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(54) Title of the Invention: Allograft tolerance without the need for systemic immune suppression

(51) INT CL: ***C12N 5/10*** (2006.01) ***A61K 35/545*** (2015.01) ***A61K 38/17*** (2006.01) ***A61K 39/395*** (2006.01)
A61L 27/38 (2006.01) ***A61P 37/06*** (2006.01) ***C07K 14/47*** (2006.01) ***C12N 5/071*** (2010.01)
C12N 15/09 (2006.01) ***C12N 15/12*** (2006.01)

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WO 2016/141480 A1 **WO 2016/081924 A1**
WO 2014/022423 A1
YOLCU ES ET AL, "Induction of Tolerance to Cardiac Allografts Using Donor SplenocytesEngineered to Display on Their Surface an Exogenous Fas Ligand Protein", THE JOURNAL OF IMMUNOLOGHY, 181 (2):931-939; 15 July 2008 (15-07-2008); ISSN:0022-1767 *whole document*
ZHAO L ET AL, "Heterelogous Expression of Mutated HLA-G Decreases Immunogencity of Human Embryonic Stem Cells and Their Epidermal Derivatives, STEM CELL RESEARCH, 13(2):342-354; September 2014(09-2014); ISSN:1873-5061 *whole document*
RONG Z ET AL, "An Effective Approach to Prevent Immune Rejectionof Human ESC-Derived Allografts", CELL STEM CELL, 14(1):121-130; January 2014 (01-2014); ISSN: 1934-5909 *whole document*

(58) Field of Search:
As for published application 2588249 A viz:
INT CL **A61K, A61L, A61P, C07K, C12N**
Other: **CAPlus; Biosis; Medline; Agricola; Scopus; Questal-Orbit; Google Scholar**
updated as appropriate

Additional Fields
Other: **WPI, EPODOC, Patent Fulltext, BIOSIS, MEDLINE**

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GB 2588249 B

FIG. 1A

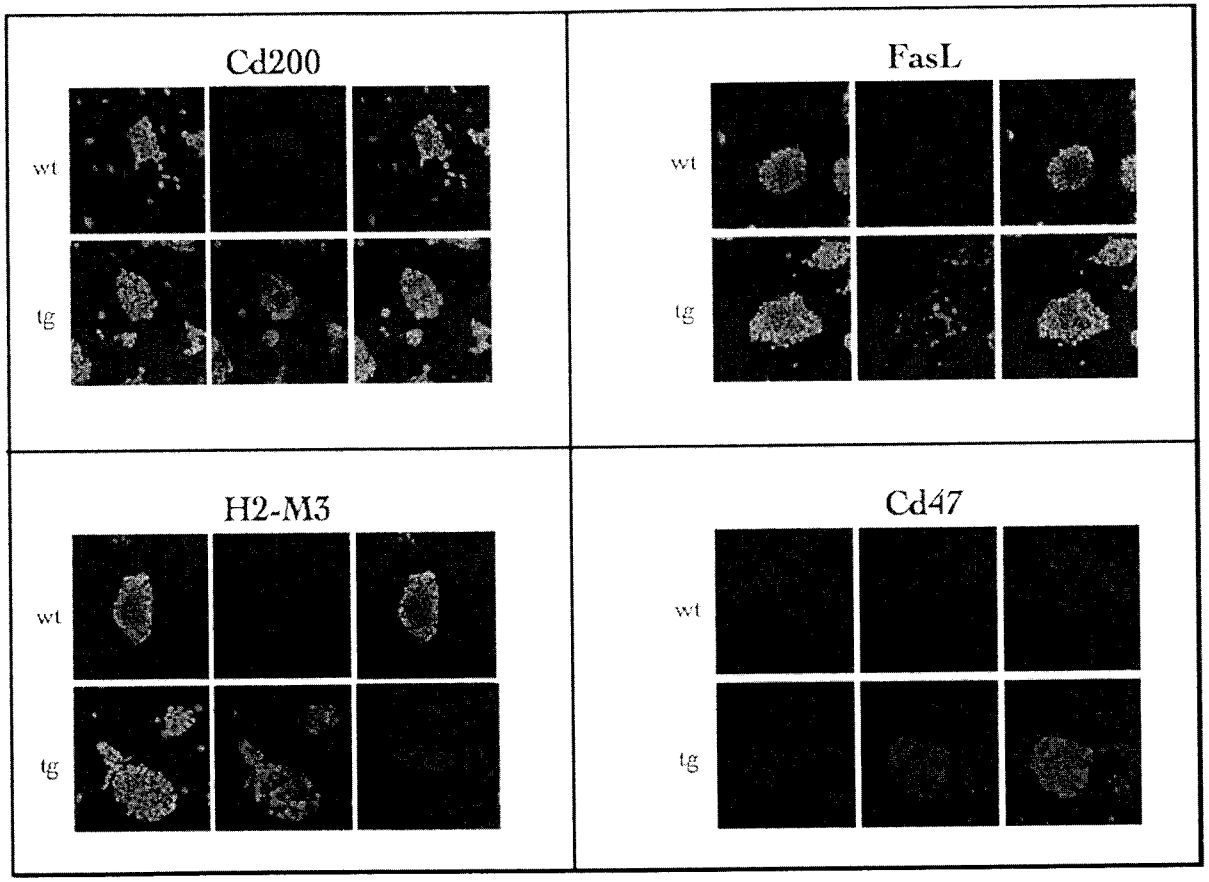


FIG. 1B

FIG. 1C

FIG. 1D

FIG. 2A

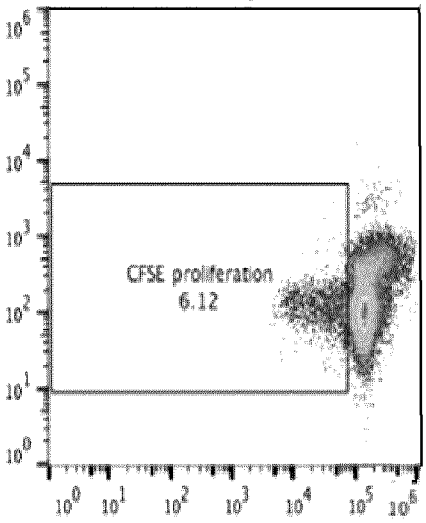


FIG. 2B

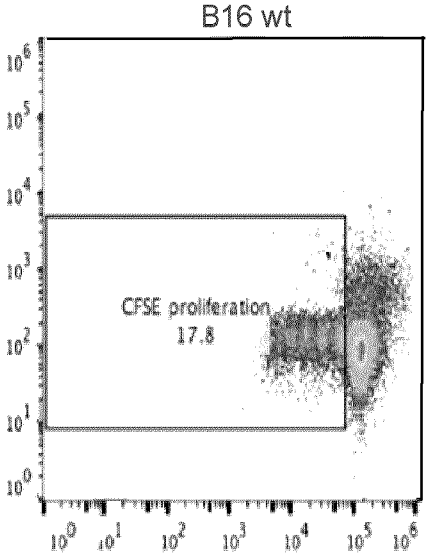


FIG. 2C

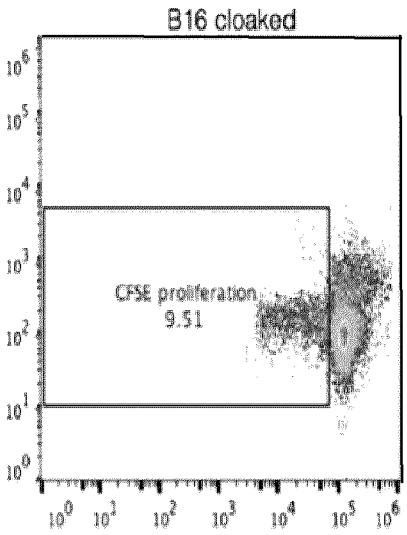


FIG. 2D

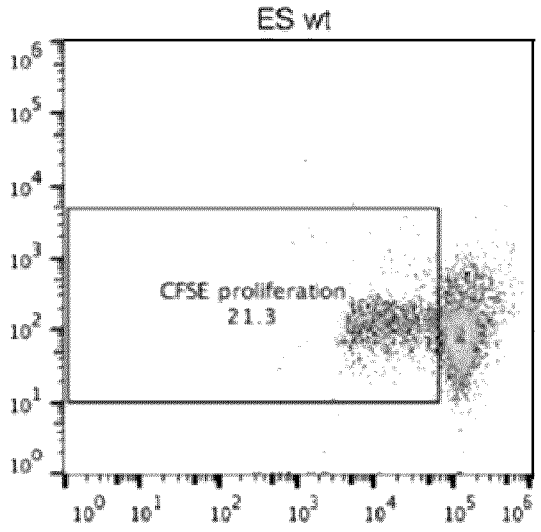


FIG. 2E

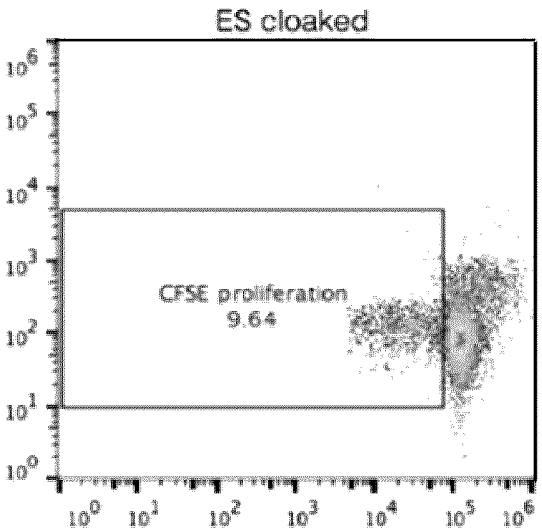


FIG. 3A

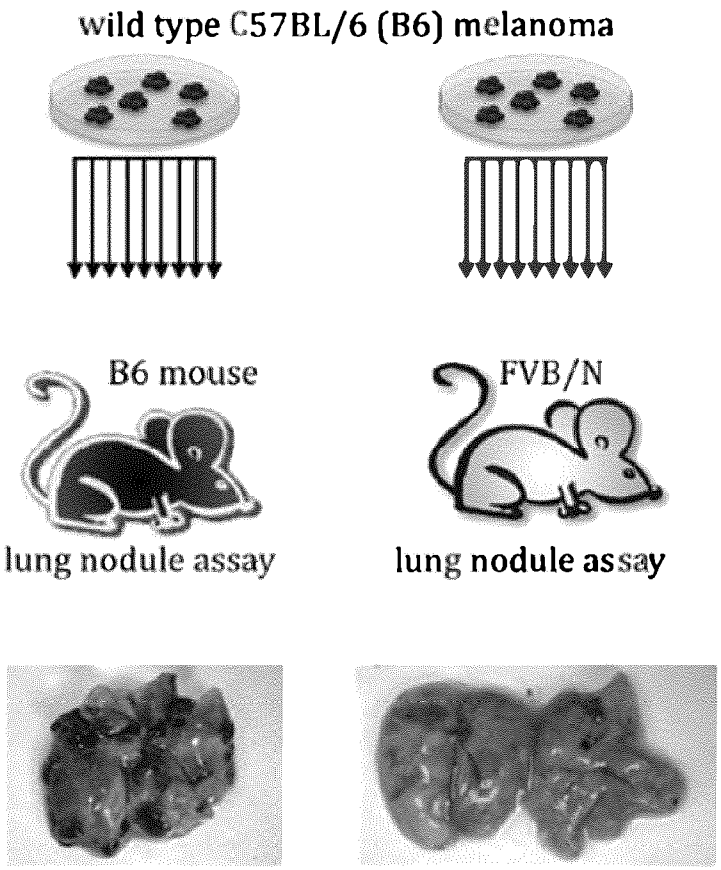

wild type C57BL/6 (B6) melanoma
B6 mouse
FVB/N
lung nodule assay
lung nodule assay
wt in B6
wt in FVB

FIG. 3B

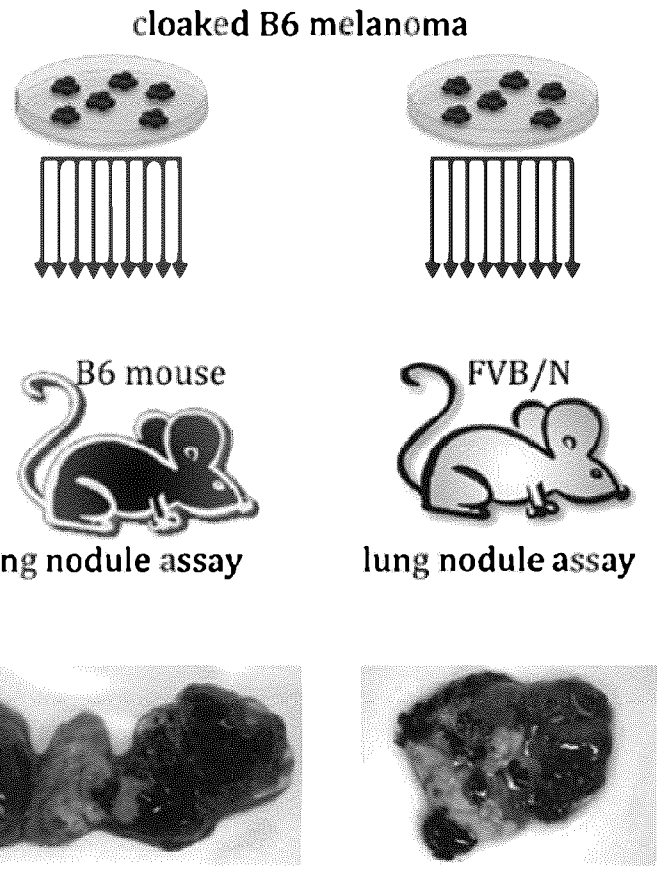

cloaked B6 melanoma
B6 mouse
FVB/N
lung nodule assay
lung nodule assay
cloaked in B6
cloaked in FVB

FIG. 4

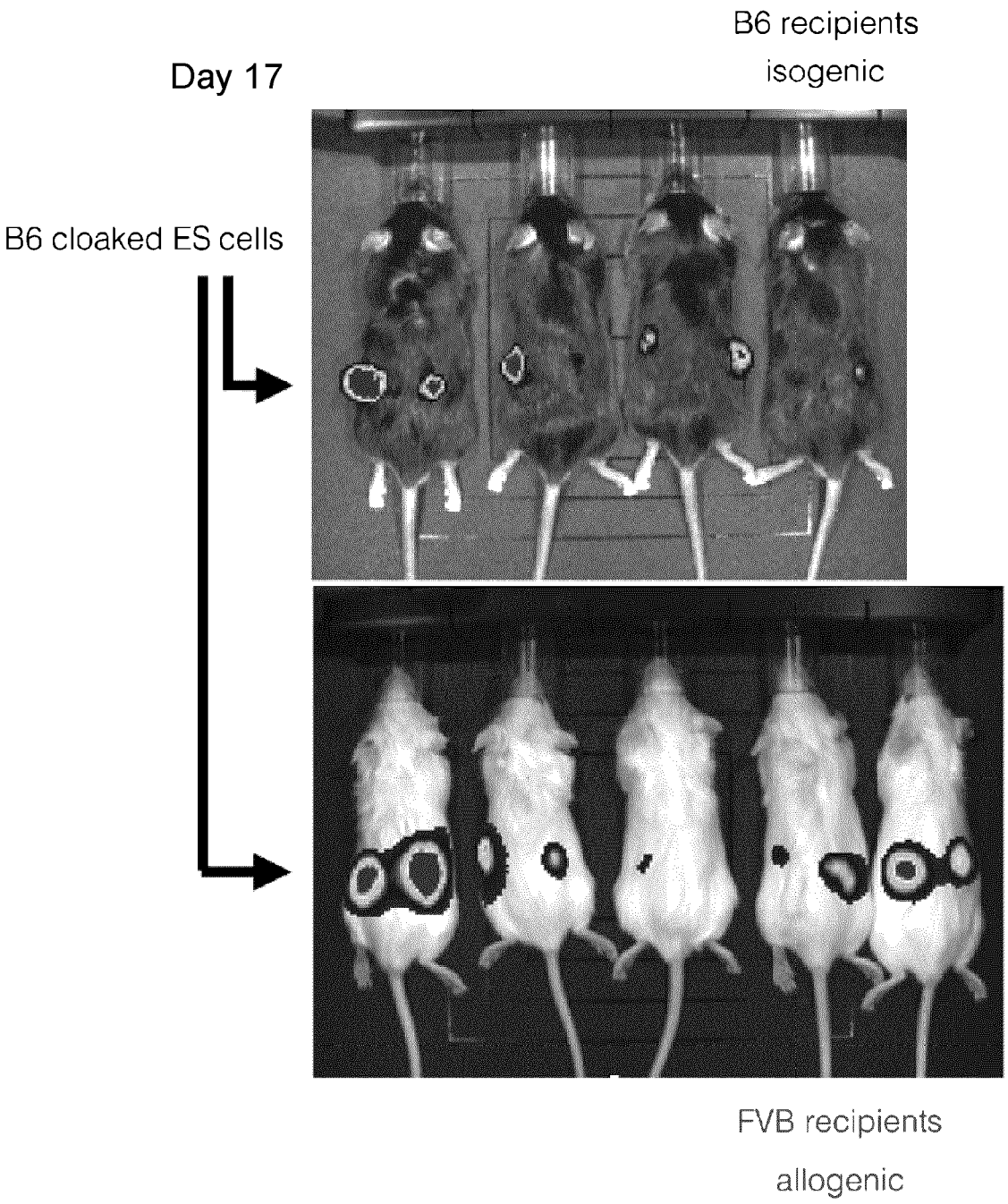

B6 recipients
isogenic
Day 17
B6 cloaked ES cells
FVB recipients
allogenic

FIG. 5A

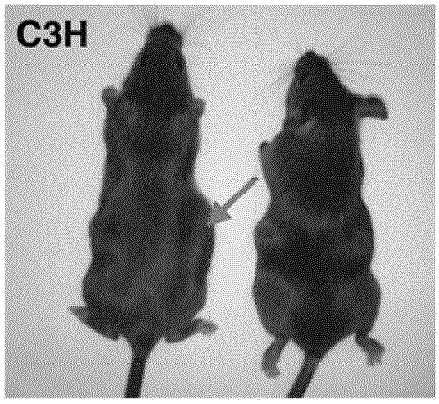


FIG. 5B

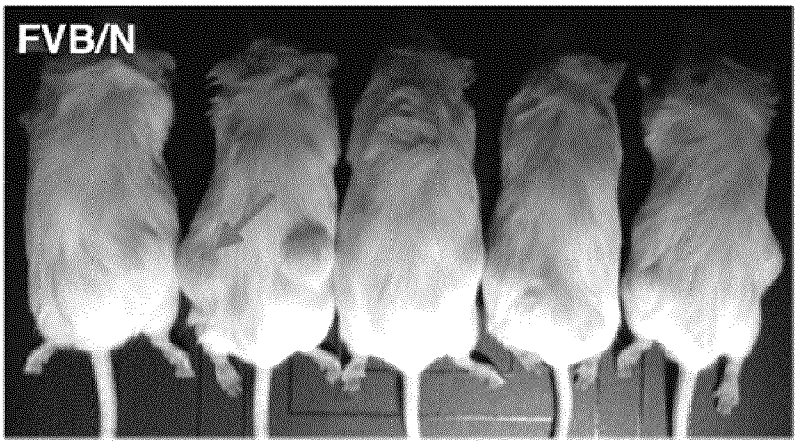


FIG. 5C

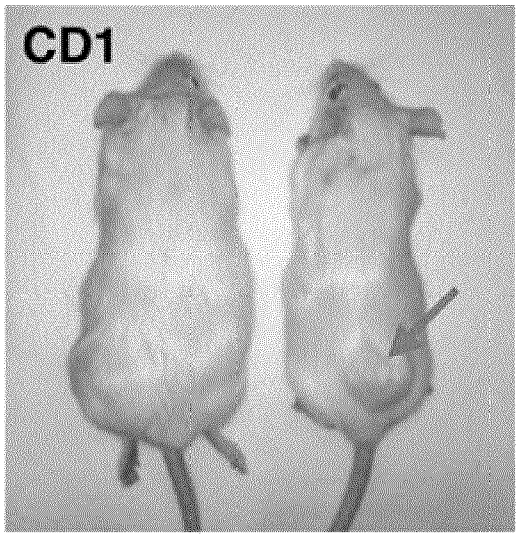


FIG. 6

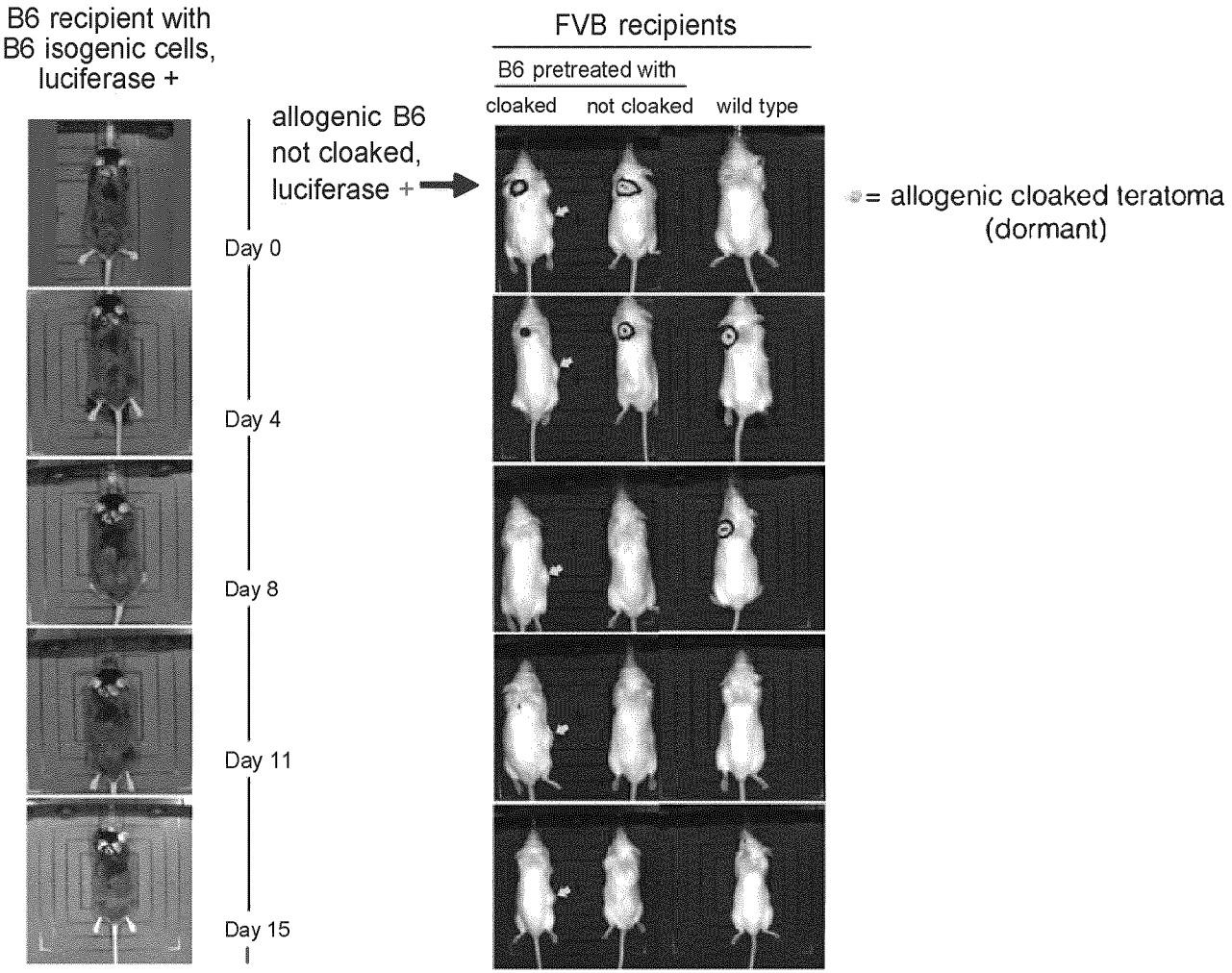

B6 recipient with
B6 isogenic cells,
luciferase +
allogenic B6
not cloaked,
luciferase +
FVB recipients
B6 pretreated with
cloaked
not cloaked
wild type
= allogenic cloaked teratoma
(dormant)
Day 0
Day 4
Day 8
Day 11
Day 15

FIG. 7

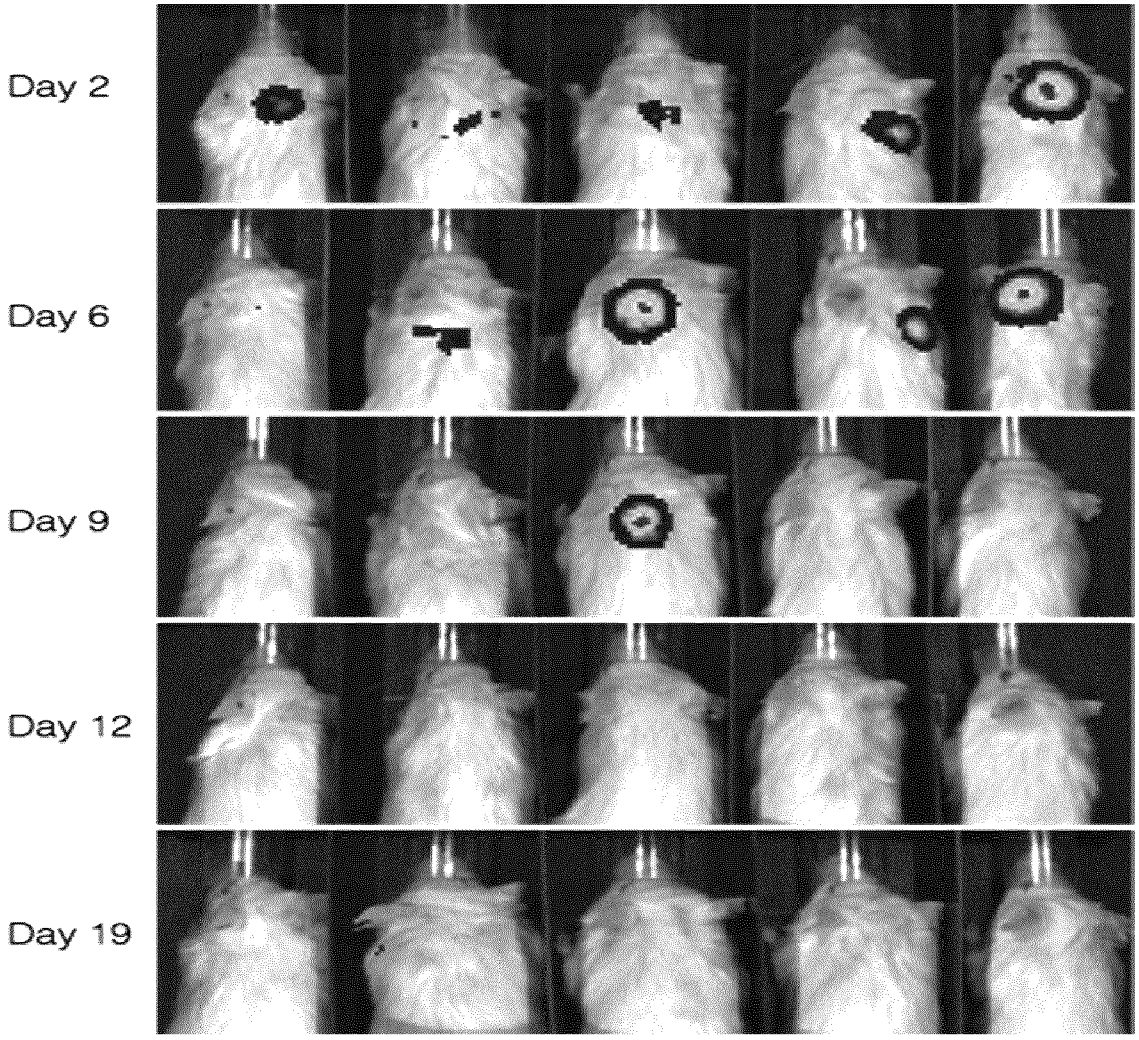


FIG. 8A

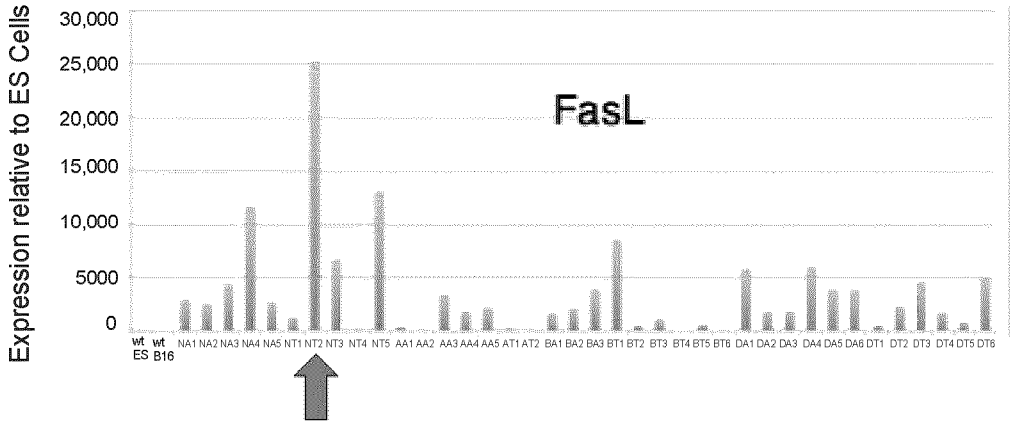


FIG. 8B

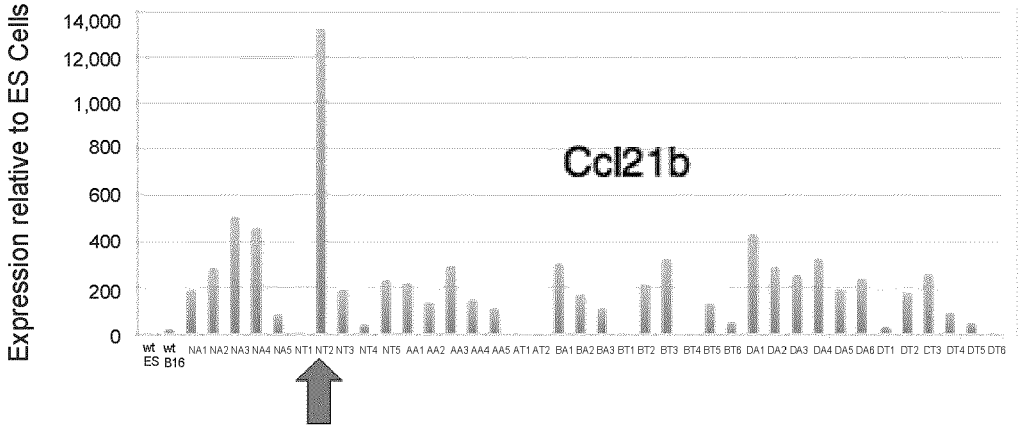


FIG. 8C

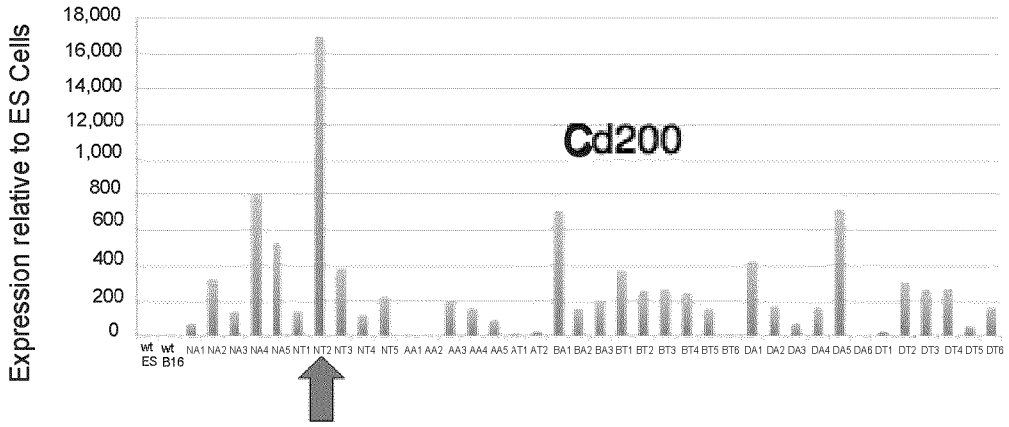


FIG. 8D

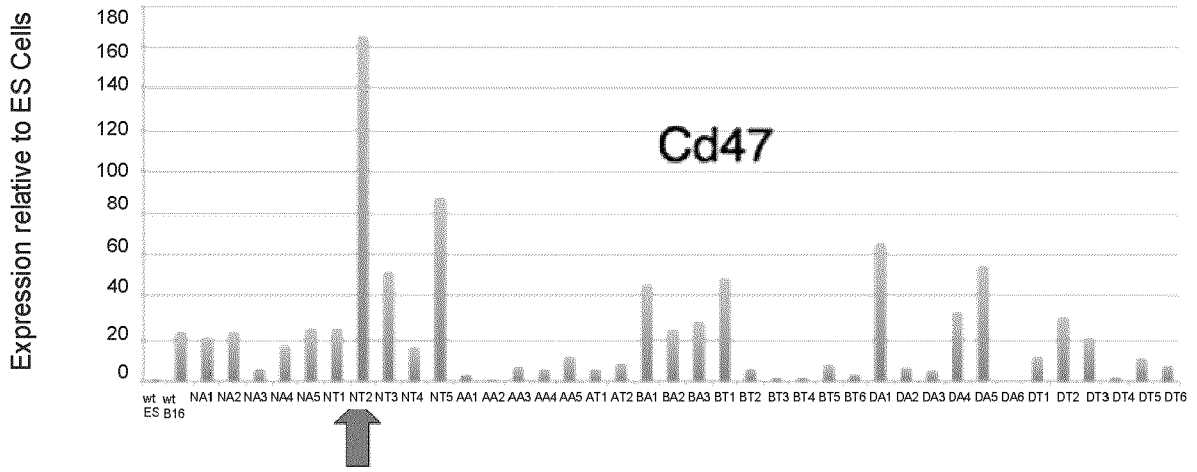


FIG. 8E

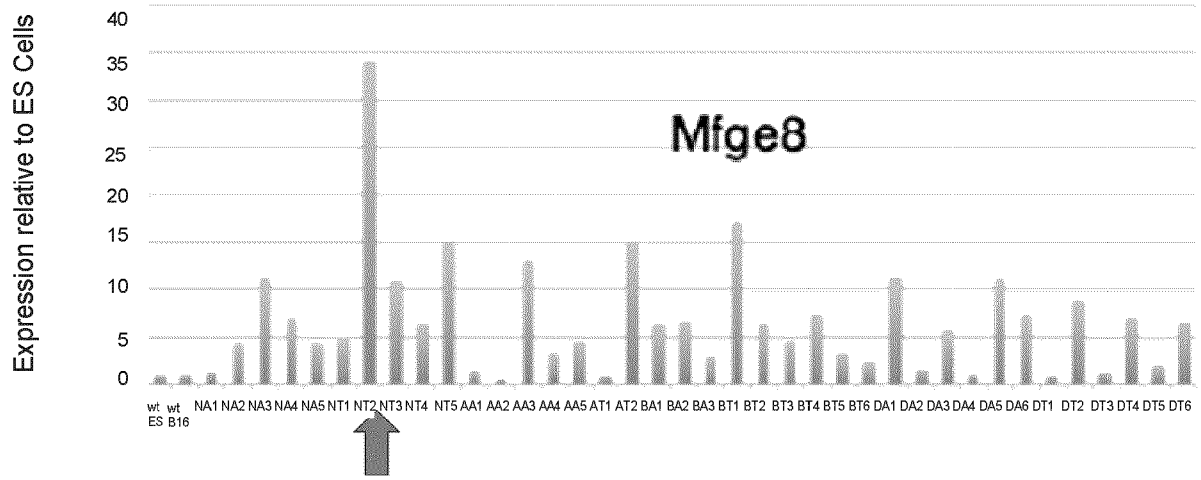


FIG. 8F

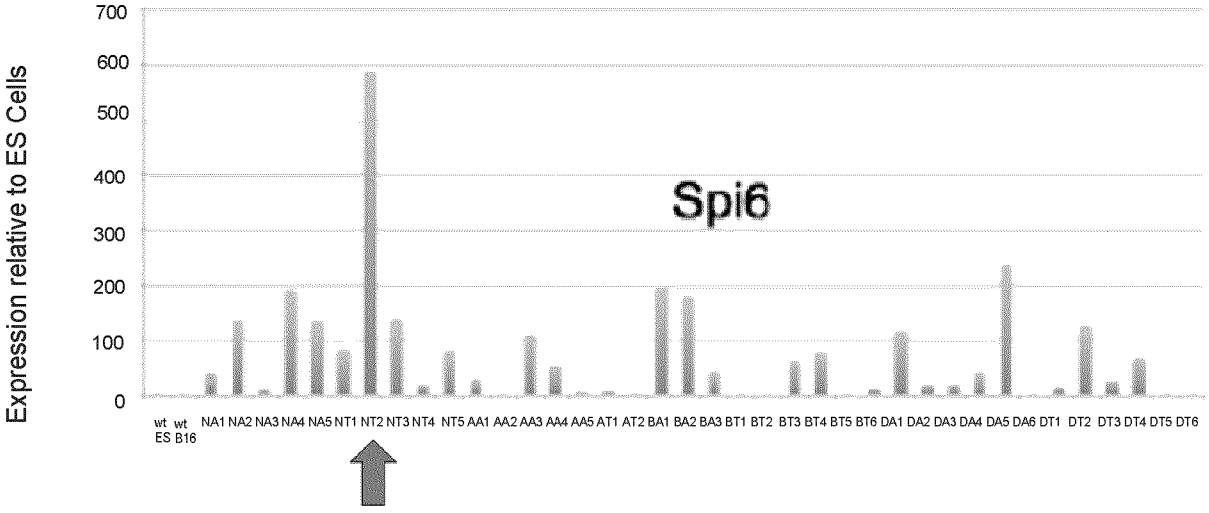


FIG. 8G

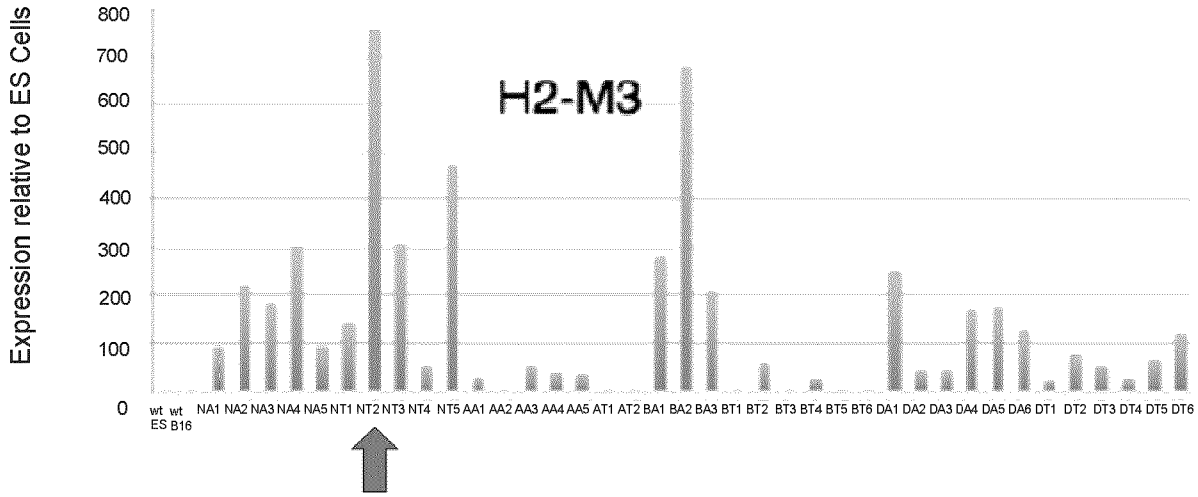

Expression relative to ES Cells
800
700
600
500
400
300
200
100
0
H2-M3
wt ES
wt B16
NA1 NA2 NA3 NA4 NA5 NT1 NT2 NT3 NT4 NT5 AA1 AA2 AA3 AA4 AA5 AT1 AT2 BA1 BA2 BA3 BT1 BT2 BT3 BT4 BT5 BT6 DA1 DA2 DA3 DA4 DA5 DA6 DT1 DT2 DT3 DT4 DT5 DT6

FIG. 8H

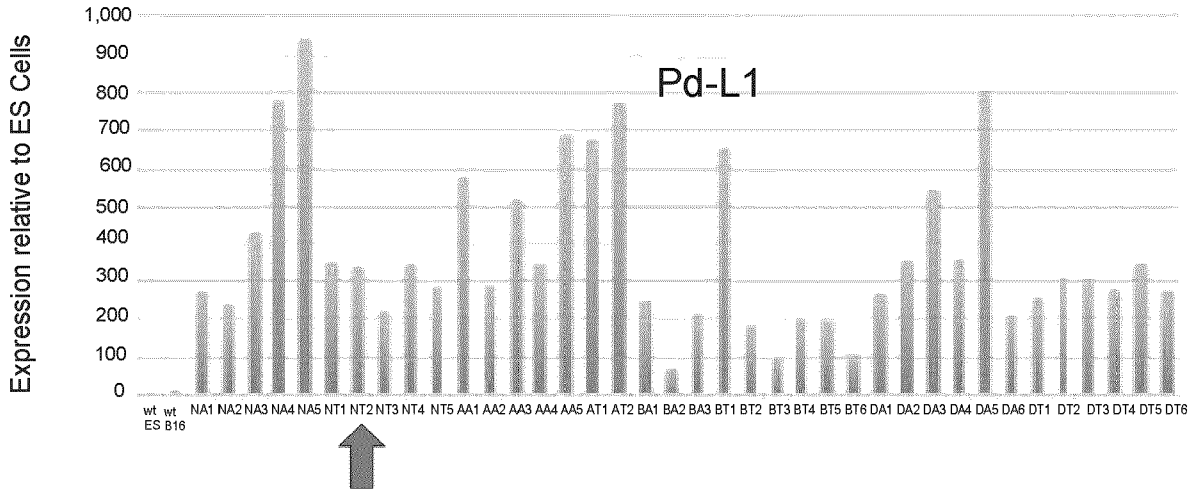

Expression relative to ES Cells
1,000
900
800
700
600
500
400
300
200
100
0
Pd-L1
wt ES
wt B16
NA1 NA2 NA3 NA4 NA5 NT1 NT2 NT3 NT4 NT5 AA1 AA2 AA3 AA4 AA5 AT1 AT2 BA1 BA2 BA3 BT1 BT2 BT3 BT4 BT5 BT6 DA1 DA2 DA3 DA4 DA5 DA6 DT1 DT2 DT3 DT4 DT5 DT6

FIG. 9

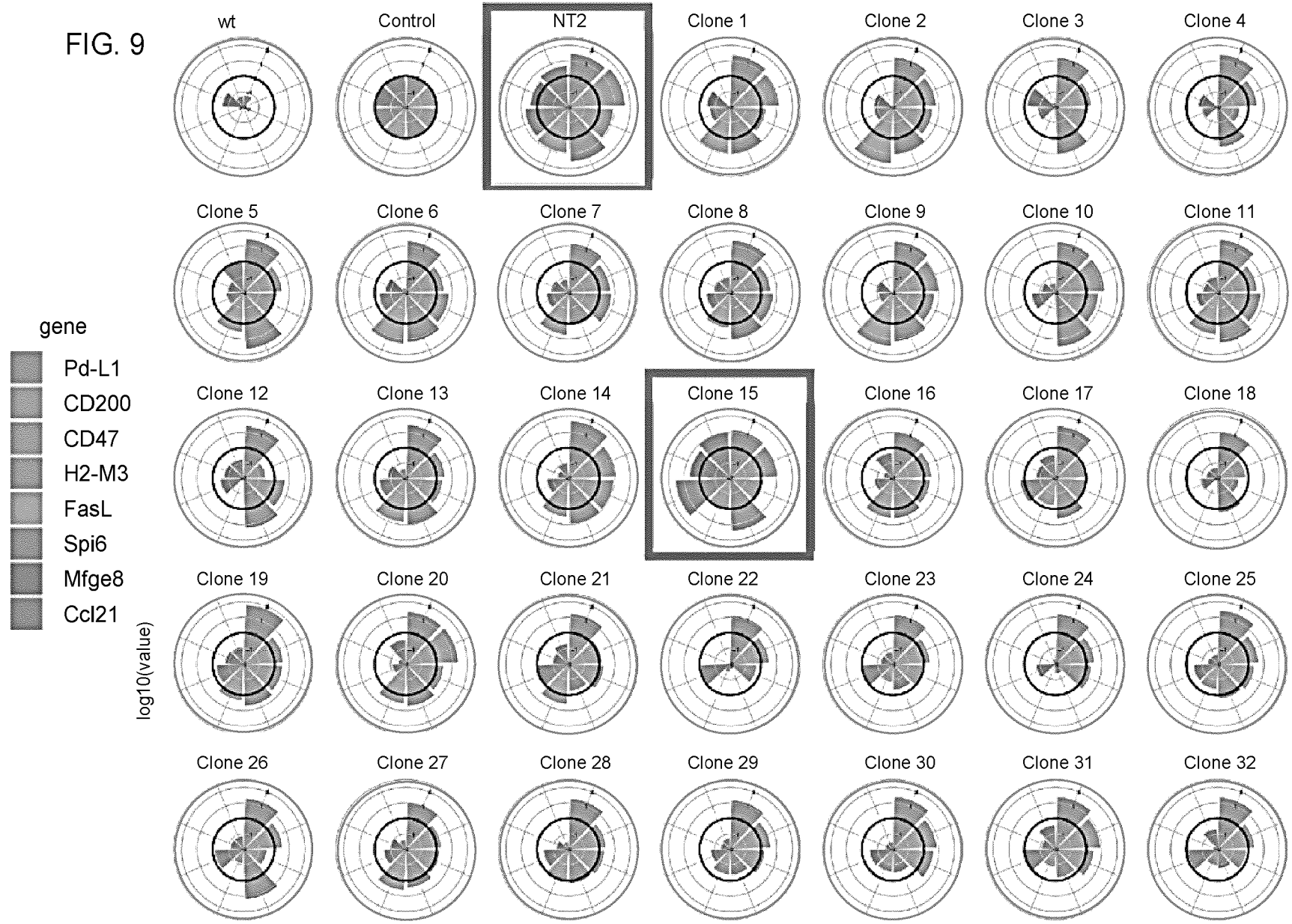

wt
Control
NT2
Clone 1
Clone 2
Clone 3
Clone 4
Clone 5
Clone 6
Clone 7
Clone 8
Clone 9
Clone 10
Clone 11
Clone 12
Clone 13
Clone 14
Clone 15
Clone 16
Clone 17
Clone 18
Clone 19
Clone 20
Clone 21
Clone 22
Clone 23
Clone 24
Clone 25
Clone 26
Clone 27
Clone 28
Clone 29
Clone 30
Clone 31
Clone 32
gene
Pd-L1
CD200
CD47
H2-M3
FasL
Spi6
Mfge8
Ccl21
log10(value)

FIG. 10

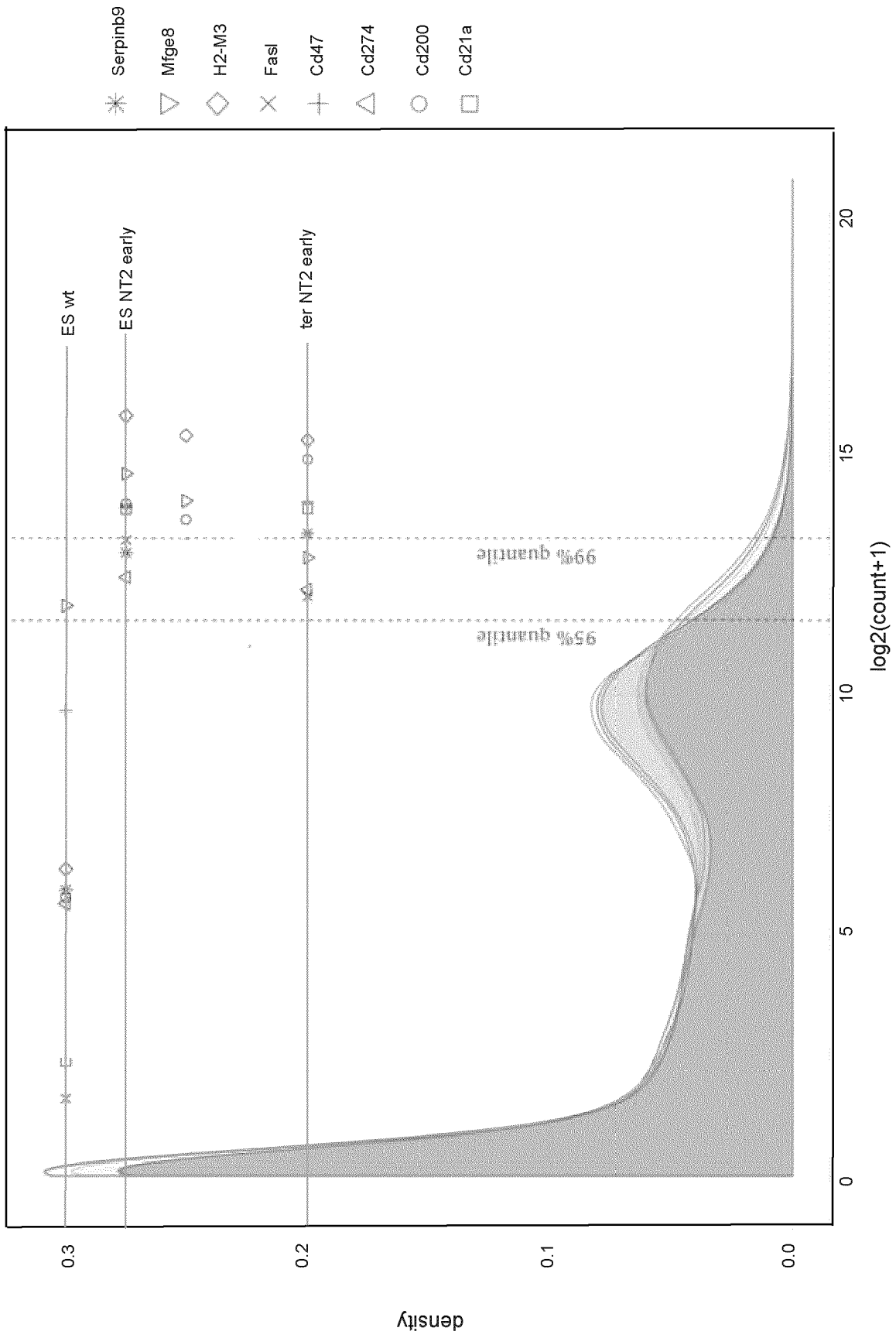

gene expression distribution
Serpinb9
Mfge8
H2-M3
Fasl
Cd47
Cd274
Cd200
Cd21a
ES wt
ES NT2 early
ter NT2 early
95% quantile
99% quantile
density
0.0
0.1
0.2
0.3
0
5
10
15
20
log2(count+1)

FIG. 11B

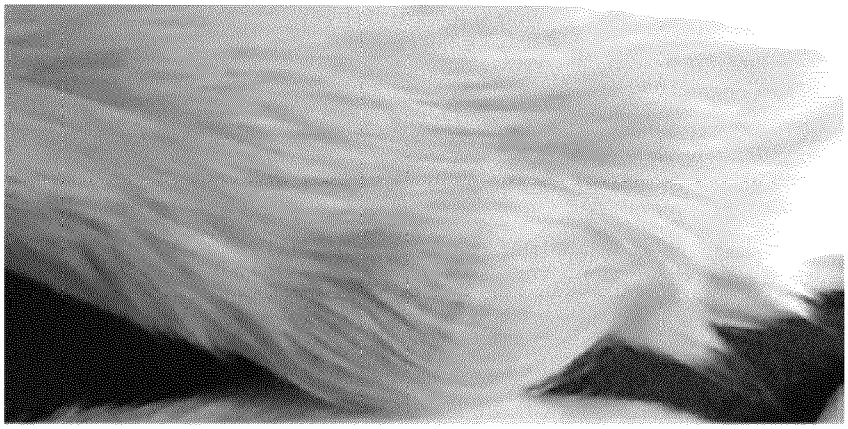


FIG. 11A

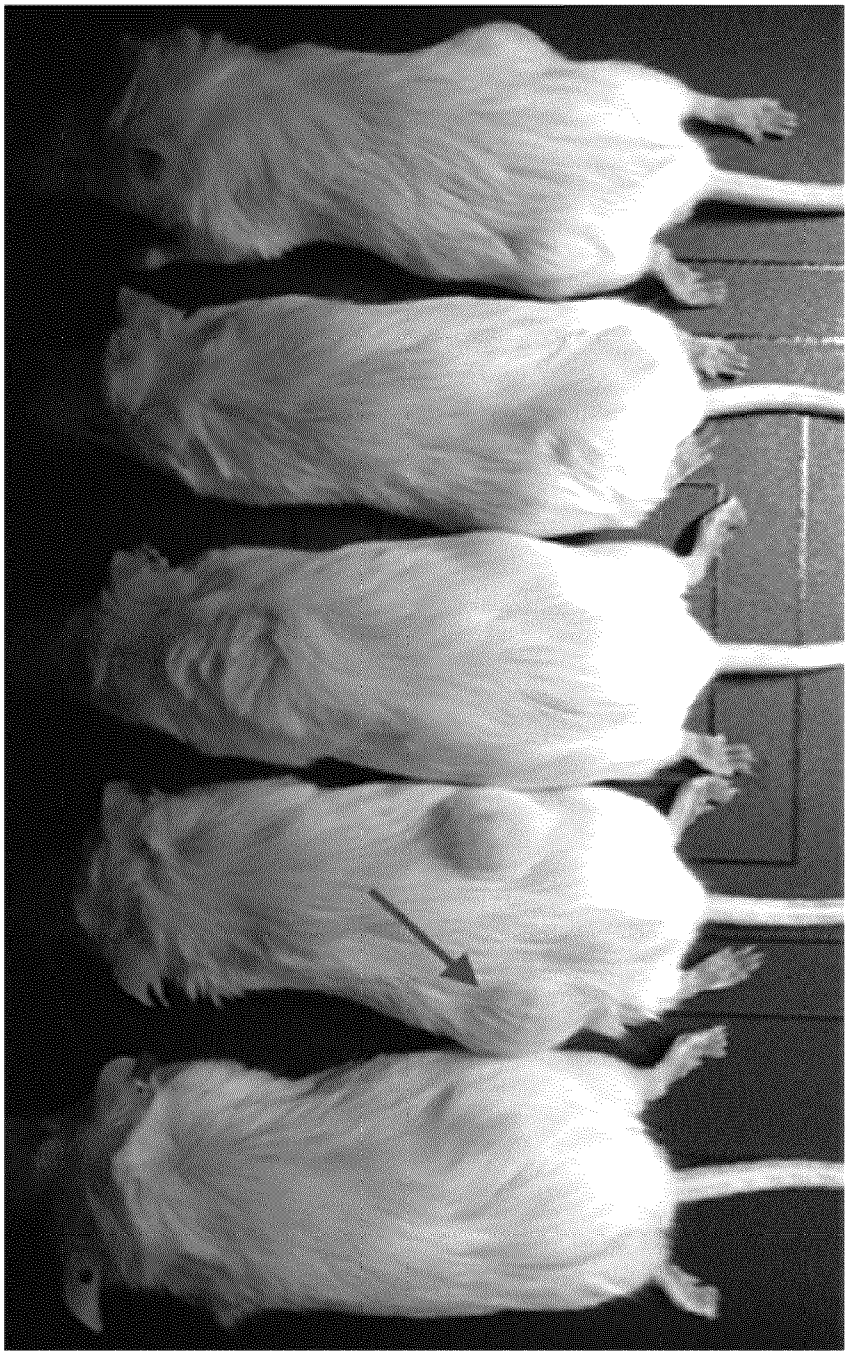


FIG. 12A

isogenic teratomas: NT2 in C57BL/6

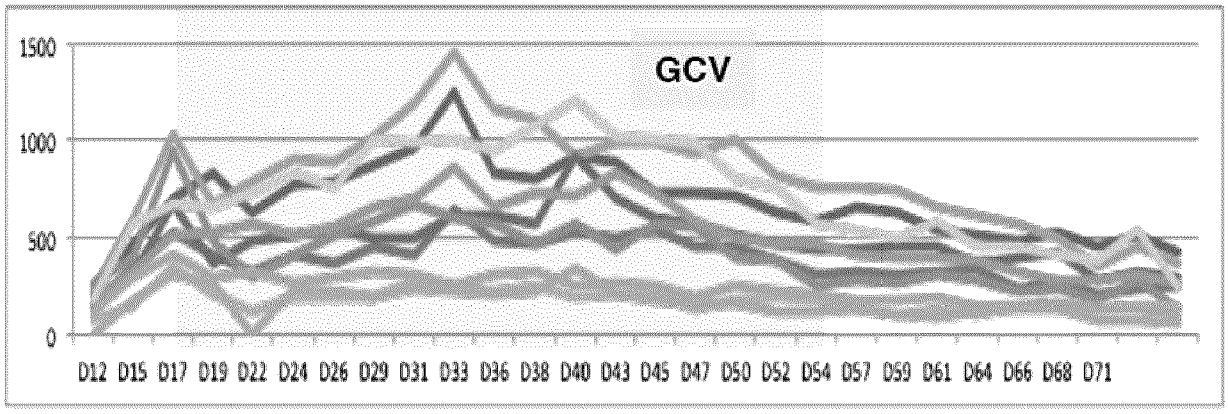


FIG. 12B

allogenic teratomas: NT2 in FVB

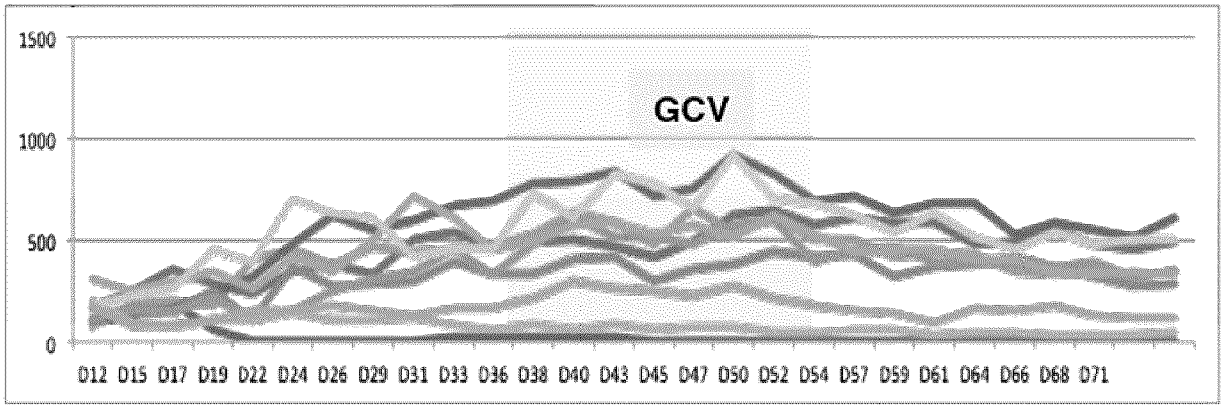


FIG. 13A

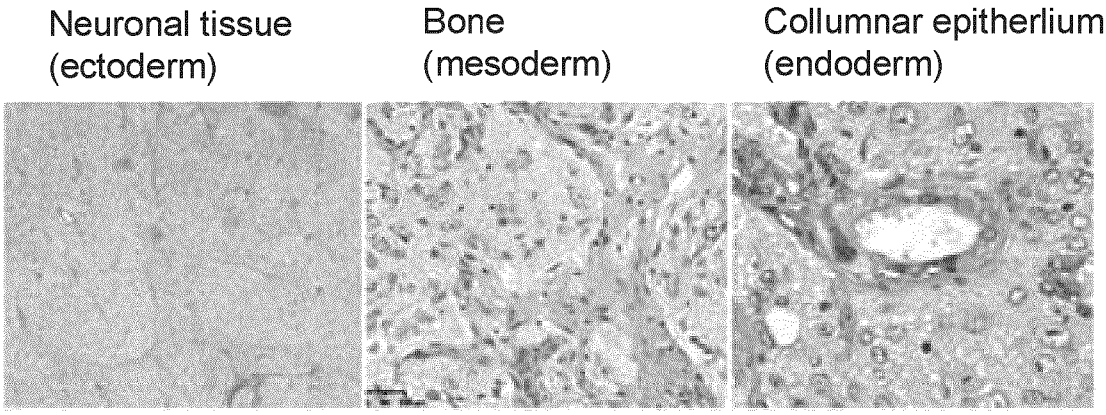


FIG. 13B

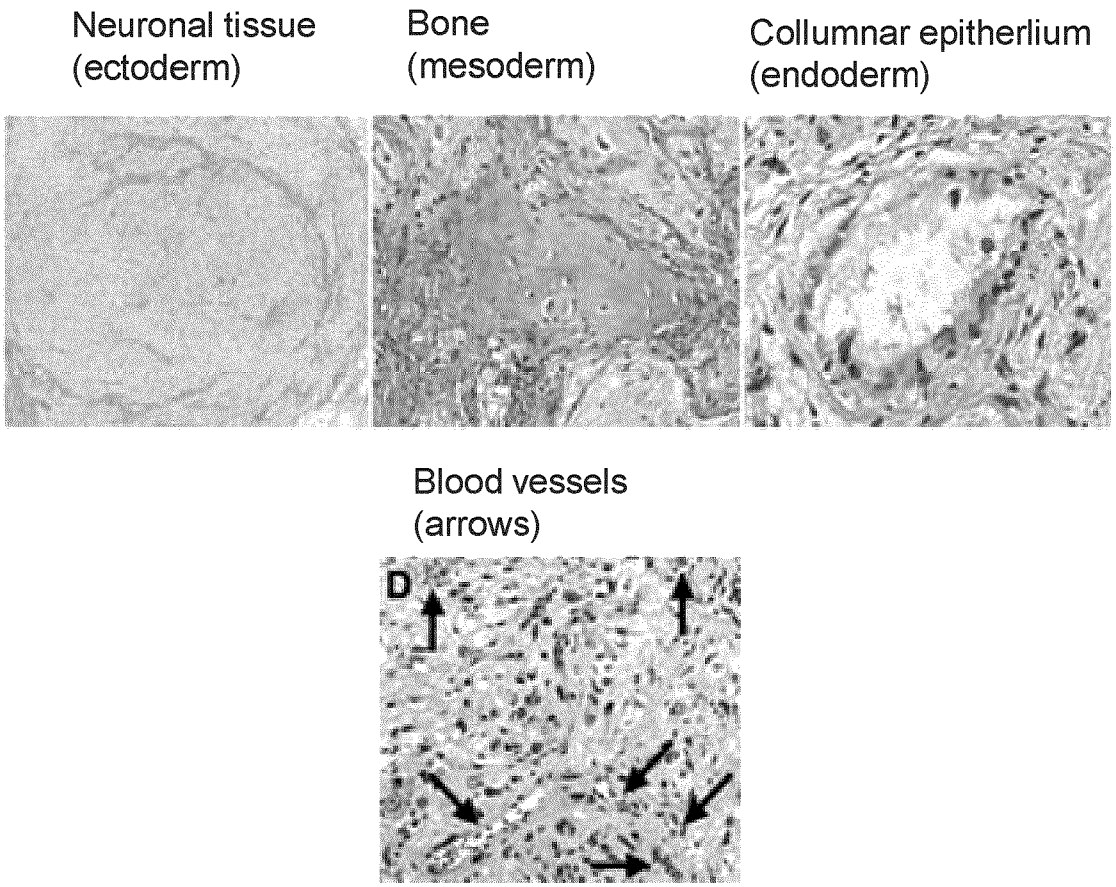


FIG. 14A

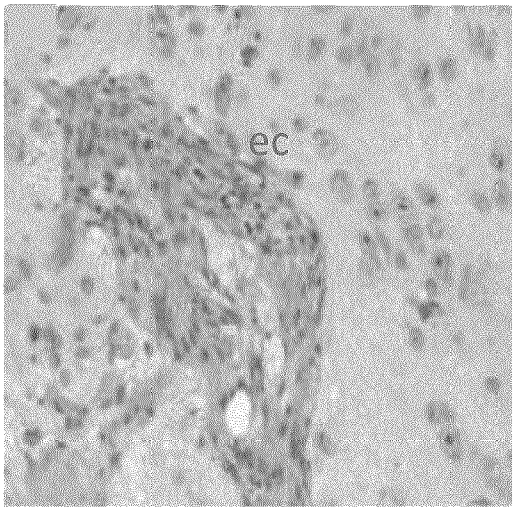


FIG. 14B

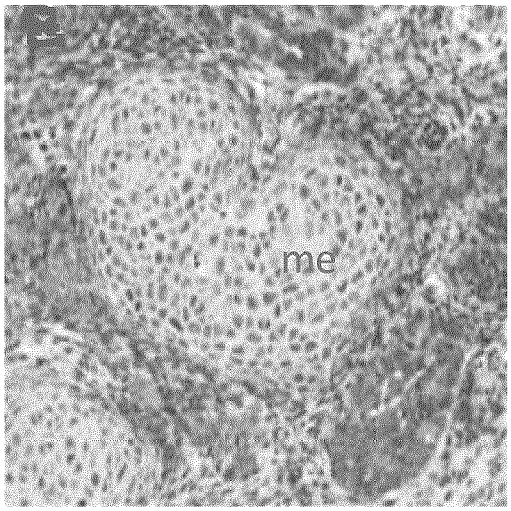


FIG. 14C

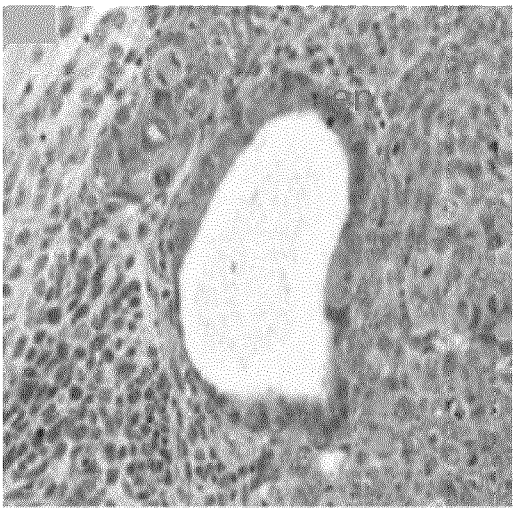


FIG. 14D

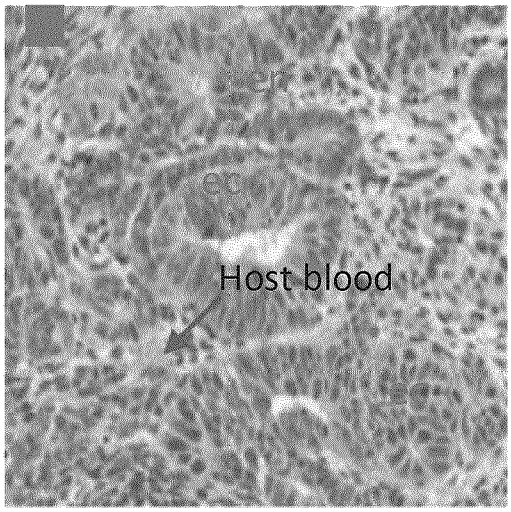


FIG. 15

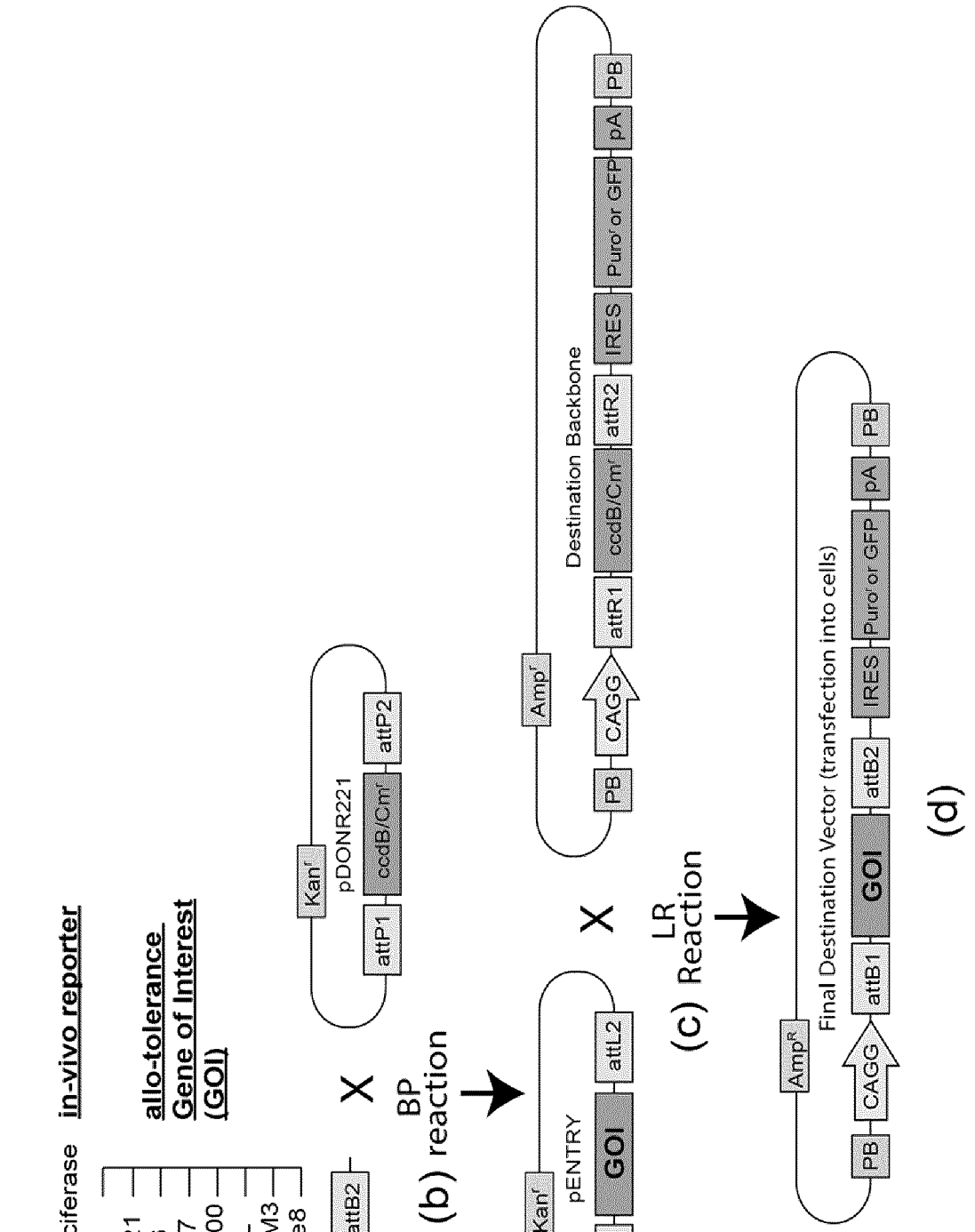

in-vivo reporter
eLuciferase
Pdl1
Ccl21
Spi6
Cd47
Cd200
FasL
H2-M3
Mfge8
allo-tolerance
Gene of Interest
(GOI)
(a)
attB1
GOI
attB2
X
Kan^r
pDONR221
attP1
ccdB/Cm^r
attP2
BP
(b) reaction
Kan^r
pENTRY
attL1
GOI
attL2
X
Amp^r
Destination Backbone
PB
CAGG
attR1
ccdB/Cm^r
attR2
IRES
Puro^r or GFP
pA
PB
LR
(c) Reaction
Amp^R
Final Destination Vector (transfection into cells)
PB
CAGG
attB1
GOI
attB2
IRES
Puro^r or GFP
pA
PB
(d)

FIG. 16A

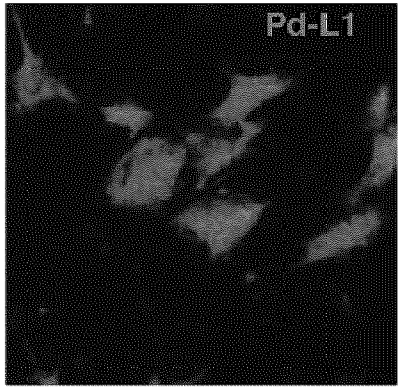


FIG. 16B

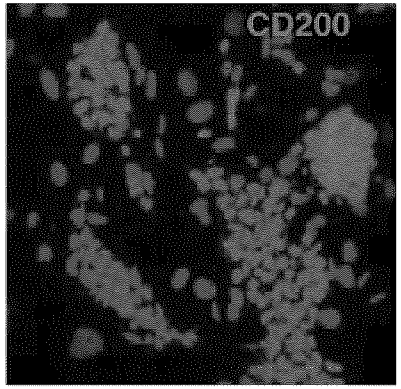


FIG. 16C

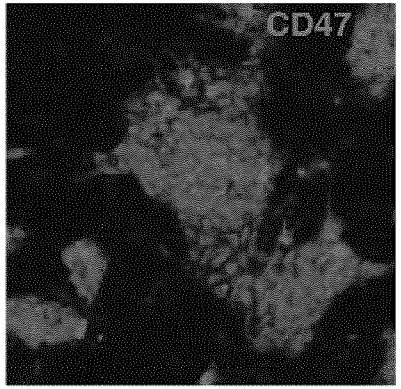


FIG. 16D

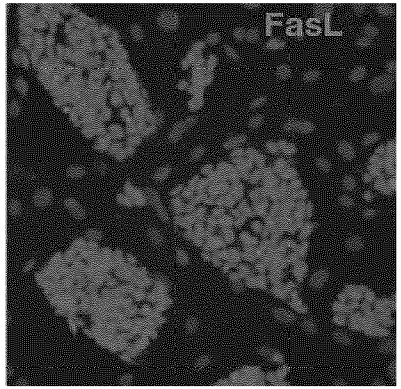


FIG. 16E

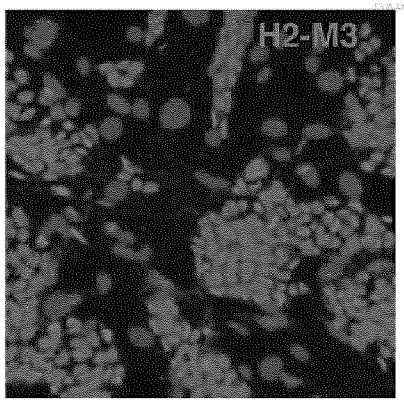


FIG. 16F

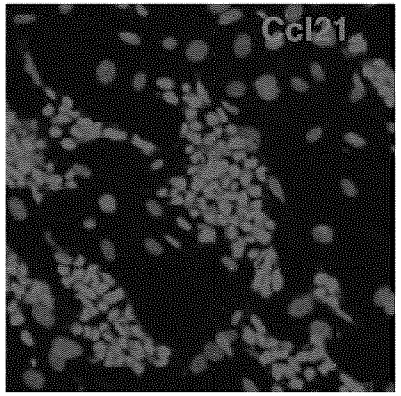


FIG. 16G

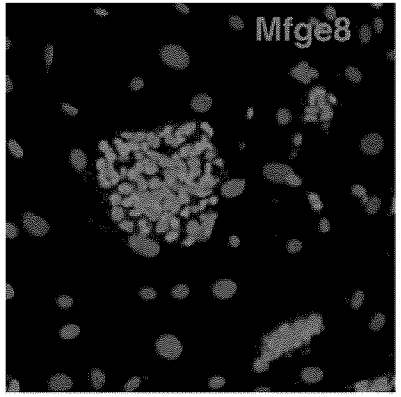


FIG. 16H

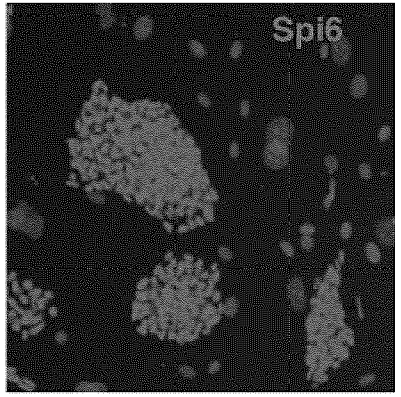


FIG. 17A

Brightfield

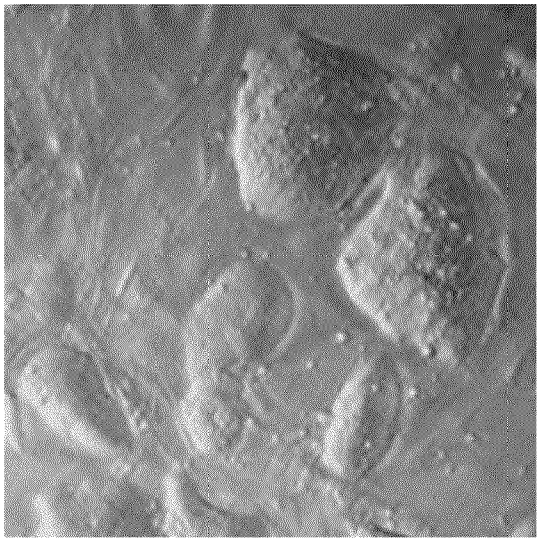


FIG. 17B

Alk. phos.

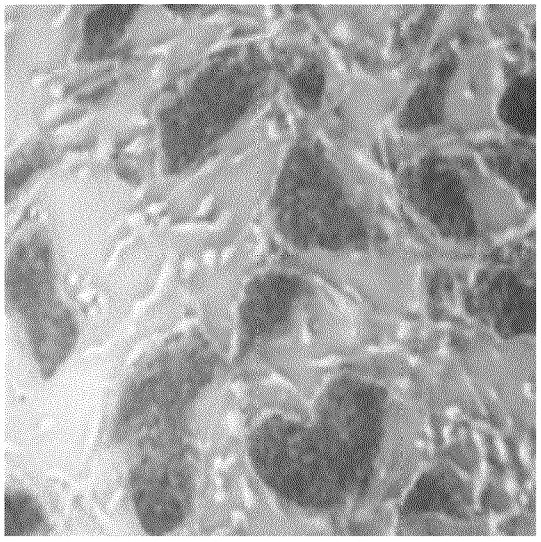


FIG. 18A

Oct4

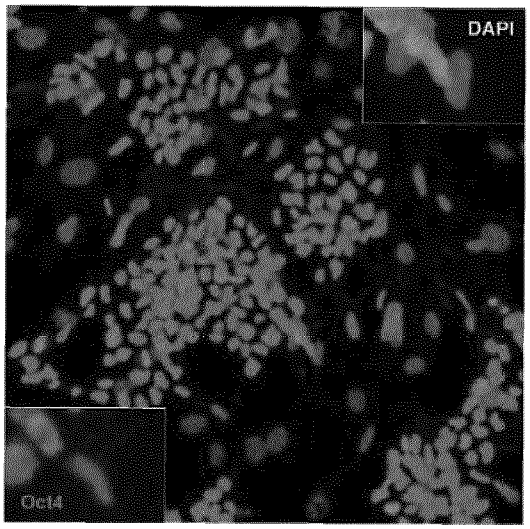


FIG. 18B

SSEA1

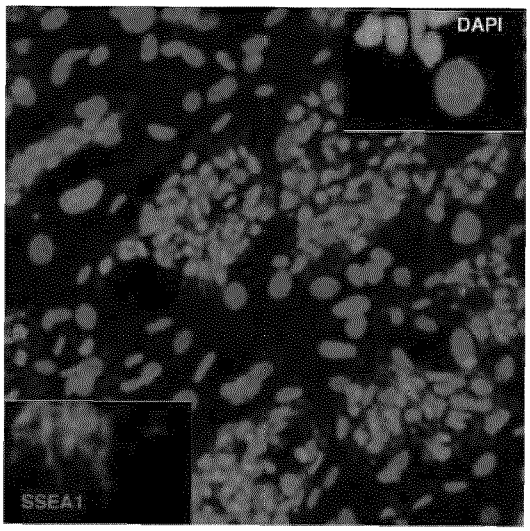
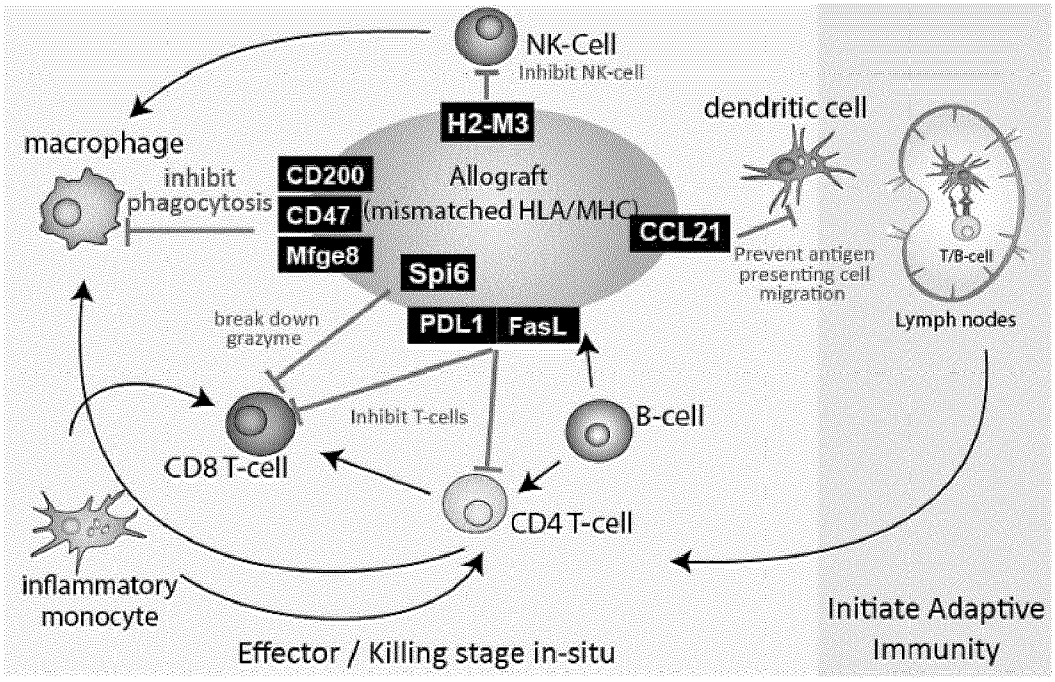


FIG. 19


NK-Cell
Inhibit NK-cell
H2-M3
macrophage
inhibit phagocytosis
CD200
CD47
Mfge8
Allograft
(mismatched HLA/MHC)
CCL21
dendritic cell
Prevent antigen presenting cell migration
T/B-cell
Lymph nodes
Spi6
break down grazyme
PDL1
FasL
Inhibit T-cells
B-cell
CD8 T-cell
CD4 T-cell
inflammatory monocyte
Effector / Killing stage in-situ
Initiate Adaptive Immunity

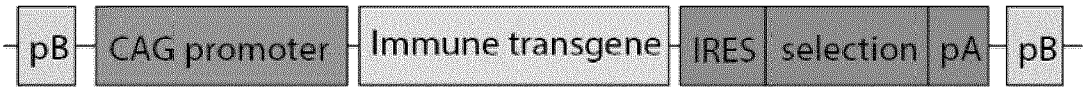
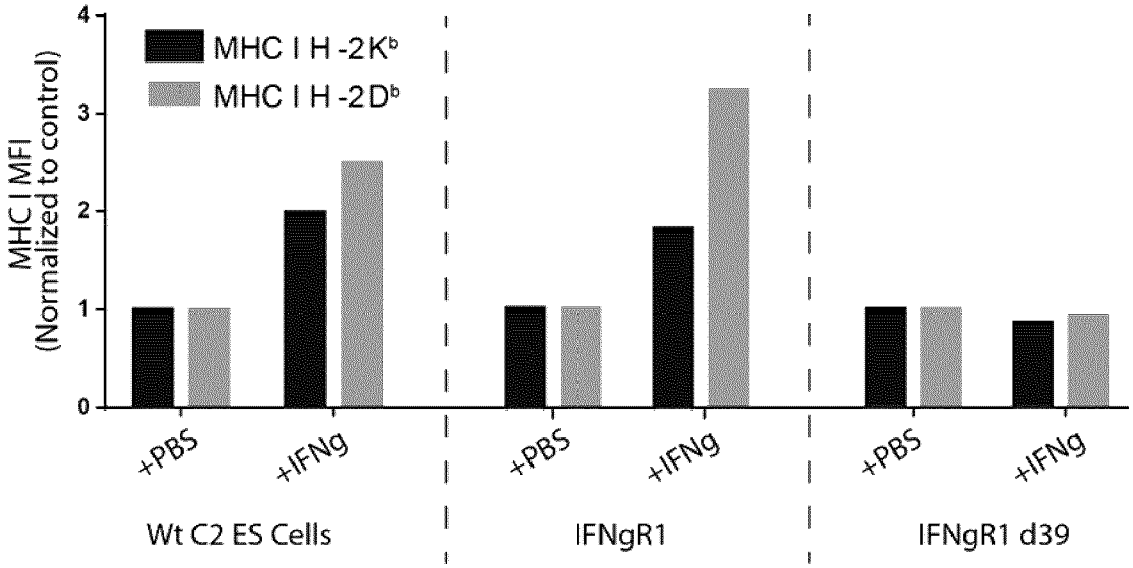

pB
CAG promoter
Immune transgene
IRES
selection
pA
pB

FIG. 20


MHC I H-2K^b
MHC I H-2D^b
MHC I MFI (Normalized to control)
0
1
2
3
4
+PBS
+IFNg
+PBS
+IFNg
+PBS
+IFNg
Wt C2 ES Cells
IFNgR1
IFNgR1 d39

ALLOGRAFT TOLERANCE WITHOUT THE NEED FOR SYSTEMIC IMMUNE SUPPRESSION

FIELD OF THE DISCLOSURE

The disclosure relates generally to the field of transplantation. The disclosure further relates to methods for generating local immunosuppression in the environment of transplanted cells.

BACKGROUND OF THE DISCLOSURE

The advent of human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells has had a paradigm-shifting effect on regenerative and translational medicine. These cells have can self-renew indefinitely in a pluripotent state while retaining the ability to differentiate into any cell type in the human body. Such properties have allowed researchers to better understand human development and the etiology of developmental disorders. They have also given modern medicine a powerful new tool against diseases that have been intractable or impossible to treat with conventional medicine, including spinal cord injury, diabetes, blindness, multiple sclerosis, and cancer, to name a few. The efficacy and range of applicable diseases for cell therapies will only increase with our growing understanding of how to control stem cell differentiation and the biology of the differentiated cell products.

With these applications come important and critical challenges. Along with cell safety, one of the most important concerns is immune rejection of cells from a different genetic background. Immune rejection remains a critical barrier because the immune system has evolved a complex set of mechanisms to recognize and eliminate "non-self" cells that express specific protein fragments - especially those from the major histocompatibility complex (MHC in mouse, HLA in humans) – that differ between donor and recipient (Yang et al., *Nat Rev Genet.* 18:309-26 (2017)). This response is almost certainty a by-product of the evolutionary pressure to protect against opportunistic infections and malignancies, which are often defined by the presence of "foreign" proteins and epitopes. Depending on the context, rejection of transplanted cells or tissues can occur over the timescale of minutes/hours (hyperacute), days/months (acute), and months/years (chronic) (LaRosa et al., *J Immunol.* 178:7503-9 (2007)). This rejection results from the complex and coordinated effects of cell types from both innate (Murphy et al., *Immunol Rev.* 241:39-48 (2011)) and adaptive immunity (Issa et al., *Expert Rev Clin Immunol.* 6:155-69 (2010)).

One of the most important pathways to rejection is the priming of the adaptive immune system and activation of CD8+ cytotoxic T-cells. This occurs after antigen presenting cells process donor-specific peptides and then activate recipient T-cells that are specific for the same peptides in secondary lymph organs (Lechler et al., *J Exp Med.* 155:31-41 (1982); Guermonprez et al., *Annu Rev Immunol.* 20:621-67 (2002); Stockwin et al., *Immunol Cell Biol.* 78:91-102 (2000)). These T-cells then migrate to and kill transplanted cells or tissues with the release of cytolytic factors like perforin and granzyme. NK-cells can also induce apoptosis in donor cells based on foreign or no MHC expression (Kitchens et al., *Transplantation.* 81:811-7 (2006); Benichou et al., *Curr Opin Organ Transplant.* 16:47-53 (2011)), and other cell types like macrophages can support rejection with the release of pro-inflammatory cytokines at

the engraftment site (Mannon, *Curr Opin Organ Transplant.* 17:20-5 (2012)). Many other cell types and subtypes also have a role in allograft rejection. Since these are the same immune pathways used to eliminate common viral and bacterial pathogens, they are – along with rejection of an allograft – highly conserved across vertebrate species.

A current solution to prevent rejection of an allograft involves the following two options: find a donor with a matched histocompatibility haplotype (mostly likely from genetically-related family), and much more commonly, use broadly-directed immunosuppressant drugs (Wiseman, *Clin J Am Soc Nephrol.* 11:332-43 (2016); Malaise et al., *Transplant Proc.* 37:2840-2 (2005)). Common drugs include those from the families of calcineurin inhibitors (Flechner et al., *Clin Transplant.* 22:1-15 (2008); Casey et al., *Curr Opin Nephrol Hypertens.* 20:610-5 (2011)), anti-proliferative agents (Hardinger et al., *World J Transplant.* 3:68-77 (2013)), mTOR inhibitors (Macdonald, *Expert Rev Clin Immunol.* 3:423-36 (2007); Neuhaus et al., *Liver Transpl.* 7:473-84 (2001)), and steroids (Steiner et al., *Semin Immunopathol.* 33:157-67 (2011)) – all of which suppress T-cell proliferation or function (particularly the former three). These drugs need to be taken every day for life, and even a single missed dose can increase the risk of rejection. Yet they do not always work, and when they do, rates of chronic rejection still continually climb over time (Demetris et al., *Ann Transplant.* 2:27-44 (1997); Libby et al., *Immunity.* 14:387-97 (2001)). Most importantly, they are systemically-acting and ultimately leave patients immunocompromised with increased rates of cancer and life-threatening infections (Gallagher et al., *J Am Soc Nephrol.* 21:852-8 (2010)). Pertaining to ES cells, these drugs have shown only marginal improvements in permitting survival across an MHC barrier (Swijnenburg et al., *Proc Natl Acad Sci U S A.* 105:12991-6 (2008); Toriumi et al., *Neurol Res.* 31:220-7 (2009)). While newer and more targeted immunosuppressant reagents are becoming available and tested in skin and cardiac (Larsen et al., *Nature.* 381:434-8 (1996)), as well as ES cell allograft settings (Pearl et al., *Cell Stem Cell.* 8:309-17 (2011)), they are still systemically-acting and therefore likely to leave hosts immune compromised.

One proposed benefit to the discovery of iPS cells was that they could be created from, and for, each patient. These cells should, in theory, be protected from immune rejection by the corresponding patient (Pearl et al., *Sci Transl Med.* 4:164ps25 (2012)). However, the induction of an iPS cell state involves epigenetic alterations and in-vitro-culture pressures that can create abnormalities and malignancies, so each cell line would need to be vigorously tested and/or genetically modified to achieve safety as well as function (Hussein et al., *Nature.* 471:58-62 (2011); Laurent et al., *Cell Stem Cell.* 8:106-18 (2011); Lister et al., *Nature.* 471:68-73 (2011)). Ultimately, the cost and time needed to create and test an iPS cell line for each individual patient makes this approach practically and economically unrealistic. Even if the costs were dramatically reduced, it would not help those patients who need immediate treatment for conditions like burns, heart attacks, strokes, and spinal cord injury (among many others). Furthermore, given recent findings, it remains controversial whether iPS cell-derived cell types are truly protected from immune rejection even when transplanted into the same host from where they were derived (Zhao et al., *Nature.* 474:212-5 (2011)).

One proposed solution in this regard has been to use naturally suppressive or regulatory immune cells, like Tregs or others, that are expanded and/or transferred before, during, or after transplant of therapeutic cells or tissues (Cobbold et al., *Cold Spring Harb Perspect Med.* 3(6) (2013); Wood et al., *Nature reviews Immunology.* 12:417-30 (2012)). These strategies have been suggested based on the recognition of suppressive immune pathways, in particular the discovery of the master regulator FoxP3 that programs a subset of CD4+ cells regulatory T-cells (Hori et al., 299:1057-61 (2003); Fontenot et al., *Nat Immunol.* 4:330-6 (2003)) and proof of their critical importance in promoting tolerance to allografts (Kendal et al., *J Exp Med.* 208:2043-53 (2011)). This thinking is in contrast to some of the first tolerance-inducing strategies which focused almost exclusively on depletion of effector T-cells with monoclonal antibodies, coupled with bone marrow transplant and the creation of donor chimerism (Cobbold et al., *Nature.* 323:164-6 (1986); Qin et al., *J Exp Med.* 169:779-94 (1989)). The importance of suppressive T-cell phenotypes was later appreciated with strategies that did not kill the cells, but blocked critical T-cell receptors in a way that left them unresponsive to allografts (Cobbold et al., *J Immunol.* 172:6003-10 (2004)), yet simultaneously able to suppress naïve T-cells of other specificities (Cobbold et al., *Immunol Rev.* 129:165-201 (1992); Qin et al., *Eur J Immunol.* 20:2737-45 (1990)). These cells, now recognized as Tregs, may promote tolerance by a number of mechanisms, including (but not limited to) the expression of suppressive factors like TGF beta (Nakamura et al., *The Journal of experimental medicine.* 194:629-44 (2001); Nakamura et al., *J Immunol.* 172:834-42 (2004)), CTLA4 (Tang et al., *J Immunol.* 181:1806-13 (2008); Walker et al., *Trends Immunol.* 36:63-70 (2015)), IL10 (O'Garra et al., *J Clin Invest.* 114:1372-8 (2004); Chaudhry et al., *Immunity.* 34:566-78 (2011)), and IL35 (Collison et al., *Nature.* 450:566-9 (2007)), as well as the preferential consumption of IL-2 (Shevach et al., *Immunity.* 30:636-45 (2009); Setoguchi et al., *J Exp Med.* 201:723-35 (2005)), manipulation or killing of antigen presenting cells (Mahnke et al., *Cell Immunol.* 250:1-13 (2007); Shevach et al., *Immunol Rev.* 212:60-73 (2006)), and depletion of local ATP (Regateiro et al., *Eur J Immunol.* 41:2955-65 (2011); Regateiro et al., *Clin Exp Immunol.* 171:1-7 (2013)) or essential amino acids (Cobbold et al., *Proc Natl Acad Sci U S A.* 106:12055-60 (2009)).

Two approaches for potential therapeutic uses of Tregs involve either *in-vitro* expansion using donor antigens coupled with transplantation, or selective *in-vivo* expansion that leverages differences between regulatory and effector T-cells. While these strategies are interesting, to date no long term of acceptance of an allograft has been demonstrated solely with the use of *in-vitro* or *in-vivo* expanded Tregs. There remain many complications and unknown facets to Treg biology, including the optimal methodology for *in-vitro* or *in-vivo* expansion, as well as the therapeutically-relevant dosage and timing. It has also been shown that antigen-specific Treg suppression can be "defeated" depending on the inflammatory context (Korn et al., *Nat Med.* 13:423-31 (2007)) and that Tregs can be killed by NK-cells (Roy et al., *J Immunol.* 180:1729-36 (2008)).

In addition to Tregs, other suppressive cell types have also been explored to induce allograft tolerance, such as antigen presenting cells like dendritic cells (DCs) (Walker et al., *Trends Immunol.*

36:63-70 (2015)). DCs are the link between innate and adaptive immunity, and they can induce both effector and suppressive immune responses depending on contexts like their maturation state and the local inflammatory cues. During allograft rejection, DCs present allograft antigens inside the binding grooves of MHC (mouse) or HLA (human) molecules on their surface, along with costimulatory molecules like CD80, CD86, and CD40 (among others), which allograft-specific T-cell clones recognize to become activated (Walker et al., *Trends Immunol.* 36:63-70 (2015)). Tolerogenic DCs can be induced from the immature state by exposure to suppressive cues, which keep expression levels of MHC and costimulatory molecules low and in turn promote naïve T-cells into anergic or even Tregulatory subtypes upon DC-Tcell interactions.

Therapeutically, one application of this biology is to expand DCs *in vitro* exposed simultaneously to specific allograft antigens of interest and immunosuppressive factors – many of which have been tested including TGF-beta, IL10, cAMP, prostaglandin E2, histamine, neuropeptides, vitamin D2, B2 agonists, HLA-G, glucosamine, as well drugs like corticosteroids, cyclosporine, tacrolimus, rapamycin, aspirin, mecophenolate mofetil, sanglifehrin, and deoxyspergualin (Hackstein et al., *Nat Rev Immunol.* 4:24-34 (2004)). Alternatively, DCs have been genetically engineered to directly express immunomodulatory factors like TGF-beta, IL-10, VEGF, FasL, CTLA4-Ig, IDO, NFKb decoy receptors, soluble TNFR, CCR7, as well as siRNA-induced silencing of IL-12 (Morelli et al., *Immunol Rev.* 196:125-46 (2003)). These cultured or engineered DCs are then transferred into recipients concomitantly with an allograft to test whether they can prolong the survival of an allograft, with the assumption that they suppress allograft-specific T-cells, or increase the number of allograft-focused Tregulatory cells.

In one prototypical approach of this kind, bone-marrow derived DCs were transduced with SOCS1 (preventing upregulation of costimulatory molecules and MHCII), which prolonged mouse cardiac allografts (Fu et al., *Cell Mol Immunol.* 6:87-95 (2009)). In another demonstration, FasL-expressing DCs were also able to prolong mouse cardiac allografts (Min et al., *J Immunol.* 164:161-7 (2000)). In general there have been many singular and combinatorial approaches using tolerogenic DCs along these lines (Bjorck et al., *J Heart Lung Transplant.* 24:1118-20 (2005): Sun et al., *PLoS One.* 7:e52096 (2012); Li et al., *J Immunol.* 178:5480-7 (2007); Xu et al., *Transplant Proc.* 38:1561-3 (2006); Lan et al., *J Immunol.* 177:5868-77 (2006); Lutz et al., *Eur J Immunol.* 30:1813-22 (2000); Fischer et al., *Transpl Immunol.* 25:20-6 (2011)), and the outcomes are highly variable depending on the type of modification to the DCs, culture conditions, timing, and type of allograft being tested (Zhou et al., *J Immunol Res.* 2016:5730674 (2016); Xia et al., *J Evid Based Med.* 7:135-46 (2014)). Almost all of these studies have been done in mouse, although recent human testing has begun including testing for safety in healthy volunteers (Dhodapkar et al., *J Exp Med.* 193:233-8 (2001); Dhodapkar et al., *Blood.* 100:174-7 (2002)) as well as a phase I clinical trial in 10 patients with diabetes (Giannoukakis et al., *Diabetes Care.* 34:2026-32 (2011)).

There remain many unknowns to both adoptive Treg and tolerogenic DC therapies, and one of the most important is the duration of their efficacy. While *in-vivo* studies show that prolonged allograft survival is possible using these two approaches (with or without additional immunosuppressive drugs), it

is not long-term, and in almost every case the allograft eventually dies. This is fitting with the fact that both Tregs and DCs have a finite time-span. Also, it is possible for tolerogenic phenotypes, especially among DCs, to "convert" and instead promote inflammatory pathways (Delamarre et al., *Semin Immunol.* 23:2-11 (2011); Schreibelt et al., *Cancer Immunol Immunother.* 59:1573-82 (2010); Satpathy et al., *Nat Immunol.* 14:937-48 (2013)). This is likely due to the highly adaptive nature of DCs, and their ability to sense and respond to a large breadth of inflammatory cues. It has also been shown that these cells can die very quickly after *in-vivo* adoptive transfer. There are also many subsets of suppressive Tregs and tolerogenic DCs that have been described, and it is still unclear which is the ideal subtype, or if it will entirely depend on the context of the allograft transplant.

Additionally, there is a huge practical and economical barrier to these kinds of approaches in that they require clinicians to manipulate and work with a complicated immune cell type *in addition* to the therapeutic one. Given their finite lifespan, it is still unclear if these cells would need to be continuously and/or repeatedly delivered to confer long-term tolerance to an allograft. This would compound the already expensive and timely methodology for culturing, expanding, or transducing the cells with critical immunomodulatory factors, and ultimately impede the uses for treatments that are extremely time-sensitive.

Another approach for inducing tolerance is the use of Hematopoietic Cell transplantation (HCT), in which recipients of an HLA-mismatched organ receive an HCT using hematopoietic cells from the same donor (Gozzo et al., *Surg Forum.* 21:281-4 (1970); Ildstad et al., *Nature.* 307:168-70 (1984); Sayegh et al., *Ann Intern Med.* 114:954-5 (1991); Huang et al., *J Clin Invest.* 105:173-81 (2000); Kawai et al., *N Engl J Med.* 358:353-61 (2008); Sachs et al., *Semin Immunol.* 23:165-73 (2011)). This results in a chimerism that can allow newly developing T and B-cells in the recipient to be tolerant of both the recipient and the donor antigens (Tomita et al., *J Immunol.* 153:1087-98 (1994); Tomita et al., *Transplantation.* 61:469-77 (1996); Tomita et al., *Transplantation.* 61:477-85 (1996); Khan et al., *Transplantation.* 62:380-7 (1996); Manilay et al., *Transplantation.* 66:96-102 (1998)). This is due to the role that hematopoietic cells play in positive and negative selection in the thymus, where they eliminate cells with an affinity for hematopoietic cell-containing antigens that might also be present in the allograft, ultimately leading to their rejection. (Griesemer et al., *Transplantation.* 90:465-74 (2010)). However, the inherent and dangerous risk of this approach is the potential for Graft vs. Host Disease (GVHD), in which transplanted hematopoietic cells recognize and systemically attack the recipient tissues as foreign (Sun et al., *PLoS One.* 7:e52096 (2012)). Since its inception, several variants of HCT to dampen rejection have been developed, including the use of nonmyeloablative strategies. These strategies use altered chemotherapy regimens, often involving lower dosages, so that the recipient receiving the HCT does not receive total ablation of their hematopoietic compartment. The most recent of these strategies, for instance, used a tolerance-promoting facilitating cell (FC)-based HCT to promote tolerance in HLA-mismatched kidney recipients while largely avoiding GHVD (Leventhal et al., *Sci Transl Med.* 4:124ra28 (2012)).

Besides the risk of GHVD and risk of a secondary HCT procedure, the general limitation to these chimerism-inducing approaches is the need to have the HLA-matched donor available for the collection of marrow. While this is easily accomplished in rodent studies, it is quite demanding in humans. Donor organs should ideally be taken from the donor as soon as possible, which leaves an incredibly short window from which to collect marrow, if at all possible. It is also an expensive and logistically demanding procedure that requires a very patient and operation-specific approach. And, as with regulatory cell approaches, it is not clear how it would be practically applied to those situations where the patient could benefit from or needs therapy immediately for treatment of acute injuries or disease.

Another approach that has been tested for reducing allorejection *in vivo* is the removal of histocompatibility molecules (Torikai et al. *Blood.* 122:1341-9 (2013)), which are the major antigenic source of "non-self" recognition in allorejection. This fits with the empirical data that HLA-matched donors and recipients have greatly improved rates of organ survival after transplantation (Opelz et al., *Rev Immunogenet.* 1:334-42 (1999)). While there have been some positive results with this approach, removal of MHC class I renders cells extremely susceptible to NK cells (Pegram et al., *Immunol Cell Biol.* 89:216-24 (2011); Raulet et al., *Nat Rev Immunol.* 6:520-31 (2006); Huntington, *Immunol Cell Biol.* 92:208-9 (2014)). It also leaves MHC-independent killing pathways among CD8+ T-cells intact (Haspot et al., *Am J Transplant.* 14:49-58 (2014)) and does not address antigenic differences (minor antigens) outside the MHC/HLA gene family (Roopenian et al., *Immunol Rev.* 190:86-94 (2002)).

An employment of this approach involved the deletion of all classical HLA class I molecules from pluripotent stem cells, coupled with the introduction of the gene encoding HLA-E, a minimally polymorphic HLA that inhibits NK-cells (Gornalusse et al., *Nat Biotechnol.* (2017)). While this approach showed short-term resistance to NK and CD8 T-cell attack in partially immune compromised humanized mice, it was not demonstrated that these cells could survive long term in a fully immune-competent host. In another approach ES cells were engineered to express PD-L1 and CTLA4-Ig, which improved survival in allogenic hosts (Rong et al., *Cell Stem Cell.* 14:121-30 (2014)), but with the severe limitation that CTLA4-Ig can lead to systemic immune suppression. It has not yet been demonstrated that a set of modifications to ES or iPS cells allows them to escape allorejection without the potential for systemic immunosuppression and without the need for immunosuppressive drugs.

It is an object of the present disclosure to mitigate and/or obviate one or more of the above deficiencies.

SUMMARY OF THE DISCLOSURE

In an aspect, a cell genetically modified to comprise at least one mechanism for providing a local immunosuppression at a transplant site when transplanted in an allogeneic host is provided. The genetically modified cell comprises: a set of transgenes, each transgene encoding a gene product that is cytoplasmic, membrane bound, or local acting, and has one or more of the following functions: a) to mitigate antigen presenting cell activation and function; b) to mitigate graft attacking leukocyte activity or cytolytic

function; c) to mitigate macrophage cytolytic function and phagocytosis of allograft cells; d) to induce apoptosis in graft attacking leukocytes; e) to mitigate local inflammatory proteins; and f) to protect against leukocyte-mediated apoptosis.

In an embodiment of the cell, the set of transgenes comprises one or more (e.g., one, two, three, four, five, six, seven, or all eight) of the following genes: PD-L1, HLA-G (or the mouse version of HLA-G, H2-M3), Cd47, Cd200, FASLG (or the mouse version of FASLG, FasL), Ccl21 (or the mouse version of Ccl21, Ccl21b), Mfge8, and Serpin B9 (or the mouse version of Serpin B9, Spi6).

In an embodiment of the cell, the set of transgenes comprises two or more of the following genes: PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6).

In an embodiment of the cell, the set of transgenes genes comprises PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) or a gene encoding a biologic that acts as an agonist of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6).

In an embodiment of the cell, the cell further comprises one or more (e.g., one, two, three, four, five, six, seven, eight, nine, ten, or all eleven) of the following transgenes: TGF-β, Cd73, Cd39, Lag3, Il1r2, Ackr2, Tnfrsf22, Tnfrs23, Tnfrsf10, Dad1, and IFNγR1 d39 or a gene encoding a biologic that acts as an agonist of TGF-β, Cd73, Cd39, Lag3, Il1r2, Ackr2, Tnfrsf22, Tnfrs23, Tnfrsf10, Dad1, or IFNγR1 d39.

In an embodiment of the cell, the TGF-β or the biologic is local acting in the graft environment. In an embodiment of the cell, the TGF-β or the biologic is local acting in the graft environment with minimal systemic effect.

In various embodiments of the cell, the cell is a stem cell, a cell amenable for genome editing, and/or a source of a therapeutic cell type (e.g., a cell that can be differentiated into a therapeutic cell type, or a cell of a desired target tissue). In various embodiments, the cell is an embryonic stem cell, a pluripotent stem cell, an induced pluripotent stem cell, a hematopoietic stem cell, a mesenchymal stem cell, an endothelial stem cell, an epithelial stem cell, an adipose stem or progenitor cell, a lung stem or progenitor cells, a mammary stem cell, an olfactory adult stem cell, a hair follicle stem cell, an intestinal stem or progenitor cell, a multipotent stem cell, an amniotic stem cell, a cord blood stem cell, a neural stem or progenitor cell, an adult stem cell, a somatic stem cell, a tissue-specific stem cell, a totipotent stem cell, a fibroblast, a monocytic precursor, a B cell, an exocrine cell, a pancreatic progenitor, an endocrine progenitor, a hepatoblast, a myoblast, a preadipocyte, a hepatocyte, a chondrocyte, a smooth muscle cell, a K562 human erythroid leukemia cell line, a bone cell, a synovial cell, a tendon cell, a ligament cell, a meniscus cell, an adipose cell, a dendritic cell, a natural killer cell, a skeletal muscle cell, a cardiac muscle cell, an erythroid-megakaryocytic cell, an eosinophil, a macrophage, a T cell, an islet beta-cell, a neuron, a cardiomyocyte, a blood cell, an exocrine progenitor, a ductal cell, an acinar cell, an alpha cell, a beta cell, a delta cell, a PP cell, a cholangiocyte, a white or brown adipocyte, a hormone-secreting cell, an epidermal keratinocyte, an epithelial cell, a kidney cell, a germ cell, a skeletal joint

synovium cell, a periosteum cell, a perichondrium cell, a cartilage cell, an endothelial cell, a pericardium cell, a meningeal cell, a keratinocyte precursor cell, a keratinocyte stem cell, a pericyte, a glial cell, an ependymal cell, a cell isolated from an amniotic or placental membrane, a serosal cell, a somatic cell, or a cell derived from skin, heart, brain or spinal cord, liver, lung, kidney, pancreas, bladder, bone marrow, spleen, intestine, or stomach.

In an embodiment of the cell, the cell is further genetically modified to comprise at least one (e.g., one, two, three, or more) mechanism for controlling cell proliferation (e.g., to reduce the tumorigenic potential of the modified cell or to reduce proliferation of a modified cell that has become tumorigenic). The genetically modified cell comprises: a genetic modification of one or more (e.g., one, two, three, or more) cell division locus/loci (CDL), the CDL being one or more loci whose transcription product(s) is expressed by dividing cells (e.g., all dividing cells containing one or more of the immunosuppressive transgenes), the genetic modification being one or more of: a) an ablation link (ALINK) system, the ALINK system comprising a DNA sequence encoding a negative selectable marker that is transcriptionally linked to a DNA sequence encoding the CDL; and b) an exogenous activator of regulation of a CDL (EARC) system, the EARC system comprising an inducible activator-based gene expression system that is operably linked to the CDL.

In an embodiment of the cell, the genetic modification of the CDL comprises performing targeted replacement of the CDL with one or more of: a) a DNA vector comprising the ALINK system; b) a DNA vector comprising the EARC system; and c) a DNA vector comprising the ALINK system and the EARC system; wherein the ALINK and/or EARC systems are each operably linked to the CDL.

In various embodiments of the cell, the ALINK genetic modification of the CDL is homozygous, heterozygous, hemizygous or compound heterozygous and/or the EARC genetic modification ensures that functional CDL modification can only be generated through EARC-modified alleles.

In various embodiments of the cell, the CDL is one or more (e.g., one, two, three, or more) of the loci recited in Table 5. In various embodiments, the CDL encodes a gene product that functions in one or more of: cell cycle, DNA replication, RNA transcription, protein translation, and metabolism. In various embodiments, the CDL is one or more of Cdk1/CDK1, Top2A/TOP2A, Cenpa/CENPA, Birc5/BIRC5, and Eef2/EEF2, preferably the CDL is Cdk1 or CDK1. In some embodiments, the CDL is Top2A. In some embodiments, the CDL is Eef2. In various embodiments, the CDL is two or more of Cdk1/CDK1, Top2A/TOP2A, Cenpa/CENPA, Birc5/BIRC5, and Eef2/EEF2, preferably the CDL is Cdk1 and Top2A or Cdk1 and Eef2.

In various embodiments of the cell, the ALINK system comprises a herpes simplex virus-thymidine kinase/ganciclovir system, a cytosine deaminase/5-fluorocytosine system, a carboxyl esterase/irinotecan system or an iCasp9/AP1903 system, preferably the ALINK system is a herpes simplex virus-thymidine kinase/ganciclovir system.

In various embodiments of the cell, the EARC system is a doxycycline inducible "dox-bridge" system, a cumate switch inducible system, an ecdysone inducible system, a radio wave inducible system, or a ligand-reversible dimerization system, preferably the EARC system is a dox-bridge system.

In an aspect, a method for providing a local immunosuppression at a transplant site in an allogeneic host is provided. The method comprises providing a cell; and expressing in the cell a set of transgenes, each transgene encoding a gene product that is cytoplasmic, membrane bound, or local acting, and has one or more of the following functions: a) to mitigate antigen presenting cell activation and function; b) to mitigate graft attacking leukocyte activity or cytolytic function; c) to mitigate macrophage cytolytic function and phagocytosis of allograft cells; d) to induce apoptosis in graft attacking leukocytes; e) to mitigate local inflammatory proteins; and f) to protect against leukocyte-mediated apoptosis.

In an embodiment of the method, the set of transgenes comprises one or more (e.g., one, two, three, four, five, six, seven, or all eight) of the following genes: PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) or a gene encoding a biologic that acts as an agonist of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, or Serpin B9 (Spi6).

In an embodiment of the method, the set of transgenes comprises two or more of the following genes: PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6).

In an embodiment of the method, the set of transgenes genes comprises PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) or a gene encoding a biologic that acts as an agonist of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6).

In an embodiment of the method, the method further comprises expressing one or more (e.g., one, two, three, four, five, six, seven, eight, nine, ten, or all eleven) of the following transgenes: TGF-β, Cd73, Cd39, Lag3, Il1r2, Ackr2, Tnfrsf22, Tnfrs23, Tnfrsf10, Dad1, and IFNγR1 d39 or a gene encoding a biologic that acts as an agonist of TGF-β, Cd73, Cd39, Lag3, Il1r2, Ackr2, Tnfrsf22, Tnfrs23, Tnfrsf10, Dad1, or IFNγR1 d39. In an embodiment, the TGF-β or the biologic is local acting in the graft environment. In an embodiment of the cell, the TGF-β or the biologic is local acting in the graft environment with minimal systemic effect.

In various embodiments of the method, the cell is a stem cell, a cell amenable to genome editing, and/or a source of a therapeutic cell type (e.g., a cell that can be differentiated into a therapeutic cell type, or a cell of a desired target tissue). In various embodiments, the cell is an embryonic stem cell, a pluripotent stem cell, an induced pluripotent stem cell, a hematopoietic stem cell, a mesenchymal stem cell, an endothelial stem cell, an epithelial stem cell, an adipose stem or progenitor cell, a lung stem or progenitor cells, a mammary stem cell, an olfactory adult stem cell, a hair follicle stem cell, an intestinal stem or progenitor cell, a multipotent stem cell, an amniotic stem cell, a cord blood stem cell, a neural stem or progenitor cell, an adult stem cell, a somatic stem cell, a tissue-specific stem cell, a totipotent

stem cell, a fibroblast, a monocytic precursor, a B cell, an exocrine cell, a pancreatic progenitor, an endocrine progenitor, a hepatoblast, a myoblast, a preadipocyte, a hepatocyte, a chondrocyte, a smooth muscle cell, a K562 human erythroid leukemia cell line, a bone cell, a synovial cell, a tendon cell, a ligament cell, a meniscus cell, an adipose cell, a dendritic cell, a natural killer cell, a skeletal muscle cell, a cardiac muscle cell, an erythroid-megakaryocytic cell, an eosinophil, a macrophage, a T cell, an islet beta-cell, a neuron, a cardiomyocyte, a blood cell, an exocrine progenitor, a ductal cell, an acinar cell, an alpha cell, a beta cell, a delta cell, a PP cell, a cholangiocyte, a white or brown adipocyte, a hormone-secreting cell, an epidermal keratinocyte, an epithelial cell, a kidney cell, a germ cell, a skeletal joint synovium cell, a periosteum cell, a perichondrium cell, a cartilage cell, an endothelial cell, a pericardium cell, a meningeal cell, a keratinocyte precursor cell, a keratinocyte stem cell, a pericyte, a glial cell, an ependymal cell, a cell isolated from an amniotic or placental membrane, a serosal cell, a somatic cell, or a cell derived from skin, heart, brain or spinal cord, liver, lung, kidney, pancreas, bladder, bone marrow, spleen, intestine, or stomach.

In various embodiments of the method, the cell is provided (e.g., injected) to or near the transplant site. In various embodiments of the method, the cell is provided (e.g., injected or implanted) into the transplant (e.g., injected or implanted into the tissue or organ transplant before, during, or after transplantation). In some embodiments, the cell in which the transgenes are expressed is a cell of the transplant (e.g., a cell of the tissue or organ that is being transplanted is modified to express one or more (e.g., one, two, three, four, five, six, seven, or all eight) of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6)).

In an aspect, a method of controlling proliferation of a cell at a transplant site in an allogeneic host is provided (e.g., to reduce the tumorigenic potential of the cell at the transplant site or to reduce proliferation of the cell that has become tumorigenic at the transplant site). The method comprises: a) genetically modifying in the cell one or more (e.g., one, two, three, or more) cell division locus/loci (CDL), the CDL being one or more loci whose transcription product(s) is expressed by dividing cells (e.g., all dividing cells containing one or more of the immunosuppressive transgenes), the genetic modification of the CDL comprising one or more of: i) an ablation link (ALINK) system, the ALINK system comprising a DNA sequence encoding a negative selectable marker that is transcriptionally linked to a DNA sequence encoding the CDL; and i) an inducible exogenous activator of regulation of a CDL (EARC) system, the EARC system comprising an inducible activator-based gene expression system that is operably linked to the CDL; b) genetically modifying the cell to comprise at least one mechanism for providing a local immunosuppression at a transplant site; c) transplanting the cell or a population of the cells at a transplantation site in an allogeneic host; and d) permitting proliferation of the genetically modified cell comprising the ALINK system by maintaining the genetically modified cell comprising the ALINK system in the absence of an inducer of the negative selectable marker or ablating and/or inhibiting proliferation of the genetically modified cell comprising the ALINK system by exposing the cell comprising the ALINK system to the inducer of the negative selectable marker; and/or permitting proliferation of the genetically

modified cell comprising the EARC system by exposing the genetically modified cell comprising the EARC system to an inducer of the inducible activator-based gene expression system or preventing or inhibiting proliferation of the genetically modified cell comprising the EARC system by maintaining the cell comprising the EARC system in the absence of the inducer of the inducible activator-based gene expression system.

In an embodiment of the method, the genetic modification of the CDL comprises performing targeted replacement of the CDL with one or more of: a) a DNA vector comprising the ALINK system; b) a DNA vector comprising the EARC system; and c) a DNA vector comprising the ALINK system and the EARC system; wherein the ALINK and/or EARC systems are each operably linked to the CDL.

In various embodiments of the method the ALINK genetic modification of the CDL is homozygous, heterozygous, hemizygous or compound heterozygous and/or the EARC genetic modification ensures that functional CDL modification can only be generated through EARC-modified alleles.

In various embodiments of the method, the CDL is one or more (e.g., one, two, three, or more) of the loci recited in Table 5. In various embodiments, the CDL encodes a gene product whose function is involved with one or more of: cell cycle, DNA replication, RNA transcription, protein translation, and metabolism. In various embodiments, the CDL is one or more of Cdk1/CDK1, Top2A/TOP2A, Cenpa/CENPA, Birc5/BIRC5, and Eef2/EEF2, preferably the CDL is Cdk1 or CDK1. In some embodiments, the CDL is Top2A. In some embodiments, the CDL is Eef2. In various embodiments, the CDL is two or more of Cdk1/CDK1, Top2A/TOP2A, Cenpa/CENPA, Birc5/BIRC5, and Eef2/EEF2, preferably the CDL is Cdk1 and Top2A or Cdk1 and Eef2.

In various embodiments of the method, the ALINK system comprises a herpes simplex virus-thymidine kinase/ganciclovir system, a cytosine deaminase/5-fluorocytosine system, a carboxyl esterase/irinotecan system or an iCasp9/AP1903 system, preferably the ALINK system is a herpes simplex virus-thymidine kinase/ganciclovir system.

In various embodiments of the method, the EARC system is a doxycycline inducible “dox-bridge” system, a cumate switch inducible system, an ecdysone inducible system, a radio wave inducible system, or a ligand-reversible dimerization system, preferably the EARC system is a dox-bridge system.

In an embodiment of the method, the genetically modified cell comprises: a set of transgenes, each transgene encoding a gene product that is cytoplasmic, membrane bound, or local acting and has one or more of the following functions: a) to mitigate antigen presenting cell activation and function; b) to mitigate graft attacking leukocyte activity or cytolytic function; c) to mitigate macrophage cytolytic function and phagocytosis of allograft cells; d) to induce apoptosis in graft attacking leukocytes; e) to mitigate local inflammatory proteins; and f) to protect against leukocyte-mediated apoptosis.

In an embodiment of the method, the set of transgenes comprises one or more (e.g., one, two, three, four, five, six, seven, or all eight) of the following genes: PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) or a gene encoding a biologic that acts as

an agonist of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, or Serpin B9 (Spi6).

In an embodiment of the method, the set of transgenes comprises two or more of the following genes: PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6).

In an embodiment of the method, the set of transgenes genes comprises PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) or a gene encoding a biologic that acts a as an agonist of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6).

In an embodiment of the method, the cell further comprises one or more (e.g., one, two, three, four, five, six, seven, eight, nine, ten, or all eleven) of the following transgenes: TGF-β, Cd73, Cd39, Lag3, Il1r2, Ackr2, Tnfrsf22, Tnfrs23, Tnfrsf10, Dad1, and IFNγR1 d39 or a gene encoding a biologic that acts as an agonist of TGF-β, Cd73, Cd39, Lag3, Il1r2, Ackr2, Tnfrsf22, Tnfrs23, Tnfrsf10, Dad1, or IFNγR1 d39.

In an embodiment of the method, the TGF-β or the biologic is local acting in the graft environment. In an embodiment, the TGF-β or the biologic is local acting in the graft environment with minimal systemic effect

In various embodiments of the method, the cell is a stem cell, a cell amenable to genome editing, and/or a source of therapeutic cell type (e.g., a cell that can be differentiated into a therapeutic cell type, or a cell of a desired target tissue). In various embodiments, the cell is an embryonic stem cell, a pluripotent stem cell, an induced pluripotent stem cell, a hematopoietic stem cell, a mesenchymal stem cell, an endothelial stem cell, an epithelial stem cell, an adipose stem or progenitor cell, a lung stem or progenitor cells, a mammary stem cell, an olfactory adult stem cell, a hair follicle stem cell, an intestinal stem or progenitor cell, a multipotent stem cell, an amniotic stem cell, a cord blood stem cell, a neural stem or progenitor cell, an adult stem cell, a somatic stem cell, a tissue-specific stem cell, a totipotent stem cell, a fibroblast, a monocytic precursor, a B cell, an exocrine cell, a pancreatic progenitor, an endocrine progenitor, a hepatoblast, a myoblast, a preadipocyte, a hepatocyte, a chondrocyte, a smooth muscle cell, a K562 human erythroid leukemia cell line, a bone cell, a synovial cell, a tendon cell, a ligament cell, a meniscus cell, an adipose cell, a dendritic cell, a natural killer cell, a skeletal muscle cell, a cardiac muscle cell, an erythroid-megakaryocytic cell, an eosinophil, a macrophage, a T cell, an islet beta-cell, a neuron, a cardiomyocyte, a blood cell, an exocrine progenitor, a ductal cell, an acinar cell, an alpha cell, a beta cell, a delta cell, a PP cell, a cholangiocyte, a white or brown adipocyte, a hormone-secreting cell, an epidermal keratinocyte, an epithelial cell, a kidney cell, a germ cell, a skeletal joint synovium cell, a periosteum cell, a perichondrium cell, a cartilage cell, an endothelial cell, a pericardium cell, a meningeal cell, a keratinocyte precursor cell, a keratinocyte stem cell, a pericyte, a glial cell, an ependymal cell, a cell isolated from an amniotic or placental membrane, a serosal cell, a somatic cell, or a

cell derived from skin, heart, brain or spinal cord, liver, lung, kidney, pancreas, bladder, bone marrow, spleen, intestine, or stomach.

In various embodiments of the method, the cell is provided (e.g., injected) to or near the transplant site. In various embodiments of the method, the cell is provided (e.g., injected or implanted) into the transplant (e.g., injected or implanted into the tissue or organ transplant before, during, or after transplantation). In some embodiments, the cell in which the transgenes are expressed is a cell of the transplant (e.g., a cell of the tissue or organ that is being transplanted is modified to express one or more (e.g., one, two, three, four, five, six, seven, or all eight) of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6)).

In various embodiments of the method, the allogeneic host is a mammal. In various embodiments of the method, the allogeneic host is a mouse or a human.

In various embodiments of the method, the host has a degenerative disease or condition that can be treated with cell therapy. In various embodiments, the disease or condition is blindness, arthritis (e.g., osteoarthritis or rheumatoid arthritis), ischemia, diabetes (e.g., Type 1 or Type 2 diabetes), multiple sclerosis, spinal cord injury, stroke, cancer, a lung disease, a blood disease, a neurological disease, such as Parkinson's disease, Alzheimer's disease, Huntington's disease, and ALS, an enzyme or hormone deficiency, a metabolic disorder (e.g., a lysosomal storage disorder, Galactosemia, Maple syrup urine disease, Phenylketonuria, a glycogen storage disease, a mitochondrial disorder, Friedrich's ataxia, a peroxisomal disorder, a metal metabolism disorder, or an organic academia), an autoimmune disease (e.g., Psoriasis, Systemic Lupus Erythematosus, Grave's disease, Inflammatory Bowel Disease, Addison's Diseases, Sjogren's Syndrome, Hashimoto's Thyroiditis, Vasculitis, Autoimmune Hepatitis, Alopecia Areata, Autoimmune pancreatitis, Crohn's Disease, Ulcerative colitis, Dermatomyositis), age-related macular degeneration, retinal dystrophy, an infectious disease, hemophilia, a degenerative disease (e.g., Charcot-Marie-Tooth disease, chronic obstructive pulmonary disease, chronic traumatic encephalopathy, Creutzfeldt-Jakob disease, Cystic Fibrosis, Cytochrome C Oxidase deficiency, Ehlers-Danlos syndrome, essential tremor, Fribrodisplasia Ossificans Progressiva, infantile neuroaxonal dystrophy, keratoconus, keratoglobus, muscular dystrophy, neuronal ceroid lipofuscinosis, a prior disease, progressive supranuclear palsy, sandhoff disease, spinal muscular atrophy, retinitis pigmentosa), or an age-related disease (e.g., atherosclerosis, cardiovascular disease (e.g., angina, myocardial infarction), cataracts, osteoporosis, or hypertension).

In some embodiments of any of the foregoing aspects, one or more (e.g., one, two, three, four, five, six, seven, or all eight) of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) is expressed at a level that is equal to or greater than the expression level of the corresponding endogenous gene in an activated leukocyte (e.g., a T cell, e.g., the expression level of the cloaking transgene is equal to the level of expression of the endogenous gene in activated leukocytes, or is 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10-fold or more higher than the level of expression of the endogenous gene in activated leukocytes). In some embodiments, all eight of PD-L1, HLA-G (H2-M3),

Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) are expressed at a level that is equal to or greater than the expression level of the corresponding endogenous gene in an activated leukocyte.

In some embodiments of any of the foregoing aspects, one or more (e.g., one, two, three, four, five, six, seven, or all eight) of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) is expressed at a level that is greater than the expression level of the corresponding endogenous gene in a wild-type stem cell (e.g., a wild-type ES cell from the same species, e.g., the expression level of the cloaking transgene is at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 500, 1,000-fold or more higher in cloaked cells compared to expression of the endogenous gene in unmodified wild-type ES cells from the same species). In some embodiments, all 8 of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) are expressed at a level that is greater (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100-fold higher or more) than the expression level of the endogenous gene in a wild-type stem cell (e.g., an embryonic stem cell from the same species as the cloaked cell). In some embodiments, one or more (e.g., one, two, three, four, five, six, seven, or all eight) of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) is expressed at a level that is in the top 5% of gene expression for all genes in the ES cell genome. In some embodiments, one or more (e.g., one, two, three, four, five, six, seven, or all eight) of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) is expressed at a level that is in the top 1% of gene expression for all genes in the ES cell genome. In some embodiments, all of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) are expressed at a level that is in the top 5% of gene expression for all genes in the ES cell genome.

In some embodiments of any of the foregoing aspects, the PD-L1 transgene encodes a protein having at least 85% identity (e.g., 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity or more) to the amino acid sequence of SEQ ID NO: 11 or SEQ ID NO: 12.

In some embodiments of any of the foregoing aspects, the HLA-G (H2-M3) transgene encodes a protein having at least 85% identity (e.g., 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity or more) to the amino acid sequence of SEQ ID NO: 16 or SEQ ID NO: 15.

In some embodiments of any of the foregoing aspects, the Cd47 transgene encodes a protein having at least 85% identity (e.g., 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity or more) to the amino acid sequence of SEQ ID NO: 3 or SEQ ID NO: 4.

In some embodiments of any of the foregoing aspects, the CD200 transgene encodes a protein having at least 85% identity (e.g., 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity or more) to the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 6.

In some embodiments of any of the foregoing aspects, the FASLG (FasL) transgene encodes a protein having at least 85% identity (e.g., 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity or more) to the amino acid sequence of SEQ ID NO: 10 or SEQ ID NO: 9.

In some embodiments of any of the foregoing aspects, the Ccl21 (Ccl21b) transgene encodes a protein having at least 85% identity (e.g., 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity or more) to the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 1.

In some embodiments of any of the foregoing aspects, the Mfge8 transgene encodes a protein having at least 85% identity (e.g., 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity or more) to the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 14.

In some embodiments of any of the foregoing aspects, the Serpin B9 (Spi6) transgene encodes a protein having at least 85% identity (e.g., 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity or more) to the amino acid sequence of SEQ ID NO: 8 or SEQ ID NO: 7.

In some embodiments of any of the foregoing aspects, the IFNγR1 d39 transgene encodes a protein having at least 85% identity (e.g., 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity or more) to the amino acid sequence of SEQ ID NO: 17.

In some embodiments of any of the foregoing aspects, the one or more transgenes is operably linked to a constitutive promoter. In some embodiments, the constitutive promoter is selected from the group consisting of the CAG promoter, the cytomegalovirus (CMV) promoter, the EF1α promoter, the PGK promoter, adenovirus late promoter, vaccinia virus 7.5K promoter, SV40 promoter, *tk* promoter of HSV, mouse mammary tumor virus (MMTV) promoter, LTR promoter of HIV, promoter of moloney virus, Epstein barr virus (EBV) promoter, and the Rous sarcoma virus (RSV) promoter.

In some embodiments of any of the foregoing aspects, the cell further comprises (e.g., the cell is further modified to include) a transgene encoding a therapeutic agent. In some embodiments, the therapeutic agent is a protein or antibody. In some embodiments, the antibody is an inhibitory antibody or agonist antibody. In some embodiments, the therapeutic agent is an agent listed in Table 2. In some embodiments, the therapeutic agent is the wild-type version of a gene that is mutated in the subject (e.g., the wild-type version of the mutated gene that is associated with the disease or condition in the subject, e.g., a genetic mutation that is associated with cancer, an enzyme or hormone deficiency, a metabolic disorder, or a degenerative disease). In some embodiments, the therapeutic agent is expressed using an inducible expression system selected from the group consisting of a tetracycline response element, a light inducible system, a radiogenetic system, a cumate switch inducible system, an ecdysone inducible system, a destabilization domain system, or a ligand-reversible dimerization system. In some embodiments, the therapeutic agent is expressed using a constitutive promoter selected from the group consisting of the CAG promoter, the cytomegalovirus (CMV) promoter, the EF1α promoter, the PGK promoter, adenovirus late promoter, vaccinia virus 7.5K promoter, SV40 promoter, *tk* promoter of HSV, mouse mammary tumor virus (MMTV) promoter, LTR promoter of HIV, promoter of moloney virus, Epstein barr virus (EBV) promoter, and the Rous sarcoma virus (RSV) promoter.

In another aspect, there is provided a population of genetically modified cells according to any of the cells described above.

In an aspect, a method for providing a local immunosuppression at a transplant site in an allogeneic host is provided. The method comprises transplanting a genetically modified cell as described above or a population of genetically modified cells as described above at a transplantation site in an allogeneic host.

In another aspect, the invention features a composition containing a cell of the invention. In some embodiments, the composition further includes a pharmaceutically acceptable excipient.

In another aspect, featured is a kit including a cell of the invention or a pharmaceutical composition of the invention.

In another aspect, featured is a method of treating a disease or condition in a subject in need thereof by administering to the subject the cell of the invention or a composition of the invention. In some embodiments, the disease or condition is blindness, arthritis (e.g., osteoarthritis or rheumatoid arthritis), ischemia, diabetes (e.g., Type 1 or Type 2 diabetes), multiple sclerosis, spinal cord injury, stroke, cancer, a lung disease, a blood disease, a neurological disease, such as Parkinson's disease, Alzheimer's disease, Huntington's disease, and ALS, an enzyme or hormone deficiency, a metabolic disorder (e.g., a lysosomal storage disorder, Galactosemia, Maple syrup urine disease, Phenylketonuria, a glycogen storage disease, a mitochondrial disorder, Friedrich's ataxia, a peroxisomal disorder, a metal metabolism disorder, or an organic academia), an autoimmune disease (e.g., Psoriasis, Systemic Lupus Erythematosus, Grave's disease, Inflammatory Bowel Disease, Addison's Diseases, Sjogren's Syndrome, Hashimoto's Thyroiditis, Vasculitis, Autoimmune Hepatitis, Alopecia Areata, Autoimmune pancreatitis, Crohn's Disease, Ulcerative colitis, Dermatomyositis), age-related macular degeneration, retinal dystrophy, an infectious disease, hemophilia, a degenerative disease (e.g., Charcot-Marie-Tooth disease, chronic obstructive pulmonary disease, chronic traumatic encephalopathy, Creutzfeldt-Jakob disease, Cystic Fibrosis, Cytochrome C Oxidase deficiency, Ehlers-Danlos syndrome, essential tremor, Fribrodisplasia Ossificans Progressiva, infantile neuroaxonal dystrophy, keratoconus, keratoglobus, muscular dystrophy, neuronal ceroid lipofuscinosis, a prior disease, progressive supranuclear palsy, sandhoff disease, spinal muscular atrophy, retinitis pigmentosa), or an age-related disease (e.g., atherosclerosis, cardiovascular disease (e.g., angina, myocardial infarction), cataracts, osteoporosis, or hypertension), or a disease or condition listed in Table 2 and/or the cell further includes a transgene encoding a corresponding therapeutic agent listed in Table 2 or the wild-type version of a gene that is mutated in the subject (e.g., the wild-type version of the mutated gene that is associated with the disease or condition in the subject, e.g., a genetic mutation associated with cancer, an enzyme or hormone deficiency, a metabolic disorder, or a degenerative disease). In some embodiments, the disease or condition is age-related macular degeneration (e.g., wet AMD) or retinal dystrophy and the therapeutic agent is a VEGF inhibitor (e.g., a soluble form of a VEGF receptors (e.g., soluble VEGFR-1 or NRP-1), platelet factor-4, prolactin, SPARC, a VEGF inhibitory antibody (e.g., bevacizumab or ranibizumab), or a soluble decoy receptor described in Holash et al., Proc Natl Acad Sci U.S.A. 99:11383-11398, 2002, e.g., VEGF-Trap$_{parental}$, VEGF-Trap$_{\Delta B1}$, VEGF-Trap$_{\Delta B2}$, VEGF-Trap$_{R1R2}$, e.g., aflibercept). In some

embodiments, the disease or condition is osteoarthritis or rheumatoid arthritis and the therapeutic agent is an anti-inflammatory biologic (e.g. a TNFα inhibitor (e.g., adalimumab, etanercept, infliximab, golimumab, or certolizumab), an interleukin-6 (IL6) receptor inhibitor (e.g., tocilizumab), an IL1 receptor inhibitor (e.g., anakinra), or another agent used to treat rheumatoid arthritis (e.g., abatacept, rituximab)). In some embodiments, the disease or condition is diabetes (e.g., Type 1 diabetes or Type 2 diabetes) and the therapeutic agent is insulin. In some embodiments, the disease or condition is hemophilia and the therapeutic agent is Factor VIII. In some embodiments, the disease or condition is a metabolic deficiency and the therapeutic agent is a transgene having the nucleic acid sequence of the wild-type version of the gene that is mutated in the subject or a transgene encoding an enzyme that is deficient in the subject.

In some embodiments of any of the foregoing aspects, the cells are differentiated into a lineage restricted cell type prior to administration to the subject. In some embodiments, the disease or condition is myocardial infarction and the cells are differentiated into cardiac muscle cells. In some embodiments, the disease or condition is blindness and the cells are differentiated into photoreceptor cells. In some embodiments, the disease or condition is spinal cord injury, Parkinson's disease, Huntington's disease, or Alzheimer's disease and the cells are dissociated into neurons. In some embodiments, the disease or condition is multiple sclerosis and the cells are differentiated into glial cells.

In some embodiments of any of the foregoing aspects, the cells are administered (e.g., injected or implanted) locally to the tissue or body site in need of cells or the therapeutic agent.

In some embodiments of any of the foregoing aspects, the cells are administered intravenously, subcutaneously, intramuscularly, percutaneously, intradermally, parenterally, intraarterially, intravascularly, or by perfusion.

In some embodiments of any of the foregoing aspects, the cells are administered by subcutaneous injection to produce a cloaked subcutaneous tissue.

In some embodiments of any of the foregoing aspects, the cells are administered as a tissue. In some embodiments, the tissue is administered with a gel, biocompatible matrix, or cellular scaffold.

In some embodiments of any of the foregoing aspects, the cells are administered in an amount of 25,000 to 5,000,000,000 cells (e.g., 2.5×10^4, 5×10^4, 7.5×10^4, 1×10^5, 2×10^5, 3×10^5, 4×10^5, 6×10^5, 6×10^5, 7×10^5, 8×10^5, 9×10^5, 1×10^6, 2×10^6, 3×10^6, 4×10^6, 5×10^6, 6×10^6, 7×10^6, 8×10^6, 9×10^6, 1×10^7, 2×10^7, 3×10^7, 4×10^7, 5×10^7, 6×10^7, 7×10^7, 8×10^7, 9×10^7, 1×10^8, 2×10^8, 3×10^8, 4×10^8, 5×10^8, 6×10^8, 7×10^8, 8×10^8, 9×10^8, 1×10^9, 2×10^9, 3×10^9, 4×10^9, or 5×10^9 cells).

In some embodiments of any of the foregoing aspects, the cells are administered in an amount of 800,000,000 to 100,000,000,000 cells (e.g., 8×10^8, 9×10^8, 1×10^9, 2×10^9, or 3×10^9, 4×10^9, 5×10^9, 6×10^9, 7×10^9, 8×10^9, 9×10^9, 1×10^{10}, 2×10^{10}, 3×10^{10}, 4×10^{10}, 5×10^{10}, 6×10^{10}, 7×10^{10}, 8×10^{10}, 9×10^{10}, or 1×10^{11}, cells).

In some embodiments of any of the foregoing methods, the method further includes administering an additional therapeutic agent. In some embodiments, the additional therapeutic agent is administered prior to administration of the cells. In some embodiments, the additional therapeutic agent is

administered after administration of the cells. In some embodiments, the additional therapeutic agent is administered concurrently with administration of the cells. In some embodiments, the additional therapeutic agent is an immunosuppressive agent, a disease-modifying anti-rheumatic drug (DMARD), a biologic response modifier (a type of DMARD), a corticosteroid, or a nonsteroidal anti-inflammatory medication (NSAID), prednisone, prednisolone, methylprednisolone, methotrexate, hydroxychloroquine, sulfasalazine, leflunomide, cyclophosphamide, azathioprine, tofacitinib, adalimumab, abatacept, anakinra, kineret, certolizumab, etanercept, golimumab, infliximab, rituximab or tocilizumab, 6-mercaptopurine, 6-thioguanine, abatacept, adalimumab, alemtuzumab, an aminosalicylate, an antibiotic, an anti-histamine, Anti-TNFα, azathioprine, belimumab, beta interferon, a calcineurin inhibitor, certolizumab, a corticosteroid, cromolyn, cyclosporin A, cyclosporine, dimethyl fumarate, etanercept, fingolimod, fumaric acid esters, glatiramer acetate, golimumab, hydroxyurea, IFNγ, IL-11, leflunomide, leukotriene receptor antagonist, long-acting beta2 agonist, mitoxantrone, mycophenolate mofetil, natalizumab, ocrelizumab, pimecrolimus, a probiotic, a retinoid, salicylic acid, short-acting beta2 agonist, sulfasalazine, tacrolimus, teriflunomide, theophylline, tocilizumab, ustekinumab, or vedolizumab, bevacuzimab, ranibizumab, or aflibercept), photodynamic therapy, photocoagulation, carbidopa-levodopa, a dopamine agonist, an MAO-B inhibitor, a catechol-O-methyltransferase inhibitor, an anticholinergic, amantadine, deep brain stimulation, an anticoagulant, an anti-platelet agent, an angiotensin-converting enzyme inhibitor, an angiotensin II receptor blocker, an angiotensin receptor neprilysin inhibitor, a beta blocker, a combined alpha and beta blocker, a calcium channel blocker, a cholesterol lowering medication, a nicotinic acid, a cholesterol absorption inhibitor, a digitalis preparation, a diuretic, a vasodilator, a dual anti-platelet therapy, a cardiac procedure, an antiviral compound, a nucleoside-analog reverse transcriptase inhibitor (NRTI), a non-nucleoside reverse transcriptase inhibitor (NNRTI), a protease inhibitor, an antibacterial compound, an antifungal compound, an antiparasitic compound, insulin, a sulfonylurea, a biguanide, a meglitinide, a thiazolidinedione, a DPP-4 inhibitor, an SGLT2 inhibitor, an alpha-glucosidase inhibitor, a bile acid sequestrant, aspirin, a dietary regimen, a clotting factor, desmopressin, a clot-preserving medication, a fibrin sealant, physical therapy, a coenzyme, a bone marrow transplant, an organ transplant, hemodialysis, hemofiltration, exchange transfusion, peritoneal dialysis, medium-chain triacylglycerols, miglustat, enzyme supplementation therapy, a checkpoint inhibitor, a chemotherapeutic drug, a biologic drug, radiation therapy, cryotherapy, hyperthermia, surgical excision or tumor tissue, or an anti-cancer vaccine.

In some embodiments of any of the foregoing methods, the method further comprises controlling proliferation of the cell. In some embodiments, the cell comprises an ALINK system, and the method of controlling proliferation comprises: i) permitting proliferation of the cell comprising the ALINK system by maintaining the cell comprising the ALINK system in the absence of an inducer of the negative selectable marker; or ii) ablating or inhibiting proliferation of the cell comprising the ALINK system by exposing the cell comprising the ALINK system to the inducer of the negative selectable marker. In some embodiments, the cell comprises an EARC system, and the method of controlling cell proliferation

comprises: i) permitting proliferation of the cell comprising the EARC system by exposing the cell comprising the EARC system to an inducer of the inducible activator-based gene expression system; or ii) preventing or inhibiting proliferation of the cell comprising the EARC system by maintaining the cell comprising the EARC system in the absence of the inducer of the inducible activator-based gene expression system.

In some embodiments of any of the foregoing methods, the cell is removed after completion of the therapy. Removal of the cell(s) can be by surgery (e.g., to remove transplanted tissue or organs, or to remove cloaked subcutaneous tissue) or by the use of the ALINK and/or EARC systems. In some embodiments, one or more (e.g., one, two, three, four, or more) ALINK and/or EARC systems are used to eliminate all of the cloaked cells.

In another aspect, the invention provides a cell of the invention or a composition of the invention for use in treating a disease or condition in a subject in need thereof. In some embodiments, disease or condition is blindness, arthritis (e.g., osteoarthritis or rheumatoid arthritis), ischemia, diabetes (e.g., Type 1 or Type 2 diabetes), multiple sclerosis, spinal cord injury, stroke, cancer, a lung disease, a blood disease, a neurological disease, such as Parkinson's disease, Alzheimer's disease, Huntington's disease, and ALS, an enzyme or hormone deficiency, a metabolic disorder (e.g., a lysosomal storage disorder, Galactosemia, Maple syrup urine disease, Phenylketonuria, a glycogen storage disease, a mitochondrial disorder, Friedrich's ataxia, a peroxisomal disorder, a metal metabolism disorder, or an organic academia), an autoimmune disease (e.g., Psoriasis, Systemic Lupus Erythematosus, Grave's disease, Inflammatory Bowel Disease, Addison's Diseases, Sjogren's Syndrome, Hashimoto's Thyroiditis, Vasculitis, Autoimmune Hepatitis, Alopecia Areata, Autoimmune pancreatitis, Crohn's Disease, Ulcerative colitis, Dermatomyositis), age-related macular degeneration, retinal dystrophy, an infectious disease, hemophilia, a degenerative disease (e.g., Charcot-Marie-Tooth disease, chronic obstructive pulmonary disease, chronic traumatic encephalopathy, Creutzfeldt-Jakob disease, Cystic Fibrosis, Cytochrome C Oxidase deficiency, Ehlers-Danlos syndrome, essential tremor, Fribrodisplasia Ossificans Progressiva, infantile neuroaxonal dystrophy, keratoconus, keratoglobus, muscular dystrophy, neuronal ceroid lipofuscinosis, a prior disease, progressive supranuclear palsy, sandhoff disease, spinal muscular atrophy, retinitis pigmentosa), or an age-related disease (e.g., atherosclerosis, cardiovascular disease (e.g., angina, myocardial infarction), cataracts, osteoporosis, or hypertension), or a disease or condition listed in Table 2.

In another aspect, the invention provides a cell of the invention or a composition of the invention for use in providing a local immunosuppression at a transplant site in an allogeneic host.

In some embodiments of any of the foregoing aspects, the cell is comprises two of the set of transgenes PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) (e.g., PD-L1 and HLA-G (H2-M3); PD-L1 and Cd47; PD-L1 and Cd200; PD-L1 and FASLG (FasL); PD-L1 and Ccl21 (Ccl21b); PD-L1 and Mfge8; PD-L1 and Serpin B9 (Spi6); HLA-G (H2-M3) and Cd47; HLA-G (H2-M3) and Cd200; HLA-G (H2-M3) and FASLG (FasL); HLA-G (H2-M3) and Ccl21 (Ccl21b);

HLA-G (H2-M3) and Mfge8; HLA-G (H2-M3) and Serpin B9 (Spi6); Cd47 and Cd200; Cd47 and FASLG (FasL); Cd47 and Ccl21 (Ccl21b); Cd47 and Mfge8; Cd47 and Serpin B9 (Spi6); Cd200 and FASLG (FasL); Cd200 and Ccl21 (Ccl21b); Cd200 and Mfge8; Cd200 and Serpin B9 (Spi6); FASLG (FasL) and Ccl21 (Ccl21b); FASLG (FasL) and Mfge8; FASLG (FasL) and Serpin B9 (Spi6); Ccl21 (Ccl21b) and Mfge8; Ccl21 (Ccl21b) and Serpin B9 (Spi6); or Mfge8 and Serpin B9 (Spi6)).

In some embodiments of any of the foregoing aspects, the cell comprises three of the set of transgenes PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) (e.g., PD-L1, HLA-G (H2-M3), and Cd47; PD-L1, HLA-G (H2-M3), and Cd200; PD-L1, HLA-G (H2-M3), and FASLG (FasL); PD-L1, HLA-G (H2-M3), and Ccl21 (Ccl21b); PD-L1, HLA-G (H2-M3), and Mfge8; PD-L1, HLA-G (H2-M3), and Serpin B9 (Spi6); PD-L1, Cd47, and Cd200; PD-L1, Cd47, and FASLG (FasL); PD-L1, Cd47, and Ccl21 (Ccl21b); PD-L1, Cd47, and Mfge8; PD-L1, Cd47, and Serpin B9; PD-L1, Cd200, and FASLG (FasL); PD-L1, Cd200, and Ccl21 (Ccl21b); PD-L1, Cd200, and Mfge8; PD-L1, Cd200, and Serpin B9 (Spi6); PD-L1, FASLG (FasL), and Ccl21 (Ccl21b); PD-L1, FASLG (FasL), and Mfge8; PD-L1, FASLG (FasL), and Serpin B9 (Spi6); PD-L1, Ccl21 (Ccl21b), and Mfge8; PD-L1, Ccl21 (Ccl21b), and Serpin B9 (Spi6); PD-L1, Mfge8, and Serpin B9 (Spi6); HLA-G (H2-M3), Cd47, and Cd200; HLA-G (H2-M3), Cd47, and FASLG (FasL); HLA-G (H2-M3), Cd47, and Ccl21 (Ccl21b); HLA-G (H2-M3), Cd47, and Mfge8; HLA-G (H2-M3), Cd47, and Serpin B9; HLA-G (H2-M3), Cd200, and FASLG (FasL); HLA-G (H2-M3), Cd200, and Ccl21 (Ccl21b); HLA-G (H2-M3), Cd200, and Mfge8; HLA-G (H2-M3), Cd200, and Serpin B9; HLA-G (H2-M3), FASLG (FasL), and Ccl21 (Ccl21b); HLA-G (H2-M3), FASLG (FasL), and Mfge8; HLA-G (H2-M3), FASLG (FasL), and Serpin B9 (Spi6); HLA-G (H2-M3), Ccl21 (Ccl21b), and Mfge8; HLA-G (H2-M3), Ccl21 (Ccl21b), and Serpin B9 (Spi6); HLA-G (H2-M3), Mfge8, and Serpin B9 (Spi6); Cd47, Cd200, and FASLG (FasL); Cd47, Cd200, and Ccl21 (Ccl21b); Cd47, Cd200, and Mfge8; Cd47, Cd200, and Serpin B9 (Spi6); Cd47, FASLG (FasL), and Ccl21 (Ccl21b); Cd47, FASLG (FasL), and Mfge8; Cd47, FASLG (FasL), and Serpin B9 (Spi6); Cd47, Ccl21 (Ccl21b), and Mfge8; Cd47, Ccl21 (Ccl21b), and Serpin B9 (Spi6); Cd47, Mfge8, and Serpin B9 (Spi6); Cd200, FASLG (FasL), and Ccl21 (Ccl21b); Cd200, FASLG (FasL), and Mfge8; Cd200, FASLG (FasL), and Serpin B9 (Spi6); Cd200, Ccl21 (Ccl21b), and Mfge8; Cd200, Ccl21 (Ccl21b), and Serpin B9 (Spi6); Cd200, Mfge8, and Serpin B9 (Spi6); FASLG (FasL), Ccl21 (Ccl21b), and Mfge8; FASLG (FasL), Ccl21 (Ccl21b), and Serpin B9 (Spi6); Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6)).

In some embodiments of any of the foregoing aspects, the cell comprises four of the set of transgenes PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) (e.g., PD-L1, HLA-G (H2-M3), Cd47, and Cd200; PD-L1, HLA-G (H2-M3), Cd47, and FASLG (FasL); PD-L1, HLA-G (H2-M3), Cd47, and Ccl21 (Ccl21b); PD-L1, HLA-G (H2-M3), Cd47, and Mfge8; PD-L1, HLA-G (H2-M3), Cd47, and Serpin B9 (Spi6); PD-L1, HLA-G (H2-M3), Cd200, and FASLG (FasL); PD-L1, HLA-G (H2-M3), Cd200, and Ccl21 (Ccl21b); PD-L1, HLA-G (H2-M3), Cd200, and Mfge8; PD-L1, HLA-G (H2-M3), Cd200, and Serpin B9 (Spi6); PD-L1, HLA-G (H2-M3), FASLG (FasL), and Ccl21 (Ccl21b); PD-L1, HLA-G (H2-M3), FASLG (FasL), and Mfge8; PD-L1, HLA-G (H2-M3), FASLG (FasL),

and Serpin B9 (Spi6); PD-L1, HLA-G (H2-M3), Ccl21(Ccl21b), and Mfge8; PD-L1, HLA-G (H2-M3), Ccl21(Ccl21b), and Serpin B9 (Spi6); PD-L1, HLA-G (H2-M3), Mfge8, and Serpin B9 (Spi6); PD-L1, Cd47, Cd200, and FASLG (FasL); PD-L1, Cd47, Cd200, and Ccl21 (Ccl21b); PD-L1, Cd47, Cd200, and Mfge8; PD-L1, Cd47, Cd200, and Serpin B9 (Spi6); PD-L1, Cd47, FASLG (FasL), and Ccl21 (Ccl21b); PD-L1, Cd47, FASLG (FasL), and Mfge8; PD-L1, Cd47, FASLG (FasL), and Serpin B9 (Spi6); PD-L1, Cd47, Ccl21 (Ccl21b), and Mfge8; PD-L1, Cd47, Ccl21 (Ccl21b), and Serpin B9 (Spi6); PD-L1, Cd47, Mfge8, and Serpin B9 (Spi6); PD-L1, Cd200, FASLG (FasL), and Ccl21 (Ccl21b); PD-L1, Cd200, FASLG (FasL), and Mfge8; PD-L1, Cd200, FASLG (FasL), and Serpin B9 (Spi6); PD-L1, Cd200, Ccl21 (Ccl21b), and Mfge8; PD-L1, Cd200, Ccl21 (Ccl21b), and Serpin B9 (Spi6); PD-L1, Cd200, Mfge8, and Serpin B9 (Spi6); PD-L1, FASLG (FasL), Ccl21 (Ccl21b), and Mfge8; PD-L1, FASLG (FasL), Ccl21 (Ccl21b), and Serpin B9 (Spi6); PD-L1, FASLG (FasL), Mfge8, and Serpin B9 (Spi6); PD-L1, Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6); HLA-G (H2-M3), Cd47, Cd200, and FASLG (FasL); HLA-G (H2-M3), Cd47, Cd200, and Ccl21 (Ccl21b); HLA-G (H2-M3), Cd47, Cd200, and Mfge8; HLA-G (H2-M3), Cd47, Cd200, and Serpin B9 (Spi6); HLA-G (H2-M3), Cd47, FASLG (FasL), and Ccl21 (Ccl21b); HLA-G (H2-M3), Cd47, FASLG (FasL), and Mfge8; HLA-G (H2-M3), Cd47, FASLG (FasL), and Serpin B9 (Spi6); HLA-G (H2-M3), Cd47, Ccl21 (Ccl21b), and Mfge8; HLA-G (H2-M3), Cd47, Ccl21 (Ccl21b), and Serpin B9 (Spi6); HLA-G (H2-M3), Cd47, Mfge8, and Serpin B9 (Spi6); HLA-G (H2-M3), Cd200, FASLG (FasL), and Ccl21 (Ccl21b); HLA-G (H2-M3), Cd200, FASLG (FasL), and Mfge8; HLA-G (H2-M3), Cd200, FASLG (FasL), and Serpin B9 (Spi6); HLA-G (H2-M3), Cd200, Ccl21 (Ccl21b), and Mfge8; HLA-G (H2-M3), Cd200, Ccl21 (Ccl21b), and Serpin B9 (Spi6); HLA-G (H2-M3), Cd200, Mfge8, and Serpin B9 (Spi6); HLA-G (H2-M3), FASLG (FasL), Ccl21 (Ccl21b), and Mfge8; HLA-G (H2-M3), FASLG (FasL), Ccl21 (Ccl21b), and Serpin B9 (Spi6); HLA-G (H2-M3), FASLG (FasL), Mfge8, and Serpin B9 (Spi6); HLA-G (H2-M3), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6); Cd47, Cd200, FASLG (FasL), and Ccl21 (Ccl21b); Cd47, Cd200, FASLG (FasL), and Mfge8; Cd47, Cd200, FASLG (FasL), and Serpin B9 (Spi6); Cd47, Cd200, Ccl21 (Ccl21b), and Mfge8; Cd47, Cd200, Ccl21 (Ccl21b), and Serpin B9 (Spi6); Cd47, Cd200, Mfge8, and Serpin B9 (Spi6); Cd47, FASLG (FasL), Ccl21 (Ccl21b), and Mfge8; Cd47, FASLG (FasL), Ccl21 (Ccl21b), and Serpin B9 (Spi6); Cd47, Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6); Cd200, FASLG (FasL), Ccl21 (Ccl21b), and Mfge8; Cd200, FASLG (FasL), Ccl21 (Ccl21b), and Serpin B9 (Spi6); Cd200, Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6); or FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6)).

In some embodiments of any of the foregoing aspects, the cell comprises five of the set of transgenes PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) (e.g., PD-L1, HLA-G (H2-M3), Cd47, Cd200, and FASLG (FasL); PD-L1, HLA-G (H2-M3), Cd47, Cd200, and Ccl21 (Ccl21b); PD-L1, HLA-G (H2-M3), Cd47, Cd200, and Mfge8; PD-L1, HLA-G (H2-M3), Cd47, Cd200, and Serpin B9 (Spi6); PD-L1, HLA-G (H2-M3), Cd47, FASLG (FasL), and Ccl21 (Ccl21b); PD-L1, HLA-G (H2-M3), Cd47, FASLG (FasL), and Mfge8; PD-L1, HLA-G (H2-M3), Cd47, FASLG (FasL), and Serpin B9 (Spi6); PD-L1, HLA-G (H2-M3), Cd47, Ccl21 (Ccl21b), and Mfge8; PD-L1, HLA-G (H2-

M3), Cd47, Ccl21 (Ccl21b), and Serpin B9 (Spi6); PD-L1, HLA-G (H2-M3), Cd47, Mfge8, and Serpin B9 (Spi6); PD-L1, HLA-G (H2-M3), Cd200, FASLG (FasL), and Ccl21 (Ccl21b); PD-L1, HLA-G (H2-M3), Cd200, FASLG (FasL), and Mfge8, PD-L1, HLA-G (H2-M3), Cd200, FASLG (FasL), and Serpin B9 (Spi6); PD-L1, HLA-G (H2-M3), Cd200, Ccl21 (Ccl21b), and Mfge8; PD-L1, HLA-G (H2-M3), Cd200, Ccl21 (Ccl21b), and Serpin B9 (Spi6); PD-L1, HLA-G (H2-M3), Cd200, Mfge8, and Serpin B9 (Spi6); PD-L1, HLA-G (H2-M3), FASLG (FasL), Ccl21 (Ccl21b), and Mfge8; PD-L1, HLA-G (H2-M3), FASLG (FasL), Ccl21 (Ccl21b), and Serpin B9 (Spi6); PD-L1, HLA-G (H2-M3), FASLG (FasL), Mfge8, and Serpin B9 (Spi6); PD-L1, HLA-G (H2-M3), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6); PD-L1, Cd47, Cd200, FASLG (FasL), and Ccl21 (Ccl21b); PD-L1, Cd47, Cd200, FASLG (FasL), and Mfge8, PD-L1, Cd47, Cd200, FASLG (FasL), and Serpin B9 (Spi6); PD-L1, Cd47, Cd200, Ccl21 (Ccl21b), and Mfge8; PD-L1, Cd47, Cd200, Ccl21 (Ccl21b), and Serpin B9 (Spi6); PD-L1, Cd47, Cd200, Mfge8, and Serpin B9 (Spi6); PD-L1, Cd47, FASLG (FasL), Ccl21 (Ccl21b), and Mfge8; PD-L1, Cd47, FASLG (FasL), Ccl21 (Ccl21b), and Serpin B9 (Spi6); PD-L1, Cd47, FASLG (FasL), Mfge8, and Serpin B9 (Spi6); PD-L1, Cd47, Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6); PD-L1, Cd200, FASLG (FasL), Ccl21 (Ccl21b), and Mfge8; PD-L1, Cd200, FASLG (FasL), Ccl21 (Ccl21b), and Serpin B9 (Spi6); PD-L1, Cd200, FASLG (FasL), Mfge8, and Serpin B9 (Spi6); PD-L1, Cd200, Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6); PD-L1, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6); HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), and Ccl21 (Ccl21b); HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), and Mfge8; HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), and Serpin B9 (Spi6); HLA-G (H2-M3), Cd47, Cd200, Ccl21 (Ccl21b), and Mfge8; HLA-G (H2-M3), Cd47, Cd200, Ccl21 (Ccl21b), and Serpin B9 (Spi6); HLA-G (H2-M3), Cd47, Cd200, Mfge8, and Serpin B9 (Spi6); HLA-G (H2-M3), Cd47, FASLG (FasL), Ccl21 (Ccl21b), and Mfge8; HLA-G (H2-M3), Cd47, FASLG (FasL), Ccl21 (Ccl21b), and Serpin B9 (Spi6); HLA-G (H2-M3), Cd47, FASLG (FasL), Mfge8, and Serpin B9 (Spi6); HLA-G (H2-M3), Cd47, Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6); HLA-G (H2-M3), Cd200, FASLG (FasL), Ccl21 (Ccl21b), and Mfge8; HLA-G (H2-M3), Cd200, FASLG (FasL), Ccl21 (Ccl21b), and Serpin B9 (Spi6); HLA-G (H2-M3), Cd200, FASLG (FasL), Mfge8, and Serpin B9 (Spi6); HLA-G (H2-M3), Cd200, Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6); HLA-G (H2-M3), FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6); Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), and Mfge8; Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), and Serpin B9 (Spi6); Cd47, Cd200, FASLG (FasL), Mfge8, and Serpin B9 (Spi6); Cd47, Cd200, Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6); Cd47, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6); or Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6)).In some embodiments of any of the foregoing aspects, the cell comprises six of the set of transgenes PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) (e.g., PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), and Ccl21 (Ccl21b); PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), and Mfge8; PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), and Serpin B9 (Spi6); PD-L1, HLA-G (H2-M3), Cd47, Cd200, Ccl21 (Ccl21b), and Mfge8; PD-L1, HLA-G (H2-M3), Cd47, Cd200, Ccl21 (Ccl21b), and Serpin B9 (Spi6); PD-L1, HLA-G (H2-M3), Cd47, Cd200, Mfge8, and Serpin B9 (Spi6); PD-L1, HLA-G (H2-M3), Cd47, FASLG

(FasL), Ccl21 (Ccl21b), and Mfge8; PD-L1, HLA-G (H2-M3), Cd47, FASLG (FasL), Ccl21 (Ccl21b), and Serpin B9 (Spi6); PD-L1, HLA-G (H2-M3), Cd47, FASLG (FasL), Mfge8, and Serpin B9 (Spi6); PD-L1, HLA-G (H2-M3), Cd200, FASLG (FasL), Ccl21 (Ccl21b), and Mfge8; PD-L1, HLA-G (H2-M3), Cd200, FASLG (FasL), Ccl21 (Ccl21b),and Serpin B9 (Spi6); PD-L1, HLA-G (H2-M3), Cd200, FASLG (FasL), Mfge8, and Serpin B9 (Spi6); PD-L1, HLA-G (H2-M3), Cd200, Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6); PD-L1, Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), and Mfge8; PD-L1, Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), and Serpin B9 (Spi6); PD-L1, Cd47, Cd200, FASLG (FasL), Mfge8, and Serpin B9 (Spi6); PD-L1, Cd47, Cd200, Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6); PD-L1, Cd47, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6); PD-L1, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6); HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), and Mfge8; HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), and Serpin B9 (Spi6); HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Mfge8, and Serpin B9 (Spi6); HLA-G (H2-M3), Cd47, Cd200, Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6); HLA-G (H2-M3), Cd47, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6); HLA-G (H2-M3), Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6); or Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6)).

In some embodiments of any of the foregoing aspects, the cell comprises seven of the set of transgenes PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) (e.g., PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), and Mfge8; PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), and Serpin B9 (Spi6); PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Mfge8, and Serpin B9 (Spi6); PD-L1, HLA-G (H2-M3), Cd47, Cd200, Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6); PD-L1, HLA-G (H2-M3), Cd47, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6); PD-L1, HLA-G (H2-M3), Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6); PD-L1, Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6); or HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6)).

In some embodiments of any of the foregoing aspects, the cell comprises all eight of the set of transgenes PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6).

In some embodiments of any of the foregoing aspects, the cell comprises one or more (e.g., one, two, three, four, five, six, or all seven) of the set of transgenes HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6).

In some embodiments of any of the foregoing aspects, the cell comprises one or more (e.g., one, two, three, four, five, six, or all seven) of the set of transgenes PD-L1, HLA-G (H2-M3), Cd47, Cd200, Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6).

In some embodiments of any of the foregoing aspects, the cell comprises one or more (e.g., one, two, three, four, five, or all six) of the set of transgenes HLA-G (H2-M3), Cd47, Cd200, Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6).

In some embodiments of any of the foregoing aspects, the cell is not modified to express PD-L1.

In some embodiments of any of the foregoing aspects, the cell is not modified to express FasL.

In some embodiments of any of the foregoing aspects, the cell is not modified to express TGF-β. In some embodiments of any of the foregoing aspects, the cell is not modified to express CTLA4 or CLTA4-Ig. In some embodiments of any of the foregoing aspects, the cell is not modified to express IDO. In some embodiments of any of the foregoing aspects, the cell is not modified to express IL-35. In some embodiments of any of the foregoing aspects, the cell is not modified to express IL-10. In some embodiments of any of the foregoing aspects, the cell is not modified to express VEGF. In some embodiments of any of the foregoing aspects, the cell is not modified to express an NFκb decoy receptor. In some embodiments of any of the foregoing aspects, the cell is not modified to express soluble TNFR. In some embodiments of any of the foregoing aspects, the cell is not modified to express CCR7. In some embodiments of any of the foregoing aspects, the cell is not modified to express SOCS1. In some embodiments of any of the foregoing aspects, the cell is not modified to express HLA-E. In some embodiments of any of the foregoing aspects, the cell is not modified to express siRNA directed to IL-12.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs.

As used herein, the term “about” refers to a value that is no more than 10% above or below the value being described. For example, the term “about 5 nM” indicates a range of from 4.5 nM to 5.5 nM.

As used herein, the term “activated leukocyte” refers to the state of a leukocyte (e.g., a granulocyte, such as a neutrophil, eosinophil, or basophil; a monocyte, or a lymphocyte, such as a B or T cell) caused by response to a perceived insult. When leukocytes become activated, they can proliferate, secrete cytokines, differentiate, present antigens, become more polarized, become more phagocytic, and/or become more cytotoxic. Factors that stimulate immune cell activation include pro-inflammatory cytokines, pathogens, and non-self antigen presentation. Activated leukocytes can be isolated from lymphoid organs. Leukocytes, such as T cells, can also be activated in vitro using anti-CD3/CD28 beads or other methods employed by those of skill in the art (see, e.g., Frauwith and Thompson, *J.Clin Invest* 109:295-299 (2002); and Trickett and Kwan, *J Immunol Methods* 275:251-255 (2003)).

As used herein, "allogeneic" means cells, tissue, DNA, or factors taken or derived from a different subject of the same species.

As used herein, the term “stem cell” refers to a cell that can differentiate into one or more specialized cells and has the capacity for self-renewal. Stem cells include pluripotent stem cells (PSCs), such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), and multipotent stem cells, such as cord blood stem cells, mesenchymal stromal cells and adult stem cells, which are found in various tissues. The term “stem cell” also includes cells amenable for genome editing, cells that can serve as a source of a therapeutic cell type (e.g., cells that can be directed to differentiate into a lineage

restricted or terminally differentiated cell that is used for cell therapy, or cells of a desired target tissue), and cells with “artificial” cell acquired stem cell properties (e.g., pluripotency or multipotency or self-renewal).

As used herein, the terms "embryonic stem cell" and "ES cell" refer to:

(i) a non-human embryo-derived totipotent or pluripotent stem cell, derived from the inner cell mass of a blastocyst that can be maintained in an in vitro culture under suitable conditions. ES cells are capable of differentiating into cells of any of the three vertebrate germ layers, e.g., the endoderm, the ectoderm, or the mesoderm. ES cells are also characterized by their ability propagate indefinitely under suitable in vitro culture conditions. See, for example, Thomson et al., Science 282:1145 (1998).

(ii) a human pluripotent embryonic stem cell obtained without the destruction of a human embryo.

As used herein, the terms "induced pluripotent stem cell," "iPS cell," and “iPSC” refer to a pluripotent stem cell that can be derived directly from a differentiated somatic cell. Human iPS cells can be generated by introducing specific sets of reprogramming factors into a non-pluripotent cell that can include, for example, Oct3/4, Sox family transcription factors (e.g., Sox1, Sox2, Sox3, Soxl5), Myc family transcription factors (e.g., c-Myc, 1-Myc, n-Myc), Kruppel-like family (KLF) transcription factors (e.g., KLF1, KLF2, KLF4, KLF5), and/or related transcription factors, such as NANOG, LIN28, and/or Glis1. Human iPS cells can also be generated, for example, by the use of miRNAs, small molecules that mimic the actions of transcription factors, or lineage specifiers. Human iPS cells are characterized by their ability to differentiate into any cell of the three vertebrate germ layers, e.g., the endoderm, the ectoderm, or the mesoderm. Human iPS cells are also characterized by their ability propagate indefinitely under suitable in vitro culture conditions. See, for example, Takahashi and Yamanaka, Cell 126:663 (2006).

As used herein, the term “mitigate antigen presenting cell activation and function” refers to a transgene that encodes a gene product whose function is to inhibit antigen presenting cell activation or the ability of an antigen presenting cell to promote the activation of graft attacking leukocytes (Fiorentino et al., *J Immunol.* 146:3444-51 (1991); Salio et al., *Eur J Immunol.* 29:3245-53 (1999)). In an embodiment, mitigation of antigen presenting cell activation and function refers to a decrease in APC activation and function of at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 100%, relative to a control (e.g., as determined using an assay for antigen presenting cell activation, such as reduced proliferation, reduced secretion of pro-inflammatory cytokines (e.g., interleukin-1 (IL-1, e.g., IL-1β), IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-18, tumor necrosis factor (TNF, e.g., TNFα), interferon gamma (IFNγ), and granulocyte macrophage colony stimulating factor (GMCSF), which can be measured using an ELISA or Western Blot analysis of culture media or a patient sample, such as a blood sample), or reduced levels of cell surface markers (e.g., CD11c, CD11b, HLA molecules (e.g., MHC-II), CD40, B7, IL-2, CD80 or CD86, which can be assessed using flow cytometry, immunohistochemistry, in situ hybridization, and other assays that allow for measurement of cell surface markers)). Antigen presenting cells include dendritic cells, B cells, and macrophages. Mast cells and neutrophils can also be induced to present antigens. Methods for determining mitigation of antigen presenting cell activation and function

are known in the art. Examples of gene products that mitigate antigen presenting cell activation and function include, but are not limited to: Ccl21 (Ccl21b) and PD-L1. Such transgenes may be referred herein to "cloaking" or "cloaked" genes.

As used herein, the term "mitigate graft attacking leukocyte activity or cytolytic function" refers to a transgene that encodes a gene product whose function is to inhibit or prevent graft attacking leukocyte activity or cytolytic function near allograft cells (MacDonald et al., *J Immunol.* 126:1671-5 (1981); Bongrand et al., *Eur J Immunol.* 13:424-9 (1983); MacDonald et al., *Eur J Immunol.* 9:466-70 (1979)). In an embodiment, mitigation of graft attacking leukocyte activity or cytolytic function refers to a decrease in leukocyte activity or cytolytic function of at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 100%, relative to a control (e.g., as determined using an assay for leukocyte activation, such as reduced proliferation, reduced secretion of pro-inflammatory cytokines (e.g., interleukin-1 (IL-1, e.g., IL-1β), IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-18, tumor necrosis factor (TNF, e.g., TNFα), interferon gamma (IFNγ), and granulocyte macrophage colony stimulating factor (GMCSF), which can be measured using an ELISA or Western Blot analysis of culture media or a patient sample, such as a blood sample), or reduced polarization (e.g., a reduction in the level of IL-12, TNF, IL-1β, IL-6, IL-23, MARCO, MHC-II, CD86, iNOS, CXCL9, and CXCL10 in a macrophage or monocyte, or a reduction in the level of a Th1-specific marker (e.g., T-bet, IL-12R, STAT4), a chemokine receptor (e.g., CCR5, CXCR6, or CXCR3); or a Th2-specific marker: (e.g., CCR3, CXCR4, STAT6, GATA3, or IL-4Rα) in a T cell, which can be assessed using flow cytometry, immunohistochemistry, situ hybridization, qPCR, or western blot analysis for cell surface markers or intracellular proteins, and ELISA or western blot analysis for secreted proteins); or as determined using an assay for cytolytic function (e.g., by incubating leukocytes with a target cell line that has been pre-coated with antibodies to a surface antigen expressed by the target cell line and measuring the number of surviving target cells with a fluorescent viability stain, or by measuring the secretion of cytolytic granules (e.g., perforin, granzymes, or other cytolytic proteins released from immune cells) from the leukocytes). Methods for determining mitigation of graft attacking leukocyte activity or cytolytic function are known in the art. Examples of gene products that mitigate graft attacking leukocyte activity or cytolytic function include, but are not limited to: PD-L1, HLA-G (H2-M3), Cd39, Cd73, and Lag3. Such transgenes may be referred herein to "cloaking" or "cloaked" genes.

As used herein, the term "mitigate macrophage cytolytic function and phagocytosis of allograft cells" refers to a transgene that encodes a gene product whose function is to inhibit or prevent macrophage cytolytic function and/or phagocytosis of allograft cells (Fish et al., *Toxicology.* 19:127-38. (1981); Sung et al., *J Biol Chem.* 260:546-54 (1985); Amash et al., *J Immunol.* 196:3331-40 (2016)). In an embodiment, mitigation of macrophage cytolytic function and phagocytosis of allograft cells refers to a decrease in macrophage cytolytic function and/or phagocytosis of allograft cells of at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 100%, relative to a control (e.g., as determined using an assay for macrophage cytolytic function (e.g., by incubating macrophages with a target cell line that has been pre-coated with antibodies to a surface antigen expressed by the target cell line and

measuring the number of surviving target cells with a fluorescent viability stain, or by measuring the secretion of cytolytic granules (e.g., perforin, granzymes, or other cytolytic proteins released from immune cells) released from the macrophages; or as determined using an assay for macrophage phagocytosis (e.g., culturing macrophages with fluorescent beads or a target cell line that has been pre-coated with antibodies to a surface antigen expressed by the target cell line and measuring fluorescence inside the immune cell or quantifying the number of beads or cells engulfed)). Methods for determining mitigation of macrophage cytolytic function and phagocytosis of allograft cells are known in the art. Examples of gene products that mitigate macrophage cytolytic function include, but are not limited to: Cd47, Cd200, Mfge8, and Il1r2. Such transgenes may be referred herein to "cloaking" or "cloaked" genes.

As used herein, the term "induce apoptosis in graft attacking leukocytes" refers to a transgene that encodes a gene product whose function is to kill graft attacking leukocytes near allograft cells (Huang et al., *Proc Natl Acad Sci U S A.* 96:14871-6 (1999); Suzuki et al., *Proc Natl Acad Sci U S A.* 97:1707-12 (2000); Simon et al., *Proc Natl Acad Sci U S A.* 98:5158-63 (2001)). In an embodiment, induction of apoptosis in graft attacking leukocytes refers to an increase in apoptosis in graft attacking leukocytes of at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 100%, relative to a control (e.g., as determined using an assay for apoptosis, such as TUNEL staining, caspase staining, or Annexin-V staining, or use of fluorescent viability stains). Methods for determining induction of apoptosis in graft attacking leukocytes are known in the art. Examples of gene products that can induce apoptosis in graft attacking leukocytes include, but are not limited to: FASLG (FasL) and Tnfsf10. Such transgenes may be referred herein to "cloaking" or "cloaked" genes.

As used herein, the term "mitigate local inflammatory proteins" refers to a transgene that encodes a gene product whose function is to inhibit the activity of local proteins, where the function of said proteins is to promote graft attacking leukocyte accumulation, and/or their cytolytic function (Felix et al., *Nat Rev Immunol.* 17:112-29 (2017)). In an embodiment, mitigation of local inflammatory proteins refers to a reduction in local inflammatory proteins of at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 100%, relative to a control (e.g., as determined using an assay for inflammatory proteins that promote leukocyte activation or migration to a site of inflammation (e.g., a chemokine, such as CCL2, CCL3, CCL5, CXCL1, CXCL2, and CXCL8, or a pro-inflammatory cytokine, such as IL-1β, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-18, TNFα, IFNγ, or GMCSF, which can be measured using an ELISA, Western blot analysis, or other techniques known in the art for measuring secreted proteins)). Methods for determining mitigation of local inflammatory proteins are known in the art. Examples of gene products that mitigate local inflammatory proteins include, but are not limited to: PD-L1, Il1r2, and Ackr2. Such transgenes may be referred herein to "cloaking" or "cloaked" genes.

As used herein, the term "protect against leukocyte-mediated apoptosis" refers to a transgene that encodes a gene product whose function is to inhibit any cell component that may induce apoptosis or cytolysis of an allograft cell (Abdullah et al., *J Immunol.* 178:3390-9 (2007)). In an embodiment, protection against leukocyte-mediated apoptosis refers to a decrease in leukocyte-mediated apoptosis of

at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 100%, relative to a control (e.g., as determined using an assay for leukocyte-mediated apoptosis (e.g., by incubating leukocytes with a target cell line that has been pre-coated with antibodies to a surface antigen expressed by the target cell line and measuring the number of surviving target cells with a fluorescent viability stain, or by measuring the secretion of cytolytic granules (e.g., perforin, granzymes, or other cytolytic proteins released from immune cells) released from the leukocyte). Methods for determining protection against leukocyte-mediated apoptosis are known in the art. Examples of gene products that protect against leukocyte-mediated apoptosis include, but are not limited to: Serpin B9 (Spi6) and Dad1. Such transgenes may be referred herein to “cloaking” or “cloaked” genes.

As used herein, the term “biologic” refers to a designed polypeptide and corresponding encoding DNA, which can be expressed as a transgene. The polypeptide may agonize or inhibit the function of an endogenous gene or inhibit or activate a biological process. Methods for determining whether a polypeptide has agonist or antagonist activity or function are generally known in the art. In an embodiment, the agonist function is at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90, 95% or 100% of the function, relative to the function of a control. In an embodiment, the antagonist function is at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90, 95% or 100% of the function, relative to the function of a control.

As used herein, the term “operably linked” refers to a first molecule joined to a second molecule, wherein the molecules are so arranged that the first molecule affects the function or expression of the second molecule. The two molecules may or may not be part of a single contiguous molecule and may or may not be adjacent. For example, a promoter is operably linked to a transcribable polynucleotide molecule if the promoter modulates transcription of the transcribable polynucleotide molecule of interest in a cell. Additionally, two portions of a transcription regulatory element are operably linked to one another if they are joined such that the transcription-activating functionality of one portion is not adversely affected by the presence of the other portion. Two transcription regulatory elements may be operably linked to one another by way of a linker nucleic acid (e.g., an intervening non-coding nucleic acid) or may be operably linked to one another with no intervening nucleotides present.

As used herein, the term "promoter" refers to a recognition site on DNA that is bound by an RNA polymerase. The polymerase drives transcription of the transgene.

“Percent (%) sequence identity” with respect to a reference polynucleotide or polypeptide sequence is defined as the percentage of nucleic acids or amino acids in a candidate sequence that are identical to the nucleic acids or amino acids in the reference polynucleotide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid or amino acid sequence identity can be achieved in various ways that are within the capabilities of one of skill in the art, for example, using publicly available computer software such as BLAST, BLAST-2, or Megalign software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any

algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For example, percent sequence identity values may be generated using the sequence comparison computer program BLAST. As an illustration, the percent sequence identity of a given nucleic acid or amino acid sequence, A, to, with, or against a given nucleic acid or amino acid sequence, B, (which can alternatively be phrased as a given nucleic acid or amino acid sequence, A that has a certain percent sequence identity to, with, or against a given nucleic acid or amino acid sequence, B) is calculated as follows:

100 multiplied by (the fraction X/Y)

where X is the number of nucleotides or amino acids scored as identical matches by a sequence alignment program (e.g., BLAST) in that program's alignment of A and B, and where Y is the total number of nucleic acids in B. It will be appreciated that where the length of nucleic acid or amino acid sequence A is not equal to the length of nucleic acid or amino acid sequence B, the percent sequence identity of A to B will not equal the percent sequence identity of B to A.

As used herein, the term "pharmaceutical composition" refers to a mixture containing a therapeutic agent, optionally in combination with one or more pharmaceutically acceptable excipients, diluents, and/or carriers, to be administered to a subject, such as a mammal, e.g., a human, in order to prevent, treat or control a particular disease or condition affecting or that may affect the subject.

As used herein, the term "pharmaceutically acceptable" refers to those compounds, materials, compositions and/or dosage forms, which are suitable for contact with the tissues of a subject, such as a mammal (e.g., a human) without excessive toxicity, irritation, allergic response and/or other problem complications commensurate with a reasonable benefit/risk ratio.

As used herein, the term "wild-type" refers to a genotype with the highest frequency for a particular gene in a given organism.

The terms "cell division locus", "cell division loci", and "CDL" as used herein, refer to a genomic locus (or loci) whose transcription product(s) is expressed by dividing cells. When a CDL comprises a single locus, absence of CDL expression in a cell (or its derivatives) means that tumour initiation and/or formation is prohibited either because the cell(s) will be ablated in the absence of CDL expression or because proliferation of the cell(s) will be blocked or compromised in the absence of CDL expression. When a CDL comprises multiple loci, absence of expression by all or subsets of the loci in a cell (or its derivatives) means that tumour initiation and/or formation is prohibited either because the cell(s) will be ablated in the absence of CDL expression or because proliferation of the cell(s) will be blocked or compromised in the absence of CDL expression. A CDL may or may not be expressed in non-dividing and/or non-proliferating cells. A CDL may be endogenous to a host cell or it may be a transgene. If a CDL is a transgene, it may be from the same or different species as a host cell or it may be of synthetic origin. In an embodiment, a CDL is a single locus that is transcribed during cell division. For example, in an embodiment, a single locus CDL is CDK1. In an embodiment, a CDL comprises two or more loci that are transcribed during cell division. For example, in an embodiment, a multi-locus CDL comprises two

MYC genes (c-Myc and N-myc) (Scognamiglio et al., 2016). In an embodiment, a multi-locus CDL comprises AURORA B and C kinases, which may have overlapping functions (Fernandez-Miranda et al., 2011). Cell division and cell proliferation are terms that may be used interchangeably herein.

The terms "normal rate of cell division", "normal cell division rate", "normal rate of cell proliferation", and "normal cell proliferation rate" as used herein, refer to a rate of cell division and/or proliferation that is typical of a non-cancerous healthy cell. A normal rate of cell division and/or proliferation may be specific to cell type. For example, it is widely accepted that the number of cells in the epidermis, intestine, lung, blood, bone marrow, thymus, testis, uterus and mammary gland is maintained by a high rate of cell division and a high rate of cell death. In contrast, the number of cells in the pancreas, kidney, cornea, prostate, bone, heart and brain is maintained by a low rate of cell division and a low rate of cell death (Pellettieri and Sánchez Alvarado, 2007).

The terms "inducible negative effector of proliferation" and "iNEP" as used herein, refer to a genetic modification that facilitates use of CDL expression to control cell division and/or proliferation by: i) inducibly stopping or blocking CDL expression, thereby prohibiting cell division and proliferation; ii) inducibly ablating at least a portion of CDL-expressing cells (i.e., killing at least a portion of proliferating cells); or iii) inducibly slowing the rate of cell division relative to a cell's normal cell division rate, such that the rate of cell division would not be fast enough to contribute to tumor formation.

The terms "ablation link" and "ALINK" as used herein, refer to an example of an iNEP, which comprises a transcriptional link between a CDL and a sequence encoding a negative selectable marker. The ALINK modification allows a user to inducibly kill proliferating host cells comprising the ALINK or inhibit the host cell's proliferation by killing at least a portion of proliferating cells by exposing the ALINK-modified cells to an inducer of the negative selectable marker. For example, a cell modified to comprise an ALINK at a CDL may be treated with an inducer (e.g., a prodrug) of the negative selectable marker in order to ablate proliferating cells or to inhibit cell proliferation by killing at least a portion of proliferating cells.

The terms "exogenous activator of regulation of CDL" and "EARC" as used herein, refer to an example of an iNEP, which comprises a mechanism or system that facilitates exogenous alteration of non-coding or coding DNA transcription or corresponding translation via an activator. An EARC modification allows a user to inducibly stop or inhibit division of cells comprising the EARC by removing from the EARC-modified cells an inducer that permits transcription and/or translation of the EARC-modified CDL. For example, an inducible activator-based gene expression system may be operably linked to a CDL and used to exogenously control expression of a CDL or CDL translation, such that the presence of a drug inducible activator and corresponding inducer drug are required for CDL transcription and/or translation. In the absence of the inducer drug, cell division and/or proliferation would be stopped or inhibited (e.g., slowed to a normal cell division rate). For example, the CDL Cdk1/CDK1 may be modified to comprise a dox-bridge, such that expression of Cdk1/CDK1 and cell division and proliferation are only possible in the presence of an inducer (e.g., doxycycline).

The term "proliferation antagonist system" as used herein, refers to a natural or engineered compound(s) whose presence inhibits (completely or partially) proliferation of a cell.

The term "dox-bridge" as used herein, refers to a mechanism for separating activity of a promoter from a target transcribed region by expressing rtTA (Gossen et al., 1995) by the endogenous or exogenous promoter and rendering the transcription of target region under the control of TRE. As used herein, "rtTA" refers to the reverse tetracycline transactivator elements of the tetracycline inducible system (Gossen et al., 1995) and "TRE" refers to a promoter consisting of *TetO* operator sequences upstream of a minimal promoter. Upon binding of rtTA to the TRE promoter in the presence of doxycycline, transcription of loci downstream of the TRE promoter increases. The rtTA sequence may be inserted in the same transcriptional unit as the CDL or in a different location of the genome, so long as the transcriptional expression's permissive or non-permissive status of the target region is controlled by doxycycline. A dox-bridge is an example of an EARC.

As used herein, the term "fail-safe cell" refers to a cell that contains one or more homozygous, heterozygous, hemizygous or compound heterozygous ALINKs or EARCs in one or more CDLs (e.g., at least two, three, four, or five CDLs). Fail-safe cells may contain either ALINKs or EARCs or both ALINK and EARC modifications (e.g., ALINK and EARC modifications in different CDLs or in a single CDL).

As used herein, the term "fail-safe" refers to a property of a cell that is unlikely to exhibit uncontrolled (e.g., tumorigenic) proliferation. A cell can be considered "fail safe" when cell proliferation is under the control of a negative regulator or inducer, and the possibility of the cell losing the activity of the system that controls proliferation due to genetic mutation is low. The fail-safe volume will depend on the number of ALINKs and the number of ALINK-targeted CDLs (e.g., a cell with homozygous modifications of two different CDLs has a higher fail safe volume (e.g., it is less likely to lose all systems that control proliferation through genetic mutation) than a cell with a heterozygous modification of a single CDL). The fail-safe property is further described in Table 3.

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DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

These and other features of the disclosure will become more apparent in the following detailed description in which reference is made to the appended drawings wherein:

FIGS. 1A-1D depict representative images showing the expression of cloaking proteins (Cd200 (Fig. 1A), FasL (Fig. 1B), H2-M3 (Fig. 1C) and Cd47 (Fig. 1D)) in C57BL/6 mouse embryonic stem cell line C2 using immunohistochemistry.

FIGS. 2A-2E are flow cytometry plots showing T-cell activation using splenocytes (FIG. 2A), wt B16 melanoma cells (FIG. 2B), cloaked B16 melanoma cells (FIG. 2C), wt ES cells (FIG. 2D), and cloaked ES cells (FIG. 2E) in a mixed lymphocyte reaction.

FIGS. 3A-3B are schematics and images showing that cloaked (FIG. 3B) B16F10 cancer cells in an allogenic model are protected from rejection compared to their WT counterparts (FIG. 3A). Representative images of uncloaked cells in C57BL/6 (n=5) and uncloaked cells in FVB/N (n=4) (FIG. 3A); cloaked cells in C57BL/6 (n=5) and cloaked cells in FVB/N (n=6) (FIG. 3B).

FIG. 4 is a schematic showing that cloaked embryonic stem cells form tumors in isogenic B6 mouse recipients (upper panel) and in FVB allogenic recipients (lower panel).

FIGS. 5A-5C are a series of photographs depicting allogenic mice bearing teratomas formed from subcutaneous injection of cloaked C57BL/6 ES cells. Red arrows indicate teratomas. FIG. 5A shows teratomas in C3H mice, FIG. 5B shows teratomas in FVB/N mice, and FIG. 5C shows teratomas in CD1 mice.

FIG. 6 is a schematic and series of images showing that animals with cloaked tissue are not immune compromised.

FIG. 7 is a series of images of FVB/N mice showing additional results showing that animals with cloaked tissues are not immune compromised.

FIGS. 8A-8H show transgene expression in clonal FailSafe containing embryonic stem cells derived from C57BL/6 mice. FIG. 8A shows FasL expression, FIG. 8B shows Ccl21b expression, FIG. 8C shows Cd200 expression, FIG. 8D shows Cd47 expression, FIG. 8E shows Mfge8 expression, FIG. 8F shows Spi6 expression, FIG. 8G H2-M3 expression, and FIG. 8H shows PD-L1 expression.

FIG. 9 is a series of graphs depicting cloaking transgene expression in ES cell clones. Each cloaking transgene is depicted in a different color. Concentric circles represent expression level on a log10 scale. The thick black circle represents 1x expression normalized to positive controls (activated leukocytes isolated from murine lymph organs), with the next outer ring representing 10x and 100x expression compared to positive controls, respectively. The innermost ring is 0.1x expression compared to positive controls. Clones NT2 and 15 (indicated with red squares) had the highest expression of the cloaking genes. These clones survived in allogenic hosts.

FIG. 10 is a graph depicting the expression of the cloaking transgenes among the whole genome gene expression level distribution for the whole genome of ES cells. All 8 cloaking transgenes in the NT2 cell line and NT2-derived teratoma had an expression level that was among the top 5% of all genes in the ES cell genome, with 5 of the cloaking transgenes having an expression level in the top 1% of all genes in the ES cell genome. The expression levels of the transgenes in the NT2 line and NT2-derived teratoma succeeded to achieve allograft tolerance.

FIGS. 11A-11B are photographs showing C57BL/6 derived teratomas in FVB/N mice. The transgenic line, NT2, resulted in 9 teratomas out of 10 injection sites. Images were taken 3 months post injection. FIG. 11B is an enlarged image of the teratoma indicated by the arrow in FIG. 11A.

FIGS. 12A-12B are graphs showing the teratoma tumor size in isogenic (FIG. 12A) and allogenic (FIG. 12B) mice treated with ganciclovir.

FIGS. 13A-13B are a series of photomicrographs showing that cloaked embryonic stem cells, injected into both isogenic (FIG. 13A) and allogenic (FIG. 13B) hosts, can differentiate into all three cell lineages.

FIGS. 14A-14D are photomicrographs showing the formation of all three germ layers in a teratoma formed from subcutaneous injection of cloaked ES cells into a mouse. FIG. 14A, FIG. 14B, and FIG. 14C show the three germ layers (ec = ectoderm, shown in FIG. 14A; en = endoderm, shown in FIG. 14C; me = mesoderm, shown in FIG. 14B). FIG. 14D shows a blood vessel, indicated by the red arrow, confirming that the tissues are well vascularized.

FIG. 15 is a schematic showing the construction of vectors that express target genes essential for allo-tolerance.

FIGS. 16A-16H are fluorescent photomicrographs showing the expression of proteins encoded by the cloaking transgenes in ES cells. FIG. 16A shows the expression of PD-L1, FIG. 16B shows the expression of CD200, FIG. 16C shows the expression of CD47, FIG. 16D shows the expression of FasL, FIG. 16E shows the expression of H2-M3, FIG. 16F shows the expression of Ccl21, FIG. 16G shows the expression of Mfge8, and FIG. 16H shows the expression of Spi6.

FIGS. 17A-17B are photomicrographs showing that cloaked ES cells have typical ES cell morphology (FIG. 17A) and express the ES cell marker alkaline phosphatase (FIG. 17B).

FIGS. 18A-18B are fluorescent photomicrographs showing the expression of markers of pluripotent ES cells (Oct4 (FIG. 18A) and SSEA1 (FIG. 18B)) in cloaked ES cells. The insets in FIGS. 18A-18B show single channel images of the fluorescent micrographs for the ES cell markers (Oct4 and SSEA) and DAPI, which labels the nucleus, to demonstrate that staining for the ES cell markers colocalizes with the cloaked cells.

FIGS. 19 is a schematic depicting the immune processes that are inhibited by the cloaking transgenes (top) and the expression cassette (bottom) used to express the cloaking transgenes in ES cells.

FIG. 20 is a series of graphs depicting the effect of interferon gamma (IFNγ) on MHC levels in ES cells. IFNγ increased MHC levels in wild-type ES cells and ES cells overexpressing the wild-type IFNγ receptor IFNγR1, but did not increase MHC levels in ES cells overexpressing a dominant negative form of the IFNγ receptor (IFNγR1 d39), indicating that IFNγR1 d39 completely inhibited the IFNγ-mediated upregulation of MHCs in ES cells.

DETAILED DESCRIPTION OF THE DISCLOSURE

Description of Cells and Methods

Featured are tools, such as genetically modified cells, and methods for providing a local immune suppression at a transplant site using the cells, e.g., when the cells are transplanted in an allogeneic host. The genetically modified cell comprises: one or a set of transgenes, each transgene encoding a gene product that is cytoplasmic, membrane bound, or local acting and whose function is to mitigate function of

the host immune system (e.g., graft attacking leukocyte and NK cell activation) or act as a defense mechanism against attacking leukocytes.

Various cytoplasmic, membrane-bound, or local acting immune factors have been found to regulate the local immune compartment and local immune populations. Immune factors like PD-L1 (Brown et al., *J Immunol.* 170:1257-66 (2003: Curiel et al., *Nat Med.* 9:562-7 (2003); Dong et al., *Nat Med.* 8:793-800 (2002)), CD47 ((Willingham et al., *Proc Natl Acad Sci U S A.* 109:6662-7 (2012); Liu et al., *PLoS One.* 10:e0137345 (2015); Demeure et al., *J Immunol.* 164:2193-9 (2000)), CD200 (Jenmalm et al., *J Immunol.* 176:191-9 (2006); Cherwinski et al., *J Immunol.* 174:1348-56 (2005); Kretz-Rommel et al., *J Immunol.* 178:5595-605 (2007)), FasL (O'Connell et al., *J Exp Med.* 184:1075-82 (1996); Ju et al., *Nature.* 373:444-8 (1995); Mazar et al., *J Biol Chem.* 284:22022-8 (2009)), and Spi6 (Medema *Proceedings of the National Academy of Sciences of the United States of America.* 98:11515-20 (2001); Zhang et al., *Immunity.* 24:451-61 (2006); Soriano et al., *Lung Cancer.* 77:38-45 (2012)) are among the very many that have been described, including their role in immune modulation. We discovered that expression of one or more of these immune regulatory factors in an allogenic cell can be used to provide local immune suppression and/or reduce allorejection in a host to which the cells are administered.

We modified allogenic cells through the use of specific immunomodulatory factors introduced into a cell or population of cells. The modified cells evade immune rejection through the simultaneous modulation of many different local immune pathways. Such genetically engineered cells can be transplanted "off the shelf" into many recipients regardless of genetic background and without rejection by the recipient's immune system. This immunomodulatory approach overcomes the requirement for systemic immunosuppression of the transplant recipient, which can be dangerous to the recipient. Thus, although an immunosuppressive agent(s) can be administered to a patient that receives the modified cells described herein, the therapy need not include the administration of an immunosuppressive agent(s). This immunomodulatory approach also overcomes the costly and impractical methodology of deriving patient-specific iPS cells, manipulating regulatory cells, or inducing chimerism through hematopoietic cell transplantation (HCT).

Cells can be genetically modified to express a set of transgenes encoding gene products that are cytoplasmic, membrane bound, or local acting, and whose function is to mitigate immune function (e.g., graft attacking leukocyte and NK cell activation) or to act as a defense mechanism against the immune response (e.g., attacking leukocytes). The set of transgenes may be selected from the genes having a role in the immune modulatory pathways described above. Such genes include, but are not limited to those provided in Table 1.

Table 1: Genes that can be expressed by allogenic cells for local immunosuppression

Gene	Function
PD-L1	Induces cell death in PD-L1 expressing T cells and macrophages
HLA-G (mouse gene: H2-M3)	Inhibits NK cells from attacking cells lacking MHC molecules

Gene	Function
Cd47	Negative regulator of macrophages and killer T cells
Cd200	Inhibits macrophage activation
FASLG (mouse gene: FasL)	Induces apoptosis in Fas expressing CD8+ T cells
Clc21 (mouse gene: Ccl21b)	Chemo-attractant for antigen presenting cells (APCs)
Mfge8	Inhibition of macrophage phagocytosis
Serpin B9 (mouse gene: Spi6)	Inhibition of granzyme/perforin attack
Dad1	Negative regulator of programmed cell death
Tnfrsf10	Induces apoptosis in leukocytes expressing the TRAIL receptor
Cd39	Converts ATP to AMP, inhibits T-cells
Cd73	Converts AMP to adenosine, inhibits T-cells, suppresses dendritic cells
Lag3	Inhibits T-cell activation, proliferation, function
Il1r2	Blocks IL-1B activity, blocks inflammation and innate cell activation
Ackr2	Decoy receptor for chemokines, prevents leukocyte accumulation
Tnfrsf22	Decoy receptor, blocks TRAIL-induced apoptosis from T-cells
Tnfrsf23	Decoy receptor, blocks TRAIL-induced apoptosis from T-cells
IFNγR1 d39	Dominant negative interferon gamma receptor 1, prevents IFNγ-mediated upregulation of MHCs in ES cells

C-C motif chemokine ligand 21(Ccl21) is expressed by local lymph nodes where it acts to attract activated antigen presenting cells (APCs). This key function offers an opportunity to "reverse" the migration of APCs by overexpressing this gene on grafted cells. Indeed, some melanomas express Ccl21 and recruit CCR7$^+$ cells that, in turn, can reorganize portions of their tumor stroma as "self". This leads to a stromal reconstruction that directs the recruitment and maintenance of Cd4$^+$ Tregs (Zindl et al., *Science.* 328:697-8 (2010)). In fact, the expression of Ccl21 on tumors can protect co-implanted Ccl21 deficient tumor cells from rejection in a syngeneic allograft setting (Shields et al., *Science.* 328:749-52(2010)). Ccl21b is the mouse ortholog of human Ccl21.

The amino acid sequences of mouse and human Ccl21 are:

Mouse Ccl21

MAQMMTLSLLSLVLALCIPWTQGSDGGGQDCCLKYSQKKIPYSIVRGYRKQEPSLGCPI PAILFLPRKHSKPELCANPEEGWVQNLMRRLDQPPAPGKQSPGCRKNRGTSKSGKKG KGSKGCKRTEQTQPSRG (SEQ ID NO: 1)

Human Ccl21

MAQSLALSLLILVLAFGIPRTQGSDGGAQDCCLKYSQRKIPAKVVRSYRKQEPSLGCSIP

AILFLPRKRSQAELCADPKELWVQQLMQHLDKTPSPQKPAQGCRKDRGASKTGKKGK GSKGCKRTERSQTPKGP (SEQ ID NO: 2)

Expression of Cd47 in umbilical cord blood can promote the development of hyporesponsive T-cells (Avice et al., *J Immunol.* 167:2459-68 (2001)). Erythrocytes also up-regulate Cd47 to avoid dendritic cell activation due to their lack of "self" presentation (van den Berg et al., *Immunity.* 43:622-4 (2015)). More recently, it was shown that expression of human Cd47 increases engraftment in a mouse model of pig-to-human hematopoietic cell transplantation (Tena et al., *Am J Transplant.* 14:2713-22 (2014)).

The amino acid sequences of mouse and human Cd47 are:

Mouse Cd47

MWPLAAALLLGSCCCGSAQLLFSNVNSIEFTSCNETVVIPCIVRNVEAQSTEEMFVKWK LNKSYIFIYDGNKNSTTTDQNFTSAKISVSDLINGIASLKMDKRDAMVGNYTCEVTELSRE GKTVIELKNRTVSWFSPNEKILIVIFPILAILLFWGKFGILTLKYKSSHTNKRIILLLVAGLVLT VIVVVGAILLIPGEKPVKNASGLGLIVISTGILILLQYNVFMTAFGMTSFTIAILITQVLGYVLA LVGLCLCIMACEPVHGPLLISGLGIIALAELLGLVYMKFVASNQRTIQPPRNR (SEQ ID NO: 3)

Human Cd47

MWPLVAALLLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEVYVKWK FKGRDIYTFDGALNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVTEL TREGETIIELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVA GLVITVIVIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYYVFSTAIGLTSFVIAILVIQVIAY ILAVVGLSLCIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLN AFKESKGMMNDE (SEQ ID NO: 4)

Cd200 is also as an important immunoregulatory molecule; increased expression can reduce the severity of allograft rejection, autoimmunity, and allergic disease (Gorczynski et al., *J Immunol.* 172:7744-9 (2004)). It has been shown that, *in vitro*, APC expression of Cd200 suppresses production of interferon gamma (IFN-γ) and cytolytic granules by activated Cd8+ T-cells (Misstear et al., *J Virol.* 86:6246-57 (2012)). Most interestingly, overexpression of Cd200 increases the survival of skin and cardiac allografts in mice by promoting of Foxp3+ Treg cells (Gorczynski et al., *Transplantation.* 98:1271-8 (2014)).

The amino acid sequences of mouse and human Cd200 are:

Mouse Cd200

MGSLVFRRPFCHLSTYSLIWGMAAVALSTAQVEVVTQDERKALHTTASLRCSLKTSQEP LIVTWQKKKAVSPENMVTYSKTHGVVIQPAYKDRINVTELGLWNSSITFWNTTLEDEGC YMCLFNTFGSQKVSGTACLTLYVQPIVHLHYNYFEDHLNITCSATARPAPAISWKGTGTG IENSTESHFHSNGTTSVTSILRVKDPKTQVGKEVICQVLYLGNVIDYKQSLDKGFWFSVP LLLSIVSLVILLVLISILLYWKRHRNQERGESSQGMQRMK (SEQ ID NO: 5)

Human Cd200

MERLVIRMPFSHLSTYSLVWVMAAVVLCTAQVQVVTQDEREQLYTPASLKCSLQNAQE ALIVTWQKKKAVSPENMVTFSENHGVVIQPAYKDKINITQLGLQNSTITFWNITLEDEGCY

MCLFNTFGFGKISGTACLTVYVQPIVSLHYKFSEDHLNITCSATARPAPMVFWKVPRSGI
ENSTVTLSHPNGTTSVTSILHIKDPKNQVGKEVICQVLHLGTVTDFKQTVNKGYWFSVPL
LLSIVSLVILLVLISILLYWKRHRNQDRGELSQGVQKMT (SEQ ID NO: 6)

Spi6 is an endogenous inhibitor of the cytotoxic effector molecule granzyme B released by activated Cd8+ T-cells (Sun et al., *J Biol Chem.* 272:15434-41 (1997)). Some data shows that Mesenchymal Stem Cells (MSCs) escape immune rejection by upregulating this molecule (El Haddad et al., *Blood.* 117:1176-83 (2011)). It has also recently been demonstrated that the ability of dendritic cells to present antigen to cytotoxic T cells without themselves being killed through contact mediated cytotoxicity is mediated by Spi6 (Lovo et al., *J Immunol.* 188:1057-63 (2012)). Spi6 is also known as Serpin B9.

The amino acid sequences of mouse Spi6 and the human counterpart, Serpin B9, are:

Mouse Spi6

MNTLSEGNGTFAIHLLKMLCQSNPSKNVCYSPASISSALAMVLLGAKGQTAVQISQALGL
NKEEGIHQGFQLLLRKLNKPDRKYSLRVANRLFADKTCEVLQTFKESSLHFYDSEMEQL
SFAEEAEVSRQHINTWVSKQTEGKIPELLSGGSVDSETRLVLINALYFKGKWHQPFNKE
YTMDMPFKINKDEKRPVQMMCREDTYNLAYVKEVQAQVLVMPYEGMELSLVVLLPDEG
VDLSKVENNLTFEKLTAWMEADFMKSTDVEVFLPKFKLQEDYDMESLFQRLGVVDVFQ
EDKADLSGMSPERNLCVSKFVHQSVVEINEEGTEAAAASAIIEFCCASSVPTFCADHPFL
FFIRHNKANSILFCGRFSSP (SEQ ID NO: 7)

Human Serpin B9

METLSNASGTFAIRLLKILCQDNPSHNVFCSPVSISSALAMVLLGAKGNTATQMAQALSL
NTEEDIHRAFQSLLTEVNKAGTQYLLRTANRLFGEKTCQFLSTFKESCLQFYHAELKELS
FIRAAEESRKHINTWVSKKTEGKIEELLPGSSIDAETRLVLVNAIYFKGKWNEPFDETYTR
EMPFKINQEEQRPVQMMYQEATFKLAHVGEVRAQLLELPYARKELSLLVLLPDDGVELS
TVEKSLTFEKLTAWTKPDCMKSTEVEVLLPKFKLQEDYDMESVLRHLGIVDAFQQGKAD
LSAMSAERDLCLSKFVHKSFVEVNEEGTEAAAASSCFVVAECCMESGPRFCADHPFLF
FIRHNRANSILFCGRFSSP (SEQ ID NO: 8)

Activated, cytotoxic, Cd8+ can kill target cells by expression of FasL, which binds to the FAS receptor and activates a caspase-mediated apoptosis in targeted cells. However, many tumors have developed a “counterattack” by upregulating FasL on their surface (Chen et al., *J Immunol.* 171:1183-91 (2003)). Selective expression of FasL in the vasculature of human and mouse solid tumors has been associated with scarce Cd8+ T-cell infiltration and a predominance of FoxP3+ Treg cells (Motz et al. *Nat Med.* 20:607-15 (2014)). Most recently, it was shown that B-lymphocytes also use the expression of FasL to kill T helper cells at the effector stage of immune responses (Lundy et al., *Front Immunol.* 6:122 (2015)). FasL is the mouse ortholog of human FASLG.

The amino acid sequences of mouse FasL and the human counterpart, FASLG, are:

Mouse FasL

MQQPMNYPCPQIFWVDSSATSSWTPPGSVFPCPSSGPRGPDQRRPPPPPPPVSPL
PPPSQPLPLPPLTPLKKKDHNTNLWLPVVFFMVLVALVGMGLGMYQLFHLQKELAEL
REFTNQSLKVSSFEKQIANPSTPSEKKELRSVAHLTGNPHSRSIPLEWEDTYGTALISG
VKYKKGSLVINEAGLYFVYSKVYFRGQSCNNQPLNHKVYMRNSKYPGDLVLMEEKRL

NYCTTGQIWAHSSYLGAVFNLTSADHLYVNISQLSLINFEESKTFFGLYKL (SEQ ID NO: 9)

Human FASLG

MQQPFNYPYPQIYWVDSSASSPWAPPGTVLPCPTSVPRRPGQRRPPPPPPPPLPP PPPPPPLPPLPLPPLKKRGNHSTGLCLLVMFFMVLVALVGLGLGMFQLFHLQKELAEL RESTSQMHTASSLEKQIGHPSPPPEKKELRKVAHLTGKSNSRSMPLEWEDTYGIVLLS GVKYKKGGLVINETGLYFVYSKVYFRGQSCNNLPLSHKVYMRNSKYPQDLVMMEGK MMSYCTTGQMWARSSYLGAVFNLTSADHLYVNVSELSLVNFEESQTFFGLYKL (SEQ ID NO: 10)

PD-L1 is a critical immune modulatory molecule that binds to Programmed Cell Death 1 (PD-1). PD-1 is expressed on T-cells, and binding to PD-L1 results in T-cell anergy (MacDonald et al., *J Immunol.* 126:1671-5 (1981)).

The amino acid sequences of mouse and human PD-L1 are:

Mouse PD-L1

MRIFAGIIFTACCHLLRAFTITAPKDLYVVEYGSNVTMECRFPVERELDLLALVVYWEKE DEQVIQFVAGEEDLKPQHSNFRGRASLPKDQLLKGNAALQITDVKLQDAGVYCCIISY GGADYKRITLKVNAPYRKINQRISVDPATSEHELICQAEGYPEAEVIWTNSDHQPVSG KRSVTTSRTEGMLLNVTSSLRVNATANDVFYCTFWRSQPGQNHTAELIIPELPATHPP QNRTHWVLLGSILLFLIVVSTVLLFLRKQVRMLDVEKCGVEDTSSKNRNDTQFEET (SEQ ID NO: 11)

Human PDL1 (CD274)

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEM EDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMIS YGGADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVL SGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPP NERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEET (SEQ ID NO: 12)

Inflammatory environments, like those induced by allograft transplants, attracts macrophages and inflammatory monocytes, among many other innate immune cells. The milk fat globule epidermal growth factor–8 (Mfge-8) is expressed by many murine tumours (Neutzner et al., *Cancer Res.* 67:6777-85 (2007)) and has been shown to contribute to local immune suppression by polarizing incoming monocytes to suppressive, M2-like macrophages (Soki et al., *J Biol Chem.* 289:24560-72 (2014)).

The amino acid sequences of mouse and human MFGE-8 are:

Mouse MFGE8

MQVSRVLAALCGMLLCASGLFAASGDFCDSSLCLNGGTCLTGQDNDIYCLCPEGFTG LVCNETERGPCSPNPCYNDAKCLVTLDTQRGDIFTEYICQCPVGYSGIHCETETNYYN LDGEYMFTTAVPNTAVPTPAPTPDLSNNLASRCSTQLGMEGGAIADSQISASSVYMG FMGLQRWGPELARLYRTGIVNAWTASNYDSKPWIQVNLLRKMRVSGVMTQGASRAG RAEYLKTFKVAYSLDGRKFEFIQDESGGDKEFLGNLDNNSLKVNMFNPTLEAQYIKLY PVSCHRGCTLRFELLGCELHGCSEPLGLKNNTIPDSQMSASSSYKTWNLRAFGWYP HLGRLDNQGKINAWTAQSNSAKEWLQVDLGTQRQVTGIITQGARDFGHIQYVASYKV AHSDDGVQWTVYEEQGSSKVFQGNLDNNSHKKNIFEKPFMARYVRVLPVSWHNRIT LRLELLGC (SEQ ID NO: 13)

Human MFGE8

MPRPRLLAALCGALLCAPSLLVALDICSKNPCHNGGLCEEISQEVRGDVFPSYTCTCL
KGYAGNHCETKCVEPLGMENGNIANSQIAASSVRVTFLGLQHWVPELARLNRAGMV
NAWTPSSNDDNPWIQVNLLRRMWVTGVVTQGASRLASHEYLKAFKVAYSLNGHEFD
FIHDVNKKHKEFVGNWNKNAVHVNLFETPVEAQYVRLYPTSCHTACTLRFELLGCELN
GCANPLGLKNNSIPDKQITASSSYKTWGLHLFSWNPSYARLDKQGNFNAWVAGSYG
NDQWLQVDLGSSKEVTGIITQGARNFGSVQFVASYKVAYSNDSANWTEYQDPRTGS
SKIFPGNWDNHSHKKNLFETPILARYVRILPVAWHNRIALRLELLGC (SEQ ID NO: 14)

The potent killing potential of NK cells is also absolutely critical in graft rejection. NK cells can kill targets cells that lack MHC class I molecules, as well as other cells within an inflammatory setting. H2-M3, the murine homologue of human HLA-G has recently been shown to have a regulatory effect on NK cells, licensing them to ignore cells that lack "self molecules" (Andrews et al., *Nat Immunol.* 13:1171-7 (2012)). This is thought to be achieved by binding of HLA-G, immunosuppressive receptors on both NK and T-cells (Carosella et al., *Adv Immunol.* 127:33-144 (2015)). H2-M3 is the mouse ortholog of human HLA-G.

The amino acid sequences of mouse H2-M3 and the human counterpart, HLA-G, are:

Mouse H2-M3

SIEEIPRMEPRAPWMEKERPEYWKELKLKVKNIAQSARANLRTLLRYYNQSEGGSHIL
QWMVSCEVGPDMRLLGAHYQAAYDGSDYITLNEDLSSWTAVDMVSQITKSRLESAG
TAEYFRAYVEGECLELLHRFLRNGKEILQRADPPKAHVAHHPRPKGDVTLRCWALGF
YPADITLTWQKDEEDLTQDMELVETRPSGDGTFQKWAAVVVPSGEEQRYTCYVHHE
GLTEPLALKWGRSSQSSVVIMV (SEQ ID NO: 15)

Human HLA-G

MVVMAPRTLFLLLSGALTLTETWAGSHSMRYFSAAVSRPGRGEPRFIAMGYVDDTQF
VRFDSDSACPRMEPRAPWVEQEGPEYWEEETRNTKAHAQTDRMNLQTLRGYYNQS
EASSHTLQWMIGCDLGSDGRLLRGYEQYAYDGKDYLALNEDLRSWTAADTAAQISKR
KCEAANVAEQRRAYLEGTCVEWLHRYLENGKEMLQRADPPKTHVTHHPVFDYEATL
RCWALGFYPAEIILTWQRDGEDQTQDVELVETRPAGDGTFQKWAAVVVPSGEEQRY
TCHVQHEGLPEPLMLRWKQSSLPTIPIMGIVAGLVVLAAVVTGAAVAAVLWRKKSSD
(SEQ ID NO: 16)

A set of transgenes that includes one or more (e.g., one, two, three, four, five, six, seven, or all eight) of PD-L1, H2-M3, Cd47, Cd200, FasL, Ccl21b, Mfge8, and Spi6A can be expressed in cells. The cells may be, for example, stem cells or a cell that is amenable to genome editing, such as a cell that can be used for therapy and/or differentiated into a therapeutic cell type. The stem cells may be, for example, embryonic stem (ES) cells or induced pluripotent stem (iPS) cells. The set of transgenes may comprise 1, 2, 3, 4, 5, 6, 7, or all 8 of these genes or may comprise at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7 of these genes. The cell may be further genetically modified to express one or more of TGF-β, Cd73, Cd39, Lag3, Il1r2, Ackr2, Tnfrsf22, Tnfrs23, Tnfrsf10, Dad1, and/or IFNγR1 d39. The TGF-β transgene may be modified to express the gene product in a membrane-bound form (i.e., such that the gene product is expressed on the cell surface of the allograft), using methods known to

those skilled in the art. For example, a method for localizing TGF-β to the membrane is to co-express TGF-β with an additional transgene encoding the LRRC32 protein or any other polypeptide that results in localization of TGF-β to the cell membrane. This protein anchors TGF-β to the membrane. (Tran DQ et al., Proc Natl Acad Sci U. S. A. 106:13445-50 (2009)).

The amino acid sequence of IFNγR1 d39 is:

MGPQAAAGRMILLVVLMLSAKVGSGALTSTEDPEPPSVPVPTNVLIKSYNLNPVVCWE
YQNMSQTPIFTVQVKVYSGSWTDSCTNISDHCCNIYGQIMYPDVSAWARVKAKVGQK
ESDYARSKEFLMCLKGKVGPPGLEIRRKKEEQLSVLVFHPEVVVNGESQGTMFGDGS
TCYTFDYTVYVEHNRSGEILHTKHTVEKEECNETLCELNISVSTLDSRYCISVDGISSF
WQVRTEKSKDVCIPPFHDDRKDSIWILVVAPLTVFTVVILVFAYWYTKKNSFKRKSIML
PKSLLSVVKSATLETKPESKYSLVTPHQPAVLESETVICEEPLSTVTAPDSPEAAEQEE
LSKETKALEAGGSTSAMTPDSPPTPTQRRSFSLLSSNQSGPCSLTAYHSRNGSDSGL
VGSGSSISDLESLPNNNSETKMAEHDPPPVRKA (SEQ ID NO: 17)

The genes may be human genes or murine genes. In an embodiment, the gene is of the same species as the recipient of the allograft recipient in which the cell is to be transplanted. In an embodiment, the gene is of any species in which the function of the gene is conserved or in which a designed biologic has the agonist function of the endogenous counterpart. Methods for introducing and expressing these transgenes in cells are described herein and are also known to those skilled in the art. Cells expressing these transgenes may be referred to as "cloaked" due to their ability to evade allorejection without systemic immunosuppression and without the need for immunosuppressive drugs.

It is contemplated herein that populations of cells derived from the above-described cloaked cells can also be used to produce a local immunosuppression when transplanted at a transplant site of an allogeneic recipient.

Before or after generating the cloaked cells of the disclosure, the cells can first be modified to be fail-safe cells. Fail-safe cells use cell division loci (CDLs) to control cell proliferation in animal cells. CDLs, as provided herein, are loci whose transcription product(s) are expressed during cell division. CDLs may be genetically modified, as described herein, to comprise a negative selectable marker and/or an inducible activator-based gene expression system, which allows a user to permit, ablate, and/or inhibit proliferation of the genetically modified cell(s) by adding or removing an appropriate inducer. Methods for making and using fail-safe cells are described, for example, in WO 2016/141480, the entire teachings of which are incorporated herein by reference. A cell may be made fail-safe first and then cloaked afterwards. Alternatively, a cell may be cloaked first and then made fail-safe afterwards.

The cell may be a vertebrate cell, for example, a mammalian cell, such as a human cell or a mouse cell. The cell may also be a vertebrate stem cell, for example, a mammalian stem cell, such as a human stem cell or a mouse stem cell. Preferably, the cell or stem cell is amenable to genetic modification. Preferably, the cell or stem cell is deemed by a user to have therapeutic value, meaning that the cell or stem cell may be used to treat a disease, disorder, defect or injury in a subject in need of treatment for same.

In some embodiments, the cell is a stem cell or progenitor cell (e.g., iPSC, embryonic stem cell, hematopoietic stem cell, mesenchymal stem cell, endothelial stem cell, epithelial stem cell, adipose stem or progenitor cells, lung stem or progenitor cells, mammary stem cells, olfactory adult stem cells, hair follicle stem cells, multipotent stem cells, amniotic stem cells, cord blood stem cells, or neural stem or progenitor cells). In some embodiments, the stem cells are adult stem cells (e.g., somatic stem cells or tissue specific stem cells). In some embodiments, the stem or progenitor cell is capable of being differentiated (e.g., the stem cell is totipotent, pluripotent, or multipotent). In some embodiments, the cell is isolated from embryonic or neonatal tissue. In some embodiments, the cell is a fibroblast, monocytic precursor, B cell, exocrine cell, pancreatic progenitor, endocrine progenitor, hepatoblast, myoblast, preadipocyte, progenitor cell, hepatocyte, chondrocyte, smooth muscle cell, K562 human erythroid leukemia cell line, bone cell, synovial cell, tendon cell, ligament cell, meniscus cell, adipose cell, dendritic cells, or natural killer cell. In some embodiments, the cell is manipulated (e.g., converted or differentiated) into a muscle cell, erythroid-megakaryocytic cell, eosinophil, iPS cell, macrophage, T cell, islet beta-cell, neuron, cardiomyocyte, blood cell, endocrine progenitor, exocrine progenitor, ductal cell, acinar cell, alpha cell, beta cell, delta cell, PP cell, hepatocyte, cholangiocyte, or brown adipocyte. In some embodiments, the cell is a muscle cell (e.g., skeletal, smooth, or cardiac muscle cell), erythroid-megakaryocytic cell, eosinophil, iPS cell, macrophage, T cell, islet beta-cell, neuron, cardiomyocyte, blood cell (e.g., red blood cell, white blood cell, or platelet), endocrine progenitor, exocrine progenitor, ductal cell, acinar cell, alpha cell, beta cell, delta cell, PP cell, hepatocyte, cholangiocyte, or white or brown adipocyte. In some embodiments, the cell is a hormone-secreting cell (e.g., a cell that secretes insulin, oxytocin, endorphin, vasopressin, serotonin, somatostatin, gastrin, secretin, glucagon, thyroid hormone, bombesin, cholecystokinin, testosterone, estrogen, or progesterone, renin, ghrelin, amylin, or pancreatic polypeptide), an epidermal keratinocyte, an epithelial cell (e.g., an exocrine secretory epithelial cell, a thyroid epithelial cell, a keratinizing epithelial cell, a gall bladder epithelial cell, or a surface epithelial cell of the cornea, tongue, oral cavity, esophagus, anal canal, distal urethra, or vagina), a kidney cell, a germ cell, a skeletal joint synovium cell, a periosteum cell, a bone cell (e.g., osteoclast or osteoblast), a perichondrium cell (e.g., a chondroblast or chondrocyte), a cartilage cell (e.g., chondrocyte), a fibroblast, an endothelial cell, a pericardium cell, a meningeal cell, a keratinocyte precursor cell, a keratinocyte stem cell, a pericyte, a glial cell, an ependymal cell, a cell isolated from an amniotic or placental membrane, or a serosal cell (e.g., a serosal cell lining body cavities). In some embodiments, the cell is a somatic cell. In some embodiments, the cells are derived from skin or other organs, e.g., heart, brain or spinal cord, liver, lung, kidney, pancreas, bladder, bone marrow, spleen, intestine, or stomach. The cells can be from humans or other mammals (e.g., rodent, non-human primate, bovine, or porcine cells). It is contemplated herein that cloaked cells may be of use in cell-based therapies wherein it may be desirable to evade allorejection at a localized transplant site.

In some embodiments, the cloaked cells described herein survive in a host without stimulating the host immune response for one week or more (e.g., one week, two weeks, one month, two months, three

months, 6 months, one year, two years, three years, four years, five years or more, e.g., for the life of the cell and/or its progeny). The cells maintain expression of the cloaking transgenes for as long as they survive in the host (e.g., if cloaking transgenes are no longer expressed, the cloaked cells may be removed by the host's immune system). In some embodiments, the cloaked cells further express a transgene encoding a protein that allows the cloaked cells to be detected *in vivo* (e.g., a fluorescent protein, such as GFP or other detectable marker).

It is contemplated herein that the combination of cloaked and fail-safe cells may be of use in cell-based therapies wherein it may be desirable to evade allorejection at a localized transplant site, while also being able to eliminate cells exhibiting undesirable growth rates, irrespective of whether such cells are generated before or after grafting the cells into a host. The combined cloaking and fail-safe technologies allows for localized immunoprotection while addressing the risk that the recipient will develop a malignancy because the cells are providing local immunosuppression.

Methods of Producing Cloaked Cells

The compositions and methods described herein can be used to reduce rejection of allogenic cells through expression of cloaking transgenes. A wide array of methods has been established for the delivery of proteins to mammalian cells and for the stable expression of genes encoding proteins in mammalian cells, which can be used to produce the cloaked cells described herein.

Polynucleotides encoding cloaking proteins or therapeutic agents

One platform that can be used to achieve therapeutically effective expression of cloaking proteins or therapeutic agents in mammalian cells is via the stable expression of a gene encoding a cloaking protein or therapeutic agent (e.g., by integration into the nuclear or mitochondrial genome of a mammalian cell, or by episomal concatemer formation in the nucleus of a mammalian cell). The gene is a polynucleotide that encodes the primary amino acid sequence of the corresponding protein. In order to introduce exogenous genes into a mammalian cell, genes can be incorporated into a vector. Vectors can be introduced into a cell by a variety of methods, including transformation, transfection, transduction, direct uptake, projectile bombardment, and by encapsulation of the vector in a liposomes. Examples of suitable methods of transfecting or transforming cells include calcium phosphate precipitation, electroporation, microinjection, infection, lipofection and direct uptake. Such methods are described in more detail, for example, in Green, et al., Molecular Cloning: A Laboratory Manual, Fourth Edition (Cold Spring Harbor University Press, New York 2014); and Ausubel, et al., Current Protocols in Molecular Biology (John Wiley & Sons, New York 2015), the disclosures of each of which are incorporated herein by reference.

Cloaking proteins or therapeutic agents can also be introduced into a mammalian cell by targeting vectors containing portions of a gene encoding a cloaking protein or therapeutic agent to cell membrane phospholipids. For example, vectors can be targeted to the phospholipids on the extracellular surface of

the cell membrane by linking the vector molecule to a VSV-G protein, a viral protein with affinity for all cell membrane phospholipids. Such a construct can be produced using methods well known to those of skill in the field.

Recognition and binding of the polynucleotide encoding a cloaking protein or therapeutic agent by mammalian RNA polymerase is important for gene expression. As such, one may include sequence elements within the polynucleotide that exhibit a high affinity for transcription factors that recruit RNA polymerase and promote the assembly of the transcription complex at the transcription initiation site. Such sequence elements include, e.g., a mammalian promoter, the sequence of which can be recognized and bound by specific transcription initiation factors and ultimately RNA polymerase.

Polynucleotides suitable for use in the compositions and methods described herein also include those that encode a cloaking protein or therapeutic agent downstream of a mammalian promoter. Promoters that are useful for the expression of a cloaking protein or therapeutic agent in mammalian cells include constitutive promoters. Constitutive promoters include the CAG promoter, the cytomegalovirus (CMV) promoter, the EF1α promoter, and the PGK promoter. Alternatively, promoters derived from viral genomes can also be used for the stable expression of these agents in mammalian cells. Examples of functional viral promoters that can be used to promote mammalian expression of these agents include adenovirus late promoter, vaccinia virus 7.5K promoter, SV40 promoter, *tk* promoter of HSV, mouse mammary tumor virus (MMTV) promoter, LTR promoter of HIV, promoter of moloney virus, Epstein barr virus (EBV) promoter, and the Rous sarcoma virus (RSV) promoter.

Once a polynucleotide encoding a cloaking protein or a therapeutic agent described herein below has been incorporated into the nuclear DNA of a mammalian cell, the transcription of this polynucleotide can be induced by methods known in the art. For example expression can be induced by exposing the mammalian cell to an external chemical reagent, such as an agent that modulates the binding of a transcription factor and/or RNA polymerase to the mammalian promoter and thus regulates gene expression. The chemical reagent can serve to facilitate the binding of RNA polymerase and/or transcription factors to the mammalian promoter, e.g., by removing a repressor protein that has bound the promoter. Alternatively, the chemical reagent can serve to enhance the affinity of the mammalian promoter for RNA polymerase and/or transcription factors such that the rate of transcription of the gene located downstream of the promoter is increased in the presence of the chemical reagent. Examples of chemical reagents that potentiate polynucleotide transcription by the above mechanisms include tetracycline and doxycycline. These reagents are commercially available (Life Technologies, Carlsbad, CA) and can be administered to a mammalian cell in order to promote gene expression according to established protocols.

Other DNA sequence elements that may be included in the nucleic acid vectors for use in the compositions and methods described herein include enhancer sequences. Enhancers represent another class of regulatory elements that induce a conformational change in the polynucleotide containing the gene of interest such that the DNA adopts a three-dimensional orientation that is favorable for binding of

transcription factors and RNA polymerase at the transcription initiation site. Thus, polynucleotides for use in the compositions and methods described herein include those that encode a cloaking protein or therapeutic agent and additionally include a mammalian enhancer sequence. Many enhancer sequences are now known from mammalian genes, and examples include enhancers from the genes that encode mammalian globin, elastase, albumin, α-fetoprotein, and insulin. Enhancers for use in the compositions and methods described herein also include those that are derived from the genetic material of a virus capable of infecting a eukaryotic cell. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. Additional enhancer sequences that induce activation of eukaryotic gene transcription are disclosed in Yaniv, et al., Nature 297:17 (1982). An enhancer may be spliced into a vector containing a polynucleotide encoding a cloaking protein or therapeutic agent, for example, at a position 5' or 3' to this gene. In a preferred orientation, the enhancer is positioned at the 5' side of the promoter, which in turn is located 5' relative to the polynucleotide encoding a cloaking protein or therapeutic agent.

The nucleic acid vectors described herein may include a Woodchuck Posttranscriptional Regulatory Element (WPRE). The WPRE acts at the transcriptional level, by promoting nuclear export of transcripts and/or by increasing the efficiency of polyadenylation of the nascent transcript, thus increasing the total amount of mRNA in the cell. The addition of the WPRE to a vector can result in a substantial improvement in the level of transgene expression from several different promoters, both in vitro and in vivo.

In some embodiments, the nucleic acid vectors for use in the compositions and methods described herein include a reporter sequence, which can be useful in verifying gene expression, for example, in specific cells and tissues. Reporter sequences that may be provided in a transgene include DNA sequences encoding β-lactamase, β-galactosidase (LacZ), alkaline phosphatase, thymidine kinase, green fluorescent protein (GFP), chloramphenicol acetyltransferase (CAT), luciferase, and others well known in the art. When associated with regulatory elements which drive their expression, the reporter sequences provide signals detectable by conventional means, including enzymatic, radiographic, colorimetric, fluorescence or other spectrographic assays, fluorescent activating cell sorting assays and immunological assays, including enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and immunohistochemistry. For example, where the marker sequence is the LacZ gene, the presence of the vector carrying the signal is detected by assays for β-galactosidase activity. Where the transgene is green fluorescent protein or luciferase, the vector carrying the signal may be measured visually by color or light production in a luminometer.

Techniques for introducing transgenes into cells

Transfection

Techniques that can be used to introduce a transgene, such as a cloaking transgene or a therapeutic transgene described herein, into a target cell (e.g., a mammalian cell) are well known in the art. For instance, electroporation can be used to permeabilize mammalian cells (e.g., human target cells) by the application of an electrostatic potential to the cell of interest. Mammalian cells, such as human cells, subjected to an external electric field in this manner are subsequently predisposed to the uptake of exogenous nucleic acids. Electroporation of mammalian cells is described in detail, e.g., in Chu et al., Nucleic Acids Research 15:1311 (1987), the disclosure of which is incorporated herein by reference. A similar technique, Nucleofection™, utilizes an applied electric field in order to stimulate the uptake of exogenous polynucleotides into the nucleus of a eukaryotic cell. Nucleofection™ and protocols useful for performing this technique are described in detail, e.g., in Distler et al., Experimental Dermatology 14:315 (2005), as well as in US 2010/0317114, the disclosures of each of which are incorporated herein by reference.

Additional techniques useful for the transfection of target cells include the squeeze-poration methodology. This technique induces the rapid mechanical deformation of cells in order to stimulate the uptake of exogenous DNA through membranous pores that form in response to the applied stress. This technology is advantageous in that a vector is not required for delivery of nucleic acids into a cell, such as a human target cell. Squeeze-poration is described in detail, e.g., in Sharei et al., Journal of Visualized Experiments 81:e50980 (2013), the disclosure of which is incorporated herein by reference.

Lipofection represents another technique useful for transfection of target cells. This method involves the loading of nucleic acids into a liposome, which often presents cationic functional groups, such as quaternary or protonated amines, towards the liposome exterior. This promotes electrostatic interactions between the liposome and a cell due to the anionic nature of the cell membrane, which ultimately leads to uptake of the exogenous nucleic acids, for instance, by direct fusion of the liposome with the cell membrane or by endocytosis of the complex. Lipofection is described in detail, for instance, in US Patent No. 7,442,386, the disclosure of which is incorporated herein by reference. Similar techniques that exploit ionic interactions with the cell membrane to provoke the uptake of foreign nucleic acids include contacting a cell with a cationic polymer-nucleic acid complex. Exemplary cationic molecules that associate with polynucleotides so as to impart a positive charge favorable for interaction with the cell membrane include activated dendrimers (described, e.g., in Dennig, Topics in Current Chemistry 228:227 (2003), the disclosure of which is incorporated herein by reference) polyethylenimine, and diethylaminoethyl (DEAE)-dextran, the use of which as a transfection agent is described in detail, for instance, in Gulick et al., Current Protocols in Molecular Biology 40:I:9.2:9.2.1 (1997), the disclosure of which is incorporated herein by reference. Magnetic beads are another tool that can be used to transfect target cells in a mild and efficient manner, as this methodology utilizes an applied magnetic field in order

to direct the uptake of nucleic acids. This technology is described in detail, for instance, in US 2010/0227406, the disclosure of which is incorporated herein by reference.

Another useful tool for inducing the uptake of exogenous nucleic acids by target cells is laserfection, also called optical transfection, a technique that involves exposing a cell to electromagnetic radiation of a particular wavelength in order to gently permeabilize the cells and allow polynucleotides to penetrate the cell membrane. The bioactivity of this technique is similar to, and in some cases found superior to, electroporation.

Impalefection is another technique that can be used to deliver genetic material to target cells. It relies on the use of nanomaterials, such as carbon nanofibers, carbon nanotubes, and nanowires. Needle-like nanostructures are synthesized perpendicular to the surface of a substrate. DNA containing the gene, intended for intracellular delivery, is attached to the nanostructure surface. A chip with arrays of these needles is then pressed against cells or tissue. Cells that are impaled by nanostructures can express the delivered gene(s). An example of this technique is described in Shalek et al., PNAS 107: 1870 (2010), the disclosure of which is incorporated herein by reference.

Magnetofection can also be used to deliver nucleic acids to target cells. The magnetofection principle is to associate nucleic acids with cationic magnetic nanoparticles. The magnetic nanoparticles are made of iron oxide, which is fully biodegradable, and coated with specific cationic proprietary molecules varying upon the applications. Their association with the gene vectors (DNA, siRNA, viral vector, etc.) is achieved by salt-induced colloidal aggregation and electrostatic interaction. The magnetic particles are then concentrated on the target cells by the influence of an external magnetic field generated by magnets. This technique is described in detail in Scherer et al., Gene Therapy 9:102 (2002), the disclosure of which is incorporated herein by reference.

Another useful tool for inducing the uptake of exogenous nucleic acids by target cells is sonoporation, a technique that involves the use of sound (typically ultrasonic frequencies) for modifying the permeability of the cell plasma membrane permeabilize the cells and allow polynucleotides to penetrate the cell membrane. This technique is described in detail, e.g., in Rhodes et al., Methods in Cell Biology 82:309 (2007), the disclosure of which is incorporated herein by reference.

Microvesicles represent another potential vehicle that can be used to modify the genome of a target cell according to the methods described herein. For instance, microvesicles that have been induced by the co-overexpression of the glycoprotein VSV-G with, e.g., a genome-modifying protein, such as a nuclease, can be used to efficiently deliver proteins into a cell that subsequently catalyzes the site-specific cleavage of an endogenous polynucleotide sequence so as to prepare the genome of the cell for the covalent incorporation of a polynucleotide of interest, such as a gene or regulatory sequence. The use of such vesicles, also referred to as Gesicles, for the genetic modification of eukaryotic cells is described in detail, e.g., in Quinn et al., Genetic Modification of Target Cells by Direct Delivery of Active Protein [abstract]. In: Methylation changes in early embryonic genes in cancer [abstract], in: Proceedings

of the 18th Annual Meeting of the American Society of Gene and Cell Therapy; 2015 May 13, Abstract No. 122.

Viral infection

In addition to achieving high rates of transcription and translation, stable expression of an exogenous gene in a mammalian cell can be achieved by integration of the polynucleotide containing the gene into the nuclear genome of the mammalian cell. A variety of vectors for the delivery and integration of polynucleotides encoding exogenous proteins into the nuclear DNA of a mammalian cell have been developed. Examples of expression vectors are disclosed in, e.g., WO 1994/011026 and are incorporated herein by reference. Expression vectors for use in the compositions and methods described herein contain a cloaking transgene or therapeutic transgene, as well as, e.g., additional sequence elements used for the expression of these agents and/or the integration of these polynucleotide sequences into the genome of a mammalian cell. Certain vectors that can be used for the expression of cloaking transgenes or therapeutic transgenes include plasmids that contain regulatory sequences, such as promoter and enhancer regions, which direct gene transcription. Other useful vectors for expression of cloaking transgenes or therapeutic transgenes contain polynucleotide sequences that enhance the rate of translation of these genes or improve the stability or nuclear export of the mRNA that results from gene transcription. These sequence elements include, e.g., 5' and 3' untranslated regions and a polyadenylation signal site in order to direct efficient transcription of the gene carried on the expression vector. The expression vectors suitable for use with the compositions and methods described herein may also contain a polynucleotide encoding a marker for selection of cells that contain such a vector. Examples of a suitable marker include genes that encode resistance to antibiotics, such as ampicillin, chloramphenicol, kanamycin, or nourseothricin.

Viral vectors for nucleic acid delivery

Viral genomes provide a rich source of vectors that can be used for the efficient delivery of a gene of interest into the genome of a target cell (e.g., a mammalian cell, such as a human cell). Viral genomes are particularly useful vectors for gene delivery because the polynucleotides contained within such genomes are typically incorporated into the nuclear genome of a mammalian cell by generalized or specialized transduction. These processes occur as part of the natural viral replication cycle, and do not require added proteins or reagents in order to induce gene integration. Examples of viral vectors include a retrovirus (e.g., Retroviridae family viral vector), adenovirus (e.g., Ad5, Ad26, Ad34, Ad35, and Ad48), parvovirus (e.g., adeno-associated viruses), coronavirus, negative strand RNA viruses such as orthomyxovirus (e.g., influenza virus), rhabdovirus (e.g., rabies and vesicular stomatitis virus), paramyxovirus (e.g. measles and Sendai), positive strand RNA viruses, such as picornavirus and alphavirus, and double stranded DNA viruses including adenovirus, herpesvirus (e.g., Herpes Simplex virus types 1 and 2, Epstein-Barr virus, cytomegalovirus), and poxvirus (e.g., vaccinia, modified vaccinia

Ankara (MVA), fowlpox and canarypox). Other viruses include Norwalk virus, togavirus, flavivirus, reoviruses, papovavirus, hepadnavirus, human papilloma virus, human foamy virus, and hepatitis virus, for example. Examples of retroviruses include: avian leukosis-sarcoma, avian C-type viruses, mammalian C-type, B-type viruses, D-type viruses, oncoretroviruses, HTLV-BLV group, lentivirus, alpharetrovirus, gammaretrovirus, spumavirus (Coffin, J. M., Retroviridae: The viruses and their replication, Virology, Third Edition (Lippincott-Raven, Philadelphia, 1996)). Other examples include murine leukemia viruses, murine sarcoma viruses, mouse mammary tumor virus, bovine leukemia virus, feline leukemia virus, feline sarcoma virus, avian leukemia virus, human T-cell leukemia virus, baboon endogenous virus, Gibbon ape leukemia virus, Mason Pfizer monkey virus, simian immunodeficiency virus, simian sarcoma virus, Rous sarcoma virus and lentiviruses. Other examples of vectors are described, for example, US Patent No. 5,801,030, the disclosure of which is incorporated herein by reference as it pertains to viral vectors for use in gene therapy.

AAV vectors for nucleic acid delivery

In some embodiments, cloaking transgenes or therapeutic transgenes described herein are incorporated into rAAV vectors and/or virions in order to facilitate their introduction into a cell. rAAV vectors useful in the compositions and methods described herein are recombinant nucleic acid constructs that include (1) a promoter, (2) a heterologous sequence to be expressed (e.g., a cloaking transgene or therapeutic transgene described herein), and (3) viral sequences that facilitate integration and expression of the heterologous genes. The viral sequences may include those sequences of AAV that are required in cis for replication and packaging (e.g., functional ITRs) of the DNA into a virion. Such rAAV vectors may also contain marker or reporter genes. Useful rAAV vectors have one or more of the AAV WT genes deleted in whole or in part, but retain functional flanking ITR sequences. The AAV ITRs may be of any serotype suitable for a particular application. Methods for using rAAV vectors are described, for example, in Tal et al., J. Biomed. Sci. 7:279 (2000), and Monahan and Samulski, Gene Delivery 7:24 (2000), the disclosures of each of which are incorporated herein by reference as they pertain to AAV vectors for gene delivery.

The transgenes and vectors described herein (e.g., a promoter operably linked to a cloaking transgene or therapeutic transgene) can be incorporated into a rAAV virion in order to facilitate introduction of the polynucleotide or vector into a cell. The capsid proteins of AAV compose the exterior, non-nucleic acid portion of the virion and are encoded by the AAV cap gene. The cap gene encodes three viral coat proteins, VP1, VP2 and VP3, which are required for virion assembly. The construction of rAAV virions has been described, for instance, in US 5,173,414; US 5,139,941; US 5,863,541; US 5,869,305; US 6,057,152; and US 6,376,237; as well as in Rabinowitz et al., J. Virol. 76:791 (2002) and Bowles et al., J. Virol. 77:423 (2003), the disclosures of each of which are incorporated herein by reference as they pertain to AAV vectors for gene delivery.

rAAV virions useful in conjunction with the compositions and methods described herein include those derived from a variety of AAV serotypes including AAV 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, rh10, rh39, rh43, and rh74. Construction and use of AAV vectors and AAV proteins of different serotypes are described, for instance, in Chao et al., Mol. Ther. 2:619 (2000); Davidson et al., Proc. Natl. Acad. Sci. USA 97:3428 (2000); Xiao et al., J. Virol. 72:2224 (1998); Halbert et al., J. Virol. 74:1524 (2000); Halbert et al., J. Virol. 75:6615 (2001); and Auricchio et al., Hum. Molec. Genet. 10:3075 (2001), the disclosures of each of which are incorporated herein by reference as they pertain to AAV vectors for gene delivery.

Also useful in conjunction with the compositions and methods described herein are pseudotyped rAAV vectors. Pseudotyped vectors include AAV vectors of a given serotype (e.g., AAV9) pseudotyped with a capsid gene derived from a serotype other than the given serotype (e.g., AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, etc.). Techniques involving the construction and use of pseudotyped rAAV virions are known in the art and are described, for instance, in Duan et al., J. Virol. 75:7662 (2001); Halbert et al., J. Virol. 74:1524 (2000); Zolotukhin et al., Methods, 28:158 (2002); and Auricchio et al., Hum. Molec. Genet. 10:3075 (2001).

AAV virions that have mutations within the virion capsid may be used to infect particular cell types more effectively than non-mutated capsid virions. For example, suitable AAV mutants may have ligand insertion mutations for the facilitation of targeting AAV to specific cell types. The construction and characterization of AAV capsid mutants including insertion mutants, alanine screening mutants, and epitope tag mutants is described in Wu et al., J. Virol. 74:8635 (2000). Other rAAV virions that can be used in methods described herein include those capsid hybrids that are generated by molecular breeding of viruses as well as by exon shuffling. See, e.g., Soong et al., Nat. Genet., 25:436 (2000) and Kolman and Stemmer, Nat. Biotechnol. 19:423 (2001).

Genome editing

In addition to the above, a variety of tools have been developed that can be used for the incorporation of a gene of interest into a target cell, such as a mammalian cell. One such method that can be used for incorporating polynucleotides encoding target genes into target cells involves the use of transposons. Transposons are polynucleotides that encode transposase enzymes and contain a polynucleotide sequence or gene of interest flanked by 5' and 3' excision sites. Once a transposon has been delivered into a cell, expression of the transposase gene commences and results in active enzymes that cleave the gene of interest from the transposon. This activity is mediated by the site-specific recognition of transposon excision sites by the transposase. In some instances, these excision sites may be terminal repeats or inverted terminal repeats. Once excised from the transposon, the gene of interest can be integrated into the genome of a mammalian cell by transposase-catalyzed cleavage of similar excision sites that exist within the nuclear genome of the cell. This allows the gene of interest to be inserted into the cleaved nuclear DNA at the complementary excision sites, and subsequent covalent ligation of the phosphodiester bonds that join the gene of interest to the DNA of the mammalian cell

genome completes the incorporation process. In certain cases, the transposon may be a retrotransposon, such that the gene encoding the target gene is first transcribed to an RNA product and then reverse-transcribed to DNA before incorporation in the mammalian cell genome. Exemplary transposon systems are the piggybac transposon (described in detail in, e.g., WO 2010/085699) and the sleeping beauty transposon (described in detail in, e.g., US 2005/0112764), the disclosures of each of which are incorporated herein by reference as they pertain to transposons for use in gene delivery to a cell of interest.

Another tool for the integration of target genes into the genome of a target cell is the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas system, a system that originally evolved as an adaptive defense mechanism in bacteria and archaea against viral infection. The CRISPR/Cas system includes palindromic repeat sequences within plasmid DNA and an associated Cas9 nuclease. This ensemble of DNA and protein directs site specific DNA cleavage of a target sequence by first incorporating foreign DNA into CRISPR loci. Polynucleotides containing these foreign sequences and the repeat-spacer elements of the CRISPR locus are in turn transcribed in a host cell to create a guide RNA, which can subsequently anneal to a target sequence and localize the Cas9 nuclease to this site. In this manner, highly site-specific cas9-mediated DNA cleavage can be engendered in a foreign polynucleotide because the interaction that brings cas9 within close proximity of the target DNA molecule is governed by RNA:DNA hybridization. As a result, one can design a CRISPR/Cas system to cleave any target DNA molecule of interest. This technique has been exploited in order to edit eukaryotic genomes (Hwang et al., Nature Biotechnology 31:227 (2013)) and can be used as an efficient means of site-specifically editing target cell genomes in order to cleave DNA prior to the incorporation of a gene encoding a target gene. The use of CRISPR/Cas to modulate gene expression has been described in, for example, US Patent No. 8,697,359, the disclosure of which is incorporated herein by reference as it pertains to the use of the CRISPR/Cas system for genome editing. Alternative methods for site-specifically cleaving genomic DNA prior to the incorporation of a gene of interest in a target cell include the use of zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). Unlike the CRISPR/Cas system, these enzymes do not contain a guiding polynucleotide to localize to a specific target sequence. Target specificity is instead controlled by DNA binding domains within these enzymes. The use of ZFNs and TALENs in genome editing applications is described, e.g., in Urnov et al., Nature Reviews Genetics 11:636 (2010); and in Joung et al., Nature Reviews Molecular Cell Biology 14:49 (2013), the disclosure of each of which are incorporated herein by reference as they pertain to compositions and methods for genome editing.

Additional genome editing techniques that can be used to incorporate polynucleotides encoding target genes into the genome of a target cell include the use of ARCUS™ meganucleases that can be rationally designed so as to site-specifically cleave genomic DNA. The use of these enzymes for the incorporation of genes encoding target genes into the genome of a mammalian cell is advantageous in view of the defined structure-activity relationships that have been established for such enzymes. Single

chain meganucleases can be modified at certain amino acid positions in order to create nucleases that selectively cleave DNA at desired locations, enabling the site-specific incorporation of a target gene into the nuclear DNA of a target cell. These single-chain nucleases have been described extensively in, for example, US Patent Nos. 8,021,867 and US 8,445,251, the disclosures of each of which are incorporated herein by reference as they pertain to compositions and methods for genome editing.

Expression of cloaking transgenes

The cloaking transgenes described herein (e.g., one of, or any combination of, PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6)) are expressed in an amount sufficient to produce a cloaking effect (e.g., in an amount sufficient to prevent rejection when injected into a subject, e.g., a mammalian subject, such as a mouse, rat, or human). Transgene expression can be considered to produce a cloaking effect if subcutaneous injection of cloaked cells generates a teratoma that is not removed by the subject's immune system. The cloaking transgenes are also expressed at a level that is sufficient to promote production of the proteins encoded by said transgenes. Protein production can be detected using routine methods known to those of skill in the art (e.g., immunohistochemistry, Western Blot analysis, or other methods that allow for visualization or proteins). Preferably, the expression of the cloaking transgenes is such that all 8 proteins encoded by the cloaking transgenes (PD-L1, H2-M3, Cd47, Cd200, FasL, Ccl21b, Mfge8, and Spi6) can be detected in cloaked cells (e.g., detected by immunohistochemistry using antibodies directed against the proteins encoded by the cloaking transgenes).

In some embodiments, cloaking transgenes are expressed at similar levels in cloaked cells to levels of endogenous gene expression in activated leukocytes, such as T cells (e.g., activated leukocytes from the same species, such as an activated leukocyte isolated from a lymph organ, for example expression in a cloaked mouse cell is similar to expression in an activated leukocyte isolated from a murine lymphoid organ). The expression of one or more cloaking transgenes (e.g., 1, 2, 3, 4, 5, 6, 7, or 8 of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6)) is greater than or equal to expression of the endogenous gene in activated leukocytes (e.g., T cells) from the same species (e.g., expression level of the cloaking transgene is equal to the level of expression of the endogenous gene in activated leukocytes, or is 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10-fold or more higher than the level of expression of the endogenous gene in activated leukocytes). In some embodiments, all 8 cloaking transgenes are expressed at a level that is greater than or equal to the expression level of the endogenous gene in an activated leukocyte from the same species. Activated leukocytes can be isolated from lymphoid organs, or leukocytes, such as T cells, can activated in vitro using anti-CD3/CD28 beads or other methods employed by those of skill in the art (see, e.g., Frauwith and Thompson, *J.Clin Invest* 109:295-299 (2002); and Trickett and Kwan, *J Immunol Methods* 275:251-255 (2003)). Transgene expression in cloaked cells can also be compared to gene expression levels reported in profiling studies of activated T cells (see, e.g., Palacios et al., PLOSone 2:e1222 (2007)). In some embodiments, cloaking

transgene expression is compared to expression of the endogenous gene in a wild-type version of the cell (e.g., a stem cell, e.g., an embryonic stem cell from the same species as the cloaked cell). The expression of one or more cloaking transgenes (e.g., 1, 2, 3, 4, 5, 6, 7, or 8 of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6)) is at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 500, 1,000-fold or more higher in cloaked cells compared to expression of the endogenous gene in unmodified wild-type cells of the same cell type as the cloaked cell (e.g., stem cells, such as embryonic stem cells from the same species). In some embodiments, all 8 cloaking transgenes are expressed at a level that is greater (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100-fold higher or more) than the expression level of the endogenous gene in a wild-type version of the cell (e.g., a stem cell, e.g., an embryonic stem cell from the same species as the cloaked cell). Gene expression can be evaluated through direct comparison to isolated ES cells, or compared to stem cell expression (e.g., ES cell expression) in the Project Grandiose dataset (www.stemformatics.org/project_grandiose). Gene expression can be measured using techniques known in the art (e.g., quantitative polymerase chain reaction (qPCR)).

Methods of Providing a Local Immunosuppression at a Transplant Site

Also featured is a method of providing local immunosuppression at a transplant site.

The method comprises providing a cell; and expressing in the cell a set of transgenes, each transgene encoding a gene product that is cytoplasmic, membrane bound, or local acting and whose function is to mitigate function of graft attacking leukocyte and NK cell activation or act as a defense mechanism against attacking leukocytes.

The set of transgenes comprises one or more (e.g., two, three, four, five, six, seven, or all eight) of the following genes: PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6). In an embodiment, the set of transgenes genes comprises Pd-L1, H2-M3, Cd47, Cd200, FasL, Ccl21b, Mfge8, and Spi6.

Optionally, the method further comprises expressing one or more of the following transgenes in the cell: TGF-β, Cd73, Cd39, Lag3, Il1r2, Ackr2, Tnfrsf22, Tnfrs23, Tnfrsf10, Dad1, and IFNγR1 d39. In an embodiment, the TGF-β or the biologic is local acting.

Techniques for introducing into animal cells various genetic modifications, such as transgenes are described herein and are generally known in the art.

In an embodiment of the method, the cell is a stem cell, a cell amenable to genome editing, and/or a source of therapeutic cell type (e.g., a cell that can be differentiated into a lineage restricted cell for cell therapy, or a cell of a desired target tissue). In an embodiment, the cell is an embryonic stem cell, an induced pluripotent stem cell, an adult stem cell, a tissue-specific stem cell, a hematopoietic stem cell, a mesenchymal stem cell, an endothelial stem cell, an epithelial stem cell, an adipose stem or progenitor cells, a lung stem or progenitor cell, a mammary stem cell, an olfactory adult stem cell, a hair follicle stem cell, a multipotent stem cell, an amniotic stem cell, a cord blood stem cell, or a neural stem or progenitor

cell. In some embodiments, the cell is derived from a target tissue, e.g., skin, heart, brain or spinal cord, liver, lung, kidney, pancreas, bladder, bone marrow, spleen, intestine, or stomach. In some embodiments, the cell is a fibroblast, an epithelial cell, or an endothelial cell. The cell may be a vertebrate cell, for example, a mammalian cell, such as a human or mouse cell. In some embodiments, the cell that is modified to express one or more (e.g., two, three, four, five, six, seven, or all eight) of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) is a cell in the tissue or organ to be transplanted. In some embodiments, the cloaked cells (e.g., cloaked stem cells) are differentiated *in vitro* using methods known by those of skill in the art into a tissue or organ for transplantation.

In some embodiments, one million to one hundred billion cloaked cells (e.g., 1×10^6, 2×10^6, 3×10^6, 4×10^6, 5×10^6, 6×10^6, 7×10^6, 8×10^6, 9×10^6, 1×10^7, 2×10^7, 3×10^7, 4×10^7, 5×10^7, 6×10^7, 7×10^7, 8×10^7, 9×10^7, 1×10^8, 2×10^8, 3×10^8, 4×10^8, 5×10^8, 6×10^8, 7×10^8, 8×10^8, 9×10^8, 1×10^9, 2×10^9, 3×10^9, 4×10^9, 5×10^9, 6×10^9, 7×10^9, 8×10^9, 9×10^9, 1×10^{10}, 2×10^{10}, 3×10^{10}, 4×10^{10}, 5×10^{10}, 6×10^{10}, 7×10^{10}, 8×10^{10}, 9×10^{10}, or 1×10^{11} cloaked cells) are administered to or near a transplant site in a subject, or into an organ or tissue to be transplanted.

Techniques for transplanting the genetically modified cells into a transplant site of an allogeneic host are described herein and are generally known in the art.

Expression of therapeutic agents by cloaked cells

The cloaked cells described herein can be further modified to express a therapeutic agent. In some embodiments, the therapeutic agent is a protein. The therapeutic protein can be a wild type form of a protein that is deficient in a subject, such as a protein that is mutated or produced in insufficient quantity (e.g., produced at low levels or not produced) by the subject's cells. In some embodiments, the therapeutic protein is an inhibitory antibody (e.g., an antibody that blocks or neutralizes protein function). The cloaked cells may be modified to produce an inhibitory antibody to treat a subject having or at risk of developing a disease or condition related to overproduction or aberrant production of a protein (e.g., production by cells that do not normally produce the protein, production of a protein at a time or in a location at which the protein is not normally produced, or production of an excessive amount of a protein). In some embodiments, the therapeutic antibody is an agonist antibody (e.g., an activating antibody). The agonist antibody can act by binding to and activating an endogenous receptor (e.g., inducing or increasing signaling downstream of receptor activation or changing the conformation of the endogenous receptor to an open or active state). The cloaked cells may be modified to produce an agonist antibody to treat a subject having or at risk of developing a disease or condition related to under activation of a receptor or signaling pathway. The cloaked cells can be modified to produce the therapeutic protein or antibody using the methods described herein or using other methods known by those of skill in the art. Cloaked cells that produce a secreted protein or antibody can be delivered as circulating cells, injected into the tissue, organ, or body site in need of the therapeutic protein or antibody, or injected

subcutaneously to produce a cloaked subcutaneous tissue. Cloaked cells that produce a transmembrane or membrane-bound protein, can be injected at or near the site of the endogenous cells that respond to the therapeutic protein.

In some embodiments, the cloaked cells described herein provide a wild-type copy of a gene that is mutated in the subject (e.g., the cloaked cell is a "wild-type cell" that does not have the genetic cause of the disease and that expresses one, two, three, four, five, six, seven or all eight of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6)). Such cells can be used to treat subjects having a disease or condition caused by a mutation in an endogenous gene (e.g., subjects having a metabolic disorder associated with one or more mutations described herein below).

A list of exemplary therapeutic agents that can be administered with or produced by cloaked cells and the associated diseases or conditions that can be treated using these therapeutic agents are provided in Table 2 below.

Table 2: Exemplary therapeutic agents that can be administered with or expressed by cloaked cells to treat disease

Disease or Condition	Therapeutic Agent
Diabetes, altered glycemic states	Insulin, insulotropin, glucagon
Skeletal growth retardation	Human growth hormone
Anemia	Erythropoietin (EPO), hemoglobins
Obesity	Ob gene translation product (leptin)
Immunodeficiency (e.g., AIDS)	Adenosine deaminase, purine nucleoside phosphorylase, CD-4
Hemophilia A	Factor VIII
Hemophilia B	Factor IX
Emphysema	α_1-antitrypsin
Hypercholesterolemia	LDL receptor protein
Pernicious anemia	Intrinsic factor
Hypoalbuminemia	Albumin
Gaucher's disease	B-glucosidase (glucocerebrosidase)
Cystic fibrosis	CF transmembrane conductance regulator
Cardiovascular disease	Tissue Plasminogen Activator (tPA), urokinase, streptokinase, antithrombin III, Apolipoproteins (e.g., APO B48, A1), Low Density lipoprotein receptor, vascular endothelial growth factor (VEGF)

Disease or Condition	Therapeutic Agent
Calcium mineral diseases	Calcitonin, parathyroid hormone (PTH), PTH-like hormone
Severe Combined Immunodeficiency (SCID)	Adenosine deaminase
Phenylketonuria	Phenylalanine hydroxylase
von Willebrand's disease	von Willebrand Factor
Cancers, cancer suppression	Tumor Necrosis Factors (TNFs), cytokines, anti-neoplastic agents (e.g., vincristine, doxorubicin, tamoxifen, methotrexate), interleukins (ILs), interferons (INFs), p53 and related, anti-BRCAs, anti-VEGF (bevacizumab), anti-Epidermal Growth Factor (EGF), oncogene anti-sense RNAs, antibodies (e.g., Rituximab; Daclizumab; Basiliximab; Palivizumab; Infliximab; Trastuzumab; Gemtuzumab ozogamicin; Alemtuzumab; Ibritumomab tiuxetan; Adalimumab; Omalizumab; Tositumomab-I-131; Efalizumab; Cetuximab; Bevacizumab; Natalizumab; Tocilizumab; Panitumumab; Ranibizumab; Eculizumab; Certolizumab pegol; Golimumab; Canakinumab; Ustekinumab; Ofatumumab; Denosumab; Motavizumab; Raxibacumab; Belimumab; Ipilimumab; Brentuximab Vedotin; Pertuzumab; Ado-trastuzumab emtansine; or Obinutuzumab), or checkpoint inhibitors (e.g., nivolumab, pidilizumab/CT-011, pembrolizumab, ipilimumab, or tremelimumab)
Peripheral vascular disease	VEGF, endothelins
Neurodegenerative states, and post neural trauma conditions	Ciliary Neurotrophic Factor (CNTF), Brain Derived Neurite Factor (BDNF), Nerve Growth Factor (NGF), tyrosine hydroxylase
Retarded fracture healing	Bone morphogenic proteins (BMP)
Lactose insufficiency	Lactase

Disease or Condition	Therapeutic Agent
Wound healing	Epidermal Growth Factors, Transforming Growth Factors, Granulocyte-Colony Stimulating Factors, Fibroblast Growth Factors, Interferons, Interleukins, Insulin-like growth Factors
Thrombosis, hypercoagulability	Antithrombins, urokinases, tPAs , hirudins, streptokinase
Diabetes insipidus	Antidiuretic hormone (ADH)
Psychiatric Disorders	Selective Serotonin Reuptake Inhibitors, anti-psychotic bio-substances
Pain Control	Endorphins
Endocrineopathies	Estrogens, Androgens, mineralocorticoids, glucocorticoids, anabolic steroids, etc.
Hypothyroidism	Thyroid hormones, thyroglobulins
Muscular dystrophy	Dystrophin
Infections (bacterial, fungal, viral)	Anti-microbial polypeptides
Shock, Sepsis	Lipid Binding Protein (LBP)
Leukemia	L-asparaginase
Disorders of digestive, pancreatic states	Pepsin, trypsin, chymotrypsin, cholecystokinin, sucrase, carboxypeptidase
Oxidative Stress, Neurodegenerative Disorders	Catalase
Hypouricasemia, Gout	Uricase
Ehlers Danlos	Elastase
Thrombocytopenia	Thrombopoietin (TPO)
SCID/ADA deficiency	Adenosine deamidase
Porphyria	Porphobilinogen deaminase
Inborn errors of carboxylic and amino acid metabolism, (e.g., glutaric acidemia)	Specific enzymes catalyzing transformations at genetic block points, (e.g., glutaryl CoA dehydrogenase)
Homocystinuria	Cystathionine B-synthase
Wilson's Disease, Menke's Disease	Specific copper transporting ATPase's
Thalassemia	β-globin
Sickle Cell Anemia	α-globin

Disease or Condition	Therapeutic Agent
Baldness	Sonic hedgehog gene products
Hashimoto's Thyroiditis,	Thyroid hormone
Wet Age-Related Macular Degeneration or Retinal Dystrophy	VEGF trap (e.g., a soluble decoy receptor described in Holash et al., Proc Natl Acad Sci U.S.A. 99:11383-11398, 2002, e.g., VEGF-Trap$_{parental}$, VEGF-Trap$_{\Delta B1}$, VEGF-Trap$_{\Delta B2}$, VEGF-Trap$_{R1R2}$, e.g., aflibercept), soluble forms of VEGF receptors (e.g., soluble VEGFR-1 or NRP-1), platelet factor-4, prolactin, SPARC, VEGF inhibitory antibodies (e.g., bevacizumab or ranibizumab).
Osteoarthritis or Rheumatoid Arthritis	TNFα inhibitors (adalimumab, etanercept, infliximab, golimumab, certolizumab), interleukin-6 (IL6) receptor inhibitors (e.g., tocilizumab), IL1 receptor inhibitors (e.g., anakinra), or other agents used to treat RA (e.g., abatacept, rituximab)
Inflammatory Bowel Disease, Crohn's disease, Ulcerative Colitis	TNFα inhibitors (adalimumab, etanercept, infliximab, golimumab, certolizumab), mesalazine, prednisone, azathioprine, methotrexate
Addison's Disease	Aldosterone, cortisol, glucocorticoids, mineralocorticoids, androgens
Hurler syndrome	Alpha-L iduronidase
Niemann-Pick disease	Sphingomyelin phosphodiesterase1 (SMPD1), NPC1 protein, or NPC2 protein
Tay-Sachs disease	beta-hexosaminidase A
Fabry disease	alpha galactosidase
Krabbe disease	Galactosylceramidase
Galactosemia	Galactokinase or galactose-1-phosphate uridyltransferase
Maple syrup urine disease	Enzymes of the branched-chain alpha-keto acid dehydrogenase complex
Phenylketonuria	Phenylalanine hydroxylase

Disease or Condition	Therapeutic Agent
Glycogen storage diseases (GSDs)	GSD0: Glycogen synthase (GYS2); GSD1/von Gierke's disease: Glucose-6-phosphatase (G6PC); GSD 2/Pompe's disease: Acid alpha-glucosidase (GAA); GSD 3/Cori's disease or Forbes' disease: Glycogen debranching enzyme (AGL); GSD 4/Andersen disease: Glycogen branching enzyme (GBE1); GSD 5/McArdle disease: Muscle glycogen phosphorylase (myophosphorylase) (PYGM); GSD 6/Hers' disease: Liver glycogen phosphorylase (PYGL) or muscle phosphoglycerate mutase (PGAM2); GSD 7/Tarui's disease: Muscle phosphofructokinase (PKFM); GSD 9: Glycogen phosphorylase kinase B (PHKA2, PHKB, PHKG2, or PHKA1), GSD 10: Enolase 3 (ENO3); GSD 11: Muscle lactate dehydrogenase (LDHA); Fanconi-Bickel syndrome: Glucose transporter 2 (GLUT2); GSD 12: Aldolase A (ALDOA); GSD 13: β-enolase (ENO3); GSD 15: Glycogenin-1 (GYG1)
Mitochondrial disorders	Leber's hereditary optic neuropathy (LHON): NADH dehydrogenase; Leigh syndrome: thiamine-diphosphate kinase, thiamine triphosphate, or pyruvate dehydrogenase ; Neuropathy, ataxia, retinitis pigmentosa, and ptosis (NARP: ATP synthase; Myoneurogenic gastrointestinal encephalopathy (MNGIE): thymidine phosphorylase (TYMP); Mitochondria myopathy, encephalomyopathy, lactic acidosis, stroke-like symptoms (MELAS): NADH dehydrogenase

Disease or Condition	Therapeutic Agent
Friedrich's ataxia	Frataxin (FXN)
Peroxisomal disorders	Zellweger syndrome: Proteins encoded by PEX1, PEX2, PEX3, PEX5, PEX6, PEX10, PEX12, PEX13, PEX14, PEX16, PEX19, or PEX26; Adrenoleukodystrophy: protein encoded by ABCD1
Metal metabolism disorders	Wilson disease: Wilson disease protein (ATP7B); Hemochromatosis: Human hemochromatosis protein (HFE)
Organic acidemias	Methylmalonic acidemia: methylmalonyl CoA mutase, methylmalonyl CoA epimerase, adenosylcobalamin Propionic academia: propionyl-CoA carboxylase
Urea cycle disorders	Ornithine transcarbamylase (OTC), deficiency: Ornithine transcarbamylase; Arginase (ARG1) deficiency: Arginase; Argininosuccinate lyase (ASL) deficiency: Argininosuccinate lyase; Argininosuccinate synthase 1 (ASS1) deficiency: Argininosuccinate synthase 1; Citrin deficiency: Citrin; Carbamoyl phosphate synthase 1 (CPSI) deficiency: Carbamoyl phosphate synthase 1; N-acetylglutamate synthase (NAGS) deficiency: N-acetylglutamate synthase; Ornithine translocase (ORNT1) deficiency: Ornithine translocase

Inducible systems for expression of therapeutic agents

If continuous administration of a therapeutic agent expressed by cloaked cells is needed to treat a disease or condition, the therapeutic agent can be expressed using a constitutive promoter described herein or known by those of skill in the art (e.g., CAG, CMV, or another constitutive promoter). If the therapeutic agent is needed intermittently (e.g., needed during a period of relapse or flare up that occurs during a disease or condition, but not needed when a subject is asymptomatic), it can be expressed by an inducible promoter, which provides the capability of expressing the therapeutic agent only when it is needed. One exemplary class of therapeutic agents that could be delivered using an inducible promoter is TNFα inhibitors. TNFα inhibitors are currently used to treat rheumatoid arthritis, but are only

administered intermittently during flare-ups of joint inflammation as constitutive administration of TNFα can lead to systemic immunosuppression. If cloaked cells are modified to express TNFα inhibitors under the control of an inducible promoter, cloaked cells can be used to deliver TNFα intermittently, thus, obviating the need for repeated injections. Other therapeutic agents that have potentially adverse effects if administered continuously can also be expressed intermittently using inducible promoters as described herein. Exemplary inducible expression systems are described below.

Tetracycline response element

One widely used inducible expression system is based on tetracycline-controlled transcriptional activation. In this system, the antibiotic tetracycline, or one of its derivatives (e.g., doxycycline), is used to reversibly activate or inhibit gene expression. To use this system, a tetracycline response element (TRE) is placed upstream of a gene of interest (e.g., a therapeutic transgene to be expressed by cloaked cells), typically along with a minimal promoter that has very low basal expression. A protein called rtTA, which also needs to be expressed by the cloaked cells, binds to the TRE and activates transcription in the presence of tetracycline or doxycycline. When tetracycline or doxycycline is removed, rtTA no longer binds to the TRE and the gene of interest is no longer expressed. Advanced versions of this system, Tet-On Advanced transactivator (rtTA2^{s}-M2) and Tet-On 3G, may be particularly useful for human therapy as they are human codon optimized and respond to low concentrations of doxycycline,

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Light inducible systems

Another method for inducible activation of gene expression involves the use of optogenetics, which uses light sensitive proteins to manipulate gene expression. A recent development in optogenetics that can be used to inducibly express therapeutic agents in cloaked cells involves a class of proteins that undergo a conformational change and dimerize in response to blue light. These proteins have been fused to DNA-binding and transcriptional components that have been shown to bind to specific promoter sequences and activate transcription when brought together by exposure to blue light (Wang et al., Nat Methods, 9:266-269, 2012). This method of inducibly activating gene expression could be used to control the production of therapeutic agents in cloaked cells that are administered subcutaneously, as blue light can be shone onto the skin near the cloaked subcutaneous tissue to induce production of a therapeutic agent by the cloaked cells.

Radiogenetics

A third method of inducibly activating gene expression (e.g., expression of a therapeutic agent by cloaked cells) involves the use of radio waves. In one version of a radio wave-inducible expression system, the TRPV1 receptor is fused to a GFP binding domain and co-expressed with a form of ferritin that is linked to GFP (Stanley et al., Nat Med 21:92-98, 2015). The GFP-ferritin binds to the GFP binding domain of the TRPV1 receptor. When a radio wave of a specific frequency is applied to the cell, ferritin

interacts with TRPV1 and allows for an influx of calcium, which activates the transcription factor NFAT. Therapeutic agents can be inducibly expressed using this system if they are operably linked to an NFAT-sensitive promoter element, such as SRE-CRE-NFATRE, and co-expressed with TRPV1-GFP and GFP-ferritin. Radio wave-induced expression provides the advantage of being able to induce expression in cells that are further from the outside of the body, as radio waves can pass through tissue. For example, radiogenetics could be used to regulate gene expression in the retina. This method could, therefore, be used to inducibly express therapeutic transgenes in cloaked cells with non-invasive and non-harmful radio waves.

Destabilization domain system

Gene expression can also be regulated using destabilization domain systems. A transgene encoding a protein of interest (e.g., a therapeutic agent described herein) can also include destabilizing domains, such that the resulting protein product includes the protein of interest fused to a destabilizing domain. Exemplary destabilizing domains include mutants of the human FK506- and rapamycin-binding protein (FKBP12), which confer instability to the proteins to which they are fused. FKBP12 mutants include N-terminal mutants F15S, V24A, H25R, E60G, and L106P, and C-terminal mutants M66T, R71G, D100G, D100N, E102G, and K105I, as characterized in Banaszynski et al., Cell 126:995 (2006), the disclosure of which is incorporated herein by reference as it pertains to FKBP12 destabilizing domains. Destabilizing domains promote protein degradation. A small molecule synthetic ligand can be used to stabilize the destabilizing domain-containing proteins when expression of the protein of interest (e.g., a therapeutic agent) is desired. The small molecule ligand Shield-1 (Shld1) can be used to stabilize FKBP12 mutant-containing proteins by protecting them from degradation. Other destabilizing domains that can be used to regulate expression proteins of interest include mutants of the *E. coli* dihydrofolate reductase (ecDHFR) and mutants of the human estrogen receptor ligand binding domain (ERLBD), which confer instability resulting in degradation when fused to a protein of interest and can be stabilized by small molecule ligand trimethoprim (TMP), or by CMP8 or 4-hydroxytamoxifen (4OHT), respectively, as described in Iwamoto et al., Chem Biol. 17:981 (2010) and Miyazaki et al., J Am Chem Soc., 134:3942 (2012), the disclosures of each of which are incorporated herein by reference as they pertain to destabilization domain systems.

Cumate switch inducible system

Another method for inducible activation of gene expression involves the use of the cumate gene-switch system. In the repressor configuration of this system, regulation is mediated by the binding of the repressor (CymR) to the operator site (CuO), placed downstream of a strong constitutive promoter. Addition of cumate, a small molecule, relieves the repression, allowing for expression of the transgene. Alternatively, a reverse-cumate-Trans-Activator (rcTA) may be inserted upstream of a minimal CMV promoter that is operably linked to a transgene encoding a therapeutic agent. A 6-times repeat of a

Cumate Operator (6xCuO) may be inserted just before the translational start (ATG) of the therapeutic transgene. In the absence of cumate, rcTA cannot bind to the 6xCuO, so the transgene encoding the therapeutic agent will not be transcribed because the 6xCuO is not active. When cumate is added, it will form a complex with rcTA, which allows for binding to 6xCuO and transcription of the transgene encoding the therapeutic agent (Mullick et al., 2006).

Ecdysone inducible system

Another example of an inducible gene expression system is the ecdysone inducible system, in which a retinoid X receptor (RXR) and an N-terminal truncation of ecdysone receptor (EcR) fused to the activation domain of Vp16 (VpEcR) are inserted in the 5' untranslated region of a gene expressed by the cloaked cell such that they are co-expressed by an endogenous promoter. An ecdysone responsive element (EcRE), with a downstream minimal promoter, can be inserted just upstream of the start codon of the transgene encoding the therapeutic agent. Co-expressed RXR and VpEcR can heterodimerize with each other. In the absence of ecdysone or synthetic drug analog muristerone A, dimerized RXR/VpEcR cannot bind to EcRE, so the transgene encoding the therapeutic agent is not transcribed. In the presence of ecdysone or muristerone A, dimerized RXR/VpEcR can bind to EcRE, such that the transgene encoding the therapeutic agent is transcribed (No et al., 1996). As ecdysone administration has no apparent effect on mammals, its use for regulating genes should be excellent for transient inducible expression of any gene.

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Ligand-reversible dimerization system

In another example, the transgene encoding a therapeutic agent can be modified so that it is functionally divided in to parts/domains, such as a 5' portion and a 3' portion, and an FKBP peptide sequence can be inserted into each domain. An IRES (internal ribosomal entry site) sequence may be placed between the two domains, which allows for simultaneous transcription of the two different domains to generate two separate proteins. In the absence of a dimerization agent, the two separate domains of the therapeutic agent will be functionally inactive. Upon introduction of a dimerization agent, such as rapamycin or AP20187, the FKBP peptides will dimerize, bringing together the 5' and 3' domains of the therapeutic agent and reconstituting an active protein (Rollins et al., 2000).

Cell-based delivery of a therapeutic agent

Treatment of Age-related Macular Degeneration or Retinal Dystrophy

In one example, cloaked cells can be modified to produce a VEGF inhibitor, such as VEGF trap (e.g., a soluble decoy receptor described in Holash et al., Proc Natl Acad Sci U.S.A. 99:11383-11398, 2002, incorporated herein by reference, such as aflibercept) to treat age-related macular degeneration (AMD) or retinal dystrophy. VEGF trap is a biologic that binds to and inhibits VEGF, an angiogenic protein that can promote the formation of aberrant blood vessels. VEGF trap is used to treat wet AMD,

which features aberrant growth of blood vessels beneath the retina that can lead to retinal detachment and progressive vision loss. To treat AMD, VEGF trap is typically delivered by regular injection into the eye. Cloaked cells can be modified to produce VEGF trap or another VEGF inhibitor by expression of a transgene encoding VEGF trap or another VEGF inhibitor operably linked to a constitutive or inducible promoter. Cloaked cells (e.g., stem cells) that express a VEGF inhibitor (e.g., VEGF trap) can be differentiated into retinal pigmented epithelium (RPE) cells before administration to the eye using methods known by those of skill in the art, or isolated RPE cells can be modified to express cloaking transgenes and a VEGF inhibitor. Twenty five thousand to one hundred thousand cloaked RPE cells (e.g., 25,000, 50,000, 75,000 or 100,000 cloaked RPE cells) expressing a VEGF inhibitor (e.g., VEGF trap) can be injected into the subretinal space of each eye to treat wet AMD or retinal dystrophy. Other VEGF inhibitors suitable for use in the compositions and methods described herein include soluble forms of VEGF receptors (e.g., soluble VEGFR-1 or NRP-1), platelet factor-4, prolactin, SPARC, and VEGF inhibitory antibodies (e.g., bevacizumab and ranibizumab).

Treatment of Parkinson's Disease

In another example, cloaked cells, such as dopaminergic neurons or cells (e.g., stem cells) that can be differentiated in vitro to produce dopaminergic neurons using methods known by those of skill in the art, can be administered to subjects suffering from Parkinson's disease, which is characterized by loss of dopaminergic neurons. Twenty five thousand to one hundred thousand cloaked dopaminergic neurons (e.g., 25,000, 50,000, 75,000 or 100,000 cloaked dopaminergic neurons) can be administered to the brain of a subject suffering from Parkinson's disease (e.g., stereotactically injected into the substantia nigra).

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Treatment of Cardiac Infarction

The cloaked cells described herein can also be used to treat cardiac infarction (e.g., myocardial infarction, commonly known as a heart attack). Cardiac infarction occurs when blood flow decreases or stops to a part of the heart, causing damage to the heart muscle. To treat subjects who have suffered a cardiac infarction, cloaked cells (e.g., stem cells) can be differentiated into cardiac muscle cells using methods known by those of skill in the art, or isolated cardiac muscle cells can be modified to express cloaking transgenes. Five hundred million to five billion cloaked cardiac muscle cells (e.g., 5×10^8, 6×10^8, 7×10^8, 8×10^8, 9×10^8, 1×10^9, 2×10^9, 3×10^9, 4×10^9, or 5×10^9 cloaked cardiac muscle cells) can be administered to a subject by injection into the cardiac muscle to treat a subject who has suffered a cardiac infarction (e.g., to replace dead or damaged cardiac muscle cells).

Treatment of Osteoarthritis and Rheumatoid Arthritis

In another example, the cloaked cells described herein can be used to treat osteoarthritis or rheumatoid arthritis. Osteoarthritis and rheumatoid arthritis (RA) are characterized by joint inflammation, and are commonly treated with anti-inflammatory therapeutics. To treat subjects suffering from

osteoarthritis or RA, cloaked cells can be modified to express anti-inflammatory biologics, such as inhibitors of TNFα (e.g., TNFα inhibitory antibodies), which are already in clinical use for the treatment of RA. Cloaked cells can be modified to produce an anti-inflammatory biologic, such as a TNFα inhibitor, by expression of a transgene encoding an anti-inflammatory biologic operably linked to a constitutive or inducible promoter. Cloaked cells (e.g., stem cells) that express an anti-inflammatory biologic (e.g., a TNFα inhibitor) can be differentiated into articular fibroblasts before administration to a joint using methods known by those of skill in the art, or isolated articular fibroblasts can be modified to express cloaking transgenes and an anti-inflammatory biologic. One million to one hundred million cloaked articular fibroblasts (e.g., 1×10^6, 2×10^6, 3×10^6, $4x\ 10^6$, 5×10^6, 6×10^6, 7×10^6, 8×10^6, 9×10^6, 1×10^7, 2×10^7, 3×10^7, $4x\ 10^7$, 5×10^7, 6×10^7, 7×10^7, 8×10^7, 9×10^7, or 1×10^8 cloaked articular fibroblasts) expressing an anti-inflammatory biologic can be injected into an arthritic or inflamed joint (depending on joint size) to treat osteoarthritis or RA. Anti-inflammatory biologics that can be expressed by cloaked cells to treat osteoarthritis or RA include TNFα inhibitors (adalimumab, etanercept, infliximab, golimumab, certolizumab), interleukin-6 (IL6) receptor inhibitors (e.g., tocilizumab), IL1 receptor inhibitors (e.g., anakinra), or other agents used to treat RA (e.g., abatacept, rituximab).

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Treatment of Diabetes

The cloaked cells can be used to treat diabetes (e.g., Type 1 or Type 2 diabetes). Type 1 diabetes results from a failure of the pancreas to produce enough insulin. Type 2 diabetes begins with insulin resistance, but a lack of insulin may develop as the disease progresses. To treat subjects suffering from diabetes, cloaked cells can be modified to express insulin, or insulin-expressing cells from a healthy subject (e.g., pancreatic beta cells from a subject without diabetes) can be modified to express one or more (e.g., one, two, three, four, five, six, seven or all eight) of cloaking transgenes PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) and administered to a subject with diabetes. Cloaked cells can be modified to produce insulin by expression of a transgene encoding insulin operably linked to a constitutive or inducible promoter. Cloaked cells (e.g., stem cells) that express insulin can be differentiated into insulin producing cells (e.g., pancreatic beta cells) prior to administration using methods known by those of skill in the art or can be administered without differentiation, or isolated pancreatic beta cells can be modified to express cloaking transgenes and, optionally, to express a transgene encoding insulin. Eight hundred million to three billion cloaked pancreatic beta cells (e.g., 8×10^8, 9×10^8, 1×10^9, 2×10^9, or 3×10^9 cloaked pancreatic beta cells) expressing insulin (e.g., expressing insulin endogenously or expressing insulin due to expression of a transgene encoding insulin) to can be injected subcutaneously in a subject to create a cloaked subcutaneous tissue that produces insulin for treating diabetes.

Treatment of Hemophilia

In another example, the cloaked cells described herein can be used to treat hemophilia. Patients with hemophilia do not produce a functional Factor VIII protein, which is a critical blood component needed for blood clotting. These patients can have severe bleeding, and the standard of care involved multiple injections per week of a purified Factor VIII protein. To treat subjects suffering from hemophilia, cloaked cells can be modified to express an additional transgene that encodes Factor VIII. Factor VIII would be expressed constitutively in cloaked cells by being operably linked to a constitutive promoter, such as CMV or CAG. Cloaked cells (e.g., stem cells) that express Factor VIII can be differentiated into cells that produce blood coagulation factors (e.g., liver sinusoidal cells or endothelial cells) prior to administration using methods known by those of skill in the art or can be administered without differentiation, or isolated Factor VIII-expressing liver sinusoidal cells or endothelial cells from a healthy subject (e.g., a subject without hemophilia) can be modified to express one or more (e.g., one, two, three, four, five, six, seven or all eight) of cloaking transgenes PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) and administered to a subject with hemophilia. Isolated Factor VIII-expressing liver sinusoidal cells or endothelial cells from a healthy subject that are modified to express one or more cloaking transgenes, can be further modified to express a transgene encoding Factor VIII, if desired to ensure that Factor VIII is expressed at high levels. Eight hundred million to three billion cloaked cells (e.g., 8×10^8, 9×10^8, 1×10^9, 2×10^9, or 3×10^9 cloaked cells) expressing Factor VIII (e.g., expressing Factor VIII endogenously or expressing Factor VIII due to expression of a transgene encoding Factor VIII) can be injected subcutaneously in a subject to create a cloaked subcutaneous tissue that produces Factor VIII for treating hemophilia.

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Treatment of Metabolic Disorders

The cloaked cells of the invention can also be used to treat inherited metabolic disorders. In most inherited metabolic disorders, a single enzyme is not produced by the body or it is produced in a form that is defective. Inherited metabolic disorders include lysosomal storage disorders, such as Hurler syndrome (deficiency in alpha-L iduronidase), Niemann-Pick disease (mutations in SMPD1, NPC1, or NPC2), Tay-Sachs disease (mutation in HEXA), Gaucher's disease (mutation in GBA gene), Fabry disease (deficiency in alpha galactosidase due to mutation in GLA), and Krabbe disease (deficiency in galactosylceramidase due to mutations in GALC); Galactosemia (deficiency in Galactokinase or galactose-1-phosphate uridyltransferase); Maple syrup urine disease (deficiency in enzyme BCKD); Phenylketonuria (deficiency in enzyme PAH); glycogen storage diseases (GSDs), such as GSD0 (deficiency in glycogen synthase (GYS2)), GSD1/von Gierke's disease (deficiency in glucose-6-phosphatase (G6PC)), GSD 2/Pompe's disease (deficiency in acid alpha-glucosidase (GAA)), GSD 3/Cori's disease or Forbes' disease (deficiency in glycogen debranching enzyme AGL), GSD 4/Andersen disease (deficiency in glycogen branching enzyme (GBE1)), GSD 5/McArdle disease (deficiency in muscle glycogen phosphorylase (PYGM)), GSD 6/Hers' disease (deficiency in liver glycogen phosphorylase (PYGL) or muscle phosphoglycerate mutase (PGAM2)), GSD 7/Tarui's disease (deficiency in muscle phosphofructokinase

(PKFM)), GSD 9 (deficiency in phosphorylase kinase (PHKA2, PHKB, PHKG2, or PHKA1)), GSD 10 (deficiency in enolase 3 (ENO3)), GSD 11 (deficiency in muscle lactate dehydrogenase (LDHA)), Fanconi-Bickel syndrome (deficiency in glucose transporter 2 (GLUT2)), GSD 12 (deficiency in aldolase A (ALDOA)), GSD 13 (deficiency in β-enolase (ENO3)), or GSD 15 (deficiency in glycogenin-1 (GYG1)); mitochondrial disorders, such as mitochondrial myopathy (Kearns-Sayre syndrome (KSS, caused by a deletion in mitochondrial DNA) and Chronic progressive external opthalmoplegia (CPEO, caused by a deletion or duplication in mitochondrial DNA or a mutation in ANT1, POLG, POLG2, or PEO1), diabetes mellitus and deafness (DAD, caused by a mutation in mitochondrial DNA at position 3243, which encodes tRNALeu(UUR)), Leber's hereditary optic neuropathy (LHON, caused by mutations in MT-ND1, MT-ND4, MT-ND4L, and MT-ND6), Leigh syndrome (associated with mutations in SURF1, MT-ATP6, MT-ND2, MT-ND3, MT-ND5, MT-ND6, BCS1L, NDUFA10, SDHA, NDUFS4, NDUFAF2, NDUFA2, NDUFAF6, COX15, NDUFS3, NDUFS8, FOXRED1, NDUFA9, NDUFA12, NDUFS7), Neuropathy, ataxia, retinitis pigmentosa, and ptosis (NARP, caused by mutations in MT-ATP6), myoneurogenic gastrointestinal encephalopathy (MNGIE, caused by mutations in TYMP), myoclonic epilepsy with ragged red fibers (MERRF, caused by mutation sin MT-TK, MT-TL1, MT-TH, MT-TS1, MT-TS2, or MT-TF), or mitochondria myopathy, encephalomyopathy, lactic acidosis, stroke-like symptoms (MELAS, caused by mutations in MT-ND1, MT-ND5, MT-TH, MT-TL1, or MT-TV); Friedrich's ataxia (mutation in FXN); peroxisomal disorders, such as Zellweger syndrome (mutations in PEX1, PEX2, PEX3, PEX5, PEX6, PEX10, PEX12, PEX13, PEX14, PEX16, PEX19, or PEX26) and adrenoleukodystrophy (mutations in ABCD1); metal metabolism disorders, such as Wilson disease (mutation in Wilson disease protein ATP7B) and hemochromatosis (mutation in human hemochromatosis protein HFE); organic acidemias, such as methylmalonic acidemia (mutations in MUT, MMAA, MMAB, MMADHC, or MCEE) and propionic academia (mutations in PCCA or PCCB); urea cycle disorders, such as ornithine transcarbamylase (OTC), deficiency, arginase (ARG1) deficiency, argininosuccinate lyase (ASL) deficiency, argininosuccinate synthase 1 (ASS1) deficiency, citrin deficiency, carbamoyl phosphate synthase 1 (CPSI) deficiency, N-acetylglutamate synthase (NAGS) deficiency, and ornithine translocase (ORNT1) deficiency.

To treat subjects suffering from a metabolic disorder, cloaked cells can be modified to express the wild-type form of the gene that is mutated in the subject or a transgene encoding the enzyme that is missing or deficient in the subject (see Table 2), or cells from a healthy subject (e.g., a subject that does not have a metabolic disorder) that express the wild-type form of the gene that is mutated in the subject or the enzyme that is deficient in the subject can be modified to express one or more (e.g., one, two, three, four, five, six, seven or all eight) of cloaking transgenes PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) and administered to a subject with a metabolic disorder. The wild-type form of the gene that is mutated in the subject or a transgene encoding the enzyme that is missing or deficient in the subject can be expressed constitutively in cloaked cells by being operably linked to a constitutive promoter, such as CMV or CAG, or can be inducibly expressed using one of the inducible expression systems described herein. Cloaked cells (e.g., stem cells) that are

modified to express the wild-type form of the gene that is mutated in the subject or the enzyme that is missing or deficient in the subject can be differentiated into cells that normally express the gene or enzyme prior to administration using methods known by those of skill in the art or can be administered without differentiation, or isolated cells from a healthy subject that express the wild-type form of the gene or enzyme that is mutated or deficient in the subject can be modified to express one or more (e.g., one, two, three, four, five, six, seven or all eight) of cloaking transgenes PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) and administered to a subject with a metabolic disorder. If the subject has not already been diagnosed as having a particular mutation prior to treatment, the subject can be evaluated using standard methods to identify the mutated gene related to the metabolic disorder, to ensure that the cloaked cells express the corresponding wild-type gene. Eight hundred million to three billion cloaked cells (e.g., 8×10^8, 9×10^8, 1×10^9, 2×10^9, or 3×10^9 cloaked cells) expressing the wild-type form of the gene that is mutated in the subject can be injected subcutaneously to create a cloaked subcutaneous tissue that produces the corresponding wild-type protein.

Methods of Controlling Division of a Cloaked Cell

In an aspect, a method of controlling proliferation of cell at a transplant site in an allogeneic host is provided (e.g., to reduce the tumorigenic potential of a cell at the transplant site or to reduce proliferation of a cell that has become tumorigenic at a transplant site).

The method comprises: providing a cell genetically modified to comprise at least one mechanism for providing a local immunosuppression at a transplant site when transplanted in an allogeneic host the cell or a population of such cells; genetically modifying in the cell a cell division locus/loci (CDL), the CDL being one or more loci whose transcription product(s) is expressed by dividing cells (e.g., all dividing cells containing one or more of the immunosuppressive transgenes), the genetic modification of the CDL comprising one or more of: a) an ablation link (ALINK) system, the ALINK system comprising a DNA sequence encoding a negative selectable marker that is transcriptionally linked to a DNA sequence encoding the CDL; and b) an inducible exogenous activator of regulation of a CDL (EARC) system, the EARC system comprising an inducible activator-based gene expression system that is operably linked to the CDL; permitting proliferation of the genetically modified cell comprising the ALINK system by maintaining the genetically modified cell comprising the ALINK system in the absence of an inducer of the negative selectable marker or ablating and/or inhibiting proliferation of the genetically modified cell comprising the ALINK system by exposing the cell comprising the ALINK system to the inducer of the negative selectable marker; and/or permitting proliferation of the genetically modified cell comprising the EARC system by exposing the genetically modified cell comprising the EARC system to an inducer of the inducible activator-based gene expression system or preventing or inhibiting proliferation of the genetically modified cell comprising the EARC system by maintaining the cell comprising the EARC system in the absence of the inducer of the inducible activator-based gene expression system; and

transplanting the cell or a population of the cells at a transplantation site in an allogeneic host. Cells that have been modified to control cell division using one or more ALINK and/or EARC systems in one or more CDLs (e.g., 2, 3, 4, or more CDLs) may be referred to as “fail-safe cells”. The number of cells that can be grown from a single fail-safe cell (clone volume) before the cell loses activity of all of the systems (e.g., ALINKs or EARCs) that control cell division through genetic mutation (e.g., the number of cell divisions it would take for a cell to “escape” from control and exhibit uncontrollable cell proliferation based on mathematical modeling) determines the fail-safe volume. The fail-safe volume will depend on the number of ALINKs and the number of ALINK-targeted CDLs. The fail-safe property is further described in Table 3.

Table 3. Fail-safe cell volumes and their relationship to a human body were calculated using mathematical modeling. The model did not take into account an event in which CDL expression was co-lost with the loss of negative selectable marker activity, compromising cell proliferation. Therefore the values are underestimates and were calculated assuming 10^6 forward mutation rate for the negative selectable marker. The estimated number of cells in a human body as $3.72x10^{13}$ was taken from (Bianconi et al., 2013).

CDL #	ALINK #	Genotype in CDLs	Fail-safe volume (#cells)	Relative (x) to a human body=$3.72x10^{13}$ cells	Estimated weight of clones
1	1	het	512	0.0000000000137	1µg
1	2	hom	16777216	0.000000451	31 mg
2	3	het, hom	1.374E+11	0.004	0.26 kg
2	4	hom, hom	1.13E+15	30	2100 kg

In various embodiments, a CDL is a locus identified as an "essential gene" as set forth in Wang et al., 2015, which is incorporated herein by reference as if set forth in its entirety. Essential genes in Wang et al., 2015, were identified by computing a score (i.e., a CRISPR score) for each gene that reflects the fitness cost imposed by inactivation of the gene. In an embodiment, a CDL has a CRISPR score (CS) of less than about –1.0 (Table 5, column 5).

In various embodiments, a CDL is a locus/loci that encodes a gene product that is relevant to cell division and/or replication (Table 5, column 6). For example, in various embodiments, a CDL is a locus/loci that encodes a gene product that is relevant to one or more of: i) cell cycle; ii) DNA replication; iii) RNA transcription and/or protein translation; and iv) metabolism (Table 5, column 7).

In an embodiment, a CDL is one or more cyclin-dependent kinases that are involved with regulating progression of the cell cycle (e.g., control of G1/S G2/M and metaphase-to-anaphase transition), such as CDK1, CDK2, CDK3, CDK4, CDK5, CDK6, CDK7, CDK8, CDK9 and/or CDK11 (Morgan, 2007). In an embodiment, a CDL is one or more cyclins that are involved with controlling progression of the cell cycle by activating one or more CDK, such as, for example, cyclinB, cyclinE, cyclinA, cyclinC, cyclinD, cyclinH, cyclinC, cyclinT, cyclinL and/or cyclinF (FUNG and POON, 2005). In an embodiment, a CDL is one or more loci involved in the anaphase-promoting complex that controls the progression of metaphase to anaphase transition in the M phase of the cell cycle (Peters, 2002). In an embodiment, a CDL is one or more loci involved with kinetochore components that control the progression of metaphase to anaphase transition in the M phase of the cell cycle (Fukagawa, 2007). In an embodiment, a CDL is one or more loci involved with microtubule components that control microtubule dynamics required for the cell cycle (Cassimeris, 1999).

In various embodiments, a CDL is a locus/loci involved with housekeeping. As used herein, the term "housekeeping gene" or "housekeeping locus" refers to one or more genes that are required for the maintenance of basic cellular function. Housekeeping genes are expressed in all cells of an organism under normal and patho-physiological conditions.

In various embodiments, a CDL is a locus/loci that encodes a gene product that is relevant to cell division and/or proliferation and has a CRISPR score of less than about -1.0. For example, in an embodiment, a CDL is a locus/loci that encodes a gene product that is relevant to one or more of: i) cell cycle; ii) DNA replication; iii) RNA transcription and/or protein translation; and iv) metabolism, and has a CRISPR score of less than about -1.0. In an embodiment, the CDL may also be a housekeeping gene.

In some embodiments, the CDL is Cdk1/CDK1, Top2A/TOP2A, Cenpa/CEPNA, Birc5/BIRC5, or Eef2/EEF2. In some embodiments, the CDL is Cdk1/CDK1. In some embodiments, the CDL is Top2A/TOP2A. In some embodiments, the CDL is Eef2/EEF2. In some embodiments, the CDLs are Cdk1/CDK1 and Top2A/TOP2A or Cdk1/CDK1 and Eef2/EEF2.

A cell can be modified to be a "fail-safe" cell by linking the expression of a CDL with that of a DNA sequence encoding a negative selectable marker, thereby allowing drug-induced ablation of mitotically active cells expressing both the CDL and the negative selectable marker. Ablation of proliferating cells may be desirable, for example, when cell proliferation is uncontrolled and/or accelerated relative to a cell's normal division rate (e.g., uncontrolled cell division exhibited by cancerous cells), or when therapeutic need for the cells has passed. Ablation of proliferating cells may be achieved via a genetic modification to the cell, referred to herein as an "ablation link" (ALINK), which links the expression of a DNA sequence encoding a negative selectable marker to that of a CDL, thereby allowing elimination or sufficient inhibition of ALINK-modified proliferating cells consequently expressing the CDL locus (sufficient inhibition being inhibition of cell expansion rate to a rate that is too low to contribute to tumour formation). In the presence of a pro-drug or other inducer of the negatively selectable system, cells expressing the negative selectable marker will stop proliferating or die, depending on the mechanism of action of the selectable marker. Cells may be modified to comprise homozygous, heterozygous, hemizygous or compound heterozygous ALINKS. In one embodiment, to improve fidelity of ablation, a negative selectable marker may be introduced into all alleles functional of a CDL. In one preferred embodiment, a negative selectable marker may be introduced into all functional alleles of a CDL. The fail-safe system can be used to eliminate all of the cloaked cells, if desired.

An ALINK may be inserted in any position of CDL, which allows co-expression of the CDL and the negative selectable marker.

In some embodiments, the ALINK system comprises a herpes simplex virus-thymidine kinase/ganciclovir system, a cytosine deaminase/5-fluorocytosine system, a carboxyl esterase/irinotecan system or an iCasp9/AP1903 system.

DNA encoding a negatively selectable marker (e.g., HSV-TK), may be inserted into a CDL (e.g., CDK1) in a host cell, such that expression of the negative selectable marker causes host cells expressing the negative selectable marker and, necessarily, the CDL, to be killed in the presence of an inducer (e.g., prodrug) of the negative selectable marker (e.g., ganciclovir (GCV)). In this example, host cells modified with the ALINK will produce thymidine kinase (TK) and the TK protein will convert GCV into GCV monophosphate, which is then converted into GCV triphosphate by cellular kinases. GCV triphosphate

incorporates into the replicating DNA during S phase, which leads to the termination of DNA elongation and cell apoptosis (Halloran and Fenton, 1998).

A modified HSV-TK gene (Preuß et al., 2010) is disclosed herein as one example of DNA encoding a negative selectable marker that may be used in an ALINK genetic modification to selectively ablate cells comprising undesirable cell division rate.

It is contemplated herein that alternative and/or additional negative selectable systems could be used in the tools and/or methods provided herein. Various negative selectable marker systems are known in the art (e.g., dCK.DM (Neschadim et al., 2012)).

For example, various negative selectable system having clinical relevance have been under active development in the field of "gene-direct enzyme/prodrug therapy" (GEPT), which aims to improve therapeutic efficacy of conventional cancer therapy with no or minimal side-effects (Hedley et al., 2007; Nawa et al., 2008). Frequently, GEPT involves the use of viral vectors to deliver a gene into cancer cells or into the vicinity of cancer cells in an area of the cancer cells that is not found in mammalian cells and that produces enzymes, which can convert a relatively non-toxic prodrug into a toxic agent.

HSV-TK/GCV, cytosine deaminase/5-fluorocytosine (CD/5-FC), and carboxyl esterase/irinotecan (CE/CPT-11) are examples of negative selectable marker systems being evaluated in GEPT pre- and clinical trials (Danks et al., 2007; Shah, 2012).

To overcome the potential immunogenicity of a Herpes Simplex Virus type 1 thymidine kinase/ganciclovir (TK/GCV) system, a "humanized" suicide system has been developed by engineering the human deoxycytidine kinase enzyme to become thymidine-active and to work as a negative selectable (suicide) system with non-toxic prodrugs: bromovinyl-deoxyuridine (BVdU), L-deoxythymidine (LdT) or L-deoxyuridine (LdU) (Neschadim et al., 2012).

The CD/5-FC negative selectable marker system is a widely used "suicide gene" system. Cytosine deaminase (CD) is a non-mammalian enzyme that may be obtained from bacteria or yeast (e.g., from *Escherichia coli* or *Saccharomyces cerevisiae*, respectively) (Ramnaraine et al., 2003). CD catalyzes conversion of cytosine into uracil and is an important member of the pyrimidine salvage pathway in prokaryotes and fungi, but it does not exist in mammalian cells. 5-fluorocytosine (5-FC) is an antifungal prodrug that causes a low level of cytotoxicity in humans (Denny, 2003). CD catalyzes conversion of 5-FC into the genotoxic agent 5-FU, which has a high level of toxicity in humans (Ireton et al., 2002).

The CE/CPT-11 system is based on the carboxyl esterase enzyme, which is a serine esterase found in a different tissues of mammalian species (Humerickhouse et al., 2000). The anti-cancer agent CPT-11 is a prodrug that is activated by CE to generate an active referred to as 7-ethyl-10-hydroxycamptothecin (SN-38), which is a strong mammalian topoisomerase I inhibitor (Wierdl et al., 2001). SN-38 induces accumulation of double-strand DNA breaks in dividing cells (Kojima et al., 1998).

Another example of a negative selectable marker system is the iCasp9/AP1903 suicide system, which is based on a modified human caspase 9 fused to a human FK506 binding protein (FKBP) to allow chemical dimerization using a small molecule AP1903, which has tested safely in humans. Administration

of the dimerizing drug induces apoptosis of cells expressing the engineered caspase 9 components. This system has several advantages, such as, for example, including low potential immunogenicity, since it consists of human gene products, the dimerizer drug only effects the cells expressing the engineered caspase 9 components (Straathof et al., 2005). The iCasp/AP1903 suicide system is being tested in clinical settings (Di Stasi et al., 2011).

It is contemplated herein that the negative selectable marker system of the ALINK system could be replaced with a proliferation antagonist system. The term "proliferation antagonist" as used herein, refers to a natural or engineered compound(s) whose presence inhibits (completely or partially) division of a cell. For example, Omomyc^{ER} is the fusion protein of MYC dominant negative Omomyc with mutant murine estrogen receptor (ER) domain. When induced with tamoxifen (TAM), the fusion protein Omomyc^{ER} localizes to the nucleus, where the dominant negative Omomyc dimerizes with C-Myc, L-Myc and N-Myc, sequestering them in complexes that are unable to bind the Myc DNA binding consensus sequences (Soucek et al., 2002). As a consequence of the lack of Myc activity, cells are unable to divide (Oricchio et al., 2014). Another example of a proliferation antagonist is A-Fos, a dominant negative to activation protein-1 (AP1) (a heterodimer of the oncogenes Fos and Jun) that inhibits DNA binding in an equimolar competition (Olive et al., 1997). A-Fos can also be fused to ER domain, rendering its nuclear localization to be induced by TAM. Omomyc^{ER} / tamoxifen or A-Fos^{ER} / tamoxifen could be a replacement for TK/GCV to be an ALINK.

A cell can also be modified to be "fail-safe" by operably linking the CDL with an EARC, such as an inducible activator-based gene expression system. Under these conditions, the CDL will only be expressed (and the cell can only divide) in the presence of the inducer of the inducible activator-based gene expression system. Under these conditions, EARC-modified cells stop dividing, significantly slow down, or die in the absence of the inducer, depending on the mechanism of action of the inducible activator-based gene expression system and CDL function. Cells may be modified to comprise homozygous or compound heterozygous EARCs or may be altered such that only EARC-modified alleles can produce functional CDLs. In an embodiment, an EARC modification may be introduced into all alleles of a CDL, for example, to provide a mechanism for cell division control.

An EARC may be inserted in any position of CDL that permits co-expression of the CDL and the activator component of the inducible system in the presence of the inducer.

In an embodiment, an "activator" based gene expression system is preferable to a "repressor" based gene expression system. For example, if a repressor is used to suppress a CDL a loss of function mutation of the repressor could release CDL expression, thereby allowing cell proliferation. In a case of an activation-based suppression of cell division, the loss of activator function (mutation) would shut down CDL expression, thereby disallowing cell proliferation.

In some embodiments, the EARC system is a dox-bridge system, a cumate switch inducible system, an ecdysone inducible system, a radio wave inducible system, or a ligand-reversible dimerization system.

A dox-bridge may be inserted into a CDL (e.g., CDK1) in a host cell, such that in the presence of an inducer (e.g., doxycycline or "DOX") the dox-bridge permits CDL expression, thereby allowing cell division and proliferation. Host cells modified with a dox-bridge EARC may comprise a reverse tetracycline Trans-Activator (rtTA) gene (Urlinger et al., 2000) under the transcriptional control of a promoter, which is active in dividing cells (e.g., in the CDL). This targeted insertion makes the CDL promoter no longer available for CDL transcription. To regain CDL transcription, a tetracycline responder element promoter (for example TRE (Agha-Mohammadi et al., 2004)) is inserted in front of the CDL transcript, which will express the CDL gene only in a situation when rtTA is expressed and doxycycline is present. When the only source of CDL expression is dox-bridged alleles, there is no CDL gene expression in the absence of doxycycline. The lack of CDL expression causes the EARC-modified cells to be compromised in their proliferation, either by death, stopping cell division, or by rendering the cell mitotic rate so slow that the EARC-modified cell could not contribute to tumor formation.

The term "dox-bridge" as used herein, refers to a mechanism for separating activity of a promoter from a target transcribed region by expressing rtTA (Gossen et al., 1995) by the endogenous or exogenous promoter and rendering the transcription of target region under the control of TRE. As used herein, "rtTA" refers to the reverse tetracycline transactivator elements of the tetracycline inducible system (Gossen et al., 1995) and "TRE" refers to a promoter consisting of *TetO* operator sequences upstream of a minimal promoter. Upon binding of rtTA to the TRE promoter in the presence of doxycycline, transcription of loci downstream of the TRE promoter increases. The rtTA sequence may be inserted in the same transcriptional unit as the CDL or in a different location of the genome, so long as the transcriptional expression's permissive or non-permissive status of the target region is controlled by doxycycline. A dox-bridge is an example of an EARC.

Introduction of an EARC system into the 5' regulatory region of a CDL is also contemplated herein.

It is contemplated herein that alternative and/or additional inducible activator-based gene expression systems could be used in the tools and or methods provided herein to produce EARC modifications. Various inducible activator-based gene expression systems are known in the art.

For example, destabilizing protein domains (Banaszynski et al., 2006) fused with an acting protein product of a coding CDL could be used in conjunction with a small molecule synthetic ligand to stabilize a CDL fusion protein when cell division and/or proliferation is desirable. In the absence of a stabilizer, destabilized-CDL-protein will be degraded by the cell, which in turn would stop proliferation. When the stabilizer compound is added, it would bind to the destabilized-CDL-protein, which would not be degraded, thereby allowing the cell to proliferate.

For example, transcription activator-like effector (TALE) technology (Maeder et al., 2013) could be combined with dimerizer-regulated expression induction (Pollock and Clackson, 2002). The TALE technology could be used to generate a DNA binding domain designed to be specific to a sequence, placed together with a minimal promoter replacing the promoter of a CDL. The TALE DNA binding

domain also extended with a drug dimerizing domain. The latter can bind to another engineered protein having corresponding dimerizing domain and a transcriptional activation domain.

For example, a reverse-cumate-Trans-Activator (rcTA) may be inserted in the 5' untranslated region of the CDL, such that it will be expressed by the endogenous CDL promoter. A 6-times repeat of a Cumate Operator (6xCuO) may be inserted just before the translational start (ATG) of CDL. In the absence of cumate in the system, rcTA cannot bind to the 6xCuO, so the CDL will not be transcribed because the 6xCuO is not active. When cumate is added, it will form a complex with rcTA, enabling binding to 6xCuO and enabling CDL transcription (Mullick et al., 2006).

For example, a retinoid X receptor (RXR) and an N-terminal truncation of ecdysone receptor (EcR) fused to the activation domain of Vp16 (VpEcR) may be inserted in the 5' untranslated region of a CDL such that they are co-expressed by an endogenous CDL promoter. Ecdysone responsive element (EcRE), with a downstream minimal promoter, may also be inserted in the CDL, just upstream of the starting codon. Co-expressed RXR and VpEcR can heterodimerize with each other. In the absence of ecdysone or a synthetic drug analog muristerone A, dimerized RXR/VpEcR cannot bind to EcRE, so the CDL is not transcribed. In the presence of ecdysone or muristerone A, dimerized RXR/VpEcR can bind to EcRE, such that the CDL is transcribed (No et al., 1996).

For example, a transient receptor potential vanilloid-1 (TRPV1), together with ferritin, may be inserted in the 5' untranslated region of a CDL and co-expressed by an endogenous CDL promoter. A promoter inducible by NFAT (NFATre) may also be inserted in the CDL, just upstream of the starting codon. In a normal environment, the NFAT promoter is not active. However, upon exposure to low-frequency radio waves, TRPV1 and ferritin create a wave of Ca^{++} entering the cell, which in turn converts cytoplasmatic-NFAT (NFATc) to nuclear-NFAT (NFATn), that ultimately will activate the NFATre and transcribe the CDL (Stanley et al., 2015).

For example, a CDL may be functionally divided in to parts/domains: 5'-CDL and 3'CDL, and a FKBP peptide sequence may be inserted into each domain. An IRES (internal ribosomal entry site) sequence may be placed between the two domains, which will be transcribed simultaneously by a CDL promoter but will generate two separate proteins. Without the presence of an inducer, the two separate CDL domains will be functionally inactive. Upon introduction of a dimerization agent, such as rapamycin or AP20187, the FKBP peptides will dimerize, bringing together the 5' and 3' CDL parts and reconstituting an active protein (Rollins et al., 2000).

In an embodiment of the method, the genetically modified cell comprises: a set of transgenes, each transgene encoding a gene product that is cytoplasmic, membrane bound, or local acting and whose function is to mitigate function of graft attacking leukocyte and NK cell activation or act as a defense mechanism against attacking leukocytes.

Methods for genetically modifying cell to comprise at least one mechanism for providing a local immunosuppression at a transplant site when transplanted in an allogeneic host the cell or a population of

such cells are described, for example, in WO 2016/141480, the entire teachings of which are incorporated herein by reference.

The set of transgenes comprises one or more of the following genes: PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6). In an embodiment, the set of transgenes genes comprises PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6).

Optionally, the method further comprises expressing one or more of the following transgenes in the cell: TGF-β, Cd73, Cd39, Lag3, Il1r2, Ackr2, Tnfrsf22, Tnfrs23, Tnfrsf10, Dad1, and IFNγR1 d39. In an embodiment, the TGF-β or the biologic is local acting in the graft environment.

Techniques for introducing into animal cells various genetic modifications, such as transgenes are described herein and are generally known in the art.

In an embodiment of the method, the cell is a stem cell, a cell amenable to genome editing, or a cell that can serve as a source of a therapeutic cell type (e.g., a cell that can be directed to differentiate into a lineage restricted or terminally differentiated cell that can be used for cell therapy, or a cell of a desired target tissue). In an embodiment, the cell is an embryonic stem cell, an induced pluripotent stem cell, an adult stem cell, a tissue-specific stem cell, a hematopoietic stem cell, a mesenchymal stem cell, an endothelial stem cell, an epithelial stem cell, an adipose stem or progenitor cells, a lung stem or progenitor cell, a mammary stem cell, an olfactory adult stem cell, a hair follicle stem cell, a multipotent stem cell, an amniotic stem cell, a cord blood stem cell, or a neural stem or progenitor cell. In some embodiments, the cell is derived from a target tissue, e.g., skin, heart, brain or spinal cord, liver, lung, kidney, pancreas, bladder, bone marrow, spleen, intestine, or stomach. In some embodiments, the cell is a fibroblast, an epithelial cell, or an endothelial cell. The cell may be a vertebrate cell, for example a mammalian cell, such as a human or mouse cell.

Techniques for transplanting the genetically modified cells into a transplant site of an allogeneic host are described herein and are generally known in the art.

In various embodiments of any of the methods of the disclosure, the host has a degenerative disease or a condition that can be treated with cell therapy. Examples of such diseases or conditions include, but are not limited to: blindness, arthritis (e.g., osteoarthritis or rheumatoid arthritis), ischemia, diabetes (e.g., Type 1 or Type 2 diabetes), multiple sclerosis, spinal cord injury, stroke, cancer, a lung disease, a blood disease, a neurological disease, such as Parkinson's disease, Alzheimer's disease, Huntington's disease, and ALS, an enzyme or hormone deficiency, a metabolic disorder (e.g., a lysosomal storage disorder, Galactosemia, Maple syrup urine disease, Phenylketonuria, a glycogen storage disease, a mitochondrial disorder, Friedrich's ataxia, a peroxisomal disorder, a metal metabolism disorder, or an organic academia), an autoimmune disease (e.g., Psoriasis, Systemic Lupus Erythematosus, Grave's disease, Inflammatory Bowel Disease, Addison's Diseases, Sjogren's Syndrome, Hashimoto's Thyroiditis, Vasculitis, Autoimmune Hepatitis, Alopecia Areata, Autoimmune pancreatitis, Crohn's Disease, Ulcerative colitis, Dermatomyositis), age-related macular degeneration, retinal

dystrophy, an infectious disease, hemophilia, a degenerative disease (e.g., Charcot-Marie-Tooth disease, chronic obstructive pulmonary disease, chronic traumatic encephalopathy, Creutzfeldt-Jakob disease, Cystic Fibrosis, Cytochrome C Oxidase deficiency, Ehlers-Danlos syndrome, essential tremor, Fribrodisplasia Ossificans Progressiva, infantile neuroaxonal dystrophy, keratoconus, keratoglobus, muscular dystrophy, neuronal ceroid lipofuscinosis, a prior disease, progressive supranuclear palsy, sandhoff disease, spinal muscular atrophy, retinitis pigmentosa), or an age-related disease (e.g., atherosclerosis, cardiovascular disease (e.g., angina, myocardial infarction), cataracts, osteoporosis, or hypertension).

Pharmaceutical Compositions

The cloaked cells described herein may be incorporated into a vehicle for administration into a patient, such as a human patient receiving a transplant or suffering from a disease or condition described herein. Pharmaceutical compositions containing cloaked cells can be prepared using methods known in the art. For example, such compositions can be prepared using, e.g., physiologically acceptable carriers, excipients or stabilizers (Remington: The Science and Practice of Pharmacology 22nd edition, Allen, L. Ed. (2013); incorporated herein by reference), and in a desired form, e.g., in the form of aqueous solutions.

The cloaked cells described herein can be administered in any physiologically compatible carrier, such as a buffered saline solution. Pharmaceutically acceptable carriers and diluents include saline, aqueous buffer solutions, solvents and/or dispersion media. The use of such carriers and diluents is well known in the art. Other examples include liquid media, for example, Dulbeccos modified eagle's medium (DMEM), sterile saline, sterile phosphate buffered saline, Leibovitz's medium (L15, Invitrogen, Carlsbad, Calif.), dextrose in sterile water, and any other physiologically acceptable liquid. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. The solution is preferably sterile and fluid to the extent that easy syringability exists. Preferably, the solution is stable under the conditions of manufacture and storage and preserved against the contaminating action of microorganisms such as bacteria and fungi through the use of, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosol, and the like. Solutions of the invention can be prepared by using a pharmaceutically acceptable carrier or diluent and, as required, other ingredients enumerated above, followed by filtered sterilization, and then incorporating the cloaked cells as described herein.

For example, a solution containing a pharmaceutical composition described herein may be suitably buffered, if necessary, and the liquid diluent first rendered isotonic with sufficient saline or

glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous, and intraperitoneal administration. In this connection, sterile aqueous media that can be employed will be known to those of skill in the art in light of the present disclosure. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations may meet sterility, pyrogenicity, general safety, and purity standards as required by FDA Office of Biologics standards.

Pharmaceutical compositions comprising cloaked cells in a semi-solid or solid carrier are typically formulated for surgical implantation at the site of transplantation or at the affected site of a disease or condition in the subject. It will be appreciated that liquid compositions also may be administered by surgical procedures. In particular embodiments, semi-solid or solid pharmaceutical compositions may comprise semi-permeable gels, matrices, cellular scaffolds and the like, which may be non-biodegradable or biodegradable. For example, in certain embodiments, it may be desirable or appropriate to sequester the cloaked cells from their surroundings, yet enable the cells to secrete and deliver biological molecules (e.g., a therapeutic agent listed in Table 2) to surrounding cells.

In other embodiments, different varieties of degradable gels and networks are utilized for the pharmaceutical compositions of the invention. For example, degradable materials include biocompatible polymers, such as poly(lactic acid), poly(lactic acid-co-glycolic acid), methylcellulose, hyaluronic acid, collagen, and the like.

In another embodiment, one or more hydrogels are used for the pharmaceutical compositions. The one or more hydrogels may include collagen, atelocollagen, fibrin constructs, hydrophilic vinyl and acrylic polymers, polysaccharides such as calcium alginate, and poly(ethylene oxide). Further, the hydrogel may be formed of poly(2-hydroxyethyl methacrylate), poly(acrylic acid), self-assembling peptides (e.g., RAD16), poly(methacrylic acid), poly(N-vinyl-2-pyrrolidinone), poly(vinyl alcohol) and their copolymers with each other and with hydrophobic monomers such as methyl methacrylate, vinyl acetate, and the like. Also preferred are hydrophilic polyurethanes containing large poly(ethylene oxide) blocks. Other preferred materials include hydrogels comprising interpenetrating networks of polymers, which may be formed by addition or by condensation polymerization, the components of which may comprise hydrophilic and hydrophobic monomers such as those just enumerated. In situ-forming degradable networks are also suitable for use in the invention (see, e.g., Anseth, K S et al. *J. Controlled Release,* 2002; 78:199-209; Wang, D. et al., *Biomaterials,* 2003; 24:3969-3980; U.S. Patent Publication 2002/0022676). These in situ forming materials are formulated as fluids suitable for injection; then may be induced to form a hydrogel by a variety of means such as change in temperature, pH, and exposure to light in situ or in vivo. In one embodiment, the construct contains fibrin glue containing gels. In another embodiment, the construct contains atelocollagen containing gels.

A polymer used to form a matrix may be in the form of a hydrogel. In general, hydrogels are cross-linked polymeric materials that can absorb more than 20% of their weight in water while maintaining a distinct three-dimensional structure. This definition includes dry cross-linked polymers that will swell in

aqueous environments, as well as water-swollen materials. A host of hydrophilic polymers can be cross-linked to produce hydrogels, whether the polymer is of biological origin, semi-synthetic or wholly synthetic. The hydrogel may be produced from a synthetic polymeric material. Such synthetic polymers can be tailored to a range of properties and predictable lot-to-lot uniformity, and represent a reliable source of material that generally is free from concerns of immunogenicity. The matrices may include hydrogels formed from self assembling peptides, such as those discussed in U.S. Pat. Nos. 5,670,483 and 5,955,343, U.S. Patent Application No. 2002/0160471, and PCT Application No. WO 02/062969.

Properties that make hydrogels valuable in drug delivery applications include the equilibrium swelling degree, sorption kinetics, solute permeability, and their in vivo performance characteristics. Permeability to compounds depends, in part, upon the swelling degree or water content and the rate of biodegradation. Since the mechanical strength of a gel may decline in proportion to the swelling degree, it is also well within the contemplation of the present invention that the hydrogel can be attached to a substrate so that the composite system enhances mechanical strength. In some embodiments, the hydrogel can be impregnated within a porous substrate, so as to gain the mechanical strength of the substrate, along with the useful delivery properties of the hydrogel.

In other embodiments, the pharmaceutical composition comprises a biocompatible matrix made of natural, modified natural or synthetic biodegradable polymers, including homopolymers, copolymers and block polymers, as well as combinations thereof.

Examples of suitable biodegradable polymers or polymer classes include any biodegradable polymers discussed within this disclosure, including but not limited to, fibrin, collagen types I, II, III, IV and V, elastin, gelatin, vitronectin, fibronectin, laminin, thrombin, poly(aminoacid), oxidized cellulose, tropoelastin, silk, ribonucleic acids, deoxyribonucleic acids; proteins, polynucleotides, gum arabic, reconstituted basement membrane matrices, starches, dextrans, alginates, hyaluron, chitin, chitosan, agarose, polysaccharides, hyaluronic acid, poly(lactic acid), poly(glycolic acid), polyethylene glycol, decellularized tissue, self-assembling peptides, polypeptides, glycosaminoglycans, their derivatives and mixtures thereof. Suitable polymers also include poly(lactide) (PLA) which can be formed of L(+) and D(−) polymers, polyhydroxybutyrate, polyurethanes, polyphoshazenes, poly(ethylene glycol)-poly(lactide-co-glycolide) co-polymer, degradable polycyanoacrylates and degradable polyurethanes. For both glycolic acid and lactic acid, an intermediate cyclic dimer is may be prepared and purified prior to polymerization. These intermediate dimers are called glycolide and lactide, respectively.

Other useful biodegradable polymers or polymer classes include, without limitation, aliphatic polyesters, poly(alkylene oxalates), tyrosine derived polycarbonates, polyiminocarbonates, polyorthoesters, polyoxaesters, polyamidoesters, polyoxaesters containing amine groups, poly(propylene fumarate), polyfumarates, polydioxanones, polycarbonates, polyoxalates, poly(alpha-hydroxyacids), poly(esters), polyurethane, poly(ester urethane), poly(ether urethane), polyanhydrides, polyacetates, polycaprolactones, poly(orthoesters), polyamino acids, polyamides and blends and copolymers thereof. Additional useful biodegradable polymers include, without limitation stereopolymers of L- and D-lactic

acid, copolymers of bis(para-carboxyphenoxy)propane and sebacic acid, sebacic acid copolymers, copolymers of caprolactone, poly(lactic acid)/poly(glycolic acid)/polyethyleneglycol copolymers, copolymers of polyurethane and poly(lactic acid), copolymers of alpha-amino acids, copolymers of alpha-amino acids and caproic acid, copolymers of alpha-benzyl glutamate and polyethylene glycol, copolymers of succinate and poly(glycols), polyphosphazene, poly(hydroxyalkanoates) and mixtures thereof. Binary and ternary systems also are contemplated.

In general, the material used to form a matrix is desirably configured so that it: (1) has mechanical properties that are suitable for the intended application; (2) remains sufficiently intact until tissue has in-grown and healed; (3) does not invoke an inflammatory or toxic response; (4) is metabolized in the body after fulfilling its purpose; (5) is easily processed into the desired final product to be formed; (6) demonstrates acceptable shelf-life; and (7) is easily sterilized.

In another embodiment, the population of cloaked cells can be administered by use of a scaffold. The composition, shape, and porosity of the scaffold may be any described above. Typically, these three-dimensional biomaterials contain the living cells attached to the scaffold, dispersed within the scaffold or incorporated in an extracellular matrix entrapped in the scaffold. Once implanted into the target region of the body, these implants become integrated with the host tissue, wherein the transplanted cells gradually become established.

Non-limiting examples of scaffolds that may be used include textile structures, such as weaves, knits, braids, meshes, non-wovens, and warped knits; porous foams, semi-porous foams, perforated films or sheets, microparticles, beads, and spheres and composite structures being a combination of the above structures. Nonwoven mats may, for example, be formed using fibers comprised of a synthetic absorbable copolymer of glycolic and lactic acids (PGA/PLA), sold under the tradename VICRYL sutures (Ethicon, Inc., Somerville, N.J.). Foams, composed of, for example, poly(epsilon-caprolactone)/poly(glycolic acid) (PCL/PGA) copolymer, formed by processes such as freeze-drying, or lyophilized, as discussed in U.S. Pat. No. 6,355,699, also may be utilized.

In another embodiment, the framework is a felt, which can be composed of a multifilament yarn made from a bioabsorbable material. The yarn can be made into a felt using standard textile processing techniques consisting of crimping, cutting, carding and needling. In another embodiment, cells are seeded onto foam scaffolds that may be used as composite structures.

The framework may be molded into a useful shape, such as to fill a tissue void. The framework can therefore be shaped to not only provide a channel for neural growth, but also provide a scaffold for the supporting and surrounding tissues, such as vascular tissue, muscle tissue, and the like. Furthermore, it will be appreciated that the population of cells may be cultured on pre-formed, non-degradable surgical or implantable devices.

Pharmaceutical compositions may include preparations made from cloaked cells that are formulated with a pharmaceutically acceptable carrier or medium. Suitable pharmaceutically acceptable carriers include any discussed within this disclosure, including but not limited to, water, salt solution (such

as Ringer's solution), alcohols, oils, gelatins, polyvinyl pyrrolidine, carbohydrates such as lactose, amylose, or starch, fatty acid esters, and hydroxymethylcellulose. Such preparations can be sterilized, and if desired, mixed with auxiliary agents such as lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, and coloring agents. Pharmaceutical carriers suitable for use in the present invention are known in the art and are described, for example, in Pharmaceutical Sciences (17th Ed., Mack Pub. Co., Easton, Pa.) and WO 96/05309.

Methods of treatment

The cloaked cells and compositions described herein may be administered to a subject in need thereof (e.g., a subject who is receiving or has received a transplant, or a subject having a disease or condition described herein) by a variety of routes, such as local administration to or near the site of a transplant, local administration to the site affected by the disease or condition (e.g., injection to a joint for treating RA, injection into the subretinal space for treating wet AMD, direct administration to the central nervous system (CNS) (e.g., intracerebral, intraventricular, intrathecal, intracisternal, or stereotactic administration) for treating a neurological disease, such as Parkinson's disease, direct injection into the cardiac muscle for treating cardiac infarction), intravenous, parenteral, intradermal, transdermal, intramuscular, intranasal, subcutaneous, percutaneous, intratracheal, intraperitoneal, intraarterial, intravascular, inhalation, perfusion, lavage, and oral administration. The most suitable route for administration in any given case will depend on the particular cells or composition administered, the patient, pharmaceutical formulation methods, administration methods (e.g., administration time and administration route), the patient's age, body weight, sex, severity of the disease being treated, the patient's diet, and the patient's excretion rate. Compositions may be administered once, or more than once (e.g., once annually, twice annually, three times annually, bi-monthly, or monthly). For local administration, the cloaked cells may be administered by any means that places the population of cells in a desired location, including catheter, syringe, shunt, stent, microcatheter, pump, implantation with a device, or implantation with a scaffold.

As described herein, before administration, the population of cells can be incubated in the presence of one or more factors, or under conditions, that stimulate stem cell differentiation into a desired cell type (e.g., a neuron, a cardiac muscle cell, an RPE cell, an insulin producing cell, a blood coagulation factor producing cell, an articular fibroblast, or other cell types described herein). Such factors are known in the art and the skilled artisan will appreciate that determination of suitable conditions for differentiation can be accomplished with routine experimentation. Such factors include growth or trophic factors, chemokines, cytokines, cellular products, demethylating agents, and other stimuli which are known to stimulate differentiation, for example, of stem cells along angiogenic, hemangiogenic, vasculogenic, skeletal muscle, vascular smooth muscle, pericyte, neuronal, or vascular endothelial pathways or lineages. Alternatively, the composition administered to the patient includes a population of cloaked cells with one or more factors that stimulate cell differentiation into a desired cell type, where the cell

differentiation occurs in vivo at the tissue site. In some embodiments, the cloaked cells can be differentiated into an organ or tissue in vitro using methods known by those of skill in the art and administered to a subject in need of an organ or tissue transplant.

In some embodiments, cells of a specific cell type are collected from the patient or from a donor (e.g., from an HLA-matched or mis-matched donor that is, e.g., free of the disease or condition), modified to express one or more (e.g., one, two, three, four, five, six, seven, or eight) cloaking transgenes, and subsequently administered to a subject. Such an approach is useful for treating subjects carrying a mutation in a particular gene, as the cloaked donor cells can endogenously express the wild-type version of the gene, or for subjects deficient in a particular secreted protein or enzyme (e.g., using cloaked donor cells that endogenously express the protein or enzyme that is deficient in the subject). This approach can also be used for treatment of subjects receiving an organ or tissue transplant, as cells in the organ or tissue transplant can be modified to express one or more (e.g., one, two, three, four, five, six, seven, or eight) of the cloaking transgenes before the transplant is performed.

Subjects that may be treated as described herein are subjects that have received a transplant, or subjects having a disease or condition described herein (e.g., wet AMD or retinal dystrophy, a neurodegenerative disease, such as Parkinson's disease, cardiac infarction, osteoarthritis or RA, diabetes, hemophilia, a metabolic disorder, or a disease or condition listed in Table 2). The cells, compositions, and methods described herein can be used to treat a disease or condition caused by or associated with loss of cells, a mutation or deficiency in a protein, or aberrant production of a protein, which could be treated using cell replacement protein or cellular therapy, production of a therapeutic protein, production of an agonist antibody, or production of an inhibitory antibody. The methods described herein may include a step of screening a subject for mutations in genes associated with deficient protein production prior to treatment with or administration of the compositions described herein. A subject can be screened for a genetic mutation using standard methods known to those of skill in the art (e.g., genetic testing). The methods described herein may also include a step of evaluating the symptoms of the disease or condition in a subject prior to treatment with or administration of the cloaked cells or compositions described herein. The subject can then be evaluated using the same diagnostic tests after administration of the cloaked cells or compositions to determine whether the subject's condition has improved. The compositions and methods described herein may be administered as a preventative treatment to patients who have received a tissue or organ transplant before the patient shows any signs of tissue or organ rejection.

The cloaked cells, compositions, and methods described herein can be used to replace dead or dying cells in a subject (e.g., to replace neurons in a subject suffering from a neurodegenerative disease, or to replace cardiac muscle cells in a subject who has had a myocardial infarction). The cloaked cells, compositions, and methods described herein can also be used to provide immunosuppression in the region of a tissue or organ transplant, or to reduce the risk of rejection of the tissue or organ transplant. Cloaked cells that express a therapeutic agent, such as a protein or agonist antibody, compositions

including such cells, or methods of administering such cells, may be used to replace or supply wild type versions of proteins that are mutated or deficient in a subject (e.g., proteins that are produced but do not function correctly due to a genetic mutation, such as truncated proteins or proteins with altered charge, polarity, or binding properties; or proteins that are not produced or that are produced in insufficient quantities, e.g., deficient protein production that is associated with a disease or condition in Table 2). Cloaked cells that express a therapeutic agent, such as an inhibitory or neutralizing antibody, compositions including such cells, or methods of administering such cells, may be used to block or neutralize proteins that are overexpressed in a subject or proteins that are aberrantly produced (e.g., proteins that are produced in at a time or in a location that differs from production of that protein in healthy subjects, e.g., aberrant protein production that is associated with a disease or condition listed in Table 2).

Treatment may include administration of cloaked cells or a composition containing cloaked cells in various unit doses. Each unit dose will ordinarily contain a predetermined-quantity of the cloaked cells described herein. The quantity to be administered, and the particular route of administration and formulation, are within the skill of those in the clinical arts. A unit dose need not be administered as a single injection but may comprise continuous infusion over a set period of time. Dosing may be performed using a catheter, syringe, shunt, stent, microcatheter, pump, implantation with a device, or implantation with a scaffold. The number of cells administered may vary depending on whether the cells are administered to a tissue, organ, or body site associated with a disease or injury, or are administered subcutaneously to produce a cloaked subcutaneous tissue. For administration to a tissue, organ, or body site, the cloaked cells may be administered to the patient at a dose of, for example 1×10^4 cells to 1×10^{10} cells (e.g., 1×10^4, 2×10^4, 3×10^4, 4×10^4, 5×10^4, 6×10^4, 7×10^4, 8×10^4, 9×10^4, 1×10^5, 2×10^5, 3×10^5, 4×10^5, 5×10^5, 6×10^5, 7×10^5, 8×10^5, 9×10^5, 1×10^6, 2×10^6, 3×10^6, 4×10^6, 5×10^6, 6×10^6, 7×10^6, 8×10^6, 9×10^6, 1×10^7, 2×10^7, 3×10^7, 4×10^7, 5×10^7, 6×10^7, 7×10^7, 8×10^7, 9×10^7, 1×10^8, 2×10^8, 3×10^8, 4×10^8, 5×10^8, 6×10^8, 7×10^8, 8×10^8, 9×10^8, 1×10^9, 2×10^9, 3×10^9, 4×10^9, 5×10^9, 6×10^9, 7×10^9, 8×10^9, 9×10^9, 1×10^{10} cells). The number of cells administered will depend on the size of the recipient tissue, organ, or body site. For example, 2.5×10^4 to 1×10^5 cells (e.g., 2.5×10^4, 3×10^4, 4×10^4, 5×10^4, 6×10^4, 7×10^4, 8×10^4, 9×10^4, or 1×10^5 cells) can be administered (e.g., injected) to the subretinal space of the eye or to a specific brain region; 1×10^6 to 1×10^8 cells (e.g., 1×10^6, 2×10^6, 3×10^6, 4×10^6, 5×10^6, 6×10^6, 7×10^6, 8×10^6, 9×10^6, 1×10^7, 2×10^7, 3×10^7, 4×10^7, 5×10^7, 6×10^7, 7×10^7, 8×10^7, 9×10^7, or 1×10^8 cells) can be administered (e.g., injected) to a joint, with the quantity of cells depending on the size of the joint; and 5×10^8 to 5×10^9 cells (e.g., 5×10^8, 6×10^8, 7×10^8, 8×10^8, 9×10^8, 1×10^9, 2×10^9, 3×10^9, 4×10^9, or 5×10^9 cells) can be administered to the cardiac muscle. For creating cloaked subcutaneous tissue, 8×10^8 cells to 3×10^9 cells (e.g., 8×10^8, 9×10^8, 1×10^9, 2×10^9, 3×10^9 cells) can be administered (e.g., injected) subcutaneously. Cloaked cells can be administered in two or more doses (e.g., two, three, four, five, or more different doses, e.g., to joints of different sizes in a patient with RA) or at the same dose two or more times (e.g., two, three, four, five, six, or more times over the course of an hour, day, week, month, or year). In some embodiments, the cloaked

cells described herein are administered as a tissue (e.g., a tissue that has been grown and/or differentiated *in vitro* from cloaked cells). In some embodiments, the cloaked tissue is administered (e.g., implanted) with a gel, biocompatible matrix, or scaffold.

The compositions described herein are administered in an amount sufficient to prevent or reduce transplant rejection or to improve symptoms of a disease or condition listed in Table 2 (e.g., to reduce symptoms of osteoarthritis or RA (e.g., reduce inflammation, joint pain, stiffness, or immobility); reduce symptoms of retinal dystrophy or wet AMD (e.g., improve vision, slow or reduce vascularization of the eye); reduce symptoms of Parkinson's disease (e.g., reduce tremors, rigidity, bradykinesia, or improve posture or gait); reduce symptoms of diabetes (e.g., improve insulin levels, reduce the need for regular insulin injections); reduce symptoms of cardiac infarction (e.g., improve heart function, reduce infarct size); reduce symptoms of hemophilia (e.g., increase level of blood coagulation factors, such as Factor VIII, reduce excessive bleeding, reduce bruising, reduce nosebleeds, reduce joint pain or swelling); or reduce symptoms of metabolic disorders (e.g., increase appetite, growth, or weight gain, or reduce lethargy, weight loss, jaundice, seizures, abdominal pain, or vomiting)). Transplant rejection may be evaluated using standard methods known by those of skill in the art and may be reduced by 5% or more (e.g., 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or more) compared to rates of transplant rejection typically observed without treatment. In some embodiments, administration of the cloaked cells or compositions described herein results in an equivalent outcome in transplant rejection as that observed in subjects administered immunosuppressive agent(s). Symptoms of diseases and conditions described herein can be evaluated using standard methods known to those of skill in the art and may be reduced (e.g., the subject's condition may be improved) by 5% or more (e.g., 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or more) compared to symptoms prior to administration of the cloaked cells or compositions described herein. These effects may occur, for example, within 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 15 weeks, 20 weeks, 25 weeks, or more, following administration of the compositions described herein. The patient may be evaluated 1 month, 2 months, 3 months, 4 months, 5 months, 6 months or more following administration of the cloaked cell or composition depending on the dose and route of administration used for treatment. Depending on the outcome of the evaluation, the patient may receive additional treatments.

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Combination therapy

In some embodiments, the cloaked cells described herein are administered in combination with one or more additional therapeutic agents. The additional therapeutic agent(s) can be administered prior to administration of the cloaked cells, after administration of the cloaked cells, or concurrently with administration of the cloaked cells. The cloaked cells and additional therapeutic agents can also be administered simultaneously via co-formulation. The cloaked cells and therapeutic agent(s) can also be administered sequentially, such that the action of the cloaked cells and therapeutic agent(s) overlaps and

their combined effect is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with the cloaked cells or therapeutic agent delivered alone or in the absence of the other. The effect of the cloaked cells and therapeutic agent(s) can be partially additive, wholly additive, or greater than additive (e.g., synergistic). Sequential or substantially simultaneous administration of cloaked cells and therapeutic agent(s) can be effected by any appropriate route including, but not limited to oral routes, intravenous routes, intramuscular routes, local routes, or subcutaneous routes. The cloaked cells and therapeutic agent(s) can be administered by the same route or by different routes. For example, cloaked cells may be administered by subcutaneous injection while the additional therapeutic agent is administered orally. The cloaked cells may be administered immediately, up to 1 hour, up to 2 hours, up to 3 hours, up to 4 hours, up to 5 hours, up to 6 hours, up to 7 hours, up to, 8 hours, up to 9 hours, up to 10 hours, up to 11 hours, up to 12 hours, up to 13 hours, 14 hours, up to hours 16, up to 17 hours, up 18 hours, up to 19 hours up to 20 hours, up to 21 hours, up to 22 hours, up to 23 hours up to 24 hours or up to 1-7, 1-14, 1-21 or 1-30 days before or after the additional therapeutic agent.

In one example, the additional therapeutic agent is an immunosuppressive agent(s) commonly given for organ or tissue transplant. The immunosuppressive agent(s) may be an agent that is given immediately after transplantation to prevent acute rejection (e.g., methylprednisolone, atgam, thymoglobulin, OKT3, basiliximab, or daclizumab) or an immunosuppressive agent(s) used for maintenance (e.g., prednisone, a calcineurin inhibitor (e.g., cyclosporine, tacrolimus), Mycophenolate Mofetil, Azathioprine or Rapamycin). Other immunosuppressive agents given after organ transplantation include corticosteroids (e.g., methylprednisolone, dexamethasone, prednisolone), cytotoxic immunosuppressants (e.g., azathioprine, chlorambucil, cyclophosphamide, mercaptopurine, methotrexate), immunosuppressant antibodies (e.g., antithymocyte globulins, basiliximab, infliximab), sirolimus derivatives (e.g., everolimus, sirolimus), and anti-proliferative agents (e.g., mycophenolate mofetil, mycophenolate sodium, and azathioprine). In this case, the cloaked cell(s) is administered to or near the transplant site, or the tissue to be transplanted is modified to express one or more (e.g., one, two, three, four, five, six, seven, or eight) cloaking transgenes, and the immunosuppressive agent(s) is administered as an additional source of immunosuppression, if needed.

For use in treating inflammatory and autoimmune related diseases or conditions, the additional agent may be a disease-modifying anti-rheumatic drug (DMARD), a biologic response modifier (a type of DMARD), a corticosteroid, or a nonsteroidal anti-inflammatory medication (NSAID). In some embodiments, the additional agent is prednisone, prednisolone, methylprednisolone, methotrexate, hydroxychloroquine, sulfasalazine, leflunomide, cyclophosphamide, azathioprine, or a biologic such as tofacitinib, adalimumab, abatacept, anakinra, kineret, certolizumab, etanercept, golimumab, infliximab, rituximab or tocilizumab. In some embodiments, the additional agent is 6-mercaptopurine, 6-thioguanine, abatacept, adalimumab, alemtuzumab (Lemtrada), an aminosalicylate (5-aminoalicylic acid, sulfasalazine, mesalamine, balsalazide, olsalazine), an antibiotic, an anti-histamine, Anti-TNFα

(infliximab, adalimumab, certolizumab pegol, natalizumab), azathioprine, belimumab, beta interferon, a calcineurin inhibitor, certolizumab, a corticosteroids, cromolyn, cyclosporin A, cyclosporine, dimethyl fumarate (tecfidera), etanercept, fingolimod (Gilenya), fumaric acid esters, glatiramer acetate (Copaxone), golimumab, hydroxyurea, IFNγ, IL-11, infliximab, leflunomide, leukotriene receptor antagonist, long-acting beta2 agonist, mitoxantrone, mycophenolate mofetil, natalizumab (tysabri), ocrelizumab, pimecrolimus, a probiotic (VSL#3), a retinoid, rituximab, salicylic acid, short-acting beta2 agonist, sulfasalazine, tacrolimus, teriflunomide (Aubagio), theophylline, tocilizumab, ustekinumab (anti-IL-12/IL-23), or vedolizumab (Anti alpha3 beta7 integrin). In this case, the cloaked cell(s) could be administered to replace a tissue or organ damaged by the inflammatory or autoimmune-related disease or condition. In another example, the cloaked cell(s) administered could be modified to express a biologic therapeutic agent (e.g., an antibody) directed to treatment of a particular inflammatory or autoimmune-related disease or condition, and the additional agent could be a compound or general anti-inflammatory agent (e.g., an NSAID or corticosteroid).

For example, if the disease is rheumatoid arthritis, the additional agent may be one or more of: prednisone, prednisolone and methylprednisolone, methotrexate, hydroxychloroquine, sulfasalazine, leflunomide, cyclophosphamide and azathioprine, tofacitinib, adalimumab, abatacept, anakinra, kineret, certolizumab, etanercept, golimumab, infliximab, rituximab or tocilizumab. The cloaked cell(s) administered could be cartilage or bone producing cells of the joints. In some embodiments, the cloaked cell(s) can be modified to produce an anti-TNFα antibody and can be administered in combination with an anti-inflammatory agent (e.g., a corticosteroid).

In another example, for use in treating AMD or retinal dystrophy, the additional therapeutic agent may be an additional biologic agent (e.g., bevacuzimab, ranibizumab, or aflibercept), photodynamic therapy, or photocoagulation. The cloaked cell(s) administered could be retinal cells (e.g., RPE cells). In some embodiments, the cloaked cell(s) can be modified to produce a VEGF inhibitor and can be administered in combination with photodynamic therapy or photocoagulation.

For use in treating Parkinson's disease, the cloaked cells described herein can be administered with carbidopa-levodopa, a dopamine agonist (e.g., pramipexole, ropinirole, rotigotine, or apomorphine), an MAO-B inhibitor (e.g., selegiline or rasagiline), a catechol-O-methyltransferase inhibitor (e.g., entacapone or tolcapone), anticholinergic (e.g., benztropine or trihexyphenidyl), amantadine, or deep brain stimulation. The cloaked cell(s) administered could be dopaminergic neurons.

Additional agents for treating cardiac infarction include anticoagulants (e.g., rivaroxaban, dabigatran, apixaban, heparin, warfarin), anti-platelet agents (e.g., aspirin, clopidogrel, dipyramidole, prasugrel, ticagrelor), angiotensin-converting enzyme inhibitors (e.g., benazepril, captopril, enalapril, fosinopril, Lisinopril, moexipril, perindopril, quinapril, Ramipril, trandolapril), angiotensin II receptor blockers (e.g., candesartan, eprosartan, irbesartan, losartan, telmisartan, valsartan), angiotensin receptor neprilysin inhibitors (e.g., sacubitril/valsartan), beta blockers (e.g., acebutelol, atenolol, betaxolol, bisoprolol, metoprolol, nadolol, propranolol, sotalol), combined alpha and beta blockers (e.g., carvedilol,

labetalol hydrochloride), calcium channel blockers (e.g., amlodipine, diltiazem, felodipine, nifedipine, nimodipine, nisoldipine, verapamil), cholesterol lowering medication (e.g., statins (e.g., atorvastatin, rosuvastatin), nicotinic acids (e.g., lovastatin), cholesterol absorption inhibitors (e.g., ezetimibe/simvastatin)), digitalis preparation (e.g., lanoxin), diuretics (e.g., amiloride, bumentanide, chlorothiazide, chlorthalidone, furosemide, hydro-chlorothiazide, indipamide, spironolactone), vasodilators (e.g., isosorbide dinitrate, nesiritide, hydralazine, nitrates, minoxidil), dual anti-platelet therapy (e.g., aspirin and a P2Y12 inhibitor), or a cardiac procedure (e.g., an angioplasty, artificial heart valve surgery, atherectomy, bypass surgery, cardiomyoplasty, heart transplant, minimally invasive heart surgery, radiofrequency ablation, stent procedure, or transmyocardial revascularization). The cloaked cell(s) administered could be cardiac muscle cells.

For use in treating infectious disease, the additional agent may be an antiviral compound (e.g., vidarabine, acyclovir, gancyclovir, valgancyclovir, nucleoside-analog reverse transcriptase inhibitor (NRTI) (e.g., AZT (Zidovudine), ddI (Didanosine), ddC (Zalcitabine), d4T (Stavudine), or 3TC (Lamivudine)), non-nucleoside reverse transcriptase inhibitor (NNRTI) (e.g., (nevirapine or delavirdine), protease inhibitor (saquinavir, ritonavir, indinavir, or nelfinavir), ribavirin, or interferon); an antibacterial compound; an antifungal compound; an antiparasitic compound. The cloaked cell(s) administered could be immune cells (e.g., cell that could assist in fighting the infectious disease, e.g., a cloaked T cell or B cell).

For use in treating diabetes, the additional agent may be insulin, a sulfonylurea (e.g., chlorpropamide, glipizide, glyburide, glimepiride), a biguanide (e.g., metformin), a meglitinide (e.g., repaglinide, nateglinide), a thiazolidinedione (e.g., rosiglitazone, pioglitazone), a DPP-4 inhibitor (sitagliptin, saxagliptin, linagliptin, alogliptin), an SGLT2 inhibitor (e.g., canagliflozin, dapagliflozin), an alpha-glucosidase inhibitor (e.g., acarbose, miglitol), a bile acid sequestrant (e.g., colesevelam), aspirin, or a dietary regimen. The cloaked cell(s) administered could be pancreatic beta cells, which can optionally be modified to express a transgene encoding insulin.

For use in treating hemophilia, the additional therapeutic agent may be a clotting factor, desmopressin, a clot-preserving medication (e.g., an anti-fibrinolytic, e.g., aprotinin, aminocaproic acid, fibrigongen, or tranexamic acid), a fibrin sealant, or physical therapy. The cloaked cell(s) administered could be liver sinusoidal cells or endothelial cells, which can optionally be modified to express a transgene encoding Factor VIII.

For treatment of a metabolic deficiency or disorder, the additional therapeutic agent may be a coenzyme (e.g., biotin, hydroxycobalamine, riboflavin, pyridoxine, folate, thiamin, ubichinone, tetrahydrobiopterine), a bone marrow transplant, an organ transplant (e.g., a liver, kidney, or heart transplant), hemodialysis, hemofiltration, exchange transfusion, peritoneal dialysis, medium-chain triacylglycerols, miglustat, enzyme supplementation therapy, or dietary restriction (e.g., low protein or phenylalanine-restricted diet for subjects with phenylketonuria), The cloaked cell(s) can be cells that carry a wild-type copy of the gene that is mutated in a subject with a metabolic disorder or cells that endogenously produce the enzyme that is deficient in subject with a metabolic disorder (e.g., a liver cell,

kidney cell, heart cell, or any other cell that carries a wild-type copy of a gene that is mutated in a subject with a metabolic disorder or produces an enzyme that is deficient in a subject with a metabolic disorder).

For use in treating cancer, the additional agent may be a checkpoint inhibitor, a chemotherapeutic drug, a biologic drug, a non-drug therapy (e.g., radiation therapy, cryotherapy, hyperthermia, or surgical excision or tumor tissue), or an anti-cancer vaccine. The cloaked cell(s) could be an immune cell that could help fight the cancer (e.g., a macrophage, natural killer cell, dendritic cell, or T cell).

Checkpoint inhibitors can be broken down into at least 4 major categories: i) agents such as antibodies that block an inhibitory pathway directly on T cells or natural killer (NK) cells (e.g., PD-1 targeting antibodies such as nivolumab, pidilizumab/CT-011, and pembrolizumab, antibodies targeting TIM-3, and antibodies targeting LAG-3, 2B4, CD160, A2aR, BTLA, CGEN-15049, or KIR), ii) agents such as antibodies that activate stimulatory pathways directly on T cells or NK cells (e.g., antibodies targeting OX40, GITR, or 4-1BB), iii) agents such as antibodies that block a suppressive pathway on immune cells or rely on antibody-dependent cellular cytotoxicity to deplete suppressive populations of immune cells (e.g., CTLA-4 targeting antibodies such as ipilimumab or tremelimumab, antibodies targeting VISTA, and antibodies targeting PD-L2 (e.g., a PDL2/Ig fusion protein such as AMP 224), Gr1, or Ly6G), and iv) agents such as antibodies or small molecules that block a suppressive pathway directly on cancer cells or that rely on antibody-dependent cellular cytotoxicity to enhance cytotoxicity to cancer cells (e.g., rituximab, antibodies or small molecules targeting PD-L1 (e.g., MPDL3280A/RG7446; MEDI4736; MSB0010718C; BMS 936559), and antibodies or small molecule inhibitors targeting B7-H3 (e.g., MGA271), B7-H4, Gal-9, or MUC1). In one embodiment, the inhibitor of checkpoint is an inhibitor (e.g., an inhibitory antibody or small molecule inhibitor) of HVEM, CD160, CHK 1, CHK2, B-7 family ligands, or a combination thereof. Such agents described herein can be designed and produced, e.g., by conventional methods known in the art (e.g., Templeton, Gene and Cell Therapy, 2015; Green and Sambrook, Molecular Cloning, 2012). In one embodiment, the inhibitor of checkpoint is an inhibitory antibody (e.g., a monospecific antibody such as a monoclonal antibody). The antibody may be, e.g., humanized or fully human. In other embodiments, the inhibitor of checkpoint is a fusion protein, e.g., an Fc-receptor fusion protein. In some embodiments, the inhibitor of checkpoint is an agent, such as an antibody, that interacts with a checkpoint protein. In other embodiments, the inhibitor of checkpoint is an agent, such as an antibody, that interacts with the ligand of a checkpoint protein.

Chemotherapeutic agents include alkylating agents, antimetabolites, folic acid analogs, pyrimidine analogs, purine analogs and related inhibitors, vinca alkaloids, epipodopyyllotoxins, antibiotics, L-asparaginase, topoisomerase inhibitors, interferons, platinum coordination complexes, anthracenedione substituted urea, methyl hydrazine derivatives, adrenocortical suppressant, adrenocorticosteroides, progestins, estrogens, antiestrogen, androgens, antiandrogen, and gonadotropin-releasing hormone analog. Also included is 5-fluorouracil (5-FU), leucovorin (LV), irenotecan, oxaliplatin, capecitabine, paclitaxel and doxetaxel. Non-limiting examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan;

aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gammall and calicheamicin omegall; dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, caminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfomithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine;; razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g., , paclitaxel; chloranbucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum coordination complexes such as cisplatin, oxaliplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (e.g., CPT-

11); topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Two or more chemotherapeutic agents can be used in a cocktail to be administered in combination with the cloaked cells described herein. Suitable dosing regimens of combination chemotherapies are known in the art.

Anti-cancer biologics include cytokines (e.g., interferon or an interleukin (e.g., IL-2)) used in cancer treatment. In other embodiments the biologic is an anti-angiogenic agent, such as an anti-VEGF agent, e.g., bevacizumab. In some embodiments the biologic is an immunoglobulin-based biologic, e.g., a monoclonal antibody (e.g., a humanized antibody, a fully human antibody, an Fc fusion protein or a functional fragment thereof) that agonizes a target to stimulate an anti-cancer response, or antagonizes an antigen important for cancer. Such agents include Rituximab; Daclizumab; Basiliximab; Palivizumab; Infliximab; Trastuzumab; Gemtuzumab ozogamicin; Alemtuzumab; Ibritumomab tiuxetan; Adalimumab; Omalizumab; Tositumomab-I-131; Efalizumab; Cetuximab; Bevacizumab; Natalizumab; Tocilizumab; Panitumumab; Ranibizumab; Eculizumab; Certolizumab pegol; Golimumab; Canakinumab; Ustekinumab; Ofatumumab; Denosumab; Motavizumab; Raxibacumab; Belimumab; Ipilimumab; Brentuximab Vedotin; Pertuzumab; Ado-trastuzumab emtansine; and Obinutuzumab. Also included are antibody-drug conjugates.

Kits

The invention also features a kit containing the cloaked cells described herein (e.g., cloaked cells expressing a set of the cloaking transgenes described herein (e.g., 1, 2, 3, 4, 5, 6, 7, or 8 of PD-L1, H2-M3, Cd47, Cd200, FasL, Ccl21b, Mfge8, and Spi6), optionally further expressing one or more of the following transgenes: TGF-β, Cd73, Cd39, Lag3, Il1r2, Ackr2, Tnfrsf22, Tnfrs23, Tnfrsf10, Dad1,and IFNγR1 d39). In some embodiments, the cloaked cells are further modified to contain one or more systems for regulating cell division (e.g., an ALINK or EARC system), and/or a therapeutic agent (e.g., a transgene encoding a protein or antibody). The cloaked cells may be provided in a pharmaceutical composition. The kit may further include a syringe for administration of the cloaked cells or pharmaceutical composition and instructions for administering the cloaked cells or pharmaceutical composition for treating a disease or condition described herein.

EXAMPLES

The following examples are provided to further illustrate some embodiments of the present invention, but are not intended to limit the scope of the invention; it will be understood by their exemplary nature that other procedures, methodologies, or techniques known to those skilled in the art may alternatively be used.

Example 1: Materials and Methods

Construction of vectors that express target genes essential for allo-tolerance

Plasmids containing the cDNA sequences of genes involved in allo-tolerance were obtained as follows:

PD-L1: Mount Sinai Hospital, clone #V102001

FasL: Mount Sinai Hospital, #75719

Cd47: Mount Sinai Hospital, #V75535

Cd200: GE Dharmacon, ID# 17470

H2-M3: Mount Sinai Hospital, clone #8188

Ccl21: Mount Sinai Hospital, clone #V77120

Mfge8: Mount Sinai Hospital, clone #V72614

Spi6: Mount Sinai Hospital, clone #V8907

Expression vectors that contain these Genes of Interest (GOI), or the luciferase enzyme, were generated using the Gateway cloning system (Thermo Fisher). Cd47, Ccl21, Mfge8 and Spi6 cDNAs were acquired in a form that contained cDNA-flanking attB sites. For H2-M3, Cd200, FasL, and PD-L1, primers were designed to amplify the cDNA sequence, and add attB sites (FIG. 15(a)). Following PCR amplification, attB-containing cDNA was recombined into pDONR221 vectors (Thermo Fisher, #1256017) by the BP (recombination between *att*B and *att*P sites) reaction to create entry (pENTRY) clones (FIG. 15(b)). The BP reaction entails mixing the attB-flanked transgene cDNA with the pDONR221 plasmid in a 1mL tube, along with buffers and the BP enzyme provided by Invitrogen, where the BP enzyme recombines the GOI into the docking site of the pDONR221 plasmid. Insertion of the transgene into the pDONR221 plasmid was verified by DNA sequencing (TCAG Sequencing Facility at the Centre for Applied Genomics, Toronto). pENTRY clones that contained the GOI were then recombined into destination vectors via the gateway LR (recombination between *att*L and *att*R sites) reaction (FIG. 15(c)). The LR reaction entails mixing the GOI-containing pDONR221 plasmid and the destination vector in a 1mL tube, along with buffers and the LR enzyme provided by Invitrogen, where the LR enzyme recombines the GOI cassette from the pDONR221 plasmid into the docking site of the destination plasmid. Destination vectors, which were used for all transgene constructions, contain a CAGG promoter followed by a Gateway entry site, internal ribosomal entry site (IRES) and either a Puromycin resistance selectable marker or a green fluorescent protein (GFP) reporter. The entire cassette is flanked by transposable PB sites. Following LR recombination, the final destination vectors containing the GOI (FIG. 15(d)) were verified by restriction enzyme digestion.

ES Cell Culture, Transfection, Selection and Cloning

Mouse ES cells derived from the inbred C57BL/6N mouse strain (Gertsenstein 2010) were cultured in DMEM high glucose supplemented with 15% fetal bovine serum (FBS, tested for compatibility with ES cell cultures) and standard amounts of Sodium Pyruvate, non-essential amino acid (NEAA),

Glutamax, Penicillin/Streptomycin, Beta-mercapto-ethanol and leukemia inhibitory factor (LIF) (Behringer et al 2014). Cells were cultured on a feeder layer of MitomycinC-inactivated Murine Embryonic Fibroblasts (MEFs). Cultures were kept in a standard cell culture incubator at 37°C and 5% CO2.

Transfection was performed using JetPRIME reagent (Polyplus, catalog #14-07) per manufacturer protocol, and was done in three steps: 1) transfection with PD-L1-IRES-GFP destination vector only, 2) transfection with all the other transgenes carrying a Puromycin selectable marker, and 3) transfection with an eLuciferase-IRES-GFP transgene.

Step 1: Following transfection with PD-L1-IRES-GFP, cells were plated at low density so that after multiple rounds of proliferation 5-6 days later, individual cell clones - existing as cell aggregates (colonies)- were selected based on the intensity of GFP expression and then expanded as a clonal cell culture. The clone with the highest and most consistent GFP expression was chosen for the next step.

Step 2: 24 hours after transfection with transgenes containing a Puromycin selection marker, Puromycin was added to the culture media. On the third day, cells were plated at clonal density and Puromycin selection was continued until individual colonies were picked and expanded as clones. A large number of these clones were screened in vivo and the one capable of forming a teratoma in an allogeneic setting was designated "NT2".

Step 3: NT2 was transfected with PB-CAG-eLuciferase-IRES-GFP as described above and plated at clonal density. GFP+ clones were picked and expanded. 10 clones with high levels of GFP expression were chosen for further studies.

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Evaluation of Transgene Expression Levels

RNA was isolated from cultures grown on 30mm culture plates, as well as from tumours grown in vivo. Cells were dissociated with Trypsin, centrifuged, and the supernatant removed. The cell pellet was immediately frozen on dry ice and stored at -80°C. Tumour tissues were dissected, immediately frozen on dry ice and stored at -80°C. RNA was isolated per standard protocols using Sigma GeneElute Total RNA Miniprep kit #RTN350. cDNA was obtained by reverse transcriptase reaction using the Qiagen Quantifast Reverse Transcriptase kit #205313. Quantitative PCR was performed using Sensifast mastermix from Bioline, #Bio-98020, gene specific primers and RNA at a 1:50 dilution. Samples were plated in 384 well plates using the Eppendorf epMotion 5070 robot and the quantitative PCR was performed on BioRad CFX384 Real-Time System C1000 Thermal cycler according to standard protocols. qPCR data was captured by BioRad CFX Manager 3.1 software and expression levels calculated with Microsoft Excel.

Teratoma Assay

Matrigel Matrix High Concentration (Corning cat# 354248) was diluted 1:1 with cold DMEM media on ice. $5x10^7$ cells were suspended into 500uL of DMEM and equal volume of Matrigel. 100ul 5x10 cells of the suspension was injected subcutaneously into each dorsal flank of B6N (isogenic) or FVB/N (allogeneic) mice. The resulting teratomas formed 2-4 weeks after injection. Teratoma size was measured

using calipers, and the volume was calculated with the formula V= (LxWxH)/2. The tumours were allowed to grow to approximately 500mm^2, a size that is well-tolerated and also well-suited for downstream experiments. All of the transgenes were delivered into cells that contain "Fail-safe system" (as described, for example, in WO 2016/141480, the entire contents of which are incorporated herein by reference. This genetic system allows for the complete inhibition of cell division with the administration of Ganciclovir (GCV). Once teratomas from the previously described experiments reached 500mm^2, mice were injected into the peritoneal cavity with 50mg/kg GCV every 2-3 days for 2-3 weeks. This treatment regimen resulted in an initial brief shrinkage of the tumours, followed by stabilization of tumour size at 400-500 mm^2 after 2-3 weeks of treatment. At the endpoint of the experiment, mice were sacrificed and tumours were dissected. A small portion of tissue was snap-frozen for RNA extraction while the rest was fixed in 4% paraformaldehyde.

Bioluminescence Imaging

Mice that developed teratomas derived from cells transfected with the eLuciferase transgene were injected with 30mg/mL VivoGlo Luciferin at 100uL / 25g body mass (Promega #P104C) 10min before imaging. Animals were anaesthetized with Isoflurane and placed in an IVIS Lumina II imager (Caliper Life Sciences) driven by Living Image software. Exposure times were set between 5 seconds and 5 minutes depending on signal intensity.

Histology

Fixed tumours were embedded in paraffin, sectioned and stained with Hematoxylin/Eosin for histological analysis at the CMHD Pathology Core. Histology images were processed with NDPview2 software.

Example 2: Generation of Cloaked Cells

Transgenes encoding the genes in Table 1 were cloned into expression vectors and sequence verified both by polymerase chain reaction (PCR), restriction enzyme digestion and sequencing, all using standard methods know in the art.

A set of constructs containing transgenes Cd47, Cd200, FasL and H2-M3 (Set 1) were transfected into mouse embryonic stem cells derived from the inventors' C57BL/6 mouse ES line (C2). The presence of the transgenes was verified by PCR and expression of the expressed proteins was documented by immunohistochemistry (FIGS. 1A-D). A second set of constructs containing transgenes Ccl21, Mfge8, TGF-β and Spi6 (Set 2) were transfected into ES cells derived from FVB/N (ES line C2).

Similar methods were used to generate cloaked B16F10 melanoma cells, except that the media used DMEM containing 10% fetal bovine serum (FBS).

Example 3: Screening Process for Inhibition of T-cell Activation

A modified in vitro Mixed Lymphocyte Reaction (MLR) assay was used to screen for the transgene combination resulting in the most efficient inhibition of T-cell activation. Cell lines transfected with Set 1 and Set 2 cloaking transgenes from Example 1 were used. Donor OT-I splenocytes were labeled with carboxyfluorescein succinimidyl ester CFSE and 60,000 cells were added to each well of the 96-well plate. ES or melanoma cells were mixed 10:1 with ova expressing cells. 10,000 of these were added to each well of splenocytes. IL-2 was added as a general activator and T-cell proliferation was measured by flow cytometry 3 days later (FIGS. 2A-2E). Cells were initially gated to include CD8+ cells only and all conditions were set up in 4 replicates.

The negative control (splenocytes only) resulted in a baseline 6.12% proliferation rate (FIG. 2A). Wildtype B16 melanoma (+10% ova expressing) cells resulted in distinct acceleration of proliferation to 17.1% (FIG. 2B), while cloaked cells reduced this proliferation to 9.51% (FIG. 2C). Similar results were obtained for wildtype (FIG. 2D) versus cloaked ES cells (FIG. 2E).

Example 4: Studies with WT and Cloaked Cancer Cells in Iso- and Allografted B16F10 Melanoma Cells

Since some of the candidate cloaking transgenes are intended to inhibit or modulate the initiation phase of the immune recognition cascade, the effect of these transgenes could be evaluated by the MLR alone as these events act on the maturation and physical migration of host APCs to local lymph nodes where they subsequently activate naïve T and B cells.

This called for an alternative assay that can screen a large number of transgene combinations in an in vivo allogeneic setting. Intraperitoneal and intravenous injection ES cells harboring a variety of transgene combinations was tried as an option. However, teratoma formation is dependent on the aggregation of a minimum number of ES cells ($1x10^5 – 5x10^6$ depending on site of injection), rendering this option not compatible with such a screen. However, the murine melanoma cancer cell line B16F10 derived from C57BL/6 mice is not limited in such a way. Intravenous injection of less than $5x10^3$ results in the efficient induction of a multitude of small cancer nodules in the lung. By limiting number of cells injected, one can anticipate that the cancer cells are trapped in the lung alveoli will form nodules derived from single or just a very small number of cells. By isolating and genotyping these nodules, the transgene can be identified.

Injection B16F10 melanoma cells into the blood-stream of C57BL/6 mice (isogenic graft control) resulted in the formation of cancer nodules in the lung (FIG. 3A, left panel). However, small melanoma nodules formed also in the lungs of the negative controls - wild type B16F10 melanoma grafted into allogeneic control FVB mice when observed at day 14 post injection. However, when the melanoma was allowed to grow for 24 days, the nodules regressed almost completely (FIG. 3A, right panel).

The above experiment was repeated, by injecting a mixture of cancer cells that expressed random combinations of the candidate cloaking genes, generated using the PiggyBac transposon system.

Lung nodules developed in the allogeneic settings contained the successful combination(s) needed to protect the allograft from recognition and rejection (FIG. 3B, right panel). The same immune cloaked cells also gave rise to an accelerated development in the isogenic host (FIG. 3B, left panel).

Example 5: Non-Cloaked Embryonic Stem Cells Do Not Form Teratoma in Allogenic Settings

As shown in Table 4, it was verified that wild-type ESCs derived from C57BL/6 mice are not capable of forming teratomas in FVB/N mice. Likewise, we have also shown that wild- type ESCs derived from the FVB/N background are not capable of forming teratomas in C57BL/6 hosts. ES cell colonies were dissociated with Trypsin, washed once with DMEM without additives and resuspended in Matrigel HC at a concentration of about 50 million cells per milliliter. Recipient mice were anaesthetized and one hundred microliter injected subcutaneously in each flank area. Developing teratomas were followed for 12 weeks and verified by palpation and measurement of volume with caliper.

Table 4: Teratomas formed in FVB/N mice and C57BL/6 hosts injected with wild- type ESCs derived from C57BL/6 mice or wild- type ESCs derived from the FVB/N background

Donor ESCs	Recipient mouse	# injection sites	# teratomas
C57BL/6	C57BL/6	18	14
C57BL/6	FVB	22	0
FVB	FVB	8	8
FVB	C57BL/6	8	0

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Example 6: Cloaked ES cells can proliferate in isogenic hosts and allogenic hosts

To verify the cloaking ability of the candidate transgenes, ESCs were transfected with the same transgenes while also adding a Luciferase transgene that can be detected by imaging. Briefly, ES cells were prepared as described above. The presence of viable cells were repeatedly measured by imaging. The images in Fig 4 were taken on day 17 post injection.

In FIG. 4, the top panel shows the proliferation of immune cloaked cells in isogenic hosts, while the lower panel shows the proliferation of immune cloaked cells in allogeneic hosts.

In another experiment, cloaked ES cells from C57BL/6 mice that had high expression of the 8 immunomodulatory transgenes (clone NT2) were injected subcutaneously into different allogenic mouse strains (C3H, FVB/N, and CD1) with mismatched MHC alleles. Red arrows indicate the teratoma that formed (FIGS. 5A-5C).

Example 7: Mice with Cloaked Tissues Are Not Immune Compromised

Non-immune cloaked (wild type) ESCs were transplanted into mice carrying an existing immune cloaked tissue and the mouse was evaluated to determine if it could effectively reject a non-immune cloaked graft (FIG. 6). The same mice were imaged several times over a period of 15 days. As shown in the left panel of FIG. 6, in isogenic mouse controls, the graft was not rejected over time. With allogenic FVB mice, the left mouse in the right panel of FIG. 6 had a pre-existing immune cloaked graft (arrows). The middle mouse in the right panel of FIG. 6 had previously been grafted with C57BL/6 allogeneic ESCs but rejected the graft (while not being bound to a theory, the rejection may have been due to pre-formed antibodies against C57BL/6 cells). The mouse on the right in the right panel of FIG. 6 had never been grafted before. All three mice successfully rejected the non-immune cloaked graft. The mouse on the right rejected the graft slower, which may have been because it did not have any preformed antibodies against C57BL/6 cells.

A similar experiment was conducted where wild type embryonic stem cells were detected up to 9 days post injection into FVB/N mice with cloaked teratomas (FIG. 7). However, at day 12, no evidence of cells remaining could be detected. Control animals were C57BL/6 mice also carrying the cloaked tumors. The signal in these mice increased over the time-course of the experiment.

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Example 8: Cloaked and Fail-Safe Embryonic Stem Cell Line

When a Fail-Safe C57BL/6 ES cell line (as described, for example, in WO/2016/141480) was co-transfected with 5 candidate cloaking transgenes (PD-L1, FasL, Cd47, Cd200 and H2-M3), none of these transgene lines resulted in teratomas in allograft settings. When the set of co-transfected genes was expanded by three additional candidate cloaking genes: Spi6, Ccl21b and Mfge8, 38 clonal lines were generated. One of these lines, NT2, created teratomas in an allogeneic recipient (FVB). The expression levels of the cloaking genes in the 38 clonal lines, including the NT2 line (see arrows in FIGS. 8A-H), were measured using quantitative PCR (FIGS. 8A-H). Of the 38 clones, NT2 was the highest overexpression of Ccl21b (16,000x), FasL (25,000x), Cd200 (1700x), Cd47 (16x), Mfge8 (34x), Spi6 (600x) and H2-M3 (750x) compared to WT ES cells. PD-L1, although not the highest level expresser among the clones, the 350x expression over ES cells was also a significant increase. The expression of these genes was also checked in the Project Grandiose dataset (www.stemformatics.org/project_grandiose) and found that Ccl21b, FasL, Cd200, PD-L1 and Spi6 expression is under the detection threshold, therefore, their relative-to-ES cells expression is very high. Based on this data these eight, highly activated genes could have a primary role in inducing immune tolerance of an allograft.

NT2 cells were injected into both C57BL/6 to create teratomas in an FVB allogenic setting (FIGS. 11A-11B) and an FVB iso C57Bl/6 isogenic setting. Allogenic teratomas (n=6) were steadily growing from day 12 to day 38. At the size of 500mm^2, ganciclovir (GCV) treatment was started to remove the proliferative component of the tumors (FIGS. 12A-12B, upper panel (FIG. 12A) isogenic teratomas;

bottom panel (FIG. 12B) allogenic teratomas)). Twenty days of treatment stopped the allograft growth. This experiment shows that: 1) Fail-safe and cloaked (NT2) cell-derived teratomas respond similarly to GCV treatment; they enter to dormancy after brief GCV exposure; 2) After GCV the teratomas remain stable. There is no sign of rejection of the dormant tissue; and 3). The dynamics of teratoma growth in FVB animals is different than in C57BL/6.

Cloaking transgenes expressed at a high level survive to form teratomas in an allogenic mouse. In our system, the cloaking transgenes are expressed under a very strong synthetic promoter, CAG (depicted in the schematic in FIG. 19). The CAG promoter is a combination of the cytomegalovirus early enhancer element, the splicer acceptor of the rabbit beta-globin gene, and also the promoter, first exon and first intron of the chicken beta-actin gene. We have performed extensive qPCR analysis on the level of transgene transcripts in many different ES cell clones, each of which has a different expression level of the transgenes. Only those ES clones that have the highest expression of cloaking transgenes survive in allogenic hosts.

As shown in FIG. 9, transcript expression level of the immunomodulatory genes relevant to the cloaking technology varied between ES cell clones. Concentric circles are depicted on a log10 scale. The thick black line is 1x, the next outer ring is 10x, and then 100x. The innermost ring is 0.1x. All values are normalized to positive controls, which were activated leukocytes isolated from murine lymph organs that naturally express the immunomodulatory transgenes. The upper left panel shows wild-type ES cells with no transgenic modifications for reference – they express little or none of the relevant immunomodulatory transgenes. By contrast, clone NT2 and clone 15 (indicated by red squares), both with high expression of the genes, survived in allogenic hosts. All other clones shown in FIG. 9 did not survive in allogenic hosts.

The high expression of the cloaking transgenes is also depicted in FIG. 10. As shown in FIG. 10, all 8 cloaking transgenes in the NT2 cell line and NT2-derived teratoma had an expression level that was among the top 5% of all genes in the ES cell genome, with 5 of the cloaking transgenes having an expression level in the top 1% of all genes in the ES cell genome. The expression of these genes is much lower in WT ES cells, as only one of the genes has an expression level among the top 5% of all genes in the genome.

Example 9: Cloaked ESCs Contribute to All Three Germ Layers in Allogenic Teratomas

It was next asked if immune cloaking would allow the full pluripotent developmental potential of ESCs to unfold in teratomas. Teratomas resulting from the injection of cloaked and uncloaked ESCs derived from C57BL/6 mice into isogenic and allogeneic hosts were analyzed by histopathology (hematoxylin and eosin staining). FIGS. 13A-13B (isogenic host neuronal, bone and columnar epithelium in upper panels (FIG. 13A); and allogenic host neuronal, bone, columnar epithelium and blood vessels in lower panels (FIG. 13B)) shows representative images obtained from both backgrounds, proving that the expression of the cloaking transgenes do not interfere with the normal developmental potential of these

ES cells and the tumors are well vascularized. Both isogenic and allogenic tissues did not show any immune cell infiltration.

In another experiment, we tested if the cloaked ES cell were truly pluripotent by testing whether they could form cells from all three germ layers – endoderm, ectoderm, and mesoderm (FIGS. 14A-14D). This was assayed by injecting between 10^6 and 10^7 cloaked ES cells subcutaneously into a mouse and allowing them to proliferate and differentiate into a tissue mass named a teratoma. The teratoma was then removed 3-4 weeks after ES cell injection, and tissue sections cut and stained with H&E. These sections were analyzed under the microscope for cell morphology to determine if all three germ layers were present.

We asked whether the 8 cloaking transgenes inserted into ES cells and expressed at high levels would disrupt their ability to form all three germ layers. They did not. FIGS. 14A-14C show the three germ layers (ec = ectoderm, shown in FIG. 14A; en = endoderm, shown in FIG. 14C; me = mesoderm, shown in FIG. 14B). FIG. 14D shows a blood vessel, which verifies that these tissues are well-vascularized.

Example 10: ES cells that express cloaking transgenes produce the proteins encoded by the transgenes

We confirmed the presence of the proteins encoded by the cloaking transgenes in NT2 ES cells (one of the clones with the highest expression) directly using fluorescent antibody-based microscopy (FIGS. 16A-16H). These data confirm that the proteins encoded by the transgenes are expressed in ES cells at easily detectable levels, which is expected based on the high levels of mRNA expression.

Example 11: ES cells that express high levels of cloaking transgenes have typical morphology and express common ES cell markers

We analyzed cloaked ES cells to determine whether they expressed markers of ES cells and retained a normal ES cell morphology. Cloaked ES cells have the typical morphology observed with healthy and pluripotent ES cells (FIG. 17A) and also stain positively for alkaline phosphatase (FIG. 17B), which is characteristic of healthy and pluripotent ES cells. Furthermore, our cloaked ES cells stained positively for the transcription factor Oct4 (FIG. 18A) as well as SSEA (FIG. 18B) using fluorescent antibodies, both common markers of normal pluripotent ES cells. These data show that ES cells that express high levels of the 8 immunomodulatory cloaking transgenes appear as normal ES cells with respect to their morphology and expression of common ES cell markers. The insets show that staining for Oct4 and SSEA1 (lower left inset) colocalizes with ES cells (visualized using DAPI in upper right insets).

Example 12: IFNγR1 d39 prevents upregulation of MHCs in ES cells

Activated T-cells secrete IFNγ, which binds to the IFNγR1/R2 complex expressed on many cell types, including tissues and cells derived from ES cells. IFNγ binding to the IFNγ receptor induces upregulation of HLA (MHC in mice) and HLA-related molecules on the cell surface, which increases the allogenicity of the allograft and the likelihood of immune rejection. Differences in HLA proteins (also called major antigens) between the donor and recipient are the primary cause of rejection in all allogenic transplants.

To evaluate whether disrupting IFNγ signaling prevents or reduces HLA upregulation, we transfected C57BL/6 ES cells with piggyback-integratable vectors containing a wild-type IFNγR1 or dominant negative IFNγR1 (IFNγR1 d39, which lacks 39 amino acids in the cytoplasmic tail) transgene. These transgenes were expressed under the control of a constitutive CAG promoter upstream of the transgene contained on the same piggyback-integrated cassette.

Wild type and transfected ES cells were then grown in culture and exposed to 100 ng/mL of IFNγ ligand for 24 hours. In wild-type ES and IFNγR1-transfected cells (left and middle panels of FIG. 20, respectively), IFNγ exposure resulted in increased expression of the H-2k^b and H-2D^b major histocompatibility surface molecules (MHC class I), but not in IFNγR1 d39 cells (right panel of FIG. 20). Exposure to PBS alone had no effect. MHC class I levels were detected by fluorescent antibody staining, and the expression level quantified by measuring the mean fluorescent intensity (MFI) by flow cytometry. These data show that overexpression of IFNγR1 d39 completely inhibits IFNγ-mediated upregulation of MHCs in ES cells, indicating that expression of IFNγR1 d39 in ES cells can be used to prevent activation of the immune system and reduce the likelihood of immune rejection. Therefore, IFNγR1 d39 is a useful immunosuppressive transgene that can be expressed by the cloaked cells described herein to reduce immune activation and transplant rejection.

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Example 13: Administration of cloaked cells expressing a VEGF inhibitor to a subject with wet AMD

According to the methods disclosed herein, a physician of skill in the art can treat a patient, such as a human patient, with wet AMD to reduce vascularization of the eye or prevent or reduce disease progression. To this end, a physician of skill in the art can administer to the human patient cloaked cells (e.g., cloaked RPE cells or cloaked stem cells that have been differentiated into RPE cells) that express one or more (e.g., one, two, three, four, five, six, seven, or all eight) of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) under the control of a constitutive promoter (e.g., CMV or CAG) and a VEGF inhibitor (e.g., VEGF-Trap, e.g., aflibercept) under the control of a constitutive promoter (e.g., CMV or CAG). The cloaked cells may be administered to the patient, for example, by local administration to the eye (e.g., injection into the subretinal space), to treat wet AMD. Twenty five thousand to one hundred thousand cloaked cells (e.g., 25,000, 50,000, 75,000, or 100,000 cloaked cells) can be administered to each affected eye.

Following administration of the cloaked cells to a patient, a practitioner of skill in the art can monitor the expression of the VEGF inhibitor, and the patient's improvement in response to the therapy, by a variety of methods. For example, a physician can monitor the patient's vision and the vascularization of the eye using standard approaches. A finding that the patient's vision improves or does not worsen, or that vascularization of the eye decreases or does not worsen compared to measurements taken prior to administration of the cloaked cells indicates that the patient is responding favorably to the treatment. Subsequent doses can be determined and administered as needed.

Example 14: Administration of cloaked dopaminergic neurons to a subject with Parkinson's disease (PD)

According to the methods disclosed herein, a physician of skill in the art can treat a patient, such as a human patient, with PD to reduce motor symptoms of PD (e.g., bradykinesia, tremors, or rigidity) or prevent or reduce disease progression. To this end, a physician of skill in the art can administer to the human patient cloaked cells (e.g., dopaminergic neurons that have been modified to express cloaking transgenes or cloaked stem cells that have been differentiated into dopaminergic neurons) that express one or more (e.g., one, two, three, four, five, six, seven, or all eight) of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) under the control of a constitutive promoter (e.g., CMV or CAG). The cloaked cells may be administered to the patient, for example, by local administration to the central nervous system (e.g., stereotactic injection into the substantia nigra), to treat PD. Twenty five thousand to one hundred thousand cloaked cells (e.g., 25,000, 50,000, 75,000, or 100,000 cloaked cells) can be administered. The patient can optionally be administered an additional therapy for PD, such as a dopamine agonist.

Following administration of the cloaked cells to a patient, a practitioner of skill in the art can monitor the patient's improvement in response to the therapy by a variety of methods. For example, a physician can monitor the patient's movement using standard neurological tests. A finding that the patient's motor symptoms improve or do not worsen compared to measurements taken prior to administration of the cloaked cells indicates that the patient is responding favorably to the treatment. Subsequent doses can be determined and administered as needed.

Example 15: Administration of cloaked cardiac muscle cells to a subject that has suffered a myocardial infarction

According to the methods disclosed herein, a physician of skill in the art can treat a patient, such as a human patient, who has recently suffered a myocardial infarction to improve cardiac function (e.g., to replace or dead or damaged cardiac muscle cells). To this end, a physician of skill in the art can administer to the human patient cloaked cells (e.g., cloaked cardiac muscle cells or cloaked stem cells that have been differentiated into cardiac muscle cells) that express one or more (e.g., one, two, three, four, five, six, seven, or all eight) of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21

(Ccl21b), Mfge8, and Serpin B9 (Spi6) under the control of a constitutive promoter (e.g., CMV or CAG). The cloaked cells may be administered to the patient, for example, by local administration to the heart (e.g., injection into the cardiac muscle), to promote recovery after the myocardial infarction. The cells can be injected into the cardiac muscle as a monotherapy, or the cells can be delivered during the performance of a bypass surgery or another open heart surgical procedure. One million to five billion cloaked cardiac muscle cells (e.g., 1×10^6, 2×10^6, 3×10^6, 4×10^6, 5×10^6, 6×10^6, 7×10^6, 8×10^6, 9×10^6, 1×10^7, 2×10^7, 3×10^7, 4×10^7, 5×10^7, 6×10^7, 7×10^7, 8×10^7, 9×10^7, or 1×10^8, 2×10^8, 3×10^8, 4×10^8, 5×10^8, 6×10^8, 7×10^8, 8×10^8, 9×10^8, 1×10^9, 2×10^9, 3×10^9, 4×10^9, or 5×10^9 cloaked cells) can be administered.

Following administration of the cloaked cells to a patient, a practitioner of skill in the art can monitor the patient's improvement in response to the therapy by a variety of methods. For example, a physician can monitor the patient's cardiac function using standard approaches (e.g., EKG, echocardiogram, angiogram, stress test, or nuclear imaging). A finding that the patient's cardiac function improves or stabilizes compared to measurements taken prior to administration of the cloaked cells indicates that the patient is responding favorably to the treatment. Subsequent doses can be determined and administered as needed.

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Example 16: Administration of cloaked cells expressing a TNFα inhibitor to a subject with RA

According to the methods disclosed herein, a physician of skill in the art can treat a patient, such as a human patient, with rheumatoid arthritis to reduce join stiffness, swelling, or pain. To this end, a physician of skill in the art can administer to the human patient cloaked cells (e.g., cloaked articular fibroblasts or cloaked stem cells that have been differentiated into articular fibroblasts) that express one or more (e.g., one, two, three, four, five, six, seven, or all eight) of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) under the control of a constitutive promoter (e.g., CMV or CAG) and a TNFα inhibitor (e.g., a TNFα inhibitory antibody, such as adalimumab) under the control of an inducible promoter (e.g., a tetracycline response element). The cloaked cells may be administered to the patient, for example, by local administration to a joint (e.g., injection into an arthritic joint, such as joint in the hand), to treat RA. One million to one hundred million cloaked articular fibroblasts expressing an anti-inflammatory biologic (e.g., 1×10^6, 2×10^6, 3×10^6, 4×10^6, 5×10^6, 6×10^6, 7×10^6, 8×10^6, 9×10^6, 1×10^7, 2×10^7, 3×10^7, 4×10^7, 5×10^7, 6×10^7, 7×10^7, 8×10^7, 9×10^7, or 1×10^8 cloaked articular fibroblasts) can be administered to each affected joint. When the patient experiences a flare up of RA symptoms, the patient can be treated with tetracycline or doxycycline to drive expression of the TNFα inhibitor. Tetracycline or doxycycline can be withdrawn when the patient's flare up has resolved.

Following administration of the cloaked cells and tetracycline or doxycycline to a patient, a practitioner of skill in the art can monitor the expression of the TNFα inhibitor, and the patient's improvement in response to the therapy, by a variety of methods. For example, a physician can monitor

the patient's joint pain, swelling, and stiffness using standard approaches. A finding that the patient's joint pain, swelling, or stiffness is reduced compared to measurements taken prior to administration of the cloaked cells indicates that the patient is responding favorably to the treatment. Subsequent doses can be determined and administered as needed.

Example 17: Administration of cloaked cells expressing insulin to a subject with Type 1 diabetes

According to the methods disclosed herein, a physician of skill in the art can treat a patient, such as a human patient, with Type 1 diabetes to increase insulin levels. To this end, a physician of skill in the art can administer to the human patient cloaked cells (e.g., cloaked stem cells, cloaked pancreatic beta cells, or cloaked stem cells that have been differentiated into pancreatic beta cells) that express one or more (e.g., one, two, three, four, five, six, seven, or all eight) of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) under the control of a constitutive promoter (e.g., CMV or CAG) and insulin under the control of a constitutive promoter (e.g., CMV or CAG). The cloaked cells may be administered to the patient, for example, by subcutaneous injection (e.g., to create a cloaked subcutaneous tissue), to treat Type 1 diabetes. One million to three billion cloaked cells expressing insulin (e.g., 1×10^6, 2×10^6, 3×10^6, 4×10^6, 5×10^6, 6×10^6, 7×10^6, 8×10^6, 9×10^6, 1×10^7, 2×10^7, 3×10^7, 4×10^7, 5×10^7, 6×10^7, 7×10^7, 8×10^7, 9×10^7, or 1×10^8, 2×10^8, 3×10^8, 4×10^8, 5×10^8, 6×10^8, 7×10^8, 8×10^8, 9×10^8, 1×10^9, 2×10^9, or 3×10^9 cloaked cells) can be administered subcutaneously.

Following administration of the cloaked cells to a patient, a practitioner of skill in the art can monitor the expression of the patient's improvement in response to the therapy by a variety of methods. For example, a physician can monitor insulin levels or symptoms of Type 1 diabetes (e.g., unintended weight loss, fatigue, or blurred vision) using standard approaches. A finding that the patient's insulin levels are increased or the symptoms of Type 1 diabetes are reduced compared to measurements taken prior to administration of the cloaked cells indicates that the patient is responding favorably to the treatment. Subsequent doses can be determined and administered as needed.

Example 18: Administration of cloaked cells expressing Factor VIII to a subject with hemophilia

According to the methods disclosed herein, a physician of skill in the art can treat a patient, such as a human patient, with hemophilia to increase the levels of a blood clotting factor or reduce excessive bleeding or bruising. To this end, a physician of skill in the art can administer to the human patient cloaked cells (e.g., cloaked stem cells, cloaked endothelial cells, or cloaked stem cells that have been differentiated into endothelial cells) that express one or more (e.g., one, two, three, four, five, six, seven, or all eight) of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) under the control of a constitutive promoter (e.g., CMV or CAG) and Factor VIII under the control of a constitutive promoter (e.g., CMV or CAG). The cloaked cells may be administered to the patient, for example, by subcutaneous injection (e.g., to create a cloaked subcutaneous tissue), to treat

hemophilia. One million to three billion cloaked cells expressing Factor VIII (e.g., 1 x 10^6, 2 x 10^6, 3 x 10^6, 4 x 10^6, 5 x 10^6, 6 x 10^6, 7 x 10^6, 8 x 10^6, 9 x 10^6, 1 x 10^7, 2 x 10^7, 3 x 10^7, 4 x 10^7, 5 x 10^7, 6 x 10^7, 7 x 10^7, 8 x 10^7, 9 x 10^7, or 1 x 10^8, 2 x 10^8, 3 x 10^8, 4 x 10^8, 5 x 10^8, 6 x 10^8, 7 x 10^8, 8 x 10^8, 9 x 10^8, 1 x 10^9, 2 x 10^9, or 3 x 10^9 cloaked cells) can be administered subcutaneously.

Following administration of the cloaked cells to a patient, a practitioner of skill in the art can monitor the expression of the patient's improvement in response to the therapy by a variety of methods. For example, a physician can monitor Factor VIII levels or symptoms of hemophilia (e.g., excessive bleeding or frequent bruising) using standard approaches. A finding that the patient's Factor VIII levels are increased or the symptoms of hemophilia are reduced compared to measurements taken prior to administration of the cloaked cells indicates that the patient is responding favorably to the treatment. Subsequent doses can be determined and administered as needed.

Example 19: Administration of cloaked cells expressing glucocerebrosidase to a subject with Gaucher's disease

According to the methods disclosed herein, a physician of skill in the art can treat a patient, such as a human patient, with Gaucher's disease to reduce the accumulation of glucocerebroside or to reduce symptoms of Gaucher's disease (e.g., fatigue, anemia, low blood platelet count, enlarged liver or spleen). To this end, a physician of skill in the art can administer to the human patient cloaked cells (e.g., cloaked stem cells) that express one or more (e.g., one, two, three, four, five, six, seven, or all eight) of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) under the control of a constitutive promoter (e.g., CMV or CAG) and glucocerebrosidase under the control of a constitutive promoter (e.g., CMV or CAG). The cloaked cells may be administered to the patient, for example, by subcutaneous injection (e.g., to create a cloaked subcutaneous tissue), to treat Gaucher's disease. One million to three billion cloaked cells expressing glucocerebrosidase (e.g., 1 x 10^6, 2 x 10^6, 3 x 10^6, 4 x 10^6, 5 x 10^6, 6 x 10^6, 7 x 10^6, 8 x 10^6, 9 x 10^6, 1 x 10^7, 2 x 10^7, 3 x 10^7, 4 x 10^7, 5 x 10^7, 6 x 10^7, 7 x 10^7, 8 x 10^7, 9 x 10^7, or 1 x 10^8, 2 x 10^8, 3 x 10^8, 4 x 10^8, 5 x 10^8, 6 x 10^8, 7 x 10^8, 8 x 10^8, 9 x 10^8, 1 x 10^9, 2 x 10^9, or 3 x 10^9 cloaked cells) can be administered subcutaneously.

Following administration of the cloaked cells to a patient, a practitioner of skill in the art can monitor the expression of the patient's improvement in response to the therapy by a variety of methods. For example, a physician can monitor accumulation of glucocerebroside or symptoms of Gaucher's disease (e.g., fatigue, anemia, low blood platelet count, enlarged liver or spleen) using standard approaches. A finding of a reduction in the patient's accumulation of glucocerebroside or symptoms of Gaucher's disease compared to measurements taken prior to administration of the cloaked cells indicates that the patient is responding favorably to the treatment. Subsequent doses can be determined and administered as needed.

Example 20: Administration of cloaked cells to a subject receiving a liver transplant

According to the methods disclosed herein, a physician of skill in the art can treat a patient, such as a human patient, who is receiving a liver transplant to reduce the risk of transplant rejection. To this end, a physician of skill in the art can administer to the human patient cloaked cells (e.g., cloaked stem cells, cloaked liver cells, or cloaked stem cells that have been differentiated into liver cells) that express one or more (e.g., one, two, three, four, five, six, seven, or all eight) of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) under the control of a constitutive promoter (e.g., CMV or CAG). The cloaked cells may be administered to the patient, for example, by injection into the liver or near the site of the transplanted liver, to reduce the risk of transplant rejection. One million to one hundred billion cloaked cells (e.g., 1×10^6, 2×10^6, 3×10^6, 4×10^6, 5×10^6, 6×10^6, 7×10^6, 8×10^6, 9×10^6, 1×10^7, 2×10^7, 3×10^7, 4×10^7, 5×10^7, 6×10^7, 7×10^7, 8×10^7, 9×10^7, 1×10^8, 2×10^8, 3×10^8, 4×10^8, 5×10^8, 6×10^8, 7×10^8, 8×10^8, 9×10^8, 1×10^9, 2×10^9, 3×10^9, 4×10^9, 5×10^9, 6×10^9, 7×10^9, 8×10^9, 9×10^9, 1×10^{10}, 2×10^{10}, 3×10^{10}, 4×10^{10}, 5×10^{10}, 6×10^{10}, 7×10^{10}, 8×10^{10}, 9×10^{10}, or 1×10^{11} cloaked cells) can be administered to or near the liver.

Following administration of the cloaked cells to a patient, a practitioner of skill in the art can monitor the expression of the patient's improvement in response to the therapy by a variety of methods. For example, a physician can monitor the patient for symptoms that predict transplant rejection using standard approaches. A finding of an equivalent outcome in transplant rejection as that observed in subjects administered immunosuppressive agent(s) indicates that the patient is responding favorably to the treatment. Subsequent doses can be determined and administered as needed.

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Example 21: Administration of cloaked and fail safe cells expressing insulin to a subject with Type 1 diabetes

According to the methods disclosed herein, a physician of skill in the art can treat a patient, such as a human patient, with Type 1 diabetes to increase insulin levels. To this end, a physician of skill in the art can administer to the human patient cloaked cells (e.g., cloaked stem cells, cloaked pancreatic beta cells, or cloaked stem cells that have been differentiated into pancreatic beta cells) that express one or more (e.g., one, two, three, four, five, six, seven, or all eight) of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) under the control of a constitutive promoter (e.g., CMV or CAG) and insulin under the control of a constitutive promoter (e.g., CMV or CAG). The cloaked cells can also be modified to allow for control of their proliferation by linking the expression of a CDL with that of a DNA sequence encoding a negative selectable marker. For example, the cloaked cells can be modified to contain homozygous ALINKS (e.g., HSV-TK systems) in two CDL loci (e.g., Cdk1 and Top2A). The cloaked cells may be administered to the patient, for example, by subcutaneous injection (e.g., to create a cloaked subcutaneous tissue), to treat Type 1 diabetes. One million to three billion cloaked cells expressing insulin (e.g., 1×10^6, 2×10^6, 3×10^6, 4×10^6, 5×10^6, 6×10^6, 7×10^6, 8×10^6, 9×10^6, 1×10^7, 2×10^7, 3×10^7, 4×10^7, 5×10^7, 6×10^7, 7×10^7, 8×10^7, 9×10^7, or 1×10^8, $2 \times$

10^8, 3 x 10^8, 4 x 10^8, 5 x 10^8, 6 x 10^8, 7 x 10^8, 8 x 10^8, 9 x 10^8, 1 x 10^9, 2 x 10^9, or 3 x 10^9 cloaked cells) can be administered subcutaneously.

Following administration of the cloaked cells to a patient, a practitioner of skill in the art can monitor the expression of the patient's improvement in response to the therapy by a variety of methods. For example, a physician can monitor insulin levels or symptoms of Type 1 diabetes (e.g., unintended weight loss, fatigue, or blurred vision) using standard approaches. A finding that the patient's insulin levels are increased or the symptoms of Type 1 diabetes are reduced compared to measurements taken prior to administration of the cloaked cells indicates that the patient is responding favorably to the treatment. Subsequent doses can be determined and administered as needed.

A practitioner of skill in the art can also monitor the size of the cloaked subcutaneous tissue. If it appears that the cloaked subcutaneous tissue is becoming tumorigenic, the practitioner can administer ganciclovir to the subject to ablate the proliferating cloaked cells. Non-proliferating cloaked cells will not express the CDLs, and, therefore, will not be ablated by ganciclovir treatment.

Example 22: Administration of cloaked and fail safe cells expressing insulin to a subject with Type 1 diabetes

According to the methods disclosed herein, a physician of skill in the art can treat a patient, such as a human patient, with Type 1 diabetes to increase insulin levels. To this end, a physician of skill in the art can administer to the human patient cloaked cells (e.g., cloaked stem cells, cloaked pancreatic beta cells, or cloaked stem cells that have been differentiated into pancreatic beta cells) that express one or more (e.g., one, two, three, four, five, six, seven, or all eight) of PD-L1, HLA-G (H2-M3), Cd47, Cd200, FASLG (FasL), Ccl21 (Ccl21b), Mfge8, and Serpin B9 (Spi6) under the control of a constitutive promoter (e.g., CMV or CAG) and insulin under the control of a constitutive promoter (e.g., CMV or CAG). The cloaked cells can also be modified to allow for control of their proliferation by linking the expression of a CDL with that of a DNA sequence encoding an inducible activator system. For example, a dox-bridge can be inserted into two CDLs (e.g., Cdk1 and Top2A) to generate homozygous modifications in both CDLs in a cloaked cell, such that in the presence of an inducer (e.g., doxycycline) the dox-bridge permits CDL expression, thereby allowing cell division and proliferation. The cloaked cells may be administered to the patient, for example, by subcutaneous injection (e.g., to create a cloaked subcutaneous tissue), to treat Type 1 diabetes. One million to three billion cloaked cells expressing insulin (e.g., 1 x 10^6, 2 x 10^6, 3 x 10^6, 4 x 10^6, 5 x 10^6, 6 x 10^6, 7 x 10^6, 8 x 10^6, 9 x 10^6, 1 x 10^7, 2 x 10^7, 3 x 10^7, 4 x 10^7, 5 x 10^7, 6 x 10^7, 7 x 10^7, 8 x 10^7, 9 x 10^7, or 1 x 10^8, 2 x 10^8, 3 x 10^8, 4 x 10^8, 5 x 10^8, 6 x 10^8, 7 x 10^8, 8 x 10^8, 9 x 10^8, 1 x 10^9, 2 x 10^9, or 3 x 10^9 cloaked cells) can be administered subcutaneously.

Following administration of the cloaked cells to a patient, a practitioner of skill in the art can monitor the expression of the patient's improvement in response to the therapy by a variety of methods. For example, a physician can monitor insulin levels or symptoms of Type 1 diabetes (e.g., unintended weight loss, fatigue, or blurred vision) using standard approaches. A finding that the patient's insulin

levels are increased or the symptoms of Type 1 diabetes are reduced compared to measurements taken prior to administration of the cloaked cells indicates that the patient is responding favorably to the treatment. Subsequent doses can be determined and administered as needed. If the practitioner determines that the subject needs a higher level of insulin, the practitioner can allow the cloaked cells to proliferate by treating the subject with doxycycline. Once the desired level of insulin is reached, treatment with doxycycline can be stopped and the cloaked cells will cease to proliferate.

Table 5: Predicted CDLs (ID refers to EntrezGene identification number; CS score refers to the CRISPR score average provided in Wang et al., 2015; function refers to the known or predicted function of the locus, predictions being based on GO terms, as set forth in the Gene Ontology Consortium website http://geneontology.org/; functional category refers to 4 categories of cell functions based on the GO term-predicted function; CDL (basis) refers to information that the inventors used to predict that a gene is a CDL, predictions being based on CS score, available gene knockout (KO) data, gene function, and experimental data provided in WO 2016 141480).

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Actr8	56249	ACTR8	93973	-1.88	chromatin remodeling	Cell cycle	CS score, function	
Alg11	207958	ALG11	440138	-1.27	dolichol-linked oligosaccharide biosynthetic process	Cell cycle	CS score, function	
Anapc11	66156	ANAPC11	51529	-2.68	protein ubiquitination involved in ubiquitin-dependent protein catabolic process	Cell cycle	CS score, function	
Anapc2	99152	ANAPC2	29882	-2.88	mitotic cell cycle	Cell cycle	CS score, mouse K.O., function	Wirth KG, et al. Genes Dev. 2004 Jan 1;18(1):88-98

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Anapc4	52206	ANAPC4	29945	-1.79	regulation of mitotic metaphase/anaphase transition	Cell cycle	CS score, function	
Anapc5	59008	ANAPC5	51433	-1.66	mitotic cell cycle	Cell cycle	CS score, function	
Aurka	20878	AURKA	6790	-2.26	meiotic spindle organization	Cell cycle	CS score, mouse K.O., function	Sasai K, et al. Oncogene. 2008 Jul 3;27(29):4122-7
Banf1	23825	BANF1	8815	-2.14	mitotic cell cycle	Cell cycle	CS score, function	
Birc5	11799	BIRC5	332	-2.24	regulation of signal transduction	Cell cycle	CS score, mouse K.O., function	Uren AG, et al. Curr Biol. 2000 Nov 2;10(21):1319-28
Bub3	12237	BUB3	9184	-3.15	mitotic sister chromatid segregation	Cell cycle	CS score, mouse K.O., function	Kalitsis P, et al. Genes Dev. 2000 Sep 15;14(18):2277-82
Casc5	76464	CASC5	57082	-1.16	mitotic cell cycle	Cell cycle	CS score, mouse K.O., function	Overbeek PA, et al. MGI Direct Data Submission. 2011
Ccna2	12428	CCNA2	890	-1.59	regulation of cyclin-dependent	Cell cycle	CS score, mouse	Kalaszczynska I, et al. Cell.

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
					protein serine/threonine kinase activity		K.O., function	2009 Jul 23;138(2): 352-65
Ccnh	66671	CCNH	902	-2.01	regulation of cyclin-dependent protein serine/threonine kinase activity	Cell cycle	CS score, function	
Cdc123	98828	CDC123	8872	-2.45	cell cycle	Cell cycle	CS score, function	
Cdc16	69957	CDC16	8881	-3.58	cell division	Cell cycle	CS score, function	
Cdc20	107995	CDC20	991	-2.97	mitotic cell cycle	Cell cycle	CS score, mouse K.O., function	Li M, et al. Mol Cell Biol. 2007 May;27(9): 3481-8
Cdc23	52563	CDC23	8697	-2.28	mitotic cell cycle	Cell cycle	CS score, function	
Cdk1	12534	CDK1	983	-2.44	cell cycle	Cell cycle	CS score, mouse K.O., function	Diril MK, et al. Proc Natl Acad Sci U S A. 2012 Mar 6;109(10): 3826-31
Cenpa	12615	CENPA	1058	-1.87	cell cycle	Cell cycle	CS score, mouse K.O., function	Howman EV, et al. Proc Natl Acad Sci U S A. 2000 Feb

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
								1;97(3):1148-53
Cenpm	66570	CENPM	79019	-2.53	mitotic cell cycle	Cell cycle	CS score, function	
Chek1	12649	CHEK1	1111	-1.67	protein phosphorylation	Cell cycle	CS score, mouse K.O., function	Takai H, et al. Genes Dev. 2000 Jun 15;14(12):1439-47
Chmp2a	68953	CHMP2A	27243	-2.40	vacuolar transport	Cell cycle	CS score, function	
Ckap5	75786	CKAP5	9793	-2.94	G2/M transition of mitotic cell cycle	Cell cycle	CS score, mouse K.O., function	Barbarese E, et al. PLoS One. 2013;8(8):e69989
Cltc	67300	CLTC	1213	-1.75	intracellular protein transport	Cell cycle	CS score, function	
Cops5	26754	COPS5	10987	-1.75	protein deneddylation	Cell cycle	CS score, mouse K.O., function	Tian L, et al. Oncogene. 2010 Nov 18;29(46):6125-37
Dctn2	69654	DCTN2	10540	-1.48	G2/M transition of mitotic cell cycle	Cell cycle	CS score, function	
Dctn3	53598	DCTN3	11258	-1.77	G2/M transition of mitotic cell cycle	Cell cycle	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Dhfr	13361	DHFR	1719	-2.84	G1/S transition of mitotic cell cycle	Cell cycle	CS score, function	
Dtl	76843	DTL	51514	-2.69	protein polyubiquitination	Cell cycle	CS score, mouse K.O., function	Liu CL, et al. J Biol Chem. 2007 Jan 12;282(2):1109-18
Dync1h1	13424	DYNC1H1	1778	-3.44	G2/M transition of mitotic cell cycle	Cell cycle	CS score, mouse K.O., function	Harada A, et al. J Cell Biol. 1998 Apr 6;141(1):51-9
Ecd	70601	ECD	11319	-3.18	regulation of glycolytic process	Cell cycle	CS score, function	
Ect2	13605	ECT2	1894	-1.80	cell morphogenesis	Cell cycle	CS score, mouse K.O., function	Hansen J, et al. Proc Natl Acad Sci U S A. 2003 Aug 19;100(17):9918-22
Ep300	328572	EP300	2033	-2.04	G2/M transition of mitotic cell cycle	Cell cycle	CS score, mouse K.O., function	Yao TP, et al. Cell. 1998 May 1;93(3):361-72
Ercc3	13872	ERCC3	2071	-2.10	nucleotide-excision repair	Cell cycle	CS score, mouse K.O., function	Andressoo JO, et al. Mol Cell Biol. 2009 Mar;29(5):1276-90
Espl1	105988	ESPL1	9700	-3.24	proteolysis	Cell cycle	CS score,	Wirth KG, et al. J

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
							mouse K.O., function	Cell Biol. 2006 Mar 13;172(6): 847-60
Fntb	110606	FNTB	2342	-2.42	phototransduction, visible light	Cell cycle	CS score, mouse K.O., function	Mijimolle N, et al. Cancer Cell. 2005 Apr;7(4):313-24
Gadd45gip1	102060	GADD45GIP1	90480	-1.81	organelle organization	Cell cycle	CS score, mouse K.O., function	Kwon MC, et al. EMBO J. 2008 Feb 20;27(4):642-53
Gins1	69270	GINS1	9837	-1.84	mitotic cell cycle	Cell cycle	CS score, mouse K.O., function	Ueno M, et al. Mol Cell Biol. 2005 Dec;25(23):10528-32
Gnb2l1	14694	GNB2L1	10399	-2.84	osteoblast differentiation	Cell cycle	CS score, function	
Gspt1	14852	GSPT1	2935	-1.77	G1/S transition of mitotic cell cycle	Cell cycle	CS score, function	
Haus1	225745	HAUS1	115106	-1.92	spindle assembly	Cell cycle	CS score, function	
Haus3	231123	HAUS3	79441	-1.38	mitotic nuclear division	Cell cycle	CS score, function	
Haus5	71909	HAUS5	23354	-2.55	spindle assembly	Cell cycle	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Haus8	76478	HAUS8	93323	-1.73	mitotic nuclear division	Cell cycle	CS score, function	
Hdac3	15183	HDAC3	8841	-2.12	histone deacetylation	Cell cycle	CS score, mouse K.O., function	Bhaskara S, et al. Mol Cell. 2008 Apr 11;30(1):61-72
Kif11	16551	KIF11	3832	-3.23	microtubule-based movement	Cell cycle	CS score, mouse K.O., function	Castillo A, et al. Biochem Biophys Res Commun. 2007 Jun 8;357(3):694-9
Kif23	71819	KIF23	9493	-1.59	microtubule-based movement	Cell cycle	CS score, function	
Kpnb1	16211	KPNB1	3837	-3.19	nucleocytoplasmic transport	Cell cycle	CS score, mouse K.O., function	Miura K, et al. Biochem Biophys Res Commun. 2006 Mar 3;341(1):132-8
Mastl	67121	MASTL	84930	-2.36	protein phosphorylation	Cell cycle	CS score, mouse K.O., function	Alvarez-Fernandez M, et al. Proc Natl Acad Sci U S A. 2013 Oct 22;110(43):17374-9

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Mau2	74549	MAU2	23383	-2.71	mitotic cell cycle	Cell cycle	CS score, mouse K.O., function	Smith TG, et al. Genesis. 2014 Jul;52(7):687-94
Mcm3	17215	MCM3	4172	-2.52	G1/S transition of mitotic cell cycle	Cell cycle	CS score, function	
Mcm4	17217	MCM4	4173	-1.87	G1/S transition of mitotic cell cycle	Cell cycle	CS score, mouse K.O., function	Shima N, et al. Nat Genet. 2007 Jan;39(1):93-8
Mcm7	17220	MCM7	4176	-2.39	G1/S transition of mitotic cell cycle	Cell cycle	CS score, function	
Mnat1	17420	MNAT1	4331	-1.22	regulation of cyclin-dependent protein serine/threonine kinase activity	Cell cycle	CS score, mouse K.O., function	Rossi DJ, et al. EMBO J. 2001 Jun 1;20(11):2844-56
Mybbp1a	18432	MYBBP1A	10514	-2.17	osteoblast differentiation	Cell cycle	CS score, mouse K.O., function	Mori S, et al. PLoS One. 2012;7(10):e39723
Ncapd2	68298	NCAPD2	9918	-2.03	mitotic chromosome condensation	Cell cycle	CS score, function	
Ncaph	215387	NCAPH	23397	-2.33	mitotic chromosome condensation	Cell cycle	CS score, mouse	Nishide K, et al. PLoS Genet.

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
							K.O., function	2014 Dec;10(12):e1004847
Ndc80	67052	NDC80	10403	-2.98	attachment of mitotic spindle microtubules to kinetochore	Cell cycle	CS score, function	
Nle1	217011	NLE1	54475	-1.88	somitogenesis	Cell cycle	CS score, mouse K.O., function	Hentges KE, et al. Gene Expr Patterns. 2006 Aug;6(6):653-65
Nsl1	381318	NSL1	25936	-1.90	mitotic cell cycle	Cell cycle	CS score, function	
Nudc	18221	NUDC	10726	-1.93	mitotic cell cycle	Cell cycle	CS score, function	
Nuf2	66977	NUF2	83540	-1.78	mitotic nuclear division	Cell cycle	CS score, function	
Nup133	234865	NUP133	55746	-2.26	mitotic cell cycle	Cell cycle	CS score, mouse K.O., function	Garcia-Garcia MJ, et al. Proc Natl Acad Sci U S A. 2005 Apr 26;102(17):5913-9
Nup160	59015	NUP160	23279	-2.64	mitotic cell cycle	Cell cycle	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Nup188	227699	NUP188	23511	-1.16	mitotic cell cycle	Cell cycle	CS score, function	
Nup214	227720	NUP214	8021	-2.70	mitotic cell cycle	Cell cycle	CS score, mouse K.O., function	van Deursen J, et al. EMBO J. 1996 Oct 15;15(20): 5574-83
n/a	n/a	NUP62	23636	-2.35	mitotic cell cycle	Cell cycle	CS score, function	
Nup85	445007	NUP85	79902	-2.47	mitotic cell cycle	Cell cycle	CS score, function	
Orc3	50793	ORC3	23595	-1.67	G1/S transition of mitotic cell cycle	Cell cycle	CS score, function	
Pafah1b1	18472	PAFAH1B1	5048	-2.34	G2/M transition of mitotic cell cycle	Cell cycle	CS score, mouse K.O., function	Cahana A, et al. Proc Natl Acad Sci U S A. 2001 May 22;98(11): 6429-34
Pcid2	234069	PCID2	55795	-1.98	negative regulation of apoptotic process	Cell cycle	CS score, function	
Pfas	237823	PFAS	5198	-2.58	purine nucleotide biosynthetic process	Cell cycle	CS score, function	
Phb2	12034	PHB2	11331	-2.98	protein import into nucleus, translocation	Cell cycle	CS score, mouse	Park SE, et al. Mol Cell Biol. 2005

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
							K.O., function	Mar;25(5): 1989-99
Pkmyt1	268930	PKMYT1	9088	-1.93	regulation of cyclin-dependent protein serine/threonine kinase activity	Cell cycle	CS score, function	
Plk1	18817	PLK1	5347	-2.83	protein phosphorylation	Cell cycle	CS score, mouse K.O., function	Lu LY, et al. Mol Cell Biol. 2008 Nov;28(22):6870-6
Pmf1	67037	PMF1	11243	-2.15	mitotic cell cycle	Cell cycle	CS score, function	
Pole2	18974	POLE2	5427	-3.08	G1/S transition of mitotic cell cycle	Cell cycle	CS score, function	
Ppat	231327	PPAT	5471	-2.15	G1/S transition of mitotic cell cycle	Cell cycle	CS score, function	
Psma6	26443	PSMA6	5687	-3.51	G1/S transition of mitotic cell cycle	Cell cycle	CS score, function	
Psma7	26444	PSMA7	5688	-2.91	G1/S transition of mitotic cell cycle	Cell cycle	CS score, function	
Psmb1	19170	PSMB1	5689	-1.63	G1/S transition of mitotic cell cycle	Cell cycle	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Psmb4	19172	PSMB4	5692	-2.91	G1/S transition of mitotic cell cycle	Cell cycle	CS score, function	
Psmd12	66997	PSMD12	5718	-1.69	G1/S transition of mitotic cell cycle	Cell cycle	CS score, function	
Psmd13	23997	PSMD13	5719	-1.57	G1/S transition of mitotic cell cycle	Cell cycle	CS score, function	
Psmd14	59029	PSMD14	10213	-3.01	G1/S transition of mitotic cell cycle	Cell cycle	CS score, function	
Psmd7	17463	PSMD7	5713	-2.18	G1/S transition of mitotic cell cycle	Cell cycle	CS score, mouse K.O., function	Soriano P, et al. Genes Dev. 1987 Jun;1(4):366-75
Racgap1	26934	RACGAP1	29127	-1.94	mitotic spindle assembly	Cell cycle	CS score, mouse K.O., function	Van de Putte T, et al. Mech Dev. 2001 Apr;102(1-2):33-44
Rad21	19357	RAD21	5885	-2.12	mitotic cell cycle	Cell cycle	CS score, function	
Rae1	66679	RAE1	8480	-2.15	mitotic cell cycle	Cell cycle	CS score, mouse K.O., function	Babu JR, et al. J Cell Biol. 2003 Feb 3;160(3):341-53

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Rcc1	100088	RCC1	1104	-2.91	G1/S transition of mitotic cell cycle	Cell cycle	CS score, function	
Rfc3	69263	RFC3	5983	-2.74	mitotic cell cycle	Cell cycle	CS score, function	
Rps27a	78294	RPS27A	6233	-2.74	G1/S transition of mitotic cell cycle	Cell cycle	CS score, function	
Rrm2	20135	RRM2	6241	-3.09	G1/S transition of mitotic cell cycle	Cell cycle	CS score, function	
Sae1	56459	SAE1	10055	-2.08	cellular protein modification process	Cell cycle	CS score, function	
Sec13	110379	SEC13	6396	-2.96	mitotic cell cycle	Cell cycle	CS score, function	
Smarcb1	20587	SMARCB1	6598	-1.98	chromatin remodeling	Cell cycle	CS score, mouse K.O., function	Guidi CJ, et al. Mol Cell Biol. 2001 May 15;21(10): 3598-603
Smc2	14211	SMC2	10592	-2.13	mitotic chromosome condensation	Cell cycle	CS score, mouse K.O., function	Nishide K, et al. PLoS Genet. 2014 Dec;10(12):e1004847
Smc4	70099	SMC4	10051	-1.47	chromosome organization	Cell cycle	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Son	20658	SON	6651	-1.99	microtubule cytoskeleton organization	Cell cycle	CS score, function	
Spc24	67629	SPC24	147841	-2.83	mitotic cell cycle	Cell cycle	CS score, function	
Spc25	66442	SPC25	57405	-1.63	mitotic cell cycle	Cell cycle	CS score, function	
Terf2	21750	TERF2	7014	-2.17	telomere maintenance	Cell cycle	CS score, mouse K.O., function	Celli GB, et al. Nat Cell Biol. 2005 Jul;7(7):712-8
Tpx2	72119	TPX2	22974	-2.08	apoptotic process	Cell cycle	CS score, mouse K.O., function	Aguirre-Portoles C, et al. Cancer Res. 2012 Mar 15;72(6):1518-28
Tubg1	103733	TUBG1	7283	-2.08	microtubule nucleation	Cell cycle	CS score, mouse K.O., function	Yuba-Kubo A, et al. Dev Biol. 2005 Jun 15;282(2):361-73
Tubgcp2	74237	TUBGCP2	10844	-2.78	microtubule cytoskeleton organization	Cell cycle	CS score, function	
Tubgcp5	233276	TUBGCP5	114791	-1.76	microtubule cytoskeleton organization	Cell cycle	CS score, function	
Tubgcp6	328580	TUBGCP6	85378	-1.52	microtubule cytoskeleton organization	Cell cycle	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Txnl4a	27366	TXNL4A	10907	-3.89	mitotic nuclear division	Cell cycle	CS score, function	
Usp39	28035	USP39	10713	-2.85	spliceosomal complex assembly	Cell cycle	CS score, function	
Wdr43	72515	WDR43	23160	-3.02	reproduction	Cell cycle	CS score, function	
Zfp830	66983	ZNF830	91603	-1.52	blastocyst growth	Cell cycle	CS score, mouse K.O., function	Houlard M, et al. Cell Cycle. 2011 Jan 1;10(1):108-17
Aatf	56321	AATF	26574	-1.46	cellular response to DNA damage stimulus	DNA replication , DNA repair	CS score, mouse K.O., function	Thomas T, et al. Dev Biol. 2000 Nov 15;227(2):324-42
Alyref	21681	ALYREF	10189	-1.92	regulation of DNA recombination	DNA replication , DNA repair	CS score, function	
Brf2	66653	BRF2	55290	-2.30	DNA-templated transcription, initiation	DNA replication , DNA repair	CS score, function	
Cdc45	12544	CDC45	8318	-3.69	DNA replication checkpoint	DNA replication , DNA repair	CS score, mouse K.O., function	Yoshida K, et al. Mol Cell Biol. 2001 Jul;21(14):4598-603
Cdc6	23834	CDC6	990	-1.87	DNA replication initiation	DNA replication , DNA repair	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Cdt1	67177	CDT1	81620	-2.74	DNA replication checkpoint	DNA replication , DNA repair	CS score, function	
Cinp	67236	CINP	51550	-1.64	DNA replication	DNA replication , DNA repair	CS score, function	
Cirh1a	21771	CIRH1A	84916	-2.62	transcription, DNA-templated	DNA replication , DNA repair	CS score, function	
Ddb1	13194	DDB1	1642	-2.14	nucleotide-excision repair, DNA damage removal	DNA replication , DNA repair	CS score, mouse K.O., function	Cang Y, et al. Cell. 2006 Dec 1;127(5):929-40
Ercc2	13871	ERCC2	2068	-2.80	DNA duplex unwinding	DNA replication , DNA repair	CS score, mouse K.O., function	de Boer J, et al. Cancer Res. 1998 Jan 1;58(1):89-94
Gabpb1	14391	GABPB1	2553	-1.74	transcription, DNA-templated	DNA replication , DNA repair	CS score, mouse K.O., function	Xue HH, et al. Mol Cell Biol. 2008 Jul;28(13):4300-9
Gtf2b	229906	GTF2B	2959	-2.76	regulation of transcription, DNA-templated	DNA replication , DNA repair	CS score, function	
Gtf2h4	14885	GTF2H4	2968	-1.93	nucleotide-excision repair, DNA damage removal	DNA replication , DNA repair	CS score, function	
Gtf3a	66596	GTF3A	2971	-2.25	regulation of transcription,	DNA replication , DNA repair	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
					DNA-templated			
Gtf3c1	233863	GTF3C1	2975	-2.45	transcription, DNA-templated	DNA replication , DNA repair	CS score, function	
Gtf3c2	71752	GTF3C2	2976	-2.09	transcription, DNA-templated	DNA replication , DNA repair	CS score, function	
Hinfp	102423	HINFP	25988	-2.35	DNA damage checkpoint	DNA replication , DNA repair	CS score, mouse K.O., function	Xie R, et al. Proc Natl Acad Sci U S A. 2009 Jul 9
n/a	n/a	HIST2H2AA3	8337	-1.71	DNA repair	DNA replication , DNA repair	CS score, function	
Ints3	229543	INTS3	65123	-3.14	DNA repair	DNA replication , DNA repair	CS score, function	
Kin	16588	KIN	22944	-1.99	DNA replication	DNA replication , DNA repair	CS score, function	
Mcm2	17216	MCM2	4171	-2.86	DNA replication initiation	DNA replication , DNA repair	CS score, function	
Mcm6	17219	MCM6	4175	-1.55	DNA replication	DNA replication , DNA repair	CS score, function	
Mcrs1	51812	MCRS1	10445	-1.23	DNA repair	DNA replication , DNA repair	CS score, function	
Med11	66172	MED11	400569	-2.39	transcription, DNA-templated	DNA replication , DNA repair	CS score, function	
Mtpap	67440	MTPAP	55149	-1.86	transcription, DNA-templated	DNA replication , DNA repair	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Myc	17869	MYC	4609	-2.49	regulation of transcription, DNA-templated	DNA replication , DNA repair	CS score, mouse K.O., function	Trumpp A, et al. Nature. 2001 Dec 13;414(6865):768-73
Ndnl2	66647	NDNL2	56160	-2.03	DNA repair	DNA replication , DNA repair	CS score, function	
Nol11	68979	NOL11	25926	-1.59	transcription, DNA-templated	DNA replication , DNA repair	CS score, function	
Nol8	70930	NOL8	55035	-1.35	DNA replication	DNA replication , DNA repair	CS score, function	
Pcna	18538	PCNA	5111	-3.60	DNA replication	DNA replication , DNA repair	CS score, mouse K.O., function	Roa S, et al. Proc Natl Acad Sci U S A. 2008 Oct 21;105(42):16248-53
Pola1	18968	POLA1	5422	-2.28	DNA-dependent DNA replication	DNA replication , DNA repair	CS score, function	
Pold2	18972	POLD2	5425	-2.51	DNA replication	DNA replication , DNA repair	CS score, function	
Pole	18973	POLE	5426	-2.90	DNA replication	DNA replication , DNA repair	CS score, function	
Polr1a	20019	POLR1A	25885	-2.62	transcription, DNA-templated	DNA replication , DNA repair	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
n/a	n/a	POLR2J2	246721	-3.08	transcription, DNA-templated	DNA replication , DNA repair	CS score, function	
Polr3a	218832	POLR3A	11128	-2.43	transcription, DNA-templated	DNA replication , DNA repair	CS score, function	
Polr3c	74414	POLR3C	10623	-2.02	transcription, DNA-templated	DNA replication , DNA repair	CS score, function	
Polr3h	78929	POLR3H	171568	-2.66	transcription, DNA-templated	DNA replication , DNA repair	CS score, function	
Prmt1	15469	PRMT1	3276	-2.40	regulation of transcription, DNA-templated	DNA replication , DNA repair	CS score, mouse K.O., function	Pawlak MR, et al. Mol Cell Biol. 2000 Jul;20(13): 4859-69
Prmt5	27374	PRMT5	10419	-2.69	regulation of transcription, DNA-templated	DNA replication , DNA repair	CS score, mouse K.O., function	Tee WW, et al. Genes Dev. 2010 Dec 15;24(24): 2772-7
Puf60	67959	PUF60	22827	-2.69	transcription, DNA-templated	DNA replication , DNA repair	CS score, function	
Rad51	19361	RAD51	5888	-2.29	DNA repair	DNA replication , DNA repair	CS score, mouse K.O., function	Tsuzuki T, et al. Proc Natl Acad Sci U S A. 1996 Jun 25;93(13): 6236-40
Rad51c	114714	RAD51C	5889	-1.62	DNA repair	DNA replication , DNA repair	CS score, mouse	Smeenk G, et al. Mutat Res. 2010

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
							K.O., function	Jul 7;689(1-2):50-58
Rbx1	56438	RBX1	9978	-2.19	DNA repair	DNA replication , DNA repair	CS score, mouse K.O., function	Tan M, et al. Proc Natl Acad Sci U S A. 2009 Apr 14;106(15):6203-8
Rfc2	19718	RFC2	5982	-2.88	DNA-dependent DNA replication	DNA replication , DNA repair	CS score, function	
Rfc4	106344	RFC4	5984	-1.92	DNA-dependent DNA replication	DNA replication , DNA repair	CS score, function	
Rfc5	72151	RFC5	5985	-2.78	DNA-dependent DNA replication	DNA replication , DNA repair	CS score, function	
Rpa1	68275	RPA1	6117	-2.61	DNA replication	DNA replication , DNA repair	CS score, mouse K.O., function	Wang Y, et al. Nat Genet. 2005 Jul;37(7):750-5
Rps3	27050	RPS3	6188	-2.75	DNA repair	DNA replication , DNA repair	CS score, function	
Rrm1	20133	RRM1	6240	-4.16	DNA replication	DNA replication , DNA repair	CS score, function	
Ruvbl1	56505	RUVBL1	8607	-3.26	DNA duplex unwinding	DNA replication , DNA repair	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Ruvbl2	20174	RUVBL2	10856	-3.91	DNA repair	DNA replication , DNA repair	CS score, function	
Sap30bp	57230	SAP30BP	29115	-2.18	regulation of transcription, DNA-templated	DNA replication , DNA repair	CS score, function	
Smc1a	24061	SMC1A	8243	-2.76	DNA repair	DNA replication , DNA repair	CS score, function	
Smc3	13006	SMC3	9126	-3.22	DNA repair	DNA replication , DNA repair	CS score, mouse K.O., function	White JK, et al. Cell. 2013 Jul 18;154(2): 452-64
Snapc4	227644	SNAPC4	6621	-2.78	regulation of transcription, DNA-templated	DNA replication , DNA repair	CS score, function	
Snapc5	330959	SNAPC5	10302	-2.24	regulation of transcription, DNA-templated	DNA replication , DNA repair	CS score, function	
Snip1	76793	SNIP1	79753	-1.78	regulation of transcription, DNA-templated	DNA replication , DNA repair	CS score, function	
Srrt	83701	SRRT	51593	-2.18	transcription, DNA-templated	DNA replication , DNA repair	CS score, mouse K.O., function	Wilson MD, et al. Mol Cell Biol. 2008 Mar;28(5): 1503-14
Ssrp1	20833	SSRP1	6749	-1.45	DNA replication	DNA replication , DNA repair	CS score, mouse K.O., function	Cao S, et al.5 mouse embryos. Mol Cell Biol. 2003

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
								Aug;23(15):5301-7
Taf10	24075	TAF10	6881	-1.38	DNA-templated transcription, initiation	DNA replication , DNA repair	CS score, mouse K.O., function	Mohan WS Jr, et al. Mol Cell Biol. 2003 Jun;23(12):4307-18
Taf1c	21341	TAF1C	9013	-1.80	chromatin silencing at rDNA	DNA replication , DNA repair	CS score, function	
Taf6	21343	TAF6	6878	-1.84	DNA-templated transcription, initiation	DNA replication , DNA repair	CS score, function	
Taf6l	67706	TAF6L	10629	-1.53	DNA-templated transcription, initiation	DNA replication , DNA repair	CS score, function	
Ticrr	77011	TICRR	90381	-2.03	DNA replication	DNA replication , DNA repair	CS score, function	
Top1	21969	TOP1	7150	-2.02	DNA topological change	DNA replication , DNA repair	CS score, mouse K.O., function	Morham SG, et al. Mol Cell Biol. 1996 Dec;16(12):6804-9
Top2a	21973	TOP2A	7153	-1.50	DNA replication	DNA replication , DNA repair	CS score, function	
Trrap	100683	TRRAP	8295	-2.36	DNA repair	DNA replication , DNA repair	CS score, mouse K.O., function	Herceg Z, et al. Nat Genet. 2001 Oct;29(2):206-11

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Zbtb11	271377	ZBTB11	27107	-2.34	transcription, DNA-templated	DNA replication , DNA repair	CS score, function	
Actl6a	56456	ACTL6A	86	-2.33	neural retina development	DNA replication, DNA repair	CS score, mouse K.O., function	Krasteva V, et al. Blood. 2012 Dec 6;120(24): 4720-32
Atr	245000	ATR	545	-2.01	double-strand break repair via homologous recombination	DNA replication, DNA repair	CS score, mouse K.O., function	de Klein A, et al. Curr Biol. 2000 Apr 20;10(8):479-82
Chd4	107932	CHD4	1108	-1.71	chromatin organization	DNA replication, DNA repair	CS score, function	
Ciao1	26371	CIAO1	9391	-1.94	chromosome segregation	DNA replication, DNA repair	CS score, function	
Ddx21	56200	DDX21	9188	-2.84	osteoblast differentiation	DNA replication, DNA repair	CS score, function	
Dnaja3	83945	DNAJA3	9093	-2.19	mitochondrion organization	DNA replication, DNA repair	CS score, mouse K.O., function	Lo JF, et al. Mol Cell Biol. 2004 Mar;24(6): 2226-36
Dnmt1	13433	DNMT1	1786	-1.97	methylation	DNA replication, DNA repair	CS score, mouse K.O., function	Lei H, et al. Development. 1996 Oct;122(10):3195-205

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Gins2	272551	GINS2	51659	-3.32	double-strand break repair via break-induced replication	DNA replication, DNA repair	CS score, function	
Gtf2h3	209357	GTF2H3	2967	-1.84	nucleotide-excision repair	DNA replication, DNA repair	CS score, function	
n/a	n/a	HIST2H2BF	440689	-1.70	chromatin organization	DNA replication, DNA repair	CS score, function	
Mms22l	212377	MMS22L	253714	-1.38	double-strand break repair via homologous recombination	DNA replication, DNA repair	CS score, function	
Mtor	56717	MTOR	2475	-1.98	double-strand break repair via homologous recombination	DNA replication, DNA repair	CS score, mouse K.O., function	Murakami M, et al. Mol Cell Biol. 2004 Aug;24(15):6710-8
Narfl	67563	NARFL	64428	-2.13	response to hypoxia	DNA replication, DNA repair	CS score, mouse K.O., function	Song D, et al. J Biol Chem. 2011 Mar 2
Ndufa13	67184	NDUFA13	51079	-1.31	positive regulation of peptidase activity	DNA replication, DNA repair	CS score, mouse K.O., function	Huang G, et al. Mol Cell Biol. 2004 Oct;24(19):8447-56
Nol12	97961	NOL12	79159	-1.61	poly(A) RNA binding	DNA replication, DNA repair	CS score, function	
Nup107	103468	NUP107	57122	-1.30	transport	DNA replication, DNA repair	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Oraov1	72284	ORAOV1	220064	-2.26	biological_process	DNA replication, DNA repair	CS score, function	
Pam16	66449	PAM16	51025	-2.13	protein import into mitochondrial matrix	DNA replication, DNA repair	CS score, function	
Pola2	18969	POLA2	23649	-2.84	protein import into nucleus, translocation	DNA replication, DNA repair	CS score, function	
Ppie	56031	PPIE	10450	-1.63	protein peptidyl-prolyl isomerization	DNA replication, DNA repair	CS score, function	
Prpf19	28000	PRPF19	27339	-3.96	generation of catalytic spliceosome for first transesterification step	DNA replication, DNA repair	CS score, mouse K.O., function	Fortschegger K, et al. Mol Cell Biol. 2007 Apr;27(8): 3123-30
Psmc5	19184	PSMC5	5705	-2.57	ER-associated ubiquitin-dependent protein catabolic process	DNA replication, DNA repair	CS score, function	
Rbbp5	213464	RBBP5	5929	-1.70	chromatin organization	DNA replication, DNA repair	CS score, function	
Rbbp6	19647	RBBP6	5930	-1.78	in utero embryonic development	DNA replication, DNA repair	CS score, mouse K.O., function	Li L, et al. Proc Natl Acad Sci U S A. 2007 May 8;104(19): 7951-6

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Rptor	74370	RPTOR	57521	-2.43	TOR signaling	DNA replication, DNA repair	CS score, mouse K.O., function	Guertin DA, et al. Dev Cell. 2006 Dec;11(6): 859-71
Rrn3	106298	RRN3	54700	-1.85	in utero embryonic development	DNA replication, DNA repair	CS score, mouse K.O., function	Yuan X, et al. Mol Cell. 2005 Jul 1;19(1):77 -87
Smg1	233789	SMG1	23049	-1.94	double-strand break repair via homologous recombination	DNA replication, DNA repair	CS score, mouse K.O., function	Roberts TL, et al. Proc Natl Acad Sci U S A. 2013 Jan 22;110(4): E285-94
Supt6	20926	SUPT6H	6830	-1.78	chromatin remodeling	DNA replication, DNA repair	CS score, mouse K.O., function	Dietrich JE, et al. EMBO Rep. 2015 Aug;16(8): 1005-21
Tada2b	231151	TADA2B	93624	-1.23	chromatin organization	DNA replication, DNA repair	CS score, function	
Tfip11	54723	TFIP11	24144	-2.19	spliceosomal complex disassembly	DNA replication, DNA repair	CS score, function	
Tonsl	66914	TONSL	4796	-3.03	double-strand break repair via homologous recombination	DNA replication, DNA repair	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Tpt1	22070	TPT1	7178	-2.05	calcium ion transport	DNA replication, DNA repair	CS score, mouse K.O., function	Susini L, et al. Cell Death Differ. 2008 Aug;15(8): 1211-20
Uba1	22201	UBA1	7317	-2.90	protein ubiquitination	DNA replication, DNA repair	CS score, function	
Vps25	28084	VPS25	84313	-2.31	protein targeting to vacuole involved in ubiquitin-dependent protein catabolic process via the multivesicular body sorting pathway	DNA replication, DNA repair	CS score, function	
Wbscr22	66138	WBSCR22	114049	-2.70	methylation	DNA replication, DNA repair	CS score, function	
Wdr5	140858	WDR5	11091	-1.99	skeletal system development	DNA replication, DNA repair	CS score, function	
Xab2	67439	XAB2	56949	-2.86	generation of catalytic spliceosome for first transesterification step	DNA replication, DNA repair	CS score, mouse K.O., function	Yonemasu R, et al. DNA Repair (Amst). 2005 Apr 4;4(4):479 -91

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Zmat2	66492	ZMAT2	153527	-2.17	histidine-tRNA ligase activity	DNA replication, DNA repair	CS score, function	
Zfp335	329559	ZNF335	63925	-1.58	in utero embryonic development	DNA replication, DNA repair	CS score, mouse K.O., function	Yang YJ, et al. Cell. 2012 Nov 21;151(5): 1097-112
Acly	104112	ACLY	47	-1.54	acetyl-CoA metabolic process	Metabolism	CS score, mouse K.O., function	Beigneux AP, et al. J Biol Chem. 2004 Mar 5;279(10): 9557-64
Adsl	11564	ADSL	158	-2.39	metabolic process	Metabolism	CS score, function	
Ahcy	269378	AHCY	191	-2.07	sulfur amino acid metabolic process	Metabolism	CS score, function	
Arl2	56327	ARL2	402	-2.29	energy reserve metabolic process	Metabolism	CS score, function	
Chka	12660	CHKA	1119	-1.64	lipid metabolic process	Metabolism	CS score, mouse K.O., function	Wu G, et al. J Biol Chem. 2008 Jan 18;283(3): 1456-62
Coasy	71743	COASY	80347	-1.82	vitamin metabolic process	Metabolism	CS score, function	
Cox4i1	12857	COX4I1	1327	-2.00	generation of precursor metabolites and energy	Metabolism	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
n/a	n/a	COX7C	1350	-1.59	generation of precursor metabolites and energy	Metabolism	CS score, function	
n/a	n/a	CTPS1	1503	-2.52	nucleobase-containing compound metabolic process	Metabolism	CS score, function	
Ddx10	77591	DDX10	1662	-2.02	metabolic process	Metabolism	CS score, function	
Ddx20	53975	DDX20	11218	-2.49	metabolic process	Metabolism	CS score, mouse K.O., function	Mouillet JF, et al. Endocrinology. 2008 May;149(5):2168-75
Dhdds	67422	DHDDS	79947	-2.86	metabolic process	Metabolism	CS score, function	
Dhx30	72831	DHX30	22907	-1.93	metabolic process	Metabolism	CS score, function	
Dhx8	217207	DHX8	1659	-2.61	metabolic process	Metabolism	CS score, function	
Dhx9	13211	DHX9	1660	-1.73	metabolic process	Metabolism	CS score, mouse K.O., function	Lee CG, et al. Proc Natl Acad Sci U S A. 1998 Nov 10;95(23):13709-13
Dlst	78920	DLST	1743	-1.93	metabolic process	Metabolism	CS score, function	
Dpagt1	13478	DPAGT1	1798	-2.80	UDP-N-acetylglucosa	Metabolism	CS score,	Marek KW, et al.

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
					mine metabolic process		mouse K.O., function	Glycobiology. 1999 Nov;9(11): 1263-71
Gfpt1	14583	GFPT1	2673	-1.81	fructose 6-phosphate metabolic process	Metabolism	CS score, function	
Gmps	229363	GMPS	8833	-1.80	purine nucleobase metabolic process	Metabolism	CS score, function	
Gpn1	74254	GPN1	11321	-1.79	metabolic process	Metabolism	CS score, function	
Gpn3	68080	GPN3	51184	-3.12	metabolic process	Metabolism	CS score, function	
Guk1	14923	GUK1	2987	-2.67	purine nucleotide metabolic process	Metabolism	CS score, function	
Hsd17b10	15108	HSD17B10	3028	-1.84	lipid metabolic process	Metabolism	CS score, function	
Lrr1	69706	LRR1	122769	-3.44	metabolic process	Metabolism	CS score, function	
Mtg2	52856	MTG2	26164	-2.04	metabolic process	Metabolism	CS score, function	
Myh9	17886	MYH9	4627	-1.70	metabolic process	Metabolism	CS score, mouse K.O., function	Matsushita T, et al. Biochem Biophys Res Commun. 2004 Dec

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
								24;325(4): 1163-71
Nampt	59027	NAMPT	10135	-2.40	vitamin metabolic process	Metabolism	CS score, mouse K.O., function	Revollo JR, et al. Cell Metab. 2007 Nov;6(5):363-75
Ncbp1	433702	NCBP1	4686	-1.62	RNA metabolic process	Metabolism	CS score, function	
Nfs1	18041	NFS1	9054	-2.40	metabolic process	Metabolism	CS score, function	
Ppcdc	66812	PPCDC	60490	-1.98	metabolic process	Metabolism	CS score, function	
Qrsl1	76563	QRSL1	55278	-1.67	metabolic process	Metabolism	CS score, function	
Rpp14	67053	RPP14	11102	-1.72	fatty acid metabolic process	Metabolism	CS score, function	
Smarca4	20586	SMARCA4	6597	-1.89	metabolic process	Metabolism	CS score, mouse K.O., function	Bultman S, et al. Mol Cell. 2000 Dec;6(6):1287-95
Snrnp200	320632	SNRNP200	23020	-2.50	metabolic process	Metabolism	CS score, function	
Srbd1	78586	SRBD1	55133	-2.35	nucleobase-containing compound metabolic process	Metabolism	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Srcap	100043597	SRCAP	10847	-1.43	metabolic process	Metabolism	CS score, function	
Ube2i	22196	UBE2I	7329	-2.55	metabolic process	Metabolism	CS score, mouse K.O., function	Nacerddine K, et al. Dev Cell. 2005 Dec;9(6):769-79
Ube2m	22192	UBE2M	9040	-2.39	metabolic process	Metabolism	CS score, function	
Vcp	269523	VCP	7415	-2.85	metabolic process	Metabolism	CS score, mouse K.O., function	Muller JM, et al. Biochem Biophys Res Commun. 2007 Mar 9;354(2):459-465
Aamp	227290	AAMP	14	-2.37	angiogenesis	Metabolism	CS score, function	
Acin1	56215	ACIN1	22985	-1.53	positive regulation of defense response to virus by host	Metabolism	CS score, function	
Aco2	11429	ACO2	50	-2.08	tricarboxylic acid cycle	Metabolism	CS score, function	
Adss	11566	ADSS	159	-2.46	purine nucleotide biosynthetic process	Metabolism	CS score, function	
Alg2	56737	ALG2	85365	-2.29	biosynthetic process	Metabolism	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Ap2s1	232910	AP2S1	1175	-2.00	intracellular protein transport	Metabolism	CS score, function	
Arcn1	213827	ARCN1	372	-1.91	intracellular protein transport	Metabolism	CS score, function	
Armc7	276905	ARMC7	79637	-2.02	molecular_function	Metabolism	CS score, function	
Atp2a2	11938	ATP2A2	488	-3.01	calcium ion transmembrane transport	Metabolism	CS score, mouse K.O., function	Andersson KB, et al. Cell Calcium. 2009 Sep;46(3): 219-25
Atp5a1	11946	ATP5A1	498	-1.99	negative regulation of endothelial cell proliferation	Metabolism	CS score, function	
Atp5d	66043	ATP5D	513	-2.21	oxidative phosphorylation	Metabolism	CS score, function	
Atp5o	28080	ATP5O	539	-1.17	ATP biosynthetic process	Metabolism	CS score, function	
Atp6v0b	114143	ATP6V0B	533	-3.01	cellular iron ion homeostasis	Metabolism	CS score, function	
Atp6v0c	11984	ATP6V0C	527	-3.84	cellular iron ion homeostasis	Metabolism	CS score, mouse K.O., function	Sun-Wada GH, et al. Dev Biol. 2000 Dec 15;228(2): 315-25
Atp6v1a	11964	ATP6V1A	523	-3.58	proton transport	Metabolism	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Atp6v1b2	11966	ATP6V1B2	526	-2.94	cellular iron ion homeostasis	Metabolism	CS score, function	
Atp6v1d	73834	ATP6V1D	51382	-2.58	transmembrane transport	Metabolism	CS score, function	
Aurkaip1	66077	AURKAIP1	54998	-1.56	organelle organization	Metabolism	CS score, function	
n/a	n/a	C1orf109	54955	-2.43	molecular_function	Metabolism	CS score, function	
n/a	n/a	C21orf59	56683	-2.77	cell projection morphogenesis	Metabolism	CS score, function	
Ccdc84	382073	CCDC84	338657	-1.86	molecular_function	Metabolism	CS score, function	
Cct2	12461	CCT2	10576	-3.23	protein folding	Metabolism	CS score, function	
Cct3	12462	CCT3	7203	-3.31	protein folding	Metabolism	CS score, function	
Cct4	12464	CCT4	10575	-2.62	protein folding	Metabolism	CS score, function	
Cct5	12465	CCT5	22948	-2.84	protein folding	Metabolism	CS score, function	
Cct7	12468	CCT7	10574	-2.47	protein folding	Metabolism	CS score, function	
Cct8	12469	CCT8	10694	-2.03	protein folding	Metabolism	CS score, function	
Cdipt	52858	CDIPT	10423	-2.53	phospholipid biosynthetic process	Metabolism	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Cenpi	102920	CENPI	2491	-1.81	centromere complex assembly	Metabolism	CS score, function	
Chordc1	66917	CHORDC1	26973	-1.52	regulation of centrosome duplication	Metabolism	CS score, mouse K.O., function	Ferretti R, et al. Dev Cell. 2010 Mar 16;18(3):486-95
Coa5	76178	COA5	493753	-2.33	mitochondrion	Metabolism	CS score, function	
Cog4	102339	COG4	25839	-1.39	Golgi vesicle transport	Metabolism	CS score, function	
Copa	12847	COPA	1314	-1.63	intracellular protein transport	Metabolism	CS score, function	
Copb1	70349	COPB1	1315	-2.30	intracellular protein transport	Metabolism	CS score, function	
Copb2	50797	COPB2	9276	-2.65	intracellular protein transport	Metabolism	CS score, function	
Cope	59042	COPE	11316	-2.93	ER to Golgi vesicle-mediated transport	Metabolism	CS score, function	
Copz1	56447	COPZ1	22818	-1.87	transport	Metabolism	CS score, function	
Coq4	227683	COQ4	51117	-1.29	ubiquinone biosynthetic process	Metabolism	CS score, function	
Cox15	226139	COX15	1355	-2.14	mitochondrial electron transport, cytochrome c to oxygen	Metabolism	CS score, mouse K.O., function	Viscomi C, et al. Cell Metab. 2011 Jul

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
								6;14(1):80-90
Cox17	12856	COX17	10063	-1.97	copper ion transport	Metabolism	CS score, mouse K.O., function	Takahashi Y, et al. Mol Cell Biol. 2002 Nov;22(21):7614-21
Cse1l	110750	CSE1L	1434	-2.31	protein export from nucleus	Metabolism	CS score, mouse K.O., function	Bera TK, et al. Mol Cell Biol. 2001 Oct;21(20):7020-4
Csnk2b	13001	CSNK2B	1460	-1.94	regulation of protein kinase activity	Metabolism	CS score, mouse K.O., function	Buchou T, et al. Mol Cell Biol. 2003 Feb;23(3):908-15
Cycs	13063	CYCS	54205	-2.36	response to reactive oxygen species	Metabolism	CS score, mouse K.O., function	Li K, et al. Cell. 2000 May 12;101(4):389-99
Dad1	13135	DAD1	1603	-2.21	protein glycosylation	Metabolism	CS score, mouse K.O., function	Brewster JL, et al. Genesis. 2000 Apr;26(4):271-8
Dap3	65111	DAP3	7818	-1.70	apoptotic process	Metabolism	CS score, mouse K.O., function	Kim HR, et al. FASEB J. 2007 Jan;21(1):188-96

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Dctn5	59288	DCTN5	84516	-2.39	antigen processing and presentation of exogenous peptide antigen via MHC class II	Metabolism	CS score, function	
Ddost	13200	DDOST	1650	-2.38	protein N-linked glycosylation via asparagine	Metabolism	CS score, function	
Dgcr8	94223	DGCR8	54487	-2.10	gene expression	Metabolism	CS score, mouse K.O., function	Ouchi Y, et al. J Neurosci. 2013 May 29;33(22): 9408-19
Dhodh	56749	DHODH	1723	-2.57	de novo' pyrimidine nucleobase biosynthetic process	Metabolism	CS score, function	
Dnlz	52838	DNLZ	728489	-1.92	protein folding	Metabolism	CS score, function	
Dnm1l	74006	DNM1L	10059	-3.25	mitochondrial fission	Metabolism	CS score, mouse K.O., function	Wakabaya shi J, et al. J Cell Biol. 2009 Sep 21;186(6): 805-16
Dnm2	13430	DNM2	1785	-3.98	endocytosis	Metabolism	CS score, mouse K.O., function	Ferguson SM, et al. Dev Cell. 2009 Dec;17(6): 811-22

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Dohh	102115	DOHH	83475	-1.76	peptidyl-lysine modification to peptidyl-hypusine	Metabolism	CS score, function	
Dolk	227697	DOLK	22845	-2.38	dolichol-linked oligosaccharide biosynthetic process	Metabolism	CS score, function	
Donson	60364	DONSON	29980	-2.30	multicellular organismal development	Metabolism	CS score, function	
Dph3	105638	DPH3	285381	-1.62	peptidyl-diphthamide biosynthetic process from peptidyl-histidine	Metabolism	CS score, mouse K.O., function	Liu S, et al. Mol Cell Biol. 2006 May;26(10):3835-41
Dtymk	21915	DTYMK	1841	-3.54	phosphorylation	Metabolism	CS score, function	
Eif2b2	217715	EIF2B2	8892	-2.24	ovarian follicle development	Metabolism	CS score, function	
Eif2s2	67204	EIF2S2	8894	-2.33	in utero embryonic development	Metabolism	CS score, mouse K.O., function	Heaney JD, et al. Hum Mol Genet. 2009 Apr 15;18(8):1395-404
Emc1	230866	EMC1	23065	-1.34	protein folding in endoplasmic reticulum	Metabolism	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Emc7	73024	EMC7	56851	-2.27	biological_process	Metabolism	CS score, function	
Eno1	13806	ENO1	2023	-2.03	glycolytic process	Metabolism	CS score, mouse K.O., function	Couldrey C, et al. Dev Dyn. 1998 Jun;212(2):284-92
Fam50a	108160	FAM50A	9130	-3.16	spermatogenesis	Metabolism	CS score, function	
Fam96b	68523	FAM96B	51647	-1.90	iron-sulfur cluster assembly	Metabolism	CS score, function	
Fdps	110196	FDPS	2224	-2.41	isoprenoid biosynthetic process	Metabolism	CS score, function	
Gapdh	14433	GAPDH	2597	-2.40	oxidation-reduction process	Metabolism	CS score, function	
Gart	14450	GART	2618	-1.87	purine nucleobase biosynthetic process	Metabolism	CS score, function	
Gemin4	276919	GEMIN4	50628	-1.56	spliceosomal snRNP assembly	Metabolism	CS score, function	
Gemin5	216766	GEMIN5	25929	-2.51	spliceosomal snRNP assembly	Metabolism	CS score, function	
Ggps1	14593	GGPS1	9453	-1.62	cholesterol biosynthetic process	Metabolism	CS score, function	
Gmppb	331026	GMPPB	29925	-3.22	biosynthetic process	Metabolism	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Gnb1l	13972	GNB1L	54584	-1.93	G-protein coupled receptor signaling pathway	Metabolism	CS score, function	
n/a	n/a	GOLGA6L1	283767	-3.15		Metabolism	CS score, function	
Gosr2	56494	GOSR2	9570	-1.13	protein targeting to vacuole	Metabolism	CS score, function	
Gpkow	209416	GPKOW	27238	-1.36	biological_process	Metabolism	CS score, function	
Gpn2	100210	GPN2	54707	-3.71	biological_process	Metabolism	CS score, function	
Gps1	209318	GPS1	2873	-2.11	inactivation of MAPK activity	Metabolism	CS score, function	
Grpel1	17713	GRPEL1	80273	-2.61	protein folding	Metabolism	CS score, function	
Grwd1	101612	GRWD1	83743	-1.90	poly(A) RNA binding	Metabolism	CS score, function	
Hmgcr	15357	HMGCR	3156	-2.94	cholesterol biosynthetic process	Metabolism	CS score, mouse K.O., function	Ohashi K, et al. J Biol Chem. 2003 Oct 31;278(44):42936-41
Hmgcs1	208715	HMGCS1	3157	-2.41	liver development	Metabolism	CS score, function	
Hspa5	14828	HSPA5	3309	-3.86	platelet degranulation	Metabolism	CS score,	Luo S, et al. Mol

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
							mouse K.O., function	Cell Biol. 2006 Aug;26(15):5688-97
Hspa9	15526	HSPA9	3313	-3.55	protein folding	Metabolism	CS score, function	
Hspd1	15510	HSPD1	3329	-1.95	response to hypoxia	Metabolism	CS score, mouse K.O., function	Christensen JH, et al. Cell Stress Chaperones. 2010 Nov;15(6):851-63
Hspe1	15528	HSPE1	3336	-3.75	osteoblast differentiation	Metabolism	CS score, function	
Hyou1	12282	HYOU1	10525	-2.06	response to ischemia	Metabolism	CS score, function	
Ipo13	230673	IPO13	9670	-2.84	intracellular protein transport	Metabolism	CS score, function	
Iscu	66383	ISCU	23479	-2.40	cellular iron ion homeostasis	Metabolism	CS score, function	
Itpk1	217837	ITPK1	3705	-1.55	phosphorylation	Metabolism	CS score, function	
Kansl2	69612	KANSL2	54934	-1.19	chromatin organization	Metabolism	CS score, function	
Kansl3	226976	KANSL3	55683	-1.53	chromatin organization	Metabolism	CS score, function	
Kri1	215194	KRI1	65095	-2.49	poly(A) RNA binding	Metabolism	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Lamtor2	83409	LAMTOR2	28956	-1.62	activation of MAPKK activity	Metabolism	CS score, mouse K.O., function	Teis D, et al. J Cell Biol. 2006 Dec 18;175(6): 861-8
Leng8	232798	LENG8	114823	-1.75	biological_process	Metabolism	CS score, function	
Ltv1	353258	LTV1	84946	-1.81	nucleoplasm	Metabolism	CS score, function	
Mak16	67920	MAK16	84549	-2.30	poly(A) RNA binding	Metabolism	CS score, function	
Mat2a	232087	MAT2A	4144	-2.34	S-adenosylmethionine biosynthetic process	Metabolism	CS score, function	
Mcm3ap	54387	MCM3AP	8888	-1.58	immune system process	Metabolism	CS score, mouse K.O., function	Yoshida M, et al. Genes Cells. 2007 Oct;12(10) :1205-13
Mdn1	100019	MDN1	23195	-1.68	protein complex assembly	Metabolism	CS score, function	
n/a	n/a	MFAP1	4236	-1.94	biological_process	Metabolism	CS score, function	
Mmgt1	236792	MMGT1	93380	-1.55	magnesium ion transport	Metabolism	CS score, function	
Mrpl16	94063	MRPL16	54948	-1.80	organelle organization	Metabolism	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Mrpl17	27397	MRPL17	63875	-1.80	mitochondrial genome maintenance	Metabolism	CS score, function	
Mrpl33	66845	MRPL33	9553	-1.62	organelle organization	Metabolism	CS score, function	
Mrpl38	60441	MRPL38	64978	-1.95	organelle organization	Metabolism	CS score, function	
Mrpl39	27393	MRPL39	54148	-1.71	organelle organization	Metabolism	CS score, function	
Mrpl45	67036	MRPL45	84311	-1.75	organelle organization	Metabolism	CS score, function	
Mrpl46	67308	MRPL46	26589	-1.83	organelle organization	Metabolism	CS score, function	
Mrpl53	68499	MRPL53	116540	-1.84	organelle organization	Metabolism	CS score, function	
Mrps22	64655	MRPS22	56945	-1.32	organelle organization	Metabolism	CS score, function	
Mrps25	64658	MRPS25	64432	-1.63	organelle organization	Metabolism	CS score, function	
Mrps35	232536	MRPS35	60488	-1.60	organelle organization	Metabolism	CS score, function	
Mrps5	77721	MRPS5	64969	-1.65	organelle organization	Metabolism	CS score, function	
Mvd	192156	MVD	4597	-3.24	isoprenoid biosynthetic process	Metabolism	CS score, function	
Mvk	17855	MVK	4598	-1.73	isoprenoid biosynthetic process	Metabolism	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Naa25	231713	NAA25	80018	-2.40	peptide alpha-N-acetyltransferase activity	Metabolism	CS score, function	
Napa	108124	NAPA	8775	-2.31	intracellular protein transport	Metabolism	CS score, function	
Nat10	98956	NAT10	55226	-2.16	biological_process	Metabolism	CS score, function	
Ndor1	78797	NDOR1	27158	-2.10	cell death	Metabolism	CS score, function	
Ndufab1	70316	NDUFAB1	4706	-1.83	fatty acid biosynthetic process	Metabolism	CS score, function	
Nol10	217431	NOL10	79954	-1.79	poly(A) RNA binding	Metabolism	CS score, function	
Nop9	67842	NOP9	161424	-1.44	biological_process	Metabolism	CS score, function	
Nrde2	217827	NRDE2	55051	-2.69	biological_process	Metabolism	CS score, function	
Nsf	18195	NSF	4905	-2.76	intra-Golgi vesicle-mediated transport	Metabolism	CS score, function	
Nubp1	26425	NUBP1	4682	-2.05	cellular iron ion homeostasis	Metabolism	CS score, function	
Nudcd3	209586	NUDCD3	23386	-1.71	molecular_function	Metabolism	CS score, function	
Nup155	170762	NUP155	9631	-1.59	nucleocytoplasmic transport	Metabolism	CS score, mouse	Zhang X, et al. Cell. 2008 Dec

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
							K.O., function	12;135(6): 1017-27
Nup93	71805	NUP93	9688	-2.11	protein import into nucleus	Metabolism	CS score, function	
Nus1	52014	NUS1	116150	-1.94	angiogenesis	Metabolism	CS score, mouse K.O., function	Park EJ, et al. Cell Metab. 2014 Sep 2;20(3):448-57
Nvl	67459	NVL	4931	-2.61	positive regulation of telomerase activity	Metabolism	CS score, function	
Ogdh	18293	OGDH	4967	-2.98	tricarboxylic acid cycle	Metabolism	CS score, function	
Osbp	76303	OSBP	5007	-2.06	lipid transport	Metabolism	CS score, function	
Pak1ip1	68083	PAK1IP1	55003	-2.28	cell proliferation	Metabolism	CS score, function	
Pfdn2	18637	PFDN2	5202	-1.32	protein folding	Metabolism	CS score, function	
Pgam1	18648	PGAM1	5223	-2.37	glycolytic process	Metabolism	CS score, function	
Pkm	18746	PKM	5315	-1.68	glycolytic process	Metabolism	CS score, mouse K.O., function	Lewis SE, et al. 1983:267-78. Plenum Publ. Corp.

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Pmpcb	73078	PMPCB	9512	-1.77	proteolysis	Metabolism	CS score, function	
Ppil2	66053	PPIL2	23759	-3.01	protein polyubiquitination	Metabolism	CS score, function	
Ppp4c	56420	PPP4C	5531	-2.89	protein dephosphorylation	Metabolism	CS score, mouse K.O., function	Toyo-oka K, et al. J Cell Biol. 2008 Mar 24;180(6): 1133-47
Prelid1	66494	PRELID1	27166	-2.27	apoptotic process	Metabolism	CS score, function	
Prpf31	68988	PRPF31	26121	-3.20	spliceosomal tri-snRNP complex assembly	Metabolism	CS score, mouse K.O., function	Bujakowska K, et al. Invest Ophthalmol Vis Sci. 2009 Dec;50(12):5927-33
Prpf6	68879	PRPF6	24148	-2.96	spliceosomal tri-snRNP complex assembly	Metabolism	CS score, function	
Psma1	26440	PSMA1	5682	-2.39	proteasomal ubiquitin-independent protein catabolic process	Metabolism	CS score, function	
Psma2	19166	PSMA2	5683	-2.23	proteasomal ubiquitin-independent protein catabolic process	Metabolism	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Psma3	19167	PSMA3	5684	-2.30	proteasomal ubiquitin-independent protein catabolic process	Metabolism	CS score, function	
Psmb2	26445	PSMB2	5690	-2.12	proteasomal ubiquitin-independent protein catabolic process	Metabolism	CS score, function	
Psmb3	26446	PSMB3	5691	-2.78	proteolysis involved in cellular protein catabolic process	Metabolism	CS score, function	
Psmb5	19173	PSMB5	5693	-1.67	proteasomal ubiquitin-independent protein catabolic process	Metabolism	CS score, function	
Psmb6	19175	PSMB6	5694	-2.42	proteasomal ubiquitin-independent protein catabolic process	Metabolism	CS score, function	
Psmb7	19177	PSMB7	5695	-2.69	proteasomal ubiquitin-independent protein catabolic process	Metabolism	CS score, function	
Psmc2	19181	PSMC2	5701	-2.35	protein catabolic process	Metabolism	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Psmc3	19182	PSMC3	5702	-2.76	ER-associated ubiquitin-dependent protein catabolic process	Metabolism	CS score, mouse K.O., function	Sakao Y, et al. Genomics. 2000 Jul 1;67(1):1-7
Psmc4	23996	PSMC4	5704	-2.36	blastocyst development	Metabolism	CS score, mouse K.O., function	Sakao Y, et al. Genomics. 2000 Jul 1;67(1):1-7
Psmd1	70247	PSMD1	5707	-1.88	regulation of protein catabolic process	Metabolism	CS score, function	
Psmd2	21762	PSMD2	5708	-2.16	regulation of protein catabolic process	Metabolism	CS score, function	
Psmd3	22123	PSMD3	5709	-2.10	regulation of protein catabolic process	Metabolism	CS score, function	
Psmd4	19185	PSMD4	5710	-1.77	ubiquitin-dependent protein catabolic process	Metabolism	CS score, mouse K.O., function	Soriano P, et al. Genes Dev. 1987 Jun;1(4):366-75
Psmd6	66413	PSMD6	9861	-2.27	proteasome-mediated ubiquitin-dependent protein catabolic process	Metabolism	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Psmg3	66506	PSMG3	84262	-2.57	molecular_function	Metabolism	CS score, function	
Ptpmt1	66461	PTPMT1	114971	-2.89	protein dephosphorylation	Metabolism	CS score, mouse K.O., function	Shen J, et al. Mol Cell Biol. 2011 Dec;31(24):4902-16
Ptpn23	104831	PTPN23	25930	-1.59	negative regulation of epithelial cell migration	Metabolism	CS score, mouse K.O., function	Gingras MC, et al. Int J Dev Biol. 2009;53(7):1069-74
Rabggta	56187	RABGGTA	5875	-3.18	protein prenylation	Metabolism	CS score, function	
Rabggtb	19352	RABGGTB	5876	-2.44	protein geranylgeranylation	Metabolism	CS score, function	
Rbm19	74111	RBM19	9904	-2.03	multicellular organismal development	Metabolism	CS score, mouse K.O., function	Zhang J, et al. BMC Dev Biol. 2008;8:115
Rfk	54391	RFK	55312	-1.56	riboflavin biosynthetic process	Metabolism	CS score, mouse K.O., function	Yazdanpanah B, et al. Nature. 2009 Aug 27;460(7259):1159-63
Rheb	19744	RHEB	6009	-1.38	signal transduction	Metabolism	CS score, mouse K.O., function	Zou J, et al. Dev Cell. 2011 Jan 18;20(1):97-108

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Riok1	71340	RIOK1	83732	-1.27	protein phosphorylation	Metabolism	CS score, function	
Rpn1	103963	RPN1	6184	-2.13	protein glycosylation	Metabolism	CS score, function	
Rtfdc1	66404	RTFDC1	51507	-2.09	biological_process	Metabolism	CS score, function	
Sacm1l	83493	SACM1L	22908	-1.80	protein dephosphorylation	Metabolism	CS score, function	
Samm50	68653	SAMM50	25813	-1.62	protein targeting to mitochondrion	Metabolism	CS score, function	
Sco2	100126824	SCO2	9997	-1.60	eye development	Metabolism	CS score, mouse K.O., function	Yang H, et al. Hum Mol Genet. 2010 Jan 1;19(1):170-80
Sdha	66945	SDHA	6389	-2.20	tricarboxylic acid cycle	Metabolism	CS score, function	
Sdhb	67680	SDHB	6390	-2.33	tricarboxylic acid cycle	Metabolism	CS score, function	
Sec61a1	53421	SEC61A1	29927	-2.42	protein transport	Metabolism	CS score, function	
Slc20a1	20515	SLC20A1	6574	-2.38	sodium ion transport	Metabolism	CS score, mouse K.O., function	Festing MH, et al. Genesis. 2009 Dec;47(12):858-63

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Slc7a6os	66432	SLC7A6OS	84138	-2.30	hematopoietic progenitor cell differentiation	Metabolism	CS score, function	
Smn1	20595	SMN1	6606	-1.58	spliceosomal complex assembly	Metabolism	CS score, mouse K.O., function	Hsieh-Li HM, et al. Nat Genet. 2000 Jan;24(1): 66-70
Smu1	74255	SMU1	55234	-3.65	molecular_function	Metabolism	CS score, function	
Snrpd1	20641	SNRPD1	6632	-2.79	spliceosomal complex assembly	Metabolism	CS score, function	
Snrpd3	67332	SNRPD3	6634	-3.62	spliceosomal complex assembly	Metabolism	CS score, function	
Snrpe	20643	SNRPE	6635	-2.74	spliceosomal complex assembly	Metabolism	CS score, function	
Spata5	57815	SPATA5	166378	-1.50	multicellular organismal development	Metabolism	CS score, function	
Spata5l1	214616	SPATA5L1	79029	-2.70	molecular_function	Metabolism	CS score, function	
Tango6	272538	TANGO6	79613	-2.29	integral component of membrane	Metabolism	CS score, function	
n/a	n/a	TBC1D3B	414059	-1.67	positive regulation of GTPase activity	Metabolism	CS score, function	
n/a	n/a	TBC1D3C	414060	-2.01	positive regulation of GTPase activity	Metabolism	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Tbcb	66411	TBCB	1155	-1.97	nervous system development	Metabolism	CS score, function	
Tbcc	72726	TBCC	6903	-3.02	cell morphogenesis	Metabolism	CS score, function	
Tbcd	108903	TBCD	6904	-1.82	microtubule cytoskeleton organization	Metabolism	CS score, function	
Tcp1	21454	TCP1	6950	-2.34	protein folding	Metabolism	CS score, function	
Telo2	71718	TELO2	9894	-2.34	regulation of TOR signaling	Metabolism	CS score, mouse K.O., function	Takai H, et al. Cell. 2007 Dec 28;131(7): 1248-59
Tex10	269536	TEX10	54881	-1.26	integral component of membrane	Metabolism	CS score, function	
Tfrc	22042	TFRC	7037	-3.40	cellular iron ion homeostasis	Metabolism	CS score, mouse K.O., function	Levy JE, et al. Nat Genet. 1999 Apr;21(4): 396-9
Timm10	30059	TIMM10	26519	-1.99	protein targeting to mitochondrion	Metabolism	CS score, function	
Timm13	30055	TIMM13	26517	-1.62	protein targeting to mitochondrion	Metabolism	CS score, function	
Timm23	53600	TIMM23	100287932	-2.00	protein targeting to mitochondrion	Metabolism	CS score, mouse K.O., function	Ahting U, et al. Biochim Biophys Acta. 2009 May;1787(5):371-6

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Timm44	21856	TIMM44	10469	-1.73	protein import into mitochondrial matrix	Metabolism	CS score, function	
Tmx2	66958	TMX2	51075	-2.29	biological_process	Metabolism	CS score, function	
Tnpo3	320938	TNPO3	23534	-1.82	splicing factor protein import into nucleus	Metabolism	CS score, function	
Trmt112	67674	TRMT112	51504	-3.70	peptidyl-glutamine methylation	Metabolism	CS score, function	
Trnau1ap	71787	TRNAU1AP	54952	-1.40	selenocysteine incorporation	Metabolism	CS score, function	
Ttc1	66827	TTC1	7265	-1.74	protein folding	Metabolism	CS score, function	
Ttc27	74196	TTC27	55622	-2.54	biological_process	Metabolism	CS score, function	
Tti1	75425	TTI1	9675	-2.91	regulation of TOR signaling	Metabolism	CS score, function	
Tti2	234138	TTI2	80185	-1.94	molecular_function	Metabolism	CS score, function	
n/a	n/a	TUBB	203068	-3.40	microtubule-based process	Metabolism	CS score, function	
Txn2	56551	TXN2	25828	-1.41	sulfate assimilation	Metabolism	CS score, mouse K.O., function	Nonn L, et al. Mol Cell Biol. 2003 Feb;23(3): 916-22

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Uqcrc1	22273	UQCRC1	7384	-1.29	oxidative phosphorylation	Metabolism	CS score, function	
Uqcrh	66576	UQCRH	7388	-1.28	oxidative phosphorylation	Metabolism	CS score, function	
Urb2	382038	URB2	9816	-2.25	molecular_function	Metabolism	CS score, function	
Vmp1	75909	VMP1	81671	-1.75	exocytosis	Metabolism	CS score, function	
n/a	n/a	VPS28	51160	-3.06	protein targeting to vacuole involved in ubiquitin-dependent protein catabolic process via the multivesicular body sorting pathway	Metabolism	CS score, function	
Vps29	56433	VPS29	51699	-2.05	intracellular protein transport	Metabolism	CS score, function	
Vps52	224705	VPS52	6293	-1.85	ectodermal cell differentiation	Metabolism	CS score, mouse K.O., function	Sugimoto M, et al. Cell Rep. 2012 Nov 29;2(5):1363-74
Wars2	70560	WARS2	10352	-1.16	vasculogenesis	Metabolism	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Wdr7	104082	WDR7	23335	-1.47	hematopoietic progenitor cell differentiation	Metabolism	CS score, function	
Wdr70	545085	WDR70	55100	-1.69	enzyme binding	Metabolism	CS score, function	
Wdr74	107071	WDR74	54663	-2.84	blastocyst formation	Metabolism	CS score, function	
Wdr77	70465	WDR77	79084	-2.19	spliceosomal snRNP assembly	Metabolism	CS score, mouse K.O., function	Zhou L, et al. J Mol Endocrinol. 2006 Oct;37(2): 283-300
Yae1d1	67008	YAE1D1	57002	-1.71	molecular_function	Metabolism	CS score, function	
Yrdc	230734	YRDC	79693	-2.33	negative regulation of transport	Metabolism	CS score, function	
Znhit2	29805	ZNHIT2	741	-2.70	metal ion binding	Metabolism	CS score, function	
Aars	234734	AARS	16	-2.48	alanyl-tRNA aminoacylation	RNA transcription, protein translation	CS score, function	
Bms1	213895	BMS1	9790	-1.36	ribosome assembly	RNA transcription, protein translation	CS score, function	
Bud31	231889	BUD31	8896	-2.46	mRNA splicing, via spliceosome	RNA transcription, protein translation	CS score, function	
Bysl	53414	BYSL	705	-2.24	maturation of SSU-rRNA from	RNA transcriptio	CS score, mouse	Aoki R, et al. FEBS Lett. 2006

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
					tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	n, protein translation	K.O., function	Nov 13;580(26):6062-8
Cars	27267	CARS	833	-2.45	tRNA aminoacylation for protein translation	RNA transcription, protein translation	CS score, function	
Cdc5l	71702	CDC5L	988	-2.09	mRNA splicing, via spliceosome	RNA transcription, protein translation	CS score, function	
Cdc73	214498	CDC73	79577	-2.58	negative regulation of transcription from RNA polymerase II promoter	RNA transcription, protein translation	CS score, mouse K.O., function	Wang P, et al. Mol Cell Biol. 2008 May;28(9):2930-40
Cebpz	12607	CEBPZ	10153	-2.11	transcription from RNA polymerase II promoter	RNA transcription, protein translation	CS score, function	
Clasrp	53609	CLASRP	11129	-1.30	mRNA processing	RNA transcription, protein translation	CS score, function	
Clp1	98985	CLP1	10978	-3.47	mRNA splicing, via spliceosome	RNA transcription, protein translation	CS score, mouse K.O., function	Hanada T, et al. Nature. 2013 Mar 28;495(7442):474-80
Cox5b	12859	COX5B	1329	-1.50	transcription initiation from RNA	RNA transcription, protein translation	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
					polymerase II promoter			
Cpsf1	94230	CPSF1	29894	-2.58	mRNA splicing, via spliceosome	RNA transcription, protein translation	CS score, function	
Cpsf2	51786	CPSF2	53981	-2.55	mRNA polyadenylation	RNA transcription, protein translation	CS score, function	
Cpsf3l	71957	CPSF3L	54973	-2.09	snRNA processing	RNA transcription, protein translation	CS score, function	
Dars	226414	DARS	1615	-2.90	translation	RNA transcription, protein translation	CS score, function	
Dbr1	83703	DBR1	51163	-3.75	RNA splicing, via transesterification reactions	RNA transcription, protein translation	CS score, function	
Ddx18	66942	DDX18	8886	-2.33	RNA secondary structure unwinding	RNA transcription, protein translation	CS score, function	
Ddx23	74351	DDX23	9416	-3.01	RNA secondary structure unwinding	RNA transcription, protein translation	CS score, function	
Ddx24	27225	DDX24	57062	-1.40	RNA secondary structure unwinding	RNA transcription, protein translation	CS score, function	
Ddx41	72935	DDX41	51428	-1.74	RNA secondary structure unwinding	RNA transcription, protein translation	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Ddx46	212880	DDX46	9879	-2.79	mRNA splicing, via spliceosome	RNA transcription, protein translation	CS score, function	
Ddx47	67755	DDX47	51202	-2.20	RNA secondary structure unwinding	RNA transcription, protein translation	CS score, function	
Ddx49	234374	DDX49	54555	-3.20	RNA secondary structure unwinding	RNA transcription, protein translation	CS score, function	
Ddx54	71990	DDX54	79039	-2.94	RNA secondary structure unwinding	RNA transcription, protein translation	CS score, function	
Ddx56	52513	DDX56	54606	-2.85	rRNA processing	RNA transcription, protein translation	CS score, function	
Dgcr14	27886	DGCR14	8220	-1.76	mRNA splicing, via spliceosome	RNA transcription, protein translation	CS score, function	
Dhx15	13204	DHX15	1665	-2.58	mRNA processing	RNA transcription, protein translation	CS score, function	
Dhx16	69192	DHX16	8449	-1.35	mRNA processing	RNA transcription, protein translation	CS score, function	
Dhx38	64340	DHX38	9785	-1.76	mRNA splicing, via spliceosome	RNA transcription, protein translation	CS score, function	
Diexf	215193	DIEXF	27042	-2.03	maturation of SSU-rRNA from	RNA transcriptio	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
					tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	n, protein translation		
Dimt1	66254	DIMT1	27292	-1.87	rRNA methylation	RNA transcriptio n, protein translation	CS score, function	
Dis3	72662	DIS3	22894	-1.77	mRNA catabolic process	RNA transcriptio n, protein translation	CS score, function	
Dkc1	245474	DKC1	1736	-2.37	box H/ACA snoRNA 3'-end processing	RNA transcriptio n, protein translation	CS score, mouse K.O., function	He J, et al. Oncogene . 2002 Oct 31;21(50): 7740-4
Dnajc17	69408	DNAJC17	55192	-2.25	negative regulation of transcription from RNA polymerase II promoter	RNA transcriptio n, protein translation	CS score, mouse K.O., function	Amendola E, et al. Endocrinol ogy. 2010 Apr;151(4) :1948-58
Ears2	67417	EARS2	124454	-1.91	tRNA aminoacylatio n for protein translation	RNA transcriptio n, protein translation	CS score, function	
Ebna1bp 2	69072	EBNA1BP 2	10969	-1.52	ribosome biogenesis	RNA transcriptio n, protein translation	CS score, function	
Eef1a1	13627	EEF1A1	1915	-3.11	translational elongation	RNA transcriptio n, protein translation	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Eef1g	67160	EEF1G	1937	-1.42	translation	RNA transcription, protein translation	CS score, function	
Eef2	13629	EEF2	1938	-3.53	translation	RNA transcription, protein translation	CS score, function	
Eftud2	20624	EFTUD2	9343	-3.79	mRNA splicing, via spliceosome	RNA transcription, protein translation	CS score, function	
Eif1ad	69860	EIF1AD	84285	-2.26	translational initiation	RNA transcription, protein translation	CS score, function	
Eif2b1	209354	EIF2B1	1967	-2.23	regulation of translational initiation	RNA transcription, protein translation	CS score, function	
Eif2b3	108067	EIF2B3	8891	-3.00	translational initiation	RNA transcription, protein translation	CS score, function	
Eif2s1	13665	EIF2S1	1965	-3.93	translation	RNA transcription, protein translation	CS score, function	
Eif3c	56347	EIF3C	8663	-2.59	formation of translation preinitiation complex	RNA transcription, protein translation	CS score, function	
n/a	n/a	EIF3CL	728689	-2.71	formation of translation preinitiation complex	RNA transcription, protein translation	CS score, function	
Eif3d	55944	EIF3D	8664	-3.23	formation of translation	RNA transcriptio	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
					preinitiation complex	n, protein translation		
Eif3f	66085	EIF3F	8665	-1.44	formation of translation preinitiation complex	RNA transcriptio n, protein translation	CS score, function	
Eif3g	53356	EIF3G	8666	-3.10	translational initiation	RNA transcriptio n, protein translation	CS score, function	
Eif3i	54709	EIF3I	8668	-2.24	formation of translation preinitiation complex	RNA transcriptio n, protein translation	CS score, function	
Eif3l	223691	EIF3L	51386	-1.28	translational initiation	RNA transcriptio n, protein translation	CS score, function	
Eif4a1	13681	EIF4A1	1973	-1.97	translational initiation	RNA transcriptio n, protein translation	CS score, function	
Eif4a3	192170	EIF4A3	9775	-4.32	RNA splicing	RNA transcriptio n, protein translation	CS score, function	
Eif4g1	208643	EIF4G1	1981	-1.79	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	RNA transcriptio n, protein translation	CS score, function	
Eif5b	226982	EIF5B	9669	-2.93	translational initiation	RNA transcriptio n, protein translation	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Eif6	16418	EIF6	3692	-2.75	mature ribosome assembly	RNA transcription, protein translation	CS score, mouse K.O., function	Gandin V, et al. Nature. 2008 Oct 2;455(7213):684-8
Elac2	68626	ELAC2	60528	-2.06	tRNA 3'-trailer cleavage, endonucleolytic	RNA transcription, protein translation	CS score, function	
Ell	13716	ELL	8178	-2.23	transcription elongation from RNA polymerase II promoter	RNA transcription, protein translation	CS score, mouse K.O., function	Mitani K, et al. Biochem Biophys Res Commun. 2000 Dec 20;279(2):563-7
Etf1	225363	ETF1	2107	-2.44	translational termination	RNA transcription, protein translation	CS score, function	
Exosc2	227715	EXOSC2	23404	-1.66	exonucleolytic trimming to generate mature 3'-end of 5.8S rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	RNA transcription, protein translation	CS score, function	
Exosc4	109075	EXOSC4	54512	-3.21	nuclear-transcribed mRNA catabolic	RNA transcription, protein translation	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
					process, deadenylation -dependent decay			
Exosc5	27998	EXOSC5	56915	-2.09	rRNA catabolic process	RNA transcriptio n, protein translation	CS score, function	
n/a	n/a	EXOSC6	118460	-3.20	nuclear-transcribed mRNA catabolic process, deadenylation -dependent decay	RNA transcriptio n, protein translation	CS score, function	
Exosc7	66446	EXOSC7	23016	-2.17	nuclear-transcribed mRNA catabolic process, deadenylation -dependent decay	RNA transcriptio n, protein translation	CS score, function	
Exosc8	69639	EXOSC8	11340	-2.08	nuclear-transcribed mRNA catabolic process, deadenylation -dependent decay	RNA transcriptio n, protein translation	CS score, function	
Fars2	69955	FARS2	10667	-1.90	tRNA aminoacylatio n for protein translation	RNA transcriptio n, protein translation	CS score, function	
Farsa	66590	FARSA	2193	-3.30	phenylalanyl-tRNA	RNA transcriptio	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
					aminoacylation	n, protein translation		
Farsb	23874	FARSB	10056	-2.49	phenylalanyl-tRNA aminoacylation	RNA transcription, protein translation	CS score, function	
Fau	14109	FAU	2197	-2.64	translation	RNA transcription, protein translation	CS score, function	
Fip1l1	66899	FIP1L1	81608	-1.93	mRNA processing	RNA transcription, protein translation	CS score, function	
Ftsj3	56095	FTSJ3	117246	-1.50	rRNA methylation	RNA transcription, protein translation	CS score, function	
Gle1	74412	GLE1	2733	-1.89	mRNA export from nucleus	RNA transcription, protein translation	CS score, function	
Gnl3l	237107	GNL3L	54552	-1.35	ribosome biogenesis	RNA transcription, protein translation	CS score, function	
Gtf2e1	74197	GTF2E1	2960	-1.22	transcriptional open complex formation at RNA polymerase II promoter	RNA transcription, protein translation	CS score, function	
Gtpbp4	69237	GTPBP4	23560	-2.25	ribosome biogenesis	RNA transcription, protein translation	CS score, function	
Hars	15115	HARS	3035	-3.49	histidyl-tRNA aminoacylation	RNA transcriptio	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
						n, protein translation		
Hars2	70791	HARS2	23438	-1.92	histidyl-tRNA aminoacylation	RNA transcription, protein translation	CS score, function	
Heatr1	217995	HEATR1	55127	-2.58	maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	RNA transcription, protein translation	CS score, function	
Hnrnpc	15381	HNRNPC	3183	-1.95	mRNA splicing, via spliceosome	RNA transcription, protein translation	CS score, mouse K.O., function	Williamson DJ, et al. Mol Cell Biol. 2000 Jun;20(11):4094-105
Hnrnpk	15387	HNRNPK	3190	-2.39	mRNA splicing, via spliceosome	RNA transcription, protein translation	CS score, function	
Hnrnpl	15388	HNRNPL	3191	-1.88	mRNA processing	RNA transcription, protein translation	CS score, mouse K.O., function	Gaudreau MC, et al. J Immunol. 2012 Jun 1;188(11):5377-88
Hnrnpu	51810	HNRNPU	3192	-2.44	mRNA splicing, via spliceosome	RNA transcription, protein translation	CS score, mouse K.O., function	Roshon MJ, et al. Transgenic Res. 2005 Apr;14(2):179-92

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
lars	105148	IARS	3376	-3.87	isoleucyl-tRNA aminoacylation	RNA transcription, protein translation	CS score, function	
lars2	381314	IARS2	55699	-2.83	tRNA aminoacylation for protein translation	RNA transcription, protein translation	CS score, function	
Imp3	102462	IMP3	55272	-3.46	rRNA processing	RNA transcription, protein translation	CS score, function	
Imp4	27993	IMP4	92856	-2.01	rRNA processing	RNA transcription, protein translation	CS score, function	
Ints1	68510	INTS1	26173	-1.93	snRNA processing	RNA transcription, protein translation	CS score, mouse K.O., function	Nakayama M, et al. FASEB J. 2006 Aug;20(10):1718-20
Ints4	101861	INTS4	92105	-1.75	snRNA processing	RNA transcription, protein translation	CS score, function	
Ints5	109077	INTS5	80789	-2.10	snRNA processing	RNA transcription, protein translation	CS score, function	
Ints8	72656	INTS8	55656	-1.35	snRNA processing	RNA transcription, protein translation	CS score, function	
Ints9	210925	INTS9	55756	-2.26	snRNA processing	RNA transcription, protein translation	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Isg20l2	229504	ISG20L2	81875	-2.27	ribosome biogenesis	RNA transcription, protein translation	CS score, function	
Kars	85305	KARS	3735	-2.76	tRNA aminoacylation for protein translation	RNA transcription, protein translation	CS score, function	
n/a	n/a	KIAA0391	9692	-1.56	tRNA processing	RNA transcription, protein translation	CS score, function	
Lars	107045	LARS	51520	-1.83	tRNA aminoacylation for protein translation	RNA transcription, protein translation	CS score, function	
Lars2	102436	LARS2	23395	-1.60	tRNA aminoacylation for protein translation	RNA transcription, protein translation	CS score, function	
Las1l	76130	LAS1L	81887	-2.12	rRNA processing	RNA transcription, protein translation	CS score, function	
Lrpprc	72416	LRPPRC	10128	-1.39	negative regulation of mitochondrial RNA catabolic process	RNA transcription, protein translation	CS score, mouse K.O., function	Ruzzenente B, et al. EMBO J. 2012 Jan 18;31(2):443-56
Lsm2	27756	LSM2	57819	-2.96	nuclear-transcribed mRNA catabolic process, deadenylation-dependent decay	RNA transcription, protein translation	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Lsm3	67678	LSM3	27258	-1.66	nuclear-transcribed mRNA catabolic process, deadenylation-dependent decay	RNA transcription, protein translation	CS score, function	
Lsm7	66094	LSM7	51690	-1.96	nuclear-transcribed mRNA catabolic process, deadenylation-dependent decay	RNA transcription, protein translation	CS score, function	
Magoh	17149	MAGOH	4116	-1.78	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	RNA transcription, protein translation	CS score, mouse K.O., function	Silver DL, et al. Nat Neurosci. 2010 May;13(5): 551-8
Mars	216443	MARS	4141	-3.24	methionyl-tRNA aminoacylation	RNA transcription, protein translation	CS score, function	
Mars2	212679	MARS2	92935	-2.31	tRNA aminoacylation for protein translation	RNA transcription, protein translation	CS score, function	
Med17	234959	MED17	9440	-1.78	regulation of transcription from RNA polymerase II promoter	RNA transcription, protein translation	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Med20	56771	MED20	9477	-2.00	regulation of transcription from RNA polymerase II promoter	RNA transcription, protein translation	CS score, function	
Med22	20933	MED22	6837	-1.86	regulation of transcription from RNA polymerase II promoter	RNA transcription, protein translation	CS score, function	
Med27	68975	MED27	9442	-1.48	regulation of transcription from RNA polymerase II promoter	RNA transcription, protein translation	CS score, function	
Med30	69790	MED30	90390	-2.21	regulation of transcription from RNA polymerase II promoter	RNA transcription, protein translation	CS score, function	
Med8	80509	MED8	112950	-1.64	regulation of transcription from RNA polymerase II promoter	RNA transcription, protein translation	CS score, function	
Mepce	231803	MEPCE	56257	-2.08	negative regulation of transcription from RNA polymerase II promoter	RNA transcription, protein translation	CS score, function	
Mettl16	67493	METTL16	79066	-2.10	rRNA base methylation	RNA transcription, protein translation	CS score, function	
Mphosph10	67973	MPHOSPH10	10199	-1.85	RNA splicing, via transesterification reactions	RNA transcription, protein translation	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Mrpl10	107732	MRPL10	124995	-1.38	translation	RNA transcription, protein translation	CS score, function	
Mrpl12	56282	MRPL12	6182	-1.56	translation	RNA transcription, protein translation	CS score, function	
Mrpl21	353242	MRPL21	219927	-1.91	translation	RNA transcription, protein translation	CS score, function	
Mrpl28	68611	MRPL28	10573	-1.50	translation	RNA transcription, protein translation	CS score, function	
Mrpl3	94062	MRPL3	11222	-1.58	translation	RNA transcription, protein translation	CS score, function	
Mrpl34	94065	MRPL34	64981	-1.66	translation	RNA transcription, protein translation	CS score, function	
Mrpl4	66163	MRPL4	51073	-2.41	translation	RNA transcription, protein translation	CS score, function	
Mrpl41	107733	MRPL41	64975	-2.15	translation	RNA transcription, protein translation	CS score, function	
Mrpl51	66493	MRPL51	51258	-1.40	translation	RNA transcription, protein translation	CS score, function	
Mrps14	64659	MRPS14	63931	-1.82	translation	RNA transcriptio	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
						n, protein translation		
Mrps15	66407	MRPS15	64960	-1.28	translation	RNA transcriptio n, protein translation	CS score, function	
Mrps16	66242	MRPS16	51021	-2.29	translation	RNA transcriptio n, protein translation	CS score, function	
Mrps18a	68565	MRPS18A	55168	-1.55	translation	RNA transcriptio n, protein translation	CS score, function	
Mrps2	118451	MRPS2	51116	-1.59	translation	RNA transcriptio n, protein translation	CS score, function	
Mrps21	66292	MRPS21	54460	-1.51	translation	RNA transcriptio n, protein translation	CS score, function	
Mrps24	64660	MRPS24	64951	-1.71	translation	RNA transcriptio n, protein translation	CS score, function	
Mrps6	121022	MRPS6	64968	-1.65	translation	RNA transcriptio n, protein translation	CS score, function	
Nars	70223	NARS	4677	-3.31	tRNA aminoacylatio n for protein translation	RNA transcriptio n, protein translation	CS score, function	
Nars2	244141	NARS2	79731	-1.32	tRNA aminoacylatio n for protein translation	RNA transcriptio n, protein translation	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Ncbp2	68092	NCBP2	22916	-3.00	mRNA cis splicing, via spliceosome	RNA transcription, protein translation	CS score, function	
Nedd8	18002	NEDD8	4738	-2.45	regulation of transcription from RNA polymerase II promoter	RNA transcription, protein translation	CS score, function	
Ngdn	68966	NGDN	25983	-2.35	maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	RNA transcription, protein translation	CS score, function	
Nhp2	52530	NHP2	55651	-1.74	rRNA pseudouridine synthesis	RNA transcription, protein translation	CS score, function	
Nip7	66164	NIP7	51388	-2.03	ribosome assembly	RNA transcription, protein translation	CS score, function	
Noc2l	57741	NOC2L	26155	-2.34	negative regulation of transcription from RNA polymerase II promoter	RNA transcription, protein translation	CS score, function	
Noc4l	100608	NOC4L	79050	-2.11	ribosome biogenesis	RNA transcription, protein translation	CS score, function	
Nol6	230082	NOL6	65083	-2.28	rRNA processing	RNA transcriptio	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
						n, protein translation		
Nol9	74035	NOL9	79707	-2.20	cleavage in ITS2 between 5.8S rRNA and LSU-rRNA of tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	RNA transcriptio n, protein translation	CS score, function	
Nop16	28126	NOP16	51491	-2.10	ribosomal large subunit biogenesis	RNA transcriptio n, protein translation	CS score, function	
Nop2	110109	NOP2	4839	-2.14	rRNA processing	RNA transcriptio n, protein translation	CS score, function	
Nop58	55989	NOP58	51602	-2.54	rRNA modification	RNA transcriptio n, protein translation	CS score, function	
Nsa2	59050	NSA2	10412	-1.78	rRNA processing	RNA transcriptio n, protein translation	CS score, function	
Nudt21	68219	NUDT21	11051	-2.36	mRNA polyadenylati on	RNA transcriptio n, protein translation	CS score, function	
Osgep	66246	OSGEP	55644	-1.98	tRNA processing	RNA transcriptio n, protein translation	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Pabpn1	54196	PABPN1	8106	-1.92	mRNA splicing, via spliceosome	RNA transcription, protein translation	CS score, function	
Pdcd11	18572	PDCD11	22984	-1.47	rRNA processing	RNA transcription, protein translation	CS score, function	
Pes1	64934	PES1	23481	-2.92	maturation of LSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	RNA transcription, protein translation	CS score, mouse K.O., function	Lerch-Gaggl A, et al. J Biol Chem. 2002 Nov 22;277(47):45347-55
Phb	18673	PHB	5245	-2.26	regulation of transcription from RNA polymerase II promoter	RNA transcription, protein translation	CS score, mouse K.O., function	He B, et al. Endocrinology. 2011 Mar;152(3):1047-56
Phf5a	68479	PHF5A	84844	-3.52	mRNA splicing, via spliceosome	RNA transcription, protein translation	CS score, function	
Pnn	18949	PNN	5411	-1.34	mRNA splicing, via spliceosome	RNA transcription, protein translation	CS score, mouse K.O., function	Joo JH, et al. Dev Dyn. 2007 Aug;236(8):2147-58
Polr1b	20017	POLR1B	84172	-3.23	transcription from RNA polymerase I promoter	RNA transcription, protein translation	CS score, mouse K.O., function	Chen H, et al. Biochem Biophys Res Commun. 2008 Jan

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
								25;365(4): 636-42
Polr1c	20016	POLR1C	9533	-2.79	transcription from RNA polymerase I promoter	RNA transcriptio n, protein translation	CS score, function	
Polr2a	20020	POLR2A	5430	-3.15	transcription from RNA polymerase II promoter	RNA transcriptio n, protein translation	CS score, function	
Polr2b	231329	POLR2B	5431	-3.09	transcription from RNA polymerase II promoter	RNA transcriptio n, protein translation	CS score, function	
Polr2c	20021	POLR2C	5432	-3.15	mRNA splicing, via spliceosome	RNA transcriptio n, protein translation	CS score, function	
Polr2d	69241	POLR2D	5433	-2.23	nuclear-transcribed mRNA catabolic process, deadenylation -dependent decay	RNA transcriptio n, protein translation	CS score, function	
Polr2f	69833	POLR2F	5435	-2.31	transcription from RNA polymerase I promoter	RNA transcriptio n, protein translation	CS score, function	
Polr2g	67710	POLR2G	5436	-2.78	nuclear-transcribed mRNA catabolic process, exonucleolytic	RNA transcriptio n, protein translation	CS score, function	
Polr2h	245841	POLR2H	5437	-1.83	transcription from RNA	RNA transcriptio	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
					polymerase I promoter	n, protein translation		
Polr2i	69920	POLR2I	5438	-2.92	maintenance of transcriptional fidelity during DNA-templated transcription elongation from RNA polymerase II promoter	RNA transcriptio n, protein translation	CS score, function	
Polr2j	20022	POLR2J	5439	-3.31	mRNA splicing, via spliceosome	RNA transcriptio n, protein translation	CS score, function	
Polr2l	66491	POLR2L	5441	-3.55	mRNA splicing, via spliceosome	RNA transcriptio n, protein translation	CS score, function	
Polr3e	26939	POLR3E	55718	-2.33	transcription from RNA polymerase III promoter	RNA transcriptio n, protein translation	CS score, function	
Pop1	67724	POP1	10940	-1.79	tRNA 5'-leader removal	RNA transcriptio n, protein translation	CS score, function	
Pop4	66161	POP4	10775	-1.87	RNA phosphodiest er bond hydrolysis	RNA transcriptio n, protein translation	CS score, function	
Ppa1	67895	PPA1	5464	-1.63	tRNA aminoacylatio n for protein translation	RNA transcriptio n, protein translation	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Ppan	235036	PPAN	56342	-1.62	ribosomal large subunit assembly	RNA transcription, protein translation	CS score, function	
Ppp2ca	19052	PPP2CA	5515	-3.01	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	RNA transcription, protein translation	CS score, mouse K.O., function	Gu P, et al. Genesis. 2012 May;50(5): 429-36
Prim1	19075	PRIM1	5557	-2.07	DNA replication, synthesis of RNA primer	RNA transcription, protein translation	CS score, function	
Prpf38b	66921	PRPF38B	55119	-2.68	mRNA processing	RNA transcription, protein translation	CS score, function	
Prpf4	70052	PRPF4	9128	-2.24	RNA splicing	RNA transcription, protein translation	CS score, function	
Prpf8	192159	PRPF8	10594	-3.43	mRNA splicing, via spliceosome	RNA transcription, protein translation	CS score, function	
Ptcd1	71799	PTCD1	26024	-1.77	tRNA 3'-end processing	RNA transcription, protein translation	CS score, function	
Pwp2	110816	PWP2	5822	-2.52	ribosomal small subunit assembly	RNA transcription, protein translation	CS score, function	
Qars	97541	QARS	5859	-3.35	tRNA aminoacylatio	RNA transcriptio	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
					n for protein translation	n, protein translation		
Ran	19384	RAN	5901	-3.09	ribosomal large subunit export from nucleus	RNA transcriptio n, protein translation	CS score, function	
Rars	104458	RARS	5917	-2.30	tRNA aminoacylatio n for protein translation	RNA transcriptio n, protein translation	CS score, function	
Rars2	109093	RARS2	57038	-1.93	arginyl-tRNA aminoacylatio n	RNA transcriptio n, protein translation	CS score, function	
Rbm25	67039	RBM25	58517	-2.15	regulation of alternative mRNA splicing, via spliceosome	RNA transcriptio n, protein translation	CS score, function	
Rbm8a	60365	RBM8A	9939	-2.97	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	RNA transcriptio n, protein translation	CS score, function	
Rbmx	19655	RBMX	27316	-1.95	regulation of alternative mRNA splicing, via spliceosome	RNA transcriptio n, protein translation	CS score, function	
Rcl1	59028	RCL1	10171	-2.08	endonucleolyt ic cleavage of tricistronic rRNA transcript (SSU-rRNA,	RNA transcriptio n, protein translation	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
					5.8S rRNA, LSU-rRNA)			
Rngtt	24018	RNGTT	8732	-2.90	transcription from RNA polymerase II promoter	RNA transcription, protein translation	CS score, function	
Rnmt	67897	RNMT	8731	-1.45	7-methylguanosine mRNA capping	RNA transcription, protein translation	CS score, function	
Rnpc3	67225	RNPC3	55599	-1.95	mRNA splicing, via spliceosome	RNA transcription, protein translation	CS score, function	
Rpap1	68925	RPAP1	26015	-2.58	transcription from RNA polymerase II promoter	RNA transcription, protein translation	CS score, function	
Rpl10	110954	RPL10	6134	-3.76	translation	RNA transcription, protein translation	CS score, function	
Rpl10a	19896	RPL10A	4736	-2.15	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	RNA transcription, protein translation	CS score, function	
Rpl11	67025	RPL11	6135	-2.99	translation	RNA transcription, protein translation	CS score, function	
Rpl12	269261	RPL12	6136	-2.64	ribosomal large subunit assembly	RNA transcription, protein translation	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Rpl13	270106	RPL13	6137	-3.28	translation	RNA transcription, protein translation	CS score, function	
Rpl14	67115	RPL14	9045	-2.92	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	RNA transcription, protein translation	CS score, function	
Rpl15	66480	RPL15	6138	-3.50	translation	RNA transcription, protein translation	CS score, function	
Rpl18	19899	RPL18	6141	-3.72	translation	RNA transcription, protein translation	CS score, function	
Rpl18a	76808	RPL18A	6142	-3.37	translation	RNA transcription, protein translation	CS score, function	
Rpl23	65019	RPL23	9349	-3.02	translation	RNA transcription, protein translation	CS score, function	
n/a	n/a	RPL23A	6147	-4.25	translation	RNA transcription, protein translation	CS score, function	
Rpl24	68193	RPL24	6152	-2.55	ribosomal large subunit assembly	RNA transcription, protein translation	CS score, mouse K.O., function	Oliver ER, et al. Development. 2004 Aug;131(16):3907-20

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Rpl26	19941	RPL26	6154	-2.88	translation	RNA transcription, protein translation	CS score, function	
Rpl27	19942	RPL27	6155	-2.25	translation	RNA transcription, protein translation	CS score, function	
Rpl27a	26451	RPL27A	6157	-2.87	translation	RNA transcription, protein translation	CS score, mouse K.O., function	Terzian T, et al. J Pathol. 2011 Aug;224(4):540-52
Rpl3	27367	RPL3	6122	-3.27	ribosomal large subunit assembly	RNA transcription, protein translation	CS score, function	
Rpl30	19946	RPL30	6156	-2.53	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	RNA transcription, protein translation	CS score, function	
Rpl31	114641	RPL31	6160	-1.92	translation	RNA transcription, protein translation	CS score, function	
Rpl32	19951	RPL32	6161	-3.70	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	RNA transcription, protein translation	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
n/a	n/a	RPL34	6164	-2.37	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	RNA transcription, protein translation	CS score, function	
Rpl35	66489	RPL35	11224	-2.25	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	RNA transcription, protein translation	CS score, function	
Rpl35a	57808	RPL35A	6165	-3.20	translation	RNA transcription, protein translation	CS score, function	
Rpl36	54217	RPL36	25873	-3.44	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	RNA transcription, protein translation	CS score, function	
Rpl37	67281	RPL37	6167	-3.02	translation	RNA transcription, protein translation	CS score, function	
Rpl37a	19981	RPL37A	6168	-2.62	nuclear-transcribed mRNA catabolic process, nonsense-	RNA transcription, protein translation	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
					mediated decay			
Rpl38	67671	RPL38	6169	-2.57	translation	RNA transcription, protein translation	CS score, mouse K.O., function	MORGAN WC, et al. J Hered. 1950 Aug;41(8): 208-15
Rpl4	67891	RPL4	6124	-2.67	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	RNA transcription, protein translation	CS score, function	
Rpl5	100503670	RPL5	6125	-3.20	translation	RNA transcription, protein translation	CS score, function	
Rpl6	19988	RPL6	6128	-3.07	translation	RNA transcription, protein translation	CS score, function	
Rpl7	19989	RPL7	6129	-2.15	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	RNA transcription, protein translation	CS score, function	
Rpl7a	27176	RPL7A	6130	-3.45	ribosome biogenesis	RNA transcription, protein translation	CS score, function	
Rpl7l1	66229	RPL7L1	285855	-1.86	maturation of LSU-rRNA from	RNA transcriptio	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
					tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	n, protein translation		
Rpl8	26961	RPL8	6132	-4.00	translation	RNA transcriptio n, protein translation	CS score, function	
Rpl9	20005	RPL9	6133	-3.57	translation	RNA transcriptio n, protein translation	CS score, function	
Rplp0	11837	RPLP0	6175	-2.61	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	RNA transcriptio n, protein translation	CS score, function	
Rpp21	67676	RPP21	79897	-2.96	tRNA processing	RNA transcriptio n, protein translation	CS score, function	
Rpp30	54364	RPP30	10556	-1.79	tRNA processing	RNA transcriptio n, protein translation	CS score, function	
Rps10	67097	RPS10	6204	-2.88	ribosomal small subunit assembly	RNA transcriptio n, protein translation	CS score, function	
Rps11	27207	RPS11	6205	-2.93	translation	RNA transcriptio n, protein translation	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Rps12	20042	RPS12	6206	-3.33	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	RNA transcription, protein translation	CS score, function	
Rps13	68052	RPS13	6207	-3.13	translation	RNA transcription, protein translation	CS score, function	
n/a	n/a	RPS14	6208	-3.18	translation	RNA transcription, protein translation	CS score, function	
Rps15	20054	RPS15	6209	-3.20	ribosomal small subunit assembly	RNA transcription, protein translation	CS score, function	
Rps15a	267019	RPS15A	6210	-3.18	translation	RNA transcription, protein translation	CS score, function	
Rps16	20055	RPS16	6217	-2.35	translation	RNA transcription, protein translation	CS score, function	
Rps17	20068	RPS17	6218	-2.69	ribosomal small subunit assembly	RNA transcription, protein translation	CS score, function	
Rps19	20085	RPS19	6223	-3.49	translation	RNA transcription, protein translation	CS score, mouse K.O., function	Matsson H, et al. Mol Cell Biol. 2004 May;24(9): 4032-7

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Rps2	16898	RPS2	6187	-2.50	translation	RNA transcription, protein translation	CS score, function	
Rps21	66481	RPS21	6227	-1.84	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	RNA transcription, protein translation	CS score, function	
Rps23	66475	RPS23	6228	-2.86	translation	RNA transcription, protein translation	CS score, function	
Rps25	75617	RPS25	6230	-2.38	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	RNA transcription, protein translation	CS score, function	
n/a	n/a	RPS3A	6189	-3.72	translation	RNA transcription, protein translation	CS score, function	
Rps4x	20102	RPS4X	6191	-3.04	translation	RNA transcription, protein translation	CS score, function	
Rps5	20103	RPS5	6193	-2.61	translation	RNA transcription, protein translation	CS score, function	
Rps6	20104	RPS6	6194	-3.31	translation	RNA transcriptio	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
						n, protein translation		
Rps7	20115	RPS7	6201	-2.97	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	RNA transcriptio n, protein translation	CS score, function	
Rps8	20116	RPS8	6202	-3.44	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	RNA transcriptio n, protein translation	CS score, function	
Rps9	76846	RPS9	6203	-3.16	translation	RNA transcriptio n, protein translation	CS score, function	
Rpsa	16785	RPSA	3921	-3.06	ribosomal small subunit assembly	RNA transcriptio n, protein translation	CS score, mouse K.O., function	Han J, et al. MGI Direct Data Submissio n. 2008
Rsl24d1	225215	RSL24D1	51187	-2.76	translation	RNA transcriptio n, protein translation	CS score, function	
Sars	20226	SARS	6301	-2.67	tRNA aminoacylatio n for protein translation	RNA transcriptio n, protein translation	CS score, function	
Sars2	71984	SARS2	54938	-2.25	seryl-tRNA aminoacylatio n	RNA transcriptio	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
						n, protein translation		
Sart1	20227	SART1	9092	-2.13	maturation of 5S rRNA	RNA transcriptio n, protein translation	CS score, function	
Sart3	53890	SART3	9733	-1.88	RNA processing	RNA transcriptio n, protein translation	CS score, function	
Sdad1	231452	SDAD1	55153	-1.96	ribosomal large subunit export from nucleus	RNA transcriptio n, protein translation	CS score, function	
Sf1	22668	SF1	7536	-3.04	mRNA splicing, via spliceosome	RNA transcriptio n, protein translation	CS score, mouse K.O., function	Shitashige M, et al. Cancer Sci. 2007 Dec;98(12):1862-7
Sf3a1	67465	SF3A1	10291	-3.18	mRNA 3'-splice site recognition	RNA transcriptio n, protein translation	CS score, function	
Sf3a2	20222	SF3A2	8175	-2.66	mRNA 3'-splice site recognition	RNA transcriptio n, protein translation	CS score, function	
Sf3a3	75062	SF3A3	10946	-2.26	RNA splicing, via transesterifica tion reactions	RNA transcriptio n, protein translation	CS score, function	
Sf3b2	319322	SF3B2	10992	-2.51	mRNA splicing, via spliceosome	RNA transcriptio n, protein translation	CS score, function	
Sf3b3	101943	SF3B3	23450	-4.13	RNA splicing, via	RNA transcriptio	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
					transesterification reactions	n, protein translation		
Sf3b4	107701	SF3B4	10262	-2.60	RNA splicing, via transesterification reactions	RNA transcription, protein translation	CS score, function	
Sfpq	71514	SFPQ	6421	-2.27	negative regulation of transcription from RNA polymerase II promoter	RNA transcription, protein translation	CS score, function	
Sin3a	20466	SIN3A	25942	-1.74	negative regulation of transcription from RNA polymerase II promoter	RNA transcription, protein translation	CS score, mouse K.O., function	Dannenberg JH, et al. Genes Dev. 2005 Jul 1;19(13):1581-95
Smg5	229512	SMG5	23381	-2.35	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	RNA transcription, protein translation	CS score, function	
Smg6	103677	SMG6	23293	-1.18	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	RNA transcription, protein translation	CS score, function	
Snrnp25	78372	SNRNP25	79622	-2.43	mRNA processing	RNA transcription, protein translation	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Snrnp27	66618	SNRNP27	11017	-1.36	mRNA processing	RNA transcription, protein translation	CS score, function	
Snrpd2	107686	SNRPD2	6633	-2.47	RNA splicing	RNA transcription, protein translation	CS score, function	
Snrpf	69878	SNRPF	6636	-3.58	mRNA splicing, via spliceosome	RNA transcription, protein translation	CS score, function	
Srrm1	51796	SRRM1	10250	-1.81	mRNA processing	RNA transcription, protein translation	CS score, function	
Srsf1	110809	SRSF1	6426	-2.75	mRNA 5'-splice site recognition	RNA transcription, protein translation	CS score, mouse K.O., function	Xu X, et al. Cell. 2005 Jan 14;120(1):59-72
Srsf2	20382	SRSF2	6427	-3.66	regulation of alternative mRNA splicing, via spliceosome	RNA transcription, protein translation	CS score, mouse K.O., function	Ding JH, et al. EMBO J. 2004 Feb 25;23(4):885-96
Srsf3	20383	SRSF3	6428	-2.28	mRNA splicing, via spliceosome	RNA transcription, protein translation	CS score, mouse K.O., function	Jumaa H, et al. Curr Biol. 1999 Aug 26;9(16):899-902
Srsf7	225027	SRSF7	6432	-2.06	mRNA splicing, via spliceosome	RNA transcription, protein translation	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Ssu72	68991	SSU72	29101	-2.57	mRNA polyadenylation	RNA transcription, protein translation	CS score, function	
Sugp1	70616	SUGP1	57794	-1.36	RNA processing	RNA transcription, protein translation	CS score, function	
Tars	110960	TARS	6897	-2.53	tRNA aminoacylation for protein translation	RNA transcription, protein translation	CS score, function	
Tars2	71807	TARS2	80222	-1.91	threonyl-tRNA aminoacylation	RNA transcription, protein translation	CS score, function	
Tbl3	213773	TBL3	10607	-2.41	maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	RNA transcription, protein translation	CS score, function	
Thoc2	331401	THOC2	57187	-2.52	mRNA processing	RNA transcription, protein translation	CS score, function	
Thoc5	107829	THOC5	8563	-1.57	mRNA processing	RNA transcription, protein translation	CS score, mouse K.O., function	Mancini A, et al. BMC Biol. 2010;8:1
Thoc7	66231	THOC7	80145	-2.23	mRNA processing	RNA transcription, protein translation	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Timeless	21853	TIMELESS	8914	-2.27	negative regulation of transcription from RNA polymerase II promoter	RNA transcription, protein translation	CS score, mouse K.O., function	Gotter AL, et al. Nat Neurosci. 2000 Aug;3(8):755-6
Tsen2	381802	TSEN2	80746	-1.41	tRNA-type intron splice site recognition and cleavage	RNA transcription, protein translation	CS score, function	
Tsr1	104662	TSR1	55720	-1.76	ribosome biogenesis	RNA transcription, protein translation	CS score, function	
Tsr2	69499	TSR2	90121	-2.82	maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	RNA transcription, protein translation	CS score, function	
Tufm	233870	TUFM	7284	-1.92	translational elongation	RNA transcription, protein translation	CS score, function	
Tut1	70044	TUT1	64852	-2.65	mRNA polyadenylation	RNA transcription, protein translation	CS score, function	
Twistnb	28071	TWISTNB	221830	-2.17	transcription from RNA polymerase I promoter	RNA transcription, protein translation	CS score, function	
U2af1	108121	U2AF1	7307	-2.41	mRNA splicing, via spliceosome	RNA transcriptio	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
						n, protein translation		
U2af2	22185	U2AF2	11338	-2.80	mRNA processing	RNA transcriptio n, protein translation	CS score, function	
Uba52	22186	UBA52	7311	-2.54	translation	RNA transcriptio n, protein translation	CS score, function	
Ubl5	66177	UBL5	59286	-2.56	mRNA splicing, via spliceosome	RNA transcriptio n, protein translation	CS score, function	
Upf1	19704	UPF1	5976	-2.63	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	RNA transcriptio n, protein translation	CS score, mouse K.O., function	Medghalc hi SM, et al. Hum Mol Genet. 2001 Jan 15;10(2):9 9-105
Upf2	326622	UPF2	26019	-2.16	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	RNA transcriptio n, protein translation	CS score, mouse K.O., function	Weischenf eldt J, et al. Genes Dev. 2008 May 15;22(10): 1381-96
Utp15	105372	UTP15	84135	-1.65	maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	RNA transcriptio n, protein translation	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Utp20	70683	UTP20	27340	-2.28	endonucleolytic cleavage in ITS1 to separate SSU-rRNA from 5.8S rRNA and LSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	RNA transcription, protein translation	CS score, function	
Utp23	78581	UTP23	84294	-2.54	rRNA processing	RNA transcription, protein translation	CS score, function	
Utp3	65961	UTP3	57050	-1.58	maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	RNA transcription, protein translation	CS score, function	
Utp6	216987	UTP6	55813	-1.99	maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	RNA transcription, protein translation	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Vars	22321	VARS	7407	-3.35	tRNA aminoacylation for protein translation	RNA transcription, protein translation	CS score, function	
Wars	22375	WARS	7453	-2.22	tryptophanyl-tRNA aminoacylation	RNA transcription, protein translation	CS score, function	
Wdr12	57750	WDR12	55759	-2.16	maturation of LSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	RNA transcription, protein translation	CS score, function	
Wdr3	269470	WDR3	10885	-2.65	maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	RNA transcription, protein translation	CS score, function	
Wdr33	74320	WDR33	55339	-2.63	mRNA polyadenylation	RNA transcription, protein translation	CS score, function	
Wdr36	225348	WDR36	134430	-2.04	rRNA processing	RNA transcription, protein translation	CS score, mouse K.O., function	Gallenberger M, et al. Hum Mol Genet. 2011 Feb 1;20(3):422-35

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Wdr46	57315	WDR46	9277	-2.41	maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	RNA transcriptio n, protein translation	CS score, function	
Wdr61	66317	WDR61	80349	-2.63	nuclear-transcribed mRNA catabolic process, exonucleolytic , 3'-5'	RNA transcriptio n, protein translation	CS score, function	
Wdr75	73674	WDR75	84128	-2.12	regulation of transcription from RNA polymerase II promoter	RNA transcriptio n, protein translation	CS score, function	
Xpo1	103573	XPO1	7514	-3.50	ribosomal large subunit export from nucleus	RNA transcriptio n, protein translation	CS score, function	
Yars	107271	YARS	8565	-2.78	tRNA aminoacylatio n for protein translation	RNA transcriptio n, protein translation	CS score, function	
Yars2	70120	YARS2	51067	-2.40	translation	RNA transcriptio n, protein translation	CS score, function	
Ythdc1	231386	YTHDC1	91746	-2.35	mRNA splice site selection	RNA transcriptio n, protein translation	CS score, function	

Name (mouse)	ID (mouse)	Name (human)	ID (human)	CS score	Function (GO term)	Functional category	CDL (basis)	Citation
Zbtb8os	67106	ZBTB8OS	339487	-2.54	tRNA splicing, via endonucleolytic cleavage and ligation	RNA transcription, protein translation	CS score, function	
Zc3h3	223642	ZC3H3	23144	-1.22	mRNA polyadenylation	RNA transcription, protein translation	CS score, function	

Although the disclosure has been described with reference to certain specific embodiments, various modifications thereof will be apparent to those skilled in the art. Any examples provided herein are included solely for the purpose of illustrating the disclosure and are not intended to limit the disclosure in any way. Any drawings provided herein are solely for the purpose of illustrating various aspects of the disclosure and are not intended to be drawn to scale or to limit the disclosure in any way. The scope of the claims appended hereto should not be limited by the preferred embodiments set forth in the above description, but should be given the broadest interpretation consistent with the present specification as a whole. The disclosures of all prior art recited herein are incorporated herein by reference in their entirety.

CLAIMS

1. A pluripotent stem cell genetically modified to comprise a set of transgenes comprising PD-L1, HLA-G or H2-M3, Cd47, Cd200, FASLG or FasL, Ccl21 or Ccl21b, Mfge8, and Serpin B9 or Spi6, wherein each of the transgenes is expressed at a level that is in the top 5% of gene expression for all genes in the genome of the pluripotent stem cell.

2. The pluripotent stem cell of claim 1, wherein:

(a) the cell is an induced pluripotent stem cell (iPSC);

(b) the cell further comprises at least one mechanism for controlling cell proliferation, the genetically modified cell comprising a genetic modification of one or more cell division locus/loci (CDL), the CDL being one or more endogenous or exogenous loci whose transcription product(s) is expressed by dividing cells, the genetic modification being one or more of:

i) an ablation link (ALINK) system that comprises a DNA sequence encoding a negative selectable marker that is transcriptionally linked to a DNA sequence encoding the CDL; and

ii) an exogenous activator of regulation of a CDL (EARC) system that comprises an inducible activator-based gene expression system that is operably linked to the CDL;

(c) the PD-L1 transgene encodes a protein having at least 85% identity to the amino acid sequence of SEQ ID NO: 11 or SEQ ID NO: 12;

(d) the HLA-G or H2-M3 transgene encodes a protein having at least 85% identity to the amino acid sequence of SEQ ID NO: 16 or SEQ ID NO: 15;

(e) the Cd47 transgene encodes a protein having at least 85% identity to the amino acid sequence of SEQ ID NO: 3 or SEQ ID NO: 4;

(f) the CD200 transgene encodes a protein having at least 85% identity to the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 6;

(g) the FASLG or FasL transgene encodes a protein having at least 85% identity to the amino acid sequence of SEQ ID NO: 10 or SEQ ID NO: 9;

(h) the Ccl21 or Ccl21b transgene encodes a protein having at least 85% identity to the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 1;

(i) the Mfge8 transgene encodes a protein having at least 85% identity to the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 14;

(j) the Serpin B9 or Spi6 transgene encodes a protein having at least 85% identity to the amino acid sequence of SEQ ID NO: 8 or SEQ ID NO: 7;

and/or

(k) the transgenes are operably linked to a constitutive promoter, optionally wherein the constitutive promoter is selected from the group consisting of the CAG promoter, the cytomegalovirus (CMV) promoter, the EF1α promoter, the PGK promoter, adenovirus late promoter, vaccinia virus 7.5K promoter, SV40 promoter, tk promoter of HSV, mouse mammary tumor virus (MMTV) promoter, LTR promoter of HIV, promoter of moloney virus, Epstein barr virus (EBV) promoter, and the Rous sarcoma virus (RSV) promoter.

3. The pluripotent stem cell of claim 2, wherein:

(a) the genetic modification of the CDL comprises performing targeted replacement of the CDL with one or more of:

i) a DNA vector comprising the ALINK system;

ii) a DNA vector comprising the EARC system; and

iii) a DNA vector comprising the ALINK system and the EARC system;

wherein the ALINK and/or EARC systems are each operably linked to the CDL;

(b) the genetic modification of the CDL comprising the ALINK system is homozygous, heterozygous, hemizygous or compound heterozygous and/or wherein the genetic modification of the CDL comprising the EARC system results in activation of the CDL solely by an inducer of the inducible activator-based gene expression system;

(c) the CDL is one or more of the loci recited in Table 5, optionally wherein the CDL encodes a gene product that functions in one or more of: cell cycle, DNA replication, RNA transcription, protein translation, and metabolism, optionally wherein the CDL is one or more of Cdk1/CDK1, Top2A/TOP2A, Cenpa/CENPA, Birc5/BIRC5, and Eef2/EEF2, preferably wherein the CDL is Cdk1 or CDK1;

(d) the ALINK system comprises a herpes simplex virus-thymidine kinase/ganciclovir system, a cytosine deaminase/5-fluorocytosine system, a carboxyl esterase/irinotecan system or an iCasp9/AP1903 system, preferably wherein the ALINK system is a herpes simplex virus-thymidine kinase/ganciclovir system; and/or

(e) the EARC system is a dox-bridge system, a cumate switch inducible system, an ecdysone inducible system, a radio wave inducible system, or a ligand-reversible dimerization system, preferably wherein the EARC system is a dox-bridge system.

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4. The pluripotent stem cell of any one of claims 1 to 4, wherein the expression level is at least 350x for PD-L1, 16,000x for Ccl21b, 25,000x for FasL, 1700x for Cd200, 16x for Cd47, 34x for Mfge8, 600x for Spi6, and 750x for H2-M3, relative to the expression level in an unmodified embryonic stem cell.

5. A population of genetically modified cells comprising the pluripotent stem cell of any one of claims 1 to 3.

6. A composition comprising the pluripotent stem cell of any one of claims 1-4, optionally wherein the composition further comprises a pharmaceutically acceptable excipient.

7. A kit comprising the pluripotent stem cell of any one of claims 1-4, the population of genetically modified cells of claim 5, or the composition of claim 6.

8. The pluripotent stem cell of any one of claims 1-4, the population of genetically modified cells of claim 5, or the composition of claim 6 for use in providing a local immunosuppression at a transplant

site in an allogeneic host.

9. The pluripotent stem cell, population of genetically modified cells, or composition for use according to claim 8, wherein:

(a) the cells are administered locally to the tissue or body site in need of cells;

(b) the cells are administered intravenously, subcutaneously, intramuscularly, percutaneously, intradermally, parenterally, intraarterially, intravascularly, or by perfusion, optionally wherein the cells are administered by subcutaneous injection to produce a cloaked subcutaneous tissue;

(c) the cells are administered as a tissue, optionally wherein the tissue is administered with a gel, biocompatible matrix, or cellular scaffold;

(d) the cells are administered in an amount of 25,000 to 5,000,000,000 cells;

(e) the cells are administered in an amount of 800,000,000 to 3,000,000,000 cells; or

(f) the cell is removed after completion of the therapy.

10. The pluripotent stem cell, population of genetically modified cells, or composition for use according to claim 8 or 9, wherein the use further comprises:

(a) administering an additional therapeutic agent; and/or

(b) controlling proliferation of the cell.

11. The pluripotent stem cell, population of genetically modified cells, or composition for use according to claim 10, wherein the additional therapeutic agent:

(a) is administered prior to administration of the cells, after administration of the cells, or concurrently with administration of the cells; and/or

(b) is an immunosuppressive agent, a disease-modifying anti-rheumatic drug (DMARD), a biologic response modifier (a type of DMARD), a corticosteroid, or a nonsteroidal anti-inflammatory medication (NSAID), prednisone, prednisolone, methylprednisolone, methotrexate, hydroxychloroquine, sulfasalazine, leflunomide, cyclophosphamide, azathioprine, tofacitinib, adalimumab, abatacept, anakinra, kineret, certolizumab, etanercept, golimumab, infliximab, rituximab or tocilizumab, 6-mercaptopurine, 6-thioguanine, abatacept, adalimumab, alemtuzumab, an aminosalicylate, an antibiotic, an anti-histamine, Anti-TNFα, azathioprine, belimumab, beta interferon, a calcineurin inhibitor, certolizumab, a corticosteroid, cromolyn, cyclosporin A, cyclosporine, dimethyl fumarate, etanercept, fingolimod, fumaric acid esters, glatiramer acetate, golimumab, hydroxyurea, IFNγ, IL-11, leflunomide, leukotriene receptor antagonist, long-acting beta2 agonist, mitoxantrone, mycophenolate mofetil, natalizumab, ocrelizumab, pimecrolimus, a probiotic, a retinoid, salicylic acid, short-acting beta2 agonist, sulfasalazine, tacrolimus, teriflunomide, theophylline, tocilizumab, ustekinumab, or vedolizumab, bevacuzimab, ranibizumab, or aflibercept), photodynamic therapy, photocoagulation, carbidopa-levodopa, a dopamine agonist, an MAO-B inhibitor, a catechol-O-methyltransferase inhibitor, an anticholindergic, amantadine, deep brain stimulation, an anticoagulant, an anti-platelet agent, an angiotensin-converting enzyme inhibitor, an angiotensin II receptor blocker, an angiotensin receptor neprilysin inhibitor, a beta blocker, a combined alpha and beta blocker, a

calcium channel blocker, a cholesterol lowering medication, a nicotinic acid, a cholesterol absorption inhibitor, a digitalis preparation, a diuretic, a vasodilator, a dual anti-platelet therapy, a cardiac procedure, an antiviral compound, a nucleoside-analog reverse transcriptase inhibitor (NRTI), a non-nucleoside reverse transcriptase inhibitor (NNRTI), a protease inhibitor, an antibacterial compound, an antifungal compound, an antiparasitic compound, insulin, a sulfonylurea, a biguanide, a meglitinide, a thiazolidinedione, a DPP-4 inhibitor, an SGLT2 inhibitor, an alpha-glucosidase inhibitor, a bile acid sequestrant, aspirin, a dietary regimen, a clotting factor, desmopressin, a clot-preserving medication, a fibrin sealant, physical therapy, a coenzyme, a bone marrow transplant, an organ transplant, hemodialysis, hemofiltration, exchange transfusion, peritoneal dialysis, medium-chain triacylglycerols, miglustat, enzyme supplementation therapy, a checkpoint inhibitor, a chemotherapeutic drug, a biologic drug, radiation therapy, cryotherapy, hyperthermia, surgical excision or tumor tissue, or an anti-cancer vaccine.

12. The pluripotent stem cell, population of genetically modified cells, or composition for use according to claim 10, wherein:

(a) the cell comprises an ALINK system, and controlling proliferation of the cell comprises:

i) permitting proliferation of the cell comprising the ALINK system by maintaining the cell comprising the ALINK system in the absence of an inducer of the negative selectable marker; or

ii) ablating or inhibiting proliferation of the cell comprising the ALINK system by exposing the cell comprising the ALINK system to the inducer of the negative selectable marker; and/or

(b) the cell comprises an EARC system, and controlling proliferation of the cell comprises:

i) permitting proliferation of the cell comprising the EARC system by exposing the cell comprising the EARC system to an inducer of the inducible activator-based gene expression system; or

ii) preventing or inhibiting proliferation of the cell comprising the EARC system by maintaining the cell comprising the EARC system in the absence of the inducer of the inducible activator-based gene expression system.

13. The pluripotent stem cell, population of genetically modified cells, or composition for use according to claim 11, wherein

the allogeneic host is a mammal, optionally wherein the allogeneic host is a mouse or a human.