Background

[0004] Viral vectors can provide an effective delivery system for transferring genetic material into a cell. Nucleic acids within many viral vectors, such as gamma-retroviral and lentiviral vectors, can integrate into the genome of a host cell. Cis-acting elements, such as viral post-transcriptional regulatory elements (PREs), including hepatitis virus PREs and variants thereof, have been used in viral vectors to enhance gene expression. Nucleic acids, expression cassettes, and vectors containing such elements are available. There is a need for improved PRE-containing nucleic acids, cassettes and vectors. Provided are embodiments that address such needs.

Summary

[0004A] In one aspect, the present invention provides a polynucleotide, comprising: a nucleic acid encoding a recombinant protein; a modified post-transcriptional regulatory element (PRE) operably linked to said nucleic acid, said modified PRE comprising a variant of a wild-type hepatitis virus X gene, said variant X gene comprising a stop codon not present in the wild-type X gene.

[0005] Provided are polynucleotides containing a modified post-transcriptional regulatory element (PRE) that contains a variant X gene that includes a stop codon and/or post-translational degradation sequence that is not present in an unmodified or wild-type hepatitis virus X gene, such as not present in an X gene from a wild-type mammalian hepatitis virus, such as a WHV or HBV. Among the provided polynucleotides are those that contain a modified WPRE containing a variant X gene that includes a stop codon and/or post-translational degradation sequence that is not present in an unmodified or wild-type WHV X gene. The provided polynucleotides include expression cassettes and viral vectors containing such modified PREs. In particular examples, the modified PRE is operably linked to a nucleic acid encoding a recombinant protein, e.g. a heterologous protein.

[0006] In another aspect, the present invention provides 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 stop codon not present in the wild-type X gene, wherein the stop codon comprises at least one stop codon selected from among: a stop codon beginning 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; and/or a stop codon beginning at a position within 32 or 30 nucleotides in the 3' direction from a position in the variant X gene corresponding to residue 411 of Woodchuck hepatitis virus (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. Provided are polynucleotides containing a modified PRE that includes 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. In some examples, at least one stop codon, e.g., the first stop codon or each of a plurality of stop codons present in the variant X gene, begins at a position within 36 or 24 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, 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 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 yet another aspect, the present invention provides 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. 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] In yet another aspect, the present invention provides 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

contains a plurality of stop codons not present in the wild-type X gene. In any of such examples, the modified PRE contains 2, 3, 4, 5 or 6 stop codons. In some examples, the plurality of stop codons comprises at least one stop codon in each reading frame present in the variant X gene. In some examples, the plurality of stop codons comprises at least two stop codons in the same reading frame.

[0009] In any of such examples of a modified PRE containing a plurality of stop codons, the first stop codon among the plurality or one or more of a plurality of stop codons begins at a position

within 36, 30 or 24 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 of a modified PRE containing a plurality of stop codons, the first stop codon among the plurality or one or more of a plurality of stop codons begins 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 or SEQ ID NO:125 and/or residue 1503 of the WHV sequence set forth as SEQ ID NO: 2. In some such examples, the variant X gene does not comprise an open reading frame of greater than at or about 39 nucleotides in length, greater than at or about 30 nucleotides in length or greater than at or about 27 nucleotides in length.

[0010] In any of such examples, the provided polynucleotides also can include a nucleic acid encoding a recombinant protein. In particular examples, the modified PRE is operably linked to the nucleic acid encoding the recombinant protein.

[0011] In some examples of any of the provided polynucleotides, 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 or within at least or at 9, 12, 15, 18 or 21 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 of any of the provided polynucleotides, 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 or within at least or at 9, 12, 15, 18, 21 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 or SEQ ID NO:125 and/or residue 1503 of the WHV sequence set forth as SEQ ID NO: 2. In some examples, the modified PRE contains at least one stop codon, such as 1, 2, 3, 4 or 5 stop codons, in which one or more stop codons of the plurality begins at a position within or within at least or at a nucleotide position corresponding to position 420, 423, 426, 429 or 432 of the sequence set forth in SEQ ID NO:1 or 125. In some such examples, the variant X gene does not comprise an open reading frame of greater than or at or about 24 nucleotides in length, greater than at or

about 21 nucleotides in length, greater than at or about 18 nucleotides in length, greater than at or about 15 nucleotides in length, or greater than at or about 12 nucleotides in length.

[0012] In some examples of any of the provided polynucleotides, 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 or within at least or at 9, 13, 17 or 21 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 of any of the provided polynucleotides, 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 or within at least or at 9, 13, 17 or 21 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 or SEQ ID NO:125 and/or residue 1503 of the WHV sequence set forth as SEQ ID NO: 2. In some examples, the modified PRE contains at least one stop codon, such as 1, 2, 3 or 4 stop codons, in which one or more of a plurality of stop codons begins at a position within or within at least or at a nucleotide position corresponding to position 420, 424, 428 or 432 of the sequence set forth in SEQ ID NO: 1 or SEQ ID NO:125. In some such examples, the variant X gene does not comprise an open reading frame of greater than at or about 24 nucleotides in length, greater than at or about 21 nucleotides in length, greater than at or about 16 nucleotides in length, or greater than at or about 12 nucleotides in length.

[0013] In some examples, the unmodified or wild-type X gene comprises the sequence of nucleotides set forth in SEQ ID NO: 221, and the variant X gene contains at least one stop codon, such as 1, 2, 3, 4 or 5 stop codons introduced therein. In some examples, a plurality of stop codons are introduced, where at least one stop codon is introduced in each reading frame therein. In some examples, a plurality of stop codons is introduced where at least two stop codons are introduced in the same reading frame present therein. In some examples, the modified PRE contains a variant X gene that contains at least one stop codon, such as 1, 2, 3 or 4 stop codons, in which one or more begins at a position within or within at least or at position 9, 13, 17 or 21 in the 3' direction from a position in the variant X gene corresponding to the 5' position of the ATG start

codon set forth in SEQ ID NO:221. In some examples, the modified PRE contains a variant X gene that comprises the sequence of nucleotides set forth in SEQ ID NO:28.

[0014] In some examples of any of the provided polynucleotides, the modified PRE contains a beta stem loop corresponding to nucleotide residues 448-470 of SEQ ID NO:1 or SEQ ID NO:125. In some examples of any of the provided polynucleotides, the modified PRE does not contain a nucleotide change in a position within the beta stem loop corresponding to nucleotides 448-470 of SEQ ID NO:1 or SEQ ID NO:125. In some examples of any of the provided polynucleotides, the stop codon or stop codons does not comprise a nucleotide in a position within the beta stem loop corresponding to one or more of nucleotides positions 448-470 of SEQ ID NO:1 or SEQ ID NO:125.

[0015] In any of such examples, the modified PRE contains a stop codon selected from among: a stop codon beginning at position 9 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 and/or at a nucleotide position corresponding to position 420 in the sequence set forth in SEQ ID NO:1 or SEQ ID NO:125; a stop codon beginning at position 13 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 and/or at a nucleotide position corresponding to position 424 in the sequence set forth in SEQ ID NO:1 or SEQ ID NO:125; a stop codon beginning at position 17 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 and/or at a nucleotide position corresponding to position 428 in the sequence set forth in SEQ ID NO:1 or SEQ ID NO:125; and/or a stop codon beginning at position 21 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 and/or at a nucleotide position corresponding to position 432 in the sequence set forth in SEQ ID NO:1 or SEQ ID NO:125.

[0016] In any of such examples, the stop codon can be an amber (TAG), ochre (TAA) or opal (TGA) stop codon. In some examples, the stop codon can be an amber (TAG) or opal (TGA) stop codon. In any of such examples, the stop codon, such as each of a plurality of stop codons, is introduced by nucleotide substitution, deletion or insertion.

[0017] In some examples of any of such polynucleotides, the variant X gene in the modified PRE is no more than 180 nucleotides in length or is no more than at or about 210 nucleotides in length. In some examples of any of such polynucleotides, the variant X gene is at least at or about 90 nucleotides in length or at least about 120 nucleotides in length or is at least at or about 180 nucleotides in length.

[0018] In some examples of any of such polynucleotides, the variant X gene can be a variant of a wild-type or unmodified mammalian hepatitis X gene or a partial or truncated portion thereof. In some examples, the wild-type or unmodified X gene can include a partial X gene encoding a truncated X protein, such as is present in a wild-type or unmodified PRE. In some examples, the wild-type or unmodified mammalian hepatitis X gene or partial X gene is or is derived from a wild-type woodchuck hepatitis virus (WHV) X gene. In some examples, the wild-type or unmodified WHV X gene contains the nucleotide sequence of SEQ ID NO:9 or is a partial X gene thereof. In some examples, the wild-type or unmodified WHV X gene contains the sequence of nucleotides set forth as nucleotides 411-592 of any of SEQ ID NOS: 1 and 12-20 or nucleotides 411-589 of SEQ ID NO:125.

[0019] In some examples of any of such polynucleotides, the modified PRE can contain at least two or at least three cis-acting post-transcriptional regulatory subelements of a wild-type hepatitis virus PRE or functional variant(s) thereof. In some such examples, the at least two or at least three subelements comprise a wild-type PRE alpha subelement or functional variant thereof, a functional variant of a wild-type PRE beta subelement, and/or a wild-type PRE gamma subelement or functional variant thereof. In some examples, the modified PRE comprises an alpha subelement of a wild-type hepatitis virus PRE or functional variant thereof and a functional variant of a wild-type PRE beta subelement. In any of such examples, the alpha subelement can comprise the sequence of SEQ ID NO: 3, SEQ ID NO:4 or SEQ ID NO:5 or a variant thereof, the beta subelement can comprise a variant of the sequence of SEQ ID NO: 6 or SEQ ID NO:7, and/or the gamma subunit can comprise the sequence of SEQ ID NO: 8 or variant thereof.

[0020] In some examples of any of such polynucleotides, the wild-type or unmodified hepatitis virus PRE can be a wild-type mammalian hepatitis PRE. In some examples, the wild-

type or unmodified mammalian hepatitis virus PRE contains the sequence of nucleotides set forth in any of SEQ ID NOS: 1 and 12-27 or is a functional portion thereof that exhibits post-transcriptional activity. In some cases, one or more N- or C-terminal amino acid residues can be deleted or removed without substantial effect on the activity of the PRE. In some examples, the wild-type or unmodified hepatitis virus PRE has the sequence set forth in SEQ ID NO:125. In some examples, the wild-type mammalian hepatitis PRE is a wild-type woodchuck hepatitis virus PRE (WPRE).

[0021] In any of such examples of the provided polynucleotides, the modified PRE can be modified with reference to a wild-type or unmodified hepatitis PRE that comprises the sequence of nucleotides set forth in SEQ ID NO:1 or SEQ ID NO:125, a sequence of nucleotides that exhibits at least 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO:1 or SEQ ID NO:125, or a functionally active portion thereof that exhibits post-transcriptional activity, so long as the variant X gene contains at least one stop codon not present in the corresponding wild-type hepatitis PRE.

[0022] In any of such examples of the provided polynucleotides, the wild-type or unmodified hepatitis PRE can contain the sequence of nucleotides set forth in any of SEQ ID NOS: 1,12-20 or 125. In some examples, the wild-type or unmodified hepatitis PRE can contain the nucleotide sequence of SEQ ID NO: 1. In some examples, the wild-type or unmodified hepatitis PRE can contain the nucleotide sequence of SEQ ID NO: 125.

[0023] In some examples of any of the polynucleotides, the variant X gene can contain a start codon beginning at a position corresponding to position 411 of SEQ ID NO: 1 or SEQ ID NO:125. In some examples, the start codon is an ATG start codon.

[0024] In some examples of any of the polynucleotides, the variant X gene can further contain a promoter operably linked to the variant X gene. In some examples, the promoter is a wild-type X-gene promoter comprising the sequence set forth in SEQ ID NO: 11 or a wild-type WHV X gene promoter sequence.

[0025] In some examples of any of the polynucleotides, the modified PRE can be selected from: a) a modified PRE comprising a sequence of nucleotides that exhibits at least 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%

sequence identity to SEQ ID NO:1 or SEQ ID NO:125, in which the modified PRE contains a variant X gene having at least one stop codon not present in SEQ ID NO:1 or SEQ ID NO:125; or b) a modified PRE comprising a portion of the sequence of nucleotides of a) in which the portion contains a variant X gene comprising the at least one stop codon and the portion exhibits post-transcriptional activity. In some examples, the variant X gene contains at least 2 stop codons, at least 3 stop codons or at least 4 stop codons. In some examples, the variant X gene comprises a stop codon in each reading frame present in said variant X gene.

[0026] In some examples, the variant X gene comprises the sequence of nucleotides set forth in any of SEQ ID NOS: 44-58 or 141-155. In some examples, the modified PRE comprises the sequence of nucleotides set forth in any of SEQ ID NOS:29-43 or 126-140.

[0027] In some examples of any of the provided polynucleotides, the modified PRE does not contain any modifications in addition to the at least one stop codon not present in the wild-type or unmodified PRE, such as not present in SEQ ID NO:1 or SEQ ID NO:125. In some examples of any of the provided polynucleotides, the modified PRE contains additional modifications in addition to the at least one stop codon as compared to the sequence of the wild-type or unmodified PRE, such as compared to SEQ ID NO:1 or SEQ ID NO:125.

[0028] In some examples of any of the provided polynucleotides, the variant X gene further contains a variant start codon comprising one or more nucleotide differences compared to a wild-type hepatitis virus X gene start codon. In some examples, the variant start codon contains one or more nucleotide differences compared to the start codon corresponding to nucleotide positions 411-413 of SEQ ID NO: 1 or SEQ ID NO:125. In some such examples, the one or more differences results in restricted or prevented translation initiation from said start codon. In some such examples, the variant X gene comprises the sequence of nucleotides set forth in any of SEQ ID NOS: 74-88 or 171-185 and/or the modified PRE comprises the sequence of nucleotides set forth in any of SEQ ID NOS:59-73 or 156-170.

[0029] In some examples of any of the provided polynucleotides, the variant X gene contains a variant promoter operably linked to the variant X gene in which the variant promoter comprises one or more nucleotide differences compared to a wild-type hepatitis virus X gene

promoter. In some examples, the variant promoter contains one or more nucleotide differences compared to the promoter set forth as SEQ ID NO: 11. In some examples, the one or more differences results in restricted or prevention of transcription from said promoter. In some such examples, the modified PRE comprises the sequence of nucleotides set forth in any of SEQ ID NOS: 89-118 or 186-215.

[0030] In some examples of any of the provided polynucleotides, upon introduction of the polynucleotide 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. In some examples of any of the provided polynucleotides, the 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.

[0031] In some examples of any of the provided polynucleotides, the modified PRE encodes an RNA that promotes nuclear RNA export and/or increases mRNA stability. In some examples of any of the provided polynucleotides, the modified PRE encodes an RNA polynucleotide that promotes nuclear RNA export and/or increases mRNA stability, wherein said promotion of nuclear RNA export and/or mRNA stability increases expression of the recombinant protein. In some examples of any of the provided polynucleotides, the modified PRE retains the post-transcriptional activity of the corresponding wild-type or unmodified hepatitis PRE and/or the PRE set forth in SEQ ID NO:1, SEQ ID NO:125, SEQ ID NO:119, SEQ ID NO:120 and/or SEQ ID NO:216. In some such examples, the modified PRE exhibits at least 75%, 80%, 85%, 90%, 95%, 100%, 110%, 120% or 130% or more of the post-transcriptional activity of the corresponding wild-type or unmodified hepatitis PRE and/or the PRE set forth in SEQ ID NO:1, SEQ ID NO:125, SEQ ID NO:119, SEQ ID NO:120 and/or SEQ ID NO:216. In some examples, the post-transcriptional activity of the modified PRE can be assessed by monitoring gene expression of an operably linked nucleic acid encoding a recombinant protein.

[0032] In some examples of any of the provided polynucleotides, the variant X gene further contains a sequence encoding a post-translational modification signal not present in the wild-type hepatitis virus X gene.

[0033] Also provided are polynucleotides containing a modified PRE that includes a variant of a wild-type or unmodified hepatitis virus X gene in which the variant X gene contains a sequence encoding a post-translational modification signal not present in the wild-type or unmodified hepatitis virus X gene. In some examples of any of the provided polynucleotides, the post-translational modification signal contains a ubiquitination site.

[0034] In some examples of any of the provided polynucleotides, the post-translational modification signal contains a first codon beginning at a position within or within at least 2, 3, 4, 5 or 6 nucleotides in the 3' direction from a position in the variant X gene corresponding to the 5' position of a start codon of the X protein open reading frame, wherein the first codon encodes a glycine, arginine, glutamic acid, phenylalanine, aspartate, cysteine, lysine, asparagine, serine, tyrosine, tryptophan, histidine, or leucine residue according to the N-end rule; and optionally a second codon beginning at a position within or within at least 2, 3, 4, 5 or 6 nucleotides in the 3' direction from a position in the variant X gene corresponding to the 5' position of a start codon of the X protein open reading frame, wherein the second codon encodes a glutamic acid or asparagine residue according to the N-end rule.

[0035] In some examples of any of the provided polynucleotides, the post-translational modification signal contains one or more PEST sequences.

[0036] In some examples of any of the provided polynucleotides, the polynucleotide contains a nucleic acid encoding a recombinant protein operably linked to the modified PRE and a variant X gene containing a sequence encoding a post-translational modification signal not present in the wild-type or unmodified hepatitis virus X gene.

[0037] In some examples of any of the provided polynucleotides, the variant X gene contains a sequence encoding a post-translational modification signal containing a beta stem loop corresponding to nucleotide residues 448-470 of SEQ ID NO:1 or SEQ ID NO:125. In some examples of any of the provided polynucleotides, the variant X gene contains a sequence encoding a post-translational modification signal that does not contain a nucleotide change in a position within the beta stem loop corresponding to nucleotides 448-470 of SEQ ID NO:1 or SEQ ID NO:125.

[0038] In some examples of any of the provided polynucleotides, the variant X gene is no more than 180 nucleotides in length or is no more than at or about 210 nucleotides in length. In some examples of any of the provided polynucleotides, the variant X gene is at least at or about 90 nucleotides in length or at least about 120 nucleotides in length or is at least at or about 180 nucleotides in length.

[0039] In some examples of any of the provided polynucleotides, the variant X gene is a variant of a wild-type or unmodified mammalian hepatitis X gene or a partial or truncated portion thereof. In some examples, the wild-type or unmodified X gene can include a partial X gene encoding a truncated X protein, such as is present in a wild-type or unmodified PRE. In some examples of any of the provided polynucleotides, the wild-type or unmodified mammalian hepatitis X gene or partial X gene is or is derived from a wild-type woodchuck hepatitis virus (WHV) X gene. In some examples, the wild-type or unmodified WHV X gene contains the nucleotide sequence of SEQ ID NO:9, the sequence of nucleotides set forth as nucleotides 1503-1928 of SEQ ID NO: 2 or is a partial X gene thereof. In some examples of any of the provided polynucleotides, the wild-type or unmodified WHV X gene contains the sequence of nucleotides set forth as nucleotides 411-592 of any of SEQ ID NOS: 1 and 12-20 or nucleotides 411-589 of SEQ ID NO:125.

[0040] In some examples of any of the provided polynucleotides, the modified PRE contains at least two or at least three cis-acting post-transcriptional regulatory subelements of a wild-type hepatitis virus PRE or functional variant(s) thereof. In some examples of any of the provided polynucleotides, the at least two or at least three subelements contain a wild-type PRE alpha subelement or functional variant thereof, a functional variant of a wild-type PRE beta subelement, and/or a wild-type PRE gamma subelement or functional variant thereof. In some examples of any of the provided polynucleotides, the modified PRE contains an alpha subelement of a wild-type hepatitis virus PRE or functional variant thereof and a functional variant of a wild-type PRE beta subelement. In some examples of any of the provided polynucleotides, the alpha subelement contains the sequence of SEQ ID NO: 3, SEQ ID NO:4 or SEQ ID NO:5 or a

variant thereof, the beta subelement contains a variant of the sequence of SEQ ID NO: 6 or SEQ ID NO:7, and/or the gamma subunit contains the sequence of SEQ ID NO: 8 or variant thereof.

[0041] In some examples of any of the provided polynucleotides, the wild-type or unmodified hepatitis virus PRE is a wild-type mammalian hepatitis PRE. In some examples of any of the provided polynucleotides, the wild-type or unmodified mammalian hepatitis virus PRE contains the sequence of nucleotides set forth in any of SEQ ID NOS: 1,12-27 or 125. In some examples of any of the provided polynucleotides, the wild-type mammalian hepatitis PRE is a wild-type woodchuck hepatitis virus PRE (WPRE).

[0042] In some examples of any of the provided polynucleotides, the modified PRE is selected from among: a) a modified PRE comprising a variant X gene of a wild-type or unmodified hepatitis PRE, the wild-type or unmodified hepatitis PRE containing the sequence of nucleotides set forth in SEQ ID NO:1 or SEQ ID NO:125 or a sequence of nucleotides that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO:1 or SEQ ID NO:125, wherein the variant X gene contains a sequence encoding a post-translational modification signal not present in the wild-type or unmodified hepatitis virus X gene; and b) a modified PRE containing a portion of the sequence of nucleotides of a), the portion containing a variant X gene containing the sequence encoding a post-translational modification signal, wherein the portion exhibits post-transcriptional activity.

[0043] In some examples of any of the provided polynucleotides, the variant X gene contains a start codon beginning at a position corresponding to position 411 of SEQ ID NO: 1 or SEQ ID NO:125. In some examples of any of the provided polynucleotides, the start codon is an ATG start codon. In some examples of any of the provided polynucleotides, the variant X gene contains a promoter operably linked to said variant X gene. In some examples of any of the provided polynucleotides, the promoter is a wild-type X-gene promoter containing the sequence set forth in SEQ ID NO: 11 or a wild-type WHV X gene promoter sequence.

[0044] In some examples of any of the provided polynucleotides, the modified PRE is selected from among: a) a modified PRE comprising a sequence of nucleotides that exhibits at least 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,

98% or 99% sequence identity to SEQ ID NO:1 or SEQ ID NO:125, the modified PRE containing a variant X gene containing a sequence encoding a post-translational modification signal not present in SEQ ID NO:1 or SEQ ID NO:125; and b) a modified PRE containing a portion of the sequence of nucleotides of a), the portion containing a variant X gene containing the sequence encoding the post-translational modification, wherein the portion exhibits post-transcriptional activity.

[0045] In some examples of any of the provided polynucleotides, the variant X gene contains up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 nucleotide changes.

[0046] In some examples of any of the provided polynucleotides, the modified PRE does not contain any modifications in addition to the sequence encoding a post-translational modification signal. In some examples of any of the provided polynucleotides, the modified PRE contains additional modification(s) in addition to the sequence encoding a post-translational modification signal not present in the wild-type or unmodified hepatitis virus X gene. In some examples of any of the provided polynucleotides, the additional modification(s) are in the variant X gene. In some examples of any of the provided polynucleotides, the additional modification(s) results in a variant X gene encoding an inactive X protein and/or a truncated X protein.

[0047] In some examples of any of the provided polynucleotides, the modified PRE encodes an RNA that promotes nuclear RNA export and/or increases mRNA stability. In some examples of any of the provided polynucleotides, the promotion of nuclear RNA export and/or mRNA stability increases expression of the recombinant protein.

[0048] In some examples of any of the provided polynucleotides, the modified PRE retains the post-transcriptional activity of the corresponding wild-type or unmodified hepatitis PRE and/or the PRE set forth in SEQ ID NO:1, SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 125 or SEQ ID NO:216. In some such examples, the modified PRE exhibits at least 75%, 80%, 85%, 90%, 95%, 100%, 110%, 120% or 130% or more of the post-transcriptional activity of the corresponding wild-type or unmodified hepatitis PRE and/or the PRE set forth in SEQ ID NO:1, SEQ ID NO:125, SEQ ID NO:119, SEQ ID NO:120 and/or SEQ ID NO:216. In some examples, the post-transcriptional activity of the modified PRE can be assessed by monitoring gene expression of an operably linked nucleic acid encoding a recombinant protein.

[0049] In some examples, any of the provided polynucleotides can further contain viral nucleic acid containing 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. In such examples, the polynucleotides contain a modified PRE and a variant Flap. In some examples, such elements are operably linked to nucleic acid encoding a recombinant protein and/or result in an increase in efficiency of gene transfer of a heterologous nucleic acid encoding a recombinant protein compared to the absence of such elements.

[0050] In yet another aspect, the present invention provides a polynucleotide, 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. Also provided are polynucleotides containing 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. In some examples, the polynucleotide containing a variant Flap can further contain nucleic acid encoding a recombinant protein. In some examples, the presence of a polynucleotide containing a variant Flap, while deleted for all or a portion of the cPPT and CTS that are involved in forming the Flap structure, nevertheless can successfully transduce or transfer into cells with the nucleic acid encoding the recombinant protein.

[0051] In some examples of any of the provided polynucleotides containing a variant Flap, the variant Flap contains a deletion of all or a portion of the nucleotide corresponding to the cPPT and the CTS. In some examples, all of the nucleotide residues corresponding to cPPT and all of the nucleotide residues corresponding to the CTS are deleted and/or the variant Flap does not contain nucleotide residues corresponding to the cPPT or nucleotide residues corresponding to the CTS. In some examples of any of the provided polynucleotides, the variant Flap contains deletion of all or a contiguous portion of nucleotides corresponding to nucleotides in the cPPT region set forth in SEQ ID NO: 123. In some

examples of any of the provided polynucleotides, the variant Flap contains deletion of all or a contiguous portion of nucleotides corresponding to nucleotides in the CTS region set forth in SEQ ID NO: 124. In some examples of any of the provided polynucleotides, the variant Flap contains deletion of all or a contiguous portion of nucleotides corresponding to nucleotides in the cPPT region set forth in SEQ ID NO:123 and

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 embodiments, 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 invention provides 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 invention provides 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 invention provides 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 invention provides a virus particle, comprising the vector as described herein. In some embodiments, also provided is a virus particle that contains any of the provided vectors.

[0059] In yet another aspect, the present invention provides a 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 invention provides 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 invention provides 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 embodiments and a pharmaceutically effective carrier.

[0062] In yet another aspect, the present invention provides a pharmaceutical composition as described herein for treating a disease or condition. In yet another aspect, the present invention provides use of a pharmaceutical composition as described herein for use in the preparation of a medicament for treating a disease or condition. 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.

Brief Description of the Figures

[0064] Figure 1 depicts the nucleic acid sequence of an exemplary WPRE set forth in SEQ ID NO:1. Nucleic acids corresponding to reported PRE subelements (Donello *et al.* (1998) *J. Virol.*, 72:5085; Smith *et al.* (1998) *Nucleic Acids Research*, 26:4818) are indicated as follows: a gamma subelement, alpha subelement, and beta subelement are indicated by solid lines with arrows indicating the beginning and end positions; nucleotides corresponding to an exemplary stem loop-forming portions of the alpha and beta subelements, respectively, are indicated with a dashed line and italics; nucleotides corresponding to a WHX protein promoter are indicated with a dashed and dotted line and by italics; and nucleotides corresponding to the truncated X gene contained within this exemplary WPRE are underlined. It is understood that the description of elements thereof are

CLAIMS

1. A polynucleotide, comprising:

a nucleic acid encoding a recombinant protein;

a modified post-transcriptional regulatory element (PRE) operably linked to said nucleic acid, said modified PRE comprising a variant of a wild-type hepatitis virus X gene, said variant X gene comprising a stop codon not present in the wild-type X gene.

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

a stop codon beginning at a position within 36 or 24 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; and/or

a stop codon beginning at a position within 36 or 24 nucleotides in the 3' direction from a position corresponding to residue 411 of Woodchuck hepatitis virus (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. 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 stop codon not present in the wild-type X gene, wherein the stop codon comprises at least one stop codon selected from among:

a stop codon beginning 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; and/or

a stop codon beginning at a position within 32 or 30 nucleotides in the 3' direction from a position in the variant X gene corresponding to residue 411 of Woodchuck hepatitis virus (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.

4. The polynucleotide of any of claims 1-3, wherein said stop codon comprises a plurality of stop codons, optionally wherein said plurality of stop codons comprises at least one stop codon in each reading frame present in said variant X gene and/or comprises at least two stop codons in the same reading frame.

5. 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.

6. The polynucleotide of claim 4 or 5, 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 the 5' position of a start codon of the wild-type X protein open reading frame; and/or

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.

7. The polynucleotide of any of claims 4-6, wherein the variant X gene comprises at least 2 stop codons, at least 3 stop codons or at least 4 stop codons.

8. The polynucleotide of any of claims 4-7, wherein the variant X gene comprises a stop codon in each reading frame present in said variant X gene.

9. The polynucleotide of any of claims 1-8, 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 the 5' position of a start codon of the X protein open reading frame; and/or

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.

10. The polynucleotide of any of claims 1-9, wherein said stop codon is selected from among:

a stop codon beginning at position 9 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 and/or at a nucleotide position corresponding to position 420 in the sequence set forth in SEQ ID NO:1;

a stop codon beginning at position 13 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 and/or at a nucleotide position corresponding to position 424 in the sequence set forth in SEQ ID NO:1;

a stop codon beginning at position 17 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 and/or at a nucleotide position corresponding to position 428 in the sequence set forth in SEQ ID NO:1;

a stop codon beginning at position 21 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 and/or at a nucleotide position corresponding to position 432 in the sequence set forth in SEQ ID NO:1.

11. The polynucleotide of claim 10, wherein said stop codon is an amber (TAG) or opal (TGA) stop codon.

12. The polynucleotide of any of claims 1-11, 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.

13. 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.

14. The polynucleotide of claim 12 or 13, wherein said post-translational modification signal comprises a ubiquitination site.

15. The polynucleotide of claim 12 or 13, wherein said post-translational modification signal comprises one or more PEST sequences.

16. The polynucleotide of any of claims 3-15, further comprising a nucleic acid encoding a recombinant protein operably linked to the modified PRE.

17. The polynucleotide of any of claims 1-16, wherein said variant X gene is a variant of a wild-type mammalian hepatitis X virus gene.

18. The polynucleotide of claim 17, wherein said wild-type mammalian hepatitis X gene is a wild-type woodchuck hepatitis virus (WHV) X gene.

19. The polynucleotide of any of claims 1-18, wherein the modified PRE comprises nucleotide modifications compared to a wild-type or unmodified hepatitis virus PRE that is a mammalian hepatitis PRE.

20. The polynucleotide of claim 19, wherein said wild-type or unmodified mammalian hepatitis PRE is a wild-type woodchuck hepatitis virus PRE (WPRE).

21. The polynucleotide of claim 19 or 20, wherein the wild-type or unmodified hepatitis virus PRE comprises: a) t 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.

22. The polynucleotide of any of claims 1-12 or 17-21, 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.

23. The polynucleotide of any of claims 1-12 or 17-22, wherein the variant X gene comprises the sequence of nucleotides set forth in any of SEQ ID NOS: 44-58 or 141-155 and/or the modified PRE comprises the sequence of nucleotides set forth in any of SEQ ID NOS:29-43 or 126-140.

24. The polynucleotide of any of claims 1-12 or 17-23, 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.

25. The polynucleotide of any of claims 1-12 or 17-24, 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.

26. The polynucleotide of any of claims 1-12 or 17-25, 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.

27. The polynucleotide of any of claims 1-26, wherein said modified PRE encodes an RNA that promotes nuclear RNA export and/or increases mRNA stability.

28. The polynucleotide of any of claims 1-27, 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.

29. A polynucleotide, 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.

30. The polynucleotide of claim 28 or 29, 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.

31. The polynucleotide of claims 28-30, 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.

32. The polynucleotide of any of claims 28-31, 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.

33. The polynucleotide of any of claims 29-32, further comprising nucleic acid encoding a recombinant protein.

34. The polynucleotide of any of claims 1-2, 16-28, or 33, wherein the recombinant protein comprises a recombinant receptor.

35. The polynucleotide of claim 34, wherein the recombinant receptor is an antigen receptor and/or a chimeric receptor.

36. An expression cassette comprising the polynucleotide of any of claims 1-2, 16-28, or 33-35 and a promoter operably linked to the nucleic acid encoding the recombinant protein.

37. A vector comprising the polynucleotide of any of claims 1-35 or the expression cassette of claim 36, optionally wherein the vector is a viral vector.

38. A cell comprising the expression cassette of claim 36 or the vector of claim 37.

39. A virus particle, comprising the vector of claim 37.

40. A method, comprising introducing the expression cassette of claim 36, the vector of claim 37, or the virus particle of claim 39 to a cell, under conditions whereby expression of the recombinant protein is effected in the cell.

41. A cell produced by the method of claim 40.

42. A pharmaceutical composition comprising the cell of claim 38 or 41, and a pharmaceutically effective carrier.

43. A pharmaceutical composition of claim 42 for treating a disease or condition.

44. Use of a pharmaceutical composition of claim 42 for use in the preparation of a medicament for treating a disease or condition.

45. The use of claim 43 or pharmaceutical composition of claim 44, wherein the disease or condition is a cancer, an autoimmune disorder or an infectious disease.