## CLAIMS

What is claimed is:

~~1. A polypeptide comprising a homing endonuclease (HE) variant that cleaves a target site in the human program cell death 1 (PD-1) gene.~~

~~2. The polypeptide of claim 1, wherein the HE variant is an LAGLIDADG homing endonuclease (LHE) variant.~~

~~3. The polypeptide of claim 1, or claim 2, wherein the polypeptide comprises a biologically active fragment of the HE variant.~~

~~4. The polypeptide of claim 3, wherein the biologically active fragment lacks the 1, 2, 3, 4, 5, 6, 7, or 8 N-terminal amino acids compared to a corresponding wild type HE.~~

~~5. The polypeptide of claim 4, wherein the biologically active fragment lacks the 4 N-terminal amino acids compared to a corresponding wild type HE.~~

~~6. The polypeptide of claim 4, wherein the biologically active fragment lacks the 8 N-terminal amino acids compared to a corresponding wild type HE.~~

~~7. The polypeptide of claim 3, wherein the biologically active fragment lacks the 1, 2, 3, 4, or 5 C-terminal amino acids compared to a corresponding wild type HE.~~

~~8. The polypeptide of claim 7, wherein the biologically active fragment lacks the C-terminal amino acid compared to a corresponding wild type HE.~~

9. The polypeptide of claim 7, wherein the biologically active fragment lacks the 2 C-terminal amino acids compared to a corresponding wild type HE.

10. The polypeptide of any one of claims 1 to 9, wherein the HE variant is a variant of an LHE selected from the group consisting of: I-AabMI, I-AaeMI, I-AniI, I-ApaMI, I-CapIII, I-CapIV, I-CkaMI, I-CpaMI, I-CpaMII, I-CpaMIII, I-CpaMIV, I-CpaMV, I-CpaV, I-CraMI, I-EjeMI, I-GpeMI, I-GpiI, I-GzeMI, I-GzeMII, I-GzeMIII, I-HjeMI, I-LtrII, I-LtrI, I-LtrWI, I-MpeMI, I-MveMI, I-NcrII, I-NcrI, I-NcrMI, I-OheMI, I-OnuI, I-OsoMI, I-OsoMII, I-OsoMIII, I-OsoMIV, I-PanMI, I-PanMII, I-PanMIII, I-PnoMI, I-ScuMI, I-SmaMI, I-SscMI, and I-Vdi141I.

11. The polypeptide of any one of claims 1 to 10, wherein the HE variant is a variant of an LHE selected from the group consisting of: I-CpaMI, I-HjeMI, I-OnuI, I-PanMI, and SmaMI.

12. The polypeptide of any one of claims 1 to 11, wherein the HE variant is an I-OnuI LHE variant.

13. The polypeptide of any one of claims 1 to 12, wherein the HE variant comprises one or more amino acid substitutions in the DNA recognition interface at amino acid positions selected from the group consisting of: 19, 24, 26, 28, 30, 32, 34, 35, 36, 37, 38, 40, 42, 44, 46, 48, 59, 68, 70, 72, 75, 76 77, 78, 80, 82, 168, 180, 182, 184, 186, 188, 189, 190, 191, 192, 193, 195, 197, 199, 201, 203, 223, 225, 227, 229, 231, 232, 234, 236, 238, and 240 of an I-OnuI LHE amino acid sequence as set forth in SEQ ID NOs: 1-5, or a biologically active fragment thereof.

14. The polypeptide of any one of claims 1 to 13, wherein the HE variant comprises at least 5, at least 15, preferably at least 25, more preferably at least 35, or even more preferably at least 40 or more amino acid substitutions in the DNA recognition interface at amino acid positions selected from the group consisting of: 19, 24, 26, 28, 30, 32, 34, 35, 36, 37, 38, 40, 42, 44, 46, 48, 59, 68, 70, 72, 75, 76 77, 78, 80, 82, 168, 180, 182, 184, 186, 188, 189, 190, 191, 192,

193, 195, 197, 199, 201, 203, 223, 225, 227, 229, 231, 232, 234, 236, 238, and 240 of an I-OnuI LHE amino acid sequence as set forth in SEQ ID NOs: 1-5, or a biologically active fragment thereof.

15. The polypeptide of any one of claims 1 to 14, wherein the HE variant cleaves a PD-1 exon 5 target site and comprises at least 5, at least 15, preferably at least 25, more preferably at least 35, or even more preferably at least 40 or more amino acid substitutions in at least one position selected from the position group consisting of positions: 26, 28, 32, 34, 35, 36, 37, 38, 40, 42, 44, 46, 48, 59, 68, 70, 72, 75, 76, 78, 80, 138, 143, 159, 168, 178, 180, 182, 184, 186, 188, 189, 190, 191, 192, 193, 197, 199, 201, 203, 207, 223, 224, 225, 227, 229, 232, 236, and 238 of any one of SEQ ID NOs: 1-5, or a biologically active fragment thereof.

16. The polypeptide of any one of claims 1 to 15, wherein the HE variant cleaves a PD-1 exon 5 target site and comprises at least 5, at least 15, preferably at least 25, more preferably at least 35, or even more preferably at least 40 or more of the following amino acid substitutions: L26Q, R28E, N32V, K34P, S35N, S36I, V37P, G38R, S40K, S40R, E42R, G44R, Q46E, T48D, N59D, V68K, A70Y, S72Q, S72R, N75S, A76Y, S78K, K80R, L138M, T143N, S159P, F168L, E178D, C180H, F182G, N184I, I186A, S188R, K189R, S190P, K191A, L192S, G193P, Q197R, V199R, S201A, S201M, T203G, K207R, Y223R, I224T, K225R, K229I, F232K, D236E, and V238E of any one of SEQ ID NOs: 1-5, or a biologically active fragment thereof.

17. The polypeptide of any one of claims 1 to 15, wherein the HE variant cleaves a PD-1 exon 5 target site and comprises at least 5, at least 15, preferably at least 25, more preferably at least 35, or even more preferably at least 40 or more of or all of the following amino acid substitutions: L26Q, R28E, N32V, K34P, S35N, S36I, V37P, G38R, S40K, E42R, G44R, Q46E, T48D, V68K, A70Y, S72Q, N75S, A76Y, S78K, K80R, L138M, T143N, S159P, F168L, C180H, F182G, N184I, I186A, S188R, K189R, S190P, K191A, L192S, G193P, Q197R,

~~V199R, S201A, T203G, K207R, Y223R, K225R, K229I, F232K, D236E, and V238E of any one of SEQ ID NOs: 1-5, or a biologically active fragment thereof.~~

~~18. The polypeptide of any one of claims 1 to 15, wherein the HE variant cleaves a PD-1 exon 5 target site and comprises at least 5, at least 15, preferably at least 25, more preferably at least 35, or even more preferably at least 40 or more of or all of the following amino acid substitutions: L26Q, R28E, N32V, K34P, S35N, S36I, V37P, G38R, S40R, E42R, G44R, Q46E, T48D, N59D, V68K, A70Y, S72R, N75S, A76Y, K80R, L138M, T143N, S159P, F168L, C180H, F182G, N184I, I186A, S188R, K189R, S190P, K191A, L192S, G193P, Q197R, V199R, S201A, T203G, K207R, Y223R, I224T, K225R, K229I, F232K, D236E, and V238E of any one of SEQ ID NOs: 1-5, or a biologically active fragment thereof.~~

~~19. The polypeptide of any one of claims 1 to 15, wherein the HE variant cleaves a PD-1 exon 5 target site and comprises at least 5, at least 15, preferably at least 25, more preferably at least 35, or even more preferably at least 40 or more of or all of the following amino acid substitutions: L26Q, R28E, N32V, K34P, S35N, S36I, V37P, G38R, S40R, E42R, G44R, Q46E, T48D, N59D, V68K, A70Y, S72Q, N75S, A76Y, K80R, L138M, T143N, S159P, F168L, E178D, C180H, F182G, N184I, I186A, S188R, K189R, S190P, K191A, L192S, G193P, Q197R, V199R, S201A, T203G, K207R, Y223R, I224T, K225R, K229I, F232K, D236E, and V238E of any one of SEQ ID NOs: 1-5, or a biologically active fragment thereof.~~

~~20. The polypeptide of any one of claims 1 to 15, wherein the HE variant cleaves a PD-1 exon 5 target site and comprises at least 5, at least 15, preferably at least 25, more preferably at least 35, or even more preferably at least 40 or more of or all of the following amino acid substitutions: L26Q, R28E, N32V, K34P, S35N, S36I, V37P, G38R, S40R, E42R, G44R, Q46E, T48D, N59D, V68K, A70Y, S72Q, N75S, A76Y, K80R, L138M, T143N, S159P, F168L, E178D, C180H, F182G, N184I, I186A, S188R, K189R, S190P, K191A, L192S, G193P, Q197R, V199R, S201A, T203G, K207R, Y223R, K225R, F232K, D236E, and V238E of any one of SEQ ID NOs: 1-5, or a biologically active fragment thereof.~~

21. The polypeptide of any one of claims 1 to 15, wherein the HE variant cleaves a PD-1 exon 5 target site and comprises at least 5, at least 15, preferably at least 25, more preferably at least 35, or even more preferably at least 40 or more of or all of the following amino acid substitutions: L26Q, R28E, N32V, K34P, S35N, S36I, V37P, G38R, S40R, E42R, G44R, Q46E, T48D, N59D, V68K, A70Y, S72Q, N75S, A76Y, K80R, L138M, T143N, S159P, F168L, E178D, C180H, F182G, N184I, I186A, S188R, K189R, S190P, K191A, L192S, G193P, Q197R, V199R, S201M, T203G, Y223R, K225R, F232K, D236E, and V238E of any one of SEQ ID NOs: 1-5, or a biologically active fragment thereof.

22. The polypeptide of any one of claims 1 to 15, wherein the HE variant cleaves a PD-1 exon 1 target site and comprises at least 5, at least 15, preferably at least 25, more preferably at least 35, or even more preferably at least 40 or more amino acid substitutions in at least one position selected from the position group consisting of positions: 26, 28, 30, 32, 34, 35, 36, 37, 38, 40, 42, 44, 46, 48, 68, 70, 72, 75, 76, 78, 80, 100, 132, 138, 143, 155, 159, 178, 180, 184, 186, 189, 190, 191, 192, 193, 195, 201, 203, 207, 223, 225, 227, 232, 236, 238, and 240 of any one of SEQ ID NOs: 1-5, or a biologically active fragment thereof.

23. The polypeptide of any one of claims 1 to 15, wherein the HE variant cleaves a PD-1 exon 1 target site and comprises at least 5, at least 15, preferably at least 25, more preferably at least 35, or even more preferably at least 40 or more of the following amino acid substitutions: L26G, R28S, R30L, N32R, K34R, S35G, S36T, V37A, V37G, G38R, S40H, E42R, G44S, Q46A, Q46T, T48V, T48M, V68I, V68S, A70T, A70Y, A70L, S72D, S72N, N75R, N75H, A76Y, S78R, S78T, K80R, K80C, K80E, K80V, T82F, T82Y, I100V, V132A, L138M, T143N, S155G, S159P, E178D, C180S, N184R, I186R, K189N, S190V, K191N, L192A, G193R, Q195R, S201E, T203S, K207R, Y223H, K225Y, K227G, F232R, D236Q, V238R, and T240E of any one of SEQ ID NOs: 1-5, or a biologically active fragment thereof.

24. The polypeptide of any one of claims 1 to 15, wherein the HE variant cleaves a PD-1 exon 1 target site and comprises at least 5, at least 15, preferably at least 25, more

preferably at least 35, or even more preferably at least 40 or more of or all of the following amino acid substitutions: L26G, R28S, R30L, N32R, K34R, S35G, S36T, V37A, G38R, S40H, E42R, G44S, Q46A, T48V, V68I, A70T, S72D, N75R, A76Y, S78R, K80R, I100V, L138M, T143N, S159P, E178D, C180S, N184R, I186R, K189N, S190V, K191N, L192A, G193R, Q195R, S201E, T203S, K207R, Y223H, K225Y, K227G, F232R, D236Q, V238R, and T240E of any one of SEQ ID NOs: 1-5, or a biologically active fragment thereof.

25. The polypeptide of any one of claims 1 to 15, wherein the HE variant cleaves a PD-1 exon 1 target site and comprises at least 5, at least 15, preferably at least 25, more preferably at least 35, or even more preferably at least 40 or more of or all of the following amino acid substitutions: L26G, R28S, R30L, N32R, K34R, S35G, S36T, V37A, V37G, G38R, S40H, E42R, G44S, Q46T, T48V, V68I, A70T, S72D, N75R, A76Y, S78R, K80C, I100V, V132A, L138M, T143N, S155G, S159P, E178D, C180S, N184R, I186R, K189N, S190V, K191N, L192A, G193R, Q195R, S201E, T203S, K207R, Y223H, K225Y, K227G, F232R, D236Q, V238R, and T240E of any one of SEQ ID NOs: 1-5, or a biologically active fragment thereof.

26. The polypeptide of any one of claims 1 to 15, wherein the HE variant cleaves a PD-1 exon 1 target site and comprises at least 5, at least 15, preferably at least 25, more preferably at least 35, or even more preferably at least 40 or more of or all of the following amino acid substitutions: L26G, R28S, R30L, N32R, K34R, S35G, S36T, V37A, G38R, S40H, E42R, G44S, Q46T, T48M, V68I, A70T, S72N, N75H, A76Y, S78T, K80R, I100V, L138M, T143N, S159P, E178D, C180S, N184R, I186R, K189N, S190V, K191N, L192A, G193R, Q195R, S201E, T203S, K207R, Y223H, K225Y, K227G, F232R, D236Q, V238R, and T240E of any one of SEQ ID NOs: 1-5, or a biologically active fragment thereof.

27. The polypeptide of any one of claims 1 to 15, wherein the HE variant cleaves a PD-1 exon 1 target site and comprises at least 5, at least 15, preferably at least 25, more preferably at least 35, or even more preferably at least 40 or more of or all of the following

amino acid substitutions: L26G, R28S, R30L, N32R, K34R, S35G, S36T, V37A, G38R, S40H, E42R, G44S, Q46T, T48M, V68S, A70Y, S72N, N75H, A76Y, K80E, T82F, L138M, T143N, S159P, E178D, C180S, N184R, I186R, K189N, S190V, K191N, L192A, G193R, Q195R, S201E, T203S, K207R, Y223H, K225Y, K227G, F232R, D236Q, V238R, and T240E of any one of SEQ ID NOs: 1-5, or a biologically active fragment thereof.

28. The polypeptide of any one of claims 1 to 15, wherein the HE variant cleaves a PD-1 exon 1 target site and comprises at least 5, at least 15, preferably at least 25, more preferably at least 35, or even more preferably at least 40 or more of or all of the following amino acid substitutions: L26G, R28S, R30L, N32R, K34R, S35G, S36T, V37A, G38R, S40H, E42R, G44S, Q46T, T48M, V68S, A70L, S72N, N75H, A76Y, K80V, T82Y, L138M, T143N, S159P, E178D, C180S, N184R, I186R, K189N, S190V, K191N, L192A, G193R, Q195R, S201E, T203S, K207R, Y223H, K225Y, K227G, F232R, D236Q, V238R, and T240E of any one of SEQ ID NOs: 1-5, or a biologically active fragment thereof.

29. The polypeptide of any one of claims 1 to 15, wherein the HE variant cleaves a PD-1 exon 1 target site and comprises at least 5, at least 15, preferably at least 25, more preferably at least 35, or even more preferably at least 40 or more of or all of the following amino acid substitutions: L26G, R28S, R30L, N32R, K34R, S35G, S36T, V37G, G38R, S40H, E42R, G44S, Q46T, T48M, V68S, A70T, S72N, N75H, A76Y, K80V, T82Y, L138M, T143N, S159P, E178D, C180S, N184R, I186R, K189N, S190V, K191N, L192A, G193R, Q195R, S201E, T203S, K207R, Y223H, K225Y, K227G, F232R, D236Q, V238R, and T240E of any one of SEQ ID NOs: 1-5, or a biologically active fragment thereof.

30. The polypeptide of any one of claims 1 to 15, wherein the HE variant cleaves a PD-1 exon 2 target site and comprises at least 5, at least 15, preferably at least 25, more preferably at least 35, or even more preferably at least 40 or more amino acid substitutions in at least one position selected from the position group consisting of positions: 24, 26, 28, 30, 32, 34, 35, 36, 37, 38, 40, 41, 42, 44, 46, 48, 68, 70, 72, 74, 75, 76, 78, 80, 82, 116, 138, 143, 159, 168,

178, 180, 182, 184, 186, 188, 189, 190, 191, 192, 193, 195, 199, 203, 207, 225, 227, 229, 232, 236, and 238 of any one of SEQ ID NOs: 1-5, or a biologically active fragment thereof.

31. The polypeptide of any one of claims 1 to 15, wherein the HE variant cleaves a PD-1 exon 2 target site and comprises at least 5, at least 15, preferably at least 25, more preferably at least 35, or even more preferably at least 40 or more amino acid substitutions in at least one position selected from the position group consisting of positions: S24C, L26Q, R28Y, R28H, R30S, N32V, N32L, K34N, K34R, S35N, S35T, S36R, V37S, V37T, G38R, G38K, S40R, T41A, E42R, G44S, G44R, Q46E, Q46A, T48E, V68I, A70N, S72I, D74N, N75T, N75R, A76S, A76R, S78R, K80S, T82G, T82R, V116L, L138M, T143N, S159P, F168L, E178D, C180N, F182Y, N184H, I186K, K189G, S190R, K191T, L192T, G193R, Q195Y, V199R, T203A, T203S, K207R, K225N, K225T, K227W, K227S, K229A, K229P, F232R, W234A, W234D, D236E, and V238R of any one of SEQ ID NOs: 1-5, or a biologically active fragment thereof.

32. The polypeptide of any one of claims 1 to 15, wherein the HE variant cleaves a PD-1 exon 2 target site and comprises at least 5, at least 15, preferably at least 25, more preferably at least 35, or even more preferably at least 40 or more of or all of the following amino acid substitutions: L26Q, R28Y, R30S, N32V, K34N, S35N, S36R, V37S, G38R, S40R, T41A, E42R, G44R, Q46A, T48E, A70N, S72I, N75T, A76S, S78R, K80S, T82G, L138M, T143N, S159P, F168L, E178D, C180N, F182Y, N184H, I186K, K189G, S190R, K191T, L192T, G193R, Q195Y, V199R, T203A, K207R, K225N, K227W, K229A, F232R, W234A, D236E, and V238R of any one of SEQ ID NOs: 1-5, or a biologically active fragment thereof.

33. The polypeptide of any one of claims 1 to 15, wherein the HE variant cleaves a PD-1 exon 2 target site and comprises at least 5, at least 15, preferably at least 25, more preferably at least 35, or even more preferably at least 40 or more of or all of the following amino acid substitutions: S24C, R28H, N32L, K34R, S35T, V37T, G38K, S40R, E42R, G44S, Q46E, T48E, V68I, A70N, S72I, D74N, N75R, A76R, S78R, K80S, T82R, V116L, L138M,

T143N, S159P, F168L, E178D, C180N, F182Y, N184H, I186K, K189G, S190R, K191T, L192T, G193R, Q195Y, V199R, T203S, K207R, K225T, K227S, K229P, F232R, W234D, D236E, and V238R of any one of SEQ ID NOs: 1-5, or a biologically active fragment thereof.

34. The polypeptide of any one of claims 1 to 33, wherein the HE variant comprises an amino acid sequence that is at least 80%, preferably at least 85%, more preferably at least 90%, or even more preferably at least 95% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 6-14 and 60-63, or a biologically active fragment thereof.

35. The polypeptide of any one of claims 1 to 34, wherein the HE variant comprises the amino acid sequence set forth in SEQ ID NO: 6, or a biologically active fragment thereof.

36. The polypeptide of any one of claims 1 to 34, wherein the HE variant comprises the amino acid sequence set forth in SEQ ID NO: 7, or a biologically active fragment thereof.

37. The polypeptide of any one of claims 1 to 34, wherein the HE variant comprises the amino acid sequence set forth in SEQ ID NO: 8, or a biologically active fragment thereof.

38. The polypeptide of any one of claims 1 to 34, wherein the HE variant comprises the amino acid sequence set forth in SEQ ID NO: 9, or a biologically active fragment thereof.

39. The polypeptide of any one of claims 1 to 34, wherein the HE variant comprises the amino acid sequence set forth in SEQ ID NO: 10, or a biologically active fragment thereof.

40. The polypeptide of any one of claims 1 to 34, wherein the HE variant comprises the amino acid sequence set forth in SEQ ID NO: 11, or a biologically active fragment thereof.

41. The polypeptide of any one of claims 1 to 34, wherein the HE variant comprises the amino acid sequence set forth in SEQ ID NO: 12, or a biologically active fragment thereof.

42. The polypeptide of any one of claims 1 to 34, wherein the HE variant comprises the amino acid sequence set forth in SEQ ID NO: 13, or a biologically active fragment thereof.

43. The polypeptide of any one of claims 1 to 34, wherein the HE variant comprises the amino acid sequence set forth in SEQ ID NO: 14, or a biologically active fragment thereof.

44. The polypeptide of any one of claims 1 to 34, wherein the HE variant comprises the amino acid sequence set forth in SEQ ID NO: 60, or a biologically active fragment thereof.

45. The polypeptide of any one of claims 1 to 34, wherein the HE variant comprises the amino acid sequence set forth in SEQ ID NO: 61, or a biologically active fragment thereof.

46. The polypeptide of any one of claims 1 to 34, wherein the HE variant comprises the amino acid sequence set forth in SEQ ID NO: 62, or a biologically active fragment thereof.

47. The polypeptide of any one of claims 1 to 34, wherein the HE variant comprises the amino acid sequence set forth in SEQ ID NO: 63, or a biologically active fragment thereof.

48. The polypeptide of any one of claims 1 to 21 and 30-39, wherein the polypeptide binds the polynucleotide sequence set forth in SEQ ID NO: 25.

49. The polypeptide of any one of claims 1 to 14, 22-29, 40-41, and 44-47 wherein the polypeptide binds the polynucleotide sequence set forth in SEQ ID NO: 30.

50. The polypeptide of any one of claims 1 to 14, 30-33, and 42-43, wherein the polypeptide binds the polynucleotide sequence set forth in SEQ ID NO: 35.

51. The polypeptide of any one of claims 1 to 50, further comprising a DNA binding domain.

52. The polypeptide of claim 51, wherein the DNA binding domain is selected from the group consisting of: a TALE DNA binding domain and a zinc finger DNA binding domain.

53. The polypeptide of claim 52, wherein the TALE DNA binding domain comprises about 9.5 TALE repeat units to about 15.5 TALE repeat units.

54. The polypeptide of claim 52 or claim 53, wherein the TALE DNA binding domain binds a polynucleotide sequence in the PD-1 gene.

55. The polypeptide of any one of claims 52 to 54, wherein the TALE DNA binding domain binds the polynucleotide sequence set forth in SEQ ID NO: 26.

56. The polypeptide of claim 55, wherein the polypeptide binds and cleaves the polynucleotide sequence set forth in SEQ ID NO: 27.

57. The polypeptide of any one of claims 52 to 54, wherein the TALE DNA binding domain binds the polynucleotide sequence set forth in SEQ ID NO: 31.

58. The polypeptide of claim 57, wherein the polypeptide binds and cleaves the polynucleotide sequence set forth in SEQ ID NO: 32.

59. The polypeptide of any one of claims 52 to 54, wherein the TALE DNA binding domain binds the polynucleotide sequence set forth in SEQ ID NO: 36.

60. The polypeptide of claim 59, wherein the polypeptide binds and cleaves the polynucleotide sequence set forth in SEQ ID NO: 37.

61. The polypeptide of claim 52, wherein the zinc finger DNA binding domain comprises 2, 3, 4, 5, 6, 7, or 8 zinc finger motifs.

62. The polypeptide of any one of claims 1 to 61, further comprising a peptide linker and an end-processing enzyme or biologically active fragment thereof.

63. The polypeptide of any one of claims 1 to 61, further comprising a viral self-cleaving 2A peptide and an end-processing enzyme or biologically active fragment thereof.

64. The polypeptide of claim 62 or claim 63, wherein the end-processing enzyme or biologically active fragment thereof has 5´ 3´ exonuclease, 5´ 3´ alkaline exonuclease, 3´ 5´ exonuclease, 5´ flap endonuclease, helicase or template-independent DNA polymerase activity.

65. The polypeptide of any one of claims 62 to 64, wherein the end-processing enzyme comprises Trex2 or a biologically active fragment thereof.

66. The polypeptide of any one of claims 1 to 65, wherein the polypeptide comprises the amino acid sequence set forth in any one of SEQ ID NOs: 15-23, and 64 or a biologically active fragment thereof.

67. The polypeptide of claim 66, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 15, or a biologically active fragment thereof.

68. The polypeptide of claim 66, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 16, or a biologically active fragment thereof.

69. The polypeptide of claim 66, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 17, or a biologically active fragment thereof.

70. The polypeptide of claim 66, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 18, or a biologically active fragment thereof.

~~71. The polypeptide of claim 66, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 19, or a biologically active fragment thereof.~~

~~72. The polypeptide of claim 66, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 20, or a biologically active fragment thereof.~~

~~73. The polypeptide of claim 66, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 21, or a biologically active fragment thereof.~~

~~74. The polypeptide of claim 66, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 22, or a biologically active fragment thereof.~~

~~75. The polypeptide of claim 66, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 23, or a biologically active fragment thereof.~~

~~76. The polypeptide of claim 66, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 64, or a biologically active fragment thereof.~~

~~77. The polypeptide of any one of claims 1 to 61, wherein the polypeptide cleaves the human PD-1 gene at a polynucleotide sequence set forth in SEQ ID NOs: 25, 27, 30, 32, 35, or 37.~~

~~78. A polynucleotide encoding the polypeptide of any one of claims 1 to 77.~~

~~79. An mRNA encoding the polypeptide of any one of claims 1 to 77.~~

~~80. A cDNA encoding the polypeptide of any one of claims 1 to 77.~~

81. A vector comprising a polynucleotide encoding the polypeptide of any one of claims 1 to 77.

82. A cell comprising the polypeptide of any one of claims 1 to 77.

83. A cell comprising a polynucleotide encoding the polypeptide of any one of claims 1 to 77.

84. A cell comprising the vector of claim 81.

85. A cell comprising one or more genome modifications introduced by the polypeptide of any one of claims 1 to 77.

86. The cell of any one of claims 82 to 85, wherein the cell comprises a polynucleotide encoding one or more of an immunopotency enhancer, an immunosuppressive signal damper, or an engineered antigen receptor.

87. The cell of claim 86, wherein the polynucleotide further comprises an RNA polymerase II promoter operably linked to the polynucleotide encoding the immunopotency enhancer, immunosuppressive signal damper, or engineered antigen receptor.

88. The cell of claim 87, wherein the RNA polymerase II promoter is selected from the group consisting of: a short EF1α promoter, a long EF1α promoter, a human ROSA 26 locus, a Ubiquitin C (UBC) promoter, a phosphoglycerate kinase-1 (PGK) promoter, a cytomegalovirus enhancer/chicken β-actin (CAG) promoter, a β-actin promoter and a myeloproliferative sarcoma virus enhancer, negative control region deleted, dl587rev primer-binding site substituted (MND) promoter.

89. The cell of any one of claims 86 to 88, wherein the polynucleotide further encodes one or more self-cleaving viral peptides operably linked to, interspersed between, and/or flanking the immunopotency enhancer, immunosuppressive signal damper, or engineered antigen receptor.

90. The cell of claim 89, wherein the self-cleaving viral peptide is a 2A peptide.

91. The cell of any one of claims 86 to 90, wherein the polynucleotide further comprises a heterologous polyadenylation signal.

92. The cell of any one of claims 86 to 91, wherein the immunosuppressive signal damper comprises an enzymatic function that counteracts an immunosuppressive factor.

93. The cell of claim 92, wherein the immunosuppressive signal damper comprises kynureninase activity.

94. The cell of any one of claims 86 to 91, wherein the immunosuppressive signal damper comprises:

(a) an exodomain that binds an immunosuppressive factor, optionally wherein the exodomain is an antibody or antigen binding fragment thereof;

(b) an exodomain that binds an immunosuppressive factor and a transmembrane domain; or

(c) an exodomain that binds an immunosuppressive factor, a transmembrane domain, and a modified endodomain that is unable to transduce immunosuppressive signals to the cell.

95. The cell of any one of claims 86 to 91, wherein the immunosuppressive signal damper is a dominant negative TGFβRII receptor.

96. The cell of any one of claims 86 to 91, wherein the immunopotency enhancer is selected from the group consisting of: a bispecific T cell engager molecule (BiTE), an immunopotentiating factor, and a flip receptor.

97. The cell of claim 96, wherein the immunopotentiating factor is selected from the group consisting of: a cytokine, a chemokine, a cytotoxin, a cytokine receptor, and variants thereof.

98. The cell of claim 97, wherein the cytokine is selected from the group consisting of IL-2, IL-7, IL-12, IL-15, IL-18, and IL-21.

99. The cell of claim 97, wherein the cytokine is selected from the group consisting of IL-2, IL-7, IL-12, IL-15, IL-18, and IL-21 and is operably linked to the endogenous PD-1 promoter.

100. The cell of claim 97, wherein the cytokine is IL-7 and is operably linked to the endogenous PD-1 promoter.

101. The cell of claim 97, wherein the cytokine is IL-12 and is operably linked to the endogenous PD-1 promoter.

102. The cell of claim 97, wherein the cytokine is IL-15 and is operably linked to the endogenous PD-1 promoter.

103. The cell of claim 96, wherein the flip receptor comprises a PD-1 exodomain and transmembrane domain; and an endodomain from CD28, CD134, CD137, CD278, and/or CD3ζ fused in frame to the C-terminal end of the PD-1 transmembrane domain.

104. The cell of claim 96, wherein the flip receptor comprises a PD-1 exodomain; a transmembrane domain isolated from a CD3 polypeptide, CD4, CD8α, CD28, CD134, or

CD137; and an endodomain from CD28, CD134, CD137, CD278, and/or CD3ζ fused in frame to the C-terminal end of the PD-1 exodomain.

105. The cell of claim 96, wherein the flip receptor comprises a PD-1 exodomain; and a transmembrane domain and endodomain isolated from a CD3 polypeptide, CD4, CD8α, CD28, CD134, or CD137 fused in frame to the C-terminal end of the PD-1 exodomain.

106. The cell of any one of claims 86 to 91, wherein the engineered antigen receptor is selected from the group consisting of: an engineered TCR, a CAR, a Daric, or a zetakine.

107. The cell of claim 106, wherein the engineered receptor is not integrated into the PD-1 gene.

108. The cell of any one of claims 86 to 91, wherein the polynucleotide encoding one or more of an immunopotency enhancer, an immunosuppressive signal damper, or an engineered antigen receptor is integrated into the PD-1 gene.

109. The cell of any one of claims 86 to 91, wherein a donor repair template comprising the polynucleotide encoding one or more of an immunopotency enhancer, an immunosuppressive signal damper, or an engineered antigen receptor is integrated into the PD-1 gene at a DNA double stranded break site introduced by the polypeptide according to any one of claims 1 to 77.

110. The cell of any one of claims 82 to 109, wherein the cell is a hematopoietic cell.

111. The cell of any one of claims 82 to 110, wherein the cell is a T cell.

112. The cell of any one of claims 82 to 111, wherein the cell is a $CD3^+$, $CD4^+$, and/or $CD8^+$ cell.

~~113. The cell of any one of claims 82 to 112, wherein the cell is an immune effector cell.~~

~~114. The cell of any one of claims 82 to 113, wherein the cell is a cytotoxic T lymphocytes (CTLs), a tumor infiltrating lymphocytes (TILs), or a helper T cells.~~

~~115. The cell of any one of claims 82 to 113, wherein the cell is a natural killer (NK) cell or natural killer T (NKT) cell.~~

~~116. The cell of any one of claims 82 to 115, wherein the source of the cell is peripheral blood mononuclear cells, bone marrow, lymph nodes tissue, cord blood, thymus issue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, or tumors.~~

~~117. The cell of any one of claims 82 to 116, wherein the cell comprises one or more modified PD-1 alleles.~~

~~118. The cell of claim 117, wherein the one or more modified PD-1 alleles are non-functional or have substantially reduced PD-1 function and/or intracellular signaling.~~

~~119. The cell of any one of claims 82 to 118, wherein the cell comprises a nucleic acid encoding an immunopotency enhancer, immunosuppressive signal damper, or an engineered antigen receptor introduced into the one or more modified PD-1 alleles.~~

~~120. The cell of any one of claims 82 to 118, wherein the cell comprises a nucleic acid encoding an immunopotency enhancer or immunosuppressive signal damper introduced into the one or more modified PD-1 alleles and the cell further comprises engineered antigen receptor that is not introduced into the one or more modifies PD-1 alleles.~~

~~121. A plurality of cells comprising one or more cells of any one of claims 82 to 120.~~

~~122. A composition comprising one or more cells according to any one of claims 82 to 121.~~

~~123. A composition comprising one or more cells according to any one of claims 82 to 121 and a physiologically acceptable carrier.~~

~~124. A method of editing a human PD-1 gene in a cell comprising: introducing a polynucleotide encoding the polypeptide of any one of claims 1 to 77 into the cell, wherein expression of the polypeptide creates a double strand break at a target site in a human PD-1 gene.~~

~~125. A method of editing a human PD-1 gene in cell comprising: introducing a polynucleotide encoding the polypeptide of any one of claims 1 to 77 into the cell, wherein expression of the polypeptide creates a double strand break at a target site in a human PD-1 gene, wherein the break is repaired by non-homologous end joining (NHEJ).~~

~~126. A method of editing a human PD-1 gene in a cell comprising: introducing a polynucleotide encoding the polypeptide of any one of claims 1 to 77 and a donor repair template into the cell, wherein expression of the polypeptide creates a double strand break at a target site in a human PD-1 gene and the donor repair template is incorporated into the human PD-1 gene by homology directed repair (HDR) at the site of the double-strand break (DSB).~~

~~127. The method of any one of claims 124 to 126, wherein the cell is a hematopoietic cell.~~

~~128. The method of any one of claims 124 to 127, wherein the cell is a T cell.~~

~~129. The method of any one of claims 124 to 128, wherein the cell is a $CD3^+$, $CD4^+$, and/or $CD8^+$ cell.~~

~~130. The method of any one of claims 124 to 129, wherein the cell is an immune effector cell.~~

~~131. The method of any one of claims 124 to 130, wherein the cell is a cytotoxic T lymphocytes (CTLs), a tumor infiltrating lymphocytes (TILs), or a helper T cells.~~

~~132. The method of any one of claims 124 to 130, wherein the cell is a natural killer (NK) cell or natural killer T (NKT) cell.~~

~~133. The method of any one of claims 124 to 132, wherein the source of the cell is peripheral blood mononuclear cells, bone marrow, lymph nodes tissue, cord blood, thymus issue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, or tumors.~~

~~134. The method of any one of claims 124 to 133, wherein the polynucleotide encoding the polypeptide is an mRNA.~~

~~135. The method of any one of claims 124 to 134, wherein a polynucleotide encoding a 3´-5´ exonuclease is introduced into the cell.~~

~~136. The method of any one of claims 124 to 134, wherein a polynucleotide encoding Trex2 or a biologically active fragment thereof is introduced into the cell.~~

~~137. The method of any one of claims 124 to 136, wherein the donor repair template encodes a PD-1 gene or portion thereof comprising one or more mutations compared to the wild type PD-1 gene.~~

~~138. The method of any one of claims 124 to 136, wherein the donor repair template encodes one or more of an immunopotency enhancer, an immunosuppressive signal damper, or an engineered antigen receptor.~~

~~139. The method of claim 138, wherein the donor repair template further comprises an RNA polymerase II promoter operably linked to the immunopotency enhancer, immunosuppressive signal damper, or engineered antigen receptor.~~

~~140. The method of claim 139, wherein the RNA polymerase II promoter is selected from the group consisting of: a short EF1α promoter, a long EF1α promoter, a human ROSA 26 locus, a Ubiquitin C (UBC) promoter, a phosphoglycerate kinase 1 (PGK) promoter, a cytomegalovirus enhancer/chicken β-actin (CAG) promoter, a β-actin promoter and a myeloproliferative sarcoma virus enhancer, negative control region deleted, dl587rev primer-binding site substituted (MND) promoter.~~

~~141. The method of any one of claims 138 to 140, wherein the donor repair template further encodes one or more self-cleaving viral peptides operably linked to, interspersed between, and/or flanking the immunopotency enhancer, immunosuppressive signal damper, or engineered antigen receptor.~~

~~142. The method of claim 141, wherein the self-cleaving viral peptide is a 2A peptide.~~

~~143. The method of any one of claims 138 to 142, wherein the donor repair template further comprises a heterologous polyadenylation signal.~~

~~144. The method of any one of claims 138 to 143, wherein the immunosuppressive signal damper comprises an enzymatic function that counteracts an immunosuppressive factor.~~

~~145. The method of claim 144, wherein the immunosuppressive signal damper comprises kynureninase activity.~~

~~146. The method of any one of claims 138 to 143, wherein the immunosuppressive signal damper comprises:~~

(a) an exodomain that binds an immunosuppressive factor, optionally wherein the exodomain is an antibody or antigen binding fragment thereof;

(b) an exodomain that binds an immunosuppressive factor and a transmembrane domain; or

(c) an exodomain that binds an immunosuppressive factor, a transmembrane domain, and a modified endodomain that is unable to transduce immunosuppressive signals to the cell.

147. The method of any one of claims 138 to 143, wherein the immunosuppressive signal damper is a dominant negative TGFβRII receptor.

148. The method of any one of claims 138 to 143, wherein the immunopotency enhancer is selected from the group consisting of: a bispecific T cell engager molecule (BiTE), an immunopotentiating factor, and a flip receptor.

149. The method of claim 148, wherein the immunopotentiating factor is selected from the group consisting of: a cytokine, a chemokine, a cytotoxin, a cytokine receptor, and variants thereof.

150. The method of claim 149, wherein the cytokine is selected from the group consisting of IL-2, IL-7, IL-12, IL-15, IL-18, and IL-21.

151. The method of claim 149, wherein the cytokine is selected from the group consisting of IL-2, IL-7, IL-12, IL-15, IL-18, and IL-21 and is operably linked to the endogenous PD-1 promoter.

152. The method of claim 149, wherein the cytokine is IL-7 and is operably linked to the endogenous PD-1 promoter.

153. The method of claim 149, wherein the cytokine is IL-12 and is operably linked to the endogenous PD-1 promoter.

~~154. The method of claim 149, wherein the cytokine is IL-15 and is operably linked to the endogenous PD-1 promoter.~~

~~155. The method of claim 148, wherein the flip receptor comprises a PD-1 exodomain and transmembrane domain; and an endodomain from CD28, CD134, CD137, CD278, and/or CD3ζ fused in frame to the C-terminal end of the PD-1 transmembrane domain.~~

~~156. The method of claim 148, wherein the flip receptor comprises a PD-1 exodomain; a transmembrane domain isolated from a CD3 polypeptide, CD4, CD8α, CD28, CD134, or CD137; and an endodomain from CD28, CD134, CD137, CD278, and/or CD3ζ fused in frame to the C-terminal end of the PD-1 exodomain.~~

~~157. The method of claim 148, wherein the flip receptor comprises a PD-1 exodomain; and a transmembrane domain and endodomain isolated from a CD3 polypeptide, CD4, CD8α, CD28, CD134, or CD137 fused in frame to the C-terminal end of the PD-1 exodomain.~~

~~158. The method of any one of claims 138 to 143, wherein the engineered antigen receptor is selected from the group consisting of: an engineered TCR, a CAR, a Daric, or a zetakine.~~

~~159. The method of any one of claims 138 to 158, wherein the donor repair template comprises a 5′ homology arm homologous to a human PD-1 gene sequence 5′ of the DSB and a 3′ homology arm homologous to a human PD-1 gene sequence 3′ of the DSB.~~

~~160. The method of claim 159, wherein the lengths of the 5′ and 3′ homology arms are independently selected from about 100 bp to about 2500 bp.~~

~~161. The method of claim 159 or claim 160, wherein the lengths of the 5′ and 3′ homology arms are independently selected from about 600 bp to about 1500 bp.~~

~~162. The method of any one of claims 159 to 161, wherein the 5´homology arm is about 1500 bp and the 3´ homology arm is about 1000 bp.~~

~~163. The method of any one of claims 159 to 162, wherein the 5´homology arm is about 600 bp and the 3´ homology arm is about 600 bp.~~

~~164. The method of any one of claims 159 to 163, wherein a viral vector is used to introduce the donor repair template into the cell.~~

~~165. The method of claim 164, wherein the viral vector is a recombinant adeno-associated viral vector (rAAV) or a retrovirus.~~

~~166. The method of claim 165, wherein the rAAV has one or more ITRs from AAV2.~~

~~167. The method of claim 165 or claim 166, wherein the rAAV has a serotype selected from the group consisting of: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, and AAV10.~~

~~168. The method of any one of claims 165 to 167, wherein the rAAV has an AAV2 or AAV6 serotype.~~

~~169. The method of claim 165, wherein the retrovirus is a lentivirus.~~

~~170. The method of claim 169, wherein the lentivirus is an integrase deficient lentivirus (IDLV).~~

~~171. A method of treating, preventing, or ameliorating at least one symptom of a cancer, infectious disease, autoimmune disease, inflammatory disease, and immunodeficiency, or~~

~~condition associated therewith, comprising administering to the subject an effective amount of the composition of claim 122 or claim 123.~~

~~172. A method of treating a solid cancer comprising administering to the subject an effective amount of the composition of claim 122 or claim 123.~~

~~173. The method of claim 172, wherein the solid cancer comprises liver cancer, pancreatic cancer, lung cancer, breast cancer, ovarian cancer, prostate cancer, testicular cancer, bladder cancer, brain cancer, sarcoma, head and neck cancer, bone cancer, thyroid cancer, kidney cancer, or skin cancer.~~

~~174. A method of treating a hematological malignancy comprising administering to the subject an effective amount of the composition of claim 122 or claim 123.~~

~~175. The method of claim 174, wherein the hematological malignancy is a leukemia, lymphoma, or multiple myeloma.~~

1. A polypeptide comprising an I-OnuI homing endonuclease (HE) variant comprising an amino acid sequence at least 95% identical to the amino acid sequence set forth in SEQ ID NO: 61, and that binds and cleaves the target polynucleotide sequence in the human program cell death 1 (PD-1) gene set forth in SEQ ID NO: 30.

2. The polypeptide of claim 1, wherein the I-OnuI HE variant comprises the following amino acid substitutions: L26G, R28S, R30L, N32R, K34R, S35G, S36T, V37A, G38R, S40H, E42R, G44S, Q46A, T48V, V68I, A70T, S72D, N75R, A76Y, S78R, K80R, I100V, L138M, T143N, S159P, E178D, C180S, N184R, I186R, K189N, S190V, K191N, L192A, G193R, Q195R, S201E, T203S, K207R, Y223H, K225Y, K227G, F232R, D236Q, V238R, and T240E of SEQ ID NO: 4.

3. The polypeptide of claim 1, wherein the I-OnuI HE variant comprises the following amino acid substitutions: L26G, R28S, R30L, N32R, K34R, S35G, S36T, V37A, G38R, S40H, E42R, G44S, Q46T, T48V, V68I, A70T, S72D, N75R, A76Y, S78R, K80C, I100V, V132A, L138M, T143N, S155G, S159P, E178D, C180S, N184R, I186R, K189N, S190V, K191N, L192A, G193R, Q195R, S201E, T203S, K207R, Y223H, K225Y, K227G, F232R, D236Q, V238R, and T240E of SEQ ID NO: 4.

4. The polypeptide of claim 1, wherein the I-OnuI HE variant comprises the following amino acid substitutions: L26G, R28S, R30L, N32R, K34R, S35G, S36T, V37A, G38R, S40H, E42R, G44S, Q46T, T48M, V68I, A70T, S72N, N75H, A76Y, S78T, K80R, I100V, L138M, T143N, S159P, E178D, C180S, N184R, I186R, K189N, S190V, K191N, L192A, G193R, Q195R, S201E, T203S, K207R, Y223H, K225Y, K227G, F232R, D236Q, V238R, and T240E of SEQ ID NO: 4.

5. The polypeptide of claim 1, wherein the I-OnuI HE variant comprises the following amino acid substitutions: L26G, R28S, R30L, N32R, K34R, S35G, S36T, V37A, G38R, S40H, E42R, G44S, Q46T, T48M, V68S, A70Y, S72N, N75H, A76Y, K80E, T82F, L138M, T143N, S159P, E178D, C180S, N184R, I186R, K189N, S190V, K191N, L192A, G193R, Q195R, S201E, T203S, K207R, Y223H, K225Y, K227G, F232R, D236Q, V238R, and T240E of SEQ ID NO: 4.

6. The polypeptide of claim 1, wherein the I-OnuI HE variant comprises the following amino acid substitutions: L26G, R28S, R30L, N32R, K34R, S35G, S36T, V37A, G38R, S40H, E42R, G44S, Q46T, T48M, V68S, A70L, S72N, N75H, A76Y, K80V, T82Y, L138M, T143N, S159P, E178D, C180S, N184R, I186R, K189N, S190V, K191N, L192A, G193R, Q195R, S201E, T203S, K207R, Y223H, K225Y, K227G, F232R, D236Q, V238R, and T240E of SEQ ID NO: 4.

7. The polypeptide of claim 1, wherein the I-OnuI HE variant comprises the following amino acid substitutions: L26G, R28S, R30L, N32R, K34R, S35G, S36T, V37G, G38R, S40H,

E42R, G44S, Q46T, T48M, V68S, A70T, S72N, N75H, A76Y, K80V, T82Y, L138M, T143N, S159P, E178D, C180S, N184R, I186R, K189N, S190V, K191N, L192A, G193R, Q195R, S201E, T203S, K207R, Y223H, K225Y, K227G, F232R, D236Q, V238R, and T240E of SEQ ID NO: 4.

8. The polypeptide of claim 1, wherein the I-OnuI HE variant comprises the amino acid sequence set forth in SEQ ID NO: 61.

9. The polypeptide of claim 1, wherein the polypeptide further comprises a TALE DNA binding domain comprising about 9.5 TALE repeat units to about 15.5 TALE repeat units.

10. The polypeptide of claim 9, wherein the TALE DNA binding domain binds a polynucleotide sequence set forth in SEQ ID NO: 31.

11. A polynucleotide encoding the polypeptide of claim 1.

12. An mRNA encoding the polypeptide of claim 1.

13. A vector comprising a polynucleotide encoding the polypeptide claim 1.