

We claim:

1. ~~———— A guide RNA (gRNA) which is a short single guide RNA (short sgRNA) comprising a conserved portion of an sgRNA comprising a hairpin region, wherein the hairpin region lacks at least 5-10 nucleotides and wherein the short sgRNA comprises a 5' end modification or a 3' end modification.~~
2. ~~———— The gRNA of claim 1, wherein the short sgRNA comprises a 5' end modification.~~
3. ~~———— The gRNA of any one of the preceding claims, wherein the short sgRNA comprises a 3' end modification.~~
4. ~~———— The gRNA of any one of the preceding claims, wherein the short sgRNA comprises a 5' end modification and a 3' end modification.~~
5. ~~———— The gRNA of any one of the preceding claims, wherein the short sgRNA comprises a 3' tail.~~
6. ~~———— The gRNA of claim 5, wherein the 3' tail comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides.~~
7. ~~———— The gRNA of claim 5, wherein the 3' tail comprises about 1-2, 1-3, 1-4, 1-5, 1-7, 1-10, at least 1-5, at least 1-3, at least 1-4, at least 1-5, at least 1-5, at least 1-7, or at least 1-10 nucleotides.~~
8. ~~———— The gRNA of any one of the preceding claims, wherein the short sgRNA does not comprise a 3' tail.~~
9. ~~———— The gRNA of any one of the preceding claims, comprising a modification in the hairpin region.~~
10. ~~———— The gRNA of any one of the preceding claims, comprising a 3' end modification, and a modification in the hairpin region.~~
11. ~~———— The gRNA of any one of the preceding claims, comprising a 3' end modification, a modification in the hairpin region, and a 5' end modification.~~
12. ~~———— The gRNA of any one of the preceding claims, comprising a 5' end modification, and a modification in the hairpin region.~~
13. ~~———— The gRNA of any one of the preceding claims, wherein the at least 5-10 lacking nucleotides are consecutive.~~
14. ~~———— The gRNA of any one of the preceding claims, wherein the at least 5-10 lacking nucleotides:~~

- ~~i. are within hairpin 1;~~
 - ~~ii. are within hairpin 1 and the “N” between hairpin 1 and hairpin 2;~~
 - ~~iii. are within hairpin 1 and the two nucleotides immediately 3’ of hairpin 1;~~
 - ~~iv. include at least a portion of hairpin 1;~~
 - ~~v. are within hairpin 2;~~
 - ~~vi. include at least a portion of hairpin 2;~~
 - ~~vii. are within hairpin 1 and hairpin 2;~~
 - ~~viii. include at least a portion of hairpin 1 and include the “N” between hairpin 1 and hairpin 2;~~
 - ~~ix. include at least a portion of hairpin 2 and include the “N” between hairpin 1 and hairpin 2;~~
 - ~~x. include at least a portion of hairpin 1, include the “N” between hairpin 1 and hairpin 2, and include at least a portion of hairpin 2;~~
 - ~~xi. are within hairpin 1 or hairpin 2, optionally including the “N” between hairpin 1 and hairpin 2;~~
 - ~~xii. are consecutive;~~
 - ~~xiii. are consecutive and include the “N” between hairpin 1 and hairpin 2;~~
 - ~~xiv. are consecutive and span at least a portion of hairpin 1 and a portion of hairpin 2;~~
 - ~~xv. are consecutive and span at least a portion of hairpin 1 and the “N” between hairpin 1 and hairpin 2; or~~
 - ~~xvi. are consecutive and span at least a portion of hairpin 1 and two nucleotides immediately 3’ of hairpin 1.~~
- ~~15. ——— The gRNA of any one of the preceding claims, further comprising a guide region.~~
- ~~16. ——— The gRNA of any one of the preceding claims, wherein the 3’ and/or 5’ end modification comprises a protective end modification, such as a modified nucleotide selected from 2’-O-methyl (2’-OMe) modified nucleotide, 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 combinations thereof.~~
- ~~17. ——— The gRNA of any one of the preceding claims, wherein the modification in the hairpin region comprises a modified nucleotide selected from 2’-O-methyl (2’-OMe)~~

modified nucleotide, a 2' fluoro (2' F) modified nucleotide, a phosphorothioate (PS) linkage between nucleotides, or combinations thereof.

18. ~~_____ The gRNA of any one of the preceding claims, wherein the 3' and/or 5' end modification comprises or further comprises a 2' O-methyl (2' OMe) modified nucleotide.~~
19. ~~_____ The gRNA of any one of the preceding claims, wherein the 3' and/or 5' end modification comprises or further comprises a 2' fluoro (2' F) modified nucleotide.~~
20. ~~_____ The gRNA of any one of the preceding claims, wherein the 3' and/or 5' end modification comprises or further comprises a phosphorothioate (PS) linkage between nucleotides.~~
21. ~~_____ The gRNA of any one of the preceding claims, wherein the 3' and/or 5' end modification comprises or further comprises an inverted abasic modified nucleotide.~~
22. ~~_____ The gRNA of any one of the preceding claims, wherein the modification in the hairpin region comprises or further comprises a 2' O-methyl (2' OMe) modified nucleotide.~~
23. ~~_____ The gRNA of any one of the preceding claims, wherein the modification in the hairpin region comprises or further comprises a 2' fluoro (2' F) modified nucleotide.~~
24. ~~_____ The gRNA of any one of the preceding claims, wherein the 3' end modification comprises any of:
 - i. ~~_____ a modification of any one or more of the last 7, 6, 5, 4, 3, 2, or 1 nucleotides;~~
 - ii. ~~_____ one modified nucleotide;~~
 - iii. ~~_____ two modified nucleotides;~~
 - iv. ~~_____ three modified nucleotides;~~
 - v. ~~_____ four modified nucleotides;~~
 - vi. ~~_____ five modified nucleotides;~~
 - vii. ~~_____ six modified nucleotides; and~~
 - viii. ~~_____ seven modified nucleotides.~~~~
25. ~~_____ The gRNA of any one of the preceding claims, wherein the at least 5-10 nucleotides comprise nucleotides 54-61 of SEQ ID NO:400, nucleotides 53-60 of SEQ ID NO:400; or nucleotides 54-58 of SEQ ID NO:400, optionally wherein the short-sgRNA comprises modifications at least H1-1 to H1-5 and H2-1 to H2-12.~~
26. ~~_____ The gRNA of any one of the preceding claims, wherein the at least 5-10 nucleotides:~~

- ~~i.—consist of 5-10 nucleotides;~~
- ~~ii.—consist of 6-10 nucleotides;~~
- ~~iii.—consist of 5 nucleotides;~~
- ~~iv.—consist of 6 nucleotides;~~
- ~~v.—consist of 7 nucleotides;~~
- ~~vi.—consist of 8 nucleotides;~~
- ~~vii.—consist of 9 nucleotides;~~
- ~~viii.—consist of 10 nucleotides;~~
- ~~ix.—consist of 5-10 consecutive nucleotides;~~
- ~~x.—consist of 6-10 consecutive nucleotides;~~
- ~~xi.—consist of 5 consecutive nucleotides;~~
- ~~xii.—consist of 6 consecutive nucleotides;~~
- ~~xiii.—consist of 7 consecutive nucleotides;~~
- ~~xiv.—consist of 8 consecutive nucleotides;~~
- ~~xv.—consist of 9 consecutive nucleotides; or~~
- ~~xvi.—consist of 10 consecutive nucleotides.~~

~~27.——The gRNA of any one of the preceding claims, wherein the 3' end modification comprises one or more of:~~

- ~~i.—a phosphorothioate (PS) linkage between nucleotides;~~
- ~~ii.—a 2'-OMe modified nucleotide;~~
- ~~iii.—a 2'-O-moe modified nucleotide;~~
- ~~iv.—a 2'-F modified nucleotide;~~
- ~~v.—an inverted abasic modified nucleotide; and~~
- ~~vi.—a combination of one or more of (i.)—(v.).~~

~~28.——The gRNA of any one of the preceding claims, wherein the short-sgRNA comprises a 3' tail comprising one or more of:~~

- ~~i.—a phosphorothioate (PS) linkage between nucleotides;~~
- ~~ii.—a 2'-OMe modified nucleotide;~~
- ~~iii.—a 2'-O-moe modified nucleotide;~~
- ~~iv.—a 2'-F modified nucleotide;~~
- ~~v.—an inverted abasic modified nucleotide; and~~
- ~~vi.—a combination of one or more of (i.)—(v.).~~

~~29.——The gRNA any one of the preceding claims, wherein the short-sgRNA comprises one or more of:~~

- ~~i. 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 PS linkages between nucleotides;~~
 - ~~ii. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, or 18 PS linkages between nucleotides;~~
 - ~~iii. about 1-3, 1-5, 1-6, 1-7, 1-8, 1-9, or 1-10 PS linkages between nucleotides;~~
 - ~~iv. about 1-3, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-12, 1-14, 1-16, 1-18, or 1-20 PS linkages between nucleotides; and~~
 - ~~v. PS linkages between each nucleotide.~~
30. ~~———— The gRNA of any one of the preceding claims, wherein the 3' end modification comprises at least one PS linkage, and wherein one or more of:~~
- ~~i. there is one PS linkage, and the linkage is between the last and second to last nucleotide;~~
 - ~~ii. there are two PS linkages between the last three nucleotides;~~
 - ~~iii. there are PS linkages between any one or more of the last four nucleotides;~~
 - ~~iv. there are PS linkages between any one or more of the last five nucleotides; and~~
 - ~~v. there are PS linkages between any one or more of the last 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides.~~
31. ~~The gRNA of claim 30, wherein the 3' end modification further comprises at least one 2'-OMe, 2'-O-moe, inverted abasic, or 2'-F modified nucleotide.~~
32. ~~———— The gRNA of any one of the preceding claims, wherein the 3' end modification comprises:~~
- ~~i. a modification of one or more of the last 1-7 nucleotides, wherein the modification is a PS linkage, inverted abasic nucleotide, 2'-OMe, 2'-O-moe, 2'-F, or combinations thereof;~~
 - ~~ii. a modification to the last nucleotide with 2'-OMe, 2'-O-moe, 2'-F, or combinations thereof, and an optional one or two PS linkages to the next nucleotide and/or the first nucleotide of the 3' tail;~~
 - ~~iii. a modification to the last and/or second to last nucleotide with 2'-OMe, 2'-O-moe, 2'-F, or combinations thereof, and optionally one or more PS linkages;~~

- iv. ~~a modification to the last, second to last, and/or third to last nucleotides with 2' OMe, 2' O-moe, 2' F, or combinations thereof, and optionally one or more PS linkages;~~
 - v. ~~a modification to the last, second to last, third to last, and/or fourth to last nucleotides with 2' OMe, 2' O-moe, 2' F, or combinations thereof, and optionally one or more PS linkages; or~~
 - vi. ~~a modification to the last, second to last, third to last, fourth to last, and/or fifth to last nucleotides with 2' OMe, 2' O-moe, 2' F, or combinations thereof, and optionally one or more PS linkages.~~
33. ~~The gRNA of any one of the preceding claims, wherein the sgRNA comprise a 3' tail, wherein the 3' tail comprises a modification of any one or more of the nucleotides present in the 3' tail.~~
34. ~~The gRNA of claim 33, wherein the 3' tail is fully modified.~~
35. ~~The gRNA of claim 33, wherein the at least 5-10 nucleotides comprise nucleotides 54-61 of SEQ ID NO:400, nucleotides 53-60 of SEQ ID NO:400; or nucleotides 54-58 of SEQ ID NO:400, optionally wherein the short sgRNA comprises modifications at least H1-1 to H1-5 and H2-1 to H2-12.~~
36. ~~The gRNA of any one of the preceding claims, wherein the short sgRNA comprises any one or more of:~~
- i. ~~the 3' end modification as shown in any one of SEQ ID Nos: 1-54;~~
 - ii. ~~(i) a 2' OMe modified nucleotide at the last nucleotide of the conserved region of an sgRNA or short sgRNA, (ii) three consecutive 2' O-moe modified nucleotides immediately 5' to the 2' OMe modified nucleotide, and (iii) three consecutive PS linkages between the last three nucleotides;~~
 - iii. ~~(i) five consecutive 2' OMe modified nucleotides from the 3' end of the 3' terminus, and (ii) three PS linkages between the last three nucleotides;~~
 - iv. ~~an inverted abasic modified nucleotide at the last nucleotide of the conserved region of an sgRNA or short sgRNA;~~
 - v. ~~(i) an inverted abasic modified nucleotide at the last nucleotide of the conserved region of an sgRNA or short sgRNA, and (ii) three consecutive 2' OMe modified nucleotides at the last three nucleotides of the conserved region of an sgRNA or short sgRNA;~~

- vi. ~~(i) 15 consecutive 2' OMe modified nucleotides from the 3' end of the 3' terminus, (ii) five consecutive 2' F modified nucleotides immediately 5' to the 2' OMe modified nucleotides, and (iii) three PS linkages between the last three nucleotides;~~
 - vii. ~~(i) alternating 2' OMe modified nucleotides and 2' F modified nucleotides at the last 20 nucleotides of the conserved region of an sgRNA or short sgRNA, and (ii) three PS linkages between the last three nucleotides;~~
 - viii. ~~(i) two or three consecutive 2' OMe modified nucleotides, and (ii) three PS linkages between the last three nucleotides;~~
 - ix. ~~one PS linkage between the last and next to last nucleotides; and~~
 - x. ~~15 or 20 consecutive 2' OMe modified nucleotides, and (ii) three PS linkages between the last three nucleotides.~~
37. ~~The gRNA of any one of the preceding claims, wherein the 5' end modification comprises any one or more of:~~
- i. ~~a modification of any one or more of nucleotides 1-7 of the guide region;~~
 - ii. ~~one modified nucleotide;~~
 - iii. ~~two modified nucleotides;~~
 - iv. ~~three modified nucleotides;~~
 - v. ~~four modified nucleotides;~~
 - vi. ~~five modified nucleotides;~~
 - vii. ~~six modified nucleotides; and~~
 - viii. ~~seven modified nucleotides.~~
38. ~~The gRNA of any one of the preceding claims, wherein the 5' end modification comprises a modification of between 1 and 7, between 1 and 5, between 1 and 4, between 1 and 3, or between 1 and 2 nucleotides.~~
39. ~~The gRNA of any one of the preceding claims, wherein the at least 5-10 nucleotides:~~
- i. ~~comprise nucleotides 54-61 of SEQ ID NO:400;~~
 - ii. ~~comprise nucleotides 53-60 of SEQ ID NO:400;~~
 - iii. ~~comprise nucleotides 54-58 of SEQ ID NO:400.~~
 - iv. ~~consist of nucleotides 54-61 of SEQ ID NO:400;~~
 - v. ~~consist of nucleotides 53-60 of SEQ ID NO:400; or~~
 - vi. ~~consist of nucleotides 54-58 of SEQ ID NO:400.~~

40. ~~The gRNA of any one of the preceding claims, wherein the 5' end modification comprises one or more of:~~
- ~~i. a phosphorothioate (PS) linkage between nucleotides;~~
 - ~~ii. a 2' OMe modified nucleotide;~~
 - ~~iii. a 2' O-moe modified nucleotide;~~
 - ~~iv. a 2' F modified nucleotide;~~
 - ~~v. an inverted-abasic modified nucleotide;~~
 - ~~vi. a deoxyribonucleotide;~~
 - ~~vii. an inosine; and~~
 - ~~viii. combinations of one or more of (i.)—(vii.).~~
41. ~~The gRNA any one of the preceding claims, wherein the 5' end modification comprises:~~
- ~~i. 1, 2, 3, 4, 5, 6, and/or 7 PS linkages between nucleotides; or~~
 - ~~ii. about 1-2, 1-3, 1-4, 1-5, 1-6, or 1-7 PS linkages between nucleotides.~~
42. ~~The gRNA of any one of the preceding claims, wherein the 5' end modification comprises at least one PS linkage, and wherein:~~
- ~~i. there is one PS linkage, and the linkage is between nucleotides 1 and 2 of the guide region;~~
 - ~~ii. there are two PS linkages, and the linkages are between nucleotides 1 and 2, and 2 and 3 of the guide region;~~
 - ~~iii. there are PS linkages between any one or more of nucleotides 1 and 2, 2 and 3, and 3 and 4 of the guide region;~~
 - ~~iv. there are PS linkages between any one or more of nucleotides 1 and 2, 2 and 3, 3 and 4, and 4 and 5 of the guide region;~~
 - ~~v. there are PS linkages between any one or more of nucleotides 1 and 2, 2 and 3, 3 and 4, 4 and 5, and 5 and 6 of the guide region;~~
 - ~~vi. there are PS linkages between any one or more of nucleotides 1 and 2, 2 and 3, 3 and 4, 4 and 5, 5 and 6, and 6 and 7 of the guide region; or~~
 - ~~vii. there are PS linkages between any one or more of nucleotides 1 and 2, 2 and 3, 3 and 4, 4 and 5, 5 and 6, 6 and 7, and 7 and 8 of the guide region.~~
43. ~~The gRNA of claim 42, wherein the 5' end modification further comprises at least one 2' OMe, 2' O-moe, inverted abasic, or 2' F modified nucleotide.~~
44. ~~The gRNA of any one of the preceding claims, wherein the short-sgRNA comprises:~~

- i. ~~a modification of one or more of nucleotides 1-7 of the variable region, wherein the modification is a PS linkage, inverted abasic nucleotide, 2'-OMe, 2'-O-moe, 2'-F, 2'-H (a deoxyribonucleotide), an inosine, and/or combinations thereof;~~
 - ii. ~~a modification to the first nucleotide of the guide region with 2'-OMe, 2'-O-moe, 2'-F, 2'-H, an inosine, or combinations thereof, and an optional PS linkage to the next nucleotide;~~
 - iii. ~~a modification to the first and/or second nucleotide of the variable region with 2'-OMe, 2'-O-moe, 2'-F, 2'-H, an inosine, or combinations thereof, and optionally one or more PS linkages;~~
 - iv. ~~a modification to the first, second, and/or third nucleotides of the variable region with 2'-OMe, 2'-O-moe, 2'-F, 2'-H, an inosine, or combinations thereof, and optionally one or more PS linkages;~~
 - v. ~~a modification to the first, second, third, and/or fourth nucleotides of the variable region with 2'-OMe, 2'-O-moe, 2'-F, 2'-H, an inosine, or combinations thereof, and optionally one or more PS linkages; or~~
 - vi. ~~a modification to the first, second, third, fourth, and/or fifth nucleotides of the variable region with 2'-OMe, 2'-O-moe, 2'-F, 2'-H, an inosine, or combinations thereof, and optionally one or more PS linkages.~~
45. ~~The gRNA of any one of the preceding claims, wherein the short-sgRNA comprises any one or more of:~~
- i. ~~a 5' end modification as shown in any one of SEQ ID Nos: 1-54;~~
 - ii. ~~2'-OMe modified nucleotides at nucleotides 1, 2, and 3 of the guide region;~~
 - iii. ~~2'-OMe modified nucleotides at nucleotides 1, 2, and 3 of the guide region and PS linkages between nucleotides 1 and 2, 2 and 3, and 3 and 4 of the guide region;~~
 - iv. ~~2'-OMe modified nucleotides at nucleotides 1, 2, 3, 4, and 5 of the guide region;~~
 - v. ~~2'-OMe modified nucleotides at nucleotides 1, 2, 3, 4, and 5 of the guide region and PS linkages between nucleotides 1 and 2, 2 and 3, 3 and 4, 4 and 5, and 5 and 6 of the guide region;~~
 - vi. ~~2'-O-moe modified nucleotides at nucleotides 1, 2, and 3 of the guide region;~~

- vii. ~~2'-O-moe-modified nucleotides at nucleotides 1, 2, and 3 of the guide region and PS linkages between nucleotides 1 and 2, 2 and 3, and 3 and 4 of the guide region;~~
 - viii. ~~an inverted abasic modified nucleotide at nucleotide 1 of the guide region;~~
 - ix. ~~an inverted abasic modified nucleotide at nucleotide 1 of the guide region and 2'-OMe-modified nucleotides at nucleotides 1, 2, and 3 of the guide region; and~~
 - x. ~~an inverted abasic modified nucleotide at nucleotide 1 of the guide region, 2'-OMe-modified nucleotides at nucleotides 1, 2, and 3 of the guide region, and PS linkages between nucleotides 1 and 2, 2 and 3, 3 and 4, 4 and 5, and 5 and 6 of the variable region.~~
46. ~~The gRNA of any one of the preceding claims, wherein the upper stem region comprises at least one modification.~~
47. ~~The gRNA of any one of the preceding claims, wherein the upper stem modification comprises any one or more of:~~
- i. ~~a modification to any one or more of US1-US12 in the upper stem region;~~
 - ii. ~~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; and~~
 - iii. ~~a modification of about 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, or 1-12 nucleotides in the upper stem region.~~
48. ~~The gRNA of claim 47, wherein the upper stem modification comprises one or more of:~~
- i. ~~a 2'-OMe-modified nucleotide;~~
 - ii. ~~a 2'-O-moe-modified nucleotide;~~
 - iii. ~~a 2'-F-modified nucleotide; and~~
 - iv. ~~combinations of one or more of (i.)-(iii.).~~
49. ~~A guide RNA which is a short-sgRNA comprising a conserved portion of an sgRNA comprising a hairpin region, wherein the hairpin region lacks at least 5-10 nucleotides and wherein the short-sgRNA comprises a 5' end modification and one or more modification in one or more of:~~
- i. ~~the upper stem region;~~
 - ii. ~~the hairpin 1 region; and~~

- iii. ~~the hairpin 2 region,~~
wherein the 5' end modification comprises a 5' protective end modification, such as at least two phosphorothioate (PS) linkages within the first seven nucleotides.
50. ~~The gRNA of claim 49, wherein at least one modification comprises a 2' O-methyl (2' OMe) modified nucleotide.~~
51. ~~The gRNA of claim 49 or claim 50, wherein at least one modification comprises a 2' fluoro (2' F) modified nucleotide.~~
52. ~~The gRNA of any one of claims 49-51, wherein at least one modification comprises a phosphorothioate (PS) bond between nucleotides.~~
53. ~~The gRNA of any one of claims 49-52, wherein the short-sgRNA comprises one or more modifications in the upper stem region.~~
54. ~~The gRNA of claim 53, comprising modifications at any one of US1 to US12.~~
55. ~~The gRNA of any one of claims 49-54, wherein the short-sgRNA comprises one or more modifications in the hairpin 1 region.~~
56. ~~The gRNA of claim 55, wherein the short-sgRNA comprises a modification at H1-1.~~
57. ~~The gRNA of any one of claims 49-56, wherein the short-sgRNA comprises one or more modifications in the hairpin 2 region.~~
58. ~~The gRNA of claim 57, wherein the short-sgRNA comprises a modification at H2-1.~~
59. ~~The gRNA of any one of claims 49-58, wherein the short-sgRNA comprises modifications at H1-1 to H1-12.~~
60. ~~The gRNA of any one of claims 49-59, wherein the short-sgRNA comprises modifications at H2-1 to H2-15.~~
61. ~~The gRNA of any one of claims 49-60, wherein the short-sgRNA comprises one or more modifications in each of the upper stem region, the hairpin 1 region, and the hairpin 2 region.~~
62. ~~The gRNA of any one of claims 49-61, wherein the short-sgRNA comprises a modified nucleotide between hairpin 1 and hairpin 2 regions.~~
63. ~~The gRNA of any one of claims 49-62, further comprising a lower stem region comprising a modification.~~
64. ~~The gRNA of any one of claims 49-63, further comprising a 3' end modification.~~

65. ~~_____ The gRNA of claim 64, wherein at least two of the last four nucleotides at the 3' end of the 3' terminus are modified.~~
66. ~~_____ The gRNA of claim 64, wherein at least two of the last four nucleotides at the 3' end of the 3' terminus are modified with 2' OMe, 2' F, or 2' O-moe.~~
67. ~~_____ The gRNA of any one of claims 64-66, further comprising phosphorothioate (PS) bonds between one or more of the last four nucleotides at the 3' end of the 3' terminus.~~
68. ~~_____ The gRNA of any one of claims 49-67, further comprising a bulge region comprising a modification.~~
69. ~~_____ The gRNA of any one of claims 49-68, further comprising a nexus region comprising a modification.~~
70. ~~_____ The gRNA of any one of claims 49-69, wherein at least the first three nucleotides at the 5' end of the variable region, and the last three nucleotides at the 3' end of the 3' terminus are modified.~~
71. ~~_____ The gRNA of any one of claims 49-70, wherein the first four nucleotides at the 5' end of the variable region, and the last four nucleotides at the 3' end of the 3' terminus are linked with phosphorothioate (PS) bonds.~~
72. ~~_____ The gRNA of any one of claims 70-71, wherein the end modifications comprise 2' OMe.~~
73. ~~_____ The gRNA of any one of claims 70-71, wherein the end modifications comprise 2' F.~~
74. ~~_____ The gRNA of any one of claims 49-73, wherein the first four nucleotides at the 5' end of the variable region and the last four nucleotides at the 3' end of the 3' terminus are linked with a PS bond, and wherein the first three nucleotides at the 5' end of the variable region and the last three nucleotides at the 3' end of the 3' terminus comprise 2' OMe modifications.~~
75. ~~_____ The gRNA of any one of claims 49-74, wherein the first four nucleotides at the 5' terminus and the last four nucleotides at the 3' terminus are linked with a PS bond, and wherein the first three nucleotides at the 5' terminus and the last three nucleotides at the 3' terminus comprise 2' OMe, 2' F, and/or 2' O-moe modifications.~~
76. ~~_____ The gRNA of any one of claims 49-75, wherein LS1, LS6, LS7, LS8, LS11, and/or LS12 are modified with 2' OMe.~~

77. ~~—The gRNA of any one of claims 49-76, wherein each of the nucleotides in the bulge region are modified with 2'-OMe.~~
78. ~~—The gRNA of any one of claims 49-77, wherein at least 50% of the nucleotides in the bulge region are modified with 2'-OMe.~~
79. ~~—The gRNA of any one of claims 49-78, wherein each of the nucleotides in the upper stem region are modified with 2'-OMe.~~
80. ~~—The gRNA of any one of claims 49-79, wherein N16, N17, and/or N18 in the nexus region are modified with 2'-OMe.~~
81. ~~—The gRNA of any one of claims 49-80, wherein N15, N16, N17, and/or N18 in the nexus region are modified.~~
82. ~~—The gRNA of claim 80 or 81, wherein the modifications in the nexus region are selected from 2'-OMe and 2'-F.~~
83. ~~—The gRNA of any one of claims 80-82, wherein N16, N17, and N18 are linked with PS bonds.~~
84. ~~—The gRNA of any one of claims 49-83, wherein each of the nucleotides remaining in the hairpin 1 region are modified with 2'-OMe.~~
85. ~~—The gRNA of any one of claims 49-84, wherein each of the nucleotides in the hairpin 2 region are modified with 2'-OMe.~~
86. ~~—A guide RNA which is a short-sgRNA comprising a conserved portion of an sgRNA comprising a hairpin region, wherein the hairpin region lacks at least 5-10 nucleotides and wherein the short-sgRNA comprises a 5' end modification and a 3' end modification, wherein the short-sgRNA further comprises any one or more of:~~
 - ~~i. at least one modification in the upper stem region; and~~
 - ~~ii. a 3' tail.~~
87. ~~—The gRNA of claim 86, wherein the upper stem modification comprises any one or more of:~~
 - ~~i. a modification of each nucleotide (US1-US12) in the upper stem region;~~
 - ~~ii. a modification to any one or more of US1-US12 in the upper stem region;~~
 - ~~iii. 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; and~~
 - ~~iv. a modification of about 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, or 1-12 nucleotides in the upper stem region.~~

~~88. The gRNA of any one of claims 86-87, wherein the 5' end modification comprises any one or more of:~~

- ~~i. a modification of any one or more of nucleotides 1-7 of the variable region;~~
- ~~ii. one modified nucleotide;~~
- ~~iii. two modified nucleotides;~~
- ~~iv. three modified nucleotides;~~
- ~~v. four modified nucleotides;~~
- ~~vi. five modified nucleotides;~~
- ~~vii. six modified nucleotides; and~~
- ~~viii. seven modified nucleotides.~~

~~89. The gRNA of any one of claims 86-88, wherein the 5' end modification comprises any one or more of:~~

- ~~i. a 5' end modification as shown in any one of SEQ ID Nos: 1-54, 401-532, 1001, 1007-1132, 1205-1212, 1322-1406, 1417-1501, 1511-1596, 3018-3059, 3063-3104, 3108-3149, 3153-3194, 3198-3239, 3243-3284, 3295-3341, 3343-3385, 3388-3430, or 3549-3552;~~
- ~~ii. 2' OMe modified nucleotides at nucleotides 1, 2, and 3 of the variable region;~~
- ~~iii. 2' OMe modified nucleotides at nucleotides 1, 2, and 3 of the variable region and PS linkages between nucleotides 1 and 2, 2 and 3, and 3 and 4 of the variable region;~~
- ~~iv. 2' OMe modified nucleotides at nucleotides 1, 2, 3, 4, and 5 of the variable region;~~
- ~~v. 2' OMe modified nucleotides at nucleotides 1, 2, 3, 4, and 5 of the variable region and PS linkages between nucleotides 1 and 2, 2 and 3, 3 and 4, 4 and 5, and 5 and 6 of the variable region;~~
- ~~vi. 2'O-moe modified nucleotides at nucleotides 1, 2, and 3 of the variable region;~~
- ~~vii. 2'O-moe modified nucleotides at nucleotides 1, 2, and 3 of the variable region and PS linkages between nucleotides 1 and 2, 2 and 3, and 3 and 4 of the variable region;~~
- ~~viii. an inverted abasic modified nucleotide at nucleotide 1 of the variable region;~~

- ix. ~~an inverted abasic modified nucleotide at nucleotide 1 of the variable region and 2' OMe modified nucleotides at nucleotides 1, 2, and 3 of the variable region; and~~
 - x. ~~an inverted abasic modified nucleotide at nucleotide 1 of the variable region, 2' OMe modified nucleotides at nucleotides 1, 2, and 3 of the variable region, and PS linkages between nucleotides 1 and 2, 2 and 3, 3 and 4, 4 and 5, and 5 and 6 of the variable region.~~
90. ~~The gRNA of claim 89, comprising 2' OMe modified nucleotides at at least nucleotides 1, 2, and 3, of the variable region and PS linkages between nucleotides 1 and 2, 2 and 3, and 3 and 4 of the variable region.~~
91. ~~The gRNA of claim 89, comprising 2' OMe modified nucleotides at at least nucleotides 1, 2, 3, and 4 of the variable region and PS linkages between nucleotides 1 and 2, 2 and 3, and 3 and 4 of the variable region.~~
92. ~~The gRNA of any one of claims 86-91, comprising a 3' end modification comprising any one or more of:~~
- i. ~~a modification of any one or more of the last 7, 6, 5, 4, 3, 2, or 1 nucleotides;~~
 - ii. ~~one modified nucleotide;~~
 - iii. ~~two modified nucleotides;~~
 - iv. ~~three modified nucleotides;~~
 - v. ~~four modified nucleotides;~~
 - vi. ~~five modified nucleotides;~~
 - vii. ~~six modified nucleotides; and~~
 - viii. ~~seven modified nucleotides.~~
93. ~~The gRNA of any one of claims 86-92, wherein the short-sgRNA comprises any one or more of:~~
- i. ~~a 3' end modification shown in any one of SEQ ID Nos: 1-54, 401-532, 1001, 1007-1132, 1205-1212, 1322-1406, 1417-1501, 1511-1596, 3018-3059, 3063-3104, 3108-3149, 3153-3194, 3198-3239, 3243-3284, 3295-3341, 3343-3385, 3388-3430, or 3549-3552;~~
 - ii. ~~(i) a 2' OMe modified nucleotide at the last nucleotide of the conserved region of an sgRNA or short-sgRNA, (ii) three consecutive 2' Ome modified nucleotides immediately 5' to the 2' OMe modified nucleotide,~~

- and (iii) three consecutive PS linkages between the last three nucleotides;
 - iii. ~~(i) five consecutive 2' OMe modified nucleotides, and (ii) three PS linkages between the last three nucleotides;~~
 - iv. ~~an inverted abasic modified nucleotide at the last nucleotide of the conserved region of an sgRNA or short sgRNA;~~
 - v. ~~(i) an inverted abasic modified nucleotide at the last nucleotide of the conserved region of an sgRNA or short sgRNA, and (ii) three consecutive 2' OMe modified nucleotides at the last three nucleotides of the conserved region of an sgRNA or short sgRNA;~~
 - vi. ~~(i) 15 consecutive 2' OMe modified nucleotides, (ii) five consecutive 2' F modified nucleotides immediately 5' to the 2' OMe modified nucleotides, and (iii) three PS linkages between the last three nucleotides;~~
 - vii. ~~(i) alternating 2' OMe modified nucleotides and 2' F modified nucleotides at the last 20 nucleotides of the conserved region of an sgRNA or short sgRNA, and (ii) three PS linkages between the last three nucleotides;~~
 - viii. ~~(i) two or three consecutive 2' OMe modified nucleotides, and (ii) three PS linkages between the last three nucleotides;~~
 - ix. ~~one PS linkage between the last and next to last nucleotides; and~~
 - x. ~~15 or 20 consecutive 2' OMe modified nucleotides, and (ii) three PS linkages between the last three nucleotides.~~
94. ~~The gRNA of any one of claims 86-93, wherein the sgRNA comprise a 3' tail, wherein the 3' tail comprises a modification of any one or more of the nucleotides present in the 3' tail.~~
95. ~~The gRNA of claim 94, wherein the 3' tail is fully modified.~~
96. ~~The gRNA of claim 94 wherein the 3' tail comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, or 1-10 nucleotides, optionally where any one or more of these nucleotides are modified.~~
97. ~~A guide RNA which is a short sgRNA comprising any of SEQ ID Nos: 1-54, 201-254, and 301-354, including the modifications of Table 1.~~
98. ~~A guide RNA which is a short sgRNA comprising nucleic acids having at least 99, 98, 97, 96, 95, 94, 93, 92, 91, 90, 85, 80, 75, or 70% identity to the nucleic~~

acids of any one of SEQ ID Nos: 1-54, 201-254, and 301-354, wherein the modification at each nucleotide of the short-sgRNA that corresponds to a nucleotide of the reference sequence identifier in Table 1, is identical to or equivalent to the modification shown in the reference sequence identifier in Table 1.

99. ——— The gRNA of any one of the preceding claims, comprising a YA modification at at least one guide region YA site.

100. ——— The gRNA of any one of the preceding claims, comprising a YA modification at at least one guide region YA site that is not a 5' end modification.

101. ——— The gRNA of any one of the preceding claims, comprising a YA modification at one or more guide region YA sites, wherein the guide region YA site is at or after nucleotide 8 from the 5' end of the 5' terminus.

102. ——— The gRNA of any one of the preceding claims comprising a YA modification at one or more guide region YA sites, wherein the short-sgRNA comprises one or more of:

- i. — a modification at one or more of H1-1 and H2-1;
- ii. — a YA modification at 1, 2, 3, 4, or 5 guide region YA sites;
- iii. — a YA modification at 1, 2, 3, 4, or 5 guide region YA sites, wherein the modification of at least one guide region YA site is different from any 5' end modification of the sgRNA;
- iv. — a YA modification at one or more guide region YA sites, wherein the guide region YA site is at or after nucleotide 8 from the 5' end of the 5' terminus;
- v. — a YA modification at one or more guide region YA sites, wherein the guide region YA site is within nucleotides 5-end, 6-end, 7-end, 8-end, 9-end, or 10-end from the 5' end of the 5' terminus;
- vi. — a YA modification at one or more guide region YA sites, wherein the guide region YA site is within 17, 16, 15, 14, 13, 12, 11, 10, or 9 nucleotides of the 3' terminal nucleotide of the guide region;
- vii. — a YA modification at a guide region YA site other than a 5' end modification;
- viii. — a YA modification at two or more guide region YA sites, wherein the guide region YA sites are at or after nucleotide 8 from the 5' end of the 5' terminus;

- ix. ~~a YA modification at two or more guide region YA sites, wherein the two guide region YA sites are within nucleotides 5-end, 6-end, 7-end, 8-end, 9-end, or 10-end from the 5'-end of the 5'-terminus;~~
 - x. ~~a YA modification at two or more guide region YA sites, wherein the guide region YA sites are within 17, 16, 15, 14, 13, 12, 11, 10, or 9 nucleotides of the 3'-terminal nucleotide of the guide region;~~
 - xi. ~~a YA modification at two or more guide region YA sites other than a 5'-end modification; and~~
 - xii. ~~a YA modification at two or more guide region YA sites, wherein the modifications of the guide region YA sites comprise a modification that at least one nucleotide located 5' of the guide region YA site does not comprise.~~
103. ~~The gRNA of any one of the preceding claims, comprising a YA modification wherein the modification comprises 2'-fluoro, 2'-H, 2'-OMe, ENA, UNA, inosine, or PS.~~
104. ~~The gRNA of any one of the preceding claims, comprising a YA modification wherein the modification alters the structure of the dinucleotide motif to reduce RNA endonuclease activity.~~
105. ~~The gRNA of any one of the preceding claims, comprising a YA modification wherein the modification interferes with recognition or cleavage of a YA site by an RNase and/or stabilizes an RNA structure.~~
106. ~~The gRNA of any one of the preceding claims, comprising a YA modification wherein the modification comprises one or more of:~~
- i. ~~a ribose modification selected from 2'-O-alkyl, 2'-F, 2'-moe, 2'-F arabinose, and 2'-H (deoxyribose);~~
 - ii. ~~a bicyclic ribose analog, such as LNA, BNA, and ENA;~~
 - iii. ~~an unlocked nucleic acid modification;~~
 - iv. ~~a base modification, such as inosine, pseudouridine, and 5'-methyleytosine; and~~
 - v. ~~an internucleoside linkage modification such as phosphorothioate.~~
107. ~~The gRNA of any one of the preceding claims, comprising a YA modification at one or more conserved region YA sites.~~
108. ~~The gRNA of any one of the preceding claims, comprising a YA modification at one or more of conserved region YA sites 2, 3, 4, and 10.~~

109. ~~—The gRNA of any one of the preceding claims, comprising a YA modification at one or more of conserved region YA sites 1 and 8.~~
110. ~~—The gRNA of any one of the preceding claims, comprising a YA modification of conserved region YA site 1.~~
111. ~~—The gRNA of any one of the preceding claims, comprising a YA modification of conserved region YA site 2.~~
112. ~~—The gRNA of any one of the preceding claims, comprising a YA modification of conserved region YA site 3.~~
113. ~~—The gRNA of any one of the preceding claims, comprising a YA modification of conserved region YA site 4.~~
114. ~~—The gRNA of any one of the preceding claims, comprising a YA modification of conserved region YA site 5.~~
115. ~~—The gRNA of any one of the preceding claims, comprising a YA modification of conserved region YA site 6.~~
116. ~~—The gRNA of any one of the preceding claims, comprising a YA modification of conserved region YA site 7.~~
117. ~~—The gRNA of any one of the preceding claims, comprising a YA modification of conserved region YA site 8.~~
118. ~~—The gRNA of any one of the preceding claims, comprising a YA modification of conserved region YA site 9.~~
119. ~~—The gRNA of any one of the preceding claims, comprising a YA modification of conserved region YA site 10.~~
120. ~~—The gRNA of any one of the preceding claims, comprising one or more of:~~
- ~~i. YA modifications of conserved region YA sites 2, 3, 4, and 10;~~
 - ~~ii. YA modifications of conserved region YA sites 2, 3, and 4;~~
 - ~~iii. YA modifications of conserved region YA sites 2, 3, and 10;~~
 - ~~iv. YA modifications of conserved region YA sites 2, 4, and 10;~~
 - ~~v. YA modifications of conserved region YA sites 3, 4, and 10;~~
 - ~~vi. YA modifications of conserved region YA sites 2 and 10;~~
 - ~~vii. YA modifications of conserved region YA sites 2 and 4;~~
 - ~~viii. YA modifications of conserved region YA sites 2 and 3;~~
 - ~~ix. YA modifications of conserved region YA sites 3 and 4;~~
 - ~~x. YA modifications of conserved region YA sites 3 and 10;~~
 - ~~xi. YA modifications of conserved region YA sites 4 and 10~~

- ~~xii. YA modifications of conserved region YA sites 1 and 5;~~
 - ~~xiii. YA modifications of conserved region YA sites 1 and 6;~~
 - ~~xiv. YA modifications of conserved region YA sites 1 and 7;~~
 - ~~xv. YA modifications of conserved region YA sites 1 and 8;~~
 - ~~xvi. YA modifications of conserved region YA sites 1 and 9;~~
 - ~~xvii. YA modifications of conserved region YA sites 8 and 5;~~
 - ~~xviii. YA modifications of conserved region YA sites 8 and 6;~~
 - ~~xix. YA modifications of conserved region YA sites 8 and 7; and~~
 - ~~xx. YA modifications of conserved region YA sites 8 and 9;~~
 - ~~xxi. optionally wherein the sgRNA further comprises YA modifications of conserved region YA sites 2, 3, 4, and/or 10.~~
- ~~121. The gRNA of any one of the preceding claims, wherein at least one modified YA site comprises a 2' OMe modification, optionally at the pyrimidine of the YA site.~~
- ~~122. The gRNA of any one of the preceding claims, wherein at least one modified YA site comprises a 2' fluoro modification, optionally at the pyrimidine of the YA site.~~
- ~~123. The gRNA of any one of the preceding claims, wherein at least one modified YA site comprises a PS modification, optionally at the pyrimidine of the YA site.~~
- ~~124. The gRNA of any one of the preceding claims, wherein the gRNA comprises a guide region that comprises modifications at at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or all of the following nucleotides: 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 13, 14, 17, and 18, optionally wherein the modifications are 2' OMe, 2' fluoro, 2' H, inosine, or phosphorothioate modifications.~~
- ~~125. The gRNA of any one of the preceding claims, wherein the short-sgRNA comprises a guide region that comprises modifications at nucleotides 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 13, 14, 17, and 18, optionally wherein the modifications are 2' OMe, 2' fluoro, 2' H, inosine, or phosphorothioate modifications.~~
- ~~126. The gRNA of claims 124-125, wherein 2' OMe modifications are not present in the guide region at nucleotides 6-11 and 13-end.~~
- ~~127. The gRNA of claims 124-126, wherein 2' fluoro modifications are not present in the guide region at nucleotides 1-7, 15, 16, and 19-end.~~
- ~~128. The gRNA of claims 124-127, wherein phosphorothioate modifications are not present in the guide region at nucleotides 4, 5, 11-14, 17, and 18.~~

129. — The gRNA of claims 124-128, wherein the guide region comprises an unmodified nucleotide 20.
130. — The gRNA of claims 124-129, wherein the guide region consists of 20 nucleotides.
131. — The gRNA of claims 124-130, wherein the guide region comprises a YA site at nucleotides 5-6 and a modification at nucleotide 5.
132. — The gRNA of claims 124-131, wherein the guide region comprises a YA site at nucleotides 12-13 and a modification at nucleotide 12.
133. — The gRNA of claims 124-132, wherein the guide region comprises a YA site at nucleotides 15-16 and a modification at nucleotide 15.
134. — The gRNA of claims 124-133, wherein the guide region comprises a YA site at nucleotides 16-17 and a modification at nucleotide 16.
135. — The gRNA of claims 124-134, wherein the guide region comprises a YA site at nucleotides 19-20 and a modification at nucleotide 19.
136. — The gRNA of claims 124-130 or 132-135, wherein the guide region does not comprise a YA site at nucleotides 5-6 and nucleotide 5 is unmodified.
137. — The gRNA of claims 124-131 or 133-136, wherein the guide region does not comprise a YA site at nucleotides 12-13 and nucleotide 12 is unmodified.
138. — The gRNA of claims 124-132 or 134-137, wherein the guide region does not comprise a YA site at nucleotides 15-16 and nucleotide 15 is unmodified.
139. — The gRNA of claims 124-133 or 135-138, wherein the guide region does not comprise a YA site at nucleotides 16-17 and nucleotide 16 is unmodified.
140. — The gRNA of claims 124-134 or 136-139, wherein the guide region does not comprise a YA site at nucleotides 19-20 and nucleotide 19 is unmodified.
141. — The gRNA of claims 124-140, wherein the short-sgRNA comprises a guide region that comprises one or more of the following:
- i. 2'-OMe and phosphorothioate modifications at nucleotide 1;
 - ii. 2'-OMe and phosphorothioate modifications at nucleotide 2;
 - iii. 2'-OMe and phosphorothioate modifications at nucleotide 3;
 - iv. a 2'-OMe modification at nucleotide 4;
 - v. a phosphorothioate modification at nucleotide 6;
 - vi. a phosphorothioate modification at nucleotide 7;
 - vii. 2'-fluoro and phosphorothioate modifications at nucleotide 8;
 - viii. 2'-fluoro and phosphorothioate modifications at nucleotide 9;

- ix. ~~2' fluoro and phosphorothioate modifications at nucleotide 10;~~
 - x. ~~a 2' fluoro modification at nucleotide 11;~~
 - xi. ~~a 2' fluoro modifications at nucleotide 13;~~
 - xii. ~~a 2' fluoro modifications at nucleotide 14;~~
 - xiii. ~~a 2' fluoro modifications at nucleotide 17; and~~
 - xiv. ~~a 2' fluoro modifications at nucleotide 18.~~
142. ~~—The gRNA of claims 124-141, wherein the guide region comprises each of the modifications set forth in the preceding claim.~~
143. ~~—The gRNA of claims 124-142, wherein the guide region comprises at least 1, 2, 3, or 4 of the following:~~
- i. ~~a 2' OMe modification at nucleotide 5 if nucleotides 5 and 6 form a YA site;~~
 - ii. ~~a 2' OMe modification at nucleotide 12 if nucleotides 12 and 13 form a YA site;~~
 - iii. ~~a phosphorothioate modification at nucleotide 15 if nucleotides 15 and 16 form a YA site;~~
 - iv. ~~a phosphorothioate modification at nucleotide 16 if nucleotides 16 and 17 form a YA site; and~~
 - v. ~~a phosphorothioate or 2' fluoro modification at nucleotide 19 if nucleotides 19 and 20 form a YA site.~~
144. ~~—The gRNA of claims 124-143, wherein the guide region comprises a YA site at nucleotides 5-6 and a 2' OMe modification at nucleotide 5.~~
145. ~~—The gRNA of claims 124-144, wherein the guide region comprises a YA site at nucleotides 12-13 and a 2' OMe modification at nucleotide 12.~~
146. ~~—The gRNA of claims 124-145, wherein the guide region comprises a YA site at nucleotides 15-16 and a phosphorothioate modification at nucleotide 15.~~
147. ~~—The gRNA of claims 124-146, wherein the guide region comprises a YA site at nucleotides 16-17 and a phosphorothioate modification at nucleotide 16.~~
148. ~~—The gRNA of claims 124-147, wherein the guide region comprises a YA site at nucleotides 19-20 and a phosphorothioate modification at nucleotide 19.~~
149. ~~—The gRNA of claims 124-148, wherein the guide region comprises a 2' fluoro modification at nucleotide 19.~~
150. ~~—The gRNA of claims 124-149, wherein the guide region comprises an unmodified nucleotide 15 or only a phosphorothioate modification at nucleotide 15.~~

- ~~151. The gRNA of claims 124-150, wherein the guide region comprises an unmodified nucleotide 16 or only a phosphorothioate modification at nucleotide 16.~~
- ~~152. A guide RNA which is a single guide RNA (sgRNA) comprising:~~
- ~~i. a YA modification at two or more guide region YA sites;~~
 - ~~ii. a YA modification at one or more of conserved region YA sites 2, 3, 4, and 10; and~~
 - ~~iii. a YA modification at one or more of conserved region YA sites 1 and 8.~~
- ~~153. A guide RNA which is a single guide RNA (sgRNA) comprising:~~
- ~~i. a YA modification at one or more guide region YA sites that are at or after nucleotide 8 from the 5' end of the 5' terminus;~~
 - ~~ii. a YA modification at one or more of conserved region YA sites 2, 3, 4, and 10; and optionally~~
 - ~~iii. a YA modification at one or more of conserved region YA sites 1 and 8.~~
- ~~154. A guide RNA which is a single guide RNA (sgRNA) comprising:~~
- ~~i. a YA modification at one or more guide region YA sites that are within 13 nucleotides of the 3' terminal nucleotide of the guide region;~~
 - ~~ii. a YA modification at one or more of conserved region YA sites 2, 3, 4, and 10; and~~
 - ~~iii. a YA modification at one or more of conserved region YA sites 1 and~~
- ~~155. A guide RNA which is a single guide RNA (sgRNA) comprising:~~
- ~~i. a 5' end modification and a 3' end modification;~~
 - ~~ii. a YA modification at one or more of conserved region YA sites 2, 3, 4, and 10; and~~
 - ~~iii. a YA modification at one or more of conserved region YA sites 1 and 8.~~
- ~~156. A guide RNA which is a single guide RNA (sgRNA) comprising:~~
- ~~i. a YA modification at at least one guide region YA site, wherein the modification of the guide region YA site comprises a modification at at~~

~~least one nucleotide located 5' of the guide region YA site does not comprise;~~

- ~~ii. a YA modification at one or more of conserved region YA sites 2, 3, 4, and 10; and~~
- ~~iii. a YA modification at one or more of conserved region YA sites 1 and 8.~~

~~157. A guide RNA which is a single guide RNA (sgRNA) comprising:~~

- ~~i. a YA modification at one or more of conserved region YA sites 2, 3, 4, and 10; and~~
- ~~ii. a YA modification at conserved region YA sites 1 and 8.~~

~~158. A guide RNA which is a single guide RNA (sgRNA) comprising:~~

- ~~i. a YA modification at one or more guide region YA sites that are at or after nucleotide 8 from the 5' end of the 5' terminus;~~
- ~~ii. a YA modification at one or more of conserved region YA sites 2, 3, 4, and 10; and~~
- ~~iii. a modification at one or more of H1-1 and H2-1.~~

~~159. A guide RNA which is a single guide RNA (sgRNA) comprising:~~

- ~~i. a YA modification at one or more of conserved region YA sites 2, 3, 4, and 10;~~
- ~~ii. a YA modification at one or more of conserved region YA sites 1, 5, 6, 7, 8, and 9; and~~
- ~~iii. a modification at one or more of H1-1 and H2-1.~~

~~160. A guide RNA which is an sgRNA comprising any one or more of the following:~~

- ~~i. a modification, such as a YA modification, at one or more nucleotides located at or after nucleotide 6 from the 5' end of the 5' terminus;~~
- ~~ii. a YA modification at one or more guide sequence YA sites;~~

- iii. ~~a modification at one or more of B3, B4, and B5, wherein B6 does not comprise a 2' OMe modification or comprises a modification other than 2' OMe;~~
- iv. ~~a modification at LS10, wherein LS10 comprises a modification other than 2' fluoro; and/or~~
- v. ~~a modification at N2, N3, N4, N5, N6, N7, N10, or N11;~~

and

wherein at least one of the following is true:

- a. ~~at least one of nucleotides 8-11, 13, 14, 17, or 18 from the 5' end of the 5' terminus does not comprise a 2' fluoro modification;~~
- b. ~~at least one of nucleotides 6-10 from the 5' end of the 5' terminus does not comprise a phosphorothioate linkage;~~
- c. ~~at least one of B2, B3, B4, or B5 does not comprise a 2' OMe modification;~~
- d. ~~at least one of LS1, LS8, or LS10 does not comprise a 2' OMe modification;~~
- e. ~~at least one of N2, N3, N4, N5, N6, N7, N10, N11, N16, or N17 does not comprise a 2' OMe modification;~~
- f. ~~H1-1 comprises a modification;~~
- g. ~~H2-1 comprises a modification; or~~
- h. ~~at least one of H1-2, H1-3, H1-4, H1-5, H1-6, H1-7, H1-8, H1-9, H1-10, H2-1, H2-2, H2-3, H2-4, H2-5, H2-6, H2-7, H2-8, H2-9, H2-10, H2-11, H2-12, H2-13, H2-14, or H2-15 does not comprise a phosphorothioate linkage.~~

161. ~~A guide RNA comprising any one or more of the following:~~

- i. ~~a modification, such as a YA modification, at one or more nucleotides located at or after nucleotide 6 from the 5' end of the 5' terminus; or~~
- ii. ~~a YA modification at one or more guide sequence YA sites;~~

wherein at least one of the following is true:

- a. ~~at least one of nucleotides 8-11, 13, 14, 17, or 18 from the 5' end of the 5' terminus does not comprise a 2' fluoro modification; or~~

- b. ~~at least one of nucleotides 6-10 from the 5' end of the 5' terminus does not comprise a phosphorothioate linkage;~~

and wherein at least one of the following is also true:

- c. ~~at least one of nucleotides 7-10 from the 5' end of the 5' terminus does not comprise a 2'-OMe modification;~~
- d. ~~nucleotide 20 from the 5' end of the 5' terminus does not comprise a 2'-OMe modification; or~~
- e. ~~the guide RNA comprises a 2'-fluoro modification at any one or more of nucleotides 1-20 from the 5' end of the 5' terminus and at least one of nucleotides 11, 12, 13, 14, 17, or 18 from the 5' end of the 5' terminus does not comprise a 2'-fluoro modification, optionally wherein nucleotide 12 from the 5' end of the 5' terminus does not comprise a 2'-fluoro modification.~~

162. ~~—A guide RNA which is an sgRNA comprising a guanosine at N14 and/or one or more of the following:~~

- i. ~~a YA modification at one or more guide region YA sites that are at or after nucleotide 8 from the 5' end of the 5' terminus;~~
- ii. ~~a YA modification at one or more of conserved region YA sites 1, 5, and 6, wherein if YA site 6 is modified at LS12 and LS9 is not modified, then the modification of LS12 is other than 2'-OMe;~~
- iii. ~~a modification at LS9, wherein if LS9 is modified and LS5, LS7, and LS12 are not modified, then the modification of LS9 is other than 2'-fluoro;~~
- iv. ~~a modification at LS12, wherein if LS12 is modified and LS9 is not modified, then the modification of LS12 is other than 2'-OMe;~~
- v. ~~a modification at LS8 or LS11, wherein at least one of LS8 and LS11 comprises a modification other than 2'-OMe; and/or~~
- vi. ~~a modification at N6, N14, or N17, wherein if N17 is modified and N6 and N14 are not modified, then the modification of N17 is other than 2'-fluoro and other than 2'-OMe;~~

and wherein at least one of the following is true:

- a. ~~at least one of nucleotides 8-11, 13-14, 17, or 18 from the 5' end of the 5' terminus does not comprise a 2' fluoro modification;~~
- b. ~~at least one of nucleotides 6-10 from the 5' end of the 5' terminus does not comprise a phosphorothioate linkage;~~
- c. ~~at least one of B2, B3, B4, or B5 does not comprise a 2' OMe modification;~~
- d. ~~at least one of LS1, LS8, or LS10 does not comprise a 2' OMe modification;~~
- e. ~~at least one of N2, N3, N4, N5, N6, N7, N10, N11, N16, or N17 does not comprise a 2' OMe modification;~~
- f. ~~H1-1 comprises a modification;~~
- g. ~~H2-1 comprises a modification; or~~
- h. ~~at least one of H1-2, H1-3, H1-4, H1-5, H1-6, H1-7, H1-8, H1-9, H1-10, H2-1, H2-2, H2-3, H2-4, H2-5, H2-6, H2-7, H2-8, H2-9, H2-10, H2-11, H2-12, H2-13, H2-14, or H2-15 does not comprise a phosphorothioate linkage.~~

163. ~~The gRNA of claims 161 or 162, comprising:~~

- i. ~~a YA modification at 1, 2, 3, 4, or 5 guide region YA sites;~~
- ii. ~~a YA modification at 1, 2, 3, 4, or 5 guide region YA sites, wherein the modification of at least one guide region YA site is different from any 5' end modification of the sgRNA;~~
- iii. ~~a YA modification at one or more guide region YA sites that are at or after nucleotide 8 from the 5' end of the 5' terminus;~~
- iv. ~~a YA modification at one or more guide region YA sites that are within nucleotides 5-end, 6-end, 7-end, 8-end, 9-end, or 10-end from the 5' end of the 5' terminus;~~
- v. ~~a YA modification at one or more guide region YA sites that are within 17, 16, 15, 14, 13, 12, 11, 10, or 9 nucleotides of the 3' terminal nucleotide of the guide region;~~
- vi. ~~a YA modification at a guide region YA site other than a 5' end modification; or~~

- vii. ~~a YA modification at a guide region YA site, wherein the modification of the guide region YA site comprises a modification at at least one nucleotide located 5' of the guide region YA site does not comprise.~~

164. ~~The gRNA of claim 163, comprising:~~

- ~~i. a YA modification at two or more guide region YA sites that are at or after nucleotide 8 from the 5' end of the 5' terminus;~~
- ~~ii. a YA modification at two or more guide region YA sites that are within nucleotides 5-end, 6-end, 7-end, 8-end, 9-end, or 10-end from the 5' end of the 5' terminus;~~
- ~~iii. a YA modification at two or more guide region YA sites that are within 17, 16, 15, 14, 13, 12, 11, 10, or 9 nucleotides of the 3' terminal nucleotide of the guide region;~~
- ~~iv. a YA modification at two or more guide region YA sites other than a 5' end modification; or~~
- ~~v. a YA modification at a two or more guide region YA sites, wherein the modifications of the guide region YA sites comprise a modification at at least one nucleotide located 5' of the guide region YA site does not comprise.~~

165. ~~The gRNA of claim 163, comprising:~~

- ~~i. a YA modification at three or more guide region YA sites that are at or after nucleotide 8 from the 5' end of the 5' terminus;~~
- ~~ii. a YA modification at three or more guide region YA sites that are within nucleotides 5-end, 6-end, 7-end, 8-end, 9-end, or 10-end from the 5' end of the 5' terminus;~~
- ~~iii. a YA modification at three or more guide region YA sites that are within 17, 16, 15, 14, 13, 12, 11, 10, or 9 nucleotides of the 3' terminal nucleotide of the guide region;~~
- ~~iv. a YA modification at three or more guide region YA sites other than a 5' end modification; or~~
- ~~v. a YA modification at a three or more guide region YA sites, wherein the modifications of the guide region YA sites comprise a modification at at~~

least one nucleotide located 5' of the guide region YA site does not comprise.

166. — The gRNA of any one of claims 161-165, comprising at least one YA modification at nucleotide 6 from the 5' end of the 5' terminus.
167. — The gRNA of any one of claims 161-166, comprising at least one YA modification at nucleotide 7 from the 5' end of the 5' terminus.
168. — The gRNA of any one of claims 161-167, comprising at least one YA modification at nucleotide 8 from the 5' end of the 5' terminus.
169. — The gRNA of any one of claims 161-168, comprising at least one YA modification at nucleotide 9 from the 5' end of the 5' terminus.
170. — The gRNA of any one of claims 161-169, comprising at least one YA modification at nucleotide 10 from the 5' end of the 5' terminus.
171. — The gRNA of any one of claims 161-170, comprising at least one YA modification at nucleotide 11 from the 5' end of the 5' terminus.
172. — The gRNA of any one of claims 161-171, comprising at least one YA modification at nucleotide 12 from the 5' end of the 5' terminus.
173. — The gRNA of any one of claims 161-172, comprising at least one YA modification at nucleotide 13 from the 5' end of the 5' terminus.
174. — The gRNA of any one of claims 161-173, comprising at least one YA modification at nucleotide 14 from the 5' end of the 5' terminus.
175. — The gRNA of any one of claims 161-174, comprising at least one YA modification at nucleotide 15 from the 5' end of the 5' terminus.
176. — The gRNA of any one of claims 161-175, comprising at least one YA modification at nucleotide 16 from the 5' end of the 5' terminus.
177. — The gRNA of any one of claims 161-176, comprising at least one YA modification at nucleotide 17 from the 5' end of the 5' terminus.
178. — The gRNA of any one of claims 161-177, comprising at least one YA modification at nucleotide 18 from the 5' end of the 5' terminus.

179. ~~The gRNA of any one of claims 161-178, comprising at least one YA modification at nucleotide 19 from the 5' end of the 5' terminus.~~
180. ~~The gRNA of any one of claims 161-179, comprising at least one YA modification at nucleotide 20 from the 5' end of the 5' terminus.~~
181. ~~The gRNA of any one of claims 161-180, wherein at least 1, 2, 3, 4, 5, 6, 7, or 8 of nucleotides 8-11, 13-14, and 17-18 from the 5' end of the 5' terminus comprise a YA modification, optionally wherein the modification comprises 2' fluoro, 2' H, 2' OMe, or PS.~~
182. ~~The gRNA of claim 181, wherein the modification is 2' fluoro.~~
183. ~~The gRNA of claim 181, wherein the modification is 2' OMe or 2' H.~~
184. ~~The gRNA of claim 181, wherein the modification is PS.~~
185. ~~The gRNA of any one of claims 161-184, wherein at least 1, 2, 3, 4, or 5 of nucleotides 6-10 from the 5' end of the 5' terminus comprise a YA modification, optionally wherein the modification comprises 2' fluoro, 2' H, 2' OMe, inosine, or PS.~~
186. ~~The gRNA of claim 185, wherein the modification is PS.~~
187. ~~The gRNA of claim 185, wherein the modification is 2' fluoro or 2' H.~~
188. ~~The gRNA of claim 185, wherein the modification is 2' OMe.~~
189. ~~The gRNA of any one of claims 161-188, comprising any one or more of the following:~~
- ~~i. 1, 2, 3, 4, 5, 6, 7, or 8 YA modifications of nucleotides 8-11, 13-14, and 17-18 from the 5' end of the 5' terminus, wherein the YA modifications are optionally 2' fluoro modifications, and a modification other than 2' fluoro at one or more of nucleotides 6-10 from the 5' terminus;~~
 - ~~ii. a YA modification other than PS at one or more of nucleotides 8-11, 13-14, and 17-18 from the 5' end of the 5' terminus, and 1, 2, 3, 4, or 5 YA modifications at nucleotides 6-10 from the 5' end of the 5' terminus, optionally wherein the modifications are PS modifications;~~

- iii. ~~1, 2, 3, 4, 5, 6, 7, or 8 YA modifications at nucleotides 8-11, 13-14, and 17-18 from the 5' end of the 5' terminus, wherein the YA modifications are optionally 2' fluoro modifications, and modifications other than 2' fluoro at nucleotides 6-10 from the 5' end of the 5' terminus;~~
- iv. ~~YA modifications other than PS at each of nucleotides 8-11, 13-14, and 17-18 from the 5' end of the 5' terminus, and 1, 2, 3, 4, or 5 YA modifications at nucleotides 6-10 from the 5' end of the 5' terminus, wherein the modifications are optionally PS modifications;~~
- v. ~~1, 2, 3, 4, 5, 6, 7, or 8 YA modifications at nucleotides 8-11, 13-14, and 17-18 from the 5' end of the 5' terminus, wherein the YA modifications are optionally 2' fluoro modifications, and one or more PS modification at any one of nucleotides 6-10 from the 5' end of the 5' terminus;~~
- vi. ~~at least one 2' fluoro modification at any one of nucleotides 8-11, 13-14, and 17-18 from the 5' end of the 5' terminus, and 1, 2, 3, 4, or 5 YA modifications of nucleotides 6-10 from the 5' end of the 5' terminus, wherein the modifications are optionally PS modifications;~~
- vii. ~~1, 2, 3, 4, 5, 6, 7, or 8 YA modifications of nucleotides 8-11, 13-14, and 17-18 from the 5' end of the 5' terminus, wherein the YA modifications are optionally 2' fluoro modifications, and a PS modification at each of nucleotides 6-10 from the 5' end of the 5' terminus; or~~
- viii. ~~a 2' fluoro modification at each of nucleotides 8-11, 13-14, and 17-18 from the 5' end of the 5' terminus, and 1, 2, 3, 4, or 5 YA modifications of nucleotides 6-10 from the 5' end of the 5' terminus, wherein the modifications are optionally PS modifications.~~

190. ~~The gRNA of any one of claims 161-189, wherein:~~

- i. ~~nucleotides 4-20 from the 5' end of the 5' terminus comprise at least 2, 3, or 4 modified YA sites including a first modified YA site comprising a 2' OMe modification and a second modified YA site comprising a 2' fluoro modification or a PS modification;~~
- ii. ~~nucleotides 4-20 from the 5' end of the 5' terminus comprise at least 2, 3, or 4 modified YA sites including a first modified YA site comprising a 2' fluoro modification and a second modified YA site comprising a 2' OMe modification or a PS modification;~~

- iii. ~~nucleotides 4-20 from the 5' end of the 5' terminus comprise at least 2, 3, or 4 modified YA sites including a first modified YA site comprising a PS modification and a second modified YA site comprising a 2' OMe modification or a 2' fluoro modification;~~
- iv. ~~nucleotides 4-20 from the 5' end of the 5' terminus comprise at least 2, 3, or 4 modified YA sites including a YA modification;~~
- v. ~~nucleotides 4-20 from the 5' end of the 5' terminus comprise at least 3 or 4 modified YA sites including a first modified YA site comprising a 2' OMe modification, a second modified YA site comprising a 2' fluoro modification, and a third modified YA site comprising a PS modification;~~
- vi. ~~nucleotides 4-20 from the 5' end of the 5' terminus comprise at least 3 or 4 modified YA sites including a first modified YA site comprising a 2' OMe modification, a second modified YA site comprising a 2' fluoro modification, a third modified YA site comprising a 2' fluoro modification, and a fourth modified YA site comprising a PS modification;~~
- vii. ~~nucleotides 4-20 from the 5' end of the 5' terminus comprise at least 3 or 4 modified YA sites including a YA modification;~~
- viii. ~~nucleotides 4-20 from the 5' end of the 5' terminus comprise at least 4 modified YA sites including a first modified YA site comprising a 2' OMe modification, a second modified YA site comprising a 2' fluoro modification, a third modified YA site comprising a PS modification, and a fourth modified YA site comprising a PS modification; or~~
- ix. ~~nucleotides 4-40 from the 5' end of the 5' terminus comprise at least 4 modified YA sites including a YA modification.~~

191. ~~The gRNA of any one of claims 161-190, wherein nucleotides 4-20 from the 5' end of the 5' terminus comprise at least 5 modified YA sites.~~

192. ~~The gRNA of any one of claims 161-191, wherein the at least 5 modified YA sites include a fifth modified YA site comprising a PS modification, optionally wherein the third modified YA site comprises a 2' fluoro modification.~~

- ~~193. — The gRNA of any one of claims 161–192, wherein the first, second, and (if applicable) third, fourth, and fifth of the at least 5 modified YA sites are arranged in the 5' to 3' direction.~~
- ~~194. — The gRNA of any one of claims 161–193, wherein the first, second, and (if applicable) third, fourth, and fifth of the at least 5 modified YA sites are not arranged in the 5' to 3' direction.~~
- ~~195. — The gRNA of any one of claims 161–194, wherein nucleotides 4–20 from the 5' end of the 5' terminus comprise at least 2, 3, 4, or 5 modified YA sites comprising a deoxyribonucleotide, optionally wherein the deoxyribonucleotide is the pyrimidine of the YA sites.~~
- ~~196. — The gRNA of any one of claims 161–195, wherein:~~
- ~~i. at least 1, 2, 3, or 4 of nucleotides 8–11 from the 5' end of the 5' terminus comprise a YA modification, which is optionally a 2' fluoro modification;~~
 - ~~ii. at least 1, 2, 3, 4, 5, 6, 7, or 8 of nucleotides 8–11, 13, 14, 17, and 18 from the 5' end of the 5' terminus comprise a YA modification, optionally wherein the YA modifications are 2' OMe if present at nucleotides 8–11 and 2' fluoro if present at nucleotides 13, 14, 17, or 18;~~
 - ~~iii. at least one or both of nucleotides 17 and 18 from the 5' end of the 5' terminus comprise a YA modification, which is optionally a 2' fluoro modification;~~
 - ~~iv. at least one or both of nucleotides 17 and 18 from the 5' end of the 5' terminus comprise a YA modification, which is optionally a 2' fluoro modification; or~~
 - ~~v. at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 of nucleotides 4–14, 17, and 18 from the 5' end of the 5' terminus comprise a YA modification, which is optionally a 2' fluoro modification.~~
- ~~197. — The gRNA of any one of claims 161–196, wherein at least 1, 2, 3, 4, 5, or 6 of nucleotides 4–10 from the 5' end of the 5' terminus comprise a YA modification, which is optionally a 2' OMe modification.~~

198. ~~The gRNA of any one of claims 161-197, wherein nucleotides 4-10 from the 5' end of the 5' terminus comprise a YA modification, which is optionally a 2' OMe modification.~~
199. ~~The gRNA of any one of claims 161-198, wherein:~~
- ~~i. at least one of nucleotides 1-3 from the 5' end of the 5' terminus comprise a 5' protective end modification, which is optionally a 2' OMe modification;~~
 - ~~ii. at least two of nucleotides 1-3 from the 5' end of the 5' terminus comprise a 5' protective end modification, which is optionally a 2' OMe modification; or~~
 - ~~iii. each of nucleotides 1-3 from the 5' end of the 5' terminus comprise a 5' protective end modification, which is optionally a 2' OMe modification.~~
200. ~~The gRNA of any one of claims 161-199, wherein at least 1, 2, 3, 4, or 5 of nucleotides 11, 13, 14, 17, and 18 from the 5' end of the 5' terminus comprise a 5' end modification, which is optionally a 2' fluoro modification.~~
201. ~~The gRNA of any one of claims 161-200, wherein nucleotide 15 from the 5' end of the 5' terminus is unmodified or modified only with phosphorothioate.~~
202. ~~The gRNA of any one of claims 161-200, wherein nucleotide 16 from the 5' terminus is unmodified or modified only with phosphorothioate.~~
203. ~~The gRNA of any one of the preceding claims, wherein nucleotide 3 from the 5' end of the 5' terminus is unmodified or modified only with phosphorothioate.~~
204. ~~The gRNA of any one of claims 161-203, which is a crRNA or dgRNA.~~
205. ~~The gRNA of any one of claims 161-203, which is an sgRNA.~~
206. ~~The gRNA of any one of claims 161-203, which is a short sgRNA.~~
207. ~~The gRNA of any one of claim 205 or 206, comprising a YA modification of conserved region YA site 1.~~
208. ~~The gRNA of any one of claims 205-207, comprising a YA modification of conserved region YA site 2.~~

- ~~209. The gRNA of any one of claims 205-208, comprising a YA modification of conserved region YA site 3.~~
- ~~210. The gRNA of any one of claims 205-209, comprising a YA modification of conserved region YA site 4.~~
- ~~211. The gRNA of any one of claims 205-210, comprising a YA modification of conserved region YA site 5.~~
- ~~212. The gRNA of any one of claims 205-211, comprising a YA modification of conserved region YA site 6.~~
- ~~213. The gRNA of any one of claims 205-212, comprising a YA modification of conserved region YA site 7.~~
- ~~214. The gRNA of any one of claims 205-213, comprising a YA modification of conserved region YA site 8.~~
- ~~215. The gRNA of any one of claims 205-214, comprising a YA modification of conserved region YA site 9.~~
- ~~216. The gRNA of any one of claims 205-215, comprising a YA modification of conserved region YA site 10.~~
- ~~217. The gRNA of any one of claims 205-216, comprising:~~
- ~~i. YA modifications of conserved region YA sites 2, 3, 4, and 10;~~
 - ~~ii. YA modifications of conserved region YA sites 2, 3, and 4;~~
 - ~~iii. YA modifications of conserved region YA sites 2, 3, and 10;~~
 - ~~iv. YA modifications of conserved region YA sites 2, 4, and 10;~~
 - ~~v. YA modifications of conserved region YA sites 3, 4, and 10;~~
 - ~~vi. YA modifications of conserved region YA sites 2 and 10;~~
 - ~~vii. YA modifications of conserved region YA sites 2 and 4;~~
 - ~~viii. YA modifications of conserved region YA sites 2 and 3;~~
 - ~~ix. YA modifications of conserved region YA sites 3 and 4;~~
 - ~~x. YA modifications of conserved region YA sites 3 and 10; or~~
 - ~~xi. YA modifications of conserved region YA sites 4 and 10.~~
- ~~218. The gRNA of any one of claims 205-217, comprising:~~

- ~~i. YA modifications of conserved region YA sites 1 and 5;~~
- ~~ii. YA modifications of conserved region YA sites 1 and 6;~~
- ~~iii. YA modifications of conserved region YA sites 1 and 7;~~
- ~~iv. YA modifications of conserved region YA sites 1 and 8;~~
- ~~v. YA modifications of conserved region YA sites 1 and 9;~~
- ~~vi. YA modifications of conserved region YA sites 8 and 5;~~
- ~~vii. YA modifications of conserved region YA sites 8 and 6;~~
- ~~viii. YA modifications of conserved region YA sites 8 and 7; or~~
- ~~ix. YA modifications of conserved region YA sites 8 and 9;~~

~~optionally wherein the sgRNA further comprises YA modifications of conserved region YA sites 2, 3, 4, and 10.~~

~~219. The gRNA of any one of claims 205-218, wherein at least one modified YA site comprises a 2' OMe modification, optionally at the pyrimidine of the YA site.~~

~~220. The gRNA of any one of claims 205-219, wherein at least one modified YA site comprises a 2' fluoro modification, optionally at the pyrimidine of the YA site.~~

~~221. The gRNA of any one of claims 205-220, wherein at least one modified YA site comprises a PS modification, optionally at the pyrimidine of the YA site.~~

~~222. The gRNA of any one of claims 205-221, wherein at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 modified YA sites comprise a 2' OMe modification, optionally at the pyrimidines of the YA sites.~~

~~223. The gRNA of any one of claims 205-222, wherein at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 modified YA sites comprise a 2' fluoro modification, optionally at the pyrimidines of the YA sites.~~

~~224. The gRNA of any one of claims 205-223, wherein at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 modified YA sites comprise a PS modification, optionally at the pyrimidines of the YA sites.~~

~~225. The gRNA of any one of claims 205-224, wherein at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 modified YA sites comprise a ribose modification at the 2' position, optionally at the pyrimidines of the YA sites, and optionally chosen from a 2' O-alkyl, 2' H, and 2' fluoro modification.~~

226. ~~The gRNA of any one of claims 205-225, wherein:~~

- ~~i. conserved region YA sites 1 and 8 comprise 2' fluoro modifications, optionally at the pyrimidines of the YA sites;~~
- ~~ii. conserved region YA sites 5 and 6; 5 and 7; 5 and 9; 6 and 7; 6 and 9; 5, 6, and 7; 5, 6, and 9; 6, 7, and 9; or 5, 6, 7, and 9 comprise 2' OMe modifications, optionally at the pyrimidines of the YA sites;~~
- ~~iii. conserved region YA site 1 comprises a 2' fluoro modification and conserved region YA sites 5 and 6; 5 and 7; 5 and 9; 6 and 7; 6 and 9; 5, 6, and 7; 5, 6, and 9; 6, 7, and 9; or 5, 6, 7, and 9 comprise 2' OMe modifications, optionally at the pyrimidines of the YA sites;~~
- ~~iv. conserved region YA site 8 comprises a 2' fluoro modification and conserved region YA sites 5 and 6; 5 and 7; 5 and 9; 6 and 7; 6 and 9; 5, 6, and 7; 5, 6, and 9; 6, 7, and 9; or 5, 6, 7, and 9 comprise 2' OMe modifications, optionally at the pyrimidines of the YA sites;~~
- ~~v. conserved region YA site 1 comprises a 2' fluoro modification at the pyrimidine of the YA sites and YA sites 5 and 6; 5 and 7; 5 and 9; 6 and 7; 6 and 9; 5, 6, and 7; 5, 6, and 9; 6, 7, and 9; or 5, 6, 7, and 9 comprise 2' OMe modifications, optionally at the pyrimidines of the YA sites;~~
- ~~vi. conserved region YA site 8 comprises a 2' fluoro modification at the pyrimidine of the YA site and YA sites 5 and 6; 5 and 7; 5 and 9; 6 and 7; 6 and 9; 5, 6, and 7; 5, 6, and 9; 6, 7, and 9; or 5, 6, 7, and 9 comprise 2' OMe modifications, optionally at the pyrimidines of the YA sites;~~
- ~~vii. conserved region YA sites 1 and 8 comprise 2' fluoro modifications and conserved region YA sites 5 and 6; 5 and 7; 5 and 9; 6 and 7; 6 and 9; 5, 6, and 7; 5, 6, and 9; 6, 7, and 9; or 5, 6, 7, and 9 comprise 2' OMe modifications, optionally at the pyrimidines of the YA sites; or~~
- ~~viii. conserved region YA sites 1 and 8 comprise 2' fluoro modifications at the pyrimidines of the YA sites and conserved region YA sites 5 and 6; 5 and 7; 5 and 9; 6 and 7; 6 and 9; 5, 6, and 7; 5, 6, and 9; 6, 7, and 9; or 5, 6, 7, and 9 comprise 2' OMe modifications, optionally at the pyrimidines of the YA sites.~~

227. ~~The gRNA of any one of claims 205-226, wherein conserved region YA sites 7 and 9 comprise YA modifications, which are optionally 2' OMe modifications.~~

228. ~~The gRNA of any one of claims 205-227, wherein conserved region YA sites 5, 6, 7, and 9 comprise YA modifications, which are optionally 2'-OMe modifications.~~
229. ~~The gRNA of any one of claims 205-228, wherein conserved region YA site 8 comprises a 2'-fluoro modification.~~
230. ~~The gRNA of any one of claims 205-229, wherein conserved region YA site 8 comprises a deoxyribonucleotide modification.~~
231. ~~The gRNA of any one of claims 205-230, wherein conserved region YA site 8 is abolished by a base substitution, optionally wherein the base substitution eliminates the uracil of YA site 8, further optionally wherein the base substitution is a uracil to guanine substitution.~~
232. ~~The gRNA of any one of claims 205-231, wherein conserved region YA site 1 comprises a 2'-fluoro modification.~~
233. ~~The gRNA of any one of claims 205-232, wherein conserved region YA site 1 comprises a PS modification.~~
234. ~~The gRNA of any one of claims 205-233, wherein 1, 2, 3, 4, 5, 6, or 7 of LS5, LS7, LS8, LS9, LS10, LS11, and LS12 comprise modifications, optionally wherein the modifications are 2'-fluoro and/or 2'-OMe modifications.~~
235. ~~The gRNA of any one of claims 205-234, wherein modifications at LS5, LS7, LS9, and LS11, if present, comprise 2'-fluoro modifications, optionally wherein each of LS5, LS7, LS9, and LS11 comprise 2'-fluoro modifications.~~
236. ~~The gRNA of any one of claims 205-235, wherein modifications at LS8, LS10, and LS12, if present, comprise 2'-OMe modifications, optionally wherein each of LS8, LS10, and LS12 comprise 2'-OMe modifications.~~
237. ~~The gRNA of any one of claims 205-236, wherein 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 of N2, N3, N4, N5, N6, N7, N10, N11, N16, and N17 comprise modifications, which are optionally 2'-OMe modifications.~~
238. ~~The gRNA of any one of claims 205-237, wherein H2-2 comprises a modification, optionally wherein H2 is otherwise unmodified.~~

239. ~~The gRNA of any one of claims 205-238, wherein H2-2 comprises a 2'-OMe modification.~~
240. ~~The gRNA of any one of claims 205-239, wherein US3, US9, and US12 comprise modifications, optionally wherein the US is otherwise unmodified.~~
241. ~~The gRNA of any one of claims 205-240, wherein US3, US9, and US12 comprise 2'-OMe modifications.~~
242. ~~The gRNA of any one of claims 205-241, wherein nucleotides 6-10 from the 5' end of the 5' terminus comprise a PS modification and nucleotides 8-11, 13, 14, 17, and 18 from the 5' end of the 5' terminus comprise a 2'-fluoro modification.~~
243. ~~The gRNA of any one of claims 205-242, wherein each guide region YA site comprises a 2'-fluoro modification, optionally excepting nucleotides 15 and/or 16 from the 5' end of the 5' terminus.~~
244. ~~The gRNA of any one of claims 205-243, wherein nucleotides 4, 8, and 11 from the 5' end of the 5' terminus comprise YA modifications, optionally wherein nucleotide 4 comprises a 2'-OMe modification and nucleotides 8 and 11 comprise a 2'-fluoro modification.~~
245. ~~The gRNA of any one of claims 205-244, wherein 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more modified YA sites comprise a YA modification at the pyrimidine position of the YA site.~~
246. ~~The gRNA of claim 245, wherein 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 modified conserved region YA sites comprise a YA modification at the pyrimidine position of the YA site.~~
247. ~~The gRNA of any one of claims 205-246, wherein 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more modified YA sites comprise a YA modification at the adenine position of the YA site.~~
248. ~~The gRNA of claim 247, wherein 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 modified conserved region YA sites comprise a YA site modification at the adenine position of the YA site.~~
249. ~~The gRNA of any one of claims 205-248, comprising:~~

- ~~i. a modification of H1-1;~~
- ~~ii. a modification of H2-1; or~~
- ~~iii. modifications of H1-1 and H2-1.~~

~~250. The gRNA of claim 249, wherein H1-1 and/or H2-1 comprises a 2'-OMe modification.~~

~~251. The gRNA of claim 250, wherein H1-1 and/or H2-1 comprises a 2' fluoro modification.~~

~~252. The gRNA of claim 251, wherein H1-1 and/or H2-1 comprises a PS modification.~~

~~253. The gRNA of any one of claims 205-252, comprising a modification at B3, optionally wherein B6 does not comprise a 2'-OMe modification or comprises a modification other than 2'-OMe.~~

~~254. The gRNA of any one of claims 205-253, comprising a modification at B4, optionally wherein B6 does not comprise a 2'-OMe modification or comprises a modification other than 2'-OMe.~~

~~255. The gRNA of any one of claims 205-254, comprising a modification at B5, optionally wherein B6 does not comprise a 2'-OMe modification or comprises a modification other than 2'-OMe.~~

~~256. The gRNA of any one of claims 205-255, comprising a modification at LS10, optionally wherein LS10 comprises a modification other than 2' fluoro.~~

~~257. The gRNA of any one of claims 205-256, comprising a modification at N2.~~

~~258. The gRNA of any one of claims 205-257, comprising a modification at N3.~~

~~259. The gRNA of any one of claims 205-258, comprising a modification at N4.~~

~~260. The gRNA of any one of claims 205-259, comprising a modification at N5.~~

~~261. The gRNA of any one of claims 205-260, comprising a modification at N6.~~

~~262. The gRNA of any one of claims 205-261, comprising a modification at N7.~~

~~263. The gRNA of any one of claims 205-262, comprising a modification at N10.~~

~~264. The gRNA of any one of claims 205-263, comprising a modification at N11.~~

~~265. The gRNA of any one of claims 205-264,, wherein:~~

- ~~i. nucleotide 8 from the 5' end of the 5' terminus does not comprise a 2' fluoro modification;~~
- ~~ii. nucleotide 9 from the 5' end of the 5' terminus does not comprise a 2' fluoro modification;~~
- ~~iii. nucleotide 10 from the 5' end of the 5' terminus does not comprise a 2' fluoro modification;~~
- ~~iv. nucleotide 11 from the 5' end of the 5' terminus does not comprise a 2' fluoro modification;~~
- ~~v. nucleotide 13 from the 5' end of the 5' terminus does not comprise a 2' fluoro modification;~~
- ~~vi. nucleotide 14 from the 5' end of the 5' terminus does not comprise a 2' fluoro modification;~~
- ~~vii. nucleotide 17 from the 5' end of the 5' terminus does not comprise a 2' fluoro modification; and/or~~
- ~~viii. nucleotide 18 from the 5' end of the 5' terminus does not comprise a 2' fluoro modification.~~

~~266. The gRNA of any one of claims 205-265, wherein:~~

- ~~i. nucleotide 6 from the 5' end of the 5' terminus does not comprise a 2' fluoro modification;~~
- ~~ii. nucleotide 7 from the 5' end of the 5' terminus does not comprise a 2' fluoro modification;~~
- ~~iii. nucleotide 8 from the 5' end of the 5' terminus does not comprise a 2' fluoro modification;~~
- ~~iv. nucleotide 9 from the 5' end of the 5' terminus does not comprise a 2' fluoro modification; and/or~~
- ~~v. nucleotide 10 from the 5' end of the 5' terminus does not comprise a 2' fluoro modification.~~

~~267. The gRNA of any one of claims 205-266, wherein:~~

- i. ~~nucleotide 6 from the 5' end of the 5' terminus does not comprise a phosphorothioate linkage;~~
- ii. ~~nucleotide 7 from the 5' end of the 5' terminus does not comprise a phosphorothioate linkage;~~
- iii. ~~nucleotide 8 from the 5' end of the 5' terminus does not comprise a phosphorothioate linkage;~~
- iv. ~~nucleotide 9 from the 5' end of the 5' terminus does not comprise a phosphorothioate linkage; and/or~~
- v. ~~nucleotide 10 from the 5' end of the 5' terminus does not comprise a phosphorothioate linkage.~~

268. ~~The gRNA of any one of claims 205-267, wherein:~~

- i. ~~nucleotide 7 from the 5' end of the 5' terminus does not comprise a 2'-OMe modification;~~
- ii. ~~nucleotide 8 from the 5' end of the 5' terminus does not comprise a 2'-OMe modification;~~
- iii. ~~nucleotide 9 from the 5' end of the 5' terminus does not comprise a 2'-OMe modification; and/or~~
- iv. ~~nucleotide 10 from the 5' end of the 5' terminus does not comprise a 2'-OMe modification.~~

269. ~~The gRNA of any one of claims 205-268, wherein nucleotide 20 from the 5' end of the 5' terminus does not comprise a 2'-OMe modification.~~

270. ~~The gRNA of any one of claims 205-269, wherein the guide RNA comprises a 2' fluoro modification at any one or more of nucleotides 1-11 and 13-20 from the 5' end of the 5' terminus and nucleotide 12 from the 5' end of the 5' terminus does not comprise a 2' fluoro modification.~~

271. ~~The gRNA of any one of claims 205-270, wherein the guide RNA comprises a 2' fluoro modification at any one or more of nucleotides 1-20 from the 5' end of the 5' terminus and:~~

- i. ~~nucleotide 11 from the 5' end of the 5' terminus does not comprise a 2' fluoro modification;~~

- ii. ~~nucleotide 12 from the 5' end of the 5' terminus does not comprise a 2' fluoro modification;~~
- iii. ~~nucleotide 13 from the 5' end of the 5' terminus does not comprise a 2' fluoro modification;~~
- iv. ~~nucleotide 14 from the 5' end of the 5' terminus does not comprise a 2' fluoro modification;~~
- v. ~~nucleotide 17 from the 5' end of the 5' terminus does not comprise a 2' fluoro modification; and/or~~
- vi. ~~nucleotide 18 from the 5' end of the 5' terminus does not comprise a 2' fluoro modification.~~

~~272. The gRNA of any one of claims 205-271, wherein:~~

- i. ~~B2 does not comprise a 2' OMe modification;~~
- ii. ~~B3 does not comprise a 2' OMe modification;~~
- iii. ~~B4 does not comprise a 2' OMe modification; and/or~~
- iv. ~~B5 does not comprise a 2' OMe modification.~~

~~273. The gRNA of any one of claims 205-272, wherein:~~

- i. ~~LS1 does not comprise a 2' OMe modification;~~
- ii. ~~LS8 does not comprise a 2' OMe modification; and/or~~
- iii. ~~LS10 does not comprise a 2' OMe modification.~~

~~274. The gRNA of any one of claims 205-273, wherein:~~

- i. ~~N2 does not comprise a 2' OMe modification;~~
- ii. ~~N3 does not comprise a 2' OMe modification;~~
- iii. ~~N4 does not comprise a 2' OMe modification;~~
- iv. ~~N5 does not comprise a 2' OMe modification;~~
- v. ~~N6 does not comprise a 2' OMe modification;~~
- vi. ~~N7 does not comprise a 2' OMe modification;~~
- vii. ~~N10 does not comprise a 2' OMe modification;~~
- viii. ~~N11 does not comprise a 2' OMe modification;~~
- ix. ~~N16 does not comprise a 2' OMe modification; and/or~~
- x. ~~N17 does not comprise a 2' OMe modification.~~

275. ~~The gRNA of any one of claims 205-274, wherein:~~

- ~~i. H1-2 does not comprise a phosphorothioate linkage;~~
- ~~ii. H1-3 does not comprise a phosphorothioate linkage;~~
- ~~iii. H1-4 does not comprise a phosphorothioate linkage;~~
- ~~iv. H1-5 does not comprise a phosphorothioate linkage;~~
- ~~v. H1-6 does not comprise a phosphorothioate linkage;~~
- ~~vi. H1-7 does not comprise a phosphorothioate linkage;~~
- ~~vii. H1-8 does not comprise a phosphorothioate linkage;~~
- ~~viii. H1-9 does not comprise a phosphorothioate linkage;~~
- ~~ix. H1-10 does not comprise a phosphorothioate linkage;~~
- ~~x. H2-1 does not comprise a phosphorothioate linkage;~~
- ~~xi. H2-2 does not comprise a phosphorothioate linkage;~~
- ~~xii. H2-3 does not comprise a phosphorothioate linkage;~~
- ~~xiii. H2-4 does not comprise a phosphorothioate linkage;~~
- ~~xiv. H2-5 does not comprise a phosphorothioate linkage;~~
- ~~xv. H2-6 does not comprise a phosphorothioate linkage;~~
- ~~xvi. H2-7 does not comprise a phosphorothioate linkage;~~
- ~~xvii. H2-8 does not comprise a phosphorothioate linkage;~~
- ~~xviii. H2-9 does not comprise a phosphorothioate linkage;~~
- ~~xix. H2-10 does not comprise a phosphorothioate linkage;~~
- ~~xx. H2-11 does not comprise a phosphorothioate linkage;~~
- ~~xxi. H2-12 does not comprise a phosphorothioate linkage;~~
- ~~xxii. H2-13 does not comprise a phosphorothioate linkage;~~
- ~~xxiii. H2-14 does not comprise a phosphorothioate linkage; and/or~~
- ~~xxiv. H2-15 does not comprise a phosphorothioate linkage.~~

276. ~~A gRNA which is an sgRNA comprising modifications at:~~

- ~~i. nucleotides 6-10 from the 5' end of the 5' terminus, which are optionally PS modifications;~~
- ~~ii. nucleotides 8-11, 13, 14, 17, and 18 from the 5' end of the 5' terminus, which are optionally 2' fluoro modifications; and~~
- ~~iii. H1-1 and H2-1, which are optionally 2' OMe modifications, or conserved region YA site 1 or 8.~~

277. ~~A gRNA which is an sgRNA comprising YA modifications at:~~
- ~~i. conserved region YA sites 1, 5, 6, 7, and 9, which are optionally 2' OMe modifications; and~~
 - ~~ii. conserved region YA site 8, which is optionally a 2' fluoro modification.~~
278. ~~A gRNA comprising YA modifications at four guide region YA sites, wherein at least one of the YA sites is at or after nucleotide 8 from the 5' end of the 5' terminus, and wherein:~~
- ~~i. the first YA site comprises a 2' OMe modification;~~
 - ~~ii. the second YA site comprises a 2' fluoro modification;~~
 - ~~iii. the third YA site comprises a 2' fluoro or PS modification; and~~
 - ~~iv. the fourth YA site comprises a PS modification,~~
- ~~optionally wherein the first, second, third, and fourth YA sites are arranged in the 5' to 3' direction.~~
279. ~~The gRNA of claim 278, wherein the third YA site comprises a PS modification.~~
280. ~~The gRNA of any one of claims 278-279, wherein the third YA site comprises a 2' fluoro modification.~~
281. ~~The gRNA of any one of claims 278-280, further comprising a fifth YA site comprising a PS modification, which is optionally 3' of the fourth YA site.~~
282. ~~The gRNA of any one of the claims 205-281, wherein conserved region YA sites 1, 5, 6, 7, and 9 comprise YA modifications, which are optionally 2' OMe modifications; and conserved region YA site 8 comprises a modification, which is optionally a 2' fluoro modification.~~
283. ~~A gRNA which is an sgRNA comprising YA modifications at:~~
- ~~i. nucleotide 4 from the 5' end of the 5' terminus, wherein the YA modification is optionally a 2' OMe modification;~~
 - ~~ii. nucleotides 6-10 from the 5' end of the 5' terminus, which are optionally PS modifications;~~
 - ~~iii. nucleotides 8-11, 13, 14, 17, and 18 from the 5' end of the 5' terminus, which are optionally 2' fluoro modifications;~~

- iv. ~~LS5, LS7, LS9, and LS11, which are optionally 2' fluoro modifications;~~
- v. ~~LS8, LS10, and LS12, which are optionally 2' OMe modifications;~~
- vi. ~~N2, N3, N4, N5, N6, N7, N10, N11, N16, and N17, which are optionally 2' OMe modifications; and~~
- vii. ~~N14, which is optionally a 2' fluoro modification.~~

284. ~~The gRNA of any one of claim 161, wherein one or more of the following are true:~~

- i. ~~nucleotide 4 from the 5' end of the 5' terminus comprises a 2' OMe modification;~~
- ii. ~~nucleotides 6-10 from the 5' end of the 5' terminus comprise PS modifications;~~
- iii. ~~nucleotides 8-11, 13, 14, 17, and 18 from the 5' end of the 5' terminus comprise 2' fluoro modifications;~~
- iv. ~~LS5, LS7, LS9, and LS11 comprise 2' fluoro modifications;~~
- v. ~~LS8, LS10, and LS12 comprise 2' OMe modifications;~~
- vi. ~~N2, N3, N4, N5, N6, N7, N10, N11, N16, and N17 comprise 2' OMe modifications; and~~
- vii. ~~N14 comprises a 2' fluoro modification.~~

285. ~~The gRNA of any one of claims 161-284, wherein at least one YA modification comprises a modification of the pyrimidine position of the YA site.~~

286. ~~The gRNA of any one of claims 161-285, wherein at least one YA modification comprises a modification of the adenine position of the YA site.~~

287. ~~The gRNA of any one of claims 161-286, wherein at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 YA sites comprise YA modifications at the pyrimidines positions of the YA sites.~~

288. ~~The gRNA of any one of claims 161-287, wherein at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 YA sites comprise YA modifications at the adenine positions of the YA sites.~~

289. ~~The gRNA of any one of claims 161-288, wherein at least one YA modification comprises a 2' OMe modification.~~

290. ~~The gRNA of any one of claims 161-289, wherein at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 YA sites comprise a 2'-OMe modification.~~
291. ~~The gRNA of any one of claims 161-290, wherein each modified conserved region YA site comprises a modification at the pyrimidine position of the YA site.~~
292. ~~The gRNA of any one of claims 161-291, wherein each modified guide region YA site, or each modified conserved region and guide region YA site, comprises a modification at the pyrimidine position of the YA site.~~
293. ~~The gRNA of any one of claims 161-292, wherein each modified conserved region YA site comprises a modification at the adenine position of the YA site.~~
294. ~~The gRNA of any one of claims 161-293, wherein each modified guide region YA site, or each modified conserved region and guide region YA site, comprises a modification at the adenine position of the YA site.~~
295. ~~The gRNA of any one of claims 161-294, which is an sgRNA comprising a modification at LS5.~~
296. ~~The gRNA of any one of claims 161-295, which is an sgRNA comprising a modification at LS7.~~
297. ~~The gRNA of any one of claims 161-296, which is an sgRNA comprising a modification at LS9, optionally wherein if LS9 is modified and LS5, LS7, and LS12 are not, then the modification of LS9 is other than 2'-fluoro.~~
298. ~~The gRNA of any one of claims 161-297, which is an sgRNA comprising a modification at LS12, optionally wherein if LS12 is modified and LS9 is not, then the modification of LS12 is other than 2'-OMe.~~
299. ~~The gRNA of any one of claims 161-298, which is an sgRNA comprising at least one YA modification that stabilizes a secondary structure, optionally wherein the secondary structure is the lower stem.~~
300. ~~The gRNA of any one of claims 161-299, which is an sgRNA comprising at least one modification of LS8 and/or LS11, optionally wherein the modification of LS8 and/or LS11 stabilizes a secondary structure.~~

301. ~~The gRNA of any one of claims 161-300, comprising a YA modification that stabilizes a secondary structure chosen from:~~
- ~~i. ENA;~~
 - ~~ii. LNA; or~~
 - ~~iii. a bicyclic ribose modification.~~
302. ~~The gRNA of any one of claims 161-301, which is an sgRNA comprising a modification at N6.~~
303. ~~The gRNA of any one of claims 161-302, which is an sgRNA comprising a modification at N14.~~
304. ~~The gRNA of any one of claims 161-303, which is an sgRNA comprising a modification at N17, optionally wherein if N17 is modified and N6 and N14 are not, then the modification of N17 is other than 2'-fluoro and other than 2'-OMe.~~
305. ~~The gRNA of any one of claims 161-304, wherein at least 1, 2, or 3 of nucleotides 1-3 from the 5' end of the 5' terminus comprise deoxyribonucleotides, optionally wherein nucleotides 1-3 from the 5' end of the 5' terminus comprise PS modifications.~~
306. ~~The gRNA of any one of claims 161-305, wherein the gRNA is an sgRNA and at least 1, 2, or 3 of nucleotides 1-3 from the 3' end of the 3' terminus comprise deoxyribonucleotides, optionally wherein nucleotides 2-3 from the 3' end of the 3' terminus comprise PS modifications.~~
307. ~~The gRNA of any one of claims 161-306, wherein the gRNA is an sgRNA and nucleotide 4 from the 3' end of the 3' terminus comprises a PS modification, optionally wherein nucleotide 4 from the 3' end of the 3' terminus comprises a 2'-OMe modification.~~
308. ~~The gRNA of any one of claims 161-307, wherein the gRNA is an sgRNA and hairpin 2 comprises deoxyribonucleotides, optionally wherein all or all but 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides of hairpin 1 and hairpin 2 are deoxyribonucleotides.~~
309. ~~The gRNA of any one of claims 161-308, wherein the gRNA is an sgRNA and hairpin 1 and hairpin 2 comprise deoxyribonucleotides, optionally wherein all or all~~

but 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 nucleotides of hairpin 1 and hairpin 2 are deoxyribonucleotides.

310. — The gRNA of any one of claims 161-309, wherein the gRNA is an sgRNA and all or all but 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 nucleotides from the beginning of hairpin 1 to the 3' end of the sgRNA are deoxyribonucleotides, optionally wherein nucleotides 1-3 from the 3' end of the 3' terminus are deoxyribonucleotides.

311. — The gRNA of any one of claims 161-310, wherein the gRNA is an sgRNA and the upper stem comprises deoxyribonucleotides.

312. — The gRNA of any one of claims 161-311, wherein the gRNA is an sgRNA and all or all but 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides of the upper stem are deoxyribonucleotides.

313. — The gRNA of any one of claims 161-312, wherein at least 1, 2, or 3 of nucleotides 1-3 from the 5' end of the 5' terminus comprise ENA, optionally wherein nucleotides 1-3 from the 5' end of the 5' terminus comprise PS modifications.

314. — The gRNA of any one of claims 161-313, wherein the gRNA is an sgRNA and at least 1, 2, or 3 of nucleotides 2-4 from the 3' end of the 3' terminus comprise ENA, optionally wherein nucleotides 2-3 from the 3' end of the 3' terminus comprise PS modifications.

315. — The gRNA of any one of claims 161-314, wherein at least 1, 2, or 3 of nucleotides 1-3 from the 5' end of the 5' terminus comprise UNA, optionally wherein nucleotides 1-3 from the 5' end of the 5' terminus comprise PS modifications.

316. — The gRNA of any one of claims 161-315, wherein the gRNA is an sgRNA and at least 1, 2, or 3 of nucleotides 2-4 from the 3' end of the 3' terminus comprise UNA, optionally wherein nucleotides 2-3 from the 3' end of the 3' terminus comprise PS modifications.

317. — The gRNA of any one of claims 161-316, wherein the gRNA is an sgRNA and nucleotide 4 from the 3' end of the 3' terminus comprises a PS modification, optionally wherein nucleotide 4 from the 3' end of the 3' terminus comprises a 2'-OMe modification.

318. ~~The gRNA of any one of claims 161-317, wherein the gRNA is an sgRNA that comprises a 3' end modification.~~
319. ~~The gRNA of any one of claims 161-318, which is an sgRNA comprising a 3' end modification, wherein the 3' end modification is a protective 3' end modification.~~
320. ~~The gRNA of any one of claims 161-319, wherein the gRNA is an sgRNA that comprises a 3' tail.~~
321. ~~The gRNA of claim 320, wherein the 3' tail comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides.~~
322. ~~The gRNA of claim 320, wherein the 3' tail comprises about 1-2, 1-3, 1-4, 1-5, 1-7, 1-10, at least 1-5, at least 1-3, at least 1-4, at least 1-5, at least 1-5, at least 1-7, or at least 1-10 nucleotides.~~
323. ~~The gRNA of any one of claims 161-322, which is an sgRNA comprising a modification in the hairpin region.~~
324. ~~The gRNA of any one of claims 161-323, which is an sgRNA comprising a 3' end modification, and a modification in the hairpin region.~~
325. ~~The gRNA of claim 323 or 324, wherein the modification in the hairpin region comprises a modified nucleotide selected from 2' O-methyl (2' O-Me) modified nucleotide, a 2' fluoro (2' F) modified nucleotide, or combinations thereof.~~
326. ~~The gRNA of any one of claims 323-325, wherein the modification in the hairpin region comprises or further comprises a 2' O-methyl (2' O-Me) modified nucleotide.~~
327. ~~The gRNA of any one of claims 323-326, wherein the modification in the hairpin region comprises or further comprises a 2' fluoro (2' F) modified nucleotide.~~
328. ~~The gRNA of any one of claims 161-327, comprising 3' and/or 5' protective end modification(s).~~
329. ~~The gRNA of claim 328, wherein the 3' and/or 5' end modification comprises a modified nucleotide selected from 2' O-methyl (2' O-Me) modified nucleotide, 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 combinations thereof.

330. ~~—The gRNA of claim 328 or 329, wherein the 3' and/or 5' end modification comprises or further comprises a 2' O methyl (2' O Me) modified nucleotide.~~

331. ~~—The gRNA of claim 328 or 329, wherein the 3' and/or 5' end modification comprises or further comprises a 2' fluoro (2' F) modified nucleotide.~~

332. ~~—The gRNA of claim 328 or 329, wherein the 3' and/or 5' end modification comprises or further comprises a phosphorothioate (PS) linkage between nucleotides.~~

333. ~~—The gRNA of claim 328 or 329, wherein the 3' and/or 5' end modification comprises or further comprises an inverted abasic modified nucleotide.~~

334. ~~—The gRNA of any one any one of claims 161-333, wherein the gRNA is an sgRNA and if the sgRNA comprises a 3' end modification, the 3' end modification comprises any one or more of the following:~~

- ~~i. a modification of any one or more of the last 7, 6, 5, 4, 3, 2, or 1 nucleotides;~~
- ~~ii. one modified nucleotide;~~
- ~~iii. two modified nucleotides;~~
- ~~iv. three modified nucleotides;~~
- ~~v. four modified nucleotides;~~
- ~~vi. five modified nucleotides;~~
- ~~vii. six modified nucleotides; and~~
- ~~viii. seven modified nucleotides.~~

335. ~~—The gRNA of claim 334, wherein the 3' end modification comprises a modification of between 1 and 7, between 1 and 5, between 1 and 4, or between 2 and 4 nucleotides.~~

336. ~~—The gRNA of any one of claims 161-335, wherein the gRNA is an sgRNA comprising a 3' end modification and the 3' end modification comprises one or more of the following:~~

- ~~i. a phosphorothioate (PS) linkage between nucleotides;~~
- ~~ii. a 2' O Me modified nucleotide;~~

- iii. ~~a 2' O-moe modified nucleotide;~~
- iv. ~~a 2' F modified nucleotide;~~
- v. ~~an inverted abasic modified nucleotide~~
- vi. ~~ENA, UNA, and/or DNA; and~~
- vii. ~~or a combination thereof.~~

337. ~~The gRNA of any one of claims 161-336, wherein the gRNA is an sgRNA comprising a 3' tail, and the 3' tail comprises any one or more of:~~

- i. ~~a phosphorothioate (PS) linkage between nucleotides;~~
- ii. ~~a 2' O-Me modified nucleotide;~~
- iii. ~~a 2' O-moe modified nucleotide;~~
- iv. ~~a 2' F modified nucleotide;~~
- v. ~~an inverted abasic modified nucleotide~~
- vi. ~~ENA, UNA, and/or DNA; and~~
- vii. ~~or a combination thereof.~~

338. ~~The gRNA of claim 336, wherein the 3' end modification comprises:~~

- i. ~~1, 2, 3, 4, 5, 6, or 7 PS linkages between nucleotides;~~
- ii. ~~about 1-3, 1-5, 1-6, or 1-7 PS linkages between nucleotides; or~~
- iii. ~~PS linkages between each nucleotide.~~

339. ~~The gRNA of any one of claims claim 326-328, wherein the 3' end modification further comprises at least one 2' O-Me, 2' O-moe, inverted abasic, or 2' F modified nucleotide.~~

340. ~~The gRNA of any one of claims 326-329, wherein the 3' end modification comprises at least one PS linkage, and wherein:~~

- i. ~~there is one PS linkage, and the linkage is between the last and second to last nucleotide;~~
- ii. ~~there are two PS linkages between the last three nucleotides;~~
- iii. ~~there are PS linkages between any one or more of the last four nucleotides;~~
- iv. ~~there are PS linkages between any one or more of the last five nucleotides; or~~

- v. ~~there are PS linkages between any one or more of the last 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides.~~

341. ~~The gRNA of any one of claims 336-340, wherein the 3' end modification comprises:~~

- i. ~~a modification of one or more of the last 1-7 nucleotides, wherein the modification is a PS linkage, inverted abasic nucleotide, 2' O-Me, 2' O-moe, 2' F, or combinations thereof;~~
- ii. ~~a modification to the last nucleotide with 2' O-Me, 2' O-moe, 2' F, or combinations thereof, and an optional one or two PS linkages to the next nucleotide and/or the first nucleotide of the 3' tail;~~
- iii. ~~a modification to the last and/or second to last nucleotide with 2' O-Me, 2' O-moe, 2' F, or combinations thereof, and optionally one or more PS linkages;~~
- iv. ~~a modification to the last, second to last, and/or third to last nucleotides with 2' O-Me, 2' O-moe, 2' F, or combinations thereof, and optionally one or more PS linkages;~~
- v. ~~a modification to the last, second to last, third to last, and/or fourth to last nucleotides with 2' O-Me, 2' O-moe, 2' F, or combinations thereof, and optionally one or more PS linkages; or~~
- vi. ~~a modification to the last, second to last, third to last, fourth to last, and/or fifth to last nucleotides with 2' O-Me, 2' O-moe, 2' F, or combinations thereof, and optionally one or more PS linkages.~~

342. ~~The gRNA of any one of claims 161-341, wherein the gRNA is an sgRNA comprising a 3' tail, wherein the 3' tail comprises a modification of any one or more of the nucleotides present in the 3' tail.~~

343. ~~The gRNA of claim 342, wherein the 3' tail is fully modified.~~

344. ~~The gRNA of claim 342, wherein the 3' tail comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, or 1-10 nucleotides, optionally where any one or more of these nucleotides are modified.~~

345. ~~The gRNA of any one of claims 336-344, wherein the 3' end modification comprises any one or more of the following:~~

- ~~i. the 3' end modification as shown in any one of SEQ ID Nos: 401-532;~~
- ~~ii. (i) a 2'O-Me modified nucleotide at the last nucleotide of the conserved region of an sgRNA or short sgRNA, (ii) three consecutive 2'O-moe modified nucleotides immediately 5' to the 2'O-Me modified nucleotide, and (iii) three consecutive PS linkages between the last three nucleotides;~~
- ~~iii. (i) five consecutive 2'O-Me modified nucleotides from the 3' terminus, and (ii) three PS linkages between the last three nucleotides;~~
- ~~iv. an inverted-abasic modified nucleotide at the last nucleotide of the conserved region of an sgRNA or short sgRNA;~~
- ~~v. (i) an inverted-abasic modified nucleotide at the last nucleotide of the conserved region of an sgRNA or short sgRNA, and (ii) three consecutive 2'O-Me modified nucleotides at the last three nucleotides of the conserved region of an sgRNA or short sgRNA;~~
- ~~vi. (i) 15 consecutive 2'O-Me modified nucleotides from the 3' terminus, (ii) five consecutive 2' F modified nucleotides immediately 5' to the 2'O-Me modified nucleotides, and (iii) three PS linkages between the last three nucleotides;~~
- ~~vii. (i) alternating 2'O-Me modified nucleotides and 2' F modified nucleotides at the last 20 nucleotides of the conserved region of an sgRNA or short sgRNA, and (ii) three PS linkages between the last three nucleotides;~~
- ~~viii. (i) two or three consecutive 2'O-Me modified nucleotides, and (ii) three PS linkages between the last three nucleotides;~~
- ~~ix. one PS linkage between the last and next to last nucleotides; and~~
- ~~x. 15 or 20 consecutive 2'O-Me modified nucleotides, and (ii) three PS linkages between the last three nucleotides.~~

~~346. The gRNA of any one of claims 161-345, comprising a 5' end modification comprising any one or more of the following:~~

- ~~i. a modification of any one or more of nucleotides 1-7 of the guide region;~~
- ~~ii. one modified nucleotide;~~
- ~~iii. two modified nucleotides;~~
- ~~iv. three modified nucleotides;~~

- v. ~~four modified nucleotides;~~
- vi. ~~five modified nucleotides;~~
- vii. ~~six modified nucleotides; and~~
- viii. ~~seven modified nucleotides.~~

347. ~~The gRNA of any one of claims 161-346, comprising a 5' end modification, wherein the 5' end modification is a protective 5' end modification.~~

348. ~~The gRNA of any one of claims 161-347, comprising a 5' end modification, wherein the 5' end modification comprises a modification of between 1 and 7, between 1 and 5, between 1 and 4, between 1 and 3, or between 1 and 2 nucleotides.~~

349. ~~The gRNA of any one of claims 161-348, comprising a 5' end modification wherein the 5' end modification comprises any one or more of the following:~~

- i. ~~modifications of 1, 2, 3, 4, 5, 6, or 7 of the first 7 nucleotides;~~
- ii. ~~modifications of about 1-3, 1-4, 1-5, 1-6, or 1-7 of the first 7 nucleotides;~~
- ~~and~~
- iii. ~~modifications at the first, second, third, fourth, fifth, sixth, and/or seventh nucleotide at the 5' end, optionally wherein the modifications are consecutive.~~

350. ~~The gRNA of any one of claims 161-349, comprising a 5' end modification, wherein the 5' end modification comprises one or more of:~~

- i. ~~a phosphorothioate (PS) linkage between nucleotides;~~
- ii. ~~a 2' O-Me modified nucleotide;~~
- iii. ~~a 2' O-moe modified nucleotide;~~
- iv. ~~a 2' F modified nucleotide;~~
- v. ~~an inverted abasic modified nucleotide~~
- vi. ~~ENA, UNA, and/or DNA; and~~
- vii. ~~combinations thereof.~~

351. ~~The gRNA any one of claims 161-350, comprising a 5' end modification wherein the 5' end modification comprises:~~

- i. ~~1, 2, 3, 4, 5, 6, and/or 7 PS linkages between nucleotides; or~~
- ii. ~~about 1-2, 1-3, 1-4, 1-5, 1-6, or 1-7 PS linkages between nucleotides.~~

352. ~~The gRNA of any one of claims 161-351, wherein the sgRNA comprises a 5' end modification and the 5' end modification comprises at least one 2' O-Me, 2' O-moe, inverted abasic, 2' H, inosine, or 2' F modified nucleotide.~~
353. ~~The gRNA of claim 352, wherein the 5' end modification comprises at least one PS linkage, and wherein:~~
- ~~i. there is one PS linkage, and the linkage is at nucleotide 1 of the guide region;~~
 - ~~ii. there are two PS linkages, and the linkages are at nucleotides 1 and 2 of the guide region;~~
 - ~~iii. there are PS linkages at any one or more of nucleotides 1, 2, and 3 of the guide region;~~
 - ~~iv. there are PS linkages at any one or more of nucleotides 1, 2, 3, and 4 of the guide region;~~
 - ~~v. there are PS linkages at any one or more of nucleotides 1, 2, 3, 4, and 5 of the guide region;~~
 - ~~vi. there are PS linkages at any one or more of nucleotides 1, 2, 3, 4, 5, and 6 of the guide region; or~~
 - ~~vii. there are PS linkages at any one or more of nucleotides 1, 2, 3, 4, 5, 6, and 7 of the guide region.~~
354. ~~The gRNA of any one of claims 352-353, wherein the 5' end modification comprises:~~
- ~~i. a modification of one or more of nucleotides 1-7 of the variable region, wherein the modification is a PS linkage, inverted abasic nucleotide, 2' O-Me, 2' O-moe, 2' F, 2' H, inosine, and/or combinations thereof;~~
 - ~~ii. a modification to the first nucleotide of the guide region with 2' O-Me, 2' O-moe, 2' F, 2' H, inosine, or combinations thereof, and an optional PS linkage to the next nucleotide;~~
 - ~~iii. a modification to the first and/or second nucleotide of the variable region with 2' O-Me, 2' O-moe, 2' F, 2' H, inosine, or combinations thereof, and optionally one or more PS linkages;~~

- ~~iv. a modification to the first, second, and/or third nucleotides of the variable region with 2' O-Me, 2' O-moe, 2' F, 2' H, inosine, or combinations thereof, and optionally one or more PS linkages;~~
- ~~v. a modification to the first, second, third, and/or fourth nucleotides of the variable region with 2' O-Me, 2' O-moe, 2' F, 2' H, inosine, or combinations thereof, and optionally one or more PS linkages; or~~
- ~~vi. a modification to the first, second, third, fourth, and/or fifth nucleotides of the variable region with 2' O-Me, 2' O-moe, 2' F, 2' H, inosine, or combinations thereof, and optionally one or more PS linkages.~~

~~355. The gRNA of any one of claims 161-354, comprising a 5' end modification, wherein the 5' end modification comprises any one or more of the following:~~

- ~~i. a 5' end modification as shown in any one of SEQ ID Nos: 1-54, 401-532, 1001, 1007-1132, 1205-1212, 1322-1406, 1417-1501, 1511-1596, 3018-3059, 3063-3104, 3108-3149, 3153-3194, 3198-3239, 3243-3284, 3295-3341, 3343-3385, 3388-3430, or 3549-3552;~~
- ~~ii. 2' OMe modified nucleotides at nucleotides 1, 2, and 3 of the guide region;~~
- ~~iii. 2' OMe modified nucleotides at nucleotides 1, 2, and 3 of the guide region and PS linkages between nucleotides 1 and 2, 2 and 3, and 3 and 4 of the guide region;~~
- ~~iv. 2' OMe modified nucleotides at nucleotides 1, 2, 3, 4, and 5 of the guide region;~~
- ~~v. 2' OMe modified nucleotides at nucleotides 1, 2, 3, 4, and 5 of the guide region and PS linkages between nucleotides 1 and 2, 2 and 3, 3 and 4, 4 and 5, and 5 and 6 of the guide region;~~
- ~~vi. 2' O-moe modified nucleotides at nucleotides 1, 2, and 3 of the guide region;~~
- ~~vii. 2' O-moe modified nucleotides at nucleotides 1, 2, and 3 of the guide region and PS linkages between nucleotides 1 and 2, 2 and 3, and 3 and 4 of the guide region;~~
- ~~viii. an inverted abasic modified nucleotide at nucleotide 1 of the guide region;~~

- ix. ~~an inverted abasic modified nucleotide at nucleotide 1 of the guide region and 2' OMe modified nucleotides at nucleotides 1, 2, and 3 of the guide region; and~~
- x. ~~an inverted abasic modified nucleotide at nucleotide 1 of the guide region, 2' OMe modified nucleotides at nucleotides 1, 2, and 3 of the guide region, and PS linkages between nucleotides 1 and 2, 2 and 3, 3 and 4, 4 and 5, and 5 and 6 of the variable region.~~

356. ~~The gRNA of any one of claims 161-355, wherein the gRNA is an sgRNA and the upper stem region comprises at least one modification.~~

357. ~~The gRNA of claim 346, wherein the upper stem modification comprises any one or more of the following:~~

- i. ~~a modification to any one or more of US1-US12 in the upper stem region;~~
- ii. ~~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; and~~
- iii. ~~a modification of about 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, or 1-12 nucleotides in the upper stem region.~~

358. ~~The gRNA of any one of claims 356-357, wherein the upper stem modification comprises one or more of:~~

- i. ~~a 2' O-Me modified nucleotide;~~
- ii. ~~a 2' H-modified nucleotide;~~
- iii. ~~a 2' F-modified nucleotide; and~~
- iv. ~~combinations thereof.~~

359. ~~The gRNA of any one of claims 161-358, wherein the gRNA is an sgRNA comprising one or more modifications in the hairpin 1 region.~~

360. ~~The gRNA of claim 359 wherein the sgRNA comprises a modification at H1-1.~~

361. ~~The gRNA of any one of claims 161-360, wherein the gRNA is an sgRNA comprising one or more modifications in the hairpin 2 region.~~

362. ~~—The gRNA of claim 361, wherein the sgRNA comprises a modification at H2-1.~~
363. ~~—The gRNA of any one of claims 161-362, wherein the gRNA is an sgRNA comprising modifications at H1-1 to H1-12.~~
364. ~~—The gRNA of any one of claims 161-363, wherein the gRNA is an sgRNA comprising modifications at H2-1 to H2-15.~~
365. ~~—The gRNA of any one of claims 161-364, wherein the gRNA is an sgRNA comprising one or more modifications in each of the upper stem region, the hairpin 1 region, and the hairpin 2 region.~~
366. ~~—The gRNA of any one of claims 161-365, wherein the gRNA is an sgRNA comprising a modified nucleotide between hairpin 1 and hairpin 2 regions.~~
367. ~~—The gRNA of any one of claims 161-366, which is an sgRNA further comprising a lower stem region comprising a modification.~~
368. ~~—The gRNA of any one of claims 161-367, further comprising a 3' end modification.~~
369. ~~—The gRNA of claim 368, wherein at least two of the last four nucleotides at the 3' end of the 3' terminus are modified.~~
370. ~~—The gRNA of claim 369, wherein at least two of the last four nucleotides at the 3' end of the 3' terminus are modified with 2'-O-Me, 2'-F, or 2'-O-moe.~~
371. ~~—The gRNA of any one of claims 368-370, further comprising phosphorothioate (PS) bonds between one or more of the last four nucleotides at the 3' end of the 3' terminus.~~
372. ~~—The gRNA of any one of claims 161-371, which is an sgRNA further comprising a bulge region comprising a modification.~~
373. ~~—The gRNA of any one of claims 161-372, which is an sgRNA further comprising a nexus region comprising a modification.~~
374. ~~—An sgRNA comprising any of SEQ ID Nos: 401-535, 601, 607-732, 801, 807-932, 1001, or 1007-1132, including the modifications of Table 1.~~

375. ~~An sgRNA comprising nucleic acids having at least 99, 98, 97, 96, 95, 94, 93, 92, 91, 90, 85, 80, 75, or 70% identity to the nucleic acids of any one of SEQ ID Nos: 401-535, 601, 607-732, 801, 807-932, 1001, or 1007-1132, wherein the modification at each nucleotide of the sgRNA that corresponds to a nucleotide of the reference sequence identifier in Table 1, is identical to or equivalent to the modification shown in the reference sequence identifier in Table 1.~~

376. ~~The gRNA of any one of claims 161-375, wherein the modification reduces gRNA degradation without significantly altering the ability of the guide to cleave a target nucleic acid.~~

377. ~~The gRNA of any one of claims 161-376, comprising a YA modification wherein the modification comprises 2'-fluoro, 2'-H, 2'-O-Me, ENA, UNA, or PS.~~

378. ~~The gRNA of any one of claims 161-377, comprising a YA modification wherein the modification alters the structure of the dinucleotide motif to reduce RNA endonuclease activity.~~

379. ~~The gRNA of any one of claims 161-378, comprising a YA modification wherein the modification interferes with recognition or cleavage of a YA site by an RNase and/or stabilizes an RNA structure.~~

380. ~~The gRNA of any one of claims 161-379, comprising a YA modification wherein the modification comprises one or more of:~~

- ~~i. a ribose modification selected from 2'-O-alkyl, 2'-F, 2'-moe, 2'-F arabinose, and 2'-H (deoxyribose);~~
- ~~ii. a bicyclic ribose analog, such as LNA, BNA, and ENA;~~
- ~~iii. an unlocked nucleic acid modification;~~
- ~~iv. a base modification, such as inosine, pseudouridine, and 5'-methylcytosine; and~~
- ~~v. an internucleoside linkage modification such as phosphorothioate.~~

381. ~~The gRNA of any one of the preceding claims, wherein the gRNA comprises a guide region that comprises a modification at nucleotide 5, optionally wherein the guide region comprises 2'-OMe modifications at nucleotides 1-4, phosphorothioate modifications at nucleotides 1-3 and 6-10, and/or 2'-F modifications at nucleotides 8-11, 13, 14, 17, and 18.~~

382. — The gRNA of any one of the preceding claims, wherein the gRNA comprises a guide region that comprises a modification at nucleotide 12, optionally wherein the guide region comprises 2' OMe modifications at nucleotides 1-4, phosphorothioate modifications at nucleotides 1-3 and 6-10, and/or 2' F modifications at nucleotides 8-11, 13, 14, 17, and 18.

383. — The gRNA of any one of the preceding claims, wherein the gRNA comprises a guide region that comprises a 2' OMe modification at nucleotide 5 and/or nucleotide 12, optionally wherein the guide region comprises 2' OMe modifications at nucleotides 1-4, phosphorothioate modifications at nucleotides 1-3 and 6-10, and/or 2' F modifications at nucleotides 8-11, 13, 14, 17, and 18.

384. — The gRNA of any one of the preceding claims, wherein the gRNA comprises a guide region that comprises a 2' F modification at nucleotide 5 and/or nucleotide 12, optionally wherein the guide region comprises 2' OMe modifications at nucleotides 1-4, phosphorothioate modifications at nucleotides 1-3 and 6-10, and/or 2' F modifications at nucleotides 8-11, 13, 14, 17, and 18.

385. — The gRNA of any one of the preceding claims, wherein the gRNA comprises a guide region that comprises a 2' H modification at nucleotide 5 and/or nucleotide 12, optionally wherein the guide region comprises 2' OMe modifications at nucleotides 1-4, phosphorothioate modifications at nucleotides 1-3 and 6-10, and/or 2' F modifications at nucleotides 8-11, 13, 14, 17, and 18.

386. — The gRNA of any one of the preceding claims, wherein the gRNA comprises a guide region that comprises a phosphorothioate modification at nucleotide 5 and/or nucleotide 12, optionally wherein the guide region comprises 2' OMe modifications at nucleotides 1-4, phosphorothioate modifications at nucleotides 1-3 and 6-10, and/or 2' F modifications at nucleotides 8-11, 13, 14, 17, and 18.

387. — The gRNA of any one of the preceding claims, wherein the gRNA comprises a guide region that comprises modifications at:

- i. — nucleotides 8-10;
- ii. — nucleotides 8 and 9;
- iii. — nucleotides 8 and 10; or
- iv. — nucleotides 9 and 10,

~~optionally wherein the guide region comprises 2' OMe modifications at nucleotides 1-4, phosphorothioate modifications at nucleotides 1-3 and 6-7, and/or 2' F modifications at nucleotides 11, 13, 14, 17, and 18.~~

388. ~~The gRNA of any one of the preceding claims, wherein the gRNA comprises a guide region that comprises 2' F modifications at:~~

- ~~i. nucleotides 8-10;~~
- ~~ii. nucleotides 8 and 9;~~
- ~~iii. nucleotides 8 and 10;~~
- ~~iv. nucleotides 9 and 10; or~~
- ~~v. nucleotide 8;~~

~~optionally wherein the guide region comprises 2' OMe modifications at nucleotides 1-4, phosphorothioate modifications at nucleotides 1-3 and 6-7, and/or 2' F modifications at nucleotides 11, 13, 14, 17, and 18.~~

389. ~~The gRNA of any one of the preceding claims, wherein the gRNA comprises a guide region that comprises 2' F modifications at:~~

- ~~i. nucleotides 8-10;~~
- ~~ii. nucleotides 8 and 9;~~
- ~~iii. nucleotides 8 and 10;~~
- ~~iv. nucleotides 9 and 10; or~~
- ~~v. nucleotide 8;~~

~~wherein nucleotides 8-10 do not comprise phosphorothioate modifications, and optionally wherein the guide region comprises 2' OMe modifications at nucleotides 1-4, phosphorothioate modifications at nucleotides 1-3 and 6-7, and/or 2' F modifications at nucleotides 11, 13, 14, 17, and 18.~~

390. ~~The gRNA of any one of the preceding claims, wherein the gRNA comprises a guide region that comprises 2' F modifications at nucleotides 8-10 and:~~

- ~~i. phosphorothioate modifications at 1, 2, or 3 of nucleotides 8-10;~~
- ~~ii. a phosphorothioate modification at nucleotide 8;~~
- ~~iii. a phosphorothioate modification at nucleotide 9;~~
- ~~iv. a phosphorothioate modification at nucleotide 10;~~
- ~~v. a phosphorothioate modification at nucleotides 8 and 9;~~

- ~~vi. a phosphorothioate modification at nucleotides 8 and 10;~~
- ~~vii. a phosphorothioate modification at nucleotides 9 and 10; or~~
- ~~viii. a phosphorothioate modification at nucleotides 8-10~~

~~optionally wherein the guide region comprises 2'-OMe modifications at nucleotides 1-4, phosphorothioate modifications at nucleotides 1-3 and 6-7, and/or 2'-F modifications at nucleotides 11, 13, 14, 17, and 18.~~

391. — The gRNA of any one of the preceding claims, wherein the gRNA comprises a guide region that comprises:

- ~~i. a 2'-F or phosphorothioate modification at nucleotides 5 and 6;~~
- ~~ii. a 2'-F modification at nucleotides 5 and 6;~~
- ~~iii. a phosphorothioate modification at nucleotides 5 and 6;~~
- ~~iv. a 2'-F modification at nucleotide 5 and a phosphorothioate modification at nucleotide 6; or~~
- ~~v. a 2'-F modification at nucleotide 6 and a phosphorothioate modification at nucleotide 5;~~

~~optionally wherein the guide region comprises 2'-OMe modifications at nucleotides 1-4, phosphorothioate modifications at nucleotides 1-3 and 7-10, and/or 2'-F modifications at nucleotides 8-11, 13, 14, 17, and 18.~~

392. — The gRNA of any one of the preceding claims, wherein the gRNA comprises a guide region that comprises 2'-F modifications at at least 1, 2, 3, 4, 5, or 6 of nucleotides 6-11, optionally wherein the guide region comprises 2'-OMe modifications at nucleotides 1-4, phosphorothioate modifications at nucleotides 1-3, and/or 2'-F modifications at nucleotides 13, 14, 17, and 18.

393. — The gRNA of any one of the preceding claims, wherein the gRNA comprises a guide region that comprises 2'-F modifications at at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 of nucleotides 1-4 and 6-11, optionally wherein the guide region comprises phosphorothioate modifications at nucleotides 1-3 and/or 2'-F modifications at nucleotides 13, 14, 17, and 18.

394. — The gRNA of any one of the preceding claims, wherein the gRNA comprises a guide region that comprises 2'-F modifications at nucleotides 6-11, optionally wherein the guide region comprises 2'-OMe modifications at nucleotides 1-4,

phosphorothioate modifications at nucleotides 1-3, and/or 2' F modifications at nucleotides 13, 14, 17, and 18.

395. — The gRNA of any one of the preceding claims, wherein the gRNA comprises a guide region that comprises 2' F modifications at nucleotides 1-4, optionally wherein the guide region comprises phosphorothioate modifications at nucleotides 1-3 and 6-10, and/or 2' F modifications at nucleotides 6-11, 13, 14, 17, and 18.

396. — The gRNA of any one of the preceding claims, wherein the gRNA comprises a guide region that comprises a 2' F modification at nucleotide 9 and not a phosphorothioate modification at nucleotide 9, optionally wherein the guide region comprises 2' OMe modifications at nucleotides 1-4, phosphorothioate modifications at nucleotides 1-3 and 6-8 and 10, and/or 2' F modifications at nucleotides 8, 10, 11, 13, 14, 17, and 18.

397. — The gRNA of any one of the preceding claims, wherein the gRNA comprises a guide region that does not comprise 2' F modifications at at least 1, 2, 3, 4, 5, 6, 7, or 8 of nucleotides 8-11, 13, 14, 17, and 18, optionally wherein the guide region comprises 2' OMe modifications at nucleotides 1-4 and/or phosphorothioate modifications at nucleotides 1-3 and 6-10.

398. — The gRNA of any one of the preceding claims, wherein the gRNA comprises a guide region that does not comprise 2' F modifications at nucleotides 8-11, 13, 14, 17, and 18, optionally wherein the guide region comprises 2' OMe modifications at nucleotides 1-4 and/or phosphorothioate modifications at nucleotides 1-3 and 6-10.

399. — The gRNA of any one of the preceding claims, wherein the gRNA comprises a guide region that comprises 2' OMe modifications at at least 1, 2, 3, or 4 of nucleotides 9, 11, 13, and 14, optionally wherein the guide region comprises 2' OMe modifications at nucleotides 1-4 and/or phosphorothioate modifications at nucleotides 1-3 and 6-10.

400. — The gRNA of any one of the preceding claims, wherein the gRNA comprises a guide region that comprises 2' OMe modifications at nucleotides 9, 11, 13, and 14, optionally wherein the guide region comprises 2' OMe modifications at nucleotides 1-4 and/or phosphorothioate modifications at nucleotides 1-3 and 6-10.

401. ~~—The gRNA of any one of the preceding claims, wherein the gRNA comprises a guide region that comprises phosphorothioate modifications at one or both of nucleotides 8 and 10, optionally wherein the guide region comprises 2' OMe modifications at nucleotides 1-4, phosphorothioate modifications at nucleotides 1-3 and 6-7, and/or 2' F modifications at nucleotides 8-11, 13, 14, 17, and 18.~~
402. ~~—The gRNA of any one of the preceding claims, wherein the gRNA comprises a guide region that comprises modifications at at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or all of the following nucleotides: 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 13, 14, 17, and 18, optionally wherein the modifications are 2' OMe, 2' fluoro, or phosphorothioate modifications.~~
403. ~~—The gRNA of any one of the the preceding claims, wherein the gRNA comprises a guide region that comprises modifications at nucleotides 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 13, 14, 17, and 18, optionally wherein the modifications are 2' OMe, 2' fluoro, or phosphorothioate modifications.~~
404. ~~—The gRNA of any one of the preceding claims, wherein 2' OMe modifications are not present in the guide region at nucleotides 6-11 and 13-end.~~
405. ~~—The gRNA of any one of the preceding claims, wherein 2' fluoro modifications are not present in the guide region at nucleotides 1-7, 15, 16, and 19-end.~~
406. ~~—The gRNA of any one of the preceding claims, wherein phosphorothioate modifications are not present in the guide region at nucleotides 4, 5, 11-14, 17, and 18.~~
407. ~~—The gRNA of any one of the preceding claims, wherein the guide region comprises an unmodified nucleotide 20.~~
408. ~~—The gRNA of any one of the preceding claims, wherein the guide region consists of 20 nucleotides.~~
409. ~~—The gRNA of any one of the preceding claims, wherein the guide region comprises a YA site at nucleotides 5-6 and a modification at nucleotide 5.~~
410. ~~—The gRNA of any one of the preceding claims, wherein the guide region comprises a YA site at nucleotides 12-13 and a modification at nucleotide 12.~~

411. ~~The gRNA of any one of the preceding claims, wherein the guide region comprises a YA site at nucleotides 15-16 and a modification at nucleotide 15.~~
412. ~~The gRNA of any one of the preceding claims, wherein the guide region comprises a YA site at nucleotides 16-17 and a modification at nucleotide 16.~~
413. ~~The gRNA of any one of the preceding claims, wherein the guide region comprises a YA site at nucleotides 19-20 and a modification at nucleotide 19.~~
414. ~~The gRNA of any one of the preceding claims, wherein the guide region does not comprise a YA site at nucleotides 5-6 and nucleotide 5 is unmodified.~~
415. ~~The gRNA of any one of the preceding claims, wherein the guide region does not comprise a YA site at nucleotides 12-13 and nucleotide 12 is unmodified.~~
416. ~~The gRNA of any one of the preceding claims, wherein the guide region does not comprise a YA site at nucleotides 15-16 and nucleotide 15 is unmodified.~~
417. ~~The gRNA of any one of the preceding claims, wherein the guide region does not comprise a YA site at nucleotides 16-17 and nucleotide 16 is unmodified.~~
418. ~~The gRNA of any one of the preceding claims, wherein the guide region does not comprise a YA site at nucleotides 19-20 and nucleotide 19 is unmodified.~~
419. ~~The gRNA of any one of the preceding claims, wherein the gRNA comprises a guide region that comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or all of the following:~~
- ~~i. 2'-OMe and phosphorothioate modifications at nucleotide 1;~~
 - ~~ii. 2'-OMe and phosphorothioate modifications at nucleotide 2;~~
 - ~~iii. 2'-OMe and phosphorothioate modifications at nucleotide 3;~~
 - ~~iv. a 2'-OMe modification at nucleotide 4;~~
 - ~~v. a phosphorothioate modification at nucleotide 6;~~
 - ~~vi. a phosphorothioate modification at nucleotide 7;~~
 - ~~vii. 2'-fluoro and phosphorothioate modifications at nucleotide 8;~~
 - ~~viii. 2'-fluoro and phosphorothioate modifications at nucleotide 9;~~
 - ~~ix. 2'-fluoro and phosphorothioate modifications at nucleotide 10;~~
 - ~~x. a 2'-fluoro modification at nucleotide 11;~~
 - ~~xi. a 2'-fluoro modifications at nucleotide 13;~~

- xii. ~~a 2' fluoro modifications at nucleotide 14;~~
- xiii. ~~a 2' fluoro modifications at nucleotide 17; and~~
- xiv. ~~a 2' fluoro modifications at nucleotide 18.~~

420. ~~—The gRNA of any one of the preceding claims, wherein the guide region comprises each of the modifications set forth in the preceding claim.~~

421. ~~—The gRNA of any one of the preceding claims, wherein the guide region comprises at least 1, 2, 3, or 4 of the following:~~

- i. ~~a 2' OMe modification at nucleotide 5 if nucleotides 5 and 6 form a YA site;~~
- ii. ~~a 2' OMe modification at nucleotide 12 if nucleotides 12 and 13 form a YA site;~~
- iii. ~~a phosphorothioate or 2' H modification at nucleotide 15 if nucleotides 15 and 16 form a YA site;~~
- iv. ~~a phosphorothioate modification at nucleotide 16 if nucleotides 16 and 17 form a YA site; and~~
- v. ~~a phosphorothioate or 2' fluoro modification at nucleotide 19 if nucleotides 19 and 20 form a YA site.~~

422. ~~—The gRNA of any one of the preceding claims, wherein the guide region comprises a YA site at nucleotides 5-6 and a 2' OMe modification at nucleotide 5.~~

423. ~~—The gRNA of any one of the preceding claims, wherein the guide region comprises a YA site at nucleotides 12-13 and a 2' OMe modification at nucleotide 12.~~

424. ~~—The gRNA of any one of the preceding claims, wherein the guide region comprises a YA site at nucleotides 15-16 and a phosphorothioate modification at nucleotide 15.~~

425. ~~—The gRNA of any one of the preceding claims, wherein the guide region comprises a YA site at nucleotides 16-17 and a phosphorothioate modification at nucleotide 16.~~

426. ~~—The gRNA of any one of the preceding claims, wherein the guide region comprises a YA site at nucleotides 19-20 and a phosphorothioate modification at nucleotide 19.~~

427. ~~—The gRNA of any one of the preceding claims, wherein the guide region comprises a 2' fluoro modification at nucleotide 19.~~
428. ~~—The gRNA of any one of the preceding claims, wherein the guide region comprises an unmodified nucleotide 15 or only a phosphorothioate modification at nucleotide 15.~~
429. ~~—The gRNA of any one of the preceding claims, wherein the guide region comprises an unmodified nucleotide 16 or only a phosphorothioate modification at nucleotide 16.~~
430. ~~—The gRNA of any one of the preceding claims, wherein the gRNA is an sgRNA comprising a conserved portion of an sgRNA comprising a hairpin region, wherein the hairpin region lacks at least 5-10 nucleotides.~~
431. ~~—The gRNA of claim 430, wherein the at least 5-10 lacking nucleotides are consecutive.~~
432. ~~—The gRNA of claim 430 or 431, wherein the at least 5-10 lacking nucleotides:~~
- ~~i. are within hairpin 1;~~
 - ~~ii. are within hairpin 1 and the “N” between hairpin 1 and hairpin 2;~~
 - ~~iii. are within hairpin 1 and the two nucleotides immediately 3' of hairpin 1;~~
 - ~~iv. include at least a portion of hairpin 1;~~
 - ~~v. are within hairpin 2;~~
 - ~~vi. include at least a portion of hairpin 2;~~
 - ~~vii. are within hairpin 1 and hairpin 2;~~
 - ~~viii. include at least a portion of hairpin 1 and include the “N” between hairpin 1 and hairpin 2;~~
 - ~~ix. include at least a portion of hairpin 2 and include the “N” between hairpin 1 and hairpin 2;~~
 - ~~x. include at least a portion of hairpin 1, include the “N” between hairpin 1 and hairpin 2, and include at least a portion of hairpin 2;~~
 - ~~xi. are within hairpin 1 or hairpin 2, optionally including the “N” between hairpin 1 and hairpin 2;~~
 - ~~xii. are consecutive;~~
 - ~~xiii. are consecutive and include the “N” between hairpin 1 and hairpin 2;~~

- ~~xiv. are consecutive and span at least a portion of hairpin 1 and a portion of hairpin 2;~~
- ~~xv. are consecutive and span at least a portion of hairpin 1 and the “N” between hairpin 1 and hairpin 2; or~~
- ~~xvi. are consecutive and span at least a portion of hairpin 1 and two nucleotides immediately 3’ of hairpin 1.~~

~~433. The gRNA of any one of claims 430-432, wherein the at least 5-10 nucleotides comprise nucleotides 54-61 of SEQ ID NO:400, nucleotides 53-60 of SEQ ID NO:400; or nucleotides 54-58 of SEQ ID NO:400, optionally wherein the sgRNA comprises modifications at least H1-1 to H1-5 and H2-1 to H2-12.~~

~~434. The gRNA of any one of claims 430-433, wherein the at least 5-10 nucleotides:~~

- ~~i. consist of 5-10 nucleotides;~~
- ~~ii. consist of 6-10 nucleotides;~~
- ~~iii. consist of 5 nucleotides;~~
- ~~iv. consist of 6 nucleotides;~~
- ~~v. consist of 7 nucleotides;~~
- ~~vi. consist of 8 nucleotides;~~
- ~~vii. consist of 9 nucleotides;~~
- ~~viii. consist of 10 nucleotides;~~
- ~~ix. consist of 5-10 consecutive nucleotides;~~
- ~~x. consist of 6-10 consecutive nucleotides;~~
- ~~xi. consist of 5 consecutive nucleotides;~~
- ~~xii. consist of 6 consecutive nucleotides;~~
- ~~xiii. consist of 7 consecutive nucleotides;~~
- ~~xiv. consist of 8 consecutive nucleotides;~~
- ~~xv. consist of 9 consecutive nucleotides; or~~
- ~~xvi. consist of 10 consecutive nucleotides.~~

~~435. The gRNA of claim 434, wherein the at least 5-10 nucleotides comprise nucleotides 54-61 of SEQ ID NO:400, nucleotides 53-60 of SEQ ID NO:400; or nucleotides 54-58 of SEQ ID NO:400, optionally wherein the sgRNA comprises modifications at least H1-1 to H1-5 and H2-1 to H2-12.~~

436. ~~The gRNA of any one of claims 430-435, wherein the at least 5-10 nucleotides:~~
- ~~i. comprise nucleotides 54-61 of SEQ ID NO:400;~~
 - ~~ii. comprise nucleotides 53-60 of SEQ ID NO:400;~~
 - ~~iii. comprise nucleotides 54-58 of SEQ ID NO:400.~~
 - ~~iv. consist of nucleotides 54-61 of SEQ ID NO:400;~~
 - ~~v. consist of nucleotides 53-60 of SEQ ID NO:400; or~~
 - ~~vi. consist of nucleotides 54-58 of SEQ ID NO:400.~~
437. ~~The gRNA of any one of the preceding claims, wherein the gRNA comprises modifications and/or unmodified nucleotides at at least 15 of nucleotides 1-20 from the 5' end of the 5' terminus that match the modification pattern at nucleotides 1-20 of any one of the gRNAs of SEQ ID NOs: 1-54, 201-254, 301-354, 401-535, 601, 607-732, 801, 807-932, 1001, 1007-1132, 1205-1212, 1322-1406, 1417-1501, 1511-1596, 3018-3059, 3063-3104, 3108-3149, 3153-3194, 3198-3239, 3243-3284, 3295-3341, 3343-3385, or 3388-3430.~~
438. ~~The gRNA of any one of the preceding claims, wherein the gRNA comprises modifications and/or unmodified nucleotides at at least 16 of nucleotides 1-20 from the 5' end of the 5' terminus that match the modification pattern at nucleotides 1-20 of any one of the gRNAs of SEQ ID NOs: 1-54, 201-254, 301-354, 401-535, 601, 607-732, 801, 807-932, 1001, 1007-1132, 1205-1212, 1322-1406, 1417-1501, 1511-1596, 3018-3059, 3063-3104, 3108-3149, 3153-3194, 3198-3239, 3243-3284, 3295-3341, 3343-3385, or 3388-3430.~~
439. ~~The gRNA of any one of the preceding claims, wherein the gRNA comprises modifications and/or unmodified nucleotides at at least 17 of nucleotides 1-20 from the 5' end of the 5' terminus that match the modification pattern at nucleotides 1-20 of a gRNA, wherein the gRNA is any one of SEQ ID NOs: 1-54, 201-254, 301-354, 401-535, 601, 607-732, 801, 807-932, 1001, 1007-1132, 1205-1212, 1322-1406, 1417-1501, 1511-1596, 3018-3059, 3063-3104, 3108-3149, 3153-3194, 3198-3239, 3243-3284, 3295-3341, 3343-3385, or 3388-3430.~~
440. ~~The gRNA of any one of the preceding claims, wherein the gRNA comprises modifications and/or unmodified nucleotides at at least 18 of nucleotides 1-20 from the 5' end of the 5' terminus that match the modification pattern at nucleotides 1-20 of any one of the gRNAs of SEQ ID NOs: 1-54, 201-254, 301-354, 401-535, 601,~~

~~607-732, 801, 807-932, 1001, 1007-1132, 1205-1212, 1322-1406, 1417-1501, 1511-1596, 3018-3059, 3063-3104, 3108-3149, 3153-3194, 3198-3239, 3243-3284, 3295-3341, 3343-3385, or 3388-3430.~~

441. ~~—The gRNA of any one of the preceding claims, wherein the gRNA comprises modifications and/or unmodified nucleotides at at least 19 of nucleotides 1-20 from the 5' end of the 5' terminus that match the modification pattern at nucleotides 1-20 of any one of the gRNAs of SEQ ID NOs: 1-54, 201-254, 301-354, 401-535, 601, 607-732, 801, 807-932, 1001, 1007-1132, 1205-1212, 1322-1406, 1417-1501, 1511-1596, 3018-3059, 3063-3104, 3108-3149, 3153-3194, 3198-3239, 3243-3284, 3295-3341, 3343-3385, or 3388-3430.~~

442. ~~—The gRNA of any one of the preceding claims, wherein the gRNA comprises modifications and/or unmodified nucleotides at nucleotides 1-20 from the 5' end of the 5' terminus that match the modification pattern at nucleotides 1-20 of any one of the gRNAs of SEQ ID NOs: 1-54, 201-254, 301-354, 401-535, 601, 607-732, 801, 807-932, 1001, 1007-1132, 1205-1212, 1322-1406, 1417-1501, 1511-1596, 3018-3059, 3063-3104, 3108-3149, 3153-3194, 3198-3239, 3243-3284, 3295-3341, 3343-3385, or 3388-3430.~~

443. ~~—The gRNA of any one of the preceding claims, wherein the gRNA comprises a modification pattern that matches at least 75% of the modification pattern of any one of the gRNAs of SEQ ID NOs: 1-54, 201-254, 301-354, 401-535, 601, 607-732, 801, 807-932, 1001, 1007-1132, 1205-1212, 1322-1406, 1417-1501, 1511-1596, 3018-3059, 3063-3104, 3108-3149, 3153-3194, 3198-3239, 3243-3284, 3295-3341, 3343-3385, or 3388-3430.~~

444. ~~—The gRNA of any one of the preceding claims, wherein the gRNA comprises the modification pattern of any one of the gRNAs in Table 1, wherein the modification pattern is the same as any one of the gRNAs of SEQ ID NOs: 1-54, 201-254, 301-354, 401-535, 601, 607-732, 801, 807-932, 1001, 1007-1132, 1205-1212, 1322-1406, 1417-1501, 1511-1596, 3018-3059, 3063-3104, 3108-3149, 3153-3194, 3198-3239, 3243-3284, 3295-3341, 3343-3385, or 3388-3430.~~

445. ~~—The gRNA of any one of claims 437-444, further comprising a sequence having at least 75% identity to the sequence of nucleotides 21-end of the gRNA.~~

446. ~~—The gRNA of any one of claims 437-444, further comprising a sequence having at least 80% identity to the sequence of nucleotides 21-end of the gRNA.~~

447. — The gRNA of any one of claims 437-444, further comprising a sequence having at least 85% identity to the sequence of nucleotides 21-end of the gRNA.
448. — The gRNA of any one of claims 437-444, further comprising a sequence having at least 90% identity to the sequence of nucleotides 21-end of the gRNA.
449. — The gRNA of any one of claims 437-444, further comprising a sequence having at least 95% identity to the sequence of nucleotides 21-end of the gRNA.
450. — The gRNA of any one of claims 437-444, further comprising a sequence having at least 98% identity to the sequence of nucleotides 21-end of the gRNA.
451. — The gRNA of any one of claims 437-444, further comprising a sequence having 100% identity to the sequence of nucleotides 21-end of the gRNA.
452. — An LNP composition comprising a gRNA of any one of the preceding claims.
453. — A composition comprising a gRNA of any one of claims 1-451 associated with a lipid nanoparticle (LNP).
454. — A composition comprising the gRNA of any one of claims 1-451, or the composition of any one of claims 452-453, further comprising a nuclease or an mRNA which encodes the nuclease.
455. — The composition of claim 454, wherein the nuclease is a Cas protein.
456. — The composition of claim 455, wherein the Cas protein is a Cas9.
457. — The composition of claim 456, wherein the Cas9 is an *S. pyogenes* Cas9 or an *S. aureus* Cas9.
458. — The composition of any one of claims 453-457, wherein the nuclease is a nickase or a dCas.
459. — The composition of any one of claims 453-458, wherein the nuclease is modified.
460. — The composition of claim 459 wherein the modified nuclease comprises a nuclear localization signal (NLS).
461. — The composition of any one of claims 452-460, comprising an mRNA which encodes the nuclease.
462. — The composition of claim 461, wherein the mRNA comprises the sequence of any one of SEQ ID NOs: 3499-3527 or 3529-3546.
463. — A pharmaceutical formulation comprising the gRNA of any one of claims 1-451 or the composition of any one of claims 452-462 and a pharmaceutically acceptable carrier.

464. ~~— 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 1-451;~~
 - ~~ii. the composition of any one of claims 452-462; and~~
 - ~~iii. the pharmaceutical formulation of claim 463.~~
465. ~~— The method of claim 464, wherein the method results in an insertion or deletion in a gene.~~
466. ~~— The method of claim 464 or claim 465, 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.~~
467. ~~— The gRNA of any one of claims 1-451, the composition of claims 452-462, or the pharmaceutical formulation of claim 463 for use in preparing a medicament for treating a disease or disorder.~~
468. ~~— Use of the gRNA of any one of claims 1-451, the composition of claims 452-462, or the pharmaceutical formulation of claim 463 in the manufacture of a medicament for treating a disease or disorder.~~

Claims

1. An invention not claimed in any divisional application.