

CLAIMS

1. A genetically modified mammalian cell comprising a transgene at a sustained transgene expression locus (STEL) in the genome, wherein the transgene is expressed at a detectable level, optionally wherein the mammalian cell is a human cell.
2. The genetically modified cell of claim 1, wherein the expression level of the transgene does not change more than 40%, more than 30%, more than 20%, or more than 10% (i) over five or more, ten or more, or 15 or more passages, or (ii) as the cell state changes, wherein the cell state is optionally state of pluripotency and/or differentiation.
3. The genetically modified cell of claim 1 or 2, wherein the STEL is selected from the gene loci listed in Table 1.
4. The genetically modified cell of claim 3, wherein the STEL is a gene locus having a mean normalized expression of more than 3.30, more than 3.50, more than 3.75, more than 4.00, more than 4.10, more than 4.20, more than 4.30, more than 4.50, more than 4.60, more than 4.70 as set forth in Table 1.
5. The genetically modified cell of claim 3, wherein the STEL is at a gene that encodes a protein involved with one or more of: ribonucleoprotein complex formation, focal adhesion, cell-substrate adherens junction, cell-substrate junction, cell anchoring, extracellular exosome, extracellular vesicle, intracellular organelle, anchoring junction, RNA binding, nucleic acid binding (e.g., rRNA or mRNA binding), and protein binding.
6. The genetically modified cell of claim 3, wherein the STEL is
a gene encoding a ribosomal protein, optionally (i) an *RPL* gene selected from *RPL13A*, *RPLP0*, *RPL10*, *RPL13*, *RPS18*, *RPL3*, *RPLP1*, *RPL15*, *RPL41*, *RPL11*, *RPL32*, *RPL18A*, *RPL19*, *RPL28*, *RPL29*, *RPL9*, *RPL8*, *RPL6*, *RPL18*, *RPL7*, *RPL7A*, *RPL21*, *RPL37A*, *RPL12*, *RPL5*, *RPL34*, *RPL35A*, *RPL30*, *RPL24*, *RPL39*, *RPL37*, *RPL14*, *RPL27A*, *RPLP2*, *RPL23A*, *RPL26*, *RPL36*, *RPL35*, *RPL23*, *RPL4*, and *RPL22*; or (ii) a *RPS* gene selected from *RPS2*, *RPS19*, *RPS14*, *RPS3A*, *RPS12*, *RPS3*, *RPS6*, *RPS23*, *RPS27A*, *RPS8*, *RPS4X*, *RPS7*, *RPS24*, *RPS27*, *RPS15A*, *RPS9*, *RPS28*, *RPS13*, *RPSA*, *RPS5*, *RPS16*, *RPS25*, *RPS15*, *RPS20*, and *RPS11*;

a gene encoding a mitochondria protein, optionally selected from *MT-CO1*, *MT-CO2*, *MT-ND4*, *MT-ND1*, and *MT-ND2*;

a gene encoding an actin protein, optionally selected from *ACTG1* and *ACTB*;

a gene encoding a eukaryotic translation factor, optionally selected from *EEF1A1*, *EEF2*, and *EIF1*;

a gene encoding a histone, such as *H3F3A* and *H3F3B*; or

a gene selected from *FTL*, *FTH1*, *TPT1*, *TMSB10*, *GAPDH*, *PTMA*, *GNB2L1*, *NACA*, *YBX1*, *NPM1*, *FAU*, *UBA52*, *HSP90AB1*, *MYL6*, *SERF2*, and *SRP14*.

7. The genetically modified cell of claim 3, wherein the STEL is a *GAPDH* gene.
8. The genetically modified cell of claim 3, wherein the STEL is a ribosomal protein gene.
9. The genetically modified cell of claim 8, wherein the STEL is a ribosomal protein L (*RPL*) gene, optionally selected from *RPL13A*, *RPL7*, and *RPLP0* genes.
10. The genetically modified cell of any one of claims 1-9, wherein the cell is a pluripotent stem cell (PSC).
11. The genetically modified mammalian cell of claim 10, wherein the PSC is a human embryonic stem cell (ESC) or a human induced PSC (iPSC).
12. The genetically modified cell of any one of claims 1-9, wherein the cell is a differentiated cell.
13. The genetically modified cell of claim 12, wherein the differentiated cell is derived from a human PSC, optionally selected from a human ESC and a human iPSC.
14. The genetically modified mammalian cell of claim 12 or 13, wherein the differentiated cell is
 - a human immune cell, optionally selected from a T cell, a T cell expressing a chimeric antigen receptor (CAR), a suppressive T cell, a myeloid cell, a dendritic cell, and an immunosuppressive macrophage;

a cell in the human nervous system, optionally selected from dopaminergic neuron, a microglial cell, an oligodendrocyte, an astrocyte, a cortical neuron, a spinal or oculomotor neuron, an enteric neuron, a Placode-derived cell, a Schwann cell, and a trigeminal or sensory neuron;

a cell in the human cardiovascular system, optionally selected from a cardiomyocyte, an endothelial cell, and a nodal cell;

a cell in the human metabolic system, optionally selected from a hepatocyte, a cholangiocyte, and a pancreatic beta cell, or

a cell in the human ocular system, optionally selected from a retinal pigment epithelial cell, a photoreceptor cone cell, a photoreceptor rod cell, a bipolar cell, and a ganglion cell.

15. The genetically modified cell of any one of the preceding claims, wherein the transgene is inserted into the 3' untranslated region of the gene locus.

16. The genetically modified cell of any one of the preceding claims, wherein the transgene sequence is linked in frame to the STEL gene sequence through a coding sequence for a self-cleaving peptide, or linked to the STEL gene sequence through an internal ribosomal entry site (IRES).

17. The genetically modified cell of any one of the preceding claims, wherein the transgene encodes a therapeutic protein, an immunomodulatory protein, a reporter protein, or a safety switch signal.

18. The genetically modified cell of any one of the preceding claims, wherein the genome of the cell further comprises an exogenous suicide gene, optionally in a STEL locus in the genome, wherein the exogenous suicide gene, once activated, causes apoptosis of the cell.

19. The genetically modified cell of claim 18, wherein the suicide gene is Herpes simplex virus (HSV) thymidine kinase (TK) gene.

20. A pharmaceutical composition comprising the genetically modified cell of any one of claims 1-19 and a pharmaceutically acceptable carrier.

21. A method of treating a human patient in need thereof, comprising introducing the genetically modified cell of any one of claims 1-19 to the patient, wherein the cell is a human cell.
22. The method of claim 21, wherein the human patient is
in need of graft transplantation, or
has inflammation, optionally neuroinflammation, an autoimmune disease, or cancer.
23. A genetically modified mammalian cell of any one of claims 1-19 for use in the method of claim 21 or 22.
24. Use of a genetically modified mammalian cell of any one of claims 1-19 for the manufacture of a medicament for use in the method of claim 21 or 22.
25. A method of generating the genetically modified mammalian cell of any one of claims 1-19, comprising
providing a cultured mammalian cell, and
introducing said transgene into a STEL site in the genome of the cultured cell.
26. The method of claim 25, wherein the introducing step is performed through CRISPR gene editing.