pigmented epithelium cell, glial progenitor cell, endothelial cell, hepatocyte, thyroid cell, skin cell, and blood cell. In some examples, the engineered primary cell is an islet cell.

[0024] In some examples, the primary islet cell has been dissociated from a primary islet cluster. In some examples, the primary islet cluster is a human primary cadaveric islet cluster. In some examples, after step a) and/or after step b) the primary islet cell is incubated under conditions for re-clustering into a modified primary islet cluster, wherein at least a portion of the incubating is carried out with motion. In some examples, the incubating further comprises a least a portion of incubating under static conditions. In some examples, the incubating comprises a first incubation under static conditions followed by the incubating with motion. In some examples, the incubating comprises the incubating with motion followed by a second incubation under static conditions. In some examples, prior to the incubating under conditions for reclustering, the method comprises selecting for islet cells that have been modified. In some examples, the selecting is by fluorescence-activated cell sorting (FACS).

[0025] In some examples, the method comprises: i) dissociating a primary islet cluster into a suspension of primary beta islet cells; ii) modifying primary beta islet cells of the suspension to reduce or eliminate the expression of one or more MHC class I and/or one or more MHC class II HLA in primary beta islet cells; iii) incubating the modified primary beta islet cells under conditions for re-clustering into a first modified primary islet cluster, wherein at least a portion of the incubating is carried out with motion; iv) dissociating the modified] primary islet cluster into a suspension of modified primary beta islet cells; v) further modifying the modified primary islet cells of the suspension to increase the expression of one or more tolerogenic factors in the primary cell; and vi) incubating the further modified primary beta islet cells under conditions for re-clustering into a second modified primary islet cluster, wherein at least a portion of the incubating is carried out with motion.

[0025a] In one aspect, the present disclosure refers to a method for modifying primary islet cells, the method comprising: i) dissociating a primary islet cluster into a suspension of primary islet cells; ii) contacting the suspension of primary islet cells with one or more first reagents to modify gene expression and incubating the suspension of primary islet cells to produce first modified islet cells, wherein the one or more first reagents comprise (1) a gene editing system comprising a genome-modifying protein or a nucleic acid encoding the genome-modifying protein for disrupting one or more target genes encoding one or more endogenous proteins and/or (2) an agent comprising an exogenous polynucleotide encoding a protein; and iii) incubating the first modified islet cells under conditions for re-clustering the cells into an islet, wherein at least a portion of the incubating is carried out with motion and iv) selecting after the incubating step iii) for islet cells that have modified gene expression relative to the primary islet cells before the contacting.

[0025b] In one aspect, the present disclosure refers to a method for modifying primary islet cells, the method comprising: i) dissociating a primary islet cluster into a suspension of primary islet cells; ii) contacting the suspension of primary islet cells with one or more first reagents to modify gene expression to produce modified islet cells, wherein the one or more first reagents comprise (1) a gene editing system comprising a genome-modifying protein for gene editing of a target gene encoding an endogenous protein and/or (2) an agent comprising an exogenous polynucleotide encoding an exogenous protein; iii) after the contacting, incubating the modified islet cells under conditions for re-clustering the cells into a first modified primary islet cluster, wherein at least a portion of the incubating is carried out with shaking comprising orbital motion; iv) dissociating the first modified primary islet cluster into a suspension of modified primary islet cells; v) further contacting the modified primary islet cells of the suspension with one or more second reagents, wherein the one or more second reagents comprise (i) a gene editing system comprising a genome-modifying protein for gene editing a target gene encoding an endogenous protein and/or (ii) an agent comprising an exogenous polynucleotide encoding an exogenous protein; and vi) incubating the further modified primary islet cells after the further contacting under conditions for re-clustering into a second modified primary islet cluster, wherein at least a portion of the incubating is carried out with shaking comprising orbital motion.

[0026] In some examples, the one or more MHC class I HLA is an HLA-A protein, an HLA-B protein, or HLA-C protein. In some examples, the one or more MHC class II HLA is an HLA-DP protein, an HLA-DR protein, or an HLA-DQ protein. In some examples, the modifying is by genetic engineering. In some examples, the motion is shaking. In some examples, the shaking comprises orbital motion. In some examples, the shaking comprises bidirectional linear movement. In some examples, the shaking is with an orbital shaker. In some examples, the incubating in (iii) and/or the incubating in vi) further comprises a least a portion of incubating under static conditions. In some examples, the

incubating in iii) and/or the incubating in vi) comprises a first incubation under static conditions followed by the incubating with motion. In some examples, the incubating comprises the incubating with motion followed by a second incubation under static conditions.

[0027] In some examples, prior to v), the method comprises selecting, from the dissociated islet cells in iv), beta islet cells that have been modified, and optionally repeating steps iii) and iv) on the selected islet cells. In some examples, after the incubating in vi), the method comprises dissociating the second modified primary islet cluster into a suspension of modified primary beta islet cells and selecting for islet cells that have been modified. In some examples, incubating the selected modified primary beta islet cells under conditions for re-clustering into a modified primary islet cluster, wherein at least a portion of the incubating is carried out with motion.

[0028] In some examples, provided herein is use of motion to promote modification of a population of cells, wherein the population of cells has been contacted with one or more reagents to modify gene expression in cells of the population before subjecting to the motion.

[0029] In some examples, provided herein is a method of enhancing modification of a population of cells, in which the method includes: i) contacting a population of cells with one or more reagents to modify gene expression in cells of the population; and ii) subjecting the population of cells to motion after contact with the one or more reagents.

[0030] In some examples, provided herein is a method of enhancing viability of a population of cells, in which the method includes: i) contacting a population of cells with one or more reagents to modify gene expression in cells of the population; and ii) subjecting the population of cells to motion after contact with the one or more reagents.

[0031] In some examples, provided herein is a method of modification of a population of cells, in which the method includes i) contacting a population of cells with one or more reagents to modify gene expression in cells of the population; and ii) subjecting the population of cells to motion after contact with the one or more reagents.

[0032] In some of any examples of a provided use or method, the population of cells are primary cells. In some f any examples of a provided use or methods, the population of cells are cells derived from stem cells. In some examples, the stem cells are selected from the group consisting of a pluripotent stem cell (PSC), an induced pluripotent stem cell, an embryonic stem cell, a hematopoietic stem cell, a mesenchymal stem cell, an endothelial stem cell, an epithelial stem cell, an adipose stem cell, a germline stem cell, a lung stem cell, a cord

blood stem cell, and a multipotent stem cell. In some examples, the stem cells are pluripotent stem cell. In some examples, the stem cells are induced pluripotent stem cells, mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs), or embryonic stem cells (ESCs). In some examples, the population of cells are selected from the group consisting of islet cells, immune cells, B cells, T cells, natural killer (NK) cells, natural killer T (NKT) cells, macrophages, endothelial cells, muscle cells, cardiac muscle cells, smooth muscle cells, skeletal muscle cells, hepatocytes, a glial progenitor cells, dopaminergic neurons, retinal pigment epithelial cells, thyroid cells, skin cells, glial progenitor cells, neural cells, cardiac cells, and blood cells.

[0033] In some of any examples of a provided use or method, the population of cells are naturally present in a 3D network.

[0034] In some of any examples of a provided use or method, the population of cells are in suspension. In some examples, the population of cells are in a vessel that has a low-attachment surface. In some examples, the population of cells are maintained in a vessel in a minimum volume of media sufficient to cover the cells. In some examples, the population of cells in suspension are produced by dissociating cells from an adherent culture or a cell cluster prior to the contacting.

[0035] In some of any examples of a provided use or method, the population of cells are islet cells. In some examples, the population of cells comprise beta islet cells. In some examples, the population of cells comprising beta islet cells are produced by dissociating a primary islet cluster into a suspension of cells comprising primary beta islet cells.

[0036] In some of any examples of a provided use or method, the contacting is carried out for less than two days prior to subjecting the cells to motion. In some examples, the contacting is carried out for 30 seconds to 24 hours prior to subjecting the cells to motion. In some examples, the contacting is carried out for 1 minute to 60 minutes, 2 minutes to 30 minutes, 5 minutes to 15 minutes prior to subjecting the cells to motion.

[0037] In some of any examples of a provided use or method, subjecting the population of cells to motion promotes the formation of cell aggregates. In some examples, subjecting the population of cells to motion forms cell clusters.

[0038] In some of any examples of a provided use or method, the method or use further includes incubating the cells under static conditions after subjecting the cells to motion. In some

[0068] In some examples, provided herein is a composition comprising any one of the populations described herein.

[0069] In some examples, provided herein is a composition comprising an engineered primary islet cluster produd by any one of the methods described herein.

[0070] In some examples, provided herein is a composition comprising a population of engineered primary islet cells, wherein the engineered primary islet cells comprise: (i) a transgene comprising an exogenous polynucleotide encoding CD47 and (ii) inactivation or disruption of both alleles of a *B2M* gene. In some examples, the population of engineered primary islet cells is a cluster of primary islet cells. In some examples, the population of engineered primary islet cells is a population of engineered primary beta islet cells.

[0070a] In one aspect, the present disclosure refers to a population of engineered primary islet cells comprising a plurality of engineered primary islet cells, wherein the engineered primary islet cell comprises modifications comprising: (i) a transgene comprising an exogenous polynucleotide encoding CD47, (ii) inactivation or disruption of both alleles of a *B2M* gene, and (iii) inactivation or disruption of both alleles of a *CIITA* gene, wherein: at least 90% of cells in the population comprise reduced expression of one or more MHC class I molecules relative to a primary islet cell that does not comprise the modifications and at least 90% of cells in the population comprise the exogenous polynucleotide encoding CD47.

[0071] In some examples, provided herein is a composition comprising a population of engineered primary T cells, wherein the engineered primary T cells comprise: (i) a transgene comprising an exogenous polynucleotide encoding CD47 and (ii) inactivation or disruption of both alleles of a *B2M* gene.

[0072] In some examples, provided herein is a composition comprising a population of engineered primary thyroid cells, wherein the engineered primary thyroid cells comprise: (i) a transgene comprising an exogenous polynucleotide encoding CD47 and (ii) inactivation or disruption of both alleles of a *B2M* gene.

[0073] In some examples, provided herein is a composition comprising a population of engineered primary skin cells, wherein the engineered primary skin cells comprise: (i) a transgene comprising an exogenous polynucleotide encoding CD47 and (ii) inactivation or disruption of both alleles of a *B2M* gene.

[0074] In some examples, provided herein is a composition comprising a population of engineered primary endothelial cells, wherein the engineered primary endothelial cells comprise: (i) a transgene comprising an exogenous polynucleotide encoding CD47 and (ii) inactivation or disruption of both alleles of a *B2M* gene.

[0075] In some examples, provided herein is a composition comprising a population of engineered primary retinal pigmented epithelium cells, wherein the engineered primary retinal pigmented epithelium cells comprise: (i) a transgene comprising an exogenous polynucleotide encoding CD47 and (ii) inactivation or disruption of both alleles of a *B2M* gene.

[0076] In some examples, engineered primary cells of the population of engineered primary cells comprise an indel in both alleles of the *B2M* gene. In some examples, the

[0082] In some examples, administering the population comprises intravenous injection, intramuscular injection, intravascular injection, or transplantation of the population. In some examples, the population is transplanted via kidney capsule transplant or intramuscular injection. In some examples, the population is derived from a donor subject, wherein the HLA type of the donor does not match the HLA type of the patient. In some examples, the population is a human cell population and the patient is a human patient. In some examples, the beta islet cells improve glucose tolerance in the subject. In some examples, the subject is a diabetic patient. In some examples, the diabetic patient has type I diabetes or type II diabetes. In some examples, glucose tolerance is improved relative to the subject's glucose tolerance prior to administration of the islet cells. In some examples, the beta islet cells reduce exogenous insulin usage in the subject. In some examples, glucose tolerance is improved as measured by HbA1c levels. In some examples, the subject is fasting. In some examples, the islet cells improve insulin secretion in the subject. In some examples, insulin secretion is improved relative to the subject's insulin secretion prior to administration of the islet cells.

[0083] In some examples of the method of treating a disease, the method further comprises administering one or more immunosuppressive agents to the patient. In some examples, the patient has been administered one or more immunosuppressive agents. In some examples, the one or more immunosuppressive agents are a small molecule or an antibody. In some examples, the one or more immunosuppressive agents are selected from the group consisting of cyclosporine, azathioprine, mycophenolic acid, mycophenolate mofetil, a corticosteroids, prednisone, methotrexate, gold salts, sulfasalazine, antimalarials, brequinar, leflunomide, mizoribine, 15-deoxyspergualine, 6-mercaptopurine, cyclophosphamide, rapamycin, tacrolimus (FK-506), OKT3, anti-thymocyte globulin, thymopentin (thymosin-α), and an immunosuppressive antibody. In some examples, the one or more immunosuppressive agents comprise cyclosporine. In some examples, the one or more immunosuppressive agents comprise mycophenolate mofetil. In some examples, the one or more immunosuppressive agents comprise a corticosteroid. In some examples, the one or more immunosuppressive agents comprise cyclophosphamide. In some examples, the one or more immunosuppressive agents comprise rapamycin. In some examples, the one or more immunosuppressive agents comprise tacrolimus (FK-506). In some examples, the one or