[7] The disclosures of all publications, patents, patent applications and published patent applications referred to herein are hereby incorporated herein by reference in their entirety.

SUMMARY

[8] The present disclosure in one example provides modified T cells (*e.g.*, allogeneic T cells), comprising: i) an exogenous Nef protein; and ii) a functional exogenous receptor comprising: (a) an extracellular ligand binding domain, (b) a transmembrane domain (*e.g.*, derived from CD8α), and (c) an intracellular signaling domain (ISD) comprising a chimeric signaling domain (CMSD), wherein the CMSD comprises one or a plurality of ITAMs ("CMSD ITAMs"), wherein the plurality of CMSD ITAMs are optionally connected by one or more linkers ("CMSD linkers"). In one aspect, the present disclosure refers to a modified T cell comprising: i) an exogenous Nef protein; and ii) a functional exogenous receptor, comprising: (a) an extracellular ligand binding domain, (b) a transmembrane domain, and (c) an intracellular signaling domain (ISD) comprising a chimeric signaling domain (CMSD), wherein the CMSD comprises one or a plurality of Immune-receptor Tyrosine-based Activation Motifs (CMSD ITAMs), wherein the plurality of CMSD ITAMs are optionally connected by one or more linkers (CMSD linkers). In some examples, the CMSD comprises one or more of the characteristics selected from the group consisting of: (a) the plurality (*e.g.*, 2, 3, 4, or more) of CMSD ITAMs are directly linked to each other; (b) the CMSD comprises two or more (*e.g.*, 2, 3, 4, or more) CMSD ITAMs connected by one or more linkers not derived from an ITAM-containing parent molecule (*e.g.*, G/S linker); (c) the CMSD comprises one or more CMSD linkers derived from an ITAM-containing parent molecule that is different from the ITAM-containing parent molecule from which one or more of the CMSD ITAMs are derived from; (d) the CMSD comprises two or more (*e.g.*, 2, 3, 4, or more) identical CMSD ITAMs; (e) at least one of the CMSD ITAMs is not derived from CD3ζ; (f) at least one of the CMSD ITAMs is not ITAM1 or ITAM2 of CD3ζ; (g) the plurality of CMSD ITAMs are each derived from a different ITAM-containing parent molecule; and/or (h) at least one of the CMSD ITAMs is derived from an ITAM-containing parent molecule selected from the group consisting of CD3ε, CD3δ, CD3γ, Igα (CD79a), Igβ (CD79b), FcεRIβ, FcεRIγ, DAP12, CNAIP/NFAM1, STAM-1, STAM-2, and Moesin. In some examples, the CMSD consists essentially of (e.g., consists of) one CMSD ITAM. In some examples, the CMSD consists essentially of (e.g., consists of) one CMSD ITAM and a CMSD N-terminal sequence and/or a CMSD C-terminal sequence that is heterologous to the ITAM-containing parent molecule (e.g., a G/S linker). In some examples, the plurality (*e.g.*, 2, 3, 4, or more) of CMSD ITAMs are directly linked to each other. In some examples, the CMSD comprises two or more (*e.g.*, 2, 3, 4, or more) CMSD ITAMs connected by one or more linkers not derived from an ITAM-containing parent molecule (*e.g.*, G/S linker). In some examples, the CMSD comprises one or more CMSD linkers derived from an ITAM-

containing parent molecule that is different from the ITAM-containing parent molecule from which one or more of the CMSD ITAMs are derived from. In some examples, the CMSD comprises two or more (*e.g.*, 2, 3, 4, or more) identical CMSD ITAMs. In some examples, at least one of the CMSD ITAMs is not derived from CD3ζ. In some examples, at least one of the CMSD ITAMs is not ITAM1 or ITAM2 of CD3ζ. In some examples, the plurality of CMSD ITAMs are each derived from a different ITAM-containing parent molecule. In some examples, at least one of the CMSD ITAMs is derived from an ITAM-containing parent molecule selected from the group consisting of CD3ε, CD3δ, CD3γ, Igα (CD79a), Igβ (CD79b), FcεRIβ, FcεRIγ, DAP12, CNAIP/NFAM1, STAM-1, STAM-2, and Moesin.

[9] In some examples according to any one of the modified T cells described above, at least one of the plurality of CMSD ITAMs is derived from an ITAM-containing parent molecule selected from the group consisting of CD3ε, CD3δ, CD3γ, CD3ζ, Igα (CD79a), Igβ (CD79b), FcεRIβ, FcεRIγ, DAP12, CNAIP/NFAM1, STAM-1, STAM-2, and Moesin. In some examples, the CMSD does not comprise ITAM1 and/or ITAM2 of CD3ζ. In some examples, the CMSD comprises ITAM3 of CD3ζ. In some examples, at least two of the CMSD ITAMs are derived from the same ITAM-containing parent molecule. In some examples, at least two of the CMSD ITAMs are different from each other. In some examples, at least one of the CMSD linkers is derived from CD3ζ. In some examples, at least one of the CMSD linkers is heterologous to the ITAM-containing parent molecule. In some examples, the heterologous CMSD linker is selected from the group consisting of SEQ ID NOs: 12-26, 103-107, and 119-126. In some examples, the heterologous CMSD linker is a G/S linker. In some examples, the CMSD comprises two or more heterologous CMSD linkers. In some examples, the two or more heterologous CMSD linker sequences are identical to each other. In some examples, the two or more heterologous CMSD linker sequences are different from each other. In some examples, the CMSD linker sequence is about 1 to about 15 amino acids long. In some examples, the heterologous CMSD linker is selected from the group consisting of SEQ ID NOs: 12-14, 18, and 120-124.

[10] In some examples according to any of the modified T cells described above, the CMSD further comprises a CMSD C-terminal sequence at the C-terminus of the most C-terminal ITAM. In some examples, the CMSD C-terminal sequence is derived from CD3ζ. In some examples, the CMSD C-terminal sequence is heterologous to the ITAM-containing parent

molecule. In some examples, the CMSD C-terminal sequence is selected from the group consisting of SEQ ID NOs: 12-26, 103-107, and 119-126. In some examples, the CMSD C-terminal sequence is about 1 to about 15 amino acids long. In some examples, the CMSD C-terminal sequence is selected from the group consisting of SEQ ID NOs: 13, 15, 120, and 122-124.

[11] In some examples according to any of the modified T cells described above, the CMSD further comprises a CMSD N-terminal sequence at the N-terminus of the most N-terminal ITAM. In some examples, the CMSD N-terminal sequence is derived from CD3ζ. In some examples, the CMSD N-terminal sequence is heterologous to the ITAM-containing parent molecule. In some examples, the CMSD N-terminal sequence is selected from the group consisting of SEQ ID NOs: 12-26, 103-107, and 119-126. In some examples, the CMSD N-terminal sequence is about 1 to about 15 amino acids long. In some examples, the CMSD N-terminal sequence is selected from the group consisting of SEQ ID NOs: 12, 16, 17, 119, 125, and 126.

[12] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – CD3ζ ITAM1 – optional first CMSD linker – CD3ζ ITAM2 – optional second CMSD linker – CD3ζ ITAM3 – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 39 or 48.

[13] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – CD3ζ ITAM1 – optional first CMSD linker – CD3ζ ITAM1 – optional second CMSD linker – CD3ζ ITAM1 – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 40 or 49.

[14] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – CD3ζ ITAM2 – optional first CMSD linker – CD3ζ ITAM2 – optional second CMSD linker – CD3ζ ITAM2 – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 41.

[15] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – CD3ζ

ITAM3 – optional first CMSD linker – CD3ζ ITAM3 – optional second CMSD linker – CD3ζ ITAM3 – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 42.

[16] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – CD3ε ITAM – optional first CMSD linker – CD3ε ITAM – optional second CMSD linker – CD3ε ITAM – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 43 or 50.

[17] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – DAP12 ITAM – optional first CMSD linker – DAP12 ITAM – optional second CMSD linker – DAP12 ITAM – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 44.

[18] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – Igα ITAM – optional first CMSD linker – Igα ITAM – optional second CMSD linker – Igα ITAM – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 45.

[19] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – Igβ ITAM – optional first CMSD linker – Igβ ITAM – optional second CMSD linker – Igβ ITAM – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 46.

[20] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – FcεRIγ ITAM – optional first CMSD linker – FcεRIγ ITAM – optional second CMSD linker – FcεRIγ ITAM – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 47.

[21] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – CD3δ ITAM – optional first CMSD linker – CD3ε ITAM – optional second CMSD linker – CD3γ

ITAM – optional third CMSD linker – DAP12 ITAM – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 51.

[22] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – CD3δ ITAM – optional first CMSD linker – CD3δ ITAM – optional second CMSD linker – CD3δ ITAM – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 132.

[23] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – CD3γ ITAM – optional first CMSD linker – CD3γ ITAM – optional second CMSD linker – CD3γ ITAM – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 133.

[24] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – FcεRIβ ITAM – optional first CMSD linker – FcεRIβ ITAM – optional second CMSD linker – FcεRIβ ITAM – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 134.

[25] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – CNAIP/NFAM1 ITAM – optional first CMSD linker – CNAIP/NFAM1 ITAM – optional second CMSD linker – CNAIP/NFAM1 ITAM – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 135.

[26] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – CD3ε ITAM – optional first CMSD linker – CD3δ ITAM – optional second CMSD linker – DAP12 ITAM – optional third CMSD linker – CD3γ ITAM – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 142.

[27] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – CD3γ ITAM – optional first CMSD linker – DAP12 ITAM – optional second CMSD linker – CD3δ

ITAM – optional third CMSD linker – CD3ε ITAM – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 143.

[28] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – DAP12 ITAM – optional first CMSD linker – CD3γ ITAM – optional second CMSD linker – CD3ε ITAM – optional third CMSD linker – CD3δ ITAM – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 144.

[29] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – CD3δ ITAM – optional first CMSD linker – CD3ε ITAM – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 147.

[30] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – CD3γ ITAM – optional first CMSD linker – DAP12 ITAM – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 148.

[31] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – CD3δ ITAM – optional first CMSD linker – CD3ε ITAM – optional second CMSD linker – CD3ε ITAM – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 149.

[32] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – CD3δ ITAM – optional first CMSD linker – CD3ε ITAM – optional second CMSD linker – CD3γ ITAM – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 150.

[33] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – DAP12 ITAM – optional first CMSD linker – CD3ε ITAM – optional second CMSD linker – CD3δ ITAM – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 151.

[34] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – DAP12 ITAM – optional first CMSD linker – CD3δ ITAM – optional second CMSD linker – CD3ε ITAM – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 152.

[35] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – CD3ε ITAM – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 145.

[36] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – CD3δ ITAM – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of SEQ ID NO: 146.

[37] In some examples according to any of the modified T cells described above, the CMSD comprises from N-terminus to C-terminus: optional CMSD N-terminal sequence – CD3δ ITAM – optional first CMSD linker – CD3ε ITAM – optional second CMSD linker – CD3γ ITAM – optional third CMSD linker – DAP12 ITAM – optional CMSD C-terminal sequence. In some examples, the CMSD comprises the sequence of any of SEQ ID NOs: 136-141.

[38] In some examples according to any of the modified T cells described above, the functional exogenous receptor is an ITAM-modified T cell receptor (TCR), an ITAM-modified chimeric antigen receptor (CAR), an ITAM-modified chimeric TCR (cTCR), or an ITAM-modified T cell antigen coupler (TAC)-like chimeric receptor.

[39] In some examples according to any of the modified T cells described above, the functional exogenous receptor is an ITAM-modified CAR. In some examples, the transmembrane domain is derived from CD8α. In some examples, the ISD further comprises a co-stimulatory signaling domain. In some examples, the co-stimulatory signaling domain is derived from 4-1BB or CD28. In some examples, the co-stimulatory signaling domain comprises the amino acid sequence of SEQ ID NO: 36. In some examples, the co-stimulatory domain is N-terminal to the CMSD. In some examples, the co-stimulatory domain is C-terminal to the CMSD.

[40] In some examples according to any of the modified T cells described above, the functional exogenous receptor is an ITAM-modified cTCR. In some examples the ITAM-modified cTCR comprises: (a) an extracellular ligand binding domain (such as antigen-binding fragments (*e.g.*, scFv, sdAb) specifically recognizing one or more epitopes of a target antigen (*e.g.*, tumor antigen such as BCMA, CD19, CD20), extracellular domains (or portion thereof) of receptors (*e.g.*, FcR), extracellular domains (or portion thereof) of ligands (*e.g.*, APRIL, BAFF)), (b) an optional receptor domain linker, (c) an optional extracellular domain of a first TCR subunit (*e.g.*, CD3ε) or a portion thereof, (d) a transmembrane domain comprising a transmembrane domain of a second TCR subunit (*e.g.*, CD3ε), and (e) an ISD comprising a CMSD (*e.g.*, CMSD comprising a sequence selected from the group consisting of SEQ ID NOs: 39-51 and 132-152), wherein the CMSD comprises one or a plurality of CMSD ITAMs, wherein the plurality of CMSD ITAMs are optionally connected by one or more CMSD linkers, and wherein the first and second TCR subunits are selected from the group consisting of TCRα, TCRβ, TCRγ, TCRδ, CD3ε, CD3γ, and CD3δ. In some examples, the first and second TCR subunits are both CD3ε. In some examples, wherein the one or plurality of CMSD ITAMs are derived from one or more of CD3ε, CD3δ, and CD3γ.

[41] In some examples according to any of the modified T cells described above, the functional exogenous receptor is an ITAM-modified TAC-like chimeric receptor. In some examples, the ITAM-modified TAC-like chimeric receptor comprises: (a) an extracellular ligand binding domain (such as antigen-binding fragments (*e.g.*, scFv, sdAb) specifically recognizing one or more epitopes of one or more target antigens (e.g., tumor antigen such as BCMA, CD19, CD20), extracellular domains (or portion thereof) of receptors (*e.g.*, FcR), extracellular domains (or portion thereof) of ligands (*e.g.*, APRIL, BAFF)), (b) an optional first receptor domain linker, (c) an extracellular TCR binding domain that specifically recognizes the extracellular domain of a first TCR subunit (*e.g.*, CD3ε), (d) an optional second receptor domain linker, (e) an optional extracellular domain of a second TCR subunit (*e.g.*, CD3ε) or a portion thereof, (f) a transmembrane domain comprising a transmembrane domain of a third TCR subunit (*e.g.*, CD3ε), and (g) an ISD comprising a CMSD (*e.g.*, CMSD comprising a sequence selected from the group consisting of SEQ ID NOs: 39-51 and 132-152), wherein the CMSD comprises one or a plurality of CMSD ITAMs, wherein the plurality of CMSD ITAMs are optionally connected by one or more CMSD linkers, and wherein the first, second, and third

TCR subunits are all selected from the group consisting of TCRα, TCRβ, TCRγ, TCRδ, CD3ε, CD3γ, and CD3δ. In some examples, the second and third TCR subunits are both CD3ε. In some examples, the one or plurality of CMSD ITAMs are derived from one or more of CD3ε, CD3δ, and CD3γ.

[42] In some examples according to any of the modified T cells described above, the extracellular ligand binding domain comprises one or more antigen-binding fragments that specifically recognizing one or more epitopes of one or more target antigens (e.g., tumor antigen). In some examples, the extracellular ligand binding domain comprises an sdAb or an scFv. In some examples, the target antigen (e.g., tumor antigen) is BCMA, CD19, or CD20.

[43] In some examples according to any of the modified T cells described above, the functional exogenous receptor further comprises a hinge domain located between the C-terminus of the extracellular ligand binding domain and the N-terminus of the transmembrane domain. In some examples, the hinge domain is derived from CD8α. In some examples, the functional exogenous receptor further comprises a signal peptide located at the N-terminus of the functional exogenous receptor, such as a signal peptide derived from CD8α.

[44] In some examples according to any of the modified T cells described above, the effector function of the functional exogenous receptor comprising the ISD that comprises the CMSD is at most about 80% (such as at most about any of 70%, 60%, 50%, 40%, 30%, 20%, 10%, or 5%) less than a functional exogenous receptor comprising an ISD that comprises an intracellular signaling domain of CD3ζ.

[45] In some examples according to any of the modified T cells described above, the effector function of the functional exogenous receptor comprising the ISD that comprises the CMSD is at least about 20% (such as at least about any of 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%) active relative to a functional exogenous receptor comprising an ISD that comprises an intracellular signaling domain of CD3ζ.

[46] In some examples according to any of the modified T cells described above, the exogenous Nef protein (*e.g.*, wildtype Nef such as wildtype SIV Nef, or mutant Nef such as mutant SIV Nef) down-modulates (*e.g.*, down-regulates cell surface expression and/or effector function of) endogenous TCR, CD3, and/or MHC I of the modified T cell, such as down-modulates (*e.g.*, down-regulates cell surface expression and/or effector function of) the

endogenous TCR, CD3, and/or MHC I by at least about 40% (such as at least about any of 50%, 60%, 70%, 80%, 90%, or 95%). In some examples, the exogenous Nef protein does not down-modulate (*e.g.*, down-regulate cell surface expression and/or effector function of) the CMSD-containing functional exogenous receptor (*e.g.*, ITAM-modified CAR, ITAM-modified TCR, ITAM-modified cTCR, or ITAM-modified TAC-like chimeric receptor). In some examples, the exogenous Nef protein down-modulates (*e.g.*, down-regulate cell surface expression and/or effector function such as signal transduction related to cytolytic activity of) the CMSD-containing functional exogenous receptor by at most about 80% (such as at most about any of 70%, 60%, 50%, 40%, 30%, 20%, 10%, or 5%).

[47] In some examples according to any of the modified T cells described above, the modified T cell expressing the exogenous Nef protein elicits no or reduced (e.g., reducing at least about 30%) graft-versus-host disease (GvHD) response in a histoincompatible individual as compared to the GvHD response elicited by a primary T cell isolated from a donor of a precursor T cell from which the modified T cell is derived.

[48] In some examples according to any of the modified T cells described above, the exogenous Nef protein is selected from the group consisting of SIV Nef, HIV1 Nef, HIV2 Nef, and subtypes thereof. In some examples, the exogenous Nef protein is a wildtype Nef, such as a wildtype Nef comprising an amino acid sequence of any one of SEQ ID NOs: 79, 80, and 84. In some examples, the exogenous Nef protein is a Nef subtype, such as HIV F2-Nef, HIV C2-Nef, or HIV HV2NZ-Nef. In some examples, the Nef subtype comprises an amino acid sequence of any one of SEQ ID NOs: 81-83 and 207-231. In some examples, the exogenous Nef protein is a mutant Nef, such as a mutant SIV Nef. In some examples, the mutant Nef comprises one or more mutations in myristoylation site, N-terminal α-helix, tyrosine-based AP recruitment, CD4 binding site, acidic cluster, proline-based repeat, PAK binding domain, COP I recruitment domain, di-leucine based AP recruitment domain, V-ATPase and Raf-1 binding domain, or any combinations thereof. In some examples, the mutant Nef comprises an amino acid sequence of any one of SEQ ID NOs: 85-89 and 198-204. In some examples, the exogenous Nef protein comprises an amino acid sequence of any of SEQ ID NOs: 79-89, 198-204, and 207-231. In some examples, the exogenous Nef protein comprises the amino acid sequence of any one of SEQ ID NOs: 235-247, wherein x and X are independently any amino acid or absent. In some examples, the exogenous Nef protein comprises an amino acid

sequence of at least about 70% (such as at least about any of 80%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to that of SEQ ID NO: 85 or 230, and comprises the amino acid sequence of any one of SEQ ID NOs: 235-247, wherein x and X are independently any amino acid or absent. In some examples, the nucleic acid encoding the exogenous Nef protein has at least about 70% (such as at least about any of 80%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to that of SEQ ID NO: 96 or 234.

[49] The present disclosure in another example provides a method of producing a modified T cell (*e.g.*, allogeneic T cell), comprising introducing into a precursor T cell a first nucleic acid encoding an exogenous Nef protein (*e.g.*, wildtype Nef such as wildtype SIV Nef, or mutant Nef such as mutant SIV Nef) and a second nucleic acid encoding a functional exogenous receptor (*e.g.*, ITAM-modified CAR, ITAM-modified TCR, ITAM-modified cTCR, or ITAM-modified TAC-like chimeric receptor), wherein the functional exogenous receptor comprises: (a) an extracellular ligand binding domain (such as antigen-binding fragments (*e.g.*, scFv, sdAb) specifically recognizing one or more epitopes of one or more target antigens (e.g., tumor antigen such as BCMA, CD19, CD20), extracellular domains (or portion thereof) of receptors (*e.g.*, FcR), extracellular domains (or portion thereof) of ligands (*e.g.*, APRIL, BAFF)), (b) a transmembrane domain (*e.g.*, derived from CD8α), and (c) an ISD comprising a CMSD (*e.g.*, CMSD comprising a sequence selected from the group consisting of SEQ ID NOs: 39-51 and 132-152), wherein the CMSD comprises one or a plurality of CMSD ITAMs, wherein the plurality of CMSD ITAMs are optionally connected by one or more CMSD linkers. In one aspect, the present disclosure refers to a method of producing a modified T cell as disclosed herein, comprising introducing into a precursor T cell a first nucleic acid encoding the exogenous Nef protein and a second nucleic acid encoding the functional exogenous receptor. In some examples, the first nucleic acid and the second nucleic acid are on separate vectors. In some examples, the first nucleic acid and the second nucleic acid are on the same vector. In some examples, the first nucleic acid and the second nucleic acid are operably linked to the same promoter. In some examples, the first nucleic acid is upstream of the second nucleic acid. In some examples, the first nucleic acid is downstream of the second nucleic acid. In some examples, the first nucleic acid and the second nucleic acid are connected via a linking sequence, such as a nucleic acid sequence encoding any of P2A, T2A, E2A, F2A, BmCPV 2A, BmIFV 2A, $(GS)_n$, $(GGGS)_n$, and $(GGGGS)_n$; or a nucleic acid sequence of any of IRES, SV40, CMV, UBC, EF1α, PGK, and CAGG; or any combinations thereof, wherein n is an integer of at least one. In some examples, the linking sequence is IRES. In some examples, the vector is a viral vector (*e.g.*, lentiviral vector). In some examples, the modified T cell expressing the

exogenous Nef protein elicits no or reduced GvHD response in a histoincompatible individual as compared to the GvHD response elicited by a primary T cell isolated from the donor of the precursor T cell. In some examples, the method further comprises isolating and/or enriching TCR-negative and functional exogenous receptor-positive T cells from the modified T cells. In some examples, the method further comprises formulating the modified T cell with at least one pharmaceutically acceptable carrier. In some examples, the plurality (*e.g.*, 2, 3, 4, or more) of CMSD ITAMs are directly linked to each other. In some examples, the CMSD comprises two or more (*e.g.*, 2, 3, 4, or more) CMSD ITAMs connected by one or more linkers not derived from an ITAM-containing parent molecule (*e.g.*, G/S linker). In some examples, the CMSD comprises one or more CMSD linkers derived from an ITAM-containing parent molecule that is different from the ITAM-containing parent molecule from which one or more of the CMSD ITAMs are derived from. In some examples, the CMSD comprises two or more (*e.g.*, 2, 3, 4, or more) identical CMSD ITAMs. In some examples, at least one of the CMSD ITAMs is not derived from CD3ζ. In some examples, at least one of the CMSD ITAMs is not ITAM1 or ITAM2 of CD3ζ. In some examples, the plurality of CMSD ITAMs are each derived from a different ITAM-containing parent molecule. In some examples, at least one of the CMSD ITAMs is derived from an ITAM-containing parent molecule selected from the group consisting of CD3ε, CD3δ, CD3γ, Igα (CD79a), Igβ (CD79b), FcεRIβ, FcεRIγ, DAP12, CNAIP/NFAM1, STAM-1, STAM-2, and Moesin. In some examples, at least one of the plurality of CMSD ITAMs is derived from an ITAM-containing parent molecule selected from the group consisting of CD3ε, CD3δ, CD3γ, CD3ζ, Igα (CD79a), Igβ (CD79b), FcεRIβ, FcεRIγ, DAP12, CNAIP/NFAM1, STAM-1, STAM-2, and Moesin.

[50] In another example, there is also provided a modified T cell (*e.g.*, allogeneic T cell) obtained by any of the methods described above. In one aspect, the present disclosure refers to a modified T cell obtained by the method as disclosed herein.

[51] In one aspect, the present disclosure refers to a vector encoding the exogenous Nef protein and the functional exogenous receptor comprised by the modified T cell as disclosed herein, comprising a first nucleic acid encoding the exogenous Nef protein and a second nucleic acid encoding the functional exogenous receptor. In a further example, there is provided a viral vector (*e.g.*, lentiviral vector) comprising a first nucleic acid encoding an exogenous Nef protein (*e.g.*, wildtype Nef such as wildtype SIV Nef, or mutant Nef such as mutant SIV Nef) and a second nucleic acid encoding a functional exogenous receptor (*e.g.*, ITAM-modified CAR, ITAM-modified TCR, ITAM-modified cTCR, or ITAM-modified TAC-like chimeric receptor), wherein the functional exogenous receptor comprises: (a) an extracellular ligand binding domain (such as antigen-binding fragments (*e.g.*, scFv, sdAb) specifically recognizing one or more epitopes of one or more target antigens (e.g.,

tumor antigen such as BCMA, CD19, CD20), extracellular domains (or portion thereof) of receptors (*e.g.*, FcR), extracellular domains (or portion thereof) of ligands (*e.g.*, APRIL, BAFF)), (b) a transmembrane domain (*e.g.*, derived from CD8α), and (c) an ISD comprising a CMSD (*e.g.*, CMSD comprising a sequence selected from the group consisting of SEQ ID NOs: 39-51 and 132-152), wherein the CMSD comprises one or a plurality of CMSD ITAMs, wherein the plurality of CMSD ITAMs are optionally connected by one or more CMSD linkers. In some examples, the first nucleic acid and the second nucleic acid are operably linked to the same promoter. In some examples, the first nucleic acid is upstream of the second nucleic acid. In some examples, the first nucleic acid is downstream of the second nucleic acid. In some examples, the first nucleic acid and the second nucleic acid are connected via a linking sequence, such as a nucleic acid sequence encoding any of P2A, T2A, E2A, F2A, BmCPV 2A, BmIFV 2A, $(GS)_n$, $(GGGS)_n$, and $(GGGGS)_n$; or a nucleic acid sequence of any of IRES, SV40, CMV, UBC, EF1α, PGK, and CAGG; or any combinations thereof, wherein n is an integer of at least one. In some examples, the linking sequence is IRES. In some examples, the plurality (*e.g.*, 2, 3, 4, or more) of CMSD ITAMs are directly linked to each other. In some examples, the CMSD comprises two or more (*e.g.*, 2, 3, 4, or more) CMSD ITAMs connected by one or more linkers not derived from an ITAM-containing parent molecule (*e.g.*, G/S linker). In some examples, the CMSD comprises one or more CMSD linkers derived from an ITAM-containing parent molecule that is different from the ITAM-containing parent molecule from which one or more of the CMSD ITAMs are derived from. In some examples, the CMSD comprises two or more (*e.g.*, 2, 3, 4, or more) identical CMSD ITAMs. In some examples, at least one of the CMSD ITAMs is not derived from CD3ζ. In some examples, at least one of the CMSD ITAMs is not ITAM1 or ITAM2 of CD3ζ. In some examples, the plurality of CMSD ITAMs are each derived from a different ITAM-containing parent molecule. In some examples, at least one of the CMSD ITAMs is derived from an ITAM-containing parent molecule selected from the group consisting of CD3ε, CD3δ, CD3γ, Igα (CD79a), Igβ (CD79b), FcεRIβ, FcεRIγ, DAP12, CNAIP/NFAM1, STAM-1, STAM-2, and Moesin. In some examples, the CMSD consists essentially of (e.g., consists of) one CMSD ITAM. In some examples, the CMSD consists essentially of (e.g., consists of) one CMSD ITAM and an N-terminal sequence and/or a C-terminal sequence that is heterologous to the ITAM-containing parent molecule (e.g., a G/S linker). In some examples, at least one of the

CMSD ITAMs is derived from an ITAM-containing parent molecule selected from the group consisting of CD3ε, CD3δ, CD3γ, CD3ζ, Igα (CD79a), Igβ (CD79b), FcεRIβ, FcεRIγ, DAP12, CNAIP/NFAM1, STAM-1, STAM-2, and Moesin.

[52] In a further example, there is provided Nef proteins (including novel, non-naturally occurring Nef proteins) and T cells expressing such Nef proteins. The T cells optionally further comprise a functional exogenous receptor (such as any of the ITAM-modified functional exogenous receptors described herein, or BCMA CAR described herein). In some examples, the Nef protein (such as a non-naturally occurring Nef protein) comprises an amino acid sequence of any one of SEQ ID NOs: 79-89, 198-204, and 207-231. In some examples, the Nef protein (such as a non-naturally occurring Nef protein) comprises the amino acid sequence of any one of SEQ ID NOs: 235-247, wherein x and X are independently any amino acid or absent. In some examples, the Nef protein (such as a non-naturally occurring Nef protein) comprises the amino acid sequence of at least about 70% (such as at least about any of 80%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to that of SEQ ID NO: 85 or 230, and comprises the amino acid sequence of any one of SEQ ID NOs: 235-247, wherein x and X are independently any amino acid or absent. In one aspect, the present disclosure refers to a non-naturally occurring Nef protein, comprising: (i) the amino acid sequence of any one of SEQ ID NOs: 85-89 and 198-204; or (ii) the amino acid sequence of at least about 70% sequence identity to that of SEQ ID NO: 85 or 230, and the amino acid sequence of any one of SEQ ID NOs: 235-247, wherein x and X are independently any amino acid or absent. In some examples, the Nef protein upon expression does not down-modulate (e.g., down-regulate cell surface expression and/or effector function) endogenous TCR, CD3, and/or MHC of a T cell. In some examples, the Nef protein upon expression down-modulates endogenous TCR, CD3, and/or MHC of a T cell by at least about 40% (such as at least about any of 50%, 60%, 70%, 80%, 90%, or 95%). In some examples, the Nef protein upon expression down-modulates endogenous TCR, CD3, and/or MHC of the T cell at least about 3% (such as at least about any of 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%) more than that of a wildtype Nef protein. In some examples, the Nef protein upon expression does not down-modulate endogenous CD4 and/or CD28 of a T cell. In some examples, the Nef protein upon expression down-modulates endogenous CD4 and/or CD28 of a T cell by at most about 50% (such as at most about any of 40%, 30%, 20%, 10%, or 5%). In some examples, the Nef protein upon expression down-modulates endogenous CD4 and/or CD28 of the T cell at least about 3% (such as at least about any of 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%) less than that of a wildtype Nef protein. In some examples, the Nef protein upon expression does not down-modulate a functional exogenous receptor (e.g., CMSD-containing functional exogenous receptor, or a BCMA CAR) of a T cell.

In some examples, the Nef protein upon expression down-modulates a functional exogenous receptor (e.g., CMSD-containing functional exogenous receptor, or a BCMA CAR) of a T cell by at most about 80% (such as at most about any of 70%, 60%, 50%, 40%, 30%, 20%, 10%, or 5%).In some examples, the Nef protein upon expression down-modulates the functional exogenous receptor of the T cell at least about 3% (such as at least about any of 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%) less than that of a wildtype Nef protein. In some examples, the Nef protein upon expression eliminates or reduces (such as reduced by at least about any of 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%) GvHD response of a donor T cell in a histoincompatible individual.

[53] Isolated nucleic acids encoding any of the exogenous Nef proteins and CMSD-containing functional exogenous receptors described herein, vectors (e.g., viral vectors) containing such nucleic acids, immune effector cells (e.g., T cell) containing such vectors, are also provided.

[54] Pharmaceutical compositions comprising any of the modified T cells (*e.g.*, allogeneic T cells) described herein, methods of treating a disease (*e.g.*, cancer, GvHD, infectious disease, transplantation rejection, autoimmune disorders, or radiation sickness) using any of the modified T cells described herein or pharmaceutical compositions thereof are also provided. In one aspect, the present disclosure refers to a pharmaceutical composition comprising the modified T cell as disclosed herein, and optionally a pharmaceutically acceptable carrier. In some examples, the individual (*e.g.*, human) for treatment is histoincompatible with the donor of the precursor T cell from which the modified T cell is derived.

[54a] In one aspect, the present disclosure refers to use of an effective amount of the modified T cell as disclosed herein, or the pharmaceutical composition as disclosed herein, in the manufacture of a medicament for treating a disease in an individual.

[55] The present disclosure further provides kits and articles of manufacture that are useful for the methods described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[56] FIG. 1A shows CAR expression rate of Jurkat-BCMA-BBz (Jurkat cells transduced with lentiviruses carrying traditional BCMA CAR “BCMA-BBz” sequence) cell culture (83.9% CAR+), Jurkat-BCMA-BBz cell culture further transduced with lentiviruses carrying wildtype SIV Nef sequence (Jurkat-BCMA-BBz-SIV Nef cells, 42.3% CAR+), Jurkat-BCMA-BBz cell culture further transduced with lentiviruses carrying SIV Nef M116 sequence (Jurkat-BCMA-BBz-SIV Nef M116 cells, 39.1% CAR+). Jurkat-BCMA-BBz cell culture further transduced with empty vector (Jurkat-BCMA-BBz-empty vector cells, 83.6% CAR+) served as control. FIG. 1B shows TCRαβ expression in control untransduced Jurkat cells (96.8% TCRαβ pos),

CLAIMS

We claim:

1. A modified T cell comprising:

i) an exogenous Nef protein; and

ii) a functional exogenous receptor, comprising:

(a) an extracellular ligand binding domain,

(b) a transmembrane domain, and

(c) an intracellular signaling domain (ISD) comprising a chimeric signaling domain (CMSD),

wherein the CMSD comprises one or a plurality of Immune-receptor Tyrosine-based Activation Motifs (CMSD ITAMs), wherein the plurality of CMSD ITAMs are optionally connected by one or more linkers (CMSD linkers).

2. The modified T cell of claim 1, wherein:

(a) the plurality of CMSD ITAMs are directly linked to each other;

(b) the CMSD comprises two or more CMSD ITAMs connected by one or more CMSD linkers not derived from an ITAM-containing parent molecule;

(c) the CMSD comprises one or more CMSD linkers derived from an ITAM-containing parent molecule that is different from the ITAM-containing parent molecule from which one or more of the CMSD ITAMs are derived from;

(d) the CMSD comprises two or more identical CMSD ITAMs;

(e) at least one of the CMSD ITAMs is not derived from CD3ζ;

(f) at least one of the CMSD ITAMs is not ITAM1 or ITAM2 of CD3ζ;

(g) the plurality of CMSD ITAMs are each derived from a different ITAM-containing parent molecule; and/or

(h) at least one of the CMSD ITAMs is derived from an ITAM-containing parent molecule selected from the group consisting of CD3ε, CD3δ, CD3γ, CD3ζ, Igα (CD79a), Igβ (CD79b), FcεRIβ, FcεRIγ, DAP12, CNAIP/NFAM1, STAM-1, STAM-2, and Moesin.

3. The modified T cell of claim 1 or 2, wherein:

i) the CMSD does not comprise ITAM1 and/or ITAM2 of CD3ζ;

ii) the CMSD comprises ITAM3 of CD3ζ;

iii) the CMSD comprises at least two CMSD ITAMs, wherein the at least two CMSD ITAMs:

(a) are derived from the same ITAM-containing parent molecule, optionally wherein the at least two CMSD ITAMs are identical to each other or are different to each other; or

(b) are different from each other and are each derived from a different ITAM-containing parent molecule;

iv) at least one of the CMSD linkers is derived from CD3ζ;

v) at least one of the CMSD linkers is heterologous to the ITAM-containing parent molecule;

vi) the CMSD further comprises a C-terminal sequence at the C-terminus of the most C-terminal CMSD ITAM (CMSD C-terminal sequence);

vii) the CMSD further comprises an N-terminal sequence at the N-terminus of the most N-terminal CMSD ITAM (CMSD N-terminal sequence); and/or

viii) the one or more CMSD linkers, the CMSD C-terminal sequence, and/or the CMSD N-terminal sequence are independently selected from the group consisting of SEQ ID NOs: 12-26, 103-107, and 119-126.

4. The modified T cell of any one of claims 1-3, wherein the CMSD comprises a sequence selected from the group consisting of SEQ ID NOs: 39-51 and 132-152.

5. The modified T cell of any one of claims 1-4, wherein the functional exogenous receptor is an ITAM-modified T cell receptor (TCR), an ITAM-modified chimeric antigen receptor (CAR), an ITAM-modified chimeric TCR (cTCR), or an ITAM-modified T cell antigen coupler (TAC)-like chimeric receptor.

6. The modified T cell of claim 5, wherein the functional exogenous receptor is an ITAM-modified CAR; optionally

i) wherein the transmembrane domain is derived from CD8α; and/or

ii) wherein the ISD further comprises a co-stimulatory signaling domain; optionally wherein:

(a) the co-stimulatory signaling domain is derived from CD137 (4-1BB) or CD28;

(b) the co-stimulatory signaling domain comprises the amino acid sequence of SEQ ID NO: 36; and/or

(c) the co-stimulatory domain is N-terminal to the CMSD or C-terminal to the CMSD.

7. The modified T cell of claim 6, wherein the ITAM-modified CAR comprises the sequence of any of SEQ ID NOs: 71, 73, 109, 153-175, 177-182, and 205.

8. The modified T cell of claim 5, wherein the functional exogenous receptor is an ITAM-modified cTCR comprising:

(a) the extracellular ligand binding domain,

(b) an optional receptor domain linker,

(c) an optional extracellular domain of a first TCR subunit or a portion thereof,

(d) the transmembrane domain, wherein the transmembrane domain comprises a transmembrane domain of a second TCR subunit, and

(e) the ISD comprising the CMSD,

wherein the first and second TCR subunits are independently selected from the group consisting of TCRα, TCRβ, TCRγ, TCRδ, CD3ε, CD3γ, and CD3δ; optionally,

wherein the first and second TCR subunits are both CD3ε; and/or

wherein the one or a plurality of CMSD ITAMs are derived from one or more of CD3ε, CD3δ, and CD3γ.

9. The modified T cell of claim 5, wherein the functional exogenous receptor is an ITAM-modified TAC-like chimeric receptor comprising:

(a) the extracellular ligand binding domain,

(b) an optional first receptor domain linker,

(c) an extracellular TCR binding domain that specifically recognizes the extracellular domain of a first TCR subunit,

(d) an optional second receptor domain linker,

(e) an optional extracellular domain of a second TCR subunit or a portion thereof,

(f) the transmembrane domain, wherein the transmembrane domain comprises a transmembrane domain of a third TCR subunit, and

(g) the ISD comprising the CMSD,

wherein the first, second, and third TCR subunits are all independently selected from the group consisting of TCRα, TCRβ, TCRγ, TCRδ, CD3ε, CD3γ, and CD3δ; optionally,

wherein the second and third TCR subunits are both CD3ε; and/or

wherein the one or a plurality of CMSD ITAMs are derived from one or more of CD3ε, CD3δ, and CD3γ.

10. The modified T cell of any one of claims 1-9, wherein

i) the extracellular ligand binding domain comprises one or more antigen-binding fragments that specifically recognize one or more epitopes of one or more target antigens; optionally

a) wherein the antigen-binding fragment is an sdAb or an scFv; and/or

b) wherein the target antigen is BCMA, CD19, and/or CD20;

ii) the functional exogenous receptor further comprises a hinge domain located between the C-terminus of the extracellular ligand binding domain and the N-terminus of the transmembrane domain;

optionally wherein the hinge domain is derived from CD8α; and/or

iii) the effector function of the functional exogenous receptor comprising the ISD that comprises the CMSD is at most about 80% less than a functional exogenous receptor comprising an ISD that comprises an intracellular signaling domain of CD3ζ.

11. The modified T cell of any one of claims 1-10, wherein

i) the exogenous Nef protein is selected from the group consisting of SIV Nef, HIV1 Nef, HIV2 Nef, subtypes thereof, and mutants thereof;

ii) the exogenous Nef protein down-modulates the endogenous TCR, CD3, and/or MHC I of the modified T cell;

iii) a) the exogenous Nef protein does not down-modulate the functional exogenous receptor; or b) the exogenous Nef protein down-modulates the functional exogenous receptor by at most about 80%; and/or

iv) the modified T cell expressing the exogenous Nef protein elicits no or reduced graft-versus-host disease (GvHD) response in a histoincompatible individual as compared to the GvHD response elicited by a primary T cell isolated from a donor of a precursor T cell from which the modified T cell is derived.

12. The modified T cell of claim 11, wherein

i) the exogenous Nef protein comprises the amino acid sequence of any of SEQ ID NOs: 235-247, wherein x and X are independently any amino acid or absent;

ii) the exogenous Nef protein comprises the amino acid sequence of at least about 70% sequence identity to that of SEQ ID NO: 85 or 230, and comprises the amino acid sequence of any one of SEQ ID NOs: 235-247, wherein x and X are independently any amino acid or absent;

iii) the exogenous Nef protein comprises the amino acid sequence of any of SEQ ID NOs: 79-89, 198-204, and 207-231; and/or

iv) the down-modulation comprises down-regulating cell surface expression of the endogenous TCR, CD3, and/or MHC I by at least about 40%.

13. A method of producing a modified T cell of any one of claims 1-12, comprising introducing into a precursor T cell a first nucleic acid encoding the exogenous Nef protein and a second nucleic acid encoding the functional exogenous receptor.

14. The method of claim 13, wherein

i) the modified T cell expressing the exogenous Nef protein elicits no or reduced GvHD response in a histoincompatible individual as compared to the GvHD response elicited by a primary T cell isolated from the donor of the precursor T cell; and/or

ii) the method further comprises isolating and/or enriching TCR-negative and/or functional exogenous receptor-positive T cells from the modified T cells.

15. A modified T cell obtained by the method of claim 13 or 14.

16. A pharmaceutical composition comprising the modified T cell of any one of claims 1-12 and 15, and optionally a pharmaceutically acceptable carrier.

17. Use of an effective amount of the modified T cell of any one of claims 1-12 and 15, or the pharmaceutical composition of claim 16, in the manufacture of a medicament for treating a disease in an individual.

18. The use of claim 17, wherein

i) the disease is cancer; and/or

ii) the individual is histoincompatible with the donor of the precursor T cell from which the modified T cell is derived.

19. A vector encoding the exogenous Nef protein and the functional exogenous receptor comprised by the modified T cell of any one of claims 1-12 and 15, comprising a first nucleic acid encoding the exogenous Nef protein and a second nucleic acid encoding the functional exogenous receptor.

20. The vector of claim 19, wherein

i) the first nucleic acid and the second nucleic acid are operably linked to the same promoter;

ii) the first nucleic acid is upstream of the second nucleic acid, or the first nucleic acid is downstream of the second nucleic acid;

iii) the first nucleic acid and the second nucleic acid are connected via a linking sequence; and/or

iv) the vector is a viral vector.

21. The vector of claim 20, wherein the linking sequence comprises:

(a) a nucleic acid sequence encoding any of P2A, T2A, E2A, F2A, BmCPV 2A, BmIFV 2A, $(GS)_n$, $(GGGS)_n$, and $(GGGGS)_n$, wherein n is an integer of at least one;

(b) a nucleic acid sequence of any of IRES, SV40, CMV, UBC, EF1α, PGK, and CAGG; or

(c) any combinations thereof.

22. A non-naturally occurring Nef protein, comprising:

(i) the amino acid sequence of any one of SEQ ID NOs: 85-89 and 198-204; or

(ii) the amino acid sequence of at least about 70% sequence identity to that of SEQ ID NO: 85 or 230, and the amino acid sequence of any one of SEQ ID NOs: 235-247, wherein x and X are independently any amino acid or absent.