

CLAIMS

What is claimed is:

1. A prime editing composition comprising a first polynucleotide encoding a DNA binding domain and a second polynucleotide encoding a DNA polymerase domain, wherein the second polynucleotide comprises a sequence (i) having at least 80% sequence identity to SEQ ID NOs: 83 or 84, or (ii) having at least 80% sequence identity to SEQ ID NOs: 91 or 92.
2. The prime editing composition of claim 1, wherein the second polynucleotide comprises a sequence having at least about 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 83 or 84, optionally wherein the second polynucleotide comprises a sequence having at least 93% sequence identity to SEQ ID NOs: 83 or 84.
3. The prime editing composition of claim 1, wherein the second polynucleotide comprises a sequence having at least about 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 91 or 92, optionally wherein the second polynucleotide comprises a sequence having at least 93% sequence identity to SEQ ID NOs: 91 or 92.
4. The prime editing composition of any one of claims 1-3, wherein the first polynucleotide encodes a CRISPR associated (Cas) protein, optionally wherein the Cas protein is a Cas9 protein that comprises a mutation in a HNH domain, optionally wherein the Cas9 protein comprises a H840A mutation compared to SEQ ID NO: 2.
5. The prime editing composition of any one of claims 1-4, wherein the first polynucleotide and the second polynucleotide are connected in a fusion polynucleotide, optionally wherein the first polynucleotide and the second polynucleotide are connected by a sequence that encodes a peptide linker, optionally wherein the polynucleotide encoding the peptide linker comprises the sequence of SEQ ID NOs: 235, 236 or 633-636, optionally wherein the first polynucleotide is connected to the 5' end of the second polynucleotide.

6. The prime editing composition of claim 5, wherein the fusion polynucleotide comprises a sequence having at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 81, 82, 108, 109, 120, 121, 126, 127, 132, 133, 138, 139, 144, 145, 150, 151, 156, 157, 162, 163, 168, 169, 174, 175, 180, 181, 186, 187, 192, 193, 198, 199, 204, 205, 210, 211, 216, 217, 222, 223, 228, 229, 241, and 242, optionally wherein the selected sequence is SEQ ID NOs: 81 or 82.
7. The prime editing composition of claim 5, wherein the fusion polynucleotide comprises a sequence having at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 89, 90, 102, 103, 114, 115, 123, 124, 129, 130, 135, 136, 141, 142, 147, 148, 153, 154, 159, 160, 165, 166, 171, 172, , 177, 178, 183, 184, 189, 190, 195, 196, 201, 202, 207, 208, 213, 214, 219, 220, 225, 226, 231, and 232, optionally wherein the selected sequence is SEQ ID NOs: 89 or 90.
8. The prime editing composition of any one of claims 1-7, wherein the first polynucleotide, the second polynucleotide, or both further comprises a sequence encoding a nuclear localization signal (NLS), optionally wherein the sequence encoding the NLS comprises the sequence of SEQ ID NOs: 239 or 240 and is connected to the 3' end of the second polynucleotide.
9. The prime editing composition of claim 8, wherein the fusion polynucleotide comprises a sequence having at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 79, 80, 94, 95, 106, 107, 118, 119, 233, and 234, optionally wherein the selected sequence is SEQ ID NOs: 79 or 80.
10. The prime editing composition of claim 8, wherein the fusion polynucleotide comprises a sequence having at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 87, 88, 97, 98, 100, 101, 112, and 113, optionally wherein the selected sequence is SEQ ID NOs: 87 or 88.

11. The prime editing composition of any one of claims 5-10, wherein the fusion polynucleotide further comprises a stop codon at the 3' end, optionally wherein the fusion polynucleotide comprises a 5' untranslated region (UTR) and a 3' UTR.
12. The prime editing composition of claim 11, wherein the 5' UTR comprises the sequence of SEQ ID NO: 268, and the 3' UTR comprises the sequence of SEQ ID NO: 273.
13. The prime editing composition of any one of claims 5-12, wherein the fusion polynucleotide comprises a sequence having at least about 90% sequence identity to SEQ ID NOs: 276, 277, 278, 279, 282, 283, 284, or 285.
14. The editing composition of claim 13, wherein the fusion polynucleotide comprises the sequence of SEQ ID NOs: 279 or 283.
15. The prime editing composition of any one of claims 11-14, wherein the fusion polynucleotide comprises the sequence of SEQ ID NOs: 274, 275, 592, or 593.
16. The prime editing composition of any one of claims 11-14, wherein the fusion polynucleotide comprises the sequence of SEQ ID NOs: 280, 281, 594, or 595.
17. The prime editing composition of any one of claims 5-16, wherein the fusion polynucleotide encodes a fusion polypeptide comprising an amino acid sequence having at least about 90% sequence identity to SEQ ID NOs: 77 or 85.
18. The prime editing composition of claim 17, wherein the fusion polynucleotide encodes a fusion polypeptide comprising an amino acid sequence of SEQ ID NOs: 77 or 85.
19. The prime editing composition of any one of claims 1-18, wherein the first polynucleotide, the second polynucleotide, and/or the fusion polynucleotide comprises DNA.
20. The prime editing composition of any one of claims 1-18, wherein the first polynucleotide, the second polynucleotide, and/or the fusion polynucleotide comprises mRNA.
21. The prime editing composition of any one of claims 1-18, wherein the sequence identities are determined by Needleman-Wunsch alignment of two sequences with Gap Costs set to Existence: 11 Extension: 1, wherein percent identity is calculated by dividing the number of identities by the length of the alignment.
22. The prime editing composition of any one of the claims 1-21, wherein the prime editing composition further comprises a prime editing guide RNA (PEgRNA) or a polynucleotide encoding the PEgRNA, optionally wherein the prime editing composition further comprises a nick guide RNA (ngRNA) or a polynucleotide encoding the ngRNA.

23. A vector comprising one or more of the polynucleotides of the prime editing composition of any one of claims 1-22, optionally wherein the vector is an AAV vector.
24. A lipid nanoparticle (LNP) comprising the prime editing composition of any one of claims 1-22.
25. A pharmaceutical composition comprising the prime editing composition of any one of claims 1-22 or the vector of claim 23, or the LNP of claim 24.
26. An ex vivo or in vitro method of editing a target gene, the method comprising contacting the target gene with the prime editing composition of any one of claims 1-22.
27. The method of claim 26, wherein the target gene is in a cell.
28. The method of claim 27, wherein the cell is a human cell.
29. The method of claim 27, wherein the cell is a (CD34+) hematopoietic stem cell or a hematopoietic stem progenitor cell.