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, wherein the at least two endogenous target genes are *SOCS1* 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, and wherein the modified immune effector cell is a T cell or tumor infiltrating lymphocyte.

2. The modified immune effector cell of claim 1, 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,

optionally 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; or

optionally 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; or

optionally wherein the protein is a Transcription activator-like effector nuclease (TALEN), a zinc-finger nuclease, or a meganuclease.

3. The modified immune effector cell of claim 2, 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,

optionally wherein the Cas protein is a Cas9 protein, and optionally wherein the Cas protein is a wild-type Cas protein comprising two enzymatically active domains, and capable of inducing double stranded DNA breaks; or

optionally wherein the Cas protein is a Cas nickase mutant comprising one enzymatically active domain and capable of inducing single stranded DNA breaks; or

optionally 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, and optionally 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).

4. The modified immune effector cell of claim 3,

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; or

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; or.

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; or

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.

5. The modified immune effector cell of claim 2,

wherein the gene-regulating system comprises at least one *SOCS1*-targeting siRNA or shRNA molecule comprising 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 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; or

wherein the gene-regulating system comprises at least one *SOCS1*-targeting siRNA or shRNA molecule comprising 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 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; or

wherein the gene-regulating system comprises at least one *SOCS1*-targeting siRNA or shRNA comprising 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 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.

6. The modified immune effector cell of claim 2,

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; or

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.

7. The modified immune effector cell of any one of claims 1-6, 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, optionally 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.

8. A modified immune effector cell, comprising reduced expression and/or function of at least two endogenous genes, wherein the reduced expression and/or function of the at least two endogenous genes enhances an effector function of the immune effector cell, wherein the at least two endogenous genes are *SOCS1* and *ZC3H12A*, and wherein the modified immune effector cell is a T cell or tumor infiltrating lymphocyte.

9. A modified immune effector cell, comprising an inactivating mutation in at least two endogenous genes, wherein the at least two endogenous genes are *SOCS1* and *ZC3H12A,* and wherein the modified immune effector cell is a T cell or tumor infiltrating lymphocyte, optionally 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, optionally wherein the deletion is a partial or complete deletion of the two or more endogenous target genes, optionally wherein the inactivating mutation is a frame shift mutation, and optionally wherein the inactivating mutation reduces the expression and/or function of the two or more endogenous target genes.

10. 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, wherein the at least two endogenous target genes are *SOCS1* and *ZC3H12A,* and wherein the modified immune effector cell is a T cell or tumor infiltrating lymphocyte, optionally wherein the at least two nucleic acid inhibitors reduce the expression and/or function of the two or more endogenous target genes, optionally 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 unmodified or control immune effector cell or 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.

11. The modified immune effector cell of any one of claims 8-10, wherein the inactivating mutation or nucleic acid inhibitors substantially inhibits the expression of the two or more endogenous target genes or the inactivating mutation or nucleic acid inhibitors substantially inhibits the function of the two or more endogenous target genes, optionally wherein the inactivating mutation or nucleic acid inhibitors enhances one or more effector functions of the modified immune effector cell, and optionally wherein the one or more effector functions are enhanced compared to an unmodified or control immune effector cell.

12. The modified immune effector cell of any one of claims 1-11, further comprising an exogenous transgene expressing an immune activating molecule, optionally 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.

13. The modified immune effector cell of any one of claims 1-12, wherein the effector function is selected from cell proliferation, cell viability, tumor infiltration, cytotoxicity, anti-tumor immune responses, and/or resistance to exhaustion.

14. The modified immune effector cell of any one of claims 1-13, further comprising an engineered immune receptor displayed on the cell surface, optionally wherein the engineered immune receptor is a chimeric antigen receptor (CAR) comprising an antigen-binding domain, a transmembrane domain, and an intracellular signaling domain, or optionally wherein the engineered immune receptor is an engineered T cell receptor (TCR).

15. The modified immune effector cell of claim 14, 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.

16. A composition, comprising the modified immune effector cell of any one of claims 1-15, optionally comprising at least 1 x 10^4, 1 x 10^5, 1 x 10^6, 1 x 10^7, 1 x 10^8, 1 x 10^9, 1 x 10^{10}, 1 x 10^{11}, or more modified immune effector cells and optionally comprising a pharmaceutically acceptable carrier or diluent.

17. A gene-regulating system capable of reducing expression of at least two endogenous target genes in a cell, wherein the at least two endogenous target genes are *SOCS1* and *ZC3H12A,* comprising (i) a nucleic acid molecule; (ii) an enzymatic protein; or (iii) a nucleic acid molecule and an enzymatic protein.

18. The gene-regulating system of claim 17, wherein the system comprises at least two guide RNA (gRNA) nucleic acid molecules and a Cas endonuclease, optionally wherein the system comprises at least one *SOCS1*-targeting gRNA molecule, at least one *ZC3H12A*-targeting gRNA molecule, and a Cas endonuclease;

optionally 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; or

optionally 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; or

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; or

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.

19. The gene-regulating system of claim 18, wherein the Cas endonuclease is a Cas9 protein,

optionally wherein the Cas endonuclease is a wild-type Cas protein comprising two enzymatically active domains, and capable of inducing double stranded DNA breaks; or

optionally wherein the Cas endonuclease is a Cas nickase mutant comprising one enzymatically active domain and capable of inducing single stranded DNA breaks; or

optionally wherein the Cas endonuclease 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, and optionally 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).

20. The gene-regulating system of claim 17, 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, optionally wherein the system comprises at least one *SOCS1*-targeting siRNA or shRNA molecule and at least one *ZC3H12A*-targeting siRNA or shRNA molecule, and

optionally wherein the at least one *SOCS1*-targeting siRNA or shRNA molecule comprises 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 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; or

optionally wherein the at least one *SOCS1*-targeting siRNA or shRNA molecule comprises 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 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; or

optionally wherein the at least one *SOCS1*-targeting siRNA or shRNA comprises 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 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.

21. The gene-regulating system of claim 20, 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, optionally 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).

22. The gene-regulating system of any one of claims 17-19, 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.

23. A kit comprising the gene-regulating system of any one of claims 17-22.

24. 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 *SOCS1* and *ZC3H12A*.

25. 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*SOCS1* and *ZC3H12A* .

26. A method of producing a modified immune effector cell, wherein the modified immune effector cell is a T cell or tumor infiltrating lymphocyte, 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, wherein the at least two endogenous genes are *SOCS1* and *ZC3H12A,* optionally wherein the gene-regulating system is selected from any one of claims 17-22, and optionally further comprising introducing a polynucleotide sequence encoding an engineered immune receptor selected from a CAR and a TCR; or

introducing an inactivating mutation in at least two endogenous target genes in an immune effector cell; or

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.

27. Use of the modified immune effector cells of any one of claims 1-15, or the composition of claim 16, in the preparation on a medicament for treating a disease or disorder in a subject,

optionally wherein the disease or disorder is a cell proliferative disorder, an inflammatory disorder, or an infectious disease; or

optionally wherein the disease or disorder is a cancer or a viral infection, optionally wherein the cancer is selected from a leukemia, a lymphoma, or a solid tumor, optionally wherein the solid tumor is a melanoma, a pancreatic tumor, a bladder tumor, or a lung tumor or metastasis, optionally wherein the cancer is a PD1 resistant or insensitive cancer, and optionally wherein the subject has previously been treated with a PD1 inhibitor or a PDL1 inhibitor.

28. The use of claim 27, wherein the modified immune effector cells are autologous to the subject.

29. The use of claim 27 or 28, wherein the subject has not undergone lymphodepletion prior to administration of the modified immune effector cells or compositions thereof.

30. A method of killing a cancerous cell comprising exposing the cancerous cell to a modified immune effector cell according to any one of claims 1-15 or the composition of claim 16, 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 exposure is *in vitro* or *ex vivo*.

31. 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, wherein the at least two endogenous genes are *SOCS1* and *ZC3H12A,* and wherein the modified immune effector cell is a T cell or tumor infiltrating lymphocyte.

32. The composition of any one of claims 1-15, the method of any one of claims 26, 30, or 31, or the use of any one of claims 27-29, wherein the modified immune effector cell is a T cell.

33. The composition of any one of claims 1-15, the method of any one of claims 26, 30, or 31, or the use of any one of claims 27-29, wherein the modified immune effector cell is a tumor infiltrating lymphocyte.