CLAIMS

1. A guide RNA (gRNA) that is a short single guide RNA (short-sgRNA) comprising a guide region and a conserved portion, wherein the conserved portion comprises from 5’ to 3’:

i) a lower stem region portion being nucleotides LS1 to LS6,

ii) a bulge region portion being nucleotides B1 to B2,

iii) an upper stem region being nucleotides US1 to US12,

iv) a bulge region portion being nucleotides B3 to B6,

v) a lower stem region portion being nucleotides LS7 to LS12,

vi) a nexus region being nucleotides N1 to N18, and

vii) a hairpin region, wherein the hairpin region comprises, from 5' to 3':

a) a hairpin 1 region being nucleotides H1-1 to H1-12, wherein the hairpin 1 region lacks 6-8 nucleotides, and wherein one or more of positions H1-1, H1-2, or H1-3 is deleted,

b) a nucleotide, and

c) a hairpin 2 region being nucleotides H2-1 to H2-15,

wherein the short-sgRNA comprises a 5’ end modification or a 3’ end modification.

2. The gRNA of claim 1, wherein:

i) Watson-Crick pairing nucleotides H1-1 and H1-12, H1-3 and H1-10, and/or H1-4 and H1-9 are deleted; and/or

ii) the hairpin 1 region lacks 6 nucleotides of H1-1 to H1-12.

3. The gRNA of claim 1 or claim 2, wherein the hairpin 1 region comprises:

i) a sequence selected from any one of AGAGCU, AGAAAU, CGAAAG, AAAAU, AAAGU, ACAGU, CAAG, or AAAU;

ii) a sequence having less than or equal to 2 mismatches to any one sequence selected from (i); or

iii) a sequence having less than or equal to 1 mismatch to any one sequence selected from (i).

4. The gRNA of any one of claims 1-3, wherein the hairpin 1 region comprises a sequence selected from any one of AGAGCU, AGAAAU, CGAAAG, AAAAU, AAAGU, ACAGU, CAAG, or AAAU.

5. The gRNA of claim 4, wherein the hairpin 1 region consists of a sequence selected from any one of AGAGCU, AGAAAU, CGAAAG, AAAAU, AAAGU, ACAGU, CAAG, or AAAU and wherein the hairpin 1 region comprises a modified nucleotide selected from a 2'-O-methyl (2'-Ome) modified nucleotide, a 2'-fluoro (2'-F) modified nucleotide, a phosphorothioate (PS) linkage between nucleotides, or a combination thereof.

6. The gRNA of any one of claims 1-5, wherein the hairpin 1 region comprises the sequence CGAAAG.

7. The gRNA of any one of claims 1-6, wherein one or more of (a)-(x) is met:

a) position H1-1 is deleted or substituted;

b) position H1-2 is deleted or substituted;

c) position H1-3 is deleted or substituted;

d) position H1-4 is deleted;

e) position H1-5 is deleted;

f) position H1-6 is deleted or substituted;

g) position H1-7 is deleted or substituted;

h) position H1-8 is deleted or substituted;

i) position H1-9 is deleted or substituted;

j) position H1-10 is deleted or substituted;

k) position H1-11 is deleted;

l) position H1-12 is deleted;

m) position H1-11 and position H1-12 are deleted;

n) position H1-7 is substituted with a G and/or position H1-8 is substituted with a C;

o) position H1-6 and/or position H1-7 are substituted;

p) position H1-6 is substituted with a C and/or position H1-7 is substituted with a U;

q) position H1-1 and/or position H1-12 are substituted;

r) position H1-1 is substituted with a C and/or position H1-12 is substituted with a G;

s) position N18 is substituted;

t) position N18 is substituted with a C;

u) position H1-12 is substituted;

v) position H1-12 is substituted with a C or an A;

w) position n is substituted; and/or

x) position n is substituted with an A.

8. The gRNA of any one of claims 1-7, wherein

i) the gRNA comprises a 5’ end modification;

ii) the gRNA comprises a 3’ end modification;

iii) the gRNA comprises a 5’ end modification and a 3’ end modification;

iv) the gRNA comprises a 3’ tail;

v) the gRNA comprises a 3’ tail comprising 1-2, 1-3, 1-4, 1-5, 1-7, or 1-10 nucleotides or 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides;

vi) the gRNA does not comprise a 3’ tail

vii) the gRNA comprises a 3’ tail comprising a modification of any one or more of the nucleotides present in the 3’ tail; or

viii) the gRNA comprises a 3’ tail and the 3’ tail is fully modified.

9. The gRNA of any one of claims 1-8, comprising a modification in the hairpin region.

10. The gRNA of any one of claims 1-9, wherein:

i) the 3’ and/or 5’ end modification comprises a protective end modification;

ii) the 3’ and/or 5’ end modification comprises a protective end modification comprising a modified nucleotide selected from a 2’-O-methyl (2’-OMe) modified nucleotide, a 2’-O-(2-methoxyethyl) (2’-O-moe) modified nucleotide, a 2’-fluoro (2’-F) modified nucleotide, a phosphorothioate (PS) linkage between nucleotides, an inverted abasic modified nucleotide, or a combination thereof; and/or

iii) the gRNA comprises a modification in the hairpin region comprising a modified nucleotide selected from a 2’-O-methyl (2’-Ome) modified

nucleotide, a 2’-fluoro (2’-F) modified nucleotide, a phosphorothioate (PS) linkage between nucleotides, or a combination thereof.

11. The gRNA of any one of claims 1-10:

i) wherein the upper stem region comprises at least one modification;

ii) wherein the upper stem region comprises at least one modification comprising one or more of:

a) a modification of any one or more of US1-US12 in the upper stem region; and

b) a modification of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or all 12 nucleotides in the upper stem region;

iii) wherein the upper stem modification comprises one or more of:

A) a 2’-OMe modified nucleotide;

B) a 2’-O-moe modified nucleotide;

C) a 2’-F modified nucleotide; and

D) combinations of one or more of (A) – (C); and/or

iv) wherein the upper stem region lacks 1-6 nucleotides.

12. The gRNA of any one of claims 1-11, comprising the nucleotide sequence of any one of:

GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGGUGC (SEQ ID NO: 281);

N_3N_xGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGGUGC (SEQ ID NO: 481);

N_{20}GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGGUGC (SEQ ID NO: 681);

ACACAAAUACCAGUCCAGCGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAGAGCUGGCACCGAGUCGGUGC (SEQ ID NO: 5);

ACACAAAUACCAGUCCAGCGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAGAAAUGGCACCGAGUCGGUGC (SEQ ID NO: 6);

ACACAAAUACCAGUCCAGCGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGGUGC (SEQ ID NO: 7);

ACACAAAUACCAGUCCAGCGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAAAAUGGCACCGAGUCGGUGC (SEQ ID NO: 8);

ACACAAAUACCAGUCCAGCGGUUUUAGAGCUAGAAAUAGCAAGUUAAA
AUAAGGCUAGUCCGUUAUCAAAAGUGGCACCGAGUCGGUGC (SEQ ID NO: 9);

ACACAAAUACCAGUCCAGCGGUUUUAGAGCUAGAAAUAGCAAGUUAAA
AUAAGGCUAGUCCGUUAUCAACAGUGGCACCGAGUCGGUGC (SEQ ID NO: 10);

ACACAAAUACCAGUCCAGCGGUUUUAGAGCUAGAAAUAGCAAGUUAAA
AUAAGGCUAGUCCGUUAUCACAAGGGCACCGAGUCGGUGC (SEQ ID NO: 11);

ACACAAAUACCAGUCCAGCGGUUUUAGAGCUAGAAAUAGCAAGUUAAA
AUAAGGCUAGUCCGUUAUCAAAAUGGCACCGAGUCGGUGC (SEQ ID NO: 12);

ACACAAAUACCAGUCCAGCGGUUUUAGAGCGAAAGCAAGUUAAAAUAA
GGCUAGUCCGUUAUCAAGAAAUGGCACCGAGUCGGUGC (SEQ ID NO: 90);

GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCA
AGAGCUGGCACCGAGUCGGUGC (SEQ ID NO: 205);

GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCA
AGAAAUGGCACCGAGUCGGUGC (SEQ ID NO: 206);

GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCA
AAAAUGGCACCGAGUCGGUGC (SEQ ID NO: 208);

GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCA
AAAGUGGCACCGAGUCGGUGC (SEQ ID NO: 209);

GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCA
ACAGUGGCACCGAGUCGGUGC (SEQ ID NO: 210);

GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCA
CAAGGGCACCGAGUCGGUGC (SEQ ID NO: 211);

GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCA
AAAUGGCACCGAGUCGGUGC (SEQ ID NO: 212);

GUUUUAGAGCGAAAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAGAA
AUGGCACCGAGUCGGUGC (SEQ ID NO: 290);

N_3N_xGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUA
UCAAGAGCUGGCACCGAGUCGGUGC (SEQ ID NO: 405);

N_3N_xGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUA
UCAAGAAAUGGCACCGAGUCGGUGC (SEQ ID NO: 406);

N_3N_xGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUA
UCAAAAAUGGCACCGAGUCGGUGC (SEQ ID NO: 408);

N_3N_xGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUA
UCAAAAGUGGCACCGAGUCGGUGC (SEQ ID NO: 409);

N_3N_xGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUA UCAACAGUGGCACCGAGUCGGUGC (SEQ ID NO: 410);

N_3N_xGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUA UCACAAGGGCACCGAGUCGGUGC (SEQ ID NO: 411);

N_3N_xGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUA UCAAAAUGGCACCGAGUCGGUGC (SEQ ID NO: 412);

N_3N_xGUUUUAGAGCGAAAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAA GAAAUGGCACCGAGUCGGUGC (SEQ ID NO: 490);

N_{20}GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAU CAAGAGCUGGCACCGAGUCGGUGC (SEQ ID NO: 605);

N_{20}GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAU CAAGAAAUGGCACCGAGUCGGUGC (SEQ ID NO: 606);

N_{20}GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAU CAAAAAUGGCACCGAGUCGGUGC (SEQ ID NO: 608);

N_{20}GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAU CAAAAGUGGCACCGAGUCGGUGC (SEQ ID NO: 609);

N_{20}GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAU CAACAGUGGCACCGAGUCGGUGC (SEQ ID NO: 610);

N_{20}GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAU CACAAGGGCACCGAGUCGGUGC (SEQ ID NO: 611);

N_{20}GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAU CAAAAUGGCACCGAGUCGGUGC (SEQ ID NO: 612);

N_{20}GUUUUAGAGCGAAAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAG AAAUGGCACCGAGUCGGUGC (SEQ ID NO: 690);

ACGCAAAUAUCAGUCCAGCGGUUUUAGAGCUAGAAAUAGCAAGUUAAA AUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGGUGC (SEQ ID NO: 809);

GCCGAGUCUGGAGAGCUGCAGUUUUAGAGCUAGAAAUAGCAAGUUAAA AUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGGUGC (SEQ ID NO: 813);

UGCUCUGUAAGCUUACCCAGGUUUUAGAGCUAGAAAUAGCAAGUUAAA AUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGGUGC (SEQ ID NO: 814);

ACACAAAUACCAGUCCAGCGGUUUUCGAGCUAGAAAUAGCAAGUGAAA AUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGGUGC (SEQ ID NO: 852);

ACACAAAUACCAGUCCAGCGGUUUUAGAGCUAGAAAUAGCAAGUUAAA AUGAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGGUGC (SEQ ID NO: 853);

ACACAAAUACCAGUCCAGCGGUUUUAGAGCUAGAAAUAGCAAGUUAAA AUAAGGCUAGUCCGUGAUCACGAAAGGGCACCGAGUCGGUGC (SEQ ID NO: 855);

ACACAAAUACCAGUCCAGCGGUUUUAGAGCUAGAAAUAGCAAGUUAAA AUAAGGCUAGUCCGUUAUCCCGAAAGGGCACCGAGUCGGUGC (SEQ ID NO: 857); or

ACACAAAUACCAGUCCAGCGGUUUUCGAGCUAGAAAUAGCAAGUGAAA AUGAGGCUAGUCCGUGAUCCCGAAAGGGCACCGAGUCGGUGC (SEQ ID NO: 859);

wherein N_x denotes x contiguous nucleotides, where x is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25.

13. The gRNA of any one of claims 1-12, comprising the modified nucleotide sequence of any one of:

GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGC UAGUCCGUUAUCACGAAAGGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 381);

$(mN^*)_3N_x$GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAA UAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 581);

$(mN^*)_3N_{17}$GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAA UAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 781);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAGCUAGAAAUAGCAAG UUAAAAUAAGGCUAGUCCGUUAUCAAGAGCUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 105);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAGCUAGAAAUAGCAAG UUAAAAUAAGGCUAGUCCGUUAUCAAGAAAUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 106);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAGCUAGAAAUAGCAAG UUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 107);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAGCUAGAAAUAGCAAG UUAAAAUAAGGCUAGUCCGUUAUCAAAAAUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 108);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAGCUAGAAAUAGCAAG UUAAAAUAAGGCUAGUCCGUUAUCAAAAGUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 109);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAGCUAGAAAUAGCAAG UUAAAAUAAGGCUAGUCCGUUAUCAACAGUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 110);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAGCUAGAAAUAGCAAG UUAAAAUAAGGCUAGUCCGUUAUCACAAGGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 111);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAGCUAGAAAUAGCAAG UUAAAAUAAGGCUAGUCCGUUAUCAAAAUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 112);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA mAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGA GUCGG*mU*mG*mC (SEQ ID NO: 181);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmGmAmAmAmG mCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAGAAAUGGCACCGAGUCGG*mU* mG*mC (SEQ ID NO: 190);

GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCA AGAGCUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 305);

GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCA AGAAAUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 306);

GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCA CGAAAGGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 307);

GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCA AAAAUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 308);

GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCA AAAGUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 309);

GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCA ACAGUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 310);

GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCA CAAGGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 311);

GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCA AAAUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 312);

GUUUUAGAmGmCmGmAmAmAmGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAAGAAAUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 390);

GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGC UAGUCCGUUAUCAmCmGmAmAmAmGmGmGmCmAmCmCmGmAmGmUmCmGmG *mU*mG*mC (SEQ ID NO: 391);

GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGC UAGUCCGUUAUCACmGmAmAmAmGmGmGmCmAmCmCmGmAmGmUmCmGmG* mU*mG*mC (SEQ ID NO: 393);

(mN*)$_3$N$_x$GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCG UUAUCAAGAGCUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 505);

(mN*)$_3$N$_x$GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCG UUAUCAAGAAAUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 506);

(mN*)$_3$N$_x$GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCG UUAUCACGAAAGGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 507);

(mN*)$_3$N$_x$GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCG UUAUCAAAAAUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 508);

(mN*)$_3$N$_x$GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCG UUAUCAAAAGUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 509);

(mN*)$_3$N$_x$GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCG UUAUCAACAGUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 510);

(mN*)$_3$N$_x$GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCG UUAUCACAAGGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 511);

(mN*)$_3$N$_x$GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCG UUAUCAAAAUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 512);

(mN*)$_3$N$_x$GUUUUAGAmGmCmGmAmAmAmGmCAAGUUAAAAUAAGGCUA GUCCGUUAUCAAGAAAUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 590);

(mN*)$_3$N$_x$GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAA
UAAGGCUAGUCCGUUAUCAmCmGmAmAmAmGmGmGmCmAmCmCmGmAmGmU
mCmGmG*mU*mG*mC (SEQ ID NO: 591);

(mN*)$_3$N$_x$GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAA
UAAGGCUAGUCCGUUAUCACmGmAmAmAmGmGmGmCmAmCmCmGmAmGmUm
CmGmG*mU*mG*mC (SEQ ID NO: 593);

(mN*)$_3$N$_{17}$GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCG
UUAUCAAGAGCUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 705);

(mN*)$_3$N$_{17}$GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCG
UUAUCAAGAAAUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 706);

(mN*)$_3$N$_{17}$GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCG
UUAUCACGAAAGGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 707);

(mN*)$_3$N$_{17}$GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCG
UUAUCAAAAAUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 708);

(mN*)$_3$N$_{17}$GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCG
UUAUCAAAAGUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 709);

(mN*)$_3$N$_{17}$GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCG
UUAUCAACAGUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 710);

(mN*)$_3$N$_{17}$GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCG
UUAUCACAAGGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 711);

(mN*)$_3$N$_{17}$GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCG
UUAUCAAAAUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 712);

(mN*)$_3$N$_{17}$GUUUUAGAmGmCmGmAmAmAmGmCAAGUUAAAAUAAGGCUA
GUCCGUUAUCAAGAAAUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 790);

(mN*)$_3$N$_{17}$GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAA
UAAGGCUAGUCCGUUAUCAmCmGmAmAmAmGmGmGmCmAmCmCmGmAmGmU
mCmGmG*mU*mG*mC (SEQ ID NO: 791);

(mN*)$_3$N$_{17}$GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAA
UAAGGCUAGUCCGUUAUCACmGmAmAmAmGmGmGmCmAmCmCmGmAmGmUm
CmGmG*mU*mG*mC (SEQ ID NO: 793);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA
mAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmCmGmAmAmAmGm
GmGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 795);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA
mAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCACmGmAmAmAmGmG
mGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 797);

mA*mC*mG*CAAAUAUCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA
mAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGA
GUCGG*mU*mG*mC (SEQ ID NO: 909);

mA*mC*mG*CAAAUAUCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA
mAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmCmGmAmAmAmGm
GmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 911);

mG*mC*mC*GAGUCUGGAGAGCUGCAGUUUUAGAmGmCmUmAmGmAmA
mAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGA
GUCGG*mU*mG*mC (SEQ ID NO: 913);

mU*mG*mC*UCUGUAAGCUUACCCAGGUUUUAGAmGmCmUmAmGmAmA
mAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGA
GUCGG*mU*mG*mC (SEQ ID NO: 914);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA
mAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCACmGmAmAmAmGmG
GCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 921);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA
mAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGmGmCmA
mCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 922);

mA*mC*ACAA*AUACC*AGUCCAGCGmGU*UUUAG*A*mGmCmUmAmGm
AmAmAmUmAmGmCmAA*G*UUAAAAU*AAG*G*C*UmAGUCmCGUUA*UCA*C*
GAAAGGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 923);

mA*mC*ACAA*AUACC*AGUCCAGCGmGU*UUUAG*A*mGmCmUmAmGm
AmAmAmUmAmGmCmAA*G*UUAAAAU*AAG*G*C*UmAGUCmCGUUA*UCA*C*
mGmAmAmAmGmGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID
NO: 924);

mA*mC*ACAA*AUACC*AGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA
mAmUmAmGmCAAGUUAAAAUAAG*G*C*UmAGUCmCGUUA*UCA*C*mGmAmA
mAmGmGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 925);

mA*mC*ACAA*AUACC*AGUCCAGCGmGU*UUUAG*A*mGmCmUmAmGm
AmAmAmUmAmGmCmAA*G*UUAAAAU*AAGGCUAGUCCGUUAUCA*C*mGmAm
AmAmGmGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 926);

mA*mC*ACAA*AUACC*AGUCCAGCGmGU*UUUAG*A*mGmCmUmAmGmAmAmAmUmAmGmCmAA*G*UUAAAAU*AAG*G*C*UmAGUCmCGUUA*UCACmGmAmAmAmGmGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 927);

mA*mC*ACAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 928);

mA*mC*mA*CAA*AUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 929);

mA*mC*mA*CAAAUACC*AGUCCAGCGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 930);

mA*mC*mA*CAAAUACCAGUCCAGCGmGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 931);

mA*mC*mA*CAAAUACCAGUCCAGCGGU*UUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 932);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAG*AmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 933);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGA*mGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 934);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCmAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 935);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAA*GUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 936);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAG*UUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 937);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA
mAmUmAmGmCAAGUUAAAAU*AAGGCUAGUCCGUUAUCACGAAAGGGCACCG
AGUCGG*mU*mG*mC (SEQ ID NO: 938);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA
mAmUmAmGmCAAGUUAAAAUAAG*GCUAGUCCGUUAUCACGAAAGGGCACCG
AGUCGG*mU*mG*mC (SEQ ID NO: 939);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA
mAmUmAmGmCAAGUUAAAAUAAGG*CUAGUCCGUUAUCACGAAAGGGCACCG
AGUCGG*mU*mG*mC (SEQ ID NO: 940);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA
mAmUmAmGmCAAGUUAAAAUAAGGC*UAGUCCGUUAUCACGAAAGGGCACCG
AGUCGG*mU*mG*mC (SEQ ID NO: 941);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA
mAmUmAmGmCAAGUUAAAAUAAGGCUmAGUCCGUUAUCACGAAAGGGCACCG
AGUCGG*mU*mG*mC (SEQ ID NO: 942);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA
mAmUmAmGmCAAGUUAAAAUAAGGCUAGUCmCGUUAUCACGAAAGGGCACCG
AGUCGG*mU*mG*mC (SEQ ID NO: 943);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA
mAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUA*UCACGAAAGGGCACCG
AGUCGG*mU*mG*mC (SEQ ID NO: 944);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA
mAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA*CGAAAGGGCACCG
AGUCGG*mU*mG*mC (SEQ ID NO: 945);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA
mAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAC*GAAAGGGCACCG
AGUCGG*mU*mG*mC (SEQ ID NO: 946);

mA*mC*ACAA*AUACC*AGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA
mAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGA
GUCGG*mU*mG*mC (SEQ ID NO: 947);

mA*mC*mA*CAAAUACCAGUCCAGCGmGU*UUUAG*A*mGmCmUmAmGm
AmAmAmUmAmGmCmAA*G*UUAAAAU*AAGGCUAGUCCGUUAUCACGAAAGG
GCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 948);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA mAmUmAmGmCAAGUUAAAAUAAG*G*C*UmAGUCmCGUUA*UCACGAAAGGGC ACCGAGUCGG*mU*mG*mC (SEQ ID NO: 949);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA mAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCA*C*GAAAGGGCACCG AGUCGG*mU*mG*mC (SEQ ID NO: 950);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUCGAmGmCmUmAmGmAmA mAmUmAmGmCAAGUGAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGA GUCGG*mU*mG*mC (SEQ ID NO: 951);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA mAmUmAmGmCAAGUUAAAAUGAGGCUAGUCCGUUAUCACGAAAGGGCACCGA GUCGG*mU*mG*mC (SEQ ID NO: 952);

mA*mC*ACAA*AUACC*AGUCCAGCGmGU*UUUAG*A*mGmCmUmAmGm AmAmAmUmAmGmCmAA*G*UUAAAAU*GAG*G*C*UmAGUCmCGUUA*UCA*C* mGmAmAmAmGmGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 953);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA mAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUGAUCACGAAAGGGCACCGA GUCGG*mU*mG*mC (SEQ ID NO: 954);

mA*mC*ACAA*AUACC*AGUCCAGCGmGU*UUUAG*A*mGmCmUmAmGm AmAmAmUmAmGmCmAA*G*UUAAAAU*AAG*G*C*UmAGUCmCGUGA*UCA*C* mGmAmAmAmGmGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 955);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA mAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCCCGAAAGGGCACCGA GUCGG*mU*mG*mC (SEQ ID NO: 956);

mA*mC*ACAA*AUACC*AGUCCAGCGmGU*UUUAG*A*mGmCmUmAmGm AmAmAmUmAmGmCmAA*G*UUAAAAU*AAG*G*C*UmAGUCmCGUUA*UCC*C* mGmAmAmAmGmGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 957);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUCGAmGmCmUmAmGmAmA mAmUmAmGmCAAGUGAAAAUGAGGCUAGUCCGUGAUCCCGAAAGGGCACCGA GUCGG*mU*mG*mC (SEQ ID NO: 958);

mA*mC*ACAA*AUACC*AGUCCAGCGmGU*UUUCG*A*mGmCmUmAmGmA
mAmAmUmAmGmCmAA*G*UGAAAAU*GAG*G*C*UmAGUCmCGUGA*UCC*C*m
GmAmAmAmGmGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID
NO: 959);

mA*mC*ACAA*AUACC*AGUCCAGCGmGU*UUU*AG*A*mGmCmUmAmGm
AmAmAmUmAmGmCmAA*G*UU*AAAAU*AAG*G*C*UmAGUCmCGUU*A*UC*A*
C*mGmAmAmAmGmGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ
ID NO: 960);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA
mAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCACmGmAmAmAmGGG
CACCGAGUCGG*mU*mG*mC (SEQ ID NO: 962);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA
mAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGmGmCmA
mCmCmGfAfGfUfCmGmG*mU*mG*mC (SEQ ID NO: 963);

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmA
mAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGmGmCmA
mCmCmGAGUCmGmG*mU*mG*mC (SEQ ID NO: 964);

mA*mC*mACAAAUACCAGUCCAGCGmGUUUUAGAmGmCmUmAmGmAmA
mAmUmAmGmCmAAGUUAAAAUAAGGCUmAGUCmCGUUAUCACmGmAmAmAm
GG*mGmCmAmCmCmGAGUCmGmG*mU*mG*mC (SEQ ID NO: 965);

mA*mC*fAfCAfA*AfUfAfCfC*AfGUCCfAfGCfGmGU*UUUAG*fA*mGmCmU
mAmGmAmAmAmUmAmGmCmAfA*G*UUfAAfAAfU*fAfAfG*fG*fC*fUmAGUCmCf
GUUA*fUfCA*C*fG*mAmAmAmGfG*mGmCmAmCmCmGfA*fG*fUfCmGmGmU*mG
*mC (SEQ ID NO: 966);

mA*mC*ACAA*AUACC*AGUCCAGCGmGU*UUUAG*A*mGmCmUmAmGm
AmAmAmUmAmGmCmAA*G*UUAAAAU*AAG*G*C*UmAGUCmCGUUA*UCA*C*
G*mAmAmAmGG*mGmCmAmCmCmGA*G*UCmGmGmU*mG*mC (SEQ ID NO:
967);

mA*mC*fAfCAfAAfUfAfCfCAfGUCCfAfGCfGmGU*UUUAG*fAmGmCmUmA
mGmAmAmAmUmAmGmCmAfAG*UUfAAfAAfUfAfAfGfGfCfUmAGUCmCfGUUA*f
UfCA*C*fGmAmAmAmGfGmGmCmAmCmCmGfAfGfUfCmGmGmU*mG*mC (SEQ ID
NO: 968);

mA*mC*ACAAAUACCAGUCCAGCGmGU*UUUAG*fA*mGmCmUmAmGmA
mAmAmUmAmGmCmAfA*G*UUfAAfAAfU*fAfAfG*fG*fC*fUmAGUCmCfGUGA*fU

fCA*C*fG*mAmAmAmGfG*mGmCmAmCmCmGfA*fG*fUfCmGmGmU*mG*mC (SEQ ID NO: 969);

mA*mC*fAfCAfA*AfUfAfCfC*AfGUCCfAfGCfGmGU*UUUfAfG*fA*mGmCmUmAmGmAmAmAmUmAmGmCmAfA*fG*fUfUfAAfAfAfU*fAfAfG*fG*fC*fUmAGfUCmCfGfUUfA*fUfCfA*fC*fG*mAmAmAmGfG*mGmCmAmCmCmGfA*fG*fUfCmGmGmU*mG*mC (SEQ ID NO: 970);

mA*mC*mA*mCAA*A*fU*fA*fC*fCAfGfUCC*fAfGCGmGUUUfUAGmAmGmCmUmAmGmAmAmAmUmAmGmCmAmAGUfUmAfAmAfAmUAmAmGmGmCmUmAGUmCmCGUfUAmUmCAmCmGmAmAmAmGmGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 971);

mA*mC*mA*mCAA*A*fU*fA*fC*fCAfGfUCC*fAfGCGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 972);

mA*mC*mA*mCAAAfU*ACfCAfGUCC*AGCGmGUUUfUAGmAmGmCmUmAmGmAmAmAmUmAmGmCmAmAGUfUAAAAmUAAGGCmUAGUCCGUfUAUmCACGAAAGGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 973);

mA*mC*mA*mCAAAfU*ACfCAfGUCC*AGCGmGUUUUAmGmAmGCmUAmGmAmAmAmUAmGCmAmAGUfUAAAAmUAAGGCmUAGUCCGUfUA*mUmCA*CmGmAmAmAmGmGGmCAmCCGfAfGfUCmGG*mU*mG*mC (SEQ ID NO: 974); or

mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGCmUAmGmAmAmAmUAmGCAAGUUAAAAUAAGGCUAGUCCGUUAUCACGAAAGGGmCAmCCGfAfGfUCmGG*mU*mG*mC (SEQ ID NO: 975);

wherein N_x denotes x contiguous nucleotides, where x is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25, $(mN*)_3$ denotes three consecutive nucleotides each having any base, a 2'-O-Me, and a 3' phosphorothioate (PS) linkage to the next nucleotide, m denotes a nucleotide that has been modified with 2'-O-Me, f denotes a nucleotide that has been modified with 2'-F, and * denotes a nucleotide that is linked to the next nucleotide with a PS bond.

14. A composition comprising the gRNA of any one of claims 1-13 further comprising a lipid nanoparticle (LNP).

15. A composition comprising the gRNA of any one of claims 1-13, or the composition of claim 14, and:

i) further comprising a nuclease or a nucleic acid which encodes the nuclease;

ii) further comprising a nuclease or a nucleic acid which encodes the nuclease, wherein the nuclease is a Cas protein;

iii) further comprising a nuclease or a nucleic acid which encodes the nuclease, wherein the nuclease is a Cas9 Cas protein;

vi) further comprising a nuclease or a nucleic acid which encodes the nuclease, wherein the nuclease is a Cas protein and the Cas protein is an *S. pyogenes* Cas9 or an *S. aureus* Cas9;

v) further comprising a nuclease or a nucleic acid which encodes the nuclease, wherein the nuclease is a nickase or a dCas;

vi) further comprising a nuclease or a nucleic acid which encodes the nuclease, wherein the nuclease is modified;

vii) further comprising a nuclease or a nucleic acid which encodes the nuclease, wherein the nuclease is modified and comprises a nuclear localization signal (NLS);

viii) further comprising a nucleic acid which encodes a nuclease; or

ix) further comprising a nucleic acid which encodes a nuclease, wherein the nucleic acid comprises the sequence of any one of SEQ ID NOs: 1099-1127 or 1129-1146.

16. A pharmaceutical formulation comprising the gRNA of any one of claims 1-13 or the composition of any one of claims 14-15 and a pharmaceutically acceptable carrier.

17. A method of modifying a target DNA comprising, delivering a Cas protein or a nucleic acid encoding a Cas protein, and any one or more of the following to a cell:

i. the gRNA of any one of claims 1-13;

ii. the composition of any one of claims 14-15; and

iii. the pharmaceutical formulation of claim 16.

18. The method of claim 17:

i) wherein the method results in an insertion or deletion in a gene; and/or

ii) further comprising delivering to the cell a template, wherein at least a part of the template incorporates into a target DNA at or near a double strand break site induced by the Cas protein.

19. Use of the gRNA of any one of claims 1-13, the composition of claims 14-15, or the pharmaceutical formulation of claim 16 in the manufacture of a medicament for treating a disease or disorder.