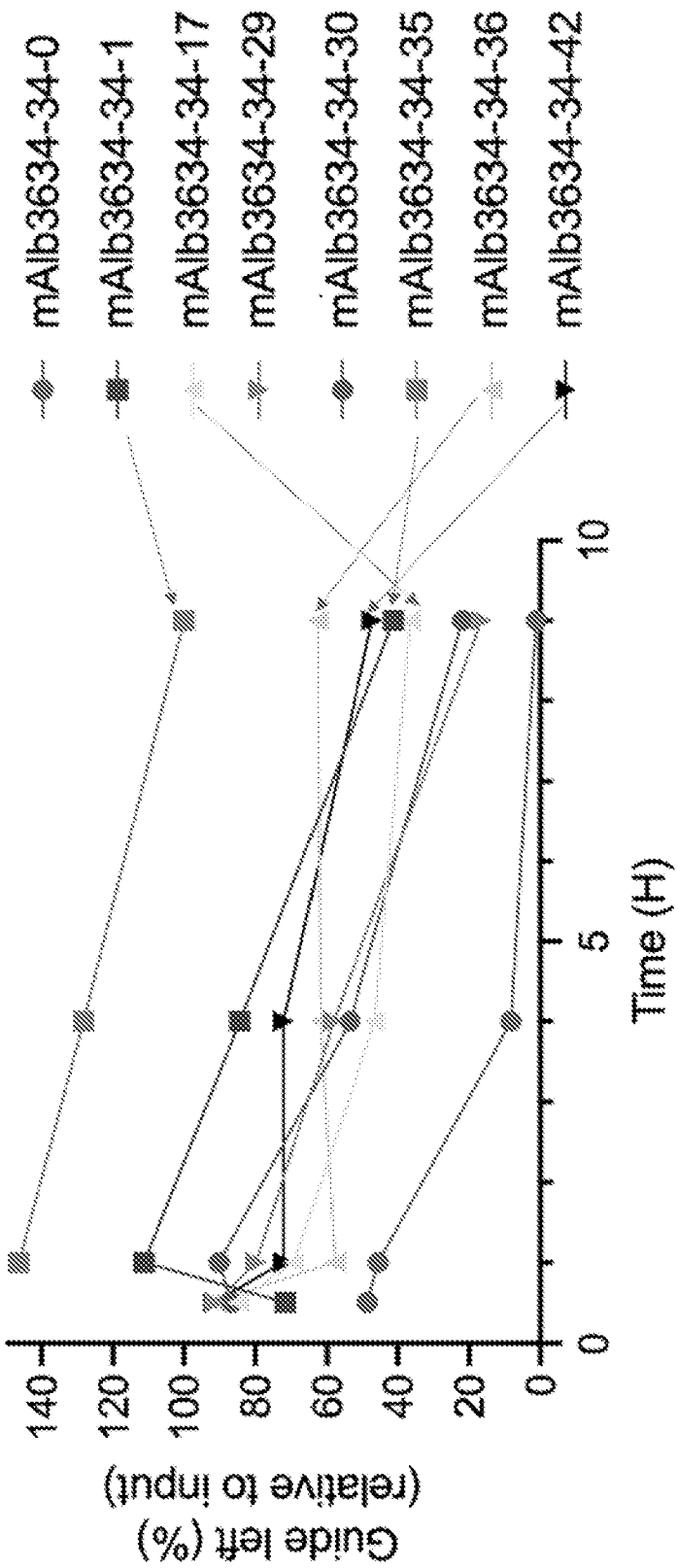

mAlb3634-34-0
mAlb3634-34-1
mAlb3634-34-17
mAlb3634-34-29
mAlb3634-34-30
mAlb3634-34-35
mAlb3634-34-36
mAlb3634-34-42
Guide left (%)
(relative to input)
140
120
100
80
60
40
20
0
0
5
10
Time (H)

FIG. 13

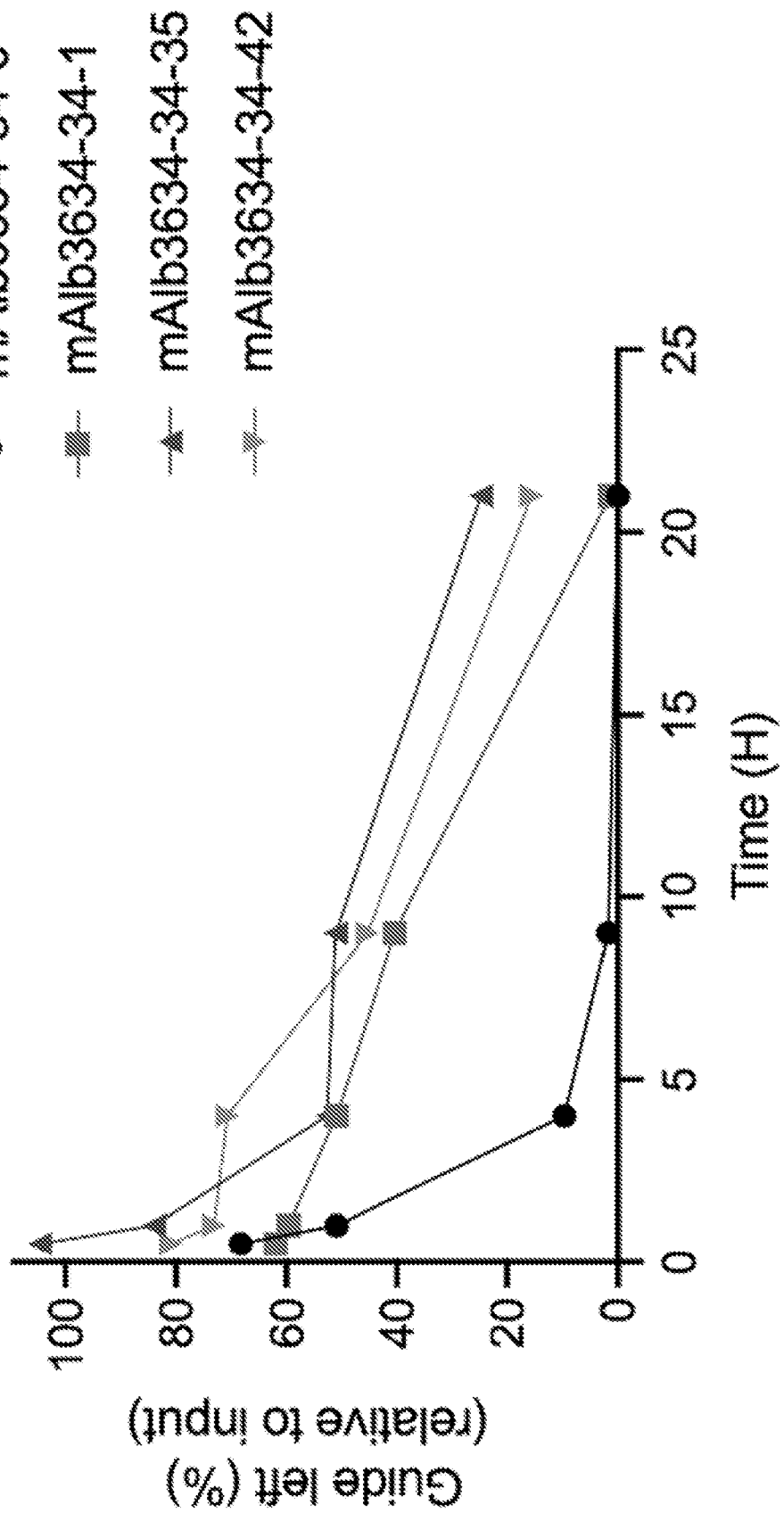

mAlb3634-34-0
mAlb3634-34-1
mAlb3634-34-35
mAlb3634-34-42
Guide left (%)
(relative to input)
100
80
60
40
20
0
0
5
10
15
20
25
Time (H)

FIG. 14

FIG. 15A

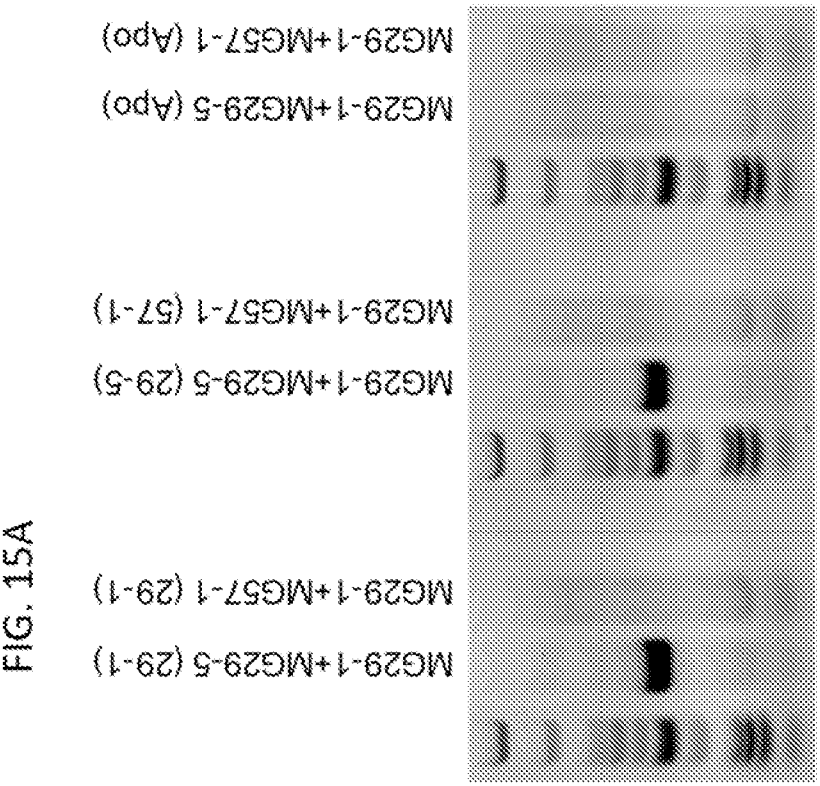
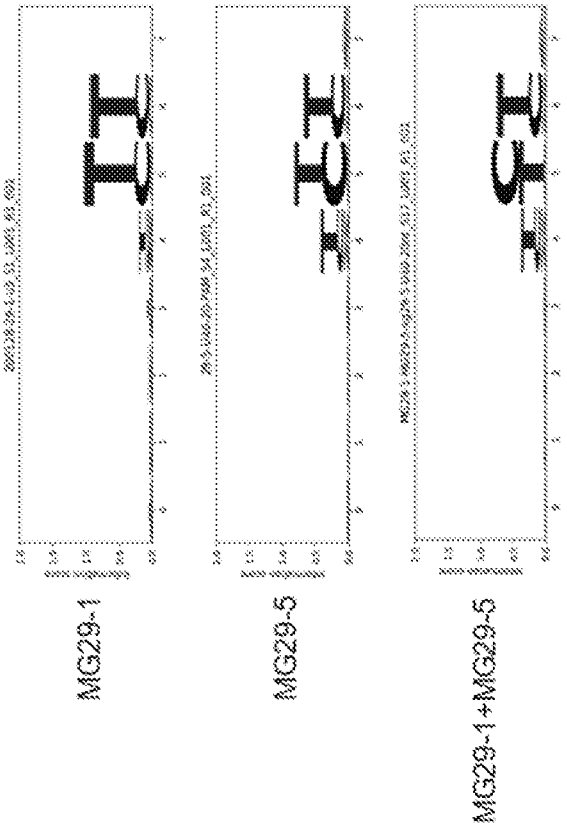


FIG. 15B



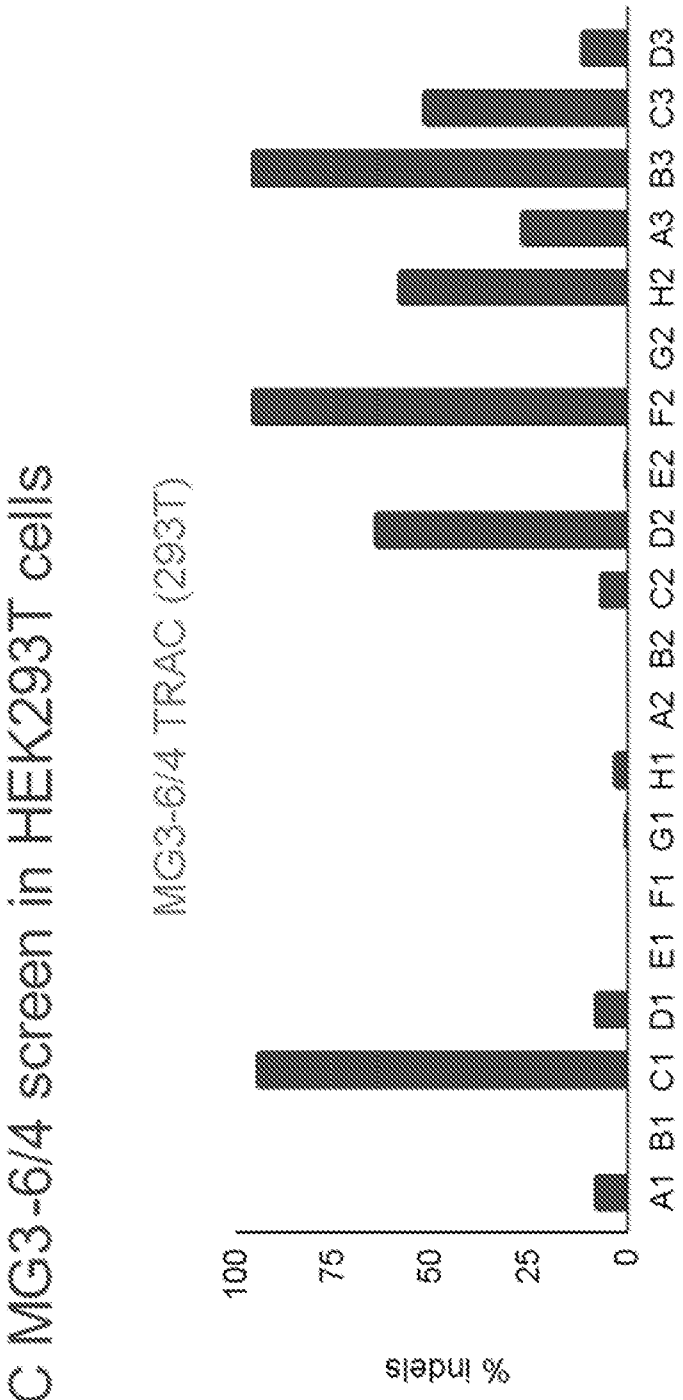

TRAC MG3-6/4 screen in HEK293T cells
MG3-6/4 TRAC (293T)
% Indels
100
75
50
25
0
A1 B1 C1 D1 E1 F1 G1 H1 A2 B2 C2 D2 E2 F2 G2 H2 A3 B3 C3 D3

FIG. 16

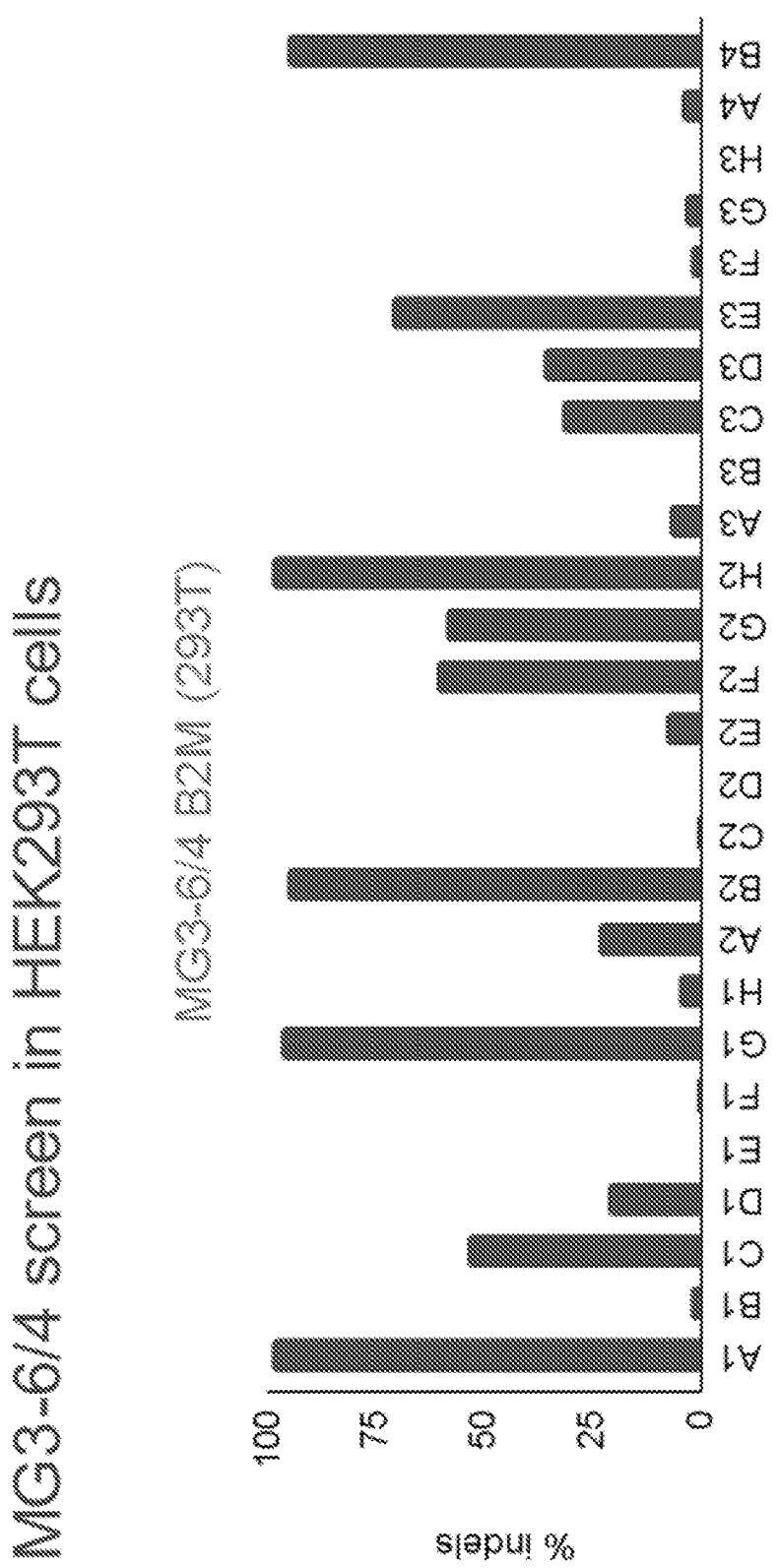

B2M MG3-6/4 screen in HEK293T cells
MG3-6/4 B2M (293T)
% Indels
100
75
50
25
0
A1
B1
C1
D1
E1
F1
G1
H1
A2
B2
C2
D2
E2
F2
G2
H2
A3
B3
C3
D3
E3
F3
G3
H3
A4
B4

FIG. 17

TRAC MG3-6/4 screen in T cells

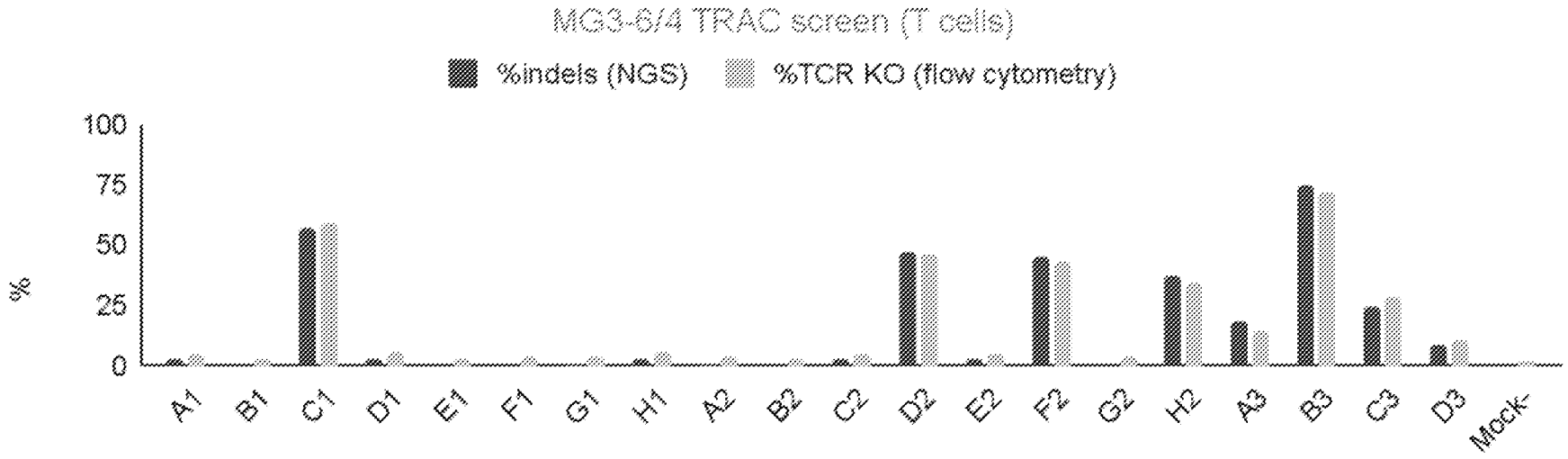


FIG. 18

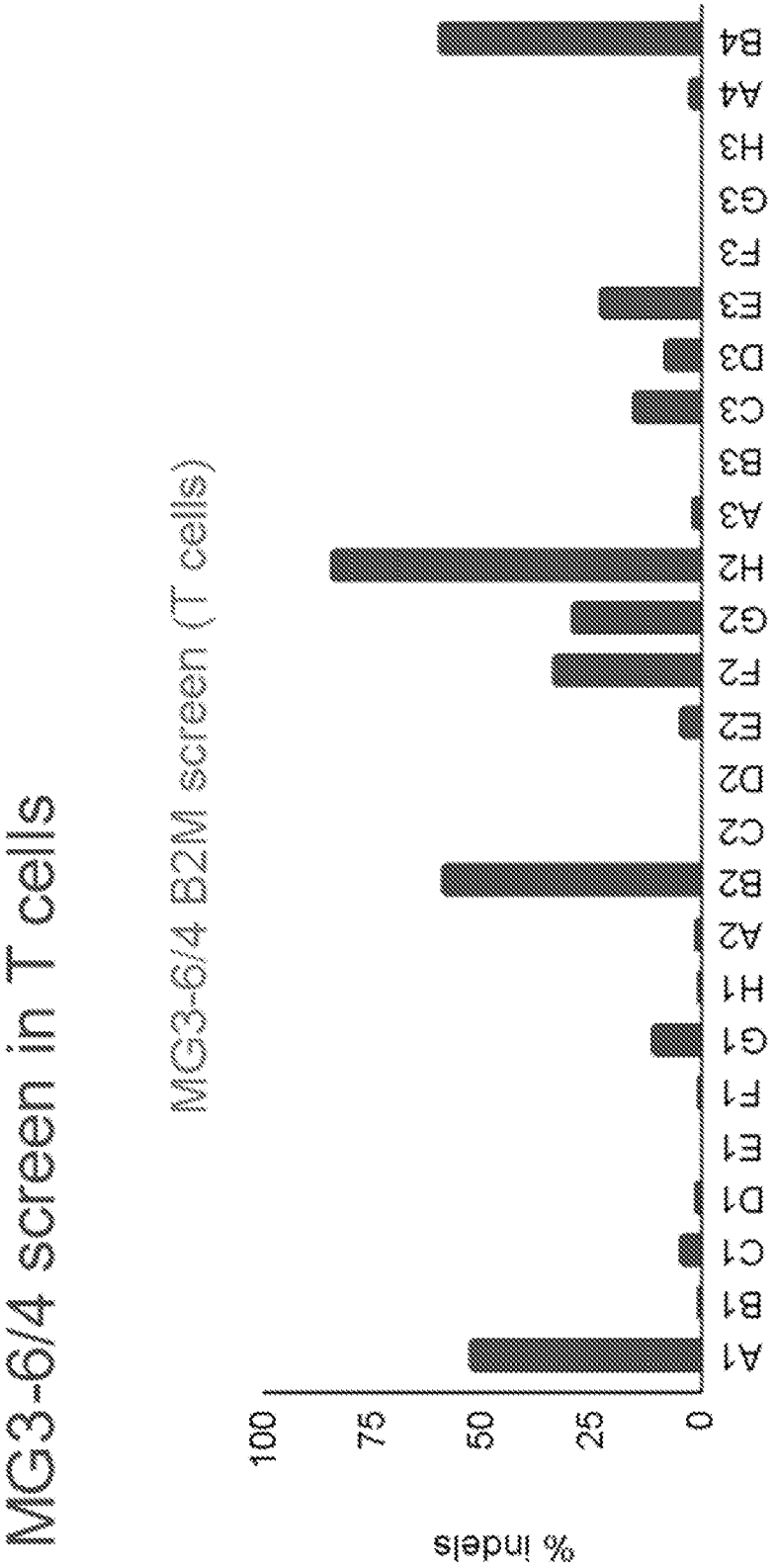

B2M MG3-6/4 screen in T cells
MG3-6/4 B2M screen (T cells)
% indels
100
75
50
25
0
A1
B1
C1
D1
E1
F1
G1
H1
A2
B2
C2
D2
E2
F2
G2
H2
A3
B3
C3
D3
E3
F3
G3
H3
A4
B4

FIG. 19

TRBC1/2 MG3-6/4 screen in T cells

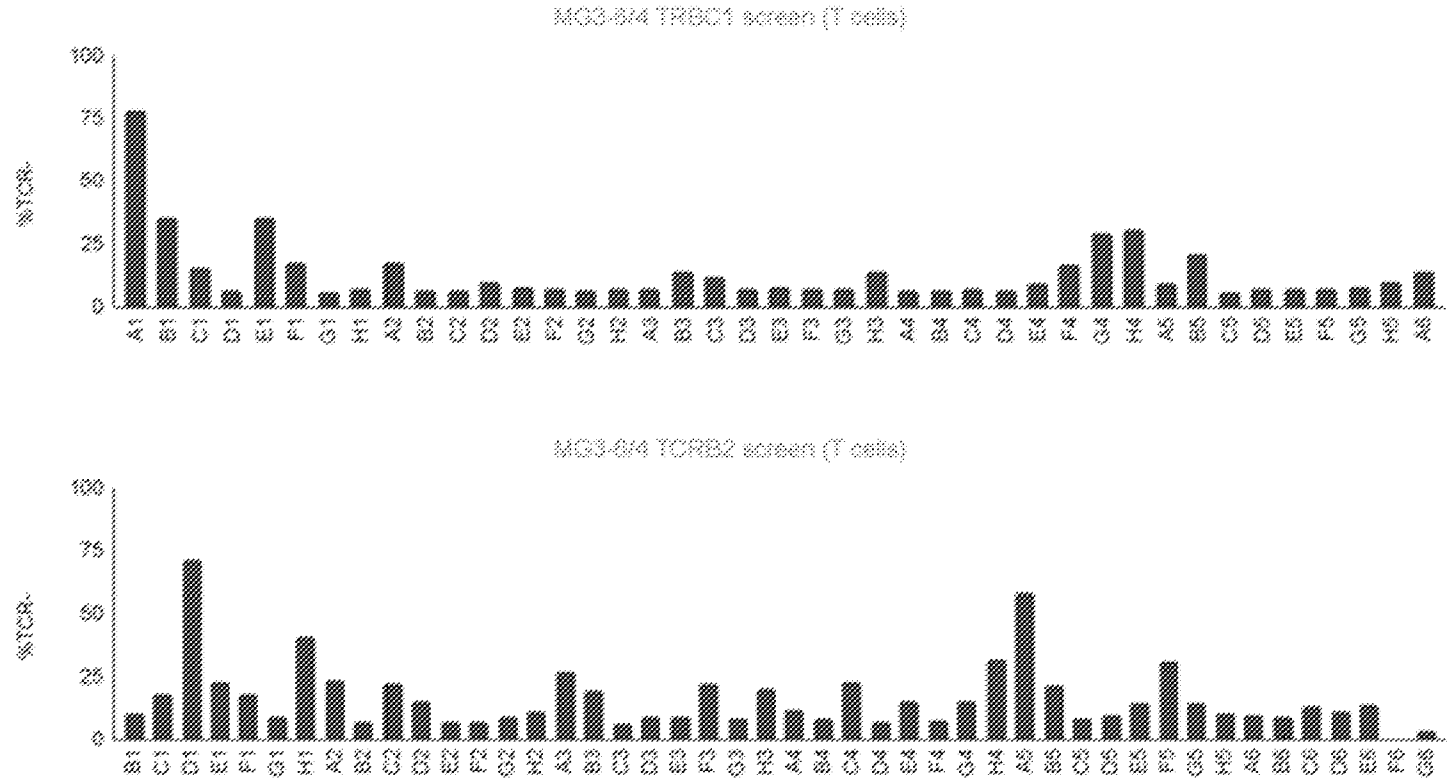


FIG. 20

ANGPTL3 MG3-6/4 screen in Hep3B cells

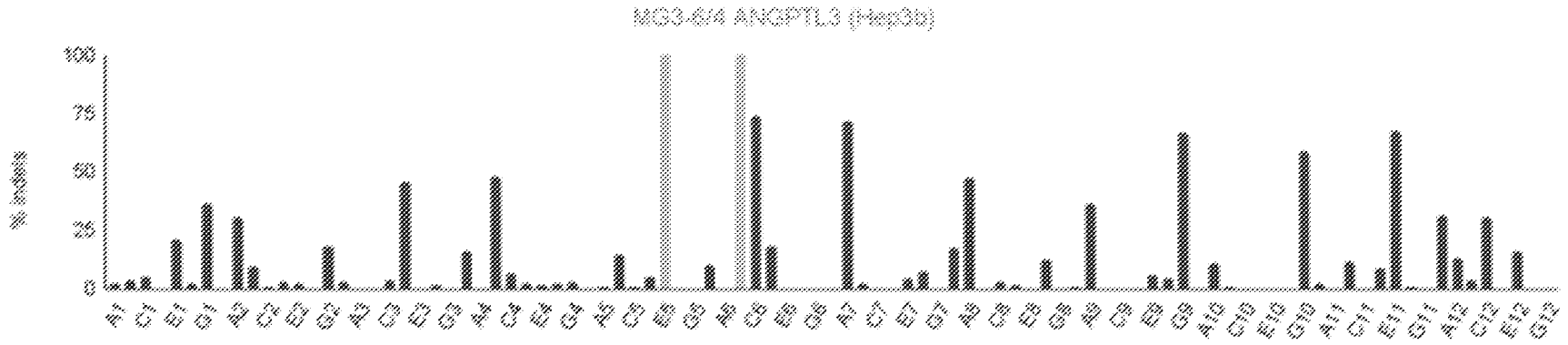


FIG. 21

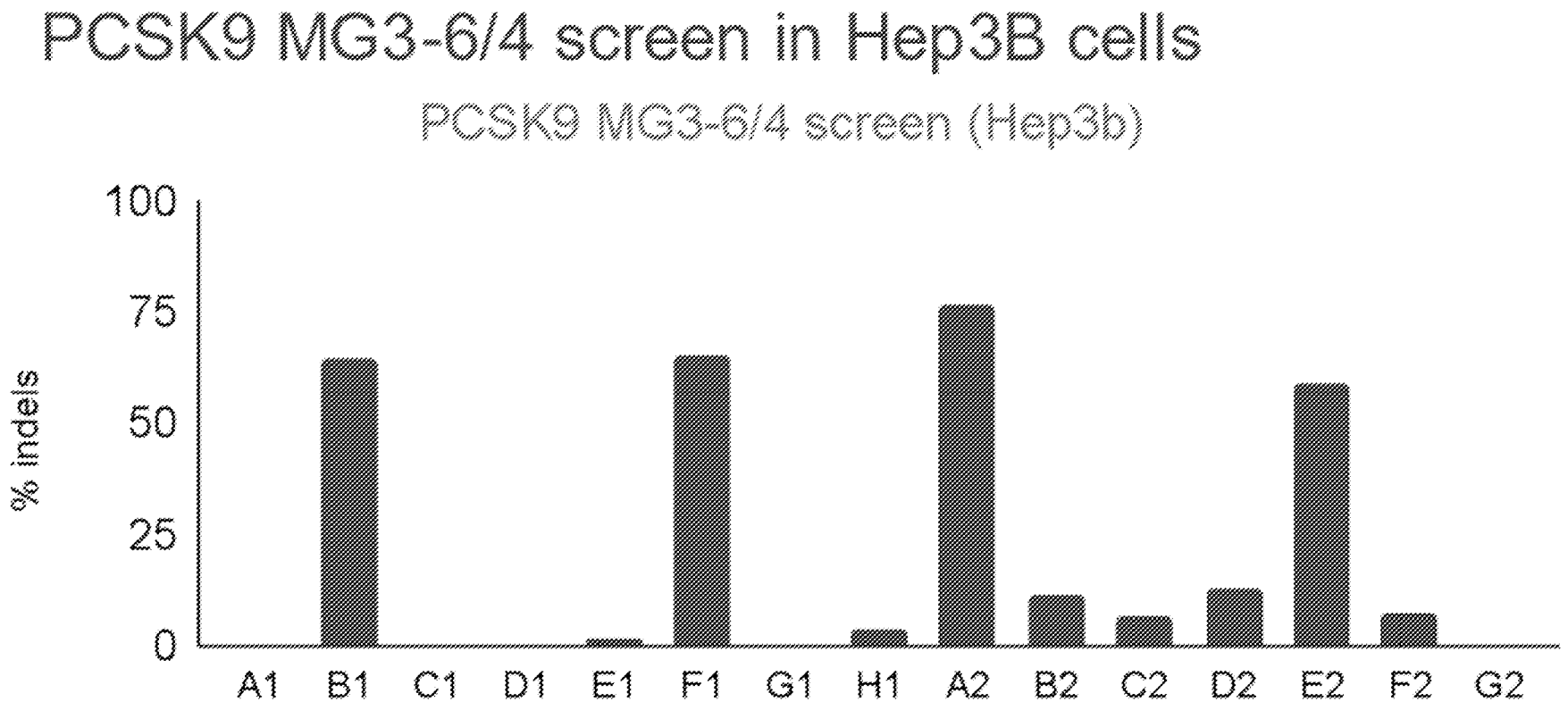

PCSK9 MG3-6/4 screen in Hep3B cells
PCSK9 MG3-6/4 screen (Hep3b)
% indels
100
75
50
25
0
A1
B1
C1
D1
E1
F1
G1
H1
A2
B2
C2
D2
E2
F2
G2

FIG. 22

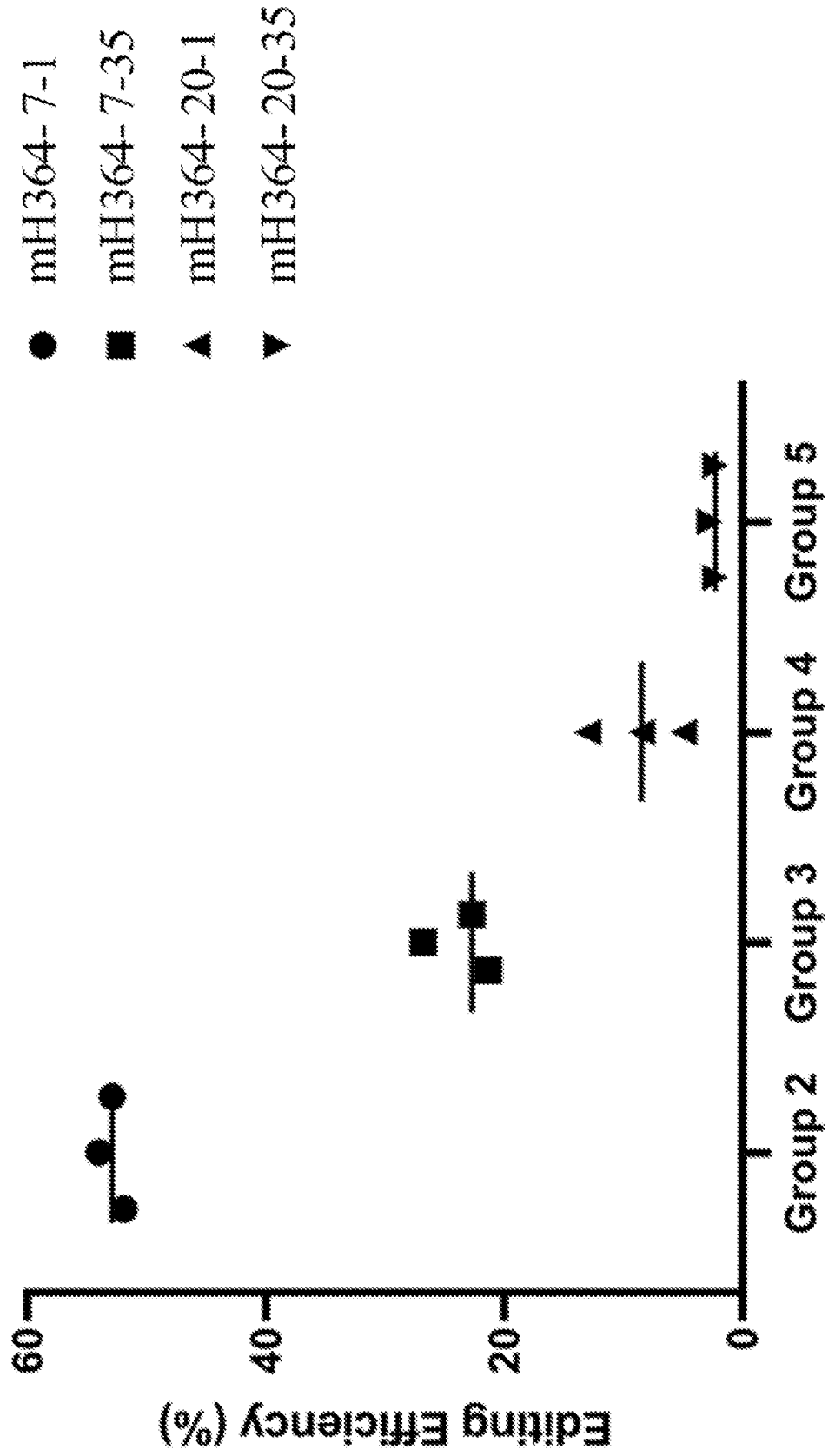
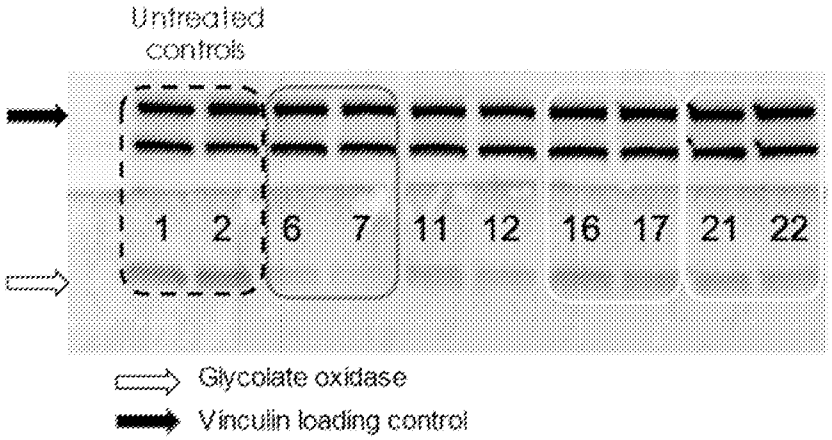

MG PH002 - Day 11- NGS Data
mH364-7-1
mH364-7-35
mH364-20-1
mH364-20-35
Editing Efficiency (%)
60
40
20
0
Group 2
Group 3
Group 4
Group 5

FIG. 23



Quantitation of Western Blot

Guide RNA	Chemistry	Mouse	GO protein % Knockdown #	Average GO protein % knockdown
mH364-7-1	1	6	65	75
		7	86	
mH364-7-35	35	11	56	58
		12	60	
mH364-20-1	1	16	4	4
		17	4	
mH364-20-35	35	21	21	24
		22	26	

GO protein levels normalized to vinculin as a % of untreated control

FIG. 24

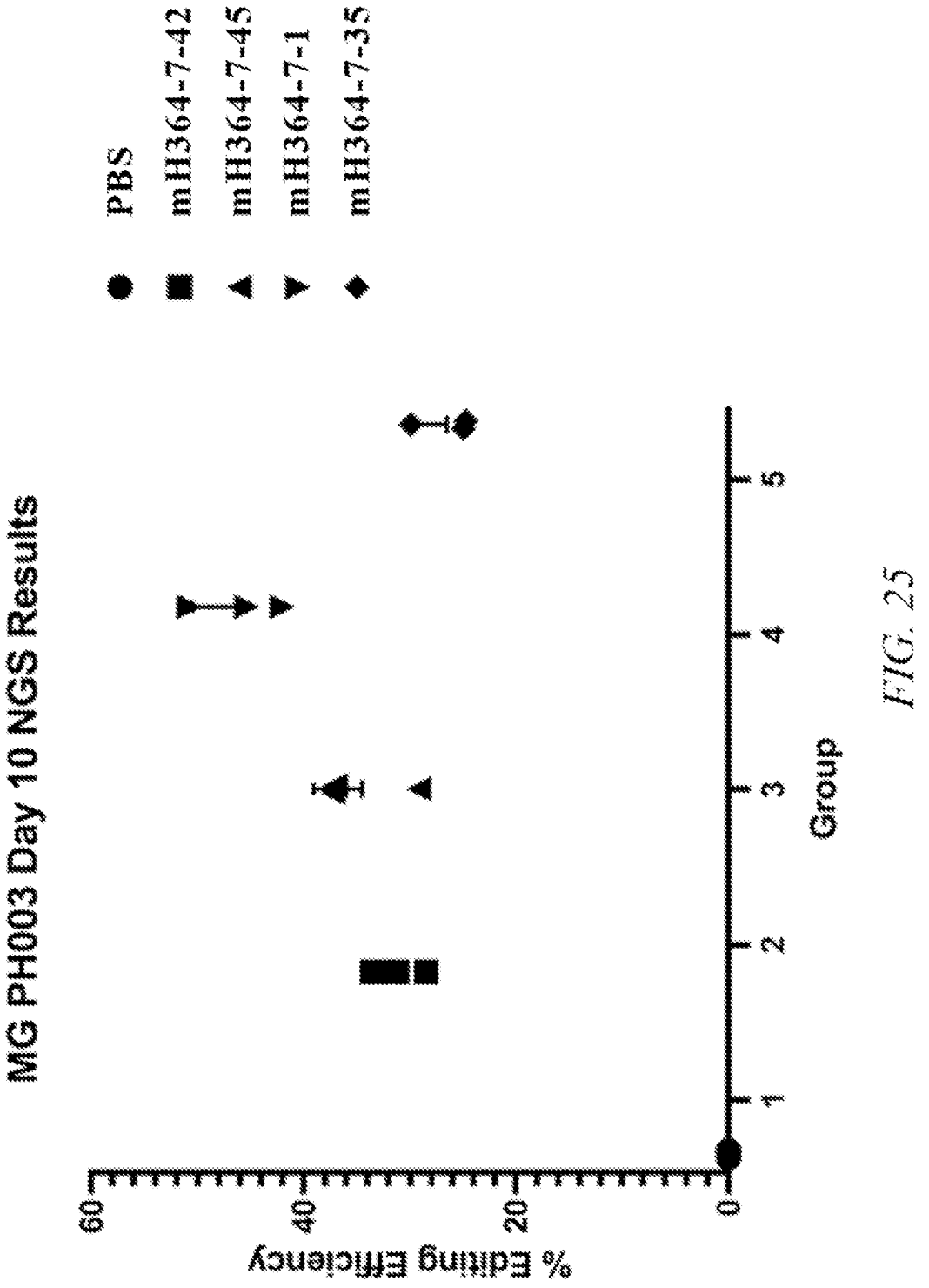

MG PH003 Day 10 NGS Results
% Editing Efficiency
0
20
40
60
Group
1
2
3
4
5
PBS
mH364-7-42
mH364-7-45
mH364-7-1
mH364-7-35

FIG. 25

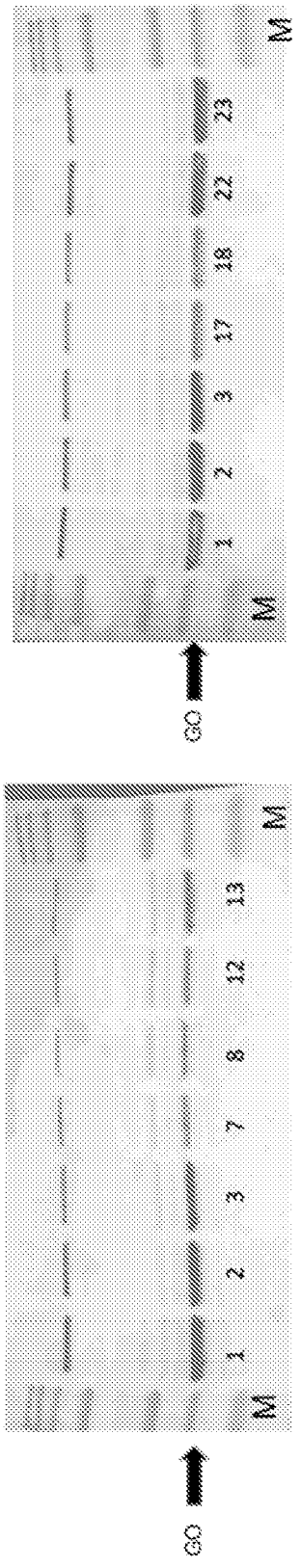


FIG. 26

NOVEL ENGINEERED AND CHIMERIC NUCLEASES

CROSS-REFERENCE

[0001] This application is related to International Application No. PCT/US2021/031136 entitled "ENZYMES WITH RUVC DOMAINS", filed on May 6, 2021, and PCT/US2020/018432, filed on Feb. 14, 2020, entitled "ENZYMES WITH RUVC DOMAINS", each of which is incorporated by reference herein in its entirety.

[0002] This application claims the benefit of U.S. Provisional Application No. 63/237,484, entitled "NOVEL ENGINEERED AND CHIMERIC NUCLEASES", filed on August 26, 2021, and U.S. Provisional Application No. 63/140,620 entitled "NOVEL ENGINEERED AND CHIMERIC NUCLEASES" filed on January 22, 2021, 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.

SUMMARY

[0004] In some aspects, the present disclosure provides for a fusion endonuclease comprising: (a) an N-terminal sequence comprising at least part of a RuvC domain, a REC domain, or an HNH domain of an endonuclease having at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NO: 696 or a variant thereof; and (b) a C-terminal sequence comprising WED, TOPO, or CTD domains of an endonuclease having at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least

83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any one of SEQ ID NOs: 697-721 or variants thereof, wherein said N-terminal sequence and said C-terminal sequence do not naturally occur together in a same reading frame. In some embodiments, the endonuclease is a Class II, type II Cas endonuclease. In some embodiments, the endonuclease is a Class II, type V Cas endonuclease. In some embodiments, said N-terminal sequence and said C-terminal sequence are derived from different organisms. In some embodiments, said N-terminal sequence further comprises RuvC-I, BH, or RuvC-II domains. In some embodiments, said C-terminal sequence further comprises a PAM-interacting domain. In some embodiments, said fusion endonuclease comprises a sequence having at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any one of SEQ ID NOs: 1-27 or 108. In some embodiments, said fusion endonuclease is configured to bind to a PAM that is not nnRGGnT (SEQ ID NO: 53). In some embodiments, said fusion endonuclease is configured to bind to a PAM that comprises any one of SEQ ID NOs:46-52 or 54-66.

[0005] In some aspects, the present disclosure provides for an endonuclease comprising an engineered amino acid sequence having at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any one of SEQ ID NOs: 1-27 or 108, or a variant thereof.

[0006] In some aspects, the present disclosure provides for an endonuclease comprising an engineered amino acid sequence having at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any one of SEQ ID NOs: 109-110, or a variant thereof.

[0007] In some aspects, the present disclosure provides for a nucleic acid comprising a sequence encoding any of the endonucleases, fusion endonucleases, or Cas enzymes described herein. In some aspects, the sequence is codon-optimized for expression in a host cell. In some embodiments, the host cell is prokaryotic, eukaryotic, mammal, or human.

[0008] In some aspects, the present disclosure provides for a vector comprising any of the nucleic acid sequences described herein.

[0009] In some aspects, the present disclosure provides for a host cell comprising any of the vectors, systems, or nucleic acids described herein. In some embodiments, the host cell is prokaryotic, eukaryotic, mammal, or human.

[0010] In some aspects, the present disclosure provides for an engineered nuclease system, comprising: (a) any of the nucleases, Cas enzymes, or fusion endonucleases described herein; and (b) an engineered guide ribonucleic structure configured to form a complex with said endonuclease comprising: a guide ribonucleic acid configured to hybridize to a target deoxyribonucleic acid sequence; wherein said guide ribonucleic acid sequence is configured to bind to said endonuclease. In some embodiments, said guide ribonucleic acid further comprises a tracr ribonucleic acid sequence configured to bind said endonuclease. In some embodiments, said endonuclease is derived from an uncultivated microorganism. In some embodiments, said 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 Cas13d endonuclease. In some embodiments, said endonuclease has less than 86% identity to a SpyCas9 endonuclease. In some embodiments, said system further comprises a source of Mg^{2+}. In some embodiments, said endonuclease comprises a sequence having at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any one of SEQ ID NOs: 8-12, 26-27, or 108, or a variant thereof. In some embodiments, said guide ribonucleic acid sequence comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to non-degenerate nucleotides of any one of SEQ ID NOs: 33, 34, 44, 45, 78, 84, or 87.

[0011] In some aspects, the present disclosure provides for an engineered nuclease comprising: (a) a class II, type II Cas enzyme RuvC or HNH domain having at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to a RuvC or HNH domain of any one of SEQ ID

NOs: 1-27, 108, or 109-110, or variants thereof; and (b) a class II, type II Cas enzyme PAM-interacting (PI) domain having at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to a PAM-interacting (PI) domain any one of SEQ ID NOs: 1-27, 108, or 109-110, or variants thereof. In some embodiments, (a) and (b) do not naturally occur together. In some embodiments, said class II, type II Cas enzyme is derived from an uncultivated microorganism. In some embodiments, said endonuclease has less than 86% identity to a SpyCas9 endonuclease. In some embodiments, said engineered nuclease comprises a sequence having at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any one of SEQ ID NOs: 1-27 or a variant thereof.

[0012] In some aspects, the present disclosure provides for an engineered nuclease system, comprising: (a) any of the endonucleases described herein; and (b) an engineered guide ribonucleic structure configured to form a complex with said endonuclease comprising: a guide ribonucleic acid sequence configured to hybridize to a target deoxyribonucleic acid sequence and configured to bind to said endonuclease. In some embodiments, said guide ribonucleic acid further comprises a tracr ribonucleic acid sequence configured to bind said endonuclease. In some embodiments, said guide ribonucleic acid sequence comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to non-degenerate nucleotides of any one of SEQ ID NOs: 28-32 or 33-44, or a variant thereof. In some embodiments, the system further comprises a PAM sequence compatible with said nuclease adjacent to said target nucleic acid site. In some embodiments, said PAM sequence is located 3′ of said target deoxyribonucleic acid sequence. In some embodiments, said PAM sequence is located 5’ of said target deoxyribonucleic acid sequence. In some embodiments, said PAM sequence comprises any one of SEQ ID NOs:46-66.

[0013] In some aspects, the present disclosure provides for a method of targeting the albumin gene, comprising introducing any of the systems described herein to a cell, wherein said guide ribonucleic acid sequence is configured to hybridize to a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least

88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity any one of SEQ ID NOs: 67-86. In some embodiments, introducing to said cell further comprises contacting said cell with a nucleic acid or vector encoding said fusion protein or said guide polynucleotide. or comprises contacting said cell with a lipid nanoparticle (LNP) comprising said vector or nucleic acid. In some embodiments, introducing to said cell further comprises contacting said cell with a ribonucleoprotein complex (RNP) comprising said fusion protein or said guide polynucleotide or comprises contacting said cell with a lipid nanoparticle (LNP) comprising said RNP.

[0014] In some aspects, the present disclosure provides for a method of targeting the HAO1 gene or locus, comprising introducing any of the systems described herein to a cell, wherein said guide ribonucleic acid sequence is configured to hybridize to a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any one of SEQ ID NOs: 611-633. In some embodiments, said guide ribonucleic acid sequence is configured to hybridize to a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any one of SEQ ID NOs: 615, 618, 620, 624, or 626. In some embodiments, said guide ribonucleic acid comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any one of SEQ ID NOs:645-684. In some embodiments, said guide ribonucleic acid comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to any one of SEQ ID NOs: 645-649, 652-656, 660-671, 674-675, or 681-684, or a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a targeting sequence of any one of SEQ ID NOs: 645-649, 652-656, 660-671, 674-675, or 681-684. In some embodiments, introducing to said cell further comprises contacting said cell with a nucleic acid or vector encoding said fusion protein or said guide polynucleotide. or comprises contacting said cell with a lipid nanoparticle (LNP) comprising

said vector or nucleic acid. In some embodiments, introducing to said cell further comprises contacting said cell with a ribonucleoprotein complex (RNP) comprising said fusion protein or said guide polynucleotide or comprises contacting said cell with a lipid nanoparticle (LNP) comprising said RNP.

[0015] In some embodiments, the present disclosure provides for a method of disrupting an HAO-1 locus in a cell, comprising introducing to said cell: (a) any of the endonucleases described herein; 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 targeting sequence configured to hybridize to a region of said HAO-1 locus, wherein said engineered guide RNA is configured to hybridize to or comprises a targeting sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NO: 611-626 or 627-633. In some embodiments, the endonuclease is a class 2, type II Cas endonuclease. In some embodiments, said class 2, type II Cas endonuclease comprises any of the fusion or engineered endonucleases described herein. In some embodiments the endonuclease comprises any of the fusion or engineered endonucleases described herein. In some embodiments, said class 2, type II Cas endonuclease comprises a sequence having at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO:10 or a variant thereof. In some embodiments, said engineered guide RNA comprises a sequence with at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to non-degenerate nucleotides of SEQ ID NO: 722. In some embodiments, said engineered guide RNA comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any one of SEQ ID NOs: 618, 620, 624, or 626, or a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to a targeting sequence of any one of SEQ

ID NOs: 618, 620, 624, or 626. In some embodiments, said engineered guide RNA comprises the nucleotide sequence of any one of the guide RNAs from Table 9 or Table 12. In some embodiments, the cell is a mammalian cell. In some embodiments, introducing to said cell further comprises contacting said cell with a nucleic acid or vector encoding said fusion protein or said guide polynucleotide. or comprises contacting said cell with a lipid nanoparticle (LNP) comprising said vector or nucleic acid. In some embodiments, introducing to said cell further comprises contacting said cell with a ribonucleoprotein complex (RNP) comprising said fusion protein or said guide polynucleotide or comprises contacting said cell with a lipid nanoparticle (LNP) comprising said RNP.

[0016] In some aspects, the present disclosure provides for a method of disrupting a TRAC locus in a cell, comprising introducing to said cell: (a) any of the endonucleases described herein; 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 targeting sequence configured to hybridize to a region of said TRAC locus, wherein said engineered guide RNA is configured to hybridize to or comprises a targeting sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NOs: 139-158; or wherein said engineered guide RNA comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to any one of SEQ ID NOs: 119-138. In some embodiments, the endonuclease is a class 2, type II Cas endonuclease. In some embodiments, said class 2, type II Cas endonuclease comprises any of the fusion or engineered endonucleases described herein. In some embodiments the endonuclease comprises any of the fusion or engineered endonucleases described herein. In some embodiments, said class 2, type II Cas endonuclease comprises any of the fusion endonucleases described herein. In some embodiments, said class 2, type II Cas endonuclease comprises the fusion endonuclease having at least 55% identity to SEQ ID NO:10 or a variant thereof. In some embodiments, said engineered guide RNA comprises a sequence with at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to non-degenerate nucleotides of SEQ ID NO: 722. In some embodiments, said engineered guide RNA comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at

least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to any one of SEQ ID NOs: 121, 132, 136, 130, 134, 135, or 137, or a sequence having at least 80% identity to a targeting sequence of any one of SEQ ID NOs: 121, 132, 136, 130, 134, 135, or 137. In some embodiments, said engineered guide RNA comprises a nucleotide sequence of any one of the guide RNAs from Table 7A. In some embodiments, introducing to said cell further comprises contacting said cell with a nucleic acid or vector encoding said fusion protein or said guide polynucleotide. or comprises contacting said cell with a lipid nanoparticle (LNP) comprising said vector or nucleic acid. In some embodiments, introducing to said cell further comprises contacting said cell with a ribonucleoprotein complex (RNP) comprising said fusion protein or said guide polynucleotide or comprises contacting said cell with a lipid nanoparticle (LNP) comprising said RNP.

[0017] In some embodiments, the present disclosure provides for a method of disrupting a B2M locus in a cell, comprising introducing to said cell: (a) any of the endonucleases described herein; 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 targeting sequence configured to hybridize to a region of said B2M locus, wherein said engineered guide RNA is configured to hybridize to or comprises a targeting sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NOs: 185-210; or wherein said engineered guide RNA comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to any one of SEQ ID NOs: 159-184. In some embodiments, the endonuclease is a class 2, type II Cas endonuclease. In some embodiments, said class 2, type II Cas endonuclease comprises any of the fusion or engineered endonucleases described herein. In some embodiments the endonuclease comprises any of the fusion or engineered endonucleases described herein. In some embodiments, said class 2, type II Cas endonuclease comprises any of the fusion endonucleases described herein. In some embodiments, said class 2, type II Cas endonuclease comprises a fusion endonuclease comprising a sequence having at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity

to SEQ ID NO: 10 or a variant thereof. In some embodiments, said engineered guide RNA comprises a sequence with at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the non-degenerate nucleotides of SEQ ID NO: 722. In some embodiments, said engineered guide RNA comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to any one of SEQ ID NOs: 159, 165, 168, 174, or 184, or a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a targeting sequence of any one of SEQ ID NOs: 159, 165, 168, 174, or 184. In some embodiments, said engineered guide RNA comprises a nucleotide sequence of any one of the guide RNAs from Table 7B. In some embodiments, introducing to said cell further comprises contacting said cell with a nucleic acid or vector encoding said fusion protein or said guide polynucleotide. or comprises contacting said cell with a lipid nanoparticle (LNP) comprising said vector or nucleic acid. In some embodiments, introducing to said cell further comprises contacting said cell with a ribonucleoprotein complex (RNP) comprising said fusion protein or said guide polynucleotide or comprises contacting said cell with a lipid nanoparticle (LNP) comprising said RNP.

[0018] In some aspects, the present disclosure provides for a method of disrupting a TRBC1 locus in a cell, comprising introducing to said cell: (a) any of the endonucleases described herein; 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 targeting sequence configured to hybridize to a region of said TRBC1 locus, wherein said engineered guide RNA is configured to hybridize to or comprises a targeting sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NOs: 252-292; or wherein the engineered guide RNA comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to any one of SEQ ID NOs: 211-251. In some embodiments, the endonuclease is a class 2, type II Cas endonuclease.

In some embodiments, said class 2, type II Cas endonuclease comprises any of the fusion or engineered endonucleases described herein. In some embodiments the endonuclease comprises any of the fusion or engineered endonucleases described herein. In some embodiments, said class 2, type II Cas endonuclease comprises any of the fusion endonucleases described herein. In some embodiments, said class 2, type II Cas endonuclease comprises a fusion endonuclease comprising a sequence having at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO:10 or a variant thereof. In some embodiments, said engineered guide RNA comprises a sequence with at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the non-degenerate nucleotides of SEQ ID NO: 722. In some embodiments, said engineered guide RNA is comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 211, 212, 215, 241, or 242, or comprises a targeting sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a targeting sequence of any one of SEQ ID NOs: 211, 212, 215, 241, or 242. In some embodiments, said engineered guide RNA comprises a nucleotide sequence of any one of the guide RNAs from Table 7C. In some embodiments, introducing to said cell further comprises contacting said cell with a nucleic acid or vector encoding said fusion protein or said guide polynucleotide. or comprises contacting said cell with a lipid nanoparticle (LNP) comprising said vector or nucleic acid. In some embodiments, introducing to said cell further comprises contacting said cell with a ribonucleoprotein complex (RNP) comprising said fusion protein or said guide polynucleotide or comprises contacting said cell with a lipid nanoparticle (LNP) comprising said RNP.

[0019] In some aspects, the present disclosure provides for a method of disrupting a TRBC2 locus in a cell, comprising introducing to said cell: (a) any of the endonucleases described herein; 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 targeting sequence configured to hybridize to a region of said TRBC2 locus, wherein said engineered guide RNA is configured to hybridize to or comprises a targeting sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at

least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NOs: 338-382; or wherein said engineered guide RNA comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to any one of SEQ ID NOs: 293-337. In some embodiments, the endonuclease is a class 2, type II Cas endonuclease. In some embodiments, said class 2, type II Cas endonuclease comprises any of the fusion or engineered endonucleases described herein. In some embodiments the endonuclease comprises any of the fusion or engineered endonucleases described herein. In some embodiments, the class 2, type II Cas endonuclease any of the fusion endonucleases described herein. In some embodiments, said class 2, type II Cas endonuclease comprises a fusion endonuclease comprising a sequence having at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO:10 or a variant thereof. In some embodiments, said engineered guide RNA comprises a sequence with at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the non-degenerate nucleotides of SEQ ID NO: 722. In some embodiments, said engineered guide RNA comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to any one of SEQ ID NOs: 296, 306, or 332, or comprises a targeting sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a targeting sequence of any one of SEQ ID Nos: 296, 306, or 332. In some embodiments, said engineered guide RNA comprises a nucleotide sequence of any one of the guide RNAs from Table 7C. In some embodiments, introducing to said cell further comprises contacting said cell with a nucleic acid or vector encoding said fusion protein or said guide polynucleotide. or comprises contacting said cell with a lipid nanoparticle (LNP) comprising said vector or nucleic acid. In some embodiments, introducing to said cell further comprises contacting said cell with a ribonucleoprotein complex

(RNP) comprising said fusion protein or said guide polynucleotide or comprises contacting said cell with a lipid nanoparticle (LNP) comprising said RNP.

[0020] In some aspects, the present disclosure provides for a method of disrupting an ANGPTL3 locus in a cell, comprising introducing to said cell: (a) any of the endonucleases described herein; 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 targeting sequence configured to hybridize to a region of said ANGPTL3 locus, wherein said engineered guide RNA is configured to hybridize to or comprises a targeting sequence having at least 80% identity to SEQ ID NOs: 478-572; or wherein said engineered guide RNA comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to any one of SEQ ID NOs: 383-477. In some embodiments, the endonuclease is a class 2, type II Cas endonuclease. In some embodiments, said class 2, type II Cas endonuclease comprises any of the fusion or engineered endonucleases described herein. In some embodiments the endonuclease comprises any of the fusion or engineered endonucleases described herein. In some embodiments, said class 2, type II Cas endonuclease comprises any of the fusion endonucleases described herein. In some embodiments, said class 2, type II Cas endonuclease comprises a fusion endonuclease having at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10 or a variant thereof. In some embodiments, said engineered guide RNA comprises a sequence with at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to a non-degenerate nucleotides of SEQ ID NO: 722. In some embodiments, said engineered guide RNA comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to any one of SEQ ID NOs: 419, 425, 431, 439, 447, 453, 461, 467, 471, or 473, or a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to any one of SEQ ID

NOs: 419, 425, 431, 439, 447, 453, 461, 467, 471, or 473. In some embodiments, said engineered guide RNA comprises a nucleotide sequence of any one of the guide RNAs from Table 7D. In some embodiments, introducing to said cell further comprises contacting said cell with a nucleic acid or vector encoding said fusion protein or said guide polynucleotide. or comprises contacting said cell with a lipid nanoparticle (LNP) comprising said vector or nucleic acid. In some embodiments, introducing to said cell further comprises contacting said cell with a ribonucleoprotein complex (RNP) comprising said fusion protein or said guide polynucleotide or comprises contacting said cell with a lipid nanoparticle (LNP) comprising said RNP.

[0021] In some aspects, the present disclosure provides for a method of disrupting a PCSK9 locus in a cell, comprising introducing to said cell: (a) any of the endonucleases described herein; 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 targeting sequence configured to hybridize to a region of said PCSK9 locus, wherein said engineered guide RNA is configured to hybridize to or comprises a targeting sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NOs: 588-602; or wherein said engineered guide RNA comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to any one of SEQ ID NOs: 573-587. In some embodiments, the endonuclease is a class 2, type II Cas endonuclease. In some embodiments, said class 2, type II Cas endonuclease comprises any of the fusion or engineered endonucleases described herein. In some embodiments the endonuclease comprises any of the fusion or engineered endonucleases described herein. In some embodiments, said class 2, type II Cas endonuclease comprises any of the fusion endonucleases described herein. In some embodiments, said class 2, type II Cas endonuclease comprises a fusion endonuclease comprising a sequence having at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10 or a variant thereof. In some embodiments, said engineered guide RNA comprises a sequence with at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at

least 99% sequence identity to the non-degenerate nucleotides of SEQ ID NO: 722. In some embodiments, said engineered guide comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to any one of SEQ ID NOs: 574, 578, 581, or 585. In some embodiments, said engineered guide RNA comprises a nucleotide sequence of any one of the guide RNAs from Table 7E. In some embodiments, introducing to said cell further comprises contacting said cell with a nucleic acid or vector encoding said fusion protein or said guide polynucleotide. or comprises contacting said cell with a lipid nanoparticle (LNP) comprising said vector or nucleic acid. In some embodiments, introducing to said cell further comprises contacting said cell with a ribonucleoprotein complex (RNP) comprising said fusion protein or said guide polynucleotide or comprises contacting said cell with a lipid nanoparticle (LNP) comprising said RNP.

[0022] In some embodiments, the present disclosure provides for a method of disrupting an albumin locus in a cell, comprising introducing to said cell: (a) any of the endonucleases described herein; 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 targeting sequence configured to hybridize to a region of said albumin locus, wherein said engineered guide RNA comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to any one of SEQ ID NOs: 67-86 or 646-695, or wherein said engineered guide RNA comprises a targeting sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a targeting sequence of any one of SEQ ID NOs: 67-86 or 646-695. In some embodiments, the endonuclease is a class 2, type II Cas endonuclease. In some embodiments, said class 2, type II Cas endonuclease comprises any of the fusion or engineered endonucleases described herein. In some embodiments the endonuclease comprises any of the fusion or engineered endonucleases described herein. In some embodiments, said class 2, type II Cas endonuclease comprises any of the type II Cas endonucleases described herein. In some embodiments, said class 2, type II Cas endonuclease comprises a fusion endonuclease having at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least

91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10 or a variant thereof. In some embodiments, said engineered guide RNA comprises a sequence with at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to non-degenerate nucleotides of SEQ ID NO: 722. In some embodiments, said engineered guide RNA is complementary to or comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to any one of SEQ ID NOs: 67, 68, 70, 71, 72, 76, 79, 80, 647, 648, 649, 653, 654, 655, 656, 673, 680, 681, or 682. In some embodiments, said engineered guide RNA comprises a nucleotide sequence of any one of the guide RNAs from Table 6. In some embodiments, introducing to said cell further comprises contacting said cell with a nucleic acid or vector encoding said fusion protein or said guide polynucleotide. or comprises contacting said cell with a lipid nanoparticle (LNP) comprising said vector or nucleic acid. In some embodiments, introducing to said cell further comprises contacting said cell with a ribonucleoprotein complex (RNP) comprising said fusion protein or said guide polynucleotide or comprises contacting said cell with a lipid nanoparticle (LNP) comprising said RNP.

[0023] In some aspects, the present disclosure provides for an endonuclease comprising an engineered amino acid sequence having at least 55% sequence identity to any one of SEQ ID NOs: 1-27, 108, or 109-110.

[0024] In some aspects, the present disclosure provides an engineered nuclease system, comprising the endonuclease described herein, and an engineered guide ribonucleic structure configured to form a complex with the endonuclease comprising: a guide ribonucleic acid sequence configured to hybridize to a target deoxyribonucleic acid sequence; and a tracr ribonucleic acid sequence configured to bind to said endonuclease. In some embodiments, the endonuclease is derived from an uncultivated microorganism. 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 Cas13d endonuclease. In some embodiments, the endonuclease has less than 86% identity to a SpyCas9 endonuclease. In some embodiments, the system further comprises a source of MG^{2+}.

[0025] In some aspects, the present disclosure provides for an engineered nuclease comprising: (a) a class II, type II Cas enzyme RuvC and HNH domain having at least 55% sequence identity

to a RuvC and HNH domain of any one of SEQ ID NOs: 1-27, 108, or 109-110; and (b) a class II, type II Cas enzyme PAM-interacting (PI) domain having at least 55% sequence identity to a PAM-interacting (PI) domain any one of SEQ ID NOs: 1-27, 108, or 109-110. In some embodiments, (a) and (b) do not naturally occur together. In some embodiments, the class II, type II Cas enzyme is derived from an uncultivated microorganism. In some embodiments, the endonuclease has less than 86% identity to a SpyCas9 endonuclease. In some embodiments, the engineered nuclease comprises a sequence having at least 55% sequence identity to any one of SEQ ID NOs: 1-27.

[0026] In some aspects, the present disclosure provides for an engineered nuclease system, comprising: an endonuclease according to any of the aspects or embodiments described herein; and an engineered guide ribonucleic structure configured to form a complex with the endonuclease comprising: a guide ribonucleic acid sequence configured to hybridize to a target deoxyribonucleic acid sequence; and a tracr ribonucleic acid sequence configured to bind to the endonuclease. In some embodiments, the guide ribonucleic acid sequence comprises a sequence having at least 80% sequence identity to non-degenerate nucleotides of any one of SEQ ID NOs: 28-32 or 33-44, or a variant thereof. In some embodiments, the system further comprises a PAM sequence compatible with the nuclease adjacent to the target nucleic acid site. In some embodiments, the PAM sequence is located 3′ of the target deoxyribonucleic acid sequence. In some embodiments, the PAM sequence comprises any one of SEQ ID NOs:46-66.

[0027] In some embodiments, the present disclosure provides for an engineered single-molecule heterologous guide polynucleotide compatible with a class II, type II enzyme according to any of the aspects or embodiments described herein, wherein the heterologous guide polynucleotide comprises chemical modifications according to any one of SEQ ID NOs: 645-684.

[0028] In some aspects, the present disclosure provides for a method of targeting the albumin gene, comprising introducing a system according to any one of the aspects or embodiments described herein to a cell, wherein the guide ribonucleic acid sequence is configured to hybridize to a sequence comprising any one of SEQ ID NOs: 67-86.

[0029] In some aspects, the present disclosure provides for a method of targeting the HAO1 gene, comprising introducing a system according to any one of the aspects or embodiments described herein to a cell, wherein the guide ribonucleic acid sequence is configured to hybridize to any one of SEQ ID NOs: 611-633. In some embodiments, the guide ribonucleic acid sequence is configured to hybridize to any one of SEQ ID NOs: 615, 618, 620, 624, or 626. In some embodiments, the guide ribonucleic acid comprises a sequence according to any one of SEQ ID NOs:645-684. In some embodiments, the guide ribonucleic acid comprises a sequence according to any one of SEQ ID NOs: 645-649, 652-656, 660-671, 674-675, or 681-684.

[0030] In some aspects, the present disclosure provides cells comprising the endonucleases described herein. In some aspects, the present disclosure provides cells comprising any nucleic acid molecule described herein. In some aspects, the present disclosure provides cells comprising any engineered nuclease system described herein.

[0031] 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.

INCORPORATION BY REFERENCE

[0032] 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

[0033] 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:

[0034] FIG. 1A – 1B depicts the natural PAM specificities of various effectors described herein. **FIG. 1A** shows a phylogenetic tree of the various effectors described herein. **FIG. 1B** is a table of the PAM specificities of natural RNA guided CRISPR-associated endonucleases.

[0035] FIG. 2 demonstrates the concept of domain swapping between RNA guided CRISPR-associated nucleases.

[0036] FIGs. 3A and **3B** depict the alignment of multiple sequences to guide the determination of an optimal breakpoint. **FIG. 3A** shows SaCas9 and SpCas9 aligned to several proteins described herein and the terminal conserved residue (an alanine residue) of these sequences are identified as the proposed C-terminus of the swapped section. **FIG. 3B** depicts the C-terminal domain of a SaCas9 protein to be swapped spans of the RuvC-III, WED, TOPO, and CTD domains. The PAM Interaction domain is composed of the TOPO domain and the CTD domain. Active site residues (D10, E477, and H701 of RuvC domain and D556, D557, and N580 of the

NHN domain) are not included in the swapped C-terminal domain.

[0037] FIG. 4 depicts the screening of chimeras with an *in vitro* PAM enrichment assay when recombining MG3-6 with various C-terminal domains from closely and distantly related nucleases. sgRNAs from N-terminal parental domains were used for RNA guided nuclease activities.

[0038] FIG. 5A – 5B depicts PAM sequences (**FIG. 5A**) and Seq Logo depictions of PAM sequences (**FIG. 5B**) of functional chimeras described herein. Given the breakpoint swapping of predicted C-terminal domains of RuvC-III, WED, TOPO and CTD, chimeras were functional if recombined with closely related nucleases. The engineered chimeras tended to preserve PAM specificities from the natural protein's PAM interacting domains, even if the natural protein was not functional in the same experiment.

[0039] FIG. 6 shows the screening of chimeras with an *in vitro* PAM enrichment assay with chimeras recombining MG3-6 with various c-terminal domains from closely and distantly related nucleases. sgRNAs from C-terminal parental domains were used for RNA guided nuclease activities. Numbers in parentheses indicate sgRNA species. Using sgRNAs from C-terminal parental domains did not rescue activities.

[0040] FIG. 7 shows predicted structures of MG3-6 and MG15-1. The WED and PI domains of MG3-6 were swapped with those of MG15-1 counterparts to generate chimera 1 (C1). Alternatively, the PI domain of MG3-6 was swapped with MG15-1's counterpart to generate chimera 2 (C2).

[0041] FIG. 8A – 8B depicts an *in vitro* PAM enrichment assay and Sanger sequencing results for PAM specificities. C1: MG3-6+MG15-1(WP) and C2: MG3-6+MG15-1(P). The engineered chimeras tend to preserve PAM specificities from the natural proteins' PAM interacting domains. PAM enrichment assay was performed in triplicate. (**FIG. 8A**) shows an agarose gel depiction of the assay indicating that sequences were cleaved in the presence of the active enzymes and (**FIG 8B**) shows SeqLogo depictions of PAM sequences determined by the assay.

[0042] FIG. 9A – 9B depicts the activity of a chimera described herein in mammalian cells. mRNA codifying for the chimera was co-transfected with 20 different sgRNAs (see e.g. SEQ ID Nos: 67-86) into Hepa 1-6 cells. Editing was assessed by Sanger sequencing and Inference of CRISPR edits (ICE). **FIG. 9A** shows the editing efficiency of the tested guides. Two biological replicates are shown. **FIG. 9B** shows the indel profiles created by representative guides.

[0043] FIG. 10 depicts the results of a guide screen in Hepa1-6 cells; guides were delivered as mRNA and gRNA using lipofectamine Messenger Max.

[0044] FIG. 11A depicts the structural portion of the MG3-6/3-4 guide. **FIG. 11B** depicts the structural portion of the MG3-6 guide.

[0045] FIG. 12 depicts the activity of chemically modified MG3-6/3-4 guides in Hepa1-6 cells when delivered as mRNA and gRNA using lipofectamine Messenger Max.

[0046] FIG. 13 depicts the stability of chemically modified MG3-6/3-4 guides over 9 hours at 37 °C.

[0047] FIG. 14 depicts the stability of chemically modified MG3-6/3-4 guides over 21 hours at 37 °C.

[0048] FIG. 15A – 15B depicts the *in vitro* screening of Type V-A chimeras. **FIG. 15A** depicts the agarose gel of amplified cleavage products for each cleavage reaction. Positive enrichment is observed with the MG29-1+MG29-5 chimera, domain swap from the same family (numbers in parentheses indicate sgRNA species). **FIG. 15B** depicts Seqlogo depictions of PAMs for parent enzymes and the chimeras derived therefrom.

[0049] FIG. 16 depicts the gene-editing outcomes at the DNA level for TRAC in HEK293T cells.

[0050] FIG. 17 depicts the gene-editing outcomes at the DNA level for B2M in HEK293T cells.

[0051] FIG. 18 depicts the gene-editing outcomes at the DNA and phenotypic levels for TRAC in T cells.

[0052] FIG. 19 depicts the gene-editing outcomes at the DNA level for B2M in T cells.

[0053] FIG. 20 depicts the gene-editing outcomes at the phenotypic level for TRBC1 and TRBC2 in T cells.

[0054] FIG. 21 depicts the gene-editing outcomes at the DNA level for ANGPTL3 in Hep3B cells.

[0055] FIG. 22 depicts the gene-editing outcomes at the DNA level for PCSK9 in Hep3B cells.

[0056] FIG. 23 depicts genome editing at the HAO-1 locus by MG3-6/3-4 in wild type mice analyzed by next generation sequencing.

[0057] FIG. 24 depicts glycolate oxidase protein levels in the liver of mice treated with MG3-6/3-4 mRNA and guide RNA targeting the HAO-1 gene.

[0058] FIG. 25 depicts genome editing at the HAO-1 locus in wild type mice treated with MG3-6/3-4 mRNA and guide RNA 7 (G7) targeting HAO-1 with 4 different chemical modifications.

[0059] FIG. 26 depicts Western blot analysis of glycolate oxidase (GO) protein levels in the liver of mice at 11 days after treatment with LNP encapsulating MG3-6/3-4 mRNA and sgRNA 7 (G7) with 4 different chemical modifications.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

[0060] The Sequence Listing filed herewith provides example polynucleotide and polypeptide sequences for use in methods, compositions, and systems according to the disclosure. Below are

example descriptions of sequences therein.

MG3-6 Chimeras

[0061] SEQ ID NOs: 1-27 show the full-length peptide sequences of MG3-6 chimeric nucleases.

[0062] SEQ ID NO: 108 shows the nucleotide sequence of an MG3-6/3-4 nuclease containing 5′ UTR, NLS, CDS, NLS, 3′ UTR, and polyA tail.

[0063] SEQ ID NOs: 28-45 and 605-610 show the nucleotide sequences of sgRNAs engineered to function with an MG3-6 chimeric nuclease.

[0064] SEQ ID NOs: 46-59 show the natural PAM specificities of various effectors.

[0065] SEQ ID NOs: 60-66 show the PAM specificities of chimeric nucleases described herein.

[0066] SEQ ID NO: 603 shows the DNA coding sequence for MG3-6/3-4.

[0067] SEQ ID NO: 604 shows the protein sequence of the MG3-6/3-4 cassette coding sequence.

MG29-1 Chimeras

[0068] SEQ ID NOs: 109-110 show the full-length peptide sequences of MG29-1 chimeric nucleases.

[0069] SEQ ID NOs: 111-113 show the nucleotide sequences of sgRNAs engineered to function with an MG29-1 chimeric nuclease.

[0070] SEQ ID NOs: 114-116 show the natural PAM specificities of various effectors.

[0071] SEQ ID NO: 117 shows the PAM specificity of a chimeric nuclease described herein.

TRAC Targeting

[0072] SEQ ID NOs: 119-138 show the nucleotide sequences of sgRNAs engineered to function with an MG3-6/3-4 nuclease in order to target TRAC.

[0073] SEQ ID NOs: 139-158 show the DNA sequences of TRAC target sites.

B2M Targeting

[0074] SEQ ID NOs: 159-184 show the nucleotide sequences of sgRNAs engineered to function with an MG3-6/3-4 nuclease in order to target B2M.

[0075] SEQ ID NOs: 185-210 show the DNA sequences of B2M target sites.

TRBC1 Targeting

[0076] SEQ ID NOs: 211-251 show the nucleotide sequences of sgRNAs engineered to function with an MG3-6/3-4 nuclease in order to target TRBC1.

[0077] SEQ ID NOs: 252-292 show the DNA sequences of TRBC1 target sites.

TRBC2 Targeting

[0078] SEQ ID NOs: 293-337 show the nucleotide sequences of sgRNAs engineered to function with an MG3-6/3-4 nuclease in order to target TRBC2.

[0079] SEQ ID NOs: 338-382 show the DNA sequences of TRBC2 target sites.

ANGPTL3 Targeting

[0080] SEQ ID NOs: 383-477 show the nucleotide sequences of sgRNAs engineered to function with an MG3-6/3-4 nuclease in order to target ANGPTL3.

[0081] SEQ ID NOs: 478-572 show the DNA sequences of ANGPTL3 target sites.

PCSK9 Targeting

[0082] SEQ ID NOs: 573-587 show the nucleotide sequences of sgRNAs engineered to function with an MG3-6/3-4 nuclease in order to target PCSK9.

[0083] SEQ ID NOs: 588-602 show the DNA sequences of PCSK9 target sites.

DETAILED DESCRIPTION

[0084] 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.

[0085] 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).

[0086] 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 or the claims, such terms are intended to be inclusive in a manner similar to the term "comprising".

[0087] 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, e.g., 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.

[0088] 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).

[0089] 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, [αS]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-rodamine-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).

[0090] 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.

[0091] 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.

[0092] 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.

[0093] 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.

[0094] 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 comprise, in some instances, a TATA-box or a CAAT box.

[0095] 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.

[0096] 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.

[0097] 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.

[0098] 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.

[0099] 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.

[00100] 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 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.

[00101] 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.

[00102] 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 example tracrRNA sequence (e.g., a tracrRNA from S. pyogenes S. aureus, etc. or SEQ ID NOs: *_*). 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 example 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 example 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 example 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.

[00103] 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".

[00104] 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 (E) 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 hmmalign with default parameters.

[00105] 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 discontiguous 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).

[00106] As used herein, the term "Wedge" (WED) domain generally refers to a domain (e.g. present in a Cas protein) interacting primarily with repeat:anti-repeat duplex of the sgRNA and PAM duplex. A WED 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.

[00107] As used herein, the term "PAM interacting domain" or "PI domain" generally refers to a domain interacting with the protospacer-adjacent motif (PAM) external to the seed sequence in a region targeted by a Cas protein. Examples of PAM-interacting domains include, but are not limited to, Topoisomerase-homology (TOPO) domains and C-terminal domains (CTD) present in Cas proteins. A PAM interacting 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.

[00108] As used herein, the term "REC domain" generally refers to a domain (e.g. present in a Cas protein) comprising at least one of two segments (REC1 or REC2) that are alpha helical domains thought to contact the guide RNA. A REC 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 PF19501 for domain REC1).

[00109] As used herein, the term "BH domain" generally refers to a domain (e.g. present in a Cas protein) that is a bridge helix between NUC and REC lobes of a Type II Cas enzyme. A BH 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 PF16593 for domain BH).

[00110] 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).

[00111] 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%, or at least about 99% identity any one of the systems 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 systems described herein lack substitution of at least one of the conserved or functional residues described herein. In some embodiments, a functional variant of any of the systems described herein lacks substitution of all of the conserved or functional residues described herein.

[00112] Conservative substitution tables providing functionally similar amino acids are available from a variety of references (see, for example, 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:

a. Alanine (A), Glycine (G);
b. Aspartic acid (D), Glutamic acid (E);
c. Asparagine (N), Glutamine (Q);
d. Arginine (R), Lysine (K);

e. Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
f. Phenylalanine (F), Tyrosine (Y), Tryptophan (W);
g. Serine (S), Threonine (T); and
h. Cysteine (C), Methionine (M).

[00113] *Overview*

[00114] 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.

[00115] 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.

[00116] Class I CRISPR-Cas systems have large, multisubunit effector complexes, and comprise Types I, III, and IV.

[00117] 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.

[00118] 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).

[00119] 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.

[00120] Class II CRISPR-Cas systems generally have single-polypeptide multidomain nuclease effectors, and comprise Types II, V and VI.

[00121] 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 documented 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.

[00122] 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 documented 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.

[00123] Type VI CRIPSR-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, in some embodiments, 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.

[00124] Because of their simpler architecture, Class II CRISPR-Cas have been most widely adopted for engineering and development as designer nuclease/genome editing applications.

[00125] 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 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.

[00126] 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) .

[00127] *Engineered nucleases*

[00128] In some aspects, the present disclosure relates to the engineering of novel nucleic acid-guided nucleases and systems. In some embodiments, the engineered nucleases are functional in

prokaryotic or eukaryotic cells for *in vitro*, *in vivo* or *ex vivo* applications. In some embodiments, the present disclosure relates to the engineering and optimization of systems, methods and compositions used for genome engineering involving sequence targeting, such as genome perturbation or gene-editing, that relate to nucleic acid-guided nuclease systems and components thereof.

[00129] In some aspects, the present disclosure provides engineered nucleases which may include nucleic acid guided nucleases, chimeric nucleases, and nuclease fusions.

[00130] *Chimeric or fusion engineered nucleases*

[00131] Chimeric engineered nucleases as described herein may comprise one or more fragments or domains, and the fragments or domains may be of a nuclease, such as nucleic acid-guided nuclease, orthologs of organisms of genus, species, or other phylogenetic groups described herein. The fragments may be from nuclease orthologs of different species. A chimeric engineered nuclease may be comprised of fragments or domains from at least two different nucleases. A chimeric engineered nuclease may be comprised of fragments or domains from nucleases from at least two different species. A chimeric engineered nuclease may be comprised of fragments or domains from at least 2, 3, 4, 5, 6, 7, 8, 9, 10, or more different nucleases or nucleases from different species. In some embodiments, a chimeric engineered nuclease comprises more than one fragment or domain from one nuclease, wherein the more than one fragment or domain are separated by fragments or domains from a second nuclease. In some examples, a chimeric engineered nuclease comprises 2 fragments, each from a different protein or nuclease. In some examples, a chimeric engineered nuclease comprises 3 fragments, each from a different protein or nuclease. In some examples, a chimeric engineered nuclease comprises 4 fragments, each from a different protein or nuclease. In some examples, a chimeric engineered nuclease comprises 5 fragments, each from a different protein or nuclease. In some examples, a chimeric engineered nuclease comprises 3 fragments, wherein at least one fragment is from a different protein or nuclease. In some examples, a chimeric engineered nuclease comprises 4 fragments, wherein at least one fragment is from a different protein or nuclease. In some examples, a chimeric engineered nuclease comprises 5 fragments, wherein at least one fragment is from a different protein or nuclease.

[00132] Junctions between fragments or domains from different nucleases or species can occur in stretches of unstructured regions. Unstructured regions may include regions which are exposed within a protein structure or are not conserved within various nuclease orthologs.

[00133] *MG Chimeric Enzymes*

[00134] The CRISPR effectors described herein have natural PAM specificities (see **FIG. 1**). In one aspect, the present disclosure provides for the enablement of novel PAM specificity by

protein engineering. This enablement of novel PAM specificity may be achieved by the domain swapping of RNA guided CRISPR-associated nucleases (see **FIG. 2**). There may be an optimal breakpoint in the process of domain swapping and recombination. The optimal breakpoint may be guided by the alignment of multiple sequences described herein (see **FIG. 3**).

[00135] In some aspects, the present disclosure provides for a fusion endonuclease comprising: (a) an N-terminal sequence comprising RuvC, REC, or HNH domains of a Cas endonuclease having at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NO: 696 or a variant thereof; and (b) a C-terminal sequence comprising WED, TOPO, or CTD domains of a Cas endonuclease having at least 55% at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any one of SEQ ID NOs: 697-721 or variants thereof. In some embodiments the fusion endonuclease comprises RuvC, REC, and HNH domains in (a). In some embodiments, the fusion endonuclease comprises RuvC and HNH domains in (a). In some embodiments, the fusion endonuclease comprises WED, TOPO, and CTD domains in (b). In some embodiments, the N-terminal sequence and the C-terminal sequence do not naturally occur together in a same reading frame. In some embodiments, the N-terminal sequence and the C-terminal sequence are derived from different organisms. In some embodiments, the N-terminal sequence further comprises RuvC-I, BH, and RuvC-II domains. In some embodiments, the C-terminal sequence further comprises a PAM-interacting domain. In some embodiments, the fusion Cas endonuclease comprises a sequence having at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity sequence identity to any one of SEQ ID NOs: 1-27 or 108. In some embodiments, the fusion endonuclease is configured to bind to a PAM that is not nnRGGnT (SEQ ID NO: 53). In some embodiments, the fusion endonuclease is configured to bind to a PAM that comprises any one of SEQ ID NOs:46-52 or 54-66.

[00136] In some aspects, the present disclosure provides an endonuclease comprising an engineered nucleic acid sequence having at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at

least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any one of SEQ ID NOs: 1-27, 108, or 109-110. In one aspect, the present disclosure provides an endonuclease comprising an engineered nucleic acid sequence having at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any one of SEQ ID NOs: 8-12, 26-27, or 108. In one aspect, the present disclosure provides an engineered nuclease system, comprising: the endonuclease described herein; and an engineered guide ribonucleic structure configured to form a complex with the endonuclease comprising: a guide ribonucleic acid sequence configured to hybridize to a target deoxyribonucleic acid sequence and configured to bind to the endonuclease. In some embodiments, and the engineered guide ribonucleic acid sequence further comprises a tracr ribonucleic acid sequence. In some embodiments, the endonuclease is derived from an uncultivated microorganism. 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 Cas13d endonuclease. In some embodiments, the endonuclease has less than 86% identity to a SpyCas9 endonuclease. In some embodiments, the system further comprises a source of Mg^{2+}.

[00137] In some aspects, the present disclosure provides for an engineered nuclease system comprising: (a) any of the endonucleases described herein (e.g. a fusion endonuclease comprising: (a) an N-terminal sequence comprising RuvC, REC, or HNH domains of a Cas endonuclease having at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NO: 696 or a variant thereof; and (b) a C-terminal sequence comprising WED, TOPO, or CTD domains of a Cas endonuclease having at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any one of SEQ ID NOs: 697-721 or variants thereof; and (b) an engineered guide ribonucleic structure configured to form a complex with the endonuclease comprising: a guide ribonucleic acid configured to hybridize to a target deoxyribonucleic acid

sequence; wherein the guide ribonucleic acid sequence is configured to bind to the endonuclease. In some embodiments, the guide ribonucleic acid further comprises a tracr ribonucleic acid sequence. In some embodiments, the endonuclease is derived from an uncultivated microorganism. 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 Cas13d endonuclease. In some embodiments, the endonuclease has less than 86% identity to a SpyCas9 endonuclease. In some embodiments, the system further comprises a source of Mg^{2+}. In some embodiments, the endonuclease comprises a sequence having at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any one of SEQ ID NOs: 8-12, 26-27, or 108. In some embodiments, the guide ribonucleic acid sequence comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to non-degenerate nucleotides of any one of SEQ ID NOs: 33, 34, 44, 45, 78, 84, or 87.

[00138] 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 int 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.

Table A-Selected Sequences Disclosed Herein

Category	SEQ ID NO:	Description	Type	Organism	Other Information	Sequence
MG3 chimeric effectors	696	MG3-6 N-terminal fragment (1-742)	protein	artificial sequence		MSTDMKNYRIGVDVGDRSVGLAAIEFDDD GLPIQKLALVTFRHDGGLDPTKNKTPMSR KETRGIARRTMRMNRERKRRLRNLDNVLE NLGYSVPEGPEPETYEAWTSRALLASIKL ASADELNEHLVRAVRHMARHRGWANPWWS LDQLEKASQEPSETFEIILARARELFGEK VPANPTLGMLGALAANNEVLLRPRDEKKR KTGYVRGTPLMFAQVRQGDQLAELRRICE VQGIEDQYEALRLGVFDHKHPYVPKERVG KDPLNPSTNRTIRASLEFQEFRILDSVAN LRVRIGSRAKRELTEAEYDAAVEFLMDYA DKEQPSWADVAEKIGVPGNRLVAPVLEDV QQKTAPYDRSSAAFEKAMGKKTEARQWWE STDDDQLRSLLIAFLVDATNDTEEAAAEA GLSELYKSWPAEEREALSNIDFEKGRVAY SQETLSKLSEYMHEYRVGLHEARKAVFGV DDTWRPPLDKLEEPTGQPAVDRVLTILRR FVLDCERQWGRPRAITVEHTRTGLMGPTQ RQKILNEQKKNRADNERIRDELRESGVDN PSRAEVRRHLIVQEQECQCLYCGTMITTT TSELDHIVPRAGGGSSRRENLAAVCRACN AKKKRELFYAWAGPVKSQETIERVRQLKA FKDSKKAKMFKNQIRRLNQTEADEPIDER SLASTSYAAVAVRERLEQHFNEGLALDDK SRVVLDVYAGAVTRESRRAGGIDERILLR GERDKNRFDVRHHAVDA
MG1 chimeric effector	697	MG1-4 C-terminal fragment	protein	artificial sequence		ICISFSRDFKYDKEIKKDIIKGFNPEIVK NAIDKIMPYPYANDKPFKGNTKPLETIYG LRTYGDKSYITQRVELNSIDKKATKIKSI IDETIKNDLLNKLKENPTEQEWKLMLQNY IHPKKQTKVKKVMISVSEGEITKDSNNRE RMGEFVDFGTKGTQHQFKHSKRHKGQILY FNEKGVVEVMPVYSNIKTTDVKDKLQNMG CKLYNKGQMFYSGCLVDIPKPFKAGSKEY PAGRYQIKTIRSDKVAELEDACGNKISTN VKYLVPAEFKKVESK
MG1 chimeric effector	698	MG1-5 C-terminal fragment	protein	artificial sequence		MCICFAPTSNAKKALSRKNILPEEIAKNP ESDDARNFFAKYLAEVVPTKVAIKKPELE QTIYSKRVIGGRQTIVKKCNVRDLAYKGQ NPKYDFDTLTKRIKDIINPVSKRVIEDFA KTEPTEAEWEDWCKYEAAIPSKNGSPTRL LRVLCKTKDDAERFKDLSKDGCGAYRKSK SHKGQFIWKDNKGNYLVAPVYIYSSKQKV YAELKNNPKCMGICDFFKTGCLVKISNEV VDEKKNRLWLKAGFYNLNSIAKEKRVYLT DVNGQEHKKIPLQHLMNAGMKRVETNTI
MG1 chimeric effector	699	MG1-6 C-terminal fragment	protein	artificial sequence		MCLCFAPTGVDSRRAKLGEILPEKLRSEK AAREFFKSYLDKIMPVDVAPKKPRLEDGI YSKRIIGGKACMVKRNNLVDLAYKSGLKP VFDIPTLIKLVDKKEKGIINPQIRKMIGE FAATNPDESAWRKWCEEVRLPSKSGLGAR VLRVLVYYGEADEYKDLSKDGCGAYRKGD

Category	SEQ ID NO:	Description	Type	Organism	Other Information	Sequence
						GHKGQVVWESVDGKYYVEPVYVHASKAGV MAALNANPKKKRICGMFNSHCTVDVGDVY NDRGDFILPAGRYMVNTILTTGRCVLTNA DGEKRNPININYLMRAGMRRVELSEL
MG1 chimeric effector	700	MG1-7 C-terminal fragment	protein	artificial sequence		MCLCFAPTGVNSKRARVDMLLPPKIRSEK EAELFFRKYLDKLIPVDVAPKKPKLEDGI YSMRTVGGKKIMARRVNLVDLAYKSGLKP VYDVSVLIKLLDKKERGIINPQIRKLVAD FARTNPSEDEWKKWCGECRLPSKNGLGTR VIRVLLNYGEPAEYKDLSKDGRGAFRRGD GHKGQIVWESTDGKYCVLPIYVHASKAKL LAELCANPKKKRICGIFTSHCMVKVGNTY NNKGELLLPEGVYMLNTIRTDGWIQLTSA NGDKSKPININYLMKAGMKKVPVKDL
MG2 chimeric effector	701	MG2-4 C-terminal fragment	protein	artificial sequence		LTLGLATALVPGIERKELRRALSLRQAKG DDATLLRSDPKLGEALRWRTEDRFEAAPL SGKLESAVRRALAEGRVVQHVPAKRQGMK VDSNFFGFVEFDETGRLRVRQKMRSPTTR RREIKTTVKNGKNLHTLSHLSLDPKSWLG APDHPLRRKQLEHGLRTENDLANPKLGNI RGMLPIRENWGIALITKDGSPRLDVIPYI NVHQWLEVLALENGGGSPVVLRKGHLVGF DAEKCPEEYCGAWMLLGVKDGRSGTTLEL IRPWMVAPRKGGTKESSAKQAIKPASGYS EKEGKASGVFLQRSADVFLKLGLRPLDHD LTGIAAF
MG2 chimeric effector	702	MG2-7 C-terminal fragment	protein	artificial sequence		VTQGLALLLFAPEDWPLLVKRNLPDSEQR HLKARYPFLDFSADKHISIQDLPEDTLHT ISERLAECRVVRHIPAKMHGIIVDQTTWG TVAAGAITTLRQKTTEKNARCDENGKRFI KTTEKKRSLLLGGPDAPDGKLAKIKGAIL VTENWGCALDPSPTVIPHFKVYPQLRALR EKNGGRPIRILRKGSLIQVKAGTYQGIWS VASIKDNADGICLDINAADKVKLENRSDD SKINVRLDSLRKSGLKILKPKLTGACPTT SSP
MG3 chimeric effector	703	MG3-1 C-terminal fragment	protein	artificial sequence		AVLTLQSPAIYRVLLTRVNLKHEHEVTGE APEWRDYEGADQAEKVLYRRWQKNIATLA ELMRQEIENNRVPVTRPIRLRKSRGAVHD ATVMKALERDLWGEWDAQAIDRLVDPELH LALRKLFTSTKSKKIDVDATSQGLPERYL ANQTVQLFDADAPSVMSPRGILRIGAGTH HARLLTWDDPKKGPQLGIQRVFAAEFGEI LKDASSNDLFEAPIPFHTMSHRDLQPKVR AAVEQGLTRQIGWITQGDELEIDPADFVG EANAFGNFLREFPERSWSIAGLKKSNTIV IRPLLLSQEGVTAAISPHAAKIVENGIEL SNSTLFTAPGTGIIRRTGLGRPRWDSGPA HLPESFNVHARMTQQSARD
MG3 chimeric effector	704	MG3-2 C-terminal fragment	protein	artificial sequence		AVLTLLDPSVAKTLAMRLDLKREQQDSGR DTRWKEFKGLTPASQERFIKWCQASECLA DMLRQQIEADRVPVVVPLRISPSNGAVHD DSVRPLTRQKIDSTWDRKSINRIVDPEIH VAMRRLLNNGTSLPEDKNRVLDLPDGNEL GPHDEVELFSTSAASIKLRRGGSAEIGGS IHHARVYAWMGAKGQLEYGMMRVFGAEFP TLTKLSGSKDILRMPIHAGSMSYRDMQDR

Category	SEQ ID NO:	Description	Type	Organism	Other Information	Sequence
						VRKPIESDIAVELGWITQGDELEILPEAH LETAGGLGDFLKSFPETQWTIDGFNDPSR LRVRPRLMSLEGRDTIDAMGHLSDTEKLK IKQALSKGLMVSASELLSHGAKIIRRDHL GRPRWRGNARPVSIELEQVANQLVNHRSV DGQ
MG3 chimeric effector	705	MG3-3 C-terminal fragment	protein	artificial sequence		AVMTLLNPSVAVTLEQRRMLKQENDYSSP RGQHDNGWRDFIGRGEASQSKFLHWKKTA VVLADLISEAIEQDTIPVVNPLRLRPQNG SVHKDTVEAVLERTVGDSWTDKQVSRIVD PNTYIAFLSLLGRKKELDADHQRLVSVSA GVKLLADERVQIFPEEAASILTPRGVVKI GDSIHHARLYGWKNQRGDIQVGMLRVFGA EFPWFMRESGVKDILRVPIPQGSQSYRDL AATTRKFIENGQATEFGWITQNDEIEISA EEYLATDKGDILSDFLGILPEIRWKVTGI EDNRRIRLRPLLLSSEAIPNMLNGRLLTQ EEHDLIALVINKGVRVVVSTFLALPSTKI IRRNNLGIPRWRGNGHLPTSLDIQRAATQ ALEGRD
MG3 chimeric effector	706	MG3-4 C-terminal fragment	protein	artificial sequence		AVMTLLNRSVALTLEQRSQLRRAFYELEL DKLDRDQLKPGEDWRNFTGLYEASQNKFS EWKKAATVLGDLLAEAIEDDAIAVVSPLR LRPQNGSVHDDTINAVKKLTLGSAWPADA VKRIVDPEIYLAMKDVLGKLKELPEDSAR SLELSDGRYIEADDEVLFFPKKAASILTP RGAAEIGNSIHHARLYSWLTKKGELKFGM LRVYGAEFPWLMRESGSRDVLHMPIHPGS QSFRGMQDGVRKAVESGEAVEFGWITQDD ELEFDPEDYIAHGGDDELNRLLRVMPERR WRVDGFYNAGTLRIRPALLSAEQLPSELQ KKVADKTLSDVELILLRAVQRGLFVAISS FLPLESLKVIRRNNLGFPRWRGNGNLPTS FEVRSSALRALGVEG
MG3 chimeric effector	707	MG3-7 C-terminal fragment	protein	artificial sequence		AVLTLLNRSVAVTLEQRRLIKQQREYSLE KSRRERDNVWRDFMGLGPAAQEKFAKWKK TAYVLADIIKEAISNDAIPVVSPLRLRPQ NGSVHLDTVDAVLERTIGDAWTVDQVHRI VNPQIYLAFAGYLGNQKALDPDSSRVLAL NDGRKLTAEDVIYVFPEKAASILTPRGVV KIGESVHHVRLYAWKNRKGKAEVGMLRVF GAEFPWLMRESGVKDVLRVPIHTGSQSYR DLSFTVRKNIEKGEAAEIGWLTQNEELEF NPESYLQEGGKDKLAKFLAFLPETRWRVD GFPMPDKLRIRPALLSREEIPEGVFRTEE QSLLEEALTKGLIIATKGLLSLPDVKVLR RNNLGIPRWRGGSYRPVSLDIQRAALAAL DEQE
MG3 chimeric effector	708	MG3-8 C-terminal fragment	protein	artificial sequence		AVMTLLNRSVALTLEQRSQLRRAFYEQGL DKLDRDQLKPEEDWRNFIGLSLASQEKFL EWKKVTTVLGDLLAEAIEDDSIAVVSPLR LRPQNGRVHKDTIAAVKKQTLGSAWSADA VKRIVDPEIYLAMKDALGKSKVLPEDSAR TLELSDGRYLEADDEVLFFPKNAASILTP RGVAEIGGSIHHARLYSWLTKKGELKIGM LRVYGAEFPWLMRESGSHDVLRMPIHPGS QSFRDMQDTTRKAVESSEAVEFAWITQND

Category	SEQ ID NO:	Description	Type	Organism	Other Information	Sequence
						ELEFEPEDYIAHGGKDELRQFLEFMPECR WRVDGFKKNYQIRIRPAMLSREQLPSDIQ RRLESKTLTENESLLLKALDTGLVVAIGG LLPLGTLKVIRRNNLGFPRWRGNGNLPTS FEVRSSALRALGVEG
MG4 chimeric effector	709	MG4-2 C-terminal fragment	protein	artificial sequence		VAIALTDPAALKSISQAASDERRGGRVSF GAVALPWVDFIGDVQAAIEAINVSHRPSR KVNGALHEETFYGPRGMDGDGRPTGYVQR KPVERLSAKEIPNIPDPAVREAVQAKLDE VGGTPAQAFKDPANHPVRKRGIPVHKVRL RLNINPVQVGSGATERHVLTGSNHHMEII EVRDAKGGKKWTGRLVHRLEAKRRALGRE TIVDRAVQAGRQFQFSLSPGDMIELTGED GERKLHVVRSISEGRIEYVDARDARKKAD IRASGDWRKPAVGSLLRLHCRKVVVTPFG EIRYAND
MG4 chimeric effector	710	MG4-5 C-terminal fragment	protein	artificial sequence		VVIALTGPGTVQALTRAALRAKELGRRLF VPLDPPWADRDSFLRDVRASVEAITVSYR VDRKVSGQLHEESNYSKPHMTVDNKGNLV EHRHIRKPLKDMSVEEVEAIVDDRVRKLV QEKLRQLGQEPKKAFADEANHPYFTTADG RLVPIHKARIRKTVATITVGPPQCPRHVA PGLNHHIEILAVRDPAGAVTHWEGELVSL FEAARRVKAGEPVVRRNHGPNKDFLFSLA KGEYVEMELQPGKRQLFRVTVISAKQIEF RLHHDARPTMLLRKTPGARVIRSPGSLFK AKARKVAVDPLGNVFPAND
MG6 chimeric effector	711	MG6-3 C-terminal fragment	protein	artificial sequence		IVVAFTNRSTLKRLSDENKRIGTAEWMDA DESGRATNDEIKRRLGGRIDLSEPWPTFR NDVEVSINNITVSHRVNRKVSGALHEETY YGPTDEPAPKNKEMMVLRKSVHQLSKKDL GLIRDETIRQIVNDEVQKRMDNGESQANA IASLEADPPFIISPKAKVPIRKVRLLMKK DPQIMHYFENKNGEEDRAALYGNNHHIAI YETSDKNGVKKQIGIVIPMMEAARRVKDG DPIVMKDYRPDHTFLYSLAKNDMIFNHED EQIYRVQKINSDGTIMFRQNNVAMKGQSD PGVYFKSGSRLGASKIKISPIGEIFPAND
MG14 chimeric effector	712	MG14-1 C-terminal fragment	protein	artificial sequence		CVIAACSPSLVIKTARINQETHWSITRGM NETQRRDAIMKALESVMPWETFANEVRAA HDFVVPTRFVPRKGKGELFEQTVYRYAGV NAQGKDIARKASSDKDIVMGNAVVSADEK SVIKVSEMLCLRLWHDPEAKKGQGAWYAD PVYKADIPALKDGTYVPRIAKAHTGRKAW KPVPESAMAKPPLEIYFGDLVQIGDFIGR FSGYNINNANWSFTDRLTRLNLSCPTVGQ LNNDLSPVVIRESPIK
MG15 chimeric effector	713	MG15-1 C-terminal fragment	protein	artificial sequence		VIIACATQGIVNKVSRYSKSRELWDYEVD METGEVLQKKNKNTKDVFPEPWLNFRYEL EQKVRVRPLDIPETADITEMEEPFVSHMP NRKIHGPAHKETIRSGRLKEEGYTISKTA LIDLKLTEDKEEIKGYYNKESDRLLYEAL KKQLQRYGGKAKEAFKEPFHKPKADGTPG PIVNKVKIMEKSTMLIPVNGGKGLASNGN MVRIDVFRAEEKGKKKYYFIPVYVADTVK EELPNRAVLAHKPYEAWKIMKEENFIFSL YPNDLIFVDAGKEIPFKAALKGSTLDPEK

Category	SEQ ID NO:	Description	Type	Organism	Other Information	Sequence
						KASRFLMYYKGADIATGSISGVNHDETYK ARGVGIQSLREIKKCCIDVLGNISFASKE KRQTFR
MG16 chimeric effector	714	MG16-1 C-terminal fragment	protein	artificial sequence		LTVALTRQSYIQRLNTLEASHEHMEKLVK EANTPYKEKKSLLEKWVALQPHFSVEEVT TQVDGILVSFRAGKRVTTPARRAVYHGGK RTIVQRGIQVPRGALTEDTIYGKLGDKFV VKYALDHPSMKPENIVDPTIRLLVENRIT ALGKKDAFKTPLYSAEGMEIKSVRCYTSL SEKGVVPIKYNEKGNAIGFAKKGNNHHVA IYKDQSGQYQEMVVSFWDAVERKLYGVPT VITNPKTVWDELLEKELPQDFLEKLPKDN WQYVLSMQENEMFVLGMEEDEFNDAIDTQ DYNTLNKHLYRVQKLSHADYTFRFHTETK VDDKYDGVENGRNTSMSLKALVRIRSFNG LFTQFPHKVKIDIMGRITKA
MG16 chimeric effector	715	MG16-2 C-terminal fragment	protein	artificial sequence		LVVACTKQSYIQRLNNLNTERDAMYQDIE AQSVEWKEKHSLLEKWIKLQPHPTVSEVT DKVDEILVSFKAGKRVATLGKRSVYKNGK KTVVQNNIIVPRGALCEESVYGQINLIEK NKPIKYLFENPSLIFKPYIKALVEERLKE YNGDTSKAISSLKNNPIYLRKDKSVVLEY GTCYKKEYVKKYSLNSIKAKDVDSIIDKH IREVVRQRLEDNNNNEKAAFASPLYADKQ KQIPIKSVRCTTGINIAAPVNYNESNDPI SFVKPGNNHHIAIYKDKDGKRQEHIVTFW HAVERKKYGMPVVITNPKEIWDLIIEKSL DLPESFLNCLPNSDWNYEISMQQNEMFVM GMSEDEFQDAIRNNDYKTLNKYLYRVQSV SESDYWLRLHIETMNDKTPEGNIIKKYYR IKSINTFFNFNPHKVKITLLGEIQSS
MG18 chimeric effector	716	MG18-1 C-terminal fragment	protein	artificial sequence		YLNAVVGNVYHEKFTKNPLRFVRSGQEYS LNLSALFQNWNIYKGGRVIWQKGEDGSLE TVRARMAKNDPMVTRYCTEGRGALYDLQP MKKSKGQLPLKSSDERLQHIDRYGGYNKL AGAYFTLAAYYKKGKRVKSIESVPLYLAA KLQRDPAALQQYLADQLGTDRVEILVPEI KLGTLFKWNGYPMTLSGRTGPQLLFRNAA ELRTNAEQEQYIKKMSRYLEKCKGRKEPL PIRPAYDKLTPEENLQLYDAFTQWLTSGI YAKRLSLQGKFLLEKRDAFAALSPEAQVR QLMEILHLFQCNPVAANLSELGGAAHAGI LLASKNIDGKVPVSIVHQSVTGYFTQEVC LNDL
MG21 chimeric effector	717	MG21-1 C-terminal fragment	protein	artificial sequence		AVIACITPGMIQKITKYAQNHERFYATAK GYVDIETGEVLTRSEYEAMDDIRFPEPWP GFRSELEARVSEHPQEAIARLKLPHYENS EEIRPIFVSRMPNHKVTGAAHLETIRSKK GGAGSTVTKTALPDLKLDKNGEIAGYYRK EDDPLLYEALKARLKAFGGDGKKAFAEPF HKPKHNGEPGPIVKKVKIQESATLTVPVN HGIAANGSMVRLDVFHVDGDGYYFVPIYT SDTVKPELPNRAVVAGRRVQEWKVMDDSY FKFSLYPKDLIRIRSKKGIKLKAVNRNAD LQEYSTNDCLCYFVKFNISTGALSVENHD RKFEQPGLGGKTLLSIEKYQVDVLGNYSP VALPEKRMKFR

Category	SEQ ID NO:	Description	Type	Organism	Other Information	Sequence
MG22 chimeric effector	718	MG22-1 C-terminal fragment	protein	artificial sequence		IAIACINRSIVNYLNNAAANQTEREDLRR AVCIPERNGQTKRQLRSPWHCFARDAENA LRQIVVSFKQNLRVATKATNSYECFDTAS GKKIRKHQSNREHYAIRKPLHKDSVYGEV ILTSIASVNLKKALLKAERILDKRLKEKI FELRKLYNYSNKQIEEHLTKVCINCPEWK NYDFKKIAVRILSNDADATHIVAIRKPLD ESFDEVKINTITDTGIQKILLNHLSRYAD DPKKAFSPEGIEDMNANIASLNGGKQHLP IYKVRVSEKDNGGYFPIGQKGNRPKKYVT TAKDTNLFFAVYADSKGKRSYKTIDLRTA IECRKQGLSVAPSINEKGDKLLFTLSPND LVYMPSEGEEANGFAIDNNLNKDQIYKMV SANNKQCFFIPHTVADFISRGEEYNSHNK IELTEDRRSIKEHCVPLKVNRLGK
MG23 chimeric effector	719	MG23-1 C-terminal fragment	protein	artificial sequence		YLNIVVGNTYSTKFTNNPLNFIKAGAKRP QDNQFKYNMDKIFDYNVISRGERAWIAGS DGSICTVKKFMSRNTVLITRKAKEVHGAL SNKATIWGKNVAKPGAYLPVKSTDLKAQD VTKYGGITSIANSGYTLAEYKVNGKTTRS LEALPVYLGRAEQLTEKTVVDYLSSSLQE SSKKKIEDIQVRKLFIPQGSKVKIDGFCY YLGGKTGDSIYLNNAVPLYLSSTSEEYLR KLLKAVENNNYNERDKNGQIILTAPKNVQ LLSSIFDKLRSKPFSNNKWNIYFSIVNGK ETKVEQLFSKLSIDKQAEVISQIVIWINS SRQNVNLSLIGGSAHSGTQALSKTVSRLN ECMLISQSITGIYEHSVDLLTI
SaCas chimeric effector	720	SaCas9 C-terminal fragment	protein	artificial sequence		LIIANADFIFKEWKKLDKAKKVMENQMFE EKQAESMPEIETEQEYKEIFITPHQIKHI KDFKDYKYSHRVDKKPNRELINDTLYSTR KDDKGNTLIVNNLNGLYDKDNDKLKKLIN KSPEKLLMYHHDPQTYQKLKLIMEQYGDE KNPLYKYYEETGNYLTKYSKKDNGPVIKK IKYYGNKLNAHLDITDDYPNSRNKVVKLS LKPYRFDVYLDNGVYKFVTVKNLDVIKKE NYYEVNSKCYEEAKKLKKISNQAEFIASF YNNDLIKINGELYRVIGVNNDLLNRIEVN MIDITYREYLENMNDKRPPRIIKTIASKT QSIKKYSTDILGNLYEVKSKKHPQIIKKG
SpCas chimeric effector	721	SpCas9 C-terminal fragment	protein	artificial sequence		YLNAVVGTALIKKYPKLESEFVYGDYKVY DVRKMIAKSEQEIGKATAKYFFYSNIMNF FKTEITLANGEIRKRPLIETNGETGEIVW DKGRDFATVRKVLSMPQVNIVKKTEVQTG GFSKESILPKRNSDKLIARKKDWDPKKYG GFDSPTVAYSVLVVAKVEKGKSKKLKSVK ELLGITIMERSSFEKNPIDFLEAKGYKEV KKDLIIKLPKYSLFELENGRKRMLASAGE LQKGNELALPSKYVNFLYLASHYEKLKGS PEDNEQKQLFVEQHKHYLDEIIEQISEFS KRVILADANLDKVLSAYNKHRDKPIREQA ENIIHLFTLTNLGAPAAFKYFDTTIDRKR YTSTKEVLDATLIHQSITGLYETRIDLSQ LGGD
MG3-6_3-4 guide	722	MG3-6_3-4 guide	Nucleotide			NNNNNNNNNNNNNNNNNNNNNGTTGAGA ATCGAAAGATTCTTAATAAGGCATCCTTC

Category	SEQ ID NO:	Description	Type	Organism	Other Information	Sequence
sgRNA scaffold		sequence scaffold	(RNA)			CGATGCTGACTTCTCACCGTCCGTTTTCC AATAGGAGCGGGCGGTATGTTTT

EXAMPLES

Example 1 – Plasmids

[00139] Chimera sequences were codon optimized for E. coli expression via Integrated DNA Technologies (IDT) website, and synthesized and cloned into pET21 vector at Twist Bioscience unless otherwise specified. To construct pET21-MG3-6+MG15-1(WP) and pET21-MG3-6+MG15-1(P), gene fragments were amplified from pMGX3-6 and pMGX15-1 using primers P441-P446. The resulting PCR products were purified by Zymo Gel DNA Recovery Kit and assembled into pAL3 (digested by ClaI and XhoI) via NEBUilder HiFi DNA assembly. DNA sequences of cloned chimeric genes were confirmed by Sanger sequencing service offered by Elim Biopharm.

Example 2 – Bioinformatic analysis

[00140] CRISPR Type II endonucleases utilized herein were predicted to have nuclease activity based on the presence of putative HNH and RuvC catalytic residues. In addition, structural predictions suggested residues involved in guide, target, and recognition of and interaction with a PAM. Based on the location of important residues, the predicted domain architecture of Type II CRISPR endonucleases comprised three RuvC domains, an HNH endonuclease domain, a recognition domain and PAM interacting domain, among others. For genomic sequences encoding a full-length Type II endonuclease next to a CRISPR array, we predicted tracrRNA sequences, which were engineered to be used by the nuclease as single guide RNAs.

[00141] A multiple sequence alignment of selected RNA guided CRISPR Type II endonuclease sequences were performed using the built-in MUSCLE aligner on Geneious Primer Software (available at https://www.geneious.com/prime) (see **FIG. 3**). Protein structures of MG3-6 and MG15-1 were predicted with DNASTAR NovaFold and displayed via Protean 3D. Details of chimeric compositions are shown in Table 1. Guided by predicted structural model information along with guide RNA optimization (see **FIG. 7**), we engineered protein variants recognizing non-canonical PAMs by concatenating domains from closely, as well as distantly related Type II CRISPR endonucleases.

Table 1 – Chimeric Compositions

Chimera	N-terminus	C-terminus	Example Sequence (SEQ ID NO:)
MG3-6+MG1-4	MG3-6 (1-742)	MG1-4 (750-1025)	1
MG3-6+MG1-5	MG3-6 (1-742)	MG1-5 (789-1077)	2
MG3-6+MG1-6	MG3-6 (1-742)	MG1-6 (773-1059)	3
MG3-6+MG1-7	MG3-6 (1-742)	MG1-7 (775-1061)	4
MG3-6+MG2-4	MG3-6 (1-742)	MG2-4 (876-1201)	5
MG3-6+MG2-7	MG3-6 (1-742)	MG2-7 (817-1080)	6
MG3-6+MG3-1	MG3-6 (1-742)	MG3-1 (684-1050)	7
MG3-6+MG3-2	MG3-6 (1-742)	MG3-2 (755-1134)	8
MG3-6+MG3-3	MG3-6 (1-742)	MG3-3 (750-1132)	9
MG3-6+MG3-4	MG3-6 (1-742)	MG3-4 (743-1134)	10
MG3-6+MG3-7	MG3-6 (1-742)	MG3-7 (751-1131)	11
MG3-6+MG3-8	MG3-6 (1-742)	MG3-8 (741-1132)	12
MG3-6+MG4-2	MG3-6 (1-742)	MG4-2 (747-1043)	13
MG3-6+MG4-5	MG3-6 (1-742)	MG4-5 (747-1055)	14
MG3-6+MG6-3	MG3-6 (1-742)	MG6-3 (709-1027)	15
MG3-6+MG14-1	MG3-6 (1-742)	MG14-1 (756-1003)	16
MG3-6+MG15-1	MG3-6 (1-742)	MG15-1 (729-1082)	17
MG3-6+MG16-1	MG3-6 (1-742)	MG16-1 (787-1154)	18
MG3-6+MG16-2	MG3-6 (1-742)	MG16-2 (796-1227)	19
MG3-6+MG18-1	MG3-6 (1-742)	MG18-1 (997-1348)	20
MG3-6+MG21-1	MG3-6 (1-742)	MG21-1 (740-1098)	21
MG3-6+MG22-1	MG3-6 (1-742)	MG22-1 (1092-1521)	22
MG3-6+MG23-1	MG3-6 (1-742)	MG23-1 (1008-1377)	23
MG3-6+SaCas9	MG3-6 (1-742)	SaCas9 (706-1053)	24
MG3-6+SpCas9	MG3-6 (1-742)	SpCas9 (988-1368)	25
MG29-1+MG29-5 (WP)	MG29-1 (1-560)	MG29-5 (556-856)	109
MG3-6+MG15-1(WP)	MG3-6 (1-840)	MG15-1 (818-1082)	26

Chimera	N-terminus	C-terminus	Example Sequence (SEQ ID NO:)
MG3-6+MG15-1(P)	MG3-6 (1-922)	MG15-1 (931-1082)	27
MG29-1+MG57-1 (WP)	MG29-1 (1-560)	MG57-1 (633-945)	110

Example 3 – *In vitro* PAM enrichment assay

[00142] The PAM sequences of nucleases utilized herein were determined via expression in either an E. coli lysate-based expression system or reconstituted *in vitro* translation (myTXTL, Arbor Biosciences or PURExpress, New England Biolabs). The E. coli codon optimized protein sequence was transcribed and translated from a PCR fragment under control of a T7 promoter. This mixture was diluted into a reaction buffer (10 mM Tris pH 7.5, 100 mM NaCl, 10 mM $MgCl_2$) with protein-specific sgRNA and a PAM plasmid library (PAM library U67/U40). The library of plasmids contained a spacer sequence matching that in the single guide followed by 8N mixed bases, a subset of which were presumed to have the correct PAM. After 1-3 h, the reaction was stopped and the DNA was recovered via a DNA clean-up kit, e.g. Zymo DCC, AMPure XP beads, QiaQuick etc. The DNA was subjected to a blunt-end ligation reaction which added adapter sequences to cleaved library plasmids while leaving intact circular plasmids unchanged. A PCR was performed with primers (LA065 and LA125) specific to the library and the adapter sequence and resolved on a gel to identify active protein complexes (see **FIG. 4** and **FIG. 6**). The resulting PCR products were further amplified by PCR using high throughput sequencing primers (TrueSeq) and KAPA HiFi HotStart with a cycling parameter of 8. Samples subjected to NGS analysis were quantified by 4200 TapeStation (Agilent Technologies) and pooled together. The NGS library was purified via AMPure XP beads and quantified with KAPA Library Quant Kit (Illumina) kit using AriaMx Real-Time PCR System (Agilent Technologies). Sequencing this library, which was a subset of the starting 8N library, revealed the sequences which contain the correct PAM (see **FIG. 5**).

Example 4 – Single guide design for *in vivo* targeting

[00143] The single guide (sgRNA) structures used herein comprised a structure of: 5′ -- 22nt protospacer- repeat – tracr -- 3′. 20 single guides targeting mouse albumin intron 1 were designed using Geneious Prime Software (https://www.geneious.com/prime/). In some instances, guides were chemically synthesized by IDT and included a chemical modification of the guide that had been optimized by IDT to improve the performance of Cas9 guides ("Alt-R" modifications).

Example 5 – *In vitro* transcription of mRNA

[00144] The coding sequences (CDS) encoding the chimeras (e.g. MG3-6+MG3-4 (SEQ ID NO: 10)) were codon-optimized for mouse and chemically synthesized at Twist biosciences. The CDS were cloned into mRNA production vector pMG010. The architecture of pMG010 comprised the sequence of elements: T7 promotor - 5′UTR – start codon – nuclear localization signal 1 – CDS – nuclear localization signal 2 – stop codon – 3′ UTR – 107 nucleotide polyA tail (SEQ ID NO: 108). A plasmid pMG010 containing the MG3-6+MG 3-4 CDS was purified from a 200 ml bacterial culture using an EndoFree Plasmid Kit (Qiagen). The vector was digested with SapI overnight in order to linearize the plasmid downstream of the polyA tail. The linearized vector was purified using phenol/chloroform DNA extraction. *In vitro* transcription was carried out using HiT7 T7 RNA polymerase (New England Biolabs) at 50°C for 1 h. *In vitro* transcribed mRNA was treated with DNase for 10 min at 37°C, and the mRNA was purified using the MEGAclear Transcription Clean-up kit (Thermo Fisher). mRNA was quantified by absorbance at 260 nm and its size and purity was assessed by automated electrophoresis (TapeStation, Agilent) and demonstrated to be of the expected size.

Example 6 – Transfection of Hepa1-6 cells and Albumin targeting

[00145] 300ng of mRNA and 350ng of each single guide RNA (sgRNA) of SEQ ID NOs: 67-86 were co-transfected into Hepa1-6 cells as follows. One Day before transfection Hepa1-6 cells were seeded into 24 wells at a density to achieve 70% confluency 24 h later. The following day 25 µl of OptiMEM media and 1.25µl of Lipofectamine Messenger Max Solution (Thermo Fisher) were mixed and vortexed for 5 s to make solution A. In a separate tube 300 ng of the MG3-6+MG3-4 chimera mRNA and 350 ng of a single guide were mixed together with 25 µl of OptiMEM to make Solution B. Solution A and B were mixed and incubated for 10 min at room temperature then added directly to the Hepa1-6 cells. Two days post transfection the media was aspirated, and genomic DNA was purified following the instructions from Purelink Genomic DNA mini kit (Thermo Fisher) (see **FIG. 9**). The results indicate that the best performing sgRNAs were those designated g87 (SEQ ID NO:72) and g34 (SEQ ID NO: 70), with appreciable editing occurring also for gRNAs g45 (SEQ ID NO: 67), g44 (SEQ ID NO: 71), g59 (SEQ ID NO: 76), g78 (SEQ ID NO: 68), g84 (SEQ ID NO: 79), and g33 (SEQ ID NO: 80).

Example 7 – Sanger sequencing of genome edited samples

[00146] Primers flanking the regions of the genome targeted by the single guide RNAs (e.g. the albumin gene) were designed. PCR amplification using primers 57F (SEQ ID NO: 97) and 1072R (SEQ ID NO: 98) was performed using Phusion Flash High-Fidelity PCR Master Mix

(Thermo Fisher) resulting in a PCR product of 1016 bp. PCR products were purified and concentrated using DNA clean & concentrator 5 (Zymo Research) and 100 ng of PCR product subjected to Sanger sequencing (ELIM Biosciences) using 8 pmoles of individual sequencing primers (132F, 282F, 446R, and 460F, SEQ ID NOs: 99-102). Sanger sequencing results were analyzed by using an algorithm called Inference of CRISPR edits (available at https://github.com/synthego-open/ice) and data was plotted using GradPrism (see **FIG. 9B**).

Example 8 – MG3-6/3-4 nuclease guide screen for mouse HAO-1 gene using mRNA transfection

[00147] Guide RNA for the MG3-6/3-4 nuclease targeting exons 1 to 4 of the mouse HAO-1 gene (encodes glycolate oxidase) were identified in silico by searching for the PAM sequence 3′ NNAAA(A/T)N 5′. A total of 23 guides with the fewest predicted off-target sites in the mouse genome were chemically synthesized as single guide RNAs. 300ng mRNA and 120ng single guide RNA were transfected into Hepa1-6 cells as follows. One day prior to transfection, Hepa1-6 cells that have been cultured for less than 10 days in DMEM, 10% FBS, 1xNEAA media, without Pen/Strep, 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 500µL. On the day of transfection, 25µL of OptiMEM media and 1.25ul 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, 300ng of the MG3-6-MG-3-4-encoding mRNA (SEQ ID NO: 108) and 120ng of the sgRNA (scaffold sequence SEQ ID NO:34) were mixed together with 25µL 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 Tables 2 and 3, while the primers used are summarized in Table 4.

Table 2: Average Activity of MG3-6/3-4 guides at mouse HAO1 delivered by mRNA Transfection

Guide Name	PAM	SEQ ID No.	Spacer Sequence	Editing Activity (Average % INDELs)
mH364-1	GCAAATG	611	GTATGACTATTACAGGTCTGGG	0
mH364-2	GAAAATG	612	AAATAGCAAAGTTTCTTACCTA	0
mH364-3	AGAAAAT	613	TAAATAGCAAAGTTTCTTACCT	0
mH364-6	CTAAAAC	614	ATTGGCATGCTGACTCTCTGTC	0
mH364-7	AGAAAAG	615	GAGCTGGCCACTGTGCGAGGTA	45.7
mH364-9	ACAAATA	616	CAGGTAAGGGGTGTCCACAGTC	0
mH364-10	TGAAAAA	617	ATTCTATGTATCTATTCTAGGA	0
mH364-11	GAAAAAC	618	TTCTATGTATCTATTCTAGGAT	31
mH364-15	CCAAATC	619	AAATTTCCCTTAGGAGAAAATG	0
mH364-16	GAAAATG	620	GTCTCCAAAATTTCCCTTAGGA	10.7
mH364-17	AGAAAAT	621	TGTCTCCAAAATTTCCCTTAGG	0
mH364-18	GGAAATT	622	TGATTTGGCATTTTCTCCTAAG	0
mH364-19	CAAAATT	623	TCAGCAAGTCCACTGTTGTCTC	0
mH364-20	CCAAAAT	624	TTCAGCAAGTCCACTGTTGTCT	25.9
mH364-22	CAAAATG	625	AGTAGAGAAATGACAAACCTCT	0
mH364-23	TCAAAAT	626	AAGTAGAGAAATGACAAACCTC	20.7

Table 3: Results of testing MG3-6/3-4 guides with a more permissive PAM design, at mouse HAO1 delivered by mRNA Transfection

Guide Name	PAM	SEQ ID No.	Spacer Sequence	Editing Activity (% INDELs)	R^2
mH364-4	AGAAACT	627	ACATCCAAGCATTTTCTAGGTA	0	1
mH364-5	TAAAACA	628	TTGGCATGCTGACTCTCTGTCC	0	1
mH364-8	ACAAAGA	629	CGCTGGATGCAACTGTACATCT	0	0.99
mH364-12	AAAAACT	630	TCTATGTATCTATTCTAGGATG	0	0.99
mH364-13	TGAAACC	631	TCTATTCTAGGATGAAAAACTT	0	0.99
mH364-14	TCAAAGT	632	AGAAAATGCCAAATCATTGGTT	0	0.99
mH364-21	GTAAAGG	633	ATTGACATCACTGCCTATTGTT	0	1

Table 4: 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	634	GTGACCAACCCTACCCGTTT
	Rev PCR	PCR_mHE1_R_-553	635	GCAAGCACCTACTGTCTCGT
	Sequencing	Seq_mHE1_F_+139	636	GTCTAGGCATACAATGTTTGCTCA
Mouse HAO1 Exon 2	Fwd PCR	HAO1_E2_F5721	637	CAACGAAGGTTCCCTCCAGG
	Rev PCR	HAO1_E2_R6271	638	GGAAGGGTGTTCGAGAAGGA
	Sequencing	5938F Seq_HAO1_E2	639	CTATGCAAGGAAAAGATTTGGCC
Mouse HAO1 Exon 3	Fwd PCR	HAO1_E3_F23198	640	TGCCCTAGACAAGCTGACAC
	Rev PCR	HAO1_E3_R23879	641	CAGATTCTGGAAGTGGCCCA
	Sequencing	HAO1_E3_F23198	642	Same as Fwd PCR Primer
Mouse HAO1 Exon 4	Fwd PCR	PCR_mHE4_F_+300	643	GGCTGGCTGAAAATAGCATCC
	Rev PCR	HAO1_E4_R31650	644	AGGTTTGGTTCCCCTCACCT
	Sequencing	PCR_mHE4_R_-149	645	TCTGCCATGAAGGCATATGGAC

Example 9 – Guide Chemistry Optimization for the MG3-6/3-4 and MG3-6 Type II nuclease

[00148] We designed 40 different chemically modified guides (named mAlb3634-34-0 to mAlb3634-34-44) and tested the activity of 39 of these guides. One guide, mH3634-34-32, failed RNA synthesis, thus it was not tested. The guide spacer sequence we chose as a model to insert various chemical modifications was mAlb3634-34 (targeting albumin intron 1) as it proved to be the most active guide in a guide screen in the mouse hepatocyte cell line Hepa1-6 cells (Table 5 and **FIG. 10**).

Table 5: Activity of chemically modified guides in Hepa1-6 cells

Guide	Editing Activity (% INDELs)
mAlb3634-13	0
mAlb3634-16	0

Guide	Editing Activity (% INDELs)
mAlb3634-19	0
mAlb3634-20	0
mAlb3634-24	0
mAlb3634-30	0
mAlb3634-45	19.5
mAlb3634-44	16.5
mAlb3634-53	0
mAlb3634-59	22
mAlb3634-64	0
mAlb3634-72	0
mAlb3634-73	0
mAlb3634-74	0
mAlb3634-78	9
mAlb3634-81	2
mAlb3634-84	15
mAlb3634-87	49
mAlb3634-34	62
mAlb3634-33	20.5

[00149] The sgRNA of MG3-6/3-4 comprises a spacer located at the 5′ end followed by the CRISPR repeat and the trans-activating CRISPR RNA (tracr). The CRISPR repeat and the tracr are identical to that of the MG3-6 nuclease (**FIG. 11a, 11b**). The CRISPR repeat and tracr form a structured RNA comprising 3 stem loops (**FIG. 11a**). We modified different areas of the stem loops by replacing the 2′ hydroxyl of the ribose with methyl groups or replacing the phosphodiester backbone by a phosphorothioate (PS). Moreover, the spacer at the 5′ of the guide was modified with a mixture of 2′-O-methyl or 2′-fluorine bases and PS bonds. The different combinations of chemical modifications designed are called mAlb3634-34-0 to mAlb3634-34-44 and the sequences are shown in Table 6.

[00150] The editing activity of 39 single guides with the exact same base sequence but different chemical modifications was evaluated in Hepa1-6 cells by co-transfection of mRNA encoding MG3-6/3-4 and the guide; the results are shown in Table 6 and **FIG. 12**.

Table 6: Sequences of chemically modified MG3-6/3-4 guides and their activity in Hepa1-6 cells when co-transfected with MG3-6/3-4 mRNA

Guide	SEQ ID No.	Sequence	Activity
mAlb3634-34-0	646	rCrUrUrArGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrUrUrU	71.8
mAlb3634-34-1	647	mC*mU*mU*rArGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU	124.5
mAlb3634-34-2	648	mC*mU*mU*rArGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrA*mU*mG*mU*mU*mU*mU	121.7
mAlb3634-34-3	649	mC*mU*mU*rArGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArGmAmAmUmCmGmAmAmAmGmAmUmUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU	120.5
mAlb3634-34-4	650	mC*mU*mU*rArGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArG*mA*mA*mU*mC*mG*mA*mA*mA*mG*mA*mU*mUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU	63.3
mAlb3634-34-5	651	mC*mU*mU*rArGrGrUrCrArGrUrGrArArGrArGrArArGrArAmGmUmUmGmAmGmAmAmUmCmGmAmAmAmGmAmUmUmCmUmUmAmArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU	0.8

Guide	SEQ ID No.	Sequence	Activity
mAlb3634-34-6	652	mC*mU*mU*rArGrGrUrCrArGrUrGrArArGrArGrArArGrArAmGmUmUmGmAmGmAmAmUmCrG*rA*rA*rA*mGmAmUmUmCmUmUmAmArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU	0.0
mAlb3634-34-7	653	mC*mU*mU*rArGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArAmGmGmCmAmUmCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU	113.0
mAlb3634-34-8	654	mC*mU*mU*rArGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArAmGmGmCmAmUmCrC*rU*rU*rC*rC*rGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU	115.6
mAlb3634-34-9	655	mC*mU*mU*rArGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArGrArArUrCmGmAmAmArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCmCmUmUmCmCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCmAmAmUmArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU	105.0
mAlb3634-34-10	656	mC*mU*mU*rArGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArGrArArUrCrG*rA*rA*rA*rGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrC*rU*rU*rC*rC*rGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrA*rA*rU*rA*rGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU	101.6
mAlb3634-34-11	657	mC*mU*mU*rArGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrC*mA*mC*mC*mG*mU*mC*mC*mG*mU*mU*mU*mU*mC*mC*mA*mA*mU*mArGrGrArGrCrGrGrGrCrGrGrUrA*mU*mG*mU*mU*mU*mU	57.0
mAlb3634-34-12	658	mC*mU*mU*rArGrGrUrCrArGrUrGrArArGrArGrArArGrArAmGmUmUmGmAmGmAmAmUmCrG*rA*rA*rA*rGrArUrUrCrU	0.0

Guide	SEQ ID No.	Sequence	Activity
		rUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrC*mA*mC*mC*mG*mU*mC*mC*mG*mU*mU*mU*mU*mC*mC*mA*mA*mU*mA*mG*mG*mA*mG*mC*mG*mG*mG*mC*mG*mG*mU*mA*mU*mG*mU*mU*mU*mU	
mAlb3634-34-13	659	mC*mU*mU*rArGrGrUrCrArGrUrGrArArGrArGrArArGrArAmGmUmUmGmAmGmAmAmUmCmGmAmAmAmGmAmUmUmCmUmUmAmArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU	0.0
mAlb3634-34-14	670	mC*mU*mU*rArGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArGmAmAmUmCmGmAmAmAmGmAmUmUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrC*mA*mC*mC*mG*mU*mC*mC*mG*mU*mU*mU*mU*mC*mC*mA*mA*mU*mA*mG*mG*mA*mG*mC*mG*mG*mG*mC*mG*mG*mU*mA*mU*mG*mU*mU*mU*mU	0.0
mAlb3634-34-15	671	mC*mU*mU*rArGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArGmAmAmUmCmGmAmAmAmGmAmUmUrCrUrUrArArUrArAmGmGmCmAmUmCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCmUmCmAmCmCmGmUmCmCmGmUmUmUmUmCmCmAmAmUmAmGmGmAmGmCmGmGmGmCmGmGmUmAmUmGmU*mU*mU*mU	34.5
mAlb3634-34-19	672	mC*mU*mU*rArGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArGrArArUrC*mG*mA*mA*mArGrArUrUrCrUrUrArA*mU*mA*mArGrGrCrArUrC*mC*mU*mU*mC*mCrGrArUrGrCrU*mG*mA*mC*mU*mU*mC*mU*mCrArCrCrGrUrCrCrGrUrUrUrUrCrC*mA*mA*mU*mArGrGrArGrCrGrGrGrCrGrGrUrA*mU*mG*mU*mU*mU*mU	0.0
mAlb3634-34-17	673	mC*mU*mU*i2FAi2FGi2FGi2FUi2FCi2FAi2FGi2FUi2FGi2FAi2FAi2FGi2FAi2FGrArArGrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU	147.7

Guide	SEQ ID No.	Sequence	Activity
mAlb3634-34-22	674	mC*mU*mU*rArGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArGrArArUrCrG*rA*rA*rA*rGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrC*rU*rU*rC*rC*rGrArUrGrCrUrGrArCrUrUrCrUrCmAmCmCmGmUmCmCmGmUmUmUmUmCmCrA*rA*rU*rA*mGmGmAmGmCmGmGmGmCmGmGmU*mA*mU*mG*mU*mU*mU*mU	44.2
mAlb3634-34-23	675	mC*mU*mU*rArGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArGrArArUrCrG*rA*rA*rA*rGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrC*rU*rU*rC*rC*rGrArUrGrCrUrG*rA*rC*rU*rU*rC*rU*rC*mAmCmCmGmUmCmCmGmUmUmUmUmCmCrA*rA*rU*rA*mGmGmAmGmCmGmGmGmCmGmGmU*mA*mU*mG*mU*mU*mU*mU	60.0
mAlb3634-34-24	676	mC*mU*mU*rArGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArGrArArUrCrG*rA*rA*rA*rGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrC*rU*rU*rC*rC*rGrArUrGrCrUrGrArCrUrUrCrUrCmAmCmCmGmUmCmCmGmUmUmUmUmCmCrA*rA*rU*rA*mGmGmAmGmCmGmGmGmCmGmGmU*mA*mU*mG*mU*mU*mU*mU	77.4
mAlb3634-34-25	677	mC*mU*mU*rArGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArGrArArUrCrG*rA*rA*rA*rGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrC*rU*rU*rC*rC*rGrArUrGrCrUrG*rA*rC*rU*rU*rC*rU*rC*mAmCmCmGmUmCmCmGmUmUmUmUmCmCrA*rA*rU*rA*mGmGmAmGmCmGmGmGmCmGmGmU*mA*mU*mG*mU*mU*mU*mU	50.5
mAlb3634-34-26	678	mC*mU*mU*mA*rGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrC*rU*rU*rC*rC*rGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrA*mU*mG*mU*mU*mU*mU	61.9
mAlb3634-34-27	679	mC*mU*mU*mA*rGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrC*rU*rU*rC*rC*rGrArUrGrCrUrGrArCrUrUrCrUrCrCrGrArUrGrCrCrUrGrArCrUrU*rC*rU*rA*rGrGrArGrCrGrGrGrCrGrGrUrA*mU*mG*mU*mU*m	67.4

Guide	SEQ ID No.	Sequence	Activity
		U*mU	
mAlb3634-34-29	680	mC*i2FU*i2FU*i2FA*rGrGrUrCrArGrUrGrArArGrArGrArArGrArAmGmUmUmGmAmGmAmAmUmCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrA*mU*mG*mU*mU*mU*mU	114.4
mAlb3634-34-30	681	mC*i2FU*i2FU*i2FA*rGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArAmGmGmCmAmUmCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrA*mU*mG*mU*mU*mU*mU	113.9
mAlb3634-34-31	682	mC*i2FU*i2FU*i2FA*rGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrA*mU*mG*mU*mU*mU*mU	100.0
mAlb3634-34-32	683	mC*mU*mU*i2FA*i2FGi2FGi2FUi2FCi2FAi2FGi2FUi2FGi2FAi2FAi2FGi2FAi2FGrArArGrArAmGmUmUmGmAmGmAmAmUmCrG*rA*rA*rA*mGmAmUmUrCrUrUrArArUrArAmGmGmCmAmUmCrC*rU*rU*rC*rC*rGrArUrGrCrUrGrArCrUrUrCrUrCmAmCmCmGmUmCmCmGmUmUmUmUmCmCrA*rA*rU*rA*mGmGmAmGmCmGmGmGmCmGmGmUmA*mU*mG*mU*mU*mU*mU	NT
mAlb3634-34-33	684	mC*mU*mU*i2FA*i2FGi2FGi2FUi2FCi2FAi2FGi2FUi2FGi2FAi2FAi2FGi2FAi2FGrArArGrArAmGmUmUmGmAmGmAmAmUmCrG*rA*rA*rA*mGmAmUmUrCrUrUrArArUrArAmGmGmCmAmUmCrC*rU*rU*rC*rC*rGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrA*rA*rU*rA*rGrGrArGrCrGrGrGrCrGrGrUrA*mU*mG*mU*mU*mU*mU	0.0
mAlb3634-34-34	685	mC*mU*mU*mA*i2FGi2FGi2FUi2FCi2FAi2FGi2FUi2FGi2FAi2FAi2FGi2FAi2FGrArArGrArArGrUrUrGrArGrArArUrCrG*rA*rA*rA*rGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrC*rU*rU	68.9

Guide	SEQ ID No.	Sequence	Activity
		*rC*rC*rGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrA*rA*rU*rA*rGrGrArGrCrGrGrGrCrGrGrUrA*mU*mG*mU*mU*mU*mU	
mAlb3634-34-35	686	mC*mU*mU*mA*rGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArGrArArUrCmG*mA*mA*mA*rGrArUrUrCrUrUrArArArUrArArGrGrCrArUrCmC*mU*mU*mC*mC*rGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCmA*mA*mU*mA*rGrGrArGrCrGrGrGrCrGrGrUrA*mU*mG*mU*mU*mU*mU	65.0
mAlb3634-34-36	687	mC*mU*mU*mA*rGrGrUrCrArGrUrGrArArGrArGrArArGrArAmGmUmUmGmAmGmAmAmUmCrG*rA*rA*rA*rGrArUrUrCrUrUrArArUrArArAmGmGmCmAmUmCrC*rU*rU*rC*rC*rGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrA*rA*rU*rA*rGrGrArGrCrGrGrGrCrGrGrUrA*mU*mG*mU*mU*mU*mU	0.0
mAlb3634-34-37	688	mC*mU*mU*mA*rGrGrUrCrArGrUrGrArArGrArGrArArGrArAmGmUmUmGmAmGmAmAmUmCrG*rA*rA*rA*rGrArUrUrCrUrUrArArUrArArAmGmGmCmAmUmCrC*rU*rU*rC*rC*rGrArUrGrCrUrGrArCrUrUrCrUrCmAmCmCmGmUmCmCmGmUmUmUmUmCmCrA*rA*rU*rA*rGrGrArGrCrGrGrGrCrGrGrUrA*mU*mG*mU*mU*mU*mU	0.0
mAlb3634-34-38	689	mC*mU*mU*mA*rGrGrUrCrArGrUrGrArArGrArGrArArGrArAmGmUmUmGmAmGmAmAmUmCrG*rA*rA*rA*rGrArUrUrCrUrUrArArUrArArAmGmGmCmAmUmCrC*rU*rU*rC*rC*rGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCmGmUmCmCmGmUmUmUmUmUmCmCrA*rA*rU*rA*mGmGmAmGmCmGmGmGmCmGrGrUrA*mU*mG*mU*mU*mU*mU	0.0
mAlb3634-34-39	690	mC*mU*mU*mA*rGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArGmAmAmUmCrG*rA*rA*rA*rGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrC*rU*rU*rC*rC*rGrArUrGrCrUrG*rA*rC*rU*rU*rC*rU*rC*rArCrCrGrUrCrCrGrUrUrUrUrCrCrA*rA*rU*rA*rGrGrArGrCrGrGrGrCrGrGrUrA*mU*mG*mU*mU*mU*mU	3.7

Guide	SEQ ID No.	Sequence	Activity
mAlb3634-34-40	691	mC*mU*mU*mA*rGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArGmAmAmUmCrG*rA*rA*rA*mGmAmUmUrCrUrUrArArUmAmAmGmGmCmAmUmCrC*rU*rU*rC*rC*mGmAmUmGmCrU*rG*rA*mCmUmUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrA*rA*rU*rA*rGrGrArGrCrGrGrGrCrGrGrUrA*mU*mG*mU*mU*mU*mU	0.0
mAlb3634-34-41	692	mC*mU*mU*mA*rGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArGmAmAmUmCrG*rA*rA*rA*rGrArUrUrCrUrUrArArUrArArAmGmGmCmAmUmCrC*rU*rU*rC*rC*rGrArUrGrCrUrGrArCrUrUrCrUrCmAmCmCmGmUmCmCmGmUmUmUmUmCmCrA*rA*rU*rA*rGrGrArGrCrGrGrGrCrGrGrUrA*mU*mG*mU*mU*mU*mU	47.1
mAlb3634-34-42	693	mC*mU*mU*mA*i2FGi2FGi2FUi2FCi2FAi2FGi2FUi2FGi2FAi2FAi2FGi2FAi2FGi2FAi2FAi2FGi2FArArGrUrUrGrArGrArArUrCrG*rA*rA*rA*rGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrC*rU*rU*rC*rC*rGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrA*rA*rU*rA*rGrGrArGrCrGrGrGrCrGrGrUrA*mU*mG*mU*mU*mU*mU	66.7
mAlb3634-34-43	694	mC*mU*mU*rArGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrAmGmGmAmGmCmGmGmGmCmGmGmUmA*mU*mG*mU*mU*mU*mU	73.8
mAlb3634-34-44	695	mC*mU*mU*rArGrGrUrCrArGrUrGrArArGrArGrArArGrArArGrUrUrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCmAmCmCmGmUmCmCmGmUmUmUmUmCmCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrA*mU*mG*mU*mU*mU*mU	84.9
(r =native ribose base, m = 2'-O methyl modified base, F = 2' Fluro modified base, * = phosphorothioate bond)			

[00151] A guide with the same base sequence and a commercially available chemical modification called AltR1/AltR2 was used as a control. The spacer sequence in these guides targets a 22-nucleotide region in albumin intron1 of the mouse genome. Guide mAlb3634-34-0

(no chemical modifications) showed 72% activity relative to the AltR1/AltR2 guide. Guide mAlb3634-34-1 showed 124% activity relative to the AltR1/AltR2 guide, showing the importance of stability of guides for editing: mAlb3634-34-1 is more stable than mAlb3634-34-0 (**FIG. 13** and **FIG. 14**). Importantly, mAlb3634-34-17 retained 147% of the activity relative to AltR1/AltR2. The incorporation of 2′-O-fluorines in the spacer greatly increased the stability of mAlb3634-34-35, and the guide retained 65% activity. mAlb3634-34-35 contains 2′-O-methyl and PS bonds in the loops of the three stem loops of the MG3-6/3-4 guide. Importantly, mAlb3634-34-42 retained 66% of activity and this guide contains as many fluorines in the spacer as mAlb3634-34-17, but it also contains PS bonds in all the loops present in the gRNA. mAlb3634-34-27 retained 67% activity and mAlb3634-34-29 retained 114% activity. Among the modifications these guides contain are PS bonds in the loop of the first stem loop and 2′-O-methyl groups in the first strand of the first stem loop for mAlb3634-34-27 and mAlb3634-34-29, respectively. When these 2 modifications were combined (2′-O-methyl in the first strand of the first stem loop and PS bonds in the loop of the first stem loop), the guides lost their activity (mAlb3634-34-33, mAlb3634-34-36, mAlb3634-34-38), showing the complexity of the gRNA/protein interaction and demonstrating that the results of simple extrapolations are difficult to predict.

[00152] In order to test the stability of these chemically modified guides compared to the guide with no chemical modification (native RNA), a stability assay using crude cell extracts was used. Crude cell extracts from mammalian cells were 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*. Hepa1-6 cells were collected by adding 3ml of cold PBS per 15cm dish of confluent cells and releasing the cells from the surface of the dish using a cell scraper. The cells were pelleted at 200g for 10 min and frozen at -80°C for future use. For the stability assays, cells were resuspended in 4 volumes of cold PBS (e.g. for a 100mg pellet, cells were resuspended in 400ul of cold PBS). Triton X-100 was added to a concentration of 0.2% (v/v), cells were 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 were set up on ice and comprised 20 µl of cell crude extract with 2 pmoles of each guide (1ul of a 2uM stock). Six reactions were 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 was incubated). Samples were incubated at 37°C from 0.5 hours up to 21 hours while the input control was left on ice for 5 minutes. After each incubation period, the reaction was stopped by adding 300ul 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 were vortexed for 15 seconds and stored at -20°C. RNA was extracted from the samples using Direct-zol RNA miniprep kit (Zymo Research) and eluted in 100ul of nuclease-free water. Detection of the modified guide was performed using Taqman RT - qPCR using the Taqman miRNA Assay technology (Thermo Fisher), and primers and probes were designed to specifically detect the sequence in the mAlb3634-34 sgRNA, which is the same for all of the guides. Data was plotted as a function of percentage of sgRNA remaining in relation to the input sample (Tables 7 and 8; **FIG. 13** and **FIG. 14**).

Table 7: Stability of MG3-6/3-4 chemically modified guides over 9 hours at 37 °C

	Percentage guide left			
Time (H)	**mAlb3634-34-0**	**mAlb3634-34-1**	**mAlb3634-34-17**	**mAlb3634-34-29**
0.5	48.6327474	71.6977624	84.9684999	91.383145
1	45.5334917	111.342162	69.2554734	79.8298386
4	8.33311673	84.3815796	46.6516496	58.2366793
9	1.23016871	41.3225159	36.6021424	16.5511114
Time (H)	**mAlb3634-34-30**	**mAlb3634-34-35**	**mAlb3634-34-36**	**mAlb3634-34-42**
0.5	86.7538687		91.7004043	91.7004043
1	90.1250463	146.40857	57.8344092	72.1964598
4	53.5886731	128.34259	61.985385	72.1964598
9	21.9912269	100	62.6332219	47.3028823

Table 8: Stability of MG3-6/3-4 chemically modified guides over 21 hours at 37 °C

	Percentage guide left			
Time (H)	**mAlb3634-34-0**	**mAlb3634-34-1**	**mAlb3634-34-35**	**mAlb3634-34-42**
0.5	68.3020128	61.98539	104.6085	80.94422
1	51.0506063	59.66679	84.08964	73.20428
4	9.67228121	51.05061	52.66805	70.71068
9	1.75790388	40.47211	51.22784	45.37596
21	0.03405136	1.447794	24.82731	15.60413

[00153] The stability assays showed that introducing three 2′-O-methyls and three PS bonds in the 5′ and 3′ end of the guides significantly improved stability (**FIG. 13** and **FIG. 14**). Adding extra 2′-fluors to the 5′ and 3′ modifications, as in mAlb3634-17 and mAlb3634-42, did not show an apparent advantage at early time points (up to 9 hr) as shown in **FIG. 13,** but a slight improvement in stability was apparent when the stability assays were run for 21 hr (**FIG. 14**). Including 2-O-methyl and PS bonds in all the loops of the stem loops (mAlb3634-35) gave an apparent larger increment in stability compared to the guide with chemical modifications on the 5′ and 3′ ends (mAlb3634-1), as seen in **FIG. 13**. However, when these results were repeated and at longer time points, this increment became less apparent at earlier time points and was became apparent at longer time points up to 21 hr, as seen in **FIG. 14**. Including 2′-O-methyl in the first strand of distinct stem loops did not provide an advantage in stability for up to 9 hr, as shown by comparing mAlb3634-0 and mAlb3634-29 and mAlb3634-30. mAlb3634-36, which has a combination of 2′-O-methyl in the first strand of all stem loops and PS bonds in the loops of all stem loops, showed an apparent increased stability at 9 hr when compared to end modified guide (mAlb3634-0). However, this guide was not active when tested via mRNA transfection in Hepa1-6 cells. In general, adding extra modifications (e.g. 2′-O-methyl, 2′-O-fluor or PS bonds) to the end modified guide did not confer a large advantage in stability at earlier time points up to 9 hr (**FIG. 13**), and a small increase in stability was apparent at longer time points (**FIG. 14**). The large size (110nt) and highly structured nature of this gRNA may make it inherently more stable than shorter or less structured guide RNA and thereby limit the benefit of chemical modifications on stability. Modifying the 5′ and 3′ ends of the guide appears to provide a good level of protection against nucleases. However adding the extra modifications in the guides might provide more benefit *in vivo*, as these types of modifications may reduce immunogenicity.

Example 10 – Protein recombination of Type V-A nucleases

[00154] To expand the capability of rapid PAM exchange beyond type II nucleases, three type V-A nucleases were chosen for protein recombination. The breakpoint was chosen based on the predicted structural information (**Table 1**). Similar to type II enzyme recombinants, the type V chimera showed activity when proteins were recombined from a closely related family. *In vitro* PAM enrichment and NGS analysis revealed a consistent result that the PAM of a chimera is inherited from C-terminal parent. It may be possible to avoid potential structural disruptions of protein recombination from distantly related families by utilizing breakpoint optimization (**FIG. 15**).

Example 11 – Analysis of gene-editing outcomes at the DNA level for TRAC in HEK293T cells

[00155] Nucleofection of MG3-6/4 RNPs (104 pmol protein/300 pmol guide) comprising sgRNAs described below in Table 7A and SEQ ID NOs: 119-158 was performed into HEK293T 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. 16**). Results indicated that sgRNAs C1, F2, and B3 were most effective at inducing indels, with appreciable editing also occurring for sgRNAs D2, H2, A3, and C3.

Table 7A: gRNAs and Targeting Sequences Used in Example 11

Category	SEQ ID NO:	Name	Sequence
MG3-6/3-4 sgRNA targeting TRAC	119	MG3-6/3-4 TRAC A1	mG*mC*mC*rGrUrGrUrArCrCrArGrCrUrGrArGrArGrArCrUrGrUrUrG rArGrArArUrCrGrArArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRAC	120	MG3-6/3-4 TRAC B1	mA*mU*mU*rCrArCrCrGrArUrUrUrUrGrArUrUrCrUrCrArArGrUrUrG rArGrArArUrCrGrArArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRAC	121	MG3-6/3-4 TRAC C1	mG*mA*mU*rUrCrUrGrArUrGrUrGrUrArUrArUrCrArCrArGrGrUrUrG rArGrArArUrCrGrArArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRAC	122	MG3-6/3-4 TRAC D1	mA*mA*mC*rArGrUrGrCrUrGrUrGrGrCrCrUrGrGrArGrCrArGrUrUrG rArGrArArUrCrGrArArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRAC	123	MG3-6/3-4 TRAC E1	mG*mG*mC*rUrGrGrGrGrArArGrArArGrGrUrGrUrCrUrUrCrGrUrUrG rArGrArArUrCrGrArArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRAC	124	MG3-6/3-4 TRAC F1	mG*mU*mU*rUrUrGrUrCrUrGrUrGrArUrArUrArCrArCrArUrGrUrUrG rArGrArArUrCrGrArArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRAC	125	MG3-6/3-4 TRAC G1	mU*mU*mA*rCrUrUrUrGrUrGrArCrArCrArUrUrUrGrUrUrUrGrUrUrG rArGrArArUrCrGrArArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA	126	MG3-6/3-4	mU*mU*mG*rUrGrArCrArCrArUrUrUrGrUrUrUrGrArGrArArGrUrUrG rArGrArArUrCrGrArArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUr

Category	SEQ ID NO:	Name	Sequence
targeting TRAC		TRAC H1	CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRAC	127	MG3-6/3-4 TRAC A2	mU*mG*mU*rGrArCrArCrArUrUrUrGrUrUrUrGrArGrArArUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRAC	128	MG3-6/3-4 TRAC B2	mA*mU*mU*rUrGrUrUrUrGrArGrArArUrCrArArArArUrCrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRAC	129	MG3-6/3-4 TRAC C2	mU*mU*mC*rCrUrGrUrGrArUrGrUrCrArArGrCrUrGrGrUrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRAC	130	MG3-6/3-4 TRAC D2	mU*mC*mC*rUrGrUrGrArUrGrUrCrArArGrCrUrGrGrUrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRAC	131	MG3-6/3-4 TRAC E2	mG*mU*mC*rArArGrCrUrGrGrUrCrGrArGrArArArArGrCrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRAC	132	MG3-6/3-4 TRAC F2	mA*mG*mC*rUrUrGrArCrArUrCrArCrArGrGrArArCrUrUrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRAC	133	MG3-6/3-4 TRAC G2	mG*mA*mC*rArUrCrArCrArGrGrArArCrUrUrUrCrUrArArArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRAC	134	MG3-6/3-4 TRAC H2	mU*mU*mA*rCrArGrArUrArCrGrArArCrCrUrArArArCrUrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRAC	135	MG3-6/3-4 TRAC A3	mA*mA*mA*rArCrCrUrGrUrCrArGrUrGrArUrUrGrGrGrUrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRAC	136	MG3-6/3-4 TRAC B3	mG*mA*mU*rUrGrGrUrUrCrCrGrArArUrCrCrUrCrCrUrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRAC	137	MG3-6/3-4 TRAC C3	mG*mG*mA*rArCrCrCrArArUrCrArCrUrGrArCrArGrGrUrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU

Category	SEQ ID NO:	Name	Sequence
MG3-6/3-4 sgRNA targeting TRAC	138	MG3-6/3-4 TRAC D3	mU*mU*mG*rArArArGrUrUrUrArGrGrUrUrCrGrUrArUrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
DNA sequence of TRAC target site	139	MG3-6/3-4 TRAC A1	GCCGTGTACCAGCTGAGAGACT
DNA sequence of TRAC target site	140	MG3-6/3-4 TRAC B1	ATTCACCGATTTTGATTCTCAA
DNA sequence of TRAC target site	141	MG3-6/3-4 TRAC C1	GATTCTGATGTGTATATCACAG
DNA sequence of TRAC target site	142	MG3-6/3-4 TRAC D1	AACAGTGCTGTGGCCTGGAGCA
DNA sequence of TRAC target site	143	MG3-6/3-4 TRAC E1	GGCTGGGGAAGAAGGTGTCTTC
DNA sequence of TRAC target site	144	MG3-6/3-4 TRAC F1	GTTTTGTCTGTGATATACACAT
DNA sequence of TRAC target site	145	MG3-6/3-4 TRAC G1	TTACTTTGTGACACATTTGTTT
DNA sequence of TRAC target site	146	MG3-6/3-4 TRAC H1	TTGTGACACATTTGTTTGAGAA
DNA sequence of TRAC target site	147	MG3-6/3-4 TRAC A2	TGTGACACATTTGTTTGAGAAT
DNA sequence of TRAC target site	148	MG3-6/3-4 TRAC B2	ATTTGTTTGAGAATCAAAATCG
DNA sequence of TRAC target site	149	MG3-6/3-4 TRAC C2	TTCCTGTGATGTCAAGCTGGTC
DNA sequence of TRAC target site	150	MG3-6/3-4 TRAC D2	TCCTGTGATGTCAAGCTGGTCG
DNA sequence of TRAC target site	151	MG3-6/3-4 TRAC E2	GTCAAGCTGGTCGAGAAAAGCT
DNA sequence	152	MG3-6/3-4	AGCTTGACATCACAGGAACTTT

Category	SEQ ID NO:	Name	Sequence
of TRAC target site		TRAC F2	
DNA sequence of TRAC target site	153	MG3-6/3-4 TRAC G2	GACATCACAGGAACTTTCTAAA
DNA sequence of TRAC target site	154	MG3-6/3-4 TRAC H2	TTACAGATACGAACCTAAACTT
DNA sequence of TRAC target site	155	MG3-6/3-4 TRAC A3	AAAACCTGTCAGTGATTGGGTT
DNA sequence of TRAC target site	156	MG3-6/3-4 TRAC B3	GATTGGGTTCCGAATCCTCCTC
DNA sequence of TRAC target site	157	MG3-6/3-4 TRAC C3	GGAACCCAATCACTGACAGGTT
DNA sequence of TRAC target site	158	MG3-6/3-4 TRAC D3	TTGAAAGTTTAGGTTCGTATCT
(r =native ribose base, m = 2'-O methyl modified base, F = 2' Fluro modified base, * = phosphorothioate bond)			

Example 12 – Analysis of gene-editing outcomes at the DNA level for B2M in HEK293T cells

[00156] Nucleofection of MG3-6/4 RNPs (104 pmol protein/300 pmol guide) comprising sgRNAs described below in Table 7B and SEQ ID NOs: 159-210 was performed into HEK293T 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. 17**). Results indicated that sgRNAs A1, G1, B2, H2, and B4 were the most effective for inducing editing, with appreciable editing also being detected for sgRNAs C1, D1, A2, H1, E2, F2, G2, A3, C3, and D3.

Table 7B: gRNAs and Targeting Sequences Used in Example 12

Category	SEQ ID NO:	Name	Sequence
MG3-6/3-4 sgRNA	159	MG3-6/3-4	mU*mC*mA*rCrGrCrUrGrGrArUrArGrCrCrUrCrCrArGrGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrCrGrUrCrCrG

Category	SEQ ID NO:	Name	Sequence
targeting B2M		B2M A1	rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting B2M	160	MG3-6/3-4 B2M B1	mG*mG*mU*rUrUrArCrUrCrArCrGrUrCrArUrCrCrArGrCrArGrUrUrGrArArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting B2M	161	MG3-6/3-4 B2M C1	mA*mC*mU*rCrArCrGrUrCrArUrCrCrArGrCrArGrArGrArArGrUrUrGrArArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting B2M	162	MG3-6/3-4 B2M D1	mU*mC*mA*rUrCrCrArGrCrArGrArGrArArUrGrGrArArArGrGrUrUrGrArArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting B2M	163	MG3-6/3-4 B2M E1	mA*mG*mA*rGrArArUrGrGrArArArGrUrCrArArArUrUrUrCrGrUrUrGrArArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting B2M	164	MG3-6/3-4 B2M F1	mC*mG*mA*rCrArUrUrGrArArGrUrUrGrArCrUrUrArCrUrGrGrUrUrGrArArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting B2M	165	MG3-6/3-4 B2M G1	mU*mU*mG*rArCrUrUrArCrUrGrArArGrArArUrGrGrArGrArGrUrUrGrArArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting B2M	166	MG3-6/3-4 B2M H1	mU*mU*mA*rCrUrGrArArGrArArUrGrGrArGrArGrArArGrUrUrGrArArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting B2M	167	MG3-6/3-4 B2M A2	mU*mA*mC*rUrGrArArGrArArUrGrGrArGrArGrArArUrGrUrUrGrArArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting B2M	168	MG3-6/3-4 B2M B2	mA*mC*mU*rGrArArGrArArUrGrGrArGrArGrArArUrUrGrUrUrGrArArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting B2M	169	MG3-6/3-4 B2M C2	mU*mC*mU*rUrUrCrUrArUrCrUrCrUrUrGrUrArCrUrArCrArGrUrUrGrArArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting B2M	170	MG3-6/3-4 B2M D2	mU*mA*mC*rUrArCrArCrUrGrArArUrUrCrArCrCrCrCrCrArGrUrUrGrArArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU

Category	SEQ ID NO:	Name	Sequence
MG3-6/3-4 sgRNA targeting B2M	171	MG3-6/3-4 B2M E2	mA*mC*mU*rArCrArCrUrGrArArUrUrCrArCrCrCrCrCrArCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting B2M	172	MG3-6/3-4 B2M F2	mC*mU*mA*rCrArCrUrGrArArUrUrCrArCrCrCrCrCrArCrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting B2M	173	MG3-6/3-4 B2M G2	mA*mU*mA*rCrUrCrArUrCrUrUrUrUrUrCrArGrUrGrGrGrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting B2M	174	MG3-6/3-4 B2M H2	mG*mA*mA*rUrUrCrArGrUrGrUrArGrUrArCrArArGrArGrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting B2M	175	MG3-6/3-4 B2M A3	mG*mA*mG*rArUrArGrArArArGrArCrCrArGrUrCrCrUrUrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting B2M	176	MG3-6/3-4 B2M B3	mC*mA*mG*rUrCrCrUrUrGrCrUrGrArArArGrArCrArArGrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting B2M	177	MG3-6/3-4 B2M C3	mA*mG*mU*rCrArArCrUrUrCrArArUrGrUrCrGrGrArUrGrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting B2M	178	MG3-6/3-4 B2M D3	mA*mA*mA*rCrCrCrArGrArCrArCrArUrArGrCrArArUrUrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting B2M	179	MG3-6/3-4 B2M E3	mA*mA*mC*rCrCrArGrArCrArCrArUrArGrCrArArUrUrCrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting B2M	180	MG3-6/3-4 B2M F3	mC*mU*mG*rCrUrGrGrArUrGrArCrGrUrGrArGrUrArArArCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting B2M	181	MG3-6/3-4 B2M G3	mA*mC*mC*rUrGrArArUrCrUrUrUrGrGrArGrUrArCrCrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA	182	MG3-6/3-4	mU*mG*mC*rUrGrCrUrUrArCrArUrGrUrCrUrCrGrArUrCrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG

Category	SEQ ID NO:	Name	Sequence
targeting B2M		B2M H3	rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting B2M	183	MG3-6/3-4 B2M A4	mG*mC*mU*rGrCrUrUrArCrArUrGrUrCrUrCrGrArUrCrUrArGrUrUrGrArGrGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting B2M	184	MG3-6/3-4 B2M B4	mC*mU*mG*rCrUrUrArCrArUrGrUrCrUrCrGrArUrCrUrArUrGrUrUrGrArGrGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
DNA sequence of B2M target site	185	MG3-6/3-4 B2M A1	TCACGCTGGATAGCCTCCAGGC
DNA sequence of B2M target site	186	MG3-6/3-4 B2M B1	GGTTTACTCACGTCATCCAGCA
DNA sequence of B2M target site	187	MG3-6/3-4 B2M C1	ACTCACGTCATCCAGCAGAGAA
DNA sequence of B2M target site	188	MG3-6/3-4 B2M D1	TCATCCAGCAGAGAATGGAAAG
DNA sequence of B2M target site	189	MG3-6/3-4 B2M E1	AGAGAATGGAAAGTCAAATTTC
DNA sequence of B2M target site	190	MG3-6/3-4 B2M F1	CGACATTGAAGTTGACTTACTG
DNA sequence of B2M target site	191	MG3-6/3-4 B2M G1	TTGACTTACTGAAGAATGGAGA
DNA sequence of B2M target site	192	MG3-6/3-4 B2M H1	TTACTGAAGAATGGAGAGAGAA
DNA sequence of B2M target site	193	MG3-6/3-4 B2M A2	TACTGAAGAATGGAGAGAGAAT
DNA sequence of B2M target site	194	MG3-6/3-4 B2M B2	ACTGAAGAATGGAGAGAGAATT
DNA sequence of B2M target site	195	MG3-6/3-4 B2M C2	TCTTTCTATCTCTTGTACTACA
DNA sequence	196	MG3-6/3-4	TACTACACTGAATTCACCCCCA

Category	SEQ ID NO:	Name	Sequence
of B2M target site		B2M D2	
DNA sequence of B2M target site	197	MG3-6/3-4 B2M E2	ACTACACTGAATTCACCCCCAC
DNA sequence of B2M target site	198	MG3-6/3-4 B2M F2	CTACACTGAATTCACCCCCACT
DNA sequence of B2M target site	199	MG3-6/3-4 B2M G2	ATACTCATCTTTTTCAGTGGGG
DNA sequence of B2M target site	200	MG3-6/3-4 B2M H2	GAATTCAGTGTAGTACAAGAGA
DNA sequence of B2M target site	201	MG3-6/3-4 B2M A3	GAGATAGAAAGACCAGTCCTTG
DNA sequence of B2M target site	202	MG3-6/3-4 B2M B3	CAGTCCTTGCTGAAAGACAAGT
DNA sequence of B2M target site	203	MG3-6/3-4 B2M C3	AGTCAACTTCAATGTCGGATGG
DNA sequence of B2M target site	204	MG3-6/3-4 B2M D3	AAACCCAGACACATAGCAATTC
DNA sequence of B2M target site	205	MG3-6/3-4 B2M E3	AACCCAGACACATAGCAATTCA
DNA sequence of B2M target site	206	MG3-6/3-4 B2M F3	CTGCTGGATGACGTGAGTAAAC
DNA sequence of B2M target site	207	MG3-6/3-4 B2M G3	ACCTGAATCTTTGGAGTACCTG
DNA sequence of B2M target site	208	MG3-6/3-4 B2M H3	TGCTGCTTACATGTCTCGATCT
DNA sequence of B2M target site	209	MG3-6/3-4 B2M A4	GCTGCTTACATGTCTCGATCTA
DNA sequence of B2M target site	210	MG3-6/3-4 B2M B4	CTGCTTACATGTCTCGATCTAT

Category	SEQ ID NO:	Name	Sequence
(r =native ribose base, m = 2'-O methyl modified base, F = 2' Fluro modified base, * = phosphorothioate bond)			

Example 13 – Analysis of gene-editing outcomes at the DNA and phenotypic levels for TRAC in T cells

[00157] Primary T cells were purified from PMBCs using a negative selection kit (Miltenyi) according to the manufacturer's recommendations. Nucleofection of MG3-6/4 RNPs (104 pmol protein/120 pmol guide) comprising sgRNAs described in Table 7A and SEQ ID NOs: 119-158 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. 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, 3 days post-nucleofection, 100,000 T cells were stained with anti-CD3 antibody for 30 minutes at 4C and analyzed on an Attune Nxt flow cytometer (**FIG. 18**). Results indicated that sgRNAs C1, D2, F2, H2, A3, B3, C3, and D3 showed appreciable editing, with the most editing performed by sgRNAs C1 and B3.

Example 14 – Analysis of gene-editing outcomes at the DNA level for B2M in T cells

[00158] Primary T cells were purified from PMBCs using a negative selection kit (Miltenyi) according to the manufacturer's recommendations. Nucleofection of MG3-6/4 RNPs (104 pmol protein/120 pmol guide) comprising sgRNAs described in Table 7B and SEQ ID NOs: 159-210 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. The amplicons were sequenced on an Illumina MiSeq machine and analyzed with a proprietary Python script to measure gene editing (**FIG. 19**).

Example 15 – Analysis of gene-editing outcomes at the phenotypic level for TRBC1 and TRBC2 in T cells

Primary T cells were purified from PBMCs using a negative selection kit (Miltenyi) according to the manufacturer's recommendations. Nucleofection of MG3-6/4 RNPs (104 pmol protein/120 pmol guide) comprising sgRNAs described below in Table 7C below and SEQ ID NOs: 211-382 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 4C and analyzed on an Attune Nxt flow cytometer (**FIG. 20**). As can be seen from the results in **FIG. 20**, the highest-performing sgRNAs for TRBC1 were A1, B1, E1, G4, H4, and B5. Similarly, the highest performing sgRNAs for TRBC2 were D1, H1, and A5.

Table 7C: gRNAs and Targeting Sequences Used in Example 15

Category	SEQ ID NO:	Name	Sequence
MG3-6/3-4 sgRNA targeting TRBC1	211	MG3-6/3-4 TRBC1 A1	mC*mA*mG*rArArGrCrArGrArGrArUrCrUrCrCrCrArCrArCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	212	MG3-6/3-4 TRBC1 B1	mC*mC*mA*rCrGrUrGrGrArGrCrUrGrArGrCrUrGrGrUrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	213	MG3-6/3-4 TRBC1 C1	mA*mG*mU*rCrCrArGrUrUrCrUrArCrGrGrGrCrUrCrUrCrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	214	MG3-6/3-4 TRBC1 D1	mG*mA*mU*rUrArGrGrUrGrArGrArCrCrArGrCrUrArCrCrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	215	MG3-6/3-4 TRBC1 E1	mA*mU*mU*rArGrGrUrGrArGrArCrCrArGrCrUrArCrCrArGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	216	MG3-6/3-4 TRBC1 F1	mU*mU*mA*rGrGrUrGrArGrArCrCrArGrCrUrArCrCrArGrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	217	MG3-6/3-4 TRBC1 G1	mU*mG*mA*rGrArCrCrArGrCrUrArCrCrArGrGrGrArArArArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	218	MG3-6/3-4 TRBC1 H1	mC*mA*mG*rGrUrArGrCrArGrArCrArArGrArCrUrArGrArUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	219	MG3-6/3-4 TRBC1 A2	mA*mG*mG*rUrArGrCrArGrArCrArArGrArCrUrArGrArUrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA	220	MG3-6/3-4	mA*mG*mC*rArGrArCrArArArGrArCrUrArGrArUrCrCrArArArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr

Category	SEQ ID NO:	Name	Sequence
targeting TRBC1		TRBC1 B2	CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	221	MG3-6/3-4 TRBC1 C2	mG*mG*mA*rArCrCrArGrCrGrCrArCrArCrCrArUrGrArArGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	222	MG3-6/3-4 TRBC1 D2	mG*mU*mG*rGrCrUrGrArCrArUrCrUrGrCrArUrGrGrCrArGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	223	MG3-6/3-4 TRBC1 E2	mG*mG*mC*rCrUrGrGrGrArGrUrCrUrGrUrGrCrCrArArCrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	224	MG3-6/3-4 TRBC1 F2	mC*mU*mG*rArCrUrUrUrArCrUrUrUrUrArArUrUrGrCrCrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	225	MG3-6/3-4 TRBC1 G2	mU*mG*mA*rCrUrUrUrArCrUrUrUrUrArArUrUrGrCrCrUrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	226	MG3-6/3-4 TRBC1 H2	mG*mA*mC*rUrUrUrArCrUrUrUrUrArArUrUrGrCrCrUrArUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	227	MG3-6/3-4 TRBC1 A3	mG*mG*mG*rArArGrGrArGrArArGrCrUrGrGrArGrUrCrArCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	228	MG3-6/3-4 TRBC1 B3	mG*mG*mA*rArGrGrArGrArArGrCrUrGrGrArGrUrCrArCrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	229	MG3-6/3-4 TRBC1 C3	mA*mA*mC*rUrCrCrUrGrGrCrUrCrUrUrArArUrArArCrCrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	230	MG3-6/3-4 TRBC1 D3	mA*mA*mC*rUrUrUrCrUrCrUrUrCrUrGrCrArGrGrUrCrArArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	231	MG3-6/3-4 TRBC1 E3	mA*mC*mU*rCrCrArCrUrUrCrCrArGrGrGrCrUrGrCrCrUrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU

Category	SEQ ID NO:	Name	Sequence
MG3-6/3-4 sgRNA targeting TRBC1	232	MG3-6/3-4 TRBC1 F3	mC*mU*mC*rCrArCrUrUrCrCrArGrGrGrCrUrGrCrCrUrUrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU	
MG3-6/3-4 sgRNA targeting TRBC1	233	MG3-6/3-4 TRBC1 G3	mU*mC*mC*rUrUrUrCrUrCrUrUrGrArCrCrUrGrCrArGrArArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU	
MG3-6/3-4 sgRNA targeting TRBC1	234	MG3-6/3-4 TRBC1 H3	mA*mG*mC*rCrArGrGrArGrUrUrGrUrGrArGrGrArUrUrGrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU	
MG3-6/3-4 sgRNA targeting TRBC1	235	MG3-6/3-4 TRBC1 A4	mA*mG*mU*rArGrUrArGrGrGrCrCrCrArUrUrGrArCrCrArCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU	
MG3-6/3-4 sgRNA targeting TRBC1	236	MG3-6/3-4 TRBC1 B4	mU*mG*mC*rArArGrUrUrArUrCrUrUrCrUrGrArGrGrCrArCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU	
MG3-6/3-4 sgRNA targeting TRBC1	237	MG3-6/3-4 TRBC1 C4	mA*mG*mU*rUrArUrCrUrUrCrUrGrArGrGrCrArCrCrUrGrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU	
MG3-6/3-4 sgRNA targeting TRBC1	238	MG3-6/3-4 TRBC1 D4	mG*mU*mU*rArUrCrUrUrCrUrGrArGrGrCrArCrCrUrGrArArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU	
MG3-6/3-4 sgRNA targeting TRBC1	239	MG3-6/3-4 TRBC1 E4	mU*mC*mA*rArGrArArCrCrArUrGrArGrArGrArGrGrGrArGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU	
MG3-6/3-4 sgRNA targeting TRBC1	240	MG3-6/3-4 TRBC1 F4	mC*mA*mA*rGrArArCrCrArUrGrArGrArGrArGrGrGrArGrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU	
MG3-6/3-4 sgRNA targeting TRBC1	241	MG3-6/3-4 TRBC1 G4	mU*mU*mA*rCrCrCrGrArGrGrUrArArArGrCrCrArCrArGrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU	
MG3-6/3-4 sgRNA targeting TRBC1	242	MG3-6/3-4 TRBC1 H4	mC*mC*mG*rArGrGrUrArArArGrCrCrArCrArGrUrCrUrGrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU	
MG3-6/3-4 sgRNA	243	MG3-6/3-4	mC*mA*mG*rUrCrUrGrArArArGrArArArGrCrArGrGrGrArGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG

Category	SEQ ID NO:	Name	Sequence
targeting TRBC1		TRBC1 A5	rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	244	MG3-6/3-4 TRBC1 B5	mA*mG*mU*rCrUrGrArArArGrArArArGrCrArGrGrGrArGrArGrUrUrG rArGrArArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	245	MG3-6/3-4 TRBC1 C5	mG*mU*mC*rUrGrArArArGrArArArGrCrArGrGrGrArGrArGrGrUrUrG rArGrArArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	246	MG3-6/3-4 TRBC1 D5	mG*mA*mA*rArGrArArArGrCrArGrGrGrArGrArGrGrArArArGrUrUrG rArGrArArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	247	MG3-6/3-4 TRBC1 E5	mG*mA*mG*rArCrCrUrUrArUrUrUrUrCrArUrArGrGrCrArArGrUrUrG rArGrArArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	248	MG3-6/3-4 TRBC1 F5	mG*mA*mU*rGrArGrArGrUrUrArCrArCrArGrGrCrCrArCrArGrUrUrG rArGrArArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	249	MG3-6/3-4 TRBC1 G5	mA*mG*mC*rUrGrCrUrUrGrGrCrUrCrUrGrUrUrGrGrGrCrUrGrUrUrG rArGrArArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	250	MG3-6/3-4 TRBC1 H5	mU*mG*mU*rUrGrGrGrCrUrGrArGrArArUrCrUrGrGrGrArGrGrUrUrG rArGrArArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC1	251	MG3-6/3-4 TRBC1 A6	mG*mG*mA*rArCrArCrCrUrUrGrUrUrCrArGrGrUrCrCrUrCrGrUrUrG rArGrArArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
DNA sequence of TRBC1 target site	252	MG3-6/3-4 TRBC1 A1	CAGAAGCAGAGATCTCCCACAC
DNA sequence of TRBC1 target site	253	MG3-6/3-4 TRBC1 B1	CCACGTGGAGCTGAGCTGGTGG
DNA sequence of TRBC1 target site	254	MG3-6/3-4 TRBC1 C1	AGTCCAGTTCTACGGGCTCTCG
DNA sequence of TRBC1 target site	255	MG3-6/3-4 TRBC1 D1	GATTAGGTGAGACCAGCTACCA

Category	SEQ ID NO:	Name	Sequence
DNA sequence of TRBC1 target site	256	MG3-6/3-4 TRBC1 E1	ATTAGGTGAGACCAGCTACCAG
DNA sequence of TRBC1 target site	257	MG3-6/3-4 TRBC1 F1	TTAGGTGAGACCAGCTACCAGG
DNA sequence of TRBC1 target site	258	MG3-6/3-4 TRBC1 G1	TGAGACCAGCTACCAGGGAAAA
DNA sequence of TRBC1 target site	259	MG3-6/3-4 TRBC1 H1	CAGGTAGCAGACAAGACTAGAT
DNA sequence of TRBC1 target site	260	MG3-6/3-4 TRBC1 A2	AGGTAGCAGACAAGACTAGATC
DNA sequence of TRBC1 target site	261	MG3-6/3-4 TRBC1 B2	AGCAGACAAGACTAGATCCAAA
DNA sequence of TRBC1 target site	262	MG3-6/3-4 TRBC1 C2	GGAACCAGCGCACACCATGAAG
DNA sequence of TRBC1 target site	263	MG3-6/3-4 TRBC1 D2	GTGGCTGACATCTGCATGGCAG
DNA sequence of TRBC1 target site	264	MG3-6/3-4 TRBC1 E2	GGCCTGGGAGTCTGTGCCAACT
DNA sequence of TRBC1 target site	265	MG3-6/3-4 TRBC1 F2	CTGACTTTACTTTTAATTGCCT
DNA sequence of TRBC1 target site	266	MG3-6/3-4 TRBC1 G2	TGACTTTACTTTTAATTGCCTA
DNA sequence of TRBC1 target site	267	MG3-6/3-4 TRBC1 H2	GACTTTACTTTTAATTGCCTAT
DNA sequence of TRBC1 target site	268	MG3-6/3-4 TRBC1 A3	GGGAAGGAGAAGCTGGAGTCAC
DNA sequence of TRBC1 target site	269	MG3-6/3-4 TRBC1 B3	GGAAGGAGAAGCTGGAGTCACC
DNA sequence	270	MG3-6/3-4	AACTCCTGGCTCTTAATAACCC

Category	SEQ ID NO:	Name	Sequence
of TRBC1 target site		TRBC1 C3	
DNA sequence of TRBC1 target site	271	MG3-6/3-4 TRBC1 D3	AACTTTCTCTTCTGCAGGTCAA
DNA sequence of TRBC1 target site	272	MG3-6/3-4 TRBC1 E3	ACTCCACTTCCAGGGCTGCCTT
DNA sequence of TRBC1 target site	273	MG3-6/3-4 TRBC1 F3	CTCCACTTCCAGGGCTGCCTTC
DNA sequence of TRBC1 target site	274	MG3-6/3-4 TRBC1 G3	TCCTTTCTCTTGACCTGCAGAA
DNA sequence of TRBC1 target site	275	MG3-6/3-4 TRBC1 H3	AGCCAGGAGTTGTGAGGATTGA
DNA sequence of TRBC1 target site	276	MG3-6/3-4 TRBC1 A4	AGTAGTAGGGCCCATTGACCAC
DNA sequence of TRBC1 target site	277	MG3-6/3-4 TRBC1 B4	TGCAAGTTATCTTCTGAGGCAC
DNA sequence of TRBC1 target site	278	MG3-6/3-4 TRBC1 C4	AGTTATCTTCTGAGGCACCTGA
DNA sequence of TRBC1 target site	279	MG3-6/3-4 TRBC1 D4	GTTATCTTCTGAGGCACCTGAA
DNA sequence of TRBC1 target site	280	MG3-6/3-4 TRBC1 E4	TCAAGAACCATGAGAGAGGGAG
DNA sequence of TRBC1 target site	281	MG3-6/3-4 TRBC1 F4	CAAGAACCATGAGAGAGGGAGA
DNA sequence of TRBC1 target site	282	MG3-6/3-4 TRBC1 G4	TTACCCGAGGTAAAGCCACAGT
DNA sequence of TRBC1 target site	283	MG3-6/3-4 TRBC1 H4	CCGAGGTAAAGCCACAGTCTGA
DNA sequence of TRBC1 target site	284	MG3-6/3-4 TRBC1 A5	CAGTCTGAAAGAAAGCAGGGAG

Category	SEQ ID NO:	Name	Sequence
DNA sequence of TRBC1 target site	285	MG3-6/3-4 TRBC1 B5	AGTCTGAAAGAAAGCAGGGAGA
DNA sequence of TRBC1 target site	286	MG3-6/3-4 TRBC1 C5	GTCTGAAAGAAAGCAGGGAGAG
DNA sequence of TRBC1 target site	287	MG3-6/3-4 TRBC1 D5	GAAAGAAAGCAGGGAGAGGAAA
DNA sequence of TRBC1 target site	288	MG3-6/3-4 TRBC1 E5	GAGACCTTATTTTCATAGGCAA
DNA sequence of TRBC1 target site	289	MG3-6/3-4 TRBC1 F5	GATGAGAGTTACACAGGCCACA
DNA sequence of TRBC1 target site	290	MG3-6/3-4 TRBC1 G5	AGCTGCTTGGCTCTGTTGGGCT
DNA sequence of TRBC1 target site	291	MG3-6/3-4 TRBC1 H5	TGTTGGGCTGAGAATCTGGGAG
DNA sequence of TRBC1 target site	292	MG3-6/3-4 TRBC1 A6	GGAACACCTTGTTCAGGTCCTC
MG3-6/3-4 sgRNA targeting TRBC2	293	MG3-6/3-4 TRBC2 A1	mA*mC*mC*rUrCrUrUrCrCrCrUrUrUrCrCrArGrArGrGrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrCrArArGrCrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	294	MG3-6/3-4 TRBC2 B1	mC*mC*mU*rCrUrUrCrCrCrUrUrUrCrCrArGrArGrGrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrCrArArGrCrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	295	MG3-6/3-4 TRBC2 C1	mC*mU*mC*rUrUrCrCrCrUrUrUrCrCrArGrArGrGrArCrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrCrArArGrCrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	296	MG3-6/3-4 TRBC2 D1	mC*mA*mG*rArArGrCrArGrArGrArUrCrUrCrCrCrArCrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrCrArArGrCrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	297	MG3-6/3-4 TRBC2 E1	mC*mC*mA*rCrGrUrGrGrArGrCrUrGrArGrCrUrGrGrUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrCrArArGrCrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA	298	MG3-6/3-4	mA*mG*mU*rCrCrArGrUrUrCrUrArCrGrGrGrCrUrCrUrCrGrGrUrUrGrArArGrArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrCrArArGrCrGrGrCrGrGrUrArUrGrU

Category	SEQ ID NO:	Name	Sequence
targeting TRBC2		TRBC2 F1	CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	299	MG3-6/3-4 TRBC2 G1	mG*mA*mU*rUrArGrGrUrGrArGrArCrCrArGrCrUrArCrCrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	300	MG3-6/3-4 TRBC2 H1	mA*mU*mU*rArGrGrUrGrArGrArCrCrArGrCrUrArCrCrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	301	MG3-6/3-4 TRBC2 A2	mU*mU*mA*rGrGrUrGrArGrArCrCrArGrCrUrArCrCrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	302	MG3-6/3-4 TRBC2 B2	mU*mG*mA*rGrArCrCrArGrCrUrArCrCrArGrGrGrArArArArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	303	MG3-6/3-4 TRBC2 C2	mU*mA*mG*rCrGrGrArCrArArGrArCrUrArGrArUrCrCrArGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	304	MG3-6/3-4 TRBC2 D2	mC*mC*mC*rCrCrArCrCrArArGrArArGrCrArUrArGrArGrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	305	MG3-6/3-4 TRBC2 E2	mU*mC*mU*rGrCrUrCrUrCrGrArArCrCrArGrGrGrCrArUrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	306	MG3-6/3-4 TRBC2 F2	mG*mG*mA*rArCrArUrCrArCrArCrArUrGrGrGrCrArUrArArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	307	MG3-6/3-4 TRBC2 G2	mC*mC*mU*rArArUrArUrArUrCrCrUrArUrCrArCrCrUrCrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	308	MG3-6/3-4 TRBC2 H2	mA*mC*mC*rArUrArArUrGrArArGrCrCrArGrArCrUrGrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	309	MG3-6/3-4 TRBC2 A3	mC*mC*mA*rUrArArUrGrArArGrCrCrArGrArCrUrGrGrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU

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MG3-6/3-4 sgRNA targeting TRBC2	310	MG3-6/3-4 TRBC2 B3	mC*mA*mU*rArArUrGrArArGrCrCrArGrArCrUrGrGrGrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	311	MG3-6/3-4 TRBC2 C3	mG*mC*mC*rArGrArCrUrGrGrGrArGrArArArArUrGrCrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	312	MG3-6/3-4 TRBC2 D3	mG*mG*mA*rGrArArArArUrGrCrArGrGrGrArArUrArUrCrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	313	MG3-6/3-4 TRBC2 E3	mG*mG*mA*rGrArCrArArCrCrArGrCrGrArGrCrCrCrUrArCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	314	MG3-6/3-4 TRBC2 F3	mU*mA*mC*rUrCrCrUrGrCrUrGrUrGrCrCrArUrArGrCrCrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	315	MG3-6/3-4 TRBC2 G3	mC*mU*mG*rUrGrCrCrArUrArGrCrCrCrCrUrGrArArArCrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	316	MG3-6/3-4 TRBC2 H3	mU*mG*mU*rGrCrCrArUrArGrCrCrCrCrUrGrArArArCrCrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	317	MG3-6/3-4 TRBC2 A4	mG*mU*mG*rCrCrArUrArGrCrCrCrCrUrGrArArArCrCrCrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	318	MG3-6/3-4 TRBC2 B4	mU*mG*mU*rUrCrUrCrUrCrUrUrCrCrArCrArGrGrUrCrArArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	319	MG3-6/3-4 TRBC2 C4	mG*mA*mA*rArGrGrArUrUrCrCrArGrArGrGrCrUrArGrCrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	320	MG3-6/3-4 TRBC2 D4	mG*mG*mA*rUrGrGrUrUrUrUrGrGrArGrCrUrArGrCrCrUrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA	321	MG3-6/3-4	mC*mC*mC*rUrGrGrUrUrCrGrArGrArGrCrArGrArGrArCrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG

Category	SEQ ID NO:	Name	Sequence
targeting TRBC2		TRBC2 E4	rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	322	MG3-6/3-4 TRBC2 F4	mA*mG*mC*rArGrArGrArCrGrGrCrGrArArArGrArUrArGrArGrUrUrG rArGrArArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	323	MG3-6/3-4 TRBC2 G4	mG*mC*mA*rGrArGrArCrGrGrCrGrArArArGrArUrArGrArGrGrUrUrG rArGrArArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	324	MG3-6/3-4 TRBC2 H4	mC*mA*mG*rArGrArCrGrGrCrGrArArArGrArUrArGrArGrArGrUrUrG rArGrArArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	325	MG3-6/3-4 TRBC2 A5	mU*mU*mA*rCrCrGrGrArGrGrUrGrArArGrCrCrArCrArGrUrGrUrUrG rArGrArArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	326	MG3-6/3-4 TRBC2 B5	mC*mG*mG*rArGrGrUrGrArArGrCrCrArCrArGrUrCrUrGrArGrUrUrG rArGrArArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	327	MG3-6/3-4 TRBC2 C5	mG*mG*mA*rGrGrUrGrArArGrCrCrArCrArGrUrCrUrGrArArGrUrUrG rArGrArArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	328	MG3-6/3-4 TRBC2 D5	mA*mC*mA*rGrUrCrUrGrArArArGrArArArArCrArGrGrGrGrUrUrG rArGrArArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	329	MG3-6/3-4 TRBC2 E5	mC*mA*mG*rUrCrUrGrArArArGrArArArArCrArGrGrGrGrArGrUrUrG rArGrArArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	330	MG3-6/3-4 TRBC2 F5	mA*mG*mU*rCrUrGrArArArGrArArArArCrArGrGrGrGrArArGrUrUrG rArGrArArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	331	MG3-6/3-4 TRBC2 G5	mG*mU*mC*rUrGrArArArGrArArArArCrArGrGrGrGrArArGrGrUrUrG rArGrArArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	332	MG3-6/3-4 TRBC2 H5	mA*mC*mA*rGrGrGrGrArArGrArArArArArUrGrGrArUrGrArGrUrUrG rArGrArArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU

Category	SEQ ID NO:	Name	Sequence
MG3-6/3-4 sgRNA targeting TRBC2	333	MG3-6/3-4 TRBC2 A6	mG*mC*mG*rArArGrUrGrGrUrCrArCrUrArUrGrArUrCrUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	334	MG3-6/3-4 TRBC2 B6	mU*mU*mA*rGrGrArArArCrCrArGrGrArCrCrCrCrArGrArArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	335	MG3-6/3-4 TRBC2 C6	mU*mA*mU*rGrGrCrUrGrGrUrCrCrUrCrArGrGrGrArGrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	336	MG3-6/3-4 TRBC2 D6	mC*mU*mA*rArGrGrUrGrUrCrArGrGrArUrCrUrGrArArGrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting TRBC2	337	MG3-6/3-4 TRBC2 E6	mG*mG*mA*rArCrArCrGrUrUrUrUrCrArGrGrUrCrCrUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
DNA sequence of TRBC2 target site	338	MG3-6/3-4 TRBC2 A1	ACCTCTTCCCTTTCCAGAGGAC
DNA sequence of TRBC2 target site	339	MG3-6/3-4 TRBC2 B1	CCTCTTCCCTTTCCAGAGGACC
DNA sequence of TRBC2 target site	340	MG3-6/3-4 TRBC2 C1	CTCTTCCCTTTCCAGAGGACCT
DNA sequence of TRBC2 target site	341	MG3-6/3-4 TRBC2 D1	CAGAAGCAGAGATCTCCCACAC
DNA sequence of TRBC2 target site	342	MG3-6/3-4 TRBC2 E1	CCACGTGGAGCTGAGCTGGTGG
DNA sequence of TRBC2 target site	343	MG3-6/3-4 TRBC2 F1	AGTCCAGTTCTACGGGCTCTCG
DNA sequence of TRBC2 target site	344	MG3-6/3-4 TRBC2 G1	GATTAGGTGAGACCAGCTACCA
DNA sequence of TRBC2 target site	345	MG3-6/3-4 TRBC2 H1	ATTAGGTGAGACCAGCTACCAG
DNA sequence	346	MG3-6/3-4	TTAGGTGAGACCAGCTACCAGG

Category	SEQ ID NO:	Name	Sequence
of TRBC2 target site		TRBC2 A2	
DNA sequence of TRBC2 target site	347	MG3-6/3-4 TRBC2 B2	TGAGACCAGCTACCAGGGAAAA
DNA sequence of TRBC2 target site	348	MG3-6/3-4 TRBC2 C2	TAGCGGACAAGACTAGATCCAG
DNA sequence of TRBC2 target site	349	MG3-6/3-4 TRBC2 D2	CCCCCACCAAGAAGCATAGAGG
DNA sequence of TRBC2 target site	350	MG3-6/3-4 TRBC2 E2	TCTGCTCTCGAACCAGGGCATG
DNA sequence of TRBC2 target site	351	MG3-6/3-4 TRBC2 F2	GGAACATCACACATGGGCATAA
DNA sequence of TRBC2 target site	352	MG3-6/3-4 TRBC2 G2	CCTAATATATCCTATCACCTCA
DNA sequence of TRBC2 target site	353	MG3-6/3-4 TRBC2 H2	ACCATAATGAAGCCAGACTGGG
DNA sequence of TRBC2 target site	354	MG3-6/3-4 TRBC2 A3	CCATAATGAAGCCAGACTGGGG
DNA sequence of TRBC2 target site	355	MG3-6/3-4 TRBC2 B3	CATAATGAAGCCAGACTGGGGA
DNA sequence of TRBC2 target site	356	MG3-6/3-4 TRBC2 C3	GCCAGACTGGGGAGAAAATGCA
DNA sequence of TRBC2 target site	357	MG3-6/3-4 TRBC2 D3	GGAGAAAATGCAGGGAATATCA
DNA sequence of TRBC2 target site	358	MG3-6/3-4 TRBC2 E3	GGAGACAACCAGCGAGCCCTAC
DNA sequence of TRBC2 target site	359	MG3-6/3-4 TRBC2 F3	TACTCCTGCTGTGCCATAGCCC
DNA sequence of TRBC2 target site	360	MG3-6/3-4 TRBC2 G3	CTGTGCCATAGCCCCTGAAACC

Category	SEQ ID NO:	Name	Sequence
DNA sequence of TRBC2 target site	361	MG3-6/3-4 TRBC2 H3	TGTGCCATAGCCCCTGAAACCC
DNA sequence of TRBC2 target site	362	MG3-6/3-4 TRBC2 A4	GTGCCATAGCCCCTGAAACCCT
DNA sequence of TRBC2 target site	363	MG3-6/3-4 TRBC2 B4	TGTTCTCTCTTCCACAGGTCAA
DNA sequence of TRBC2 target site	364	MG3-6/3-4 TRBC2 C4	GAAAGGATTCCAGAGGCTAGCT
DNA sequence of TRBC2 target site	365	MG3-6/3-4 TRBC2 D4	GGATGGTTTTGGAGCTAGCCTC
DNA sequence of TRBC2 target site	366	MG3-6/3-4 TRBC2 E4	CCCTGGTTCGAGAGCAGAGACG
DNA sequence of TRBC2 target site	367	MG3-6/3-4 TRBC2 F4	AGCAGAGACGGCGAAAGATAGA
DNA sequence of TRBC2 target site	368	MG3-6/3-4 TRBC2 G4	GCAGAGACGGCGAAAGATAGAG
DNA sequence of TRBC2 target site	369	MG3-6/3-4 TRBC2 H4	CAGAGACGGCGAAAGATAGAGA
DNA sequence of TRBC2 target site	370	MG3-6/3-4 TRBC2 A5	TTACCGGAGGTGAAGCCACAGT
DNA sequence of TRBC2 target site	371	MG3-6/3-4 TRBC2 B5	CGGAGGTGAAGCCACAGTCTGA
DNA sequence of TRBC2 target site	372	MG3-6/3-4 TRBC2 C5	GGAGGTGAAGCCACAGTCTGAA
DNA sequence of TRBC2 target site	373	MG3-6/3-4 TRBC2 D5	ACAGTCTGAAAGAAAACAGGGG
DNA sequence of TRBC2 target site	374	MG3-6/3-4 TRBC2 E5	CAGTCTGAAAGAAAACAGGGGA
DNA sequence	375	MG3-6/3-4	AGTCTGAAAGAAAACAGGGGAA

Category	SEQ ID NO:	Name	Sequence
of TRBC2 target site		TRBC2 F5	
DNA sequence of TRBC2 target site	376	MG3-6/3-4 TRBC2 G5	GTCTGAAAGAAAACAGGGGAAG
DNA sequence of TRBC2 target site	377	MG3-6/3-4 TRBC2 H5	ACAGGGGAAGAAAAATGGATGA
DNA sequence of TRBC2 target site	378	MG3-6/3-4 TRBC2 A6	GCGAAGTGGTCACTATGATCTT
DNA sequence of TRBC2 target site	379	MG3-6/3-4 TRBC2 B6	TTAGGAAACCAGGACCCCAGAA
DNA sequence of TRBC2 target site	380	MG3-6/3-4 TRBC2 C6	TATGGCTGGTCCTCAGGGAGAC
DNA sequence of TRBC2 target site	381	MG3-6/3-4 TRBC2 D6	CTAAGGTGTCAGGATCTGAAGG
DNA sequence of TRBC2 target site	382	MG3-6/3-4 TRBC2 E6	GGAACACGTTTTTCAGGTCCTC
(r =native ribose base, m = 2'-O methyl modified base, F = 2' Fluro modified base, * = phosphorothioate bond)			

Example 16 – Analysis of gene-editing outcomes at the DNA level for ANGPTL3 in Hep3B cells

[00159] Nucleofection of MG3-6/4 RNPs (104 pmol protein/120 pmol guide) comprising sgRNAs described below in Table 7D below and SEQ ID NOs: 383-572 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. The amplicons were sequenced on an Illumina MiSeq machine and analyzed with a proprietary Python script to measure gene editing **(FIG. 21)**. The results indicate that sgRNA E5, C6, A7, A8, A9, G9, G10, E11, A12, and C12 are the highest performing sgRNAs in this assay.

Table 7D: gRNAs and Targeting Sequences Used in Example 16

Category	SEQ ID NO:	Name	Sequence
MG3-6/3-4 sgRNA targeting ANGPTL3	383	MG3-6/3-4 ANGPTL3 A1	mU*mU*mG*rUrUrCrCrUrCrUrArGrUrUrArUrUrUrCrCrUrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	384	MG3-6/3-4 ANGPTL3 B1	mA*mU*mU*rUrGrArUrUrCrUrCrUrArUrCrUrCrCrArGrArGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	385	MG3-6/3-4 ANGPTL3 C1	mU*mU*mU*rGrArUrUrCrUrCrUrArUrCrUrCrCrArGrArGrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	386	MG3-6/3-4 ANGPTL3 D1	mA*mA*mG*rArUrUrUrGrCrUrArUrGrUrUrArGrArCrGrArUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	387	MG3-6/3-4 ANGPTL3 E1	mA*mG*mA*rUrUrUrGrCrUrArUrGrUrUrArGrArCrGrArUrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	388	MG3-6/3-4 ANGPTL3 F1	mG*mA*mU*rUrUrGrCrUrArUrGrUrUrArGrArCrGrArUrGrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	389	MG3-6/3-4 ANGPTL3 G1	mA*mC*mU*rUrUrGrUrCrCrArUrArArGrArCrGrArArGrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	390	MG3-6/3-4 ANGPTL3 H1	mA*mG*mG*rGrCrCrArArArUrUrArArArUrGrArCrArUrArUrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	391	MG3-6/3-4 ANGPTL3 A2	mG*mG*mG*rCrCrArArArUrUrArArArUrGrArCrArUrArUrUrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	392	MG3-6/3-4 ANGPTL3 B2	mU*mA*mU*rGrArUrCrUrArUrCrGrCrUrGrCrArArArCrCrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	393	MG3-6/3-4 ANGPTL3 C2	mA*mU*mG*rArUrCrUrArUrCrGrCrUrGrCrArArArCrCrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting	394	MG3-6/3-4	mC*mA*mA*rArCrCrArGrUrGrArArArUrCrArArArGrArArGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG

Category	SEQ ID NO:	Name	Sequence
ANGPTL 3		ANGPT L3 D2	rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	395	MG3-6/3-4 ANGPT L3 E2	mA*mA*mA*rCrCrArGrUrGrArArArUrCrArArArGrArArGrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	396	MG3-6/3-4 ANGPT L3 F2	mA*mC*mA*rArGrUrCrArArArArArUrGrArArGrArGrGrUrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	397	MG3-6/3-4 ANGPT L3 G2	mG*mA*mA*rUrArUrGrUrCrArCrUrUrGrArArCrUrCrArArCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	398	MG3-6/3-4 ANGPT L3 H2	mU*mC*mA*rCrUrUrGrArArCrUrCrArArCrUrCrArArArArCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	399	MG3-6/3-4 ANGPT L3 A3	mU*mC*mA*rArArArCrUrUrGrArArArGrCrCrUrCrCrUrArGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	400	MG3-6/3-4 ANGPT L3 B3	mC*mA*mA*rArArCrUrUrGrArArArGrCrCrUrCrCrUrArGrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	401	MG3-6/3-4 ANGPT L3 C3	mA*mA*mA*rArCrUrUrGrArArArGrCrCrUrCrCrUrArGrArArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	402	MG3-6/3-4 ANGPT L3 D3	mA*mA*mA*rCrUrUrGrArArArGrCrCrUrCrCrUrArGrArArGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	403	MG3-6/3-4 ANGPT L3 E3	mA*mA*mC*rUrUrGrArArArGrCrCrUrCrCrUrArGrArArGrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	404	MG3-6/3-4 ANGPT L3 F3	mG*mU*mU*rCrUrGrGrArGrUrUrUrCrArGrGrUrUrGrArUrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	405	MG3-6/3-4 ANGPT L3 G3	mC*mA*mC*rUrGrGrUrUrUrGrCrArGrCrGrArUrArGrArUrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU

Category	SEQ ID NO:	Name	Sequence
MG3-6/3-4 sgRNA targeting ANGPTL3	406	MG3-6/3-4 ANGPTL3 H3	mA*mC*mU*rGrGrUrUrUrGrCrArGrCrGrArUrArGrArUrCrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	407	MG3-6/3-4 ANGPTL3 A4	mC*mG*mA*rUrArGrArUrCrArUrArArArArArGrArCrUrGrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	408	MG3-6/3-4 ANGPTL3 B4	mC*mC*mC*rArArCrUrGrArArGrGrArGrGrCrCrArUrUrGrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	409	MG3-6/3-4 ANGPTL3 C4	mC*mC*mA*rArCrUrGrArArGrGrArGrGrCrCrArUrUrGrGrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	410	MG3-6/3-4 ANGPTL3 D4	mC*mU*mU*rGrArUrUrUrUrGrGrCrUrCrUrGrGrArGrArUrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	411	MG3-6/3-4 ANGPTL3 E4	mU*mU*mU*rUrGrGrCrUrCrUrGrGrArGrArUrArGrArGrArArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	412	MG3-6/3-4 ANGPTL3 F4	mU*mC*mU*rGrGrArGrArUrArGrArGrArArUrCrArArArUrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	413	MG3-6/3-4 ANGPTL3 G4	mG*mA*mA*rUrUrGrUrCrUrUrGrArUrCrArArUrUrCrUrGrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	414	MG3-6/3-4 ANGPTL3 H4	mA*mA*mU*rUrGrUrCrUrUrGrArUrCrArArUrUrCrUrGrGrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	415	MG3-6/3-4 ANGPTL3 A5	mG*mG*mA*rGrGrArArArUrArArCrUrArGrArGrGrArArCrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	416	MG3-6/3-4 ANGPTL3 B5	mG*mA*mG*rGrArArArUrArArCrUrArGrArGrGrArArCrArArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting	417	MG3-6/3-4	mA*mC*mU*rCrUrCrUrArUrArUrCrCrArGrArCrUrUrUrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG

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ANGPTL 3		ANGPT L3 C5	rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	418	MG3-6/3-4 ANGPT L3 D5	mC*mU*mC*rUrCrUrArUrArUrCrCrArGrArCrUrUrUrUrGrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	419	MG3-6/3-4 ANGPT L3 E5	mU*mC*mU*rCrUrArUrArUrCrCrArGrArCrUrUrUrUrGrUrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	420	MG3-6/3-4 ANGPT L3 F5	mA*mA*mC*rArArUrUrArArArCrCrArArCrArGrCrArUrArGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	421	MG3-6/3-4 ANGPT L3 G5	mA*mU*mU*rArArArCrCrArArCrArGrCrArUrArGrUrCrArArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	422	MG3-6/3-4 ANGPT L3 H5	mA*mA*mC*rCrArArCrArGrCrArUrArGrUrCrArArArUrArArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	423	MG3-6/3-4 ANGPT L3 A6	mA*mC*mC*rArArCrArGrCrArUrArGrUrCrArArArUrArArArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	424	MG3-6/3-4 ANGPT L3 B6	mG*mA*mU*rGrCrUrArUrUrArUrCrUrUrGrUrUrUrUrUrCrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	425	MG3-6/3-4 ANGPT L3 C6	mA*mG*mG*rArCrUrArGrUrArUrUrCrArArGrArArCrCrCrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	426	MG3-6/3-4 ANGPT L3 D6	mG*mG*mA*rCrUrArGrUrArUrUrCrArArGrArArCrCrCrArCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	427	MG3-6/3-4 ANGPT L3 E6	mA*mA*mG*rArArCrUrArCrUrCrCrCrUrUrUrCrUrUrCrArGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	428	MG3-6/3-4 ANGPT L3 F6	mA*mC*mU*rArCrUrCrCrCrUrUrUrCrUrUrCrArGrUrUrGrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU

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MG3-6/3-4 sgRNA targeting ANGPTL3	429	MG3-6/3-4 ANGPTL3 G6	mC*mU*mA*rCrUrCrCrCrUrUrUrCrUrUrCrArGrUrUrGrArArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	430	MG3-6/3-4 ANGPTL3 H6	mC*mC*mU*rUrUrCrUrUrCrArGrUrUrGrArArUrGrArArArUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	431	MG3-6/3-4 ANGPTL3 A7	mG*mG*mU*rGrCrUrCrUrUrGrGrCrUrUrGrGrArArGrArUrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	432	MG3-6/3-4 ANGPTL3 B7	mG*mU*mG*rCrUrCrUrUrGrGrCrUrUrGrGrArArGrArUrArGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	433	MG3-6/3-4 ANGPTL3 C7	mA*mU*mA*rGrArGrArArArUrUrUrCrUrGrUrGrGrGrUrUrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	434	MG3-6/3-4 ANGPTL3 D7	mG*mA*mA*rUrArCrUrArGrUrCrCrUrUrCrUrGrArGrCrUrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	435	MG3-6/3-4 ANGPTL3 E7	mU*mU*mA*rUrUrGrArUrUrCrUrArGrGrCrArUrUrCrCrUrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	436	MG3-6/3-4 ANGPTL3 F7	mG*mU*mC*rUrArCrUrGrUrGrArUrGrUrUrArUrArUrCrArGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	437	MG3-6/3-4 ANGPTL3 G7	mC*mU*mG*rArUrArUrArArCrArUrCrArCrArGrUrArGrArCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	438	MG3-6/3-4 ANGPTL3 H7	mU*mG*mA*rUrArUrArArCrArUrCrArCrArGrUrArGrArCrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	439	MG3-6/3-4 ANGPTL3 A8	mG*mA*mU*rArUrArArCrArUrCrArCrArGrUrArGrArCrArUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting	440	MG3-6/3-4	mC*mA*mC*rUrUrGrUrArUrGrUrUrCrArCrCrUrCrUrGrUrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG

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ANGPTL 3		ANGPTL3 B8	rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	441	MG3-6/3-4 ANGPTL3 C8	mU*mA*mU*rArArArUrGrGrUrGrGrUrArCrArUrUrCrArGrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	442	MG3-6/3-4 ANGPTL3 D8	mU*mG*mG*rUrArCrArUrUrCrArGrCrArGrGrArArUrGrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	443	MG3-6/3-4 ANGPTL3 E8	mG*mU*mC*rCrArUrGrGrArCrArUrUrArArUrUrCrArArCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	444	MG3-6/3-4 ANGPTL3 F8	mU*mU*mC*rArArCrArUrCrGrArArUrArGrArUrGrGrArUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	445	MG3-6/3-4 ANGPTL3 G8	mA*mU*mA*rGrArUrGrGrArUrCrArCrArArArArCrUrUrCrArGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	446	MG3-6/3-4 ANGPTL3 H8	mU*mU*mC*rArArUrGrArArArCrGrUrGrGrGrArGrArArCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	447	MG3-6/3-4 ANGPTL3 A9	mA*mG*mU*rCrCrCrCrUrUrArCrCrArUrCrArArGrCrCrUrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	448	MG3-6/3-4 ANGPTL3 B9	mU*mU*mU*rGrUrGrArUrCrCrArUrCrUrArUrUrUrCrGrArUrGrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	449	MG3-6/3-4 ANGPTL3 C9	mU*mG*mA*rArUrUrArArUrGrUrCrCrArUrGrGrArCrUrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	450	MG3-6/3-4 ANGPTL3 D9	mU*mU*mU*rArCrGrArArUrUrGrArGrUrUrGrGrArArGrArCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	451	MG3-6/3-4 ANGPTL3 E9	mG*mG*mC*rArArUrGrUrCrCrCrCrArArUrGrCrArArUrCrCrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU

Category	SEQ ID NO:	Name	Sequence
MG3-6/3-4 sgRNA targeting ANGPTL3	452	MG3-6/3-4 ANGPTL3 F9	mG*mC*mA*rArUrGrUrCrCrCrCrArArUrGrCrArArUrCrCrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	453	MG3-6/3-4 ANGPTL3 G9	mG*mU*mU*rUrUrCrUrArCrUrUrGrGrGrArUrCrArCrArArArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	454	MG3-6/3-4 ANGPTL3 H9	mC*mC*mU*rUrUrUrGrCrUrUrUrGrUrGrArUrCrCrCrArArGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	455	MG3-6/3-4 ANGPTL3 A10	mC*mU*mU*rUrUrGrCrUrUrUrGrUrGrArUrCrCrCrArArGrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	456	MG3-6/3-4 ANGPTL3 B10	mU*mU*mG*rUrGrArUrCrCrCrArArGrUrArGrArArArArCrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	457	MG3-6/3-4 ANGPTL3 C10	mA*mG*mU*rUrGrGrUrUrUrCrGrUrGrArUrUrUrCrCrCrArArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	458	MG3-6/3-4 ANGPTL3 D10	mG*mU*mU*rGrGrUrUrUrCrGrUrGrArUrUrUrCrCrCrArArGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	459	MG3-6/3-4 ANGPTL3 E10	mG*mU*mU*rUrCrGrUrGrArUrUrUrCrCrCrArArGrUrArArArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	460	MG3-6/3-4 ANGPTL3 F10	mU*mU*mC*rCrArGrUrCrUrUrCrCrArArCrUrCrArArUrUrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	461	MG3-6/3-4 ANGPTL3 G10	mA*mG*mU*rArUrArUrCrUrUrCrUrCrUrArGrGrCrCrCrArArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL3	462	MG3-6/3-4 ANGPTL3 H10	mG*mU*mA*rUrArUrCrUrUrCrUrCrUrArGrGrCrCrCrArArCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting	463	MG3-6/3-4	mU*mC*mU*rArGrGrCrCrCrArArCrCrArArArArUrUrCrUrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG

Category	SEQ ID NO:	Name	Sequence
ANGPTL 3		ANGPT L3 A11	rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	464	MG3-6/3-4 ANGPT L3 B11	mC*mU*mA*rGrGrCrCrCrArArCrCrArArArArUrUrCrUrCrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	465	MG3-6/3-4 ANGPT L3 C11	mG*mC*mC*rCrArArCrCrArArArArUrUrCrUrCrCrUrGrArArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	466	MG3-6/3-4 ANGPT L3 D11	mU*mG*mG*rUrGrGrUrGrGrCrArUrGrArUrGrArGrUrGrUrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	467	MG3-6/3-4 ANGPT L3 E11	mG*mG*mU*rGrGrUrGrGrCrArUrGrArUrGrArGrUrGrUrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	468	MG3-6/3-4 ANGPT L3 F11	mU*mG*mA*rUrGrArGrUrGrUrGrGrArGrArArArArCrArArCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	469	MG3-6/3-4 ANGPT L3 G11	mU*mG*mU*rGrGrArGrArArArArCrArArCrCrUrArArArUrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	470	MG3-6/3-4 ANGPT L3 H11	mG*mG*mU*rArArArUrArUrArArCrArArArCrCrArArGrArGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	471	MG3-6/3-4 ANGPT L3 A12	mG*mA*mA*rGrArGrGrArUrUrArUrCrUrUrGrGrArArGrUrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	472	MG3-6/3-4 ANGPT L3 B12	mA*mA*mG*rArGrGrArUrUrArUrCrUrUrGrGrArArGrUrCrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	473	MG3-6/3-4 ANGPT L3 C12	mU*mC*mA*rArArArUrGrGrArArGrGrUrUrArUrArCrUrCrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	474	MG3-6/3-4 ANGPT L3 D12	mC*mA*mA*rArArUrGrGrArArGrGrUrUrArUrArCrUrCrUrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU

Category	SEQ ID NO:	Name	Sequence
MG3-6/3-4 sgRNA targeting ANGPTL 3	475	MG3-6/3-4 ANGPTL3 E12	mA*mU*mG*rUrUrGrArUrCrCrArUrCrCrArArCrArGrArUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	476	MG3-6/3-4 ANGPTL3 F12	mC*mA*mU*rCrCrArArCrArGrArUrUrCrArGrArArArGrCrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
MG3-6/3-4 sgRNA targeting ANGPTL 3	477	MG3-6/3-4 ANGPTL3 G12	mG*mC*mC*rUrCrArGrUrUrCrArUrUrCrArArArGrCrUrUrUrGrUrUrGrArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArArGrGrCrArUrCrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrGrUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU*mU*mU
DNA sequence of ANGPTL 3 target site	478	MG3-6/3-4 ANGPTL3 A1	TTGTTCCTCTAGTTATTTCCTC
DNA sequence of ANGPTL 3 target site	479	MG3-6/3-4 ANGPTL3 B1	ATTTGATTCTCTATCTCCAGAG
DNA sequence of ANGPTL 3 target site	480	MG3-6/3-4 ANGPTL3 C1	TTTGATTCTCTATCTCCAGAGC
DNA sequence of ANGPTL 3 target site	481	MG3-6/3-4 ANGPTL3 D1	AAGATTTGCTATGTTAGACGAT
DNA sequence of ANGPTL 3 target site	482	MG3-6/3-4 ANGPTL3 E1	AGATTTGCTATGTTAGACGATG
DNA sequence of ANGPTL 3 target site	483	MG3-6/3-4 ANGPTL3 F1	GATTTGCTATGTTAGACGATGT
DNA sequence of ANGPTL 3 target site	484	MG3-6/3-4 ANGPTL3 G1	ACTTTGTCCATAAGACGAAGGG
DNA sequence	485	MG3-6/3-4	AGGGCCAAATTAATGACATATT

Category	SEQ ID NO:	Name	Sequence
of ANGPTL 3 target site		ANGPTL3 H1	
DNA sequence of ANGPTL 3 target site	486	MG3-6/3-4 ANGPTL3 A2	GGGCCAAATTAATGACATATTT
DNA sequence of ANGPTL 3 target site	487	MG3-6/3-4 ANGPTL3 B2	TATGATCTATCGCTGCAAACCA
DNA sequence of ANGPTL 3 target site	488	MG3-6/3-4 ANGPTL3 C2	ATGATCTATCGCTGCAAACCAG
DNA sequence of ANGPTL 3 target site	489	MG3-6/3-4 ANGPTL3 D2	CAAACCAGTGAAATCAAAGAAG
DNA sequence of ANGPTL 3 target site	490	MG3-6/3-4 ANGPTL3 E2	AAACCAGTGAAATCAAAGAAGA
DNA sequence of ANGPTL 3 target site	491	MG3-6/3-4 ANGPTL3 F2	ACAAGTCAAAAATGAAGAGGTA
DNA sequence of ANGPTL 3 target site	492	MG3-6/3-4 ANGPTL3 G2	GAATATGTCACTTGAACTCAAC
DNA sequence of ANGPTL 3 target site	493	MG3-6/3-4 ANGPTL3 H2	TCACTTGAACTCAACTCAAAAC
DNA sequence of ANGPTL 3 target site	494	MG3-6/3-4 ANGPTL3 A3	TCAAAACTTGAAAGCCTCCTAG

Category	SEQ ID NO:	Name	Sequence
DNA sequence of ANGPTL 3 target site	495	MG3-6/3-4 ANGPTL3 B3	CAAAACTTGAAAGCCTCCTAGA
DNA sequence of ANGPTL 3 target site	496	MG3-6/3-4 ANGPTL3 C3	AAAACTTGAAAGCCTCCTAGAA
DNA sequence of ANGPTL 3 target site	497	MG3-6/3-4 ANGPTL3 D3	AAACTTGAAAGCCTCCTAGAAG
DNA sequence of ANGPTL 3 target site	498	MG3-6/3-4 ANGPTL3 E3	AACTTGAAAGCCTCCTAGAAGA
DNA sequence of ANGPTL 3 target site	499	MG3-6/3-4 ANGPTL3 F3	GTTCTGGAGTTTCAGGTTGATT
DNA sequence of ANGPTL 3 target site	500	MG3-6/3-4 ANGPTL3 G3	CACTGGTTTGCAGCGATAGATC
DNA sequence of ANGPTL 3 target site	501	MG3-6/3-4 ANGPTL3 H3	ACTGGTTTGCAGCGATAGATCA
DNA sequence of ANGPTL 3 target site	502	MG3-6/3-4 ANGPTL3 A4	CGATAGATCATAAAAAGACTGA
DNA sequence of ANGPTL 3 target site	503	MG3-6/3-4 ANGPTL3 B4	CCCAACTGAAGGAGGCCATTGG
DNA sequence of ANGPTL	504	MG3-6/3-4 ANGPTL3 C4	CCAACTGAAGGAGGCCATTGGC

Category	SEQ ID NO:	Name	Sequence
3 target site			
DNA sequence of ANGPTL 3 target site	505	MG3-6/3-4 ANGPTL3 D4	CTTGATTTTGGCTCTGGAGATA
DNA sequence of ANGPTL 3 target site	506	MG3-6/3-4 ANGPTL3 E4	TTTTGGCTCTGGAGATAGAGAA
DNA sequence of ANGPTL 3 target site	507	MG3-6/3-4 ANGPTL3 F4	TCTGGAGATAGAGAATCAAATG
DNA sequence of ANGPTL 3 target site	508	MG3-6/3-4 ANGPTL3 G4	GAATTGTCTTGATCAATTCTGG
DNA sequence of ANGPTL 3 target site	509	MG3-6/3-4 ANGPTL3 H4	AATTGTCTTGATCAATTCTGGA
DNA sequence of ANGPTL 3 target site	510	MG3-6/3-4 ANGPTL3 A5	GGAGGAAATAACTAGAGGAACA
DNA sequence of ANGPTL 3 target site	511	MG3-6/3-4 ANGPTL3 B5	GAGGAAATAACTAGAGGAACAA
DNA sequence of ANGPTL 3 target site	512	MG3-6/3-4 ANGPTL3 C5	ACTCTCTATATCCAGACTTTTG
DNA sequence of ANGPTL 3 target site	513	MG3-6/3-4 ANGPTL3 D5	CTCTCTATATCCAGACTTTTGT
DNA sequence of	514	MG3-6/3-4	TCTCTATATCCAGACTTTTGTA

Category	SEQ ID NO:	Name	Sequence
ANGPTL 3 target site		ANGPTL3 E5	
DNA sequence of ANGPTL 3 target site	515	MG3-6/3-4 ANGPTL3 F5	AACAATTAAACCAACAGCATAG
DNA sequence of ANGPTL 3 target site	516	MG3-6/3-4 ANGPTL3 G5	ATTAAACCAACAGCATAGTCAA
DNA sequence of ANGPTL 3 target site	517	MG3-6/3-4 ANGPTL3 H5	AACCAACAGCATAGTCAAATAA
DNA sequence of ANGPTL 3 target site	518	MG3-6/3-4 ANGPTL3 A6	ACCAACAGCATAGTCAAATAAA
DNA sequence of ANGPTL 3 target site	519	MG3-6/3-4 ANGPTL3 B6	GATGCTATTATCTTGTTTTTCT
DNA sequence of ANGPTL 3 target site	520	MG3-6/3-4 ANGPTL3 C6	AGGACTAGTATTCAAGAACCCA
DNA sequence of ANGPTL 3 target site	521	MG3-6/3-4 ANGPTL3 D6	GGACTAGTATTCAAGAACCCAC
DNA sequence of ANGPTL 3 target site	522	MG3-6/3-4 ANGPTL3 E6	AAGAACTACTCCCTTTCTTCAG
DNA sequence of ANGPTL 3 target site	523	MG3-6/3-4 ANGPTL3 F6	ACTACTCCCTTTCTTCAGTTGA
DNA sequence	524	MG3-6/3-4	CTACTCCCTTTCTTCAGTTGAA

Category	SEQ ID NO:	Name	Sequence
of ANGPTL 3 target site		ANGPTL3 G6	
DNA sequence of ANGPTL 3 target site	525	MG3-6/3-4 ANGPTL3 H6	CCTTTCTTCAGTTGAATGAAAT
DNA sequence of ANGPTL 3 target site	526	MG3-6/3-4 ANGPTL3 A7	GGTGCTCTTGGCTTGGAAGATA
DNA sequence of ANGPTL 3 target site	527	MG3-6/3-4 ANGPTL3 B7	GTGCTCTTGGCTTGGAAGATAG
DNA sequence of ANGPTL 3 target site	528	MG3-6/3-4 ANGPTL3 C7	ATAGAGAAATTTCTGTGGGTTC
DNA sequence of ANGPTL 3 target site	529	MG3-6/3-4 ANGPTL3 D7	GAATACTAGTCCTTCTGAGCTG
DNA sequence of ANGPTL 3 target site	530	MG3-6/3-4 ANGPTL3 E7	TTATTGATTCTAGGCATTCCTG
DNA sequence of ANGPTL 3 target site	531	MG3-6/3-4 ANGPTL3 F7	GTCTACTGTGATGTTATATCAG
DNA sequence of ANGPTL 3 target site	532	MG3-6/3-4 ANGPTL3 G7	CTGATATAACATCACAGTAGAC
DNA sequence of ANGPTL 3 target site	533	MG3-6/3-4 ANGPTL3 H7	TGATATAACATCACAGTAGACA

Category	SEQ ID NO:	Name	Sequence
DNA sequence of ANGPTL 3 target site	534	MG3-6/3-4 ANGPTL3 A8	GATATAACATCACAGTAGACAT
DNA sequence of ANGPTL 3 target site	535	MG3-6/3-4 ANGPTL3 B8	CACTTGTATGTTCACCTCTGTT
DNA sequence of ANGPTL 3 target site	536	MG3-6/3-4 ANGPTL3 C8	TATAAATGGTGGTACATTCAGC
DNA sequence of ANGPTL 3 target site	537	MG3-6/3-4 ANGPTL3 D8	TGGTACATTCAGCAGGAATGCC
DNA sequence of ANGPTL 3 target site	538	MG3-6/3-4 ANGPTL3 E8	GTCCATGGACATTAATTCAACA
DNA sequence of ANGPTL 3 target site	539	MG3-6/3-4 ANGPTL3 F8	TTCAACATCGAATAGATGGATC
DNA sequence of ANGPTL 3 target site	540	MG3-6/3-4 ANGPTL3 G8	ATAGATGGATCACAAAACTTCA
DNA sequence of ANGPTL 3 target site	541	MG3-6/3-4 ANGPTL3 H8	TTCAATGAAACGTGGGAGAACT
DNA sequence of ANGPTL 3 target site	542	MG3-6/3-4 ANGPTL3 A9	AGTCCCCTTACCATCAAGCCTC
DNA sequence of ANGPTL	543	MG3-6/3-4 ANGPTL3 B9	TTTGTGATCCATCTATTCGATG

Category	SEQ ID NO:	Name	Sequence
3 target site			
DNA sequence of ANGPTL 3 target site	544	MG3-6/3-4 ANGPTL3 C9	TGAATTAATGTCCATGGACTAC
DNA sequence of ANGPTL 3 target site	545	MG3-6/3-4 ANGPTL3 D9	TTTACGAATTGAGTTGGAAGAC
DNA sequence of ANGPTL 3 target site	546	MG3-6/3-4 ANGPTL3 E9	GGCAATGTCCCCAATGCAATCC
DNA sequence of ANGPTL 3 target site	547	MG3-6/3-4 ANGPTL3 F9	GCAATGTCCCCAATGCAATCCC
DNA sequence of ANGPTL 3 target site	548	MG3-6/3-4 ANGPTL3 G9	GTTTTCTACTTGGGATCACAAA
DNA sequence of ANGPTL 3 target site	549	MG3-6/3-4 ANGPTL3 H9	CCTTTTGCTTTGTGATCCCAAG
DNA sequence of ANGPTL 3 target site	550	MG3-6/3-4 ANGPTL3 A10	CTTTTGCTTTGTGATCCCAAGT
DNA sequence of ANGPTL 3 target site	551	MG3-6/3-4 ANGPTL3 B10	TTGTGATCCCAAGTAGAAAACA
DNA sequence of ANGPTL 3 target site	552	MG3-6/3-4 ANGPTL3 C10	AGTTGGTTTCGTGATTTCCCAA
DNA sequence of	553	MG3-6/3-4	GTTGGTTTCGTGATTTCCCAAG

Category	SEQ ID NO:	Name	Sequence
ANGPTL 3 target site		ANGPT L3 D10	
DNA sequence of ANGPTL 3 target site	554	MG3-6/3-4 ANGPT L3 E10	GTTTCGTGATTTCCCAAGTAAA
DNA sequence of ANGPTL 3 target site	555	MG3-6/3-4 ANGPT L3 F10	TTCCAGTCTTCCAACTCAATTC
DNA sequence of ANGPTL 3 target site	556	MG3-6/3-4 ANGPT L3 G10	AGTATATCTTCTCTAGGCCCAA
DNA sequence of ANGPTL 3 target site	557	MG3-6/3-4 ANGPT L3 H10	GTATATCTTCTCTAGGCCCAAC
DNA sequence of ANGPTL 3 target site	558	MG3-6/3-4 ANGPT L3 A11	TCTAGGCCCAACCAAAATTCTC
DNA sequence of ANGPTL 3 target site	559	MG3-6/3-4 ANGPT L3 B11	CTAGGCCCAACCAAAATTCTCC
DNA sequence of ANGPTL 3 target site	560	MG3-6/3-4 ANGPT L3 C11	GCCCAACCAAAATTCTCCTGAA
DNA sequence of ANGPTL 3 target site	561	MG3-6/3-4 ANGPT L3 D11	TGGTGGTGGCATGATGAGTGTG
DNA sequence of ANGPTL 3 target site	562	MG3-6/3-4 ANGPT L3 E11	GGTGGTGGCATGATGAGTGTGG
DNA sequence	563	MG3-6/3-4	TGATGAGTGTGGAGAAAACAAC

Category	SEQ ID NO:	Name	Sequence
of ANGPTL 3 target site		ANGPTL3 F11	
DNA sequence of ANGPTL 3 target site	564	MG3-6/3-4 ANGPTL3 G11	TGTGGAGAAAACAACCTAAATG
DNA sequence of ANGPTL 3 target site	565	MG3-6/3-4 ANGPTL3 H11	GGTAAATATAACAAACCAAGAG
DNA sequence of ANGPTL 3 target site	566	MG3-6/3-4 ANGPTL3 A12	GAAGAGGATTATCTTGGAAGTC
DNA sequence of ANGPTL 3 target site	567	MG3-6/3-4 ANGPTL3 B12	AAGAGGATTATCTTGGAAGTCT
DNA sequence of ANGPTL 3 target site	568	MG3-6/3-4 ANGPTL3 C12	TCAAAATGGAAGGTTATACTCT
DNA sequence of ANGPTL 3 target site	569	MG3-6/3-4 ANGPTL3 D12	CAAAATGGAAGGTTATACTCTA
DNA sequence of ANGPTL 3 target site	570	MG3-6/3-4 ANGPTL3 E12	ATGTTGATCCATCCAACAGATT
DNA sequence of ANGPTL 3 target site	571	MG3-6/3-4 ANGPTL3 F12	CATCCAACAGATTCAGAAAGCT
DNA sequence of ANGPTL 3 target site	572	MG3-6/3-4 ANGPTL3 G12	GCCTCAGTTCATTCAAAGCTTT

Category	SEQ ID NO:	Name	Sequence
(r =native ribose base, m = 2'-O methyl modified base, F = 2' Fluro modified base, * = phosphorothioate bond)			

Example 17 – Analysis of gene-editing outcomes at the DNA level for PCSK9 in Hep3B cells

[00160] Nucleofection of MG3-6/4 RNPs (104 pmol protein/120 pmol guide) comprising sgRNAs described below in Table 7E below and SEQ ID NOs: 573-602 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. The amplicons were sequenced on an Illumina MiSeq machine and analyzed with a proprietary Python script to measure gene editing **(FIG. 22)**. Results indicate that the highest editing performance was achieved with sgRNAs B1, F1, A2, and E2, with appreciable editing also occurring with D2, C2, B2, H1, and F2.

Table 7E: gRNAs and Targeting Sequences Used in Example 17

Category	SEQ ID NO:	Name	Sequence
MG3-6/3-4 sgRNA targeting PCSK9	573	MG3-6/3-4 PCSK9 A1	mA*mC*mC*rCrCrUrCrCrArCrGrGrUrArCrCrGrGrGrCrGrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting PCSK9	574	MG3-6/3-4 PCSK9 B1	mA*mC*mC*rArGrCrArUrArCrArGrArGrUrGrArCrCrArCrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting PCSK9	575	MG3-6/3-4 PCSK9 C1	mC*mC*mA*rGrCrArUrArCrArGrArGrUrGrArCrCrArCrCrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting PCSK9	576	MG3-6/3-4 PCSK9 D1	mC*mA*mG*rGrGrUrCrArUrGrGrUrCrArCrCrGrArCrUrUrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting PCSK9	577	MG3-6/3-4 PCSK9 E1	mC*mC*mU*rCrCrCrArGrGrCrCrUrGrGrArGrUrUrUrArUrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting PCSK9	578	MG3-6/3-4 PCSK9 F1	mC*mU*mC*rCrCrArGrGrCrCrUrGrGrArGrUrUrUrArUrUrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU

Category	SEQ ID NO:	Name	Sequence
MG3-6/3-4 sgRNA targeting PCSK9	579	MG3-6/3-4 PCSK9 G1	mC*mA*mG*rGrCrUrGrGrArCrCrArGrCrUrGrGrCrUrUrUrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting PCSK9	580	MG3-6/3-4 PCSK9 H1	mG*mG*mU*rGrGrCrCrCrCrArArCrUrGrUrGrArUrGrArCrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting PCSK9	581	MG3-6/3-4 PCSK9 A2	mG*mC*mC*rCrCrGrCrCrGrCrUrUrCrCrCrArCrUrCrCrUrGrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting PCSK9	582	MG3-6/3-4 PCSK9 B2	mA*mG*mU*rGrUrGrCrUrGrArCrCrArUrArCrArGrUrCrCrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting PCSK9	583	MG3-6/3-4 PCSK9 C2	mC*mC*mU*rGrCrArArArArCrArGrCrUrGrCrCrArArCrCrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting PCSK9	584	MG3-6/3-4 PCSK9 D2	mC*mU*mG*rCrArArArArCrArGrCrUrGrCrCrArArCrCrUrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting PCSK9	585	MG3-6/3-4 PCSK9 E2	mA*mA*mU*rGrGrCrGrUrArGrArCrArCrCrCrUrCrArCrCrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting PCSK9	586	MG3-6/3-4 PCSK9 F2	mU*mC*mC*rUrGrCrUrGrCrCrArUrGrCrCrCrCrArGrGrUrCrGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
MG3-6/3-4 sgRNA targeting PCSK9	587	MG3-6/3-4 PCSK9 G2	mU*mG*mG*rArArUrGrCrArArArGrUrCrArArGrGrArGrCrArGrUrUrG rArGrArArUrCrGrArArArGrArUrUrCrUrUrArArUrArArGrGrCrArUr CrCrUrUrCrCrGrArUrGrCrUrGrArCrUrUrCrUrCrArCrCrGrUrCrCrG rUrUrUrUrCrCrArArUrArGrGrArGrCrGrGrGrCrGrGrUrArUrGrU*mU *mU*mU
DNA sequence of PCSK9 target site	588	MG3-6/3-4 PCSK9 A1	ACCCCTCCACGGTACCGGGCGG
DNA sequence of PCSK9 target site	589	MG3-6/3-4 PCSK9 B1	ACCAGCATACAGAGTGACCACC
DNA sequence of PCSK9 target site	590	MG3-6/3-4 PCSK9 C1	CCAGCATACAGAGTGACCACCG

Category	SEQ ID NO:	Name	Sequence
DNA sequence of PCSK9 target site	591	MG3-6/3-4 PCSK9 D1	CAGGGTCATGGTCACCGACTTC
DNA sequence of PCSK9 target site	592	MG3-6/3-4 PCSK9 E1	CCTCCCAGGCCTGGAGTTTATT
DNA sequence of PCSK9 target site	593	MG3-6/3-4 PCSK9 F1	CTCCCAGGCCTGGAGTTTATTC
DNA sequence of PCSK9 target site	594	MG3-6/3-4 PCSK9 G1	CAGGCTGGACCAGCTGGCTTTT
DNA sequence of PCSK9 target site	595	MG3-6/3-4 PCSK9 H1	GGTGGCCCCAACTGTGATGACC
DNA sequence of PCSK9 target site	596	MG3-6/3-4 PCSK9 A2	GCCCCGCCGCTTCCCACTCCTG
DNA sequence of PCSK9 target site	597	MG3-6/3-4 PCSK9 B2	AGTGTGCTGACCATACAGTCCT
DNA sequence of PCSK9 target site	598	MG3-6/3-4 PCSK9 C2	CCTGCAAAACAGCTGCCAACCT
DNA sequence of PCSK9 target site	599	MG3-6/3-4 PCSK9 D2	CTGCAAAACAGCTGCCAACCTG
DNA sequence of PCSK9 target site	600	MG3-6/3-4 PCSK9 E2	AATGGCGTAGACACCCTCACCC
DNA sequence of PCSK9 target site	601	MG3-6/3-4 PCSK9 F2	TCCTGCTGCCATGCCCCAGGTC
DNA sequence of PCSK9 target site	602	MG3-6/3-4 PCSK9 G2	TGGAATGCAAAGTCAAGGAGCA
(r =native ribose base, m = 2'-O methyl modified base, F = 2' Fluro modified base, * = phosphorothioate bond)			

Example 18 – *In vivo* gene editing in the liver of mice by the chimeric nuclease MG3-6/3-4 delivered by systemic administration of a lipid nanoparticle

[00161] To evaluate the ability of the MG3-6/3-4 chimeric Type II nuclease to edit the genome *in vivo* in a living animal, a lipid nanoparticle was used to deliver an mRNA encoding the MG3-6/3-4 nuclease (e.g. RNA version of SEQ ID NO: 603) and single guide RNAs (sgRNA) that target different parts of the coding sequence of the mouse HAO-1 gene (e.g. described in the tables below). The HAO-1 gene encodes glycolate oxidase which is an enzyme involved in glycolate metabolism and is expressed primarily in hepatocytes in the liver. A screen of sgRNAs that target the HAO-1 coding sequence was performed in the mouse liver cell line Hepa1-6 to identify active guides. The sgRNAs mH364-7 and mH364-20, which exhibited 46% and 26% editing in Hepa1-6 cells when transfected with the mRNA encoding the MG3-6/3-4 nuclease, were selected for testing in mice. mH364-7 targets exon 2 and mH364-20 targets exon 4.

[00162] A number of chemical modifications of the native RNA structure were incorporated into these sgRNAs. These chemical modifications were selected based on their ability to improve the stability of the sgRNA *in vitro* when incubated in extracts from mammalian cells without negatively impacting editing activity. For initial testing in mice, sgRNAs mH364-7 and mH364-20 incorporating chemistry 1 and chemistry 35 were selected for testing and designated as mH364-7-1, mH364-20-1, mH364-7-35, mH364-20-35. The sequences of these guides including the chemical modifications are shown below in **Table 9**.

Table 9: Sequences and chemical modifications of guide RNA tested *in vivo* in mice

Guide name	Sequence
mH364-7-1	mG*mA*mG*CUGGCCACUGUGCGAGGUAGUUGAGAAUCGAAAG AUUCUUAAUAAGGCAUCCUUCCGAUGCUGACUUCUCACCGUCC GUUUUCCAAUAGGAGCGGGCGGUAUGU*mU*mU*mU
mH364-20-1	mU*mU*mC*AGCAAGUCCACUGUUGUCUGUUGAGAAUCGAAAG AUUCUUAAUAAGGCAUCCUUCCGAUGCUGACUUCUCACCGUCC GUUUUCCAAUAGGAGCGGGCGGUAUGU*mU*mU*mU
mH364-7-35	mG*mA*mG*mC*UGGCCACUGUGCGAGGUAGUUGAGAAUCmG*m A*mA*mA*GAUUCUUAAUAAGGCAUCmC*mU*mU*mC*mC*GAU GCUGACUUCUCACCGUCCGUUUUCCmA*mA*mU*mA*GGAGCGG GCGGUA*mU*mG*mU*mU*mU*mU
mH364-20-35	mU*mU*mC*mA*GCAAGUCCACUGUUGUCUGUUGAGAAUCmG*m A*mA*mA*GAUUCUUAAUAAGGCAUCmC*mU*mU*mC*mC*GAU GCUGACUUCUCACCGUCCGUUUUCCmA*mA*mU*mA*GGAGCGG GCGGUA*mU*mG*mU*mU*mU*mU

m: 2′-O methyl modified base, *: phosphorothioate backbone

[00163] The mRNA encoding the MG3-6/3-4 nuclease was generated by *in vitro* transcription of a linearized plasmid template using T7 RNA polymerase, nucleotides, and enzymes purchased from New England Biolabs or Trilink Biotechnologies.

[00164] The DNA sequence (**SEQ ID No: 603**) that was transcribed into RNA comprised the following elements in order from 5′ to 3′: the T7 RNA polymerase promoter, a 5′ untranslated region (5′ UTR), a nuclear localization signal, a short linker, the coding sequence for the MG3-6/3-4 nuclease, a short linker, a nuclear localization signal, and a 3′ untranslated region and an approximately 100 nucleotide polyA tail (not included in SEQ ID No: 603).

[00165] The protein sequence encoded in the synthetic mRNA encoded in this MG3-6/3-4 cassette comprises the following elements from 5′ to 3′: the nuclear localization signal from SV40, a five amino acid linker (GGGS), the protein coding sequence of the MG3-6/3-4 nuclease from which the initiating methionine codon was removed, a 3 amino acid linker (SGG) and the nuclear localization signal from nucleoplasmin. The DNA sequence of the protein coding region of this cassette was modified to reflect the codon usage in humans using a commercially available algorithm. An approximately 100-nucleotide polyA tail was encoded in the plasmid used for *in vitro* transcription and the mRNA was co-transcriptionally capped using the CleanCAP (™) reagent purchased from Trilink Biotechnologies. Uridine in the mRNA was replaced with N1-methyl pseudouridine.

[00166] The lipid nanoparticle (LNP) formulation used to deliver the MG3-6/3-4 mRNA and the guide RNA is based on LNP formulations described in the literature including Kauffman *et al* (Nano Lett. 2015, 15, 11, 7300–7306 (https://doi.org/10.1021/acs.nanolett.5b024970). The four lipid components were dissolved in ethanol and mixed in an appropriate molar ratio to make the lipid working mix. The mRNA and the guide RNA were either mixed prior to formulation at a 1:1 mass ratio or formulated in separate LNP that were later co-injected into mice at a 1:1 mass ratio of the two RNA's. In either case, the RNA was diluted in 100 mM Sodium Acetate (pH 4.0) to make the RNA working stock. The lipid working stock and the RNA working stock were mixed in a microfluidics device (Ignite NanoAssembler, Precision Nanosystems) at a flow rate ratio of 1:3, respectively and a flow rate of 12 mLs/min. The LNP were dialyzed against phosphate buffered saline (PBS) for 2 to 16 hours and then concentrated using Amicon spin concentrators (Millipore) until the reduced volume was achieved. The concentration of RNA in the LNP formulation was measured using the Ribogreen reagent (Thermo Fisher). The diameter and polydispersity (PDI) of the LNP were determined by dynamic light scattering. Representative LNP had diameters ranged from 65 nm to 120 nm with PDI of 0.05 to 0.20. LNP were injected intravenously into 8- to 12-week-old C57Bl6 wild type mice via the tail vein (0.1 mL per mouse) at a total RNA dose of 1 mg RNA per kg body weight. Eleven days post-dosing,

3 of the 5 mice in each group were sacrificed and the liver was collected and homogenized using a bead beater (Omni International) in a digestion buffer supplied in the PureLink Genomic DNA Isolation Kit (Thermo Fisher Scientific). Genomic DNA was purified from the resulting homogenate using the PureLink Genomic DNA Isolation Kit (Thermo Fisher Scientific) and quantified by measuring the absorbance at 260 nm. Genomic DNA purified from mice injected with buffer alone was used as a control. At 28 days post-dosing, the remaining 2 mice in each group were sacrificed and the liver was collected and homogenized using a bead beater (Omni International) in a digestion buffer supplied in the PureLink Genomic DNA Isolation Kit (Thermo Fisher Scientific). Genomic DNA was purified from the resulting homogenate using the PureLink Genomic DNA Isolation Kit (Thermo Fisher Scientific) and quantified by measuring the absorbance at 260 nm. Genomic DNA purified from mice injected with buffer alone was used as a control.

[00167] The liver genomic DNA was then PCR amplified using a first set of primers flanking the region targeted by the two guides. The PCR primers used are shown below in **Table 10**.

Table 10: Sequences of PCR primers and Next Generation Sequencing primers used to analyze *in vivo* genome editing in mice

Primer Set Name	Purpose	Left Primer Sequence	Right Primer Sequence
mHAO1-NGS-P4	Amplify the target site in HAO1 exon 2 for guide mH364-7	GTAAAGAAAAACAAG GAATGTAAT	ATCTGTCAACTTCTG TTTTAGGAC
mHAO1-NGS-P5	Amplify the target site in HAOI exon 4 for guide mH364-20	GCAAAGTAGAGAAATG ACAAACC	ACCAAGTCAGATATA AACTGTCT

[00168] The 5′ end of these primers comprise conserved regions complementary to the PCR primers used in the second PCR, followed by 5 Ns in order to give sequence diversity and improve MiSeq sequencing quality, and end with sequences complementary to the target region in the mouse genome. PCR was performed using Q5® Hot Start High-Fidelity 2X Master Mix (New England Biolabs) on 100 ng of genomic DNA and an annealing temperature of 60 °C for a total of 30 cycles. This was followed by a 2nd round of 10 cycles of PCR using primers designed to add unique dual Illumina barcodes (IDT) for next generation sequencing on a MiSeq

instrument. Each sample was sequenced to a depth of greater than 10,000 reads using 150bp paired end reads. Reads were merged to generate a single 250 bp sequence from which Indel percentage and INDEL profile was calculated using a proprietary Python Script.

[00169] The results of the NGS analysis of INDELS from mice at day 11 post dosing are shown in **Table 11** for individual mice and are summarized in **FIG. 32**.

Table 11: Genome editing at the HAO-1 locus by MG3-6/3-4 in the whole liver of wild type mice at day 11 post LNP dosing analyzed by next generation sequencing.

Animal #	Guide RNA	Total NGS reads	Indel %	% of Indels OOF	Mean INDELS	Mean total OOF%
1	PBS control	210962	0.09	100	0.2	0.2
2	PBS control	259982	0.29	99.87		
3	PBS control	211193	0.08	100		
6	364mHA-G7-1	164396	54.06	87.02	53.0	46.0
7	364mHA-G7-1	163409	51.93	85.9		
8	364mHA-G7-1	183054	52.94	87.6		
11	364mHA-G7-35	38835	22.71	91.57	23.6	21.1
12	364mHA-G7-35	269963	26.83	89.59		
13	364mHA-G7-35	190007	21.32	87.11		
16	364mHA-G20-1	227766	8.53	88.62	8.9	7.5
17	364mHA-G20-1	202915	5.01	90.36		
18	364mHA-G20-1	236757	13.06	80.52		
21	364mHA-G20-35	177059	2.78	80.98	2.5	2.0
22	364mHA-G20-35	163515	2.29	67.62		
23	364mHA-G20-35	136634	2.31	89.32		

Data for individual mice is shown. All mice that received guide RNA LNP also received LNP encapsulating the MG3-6/3-4 mRNA. % of indels OOF is the percentage of all the INDELS that resulted in a sequence where the HAO1 coding sequence is out of frame. The mean total OOF% is the average percentage of all alleles in which the

HAO1 coding sequence is out of frame. The total number of NGS sequencing reads is given.

[00170] Group 2 mice received LNP encapsulating guide RNA mH364-7-1. Group 3 mice received LNP encapsulating guide RNAmH364-7-35. Group 4 mice received LNP encapsulating guide RNA mH364-20-1. Group 5 mice received LNP encapsulating guide RNAmH364-20-35. All mice in groups 2 to 5 also received LNP encapsulating the MG3-6/3-4 mRNA that was mixed with the guide RNA containing LNP at a 1:1 RNA mass ratio prior to injection. No INDELS were detected in the liver of mice injected with PBS buffer (see **Table 11**). Mice injected with LNPs encapsulating guide 364mHA-G7-1 and MG3-6/3-4 mRNA exhibited INDELS at the target site in HAO-1 at a mean frequency of 53.0 %. Mice injected with LNPs encapsulating guide 364mHA-G7-35 and MG3-6/3-4 mRNA exhibited INDELS at the target site in HAO-1 at a mean frequency of 23.6 %. Mice injected with LNPs encapsulating guide 364mHA-G20-1 and MG3-6/3-4 mRNA exhibited INDELS at the target site in HAO-1 at a mean frequency of 8.9 %. Mice injected with LNPs encapsulating guide 364mHA-G20-35 and MG3-6/3-4 mRNA exhibited indels at the target site in HAO-1 at a mean frequency of 2.5%. These data demonstrate that the guides with spacer 7 (364mHA-G7-1 and 364mHA-G7-35) are significantly more potent *in vivo* than the guides with spacer 20 (364mHA-G20-1 and 364mHA-G20-35) when guides with the same chemical modifications are compared. This is consistent with the higher level of editing observed with these 2 guide sequences in Hepa1-6 cells by mRNA-based transfection (mH364-7 exhibited 46% INDELS and mH364-20 26% INDELS in Hepa1-6 cells). Guide chemistry #1 resulted in higher levels of editing than chemistry #35 for both guide 7 (2.2-fold higher editing with chemistry #1) and guide 20 (3.5-fold higher editing with chemistry #1). These data demonstrate that the MG3-6/3-4 nuclease can edit *in vivo* in mice at the target site specified by the sgRNA. Moreover, an sgRNA with a set of chemical modifications designated chemistry #1 was able to promote editing at 53% of the genomic DNA in whole liver when delivered using an LNP. The LNP used in these studies is taken up via binding of apolipoprotein E (apoE) to the LNP which is a ligand for binding to the low-density lipoprotein receptor (see e.g. Yan et al, Biochem Biophys Res Commun 2005 328(1):57-62.doi: 10.1016/j.bbrc.2004.12.137, Akinc et al Mol Ther 2010 (7):1357-64, doi: 10.1038/mt.2010.85).

[00171] The liver is composed of a number of different cell types. In the liver of mice, the hepatocytes make up about 52% of all cells (and 35% of hepatocytes contain two nuclei), with Kupffer cells (18%), Ito cells (8%), and endothelial cells (22%) making up the remaining cells (Histochem Cell Biol 131, 713–726 https://doi.org/10.1007/s00418-009-0577-1). By extrapolation, without wishing to be bound by theory, about 60% [((52 + (0.35 x 52)) / (48+(52+ (0.35 x 52)))] of the total nuclei in the mouse liver are predicted to be derived from hepatocytes.

Because the LDL receptor is expressed mainly on hepatocytes in the liver (see e.g. https://www.proteinatlas.org/ENSG00000130164-LDLR/tissue/liver#imid_2815831), the LNP used in the mouse studies described herein is expected to be taken up primarily by hepatocytes. Because hepatocyte nuclei make up about 60% of all nuclei in the whole liver of mice, it can be predicted that if all the hepatocyte nuclei were edited, the level of INDELS measured in the whole liver are predicted to be about 60%. The finding that LNP delivery of MG3-6/3-4 was able to achieve INDEL rates of 53% suggests that the majority of hepatocyte nuclei were edited.

[00172] The HAO1 gene encodes the protein glycolate oxidase (GO), an intracellular enzyme involved in glycolate metabolism. To determine if the observed gene editing in the HAO1 gene resulted in a reduction in the expression of the GO protein in the liver, we extracted total protein from a separate lobe of the liver from mice in the same study. The GO protein was detected using a Western blot assay with commercially available antibodies against the mouse GO protein. The protein vinculin was used as a loading control on the Western blot, as Vinculin levels are predicted to not be impacted by gene editing of the HAO1 gene. As shown in **FIG. 24**, the level of GO protein was significantly reduced in the livers of mice treated with LNP encapsulating MG3-6/3-4 mRNA and sgRNA targeting HAO1. Quantification of the Western blot using image analysis software (Biorad) and normalization of GO to the level of vinculin demonstrated that GO levels were reduced by an average of 75%, 58%, 4%, and 24% in mice treated with sgRNA mH364-7-1, mH364-7-35, mH364-20-1, and mH364-20-35, respectively. The degree of GO protein reduction correlates with the INDEL frequency in these groups of mice (see **Table 11**). These data demonstrate that the MG3-6/3-4 nuclease combined with an appropriately designed sgRNA can be used to create indels in a gene of interest *in vivo* in a living mammal and reduce (knockdown) the production of the protein encoded by that gene. Reducing the expression of specific genes can be therapeutically beneficial in specific diseases. In the case of the HAO1 gene that encodes the GO protein, reduction of the levels of GO protein in the liver is expected to be beneficial in patients with the hereditary disease primary hyperoxaluria type I (Martin-Higueras, Mol. Ther. 24, 719–725). Thus, the MG3-6/3-4 nuclease, together with an appropriate sgRNA containing appropriate chemical modifications targeting the HAO1 gene, is a potential approach for the treatment of primary hyperoxaluria type I.

Example 19 – Comparison of MG3-6/3-4 gene editing efficiency in mice using the same guide RNA sequence with four different chemical modifications

[00173] The impact of chemical modifications to the sgRNA upon *in vivo* editing efficiency was further investigated by testing 4 different guide chemistries introduced into the same guide RNA

sequence. Guide RNA 7 that targets the mouse HAO1 gene was synthesized with chemical modifications #1, #35, #42, or #45. The sequences of these guides are shown below in **Table 12**.

Table 12: Sequences of MG3-6/3-4 sgRNA guide 7 targeting mouse HAO1

Guide name	Sequence
mH364-7-1	mG*mA*mG*CUGGCCACUGUGCGAGGUAGUUGAGAAUCGAAAGAUUCUUAAUAAGGCAUCCUUCCGAUGCUGACUUCUCACCGUCCGUUUUCCAAUAGGAGCGGGCGGUAUGU*mU*mU*mU
mH364-7-35	mG*mA*mG*mC*UGGCCACUGUGCGAGGUAGUUGAGAAUCmG*mA*mA*mA*GAUUCUUAAUAAGGCAUCmC*mU*mU*mC*mC*GAUGCUGACUUCUCACCGUCCGUUUUCCmA*mA*mU*mA*GGAGCGGGCGGUA*mU*mG*mU*mU*mU*mU
mH364-7-42	mG*mA*mG*mC*fUfGfGfCfCfAfCfUfGfUfGfCfGfAfGfGfUAGUUGAGAAUCG*A*A*A*GAUUCUUAAUAAGGCAUCC*U*U*C*C*GAUGCUGACUUCUCACCGUCCGUUUUCCA*A*U*A*GGAGCGGGCGGUA*mU*mG*mU*mU*mU*mU
mH364-7-45	mG*mA*mG*mC*fUfGfGfCfCfAfCfUfGfUfGfCfGfAfGfGfUAGUUGAGAAUCmG*mA*mA*mA*GAUUCUUAAUAAGGCAUCmC*mU*mU*mC*mC*GAUGCUGACUUCUCACCGUCCGUUUUCCmA*mA*mU*mA*GGAGCGGGCGGUA*mU*mG*mU*mU*mU*mU
m: 2′-O methyl modified base, *: phosphorothioate backbone	

[00174] The mRNA encoding MG3-6/3-4 nuclease was generated by *in vitro* transcription of a linearized plasmid template using T7 RNA polymerase, nucleotides, and enzymes purchased from New England Biolabs or Trilink Biotechnologies. The DNA sequence that was transcribed into RNA comprised the following elements in order from 5′ to 3′: the T7 RNA polymerase promoter, a 5′ untranslated region (5′ UTR), a nuclear localization signal, a short linker, the coding sequence for the MG3-6/3-4 nuclease, a short linker, a nuclear localization signal, and a 3′ untranslated region (**SEQ ID No: 603**) and an approximately 100 nucleotide polyA tail (not included in SEQ ID No: 603)

[00175] The protein sequence encoded in the synthetic mRNA encoded in this MG3-6/3-4 cassette comprises the following elements from 5′ to 3′: the nuclear localization signal from SV40, a five amino acid linker (GGGS), the protein coding sequence of the MG3-6/3-4 nuclease from which the initiating methionine codon was removed, a 3 amino acid linker (SGG), and the nuclear localization signal from nucleoplasmin. The DNA sequence of the protein coding region of this cassette was modified to reflect the codon usage in humans using a commercially available algorithm. An approximately 100 nucleotide polyA tail was encoded in the plasmid

used for *in vitro* transcription, and the mRNA was co-transcriptionally capped using the CleanCAP (™) reagent purchased from Trilink Biotechnologies. Uridine in the mRNA was replaced with N1-methyl pseudouridine. The lipid nanoparticle (LNP) formulation used to deliver the MG3-6/3-4 mRNA and the guide RNA is based on LNP formulations described in the literature including Kauffman et al (*Nano Lett.* 2015, 15, 11, 7300–7306, https://doi.org/10.1021/acs.nanolett.5b024970). The four lipid components were dissolved in ethanol and mixed in an appropriate molar ratio to make the lipid working mix. The mRNA and the guide RNA were either mixed prior to formulation at a 1:1 mass ratio or formulated in separate LNP that were later co-injected into mice at a 1:1 mass ratio of the two RNA's. In either case, the RNA was diluted in 100 mM Sodium Acetate (pH 4.0) to make the RNA working stock. The lipid working stock and the RNA working stock were mixed in a microfluidics device (Ignite NanoAssembler, Precision Nanosystems) at a flow rate ratio of 1:3, respectively, and a flow rate of 12 mLs/min. The LNP were dialyzed against phosphate buffered saline (PBS) for 2 to 16 hours and then concentrated using Amicon spin concentrators (Milipore) until the reduced volume was achieved. The concentration of RNA in the LNP formulation was measured using the Ribogreen reagent (Thermo Fisher). The diameter and polydispersity (PDI) of the LNP were determined by dynamic light scattering. Representative LNP had diameters ranged from 65 nm to 120 nm with PDI of 0.05 to 0.20. LNP were injected intravenously into 8- to 12-week-old C57Bl6 wild type mice via the tail vein (0.1 mL per mouse) at a total RNA dose of 1 mg RNA per kg body weight. Ten days post-dosing, 3 of the 5 mice in each group were sacrificed and the liver was collected and homogenized using a bead beater (Omni International) in a digestion buffer supplied in the PureLink Genomic DNA Isolation Kit (Thermo Fisher Scientific). Genomic DNA was purified from the resulting homogenate using the PureLink Genomic DNA Isolation Kit (Thermo Fisher Scientific) and quantified by measuring the absorbance at 260 nm. Genomic DNA purified from mice injected with buffer alone was used as a control. At 28 days post-dosing, the remaining 2 mice in each group were sacrificed and the liver was collected and homogenized using a bead beater (Omni International) in a digestion buffer supplied in the PureLink Genomic DNA Isolation Kit (Thermo Fisher Scientific). Genomic DNA was purified from the resulting homogenate using the PureLink Genomic DNA Isolation Kit (Thermo Fisher Scientific) and quantified by measuring the absorbance at 260 nm. Genomic DNA purified from mice injected with buffer alone was used as a control.

[00176] The liver genomic DNA was then PCR amplified using a first set of primers flanking the region targeted by the two guides . The PCR primers used are shown in **Table 10**. The 5′ end of these primers comprise conserved regions complementary to the PCR primers used in the

second PCR, followed by 5 Ns in order to give sequence diversity and improve MiSeq sequencing quality, and end with sequences complementary to the target region in the mouse genome. PCR was performed using Q5® Hot Start High-Fidelity 2X Master Mix (New England Biolabs) on 100 ng of genomic DNA and an annealing temperature of 60 °C for a total of 30 cycles. This was followed by a 2nd round of 10 cycles of PCR using primers designed to add unique dual Illumina barcodes (IDT) for next generation sequencing on a MiSeq instrument. Each sample was sequenced to a depth of greater than 10,000 reads using 150bp paired end reads. Reads were merged to generate a single 250 bp sequence from which Indel percentage and INDEL profile was calculated using a proprietary Python Script.

[00177] The editing results are summarized in **FIG. 25** and tabulated in **Table 13**.

Table 13: Genome editing frequencies in the HAO1 gene in the whole liver of individual mice treated with LNP encapsulating MG3-6/3-4 mRNA and guide RNA 7 targeting the HAO-1 gene with chemical modifications 42 (mH364-7-42), 45 (mH364-7-45), 1 (mH364-7-1), and 35 (mH364-7-35)

DAY	mH364 Guide 7 chemistry	Mouse	INDEL %	Mean Group INDELS	Stdev
10	PBS control	1	0.01	0.0	0.0
10	PBS control	2	0.01		
10	PBS control	3	0.01		
28	PBS control	4	0.02		
28	PBS control	5	0.02		
10	42	6	33.54	32.4	2.5
10	42	7	28.48		
10	42	8	31.3		
28	42	9	34.43		
28	42	10	34.19		

DAY	mH364 Guide 7 chemistry	Mouse	INDEL %	Mean Group INDELS	Stdev
10	45	11	29.22	32.1	5.8
10	45	12	37.04		
10	45	13	37.24		
28	45	14	33.57		
28	45	15	23.63		
10	1	16	42.04	46.1	3.1
10	1	17	45.38		
10	1	18	50.8		
28	1	19	46.31		
28	1	20	45.98		
10	35	21	24.95	26.6	2.3
10	35	22	29.93		
10	35	23	24.75		
28	35	24	28.14		
28	35	25	25.22		

[00178] Control mice injected with PBS buffer did not contain measurable INDELS at the target site for guide 7. The mean INDEL frequency in mice that received LNP containing guides mH364-7-1, mH364-7-35, mH364-7-42, and mH364-7-45 was 46.1%, 26.6%, 32.4%, and 32.1%, respectively, demonstrating that guide RNA chemistry #1 was the most potent followed by #42 and #45, with chemistry #35 being the least potent. These data suggest that chemical modifications to the bases and backbone at the 5′ and 3′ ends of the guide RNA provided the

highest *in vivo* potency amongst the chemistries tested. Additional modifications of internal bases did not improve *in vivo* potency. These findings are in contrast with published data for the spCas9 sgRNA where modifications of bases or the backbone at both the ends of the sgRNA and at internal sequences was required for optimal *in vivo* editing (Yin et al, Nature Biotechnology, doi:10.1038/nbt.4005) and modifications of just the 5′ and 3′ ends of the sgRNA enabled low levels of editing (20% INDELS) in the liver using delivery in a similar LNP.

[00179] Total RNA was purified from a separate lobe of the liver from the same mice described in **Table 13** and used to measure level of HAO-1 mRNA by digital droplet PCR (dd-PCR). The PBS injected mice were used as controls and the levels of HAO-1 mRNA in the livers of edited mice were compared to these controls. The dd-PCR assay was designed and optimized using standard techniques. ddPCR is a highly accurate method for determining the absolute copy number of a specific nucleic acid in a complex mixture (e.g. Taylor et al *Sci Rep* **7,** 2409 (2017). doi.10.1038/s41598-017-02217-x). The total liver RNA was first converted to cDNA by reverse transcription then quantified in the dd-PCR assay using GAPDH as an internal control to normalize between samples. As shown in **Table 14**, the level of HAO1 mRNA in the individual mice treated with LNP encapsulating MG3-6/3-4 mRNA and sgRNA targeting the mouse HAO1 gene was decreased, and the magnitude of decrease was correlated with the INDEL frequency.

Table 14: HAO1 mRNA levels in the whole liver of individual mice treated with LNP encapsulating MG3-6/3-4 mRNA and guide RNA 7 targeting the HAO-1 gene with chemical modifications 42 (mH364-7-42), 45 (mH364-7-45), 1 (mH364-7-1), and 35 (mH364-7-35).

Harvest Day	mH364 Guide 7 chemistry	Mouse	% Decrease in HAO mRNA	Mean Group % decrease in HAO mRNA	Stdev
10	42	6	47.4	35.5	8.8
10	42	7	42.4		
10	42	8	29.0		
28	42	9	29.6		
28	42	10	28.9		
10	45	11	20.3	38.0	10.2

Harvest Day	mH364 Guide 7 chemistry	Mouse	% Decrease in HAO mRNA	Mean Group % decrease in HAO mRNA	Stdev
10	45	12	38.6		
10	45	13	41.8		
28	45	14	45.9		
28	45	15	43.2		
10	1	16	57.0	60.0	3.9
10	1	17	54.7		
10	1	18	62.5		
28	1	19	63.1		
28	1	20	62.6		
10	35	21	18.3	23.4	20.8
10	35	22	-2.5		
10	35	23	14.8		
28	35	24	52.6		
28	35	25	33.8		

The same mice in Table 10 were analyzed

[00180] The largest reduction in HAO1 mRNA was seen in the group of mice treated with sgRNA mH364-7-1, while the smallest reduction of HAO-1 mRNA was observed in mice treated with sgRNA mH364-7-35. A reduction in HAO1 mRNA can occur when frameshift mutations are introduced into the coding sequence of a gene via a mechanism called nonsense mediated decay (Brogna et al, *Nat Struct Mol Biol* **16,** 107–113 (2009), doi.10.1038/nsmb.1550). The observation of reduced HAO-1 mRNA in the liver of mice edited

at the HAO-1 gene with MG3-6/3-4 is consistent with the presence of INDELS that result in a high rate of frame shifts as shown in **Table 15**.

Table 15: Analysis of the frequency of edits that result in frame shifts in the liver of mice treated with LNP encapsulating MG3-6/3-4 mRNA and sgRNA number 7 (G7) that targets the HAO-1 gene

Treatment	Mean INDELS	Stdev of INDELS	Mean OOF % total	Stdev OFF % total
PBS control	**0.0**	0.0	0.0	0.0
mH364-7-42	**31.1**	2.1	28.6	1.7
mH364-7-45	**34.5**	3.7	31.2	3.2
mH364-7-1	**46.1**	3.6	41.9	3.4
mH364-7-35	**26.5**	2.4	24.3	2.5

The out of frame percentage (OOF%) was calculated by analyzing the NGS data using a custom algorithm

[00181] In **Table 15**, the mean frequency of INDELS that result in a frame shift in the HAO1 coding sequence were determined from the NGS data. This analysis shows that the majority of the INDELS resulted in a frameshift for all four of the sgRNA tested.

[00182] The HAO1 gene encodes the protein glycolate oxidase (GO) that is an intracellular enzyme involved in glycolate metabolism. To determine if the observed gene editing in the HAO1 gene resulted in a reduction in the expression of the GO protein in the liver, we extracted total protein from a separate lobe of the liver from mice in the same study described in **FIG. 25** and **Tables 13 to 15**. The GO protein was detected using a Western blot assay with commercially available antibodies against the mouse GO protein. Equal amounts of protein were loaded on the Western blot. As shown in **FIG. 25**, the level of GO protein was reduced in the livers of mice treated with LNP encapsulating MG3-6/3-4 mRNA and sgRNA targeting HAO1. Guides mH364-7-42 (mice 7,8), mH364-7-45 (mice 12, 13), and mH364-7-1 (mice 17,18) resulted in clear reductions in GO protein. Guide mH364-7-35 (mice 22,23) which had the lowest levels of INDELS among the 4 guides tested, did not appreciably reduce GO protein levels. These data demonstrate that the MG3-6/3-4 nuclease combined with an appropriately designed sgRNA can be used to create INDELS in a gene of interest *in vivo* in a living mammal and reduce (knockdown) the production of the protein encoded by that gene. Reducing the expression of specific genes can be therapeutically beneficial in specific diseases. In the case of

the HAO1 gene that encodes the GO protein, reduction of the levels of GO protein in the liver is expected to be beneficial in patients with the hereditary disease primary hyperoxaluria type I (Martin-Higueras, Mol. Ther. 24, 719–725). Thus the MG3-6/3-4 nuclease, together with an appropriate sgRNA containing appropriate chemical modifications targeting the HAO1 gene, is a potential approach for the treatment of primary hyperoxaluria type I.

[00183] 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.

CLAIMS

1. A fusion endonuclease comprising:

(a) an N-terminal sequence comprising a RuvC domain, a REC domain, or an HNH domain of an endonuclease having at least 80% sequence identity to SEQ ID NO: 696; and

(b) a C-terminal sequence comprising WED, TOPO, or CTD domains of an endonuclease having at least 80% sequence identity to SEQ ID NO: 708, wherein said N-terminal sequence and said C-terminal sequence do not naturally occur together in a same reading frame.

2. The fusion endonuclease of claim 1, wherein said N-terminal sequence and said C-terminal sequence are derived from different organisms.
3. The fusion endonuclease of claim 1, wherein said N-terminal sequence further comprises RuvC-I, BH, or RuvC-II domains of an endonuclease having at least 80% sequence identity to SEQ ID NO:696.
4. The fusion endonuclease of claim 1, wherein said C-terminal sequence further comprises a PAM-interacting domain.
5. The fusion endonuclease of claim 1, wherein said fusion endonuclease comprises a sequence having at least 80% sequence identity to SEQ ID NO: 12.
6. The fusion endonuclease claim 1, wherein said fusion endonuclease is configured to have selectivity for a PAM that is not nnRGGnT (SEQ ID NO: 53).
7. The fusion endonuclease of claim 6, wherein said fusion endonuclease is configured to have selectivity for a PAM of SEQ ID NO: 62.
8. The fusion endonuclease of claim 1, wherein said fusion endonuclease is a class II, type II Cas endonuclease.
9. The fusion endonuclease of claim 8, wherein said class II, type II Cas endonuclease is derived from an uncultivated microorganism.
10. The fusion endonuclease of claim 1, wherein said fusion endonuclease has less than 86% identity to a SpyCas9 endonuclease.
11. An engineered nuclease system, comprising:

(a) the fusion endonuclease of claim 1; and

(b) an engineered guide ribonucleic acid structure configured to form a complex with said fusion endonuclease comprising:

a guide ribonucleic acid sequence configured to hybridize to a target deoxyribonucleic acid sequence.

12. The engineered nuclease system of claim 11, wherein said engineered guide ribonucleic acid structure further comprises a tracr ribonucleic acid sequence configured to bind said fusion endonuclease.
13. The engineered nuclease system of claim 11, wherein said fusion endonuclease is derived from an uncultivated microorganism.
14. The engineered nuclease system of claim 11, wherein said fusion 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 Cas13d endonuclease.
15. The engineered nuclease system of claim 11, wherein said fusion endonuclease has less than 86% identity to a SpyCas9 endonuclease.
16. The engineered nuclease system of claim 11, wherein said fusion endonuclease comprises a sequence having at least 80% sequence identity to SEQ ID NO: 12.
17. The engineered nuclease system of claim 13, wherein said engineered guide ribonucleic acid structure comprises a sequence having at least 80% identity to non-degenerate nucleotides of SEQ ID NO: 35.