COMPOSITIONS AND METHODS FOR TREATING OR PREVENTING AUTOIMMUNE DISEASES

Sequence Listing

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Field of the Invention

The disclosure relates to methods for treating autoimmune diseases by way of regulatory T cells derived from genetically modified pluripotent hematopoietic cells, as well as compositions that may be used in such methods.

Background of the Invention

Regulatory T (Treg) cells are a subset of T cells that play a critical role in suppressing the immune response, thereby maintaining homeostasis and self-tolerance. Treg deficiency or dysfunction is implicated in the pathology of several autoimmune diseases, and Treg cell therapy has been investigated as a potential therapeutic paradigm for these diseases. The development of Treg cell therapies has been hindered by difficulties associated with durability, stability, feasibility, manufacturing, and dosage of Treg cells. There remains a need for improved Treg cell therapies for the treatment of autoimmune diseases.

Summary

The present disclosure relates to compositions and methods for the treatment of autoimmune diseases. In a first example, the disclosure provides a method of treating or preventing an autoimmune disease in a patient (e.g., a mammalian patient, such as a human patient) in need thereof by administering to the patient a population of pluripotent cells that include a nucleic acid cassette that encodes an autoantigen-binding protein. The nucleic acid cassette may be operably linked to one or more lineage-specific transcription regulatory elements that are active in CD4+CD25+ regulatory T (Treg) cells (i.e., preferentially active in cells of the Treg lineage as compared to other cell types (e.g., other hematopoietic cells)). In one aspect, the present disclosure refers to use of a population of pluripotent hematopoietic cells comprising a nucleic acid cassette that encodes an autoantigen-binding protein in the manufacture of a medicament for treating or preventing an autoimmune disease in a patient in need thereof, wherein the nucleic acid cassette is operably linked to one or more lineage-specific transcription regulatory elements that are active in CD4+CD25+ regulatory T (Treg) cells.

In a further example, the disclosure provides a method of suppressing activity and/or proliferation of a population of autoreactive effector immune cells in a patient (e.g., a mammalian patient, such as a human patient) diagnosed as having an autoimmune disease, the method including the step of administering to the patient a population of pluripotent cells that include a nucleic acid cassette that encodes an autoantigen-binding protein. The nucleic acid cassette may be operably linked to one or more lineage-specific transcription regulatory elements that are active in CD4+CD25+ Treg cells (i.e., specifically active in cells of the Treg lineage and not active in other cell types (e.g., other hematopoietic cells)). In one aspect, the present disclosure refers to use of a population of pluripotent hematopoietic cells comprising a nucleic acid cassette that encodes an autoantigen-binding protein in the manufacture of a medicament for suppressing activity and/or proliferation of a population of autoreactive effector immune cells in a patient diagnosed as having an autoimmune disease, wherein the nucleic acid cassette is operably linked to one or more lineage-specific transcription regulatory elements that are active in CD4+CD25+ Treg cells.

In another example, the disclosure provides a method of inducing apoptosis of an autoreactive effector immune cell in a patient (e.g., a mammalian patient, such as a human patient) diagnosed as having an autoimmune disease, the method including the step of administering to the patient a population of pluripotent cells that include a nucleic acid cassette that encodes an autoantigen-binding protein. The

nucleic acid cassette may be operably linked to one or more lineage-specific transcription regulatory elements that are active in CD4+CD25+ Treg cells (i.e., specifically active in cells of the Treg lineage and not active in other cell types (e.g., other hematopoietic cells)). In one aspect, the present disclosure refers to use of a population of pluripotent hematopoietic cells comprising a nucleic acid cassette that encodes an autoantigen-binding protein in the manufacture of a medicament for inducing apoptosis of an autoreactive effector immune cell in a patient diagnosed as having an autoimmune disease, wherein the nucleic acid cassette is operably linked to one or more lineage-specific transcription regulatory elements that are active in CD4+CD25+ Treg cells.

In another example, the disclosure provides a method of protecting endogenous tissue from an autoimmune response in a patient (e.g., a mammalian patient, such as a human patient) diagnosed as having an autoimmune disease, the method including the step of administering to the patient a population of pluripotent cells that include a nucleic acid cassette that encodes an autoantigen-binding protein. The nucleic acid cassette may be operably linked to one or more lineage-specific transcription regulatory elements that are active in CD4+CD25+ Treg cells (i.e., specifically active in cells of the Treg lineage and not active in other cell types (e.g., other hematopoietic cells)). In one aspect, the present disclosure refers to use of a population of pluripotent hematopoietic cells comprising a nucleic acid cassette that encodes an autoantigen-binding protein in the manufacture of a medicament for protecting endogenous tissue from an autoimmune response in a patient diagnosed as having an autoimmune disease, wherein the nucleic acid cassette is operably linked to one or more lineage-specific transcription regulatory elements that are active in CD4+CD25+ Treg cells.

In another example, the disclosure provides a method of reducing inflammation in a patient (e.g., a mammalian patient, such as a human patient) diagnosed as having an autoimmune disease, the method including the step of administering to the patient a population of pluripotent cells that include a nucleic acid cassette that encodes an autoantigen-binding protein. The nucleic acid cassette may be operably linked to one or more lineage-specific transcription regulatory elements that are active in CD4+CD25+ Treg cells (i.e., specifically active in cells of the Treg lineage and not active in other cell types (e.g., other hematopoietic cells)). In one aspect, the present disclosure refers to use of a population of pluripotent hematopoietic cells comprising a nucleic acid cassette that encodes an autoantigen-binding protein in the manufacture of a medicament for reducing inflammation in a patient diagnosed as having an autoimmune disease, wherein the nucleic acid cassette is operably linked to one or more lineage-specific transcription regulatory elements that are active in CD4+CD25+ Treg cells.

In some examples of any of the above aspects, the pluripotent cells are pluripotent hematopoietic cells (e.g., hematopoietic stem cells (HSCs) or hematopoietic progenitor cells (HPCs)). In some examples, the pluripotent hematopoietic cells are embryonic stem cells. In some examples, the pluripotent hematopoietic cells are induced pluripotent stem cells. In some examples, the pluripotent hematopoietic cells are lymphoid progenitor cells. In some examples, the pluripotent hematopoietic cells are CD34+ cells (e.g., HSCs).

In some examples, the population of pluripotent hematopoietic cells is administered systemically to the patient. For example, the population of pluripotent hematopoietic cells may be administered to the patient by way of intravenous injection. In some examples, the population of pluripotent hematopoietic cells is administered locally to the patient.

In some examples, the pluripotent hematopoietic cells are autologous with respect to the patient. In some examples, the pluripotent hematopoietic cells are allogeneic with respect to the patient (e.g., HLA-matched allogeneic cells).

In some examples, the pluripotent hematopoietic cells (e.g., HSCs, HPCs, embryonic stem cells, induced pluripotent stem cells, lymphoid progenitor cells and/or CD34+ cells) are transduced ex vivo with a viral vector that includes the nucleic acid cassette that encodes the autoantigen-binding protein.

In some examples, the pluripotent hematopoietic cells are transduced with a viral vector selected from the group consisting of a Retroviridae family virus, an adenovirus, a parvovirus, a coronavirus, a rhabdovirus, a paramyxovirus, a picornavirus, an alphavirus, a herpes virus, and a poxvirus. In some examples, the viral vector is a Retroviridae family viral vector. In some examples, the Retroviridae family viral vector is a lentiviral vector. In some examples, the Retroviridae family viral vector is an alpharetroviral vector or a gammaretroviral vector.

In some examples, the Retroviridae family viral vector includes a central polypurine tract, a woodchuck hepatitis virus post-transcriptional regulatory element, a 5'-LTR, HIV signal sequence, HIV Psi signal 5'-splice site, delta-GAG element, 3'-splice site, and a 3'-self inactivating LTR.

In some examples, the viral vector is a pseudotyped viral vector. In some examples, the pseudotyped viral vector is selected from the group consisting of a pseudotyped adenovirus, a pseudotyped parvovirus, a pseudotyped coronavirus, a pseudotyped rhabdovirus, a pseudotyped paramyxovirus, a pseudotyped picornavirus, a pseudotyped alphavirus, a pseudotyped herpes virus, a pseudotyped poxvirus, and a pseudotyped Retroviridae family virus. In some examples, the pseudotyped viral vector is a pseudotyped lentiviral vector.

In some examples, the pseudotyped viral vector includes an envelope protein from a virus selected from vesicular stomatitis virus (VSV), RD114 virus, murine leukemia virus (MLV), feline leukemia virus (FeLV), Venezuelan equine encephalitis virus (VEE), human foamy virus (HFV), walleye dermal sarcoma virus (WDSV), Semliki Forest virus (SFV), Rabies virus, avian leukosis virus (ALV), bovine immunodeficiency virus (BIV), bovine leukemia virus (BLV), Epstein-Barr virus (EBV), Caprine arthritis encephalitis virus (CAEV), Sin Nombre virus (SNV), Cherry Twisted Leaf virus (ChTLV), Simian T-cell leukemia virus (STLV), Mason-Pfizer monkey virus (MPMV), squirrel monkey retrovirus (SMRV), Rous-associated virus (RAV), Fujinami sarcoma virus (FuSV), avian carcinoma virus (MH2), avian encephalomyelitis virus (AEV), Alfa mosaic virus (AMV), avian sarcoma virus CT10, and equine infectious anemia virus (EIAV).

In some examples, the pseudotyped viral vector includes a VSV-G envelope protein.

In some examples, the pluripotent hematopoietic cells (e.g., HSCs, HPCs, embryonic stem cells, induced pluripotent stem cells, lymphoid progenitor cells and/or CD34+ cells) are transfected ex vivo with a polynucleotide that includes the nucleic acid cassette that encodes the autoantigen-binding protein.

In some examples, the pluripotent hematopoietic cells are transfected using a cationic polymer, diethylaminoethyldextran, polyethylenimine, a cationic lipid, a liposome, calcium phosphate, an activated dendrimer, and/or a magnetic bead. In some examples, the pluripotent hematopoietic cells are transfected by way of electroporation, Nucleofection, squeeze-poration, sonoporation, optical transfection, Magnetofection, and/or impalefection.

In some examples, the nucleic acid cassette is part of a transposable element. In some examples, the nucleic acid cassette includes a transposase recognition and cleavage element for incorporation into a deoxyribonucleic acid (DNA) molecule of a pluripotent hematopoietic cell. In some examples, the DNA molecule is a nuclear or mitochondrial DNA molecule and the transposase recognition and cleavage element promotes incorporation into the nuclear or mitochondrial DNA molecule.

In some examples, the pluripotent hematopoietic cells are obtained by delivering to the cells a nuclease that catalyzes a single-strand break or a double-strand break at a target position within the genome of the cell. In some examples, the nuclease is delivered to the cells in combination with a guide RNA (gRNA) that hybridizes to the target position within the genome of the cell. In some examples, the nuclease is a clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein. For example, in some examples, the CRISPR-associated protein is CRISPR-

associated protein 9 (Cas9) or CRISPR-associated protein 12a (Cas12a). In some examples, the nuclease is a transcription activator-like effector nuclease, a meganuclease, or a zinc finger nuclease.

In some examples, while the cells are contacted with the nuclease, the cells are additionally contacted with a template polynucleotide that includes the nucleic acid cassette that encodes the autoantigen-binding protein. In some examples, the template polynucleotide includes a 5' homology arm and a 3' homology arm having nucleic acid sequences that are sufficiently similar to the nucleic acid sequences located 5' to the target position and 3' to the target position, respectively, to promote homologous recombination.

In some examples, the nuclease, gRNA, and/or template polynucleotide are delivered to the cells by contacting the cells with a viral vector that encodes the nuclease, gRNA, and/or template polynucleotide.

In some examples, the viral vector that encodes the nuclease, gRNA, and/or template polynucleotide is an AAV, an adenovirus, a parvovirus, a coronavirus, a rhabdovirus, a paramyxovirus, a picornavirus, an alphavirus, a herpes virus, a poxvirus, or a Retroviridae family virus.

In some examples, the viral vector that encodes the nuclease, gRNA, and/or template polynucleotide is a Retroviridae family virus. In some examples, the Retroviridae family virus is a lentiviral vector, alpharetroviral vector, or gammaretroviral vector. In some examples, the Retroviridae family virus that encodes the nuclease, gRNA, and/or template polynucleotide includes a central polypurine tract, a woodchuck hepatitis virus post-transcriptional regulatory element, a 5'-LTR, HIV signal sequence, HIV Psi signal 5'-splice site, delta-GAG element, 3'-splice site, and a 3'-self inactivating LTR.

In some examples, the viral vector that encodes the nuclease, gRNA, and/or template polynucleotide is an integration-deficient lentiviral vector. In some examples, the viral vector that encodes the nuclease, gRNA, and/or template polynucleotide is an AAV selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, and AAVrh74.

In some examples, the one or more lineage-specific transcription regulatory elements include a Foxp3 promoter.

In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 1. In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 1. In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 1. In some examples, the Foxp3 promoter has the nucleic acid sequence of SEQ ID NO: 1.

SEQ ID NO: 1:

TTCCCATCCACACATAGAGCTTCAGATTCTCTTTCTTTCCCCAGAGACCCTCAAATATCCTCTCACTC
ACAGAATGGTGTCTCTGCCTGCCTCGGGTTGGCCCTGTGATTTATTTTAGTTCTTTTCCCTTGTTTTTT
TTTTTTCAAACTCTATACACTTTTGTTTTAAAAACTGTGGTTTCTCATGAGCCCTATTATCTCATTGATA
CCTCTCACCTCTGTGGTGAGGGGAAGAAATCATATTTTCAGATGACTCGTAAAGGGCAAAGAAAAAA
ACCCAAAATTTCAAAATTTCCGTTTAAGTCTCATAATCAAGAAAAGGAGAAACACAGAGAGAGAGAAA

AAAAAAACTATGAGAACCCCCCCCCACCCCGTGATTATCAGCGCACACACTCATCGAAAAAAATTTG
GATTATTAGAAGAGAGAGGTCTGCGGCTTCCACACCGTACAGCGTGGTTTTTCTTCTCGGTATAAAA
GCAAAGTTGTTTTTGATACGTGACAGTTTCCCACAAGCCAGGCTGATCCTTTTCTGTCAGTCCACTTC
ACCAAGGTGAGTGTCCCTGCTCTCCCCTACCAGATGTGGGCCCCATTGGAGGAGATGGCAGGGAG
GTAGGCACGGCGGGGGGGTCAGGGGCCCTCTGGTACAGTGGGATGTACCCAGCTACCGTGATTCC
AGCCAGGTAAGGTCT

In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 2. In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 2. In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 2. In some examples, the Foxp3 promoter has the nucleic acid sequence of SEQ ID NO: 2.

SEQ ID NO: 2:
GCTTCAGATTCTCTTTCTTTCCCCAGAGACCCTCAAATATCCTCTCACTCACAGAATGGTGTCTCTGC
CTGCCTCGGGTTGGCCCTGTGATTTATTTTAGTTCTTTTCCCTTGTTTTTTTTTTTTCAAACTCTATACA
CTTTTGTTTTAAAAACTGTGGTTTCTCATGAGCCCTATTATCTCATTGATACCTCTCACCTCTGTGGTG
AGGGGAAGAAATCATATTTTCAGATGACTCGTAAAGGGCAAAGAAAAAAACCCAAAATTTCAAAATTT
CCGTTTAAGTCTCATAATCAAGAAAAGGAGAAACACAGAGAGAGAGAAAAAAAAAACTATGAGAACC
CCCCCCCACCCCGTGATTATCAGCGCACACACTCATCGAAAAAAATTTGGATTATTAGAAGAGAGAG
GTCTGCGGCTTCCACACCGTACAGCGTGGTTTTTCTTCTCGGTATAAAAGCAAAGTTGTTTTTGATAC
GTGAC

In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 3. In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 3. In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 3. In some examples, the Foxp3 promoter has the nucleic acid sequence of SEQ ID NO: 3.

SEQ ID NO: 3
GCTTCAGATCCCTTCTTCTGTTCAACCCAGCGATCCTCCAACGTCTCACAAACACAATGCTGTCTCTA
CCTGCCTCGGGATGCCTTTGTGATTTGACTTATTTTCCCTCAGTTTTTTTTTTCTGACTCTACACACTT
TTGTTTAAGAAATTGTGGTTTCTCATGAGCCCTGTTATCTCATTGATACCTTTTACCTCTGTGGTGAGG
GGAAGAAATCATATTTTCAGATGACTTGTAAAGGGCAAAGAAAAAACCCAAAATTTCAAAATTTCCGTT
TAAGTCTCATAAGAAAAGAATAAACAAAGTAAGAGAGCAAAGAAAAAAAAACTACAAGAACCCCCCCC

CCACCCTGCAATTATCAGCACACACACTCATCAAAAAAAAATTGGATTATTAGAAGAGCGAGGTCTGC
GGCTTCCAC

In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 4. In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 4. In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 4. In some examples, the Foxp3 promoter has the nucleic acid sequence of SEQ ID NO: 4.

SEQ ID NO: 4
GCTTCAGATTCTCTTTCTTTCCCCAGAGACCCTCAAATATCCTCTCACTCACAGAATGGTGTCTCTGC
CTGCCTCGGGTTGGCCCTGTGATTTATTTTAGTTCTTTTCCCTTGTTTTTTTTTTTTCAAACTCTATACA
CTTTTGTTTTAAAAACTGTGGTTTCTCATGAGCCCTATTATCTCATTGATACCTCTCACCTCTGTGGTG
AGGGGAAGAAATCATATTTTCAGATGACTCGTAAAGGGCAAAGAAAAAAACCCAAAATTTCAAAATTT
CCGTTTAAGTCTCATAATCAAGAAAAGGAGAAACACAGAGAGAGAGAAAAAAAAAACTATGAGAACC
CCCCCCCACCCCGTGATTATCAGCGCACACACTCATCGAAAAAAATTTGGATTATTAGAAGAGAGAG
GTCTGCGGCTTCCAC

In some examples, the Foxp3 promoter specifically binds transcription factor Nr4a and/or Foxo.

In some examples, the one or more lineage-specific transcription regulatory elements include a CNS1 enhancer.

In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 5. In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 5. In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 5. In some examples, the CNS1 enhancer has the nucleic acid sequence of SEQ ID NO: 5.

SEQ ID NO: 5
TTTAAGTCTTTTGCACTTGAAAATGAGATAACTGTTCACCCCATGTTGGCTTCCAGTCTCCTTTATGGC
TTCATTTTTTCCATTTACTGCAGAGGTCAAAAGTGTGGGTATGGGAGCCAGACTGTCTGGAACAACCT
AGCCTCAACTCAA

In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or

100% identical) to the nucleic acid sequence of SEQ ID NO: 6. In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 6. In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 6. In some examples, the CNS1 enhancer has the nucleic acid sequence of SEQ ID NO: 6.

SEQ ID NO: 6
AAGTCCTTTCCTACTTGAAAATGAGATAAATGTTCACCTATGTTGGCTTCTAGTCTCTTTTATGGCTTC
ATTTTTTCCATTTACTATAGAGGTTAAGAGTGTGGGTACTGGAGCCAGACTGTCTGGGACAAACCCA
GCGTCACCCCAA

In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 7. In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 7. In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 7. In some examples, the CNS1 enhancer has the nucleic acid sequence of SEQ ID NO: 7.

SEQ ID NO: 7
TAGATTACTCTTTTCTTGTGGGGCTTCTGTGTATGGTTTTGTGTTTTAAGTCTTTTGCACTTGAAAATG
AGATAACTGTTCACCCCATGTTGGCTTCCAGTCTCCTTTATGGCTTCATTTTTTCCATTTACTGCAGAG
GTCAAAAGTGTGGGTATGGGAGCCAGACTGTCTGGAACAACCTAGCCTCAACTCAAGTCATCTGTGT
GAATTTTACCCAGGCTCTTAACCTCTCTGTACCTCCATTTCCTCGTATGTACTGTGATGATTATAACAG
TACCTACCTCAGAGGATCTTTCTGAGGATTATTTTTATTAATGATGGTAGGTGCTCAGCACAAGGCC

In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 8. In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 8. In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 8. In some examples, the CNS1 enhancer has the nucleic acid sequence of SEQ ID NO: 8.

SEQ ID NO: 8
TAGGTTAGTCTTTTTTTCTGTGGCTTCTGTCTCTGGTTTTGTGCTTAGAAAGTCCTTTCCTACTTGAAA
ATGAGATAAATGTTCACCTATGTTGGCTTCTAGTCTCTTTTATGGCTTCATTTTTTCCATTTACTATAGA
GGTTAAGAGTGTGGGTACTGGAGCCAGACTGTCTGGGACAAACCCAGCGTCACCCCAAGCCCTATG

TGTGATTTTTAGCCAGGCACTTAACCTCTCCATACCTCCATTTCCTCATATGTACTGCAATGGTTATAA
TAGTACCTTCCTCAGGAGTCTTTGTTTAGATTAAAATTTTTAACCACAGTAAATACTTAGCACAAGGCC

In some examples, the CNS1 enhancer specifically binds transcription factor AP-1, NFAT, Smad3, and/or Foxo.

In some examples, the one or more lineage-specific transcription regulatory elements include a CNS2 enhancer.

In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 9. In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 9. In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 9. In some examples, the CNS2 enhancer has the nucleic acid sequence of SEQ ID NO: 9.

SEQ ID NO: 9
CAGATGGACGTCACCTACCACATCCGCTAGCACCCACATCACCCTACCTGGGCCTATCCGGCTACA
GGATAGACTAGCCACTTCTCGGAACGAAACCTGTGGGGTAGATTATCTGCCCCCTTCTCTTCCTCCT
TGTTGCCGATGAAGCCCAATGCATCCGGCCGCCATGACGTCAATGGCAGAAAAATCTGGCCAAGTT
CAGGTTGTGACAACAGGGCCCAGATGTAGACCCCGATAGGAAAACATATTCTATGTCCCAGAAACAA
CCTCCATACAGCTTCTAAGAAACAGTCAAACAGGAACGCCCCAACAGACAGTGCAGGAAGCTGGCT
GGCCAGCCCAGCCCTCCAGGTCCCTAGTACCACTAGACAGACCATATCCAATTCAGGTCCTCTTTCT
GAGA

In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 10. In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 10. In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 10. In some examples, the CNS2 enhancer has the nucleic acid sequence of SEQ ID NO: 10.

SEQ ID NO: 10
CAGATGGACATCACCTACCACATCCACCAGCACCCATGTCACCCCACCTGGGCCAAGCCTGCTGCA
GGACAGGGCAGCCAGTTCTCGGAACGAAACCTGTGGGGTGGGGTATCTGCCCTCTTCTCTTCCTCC
GTGGTGTCGATGAAGCCCGGCGCATCCGGCCGCCATGACGTCAATGGCGGAAAAATCTGGGCAAG
TCGGGGGCTGTGACAACAGGGCCCAGATGCAGACCCCGATATGAAAACATAATCTGTGTCCCAGAA
ACATCCCCCATTCAGCTTCTGAGAAACCCAGTCAGAAAGGGACGTCCCAACAGACAGTGCAGGAAG
CCGGCTGCCCAGCCCGGCCCTCTAGGTCCTCTACCCCCAGACAGATCATCTCCA

In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 11. In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 11. In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 11. In some examples, the CNS2 enhancer has the nucleic acid sequence of SEQ ID NO: 11.

SEQ ID NO: 11

TGGGTTTTGCATGGTAGCCAGATGGACGTCACCTACCACATCCGCTAGCACCCACATCACCCTACCT
GGGCCTATCCGGCTACAGGATAGACTAGCCACTTCTCGGAACGAAACCTGTGGGGTAGATTATCTG
CCCCCTTCTCTTCCTCCTTGTTGCCGATGAAGCCCAATGCATCCGGCCGCCATGACGTCAATGGCAG
AAAAATCTGGCCAAGTTCAGGTTGTGACAACAGGGCCCAGATGTAGACCCCGATAGGAAAACATATT
CTATGTCCCAGAAACAACCTCCATACAGCTTCTAAGAAACAGTCAAACAGGAACGCCCCAACAGACA
GTGCAGGAAGCTGGCTGGCCAGCCCAGCCCTCCAGGTCCCTAGTACCACTAGACAGACCATATCCA
ATTCAGGTCCTCTTTCTGAGAATGTA

In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 12. In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 12. In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 12. In some examples, the CNS2 enhancer has the nucleic acid sequence of SEQ ID NO: 12.

SEQ ID NO: 12

GGGCTTGTCATAGTGGCCAGATGGACATCACCTACCACATCCACCAGCACCCATGTCACCCCACCT
GGGCCAAGCCTGCTGCAGGACAGGGCAGCCAGTTCTCGGAACGAAACCTGTGGGGTGGGGTATCT
GCCCTCTTCTCTTCCTCCGTGGTGTCGATGAAGCCCGGCGCATCCGGCCGCCATGACGTCAATGGC
GGAAAAATCTGGGCAAGTCGGGGGCTGTGACAACAGGGCCCAGATGCAGACCCCGATATGAAAACA
TAATCTGTGTCCCAGAAACATCCCCCATTCAGCTTCTGAGAAACCCAGTCAGAAAGGGACGTCCCAA
CAGACAGTGCAGGAAGCCGGCTGCCCAGCCCGGCCCTCTAGGTCCTCTACCCCCAGACAGATCATC
TCCATGTCCCTGTCTGAGAATGTA

In some examples, the CNS2 enhancer specifically binds transcription factor Runx, Foxp3, Ets-1, CREB, Stat5, NFAT, and/or c-Rel.

In some examples, the one or more lineage-specific transcription regulatory elements include a CNS3 enhancer.

In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 13. In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 13. In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 13. In some examples, the CNS3 enhancer has the nucleic acid sequence of SEQ ID NO: 13.

SEQ ID NO: 13

CCCGGGGCCCAGAATGGGGTAAGCAGGGTGGGGTACTTGGGCCTATAGGTGTCGACCTTTACTGT
GGCATGTGGCGGGGGGGGGGGGGGGGGCTGGGGCACAGGAAGTGGTTTATGGGTCCCAGGCAAG
TCTGACTTATGCAGATATTGCAGGGCCAAGAAAATCCCCACTCTCCAGGCTTCAGAGATTCAAGGCT
TTCCCCACCCC

In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 14. In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 14. In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 14. In some examples, the CNS3 enhancer has the nucleic acid sequence of SEQ ID NO: 14.

SEQ ID NO: 14

CCCTGGGCCCAGGATGGGGCAGGCAGGGTGGGGTACCTGGACCTACAGGTGCCGACCTTTACTGT
GGCACTGGGCGGGAGGGGGGCTGGCTGGGGCACAGGAAGTGGTTTCTGGGTCCCAGGCAAGTCT
GTGACTTATGCAGATGTTGCAGGGCCAAGAAAATCCCCACCTGCCAGGCCTCAGAGATTGGAGGCT
CTCCCC

In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 15. In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 15. In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 15. In some examples, the CNS3 enhancer has the nucleic acid sequence of SEQ ID NO: 15.

SEQ ID NO: 15

GTGAGGCCCGGGGCCCAGAATGGGGTAAGCAGGGTGGGGTACTTGGGCCTATAGGTGTCGACCTT
TACTGTGGCATGTGGCGGGGGGGGGGGGGGGGGCTGGGGCACAGGAAGTGGTTTATGGGTCCCA
GGCAAGTCTGACTTATGCAGATATTGCAGGGCCAAGAAAATCCCCACTCTCCAGGCTTCAGAGATTC
AAGGCTTTCCCCACCCCTCCCAATCCTCATCCCGATAG

In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 16. In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 16. In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 16. In some examples, the CNS3 enhancer has the nucleic acid sequence of SEQ ID NO: 16.

SEQ ID NO: 16

GTGAGGCCCTGGGCCCAGGATGGGGCAGGCAGGGTGGGGTACCTGGACCTACAGGTGCCGACCT
TTACTGTGGCACTGGGCGGGAGGGGGGCTGGCTGGGGCACAGGAAGTGGTTTCTGGGTCCCAGGC
AAGTCTGTGACTTATGCAGATGTTGCAGGGCCAAGAAAATCCCCACCTGCCAGGCCTCAGAGATTG
GAGGCTCTCCCCGACCTCCCAATCC

In some examples, the CNS3 enhancer specifically binds transcription factor Foxo and/or c-Rel.

In some examples, the one or more lineage-specific transcription regulatory elements include a CNS0 enhancer.

In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 17. In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 17. In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 17. In some examples, the CNS0 enhancer has the nucleic acid sequence of SEQ ID NO: 17.

SEQ ID NO: 17

TCCCCTGAGGTCCACCACCATTTCCCCAGAGGGCTGGATCACGGGGGGTAGCTATTCTTCAACAGC
ACTTCAAATCAGCAGCAGCACACAGGCCTTAAAACAATAATAAGTTGAAATGTATTTGCTAGGAAAGT
CACCGACCTACAAAGAAAACCTTATCGCTGATCTAGCAGCGCACACCAGCCTCCCCTTTGCAAGAGC
TGAGATCAAAAGATAAAGAAGCTATCAAAAAGCCATCTGCCCACTTAAAATAACATCTCAAGTCACGT
TGGGAACCACAAACATGGGGCCAGCTACCAAAACAATTGTCTAAATGAACTACTTCAATTTCTCCTTA

AAACCACCCATGTATTTTAAAAGAAAAACACCCTCTCCACCCACCTTGGCACGGCAAGGTTTTGATTT
GTCTGTTCCCTTCCTTTCACATTCTTGAAAATGACCAAACTT

In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 18. In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 18. In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 18. In some examples, the CNS0 enhancer has the nucleic acid sequence of SEQ ID NO: 18.

SEQ ID NO: 18
AGTTTGGTCATTTTAAAGAGTGTGAAAGGCAGAGAACAGAGAAATCAAAACCTTGCAGGGCCAAGGT
GGGTGGAGAGGGTGTTTTTCTTTTAACATACATGGGCGGTTTTAAGGAGAAATTGAAGCAGCCTGTT
CAGACAATTGTTTTGGTATCTGGCCCCAGGTCTGTGGTTCCTAACATGACTTGTGATATTATTTTAAG
TGGGCAGATGGCTTTTTGATAGCTTCTTTATCTTTCGATCTCAGCTCTTGCAAAGGGGAGGTTGGTG
CTCATTGCAAGATCAGCGATAAGGGTTTCTTTGTAGGTCGGTGGCTTTCTTGGTGAGTACATTTCAAC
ATATTATTGTTTTAGAACCTGTGTGCTGCCAGTGACTTGCAGCACTGTTGAAGACTAGCCACCCTTTG
TGACCTAGCCCTCTTGGGAAATGGCGGAGGATCTCAGGG

In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 19. In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 19. In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 19. In some examples, the CNS0 enhancer has the nucleic acid sequence of SEQ ID NO: 19.

SEQ ID NO: 19
CAGTGGGCCTGTGGCCAACGATTCTGAAGCCCTCTACAGCAGGCCTCCCACAGATAAGAAAAGGGA
TCCCCTGAGGTCCACCACCATTTCCCCAGAGGGCTGGATCACGGGGGGTAGCTATTCTTCAACAGC
ACTTCAAATCAGCAGCAGCACACAGGCCTTAAAACAATAATAAGTTGAAATGTATTTGCTAGGAAAGT
CACCGACCTACAAAGAAAACCTTATCGCTGATCTAGCAGCGCACACCAGCCTCCCCTTTGCAAGAGC
TGAGATCAAAAGATAAAGAAGCTATCAAAAAGCCATCTGCCCACTTAAAATAACATCTCAAGTCACGT
TGGGAACCACAAACATGGGGCCAGCTACCAAAACAATTGTCTAAATGAACTACTTCAATTTCTCCTTA
AAACCACCCATGTATTTTAAAAGAAAAACACCCTCTCCACCCACCTTGGCACGGCAAGGTTTTGATTT
GTCTGTTCCCTTCCTTTCACATTCTTGAAAATGACCAAACTTCAGTACTCAACTGTCTTATCTTCCAGA
AAGGGCTCCCACAACTGCCGATGGAATAAGAAGTGATTGAAATGCAGGCGATTCTGGGGGC

In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 20. In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 20. In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 20. In some examples, the CNS0 enhancer has the nucleic acid sequence of SEQ ID NO: 20.

SEQ ID NO: 20

CGGCTGTCATGGGAACCCTGTCTGTAAGATGCGACAGTTTGGGTAAAGGAGTTTGGTCATTTTAAAG
AGTGTGAAAGGCAGAGAACAGAGAAATCAAAACCTTGCAGGGCCAAGGTGGGTGGAGAGGGTGTTT
TTCTTTTAACATACATGGGCGGTTTTAAGGAGAAATTGAAGCAGCCTGTTCAGACAATTGTTTTGGTA
TCTGGCCCCAGGTCTGTGGTTCCTAACATGACTTGTGATATTATTTTAAGTGGGCAGATGGCTTTTTG
ATAGCTTCTTTATCTTTCGATCTCAGCTCTTGCAAAGGGGAGGTTGGTGCTCATTGCAAGATCAGCGA
TAAGGGTTTCTTTGTAGGTCGGTGGCTTTCTTGGTGAGTACATTTCAACATATTATTGTTTTAGAACCT
GTGTGCTGCCAGTGACTTGCAGCACTGTTGAAGACTAGCCACCCTTTGTGACCTAGCCCTCTTGGGA
AATGGCGGAGGATCTCAGGGTATATCCCTTACCTGTGGGAGCCCTATCAGAGGGCTTC

In some examples, the CNS0 enhancer specifically binds transcription factor Satb1 and/or Stat5.

In some examples, the nucleic acid cassette is operably linked to a riboswitch. In some examples, binding of a ligand to the riboswitch induces expression of the nucleic acid cassette. In some examples, binding of a ligand to the riboswitch represses expression of the nucleic acid cassette.

In some examples, the autoantigen-binding protein is a single-chain polypeptide. In some examples, the autoantigen-binding protein is a chimeric antigen receptor (CAR).

In some examples, the chimeric antigen receptor includes an antigen recognition domain, a hinge domain, a transmembrane domain, and one or more intracellular signaling domains.

In some examples, the one or more intracellular signaling domains include one or more primary intracellular signaling domains and optionally one or more costimulatory intracellular signaling domains.

In some examples, the antigen recognition domain is a single-chain antibody fragment (e.g., a single-chain Fv molecule (scFv)).

In some examples, the hinge domain is a CD28, CD8, IgG1/IgG4, CD4, CD7, or IgD hinge domain.

In some examples, the hinge domain is a CD28 hinge domain.

In some examples, the transmembrane domain includes a CD28, CD3 zeta, CD8, FcRIγ, CD4, CD7, OX40, or MHC (H2-Kb) transmembrane domain.

In some examples, the transmembrane domain includes a CD28 transmembrane domain.

In some examples, the one or more primary intracellular signaling domains are selected from the group consisting of a CD3 zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (ICOS), CD66d, DAP10, and a DAP12 intracellular signaling domain.

In some examples, at least one of the one or more primary intracellular signaling domains is a CD3 zeta intracellular signaling domain.

In some examples, the one or more costimulatory intracellular signaling domains are selected from the group consisting of a CD27, CD28, 4-1BB (CD137), OX40, GITR, CD30, CD40, ICOS, BAFFR, HVEM, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, SLAMF7, NKp80, CD160, B7-H3, CD83, CDS, ICAM-1, LFA-1 (CD11a/CD18), an MHC class I molecule, BTLA, and a Toll ligand receptor intracellular signaling domain.

In some examples, at least one of the one or more co-stimulatory intracellular signaling domains is a CD28 intracellular signaling domain.

In some examples, the chimeric antigen receptor includes an N-terminal leader sequence. In some examples, the antigen recognition domain includes an N-terminal leader sequence. In some examples, the N-terminal leader sequence of the antigen recognition domain is cleaved from the antigen recognition domain during cellular processing and localization of the chimeric antigen receptor to the cellular membrane.

In some examples, the autoantigen-binding protein is a multi-chain protein. In some examples, the autoantigen-binding protein is a full-length antibody, a dual-variable immunoglobulin domain, a diabody, a triabody, a nanobody, an antibody-like protein scaffold, a Fab fragment, or a $F(ab')_2$ molecule.

In some examples, the autoimmune disease is type 1 diabetes, Alopecia Areata, Ankylosing Spondylitis, Antiphospholipid Syndrome, Autoimmune Addison's Disease, Autoimmune Hemolytic Anemia, Autoimmune Hepatitis, Behcet's Disease, Bullous Pemphigoid, Cardiomyopathy, Celiac Sprue-Dermatitis, Chronic Fatigue Immune Dysfunction Syndrome (CFIDS), Chronic Inflammatory Demyelinating Polyneuropathy, Churg-Strauss Syndrome, Cicatricial Pemphigoid, CREST Syndrome, Cold Agglutinin Disease, Crohn's Disease, Essential Mixed Cryoglobulinemia, Fibromyalgia-Fibromyositis, Graves' Disease, Guillain-Barré, Hashimoto's Thyroiditis, Hypothyroidism, Idiopathic Pulmonary Fibrosis, Idiopathic Thrombocytopenia Purpura (ITP), IgA Nephropathy, Juvenile Arthritis, Lichen Planus, Lupus, Ménière's Disease, Mixed Connective Tissue Disease, Multiple Sclerosis, Myasthenia Gravis, Neuromyelitis Optica, Pemphigus Vulgaris, Pernicious Anemia, Polyarteritis Nodosa, Polychondritis, Polyglandular Syndromes, Polymyalgia Rheumatica, Polymyositis and Dermatomyositis, Primary Agammaglobulinemia, Primary Biliary Cirrhosis, Psoriasis, Raynaud's Phenomenon, Reiter's Syndrome, Rheumatic Fever, Rheumatoid Arthritis, Sarcoidosis, Scleroderma, Sjögren's Syndrome, Stiff-Man Syndrome, Takayasu Arteritis, Temporal Arteritis/Giant Cell Arteritis, Ulcerative Colitis, Uveitis, Vasculitis, Vitiligo, or Wegener's Granulomatosis.

In some examples, the autoantigen is myelin oligodendrocyte glycoprotein, aquaporin 4, actin, tubulin, myosin, tropomyosin, vimentin, fibronectin, collagen I, collagen II, collagen III, collagen IV, collagen V, heparin, laminin, collagenase, cardiolipin, glucocerebroside, phosphatidylethanolamine, cholesterol, enolase, aldolase, acid phosphatase, annexin 33 kDa, annexin 67 kDa, cytochrome P450C, catalase, peroxidase, tyrosinase, ribonuclease, histone II A, double-stranded DNA, single-stranded DNA,

transferrin, fetuin, factor II, factor VII, fibrin, fibrinogen, C1, C1q, interleukin 2, interleukin 10, interleukin 4, interferon-γ, TNFαR, HSP60, HSP65, GAD, insulin, IA-2, ZnT8, MBP, AchR, myoglobulin, thyroglobulin, hemoglobin A, spectrin, TB PPD, LPS, MuSK, LRP4, the Fc portion of immunoglobin, citrullinated peptides, carbamylated peptides, the thyrotrophin receptor, or a protein expressed in the thyroid gland.

In some examples, the autoimmune disease is multiple sclerosis and the autoantigen is myelin oligodendrocyte glycoprotein.

In some examples, the autoimmune disease is type 1 diabetes and the autoantigen is insulin, GAD-65, IA-2, or ZnT8.

In some examples, the autoimmune disease is rheumatoid arthritis and the autoantigen is collagen II, the Fc portion of immunoglobin, citrullinated peptides, carbamylated peptides, or HSP65.

In some examples, the autoimmune disease is myasthenia gravis and the autoantigen is AChR, MuSK, or LRP4.

In some examples, the autoimmune disease is lupus and the autoantigen is histone II A.

In some examples, the autoimmune disease is hypothyroidism and the autoantigen is a protein expressed in the thyroid gland.

In some examples, the autoimmune disease is Graves' disease and the autoantigen is the thyrotrophin receptor.

In some examples, the autoimmune disease is pemphigus vulgaris and the autoantigen is double-stranded DNA.

In some examples, the autoimmune disease is psoriasis and the autoantigen is double-stranded DNA.

In some examples, the autoimmune disease is neuromyelitis optica and the autoantigen is aquaporin 4.

In some examples, prior to administering the population of pluripotent hematopoietic cells to the patient, a population of precursor cells is isolated from the patient or a donor, and the precursor cells are expanded and genetically modified ex vivo to yield the population of cells being administered to the patient. In some examples, the precursor cells are CD34+ HSCs, and the precursor cells are expanded without substantial loss of HSC functional potential. In some examples, prior to isolation of the precursor cells from the patient or donor, the patient or donor is administered one or more pluripotent hematopoietic cell mobilization agents.

In some examples, prior to administering the population of pluripotent hematopoietic cells to the patient, a population of endogenous pluripotent hematopoietic cells is ablated in the patient by administration of one or more conditioning agents to the patient.

In some examples, the method includes ablating a population of endogenous pluripotent hematopoietic cells in the patient by administering to the patient one or more conditioning agents prior to administering the population of pluripotent hematopoietic cells to the patient.

In some examples, the one or more conditioning agents are non-myeloablative conditioning agents. In some examples, the one or more conditioning agents deplete a population of CD34+ cells in the patient. In some examples, the depleted CD34+ cells are lymphoid progenitor cells. In some examples, the one or more conditioning agents include an antibody or antigen-binding fragment thereof. In some examples, the antibody or antigen-binding fragment thereof binds to CD117, HLA-

DR, CD34, CD90, CD45, or CD133. In some examples, the antibody or antigen-binding fragment thereof binds to CD117. In some examples, the antibody or antigen-binding fragment thereof is conjugated to a cytotoxin.

In some examples, upon administration of the population of pluripotent hematopoietic cells to the patient, the administered cells, or progeny thereof, differentiate into CD4+CD25+ Treg cells.

In some examples, the patient is a mammal and the cells are mammalian cells. In some examples, the mammal is a human and the cells are human cells.

In another example, the disclosure provides a pharmaceutical composition that includes (i) a population of pluripotent cells (e.g., pluripotent hematopoietic cells) that include a nucleic acid cassette that encodes an autoantigen-binding protein. The nucleic acid cassette may be operably linked to one or more lineage-specific transcription regulatory elements that are active in CD4+CD25+ Treg cells (i.e., specifically active in cells of the Treg lineage and not active in other cell types (e.g., other hematopoietic cells)), and (ii) one or more pharmaceutically acceptable excipients, carriers, or diluents. In one aspect, the present disclosure refers to a pharmaceutical composition comprising (i) a population of pluripotent hematopoietic cells comprising a nucleic acid cassette that encodes an autoantigen-binding protein, wherein the nucleic acid cassette is operably linked to one or more lineage-specific transcription regulatory elements that are active in CD4+CD25+ Treg cells, and (ii) one or more pharmaceutically acceptable excipients, carriers, or diluents.

In some examples of any of the above aspects, the pluripotent cells are pluripotent hematopoietic cells (e.g., HSCs or HPCs). In some examples, the pluripotent hematopoietic cells are embryonic stem cells. In some examples, the pluripotent hematopoietic cells are induced pluripotent stem cells. In some examples, the pluripotent hematopoietic cells are lymphoid progenitor cells. In some examples, the pluripotent hematopoietic cells are CD34+ cells (e.g., HSCs).

In some examples, the pluripotent hematopoietic cells are transduced ex vivo with a viral vector that includes the nucleic acid cassette that encodes the autoantigen-binding protein.

In some examples, the viral vector is selected from the group consisting of a Retroviridae family virus, an adenovirus, a parvovirus, a coronavirus, a rhabdovirus, a paramyxovirus, a picornavirus, an alphavirus, a herpes virus, and a poxvirus. In some examples, the viral vector is a Retroviridae family viral vector. In some examples, the Retroviridae family viral vector is a lentiviral vector. In some examples, the Retroviridae family viral vector is an alpharetroviral vector or a gammaretroviral vector.

In some examples, the Retroviridae family viral vector includes a central polypurine tract, a woodchuck hepatitis virus post-transcriptional regulatory element, a 5'-LTR, HIV signal sequence, HIV Psi signal 5'-splice site, delta-GAG element, 3'-splice site, and a 3'-self inactivating LTR.

In some examples, the viral vector is a pseudotyped viral vector. In some examples, the pseudotyped viral vector is selected from the group consisting of a pseudotyped adenovirus, a pseudotyped parvovirus, a pseudotyped coronavirus, a pseudotyped rhabdovirus, a pseudotyped paramyxovirus, a pseudotyped picornavirus, a pseudotyped alphavirus, a pseudotyped herpes virus, a pseudotyped poxvirus, and a pseudotyped Retroviridae family virus. In some examples, the pseudotyped viral vector is a pseudotyped lentiviral vector.

In some examples, the pseudotyped viral vector includes an envelope protein from a virus selected from vesicular stomatitis virus (VSV), RD114 virus, murine leukemia virus (MLV), feline leukemia virus (FeLV), Venezuelan equine encephalitis virus (VEE), human foamy virus (HFV), walleye dermal sarcoma virus (WDSV), Semliki Forest virus (SFV), Rabies virus, avian leukosis virus (ALV), bovine immunodeficiency virus (BIV), bovine leukemia virus (BLV), Epstein-Barr virus (EBV), Caprine arthritis encephalitis virus (CAEV), Sin Nombre virus (SNV), Cherry Twisted Leaf virus (ChTLV), Simian T-cell

leukemia virus (STLV), Mason-Pfizer monkey virus (MPMV), squirrel monkey retrovirus (SMRV), Rous-associated virus (RAV), Fujinami sarcoma virus (FuSV), avian carcinoma virus (MH2), avian encephalomyelitis virus (AEV), Alfa mosaic virus (AMV), avian sarcoma virus CT10, and equine infectious anemia virus (EIAV).

In some examples, the pseudotyped viral vector includes a VSV-G envelope protein.

In some examples, the pluripotent hematopoietic cells are transfected ex vivo with a polynucleotide that includes the nucleic acid cassette that encodes the autoantigen-binding protein.

In some examples, the pluripotent hematopoietic cells are transfected using a cationic polymer, diethylaminoethyldextran, polyethylenimine, a cationic lipid, a liposome, calcium phosphate, an activated dendrimer, and/or a magnetic bead. In some examples, the pluripotent hematopoietic cells are transfected by way of electroporation, Nucleofection, squeeze-poration, sonoporation, optical transfection, Magnetofection, and/or impalefection.

In some examples, the nucleic acid cassette is part of a transposable element. In some examples, the nucleic acid cassette includes a transposase recognition and cleavage element for incorporation into a deoxyribonucleic acid (DNA) molecule of a pluripotent hematopoietic cell. In some examples, the DNA molecule is a nuclear or mitochondrial DNA molecule and the transposase recognition and cleavage element promotes incorporation into the nuclear or mitochondrial DNA molecule.

In some examples, the pluripotent hematopoietic cells are obtained by delivering to the cells a nuclease that catalyzes a single-strand break or a double-strand break at a target position within the genome of the cell. In some examples, the nuclease is delivered to the cells in combination with a guide RNA (gRNA) that hybridizes to the target position within the genome of the cell. In some examples, the nuclease is a clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein. In some examples, the CRISPR-associated protein is CRISPR-associated protein 9 (Cas9) or CRISPR-associated protein 12a (Cas12a). In some examples, the nuclease is a transcription activator-like effector nuclease, a meganuclease, or a zinc finger nuclease.

In some examples, while the cells are contacted with the nuclease, the cells are additionally contacted with a template polynucleotide that includes the nucleic acid cassette that encodes the autoantigen-binding protein. In some examples, the template polynucleotide includes a 5' homology arm and a 3' homology arm having nucleic acid sequences that are sufficiently similar to the nucleic acid sequences located 5' to the target position and 3' to the target position, respectively, to promote homologous recombination.

In some examples, the nuclease, gRNA, and/or template polynucleotide are delivered to the cells by contacting the cells with a viral vector that encodes the nuclease, gRNA, and/or template polynucleotide.

In some examples, the viral vector that encodes the nuclease, gRNA, and/or template polynucleotide is an AAV, an adenovirus, a parvovirus, a coronavirus, a rhabdovirus, a paramyxovirus, a picornavirus, an alphavirus, a herpes virus, a poxvirus, or a Retroviridae family virus.

In some examples, the viral vector that encodes the nuclease, gRNA, and/or template polynucleotide is a Retroviridae family virus. In some examples, the Retroviridae family virus is a lentiviral vector, alpharetroviral vector, or gammaretroviral vector. In some examples, the Retroviridae

family virus that encodes the nuclease, gRNA, and/or template polynucleotide includes a central polypurine tract, a woodchuck hepatitis virus post-transcriptional regulatory element, a 5'-LTR, HIV signal sequence, HIV Psi signal 5'-splice site, delta-GAG element, 3'-splice site, and a 3'-self inactivating LTR.

In some examples, the viral vector that encodes the nuclease, gRNA, and/or template polynucleotide is an integration-deficient lentiviral vector. In some examples, the viral vector that encodes the nuclease, gRNA, and/or template polynucleotide is an AAV selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, and AAVrh74.

In some examples, the one or more lineage-specific transcription regulatory elements include a Foxp3 promoter.

In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 1. In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 1. In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 1. In some examples, the Foxp3 promoter has the nucleic acid sequence of SEQ ID NO: 1.

In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 2. In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 2. In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 2. In some examples, the Foxp3 promoter has the nucleic acid sequence of SEQ ID NO: 2.

In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 3. In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 3. In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 3. In some examples, the Foxp3 promoter has the nucleic acid sequence of SEQ ID NO: 3.

In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 4. In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 4. In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 4. In some examples, the Foxp3 promoter has the nucleic acid sequence of SEQ ID NO: 4.

In some examples, the Foxp3 promoter specifically binds transcription factor Nr4a and/or Foxo.

In some examples, the one or more lineage-specific transcription regulatory elements include a CNS1 enhancer.

In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 5. In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 5. In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 5. In some examples, the CNS1 enhancer has the nucleic acid sequence of SEQ ID NO: 5.

In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 6. In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 6. In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 6. In some examples, the CNS1 enhancer has the nucleic acid sequence of SEQ ID NO: 6.

In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 7. In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 7. In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 7. In some examples, the CNS1 enhancer has the nucleic acid sequence of SEQ ID NO: 7.

In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 8. In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 8. In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 8. In some examples, the CNS1 enhancer has the nucleic acid sequence of SEQ ID NO: 8.

In some examples, the CNS1 enhancer specifically binds transcription factor AP-1, NFAT, Smad3, and/or Foxo.

In some examples, the one or more lineage-specific transcription regulatory elements include a CNS2 enhancer.

In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 9. In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 9. In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 9. In some examples, the CNS2 enhancer has the nucleic acid sequence of SEQ ID NO: 9.

In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 10. In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 10. In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 10. In some examples, the CNS2 enhancer has the nucleic acid sequence of SEQ ID NO: 10.

In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 11. In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 11. In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 11. In some examples, the CNS2 enhancer has the nucleic acid sequence of SEQ ID NO: 11.

In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 12. In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 12. In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 12. In some examples, the CNS2 enhancer has the nucleic acid sequence of SEQ ID NO: 12.

In some examples, the CNS2 enhancer specifically binds transcription factor Runx, Foxp3, Ets-1, CREB, Stat5, NFAT, and/or c-Rel.

In some examples, the one or more lineage-specific transcription regulatory elements include a CNS3 enhancer.

In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 13. In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 13. In some

examples, the CNS3 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 13. In some examples, the CNS3 enhancer has the nucleic acid sequence of SEQ ID NO: 13.

In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 14. In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 14. In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 14. In some examples, the CNS3 enhancer has the nucleic acid sequence of SEQ ID NO: 14.

In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 15. In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 15. In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 15. In some examples, the CNS3 enhancer has the nucleic acid sequence of SEQ ID NO: 15.

In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 16. In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 16. In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 16. In some examples, the CNS3 enhancer has the nucleic acid sequence of SEQ ID NO: 16.

In some examples, the CNS3 enhancer specifically binds transcription factor Foxo and/or c-Rel.

In some examples, the one or more lineage-specific transcription regulatory elements include a CNS0 enhancer.

In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 17. In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 17. In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 17. In some examples, the CNS0 enhancer has the nucleic acid sequence of SEQ ID NO: 17.

In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or

100% identical) to the nucleic acid sequence of SEQ ID NO: 18. In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 18. In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 18. In some examples, the CNS0 enhancer has the nucleic acid sequence of SEQ ID NO: 18.

In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 19. In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 19. In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 19. In some examples, the CNS0 enhancer has the nucleic acid sequence of SEQ ID NO: 19.

In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 20. In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 20. In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 20. In some examples, the CNS0 enhancer has the nucleic acid sequence of SEQ ID NO: 20.

In some examples, the CNS0 enhancer specifically binds transcription factor Satb1 and/or Stat5.

In some examples, the nucleic acid cassette is operably linked to a riboswitch. In some examples, binding of a ligand to the riboswitch induces expression of the nucleic acid cassette.

In some examples, the autoantigen-binding protein is a single-chain polypeptide. In some examples, the autoantigen-binding protein is a chimeric antigen receptor.

In some examples, the chimeric antigen receptor includes an antigen recognition domain, a hinge domain, a transmembrane domain, and one or more intracellular signaling domains.

In some examples, the one or more intracellular signaling domains include one or more primary intracellular signaling domains and optionally one or more costimulatory intracellular signaling domains.

In some examples, the antigen recognition domain is a single-chain antibody fragment (e.g., a single-chain Fv molecule (scFv)).

In some examples, the hinge domain is a CD28, CD8, IgG1/IgG4, CD4, CD7, or IgD hinge domain.

In some examples, the hinge domain is a CD28 hinge domain.

In some examples, the transmembrane domain includes a CD28, CD3 zeta, CD8, FcRIγ, CD4, CD7, OX40, or MHC (H2-Kb) transmembrane domain.

In some examples, the transmembrane domain includes a CD28 transmembrane domain.

In some examples, the one or more primary intracellular signaling domains are selected from the group consisting of a CD3 zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (ICOS), CD66d, DAP10, and a DAP12 intracellular signaling domain.

In some examples, at least one of the one or more primary intracellular signaling domains is a CD3 zeta intracellular signaling domain.

In some examples, the one or more costimulatory intracellular signaling domains are selected from the group consisting of a CD27, CD28, 4-1BB (CD137), OX40, GITR, CD30, CD40, ICOS, BAFFR, HVEM, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, SLAMF7, NKp80, CD160, B7-H3, CD83, CDS, ICAM-1, LFA-1 (CD11a/CD18), an MHC class I molecule, BTLA, and a Toll ligand receptor intracellular signaling domain.

In some examples, at least one of the one or more co-stimulatory intracellular signaling domains is a CD28 intracellular signaling domain.

In some examples, the chimeric antigen receptor includes an N-terminal leader sequence. In some examples, the antigen recognition domain includes an N-terminal leader sequence. In some examples, the N-terminal leader sequence of the antigen recognition domain is cleaved from the antigen recognition domain during cellular processing and localization of the chimeric antigen receptor to the cellular membrane.

In some examples, the autoantigen-binding protein is a multi-chain protein. In some examples, the autoantigen-binding protein is a full-length antibody, a dual-variable immunoglobulin domain, a diabody, a triabody, an antibody-like protein scaffold, a Fab fragment, or a $F(ab')_2$ molecule.

In some examples, the autoantigen is myelin oligodendrocyte glycoprotein, aquaporin 4, actin, tubulin, myosin, tropomyosin, vimentin, fibronectin, collagen I, collagen II, collagen III, collagen IV, collagen V, heparin, laminin, collagenase, cardiolipin, glucocerebroside, phosphatidylethanolamine, cholesterol, enolase, aldolase, acid phosphatase, annexin 33 kDa, annexin 67 kDa, cytochrome P450C, catalase, peroxidase, tyrosinase, ribonuclease, histone II A, double stranded DNA, single stranded DNA, transferrin, fetuin, factor II, factor VII, fibrin, fibrinogen, C1, C1q, interleukin 2, interleukin 10, interleukin 4, interferon-γ, TNFαR, HSP60, HSP65, GAD, insulin, IA-2, ZnT8, MBP, AchR, myoglobulin, thyroglobulin, hemoglobin A, spectrin, TB PPD, LPS, MuSK, LRP4, the Fc portion of immunoglobin, citrullinated peptides, carbamylated peptides, the thyrotrophin receptor, or a protein expressed in the thyroid gland.

In another example, the disclosure provides a kit including a pharmaceutical composition as described herein. The kit may further include a package insert instructing a user of the kit to administer the pharmaceutical composition to a human patient having an autoimmune disease. The package insert may instruct a user of the kit to perform a method as described herein. In one aspect, the present disclosure refers to a kit comprising the pharmaceutical composition as described herein, wherein the kit further comprises a package insert instructing a user of the kit to administer the pharmaceutical composition to a human patient having an autoimmune disease.

In another example, the disclosure provides a nucleic acid cassette encoding an autoantigen-binding protein. The nucleic acid cassette may be operably linked to one or more lineage-specific transcription regulatory elements that are active in CD4+CD25+ Treg cells (i.e., specifically active in cells of the Treg lineage and not active in other cell types (e.g., other hematopoietic cells)). In one aspect, the present disclosure refers to a nucleic acid cassette encoding an autoantigen-binding protein, wherein the nucleic acid cassette is operably linked to one or more lineage-specific transcription regulatory elements that are active in CD4+CD25+ Treg cells.

In some examples, the one or more lineage-specific transcription regulatory elements include a Foxp3 promoter.

In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or

100% identical) to the nucleic acid sequence of SEQ ID NO: 1. In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 1. In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 1. In some examples, the Foxp3 promoter has the nucleic acid sequence of SEQ ID NO: 1.

In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 2. In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 2. In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 2. In some examples, the Foxp3 promoter has the nucleic acid sequence of SEQ ID NO: 2.

In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 3. In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 3. In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 3. In some examples, the Foxp3 promoter has the nucleic acid sequence of SEQ ID NO: 3.

In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 4. In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 4. In some examples, the Foxp3 promoter has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 4. In some examples, the Foxp3 promoter has the nucleic acid sequence of SEQ ID NO: 4.

In some examples, the Foxp3 promoter specifically binds transcription factor Nr4a and/or Foxo.

In some examples, the one or more lineage-specific transcription regulatory elements include a CNS1 enhancer.

In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 5. In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 5. In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%,

96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 5. In some examples, the CNS1 enhancer has the nucleic acid sequence of SEQ ID NO: 5.

In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 6. In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 6. In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 6. In some examples, the CNS1 enhancer has the nucleic acid sequence of SEQ ID NO: 6.

In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 7. In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 7. In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 7. In some examples, the CNS1 enhancer has the nucleic acid sequence of SEQ ID NO: 7.

In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 8. In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 8. In some examples, the CNS1 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 8. In some examples, the CNS1 enhancer has the nucleic acid sequence of SEQ ID NO: 8.

In some examples, the CNS1 enhancer specifically binds transcription factor AP-1, NFAT, Smad3, and/or Foxo.

In some examples, the one or more lineage-specific transcription regulatory elements include a CNS2 enhancer.

In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 9. In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 9. In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 9. In some examples, the CNS2 enhancer has the nucleic acid sequence of SEQ ID NO: 9.

In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 10. In some examples, the CNS2

enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 10. In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 10. In some examples, the CNS2 enhancer has the nucleic acid sequence of SEQ ID NO: 10.

In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 11. In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 11. In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 11. In some examples, the CNS2 enhancer has the nucleic acid sequence of SEQ ID NO: 11.

In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 12. In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 12. In some examples, the CNS2 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 12. In some examples, the CNS2 enhancer has the nucleic acid sequence of SEQ ID NO: 12.

In some examples, the CNS2 enhancer specifically binds transcription factor Runx, Foxp3, Ets-1, CREB, Stat5, NFAT, and/or c-Rel.

In some examples, the one or more lineage-specific transcription regulatory elements include a CNS3 enhancer.

In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 13. In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 13. In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 13. In some examples, the CNS3 enhancer has the nucleic acid sequence of SEQ ID NO: 13.

In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 14. In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 14. In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 14. In some examples, the CNS3 enhancer has the nucleic acid sequence of SEQ ID NO: 14.

In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 15. In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 15. In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 15. In some examples, the CNS3 enhancer has the nucleic acid sequence of SEQ ID NO: 15.

In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 16. In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 16. In some examples, the CNS3 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 16. In some examples, the CNS3 enhancer has the nucleic acid sequence of SEQ ID NO: 16.

In some examples, the CNS3 enhancer specifically binds transcription factor Foxo and/or c-Rel.

In some examples, the one or more lineage-specific transcription regulatory elements include a CNS0 enhancer.

In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 17. In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 17. In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 17. In some examples, the CNS0 enhancer has the nucleic acid sequence of SEQ ID NO: 17.

In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 18. In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 18. In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 18. In some examples, the CNS0 enhancer has the nucleic acid sequence of SEQ ID NO: 18.

In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 19. In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 19. In some

examples, the CNS0 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 19. In some examples, the CNS0 enhancer has the nucleic acid sequence of SEQ ID NO: 19.

In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 85% identical (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 20. In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 90% identical (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 20. In some examples, the CNS0 enhancer has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of SEQ ID NO: 20. In some examples, the CNS0 enhancer has the nucleic acid sequence of SEQ ID NO: 20.

In some examples, the CNS0 enhancer specifically binds transcription factor Satb1 and/or Stat5.

In some examples, the nucleic acid cassette is operably linked to a riboswitch. In some examples, binding of a ligand to the riboswitch induces expression of the nucleic acid cassette.

In some examples, the autoantigen-binding protein is a single-chain polypeptide. In some examples, the autoantigen-binding protein is a chimeric antigen receptor.

In some examples, the chimeric antigen receptor includes an antigen recognition domain, a hinge domain, a transmembrane domain, and one or more intracellular signaling domains.

In some examples, the one or more intracellular signaling domains include one or more primary intracellular signaling domains and optionally one or more costimulatory intracellular signaling domains.

In some examples, the antigen recognition domain is a single-chain antibody fragment (e.g., a single-chain Fv molecule (scFv)).

In some examples, the hinge domain is a CD28, CD8, IgG1/IgG4, CD4, CD7, or IgD hinge domain.

In some examples, the hinge domain is a CD28 hinge domain.

In some examples, the transmembrane domain includes a CD28, CD3 zeta, CD8, FcRIγ, CD4, CD7, OX40, or MHC (H2-Kb) transmembrane domain.

In some examples, the transmembrane domain includes a CD28 transmembrane domain.

In some examples, the one or more primary intracellular signaling domains are selected from the group consisting of a CD3 zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (ICOS), CD66d, DAP10, and a DAP12 intracellular signaling domain.

In some examples, at least one of the one or more primary intracellular signaling domains is a CD3 zeta intracellular signaling domain.

In some examples, the one or more costimulatory intracellular signaling domains are selected from the group consisting of a CD27, CD28, 4-1BB (CD137), OX40, GITR, CD30, CD40, ICOS, BAFFR, HVEM, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, SLAMF7, NKp80, CD160, B7-H3, CD83, CDS, ICAM-1, LFA-1 (CD11a/CD18), an MHC class I molecule, BTLA, and a Toll ligand receptor intracellular signaling domain.

In some examples, at least one of the one or more co-stimulatory intracellular signaling domains is a CD28 intracellular signaling domain.

In some examples, the chimeric antigen receptor includes an N-terminal leader sequence. In some examples, the antigen recognition domain includes an N-terminal leader sequence. In some examples, the N-terminal leader sequence of the antigen recognition domain is cleaved from the antigen recognition domain during cellular processing and localization of the chimeric antigen receptor to the cellular membrane.

In some examples, the autoantigen-binding protein is a multi-chain protein. In some examples, the autoantigen-binding protein is a full-length antibody, a dual-variable immunoglobulin domain, a diabody, a triabody, an antibody-like protein scaffold, a Fab fragment, or a $F(ab')_2$ molecule.

In some examples, the autoantigen is myelin oligodendrocyte glycoprotein, aquaporin 4, actin, tubulin, myosin, tropomyosin, vimentin, fibronectin, collagen I, collagen II, collagen III, collagen IV, collagen V, heparin, laminin, collagenase, cardiolipin, glucocerebroside, phosphatidylethanolamine, cholesterol, enolase, aldolase, acid phosphatase, annexin 33 kDa, annexin 67 kDa, cytochrome P450C, catalase, peroxidase, tyrosinase, ribonuclease, histone II A, double stranded DNA, single stranded DNA, transferrin, fetuin, factor II, factor VII, fibrin, fibrinogen, C1, C1q, interleukin 2, interleukin 10, interleukin 4, interferon-γ, TNFαR, HSP60, HSP65, GAD, insulin, IA-2, ZnT8, MBP, AchR, myoglobulin, thyroglobulin, hemoglobin A, spectrin, TB PPD, LPS, MuSK, LRP4, the Fc portion of immunoglobin, citrullinated peptides, carbamylated peptides, the thyrotrophin receptor, or a protein expressed in the thyroid gland.

In another example, the present disclosure provides a viral vector that includes a nucleic acid cassette as described herein. In one aspect, the present disclosure refers to a viral vector comprising the nucleic acid cassette as described herein.

In some examples, the viral vector is selected from the group consisting of a Retroviridae family virus, an adenovirus, a parvovirus, a coronavirus, a rhabdovirus, a paramyxovirus, a picornavirus, an alphavirus, a herpes virus, and a poxvirus. In some examples, the viral vector is a Retroviridae family viral vector. In some examples, the Retroviridae family viral vector is a lentiviral vector. In some examples, the Retroviridae family viral vector is an alpharetroviral vector or a gammaretroviral vector.

In some examples, the Retroviridae family viral vector includes a central polypurine tract, a woodchuck hepatitis virus post-transcriptional regulatory element, a 5'-LTR, HIV signal sequence, HIV Psi signal 5'-splice site, delta-GAG element, 3'-splice site, and a 3'-self inactivating LTR.

In some examples, the viral vector is a pseudotyped viral vector. In some examples, the pseudotyped viral vector is selected from the group consisting of a pseudotyped adenovirus, a pseudotyped parvovirus, a pseudotyped coronavirus, a pseudotyped rhabdovirus, a pseudotyped paramyxovirus, a pseudotyped picornavirus, a pseudotyped alphavirus, a pseudotyped herpes virus, a pseudotyped poxvirus, and a pseudotyped Retroviridae family virus. In some examples, the pseudotyped viral vector is a pseudotyped lentiviral vector.

In some examples, the pseudotyped viral vector includes an envelope protein from a virus selected from vesicular stomatitis virus (VSV), RD114 virus, murine leukemia virus (MLV), feline leukemia virus (FeLV), Venezuelan equine encephalitis virus (VEE), human foamy virus (HFV), walleye dermal sarcoma virus (WDSV), Semliki Forest virus (SFV), Rabies virus, avian leukosis virus (ALV), bovine immunodeficiency virus (BIV), bovine leukemia virus (BLV), Epstein-Barr virus (EBV), Caprine arthritis

encephalitis virus (CAEV), Sin Nombre virus (SNV), Cherry Twisted Leaf virus (ChTLV), Simian T-cell leukemia virus (STLV), Mason-Pfizer monkey virus (MPMV), squirrel monkey retrovirus (SMRV), Rous-associated virus (RAV), Fujinami sarcoma virus (FuSV), avian carcinoma virus (MH2), avian encephalomyelitis virus (AEV), Alfa mosaic virus (AMV), avian sarcoma virus CT10, and equine infectious anemia virus (EIAV).

In some examples, the pseudotyped viral vector includes a VSV-G envelope protein.

Brief Description of the Drawings

FIGS. 1A and 1B are schematics of lentiviral vector constructs designed to allow expression of chimeric antigen receptors (CAR) under the control of a constitutive promoter for PoC studies. FIG. 1A is a schematic showing basic components of lentiviral construct design. Single chain variable fragments (scFv) were generated by linking heavy and light chain sequences from antibodies with known antigen specificity. A His-tag was introduced to facilitate detection of CARs. Second generation CAR signaling domains were chosen for compatibility with regulatory T cell function. For PoC studies, an scFv (FIG. 1B) with specificity for an irrelevant antigen (Ag) was selected to allow optimisation of *in vitro* assays and to test the safety and function of CAR biology *in vivo.* RRE (Rev response element); cPPT (central polypurine tract); EFS (elongation factor 1a short binding sequence); VL (Variable light chain; VH (Variable heavy chain); Woodchuck hepatitis virus post transcriptional regulatory element (WPRE).

FIGS. 2A – 2C are a series of graphs demonstrating the expression of an antigen-specific CAR in a human T cell line. Jurkat T cells were transduced with a lentiviral vector (MOI5) to express an antigen-specific CAR (aAg-CAR). FIG. 2A is a set of graphs showing CAR expression, after 72 hours, as assessed by flow cytometry (FC) by incubating cells with 50,000pg/ml biotinylated CAR ligand (whole protein) before staining with a streptavidin-PE conjugate. FC plots are gated on live Jurkat T cells, depicting untransduced cells (negative control) and transduced cells. A titration of CAR ligand was used to assess receptor expression, quantified as mean fluorescence intensity (MFI). FIG. 2B is a set of graphs showing an MOI titration used to generate a library of Jurkat T cells expressing different levels of Ag-specific CAR. Transgene vector copy number (VCN) (left graph) was measured by ddPCR while % CAR^+ cells was quantified as outlined in (a). FIG. 2C is a graph showing increasing CAR expression with increasing VCN was confirmed by assessing CAR expression by FC, quantified as MFI as a measure of the MOI used.

FIGS. 3A and 3B are graphs demonstrating confirmation of antigen-specific CAR function *in vitro* in a human T cell line. Transduced Jurkat T cells expressing different levels of aAg-CAR (transduction efficiencies shown in FIGS. 2A – 2C) were treated with increasing amounts of CAR ligand *in vitro* for 24hrs. FIG. 3A is a set of graphs showing CAR function as assessed by FC analysis of expressed T cell activation markers, CD69 (left graph) and CD25 (right graph), quantified as mean fluorescence intensity (MFI). FIG. 3B is a graph showing the results of an experiment in which supernatants from cultured Jurkat T cells were collected and assessed for IL-2 production by enzyme-linked immunosorbent assay (ELISA). Data points represent mean +/- SEM (n=3).

FIGS. 4A – 4D are graphs showing that transduced primary murine T cells express a functional antigen-specific CAR. Purified, $CD4^+CD25^-$ naïve splenic T cells, were activated *in vitro* using CD3/CD28 microbeads before addition of lentiviral vectors (MOI10) for expression of aAg-CAR. FIG. 4A is a set of