Claims

1. A single guide RNA (sgRNA) comprising

(i) an upper stem region and a hairpin region,

wherein (a) each nucleotide in the upper stem region is modified with 2′-O-Me;

(b) the hairpin region comprises a hairpin 1 region and a hairpin 2 region and each nucleotide in the hairpin 2 region is modified with 2′-O-Me; or both (a) and (b); and

(ii) a 5′ end modification comprising at least two phosphorothioate (PS) linkages within the first seven nucleotides at the 5′ end of the 5′ terminus.

2. The sgRNA of claim 1, wherein

a. the sgRNA comprises one or more modifications in the upper stem region; and/or

b. the sgRNA comprises one or more modifications in the hairpin 1 region; and/or

c. the sgRNA comprises one or more modifications in the hairpin 2 region.

3. The sgRNA of claim 1, wherein the hairpin region comprises a hairpin 1 region and a hairpin 2 region, and the sgRNA comprises one or more modifications in each of the upper stem region, the hairpin 1 region, and the hairpin 2 region.

4. The sgRNA of claim 1, wherein the hairpin region comprises a hairpin 1 region and a hairpin 2 region, and the sgRNA comprises a modified nucleotide between the hairpin 1 and hairpin 2 regions.

5. The sgRNA of claim 1, further comprising a lower stem region comprising a modification.

6. The sgRNA of claim 1, further comprising a 3′ terminus region comprising a modification.

7. The sgRNA of claim 6, further comprising a 3′ end modification in the 3′ terminus.

8. The sgRNA of claim 7, wherein at least two of the last four nucleotides at the 3′ end of the 3′ terminus are modified, optionally with 2′-O-Me, 2′-F, or 2′-O-moe.

9. The sgRNA of claim 7, further comprising phosphorothioate (PS) bonds between one or more of the last four nucleotides at the 3′ end of the 3′ terminus.

10. The sgRNA of claim 1, further comprising a bulge region comprising a modification and/or a *nexus* region comprising a modification.

11. The sgRNA of claim 1, wherein at least the first three nucleotides at the 5′ end of the 5′ terminus, and the last three nucleotides at the 3′ end of the 3′ terminus are modified.

12. The sgRNA of claim 1, wherein the first four nucleotides at the 5′ end of the 5′ terminus, and the last four nucleotides at the 3′ end of the 3′ terminus are linked with phosphorothioate (PS) bonds.

13. The sgRNA of claim 12, wherein the end modifications comprise 2′-O-Me or 2′-F.

14. The sgRNA of claim 1, wherein the first four nucleotides at the 5′ end of the 5′ terminus 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 5′ terminus and the last three or four nucleotides at the 3′ end of the 3′ terminus comprise 2′-O-Me modifications.

15. The sgRNA of claim 1, 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′-O-Me, 2′-F, and/or 2′-O-moe modifications.

16. The sgRNA of claim 1, wherein the lower stem region comprises nucleotides LS1 to LS12 from the 5′ end to the 3′ end thereof, and LS1, LS6, LS7, LS8, LS11, and/or LS12 are modified with 2′-O-Me and/or wherein the sgRNA comprises a bulge region and each nucleotide in the bulge region is modified with 2′-O-Me and/or wherein at least 50% of the nucleotides in the bulge region are modified with 2′-O-Me and/or wherein each nucleotide in the upper stem region is modified with 2′-O-Me and/or wherein the sgRNA further comprises a *nexus* region comprising nucleotides N1 through N18 from the 5′ end to the 3′ end thereof, and N16, N17, and/or N18 in the *nexus* region are modified with 2′-O-Me and/or wherein N15, N16, N17, and/or N18 in the *nexus* region are modified.

17. The sgRNA of claim 16, wherein the modifications in the *nexus* region are selected from 2′-O-Me and 2′F.

18. The sgRNA of claim 1, wherein each of the nucleotides in the hairpin 1 region and/or in the hairpin 2 region are modified with 2′-O-Me.

19. A composition comprising an sgRNA of claim 1, further comprising a lipid nanoparticle (LNP) and/or further comprising a nuclease or an mRNA which encodes the nuclease.

20. A pharmaceutical formulation comprising the sgRNA of claim 1 and a pharmaceutically acceptable carrier.

21. An *in vitro* or *ex vivo* method of modifying a target DNA comprising, delivering a Cas protein or a nucleic acid encoding a Cas protein, and the sgRNA of claims 1-18 and 22-27 or the composition of claim 19 to a cell.

22. The sgRNA of claim 1, wherein the upper stem region comprises US1 to US12 from the 5′ end to the 3′ end thereof, and the hairpin region comprises a hairpin 1 region and a hairpin 2 region, wherein the hairpin 1 region comprises H1-1 to H1-12 from the 5′ end to the 3′ end thereof and the hairpin 2 region comprises H2-1 to H2-15 from the 5′ end to the 3′ end thereof, and wherein the sgRNA is a sgRNA comprises 2′-O-Me modified nucleotides consisting of 2′-O-Me modified nucleotides at:

a. the first three nucleotides at the 5′ end of the 5′ terminus;

b. each of nucleotides US1, US2, US3, US4, US5, US6, US7, USB, US9, US10, US11 and US12;

c. each of nucleotides H1-1, H1-2, H1-3, H1-4, H1-5, H1-6, H1-7, H1-8, H1-9, H1-10, H1-11, and H1-12;

d. the nucleotide between hairpin 1 and hairpin 2;

e. each of nucleotides 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, and H2-15; and

f. the last four nucleotides at the 3′ end of the 3′ terminus; and

three PS bonds linking the first four nucleotides at the 5′ end of the 5′ terminus and three PS bonds linking the last four nucleotides at the 3′ end of the 3′ terminus.

23. The sgRNA of claim 1, wherein the sgRNA comprises any of SEQ ID NOs: 235, 236, 240, 265-283, 309-327, or 331.

24. The sgRNA of claim 1, wherein the sgRNA comprises SEQ ID NO: 242 or 358.

25. The sgRNA of claim 1, wherein the sgRNA comprises 2′-O-Me modified nucleotides consisting of 2′-O-Me modified nucleotides at:

a. the first three nucleotides at the 5′ end of the 5′ terminus;

b. each nucleotide in the upper stem region;

c. each nucleotide in the hairpin 1 region;

d. the nucleotide between hairpin 1 and hairpin 2;

e. each nucleotide in the hairpin 2 region; and

f. the last four nucleotides at the 3′ end of the 3′ terminus.

26. The sgRNA of claim 1, wherein the first four nucleotides at the 5′ end of the 5′ terminus 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 5′ terminus and the last three nucleotides at the 3′ end of the 3′ terminus are modified with 2′-O-Me, and wherein each nucleotide in the upper stem region are modified with 2′-O-Me.

27. The sgRNA of claim 1, wherein the first four nucleotides at the 5′ end of the 5′ terminus and the last four nucleotides at the 3′ end of the 3′terminus are linked with a PS bond, wherein the first three nucleotides at the 5′ end of the 5′ terminus and the last four nucleotides at the 3′ end of the 3′ terminus are modified with 2′-O-Me, and wherein each nucleotide in the upper stem region are modified with 2′-O-Me.

28. The sgRNA of any one of claims 1-18 and 22-27, the composition of claim 19, or the pharmaceutical formulation of claim 20 for use in therapy.

29. The sgRNA of any one of claims 1-18 and 22-27, the composition of claim 19, or the pharmaceutical formulation of claim 20 for use as a medicament.