CLAIMS

What is claimed is:

1. A composition comprising:

(i) a first nucleotide sequence encoding a N-terminal portion of a Cas9 protein fused at its C-terminus to an intein-N; and

(ii) a second nucleotide sequence encoding an intein-C fused to the N-terminus of a C-terminal portion of the Cas9 protein,

wherein the first nucleotide sequence or second nucleotide sequence is operably linked to a nucleotide sequence encoding at least one bipartite nuclear localization signal.

2. The composition of claim 1, wherein the N-terminal portion of the Cas9 protein comprises a portion of any one of SEQ ID NOs: 1-275 and 394-397 that corresponds to amino acids 1-573 or 1-637 of SEQ ID NO: 1.

3. The composition of claim 1 or claim 2, wherein the C-terminal portion of the Cas9 protein comprises a portion of any one of SEQ ID NOs: 1-275 and 394-397 that corresponds to amino acids 574-1368 or 638-1368 of SEQ ID NO: 1.

4. The composition of any one of claims 1-3, wherein the intein-N comprises the amino acid sequence as set forth in any one of SEQ ID NOs: 350-351 amd 354-355.

5. The composition of any one of claims 1-4, wherein the intein-C comprises the amino acid sequence as set forth in any one of SEQ ID NOs: 352-353 and 356-357.

6. The composition of any one of claims 1-5, wherein the first nucleotide sequence or the second nucleotide sequence further comprises a nucleotide encoding a guide RNA (gRNA) operably linked to a promoter.

7. The composition of any one of claims 1-6, wherein the first nucleotide sequence or the second nucleotide sequence further comprises a transcriptional terminator.

8. The composition of claim 7, wherein the transcriptional terminator is the transcriptional terminator from a bGH gene, hGH gene, or SV40 gene.

9. The composition of claim 8, wherein the transcriptional terminator is the transcriptional terminator from a bGH gene.

10. The composition of any one of claims 1-9, wherein the first nucleotide sequence or the second nucleotide sequence further comprises a woodchuck hepatitis posttranscriptional regulatory element (WPRE) inserted 5′ of the transcriptional terminator.

11. The composition of any one of claims 1-10, wherein the bipartite nuclear localization signal comprises an amino acid sequence selected from the group consisting of: KRPAATKKAGQAKKKK (SEQ ID NO: 344), KKTELQTTNAENKTKKL(SEQ ID NO: 345), KRGINDRNFWRGENGRKTR(SEQ ID NO: 346), and RKSGKIAAIVVKRPRK(SEQ ID NO: 347).

12. The composition of claim 11, wherein the bipartite nuclear localization signal comprises the amino acid sequence as set forth in SEQ ID NO: 344.

13. The composition of any one of claims 1-12, wherein the Cas9 protein is a catalytically inactive Cas9 (dCas9) or a Cas9 nickase (nCas9), and wherein the first nucleotide sequence of (i) further comprises a nucleotide sequence encoding a nucleobase modifying enzyme fused to the N-terminus of the N-terminal portion of the Cas9 protein.

14. The composition of any one of claims 1-12, wherein the Cas9 protein is a catalytically inactive Cas9 (dCas9) or a Cas9 nickase (nCas9), and wherein the second nucleotide sequence of (ii) further comprises a nucleotide sequence encoding a nucleobase modifying enzyme fused to the C-terminus of the C-terminal portion of the Cas9 protein.

15. The composition of claim 13 or 14, wherein the nucleobase modifying enzyme is a deaminase.

16. The composition of claim 15, wherein the deaminase is a cytosine deaminase.

17. The composition of claim 15, wherein the deaminase is an adenosine deaminase.

18. The composition of any one of claims 14-17, wherein the second nucleotide sequence of (ii) further comprises a nucleotide sequence encoding a uracil glycosylase inhibitor (UGI) at the 3′ end of the second nucleotide sequence.

19. The composition of any one of claims 13-17, wherein the first nucleotide sequence of (i) further comprises a nucleotide sequence encoding a uracil glycosylase inhibitor (UGI) at the 5′ end of the first nucleotide sequence.

20. The composition of claim 18 or 19, wherein the UGI comprises the amino acids sequence of SEQ ID NOs: 299-302.

21. The composition of any one of claims 1-20, wherein the first nucleotide sequence and the second nucleotide sequence are on different vectors.

22. The composition of claim 21, wherein each of the different vectors is a genome of a recombinant adeno-associated virus (rAAV).

23. The composition of claim 22, wherein each vector is packaged in a rAAV particle.

24. A composition, comprising:

(i) a first recombinant adeno associated virus (rAAV) particle comprising a first nucleotide sequence encoding a N-terminal portion of a Cas9 protein fused at its C-terminus to an intein-N; and

(ii) a second recombinant adeno associated virus (rAAV) particle comprising a second nucleotide sequence encoding an intein-C fused to the N-terminus of a C-terminal portion of the Cas9 protein,

wherein the first nucleotide sequence or second nucleotide sequence is operably linked to a nucleotide sequence encoding at least one bipartite nuclear localization signal.

25. A cell comprising the composition of any one of claims 1-24.

26. The cell of claim 25, wherein the N-terminal portion of the Cas9 protein and the C-terminal portion of the Cas9 protein are joined together to form the Cas9 protein.

27. The cell of claim 25 or claim 26, wherein the cell is a prokaryotic cell.

28. The cell of claim 27, wherein the cell is a bacterial cell.

29. The cell of claim 25 or 26, wherein the cell is a eukaryotic cell.

30. The cell of claim 29, wherein the cell is a yeast cell, a plant cell, or a mammalian cell.

31. The cell of claim 29, wherein the cell is a human cell.

32. A kit comprising the composition of any one of claims 1-24.

33. A composition comprising:

(i) a first nucleotide sequence encoding a N-terminal portion of a nucleobase editor fused at its C-terminus to an intein-N; and

(ii) a second nucleotide sequence encoding an intein-C fused to the N-terminus of a C-terminal portion of the nucleobase editor.

34. The composition of claim 33, wherein the intein-N comprises the amino acid sequence as set forth in any one of SEQ ID NO: 350-351 and 354-355.

35. The composition of claim 33 or 34, wherein the intein-C comprises the amino acid sequence as set forth in any one of SEQ ID NO: 352-353 and 356-357.

36. The composition of any one of claims 33-35, wherein the first nucleotide sequence or the second nucleotide sequence further comprises a nucleotide encoding a guide RNA (gRNA) operably linked to a promoter.

37. The composition of any one of claims 33-36, wherein the first nucleotide sequence or the second nucleotide sequence further comprises a transcriptional terminator.

38. The composition of claim 37, wherein the transcriptional terminator is a transcriptional terminator from a bGH gene, hGH gene, or SV40 gene.

39. The composition of claim 38, wherein the transcriptional terminal is from a bGH gene.

40. The composition of any one of claims 33-39, wherein the first nucleotide sequence or the second nucleotide sequence further comprises a woodchuck hepatitis posttranscriptional regulatory element (WPRE) inserted 5′ of the transcriptional terminator.

41. The composition of any one of claims 33-40, wherein the first nucleotide sequence or second nucleotide sequence are operably linked to a nucleotide sequence encoding at least one bipartite nuclear localization signal.

42. The composition of any one of claims 33-41, wherein the bipartite nuclear localization signal comprises an amino acid sequence selected from the group consisting of: KRPAATKKAGQAKKKK (SEQ ID NO: 344), KKTELQTTNAENKTKKL(SEQ ID NO: 345), KRGINDRNFWRGENGRKTR(SEQ ID NO: 346), and RKSGKIAAIVVKRPRK(SEQ ID NO: 347).

43. The composition of claim 42, wherein the bipartite nuclear localization signal comprises the amino acid sequence as set forth in SEQ ID NO: 344.

44. The composition of any one of claims 33-43, wherein the nucleobase editor comprises a cytosine deaminase fused to the N-terminus of a catalytically inactive Cas9 or a Cas9 nickase.

45. The composition of claim 44, wherein the cytosine deaminase is selected from the group consisting of: APOBEC1, APOBEC3, AID, and pmCDA1.

46. The composition of claim 44 or 45, wherein the nucleobase editor further comprises a uracil glycosylase inhibitor (UGI).

47. The composition of claim 46, wherein the UGI comprises the amino acids sequence of SEQ ID NOs: 299-302.

48. The composition of any one of claims 33-47, wherein the first nucleotide sequence and the second nucleotide sequence are on different vectors.

49. The composition of claim 48, wherein each of the different vectors r is a genome of a recombinant adeno-associated virus (rAAV).

50. The composition of claim 49, wherein the vector is packaged in a rAAV particle.

51. A composition comprising:

(i) a first recombinant adeno associated virus (rAAV) particle comprising a first nucleotide sequence encoding a N-terminal portion of a nucleobase editor fused at its C-terminus to an intein-N; and

(ii) a second recombinant adeno associated virus (rAAV) particle comprising a second nuclei acid encoding an intein-C fused to the N-terminus of a C-terminal portion of the nucleobase editor.

52. A cell comprising the composition of any one of claims 33-51.

53. The cell of claim 52, wherein the N-terminal portion of the nucleobase editor and the C-terminal portion of the nucleobase editor are joined together to form the nucleobase editor.

54. The cell of claim 52 or claim 53, wherein the cell is a prokaryotic cell.

55. The cell of claim 54, wherein the cell is a bacterial cell.

56. The cell of claim 52 or claim 53, wherein the cell is an eukaryotic cell.

57. The cell of claim 56, wherein the cell is a yeast cell, a plant cell, or a mammalian cell.

58. The cell of claim 56, wherein the cell is a human cell.

59. A kit comprising the composition of any one of claims 33-51.

60. An *in vitro* or *ex vivo* method comprising:

contacting a cell with the composition of any one of claims 1-24, wherein the contacting results in the delivery of the first nucleotide sequence and the second nucleotide sequence into the cell, and wherein the N-terminal portion of the Cas9 protein and the C-terminal portion of the Cas9 protein are joined to form a Cas9 protein.

61. An *in vitro* or *ex vivo* method comprising:

contacting a cell with the composition of any one of claims 33-51, wherein the contacting results in the delivery of the first nucleotide sequence and the second nucleotide sequence into the cell, and wherein the N-terminal portion of the nucleobase editor and the C-terminal portion of the nucleobase editor are joined to form a nucleobase editor.

62. The composition of any one of claims 1-24 and 33-51 for use in therapy.

63. Use of the composition of any one of claims 1-24 amd 33-51 in the preparation of a medicament for the treatment of a disease or disorder selected from the group consisting of: cystic fibrosis, phenylketonuria, epidermolytic hyperkeratosis (EHK), chronic obstructive pulmonary disease (COPD), Charcot-Marie-Toot disease type 4J. , neuroblastoma (NB), von Willebrand disease (vWD), myotonia congenital, hereditary renal amyloidosis, dilated cardiomyopathy, hereditary lymphedema, familial Alzheimer's disease, prion disease, chronic infantile neurologic cutaneous articular syndrome (CINCA), and desmin-related myopathy (DRM).