stop codons present in the variant X gene, begins at a position within 36 or 24 nucleotides in the 3' direction from a position corresponding to residue 411 of WHV PRE sequence set forth in SEQ ID NO:1 or SEQ ID NO:125 and/or residue 1503 of the sequence set forth in SEQ ID NO:2. In some such examples, the variant X gene does not contain an open reading frame of greater than at or about 39 nucleotides in length or greater than at or about 27 nucleotides in length.

[0007] In one aspect, the present disclosure refers to a polynucleotide, comprising a modified post-transcriptional regulatory element (PRE), said modified PRE comprising a variant of a wild-type hepatitis virus X gene, said variant X gene comprising a plurality of stop codons not present in the wild-type X gene; and a nucleic acid encoding a recombinant protein operably linked to the modified PRE. Provided are polynucleotides containing a modified PRE that contains a variant of a wild-type hepatitis virus X gene in which the variant X gene contains a stop codon, such as at least one stop codon, not present in the wild-type X gene and beginning at a position within 32 or 30 nucleotides of a start codon of the X gene. In some examples, the at least one stop codon, e.g. the first stop codon or one or more of a plurality of stop codons present in the variant X gene, begins at a position within 32 or 30 nucleotides in the 3' direction from a position in the variant X gene corresponding to the 5' position of a start codon of the wild-type X protein open reading frame. In some examples, the at least one stop codon, e.g. the first stop codon or one or more of a plurality of stop codons present in the variant X gene, begins at a position within 32 or 30 nucleotides in the 3' direction from a position corresponding to residue 411 of WHV PRE sequence set forth in SEQ ID NO:1 or SEQ ID NO:125 and/or residue 1503 of the sequence set forth in SEQ ID NO:2. In some such examples, the variant X gene has an open reading frame of less than or equal to 33 nucleotides in length.

[0008] Also disclosed is a polynucleotide, comprising a modified post-transcriptional regulatory element (PRE), said modified PRE comprising a variant of a wild-type hepatitis virus X gene, said variant X gene comprising a sequence encoding a post-translational modification signal not present in the wild-type hepatitis virus X gene. In any of such examples of a modified PRE, the at least one stop codon not present in the wild-type X gene includes a plurality of stop codons. Provided are polynucleotides comprising a modified PRE that contains a variant of a wild-type hepatitis virus X gene in which the variant X gene

deletion of all or a contiguous portion of nucleotides corresponding to nucleotides in the CTS region set forth in SEQ ID NO:124.

[0052] In any of such examples of a polynucleotide containing a variant Flap, the variant Flap is modified or comprises modifications as compared to a sequence containing a wild-type or unmodified Flap. In some examples, the wild-type or unmodified Flap sequence contains from or from about 80 to 200 contiguous nucleotides that includes the cPPT or CTS regions of a retrovirus, which optionally is a lentivirus. In some examples, the retrovirus is a lentivirus. In some examples, the lentivirus is HIV-1. In some examples, the wild-type or unmodified Flap contains a) the sequence of nucleotides set forth in SEQ ID NO:121; b) a sequence of nucleotides comprising at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the sequence of nucleotides set forth in SEQ ID NO:121 that contains the cPPT and CTS regions; or c) a contiguous portion of a) or b) that includes the cPPT and CTS regions.

[0053] In some examples, the viral nucleic acid containing the variant Flap is or comprises a sequence of nucleotides that exhibits at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 85%, 90% or 95% sequence identity to SEQ ID NO:121, said variant Flap lacking all or a portion of the cPPT and CTS regions. In some examples of any of the provided polynucleotides, the viral nucleic acid is or contains the sequence set forth in SEQ ID NO:122.

[0054] In some examples of any of the provided polynucleotides , the polynucleotide contains a) a variant Flap that is or comprises the sequence of SEQ ID NO: 122 or a sequence having at least at or about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:122, said variant Flap lacking all or a portion of the cPPT and CTS regions of the corresponding wild-type or unmodified Flap or of the sequence set forth in SEQ ID NO:121; and b) a modified PRE that is or comprises the sequence of SEQ ID NO:29 or 126 or a sequence having at least at or about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:29 or 126, said modified PRE containing at least one stop codon in the variant X gene of the modified PRE not present in a wild-type or unmodified X gene or not present

in a wild-type or unmodified PRE containing an X gene, such as not present in SEQ ID NO:1 or SEQ ID NO:125.

[0055] In some examples of any of the provided polynucleotides, the modified WPRE and/or variant Flap is operably linked to nucleotides encoding a recombinant protein that is or contains a recombinant receptor. In some examples of any of the provided polynucleotides, the recombinant receptor is an antigen receptor and/or a chimeric receptor. In some examples of any of the provided polynucleotides, the recombinant receptor is a functional non-TCR antigen receptor or a transgenic TCR. In some examples of any of the provided polynucleotides, the recombinant receptor is a chimeric antigen receptor (CAR).

[0056] In yet another aspect, the present disclosure refers to an expression cassette comprising the polynucleotide as described herein and a promoter operably linked to the nucleic acid encoding the recombinant protein. In yet another aspect, the present disclosure refers to a vector comprising the polynucleotide as described herein or the expression cassette as described herein, optionally wherein the vector is a viral vector. In some examples, also provided is an expression cassette that contains any of the provided polynucleotides and a promoter operably linked to a nucleic acid encoding a recombinant protein. In some examples, also provided is a vector that contains any of the provided polynucleotides or expression cassettes. In some examples of any of the provided vectors, the vector is a viral vector. In some examples of any of the provided vectors, the vector is a retroviral vector. In some examples of any of the provided vectors, the vector is a lentiviral vector. In some examples, the viral vector is a lentiviral vector that is derived from HIV-1.

[0057] In yet another aspect, the present disclosure refers to a cell comprising the expression cassette as described herein or the vector as described herein. In some examples, also provided is a cell that contains any of the provided polynucleotides, expression cassettes or any of the provided vectors. In some examples of any of the provided cells, the cell is a T cell, a natural killer (NK) cell, an iPS cell, or an iPS-derived cell.

[0058] In yet another aspect, the present disclosure refers to a virus particle, comprising the vector as described herein. In some examples, also provided is a virus particle that contains any of the provided vectors.

[0059] In yet another aspect, the present disclosure refers to an *in vitro* method, comprising introducing the expression cassette as described herein, the vector as described herein, or the virus particle as described herein to a cell, under conditions whereby expression of the recombinant protein is effected in the cell. In some examples, also provided are methods that include introducing any of the provided polynucleotides, expression cassettes, vectors, or virus particles into a cell, under conditions whereby expression of the recombinant protein is effected in the cell. In some examples of any of the provided methods, the introduction is effected by transducing the cell with the vector or virus particle. In some examples, the introduction is effected by transfecting the cell with the vector; and/or the introduction is effected by electroporation of the cell with the

vector. In some examples of any of the provided methods, the recombinant protein is expressed at a level that is increased compared to that achieved by introducing the vector in the absence of the modified PRE or a corresponding vector that does not contain the modified PRE or a PRE.

[0060] In yet another aspect, the present disclosure refers to a cell produced by the method as described herein. Also provided is a cell or cells produced by any of the provided methods.

[0061] In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising the cell as described herein, and a pharmaceutically effective carrier. Also provided is a pharmaceutical composition that contains a cell of any of the provided examples and a pharmaceutically effective carrier.

[0062] In yet another aspect, the present disclosure refers to a pharmaceutical composition as described herein for treating a disease or condition. In yet another aspect, the present disclosure refers to use of a pharmaceutical composition as described herein for use in the preparation of a medicament for treating a disease or condition, wherein the disease or condition is a cancer, an autoimmune disorder or an infectious disease. Also provided are methods of treatment that include administering to a subject having a disease or condition any of the provided polynucleotides, vectors, virus particles, cells, or pharmaceutical compositions.

[0063] In some examples of any of the provided methods of treatment, the method results in expression of the recombinant protein encoded by the polynucleotide. In some examples, the recombinant protein comprises a recombinant receptor that specifically binds to a ligand expressed by the disease or condition or a cell or tissue thereof. In some examples of any of the provided methods, the receptor is an antigen receptor and the ligand is an antigen specific for and/or associated with the disease or condition. In some examples of any of the provided methods, the disease or condition is a cancer, and autoimmune disorder, or an infectious disease.

CLAIMS

1. A polynucleotide, comprising a modified post-transcriptional regulatory element (PRE), said modified PRE comprising a variant of a wild-type hepatitis virus X gene, said variant X gene comprising a plurality of stop codons not present in the wild-type X gene; and a nucleic acid encoding a recombinant protein operably linked to the modified PRE.

2. The polynucleotide of claim 1, wherein the plurality of stop codons comprises at least one stop codon selected from among:

a stop codon beginning at a position within 36, 30 or 24 nucleotides in the 3' direction from a position in the variant X gene corresponding to residue 411 of WHV post-transcriptional regulatory element (WPRE) sequence set forth in SEQ ID NO: 1 and/or residue 1503 of the WHV sequence set forth as SEQ ID NO: 2.

3. The polynucleotide of claim 1 or claim 2, wherein the variant X gene comprises at least 2 stop codons, at least 3 stop codons or at least 4 stop codons.

4. The polynucleotide of any of claims 1-3, wherein the variant X gene comprises a stop codon in each reading frame present in said variant X gene.

5. The polynucleotide of any of claims 1-4, wherein the variant X gene comprises at least two stop codons in the same reading frame.

6. The polynucleotide of any of claims 1-5, wherein the stop codon comprises at least one stop codon selected from among:

a stop codon beginning at a position within or within at least 9, 12, 15, or 18 nucleotides in the 3' direction from a position in the variant X gene corresponding to residue 411 of WHV post-

transcriptional regulatory element (WPRE) sequence set forth in SEQ ID NO: 1 and/or residue 1503 of the WHV sequence set forth as SEQ ID NO: 2.

7. The polynucleotide of any of claims 1-6, wherein said stop codon is selected from among:

a stop codon beginning at a nucleotide position corresponding to position 420 in the sequence set forth in SEQ ID NO:1;

a stop codon beginning at a nucleotide position corresponding to position 424 in the sequence set forth in SEQ ID NO:1;

a stop codon beginning at a nucleotide position corresponding to position 428 in the sequence set forth in SEQ ID NO:1; and

a stop codon beginning at a nucleotide position corresponding to position 432 in the sequence set forth in SEQ ID NO:1.

8. The polynucleotide of claim 7, wherein said stop codon is an amber (TAG) or opal (TGA) stop codon.

9. The polynucleotide of any of claims 1-8, wherein the variant X gene further comprises a sequence encoding a post-translational modification signal not present in the wild-type hepatitis virus X gene.

10. The polynucleotide of claim 9, wherein said post-translational modification signal comprises a ubiquitination site.

11. The polynucleotide of claim 9, wherein said post-translational modification signal comprises one or more PEST sequences.

12. The polynucleotide of any of claims 1-11, wherein said variant X gene is a variant of a wild-type mammalian hepatitis virus X gene.

13. The polynucleotide of claim 12, wherein said wild-type mammalian hepatitis X gene is a wild-type woodchuck hepatitis virus (WHV) X gene.

14. The polynucleotide of any of claims 1-13, wherein the modified PRE comprises nucleotide modifications compared to a wild-type or unmodified hepatitis virus PRE that is a mammalian hepatitis PRE.

15. The polynucleotide of claim 14, wherein said wild-type or unmodified mammalian hepatitis PRE is a wild-type woodchuck hepatitis virus PRE (WPRE).

16. The polynucleotide of claim 14 or 15, wherein the wild-type or unmodified hepatitis virus PRE comprises:

a) the sequence of nucleotides set forth in SEQ ID NO:1 or 125 or a sequence of nucleotides that exhibits at least 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:1 or 125; or

b) a portion of the sequence of nucleotides of a), wherein the portion exhibits post-transcriptional activity.

17. The polynucleotide of any of claims 1-16, wherein the modified PRE is selected from among:

a) a modified PRE comprising a sequence of nucleotides that exhibits at least 85% sequence identity to SEQ ID NO:1 or 125, said modified PRE containing a variant X gene comprising at least one stop codon not present in SEQ ID NO:1 or 125; and

b) a modified PRE comprising a portion of the sequence of nucleotides of a), said portion comprising a variant X gene comprising the at least one stop codon, wherein the portion exhibits post-transcriptional activity.

18. The polynucleotide of any of claims 1-17, wherein the variant X gene comprises the sequence of nucleotides set forth in any of SEQ ID NOS: 44-54 or 141-151 and/or the modified PRE comprises the sequence of nucleotides set forth in any of SEQ ID NOS:29-39 or 126-136.

19. The polynucleotide of any of claims 1-18, wherein said variant X gene further comprises a variant of a start codon comprising one or more nucleotide differences compared to a wild-type hepatitis virus X gene start codon and/or compared to the start codon corresponding to nucleotide positions 411-413 of SEQ ID NO: 1, wherein the one or more nucleotide differences results in restricted or prevented translation initiation from said start codon.

20. The polynucleotide of any of claims 1-19, wherein said variant X gene comprises a variant promoter operably linked to said variant X gene, said variant promoter comprising one or more nucleotide differences compared to a wild-type hepatitis virus X gene promoter and/or compared to a promoter of SEQ ID NO: 11, wherein said one or more differences results in restricted or prevention of transcription from said promoter.

21. The polynucleotide of any of claims 1-20, wherein:

upon introduction into a eukaryotic cell, no polypeptide of a length greater than 12, 11, 10, 9, or 8 amino acids in length encoded by said variant X gene is produced; and/or

said polynucleotide is incapable of producing a polypeptide of a length greater than 12, 11, 10, 9, or 8 amino acids in length encoded by said variant X gene.

22. The polynucleotide of any of claims 1-21, wherein said modified PRE encodes an RNA that promotes nuclear RNA export and/or increases mRNA stability.

23. The polynucleotide of any of claims 1-22, further comprising a viral nucleic acid comprising a variant Flap, wherein the variant Flap contains a deletion of all or a portion of the

nucleotides corresponding to the central polypurine tract (cPPT) and/or the central termination sequence (CTS) regions of a wild-type or unmodified Flap sequence.

24. The polynucleotide of claim 23, wherein the wild-type or unmodified Flap sequence comprises from or from about 80 to 200 contiguous nucleotides comprising the cPPT or CTS regions of a retrovirus, which optionally is a lentivirus, optionally HIV-1.

25. The polynucleotide of claim 23 or claim 24, wherein the variant Flap comprises deletion of all or a contiguous portion of nucleotides corresponding to nucleotides in the cPPT region set forth in SEQ ID NO:123 and comprises deletion of all or a contiguous portion of nucleotides corresponding to nucleotides in the CTS region set forth in SEQ ID NO:124, and/or

wherein the viral nucleic acid comprising the variant Flap comprises a sequence of nucleotides that exhibits at least 70% sequence identity to SEQ ID NO:121 , said variant Flap lacking all or a portion of the cPPT and CTS regions.

26. The polynucleotide of any of claims 23-25, comprising:

a variant Flap comprising the sequence of SEQ ID NO: 122 or a sequence having at least at or about 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:122; and

a modified PRE comprising the sequence of SEQ ID NO:29 or 126 or a sequence having at least at or about 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:29 or 126.

27. The polynucleotide of any of claims 11-26, wherein the recombinant protein comprises a recombinant receptor.

28. The polynucleotide of claim 27, wherein the recombinant receptor is an antigen receptor and/or a chimeric receptor.

29. An expression cassette comprising the polynucleotide of any of claims 11-28 and a promoter operably linked to the nucleic acid encoding the recombinant protein.

30. A vector comprising the polynucleotide of any of claims 1-28 or the expression cassette of claim 29, optionally wherein the vector is a viral vector.

31. A cell comprising the expression cassette of claim 29 or the vector of claim 30.

32. A virus particle, comprising the vector of claim 30.

33. An *in vitro* method, comprising introducing the expression cassette of claim 29, the vector of claim 30, or the virus particle of claim 32 to a cell, under conditions whereby expression of the recombinant protein is effected in the cell.

34. A cell produced by the method of claim 33.

35. A pharmaceutical composition comprising the cell of claim 31 or claim 34, and a pharmaceutically effective carrier.

36. A pharmaceutical composition of claim 35 for use in treating a disease or condition.

37. The pharmaceutical composition of claim 36, wherein the disease or condition is a cancer, an autoimmune disorder or an infectious disease.

38. Use of a pharmaceutical composition of claim 35 in the preparation of a medicament for treating a disease or condition, wherein the disease or condition is a cancer, an autoimmune disorder or an infectious disease.