WHAT IS CLAIMED IS:

1. A polynucleotide encoding a Ubiquitin Protein Ligase E3A (Ube3a) polypeptide or a biological equivalent thereof, wherein the Ube3a polypeptide or biological equivalent thereof has an amino acid sequence that differs from a wildtype Ube3a protein at least by addition of one or more glycosylation sites.

2. The polynucleotide of claim 1, comprising a nucleotide (nt) sequence selected from any one of the following: nt61 to nt2619 of SEQ ID NO: 13, nt61 to nt2688 of SEQ ID NO: 15, nt61 to nt2679 of SEQ ID NO: 17, nt61 to nt2673 of SEQ ID NO: 25, nt61 to nt2673 of SEQ ID NO: 27, nt61 to nt2610 of SEQ ID NO: 29; SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, or a sequence having at least about 90%, or at least about 95%, or at least about 99% identity to each thereof.

3. The polynucleotide of claim 1 or 2, further comprising a regulatory sequence which directs expression of the Ube3a polypeptide or the biological equivalent thereof, optionally wherein the regulatory sequence comprises one or more of the following: a promoter, an intron, an enhancer, a polyadenylation signal, a terminator, a silencer, a TATA box, or a Woodchuck Hepatitis Virus (WHP) Posttranscriptional Regulatory Element (WPRE), optionally wherein the promoter is selected from the following: an MNDU3 promoter, a CMV promoter, a PGK promoter, a MNDU promoter, or an EF1 alpha promoter, optionally wherein the MNDU3 promoter comprises a sequence of SEQ ID NO: 3, wherein the CMV promoter comprises a sequence selected from SEQ ID NO: 1, 2, or 34, wherein the PKG promoter comprise a sequence of SEQ ID NO: 4, wherein the MNDU promoter comprises a sequence of SEQ ID NO: 5, and wherein the EF1alpha promoter comprises a sequence of SEQ ID NO: 6.

4. The polynucleotide of any one of claims 1 to 3, further comprising one or more of the following: a polypurine tract sequence (PPT), a central PPT (cPPT), an R region, a U5, an encapsidation signal (Psi), a Rev-Responsive Element (RRE), a full-length U3 or a fragment thereof, a detectable or selection maker, a polynucleotide encoding a detectable or selection polypeptide, a regulatory sequence directing expression of the detectable or selection polypeptide, or a coding sequence for a self-cleaving peptide located between the coding

sequence for the detectable or selection polypeptide and the sequence encoding the Ube3a polypeptide or the biological equivalent thereof.

5. A recombinant Ubiquitin Protein Ligase E3A (Ube3a) polypeptide or a biological equivalent thereof whose amino acid sequence differs from a wildtype Ube3a protein at least by addition of one or more glycosylation sites.

6. The polynucleotide or recombinant Ube3a polypeptide or the biological equivalent thereof of any one of claims 1 to 5, wherein the glycosylation is an N-linked glycosylation and wherein the glycosylation site comprise a consensus sequence of NXaaT or NXaaS, wherein Xaa is any amino acid residue optionally except proline (P).

7. The polynucleotide or recombinant Ube3a polypeptide or the biological equivalent thereof of any one of claims 1 to 6, wherein the glycosylation sites are at amino acid (aa) positions of the polypeptide or protein corresponding to one or more of those selected from the following:

aa62 to aa64 of SEQ ID NO: 14, aa96 to aa98 of SEQ ID NO: 14, aa102 to aa104 of SEQ ID NO: 14, aa219 to aa221 of SEQ ID NO: 14, aa354 to aa356 of SEQ ID NO: 14, aa591 to aa593 of SEQ ID NO: 14, aa622 to aa624 of SEQ ID NO: 14, aa805 to aa807 of SEQ ID NO: 14;

aa85 to aa87 of SEQ ID NO: 16, aa119 to aa121 of SEQ ID NO: 16, aa125 to aa127 of SEQ ID NO: 16, aa242 to aa244 of SEQ ID NO: 16, aa377 to aa379 of SEQ ID NO: 16, aa614 to aa616 of SEQ ID NO: 16, aa645 to aa647 of SEQ ID NO: 16, aa828 to aa830 of SEQ ID NO: 16;

aa82 to aa84 of SEQ ID NO: 18, aa116 to aa118 of SEQ ID NO: 18, aa 122 to aa124 of SEQ ID NO: 18, aa239 to aa241 of SEQ ID NO: 18, aa374 to aa376 of SEQ ID NO: 18, aa611 to aa613 of SEQ ID NO: 18, aa642 to aa644 of SEQ ID NO: 18, aa825 to aa827 of SEQ ID NO: 18;

aa83 to aa85 of SEQ ID NO: 26 or 28, aa117 to aa119 of SEQ ID NO: 26 or 28, aa123 to aa125 of SEQ ID NO: 26 or 28, aa 237 to aa239 of SEQ ID NO: 26 or 28, aa372 to aa374 of

SEQ ID NO: 26 or 28, aa609 to aa611 of SEQ ID NO: 26 or 28, aa640 to aa642 of SEQ ID NO: 26 or 28, aa823 to aa825 of SEQ ID NO: 26 or 28;

aa62 to aa64 of SEQ ID NO: 30, aa96 to aa98 of SEQ ID NO: 30, aa102 to aa104 of SEQ ID NO: 30, aa216 to aa218 of SEQ ID NO: 30, aa351 to aa353 of SEQ ID NO: 30, aa588 to aa590 of SEQ ID NO: 30, aa619 to aa621 of SEQ ID NO: 30, or aa802 to aa804 of SEQ ID NO: 30.

8. The polynucleotide or recombinant Ube3a polypeptide or the biological equivalent thereof of any one of claims 1 to 7, wherein the Ube3a polypeptide or the biological equivalent thereof comprises eight glycosylation sites at an amino acid (aa) positions corresponding to the eight aa positions as identified in any one of the following (a) to (e):

(a) aa62 to aa64 of SEQ ID NO: 14, aa96 to aa98 of SEQ ID NO: 14, aa102 to aa104 of SEQ ID NO: 14, aa219 to aa221 of SEQ ID NO: 14, aa354 to aa356 of SEQ ID NO: 14, aa591 to aa 593 of SEQ ID NO: 14, aa622 to aa624 of SEQ ID NO: 14, aa 805 to aa 807 of SEQ ID NO: 14;

(b) aa85 to aa87 of SEQ ID NO: 16, aa119 to aa121 of SEQ ID NO: 16, aa125 to aa127 of SEQ ID NO: 16, aa242 to aa244 of SEQ ID NO: 16, aa377 to aa379 of SEQ ID NO: 16, aa614 to aa616 of SEQ ID NO: 16, aa645 to aa647 of SEQ ID NO: 16, aa828 to aa830 of SEQ ID NO: 16;

(c) aa82 to aa84 of SEQ ID NO: 18, aa116 to aa118 of SEQ ID NO: 18, aa 122 to aa124 of SEQ ID NO: 18, aa239 to aa241 of SEQ ID NO: 18, aa374 to aa376 of SEQ ID NO: 18, aa611 to aa613 of SEQ ID NO: 18, aa642 to aa644 of SEQ ID NO: 18, aa825 to aa827 of SEQ ID NO: 18;

(d) aa83 to aa85 of SEQ ID NO: 26 or 28, aa117 to aa119 of SEQ ID NO: 26 or 28, aa123 to aa125 of SEQ ID NO: 26 or 28, aa 237 to aa239 of SEQ ID NO: 26 or 28, aa372 to aa374 of SEQ ID NO: 26 or 28, aa609 to aa611 of SEQ ID NO: 26 or 28, aa640 to aa642 of SEQ ID NO: 26 or 28, aa823 to aa825 of SEQ ID NO: 26 or 28;

(e) aa62 to aa64 of SEQ ID NO: 30, aa96 to aa98 of SEQ ID NO: 30, aa102 to aa104 of SEQ ID NO: 30, aa216 to aa218 of SEQ ID NO: 30, aa351 to aa353 of SEQ ID NO: 30, aa588 to aa590 of SEQ ID NO: 30, aa619 to aa621 of SEQ ID NO: 30, or aa802 to aa804 of SEQ ID NO: 30.

9. The polynucleotide or recombinant Ube3a polypeptide or the biological equivalent thereof of any one of claims 1 to 8, wherein the Ube3a polypeptide or the biological equivalent thereof comprises one or more of mutated amino acid residue(s) at aa position(s) corresponding to one or more of those selected from the following, thereby forming one or more of glycosylation site(s):

aa64 of SEQ ID NO: 14, aa98 of SEQ ID NO: 14, aa104 of SEQ ID NO: 14, aa221 of SEQ ID NO: 14, aa356 of SEQ ID NO: 14, aa591 of SEQ ID NO: 14, aa624 of SEQ ID NO: 14, aa805 of SEQ ID NO: 14, aa 806 of SEQ ID NO: 14;

aa87 of SEQ ID NO: 16, aa121 of SEQ ID NO: 16, aa127 of SEQ ID NO: 16, aa244 of SEQ ID NO: 16, aa379 of SEQ ID NO: 16, aa614 of SEQ ID NO: 16, aa647 of SEQ ID NO: 16, aa828 of SEQ ID NO: 16, aa829 of SEQ ID NO: 16;

aa84 of SEQ ID NO: 18, aa118 of SEQ ID NO: 18, aa124 of SEQ ID NO: 18, aa241 of SEQ ID NO: 18, aa376 of SEQ ID NO: 18, aa611 of SEQ ID NO: 18, aa644 of SEQ ID NO: 18, aa825 of SEQ ID NO: 18, aa826 of SEQ ID NO: 18;

aa85 of SEQ ID NO: 26 or 28, aa119 of SEQ ID NO: 26 or 28, aa125 of SEQ ID NO: 26 or 28, aa239 of SEQ ID NO: 26 or 28, aa374 of SEQ ID NO: 26 or 28, aa609 of SEQ ID NO: 26 or 28, aa610 of SEQ ID NO: 26 or 28, aa642 of SEQ ID NO: 26 or 28, aa823 of SEQ ID NO: 26 or 28;

aa64 of SEQ ID NO: 30, aa98 of SEQ ID NO: 30, aal04 of SEQ ID NO: 30, aa218 of SEQ ID NO: 30, aa353 of SEQ ID NO: 30, aa588 of SEQ ID NO: 30, aa589 of SEQ ID NO: 30, aa621 of SEQ ID NO: 30, or aa802 of SEQ ID NO: 30,

optionally wherein any one or any two or all three amino acid residue(s) in at least one of the glycosylation sites is or are mutated compared to a wild type Ube3a polypeptide or protein, thereby constituting the glycosylation site.

10. The polynucleotide or recombinant Ube3a polypeptide or the biological equivalent thereof of any one of claims 1 to 9, wherein the Ube3a polypeptide or the biological equivalent thereof comprises nine mutated amino acid residues at aa positions corresponding to those identified in any one of the following (a) to (e), thereby forming eight glycosylation sites:

(a) aa64 of SEQ ID NO: 14, aa98 of SEQ ID NO: 14, aa104 of SEQ ID NO: 14, aa221 of SEQ ID NO: 14, aa356 of SEQ ID NO: 14, aa591 of SEQ ID NO: 14, aa624 of SEQ ID NO: 14, aa805 of SEQ ID NO: 14, and aa806 of SEQ ID NO: 14;

(b) aa87 of SEQ ID NO: 16, aa121 of SEQ ID NO: 16, aa127 of SEQ ID NO: 16, aa244 of SEQ ID NO: 16, aa379 of SEQ ID NO: 16, aa614 of SEQ ID NO: 16, aa647 of SEQ ID NO: 16, aa828 of SEQ ID NO: 16, and aa829 of SEQ ID NO: 16;

(c) aa84 of SEQ ID NO: 18, aa118 of SEQ ID NO: 18, aa124 of SEQ ID NO: 18, aa241 of SEQ ID NO: 18, aa376 of SEQ ID NO: 18, aa611 of SEQ ID NO: 18, aa644 of SEQ ID NO: 18, aa825 of SEQ ID NO: 18, and aa826 of SEQ ID NO: 18;

(d) aa85 of SEQ ID NO: 26 or 28, aa119 of SEQ ID NO: 26 or 28, aa125 of SEQ ID NO: 26 or 28, aa239 of SEQ ID NO: 26 or 28, aa374 of SEQ ID NO: 26 or 28, aa609 of SEQ ID NO: 26 or 28, aa610 of SEQ ID NO: 26 or 28, aa642 of SEQ ID NO: 26 or 28, and aa823 of SEQ ID NO: 26 or 28; or

(e) aa64 of SEQ ID NO: 30, aa98 of SEQ ID NO: 30, aa104 of SEQ ID NO: 30, aa218 of SEQ ID NO: 30, aa353 of SEQ ID NO: 30, aa588 of SEQ ID NO: 30, aa589 of SEQ ID NO: 30, aa621 of SEQ ID NO: 30, and aa802 of SEQ ID NO: 30.

11. The polynucleotide or recombinant Ube3a polypeptide or the biological equivalent thereof of any one of claims 1 to 10 , wherein the Ube3a polypeptide or the biological equivalent thereof comprises eight mutated amino acid residues at aa positions corresponding to those identified in any one of the following (a) to (e), thereby forming seven glycosylation sites:

(a) aa64 of SEQ ID NO: 14, aa98 of SEQ ID NO: 14, aa221 of SEQ ID NO: 14, aa356 of SEQ ID NO: 14, aa591 of SEQ ID NO: 14, aa624 of SEQ ID NO: 14, aa805 of SEQ ID NO: 14, and aa806 of SEQ ID NO: 14;

(b) aa87 of SEQ ID NO: 16, aa121 of SEQ ID NO: 16, aa244 of SEQ ID NO: 16, aa379 of SEQ ID NO: 16, aa614 of SEQ ID NO: 16, aa647 of SEQ ID NO: 16, aa828 of SEQ ID NO: 16, and aa829 of SEQ ID NO: 16;

(c) aa84 of SEQ ID NO: 18, aa118 of SEQ ID NO: 18, aa241 of SEQ ID NO: 18, aa376 of SEQ ID NO: 18, aa611 of SEQ ID NO: 18, aa644 of SEQ ID NO: 18, aa825 of SEQ ID NO: 18, and aa826 of SEQ ID NO: 18;

(d) aa85 of SEQ ID NO: 26 or 28, aa119 of SEQ ID NO: 26 or 28, aa239 of SEQ ID NO: 26 or 28, aa374 of SEQ ID NO: 26 or 28, aa609 of SEQ ID NO: 26 or 28, aa610 of SEQ ID NO: 26 or 28, aa642 of SEQ ID NO: 26 or 28, and aa823 of SEQ ID NO: 26 or 28; or

(e) aa64 of SEQ ID NO: 30, aa98 of SEQ ID NO: 30, aa218 of SEQ ID NO: 30, aa353 of SEQ ID NO: 30, aa588 of SEQ ID NO: 30, aa589 of SEQ ID NO: 30, aa621 of SEQ ID NO: 30, and aa802 of SEQ ID NO: 30.

12. The polynucleotide or recombinant Ube3a polypeptide or the biological equivalent thereof of claim any one of claims 1 to 11, wherein the formed glycosylation site comprises a consensus sequence of NXaaT or NXaaS, wherein Xaa is any amino acid residue optionally except proline (P).

13. The polynucleotide or recombinant Ube3a polypeptide or the biological equivalent thereof of any one of claims 1 to 12, wherein the mutated amino acid residue(s) is/are selected from one or more of:

T or S at an aa position corresponding to aa64 of SEQ ID NO: 14, T or S at an aa position corresponding to aa98 of SEQ ID NO: 14, T or S at an aa position corresponding to aa104 of SEQ ID NO: 14, T or S at an aa position corresponding to aa221 of SEQ ID NO: 14, T or S at an aa position corresponding to aa356 of SEQ ID NO: 14, N at an aa position corresponding to aa591 of SEQ ID NO: 14, T or S at an aa position corresponding to aa624 of SEQ ID NO: 14, N at an aa position corresponding to aa805 of SEQ ID NO: 14, and N at an aa position corresponding to aa806 of SEQ ID NO: 14;

T or S at an aa position corresponding to aa87 of SEQ ID NO: 16, T or S at an aa position corresponding to aa121 of SEQ ID NO: 16, T or S at an aa position corresponding to aa127 of SEQ ID NO: 16, T or S at an aa position corresponding to aa244 of SEQ ID NO: 16, T or S at an aa position corresponding to aa379 of SEQ ID NO: 16, N at an aa position corresponding to aa614 of SEQ ID NO: 16, T or S at an aa position corresponding to aa647 of SEQ ID NO: 16, N at an aa position corresponding to aa828 of SEQ ID NO: 16, and N at an aa position corresponding to aa829 of SEQ ID NO: 16;

T or S at an aa position corresponding to aa84 of SEQ ID NO: 18, T or S at an aa position corresponding to aa118 of SEQ ID NO: 18, T or S at an aa position corresponding to aa124 of

SEQ ID NO: 18, T or S at an aa position corresponding to aa241 of SEQ ID NO: 18, T or S at an aa position corresponding to aa376 of SEQ ID NO: 18, N at an aa position corresponding to aa611 of SEQ ID NO: 18, T or S at an aa position corresponding to aa644 of SEQ ID NO: 18, N at an aa position corresponding to aa825 of SEQ ID NO: 18, and N at an aa position corresponding to aa826 of SEQ ID NO: 18;

T or S at an aa position corresponding to aa85 of SEQ ID NO: 26 or 28, T or S at an aa position corresponding to aa119 of SEQ ID NO: 26 or 28, T or S at an aa position corresponding to aa125 of SEQ ID NO: 26 or 28, T or S at an aa position corresponding to aa239 of SEQ ID NO: 26 or 28, T or S at an aa position corresponding to aa374 of SEQ ID NO: 26 or 28, N at an aa position corresponding to aa609 of SEQ ID NO: 26 or 28, N at an aa position corresponding to aa610 of SEQ ID NO: 26 or 28, T or S at an aa position corresponding to aa642 of SEQ ID NO: 26 or 28, and N at an aa position corresponding to aa823 of SEQ ID NO: 26 or 28; or

T or S at an aa position corresponding to aa64 of SEQ ID NO: 30, T or S at an aa position corresponding to aa98 of SEQ ID NO: 30, T or S at an aa position corresponding to aa104 of SEQ ID NO: 30, T or S at an aa position corresponding to aa218 of SEQ ID NO: 30, T or S at an aa position corresponding to aa353 of SEQ ID NO: 30, N at an aa position corresponding to aa588 of SEQ ID NO: 30, N at an aa position corresponding to aa589 of SEQ ID NO: 30, T or S at an aa position corresponding to aa621 of SEQ ID NO: 30, and N at an aa position corresponding to aa802 of SEQ ID NO: 30.

14. The polynucleotide or recombinant Ube3a polypeptide or the biological equivalent thereof of any one of claims 1 to 13, wherein the Ube3a polypeptide or the biological equivalent thereof comprises one or more non-naturally occurring glycosylation sites.

15. The polynucleotide or recombinant Ube3a polypeptide or the biological equivalent thereof of any one of claims 1 to 14, wherein the Ube3a polypeptide or the biological equivalent thereof comprises an amino acid sequence selected from the following:

aa21 to aa 872 of SEQ ID NO: 14, aa21 to aa 895 of SEQ ID NO: 16, aa21 to aa 892 of SEQ ID NO: 18, aa21 to aa 890 of SEQ ID NO: 26, aa21 to aa 890 of SEQ ID NO: 28, aa21 to aa 869 of SEQ ID NO: 30, or a sequence having at least about 90%, or at least about 95%, or at least about 99% identity to each thereof.

16. The polynucleotide or recombinant Ube3a polypeptide or biological equivalent thereof of any one of claims 1 to 15, wherein the Ube3a polypeptide or the biological equivalent thereof is derived from a wildtype human Ube3a protein or a wildtype murine Ube3a protein, optionally wherein the wildtype human Ube3a protein has an amino acid sequence that is or comprises any one of SEQ ID NOs: 8, 10, or 12, or a sequence having at least about 90%, or at least 95%, or at least about 99% identity to each thereof, and wherein the wildtype murine Ube3a protein has an amino acid sequence that is or comprises any one of SEQ ID NOs: 20, 22, or 24, or a sequence having at least about 90%, or at least 95%, or at least 99% identity to each thereof.

17. The polynucleotide or recombinant Ube3a polypeptide or the biological equivalent thereof of any one of claims 1 to 16, wherein the Ube3a polypeptide or the biological equivalent thereof further comprises a signal peptide, optionally wherein the signal peptide is a secretion signal, optionally wherein the signal peptide is selected from the following: an antibody heavy/light chain secretion signal, a twin-arginine transport protein secretion signal, an Interleukin-2 (IL2) secretion signal, an Interleukin-4 (IL4) secretion signal, Interleukin-10 (IL10) secretion signal, an Interleukin-3 (IL3) secretion signal, an Interleukin-7 (IL7) secretion signal, an human IL2 secretion signal, a human OSM secretion signal, a VSV-G secretion signal, a Mouse Ig Kappa secretion signal, a Human IgG2 H secretion signal, a BM40 secretion signal, a Secrecon secretion signal, a Human IgKVIII secretion signal, a CD33 secretion signal, a tPA secretion signal, a Human Chymotrypsinogen secretion signal, a Human trypsinogen-2 secretion signal, a Gaussia luc secretion signal, a Albumin(HSA) secretion signal, an Influenza Haemagglutinin secretion signal, a Human insulin secretion signal, or a Silkworm Fibroin LC, optionally wherein the signal peptide has an amino acid sequence of aa1 to aa20 of SEQ ID NO: 14.

18. The polynucleotide or recombinant Ube3a polypeptide or the biological equivalent thereof of any one of claims 1 to 17, wherein the Ube3a polypeptide or the biological equivalent thereof has an amino acid sequence selected from any one of SEQ ID NOs: 14, 16, 18, 26, 28 and 30, or a sequence having at least about 90%, or at least about 95%, or at least about 99% identity to each thereof.

19. The polynucleotide or recombinant Ube3a polypeptide or the biological equivalent thereof of any one of claims 1 to 18, wherein the Ube3a polypeptide or the biological equivalent thereof further comprises a self-cleaving peptide and a detectable or selection polypeptide.

20. A polynucleotide that is reverse or complement or reverse-complement to the polynucleotide of any one of claims 1 to 4 and 6 to 19.

21. A vector comprising the polynucleotide of any one of claims 1 to 4 and 6 to 20.

22. The vector of claim 21, wherein the vector is a viral vector or a non-viral vector.

23. The vector of claim 22, wherein the viral vector is selected from a retroviral vector, an adenoviral vector, an adeno-associated viral vector, or a Herpes viral vector, optionally wherein the retroviral vector is a lentiviral vector, optionally wherein the lentiviral vector is a self-inactivating lentiviral vector, optionally having a U3 region lacking a TATA box and optionally one or more of transcription factor binding site(s), optionally wherein the lentiviral vector is derived from human immunodeficiency virus (HIV).

24. The vector of claims 22, wherein non-viral vector is a plasmid.

25. The vector of any one of claims 21 to 24, further comprising a regulatory sequence operatively linked to the polynucleotide and directing the replication of the polynucleotide.

26. An isolated or engineered cell comprising one or more of the following: a polynucleotide of any one of claims 1 to 4 and 6 to 20, a vector of any one of claims 21 to 25, or a recombinant Ube3a polypeptide or the biological equivalent thereof of any one of claims 5 to 19, thereby expressing and secreting a recombinant Ube3a polypeptide or a biological equivalent thereof of any one of 5 to 19, optionally wherein at least one or more of the glycosylation sites of the recombinant Ube3a polypeptide are glycosylated in the isolated or engineered cell, optionally wherein the isolated or engineered cell is a mammalian cell, optionally wherein the mammalian cell is a murine cell or a human cell.

27. The isolated or engineered cell of claim 26, which is selected from a stem cell, a progenitor cell, an induced pluripotent stem cell (iPSC), an embryonic stem cell, an adult or somatic stem cell, a mesenchymal stem cell, a neural stem cell, or a progeny of each thereof.

28. The isolated or engineered cell of claim 26 or 27, which is a hematopoietic stem cell or a progeny thereof, optionally wherein the hematopoietic stem cell or a progeny thereof is CD34+.

29. The isolated or engineered cell of any one of claims 26 to 28, further comprising a detectable maker.

30. An isolated cell population comprising a cell of any one of claims 26 to 29 or a progeny thereof, optionally wherein the cell population expresses CD4, CD14 and HLADR, optionally wherein at least 90% of the cells in the population are CD4+, at least 95% of the cell in the population are CD14+ and at least 95% of the cells in the population are HLADR+.

31. The isolated cell population of any one of claims 26 to 30, deriving macrophages under a suitable condition.

32. The isolated cell population of any one of claims 26 to 31, comprising substantially macrophages.

33. The isolated cell population of any one of claims 26 to 32, which is substantially homogenous.

34. The isolated cell population of any one of claims 26 to 33, wherein the cell of the population further comprises a detectable maker.

35. Use of one or more of the following: a polynucleotide of any one of claims 1 to 4 and 6 to 20, a vector of any one of claims 21 to 25, a recombinant Ube3a polypeptide or the biological equivalent thereof of any one of 5 to 19, a cell of any one of claims 26 to 29, or a cell population

of any one of claims 30 to 34 in the manufacture of a medicament for treating, preventing or reversing Angelman syndrome in a subject carrying a defective Ube3a allele, wherein the polynucleotide, vector, recombinant Ube3a polypeptide or the biological equivalent thereof, cell, or cell population is to be locally or systemically administered to the subject.

36. Use of one or more of the following: a polynucleotide of any one of claims 1 to 4 and 6 to 20, a vector of any one of claims 21 to 25, a recombinant Ube3a polypeptide or the biological equivalent thereof of any one of 5 to 19, a cell of any one of claims 26 to 29, or a cell population of any one of claims 30 to 34, in the manufacture of a medicament for expressing a Ube3a polypeptide or a biological equivalent thereof in a subject, wherein the polynucleotide, vector, recombinant Ube3a polypeptide or the biological equivalent thereof, cell, or cell population is to be administered to the subject, optionally wherein the subject carries a defective Ube3a gene.

37. The use of claim 35 or 36, wherein the cell or cell population is to be administered at a dose of about 1.0×10^4 to about 1×10^{15} cells/kg body weight of the subject, optionally wherein the subject is symptomatic or asymptomatic for Angelman syndrome, optionally wherein the subject is selected from the group consisting of a mammal, a fetus, an infant, a prepubescent subject, and an adult.

38. An isolated or engineered cell comprising one or more of the following: a polynucleotide of any one of claims 1 to 4 and 6 to 20, a vector of any one of claims 21 to 25, a recombinant Ube3a polypeptide or the biological equivalent thereof of any one of 5 to 19, thereby producing the polynucleotide, the vector, or the recombinant Ube3a polypeptide or the biological equivalent thereof, optionally wherein at least one or more of the glycosylation sites of the produced recombinant Ube3a polypeptide or the biological equivalent thereof are glycosylated in the isolated or engineered cell, optionally wherein the isolated or engineered cell is a eukaryotic or a prokaryotic cell, optionally, wherein the isolated or engineered cell is a mammalian cell.

39. A clonal population of the cells of claim 38.

40. A viral packaging system comprising: (a) the vector of any one of claims 21 to 25; (b) one or more packaging plasmids; and (c) an envelope plasmid.

41. The viral packaging system of claim 40, further comprising (d) a packaging cell line, optionally wherein the packaging cell line is an HEK-293 cell line.

42. A method for producing a viral particle, comprising transducing a packaging cell line with the system of claim 40 under conditions suitable to package the viral particle, optionally wherein the packaging cell line is an HEK-293 cell line.

43. An *in vitro* method to express a secreted Ube3a polypeptide or the biological equivalent thereof comprising growing a cell of any one of claims 26 to 29 and 38 under conditions that allow for the expression of the recombinant Ube3a polypeptide or a biological equivalent thereof, optionally wherein at least one or more of the glycosylation sites of the expressed recombinant Ube3a polypeptide or the biological equivalent thereof are glycosylated in the cell of any one of claims 26 to 29 and 38.

44. A composition comprising a carrier and one or more of: a polynucleotide of any one of claims 1 to 4 and 6 to 20, a vector of any one of claims 21 to 25, a recombinant Ube3a polypeptide or the biological equivalent thereof of any one of claims 5 to 19, a cell of any one of claims 26 to 29 and 38, a cell population of any one of claims 30 to 34, or a clonal population of claim 39, optionally wherein the carrier is a pharmaceutically acceptable carrier.

45. A kit comprising one or more of: a polynucleotide of any one of claims 1 to 4 and 6 to 20, a vector of any one of claims 21 to 25, a recombinant Ube3a polypeptide or the biological equivalent thereof of any one of claims 5 to 19, a cell of any one of claims 26 to 29 and 38, a cell population of any one of claims 30 to 34, or a clonal population of claim 39, and optionally instructions for use.

46. The polynucleotide of any one of claims 1 to 4 and 6 to 20, the vector of any one of claims 21 to 25, the recombinant Ube3a polypeptide or the biological equivalent thereof of any

one of claims 5 to 19, the cell of any one of claims 26 to 29 and 38, the cell population of any one of claims 30 to 34, or the clonal population of claim 39 for use in therapy.

UBE3A FOR THE TREATMENT OF ANGELMAN SYNDROME

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. § 119(e) of U.S. Provisional Application Nos. 62/890,364 and 62/945,062, filed August 22, 2019 and December 6, 2019, respectively, the contents of each of which is incorporated by reference in its entireties into the present application.

BACKGROUND

[0002] Angelman syndrome (AS) is a genetic neurological disorder with characteristics including delayed development, intellectual disability, severe speech impairment, and problems with movement and balance. Most patients have recurrent seizures and a smaller head size. Most patients display delayed development and other common symptoms appear in early childhood. Children with AS typically have a happy and excitable demeanor. Other symptoms include hyperactivity, a short attention span, and a fascination with water. As patients get older, people with Angelman syndrome become less excitable and sleeping problems improve. However, these patients continue to have intellectual disability, speech impairment, and seizures for the rest of their lives.

[0003] AS is caused by the loss of function of a gene called *UBE3A*. People inherit one copy of the *UBE3A* gene ("Ube3a") from each parent. Both copies of Ube3a are active in many of the body's tissues. In the brain, however, only the maternal copy is active. This parent-specific activation of a gene is caused by a process called genomic imprinting. If the maternal copy of *UBE3A* is lost because of a deletion or mutation, a person will lack expression of Ube3a in some parts of the brain. No effective therapies are available to treat AS. This disclosure provides a gene therapy to replace or diminish the symptoms and causes of AS.

SUMMARY OF THE DISCLOSURE

[0003a] In one aspect, the present disclosure refers to a polynucleotide encoding a Ubiquitin Protein Ligase E3A (Ube3a) polypeptide or a biological equivalent thereof, wherein the

Ube3a polypeptide or biological equivalent thereof has an amino acid sequence that differs from a wildtype Ube3a protein at least by addition of one or more glycosylation sites.

[0003b] In another aspect, the present disclosure refers to a recombinant Ubiquitin Protein Ligase E3A (Ube3a) polypeptide or a biological equivalent thereof whose amino acid sequence differs from a wildtype Ube3a protein at least by addition of one or more glycosylation sites.

[0003c] In another aspect, the present disclosure refers to a polynucleotide that is reverse or complement or reverse-complement to the polynucleotide as described herein.

[0003d] In another aspect, the present disclosure refers to a vector comprising the polynucleotide as described herein.

[0003e] In another aspect, the present disclosure refers to an isolated or engineered cell comprising one or more of the following: a polynucleotide as described herein, a vector as described herein, or a recombinant Ube3a polypeptide or the biological equivalent thereof as described herein, thereby expressing and secreting a recombinant Ube3a polypeptide or a biological equivalent thereof as described herein, optionally wherein at least one or more of the glycosylation sites of the recombinant Ube3a polypeptide are glycosylated in the isolated or engineered cell, optionally wherein the isolated or engineered cell is a mammalian cell, optionally wherein the mammalian cell is a murine cell or a human cell.

[0003f] In yet another aspect, the present disclosure refers to an isolated cell population comprising a cell as described herein or a progeny thereof, optionally wherein the cell population expresses CD4, CD14 and HLADR, optionally wherein at least 90% of the cells in the population are CD4+, at least 95% of the cell in the population are CD14+ and at least 95% of the cells in the population are HLADR+.

[0003g] In yet another aspect, the present disclosure refers to a use of one or more of the following: a polynucleotide as described herein, a vector as described herein, a recombinant Ube3a polypeptide or the biological equivalent thereof as described herein, a cell as described herein, or a cell population as described herein in the manufacture of a medicament for treating, preventing or reversing Angelman syndrome in a subject carrying a defective Ube3a allele, wherein the polynucleotide, vector, recombinant Ube3a polypeptide or the biological

equivalent thereof, cell, or cell population is to be locally or systemically administered to the subject.

[0003h] In yet another aspect, the present disclosure refers to a use of one or more of the following: a polynucleotide as described herein, a vector as described herein, a recombinant Ube3a polypeptide or the biological equivalent thereof as described herein, a cell as described herein, or a cell population as described herein, in the manufacture of a medicament for expressing a Ube3a polypeptide or a biological equivalent thereof in a subject, wherein the polynucleotide, vector, recombinant Ube3a polypeptide or the biological equivalent thereof, cell, or cell population is to be administered to the subject, optionally wherein the subject carries a defective Ube3a gene.

[0003i] In yet another aspect, the present disclosure refers to an isolated or engineered cell comprising one or more of the following: a polynucleotide as described herein, a vector as described herein, a recombinant Ube3a polypeptide or the biological equivalent thereof as described herein, thereby producing the polynucleotide, the vector, or the recombinant Ube3a polypeptide or the biological equivalent thereof, optionally wherein at least one or more of the glycosylation sites of the produced recombinant Ube3a polypeptide or the biological equivalent thereof are glycosylated in the isolated or engineered cell, optionally wherein the isolated or engineered cell is a eukaryotic or a prokaryotic cell, optionally, wherein the isolated or engineered cell is a mammalian cell.

[0003j] In yet another aspect, the present disclosure refers to a clonal population of the cells as described herein.

[0003k] In yet another aspect, the present disclosure refers to a viral packaging system comprising: (a) the vector as described herein; (b) one or more packaging plasmids; and (c) an envelope plasmid.

[0003l] In yet another aspect, the present disclosure refers to a method for producing a viral particle, comprising transducing a packaging cell line with the system as described herein under conditions suitable to package the viral particle, optionally wherein the packaging cell line is an HEK-293 cell line.

[0003m] In yet another aspect, the present disclosure refers to an *in vitro* method to express a secreted Ube3a polypeptide or the biological equivalent thereof comprising growing a cell as

described herein under conditions that allow for the expression of the recombinant Ube3a polypeptide or a biological equivalent thereof, optionally wherein at least one or more of the glycosylation sites of the expressed recombinant Ube3a polypeptide or the biological equivalent thereof are glycosylated in the cell as described herein.

[0003n] In yet another aspect, the present disclosure refers to a composition comprising a carrier and one or more of: a polynucleotide as described herein, a vector as described herein, a recombinant Ube3a polypeptide or the biological equivalent thereof as described herein, a cell as described herein, a cell population as described herein, or a clonal population as described herein, optionally wherein the carrier is a pharmaceutically acceptable carrier.

[0003o] In yet another aspect, the present disclosure refers to a kit comprising one or more of: a polynucleotide as described herein, a vector as described herein, a recombinant Ube3a polypeptide or the biological equivalent thereof as described herein, a cell as described herein, a cell population as described herein, or a clonal population as described herein, and optionally instructions for use.

[0003p] In yet another aspect, the present disclosure refers to the polynucleotide as described herein, the vector as described herein, the recombinant Ube3a polypeptide or the biological equivalent thereof as described herein, the cell as described herein, the cell population as described herein, or the clonal population as described herein for use in therapy.

[0004] Thus, in one example, provided herein is a recombinant polynucleotide encoding a Ubiquitin Protein Ligase E3A (Ube3a) protein having one or more naturally occurring or non-occurring glycosylation sites, for example, for use in gene therapy and research. In one example, the Ube3a protein has one or more naturally occurring or non-naturally occurring glycosylation sites, or two or more naturally occurring or non-naturally occurring

glycosylation sites, or three or more naturally occurring or non-naturally occurring glycosylation sites. In another example, the Ube3a protein has four or more naturally occurring or non-naturally occurring glycosylation sites. In another example, the Ube3a protein has five or more naturally occurring or non-naturally occurring glycosylation sites. In another example, the Ube3a protein has six or more naturally occurring or non-naturally occurring glycosylation sites. In one example, the Ube3a protein has seven or more, or eight or more naturally occurring or non-naturally occurring glycosylation sites. In one example, the Ube3a protein has naturally occurring and non-naturally occurring glycosylation sites. In one example, the Ube3a protein is non-naturally occurring as disclosed herein, or an equivalent or complement thereof. In one example, the Ube3a protein is naturally occurring but contains one or more naturally occurring or non-naturally occurring glycosylation sites. In another example, the protein is naturally occurring but in each example, the protein has one or more non-naturally occurring glycosylation sites. In one example, the protein is a non-naturally occurring Ube3a protein or polypeptide having one or more non-naturally occurring glycosylation sites. Additionally or alternatively, one or more of the glycosylation sites are non-naturally occurring.

[0005] Also provided are Ube3a proteins having one or more glycosylation sites, or two or more glycosylation sites, or three or more glycosylation sites. In another example, the Ube3a protein has four or more glycosylation sites. In another example, the Ube3a protein has five or more glycosylation sites. In another example, the Ube3a protein has six or more glycosylation sites. In one example, the Ube3a protein has seven or more, or eight or more glycosylation sites. In one example, the protein is non-naturally occurring and contains one or more glycosylation sites. In another example, the protein is naturally occurring but in each example, the protein has one or more glycosylation sites. In one example, the protein is a non-naturally occurring Ube3a protein or polypeptide having one or more non-naturally occurring glycosylation sites. Additionally or alternatively, one or more of the glycosylation sites are non-naturally occurring. In a further example, the proteins further comprise a cell penetrating domain. In yet a further example, the proteins comprise a secretion signal. Additionally or alternatively, the protein further comprises a detectable or purification marker.

[0006] In one example, the protein is encoded by a polynucleotide provided herein or its complement or equivalent thereof, for example, in the Sequence Listing section of this

document as well as equivalents of each thereof. In one example, the polynucleotides also are non-naturally occurring and may optionally comprise a polynucleotide encoding a cell penetrating domain.

[0007] In a further example, the polynucleotide further comprises a promoter operatively linked to the polynucleotide. Non-limiting examples of such include pol II promoters selected from the group of an MNDU3 promoter, a CMV promoter, a PGK promoter, and an EF1alpha promoter. The promoter can be operatively linked to the coding polynucleotides to drive expression in a suitable host system. In a further example, the polynucleotide further comprises a polynucleotide encoding a secretion signal located 5' to the polynucleotide encoding the modified Ube3a protein. Non-limiting examples of secretion signals include a single chain fragment variable secretion signal, a twin-arginine transport protein secretion signal, an IL-4 secretion signal, an IL-2 secretion signal and an IL-10 secretion signal. An exemplary secretion signal polynucleotide is provided below along with equivalents thereof. The polynucleotides can further comprise a polynucleotide that is, or encodes a detectable or purification marker.

[0008] Also provided are vectors comprising the recombinant polynucleotides as described herein. The vectors include vectors for expression in prokaryotic and eukaryotic host cell systems, e.g., a plasmid, or a viral vector such as, baculovirus, a retroviral vector, an adenoviral vector, an AAV vector, or a lentiviral vector. Exemplary vector maps are shown in FIGS. 3A to 3D. In some examples, the recombinant polynucleotide is flanked by inverted terminal repeats (ITRs) of viral vectors, e.g., adenoviral or lentiviral vectors.

[0009] Non-viral vectors may include a plasmid that comprises a heterologous polynucleotide capable of being delivered to a target cell, either in vitro, in vivo or ex-vivo. The heterologous polynucleotide can comprise the mutated Ube3a gene (such as a recombinant polynucleotide as disclosed herein) and can be operably linked to one or more regulatory elements and may control the transcription of the mutated Ube3a gene. As used herein, a vector need not be capable of replication in the ultimate target cell or subject. The term vector may include expression vector and cloning vector. In one example, the vector is the pCCLc plasmid vector.

[0010] The polynucleotides and vectors can be contained in a host cell system for delivery or

expression of the polynucleotides. The cell can be a prokaryotic or a eukaryotic cell. In one example, the host cell is a mammalian cell, e.g., a canine, feline, bovine, equine, murine, rat or human. The mammalian cell can be selected from a stem cell, e.g., an induced pluripotent stem cell (iPSC), an embryonic stem cell, an adult or somatic stem cell. In one example, the stem cell is a mesenchymal stem cell such as for example, a hematopoietic stem cell or a neuronal stem cell. In another example, the stem cell is a mesenchymal stem cell optionally identified by expressing the CD34+ marker.

[0011] Also provided are populations of the cells, that can be heterologous (of different species or having different vectors and polynucleotides) or substantially homogeneous as well as clonal.

[0012] Further provided are compositions comprising one or more of the polynucleotides, protein or polypeptide, vectors, host cells or populations as described herein, and a carrier. In one example, the carrier is a pharmaceutically acceptable carrier.

[0013] Also provided is a viral packaging system comprising: (a) the viral vector as described herein; (b) a packaging plasmid; and (c) an envelope plasmid. In a further example, the system further comprises (d) a packaging cell line, such as, for example, the HEK-293 cell line. The packaging system can be used to transduce a packaging cell line under conditions suitable to package the viral vector.

[0014] Also provided is a method to express a secreted modified Ube3a protein comprising, or alternatively consisting essentially of, or yet further consisting of, growing the host cell as described herein under conditions that allow for the expression of the Ube3a protein. The method can be practiced in vitro, ex vivo or in vivo. Further provided is a method to express modified Ube3a in a subject, comprising or alternatively consisting essentially of, or yet further consisting of, administering an effective amount of the vector or host cell as described herein to the subject, thereby expressing modified Ube3a in the subject. In one example, the subject is a mammal, e.g., a human patient. In a further example, the subject is deficient or carries a defective Ube3a gene. In another example, the mammal is asymptomatic for Angelman syndrome. In another example, the subject is a fetus, an infant or a pre-pubescent subject that is or is not, symptomatic for Angelman syndrome (which is also referred to

herein as AS). In an additional example, the subject is an adult, optionally, an adult human, further optionally, an adult human greater than 18 years of age.

[0015] Further provided is a method to treat Angelman syndrome in a subject carrying a defective Ube3a gene or allele, the method comprising, or alternatively consisting essentially of, or yet further consisting of, administering, for example an effective amount of, one or more of: the polynucleotide, the vector, the host cell, or the Ube3a protein or polypeptide as described herein to the subject, thereby treating Angelman syndrome. In a further example, the subject is deficient or carries a defective Ube3A gene. In one example, the subject is a mammal, e.g. a human patient. In one example, the mammal is symptomatic for Angelman syndrome. In another example, the mammal is asymptomatic for Angelman syndrome. In another example, the subject is a fetus, an infant or a pre-pubescent subject that is or is not, symptomatic for AS. In an additional example, the subject is an adult, optionally, an adult human, further optionally, an adult human greater than 18 years of age.

[0016] Further provided is a kit comprising one or more of: the polynucleotide, the protein or polypeptide, the vector, the host cell, or the population as described herein and optionally, instructions for use.

[0017] The foregoing general description and following detailed description are exemplary and explanatory and are intended to provide further explanation of the disclosure as claimed. Other objects, advantages, and novel features will be readily apparent to those skilled in the art from the following brief description of the drawings and detailed description of the disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] FIGS. 1A – 1F show schematic of Ube3a expressing lentiviral vectors. **FIG. 1A** depicts a self-inactivating lentiviral vector, CCLc-X backbone was used. It is a parental vector, without the transgene expressing Ube3a polynucleotide. An exemplified sequence of a CCLc-MNDU3-X vector is provided as SEQ ID NO: 35. **FIG. 1B** depicts an EGFP vector used as an empty vector control. **FIG. 1C** shows that the modified mouse Ube3a isoform 3 was cloned under the control of the MNDU3 promoter. EGFP was cloned downstream under the control of a PGK promoter. **FIG. 1D** shows that the modified human isoform 1 was cloned under the control of the MNDU3 promoter. EGFP was cloned downstream under the