

CLAIMS

What is claimed is:

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: 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 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 any one of SEQ ID NOs: 11, 12, and 60-63.

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.
14. The polypeptide of claim 1, wherein the I-OnuI HE variant comprises the amino acid sequence set forth in SEQ ID NO: 11.
15. The polypeptide of claim 1, wherein the I-OnuI HE variant comprises the amino acid sequence set forth in SEQ ID NO: 12.
16. The polypeptide of claim 1, wherein the I-OnuI HE variant comprises the amino acid sequence set forth in SEQ ID NO: 60.
17. The polypeptide of claim 1, wherein the I-OnuI HE variant comprises the amino acid sequence set forth in SEQ ID NO: 61.
18. The polypeptide of claim 1, wherein the I-OnuI HE variant comprises the amino acid sequence set forth in SEQ ID NO: 62.
19. The polypeptide of claim 1, wherein the I-OnuI HE variant comprises the amino acid sequence set forth in SEQ ID NO: 63.