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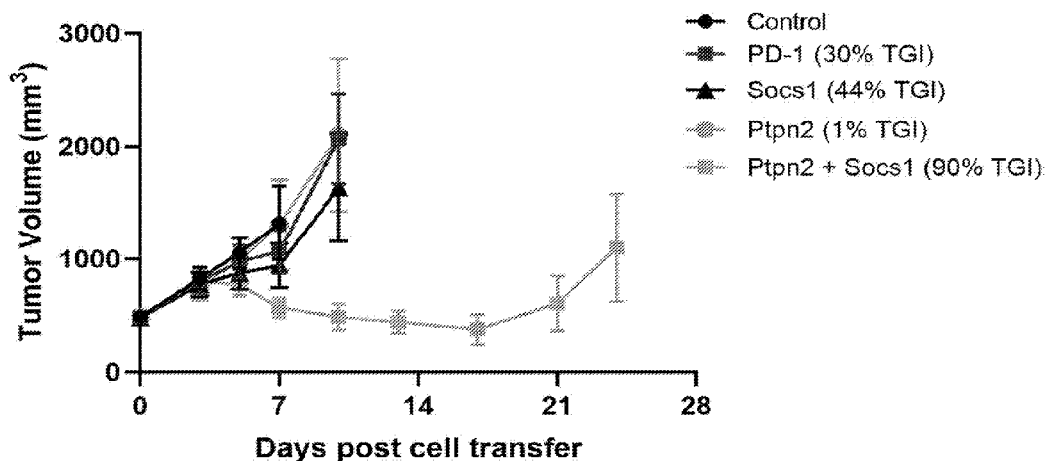
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Fig. 1



(57) Abstract: The present disclosure provides methods and compositions related to the modification of immune effector cells to increase therapeutic efficacy. In some embodiments, immune effector cells modified to reduce expression of one or more endogenous target genes, or to reduce one or more functions of an endogenous protein to enhance effector functions of the immune cells are provided. In some embodiments, immune effector cells further modified by introduction of transgenes conferring antigen specificity, such as exogenous T cell receptors (TCRs) or chimeric antigen receptors (CARs) are provided. Methods of treating a cell proliferative disorder, such as a cancer, using the modified immune effector cells described herein are also provided.





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**COMBINATION GENE TARGETS FOR IMPROVED IMMUNOTHERAPY****CROSS-REFERENCE TO RELATED APPLICATIONS**

**[0001]** This application claims priority to U.S. Provisional Patent Application No. 62/800,999, filed on February 4, 2019; and U.S. Provisional Patent Application No. 62/818,677, filed on March 14, 2019, both of which are incorporated herein by reference in their entireties.

**DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY**

**[0002]** The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing (filename: 700061\_KSQW-014\_ST25.txt; date recorded: February 4, 2020; file size: 945 kilobytes).

**FIELD**

**[0003]** The disclosure relates to methods, compositions, and components for editing a target nucleic acid sequence, or modulating expression of a target nucleic acid sequence, and applications thereof in connection with immunotherapy, including use with receptor-engineered immune effector cells, in the treatment of cell proliferative diseases, inflammatory diseases, and/or infectious diseases.

**BACKGROUND**

**[0004]** Adoptive cell transfer utilizing genetically modified T cells, in particular CAR-T cells, has entered clinical testing as a therapeutic for solid and hematologic malignancies. Results to date have been mixed. In hematologic malignancies (especially lymphoma, CLL and ALL), the majority of patients in several Phase 1 and 2 trials exhibited at least a partial response, with some exhibiting complete responses (Kochenderfer *et al.*, 2012 Blood 1 19, 2709-2720). In 2017, the FDA approved two CAR-T therapies, Kymriah™ and Yescarta™, both for the treatment of hematological cancers. However, in most tumor types (including melanoma, renal cell carcinoma and colorectal cancer), fewer responses have been observed (Johnson *et al.*, 2009 Blood 1 14, 535-546; Lamers *et al.*, 2013 Mol. Ther. 21, 904-912; Warren *et al.*, 1998 Cancer Gene Ther. 5, S1-S2). As such, there is considerable room for improvement with adoptive T cell therapies, as success has largely been limited to CAR-T cells approaches targeting hematological malignancies of the B cell lineage.

**SUMMARY**

**[0005]** There exists a need to improve the efficacy of adoptive transfer of modified immune cells in cancer treatment, in particular increasing the efficacy of adoptive cell therapies against solid

malignancies, as reduced responses have been observed in these tumor types (melanoma, renal cell carcinoma and colorectal cancer; Yong, 2017, Imm Cell Biol., 95:356-363). In addition, even in hematological malignancies where a benefit of adoptive transfer has been observed, not all patients respond and relapses occur with a greater than desired frequency, likely as a result of diminished function of the adoptively transferred T cells.

**[0006]** Factors limiting the efficacy of genetically modified immune cells as cancer therapeutics include: (1) cell proliferation, *e.g.*, limited proliferation of T cells following adoptive transfer; (2) cell survival, *e.g.*, induction of T cell apoptosis by factors in the tumor environment; and (3) cell function, *e.g.*, inhibition of cytotoxic T cell function by inhibitory factors secreted by host immune cells and cancer cells and exhaustion of immune cells during manufacturing processes and/or after transfer.

**[0007]** Particular features thought to increase the anti-tumor effects of an immune cell include a cell's ability to: 1) proliferate in the host following adoptive transfer; 2) infiltrate a tumor; 3) persist in the host and/or exhibit resistance to immune cell exhaustion; and 4) function in a manner capable of killing tumor cells. The present disclosure provides immune cells comprising decreased expression and/or function of one or more endogenous target genes wherein the modified immune cells demonstrate an enhancement of one or more effector functions including increased proliferation, increased infiltration into tumors, persistence of the immune cells in a subject, and/or increased resistance to immune cell exhaustion. The present disclosure also provides methods and compositions for modification of immune effector cells to elicit enhanced immune cell activity towards a tumor cell, as well as methods and compositions suitable for use in the context of adoptive immune cell transfer therapy.

**[0008]** In some embodiments, the present disclosure provides a modified immune effector cell comprising a gene-regulating system capable of reducing the expression and/or function of at least two endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*, wherein the reduced expression and/or function of the at least two endogenous target genes enhances an effector function of the immune effector cell. In some embodiments, the at least two target genes are *SOCS1* and *PTPN2*. In some embodiments, the at least two target genes are *SOCS1* and *ZC3H12A*. In some embodiments, the at least two target genes are *PTPN2* and *ZC3H12A*. In some embodiments, the gene regulating system is further capable of reducing the expression and/or function of CBLB.



[0009] In some embodiments, the gene-regulating system comprises (i) a nucleic acid molecule; (ii) an enzymatic protein; or (iii) a nucleic acid molecule and an enzymatic protein. In some embodiments, the gene-regulating system comprises a nucleic acid molecule selected from an siRNA, an shRNA, a microRNA (miR), an antagomiR, or an antisense RNA. In some embodiments, the gene-regulating system comprises an enzymatic protein, and wherein the enzymatic protein has been engineered to specifically bind to a target sequence in one or more of the endogenous genes. In some embodiments, the protein is a Transcription activator-like effector nuclease (TALEN), a zinc-finger nuclease, or a meganuclease. In some embodiments, the gene-regulating system comprises a nucleic acid molecule and an enzymatic protein, wherein the nucleic acid molecule is a guide RNA (gRNA) molecule and the enzymatic protein is a Cas protein or Cas ortholog. In some embodiments, the Cas protein is a Cas9 protein. In some embodiments, the Cas protein is a wild-type Cas protein comprising two enzymatically active domains, and capable of inducing double stranded DNA breaks. In some embodiments, the Cas protein is a Cas nickase mutant comprising one enzymatically active domain and capable of inducing single stranded DNA breaks. In some embodiments, the Cas protein is a deactivated Cas protein (dCas) and is associated with a heterologous protein capable of modulating the expression of the one or more endogenous target genes. In some embodiments, the heterologous protein is selected from the group consisting of MAX-interacting protein 1 (MXI1), Krüppel-associated box (KRAB) domain, methyl-CpG binding protein 2 (MECP2), and four concatenated mSin3 domains (SID4X).

[0010] In some embodiments, the at least two endogenous genes are *SOCS1* and *PTPN2*, and wherein the gene-regulating system comprises at least one *SOCS1*-targeting gRNA molecule comprising a targeting domain sequence complementary to a nucleic acid sequence defined by any one of the set of genome coordinates shown in Tables 3 and 4 and at least one *PTPN2*-targeting gRNA molecule comprising a targeting domain sequence complementary to a nucleic acid sequence defined by any one of the set of genome coordinates shown in Tables 5 and 6. In some embodiments, the at least two endogenous genes are *SOCS1* and *PTPN2*, and wherein the gene-regulating system comprises at least one *SOCS1*-targeting gRNA molecule comprising a targeting domain sequence that binds to a nucleic acid sequence defined by any one of the set of genome coordinates shown in Tables 3 and 4 and at least one *PTPN2*-targeting gRNA molecule comprising a targeting domain sequence that binds to a nucleic acid sequence defined by any one of the set of genome coordinates shown in Tables 5 and 6. In some embodiments, the at least two endogenous genes are *SOCS1* and *PTPN2*, and wherein the gene-regulating system comprises at least one *SOCS1*-targeting gRNA molecule

comprising a targeting domain sequence that binds to a target DNA sequence selected from the group consisting of SEQ ID NOs: 7-151 and at least one *PTPN2*-targeting gRNA molecule comprising a targeting domain sequence that binds to a target DNA sequence selected from the group consisting of SEQ ID NOs: 185-207. In some embodiments, the at least two endogenous genes are *SOCS1* and *PTPN2*, and wherein the gene-regulating system comprises at least one *SOCS1*-targeting gRNA molecule comprising a targeting domain sequence encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 7-151 and at least one *PTPN2*-targeting gRNA molecule comprising a targeting domain sequence encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 185-207.

**[0011]** In some embodiments, the at least two endogenous genes are *SOCS1* and *ZC3H12A*, and wherein the gene-regulating system comprises at least one *SOCS1*-targeting gRNA molecule comprising a targeting domain sequence complementary to a nucleic acid sequence defined by any one of the set of genome coordinates shown in Tables 3 and 4, and at least one *ZC3H12A*-targeting gRNA molecule comprising a targeting domain sequence complementary to a nucleic acid sequence defined by any one of the set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least two endogenous genes are *SOCS1* and *ZC3H12A*, and wherein the gene-regulating system comprises at least one *SOCS1*-targeting gRNA molecule comprising a targeting domain sequence that binds to a nucleic acid sequence defined by any one of the set of genome coordinates shown in Tables 3 and 4 and at least one *ZC3H12A*-targeting gRNA molecule comprising a targeting domain sequence that binds to a nucleic acid sequence defined by any one of the set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least two endogenous genes are *SOCS1* and *ZC3H12A*, and wherein the gene-regulating system comprises at least one *SOCS1*-targeting gRNA molecule comprising a targeting domain sequence that binds to a target DNA sequence selected from the group consisting of SEQ ID NOs: 7-151 and at least one *ZC3H12A*-targeting gRNA molecule comprising a targeting domain sequence that binds to a target DNA sequence selected from the group consisting of SEQ ID NOs: 208-230. In some embodiments, the at least two endogenous genes are *SOCS1* and *ZC3H12A*, and wherein the gene-regulating system comprises at least one *SOCS1*-targeting gRNA molecule comprising a targeting domain sequence encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 7-151 and at least one *ZC3H12A*-targeting gRNA molecule comprising a targeting domain sequence encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 208-230.

**[0012]** In some embodiments, the at least two endogenous genes are *PTPN2* and *ZC3H12A*, and wherein the gene-regulating system comprises at least one *PTPN2*-targeting gRNA molecule comprising a targeting domain sequence complementary to a nucleic acid sequence defined by any one of the set of genome coordinates shown in Tables 5 and 6 and at least one *ZC3H12A*-targeting gRNA molecule comprising a targeting domain sequence complementary to a nucleic acid sequence defined by any one of the set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least two endogenous genes are *PTPN2* and *ZC3H12A*, and wherein the gene-regulating system comprises at least one *PTPN2*-targeting gRNA molecule comprising a targeting domain sequence that binds to a nucleic acid sequence defined by any one of the set of genome coordinates shown in Tables 5 and 6 and at least one *ZC3H12A*-targeting gRNA molecule comprising a targeting domain sequence that binds to a nucleic acid sequence defined by any one of the set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least two endogenous genes are *PTPN2* and *ZC3H12A*, and wherein the gene-regulating system comprises at least one *PTPN2*-targeting gRNA molecule comprising a targeting domain sequence that binds to a target DNA sequence selected from the group consisting of SEQ ID NOs: 185-207 and at least one *ZC3H12A*-targeting gRNA molecule comprising a targeting domain sequence that binds to a target DNA sequence selected from the group consisting of SEQ ID NOs: 208-230. In some embodiments, the at least two endogenous genes are *PTPN2* and *ZC3H12A*, and wherein the gene-regulating system comprises at least one *PTPN2*-targeting gRNA molecule comprising a targeting domain sequence encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 185-207 and at least one *ZC3H12A*-targeting gRNA molecule comprising a targeting domain sequence encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 208-230.

**[0013]** In some embodiments, the at least two endogenous genes are *SOCS1* and *PTPN2*, and wherein the gene-regulating system comprises at least one *SOCS1*-targeting siRNA or shRNA molecule comprising about 19-30 nucleotides that are complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 3 and 4 and at least one *PTPN2*-targeting siRNA or shRNA molecule comprising about 19-30 nucleotides that are complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6. In some embodiments, the at least two endogenous genes are *SOCS1* and *PTPN2*, and wherein the gene-regulating system comprises at least one *SOCS1*-targeting siRNA or shRNA molecule comprising about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 3 and 4 and at

least one *PTPN2*-targeting siRNA or shRNA molecule comprising about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6. In some embodiments, the at least two endogenous genes are *SOCS1* and *PTPN2*, wherein the gene-regulating system comprises at least one *SOCS1*-targeting siRNA or shRNA comprising about 19-30 nucleotides that bind to an RNA sequence encoded by a DNA sequence selected from the group consisting of SEQ ID NOs: 7-151 and at least one *PTPN2*-targeting siRNA or shRNA comprising about 19-30 nucleotides that bind to an RNA sequence encoded by a DNA sequence selected from the group consisting of SEQ ID NOs: 185-207.

**[0014]** In some embodiments, the at least two endogenous genes are *SOCS1* and *ZC3H12A*, wherein the gene-regulating system comprises at least one *SOCS1*-targeting siRNA or shRNA molecule comprising about 19-30 nucleotides that are complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 3 and 4 and at least one *ZC3H12A*-targeting siRNA or shRNA molecule comprising about 19-30 nucleotides that are complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least two endogenous genes are *SOCS1* and *ZC3H12A*, wherein the gene-regulating system comprises at least one *SOCS1*-targeting siRNA or shRNA molecule comprising about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Table 3 and 4 and at least one *ZC3H12A*-targeting siRNA or shRNA molecule comprising about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least two endogenous genes are *SOCS1* and *ZC3H12A*, wherein the gene-regulating system comprises at least one *SOCS1*-targeting siRNA or shRNA comprising about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence selected from the group consisting of SEQ ID NOs: 7-151 and at least one *ZC3H12A*-targeting siRNA or shRNA comprising about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence selected from the group consisting of SEQ ID NOs: 208-230.

**[0015]** In some embodiments, the at least two endogenous genes are *PTPN2* and *ZC3H12A*, wherein the gene-regulating system comprises at least one *PTPN2*-targeting siRNA or shRNA molecule comprising about 19-30 nucleotides that are complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6 and at least one *ZC3H12A*-targeting siRNA or shRNA molecule comprising about 19-30 nucleotides that are complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome

coordinates shown in Tables 7 and 8. In some embodiments, the at least two endogenous genes are *PTPN2* and *ZC3H12A*, wherein the gene-regulating system comprises at least one *PTPN2*-targeting siRNA or shRNA molecule comprising about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6 and at least one *ZC3H12A*-targeting siRNA or shRNA molecule comprising about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least two endogenous genes are *PTPN2* and *ZC3H12A*, wherein the gene-regulating system comprises at least one *PTPN2*-targeting siRNA or shRNA comprising about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence selected from the group consisting of SEQ ID NOs: 185-207 and at least one *ZC3H12A*-targeting siRNA or shRNA comprising about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence selected from the group consisting of SEQ ID NOs: 208-230.

**[0016]** In some embodiments, the at least two endogenous genes are *SOCS1* and *PTPN2*, and wherein the gene-regulating system comprises at least one *SOCS1*-targeting TALEN, zinc finger, or meganuclease protein that binds to a target DNA sequence defined by a set of genome coordinates shown in Tables 3 and 4 and at least one *PTPN2*-targeting TALEN, zinc finger, or meganuclease protein that binds to a target DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6. In some embodiments, the at least two endogenous genes are *SOCS1* and *PTPN2*, wherein the gene-regulating system comprises at least one *SOCS1*-targeting TALEN, zinc finger, or meganuclease protein that binds to a DNA sequence selected from SEQ ID NOs: 7-151 and at least one *PTPN2*-targeting TALEN, zinc finger, or meganuclease protein that binds to a DNA sequence selected from SEQ ID NOs: 185-207.

**[0017]** In some embodiments, the at least two endogenous genes are *SOCS1* and *ZC3H12A*, wherein the gene-regulating system comprises at least one *SOCS1*-targeting TALEN, zinc finger, or meganuclease protein that binds to a target DNA sequence defined by a set of genome coordinates shown in Tables 3 and 4 and at least one *ZC3H12A*-targeting TALEN, zinc finger, or meganuclease protein that binds to a target DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least two endogenous genes are *SOCS1* and *ZC3H12A*, wherein the gene-regulating system comprises at least one *SOCS1*-targeting TALEN, zinc finger, or meganuclease protein that binds to a DNA sequence selected from SEQ ID NOs: 7-151 and at least one *ZC3H12A*-targeting TALEN, zinc finger, or meganuclease protein that binds to a DNA sequence selected from SEQ ID NOs: 208-230.

**[0018]** In some embodiments, the at least two endogenous genes are *PTPN2* and *ZC3H12A*, wherein the gene-regulating system comprises at least one *PTPN2*-targeting TALEN, zinc finger, or meganuclease protein that binds to a target DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6 and at least one *ZC3H12A*-targeting TALEN, zinc finger, or meganuclease protein that binds to a target DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least two endogenous genes are *PTPN2* and *ZC3H12A*, wherein the gene-regulating system comprises at least one *PTPN2*-targeting TALEN, zinc finger, or meganuclease protein that binds to a DNA sequence selected from SEQ ID NOs: 185-207 and at least one *ZC3H12A*-targeting TALEN, zinc finger, or meganuclease protein that binds to a DNA sequence selected from SEQ ID NOs: 208-230.

**[0019]** In some embodiments, the gene-regulating system is introduced to the immune effector cell by transfection, transduction, electroporation, or physical disruption of the cell membrane by a microfluidics device. In some embodiments, the gene-regulating system is introduced as a polynucleotide encoding one or more components of the system, a protein, or a ribonucleoprotein (RNP) complex.

**[0020]** In some embodiments, the present disclosure provides a modified immune effector cell, comprising reduced expression and/or function of at least two endogenous genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*, wherein the reduced expression and/or function of the at least two endogenous genes enhances an effector function of the immune effector cell. In some embodiments, the at least two target genes are *SOCS1* and *PTPN2*. In some embodiments, the at least two target genes are *SOCS1* and *ZC3H12A*. In some embodiments, the at least two target genes are *PTPN2* and *ZC3H12A*.

**[0021]** In some embodiments, the present disclosure provides a modified immune effector cell, comprising an inactivating mutation in at least two endogenous genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*. In some embodiments, the immune effector cell is a tumor infiltrating lymphocyte (TIL) or a CAR-T cell. In some embodiments, the at least two target genes are *SOCS1* and *PTPN2*. In some embodiments, the at least two target genes are *SOCS1* and *ZC3H12A*. In some embodiments, the at least two target genes are *PTPN2* and *ZC3H12A*. In some embodiments, the inactivating mutation comprises a deletion, substitution, or insertion of one or more nucleotides in the genomic sequences of the two or more endogenous genes. In some embodiments, the deletion is a partial or complete deletion of the two or more endogenous target genes. In some embodiments, the inactivating

mutation is a frame shift mutation. In some embodiments, the inactivating mutation reduces the expression and/or function of the two or more endogenous target genes.

**[0022]** In some embodiments, the present disclosure provides a modified immune effector cell, comprising one or more exogenous polynucleotides encoding at least two nucleic acid inhibitors of at least two endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*. In some embodiments, the immune effector cell is a tumor infiltrating lymphocyte (TIL) or a CAR-T cell. In some embodiments, the at least two target genes are *SOCS1* and *PTPN2*. In some embodiments, the at least two target genes are *SOCS1* and *ZC3H12A*. In some embodiments, the at least two target genes are *PTPN2* and *ZC3H12A*. In some embodiments, the at least two nucleic acid inhibitors reduce the expression and/or function of the two or more endogenous target genes. In some embodiments, the expression of the two or more endogenous target genes is reduced by at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% compared to an un-modified or control immune effector cell. In some embodiments, the function of the two or more endogenous target genes is reduced by at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% compared to an un-modified or control immune effector cell. In some embodiments, the inactivating mutation or nucleic acid inhibitors substantially inhibits the expression of the two or more endogenous target genes. In some embodiments, the inactivating mutation or nucleic acid inhibitors substantially inhibits the function of the two or more endogenous target genes. In some embodiments, the inactivating mutation or nucleic acid inhibitors enhances one or more effector functions of the modified immune effector cell. In some embodiments, the one or more effector functions are enhanced compared to an un-modified or control immune effector cell.

**[0023]** In some embodiments, the immune effector cell is a T cell, a natural killer (NK) cell, an NKT cell, or a tumor infiltrating lymphocyte (TIL). In some embodiments, the modified immune effector cell further comprises an exogenous transgene expressing an immune activating molecule. In some embodiments, the immune activating molecule is selected from the group consisting of a cytokine, a chemokine, a co-stimulatory molecule, an activating peptide, an antibody, or an antigen-binding fragment thereof.

**[0024]** In some embodiments, the effector function is selected from cell proliferation, cell viability, tumor infiltration, cytotoxicity, anti-tumor immune responses, and/or resistance to exhaustion.

**[0025]** In some embodiments, the modified immune effector cell further comprises an engineered immune receptor displayed on the cell surface. In some embodiments, the engineered immune receptor is a chimeric antigen receptor (CAR) comprising an antigen-binding domain, a transmembrane domain, and an intracellular signaling domain. In some embodiments, the engineered immune receptor is an engineered T cell receptor (TCR). In some embodiments, the engineered immune receptor is capable of specifically binding to an antigen expressed on the surface of a target cell, wherein the antigen is a tumor-associated antigen.

**[0026]** In some embodiments, the present disclosure provides a composition comprising a modified immune effector cell described herein. In some embodiments, the composition comprises at least  $1 \times 10^4$ ,  $1 \times 10^5$ ,  $1 \times 10^6$ ,  $1 \times 10^7$ ,  $1 \times 10^8$ ,  $1 \times 10^9$ ,  $1 \times 10^{10}$ ,  $1 \times 10^{11}$ , or more modified immune effector cells. In some embodiments, the composition comprises a pharmaceutically acceptable carrier or diluent. In some embodiments, the composition comprises autologous immune effector cells. In some embodiments, the composition comprises allogeneic immune effector cells.

**[0027]** In some embodiments, the present disclosure provides a gene-regulating system capable of reducing expression of at least two endogenous target genes in a cell selected from *SOCS1*, *PTPN2*, and *ZC3H12A*, comprising (i) a nucleic acid molecule; (ii) an enzymatic protein; or (iii) a nucleic acid molecule and an enzymatic protein. In some embodiments, the at least two target genes are *SOCS1* and *PTPN2*. In some embodiments, the at least two target genes are *SOCS1* and *ZC3H12A*. In some embodiments, the at least two endogenous genes are *PTPN2* and *ZC3H12A*.

**[0028]** In some embodiments, the system comprises at least two guide RNA (gRNA) nucleic acid molecules and a Cas endonuclease. In some embodiments, the at least two target genes are *SOCS1* and *PTPN2*, and wherein the system comprises at least one *SOCS1*-targeting guide RNA (gRNA) molecule, at least one *PTPN2*-targeting gRNA molecule, and a Cas endonuclease. In some embodiments, the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 5 and 6. In some embodiments, the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 5 and 6. In some embodiments, the at least one *SOCS1*-targeting gRNA



molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 7-151 and the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 185-207. In some embodiments, the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 7-151 and the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 185-207.

**[0029]** In some embodiments, the at least two target genes are *SOCS1* and *ZC3H12A*, and wherein the system comprises at least one *SOCS1*-targeting gRNA molecule, at least one *ZC3H12A*-targeting gRNA molecule, and a Cas endonuclease. In some embodiments, the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 7-151 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 208-230. In some embodiments, the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 7-151 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 208-230.

**[0030]** In some embodiments, the at least two endogenous genes are *PTPN2* and *ZC3H12A*, wherein the system comprises at least one *PTPN2*-targeting gRNA molecule, at least one *ZC3H12A*-targeting gRNA molecule, and a Cas endonuclease. In some embodiments, the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 5 and 6 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence complementary to a

nucleic acid sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 5 and 6 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 185-207 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 208-230. In some embodiments, the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 185-207 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 208-230.

**[0031]** In some embodiments, the Cas protein is a Cas9 protein. In some embodiments, the Cas protein is a wild-type Cas protein comprising two enzymatically active domains, and capable of inducing double stranded DNA breaks. In some embodiments, the Cas protein is a Cas nickase mutant comprising one enzymatically active domain and capable of inducing single stranded DNA breaks. In some embodiments, the Cas protein is a deactivated Cas protein (dCas) and is associated with a heterologous protein capable of modulating the expression of the one or more endogenous target genes. In some embodiments, the heterologous protein is selected from the group consisting of MAX-interacting protein 1 (MXI1), Krüppel-associated box (KRAB) domain, and four concatenated mSin3 domains (SID4X).

**[0032]** In some embodiments, the system comprises at least two nucleic acid molecules and wherein the at least two nucleic acid molecules are selected from an siRNA, an shRNA, a microRNA (miR), an antagomiR, or an antisense RNA. In some embodiments, the at least two target genes are *SOCS1* and *PTPN2*, and wherein the system comprises at least one *SOCS1*-targeting guide siRNA or shRNA molecule and at least one *PTPN2*-targeting siRNA or shRNA molecule. In some embodiments, the *SOCS1*-targeting siRNA or shRNA molecule comprises about 19-30 nucleotides that are complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *PTPN2*-targeting siRNA or shRNA molecule comprises about 19-30 nucleotides that are complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6. In some embodiments, the

at least one *SOCS1*-targeting siRNA or shRNA molecule comprises about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *PTPN2*-targeting siRNA or shRNA molecule comprises about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6. In some embodiments, the at least one *SOCS1*-targeting siRNA or shRNA comprises about 19-30 nucleotides that bind to an RNA sequence encoded by a DNA sequence selected from SEQ ID NOs: 7-151 and the at least one *PTPN2*-targeting siRNA or shRNA comprises about 19-30 nucleotides that bind to an RNA sequence encoded by a DNA sequence selected from SEQ ID NOs: 185-207.

**[0033]** In some embodiments, the at least two target genes are *SOCS1* and *ZC3H12A*, and wherein the system comprises at least one *SOCS1*-targeting guide siRNA or shRNA molecule and at least one *ZC3H12A*-targeting siRNA or shRNA molecule. In some embodiments, the at least one *SOCS1*-targeting siRNA or shRNA molecule comprises about 19-30 nucleotides that are complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule comprises about 19-30 nucleotides that are complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least one *SOCS1*-targeting siRNA or shRNA molecule comprises about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule comprises about 19-30 nucleotides and binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least one *SOCS1*-targeting siRNA or shRNA comprises about 19-30 nucleotides that bind to an RNA sequence encoded by a DNA sequence selected from SEQ ID NOs: 7-151 and the at least one *ZC3H12A*-targeting siRNA or shRNA comprises about 19-30 nucleotides that bind to an RNA sequence encoded by a DNA sequence selected from SEQ ID NOs: 208-230.

**[0034]** In some embodiments, the at least two target genes are *PTPN2* and *ZC3H12A*, and wherein the system comprises at least one *PTPN2*-targeting guide siRNA or shRNA molecule and at least one *ZC3H12A*-targeting siRNA or shRNA molecule. In some embodiments, the at least one *PTPN2*-targeting siRNA or shRNA molecule comprises about 19-30 nucleotides that are complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6 and the at least one *ZC3H12A*-targeting siRNA or shRNA

molecule comprises about 19-30 nucleotides that are complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least one *PTPN2*-targeting siRNA or shRNA molecule comprises about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6 and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule comprises about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least one *PTPN2*-targeting siRNA or shRNA comprises about 19-30 nucleotides that bind to an RNA sequence encoded by a DNA sequence selected from SEQ ID NOs: 185-207 and the at least one *ZC3H12A*-targeting siRNA or shRNA comprises about 19-30 nucleotides that bind to an RNA sequence encoded by a DNA sequence selected from SEQ ID NOs: 208-230.

**[0035]** In some embodiments, the gene-regulating system comprises an enzymatic protein, and wherein the enzymatic protein has been engineered to specifically bind to a target sequence in one or more of the endogenous genes. In some embodiments, the system comprises a protein comprising a DNA binding domain and an enzymatic domain and is selected from a zinc finger nuclease and a transcription-activator-like effector nuclease (TALEN). In some embodiments, the system comprises one or more vectors encoding at least one gRNA targeting a first target gene, at least one gRNA targeting a second target gene, and a Cas endonuclease protein, wherein the first target gene is *SOCS1* and the least one *SOCS1*-targeting gRNA comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 7-151, and wherein the second target gene is *PTPN2* and the at least one *PTPN2*-targeting gRNA comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 185-207.

**[0036]** In some embodiments, the gene-regulating system comprises one or more vectors encoding at least one gRNA targeting a first target gene, at least one gRNA targeting a second target gene, and a Cas endonuclease protein, wherein the first target gene is *SOCS1* and the least one *SOCS1*-targeting gRNA comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 7-151, and wherein the second target gene is *ZC3H12A* and the at least one *ZC3H12A*-targeting gRNA comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 208-230.

**[0037]** In some embodiments, the gene-regulating system comprises one or more vectors encoding at least one gRNA targeting a first target gene, at least one gRNA targeting a second target gene, and a Cas endonuclease protein, wherein the first target gene is *PTPN2* and the *PTPN2*-targeting

gRNA comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 185-207, and wherein the second target gene is *ZC3H12A* and the at least one *ZC2H12A*-targeting gRNA comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 208-230.

**[0038]** In some embodiments, the at least one gRNA targeting the first target gene, the at least one gRNA targeting the second target gene, and the Cas endonuclease protein are encoded by one vector. In some embodiments, the at least one gRNA targeting the first target gene and the at least one gRNA targeting the second target gene are encoded by a first vector, and the Cas endonuclease protein is encoded by a second vector. In some embodiments, the at least one gRNA targeting the first target gene is encoded by a first vector, the at least one gRNA targeting the second target gene is encoded by a second vector, and the Cas endonuclease protein is encoded by a third vector.

**[0039]** In some embodiments, the gene-regulating system comprises (i) one or more vectors encoding at least one *SOCS1*-targeting gRNA comprising a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 7-151 and at least one *PTPN2*-targeting gRNA comprising a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 185-207; and (ii) an mRNA molecule encoding the Cas endonuclease protein.

**[0040]** In some embodiments, the gene-regulating system comprises (i) one or more vectors encoding at least one *SOCS1*-targeting gRNA comprising a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 7-151 and at least one *ZC2H12A*-targeting gRNA comprising a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 208-230; and (ii) an mRNA molecule encoding the Cas endonuclease protein.

**[0041]** In some embodiments, the gene-regulating system comprises (i) one or more vectors encoding at least one *PTPN2*-targeting gRNA comprising a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 185-207 and at least one *ZC2H12A*-targeting gRNA comprising a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 208-230; and (ii) an mRNA molecule encoding the Cas endonuclease protein.

**[0042]** In some embodiments, the at least one gRNA targeting the first target gene and the at least one gRNA targeting the second target gene are encoded by one vector. In some embodiments, the at least one gRNA targeting the first target gene is encoded by a first vector and the at least one gRNA targeting the second target gene is encoded by a second vector.

**[0043]** In some embodiments, the gene-regulating system comprises (i) at least one *SOCS1*-targeting gRNA comprising a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 7-151 complexed to a first Cas endonuclease protein to form a first ribonucleoprotein (RNP) complex; and (ii) at least one *PTPN2*-targeting gRNA comprising a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 185-207 complexed to a second Cas endonuclease protein to form a second RNP complex.

**[0044]** In some embodiments, the gene-regulating system comprises (i) at least one *SOCS1*-targeting gRNA comprising a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 7-151 complexed to a first Cas endonuclease protein to form a first RNP complex; and (ii) at least one *ZC2H12A*-targeting gRNA comprising a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 208-230 complexed to a second Cas endonuclease protein to form a second RNP complex.

**[0045]** In some embodiments, the gene-regulating system comprises (i) at least one *PTPN2*-targeting gRNA comprising a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 185-207 complexed to a first Cas endonuclease protein to form a first RNP complex; and (ii) at least one *ZC2H12A*-targeting gRNA comprising a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 208-230 complexed to a second Cas endonuclease protein to form a second RNP complex.

**[0046]** In some embodiments, the present disclosure provides a kit comprising a gene-regulating system described herein.

**[0047]** In some embodiments, the present disclosure provides a composition comprising a plurality of gRNA molecules, wherein the plurality of gRNA molecules comprises at least one gRNA molecule targeting a first target gene and at least one gRNA molecule targeting a second target gene, wherein the first and second target gene are selected from *SOCS1*, *PTPN2*, and *ZC3H12A*. In some embodiments, the first target gene is *SOCS1* and the second target gene is *PTPN2*. In some embodiments, the plurality of gRNA molecules comprises at least one *SOCS1*-targeting gRNA molecule comprising a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 3 and 4 and at least one *PTPN2*-targeting gRNA molecule comprising a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 5 and 6. In some embodiments, the plurality of gRNA molecules comprises at least one *SOCS1*-targeting gRNA molecule comprising a targeting domain

sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 5 and 6. In some embodiments, the plurality of gRNA molecules comprises at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 7-151 and the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 185-207. In some embodiments, the plurality of gRNA molecules comprises at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 7-151 and the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 185-207.

**[0048]** In some embodiments, the first target gene is *SOCS1* and the second target gene is *ZC3H12A*. In some embodiments, the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 7-151 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 208-230. In some embodiments, the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 7-151 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 208-230.

**[0049]** In some embodiments, the first target gene is *PTPN2* and the second target gene is *ZC3H12A*. In some embodiments, the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 5 and 6 and the at least one *ZC3H12A*-targeting gRNA molecule

comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 5 and 6 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 185-207 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 208-230. In some embodiments, the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 185-207 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 208-230.

**[0050]** In some embodiments, the gRNAs are modular gRNA molecules. In some embodiments, the gRNAs are dual gRNA molecules. In some embodiments, the gRNA targeting domains are 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26 or more nucleotides in length. In some embodiments, the gRNAs comprise a modification at or near the 5' end (e.g., within 1-10, 1-5, or 1-2 nucleotides of the 5' end) and/or a modification at or near the 3' end (e.g., within 1-10, 1-5, or 1-2 nucleotides of the 3' end). In some embodiments, the modified gRNAs exhibit increased resistance to nucleases when introduced into an immune effector cell. In some embodiments, the modified gRNAs do not induce an innate immune response when introduced into an immune effector cell or induce a decreased innate immune response compared to an unmodified gRNA when introduced into an immune effector cell.

**[0051]** In some embodiments, the present disclosure provides a polynucleotide molecule encoding a plurality of gRNA molecules, wherein the plurality of gRNA molecules comprises at least one gRNA molecule targeting a first target gene and at least one gRNA molecule targeting a second target gene, wherein the first and second target gene are selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.

**[0052]** In some embodiments, the first target gene is *SOCS1* and the second target gene is *PTPN2*. In some embodiments, the plurality of gRNA molecules comprises at least one *SOCS1*-targeting gRNA molecule comprising a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 3 and 4 and at least one *PTPN2*-targeting gRNA molecule comprising a targeting domain sequence complementary to a nucleic acid



sequence defined by a set of genome coordinates shown in Tables 5 and 6. In some embodiments, the plurality of gRNA molecules comprises at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 5 and 6. In some embodiments, the plurality of gRNA molecules comprises at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 7-151 and the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 185-207. In some embodiments, the plurality of gRNA molecules comprises at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 7-151 and the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 185-207.

**[0053]** In some embodiments, the first target gene is *SOCS1* and the second target gene is *ZC3H12A*. In some embodiments, the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 7-151 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 208-230. In some embodiments, the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 7-151 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 208-230.

**[0054]** In some embodiments, the first target gene is *PTPN2* and the second target gene is *ZC3H12A*. In some embodiments, the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 5 and 6 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 5 and 6 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 185-207 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 208-230. In some embodiments, at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 185-207 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 208-230.

**[0055]** In some embodiments, the present disclosure provides a polynucleotide molecule encoding a plurality of siRNA or shRNA molecules, wherein the plurality of siRNA or shRNA molecules comprises at least one siRNA or shRNA molecule targeting a first target gene and at least one siRNA or shRNA molecule targeting a second target gene, wherein the first and second target gene are selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.

**[0056]** In some embodiments, the first target gene is *SOCS1* and the second target gene is *PTPN2*. In some embodiments, the plurality of siRNA or shRNA molecules comprises at least one *SOCS1*-targeting siRNA or shRNA molecule comprising a targeting domain sequence that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 3 and 4 and at least one *PTPN2*-targeting siRNA or shRNA molecule comprising a targeting domain sequence complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6. In some embodiments, the plurality of siRNA or shRNA molecules comprises at least one *SOCS1*-targeting siRNA or shRNA molecule comprising a targeting domain sequence that binds to an RNA sequence encoded by a DNA sequence selected from SEQ ID NOs: 7-151 and the at least one *PTPN2*-targeting siRNA or shRNA molecule

comprising a targeting domain sequence that binds to an RNA sequence encoded by a DNA sequence selected from SEQ ID NOs: 185-207.

**[0057]** In some embodiments, the first target gene is *SOCS1* and the second target gene is *ZC3H12A*. In some embodiments, the at least one *SOCS1*-targeting siRNA or shRNA molecule comprising a targeting domain sequence that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule comprising a targeting domain sequence that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least one *SOCS1*-targeting siRNA or shRNA molecule comprising a targeting domain sequence that binds to an RNA sequence encoded by a DNA sequence selected from SEQ ID NOs: 7-151 and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule comprising a targeting domain sequence that binds to an RNA sequence encoded by a DNA sequence selected from SEQ ID NOs: 208-230.

**[0058]** In some embodiments, the first target gene is *PTPN2* and the second target gene is *ZC3H12A*. In some embodiments, the at least one *PTPN2*-targeting siRNA or shRNA molecule comprising a targeting domain sequence that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6 and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule comprising a targeting domain sequence that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the at least one *PTPN2*-targeting siRNA or shRNA molecule comprising a targeting domain sequence that binds to an RNA sequence encoded by a DNA sequence selected from SEQ ID NOs: 185-207 and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule comprising a targeting domain sequence that binds to an RNA sequence encoded by a DNA sequence selected from SEQ ID NOs: 208-230.

**[0059]** In some embodiments, the present disclosure provides a polynucleotide molecule encoding at least one TALEN, zinc finger, or meganuclease protein targeting a first target gene and at least one TALEN, zinc finger, or meganuclease protein targeting a second target gene, wherein the first and second target gene are selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.

**[0060]** In some embodiments, the first target gene is *SOCS1* and the second target gene is *PTPN2*. In some embodiments, the polynucleotide encodes at least one *SOCS1*-targeting TALEN, zinc finger, or meganuclease protein comprising a targeting domain sequence that binds to a DNA

sequence defined by a set of genome coordinates shown in Tables 3 and 4 and at least one *PTPN2*-targeting TALEN, zinc finger, or meganuclease protein comprising a targeting domain sequence that binds to a DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6. In some embodiments, the polynucleotide encodes at least one *SOCS1*-targeting TALEN, zinc finger, or meganuclease protein comprising a targeting domain sequence that binds to a DNA sequence selected from SEQ ID NOs: 7-151 and at least one *PTPN2*-targeting TALEN, zinc finger, or meganuclease protein comprising a targeting domain sequence that binds to a DNA sequence selected from SEQ ID NOs: 185-207.

**[0061]** In some embodiments, the first target gene is *SOCS1* and the second target gene is *ZC3H12A*. In some embodiments, the polynucleotide encodes at least one *SOCS1*-targeting TALEN, zinc finger, or meganuclease protein comprising a targeting domain sequence that binds to a DNA sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *ZC3H12A*-targeting TALEN, zinc finger, or meganuclease protein comprising a targeting domain sequence that binds to a DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the polynucleotide encodes at least one *SOCS1*-targeting TALEN, zinc finger, or meganuclease protein comprising a targeting domain sequence that binds to a DNA sequence selected from SEQ ID NOs: 7-151 and at least one *ZC3H12A*-targeting TALEN, zinc finger, or meganuclease protein comprising a targeting domain sequence that binds to a DNA sequence selected from SEQ ID NOs: 208-230.

**[0062]** In some embodiments, the first target gene is *PTPN2* and the second target gene is *ZC3H12A*. In some embodiments, the polynucleotide encodes at least one *PTPN2*-targeting TALEN, zinc finger, or meganuclease protein comprising a targeting domain sequence that binds to a DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6 and the at least one *ZC3H12A*-targeting TALEN, zinc finger, or meganuclease protein comprising a targeting domain sequence that binds to a DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8. In some embodiments, the polynucleotide encodes at least one *PTPN2*-targeting TALEN, zinc finger, or meganuclease protein comprising a targeting domain sequence that binds to a DNA sequence selected from SEQ ID NOs: 185-207 and at least one *ZC3H12A*-targeting TALEN, zinc finger, or meganuclease protein comprising a targeting domain sequence that binds to a DNA sequence selected from SEQ ID NOs: 208-230.

**[0063]** In some embodiments, the present disclosure provides a composition comprising the polynucleotide described herein.

**[0064]** In some embodiments, the present disclosure provides a kit comprising the polynucleotide or composition described herein.

**[0065]** In some embodiments, the present disclosure provides a method of producing a modified immune effector cell comprising: introducing a gene-regulating system into the immune effector cell, wherein the gene-regulating system is capable of reducing expression and/or function of at least two endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.

**[0066]** In some embodiments, the present disclosure provides a method of producing a modified immune effector cell comprising: obtaining an immune effector cell from a subject; introducing a gene-regulating system into the immune effector cell, wherein the gene-regulating system is capable of reducing expression and/or function of at least two endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*; and culturing the immune effector cell such that the expression and/or function of one or more endogenous target genes is reduced compared to an immune effector cell that has not been modified. In some embodiments, the gene-regulating system is one selected from those described herein. In some embodiments, the method further comprises introducing a polynucleotide sequence encoding an engineered immune receptor selected from a CAR and a TCR. In some embodiments, the gene-regulating system and/or the polynucleotide encoding the engineered immune receptor are introduced to the immune effector cell by transfection, transduction, electroporation, or physical disruption of the cell membrane by a microfluidics device. In some embodiments, the gene-regulating system is introduced as a polynucleotide sequence encoding one or more components of the system, as a protein, or as a ribonucleoprotein (RNP) complex.

**[0067]** In some embodiments, the present disclosure provides a method of producing a modified immune effector cell comprising: expanding a population of immune effector cells in a first round expansion and a second round of expansion; and introducing a gene-regulating system into the population of immune effector cells, wherein the gene-regulating system is capable of reducing expression and/or function of at least two endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.

**[0068]** In some embodiments, the present disclosure provides a method of producing a modified immune effector cell comprising: obtaining a population of immune effector cells; expanding the population of immune effector cells in a first round expansion and a second round of expansion; introducing a gene-regulating system into the population of immune effector cells, wherein the gene-regulating system is capable of reducing expression and/or function of at least two

endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*; and culturing the immune effector cell such that the expression and/or function of one or more endogenous target genes is reduced compared to an immune effector cell that has not been modified. In some embodiments, the gene-regulating system is introduced to the population of immune effector cells prior to the first and second rounds of expansion. In some embodiments, the gene-regulating system is introduced to the population of immune effector cells after the first round of expansion and prior to the second round of expansion. In some embodiments, the gene-regulating system is introduced to the population of immune effector cells after the first and second rounds of expansion. In some embodiments, the gene-regulating system is one selected from those described herein.

**[0069]** In some embodiments, the present disclosure provides a method of treating a disease or disorder in a subject in need thereof comprising administering to the subject an effective amount of the modified immune effector cells described herein or composition thereof. In some embodiments, the disease or disorder is a cell proliferative disorder, an inflammatory disorder, or an infectious disease. In some embodiments, the disease or disorder is a cancer or a viral infection. In some embodiments, the cancer is selected from a leukemia, a lymphoma, or a solid tumor. In some embodiments, the solid tumor is a melanoma, a pancreatic tumor, a bladder tumor, or a lung tumor or metastasis. In some embodiments, the cancer is a PD1 resistant or insensitive cancer. In some embodiments, the subject has previously been treated with a PD1 inhibitor or a PDL1 inhibitor. In some embodiments, the modified immune effector cells are autologous to the subject. In some embodiments, the modified immune effector cells are allogenic to the subject.

**[0070]** In some embodiments, the method further comprises administering to the subject an antibody or binding fragment thereof that specifically binds to and inhibits the function of the protein encoded by *NRP1*, *HAVCR2*, *LAG3*, *TIGIT*, *CTLA4*, or *PDCD1*. In some embodiments, the subject has not undergone lymphodepletion prior to administration of the modified immune effector cells or compositions thereof. In some embodiments, administration of the modified immune effector cells or compositions thereof to the subject is not accompanied by high dose IL-2 treatment. In some embodiments, administration of the modified immune effector cells or compositions thereof to the subject is not accompanied by any IL-2 treatment. In some embodiments, the subject has not undergone any IL-2 treatment prior to administration of the modified immune effector cells or compositions thereof. In some embodiments, the subject has not undergone any high dose IL-2 treatment prior to administration of the modified immune effector cells or compositions thereof. In some embodiments, the subject has not undergone lymphodepletion prior to administration of the

modified immune effector cells or compositions thereof and the administration of modified immune effector cells or compositions thereof to the subject is not accompanied by high dose IL-2 treatment. In some embodiments, the subject has not undergone lymphodepletion prior to administration of the modified immune effector cells or compositions thereof and the administration of modified immune effector cells or compositions thereof to the subject is not accompanied by any IL-2 treatment. In some embodiments, the subject has not undergone lymphodepletion or high dose IL-2 treatment prior to administration of the modified immune effector cells or compositions thereof. In some embodiments, the subject has not undergone lymphodepletion or any IL-2 treatment prior to administration of the modified immune effector cells or compositions thereof.

**[0071]** In some embodiments, the present disclosure provides a method of killing a cancerous cell comprising exposing the cancerous cell to a modified immune effector cell described herein or composition thereof, wherein exposure to the modified immune effector cell results in increased killing of the cancerous cells compared to exposure to an immune effector cell that has not been modified. In some embodiments, the exposure is in vitro, in vivo, or ex vivo.

**[0072]** In some embodiments, the present disclosure provides a method of enhancing one or more effector functions of an immune effector cell comprising introducing a gene-regulating system into the immune effector cell, wherein the gene-regulating system is capable of reducing the expression and/or function of at least two endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.

**[0073]** In some embodiments, the present disclosure provides a method of enhancing one or more effector functions of an immune effector cell comprising: introducing a gene-regulating system into the immune effector cell, wherein the gene-regulating system is capable of reducing the expression and/or function of at least two endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*; and culturing the immune effector cell such that the expression and/or function of one or more endogenous target genes is reduced compared to an immune effector cell that has not been modified, wherein the modified immune effector cell demonstrates one or more enhanced effector functions compared to the immune effector cell that has not been modified. In some embodiments, the one or more effector functions are selected from cell proliferation, cell viability, cytotoxicity, tumor infiltration, increased cytokine production, anti-tumor immune responses, and/or resistance to exhaustion. In some embodiments, the gene-regulating system is one described herein.

[0074] In some embodiments, the present disclosure provides a method of producing a modified immune effector cell comprising introducing an inactivating mutation in at least two endogenous target genes in an immune effector cell, wherein the at least two endogenous target genes are selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.

[0075] In some embodiments, the present disclosure provides a method of producing a modified immune effector cell comprising: expanding a population of immune effector cells in a first round expansion and a second round of expansion; and introducing an inactivating mutation in at least two endogenous target genes in the population of immune effector cells, wherein the at least two endogenous target genes are selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.

[0076] In some embodiments, the present disclosure provides a method of producing a modified immune effector cell comprising introducing one or more exogenous polynucleotides encoding at least two nucleic acid inhibitors of at least two endogenous target genes in an immune effector cell, wherein the at least two endogenous target genes are selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.

[0077] In some embodiments, the present disclosure provides a method of producing a modified immune effector cell comprising: expanding a population of immune effector cells in a first round expansion and a second round of expansion; and introducing one or more exogenous polynucleotides encoding at least two nucleic acid inhibitors of at least two endogenous target genes in the population of immune effector cells, wherein the at least two endogenous target genes are selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.

[0078] In some embodiments, the methods further comprise introducing a polynucleotide sequence encoding an engineered immune receptor selected from a CAR and a TCR. In some embodiments, the inactivating mutation is introduced by the nucleic acid gene-regulating system of any one of the preceding claims. In some embodiments, the at least two nucleic acid inhibitors are comprised in a gene-regulating system described herein.

In some embodiments, the disclosure provides a method of killing a cancerous cell in a subject in need thereof comprising administering to the subject a therapeutically effective amount of modified immune effector cell according to any one of claims 1-57 or the composition of any one of claims 79-83, wherein exposure to the modified immune effector cell results in increased killing of the cancerous cells compared to exposure to an immune effector cell that has not been modified, wherein the number of modified immune effector cells necessary to comprise a therapeutically effective amount is at least



ten fold less or at least 100 fold less than the number of non-modified immune effector cells necessary to comprise a therapeutically effective amount. In some embodiments, the number of modified immune effector cells necessary to comprise a therapeutically effective amount is at least  $1 \times 10^3$ ,  $5 \times 10^3$ ,  $1 \times 10^4$ ,  $5 \times 10^4$ ,  $1 \times 10^5$ ,  $5 \times 10^5$ ,  $1 \times 10^6$ ,  $2 \times 10^6$ ,  $3 \times 10^6$ ,  $4 \times 10^6$ ,  $5 \times 10^6$ ,  $1 \times 10^7$ ,  $5 \times 10^7$ ,  $1 \times 10^8$ ,  $5 \times 10^8$ ,  $1 \times 10^9$  cells.

### BRIEF DESCRIPTION OF THE FIGURES

**[0079]** Fig. 1 shows the anti-tumor efficacy of *Pptn2/Socs1* dual-edited transgenic T cells in a B16-Ova murine tumor model.

**[0080]** Fig. 2 shows the anti-tumor efficacy of *Zc3h12a/Socs1* dual-edited transgenic T cells in a B16-Ova murine tumor model.

**[0081]** Fig. 3 shows the anti-tumor efficacy of *Zc3h12a/Socs1* dual-edited TILs in a B16-Ova murine tumor model.

**[0082]** Fig. 4 shows the anti-tumor efficacy of *Pd1/Lag3* dual-edited transgenic T cells in a B16-Ova murine tumor model.

**[0083]** Fig. 5 shows the increase in pSTAT5 levels in primary human CD8 T cells in response to IL-2 signaling after deletion of *SOCS1*.

**[0084]** Fig. 6 shows the increase pSTAT1 levels in Jurkat T cells in response to IFN $\gamma$  stimulation after genetic knockdown of *PTPN2*.

**[0085]** Figs. 7A-7D show the production of IFN $\gamma$  (Fig. 7A) and TNF $\alpha$  (Fig. 7B) by dual *SOCS1/PTPN2*-edited human TILs as well as the ability of CD8 T cells within TIL populations to degranulate as measured by positivity (Fig. 7C) and intensity (Fig. 7D) of CD107a staining upon stimulation with phorbol 12-myristate 13-acetate (PMA) and ionomycin.

**[0086]** Figs. 8A-8D show the ability of *Ptpn2<sup>-/-</sup>/Socs1<sup>-/-</sup>* OT1s to completely regress 100mm<sup>3</sup> B16Ova tumors in seventeen out of eighteen mice. (Fig. 8A). Also shown is the ability of the mice who previously regressed a B16Ova tumor to completely resist subsequent inoculation with B16Ova and partially resist subsequent parental B16F10 tumor inoculation. (Fig. 8B). The OT1 population in peripheral blood was tracked over the course of the rechallenge study (Fig. 8C) along with their memory phenotype (Fig. 8D).

**[0087]** Figs. 9A-9E show the ability of *Ptpn2<sup>-/-</sup>/Socs1<sup>-/-</sup>* OT1s to completely regress larger 343mm<sup>3</sup> B16Ova tumors in eight out of eight mice. (Fig. 9A). In a separate cohort of mice total OT1

infiltration into the tumor (Fig. 9B) and their ability to produce granzyme B (Fig. 9C) is presented. Also shown is the ability of the mice who previously regressed a B16Ova tumor to completely resist subsequent inoculation with B16Ova and partially resist subsequent parental B16F10 tumor inoculation. (Fig. 9D). The OT1 population in peripheral blood was tracked over the course of the rechallenge study along with their memory phenotype. (Fig. 9E).

**[0088]** Figs. 10A-G shows tumor volume measurements after increasing doses of *Ptpn2/Socs1* dual-edited or control edited mouse OT1 CD8<sup>+</sup> T cells adoptively transferred into the large tumor B16Ova model. Figs. 10A-10D show control cells administered at  $4.1 \times 10^4$ ,  $4.1 \times 10^5$ ,  $4.1 \times 10^6$  and  $4.1 \times 10^7$ , respectively. Figs. 10E-10G show *Ptpn2/Socs1* dual-edited cells administered at  $4.1 \times 10^4$ ,  $4.1 \times 10^5$ ,  $4.1 \times 10^6$  and  $4.1 \times 10^7$ , respectively. Each line represents the results for a separate animal.

### DETAILED DESCRIPTION

**[0089]** The present disclosure provides methods and compositions related to the modification of immune effector cells to increase their therapeutic efficacy in the context of immunotherapy. In some embodiments, immune effector cells are modified by the methods of the present disclosure to reduce the expression and/or function of two or more endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A* such that one or more effector functions of the immune cells are enhanced. In some embodiments, the immune effector cells are further modified by introduction of transgenes conferring antigen specificity, such as introduction of T cell receptor (TCR) or chimeric antigen receptor (CAR) expression constructs. In some embodiments, the present disclosure provides compositions and methods for modifying the immune effector cells, such as compositions of gene-regulating systems capable of reducing the expression and/or function of two or more endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*. In some embodiments, the present disclosure provides methods of treating a cell proliferative disorder, such as a cancer, comprising administration of the modified immune effector cells described herein to a subject in need thereof.

### I. Definitions

**[0090]** As used in this specification and the appended claims, the singular forms “a,” “an” and “the” include plural references unless the content clearly dictates otherwise.

**[0091]** As used in this specification, the term “and/or” is used in this disclosure to mean either “and” or “or” unless indicated otherwise.

**[0092]** Throughout this specification, unless the context requires otherwise, the words “comprise”, or variations such as “comprises” or “comprising”, will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

**[0093]** As used in this application, the terms “about” and “approximately” are used as equivalents. Any numerals used in this application with or without about/approximately are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art. In certain embodiments, the term “approximately” or “about” refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

**[0094]** “Decrease” or “reduce” refers to a decrease or a reduction in a particular value of at least 5%, for example, a 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% decrease as compared to a reference value. A decrease or reduction in a particular value may also be represented as a fold-change in the value compared to a reference value, for example, at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000-fold, or more, decrease as compared to a reference value.

**[0095]** “Increase” refers to an increase in a particular value of at least 5%, for example, a 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, 100%, 200%, 300%, 400%, 500%, or more increase as compared to a reference value. An increase in a particular value may also be represented as a fold-change in the value compared to a reference value, for example, at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000-fold or more, increase as compared to the level of a reference value.

**[0096]** The terms “peptide,” “polypeptide,” and “protein” are used interchangeably herein, and refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones.

**[0097]** The terms “polynucleotide” and “nucleic acid,” used interchangeably herein, refer to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. Thus, this term includes, but is not limited to, single-, double-, or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer comprising purine and pyrimidine bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases. “Oligonucleotide” generally refers to polynucleotides of between about 5 and about 100 nucleotides of single- or double-stranded DNA. However, for the purposes of this disclosure, there is no upper limit to the length of an oligonucleotide. Oligonucleotides are also known as “oligomers” or “oligos” and may be isolated from genes, or chemically synthesized by methods known in the art. The terms “polynucleotide” and “nucleic acid” should be understood to include, as applicable to the embodiments being described, single-stranded (such as sense or antisense) and double-stranded polynucleotides.

**[0098]** “Fragment” refers to a portion of a polypeptide or polynucleotide molecule containing less than the entire polypeptide or polynucleotide sequence. In some embodiments, a fragment of a polypeptide or polynucleotide comprises at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% of the entire length of the reference polypeptide or polynucleotide. In some embodiments, a polypeptide or polynucleotide fragment may contain 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, or more nucleotides or amino acids.

**[0099]** The term “sequence identity” refers to the percentage of bases or amino acids between two polynucleotide or polypeptide sequences that are the same, and in the same relative position. As such one polynucleotide or polypeptide sequence has a certain percentage of sequence identity compared to another polynucleotide or polypeptide sequence. For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. The term “reference sequence” refers to a molecule to which a test sequence is compared.

**[00100]** “Complementary” refers to the capacity for pairing, through base stacking and specific hydrogen bonding, between two sequences comprising naturally or non-naturally occurring bases or analogs thereof. For example, if a base at one position of a nucleic acid is capable of hydrogen bonding with a base at the corresponding position of a target, then the bases are considered to be complementary to each other at that position. Nucleic acids can comprise universal bases, or inert abasic spacers that provide no positive or negative contribution to hydrogen bonding. Base pairings may include both canonical Watson-Crick base pairing and non-Watson-Crick base pairing (*e.g.*,

Wobble base pairing and Hoogsteen base pairing). It is understood that for complementary base pairings, adenosine-type bases (A) are complementary to thymidine-type bases (T) or uracil-type bases (U), that cytosine-type bases (C) are complementary to guanosine-type bases (G), and that universal bases such as such as 3-nitropyrrole or 5-nitroindole can hybridize to and are considered complementary to any A, C, U, or T. Nichols *et al.*, Nature, 1994;369:492-493 and Loakes *et al.*, Nucleic Acids Res., 1994;22:4039-4043. Inosine (I) has also been considered in the art to be a universal base and is considered complementary to any A, C, U, or T. *See* Watkins and SantaLucia, Nucl. Acids Research, 2005; 33 (19): 6258-6267.

**[00101]** As referred to herein, a “complementary nucleic acid sequence” is a nucleic acid sequence comprising a sequence of nucleotides that enables it to non-covalently bind to another nucleic acid in a sequence-specific, antiparallel, manner (*i.e.*, a nucleic acid specifically binds to a complementary nucleic acid) under the appropriate *in vitro* and/or *in vivo* conditions of temperature and solution ionic strength.

**[00102]** Methods of sequence alignment for comparison and determination of percent sequence identity and percent complementarity are well known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the homology alignment algorithm of Needleman and Wunsch, (1970) J. Mol. Biol. 48:443, by the search for similarity method of Pearson and Lipman, (1988) Proc. Nat'l. Acad. Sci. USA 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), by manual alignment and visual inspection (see, *e.g.*, Brent *et al.*, (2003) Current Protocols in Molecular Biology), by use of algorithms known in the art including the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.*, (1977) Nuc. Acids Res. 25:3389-3402; and Altschul *et al.*, (1990) J. Mol. Biol. 215:403-410, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information.

**[00103]** Herein, the term “hybridize” refers to pairing between complementary nucleotide bases (*e.g.*, adenine (A) forms a base pair with thymine (T) in a DNA molecule and with uracil (U) in an RNA molecule, and guanine (G) forms a base pair with cytosine (C) in both DNA and RNA molecules) to form a double-stranded nucleic acid molecule. (*See, e.g.*, Wahl and Berger (1987) Methods Enzymol. 152:399; Kimmel, (1987) Methods Enzymol. 152:507). In addition, it is also known in the art that for hybridization between two RNA molecules (*e.g.*, dsRNA), guanine (G) base pairs with uracil (U). For example, G/U base-pairing is partially responsible for the degeneracy (*i.e.*,

redundancy) of the genetic code in the context of tRNA anti-codon base-pairing with codons in mRNA. In the context of this disclosure, a guanine (G) of a protein-binding segment (dsRNA duplex) of a guide RNA molecule is considered complementary to a uracil (U), and vice versa. As such, when a G/U base-pair can be made at a given nucleotide position a protein-binding segment (dsRNA duplex) of a guide RNA molecule, the position is not considered to be non-complementary, but is instead considered to be complementary. It is understood in the art that the sequence of polynucleotide need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. Moreover, a polynucleotide may hybridize over one or more segments such that intervening or adjacent segments are not involved in the hybridization event (*e.g.*, a loop structure or hairpin structure). A polynucleotide can comprise at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or 100% sequence complementarity to a target region within the target nucleic acid sequence to which they are targeted.

**[00104]** The term “modified” refers to a substance or compound (*e.g.*, a cell, a polynucleotide sequence, and/or a polypeptide sequence) that has been altered or changed as compared to the corresponding unmodified substance or compound.

**[00105]** The term “naturally-occurring” as used herein as applied to a nucleic acid, a polypeptide, a cell, or an organism, refers to a nucleic acid, polypeptide, cell, or organism that is found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by a human in the laboratory is naturally occurring.

**[00106]** “Isolated” refers to a material that is free to varying degrees from components which normally accompany it as found in its native state.

**[00107]** An “expression cassette” or “expression construct” refers to a DNA polynucleotide sequence operably linked to a promoter. “Operably linked” refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. For instance, a promoter is operably linked to a polynucleotide sequence if the promoter affects the transcription or expression of the polynucleotide sequence.

**[00108]** The term “recombinant vector” as used herein refers to a polynucleotide molecule capable transferring or transporting another polynucleotide inserted into the vector. The inserted polynucleotide may be an expression cassette. In some embodiments, a recombinant vector may be viral vector or a non-viral vector (*e.g.*, a plasmid).

**[00109]** The term “sample” refers to a biological composition (*e.g.*, a cell or a portion of a tissue) that is subjected to analysis and/or genetic modification. In some embodiments, a sample is a “primary sample” in that it is obtained directly from a subject; in some embodiments, a “sample” is the result of processing of a primary sample, for example to remove certain components and/or to isolate or purify certain components of interest.

**[00110]** The term “subject” includes animals, such as *e.g.* mammals. In some embodiments, the mammal is a primate. In some embodiments, the mammal is a human. In some embodiments, subjects are livestock such as cattle, sheep, goats, cows, swine, and the like; or domesticated animals such as dogs and cats. In some embodiments (*e.g.*, particularly in research contexts) subjects are rodents (*e.g.*, mice, rats, hamsters), rabbits, primates, or swine such as inbred pigs and the like. The terms “subject” and “patient” are used interchangeably herein.

**[00111]** “Administration” refers herein to introducing an agent or composition into a subject.

**[00112]** “Treating” as used herein refers to delivering an agent or composition to a subject to affect a physiologic outcome.

**[00113]** As used herein, the term “effective amount” refers to the minimum amount of an agent or composition required to result in a particular physiological effect. The effective amount of a particular agent may be represented in a variety of ways based on the nature of the agent, such as mass/volume, # of cells/volume, particles/volume, (mass of the agent)/(mass of the subject), # of cells/(mass of subject), or particles/(mass of subject). The effective amount of a particular agent may also be expressed as the half-maximal effective concentration (EC<sub>50</sub>), which refers to the concentration of an agent that results in a magnitude of a particular physiological response that is half-way between a reference level and a maximum response level.

**[00114]** “Population” of cells refers to any number of cells greater than 1, but is preferably at least  $1 \times 10^3$  cells, at least  $1 \times 10^4$  cells, at least  $1 \times 10^5$  cells, at least  $1 \times 10^6$  cells, at least  $1 \times 10^7$  cells, at least  $1 \times 10^8$  cells, at least  $1 \times 10^9$  cells, at least  $1 \times 10^{10}$  cells, or more cells. A population of cells may refer to an *in vitro* population (*e.g.*, a population of cells in culture) or an *in vivo* population (*e.g.*, a population of cells residing in a particular tissue).

**[00115]** General methods in molecular and cellular biochemistry can be found in such standard textbooks as Molecular Cloning: A Laboratory Manual, 3rd Ed. (Sambrook *et al.*, HaRBor Laboratory Press 2001); Short Protocols in Molecular Biology, 4th Ed. (Ausubel *et al.* eds., John Wiley & Sons 1999); Protein Methods (Bollag *et al.*, John Wiley & Sons 1996); Nonviral Vectors for Gene Therapy

(Wagner *et al.* eds., Academic Press 1999); Viral Vectors (Kaplift & Loewy eds., Academic Press 1995); Immunology Methods Manual (I. Lefkovits ed., Academic Press 1997); and Cell and Tissue Culture: Laboratory Procedures in Biotechnology (Doyle & Griffiths, John Wiley & Sons 1998), the disclosures of which are incorporated herein by reference.

## II. Modified Immune Effector Cells

**[00116]** In some embodiments, the present disclosure provides modified immune effector cells comprising reduced expression and/or function of two or more endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*. In some embodiments, the present disclosure provides modified immune effector cells comprising a gene-regulating system capable of reducing the expression and/or function of two or more endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*. Herein, the term “modified immune effector cells” encompasses immune effector cells comprising one or more genomic modifications resulting in the reduced expression and/or function of two or more endogenous target genes as well as immune effector cells comprising a gene-regulating system capable of reducing the expression and/or function of two or more endogenous target genes. Herein, an “un-modified immune effector cell” or “control immune effector cell” refers to a cell or population of cells wherein the genomes have not been modified and that does not comprise a gene-regulating system or comprises a control gene-regulating system (*e.g.*, an empty vector control, a non-targeting gRNA, a scrambled siRNA, etc.).

**[00117]** The term “immune effector cell” refers to cells involved in mounting innate and adaptive immune responses, including but not limited to lymphocytes (such as T-cells (including thymocytes) and B-cells), natural killer (NK) cells, NKT cells, macrophages, monocytes, eosinophils, basophils, neutrophils, dendritic cells, and mast cells. In some embodiments, the modified immune effector cell is a T cell, such as a CD4<sup>+</sup> T cell, a CD8<sup>+</sup> T cell (also referred to as a cytotoxic T cell or CTL), a regulatory T cell (Treg), a Th1 cell, a Th2 cell, or a Th17 cell.

**[00118]** In some embodiments, the modified immune effector cell is a T cell that has been isolated from a tumor sample (referred to herein as “tumor infiltrating lymphocytes” or “TILs”). Without wishing to be bound by theory, it is thought that TILs possess increased specificity to tumor antigens (Radvanyi *et al.*, 2012 Clin Canc Res 18:6758-6770) and can therefore mediate tumor antigen-specific immune response (*e.g.*, activation, proliferation, and cytotoxic activity against the cancer cell) leading to cancer cell destruction (Brudno *et al.*, 2018 Nat Rev Clin Onc 15:31-46)) without the introduction of an exogenous engineered receptor. Therefore, in some embodiments, TILs



are isolated from a tumor in a subject, expanded *ex vivo*, and re-infused into a subject. In some embodiments, TILs are modified to express one or more exogenous receptors specific for an autologous tumor antigen, expanded *ex vivo*, and re-infused into the subject. Such embodiments can be modeled using *in vivo* mouse models wherein mice have been transplanted with a cancer cell line expressing a cancer antigen (*e.g.*, CD19) and are treated with modified T cells that express an exogenous receptor that is specific for the cancer antigen (*See e.g.*, Examples 6-9).

**[00119]** In some embodiments, the modified immune effector cell is an animal cell or is derived from an animal cell, including invertebrate animals and vertebrate animals (*e.g.*, fish, amphibian, reptile, bird, or mammal). In some embodiments, the modified immune effector cell is a mammalian cell or is derived from a mammalian cell (*e.g.*, a pig, a cow, a goat, a sheep, a rodent, a non-human primate, a human, etc.). In some embodiments, the modified immune effector cell is a rodent cell or is derived from a rodent cell (*e.g.*, a rat or a mouse). In some embodiments, the modified immune effector cell is a human cell or is derived from a human cell.

**[00120]** In some embodiments, the modified immune effector cells comprise one or more modifications (*e.g.*, insertions, deletions, or mutations of one or more nucleic acids) in the genomic DNA sequence of an endogenous target gene resulting in the reduced expression and/or function the endogenous gene. In such embodiments, the modified immune effector cells comprise a “modified endogenous target gene.” In some embodiments, the modifications in the genomic DNA sequence reduce or inhibit mRNA transcription, thereby reducing the expression level of the encoded mRNA transcript and protein. In some embodiments, the modifications in the genomic DNA sequence reduce or inhibit mRNA translation, thereby reducing the expression level of the encoded protein. In some embodiments, the modifications in the genomic DNA sequence encode a modified endogenous protein with reduced or altered function compared to the unmodified (*i.e.*, wild-type) version of the endogenous protein (*e.g.*, a dominant-negative mutant, described *infra*). In some embodiments, the modified immune effector cells comprise at least two modified endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.

**[00121]** In some embodiments, the modified immune effector cells comprise one or more genomic modifications at a genomic location other than an endogenous target gene that result in the reduced expression and/or function of the endogenous target gene or that result in the expression of a modified version of an endogenous protein. For example, in some embodiments, a polynucleotide sequence encoding a gene regulating system is inserted into one or more locations in the genome, thereby reducing the expression and/or function of an endogenous target gene upon the expression of

the gene-regulating system. In some embodiments, a polynucleotide sequence encoding a modified version of an endogenous protein is inserted at one or more locations in the genome, wherein the function of the modified version of the protein is reduced compared to the un-modified or wild-type version of the protein (*e.g.*, a dominant-negative mutant, described *infra*).

**[00122]** In some embodiments, the modified immune effector cells described herein comprise two or more modified endogenous target genes, wherein the one or more modifications result in a reduced expression and/or function of a gene product (*i.e.*, an mRNA transcript or a protein) encoded by the endogenous target gene compared to an unmodified immune effector cell. For example, in some embodiments, a modified immune effector cell demonstrates reduced expression of an mRNA transcript and/or reduced expression of a protein. In some embodiments, the expression of the gene product in a modified immune effector cell is reduced by at least 5% compared to the expression of the gene product in an unmodified immune effector cell. In some embodiments, the expression of the gene product in a modified immune effector cell is reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more compared to the expression of the gene product in an unmodified immune effector cell. In some embodiments, the modified immune effector cells described herein demonstrate reduced expression and/or function of gene products encoded by a plurality (*e.g.*, two or more) of endogenous target genes compared to the expression of the gene products in an unmodified immune effector cell. For example, in some embodiments, a modified immune effector cell demonstrates reduced expression and/or function of gene products from 2, 3, 4, 5, 6, 7, 8, 9, 10, or more endogenous target genes compared to the expression of the gene products in an unmodified immune effector cell.

**[00123]** In some embodiments, the present disclosure provides a modified immune effector cell wherein two or more endogenous target genes, or a portion thereof, are deleted (*i.e.*, “knocked-out”) such that the modified immune effector cell does not express the mRNA transcript or protein. In some embodiments, a modified immune effector cell comprises deletion of a plurality of endogenous target genes, or portions thereof. In some embodiments, a modified immune effector cell comprises deletion of 2, 3, 4, 5, 6, 7, 8, 9, 10, or more endogenous target genes.

**[00124]** In some embodiments, the modified immune effector cells described herein comprise one or more modified endogenous target genes, wherein the one or more modifications to the target DNA sequence result in expression of a protein with reduced or altered function (*e.g.*, a “modified endogenous protein”) compared to the function of the corresponding protein expressed in an unmodified immune effector cell (*e.g.*, a “unmodified endogenous protein”). In some embodiments,

the modified immune effector cells described herein comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, or more modified endogenous target genes encoding 2, 3, 4, 5, 6, 7, 8, 9, 10, or more modified endogenous proteins. In some embodiments, the modified endogenous protein demonstrates reduced or altered binding affinity for another protein expressed by the modified immune effector cell or expressed by another cell; reduced or altered signaling capacity; reduced or altered enzymatic activity; reduced or altered DNA-binding activity; or reduced or altered ability to function as a scaffolding protein.

**[00125]** In some embodiments, the modified endogenous target gene comprises one or more dominant negative mutations. As used herein, a “dominant-negative mutation” refers to a substitution, deletion, or insertion of one or more nucleotides of a target gene such that the encoded protein acts antagonistically to the protein encoded by the unmodified target gene. The mutation is dominant-negative because the negative phenotype confers genic dominance over the positive phenotype of the corresponding unmodified gene. A gene comprising one or more dominant-negative mutations and the protein encoded thereby are referred to as a “dominant-negative mutants”, *e.g.* dominant-negative genes and dominant-negative proteins. In some embodiments, the dominant negative mutant protein is encoded by an exogenous transgene inserted at one or more locations in the genome of the immune effector cell.

**[00126]** Various mechanisms for dominant negativity are known. Typically, the gene product of a dominant negative mutant retains some functions of the unmodified gene product but lacks one or more crucial other functions of the unmodified gene product. This causes the dominant-negative mutant to antagonize the unmodified gene product. For example, as an illustrative embodiment, a dominant-negative mutant of a transcription factor may lack a functional activation domain but retain a functional DNA binding domain. In this example, the dominant-negative transcription factor cannot activate transcription of the DNA as the unmodified transcription factor does, but the dominant-negative transcription factor can indirectly inhibit gene expression by preventing the unmodified transcription factor from binding to the transcription-factor binding site. As another illustrative embodiment, dominant-negative mutations of proteins that function as dimers are known. Dominant-negative mutants of such dimeric proteins may retain the ability to dimerize with unmodified protein but be unable to function otherwise. The dominant-negative monomers, by dimerizing with unmodified monomers to form heterodimers, prevent formation of functional homodimers of the unmodified monomers. Dominant negative mutations of the *SOCS1* gene are known in the art and include the murine F59D mutant (*See e.g.*, Hanada *et al.*, J Biol Chem, 276:44:2 (2001), 40746-40754;

and Suzuki *et al.*, J Exp Med, 193:4 (2001), 471-482), and the human F58D mutant, identified by sequence alignments of the human and murine SOCS1 amino acid sequences.

**[00127]** In some embodiments, the modified immune effector cells comprise a gene-regulating system capable of reducing the expression and/or function of two or more endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*. The gene-regulating system can reduce the expression and/or function of the endogenous target genes modifications by a variety of mechanisms including by modifying the genomic DNA sequence of the endogenous target gene (*e.g.*, by insertion, deletion, or mutation of one or more nucleic acids in the genomic DNA sequence); by regulating transcription of the endogenous target gene (*e.g.*, inhibition or repression of mRNA transcription); and/or by regulating translation of the endogenous target gene (*e.g.*, by mRNA degradation).

**[00128]** In some embodiments, the modified immune effector cells described herein comprise a gene-regulating system comprising:

(a) two or more nucleic acid molecules capable of reducing the expression and/or modifying the function of a gene product encoded by two or more endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;

(b) one or more polynucleotides encoding two or more nucleic acid molecules that are capable of reducing the expression and/or modifying the function of the gene products encoded by two or more endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;

(c) two or more proteins capable of reducing the expression and/or modifying the function of the gene products encoded by two or more endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;

(d) one or more polynucleotides encoding two or more proteins that are capable of reducing the expression and/or modifying the function of a gene product encoded by two or more endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;

(e) two or more guide RNAs (gRNAs) capable of binding to a target DNA sequence in two or more endogenous genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;

(f) one or more polynucleotides encoding two or more gRNAs capable of binding to a target DNA sequence in two or more endogenous genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;

- (g) one or more site-directed modifying polypeptides capable of interacting with a gRNA and modifying a target DNA sequence in an endogenous gene selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;
- (h) one or more polynucleotides encoding a site-directed modifying polypeptide capable of interacting with a gRNA and modifying a target DNA sequence in an endogenous gene selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;
- (i) two or more guide DNAs (gDNAs) capable of binding to a target DNA sequence in two or more endogenous genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;
- (j) one or more polynucleotides encoding two or more gDNAs capable of binding to a target DNA sequence in two or more endogenous genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;
- (k) one or more site-directed modifying polypeptides capable of interacting with a gDNA and modifying a target DNA sequence in an endogenous gene selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;
- (l) one or more polynucleotides encoding a site-directed modifying polypeptide capable of interacting with a gDNA and modifying a target DNA sequence in an endogenous gene selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;
- (m) two or more gRNAs capable of binding to a target mRNA sequence encoded by two or more endogenous genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;
- (n) one or more polynucleotides encoding two or more gRNAs capable of binding to a target mRNA sequence encoded by two or more endogenous genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;
- (o) one or more site-directed modifying polypeptides capable of interacting with a gRNA and modifying a target mRNA sequence encoded by an endogenous gene selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;
- (p) one or more polynucleotides encoding a site-directed modifying polypeptide capable of interacting with a gRNA and modifying a target mRNA sequence encoded by an endogenous gene selected from *SOCS1*, *PTPN2*, and *ZC3H12A*; or
- (q) any combination of the above.

**[00129]** In some embodiments, one or more polynucleotides encoding the gene-regulating system are inserted into the genome of the immune effector cell. In some embodiments, one or more polynucleotides encoding the gene-regulating system are expressed episomally and are not inserted into the genome of the immune effector cell.

**[00130]** In some embodiments, the modified immune effector cells described herein comprise reduced expression and/or function of two or more endogenous target genes and further comprise one or more exogenous transgenes inserted at one or more genomic loci (*e.g.*, a genetic “knock-in”). In some embodiments, the one or more exogenous transgenes encode detectable tags, safety-switch systems, chimeric switch receptors, and/or engineered antigen-specific receptors.

**[00131]** In some embodiments, the modified immune effector cells described herein further comprise an exogenous transgene encoding a detectable tag. Examples of detectable tags include but are not limited to, FLAG tags, poly-histidine tags (*e.g.* 6xHis), SNAP tags, Halo tags, cMyc tags, glutathione-S-transferase tags, avidin, enzymes, fluorescent proteins, luminescent proteins, chemiluminescent proteins, bioluminescent proteins, and phosphorescent proteins. In some embodiments the fluorescent protein is selected from the group consisting of blue/UV proteins (such as BFP, TagBFP, mTagBFP2, Azurite, EBFP2, mKalama1, Sirius, Sapphire, and T-Sapphire); cyan proteins (such as CFP, eCFP, Cerulean, SCFP3A, mTurquoise, mTurquoise2, monomeric Midoriishi-Cyan, TagCFP, and mTFP1); green proteins (such as: GFP, eGFP, meGFP (A208K mutation), Emerald, Superfolder GFP, Monomeric Azami Green, TagGFP2, mUKG, mWasabi, Clover, and mNeonGreen); yellow proteins (such as YFP, eYFP, Citrine, Venus, SYFP2, and TagYFP); orange proteins (such as Monomeric Kusabira-Orange, mKOκ, mKO2, mOrange, and mOrange2); red proteins (such as RFP, mRaspberry, mCherry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mApple, mRuby, and mRuby2); far-red proteins (such as mPlum, HcRed-Tandem, mKate2, mNeptune, and NirFP); near-infrared proteins (such as TagRFP657, IFP1.4, and iRFP); long stokes shift proteins (such as mKeima Red, LSS-mKate1, LSS-mKate2, and mBeRFP); photoactivatable proteins (such as PA-GFP, PAmCherry1, and PAtagRFP); photoconvertible proteins (such as Kaede (green), Kaede (red), KikGR1 (green), KikGR1 (red), PS-CFP2, PS-CFP2, mEos2 (green), mEos2 (red), mEos3.2 (green), mEos3.2 (red), PSmOrange, and PSmOrange); and photoswitchable proteins (such as Dronpa). In some embodiments, the detectable tag can be selected from AmCyan, AsRed, DsRed2, DsRed Express, E2-Crimson, HcRed, ZsGreen, ZsYellow, mCherry, mStrawberry, mOrange, mBanana, mPlum, mRaspberry, tdTomato, DsRed Monomer, and/or AcGFP, all of which are available from Clontech.

**[00132]** In some embodiments, the modified immune effector cells described herein further comprise an exogenous transgene encoding a safety-switch system. Safety-switch systems (also referred to in the art as suicide gene systems) comprise exogenous transgenes encoding for one or more proteins that enable the elimination of a modified immune effector cell after the cell has been administered to a subject. Examples of safety-switch systems are known in the art. For example, safety-switch systems include genes encoding for proteins that convert non-toxic pro-drugs into toxic compounds such as the Herpes simplex thymidine kinase (*Hsv-tk*) and ganciclovir (GCV) system (*Hsv-tk/GCV*). *Hsv-tk* converts non-toxic GCV into a cytotoxic compound that leads to cellular apoptosis. As such, administration of GCV to a subject that has been treated with modified immune effector cells comprising a transgene encoding the *Hsv-tk* protein can selectively eliminate the modified immune effector cells while sparing endogenous immune effector cells. (See e.g., Bonini *et al.*, Science, 1997, 276(5319):1719-1724; Ciceri *et al.*, Blood, 2007, 109(11):1828-1836; Bondanza *et al.*, Blood 2006, 107(5):1828-1836).

**[00133]** Additional safety-switch systems include genes encoding for cell-surface markers, enabling elimination of modified immune effector cells by administration of a monoclonal antibody specific for the cell-surface marker via ADCC. In some embodiments, the cell-surface marker is CD20 and the modified immune effector cells can be eliminated by administration of an anti-CD20 monoclonal antibody such as Rituximab (See e.g., Introna *et al.*, Hum Gene Ther, 2000, 11(4):611-620; Serafini *et al.*, Hum Gene Ther, 2004, 14, 63-76; van Meerten *et al.*, Gene Ther, 2006, 13, 789-797). Similar systems using EGF-R and Cetuximab or Panitumumab are described in International PCT Publication No. WO 2018006880. Additional safety-switch systems include transgenes encoding pro-apoptotic molecules comprising one or more binding sites for a chemical inducer of dimerization (CID), enabling elimination of modified immune effector cells by administration of a CID which induces oligomerization of the pro-apoptotic molecules and activation of the apoptosis pathway. In some embodiments, the pro-apoptotic molecule is Fas (also known as CD95) (Thomis *et al.*, Blood, 2001, 97(5), 1249-1257). In some embodiments, the pro-apoptotic molecule is caspase-9 (Straathof *et al.*, Blood, 2005, 105(11), 4247-4254).

**[00134]** In some embodiments, the modified immune effector cells described herein further comprise an exogenous transgene encoding a chimeric switch receptor. Chimeric switch receptors are engineered cell-surface receptors comprising an extracellular domain from an endogenous cell-surface receptor and a heterologous intracellular signaling domain, such that ligand recognition by the extracellular domain results in activation of a different signaling cascade than that activated by the

wild type form of the cell-surface receptor. In some embodiments, the chimeric switch receptor comprises the extracellular domain of an inhibitory cell-surface receptor fused to an intracellular domain that leads to the transmission of an activating signal rather than the inhibitory signal normally transduced by the inhibitory cell-surface receptor. In particular embodiments, extracellular domains derived from cell-surface receptors known to inhibit immune effector cell activation can be fused to activating intracellular domains. Engagement of the corresponding ligand will then activate signaling cascades that increase, rather than inhibit, the activation of the immune effector cell. For example, in some embodiments, the modified immune effector cells described herein comprise a transgene encoding a PD1-CD28 switch receptor, wherein the extracellular domain of PD1 is fused to the intracellular signaling domain of CD28 (*See e.g.*, Liu *et al.*, Cancer Res 76:6 (2016), 1578-1590 and Moon *et al.*, Molecular Therapy 22 (2014), S201). In some embodiments, the modified immune effector cells described herein comprise a transgene encoding the extracellular domain of CD200R and the intracellular signaling domain of CD28 (*See Oda et al.*, Blood 130:22 (2017), 2410-2419).

**[00135]** In some embodiments, the modified immune effector cells described herein further comprise an engineered antigen-specific receptor recognizing a protein target expressed by a target cell, such as a tumor cell or an antigen presenting cell (APC), referred to herein as “modified receptor-engineered cells” or “modified RE-cells”. The term “engineered antigen receptor” refers to a non-naturally occurring antigen-specific receptor such as a chimeric antigen receptor (CAR) or a recombinant T cell receptor (TCR). In some embodiments, the engineered antigen receptor is a CAR comprising an extracellular antigen binding domain fused via hinge and transmembrane domains to a cytoplasmic domain comprising a signaling domain. In some embodiments, the CAR extracellular domain binds to an antigen expressed by a target cell in an MHC-independent manner leading to activation and proliferation of the RE cell. In some embodiments, the extracellular domain of a CAR recognizes a tag fused to an antibody or antigen-binding fragment thereof. In such embodiments, the antigen-specificity of the CAR is dependent on the antigen-specificity of the labeled antibody, such that a single CAR construct can be used to target multiple different antigens by substituting one antibody for another (*See e.g.*, US Patent Nos. 9,233,125 and 9,624,279; US Patent Application Publication Nos. 20150238631 and 20180104354). In some embodiments, the extracellular domain of a CAR may comprise an antigen binding fragment derived from an antibody. Antigen binding domains that are useful in the present disclosure include, for example, scFvs; antibodies; antigen binding regions of antibodies; variable regions of the heavy/light chains; and single chain antibodies.



**[00136]** In some embodiments, the intracellular signaling domain of a CAR may be derived from the TCR complex zeta chain (such as CD3 $\zeta$  signaling domains), Fc $\gamma$ RIII, Fc $\epsilon$ RI, or the T-lymphocyte activation domain. In some embodiments, the intracellular signaling domain of a CAR further comprises a costimulatory domain, for example a 4-1BB, CD28, CD40, MyD88, or CD70 domain. In some embodiments, the intracellular signaling domain of a CAR comprises two costimulatory domains, for example any two of 4-1BB, CD28, CD40, MyD88, or CD70 domains. Exemplary CAR structures and intracellular signaling domains are known in the art (*See e.g.*, WO 2009/091826; US 20130287748; WO 2015/142675; WO 2014/055657; and WO 2015/090229, incorporated herein by reference).

**[00137]** CARs specific for a variety of tumor antigens are known in the art, for example CD171-specific CARs (Park *et al.*, Mol Ther (2007) 15(4):825-833), EGFRvIII-specific CARs (Morgan *et al.*, Hum Gene Ther (2012) 23(10):1043-1053), EGF-R-specific CARs (Kobold *et al.*, J Natl Cancer Inst (2014) 107(1):364), carbonic anhydrase K-specific CARs (Lamers *et al.*, Biochem Soc Trans (2016) 44(3):951-959), FR- $\alpha$ -specific CARs (Kershaw *et al.*, Clin Cancer Res (2006) 12(20):6106-6015), HER2-specific CARs (Ahmed *et al.*, J Clin Oncol (2015) 33(15):1688-1696; Nakazawa *et al.*, Mol Ther (2011) 19(12):2133-2143; Ahmed *et al.*, Mol Ther (2009) 17(10):1779-1787; Luo *et al.*, Cell Res (2016) 26(7):850-853; Morgan *et al.*, Mol Ther (2010) 18(4):843-851; Grada *et al.*, Mol Ther Nucleic Acids (2013) 9(2):32), CEA-specific CARs (Katz *et al.*, Clin Cancer Res (2015) 21(14):3149-3159), IL13R $\alpha$ 2-specific CARs (Brown *et al.*, Clin Cancer Res (2015) 21(18):4062-4072), GD2-specific CARs (Louis *et al.*, Blood (2011) 118(23):6050-6056; Caruana *et al.*, Nat Med (2015) 21(5):524-529), ErbB2-specific CARs (Wilkie *et al.*, J Clin Immunol (2012) 32(5):1059-1070), VEGF-R-specific CARs (Chinnasamy *et al.*, Cancer Res (2016) 22(2):436-447), FAP-specific CARs (Wang *et al.*, Cancer Immunol Res (2014) 2(2):154-166), MSLN-specific CARs (Moon *et al.*, Clin Cancer Res (2011) 17(14):4719-30), NKG2D-specific CARs (VanSeggelen *et al.*, Mol Ther (2015) 23(10):1600-1610), CD19-specific CARs (Axicabtagene ciloleucel (Yescarta<sup>®</sup>) and Tisagenlecleucel (Kymriah<sup>®</sup>). *See also*, Li *et al.*, J Hematol and Oncol (2018) 11(22), reviewing clinical trials of tumor-specific CARs. Exemplary CARs suitable for use according to the present disclosure are described below in Table 1.

**Table 1: Exemplary CAR constructs**

| CAR Ref ID | Target     | Ag-binding domain   | Intracellular Domain | Transmembrane Domain | AA SEQ ID | NA SEQ ID |
|------------|------------|---------------------|----------------------|----------------------|-----------|-----------|
| KSQCAR017  | human EGFR | Cetuximab H225 scFv | CD3 zeta             | CD8a hinge           | 264       | 265       |
| KSQCAR1909 | human CD19 | FMC63 scFv          | CD3 zeta             | CD8a hinge           | 266       | 267       |
| KSQCAR010  | human HER2 | Herceptin scFv      | CD3 zeta             | CD8a hinge           | 268       | 269       |

**[00138]** In some embodiments, the engineered antigen receptor is a recombinant TCR. Recombinant TCRs comprise TCR $\alpha$  and/or TCR $\beta$  chains that have been isolated and cloned from T cell populations recognizing a particular target antigen. For example, TCR $\alpha$  and/or TCR $\beta$  genes (*i.e.*, *TRAC* and *TRBC*) can be cloned from T cell populations isolated from individuals with particular malignancies or T cell populations that have been isolated from humanized mice immunized with specific tumor antigens or tumor cells. Recombinant TCRs recognize antigen through the same mechanisms as their endogenous counterparts (*e.g.*, by recognition of their cognate antigen presented in the context of major histocompatibility complex (MHC) proteins expressed on the surface of a target cell). This antigen engagement stimulates endogenous signal transduction pathways leading to activation and proliferation of the TCR-engineered cells.

**[00139]** Recombinant TCRs specific for tumor antigens are known in the art, for example WT1-specific TCRs (JTCR016, Juno Therapeutics; WT1-TCRc4, described in US Patent Application Publication No. 20160083449), MART-1 specific TCRs (including the DMF4T clone, described in Morgan *et al.*, Science 314 (2006) 126-129); the DMF5T clone, described in Johnson *et al.*, Blood 114 (2009) 535-546); and the ID3T clone, described in van den Berg *et al.*, Mol. Ther. 23 (2015) 1541-1550), gp100-specific TCRs (Johnson *et al.*, Blood 114 (2009) 535-546), CEA-specific TCRs (Parkhurst *et al.*, Mol Ther. 19 (2011) 620-626), NY-ESO and LAGE-1 specific TCRs (1G4T clone, described in Robbins *et al.*, J Clin Oncol 26 (2011) 917-924; Robbins *et al.*, Clin Cancer Res 21 (2015) 1019-1027; and Rapoport *et al.*, Nature Medicine 21 (2015) 914-921), and MAGE-A3-specific TCRs (Morgan *et al.*, J Immunother 36 (2013) 133-151) and Linette *et al.*, Blood 122 (2013) 227-242). (*See also*, Debets *et al.*, Seminars in Immunology 23 (2016) 10-21).

**[00140]** To generate the recombinant TCRs, the native TRAC (SEQ ID NO: 260) and TRBC (SEQ ID NOs: 262) protein sequences are fused to the C-terminal ends of TCR- $\alpha$  and TCR- $\beta$  chain variable regions specific for a protein or peptide of interest. For example, the engineered TCR can

recognize an amino acid sequence comprising the NY-ESO peptide (SLLMWITQC, SEQ ID NO: 239), such as the 1G4 TCR or the 95:LY TCR (Robbins *et al*, Journal of Immunology 2008 180:6116-6131). In such illustrative embodiments, the paired 1G4-TCR  $\alpha/\beta$  chains comprise SEQ ID NOs: 249 and 248, respectively and the paired 95:LY-TCR  $\alpha/\beta$  chains comprise SEQ ID NOs: 252 and 251, respectively. The recombinant TCR can recognize the MART-1 peptide (AAGIGILTV, SEQ ID NO: 240), such as the DMF4 and DMF5 TCRs (Robbins *et al*, Journal of Immunology 2008 180:6116-6131). In such illustrative embodiments, the paired DMF4-TCR  $\alpha/\beta$  chains comprise SEQ ID NOs: 255 and 254, respectively and the paired DMF5-TCR  $\alpha/\beta$  chains comprise SEQ ID NOs: 258 and 257, respectively. The recombinant TCR can recognize the WT-1 peptide (RMFPNAPYL, SEQ ID NO: 241), such as the DLT TCR (Robbins *et al*, Journal of Immunology 2008 180:6116-6131). In such illustrative embodiments, the paired high-affinity DLT-TCR  $\alpha/\beta$  chains comprise SEQ ID NOs: 246 and 245, respectively.

**[00141]** Codon-optimized DNA sequences encoding the recombinant TCR $\alpha$  and TCR $\beta$  chain proteins can be generated such that expression of both TCR chains is driven off of a single promoter in a stoichiometric fashion. In such embodiment, the P2A sequence (SEQ ID NO: 238) can be inserted between the DNA sequences encoding the TCR $\beta$  and the TCR $\alpha$  chain, such that the expression cassettes encoding the recombinant TCR chains comprise the following format: TCR $\beta$  – P2A – TCR $\alpha$ . As an illustrative embodiment, the protein sequence of the 1G4 NY-ESO-specific TCR expressed from such a cassette would comprise SEQ ID NO: 250, the protein sequence of the 95:LY NY-ESO-specific TCR expressed from such a cassette would comprise SEQ ID NO: 23, the protein sequence of the DMF4 MART1-specific TCR expressed from such a cassette would comprise SEQ ID NO: 256, the protein sequence of the DMF5 MART1-specific TCR expressed from such a cassette would comprise SEQ ID NO: 259, and the protein sequence of the DLT WT1-specific TCR expressed from such a cassette would comprise SEQ ID NO: 247.

**[00142]** In some embodiments, the engineered antigen receptor is directed against a target antigen selected from a cluster of differentiation molecule, such as CD3, CD4, CD8, CD16, CD24, CD25, CD33, CD34, CD45, CD64, CD71, CD78, CD80 (also known as B7-1), CD86 (also known as B7-2), CD96, , CD116, CD117, CD123, CD133, and CD138, CD371 (also known as CLL1); a tumor-associated surface antigen, such as 5T4, BCMA (also known as CD269 and TNFRSF17, UniProt# Q02223), carcinoembryonic antigen (CEA), carbonic anhydrase 9 (CAIX or MN/CAIX), CD19, CD20, CD22, CD30, CD40, disialogangliosides such as GD2, ELF2M, ductal-epithelial mucin, ephrin B2, epithelial cell adhesion molecule (EpCAM), ErbB2 (HER2/neu), FCRL5 (UniProt# Q68SN8),

FKBP11 (UniProt# Q9NYL4), glioma-associated antigen, glycosphingolipids, gp36, GPRC5D (UniProt# Q9NZD1), mut hsp70-2, intestinal carboxyl esterase, IGF-I receptor, ITGA8 (UniProt# P53708), KAMP3, LAGE-1a, MAGE, mesothelin, neutrophil elastase, NKG2D, Nkp30, NY-ESO-1, PAP, prostase, prostate-carcinoma tumor antigen-1 (PCTA-1), prostate specific antigen (PSA), PSMA, prostein, RAGE-1, ROR1, RU1 (SFMBT1), RU2 (DCDC2), SLAMF7 (UniProt# Q9NQ25), survivin, TAG-72, and telomerase; a major histocompatibility complex (MHC) molecule presenting a tumor-specific peptide epitope; tumor stromal antigens, such as the extra domain A (EDA) and extra domain B (EDB) of fibronectin; the A1 domain of tenascin-C (TnC A1) and fibroblast associated protein (FAP); cytokine receptors, such as epidermal growth factor receptor (EGFR), EGFR variant III (EGFRvIII), TGF $\beta$ -R or components thereof such as endoglin; a major histocompatibility complex (MHC) molecule; a virus-specific surface antigen such as an HIV-specific antigen (such as HIV gp120); an EBV-specific antigen, a CMV-specific antigen, a HPV-specific antigen, a Lassa virus-specific antigen, an Influenza virus-specific antigen as well as any derivate or variant of these surface antigens.

**[00143]** In some embodiments, the present disclosure provides modified immune effector cells comprising reduced expression and/or function of *SOCS1* and *PTPN2* or a gene-regulating system capable of reducing the expression and/or function of *SOCS1* and *PTPN2* and further comprising a CAR or recombinant TCR expressed on the cell surface. In some embodiments, the modified immune effector cells comprise reduced expression and/or function of *SOCS1* and *PTPN2* or a gene-regulating system capable of reducing the expression and/or function of *SOCS1* and *PTPN2* and further comprising a recombinant expression vector encoding a CAR or a recombinant TCR.

**[00144]** In some embodiments, the present disclosure provides modified immune effector cells comprising reduced expression and/or function of *SOCS1* and *ZC3H12A* or a gene-regulating system capable of reducing the expression and/or function of *SOCS1* and *ZC3H12A* and further comprising a CAR or recombinant TCR expressed on the cell surface. In some embodiments, the modified immune effector cells comprise reduced expression and/or function of *SOCS1* and *ZC3H12A* or a gene-regulating system capable of reducing the expression and/or function of *SOCS1* and *ZC3H12A* and further comprising a recombinant expression vector encoding a CAR or a recombinant TCR.

**[00145]** In some embodiments, the present disclosure provides modified immune effector cells comprising reduced expression and/or function of *PTPN2* and *ZC3H12A* or a gene-regulating system capable of reducing the expression and/or function of *PTPN2* and *ZC3H12A* and further comprising a CAR or recombinant TCR expressed on the cell surface. In some embodiments, the modified

immune effector cells comprise reduced expression and/or function of *PTPN2* and *ZC3H12A* or a gene-regulating system capable of reducing the expression and/or function of *PTPN2* and *ZC3H12A* and further comprising a recombinant expression vector encoding a CAR or a recombinant TCR.

**[00146]** In some embodiments, the present disclosure provides modified immune effector cells comprising reduced expression and/or function of *SOCS1* and *PTPN2* or a gene-regulating system capable of reducing the expression and/or function of *SOCS1* and *PTPN2*, wherein the immune effector cell is a TIL. In some embodiments, the present disclosure provides modified immune effector cells comprising reduced expression and/or function of *SOCS1* and *ZC3H12A* or a gene-regulating system capable of reducing the expression and/or function of *SOCS1* and *ZC3H12A*, wherein the immune effector cell is a TIL. In some embodiments, the present disclosure provides modified immune effector cells comprising reduced expression and/or function of *PTPN2* and *ZC3H12A* or a gene-regulating system capable of reducing the expression and/or function of *PTPN2* and *ZC3H12A*, wherein the immune effector cell is a TIL.

#### ***A. Effector functions***

**[00147]** In some embodiments, the modified immune effector cells described herein comprise reduced expression and/or function (or a gene-regulating system capable of reducing the expression and/or function) of two or more endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A* and demonstrate an increase in one or more immune cell effector functions. Herein, the term “effector function” refers to functions of an immune cell related to the generation, maintenance, and/or enhancement of an immune response against a target cell or target antigen. In some embodiments, the modified immune effector cells described herein demonstrate one or more of the following characteristics compared to an unmodified immune effector cell: increased infiltration or migration in to a tumor, increased proliferation, increased or prolonged cell viability, increased resistance to inhibitory factors in the surrounding microenvironment such that the activation state of the cell is prolonged or increased, increased production of pro-inflammatory immune factors (*e.g.*, pro-inflammatory cytokines, chemokines, and/or enzymes), increased cytotoxicity, and/or increased resistance to exhaustion.

**[00148]** In some embodiments, the modified immune effector cells described herein demonstrate increased infiltration into a tumor compared to an unmodified immune effector cell. In some embodiments, increased tumor infiltration by modified immune effector cells refers to an increase the number of modified immune effector cells infiltrating into a tumor during a given period

of time compared to the number of unmodified immune effector cells that infiltrate into a tumor during the same period of time. In some embodiments, the modified immune effector cells demonstrate a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, or more fold increase in tumor infiltration compared to an unmodified immune cell. Tumor infiltration can be measured by isolating one or more tumors from a subject and assessing the number of modified immune cells in the sample by flow cytometry, immunohistochemistry, and/or immunofluorescence.

**[00149]** In some embodiments, the modified immune effector cells described herein demonstrate an increase in cell proliferation compared to an unmodified immune effector cell. In these embodiments, the result is an increase in the number of modified immune effector cells present compared to unmodified immune effector cells after a given period of time. For example, in some embodiments, modified immune effector cells demonstrate increased rates of proliferation compared to unmodified immune effector cells, wherein the modified immune effector cells divide at a more rapid rate than unmodified immune effector cells. In some embodiments, the modified immune effector cells demonstrate a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, or more fold increase in the rate of proliferation compared to an unmodified immune cell. In some embodiments, modified immune effector cells demonstrate prolonged periods of proliferation compared to unmodified immune effector cells, wherein the modified immune effector cells and unmodified immune effector cells divide at similar rates, but wherein the modified immune effector cells maintain the proliferative state for a longer period of time. In some embodiments, the modified immune effector cells maintain a proliferative state for 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, or more times longer than an unmodified immune cell.

**[00150]** In some embodiments, the modified immune effector cells described herein demonstrate increased or prolonged cell viability compared to an unmodified immune effector cell. In such embodiments, the result is an increase in the number of modified immune effector cells or present compared to unmodified immune effector cells after a given period of time. For example, in some embodiments, modified immune effector cells described herein remain viable and persist for 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, or more times longer than an unmodified immune cell.

**[00151]** In some embodiments, the modified immune effector cells described herein demonstrate increased resistance to inhibitory factors compared to an unmodified immune effector

cell. Exemplary inhibitory factors include signaling by immune checkpoint molecules (*e.g.*, PD1, PDL1, CTLA4, LAG3, IDO) and/or inhibitory cytokines (*e.g.*, IL-10, TGF $\beta$ ).

**[00152]** In some embodiments, the modified T cells described herein demonstrate increased resistance to T cell exhaustion compared to an unmodified T cell. T cell exhaustion is a state of antigen-specific T cell dysfunction characterized by decreased effector function and leading to subsequent deletion of the antigen-specific T cells. In some embodiments, exhausted T cells lack the ability to proliferate in response to antigen, demonstrate decreased cytokine production, and/or demonstrate decreased cytotoxicity against target cells such as tumor cells. In some embodiments, exhausted T cells are identified by altered expression of cell surface markers and transcription factors, such as decreased cell surface expression of CD122 and CD127; increased expression of inhibitory cell surface markers such as PD1, LAG3, CD244, CD160, TIM3, and/or CTLA4; and/or increased expression of transcription factors such as Blimp1, NFAT, and/or BATF. In some embodiments, exhausted T cells demonstrate altered sensitivity cytokine signaling, such as increased sensitivity to TGF $\beta$  signaling and/or decreased sensitivity to IL-7 and IL-15 signaling. T cell exhaustion can be determined, for example, by co-culturing the T cells with a population of target cells and measuring T cell proliferation, cytokine production, and/or lysis of the target cells. In some embodiments, the modified immune effector cells described herein are co-cultured with a population of target cells (*e.g.*, autologous tumor cells or cell lines that have been engineered to express a target tumor antigen) and effector cell proliferation, cytokine production, and/or target cell lysis is measured. These results are then compared to the results obtained from co-culture of target cells with a control population of immune cells (such as unmodified immune effector cells or immune effector cells that have a control modification).

**[00153]** In some embodiments, resistance to T cell exhaustion is demonstrated by increased production of one or more cytokines (*e.g.*, IFN $\gamma$ , TNF $\alpha$ , or IL-2) from the modified immune effector cells compared to the cytokine production observed from the control population of immune cells. In some embodiments, a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5, 6, 7, 8, 9, 10, 15, 20, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100 or more fold increase in cytokine production from the modified immune effector cells compared to the cytokine production from the control population of immune cells is indicative of an increased resistance to T cell exhaustion. In some embodiments, resistance to T cell exhaustion is demonstrated by increased proliferation of the modified immune effector cells compared to the proliferation observed from the control population of immune cells. In some embodiments, a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5, 6, 7, 8, 9,

10, 15, 20, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100 or more fold increase in proliferation of the modified immune effector cells compared to the proliferation of the control population of immune cells is indicative of an increased resistance to T cell exhaustion. In some embodiments, resistance to T cell exhaustion is demonstrated by increased target cell lysis by the modified immune effector cells compared to the target cell lysis observed by the control population of immune cells. In some embodiments, a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5, 6, 7, 8, 9, 10, 15, 20, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100 or more fold increase in target cell lysis by the modified immune effector cells compared to the target cell lysis by the control population of immune cells is indicative of an increased resistance to T cell exhaustion.

**[00154]** In some embodiments, exhaustion of the modified immune effector cells compared to control populations of immune cells is measured during the *in vitro* or *ex vivo* manufacturing process. For example, in some embodiments, TILs isolated from tumor fragments are modified according to the methods described herein and then expanded in one or more rounds of expansion to produce a population of modified TILs. In such embodiments, the exhaustion of the modified TILs can be determined immediately after harvest and prior to a first round of expansion, after the first round of expansion but prior to a second round of expansion, and/or after the first and the second round of expansion. In some embodiments, exhaustion of the modified immune effector cells compared to control populations of immune cells is measured at one or more time points after transfer of the modified immune effector cells into a subject. For example, in some embodiments, the modified cells are produced according to the methods described herein and administered to a subject. Samples can then be taken from the subject at various time points after the transfer to determine exhaustion of the modified immune effector cells *in vivo* over time.

**[00155]** In some embodiments, the modified immune effector cells described herein demonstrate increased expression or production of pro-inflammatory immune factors compared to an unmodified immune effector cell. Examples of pro-inflammatory immune factors include cytolytic factors, such as granzyme B, perforin, and granulysin; and pro-inflammatory cytokines such as interferons (IFN $\alpha$ , IFN $\beta$ , IFN $\gamma$ ), TNF $\alpha$ , IL-1 $\beta$ , IL-12, IL-2, IL-17, CXCL8, and/or IL-6.

**[00156]** In some embodiments, the modified immune effector cells described herein demonstrate increased cytotoxicity against a target cell compared to an unmodified immune effector cell. In some embodiments, the modified immune effector cells demonstrate a 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, or more fold increase in cytotoxicity against a target cell compared to an unmodified immune cell.



In some embodiments, the modified immune effector cells described herein produce a TIL population that persists with both the central memory phenotype (T<sub>cm</sub> cells) and effector memory phenotype (T<sub>em</sub> cells). These phenotypes provide durable anti-tumor memory and invoke epitope spreading.

[00157] Assays for measuring immune effector function are known in the art. For example, tumor infiltration can be measured by isolating tumors from a subject and determining the total number and/or phenotype of the lymphocytes present in the tumor by flow cytometry, immunohistochemistry, and/or immunofluorescence. Cell-surface receptor expression can be determined by flow cytometry, immunohistochemistry, immunofluorescence, Western blot, and/or qPCR. Cytokine and chemokine expression and production can be measured by flow cytometry, immunohistochemistry, immunofluorescence, Western blot, ELISA, and/or qPCR. Responsiveness or sensitivity to extracellular stimuli (*e.g.*, cytokines, inhibitory ligands, or antigen) can be measured by assaying cellular proliferation and/or activation of downstream signaling pathways (*e.g.*, phosphorylation of downstream signaling intermediates) in response to the stimuli. Cytotoxicity can be measured by target-cell lysis assays known in the art, including *in vitro* or *ex vivo* co-culture of the modified immune effector cells with target cells and *in vivo* murine tumor models, such as those described throughout the Examples.

### ***B. Regulation of endogenous pathways and genes***

[00158] In some embodiments, the modified immune effector cells described herein demonstrate a reduced expression and/or function of two or more endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*. Further details on the endogenous target genes are provided below in Table 2. In such embodiments, the reduced expression or function of the two or more endogenous target genes enhances one or more effector functions of the immune cell.

[00159] In some embodiments, the modified effector cells described herein comprise reduced expression and/or function of the Suppressors of cytokine signaling SOCS 1 (*SOCS1*) gene. The SOCS1 protein comprises C-terminal SOCS box motifs, an SH2-domain, an ESS domain, and an N-terminal KIR domain. The 12 amino-acid residues called the kinase inhibitory region (KIR) has been found to be critical in the ability of SOCS1 to negatively regulate JAK1, TYK2 and JAK2 tyrosine kinase function.

[00160] In some embodiments, the modified effector cells described herein comprise reduced expression and/or function of the *PTPN2* gene. The protein tyrosine phosphatase family (PTP) dephosphorylate phospho-tyrosine residues by their phosphatase catalytic domain. PTPN2 functions

as a brake on both TCRs and cytokines, which signal through JAK/STAT signaling complexes, and thus serves as a checkpoint on both Signals 1 and 3. Following T Cell engagement with antigen and activation of the TCR, positive signals are amplified downstream by the kinases Lck and Fyn by phosphorylation of tyrosine residues. PTPN2 serves to dephosphorylate both Lck and Fyn and thus attenuate TCR signaling. In addition, following T cell encounter with cytokines and signaling through common  $\gamma$  chain receptor complex, which transmit positive signals through JAK/STAT signaling, PTPN2 also attenuates by dephosphorylation of STAT1 and STAT3. The sum functional impact of PTPN2 loss on T cell function is a lowering of the activation threshold needed for fulminant T cell activation through the TCR, and a hypersensitivity to growth and differentiation-enhancing cytokines. The protein tyrosine phosphatase family (PTP) dephosphorylate phospho-tyrosine residues by their phosphatase catalytic domain. PTPN2 functions as a brake on both TCRs and cytokines, which signal through JAK/STAT signaling complexes, and thus serves as a checkpoint on both Signals 1 and 3. Following T Cell engagement with antigen and activation of the TCR, positive signals are amplified downstream by the kinases Lck and Fyn by phosphorylation of tyrosine residues. PTPN2 serves to dephosphorylate both Lck and Fyn and thus attenuate TCR signaling. In addition, following T cell encounter with cytokines and signaling through common  $\gamma$ c chain receptor complex, which transmit positive signals through JAK/STAT signaling, PTPN2 also attenuates by dephosphorylation of STAT1 and STAT3. The sum functional impact of PTPN2 loss on T cell function is a lowering of the activation threshold needed for fulminant T cell activation through the TCR, and a hypersensitivity to growth and differentiation-enhancing cytokines.

**[00161]** In addition, in genetically engineered mouse (GEM) models, deletion of *PTPN2* in the whole mouse increases cytokine levels, lymphocytic infiltration in nonlymphoid tissues and early signs of rheumatoid arthritis-like symptoms; these mice do not survive past 5 weeks of age. Thus, *PTPN2* has been identified as critical for postnatal development in mice. Consistent with this autoimmune phenotype, deletion of *Ptpn2* in the T cell lineage from birth also results in an increase in lymphocytic infiltration in non-lymphoid tissues. Importantly, an inducible knockout of *Ptpn2* in adult mouse T cells did not result in any autoimmune manifestations of its role in autoimmunity, *Ptpn2* deletion was identified to associate with a small percentage of T cell acute lymphoblastic leukemia in humans (ALL); and to enhance skin tumor development in a two-stage chemically-induced carcinogenicity mouse model. These data have led some to postulate that PTPN2 may be a tumor suppressor protein.

**[00162]** In some embodiments, the modified effector cells described herein comprise reduced expression and/or function of the *ZC3H12A* gene. *ZC3H12A*, also referred to as MCPIP1 and REGNASE-1, is an RNase that possesses a RNase domain just upstream of a CCCH-type zinc-finger motif. Through its nuclease activity, *ZC3H12A* targets and destabilizes the mRNAs of transcripts, such as IL-6, by binding a conserved stem loop structure within the 3' UTR of these genes. In T cells, *ZC3H12A* controls the transcript levels of a number of pro-inflammatory genes, including *c-Rel*, *OX40* and *IL-2*. REGNASE-1 activation is transient and is subject to negative feedback mechanisms including proteasome-mediated degradation or mucosa-associated lymphoid tissue 1 (MALT1) mediated cleavage. The major function of REGNASE-1 is promoting mRNA decay via its ribonuclease activity by specifically targeting a subset of genes in different cell types. In monocytes, REGNASE-1 downregulates IL-6 and IL-12B mRNAs, thus mitigating inflammation, whereas in T cells, it restricts T-cell activation by targeting *c-Rel*, *Ox40* and *IL-2* transcripts. In cancer cells, REGNASE-1 promotes apoptosis by inhibiting anti-apoptotic genes including *BCL2L1*, *BCL2A1*, *RELB* and *BCL3*.

**Table 2: Endogenous target genes**

| Gene Symbol    | Gene Name                                         | Human UniProt Ref. | Human NCBI Ref          | Murine UniProt Ref. | Murine NCBI Ref            |
|----------------|---------------------------------------------------|--------------------|-------------------------|---------------------|----------------------------|
| <i>SOCS1</i>   | suppressor of cytokine signaling 1                | O15524             | 8651<br>(SEQ ID NO: 1)  | O35716              | 12703<br>(SEQ ID NO: 2)    |
| <i>PTPN2</i>   | protein tyrosine phosphatase, non-receptor type 2 | P17706             | 5771<br>(SEQ ID NO: 3)  | Q06180              | 19255<br>(SEQ ID NO: 4)    |
| <i>ZC3H12A</i> | Endoribonuclease <i>ZC3H12A</i>                   | Q5D1E8             | 80149<br>(SEQ ID NO: 5) | Q5D1E7              | 230738<br>(SEQ ID NO: 6)   |
| <i>CBLB</i>    | Cbl proto-oncogene B                              | Q13191             | 868<br>(SEQ ID NO: 823) | Q3TTA7              | 208650<br>(SEQ ID NO: 824) |

**[00163]** In some embodiments, the modified immune effector cells comprise reduced expression and/or function of *SOCS1* and reduced expression and/or function of *PTPN2*. In some embodiments, the modified immune effector cells comprise reduced expression and/or function of *SOCS1* and reduced expression and/or function of *ZC3H12A*. In some embodiments, the modified immune effector cells comprise reduced expression and/or function of *PTPN2* and reduced expression

and/or function of *ZC3H12A*. In some embodiments, the modified immune effector cells comprise reduced expression and/or function of at least two genes endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A* and further comprise reduced expression and/or function of *CBLB*.

### III. Gene-Regulating Systems

[00164] Herein, the term “gene-regulating system” refers to a protein, nucleic acid, or combination thereof that is capable of modifying an endogenous target DNA sequence when introduced into a cell, thereby regulating the expression or function of the encoded gene product. Numerous gene editing systems suitable for use in the methods of the present disclosure are known in the art including, but not limited to, shRNAs, siRNAs, zinc-finger nuclease systems, TALEN systems, and CRISPR/Cas systems.

[00165] As used herein, “regulate,” when used in reference to the effect of a gene-regulating system on an endogenous target gene encompasses any change in the sequence of the endogenous target gene, any change in the epigenetic state of the endogenous target gene, and/or any change in the expression or function of the protein encoded by the endogenous target gene.

[00166] In some embodiments, the gene-regulating system may mediate a change in the sequence of the endogenous target gene, for example, by introducing one or more mutations into the endogenous target sequence, such as by insertion or deletion of one or more nucleic acids in the endogenous target sequence. Exemplary mechanisms that can mediate alterations of the endogenous target sequence include, but are not limited to, non-homologous end joining (NHEJ) (*e.g.*, classical or alternative), microhomology-mediated end joining (MMEJ), homology-directed repair (*e.g.*, endogenous donor template mediated), SDSA (synthesis dependent strand annealing), single strand annealing or single strand invasion.

[00167] In some embodiments, the gene-regulating system may mediate a change in the epigenetic state of the endogenous target sequence. For example, in some embodiments, the gene-regulating system may mediate covalent modifications of the endogenous target gene DNA (*e.g.*, cytosine methylation and hydroxymethylation) or of associated histone proteins (*e.g.* lysine acetylation, lysine and arginine methylation, serine and threonine phosphorylation, and lysine ubiquitination and sumoylation).

[00168] In some embodiments, the gene-regulating system may mediate a change in the expression of the protein encoded by the endogenous target gene. In such embodiments, the gene-regulating system may regulate the expression of the encoded protein by modifications of the

endogenous target DNA sequence, or by acting on the mRNA product encoded by the DNA sequence. In some embodiments, the gene-regulating system may result in the expression of a modified endogenous protein. In such embodiments, the modifications to the endogenous DNA sequence mediated by the gene-regulating system result in the expression of an endogenous protein demonstrating a reduced function as compared to the corresponding endogenous protein in an unmodified immune effector cell. In such embodiments, the expression level of the modified endogenous protein may be increased, decreased or may be the same, or substantially similar to, the expression level of the corresponding endogenous protein in an unmodified immune cell.

#### ***A. Nucleic acid-based gene-regulating systems***

**[00169]** In some embodiments, the present disclosure provides nucleic acid gene-regulating systems comprising two or more nucleic acids capable of reducing the expression and/or function of at least two endogenous genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*. In some embodiments, the present disclosure provides modified immune effector cells comprising such gene-regulating systems. As used herein, a nucleic acid-based gene-regulating system is a system comprising one or more nucleic acid molecules that is capable of regulating the expression of an endogenous target gene without the requirement for an exogenous protein. In some embodiments, the nucleic acid-based gene-regulating system comprises an RNA interference molecule or antisense RNA molecule that is complementary to a target nucleic acid sequence.

**[00170]** An “antisense RNA molecule” refers to an RNA molecule, regardless of length, that is complementary to an mRNA transcript. Antisense RNA molecules refer to single stranded RNA molecules that can be introduced to a cell, tissue, or subject and result in decreased expression of an endogenous target gene product through mechanisms that do not rely on endogenous gene silencing pathways, but rather rely on RNaseH-mediated degradation of the target mRNA transcript. In some embodiments, an antisense nucleic acid comprises a modified backbone, for example, phosphorothioate, phosphorodithioate, or others known in the art, or may comprise non-natural internucleoside linkages. In some embodiments, an antisense nucleic acid can comprise locked nucleic acids (LNA).

**[00171]** “RNA interference molecule” as used herein refers to an RNA polynucleotide that mediates the decreased the expression of an endogenous target gene product by degradation of a target mRNA through endogenous gene silencing pathways (*e.g.*, Dicer and RNA-induced silencing complex (RISC)). Exemplary RNA interference agents include micro RNAs (also referred to herein

as “miRNAs”), short hair-pin RNAs (shRNAs), small interfering RNAs (siRNAs), RNA aptamers, and morpholinos.

**[00172]** In some embodiments, the nucleic acid-based gene-regulating system comprises one or more miRNAs. miRNAs are naturally occurring, small non-coding RNA molecules of about 21-25 nucleotides in length. miRNAs are at least partially complementary to one or more target mRNA molecules. miRNAs can downregulate (*e.g.*, decrease) expression of an endogenous target gene product through translational repression, cleavage of the mRNA, and/or deadenylation.

**[00173]** In some embodiments, the nucleic acid-based gene-regulating system comprises one or more shRNAs. shRNAs are single stranded RNA molecules of about 50-70 nucleotides in length that form stem-loop structures and result in degradation of complementary mRNA sequences. shRNAs can be cloned in plasmids or in non-replicating recombinant viral vectors to be introduced intracellularly and result in the integration of the shRNA-encoding sequence into the genome. As such, an shRNA can provide stable and consistent repression of endogenous target gene translation and expression.

**[00174]** In some embodiments, nucleic acid-based gene-regulating system comprises one or more siRNAs. siRNAs refer to double stranded RNA molecules typically about 21-23 nucleotides in length. The siRNA associates with a multi protein complex called the RNA-induced silencing complex (RISC), during which the “passenger” sense strand is enzymatically cleaved. The antisense “guide” strand contained in the activated RISC then guides the RISC to the corresponding mRNA because of sequence homology and the same nuclease cuts the target mRNA, resulting in specific gene silencing. Optimally, an siRNA is 18, 19, 20, 21, 22, 23 or 24 nucleotides in length and has a 2 base overhang at its 3' end. siRNAs can be introduced to an individual cell and/or culture system and result in the degradation of target mRNA sequences. siRNAs and shRNAs are further described in Fire *et al.*, Nature, 391:19, 1998 and US Patent Nos. 7,732,417; 8,202,846; and 8,383,599.

**[00175]** In some embodiments, the nucleic acid-based gene-regulating system comprises one or more morpholinos. “Morpholino” as used herein refers to a modified nucleic acid oligomer wherein standard nucleic acid bases are bound to morpholine rings and are linked through phosphorodiamidate linkages. Similar to siRNA and shRNA, morpholinos bind to complementary mRNA sequences. However, morpholinos function through steric-inhibition of mRNA translation and alteration of mRNA splicing rather than targeting complementary mRNA sequences for degradation.

**[00176]** In some embodiments, the nucleic acid-based gene-regulating system comprises a nucleic acid molecule (*e.g.*, an siRNA, an shRNA, an RNA aptamer, or a morpholino) that binds to a target RNA sequence that is at least 90% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Tables 3-8. Throughout this application, the referenced genomic coordinates are based on genomic annotations in the GRCh38 (also referred to as hg38) assembly of the human genome from the Genome Reference Consortium, available at the National Center for Biotechnology Information website. Tools and methods for converting genomic coordinates between one assembly and another are known in the art and can be used to convert the genomic coordinates provided herein to the corresponding coordinates in another assembly of the human genome, including conversion to an earlier assembly generated by the same institution or using the same algorithm (*e.g.*, from GRCh38 to GRCh37), and conversion an assembly generated by a different institution or algorithm (*e.g.*, from GRCh38 to NCBI33, generated by the International Human Genome Sequencing Consortium). Available methods and tools known in the art include, but are not limited to, NCBI Genome Remapping Service, available at the National Center for Biotechnology Information website, UCSC LiftOver, available at the UCSC Genome Brower website, and Assembly Converter, available at the Ensembl.org website.

**[00177]** In some embodiments, the nucleic acid-based gene-regulating system comprises at least two nucleic acid molecules (*e.g.*, an siRNA, an shRNA, an RNA aptamer, or a morpholino), wherein at least one nucleic acid molecule is a *SOCS1*-targeting nucleic acid molecule. In some embodiments, the at least one *SOCS1*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2). In some embodiments, the at least one *SOCS1*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2). In some embodiments, the least one *SOCS1*-targeting nucleic acid molecule is an siRNA or an shRNA molecule. In some embodiments, the at least one *SOCS1*-targeting siRNA or an shRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2). In some embodiments, the at least one *SOCS1*-targeting siRNA or an shRNA molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2).

**[00178]** In some embodiments, the at least one *SOCS1*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4. In some embodiments, the at least one *SOCS1*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4. In some embodiments, the at least one *SOCS1*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by one of SEQ ID NOs: 7-151. In some embodiments, the at least one *SOCS1*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by one of SEQ ID NOs: 7-151.

**[00179]** In some embodiments, the at least one *SOCS1*-targeting nucleic acid molecule is a *SOCS1*-targeting shRNA or siRNA molecule. In some embodiments, the at least one *SOCS1*-targeting shRNA or siRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4. In some embodiments, the at least one *SOCS1*-targeting shRNA or siRNA molecule binds to a target RNA sequence that is 100% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4. In some embodiments, the at least one *SOCS1*-targeting shRNA or siRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by one of SEQ ID NOs: 7-151. In some embodiments, the at least one *SOCS1*-targeting shRNA or siRNA molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by one of SEQ ID NOs: 7-151.

**[00180]** In some embodiments, the nucleic acid-based gene-regulating system comprises at least one *SOCS1*-targeting siRNA molecule or shRNA molecule selected from those known in the art. For example, in some embodiments, the *SOCS1*-targeting nucleic acid molecule is a *SOCS1*-targeting shRNA molecule that binds to a target sequence selected from SEQ ID NOs: 152-171 shown in Table A (See US Patent No. 9,944,931). In some embodiments, the *SOCS1*-targeting shRNA molecule is encoded by a nucleic acid sequence selected from SEQ ID NOs: 172-174 shown in Table A (See US Patent No. 8,324,369). In some embodiments, the *SOCS1*-targeting nucleic acid molecule is a *SOCS1*-targeting siRNA comprising a nucleic acid sequence selected from SEQ ID NOs: 175-184 shown in Table B (See International PCT Publication Nos. WO 2017120996; WO 2018137295; WO 2017120998; and WO 2018137293).



**Table 3: *SOCS1* Human Genome Coordinates**

| <b>Target</b> | <b>Coordinates</b>      | <b>Target</b> | <b>Coordinates</b>      |
|---------------|-------------------------|---------------|-------------------------|
| <i>SOCS1</i>  | chr16:11255187-11255206 | <i>SOCS1</i>  | chr16:11254923-11254942 |
| <i>SOCS1</i>  | chr16:11255238-11255257 | <i>SOCS1</i>  | chr16:11255431-11255450 |
| <i>SOCS1</i>  | chr16:11255058-11255077 | <i>SOCS1</i>  | chr16:11255463-11255482 |
| <i>SOCS1</i>  | chr16:11255158-11255177 | <i>SOCS1</i>  | chr16:11255343-11255362 |
| <i>SOCS1</i>  | chr16:11255239-11255258 | <i>SOCS1</i>  | chr16:11255088-11255107 |
| <i>SOCS1</i>  | chr16:11255237-11255256 | <i>SOCS1</i>  | chr16:11254834-11254853 |
| <i>SOCS1</i>  | chr16:11255019-11255038 | <i>SOCS1</i>  | chr16:11254922-11254941 |
| <i>SOCS1</i>  | chr16:11255066-11255085 | <i>SOCS1</i>  | chr16:11255098-11255117 |
| <i>SOCS1</i>  | chr16:11255238-11255257 | <i>SOCS1</i>  | chr16:11254993-11255012 |
| <i>SOCS1</i>  | chr16:11255168-11255187 | <i>SOCS1</i>  | chr16:11254840-11254859 |
| <i>SOCS1</i>  | chr16:11255079-11255098 | <i>SOCS1</i>  | chr16:11255400-11255419 |
| <i>SOCS1</i>  | chr16:11255287-11255306 | <i>SOCS1</i>  | chr16:11254920-11254939 |
| <i>SOCS1</i>  | chr16:11255249-11255268 | <i>SOCS1</i>  | chr16:11254966-11254985 |
| <i>SOCS1</i>  | chr16:11255186-11255205 | <i>SOCS1</i>  | chr16:11254860-11254879 |
| <i>SOCS1</i>  | chr16:11255236-11255255 | <i>SOCS1</i>  | chr16:11254980-11254999 |
| <i>SOCS1</i>  | chr16:11255116-11255135 | <i>SOCS1</i>  | chr16:11254857-11254876 |
| <i>SOCS1</i>  | chr16:11255070-11255089 | <i>SOCS1</i>  | chr16:11254874-11254893 |
| <i>SOCS1</i>  | chr16:11255117-11255136 | <i>SOCS1</i>  | chr16:11255028-11255047 |
| <i>SOCS1</i>  | chr16:11255283-11255302 | <i>SOCS1</i>  | chr16:11254956-11254975 |
| <i>SOCS1</i>  | chr16:11255442-11255461 | <i>SOCS1</i>  | chr16:11254908-11254927 |
| <i>SOCS1</i>  | chr16:11255209-11255228 | <i>SOCS1</i>  | chr16:11255337-11255356 |
| <i>SOCS1</i>  | chr16:11254932-11254951 | <i>SOCS1</i>  | chr16:11254836-11254855 |
| <i>SOCS1</i>  | chr16:11254966-11254985 | <i>SOCS1</i>  | chr16:11254842-11254861 |
| <i>SOCS1</i>  | chr16:11254950-11254969 | <i>SOCS1</i>  | chr16:11254865-11254884 |
| <i>SOCS1</i>  | chr16:11255049-11255068 | <i>SOCS1</i>  | chr16:11254830-11254849 |
| <i>SOCS1</i>  | chr16:11255155-11255174 | <i>SOCS1</i>  | chr16:11255401-11255420 |
| <i>SOCS1</i>  | chr16:11255460-11255479 | <i>SOCS1</i>  | chr16:11254864-11254883 |
| <i>SOCS1</i>  | chr16:11255037-11255056 | <i>SOCS1</i>  | chr16:11255311-11255330 |
| <i>SOCS1</i>  | chr16:11255154-11255173 | <i>SOCS1</i>  | chr16:11255343-11255362 |
| <i>SOCS1</i>  | chr16:11255115-11255134 | <i>SOCS1</i>  | chr16:11255342-11255361 |
| <i>SOCS1</i>  | chr16:11254985-11255004 | <i>SOCS1</i>  | chr16:11255272-11255291 |
| <i>SOCS1</i>  | chr16:11255013-11255032 | <i>SOCS1</i>  | chr16:11254866-11254885 |
| <i>SOCS1</i>  | chr16:11255016-11255035 | <i>SOCS1</i>  | chr16:11255310-11255329 |
| <i>SOCS1</i>  | chr16:11255139-11255158 | <i>SOCS1</i>  | chr16:11255336-11255355 |
| <i>SOCS1</i>  | chr16:11255248-11255267 | <i>SOCS1</i>  | chr16:11255416-11255435 |
| <i>SOCS1</i>  | chr16:11255217-11255236 | <i>SOCS1</i>  | chr16:11255402-11255421 |
| <i>SOCS1</i>  | chr16:11254994-11255013 | <i>SOCS1</i>  | chr16:11255467-11255486 |
| <i>SOCS1</i>  | chr16:11254965-11254984 | <i>SOCS1</i>  | chr16:11254873-11254892 |
| <i>SOCS1</i>  | chr16:11255219-11255238 | <i>SOCS1</i>  | chr16:11255265-11255284 |
| <i>SOCS1</i>  | chr16:11255173-11255192 | <i>SOCS1</i>  | chr16:11254820-11254839 |
| <i>SOCS1</i>  | chr16:11255210-11255229 | <i>SOCS1</i>  | chr16:11254848-11254867 |

| Target       | Coordinates             | Target       | Coordinates             |
|--------------|-------------------------|--------------|-------------------------|
| <i>SOCS1</i> | chr16:11255062-11255081 | <i>SOCS1</i> | chr16:11255317-11255336 |
| <i>SOCS1</i> | chr16:11255259-11255278 | <i>SOCS1</i> | chr16:11255351-11255370 |
| <i>SOCS1</i> | chr16:11255230-11255249 | <i>SOCS1</i> | chr16:11254811-11254830 |
| <i>SOCS1</i> | chr16:11255084-11255103 | <i>SOCS1</i> | chr16:11255353-11255372 |
| <i>SOCS1</i> | chr16:11255175-11255194 | <i>SOCS1</i> | chr16:11255350-11255369 |
| <i>SOCS1</i> | chr16:11255419-11255438 | <i>SOCS1</i> | chr16:11255309-11255328 |
| <i>SOCS1</i> | chr16:11254903-11254922 | <i>SOCS1</i> | chr16:11255390-11255409 |
| <i>SOCS1</i> | chr16:11255089-11255108 | <i>SOCS1</i> | chr16:11255478-11255497 |
| <i>SOCS1</i> | chr16:11255379-11255398 | <i>SOCS1</i> | chr16:11255330-11255349 |
| <i>SOCS1</i> | chr16:11255206-11255225 | <i>SOCS1</i> | chr16:11254875-11254894 |
| <i>SOCS1</i> | chr16:11255090-11255109 | <i>SOCS1</i> | chr16:11255124-11255143 |
| <i>SOCS1</i> | chr16:11255208-11255227 | <i>SOCS1</i> | chr16:11255352-11255371 |
| <i>SOCS1</i> | chr16:11254956-11254975 | <i>SOCS1</i> | chr16:11254872-11254891 |
| <i>SOCS1</i> | chr16:11255118-11255137 | <i>SOCS1</i> | chr16:11255331-11255350 |
| <i>SOCS1</i> | chr16:11254906-11254925 | <i>SOCS1</i> | chr16:11255315-11255334 |
| <i>SOCS1</i> | chr16:11255167-11255186 | <i>SOCS1</i> | chr16:11255482-11255501 |
| <i>SOCS1</i> | chr16:11254835-11254854 | <i>SOCS1</i> | chr16:11254995-11255014 |
| <i>SOCS1</i> | chr16:11255292-11255311 | <i>SOCS1</i> | chr16:11255316-11255335 |
| <i>SOCS1</i> | chr16:11255416-11255435 | <i>SOCS1</i> | chr16:11255308-11255327 |
| <i>SOCS1</i> | chr16:11255136-11255155 | <i>SOCS1</i> | chr16:11255321-11255340 |
| <i>SOCS1</i> | chr16:11254964-11254983 | <i>SOCS1</i> | chr16:11255322-11255341 |
| <i>SOCS1</i> | chr16:11254896-11254915 | <i>SOCS1</i> | chr16:11255330-11255349 |
| <i>SOCS1</i> | chr16:11254940-11254959 | <i>SOCS1</i> | chr16:11255368-11255387 |
| <i>SOCS1</i> | chr16:11255349-11255368 | <i>SOCS1</i> | chr16:11255377-11255396 |
| <i>SOCS1</i> | chr16:11254992-11255011 | <i>SOCS1</i> | chr16:11255380-11255399 |

**Table 4: *Socs1* Murine Genome Coordinates**

| Target       | Coordinates             |
|--------------|-------------------------|
| <i>Socs1</i> | chr16:10784479-10784498 |
| <i>Socs1</i> | chr16:10784409-10784428 |
| <i>Socs1</i> | chr16:10784456-10784475 |
| <i>Socs1</i> | chr16:10784322-10784341 |
| <i>Socs1</i> | chr16:10784548-10784567 |
| <i>Socs1</i> | chr16:10784596-10784615 |
| <i>Socs1</i> | chr16:10784264-10784283 |
| <i>Socs1</i> | chr16:10784628-10784647 |
| <i>Socs1</i> | chr16:10784526-10784545 |
| <i>Socs1</i> | chr16:10784508-10784527 |
| <i>Socs1</i> | chr16:10784565-10784584 |
| <i>Socs1</i> | chr16:10784474-10784493 |
| <i>Socs1</i> | chr16:10784293-10784312 |

**Table A: Exemplary shRNA Target Sequences**

| Sequence               | SEQ ID |
|------------------------|--------|
| TTTCGAGCTGCTGGAGCACTA  | 152    |
| TCGAGCTGCTGGAGCACTACG  | 153    |
| TCGCCAACGGAAGCTGCTTCTT | 154    |
| ACTTCTGGCTGGAGACCTCAT  | 155    |
| GCGAGACCTTCGACTGCCTTT  | 156    |
| CGACACTCACTTCCGCACCTT  | 157    |
| CTACCTGAGTTCCTTCCCCTT  | 158    |
| TTCCGCTCCCACTCCGATTAC  | 159    |
| TAACCCGGTACTCCGTGACTA  | 160    |
| TACTCCGTGACTACCTGAGTT  | 161    |
| CTTCCGCTCCCACTCCGATTA  | 162    |
| GCGCGACAGTCGCCAACGGAA  | 163    |
| TGGACGCCTGCGGCTTCTATT  | 164    |
| CGCATCCCTCTTAACCCGGTA  | 165    |
| TACATATTCCCAGTATCTTTG  | 166    |
| GCGCCTTATTATTTCTTATTA  | 167    |
| CCGTGACTACCTGAGTTCCTT  | 168    |
| GGAGGGTCTCTGGCTTCATTT  | 169    |
| TTCGCGCTCAGCGTGAAGATG  | 170    |
| ATCCCTCTTAACCCGGTACTC  | 171    |
| CACGCACTTCCGCACATTC    | 172    |
| TTCCGTTCGCACGCCGATT    | 173    |
| GAGCTTCGACTGCCTCTTC    | 174    |

**Table B: Exemplary siRNA Target Sequences**

| Sequence                | SEQ ID |
|-------------------------|--------|
| CGCACUUCGACACAUCUGUUCG  | 175    |
| GGGGAGGGUCUCUGGCUUUAUUU | 176    |
| CAGCAUUAACUGGGAUGCCGUGU | 177    |
| CCAGGACCUGAACUCGCACCUCC | 178    |
| UACAUAUACCCAGUAUCUUUGCA | 179    |
| GCCGACAAUGCAGUCUCCACAGC | 180    |
| CCCCUGGUUGUUGUAGCAGCUUA | 181    |
| CUGCUGUGCAGAAUCCUAUUUUA | 182    |
| UGGGAUGCCGUGUUAUUUUGUUA | 183    |
| UCGCACCUCCUACCUCUUAUGU  | 184    |

**[00181]** In some embodiments, the nucleic acid-based gene-regulating system comprises at least two nucleic acid molecules (*e.g.*, an siRNA, an shRNA, an RNA aptamer, or a morpholino),

wherein at least one nucleic acid molecule is a *PTPN2*-targeting nucleic acid molecule. In some embodiments, the at least one *PTPN2*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4). In some embodiments, the at least one *PTPN2*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4). In some embodiments, the at least one *PTPN2*-targeting nucleic acid molecule is an siRNA or an shRNA molecule. In some embodiments, the at least one *PTPN2*-targeting siRNA or an shRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4). In some embodiments, the at least one *PTPN2*-targeting siRNA or an shRNA molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4).

**[00182]** In some embodiments, the at least one *PTPN2*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6. In some embodiments, the at least one *PTPN2*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6. In some embodiments, the at least one *PTPN2*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by one of SEQ ID NOs: 185-207. In some embodiments, the at least one *PTPN2*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by one of SEQ ID NOs: 185-207.

**[00183]** In some embodiments, the at least one *PTPN2*-targeting nucleic acid molecule is a *SOCS1*-targeting shRNA or siRNA molecule. In some embodiments, the at least one *PTPN2*-targeting shRNA or siRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6. In some embodiments, the at least one *PTPN2*-targeting shRNA or siRNA molecule binds to a target RNA sequence that is 100% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6. In some embodiments, the at least one *PTPN2*-targeting shRNA or siRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded

by one of SEQ ID NOs: 185-207. In some embodiments, the at least one *PTPN2*-targeting shRNA or siRNA molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by one of SEQ ID NOs: 185-207.

**Table 5: *PTPN2* Human Genome Coordinates**

|              |                         |
|--------------|-------------------------|
| <i>PTPN2</i> | Chr18:12859218-12859237 |
| <i>PTPN2</i> | Chr18:12884109-12884128 |
| <i>PTPN2</i> | Chr18:12817227-12817246 |
| <i>PTPN2</i> | Chr18:12817234-12817253 |
| <i>PTPN2</i> | Chr18:12884091-12884110 |
| <i>PTPN2</i> | Chr18:12884121-12884140 |
| <i>PTPN2</i> | Chr18:12831010-12831029 |
| <i>PTPN2</i> | Chr18:12817208-12817227 |
| <i>PTPN2</i> | Chr18:12817158-12817177 |
| <i>PTPN2</i> | Chr18:12831016-12831035 |
| <i>PTPN2</i> | Chr18:12817228-12817247 |
| <i>PTPN2</i> | Chr18:12830964-12830983 |
| <i>PTPN2</i> | Chr18:12801972-12801991 |
| <i>PTPN2</i> | Chr18:12836818-12836837 |
| <i>PTPN2</i> | Chr18:12817215-12817234 |
| <i>PTPN2</i> | Chr18:12802018-12802037 |
| <i>PTPN2</i> | Chr18:12884116-12884135 |
| <i>PTPN2</i> | Chr18:12840739-12840758 |
| <i>PTPN2</i> | Chr18:12802004-12802023 |
| <i>PTPN2</i> | Chr18:12840767-12840786 |
| <i>PTPN2</i> | Chr18:12817197-12817216 |
| <i>PTPN2</i> | Chr18:12884108-12884127 |
| <i>PTPN2</i> | Chr18:12817221-12817240 |
| <i>PTPN2</i> | Chr18:12836820-12836839 |
| <i>PTPN2</i> | Chr18:12884124-12884143 |
| <i>PTPN2</i> | Chr18:12830996-12831015 |
| <i>PTPN2</i> | Chr18:12830942-12830961 |
| <i>PTPN2</i> | Chr18:12884112-12884131 |
| <i>PTPN2</i> | Chr18:12817193-12817212 |
| <i>PTPN2</i> | Chr18:12859205-12859224 |
| <i>PTPN2</i> | Chr18:12817202-12817221 |
| <i>PTPN2</i> | Chr18:12859216-12859235 |
| <i>PTPN2</i> | Chr18:12859215-12859234 |
| <i>PTPN2</i> | Chr18:12817201-12817220 |
| <i>PTPN2</i> | Chr18:12802134-12802153 |
| <i>PTPN2</i> | Chr18:12884075-12884094 |
| <i>PTPN2</i> | Chr18:12884115-12884134 |

|              |                         |
|--------------|-------------------------|
| <i>PTPN2</i> | Chr18:12840757-12840776 |
| <i>PTPN2</i> | Chr18:12814205-12814224 |
| <i>PTPN2</i> | Chr18:12840777-12840796 |
| <i>PTPN2</i> | Chr18:12814277-12814296 |
| <i>PTPN2</i> | Chr18:12840746-12840765 |
| <i>PTPN2</i> | Chr18:12801989-12802008 |
| <i>PTPN2</i> | Chr18:12819237-12819256 |
| <i>PTPN2</i> | Chr18:12814348-12814367 |
| <i>PTPN2</i> | Chr18:12794428-12794447 |
| <i>PTPN2</i> | Chr18:12831005-12831024 |
| <i>PTPN2</i> | Chr18:12825890-12825909 |
| <i>PTPN2</i> | Chr18:12840723-12840742 |
| <i>PTPN2</i> | Chr18:12840747-12840766 |
| <i>PTPN2</i> | Chr18:12802068-12802087 |
| <i>PTPN2</i> | Chr18:12840716-12840735 |
| <i>PTPN2</i> | Chr18:12840773-12840792 |
| <i>PTPN2</i> | Chr18:12831012-12831031 |
| <i>PTPN2</i> | Chr18:12814240-12814259 |
| <i>PTPN2</i> | Chr18:12802130-12802149 |
| <i>PTPN2</i> | Chr18:12794454-12794473 |
| <i>PTPN2</i> | Chr18:12817208-12817227 |
| <i>PTPN2</i> | Chr18:12819226-12819245 |
| <i>PTPN2</i> | Chr18:12825889-12825908 |
| <i>PTPN2</i> | Chr18:12840782-12840801 |
| <i>PTPN2</i> | Chr18:12836812-12836831 |
| <i>PTPN2</i> | Chr18:12817298-12817317 |
| <i>PTPN2</i> | Chr18:12817324-12817343 |
| <i>PTPN2</i> | Chr18:12819268-12819287 |
| <i>PTPN2</i> | Chr18:12817303-12817322 |
| <i>PTPN2</i> | Chr18:12825927-12825946 |
| <i>PTPN2</i> | Chr18:12817220-12817239 |
| <i>PTPN2</i> | Chr18:12825901-12825920 |
| <i>PTPN2</i> | Chr18:12814222-12814241 |
| <i>PTPN2</i> | Chr18:12831000-12831019 |
| <i>PTPN2</i> | Chr18:12840738-12840757 |
| <i>PTPN2</i> | Chr18:12802057-12802076 |
| <i>PTPN2</i> | Chr18:12802069-12802088 |
| <i>PTPN2</i> | Chr18:12884123-12884142 |
| <i>PTPN2</i> | Chr18:12814294-12814313 |
| <i>PTPN2</i> | Chr18:12817283-12817302 |
| <i>PTPN2</i> | Chr18:12830945-12830964 |
| <i>PTPN2</i> | Chr18:12817284-12817303 |
| <i>PTPN2</i> | Chr18:12817256-12817275 |

|              |                         |
|--------------|-------------------------|
| <i>PTPN2</i> | Chr18:12884062-12884081 |
| <i>PTPN2</i> | Chr18:12814295-12814314 |
| <i>PTPN2</i> | Chr18:12817313-12817332 |
| <i>PTPN2</i> | Chr18:12814255-12814274 |
| <i>PTPN2</i> | Chr18:12814253-12814272 |
| <i>PTPN2</i> | Chr18:12814257-12814276 |
| <i>PTPN2</i> | Chr18:12814256-12814275 |
| <i>PTPN2</i> | Chr18:12840753-12840772 |
| <i>PTPN2</i> | Chr18:12830957-12830976 |
| <i>PTPN2</i> | Chr18:12802093-12802112 |
| <i>PTPN2</i> | Chr18:12817333-12817352 |
| <i>PTPN2</i> | Chr18:12794479-12794498 |
| <i>PTPN2</i> | Chr18:12814223-12814242 |
| <i>PTPN2</i> | Chr18:12802089-12802108 |
| <i>PTPN2</i> | Chr18:12794463-12794482 |
| <i>PTPN2</i> | Chr18:12794436-12794455 |
| <i>PTPN2</i> | Chr18:12794416-12794435 |
| <i>PTPN2</i> | Chr18:12817235-12817254 |
| <i>PTPN2</i> | Chr18:12836793-12836812 |
| <i>PTPN2</i> | Chr18:12801986-12802005 |
| <i>PTPN2</i> | Chr18:12817165-12817184 |
| <i>PTPN2</i> | Chr18:12817179-12817198 |
| <i>PTPN2</i> | Chr18:12794425-12794444 |
| <i>PTPN2</i> | Chr18:12802146-12802165 |

**Table 6: *Ptpn2* Murine Genome Coordinates**

| <b>Target</b> | <b>Coordinates</b>      |
|---------------|-------------------------|
| <i>Ptpn2</i>  | Chr18:67680998-67681017 |
| <i>Ptpn2</i>  | Chr18:67677801-67677820 |
| <i>Ptpn2</i>  | Chr18:67680904-67680923 |
| <i>Ptpn2</i>  | Chr18:67681553-67681572 |
| <i>Ptpn2</i>  | Chr18:67688965-67688984 |
| <i>Ptpn2</i>  | Chr18:67680958-67680977 |
| <i>Ptpn2</i>  | Chr18:67688944-67688963 |
| <i>Ptpn2</i>  | Chr18:67677855-67677874 |
| <i>Ptpn2</i>  | Chr18:67677734-67677753 |
| <i>Ptpn2</i>  | Chr18:67680967-67680986 |
| <i>Ptpn2</i>  | Chr18:67688912-67688931 |
| <i>Ptpn2</i>  | Chr18:67680881-67680900 |
| <i>Ptpn2</i>  | Chr18:67681529-67681548 |

**[00184]** In some embodiments, the nucleic acid-based gene-regulating system comprises at least two nucleic acid molecules (*e.g.*, an siRNA, an shRNA, an RNA aptamer, or a morpholino), wherein at least one nucleic acid molecule is a *ZC3H12A*-targeting nucleic acid molecule. In some embodiments, the at least one *ZC3H12A*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6). In some embodiments, the at least one *ZC3H12A*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6). In some embodiments, the at least one *ZC3H12A*-targeting nucleic acid molecule is an siRNA or an shRNA molecule. In some embodiments, the at least one *ZC3H12A*-targeting siRNA or an shRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6). In some embodiments, the at least one *ZC3H12A*-targeting siRNA or an shRNA molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6).

**[00185]** In some embodiments, the at least one *ZC3H12A*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8. In some embodiments, the at least one *ZC3H12A*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8. In some embodiments, the at least one *ZC3H12A*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by one of SEQ ID NOs: 208-230. In some embodiments, the at least one *ZC3H12A*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by one of SEQ ID NOs: 208-230.

**[00186]** In some embodiments, the at least one *ZC3H12A*-targeting nucleic acid molecule is a *SOCS1*-targeting shRNA or siRNA molecule. In some embodiments, the at least one *ZC3H12A*-targeting shRNA or siRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8. In some embodiments, the at least one *ZC3H12A*-targeting shRNA or siRNA molecule binds to a target RNA sequence that is 100% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8. In



some embodiments, the at least one *ZC3H12A*-targeting shRNA or siRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by one of SEQ ID NOs: 208-230. In some embodiments, the at least one *ZC3H12A*-targeting shRNA or siRNA molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by one of SEQ ID NOs: 208-230. In some embodiments, the *ZC3H12A*-targeting nucleic acid molecule is a *ZC3H12A*-targeting shRNA molecule encoded by a nucleic acid sequence selected from SEQ ID NOs: 234-237 (See Huang *et al.*, J Biol Chem (2015) 290(34), 20782-20792). In some embodiments, the *ZC3H12A*-targeting nucleic acid molecule is a *ZC3H12A*-targeting siRNA comprising a nucleic acid sequence selected from SEQ ID NOs: 231-233 (See Liu *et al.*, Scientific Reports (2016), 6, Article # 24073 and Mino *et al.*, Cell (2015) 161(5), 1058-1073).

**Table 7: *ZC3H12A* Human Genome Coordinates**

| Target         | Coordinates            |
|----------------|------------------------|
| <i>ZC3H12A</i> | Chr1:37481708-37481727 |
| <i>ZC3H12A</i> | Chr1:37475808-37475827 |
| <i>ZC3H12A</i> | Chr1:37475809-37475828 |
| <i>ZC3H12A</i> | Chr1:37475684-37475703 |
| <i>ZC3H12A</i> | Chr1:37481823-37481842 |
| <i>ZC3H12A</i> | Chr1:37480415-37480434 |
| <i>ZC3H12A</i> | Chr1:37475756-37475775 |
| <i>ZC3H12A</i> | Chr1:37481692-37481711 |
| <i>ZC3H12A</i> | Chr1:37481648-37481667 |
| <i>ZC3H12A</i> | Chr1:37480284-37480303 |
| <i>ZC3H12A</i> | Chr1:37481779-37481798 |
| <i>ZC3H12A</i> | Chr1:37475827-37475846 |
| <i>ZC3H12A</i> | Chr1:37481747-37481766 |
| <i>ZC3H12A</i> | Chr1:37482445-37482464 |
| <i>ZC3H12A</i> | Chr1:37475631-37475650 |
| <i>ZC3H12A</i> | Chr1:37480274-37480293 |
| <i>ZC3H12A</i> | Chr1:37482967-37482986 |
| <i>ZC3H12A</i> | Chr1:37482922-37482941 |
| <i>ZC3H12A</i> | Chr1:37480273-37480292 |
| <i>ZC3H12A</i> | Chr1:37482886-37482905 |
| <i>ZC3H12A</i> | Chr1:37483185-37483204 |
| <i>ZC3H12A</i> | Chr1:37475817-37475836 |
| <i>ZC3H12A</i> | Chr1:37483033-37483052 |
| <i>ZC3H12A</i> | Chr1:37480408-37480427 |
| <i>ZC3H12A</i> | Chr1:37483026-37483045 |

| Target         | Coordinates            |
|----------------|------------------------|
| <i>ZC3H12A</i> | Chr1:37483463-37483482 |
| <i>ZC3H12A</i> | Chr1:37480362-37480381 |
| <i>ZC3H12A</i> | Chr1:37482962-37482981 |
| <i>ZC3H12A</i> | Chr1:37475775-37475794 |
| <i>ZC3H12A</i> | Chr1:37475509-37475528 |
| <i>ZC3H12A</i> | Chr1:37475722-37475741 |
| <i>ZC3H12A</i> | Chr1:37475818-37475837 |
| <i>ZC3H12A</i> | Chr1:37482966-37482985 |
| <i>ZC3H12A</i> | Chr1:37480388-37480407 |
| <i>ZC3H12A</i> | Chr1:37483142-37483161 |
| <i>ZC3H12A</i> | Chr1:37482448-37482467 |
| <i>ZC3H12A</i> | Chr1:37483049-37483068 |
| <i>ZC3H12A</i> | Chr1:37482905-37482924 |
| <i>ZC3H12A</i> | Chr1:37482733-37482752 |
| <i>ZC3H12A</i> | Chr1:37480423-37480442 |
| <i>ZC3H12A</i> | Chr1:37482456-37482475 |
| <i>ZC3H12A</i> | Chr1:37483551-37483570 |
| <i>ZC3H12A</i> | Chr1:37481767-37481786 |
| <i>ZC3H12A</i> | Chr1:37475715-37475734 |
| <i>ZC3H12A</i> | Chr1:37483377-37483396 |
| <i>ZC3H12A</i> | Chr1:37475593-37475612 |
| <i>ZC3H12A</i> | Chr1:37475875-37475894 |
| <i>ZC3H12A</i> | Chr1:37475534-37475553 |
| <i>ZC3H12A</i> | Chr1:37482764-37482783 |
| <i>ZC3H12A</i> | Chr1:37475869-37475888 |
| <i>ZC3H12A</i> | Chr1:37483437-37483456 |
| <i>ZC3H12A</i> | Chr1:37475598-37475617 |
| <i>ZC3H12A</i> | Chr1:37482438-37482457 |
| <i>ZC3H12A</i> | Chr1:37483257-37483276 |
| <i>ZC3H12A</i> | Chr1:37483263-37483282 |
| <i>ZC3H12A</i> | Chr1:37482545-37482564 |
| <i>ZC3H12A</i> | Chr1:37483015-37483034 |
| <i>ZC3H12A</i> | Chr1:37481595-37481614 |
| <i>ZC3H12A</i> | Chr1:37482923-37482942 |
| <i>ZC3H12A</i> | Chr1:37483143-37483162 |
| <i>ZC3H12A</i> | Chr1:37482348-37482367 |
| <i>ZC3H12A</i> | Chr1:37483018-37483037 |
| <i>ZC3H12A</i> | Chr1:37482612-37482631 |
| <i>ZC3H12A</i> | Chr1:37475613-37475632 |
| <i>ZC3H12A</i> | Chr1:37475563-37475582 |
| <i>ZC3H12A</i> | Chr1:37475535-37475554 |
| <i>ZC3H12A</i> | Chr1:37482843-37482862 |

| Target         | Coordinates            |
|----------------|------------------------|
| <i>ZC3H12A</i> | Chr1:37480424-37480443 |
| <i>ZC3H12A</i> | Chr1:37482606-37482625 |
| <i>ZC3H12A</i> | Chr1:37483098-37483117 |
| <i>ZC3H12A</i> | Chr1:37483508-37483527 |
| <i>ZC3H12A</i> | Chr1:37483559-37483578 |
| <i>ZC3H12A</i> | Chr1:37483256-37483275 |
| <i>ZC3H12A</i> | Chr1:37475936-37475955 |
| <i>ZC3H12A</i> | Chr1:37475607-37475626 |
| <i>ZC3H12A</i> | Chr1:37475809-37475828 |
| <i>ZC3H12A</i> | Chr1:37483186-37483205 |
| <i>ZC3H12A</i> | Chr1:37481747-37481766 |
| <i>ZC3H12A</i> | Chr1:37482734-37482753 |
| <i>ZC3H12A</i> | Chr1:37483278-37483297 |
| <i>ZC3H12A</i> | Chr1:37482332-37482351 |
| <i>ZC3H12A</i> | Chr1:37483109-37483128 |
| <i>ZC3H12A</i> | Chr1:37475633-37475652 |
| <i>ZC3H12A</i> | Chr1:37482591-37482610 |
| <i>ZC3H12A</i> | Chr1:37483271-37483290 |
| <i>ZC3H12A</i> | Chr1:37483603-37483622 |
| <i>ZC3H12A</i> | Chr1:37482504-37482523 |
| <i>ZC3H12A</i> | Chr1:37483252-37483271 |
| <i>ZC3H12A</i> | Chr1:37483119-37483138 |
| <i>ZC3H12A</i> | Chr1:37482343-37482362 |
| <i>ZC3H12A</i> | Chr1:37483144-37483163 |
| <i>ZC3H12A</i> | Chr1:37483213-37483232 |
| <i>ZC3H12A</i> | Chr1:37482981-37483000 |
| <i>ZC3H12A</i> | Chr1:37482789-37482808 |
| <i>ZC3H12A</i> | Chr1:37483159-37483178 |
| <i>ZC3H12A</i> | Chr1:37482349-37482368 |
| <i>ZC3H12A</i> | Chr1:37483602-37483621 |
| <i>ZC3H12A</i> | Chr1:37481596-37481615 |
| <i>ZC3H12A</i> | Chr1:37482537-37482556 |
| <i>ZC3H12A</i> | Chr1:37482370-37482389 |
| <i>ZC3H12A</i> | Chr1:37475546-37475565 |
| <i>ZC3H12A</i> | Chr1:37482598-37482617 |
| <i>ZC3H12A</i> | Chr1:37483146-37483165 |
| <i>ZC3H12A</i> | Chr1:37475812-37475831 |
| <i>ZC3H12A</i> | Chr1:37483400-37483419 |
| <i>ZC3H12A</i> | Chr1:37475703-37475722 |
| <i>ZC3H12A</i> | Chr1:37483418-37483437 |
| <i>ZC3H12A</i> | Chr1:37480284-37480303 |
| <i>ZC3H12A</i> | Chr1:37482800-37482819 |

| Target         | Coordinates            |
|----------------|------------------------|
| <i>ZC3H12A</i> | Chr1:37475721-37475740 |
| <i>ZC3H12A</i> | Chr1:37482715-37482734 |
| <i>ZC3H12A</i> | Chr1:37480281-37480300 |
| <i>ZC3H12A</i> | Chr1:37482491-37482510 |
| <i>ZC3H12A</i> | Chr1:37483497-37483516 |
| <i>ZC3H12A</i> | Chr1:37475899-37475918 |
| <i>ZC3H12A</i> | Chr1:37475889-37475908 |
| <i>ZC3H12A</i> | Chr1:37482375-37482394 |
| <i>ZC3H12A</i> | Chr1:37475741-37475760 |
| <i>ZC3H12A</i> | Chr1:37482900-37482919 |
| <i>ZC3H12A</i> | Chr1:37482442-37482461 |
| <i>ZC3H12A</i> | Chr1:37481644-37481663 |
| <i>ZC3H12A</i> | Chr1:37482464-37482483 |
| <i>ZC3H12A</i> | Chr1:37482994-37483013 |
| <i>ZC3H12A</i> | Chr1:37483437-37483456 |
| <i>ZC3H12A</i> | Chr1:37482736-37482755 |
| <i>ZC3H12A</i> | Chr1:37482538-37482557 |
| <i>ZC3H12A</i> | Chr1:37483515-37483534 |
| <i>ZC3H12A</i> | Chr1:37475874-37475893 |
| <i>ZC3H12A</i> | Chr1:37483145-37483164 |
| <i>ZC3H12A</i> | Chr1:37482587-37482606 |
| <i>ZC3H12A</i> | Chr1:37475482-37475501 |
| <i>ZC3H12A</i> | Chr1:37475844-37475863 |
| <i>ZC3H12A</i> | Chr1:37480415-37480434 |
| <i>ZC3H12A</i> | Chr1:37481709-37481728 |
| <i>ZC3H12A</i> | Chr1:37483366-37483385 |
| <i>ZC3H12A</i> | Chr1:37475627-37475646 |
| <i>ZC3H12A</i> | Chr1:37482447-37482466 |
| <i>ZC3H12A</i> | Chr1:37481758-37481777 |
| <i>ZC3H12A</i> | Chr1:37483560-37483579 |
| <i>ZC3H12A</i> | Chr1:37475869-37475888 |
| <i>ZC3H12A</i> | Chr1:37481655-37481674 |
| <i>ZC3H12A</i> | Chr1:37481645-37481664 |
| <i>ZC3H12A</i> | Chr1:37483016-37483035 |
| <i>ZC3H12A</i> | Chr1:37475838-37475857 |
| <i>ZC3H12A</i> | Chr1:37482850-37482869 |
| <i>ZC3H12A</i> | Chr1:37475510-37475529 |
| <i>ZC3H12A</i> | Chr1:37483510-37483529 |
| <i>ZC3H12A</i> | Chr1:37483064-37483083 |
| <i>ZC3H12A</i> | Chr1:37483149-37483168 |
| <i>ZC3H12A</i> | Chr1:37483449-37483468 |
| <i>ZC3H12A</i> | Chr1:37483264-37483283 |

| Target         | Coordinates            |
|----------------|------------------------|
| <i>ZC3H12A</i> | Chr1:37475508-37475527 |
| <i>ZC3H12A</i> | Chr1:37480415-37480434 |
| <i>ZC3H12A</i> | Chr1:37482918-37482937 |
| <i>ZC3H12A</i> | Chr1:37482474-37482493 |
| <i>ZC3H12A</i> | Chr1:37483232-37483251 |
| <i>ZC3H12A</i> | Chr1:37475732-37475751 |
| <i>ZC3H12A</i> | Chr1:37481602-37481621 |
| <i>ZC3H12A</i> | Chr1:37480289-37480308 |
| <i>ZC3H12A</i> | Chr1:37483165-37483184 |
| <i>ZC3H12A</i> | Chr1:37483248-37483267 |
| <i>ZC3H12A</i> | Chr1:37483078-37483097 |
| <i>ZC3H12A</i> | Chr1:37483017-37483036 |
| <i>ZC3H12A</i> | Chr1:37483174-37483193 |
| <i>ZC3H12A</i> | Chr1:37482857-37482876 |
| <i>ZC3H12A</i> | Chr1:37475578-37475597 |
| <i>ZC3H12A</i> | Chr1:37480329-37480348 |
| <i>ZC3H12A</i> | Chr1:37480288-37480307 |
| <i>ZC3H12A</i> | Chr1:37481600-37481619 |
| <i>ZC3H12A</i> | Chr1:37483212-37483231 |
| <i>ZC3H12A</i> | Chr1:37483337-37483356 |
| <i>ZC3H12A</i> | Chr1:37475542-37475561 |
| <i>ZC3H12A</i> | Chr1:37483197-37483216 |
| <i>ZC3H12A</i> | Chr1:37482730-37482749 |
| <i>ZC3H12A</i> | Chr1:37475599-37475618 |
| <i>ZC3H12A</i> | Chr1:37483262-37483281 |
| <i>ZC3H12A</i> | Chr1:37482790-37482809 |
| <i>ZC3H12A</i> | Chr1:37482719-37482738 |
| <i>ZC3H12A</i> | Chr1:37482860-37482879 |
| <i>ZC3H12A</i> | Chr1:37483443-37483462 |
| <i>ZC3H12A</i> | Chr1:37483558-37483577 |
| <i>ZC3H12A</i> | Chr1:37481599-37481618 |
| <i>ZC3H12A</i> | Chr1:37475845-37475864 |
| <i>ZC3H12A</i> | Chr1:37475730-37475749 |
| <i>ZC3H12A</i> | Chr1:37482524-37482543 |
| <i>ZC3H12A</i> | Chr1:37482849-37482868 |
| <i>ZC3H12A</i> | Chr1:37475529-37475548 |
| <i>ZC3H12A</i> | Chr1:37475664-37475683 |
| <i>ZC3H12A</i> | Chr1:37482972-37482991 |
| <i>ZC3H12A</i> | Chr1:37483321-37483340 |
| <i>ZC3H12A</i> | Chr1:37482984-37483003 |
| <i>ZC3H12A</i> | Chr1:37475807-37475826 |
| <i>ZC3H12A</i> | Chr1:37483213-37483232 |

| Target         | Coordinates            |
|----------------|------------------------|
| <i>ZC3H12A</i> | Chr1:37482427-37482446 |
| <i>ZC3H12A</i> | Chr1:37483104-37483123 |
| <i>ZC3H12A</i> | Chr1:37482879-37482898 |
| <i>ZC3H12A</i> | Chr1:37483409-37483428 |
| <i>ZC3H12A</i> | Chr1:37482752-37482771 |
| <i>ZC3H12A</i> | Chr1:37480391-37480410 |
| <i>ZC3H12A</i> | Chr1:37475694-37475713 |
| <i>ZC3H12A</i> | Chr1:37482458-37482477 |
| <i>ZC3H12A</i> | Chr1:37475774-37475793 |
| <i>ZC3H12A</i> | Chr1:37475574-37475593 |
| <i>ZC3H12A</i> | Chr1:37475803-37475822 |
| <i>ZC3H12A</i> | Chr1:37481605-37481624 |
| <i>ZC3H12A</i> | Chr1:37482437-37482456 |
| <i>ZC3H12A</i> | Chr1:37482825-37482844 |
| <i>ZC3H12A</i> | Chr1:37483595-37483614 |
| <i>ZC3H12A</i> | Chr1:37483510-37483529 |
| <i>ZC3H12A</i> | Chr1:37483283-37483302 |
| <i>ZC3H12A</i> | Chr1:37482446-37482465 |
| <i>ZC3H12A</i> | Chr1:37475700-37475719 |
| <i>ZC3H12A</i> | Chr1:37475721-37475740 |
| <i>ZC3H12A</i> | Chr1:37475628-37475647 |
| <i>ZC3H12A</i> | Chr1:37482848-37482867 |
| <i>ZC3H12A</i> | Chr1:37483134-37483153 |
| <i>ZC3H12A</i> | Chr1:37475543-37475562 |
| <i>ZC3H12A</i> | Chr1:37482799-37482818 |
| <i>ZC3H12A</i> | Chr1:37483296-37483315 |
| <i>ZC3H12A</i> | Chr1:37483332-37483351 |
| <i>ZC3H12A</i> | Chr1:37483600-37483619 |
| <i>ZC3H12A</i> | Chr1:37482410-37482429 |
| <i>ZC3H12A</i> | Chr1:37481718-37481737 |
| <i>ZC3H12A</i> | Chr1:37483395-37483414 |
| <i>ZC3H12A</i> | Chr1:37482428-37482447 |
| <i>ZC3H12A</i> | Chr1:37475562-37475581 |
| <i>ZC3H12A</i> | Chr1:37483500-37483519 |
| <i>ZC3H12A</i> | Chr1:37475827-37475846 |
| <i>ZC3H12A</i> | Chr1:37483586-37483605 |
| <i>ZC3H12A</i> | Chr1:37483089-37483108 |
| <i>ZC3H12A</i> | Chr1:37483419-37483438 |
| <i>ZC3H12A</i> | Chr1:37480285-37480304 |
| <i>ZC3H12A</i> | Chr1:37483256-37483275 |
| <i>ZC3H12A</i> | Chr1:37483420-37483439 |
| <i>ZC3H12A</i> | Chr1:37475691-37475710 |

| Target         | Coordinates            |
|----------------|------------------------|
| <i>ZC3H12A</i> | Chr1:37483419-37483438 |
| <i>ZC3H12A</i> | Chr1:37475918-37475937 |
| <i>ZC3H12A</i> | Chr1:37475589-37475608 |
| <i>ZC3H12A</i> | Chr1:37482362-37482381 |
| <i>ZC3H12A</i> | Chr1:37482566-37482585 |
| <i>ZC3H12A</i> | Chr1:37482963-37482982 |
| <i>ZC3H12A</i> | Chr1:37483420-37483439 |
| <i>ZC3H12A</i> | Chr1:37483139-37483158 |
| <i>ZC3H12A</i> | Chr1:37483619-37483638 |
| <i>ZC3H12A</i> | Chr1:37481764-37481783 |
| <i>ZC3H12A</i> | Chr1:37475650-37475669 |
| <i>ZC3H12A</i> | Chr1:37483405-37483424 |
| <i>ZC3H12A</i> | Chr1:37483037-37483056 |
| <i>ZC3H12A</i> | Chr1:37483211-37483230 |
| <i>ZC3H12A</i> | Chr1:37475537-37475556 |
| <i>ZC3H12A</i> | Chr1:37475756-37475775 |
| <i>ZC3H12A</i> | Chr1:37482403-37482422 |
| <i>ZC3H12A</i> | Chr1:37482455-37482474 |
| <i>ZC3H12A</i> | Chr1:37480311-37480330 |
| <i>ZC3H12A</i> | Chr1:37482586-37482605 |
| <i>ZC3H12A</i> | Chr1:37483099-37483118 |
| <i>ZC3H12A</i> | Chr1:37483342-37483361 |
| <i>ZC3H12A</i> | Chr1:37481823-37481842 |
| <i>ZC3H12A</i> | Chr1:37482777-37482796 |
| <i>ZC3H12A</i> | Chr1:37482412-37482431 |
| <i>ZC3H12A</i> | Chr1:37483604-37483623 |
| <i>ZC3H12A</i> | Chr1:37483438-37483457 |
| <i>ZC3H12A</i> | Chr1:37482445-37482464 |
| <i>ZC3H12A</i> | Chr1:37483331-37483350 |
| <i>ZC3H12A</i> | Chr1:37483111-37483130 |
| <i>ZC3H12A</i> | Chr1:37482847-37482866 |
| <i>ZC3H12A</i> | Chr1:37483249-37483268 |
| <i>ZC3H12A</i> | Chr1:37481754-37481773 |
| <i>ZC3H12A</i> | Chr1:37475684-37475703 |
| <i>ZC3H12A</i> | Chr1:37482519-37482538 |
| <i>ZC3H12A</i> | Chr1:37482475-37482494 |
| <i>ZC3H12A</i> | Chr1:37482613-37482632 |
| <i>ZC3H12A</i> | Chr1:37482939-37482958 |
| <i>ZC3H12A</i> | Chr1:37475541-37475560 |
| <i>ZC3H12A</i> | Chr1:37481763-37481782 |
| <i>ZC3H12A</i> | Chr1:37483231-37483250 |
| <i>ZC3H12A</i> | Chr1:37482953-37482972 |

| Target         | Coordinates            |
|----------------|------------------------|
| <i>ZC3H12A</i> | Chr1:37482407-37482426 |
| <i>ZC3H12A</i> | Chr1:37475808-37475827 |
| <i>ZC3H12A</i> | Chr1:37481620-37481639 |
| <i>ZC3H12A</i> | Chr1:37475592-37475611 |
| <i>ZC3H12A</i> | Chr1:37483156-37483175 |
| <i>ZC3H12A</i> | Chr1:37480329-37480348 |
| <i>ZC3H12A</i> | Chr1:37475573-37475592 |
| <i>ZC3H12A</i> | Chr1:37483198-37483217 |
| <i>ZC3H12A</i> | Chr1:37483557-37483576 |
| <i>ZC3H12A</i> | Chr1:37482892-37482911 |
| <i>ZC3H12A</i> | Chr1:37483334-37483353 |
| <i>ZC3H12A</i> | Chr1:37481708-37481727 |
| <i>ZC3H12A</i> | Chr1:37483063-37483082 |
| <i>ZC3H12A</i> | Chr1:37482998-37483017 |
| <i>ZC3H12A</i> | Chr1:37482942-37482961 |
| <i>ZC3H12A</i> | Chr1:37475508-37475527 |
| <i>ZC3H12A</i> | Chr1:37482371-37482390 |
| <i>ZC3H12A</i> | Chr1:37483119-37483138 |
| <i>ZC3H12A</i> | Chr1:37482798-37482817 |
| <i>ZC3H12A</i> | Chr1:37475859-37475878 |
| <i>ZC3H12A</i> | Chr1:37483401-37483420 |
| <i>ZC3H12A</i> | Chr1:37482851-37482870 |
| <i>ZC3H12A</i> | Chr1:37475524-37475543 |
| <i>ZC3H12A</i> | Chr1:37475601-37475620 |
| <i>ZC3H12A</i> | Chr1:37475815-37475834 |
| <i>ZC3H12A</i> | Chr1:37482801-37482820 |
| <i>ZC3H12A</i> | Chr1:37475544-37475563 |
| <i>ZC3H12A</i> | Chr1:37483010-37483029 |
| <i>ZC3H12A</i> | Chr1:37483077-37483096 |
| <i>ZC3H12A</i> | Chr1:37482404-37482423 |
| <i>ZC3H12A</i> | Chr1:37475692-37475711 |
| <i>ZC3H12A</i> | Chr1:37483596-37483615 |
| <i>ZC3H12A</i> | Chr1:37483372-37483391 |
| <i>ZC3H12A</i> | Chr1:37481596-37481615 |
| <i>ZC3H12A</i> | Chr1:37480370-37480389 |
| <i>ZC3H12A</i> | Chr1:37480377-37480396 |
| <i>ZC3H12A</i> | Chr1:37483381-37483400 |
| <i>ZC3H12A</i> | Chr1:37482899-37482918 |
| <i>ZC3H12A</i> | Chr1:37480373-37480392 |
| <i>ZC3H12A</i> | Chr1:37481847-37481866 |
| <i>ZC3H12A</i> | Chr1:37483330-37483349 |
| <i>ZC3H12A</i> | Chr1:37483065-37483084 |



| Target         | Coordinates            |
|----------------|------------------------|
| <i>ZC3H12A</i> | Chr1:37482499-37482518 |
| <i>ZC3H12A</i> | Chr1:37483105-37483124 |
| <i>ZC3H12A</i> | Chr1:37475631-37475650 |
| <i>ZC3H12A</i> | Chr1:37483530-37483549 |
| <i>ZC3H12A</i> | Chr1:37483407-37483426 |
| <i>ZC3H12A</i> | Chr1:37483308-37483327 |
| <i>ZC3H12A</i> | Chr1:37482853-37482872 |
| <i>ZC3H12A</i> | Chr1:37482934-37482953 |
| <i>ZC3H12A</i> | Chr1:37475591-37475610 |
| <i>ZC3H12A</i> | Chr1:37475826-37475845 |
| <i>ZC3H12A</i> | Chr1:37475865-37475884 |
| <i>ZC3H12A</i> | Chr1:37481784-37481803 |
| <i>ZC3H12A</i> | Chr1:37480322-37480341 |
| <i>ZC3H12A</i> | Chr1:37475664-37475683 |
| <i>ZC3H12A</i> | Chr1:37475757-37475776 |
| <i>ZC3H12A</i> | Chr1:37483385-37483404 |
| <i>ZC3H12A</i> | Chr1:37482933-37482952 |
| <i>ZC3H12A</i> | Chr1:37475866-37475885 |
| <i>ZC3H12A</i> | Chr1:37475843-37475862 |
| <i>ZC3H12A</i> | Chr1:37475797-37475816 |
| <i>ZC3H12A</i> | Chr1:37475642-37475661 |
| <i>ZC3H12A</i> | Chr1:37483270-37483289 |
| <i>ZC3H12A</i> | Chr1:37483024-37483043 |
| <i>ZC3H12A</i> | Chr1:37483201-37483220 |
| <i>ZC3H12A</i> | Chr1:37482447-37482466 |
| <i>ZC3H12A</i> | Chr1:37483253-37483272 |
| <i>ZC3H12A</i> | Chr1:37483429-37483448 |
| <i>ZC3H12A</i> | Chr1:37483195-37483214 |
| <i>ZC3H12A</i> | Chr1:37481648-37481667 |
| <i>ZC3H12A</i> | Chr1:37483424-37483443 |
| <i>ZC3H12A</i> | Chr1:37475580-37475599 |
| <i>ZC3H12A</i> | Chr1:37482980-37482999 |
| <i>ZC3H12A</i> | Chr1:37480408-37480427 |
| <i>ZC3H12A</i> | Chr1:37483405-37483424 |
| <i>ZC3H12A</i> | Chr1:37475740-37475759 |
| <i>ZC3H12A</i> | Chr1:37480387-37480406 |
| <i>ZC3H12A</i> | Chr1:37483507-37483526 |
| <i>ZC3H12A</i> | Chr1:37483110-37483129 |
| <i>ZC3H12A</i> | Chr1:37483325-37483344 |
| <i>ZC3H12A</i> | Chr1:37481692-37481711 |
| <i>ZC3H12A</i> | Chr1:37475826-37475845 |
| <i>ZC3H12A</i> | Chr1:37483098-37483117 |

| Target         | Coordinates            |
|----------------|------------------------|
| <i>ZC3H12A</i> | Chr1:37481758-37481777 |
| <i>ZC3H12A</i> | Chr1:37480320-37480339 |
| <i>ZC3H12A</i> | Chr1:37483380-37483399 |
| <i>ZC3H12A</i> | Chr1:37483011-37483030 |
| <i>ZC3H12A</i> | Chr1:37483509-37483528 |
| <i>ZC3H12A</i> | Chr1:37483509-37483528 |
| <i>ZC3H12A</i> | Chr1:37482768-37482787 |
| <i>ZC3H12A</i> | Chr1:37475804-37475823 |
| <i>ZC3H12A</i> | Chr1:37475808-37475827 |
| <i>ZC3H12A</i> | Chr1:37475859-37475878 |
| <i>ZC3H12A</i> | Chr1:37482973-37482992 |
| <i>ZC3H12A</i> | Chr1:37475634-37475653 |
| <i>ZC3H12A</i> | Chr1:37475854-37475873 |
| <i>ZC3H12A</i> | Chr1:37480334-37480353 |
| <i>ZC3H12A</i> | Chr1:37480414-37480433 |
| <i>ZC3H12A</i> | Chr1:37480316-37480335 |
| <i>ZC3H12A</i> | Chr1:37482971-37482990 |
| <i>ZC3H12A</i> | Chr1:37482781-37482800 |
| <i>ZC3H12A</i> | Chr1:37483173-37483192 |
| <i>ZC3H12A</i> | Chr1:37482391-37482410 |
| <i>ZC3H12A</i> | Chr1:37482392-37482411 |
| <i>ZC3H12A</i> | Chr1:37482936-37482955 |
| <i>ZC3H12A</i> | Chr1:37483408-37483427 |
| <i>ZC3H12A</i> | Chr1:37481779-37481798 |
| <i>ZC3H12A</i> | Chr1:37483206-37483225 |
| <i>ZC3H12A</i> | Chr1:37482561-37482580 |
| <i>ZC3H12A</i> | Chr1:37481745-37481764 |
| <i>ZC3H12A</i> | Chr1:37475802-37475821 |
| <i>ZC3H12A</i> | Chr1:37483494-37483513 |
| <i>ZC3H12A</i> | Chr1:37483371-37483390 |
| <i>ZC3H12A</i> | Chr1:37482552-37482571 |
| <i>ZC3H12A</i> | Chr1:37475491-37475510 |
| <i>ZC3H12A</i> | Chr1:37482479-37482498 |
| <i>ZC3H12A</i> | Chr1:37483140-37483159 |
| <i>ZC3H12A</i> | Chr1:37483313-37483332 |
| <i>ZC3H12A</i> | Chr1:37483458-37483477 |
| <i>ZC3H12A</i> | Chr1:37483320-37483339 |
| <i>ZC3H12A</i> | Chr1:37483204-37483223 |
| <i>ZC3H12A</i> | Chr1:37475792-37475811 |
| <i>ZC3H12A</i> | Chr1:37483475-37483494 |
| <i>ZC3H12A</i> | Chr1:37475577-37475596 |
| <i>ZC3H12A</i> | Chr1:37475787-37475806 |

| Target         | Coordinates            |
|----------------|------------------------|
| <i>ZC3H12A</i> | Chr1:37483574-37483593 |
| <i>ZC3H12A</i> | Chr1:37480284-37480303 |
| <i>ZC3H12A</i> | Chr1:37482369-37482388 |
| <i>ZC3H12A</i> | Chr1:37483384-37483403 |
| <i>ZC3H12A</i> | Chr1:37483425-37483444 |
| <i>ZC3H12A</i> | Chr1:37482582-37482601 |
| <i>ZC3H12A</i> | Chr1:37483153-37483172 |
| <i>ZC3H12A</i> | Chr1:37482935-37482954 |
| <i>ZC3H12A</i> | Chr1:37483378-37483397 |
| <i>ZC3H12A</i> | Chr1:37482952-37482971 |
| <i>ZC3H12A</i> | Chr1:37483399-37483418 |
| <i>ZC3H12A</i> | Chr1:37483309-37483328 |
| <i>ZC3H12A</i> | Chr1:37483200-37483219 |
| <i>ZC3H12A</i> | Chr1:37481641-37481660 |
| <i>ZC3H12A</i> | Chr1:37481656-37481675 |
| <i>ZC3H12A</i> | Chr1:37483036-37483055 |
| <i>ZC3H12A</i> | Chr1:37483474-37483493 |
| <i>ZC3H12A</i> | Chr1:37483004-37483023 |
| <i>ZC3H12A</i> | Chr1:37481846-37481865 |
| <i>ZC3H12A</i> | Chr1:37483205-37483224 |
| <i>ZC3H12A</i> | Chr1:37483406-37483425 |
| <i>ZC3H12A</i> | Chr1:37480336-37480355 |
| <i>ZC3H12A</i> | Chr1:37481716-37481735 |
| <i>ZC3H12A</i> | Chr1:37480335-37480354 |
| <i>ZC3H12A</i> | Chr1:37481659-37481678 |
| <i>ZC3H12A</i> | Chr1:37475809-37475828 |
| <i>ZC3H12A</i> | Chr1:37482565-37482584 |
| <i>ZC3H12A</i> | Chr1:37482491-37482510 |
| <i>ZC3H12A</i> | Chr1:37483379-37483398 |
| <i>ZC3H12A</i> | Chr1:37481654-37481673 |
| <i>ZC3H12A</i> | Chr1:37482567-37482586 |
| <i>ZC3H12A</i> | Chr1:37481614-37481633 |
| <i>ZC3H12A</i> | Chr1:37482562-37482581 |
| <i>ZC3H12A</i> | Chr1:37475868-37475887 |
| <i>ZC3H12A</i> | Chr1:37482557-37482576 |
| <i>ZC3H12A</i> | Chr1:37483511-37483530 |
| <i>ZC3H12A</i> | Chr1:37475615-37475634 |
| <i>ZC3H12A</i> | Chr1:37483333-37483352 |
| <i>ZC3H12A</i> | Chr1:37482840-37482859 |
| <i>ZC3H12A</i> | Chr1:37483545-37483564 |
| <i>ZC3H12A</i> | Chr1:37482830-37482849 |
| <i>ZC3H12A</i> | Chr1:37482444-37482463 |

| Target         | Coordinates            |
|----------------|------------------------|
| <i>ZC3H12A</i> | Chr1:37482571-37482590 |
| <i>ZC3H12A</i> | Chr1:37482553-37482572 |
| <i>ZC3H12A</i> | Chr1:37483543-37483562 |
| <i>ZC3H12A</i> | Chr1:37483542-37483561 |
| <i>ZC3H12A</i> | Chr1:37482575-37482594 |
| <i>ZC3H12A</i> | Chr1:37475855-37475874 |
| <i>ZC3H12A</i> | Chr1:37482572-37482591 |

**Table 8: *Zc3h12a* Murine Genome Coordinates**

| Target         | Coordinates              |
|----------------|--------------------------|
| <i>Zc3h12a</i> | Chr1:125122335-125122354 |
| <i>Zc3h12a</i> | Chr1:125121083-125121102 |
| <i>Zc3h12a</i> | Chr1:125120961-125120980 |
| <i>Zc3h12a</i> | Chr1:125122390-125122409 |
| <i>Zc3h12a</i> | Chr1:125120373-125120392 |
| <i>Zc3h12a</i> | Chr1:125122250-125122269 |
| <i>Zc3h12a</i> | Chr1:125122375-125122394 |
| <i>Zc3h12a</i> | Chr1:125120975-125120994 |

[00187] In some embodiments, the at least one *SOCS1*-, *PTPN2*-, or *ZC3H12A*-targeting siRNA molecule or shRNA molecule is obtained from a commercial suppliers such as Sigma Aldrich®, Dharmacon®, ThermoFisher®, and the like. In some embodiments, the at least one *SOCS1*-, *PTPN2*-, or *ZC3H12A*-targeting siRNA molecule is one shown in Table 9. In some embodiments, the at least one *SOCS1*-, *PTPN2*-, or *ZC3H12A*-targeting shRNA molecule is one shown in Table 10.

**Table 9: Exemplary *SOCS1*, *PTPN2*, and *ZC3H12A* siRNAs**

| Target Gene    | siRNA construct                                                                    |
|----------------|------------------------------------------------------------------------------------|
| <i>SOCS1</i>   | MISSION® esiRNA targeting mouse <i>Socs1</i> (SigmaAldrich# EMU203261)             |
|                | Rosetta Predictions human (SigmaAldrich# NM_003745)                                |
|                | Rosetta Predictions murine (SigmaAldrich# NM_009896)                               |
| <i>PTPN2</i>   | MISSION® esiRNA human <i>PTPN2</i> (esiRNA1) (SigmaAldrich# EHU113971)             |
|                | human Rosetta Predictions (SigmaAldrich# NM_002828)                                |
|                | human Rosetta Predictions (SigmaAldrich# NM_080422)                                |
|                | human Rosetta Predictions (SigmaAldrich# NM_080423)                                |
|                | murine Rosetta Predictions (SigmaAldrich# NM_001127177)                            |
| <i>ZC3H12A</i> | MISSION® esiRNA targeting human <i>ZC3H12A</i> (esiRNA1) (SigmaAldrich# EHU009491) |
|                | MISSION® esiRNA targeting mouse <i>Zc3h12a</i> (esiRNA1) (SigmaAldrich# EMU048551) |

| Target Gene | siRNA construct                                     |
|-------------|-----------------------------------------------------|
|             | Rosetta Predictions human (SigmaAldrich# NM_025079) |
|             | Rosetta Predictions mouse (SigmaAldrich# NM_153159) |

**Table 10: Exemplary *SOCS1*, *PTPN2*, and *ZC3H12A* shRNAs**

| Target Gene    | shRNA construct                                                    |
|----------------|--------------------------------------------------------------------|
| <i>SOCS1</i>   | MISSION® shRNA Plasmid DNA human (SigmaAldrich# SHCLND-NM_003745)  |
|                | MISSION® shRNA Plasmid DNA murine (SigmaAldrich# SHCLND-NM_009896) |
| <i>PTPN2</i>   | MISSION® shRNA Plasmid human (SigmaAldrich# SHCLND-NM_002827)      |
|                | MISSION® shRNA Plasmid murine (SigmaAldrich# SHCLND-NM_011201)     |
| <i>ZC3H12A</i> | MISSION® shRNA Plasmid DNA human (SigmaAldrich# SHCLND-NM_025079)  |
|                | MISSION® shRNA Plasmid DNA mouse (SigmaAldrich# SHCLND-NM_153159)  |

**[00188]** In some embodiments, the nucleic acid-based gene-regulating system comprises at least two nucleic acid molecules (*e.g.*, an siRNA, an shRNA, an RNA aptamer, or a morpholino), wherein at least one nucleic acid molecule is a *SOCS1*-targeting nucleic acid molecule and at least one nucleic acid molecule is a *PTPN2*-targeting nucleic acid molecule. In some embodiments, the at least one *SOCS1*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2) and the at least one *PTPN2*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4). In some embodiments, the at least one *SOCS1*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2) and the at least one *PTPN2*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4).

**[00189]** In some embodiments, the at least one *SOCS1*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4 and the at least one *PTPN2*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6. In some embodiments, the at least one

*SOCS1*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4 and the at least one *PTPN2*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6.

**[00190]** In some embodiments, the at least one *SOCS1*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by one of SEQ ID NOs: 7-151 and the at least one *PTPN2*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by one of SEQ ID NOs: 185-207. In some embodiments, the at least one *SOCS1*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by one of SEQ ID NOs: 7-151 and the at least one *PTPN2*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by one of SEQ ID NOs: 185-207.

**[00191]** In some embodiments, the nucleic acid-based gene-regulating system comprises at least two siRNA or shRNA molecules, wherein at least one siRNA or shRNA molecule is a *SOCS1*-targeting siRNA or shRNA molecule and at least one siRNA or shRNA molecule is a *PTPN2*-targeting siRNA or shRNA molecule. In some embodiments, the at least one *SOCS1*-targeting nucleic acid molecule is an siRNA or an shRNA molecule and at least one *PTPN2*-targeting nucleic acid molecule is an siRNA or shRNA molecule. In some embodiments, the at least one *SOCS1*-targeting siRNA or an shRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2) and the at least one *PTPN2*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4). In some embodiments, the at least one *SOCS1*-targeting siRNA or an shRNA molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2) and the at least one *PTPN2*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4).

**[00192]** In some embodiments, the at least one *SOCS1*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a RNA

sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4 and the at least one *PTPN2*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6. In some embodiments, the at least one *SOCS1*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is 100% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4 and the at least one *PTPN2*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is 100% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6.

**[00193]** In some embodiments, the at least one *SOCS1*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by one of SEQ ID NOs: 7-151 and the at least one *PTPN2*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by one of SEQ ID NOs: 185-207. In some embodiments, the at least one *SOCS1*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by one of SEQ ID NOs: 7-151 and the at least one *PTPN2*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by one of SEQ ID NOs: 185-207.

**[00194]** In some embodiments, the nucleic acid-based gene-regulating system comprises at least two nucleic acid molecules (*e.g.*, an siRNA, an shRNA, an RNA aptamer, or a morpholino), wherein at least one nucleic acid molecule is a *SOCS1*-targeting nucleic acid molecule and at least one nucleic acid molecule is a *ZC3H12A*-targeting nucleic acid molecule. In some embodiments, the at least one *SOCS1*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2) and the at least one *ZC3H12A*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6). In some embodiments, the at least one *SOCS1*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2) and the at least one *ZC3H12A*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6).

**[00195]** In some embodiments, the at least one *SOCS1*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4 and the at least one *ZC3H12A*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8. In some embodiments, the at least one *SOCS1*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4 and the at least one *ZC3H12A*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8.

**[00196]** In some embodiments, the at least one *SOCS1*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by one of SEQ ID NOs: 7-151 and the at least one *ZC3H12A*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by one of SEQ ID NOs: 208-230. In some embodiments, the at least one *SOCS1*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by one of SEQ ID NOs: 7-151 and the at least one *ZC3H12A*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by one of SEQ ID NOs: 208-230.

**[00197]** In some embodiments, the nucleic acid-based gene-regulating system comprises at least two siRNA or shRNA molecules, wherein at least one siRNA or shRNA molecule is a *SOCS1*-targeting siRNA or shRNA molecule and at least one siRNA or shRNA molecule is a *ZC3H12A*-targeting siRNA or shRNA molecule. In some embodiments, the least one *SOCS1*-targeting nucleic acid molecule is an siRNA or an shRNA molecule and at least one *ZC3H12A*-targeting nucleic acid molecule is an siRNA or shRNA molecule. In some embodiments, the at least one *SOCS1*-targeting siRNA or an shRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2) and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6). In some embodiments, the at least one *SOCS1*-targeting siRNA or an shRNA molecule binds to a target RNA sequence that



is 100% identical to an RNA sequence encoded by the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2) and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6).

**[00198]** In some embodiments, the at least one *SOCS1*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4 and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8. In some embodiments, the at least one *SOCS1*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is 100% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4 and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is 100% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8.

**[00199]** In some embodiments, the at least one *SOCS1*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by one of SEQ ID NOs: 7-151 and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by one of SEQ ID NOs: 208-230. In some embodiments, the at least one *SOCS1*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by one of SEQ ID NOs: 7-151 and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by one of SEQ ID NOs: 208-230.

**[00200]** In some embodiments, the nucleic acid-based gene-regulating system comprises at least two nucleic acid molecules (*e.g.*, an siRNA, an shRNA, an RNA aptamer, or a morpholino), wherein at least one nucleic acid molecule is a *PTPN2*-targeting nucleic acid molecule and at least one nucleic acid molecule is a *ZC3H12A*-targeting nucleic acid molecule. In some embodiments, the at least one *PTPN2*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4) and the at least one *ZC3H12A*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an

RNA sequence encoded by the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6). In some embodiments, the at least one *PTPN2*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4) and the at least one *ZC3H12A*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6).

**[00201]** In some embodiments, the at least one *PTPN2*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6 and the at least one *ZC3H12A*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8. In some embodiments, the at least one *PTPN2*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6 and the at least one *ZC3H12A*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8.

**[00202]** In some embodiments, the at least one *PTPN2*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by one of SEQ ID NOs: 185-207 and the at least one *ZC3H12A*-targeting nucleic acid molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by one of SEQ ID NOs: 208-230. In some embodiments, the at least one *PTPN2*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by one of SEQ ID NOs: 185-207 and the at least one *ZC3H12A*-targeting nucleic acid molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by one of SEQ ID NOs: 208-230.

**[00203]** In some embodiments, the nucleic acid-based gene-regulating system comprises at least two siRNA or shRNA molecules, wherein at least one siRNA or shRNA molecule is a *PTPN2*-targeting siRNA or shRNA molecule and at least one siRNA or shRNA molecule is a *ZC3H12A*-targeting siRNA or shRNA molecule. In some embodiments, the at least one *PTPN2*-targeting siRNA or an shRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ

ID NO: 4) and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6). In some embodiments, the at least one *PTPN2*-targeting siRNA or an shRNA molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4) and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6).

**[00204]** In some embodiments, the at least one *PTPN2*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6 and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8. In some embodiments, the at least one *PTPN2*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is 100% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6 and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is 100% identical to a RNA sequence encoded by a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8.

**[00205]** In some embodiments, the at least one *PTPN2*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by one of SEQ ID NOs: 185-207 and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to an RNA sequence encoded by one of SEQ ID NOs: 208-230. In some embodiments, the at least one *PTPN2*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by one of SEQ ID NOs: 185-207 and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule binds to a target RNA sequence that is 100% identical to an RNA sequence encoded by one of SEQ ID NOs: 208-230.

### ***B. Protein-based gene-regulating systems***

**[00206]** In some embodiments, the present disclosure provides protein gene-regulating systems comprising two or more proteins capable of reducing the expression and/or function of at least two

endogenous genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*. In some embodiments, the present disclosure provides modified immune effector cells comprising such gene-regulating systems. In some embodiments, a protein-based gene-regulating system is a system comprising one or more proteins capable of regulating the expression of an endogenous target gene in a sequence specific manner without the requirement for a nucleic acid guide molecule. In some embodiments, the protein-based gene-regulating system comprises a protein comprising one or more zinc-finger binding domains and an enzymatic domain. In some embodiments, the protein-based gene-regulating system comprises a protein comprising a Transcription activator-like effector nuclease (TALEN) domain and an enzymatic domain. Such embodiments are referred to herein as “TALENs”.

### 1. Zinc finger systems

[00207] In some embodiments, the present disclosure provides zinc finger gene-regulating systems comprising two or more zinc finger fusion proteins capable of reducing the expression and/or function of at least two endogenous genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*. In some embodiments, the present disclosure provides modified immune effector cells comprising such gene-regulating systems. Herein, zinc finger-based systems comprise a fusion protein with two protein domains: a zinc finger DNA binding domain and an enzymatic domain. A “zinc finger DNA binding domain”, “zinc finger protein”, or “ZFP” is a protein, or a domain within a larger protein, that binds DNA in a sequence-specific manner through one or more zinc fingers, which are regions of amino acid sequence within the binding domain whose structure is stabilized through coordination of a zinc ion. The zinc finger domain, by binding to a target DNA sequence, directs the activity of the enzymatic domain to the vicinity of the sequence and, hence, induces modification of the endogenous target gene in the vicinity of the target sequence. A zinc finger domain can be engineered to bind to virtually any desired sequence. Accordingly, after identifying a target genetic locus containing a target DNA sequence at which cleavage or recombination is desired (*e.g.*, a target locus in a target gene referenced in Tables 2 or 3), one or more zinc finger binding domains can be engineered to bind to one or more target DNA sequences in the target genetic locus. Expression of a fusion protein comprising a zinc finger binding domain and an enzymatic domain in a cell affects modification in the target genetic locus.

[00208] In some embodiments, a zinc finger binding domain comprises one or more zinc fingers. Miller *et al.* (1985) EMBO J. 4:1609-1614; Rhodes (1993) Scientific American February:56-65; U.S. Pat. No. 6,453,242. Typically, a single zinc finger domain is about 30 amino acids in length. An individual zinc finger binds to a three-nucleotide (*i.e.*, triplet) sequence (or a four-nucleotide

sequence which can overlap, by one nucleotide, with the four-nucleotide binding site of an adjacent zinc finger). Therefore, the length of a sequence to which a zinc finger binding domain is engineered to bind (*e.g.*, a target sequence) will determine the number of zinc fingers in an engineered zinc finger binding domain. For example, for ZFPs in which the finger motifs do not bind to overlapping subsites, a six-nucleotide target sequence is bound by a two-finger binding domain; a nine-nucleotide target sequence is bound by a three-finger binding domain, etc. Binding sites for individual zinc fingers (*i.e.*, subsites) in a target site need not be contiguous, but can be separated by one or several nucleotides, depending on the length and nature of the amino acids sequences between the zinc fingers (*i.e.*, the inter-finger linkers) in a multi-finger binding domain. In some embodiments, the DNA-binding domains of individual ZFNs comprise between three and six individual zinc finger repeats and can each recognize between 9 and 18 basepairs.

**[00209]** Zinc finger binding domains can be engineered to bind to a sequence of choice. See, for example, Beerli *et al.* (2002) *Nature Biotechnol.* 20:135-141; Pabo *et al.* (2001) *Ann. Rev. Biochem.* 70:313-340; Isalan *et al.* (2001) *Nature Biotechnol.* 19:656-660; Segal *et al.* (2001) *Curr. Opin. Biotechnol.* 12:632-637; Choo *et al.* (2000) *Curr. Opin. Struct. Biol.* 10:411-416. An engineered zinc finger binding domain can have a novel binding specificity, compared to a naturally-occurring zinc finger protein. Engineering methods include, but are not limited to, rational design and various types of selection.

**[00210]** Selection of a target DNA sequence for binding by a zinc finger domain can be accomplished, for example, according to the methods disclosed in U.S. Pat. No. 6,453,242. It will be clear to those skilled in the art that simple visual inspection of a nucleotide sequence can also be used for selection of a target DNA sequence. Accordingly, any means for target DNA sequence selection can be used in the methods described herein. A target site generally has a length of at least 9 nucleotides and, accordingly, is bound by a zinc finger binding domain comprising at least three zinc fingers. However binding of, for example, a 4-finger binding domain to a 12-nucleotide target site, a 5-finger binding domain to a 15-nucleotide target site or a 6-finger binding domain to an 18-nucleotide target site, is also possible. As will be apparent, binding of larger binding domains (*e.g.*, 7-, 8-, 9-finger and more) to longer target sites is also possible.

**[00211]** In some embodiments, the protein-based gene-regulating system comprises at least two zinc finger fusion proteins (ZFP), wherein at least one ZFP comprises a *SOCS1*-targeting zinc finger binding domain. In some embodiments, the at least one *SOCS1*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a target

DNA sequence in the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2). In some embodiments, the at least one *SOCS1*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to a target DNA sequence in the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2).

**[00212]** In some embodiments, the at least one *SOCS1*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4. In some embodiments, the at least one *SOCS1*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4. In some embodiments, the at least one *SOCS1*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 7-151. In some embodiments, the at least one *SOCS1*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 7-151. Exemplary *SOCS1* target DNA sequences are shown in Tables 12 and 13.

**[00213]** In some embodiments, the protein-based gene-regulating system comprises at least two zinc finger fusion proteins (ZFP), wherein at least one ZFP comprises a *PTPN2*-targeting zinc finger binding domain. In some embodiments, the at least one *PTPN2*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a target DNA sequence in the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4). In some embodiments, the at least one *PTPN2*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to a target DNA sequence in the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4).

**[00214]** In some embodiments, the at least one *PTPN2*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6. In some embodiments, the at least one *PTPN2*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6. In some embodiments, the at least one *PTPN2*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 185-207. In some embodiments, the at least one *PTPN2*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 185-207. Exemplary *PTPN2* target DNA sequences are shown in Tables 14 and 15.

**[00215]** In some embodiments, the protein-based gene-regulating system comprises at least two zinc finger fusion proteins (ZFP), wherein at least one ZFP comprises a *ZC3H12A*-targeting zinc finger binding domain. In some embodiments, the at least one *ZC3H12A*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a target DNA sequence in the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6). In some embodiments, the at least one *ZC3H12A*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to a target DNA sequence in the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6).

**[00216]** In some embodiments, the at least one *ZC3H12A*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8. In some embodiments, the at least one *ZC3H12A*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8. In some embodiments, the at least one *ZC3H12A*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 208-230. In some embodiments, the at least one *ZC3H12A*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 208-230. Exemplary *ZC3H12A* target DNA sequences are shown in Tables 16 and 17.

**[00217]** In some embodiments, the at least one *SOCS1*-, *PTPN2*-, or *ZC3H12A*-targeting ZFP is obtained from a commercial suppliers such as Sigma Aldrich, Dharmacon, ThermoFisher, and the like. For example, in some embodiments, the at least one *SOCS1*, *PTPN2*, or *ZC3H12A* ZFP is one shown in Table 11.

**Table 11: Exemplary *SOCS1*, *PTPN2*, and *ZC3H12A* Zinc Finger Systems**

| Target Gene    | Zinc Finger System                                                                                   |
|----------------|------------------------------------------------------------------------------------------------------|
| <i>SOCS1</i>   | CompoZr® Knockout ZFN plasmid Human <i>SOCS1</i> (NM_003745) (SigmaAldrich# CKOZFND20320)            |
|                | CompoZr® Knockout ZFN plasmid Mouse <i>Socs1</i> (NM_009896.2) (SigmaAldrich# CKOZFND41801)          |
| <i>PTPN2</i>   | PTPN2 CompoZr® Knockout ZFN human plasmid <i>PTPN2</i> (NM_002828) (SigmaAldrich# CKOZFND17697)      |
|                | CompoZr® Knockout ZFN murine plasmid Ptpn2 (NM_008977.3) (SigmaAldrich# CKOZFND39632)                |
| <i>ZC3H12A</i> | CompoZr® Knockout ZFN Kit, ZFN plasmid Human <i>ZC3H12A</i> (NM_025079) (SigmaAldrich# CKOZFND23094) |

| Target Gene | Zinc Finger System                                                                                     |
|-------------|--------------------------------------------------------------------------------------------------------|
|             | CompoZr® Knockout ZFN Kit, ZFN plasmid mouse <i>Zc3h12a</i> (NM 153159.2) (SigmaAldrich# CKOZFND44851) |

**[00218]** In some embodiments, the protein-based gene-regulating system comprises at least two ZFPs, wherein at least one ZFP comprises a *SOCS1*-targeting zinc finger binding domain and at least one ZFP comprises a *PTPN2*-targeting zinc finger binding domain. In some embodiments, the at least one *SOCS1*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence in the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2) and the at least one *PTPN2*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence in the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4). In some embodiments, the at least one *SOCS1*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to a DNA sequence in the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2) and the at least one *PTPN2*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to a DNA sequence in the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4).

**[00219]** In some embodiments, the at least one *SOCS1*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4 and the at least one *PTPN2*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6. In some embodiments, the at least one *SOCS1*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4 and the at least one *PTPN2*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6.

**[00220]** In some embodiments, the at least one *SOCS1*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 7-151 and the at least one *PTPN2*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 185-207. In some embodiments, the at least one *SOCS1*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 7-151 and the at least one *PTPN2*-



targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 185-207.

**[00221]** In some embodiments, the protein-based gene-regulating system comprises at least two ZFPs, wherein at least one ZFP comprises a *SOCS1*-targeting zinc finger binding domain and at least one ZFP comprises a *ZC3H12A*-targeting zinc finger binding domain. In some embodiments, the at least one *SOCS1*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence in the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2) and the at least one *ZC3H12A*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence in the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6). In some embodiments, the at least one *SOCS1*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to a DNA sequence in the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2) and the at least one *ZC3H12A*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to a DNA sequence in the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6).

**[00222]** In some embodiments, the at least one *SOCS1*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4 and the at least one *ZC3H12A*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8. In some embodiments, the at least one *SOCS1*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4 and the at least one *ZC3H12A*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8.

**[00223]** In some embodiments, the at least one *SOCS1*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 7-151 and the at least one *ZC3H12A*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 208-230. In some embodiments, the at least one *SOCS1*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 7-151 and the at least one

*ZC3H12A*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 208-230.

**[00224]** In some embodiments, the protein-based gene-regulating system comprises at least two ZFPs, wherein at least one ZFP comprises a *PTPN2*-targeting zinc finger binding domain and at least one ZFP comprises a *ZC3H12A*-targeting zinc finger binding domain. In some embodiments, the at least one *PTPN2*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence in the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4) and the at least one *ZC3H12A*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence in the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6). In some embodiments, the at least one *PTPN2*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to a DNA sequence in the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4) and the at least one *ZC3H12A*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to a DNA sequence in the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6).

**[00225]** In some embodiments, the at least one *PTPN2*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6 and the at least one *ZC3H12A*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8. In some embodiments, the at least one *PTPN2*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6 and the at least one *ZC3H12A*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8.

**[00226]** In some embodiments, the at least one *PTPN2*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 185-207 and the at least one *ZC3H12A*-targeting zinc finger binding domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 208-230. In some embodiments, the at least one *PTPN2*-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 185-207 and the at least one

ZC3H12A-targeting zinc finger binding domain binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 208-230.

**[00227]** The enzymatic domain portion of the zinc finger fusion proteins can be obtained from any endo- or exonuclease. Exemplary endonucleases from which an enzymatic domain can be derived include, but are not limited to, restriction endonucleases and homing endonucleases. *See*, for example, 2002-2003 Catalogue, New England Biolabs, Beverly, Mass.; and Belfort *et al.* (1997) *Nucleic Acids Res.* 25:3379-3388. Additional enzymes which cleave DNA are known (*e.g.*, 51 Nuclease; mung bean nuclease; pancreatic DNaseI; micrococcal nuclease; yeast HO endonuclease; *see also* Linn *et al.* (eds.) *Nucleases*, Cold Spring Harbor Laboratory Press, 1993). One or more of these enzymes (or functional fragments thereof) can be used as a source of cleavage domains.

**[00228]** Exemplary restriction endonucleases (restriction enzymes) suitable for use as an enzymatic domain of the ZFPs described herein are present in many species and are capable of sequence-specific binding to DNA (at a recognition site), and cleaving DNA at or near the site of binding. Certain restriction enzymes (*e.g.*, Type IIS) cleave DNA at sites removed from the recognition site and have separable binding and cleavage domains. For example, the Type IIS enzyme FokI catalyzes double-stranded cleavage of DNA, at 9 nucleotides from its recognition site on one strand and 13 nucleotides from its recognition site on the other. *See*, for example, U.S. Pat. Nos. 5,356,802; 5,436,150 and 5,487,994; as well as Li *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:4275-4279; Li *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:2764-2768; Kim *et al.* (1994a) *Proc. Natl. Acad. Sci. USA* 91:883-887; Kim *et al.* (1994b) *J. Biol. Chem.* 269:31,978-31,982. Thus, in one embodiment, fusion proteins comprise the enzymatic domain from at least one Type IIS restriction enzyme and one or more zinc finger binding domains.

**[00229]** An exemplary Type IIS restriction enzyme, whose cleavage domain is separable from the binding domain, is FokI. This particular enzyme is active as a dimer. Bitinaite *et al.* (1998) *Proc. Natl. Acad. Sci. USA* 95: 10,570-10,575. Thus, for targeted double-stranded DNA cleavage using zinc finger-FokI fusions, two fusion proteins, each comprising a FokI enzymatic domain, can be used to reconstitute a catalytically active cleavage domain. Alternatively, a single polypeptide molecule containing a zinc finger binding domain and two FokI enzymatic domains can also be used. Exemplary ZFPs comprising FokI enzymatic domains are described in US Patent No. 9,782,437.

## 2. TALEN systems

**[00230]** In some embodiments, the present disclosure provides TALEN gene-regulating systems comprising two or more TALEN fusion proteins capable of reducing the expression and/or function of at least two endogenous genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*. In some embodiments, the present disclosure provides modified immune effector cells comprising such gene-regulating systems. TALEN-based systems comprise a TALEN fusion protein comprising a TAL effector DNA binding domain and an enzymatic domain. They are made by fusing a TAL effector DNA-binding domain to a DNA cleavage domain (a nuclease which cuts DNA strands). The FokI restriction enzyme described above is an exemplary enzymatic domain suitable for use in TALEN-based gene-regulating systems.

**[00231]** TAL effectors are proteins that are secreted by *Xanthomonas* bacteria via their type III secretion system when they infect plants. The DNA binding domain contains a repeated, highly conserved, 33–34 amino acid sequence with divergent 12th and 13th amino acids. These two positions, referred to as the Repeat Variable Diresidue (RVD), are highly variable and strongly correlated with specific nucleotide recognition. Therefore, the TAL effector domains can be engineered to bind specific target DNA sequences by selecting a combination of repeat segments containing the appropriate RVDs. The nucleic acid specificity for RVD combinations is as follows: HD targets cytosine, NI targets adenine, NG targets thymine, and NN targets guanine (though, in some embodiments, NN can also bind adenine with lower specificity).

**[00232]** Methods and compositions for assembling the TAL-effector repeats are known in the art. *See e.g.*, Cermak *et al*, Nucleic Acids Research, 39:12, 2011, e82. Plasmids for constructions of the TAL-effector repeats are commercially available from Addgene.

**[00233]** In some embodiments, the protein-based gene-regulating system comprises at least two TALEN fusion proteins, wherein at least one TALEN fusion protein comprises a *SOCS1*-targeting TAL effector domain. In some embodiments, the at least one *SOCS1*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a target DNA sequence in the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2). In some embodiments, the at least one *SOCS1*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to a target DNA sequence in the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 1).

**[00234]** In some embodiments, the at least one *SOCS1*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4. In some embodiments, the at least one *SOCS1*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4. In some embodiments, the at least one *SOCS1*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 7-151. In some embodiments, the at least one *SOCS1*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 7-151. Exemplary *SOCS1* target DNA sequences are shown in Tables 12 and 13.

**[00235]** In some embodiments, the protein-based gene-regulating system comprises at least two TALEN fusion proteins, wherein at least one TALEN fusion protein comprises a *PTPN2*-targeting TAL effector domain. In some embodiments, the at least one *PTPN2*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a target DNA sequence in the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4). In some embodiments, the at least one *PTPN2*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to a target DNA sequence in the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4).

**[00236]** In some embodiments, the at least one *PTPN2*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6. In some embodiments, the at least one *PTPN2*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6. In some embodiments, the at least one *PTPN2*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 185-207. In some embodiments, the at least one *PTPN2*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 185-207. Exemplary *PTPN2* target DNA sequences are shown in Tables 14 and 15.

**[00237]** In some embodiments, the protein-based gene-regulating system comprises at least two TALEN fusion proteins, wherein at least one TALEN fusion protein comprises a *ZC3H12A*-targeting TAL effector domain. In some embodiments, the at least one *ZC3H12A*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a

target DNA sequence in the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6). In some embodiments, the at least one *ZC3H12A*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to a target DNA sequence in the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6).

**[00238]** In some embodiments, the at least one *ZC3H12A*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8. In some embodiments, the at least one *ZC3H12A*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8. In some embodiments, the at least one *ZC3H12A*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 208-230. In some embodiments, the at least one *ZC3H12A*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 208-230. Exemplary *ZC3H12A* target DNA sequences are shown in Tables 16 and 17.

**[00239]** In some embodiments, the protein-based gene-regulating system comprises at least two TAL fusion proteins, wherein at least one TALEN fusion protein comprises a *SOCS1*-targeting TAL effector domain and at least one TALEN fusion protein comprises a *PTPN2*-targeting TAL effector domain. In some embodiments, the at least one *SOCS1*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence in the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2) and the at least one *PTPN2*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4). In some embodiments, the at least one *SOCS1*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to a DNA sequence in the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2) and the at least one *PTPN2*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to a DNA sequence in the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4).

**[00240]** In some embodiments, the at least one *SOCS1*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4 and the at least one *PTPN2*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 5 or

Table 6. In some embodiments, the at least one *SOCS1*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4 and the at least one *PTPN2*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6.

**[00241]** In some embodiments, the at least one *SOCS1*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 7-151 and the at least one *PTPN2*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 185-207. In some embodiments, the at least one *SOCS1*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 7-151 and the at least one *PTPN2*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 185-207.

**[00242]** In some embodiments, the protein-based gene-regulating system comprises at least two TALEN fusion proteins, wherein at least one TALEN fusion protein comprises a *SOCS1*-targeting TAL effector domain and at least one TALEN fusion protein comprises a *ZC3H12A*-targeting TAL effector domain. In some embodiments, the at least one *SOCS1*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence in the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2) and the at least one *ZC3H12A*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence in the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6). In some embodiments, the at least one *SOCS1*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to a DNA sequence in the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2) and the at least one *ZC3H12A*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to a DNA sequence in the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6).

**[00243]** In some embodiments, the at least one *SOCS1*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4 and the at least one *ZC3H12A*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8. In some embodiments, the at least one *SOCS1*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic

coordinates shown in Table 3 or Table 4 and the at least one *ZC3H12A*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8.

**[00244]** In some embodiments, the at least one *SOCS1*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 7-151 and the at least one *ZC3H12A*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 208-230. In some embodiments, the at least one *SOCS1*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 7-151 and the at least one *ZC3H12A*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 208-230.

**[00245]** In some embodiments, the protein-based gene-regulating system comprises at least two TALEN fusion proteins, wherein at least one TALEN fusion protein comprises a *PTPN2*-targeting TAL effector domain and at least one TALEN fusion protein comprises a *ZC3H12A*-targeting TAL effector domain. In some embodiments, the at least one *PTPN2*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence in the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4) and the at least one *ZC3H12A*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence in the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6). In some embodiments, the at least one *PTPN2*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to a DNA sequence in the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4) and the at least one *ZC3H12A*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to a DNA sequence in the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6).

**[00246]** In some embodiments, the at least one *PTPN2*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6 and the at least one *ZC3H12A*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8. In some embodiments, the at least one *PTPN2*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6 and the at least one *ZC3H12A*-targeting TAL effector domain



binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8.

**[00247]** In some embodiments, the at least one *PTPN2*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 185-207 and the at least one *ZC3H12A*-targeting TAL effector domain binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 208-230. In some embodiments, the at least one *PTPN2*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 185-207 and the at least one *ZC3H12A*-targeting TAL effector domain binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 208-230.

### ***C. Combination nucleic acid/protein-based gene-regulating systems***

**[00248]** Combination gene-regulating systems comprise a site-directed modifying polypeptide and a nucleic acid guide molecule. Herein, a “site-directed modifying polypeptide” refers to a polypeptide that binds to a nucleic acid guide molecule, is targeted to a target nucleic acid sequence, (for example, an endogenous target DNA or RNA sequence) by the nucleic acid guide molecule to which it is bound, and modifies the target nucleic acid sequence (*e.g.*, cleavage, mutation, or methylation of a target nucleic acid sequence).

**[00249]** A site-directed modifying polypeptide comprises two portions, a portion that binds the nucleic acid guide and an activity portion. In some embodiments, a site-directed modifying polypeptide comprises an activity portion that exhibits site-directed enzymatic activity (*e.g.*, DNA methylation, DNA or RNA cleavage, histone acetylation, histone methylation, etc.), wherein the site of enzymatic activity is determined by the guide nucleic acid. In some cases, a site-directed modifying polypeptide comprises an activity portion that has enzymatic activity that modifies the endogenous target nucleic acid sequence (*e.g.*, nuclease activity, methyltransferase activity, demethylase activity, DNA repair activity, DNA damage activity, deamination activity, dismutase activity, alkylation activity, depurination activity, oxidation activity, pyrimidine dimer forming activity, integrase activity, transposase activity, recombinase activity, polymerase activity, ligase activity, helicase activity, photolyase activity or glycosylase activity). In other cases, a site-directed modifying polypeptide comprises an activity portion that has enzymatic activity that modifies a polypeptide (*e.g.*, a histone) associated with the endogenous target nucleic acid sequence (*e.g.*, methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, kinase activity,

phosphatase activity, ubiquitin ligase activity, deubiquitinating activity, adenylation activity, deadenylation activity, SUMOylating activity, deSUMOylating activity, ribosylation activity, deribosylation activity, myristoylation activity or demyristoylation activity). In some embodiments, a site-directed modifying polypeptide comprises an activity portion that modulates transcription of a target DNA sequence (*e.g.*, to increase or decrease transcription). In some embodiments, a site-directed modifying polypeptide comprises an activity portion that modulates expression or translation of a target RNA sequence (*e.g.*, to increase or decrease transcription).

**[00250]** The nucleic acid guide comprises two portions: a first portion that is complementary to, and capable of binding with, an endogenous target nucleic sequence (referred to herein as a “nucleic acid-binding segment”), and a second portion that is capable of interacting with the site-directed modifying polypeptide (referred to herein as a “protein-binding segment”). In some embodiments, the nucleic acid-binding segment and protein-binding segment of a nucleic acid guide are comprised within a single polynucleotide molecule. In some embodiments, the nucleic acid-binding segment and protein-binding segment of a nucleic acid guide are each comprised within separate polynucleotide molecules, such that the nucleic acid guide comprises two polynucleotide molecules that associate with each other to form the functional guide.

**[00251]** The nucleic acid guide mediates the target specificity of the combined protein/nucleic acid gene-regulating systems by specifically hybridizing with a target nucleic acid sequence. In some embodiments, the target nucleic acid sequence is an RNA sequence, such as an RNA sequence comprised within an mRNA transcript of a target gene. In some embodiments, the target nucleic acid sequence is a DNA sequence comprised within the DNA sequence of a target gene. Reference herein to a target gene encompasses the full-length DNA sequence for that particular gene which comprises a plurality of target genetic loci (*i.e.*, portions of a particular target gene sequence (*e.g.*, an exon or an intron)). Within each target genetic loci are shorter stretches of DNA sequences referred to herein as “target DNA sequences” that can be modified by the gene-regulating systems described herein. Further, each target genetic loci comprises a “target modification site,” which refers to the precise location of the modification induced by the gene-regulating system (*e.g.*, the location of an insertion, a deletion, or mutation, the location of a DNA break, or the location of an epigenetic modification). The gene-regulating systems described herein may comprise 2 or more nucleic acid guides (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more nucleic acid guides).

**[00252]** In some embodiments, the combined protein/nucleic acid gene-regulating systems comprise site-directed modifying polypeptides derived from Argonaute (Ago) proteins (*e.g.*, *T.*

*thermophiles* Ago or TtAgo). In such embodiments, the site-directed modifying polypeptide is a *T. thermophiles* Ago DNA endonuclease and the nucleic acid guide is a guide DNA (gDNA) (See, Swarts *et al.*, Nature 507 (2014), 258-261). In some embodiments, the present disclosure provides a polynucleotide encoding a gDNA. In some embodiments, a gDNA-encoding nucleic acid is comprised in an expression vector, *e.g.*, a recombinant expression vector. In some embodiments, the present disclosure provides a polynucleotide encoding a TtAgo site-directed modifying polypeptide or variant thereof. In some embodiments, the polynucleotide encoding a TtAgo site-directed modifying polypeptide is comprised in an expression vector, *e.g.*, a recombinant expression vector.

**[00253]** In some embodiments, the gene editing systems described herein are CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas (CRISPR Associated) nuclease systems. In some embodiments, the CRISPR/Cas system is a Class 2 system. Class 2 CRISPR/Cas systems are divided into three types: Type II, Type V, and Type VI systems. In some embodiments, the CRISPR/Cas system is a Class 2 Type II system, utilizing the Cas9 protein. In such embodiments, the site-directed modifying polypeptide is a Cas9 DNA endonuclease (or variant thereof) and the nucleic acid guide molecule is a guide RNA (gRNA). In some embodiments, the CRISPR/Cas system is a Class 2 Type V system, utilizing the Cas12 proteins (*e.g.*, Cas12a (also known as Cpf1), Cas12b (also known as C2c1), Cas12c (also known as C2c3), Cas12d (also known as CasY), and Cas12e (also known as CasX)). In such embodiments, the site-directed modifying polypeptide is a Cas12 DNA endonuclease (or variant thereof) and the nucleic acid guide molecule is a gRNA. In some embodiments, the CRISPR/Cas system is a Class 2 and Type VI system, utilizing the Cas13 proteins (*e.g.*, Cas13a (also known as C2c2), Cas13b, and Cas13c). (See, Pyzocha *et al.*, ACS Chemical Biology, 13(2), 347-356). In such embodiments, the site-directed modifying polypeptide is a Cas13 RNA ribonuclease and the nucleic acid guide molecule is a gRNA.

**[00254]** A Cas polypeptide refers to a polypeptide that can interact with a gRNA molecule and, in concert with the gRNA molecule, home or localize to a target DNA or target RNA sequence. Cas polypeptides include naturally occurring Cas proteins and engineered, altered, or otherwise modified Cas proteins that differ by one or more amino acid residues from a naturally-occurring Cas sequence.

**[00255]** A guide RNA (gRNA) comprises two segments, a DNA-binding segment and a protein-binding segment. In some embodiments, the protein-binding segment of a gRNA is comprised in one RNA molecule and the DNA-binding segment is comprised in another separate RNA molecule. Such embodiments are referred to herein as “double-molecule gRNAs” or “two-molecule gRNA” or “dual gRNAs.” In some embodiments, the gRNA is a single RNA molecule and is referred to herein

as a “single-guide RNA” or an “sgRNA.” The term “guide RNA” or “gRNA” is inclusive, referring both to two-molecule guide RNAs and sgRNAs.

**[00256]** The protein-binding segment of a gRNA comprises, in part, two complementary stretches of nucleotides that hybridize to one another to form a double stranded RNA duplex (dsRNA duplex), which facilitates binding to the Cas protein. The nucleic acid-binding segment (or “nucleic acid-binding sequence”) of a gRNA comprises a nucleotide sequence that is complementary to and capable of binding to a specific target nucleic acid sequence. The protein-binding segment of the gRNA interacts with a Cas polypeptide and the interaction of the gRNA molecule and site-directed modifying polypeptide results in Cas binding to the endogenous nucleic acid sequence and produces one or more modifications within or around the target nucleic acid sequence. The precise location of the target modification site is determined by both (i) base-pairing complementarity between the gRNA and the target nucleic acid sequence; and (ii) the location of a short motif, referred to as the protospacer adjacent motif (PAM), in the target DNA sequence (referred to as a protospacer flanking sequence (PFS) in target RNA sequences). The PAM/PFS sequence is required for Cas binding to the target nucleic acid sequence. A variety of PAM/PFS sequences are known in the art and are suitable for use with a particular Cas endonuclease (*e.g.*, a Cas9 endonuclease). (*See e.g.*, Nat Methods. 2013 Nov; 10(11): 1116–1121 and Sci Rep. 2014; 4: 5405). In some embodiments, the PAM sequence is located within 50 base pairs of the target modification site in a target DNA sequence. In some embodiments, the PAM sequence is located within 10 base pairs of the target modification site in a target DNA sequence. The DNA sequences that can be targeted by this method are limited only by the relative distance of the PAM sequence to the target modification site and the presence of a unique 20 base pair sequence to mediate sequence-specific, gRNA-mediated Cas binding. In some embodiments, the PFS sequence is located at the 3' end of the target RNA sequence. In some embodiments, the target modification site is located at the 5' terminus of the target locus. In some embodiments, the target modification site is located at the 3' end of the target locus. In some embodiments, the target modification site is located within an intron or an exon of the target locus.

**[00257]** In some embodiments, the present disclosure provides a polynucleotide encoding a gRNA. In some embodiments, a gRNA-encoding nucleic acid is comprised in an expression vector, *e.g.*, a recombinant expression vector. In some embodiments, the present disclosure provides a polynucleotide encoding a site-directed modifying polypeptide. In some embodiments, the polynucleotide encoding a site-directed modifying polypeptide is comprised in an expression vector, *e.g.*, a recombinant expression vector.

### 1. Cas proteins

**[00258]** In some embodiments, the site-directed modifying polypeptide is a Cas protein. Cas molecules of a variety of species can be used in the methods and compositions described herein, including Cas molecules derived from *S. pyogenes*, *S. aureus*, *N. meningitidis*, *S. thermophiles*, *Acidovorax avenae*, *Actinobacillus pleuropneumoniae*, *Actinobacillus succinogenes*, *Actinobacillus suis*, *Actinomyces sp.*, *Cycliphilus denitrificans*, *Aminomonas paucivorans*, *Bacillus cereus*, *Bacillus smithii*, *Bacillus thuringiensis*, *Bacteroides sp.*, *Blastopirellula marina*, *Bradyrhizobium sp.*, *Brevibacillus laterosporus*, *Campylobacter coli*, *Campylobacter jejuni*, *Campylobacter lari*, *Candidatus puniceispirillum*, *Clostridium cellulolyticum*, *Clostridium perfringens*, *Corynebacterium accolens*, *Corynebacterium diphtheria*, *Corynebacterium matruchotii*, *Dinoroseobacter shibae*, *Eubacterium dolichum*, *Gammaproteobacterium*, *Gluconacetobacter diazotrophicus*, *Haemophilus parainfluenzae*, *Haemophilus sputum*, *Helicobacter canadensis*, *Helicobacter cinaedi*, *Helicobacter mustelae*, *Ilyobacter polytropus*, *Kingella kingae*, *Lactobacillus crispatus*, *Listeria ivanovii*, *Listeria monocytogenes*, *Listeriaceae bacterium*, *Methylocystis sp.*, *Methylosinus trichosporium*, *Mobiluncus mulieris*, *Neisseria bacilliformis*, *Neisseria cinerea*, *Neisseria flavescens*, *Neisseria lactamica*, *Neisseria meningitidis*, *Neisseria sp.*, *Neisseria wadsworthii*, *Nitrosomonas sp.*, *Parvibaculum lavamentivorans*, *Pasteurella multocida*, *Phascolarctobacterium succinatutens*, *Ralstonia syzygii*, *Rhodopseudomonas palustris*, *Rhodovulum sp.*, *Simonsiella muelleri*, *Sphingomonas sp.*, *Sporolactobacillus vineae*, *Staphylococcus aureus*, *Staphylococcus lugdunensis*, *Streptococcus sp.*, *Subdoligranulum sp.*, *Tistrella mobilis*, *Treponema sp.*, or *Verminephrobacter eiseniae*.

**[00259]** In some embodiments, the Cas protein is a naturally-occurring Cas protein. In some embodiments, the Cas endonuclease is selected from the group consisting of C2C1, C2C3, Cpf1 (also referred to as Cas12a), Cas12b, Cas12c, Cas12d, Cas12e, Cas13a, Cas13b, Cas13c, Cas13d, Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas10, Csy1, Csy2, Csy3, Cse1, Cse2, Cse1, Cse2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, and Csf4.

**[00260]** In some embodiments, the Cas protein is an endoribonuclease such as a Cas13 protein. In some embodiments, the Cas13 protein is a Cas13a (Abudayyeh *et al.*, Nature 550 (2017), 280-284), Cas13b (Cox *et al.*, Science (2017) 358:6336, 1019-1027), Cas13c (Cox *et al.*, Science (2017) 358:6336, 1019-1027), or Cas13d (Zhang *et al.*, Cell 175 (2018), 212-223) protein.

**[00261]** In some embodiments, the Cas protein is a wild-type or naturally occurring Cas9 protein or a Cas9 ortholog. Wild-type Cas9 is a multi-domain enzyme that uses an HNH nuclease domain to cleave the target strand of DNA and a RuvC-like domain to cleave the non-target strand. Binding of WT Cas9 to DNA based on gRNA specificity results in double-stranded DNA breaks that can be repaired by non-homologous end joining (NHEJ) or homology-directed repair (HDR). Exemplary naturally occurring Cas9 molecules are described in Chylinski *et al.*, RNA Biology 2013 10:5, 727-737 and additional Cas9 orthologs are described in International PCT Publication No. WO 2015/071474. Such Cas9 molecules include Cas9 molecules of a cluster 1 bacterial family, cluster 2 bacterial family, cluster 3 bacterial family, cluster 4 bacterial family, cluster 5 bacterial family, cluster 6 bacterial family, a cluster 7 bacterial family, a cluster 8 bacterial family, a cluster 9 bacterial family, a cluster 10 bacterial family, a cluster 11 bacterial family, a cluster 12 bacterial family, a cluster 13 bacterial family, a cluster 14 bacterial family, a cluster 15 bacterial family, a cluster 16 bacterial family, a cluster 17 bacterial family, a cluster 18 bacterial family, a cluster 19 bacterial family, a cluster 20 bacterial family, a cluster 21 bacterial family, a cluster 22 bacterial family, a cluster 23 bacterial family, a cluster 24 bacterial family, a cluster 25 bacterial family, a cluster 26 bacterial family, a cluster 27 bacterial family, a cluster 28 bacterial family, a cluster 29 bacterial family, a cluster 30 bacterial family, a cluster 31 bacterial family, a cluster 32 bacterial family, a cluster 33 bacterial family, a cluster 34 bacterial family, a cluster 35 bacterial family, a cluster 36 bacterial family, a cluster 37 bacterial family, a cluster 38 bacterial family, a cluster 39 bacterial family, a cluster 40 bacterial family, a cluster 41 bacterial family, a cluster 42 bacterial family, a cluster 43 bacterial family, a cluster 44 bacterial family, a cluster 45 bacterial family, a cluster 46 bacterial family, a cluster 47 bacterial family, a cluster 48 bacterial family, a cluster 49 bacterial family, a cluster 50 bacterial family, a cluster 51 bacterial family, a cluster 52 bacterial family, a cluster 53 bacterial family, a cluster 54 bacterial family, a cluster 55 bacterial family, a cluster 56 bacterial family, a cluster 57 bacterial family, a cluster 58 bacterial family, a cluster 59 bacterial family, a cluster 60 bacterial family, a cluster 61 bacterial family, a cluster 62 bacterial family, a cluster 63 bacterial family, a cluster 64 bacterial family, a cluster 65 bacterial family, a cluster 66 bacterial family, a cluster 67 bacterial family, a cluster 68 bacterial family, a cluster 69 bacterial family, a cluster 70 bacterial family, a cluster 71 bacterial family, a cluster 72 bacterial family, a cluster 73 bacterial family, a cluster 74 bacterial family, a cluster 75 bacterial family, a cluster 76 bacterial family, a cluster 77 bacterial family, or a cluster 78 bacterial family.

**[00262]** In some embodiments, the naturally occurring Cas9 polypeptide is selected from the group consisting of SpCas9, SpCas9-HF1, SpCas9-HF2, SpCas9-HF3, SpCas9-HF4, SaCas9, FnCpf, FnCas9, eSpCas9, and NmeCas9. In some embodiments, the Cas9 protein comprises an amino acid sequence having at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a Cas9 amino acid sequence described in Chylinski *et al.*, RNA Biology 2013 10:5, 727-737; Hou *et al.*, PNAS Early Edition 2013, 1-6).

**[00263]** In some embodiments, the Cas polypeptide comprises one or more of the following activities:

- (a) a nickase activity, *i.e.*, the ability to cleave a single strand, *e.g.*, the non-complementary strand or the complementary strand, of a nucleic acid molecule;
- (b) a double stranded nuclease activity, *i.e.*, the ability to cleave both strands of a double stranded nucleic acid and create a double stranded break, which in an embodiment is the presence of two nickase activities;
- (c) an endonuclease activity;
- (d) an exonuclease activity; and/or
- (e) a helicase activity, *i.e.*, the ability to unwind the helical structure of a double stranded nucleic acid.

**[00264]** In some embodiments, the Cas polypeptide is fused to heterologous proteins that recruit DNA-damage signaling proteins, exonucleases, or phosphatases to further increase the likelihood or the rate of repair of the target sequence by one repair mechanism or another. In some embodiments, a WT Cas polypeptide is co-expressed with a nucleic acid repair template to facilitate the incorporation of an exogenous nucleic acid sequence by homology-directed repair.

**[00265]** In some embodiments, different Cas proteins (*i.e.*, Cas9 proteins from various species) may be advantageous to use in the various provided methods in order to capitalize on various enzymatic characteristics of the different Cas proteins (*e.g.*, for different PAM sequence preferences; for increased or decreased enzymatic activity; for an increased or decreased level of cellular toxicity; to change the balance between NHEJ, homology-directed repair, single strand breaks, double strand breaks, etc.).

**[00266]** In some embodiments, the Cas protein is a Cas9 protein derived from *S. pyogenes* and recognizes the PAM sequence motif NGG, NAG, NGA (Mali *et al.*, Science 2013; 339(6121): 823-

826). In some embodiments, the Cas protein is a Cas9 protein derived from *S. thermophiles* and recognizes the PAM sequence motif NGGNG and/or NNAGAAW (W = A or T) (See, *e.g.*, Horvath *et al*, Science, 2010; 327(5962): 167-170, and Deveau *et al*, J Bacteriol 2008; 190(4): 1390-1400). In some embodiments, the Cas protein is a Cas9 protein derived from *S. mutans* and recognizes the PAM sequence motif NGG and/or NAAR (R = A or G) (See, *e.g.*, Deveau *et al*, J BACTERIOL 2008; 190(4): 1390-1400). In some embodiments, the Cas protein is a Cas9 protein derived from *S. aureus* and recognizes the PAM sequence motif NNGRR (R = A or G). In some embodiments, the Cas protein is a Cas9 protein derived from *S. aureus* and recognizes the PAM sequence motif N GRRT (R = A or G). In some embodiments, the Cas protein is a Cas9 protein derived from *S. aureus* and recognizes the PAM sequence motif N GRRV (R = A or G). In some embodiments, the Cas protein is a Cas9 protein derived from *N. meningitidis* and recognizes the PAM sequence motif N GATT or N GCTT (R = A or G, V = A, G or C) (See, *e.g.*, Hou *et al*, PNAS 2013, 1-6). In the aforementioned embodiments, N can be any nucleotide residue, *e.g.*, any of A, G, C or T. In some embodiments, the Cas protein is a Cas13a protein derived from *Leptotrichia shahii* and recognizes the PFS sequence motif of a single 3' A, U, or C.

**[00267]** In some embodiments, a polynucleotide encoding a Cas protein is provided. In some embodiments, the polynucleotide encodes a Cas protein that is at least 90% identical to a Cas protein described in International PCT Publication No. WO 2015/071474 or Chylinski *et al.*, RNA Biology 2013 10:5, 727-737. In some embodiments, the polynucleotide encodes a Cas protein that is at least 95%, 96%, 97%, 98%, or 99% identical to a Cas protein described in International PCT Publication No. WO 2015/071474 or Chylinski *et al.*, RNA Biology 2013 10:5, 727-737. In some embodiments, the polynucleotide encodes a Cas protein that is 100% identical to a Cas protein described in International PCT Publication No. WO 2015/071474 or Chylinski *et al.*, RNA Biology 2013 10:5, 727-737.

## 2. Cas Mutants

**[00268]** In some embodiments, the Cas polypeptides are engineered to alter one or more properties of the Cas polypeptide. For example, in some embodiments, the Cas polypeptide comprises altered enzymatic properties, *e.g.*, altered nuclease activity, (as compared with a naturally occurring or other reference Cas molecule) or altered helicase activity. In some embodiments, an engineered Cas polypeptide can have an alteration that alters its size, *e.g.*, a deletion of amino acid sequence that reduces its size without significant effect on another property of the Cas polypeptide. In some embodiments, an engineered Cas polypeptide comprises an alteration that affects PAM recognition.



For example, an engineered Cas polypeptide can be altered to recognize a PAM sequence other than the PAM sequence recognized by the corresponding wild-type Cas protein.

**[00269]** Cas polypeptides with desired properties can be made in a number of ways, including alteration of a naturally occurring Cas polypeptide or parental Cas polypeptide, to provide a mutant or altered Cas polypeptide having a desired property. For example, one or more mutations can be introduced into the sequence of a parental Cas polypeptide (*e.g.*, a naturally occurring or engineered Cas polypeptide). Such mutations and differences may comprise substitutions (*e.g.*, conservative substitutions or substitutions of non-essential amino acids); insertions; or deletions. In some embodiments, a mutant Cas polypeptide comprises one or more mutations (*e.g.*, at least 1, 2, 3, 4, 5, 10, 15, 20, 30, 40 or 50 mutations) relative to a parental Cas polypeptide.

**[00270]** In an embodiment, a mutant Cas polypeptide comprises a cleavage property that differs from a naturally occurring Cas polypeptide. In some embodiments, the Cas is a deactivated Cas (dCas) mutant. In such embodiments, the Cas polypeptide does not comprise any intrinsic enzymatic activity and is unable to mediate target nucleic acid cleavage. In such embodiments, the dCas may be fused with a heterologous protein that is capable of modifying the target nucleic acid in a non-cleavage based manner. For example, in some embodiments, a dCas protein is fused to transcription activator or transcription repressor domains (*e.g.*, the Kruppel associated box (KRAB or SKD); the Mad mSIN3 interaction domain (SID or SID4X); the ERF repressor domain (ERD); the MAX-interacting protein 1 (MXI1); methyl-CpG binding protein 2 (MECP2); etc.). In some such cases, the dCas fusion protein is targeted by the gRNA to a specific location (*i.e.*, sequence) in the target nucleic acid and exerts locus-specific regulation such as blocking RNA polymerase binding to a promoter (which selectively inhibits transcription activator function), and/or modifying the local chromatin status (*e.g.*, when a fusion sequence is used that modifies the target DNA or modifies a polypeptide associated with the target DNA). In some cases, the changes are transient (*e.g.*, transcription repression or activation). In some cases, the changes are inheritable (*e.g.*, when epigenetic modifications are made to the target DNA or to proteins associated with the target DNA, *e.g.*, nucleosomal histones).

**[00271]** In some embodiments, the dCas is a dCas13 mutant (Konermann *et al.*, Cell 173 (2018), 665-676). These dCas13 mutants can then be fused to enzymes that modify RNA, including adenosine deaminases (*e.g.*, ADAR1 and ADAR2). Adenosine deaminases convert adenine to inosine, which the translational machinery treats like guanine, thereby creating a functional A → G change in the RNA sequence. In some embodiments, the dCas is a dCas9 mutant.

**[00272]** In some embodiments, the mutant Cas9 is a Cas9 nickase mutant. Cas9 nickase mutants comprise only one catalytically active domain (either the HNH domain or the RuvC domain). The Cas9 nickase mutants retain DNA binding based on gRNA specificity, but are capable of cutting only one strand of DNA resulting in a single-strand break (*e.g.* a “nick”). In some embodiments, two complementary Cas9 nickase mutants (*e.g.*, one Cas9 nickase mutant with an inactivated RuvC domain, and one Cas9 nickase mutant with an inactivated HNH domain) are expressed in the same cell with two gRNAs corresponding to two respective target sequences; one target sequence on the sense DNA strand, and one on the antisense DNA strand. This dual-nickase system results in staggered double stranded breaks and can increase target specificity, as it is unlikely that two off-target nicks will be generated close enough to generate a double stranded break. In some embodiments, a Cas9 nickase mutant is co-expressed with a nucleic acid repair template to facilitate the incorporation of an exogenous nucleic acid sequence by homology-directed repair.

**[00273]** In some embodiments, the Cas polypeptides described herein can be engineered to alter the PAM/PFS specificity of the Cas polypeptide. In some embodiments, a mutant Cas polypeptide has a PAM/PFS specificity that is different from the PAM/PFS specificity of the parental Cas polypeptide. For example, a naturally occurring Cas protein can be modified to alter the PAM/PFS sequence that the mutant Cas polypeptide recognizes to decrease off target sites, improve specificity, or eliminate a PAM/PFS recognition requirement. In some embodiments, a Cas protein can be modified to increase the length of the PAM/PFS recognition sequence. In some embodiments, the length of the PAM recognition sequence is at least 4, 5, 6, 7, 8, 9, 10 or 15 amino acids in length. Cas polypeptides that recognize different PAM/PFS sequences and/or have reduced off-target activity can be generated using directed evolution. Exemplary methods and systems that can be used for directed evolution of Cas polypeptides are described, *e.g.*, in Esvelt *et al.* Nature 2011, 472(7344): 499-503.

**[00274]** Exemplary Cas mutants are described in International PCT Publication No. WO 2015/161276 and Konermann *et al.*, Cell 173 (2018), 665-676 which are incorporated herein by reference in their entireties.

### 3. gRNAs

**[00275]** The present disclosure provides guide RNAs (gRNAs) that direct a site-directed modifying polypeptide to a specific target nucleic acid sequence. A gRNA comprises a nucleic acid-targeting segment and protein-binding segment. The nucleic acid-targeting segment of a gRNA comprises a nucleotide sequence that is complementary to a sequence in the target nucleic acid

sequence. As such, the nucleic acid-targeting segment of a gRNA interacts with a target nucleic acid in a sequence-specific manner via hybridization (*i.e.*, base pairing), and the nucleotide sequence of the nucleic acid-targeting segment determines the location within the target nucleic acid that the gRNA will bind. The nucleic acid-targeting segment of a gRNA can be modified (*e.g.*, by genetic engineering) to hybridize to any desired sequence within a target nucleic acid sequence.

**[00276]** The protein-binding segment of a guide RNA interacts with a site-directed modifying polypeptide (*e.g.* a Cas protein) to form a complex. The guide RNA guides the bound polypeptide to a specific nucleotide sequence within target nucleic acid via the above-described nucleic acid-targeting segment. The protein-binding segment of a guide RNA comprises two stretches of nucleotides that are complementary to one another and which form a double stranded RNA duplex.

**[00277]** In some embodiments, a gRNA comprises two separate RNA molecules. In such embodiments, each of the two RNA molecules comprises a stretch of nucleotides that are complementary to one another such that the complementary nucleotides of the two RNA molecules hybridize to form the double-stranded RNA duplex of the protein-binding segment. In some embodiments, a gRNA comprises a single RNA molecule (sgRNA).

**[00278]** The specificity of a gRNA for a target locus is mediated by the sequence of the nucleic acid-binding segment, which comprises about 20 nucleotides that are complementary to a target nucleic acid sequence within the target locus. In some embodiments, the corresponding target nucleic acid sequence is approximately 20 nucleotides in length. In some embodiments, the nucleic acid-binding segments of the gRNA sequences of the present disclosure are at least 90% complementary to a target nucleic acid sequence within a target locus. In some embodiments, the nucleic acid-binding segments of the gRNA sequences of the present disclosure are at least 95%, 96%, 97%, 98%, or 99% complementary to a target nucleic acid sequence within a target locus. In some embodiments, the nucleic acid-binding segments of the gRNA sequences of the present disclosure are 100% complementary to a target nucleic acid sequence within a target locus. In some embodiments, the target nucleic acid sequence is an RNA target sequence. In some embodiments, the target nucleic acid sequence is a DNA target sequence.

**[00279]** In some embodiments, the target nucleic acid sequence within the target locus must be changed. For example, changes in the target nucleic acid sequence may occur because the Cas protein being used is changed and the new Cas protein has a different PAM. The description provides many examples of target nucleic acid sequences for gRNAs in the specification and tables provided herein.

Any of these target nucleic acid sequences can be changed by moving the target nucleic acid sequence 5' or 3' within the target locus within a given gene. In some embodiments, the target nucleic acid sequence is moved, at most, 100 bp 5' or 3' within the target locus within a given gene. In other embodiments, the target nucleic acid sequence is moved, at most, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90 or 95 bp 5' or 3' within the target locus within a given gene.

**[00280]** In some embodiments, the gene-regulating system comprises at least two gRNA molecules, wherein at least one gRNA molecule comprises a *SOCS1*-targeting nucleic acid-binding segment (*i.e.*, a *SOCS1*-targeting gRNA). In some embodiments, the nucleic acid-binding segment of the at least one *SOCS1*-targeting gRNA molecules binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence encoded by the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2). In some embodiments, the nucleic acid-binding segment of the at least one *SOCS1*-targeting gRNA molecule binds to a target DNA sequence that is 100% identical to a DNA sequence encoded by the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2).

**[00281]** In some embodiments, the nucleic acid-binding segment of the at least one *SOCS1*-targeting gRNA molecules binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4. In some embodiments, the nucleic acid-binding segment of the at least one *SOCS1*-targeting gRNA molecules binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4. In some embodiments, the nucleic acid-binding segment of the at least one *SOCS1*-targeting gRNA molecules binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 7-151. In some embodiments, the nucleic acid-binding segment of the at least one *SOCS1*-targeting gRNA molecules binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 7-151. Exemplary *SOCS1* target DNA sequences are shown in Tables 12 and 13.

**[00282]** In some embodiments, the nucleic acid-binding segment of the at least one *SOCS1*-targeting gRNA molecules is encoded by a DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 7-151. In some embodiments, the nucleic acid-binding segment of the at least one *SOCS1*-targeting gRNA molecules is encoded by a DNA sequence that is 100% identical to one of SEQ ID NOs: 7-151. Exemplary DNA sequences encoding the nucleic acid-binding segment of the *SOCS1*-targeting gRNAs are shown in Tables 12 and 13.

**Table 12: Exemplary human *SOCS1* gRNA sequences**

| Target         | Sequence              | SEQ ID |
|----------------|-----------------------|--------|
| hSOCS1_gRNA_1  | GCGGCTGCGCGCCGAGCCCG  | 20     |
| hSOCS1_gRNA_2  | GGACGCCTGCGGATTCTACT  | 21     |
| hSOCS1_gRNA_3  | GGCTGCCATCCAGGTGAAAG  | 22     |
| hSOCS1_gRNA_4  | GCGGCTGTCGCGCACCAGGA  | 23     |
| hSOCS1_gRNA_5  | TGGACGCCTGCGGATTCTAC  | 24     |
| hSOCS1_gRNA_6  | GACGCCTGCGGATTCTACTG  | 25     |
| hSOCS1_gRNA_7  | AGTGCTCCAGCAGCTCGAAG  | 26     |
| hSOCS1_gRNA_8  | GCCGGCCGCTTTCACCTGGA  | 27     |
| hSOCS1_gRNA_9  | AGTAGAATCCGCAGGCGTCC  | 28     |
| hSOCS1_gRNA_10 | CGCACCAGGAAGGTGCCCAC  | 29     |
| hSOCS1_gRNA_11 | GGCCGGCCTGAAAGTGCACG  | 30     |
| hSOCS1_gRNA_12 | TCCGTTCGCACGCCGATTAC  | 31     |
| hSOCS1_gRNA_13 | AGCGCGCTCCTGGACGCCTG  | 32     |
| hSOCS1_gRNA_14 | CGGCTGCGCGCCGAGCCCGT  | 33     |
| hSOCS1_gRNA_15 | ACGCCTGCGGATTCTACTGG  | 34     |
| hSOCS1_gRNA_16 | CGAGGCCATCTTCACGCTAA  | 35     |
| hSOCS1_gRNA_17 | TCAGGCCGGCCGCTTTCACC  | 36     |
| hSOCS1_gRNA_18 | CTTAGCGTGAAGATGGCCTC  | 37     |
| hSOCS1_gRNA_19 | GCCGGTAATCGGCGTGCGAA  | 38     |
| hSOCS1_gRNA_20 | CTGCATTGTGCGGCTGCCACC | 39     |
| hSOCS1_gRNA_21 | GTGCGCCCCGTGCACGCTCA  | 40     |
| hSOCS1_gRNA_22 | GCTGTGCCGCCAGCGCATCG  | 41     |
| hSOCS1_gRNA_23 | CACGCGGCGCTGGCGCAGCG  | 42     |
| hSOCS1_gRNA_24 | GCTCCTGCAGCGGCCGCACG  | 43     |
| hSOCS1_gRNA_25 | AGCTCTCGCGGCTGCCATCC  | 44     |
| hSOCS1_gRNA_26 | TGGTGCGCGACAGCCGCCAG  | 45     |
| hSOCS1_gRNA_27 | GATGGTAGCACACAACCAGG  | 46     |
| hSOCS1_gRNA_28 | AGAGGCAGTCGAAGCTCTCG  | 47     |
| hSOCS1_gRNA_29 | GCTGGCGGCTGTCGCGCACC  | 48     |
| hSOCS1_gRNA_30 | CCGAGGCCATCTTCACGCTA  | 49     |
| hSOCS1_gRNA_31 | GGGGCCCCCAGCATGCGGCG  | 50     |
| hSOCS1_gRNA_32 | GCTGCTGGAGCACTACGTGG  | 51     |
| hSOCS1_gRNA_33 | CGAGCTGCTGGAGCACTACG  | 52     |
| hSOCS1_gRNA_34 | CGAAAAAGCAGTTCCGCTGG  | 53     |
| hSOCS1_gRNA_35 | GCAGGCGTCCAGGAGCGCGC  | 54     |
| hSOCS1_gRNA_36 | GGGGCCCCTGAGCGTGCACG  | 55     |
| hSOCS1_gRNA_37 | GCGGCGCCGCGCCGCATGCT  | 56     |
| hSOCS1_gRNA_38 | GCACGCGGCGCTGGCGCAGC  | 57     |
| hSOCS1_gRNA_39 | TGGGGGCCCCTGAGCGTGCA  | 58     |
| hSOCS1_gRNA_40 | CAGGAAGGTGCCCACGGGCT  | 59     |

|                |                       |     |
|----------------|-----------------------|-----|
| hSOCS1 gRNA 41 | TGCGCCCCGTGCACGCTCAG  | 60  |
| hSOCS1 gRNA 42 | GCCATCCAGGTGAAAGCGGC  | 61  |
| hSOCS1 gRNA 43 | CACGCGCGCCAGCGCGCTCC  | 62  |
| hSOCS1 gRNA 44 | GGGCCCCCAGTAGAATCCGC  | 63  |
| hSOCS1 gRNA 45 | ATCCGCGTGCACCTTTCAGGC | 64  |
| hSOCS1 gRNA 46 | CGAGCCCGTGGGCACCTTCC  | 65  |
| hSOCS1 gRNA 47 | CCACAGCAGCAGAGCCCCGA  | 66  |
| hSOCS1 gRNA 48 | AGCCAGGTTCTCGCGGCCCA  | 67  |
| hSOCS1 gRNA 49 | AAAGTGCACGCGGATGCTCG  | 68  |
| hSOCS1 gRNA 50 | CTCTTCCTCCTCCTCGCCCCG | 69  |
| hSOCS1 gRNA 51 | GCGTGCACGGGGCGCACGAG  | 70  |
| hSOCS1 gRNA 52 | AAGTGCACGCGGATGCTCGT  | 71  |
| hSOCS1 gRNA 53 | CGTGCGCCCCGTGCACGCTC  | 72  |
| hSOCS1 gRNA 54 | GCAGCGGCCGCACGCGGCGC  | 73  |
| hSOCS1 gRNA 55 | CCTTAGCGTGAAGATGGCCT  | 74  |
| hSOCS1 gRNA 56 | CAGGTTCTCGCGGCCACGG   | 75  |
| hSOCS1 gRNA 57 | GCGCACCAGGAAGGTGCCCA  | 76  |
| hSOCS1 gRNA 58 | GCTGCCGGTCAAATCTGGAA  | 77  |
| hSOCS1 gRNA 59 | CGGCGTGCGAACGGAATGTG  | 78  |
| hSOCS1 gRNA 60 | CAGCAGCAGAGCCCCGACGG  | 79  |
| hSOCS1 gRNA 61 | GGGCGAAAAAGCAGTTCCGC  | 80  |
| hSOCS1 gRNA 62 | CGCACGCGGCGCTGGCGCAG  | 81  |
| hSOCS1 gRNA 63 | GGATGCGAGCCAGGTTCTCG  | 82  |
| hSOCS1 gRNA 64 | TGGCGGCACAGCTCCTGCAG  | 83  |
| hSOCS1 gRNA 65 | GCGCCCCGCGGCCGTGCCCCG | 84  |
| hSOCS1 gRNA 66 | GGCGCCGCGCCGCATGCTGG  | 85  |
| hSOCS1 gRNA 67 | CGGTGGCCACGATGCGCTGG  | 86  |
| hSOCS1 gRNA 68 | TGCTGTGGAGACTGCATTGT  | 87  |
| hSOCS1 gRNA 69 | TAGGATGGTAGCACACAACC  | 88  |
| hSOCS1 gRNA 70 | GCGGCCGTGCCCCGCGGTCC  | 89  |
| hSOCS1 gRNA 71 | GAGCATCCGCGTGCACCTTC  | 90  |
| hSOCS1 gRNA 72 | CGCTGCCGGTCAAATCTGGA  | 91  |
| hSOCS1 gRNA 73 | CAGCGCATCGTGGCCACCGT  | 92  |
| hSOCS1 gRNA 74 | GCGGATGCTCGTGGGTCCCG  | 93  |
| hSOCS1 gRNA 75 | CGGCGCCGCGCCGCATGCTG  | 94  |
| hSOCS1 gRNA 76 | CGGTCAAATCTGGAAGGGGA  | 95  |
| hSOCS1 gRNA 77 | AGGAAGGTTCTGGCCGCCGT  | 96  |
| hSOCS1 gRNA 78 | CCACGGTGGCCACGATGCGC  | 97  |
| hSOCS1 gRNA 79 | CGCTGCGCCAGCGCCGCGTG  | 98  |
| hSOCS1 gRNA 80 | AGGAGCTCAGGTAGTCGCGG  | 99  |
| hSOCS1 gRNA 81 | GCAGCGGGGCCCCCAGCATG  | 100 |
| hSOCS1 gRNA 82 | GGAAGGAGCTCAGGTAGTCG  | 101 |
| hSOCS1 gRNA 83 | TCGCGGAGGACGGGGTTGAG  | 102 |

|                 |                       |     |
|-----------------|-----------------------|-----|
| hSOCS1 gRNA 84  | CGACTGCCTCTTCGAGCTGC  | 103 |
| hSOCS1 gRNA 85  | GCGCCGCGTGCGGCCGCTGC  | 104 |
| hSOCS1 gRNA 86  | CACCGTGGGCCGCGAGAACC  | 105 |
| hSOCS1 gRNA 87  | GTGCCCCGCGGTCCCGGCC   | 106 |
| hSOCS1 gRNA 88  | CTGCCGTTCAAATCTGGAAG  | 107 |
| hSOCS1 gRNA 89  | CTTCCCCTTCCAGATTTGAC  | 108 |
| hSOCS1 gRNA 90  | CTCAGGTAGTCGCGGAGGAC  | 109 |
| hSOCS1 gRNA 91  | CGGGCGCTGCCGGTCAAATC  | 110 |
| hSOCS1 gRNA 92  | GGAAGGTTCTGGCCGCCGTC  | 111 |
| hSOCS1 gRNA 93  | GCTCAGGTAGTCGCGGAGGA  | 112 |
| hSOCS1 gRNA 94  | GCGGAAGTGCGTGTCGCCGG  | 113 |
| hSOCS1 gRNA 95  | GGACCGCGGGGCACGGCCGC  | 114 |
| hSOCS1 gRNA 96  | GGGACCGCGGGGCACGGCCG  | 115 |
| hSOCS1 gRNA 97  | GCGCGTGATGCGCCGGTAAT  | 116 |
| hSOCS1 gRNA 98  | TCAGGTAGTCGCGGAGGACG  | 117 |
| hSOCS1 gRNA 99  | TGCGGAAGTGCGTGTCGCCG  | 118 |
| hSOCS1 gRNA 100 | GGGGCCGGGACCGCGGGGCA  | 119 |
| hSOCS1 gRNA 101 | CCGTCGGGGCTCTGCTGCTG  | 120 |
| hSOCS1 gRNA 102 | GAAGGTTCTGGCCGCCGTCG  | 121 |
| hSOCS1 gRNA 103 | GTGTGCTACCATCCTACAGA  | 122 |
| hSOCS1 gRNA 104 | GTCGCGGAGGACGGGGTTGA  | 123 |
| hSOCS1 gRNA 105 | CGCTGGCGCGCGTGATGCGC  | 124 |
| hSOCS1 gRNA 106 | GCGTGACGGCGGGCGCTGC   | 125 |
| hSOCS1 gRNA 107 | TCTGGAAGGGGAAGGAGCTC  | 126 |
| hSOCS1 gRNA 108 | GTGCGTGTCGCCGGGGGCCG  | 127 |
| hSOCS1 gRNA 109 | GGGCACGGCCGCGGGCGCGC  | 128 |
| hSOCS1 gRNA 110 | GTTAATGCTGCGTGACGGC   | 129 |
| hSOCS1 gRNA 111 | GCACGGCCGCGGGCGCGCGG  | 130 |
| hSOCS1 gRNA 112 | GGGGCACGGCCGCGGGCGCG  | 131 |
| hSOCS1 gRNA 113 | GTGCGGAAGTGCGTGTCGCC  | 132 |
| hSOCS1 gRNA 114 | GAGGAAGAGGAGGAAGGTTC  | 133 |
| hSOCS1 gRNA 115 | GGCTGGCCCCCTTCTGTAGGA | 134 |
| hSOCS1 gRNA 116 | GGGGCCGGGGCCGGGACCGC  | 135 |
| hSOCS1 gRNA 117 | CGCGGAGGACGGGGTTGAGG  | 136 |
| hSOCS1 gRNA 118 | TTTCGCCCTTAGCGTGAAGA  | 137 |
| hSOCS1 gRNA 119 | GGCACGGCCGCGGGCGCGCG  | 138 |
| hSOCS1 gRNA 120 | AGTCGCGGAGGACGGGGTTG  | 139 |
| hSOCS1 gRNA 121 | GGGCCGGGGCCGGGACCGCG  | 140 |
| hSOCS1 gRNA 122 | AAGTGCGTGTCGCCGGGGGC  | 141 |
| hSOCS1 gRNA 123 | CTCCGGCTGGCCCCTTCTGT  | 142 |
| hSOCS1 gRNA 124 | GGCGGCGCCGCGCCGCATGC  | 143 |
| hSOCS1 gRNA 125 | AGTGCGTGTCGCCGGGGGCC  | 144 |
| hSOCS1 gRNA 126 | TGTGCGGAAGTGCGTGTCGC  | 145 |

|                 |                      |     |
|-----------------|----------------------|-----|
| hSOCS1 gRNA 127 | GTGTCGCCGGGGGCCGGGGC | 146 |
| hSOCS1 gRNA 128 | TGTCGCCGGGGGCCGGGGCC | 147 |
| hSOCS1 gRNA 129 | GCGGTCCCGGCCCGGCCCC  | 148 |
| hSOCS1 gRNA 130 | CGCGGGGGCCGCGGGCGAGG | 149 |
| hSOCS1 gRNA 131 | CGCGGGCGAGGAGGAGGAAG | 150 |
| hSOCS1 gRNA 132 | GGGCGAGGAGGAGGAAGAGG | 151 |

**Table 13: Exemplary murine *Socs1* gRNA sequences**

| Target         | Sequence             | SEQ ID |
|----------------|----------------------|--------|
| mSocs1 gRNA 1  | GAAGTGCACGCGGATGCTCG | 7      |
| mSocs1 gRNA 2  | AGTGCTCCAGCAGCTCGAAA | 8      |
| mSocs1 gRNA 3  | GCCGGCCGCTTCCACTTGGA | 9      |
| mSocs1 gRNA 4  | GCTGTGTCGCCAGCGCATCG | 10     |
| mSocs1 gRNA 5  | GCGACTGTCGCGCACCAAGA | 11     |
| mSocs1 gRNA 6  | GCGTGCACGGGGCGCACGAG | 12     |
| mSocs1 gRNA 7  | TCACGGAGTACCGGGTTAAG | 13     |
| mSocs1 gRNA 8  | GGACGCCTGCGGCTTCTATT | 14     |
| mSocs1 gRNA 9  | GCGCGAAGAAGCAGTTCCGT | 15     |
| mSocs1 gRNA 10 | GCTCAGCGTGAAGATGGCTT | 16     |
| mSocs1 gRNA 11 | CGAGCCCGTGGGCACCTTCT | 17     |
| mSocs1 gRNA 12 | ATCCGCGTGCACTTCCAGGC | 18     |
| mSocs1 gRNA 13 | CGCCAGGTTCTCGCGACCCA | 19     |

**[00283]** In some embodiments, the gene-regulating system comprises at least two gRNA molecules, wherein at least one gRNA molecule comprises a *PTPN2*-targeting nucleic acid-binding segment (*i.e.*, a *PTPN2*-targeting gRNA). In some embodiments, the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA molecules binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence encoded by the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4). In some embodiments, the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA molecules binds to a target DNA sequence that is 100% identical to a DNA sequence encoded by the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4).

**[00284]** In some embodiments, the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA molecules binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6. In some embodiments, the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA



molecules binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6. In some embodiments, the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA molecules binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 185-207. In some embodiments, the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA molecules binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 185-207. Exemplary *PTPN2* target DNA sequences are shown in Tables 14 and 15.

**[00285]** In some embodiments, the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA molecules is encoded by a DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 185-207. In some embodiments, the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA molecules is encoded by a DNA sequence that is 100% identical to one of SEQ ID NOs: 185-207. In some embodiments, the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA molecules is encoded by a DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 272-375. In some embodiments, the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA molecules is encoded by a DNA sequence that is 100% identical to one of SEQ ID NOs: 272-375. In some embodiments, the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA molecules is encoded by a DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 272-308. In some embodiments, the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA molecules is encoded by a DNA sequence that is 100% identical to one of SEQ ID NOs: 272-308. Exemplary DNA sequences encoding the nucleic acid-binding segment of the *PTPN2*-targeting gRNAs are shown in Tables 14 and 15.

**Table 14: Exemplary human *PTPN2* gRNA sequences**

| Target         | Sequence             | SEQ ID |
|----------------|----------------------|--------|
| hPTPN2_gRNA_1  | CCATGCCCACCACCATCGAG | 185    |
| hPTPN2_gRNA_2  | TCTACGGAAACGTATTCGAG | 186    |
| hPTPN2_gRNA_3  | TTTAGTATATTGAGAACTTG | 187    |
| hPTPN2_gRNA_4  | GCACTACAGTGGATCACCGC | 188    |
| hPTPN2_gRNA_5  | TGTCATGCTGAACCGCATTG | 189    |
| hPTPN2_gRNA_6  | GGAAACTTGGCCACTCTATG | 190    |
| hPTPN2_gRNA_7  | GTATTTGAAATTATTAATGC | 191    |
| hPTPN2_gRNA_8  | CAGTTTAGTTGACATAGAAG | 192    |
| hPTPN2_gRNA_9  | GGGTCTGAATAAGACCCATT | 193    |
| hPTPN2_gRNA_10 | CCATGACTATCCTCATAGAG | 194    |

| Target         | Sequence              | SEQ ID |
|----------------|-----------------------|--------|
| hPTPN2_gRNA_11 | CCATGACTATCCTCATAGAG  | 272    |
| hPTPN2_gRNA_12 | CTCTTCGAACTCCCGCTCGA  | 273    |
| hPTPN2_gRNA_13 | GAACCCTGACCATGGGCCTG  | 274    |
| hPTPN2_gRNA_14 | GCTCCTTGAACCCTGACCAT  | 275    |
| hPTPN2_gRNA_15 | AGTTGGATACTCAGCGTCGC  | 276    |
| hPTPN2_gRNA_16 | CCGCTCGATGGTGGTGGGCA  | 277    |
| hPTPN2_gRNA_17 | CAGAAATGGCAGCATGTGTT  | 278    |
| hPTPN2_gRNA_18 | GCACTACAGTGGATCACCGC  | 279    |
| hPTPN2_gRNA_19 | GGTAGACACTTGTCTTGTTT  | 280    |
| hPTPN2_gRNA_20 | TGGCAGCATGTGTTAGGAAG  | 281    |
| hPTPN2_gRNA_21 | AGGCCCATGGTCAGGGTTCA  | 282    |
| hPTPN2_gRNA_22 | G TTCAGCATGACAACTGCTT | 283    |
| hPTPN2_gRNA_23 | CAATGGAGGAGAACAGTGAG  | 284    |
| hPTPN2_gRNA_24 | CTCTTCTATGTCAACTAAAC  | 285    |
| hPTPN2_gRNA_25 | AGTGGATCACCGCAGGCCCA  | 286    |
| hPTPN2_gRNA_26 | CTGACAGGTGACCGATGTAC  | 287    |
| hPTPN2_gRNA_27 | AACTCCCGCTCGATGGTGGT  | 288    |
| hPTPN2_gRNA_28 | GTCTCCCTGATCCATCCAGT  | 289    |
| hPTPN2_gRNA_29 | TAGAGGAAAGTCCTGTACAT  | 290    |
| hPTPN2_gRNA_30 | ATGTATGGAAAGGATGGTAA  | 291    |
| hPTPN2_gRNA_31 | GCCCAATGCCTGCACTACAG  | 292    |
| hPTPN2_gRNA_32 | CGAGCGGGAGTTCGAAGAGT  | 293    |
| hPTPN2_gRNA_33 | TCACCGCAGGCCCATGGTCA  | 294    |
| hPTPN2_gRNA_34 | CAGTTTAGTTGACATAGAAG  | 295    |
| hPTPN2_gRNA_35 | CCATGCCACCAACCATCGAG  | 296    |
| hPTPN2_gRNA_36 | GCCAAACCATAAGCCAGAAA  | 297    |
| hPTPN2_gRNA_37 | CCGATTCTTTCTCCACAATG  | 298    |
| hPTPN2_gRNA_38 | TTCGAACTCCCGCTCGATGG  | 299    |
| hPTPN2_gRNA_39 | AGTGCAGGCATTGGGCGCTC  | 300    |
| hPTPN2_gRNA_40 | GGAAACTTGGCCACTCTATG  | 301    |
| hPTPN2_gRNA_41 | ATCCACTGTAGTGCAGGCAT  | 302    |
| hPTPN2_gRNA_42 | CACTCTATGAGGATAGTCAT  | 303    |
| hPTPN2_gRNA_43 | CCACTCTATGAGGATAGTCA  | 304    |
| hPTPN2_gRNA_44 | TCCACTGTAGTGCAGGCATT  | 305    |
| hPTPN2_gRNA_45 | AAGTTCTTTCCATCGTTTCT  | 306    |
| hPTPN2_gRNA_46 | TCGCTGGCAGCCGCTGTACT  | 307    |
| hPTPN2_gRNA_47 | GAAGTCCCGCTCGATGGTGG  | 308    |
| hPTPN2_gRNA_48 | AGGATGGTAAAGGCACCAAC  | 309    |
| hPTPN2_gRNA_49 | AAAGGGAGATTCTAGTATAC  | 310    |
| hPTPN2_gRNA_50 | AGAATTTAGGATGTATGGAA  | 311    |
| hPTPN2_gRNA_51 | GGGTCTGAATAAGACCCATT  | 312    |
| hPTPN2_gRNA_52 | GGCACCAACTGGATGGATCA  | 313    |

| Target         | Sequence             | SEQ ID |
|----------------|----------------------|--------|
| hPTPN2_gRNA_53 | CTCTAAAATGCAAGATACAA | 314    |
| hPTPN2_gRNA_54 | GTATTTGAAATTATTAATGC | 315    |
| hPTPN2_gRNA_55 | CCTTTCTTGCAGATGGAAAA | 316    |
| hPTPN2_gRNA_56 | CTGCACCTTCTGAGCTGTGG | 317    |
| hPTPN2_gRNA_57 | ATGCTGCCATTTCTGGCTTA | 318    |
| hPTPN2_gRNA_58 | TTTCTTTAAACAGCATCTCT | 319    |
| hPTPN2_gRNA_59 | AGACATGGAATGCAGAATGC | 320    |
| hPTPN2_gRNA_60 | AGGCACCAACTGGATGGATC | 321    |
| hPTPN2_gRNA_61 | TAATGACTGAAAAATACAAT | 322    |
| hPTPN2_gRNA_62 | GAATGCAGAATGCAGGAAAT | 323    |
| hPTPN2_gRNA_63 | TTTAGGATGTATGGAAAGGA | 324    |
| hPTPN2_gRNA_64 | CTAACACATGCTGCCATTTC | 325    |
| hPTPN2_gRNA_65 | TCATACATGGCTATAATAGA | 326    |
| hPTPN2_gRNA_66 | ACGATGGAAAGAACTTTCTA | 327    |
| hPTPN2_gRNA_67 | ACGTATTCGAGAGGACAGAA | 328    |
| hPTPN2_gRNA_68 | GCGGTGATCCACTGTAGTGC | 329    |
| hPTPN2_gRNA_69 | TATTAATGCTGGATGTTAAA | 330    |
| hPTPN2_gRNA_70 | GAGATGCTGTTTAAAGAAAC | 331    |
| hPTPN2_gRNA_71 | CAGCAAGAATTTAGGATGTA | 332    |
| hPTPN2_gRNA_72 | TTGACATAGAAGAGGCACAA | 333    |
| hPTPN2_gRNA_73 | GATTCAGGGACTCCAAAATC | 334    |
| hPTPN2_gRNA_74 | CTCACTTTCATTATACTACC | 335    |
| hPTPN2_gRNA_75 | TTTAGTATATTGAGAACTTG | 336    |
| hPTPN2_gRNA_76 | AGGGACTCCAAAATCTGGCC | 337    |
| hPTPN2_gRNA_77 | AGGTAAATGTGCACAGTAC  | 338    |
| hPTPN2_gRNA_78 | ATCACCGCAGGCCCATGGTC | 339    |
| hPTPN2_gRNA_79 | AGCATCTCTTGGTCATCTGT | 340    |
| hPTPN2_gRNA_80 | GAAGGAGCAAAATGTATAAA | 341    |
| hPTPN2_gRNA_81 | GCCATTTCTGGCTTATGGTT | 342    |
| hPTPN2_gRNA_82 | CTGGATGGATCAGGGAGACA | 343    |
| hPTPN2_gRNA_83 | AAATACAATGGGAACAGAAT | 344    |
| hPTPN2_gRNA_84 | ATAATGACTGAAAAATACAA | 345    |
| hPTPN2_gRNA_85 | CATGCCCACCACCATCGAGC | 346    |
| hPTPN2_gRNA_86 | AACATGAGAAAATACCGAAT | 347    |
| hPTPN2_gRNA_87 | AGAAATGAAGCTGGTGATTC | 348    |
| hPTPN2_gRNA_88 | CCGCATTGTGGAGAAAGAAT | 349    |
| hPTPN2_gRNA_89 | GAAATGAAGCTGGTGATTCA | 350    |
| hPTPN2_gRNA_90 | TTGTTTAAAGTGAGAGAATC | 351    |
| hPTPN2_gRNA_91 | CCGCGACTCACCAAGTACAG | 352    |
| hPTPN2_gRNA_92 | GAACATGAGAAAATACCGAA | 353    |
| hPTPN2_gRNA_93 | TATACTACCTGGCCAGATTT | 354    |
| hPTPN2_gRNA_94 | TATGAGAATCTCAGTTGATC | 355    |

| Target          | Sequence             | SEQ ID |
|-----------------|----------------------|--------|
| hPTPN2_gRNA_95  | TCAACTGAGATTCTCATACA | 356    |
| hPTPN2_gRNA_96  | TGAGAATCTCAGTTGATCTG | 357    |
| hPTPN2_gRNA_97  | ATGAGAATCTCAGTTGATCT | 358    |
| hPTPN2_gRNA_98  | TGGTAAAGGCACCAACTGGA | 359    |
| hPTPN2_gRNA_99  | TGTCATGCTGAACCGCATTG | 360    |
| hPTPN2_gRNA_100 | TTTGGTGAATGATCAAAGGC | 361    |
| hPTPN2_gRNA_101 | ATGAAAGTGAGATATTGTTC | 362    |
| hPTPN2_gRNA_102 | TATTTCTCATAGTGCTCTA  | 363    |
| hPTPN2_gRNA_103 | AGAAGGAGCAAAATGTATAA | 364    |
| hPTPN2_gRNA_104 | TTTGTTTGGTGAATGATCAA | 365    |
| hPTPN2_gRNA_105 | TCTACGGAAACGTATTCGAG | 366    |
| hPTPN2_gRNA_106 | AAAGGCCACCACAGCTCAGA | 367    |
| hPTPN2_gRNA_107 | AGGTGCAGCAGATGAAACAG | 368    |
| hPTPN2_gRNA_108 | GGCTCCTTGAACCTGACCA  | 369    |
| hPTPN2_gRNA_109 | AAGGAGTTACATCTTAACAC | 370    |
| hPTPN2_gRNA_110 | TAAAATGCAAGATACAATGG | 371    |
| hPTPN2_gRNA_111 | ACAAGTGTCTACCAGAGAGA | 372    |
| hPTPN2_gRNA_112 | GCGCTCTGGCACCTTCTCTC | 373    |
| hPTPN2_gRNA_113 | CTGCTGCACCTTCTGAGCTG | 374    |
| hPTPN2_gRNA_114 | TCTTCCCTACCTAGAAACGA | 375    |

Table 15: Exemplary murine *Ptpn2* gRNA sequences

| Target         | Sequence              | SEQ ID |
|----------------|-----------------------|--------|
| mPTPN2_gRNA_1  | AATCTGGCCAGGTGGTATAA  | 195    |
| mPTPN2_gRNA_2  | AATATGAGAAAGTATCGAAT  | 196    |
| mPTPN2_gRNA_3  | ATCACTGCAGGTCCATGGTC  | 197    |
| mPTPN2_gRNA_4  | ATGTGCACAGTACTGGCCAA  | 198    |
| mPTPN2_gRNA_5  | GGCAGCATGTGTTCGGAAGT  | 199    |
| mPTPN2_gRNA_6  | AAGAAGTTTAGAAATGAAGC  | 200    |
| mPTPN2_gRNA_7  | GCCACACCATGAGCCAGAAA  | 201    |
| mPTPN2_gRNA_8  | CCTTTCTTGCAGATGGAAAA  | 202    |
| mPTPN2_gRNA_9  | GTA CTTTGCTCCTTCTATTA | 203    |
| mPTPN2_gRNA_10 | AGAAATGAAGCTGGTGACTC  | 204    |
| mPTPN2_gRNA_11 | GTTTAGCATGACAACTGCTT  | 205    |
| mPTPN2_gRNA_12 | GCCCGATGCCCGCACTGCAA  | 206    |
| mPTPN2_gRNA_13 | TGACAGAGAAATGGTGT TTA | 207    |

[00286] In some embodiments, the gene-regulating system comprises at least two gRNA molecules, wherein at least one gRNA molecule comprises a *ZC3H12A*-targeting nucleic acid-binding segment (*i.e.*, a *ZC3H12A*-targeting gRNA). In some embodiments, the nucleic acid-binding segment

of the at least one *ZC3H12A*-targeting gRNA molecules binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence encoded by the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6). In some embodiments, the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA molecules binds to a target DNA sequence that is 100% identical to a DNA sequence encoded by the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6).

**[00287]** In some embodiments, the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA molecules binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8. In some embodiments, the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA molecules binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8. In some embodiments, the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA molecules binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 208-230. In some embodiments, the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA molecules binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 208-230. Exemplary *ZC3H12A* target DNA sequences are shown in Tables 16 and 17.

**[00288]** In some embodiments, the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA molecules is encoded by a DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 208-230. In some embodiments, the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA molecules is encoded by a DNA sequence that is 100% identical to one of SEQ ID NOs: 208-230. In some embodiments, the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA molecules is encoded by a DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 376-812. In some embodiments, the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA molecules is encoded by a DNA sequence that is 100% identical to one of SEQ ID NOs: 376-812. In some embodiments, the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA molecules is encoded by a DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 376-575. In some embodiments, the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA molecules is encoded by a DNA sequence that is 100% identical to one of SEQ ID NOs: 376-575. Exemplary DNA sequences encoding the nucleic acid-binding segment of the *ZC3H12A*-targeting gRNAs are shown in Tables 16 and 17.

**Table 16: Exemplary human *ZC3H12A* gRNA sequences**

| Target           | Sequence              | SEQ ID |
|------------------|-----------------------|--------|
| hZC2H12A_gRNA_1  | AAGCTGGCCTACGAGTCTGA  | 216    |
| hZC2H12A_gRNA_2  | GCGGGACTAGAGGGAGCTGA  | 217    |
| hZC2H12A_gRNA_3  | CAGCTCCCTCTAGTCCCGCG  | 218    |
| hZC2H12A_gRNA_4  | CAGGACGCTGTGGATCTCCG  | 219    |
| hZC2H12A_gRNA_5  | AACATACTTGTCATTGACGA  | 220    |
| hZC2H12A_gRNA_6  | CTCACCTGTGATGGGCACGT  | 221    |
| hZC2H12A_gRNA_7  | AACACGGGACAGCCACCGAG  | 222    |
| hZC2H12A_gRNA_8  | CGACAGATTTCATTGTGAAGC | 223    |
| hZC2H12A_gRNA_9  | ACACCATCACGACGCGTGGG  | 224    |
| hZC2H12A_gRNA_10 | TCCCAGCCATGGGAACAAGG  | 225    |
| hZC2H12A_gRNA_11 | GGAGTGGAAAGCGCTTCATCG | 226    |
| hZC2H12A_gRNA_12 | TTAGGGGTGCCACCACCCCG  | 227    |
| hZC2H12A_gRNA_13 | GACACATACCGTGACCTCCA  | 228    |
| hZC2H12A_gRNA_14 | CCGGCCCAGTGGGTCATCAG  | 229    |
| hZC2H12A_gRNA_15 | CCTGGAAGTGCAGATGAAGG  | 230    |
| hZC3H12A_gRNA_16 | GTCCTCTCCCTCCCAGCCAT  | 376    |
| hZC3H12A_gRNA_17 | TCCCCAGGGTCCCGCCAAGA  | 377    |
| hZC3H12A_gRNA_18 | AGTGAGCAGTGCAGCCTGGA  | 378    |
| hZC3H12A_gRNA_19 | TGTCCTCTCCCTCCCAGCCA  | 379    |
| hZC3H12A_gRNA_20 | CTGGACTGGGATGAAGGTGA  | 380    |
| hZC3H12A_gRNA_21 | GGGGTGGGCCCCGGCTCACCA | 381    |
| hZC3H12A_gRNA_22 | CACCACCCCGCGGGACTAGA  | 382    |
| hZC3H12A_gRNA_23 | CTGCTGCCACTGCCCCCGCT  | 383    |
| hZC3H12A_gRNA_24 | CGGCCCCGACGTGCCCATCAC | 384    |
| hZC3H12A_gRNA_25 | CACTGCCCCCGCTAGGTGCG  | 385    |
| hZC3H12A_gRNA_26 | ATACACGCTGGCCTGCTCCT  | 386    |
| hZC3H12A_gRNA_27 | CAAACACTGTGATGTCTGTG  | 387    |
| hZC3H12A_gRNA_28 | GCGGGACCCTGGGGATGCCT  | 388    |
| hZC3H12A_gRNA_29 | GCGGGAGCGCCAGACCTCAC  | 389    |
| hZC3H12A_gRNA_30 | AGGACAGGCTTCTCTCCACA  | 390    |
| hZC3H12A_gRNA_31 | GCAGACACCAACACGGTGCT  | 391    |
| hZC3H12A_gRNA_32 | CCACCACCCCGCGGGACTAG  | 392    |
| hZC3H12A_gRNA_33 | ATCCCCAGGGTCCCGCCAAG  | 393    |
| hZC3H12A_gRNA_34 | CCTGGAGGAAGGAGCAGCCT  | 394    |
| hZC3H12A_gRNA_35 | AGAGCCAGATGTCGGAACCT  | 395    |
| hZC3H12A_gRNA_36 | ATGACCCACTGGGCCGCGCAC | 396    |
| hZC3H12A_gRNA_37 | GCAGCTTTGGGCCACAGAC   | 397    |
| hZC3H12A_gRNA_38 | ACTCTCTGTTAGCAGAGAGC  | 398    |
| hZC3H12A_gRNA_39 | CCAGGAAGGAAATGCACCTA  | 399    |
| hZC3H12A_gRNA_40 | AGGCACCACTCACCTGTGAT  | 400    |
| hZC3H12A_gRNA_41 | CTGGGCCCGTGCCGGCCCAG  | 401    |

| Target           | Sequence             | SEQ ID |
|------------------|----------------------|--------|
| hZC3H12A_gRNA_42 | CAGCCAGCTGCTGGGGGTCC | 402    |
| hZC3H12A_gRNA_43 | TCCACTCCTGCCGCTCGCCT | 403    |
| hZC3H12A_gRNA_44 | CGTCCAGGCAGACACCAACA | 404    |
| hZC3H12A_gRNA_45 | CCCACCCACATCAGTCCTTC | 405    |
| hZC3H12A_gRNA_46 | GCCAGCTCTTGACCCGGCCT | 406    |
| hZC3H12A_gRNA_47 | CTGCCCTCCTTTTCCTCTTC | 407    |
| hZC3H12A_gRNA_48 | CCAGCCCCACCATGAGTCTG | 408    |
| hZC3H12A_gRNA_49 | GCCGATTCTTCCACCCAGAG | 409    |
| hZC3H12A_gRNA_50 | CTCCCAGAAGAGGAAAAGGA | 410    |
| hZC3H12A_gRNA_51 | GTGGGGCAGGGCAGGCAGCC | 411    |
| hZC3H12A_gRNA_52 | GGGTCAAGAGCTGGCCGCTG | 412    |
| hZC3H12A_gRNA_53 | ATGCCCCCTGATGACCCACT | 413    |
| hZC3H12A_gRNA_54 | AGCCTTCTCTGCCTTTGGCC | 414    |
| hZC3H12A_gRNA_55 | CTCTGCCTTTGGCCGGGCCA | 415    |
| hZC3H12A_gRNA_56 | GGAACCCAGCCTGCCCTCCC | 416    |
| hZC3H12A_gRNA_57 | GGCAGGAGCCTCGCACCTAG | 417    |
| hZC3H12A_gRNA_58 | TCCCAGACCAGCACATCCTG | 418    |
| hZC3H12A_gRNA_59 | GTGAGCAGTGCAGCCTGGAT | 419    |
| hZC3H12A_gRNA_60 | GAGCCAGATGTCGGAACCTT | 420    |
| hZC3H12A_gRNA_61 | GGCCGATGGCAAGCCTTGCT | 421    |
| hZC3H12A_gRNA_62 | AGGAGCCTCGCACCTAGCGG | 422    |
| hZC3H12A_gRNA_63 | AGGTCCCCAAGAGGAAAACA | 423    |
| hZC3H12A_gRNA_64 | CGCTGAGGAGGCCTCGGCCC | 424    |
| hZC3H12A_gRNA_65 | GAGGACAGCCACAGCCGTCA | 425    |
| hZC3H12A_gRNA_66 | CAGCCCCACCATGAGTCTGT | 426    |
| hZC3H12A_gRNA_67 | ACCCCCCAGAGCCCCAAGCA | 427    |
| hZC3H12A_gRNA_68 | GAGGCACCACTCACCTGTGA | 428    |
| hZC3H12A_gRNA_69 | CCAAGAGGAAAACAGGGCAC | 429    |
| hZC3H12A_gRNA_70 | GTACGTCTCCCAGGATTGCC | 430    |
| hZC3H12A_gRNA_71 | CACAGCCTCCACCAGGTGCG | 431    |
| hZC3H12A_gRNA_72 | GATCTCGGCAGCCAGCTGCT | 432    |
| hZC3H12A_gRNA_73 | CAGCCTTCTCTGCCTTTGGC | 433    |
| hZC3H12A_gRNA_74 | CAGAAGTGACACTTACCTCA | 434    |
| hZC3H12A_gRNA_75 | GCTGGCCGCTGAGGAGGCCT | 435    |
| hZC3H12A_gRNA_76 | CAGCTCCCTCTAGTCCCGCG | 436    |
| hZC3H12A_gRNA_77 | CGGGGTGGGCCCGGCTCACC | 437    |
| hZC3H12A_gRNA_78 | GACACATACCGTGACCTCCA | 438    |
| hZC3H12A_gRNA_79 | CAGGAAGGAAATGCACCTAT | 439    |
| hZC3H12A_gRNA_80 | AGTGGCCAGCACCCATGGCC | 440    |
| hZC3H12A_gRNA_81 | CTCTCCTATTCTTCCCAGCA | 441    |
| hZC3H12A_gRNA_82 | GCCCCAGTCCAGGCAATCCT | 442    |
| hZC3H12A_gRNA_83 | CACCTTCATCTGCAGTTCCA | 443    |

| Target            | Sequence              | SEQ ID |
|-------------------|-----------------------|--------|
| hZC3H12A_gRNA_84  | GGCACAGGCAGACAGGTGAG  | 444    |
| hZC3H12A_gRNA_85  | AGCACCCATGGCCCGGCCAA  | 445    |
| hZC3H12A_gRNA_86  | CCACAGGCAGCTTACTCACT  | 446    |
| hZC3H12A_gRNA_87  | TTCCTGTGCTCCAAAGTGAG  | 447    |
| hZC3H12A_gRNA_88  | ACCGCAGCCTTCTCTGCCTT  | 448    |
| hZC3H12A_gRNA_89  | GGGAGCCAATGCCCCGAGTCC | 449    |
| hZC3H12A_gRNA_90  | TTCCCAGCAAGGCTTGCCAT  | 450    |
| hZC3H12A_gRNA_91  | AGCCAGATGTTCGGAACCTTG | 451    |
| hZC3H12A_gRNA_92  | TACACGGGCTACAGTCCCTA  | 452    |
| hZC3H12A_gRNA_93  | TCTGTGTAGACCCTCTTGG   | 453    |
| hZC3H12A_gRNA_94  | AAGCTGCCCCCAGCGCTCTG  | 454    |
| hZC3H12A_gRNA_95  | CTTTGGGGGGTTCGAGGAGG  | 455    |
| hZC3H12A_gRNA_96  | GGGCCGATGGCAAGCCTTGC  | 456    |
| hZC3H12A_gRNA_97  | CACAGGCAGCTTACTCACTG  | 457    |
| hZC3H12A_gRNA_98  | CCCAGACCAGCACATCCTGC  | 458    |
| hZC3H12A_gRNA_99  | AGGCTGGGTTCATACCATA   | 459    |
| hZC3H12A_gRNA_100 | GGACTTCTAATTGCTGAGAA  | 460    |
| hZC3H12A_gRNA_101 | CTCAAATTCCCACAGACTCA  | 461    |
| hZC3H12A_gRNA_102 | AAAACAGGGCACAGGCAGAC  | 462    |
| hZC3H12A_gRNA_103 | CCAGATGTTCGGAACCTTGGG | 463    |
| hZC3H12A_gRNA_104 | CTCCCTCTAGTCCCAGCGGG  | 464    |
| hZC3H12A_gRNA_105 | AGCCCCCAGTGCAGAGCCCA  | 465    |
| hZC3H12A_gRNA_106 | CCTGGACGCCCAGCTTCTGC  | 466    |
| hZC3H12A_gRNA_107 | CAGGGGCTGGCAGGAGCCCG  | 467    |
| hZC3H12A_gRNA_108 | CCTTGTTCCCATGGCTGGGA  | 468    |
| hZC3H12A_gRNA_109 | CTCATCTGCCACAGAGCGCT  | 469    |
| hZC3H12A_gRNA_110 | GGCAGACACCAACACGGTGC  | 470    |
| hZC3H12A_gRNA_111 | TCCCTCTTGATTCTCTTCC   | 471    |
| hZC3H12A_gRNA_112 | CCCTCCCAGCCATGGGAACA  | 472    |
| hZC3H12A_gRNA_113 | GCGTAAGAAGCCACTCACTT  | 473    |
| hZC3H12A_gRNA_114 | TGTGTTTCCCCCGCACCTGG  | 474    |
| hZC3H12A_gRNA_115 | CTGAGACCAGTGGTCATCGA  | 475    |
| hZC3H12A_gRNA_116 | GGGCAGCGACCTGAGACCAG  | 476    |
| hZC3H12A_gRNA_117 | AGCAATTAGAAGTCCCTGCA  | 477    |
| hZC3H12A_gRNA_118 | TGGGTGAGCTGGTGAAACAC  | 478    |
| hZC3H12A_gRNA_119 | CTGTTAGCAGAGAGCTGGAC  | 479    |
| hZC3H12A_gRNA_120 | CCCCTGATGACCCACTGGGC  | 480    |
| hZC3H12A_gRNA_121 | GTTACACCATCACGACGCG   | 481    |
| hZC3H12A_gRNA_122 | TGTCCAGGCTGGGCCCCGTGC | 482    |
| hZC3H12A_gRNA_123 | ACACAGACCTATGCCCCATC  | 483    |
| hZC3H12A_gRNA_124 | GGCTGCCTGCCCTGCCCCAC  | 484    |
| hZC3H12A_gRNA_125 | CCATAGGTGCATTTCCTTCC  | 485    |



| Target            | Sequence               | SEQ ID |
|-------------------|------------------------|--------|
| hZC3H12A_gRNA_126 | CAGGCTGGGTTCATACCAT    | 486    |
| hZC3H12A_gRNA_127 | GCCCCATCACAGCCTCCACC   | 487    |
| hZC3H12A_gRNA_128 | TGCCCTCCTTTTCCTCTTCT   | 488    |
| hZC3H12A_gRNA_129 | GCCAGATGTCGGAACCTTGG   | 489    |
| hZC3H12A_gRNA_130 | CAGGCAGACAGGTGAGAGGA   | 490    |
| hZC3H12A_gRNA_131 | CCAGGAGTCTGAGCTATGAG   | 491    |
| hZC3H12A_gRNA_132 | GCTCCAGGTGTTGGGAGCCTTA | 492    |
| hZC3H12A_gRNA_133 | CTCACCTGTGATGGGCACGT   | 493    |
| hZC3H12A_gRNA_134 | AGCTGGCCTACGAGTCTGAC   | 494    |
| hZC3H12A_gRNA_135 | GTGGGTGGGGGCAGTGGGTGA  | 495    |
| hZC3H12A_gRNA_136 | CATCTGCAGTTCCAGGGCCG   | 496    |
| hZC3H12A_gRNA_137 | GATGACCCACTGGGCCGGCA   | 497    |
| hZC3H12A_gRNA_138 | TGACCTCCAAGGCGAGCGGC   | 498    |
| hZC3H12A_gRNA_139 | GGATCTCGGCAGCCAGCTGC   | 499    |
| hZC3H12A_gRNA_140 | TCCTTTTCCTCTTCTGGGAG   | 500    |
| hZC3H12A_gRNA_141 | CACGACGCGTGGGTGGCAAG   | 501    |
| hZC3H12A_gRNA_142 | TTCACACCATCACGACGCGT   | 502    |
| hZC3H12A_gRNA_143 | GCAGGAGCCTCGCACCTAGC   | 503    |
| hZC3H12A_gRNA_144 | CACCCCTAAGGCTCCCAACC   | 504    |
| hZC3H12A_gRNA_145 | TTGTCCTTGCTTGGGGCTCT   | 505    |
| hZC3H12A_gRNA_146 | CAGGACAGGCTTCTCTCCAC   | 506    |
| hZC3H12A_gRNA_147 | CACCTGGTGGAGGCTGTGAT   | 507    |
| hZC3H12A_gRNA_148 | CGTCTGTGGGAGCCAGTCTG   | 508    |
| hZC3H12A_gRNA_149 | CCCCCAAAGTTCCGACATC    | 509    |
| hZC3H12A_gRNA_150 | AGGCAGCCTGGCCAAGGAGC   | 510    |
| hZC3H12A_gRNA_151 | TCTGCCTTTGGCCGGGCCAT   | 511    |
| hZC3H12A_gRNA_152 | GGACAGGCTTCTCTCCACAG   | 512    |
| hZC3H12A_gRNA_153 | ACGTGCCCATCACAGGTGAG   | 513    |
| hZC3H12A_gRNA_154 | AGAGAGTGAGCAGTGCAGCC   | 514    |
| hZC3H12A_gRNA_155 | CGCAGGAAGTTGTCCAGGCT   | 515    |
| hZC3H12A_gRNA_156 | GGCTGGGAGCTCAGATCCAT   | 516    |
| hZC3H12A_gRNA_157 | CAGCTCACCCAGCACCGTGT   | 517    |
| hZC3H12A_gRNA_158 | CCAGCACATCCTGCGGGAAC   | 518    |
| hZC3H12A_gRNA_159 | GACCTCCTTGTTCCCATGGC   | 519    |
| hZC3H12A_gRNA_160 | GGGGTTCGAGGAGGAGGCC    | 520    |
| hZC3H12A_gRNA_161 | CAGAGAAGGCTGCGGTGGCT   | 521    |
| hZC3H12A_gRNA_162 | GGGAGTGAGTCCAGCGTCTG   | 522    |
| hZC3H12A_gRNA_163 | CAGGAGCCTCGCACCTAGCG   | 523    |
| hZC3H12A_gRNA_164 | GGAGGAGGCCCTGGTGAGCC   | 524    |
| hZC3H12A_gRNA_165 | CAAGCAAGGACAAAAATGGC   | 525    |
| hZC3H12A_gRNA_166 | CGTCAGGGCACCCCAAGGCC   | 526    |
| hZC3H12A_gRNA_167 | GCTGGCAGTGAACCTGGTTTC  | 527    |

| Target            | Sequence              | SEQ ID |
|-------------------|-----------------------|--------|
| hZC3H12A_gRNA_168 | ACCTCCTTGTTCCCATGGCT  | 528    |
| hZC3H12A_gRNA_169 | TCCCGCAGGATGTGCTGGTC  | 529    |
| hZC3H12A_gRNA_170 | AGGGACTGTAGCCCGTGTA   | 530    |
| hZC3H12A_gRNA_171 | CCAGTACTCTCGAGGTGGAA  | 531    |
| hZC3H12A_gRNA_172 | AATTCCCACAGACTCATGGT  | 532    |
| hZC3H12A_gRNA_173 | CCCACCCCGAGCCCCTTACA  | 533    |
| hZC3H12A_gRNA_174 | GTGCATTTCCTTCCTGGAAG  | 534    |
| hZC3H12A_gRNA_175 | TCAGCGGCCAGCTCTTGACC  | 535    |
| hZC3H12A_gRNA_176 | GGCCCGGCCAAAGGCAGAGA  | 536    |
| hZC3H12A_gRNA_177 | ACAGAGCGCTGGGGGCAGCT  | 537    |
| hZC3H12A_gRNA_178 | TCTTGATTCTCTTCCAGGA   | 538    |
| hZC3H12A_gRNA_179 | GCAAGGACAAAAATGGCCGG  | 539    |
| hZC3H12A_gRNA_180 | CAGGGCAGGCAGCCTGGCCA  | 540    |
| hZC3H12A_gRNA_181 | ATCTCGGCAGCCAGCTGCTG  | 541    |
| hZC3H12A_gRNA_182 | CCCGCAGGATGTGCTGGTCT  | 542    |
| hZC3H12A_gRNA_183 | GGCTCCAGGTTGGGAGCCTT  | 543    |
| hZC3H12A_gRNA_184 | CAACACGGTGCTGGGTGAGC  | 544    |
| hZC3H12A_gRNA_185 | GCAGCCGTGTCCCTATGGTA  | 545    |
| hZC3H12A_gRNA_186 | TGTCCTTGCTTGGGGCTCTG  | 546    |
| hZC3H12A_gRNA_187 | TCATGGTGGGGCTGGCTTCC  | 547    |
| hZC3H12A_gRNA_188 | GAAGCTGGGCTATTCATCCA  | 548    |
| hZC3H12A_gRNA_189 | GACCCTCTTGGCGGGACCCT  | 549    |
| hZC3H12A_gRNA_190 | GGAAAGGCAGGGGGCGCGGG  | 550    |
| hZC3H12A_gRNA_191 | AGGTCTGTGTTAGACCCTCT  | 551    |
| hZC3H12A_gRNA_192 | CTCAGCTCCCTCTAGTCCCG  | 552    |
| hZC3H12A_gRNA_193 | TAGGGACTGTAGCCCGTGTA  | 553    |
| hZC3H12A_gRNA_194 | AGGGGGCATAAACCTGCAGA  | 554    |
| hZC3H12A_gRNA_195 | CTCCCAGGATTGCCTGGACT  | 555    |
| hZC3H12A_gRNA_196 | GGGATGAAGGTGAAGGCCGC  | 556    |
| hZC3H12A_gRNA_197 | TGCAGAGCCCAGGGGCTGGC  | 557    |
| hZC3H12A_gRNA_198 | GAATCGGCACTTGATCCCAT  | 558    |
| hZC3H12A_gRNA_199 | CCGAGGCTGCTCCTTCCTCC  | 559    |
| hZC3H12A_gRNA_200 | CCAGCTTCTGCAGGACGCTG  | 560    |
| hZC3H12A_gRNA_201 | GGGCCGGCACGGGCCAGCC   | 561    |
| hZC3H12A_gRNA_202 | TGAGGTCTGGCGCTCCCGCT  | 562    |
| hZC3H12A_gRNA_203 | TTGGGGTGCCCTGACGGCTG  | 563    |
| hZC3H12A_gRNA_204 | ACTAGAGGGAGCTGAGGGCA  | 564    |
| hZC3H12A_gRNA_205 | CCAGTTCCCGCAGGATGTGC  | 565    |
| hZC3H12A_gRNA_206 | TATGCCCCCTGATGACCCAC  | 566    |
| hZC3H12A_gRNA_207 | GTGAGAGGAGAGCATTTGGCA | 567    |
| hZC3H12A_gRNA_208 | AGCTTACTCACTGGGGTGCT  | 568    |
| hZC3H12A_gRNA_209 | ATCACAGCCTCCACCAGGTG  | 569    |

| Target            | Sequence              | SEQ ID |
|-------------------|-----------------------|--------|
| hZC3H12A_gRNA_210 | ACTGAAGTGGCCAGCACCCA  | 570    |
| hZC3H12A_gRNA_211 | GCCGGCCCAGTGGGTCATCA  | 571    |
| hZC3H12A_gRNA_212 | CCTGCAGAAAGCTGGGCGTCC | 572    |
| hZC3H12A_gRNA_213 | GCACCGTGTGTGGTGTCTGCC | 573    |
| hZC3H12A_gRNA_214 | GGCCCTGGAAGTGCAGATGA  | 574    |
| hZC3H12A_gRNA_215 | GTCCTTGCTTGGGGCTCTGG  | 575    |
| hZC3H12A_gRNA_216 | CTCCCTGGAGAGCCAGATGT  | 576    |
| hZC3H12A_gRNA_217 | AAATTCCCACAGACTCATGG  | 577    |
| hZC3H12A_gRNA_218 | TCATCTGCCACAGAGCGCTG  | 578    |
| hZC3H12A_gRNA_219 | AGTCGGCAGGGACACTGAAG  | 579    |
| hZC3H12A_gRNA_220 | ACTCTCGAGGTGGAAAGGCA  | 580    |
| hZC3H12A_gRNA_221 | CCCAGTGAGTAAGCTGCCTG  | 581    |
| hZC3H12A_gRNA_222 | AGAGGGTGCAAAGAACTCTC  | 582    |
| hZC3H12A_gRNA_223 | CACGATCCCGTCAGACTCGT  | 583    |
| hZC3H12A_gRNA_224 | TCTGCACTGGGGGCTCCTGA  | 584    |
| hZC3H12A_gRNA_225 | CAGGGGGCATAAACCTGCAG  | 585    |
| hZC3H12A_gRNA_226 | TGAGGACAGCCACAGCCGTC  | 586    |
| hZC3H12A_gRNA_227 | GTTTCCCCCGCACCTGGTGG  | 587    |
| hZC3H12A_gRNA_228 | TTAGGGGTGCCACCACCCCG  | 588    |
| hZC3H12A_gRNA_229 | ACTGGGGTGCTGGGACTTGT  | 589    |
| hZC3H12A_gRNA_230 | CTCACTCCCGTACGTCTCCC  | 590    |
| hZC3H12A_gRNA_231 | AGGGGCTGGCAGGAGCCCGT  | 591    |
| hZC3H12A_gRNA_232 | TCCTTGTTCCCATGGCTGGG  | 592    |
| hZC3H12A_gRNA_233 | GCCAAAGGCAGAGAAGGCTG  | 593    |
| hZC3H12A_gRNA_234 | CACGGGCTCCTGCCAGCCCC  | 594    |
| hZC3H12A_gRNA_235 | CCACAGCGTCCTGCAGAAGC  | 595    |
| hZC3H12A_gRNA_236 | ACGGGCTCCTGCCAGCCCCT  | 596    |
| hZC3H12A_gRNA_237 | ATGGGAGCAACGTGGCCATG  | 597    |
| hZC3H12A_gRNA_238 | CCCAAGGCCGGGTCAAGAGC  | 598    |
| hZC3H12A_gRNA_239 | AATTGCTGAGAAGGGGCCGA  | 599    |
| hZC3H12A_gRNA_240 | GGGCAGGAGTGAGGAGGGCC  | 600    |
| hZC3H12A_gRNA_241 | GGCGGGACCCTGGGGATGCC  | 601    |
| hZC3H12A_gRNA_242 | GGGGCTGGCAGGAGCCCGTG  | 602    |
| hZC3H12A_gRNA_243 | TTCCGACATCTGGCTCTCCA  | 603    |
| hZC3H12A_gRNA_244 | GTGCTGCCCTTGCCAGCCAC  | 604    |
| hZC3H12A_gRNA_245 | ACTCCTGCCGCTCGCCTTGG  | 605    |
| hZC3H12A_gRNA_246 | GTGGACTTCTTCCGGAAGCT  | 606    |
| hZC3H12A_gRNA_247 | CCAGTGACAGAGCCCAGGGGC | 607    |
| hZC3H12A_gRNA_248 | GGGGCAGTGGCAGCAGCTTT  | 608    |
| hZC3H12A_gRNA_249 | GGGACTGTAGCCCGTGTAAG  | 609    |
| hZC3H12A_gRNA_250 | CCACAGACTCATGGTGGGGC  | 610    |
| hZC3H12A_gRNA_251 | AACACGGGACAGCCACCGAG  | 611    |

| Target            | Sequence              | SEQ ID |
|-------------------|-----------------------|--------|
| hZC3H12A_gRNA_252 | GCAAAGAACTCTCTGGAGGT  | 612    |
| hZC3H12A_gRNA_253 | TGGGCCCCGTGCCGGCCAGT  | 613    |
| hZC3H12A_gRNA_254 | CTCCTGCCGGGGCATCCTGC  | 614    |
| hZC3H12A_gRNA_255 | AGGCAGACAGGTGAGAGGAA  | 615    |
| hZC3H12A_gRNA_256 | AGGCAATCCTGGGAGACGTA  | 616    |
| hZC3H12A_gRNA_257 | TCAGACCAGTACTCTCGAGG  | 617    |
| hZC3H12A_gRNA_258 | AACATACTTGTCATTGACGA  | 618    |
| hZC3H12A_gRNA_259 | GGCAGCTTGCCGCTCTGGG   | 619    |
| hZC3H12A_gRNA_260 | GAGTTCTTTGCACCCTCTGC  | 620    |
| hZC3H12A_gRNA_261 | GCCACAGGCAGCTTACTCAC  | 621    |
| hZC3H12A_gRNA_262 | AGGCTGCCTGCCCTGCCCCA  | 622    |
| hZC3H12A_gRNA_263 | CCGGCCCAGTGGGTCATCAG  | 623    |
| hZC3H12A_gRNA_264 | CTCTCGAGGTGGAAAGGCAG  | 624    |
| hZC3H12A_gRNA_265 | GATTGCCTGGACTCGGGCAT  | 625    |
| hZC3H12A_gRNA_266 | TCCTTGCTTGGGGCTCTGGG  | 626    |
| hZC3H12A_gRNA_267 | GCAGAGAAGGCTGCGGTGGC  | 627    |
| hZC3H12A_gRNA_268 | ACCGTGACCTCCAAGGCGAG  | 628    |
| hZC3H12A_gRNA_269 | CAGGACGCTGTGGATCTCCG  | 629    |
| hZC3H12A_gRNA_270 | AGGAAGCAGCCGTGTCCCTA  | 630    |
| hZC3H12A_gRNA_271 | ACGCAGGAAGTTGTCCAGGC  | 631    |
| hZC3H12A_gRNA_272 | GAGGTCCCCAAGAGGAAAAC  | 632    |
| hZC3H12A_gRNA_273 | CCCCCAGCTTCTTCCCATCC  | 633    |
| hZC3H12A_gRNA_274 | ATTCCCACAGACTCATGGTG  | 634    |
| hZC3H12A_gRNA_275 | TCCAAGGCGAGCGGCAGGAG  | 635    |
| hZC3H12A_gRNA_276 | GCTGGGAGCTCAGATCCATA  | 636    |
| hZC3H12A_gRNA_277 | TGGGGGCCAGGCATCCCCA   | 637    |
| hZC3H12A_gRNA_278 | GGGTGCAAAGAACTCTCTGG  | 638    |
| hZC3H12A_gRNA_279 | GCGGGACTAGAGGGAGCTGA  | 639    |
| hZC3H12A_gRNA_280 | ACTGGAGAAGAAGAAGATCC  | 640    |
| hZC3H12A_gRNA_281 | CCAGCTCTTGACCCGGCCTT  | 641    |
| hZC3H12A_gRNA_282 | GAACCTTGGGGGGTTTCGAGG | 642    |
| hZC3H12A_gRNA_283 | GAAACCAGTTCCTGACCAGC  | 643    |
| hZC3H12A_gRNA_284 | ACAGCCGTCAGGGCACCCCA  | 644    |
| hZC3H12A_gRNA_285 | CCACCCCGAGCCCCTTACAC  | 645    |
| hZC3H12A_gRNA_286 | TCTCGGCAGCCAGCTGCTGG  | 646    |
| hZC3H12A_gRNA_287 | AGAGAGCTGGACTGGGATGA  | 647    |
| hZC3H12A_gRNA_288 | CCTTTCCACCTCGAGAGTAC  | 648    |
| hZC3H12A_gRNA_289 | AAGCTGGCCTACGAGTCTGA  | 649    |
| hZC3H12A_gRNA_290 | GTCTGTGGGAGCCAGTCTGT  | 650    |
| hZC3H12A_gRNA_291 | AGACCTATGCCCCATCAGGC  | 651    |
| hZC3H12A_gRNA_292 | TGGGAAGAAGCTGGGGGCC   | 652    |
| hZC3H12A_gRNA_293 | CTGTGGAGAGAAGCCTGTCC  | 653    |

| Target            | Sequence             | SEQ ID |
|-------------------|----------------------|--------|
| hZC3H12A_gRNA_294 | GGGACTTCTAATTGCTGAGA | 654    |
| hZC3H12A_gRNA_295 | GGACTCGGGCATTGGCTCCC | 655    |
| hZC3H12A_gRNA_296 | CATCTGCCACAGAGCGCTGG | 656    |
| hZC3H12A_gRNA_297 | CTTCTGGGAGTGGAGGCTCC | 657    |
| hZC3H12A_gRNA_298 | GCCCCCAGTGCAGAGCCCAG | 658    |
| hZC3H12A_gRNA_299 | TTTGTCTTGCTTGGGGCTC  | 659    |
| hZC3H12A_gRNA_300 | GTGGGGCTGGCTTCCAGGAC | 660    |
| hZC3H12A_gRNA_301 | TCAAGAGCTGGCCGCTGAGG | 661    |
| hZC3H12A_gRNA_302 | CCTCTAGTCCCGCGGGGTGG | 662    |
| hZC3H12A_gRNA_303 | GCTCATCTGCCACAGAGCGC | 663    |
| hZC3H12A_gRNA_304 | CATGAGTCTGTGGGAATTTG | 664    |
| hZC3H12A_gRNA_305 | TGCGAGGCTCCTGCCTGATG | 665    |
| hZC3H12A_gRNA_306 | GGAGTGAGTCCAGCGTCTGT | 666    |
| hZC3H12A_gRNA_307 | TGCAAAGAACTCTCTGGAGG | 667    |
| hZC3H12A_gRNA_308 | CACAGCGTCCTGCAGAAGCT | 668    |
| hZC3H12A_gRNA_309 | CAGCTTACTCACTGGGGTGC | 669    |
| hZC3H12A_gRNA_310 | ACTGATGTGGGTGGGGGCAG | 670    |
| hZC3H12A_gRNA_311 | GCAGGATGTGCTGGTCTGGG | 671    |
| hZC3H12A_gRNA_312 | TCACAGTGTTTGTGCCATCC | 672    |
| hZC3H12A_gRNA_313 | GTTTGTGCCATCCTGGAGGA | 673    |
| hZC3H12A_gRNA_314 | TCCTGAAGGACTGATGTGGG | 674    |
| hZC3H12A_gRNA_315 | TGTTAGCAGAGAGCTGGACT | 675    |
| hZC3H12A_gRNA_316 | CAGTGTTTGTGCCATCCTGG | 676    |
| hZC3H12A_gRNA_317 | AGTCTGTCAGGGCCTCTGGG | 677    |
| hZC3H12A_gRNA_318 | TCTCGAGGTGGAAAGGCAGG | 678    |
| hZC3H12A_gRNA_319 | AGACTGGCTCCCACAGACGC | 679    |
| hZC3H12A_gRNA_320 | AGCCACTCACTTTGGAGCAC | 680    |
| hZC3H12A_gRNA_321 | TCCCAGGATTGCCTGGACTC | 681    |
| hZC3H12A_gRNA_322 | CCTGGAAGTGCAGATGAAGG | 682    |
| hZC3H12A_gRNA_323 | GGGGCGCTTCCCACAGCTCC | 683    |
| hZC3H12A_gRNA_324 | CAGCCCCTGGGCTCTGCACT | 684    |
| hZC3H12A_gRNA_325 | GCGCGGGTGGGTAGTCGGCA | 685    |
| hZC3H12A_gRNA_326 | GCCCCAAGCAAGGACAAAAA | 686    |
| hZC3H12A_gRNA_327 | AGCCTGGATGGGAAGAAGCT | 687    |
| hZC3H12A_gRNA_328 | CAGCTCTTGACCCGGCCTTG | 688    |
| hZC3H12A_gRNA_329 | TAGGGGTGCCACCACCCCGC | 689    |
| hZC3H12A_gRNA_330 | TCCACTCCCAGAAGAGGAAA | 690    |
| hZC3H12A_gRNA_331 | GGAAGCGCTTCATCGAGGAG | 691    |
| hZC3H12A_gRNA_332 | GCATCCTGCTGGCAGTGAAC | 692    |
| hZC3H12A_gRNA_333 | TGGATGAATAGCCCAGCTTC | 693    |
| hZC3H12A_gRNA_334 | ACACGGGACAGCCACCGAGC | 694    |
| hZC3H12A_gRNA_335 | GGGCTCCTGAAGGACTGATG | 695    |

| Target            | Sequence              | SEQ ID |
|-------------------|-----------------------|--------|
| hZC3H12A_gRNA_336 | CAGCCTGGATGGGAAGAAGC  | 696    |
| hZC3H12A_gRNA_337 | TTTTCCTCTTCTGGGAGTGG  | 697    |
| hZC3H12A_gRNA_338 | CTCCAGGTTGGGAGCCTTAG  | 698    |
| hZC3H12A_gRNA_339 | GGGAGCTGAGGGCAGGGGTC  | 699    |
| hZC3H12A_gRNA_340 | AGATGAAGGTGGACTTCTTC  | 700    |
| hZC3H12A_gRNA_341 | TTTGGCCGGGCCATGGGTGC  | 701    |
| hZC3H12A_gRNA_342 | CTCGCACCTAGCGGGGGCAG  | 702    |
| hZC3H12A_gRNA_343 | CCCGTGTAAGGGGCTCGGGG  | 703    |
| hZC3H12A_gRNA_344 | TGCCGGCCCAGTGGGTCATC  | 704    |
| hZC3H12A_gRNA_345 | AAAGGCAGAGAAGGCTGCGG  | 705    |
| hZC3H12A_gRNA_346 | AGGAGCCCGTGGGGCAGGGC  | 706    |
| hZC3H12A_gRNA_347 | TAAGGGGCTCGGGGTGGGCC  | 707    |
| hZC3H12A_gRNA_348 | ACACCATCACGACGCGTGGG  | 708    |
| hZC3H12A_gRNA_349 | CTGGCAGGAGCCCGTGGGGC  | 709    |
| hZC3H12A_gRNA_350 | CCGGCCTTGGGGTGCCCTGA  | 710    |
| hZC3H12A_gRNA_351 | CTGTGTTAGACCCTCTTGGC  | 711    |
| hZC3H12A_gRNA_352 | GTGATGGGCACGTCGGGCCG  | 712    |
| hZC3H12A_gRNA_353 | GCCCCTGGGCTCTGCACTGG  | 713    |
| hZC3H12A_gRNA_354 | CTGGGTGAGCTGGTGAAACA  | 714    |
| hZC3H12A_gRNA_355 | GGCTGCTCCTTCCTCCAGGA  | 715    |
| hZC3H12A_gRNA_356 | ACAGCCTCCACCAGGTGCGG  | 716    |
| hZC3H12A_gRNA_357 | TGCCCCGAGTCCAGGCAATCC | 717    |
| hZC3H12A_gRNA_358 | AGGTGGAAAGGCAGGGGGCG  | 718    |
| hZC3H12A_gRNA_359 | CGACAGATTTCATTGTGAAGC | 719    |
| hZC3H12A_gRNA_360 | GCGGGGTGGTGGCACCCCTA  | 720    |
| hZC3H12A_gRNA_361 | GGCAATCCTGGGAGACGTAC  | 721    |
| hZC3H12A_gRNA_362 | GCCGCTCGCCTTGGAGGTCA  | 722    |
| hZC3H12A_gRNA_363 | TCACTGCCAGCAGGATGCCC  | 723    |
| hZC3H12A_gRNA_364 | CCTGAAGGACTGATGTGGGT  | 724    |
| hZC3H12A_gRNA_365 | GTGCGAGGCTCCTGCCTGAT  | 725    |
| hZC3H12A_gRNA_366 | GCACCTGGTGGAGGCTGTGA  | 726    |
| hZC3H12A_gRNA_367 | TCACAGCCTCCACCAGGTGC  | 727    |
| hZC3H12A_gRNA_368 | GCCGCTCTGGGTGGAAGAAT  | 728    |
| hZC3H12A_gRNA_369 | GACTAGAGGGAGCTGAGGGC  | 729    |
| hZC3H12A_gRNA_370 | TCAGCTCCCTCTAGTCCCGC  | 730    |
| hZC3H12A_gRNA_371 | GGAGCCTCCACTCCCAGAAG  | 731    |
| hZC3H12A_gRNA_372 | AGACCCTCTTGGCGGGACCC  | 732    |
| hZC3H12A_gRNA_373 | CCACCTTCATCTGCAGTTCC  | 733    |
| hZC3H12A_gRNA_374 | GGGAGTGGAGGCTCCAGGTT  | 734    |
| hZC3H12A_gRNA_375 | CAGTGAAGTGGTTTCTGGAG  | 735    |
| hZC3H12A_gRNA_376 | TCACCTGTGATGGGCACGTC  | 736    |
| hZC3H12A_gRNA_377 | TGCCAGCAGGATGCCCCGGC  | 737    |

| Target            | Sequence              | SEQ ID |
|-------------------|-----------------------|--------|
| hZC3H12A_gRNA_378 | ACCTCTTGGCGGGACCCTG   | 738    |
| hZC3H12A_gRNA_379 | TGGGGGCAGCTTGGCCGCTC  | 739    |
| hZC3H12A_gRNA_380 | AGGAGGAGGCCCTGGTGAGC  | 740    |
| hZC3H12A_gRNA_381 | CTGGAGGTGGGAGCCATGCA  | 741    |
| hZC3H12A_gRNA_382 | TCTGGAGGTGGGAGCCATGC  | 742    |
| hZC3H12A_gRNA_383 | CCTGGATGGGAAGAAGCTGG  | 743    |
| hZC3H12A_gRNA_384 | CCAGCCCCTGGGCTCTGCAC  | 744    |
| hZC3H12A_gRNA_385 | GGAGTGGGAAGCGCTTCATCG | 745    |
| hZC3H12A_gRNA_386 | TGTAGCCCGTGTAAGGGGCT  | 746    |
| hZC3H12A_gRNA_387 | GGAGTGAGGAGGGCCGGGGA  | 747    |
| hZC3H12A_gRNA_388 | GAGGTCACGGTATGTGTCTG  | 748    |
| hZC3H12A_gRNA_389 | CTAGAGGGAGCTGAGGGCAG  | 749    |
| hZC3H12A_gRNA_390 | TGGTGTGTTTCCCCCGCACC  | 750    |
| hZC3H12A_gRNA_391 | CTGATGTGGGTGGGGGCAGT  | 751    |
| hZC3H12A_gRNA_392 | AGGGCCGGGAGGGCAGGCT   | 752    |
| hZC3H12A_gRNA_393 | TGAGCTATGAGTGGCCCCTG  | 753    |
| hZC3H12A_gRNA_394 | TCTTACGCAGGAAGTTGTCC  | 754    |
| hZC3H12A_gRNA_395 | GTTCCGACATCTGGCTCTCC  | 755    |
| hZC3H12A_gRNA_396 | AGGGGGCGCGGGTGGGTAGT  | 756    |
| hZC3H12A_gRNA_397 | CGCTGGCCTGCTCCTTGGCC  | 757    |
| hZC3H12A_gRNA_398 | GAAAGGCAGGGGGCGCGGGT  | 758    |
| hZC3H12A_gRNA_399 | TAGCCCGTGTAAGGGGCTCG  | 759    |
| hZC3H12A_gRNA_400 | CTGAGGGCAGGGGTCCGGTG  | 760    |
| hZC3H12A_gRNA_401 | ACACAGCTTAGTATACACGC  | 761    |
| hZC3H12A_gRNA_402 | CCGTCAGGGCACCCCAAGGC  | 762    |
| hZC3H12A_gRNA_403 | GGCAGGGGTCCGGTGAGGTC  | 763    |
| hZC3H12A_gRNA_404 | GGACTTGTAGGAGAGGATCT  | 764    |
| hZC3H12A_gRNA_405 | TCCCAGCCATGGGAACAAGG  | 765    |
| hZC3H12A_gRNA_406 | GACTTCTAATTGCTGAGAAG  | 766    |
| hZC3H12A_gRNA_407 | GGCTCCTGAAGGACTGATGT  | 767    |
| hZC3H12A_gRNA_408 | TGGCAGGAGCCCGTGGGGCA  | 768    |
| hZC3H12A_gRNA_409 | AGACAGGTGAGAGGAAGGGC  | 769    |
| hZC3H12A_gRNA_410 | TCGGAACCTTGGGGGGTTCG  | 770    |
| hZC3H12A_gRNA_411 | GCCTGGATGGGAAGAAGCTG  | 771    |
| hZC3H12A_gRNA_412 | TGAAGGACTGATGTGGGTGG  | 772    |
| hZC3H12A_gRNA_413 | CTGGGGGCCCCAGGCATCCCC | 773    |
| hZC3H12A_gRNA_414 | GAGCCCCCAGTGCAGAGCCC  | 774    |
| hZC3H12A_gRNA_415 | GGCGCGGGTGGGTAGTCGGC  | 775    |
| hZC3H12A_gRNA_416 | CCGTGTAAGGGGCTCGGGGT  | 776    |
| hZC3H12A_gRNA_417 | GTCGTGATGGTGTGAACACC  | 777    |
| hZC3H12A_gRNA_418 | ACGACGCGTGGGTGGCAAGC  | 778    |
| hZC3H12A_gRNA_419 | GGGGGCAGTGGCAGCAGCTT  | 779    |

| Target            | Sequence              | SEQ ID |
|-------------------|-----------------------|--------|
| hZC3H12A_gRNA_420 | AGCGTGTATACTAAGCTGTG  | 780    |
| hZC3H12A_gRNA_421 | GCTCCTGCCTGATGGGGCAT  | 781    |
| hZC3H12A_gRNA_422 | GTCTGTCAGGGCCTCTGGGA  | 782    |
| hZC3H12A_gRNA_423 | GTAGCCCGTGTAAGGGGCTC  | 783    |
| hZC3H12A_gRNA_424 | AGCCCCTGGGCTCTGCACTG  | 784    |
| hZC3H12A_gRNA_425 | GTGAACTGGTTTCTGGAGCG  | 785    |
| hZC3H12A_gRNA_426 | CTACGAGTCTGACGGGATCG  | 786    |
| hZC3H12A_gRNA_427 | AGTGAACCTGGTTTCTGGAGC | 787    |
| hZC3H12A_gRNA_428 | ACGCGTGGGTGGCAAGCGGG  | 788    |
| hZC3H12A_gRNA_429 | CGCGGGACTAGAGGGAGCTG  | 789    |
| hZC3H12A_gRNA_430 | GGCAGGAGTGAGGAGGGCCG  | 790    |
| hZC3H12A_gRNA_431 | AAGTGAGTGGCTTCTTACGC  | 791    |
| hZC3H12A_gRNA_432 | CTGAAGGACTGATGTGGGTG  | 792    |
| hZC3H12A_gRNA_433 | TTGCCACCCACGCGTCGTGA  | 793    |
| hZC3H12A_gRNA_434 | AGGGCAGGAGTGAGGAGGGC  | 794    |
| hZC3H12A_gRNA_435 | TCTTCTTCTCCAGTTCCCGC  | 795    |
| hZC3H12A_gRNA_436 | AGGAGTGAGGAGGGCCGGGG  | 796    |
| hZC3H12A_gRNA_437 | ACTCCCAGAAGAGGAAAAGG  | 797    |
| hZC3H12A_gRNA_438 | TGAGGAGGGCCGGGGAGGGC  | 798    |
| hZC3H12A_gRNA_439 | ACCTGGTGGAGGCTGTGATG  | 799    |
| hZC3H12A_gRNA_440 | CAGGGCCGAGGCCTCCTCAG  | 800    |
| hZC3H12A_gRNA_441 | TACTCTCGAGGTGGAAAGGC  | 801    |
| hZC3H12A_gRNA_442 | TTGGGGCTCTGGGGGGTGAG  | 802    |
| hZC3H12A_gRNA_443 | GCTCCTGGACCCCCAGCAGC  | 803    |
| hZC3H12A_gRNA_444 | GGGGGGTGAGAGGAGAGCAT  | 804    |
| hZC3H12A_gRNA_445 | CGGCCCACTGGGTCATCAGG  | 805    |
| hZC3H12A_gRNA_446 | AGGAAGGGCAGGAGTGAGGA  | 806    |
| hZC3H12A_gRNA_447 | GAGGGCCGGGGAGGGCAGGC  | 807    |
| hZC3H12A_gRNA_448 | TGCTGGGGGTCCAGGAGCTG  | 808    |
| hZC3H12A_gRNA_449 | GCTGGGGGTCCAGGAGCTGT  | 809    |
| hZC3H12A_gRNA_450 | TGAGAGGAAGGGCAGGAGTG  | 810    |
| hZC3H12A_gRNA_451 | TGGGAGTGAGGCTCCAGGT   | 811    |
| hZC3H12A_gRNA_452 | GAGGAAGGGCAGGAGTGAGG  | 812    |

Table 17: Exemplary murine *Zc3h12a* gRNA sequences

| Target          | Sequence             | SEQ ID |
|-----------------|----------------------|--------|
| mZc3h12a_gRNA_1 | GCTGGCTGTGAACTGGTTTC | 208    |
| mZc3h12a_gRNA_2 | CTAGTTCCCGAAGGATGTGC | 209    |
| mZc3h12a_gRNA_3 | ATTGGAGACCACCACTCCGT | 210    |
| mZc3h12a_gRNA_4 | TTCCCTCCTCTGCCAGCCAT | 211    |



| Target          | Sequence             | SEQ ID |
|-----------------|----------------------|--------|
| mZc3h12a gRNA 5 | CGAAGGAAGTTGTCCAGGCT | 212    |
| mZc3h12a gRNA 6 | ATACCTGTGATAGGCACATC | 213    |
| mZc3h12a gRNA 7 | GACTTCCTTGTTCCCATGGC | 214    |
| mZc3h12a gRNA 8 | GGCCTTCGAATCCGACGGAG | 215    |

**[00289]** In some embodiments, the gene-regulating system comprises at least two gRNA molecules, wherein at least one gRNA molecule comprises a *SOCS1*-targeting nucleic acid-binding segment (*i.e.*, a *SOCS1*-targeting gRNA) and at least one gRNA molecule comprises a *PTPN2*-targeting nucleic acid-binding segment (*i.e.*, a *PTPN2*-targeting gRNA). In some embodiments, the nucleic acid-binding segment of the at least one *SOCS1*-targeting gRNA binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence in the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2) and the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence in the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4). In some embodiments, the nucleic acid-binding segment of the at least one *SOCS1*-targeting gRNA binds to a target DNA sequence that is 100% identical to a DNA sequence in the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2) and the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA binds to a target DNA sequence that is 100% identical to a DNA sequence in the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4).

**[00290]** In some embodiments, the nucleic acid-binding segment of the at least one *SOCS1*-targeting gRNA binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4 and the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6. In some embodiments, the nucleic acid-binding segment of the at least one *SOCS1*-targeting gRNA binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4 and the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6.

**[00291]** In some embodiments, the nucleic acid-binding segment of the at least one *SOCS1*-targeting gRNA binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical

to one of SEQ ID NOs: 7-151 and the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 185-207. In some embodiments, the nucleic acid-binding segment of the at least one *SOCS1*-targeting gRNA binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 7-151 and the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 185-207. Exemplary *SOCS1* target DNA sequences are shown in Tables 12 and 13 and exemplary *PTPN2* target DNA sequences are shown in Tables 14 and 15.

**[00292]** In some embodiments, the nucleic acid-binding segment of the at least one *SOCS1*-targeting gRNA is encoded by a DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 7-151 and the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA is encoded by a DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 185-207. In some embodiments, the nucleic acid-binding segment of the at least one *SOCS1*-targeting gRNA is encoded by a DNA sequence that is 100% identical to one of SEQ ID NOs: 7-151 and the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA is encoded by a DNA sequence that is 100% identical to one of SEQ ID NOs: 185-207. Exemplary DNA sequences encoding the nucleic acid-binding segment of the *SOCS1*-targeting gRNAs are shown in Tables 12 and 13 and exemplary DNA sequences encoding the nucleic acid-binding segment of the *PTPN2*-targeting gRNAs are shown in Tables 14 and 15.

**[00293]** In some embodiments, the gene-regulating system comprises at least two gRNA molecules, wherein at least one gRNA molecule comprises a *SOCS1*-targeting nucleic acid-binding segment (*i.e.*, a *SOCS1*-targeting gRNA) and at least one gRNA molecule comprises a *ZC3H12A*-targeting nucleic acid-binding segment (*i.e.*, a *ZC3H12A*-targeting gRNA). In some embodiments, the nucleic acid-binding segment of the at least one *SOCS1*-targeting gRNA binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence in the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2) and the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence in the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6). In some embodiments, the nucleic acid-binding segment of the at least one *SOCS1*-targeting gRNA binds to a target DNA sequence that is 100% identical to a DNA sequence in the *SOCS1* gene (SEQ ID NO: 1) or the *Socs1* gene (SEQ ID NO: 2) and the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA binds to a target DNA sequence that is 100%

identical to a DNA sequence in the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6).

**[00294]** In some embodiments, the nucleic acid-binding segment of the at least one *SOCS1*-targeting gRNA binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4 and the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8. In some embodiments, the nucleic acid-binding segment of the at least one *SOCS1*-targeting gRNA binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 3 or Table 4 and the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8.

**[00295]** In some embodiments, the nucleic acid-binding segment of the at least one *SOCS1*-targeting gRNA binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 7-151 and the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 208-230. In some embodiments, the nucleic acid-binding segment of the at least one *SOCS1*-targeting gRNA binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 7-151 and the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 208-230. Exemplary *SOCS1* target DNA sequences are shown in Tables 12 and 13 and exemplary *ZC3H12A* target DNA sequences are shown in Tables 16 and 17.

**[00296]** In some embodiments, the nucleic acid-binding segment of the at least one *SOCS1*-targeting gRNA is encoded by a DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 7-151 and the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA is encoded by a DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 208-230. In some embodiments, the nucleic acid-binding segment of the at least one *SOCS1*-targeting gRNA is encoded by a DNA sequence that is 100% identical to one of SEQ ID NOs: 7-151 and the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA is encoded by a DNA sequence that is 100% identical to one of SEQ ID NOs: 208-230. Exemplary DNA sequences encoding the nucleic acid-binding segment of the *SOCS1*-targeting gRNAs are shown in

Tables 12 and 13 and exemplary DNA sequences encoding the nucleic acid-binding segment of the *ZC3H12A*-targeting gRNAs are shown in Tables 16 and 17.

**[00297]** In some embodiments, the gene-regulating system comprises at least two gRNA molecules, wherein at least one gRNA molecule comprises a *PTPN2*-targeting nucleic acid-binding segment (*i.e.*, a *PTPN2*-targeting gRNA) and at least one gRNA molecule comprises a *ZC3H12A*-targeting nucleic acid-binding segment (*i.e.*, a *ZC3H12A*-targeting gRNA). In some embodiments, the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence in the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4) and the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence in the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6). In some embodiments, the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA binds to a target DNA sequence that is 100% identical to a DNA sequence in the *PTPN2* gene (SEQ ID NO: 3) or the *Ptpn2* gene (SEQ ID NO: 4) and the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA binds to a target DNA sequence that is 100% identical to a DNA sequence in the *ZC3H12A* gene (SEQ ID NO: 5) or the *Zc3h12a* gene (SEQ ID NO: 6).

**[00298]** In some embodiments, the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6 and the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8. In some embodiments, the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 5 or Table 6 and the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA binds to a target DNA sequence that is 100% identical to a DNA sequence defined by a set of genomic coordinates shown in Table 7 or Table 8.

**[00299]** In some embodiments, the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 185-207 and the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA binds to a target DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical

to one of SEQ ID NOs: 208-230. In some embodiments, the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 185-207 and the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA binds to a target DNA sequence that is 100% identical to one of SEQ ID NOs: 208-230. Exemplary *PTPN2* target DNA sequences are shown in Tables 14 and 15 and exemplary *ZC3H12A* target DNA sequences are shown in Tables 16 and 17.

**[00300]** In some embodiments, the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA is encoded by a DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 185-207 and the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA is encoded by a DNA sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs: 208-230. In some embodiments, the nucleic acid-binding segment of the at least one *PTPN2*-targeting gRNA is encoded by a DNA sequence that is 100% identical to one of SEQ ID NOs: 185-207 and the nucleic acid-binding segment of the at least one *ZC3H12A*-targeting gRNA is encoded by a DNA sequence that is 100% identical to one of SEQ ID NOs: 208-230. Exemplary DNA sequences encoding the nucleic acid-binding segment of the *PTPN2*-targeting gRNAs are shown in Tables 14 and 15 and exemplary DNA sequences encoding the nucleic acid-binding segment of the *ZC3H12A*-targeting gRNAs are shown in Tables 16 and 17.

**[00301]** In some embodiments, the nucleic acid-binding segments of the gRNA sequences described herein are designed to minimize off-target binding using algorithms known in the art (*e.g.*, Cas-OFF finder) to identify target sequences that are unique to a particular target locus or target gene.

**[00302]** In some embodiments, the gRNAs described herein can comprise one or more modified nucleosides or nucleotides which introduce stability toward nucleases. In such embodiments, these modified gRNAs may elicit a reduced innate immune response as compared to a non-modified gRNA. The term “innate immune response” includes a cellular response to exogenous nucleic acids, including single stranded nucleic acids, generally of viral or bacterial origin, which involves the induction of cytokine expression and release, particularly the interferons, and cell death.

**[00303]** In some embodiments, the gRNAs described herein are modified at or near the 5' end (*e.g.*, within 1-10, 1-5, or 1-2 nucleotides of their 5' end). In some embodiments, the 5' end of a gRNA is modified by the inclusion of a eukaryotic mRNA cap structure or cap analog (*e.g.*, a G(5')ppp(5')G cap analog, a m7G(5')ppp(5')G cap analog, or a 3'-O-Me-m7G(5')ppp(5')G anti reverse cap analog (ARCA)). In some embodiments, an *in vitro* transcribed gRNA is modified by treatment with a

phosphatase (*e.g.*, calf intestinal alkaline phosphatase) to remove the 5' triphosphate group. In some embodiments, a gRNA comprises a modification at or near its 3' end (*e.g.*, within 1-10, 1-5, or 1-2 nucleotides of its 3' end). For example, in some embodiments, the 3' end of a gRNA is modified by the addition of one or more (*e.g.*, 25-200) adenine (A) residues.

**[00304]** In some embodiments, modified nucleosides and modified nucleotides can be present in a gRNA, but also may be present in other gene-regulating systems, *e.g.*, mRNA, RNAi, or siRNA-based systems. In some embodiments, modified nucleosides and nucleotides can include one or more of:

- (a) alteration, *e.g.*, replacement, of one or both of the non-linking phosphate oxygens and/or of one or more of the linking phosphate oxygens in the phosphodiester backbone linkage;
- (b) alteration, *e.g.*, replacement, of a constituent of the ribose sugar, *e.g.*, of the 2' hydroxyl on the ribose sugar;
- (c) wholesale replacement of the phosphate moiety with “dephospho” linkers;
- (d) modification or replacement of a naturally occurring nucleobase;
- (e) replacement or modification of the ribose-phosphate backbone;
- (f) modification of the 3' end or 5' end of the oligonucleotide, *e.g.*, removal, modification or replacement of a terminal phosphate group or conjugation of a moiety; and
- (g) modification of the sugar.

**[00305]** In some embodiments, the modifications listed above can be combined to provide modified nucleosides and nucleotides that can have two, three, four, or more modifications. For example, in some embodiments, a modified nucleoside or nucleotide can have a modified sugar and a modified nucleobase. In some embodiments, every base of a gRNA is modified. In some embodiments, each of the phosphate groups of a gRNA molecule are replaced with phosphorothioate groups.

**[00306]** In some embodiments, a software tool can be used to optimize the choice of gRNA within a user's target sequence, *e.g.*, to minimize total off-target activity across the genome. Off target activity may be other than cleavage. For example, for each possible gRNA choice using *S. pyogenes* Cas9, software tools can identify all potential off-target sequences (preceding either NAG or NGG PAMs) across the genome that contain up to a certain number (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) of

mismatched base-pairs. The cleavage efficiency at each off-target sequence can be predicted, *e.g.*, using an experimentally-derived weighting scheme. Each possible gRNA can then be ranked according to its total predicted off-target cleavage; the top-ranked gRNAs represent those that are likely to have the greatest on-target and the least off-target cleavage. Other functions, *e.g.*, automated reagent design for gRNA vector construction, primer design for the on-target Surveyor assay, and primer design for high-throughput detection and quantification of off-target cleavage via next-generation sequencing, can also be included in the tool.

#### **IV. Methods of producing modified immune effector cells**

**[00307]** In some embodiments, the present disclosure provides methods for producing modified immune effector cells. In some embodiments, the methods comprise introducing a gene-regulating system into a population of immune effector cells wherein the gene-regulating system is capable of reducing expression and/or function of two or more endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.

**[00308]** The components of the gene-regulating systems described herein, *e.g.*, a nucleic acid-, protein-, or nucleic acid/protein-based system can be introduced into target cells in a variety of forms using a variety of delivery methods and formulations. In some embodiments, a polynucleotide encoding one or more components of the system is delivered by a recombinant vector (*e.g.*, a viral vector or plasmid). In some embodiments, where the system comprises more than a single component, a vector may comprise a plurality of polynucleotides, each encoding a component of the system. In some embodiments, where the system comprises more than a single component, a plurality of vectors may be used, wherein each vector comprises a polynucleotide encoding a particular component of the system. In some embodiments, a vector may also comprise a sequence encoding a signal peptide (*e.g.*, for nuclear localization, nucleolar localization, mitochondrial localization), fused to the polynucleotide encoding the one or more components of the system. For example, a vector may comprise a nuclear localization sequence (*e.g.*, from SV40) fused to the polynucleotide encoding the one or more components of the system. In some embodiments, the introduction of the gene-regulating system to the cell occurs *in vitro*. In some embodiments, the introduction of the gene-regulating system to the cell occurs *in vivo*. In some embodiments, the introduction of the gene-regulating system to the cell occurs *ex vivo*.

**[00309]** In some embodiments, the recombinant vector comprising a polynucleotide encoding one or more components of a gene-regulating system described herein is a viral vector. Suitable viral

vectors include, but are not limited to, viral vectors based on vaccinia virus; poliovirus; adenovirus (see, *e.g.*, Li *et al.*, Invest Ophthalmol Vis Sci 35:2543 2549, 1994; Borrás *et al.*, Gene Ther 6:515 524, 1999; Li and Davidson, PNAS 92:7700 7704, 1995; Sakamoto *et al.*, H Gene Ther 5:1088 1097, 1999; WO 94/12649, WO 93/03769; WO 93/19191 ; WO 94/28938; WO 95/11984 and WO 95/00655); adeno-associated virus (see, *e.g.*, U.S. Patent No. 7,078,387; Ali *et al.*, Hum Gene Ther 9:81 86, 1998, Flannery *et al.*, PNAS 94:6916 6921 , 1997; Bennett *et al.*, Invest Ophthalmol Vis Sci 38:2857 2863, 1997; Jomary *et al.*, Gene Ther 4:683 690, 1997, Rolling *et al.*, Hum Gene Ther 10:641 648, 1999; Ali *et al.*, Hum Mol Genet 5:591 594, 1996; Srivastava in WO 93/09239, Samulski *et al.*, J. Vir. (1989) 63:3822-3828; Mendelson *et al.*, Virol. (1988) 166:154-165; and Flotte *et al.*, PNAS (1993) 90:10613-10617); SV40; herpes simplex virus; human immunodeficiency virus (see, *e.g.*, Miyoshi *et al.*, PNAS 94:10319 23, 1997; Takahashi *et al.*, J Virol 73:7812 7816, 1999); a retroviral vector (*e.g.*, Murine Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, a lentivirus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus); and the like.

**[00310]** In some embodiments, the recombinant vector comprising a polynucleotide encoding one or more components of a gene-regulating system described herein is a plasmid. Numerous suitable plasmid expression vectors are known to those of skill in the art, and many are commercially available. The following vectors are provided by way of example; for eukaryotic host cells: pXT1, pSG5 (Stratagene), pSVK3, pBPV, pMSG, and pSVLSV40 (Pharmacia). However, any other plasmid vector may be used so long as it is compatible with the host cell. Depending on the cell type and gene-regulating system utilized, any of a number of suitable transcription and translation control elements, including constitutive and inducible promoters, transcription enhancer elements, transcription terminators, etc. may be used in the expression vector (see *e.g.*, Bitter *et al.* (1987) Methods in Enzymology, 153:516-544).

**[00311]** In some embodiments, a polynucleotide sequence encoding one or more components of a gene-regulating system described herein is operably linked to a control element, *e.g.*, a transcriptional control element, such as a promoter. The transcriptional control element may be functional in either a eukaryotic cell (*e.g.*, a mammalian cell) or a prokaryotic cell (*e.g.*, bacterial or archaeal cell). In some embodiments, a polynucleotide sequence encoding one or more components of a gene-regulating system described herein is operably linked to multiple control elements that allow expression of the polynucleotide in both prokaryotic and eukaryotic cells. Depending on the cell type and gene-regulating system utilized, any of a number of suitable transcription and translation control



elements, including constitutive and inducible promoters, transcription enhancer elements, transcription terminators, etc. may be used in the expression vector (see *e.g.*, Bitter *et al.* (1987) *Methods in Enzymology*, 153:516-544).

**[00312]** Non-limiting examples of suitable eukaryotic promoters (promoters functional in a eukaryotic cell) include those from cytomegalovirus (CMV) immediate early, herpes simplex virus (HSV) thymidine kinase, early and late SV40, long terminal repeats (LTRs) from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art. The expression vector may also contain a ribosome binding site for translation initiation and a transcription terminator. The expression vector may also include appropriate sequences for amplifying expression. The expression vector may also include nucleotide sequences encoding protein tags (*e.g.*, 6xHis tag, hemagglutinin tag, green fluorescent protein, etc.) that are fused to the site-directed modifying polypeptide, thus resulting in a chimeric polypeptide.

**[00313]** In some embodiments, a polynucleotide sequence encoding one or more components of a gene-regulating system described herein is operably linked to an inducible promoter. In some embodiments, a polynucleotide sequence encoding one or more components of a gene-regulating system described herein is operably linked to a constitutive promoter.

**[00314]** Methods of introducing polynucleotides and recombinant vectors into a host cell are known in the art, and any known method can be used to introduce components of a gene-regulating system into a cell. Suitable methods include *e.g.*, viral or bacteriophage infection, transfection, conjugation, protoplast fusion, lipofection, electroporation, calcium phosphate precipitation, polyethyleneimine (PEI)-mediated transfection, DEAE-dextran mediated transfection, liposome-mediated transfection, particle gun technology, calcium phosphate precipitation, direct micro injection, nanoparticle-mediated nucleic acid delivery (see, *e.g.*, Panyam *et al.*, *Adv Drug Deliv Rev.* 2012 Sep 13. pii: S0169-409X(12)00283-9), microfluidics delivery methods (*See e.g.*, International PCT Publication No. WO 2013/059343), and the like. In some embodiments, delivery via electroporation comprises mixing the cells with the components of a gene-regulating system in a cartridge, chamber, or cuvette and applying one or more electrical impulses of defined duration and amplitude. In some embodiments, cells are mixed with components of a gene-regulating system in a vessel connected to a device (*e.g.*, a pump) which feeds the mixture into a cartridge, chamber, or cuvette wherein one or more electrical impulses of defined duration and amplitude are applied, after which the cells are delivered to a second vessel.

**[00315]** In some embodiments, one or more components of a gene-regulating system, or polynucleotide sequence encoding one or more components of a gene-regulating system described herein are introduced to a cell in a non-viral delivery vehicle, such as a transposon, a nanoparticle (*e.g.*, a lipid nanoparticle), a liposome, an exosome, an attenuated bacterium, or a virus-like particle. In some embodiments, the vehicle is an attenuated bacterium (*e.g.*, naturally or artificially engineered to be invasive but attenuated to prevent pathogenesis including *Listeria monocytogenes*, certain *Salmonella* strains, *Bifidobacterium longum*, and modified *Escherichia coli*), bacteria having nutritional and tissue-specific tropism to target specific cells, and bacteria having modified surface proteins to alter target cell specificity. In some embodiments, the vehicle is a genetically modified bacteriophage (*e.g.*, engineered phages having large packaging capacity, less immunogenicity, containing mammalian plasmid maintenance sequences and having incorporated targeting ligands). In some embodiments, the vehicle is a mammalian virus-like particle. For example, modified viral particles can be generated (*e.g.*, by purification of the “empty” particles followed by *ex vivo* assembly of the virus with the desired cargo). The vehicle can also be engineered to incorporate targeting ligands to alter target tissue specificity. In some embodiments, the vehicle is a biological liposome. For example, the biological liposome is a phospholipid-based particle derived from human cells (*e.g.*, erythrocyte ghosts, which are red blood cells broken down into spherical structures derived from the subject and wherein tissue targeting can be achieved by attachment of various tissue or cell-specific ligands), secretory exosomes, or subject derived membrane-bound nanovesicles (30 -100 nm) of endocytic origin (*e.g.*, can be produced from various cell types and can therefore be taken up by cells without the need for targeting ligands).

**[00316]** In some embodiments, the methods of modified immune effector cells described herein comprise obtaining a population of immune effector cells from a sample. In some embodiments, a sample comprises a tissue sample, a fluid sample, a cell sample, a protein sample, or a DNA or RNA sample. In some embodiments, a tissue sample may be derived from any tissue type including, but not limited to skin, hair (including roots), bone marrow, bone, muscle, salivary gland, esophagus, stomach, small intestine (*e.g.*, tissue from the duodenum, jejunum, or ileum), large intestine, liver, gallbladder, pancreas, lung, kidney, bladder, uterus, ovary, vagina, placenta, testes, thyroid, adrenal gland, cardiac tissue, thymus, spleen, lymph node, spinal cord, brain, eye, ear, tongue, cartilage, white adipose tissue, or brown adipose tissue. In some embodiments, a tissue sample may be derived from a cancerous, pre-cancerous, or non-cancerous tumor. In some embodiments, a fluid sample comprises buccal swabs, blood, plasma, oral mucous, vaginal mucous, peripheral blood, cord blood, saliva,

semen, urine, ascites fluid, pleural fluid, spinal fluid, pulmonary lavage, tears, sweat, semen, seminal fluid, seminal plasma, prostatic fluid, pre-ejaculatory fluid (Cowper's fluid), excreta, cerebrospinal fluid, lymph, cell culture media comprising one or more populations of cells, buffered solutions comprising one or more populations of cells, and the like.

**[00317]** In some embodiments, the sample is processed to enrich or isolate a particular cell type, such as an immune effector cell, from the remainder of the sample. In certain embodiments, the sample is a peripheral blood sample which is then subject to leukopheresis to separate the red blood cells and platelets and to isolate lymphocytes. In some embodiments, the sample is a leukopak from which immune effector cells can be isolated or enriched. In some embodiments, the sample is a tumor sample that is further processed to isolate lymphocytes present in the tumor (*i.e.*, to isolate tumor infiltrating lymphocytes).

**[00318]** In some embodiments, the isolated immune effector cells are expanded in culture to produce an expanded population of immune effector cells. One or more activating or growth factors may be added to the culture system during the expansion process. For example, in some embodiments, one or more cytokines (such as IL-2, IL-15, and/or IL-7) can be added to the culture system to enhance or promote cell proliferation and expansion. In some embodiments, one or more activating antibodies, such as an anti-CD3 antibody, may be added to the culture system to enhance or promote cell proliferation and expansion. In some embodiments, the immune effector cells may be co-cultured with feeder cells during the expansion process. In some embodiments, the methods provided herein comprise one or more expansion phases. For example, in some embodiments, the immune effector cells can be expanded after isolation from a sample, allowed to rest, and then expanded again. In some embodiments, the immune effector cells can be expanded in one set of expansion conditions followed by a second round of expansion in a second, different, set of expansion conditions. Methods for *ex vivo* expansion of immune cells are known in the art, for example, as described in US Patent Application Publication Nos. 20180282694 and 20170152478 and US Patent Nos. 8,383,099 and 8,034,334.

**[00319]** At any point during the culture and expansion process, the gene-regulating systems described herein can be introduced to the immune effector cells to produce a population of modified immune effector cells. In some embodiments, the gene-regulating system is introduced to the population of immune effector cells immediately after enrichment from a sample. In some embodiments, the gene-regulating system is introduced to the population of immune effector cells before, during, or after the one or more expansion process. In some embodiments, the gene-regulating

system is introduced to the population of immune effector cells immediately after enrichment from a sample or harvest from a subject, and prior to any expansion rounds. In some embodiments, the gene-regulating system is introduced to the population of immune effector cells after a first round of expansion and prior to a second round of expansion. In some embodiments, the gene-regulating system is introduced to the population of immune effector cells after a first and a second round of expansion.

**[00320]** In some embodiments, the modified immune effector cells produced by the methods described herein may be used immediately. Alternatively, the cells may be frozen at liquid nitrogen temperatures and stored for long periods of time, being thawed and capable of being reused. In such cases, the cells will usually be frozen in 10% dimethylsulfoxide (DMSO), 50% serum, 40% buffered medium, or some other such solution as is commonly used in the art to preserve cells at such freezing temperatures, and thawed in a manner as commonly known in the art for thawing frozen cultured cells.

**[00321]** In some embodiments, the modified immune effector cells may be cultured *in vitro* under various culture conditions. The cells may be expanded in culture, *i.e.* grown under conditions that promote their proliferation. Culture medium may be liquid or semi-solid, *e.g.* containing agar, methylcellulose, etc. The cell population may be suspended in an appropriate nutrient medium, such as Iscove's modified DMEM or RPMI 1640, normally supplemented with fetal calf serum (about 5-10%), L-glutamine, a thiol, particularly 2-mercaptoethanol, and antibiotics, *e.g.* penicillin and streptomycin. The culture may contain growth factors to which the regulatory T cells are responsive. Growth factors, as defined herein, are molecules capable of promoting survival, growth and/or differentiation of cells, either in culture or in the intact tissue, through specific effects on a transmembrane receptor. Growth factors include polypeptides and non-polypeptide factors.

#### ***A. Producing modified immune effector cells using CRISPR/Cas Systems***

**[00322]** In some embodiments, a method of producing a modified immune effector cell involves contacting a target DNA sequence with a complex comprising a gRNA and a Cas polypeptide. As discussed above, a gRNA and Cas polypeptide form a complex, wherein the DNA-binding domain of the gRNA targets the complex to a target DNA sequence and wherein the Cas protein (or heterologous protein fused to an enzymatically inactive Cas protein) modifies target DNA sequence. In some embodiments, this complex is formed intracellularly after introduction of the gRNA and Cas protein (or polynucleotides encoding the gRNA and Cas proteins) to a cell. In some embodiments, the nucleic acid encoding the Cas protein is a DNA nucleic acid and is introduced to

the cell by transduction. In some embodiments, the Cas9 and gRNA components of a CRISPR/Cas gene editing system are encoded by a single polynucleotide molecule. In some embodiments, the polynucleotide encoding the Cas protein and gRNA component are comprised in a viral vector and introduced to the cell by viral transduction. In some embodiments, the Cas9 and gRNA components of a CRISPR/Cas gene editing system are encoded by different polynucleotide molecules. In some embodiments, the polynucleotide encoding the Cas protein is comprised in a first viral vector and the polynucleotide encoding the gRNA is comprised in a second viral vector. In some aspects of this embodiment, the first viral vector is introduced to a cell prior to the second viral vector. In some aspects of this embodiment, the second viral vector is introduced to a cell prior to the first viral vector. In such embodiments, integration of the vectors results in sustained expression of the Cas9 and gRNA components. However, sustained expression of Cas9 may lead to increased off-target mutations and cutting in some cell types. Therefore, in some embodiments, an mRNA nucleic acid sequence encoding the Cas protein may be introduced to the population of cells by transfection. In such embodiments, the expression of Cas9 will decrease over time, and may reduce the number of off target mutations or cutting sites.

**[00323]** In some embodiments, this complex is formed in a cell-free system by mixing the gRNA molecules and Cas proteins together and incubating for a period of time sufficient to allow complex formation. This pre-formed complex, comprising the gRNA and Cas protein and referred to herein as a CRISPR-ribonucleoprotein (CRISPR-RNP) can then be introduced to a cell in order to modify a target DNA sequence.

#### ***B. Producing modified immune effector cells using shRNA systems***

**[00324]** In some embodiments, a method of producing a modified immune effector cell introducing into the cell one or more DNA polynucleotides encoding one or more shRNA molecules with sequence complementary to the mRNA transcript of a target gene. The immune effector cell can be modified to produce the shRNA by introducing specific DNA sequences into the cell nucleus via a small gene cassette. Both retroviruses and lentiviruses can be used to introduce shRNA-encoding DNAs into immune effector cells. The introduced DNA can either become part of the cell's own DNA or persist in the nucleus, and instructs the cell machinery to produce shRNAs. shRNAs may be processed by Dicer or AGO2-mediated slicer activity inside the cell to induce RNAi mediated gene knockdown.

## V. Compositions and Kits

**[00325]** The term “composition” as used herein refers to a formulation of a gene-regulating system or a modified immune effector cell described herein that is capable of being administered or delivered to a subject or cell. Typically, formulations include all physiologically acceptable compositions including derivatives and/or prodrugs, solvates, stereoisomers, racemates, or tautomers thereof with any physiologically acceptable carriers, diluents, and/or excipients. A “therapeutic composition” or “pharmaceutical composition” (used interchangeably herein) is a composition of a gene-regulating system or a modified immune effector cell capable of being administered to a subject for the treatment of a particular disease or disorder or contacted with a cell for modification of one or more endogenous target genes.

**[00326]** The phrase “pharmaceutically acceptable” is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

**[00327]** As used herein “pharmaceutically acceptable carrier, diluent or excipient” includes without limitation any adjuvant, carrier, excipient, glidant, sweetening agent, diluent, preservative, dye/colorant, flavor enhancer, surfactant, wetting agent, dispersing agent, suspending agent, stabilizer, isotonic agent, solvent, surfactant, and/or emulsifier which has been approved by the United States Food and Drug Administration as being acceptable for use in humans and/or domestic animals. Exemplary pharmaceutically acceptable carriers include, but are not limited to, to sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; tragacanth; malt; gelatin; talc; cocoa butter, waxes, animal and vegetable fats, paraffins, silicones, bentonites, silicic acid, zinc oxide; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen- free water; isotonic saline; Ringer’s solution; ethyl alcohol; phosphate buffer solutions; and any other compatible substances employed in pharmaceutical formulations. Except insofar as any conventional media and/or agent is incompatible with the agents of the present disclosure, its use in therapeutic compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions.

**[00328]** “Pharmaceutically acceptable salt” includes both acid and base addition salts. Pharmaceutically-acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as, but not limited to, acetic acid, 2,2-dichloroacetic acid, adipic acid, alginic acid, ascorbic acid, aspartic acid, benzenesulfonic acid, benzoic acid, 4-acetamidobenzoic acid, camphoric acid, camphor-10-sulfonic acid, capric acid, caproic acid, caprylic acid, carbonic acid, cinnamic acid, citric acid, cyclamic acid, dodecylsulfuric acid, ethane-1,2-disulfonic acid, ethanesulfonic acid, 2-hydroxyethanesulfonic acid, formic acid, fumaric acid, galactaric acid, gentisic acid, glucoheptonic acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, 2-oxo-glutaric acid, glycerophosphoric acid, glycolic acid, hippuric acid, isobutyric acid, lactic acid, lactobionic acid, lauric acid, maleic acid, malic acid, malonic acid, mandelic acid, methanesulfonic acid, mucic acid, naphthalene-1,5-disulfonic acid, naphthalene-2-sulfonic acid, 1-hydroxy-2-naphthoic acid, nicotinic acid, oleic acid, orotic acid, oxalic acid, palmitic acid, pamoic acid, propionic acid, pyroglutamic acid, pyruvic acid, salicylic acid, 4-aminosalicylic acid, sebacic acid, stearic acid, succinic acid, tartaric acid, thiocyanic acid, p-toluenesulfonic acid, trifluoroacetic acid, undecylenic acid, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Salts derived from organic bases include, but are not limited to, salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as ammonia, isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, diethanolamine, ethanolamine, deanol, 2-dimethylaminoethanol, 2-diethylaminoethanol, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, benethamine, benzathine, ethylenediamine, glucosamine, methylglucamine, theobromine, triethanolamine, tromethamine, purines, piperazine, piperidine, N-ethylpiperidine, polyamine resins and the like. Particularly preferred organic bases are isopropylamine, diethylamine, ethanolamine, trimethylamine, dicyclohexylamine, choline and caffeine.

**[00329]** Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

**[00330]** Examples of pharmaceutically-acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

**[00331]** Further guidance regarding formulations that are suitable for various types of administration can be found in Remington's Pharmaceutical Sciences, Mace Publishing Company, Philadelphia, Pa., 17th ed. (1985). For a brief review of methods for drug delivery, see, Langer, Science 249:1527-1533 (1990).

**[00332]** In some embodiments, the present disclosure provides kits for carrying out a method described herein. In some embodiments, a kit can include:

(a) two or more nucleic acid molecules capable of reducing the expression or modifying the function of a gene product encoded by two or more endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;

(b) one or more polynucleotides encoding two or more nucleic acid molecules that are capable of reducing the expression or modifying the function of a gene product encoded by two or more endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;

(c) two or more proteins capable of reducing the expression or modifying the function of a gene product encoded by two or more endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;

(d) one or more polynucleotides encoding two or more modifying proteins that is capable of reducing the expression or modifying the function of a gene product encoded by two or more endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;

(e) two or more gRNAs capable of binding to a target DNA sequence in two or more endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;

(f) one or more polynucleotides encoding two or more gRNAs capable of binding to a target DNA sequence in two or more endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;



- (g) one or more site-directed modifying polypeptides capable of interacting with a gRNA and modifying a target DNA sequence in an endogenous gene;
- (h) one or more polynucleotides encoding a site-directed modifying polypeptide capable of interacting with a gRNA and modifying a target DNA sequence in two or more endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;
- (i) two or more guide DNAs (gDNAs) capable of binding to a target DNA sequence in two or more endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;
- (j) one or more polynucleotides encoding two or more gDNAs capable of binding to a target DNA sequence in two or more endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;
- (k) one or more site-directed modifying polypeptides capable of interacting with a gDNA and modifying a target DNA sequence in an endogenous gene;
- (l) one or more polynucleotides encoding a site-directed modifying polypeptide capable of interacting with a gDNA and modifying a target DNA sequence in an endogenous gene;
- (m) two or more gRNAs capable of binding to a target mRNA sequence encoded by two or more endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;
- (n) one or more polynucleotides encoding two or more gRNAs capable of binding to a target mRNA sequence encoded by two or more endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*;
- (o) one or more site-directed modifying polypeptides capable of interacting with a gRNA and modifying a target mRNA sequence encoded by an endogenous gene;
- (p) one or more polynucleotides encoding a site-directed modifying polypeptide capable of interacting with a gRNA and modifying a target mRNA sequence encoded by an endogenous gene;
- (q) a modified immune effector cell described herein; or
- (r) any combination of the above.

**[00333]** In some embodiments, the kits described herein further comprise one or more immune checkpoint inhibitors. Several immune checkpoint inhibitors are known in the art and have received FDA approval for the treatment of one or more cancers. For example, FDA-approved PD-L1 inhibitors

include Atezolizumab (Tecentriq<sup>®</sup>, Genentech), Avelumab (Bavencio<sup>®</sup>, Pfizer), and Durvalumab (Imfinzi<sup>®</sup>, AstraZeneca); FDA-approved PD-1 inhibitors include Pembrolizumab (Keytruda<sup>®</sup>, Merck) and Nivolumab (Opdivo<sup>®</sup>, Bristol-Myers Squibb); and FDA-approved CTLA4 inhibitors include Ipilimumab (Yervoy<sup>®</sup>, Bristol-Myers Squibb). Additional inhibitory immune checkpoint molecules that may be the target of future therapeutics include A2AR, B7-H3, B7-H4, BTLA, IDO, LAG3 (*e.g.*, BMS-986016, under development by BSM), KIR (*e.g.*, Lirilumab, under development by BSM), TIM3, TIGIT, and VISTA.

**[00334]** In some embodiments, the kits described herein comprise one or more components of a gene-regulating system (or one or more polynucleotides encoding the one or more components) and one or more immune checkpoint inhibitors known in the art (*e.g.*, a PD1 inhibitor, a CTLA4 inhibitor, a PDL1 inhibitor, *etc.*). In some embodiments, the kits described herein comprise one or more components of a gene-regulating system (or one or more polynucleotides encoding the one or more components) and an anti-PD1 antibody (*e.g.*, Pembrolizumab or Nivolumab). In some embodiments, the kits described herein comprise a modified immune effector cell described herein (or population thereof) and one or more immune checkpoint inhibitors known in the art (*e.g.*, a PD1 inhibitor, a CTLA4 inhibitor, a PDL1 inhibitor, *etc.*). In some embodiments, the kits described herein comprise a modified immune effector cell described herein (or population thereof) and an anti-PD1 antibody (*e.g.*, Pembrolizumab or Nivolumab).

**[00335]** In some embodiments, the kit comprises one or more components of a gene-regulating system (or one or more polynucleotides encoding the one or more components) and a reagent for reconstituting and/or diluting the components. In some embodiments, a kit comprising one or more components of a gene-regulating system (or one or more polynucleotides encoding the one or more components) and further comprises one or more additional reagents, where such additional reagents can be selected from: a buffer for introducing the gene-regulating system into a cell; a wash buffer; a control reagent; a control expression vector or RNA polynucleotide; a reagent for *in vitro* production of the gene-regulating system from DNA, and the like. Components of a kit can be in separate containers or can be combined in a single container.

**[00336]** In addition to above-mentioned components, in some embodiments a kit further comprises instructions for using the components of the kit to practice the methods of the present disclosure. The instructions for practicing the methods are generally recorded on a suitable recording medium. For example, the instructions may be printed on a substrate, such as paper or plastic, *etc.* As such, the instructions may be present in the kits as a package insert or in the labeling of the container

of the kit or components thereof (*i.e.*, associated with the packaging or sub-packaging). In other embodiments, the instructions are present as an electronic storage data file present on a suitable computer readable storage medium, *e.g.* CD-ROM, diskette, flash drive, etc. In yet other embodiments, the actual instructions are not present in the kit, but means for obtaining the instructions from a remote source, *e.g.* via the internet, are provided. An example of this embodiment is a kit that includes a web address where the instructions can be viewed and/or from which the instructions can be downloaded. As with the instructions, this means for obtaining the instructions is recorded on a suitable substrate.

## VI. Therapeutic Methods and Applications

**[00337]** In some embodiments, the modified immune effector cells and gene-regulating systems described herein may be used in a variety of therapeutic applications. For example, in some embodiments the modified immune effector cells and/or gene-regulating systems described herein may be administered to a subject for purposes such as gene therapy, *e.g.* to treat a disease, for use as an antiviral, for use as an anti-pathogenic, for use as an anti-cancer therapeutic, or for biological research.

**[00338]** In some embodiments, the subject may be a neonate, a juvenile, or an adult. Of particular interest are mammalian subjects. Mammalian species that may be treated with the present methods include canines and felines; equines; bovines; ovines; etc. and primates, particularly humans. Animal models, particularly small mammals (*e.g.* mice, rats, guinea pigs, hamsters, rabbits, *etc.*) may be used for experimental investigations.

**[00339]** Administration of the modified immune effector cells described herein, populations thereof, and compositions thereof can occur by injection, irrigation, inhalation, consumption, electro-osmosis, hemodialysis, iontophoresis, and other methods known in the art. In some embodiments, administration route is local or systemic. In some embodiments administration route is intraarterial, intracranial, intradermal, intraduodenal, intramammary, intrameningeal, intraperitoneal, intrathecal, intratumoral, intravenous, intravitreal, ophthalmic, parenteral, spinal, subcutaneous, ureteral, urethral, vaginal, or intrauterine.

**[00340]** In some embodiments, the administration route is by infusion (*e.g.*, continuous or bolus). Examples of methods for local administration, that is, delivery to the site of injury or disease, include through an Ommaya reservoir, *e.g.* for intrathecal delivery (*See e.g.*, US Patent Nos. 5,222,982 and 5,385,582, incorporated herein by reference); by bolus injection, *e.g.* by a syringe, *e.g.* into a joint;

by continuous infusion, *e.g.* by cannulation, such as with convection (*See e.g.*, US Patent Application Publication No. 2007-0254842, incorporated herein by reference); or by implanting a device upon which the cells have been reversibly affixed (see *e.g.* US Patent Application Publication Nos. 2008-0081064 and 2009-0196903, incorporated herein by reference). In some embodiments, the administration route is by topical administration or direct injection. In some embodiments, the modified immune effector cells described herein may be provided to the subject alone or with a suitable substrate or matrix, *e.g.* to support their growth and/or organization in the tissue to which they are being transplanted.

**[00341]** In some embodiments, at least  $1 \times 10^3$  cells are administered to a subject. In some embodiments, at least  $5 \times 10^3$  cells,  $1 \times 10^4$  cells,  $5 \times 10^4$  cells,  $1 \times 10^5$  cells,  $5 \times 10^5$  cells,  $1 \times 10^6$ ,  $2 \times 10^6$ ,  $3 \times 10^6$ ,  $4 \times 10^6$ ,  $5 \times 10^6$ ,  $1 \times 10^7$ ,  $1 \times 10^8$ ,  $5 \times 10^8$ ,  $1 \times 10^9$ ,  $5 \times 10^9$ ,  $1 \times 10^{10}$ ,  $5 \times 10^{10}$ ,  $1 \times 10^{11}$ ,  $5 \times 10^{11}$ ,  $1 \times 10^{12}$ ,  $5 \times 10^{12}$ , or more cells are administered to a subject. In some embodiments, between about  $1 \times 10^7$  and about  $1 \times 10^{12}$  cells are administered to a subject. In some embodiments, between about  $1 \times 10^8$  and about  $1 \times 10^{12}$  cells are administered to a subject. In some embodiments, between about  $1 \times 10^9$  and about  $1 \times 10^{12}$  cells are administered to a subject. In some embodiments, between about  $1 \times 10^{10}$  and about  $1 \times 10^{12}$  cells are administered to a subject. In some embodiments, between about  $1 \times 10^{11}$  and about  $1 \times 10^{12}$  cells are administered to a subject. In some embodiments, between about  $1 \times 10^7$  and about  $1 \times 10^{11}$  cells are administered to a subject. In some embodiments, between about  $1 \times 10^7$  and about  $1 \times 10^{10}$  cells are administered to a subject. In some embodiments, between about  $1 \times 10^7$  and about  $1 \times 10^9$  cells are administered to a subject. In some embodiments, between about  $1 \times 10^7$  and about  $1 \times 10^8$  cells are administered to a subject. The number of administrations of treatment to a subject may vary. In some embodiments, introducing the modified immune effector cells into the subject may be a one-time event. In some embodiments, such treatment may require an on-going series of repeated treatments. In some embodiments, multiple administrations of the modified immune effector cells may be required before an effect is observed. The exact protocols depend upon the disease or condition, the stage of the disease and parameters of the individual subject being treated.

**[00342]** In some embodiments, the gene-regulating systems described herein are employed to modify cellular DNA or RNA *in vivo*, such as for gene therapy or for biological research. In such embodiments, a gene-regulating system may be administered directly to the subject, such as by the methods described *supra*. In some embodiments, the gene-regulating systems described herein are employed for the *ex vivo* or *in vitro* modification of a population of immune effector cells. In such

embodiments, the gene-regulating systems described herein are administered to a sample comprising immune effector cells.

**[00343]** In some embodiments, the modified immune effector cells described herein are administered to a subject. In some embodiments, the modified immune effector cells described herein administered to a subject are autologous immune effector cells. The term “autologous” in this context refers to cells that have been derived from the same subject to which they are administered. For example, immune effector cells may be obtained from a subject, modified *ex vivo* according to the methods described herein, and then administered to the same subject in order to treat a disease. In such embodiments, the cells administered to the subject are autologous immune effector cells. In some embodiments, the modified immune effector cells, or compositions thereof, administered to a subject are allogenic immune effector cells. The term “allogenic” in this context refers to cells that have been derived from one subject and are administered to another subject. For example, immune effector cells may be obtained from a first subject, modified *ex vivo* according to the methods described herein and then administered to a second subject in order to treat a disease. In such embodiments, the cells administered to the subject are allogenic immune effector cells.

**[00344]** In some embodiments, the modified immune effector cells described herein are administered to a subject in order to treat a disease. In some embodiments, treatment comprises delivering an effective amount of a population of cells (*e.g.*, a population of modified immune effector cells) or composition thereof to a subject in need thereof. In some embodiments, treating refers to the treatment of a disease in a mammal, *e.g.*, in a human, including (a) inhibiting the disease, *i.e.*, arresting disease development or preventing disease progression; (b) relieving the disease, *i.e.*, causing regression of the disease state or relieving one or more symptoms of the disease; and (c) curing the disease, *i.e.*, remission of one or more disease symptoms. In some embodiments, treatment may refer to a short-term (*e.g.*, temporary and/or acute) and/or a long-term (*e.g.*, sustained) reduction in one or more disease symptoms. In some embodiments, treatment results in an improvement or remediation of the symptoms of the disease. The improvement is an observable or measurable improvement, or may be an improvement in the general feeling of well-being of the subject.

**[00345]** The effective amount of a modified immune effector cell administered to a particular subject will depend on a variety of factors, several of which will differ from patient to patient including the disorder being treated and the severity of the disorder; activity of the specific agent(s) employed; the age, body weight, general health, sex and diet of the patient; the timing of administration, route of

administration; the duration of the treatment; drugs used in combination; the judgment of the prescribing physician; and like factors known in the medical arts.

**[00346]** In some embodiments, the effective amount of a modified immune effector cell may be the number of cells required to result in at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, or more fold decrease in tumor mass or volume, decrease in the number of tumor cells, or decrease in the number of metastases. In some embodiments, the effective amount of a modified immune effector cell may be the number of cells required to achieve an increase in life expectancy, an increase in progression-free or disease-free survival, or amelioration of various physiological symptoms associated with the disease being treated. In some embodiments, an effective amount of modified immune effector cells will be at least  $1 \times 10^3$ , for example  $5 \times 10^3$ ,  $1 \times 10^4$ ,  $5 \times 10^4$ ,  $1 \times 10^5$ ,  $5 \times 10^5$ ,  $1 \times 10^6$ ,  $2 \times 10^6$ ,  $3 \times 10^6$ ,  $4 \times 10^6$ ,  $5 \times 10^6$ ,  $1 \times 10^7$ ,  $1 \times 10^8$ ,  $5 \times 10^8$ ,  $1 \times 10^9$ ,  $5 \times 10^9$ ,  $1 \times 10^{10}$ ,  $5 \times 10^{10}$ ,  $1 \times 10^{11}$ ,  $5 \times 10^{11}$ ,  $1 \times 10^{12}$ ,  $5 \times 10^{12}$ , or more cells.

**[00347]** In some embodiments, the modified immune effector cells and gene-regulating systems described herein may be used in the treatment of a cell-proliferative disorder, such as a cancer. Cancers that may be treated using the compositions and methods disclosed herein include cancers of the blood and solid tumors. For example, cancers that may be treated using the compositions and methods disclosed herein include, but are not limited to, adenoma, carcinoma, sarcoma, leukemia or lymphoma. In some embodiments, the cancer is chronic lymphocytic leukemia (CLL), B cell acute lymphocytic leukemia (B-ALL), acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), non-Hodgkin's lymphoma (NHL), diffuse large cell lymphoma (DLCL), diffuse large B cell lymphoma (DLBCL), Hodgkin's lymphoma, multiple myeloma, renal cell carcinoma (RCC), neuroblastoma, colorectal cancer, breast cancer, ovarian cancer, melanoma, sarcoma, prostate cancer, lung cancer including but not limited to NSCLC esophageal cancer, hepatocellular carcinoma, pancreatic cancer, astrocytoma, mesothelioma, head and neck cancer, medulloblastoma, bladder cancer, and liver cancer.

**[00348]** As described above, several immune checkpoint inhibitors are currently approved for use in a variety of oncologic indications (*e.g.*, CTLA4 inhibitors, PD1 inhibitors, PDL1 inhibitors, etc.). In some embodiments, administration of a modified immune effector cell comprising reduced expression and/or function of an endogenous target gene described herein results in an enhanced therapeutic effect (*e.g.*, a more significant reduction in tumor growth, an increase in tumor infiltration by lymphocytes, an increase in the length of progression free survival, etc.) than is observed after treatment with an immune checkpoint inhibitor.

**[00349]** Further, some oncologic indications are non-responsive (*i.e.*, are insensitive) to treatment with immune checkpoint inhibitors. Further still, some oncologic indications that are initially responsive (*i.e.*, sensitive) to treatment with immune checkpoint inhibitors develop an inhibitor-resistant phenotype during the course of treatment. Therefore, in some embodiments, the modified immune effector cells described herein, or compositions thereof, are administered to treat a cancer that is resistant (or partially resistant) or insensitive (or partially insensitive) to treatment with one or more immune checkpoint inhibitors. In some embodiments, administration of the modified immune effector cells or compositions thereof to a subject suffering from a cancer that is resistant (or partially resistant) or insensitive (or partially insensitive) to treatment with one or more immune checkpoint inhibitors results in treatment of the cancer (*e.g.*, reduction in tumor growth, an increase in the length of progression free survival, etc.). In some embodiments, the cancer is resistant (or partially resistant) or insensitive (or partially insensitive) to treatment with a PD1 inhibitor.

**[00350]** In some embodiments, the modified immune effector cells or compositions thereof are administered in combination with an immune checkpoint inhibitor. In some embodiments, administration of the modified immune effector cells in combination with the immune checkpoint inhibitor results in an enhanced therapeutic effect in a cancer that is resistant, refractory, or insensitive to treatment by an immune checkpoint inhibitor than is observed by treatment with either the modified immune effector cells or the immune checkpoint inhibitor alone. In some embodiments, administration of the modified immune effector cells in combination with the immune checkpoint inhibitor results in an enhanced therapeutic effect in a cancer that is partially resistant, partially refractory, or partially insensitive to treatment by an immune checkpoint inhibitor than is observed by treatment with either the modified immune effector cells or the immune checkpoint inhibitor alone. In some embodiments, the cancer is resistant (or partially resistant), refractory (or partially refractory), or insensitive (or partially insensitive) to treatment with a PD1 inhibitor.

**[00351]** In some embodiments, administration of a modified immune effector cell described herein or composition thereof in combination with an anti-PD1 antibody results in an enhanced therapeutic effect in a cancer that is resistant or insensitive to treatment by the anti-PD1 antibody alone. In some embodiments, administration of a modified immune effector cell described herein or composition thereof in combination with an anti-PD1 antibody results in an enhanced therapeutic effect in a cancer that is partially resistant or partially insensitive to treatment by the anti-PD1 antibody alone.

**[00352]** Cancers that demonstrate resistance or sensitivity to immune checkpoint inhibition are known in the art and can be tested in a variety of *in vivo* and *in vitro* models. For example, some melanomas are sensitive to treatment with an immune checkpoint inhibitor such as an anti-PD1 antibody and can be modeled in an *in vivo* B16-Ova tumor model (*See* Example 5). Further, some colorectal cancers are known to be resistant to treatment with an immune checkpoint inhibitor such as an anti-PD1 antibody and can be modeled in a PMEL/MC38-gp100 model (*See* Example 5). Further still, some lymphomas are known to be insensitive to treatment with an immune checkpoint inhibitor such as an anti-PD1 antibody and can be modeled in various models by adoptive transfer or subcutaneous administration of lymphoma cell lines, such as Raji cells (*See* Example 6-9).

**[00353]** Current adoptive cell therapy, including TIL therapy, includes lymphodepletion 7 days prior to TIL infusion using Cy/Flu based treatment. The lymphodepletion is believed necessary to deplete the endogenous Treg population, to boost endogenous IL-7 and IL-15 production and to create physical space for the TIL infusion. This lymphodepletion is associated with severe grade 3, 4, and sometimes 5 adverse events and can significantly impact patient outcome. In addition, current therapy includes an infusion of high dose IL-2 5 days prior to TIL infusion in order to boost function and survival of the transferred TILs. However, the high dose IL-2 infusion is associated with severe grade 3 and 4 adverse events, including capillary leak syndrome. In some embodiments, the modified immune effector cells described herein are transferred to a recipient host that has not undergone lymphodepletion treatment and/or are transferred to a recipient host in the absence of high dose IL-2 treatment. Without wishing to be bound by theory, it is possible that the modified immune effector cells described herein (*e.g.*, modified TILs) demonstrate increased sensitivity to IL-7, IL-15 and/or IL-2, therefore allowing for increased steps enhanced competitive fitness, survival, and/or persistence of the modified cells such that lymphodepletion and/or high dose IL-2 is not required.

**[00354]** In some embodiments, the modified immune effector cells and gene-regulating systems described herein may be used in the treatment of a viral infection. In some embodiments, the virus is selected from one of adenoviruses, herpesviruses (including, for example, herpes simplex virus and Epstein Barr virus, and herpes zoster virus), poxviruses, papovaviruses, hepatitis viruses, (including, for example, hepatitis B virus and hepatitis C virus), papilloma viruses, orthomyxoviruses (including, for example, influenza A, influenza B, and influenza C), paramyxoviruses, coronaviruses, picornaviruses, reoviruses, togaviruses, flaviviruses, bunyaviridae, rhabdoviruses, rotavirus, respiratory syncytial virus, human immunodeficiency virus, or retroviruses.



## EXAMPLES

### EXAMPLE 1: MATERIALS AND METHODS

**[00355]** The experiments described herein utilize the CRISPR/Cas9 system to reduce expression of two or more of *SOCS1*, *PTPN2*, and *ZC3H12A* in different T cell populations.

**[00356]** gRNA-Cas9 RNPs: Unless otherwise indicated, the following experiments use dual gRNA molecules formed by duplexing 200  $\mu$ M tracrRNA (IDT Cat# 1072534) with 200  $\mu$ M of target-specific crRNA (IDT) in nuclease free duplex buffer (IDT Cat#11-01-03-01) for 5 min at 95° C, to form 100  $\mu$ M of tracrRNA:crRNA duplex, where the tracrRNA and crRNA are present at a 1:1 ratio. Alternatively, single-molecule gRNAs (sgRNAs) (IDT) were resuspended at 100  $\mu$ M in nuclease free duplex buffer (IDT). Cas9 was expressed in target cells by introduction of either Cas9 mRNA or a Cas9 protein. Unless otherwise indicated, the Cas9 protein derived from *S. pyogenes* (IDT Cat# 1074182) was used in the following experiments. For Human RNPs, gRNA-Cas9 ribonucleoproteins (RNPs) were formed by combining 1.2  $\mu$ L of 100  $\mu$ M tracrRNA:crRNA duplex or gRNAs with 1  $\mu$ L of 20  $\mu$ M Cas9 protein and 0.8  $\mu$ L of PBS. For Mouse RNPs, gRNA-Cas9 ribonucleoproteins (RNPs) were formed by combining 1 Volume of 44  $\mu$ M tracrRNA:crRNA duplex or gRNA with 1 Volume of 36  $\mu$ M Cas9 in Invitrogen Buffer T. For both, mixtures were incubated at RT for 20 minutes to form the RNP complexes. gRNAs used in the following experiments are provided in Table 18 below.

**Table 18:**

| Target Gene    | Guide ID   | Sequence             | SEQ ID |
|----------------|------------|----------------------|--------|
| <i>Pdcd1</i>   | Nm.Pdcd1   | CGGAGGATCTTATGCTGAAC | 270    |
| <i>Cblb</i>    | Nm.Cblb    | CCTTATCTTCAGTCACATGC | 271    |
| <i>Zc3h12a</i> | Nm.Zc3h12a | TTCCCTCCTCTGCCAGCCAT | 211    |
| <i>Socs1</i>   | Nm.Socs1   | GCCGGCCGCTTCCACTTGGA | 9      |
| <i>Ptpn2</i>   | Nm.Ptpn2   | CCTTTCTTGCAGATGGAAAA | 202    |

**[00357]** CAR Expression Constructs: A Chimeric antigen receptor (CAR) specific for human CD19, was generated. Briefly, the 22 amino acid signal peptide of the human granulocyte-macrophage colony stimulating factor receptor subunit alpha (GMSCF-R $\alpha$ ) was fused to an antigen-specific scFv domain specifically binding to CD19 (clone FMC63). The human CD8 $\alpha$  stalk was used as a transmembrane domain. The intracellular signaling domains of the CD3 $\xi$  chain were fused to the

cytoplasmic end of the CD8 $\alpha$  stalk. The full length CAR construct is provided in SEQ ID NO: 813 and nucleic acid sequences of the full length CAR constructs is provided in SEQ ID NO: 814.

**[00358]** Engineered TCRs Expression Constructs: A recombinant T cell receptor (TCR) specific for the NY-ESO-1 peptide (in the context of HLA-A\*02:01) was generated. Paired TCR- $\alpha$ :TCR- $\beta$  variable region protein sequences encoding the 1G4 TCR specific for the NY-ESO-1 peptide comprising the sequence of SLLMWITQ (SEQ ID NO: 815), presented by HLA-A\*02:01, were identified from the literature (Robbins *et al*, Journal of Immunology 2008 180:6116-6131). The NY-ESO-1 peptide can have an additional cysteine or valine at its C-terminal end. TCR $\alpha$  chains were composed of V and J gene segments and CDR3 $\alpha$  sequences and TCR $\beta$  chains were composed of V, D, and J gene segment and CDR3- $\beta$  sequences. The native TRAC (SEQ ID NO: 816) and TRBC (SEQ ID NOs: 817) protein sequences were fused to the C-terminal ends of the  $\alpha$  and  $\beta$  chain variable regions, respectively, to produce 95:LY 1G4-TCR  $\alpha/\beta$  chains (SEQ ID NOs: 818 and 819, respectively).

**[00359]** Codon-optimized DNA sequences encoding the engineered TCR $\alpha$  and TCR $\beta$  chain proteins were generated where the P2A sequence (SEQ ID NO: 820) was inserted between the DNA sequences encoding the TCR $\beta$  and the TCR $\alpha$  chain, such that expression of both TCR chains was driven off of a single promoter in a stoichiometric fashion. The expression cassettes encoding the engineered TCR chains therefore comprised the following format: TCR $\beta$  – P2A – TCR $\alpha$ . Final protein sequences for each TCR construct are provided in SEQ ID NO: 821 (95:LY 1G4). This TCR construct is referred to hereafter as “TCR2”.

**[00360]** Lentiviral Expression Constructs: The CAR and engineered TCR expression constructs described above were then inserted into a plasmid comprising an SFFV promoter driving expression of the engineered receptor, a T2A sequence, and a puromycin resistance cassette. Lentiviral constructs comprising an engineered CAR expression construct may further comprise an sgRNA targeting the endogenous TRAC gene, which encodes the constant region of the  $\alpha$  chain of the T cell receptor.

**[00361]** Lentiviruses encoding the engineered receptors described above were generated as follows. Briefly,  $289 \times 10^6$  of LentiX-293T cells were plated out in a 5-layer CellSTACK 24 hours prior to transfection. Serum-free OptiMEM and TransIT-293 were combined and incubated for 5 minutes before combining helper plasmids (58  $\mu$ g VSVG and 115  $\mu$ g PAX2-Gag-Pol) with 231  $\mu$ g of an engineered receptor- and sgRNA-expressing plasmid described above. After 20 minutes, this mixture was added to the LentiX-293T cells with fresh media. Media was replaced 18 hours after

transfection and viral supernatants were collected 48 hours post-transfection. Supernatants were treated with Benzonase® nuclease and passed through a 0.45 µm filter to isolate the viral particles. Virus particles were then concentrated by Tangential Flow Filtration (TFF), aliquoted, tittered, and stored at -80° C.

**[00362]**      Human T cell Isolation and Activation: Total human PBMCs were isolated from fresh leukopheresis by Ficoll gradient centrifugation. CD8<sup>+</sup> T-cells were then purified from total PBMCs using a CD8<sup>+</sup> T-cell isolation kit (Stemcell Technologies Cat # 17953). For T cell activation, CD8<sup>+</sup> T cells were plated at  $2 \times 10^6$  cells/mL in X-VIVO 15 T Cell Expansion Medium (Lonza, Cat# 04-418Q) in a T175 flask, with 6.25 µL/mL of ImmunoCult T-cell activators (anti-CD3/CD28/CD2, StemCell Technologies, Vancouver BC, Canada) and 10 ng/mL human IL2. T-cells were activated for 18 hours prior to transduction with lentiviral constructs.

**[00363]**      Lentiviral transduction of T cells: T-cells activated 18 hours prior were seeded at  $5 \times 10^6$  cells per well in a 6 well plate, in 1.5 mL volume of X-VIVO 15 media, 10 ng/mL human IL-2 and 12.5 µL Immunocult Human CD3/CD28/CD2 T-cell Activator. Lentivirus expressing the engineered receptors was added at an MOI capable of infecting 80% of all cells. 25 µL of Retronectin (1 mg/mL) was added to each well. XVIVO-15 media was added to a final volume of 2.0 mL per well. Plates were spun at 600 x g for 1.5 hours at room temperature. One day later, cells were washed and seeded at  $1 \times 10^6$  cells/mL in X-VIVO 15, 10 ng/mL IL2 + T-cell activators.

**[00364]**      Electroporation of human PBMC-derived T cells: 3 days after T cell activation, T cells were harvested and resuspended in nucleofection buffer (18% supplement 1, 82% P3 buffer from the Amaxa P3 primary cell 4D-Nucleofector X kit S) at a concentration of  $100 \times 10^6$  cells/mL. 1.5 µL of sgRNA/Cas9 RNP complexes (containing 120 pmol of crRNA:tracrRNA duplex and 20 pmol of Cas9 nuclease) and 2.1 µL (100 pmol) of electroporation enhancer were added per 20 µL of cell solution. 25 µL of the cell/RNP/enhancer mixture was then added to each electroporation well. Cells were electroporated using the Lonza electroporator with the “EO-115” program. After electroporation, 80 µL of warm X-VIVO 15 media was added to each well and cells were pooled into a culture flask at a density of  $2 \times 10^6$  cells/mL in X-VIVO 15 media containing IL-2 (10 ng/mL). On Day 4, cells were washed, counted, and seeded at densities of 50-100 x  $10^6$  cells/L in X-VIVO 15 media containing IL-2 (10 ng/mL) in G-Rex6M well plates or G-Rex100M, depending on the number of cells available. On Days 6 and 8, 10 ng/mL of fresh recombinant human IL-2 was added to the cultures.

**[00365]**      Human TIL Isolation and Activation: Tumor infiltrating lymphocytes can also be modified by the methods described herein. In such cases, tumors are surgically resected from human patients and diced with scalpel blades into 1 mm<sup>3</sup> pieces, with a single piece of tumor placed into each well of a 24 plate. 2 mL of complete TIL media (RPMI + 10% heat inactivated human male AB serum, 1 mM pyruvate, 20 µg/mL gentamycin, 1X glutamax) supplemented with 6000 U/mL of recombinant human IL-2 is added to each well of isolated TILs. 1 mL of media is removed from the well and replaced with fresh media and IL-2 up to 3 times a week as needed. As wells reach confluence, they are split 1:1 in new media + IL-2. After 4-5 weeks of culture, the cells are harvested for rapid expansion.

**[00366]**      TIL Rapid Expansion: TILs are rapidly expanded by activating 500,000 TILs with  $26 \times 10^6$  allogeneic, irradiated (5000cGy) PBMC feeder cells in 20 mL TIL media + 20 mL of Aim-V media (Invitrogen) + 30 ng/mL OKT3 mAb. 48 hours later (Day 2), 6000 U/mL IL-2 is added to the cultures. On day 5, 20 mL of media is removed, and 20 mL fresh media (+ 30 ng/ml OKT3) is added. On Day 7, cells are counted, and reseeded at  $60 \times 10^6$  cells/L in G-Rex6M well plates (Wilson Wolf, Cat# 80660M) or G-Rex100M (Wilson Wolf, Cat# 81100S), depending on the number of cells available. 6000 U/mL fresh IL-2 is added on Day 9 and 3000 U/mL fresh IL-2 is added on Day 12. TILs are harvested on Day 14. Expanded cells are then slow-frozen in Cryostor CS-10 (Stemcell Technologies Cat #07930) using Coolcell Freezing containers (Corning) and stored long term in liquid nitrogen.

**[00367]**      Mice: Wild type CD8<sup>+</sup> T cells were derived from C57BL/6J mice (The Jackson Laboratory, Bar Harbor ME). Ovalbumin (Ova)-specific CD8<sup>+</sup> T cells were derived from OT1 mice (C57BL/6-Tg(Tcr $\alpha$ Tcr $\beta$ ) 1100Mjb/J; Jackson Laboratory). OT1 mice comprise a transgenic TCR that recognizes residues 257-264 of the ovalbumin (Ova) protein. gp100-specific CD8<sup>+</sup> T cells were derived from PMEL mice (B6.Cg-Thy1<sup>lcr</sup>/CyTg(Tcr $\alpha$ Tcr $\beta$ ) 8Rest/J; The Jackson Laboratory, Bar Harbor ME Cat # 005023). Mice constitutively expressing the Cas9 protein were obtained from Jackson labs (B6J.129(Cg)-Gt(ROSA)26Sortm1.1(CAG-cas9\*,-EGFP)Fezh/J; The Jackson Laboratory, Bar Harbor ME Strain # 026179), TCR-transgenic mice constitutively expressing Cas9 were obtained by breeding of OT1 mice with Cas9 mice.

**[00368]**      Murine T cell Isolation and Activation: Spleens from transgenic mice were harvested and reduced to a single cell suspension using the GentleMACS system, according to the manufacturer's recommendations. Purified CD8<sup>+</sup> T cells were obtained using the EasySep Mouse CD8<sup>+</sup> T Cell Isolation Kit (Catalog # 19853). CD8 T cells were cultured at  $1 \times 10^6$  cells/mL in

complete T cell media (RPMI + 10% heat inactivated FBS, 20 mM HEPES, 100 U/mL Penicillin, 100 µg/mL Streptomycin, 50 µM Beta-Mercaptoethanol) supplemented with 2ng/mL of Recombinant Mouse IL-2 (Biolegend Catalog # 575406) and activated with anti-CD3/anti-CD28 beads (Dynabeads™ Mouse T-Activator CD3/CD28 for T-Cell Expansion and Activation Cat # 11456D).

**[00369]** Electroporation of mouse T cells: Murine T-cells activated 48 hours prior were harvested, activation beads were removed and cells were washed and resuspended in Neon nucleofection buffer T. Up to  $20 \times 10^6$  cells resuspended in 90 µL (for single edits) or 80 µL (for combination edits) Buffer T can be electroporated using one Neon™ 100-µL tip. 10 µL of each sgRNA/Cas9 RNP complexes and 20 µL of 10.8 µM electroporation enhancer were added per tip. The T cell/RNP/enhancer mixture was loaded into the Neon™ tips and cells were electroporated on the Neon Transfection System using a single pulse of 20ms at 1700V. Immediately after electroporation, the cells were transferred into a culture flask at a density of  $1.6 \times 10^6$  cells/mL in warm complete T cell media supplemented with 2 ng/mL of Recombinant Mouse IL-2. Edited murine CD8 T cells were further cultured at  $1 \times 10^6$  cells/mL in complete T cell media supplemented with IL-2 for an additional 2 days. On Day 4, cells were harvested counted and resuspended in PBS for injection *in vivo*.

**[00370]** Generation and editing of murine TILs: To generate TILs, donor CD45.1 Pep Boy mice (B6.SJL-*Ptprca*<sup>a</sup> *Pepc*<sup>b</sup>/BoyJ) were injected subcutaneously with  $0.5 \times 10^6$  B16-Ova cells. On Day 14 post-tumor cell inoculation, tumors were harvested to generate edited CD45.1 Tumor Infiltrating Lymphocytes (TILs) to infuse into the second cohort of mice. B16-OVA tumors (200-600mm<sup>3</sup>) were harvested, diced and reduced to a single cell suspension using the GentleMACS system and mouse Tumor Dissociation Kit (Miltenyi Biotech Catalog # 130-096-730), according to the manufacturer's recommendations. Tumor suspension were filtered over 70 µm cell strainers and TILs were enriched using CD4/CD8 (TIL) Microbeads (Miltenyi Biotech Catalog # 130-116-480). Isolated TILs were cultured in 6 well plates at  $1.5 \times 10^6$  cells/mL in complete mTIL media (RPMI + 10% heat inactivated FBS, 20 mM HEPES, 100 U/mL Penicillin, 100 µg/mL Streptomycin, 50 µM Beta-Mercaptoethanol) supplemented with 3000 U/mL of recombinant human IL-2 (Peprotech Catalog # 200-02). On Day 3 cells were harvested, washed and resuspended in nucleofection buffer T and electroporated with RNPs using the Neon Transfection System. After electroporation, TILs were cultured in 6 well plates at  $1.5 \times 10^6$  cells/mL in complete mTIL media supplemented with 3000 U/mL of recombinant human IL-2. On Day 5 and 7, cells were resuspended in fresh complete mTIL media supplemented with 3000 U/mL of recombinant human IL-2 and plated in flasks at a density of  $1 \times 10^6$  cells /mL. On Day 8, cells were harvested counted and resuspended in PBS for injection *in vivo*.

**EXAMPLE 2: SCREEN FOR DUAL-EDIT COMBINATIONS**

[00371] A double sgRNA library was constructed in a retroviral backbone. The library consisted of two U6 promoters (one human and one mouse), each driving expression of a single guide RNA (guide+tracr, sgRNA). The guides were cloned as pools to provide random pairings between guides, such that every sgRNA would be paired with every other sgRNA. The final double guide library was transfected into Phoenix-Eco 293T cells to generate murine ecotropic retrovirus. TCR transgenic OT1 cells expressing Cas9 were infected with the sgRNA-expressing virus to edit the two loci targeted by each of the sgRNAs. The edited transgenic T-cells were then transferred into mice bearing  $> 400 \text{ mm}^3$  B16-Ova tumors allografts. After two weeks, the tumors were excised and digested into single cell suspension using Miltenyi Tumor Dissociation Kit. gDNA was extracted from the cell pellet using a Qiagen QIAmp DNA and blood kit and the retroviral inserts were recovered by PCR using primers corresponding to the retroviral backbone sequences. The resulting PCR product were then sequenced to identify the sgRNAs present in the tumors two weeks after transfer. The representation of guide pairs in the final isolated cell populations was compared to the initial plasmid population and the population of infected transgenic T-cells before injection into the mouse. The frequency of sgRNA pairs that improved T-cells fitness and/or tumor infiltration were expected to increase over time, while combinations that impaired fitness were expected to decrease over time. Table 19 below shows the median fold change of sgRNA frequency in the final cell population compared to the sgRNA frequency in the initial cell population transferred *in vivo*.

**Table 19:**

| Mouse Gene 1 | Mouse Gene 2   | Avg(Tmedian.Ifoldch.all) |
|--------------|----------------|--------------------------|
| <i>CBLB</i>  | <i>ZAP70</i>   | 0                        |
| <i>CBLB</i>  | <i>LAG3</i>    | 0.08                     |
| <i>CBLB</i>  | <i>CBLB</i>    | 0.17                     |
| <i>CBLB</i>  | <i>TIGIT</i>   | 0.31                     |
| <i>CBLB</i>  | <i>TGFBRI</i>  | 0.15                     |
| <i>CBLB</i>  | <i>CTLA4</i>   | 0.21                     |
| <i>CBLB</i>  | <i>PTPN2</i>   | 1.48                     |
| <i>CBLB</i>  | <i>TGFBRI</i>  | 0.75                     |
| <i>CBLB</i>  | <i>ZC3H12A</i> | 8.9                      |
| <i>PDCD1</i> | <i>ZAP70</i>   | 0                        |
| <i>PDCD1</i> | <i>LAG3</i>    | 0.02                     |
| <i>PDCD1</i> | <i>PDCD1</i>   | 0.02                     |
| <i>PDCD1</i> | <i>TIGIT</i>   | 0.02                     |
| <i>PDCD1</i> | <i>TGFBRI</i>  | 0.02                     |

|                 |                 |      |
|-----------------|-----------------|------|
| <i>PDCD1</i>    | <i>CTLA4</i>    | 0.59 |
| <i>PDCD1</i>    | <i>PTPN2</i>    | 0.07 |
| <i>PDCD1</i>    | <i>TGFBR2</i>   | 0.07 |
| <i>PDCD1</i>    | <i>ZC3H12A</i>  | 1.33 |
| <i>PTPN2</i>    | <i>ZAP70</i>    | 0    |
| <i>PTPN2</i>    | <i>LAG3</i>     | 0.09 |
| <i>PTPN2</i>    | <i>PTPN2</i>    | 0.04 |
| <i>PTPN2</i>    | <i>TIGIT</i>    | 0.38 |
| <i>PTPN2</i>    | <i>TGFBR1</i>   | 0.22 |
| <i>PTPN2</i>    | <i>CTLA4</i>    | 0.03 |
| <i>PTPN2</i>    | <i>TGFBR2</i>   | 0.23 |
| <i>PTPN2</i>    | <i>ZC3H12A</i>  | 9.4  |
| <i>TGFBR1</i>   | <i>ZAP70</i>    | 0    |
| <i>TGFBR1</i>   | <i>LAG3</i>     | 0    |
| <i>TGFBR1</i>   | <i>TIGIT</i>    | 0.03 |
| <i>TGFBR1</i>   | <i>TGFBR1</i>   | 0.06 |
| <i>TGFBR1</i>   | <i>CTLA4</i>    | 0.01 |
| <i>TGFBR1</i>   | <i>TGFBR2</i>   | 0.07 |
| <i>TGFBR1</i>   | <i>ZC3H12A</i>  | 3.33 |
| <i>ZC3H12A</i>  | <i>ZAP70</i>    | 0.01 |
| <i>ZC3H12A</i>  | <i>LAG3</i>     | 0.73 |
| <i>ZC3H12A</i>  | <i>TIGIT</i>    | 2.53 |
| <i>ZC3H12A</i>  | <i>CTLA4</i>    | 0.61 |
| <i>ZC3H12A</i>  | <i>ZC3H12A</i>  | 1.14 |
| <i>Havcr2</i>   | <i>Havcr2</i>   | 0.02 |
| <i>Havcr2</i>   | <i>LAG3</i>     | 0.01 |
| <i>Havcr2</i>   | <i>Olfr1389</i> | 0    |
| <i>Havcr2</i>   | <i>Olfr453</i>  | 0.01 |
| <i>Havcr2</i>   | <i>PDCD1</i>    | 0.02 |
| <i>LAG3</i>     | <i>Olfr1389</i> | 0    |
| <i>LAG3</i>     | <i>Olfr453</i>  | 0.02 |
| <i>LAG3</i>     | <i>PDCD1</i>    | 0.02 |
| <i>Olfr1389</i> | <i>Olfr1389</i> | 0.01 |
| <i>Olfr1389</i> | <i>Olfr453</i>  | 0    |
| <i>Olfr1389</i> | <i>PDCD1</i>    | 0.02 |
| <i>Olfr1389</i> | <i>PTPN2</i>    | 0.03 |
| <i>Olfr1389</i> | <i>ZC3H12A</i>  | 0.78 |
| <i>Olfr453</i>  | <i>Olfr453</i>  | 0.01 |
| <i>Olfr453</i>  | <i>PDCD1</i>    | 0.01 |
| <i>Olfr453</i>  | <i>PTPN2</i>    | 0.46 |
| <i>Olfr453</i>  | <i>ZC3H12A</i>  | 1.58 |

### EXAMPLE 3: EFFICACY OF *PTPN2/SOCS1* DUAL-EDITED TRANSGENIC T CELLS IN MURINE SYNGENEIC TUMOR MODELS

**[00372]** OT1 T cell and B16-Ova Tumor Cell Model: Anti-tumor efficacy of dual-edited *Ptpn2/Socs1* CD8<sup>+</sup> T cells was evaluated in mice using the B16Ova subcutaneous syngeneic tumor model. 6-8 week old female C57BL/6J mice from Jackson labs were injected subcutaneously in the right flank with  $0.5 \times 10^6$  B16-Ova tumor cells. When tumors in the entire cohort of mice reached an average volume of approximately 485 mm<sup>3</sup> at 15 days after inoculation, the mice were randomized into five groups of 10 mice each and injected intravenously with edited murine OT1 CD8<sup>+</sup> T cells via tail vein injection. Prior to injection, these cells were edited by electroporation with gRNA/Cas9 RNP complexes comprising (1) a non-targeting control gRNA; (2) a single gRNA targeting the *PD1* gene (SEQ ID NO: 270); (3) a single gRNA targeting the *Ptpn2* gene (SEQ ID NO: 202); (4) a single gRNA targeting the *Socs1* gene (SEQ ID NO: 9); (5) 2 gRNAs, one targeting each of the *Socs1* and *Ptpn2* genes. Editing efficiency of the gRNA/Cas9 complex targeting the *Ptpn2* and *Socs1* genes was assessed by next-generation sequencing and determined to be 70% and 82%, respectively. Body weight and tumor volume was measured at least twice per week. Tumor volume was calculated as mean and standard error of the mean for each treatment group. The percentage tumor growth inhibition (TGI) was calculated according to the following formula:

$$\% \text{ TGI} = (\text{Ptpn2/Socs1 } TV_{\text{final}} - \text{Ptpn2/Socs1 } TV_{\text{initial}}) / (\text{Control } TV_{\text{final}} - \text{Control } TV_{\text{initial}}),$$

where TV = mean tumor volume, *final* = Day 7, and *initial* = day of edited mouse OT1 CD8<sup>+</sup> T cell transfer.

**[00373]** The data in Fig. 1 show that compared to a control guide, adoptive transfer of *Ptpn2/Socs1* dual-edited mouse OT1 CD8<sup>+</sup> T cells resulted in an anti-tumor response in the B16Ova subcutaneous mouse model. Further, the %TGI observed on Day 7 after T cell transfer was increased in the *Ptpn2/Socs1* dual-edited group (TGI = 90%) compared to either of the *Ptpn2*-single edited (TGI = 1%) or *Socs1*-single edited (TGI = 44%) groups. Additionally, the %TGI of the *Ptpn2/Socs1* dual-edited group was also increased compared to the %TGI observed with PD1-edited T cells (TGI = 30%). A summary of the efficacy of dual-edited and single-edited T cells in the B16-Ova model is provided in Table 20 below.



**Table 20:**

| <b>Gene Target</b>      | <b>PD-1 sensitive</b>          | <b>PD-1 resistant</b>          |
|-------------------------|--------------------------------|--------------------------------|
|                         | B16-Ova, normal*<br>(melanoma) | B16-Ova, large**<br>(melanoma) |
| <i>Ptpn2</i>            | ++                             | -                              |
| <i>Socs1</i>            | ++                             | -                              |
| Dual <i>Ptpn2/Socs1</i> | +++                            | +++                            |
| <i>Pdcd-1</i>           | ++                             | -                              |

\*100 mm<sup>3</sup>\*\*>500 mm<sup>3</sup>

NT = not tested; (-) = no efficacy observed; (+) = modest responses in majority of animals; (++) = strong responses in majority of animals; (+++) = strong responses, including some complete responses, in all animals treated

**[00374]** Subsequent studies in the PD-1 resistant large tumor B16Ova model were performed as described above with initial starting tumor volumes of approximately 343mm<sup>3</sup> at 15 days after inoculation. Mice that completely rejected the original large B16Ova tumor were then rechallenged subcutaneously in the left flank with either 0.5 x 10<sup>6</sup> B16-Ova tumor cells (n=6) or 0.3 x 10<sup>6</sup> B16F10 tumor cells (n=6) on day 106 after T cell transfer. Editing efficiency of the gRNA/Cas9 complex targeting the *Ptpn2* and *Socs1* genes was assessed by next-generation sequencing and determined to be 75.4% and 86.5%, respectively. Body weight and tumor volume was measured at least twice per week. Tumor volume was calculated as described above. At various timepoints before and after rechallenge the mice were bled via tail stick and samples were analyzed via flow cytometry to track the OT1 CD8<sup>+</sup> T cells and their phenotype in peripheral blood.

**[00375]** A separate cohort of mice was inoculated and euthanized on day six. Tumor, spleen and blood was analyzed via flow cytometry for total OT1 population, cytokine production and other target related readouts.

**[00376]** The data in Fig. 9A shows that adoptive transfer of *Ptpn2/Socs1* dual-edited mouse OT1 CD8<sup>+</sup> T cells resulted in eight out of eight mice achieving complete responses against the large B16OVA tumors. Mice treated with *Ptpn2/Socs1* dual-edited mouse OT1 CD8<sup>+</sup> T cells showed an increase in the number of OT1 cells in the B16Ova tumor on day six compared to single edited OT1 CD8 T cells (Fig. 9B) and also showed an increase in granzyme B production (Fig. 9C). Fig. 9D shows that all mice that previously had rejected a large B16Ova tumor were subsequently able to reject a second inoculation of B16Ova compared to naïve mice. Two out of six mice that previously had rejected a large B16Ova tumor were also able to completely reject a second inoculation with parental B16F10 that does not express neoantigen. Characterization of *Ptpn2/Socs1* dual-edited mouse OT1 CD8<sup>+</sup> T cells during the B16Ova rechallenge showed an expansion of these cells from a central

memory phenotype to an effector phenotype eight days after B16Ova rechallenge. This was followed by a contraction back to a central memory phenotype (Fig. 9E).

**[00377]** PMEL T cell and MC38-gp100 tumor cell Model: Additional experiments are performed to assess the effect of *Ptpn2/Socs1* dual-edited T cells in a MC38 subcutaneous syngeneic tumor model of colorectal cancer (which is insensitive to treatment with anti-PD1 antibodies). Briefly, 6-8 week old female C57BL/6J mice from Jackson labs are injected subcutaneously with  $1 \times 10^6$  MC38 tumor cells expressing gp100. When tumors reached a volume of approximately 100 mm<sup>3</sup>, mice are randomized into groups of 10 and injected intravenously with edited murine PMEL CD8<sup>+</sup> T cells via tail vein. Prior to injection, these cells are edited by electroporation with gRNA/Cas9 RNP complexes comprising (1) a non-targeting control gRNA; (2) a single gRNA targeting the PD1 gene; (3) a single gRNA targeting the *Ptpn2* gene; (4) a single gRNA targeting the *Socs1* gene; (5) 2 gRNAs, one targeting each of the *Socs1* and *Ptpn2* genes. PMEL CD8<sup>+</sup> T cells were edited according to the method described here in Example 4. The editing efficiency of the dual gRNA/Cas9 complex targeting the *Socs1* and *Ptpn2* genes was assessed using by NGS and was determined to be 65% and 47%, respectively. Body weight and tumor volume are measured at least twice per week. Tumor volume was calculated as mean and standard error of the mean for each treatment group and the % TGI for each group is calculated as described above. These experiments show an enhanced anti-tumor efficacy of *Ptpn2/Socs1* dual-edited T cells compared to control or single-edited T cell treatment.

#### **EXAMPLE 4: EFFICACY OF *PTPN2/SOCS1* DUAL-EDITED TRANSGENIC T CELLS IN A MURINE SYNGENEIC MODEL OF METASTATIC LUNG CANCER**

**[00378]** Anti-tumor efficacy of *Ptpn2/Socs1* dual-edited T cells was evaluated in mice using the aggressive metastatic B16-F10 syngeneic tumor model with disease manifesting as lung metastasis. Briefly, 6-8 week old female C57BL/6J mice from Jackson labs were injected intravenously with  $0.5 \times 10^6$  B16-F10 tumor cells. Prior to inoculation, mice were weighed and randomly assigned to treatment groups. 3 days post-tumor cell inoculation, mice were injected intravenously with edited mouse PMEL CD8<sup>+</sup> T cells via tail vein. Prior to T cell injection, these cells were edited by electroporation with gRNA/Cas9 RNP complexes comprising (1) a non-targeting control gRNA; (2) a single gRNA targeting the *Ptpn2* gene (SEQ ID NO: 202); (3) a single gRNA targeting the *Socs1* gene (SEQ ID NO: 9); (4) 2 gRNAs, one targeting each of the *Socs1* and *Ptpn2* genes. The editing efficiency of the dual gRNA/Cas9 complex targeting the *Socs1* and *Ptpn2* genes was assessed using by NGS and was determined to be 65% and 47%, respectively. Body weight was monitored at least twice per week. At D15 post-tumor cell inoculation (D12 post-T cell transfer), lungs

of each mouse were perfused and fixed with 10% para-formaldehyde. After overnight fixation, lungs were transferred to 70% EtOH for further preservation.

**[00379]** Tumor efficacy was evaluated by visually assessing the B16-F10 tumor burden which can be seen as black colonies of cancer cells on the lungs. Large numbers of metastatic colonies were observed in all lungs from the untreated group and from mice treated with control-edited PMEL CD8+ T cells, signifying significant disease progression in these groups. Partial efficacy was seen in mice treated with *Socs1* single-edited cells with evidence of a partial reduction of metastatic burden, while *Ptpn2* single-edited cells had a minimal efficacy. However, treatment with *Ptpn2/Socs1* dual-edited cells resulted in strong anti-tumor efficacy with a near complete inhibition of tumor formation. A summary of the efficacy of dual-edited and single-edited T cells in the B16-F10 model is provided in Table 21 below.

**Table 21:**

| Target Gene             | PD-1 resistant - B16F10 (lung) |
|-------------------------|--------------------------------|
| <i>Ptpn2</i>            | +                              |
| <i>Socs1</i>            | ++                             |
| Dual <i>Ptpn2/Socs1</i> | +++                            |
| <i>Pdcd1</i>            | -                              |

(-) = no efficacy observed; (+) = modest responses in majority of animals; (++) = strong responses in majority of animals; (+++) = strong responses, including some complete responses, in all animals treated

**EXAMPLE 5: EFFICACY OF *PTPN2/SOCS1* DUAL-EDITED TRANSGENIC T CELLS IN A XENOGRAFT MODEL OF MELANOMA**

**[00380]** Anti-tumor efficacy of *Ptpn2/Socs1* dual-edited T cells is evaluated in mice using the A375 xenograft tumor model. Briefly, 6-8 week old NSG mice from Jackson labs are injected subcutaneously with  $5 \times 10^6$  A375 cells (expressing the NY-ESO-1 antigen). When tumors reach a volume of approximately 200 mm<sup>3</sup>, mice are randomized into groups of 8 and injected intravenously with up to  $30 \times 10^6$  edited cells, which were additionally lentivirally transduced to express TCR2, via tail vein. Prior to T cell injection, these cells are edited by electroporation with gRNA/Cas9 RNP complexes comprising (1) a non-targeting control gRNA; (2) a single gRNA targeting the PD1 gene; (3) a single gRNA targeting the *Ptpn2* gene; (4) a single gRNA targeting the *Socs1* gene; (5) 2 gRNAs, one targeting each of the *Socs1* and *Ptpn2* genes. Body weight and tumor volume are measured at least twice per week. Tumor volume is calculated as mean and standard error of the mean for each treatment group and % TGI will be calculated as described above. These data demonstrate that

treatment with *Ptpn2/Socs1* dual-edited T cells enhances anti-tumor efficacy in a NY-ESO-1 tumor model compared to the anti-tumor efficacy observed after treatment with either *Ptpn2*-single edited or *Socs1*-single edited cells.

#### EXAMPLE 6: EFFICACY OF *PTPN2/SOCS1* DUAL-EDITED TUMOR INFILTRATING LYMPHOCYTES

**[00381]** Anti-tumor efficacy of *Ptpn2/Socs1* dual-edited tumor infiltrating lymphocytes (TILs) was evaluated in an exploratory mouse model. Two mice cohorts were used in this experiment: a donor cohort of CD45.1 Pep Boy mice (B6.SJL-*Ptprc<sup>a</sup> Pepc<sup>b</sup>*/BoyJ) and a recipient cohort of CD45.2 C57BL/6J mice (Jackson labs), each comprised of 6-8 week old female mice.

**[00382]** To generate TILs, donor CD45.1 Pep Boy mice were injected subcutaneously with  $0.5 \times 10^6$  B16-Ova cells. On Day 14 post-tumor cell inoculation, tumors were harvested to generate edited CD45.1 Tumor Infiltrating Lymphocytes (TILs) to infuse into the second cohort of mice as described above in Example 1. These TIL cells were edited by electroporation of gRNA/Cas9 complexes comprise (1) a non-targeting control gRNA; (2) a single gRNA targeting the *Ptpn2* gene; (3) a single gRNA targeting the *Socs1* gene; or (4) 2 gRNAs, one targeting each of the *Socs1* and *Ptpn2* genes. The editing efficiency of the dual gRNA/Cas9 complex targeting the *Socs1* and *Ptpn2* genes was assessed using by NGS and was determined to be 77.8% and 87.6%, respectively. For details for *Ptpn2* + *Socs1* editing see Example 10 and Example 15.

**[00383]** Recipient CD45.2 C57BL/6J mice were injected subcutaneously with  $0.5 \times 10^6$  B16-Ova tumor cells. When tumors reach a volume of approximately  $100 \text{ mm}^3$ , mice were randomized into groups of 10 and injected intravenously with edited CD45.1 TILs via tail vein. In additional experiments, human IL-2 can be delivered simultaneously. Body weight and tumor volume were measured at least twice per week. Tumor volume was calculated as mean and standard error of the mean for each treatment group and the % TGI is calculated according to the following formula:

$$\% \text{ TGI} = (\text{Ptpn2/Socs1 } TV_{\text{final}}) - \text{Ptpn2/Socs1 } TV_{\text{initial}} / (\text{Control } TV_{\text{final}} - \text{Control } TV_{\text{initial}}),$$

where TV = mean tumor volume, *final* = Day 17, and *initial* = day of edited TIL transfer.

**[00384]** In this preliminary experiment using this exploratory model, treatment with *Ptpn2/Socs1* dual edited TILs leads to enhanced tumor efficacy.

Table 22.

| Gene Target               | Non-Lymphodepleted<br>mTIL model |
|---------------------------|----------------------------------|
| <i>Ptpn2</i>              | -                                |
| <i>Socs1</i>              | -                                |
| Dual <i>Ptpn2/Socs1</i>   | -                                |
| <i>Pdcd-1</i>             | -                                |
| Dual <i>ZC3H12A/Socs1</i> | +++                              |

(-) = no efficacy observed; (+) = modest responses in majority of animals; (++) = strong responses in majority of animals; (+++) = strong responses, including some complete responses, in all animals treated

#### EXAMPLE 7: EFFICACY OF *ZC3H12A/SOCS1* DUAL-EDITED TRANSGENIC T CELLS IN MURINE SYNGENEIC TUMOR MODELS

**[00385]** *OT1 T cell and B16-Ova Tumor cell Model:* Anti-tumor efficacy of *Zc3h12a/Socs1* dual-edited transgenic CD8<sup>+</sup> T cells was evaluated in mice using the B16Ova subcutaneous syngeneic tumor model. 6-8 week old female C57BL/6J mice from Jackson labs were injected subcutaneously with  $0.5 \times 10^6$  B16-Ova tumor cells. When tumors in the entire cohort of mice reached an average volume of approximately 485 mm<sup>3</sup>, the mice were randomized into five groups of 10 mice each and injected intravenously with edited murine OT1 CD8<sup>+</sup> T cells via tail vein injection. Prior to injection, these cells were edited by electroporation with gRNA/Cas9 RNP complexes comprising (1) a non-targeting control gRNA; (2) a single gRNA targeting the *Zc3h12a* gene (SEQ ID NO: 211); (3) a single gRNA targeting the *Socs1* gene (SEQ ID NO: 9); or (4) 2 gRNAs, one targeting each of the *Zc3h12a* and *Socs1* genes. The editing efficiency of the dual gRNA/Cas9 complex targeting the *Zc3h12a* and *Socs1* genes was assessed by NGS and determined to be 86% and 84%, respectively. Body weight and tumor volume were measured at least twice per week. Tumor volume was calculated as mean and standard error of the mean for each treatment group. The percentage tumor growth inhibition (TGI) was calculated using the following formula:

$$\% \text{ TGI} = (\text{Zc3h12a/Socs1 TV}_{\text{final}}) - \text{Zc3h12a/Socs1 TV}_{\text{initial}} / (\text{Control TV}_{\text{final}} - \text{Control TV}_{\text{initial}}),$$

where TV = mean tumor volume, *final* = Day 10, and *initial* = day 0 of edited T cell transfer.

**[00386]** The data in Fig. 2 show that compared to a control guide, adoptive transfer of *Zc3h12a/Socs1* dual-edited mouse OT1 CD8<sup>+</sup> T cells resulted in an enhanced anti-tumor response in

the B16Ova subcutaneous mouse model. This effect was maintained out to day 140 before the study was terminated.

**[00387]** PMEL T cell and MC38-gp100 tumor cell Model: Additional experiments are performed to assess the effect of *Zc3h12a/Socs1* dual-edited T cells in a MC38 subcutaneous syngeneic tumor model of colorectal cancer (which is insensitive to treatment with anti-PD1 antibodies). Briefly, 6-8 week old female C57BL/6J mice from Jackson labs are injected subcutaneously with  $1 \times 10^6$  MC38 tumor cells expressing gp100. When tumors reach a volume of approximately  $100 \text{ mm}^3$ , mice are randomized into groups of 10 and injected intravenously with edited murine PMEL CD8<sup>+</sup> T cells via tail vein. Prior to injection, these cells are edited by electroporation with gRNA/Cas9 RNP complexes comprising (1) a non-targeting control gRNA; (2) a single gRNA targeting the PD1 gene; (3) a single gRNA targeting the *Zc3h12a* gene; (4) a single gRNA targeting the *Socs1* gene; (5) 2 gRNAs, one targeting each of the *Socs1* and *Zc3h12a* genes. Body weight and tumor volume will be measured at least twice per week. Tumor volume is calculated as mean and standard error of the mean for each treatment group and the % TGI for each group is calculated as described above. These experiments are expected to show an enhanced anti-tumor efficacy of *Ptpn2/Socs1* dual-edited T cells compared to control or single-edited T cell treatment.

**EXAMPLE 8: EFFICACY OF *ZC3H12A/SOCS1* DUAL-EDITED TRANSGENIC T CELLS IN A MURINE SYNGENEIC MODEL OF METASTATIC CANCER**

**[00388]** Anti-tumor efficacy of *Zc3h12a/Socs1* dual-edited T cells are evaluated in mice using the aggressive metastatic B16-F10 syngeneic tumor model with disease manifesting as lung metastasis. Briefly, 6-8 week old female C57BL/6J mice from Jackson labs are injected intravenously with  $0.5 \times 10^6$  B16-F10 tumor cells. Prior to inoculation, mice are weighed and randomly assigned to treatment groups. 3 days post-tumor cell inoculation, mice are injected intravenously with edited mouse PMEL CD8<sup>+</sup> T cells via tail vein. Prior to T cell injection, these cells are edited by electroporation with gRNA/Cas9 RNP complexes comprising (1) a non-targeting control gRNA; (2) a single gRNA targeting the *Zc3h12a* gene; (3) a single gRNA targeting the *Socs1* gene; (4) 2 gRNAs, one targeting each of the *Zc3h12a* and *Socs1* genes. The editing efficiency of the dual gRNA/Cas9 complex targeting the *Zc3h12a* and *Socs1* genes is assessed using by NGS. Body weight is monitored at least twice per week. At D15 post-tumor cell inoculation (D12 post-T cell transfer), lungs of each mouse are perfused and fixed with 10% para-formaldehyde. After overnight fixation, lungs are transferred to 70% EtOH for further preservation. Tumor efficacy is evaluated by visually assessing the B16-F10 tumor burden which can be seen as black colonies of cancer cells on the lungs. These

data are expected to show an enhanced anti-tumor efficacy of *Zc3h12a/Socs1* dual-edited T cells compared to control or single-edited T cell treatment.

**Table 23:**

| Target Gene    | PD-1 resistant - B16F10 (lung) |
|----------------|--------------------------------|
| <i>Zc3h12a</i> | +++                            |
| <i>Socs1</i>   | ++                             |
| <i>Pdcd1</i>   | -                              |

(-) = no efficacy observed; (+) = modest responses in majority of animals; (++) = strong responses in majority of animals; (+++) = strong responses, including some complete responses, in all animals treated

**EXAMPLE 9: EFFICACY OF *ZC3H12A/SOCS1* DUAL-EDITED TRANSGENIC T CELLS IN A XENOGRAFT MODEL OF MELANOMA**

**[00389]** Anti-tumor efficacy of *Zc3h12a/Socs1* dual-edited T cells is evaluated in mice using the A375 xenograft tumor model. Briefly, 6-8 week old NSG mice from Jackson labs are injected subcutaneously with  $5 \times 10^6$  A375 cells (expressing the NY-ESO-1 antigen). When tumors reach a volume of approximately 400 mm<sup>3</sup>, mice are randomized into groups of 8 and injected intravenously with  $30 \times 10^6$  edited TCR2 cells via tail vein. Prior to T cell injection, these cells are edited by electroporation with gRNA/Cas9 RNP complexes comprising (1) a non-targeting control gRNA; (2) a single gRNA targeting the PD1 gene; (3) a single gRNA targeting the *Zc3h12a* gene; (4) a single gRNA targeting the *Socs1* gene; (5) 2 gRNAs, one targeting each of the *Socs1* and *Zc3h12a* genes. Body weight and tumor volume are measured at least twice per week. Tumor volume is calculated as mean and standard error of the mean for each treatment group and % TGI will be calculated as described above. These data demonstrate that treatment with *Zc3h12a/Socs1* dual-edited T cells is expected to lead to enhanced anti-tumor efficacy in a NY-ESO-1 tumor model compared to the anti-tumor efficacy observed after treatment with either *Zc3h12a*-single edited or *Socs1*-single edited cells.

**EXAMPLE 10: EFFICACY OF *ZC3H12A/SOCS1* DUAL-EDITED TUMOR INFILTRATING LYMPHOCYTES**

**[00390]** Anti-tumor efficacy of *Zc3h12a/Socs1* dual-edited tumor infiltrating lymphocytes (TILs) was evaluated in non-lymphodepleted mice using the B16Ova subcutaneous syngeneic tumor model. Two mice cohorts were used in this experiment: a donor cohort of CD45.1 Pep Boy mice (B6.SJL-*Ptprc<sup>a</sup> Pepc<sup>b</sup>*/BoyJ) and a recipient cohort of CD45.2 C57BL/6J mice (Jackson labs), each comprised of 6-8 week old female mice.

**[00391]** To generate TILs, donor CD45.1 Pep Boy mice are injected subcutaneously with  $0.5 \times 10^6$  B16-Ova cells. On Day 14 post-tumor cell inoculation, tumors were harvested to generate edited CD45.1 Tumor Infiltrating Lymphocytes (TILs) to infuse into the second cohort of mice as described above in Example 1. These TIL cells were edited by electroporation of gRNA/Cas9 complexes comprise (1) a non-targeting control gRNA; (2) a single gRNA targeting the *Zc3h12a* gene (SEQ ID NO: 211); (3) a single gRNA targeting the *Socs1* gene (SEQ ID NO: 9); or (4) 2 gRNAs, one targeting each of the *Socs1* and *Zc3h12a* genes. The editing efficiency of the dual gRNA/Cas9 complex targeting the *Zc3h12a* and *Socs1* genes was assessed using by NGS and was determined to be 82% and 84%, respectively.

**[00392]** Recipient CD45.2 C57BL/6J mice were injected subcutaneously with  $0.5 \times 10^6$  B16-Ova tumor cells. When tumors reached a volume of approximately  $100 \text{ mm}^3$  mice were randomized into groups of 10 and injected intravenously with edited CD45.1 TILs via tail vein. Body weight and tumor volume was measured at least twice per week. Tumor volume was calculated as mean and standard error of the mean for each treatment group and the % TGI is calculated according to the following formula:

$$\% \text{ TGI} = (Zc3h12a/Socs1 \text{ TV}_{final}) - Zc3h12a/Socs1 \text{ TV}_{initial}) / (\text{Control TV}_{final} - \text{Control TV}_{initial}),$$

where TV = mean tumor volume, *final* = Day 17 and *initial* = day of edited TIL transfer.

**[00393]** The data in Fig. 3 show that compared to a control guide, adoptive transfer of *Zc3h12a/Socs1* dual-edited mouse TILs resulted in an enhanced anti-tumor response in the B16Ova subcutaneous mouse model (TGI = 97%) compared to treatment with either *Zc3h12a* single-edited TILs (TGI = 47%) or *Socs1* single-edited TILs (TGI = 32%).

#### **EXAMPLE 11: EFFICACY OF PD1/LAG3 DUAL-EDITED TRANSGENIC T CELLS IN A B16-OVA MURINE TUMOR MODEL**

**[00394]** Anti-tumor efficacy of PD-1/Lag3 dual-edited T cells was evaluated in mice using the B16Ova subcutaneous syngeneic tumor model. 6-8 week old female C57BL/6J mice from Jackson labs were injected subcutaneously with  $0.5 \times 10^6$  B16Ova tumor cells. When tumors in the entire cohort of mice reached an average volume of approximately  $485 \text{ mm}^3$ , the mice were randomized into groups of 10 and injected intravenously with edited mouse OT1 CD8+ T cells via tail vein. Prior to injection these cells were edited by electroporation with gRNA/Cas9 RNP complexes comprising (1) a non-targeting control gRNA; (2) a single gRNA targeting the PD1 gene (SEQ ID NO: 270); (3) a



single gRNA targeting the Lag3 gene; (4) 2 gRNAs, one targeting each of the PD1 and Lag3 genes. The editing efficiency of the dual gRNA/Cas9 complex targeting the Pdccl1 and Lag3 genes was assessed using by NGS and was determined to be 58.8% and 89.4%, respectively. Body weight and tumor volume was measured at least twice per week. Tumor volume was calculated as mean and standard error of the mean for each treatment group. The percentage tumor growth inhibition (TGI) was calculated using the following formula:

$$\% \text{ TGI} = (\text{PD1/Lag3 TV}_{\text{final}} - \text{PD1/Lag3 TV}_{\text{initial}}) / (\text{Control TV}_{\text{final}} - \text{Control TV}_{\text{initial}}),$$

where TV = mean tumor volume, *final* = Day 10 and *initial* = day of edited mouse OT1 CD8+ T cell transfer.

**[00395]** The data in Fig. 4 show adoptive transfer of PD-1 single-edited T cells resulted in a TGI of 70% and adoptive transfer of Lag3 single-edited T cells resulted in a TGI of 36%. Surprisingly, combination edits of PD1 and Lag3 did not result in enhanced tumor growth inhibition and demonstrated a TGI of 38%.

#### **EXAMPLE 12: VALIDATION OF DUAL-EDITED CAR-T AND TCR TRANSGENIC T CELL EFFICACY AND FUNCTION**

**[00396]** Experiments were performed to validate the effects of editing two of *PTPN2*, *ZC3H12A*, and/or *SOCS1* on the anti-tumor efficacy of CAR T cells and T cells engineered to express an artificial TCR. The engineered T cells described in Table 24 were edited as described in Example 1 to reduce expression of *PTPN2*, *ZC3H12A*, and/or *SOCS1*. These edited T cells were then evaluated in subcutaneous xenograft models using the indicated cell type.

**Table 24: Engineered Receptor Specificity and Target Cell Lines**

| <b>Receptor Specificity</b> | <b>Target Cell Line</b>                                      |
|-----------------------------|--------------------------------------------------------------|
| CD19                        | Raji, Daudi, NALM-6, NALM-16, RAMOS, JeKo1                   |
| BCMA                        | Multiple Myeloma cell lines NCI-H929, U266-B1, and RPMI-8226 |
| NYESO                       | A375                                                         |
| MART1                       | SKMEL5, WM2664, IGR1                                         |
| HER2+                       | BT474                                                        |

**[00397]** Briefly, 6-8 week old female NSG mice from Jackson labs were injected subcutaneously with  $1 \times 10^6$  Raji cells. When tumors reached a volume of approximately 200 mm<sup>3</sup>, mice were randomized into groups of 8 and injected intravenously with  $3 \times 10^6 - 10 \times 10^6$  edited engineered CAR T cells targeting CD19 via tail vein. Prior to injection the adoptively transferred cells

were edited with either a control gRNA or a gRNA targeting *PTPN2*, *ZC3H12A*, and/or *SOCS1*. Body weight and tumor volume were measured at least twice per week. Tumor volume was calculated as mean and standard error of the mean for each treatment group. The results of these experiments (Table 25) show enhanced anti-tumor efficacy of  $10 \times 10^6$  *PTPN2*<sup>-/-</sup> and *SOCS1*<sup>-/-</sup> dual edited engineered T cells or as compared to a control guide, measured by tumor volume and number of complete responses at the end of the study (eight out of eight for *PTPN2*<sup>-/-</sup>/*SOCS1*<sup>-/-</sup> CAR T cells vs one out of eight for control edited CAR T cells)

**Table 25**

| Target Gene                 | Raji – CD19 CAR T Model |
|-----------------------------|-------------------------|
| <i>PTPN2</i> + <i>SOCS1</i> | +++                     |
| <i>Pdcd1</i>                | -                       |

(-) = no efficacy observed; (+) = modest responses in majority of animals; (++) = strong responses in majority of animals; (+++) = strong responses, including some complete responses, in all animals treated

**[00398]** Additional experiments are performed to validate the effects of editing *PTPN2*, *ZC3H12A*, and/or *SOCS1* on engineered T cell cytokine production. Briefly, the engineered T cells described in Table 25 above are generated from human T cells, and two or more of *PTPN2*, *ZC3H12A*, and *SOCS1* are edited by electroporation using guide RNAs complexed to Cas9 in an RNP format. CAR-Ts are co-cultured with the corresponding cell line indicated in Table 22 *in vitro* at a 1:0, 0.3:1, 1:1, 3:1 and 10:1 ratio. After 24 hours, total cell counts of engineered T cells are determined, and supernatants saved for cytokine analyses. The results of these experiments are expected to show enhanced accumulation of and increased levels of cytokine production from dual-edited CAR T cells compared to control edited cells.

#### **EXAMPLE 13: IN VITRO ASSESSMENT OF DUAL-EDITED IMMUNE CELL FUNCTION**

**[00399]** To assess for *SOCS1*-, *PTPN2*-, and *ZC3H12A*-dependent pharmacology, assays are developed that quantify the dependent biology of each target. These assays are also intended to be used to assess target-dependent pharmacology in double edited TILs. The activity of sgRNAs targeting *SOCS1*, *PTPN2*, and *ZC3H12A* in TILs are assessed in these assays. For example, cells in which both *SOCS1* and *PTPN2* are inactivated should demonstrate activity in assays measuring both *SOCS1* and *PTPN2* pharmacology.

**[00400]** In addition to the negative role of PTPN2 on T cell receptor (TCR) signaling, both SOCS1 and PTPN2 are negative regulators of JAK/STAT signaling. Therefore, SOCS1-dependent and PTPN2-dependent pharmacology can be measured by increases in JAK/STAT signaling.

**[00401]** SOCS1 negatively regulates cytokine signaling in T cells, in part by inhibiting JAK1, a kinase involved in STAT5 phosphorylation and cytokine signal transduction. Upon IL-2 signaling through the IL-2 receptor complex, STAT5 is phosphorylated in a JAK1-dependent manner. Therefore, levels of pSTAT5 and activation of downstream signaling pathways upon IL-2 stimulation may serve as an assay measuring *SOCS1*-dependent pharmacology in TILs. Indeed, deletion of *SOCS1* lead to an increase in pSTAT5 levels in primary human CD8 T cells in response to IL-2 signaling (Fig. 5).

**[00402]** PTPN2 also acts as a negative regulator of cytokine signaling, including IL-2 and IFN $\gamma$ , by directly dephosphorylating STAT proteins such as pSTAT1 and pSTAT3. Therefore, levels of pSTAT1 and pSTAT3 and activation of downstream signaling pathways may serve as an assay measuring *PTPN2*-dependent pharmacology in TILs. Indeed, Cas9-mediated genetic knockdown of *PTPN2* leads to increased pSTAT1 levels in Jurkat T cells in response to IFN $\gamma$  stimulation (Fig. 6). PTPN2 is also a negative regulator of TCR signaling. Both LCK and FYN transmit positive signaling downstream of the TCR, and are direct targets of PTPN2 phosphatase activity following TCR activation. Therefore, the impact of genetic inactivation of *PTPN2* on proximal T cell receptor signaling may be assessed by quantifying pLCK and pFYN following TCR stimulation.

**[00403]** In conclusion, direct assessment of *SOCS1* and *PTPN2* pharmacology in dual-edited cells can be conducted using 1) cytokine stimulation and pSTAT assays and 2) TCR activation and downstream signaling assays.

**[00404]** To determine the impact of genetic inactivation of *SOCS1* and *PTPN2* on cell function in vitro, multiple parameters may be assessed that correlate with T cell function. These include cytokine production (*e.g.*, IL-6 and IL-12), baseline cell surface phenotypes and activated cell surface phenotypes, T cell differentiation state, and tumor-killing ability.

#### **EXAMPLE 14: MANUFACTURING OF DUAL-EDITED TUMOR INFILTRATING LYMPHOCYTES**

**[00405]** Dual-edited TILs are manufactured following established protocols used previously in FDA-approved clinical trials for the isolation and expansion of TILs.

**[00406]** Following removal of tumor tissue, the tumor is both fragmented into 2 mm<sup>3</sup> pieces and mechanically/enzymatically homogenized and cultured in 6,000 IU/mL recombinant human IL-2 for up to 6 weeks or until the cell numbers reach or exceed 1 x 10<sup>8</sup>; this is defined as the pre-rapid expansion phase (pre-REP) of TIL manufacturing. Upon completion of the pre-REP stage TILs are electroporated with gRNA/Cas9 RNP complexes targeting *SOCS1*, *PTPN2*, and/or *ZC3H12A* under cGMP conditions. Cells may be also electroporated prior to or during the pre-REP process. Following electroporation, 50 x 10<sup>6</sup> cells are transferred into a 1 L G-Rex<sup>TM</sup> culture flask with a 1:100 ratio of TIL:irradiated feeder cells for approximately 2 weeks. This portion of manufacturing is defined as the rapid expansion phase (REP). After the REP phase, TIL's are harvested, washed, and suspended in a solution for immediate infusion into the patient.

**[00407]** Using methods similar to those above, edited tumor infiltrating lymphocytes were generated at miniaturized research scale in three independent donors. *SOCS1* single edited, *PTPN2* single edited, *ZC3H12A* single edited, *SOCS1/PTPN2* dual edited, and *SOCS1/ZC3H12A* dual edited cells were produced. Briefly, after the pre-REP expansion of TIL in IL-2, TILs were taken and resuspended in Maxcyte electroporation buffer (Maxcyte) at a concentration of 30M cells/ml. Per 20 µl of cells in electroporation buffer, 5 µl of RNP solution was added. Per 5 µl reaction, RNP solution was composed of 0.85 µl 61 µM sNLS-spCas9-sNLS (Aldevron), 1.75 µl of PBS, and 2.4 µl of 100 µM total sgRNA solution. sgRNA solutions were comprised of either 2.4 µl of a single sgRNA, or 1.2 µl each of 2 different sgRNAs. The guides used were as follows: *SOCS1* – GACGCCTGCGGATTCTACTG (SEQ ID 25), *PTPN2* – GGAAACTTGGCCACTCTATG (SEQ ID 190), and *ZC3H12A* – CAGGACGCTGTGGATCTCCG (SEQ ID NO: 219).

**[00408]** Cell/RNP solutions were loaded into Maxcyte processing assemblies (Cat# OC-25X3 or OC-100X2), and subsequently electroporated using a Maxcyte STX, using the program “Optimization #9”. Cells were recovered from the processing assemblies and added to 2X the volume complete REP media (50:50 mix of AIMV media (Gibco #12055) and RPMI 1640 (Gibco #11875), supplemented with 5% heat inactivated human AB serum (Valley Biomedical). Cells were allowed to recover at 37C for 20 minutes.

**[00409]** Subsequently, TILs were seeded into the REP by transferring them to either 6-well (10cm<sup>2</sup> surface area per well) or 24-well (2cm<sup>2</sup> surface area per well) Grex flasks at a density of 50,000 TIL per cm<sup>2</sup>. Flasks additionally contained irradiated PBMC feeder cells at a density of 5M per cm<sup>2</sup>, 6000U/ml of recombinant human IL-2, and 30ng/ml OKT3. REP was carried on for 14 days, during which cells were fed IL-2, fresh media containing IL-2, and/or cells were split. At day

14 of the REP, cells were harvested and editing efficiency was determined by amplicon sequencing of the genomic DNA of the cut site. Editing efficiencies were as noted in **Table 26** (numbers reflect the percentage of DNA reads that demonstrated a mutation from the expected wild-type sequence):

**Table 26.**

| Donor    | Sample                         | Target Locus 1 | % Edited | Target Locus 2 | % Edited |
|----------|--------------------------------|----------------|----------|----------------|----------|
| <b>A</b> | <i>ZC3H12A/SOCS1</i> dual edit | <i>SOCS1</i>   | 98%      | <i>ZC3H12A</i> | 99%      |
|          | <i>SOCS1/PTPN2</i> dual edit   | <i>SOCS1</i>   | 98%      | <i>PTPN2</i>   | 97%      |
| <b>B</b> | <i>ZC3H12A/SOCS1</i> dual edit | <i>SOCS1</i>   | 98%      | <i>ZC3H12A</i> | 99%      |
|          | <i>SOCS1/PTPN2</i> dual edit   | <i>SOCS1</i>   | 96%      | <i>PTPN2</i>   | 92%      |
| <b>C</b> | <i>ZC3H12A/SOCS1</i> dual edit | <i>SOCS1</i>   | 98%      | <i>ZC3H12A</i> | 98%      |
|          | <i>SOCS1/PTPN2</i> dual edit   | <i>SOCS1</i>   | 84%      | <i>PTPN2</i>   | 71%      |

**EXAMPLE 15: EFFICACY OF DUAL-EDITED TUMOR INFILTRATING LYMPHOCYTES IN LYMPHODEPLETED SYSTEM**

**[00410]** Anti-tumor efficacy of *Ptpn2/Socs1*, *Ptpn2/Zc3h12a*, or *Socs1/Zc3h12a* dual-edited tumor infiltrating lymphocytes (TILs) was evaluated in mice using the B16Ova subcutaneous syngeneic tumor model with lymphodepletion in contrast to Example 6 and 10. Two mice cohorts were used in this experiment: a donor cohort of CD45.1 Pep Boy mice (B6.SJL-*Ptprc<sup>a</sup> Pepc<sup>b</sup>*/BoyJ) and a recipient cohort of CD45.2 C57BL/6J mice (Jackson labs), each comprised of 6-8 week old female mice.

**[00411]** To generate TILs, donor CD45.1 Pep Boy mice were injected subcutaneously with  $0.5 \times 10^6$  B16-Ova cells. On Day 14 post-tumor cell inoculation, tumors were harvested to generate edited CD45.1 Tumor Infiltrating Lymphocytes (TILs) to infuse into the second cohort of mice as described above in Example 1. These TIL cells were edited by electroporation of gRNA/Cas9 complexes comprise (1) a non-targeting control gRNA; (2) a single gRNA targeting the *Ptpn2*, *Socs1*, or (3) 2 gRNAs, one targeting each of the *Socs1* and *Ptpn2* genes or the *Socs1* and *Zc3h12a* genes. The editing efficiency of the dual gRNA/Cas9 complex targeting the *Socs1* and *Ptpn2* genes was assessed using by NGS and was determined to be 85% and 71%, respectively. The editing efficiency of the dual gRNA/Cas9 complex targeting the *Socs1* and *Zc3h12a* genes was assessed using NGS and was

determined to be 94% and 90%, respectively. In additional experiments, TIL can be edited by electroporation of gRNA/Cas9 complexes targeting each of the *Ptpn2* and *Zc3h12a* genes.

**[00412]** Recipient CD45.2 C57BL/6J mice were injected subcutaneously with  $0.5 \times 10^6$  B16-Ova tumor cells. When tumors reach a volume of approximately 100 mm<sup>3</sup> mice were randomized into groups of 10 and injected intraperitoneal with cyclophosphamide (200mg/kg) to induce lymphodepletion. The following day mice were injected intravenously with edited CD45.1 TILs via tail vein. In additional experiments, mice can be injected intraperitoneally with recombinant human IL-2 (720,000 IU/Kg) twice daily for up to a maximum of 4 days. Body weight and tumor volume were measured at least twice per week. Tumor volume is calculated as mean and standard error of the mean for each treatment group and the % TGI is calculated on Day 17 according to the following formula:

$$\% \text{ TGI} = (\text{Combo TV}_{\text{final}} - \text{Combo TV}_{\text{initial}}) / (\text{Control TV}_{\text{final}} - \text{Control TV}_{\text{initial}}),$$

where TV = mean tumor volume, *final* = Day 17 and *initial* = day of edited TIL transfer.

**[00413]** These data demonstrate that treatment with dual-edited TILs leads to enhanced anti-tumor efficacy compared to the anti-tumor efficacy observed after treatment with single edited TILs in a lymphodepleted system.

**Table 27**

| Gene Target               | Lymphodepleted mTIL model |
|---------------------------|---------------------------|
| <i>Ptpn2</i>              | +                         |
| <i>Socs1</i>              | -                         |
| Dual <i>Ptpn2/Socs1</i>   | +                         |
| <i>Pdcd-1</i>             | -                         |
| Dual <i>ZC3H12A/Socs1</i> | ++                        |

(-) = no efficacy observed; (+) = modest responses in majority of animals; (++) = strong responses in the majority of animals; (+++) = strong responses, including some complete responses, in all animals treated

#### **EXAMPLE 16: FUNCTIONAL CHARACTERIZATION OF *SOCS1/PTPN2* DUAL-EDITED TIL**

**[00414]** *SOCS1/PTPN2* dual-edited TILs, and control TILs (edited at the OR1A1 locus, which is not expressed in T cells) were generated using methods as described in Example 14. The ability of TILs to produce inflammatory cytokines was assessed. Briefly, 200,000 viable TILs from 5 unique donors were seeded into the wells of a 96-well plate. The volume of media in the well was 200  $\mu$ l, comprised of 180  $\mu$ l of REP media (a 50:50 mix of AIM V (Gibco) and RPMI 1640 (Gibco),

supplemented with 5% heat inactivated human AB serum (Valley Biomedical)) and 20  $\mu$ l of anti-CD3 activation tetramer (Stemcell Technologies, custom reagent). The TILs were incubated at 37 degrees Celsius for 18 hours in a 5% CO<sub>2</sub> humidified chamber. After incubation, culture supernatants were harvested and the levels of IFN $\gamma$  and TNF $\alpha$  in the supernatants were measured using by V-plex cytokine plates and a Quickplex SQ 120 machine (Mesoscale Diagnostics). Dual *SOCS1/PTPN2*-edited TIL demonstrated a comparable ability to produce IFN $\gamma$  (Fig. 7A) and an increased ability to produce TNF $\alpha$  (Fig. 7B) compared to controlled edited TILs.

**[00415]** The ability of *SOCS1/PTPN2* dual-edited TILs to undergo degranulation upon stimulation was also assessed. 500,000 TILs were stimulated in a 96 well plate with 1/500 dilution of Cell Stimulation Cocktail (Invitrogen) in the presence of golgiplug (BD) and fluorescent anti-CD107a antibody (BD) for 4 hours. Cells were subsequently stained for T cell markers, and CD107a positivity and fluorescence intensity on T cells was assessed by flow cytometry. Dual *SOCS1/PTPN2*-edited TILs demonstrated increase in degranulation (Fig. 7C) and CD107a intensity (Fig. 7D) as compared to control edited TIL.

#### **EXAMPLE 17 – EFFICACY, MECHANISM OF ACTION AND RECHALLENGE OF *PTPN2/SOCS1* DUAL-EDITED TRANSGENIC T CELLS IN MURINE SYNGENEIC TUMOR MODELS**

**[00416]** *OT1 T cell and B16-Ova tumor cell model:* Anti-tumor efficacy of dual-edited *Ptpn2/Socs1* CD8<sup>+</sup> T cells was evaluated in mice using the B16Ova subcutaneous syngeneic tumor model. 6-8 week old female C57BL/6J mice from Jackson labs were injected subcutaneously in the right flank with 0.5 x 10<sup>6</sup> B16-Ova tumor cells. When tumors in the entire cohort of mice reached an average volume of approximately 100 mm<sup>3</sup>, the mice were randomized into five groups of 10 to 20 mice each and injected intravenously with edited murine OT1 CD8<sup>+</sup> T cells via tail vein injection. Prior to injection, these cells were edited by electroporation with gRNA/Cas9 RNP complexes comprising (1) a non-targeting control gRNA; (2) a single gRNA targeting the PD1 gene (SEQ ID NO: 270); (3) a single gRNA targeting the *Ptpn2* gene (SEQ ID NO: 202); (4) a single gRNA targeting the *Socs1* gene (SEQ ID NO: 9); (5) 2 gRNAs, one targeting each of the *Socs1* and *Ptpn2* genes. Editing efficiency of the gRNA/Cas9 complex targeting the *Ptpn2* and *Socs1* genes was assessed by next-generation sequencing and determined to be 80.3% and 87.6%, respectively. Body weight and tumor volume was measured at least twice per week. Tumor volume was calculated as mean and standard error of the mean for each treatment group. The percentage tumor growth inhibition (TGI) was calculated according to the following formula:

$$\% \text{ TGI} = (\text{Ptpn2/Socs1 } TV_{\text{final}} - \text{Ptpn2/Socs1 } TV_{\text{initial}}) / (\text{Control } TV_{\text{final}} - \text{Control } TV_{\text{initial}}),$$

where TV = mean tumor volume, *final* = Day 7, and *initial* = day of edited mouse OT1 CD8+ T cell transfer.

**[00417]** Mice that completely rejected the original large B16Ova tumor were then rechallenged subcutaneously in the left flank with either  $0.5 \times 10^6$  B16-Ova tumor cells (n=6) or  $0.3 \times 10^6$  B16F10 tumor cells (n=5) on day 76. Body weight and tumor volume was measured at least twice per week. Tumor volume was calculated as described above. At various timepoints before and after rechallenge the mice were bled via tail stick and samples were analyzed via flow cytometry to track the OT1 CD8+ T cells and their phenotype in peripheral blood.

**[00418]** The data in Fig. 8A shows that adoptive transfer of *Ptpn2/Socs1* dual-edited mouse OT1 CD8+ T cells resulted in seventeen out of eighteen mice achieving complete responses against the B16OVA tumors. Fig. 8B shows that all mice that previously had rejected a B16Ova tumor were subsequently able to reject a second inoculation of B16Ova compared to naïve mice. Two out of five mice that previously had rejected a B16Ova tumor were also able to completely reject a second inoculation with parental B16F10 that does not express neoantigen. Fig. 8C OT1 shows CD8+ T cells underwent a rapid expansion in peripheral blood eight days after rechallenge. Fig. 8D displays characterization of these same OT1s shifting from a central memory phenotype prior to rechallenge to an effector phenotype eight days after the second inoculation of B16Ova. This was followed by a contraction back to a central memory phenotype out to day 84 post rechallenge.

#### **EXAMPLE 18. INCREASED POTENCY OF *PTPN2/SOCS1* DUAL-EDITED MOUSE T CELLS**

**[00419]** To assess the relative potency of *Ptpn2/Socs1* dual-edited mouse OT1 CD8+ T cells in the PD-1 resistant large tumor B16Ova model, four different doses were tested versus their control edited equivalents. These studies were initiated as described above in Example 3 with initial starting tumor volumes of approximately  $355\text{mm}^3$  at which point either *Ptpn2/Socs1* dual-edited or control edited mouse OT1 CD8+ T cells were adoptively transferred intravenously at doses of  $4.1 \times 10^4$ ,  $4.1 \times 10^5$ ,  $4.1 \times 10^6$ , or  $4.1 \times 10^7$  cells per mouse. Editing efficiency of the gRNA/Cas9 complex targeting the *Ptpn2* and *Socs1* genes was assessed by next-generation sequencing and determined to be 66.4% and 86.5%, respectively. Body weight and tumor volume was measured at least twice per week. Tumor volume was calculated as described above. As shown in Fig. 10, adoptive transfer of control edited mouse OT1 CD8+ T cells resulted in delayed tumor growth only at the highest dose,  $4.1 \times 10^7$



T-cells per mouse, with no complete responses observed. In mice dosed with an equivalent number of *Ptpn2/Socs1* dual-edited mouse OT1 CD8<sup>+</sup> T cells nine out of ten complete tumor regressions were observed. Additionally, at lower doses of *Ptpn2/Socs1* dual-edited mouse OT1 CD8<sup>+</sup> T cells significant anti-tumor activity was observed with one out of ten mice showing complete tumor response in the  $4.1 \times 10^5$  group. Taken together, these data demonstrate that *Ptpn2/Socs1* dual-edited mouse T cells were approximately 10-100x as potent as control edited mouse OT1 CD8<sup>+</sup> T cells.

#### INCORPORATION BY REFERENCE

**[00420]** All references, articles, publications, patents, patent publications, and patent applications cited herein are incorporated by reference in their entireties for all purposes. However, mention of any reference, article, publication, patent, patent publication, and patent application cited herein is not, and should not be taken as, an acknowledgment or any form of suggestion that they constitute valid prior art or form part of the common general knowledge in any country in the world.

## CLAIMS

1. A modified immune effector cell comprising a gene-regulating system capable of reducing expression and/or function of at least two endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*, wherein the reduced expression and/or function of the at least two endogenous target genes enhances an effector function of the immune effector cell.
2. The modified immune effector cell of claim 1, wherein the at least two target genes are *SOCS1* and *PTPN2*.
3. The modified immune effector cell of claim 1, wherein the at least two target genes are *SOCS1* and *ZC3H12A*.
4. The modified immune effector cell of claim 1, wherein the at least two target genes are *PTPN2* and *ZC3H12A*.
5. The modified immune effector cell of any one of claims 1-4, wherein the gene regulating system is further capable of reducing the expression and/or function of *CBLB*.
6. The modified immune effector cell of any one of claims 1-5, wherein the gene-regulating system comprises (i) a nucleic acid molecule; (ii) an enzymatic protein; or (iii) a nucleic acid molecule and an enzymatic protein.
7. The modified immune effector cell of claim 6, wherein the gene-regulating system comprises a nucleic acid molecule selected from an siRNA, an shRNA, a microRNA (miR), an antagomiR, or an antisense RNA.
8. The modified immune effector cell of claim 6, wherein the gene-regulating system comprises an enzymatic protein, and wherein the enzymatic protein has been engineered to specifically bind to a target sequence in one or more of the endogenous genes.
9. The modified immune effector cell of claim 8, wherein the protein is a Transcription activator-like effector nuclease (TALEN), a zinc-finger nuclease, or a meganuclease.

10. The modified immune effector cell of claim 6, wherein the gene-regulating system comprises a nucleic acid molecule and an enzymatic protein, wherein the nucleic acid molecule is a guide RNA (gRNA) molecule and the enzymatic protein is a Cas protein or Cas ortholog.
11. The modified immune effector cell of claim 10, wherein the Cas protein is a Cas9 protein.
12. The modified immune effector cell of claim 10, wherein the Cas protein is a wild-type Cas protein comprising two enzymatically active domains, and capable of inducing double stranded DNA breaks.
13. The modified immune effector cell of claim 10, wherein the Cas protein is a Cas nickase mutant comprising one enzymatically active domain and capable of inducing single stranded DNA breaks.
14. The modified immune effector cell of claim 10, wherein the Cas protein is a deactivated Cas protein (dCas) and is associated with a heterologous protein capable of modulating the expression of the one or more endogenous target genes.
15. The modified immune effector cell of claim 14, wherein the heterologous protein is selected from the group consisting of MAX-interacting protein 1 (MXI1), Krüppel-associated box (KRAB) domain, methyl-CpG binding protein 2 (MECP2), and four concatenated mSin3 domains (SID4X).
16. The modified immune effector cell of any one of claims 10-15, wherein the at least two endogenous genes are *SOCS1* and *PTPN2*, and wherein the gene-regulating system comprises at least one *SOCS1*-targeting gRNA molecule comprising a targeting domain sequence complementary to a nucleic acid sequence defined by any one of the set of genome coordinates shown in Tables 3 and 4 and at least one *PTPN2*-targeting gRNA molecule comprising a targeting domain sequence complementary to a nucleic acid sequence defined by any one of the set of genome coordinates shown in Tables 5 and 6.

17. The modified immune effector cell of any one of claims 10-15, wherein the at least two endogenous genes are *SOCS1* and *PTPN2*, and wherein the gene-regulating system comprises at least one *SOCS1*-targeting gRNA molecule comprising a targeting domain sequence that binds to a nucleic acid sequence defined by any one of the set of genome coordinates shown in Tables 3 and 4 and at least one *PTPN2*-targeting gRNA molecule comprising a targeting domain sequence that binds to a nucleic acid sequence defined by any one of the set of genome coordinates shown in Tables 5 and 6.

18. The modified immune effector cell of any one of claims 10-15, wherein the at least two endogenous genes are *SOCS1* and *PTPN2*, and wherein the gene-regulating system comprises at least one *SOCS1*-targeting gRNA molecule comprising a targeting domain sequence that binds to a target DNA sequence selected from the group consisting of SEQ ID NOs: 7-151 and at least one *PTPN2*-targeting gRNA molecule comprising a targeting domain sequence that binds to a target DNA sequence selected from the group consisting of SEQ ID NOs: 185-207.

19. The modified immune effector cell of any one of claims 10-15, wherein the at least two endogenous genes are *SOCS1* and *PTPN2*, and wherein the gene-regulating system comprises at least one *SOCS1*-targeting gRNA molecule comprising a targeting domain sequence encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 7-151 and at least one *PTPN2*-targeting gRNA molecule comprising a targeting domain sequence encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 185-207.

20. The modified immune effector cell of any one of claims 10-15, wherein the at least two endogenous genes are *SOCS1* and *ZC3H12A*, and wherein the gene-regulating system comprises at least one *SOCS1*-targeting gRNA molecule comprising a targeting domain sequence complementary to a nucleic acid sequence defined by any one of the set of genome coordinates shown in Tables 3 and 4, and at least one *ZC3H12A*-targeting gRNA molecule comprising a targeting domain sequence complementary to a nucleic acid sequence defined by any one of the set of genome coordinates shown in Tables 7 and 8.

21. The modified immune effector cell of any one of claims 10-15, wherein the at least two endogenous genes are *SOCS1* and *ZC3H12A*, and wherein the gene-regulating system comprises at least one *SOCS1*-targeting gRNA molecule comprising a targeting domain sequence that binds to a

nucleic acid sequence defined by any one of the set of genome coordinates shown in Tables 3 and 4 and at least one *ZC3H12A*-targeting gRNA molecule comprising a targeting domain sequence that binds to a nucleic acid sequence defined by any one of the set of genome coordinates shown in Tables 7 and 8.

22. The modified immune effector cell of any one of claims 10-15, wherein the at least two endogenous genes are *SOCS1* and *ZC3H12A*, and wherein the gene-regulating system comprises at least one *SOCS1*-targeting gRNA molecule comprising a targeting domain sequence that binds to a target DNA sequence selected from the group consisting of SEQ ID NOs: 7-151 and at least one *ZC3H12A*-targeting gRNA molecule comprising a targeting domain sequence that binds to a target DNA sequence selected from the group consisting of SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

23. The modified immune effector cell of any one of claims 10-15, wherein the at least two endogenous genes are *SOCS1* and *ZC3H12A*, and wherein the gene-regulating system comprises at least one *SOCS1*-targeting gRNA molecule comprising a targeting domain sequence encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 7-151 and at least one *ZC3H12A*-targeting gRNA molecule comprising a targeting domain sequence encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

24. The modified immune effector cell of any one of claims 10-15, wherein the at least two endogenous genes are *PTPN2* and *ZC3H12A*, and wherein the gene-regulating system comprises at least one *PTPN2*-targeting gRNA molecule comprising a targeting domain sequence complementary to a nucleic acid sequence defined by any one of the set of genome coordinates shown in Tables 5 and 6 and at least one *ZC3H12A*-targeting gRNA molecule comprising a targeting domain sequence complementary to a nucleic acid sequence defined by any one of the set of genome coordinates shown in Tables 7 and 8.

25. The modified immune effector cell of any one of claims 10-15, wherein the at least two endogenous genes are *PTPN2* and *ZC3H12A*, and wherein the gene-regulating system comprises at least one *PTPN2*-targeting gRNA molecule comprising a targeting domain sequence that binds to a

nucleic acid sequence defined by any one of the set of genome coordinates shown in Tables 5 and 6 and at least one *ZC3H12A*-targeting gRNA molecule comprising a targeting domain sequence that binds to a nucleic acid sequence defined by any one of the set of genome coordinates shown in Tables 7 and 8.

26. The modified immune effector cell of any one of claims 10-15, wherein the at least two endogenous genes are *PTPN2* and *ZC3H12A*, and wherein the gene-regulating system comprises at least one *PTPN2*-targeting gRNA molecule comprising a targeting domain sequence that binds to a target DNA sequence selected from the group consisting of SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308 and at least one *ZC3H12A*-targeting gRNA molecule comprising a targeting domain sequence that binds to a target DNA sequence selected from the group consisting of SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

27. The modified immune effector cell of any one of claims 10-15, wherein the at least two endogenous genes are *PTPN2* and *ZC3H12A*, and wherein the gene-regulating system comprises at least one *PTPN2*-targeting gRNA molecule comprising a targeting domain sequence encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308 and at least one *ZC3H12A*-targeting gRNA molecule comprising a targeting domain sequence encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

28. The modified immune effector cell of claim 7, wherein the at least two endogenous genes are *SOCS1* and *PTPN2*, and wherein the gene-regulating system comprises at least one *SOCS1*-targeting siRNA or shRNA molecule comprising about 19-30 nucleotides that are complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 3 and 4 and at least one *PTPN2*-targeting siRNA or shRNA molecule comprising about 19-30 nucleotides that are complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6.

29. The modified immune effector cell of claim 7, wherein the at least two endogenous genes are *SOCS1* and *PTPN2*, and wherein the gene-regulating system comprises at least one *SOCS1*-targeting siRNA or shRNA molecule comprising about 19-30 nucleotides that binds to an RNA sequence

encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 3 and 4 and at least one *PTPN2*-targeting siRNA or shRNA molecule comprising about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6.

30. The modified immune effector cell of claim 7, wherein the at least two endogenous genes are *SOCS1* and *PTPN2*, wherein the gene-regulating system comprises at least one *SOCS1*-targeting siRNA or shRNA comprising about 19-30 nucleotides that bind to an RNA sequence encoded by a DNA sequence selected from the group consisting of SEQ ID NOs: 7-151 and at least one *PTPN2*-targeting siRNA or shRNA comprising about 19-30 nucleotides that bind to an RNA sequence encoded by a DNA sequence selected from the group consisting of SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308.

31. The modified immune effector cell of claim 7, wherein the at least two endogenous genes are *SOCS1* and *ZC3H12A*, wherein the gene-regulating system comprises at least one *SOCS1*-targeting siRNA or shRNA molecule comprising about 19-30 nucleotides that are complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 3 and 4 and at least one *ZC3H12A*-targeting siRNA or shRNA molecule comprising about 19-30 nucleotides that are complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8.

32. The modified immune effector cell of claim 7, wherein the at least two endogenous genes are *SOCS1* and *ZC3H12A*, wherein the gene-regulating system comprises at least one *SOCS1*-targeting siRNA or shRNA molecule comprising about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Table 3 and 4 and at least one *ZC3H12A*-targeting siRNA or shRNA molecule comprising about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8.

33. The modified immune effector cell of claim 7, wherein the at least two endogenous genes are *SOCS1* and *ZC3H12A*, wherein the gene-regulating system comprises at least one *SOCS1*-targeting siRNA or shRNA comprising about 19-30 nucleotides that binds to an RNA sequence encoded by a

DNA sequence selected from the group consisting of SEQ ID NOs: 7-151 and at least one *ZC3H12A*-targeting siRNA or shRNA comprising about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence selected from the group consisting of SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

34. The modified immune effector cell of claim 7, wherein the at least two endogenous genes are *PTPN2* and *ZC3H12A*, wherein the gene-regulating system comprises at least one *PTPN2*-targeting siRNA or shRNA molecule comprising about 19-30 nucleotides that are complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6 and at least one *ZC3H12A*-targeting siRNA or shRNA molecule comprising about 19-30 nucleotides that are complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8.

35. The modified immune effector cell of claim 7, wherein the at least two endogenous genes are *PTPN2* and *ZC3H12A*, wherein the gene-regulating system comprises at least one *PTPN2*-targeting siRNA or shRNA molecule comprising about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6 and at least one *ZC3H12A*-targeting siRNA or shRNA molecule comprising about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8.

36. The modified immune effector cell of claim 7, wherein the at least two endogenous genes are *PTPN2* and *ZC3H12A*, wherein the gene-regulating system comprises at least one *PTPN2*-targeting siRNA or shRNA comprising about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence selected from the group consisting of SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308 and at least one *ZC3H12A*-targeting siRNA or shRNA comprising about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence selected from the group consisting of SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

37. The modified immune effector cell of claim 9, wherein the at least two endogenous genes are *SOCS1* and *PTPN2*, and wherein the gene-regulating system comprises at least one *SOCS1*-targeting TALEN, zinc finger, or meganuclease protein that binds to a target DNA sequence defined by a set of



genome coordinates shown in Tables 3 and 4 and at least one *PTPN2*-targeting TALEN, zinc finger, or meganuclease protein that binds to a target DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6.

38. The modified immune effector cell of claim 9, wherein the at least two endogenous genes are *SOCS1* and *PTPN2*, wherein the gene-regulating system comprises at least one *SOCS1*-targeting TALEN, zinc finger, or meganuclease protein that binds to a DNA sequence selected from SEQ ID NOs: 7-151 and at least one *PTPN2*-targeting TALEN, zinc finger, or meganuclease protein that binds to a DNA sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308.

39. The modified immune effector cell of claim 9, wherein the at least two endogenous genes are *SOCS1* and *ZC3H12A*, wherein the gene-regulating system comprises at least one *SOCS1*-targeting TALEN, zinc finger, or meganuclease protein that binds to a target DNA sequence defined by a set of genome coordinates shown in Tables 3 and 4 and at least one *ZC3H12A*-targeting TALEN, zinc finger, or meganuclease protein that binds to a target DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8.

40. The modified immune effector cell of claim 9, wherein the at least two endogenous genes are *SOCS1* and *ZC3H12A*, wherein the gene-regulating system comprises at least one *SOCS1*-targeting TALEN, zinc finger, or meganuclease protein that binds to a DNA sequence selected from SEQ ID NOs: 7-151 and at least one *ZC3H12A*-targeting TALEN, zinc finger, or meganuclease protein that binds to a DNA sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

41. The modified immune effector cell of claim 9, wherein the at least two endogenous genes are *PTPN2* and *ZC3H12A*, wherein the gene-regulating system comprises at least one *PTPN2*-targeting TALEN, zinc finger, or meganuclease protein that binds to a target DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6 and at least one *ZC3H12A*-targeting TALEN, zinc finger, or meganuclease protein that binds to a target DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8.

42. The modified immune effector cell of claim 9, wherein the at least two endogenous genes are *PTPN2* and *ZC3H12A*, wherein the gene-regulating system comprises at least one *PTPN2*-targeting TALEN, zinc finger, or meganuclease protein that binds to a DNA sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308 and at least one *ZC3H12A*-targeting TALEN, zinc finger, or meganuclease protein that binds to a DNA sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.
43. The modified immune effector cell of any one of claims 1-42, wherein the gene-regulating system is introduced to the immune effector cell by transfection, transduction, electroporation, or physical disruption of the cell membrane by a microfluidics device.
44. The modified immune effector cell of claim 43, wherein the gene-regulating system is introduced as a polynucleotide encoding one or more components of the system, a protein, or a ribonucleoprotein (RNP) complex.
45. A modified immune effector cell, comprising reduced expression and/or function of at least two endogenous genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*, wherein the reduced expression and/or function of the at least two endogenous genes enhances an effector function of the immune effector cell.
46. The modified immune effector cell of claim 45, wherein the at least two target genes are *SOCS1* and *PTPN2*.
47. The modified immune effector cell of claim 45, wherein the at least two target genes are *SOCS1* and *ZC3H12A*.
48. The modified immune effector cell of claim 45, wherein the at least two target genes are *PTPN2* and *ZC3H12A*.
49. A modified immune effector cell, comprising an inactivating mutation in at least two endogenous genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.

50. The modified immune effector cell of claim 49, wherein the immune effector cell is a tumor infiltrating lymphocyte (TIL) or a CAR-T cell.
51. The modified immune effector cell of claim 49 or claim 50, wherein the at least two target genes are *SOCS1* and *PTPN2*.
52. The modified immune effector cell of claim 49 or claim 50, wherein the at least two target genes are *SOCS1* and *ZC3H12A*.
53. The modified immune effector cell of claim 49 or claim 50, wherein the at least two target genes are *PTPN2* and *ZC3H12A*.
54. The modified cell of any one of claims 49-53, wherein the inactivating mutation comprises a deletion, substitution, or insertion of one or more nucleotides in the genomic sequences of the two or more endogenous genes.
55. The modified immune effector cell of claim 54, wherein the deletion is a partial or complete deletion of the two or more endogenous target genes.
56. The modified immune effector cell of claim 54, wherein the inactivating mutation is a frame shift mutation.
57. The modified immune effector cell of any one of claims 49-56, wherein the inactivating mutation reduces the expression and/or function of the two or more endogenous target genes.
58. The modified immune effector cell of any one of claims 45-57, further comprising reduced expression and/or function of *CBLB*.
59. A modified immune effector cell, comprising one or more exogenous polynucleotides encoding at least two nucleic acid inhibitors of at least two endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.

60. The modified immune effector cell of claim 59, wherein the immune effector cell is a tumor infiltrating lymphocyte (TIL) or a CAR-T cell.
61. The modified immune effector cell of claim 59 or claim 60, wherein the at least two target genes are *SOCS1* and *PTPN2*.
62. The modified immune effector cell of claim 59 or claim 60, wherein the at least two target genes are *SOCS1* and *ZC3H12A*.
63. The modified immune effector cell of claim 59 or claim 60, wherein the at least two target genes are *PTPN2* and *ZC3H12A*.
64. The modified immune effector cell of any one of claims 59-63, wherein the at least two nucleic acid inhibitors reduce the expression and/or function of the two or more endogenous target genes.
65. The modified immune effector cell of claim 58 or claim 64, wherein the expression of the two or more endogenous target genes is reduced by at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% compared to an un-modified or control immune effector cell.
66. The modified immune effector cell of claim 65, wherein the function of the two or more endogenous target genes is reduced by at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% compared to an un-modified or control immune effector cell.
67. The modified immune effector cell of claim 65, wherein the inactivating mutation or nucleic acid inhibitors substantially inhibits the expression of the two or more endogenous target genes.
68. The modified immune effector cell of claim 65, wherein the inactivating mutation or nucleic acid inhibitors substantially inhibits the function of the two or more endogenous target genes.
69. The modified immune effector cell of any one of claims 50-68, wherein the inactivating mutation or nucleic acid inhibitors enhances one or more effector functions of the modified immune effector cell.

70. The modified immune effector cell of claim 69, wherein the one or more effector functions are enhanced compared to an un-modified or control immune effector cell.
71. The modified immune effector cell of any one of claims 1-70, wherein the immune effector cell is a T cell, a natural killer (NK) cell, an NKT cell, or a tumor infiltrating lymphocyte (TIL).
72. The modified immune effector cell of any one of claims 1-71, further comprising an exogenous transgene expressing an immune activating molecule.
73. The modified immune effector cell of claim 1, wherein the immune activating molecule is selected from the group consisting of a cytokine, a chemokine, a co-stimulatory molecule, an activating peptide, an antibody, or an antigen-binding fragment thereof.
74. The modified immune effector cell of any one of claims 1-73, wherein the effector function is selected from cell proliferation, cell viability, tumor infiltration, cytotoxicity, anti-tumor immune responses, and/or resistance to exhaustion.
75. The modified immune effector cell of any one of claims 1-74, further comprising an engineered immune receptor displayed on the cell surface.
76. The modified immune effector cell of claim 75, wherein the engineered immune receptor is a chimeric antigen receptor (CAR) comprising an antigen-binding domain, a transmembrane domain, and an intracellular signaling domain.
77. The modified immune effector cell of claim 76, wherein the engineered immune receptor is an engineered T cell receptor (TCR).
78. The modified immune effector cell of any one of claims 75-77, wherein the engineered immune receptor is capable of specifically binding to an antigen expressed on the surface of a target cell, wherein the antigen is a tumor-associated antigen.

79. A composition, comprising the modified immune effector cells of any one of claims 1-78.
80. The composition of claim 79, comprising at least  $1 \times 10^4$ ,  $1 \times 10^5$ ,  $1 \times 10^6$ ,  $1 \times 10^7$ ,  $1 \times 10^8$ ,  $1 \times 10^9$ ,  $1 \times 10^{10}$ ,  $1 \times 10^{11}$ , or more modified immune effector cells.
81. The composition of claim 79 or 80, wherein the composition comprises a pharmaceutically acceptable carrier or diluent.
82. The composition of any one of claims 79-81, comprising autologous immune effector cells
83. The composition of any one of claims 79-81, comprising allogeneic immune effector cells.
84. A gene-regulating system capable of reducing expression of at least two endogenous target genes in a cell selected from *SOCS1*, *PTPN2*, and *ZC3H12A*, comprising (i) a nucleic acid molecule; (ii) an enzymatic protein; or (iii) a nucleic acid molecule and an enzymatic protein.
85. The gene regulating system of claim 84, wherein the at least two target genes are *SOCS1* and *PTPN2*.
86. The gene regulating system of claim 84, wherein the at least two target genes are *SOCS1* and *ZC3H12A*.
87. The gene regulating system of claim 84, wherein the at least two endogenous genes are *PTPN2* and *ZC3H12A*.
88. The gene-regulating system of any one of claims 84-87, wherein the system comprises at least two guide RNA (gRNA) nucleic acid molecules and a Cas endonuclease.
89. The gene-regulating system of claim 88, wherein the at least two target genes are *SOCS1* and *PTPN2*, and wherein the system comprises at least one *SOCS1*-targeting guide RNA (gRNA) molecule, at least one *PTPN2*-targeting gRNA molecule, and a Cas endonuclease.

90. The gene-regulating system of claim 89, wherein the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 5 and 6.

91. The gene-regulating system of claim 89, wherein the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 5 and 6.

92. The gene-regulating system of claim 89, wherein the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 7-151 and the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308.

93. The gene-regulating system of claim 89, wherein the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 7-151 and the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308.

94. The gene-regulating system of claim 89, wherein the at least two target genes are *SOCS1* and *ZC3H12A*, and wherein the system comprises at least one *SOCS1*-targeting gRNA molecule, at least one *ZC3H12A*-targeting gRNA molecule, and a Cas endonuclease.

95. The gene-regulating system of claim 94, wherein the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *ZC3H12A*-targeting gRNA

molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 7 and 8.

96. The gene-regulating system of claim 94, wherein the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 7 and 8.

97. The gene-regulating system of claim 94, wherein the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 7-151 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

98. The gene-regulating system of claim 94, wherein the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 7-151 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

99. The gene-regulating system of claim 88, wherein the at least two endogenous genes are *PTPN2* and *ZC3H12A*, wherein the system comprises at least one *PTPN2*-targeting gRNA molecule, at least one *ZC3H12A*-targeting gRNA molecule, and a Cas endonuclease.

100. The gene-regulating system of claim 99, wherein the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 5 and 6 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 7 and 8.



101. The gene-regulating system of claim 99, wherein the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 5 and 6 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 7 and 8.

102. The gene-regulating system of claim 99, wherein the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

103. The gene-regulating system of claim 99, wherein the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

104. The gene-regulating system of any one of claims 88-103, wherein the Cas protein is a Cas9 protein.

105. The gene-regulating system of any one of claims 88-103, wherein the Cas protein is a wild-type Cas protein comprising two enzymatically active domains, and capable of inducing double stranded DNA breaks.

106. The gene-regulating system of any one of claims 88-103, wherein the Cas protein is a Cas nickase mutant comprising one enzymatically active domain and capable of inducing single stranded DNA breaks.

107. The gene-regulating system of any one of claims 88-103, wherein the Cas protein is a deactivated Cas protein (dCas) and is associated with a heterologous protein capable of modulating the expression of the one or more endogenous target genes.

108. The gene-regulating system of claim 107, wherein the heterologous protein is selected from the group consisting of MAX-interacting protein 1 (MXI1), Krüppel-associated box (KRAB) domain, and four concatenated mSin3 domains (SID4X).

109. The gene-regulating system of claim 84-87, wherein the system comprises at least two nucleic acid molecules and wherein the at least two nucleic acid molecules are selected from an siRNA, an shRNA, a microRNA (miR), an antagomiR, or an antisense RNA.

110. The gene-regulating system of claim 109, wherein the at least two target genes are *SOCS1* and *PTPN2*, and wherein the system comprises at least one *SOCS1*-targeting guide siRNA or shRNA molecule and at least one *PTPN2*-targeting siRNA or shRNA molecule.

111. The gene-regulating system of claim 110, wherein the *SOCS1*-targeting siRNA or shRNA molecule comprises about 19-30 nucleotides that are complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *PTPN2*-targeting siRNA or shRNA molecule comprises about 19-30 nucleotides that are complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6.

112. The gene-regulating system of claim 110, wherein the at least one *SOCS1*-targeting siRNA or shRNA molecule comprises about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *PTPN2*-targeting siRNA or shRNA molecule comprises about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6.

113. The gene-regulating system of claim 110, wherein the at least one *SOCS1*-targeting siRNA or shRNA comprises about 19-30 nucleotides that bind to an RNA sequence encoded by a DNA sequence

selected from SEQ ID NOs: 7-151 and the at least one *PTPN2*-targeting siRNA or shRNA comprises about 19-30 nucleotides that bind to an RNA sequence encoded by a DNA sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308.

114. The gene-regulating system of claim 109, wherein the at least two target genes are *SOCS1* and *ZC3H12A*, and wherein the system comprises at least one *SOCS1*-targeting guide siRNA or shRNA molecule and at least one *ZC3H12A*-targeting siRNA or shRNA molecule.

115. The gene-regulating system of claim 114, wherein the at least one *SOCS1*-targeting siRNA or shRNA molecule comprises about 19-30 nucleotides that are complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule comprises about 19-30 nucleotides that are complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8.

116. The gene-regulating system of claim 114, wherein the at least one *SOCS1*-targeting siRNA or shRNA molecule comprises about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule comprises about 19-30 nucleotides and binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8.

117. The gene-regulating system of claim 114, wherein the at least one *SOCS1*-targeting siRNA or shRNA comprises about 19-30 nucleotides that bind to an RNA sequence encoded by a DNA sequence selected from SEQ ID NOs: 7-151 and the at least one *ZC3H12A*-targeting siRNA or shRNA comprises about 19-30 nucleotides that bind to an RNA sequence encoded by a DNA sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

118. The gene-regulating system of claim 109, wherein the at least two target genes are *PTPN2* and *ZC3H12A*, and wherein the system comprises at least one *PTPN2*-targeting guide siRNA or shRNA molecule and at least one *ZC3H12A*-targeting siRNA or shRNA molecule.

119. The gene-regulating system of claim 118, wherein the at least one *PTPN2*-targeting siRNA or shRNA molecule comprises about 19-30 nucleotides that are complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6 and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule comprises about 19-30 nucleotides that are complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8.

120. The gene-regulating system of claim 118, wherein the at least one *PTPN2*-targeting siRNA or shRNA molecule comprises about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6 and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule comprises about 19-30 nucleotides that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8.

121. The gene-regulating system of claim 118, wherein the at least one *PTPN2*-targeting siRNA or shRNA comprises about 19-30 nucleotides that bind to an RNA sequence encoded by a DNA sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308 and the at least one *ZC3H12A*-targeting siRNA or shRNA comprises about 19-30 nucleotides that bind to an RNA sequence encoded by a DNA sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

122. The gene-regulating system of claim 84-87, wherein the gene-regulating system comprises an enzymatic protein, and wherein the enzymatic protein has been engineered to specifically bind to a target sequence in one or more of the endogenous genes

123. The gene-regulating system of claim 122, wherein the system comprises a protein comprising a DNA binding domain and an enzymatic domain and is selected from a zinc finger nuclease and a transcription-activator-like effector nuclease (TALEN).

124. The gene-regulating system of any one of claims 84-108, comprising one or more vectors encoding at least one gRNA targeting a first target gene, at least one gRNA targeting a second target gene, and a Cas endonuclease protein,

wherein the first target gene is *SOCS1* and the least one *SOCS1*-targeting gRNA comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 7-151, and

wherein the second target gene is *PTPN2* and the at least one *PTPN2*-targeting gRNA comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308.

125. The gene-regulating system of any one of claims 84-108, comprising one or more vectors encoding at least one gRNA targeting a first target gene, at least one gRNA targeting a second target gene, and a Cas endonuclease protein,

wherein the first target gene is *SOCS1* and the least one *SOCS1*-targeting gRNA comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 7-151, and

wherein the second target gene is *ZC3H12A* and the at least one *ZC3H12A*-targeting gRNA comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

126. The gene-regulating system of any one of claims 84-108, comprising one or more vectors encoding at least one gRNA targeting a first target gene, at least one gRNA targeting a second target gene, and a Cas endonuclease protein,

wherein the first target gene is *PTPN2* and the *PTPN2*-targeting gRNA comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308, and

wherein the second target gene is *ZC3H12A* and the at least one *ZC2H12A*-targeting gRNA comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

127. The gene-regulating system of any one of claims 124-126, wherein the at least one gRNA targeting the first target gene, the at least one gRNA targeting the second target gene, and the Cas endonuclease protein are encoded by one vector.

128. The gene-regulating system of any one of claims 124-126, wherein the at least one gRNA targeting the first target gene and the at least one gRNA targeting the second target gene are encoded by a first vector, and the Cas endonuclease protein is encoded by a second vector.

129. The gene-regulating system of any one of claims 124-126, wherein the at least one gRNA targeting the first target gene is encoded by a first vector, the at least one gRNA targeting the second target gene is encoded by a second vector, and the Cas endonuclease protein is encoded by a third vector.

130. The gene-regulating system of any one of claims 84-108, comprising:

(i) one or more vectors encoding at least one *SOCS1*-targeting gRNA comprising a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 7-151 and at least one *PTPN2*-targeting gRNA comprising a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308; and

(ii) an mRNA molecule encoding the Cas endonuclease protein.

131. The gene-regulating system of any one of claims 84-108, comprising:

(i) one or more vectors encoding at least one *SOCS1*-targeting gRNA comprising a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 7-151 and at least one *ZC2H12A*-targeting gRNA comprising a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575; and

(ii) an mRNA molecule encoding the Cas endonuclease protein.

132. The gene-regulating system of any one of claims 84-108, comprising:

(i) one or more vectors encoding at least one *PTPN2*-targeting gRNA comprising a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308 and at least one *ZC2H12A*-targeting gRNA comprising a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575; and

(ii) an mRNA molecule encoding the Cas endonuclease protein.

133. The gene-regulating system of any one of claims 130-132, wherein the at least one gRNA targeting the first target gene and the at least one gRNA targeting the second target gene are encoded by one vector.

134. The gene-regulating system of any one of claims 130-132, wherein the at least one gRNA targeting the first target gene is encoded by a first vector and the at least one gRNA targeting the second target gene is encoded by a second vector.

135. The gene-regulating system of any one of claims 84-108, comprising:

(i) at least one *SOCS1*-targeting gRNA comprising a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 7-151 complexed to a first Cas endonuclease protein to form a first ribonucleoprotein (RNP) complex; and

(ii) at least one *PTPN2*-targeting gRNA comprising a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308 complexed to a second Cas endonuclease protein to form a second RNP complex.

136. The gene-regulating system of any one of claims 84-108, comprising:

(i) at least one *SOCS1*-targeting gRNA comprising a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 7-151 complexed to a first Cas endonuclease protein to form a first RNP complex; and

(ii) at least one *ZC2H12A*-targeting gRNA comprising a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575 complexed to a second Cas endonuclease protein to form a second RNP complex.

137. The gene-regulating system of any one of claims 84-108, comprising:

(i) at least one *PTPN2*-targeting gRNA comprising a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308 complexed to a first Cas endonuclease protein to form a first RNP complex; and

(ii) at least one *ZC2H12A*-targeting gRNA comprising a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or

SEQ ID NOs: 376-575 complexed to a second Cas endonuclease protein to form a second RNP complex.

138. A kit comprising the gene-regulating system of any one of claims 84-137.

139. A composition comprising a plurality of gRNA molecules, wherein the plurality of gRNA molecules comprises at least one gRNA molecule targeting a first target gene and at least one gRNA molecule targeting a second target gene, wherein the first and second target gene are selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.

140. The composition of claim 139, wherein the first target gene is *SOCS1* and the second target gene is *PTPN2*.

141. The composition of claim 140, wherein the plurality of gRNA molecules comprises at least one *SOCS1*-targeting gRNA molecule comprising a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 3 and 4 and at least one *PTPN2*-targeting gRNA molecule comprising a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 5 and 6.

142. The composition of claim 140, wherein the plurality of gRNA molecules comprises at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 5 and 6.

143. The composition of claim 140, wherein the plurality of gRNA molecules comprises at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 7-151 and the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308.



144. The composition of claim 140, wherein the plurality of gRNA molecules comprises at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 7-151 and the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308.

145. The composition of claim 139, wherein the first target gene is *SOCS1* and the second target gene is *ZC3H12A*.

146. The composition of claim 145, wherein the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 7 and 8.

147. The composition of claim 145, wherein the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 7 and 8.

148. The composition of claim 145, wherein the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 7-151 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

149. The composition of claim 145, wherein the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 7-151 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

150. The composition of claim 139, wherein the first target gene is *PTPN2* and the second target gene is *ZC3H12A*.

151. The composition of claim 150, wherein the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 5 and 6 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 7 and 8.

152. The composition of claim 150, wherein the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 5 and 6 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 7 and 8.

153. The composition of claim 150, wherein the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

154. The composition of claim 150, wherein the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

155. The composition of any one of claims 139-154, wherein the gRNAs are modular gRNA molecules.

156. The composition of any one of claims 139-154, wherein the gRNAs are dual gRNA molecules.

157. The composition of any one of claims 139-156, wherein the gRNA targeting domains are 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26 or more nucleotides in length.

158. The composition of any one of claims 139-157, wherein the gRNAs comprise a modification at or near the 5' end (*e.g.*, within 1-10, 1-5, or 1-2 nucleotides of the 5' end) and/or a modification at or near the 3' end (*e.g.*, within 1-10, 1-5, or 1-2 nucleotides of the 3' end).

159. The composition of claim 158, wherein the modified gRNAs exhibit increased resistance to nucleases when introduced into an immune effector cell.

160. The composition of claim 158 or 159, wherein the modified gRNAs do not induce an innate immune response when introduced into an immune effector cell or induce a decreased innate immune response compared to an unmodified gRNA when introduced into an immune effector cell.

161. A polynucleotide molecule encoding a plurality of gRNA molecules, wherein the plurality of gRNA molecules comprises at least one gRNA molecule targeting a first target gene and at least one gRNA molecule targeting a second target gene, wherein the first and second target gene are selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.

162. The polynucleotide of claim 161, wherein the first target gene is *SOCS1* and the second target gene is *PTPN2*.

163. The polynucleotide of claim 162, wherein the plurality of gRNA molecules comprises at least one *SOCS1*-targeting gRNA molecule comprising a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 3 and 4 and at least one *PTPN2*-targeting gRNA molecule comprising a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 5 and 6.

164. The polynucleotide of claim 162, wherein the plurality of gRNA molecules comprises at least one *SOCS1*-targeting gRNA molecule comprising a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one

*PTPN2*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 5 and 6.

165. The polynucleotide of claim 162, wherein the plurality of gRNA molecules comprises at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 7-151 and the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308.

166. The polynucleotide of claim 162, wherein the plurality of gRNA molecules comprises at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 7-151 and the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308.

167. The polynucleotide of claim 161, wherein the first target gene is *SOCS1* and the second target gene is *ZC3H12A*.

168. The polynucleotide of claim 167, wherein the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 7 and 8.

169. The polynucleotide of claim 167, wherein the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 7 and 8.

170. The polynucleotide of claim 167, wherein the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID

NOs: 7-151 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

171. The polynucleotide of claim 167, wherein the at least one *SOCS1*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 7-151 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

172. The polynucleotide of claim 161, wherein the first target gene is *PTPN2* and the second target gene is *ZC3H12A*.

173. The polynucleotide of claim 172, wherein the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 5 and 6 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence complementary to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 7 and 8.

174. The polynucleotide of claim 172, wherein the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 5 and 6 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence that binds to a nucleic acid sequence defined by a set of genome coordinates shown in Tables 7 and 8.

175. The polynucleotide of claim 172, wherein the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence that binds to a target DNA sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

176. The polynucleotide of claim 172, wherein the at least one *PTPN2*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308 and the at least one *ZC3H12A*-targeting gRNA molecule comprises a targeting domain sequence encoded by a nucleic acid sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

177. A polynucleotide molecule encoding a plurality of siRNA or shRNA molecules, wherein the plurality of siRNA or shRNA molecules comprises at least one siRNA or shRNA molecule targeting a first target gene and at least one siRNA or shRNA molecule targeting a second target gene, wherein the first and second target gene are selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.

178. The polynucleotide of claim 177, wherein the first target gene is *SOCS1* and the second target gene is *PTPN2*.

179. The polynucleotide of claim 178, wherein the plurality of siRNA or shRNA molecules comprises at least one *SOCS1*-targeting siRNA or shRNA molecule comprising a targeting domain sequence that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 3 and 4 and at least one *PTPN2*-targeting siRNA or shRNA molecule comprising a targeting domain sequence complementary to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6.

180. The polynucleotide of claim 178, wherein the plurality of siRNA or shRNA molecules comprises at least one *SOCS1*-targeting siRNA or shRNA molecule comprising a targeting domain sequence t that binds to an RNA sequence encoded by a DNA sequence selected from SEQ ID NOs: 7-151 and the at least one *PTPN2*-targeting siRNA or shRNA molecule comprising a targeting domain sequence that binds to an RNA sequence encoded by a DNA sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308.

181. The polynucleotide of claim 177, wherein the first target gene is *SOCS1* and the second target gene is *ZC3H12A*.

182. The polynucleotide of claim 181, wherein the at least one *SOCS1*-targeting siRNA or shRNA molecule comprising a targeting domain sequence that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule comprising a targeting domain sequence that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8.

183. The polynucleotide of claim 181, wherein the at least one *SOCS1*-targeting siRNA or shRNA molecule comprising a targeting domain sequence that binds to an RNA sequence encoded by a DNA sequence selected from SEQ ID NOs: 7-151 and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule comprising a targeting domain sequence that binds to an RNA sequence encoded by a DNA sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

184. The polynucleotide of claim 177, wherein the first target gene is *PTPN2* and the second target gene is *ZC3H12A*.

185. The polynucleotide of claim 184, wherein the at least one *PTPN2*-targeting siRNA or shRNA molecule comprising a targeting domain sequence that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6 and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule comprising a targeting domain sequence that binds to an RNA sequence encoded by a DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8.

186. The polynucleotide of claim 184, wherein the at least one *PTPN2*-targeting siRNA or shRNA molecule comprising a targeting domain sequence that binds to an RNA sequence encoded by a DNA sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308 and the at least one *ZC3H12A*-targeting siRNA or shRNA molecule comprising a targeting domain sequence that binds to an RNA sequence encoded by a DNA sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.

187. A polynucleotide molecule encoding at least one TALEN, zinc finger, or meganuclease protein targeting a first target gene and at least one TALEN, zinc finger, or meganuclease protein targeting a

second target gene, wherein the first and second target gene are selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.

188. The polynucleotide of claim 187, wherein the first target gene is *SOCS1* and the second target gene is *PTPN2*.

189. The polynucleotide of claim 188, encoding at least one *SOCS1*-targeting TALEN, zinc finger, or meganuclease protein comprising a targeting domain sequence that binds to a DNA sequence defined by a set of genome coordinates shown in Tables 3 and 4 and at least one *PTPN2*-targeting TALEN, zinc finger, or meganuclease protein comprising a targeting domain sequence that binds to a DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6.

190. The polynucleotide of claim 188, encoding at least one *SOCS1*-targeting TALEN, zinc finger, or meganuclease protein comprising a targeting domain sequence that binds to a DNA sequence selected from SEQ ID NOs: 7-151 and at least one *PTPN2*-targeting TALEN, zinc finger, or meganuclease protein comprising a targeting domain sequence that binds to a DNA sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308.

191. The polynucleotide of claim 187, wherein the first target gene is *SOCS1* and the second target gene is *ZC3H12A*.

192. The polynucleotide of claim 191, encoding at least one *SOCS1*-targeting TALEN, zinc finger, or meganuclease protein comprising a targeting domain sequence that binds to a DNA sequence defined by a set of genome coordinates shown in Tables 3 and 4 and the at least one *ZC3H12A*-targeting TALEN, zinc finger, or meganuclease protein comprising a targeting domain sequence that binds to a DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8.

193. The polynucleotide of claim 191, encoding at least one *SOCS1*-targeting TALEN, zinc finger, or meganuclease protein comprising a targeting domain sequence that binds to a DNA sequence selected from SEQ ID NOs: 7-151 and at least one *ZC3H12A*-targeting TALEN, zinc finger, or meganuclease protein comprising a targeting domain sequence that binds to a DNA sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.



194. The polynucleotide of claim 187, wherein the first target gene is *PTPN2* and the second target gene is *ZC3H12A*.
195. The polynucleotide of claim 194, encoding least one *PTPN2*-targeting TALEN, zinc finger, or meganuclease protein comprising a targeting domain sequence that binds to a DNA sequence defined by a set of genome coordinates shown in Tables 5 and 6 and the at least one *ZC3H12A*-targeting TALEN, zinc finger, or meganuclease protein comprising a targeting domain sequence that binds to a DNA sequence defined by a set of genome coordinates shown in Tables 7 and 8.
196. The polynucleotide of claim 194, encoding at least one *PTPN2*-targeting TALEN, zinc finger, or meganuclease protein comprising a targeting domain sequence that binds to a DNA sequence selected from SEQ ID NOs: 185-207, SEQ ID NOs: 272-375, or SEQ ID NOs: 272-308 and at least one *ZC3H12A*-targeting TALEN, zinc finger, or meganuclease protein comprising a targeting domain sequence that binds to a DNA sequence selected from SEQ ID NOs: 208-230, SEQ ID NOs: 376-812, or SEQ ID NOs: 376-575.
197. A composition comprising the polynucleotide of any one of claims 161-196.
198. A kit comprising the polynucleotide of any one of claims 161-196 or the composition of claim 176.
199. A method of producing a modified immune effector cell comprising:  
introducing a gene-regulating system into the immune effector cell, wherein the gene-regulating system is capable of reducing expression and/or function of at least two endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.
200. A method of producing a modified immune effector cell comprising:  
obtaining an immune effector cell from a subject;  
introducing a gene-regulating system into the immune effector cell, wherein the gene-regulating system is capable of reducing expression and/or function of at least two endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*; and

culturing the immune effector cell such that the expression and/or function of one or more endogenous target genes is reduced compared to an immune effector cell that has not been modified.

201. The method of claim 199 or 200, wherein the gene-regulating system is one selected from claims 84-137.

202. The method of any one of claims 199-201, further comprising introducing a polynucleotide sequence encoding an engineered immune receptor selected from a CAR and a TCR.

203. The method of claim 202, wherein the gene-regulating system and/or the polynucleotide encoding the engineered immune receptor are introduced to the immune effector cell by transfection, transduction, electroporation, or physical disruption of the cell membrane by a microfluidics device.

204. The method of any one of claims 199-203, wherein the gene-regulating system is introduced as a polynucleotide sequence encoding one or more components of the system, as a protein, or as a ribonucleoprotein (RNP) complex.

205. A method of producing a modified immune effector cell comprising:  
expanding a population of immune effector cells in a first round expansion and a second round of expansion; and  
introducing a gene-regulating system into the population of immune effector cells, wherein the gene-regulating system is capable of reducing expression and/or function of at least two endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.

206. A method of producing a modified immune effector cell comprising:  
obtaining a population of immune effector cells;  
expanding the population of immune effector cells in a first round expansion and a second round of expansion;  
introducing a gene-regulating system into the population of immune effector cells, wherein the gene-regulating system is capable of reducing expression and/or function of at least two endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*; and

culturing the immune effector cell such that the expression and/or function of one or more endogenous target genes is reduced compared to an immune effector cell that has not been modified.

207. The method of claim 205 or 206, wherein the gene-regulating system is introduced to the population of immune effector cells prior to the first and second rounds of expansion.

208. The method of claim 205 or 206, wherein the gene-regulating system is introduced to the population of immune effector cells after the first round of expansion and prior to the second round of expansion.

209. The method of claim 205 or 206, wherein the gene-regulating system is introduced to the population of immune effector cells after the first and second rounds of expansion.

210. The method of any one of claims 204-209, wherein the gene-regulating system is one selected from claims 84-137.

211. A method of treating a disease or disorder in a subject in need thereof comprising administering to the subject an effective amount of the modified immune effector cells of any one of claims 1-78, or the composition of any one of claims 79-83.

212. The method of claim 211, wherein the disease or disorder is a cell proliferative disorder, an inflammatory disorder, or an infectious disease.

213. The method of claim 211, wherein the disease or disorder is a cancer or a viral infection.

214. The method of claim 213, wherein the cancer is selected from a leukemia, a lymphoma, or a solid tumor.

215. The method of claim 214, wherein the solid tumor is a melanoma, a pancreatic tumor, a bladder tumor, or a lung tumor or metastasis.

216. The method of any one of claims 213-215, wherein the cancer is a PD1 resistant or insensitive cancer.
217. The method of any one of claims 211-216, wherein the subject has previously been treated with a PD1 inhibitor or a PDL1 inhibitor.
218. The method of any one of claims 211-217, wherein the modified immune effector cells are autologous to the subject.
219. The method of any one of claims 211-217, wherein the modified immune effector cells are allogenic to the subject.
220. The method of any one of claims 211-219, further comprising administering to the subject an antibody or binding fragment thereof that specifically binds to and inhibits the function of the protein encoded by *NRP1*, *HAVCR2*, *LAG3*, *TIGIT*, *CTLA4*, or *PDCD1*.
221. The method of any one of claims 211-220, wherein the subject has not undergone lymphodepletion prior to administration of the modified immune effector cells or compositions thereof.
222. The method of any one of claims 211-220, wherein administration of the modified immune effector cells or compositions thereof to the subject is not accompanied by high dose IL-2 treatment.
223. The method of any one of claims 211-220, wherein administration of the modified immune effector cells or compositions thereof to the subject is not accompanied by any IL-2 treatment.
224. The method of claims 221 or 222, wherein the subject has not undergone lymphodepletion prior to administration of the modified immune effector cells or compositions thereof and the modified immune effector cells or compositions thereof to the subject is not accompanied by high dose IL-2 treatment.

225. A method of killing a cancerous cell comprising exposing the cancerous cell to a modified immune effector cell according to any one of claims 1-78 or the composition of any one of claims 79-83, wherein exposure to the modified immune effector cell results in increased killing of the cancerous cells compared to exposure to an immune effector cell that has not been modified.

226. The method of claim 225, wherein the exposure is *in vitro*, *in vivo*, or *ex vivo*.

227. A method of enhancing one or more effector functions of an immune effector cell comprising introducing a gene-regulating system into the immune effector cell, wherein the gene-regulating system is capable of reducing the expression and/or function of at least two endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.

228. A method of enhancing one or more effector functions of an immune effector cell comprising:  
introducing a gene-regulating system into the immune effector cell, wherein the gene-regulating system is capable of reducing the expression and/or function of at least two endogenous target genes selected from *SOCS1*, *PTPN2*, and *ZC3H12A*; and

culturing the immune effector cell such that the expression and/or function of one or more endogenous target genes is reduced compared to an immune effector cell that has not been modified,

wherein the modified immune effector cell demonstrates one or more enhanced effector functions compared to the immune effector cell that has not been modified.

229. The method of claim 227 or 228, wherein the one or more effector functions are selected from cell proliferation, cell viability, cytotoxicity, tumor infiltration, increased cytokine production, anti-tumor immune responses, and/or resistance to exhaustion.

230. The method of any one of claims 227-228, wherein the gene-regulating system is one selected from claims 85-137.

231. A method of producing a modified immune effector cell comprising introducing an inactivating mutation in at least two endogenous target genes in an immune effector cell, wherein the at least two endogenous target genes are selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.

232. A method of producing a modified immune effector cell comprising:  
expanding a population of immune effector cells; and  
introducing an inactivating mutation in at least two endogenous target genes in the population of immune effector cells, wherein the at least two endogenous target genes are selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.
233. A method of producing a modified immune effector cell comprising introducing one or more exogenous polynucleotides encoding at least two nucleic acid inhibitors of at least two endogenous target genes in an immune effector cell, wherein the at least two endogenous target genes are selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.
234. A method of producing a modified immune effector cell comprising:  
expanding a population of immune effector cells; and  
introducing one or more exogenous polynucleotides encoding at least two nucleic acid inhibitors of at least two endogenous target genes in the population of immune effector cells, wherein the at least two endogenous target genes are selected from *SOCS1*, *PTPN2*, and *ZC3H12A*.
235. The method of claim 231 or 234, wherein the population of immune effector cells is expanded in a first and/or second round of expansion
236. The method of any one of claims 231-235, further comprising introducing a polynucleotide sequence encoding an engineered immune receptor selected from a CAR and a TCR.
237. The method of claim 231 or claim 232, wherein inactivating mutation is introduced by the gene-regulating system of any one of claims 85-137.
238. The method of claim 233 or claim 234, wherein the at least two nucleic acid inhibitors are comprised in the gene-regulating system of any one of claims 85-137.
239. A method of killing a cancerous cell in a subject in need thereof comprising administering to the subject a therapeutically effective amount of modified immune effector cell according to any one

of claims 1-78 or the composition of any one of claims 79-83, wherein exposure to the modified immune effector cell results in increased killing of the cancerous cells compared to exposure to an immune effector cell that has not been modified, wherein the number of modified immune effector cells necessary to comprise a therapeutically effective amount is at least ten fold less than the number of non-modified immune effector cells necessary to comprise a therapeutically effective amount.

240. The method of claim 239, wherein the number of modified immune effector cells necessary to comprise a therapeutically effective amount is at least  $1 \times 10^3$ ,  $5 \times 10^3$ ,  $1 \times 10^4$ ,  $5 \times 10^4$ ,  $1 \times 10^5$ ,  $5 \times 10^5$ ,  $1 \times 10^6$ ,  $2 \times 10^6$ ,  $3 \times 10^6$ ,  $4 \times 10^6$ ,  $5 \times 10^6$ , or  $1 \times 10^7$ ,  $5 \times 10^7$ ,  $1 \times 10^8$ ,  $5 \times 10^8$ ,  $1 \times 10^9$  cells.

Fig. 1

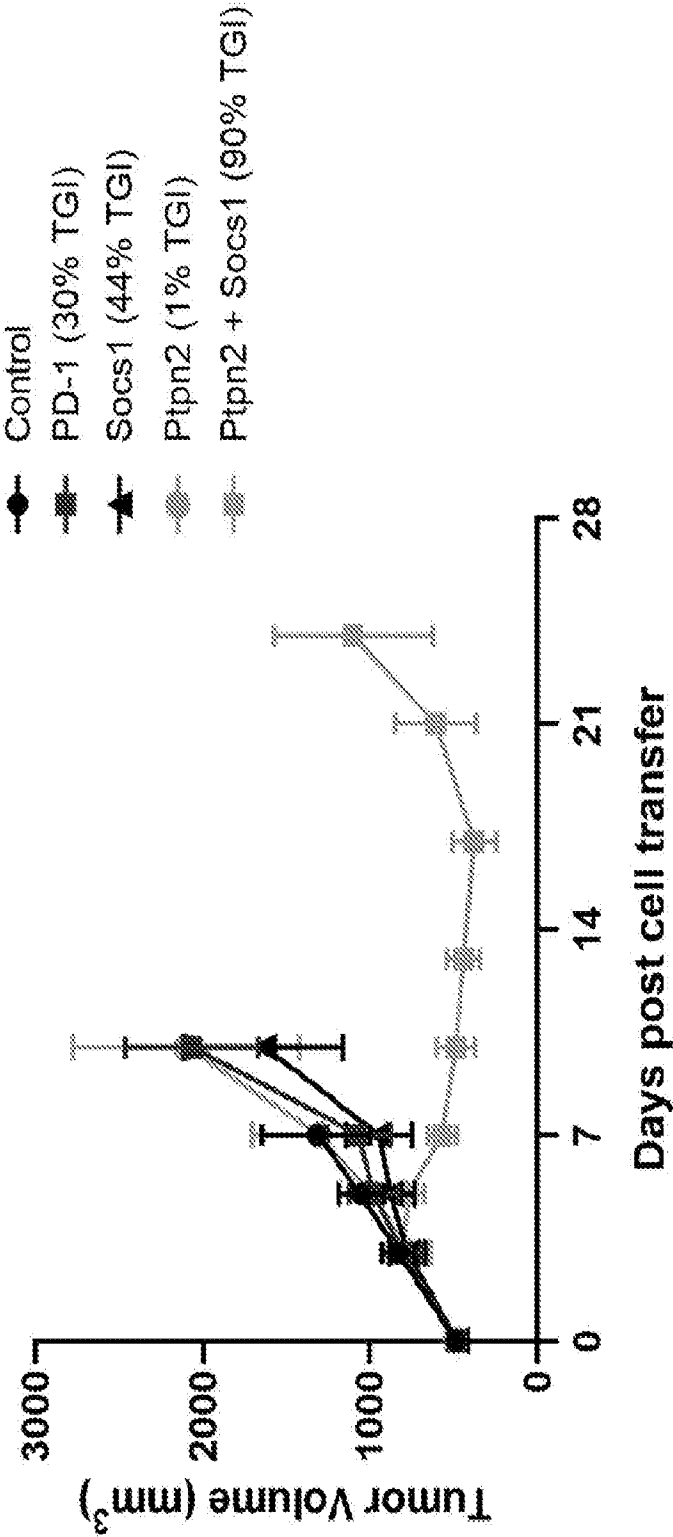




**Fig. 2**

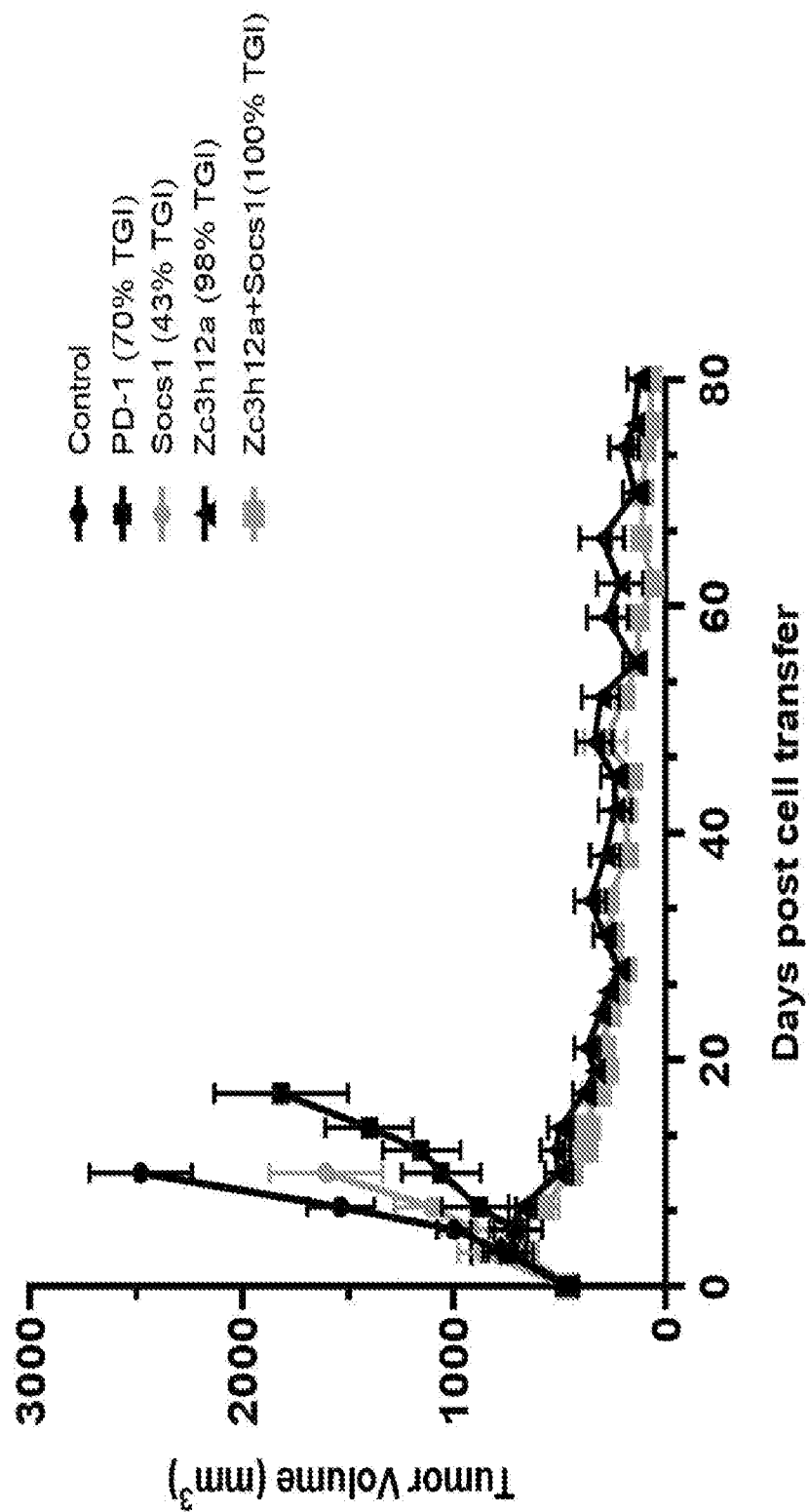
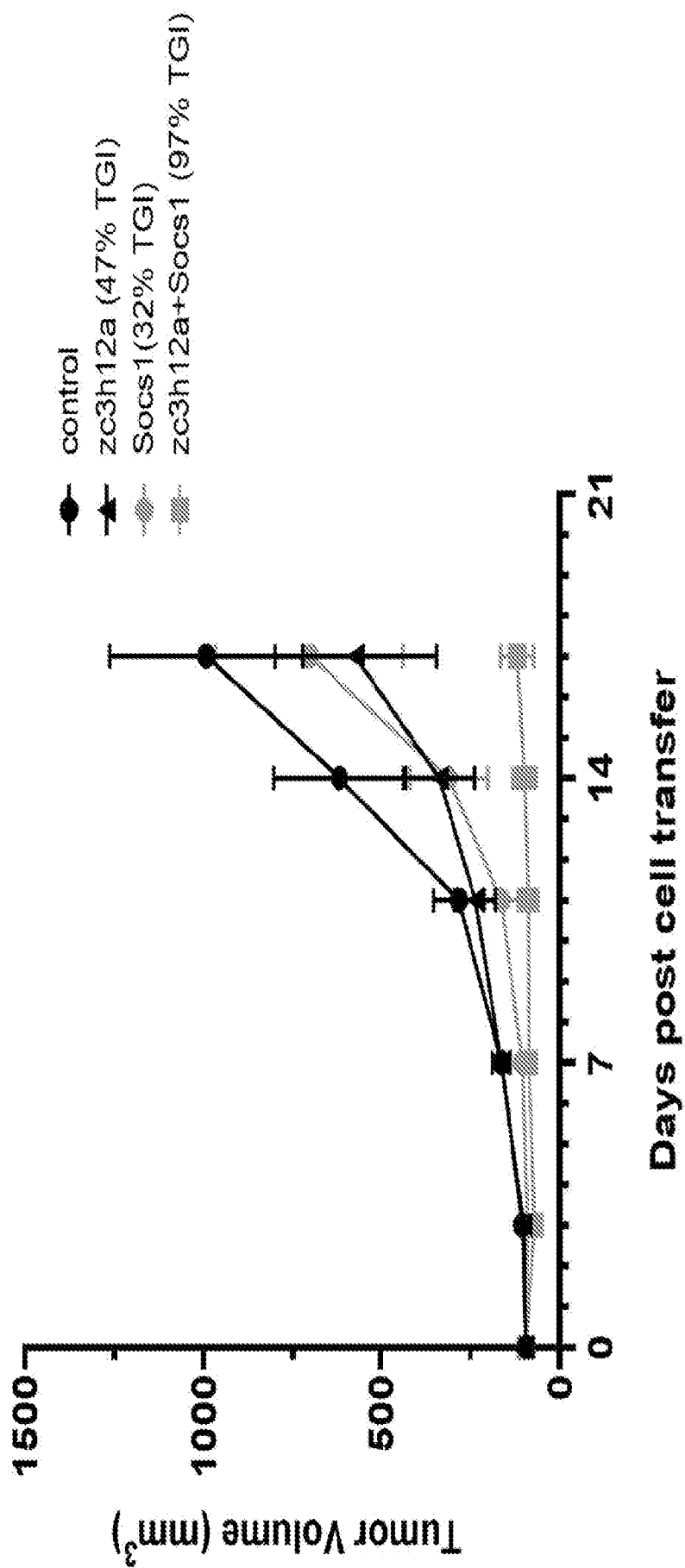


Fig. 3



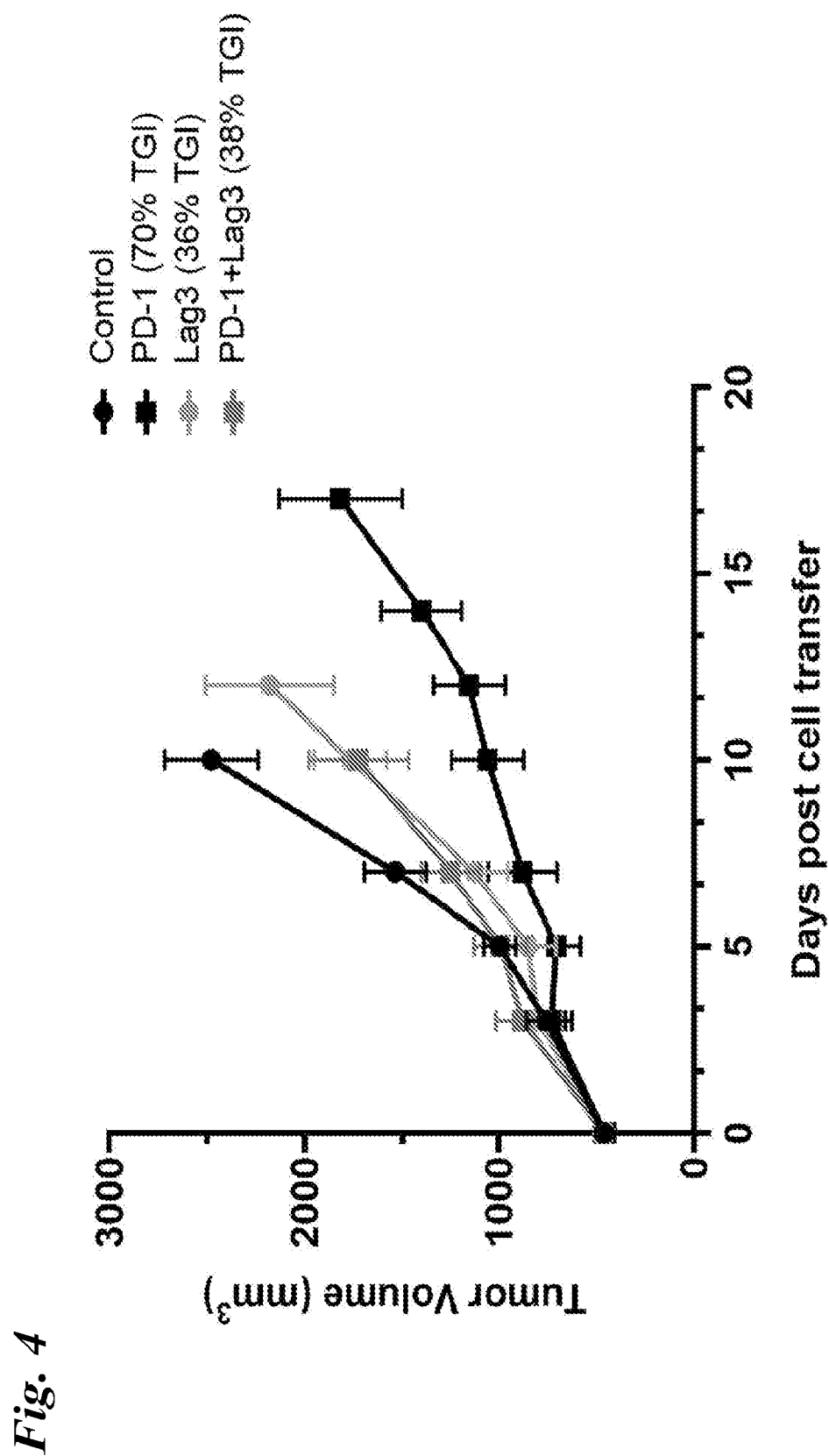


Fig. 5

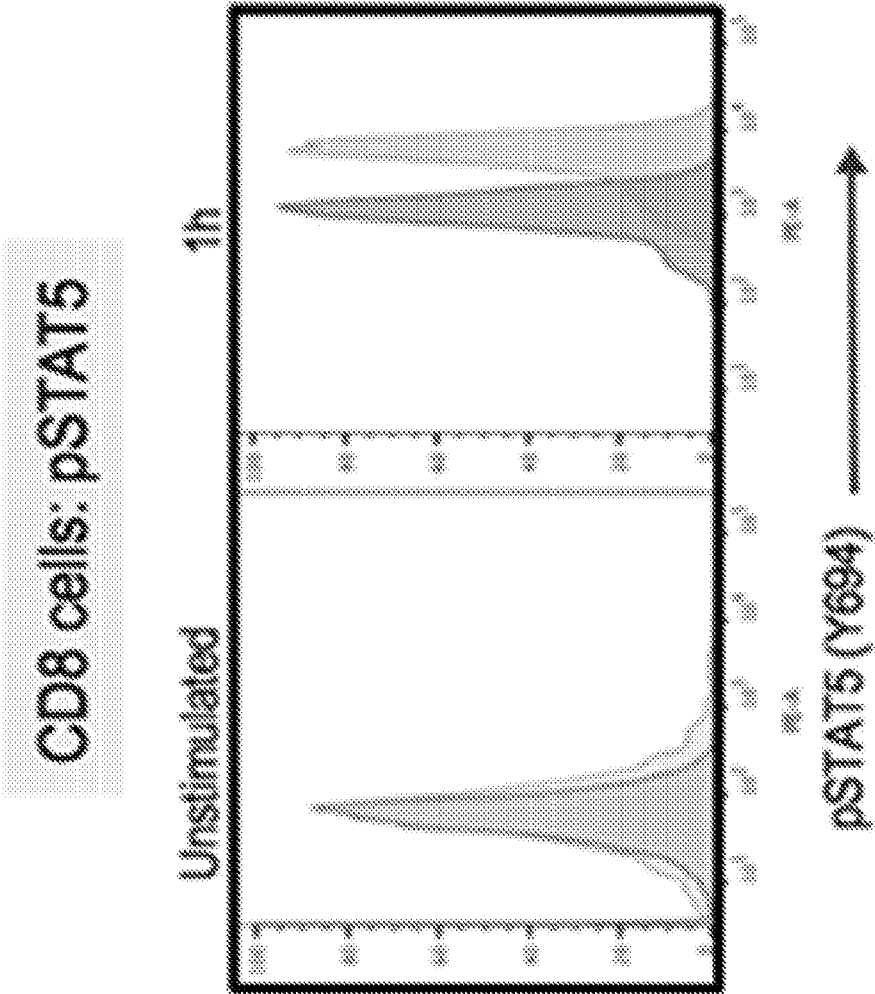


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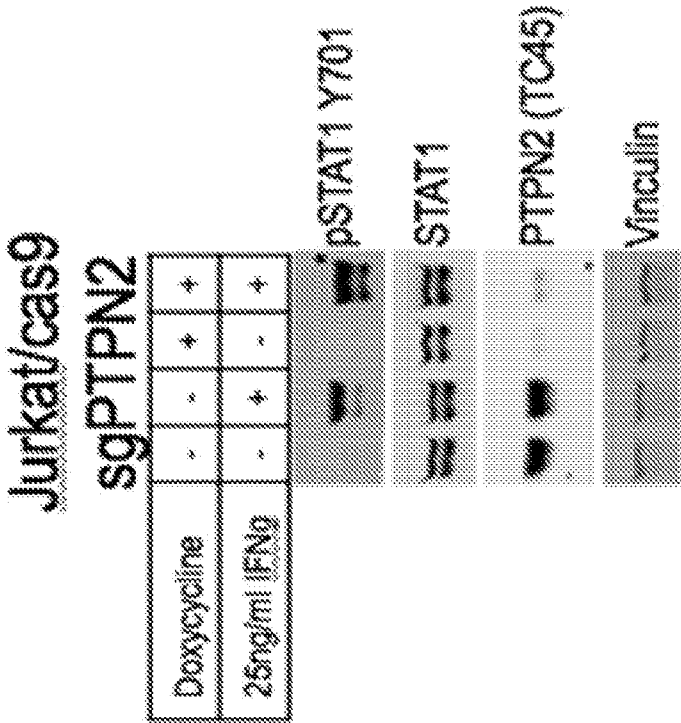
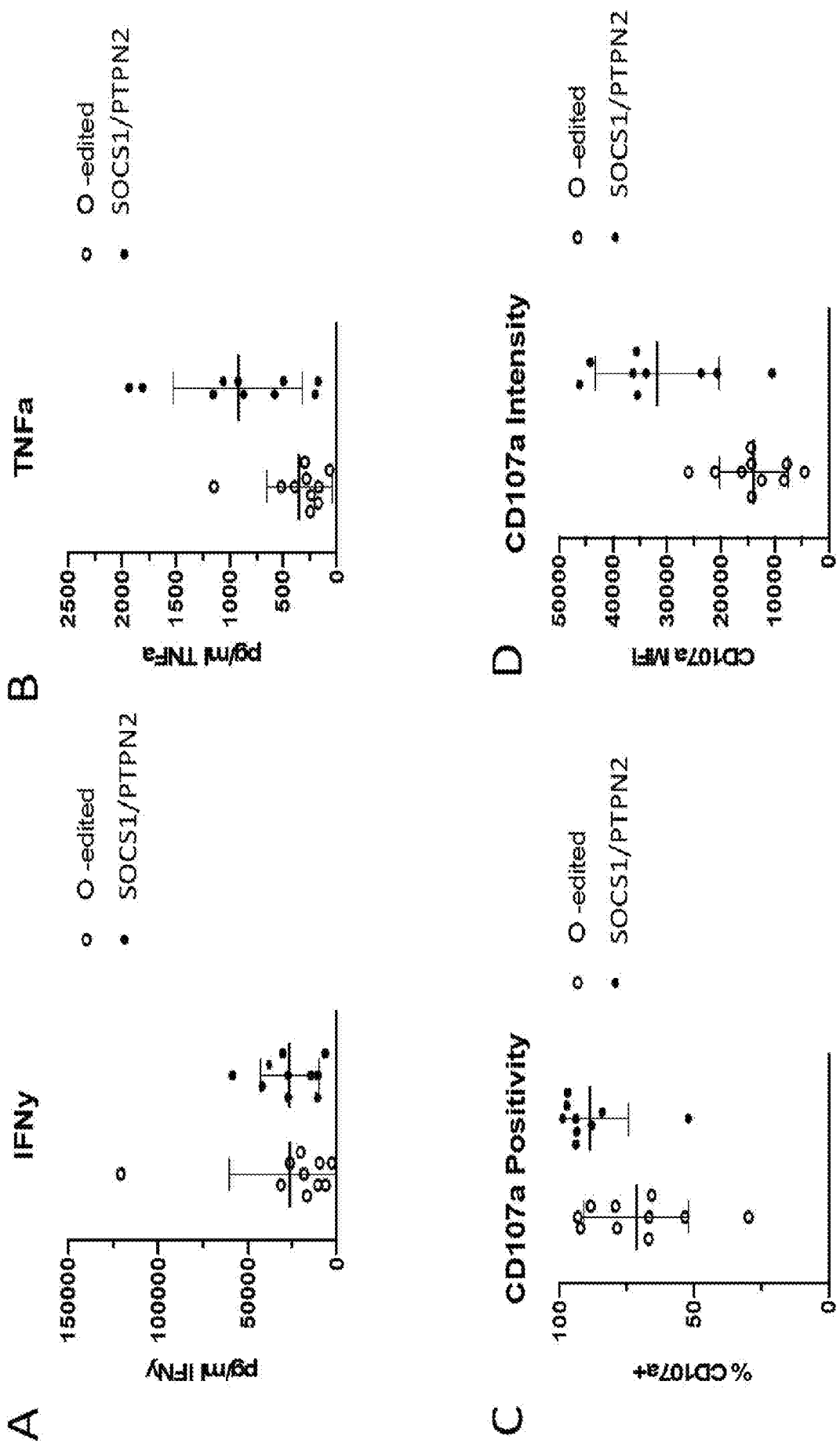


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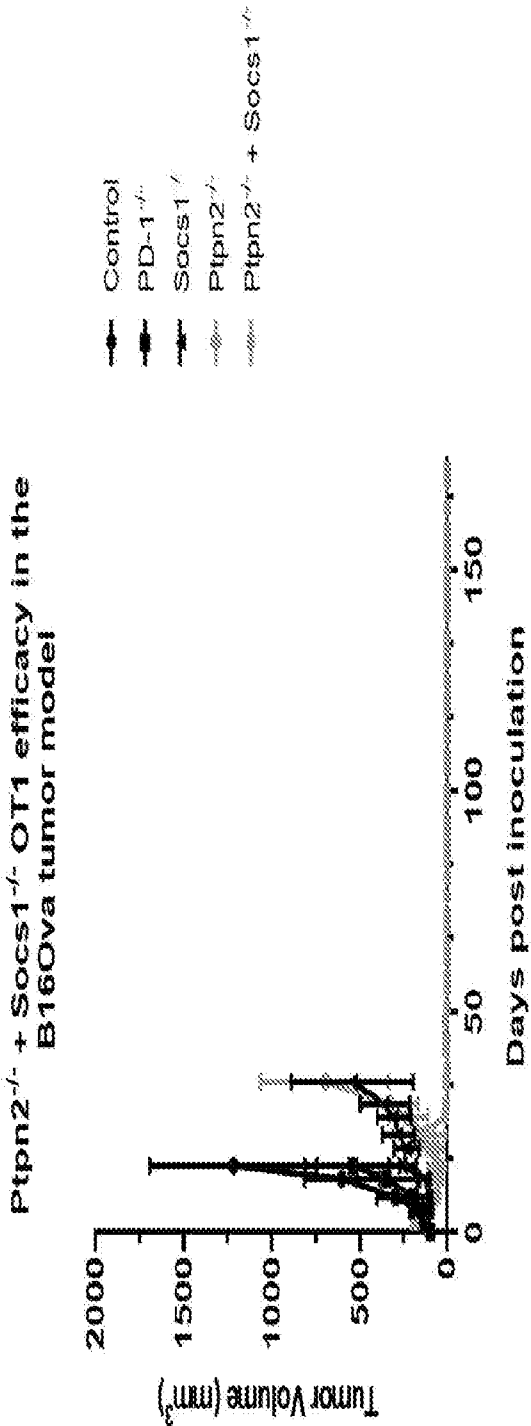


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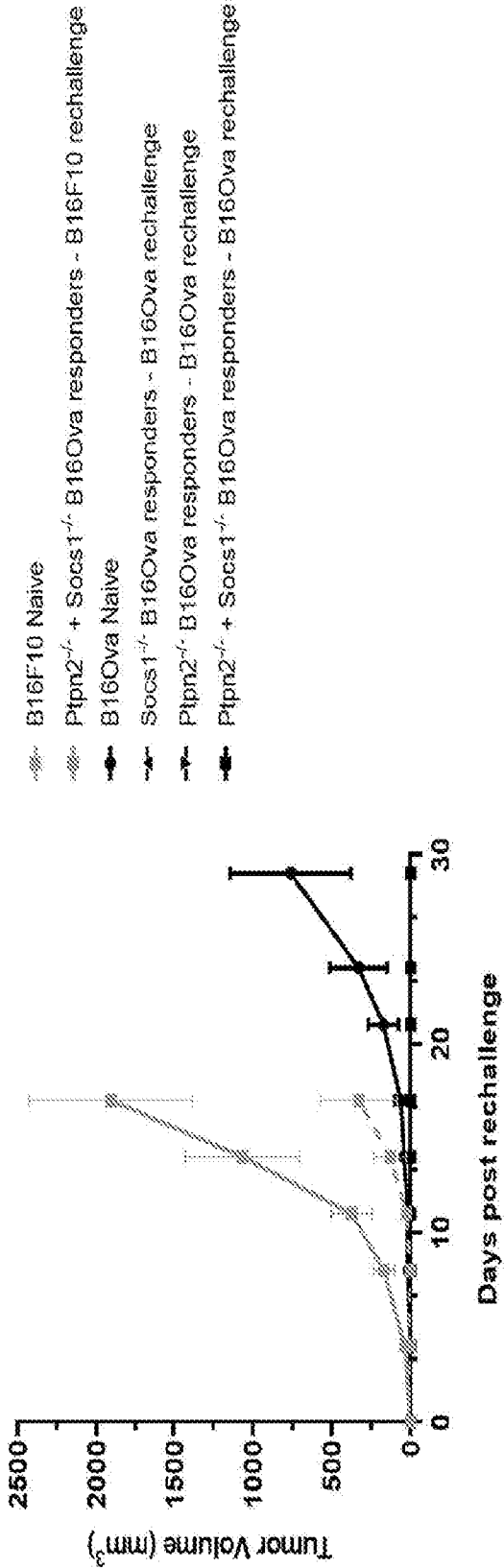


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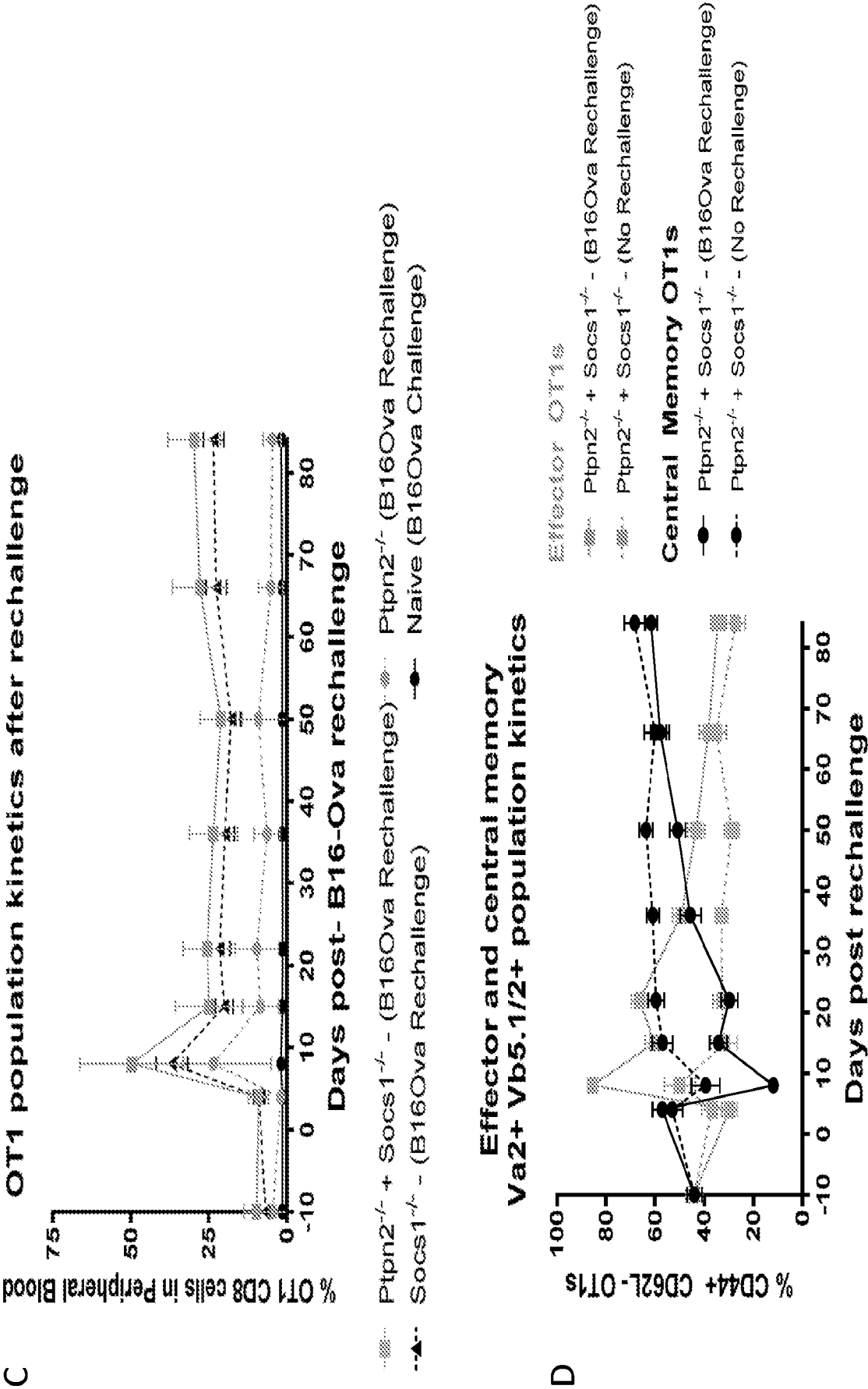


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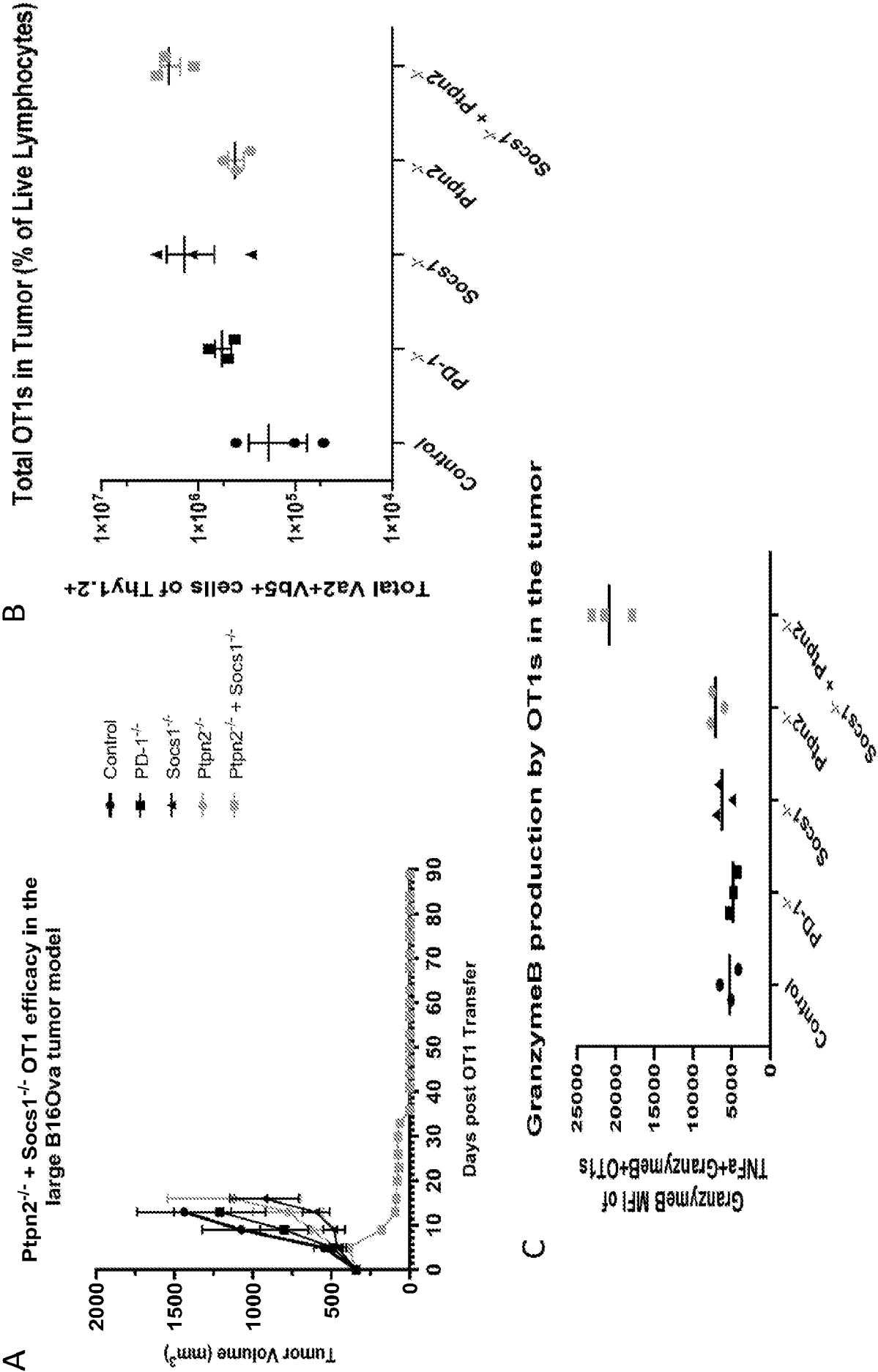
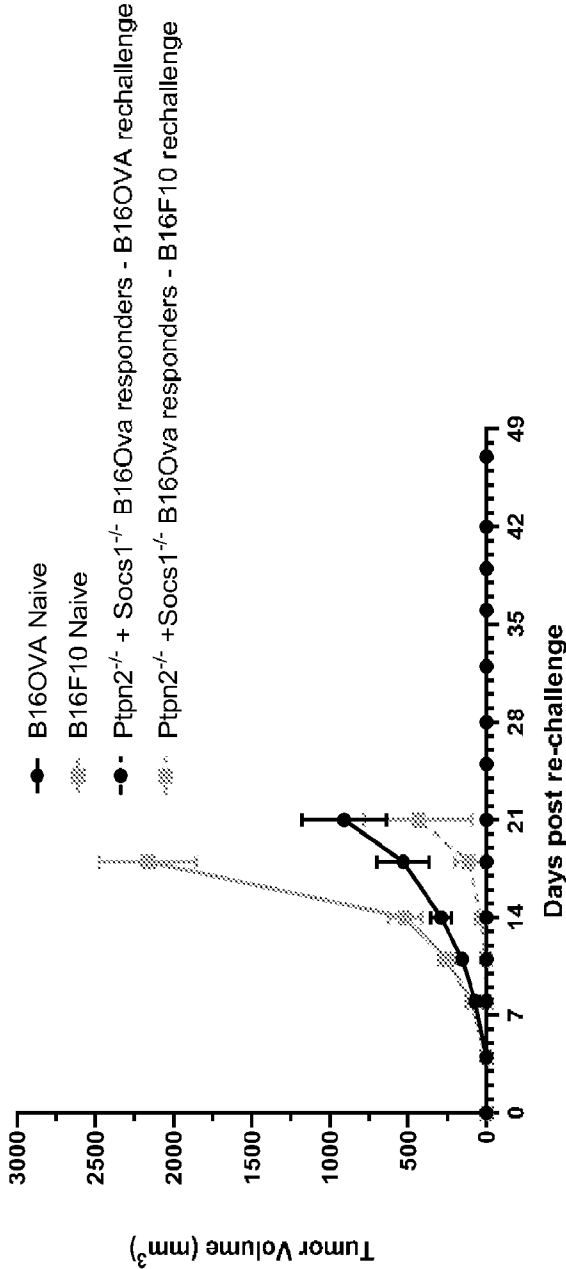




Fig. 9

Rechallenge Efficacy

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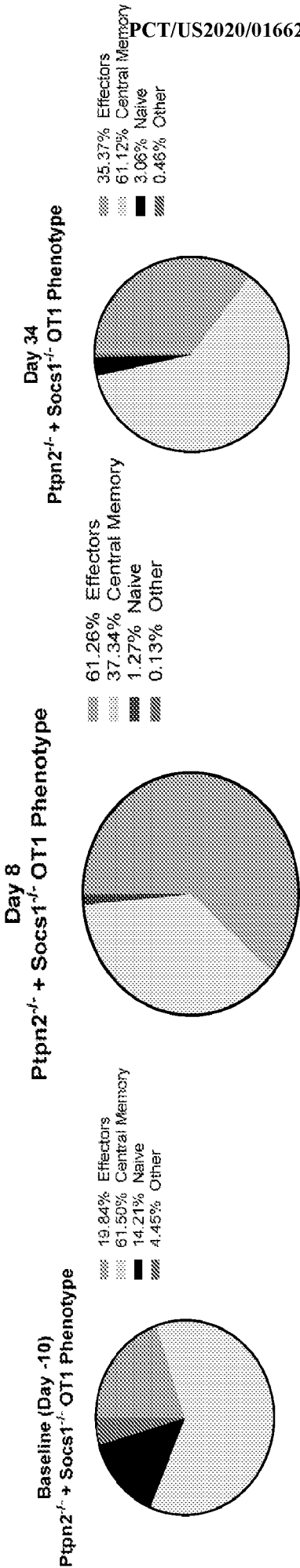


**Fig. 10**

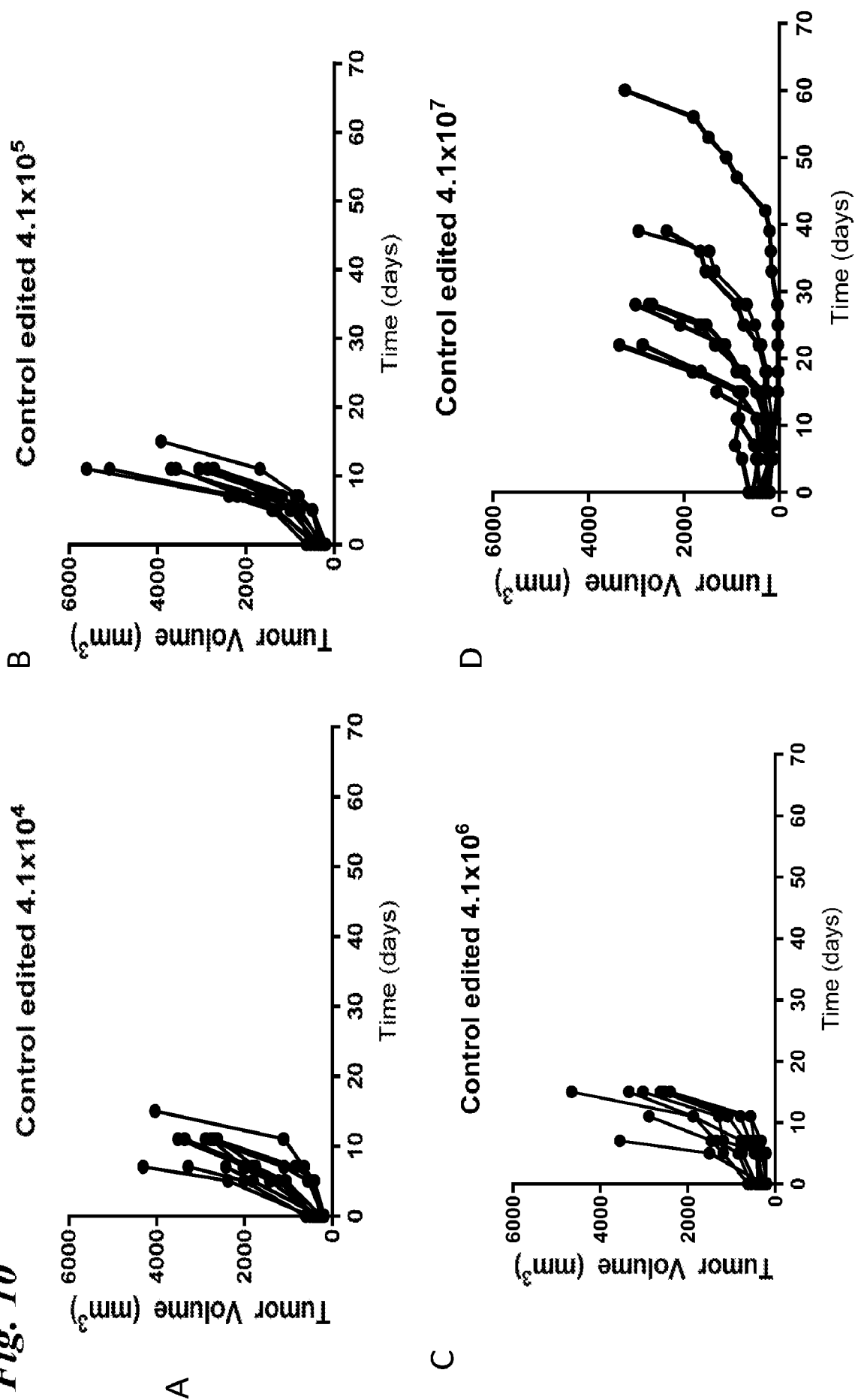
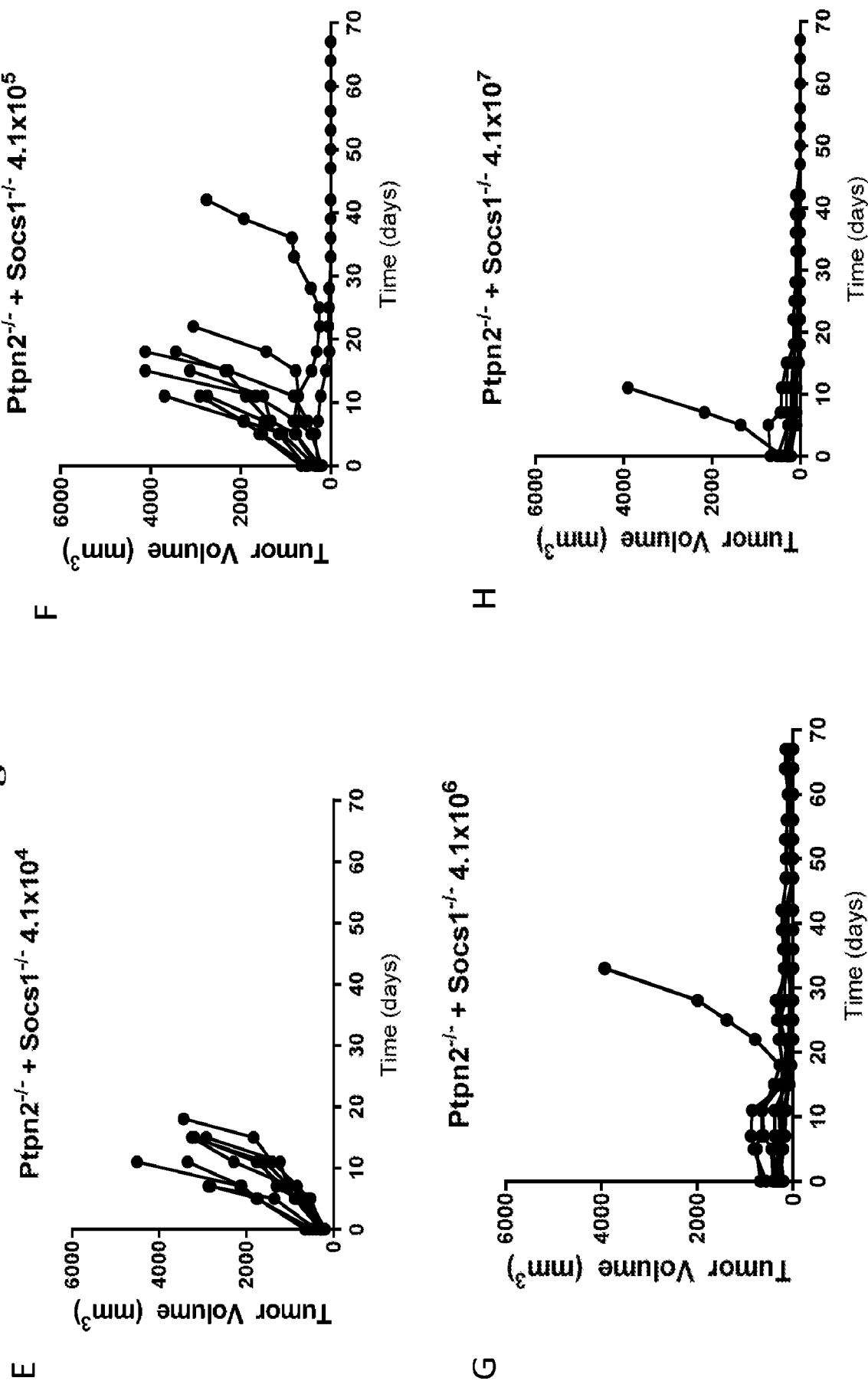


**Fig. 10**



## SEQUENCE LISTING

&lt;110&gt; KSQ Therapeutics, Inc.

&lt;120&gt; COMBINATION GENE TARGETS FOR IMPROVED IMMUNOTHERAPY

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cctggaactg cagatgaagg 20

<210> 231  
<211> 25  
<212> RNA  
<213> Artificial Sequence

<220>  
<223> Zc3H12A\_siRNA\_1

<400> 231  
ccuggacaac uuccuucgua agaaa 25

<210> 232  
<211> 19  
<212> RNA  
<213> Artificial Sequence

<220>  
<223> Zc3H12A\_siRNA\_2

<400> 232  
gugucccuau ggaaggaaa 19

<210> 233  
<211> 19  
<212> RNA  
<213> Artificial Sequence

<220>  
<223> Zc3H12A\_siRNA\_3

<400> 233  
caacuuccuu cguaagaaa 19

<210> 234  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Zc3H12A\_shRNA\_1

<400> 234  
agcgaggcca cacagatatt a 21

<210> 235  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Zc3H12A\_shRNA\_2

<400> 235  
gctatgatga ccgcttcatt g 21

<210> 236  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Zc3H12A\_shRNA\_3

<400> 236  
tgggtctgagc cgtacccatt a 21

<210> 237  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Zc3H12A\_shRNA\_4

<400> 237  
ctgtgtacag aggcgagatt t 21

<210> 238  
<211> 22  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> P2A\_linker

<400> 238

Gly Ser Gly Ala Thr Asn Phe Ser Leu Leu Lys Gln Ala Gly Asp Val  
1 5 10 15

Glu Glu Asn Pro Gly Pro  
20

<210> 239  
<211> 8  
<212> PRT  
<213> Homo sapiens

<400> 239

Ser Leu Leu Met Trp Ile Thr Gln  
1 5

<210> 240  
<211> 9  
<212> PRT  
<213> Homo sapiens

<400> 240

Ala Ala Gly Ile Gly Ile Leu Thr Val  
1 5

<210> 241  
<211> 9  
<212> PRT  
<213> Homo sapiens

&lt;400&gt; 241

Arg Met Phe Pro Asn Ala Pro Tyr Leu  
1 5

&lt;210&gt; 242

&lt;211&gt; 310

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 242

Met Ser Asn Gln Val Leu Cys Cys Val Val Leu Cys Phe Leu Gly Ala  
1 5 10 15

Asn Thr Val Asp Gly Gly Ile Thr Gln Ser Pro Lys Tyr Leu Phe Arg  
20 25 30

Lys Glu Gly Gln Asn Val Thr Leu Ser Cys Glu Gln Asn Leu Asn His  
35 40 45

Asp Ala Met Tyr Trp Tyr Arg Gln Asp Pro Gly Gln Gly Leu Arg Leu  
50 55 60

Ile Tyr Tyr Ser Gln Ile Val Asn Asp Phe Gln Lys Gly Asp Ile Ala  
65 70 75 80

Glu Gly Tyr Ser Val Ser Arg Glu Lys Lys Glu Ser Phe Pro Leu Thr  
85 90 95

Val Thr Ser Ala Gln Lys Asn Pro Thr Ala Phe Tyr Leu Cys Ala Ser  
100 105 110

Ser Pro Gly Ala Leu Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu  
115 120 125

Thr Val Thr Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val  
130 135 140

Phe Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu  
145 150 155 160

Val Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser Trp  
165 170 175

Trp Val Asn Gly Lys Glu Val His Ser Gly Val Cys Thr Asp Pro Gln  
180 185 190

Pro Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu Ser  
195 200 205

Ser Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn His  
210 215 220

Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp  
 225 230 235 240

Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala  
 245 250 255

Trp Gly Arg Ala Asp Cys Gly Phe Thr Ser Glu Ser Tyr Gln Gln Gly  
 260 265 270

Val Leu Ser Ala Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys Ala Thr  
 275 280 285

Leu Tyr Ala Val Leu Val Ser Ala Leu Val Leu Met Ala Met Val Lys  
 290 295 300

Arg Lys Asp Ser Arg Gly  
 305 310

<210> 243  
 <211> 270  
 <212> PRT  
 <213> Homo sapiens

<400> 243

Met Thr Ser Ile Arg Ala Val Phe Ile Phe Leu Trp Leu Gln Leu Asp  
 1 5 10 15

Leu Val Asn Gly Glu Asn Val Glu Gln His Pro Ser Thr Leu Ser Val  
 20 25 30

Gln Glu Gly Asp Ser Ala Val Ile Lys Cys Thr Tyr Ser Asp Ser Ala  
 35 40 45

Ser Asn Tyr Phe Pro Trp Tyr Lys Gln Glu Leu Gly Lys Arg Pro Gln  
 50 55 60

Leu Ile Ile Asp Ile Arg Ser Asn Val Gly Glu Lys Lys Asp Gln Arg  
 65 70 75 80

Ile Ala Val Thr Leu Asn Lys Thr Ala Lys His Phe Ser Leu His Ile  
 85 90 95

Thr Glu Thr Gln Pro Glu Asp Ser Ala Val Tyr Phe Cys Ala Ala Thr  
 100 105 110

Glu Asp Tyr Gln Leu Ile Trp Gly Ala Gly Thr Lys Leu Ile Ile Lys  
 115 120 125

Pro Asp Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser  
 130 135 140

Lys Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln

145 150 155 160

Thr Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys  
165 170 175

Cys Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val  
180 185 190

Ala Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn  
195 200 205

Ser Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser Cys  
210 215 220

Asp Val Lys Leu Val Glu Lys Ser Phe Glu Thr Asp Thr Asn Leu Asn  
225 230 235 240

Phe Gln Asn Leu Ser Val Ile Gly Phe Arg Ile Leu Leu Leu Lys Val  
245 250 255

Ala Gly Phe Asn Leu Leu Met Thr Leu Arg Leu Trp Ser Ser  
260 265 270

<210> 244

<211> 602

<212> PRT

<213> Artificial Sequence

<220>

<223> Native\_WT1\_TCRa\_TCRb

<400> 244

Met Ser Asn Gln Val Leu Cys Cys Val Val Leu Cys Phe Leu Gly Ala  
1 5 10 15

Asn Thr Val Asp Gly Gly Ile Thr Gln Ser Pro Lys Tyr Leu Phe Arg  
20 25 30

Lys Glu Gly Gln Asn Val Thr Leu Ser Cys Glu Gln Asn Leu Asn His  
35 40 45

Asp Ala Met Tyr Trp Tyr Arg Gln Asp Pro Gly Gln Gly Leu Arg Leu  
50 55 60

Ile Tyr Tyr Ser Gln Ile Val Asn Asp Phe Gln Lys Gly Asp Ile Ala  
65 70 75 80

Glu Gly Tyr Ser Val Ser Arg Glu Lys Lys Glu Ser Phe Pro Leu Thr  
85 90 95

Val Thr Ser Ala Gln Lys Asn Pro Thr Ala Phe Tyr Leu Cys Ala Ser  
100 105 110

Ser Pro Gly Ala Leu Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu  
115 120 125

Thr Val Thr Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val  
130 135 140

Phe Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu  
145 150 155 160

Val Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser Trp  
165 170 175

Trp Val Asn Gly Lys Glu Val His Ser Gly Val Cys Thr Asp Pro Gln  
180 185 190

Pro Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu Ser  
195 200 205

Ser Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn His  
210 215 220

Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp  
225 230 235 240

Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala  
245 250 255

Trp Gly Arg Ala Asp Cys Gly Phe Thr Ser Glu Ser Tyr Gln Gln Gly  
260 265 270

Val Leu Ser Ala Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys Ala Thr  
275 280 285

Leu Tyr Ala Val Leu Val Ser Ala Leu Val Leu Met Ala Met Val Lys  
290 295 300

Arg Lys Asp Ser Arg Gly Gly Ser Gly Ala Thr Asn Phe Ser Leu Leu  
305 310 315 320

Lys Gln Ala Gly Asp Val Glu Glu Asn Pro Gly Pro Met Thr Ser Ile  
325 330 335

Arg Ala Val Phe Ile Phe Leu Trp Leu Gln Leu Asp Leu Val Asn Gly  
340 345 350

Glu Asn Val Glu Gln His Pro Ser Thr Leu Ser Val Gln Glu Gly Asp  
355 360 365

Ser Ala Val Ile Lys Cys Thr Tyr Ser Asp Ser Ala Ser Asn Tyr Phe  
370 375 380

Pro Trp Tyr Lys Gln Glu Leu Gly Lys Arg Pro Gln Leu Ile Ile Asp

385 390 395 400

Ile Arg Ser Asn Val Gly Glu Lys Lys Asp Gln Arg Ile Ala Val Thr  
405 410 415

Leu Asn Lys Thr Ala Lys His Phe Ser Leu His Ile Thr Glu Thr Gln  
420 425 430

Pro Glu Asp Ser Ala Val Tyr Phe Cys Ala Ala Thr Glu Asp Tyr Gln  
435 440 445

Leu Ile Trp Gly Ala Gly Thr Lys Leu Ile Ile Lys Pro Asp Ile Gln  
450 455 460

Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser Lys Ser Ser Asp  
465 470 475 480

Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln Thr Asn Val Ser  
485 490 495

Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys Cys Val Leu Asp  
500 505 510

Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val Ala Trp Ser Asn  
515 520 525

Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn Ser Ile Ile Pro  
530 535 540

Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser Cys Asp Val Lys Leu  
545 550 555 560

Val Glu Lys Ser Phe Glu Thr Asp Thr Asn Leu Asn Phe Gln Asn Leu  
565 570 575

Ser Val Ile Gly Phe Arg Ile Leu Leu Leu Lys Val Ala Gly Phe Asn  
580 585 590

Leu Leu Met Thr Leu Arg Leu Trp Ser Ser  
595 600

<210> 245  
<211> 310  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> DLT\_WT1\_TCRb

<400> 245

Met Ser Asn Gln Val Leu Cys Cys Val Val Leu Cys Phe Leu Gly Ala  
1 5 10 15



Asn Thr Val Asp Gly Gly Ile Thr Gln Ser Pro Lys Tyr Leu Phe Arg  
20 25 30

Lys Glu Gly Gln Asn Val Thr Leu Ser Cys Glu Gln Asn Leu Asn His  
35 40 45

Asp Ala Met Tyr Trp Tyr Arg Gln Asp Pro Gly Gln Gly Leu Arg Leu  
50 55 60

Ile Tyr Tyr Ser Gln Ile Val Asn Asp Phe Gln Lys Gly Asp Ile Ala  
65 70 75 80

Glu Gly Tyr Ser Val Ser Arg Glu Lys Lys Glu Ser Phe Pro Leu Thr  
85 90 95

Val Thr Ser Ala Gln Lys Asn Pro Thr Ala Phe Tyr Leu Cys Ala Ser  
100 105 110

Ser Pro Gly Ala Leu Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu  
115 120 125

Thr Val Thr Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val  
130 135 140

Phe Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu  
145 150 155 160

Val Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser Trp  
165 170 175

Trp Val Asn Gly Lys Glu Val His Ser Gly Val Cys Thr Asp Pro Gln  
180 185 190

Pro Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu Ser  
195 200 205

Ser Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn His  
210 215 220

Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp  
225 230 235 240

Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala  
245 250 255

Trp Gly Arg Ala Asp Cys Gly Phe Thr Ser Glu Ser Tyr Gln Gln Gly  
260 265 270

Val Leu Ser Ala Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys Ala Thr  
275 280 285

Leu Tyr Ala Val Leu Val Ser Ala Leu Val Leu Met Ala Met Val Lys

290

295

300

Arg Lys Asp Ser Arg Gly  
305 310

<210> 246  
<211> 270  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> DLT\_WT1\_TCRa

<400> 246

Met Thr Ser Ile Arg Ala Val Phe Ile Phe Leu Trp Leu Gln Leu Asp  
1 5 10 15

Leu Val Asn Gly Glu Asn Val Glu Gln His Pro Ser Thr Leu Ser Val  
20 25 30

Gln Glu Gly Asp Ser Ala Val Ile Lys Cys Thr Tyr Ser Asp Ser Ala  
35 40 45

Ser Asn Tyr Phe Pro Trp Tyr Lys Gln Glu Leu Gly Lys Arg Pro Gln  
50 55 60

Leu Ile Ile Asp Ile Arg Ser Asn Val Gly Glu Lys Lys Asp Gln Arg  
65 70 75 80

Ile Ala Val Thr Leu Asn Lys Thr Ala Lys His Phe Ser Leu His Ile  
85 90 95

Thr Glu Thr Gln Pro Glu Asp Ser Ala Val Tyr Phe Cys Ala Ala Thr  
100 105 110

Glu Asp Leu Thr Leu Ile Trp Gly Ala Gly Thr Lys Leu Ile Ile Lys  
115 120 125

Pro Asp Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser  
130 135 140

Lys Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln  
145 150 155 160

Thr Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys  
165 170 175

Cys Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val  
180 185 190

Ala Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn  
195 200 205

Ser Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser Cys  
210 215 220

Asp Val Lys Leu Val Glu Lys Ser Phe Glu Thr Asp Thr Asn Leu Asn  
225 230 235 240

Phe Gln Asn Leu Ser Val Ile Gly Phe Arg Ile Leu Leu Leu Lys Val  
245 250 255

Ala Gly Phe Asn Leu Leu Met Thr Leu Arg Leu Trp Ser Ser  
260 265 270

<210> 247  
<211> 602  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> DLT\_WT1\_TCRa\_TCRb

<400> 247

Met Ser Asn Gln Val Leu Cys Cys Val Val Leu Cys Phe Leu Gly Ala  
1 5 10 15

Asn Thr Val Asp Gly Gly Ile Thr Gln Ser Pro Lys Tyr Leu Phe Arg  
20 25 30

Lys Glu Gly Gln Asn Val Thr Leu Ser Cys Glu Gln Asn Leu Asn His  
35 40 45

Asp Ala Met Tyr Trp Tyr Arg Gln Asp Pro Gly Gln Gly Leu Arg Leu  
50 55 60

Ile Tyr Tyr Ser Gln Ile Val Asn Asp Phe Gln Lys Gly Asp Ile Ala  
65 70 75 80

Glu Gly Tyr Ser Val Ser Arg Glu Lys Lys Glu Ser Phe Pro Leu Thr  
85 90 95

Val Thr Ser Ala Gln Lys Asn Pro Thr Ala Phe Tyr Leu Cys Ala Ser  
100 105 110

Ser Pro Gly Ala Leu Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu  
115 120 125

Thr Val Thr Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val  
130 135 140

Phe Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu  
145 150 155 160

Val Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser Trp  
165 170 175

Trp Val Asn Gly Lys Glu Val His Ser Gly Val Cys Thr Asp Pro Gln  
180 185 190

Pro Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu Ser  
195 200 205

Ser Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn His  
210 215 220

Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp  
225 230 235 240

Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala  
245 250 255

Trp Gly Arg Ala Asp Cys Gly Phe Thr Ser Glu Ser Tyr Gln Gln Gly  
260 265 270

Val Leu Ser Ala Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys Ala Thr  
275 280 285

Leu Tyr Ala Val Leu Val Ser Ala Leu Val Leu Met Ala Met Val Lys  
290 295 300

Arg Lys Asp Ser Arg Gly Gly Ser Gly Ala Thr Asn Phe Ser Leu Leu  
305 310 315 320

Lys Gln Ala Gly Asp Val Glu Glu Asn Pro Gly Pro Met Thr Ser Ile  
325 330 335

Arg Ala Val Phe Ile Phe Leu Trp Leu Gln Leu Asp Leu Val Asn Gly  
340 345 350

Glu Asn Val Glu Gln His Pro Ser Thr Leu Ser Val Gln Glu Gly Asp  
355 360 365

Ser Ala Val Ile Lys Cys Thr Tyr Ser Asp Ser Ala Ser Asn Tyr Phe  
370 375 380

Pro Trp Tyr Lys Gln Glu Leu Gly Lys Arg Pro Gln Leu Ile Ile Asp  
385 390 395 400

Ile Arg Ser Asn Val Gly Glu Lys Lys Asp Gln Arg Ile Ala Val Thr  
405 410 415

Leu Asn Lys Thr Ala Lys His Phe Ser Leu His Ile Thr Glu Thr Gln  
420 425 430

Pro Glu Asp Ser Ala Val Tyr Phe Cys Ala Ala Thr Glu Asp Leu Thr  
435 440 445

Leu Ile Trp Gly Ala Gly Thr Lys Leu Ile Ile Lys Pro Asp Ile Gln  
 450 455 460

Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser Lys Ser Ser Asp  
 465 470 475 480

Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln Thr Asn Val Ser  
 485 490 495

Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys Cys Val Leu Asp  
 500 505 510

Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val Ala Trp Ser Asn  
 515 520 525

Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn Ser Ile Ile Pro  
 530 535 540

Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser Cys Asp Val Lys Leu  
 545 550 555 560

Val Glu Lys Ser Phe Glu Thr Asp Thr Asn Leu Asn Phe Gln Asn Leu  
 565 570 575

Ser Val Ile Gly Phe Arg Ile Leu Leu Leu Lys Val Ala Gly Phe Asn  
 580 585 590

Leu Leu Met Thr Leu Arg Leu Trp Ser Ser  
 595 600

<210> 248  
 <211> 311  
 <212> PRT  
 <213> Homo sapiens

<400> 248

Met Ser Ile Gly Leu Leu Cys Cys Ala Ala Leu Ser Leu Leu Trp Ala  
 1 5 10 15

Gly Pro Val Asn Ala Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu  
 20 25 30

Lys Thr Gly Gln Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His  
 35 40 45

Glu Tyr Met Ser Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu  
 50 55 60

Ile His Tyr Ser Val Gly Ala Gly Ile Thr Asp Gln Gly Glu Val Pro  
 65 70 75 80

Asn Gly Tyr Asn Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg  
 85 90 95

Leu Leu Ser Ala Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser  
100 105 110

Ser Tyr Val Gly Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg  
115 120 125

Leu Thr Val Leu Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala  
130 135 140

Val Phe Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr  
145 150 155 160

Leu Val Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser  
165 170 175

Trp Trp Val Asn Gly Lys Glu Val His Ser Gly Val Cys Thr Asp Pro  
180 185 190

Gln Pro Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu  
195 200 205

Ser Ser Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn  
210 215 220

His Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu  
225 230 235 240

Trp Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu  
245 250 255

Ala Trp Gly Arg Ala Asp Cys Gly Phe Thr Ser Glu Ser Tyr Gln Gln  
260 265 270

Gly Val Leu Ser Ala Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys Ala  
275 280 285

Thr Leu Tyr Ala Val Leu Val Ser Ala Leu Val Leu Met Ala Met Val  
290 295 300

Lys Arg Lys Asp Ser Arg Gly  
305 310

<210> 249

<211> 274

<212> PRT

<213> Homo sapiens

<400> 249

Met Glu Thr Leu Leu Gly Leu Leu Ile Leu Trp Leu Gln Leu Gln Trp  
1 5 10 15

Val Ser Ser Lys Gln Glu Val Thr Gln Ile Pro Ala Ala Leu Ser Val  
20 25 30

Pro Glu Gly Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala  
35 40 45

Ile Tyr Asn Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr  
50 55 60

Ser Leu Leu Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg  
65 70 75 80

Leu Asn Ala Ser Leu Asp Lys Ser Ser Gly Arg Ser Thr Leu Tyr Ile  
85 90 95

Ala Ala Ser Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg  
100 105 110

Pro Thr Ser Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser  
115 120 125

Leu Ile Val His Pro Tyr Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln  
130 135 140

Leu Arg Asp Ser Lys Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp  
145 150 155 160

Phe Asp Ser Gln Thr Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr  
165 170 175

Ile Thr Asp Lys Cys Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser  
180 185 190

Asn Ser Ala Val Ala Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn  
195 200 205

Ala Phe Asn Asn Ser Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro  
210 215 220

Glu Ser Ser Cys Asp Val Lys Leu Val Glu Lys Ser Phe Glu Thr Asp  
225 230 235 240

Thr Asn Leu Asn Phe Gln Asn Leu Ser Val Ile Gly Phe Arg Ile Leu  
245 250 255

Leu Leu Lys Val Ala Gly Phe Asn Leu Leu Met Thr Leu Arg Leu Trp  
260 265 270

Ser Ser

<210> 250

<211> 607  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> IG4\_NyESO\_TCRa\_TCRb

<400> 250

Met Ser Ile Gly Leu Leu Cys Cys Ala Ala Leu Ser Leu Leu Trp Ala  
1 5 10 15

Gly Pro Val Asn Ala Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu  
20 25 30

Lys Thr Gly Gln Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His  
35 40 45

Glu Tyr Met Ser Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu  
50 55 60

Ile His Tyr Ser Val Gly Ala Gly Ile Thr Asp Gln Gly Glu Val Pro  
65 70 75 80

Asn Gly Tyr Asn Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg  
85 90 95

Leu Leu Ser Ala Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser  
100 105 110

Ser Tyr Val Gly Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg  
115 120 125

Leu Thr Val Leu Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala  
130 135 140

Val Phe Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr  
145 150 155 160

Leu Val Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser  
165 170 175

Trp Trp Val Asn Gly Lys Glu Val His Ser Gly Val Cys Thr Asp Pro  
180 185 190

Gln Pro Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu  
195 200 205

Ser Ser Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn  
210 215 220

His Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu  
225 230 235 240



Trp Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu  
245 250 255

Ala Trp Gly Arg Ala Asp Cys Gly Phe Thr Ser Glu Ser Tyr Gln Gln  
260 265 270

Gly Val Leu Ser Ala Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys Ala  
275 280 285

Thr Leu Tyr Ala Val Leu Val Ser Ala Leu Val Leu Met Ala Met Val  
290 295 300

Lys Arg Lys Asp Ser Arg Gly Gly Ser Gly Ala Thr Asn Phe Ser Leu  
305 310 315 320

Leu Lys Gln Ala Gly Asp Val Glu Glu Asn Pro Gly Pro Met Glu Thr  
325 330 335

Leu Leu Gly Leu Leu Ile Leu Trp Leu Gln Leu Gln Trp Val Ser Ser  
340 345 350

Lys Gln Glu Val Thr Gln Ile Pro Ala Ala Leu Ser Val Pro Glu Gly  
355 360 365

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn  
370 375 380

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu  
385 390 395 400

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala  
405 410 415

Ser Leu Asp Lys Ser Ser Gly Arg Ser Thr Leu Tyr Ile Ala Ala Ser  
420 425 430

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Thr Ser  
435 440 445

Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val  
450 455 460

His Pro Tyr Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp  
465 470 475 480

Ser Lys Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser  
485 490 495

Gln Thr Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp  
500 505 510

Lys Cys Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala

515

520

525

Val Ala Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn  
530 535 540

Asn Ser Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser  
545 550 555 560

Cys Asp Val Lys Leu Val Glu Lys Ser Phe Glu Thr Asp Thr Asn Leu  
565 570 575

Asn Phe Gln Asn Leu Ser Val Ile Gly Phe Arg Ile Leu Leu Leu Lys  
580 585 590

Val Ala Gly Phe Asn Leu Leu Met Thr Leu Arg Leu Trp Ser Ser  
595 600 605

<210> 251  
<211> 311  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> 95LY\_NyESO\_TCRb

<400> 251

Met Ser Ile Gly Leu Leu Cys Cys Ala Ala Leu Ser Leu Leu Trp Ala  
1 5 10 15

Gly Pro Val Asn Ala Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu  
20 25 30

Lys Thr Gly Gln Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His  
35 40 45

Glu Tyr Met Ser Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu  
50 55 60

Ile His Tyr Ser Val Gly Ala Gly Ile Thr Asp Gln Gly Glu Val Pro  
65 70 75 80

Asn Gly Tyr Asn Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg  
85 90 95

Leu Leu Ser Ala Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser  
100 105 110

Ser Tyr Val Gly Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg  
115 120 125

Leu Thr Val Leu Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala  
130 135 140

Val Phe Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr  
 145 150 155 160

Leu Val Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser  
 165 170 175

Trp Trp Val Asn Gly Lys Glu Val His Ser Gly Val Cys Thr Asp Pro  
 180 185 190

Gln Pro Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu  
 195 200 205

Ser Ser Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn  
 210 215 220

His Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu  
 225 230 235 240

Trp Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu  
 245 250 255

Ala Trp Gly Arg Ala Asp Cys Gly Phe Thr Ser Glu Ser Tyr Gln Gln  
 260 265 270

Gly Val Leu Ser Ala Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys Ala  
 275 280 285

Thr Leu Tyr Ala Val Leu Val Ser Ala Leu Val Leu Met Ala Met Val  
 290 295 300

Lys Arg Lys Asp Ser Arg Gly  
 305 310

<210> 252  
 <211> 274  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> 95LY\_NyES0\_TCRa

<400> 252

Met Glu Thr Leu Leu Gly Leu Leu Ile Leu Trp Leu Gln Leu Gln Trp  
 1 5 10 15

Val Ser Ser Lys Gln Glu Val Thr Gln Ile Pro Ala Ala Leu Ser Val  
 20 25 30

Pro Glu Gly Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala  
 35 40 45

Ile Tyr Asn Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr  
 50 55 60

Ser Leu Leu Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg  
65 70 75 80

Leu Asn Ala Ser Leu Asp Lys Ser Ser Gly Arg Ser Thr Leu Tyr Ile  
85 90 95

Ala Ala Ser Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg  
100 105 110

Pro Leu Tyr Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser  
115 120 125

Leu Ile Val His Pro Tyr Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln  
130 135 140

Leu Arg Asp Ser Lys Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp  
145 150 155 160

Phe Asp Ser Gln Thr Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr  
165 170 175

Ile Thr Asp Lys Cys Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser  
180 185 190

Asn Ser Ala Val Ala Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn  
195 200 205

Ala Phe Asn Asn Ser Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro  
210 215 220

Glu Ser Ser Cys Asp Val Lys Leu Val Glu Lys Ser Phe Glu Thr Asp  
225 230 235 240

Thr Asn Leu Asn Phe Gln Asn Leu Ser Val Ile Gly Phe Arg Ile Leu  
245 250 255

Leu Leu Lys Val Ala Gly Phe Asn Leu Leu Met Thr Leu Arg Leu Trp  
260 265 270

Ser Ser

<210> 253  
<211> 607  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> 95LY\_NyESO\_TCRa\_TCRb

<400> 253

Met Ser Ile Gly Leu Leu Cys Cys Ala Ala Leu Ser Leu Leu Trp Ala  
1 5 10 15

Gly Pro Val Asn Ala Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu  
20 25 30

Lys Thr Gly Gln Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His  
35 40 45

Glu Tyr Met Ser Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu  
50 55 60

Ile His Tyr Ser Val Gly Ala Gly Ile Thr Asp Gln Gly Glu Val Pro  
65 70 75 80

Asn Gly Tyr Asn Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg  
85 90 95

Leu Leu Ser Ala Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser  
100 105 110

Ser Tyr Val Gly Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg  
115 120 125

Leu Thr Val Leu Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala  
130 135 140

Val Phe Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr  
145 150 155 160

Leu Val Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser  
165 170 175

Trp Trp Val Asn Gly Lys Glu Val His Ser Gly Val Cys Thr Asp Pro  
180 185 190

Gln Pro Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu  
195 200 205

Ser Ser Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn  
210 215 220

His Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu  
225 230 235 240

Trp Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu  
245 250 255

Ala Trp Gly Arg Ala Asp Cys Gly Phe Thr Ser Glu Ser Tyr Gln Gln  
260 265 270

Gly Val Leu Ser Ala Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys Ala  
275 280 285

Thr Leu Tyr Ala Val Leu Val Ser Ala Leu Val Leu Met Ala Met Val  
290 295 300

Lys Arg Lys Asp Ser Arg Gly Gly Ser Gly Ala Thr Asn Phe Ser Leu  
305 310 315 320

Leu Lys Gln Ala Gly Asp Val Glu Glu Asn Pro Gly Pro Met Glu Thr  
325 330 335

Leu Leu Gly Leu Leu Ile Leu Trp Leu Gln Leu Gln Trp Val Ser Ser  
340 345 350

Lys Gln Glu Val Thr Gln Ile Pro Ala Ala Leu Ser Val Pro Glu Gly  
355 360 365

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn  
370 375 380

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu  
385 390 395 400

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala  
405 410 415

Ser Leu Asp Lys Ser Ser Gly Arg Ser Thr Leu Tyr Ile Ala Ala Ser  
420 425 430

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Leu Tyr  
435 440 445

Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val  
450 455 460

His Pro Tyr Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp  
465 470 475 480

Ser Lys Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser  
485 490 495

Gln Thr Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp  
500 505 510

Lys Cys Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala  
515 520 525

Val Ala Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn  
530 535 540

Asn Ser Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser  
545 550 555 560

Cys Asp Val Lys Leu Val Glu Lys Ser Phe Glu Thr Asp Thr Asn Leu  
565 570 575

Asn Phe Gln Asn Leu Ser Val Ile Gly Phe Arg Ile Leu Leu Leu Lys  
580 585 590

Val Ala Gly Phe Asn Leu Leu Met Thr Leu Arg Leu Trp Ser Ser  
595 600 605

<210> 254  
<211> 311  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> DMF4\_MART\_TCRb

<400> 254

Met Gly Thr Arg Leu Phe Phe Tyr Val Ala Leu Cys Leu Leu Trp Thr  
1 5 10 15

Gly His Met Asp Ala Gly Ile Thr Gln Ser Pro Arg His Lys Val Thr  
20 25 30

Glu Thr Gly Thr Pro Val Thr Leu Arg Cys His Gln Thr Glu Asn His  
35 40 45

Arg Tyr Met Tyr Trp Tyr Arg Gln Asp Pro Gly His Gly Leu Arg Leu  
50 55 60

Ile His Tyr Ser Tyr Gly Val Lys Asp Thr Asp Lys Gly Glu Val Ser  
65 70 75 80

Asp Gly Tyr Ser Val Ser Arg Ser Lys Thr Glu Asp Phe Leu Leu Thr  
85 90 95

Leu Glu Ser Ala Thr Ser Ser Gln Thr Ser Val Tyr Phe Cys Ala Ile  
100 105 110

Ser Glu Val Gly Val Gly Gln Pro Gln His Phe Gly Asp Gly Thr Arg  
115 120 125

Leu Ser Ile Leu Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala  
130 135 140

Val Phe Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr  
145 150 155 160

Leu Val Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser  
165 170 175

Trp Trp Val Asn Gly Lys Glu Val His Ser Gly Val Cys Thr Asp Pro  
180 185 190

Gln Pro Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu  
195 200 205

Ser Ser Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn  
210 215 220

His Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu  
225 230 235 240

Trp Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu  
245 250 255

Ala Trp Gly Arg Ala Asp Cys Gly Phe Thr Ser Glu Ser Tyr Gln Gln  
260 265 270

Gly Val Leu Ser Ala Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys Ala  
275 280 285

Thr Leu Tyr Ala Val Leu Val Ser Ala Leu Val Leu Met Ala Met Val  
290 295 300

Lys Arg Lys Asp Ser Arg Gly  
305 310

<210> 255  
<211> 268  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> DMF4\_MART\_TCRa

<400> 255

Met Leu Leu Glu His Leu Leu Ile Ile Leu Trp Met Gln Leu Thr Trp  
1 5 10 15

Val Ser Gly Gln Gln Leu Asn Gln Ser Pro Gln Ser Met Phe Ile Gln  
20 25 30

Glu Gly Glu Asp Val Ser Met Asn Cys Thr Ser Ser Ser Ile Phe Asn  
35 40 45

Thr Trp Leu Trp Tyr Lys Gln Glu Pro Gly Glu Gly Pro Val Leu Leu  
50 55 60

Ile Ala Leu Tyr Lys Ala Gly Glu Leu Thr Ser Asn Gly Arg Leu Thr  
65 70 75 80

Ala Gln Phe Gly Ile Thr Arg Lys Asp Ser Phe Leu Asn Ile Ser Ala  
85 90 95

Ser Ile Pro Ser Asp Val Gly Ile Tyr Phe Cys Ala Gly Gly Thr Gly  
100 105 110



Asn Gln Phe Tyr Phe Gly Thr Gly Thr Ser Leu Thr Val Ile Pro Asn  
 115 120 125

Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser Lys Ser  
 130 135 140

Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln Thr Asn  
 145 150 155 160

Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys Cys Val  
 165 170 175

Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val Ala Trp  
 180 185 190

Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn Ser Ile  
 195 200 205

Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser Cys Asp Val  
 210 215 220

Lys Leu Val Glu Lys Ser Phe Glu Thr Asp Thr Asn Leu Asn Phe Gln  
 225 230 235 240

Asn Leu Ser Val Ile Gly Phe Arg Ile Leu Leu Leu Lys Val Ala Gly  
 245 250 255

Phe Asn Leu Leu Met Thr Leu Arg Leu Trp Ser Ser  
 260 265

<210> 256  
 <211> 601  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> DMF4\_MART\_TCRa\_TCRb

<400> 256

Met Gly Thr Arg Leu Phe Phe Tyr Val Ala Leu Cys Leu Leu Trp Thr  
 1 5 10 15

Gly His Met Asp Ala Gly Ile Thr Gln Ser Pro Arg His Lys Val Thr  
 20 25 30

Glu Thr Gly Thr Pro Val Thr Leu Arg Cys His Gln Thr Glu Asn His  
 35 40 45

Arg Tyr Met Tyr Trp Tyr Arg Gln Asp Pro Gly His Gly Leu Arg Leu  
 50 55 60

Ile His Tyr Ser Tyr Gly Val Lys Asp Thr Asp Lys Gly Glu Val Ser

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 65  |     | 70  |     | 75  |     | 80  |     |     |     |     |     |     |     |     |     |
| Asp | Gly | Tyr | Ser | Val | Ser | Arg | Ser | Lys | Thr | Glu | Asp | Phe | Leu | Leu | Thr |
|     |     |     | 85  |     |     |     |     |     | 90  |     |     |     |     | 95  |     |
| Leu | Glu | Ser | Ala | Thr | Ser | Ser | Gln | Thr | Ser | Val | Tyr | Phe | Cys | Ala | Ile |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |
| Ser | Glu | Val | Gly | Val | Gly | Gln | Pro | Gln | His | Phe | Gly | Asp | Gly | Thr | Arg |
|     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |
| Leu | Ser | Ile | Leu | Glu | Asp | Leu | Lys | Asn | Val | Phe | Pro | Pro | Glu | Val | Ala |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |
| Val | Phe | Glu | Pro | Ser | Glu | Ala | Glu | Ile | Ser | His | Thr | Gln | Lys | Ala | Thr |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |
| Leu | Val | Cys | Leu | Ala | Thr | Gly | Phe | Tyr | Pro | Asp | His | Val | Glu | Leu | Ser |
|     |     |     | 165 |     |     |     |     |     | 170 |     |     |     |     | 175 |     |
| Trp | Trp | Val | Asn | Gly | Lys | Glu | Val | His | Ser | Gly | Val | Cys | Thr | Asp | Pro |
|     |     | 180 |     |     |     |     |     | 185 |     |     |     |     | 190 |     |     |
| Gln | Pro | Leu | Lys | Glu | Gln | Pro | Ala | Leu | Asn | Asp | Ser | Arg | Tyr | Cys | Leu |
|     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |
| Ser | Ser | Arg | Leu | Arg | Val | Ser | Ala | Thr | Phe | Trp | Gln | Asn | Pro | Arg | Asn |
|     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |
| His | Phe | Arg | Cys | Gln | Val | Gln | Phe | Tyr | Gly | Leu | Ser | Glu | Asn | Asp | Glu |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |
| Trp | Thr | Gln | Asp | Arg | Ala | Lys | Pro | Val | Thr | Gln | Ile | Val | Ser | Ala | Glu |
|     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |
| Ala | Trp | Gly | Arg | Ala | Asp | Cys | Gly | Phe | Thr | Ser | Glu | Ser | Tyr | Gln | Gln |
|     |     | 260 |     |     |     |     |     | 265 |     |     |     |     | 270 |     |     |
| Gly | Val | Leu | Ser | Ala | Thr | Ile | Leu | Tyr | Glu | Ile | Leu | Leu | Gly | Lys | Ala |
|     | 275 |     |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |
| Thr | Leu | Tyr | Ala | Val | Leu | Val | Ser | Ala | Leu | Val | Leu | Met | Ala | Met | Val |
|     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |
| Lys | Arg | Lys | Asp | Ser | Arg | Gly | Gly | Ser | Gly | Ala | Thr | Asn | Phe | Ser | Leu |
| 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |
| Leu | Lys | Gln | Ala | Gly | Asp | Val | Glu | Glu | Asn | Pro | Gly | Pro | Met | Leu | Leu |
|     |     |     | 325 |     |     |     |     |     | 330 |     |     |     |     | 335 |     |
| Glu | His | Leu | Leu | Ile | Ile | Leu | Trp | Met | Gln | Leu | Thr | Trp | Val | Ser | Gly |
|     |     | 340 |     |     |     |     |     | 345 |     |     |     |     | 350 |     |     |

Gln Gln Leu Asn Gln Ser Pro Gln Ser Met Phe Ile Gln Glu Gly Glu  
 355 360 365  
 Asp Val Ser Met Asn Cys Thr Ser Ser Ser Ile Phe Asn Thr Trp Leu  
 370 375 380  
 Trp Tyr Lys Gln Glu Pro Gly Glu Gly Pro Val Leu Leu Ile Ala Leu  
 385 390 395 400  
 Tyr Lys Ala Gly Glu Leu Thr Ser Asn Gly Arg Leu Thr Ala Gln Phe  
 405 410 415  
 Gly Ile Thr Arg Lys Asp Ser Phe Leu Asn Ile Ser Ala Ser Ile Pro  
 420 425 430  
 Ser Asp Val Gly Ile Tyr Phe Cys Ala Gly Gly Thr Gly Asn Gln Phe  
 435 440 445  
 Tyr Phe Gly Thr Gly Thr Ser Leu Thr Val Ile Pro Asn Ile Gln Asn  
 450 455 460  
 Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser Lys Ser Ser Asp Lys  
 465 470 475 480  
 Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln Thr Asn Val Ser Gln  
 485 490 495  
 Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys Cys Val Leu Asp Met  
 500 505 510  
 Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val Ala Trp Ser Asn Lys  
 515 520 525  
 Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn Ser Ile Ile Pro Glu  
 530 535 540  
 Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser Cys Asp Val Lys Leu Val  
 545 550 555 560  
 Glu Lys Ser Phe Glu Thr Asp Thr Asn Leu Asn Phe Gln Asn Leu Ser  
 565 570 575  
 Val Ile Gly Phe Arg Ile Leu Leu Leu Lys Val Ala Gly Phe Asn Leu  
 580 585 590  
 Leu Met Thr Leu Arg Leu Trp Ser Ser  
 595 600

&lt;210&gt; 257

&lt;211&gt; 310

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; DMF5\_MART\_TCRb

&lt;400&gt; 257

Met Arg Ile Arg Leu Leu Cys Cys Val Ala Phe Ser Leu Leu Trp Ala  
1 5 10 15

Gly Pro Val Ile Ala Gly Ile Thr Gln Ala Pro Thr Ser Gln Ile Leu  
20 25 30

Ala Ala Gly Arg Arg Met Thr Leu Arg Cys Thr Gln Asp Met Arg His  
35 40 45

Asn Ala Met Tyr Trp Tyr Arg Gln Asp Leu Gly Leu Gly Leu Arg Leu  
50 55 60

Ile His Tyr Ser Asn Thr Ala Gly Thr Thr Gly Lys Gly Glu Val Pro  
65 70 75 80

Asp Gly Tyr Ser Val Ser Arg Ala Asn Thr Asp Asp Phe Pro Leu Thr  
85 90 95

Leu Ala Ser Ala Val Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser  
100 105 110

Ser Leu Ser Phe Gly Thr Glu Ala Phe Phe Gly Gln Gly Thr Arg Leu  
115 120 125

Thr Val Val Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val  
130 135 140

Phe Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu  
145 150 155 160

Val Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser Trp  
165 170 175

Trp Val Asn Gly Lys Glu Val His Ser Gly Val Cys Thr Asp Pro Gln  
180 185 190

Pro Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu Ser  
195 200 205

Ser Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn His  
210 215 220

Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp  
225 230 235 240

Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala  
245 250 255



165

170

175

Asp Lys Cys Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser  
 180 185 190

Ala Val Ala Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe  
 195 200 205

Asn Asn Ser Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser  
 210 215 220

Ser Cys Asp Val Lys Leu Val Glu Lys Ser Phe Glu Thr Asp Thr Asn  
 225 230 235 240

Leu Asn Phe Gln Asn Leu Ser Val Ile Gly Phe Arg Ile Leu Leu Leu  
 245 250 255

Lys Val Ala Gly Phe Asn Leu Leu Met Thr Leu Arg Leu Trp Ser Ser  
 260 265 270

&lt;210&gt; 259

&lt;211&gt; 604

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; DMF5\_MART\_TCRa\_TCRb

&lt;400&gt; 259

Met Arg Ile Arg Leu Leu Cys Cys Val Ala Phe Ser Leu Leu Trp Ala  
 1 5 10 15

Gly Pro Val Ile Ala Gly Ile Thr Gln Ala Pro Thr Ser Gln Ile Leu  
 20 25 30

Ala Ala Gly Arg Arg Met Thr Leu Arg Cys Thr Gln Asp Met Arg His  
 35 40 45

Asn Ala Met Tyr Trp Tyr Arg Gln Asp Leu Gly Leu Gly Leu Arg Leu  
 50 55 60

Ile His Tyr Ser Asn Thr Ala Gly Thr Thr Gly Lys Gly Glu Val Pro  
 65 70 75 80

Asp Gly Tyr Ser Val Ser Arg Ala Asn Thr Asp Asp Phe Pro Leu Thr  
 85 90 95

Leu Ala Ser Ala Val Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser  
 100 105 110

Ser Leu Ser Phe Gly Thr Glu Ala Phe Phe Gly Gln Gly Thr Arg Leu  
 115 120 125

Thr Val Val Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val  
130 135 140

Phe Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu  
145 150 155 160

Val Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser Trp  
165 170 175

Trp Val Asn Gly Lys Glu Val His Ser Gly Val Cys Thr Asp Pro Gln  
180 185 190

Pro Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu Ser  
195 200 205

Ser Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn His  
210 215 220

Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp  
225 230 235 240

Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala  
245 250 255

Trp Gly Arg Ala Asp Cys Gly Phe Thr Ser Glu Ser Tyr Gln Gln Gly  
260 265 270

Val Leu Ser Ala Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys Ala Thr  
275 280 285

Leu Tyr Ala Val Leu Val Ser Ala Leu Val Leu Met Ala Met Val Lys  
290 295 300

Arg Lys Asp Ser Arg Gly Gly Ser Gly Ala Thr Asn Phe Ser Leu Leu  
305 310 315 320

Lys Gln Ala Gly Asp Val Glu Glu Asn Pro Gly Pro Met Lys Ser Leu  
325 330 335

Arg Val Leu Leu Val Ile Leu Trp Leu Gln Leu Ser Trp Val Trp Ser  
340 345 350

Gln Gln Lys Glu Val Glu Gln Asn Ser Gly Pro Leu Ser Val Pro Glu  
355 360 365

Gly Ala Ile Ala Ser Leu Asn Cys Thr Tyr Ser Asp Arg Gly Ser Gln  
370 375 380

Ser Phe Phe Trp Tyr Arg Gln Tyr Ser Gly Lys Ser Pro Glu Leu Ile  
385 390 395 400

Met Phe Ile Tyr Ser Asn Gly Asp Lys Glu Asp Gly Arg Phe Thr Ala

405

410

415

Gln Leu Asn Lys Ala Ser Gln Tyr Val Ser Leu Leu Ile Arg Asp Ser  
420 425 430

Gln Pro Ser Asp Ser Ala Thr Tyr Leu Cys Ala Val Asn Phe Gly Gly  
435 440 445

Gly Lys Leu Ile Phe Gly Gln Gly Thr Glu Leu Ser Val Lys Pro Asn  
450 455 460

Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser Lys Ser  
465 470 475 480

Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln Thr Asn  
485 490 495

Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys Cys Val  
500 505 510

Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val Ala Trp  
515 520 525

Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn Ser Ile  
530 535 540

Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser Cys Asp Val  
545 550 555 560

Lys Leu Val Glu Lys Ser Phe Glu Thr Asp Thr Asn Leu Asn Phe Gln  
565 570 575

Asn Leu Ser Val Ile Gly Phe Arg Ile Leu Leu Leu Lys Val Ala Gly  
580 585 590

Phe Asn Leu Leu Met Thr Leu Arg Leu Trp Ser Ser  
595 600

&lt;210&gt; 260

&lt;211&gt; 142

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 260

Pro Asn Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser  
1 5 10 15

Lys Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln  
20 25 30

Thr Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys  
35 40 45



Thr Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val  
50 55 60

Ala Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn  
65 70 75 80

Ser Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser Cys  
85 90 95

Asp Val Lys Leu Val Glu Lys Ser Phe Glu Thr Asp Thr Asn Leu Asn  
100 105 110

Phe Gln Asn Leu Ser Val Ile Gly Phe Arg Ile Leu Leu Leu Lys Val  
115 120 125

Ala Gly Phe Asn Leu Leu Met Thr Leu Arg Leu Trp Ser Ser  
130 135 140

<210> 261  
<211> 142  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> cys\_TRAC

<400> 261

Pro Asn Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser  
1 5 10 15

Lys Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln  
20 25 30

Thr Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys  
35 40 45

Cys Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val  
50 55 60

Ala Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn  
65 70 75 80

Ser Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser Cys  
85 90 95

Asp Val Lys Leu Val Glu Lys Ser Phe Glu Thr Asp Thr Asn Leu Asn  
100 105 110

Phe Gln Asn Leu Ser Val Ile Gly Phe Arg Ile Leu Leu Leu Lys Val  
115 120 125

Ala Gly Phe Asn Leu Leu Met Thr Leu Arg Leu Trp Ser Ser  
130 135 140

<210> 262  
<211> 178  
<212> PRT  
<213> Homo sapiens

<400> 262

Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val Phe Glu Pro Ser  
1 5 10 15

Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu Val Cys Leu Ala  
20 25 30

Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser Trp Trp Val Asn Gly  
35 40 45

Lys Glu Val His Ser Gly Val Ser Thr Asp Pro Gln Pro Leu Lys Glu  
50 55 60

Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu Ser Ser Arg Leu Arg  
65 70 75 80

Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn His Phe Arg Cys Gln  
85 90 95

Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp Thr Gln Asp Arg  
100 105 110

Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala Trp Gly Arg Ala  
115 120 125

Asp Cys Gly Phe Thr Ser Glu Ser Tyr Gln Gln Gly Val Leu Ser Ala  
130 135 140

Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys Ala Thr Leu Tyr Ala Val  
145 150 155 160

Leu Val Ser Ala Leu Val Leu Met Ala Met Val Lys Arg Lys Asp Ser  
165 170 175

Arg Gly

<210> 263  
<211> 178  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> cys\_TRBC

<400> 263

Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val Phe Glu Pro Ser  
1 5 10 15

Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu Val Cys Leu Ala  
20 25 30

Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser Trp Trp Val Asn Gly  
35 40 45

Lys Glu Val His Ser Gly Val Cys Thr Asp Pro Gln Pro Leu Lys Glu  
50 55 60

Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu Ser Ser Arg Leu Arg  
65 70 75 80

Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn His Phe Arg Cys Gln  
85 90 95

Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp Thr Gln Asp Arg  
100 105 110

Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala Trp Gly Arg Ala  
115 120 125

Asp Cys Gly Phe Thr Ser Glu Ser Tyr Gln Gln Gly Val Leu Ser Ala  
130 135 140

Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys Ala Thr Leu Tyr Ala Val  
145 150 155 160

Leu Val Ser Ala Leu Val Leu Met Ala Met Val Lys Arg Lys Asp Ser  
165 170 175

Arg Gly

<210> 264  
<211> 444  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> KSQCAR017\_aa

<400> 264

Met Leu Leu Leu Val Thr Ser Leu Leu Leu Cys Glu Leu Pro His Pro  
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Ala Phe Leu Leu Ile Pro Asp Ile Leu Leu Thr Gln Ser Pro Val Ile  
20 25 30

Leu Ser Val Ser Pro Gly Glu Arg Val Ser Phe Ser Cys Arg Ala Ser  
35 40 45

Gln Ser Ile Gly Thr Asn Ile His Trp Tyr Gln Gln Arg Thr Asn Gly

50

55

60

Ser Pro Arg Leu Leu Ile Lys Tyr Ala Ser Glu Ser Ile Ser Gly Ile  
65 70 75 80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser  
85 90 95

Ile Asn Ser Val Glu Ser Glu Asp Ile Ala Asp Tyr Tyr Cys Gln Gln  
100 105 110

Asn Asn Asn Trp Pro Thr Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu  
115 120 125

Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
130 135 140

Gln Val Gln Leu Lys Gln Ser Gly Pro Gly Leu Val Gln Pro Ser Gln  
145 150 155 160

Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Asn Tyr  
165 170 175

Gly Val His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu  
180 185 190

Gly Val Ile Trp Ser Gly Gly Asn Thr Asp Tyr Asn Thr Pro Phe Thr  
195 200 205

Ser Arg Leu Ser Ile Asn Lys Asp Asn Ser Lys Ser Gln Val Phe Phe  
210 215 220

Lys Met Asn Ser Leu Gln Ser Asn Asp Thr Ala Ile Tyr Tyr Cys Ala  
225 230 235 240

Arg Ala Leu Thr Tyr Tyr Asp Tyr Glu Phe Ala Tyr Trp Gly Gln Gly  
245 250 255

Thr Leu Val Thr Val Ser Ala Thr Thr Thr Pro Ala Pro Arg Pro Pro  
260 265 270

Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu  
275 280 285

Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp  
290 295 300

Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly  
305 310 315 320

Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Arg Val Lys Phe  
325 330 335

Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu  
 340 345 350

Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp  
 355 360 365

Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys  
 370 375 380

Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala  
 385 390 395 400

Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys  
 405 410 415

Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr  
 420 425 430

Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg  
 435 440

<210> 265  
 <211> 1328  
 <212> DNA  
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<220>  
 <223> KSQCAR017\_na

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 gtgtcatttt catgccgggc gagtcaatcc atcgggacga atattcactg gtatcagcaa 180  
 aggactaacg gctcaccacg ccttctcatc aagtatgcca gtgagtccat aagtggcatt 240  
 ccctctagat tctcaggatc aggcagtggc acggacttca cattgtcaat taatagcgta 300  
 gaaagtgagg acatagcaga ttattattgc caacaaaaca ataactggcc taccacattc 360  
 ggtgcaggca ccaagcttga gttgaagggg ggtggtggtt ctggaggcgg tgggagcggg 420  
 ggtggtgggt cacaagtga gcttaagcaa agcggaccag gtctggtcca acctagccag 480  
 tcactgtcaa tcacatgtac ggtatccggc tttagtctga caaattatgg cgtccactgg 540  
 gtaaggcaat cccctggaaa gggcctcgag tggttggggg tgatttggag cggaggaaac 600  
 accgactata ataccctttt cacctccaga ctgtccataa acaaggacaa ctctaaaagt 660  
 caggtattct tcaaaatgaa cagtctgcaa agtaatgaca cagcgatata ttattgcgcg 720  
 agagccctta catactacga ttacgagttc gcttattggg gacaaggaac gttggtgacg 780  
 gtgtctgcca caaccactcc tgctcccagg ccaccaacac cggcgcctac catagcgtca 840  
 cagccgctta gtctcaggcc ggaagcgtgt cgccccgcag ccggtggggc ggtccacaca 900

cgcgggctgg atttcgcatg cgatatatac atctgggcac cccttgccgg gacctgcggt 960  
gttttgctct tgtctctcgt aatcacgctg tactgtcggg ttaagttttc aagatctgcg 1020  
gatgccccgg cataccaaca agggcagaat cagttgtaca acgaactgaa cttgggcaga 1080  
cgcgaggagt atgatgtctt ggacaagagg cgggggcgcg acccggaat ggggtggcaaa 1140  
ccacggcgca agaaccaca agaggggctt tacaacgaat tgcagaaaga caagatggcc 1200  
gaggcataca gcgagattgg catgaaagga gagaggagga ggggaaagg gcatgatggc 1260  
ctttatcagg gcctttctac tgccaccaag gacacatacg acgcactgca catgcaggca 1320  
ttgccacc 1328

<210> 266  
<211> 446  
<212> PRT  
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<220>  
<223> KSQCAR1909\_aa

<400> 266

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20 25 30

Leu Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser  
35 40 45

Gln Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly  
50 55 60

Thr Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val  
65 70 75 80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr  
85 90 95

Ile Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln  
100 105 110

Gly Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile  
115 120 125

Thr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
130 135 140

Glu Val Lys Leu Gln Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln  
145 150 155 160

Ser Leu Ser Val Thr Cys Thr Val Ser Gly Val Ser Leu Pro Asp Tyr  
165 170 175

Gly Val Ser Trp Ile Arg Gln Pro Pro Arg Lys Gly Leu Glu Trp Leu  
180 185 190

Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr Tyr Asn Ser Ala Leu Lys  
195 200 205

Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser Lys Ser Gln Val Phe Leu  
210 215 220

Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Ile Tyr Tyr Cys Ala  
225 230 235 240

Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala Met Asp Tyr Trp Gly Gln  
245 250 255

Gly Thr Ser Val Thr Val Ser Ser Thr Thr Thr Pro Ala Pro Arg Pro  
260 265 270

Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro  
275 280 285

Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu  
290 295 300

Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys  
305 310 315 320

Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Leu Arg Val  
325 330 335

Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn  
340 345 350

Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val  
355 360 365

Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg  
370 375 380

Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys  
385 390 395 400

Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg  
405 410 415

Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys  
420 425 430

Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg  
435 440 445

<210> 267  
 <211> 1338  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> KSQCAR1909\_na

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 gtgacaatca gttgccgagc ttcccaggac atatctaaat atttgaattg gtatcagcaa 180  
 aagccagatg gtacggttaa acttcttatac taccacacct ccaggctcca ttctgggggt 240  
 ccgagccgat tctctggatc tggctcaggg accgattatt ctttgactat ttccaatttg 300  
 gagcaggaag acatcgcaac ctatttctgc caacaaggaa atacgctgcc atacacctc 360  
 ggcgggggga ccaaactcga gattactggg ggtgggggga gtggaggagg gggttccggt 420  
 ggagggtgggt cagaagtcaa gctgcaagag agtggggccg ggttggttgc tcctagccaa 480  
 tccttgagtg taacatgcac cgttagcgga gtttcacttc ctgactacgg tgttagctgg 540  
 ataagacagc ccccgaggaa gggctctgaa tggctggggg tcatttgggg cagtgcagcg 600  
 acatattaca acagtgcctt gaaatccagg cttacgatca taaaagacaa tagtaaaagc 660  
 caagtgttcc tcaagatgaa ctctcttcag accgacgaca cagccatcta ctattgcgca 720  
 aaacattatt attatggagg tagttacgct atggactatt ggggccaggg gacttcagtg 780  
 acggtgagta gtaccacgac tccggcaccg agaccaccaa caccagcccc aacaattgcc 840  
 tcacagccct tgagccttag acccgaggcc tgtagggccc ccgcaggagg ggcagttcat 900  
 acgcgaggat tggactttgc atgtgacatc tatatctggg cgccacttgc gggaacttgc 960  
 ggtgtccttt tgctctcatt ggtcattacc ctctattggt tgagagtaaa attttcccg 1020  
 tccgctgatg cgctgcata ccagcaaggt cagaaccaac tctacaatga gcttaacctc 1080  
 ggtagaagag aggaatatga cgtcttgat aagaggagag gccgagacc agaaatgggg 1140  
 ggaaagccgc gccgaagaa tccacaagaa ggtctttaca atgaactgca gaaggacaaa 1200  
 atggccgaag cgtatagcga gataggaatg aaaggcgaac ggagacgggg caaggggcat 1260  
 gacgggcttt accaaggact tagcacagcg acgaaggata catacgacgc actgcatatg 1320  
 caagcgctgc caccgcgc 1338

<210> 268  
 <211> 496  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> KSQCAR010\_aa

<400> 268

Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser  
 1 5 10 15



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|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|-----|------------|------------|
| Val        | Ile        | Met        | Ser<br>20  | Arg        | Gly        | Ala        | Thr        | Gly<br>25  | Gly        | Ala        | Thr        | Thr        | Thr | Thr        | Cys        |
| Ala        | Gly        | Gly<br>35  | Thr        | Gly        | Cys        | Ala        | Gly<br>40  | Ala        | Thr        | Thr        | Thr        | Thr        | Cys | Ala        | Gly        |
| Cys        | Thr        | Thr        | Cys        | Cys        | Thr        | Gly<br>55  | Cys        | Thr        | Ala        | Ala        | Thr        | Cys        | Ala | Gly        | Thr        |
| Gly<br>65  | Cys        | Cys        | Thr        | Cys        | Ala<br>70  | Asp        | Ile        | Gln        | Met        | Thr<br>75  | Gln        | Ser        | Pro | Ser        | Ser<br>80  |
| Leu        | Ser        | Ala        | Ser        | Val<br>85  | Gly        | Asp        | Arg        | Val        | Thr<br>90  | Ile        | Thr        | Cys        | Arg | Ala        | Ser        |
| Gln        | Asp        | Val        | Asn<br>100 | Thr        | Ala        | Val        | Ala        | Trp<br>105 | Tyr        | Gln        | Gln        | Lys        | Pro | Gly        | Lys        |
| Ala        | Pro        | Lys<br>115 | Leu        | Leu        | Ile        | Tyr        | Ser        | Ala        | Ser        | Phe        | Leu        | Tyr        | Ser | Gly        | Val        |
| Pro        | Ser        | Arg        | Phe        | Ser        | Gly        | Ser<br>135 | Arg        | Ser        | Gly        | Thr        | Asp<br>140 | Phe        | Thr | Leu        | Thr        |
| Ile<br>145 | Ser        | Ser        | Leu        | Gln        | Pro<br>150 | Glu        | Asp        | Phe        | Ala        | Thr<br>155 | Tyr        | Tyr        | Cys | Gln        | Gln<br>160 |
| His        | Tyr        | Thr        | Thr        | Pro<br>165 | Pro        | Thr        | Phe        | Gly        | Gln<br>170 | Gly        | Thr        | Lys        | Val | Glu<br>175 | Ile        |
| Lys        | Arg        | Thr        | Gly<br>180 | Ser        | Thr        | Ser        | Gly        | Ser<br>185 | Gly        | Lys        | Pro        | Gly        | Ser | Gly        | Glu        |
| Gly        | Ser        | Glu<br>195 | Val        | Gln        | Leu        | Val        | Glu<br>200 | Ser        | Gly        | Gly        | Gly        | Leu        | Val | Gln        | Pro        |
| Gly        | Gly<br>210 | Ser        | Leu        | Arg        | Leu        | Ser<br>215 | Cys        | Ala        | Ala        | Ser        | Gly<br>220 | Phe        | Asn | Ile        | Lys        |
| Asp<br>225 | Thr        | Tyr        | Ile        | His        | Trp<br>230 | Val        | Arg        | Gln        | Ala        | Pro<br>235 | Gly        | Lys        | Gly | Leu        | Glu<br>240 |
| Trp        | Val        | Ala        | Arg        | Ile<br>245 | Tyr        | Pro        | Thr        | Asn        | Gly<br>250 | Tyr        | Thr        | Arg        | Tyr | Ala        | Asp        |
| Ser        | Val        | Lys        | Gly<br>260 | Arg        | Phe        | Thr        | Ile        | Ser<br>265 | Ala        | Asp        | Thr        | Ser        | Lys | Asn        | Thr        |
| Ala        | Tyr<br>275 | Leu        | Gln        | Met        | Asn        | Ser        | Leu<br>280 | Arg        | Ala        | Glu        | Asp        | Thr<br>285 | Ala | Val        | Tyr        |

Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Val Trp  
290 295 300

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Thr Thr Thr Pro Ala Pro  
305 310 315 320

Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu  
325 330 335

Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg  
340 345 350

Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly  
355 360 365

Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Leu  
370 375 380

Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly  
385 390 395 400

Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr  
405 410 415

Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys  
420 425 430

Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys  
435 440 445

Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg  
450 455 460

Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala  
465 470 475 480

Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg  
485 490 495

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gcgaccacga ctacctgcgc aggttgtacc acctgttgta ctggatgtac tgctgcgaca 180  
tgtgcgggga cgggttgttg cacttgtgctg gacatacaaa tgacgcaaag cccgtccagc 240

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ctgtctgcat cagttgggga tagagtgacg attacatgta gagcaagcca ggatgttaac      300
accgccgtag cgtggtacca acaaaaacca ggtaaagccc cgaagctgct catatactcc      360
gccagctttc tgtattcagg cgttcccagt cggttcagcg gcagcagatc agggacggat      420
tttacgtca ctatctcttc ccttcagcct gaagattttg ctacctatta ttgtcagcag      480
cattacacga ctcccccaac ttttggtcag gggactaaag ttgaaatcaa acgaacgggc      540
tccacctcag gtagcggtaa gccaggcagc ggagaagggt ctgaagtcca gttggttgag      600
agtggaggcg gtcttggtca acccggtggc agcttgcgac tgagctgtgc agcgtctggc      660
ttcaacataa aagatactta tattcattgg gtaagacagg ctcttggtaa agggctggaa      720
tgggtggcac gaatatatcc gactaacggt tataccagat acgccgattc tgttaagggc      780
aggttcacia taagcgccga cacaagtaag aatacggcgt atctgcagat gaattcactt      840
cgagctgaag acacagcggt atactactgc tccaggtggg gtggggatgg tttttatgcg      900
atggacgttt ggggtcaagg aacactggta actgttagtt ctaccacgac acctgctcct      960
aggcccccca caccggcacc tacgatcgct tcccagccgc ttagcctccg cccggaggca     1020
tgccggcccc ctgcgggggg agcggtagat actcgcgggg tggacttcgc ttgcgacatc     1080
tacatttggg caccactggc aggcacatgt ggcgttctgt tgcttagtct ggttattaca     1140
ctgtattgcc tgcgagttaa attctccgc agcgtgatg cggccgccta tcagcaaggt     1200
caaaaccagc tgtataatga gcttaatttg ggacgccgag aagagtatga cgtccttgac     1260
aagaggcgcg ggcgcgatcc ggagatgggt ggtaaaccgc gccggaaaaa ccccaggaa     1320
ggcctttaca atgagctcca aaaagataaa atggcagagg catactctga aataggaatg     1380
aagggcgaga gacgccgggg taaggacac gatggccttt atcaagggt tagtacagcc     1440
acgaaggata cgtatgacgc tctgcacatg caggctcttc ccccgaga                     1488

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<210> 270  
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<212> DNA  
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<400> 270
cggaggatct tatgctgaac                                     20

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<210> 271  
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<400> 271
ccttatcttc agtcacatgc                                     20

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<210> 272  
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ccatgactat cctcatagag                                     20

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gaaccctgac catgggcctg 20

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<210> 276  
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<400> 276  
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ccgctcgatg gtggtgggca 20

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cagaaatggc agcatgtgtt 20

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<400> 279  
gcactacagt ggatcaccgc 20

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<400> 280  
ggtagacact tgtcttgttt 20

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<400> 281  
tggcagcatg tgtaggaag 20

<210> 282  
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<400> 282  
aggcccatgg tcagggttca 20

<210> 283  
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<400> 283  
gttcagcatg acaactgctt 20

<210> 284  
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<400> 284  
caatggagga gaacagtgag 20

<210> 285  
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<400> 285  
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<210> 286  
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<400> 286  
agtggatcac cgcaggccca 20

<210> 287  
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<400> 287  
ctgacaggtg accgatgtac 20

<210> 288

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<400> 288  
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<210> 289  
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<400> 290  
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<400> 291  
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<400> 298  
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<400> 299  
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<210> 300  
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<400> 300  
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<400> 301  
ggaaacttgg ccactctatg 20

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<400> 302  
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<400> 303  
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<210> 304  
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<400> 304  
ccactctatg aggatagtcac  
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<210> 305  
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<400> 813

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Leu Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser  
35 40 45

Gln Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly  
50 55 60

Thr Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val  
65 70 75 80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr  
85 90 95

Ile Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln  
100 105 110

Gly Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile  
115 120 125

Thr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
130 135 140

Glu Val Lys Leu Gln Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln  
145 150 155 160

Ser Leu Ser Val Thr Cys Thr Val Ser Gly Val Ser Leu Pro Asp Tyr  
165 170 175

Gly Val Ser Trp Ile Arg Gln Pro Pro Arg Lys Gly Leu Glu Trp Leu  
180 185 190

Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr Tyr Asn Ser Ala Leu Lys  
195 200 205

Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser Lys Ser Gln Val Phe Leu  
210 215 220

Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Ile Tyr Tyr Cys Ala  
225 230 235 240

Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala Met Asp Tyr Trp Gly Gln  
245 250 255

Gly Thr Ser Val Thr Val Ser Ser Thr Thr Thr Pro Ala Pro Arg Pro  
260 265 270

Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro  
275 280 285

Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu  
290 295 300

Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys  
305 310 315 320

Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Leu Arg Val  
325 330 335

Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn  
340 345 350

Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val  
355 360 365

Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg  
370 375 380

Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys  
 385 390 395 400

Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg  
 405 410 415

Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys  
 420 425 430

Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg  
 435 440 445

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 acatattaca acagtgcctt gaaatccagg cttacgatca taaaagacaa tagtaaaagc 660  
 caagtgttcc tcaagatgaa ctctcttcag accgacgaca cagccatcta ctattgcgca 720  
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 ggaaagccgc gccgaagaa tccacaagaa ggtctttaca atgaactgca gaaggacaaa 1200  
 atggccgaag cgtatagcga gataggaatg aaaggcgaac ggagacgggg caaggggcat 1260  
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caagcgctgc caccgcgc

1338

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Ser Leu Leu Met Trp Ile Thr Gln  
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<210> 816  
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<212> PRT  
<213> Human

&lt;400&gt; 816

Pro Asn Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser  
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Lys Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln  
20 25 30

Thr Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys  
35 40 45

Thr Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val  
50 55 60

Ala Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn  
65 70 75 80

Ser Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser Cys  
85 90 95

Asp Val Lys Leu Val Glu Lys Ser Phe Glu Thr Asp Thr Asn Leu Asn  
100 105 110

Phe Gln Asn Leu Ser Val Ile Gly Phe Arg Ile Leu Leu Leu Lys Val  
115 120 125

Ala Gly Phe Asn Leu Leu Met Thr Leu Arg Leu Trp Ser Ser  
130 135 140

<210> 817  
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<212> PRT  
<213> Human

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Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val Phe Glu Pro Ser  
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Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu Val Cys Leu Ala

20

25

30

Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser Trp Trp Val Asn Gly  
 35 40 45

Lys Glu Val His Ser Gly Val Ser Thr Asp Pro Gln Pro Leu Lys Glu  
 50 55 60

Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu Ser Ser Arg Leu Arg  
 65 70 75 80

Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn His Phe Arg Cys Gln  
 85 90 95

Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp Thr Gln Asp Arg  
 100 105 110

Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala Trp Gly Arg Ala  
 115 120 125

Asp Cys Gly Phe Thr Ser Glu Ser Tyr Gln Gln Gly Val Leu Ser Ala  
 130 135 140

Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys Ala Thr Leu Tyr Ala Val  
 145 150 155 160

Leu Val Ser Ala Leu Val Leu Met Ala Met Val Lys Arg Lys Asp Ser  
 165 170 175

Arg Gly

<210> 818

<211> 274

<212> PRT

<213> Artificial Sequence

<220>

<223> 95L TCRA

<400> 818

Met Glu Thr Leu Leu Gly Leu Leu Ile Leu Trp Leu Gln Leu Gln Trp  
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Val Ser Ser Lys Gln Glu Val Thr Gln Ile Pro Ala Ala Leu Ser Val  
 20 25 30

Pro Glu Gly Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala  
 35 40 45

Ile Tyr Asn Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr  
 50 55 60

Ser Leu Leu Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 65  |     |     |     | 70  |     |     |     | 75  |     |     |     | 80  |     |     |     |
| Leu | Asn | Ala | Ser | Leu | Asp | Lys | Ser | Ser | Gly | Arg | Ser | Thr | Leu | Tyr | Ile |
|     |     |     | 85  |     |     |     |     | 90  |     |     |     | 95  |     |     |     |
| Ala | Ala | Ser | Gln | Pro | Gly | Asp | Ser | Ala | Thr | Tyr | Leu | Cys | Ala | Val | Arg |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     | 110 |     |     |     |
| Pro | Leu | Tyr | Gly | Gly | Ser | Tyr | Ile | Pro | Thr | Phe | Gly | Arg | Gly | Thr | Ser |
|     |     |     | 115 |     |     |     | 120 |     |     |     | 125 |     |     |     |     |
| Leu | Ile | Val | His | Pro | Tyr | Ile | Gln | Asn | Pro | Asp | Pro | Ala | Val | Tyr | Gln |
|     |     |     | 130 |     |     |     | 135 |     |     |     | 140 |     |     |     |     |
| Leu | Arg | Asp | Ser | Lys | Ser | Ser | Asp | Lys | Ser | Val | Cys | Leu | Phe | Thr | Asp |
|     |     |     | 145 |     |     |     | 150 |     |     |     | 155 |     |     | 160 |     |
| Phe | Asp | Ser | Gln | Thr | Asn | Val | Ser | Gln | Ser | Lys | Asp | Ser | Asp | Val | Tyr |
|     |     |     | 165 |     |     |     |     |     |     |     | 170 |     |     | 175 |     |
| Ile | Thr | Asp | Lys | Cys | Val | Leu | Asp | Met | Arg | Ser | Met | Asp | Phe | Lys | Ser |
|     |     |     | 180 |     |     |     |     |     |     |     | 185 |     |     | 190 |     |
| Asn | Ser | Ala | Val | Ala | Trp | Ser | Asn | Lys | Ser | Asp | Phe | Ala | Cys | Ala | Asn |
|     |     |     | 195 |     |     |     | 200 |     |     |     | 205 |     |     |     |     |
| Ala | Phe | Asn | Asn | Ser | Ile | Ile | Pro | Glu | Asp | Thr | Phe | Phe | Pro | Ser | Pro |
|     |     |     | 210 |     |     |     | 215 |     |     |     | 220 |     |     |     |     |
| Glu | Ser | Ser | Cys | Asp | Val | Lys | Leu | Val | Glu | Lys | Ser | Phe | Glu | Thr | Asp |
|     |     |     | 225 |     |     |     | 230 |     |     |     | 235 |     |     | 240 |     |
| Thr | Asn | Leu | Asn | Phe | Gln | Asn | Leu | Ser | Val | Ile | Gly | Phe | Arg | Ile | Leu |
|     |     |     | 245 |     |     |     |     |     |     |     | 250 |     |     | 255 |     |
| Leu | Leu | Lys | Val | Ala | Gly | Phe | Asn | Leu | Leu | Met | Thr | Leu | Arg | Leu | Trp |
|     |     |     | 260 |     |     |     |     |     |     |     | 265 |     |     | 270 |     |

Ser Ser

|       |                     |
|-------|---------------------|
| <210> | 819                 |
| <211> | 311                 |
| <212> | PRT                 |
| <213> | Artificial Sequence |

<220>  
<223> 95L TCRb

<400> 819

Met Ser Ile Gly Leu Leu Cys Cys Ala Ala Leu Ser Leu Leu Trp Ala  
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Gly Pro Val Asn Ala Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu

20

25

30

Lys Thr Gly Gln Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His  
35 40 45

Glu Tyr Met Ser Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu  
50 55 60

Ile His Tyr Ser Val Gly Ala Gly Ile Thr Asp Gln Gly Glu Val Pro  
65 70 75 80

Asn Gly Tyr Asn Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg  
85 90 95

Leu Leu Ser Ala Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser  
100 105 110

Ser Tyr Val Gly Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg  
115 120 125

Leu Thr Val Leu Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala  
130 135 140

Val Phe Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr  
145 150 155 160

Leu Val Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser  
165 170 175

Trp Trp Val Asn Gly Lys Glu Val His Ser Gly Val Cys Thr Asp Pro  
180 185 190

Gln Pro Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu  
195 200 205

Ser Ser Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn  
210 215 220

His Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu  
225 230 235 240

Trp Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu  
245 250 255

Ala Trp Gly Arg Ala Asp Cys Gly Phe Thr Ser Glu Ser Tyr Gln Gln  
260 265 270

Gly Val Leu Ser Ala Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys Ala  
275 280 285

Thr Leu Tyr Ala Val Leu Val Ser Ala Leu Val Leu Met Ala Met Val  
290 295 300



Lys Arg Lys Asp Ser Arg Gly  
305 310

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<212> PRT  
<213> Artificial Sequence

<220>  
<223> P2A linker sequence

<400> 820

Gly Ser Gly Ala Thr Asn Phe Ser Leu Leu Lys Gln Ala Gly Asp Val  
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Glu Glu Asn Pro Gly Pro  
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<210> 821  
<211> 607  
<212> PRT  
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<220>  
<223> 95L IG4 TCR

<400> 821

Met Ser Ile Gly Leu Leu Cys Cys Ala Ala Leu Ser Leu Leu Trp Ala  
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Gly Pro Val Asn Ala Gly Val Thr Gln Thr Pro Lys Phe Gln Val Leu  
20 25 30

Lys Thr Gly Gln Ser Met Thr Leu Gln Cys Ala Gln Asp Met Asn His  
35 40 45

Glu Tyr Met Ser Trp Tyr Arg Gln Asp Pro Gly Met Gly Leu Arg Leu  
50 55 60

Ile His Tyr Ser Val Gly Ala Gly Ile Thr Asp Gln Gly Glu Val Pro  
65 70 75 80

Asn Gly Tyr Asn Val Ser Arg Ser Thr Thr Glu Asp Phe Pro Leu Arg  
85 90 95

Leu Leu Ser Ala Ala Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser  
100 105 110

Ser Tyr Val Gly Asn Thr Gly Glu Leu Phe Phe Gly Glu Gly Ser Arg  
115 120 125

Leu Thr Val Leu Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala  
130 135 140

Val Phe Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr  
145 150 155 160

Leu Val Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser  
165 170 175

Trp Trp Val Asn Gly Lys Glu Val His Ser Gly Val Cys Thr Asp Pro  
180 185 190

Gln Pro Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu  
195 200 205

Ser Ser Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn  
210 215 220

His Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu  
225 230 235 240

Trp Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu  
245 250 255

Ala Trp Gly Arg Ala Asp Cys Gly Phe Thr Ser Glu Ser Tyr Gln Gln  
260 265 270

Gly Val Leu Ser Ala Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys Ala  
275 280 285

Thr Leu Tyr Ala Val Leu Val Ser Ala Leu Val Leu Met Ala Met Val  
290 295 300

Lys Arg Lys Asp Ser Arg Gly Gly Ser Gly Ala Thr Asn Phe Ser Leu  
305 310 315 320

Leu Lys Gln Ala Gly Asp Val Glu Glu Asn Pro Gly Pro Met Glu Thr  
325 330 335

Leu Leu Gly Leu Leu Ile Leu Trp Leu Gln Leu Gln Trp Val Ser Ser  
340 345 350

Lys Gln Glu Val Thr Gln Ile Pro Ala Ala Leu Ser Val Pro Glu Gly  
355 360 365

Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn  
370 375 380

Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu  
385 390 395 400

Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala  
405 410 415

Ser Leu Asp Lys Ser Ser Gly Arg Ser Thr Leu Tyr Ile Ala Ala Ser  
420 425 430

Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Pro Leu Tyr  
435 440 445

Gly Gly Ser Tyr Ile Pro Thr Phe Gly Arg Gly Thr Ser Leu Ile Val  
450 455 460

His Pro Tyr Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp  
465 470 475 480

Ser Lys Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser  
485 490 495

Gln Thr Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp  
500 505 510

Lys Cys Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala  
515 520 525

Val Ala Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn  
530 535 540

Asn Ser Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser  
545 550 555 560

Cys Asp Val Lys Leu Val Glu Lys Ser Phe Glu Thr Asp Thr Asn Leu  
565 570 575

Asn Phe Gln Asn Leu Ser Val Ile Gly Phe Arg Ile Leu Leu Leu Lys  
580 585 590

Val Ala Gly Phe Asn Leu Leu Met Thr Leu Arg Leu Trp Ser Ser  
595 600 605

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<210> 823  
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cgcccagagc ggagaagcaa cacccttcca gccactactc tgttgctatg aaacaaaaga 120  
ctaggaaggt aagcgaagca ggaagtccc cctttcagcc ccagatggct ttggctcccg 180

|             |            |             |             |            |             |      |
|-------------|------------|-------------|-------------|------------|-------------|------|
| gtccccgacgc | tcacgcccac | ctgccacccc  | ttattggtta  | gctccttcgt | gccttgcatc  | 240  |
| ccttcgtcca  | tttctttcc  | cgtttccact  | ttcgttcccc  | ggagagtcca | ggcccccat   | 300  |
| ttctctccga  | gtccaattcc | tttctgacgt  | gctgcgagcg  | ggccttcctc | tggggccatt  | 360  |
| gtgtactgtg  | gaggacgtgg | acggggcggc  | agcgcgagga  | ggacggggcg | ctcccgtcca  | 420  |
| ttggctgcac  | ccgctgtcgg | gcagtgcgcg  | gttgcgcggc  | attggctcag | cggcgcccgg  | 480  |
| ccccgcccc   | gccccgccc  | gccccgagcc  | tgggtcctgt  | gtgtgccaca | ggggtggggt  | 540  |
| gtccagcgag  | cggctctctc | ctcctgctag  | tgctgctgcg  | gcgtcccgcg | gcctccccga  | 600  |
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| agagaactac gtctcttctg ccagactctt tgcactctt tgaatcagga agtatgttct     | 12180 |
| agtcacttat aatttcctag ctctaagctc agtacctggc atatagtagt tcaagcaatt    | 12240 |
| tccacctgat aaagcaataa attcccacat ggaattacag ttttaataa agcaaatgag     | 12300 |
| aaaaaagttc agataaagaa tagtgaatta aatatttgta aaacataaac gtttctgtca    | 12360 |
| aagaatattt atgtggaatg tctggaaaaa aaatacctga cagaaatttt gtatgtatca    | 12420 |
| gttaaaaagt tctctagtag aggaaatgaa tttgaagcta cctgggtccc aaactagaag    | 12480 |
| aaaataatgt atcagctgtg gacatgttac accaagtttt atgttgctga cattaaacta    | 12540 |
| tgcctgctgt agaattgttg tggcaatatt cttggatttt atgtatactt gtgatagtat    | 12600 |

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| ttttggttta | aataatttaa | aaacatatt   | ctttttcaag | caggatttgt | taaataattg | 12660 |
| gcatatttgg | gacttaatct | ttagggcaga  | atgaactctg | attatactaa | cgtatcacgt | 12720 |
| attaaaaagg | gggtaaaaaa | agctctgtca  | gccattttca | ctatactttg | taaagatttg | 12780 |
| ctgttattta | gtaaatgaga | tgacatctta  | cctttccaag | gactttcttg | gagataaaaa | 12840 |
| tactattatt | actttttaac | taagcctata  | cctttggatt | attaatttga | atacttgata | 12900 |
| ctttagagtt | caattgtaaa | aataaaggaa  | ataatacaat | caaactat   | gtgaaaaaaa | 12960 |
| aatagcagtc | aaccaatcca | ttaatttgtt  | ggttatgtac | tcagcaattc | tgctttaagg | 13020 |
| cattgtttac | aaagaggaag | aatataagat  | catgccctgg | gttaatgtga | aaaacatctt | 13080 |
| aaattagagg | ctgatggttg | gaaatttgaa  | ccagattttt | ctctccatct | actccctctg | 13140 |
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| cccaaagtgc | tggggttaca | ggtgtgagcc  | actgcgctg  | gccaatctt  | ttaagaaagt | 13500 |
| aagtgtgata | gtgtgtataa | gagtaccctt  | taaatttgtt | agttccttga | tttattgtgt | 13560 |
| tcgttatgct | tgaagttatt | atagttcgat  | tgtttatttg | tcccaggcac | ttttatgggt | 13620 |
| gcccagaggc | agatccatgt | tttatgtccc  | ctgaaactta | tatgatttga | aagaatcttt | 13680 |
| ctaaatataa | agaatacgaa | agtacaaaca  | tcaaatgtt  | tatgaaagt  | aatttttttt | 13740 |
| agaatgagaa | aatccacaa  | agcactatag  | tttttaaaaa | gccggtatat | ctacaagtga | 13800 |
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| gttaccgcat | atacttcag  | ttttgggtgt  | catgaaacac | attcaaattg | ccagatttga | 13920 |
| attggatttc | actgttccca | tggtaggcta  | gtcagcattg | tgggcacctc | aggtatttct | 13980 |
| aaaacatct  | tttaaaaaaa | gttctattac  | agatttaaaa | aatattgtat | ttatttaagg | 14040 |
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| caggaggctt | tagccaatta | tgtttaagat  | attatactct | taggccgggt | acggtggcgc | 14640 |
| acacctgtaa | tcccagcact | ttggggaggcc | gaggcgggtg | gatcacaagg | tcaggagttc | 14700 |

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| tgaacccggg  | aggcagaggt  | tgacgtgagc  | tgagatcgtg  | tcactacact  | ccagcctggg  | 14880 |
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| gagaaggccc  | atttcctcat  | ttttatttaa  | aaaaaaagac  | tatcagtgat  | atacagtgat  | 15600 |
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| ctcagtgaga  | atgagtactt  | taaaatctac  | attgatagcc  | ttatgaaaaa  | gtcaaaacgg  | 16080 |
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| actgaatact  | gtgggcagtt  | gtaacacaa   | ggttaagtatg | tgtgtatcta  | aatataccta  | 16500 |
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| gtaatcccag  | cactttgaga  | ggctgagggtg | ggcggatcac  | ttgaggtcgg  | gagttcgaga  | 16620 |
| ccagcctgac  | caacatggag  | aaaccctgtc  | tctactaaaa  | atacaaaatt  | agcccggcgt  | 16680 |
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| gatcatcaat  | attattgtct | tccacctcca  | catcttattc | cactggaagg  | tcacacaata | 17280 |
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| atagtagcta  | gttcatgaga | cagtattctc  | aatgttttaa | tagacttcat  | tttcagagtc | 17880 |
| attttaggtt  | tacaggaaaa | ttattcagaa  | attacagttc | ccataaatct  | cctctgcca  | 17940 |
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| agacatagtt  | tgattgaatc | tgcggatgtg  | gtagatgtgg | gtgggagatg  | taacttccat | 18780 |
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| tgtgtcaaca | ataaatgcgg | attgttatac | atttaagggg | agatgagaaa  | aaatttaggt | 21000 |
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| cccgaat    | aaagcaat   | tacataaat  | taataaatag | aaagataaag  | aagtaa     | 21120 |
| cattcaatac | atttcctagt | tacttaggtc | ctgtgtagaa | ggagagaggt  | agaaattata | 21180 |
| gtcacataat | tgtatatgct | actgaagtag | atatttaggg | cacagtga    | gtcttttgcc | 21240 |
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| catagaaaaa | ttagctctag | aagaagctag | cctgtattta | aaaggcttca  | ggagcatccg | 22260 |
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| ggctctaaaa | tgtgttcaat | tttttcttgt  | gaatatgttt | ctgcaaaatt | atcctctcat | 175200 |
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| aggacaaaaa  | ctttgtgttc  | tctatctccc  | ataacatctt  | actgtatttg | atatatacta  | 177300     |        |
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| gataccctgt  | aattgaaaaa | tgaacataag  | tgataaagta | gcttctttga  | cttgaatatg  | 184020 |
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| ccagcacttt  | gggagggtga | ggtaggcaga | tcacttgagg  | ccaggagttt | gagaccagcc  | 186840 |
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| cctttacaaa  | cacattaata | actgtacttg | tcgtgtaccc  | aaggcctttg | ctaaagtcac  | 187140 |
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| ctctagttct  | ttgaatgttc | ctgtcagaat | tgtaatggaa  | ttattgaaag | ttcaataatt  | 187260 |
| tttcttcttt  | tatgtgtctg | agcttattth | tattacaagg  | aaatthtaag | ttacagaagc  | 187320 |
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| agcaaaactc tgtcttgtgc atcttcagat gtgttaataa gaaaaatatg attaaagaaa  | 189720 |
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| gaataaacta tatgggataa tctaaactat tctttgacat taaattatat gtatttgagg  | 190560 |
| tgattttcca gttaaaggct gtgaactctt tttgctacac agaaaatcct gttttcatag  | 190620 |

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| agtttgcatg  | gggtgtgttg  | tacgtatgta | tgtatgtata  | tgtgtgcatg  | tgtgtgtttc  | 190800 |
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| aaacctgctg  | agtgaatttc  | tttataagca | gcaagagaaa  | atcaattatt  | acttttggtta | 190920 |
| gatgctgtgt  | attttgatta  | atgaagtctt | gctgctgata  | tcgacctttc  | tttaaagtca  | 190980 |
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| catagtctca  | ttgaacattc  | aaaacctcct | ggctccagta  | gccggccatc  | ctcaggacag  | 191100 |
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| caaaaatatt  | tccaaaacca  | tactatcaaa | taaatgttag  | tgattcatta  | gtctctacct  | 191220 |
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| gtaactattt  | aatattatta  | tttacatatg | tatatattat  | ataatattat  | ttgtttaata  | 191760 |
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| aaatgttata  | tggaatctgc  | tgataactaa | taacagttcc  | agtctgtctt  | gtagtgata   | 191940 |
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| acaatgagtc attccaaatc ttagttactt aaatttcctg atgctaagtt caagatagtt   | 193020 |
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| gaattgcagg cgtgaaccac tgcacccagc ccttttatga ctttcattgt gacaattatt   | 193980 |
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| ttgagtattg ttttgcaaag tatttaatac attatactgt ctaaagaaat gtaaaagatt   | 194160 |
| cattatagca gttctcccta agaattgact ttattaagaa tatgctataa atgctaaagt   | 194220 |
| ttaactcagt agtttttagaa ttataaaagg taaataaaag ggactgcaa atttaacaa    | 194280 |
| actctgtgag cattttaccg ttgttttcgt gtgtcagtct gtaaaatctt agtctggtgt   | 194340 |
| aatgatacat cttttctaaa cattattgag atttggtttc ctgaggaaat aattatttct   | 194400 |
| aattaaagac aaatgatagg tgtcttagga agagagggtg aattttctca tgatattata   | 194460 |
| ctaagtacct aatcaagcct acatttttat tccagcattc agtgccctgc acagttgggc   | 194520 |
| caggttcagc ttttcagcct tatagctcat tgacttctat atagtagtga ttgtcaaatt   | 194580 |
| gcagtgtcct atgtctctgc aaaagtcct cagtactgtt gtaggatcaa gggggcaggg    | 194640 |
| caagcagagg cagggtctct attcttcttt tatctctttt ctattttagg ccttcattga   | 194700 |
| agatttcttt tggcagaata gtaatgccta cagctgtaca tgagccctca gttttggaca   | 194760 |

|             |             |             |            |             |            |        |
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| ccaatcccaa  | ggctaccact  | agtgtatatg  | tgcttttttc | catattacaa  | aaaagtactt | 194880 |
| cctttttgtc  | ggtagattga  | agtagacacc  | cctacctcta | ctttgtgcca  | ggtcagggct | 194940 |
| caagaacaaa  | agcaaagact  | gtatttaagt  | cctgctcata | accataacta  | ggtgtctttg | 195000 |
| tgcagtgcac  | aaaatacagt  | gtttttgtca  | gtctttatgc | tacctttatt  | ttgctgtttc | 195060 |
| tctaacctgt  | aatctcactt  | gaagatctta  | tctaattcta | aagtcacagt  | tcaactctga | 195120 |
| aagctagcca  | aattgacttt  | attaaagata  | aatggaagtt | ttattcttgg  | atccagaaat | 195180 |
| gcaactgcat  | aagtaaaata  | tgaagaaaat  | ccagatcatt | agcaacaatt  | gttgaaaagg | 195240 |
| ttttaggggt  | ttgtttgact  | acaatcttta  | taaagcatca | tcatgatgag  | aaagcgcag  | 195300 |
| ccatttgggg  | cttgaactca  | cagaattcaa  | atctgaggag | tgaagtcctc  | cacagtactc | 195360 |
| agcctttgtg  | gagaccactg  | tgctcagatg  | ttgaagcatt | aatgggctag  | agcagcaatt | 195420 |
| ctcaaattgt  | gttctccgga  | ttcacaacat  | tagcatctcc | tggggatttg  | ctttaaaatg | 195480 |
| aaaattcaag  | acttaataaa  | tcagaaaccc  | tgggaatggg | actcagtggg  | cacaagcctt | 195540 |
| cctggtgatt  | ctgatgcatg  | ttcaagtttg  | aaatatactg | ggctggggta  | ttctcaaaaa | 195600 |
| agtgaacca   | ggatgataaa  | gtaaaactaga | gtggattaaa | ggaactgaga  | attggctcag | 195660 |
| agaagagaag  | accttggatg  | tagacgagag  | ttgtcttcag | ttacagtatt  | tgggaaagaa | 195720 |
| ggattatatt  | ttttccatgt  | cacttcagaa  | ggtaggagta | agatcaatgg  | gaaatgttaa | 195780 |
| gaatgaaaat  | tgcatctatc  | ctacaaagaa  | gtttcctaaa | tcattacgaa  | ttgttctacc | 195840 |
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| tgggtgacag  | aacaagacgc  | tctctcaaaa  | aaaaaaaaaa | aaaaatccat  | acttcctcct | 196500 |
| cctaatacatt | gataggtatc  | cttccttttt  | ttttgttgtt | ttttgtttta  | gtttgttttt | 196560 |
| gttttttgcc  | tacgagtatg  | taagttcaca  | tttatgtaaa | atttatttct  | catagtatat | 196620 |
| gctatttata  | accattttcc  | agttaatatg  | tcatgagcaa | tgtatatagt  | tgttaaatat | 196680 |
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| catttcctcc  | cagtcttccc  | ttagtttttt  | ataatctctc | aaataagtac  | taaattttca | 196800 |
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| atcatttcag tttgttttaa tcctgtggat ataataat tcttttagtt ttgaagaatt    | 196980 |
| tattttagaa aagctttatt actttacaaa gttttcttat caggggacaa ttgtaatta   | 197040 |
| ttatactctg tgtagcaatt gtgttatatc taatctcttt ccctttaaag cctgaatgct  | 197100 |
| tccaacttta taatttgctt tagagagccc agttttaccct atggctgaca aatagcaaac | 197160 |
| ttaggatatg atgcaagata gaacatgata atgattcaaa acagaacat aagcaggtat   | 197220 |
| ttttgctatt tataataata ttttgagtgt atctagtacc ttttaaataa tatagtttaa  | 197280 |
| tggaagaata tagtacagtg gtatatatta tagtctatgt atggctctga agtgtatcca  | 197340 |
| gagtttactt ttttaacctta aataaataga tgattagaaa tagcattaag tgtatttgac | 197400 |
| aaacctataa atttcccgtg aagaaataag caagtgtatg tattgtaaaa gacagtcttt  | 197460 |
| cctctgactt aatgttgtat caggggcatt tagagtctac aagaacctaa agtaaatttt  | 197520 |
| atgaaatata tagtgcaaaa tctttgggaa atttcaaaat ttatgctgaa atcaccatat  | 197580 |
| ttgtttcctt tggtttctct aacaaaccaa aagatcaaca ttgtctcatg cgatttatct  | 197640 |
| tctcattcct tcctaaaaat atttctggaa aattaatgga gctggaccac caattcttca  | 197700 |
| aatattgaaa actcaaaatc taagagttat tttaatcttg aaattaatga aaacaagtta  | 197760 |
| aagagccttg tctaagtcaa aaagtcctca gcaaagctgg tgataattat tatactcaa   | 197820 |
| aaaagggtgaa aagataatgg cttcaaatcc tgccttacat tatccagtca aaaactaata | 197880 |
| taaaaatagt caatatatta gcctatgttt tcatcgtaa taatcaaata cttacatatt   | 197940 |
| attaatgtct aaggagtggt aagttaaaac aatgaaattc aatcggtct tctttaatat   | 198000 |
| ttacaatttt tgctttacat tagcaattca ggtttctact attttaaaat tgtgaacaat  | 198060 |
| ctgaatttgt gtggatttat ttgtggtagt taaaagtaaa tattaagtgc aagctatttg  | 198120 |
| ccaagagtgt tctaggtgct atgtttgatt taggaggtat aattcaaaca tgagaagtag  | 198180 |
| gaaaagatcc ctcagatccc tgtcctcaga agaattgacg gtactttcag agatatcgac  | 198240 |
| aacataaaag tactatgaga ggaaagcagc ggtgcctgag gtgggagtga tagctagata  | 198300 |
| aattattagt gaaccaaggt tttcagatgt aatgtttgtc tactcaaaaa gggaatcaat  | 198360 |
| tctctaagaa tttaaaccca tataatacta tgaatgttaa accagttggg tttatggtat  | 198420 |
| taatgattta atacagcaac actgacaaaa tctctttttc tccctttagt tccagttagt  | 198480 |
| aaatatcata ttttttacta tttcagatgc cccgtctctg actgccaagg tggtaaatta  | 198540 |
| tcacatttta ctgtatatc aaatgttaat tactaaatgg cataatttta acaattaaat   | 198600 |
| ttactatttt gtgagccaac taacaacaat gatgttaagc ataggatgcc caatataata  | 198660 |
| ataagcacct tgtatgtaca gaagatttta catgcacaaa ttcatttaat ccacacaaat  | 198720 |
| ccgaagtttc aggtctgttt taaaaatttt gtatgccccaa tgcttagaga gatcaagtaa | 198780 |
| tttgataata ctcttatagg gaggatccaa acccatgggt ttatgtatat ttgtttgttt  | 198840 |
| gttttttgct tttacttttag aacagtttct atacattgta caggttggat tgggacagaa | 198900 |

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|-------------|-------------|-------------|------------|-------------|-------------|--------|
| aataaaat    | agaagctgtt  | tttattgtca  | acaatcttat | aattaaatat  | atctgcaaaa  | 198960 |
| tataaatata  | aaacaagtag  | tctttattat  | ctggaaataa | atgtgaagag  | catattttaag | 199020 |
| caagtgatga  | ataaattaga  | aaatatgcag  | gcagtaaaaa | atggttaata  | tcagtcatga  | 199080 |
| atttaatttt  | ctaattttatt | tttgaaagcc  | ataatcttca | taaaataatt  | ctagaatttt  | 199140 |
| tttcttcagc  | caatcaacag  | agatcatcct  | tttaatttga | aacttgtttt  | tattttttaag | 199200 |
| atccctttgt  | tgatctagca  | agtgccaag   | ttcctttgcc | tcctgctaga  | aggttaccag  | 199260 |
| gtgaaaatgt  | caaaaactaac | agaacatcac  | aggactatga | tcagcttcct  | tcatgttcag  | 199320 |
| gtgagttggc  | tagtaacagt  | aacaataata  | ccttattgtt | atatagtgt   | ttttgctttt  | 199380 |
| cagaacacct  | tcttattcat  | tatataat    | aatgaatgtt | gtccacccaa  | gaaagtattt  | 199440 |
| tgatcatcat  | tgcattttgc  | ttataaagct  | ggcattgtga | gaggaatggc  | tacagaactg  | 199500 |
| cctcattgaa  | aagaaaaata  | ttaagcttaa  | tattatcttt | gatcactcag  | tgagagccact | 199560 |
| tctttgattc  | aaaatgtatt  | cattatctaa  | ctttcatttt | gtctcagatt  | taggaaatga  | 199620 |
| ggaaaatctc  | ttgtacatt   | gcaatgctgt  | tttatagcaa | tctctatgga  | attaatactg  | 199680 |
| gcaccaaact  | attcattttc  | ttagcttatt  | tagggctcca | accagtgtctg | cactgacagc  | 199740 |
| catcaaatag  | aaggatgtac  | catttttgta  | aatacaagtc | atactcacia  | ccgttaataa  | 199800 |
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| taaagtatta  | acactttgga  | gataagaatg  | ttgtctgggt | cactgcttgt  | cctagttagt  | 200040 |
| tcaggctact  | ataacacaat  | accatagact  | ggattgccta | aacagcaaac  | atttatatct  | 200100 |
| cacagttctg  | gaagctggga  | agtccaagat  | caagctacca | acattcagtg  | tctagttagg  | 200160 |
| gccctcttcc  | aggttcatag  | ataactgcct  | tcatactgtg | tcctcacaag  | ggcaaggag   | 200220 |
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| caaatcacia accttgttga aatatccccg aatagccatt tgccagaagc ctgaactggc   | 201360 |
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| tcaaataattt caatctactc cctaaattct ctttaciaac acagtcaacc acagaaaata  | 201480 |
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| acatgtctct tctatctatg tgtgaggttt tacaaatttc tcagtttgct tttctcttgt   | 202200 |
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| ttaaaagacc ttattgccaa ataagccaat ctattataa aataaaggag atctcattac    | 202440 |
| aaaatgaatt aatttcaata ctaccattag ttactagct taaaggagaa ttgttggaa     | 202500 |
| gttgaatata ttttatatta ggaccaagta ttcaatagtt tatctctgtt aagatctcaa   | 202560 |
| ttggaaatcg tggcttctc tcacgcaagg gttggaattt taaggtagat tatgtgtctt    | 202620 |
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| tccttaciaa tctaagatta aataaccatt ttctggatat atttaaaaca gtgtatgtga   | 202800 |
| agattttttt atttgtaatt tagtttatta aatcatgtcc ttctataggc agtatttctt   | 202860 |
| tttttttgag atggagtctc gctctgtcgc ccaggctgga gtgcagtggc gtgatctcag   | 202920 |
| ctcccagcaa tttccacctc ccaggttcaa gtagtttca tgcctcagct tcccagtag     | 202980 |
| ctgggattac aggcattcac catcatgccc ggctaatttt tgtattttta gtagagatgg   | 203040 |

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| gcctcctaaa  | gtgctgggat | tacaggcttg | agccactgcg  | cgtggccagc  | agtatttcta  | 203160 |
| tagaggcagt  | aattcaacct | tatggcataa | aatgaatcc   | cagaattaat  | tgatccaact  | 203220 |
| tctagtagaa  | taacaaagtt | aatcataatg | tgtttgagat  | ttctttttatt | ccttgatcaa  | 203280 |
| ttaagtgtat  | ttatatTTTT | ttctaattct | gttattgtta  | tagttgagaa  | ggtcagtagt  | 203340 |
| ctaagagttt  | tgatatctgt | ccaagaatt  | gtcactgggt  | tactaagtaa  | atttagacag  | 203400 |
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| ttttttttga  | gacgagtctc | actctgttgc | ccaggctaga  | gtgcagtggc  | acgatcttgg  | 203580 |
| ctcactgcaa  | cctccacctc | ctgggtttta | gcagttctgc  | ctcagcctcc  | tgagtagctg  | 203640 |
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| atattcatgt  | tatttttttt | aataatgtat | attttatatt  | atctgtttat  | aattatacat  | 203940 |
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| tgaaatatat  | atatgtgtgt | gtgtgtgtat | atatatgtgt  | gtgtgtgtgt  | atatatatat  | 204120 |
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| ctgcgcctgg  | cctgaaatag | atactattaa | tgtcaattta  | tagaagaaag  | aaagacataa  | 204480 |
| caaagtttag  | taactaactc | aagctgctgc | agaaagtgtg  | agcctgatag  | aatttacatc  | 204540 |
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| gttttagaaa  | tagtaaaact | gtactagaaa | tttcaaaatc  | tagattctga  | ttattttttca | 204780 |
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