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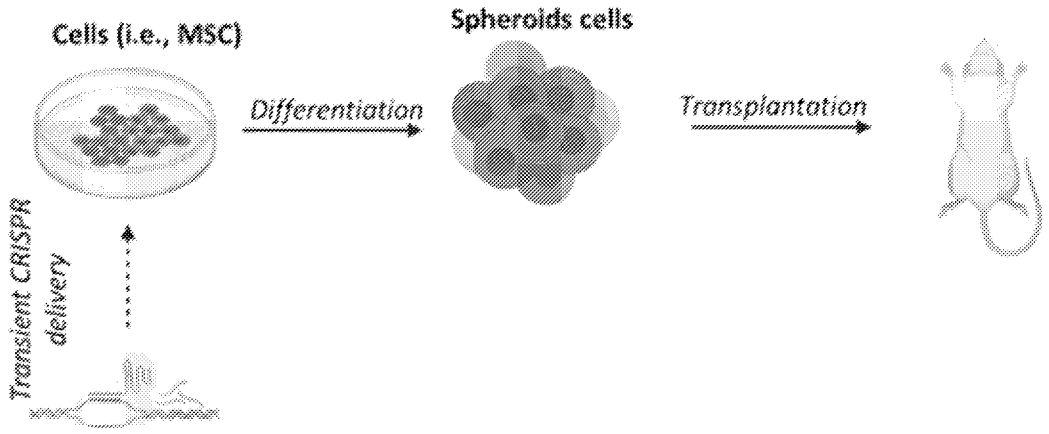

FIG. 8

(57) Abstract: The present disclosure describes systems and methods for modifying a cell. The cell can be an adult-derived stem cell. The method can comprise contacting the cell with a heterologous polypeptide comprising a first actuator moiety configured to complex with a target polynucleotide in the cell. The first actuator moiety can be linked to an epigenetic modifier to effect a modification in an expression profile of a target gene in the cell. The modified expression profile of the target gene can effect regulation of one or more characteristics of the cell.

WO 2022/133062 A1

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SYSTEMS AND METHODS FOR ENGINEERING CHARACTERISTICS OF A CELL

CROSS REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 63/126,419, filed December 16, 2020, which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] A genetic makeup of a cell can be altered via various genetic engineering methods to engineer the cell's characteristics, such as, for example, growth, migration, proliferation, cytokine secretion, size, and de-differentiation. In some cases, a heterologous gene can be knocked-in to a genome of the cell to induce a change in the characteristics of the cell. In some cases, an endogenous gene of the cell can be knocked-out to induce a change in the characteristics of the cell. In some cases, such modifications to the genetic makeup of the cell can be permanent.

SUMMARY

[0003] The present disclosure provides methods and systems for engineering (or modifying) cells, such as stem cells, to control their characteristics. Some aspects of the present disclosure provides systems and methods for engineering stem cells (e.g., adult-derived stem cells, such as mesenchymal stem cells) to modulate their characteristics, such as cell fate (e.g., differentiation into a particular cell type) or senescence (e.g., rejuvenation into a younger phenotype). Some aspects of the present disclosure provides inducing epigenetic modifications to a genetic makeup of a cell to modify the cell's characteristics. In some cases, the epigenetic modification can be permanent. In some cases, the epigenetic modification can be transient (e.g., reversed). Some aspects of the present disclosure provides methods of using the engineered cells disclosed herein for treating a subject in need thereof, such as tissue repair or regeneration.

[0004] In an aspect, the present disclosure provides a method of modifying a cell, the method comprising transiently contacting the cell with a heterologous polypeptide comprising an actuator moiety configured to complex with a target polynucleotide in the cell, wherein the actuator moiety is linked to an epigenetic modifier to modify an expression profile of a target gene in the cell, to effect a change in a characteristic of the cell that is sustained for at least about 3 days as compared to a control cell, the characteristic being different than the modified expression profile of the target gene, wherein the characteristic of the cell comprises one or more members selected from the group consisting of (1) cell morphology, (2) cell metabolism, (3) chromatin accessibility of an additional gene operatively coupled to the target gene, (4) a degree of methylation of the additional gene, (5) telomere length, and (6) cell fate.

[0005] In some embodiments of any one of the methods disclosed herein, the transiently contacting comprises contacting the cell with a heterologous polynucleotide encoding the heterologous polypeptide. In some embodiments of any one of the methods disclosed herein, the heterologous polynucleotide is not

integrated into a genome of the cell. In some embodiments of any one of the methods disclosed herein, the transiently contacting comprises delivering the heterologous polypeptide into the cell.

[0006] In some embodiments of any one of the methods disclosed herein, the transiently contacting is characterized by having a peak level of the heterologous polypeptide in the cell in less than about 3 days. In some embodiments of any one of the methods disclosed herein, the transiently contacting is characterized by having a peak level of the heterologous polypeptide in the cell in less than about 2 days. In some embodiments of any one of the methods disclosed herein, the transiently contacting is characterized by having a peak level of the heterologous polypeptide in the cell in less than about 1 day. In some embodiments of any one of the methods disclosed herein, the change in the characteristic of the cell is sustained for at least about 5 days. In some embodiments of any one of the methods disclosed herein, the change in the characteristic of the cell is sustained for at least about 2 weeks. In some embodiments of any one of the methods disclosed herein, the change in the characteristic of the cell is sustained for at least about 1 month.

[0007] In some embodiments of any one of the methods disclosed herein, the expression profile is transiently modified. In some embodiments of any one of the methods disclosed herein, the cell exhibits a maximum change within the transiently modified expression profile of the target gene in less than about 3 days upon the contacting. In some embodiments of any one of the methods disclosed herein, the cell exhibits a maximum change within the transiently modified expression profile of the target gene in less than about 1 day upon the contacting.

[0008] In some embodiments of any one of the methods disclosed herein, the characteristic of the cell comprises (1) the cell morphology, wherein the cell morphology is ascertained by cell roundness or projection area of the cell. In some embodiments of any one of the methods disclosed herein, the characteristic of the cell comprises (2) the cell metabolism, wherein the cell metabolism is ascertained by a number of mitochondria per cell or a level of ATP release from the cell. In some embodiments of any one of the methods disclosed herein, the characteristic of the cell comprises (3) the chromatin accessibility of the additional gene. In some embodiments of any one of the methods disclosed herein, the characteristic of the cell comprises (4) the degree of methylation of the additional gene. In some embodiments of any one of the methods disclosed herein, the characteristic of the cell comprises (5) the telomere length. In some embodiments of any one of the methods disclosed herein, the characteristic of the cell comprises (6) the cell fate.

[0009] In some embodiments of any one of the methods disclosed herein, the cell fate comprises differentiation of the cell into a specific cell type, as ascertained by a change in a presence a biomarker that is different than the target gene. In some embodiments of any one of the methods disclosed herein, the biomarker is an additional gene.

[0010] In some embodiments of any one of the methods disclosed herein, upon at least about 3 days following the transiently contacting, an expression level of the additional gene is greater than that of a control cell by at least about 10-fold. In some embodiments of any one of the methods disclosed herein,

upon at least about 6 days following the transiently contacting, an expression level of the additional gene is greater than that of a control cell by at least about 15-fold. In some embodiments of any one of the methods disclosed herein, upon at least about 9 days following the transiently contacting, an expression level of the additional gene is greater than that of a control cell by at least about 15-fold.

[0011] In some embodiments of any one of the methods disclosed herein, the differentiation comprises osteogenic differentiation, and the additional gene comprises osteopontin (SPP1). In some embodiments of any one of the methods disclosed herein, the biomarker is an extracellular biomarker. In some embodiments of any one of the methods disclosed herein, the differentiation comprises osteogenic differentiation, and the extracellular biomarker comprises calcium deposit.

[0012] In some embodiments of any one of the methods disclosed herein, upon at least about 6 days following the transiently contacting, a level of the calcium deposit is greater than that of a control cell by at least about 2-fold. In some embodiments of any one of the methods disclosed herein, upon at least about 12 days following the transiently contacting, a level of the calcium deposit is greater than that of a control cell by at least about 2-fold. In some embodiments of any one of the methods disclosed herein, upon at least about 12 days following the transiently contacting, a level of the calcium deposit is greater than that of a control cell by at least about 5-fold.

[0013] In some embodiments of any one of the methods disclosed herein, the cell fate comprises de-differentiation of the cell into a less-committed cell type, as ascertained by a change in an expression level of an additional gene that is different than the target gene. In some embodiments of any one of the methods disclosed herein, the target gene comprises a plurality of different genes.

[0014] In some embodiments of any one of the methods disclosed herein, the actuator moiety comprises a heterologous endonuclease. In some embodiments of any one of the methods disclosed herein, the heterologous endonuclease is directed by a guide ribonucleic acid (RNA) to specifically bind the endogenous target gene. In some embodiments of any one of the methods disclosed herein, the heterologous endonuclease substantially lacks DNA cleavage activity.

[0015] In some embodiments of any one of the methods disclosed herein, the epigenetic modifier comprises one or more members selected from the group consisting of a chromatin remodeling protein, chromatin reader protein, nuclear protein, transcription factor protein, mediator (or coactivator) protein, DNA binding protein, RNA binding protein, DNA methylation protein, histone acetylation protein, histone methylation protein, a peptide domain fragment thereof, and a modification thereof. In some embodiments of any one of the methods disclosed herein, the chromatin remodeling protein comprises one or more members selected from the group consisting of SMARCB1, SMARCA4, PBRM1, ARID1A, ARID1B, ARID2, SMARCD1, SMARCE1, ATRX, DAXX, and CHD (e.g., CHD1, CHD2, CHD3, CHD4, CHD5, CHD6, CHD7, CHD8). In some embodiments of any one of the methods disclosed herein, the DNA methylation protein comprises one or more members selected from the group consisting of DNMT3A, DNMT1, TET (e.g., TET1, TET2, TET3), MBD1, and MBD4.

[0016] In some embodiments of any one of the methods disclosed herein, the histone acetylation

protein comprises one or more members selected from the group consisting of EP300, CREBBP, HDAC2, HDAC4, and HDAC9. In some embodiments of any one of the methods disclosed herein, the histone methylation protein comprises one or more members selected from the group consisting of MLL (e.g., MLL1, MLL2, MLL3, MLL4), SETD1A, PRDM9, EZH2, NSD1, NSD2, SETD2, KDM5C, KDM6A, and KDM2B.

[0017] In some embodiments of any one of the methods disclosed herein, the modification comprises at least one mutation relative to a wild type epigenetic modifier. In some embodiments of any one of the methods disclosed herein, the epigenetic modifier is less than about 100 amino acids in length. In some embodiments of any one of the methods disclosed herein, the epigenetic modifier is between about 100 amino acids and about 700 amino acids in length. In some embodiments of any one of the methods disclosed herein, the epigenetic modifier is a synthetic polypeptide.

[0018] In some embodiments of any one of the methods disclosed herein, wherein the target gene comprises a positive regulator of the differentiation selected from the group consisting of: Runx2, SP7/OSX, DLX5, BMPR1B, BMPR2, MSX2, DLX2, DLX3, DLX6, MSX1, ATF4, VDR, PDLIM7, ETS1, FOSL1, WNT9A, BMP2, BMP4, BMP6, BMP7, SMAD1, SMAD5, SMAD8, IGF, Pax7, and MyoD. In some embodiments of any one of the methods disclosed herein, the target gene comprises a negative regulator of the differentiation selected from the group consisting of: PPARg, LIPE, DKK1, DKK2, TWIST1, SMURF1, TGFBR1, TGFBR2.

[0019] In some embodiments of any one of the methods disclosed herein, the cell is a stem cell. In some embodiments of any one of the methods disclosed herein, the cell is an embryonic stem cell or an adult-derived stem cell. In some embodiments of any one of the methods disclosed herein, the adult-derived stem cell comprises a hematopoietic stem cell (HSC), a neuronal stem cells (NSC), a mesenchymal stem cells (MSC), an induced pluripotent stem cell (iPSC), a muscle stem cell (MuSC), a skeletal stem cell, or a skin stem cell. In some embodiments of any one of the methods disclosed herein, the cell is a committed cell comprising a fibroblast, a neuron, a glial cell, an osteoblast, or a chondrocyte.

[0020] In some embodiments of any one of the methods disclosed herein, the transiently contacting occurs in vivo. In some embodiments of any one of the methods disclosed herein, the transiently contacting occurs in vitro or ex vivo.

[0021] In some embodiments, the present disclosure provides a composition comprising the heterologous polypeptide or a heterologous encoding the heterologous polypeptide of any one of the methods disclosed herein, wherein the heterologous polypeptide is for modifying the cell. In some embodiments, the present disclosure provides a kit comprising the composition comprising the heterologous polypeptide or a heterologous encoding the heterologous polypeptide of any one of the methods disclosed herein, wherein the heterologous polypeptide is for modifying the cell, and instructions directing (i) contacting the cell with the composition or (ii) administration of the modified cell to a subject.

[0022] In some embodiments, the present disclosure provides a novel cell population derived from an

initial cell population, wherein the initial cell population is contacted by the heterologous polypeptide of any of the methods described herein in order to generate the novel cell population. In some embodiments of any one of the novel cell population disclosed herein, the cell population is transiently contacted by the heterologous polypeptide. In some embodiments of any one of the novel cell population disclosed herein, the cell population comprises a plurality of embryonic stem cells or a plurality of adult-derived stem cells. In some embodiments of any one of the novel cell population disclosed herein, the cell is a committed cell comprising a fibroblast, a neuron, a glial cell, an osteoblast, or a chondrocyte.

[0023] In some embodiments, the present disclosure provides a method of treating a subject in need thereof, comprising administering to the subject any one of the novel cell population disclosed herein.

[0024] In another aspect, the present disclosure provides a method for modifying an adult-derived stem cell, the method comprising contacting the adult-derived stem cell with a heterologous polypeptide comprising a first actuator moiety configured to complex with a target polynucleotide in the adult-derived stem cell, wherein the first actuator moiety is linked to an epigenetic modifier to effect a modification in an expression profile of a target gene in the adult-derived stem cell, wherein, upon the contacting, the adult-derived stem cell exhibits a maximum change within the modified expression profile of the target gene in less than about 3 days.

[0025] In some embodiments of any one of the methods disclosed herein, the adult-derived stem cell exhibits the maximum change in less than about 2 days. In some embodiments of any one of the methods disclosed herein, the adult-derived stem cell exhibits the maximum change in less than about 36 hours. In some embodiments of any one of the methods disclosed herein, the adult-derived stem cell exhibits the maximum change at about 24 hours upon the contacting.

[0026] In some embodiments of any one of the methods disclosed herein, the maximum change yields a local maximum level within the modified expression profile. In some embodiments of any one of the methods disclosed herein, the local maximum is at least about 5-fold greater as compared to the control stem cell. In some embodiments of any one of the methods disclosed herein, the local maximum is at least about 10-fold greater as compared to the control stem cell. In some embodiments of any one of the methods disclosed herein, the local maximum level is reversed by at least about 60% within about 4 days upon the contacting.

[0027] In some embodiments of any one of the methods disclosed herein, the maximum change yields a local minimum level within the modified expression profile. In some embodiments of any one of the methods disclosed herein, the local minimum is at least about 5-fold lower as compared to the control stem cell. In some embodiments of any one of the methods disclosed herein, the local minimum is at least about 10-fold lower as compared to the control stem cell. In some embodiments of any one of the methods disclosed herein, the maximum change within the modified expression profile is lost within about 4 days upon the contacting.

[0028] In another aspect, the present disclosure provides a method for modifying an adult-derived stem cell, the method comprising contacting the adult-derived stem cell with a heterologous polypeptide

comprising a first actuator moiety configured to complex with a target polynucleotide in the adult-derived stem cell, wherein the first actuator moiety is linked to an epigenetic modifier to effect a modification of an expression profile of a target gene in the adult-derived stem cell, wherein, upon reduction or termination of the contacting, the adult-derived stem cell sustains at least a portion of the modified expression profile for at least about 1 week.

[0029] In some embodiments of any one of the methods disclosed herein, the reduction or termination of the contacting comprises removal of the heterologous polypeptide or a gene encoding the heterologous polypeptide from the adult-derived stem cell. In some embodiments of any one of the methods disclosed herein, the at least the portion of the modified expression profile is sustained for at least about 2 weeks. In some embodiments of any one of the methods disclosed herein, the at least the portion of the modified expression profile is sustained for at least about 1 month. In some embodiments of any one of the methods disclosed herein, the at least the portion of the modified expression profile is sustained for at least about 3 months. In some embodiments of any one of the methods disclosed herein, at least 5% of the modified expression profile is sustained for at least about 1 week. In some embodiments of any one of the methods disclosed herein, at least 5% of the modified expression profile is sustained for at least about 1 week.

[0030] In another aspect, the present disclosure provides a method for modifying an adult-derived stem cell, the method comprising (a) contacting the adult-derived stem cell with a first heterologous polypeptide comprising a first actuator moiety configured to complex with a first target polynucleotide of the adult-derived stem cell, wherein the first actuator moiety is linked to a first epigenetic modifier to effect a modification of an expression profile of a target gene in the adult-derived stem cell, to generate a modified cell; and (b) contacting the modified cell with a second heterologous polypeptide comprising a second actuator moiety configured to complex with a second target polynucleotide of the modified cell, wherein the second actuator moiety is linked to a second epigenetic modifier to reverse all or part of the modified expression profile of the target gene in the modified cell, wherein the first and second actuator moieties are different, and wherein the first and second epigenetic modifiers are different.

[0031] In some embodiments of any one of the methods disclosed herein, contacting the modified cell with a second heterologous polypeptide comprising a second actuator moiety configured to complex with a second target polynucleotide of the modified cell is performed at least about 24 hours subsequent to contacting the adult-derived stem cell with a first heterologous polypeptide comprising a first actuator moiety configured to complex with a first target polynucleotide of the adult-derived stem cell. In some embodiments of any one of the methods disclosed herein, contacting the modified cell with a second heterologous polypeptide comprising a second actuator moiety configured to complex with a second target polynucleotide of the modified cell is performed at least about 5 days subsequent to contacting the adult-derived stem cell with a first heterologous polypeptide comprising a first actuator moiety configured to complex with a first target polynucleotide of the adult-derived stem cell. In some embodiments of any one of the methods disclosed herein, contacting the modified cell with a second heterologous polypeptide

comprising a second actuator moiety configured to complex with a second target polynucleotide of the modified cell is performed at least about 10 days subsequent to contacting the adult-derived stem cell with a first heterologous polypeptide comprising a first actuator moiety configured to complex with a first target polynucleotide of the adult-derived stem cell.

[0032] In some embodiments of any one of the methods disclosed herein, prior to contacting the modified cell with a second heterologous polypeptide comprising a second actuator moiety configured to complex with a second target polynucleotide of the modified cell, a resulting expression level of the target gene is sustained. In some embodiments of any one of the methods disclosed herein, contacting the adult-derived stem cell with a first heterologous polypeptide comprising a first actuator moiety configured to complex with a first target polynucleotide of the adult-derived stem cell, comprises transiently contacting the cell with the first heterologous polypeptide. In some embodiments of any one of the methods disclosed herein, contacting the modified cell with a second heterologous polypeptide comprising a second actuator moiety configured to complex with a second target polynucleotide of the modified cell comprises transiently contacting the cell with the second heterologous polypeptide.

[0033] In some embodiments of any one of the methods disclosed herein, contacting the modified cell with a second heterologous polypeptide comprising a second actuator moiety configured to complex with a second target polynucleotide of the modified cell effects at least about 70% reversal of the modified expression profile of the target gene. In some embodiments of any one of the methods disclosed herein, contacting the modified cell with a second heterologous polypeptide comprising a second actuator moiety configured to complex with a second target polynucleotide of the modified cell effects at least about 80% reversal of the modified expression profile of the target gene. In some embodiments of any one of the methods disclosed herein, contacting the modified cell with a second heterologous polypeptide comprising a second actuator moiety configured to complex with a second target polynucleotide of the modified cell effects at least about 90% reversal of the modified expression profile of the target gene. In some embodiments of any one of the methods disclosed herein, contacting the modified cell with a second heterologous polypeptide comprising a second actuator moiety configured to complex with a second target polynucleotide of the modified cell effects at least about 99% reversal of the modified expression profile of the target gene.

[0034] In some embodiments of any one of the methods disclosed herein, subsequent to contacting the modified cell with a second heterologous polypeptide comprising a second actuator moiety configured to complex with a second target polynucleotide of the modified cell, the modified expression profile of the target gene is reversed in less than about 1 week. In some embodiments of any one of the methods disclosed herein, subsequent to contacting the modified cell with a second heterologous polypeptide comprising a second actuator moiety configured to complex with a second target polynucleotide of the modified cell, the modified expression profile of the target gene is reversed in less than about 4 days. In some embodiments of any one of the methods disclosed herein, subsequent to contacting the modified cell with a second heterologous polypeptide comprising a second actuator moiety configured to complex with

a second target polynucleotide of the modified cell, the modified expression profile of the target gene is reversed in less than about 2 days.

[0035] In some embodiments of any one of the methods disclosed herein, the first target polynucleotide and the second target polynucleotide are different. In some embodiments of any one of the methods disclosed herein, the first target polynucleotide and the second target polynucleotide are the same.

[0036] In some embodiments of any one of the methods disclosed herein, a maximum change within the modified expression profile of the target gene is at least about 3-fold greater as compared to a control cell. In some embodiments of any one of the methods disclosed herein, a maximum change within the modified expression profile of the target gene is at least about 3-fold lower as compared to a control cell.

[0037] In some embodiments of any one of the methods disclosed herein, wherein the first actuator moiety comprises a heterologous endonuclease. In some embodiments of any one of the methods disclosed herein, the heterologous endonuclease is directed by a guide ribonucleic acid (RNA) to specifically bind the endogenous target gene. In some embodiments of any one of the methods disclosed herein, the heterologous endonuclease substantially lacks DNA cleavage activity.

[0038] In some embodiments of any one of the methods disclosed herein, the epigenetic modifier comprises one or more members selected from the group consisting of a chromatin remodeling protein, chromatin reader protein, nuclear protein, transcription factor protein, mediator (or coactivator) protein, DNA binding protein, RNA binding protein, DNA methylation protein, histone acetylation protein, histone methylation protein, a peptide domain fragment thereof, and a modification thereof. In some embodiments of any one of the methods disclosed herein, the chromatin remodeling protein comprises one or more members selected from the group consisting of SMARCB1, SMARCA4, PBRM1, ARID1A, ARID1B, ARID2, SMARCD1, SMARCE1, ATRX, DAXX, and CHD (e.g., CHD1, CHD2, CHD3, CHD4, CHD5, CHD6, CHD7, CHD8). In some embodiments of any one of the methods disclosed herein, the DNA methylation protein comprises one or more members selected from the group consisting of DNMT3A, DNMT1, TET (e.g., TET1, TET2, TET3), MBD1, and MBD4. In some embodiments of any one of the methods disclosed herein, the histone acetylation protein comprises one or more members selected from the group consisting of EP300, CREBBP, HDAC2, HDAC4, and HDAC9. In some embodiments of any one of the methods disclosed herein, the histone methylation protein comprises one or more members selected from the group consisting of MLL (e.g., MLL1, MLL2, MLL3, MLL4), SETD1A, PRDM9, EZH2, NSD1, NSD2, SETD2, KDM5C, KDM6A, and KDM2B.

[0039] In some embodiments of any one of the methods disclosed herein, the modification comprises at least one mutation relative to a wild type epigenetic modifier. In some embodiments of any one of the methods disclosed herein, the epigenetic modifier is less than about 100 amino acids in length. In some embodiments of any one of the methods disclosed herein, the epigenetic modifier is between about 100 amino acids and about 700 amino acids in length. In some embodiments of any one of the methods disclosed herein, the epigenetic modifier is a synthetic polypeptide.

[0040] In some embodiments of any one of the methods disclosed herein, the modification of the expression profile of the target gene effects differentiation of the adult-derived stem cell into a specific cell type. In some embodiments of any one of the methods disclosed herein, the target gene comprises a positive regulator of the differentiation selected from the group consisting of: Runx2, SP7/OSX, DLX5, BMPR1B, BMPR2, MSX2, DLX2, DLX3, DLX6, MSX1, ATF4, VDR, PDLIM7, ETS1, FOSL1, WNT9A, BMP2, BMP4, BMP6, BMP7, SMAD1, SMAD5, SMAD8, IGF, Pax7, and MyoD. In some embodiments of any one of the methods disclosed herein, the target gene comprises a negative regulator of the differentiation selected from the group consisting of: PPARg, LIPE, DKK1, DKK2, TWIST1, SMURF1, TGFBR1, TGFBR2.

[0041] In some embodiments of any one of the methods disclosed herein, the adult-derived stem cell comprises a hematopoietic stem cell (HSC), a neuronal stem cells (NSC), a mesenchymal stem cells (MSC), an induced pluripotent stem cell (iPSC), a muscle stem cell (MuSC), a skeletal stem cell, or a skin stem cell.

[0042] In some embodiments, the present disclosure provides a composition comprising the heterologous polypeptide or a heterologous encoding the heterologous polypeptide of any one the methods disclosed herein, wherein the heterologous polypeptide is for modifying the adult-derived stem cell. In some embodiments, the present disclosure provides a kit comprising the composition comprising the heterologous polypeptide or a heterologous encoding the heterologous polypeptide of any one the methods disclosed herein, and instructions directing (i) contacting the adult-derived stem cell with the composition or (ii) administration of the modifying adult-derived stem cell to a subject.

[0043] In some embodiments, the present disclosure provides a novel cell population derived from an adult-derived stem cell population, wherein the adult-derived stem cell population is contacted by the heterologous polypeptide of any one of the methods disclosed herein, to generate the novel cell population. In some embodiments of any one of the methods disclosed herein, the adult-derived stem cell population is transiently contacted by the heterologous polypeptide, to generate the novel cell population. In some embodiments, the present disclosure provides a method of treatment of a subject in need thereof, comprising administering to the subject the novel cell population derived from an adult-derived stem cell population, wherein the adult-derived stem cell population is contacted by the heterologous polypeptide of any one of the methods disclosed herein.

[0044] In another aspect, the present disclosure provides a system for inducing differentiation of an adult-derived stem cell, the system comprising a first heterologous polypeptide comprising an actuator moiety configured to form a first complex with a first target gene, to modify an expression profile of the first target gene; and a second heterologous polypeptide comprising an actuator moiety configured to form a second complex with a second target gene, to modify an expression profile of the second target gene, wherein the modification of the expression profile of the first target gene and the expression profile of the second target gene effects differentiation of the adult-derived stem cell into a target cell type, wherein the modification comprises either (i) enhancing the expression profile of the first target gene and the

expression profile of the second target gene or (ii) decreasing the expression profile of the first target gene and the expression profile of the second target gene.

[0045] In some embodiments of any one of the systems disclosed herein, the modification of the expression profile of the target gene effects differentiation of the adult-derived stem cell into a specific cell type. In some embodiments of any one of the systems disclosed herein, the specific cell type is derived from osteogenesis. In some embodiments of any one of the systems disclosed herein, the specific cell type derived from osteogenesis is effected by activating BMP6 and TGFB3. In some embodiments of any one of the systems disclosed herein, the specific cell type is derived from chondrogenesis. In some embodiments of any one of the systems disclosed herein, the specific cell type derived from chondrogenesis is effected by activating RUNX2, OSX, and DLX5.

[0046] In some embodiments of any one of the systems disclosed herein, the target gene comprises a positive regulator of differentiation selected from the group consisting of: Runx2, SP7/OSX, DLX5, BMPR1B, BMPR2, MSX2, DLX2, DLX3, DLX6, MSX1, ATF4, VDR, PDLIM7, ETS1, FOSL1, WNT9A, BMP2, BMP4, BMP6, BMP7, SMAD1, SMAD5, SMAD8, IGF, Pax7, and MyoD. In some embodiments of any one of the systems disclosed herein, the target gene comprises a negative regulator of the differentiation selected from the group consisting of: PPARg, LIPE, DKK1, DKK2, TWIST1, SMURF1, TGFBR1, TGFBR2.

[0047] In some embodiments of any one of the systems disclosed herein, the adult-derived stem cell comprises a mesenchymal stem cell (MSC) or an induced pluripotent stem cell (iPSC). In some embodiments of any one of the systems disclosed herein, the adult-derived stem cell exhibits a maximum change within the modified expression profile of the target gene in less than about 3 days. In some embodiments of any one of the systems disclosed herein, the modification is transient.

[0048] In another aspect, the present disclosure provides a system for inducing differentiation of an adult-derived stem cell, the system comprising a first heterologous polypeptide comprising an actuator moiety configured to form a first complex with a first target gene, to modify an expression profile of the first target gene; and a first heterologous polypeptide comprising an actuator moiety configured to form a second complex with a second target gene, to modify an expression profile of the second target gene, wherein the modification of the expression profile of the first target gene and the expression profile of the second target gene effects differentiation of the adult-derived stem cell into a target cell type, wherein the first target gene and the second target gene are different, and wherein the first target gene or and/or the second target gene is not (i) Sox9 or (ii) PPARγ.

[0049] In some embodiments of any one of the systems disclosed herein, the modification of the expression profile of the target gene effects differentiation of the adult-derived stem cell into a specific cell type. In some embodiments of any one of the systems disclosed herein, the specific cell type is derived from osteogenesis. In some embodiments of any one of the systems disclosed herein, the specific cell type derived from osteogenesis is effected by activating BMP6 and TGFB3. In some embodiments of any one of the systems disclosed herein, the specific cell type is derived from chondrogenesis. In some

embodiments of any one of the systems disclosed herein, the specific cell type derived from chondrogenesis is effected by activating RUNX2, OSX, and DLX5.

[0050] In some embodiments of any one of the systems disclosed herein, the target gene comprises a positive regulator of differentiation selected from the group consisting of: Runx2, SP7/OSX, DLX5, BMPR1B, BMPR2, MSX2, DLX2, DLX3, DLX6, MSX1, ATF4, VDR, PDLIM7, ETS1, FOSL1, WNT9A, BMP2, BMP4, BMP6, BMP7, SMAD1, SMAD5, SMAD8, IGF, Pax7, and MyoD. In some embodiments of any one of the systems disclosed herein, the target gene comprises a negative regulator of the differentiation selected from the group consisting of: PPARg, LIPE, DKK1, DKK2, TWIST1, SMURF1, TGFBR1, TGFBR2.

[0051] In some embodiments of any one of the systems disclosed herein, the adult-derived stem cell comprises a mesenchymal stem cell (MSC) or an induced pluripotent stem cell (iPSC). In some embodiments of any one of the systems disclosed herein, the adult-derived stem cell exhibits a maximum change within the modified expression profile of the target gene in less than about 3 days. In some embodiments of any one of the systems disclosed herein, the modification is transient.

[0052] In another aspect, the present disclosure provides a system for inducing differentiation of an adult-derived stem cell, the system comprising a heterologous polypeptide comprising an actuator moiety configured to form a complex with a target polynucleotide in the adult-derived stem cell, wherein the actuator moiety is operatively linked to an epigenetic modifier to modify an expression profile of a target gene in the adult-derived stem cell, and wherein the modification of the expression profile of the target gene induced by the formation of the complex sufficiently effects chondrogenic differentiation of the adult-derived stem cell.

[0053] In some embodiments of any one of the systems disclosed herein, the modification of the expression profile of the target gene induced by the formation of the complex sufficiently induces expression of one or more chondrogenic differentiation markers. In some embodiments of any one of the systems disclosed herein, the system occurs in an environment that is substantially free of an additional chondrogenic factor in medium. In some embodiments of any one of the systems disclosed herein, chondrogenic differentiation is effected by activating RUNX2, OSX, and DLX5.

[0054] In some embodiments of any one of the systems disclosed herein, the target gene comprises a positive regulator of the differentiation selected form the group consisting of: Runx2, SP7/OSX, DLX5, BMPR1B, BMPR2, MSX2, DLX2, DLX3, DLX6, MSX1, ATF4, VDR, PDLIM7, ETS1, FOSL1, WNT9A, BMP2, BMP4, BMP6, BMP7, SMAD1, SMAD5, SMAD8, IGF, Pax7, and MyoD. In some embodiments of any one of the systems disclosed herein, the target gene comprises a negative regulator of the differentiation selected from the group consisting of: PPARg, LIPE, DKK1, DKK2, TWIST1, SMURF1, TGFBR1, TGFBR2.

[0055] In some embodiments of any one of the systems disclosed herein, the adult-derived stem cell comprises a mesenchymal stem cell (MSC) or an induced pluripotent stem cell (iPSC). In some embodiments of any one of the systems disclosed herein, the adult-derived stem cell exhibits a maximum

change within the modified expression profile of the target gene in less than about 3 days. In some embodiments of any one of the systems disclosed herein, the modification is transient.

[0056] In another aspect, the present disclosure provides a system for inducing osteogenic differentiation of an adult-derived stem cell, the system comprising a heterologous polypeptide comprising an actuator moiety configured to form a complex with a target polynucleotide in the adult-derived stem cell, wherein the actuator moiety is operatively linked to an epigenetic modifier to modify an expression profile of a target gene in the adult-derived stem cell, and wherein the modification of the expression profile of the target gene induced by the formation of the complex effects osteogenic differentiation of the adult-derived stem cell.

[0057] In some embodiments of any one of the systems disclosed herein, the modification of the expression profile of the target gene induced by the formation of the complex sufficiently induces osteogenic differentiation of the adult-derived stem cell. In some embodiments of any one of the systems disclosed herein, the modification of the expression profile of the target gene induced by the formation of the complex sufficiently induces expression of one or more osteogenic differentiation markers. In some embodiments of any one of the systems disclosed herein, the system occurs in an environment that is substantially free of dexamethasone in medium. In some embodiments of any one of the systems disclosed herein, the osteogenic differentiation is effected by activating BMP6 and TGFB3.

[0058] In some embodiments of any one of the systems disclosed herein, the target gene comprises a positive regulator of the differentiation selected form the group consisting of: Runx2, SP7/OSX, DLX5, BMPR1B, BMPR2, MSX2, DLX2, DLX3, DLX6, MSX1, ATF4, VDR, PDLIM7, ETS1, FOSL1, WNT9A, BMP2, BMP4, BMP6, BMP7, SMAD1, SMAD5, SMAD8, IGF, Pax7, and MyoD. In some embodiments of any one of the systems disclosed herein, the target gene comprises a negative regulator of the differentiation selected from the group consisting of: PPARg, LIPE, DKK1, DKK2, TWIST1, SMURF1, TGFBR1, TGFBR2.

[0059] In some embodiments of any one of the systems disclosed herein, the adult-derived stem cell comprises a mesenchymal stem cell (MSC) or an induced pluripotent stem cell (iPSC). In some embodiments of any one of the systems disclosed herein, the adult-derived stem cell exhibits a maximum change within the modified expression profile of the target gene in less than about 3 days. In some embodiments of any one of the systems disclosed herein, the modification is transient.

[0060] In another aspect, the present disclosure provides a method for inducing differentiation of an adult-derived stem cell, the method comprising contacting a cell with a first heterologous polypeptide comprising an actuator moiety configured to form a first complex with a first target gene, to modify an expression profile of the first target gene; and contacting a cell with a second heterologous polypeptide comprising an actuator moiety configured to form a second complex with a second target gene, to modify an expression profile of the second target gene, wherein the modification of the expression profile of the first target gene and the expression profile of the second target gene effects differentiation of the adult-derived stem cell into a target cell type, wherein the modification comprises either (i) enhancing the

expression profile of the first target gene and the expression profile of the second target gene or (ii) decreasing the expression profile of the first target gene and the expression profile of the second target gene.

[0061] In some embodiments of any one of the methods disclosed herein, the modification of the expression profile of the target gene effects differentiation of the adult-derived stem cell into a specific cell type. In some embodiments of any one of the methods disclosed herein, the specific cell type is derived from osteogenesis. In some embodiments of any one of the methods disclosed herein, the specific cell type derived from osteogenesis is effected by activating BMP6 and TGFB3. In some embodiments of any one of the methods disclosed herein, the specific cell type is derived from chondrogenesis. In some embodiments of any one of the methods disclosed herein, the specific cell type derived from chondrogenesis is effected by activating RUNX2, OSX, and DLX5.

[0062] In some embodiments of any one of the methods disclosed herein, the target gene comprises a positive regulator of differentiation selected from the group consisting of: Runx2, SP7/OSX, DLX5, BMPR1B, BMPR2, MSX2, DLX2, DLX3, DLX6, MSX1, ATF4, VDR, PDLIM7, ETS1, FOSL1, WNT9A, BMP2, BMP4, BMP6, BMP7, SMAD1, SMAD5, SMAD8, IGF, Pax7, and MyoD. In some embodiments of any one of the methods disclosed herein, the target gene comprises a negative regulator of the differentiation selected from the group consisting of: PPARg, LIPE, DKK1, DKK2, TWIST1, SMURF1, TGFBR1, TGFBR2.

[0063] In some embodiments of any one of the methods disclosed herein, the adult-derived stem cell comprises a mesenchymal stem cell (MSC) or an induced pluripotent stem cell (iPSC). In some embodiments of any one of the methods disclosed herein, the adult-derived stem cell exhibits a maximum change within the modified expression profile of the target gene in less than about 3 days. In some embodiments of any one of the methods disclosed herein, the modification is transient.

[0064] In another aspect, the present disclosure provides a method for inducing differentiation of an adult-derived stem cell, the method comprising contacting a cell with a first heterologous polypeptide comprising an actuator moiety configured to form a first complex with a first target gene, to modify an expression profile of the first target gene; and contacting a cell with a second heterologous polypeptide comprising an actuator moiety configured to form a second complex with a second target gene, to modify an expression profile of the second target gene, wherein the modification of the expression profile of the first target gene and the expression profile of the second target gene effects differentiation of the adult-derived stem cell into a target cell type wherein the first target gene and the second target gene are different, and wherein the first target gene or and/or the second target gene is not (i) Sox9 or (ii) PPARγ.

[0065] In some embodiments of any one of the methods disclosed herein, the modification of the expression profile of the target gene effects differentiation of the adult-derived stem cell into a specific cell type. In some embodiments of any one of the methods disclosed herein, the specific cell type is derived from osteogenesis. In some embodiments of any one of the methods disclosed herein, the specific cell type derived from osteogenesis is effected by activating BMP6 and TGFB3. In some embodiments of

any one of the methods disclosed herein, the specific cell type is derived from chondrogenesis. In some embodiments of any one of the methods disclosed herein, the specific cell type derived from chondrogenesis is effected by activating RUNX2, OSX, and DLX5.

[0066] In some embodiments of any one of the methods disclosed herein, the target gene comprises a positive regulator of differentiation selected from the group consisting of: Runx2, SP7/OSX, DLX5, BMPR1B, BMPR2, MSX2, DLX2, DLX3, DLX6, MSX1, ATF4, VDR, PDLIM7, ETS1, FOSL1, WNT9A, BMP2, BMP4, BMP6, BMP7, SMAD1, SMAD5, SMAD8, IGF, Pax7, and MyoD. In some embodiments of any one of the methods disclosed herein, the target gene comprises a negative regulator of the differentiation selected from the group consisting of: PPARg, LIPE, DKK1, DKK2, TWIST1, SMURF1, TGFBR1, TGFBR2.

[0067] In some embodiments of any one of the methods disclosed herein, the adult-derived stem cell comprises a mesenchymal stem cell (MSC) or an induced pluripotent stem cell (iPSC). In some embodiments of any one of the methods disclosed herein, the adult-derived stem cell exhibits a maximum change within the modified expression profile of the target gene in less than about 3 days. In some embodiments of any one of the methods disclosed herein, the modification is transient.

[0068] In another aspect, the present disclosure provides a method for inducing differentiation of an adult-derived stem cell, the method comprising contacting a cell with a heterologous polypeptide comprising an actuator moiety configured to form a complex with a target polynucleotide in the adult-derived stem cell, wherein the actuator moiety is operatively linked to an epigenetic modifier to modify an expression profile of a target gene in the adult-derived stem cell, and wherein the modification of the expression profile of the target gene induced by the formation of the complex sufficiently effects chondrogenic differentiation of the adult-derived stem cell.

[0069] In some embodiments of any one of the methods disclosed herein, the modification of the expression profile of the target gene induced by the formation of the complex sufficiently induces expression of one or more chondrogenic differentiation markers. In some embodiments of any one of the methods disclosed herein, the method occurs in an environment that is substantially free of an additional chondrogenic factor in medium. In some embodiments of any one of the methods disclosed herein, chondrogenic differentiation is effected by activating RUNX2, OSX, and DLX5.

[0070] In some embodiments of any one of the methods disclosed herein, the target gene comprises a positive regulator of the differentiation selected form the group consisting of: Runx2, SP7/OSX, DLX5, BMPR1B, BMPR2, MSX2, DLX2, DLX3, DLX6, MSX1, ATF4, VDR, PDLIM7, ETS1, FOSL1, WNT9A, BMP2, BMP4, BMP6, BMP7, SMAD1, SMAD5, SMAD8, IGF, Pax7, and MyoD. In some embodiments of any one of the methods disclosed herein, the target gene comprises a negative regulator of the differentiation selected from the group consisting of: PPARg, LIPE, DKK1, DKK2, TWIST1, SMURF1, TGFBR1, TGFBR2.

[0071] In some embodiments of any one of the methods disclosed herein, the adult-derived stem cell comprises a mesenchymal stem cell (MSC) or an induced pluripotent stem cell (iPSC). In some

embodiments of any one of the methods disclosed herein, the adult-derived stem cell exhibits a maximum change within the modified expression profile of the target gene in less than about 3 days. In some embodiments of any one of the methods disclosed herein, the modification is transient.

[0072] In another aspect, the present disclosure provides a method for inducing osteogenic differentiation of an adult-derived stem cell, the method comprising contacting a cell with a heterologous polypeptide comprising an actuator moiety configured to form a complex with a target polynucleotide in the adult-derived stem cell, wherein the actuator moiety is operatively linked to an epigenetic modifier to modify an expression profile of a target gene in the adult-derived stem cell, and wherein the modification of the expression profile of the target gene induced by the formation of the complex effects osteogenic differentiation of the adult-derived stem cell.

[0073] In some embodiments of any one of the methods disclosed herein, the modification of the expression profile of the target gene induced by the formation of the complex sufficiently induces osteogenic differentiation of the adult-derived stem cell. In some embodiments of any one of the methods disclosed herein, the modification of the expression profile of the target gene induced by the formation of the complex sufficiently induces expression of one or more osteogenic differentiation markers. In some embodiments of any one of the methods disclosed herein, the method occurs in an environment that is substantially free of dexamethasone in medium. In some embodiments of any one of the methods disclosed herein, the osteogenic differentiation is effected by activating BMP6 and TGFB3.

[0074] In some embodiments of any one of the methods disclosed herein, the target gene comprises a positive regulator of the differentiation selected form the group consisting of: Runx2, SP7/OSX, DLX5, BMPR1B, BMPR2, MSX2, DLX2, DLX3, DLX6, MSX1, ATF4, VDR, PDLIM7, ETS1, FOSL1, WNT9A, BMP2, BMP4, BMP6, BMP7, SMAD1, SMAD5, SMAD8, IGF, Pax7, and MyoD. In some embodiments of any one of the methods disclosed herein, the target gene comprises a negative regulator of the differentiation selected from the group consisting of: PPARg, LIPE, DKK1, DKK2, TWIST1, SMURF1, TGFBR1, TGFBR2.

[0075] In some embodiments of any one of the methods disclosed herein, the adult-derived stem cell comprises a mesenchymal stem cell (MSC) or an induced pluripotent stem cell (iPSC). In some embodiments of any one of the methods disclosed herein, the adult-derived stem cell exhibits a maximum change within the modified expression profile of the target gene in less than about 3 days. In some embodiments of any one of the methods disclosed herein, the modification is transient.

[0076] In certain aspects, the present disclosure provides a method for modifying an adult-derived stem cell, the method comprising:

contacting the adult-derived stem cell with a heterologous polypeptide comprising a first actuator moiety configured to complex with a target polynucleotide in the adult-derived stem cell, wherein the first actuator moiety is operatively linked to an epigenetic modifier to effect a modification in an expression profile of a target gene in the adult-derived stem cell,

wherein, upon the contacting, the adult-derived stem cell exhibits a maximum change within

the modified expression profile of the target gene in less than about 3 days.

[0077] In practicing the method described hereinabove (or described elsewhere herein) for modifying the adult-derived stem cell, the adult-derived stem cell may exhibit the maximum change in less than about 2 days. In some embodiments, the adult-derived stem cell exhibits the maximum change in less than about 1 day. In some embodiments, the adult-derived stem cell exhibits the maximum change in less than about 12 hours. In some embodiments, the maximum change yields a local maximum level within the modified expression profile. In some embodiments, the local maximum is at least about 5-fold greater as compared to the control stem cell. In some embodiments, the local maximum is at least about 10-fold greater as compared to the control stem cell. In some embodiments, the local maximum level is reversed by at least about 60% within about 4 days upon the contacting. In some embodiments, the maximum change yields a local minimum level within the modified expression profile. In some embodiments, the local minimum is at least about 5-fold lower as compared to the control stem cell. In some embodiments, the local minimum is at least about 10-fold lower as compared to the control stem cell. In some embodiments, the maximum change within the modified expression profile is lost within about 4 days upon the contacting.

[0078] In certain aspects, the present disclosure provides a method for modifying an adult-derived stem cell, comprising:

contacting the adult-derived stem cell with a heterologous polypeptide comprising a first actuator moiety configured to complex with a target polynucleotide in the adult-derived stem cell, wherein the first actuator moiety is operatively linked to an epigenetic modifier to effect a modification of an expression profile of a target gene in the adult-derived stem cell,

wherein, upon reduction or termination of the contacting, the adult-derived stem cell sustains the modified expression profile for at least about 1 week.

[0079] In practicing the method described in the immediately preceding paragraph (or described elsewhere herein) for modifying the adult-derived stem cell, the reduction or termination of the contacting may comprise removal of the heterologous polypeptide or a gene encoding the heterologous polypeptide from the adult-derived stem cell. In some embodiments, the modified expression profile is sustained for at least about 2 weeks. In some embodiments, the modified expression profile is sustained for at least about 1 month. In some embodiments, the modified expression profile is sustained for at least about 3 months.

[0080] In certain aspects, the present disclosure provides a method for modifying an adult-derived stem cell, comprising:

(a) contacting the adult-derived stem cell with a first heterologous polypeptide comprising a first actuator moiety configured to complex with a first target polynucleotide of the adult-derived stem cell, wherein the first actuator moiety is operatively linked to a first epigenetic modifier to effect a modification of an expression profile of a target gene in the adult-derived stem cell, to generate a modified cell; and

(b) contacting the modified cell with a second heterologous polypeptide comprising a second

actuator moiety configured to complex with a second target polynucleotide of the modified cell, wherein the second actuator moiety is operatively linked to a second epigenetic modifier to reverse all or part of the modified expression profile of the target gene in the modified cell,

wherein the first and second actuator moieties are different, and

wherein the first and second epigenetic modifiers are different.

[0081] In practicing the method described in the immediately preceding paragraph (or described elsewhere herein) for modifying the adult-derived stem cell, (b) may be performed at least about 24 hours subsequent to (a). In some embodiments, (b) is performed at least about 5 days subsequent to (a). In some embodiments, (b) is performed at least about 10 days subsequent to (a). In some embodiments, prior to (b), a resulting expression level of the target gene is sustained. In some embodiments, (a) comprises transiently contacting the cell with the first heterologous polypeptide. In some embodiments, (b) comprises transiently contacting the cell with the second heterologous polypeptide. In some embodiments, (b) effects at least about 70% reversal of the modified expression profile of the target gene. In some embodiments, (b) effects at least about 80% reversal of the modified expression profile of the target gene. In some embodiments, (b) effects at least about 90% reversal of the modified expression profile of the target gene. In some embodiments, (b) effects at least about 99% reversal of the modified expression profile of the target gene. In some embodiments, subsequent to (b), the modified expression profile of the target gene is reversed in less than about 1 week. In some embodiments, subsequent to (b), the modified expression profile of the target gene is reversed in less than about 4 days. In some embodiments, subsequent to (b), the modified expression profile of the target gene is reversed in less than about 2 days. In some embodiments, the first target polynucleotide and the second target polynucleotide are different. In some embodiments, the first target polynucleotide and the second target polynucleotide are the same. In some embodiments, a maximum change within the modified expression profile of the target gene is at least about 3-fold greater as compared to a control cell. In some embodiments, a maximum change within the modified expression profile of the target gene is at least about 3-fold lower as compared to a control cell.

[0082] In practicing any of the method for modifying the adult-derived stem cell as described herein, the first actuator moiety may comprise a heterologous endonuclease. In some embodiments, the heterologous endonuclease is directed by a guide ribonucleic acid (RNA) to specifically bind the endogenous target gene. In some embodiments, the heterologous endonuclease substantially lacks DNA cleavage activity.

[0083] In practicing any of the method for modifying the adult-derived stem cell as described herein, the epigenetic modifier may comprise one or more members selected from the group consisting of a chromatin remodeling protein, chromatin reader protein, nuclear protein, transcription factor protein, mediator (or coactivator) protein, DNA binding protein, RNA binding protein, DNA methylation protein, histone acetylation protein, histone methylation protein, a peptide domain fragment thereof, and a modification thereof. In some embodiments, the chromatin remodeling protein comprises one or more

members selected from the group consisting of SMARCB1, SMARCA4, PBRM1, ARID1A, ARID1B, ARID2, SMARCD1, SMARCE1, ATRX, DAXX, and CHD (e.g., CHD1, CHD2, CHD3, CHD4, CHD5, CHD6, CHD7, CHD8). In some embodiments, the DNA methylation protein comprises one or more members selected from the group consisting of DNMT3A, DNMT1, TET (e.g., TET1, TET2, TET3), MBD1, and MBD4. In some embodiments, the histone acetylation protein comprises one or more members selected from the group consisting of EP300, CREBBP, HDAC2, HDAC4, and HDAC9. In some embodiments, the histone methylation protein comprises one or more members selected from the group consisting of MLL (e.g., MLL1, MLL2, MLL3, MLL4), SETD1A, PRDM9, EZH2, NSD1, NSD2, SETD2, KDM5C, KDM6A, and KDM2B. In some embodiments, the modification comprises at least one mutation relative to a wild type epigenetic modifier. In some embodiments, the epigenetic modifier is less than about 100 amino acids in length. In some embodiments, the epigenetic modifier is between about 100 amino acids and about 700 amino acids in length. In some embodiments, the epigenetic modifier is a synthetic polypeptide.

[0084] In practicing any of the method for modifying the adult-derived stem cell as described herein, the modification of the expression profile of the target gene may effect differentiation of the adult-derived stem cell into a specific cell type. In some embodiments, the target gene comprises a positive regulator of the differentiation selected from the group consisting of: Runx2, SP7/OSX, DLX5, BMPR1B, BMPR2, MSX2, DLX2, DLX3, DLX6, MSX1, ATF4, VDR, PDLIM7, ETS1, FOSL1, WNT9A, BMP2, BMP4, BMP6, BMP7, SMAD1, SMAD5, SMAD8, IGF, Pax7, and MyoD. In some embodiments, the target gene comprises a negative regulator of the differentiation selected from the group consisting of: PPARg, LIPE, DKK1, DKK2, TWIST1, SMURF1, TGFBR1, TGFBR2.

[0085] In practicing any of the method for modifying the adult-derived stem cell as described herein, the adult-derived stem cell may comprise a hematopoietic stem cell (HSC), a neuronal stem cells (NSC), a mesenchymal stem cells (MSC), an induced pluripotent stem cell (iPSC), a muscle stem cell (MuSC), a skeletal stem cell, or a skin stem cell.

[0086] Some aspects of the present disclosure provide a composition, the composition comprising the heterologous polypeptide as described herein or a heterologous encoding the heterologous polypeptide as described herein, wherein the heterologous polypeptide is for modifying the adult-derived stem cell.

[0087] Some aspects of the present disclosure provide a kit, the kit comprising: the composition described hereinabove (or described elsewhere herein); and instructions directing (i) contacting the adult-derived stem cell with the composition or (ii) administration of the modifying adult-derived stem cell to a subject.

[0088] Some aspects of the present disclosure provide a novel cell population derived from an adult-derived stem cell population, wherein the adult-derived stem cell population is contacted by the heterologous polypeptide as described herein, to generate the novel cell population. In some embodiments, the adult-derived stem cell population is transiently contacted by the heterologous polypeptide.

[0089] Some aspects of the present disclosure provide a method of treatment of a subject in need thereof, the comprising administering to the subject the novel cell population described hereinabove (or described elsewhere herein).

[0090] In certain aspects, the present disclosure provides a method of modifying a cell, the method comprising:

transiently contacting the cell with a heterologous polypeptide comprising an actuator moiety configured to complex with a target polynucleotide in the cell, wherein the actuator moiety is operatively linked to an epigenetic modifier to modify an expression profile of a target gene in the cell, to effect a change in a characteristic of the cell that is sustained for at least about 3 days as compared to a control cell, the characteristic being different than the modified expression profile of the target gene,

wherein the characteristic of the cell comprises one or more members selected from the group consisting of (1) cell morphology, (2) cell metabolism, (3) chromatin accessibility of an additional gene operatively coupled to the target gene, (4) a degree of methylation of the additional gene, (5) telomere length, and (6) cell fate.

[0091] In practicing any of the methods for modifying the cell as described herein, the transient contacting comprises contacting the cell with a heterologous polynucleotide encoding the heterologous polypeptide. In some embodiments, the heterologous polynucleotide is not integrated into a genome of the cell. In some embodiments, the transient contacting comprises delivering the heterologous polypeptide into the cell. In some embodiments, the transient contacting is characterized by having a peak level of the heterologous polypeptide in the cell in less than about 3 days. In some embodiments, the transient contacting is characterized by having a peak level of the heterologous polypeptide in the cell in less than about 2 days. In some embodiments, the transient contacting is characterized by having a peak level of the heterologous polypeptide in the cell in less than about 1 day.

[0092] In practicing any of the method for modifying the cell as described herein, the change in the characteristic of the cell is sustained for at least about 5 days. In some embodiments, the change in the characteristic of the cell is sustained for at least about 2 weeks. In some embodiments, the change in the characteristic of the cell is sustained for at least about 1 month. In some embodiments, the expression profile is transiently modified. In some embodiments, the cell exhibits a maximum change within the transiently modified expression profile of the target gene in less than about 3 days upon the contacting. In some embodiments, the cell exhibits a maximum change within the transiently modified expression profile of the target gene in less than about 1 day upon the contacting. In some embodiments, in (1), the change in the characteristic of the cell comprises (i) either increased or decreased cell roundness or (ii) either increased or decreased projection area of the cell. In some embodiments, in (2), the change in the characteristic of the cell comprises (i) either increased or decreased number of mitochondria per cell or (ii) either increased or decreased level of ATP release from the cell. In some embodiments, in (3), the change in the characteristic of the cell comprises either increased or decreased chromatin accessibility of the additional gene. In some embodiments, in (4), the change in the characteristic of the cell comprises either

increased or decreased degree of methylation of the additional gene. In some embodiments, in (5), the change in the characteristic of the cell comprises either increased or decreased telomere length. In some embodiments, in (6), the change in the characteristic of the cell comprises a modified expression of an additional gene indicative of (i) differentiation of the cell to a more committed cell type, (ii) trans-differentiation into a of the cell to different cell type, or (iii) dedifferentiation of the cell to a less committed cell type.

[0093] In practicing any of the method for modifying the cell as described herein, the actuator moiety comprises a heterologous endonuclease. In some embodiments, the heterologous endonuclease is directed by a guide ribonucleic acid (RNA) to specifically bind the endogenous target gene. In some embodiments, the heterologous endonuclease substantially lacks DNA cleavage activity.

[0094] In practicing any of the method for modifying the cell as described herein, the epigenetic modifier comprises one or more members selected from the group consisting of a chromatin remodeling protein, chromatin reader protein, nuclear protein, transcription factor protein, mediator (or coactivator) protein, DNA binding protein, RNA binding protein, DNA methylation protein, histone acetylation protein, histone methylation protein, a peptide domain fragment thereof, and a modification thereof. In some embodiments, the chromatin remodeling protein comprises one or more members selected from the group consisting of SMARCB1, SMARCA4, PBRM1, ARID1A, ARID1B, ARID2, SMARCD1, SMARCE1, ATRX, DAXX, and CHD (e.g., CHD1, CHD2, CHD3, CHD4, CHD5, CHD6, CHD7, CHD8). In some embodiments, the DNA methylation protein comprises one or more members selected from the group consisting of DNMT3A, DNMT1, TET (e.g., TET1, TET2, TET3), MBD1, and MBD4. In some embodiments, the histone acetylation protein comprises one or more members selected from the group consisting of EP300, CREBBP, HDAC2, HDAC4, and HDAC9. In some embodiments, the histone methylation protein comprises one or more members selected from the group consisting of MLL (e.g., MLL1, MLL2, MLL3, MLL4), SETD1A, PRDM9, EZH2, NSD1, NSD2, SETD2, KDM5C, KDM6A, and KDM2B. In some embodiments, wherein the modification comprises at least one mutation relative to a wild type epigenetic modifier. In some embodiments, the epigenetic modifier is less than about 100 amino acids in length. In some embodiments, the epigenetic modifier is between about 100 amino acids and about 700 amino acids in length. In some embodiments, the epigenetic modifier is a synthetic polypeptide.

[0095] In practicing any of the method for modifying the cell as described herein, the target gene comprises a positive regulator of the differentiation selected from the group consisting of: Runx2, SP7/OSX, DLX5, BMPR1B, BMPR2, MSX2, DLX2, DLX3, DLX6, MSX1, ATF4, VDR, PDLIM7, ETS1, FOSL1, WNT9A, BMP2, BMP4, BMP6, BMP7, SMAD1, SMAD5, SMAD8, IGF, Pax7, and MyoD. In some embodiments, the target gene comprises a negative regulator of the differentiation selected from the group consisting of: PPARg, LIPE, DKK1, DKK2, TWIST1, SMURF1, TGFBR1, TGFBR2.

[0096] In practicing any of the method for modifying the cell as described herein, the cell is a stem

cell. In some embodiments, the cell is an embryonic stem cell or an adult-derived stem cell. In some embodiments, the adult-derived stem cell comprises a hematopoietic stem cell (HSC), a neuronal stem cells (NSC), a mesenchymal stem cells (MSC), an induced pluripotent stem cell (iPSC), a muscle stem cell (MuSC), a skeletal stem cell, or a skin stem cell. In some embodiments, the cell is a committed cell comprising a fibroblast, a neuron, a glial cell, an osteoblast, or a chondrocyte.

[0097] Some aspects of the present disclosure provide a composition, the composition comprising the heterologous polypeptide as described herein or a heterologous encoding the heterologous polypeptide as described herein, wherein the heterologous polypeptide is for modifying the cell.

[0098] Some aspects of the present disclosure provide a kit, the kit comprising: the composition as described hereinabove (or described elsewhere herein); and instructions directing (i) contacting the cell with the composition or (ii) administration of the modified cell to a subject.

[0099] Some aspects of the present disclosure provide a novel cell population derived from an initial cell population, wherein the initial cell population is contacted by the heterologous polypeptide as described herein, to generate the novel cell population. In some embodiments, the cell population is transiently contacted by the heterologous polypeptide. In some embodiments, the cell population comprises a plurality of embryonic stem cells or a plurality of adult-derived stem cells. In some embodiments, the cell is a committed cell comprising a fibroblast, a neuron, a glial cell, an osteoblast, or a chondrocyte.

[0100] Some aspects of the present disclosure provide a method of treatment of a subject in need thereof, the method comprising administering to the subject the novel cell population described hereinabove (or described elsewhere herein).

[0101] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[0102] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

BRIEF DESCRIPTION OF THE DRAWINGS

[0103] The novel features of the invention are set forth with particularity in the appended claims. A

better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings (also "Figure" and "FIG." herein), of which:

[0104] FIGs. 1A-1F schematically illustrate different expression profiles of a target gene that is modulated upon by an epigenetic modification of a target polynucleotide. FIG. 1A illustrates sustained enhancement in an expression profile. FIG. 1B illustrates temporary enhancement in an expression profile. FIG. 1C illustrates partially sustained enhancement in an expression profile. FIG. 1D illustrates sustained repression in an expression profile. FIG. 1E illustrates temporary repression in an expression profile. FIG. 1F illustrates partially sustained repression in an expression profile.

[0105] FIG. 2 schematically illustrates an example process of a method for inducing enhanced skeletal repair.

[0106] FIG. 3A illustrates the Runx2 signaling pathway that is turned on during osteogenesis.

[0107] FIG. 3B illustrates role of Runx2 in promoting osteoblast/osteocyte differentiation from various mesenchymal stem cells.

[0108] FIG. 4A illustrates an example in vitro method of inducing osteoblast differentiation of mesenchymal stem cells.

[0109] FIGs. 4B and 4C show alizarin red staining imaging (4B) and brightfield imaging (4C), of mesenchymal stem cells after osteogenic differentiation, to visualize mineralization;

[0110] FIGs. 5A and 5B show different expression profiles of Runx2 upon activation of endogenous Runx2 (5A) or Runx2 mRNA transfection (5B).

[0111] FIG. 6 shows an example process of incorporating modified mesenchymal stem cells onto a collagen scaffold for implantation *in vivo* in mice.

[0112] FIG. 7 shows qRT-PCR absolute quantification of human *BMP6* in human Adipose Derived Mesenchymal Stem Cells harvested 1 to 7 Days post reprogramming with CRISPRa-BMP6 reprogramming (Black) or negative control (White).

[0113] FIG. 8 schematically illustrates an example process of stem cell reprogramming. Stem Cells (i.e. MSC) are first reprogrammed by transient delivery of CRISPRa or CRISPRi. Next, cells differentiate into specific lineage and are transplanted into animals in a spheroid format.

[0114] FIG. 9A-D shows reprogramming of adipose-derived mesenchymal stem cells (AD-MSC) into osteocyte-like cells by transient CRISPRa delivery. FIG. 9A shows calcium deposit accumulation by bright field images of AD-MSC with and without reprogramming at 7 and 14 days after transient RunX2, Osx and DLx5 (ROD)-CRISPR reprogramming. FIG. 9B shows relative quantification of calcium deposit through Osteoimage Mineralization Assay (Lonza) of AD-MSC with and without reprogramming at 7 and 14 days after transient ROD-CRISPR reprogramming. FIG. 9C-D show qRT-PCR relative quantification analysis of human *Col11a1* (FIG. 9C) and *SPP1* (FIG. 9D) in microspheroids culture of human AD-MSC harvested 7 and 10 Days post reprogramming with ROD-CRISPRa (light grey) or negative control (dark grey)

[0115] FIG. 10A-D shows evaluation of MSC reprogrammed with CRISPRa *in vivo*. FIG. 10A shows the average radiance (FIG. 10A) of bioluminescent imaging (BLI) in MSC and MSC reprogrammed with ROD-CRISPRa expressing firefly luciferase gene reporter 7 days post transplantation in 5mm Calvarial defects generated in nude rats (FIG. 10B). FIG. 10C shows microCT imaging of bone regrowth 1 month post 5mm Calvarial defects formation and transplantation of 150,000 or 600,000 human primary osteoblasts. FIG. 10D shows newly formed bone localization by Von Kossa Staining 1 month post 5mm Calvarial Defects formation and transplantation of 150,000 or 600,000 human primary osteoblasts.

[0116] FIG. 11A-G shows CRISPR reprogramming of AD-MSC into chondrocyte-like cells through upregulation of growth factors BMP6 and TGFB3. FIG. 11A-B show qRT-PCR Relative Quantification analysis of human *Col2a1* (FIG. 11A) and *ACAN* (FIG. 11B) in microspheroid culture of human Adipose Derived Mesenchymal Stem Cells harvested **14 Days** following reprogramming with permanent delivery of BP-CRISPRa (Light Gray) or transient delivery of BP-CIRSPRa (Dark Gray) or negative control (Black). FIG. 11C shows the average radiance of bioluminescent imaging (BLI) in MSC and MSC reprogrammed with permanent delivery of BP-CRISPRa or transient delivery of BP-CIRSPRa expressing firefly luciferase gene reporter 1 month post transplantation in an animal model of MIA induced OsteoArthritis. FIG. 11D-G show gait phenotype analysis by stride distance in a no cell-control (FIG. 11D), WT MSC (FIG. 11E), and MSC reprogrammed with permanent delivery of BP-CRISPRa (FIG. 11F) or transient delivery of BP-CIRSPRa (FIG. 11G) at different timepoints post transplantation in an animal model of MIA induced OsteoArthritis.

DETAILED DESCRIPTION

[0117] While various embodiments of the invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions may occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed.

[0118] As used in the specification and claims, the singular forms "a," "an," and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a chimeric transmembrane receptor" includes a plurality of chimeric transmembrane receptors.

[0119] The term "about" or "approximately" generally mean within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, up to 10%, up to 5%, or up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2- fold, of a value. Where particular values are described in the application and claims, unless otherwise stated, the term "about" meaning

within an acceptable error range for the particular value should be assumed.

[0120] The use of the alternative (e.g., "or") should be understood to mean either one, both, or any combination thereof of the alternatives. The term "and/or" should be understood to mean either one, or both of the alternatives.

[0121] The terms "engineered" and "modified" are used interchangeably herein. The terms "engineering" and "modifying" are used interchangeably herein. The terms "engineered cell" or "modified cell" are used interchangeably herein. The terms "engineered characteristic" and "modified characteristic" are used interchangeably herein. The terms "engineered fate" and "modified fate" are used interchangeably herein.

[0122] The term "cell" generally refers to a biological cell. A cell can be the basic structural, functional and/or biological unit of a living organism. A cell can originate from any organism having one or more cells. Some non-limiting examples include: a prokaryotic cell, eukaryotic cell, a bacterial cell, an archaeal cell, a cell of a single-cell eukaryotic organism, a protozoa cell, a cell from a plant (e.g. cells from plant crops, fruits, vegetables, grains, soy bean, corn, maize, wheat, seeds, tomatoes, rice, cassava, sugarcane, pumpkin, hay, potatoes, cotton, cannabis, tobacco, flowering plants, conifers, gymnosperms, ferns, clubmosses, hornworts, liverworts, mosses), an algal cell, (e.g., Botryococcus braunii, Chlamydomonas reinhardtii, Nannochloropsis gaditana, Chlorella pyrenoidosa, Sargassum patens C. Agardh, and the like), seaweeds (e.g. kelp), a fungal cell (e.g., a yeast cell, a cell from a mushroom), an animal cell, a cell from an invertebrate animal (e.g. fruit fly, cnidarian, echinoderm, nematode, etc.), a cell from a vertebrate animal (e.g., fish, amphibian, reptile, bird, mammal), a cell from a mammal (e.g., a pig, a cow, a goat, a sheep, a rodent, a rat, a mouse, a non-human primate, a human, etc.), and etcetera. Sometimes a cell is not originating from a natural organism (e.g. a cell can be a synthetically made, sometimes termed an artificial cell).

[0123] The term "reprogramming," "dedifferentiation," "increasing cell potency," or "increasing developmental potency," as used interchangeable herein, generally refers to a method of increasing the potency of a cell or dedifferentiating the cell to a less differentiated state. For example, a cell that has an increased cell potency has more developmental plasticity (i.e., can differentiate into more cell types) compared to the same cell in the non-reprogrammed state. In other words, a reprogrammed cell is one that is in a less differentiated state than the same cell in a non-reprogrammed state.

[0124] The term "differentiation" generally refers to a process by which an unspecialized ("uncommitted") or less specialized cell acquires the features of a specialized cell such as, e.g., an osteoblast, chondrocyte, myocyte, or neuron. A differentiated or differentiation-induced cell is one that has taken on a more specialized ("committed") position within the lineage of a cell. The term "committed" generally refers to a cell that has proceeded in the differentiation pathway to a point where, under normal circumstances, it will continue to differentiate into a specific cell type or subset of cell types, and cannot, under normal circumstances, differentiate into a different cell type or revert to a less differentiated cell type.

[0125] The term "adulted-derived stem cell" as used herein generally refers to undifferentiated cells derived from one or more adult tissues of an organism. In some cases, an adult-derived stem cell can be obtained from an adult tissue of an organism, such as, for example, a hematopoietic stem cell (HSC) from the blood, a neuronal stem cell (NSC) from the nervous system, a mesenchymal stem cell (MSC) from the bone marrow, a muscle stem cell (MuSC) from the muscle tissue, dental pulp stem cell, a skeletal stem cell, or a skin stem cell. In some cases, such adult-derived stem cells can be interchangeably referred to as isolated stem cells. In some cases, an adult-derived stem cell can be engineered cell that is derived from a cell that is obtained from an adult tissue of an organism. In some examples, an adult-derived stem cell can be a progeny (e.g., expanded ex vivo or in vitro) of a stem cell that is obtained from an adult tissue of an organism. In some examples, a differentiated adult cell (e.g., a fibroblast) can be isolated from an adult tissue, then induced (e.g., genetically engineered) to be differentiated into a stem cell (e.g., induced pluripotent stem cell). An adult-derived stem cell be pluripotent.

[0126] The term "pluripotent" generally refers to the ability of a cell to form all lineages of the body or soma (i.e., the embryo proper). For example, embryonic stem cells are a type of pluripotent stem cells that are able to form cells from each of the three germs layers, the ectoderm, the mesoderm, and the endoderm. Pluripotency can be a continuum of developmental potencies ranging from the incompletely or partially pluripotent cell (e.g., an epiblast stem cell), which is unable to give rise to a complete organism to the more primitive, more pluripotent cell, which is able to give rise to a complete organism (e.g., an embryonic stem cell).

[0127] A pluripotent cell can exhibit one or more pluripotency characteristics. Non-limiting examples of a pluripotency characteristic can include (i) pluripotent stem cell morphology; (ii) the potential for unlimited self-renewal; (iii) expression of pluripotent stem cell markers including, but not limited to SSEA1 (mouse only), SSEA3/4, SSEA5, TRA1-60/81, TRA1-85, TRA2-54, GCTM-2, TG343, TG30, CD9, CD29, CD133/prominin, CD140a, CD56, CD73, CD90, CD105, OCT4, NANOG, SOX2, CD30 and/or CD50; (iv) ability to differentiate to all three somatic lineages (ectoderm, mesoderm and endoderm); (v) teratoma formation consisting of the three somatic lineages; and (vi) formation of embryoid bodies consisting of cells from the three somatic lineages.

[0128] The term "mesenchymal stem cell" (MSC) generally refers to a stem cell originally derived from the mesenchyme. A mesenchymal stem cell can be capable of differentiating into at least two or more of an osteoblast, a chondrocyte, an adipocyte, or a myocyte. A mesenchymal stem cell can be isolated from any type of adult tissue. In some cases, a mesenchymal stem cell can be isolated from bone marrow, adipose tissue, umbilical cord, or peripheral blood. In some examples, a mesenchymal stem cell can be obtained from bone marrow or lipoaspirates, themselves obtained from an adipose tissue.

[0129] The term "induced pluripotent stem cells" (iPSCs) generally refers to stem cells that are derived from differentiated cells (e.g., differentiated adult, neonatal, or fetal cells) that have been induced or changed (i.e., reprogrammed) into cells capable of differentiating into tissues of all three germ or dermal layers: mesoderm, endoderm, and ectoderm. The iPSCs produced do not refer to cells as they are

found in nature. In some cases, iPSCs can be engineered to differentiation directly into committed cells (e.g., neurons, osteoblasts, chondrocytes, islet cells, etc.). In some cases, iPSCs can be engineered to differentiate first into tissue-specific stem cells (e.g., neuron progenitor cells, muscle stem cells), which can be further induced to differentiate into committed cells (e.g., neurons, myoblasts).

[0130] The term "isolated stem cells" generally refers to any type of stem cells disclosed herein (e.g., ESCs, HSCs, MSCs, etc.) that are isolated from a multicellular organism. For example, HSCs can be isolated from a mammal's body, such as a human body. In another example, an embryonic stem cells can be isolated from an embryo.

[0131] The term "isolated" generally refers to a cell or a population of cells, which has been separated from its original environment. For example, a new environment of the isolated cells is substantially free of at least one component as found in the environment in which the "un-isolated" reference cells exist. An isolated cell can be a cell that is removed from some or all components as it is found in its natural environment, for example, isolated from a tissue or biopsy sample. The term also includes a cell that is removed from at least one, some or all components as the cell is found in non-naturally occurring environments, for example, isolated form a cell culture or cell suspension. Therefore, an isolated cell is partly or completely separated from at least one component, including other substances, cells or cell populations, as it is found in nature or as it is grown, stored or subsisted in non-naturally occurring environments.

[0132] The term "hematopoietic stem and progenitor cells," "hematopoietic stem cells," "hematopoietic progenitor cells," or "hematopoietic precursor cells," as used interchangeably herein, generally refers to cells which are committed to a hematopoietic lineage but are capable of further hematopoietic differentiation (e.g., into T cells, NK cells, etc.) and include, multipotent hematopoietic stem cells (hematoblasts), myeloid progenitors, megakaryocyte progenitors, erythrocyte progenitors, and lymphoid progenitors. Hematopoietic stem and progenitor cells (HSCs) are multipotent stem cells that give rise to all the blood cell types including myeloid (monocytes and macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, dendritic cells), and lymphoid lineages (T cells, B cells, NK cells). In some cases, HSCs can be CD34+ hematopoietic cells capable of giving rise to both mature myeloid and lymphoid cell types including T cells, NK cells and B cells.

[0133] The term "nucleotide," as used herein, generally refers to a base-sugar-phosphate combination. A nucleotide can comprise a synthetic nucleotide. A nucleotide can comprise a synthetic nucleotide analog. Nucleotides can be monomeric units of a nucleic acid sequence (e.g. deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)). The term nucleotide can include ribonucleoside triphosphates adenosine triphosphate (ATP), uridine triphosphate (UTP), cytosine triphosphate (CTP), guanosine triphosphate (GTP) and deoxyribonucleoside triphosphates such as dATP, dCTP, dITP, dUTP, dGTP, dTTP, or derivatives thereof. Such derivatives can include, for example, [αS]dATP, 7-deaza-dGTP and 7-deaza-dATP, and nucleotide derivatives that confer nuclease resistance on the nucleic acid molecule containing them. The term nucleotide as used herein can refer to dideoxyribonucleoside triphosphates

(ddNTPs) and their derivatives. Illustrative examples of dideoxyribonucleoside triphosphates can include, but are not limited to, ddATP, ddCTP, ddGTP, ddITP, and ddTTP. A nucleotide may be unlabeled or detectably labeled by well-known techniques. Labeling can also be carried out with quantum dots. Detectable labels can include, for example, radioactive isotopes, fluorescent labels, chemiluminescent labels, bioluminescent labels and enzyme labels. Fluorescent labels of nucleotides may include but are not limited fluorescein, 5-carboxyfluorescein (FAM), 2′7′-dimethoxy-4′5-dichloro-6-carboxyfluorescein (JOE), rhodamine, 6-carboxyrhodamine (R6G), N,N,N′,N′-tetramethyl-6-carboxyrhodamine (TAMRA), 6-carboxy-X-rhodamine (ROX), 4-(4′dimethylaminophenylazo) benzoic acid (DABCYL), Cascade Blue, Oregon Green, Texas Red, Cyanine and 5-(2′-aminoethyl)aminonaphthalene-1-sulfonic acid (EDANS). Specific examples of fluorescently labeled nucleotides can include [R6G]dUTP, [TAMRA]dUTP, [R110]dCTP, [R6G] dCTP, [TAMRA] dCTP, [JOE] ddATP, [R6G] ddATP, [FAM] ddCTP, [R110]ddCTP, [TAMRA]ddGTP, [ROX]ddTTP, [dR6G]ddATP, [dR110]ddCTP, [dTAMRA]ddGTP, and [dROX]ddTTP available from Perkin Elmer, Foster City, Calif. FluoroLink DeoxyNucleotides, FluoroLink Cy3-dCTP, FluoroLink Cy5-dCTP, FluoroLink Fluor X-dCTP, FluoroLink Cy3-dUTP, and FluoroLink Cy5-dUTP available from Amersham, Arlington Heights, Ill.; Fluorescein-15-dATP, Fluorescein-12-dUTP, Tetramethyl-rodamine-6-dUTP, IR770-9-dATP, Fluorescein-12-ddUTP, Fluorescein-12-UTP, and Fluorescein-15-2′-dATP available from Boehringer Mannheim, Indianapolis, Ind.; and Chromosome Labeled Nucleotides, BODIPY-FL-14-UTP, BODIPY-FL-4-UTP, BODIPY-TMR-14-UTP, BODIPY-TMR-14-dUTP, BODIPY-TR-14-UTP, BODIPY-TR-14-dUTP, Cascade Blue-7-UTP, Cascade Blue-7-dUTP, fluorescein-12-UTP, fluorescein-12-dUTP, Oregon Green 488-5-dUTP, Rhodamine Green-5-UTP, Rhodamine Green-5-dUTP, tetramethylrhodamine-6-UTP, tetramethylrhodamine-6-dUTP, Texas Red-5-UTP, Texas Red-5-dUTP, and Texas Red-12-dUTP available from Molecular Probes, Eugene, Oreg. Nucleotides can also be labeled or marked by chemical modification. A chemically-modified single nucleotide can be biotin-dNTP. Some non-limiting examples of biotinylated dNTPs can include, biotin-dATP (e.g., bio-N6-ddATP, biotin-14-dATP), biotin-dCTP (e.g., biotin-11-dCTP, biotin-14-dCTP), and biotin-dUTP (e.g. biotin-11-dUTP, biotin-16-dUTP, biotin-20-dUTP).

[0134] The term “polynucleotide,” “oligonucleotide,” or “nucleic acid,” as used interchangeably herein, generally refers to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof, either in single-, double-, or multi-stranded form. A polynucleotide can be exogenous or endogenous to a cell. A polynucleotide can exist in a cell-free environment. A polynucleotide can be a gene or fragment thereof. A polynucleotide can be DNA. A polynucleotide can be RNA. A polynucleotide can have any three dimensional structure, and can perform any function, known or unknown. A polynucleotide can comprise one or more analogs (e.g. altered backbone, sugar, or nucleobase). If present, modifications to the nucleotide structure can be imparted before or after assembly of the polymer. Some non-limiting examples of analogs include: 5-bromouracil, peptide nucleic acid, xeno nucleic acid, morpholinos, locked nucleic acids, glycol nucleic acids, threose nucleic acids,

dideoxynucleotides, cordycepin, 7-deaza-GTP, florophores (e.g. rhodamine or flurescein linked to the sugar), thiol containing nucleotides, biotin linked nucleotides, fluorescent base analogs, CpG islands, methyl-7-guanosine, methylated nucleotides, inosine, thiouridine, pseudourdine, dihydrouridine, queuosine, and wyosine. Non-limiting examples of polynucleotides include coding or non-coding regions of a gene or gene fragment, loci (locus) defined from linkage analysis, exons, introns, messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), short interfering RNA (siRNA), short-hairpin RNA (shRNA), micro-RNA (miRNA), ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, cell-free polynucleotides including cell-free DNA (cfDNA) and cell-free RNA (cfRNA), nucleic acid probes, and primers. The sequence of nucleotides can be interrupted by non-nucleotide components.

[0135] The term "gene" generally refers to a nucleic acid (e.g., DNA such as genomic DNA and cDNA) and its corresponding nucleotide sequence that is involved in encoding an RNA transcript. The term as used herein with reference to genomic DNA includes intervening, non-coding regions as well as regulatory regions and can include 5′ and 3′ ends. In some uses, the term encompasses the transcribed sequences, including 5′ and 3′ untranslated regions (5′-UTR and 3′-UTR), exons and introns. In some genes, the transcribed region will contain "open reading frames" that encode polypeptides. In some uses of the term, a "gene" comprises only the coding sequences (e.g., an "open reading frame" or "coding region") necessary for encoding a polypeptide. In some cases, genes do not encode a polypeptide, for example, ribosomal RNA genes (rRNA) and transfer RNA (tRNA) genes. In some cases, the term "gene" includes not only the transcribed sequences, but in addition, also includes non-transcribed regions including upstream and downstream regulatory regions, enhancers and promoters. A gene can refer to an "endogenous gene" or a native gene in its natural location in the genome of an organism. A gene can refer to an "exogenous gene" or a non-native gene. A non-native gene can refer to a gene not normally found in the host organism but which is introduced into the host organism by gene transfer. A non-native gene can also refer to a gene not in its natural location in the genome of an organism. A non-native gene can also refer to a naturally occurring nucleic acid or polypeptide sequence that comprises mutations, insertions and/or deletions (e.g., non-native sequence).

[0136] The term "expression" generally refers to one or more processes by which a polynucleotide is transcribed from a DNA template (such as into an mRNA or other RNA transcript) and/or the process by which a transcribed mRNA is subsequently translated into peptides, polypeptides, or proteins. Transcripts and encoded polypeptides can be collectively referred to as "gene product." If the polynucleotide is derived from genomic DNA, expression can include splicing of the mRNA in a eukaryotic cell. "Up-regulated," with reference to expression, generally refers to an increased expression level of a polynucleotide (e.g., RNA such as mRNA) and/or polypeptide sequence relative to its expression level in a wild-type state while "down-regulated" generally refers to a decreased expression level of a polynucleotide (e.g., RNA such as mRNA) and/or polypeptide sequence relative to its expression in a wild-type state. Expression of a transfected gene can occur transiently or stably in a cell. During

"transient expression" the transfected gene is not transferred to the daughter cell during cell division. Since its expression is restricted to the transfected cell, expression of the gene is lost over time. In contrast, stable expression of a transfected gene can occur when the gene is co-transfected with another gene that confers a selection advantage to the transfected cell. Such a selection advantage may be a resistance towards a certain toxin that is presented to the cell.

[0137] The term "expression profile" generally refers to quantitative (e.g., abundance) and qualitative expression of one or more genes in a sample (e.g., a cell). The one or more genes can be expressed and ascertained in the form of a nucleic acid molecule (e.g., an mRNA or other RNA transcript). Alternatively or in addition to, the one or more genes can be expressed and ascertained in the form of a polypeptide (e.g., a protein measured via Western blot). An expression profile of a gene may be defined as a shape of an expression level of the gene over a time period (e.g., at least or up to about 1 hour, at least or up to about 2 hours, at least or up to about 3 hours, at least or up to about 4 hours, at least or up to about 5 hours, at least or up to about 6 hours, at least or up to about 7 hours, at least or up to about 8 hours, at least or up to about 9 hours, at least or up to about 10 hours, at least or up to about 11 hours, at least or up to about 12 hours, at least or up to about 16 hours, at least or up to about 18 hours, at least or up to about 24 hours, at least or up to about 36 hours, at least or up to about 48 hours, at least up to about 3 days, at least up to about 4 days, at least up to about 5 days, at least up to about 6 days, at least up to about 7 days, at least up to about 8 days, at least up to about 9 days, at least up to about 10 days, at least up to about 11 days, at least up to about 12 days, at least up to about 13 days, at least up to about 14 days, etc.). Alternatively, an expression profile of a gene may be defined as an expression level of the gene at a time point of interest (e.g., the expression level of the gene measured at least or up to about 1 hour, at least or up to about 2 hours, at least or up to about 3 hours, at least or up to about 4 hours, at least or up to about 5 hours, at least or up to about 6 hours, at least or up to about 7 hours, at least or up to about 8 hours, at least or up to about 9 hours, at least or up to about 10 hours, at least or up to about 11 hours, at least or up to about 12 hours, at least or up to about 16 hours, at least or up to about 18 hours, at least or up to about 24 hours, at least or up to about 36 hours, at least or up to about 48 hours, at least up to about 3 days, at least up to about 4 days, at least up to about 5 days, at least upto about 6 days, at least up to about 7 days, at least up to about 8 days, at least up to about 9 days, at least up to about 10 days, at least up to about 11 days, at least up to about 12 days, at least up to about 13 days, or at least up to about 14 days after treating a cell to induce such expression level.)

[0138] The term "peptide," "polypeptide," or "protein," as used interchangeably herein, generally refers to a polymer of at least two amino acid residues joined by peptide bond(s). This term does not connote a specific length of polymer, nor is it intended to imply or distinguish whether the peptide is produced using recombinant techniques, chemical or enzymatic synthesis, or is naturally occurring. The terms apply to naturally occurring amino acid polymers as well as amino acid polymers comprising at least one modified amino acid. In some cases, the polymer can be interrupted by non-amino acids. The terms include amino acid chains of any length, including full length proteins, and proteins with or without

secondary and/or tertiary structure (e.g., domains). The terms also encompass an amino acid polymer that has been modified, for example, by disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, oxidation, and any other manipulation such as conjugation with a labeling component. The terms "amino acid" and "amino acids," as used herein, generally refer to natural and non-natural amino acids, including, but not limited to, modified amino acids and amino acid analogues. Modified amino acids can include natural amino acids and non-natural amino acids, which have been chemically modified to include a group or a chemical moiety not naturally present on the amino acid. Amino acid analogues can refer to amino acid derivatives. The term "amino acid" includes both D-amino acids and L-amino acids.

[0139] The term "derivative," "variant," or "fragment," as used herein with reference to a polypeptide, generally refers to a polypeptide related to a wild type polypeptide, for example either by amino acid sequence, structure (e.g., secondary and/or tertiary), activity (e.g., enzymatic activity) and/or function. Derivatives, variants and fragments of a polypeptide can comprise one or more amino acid variations (e.g., mutations, insertions, and deletions), truncations, modifications, or combinations thereof compared to a wild type polypeptide.

[0140] The term "engineered," "chimeric," or "recombinant," as used herein with respect to a polypeptide molecule (e.g., a protein), generally refers to a polypeptide molecule having a heterologous amino acid sequence or an altered amino acid sequence as a result of the application of genetic engineering techniques to nucleic acids which encode the polypeptide molecule, as well as cells or organisms which express the polypeptide molecule. The term "engineered" or "recombinant," as used herein with respect to a polynucleotide molecule (e.g., a DNA or RNA molecule), generally refers to a polynucleotide molecule having a heterologous nucleic acid sequence or an altered nucleic acid sequence as a result of the application of genetic engineering techniques. Genetic engineering techniques include, but are not limited to, PCR and DNA cloning technologies; transfection, transformation and other gene transfer technologies; homologous recombination; site-directed mutagenesis; and gene fusion. In some cases, an engineered or recombinant polynucleotide (e.g., a genomic DNA sequence) can be modified or altered by a gene editing moiety.

[0141] The term "actuator moiety," as used herein, generally refers to a moiety which can regulate expression or activity of a gene and/or edit a nucleic acid sequence, whether exogenous or endogenous. An actuator moiety can be a heterologous moiety (e.g., a heterologous polypeptide) to any of the cells disclosed herein (e.g., engineered cells). An actuator moiety can regulate expression of a gene at the transcription level and/or the translation level. An actuator moiety can regulate gene expression at the transcription level, for example, by regulating the production of mRNA from DNA, such as chromosomal DNA or cDNA. In some embodiments, an actuator moiety recruits or comprises at least one functional moiety (e.g., a transcription factor, an epigenetic modifier) that binds to a specific DNA sequence, thereby controlling the rate of transcription of genetic information from DNA to mRNA. An actuator moiety can itself bind to DNA and regulate transcription by physical obstruction, for example preventing proteins

such as RNA polymerase and other associated proteins from assembling on a DNA template. An actuator moiety can regulate expression of a gene at the translation level, for example, by regulating the production of protein from mRNA template. In some embodiments, an actuator moiety regulates gene expression by affecting the stability of an mRNA transcript. In some embodiments, an actuator moiety regulates expression of a gene by editing a nucleic acid sequence (e.g., a region of a genome). In some embodiments, an actuator moiety regulates expression of a gene by editing an mRNA template. Editing a nucleic acid sequence can, in some cases, alter the underlying template for gene expression.

[0142] The term "epigenetic modifier," as used herein, generally refers to a protein or catalytic domain thereof having enzymatic activity that results in the epigenetic modification of DNA, for example chromosomal DNA or a heterologous DNA. Epigenetic modifications can include, but are not limited to DNA modification (e.g., methylation or demethylation); histone modifications, such as histone methylation (e.g., mono-, di- and tri-methylation) and deacetylation, histone acetylation and deacetylation, as well as histone ubiquitylation, phosphorylation, and sumoylation; and microRNA modification.

[0143] The term "enhanced expression," "increased expression," or "upregulated expression" generally refers to production of a moiety of interest (e.g., a polynucleotide or a polypeptide) to a level that is above a normal level of expression of the moiety of interest in a host strain (e.g., a host cell). The normal level of expression can be substantially zero (or null) or higher than zero. The moiety of interest can comprise an endogenous gene or polypeptide construct of the host strain. The moiety of interest can comprise a heterologous gene or polypeptide construct that is introduced to or into the host strain. For example, a heterologous gene encoding a polypeptide of interest can be knocked-in (KI) to a genome of the host strain for enhanced expression of the polypeptide of interest in the host strain.

[0144] The term "enhanced activity," "increased activity," or "upregulated activity" generally refers to activity of a moiety of interest (e.g., a polynucleotide or a polypeptide) that is modified to a level that is above a normal level of activity of the moiety of interest in a host strain (e.g., a host cell). The normal level of activity can be substantially zero (or null) or higher than zero. The moiety of interest can comprise a polypeptide construct of the host strain. The moiety of interest can comprise a heterologous polypeptide construct that is introduced to or into the host strain. For example, a heterologous gene encoding a polypeptide of interest can be knocked-in (KI) to a genome of the host strain for enhanced activity of the polypeptide of interest in the host strain.

[0145] The term "reduced expression," "decreased expression," or "downregulated expression" generally refers to a production of a moiety of interest (e.g., a polynucleotide or a polypeptide) to a level that is below a normal level of expression of the moiety of interest in a host strain (e.g., a host cell). The normal level of expression is higher than zero. The moiety of interest can comprise an endogenous gene or polypeptide construct of the host strain. In some cases, the moiety of interest can be knocked-out or knocked-down in the host strain. In some examples, reduced expression of the moiety of interest can include a complete inhibition of such expression in the host strain.

[0146] The term "reduced activity," "decreased activity," or "downregulated activity" generally

refers to activity of a moiety of interest (e.g., a polynucleotide or a polypeptide) that is modified to a level that is below a normal level of activity of the moiety of interest in a host strain (e.g., a host cell). The normal level of activity is higher than zero. The moiety of interest can comprise an endogenous gene or polypeptide construct of the host strain. In some cases, the moiety of interest can be knocked-out or knocked-down in the host strain. In some examples, reduced activity of the moiety of interest can include a complete inhibition of such activity in the host strain.

[0147] The term "senescence" as used herein generally refers to a state of a cell characterized by reduced level of or permanent cessation of DNA replication and/or cell growth, neither of which may not be reversible (e.g., by growth factors). A cell can exhibit senescence, for example, at the end of the proliferative lifespan of normal cells; in normal or tumor cells in response to cytotoxic drugs; upon DNA damage; or other cellular insult. In some cases, senescence can be characterized by certain morphological features, e.g., increased size, flattened morphology increased granularity, and expression or activity of a senescence-associated gene or a gene product thereof (e.g., activity of senescence-associated β-galactosidase activity (SA-β-gal)).

[0148] The term "senescence-associated gene" as used herein generally refers to one or more genes, wherein the expression of which is modulated (either induced or repressed) when a cell is in the state of senescence (e.g., when the cell expresses a senescent phenotype). In some cases, a senescence-associated gene can encode a polypeptide that is secreted by a cell. In some cases, a senescence-associated gene can encode a polypeptide that is presented on a cell surface.

[0149] The term "subject," "individual," or "patient," as used interchangeably herein, generally refers to a vertebrate, preferably a mammal such as a human. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets. Tissues, cells and their progeny of a biological entity obtained in vivo or cultured in vitro are also encompassed.

[0150] The term "treatment" or "treating" generally refers to an approach for obtaining beneficial or desired results including but not limited to a therapeutic benefit and/or a prophylactic benefit. For example, a treatment can comprise administering a system or cell population disclosed herein. By therapeutic benefit is meant any therapeutically relevant improvement in or effect on one or more diseases, conditions, or symptoms under treatment. For prophylactic benefit, a composition can be administered to a subject at risk of developing a particular disease, condition, or symptom, or to a subject reporting one or more of the physiological symptoms of a disease, even though the disease, condition, or symptom may not have yet been manifested.

[0151] The term "effective amount" or "therapeutically effective amount" generally refers to the quantity of a composition, for example a composition comprising heterologous polypeptides, heterologous polynucleotides, and/or modified cells (e.g., modified stem cells), that is sufficient to result in a desired activity upon administration to a subject in need thereof. Within the context of the present disclosure, the term "therapeutically effective" generally refers to that quantity of a composition that is sufficient to delay the manifestation, arrest the progression, relieve or alleviate at least one symptom of a disorder treated by

the methods of the present disclosure.

[0152] *A. Overview*

[0153] Controlling or engineering a characteristic of a cell (e.g., a stem cell, a differentiated cell) can involve treating the cell with, for example, small molecules (e.g., dexamethasone), growth factors (e.g., BMP-2 for osteogenesis, TGF beta for chondrogenesis), or oligonucleotides (e.g., antisense oligonucleotide, siRNA). In some cases, endonuclease (e.g., Cas) can be used to genetically manipulate one or more target genetic loci (e.g., insertion, deletion) of the cell to modify its characteristic. In some cases, new heterologous genes are introduced to the cell, and gene products (e.g., transcription factors, growth factors, et c.) can in effect control the cell's characteristic.

[0154] However, aforementioned methods of manipulating cell's characteristic can be limited. For example, treating cells with exogenous factors such as small compounds or polypeptide/polynucleotide molecules can be limiting due to (1a) the short half-life of the exogenous factors, thus often requiring replacement of the culture media to replenish the cell with fresh exogenous factors and/or (1b) inducible non-physiological levels of expression that induces off-targets and pleiotropy effects (1c) availability of such exogenous factors to modulate a target cell characteristic. In another example, insertion and/or deletion of one or more nucleotides of the cell's genome can be limiting due to concerns about (2a) off-target effects (e.g., off-target modifications) and/or (2b) DNA damage-induced cell apoptosis or carcinogenesis, (2c) having a permanent change to the genetic makeup of the cell's genome that is irreversible when the factor is no longer needed. In a different example, introducing heterologous transcription factors alone can lack target specificity and thus can exhibit limited effect on modulating the characteristic of the cell.

[0155] Thus, there remains a significant unmet need for alternative systems and methods to engineer cells, such as stem cells, to control their characteristic.

[0156] The present disclosure describes systems and methods for modifying a cell (e.g., a stem cell, such as an MSC), to control its characteristic. Cells can be contacted by an epigenetic modifier to induce a modification in a target polynucleotide (e.g., at least 1, 2, 3, 4, 5, or more target polynucleotides, such as genomic loci, of the cell, thereby to control its characteristic. The epigenetic modification of the target polynucleotide may not and need not be an insertion and/or deletion (indel), non-homology end joining (NHEJ) modification, or homology directed repair (HDR) medication. The epigenetic modification of the target polynucleotide may not and need not involve cleavage (e.g., single strand break, double strand break) of the target polynucleotide. The epigenetic modification of the target polynucleotide may not and need not result in a substitution of a nucleotide of the target polynucleotide. The epigenetic modification can modify an expression profile of a target gene (e.g., at least 1, 2, 3, 4, 5, or more target genes) in the cell, thereby to control its characteristic. In some cases, the epigenetic modification can be permanent. Alternatively, the epigenetic modification may not and need not be permanent, e.g., reversible or temporary, yet still sufficient to modulate the cell's characteristic.

[0157] Non-limiting examples of cell characteristic that can be modulated by the systems and

methods disclosed herein can include growth, migration, proliferation, cytokine secretion, size, de-differentiation, differentiation (e.g., into a particular cell type or tissue type), and rejuvenation into a younger phenotype (e.g., downregulating one or more senescent features of the cell, upregulating one or more "young" features of the cell).

[0158] In some aspects, the target polynucleotide that is epigenetically modified and the target gene with modified expression profile can be the same. Alternatively, the target polynucleotide that is epigenetically modified and the target gene with modified expression profile can be different. In some cases, the target polynucleotide that is epigenetically modified can be operatively coupled to the target gene with modified expression profile. For example, the target polynucleotide can be a promoter of the target gene that encodes a target gene product (e.g., a protein, such as a transcription factor or a growth factor).

[0159] In some aspects, a permanent modification of the expression profile of the target gene can be sufficient to effect a specific change in the cell's characteristic. Such may utilize, for example, a permanent epigenetic modification of a target polynucleotide of the cell.

[0160] In some aspects, induction and a subsequent reversal of the modification of the expression profile of the target gene can be sufficient to effect a specific change in the cell's characteristic. Thus, modulating the cell's characteristic can require two separate steps comprising (i) a first epigenetic modification step to induce epigenetic modification of a target polynucleotide and (ii) a separate and second epigenetic modification step to reverse some or all of the induced epigenetic modification of the same target polynucleotide. Alternatively or in addition to, modulating the cell's characteristic can require two separate steps comprising (i) a first epigenetic modification step to induce epigenetic modification of a first target polynucleotide and (ii) a separate and second epigenetic modification step to induce epigenetic modification of a second target polynucleotide that is different than the first target polynucleotide. In an example, the first target polynucleotide can be downstream of the second target polynucleotide, or vice versa, within the same gene (e.g., within the same promoter sequence of a target gene). In another example, the first target polynucleotide can be in a promoter sequence of a target gene, and the second target polynucleotide can be in an exon sequence of the target gene.

[0161] In some aspects, a single epigenetic modification of a target polynucleotide in the cell can be sufficient to (i) transiently modify the expression profile of the target gene of the cell and (ii) effect a specific change in the cell's characteristic. For example, the single epigenetic modification of the target polynucleotide can be sufficient to induce a pulsing effect in the expression profile of the target gene.

[0162] In some aspects, a non-permanent modification of an expression profile of a target gene, as disclosed herein, can exhibit various expression profile shapes (FIG. 1). The non-permanently modified expression profile of the target gene can comprise an increased expression region and a decreased expression region (e.g., an increased expression of the target gene that is followed by a decrease, or a decreased expression of the target gene that is followed by an increase). A rate of change (e.g., slope) of the increased expression region can be the same as an absolute value (or inverse) of a rate of change of the

decreased expression region (e.g., indicated by an expression profile shape of an equilateral triangle or a rectangle). Alternatively, a rate of change of the increased expression region can be different than an absolute value of a rate of change of the decreased expression region. In an example, a rate of change of the increased expression region can be greater than an absolute value of a rate of change of the decreased expression region. In an example, a rate of change of the increased expression region can be less than an absolute value of a rate of change of the decreased expression region.

[0163] The non-permanently modified expression profile of the target gene can exhibit different "pulse" shapes, e.g., a rectangular pulse, a cosine squared (raised cosine) pulse, Dirac pulse, sinc pulse, and Gaussian pulse. Such "pulse" shapes can be positive (e.g., exhibiting an increase and a subsequent decrease in the expression profile) or negative (e.g., exhibiting a decrease and a subsequent increase in the expression profile).

[0164] In some aspects, modifying expression profile of a target gene can effect modification of one or more additional target genes (e.g., via action of a gene product of the target gene). Controlling the expression profile of the first target gene (e.g., via one or more epigenetic modifications as disclosed herein) can induce an expression profile of the one or more additional target genes that is different in comparison to a control cell (e.g., a natural expression profile of the one or more additional target genes when naturally expressed by the control cell in absence of any non-natural epigenetic modification).

[0165] FIGs. 1A-1f schematically illustrate different expression profiles of a target gene that is modulated upon an epigenetic modification of a target polynucleotide, as disclosed herein. Y axes indicate time, and X axes indicate expression levels of a gene of interest (e.g., relative to a house keeping gene, such as GAPDH). FIGs. 1A-1C illustrate expression levels that are initially increased. FIGs. 1D-1F illustrate expression levels that are initially decreased. FIGs. 1A and 1D show three graphs, each illustrating a permanently modified expression profile of the target gene, wherein a peak of the modified expression profile remains permanent. FIGs. 1B and 1E show three graphs, each illustrating a non-permanently modified expression profile of the target gene, wherein expression levels of the target gene prior to and subsequent to the modification are about the same (i.e., reversed). FIGs. 1C and 1F show three graphs, each illustrating a permanently modified expression profile of the target gene, wherein a peak of the modified expression profile does not remain permanent, but expression levels of the target gene prior to and subsequent to the modification are different (i.e., partially reversed).

[0166] In some aspects, an epigenetic modification of a target polynucleotide, as disclosed herein, can be permanent. In such cases, the epigenetic modification of the target polynucleotide can remain about the same for at least about 24 hours, at least about 2 days, at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about 7 days, at least about 2 weeks, at least about 3 weeks, at least about 4 weeks, at least about 2 months, at least about 3 months, or at least or up to about 5 months, as ascertained by, e.g., enzyme-linked immunosorbent assay (ELISA), Western blotting, or chromatin immunoprecipitation (ChIP), as disclosed herein.

[0167] In some aspects, an epigenetic modification of a target polynucleotide, as disclosed herein,

can be reversed or transient. In such cases, the epigenetic modification of the target polynucleotide can last for at most about 6 months, at most about 3 months, at most about 2 months, at most about 4 weeks, at most about 3 weeks, at most about 2 weeks, at most about 7 days, at most about 6 days, at most about 5 days, at most about 4 days, at most about 3 days, at most about 2 days, at most about 24 hours, at most about 12 hours, at most about 10 hours, at most about 9 hours, at most about 8 hours, at most about 7 hours, at most about 6 hours, at most about 5 hours, at most about 4 hours, at most about 3 hours, at most about 2 hours, or at most about 1 hour, or less, as ascertained by, e.g., ELISA, Western blotting, or ChIP, as disclosed herein.

[0168] In some aspects, a modification of an expression profile of a target gene, as disclosed herein, can be permanent. In such cases, the expression level of the target gene can remain about the same for at least or up to about 12 hours, at least or up to about 24 hours, at least or up to about 2 days, at least or up to about 3 days, at least or up to about 4 days, at least or up to about 5 days, at least or up to about 6 days, at least or up to about 7 days, at least or up to about 2 weeks, at least or up to about 3 weeks, at least or up to about 4 weeks, at least or up to about 2 months, at least or up to about 3 months, at least or up to about 4 months, or at least or up to about 5 months, as ascertained by, e.g., ELISA, Western blotting, or polymerase chain reaction (PCR) assays, as disclosed herein.

[0169] In some aspects, a modification of an expression profile of a target gene, as disclosed herein, can be reversed or transient. In such cases, the modified expression profile of the target gene can last for at most about 6 months, at most about 3 months, at most about 2 months, at most about 4 weeks, at most about 3 weeks, at most about 2 weeks, at most about 7 days, at most about 6 days, at most about 5 days, at most about 4 days, at most about 3 days, at most about 2 days, at most about 24 hours, at most about 12 hours, at most about 10 hours, at most about 9 hours, at most about 8 hours, at most about 7 hours, at most about 6 hours, at most about 5 hours, at most about 4 hours, at most about 3 hours, at most about 2 hours, or at most about 1 hour, or less, as ascertained by, e.g., ELISA, Western blotting, or PCR, as disclosed herein.

[0170] In some aspects, the cell that is modified by the systems and methods disclosed herein can be a stem cell. In some cases, the stem cell can be in an adult-derived stem cell, as disclosed herein. In some aspects, the cell that is modified by the systems and methods disclosed herein can be a differentiated cell (e.g., a tissue-specific, non-pluripotent cell).

[0171] In some aspects, any of the target gene that is modified by the systems and methods disclosed herein can be an endogenous gene of the cell. In some aspects, any of the target gene that is modified by the systems and methods disclosed herein can be a heterologous gene of the cell (e.g., a knocked-in gene).

[0172] ***B. Modified target gene expression and cell characteristic***

[0173] In some aspects, a modified expression profile of a target gene, as disclosed herein, can be an augmented expression level of the target gene. In some cases, a modified expression profile of a target gene, as disclosed herein, can be a decreased expression level of the target gene. In some cases, a modified expression profile of a target gene, as disclosed herein, can be a combination of (i) an

augmented expression level of the target gene and (ii) a decreased expression level of the target gene, in any order.

[0174] In one aspect, the present disclosure provides a method for modifying a cell (e.g., a stem cell, such as an adult-derived stem cell). The method can comprise contacting the cell with a heterologous polypeptide comprising a first actuator moiety configured to complex with a target polynucleotide in the cell. In some cases, the first actuator moiety can be operatively linked to an epigenetic modifier to promote an epigenetic modification of the target polynucleotide, which epigenetic modification effects a modification in an expression profile of a target gene the cell. Upon the contacting between the cell and the heterologous polypeptide (e.g., contacting between the heterologous polypeptide and the target polynucleotide), the cell can exhibit a maximum change within the modified expression profile of the target gene in less than about 7 days. In some cases, the method as disclosed herein can artificially induce a rapid expression of a target gene of the cell.

[0175] In one aspect, the present disclosure provides a method for modifying a cell (e.g., a stem cell, a differentiated cell). The method can comprise contacting the cell with a heterologous polypeptide comprising an actuator moiety configured to complex with a target polynucleotide in the cell. In some cases, the first actuator moiety can be operatively linked to an epigenetic modifier to promote an epigenetic modification of the target polynucleotide, which epigenetic modification effects a modification in an expression profile of a target gene the cell. In some cases, the modified expression profile of the target gene, as a result of the contacting, can effect a change in a characteristic of the cell. The characteristic of the cell may not and need not be the same as the modified expression profile of the target gene. In other words, the characteristic of the cell may be a feature of the cell other than the expression profile of the target gene.

[0176] In some cases, the contacting of the cell by the heterologous polypeptide can be a transient contacting, as disclosed herein throughout the present disclosure. In some examples, if a heterologous gene encoding the heterologous polynucleotide is not integrated into the genome of the cell, a use of a predetermined amount of the heterologous polypeptide or the gene encoding thereof may be understood to a transient contacting between the cell and the heterologous polypeptide.

[0177] In an example, a predetermined amount of recombinant form of the heterologous polypeptide can be delivered to the cell, and because the cell does not comprise a heterologous gene encoding the actuator moiety, any of the actuator moiety that is introduced (e.g., delivered) to the cell will eventually be degraded by the cell over time. Thus, the contacting of the cell by the heterologous polypeptide comprising the actuator moiety can be reduced or terminated by virtue of not introducing or adding any additional amount of the heterologous polypeptide to the cell. In such a case, duration of the contacting (or transient contacting) by the heterologous polypeptide to the cell may be dependent at least in part on the half-life of the heterologous polypeptide in the environment (e.g., blood or media encapsulating the cell). Such process can be described to promote (i) a transient contacting by the heterologous polypeptide to the cell, or (ii) indirectly reduce or terminate the contacting by the heterologous polypeptide to the cell.

[0178] In another example, the removal may comprise physical pull-down of either the heterologous polypeptide or a heterologous polynucleotide encoding thereof can be physically pulled-down from the environment of the cell. After a predetermined period of culturing the celli in vitro or in vivo with the heterologous polypeptide or the heterologous polypeptide/polynucleotide, the heterologous polypeptide/polynucleotide can be pulled down (e.g., using magnetic beads, wherein the heterologous polypeptide/polynucleotide and the beads are designed to recognize and couple to one another) to substantially stop any further activity of the heterologous polypeptide/polynucleotide. Such process can be described to promote (i) a transient contacting by the heterologous polypeptide to the cell, or (ii) directly reduce or terminate the contacting by the heterologous polypeptide to the cell.

[0179] In another example, the removal may comprise destruction of the heterologous polynucleotide by the host cell. If the transfected gene is not genomically integrated, the gene and/or the protein from the gene can be degraded by the host cell. Alternatively, the removal may comprise manual destruction or deactivation of the heterologous polynucleotide through the use of genetic, molecular or cellular laboratory techniques (e.g., CRISPR/Cas editing or RNAi).

[0180] In some cases, upon the contacting between the cell (e.g., a stem cell, such as an adult-derived stem cell) and the heterologous polypeptide, as disclosed herein, the cell can exhibit a maximum change within the modified expression profile of the target gene in less than about 7 days, less than about 6 days, less than about 5 days, less than about 4 days, less than about 3 days, less than about 2 days, less than about 1 day, less than about 20 hours, less than about 16 hours, less than about 12 hours, less than about 10 hours, less than about 9 hours, less than about 8 hours, less than about 7 hours, less than about 6 hours, less than about 5 hours, less than about 4 hours, less than about 3 hours, less than about 2 hours, or less than about 1 hour, or less. For example, the cell can exhibit the maximum change within the modified expression profile of the target gene in less than about 3 days. In another example, the cell can exhibit the maximum change within the modified expression profile of the target gene in less than about 2 days. In another example, the cell can exhibit the maximum change within the modified expression profile of the target gene in less than about 1 day. In a different example, the cell can exhibit the maximum change within the modified expression profile of the target gene in less than about 12 hours.

[0181] In some cases, the modified expression profile can comprise a maximum change, which can be defined as the greatest change of the expression level of the target gene within the modified expression profile, relative to an initial expression level of the target gene prior to the contacting by the heterologous polypeptide to the cell. In an example, the maximum change can be a value of a local maximum (or a peak increase) within a modified expression profile observed. In another example, the maximum change can be a value of a local minimum (or a peak decrease) within a modified expression profile observed.

[0182] In some cases, the maximum change within the modified expression profile of the target gene as disclosed herein can yield a local maximum level within the modified expression profile.

[0183] In some cases, the local maximum level of the expression of the target gene in the cell (e.g., a stem cell, such as an adult-derived stem cell) can be greater than that of a control cell (or an initial

expression level of the target gene prior to the epigenetic modification as disclosed herein) by at least or up to about 0.1-fold, at least or up to about 0.2-fold, at least or up to about 0.3-fold, at least or up to about 0.4-fold, at least or up to about 0.5-fold, at least or up to about 0.6-fold, at least or up to about 0.7-fold, at least or up to about 0.8-fold, at least or up to about 0.9-fold, at least or up to about 1-fold, at least or up to about 2-fold, at least or up to about 3-fold, at least or up to about 4-fold, at least or up to about 5-fold, at least or up to about 6-fold, at least or up to about 7-fold, at least or up to about 8-fold, at least or up to about 9-fold, at least or up to about 10-fold, at least or up to about 20-fold, at least or up to about 30-fold, at least or up to about 40-fold, at least or up to about 50-fold, at least or up to about 60-fold, at least or up to about 70-fold, at least or up to about 80-fold, at least or up to about 90-fold, at least or up to about 100-fold, at least or up to about 500-fold, at least or up to about 1,000-fold, at least or up to about 5,000-fold, or at least or up to about 10,000-fold. In an example, the local maximum level of the expression of the target gene in the cell can be greater than that of a control cell (or the initial expression level of the target gene) by at least about 5-fold. In an example, the local maximum level of the expression of the target gene in the cell can be greater than that of a control cell (or the initial expression level of the target gene) by at least about 10-fold. In an example, the local maximum level of the expression of the target gene in the cell can be greater than that of a control cell (or the initial expression level of the target gene) by at least about 20-fold.

[0184] In some cases, the local maximum level of the modified expression profile of the target gene of the cell (e.g., a stem cell, such as an adult-derived stem cell) disclosed herein can be reversed partially or completely. The local maximum level of the modified expression profile of the target gene can be reversed (i.e., reduced) by at least or up to about 5%, at least or up to about 10%, at least or up to about 20%, at least or up to about 30%, at least or up to about 40%, at least or up to about 50%, at least or up to about 60%, at least or up to about 70%, at least or up to about 80%, at least or up to about 90%, at least or up to about 95%, at least or up to about 99%, or about 100%. In an example, the local maximum level of the modified expression profile of the target gene can be reversed by at least about 50%. In another example, the local maximum level of the modified expression profile of the target gene can be reversed by at least about 60%. In a different example, the local maximum level of the modified expression profile of the target gene can be reversed by at least about 70%.

[0185] In some cases, any of the reversal (i.e., reduction) of the local maximum level of the modified expression profile of the target gene, as disclosed herein, can occur within at least or up to about 12 hours, at least or up to about 24 hours, at least or up to about 2 days, at least or up to about 3 days, at least or up to about 4 days, at least or up to about 5 days, at least or up to about 6 days, at least or up to about 7 days, at least or up to about 2 weeks, at least or up to about 4 weeks, or at least or up to about 4 weeks upon the contacting by the heterologous polypeptide to the cell. In an example, any of the reversal of the local maximum level of the modified expression profile of the target gene can occur within about 6 days upon the contacting. In another example, any of the reversal of the local maximum level of the modified expression profile of the target gene can occur within about 4 days upon the contacting. In a different

example, any of the reversal of the local maximum level of the modified expression profile of the target gene can occur within about 2 days upon the contacting.

[0186] In some cases, the local maximum level of the modified expression profile of the target gene of the cell (e.g., a stem cell, such as an adult-derived stem cell) disclosed herein can be reversed (i.e., reduced) by between about 40% and about 80%, within between about 2 days and about 6 days upon the contacting. In some examples, the local maximum level of the modified expression profile of the target gene of the cell can be reversed (i.e., reduced) by between about 50% and about 70%, within between about 3 days and about 5 days upon the contacting. In some examples, the local maximum level of the modified expression profile of the target gene of the cell can be reversed (i.e., reduced) by between about 50% and about 70%, within about 4 days upon the contacting. In some examples, the local maximum level of the modified expression profile of the target gene of the cell can be reversed (i.e., reduced) by about 60%, within between about 3 days and about 5 days upon the contacting. In some examples, the local maximum level of the modified expression profile of the target gene of the cell can be reversed (i.e., reduced) by about 40% within about 4 days upon the contacting.

[0187] In some cases, the maximum change within the modified expression profile of the target gene as disclosed herein can yield a local minimum level within the modified expression profile.

[0188] In some cases, the local minimum level of the expression of the target gene in the cell (e.g., a stem cell, such as an adult-derived stem cell) can be less than that of a control cell (or an initial expression level of the target gene prior to the epigenetic modification as disclosed herein) by at least or up to about 0.1-fold, at least or up to about 0.2-fold, at least or up to about 0.3-fold, at least or up to about 0.4-fold, at least or up to about 0.5-fold, at least or up to about 0.6-fold, at least or up to about 0.7-fold, at least or up to about 0.8-fold, at least or up to about 0.9-fold, at least or up to about 1-fold, at least or up to about 2-fold, at least or up to about 3-fold, at least or up to about 4-fold, at least or up to about 5-fold, at least or up to about 6-fold, at least or up to about 7-fold, at least or up to about 8-fold, at least or up to about 9-fold, at least or up to about 10-fold, at least or up to about 20-fold, at least or up to about 30-fold, at least or up to about 40-fold, at least or up to about 50-fold, at least or up to about 60-fold, at least or up to about 70-fold, at least or up to about 80-fold, at least or up to about 90-fold, at least or up to about 100-fold, at least or up to about 500-fold, at least or up to about 1,000-fold, at least or up to about 5,000-fold, or at least or up to about 10,000-fold. In an example, the local minimum level of the expression of the target gene in the cell can be greater than that of a control cell (or the initial expression level of the target gene) by at least about 5-fold. In an example, the local minimum level of the expression of the target gene in the cell can be less than that of a control cell (or the initial expression level of the target gene) by at least about 10-fold. In an example, the local minimum level of the expression of the target gene in the cell can be less than that of a control cell (or the initial expression level of the target gene) by at least about 20-fold.

[0189] In some cases, the maximum change (e.g., maximum increase or decrease, local maximum or minimum observed) within the modified expression profile of the target gene of the cell (e.g., a stem cell,

such as an adult-derived stem cell) as disclosed herein can be lost (i.e., reversed completely) within at least or up to about 12 hours, at least or up to about 24 hours, at least or up to about 2 days, at least or up to about 3 days, at least or up to about 4 days, at least or up to about 5 days, at least or up to about 6 days, at least or up to about 7 days, at least or up to about 2 weeks, at least or up to about 4 weeks, or at least or up to about 2 months upon the contacting by the heterologous polypeptide to the cell. In an example, the maximum change within the modified expression profile of the target gene can be lost (i.e., reversed completely) within about 6 days upon the contacting. In another example, the maximum change within the modified expression profile of the target gene can be lost (i.e., reversed completely) within about 4 days upon the contacting. In a different example, the maximum change within the modified expression profile of the target gene can be lost (i.e., reversed completely) within about 2 days upon the contacting.

[0190] In one aspect, the present disclosure provides a method for modifying a cell (e.g., a stem cell, such as an adult-derived stem cell). The method can comprise contacting the cell with a heterologous polypeptide comprising a first actuator moiety configured to complex with a target polynucleotide in the cell. In some cases, the first actuator moiety can be operatively linked to an epigenetic modifier to promote an epigenetic modification of the target polynucleotide, which epigenetic modification effects a modification in an expression profile of a target gene the cell. In some cases, upon reduction or termination of the contacting between the cell and the heterologous polypeptide (e.g., contacting between the heterologous polypeptide and the target polynucleotide), the cell can sustain at least a portion of the modified expression profile of the target gene for a period of time. In some examples, upon reduction or termination of the contacting, the cell can sustain a final expression level of the modified expression profile of the target gene for a period of time.

[0191] In some cases, the reduction or termination of the contacting between the cell and (e.g., a stem cell, such as an adult-derived stem cell) the heterologous polypeptide can comprise removal (e.g., direct or indirect, as aforementioned in the present disclosure) of the heterologous polypeptide or a gene encoding the heterologous polypeptide from the adult-derived stem cell.

[0192] In some cases, a detectable amount of the actuator moiety within the cell (e.g., a stem cell, such as an adult-derived stem cell) as disclosed herein can be reduced by about 50% within at least or up to about 2 hours, at least or up to about 4 hours, at least or up to about 8 hours, at least or up to about 12 hours, at least or up to about 24 hours, at least or up to about 2 days, at least or up to about 3 days, at least or up to about 4 days, at least or up to about 5 days, at least or up to about 6 days, or at least or up to about 7 days upon the contacting by the heterologous polypeptide to the cell.

[0193] In some cases, upon reduction or termination of the contacting between the cell and the heterologous polypeptide, as disclosed herein, the cell (e.g., a stem cell, such as an adult-derived stem cell) can sustain at least a portion of the modified expression profile of the target gene for at least or up to about 3 days, at least or up to about 4 days, at least or up to about 5 days, at least or up to about 6 days, at least or up to about 7 days, at least or up to about 2 weeks, at least or up to about 4 weeks, at least or up to about 2 months, at least or up to about 4 months, or at least or up to about 6 months. In an example, upon

reduction or termination of the contacting, the cell can sustain at least a portion of the modified expression profile of the target gene for at least about 1 week. In an example, upon reduction or termination of the contacting, the cell can sustain at least a portion of the modified expression profile of the target gene for at least about 2 weeks. In an example, upon reduction or termination of the contacting, the cell can sustain at least a portion of the modified expression profile of the target gene for at least about 1 month. In an example, upon reduction or termination of the contacting, the cell can sustain at least a portion of the modified expression profile of the target gene for at least about 3 months.

[0194] In some cases, upon reduction or termination of the contacting between the cell and the heterologous polypeptide, as disclosed herein, the cell (e.g., a stem cell, such as an adult-derived stem cell) can sustain at least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 11%, at least about 12%, at least about 13%, at least about 14%, at least about 15%, at least about 16%, at least about 17%, at least about 18%, at least about 19%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or more of the modified expression profile of the target gene for at least or up to about 3 days, at least or up to about 4 days, at least or up to about 5 days, at least or up to about 6 days, at least or up to about 7 days, at least or up to about 2 weeks, at least or up to about 4 weeks, at least or up to about 2 months, at least or up to about 4 months, or at least or up to about 6 months.

[0195] In some examples, upon reduction or termination of the contacting between the cell and the heterologous polypeptide, the cell as disclosed herein can sustain at least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 11%, at least about 12%, at least about 13%, at least about 14%, at least about 15%, or more of the modified expression profile of the target gene for at least or up to about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days. Without wishing to be bound by theory, when some but not all of the initially modified expression profile of the target gene (e.g., within 24 hours of contacting the cell and the heterologous polypeptide as disclosed herein) is sustained for a prolonged period of time (e.g., at least about 1 week), the sustained expression level of the target gene can be sufficient to elicit a desired effect in the cell (e.g., differentiation, cell function, etc.). Such initially modified expression level of the target gene may be induced at such intense level (e.g., high for gene activation, low for gene inactivation), in order to yield the subsequently prolonged expression of the target gene at a lower degree than the initially modified level but still sufficient and/or desirable to yield the desired effect in the cell.

[0196] In some cases, upon reduction or termination of the contacting, the cell can sustain a final expression level of the modified expression profile of the target gene for at least or up to about 1 day, at least or up to about 2 days, at least or up to about 3 days, at least or up to about 4 days, at least or up to

about 5 days, at least or up to about 6 days, at least or up to about 7 days, at least up to about 8 days, at least up to about 9 days, at least up to about 10 days, at least up to about 11 days, at least up to about 12 days, at least up to about 13 days, at least or up to about 2 weeks, at least or up to about 4 weeks, at least or up to about 2 months, at least or up to about 4 months, or at least or up to about 6 months. In an example, upon reduction or termination of the contacting, the cell can sustain the final expression level of the target gene for at least about 1 week. In an example, upon reduction or termination of the contacting, the cell can sustain the final expression level of the target gene for at least about 2 weeks. In an example, upon reduction or termination of the contacting, the cell can sustain the final expression level of the target gene for at least about 1 month. In an example, upon reduction or termination of the contacting, the cell can sustain the final expression level of the target gene for at least about 3 months.

[0197] In one aspect, the present disclosure provides a method for modifying a cell (e.g., a stem cell, such as an adult-derived stem cell). The method can comprise contacting the cell with a first heterologous polypeptide comprising a first actuator moiety configured to complex with a first target polynucleotide in the cell. In some cases, the first actuator moiety can be operatively linked to a first epigenetic modifier to effect a modification (e.g., an epigenetic modification) in an expression profile of a target gene the cell, thereby to generate a modified cell. The method can further comprise contacting the modified cell with a second heterologous polypeptide comprising a second actuator moiety. In some cases, the second actuator moiety can be operatively linked to a second epigenetic modifier to reverse all or part of (e.g., at least a portion of) the modified expression profile of the target gene in the modified cell. In some cases, the method as disclosed herein and reversibly witch on and off an expression profile of a target gene in the cell via action of two or more epigenetic modifications.

[0198] In one aspect, the present disclosure provides a method for modifying a cell (e.g., a stem cell, such as an adult-derived stem cell). The method can comprise contacting the cell with a first heterologous polypeptide comprising a first actuator moiety to modify an expression profile of an endogenous target gene in the adult-derived stem cell, thereby to generate a modified cell. The method can further comprise, at least about 1 week subsequent to the contacting of the cell by the first heterologous polypeptide, contacting the modified cell with a second heterologous polypeptide comprising a second actuator moiety, to reverse all or part of the modified expression profile of the endogenous target gene in the modified cell.

[0199] In one aspect, the present disclosure provides a method for modifying a cell (e.g., a stem cell, such as an adult-derived stem cell). The method can comprise contacting the cell with a first heterologous polypeptide comprising a first actuator moiety to modify an expression profile of an endogenous target gene in the adult-derived stem cell, thereby to generate a modified cell. In some cases, a maximum change, as disclosed herein, within the modified expression profile of the target gene can be greater than about 3-fold as compared to a control cell. The method can further comprise, subsequent to the contacting of the cell by the first heterologous polypeptide, contacting the modified cell with a second heterologous polypeptide comprising a second actuator moiety, to reverse all or part of the modified expression profile of the endogenous target gene in the modified cell.

[0200] In some cases, (i) the first actuator moiety as disclosed herein can be configured to complex with a first target polynucleotide of the cell (e.g., a stem cell, such as an adult-derived stem cell) that is operatively linked to the endogenous target gene, and (ii) the second actuator moiety as disclosed herein can be configured to complex with a second target polynucleotide of the cell that is also operatively linked to the endogenous target gene. In some cases, the first actuator moiety can be operatively linked to a first epigenetic modifier to induce epigenetic modification of the first target polynucleotide, thereby to effect the modified expression profile of the endogenous target gene. In some cases, the second actuator moiety can be operatively linked to a second epigenetic modifier to induce epigenetic modification of the second target polynucleotide, thereby to effect the reversal of all or part of the modified expression profile of the endogenous target gene.

[0201] In some cases, the first and second actuator moieties, as disclosed herein, can be different. Alternatively, the first and second actuator moieties, as disclosed herein, can be the same. In some cases, the first and second epigenetic modifiers, as disclosed herein, can be different. Alternatively, the first and second epigenetic modifiers, as disclosed herein, can be the same. In an example, the first and second actuator moieties are different and the first and second epigenetic modifiers are different.

[0202] In some cases, (1) the contacting of the cell by the first heterologous polypeptide comprising the first actuator moiety to generate the modified cell and (2) the contacting of the modified cell by the second heterologous polypeptide comprising the second actuator moiety, as disclosed herein, can be performed

[0203] In some cases, the contacting of the modified cell by the second heterologous polypeptide comprising the second actuator moiety, as disclosed herein, can be performed at least or up to about 12 hours, at least or up to about 24 hours, at least or up to about 2 days, at least or up to about 3 days, at least or up to about 4 days, at least or up to about 5 days, at least or up to about 6 days, at least or up to about 7 days, at least or up to about 8 days, at least or up to about 9 days, at least or up to about 10 days, at least or up to about 11 days, at least or up to about 12 days, at least or up to about 13 days, at least or up to about 14 days, at least or up to about 3 weeks, or at least or up to about 4 weeks subsequent to the contacting of the cell by the first heterologous polypeptide comprising the first actuator moiety for generating the modified cell.

[0204] In some cases, subsequent to contacting of the modified cell by the second heterologous polypeptide comprising the second actuator moiety, as disclosed herein, a resulting modified expression level of the target gene can be sustained for at least or up to about 1 day, at least or up to about 2 days, at least or up to about 3 days, at least or up to about 4 days, at least or up to about 5 days, at least or up to about 6 days, at least or up to about 7 days, at least or up to about 2 weeks, at least or up to about 4 weeks, at least or up to about 2 months, at least or up to about 4 months, or at least or up to about 6 months.

[0205] In some cases, the cell as disclosed herein is transiently contacted by the first heterologous polypeptide comprising the first actuator moiety. For example, the cell may not comprise a heterologous gene encoding the first heterologous polypeptide, and the transient contacting of the cell by the such

heterologous polypeptide can be determined by denaturation or degradation of the heterologous polypeptide, as disclosed herein.

[0206] In some cases, the modified cell as disclosed herein is transiently contacted by the second heterologous polypeptide comprising the second actuator moiety. For example, the cell may not comprise a heterologous gene encoding the second heterologous polypeptide, and the transient contacting of the modified cell by the second heterologous polypeptide can be determined by denaturation or degradation of such heterologous polypeptide, as disclosed herein.

[0207] As used throughout the present disclosure, the term "transiently contacting" or "transient contact" generally refers to a contact that is not permanent. In some cases, transiently contacting a cell with a heterologous moiety (e.g., a heterologous polypeptide, a heterologous polynucleotide encoding the heterologous polypeptide, a functional heterologous polynucleotide that is functional and that is not encoding any particular polypeptide, etc.) can be achieved by transfecting the cell with the heterologous moiety with a transfection agent (e.g., a cationic polymer, a virus that does not integrate into a genome of the cell, such as a baculovirus).

[0208] In some cases, subsequent to the transient contacting of the modified cell by a heterologous polypeptide, as disclosed herein, the resulting expression level of the additional gene can be sustained for at least or up to about 1 day, at least or up to about 2 days, at least or up to about 3 days, at least or up to about 4 days, at least or up to about 5 days, at least or up to about 6 days, at least or up to about 7 days, at least or up to about 8 days, at least or up to about 9 days, at least or up to about 10 days, at least or up to about 11 days, at least or up to about 12 days, at least or up to about 13, days, at least or up to about 2 weeks, at least or up to about 4 weeks, at least or up to about 2 months, at least or up to about 4 months, or at least or up to about 6 months. In some cases, subsequent to the transient contacting of the modified cell by a heterologous polypeptide, as disclosed herein, the resulting expression level of the additional gene is greater than that of a control cell by at least or up to about 0.1-fold, at least or up to about 0.2-fold, at least or up to about 0.3-fold, at least or up to about 0.4-fold, at least or up to about 0.5-fold, at least or up to about 0.6-fold, at least or up to about 0.7-fold, at least or up to about 0.8-fold, at least or up to about 0.9-fold, at least or up to about 1-fold, at least or up to about 2-fold, at least or up to about 3-fold, at least or up to about 4-fold, at least or up to about 5-fold, at least or up to about 6-fold, at least or up to about 7-fold, at least or up to about 8-fold, at least or up to about 9-fold, at least or up to about 10-fold, at least or up to about 20-fold, at least or up to about 30-fold, at least or up to about 40-fold, at least or up to about 50-fold, at least or up to about 60-fold, at least or up to about 70-fold, at least or up to about 80-fold, at least or up to about 90-fold, at least or up to about 100-fold, at least or up to about 500-fold, at least or up to about 1,000-fold, at least or up to about 5,000-fold, or at least or up to about 10,000-fold. In some cases, subsequent to the transient contacting of the modified cell by a heterologous polypeptide, as disclosed herein, the resulting expression level of the additional gene is less than that of a control cell by at least or up to about 0.1-fold, at least or up to about 0.2-fold, at least or up to about 0.3-fold, at least or up to about 0.4-fold, at least or up to about 0.5-fold, at least or up to about 0.6-fold, at least or up to about

0.7-fold, at least or up to about 0.8-fold, at least or up to about 0.9-fold, at least or up to about 1-fold, at least or up to about 2-fold, at least or up to about 3-fold, at least or up to about 4-fold, at least or up to about 5-fold, at least or up to about 6-fold, at least or up to about 7-fold, at least or up to about 8-fold, at least or up to about 9-fold, at least or up to about 10-fold, at least or up to about 20-fold, at least or up to about 30-fold, at least or up to about 40-fold, at least or up to about 50-fold, at least or up to about 60-fold, at least or up to about 70-fold, at least or up to about 80-fold, at least or up to about 90-fold, at least or up to about 100-fold, at least or up to about 500-fold, at least or up to about 1,000-fold, at least or up to about 5,000-fold, or at least or up to about 10,000-fold.

[0209] In some cases, the contacting of the modified cell by the second heterologous polypeptide comprising the second actuator moiety, as disclosed herein, can effect reversal of the modified expression profile of the target gene by at least or up to about 40%, at least or up to about 50%, at least or up to about 60%, at least or up to about 70%, at least or up to about 80%, at least or up to about 90%, at least or up to about 95%, at least or up to about 99%, or about 100%.

[0210] A 100% reversal or a full reversal of any of the modified expression profile, as disclosed herein, may be indicated by exhibiting a final expression profile of the target gene that is about the same as an initial expression profile before performing any of the methods as disclosed herein.

[0211] In some cases, the modified expression profile of the target gene via action of the first heterologous polypeptide, as disclosed herein, can be fully reversed within less than about 10 days, less than about 9 days, less than about 8 days, less than about 7 days, less than about 6 days, less than about 5 days, less than about 4 days, less than about 3 days, less than about 2 days, less than about 1 day, or less than about 12 hours subsequent to the contacting of the modified cell by the second heterologous polypeptide comprising the second actuator moiety.

[0212] In some cases, the first target polynucleotide (that is complexed by the first actuator moiety) and the second target polynucleotide (that is complexed by the second actuator moiety), as disclosed herein, can be different. For example, the second epigenetic modifier operatively linked to (or fused to) the second actuator moiety may serve to reverse any epigenetic modification induced by the first epigenetic modifier operatively linked to (or fused to) the first actuator moiety. Alternatively, the first target polynucleotide (that is complexed by the first actuator moiety) and the second target polynucleotide (that is complexed by the second actuator moiety) can be different. For example, the first epigenetic modifier operatively linked to (or fused to) the first actuator moiety may induce a first epigenetic modification at a first genomic site, and the second epigenetic modifier operatively linked to (or fused to) the second actuator moiety may not reverse the first epigenetic modification, but induce a different second epigenetic modification that still effects full or partial reversal of the modified expression profile of the target gene in the cell.

[0213] In some cases, the maximum change within the modified expression profile of the target gene can yield a local maximum level within the modified expression profile, as disclosed herein.

[0214] In some cases, the maximum change within the modified expression profile of the target gene

can yield a local minimum level within the modified expression profile, as disclosed herein.

[0215] In some cases, the target gene as disclosed herein comprises a positive regulator of the differentiation comprising one or more members selected from the group consisting of: Runx2, SP7/OSX, DLX5, BMPR1B, BMPR2, MSX2, DLX2, DLX3, DLX6, MSX1, ATF4, VDR, PDLIM7, ETS1, FOSL1, WNT9A, BMP2, BMP4, BMP6, BMP7, SMAD1, SMAD5, SMAD8, IGF, Pax7, and MyoD. Alternatively, the target gene comprises a negative regulator of the differentiation selected from the group consisting of: PPARg, LIPE, DKK1, DKK2, TWIST1, SMURF1, TGFBR1, and TGFBR2.

[0216] In some embodiments of any one of the methods disclosed herein, the modification of the expression profile of the target gene can effect initiation of differentiation of the stem cell (e.g., adult-derived stem cell, such as a MSC) into a specific cell type. In some embodiments of any one of the methods disclosed herein, the modification of the expression profile of the target gene can effect differentiation of the adult-derived stem cell into a specific cell type, as disclosed herein.

[0217] In some embodiments of any one of the methods disclosed herein, the modification of the expression profile of the target gene can effect a change in a characteristic of the cell, such as (1) cell morphology, (2) cell metabolism, (3) chromatin accessibility of the target gene and/or an additional gene operatively coupled to the target gene, (4) a degree of methylation of the target gene and/or the additional gene, (5) telomere length, (6) cell fate, rate of protein synthesis, rate of proteolysis, and rate of protein complex aggregation, as further described below.

[0218] In some cases, the modified expression profile of the target gene, as disclosed herein, can effect a change in a characteristic of the cell comprising (1) cell morphology. In some cases, the change in the characteristic of the cell can comprise either increased or decreased cell roundness (or cell circularity). Alternatively or in addition to, the change in the characteristic of the cell can comprise either increased or decreased projection area of the cell. In some examples, images or videos of the cell can be analyzed by one or more computer programs (e.g., ImageJ) to determine the cell circularity or projection area.

[0219] Cell circularity can be defined as, for example, $4 \cdot \pi \cdot$cell projection area/(cell perimeter squared). Without wishing to be bound by theory, an increase in cell circularity may suggest a more stem-like cell state, while a decrease in cell-circularity may suggest a more differentiated cell state. In some examples, upon the modification of the expression profile of the target gene of the cell, as disclosed herein, the cell's circularity can be increased by at least or up to about 0.1-fold, at least or up to about 0.2-fold, at least or up to about 0.3-fold, at least or up to about 0.4-fold, at least or up to about 0.5-fold, at least or up to about 0.6-fold, at least or up to about 0.7-fold, at least or up to about 0.8-fold, at least or up to about 0.9-fold, at least or up to about 1-fold, at least or up to about 2-fold, at least or up to about 3-fold, at least or up to about 4-fold, at least or up to about 5-fold, at least or up to about 6-fold, at least or up to about 7-fold, at least or up to about 8-fold, at least or up to about 9-fold, at least or up to about 10-fold, at least or up to about 20-fold, at least or up to about 30-fold, at least or up to about 40-fold, at least or up to about 50-fold, at least or up to about 60-fold, at least or up to about 70-fold, at least or up to about 80-fold,

at least or up to about 90-fold, at least or up to about 100-fold, at least or up to about 500-fold, at least or up to about 1,000-fold, at least or up to about 5,000-fold, or at least or up to about 10,000-fold, as compared to a control cell. In some examples, upon the modification of the expression profile of the target gene of the cell, as disclosed herein, the cell's circularity can be decreased by at least or up to about 0.1-fold, at least or up to about 0.2-fold, at least or up to about 0.3-fold, at least or up to about 0.4-fold, at least or up to about 0.5-fold, at least or up to about 0.6-fold, at least or up to about 0.7-fold, at least or up to about 0.8-fold, at least or up to about 0.9-fold, at least or up to about 1-fold, at least or up to about 2-fold, at least or up to about 3-fold, at least or up to about 4-fold, at least or up to about 5-fold, at least or up to about 6-fold, at least or up to about 7-fold, at least or up to about 8-fold, at least or up to about 9-fold, at least or up to about 10-fold, at least or up to about 20-fold, at least or up to about 30-fold, at least or up to about 40-fold, at least or up to about 50-fold, at least or up to about 60-fold, at least or up to about 70-fold, at least or up to about 80-fold, at least or up to about 90-fold, at least or up to about 100-fold, at least or up to about 500-fold, at least or up to about 1,000-fold, at least or up to about 5,000-fold, or at least or up to about 10,000-fold, as compared to a control cell.

[0220] Cell projection area can be defined as, for example, a number of pixels occupied by a cell in an image or video of the cell when analyzed by a computer system. Without wishing to be bound by theory, a decrease in cell projection area may suggest a more stem-like cell state, while an increase in cell projection area may suggest a more differentiated cell state. In some examples, upon the modification of the expression profile of the target gene of the cell, as disclosed herein, the cell's projection area can be increased by at least or up to about 0.1-fold, at least or up to about 0.2-fold, at least or up to about 0.3-fold, at least or up to about 0.4-fold, at least or up to about 0.5-fold, at least or up to about 0.6-fold, at least or up to about 0.7-fold, at least or up to about 0.8-fold, at least or up to about 0.9-fold, at least or up to about 1-fold, at least or up to about 2-fold, at least or up to about 3-fold, at least or up to about 4-fold, at least or up to about 5-fold, at least or up to about 6-fold, at least or up to about 7-fold, at least or up to about 8-fold, at least or up to about 9-fold, at least or up to about 10-fold, at least or up to about 20-fold, at least or up to about 30-fold, at least or up to about 40-fold, at least or up to about 50-fold, at least or up to about 60-fold, at least or up to about 70-fold, at least or up to about 80-fold, at least or up to about 90-fold, at least or up to about 100-fold, at least or up to about 500-fold, at least or up to about 1,000-fold, at least or up to about 5,000-fold, or at least or up to about 10,000-fold, as compared to a control cell. In some examples, upon the modification of the expression profile of the target gene of the cell, as disclosed herein, the cell's projection area can be decreased by at least or up to about 0.1-fold, at least or up to about 0.2-fold, at least or up to about 0.3-fold, at least or up to about 0.4-fold, at least or up to about 0.5-fold, at least or up to about 0.6-fold, at least or up to about 0.7-fold, at least or up to about 0.8-fold, at least or up to about 0.9-fold, at least or up to about 1-fold, at least or up to about 2-fold, at least or up to about 3-fold, at least or up to about 4-fold, at least or up to about 5-fold, at least or up to about 6-fold, at least or up to about 7-fold, at least or up to about 8-fold, at least or up to about 9-fold, at least or up to about 10-fold, at least or up to about 20-fold, at least or up to about 30-fold, at least or up to about 40-fold, at least or up to

about 50-fold, at least or up to about 60-fold, at least or up to about 70-fold, at least or up to about 80-fold, at least or up to about 90-fold, at least or up to about 100-fold, at least or up to about 500-fold, at least or up to about 1,000-fold, at least or up to about 5,000-fold, or at least or up to about 10,000-fold, as compared to a control cell.

[0221] In some cases, the modified expression profile of the target gene, as disclosed herein, can effect a change in a characteristic of the cell comprising (2) cell metabolism. In some cases, the change in the characteristic of the cell can comprise either increased or decreased number of mitochondria per cell. Alternatively or in addition to, the change in the characteristic of the cell can comprise either an increase or a decrease in circularity of the mitochondria of the cell. Yet in another alternative or in an additional embodiment, the change in the characteristic of the cell can comprise either increased or decreased level of adenosine triphosphate (ATP) release from the cell.

[0222] Non-limiting methods of ascertaining the number of mitochondria per cell can include mitochondrial biogenesis assay (e.g., via ELISA, Western blot, for flow cytometry), mitochondrial viability assay (e.g., mitochondrial membrane potential assay), and OXPHOS protein expression analysis. Without wishing to be bound by theory, an increase in the number of mitochondria per cell, an increase in the membrane potential or activity of mitochondria, an enhancement in ATP/NADH metabolism of cell(s), or any combination thereof, may suggest a more stem-like cell state, while a decrease in the number of mitochondria per cell, a reduction in the membrane potential or activity of mitochondria, a decrease in ATP/NADH metabolism of cell(s), or any combination thereof, may suggest a more differentiated cell state. In some examples, upon the modification of the expression profile of the target gene of the cell, as disclosed herein, the number of mitochondria per cell can be increased by at least or up to about 0.1-fold, at least or up to about 0.2-fold, at least or up to about 0.3-fold, at least or up to about 0.4-fold, at least or up to about 0.5-fold, at least or up to about 0.6-fold, at least or up to about 0.7-fold, at least or up to about 0.8-fold, at least or up to about 0.9-fold, at least or up to about 1-fold, at least or up to about 2-fold, at least or up to about 3-fold, at least or up to about 4-fold, at least or up to about 5-fold, at least or up to about 6-fold, at least or up to about 7-fold, at least or up to about 8-fold, at least or up to about 9-fold, at least or up to about 10-fold, at least or up to about 20-fold, at least or up to about 30-fold, at least or up to about 40-fold, at least or up to about 50-fold, at least or up to about 60-fold, at least or up to about 70-fold, at least or up to about 80-fold, at least or up to about 90-fold, at least or up to about 100-fold, at least or up to about 500-fold, at least or up to about 1,000-fold, at least or up to about 5,000-fold, or at least or up to about 10,000-fold, as compared to a control cell. In some examples, upon the modification of the expression profile of the target gene of the cell, as disclosed herein, the number of mitochondria per cell can be decreased by at least or up to about 0.1-fold, at least or up to about 0.2-fold, at least or up to about 0.3-fold, at least or up to about 0.4-fold, at least or up to about 0.5-fold, at least or up to about 0.6-fold, at least or up to about 0.7-fold, at least or up to about 0.8-fold, at least or up to about 0.9-fold, at least or up to about 1-fold, at least or up to about 2-fold, at least or up to about 3-fold, at least or up to about 4-fold, at least or up to about 5-fold, at least or up to about 6-fold, at least or up to about 7-

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[0223] The shape of mitochondria can be measured based on images and/or videos of the cell, as disclosed herein, and circularity of the cell can be determined. Without wishing to be bound by theory, an increase in mitochondrial length or volume, a decrease in mitochondrial circularity, or any combination thereof, may suggest a more stem-like cell state, while a decrease in mitochondrial length or volume, an increase in mitochondrial circularity, or any combination thereof, may suggest a more differentiated cell state. In some examples, upon the modification of the expression profile of the target gene of the cell, as disclosed herein, the mitochondrial circularity can be increased by at least or up to about 0.1-fold, at least or up to about 0.2-fold, at least or up to about 0.3-fold, at least or up to about 0.4-fold, at least or up to about 0.5-fold, at least or up to about 0.6-fold, at least or up to about 0.7-fold, at least or up to about 0.8-fold, at least or up to about 0.9-fold, at least or up to about 1-fold, at least or up to about 2-fold, at least or up to about 3-fold, at least or up to about 4-fold, at least or up to about 5-fold, at least or up to about 6-fold, at least or up to about 7-fold, at least or up to about 8-fold, at least or up to about 9-fold, at least or up to about 10-fold, at least or up to about 20-fold, at least or up to about 30-fold, at least or up to about 40-fold, at least or up to about 50-fold, at least or up to about 60-fold, at least or up to about 70-fold, at least or up to about 80-fold, at least or up to about 90-fold, at least or up to about 100-fold, at least or up to about 500-fold, at least or up to about 1,000-fold, at least or up to about 5,000-fold, or at least or up to about 10,000-fold, as compared to a control cell. In some examples, upon the modification of the expression profile of the target gene of the cell, as disclosed herein, the mitochondria's circularity can be decreased by at least or up to about 0.1-fold, at least or up to about 0.2-fold, at least or up to about 0.3-fold, at least or up to about 0.4-fold, at least or up to about 0.5-fold, at least or up to about 0.6-fold, at least or up to about 0.7-fold, at least or up to about 0.8-fold, at least or up to about 0.9-fold, at least or up to about 1-fold, at least or up to about 2-fold, at least or up to about 3-fold, at least or up to about 4-fold, at least or up to about 5-fold, at least or up to about 6-fold, at least or up to about 7-fold, at least or up to about 8-fold, at least or up to about 9-fold, at least or up to about 10-fold, at least or up to about 20-fold, at least or up to about 30-fold, at least or up to about 40-fold, at least or up to about 50-fold, at least or up to about 60-fold, at least or up to about 70-fold, at least or up to about 80-fold, at least or up to about 90-fold, at least or up to about 100-fold, at least or up to about 500-fold, at least or up to about 1,000-fold, at least or up to about 5,000-fold, or at least or up to about 10,000-fold, as compared to a control cell.

[0224] Non-limiting methods of ascertaining a level of ATM release from the cell include luciferin-based luminescent ATP detection assay using extracellular supernatant and/or intracellular supernatant. Without wishing to be bound by theory, an increase in ATP release or production (e.g., related to plasma

ATP, erythrocyte-mediated ATP, etc.) by the cell may suggest a more stem-like cell state, while an decrease in ATP release or production by the cell may suggest a more differentiated cell state. In some examples, upon the modification of the expression profile of the target gene of the cell, as disclosed herein, the ATP release by the cell can be increased by at least or up to about 0.1-fold, at least or up to about 0.2-fold, at least or up to about 0.3-fold, at least or up to about 0.4-fold, at least or up to about 0.5-fold, at least or up to about 0.6-fold, at least or up to about 0.7-fold, at least or up to about 0.8-fold, at least or up to about 0.9-fold, at least or up to about 1-fold, at least or up to about 2-fold, at least or up to about 3-fold, at least or up to about 4-fold, at least or up to about 5-fold, at least or up to about 6-fold, at least or up to about 7-fold, at least or up to about 8-fold, at least or up to about 9-fold, at least or up to about 10-fold, at least or up to about 20-fold, at least or up to about 30-fold, at least or up to about 40-fold, at least or up to about 50-fold, at least or up to about 60-fold, at least or up to about 70-fold, at least or up to about 80-fold, at least or up to about 90-fold, at least or up to about 100-fold, at least or up to about 500-fold, at least or up to about 1,000-fold, at least or up to about 5,000-fold, or at least or up to about 10,000-fold, as compared to a control cell. In some examples, upon the modification of the expression profile of the target gene of the cell, as disclosed herein, the ATP release by the cell can be decreased by at least or up to about 0.1-fold, at least or up to about 0.2-fold, at least or up to about 0.3-fold, at least or up to about 0.4-fold, at least or up to about 0.5-fold, at least or up to about 0.6-fold, at least or up to about 0.7-fold, at least or up to about 0.8-fold, at least or up to about 0.9-fold, at least or up to about 1-fold, at least or up to about 2-fold, at least or up to about 3-fold, at least or up to about 4-fold, at least or up to about 5-fold, at least or up to about 6-fold, at least or up to about 7-fold, at least or up to about 8-fold, at least or up to about 9-fold, at least or up to about 10-fold, at least or up to about 20-fold, at least or up to about 30-fold, at least or up to about 40-fold, at least or up to about 50-fold, at least or up to about 60-fold, at least or up to about 70-fold, at least or up to about 80-fold, at least or up to about 90-fold, at least or up to about 100-fold, at least or up to about 500-fold, at least or up to about 1,000-fold, at least or up to about 5,000-fold, or at least or up to about 10,000-fold, as compared to a control cell.

[0225] In some cases, the modified expression profile of the target gene, as disclosed herein, can effect a change in a characteristic of the cell comprising (3) chromatin accessibility of the target gene and/or an additional gene operatively coupled to the target gene. In some cases, the change in the characteristic of the cell can comprise either more open chromatin or less open (closed) chromatin at or near the target gene and/or the additional gene. For example, epigenetic modification of a target gene encoding a transcription factor (e.g., Runx2 or osteoblast differentiation) can effect opening of the chromatin near an additional gene (e.g., collagen, BMP-2, alkaline phosphatase, osteopontin, etc.) that is operatively coupled to the target gene, to promote activation of the additional gene. In a different example, epigenetic modification of a target gene encoding a transcription factor (e.g., Runx2 for osteoblast differentiation) can effect closing of the chromatin near an additional gene (e.g., Myc) that is operatively coupled to the target gene, to promote suppression of the additional gene.

[0226] Non-limiting methods of ascertaining chromatin accessibility of a gene of interest include (i)

RNA sequencing, (ii) Assay for Transposase-Accessible Chromatin using sequencing (ATAC seq), (iii) chromatin immunoprecipitation (ChIP) sequencing, (iv) chromosome conformation capture (i.e., 2C) assay, or (v) cell-free DNA (cfDNA) sequencing, (vi) Trac-looping. For example, a chromatin accessibility intensity, as measure by using any one method of (i)-(vi) or a combination thereof, may change (e.g., increase or decrease) by about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50%, or more, indicating a change in the chromatin accessibility of target genomic locus (or loci).

[0227] In some cases, the modified expression profile of the target gene, as disclosed herein, can effect a change in a characteristic of the cell comprising (4) a degree of methylation of the target gene or the additional gene. In some cases, the change in the characteristic of the cell can comprise either increased or decreased degree of methylation of the target gene or the additional gene. For example, epigenetic modification of a target gene encoding a transcription factor (e.g., Runx2 or osteoblast differentiation) can effect demethylation of an additional gene (e.g., collagen, BMP-2, alkaline phosphatase, osteopontin, etc.) that is operatively coupled to the target gene, to promote activation of the additional gene. In a different example, epigenetic modification of a target gene encoding a transcription factor (e.g., Runx2 for osteoblast differentiation) can effect methylation of an additional gene (e.g., Myc) that is operatively coupled to the target gene, to promote suppresion of the additional gene.

[0228] Non-limiting methods of ascertaining methylation of a gene of interest include bisulfite conversion assay, high resolution melt (HRM) analysis, and methylated DNA immunoprecipitation. Without wishing to be bound by theory, an increase in methylation of a gene of interest may suggest suppression of the gene of interest, while a decrease in methylation (i.e., demethylation) of the gene of interest may suggest activation of the gene of interest. In some examples, upon the modification of the expression profile of the target gene of the cell, as disclosed herein, a degree of methylation of the target gene or the additional gene can be increased by at least or up to about 0.1-fold, at least or up to about 0.2-fold, at least or up to about 0.3-fold, at least or up to about 0.4-fold, at least or up to about 0.5-fold, at least or up to about 0.6-fold, at least or up to about 0.7-fold, at least or up to about 0.8-fold, at least or up to about 0.9-fold, at least or up to about 1-fold, at least or up to about 2-fold, at least or up to about 3-fold, at least or up to about 4-fold, at least or up to about 5-fold, at least or up to about 6-fold, at least or up to about 7-fold, at least or up to about 8-fold, at least or up to about 9-fold, at least or up to about 10-fold, at least or up to about 20-fold, at least or up to about 30-fold, at least or up to about 40-fold, at least or up to about 50-fold, at least or up to about 60-fold, at least or up to about 70-fold, at least or up to about 80-fold, at least or up to about 90-fold, at least or up to about 100-fold, at least or up to about 500-fold, at least or up to about 1,000-fold, at least or up to about 5,000-fold, or at least or up to about 10,000-fold, as compared to a control cell. In some examples, upon the modification of the expression profile of the target gene of the cell, as disclosed herein, a degree of methylation of the target gene or the additional gene can be decreased by at least or up to about 0.1-fold, at least or up to about 0.2-fold, at least or up to about 0.3-fold, at least or up to about 0.4-fold, at least or up to about 0.5-fold, at least or up to about 0.6-fold, at least or up to about 0.7-fold, at least or up to about 0.8-fold, at least or up to about 0.9-fold, at least

or up to about 1-fold, at least or up to about 2-fold, at least or up to about 3-fold, at least or up to about 4-fold, at least or up to about 5-fold, at least or up to about 6-fold, at least or up to about 7-fold, at least or up to about 8-fold, at least or up to about 9-fold, at least or up to about 10-fold, at least or up to about 20-fold, at least or up to about 30-fold, at least or up to about 40-fold, at least or up to about 50-fold, at least or up to about 60-fold, at least or up to about 70-fold, at least or up to about 80-fold, at least or up to about 90-fold, at least or up to about 100-fold, at least or up to about 500-fold, at least or up to about 1,000-fold, at least or up to about 5,000-fold, or at least or up to about 10,000-fold, as compared to a control cell.

[0229] In some cases, the modified expression profile of the target gene, as disclosed herein, can effect a change in a characteristic of the cell comprising (5) telomere length. In some cases, the change in the characteristic of the cell comprises either increased or decreased telomere length.

[0230] Non-limiting methods of ascertaining telomere length include Q-PCR (Quantitative Polymerase Chain Reaction), TRF (Terminal Restriction Fragment) analysis, a Q-FISH (Quantitative Fluorescence In Situ Hybridization) methods, STELA (Single TElomere Length Analysis) and TeSLA (Telomere Shortest Length Assay). Without wishing to be bound by theory, a decrease in telomere length may suggest a more stem-like cell state, while an increase in telomere may suggest a more differentiated cell state. Without wishing to be bound by theory, a decrease in telomere length may suggest a more "young" state, while an increase in telomere may suggest a more senescent cell state. In some examples, upon the modification of the expression profile of the target gene of the cell, as disclosed herein, the telomere length (e.g., average telomere length) of the cell can be increased by at least or up to about 0.1-fold, at least or up to about 0.2-fold, at least or up to about 0.3-fold, at least or up to about 0.4-fold, at least or up to about 0.5-fold, at least or up to about 0.6-fold, at least or up to about 0.7-fold, at least or up to about 0.8-fold, at least or up to about 0.9-fold, at least or up to about 1-fold, at least or up to about 2-fold, at least or up to about 3-fold, at least or up to about 4-fold, at least or up to about 5-fold, at least or up to about 6-fold, at least or up to about 7-fold, at least or up to about 8-fold, at least or up to about 9-fold, at least or up to about 10-fold, at least or up to about 20-fold, at least or up to about 30-fold, at least or up to about 40-fold, at least or up to about 50-fold, at least or up to about 60-fold, at least or up to about 70-fold, at least or up to about 80-fold, at least or up to about 90-fold, at least or up to about 100-fold, at least or up to about 500-fold, at least or up to about 1,000-fold, at least or up to about 5,000-fold, or at least or up to about 10,000-fold, as compared to a control cell. In some examples, upon the modification of the expression profile of the target gene of the cell, as disclosed herein, the telomere length (e.g., average telomere length) can be decreased by at least or up to about 0.1-fold, at least or up to about 0.2-fold, at least or up to about 0.3-fold, at least or up to about 0.4-fold, at least or up to about 0.5-fold, at least or up to about 0.6-fold, at least or up to about 0.7-fold, at least or up to about 0.8-fold, at least or up to about 0.9-fold, at least or up to about 1-fold, at least or up to about 2-fold, at least or up to about 3-fold, at least or up to about 4-fold, at least or up to about 5-fold, at least or up to about 6-fold, at least or up to about 7-fold, at least or up to about 8-fold, at least or up to about 9-fold, at least or up to about 10-fold, at least or up to about 20-fold, at least or up to about 30-fold, at least or up to about 40-fold, at least or up to about

50-fold, at least or up to about 60-fold, at least or up to about 70-fold, at least or up to about 80-fold, at least or up to about 90-fold, at least or up to about 100-fold, at least or up to about 500-fold, at least or up to about 1,000-fold, at least or up to about 5,000-fold, or at least or up to about 10,000-fold, as compared to a control cell.

[0231] In some cases, the modified expression profile of the target gene, as disclosed herein, can effect a change in a characteristic of the cell comprising (6) cell fate. In some cases, the change in the characteristic of the cell comprises a modified expression of an additional gene (i.e., a gene marker) indicative of (i) differentiation of the cell to a more committed cell type, (ii) trans-differentiation into a of the cell to different cell type, or (iii) dedifferentiation of the cell to a less committed cell type.

[0232] In some examples, upon the modification of the expression profile of the target gene of the cell, as disclosed herein, the cell may exhibit differentiation into a more committed cell type. In some examples, upon the modification of the expression profile of the target gene of the cell, the cell may exhibit trans-differentiation into a different cell type. In some examples, upon the modification of the expression profile of the target gene of the cell, the cell may exhibit dedifferentiation into a less committed cell type, e.g., a stem cell (e.g., iPSC).

[0233] In some cases, the modified expression profile of the target gene, as disclosed herein, can effect a change in a characteristic of the cell comprising: protein synthesis rate. In some cases, the change in the characteristic of the cell comprises either an increased or decreased rate of protein synthesis within cell(s).

[0234] In some cases, the modified expression profile of the target gene, as disclosed herein, can effect a change in a characteristic of the cell comprising: rate of proteolysis (i.e. degradation of polypeptide or protein). In some cases, the change in the characteristic of the cell comprises either increased or decreased rate of proteolysis within cell(s).

[0235] In some cases, the modified expression profile of the target gene, as disclosed herein, can effect a change in a characteristic of the cell comprising: aggregation rate of protein complexes. In some cases, the change in the characteristic of the cell comprises either increased or decreased rate of protein complex aggregation within cell(s).

[0236] In some cases, the modified expression profile of the target gene, as disclosed herein, can effect a change in a characteristic of the cell comprising two or more members selected from the group consisting of (1) cell morphology, (2) cell metabolism, (3) chromatin accessibility of the gene and/or an additional gene operatively coupled to the target gene, (4) a degree of methylation of the additional gene, (5) telomere length, (6) cell fate, rate of protein synthesis, rate of proteolysis, and rate of protein complex aggregation.

[0237] In some cases, the modified expression profile of the target gene, as disclosed herein, can effect a change in a characteristic of the cell comprising three or more members selected from the group consisting of (1) cell morphology, (2) cell metabolism, (3) chromatin accessibility of the gene and/or of an additional gene operatively coupled to the target gene, (4) a degree of methylation of the additional gene,

(5) telomere length, (6) cell fate, rate of protein synthesis, rate of proteolysis, and rate of protein complex aggregation.

[0238] In some cases, the modified expression profile of the target gene, as disclosed herein, can effect a change in a characteristic of the cell comprising four or more members selected from the group consisting of (1) cell morphology, (2) cell metabolism, (3) chromatin accessibility of the gene and/or an additional gene operatively coupled to the target gene, (4) a degree of methylation of the additional gene, (5) telomere length, (6) cell fate, rate of protein synthesis, rate of proteolysis, and rate of protein complex aggregation.

[0239] In some cases, the modified expression profile of the target gene, as disclosed herein, can effect a change in a characteristic of the cell comprising five or more members selected from the group consisting of (1) cell morphology, (2) cell metabolism, (3) chromatin accessibility of the gene and/or an additional gene operatively coupled to the target gene, (4) a degree of methylation of the additional gene, (5) telomere length, (6) cell fate, rate of protein synthesis, rate of proteolysis, and rate of protein complex aggregation.

[0240] In some cases, the modified expression profile of the target gene, as disclosed herein, can effect a change in a characteristic of the cell comprising one or more members (such as two or more, three or more, four or more, five or more, or all six members) selected from the group consisting of (1) cell morphology, (2) cell metabolism, (3) chromatin accessibility of the gene and/or an additional gene operatively coupled to the target gene, (4) a degree of methylation of the additional gene, (5) telomere length, and (6) cell fate.

[0241] In some cases, the change in the characteristic of the cell, as disclosed herein, can be sustained as compared to a control cell (e.g., without any epigenetic modification, as disclosed herein). The change in the characteristic of the cell, as disclosed herein, can be sustained for at least or up to about 12 hours, at least or up to about 24 hours, at least or up to about 2 days, at least or up to about 3 days, at least or up to about 4 days, at least or up to about 5 days, at least or up to about 6 days, at least or up to about 7 days, at least or up to about 2 weeks, at least or up to about 4 weeks, at least or up to about 2 months, at least or up to about 4 months, or at least or up to about 6 months.

[0242] In some cases, the modified expression profile of the target gene as disclosed herein can be an augmented expression level of the target gene. In some cases, the modified expression profile of the target gene as disclosed herein can be a decreased expression level of the target gene. In some cases, the modified expression profile of the target gene as disclosed herein can be a combination of (i) an augmented expression level of the target gene and (ii) a decreased expression level of the target gene, in any order.

[0243] In some cases, a predetermined amount of recombinant form of the heterologous polypeptide or a heterologous polynucleotide encoding thereof can be delivered to the cell as disclosed herein, thereby promoting a transient contacting by the heterologous polypeptide to the cell. As aforementioned, the transient contacting may be dependent at least in part on the half-life of the heterologous

polypeptide/polynucleotide in the environment (e.g., blood or media encapsulating the cell). In such a case, the heterologous polynucleotide may not and need not be integrated into the genome of the cell.

[0244] A control cell can be a cell that has not been contacted by the heterologous polypeptide as disclosed herein.

[0245] *C. Cells*

[0246] In some embodiments of any of the methods disclosed herein, the method can be applied to modify a target cell (e.g., modify expression profile of a target gene of the target cell), such as a stem cell, in vitro, ex vivo, or in vivo. In some cases, the stem cell can be an adult-derived stem cell, as disclosed herein (e.g., MSCs), and may not and need not include an embryonic stem cell. Alternatively, the stem cell can be either an adult-derive stem cell or an embryonic stem cell.

[0247] In some embodiments of any of the methods disclosed herein, the method can be applied to modify a target cell (e.g., modify expression profile of a target gene of the target cell), such as a committed cell type. Non-limiting examples cells are provided elsewhere in the present disclosure. In some examples, the committed cell can comprise one or more members selected form the group consisting of a fibroblast, a neuron, a glial cell, an osteoblast, and a chondrocyte.

[0248] Additional examples of a target cell that can be subjected to any of the methods as disclosed herein are provided in the present disclosure.

[0249] Whether stem cell or committed cell, the subject cell can be treated by any of the method disclosed herein in vitro or ex vivo, then administered to the subject, e.g., to treat a condition of the subject. For example, any subject modified cell product can be administered to the subject to treat a condition of a bodily tissue of the subject. In some cases, the cell can be resident inside the subject's body, and any of the heterologous polypeptide (e.g., comprising the actuator moiety that is operatively coupled to the epigenetic modifier) or a heterologous gene encoding thereof can be administered to the subject, to thereby promote the contacting between the heterologous polypeptide and the cell.

[0250] Whether stem cell or committed cell, any modification thereof can, for example, treat a condition of the bodily tissue of the subject (e.g., MuSCs can be modified in vivo to promote enhanced muscle regeneration). In another example, the subject cell can reside in a first bodily tissue and upon modification of the subject cell, as disclosed herein, the resulting modified cell can treat a condition of a second bodily tissue of the subject (e.g., MSCs in bone marrow can be modified and used to promote bone repair/regeneration).

[0251] In some cases, the stem cell or committed cell, as disclosed herein, can be an autologous cell or derived from the autologous cell of the subject. The autologous cell can be obtained from the subject having a condition or is suspected of having the condition. Alternatively, the autologous cell can be obtained from the subject before the subject is found to have the condition. In some cases, the autologous cell can be an allogeneic cell, e.g., a universal stem cell with reduced immunogenicity and with reduced amount or no need for immunosuppressive drugs. The autologous cell can be obtained from a healthy donor.

[0252] In some aspects, the present disclosure provides a novel cell population derived from an initial cell population, wherein the initial cell population has been contacted by the heterologous polypeptide or a heterologous encoding the heterologous polypeptide via any of the methods as disclosed herein, thereby to generate the novel cell population. In some examples, the cell population can be transiently contacted by the heterologous polypeptide, as aforementioned herein. In some examples, the cell population can comprise a plurality of embryonic stem cells or a plurality of adult-derived stem cells. In some examples, the cell cam be a committed cell comprising a fibroblast, a neuron, a glial cell, an osteoblast, or a chondrocyte.

[0253] ***D. Composition***

[0254] In some aspects, the present disclosure provides a composition comprising any of the heterologous polypeptide or a heterologous encoding the heterologous polypeptide, as disclosed herein, for use in any of the methods as disclosed herein. The subject composition can be usable for modifying a cell in vitro, ex vivo, or in vivo. In some aspects, the present disclosure provides a composition comprising a modified cell or a population of modified cells (e.g., modified stem cells, modified committed cells) that has been contacted by the heterologous polypeptide or a heterologous encoding the heterologous polypeptide, as disclosed herein. The subject composition can be usable for treating or enhancing a condition of a subject, as disclosed herein. In some aspects, the present disclosure provides a composition comprising a cell or a population of cells and the heterologous polypeptide or a heterologous encoding the heterologous polypeptide for modifying the cell or the population of cells, as disclosed herein. Such composition can comprise a single dose that comprises both (i) the cell or the population of cells and (ii) the heterologous polypeptide/polynucleotide. Alternatively, the composition can comprise multiple doses comprising (i) a first does that comprises the cell or the population of cells and (ii) a second dose that comprises the heterologous polypeptide/polynucleotide.

[0255] The composition as disclosed herein can comprise an active ingredient (e.g., the heterologous polypeptide, unmodified cells, modified cells, as disclosed herein) and an additional ingredient (e.g., excipient). If necessary and/or desirable, the composition can be divided, shaped and/or packaged into a desired single- or multi-dose unit or single-or multi-implantation unit.

[0256] In some cases, the additional ingredient of the composition as disclosed herein can comprise an excipient. Non-limiting examples of the excipient can include solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, lipidoids, liposomes, lipid nanoparticles, polymers, lipoplexes, core-shell nanoparticles, peptides, proteins, hyaluronidase, nanoparticle mimics, inert diluents, buffering agents, lubricating agents, oils, and combinations thereof. In some examples, the composition as disclosed herein can include one or more excipients, each in an amount that together increases the stability of (i) the heterologous polypeptide or the heterologous gene encoding thereof and/or (ii) cells or modified cells.

[0257] In some aspects, the present disclosure provides a kit comprising such composition and

instructions directing (i) contacting the cell with the composition or (ii) administration of cells modified by any of the methods disclosed herein to a subject. The subject may have or may be suspected of having a condition, such as a disease or tissue degeneration.

[0258] In some embodiments, any of the heterologous polypeptide, heterologous gene encoding the heterologous polypeptide, or modified cell, as disclosed herein, can be administered to the subject via orally, intraperitoneally, intravenously, intraarterially, transdermally, intramuscularly, liposomally, via local delivery by catheter or stent, subcutaneously, intraadiposally, or intrathecally.

[0259] The compositions (e.g., pharmaceutical compositions) disclosed herein can be suitable for administration to humans. In addition, such compositions can be suitable for administration to any other animal, e.g., to non-human animals, e.g. non-human mammals. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and/or perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions is contemplated include, but are not limited to, humans and/or other primates; mammals, including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, dogs, mice, and/or rats; and/or birds, including commercially relevant birds such as poultry, chickens, ducks, geese, and/or turkeys.

[0260] ***E. Target gene***

[0261] In some embodiments of any one of the methods and systems disclosed herein, the target gene of the cell (e.g., a stem cell, such as an adult-derived stem cell) as disclosed herein can encode a transcription factor. Thus, the epigenetic modification of a target polynucleotide, as disclosed herein, can modulate an expression profile of the transcription factor in the cell. In some cases, the transcription factor can effect differentiation of the cell into a differentiated phenotype. In some examples, inducing the epigenetic modification of the target polynucleotide, thereby modulating expression profile of the transcription factor, can be sufficient to induce differentiation of the target cell (e.g., from a mesenchymal stem cell into an osteoblast) without aid of any ectopic growth factor (e.g., BMP-2 or BMP-4).

[0262] In some embodiments of any one of the methods and systems disclosed herein, the target gene of the cell (e.g., a stem cell, such as an adult-derived stem cell) as disclosed herein can encode a growth factor. Non-limiting examples of a growth factor include Vascular Endothelial Growth Factor (VEGF) (e.g., VEGF-121, VEGF-165, VEGF-183, VEGF-189, and VEGF-206), Transforming Growth Factor (TGF) such as TGF Beta (TGF-β) (e.g., TGF-beta-1, TGF-beta-2, TGF-beta-3, TGF-beta-4, and TGF-beta-5), Fibroblast Growth Factors (FGF) (e.g., FGF1, FGF2, FGF3 (INT2), FGF4, FGF5, FGF6, FGF7 (KGF), FGF8 (AIGF), FGF9, FGF10, FGF11, FGF12, FGF13, FGF14, FGF16, FGF17, FGF18, FGF19, FGF20, FGF21, FGF22, and FGF2), growth/differentiation factors (GDF) (e.g., GDF-1, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-9B, GDF-10, GDF-11, and GDF-15), Epidermal Growth Factors (EGF) (e.g., Epiregulin (EPR), Epigen, Betacellulin (BTC), neuregulin such as NRG1, NRG2, NRG3, and NRG4), angiogenin (Agn), TNF-alpha, insulin-like growth factor-1 (IGF-1), transforming growth factor α

(TGF-α), platelet-derived growth factors (PDGF), Placental Growth Factors (PGF), Heparin-binding EGF-like Growth Factors (HEGF), Hepatocyte Growth Factors (LGF), Interferon-gamma (IFN-gamma), Bone Morphogenic Proteins (BMP) (e.g., BMP-2-alpha, BMP-2-beta, BMP-3, BMP-3b, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8, BMP-8b, BMP-9, BMP-10, BMP-11, BMP-12, BMP-13, BMP-14, BMP-15), Dentin matrix acidic phosphoprotein (e.g., DMP-1, DMP-4), and nerve growth factor (NGF).

[0263] In some embodiments of any one of the methods and systems disclosed herein, the target gene of the cell (e.g., a stem cell, such as an adult-derived stem cell) as disclosed herein can encode a transcription factor. In some cases, non-limiting examples of the transcription factor encoded by the target gene as disclosed herein can include MyoD; MyoG, Myf5, Mrf4, Ngn family (e.g. Ngnl-3), NeuroD family (e.g. NeuroDl-3), Ascl family (e.g. Ascll-2), Hb9, Zicl, Brn2, Mytll, Nurrl, Lmxla, Gata family (e.g. Gatal, 2, 4, 5, 6), Tbx5, Mef2 family (e.g. Mef2a,b,c), Mespl, Hnf/FoxA family (e.g. Hnf4a, FoxA2), Pdxl, MafA, Runx family (e.g. Runx2, Runxltl), Mitf, Spil, Nkx family (e.g. Nkx2.1, 2.2), C/EBP family (e.g. C/EBPa, β), Prdm family (e.g. Prdml, 16), PPARy, Scl, Lmo2, Ldbl, E2A, Ebf, Sox9, Hlf, Prdm5, Pbxl, Zfp37, Isll, Lhx3, Phox2a, Fezf2, Olig family (e.g. 1 and 2), Elf5, Irf2, Elfl, Tgifl, Etsl, Sox family (e.g. Sox4, 6, 9, 17), Bach2, Cdx2, Smydl, Pax family (e.g. Pax3, 6, 7), Klf family (e.g. Klf4, Klf5), and basic helix-loop-helix factors.

[0264] In some cases, the target gene as disclosed herein can encode a myogenic factor (e.g., a transcription factor) that effects (or promotes) myoblast differentiation. Non-limiting examples of such transcription factor can include MyoD, MyoG, Myf5, Mrf4, Gata4, Tbx5, Mef2a, Mef2b, Mef2c, Mesp1, Pax3, Pax7, and Smyd1.

[0265] In some cases, the target gene as disclosed herein can encode a neuronal factor (e.g., a growth factor, a transcription factor) that effects (or promotes) neuronal differentiation. Non-limiting examples of such transcription factor can include Ngn1, Ngn2, Ngn3, NeuroD1, NeuroD2, NEuroD3, Ascl1, Ascl2, Zic1, Brn2, Nurr1, Mytl1, Lmx1a, Hlf, Zfp37, Phox2a, and Fezf2.

[0266] In some cases, the target gene as disclosed herein can encode a neuronal and/or pancreatic factor (e.g., a transcription factor) that effects (or promotes) motor neuron and/or pancreatic cell differentiation. Non-limiting examples of such transcription factor can include Hb9, Isl1, Ngn2, and Lhx3.

[0267] In some cases, the target gene as disclosed herein can encode an Erythroid factor (e.g., a growth factor, transcription factor) that effects (or promotes) Erythroid differentiation. Non-limiting examples of such transcription factor can include Gata1 and Ldb1.

[0268] In some cases, the target gene as disclosed herein can encode a hematopoietic and/or endocrine lineage factor (e.g., a growth factor, transcription factor) that effects (or promotes) hematopoietic and/or endocrine lineage differentiation. Non-limiting examples of such endocrine lineage factor can include Gata2.

[0269] In some cases, the target gene as disclosed herein can encode a myocardial and/or endodermal lineage factor that effects (or promotes) myocardial and/or endodermal lineage differentiation. Non-

limiting examples of such myocardial and/or endodermal lineage factor can include Gata5.

[0270] In some cases, the target gene as disclosed herein can encode an endodermal and/or mesodermal lineage factor that effects (or promotes) Endodermal and/or mesodermal lineage differentiation. Non-limiting examples of such endodermal and/or mesodermal lineage factor can include Gata6.

[0271] In some cases, the target gene as disclosed herein can encode a hepatocyte, liver, kidney, and/or intestinal cell factor that effects (or promotes) hepatocyte, liver, kidney, and/or intestinal cell differentiation. Non-limiting examples of such hepatocyte, liver, kidney, and/or intestinal cell factor can include Hnf4α.

[0272] In some cases, the target gene as disclosed herein can encode a hepatocyte factor that effects (or promotes) hepatocyte differentiation. Non-limiting examples of such hepatocyte factor can include Hnf1α, Hnf4α, Hnf1β, FoxA2, and LRH-1.

[0273] In some cases, the target gene as disclosed herein can encode a pancreatic cell factor that effects (or promotes) pancreatic differentiation. Non-limiting examples of such pancreatic factor can include Pdx1 and MafA.

[0274] In some cases, the target gene as disclosed herein can encode an osteogenic factor (e.g., a growth factor, a transcription factor) that effects (or promotes) osteoblast differentiation. Non-limiting examples of such osteogenic factor can include Runx2, Prdm5, Pbx1, Osx, DLx5, Bmp2, Bmp4, Bmp7, Fgf2, Col11a1, Ctsk, Tbp, Pou5f1, Nanog, Sp7, Alp, Oc, Sost, Gapdh, Cbfa1, Pebp2, Aml3, Osf2, Mmp1, Bsp, Foxc1, Opn, C-fos, Spp1, and Dmp1. Non-limiting examples of such transcription factor can include Runx2, Prdm5, and Pbx1. Osteogenic differentiation can comprise a biomarker such as increased levels of calcium deposits. Calcium deposits can increase by at least or up to about 0.1-fold, at least or up to about 0.2-fold, at least or up to about 0.3-fold, at least or up to about 0.4-fold, at least or up to about 0.5-fold, at least or up to about 0.6-fold, at least or up to about 0.7-fold, at least or up to about 0.8-fold, at least or up to about 0.9-fold, at least or up to about 1-fold, at least or up to about 2-fold, at least or up to about 3-fold, at least or up to about 4-fold, at least or up to about 5-fold, at least or up to about 6-fold, at least or up to about 7-fold, at least or up to about 8-fold, at least or up to about 9-fold, at least or up to about 10-fold, at least or up to about 20-fold, at least or up to about 30-fold, at least or up to about 40-fold, at least or up to about 50-fold, at least or up to about 60-fold, at least or up to about 70-fold, at least or up to about 80-fold, at least or up to about 90-fold, or at least or up to about 100-fold over a control.

[0275] In some cases, the target gene as disclosed herein can encode a melanocyte factor that effects (or promotes) melanocyte differentiation. Non-limiting examples of such melanocyte factor can include Mitf.

[0276] In some cases, the target gene as disclosed herein can encode a myeloid and/or B lymphoid factor that effects (or promotes) myeloid and/or B lymphoid differentiation. Non-limiting examples of such myeloid and/or B lymphoid factor can include Spi1.

[0277] In some cases, the target gene as disclosed herein can encode a thyroid factor that effects (or

promotes) thyroid differentiation. Non-limiting examples of such thyroid factor can include Nkx2.1.

[0278] In some cases, the target gene as disclosed herein can encode a adipocyte factor that effects (or promotes) adipocyte differentiation. Non-limiting examples of such adipocyte factor can include C/EBPα, C/EBPβ, Prdm16, and PPARγ.

[0279] In some cases, the target gene as disclosed herein can encode a trophoblast and/or plasma cell factor that effects (or promotes) trophoblast and/or plasma cell differentiation. Non-limiting examples of such trophoblast and/or plasma cell factor can include Prdm1.

[0280] In some cases, the target gene as disclosed herein can encode an osteoblast, neuron, and/or hematopoietic factor that effects (or promotes) osteoblast, neuron, and/or hematopoietic differentiation. Non-limiting examples of such osteoblast, neuron, and/or hematopoietic factor can include Scl.

[0281] In some cases, the target gene as disclosed herein can encode a hematopoietic factor that effects (or promotes) hematopoietic differentiation. Non-limiting examples of such hematopoietic factor can include Lmo2, Runx1t1, Back2, Irf2, and Sox4.

[0282] In some cases, the target gene as disclosed herein can encode a lymphocyte factor that effects (or promotes) lymphocyte differentiation. Non-limiting examples of such lymphocyte factor can include E2A and Elf1.

[0283] In some cases, the target gene as disclosed herein can encode a B cell factor that effects (or promotes) B cell differentiation. Non-limiting examples of such B cell factor can include Ebf.

[0284] In some cases, the target gene as disclosed herein can encode a chondrogenic factor (e.g., a growth factor, a transcription factor) that effects (or promotes) chondrocyte differentiation. Non-limiting examples of such osteogenic factor can include Sox9, Col2a1, Col10a1, Aggrecan, Comp, TgfB1, Bmp2, Gdf5, Igf1, and Ncam1. Non-limiting examples of such transcription factor can include Sox9.

[0285] In some cases, the target gene as disclosed herein can encode a motor neuron factor that effects (or promotes) motor neuron differentiation. Non-limiting examples of such motor neuron factor can include Lhx3.

[0286] In some cases, the target gene as disclosed herein can encode a oligodendrocyte factor that effects (or promotes) oligodendrocyte differentiation. Non-limiting examples of such oligodendrocyte factor can include Olig1 and Olig2.

[0287] In some cases, the target gene as disclosed herein can encode a epithelial cell factor that effects (or promotes) epithelial cell differentiation. Non-limiting examples of such epithelial cell factor can include Elf5 and Klf4.

[0288] In some cases, the target gene as disclosed herein can encode a myeloid factor that effects (or promotes) myeloid differentiation. Non-limiting examples of such myeloid factor can include Tgif1.

[0289] In some cases, the target gene as disclosed herein can encode a hematopoietic and/or epithelial factor that effects (or promotes) hematopoietic and/or epithelial differentiation. Non-limiting examples of such hematopoietic and/or epithelial factor can include Ets1.

[0290] In some cases, the target gene as disclosed herein can encode a intestinal cell factor that

effects (or promotes) intestinal cell differentiation. Non-limiting examples of such intestinal cell factor can include Cdx2.

[0291] In some cases, the target gene as disclosed herein can encode a eye cell factor that effects (or promotes) eye cell differentiation. Non-limiting examples of such eye cell factor can include Pax6.

[0292] In some embodiments of any one of the methods and systems disclosed herein, the target gene of the cell (e.g., a stem cell, such as an adult-derived stem cell) as disclosed herein can be a signaling biochemical pathway-associated gene, as shown in TABLE 1 below.

TABLE 1.

CELLULAR FUNCTION	GENES
PI3K/AKT Signaling	PRKCE; ITGAM; ITGA5; IRAK1; PRKAA2; EIF2AK2; PTEN; EIF4E; PRKCZ; GRK6; MAPK1; TSC1; PLK1; AKT2; IKBKB; PIK3CA; CDK8; CDKN1B; NFKB2; BCL2; PIK3CB; PPP2R1A; MAPK8; BCL2L1; MAPK3; TSC2; ITGA1; KRAS; EIF4EBP1; RELA; PRKCD; NOS3; PRKAA1; MAPK9; CDK2; PPP2CA; PIM1; ITGB7; YWHAZ; ILK; TP53; RAF1; IKBKG; RELB; DYRK1A; CDKN1A; ITGB1; MAP2K2; JAK1; AKT1; JAK2; PIK3R1; CHUK; PDPK1; PPP2R5C; CTNNB1; MAP2K1; NFKB1; PAK3; ITGB3; CCND1; GSK3A; FRAP1; SFN; ITGA2; TTK; CSNK1A1; BRAF; GSK3B; AKT3; FOXO1; SGK; HSP90AA1; RP S6KB1
ERK/MAPK Signaling	PRKCE; ITGAM; ITGA5; HSPB1; IRAK1; PRKAA2; EIF2AK2; RAC1; RAP1A; TLN1; EIF4E; ELK1; GRK6; MAPK1; RAC2; PLK1; AKT2; PIK3CA; CDK8; CREB1; PRKCI; PTK2; FOS; RPS6KA4; PIK3CB; PPP2R1A; PIK3C3; MAPK8; MAPK3; ITGA1; ETS1; KRAS; MYCN; EIF4EBP1; PPARG; PRKCD; PRKAA1; MAPK9; SRC; CDK2; PPP2CA; PIM1; PIK3C2A; ITGB7; YWHAZ; PPP1CC; KSR1; PXN; RAF1; FYN; DYRK1A; ITGB1; MAP2K2; PAK4; PIK3R1; STAT3; PPP2R5C; MAP2K1; PAK3; ITGB3; ESR1; ITGA2; MYC; TTK; CSNK1A1; CRKL; BRAF; ATF4; PRKCA; SRF; STAT1; SGK
Glucocorticoid Receptor Signaling	RAC1; TAF4B; EP300; SMAD2; TRAF6; PCAF; ELK1; MAPK1; SMAD3; AKT2; IKBKB; NCOR2; UBE2I; PIK3CA; CREB1; FOS; HSPA5; NFKB2; BCL2; MAP3K14; STAT5B; PIK3CB; PIK3C3; MAPK8; BCL2L1; MAPK3; T5C22D3 ; MAPK10; NRIP1; KRAS; MAPK13; RELA; STAT5A; MAPK9;

CELLULAR FUNCTION	GENES
	NOS2A; PBX1; NR3C1; PIK3C2A; CDKN1C; TRAF2; SERPINE1; NCOA3; MAPK14; TNF; RAF1; IKBKG; MAP3K7; CREBBP; CDKN1A; MAP2K2; JAK1; IL8; NCOA2; AKT1; JAK2; PIK3R1; CHUK; STAT3; MAP2K1; NFKB1; TGFBR1; ESR1; SMAD4; CEBPB; JUN; AR; AKT3; CCL2; IVINIP1; STAT1; IL6; HSP90AA1
Axonal Guidance Signaling	PRKCE; ITGAM; ROCK1; ITGA5; CXCR4; ADAM12; IGF1; RAC1; RAP1A; E1F4E; PRKCZ; NRP1; NTRK2; ARHGEF7; SMO; ROCK2; MAPK1; PGF; RAC2; PTPN11; GNAS; AKT2; PIK3CA; ERBB2; PRKCI; PTK2; CFL1; GNAQ; PIK3CB; CXCL12; PIK3C3; WNT11; PRKD1; GNB2L1; ABL1; MAPK3; ITGA1; KRAS; RHOA; PRKCD; PIK3C2A; ITGB7; GLI2; PXN; VASP; RAF1; FYN; ITGB1; MAP2K2; PAK4; ADAM17; AKT1; PIK3R1; GLI1; WNT5A; ADAM10; MAP2K1; PAK3; ITGB3; CDC42; VEGFA; ITGA2; EPHA8; CRKL; RND1; GSK3B; AKT3; PRKCA
Ephrin Receptor Signaling	PRKCE; ITGAM; ROCK1; ITGA5; CXCR4; IRAK1; PRKAA2; EIF2AK2; RAC1; RAP1A; GRK6; ROCK2; MAPK1; PGF; RAC2; PTPN11; GNAS; PLK1; AKT2; DOK1; CDK8; CREB1; PTK2; CFL1; GNAQ; MAP3K14; CXCL12; MAPK8; GNB 2L1; ABL1; MAPK3; ITGA1; KRAS; RHOA; PRKCD; PRKAA1; MAPK9; SRC; CDK2; PIM1; ITGB7; PXN; RAF1; FYN; DYRK1A; ITGB1; MAP2K2; PAK4, AKT1; JAK2; STAT3; ADAM10; MAP2K1; PAK3; ITGB3; CDC42; VEGFA; ITGA2; EPHA8; TTK; CSNK1A1; CRKL; BRAF; PTPN13; ATF4; AKT3; SGK
Actin Cytoskeleton Signaling	ACTN4; PRKCE; ITGAM; ROCK1; ITGA5; IRAK1; PRKAA2; EIF2AK2; RAC1; INS; ARHGEF7; GRK6; ROCK2; MAPK1; RAC2; PLK1; AKT2; PIK3CA; CDK8; PTK2; CFL1; PIK3CB; MYH9; DIAPH1; PIK3C3; MAPK8; F2R; MAPK3; SLC9A1; ITGA1; KRAS; RHOA; PRKCD; PRKAA1; MAPK9; CDK2; PIM1; PIK3C2A; ITGB7; PPP1CC; PXN; VIL2; RAF1; GSN; DYRK1A; ITGB1; MAP2K2; PAK4; PIP5K1A; PIK3R1; MAP2K1; PAK3; ITGB3; CDC42; APC; ITGA2; TTK; CSNK1A1; CRKL; BRAF; VAV3; SGK

CELLULAR FUNCTION	GENES
Huntington's Disease Signaling	PRKCE; IGF1; EP300; RCOR1; PRKCZ; HDAC4; TGM2; MAPK1; CAPNS1; AKT2; EGFR; NCOR2; SP1; CAPN2; PIK3CA; HDAC5; CREB1; PRKC1; HSPA5; REST; GNAQ; PIK3CB; PIK3C3; MAPK8; IGF1R; PRKD1; GNB2L1; BCL2L1; CAPN1; MAPK3; CASP8; HDAC2; HDAC7A; PRKCD; HDAC11; MAPK9; HDAC9; PIK3C2A; HDAC3; TP53; CASP9; CREBBP; AKT1; PIK3R1; PDPK1; CASP1; APAF1; FRAP1; CASP2; JUN; BAX; ATF4; AKT3; PRKCA; CLTC; SGK; HDAC6; CASP3
Apoptosis Signaling	PRKCE; ROCK1; BID; IRAK1; PRKAA2; EIF2AK2; BAK1; BIRC4; GRK6; MAPK1; CAPNS1; PLK1; AKT2; IKBKB; CAPN2; CDK8; FAS; NFKB2; BCL2; MAP3K14; MAPK8; BCL2L1; CAPN1; MAPK3; CASP8; KRAS; RELA; PRKCD; PRKAA1; MAPK9; CDK2; PIM1; TP53; TNF; RAF1; IKBKG; RELB; CASP9; DYRK1A; MAP2K2; CHUK; APAF1; MAP2K1; NFKB1; PAK3; LMNA; CASP2; BIRC2; TTK; CSNK1A1; BRAF; BAX; PRKCA; SGK; CASP3; BIRC3; PARP1
B Cell Receptor Signaling	RAC1; PTEN; LYN; ELK1; MAPK1; RAC2; PTPN11; AKT2; IKBKB; PIK3CA; CREB1; SYK; NFKB2; CAMK2A; MAP3K14; PIK3CB; PIK3C3; MAPK8; BCL2L1; ABL1; MAPK3; ETS1; KRAS; MAPK13; RELA; PTPN6; MAPK9; EGR1; PIK3C2A; BTK; MAPK14; RAF1; IKBKG; RELB; MAP3K7; MAP2K2; AKT1; PIK3R1; CHUK; MAP2K1; NFKB1; CDC42; GSK3A; FRAP1; BCL6; BCL10; JUN; GSK3B; ATF4; AKT3; VAV3; RPS6KB1
Leukocyte Extravasation Signaling	ACTN4; CD44; PRKCE; ITGAM; ROCK1; CXCR4; CYBA; RAC1; RAP1A; PRKCZ; ROCK2; RAC2; PTPN11; NIMP14; PIK3CA; PRKCI; PTK2; PIK3CB; CXCL12; PIK3C3; MAPK8; PRKD1; ABL1; MAPK10; CYBB; MAPK13; RHOA; PRKCD; MAPK9; SRC; PIK3C2A; BTK; MAPK14; NOX1; PXN; VIL2; VASP; ITGB1; MAP2K2; CTNND1; PIK3R1; CTNNB1; CLDN1; CDC42; F11R; ITK; CRKL; VAV3; CTTN; PRKCA; MNIP1; MMP9

CELLULAR FUNCTION	GENES
Integrin Signaling	ACTN4; ITGAM; ROCK1; ITGA5; RAC1; PTEN; RAP1A; TLN1; ARHGEF7; MAPK1; RAC2; CAPNS1; AKT2; CAPN2; PIK3CA; PTK2; PIK3CB; PIK3C3; MAPK8; CAV1; CAPN1; ABL1; MAPK3; ITGA1; KRAS; RHOA; SRC; PIK3C2A; ITGB7; PPP1CC; ILK; PXN; VASP; RAF1; FYN; ITGB1; MAP2K2; PAK4; AKT1; PIK3R1; TNK2; MAP2K1; PAK3; ITGB3; CDC42; RND3; ITGA2; CRKL; BRAF; GSK3B; AKT3
Acute Phase Response Signaling	IRAK1; SOD2; MYD88; TRAF6; ELK1; MAPK1; PTPN11; AKT2; IKBKB; PIK3CA; FOS; NFKB2; MAP3K14; PIK3CB; MAPK8; RIPK1; MAPK3; IL6ST; KRAS; MAPK13; IL6R; RELA; SOCS1; MAPK9; FTL; NR3C1; TRAF2; SERPINE1; MAPK14; TNF; RAF1; PDK1; IKBKG; RELB; MAP3K7; MAP2K2; AKT1; JAK2; PIK3R1; CHUK; STAT3; MAP2K1; NFKB1; FRAP1; CEBPB; JUN; AKT3; IL1R1; IL6
PTEN Signaling	ITGAM; ITGA5; RAC1; PTEN; PRKCZ; BCL2L11; MAPK1; RAC2; AKT2; EGFR; IKBKB; CBL; PIK3CA; CDKN1B; PTK2; NFKB2; BCL2; PIK3CB; BCL2L1; MAPK3; ITGA1; KRAS; ITGB7; ILK; PDGFRB; INSR; RAF1; IKBKG; CASP9; CDKN1A; ITGB1; MAP2K2; AKT1; PIK3R1; CHUK; PDGFRA; PDPK1; MAP2K1; NFKB1; ITGB3; CDC42; CCND1; GSK3A; ITGA2; GSK3B; AKT3; FOXO1; CASP3; RPS6KB1
p53 Signaling	PTEN; EP300; BBC3; PCAF; FASN; BRCA1; GADD45A; BIRC5; AKT2; PIK3CA; CHEK1; TP53INP1; BCL2; PIK3CB; PIK3C3; MAPK8; THBS1; ATR; BCL2L1; E2F1; PMAIP1; CHEK2; TNFRSF10B; TP73; RB1; HDAC9; CDK2; PIK3C2A; MAPK14; TP53; LRDD; CDKN1A; HIPK2; AKT1; PIK3R1; RRM2B; APAF1; CTNNB1; SIRT1; CCND1; PRKDC; ATM; SFN; CDKN2A; JUN; SNAI2; GSK3B; BAX; AKT3
Aryl Hydrocarbon Receptor Signaling	HSPB1; EP300; FASN; TGM2; RXRA; MAPK1; NQO1; NCOR2; SP1; ARNT; CDKN1B; FOS; CHEK1; SMARCA4; NFKB2; MAPK8; ALDH1A1; ATR; E2F1; MAPK3; NRIP1; CHEK2; RELA; TP73; GSTP1; RB1; SRC; CDK2; AHR; NFE2L2; NCOA3; TP53; TNF; CDKN1A; NCOA2; APAF1;

CELLULAR FUNCTION	GENES
	NFKB1; CCND1; ATM; ESR1; CDKN2A; MYC; JUN; ESR2; BAX; IL6; CYP1B1; HSP90AA1
Xenobiotic Metabolism Signaling	PRKCE; EP300; PRKCZ; RXRA; MAPK1; NQO1; NCOR2; PIK3CA; ARNT; PRKCI; NFKB2; CAMK2A; PIK3CB; PPP2R1A; PIK3C3; MAPK8; PRKD1; ALDH1A1; MAPK3; NRIP1; KRAS; MAPK13; PRKCD; GSTP1; MAPK9; NOS2A; ABCB1; AHR; PPP2CA; FTL; NFE2L2; PIK3C2A; PPARGC1A; MAPK14; TNF; RAF1; CREBBP; MAP2K2; PIK3R1; PPP2R5C; MAP2K1; NFKB1; KEAP1; PRKCA; EIF2AK3; IL6; CYP1B1; HSP90AA1
SAPK/JNK Signaling	PRKCE; IRAK1; PRKAA2; EIF2AK2; RAC1; ELK1; GRK6; MAPK1; GADD45A; RAC2; PLK1; AKT2; PIK3CA; FADD; CDK8; PIK3CB; PIK3C3; MAPK8; RIPK1; GNB2L1; IRS1; MAPK3; MAPK10; DAXX; KRAS; PRKCD; PRKAA1; MAPK9; CDK2; PIM1; PIK3C2A; TRAF2; TP53; LCK; MAP3K7; DYRK1A; MAP2K2; PIK3R1; MAP2K1; PAK3; CDC42; JUN; TTK; CSNK1A1; CRKL; BRAF; SGK
PPAr/RXR Signaling	PRKAA2; EP300; INS; SMAD2; TRAF6; PPARA; FASN; RXRA; MAPK1; SMAD3; GNAS; IKBKB; NCOR2; ABCA1; GNAQ; NFKB2; MAP3K14; STAT5B; MAPK8; IRSI; MAPK3; KRAS; RELA; PRKAA1; PPARGC1A; NCOA3; MAPK14; INSR; RAF1; IKBKG; RELB; MAP3K7; CREBBP; MAP2K2; JAK2; CHUK; MAP2K1; NFKB1; TGFBR1; SMAD4; JUN; IL1R1; PRKCA; IL6; HSP90AA1; ADIPOQ
NF-KB Signaling	IRAK1; EIF2AK2; EP300; INS; MYD88; PRKCZ: TRAF6; TBK1; AKT2; EGFR; IKBKB; PIK3CA; BTRC; NFKB2; MAP3K14; PIK3CB; PIK3C3; MAPK8; RIPK1; HDAC2; KRAS; RELA; PIK3C2A; TRAF2; TLR4: PDGFRB; TNF; INSR; LCK; IKBKG; RELB; MAP3K7; CREBBP; AKT1; PIK3R1; CHUK; PDGFRA; NFKB1; TLR2; BCL10; GSK3B; AKT3; TNFAIP3; IL1R1
Neuregulin Signaling	ERBB4; PRKCE; ITGAM; ITGA5: PTEN; PRKCZ; ELK1; MAPK1; PTPN11; AKT2; EGFR; ERBB2; PRKCI; CDKN1B; STAT5B; PRKD1; MAPK3; ITGA1; KRAS; PRKCD; STAT5A; SRC; ITGB7; RAF1; ITGB1; MAP2K2; ADAM17; AKT1;

CELLULAR FUNCTION	GENES
	PIK3R1; PDPK1; MAP2K1; ITGB3; EREG; FRAP1; PSEN1; ITGA2; MYC; NRG1; CRKL; AKT3; PRKCA; HSP90AA1; RPS6KB1
Wnt & Beta catenin Signaling	CD44; EP300; LRP6; DVL3; CSNK1E; GJA1; SMO; AKT2; PIN1; CDH1; BTRC; GNAQ; MARK2; PPP2R1A; WNT11; SRC; DKK1; PPP2CA; SOX6; SFRP2: ILK; LEF1; SOX9; TP53; MAP3K7; CREBBP; TCF7L2; AKT1; PPP2R5C; WNT5A; LRP5; CTNNB1; TGFBA1; CCND1; GSK3A; DVL1; APC; CDKN2A; MYC; CSNK1A1; GSK3B; AKT3; SOX2
Insulin Receptor	PTEN; INS; EIF4E; PTPN1; PRKCZ; MAPK1; TSC1; PTPN11; AKT2; CBL; PIK3CA; PRKCI; PIK3CB; PIK3C3; MAPK8; IRS1; MAPK3; TSC2; KRAS; EIF4EBP1; SLC2A4; PIK3C2A; PPP1CC; INSR; RAF1; FYN; MAP2K2; JAK1; AKT1; JAK2; PIK3R1; PDPK1; MAP2K1; GSK3A; FRAP1; CRKL; GSK3B; AKT3; FOXO1; SGK; RPS6KB1
IL-6 Signaling	HSPB1; TRAF6; MAPKAPK2; ELK1; MAPK1; PTPN11; IKBKB; FOS; NFKB2: MAP3K14; MAPK8; MAPK3; MAPK10; IL6ST; KRAS; MAPK13; IL6R; RELA; SOCS1; MAPK9; ABCB1; TRAF2; MAPK14; TNF; RAF1; IKBKG; RELB; MAP3K7; MAP2K2; IL8; JAK2; CHUK; STAT3; MAP2K1; NFKB1; CEBPB; JUN; IL1R1; SRF; IL6
Hepatic Cholestasis	PRKCE; IRAK1; INS; MYD88; PRKCZ; TRAF6; PPARA; RXRA; IKBKB; PRKCI; NFKB2; MAP3K14; MAPK8; PRKD1; MAPK10; RELA; PRKCD; MAPK9; ABCB1; TRAF2; TLR4; TNF; INSR; IKBKG; RELB; MAP3K7; IL8; CHUK; NR1H2; TJP2; NFKB1; ESR1; SREBF1; FGFR4; JUN; IL1R1; PRKCA; IL6
IGF-1 Signaling	IGF1; PRKCZ; ELK1; MAPK1; PTPN11; NEDD4; AKT2; PIK3CA; PRKCI; PTK2; FOS; PIK3CB; PIK3C3; MAPK8; IGF1R; IRS1; MAPK3; IGFBP7; KRAS; PIK3C2A; YWHAZ; PXN; RAF1; CASP9; MAP2K2; AKT1; PIK3R1; PDPK1; MAP2K1; IGFBP2; SFN; JUN; CYR61; AKT3; FOXO1; SRF; CTGF; RPS6KB1

CELLULAR FUNCTION	GENES
NRF2-Mediated Oxidative Stress Response	PRKCE; EP300; SOD2; PRKCZ; MAPK1; SQSTM1; NQO1; PIK3CA; PRKCI; FOS; PIK3CB; PIK3C3; MAPK8; PRKD1; MAPK3; KRAS; PRKCD; GSTP1; MAPK9; FTL; NFE2L2; PIK3C2A; MAPK14; RAF1; MAP3K7; CREBBP; MAP2K2; AKT1; PIK3R1; MAP2K1; PPIB; JUN; KEAP1; GSK3B; ATF4; PRKCA; EIF2AK3; HSP9OAA1
Hepatic Fibrosis/Hepatic Stellate Cell Activation	EDN1; IGF1; KDR; FLT1; SMAD2; FGFR1; MET; PGF; SMAD3; EGFR; FAS; CSF1; NFKB2; BCL2; MYH9; IGF1R; IL6R; RELA; TLR4; PDGFRB; TNF; RELB; IL8; PDGFRA; NFKB1; TGFBR1; SMAD4; VEGFA; BAX; IL1R1; CCL2; HGF; IVIMPI; STAT1; IL6; CTGF; MMP9
PPAR Signaling	EP300; INS; TRAF6; PPARA; RXRA; MAPK1; IKBKB; NCOR2; FOS; NFKB2; MAP3K14; STAT5B; MAPK3; NRIP1; KRAS; PPARG; RELA; STAT5A; TRAF2; PPARGC1A; PDGFRB; TNF; INSR; RAF1; IKBKG; RELB; MAP3K7; CREBBP; MAP2K2; CHUK; PDGFRA; MAP2K1; NFKB1; JUN; IL1R1; HSP90AA1
Fc Epsilon RI Signaling	PRKCE; RAC1; PRKCZ; LYN; MAPK1; RAC2; PTPN11; AKT2; PIK3CA; SYK; PRKCI; PIK3CB; PIK3C3; MAPK8; PRKD1; MAPK3; MAPK10; KRAS; MAPK13; PRKCD; MAPK9; PIK3C2A; BTK; MAPK14; TNF; RAF1; FYN; MAP2K2; AKT1; PIK3R1; PDPK1; MAP2K1; AKT3; VAV3; PRKCA
G-Protein Coupled Receptor Signaling	PRKCE; RAP1A; RGS16; MAPK1; GNAS; AKT2; IKBKB; PIK3CA; CREB1; GNAQ; NFKB2; CAMK2A; PIK3CB; PIK3C3; MAPK3; KRAS; RELA; SRC; PIK3C2A; RAF1; IKBKG; RELB; FYN; MAP2K2; AKT1; PIK3R1; CHUK; PDPK1; STAT3; MAP2K1; NFKB1; BRAF; ATF4; AKT3; PRKCA
Inositol Phosphate Metabolism	PRKCE; IRAK1; PRKAA2; EIF2AK2; PTEN; GRK6; MAPK1; PLK1; AKT2; PIK3CA; CDK8; PIK3CB; PIK3C3; MAPK8; MAPK3; PRKCD; PRKAA1; MAPK9; CDK2; PIM1; PIK3C2A; DYRK1A; MAP2K2; PIP5K1A; PIK3R1; MAP2K1; PAK3; ATM; TTK; CSNK1A1; BRAF; SGK

CELLULAR FUNCTION	GENES
PDGF Signaling	EIF2AK2; ELK1; ABL2; MAPK1; PIK3CA; FOS; PIK3CB; PIK3C3; MAPK8; CAV1; ABL1; MAPK3; KRAS; SRC; PIK3C2A; PDGFRB; RAF1; MAP2K2; JAK1; JAK2; PIK3R1; PDGFRA; STAT3; SPHK1; MAP2K1; MYC; JUN; CRKL; PRKCA; SRF; STAT1; SPHK2
VEGF Signaling	ACTN4; ROCK1; KDR; FLT1; ROCK2; MAPK1; PGF; AKT2; PIK3CA; ARNT; PTK2; BCL2; PIK3CB; PIK3C3; BCL2L1; MAPK3; KRAS; HIF1A; NOS3; PIK3C2A; PXN; RAF1; MAP2K2; ELAVL1; AKT1; PIK3R1; MAP2K1; SFN; VEGFA; AKT3; FOXO1; PRKCA
Natural Killer Cell Signaling	PRKCE; RAC1; PRKCZ; MAPK1; RAC2; PTPN11; KIR2DL3; AKT2; PIK3CA; SYK; PRKCI; PIK3CB; PIK3C3; PRKD1; MAPK3; KRAS; PRKCD; PTPN6; PIK3C2A; LCK; RAF1; FYN; MAP2K2; PAK4; AKT1; PIK3R1; MAP2K1; PAK3; AKT3; VAV3; PRKCA
Cell Cycle: G1/S Checkpoint Regulation	HDAC4; SMAD3; SUV39H1; HDAC5; CDKN1B; BTRC; ATR; ABL1; E2F1; HDAC2; HDAC7A; RB1; HDAC11; HDAC9; CDK2; E2F2; HDAC3; TP53; CDKN1A; CCND1; E2F4; ATM; RBL2; SMAD4; CDKN2A; MYC; NRG1; GSK3B; RBL1; HDAC6
T Cell Receptor Signaling	RAC1; ELK1; MAPK1; IKBKB; CBL; PIK3CA; FOS; NFKB2; PIK3CB; PIK3C3; MAPK8; MAPK3; KRAS; RELA, PIK3C2A; BTK; LCK; RAF1; IKBKG; RELB, FYN; MAP2K2; PIK3R1; CHUK; MAP2K1; NFKB1; ITK; BCL10; JUN; VAV3
Death Receptor Signaling	CRADD; HSPB1; BID; BIRC4; TBK1; IKBKB; FADD; FAS; NFKB2; BCL2; MAP3K14; MAPK8; RIPK1; CASP8; DAXX; TNFRSF10B; RELA; TRAF2; TNF; IKBKG; RELB; CASP9; CHUK; APAF1; NFKB1; CASP2; BIRC2; CASP3; BIRC3
FGF Signaling	RAC1; FGFR1; MET; MAPKAPK2; MAPK1; PTPN11; AKT2; PIK3CA; CREB1; PIK3CB; PIK3C3; MAPK8; MAPK3; MAPK13; PTPN6; PIK3C2A; MAPK14; RAF1; AKT1; PIK3R1; STAT3; MAP2K1; FGFR4; CRKL; ATF4; AKT3; PRKCA; HGF
GM-CSF Signaling	LYN; ELK1; MAPK1; PTPN11; AKT2; PIK3CA; CAMK2A; STAT5B; PIK3CB; PIK3C3; GNB2L1; BCL2L1; MAPK3;

CELLULAR FUNCTION	GENES
	ETS1; KRAS; RUNX1; PIM1; PIK3C2A; RAF1; MAP2K2; AKT1; JAK2; PIK3R1; STAT3; MAP2K1; CCND1; AKT3; STAT1
Amyotrophic Lateral Sclerosis Signaling	BID; IGF1; RAC1; BIRC4; PGF; CAPNS1; CAPN2; PIK3CA; BCL2; PIK3CB; PIK3C3; BCL2L1; CAPN1; PIK3C2A; TP53; CASP9; PIK3R1; RAB5A; CASP1; APAF1; VEGFA; BIRC2; BAX; AKT3; CASP3; BIRC3
JAK/Stat Signaling	PTPN1; MAPK1; PTPN11; AKT2; PIK3CA; STAT5B; PIK3CB; PIK3C3; MAPK3; KRAS; SOCS1; STAT5A; PTPN6; PIK3C2A; RAF1; CDKN1A; MAP2K2; JAK1; AKT1; JAK2; PIK3R1; STAT3; MAP2K1; FRAP1; AKT3; STAT1
Nicotinate and Nicotinamide Metabolism	PRKCE; IRAK1; PRKAA2; EIF2AK2; GRK6; MAPK1; PLK1; AKT2; CDK8; MAPK8; MAPK3; PRKCD; PRKAA1; PBEF1; MAPK9; CDK2; PIM1; DYRK1A; MAP2K2; MAP2K1; PAK3; NT5E; TTK; CSNK1A1; BRAF; SGK
Chemokine Signaling	CXCR4; ROCK2; MAPK1; PTK2; FOS; CFL1; GNAQ; CAMK2A; CXCL12; MAPK8; MAPK3; KRAS; MAPK13; RHOA; CCR3; SRC; PPP1CC; MAPK14; NOX1; RAF1; MAP2K2; MAP2K1; JUN; CCL2; PRKCA
IL-2 Signaling	ELK1; MAPK1; PTPN11; AKT2; PIK3CA; SYK; FOS; STAT5B; PIK3CB; PIK3C3; MAPK8; MAPK3; KRAS; SOCS1; STAT5A; PIK3C2A; LCK; RAF1; MAP2K2; JAK1; AKT1; PIK3R1; MAP2K1; JUN; AKT3:
Synaptic Long Term Depression	PRKCE; IGF1; PRKCZ; PRDX6; LYN; MAPK1; GNAS; PRKCI; GNAQ; PPP2R1A; IGF1R; PRKD1; MAPK3; KRAS; GRN; PRKCD; NOS3; NOS2A; PPP2CA; YWHAZ; RAF1; MAP2K2; PPP2R5C; MAP2K1; PRKCA;
Estrogen Receptor Signaling	TAF4B; EP300; CARM1; PCAF; MAPK1; NCOR2; SMARCA4; MAPK3; NRIP1; KRAS; SRC; NR3C1; HDAC3; PPARGC1A; RBM9; NCOA3; RAF1; CREBBP; MAP2K2; NCOA2; MAP2K1; PRKDC; ESR1; ESR2
Protein Ubiquitination Pathway	TRAF6; SMURF1; BIRC4; BRCA1; UCHL1; NEDD4; CBL; UBE2I; BTRC; HSPA5; USP7; USP10; FBW7; USP9X;

CELLULAR FUNCTION	GENES
	STUB1; USP22; B2M; BIRC2; PARK2; USP8; USP1; VHL; HSP90AA1; BIRC3
IL-10 Signaling	TRAF6; CCR1; ELK1; IKBKB; SP1; FOS; NFKB2; MAP3K14; MAPK8; MAPK13; RELA; MAPK14; TNF; IKBKG; RELB; MAP3K7; JAK1; CHUK; STAT3; NFKB1; JUN; IL1R1; IL6
VDR/RXR Activation	PRKCE; EP300; PRKCZ; RXRA; GADD45A; HES1; NCOR2; SP1; PRKC1; CDKN1B ; PRKD1; PRKCD; RUNX2; KLF4; YY1; NCOA3; CDKN1A; NCOA2; SPP1; LRP5; CEBPB; FOXO1; PRKCA
TGF-beta Signaling	EP300; SMAD2; SMURF1; MAPK1; SMAD3; SMAD1; FOS; MAPK8; MAPK3; KRAS; MAPK9; RUNX2; SERPINE1; RAF1; MAP3K7; CREBBP; MAP2K2; MAP2K1; TGFBR1; SMAD4; JUN; SMAD5
Toll-like Receptor Signaling	IRAK1; EIF2AK2; MYD88; TRAF6; PPARA; ELK1; IKBKB; FOS; NFKB2; MAP3K14; MAPK8; MAPK13; RELA; TLR4; MAPK14; IKBKG; RELB; MAP3K7; CHUK; NFKB1; TLR2; JUN
p38 MAPK Signaling	HSPB1; IRAK1; TRAF6; MAPKAPK2; ELK1; FADD; FAS; CREB1; DDIT3; RPS6KA4; DAXX; MAPK13; TRAF2; MAPK14; TNF; MAP3K7; TGFBR1; MYC; ATF4; IL1R1; SRF; STAT1
Neurotrophin/TRK Signaling	NTRK2; MAPK1; PTPN11; PIK3CA; CREB1; FOS; PIK3CB; PIK3C3; MAPK8; MAPK3; KRAS; PIK3C2A; RAF1; MAP2K2; AKT1; PIK3R1; PDPK1; MAP2K1; CDC42; JUN; ATF4
FXR/RXR Activation	INS; PPARA; FASN; RXRA; AKT2; SDC1; MAPK8; APOB; MAPK10; PPARG; MTTP; MAPK9; PPARGC1A; TNF; CREBBP; AKT1; SREBF1; FGFR4; AKT3; FOXO1
Synaptic Long-Term Potentiation	PRKCE; RAP1A; EP300; PRKCZ; MAPK1; CREB1; PRKCI; GNAQ; CAMK2A; PRKD1; MAPK3; KRAS; PRKCD; PPP1CC; RAF1; CREBBP; MAP2K2; MAP2K1; ATF4; PRKCA

CELLULAR FUNCTION	GENES
Calcium Signaling	RAP1A; EP300; HDAC4; MAPK1; HDAC5; CREB1; CAMK2A; MYH9; MAPK3; HDAC2; HDAC7A; HDAC11; HDAC9; HDAC3; CREBBP; CALR; CAMKK2; ATF4; HDAC6
EGF Signaling	ELK1; MAPK1; EGFR; PIK3CA; FOS; PIK3CB; PIK3C3; MAPK8; MAPK3; PIK3C2A; RAF1; JAK1; PIK3R1; STAT3; MAP2K1; JUN; PRKCA; SRF; STAT1
Hypoxia Signaling in the Cardiovascular System	EDN1; PTEN; EP300; NQO1; UBE2I; CREB1; ARNT; HIF1A; SLC2A4; NOS3; TP53; LDHA; AKT1; ATM; VEGFA; JUN; ATF4; VHL; HSP90AA1
LPS/IL-1 Mediated Inhibition of RXR	IRAK1; MYD88; TRAF6; PPARA; RXRA; ABCA1, MAPK8; ALDH1A1; GSTP1; MAPK9; ABCB1; TRAF2;
Function LXR/RXR Activation	TLR4; TNF; MAP3K7; NR1H2; SREBF1; JUN; IL1R1 FASN; RXRA; NCOR2; ABCA1; NFKB2; IRF3; RELA; NOS2A; TLR4; TNF; RELB; LDLR; NR1H2; NFKB1; SREBF1; IL1R1; CCL2; IL6; MMP9
Amyloid Processing	PRKCE; CSNK1E; MAPK1; CAPNS1; AKT2; CAPN2; CAPN1; MAPK3; MAPK13; MAPT; MAPK14; AKT1; PSEN1; CSNK1A1; GSK3B; AKT3; APP
IL-4 Signaling	AKT2; PIK3CA; PIK3CB; PIK3C3; IRS1; KRAS; SOCS1; PTPN6; NR3C1; PIK3C2A; JAK1; AKT1; JAK2; PIK3R1; FRAP1; AKT3; RP S6KB1
Cell Cycle: G2/M DNA Damage Checkpoint Regulation	EP300; PCAF; BRCA1; GADD45A; PLK1; BTRC; CHEK1; ATR; CHEK2; YWHAZ; TP53; CDKN1A; PRKDC; ATM; SFN; CDKN2A
Nitric Oxide Signaling in the Cardiovascular System	KDR; FLT1; PGF; AKT2; PIK3CA; PIK3CB; PIK3C3; CAV1; PRKCD; NOS3; PIK3C2A; AKT1; PIK3R1; VEGFA; AKT3; HSP90AA1
Purine Metabolism	NME2; SMARCA4; MYH9; RRM2; ADAR; EIF2AK4; PKM2; ENTPD1; RAD51; RRM2B; TJP2; RAD51C; NT5E; POLD1; NME1
cAMP-mediated Signaling	RAP1A; MAPK1; GNAS; CREB1; CAMK2A; MAPK3; SRC; RAF1; MAP2K2; STAT3; MAP2K1; BRAF; ATF4

CELLULAR FUNCTION	GENES
Mitochondrial Dysfunction	SOD2; MAPK8; CASP8; MAPK10; MAPK9; CASP9; PARK7; PSEN1; PARK2; APP; CASP3
Notch Signaling	HES1; JAG1; NUMB; NOTCH4; ADAM17; NOTCH2; PSEN1; NOTCH3; NOTCH1; DLL4
Endoplasmic Reticulum Stress Pathway	HSPA5; MAPK8; XBP1; TRAF2; ATF6; CASP9; ATF4; EIF2AK3; CASP3
Pyrimidine Metabolism	NME2; AICDA; RRM2; EIF2AK4; ENTPD1; RRM2B; NT5E; POLD1; NME1
Parkinson's Signaling	UCHL1; MAPK8; MAPK13; MAPK14; CASP9; PARK7; PARK2; CASP3
Cardiac & Beta Adrenergic Signaling	GNAS; GNAQ; PPP2R1A; GNB2L1; PPP2CA; PPP1CC; PPP2R5C
Glycolysis/ Gluconeogenesis	HK2; GCK; GPI; ALDH1A1; PKM2; LDHA; HK1
Interferon Signaling	IRF1; SOCS1; JAK1; JAK2; IFITM1; STAT1; IFIT3
Sonic Hedgehog Signaling	ARRB2; SMO; GLI2; DYRK1A; GLI1; GSK3B; DYRKIB
Glycerophospholipid Metabolism	PLD1; GRN; GPAM; YWHAZ; SPHK1; SPHK2
Phospholipid Degradation	PRDX6; PLD1; GRN; YWHAZ; SPHK1; SPHK2
Tryptophan Metabolism	SIAH2; PRMT5; NEDD4; ALDH1A1; CYP1B1; SIAH1
Lysine Degradation	SUV39H1; EHMT2; NSD1; SETD7; PPP2R5C
Nucleotide Excision Repair Pathway	ERCC5; ERCC4; XPA; XPC; ERCC1
Starch and Sucrose Metabolism	UCHL1; HK2; GCK; GPI; HK1
Aminosugars Metabolism	NQO1; HK2; GCK; HK1

CELLULAR FUNCTION	GENES
Arachidonic Acid Metabolism	PRDX6; GRN; YWHAZ; CYP1B1
Circadian Rhythm Signaling	CSNK1E; CREB1; ATF4; NR1D1
Coagulation System	BDKRB1; F2R; SERPINE1; F3
Dopamine Receptor Signaling	PPP2R1A; PPP2CA; PPP1CC; PPP2R5C
Glutathione Metabolism	IDH2; GSTP1; ANPEP; IDH1
Glycerolipid Metabolism	ALDH1A1; GPAM; SPHK1; SPHK2
Linoleic Acid Metabolism	PRDX6; GRN; YWHAZ; CYP1B1
Methionine Metabolism	DNMT1; DNWIT3B; AHCY; DNWIT3A
Pyruvate Metabolism	GLO1; ALDH1A1; PKM2; LDHA
Arginine and Proline Metabolism	ALDH1A1; NOS3; NOS2A
Eicosanoid Signaling	PRDX6; GRN; YWHAZ
Fructose and Mannose Metabolism	HK2; GCK; HK1
Galactose Metabolism	HK2; GCK; HK1
Stilbene, Coumarine and Lignin Biosynthesis	PRDX6; PRDX1; TYR
Lignin Biosynthesis Antigen Presentation Pathway	CALR; B2M

CELLULAR FUNCTION	GENES
Biosynthesis of Steroids	NQO1; DHCR7
Butanoate Metabolism	ALDH1A1; NLGN1
Citrate Cycle	IDH2; IDH1
Fatty Acid Metabolism	ALDH1A1; CYP1B1
Glycerophospholipid Metabolism	PRDX6; CHKA
Histidine Metabolism	PRMT5; ALDH1A1
Inositol Metabolism	ERO1L; APEX1
Metabolism of Xenobiotics by Cytochrome p450	GSTP1; CYP1B1
Methane Metabolism	PRDX6; PRDX1
Phenylalanine Metabolism	PRDX6; PRDX1
Propanoate Metabolism	ALDH1A1; LDHA
Selenoamino Acid Metabolism	PRMT5; AHCY
Sphingolipid Metabolism	SPHK1; SPHK2
Aminophosphonate Metabolism	PRMT5
Androgen and Estrogen Metabolism	PRMT5
Ascorbate and Aldarate Metabolism	ALDH1A1

CELLULAR FUNCTION	GENES
Bile Acid Biosynthesis	ALDH1A1
Cysteine Metabolism	LDHA
Fatty Acid Biosynthesis	FASN
Glutamate Receptor Signaling	GNB2L1
NRF2-mediated Oxidative Stress Response	PRDX1
Pentose Phosphate Pathway	GPI
Pentose and Glucuronate Interconversions	UCHL1
Retinol Metabolism	ALDH1A1
Riboflavin Metabolism	TYR
Tyrosine Metabolism	PRMT5, TYR
Ubiquinone Biosynthesis	PRMT5
Valine, Leucine and Isoleucine Degradation	ALDH1A1
Glycine, Serine and Threonine Metabolism	CHKA
Lysine Degradation	ALDH1A1
Pain/Taste	TRPM5; TRPA1

CELLULAR FUNCTION	GENES
Pain	TRPM7; TRPCS; TRPC6; TRPC1; Cnr1; cnr2; Grk2; Trpa1; Pomc; Cgrp; Crf; Pka; Era; Nr2b; TRPM5; Prkaca; Prkacb; Prkar1a; Prkar2a
Mitochondrial Function	AIF; CytC; SMAC (Diablo); Aifm-1; Aifm-2
Developmental Neurology	BMP-4; Chordin (Chrd); Noggin (Nog); WNT (Wnt2; Wnt2b; Wnt3a; Wnt4; Wnt5a; Wnt6; Wnt7b; Wnt8b; Wnt9a; Wnt9b; Wnt10a; Wnt10b; Wnt16); beta-catenin; Dkk-1; Frizzled related proteins; Otx-2; Gbx2; FGF-8; Reelin; Dab1; unc-86 (Pou4fl or Brn3a); Numb; Rein

[0293] ***F. Epigenetic modification or gene editing***

[0294] The actuator moiety as disclosed herein can be operatively coupled to at least one functional moiety. In some cases, the actuator moiety can be fused to at least one functional moiety, to form a fusion moiety. In some cases, the actuator moiety can comprise a first coupling moiety (e.g., a polynucleotide) and the at least one functional moiety can comprise a second coupling moiety (e.g., a second polynucleotide having complementarity to the first polynucleotide), such that the actuator moiety and the at least one functional moiety can be coupled to one another. In some cases, the at least one functional moiety can be an epigenetic modifier, as disclosed herein, to effect epigenetic modification of DNA (e.g., chromosomal DNA of any of the engineered cell as disclosed herein), to effect, e.g., modification of an expression profile of a target gene in a cell.

[0295] Non-limiting examples of a function of the at least one functional moiety as disclosed herein can include methyltransferase activity, demethylase activity, dismutase activity, alkylation activity, depurination activity, oxidation activity, pyrimidine dimer forming activity, integrase activity, transposase activity, recombinase activity, polymerase activity, ligase activity, helicase activity, photolyase activity or glycosylase activity, acetyltransferase activity, deacetylase activity, kinase activity, phosphatase activity, ubiquitin ligase activity, deubiquitinating activity, adenylation activity, deadenylation activity, SUMOylating activity, deSUMOylating activity, ribosylation activity, deribosylation activity, myristoylation activity, remodeling activity, protease activity, oxidoreductase activity, transferase activity, hydrolase activity, lyase activity, isomerase activity, synthase activity, synthetase activity, and demyristoylation activity. For example, a fusion protein can be a fusion in a Cas protein and an functional moiety to induce epigenetic modification (e.g., methylation, demethylation, acetylation, deacetylation, etc.).

[0296] Non-limiting examples of the at least one functional moiety as disclosed herein can include methyltransferase, demethylase, dismutase, alkylation enzyme, depurination enzyme, oxidation enzyme, pyrimidine dimer forming enzyme, integrase, transposase, recombinase, polymerase, ligase, helicase,

photolyase or glycosylase, acetyltransferase, deacetylase, kinase, phosphatase, ubiquitin ligase, deubiquitinating enzyme, adenylation enzyme, deadenylation enzyme, SUMOylating enzyme, deSUMOylating enzyme, ribosylation enzyme, deribosylation enzyme, myristoylation enzyme, remodeling enzyme, protease, oxidoreductase, transferase, hydrolase, lyase, isomerase, synthase, synthetase, and demyristoylation enzyme.

[0297] In some cases, the at least one functional moiety as disclosed herein can be an epigenetic modifier. The epigenetic modifier can comprise one or more members selected from the group consisting of a chromatin remodeling protein, chromatin reader protein, nuclear protein, transcription factor protein, mediator (or coactivator) protein, DNA binding protein, RNA binding protein, DNA methylation protein, histone acetylation protein, histone methylation protein, a peptide domain fragment thereof, and a modification thereof. Non-limiting examples of the epigenetic modifier are provided herein. In some cases, the modification can comprise at least one mutation relative to a wild type epigenetic modifier.

[0298] Non-limiting examples of an epigenetic modifier can include ACTL6A, ACTL6B, ACTR5, ACTR8, ARID1A, ARID1B, ARID2, ASH1L, ATRX, CAR,1, CDY1. CDYL, CHD1, CHD2, CHD3, CHD4, CHD5, CHD6, CHD7, CHD8, CHRAC1, CREBBP, DAXX, DNMT1, DNMT3A, DNMT3B, DOTL1, EHMT1, EHMT2, ELP3, EP300, EZH1, EZH2, FBXO10, GTF3C4, HAT1, HDAC1, HDAC2, HDAC3, HDAC4, HDAC5, HDAC6, HDAC7, HDAC8, HDAC9, HDAC10, HDAC11, HIF1AN, HINFP, HR, HSPBAP1, ING1, ING2, ING3, ING4, ING5, INO80, INO80B, INO80C, INO80D, INO80E, JARID2, JMJD1C, JMJD4, JMJD5, JMJD6, JMJD7, JMJD8, KAT2A, KAT2B, KAT5, KAT6A, KAT6B, KAT7, KAT8, KDM1A, KDM1B, KDM2A, KDM2B, KDM3A, KDM4A, KDM4B, KDM4C, KDM4D, KDM5A, KDM5B, KDM5C, KDM5D, KDM6A, KDM6B, KDM7A, KDM8, KMT2A, KMT2B, KMT2C, KMT2D, KMT2E, MBD1, MBD2, MBD3, MBD4, MCRS1, MECEP2, MGEA5, MINA, MLL1, MLL2, MLL3, MLL4, MTA1, MTA2, MTA3, NAT10, NCOA1, NCOA3, KFRKB, NSD1, NSD2, PBRM1, PHF2, PHF8, PRDM2, PRDM9, PRMT1, PRMT2, PRMT3, PRMT5, PRMT6, PRMT7, PRMT8, PRMT9, RBBP4, RBBP7, RING1, RUVBL1, RUVBL2, SETD1A, SETD1B, SETD2, SETD3, SETD4, SETD5, SETD6, SETD7, SETD8, SETD9, SETDB1, SETDB2, SETMAR, SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, SIRT7, SMARCA1, SMARCA4, SMARCA5, SMARCB1, SMARCC1, SMARCC2, SMARCD1, SMARCD2, SMARCD3, SMARCE1, SMYD1. SMYD2, SMYD3, SMYD4, SMYD5, SUV39H1, SUV39H2, SUV420H1, SUV420H2, TAF1, TET1, TET2, TET3, TFPT, UCHL5, UTY, WHSC1, WHSC1L1, YY1, functional variants thereof, fragments thereof, and combinations thereof.

[0299] In some cases, the epigenetic modifier as disclosed herein can comprise a chromatin remodeling protein comprising one or more members selected from the group consisting of SMARCB1, SMARCA4, PBRM1, ARID1A, ARID1B, ARID2, SMARCD1, SMARCE1, ATRX, DAXX, and CHD (e.g., CHD1, CHD2, CHD3, CHD4, CHD5, CHD6, CHD7, CHD8).

[0300] In some cases, the epigenetic modifier as disclosed herein can comprise a DNA methylation protein comprising one or more members selected from the group consisting of DNMT3A, DNMT1, TET

(e.g., TET1, TET2, TET3), MBD1, and MBD4.

[0301] In some cases, the epigenetic modifier as disclosed herein can comprise a histone acetylation protein comprising one or more members selected from the group consisting of EP300, CREBBP, HDAC2, HDAC4, and HDAC9.

[0302] In some cases, the epigenetic modifier as disclosed herein can comprise a histone methylation protein comprising one or more members selected from the group consisting of MLL (e.g., MLL1, MLL2, MLL3, MLL4), SETD1A, PRDM9, EZH2, NSD1, NSD2, SETD2, KDM5C, KDM6A, and KDM2B.

[0303] In some cases, the epigenetic modifier as disclosed herein can be less than or equal to about 200, less than or equal to about 180, less than or equal to about 160, less than or equal to about 150, less than or equal to about 140, less than or equal to about 130, less than or equal to about 120, less than or equal to about 110, less than or equal to about 100, less than or equal to about 90, less than or equal to about 80, less than or equal to about 70, less than or equal to about 60, or less than or equal to about 50 amino acids in lengths. In an example, the epigenetic modifier can be less than or equal to about 100 amino acids in length.

[0304] In some cases, the epigenetic modifier as disclosed herein can be about 10 amino acids in length to about 1,000 amino acids in length. In some cases, the epigenetic modifier can be at least about 10 amino acids in length. In some cases, the epigenetic modifier can be at most about 1,000 amino acids in length. In some cases, the epigenetic modifier can be about 10 amino acids in length to about 50 amino acids in length, about 10 amino acids in length to about 100 amino acids in length, about 10 amino acids in length to about 200 amino acids in length, about 10 amino acids in length to about 300 amino acids in length, about 10 amino acids in length to about 400 amino acids in length, about 10 amino acids in length to about 500 amino acids in length, about 10 amino acids in length to about 600 amino acids in length, about 10 amino acids in length to about 700 amino acids in length, about 10 amino acids in length to about 800 amino acids in length, about 10 amino acids in length to about 1,000 amino acids in length, about 50 amino acids in length to about 100 amino acids in length, about 50 amino acids in length to about 200 amino acids in length, about 50 amino acids in length to about 300 amino acids in length, about 50 amino acids in length to about 400 amino acids in length, about 50 amino acids in length to about 500 amino acids in length, about 50 amino acids in length to about 600 amino acids in length, about 50 amino acids in length to about 700 amino acids in length, about 50 amino acids in length to about 800 amino acids in length, about 50 amino acids in length to about 1,000 amino acids in length, about 100 amino acids in length to about 200 amino acids in length, about 100 amino acids in length to about 300 amino acids in length, about 100 amino acids in length to about 400 amino acids in length, about 100 amino acids in length to about 500 amino acids in length, about 100 amino acids in length to about 600 amino acids in length, about 100 amino acids in length to about 700 amino acids in length, about 100 amino acids in length to about 800 amino acids in length, about 100 amino acids in length to about 1,000 amino acids in length, about 200 amino acids in length to about 300 amino acids in length, about 200 amino acids in length to about 400 amino acids in length, about 200 amino acids in length to about 500 amino

acids in length, about 200 amino acids in length to about 600 amino acids in length, about 200 amino acids in length to about 700 amino acids in length, about 200 amino acids in length to about 800 amino acids in length, about 200 amino acids in length to about 1,000 amino acids in length, about 300 amino acids in length to about 400 amino acids in length, about 300 amino acids in length to about 500 amino acids in length, about 300 amino acids in length to about 600 amino acids in length, about 300 amino acids in length to about 700 amino acids in length, about 300 amino acids in length to about 800 amino acids in length, about 300 amino acids in length to about 1,000 amino acids in length, about 400 amino acids in length to about 500 amino acids in length, about 400 amino acids in length to about 600 amino acids in length, about 400 amino acids in length to about 700 amino acids in length, about 400 amino acids in length to about 800 amino acids in length, about 400 amino acids in length to about 1,000 amino acids in length, about 500 amino acids in length to about 600 amino acids in length, about 500 amino acids in length to about 700 amino acids in length, about 500 amino acids in length to about 800 amino acids in length, about 500 amino acids in length to about 1,000 amino acids in length, about 600 amino acids in length to about 700 amino acids in length, about 600 amino acids in length to about 800 amino acids in length, about 600 amino acids in length to about 1,000 amino acids in length, about 700 amino acids in length to about 800 amino acids in length, about 700 amino acids in length to about 1,000 amino acids in length, or about 800 amino acids in length to about 1,000 amino acids in length. In some cases, the epigenetic modifier can be about 10 amino acids in length, about 50 amino acids in length, about 100 amino acids in length, about 200 amino acids in length, about 300 amino acids in length, about 400 amino acids in length, about 500 amino acids in length, about 600 amino acids in length, about 700 amino acids in length, about 800 amino acids in length, or about 1,000 amino acids in length.

[0305] In some cases, the epigenetic modifier as disclosed herein can be a synthetic polypeptide.

[0306] The actuator moiety as disclosed herein can comprise a nuclease, such as an endonuclease (e.g., Cas). The endonuclease can be heterologous to any of the cells disclosed herein.

[0307] The actuator moiety as disclosed herein can comprise a Cas endonuclease, zinc finger nuclease (ZFN), zinc finger associate gene regulation polypeptides, transcription activator-like effector nuclease (TALEN), transcription activator-like effector associated gene regulation polypeptides, meganuclease, natural master transcription factors, epigenetic modifying enzymes, recombinase, flippase, transposase, RNA-binding proteins (RBP), an Argonaute protein, any derivative thereof, any variant thereof, or any fragment thereof. In some embodiments, the actuator moiety comprises a Cas protein, and the system further comprises a guide RNA (gRNA) which complexes with the Cas protein. In some embodiments, the actuator moiety comprises an RBP complexed with a gRNA which is able to form a complex with a Cas protein. In some embodiments, the gRNA comprises a targeting segment which exhibits at least 80% sequence identity to a target polynucleotide. In some embodiments, the Cas protein substantially lacks DNA cleavage activity (i.e., dead Cas, deactivated Cas, or dCas). For example, the Cas protein is mutated and/or modified yo yield a nuclease deficient protein or a protein with decreased nuclease activity relative to a wild-type Cas protein. A nuclease deficient protein can retain the ability to

bind DNA, but may lack or have reduced nucleic acid cleavage activity.

[0308] In some cases, a suitable actuator moiety comprises CRISPR-associated (Cas) proteins or Cas nucleases including type I CRISPR-associated (Cas) polypeptides, type II CRISPR-associated (Cas) polypeptides, type III CRISPR-associated (Cas) polypeptides, type IV CRISPR-associated (Cas) polypeptides, type V CRISPR-associated (Cas) polypeptides, and type VI CRISPR-associated (Cas) polypeptides; zinc finger nucleases (ZFN); transcription activator-like effector nucleases (TALEN); meganucleases; RNA-binding proteins (RBP); CRISPR-associated RNA binding proteins; recombinases; flippases; transposases; Argonaute (Ago) proteins (e.g., prokaryotic Argonaute (pAgo), archaeal Argonaute (aAgo), and eukaryotic Argonaute (eAgo)); any derivative thereof, any variant thereof; and any fragment thereof.

[0309] A Cas protein referred to herein can be a type of protein or polypeptide. A Cas protein can refer to a nuclease. A Cas protein can refer to an endoribonuclease. A Cas protein can refer to any modified (e.g., shortened, mutated, lengthened) polypeptide sequence or homologue of the Cas protein. A Cas protein can be codon optimized. A Cas protein can be a codon-optimized homologue of a Cas protein. A Cas protein can be enzymatically inactive, partially active, constitutively active, fully active, inducible active and/or more active, (e.g. more than the wild type homologue of the protein or polypeptide.). A Cas protein can be Cas9. A Cas protein can be Cpf1. A Cas protein can be C2c2. A Cas protein (e.g., variant, mutated, enzymatically inactive and/or conditionally enzymatically inactive site-directed polypeptide) can bind to a target nucleic acid. A Cas protein (e.g., variant, mutated, enzymatically inactive and/or conditionally enzymatically inactive endoribonuclease) can bind to a target RNA or DNA.

[0310] Non-limiting examples of Cas proteins include c2c1, C2c2, c2c3, Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas5e (CasD), Cas6, Cas6e, Cas6f, Cas7, Cas8a, Cas8a1, Cas8a2, Cas8b, Cas8c, Cas9 (Csn1 or Csx12), Cas10, Cas10d, Cas1O, Cas1Od, CasF, CasG, CasH, Cpf1, Csy1, Csy2, Csy3, Cse1 (CasA), Cse2 (CasB), Cse3 (CasE), Cse4 (CasC), Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx1O, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, and Cul966, and homologs or modified versions thereof.

[0311] In some cases, a nuclease disclosed herein (e.g., Cas) can be a nucleic acid-guided nuclease (e.g., an RNA guided endonuclease). The term "guide nucleic acid" generally refers to a nucleic acid that can hybridize to another nucleic acid. A guide nucleic acid can be RNA. A guide nucleic acid can be DNA. The guide nucleic acid can be programmed to bind to a sequence of nucleic acid site-specifically. The nucleic acid to be targeted, or the target nucleic acid, can comprise nucleotides. The guide nucleic acid can comprise nucleotides. A portion of the target nucleic acid can be complementary to a portion of the guide nucleic acid. The strand of a double-stranded target polynucleotide that is complementary to and hybridizes with the guide nucleic acid can be called the complementary strand. The strand of the double-stranded target polynucleotide that is complementary to the complementary strand, and therefore may not be complementary to the guide nucleic acid can be called noncomplementary strand. A guide nucleic acid can comprise a polynucleotide chain and can be called a "single guide nucleic acid." A guide nucleic acid

can comprise two polynucleotide chains and can be called a "double guide nucleic acid." If not otherwise specified, the term "guide nucleic acid" can be inclusive, referring to both single guide nucleic acids and double guide nucleic acids.

[0312] A guide nucleic acid can comprise a segment that can be referred to as a "nucleic acid-targeting segment" or a "nucleic acid-targeting sequence." A nucleic acid-targeting segment can comprise a sub-segment that can be referred to as a "protein binding segment" or "protein binding sequence" or "Cas protein binding segment."

[0313] A guide nucleic acid can comprise two separate nucleic acid molecules, which can be referred to as a double guide nucleic acid. A guide nucleic acid can comprise a single nucleic acid molecule, which can be referred to as a single guide nucleic acid (e.g., sgRNA). In some cases, the guide nucleic acid is a single guide nucleic acid comprising a fused CRISPR RNA (crRNA) and a transactivating crRNA (tracrRNA). In some cases, the guide nucleic acid is a single guide nucleic acid comprising a crRNA. In some cases, the guide nucleic acid is a single guide nucleic acid comprising a crRNA but lacking a tracrRNA. In some cases, the guide nucleic acid is a double guide nucleic acid comprising non-fused crRNA and tracrRNA. An exemplary double guide nucleic acid can comprise a crRNA-like molecule and a tracrRNA-like molecule. An exemplary single guide nucleic acid can comprise a crRNA-like molecule. An exemplary single guide nucleic acid can comprise a fused crRNA-like and tracrRNA-like molecules.

[0314] The term "crRNA," as used herein, generally refers to a nucleic acid with at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% sequence identity and/or sequence similarity to a wild type exemplary crRNA (e.g., a crRNA from S. pyogenes). crRNA can generally refer to a nucleic acid with at most about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% sequence identity and/or sequence similarity to a wild type exemplary crRNA (e.g., a crRNA from S. pyogenes). crRNA can refer to a modified form of a crRNA that can comprise a nucleotide change such as a deletion, insertion, or substitution, variant, mutation, or chimera. A crRNA can be a nucleic acid having at least about 60% sequence identity to a wild type exemplary crRNA (e.g., a crRNA from S. pyogenes) sequence over a stretch of at least 6 contiguous nucleotides. For example, a crRNA sequence can be at least about 60% identical, at least about 65% identical, at least about 70% identical, at least about 75% identical, at least about 80% identical, at least about 85% identical, at least about 90% identical, at least about 95% identical, at least about 98% identical, at least about 99% identical, or 100% identical to a wild type exemplary crRNA sequence (e.g., a crRNA from S. pyogenes) over a stretch of at least 6 contiguous nucleotides.

[0315] The term "tracrRNA," as used herein, generally refers to a nucleic acid with at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% sequence identity and/or sequence similarity to a wild type exemplary tracrRNA sequence (e.g., a tracrRNA from S. pyogenes). tracrRNA can refer to a nucleic acid with at most about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% sequence identity and/or sequence similarity to a wild type exemplary tracrRNA sequence (e.g., a tracrRNA from S. pyogenes). tracrRNA can refer to a modified form of a tracrRNA that can comprise a

nucleotide change such as a deletion, insertion, or substitution, variant, mutation, or chimera. A tracrRNA can refer to a nucleic acid that can be at least about 60% identical to a wild type exemplary tracrRNA (e.g., a tracrRNA from S. pyogenes) sequence over a stretch of at least 6 contiguous nucleotides. For example, a tracrRNA sequence can be at least about 60% identical, at least about 65% identical, at least about 70% identical, at least about 75% identical, at least about 80% identical, at least about 85% identical, at least about 90% identical, at least about 95% identical, at least about 98% identical, at least about 99% identical, or 100% identical to a wild type exemplary tracrRNA (e.g., a tracrRNA from S. pyogenes) sequence over a stretch of at least 6 contiguous nucleotides.

[0316] A crRNA can comprise the nucleic acid-targeting segment (e.g., spacer region) of the guide nucleic acid and a stretch of nucleotides that can form one half of a double-stranded duplex of the Cas protein-binding segment of the guide nucleic acid.

[0317] A tracrRNA can comprise a stretch of nucleotides that forms the other half of the double-stranded duplex of the Cas protein-binding segment of the gRNA. A stretch of nucleotides of a crRNA can be complementary to and hybridize with a stretch of nucleotides of a tracrRNA to form the double-stranded duplex of the Cas protein-binding domain of the guide nucleic acid.

[0318] The crRNA and tracrRNA can hybridize to form a guide nucleic acid. The crRNA can also provide a single-stranded nucleic acid targeting segment (e.g., a spacer region) that hybridizes to a target nucleic acid recognition sequence (e.g., protospacer). The sequence of a crRNA, including spacer region, or tracrRNA molecule can be designed to be specific to the species in which the guide nucleic acid is to be used.

[0319] RNA or DNA viral based systems can be used to deliver one or more genes that encode the actuator moiety, with or without the additional functional moiety (e.g., epigenetic modifier), to the cell of the present disclosure, to engineer the cell. Viral vectors can be used to treat cells in vitro, and the modified cells can optionally be administered (ex vivo). Alternatively, viral vectors can be administered directly (in vivo) to the subject. Viral based systems can include retroviral, lentivirus, adenoviral, adeno-associated and herpes simplex virus vectors for gene transfer. Integration in the host genome can occur with the retrovirus, lentivirus, and adeno-associated virus gene transfer methods, which can result in long term expression of the inserted transgene.

[0320] In some cases, non-viral delivery methods can be used to deliver (i) the actuator moiety, with or without the additional functional moiety (e.g., epigenetic modifier) or (ii) one or more genes encoding thereof to the cell of the present disclosure, to engineer the cell. Methods of non-viral delivery of such cargo can include lipofection, nucleofection, microinjection, biolistics, virosomes, liposomes, immunoliposomes, exosomes, polycation or lipid:cargo conjugates (or aggregates), naked polypeptide (e.g., recombinant polypeptides), naked DNA, artificial virions, and agent-enhanced uptake of polypeptide or DNA. Cationic and neutral lipids that are suitable for efficient receptor-recognition lipo-delivery of polynucleotides or polypeptides can be used.

[0321] In some examples, any of the heterologous polypeptide comprising the actuator moiety (e.g.,

dCas-epigenetic modifier fusion polypeptide and sgRNA polynucleotide complex, i.e. dCas-sgRNA ribonucleoprotein (RNP)) can be delivered to the cell via any of the non-viral delivery methods disclosed herein. In another example, mRNA or DNA encoding such heterologous polypeptide comprising the actuator moiety can be delivered to the cell via any of the non-viral delivery methods disclosed herein.

[0322] ***G. Scaffolds***

[0323] In some aspects, any subject (i) heterologous polypeptides and/or cells (e.g., modified cells) or (ii) compositions comprising thereof, as disclosed herein, can be administered to the subject in need thereof via a scaffold. The (i) heterologous polypeptides and/or cells or (ii) composition comprising thereof can be incorporated with the scaffold. The scaffold can serve as a carrier or delivery vehicle. The scaffold can be a solid (e.g., a porous collagen scaffold), semi-solid, liquid, gel (e.g., hyaluronic acid hydrogel), etc. For example, the scaffold can comprise structures (e.g., fibrous structures, meshes, woven structures, non-woven structures, knitted structures, three-dimensional woven structures) for growth, repair, or regeneration of at least a portion of a bodily tissue (e.g., ingrowth of blood vessels and cells for bone generation). The scaffold can comprise polymers, e.g., natural polymers or synthetic materials. The polymers can be biocompatible and/or biodegradable.

[0324] Non-limiting examples of suitable biocompatible, biodegradable synthetic polymers can include aliphatic polyesters, poly(amino acids), copoly(ether-esters), polyalkylenes oxalates, polyamides, poly(iminocarbonates), polyorthoesters, polyoxaesters, polyamidoesters, polyoxaesters containing amine groups, and poly(anhydrides). Such synthetic polymers can be homopolymers or copolymers (e.g., random, block, segmented, graft) of a plurality of different monomers, e.g., two or more of lactic acid, lactide, glycolic acid, glycolide, epsilon-caprolactone, trimethylene carbonate, p-dioxanone, etc. In an example, the scaffold can be comprised of a polymer comprising glycolic acid and lactic acid, such as those with a ratio of glycolic acid to lactic acid of 90/10 or 5/95.

[0325] Non-limiting examples of naturally occurring biocompatible, biodegradable polymers can include glycoproteins, proteoglycans, polysaccharides, glycosamineoglycan (GAG) and fragment(s) derived from these components, elastin, laminins, decrorin, fibrinogen/fibrin, fibronectins, osteopontin, tenascins, hyaluronic acid, collagen, chondroitin sulfate, heparin, heparan sulfate, ORC, carboxymethyl cellulose, and chitin.

[0326] ***H. Therapeutic applications***

[0327] As disclosed herein, non-limiting examples of the bodily tissue can comprise one or more members selected from the group consisting of blood, plasma, serum, urine, perilymph fluid, feces, saliva, semen, amniotic fluid, cerebrospinal fluid, bile, sweat, tears, sputum, synovial fluid, vomit, bone, heart, thymus, artery, blood vessel, lung, muscle, stomach, intestine, liver, pancreas, spleen, kidney, gall bladder, thyroid gland, adrenal gland, mammary gland, ovary, prostate gland, testicle, skin, adipose, eye, brain, infected tissue, diseased tissue, malignant tissue, calcified tissue, and healthy tissue.

[0328] The heterologous polypeptides or modified cells of the present disclosure can be used (e.g., administered) to treat a subject in need thereof. The subject can have or can be suspected of having a

condition, such as a disease (e.g., cancer, tumor, tissue degeneration, fibrosis, tissue damage from trauma or aging, etc.). For example, a cell (e.g., a stem cell or a committed cell) can be obtained from the subject, and such cell can be cultured ex vivo and modified in accordance with any of the methods disclosed herein. Subsequently, the modified cell can be administered to the subject for treatment of the condition.

[0329] The subject can be treated (e.g., administered with) a population of the modified cells (e.g., engineered adult-derived stem cells, such as MSCs or iPSCs) of the present disclosure for at least or up to about 1 dose, at least or up to about 2 doses, at least or up to about 3 doses, at least or up to about 4 doses, at least or up to about 5 doses, at least or up to about 6 doses, at least or up to about 7 doses, at least or up to about 8 doses, at least or up to about 9 doses, or at least or up to about 10 doses.

[0330] The subject can be treated (e.g., administered with) the heterologous polypeptide (e.g., comprising an actuator moiety fused to an epigenetic modifier) or a heterologous gene encoding thereof, as disclosed herein, for at least or up to about 1 dose, at least or up to about 2 doses, at least or up to about 3 doses, at least or up to about 4 doses, at least or up to about 5 doses, at least or up to about 6 doses, at least or up to about 7 doses, at least or up to about 8 doses, at least or up to about 9 doses, or at least or up to about 10 doses.

[0331] Any one of the methods disclosed herein can be utilized to treat a target cell, a target tissue (i.e., a bodily tissue), a target condition, or a target disease of a subject.

[0332] A target disease can be a viral, bacterial, and/or parasitic infection; inflammatory and/or autoimmune disease; neoplasm such as a cancer and/or tumor; injuries due to accidents or trauma; or degeneration of a bodily. Non-limiting examples of such diseases can include infection by a bacteria, including, for example, disease, such as, infections of the upper respiratory tract (e.g., otitis media, bacterial tracheitis, acute epiglottitis, thyroiditis), lower respiratory (e.g., empyema, lung abscess), cardiac (e.g., infective endocarditis), gastrointestinal (e.g., secretory diarrhoea, splenic absces, retroperitoneal abscess), CNS (e.g., cerebral abscess), eye (e.g., blepharitis, conjunctivitis, keratitis, endophthalmitis, preseptal and orbital cellulitis, darcryocystitis), kidney and urinary tract (e.g., epididymitis, intrarenal and perinephric absces, toxic shock syndrome), skin (e.g., impetigo, folliculitis, cutaneous abscesses, cellulitis, wound infection, bacterial myositis) bone and joint (e.g., broken bones, arthritis, septic arthritis, osteomyelitis, spinal disc damage).

[0333] A target cell can be isolated from subject when subjected to any of the methods disclosed herein. Alternatively, a target cell can be found in a bodily tissue within a subject when subjected to any of the methods disclosed herein. A target cell can be a diseased cell. A diseased cell can have altered metabolic, gene expression, and/or morphologic features. A diseased cell can be a cancer cell, a diabetic cell, and an apoptotic cell. A diseased cell can be a cell from a diseased subject. Exemplary diseases can include blood disorders, cancers, metabolic disorders, eye disorders, organ disorders, musculoskeletal disorders, cardiac disease, and the like.

[0334] A target cell can include a wide variety of cell types. A target cell can be in vitro. A target cell can be in vivo. A target cell can be ex vivo. A target cell can be an isolated cell. A target cell can be a cell

inside of an organism. A target cell can be an organism. A target cell can be a cell in a cell culture. A target cell can be one of a collection of cells. A target cell can be a mammalian cell or derived from a mammalian cell. A target cell can be a rodent cell or derived from a rodent cell. A target cell can be a human cell or derived from a human cell. A target cell can be a prokaryotic cell or derived from a prokaryotic cell. A target cell can be a bacterial cell or can be derived from a bacterial cell. A target cell can be an archaeal cell or derived from an archaeal cell. A target cell can be a eukaryotic cell or derived from a eukaryotic cell. A target cell can be a pluripotent stem cell. A target cell can be a plant cell or derived from a plant cell. A target cell can be an animal cell or derived from an animal cell. A target cell can be an invertebrate cell or derived from an invertebrate cell. A target cell can be a vertebrate cell or derived from a vertebrate cell. A target cell can be a microbe cell or derived from a microbe cell. A target cell can be a fungi cell or derived from a fungi cell. A target cell can be from a specific organ or tissue.

[0335] A target cell can be a stem cell or progenitor cell. Target cells can include stem cells (e.g., MSCs.) and progenitor cells (e.g., cardiac progenitor cells, neural progenitor cells, etc.). Target cells can include mammalian stem cells and progenitor cells, including rodent stem cells, rodent progenitor cells, human stem cells, human progenitor cells, etc. Clonal cells can comprise the progeny of a cell. A target cell can comprise a target nucleic acid. A target cell can be in a living organism. A target cell can be a genetically modified cell. A target cell can be a host cell.

[0336] A target cell can be a totipotent stem cell, however, in some embodiments of this disclosure, the term "cell" may be used but may not refer to a totipotent stem cell. A target cell can be a plant cell, but in some embodiments of this disclosure, the term "cell" may be used but may not refer to a plant cell. A target cell can be a pluripotent cell. For example, a target cell can be a pluripotent hematopoietic cell that can differentiate into other cells in the hematopoietic cell lineage but may not be able to differentiate into any other non-hematopoietic cell. A target cell may be able to develop into a whole organism. A target cell may or may not be able to develop into a whole organism. A target cell may be a whole organism.

[0337] A target cell can be a primary cell. For example, cultures of primary cells can be passaged 0 times, 1 time, 2 times, 4 times, 5 times, 10 times, 15 times or more. Cells can be unicellular organisms. Cells can be grown in culture.

[0338] If the target cells are primary cells, they may be harvested from an individual by any method. For example, leukocytes may be harvested by apheresis, leukocytapheresis, density gradient separation, etc. Cells from tissues such as skin, muscle, bone marrow, spleen, liver, pancreas, lung, intestine, stomach, etc. can be harvested by biopsy. An appropriate solution may be used for dispersion or suspension of the harvested cells. Such solution can generally be a balanced salt solution, (e.g. normal saline, phosphate-buffered saline (PBS), Hank's balanced salt solution, etc.), conveniently supplemented with fetal calf serum or other naturally occurring factors, in conjunction with an acceptable buffer at low concentration. Buffers can include HEPES, phosphate buffers, lactate buffers, etc. Cells may be used immediately, or they may be stored (e.g., by freezing). Frozen cells can be thawed and can be capable of being reused. Cells can be frozen in a DMSO, serum, medium buffer (e.g., 10% DMSO, 50% serum, 40%

buffered medium), and/or some other such common solution used to preserve cells at freezing temperatures.

[0339] Non-limiting examples of cells which can be target cells include, but are not limited to, lymphoid cells, such as B cell, T cell (Cytotoxic T cell, Natural Killer T cell, Regulatory T cell, T helper cell), Natural killer cell, cytokine induced killer (CIK) cells; myeloid cells, such as granulocytes (Basophil granulocyte, Eosinophil granulocyte, Neutrophil granulocyte/Hypersegmented neutrophil), Monocyte/Macrophage, Red blood cell (Reticulocyte), Mast cell, Thrombocyte/Megakaryocyte, Dendritic cell; cells from the endocrine system, including thyroid (Thyroid epithelial cell, Parafollicular cell), parathyroid (Parathyroid chief cell, Oxyphil cell), adrenal (Chromaffin cell), pineal (Pinealocyte) cells; cells of the nervous system, including glial cells (Astrocyte, Microglia), Magnocellular neurosecretory cell, Stellate cell, Boettcher cell, and pituitary (Gonadotrope, Corticotrope, Thyrotrope, Somatotrope, Lactotroph); cells of the Respiratory system, including Pneumocyte (Type I pneumocyte, Type II pneumocyte), Clara cell, Goblet cell, Dust cell; cells of the circulatory system, including Myocardiocyte, Pericyte; cells of the digestive system, including stomach (Gastric chief cell, Parietal cell), Goblet cell, Paneth cell, G cells, D cells, ECL cells, I cells, K cells, S cells; enteroendocrine cells, including enterochromaffm cell, APUD cell, liver (Hepatocyte, Kupffer cell), Cartilage/bone/muscle; bone cells, including Osteoblast, Osteocyte, Osteoclast, teeth (Cementoblast, Ameloblast); cartilage cells, including Chondroblast, Chondrocyte; skin cells, including Trichocyte, Keratinocyte, Melanocyte (Nevus cell); muscle cells, including Myocyte; urinary system cells, including Podocyte, Juxtaglomerular cell, Intraglomerular mesangial cell/Extraglomerular mesangial cell, Kidney proximal tubule brush border cell, Macula densa cell; reproductive system cells, including Spermatozoon, Sertoli cell, Leydig cell, Ovum; and other cells, including Adipocyte, Fibroblast, Tendon cell, Epidermal keratinocyte (differentiating epidermal cell), Epidermal basal cell (stem cell), Keratinocyte of fingernails and toenails, Nail bed basal cell (stem cell), Medullary hair shaft cell, Cortical hair shaft cell, Cuticular hair shaft cell, Cuticular hair root sheath cell, Hair root sheath cell of Huxley's layer, Hair root sheath cell of Henle's layer, External hair root sheath cell, Hair matrix cell (stem cell), Wet stratified barrier epithelial cells, Surface epithelial cell of stratified squamous epithelium of cornea, tongue, oral cavity, esophagus, anal canal, distal urethra and vagina, basal cell (stem cell) of epithelia of cornea, tongue, oral cavity, esophagus, anal canal, distal urethra and vagina, Urinary epithelium cell (lining urinary bladder and urinary ducts), Exocrine secretory epithelial cells, Salivary gland mucous cell (polysaccharide-rich secretion), Salivary gland serous cell (glycoprotein enzyme-rich secretion), Von Ebner's gland cell in tongue (washes taste buds), Mammary gland cell (milk secretion), Lacrimal gland cell (tear secretion), Ceruminous gland cell in ear (wax secretion), Eccrine sweat gland dark cell (glycoprotein secretion), Eccrine sweat gland clear cell (small molecule secretion). Apocrine sweat gland cell (odoriferous secretion, sex-hormone sensitive), Gland of Moll cell in eyelid (specialized sweat gland), Sebaceous gland cell (lipid-rich sebum secretion), Bowman's gland cell in nose (washes olfactory epithelium), Brunner's gland cell in duodenum (enzymes and alkaline mucus), Seminal vesicle cell (secretes seminal fluid components, including fructose for swimming

sperm), Prostate gland cell (secretes seminal fluid components), Bulbourethral gland cell (mucus secretion), Bartholin's gland cell (vaginal lubricant secretion), Gland of Littre cell (mucus secretion), Uterus endometrium cell (carbohydrate secretion), Isolated goblet cell of respiratory and digestive tracts (mucus secretion), Stomach lining mucous cell (mucus secretion), Gastric gland zymogenic cell (pepsinogen secretion), Gastric gland oxyntic cell (hydrochloric acid secretion), Pancreatic acinar cell (bicarbonate and digestive enzyme secretion), Paneth cell of small intestine (lysozyme secretion), Type II pneumocyte of lung (surfactant secretion), Clara cell of lung, Hormone secreting cells, Anterior pituitary cells, Somatotropes, Lactotropes, Thyrotropes, Gonadotropes, Corticotropes, Intermediate pituitary cell, Magnocellular neurosecretory cells, Gut and respiratory tract cells, Thyroid gland cells, thyroid epithelial cell, parafollicular cell, Parathyroid gland cells, Parathyroid chief cell, Oxyphil cell, Adrenal gland cells, chromaffin cells, Ley dig cell of testes, Theca interna cell of ovarian follicle, Corpus luteum cell of ruptured ovarian follicle, Granulosa lutein cells, Theca lutein cells, Juxtaglomerular cell (renin secretion), Macula densa cell of kidney, Metabolism and storage cells, Barrier function cells (Lung, Gut, Exocrine Glands and Urogenital Tract), Kidney, Type I pneumocyte (lining air space of lung), Pancreatic duct cell (centroacinar cell), Nonstriated duct cell (of sweat gland, salivary gland, mammary gland, etc.), Duct cell (of seminal vesicle, prostate gland, etc.), Epithelial cells lining closed internal body cavities, Ciliated cells with propulsive function, Extracellular matrix secretion cells, Contractile cells; Skeletal muscle cells, stem cell, Heart muscle cells, Blood and immune system cells, Erythrocyte (red blood cell), Megakaryocyte (platelet precursor), Monocyte, Connective tissue macrophage (various types), Epidermal Langerhans cell, Osteoclast (in bone), Dendritic cell (in lymphoid tissues), Microglial cell (in central nervous system), Neutrophil granulocyte, Eosinophil granulocyte, Basophil granulocyte, Mast cell, Helper T cell, Suppressor T cell, Cytotoxic T cell, Natural Killer T cell, B cell, Natural killer cell, Reticulocyte, Stem cells and committed progenitors for the blood and immune system (various types), Pluripotent stem cells, Totipotent stem cells, Induced pluripotent stem cells, adult stem cells, Sensory transducer cells, Autonomic neuron cells, Sense organ and peripheral neuron supporting cells, Central nervous system neurons and glial cells, Lens cells, Pigment cells, Melanocyte, Retinal pigmented epithelial cell, Germ cells, Oogonium/Oocyte, Spermatid, Spermatocyte, Spermatogonium cell (stem cell for spermatocyte), Spermatozoon, Nurse cells, Ovarian follicle cell, Sertoli cell (in testis), Thymus epithelial cell, Interstitial cells, and Interstitial kidney cells.

[0340] In some embodiments, any of the heterologous polypeptide, heterologous gene encoding the heterologous polypeptide, or modified cell, as disclosed herein, can promote healing of a diseased or damaged bodily tissue of a subject. In some cases, an area of volume of a new portion of the bodily tissue formed upon treatment by the heterologous polypeptide, heterologous gene encoding the heterologous polypeptide, or modified cell, as disclosed herein, can be greater than that upon a control treatment by at least or up to about 0.1-fold, at least or up to about 0.2-fold, at least or up to about 0.3-fold, at least or up to about 0.4-fold, at least or up to about 0.5-fold, at least or up to about 0.6-fold, at least or up to about 0.7-fold, at least or up to about 0.8-fold, at least or up to about 0.9-fold, at least or up to about 1-fold, at

least or up to about 2-fold, at least or up to about 3-fold, at least or up to about 4-fold, at least or up to about 5-fold, at least or up to about 6-fold, at least or up to about 7-fold, at least or up to about 8-fold, at least or up to about 9-fold, at least or up to about 10-fold, at least or up to about 20-fold, at least or up to about 30-fold, at least or up to about 40-fold, at least or up to about 50-fold, at least or up to about 60-fold, at least or up to about 70-fold, at least or up to about 80-fold, at least or up to about 90-fold, or at least or up to about 100-fold. In some cases, a rate of growth of a new portion of the bodily tissue upon treatment by the heterologous polypeptide, heterologous gene encoding the heterologous polypeptide, or modified cell, as disclosed herein, can be greater than that upon a control treatment by at least or up to about 0.1-fold, at least or up to about 0.2-fold, at least or up to about 0.3-fold, at least or up to about 0.4-fold, at least or up to about 0.5-fold, at least or up to about 0.6-fold, at least or up to about 0.7-fold, at least or up to about 0.8-fold, at least or up to about 0.9-fold, at least or up to about 1-fold, at least or up to about 2-fold, at least or up to about 3-fold, at least or up to about 4-fold, at least or up to about 5-fold, at least or up to about 6-fold, at least or up to about 7-fold, at least or up to about 8-fold, at least or up to about 9-fold, at least or up to about 10-fold, at least or up to about 20-fold, at least or up to about 30-fold, at least or up to about 40-fold, at least or up to about 50-fold, at least or up to about 60-fold, at least or up to about 70-fold, at least or up to about 80-fold, at least or up to about 90-fold, or at least or up to about 100-fold.

[0341] In some embodiments, any of the heterologous polypeptide, heterologous gene encoding the heterologous polypeptide, or modified cell, as disclosed herein, can enhance function of a bodily tissue of a subject. In some cases, a function of the bodily tissue upon treatment by the heterologous polypeptide, heterologous gene encoding the heterologous polypeptide, or modified cell, as disclosed herein, can be greater than that upon a control treatment by at least or up to about 0.1-fold, at least or up to about 0.2-fold, at least or up to about 0.3-fold, at least or up to about 0.4-fold, at least or up to about 0.5-fold, at least or up to about 0.6-fold, at least or up to about 0.7-fold, at least or up to about 0.8-fold, at least or up to about 0.9-fold, at least or up to about 1-fold, at least or up to about 2-fold, at least or up to about 3-fold, at least or up to about 4-fold, at least or up to about 5-fold, at least or up to about 6-fold, at least or up to about 7-fold, at least or up to about 8-fold, at least or up to about 9-fold, at least or up to about 10-fold, at least or up to about 20-fold, at least or up to about 30-fold, at least or up to about 40-fold, at least or up to about 50-fold, at least or up to about 60-fold, at least or up to about 70-fold, at least or up to about 80-fold, at least or up to about 90-fold, or at least or up to about 100-fold.

[0342] In some embodiments, any of the heterologous polypeptide, heterologous gene encoding the heterologous polypeptide, or modified cell, as disclosed herein, can be used to treat a subject having a metabolic disorder. Non-limiting examples of the metabolic disease can include cystic fibrosis, phenylketonuria (PKU), diabetes (e.g., type II diabetes), insulin resistance syndrome, metabolic syndrome (i.e., dysmetabolic syndrome X), hyperlipidemia, gout, rickets, and obesity. In some cases, subjects having a metabolic disorder can exhibit an imbalance in one or more enzyme expression profiles (e.g., enzyme deficiency). Thus, the heterologous polypeptide or heterologous gene encoding the heterologous polypeptide can be used to treat target cells relevant to the metabolic disorder (e.g., islet cells for diabetes)

to artificially modulate and control the expression profile of the appropriate enzymes (e.g., insulate for diabetes). In addition, variations of the heterologous polypeptide or heterologous gene encoding the heterologous polypeptide can be used to reversibly turn on and turn off the enzyme expression, to reduce adverse side effects.

[0343] In some embodiments, any of the heterologous polypeptide, heterologous gene encoding the heterologous polypeptide, or modified cell, as disclosed herein, can be used to enhance adoptive cell therapies (e.g., cellular immunotherapy) for a subject having or is suspected of having a cancer. Autologous or allogeneic immune cells (e.g., T cells, NK cells) can be engineered with enhanced function (e.g., with modified T-cell receptors to recognize a target antigen, or a chimeric antigen receptor (CAR) to recognize a specific target antigen) can be administered to the subject for cellular immunotherapy.

[0344] In some cases, it may be useful to temporarily modulate expression of one or more cytokines in the therapeutic immune cells. Thus, the immune cells can be treated with any of the heterologous polypeptide or the heterologous gene encoding the heterologous polypeptide, in vitro and/or in vivo, to reversibly turn on and off secretion of proteins (e.g., cytokines) that (i) can be beneficial during the initial stage of the cellular immunotherapy but (ii) can elicit side effects or drawbacks if secreted for a prolonged period of time.

[0345] In some cases, it may be useful to maintain cellular fate (e.g., to remain as a particular T cell type, such as central memory T (TCM) cells) for optimal cellular immunotherapy. Thus, the immune cells can be treated with any of the heterologous polypeptide or the heterologous gene encoding the heterologous polypeptide, in vitro and/or in vivo, to modulate expression profile of one or more transcription factors to maintain the cellular fate (e.g., suppression of one or more transcription factors that promotes transition from TCM cells to memory T cells (TEM).

[0346] Non-limiting examples of cancer can include Acanthoma, Acinic cell carcinoma, Acoustic neuroma, Acral lentiginous melanoma, Acrospiroma, Acute eosinophilic leukemia, Acute lymphoblastic leukemia, Acute megakaryoblastic leukemia, Acute monocytic leukemia, Acute myeloblastic leukemia with maturation, Acute myeloid dendritic cell leukemia, Acute myeloid leukemia, Acute promyelocytic leukemia, Adamantinoma, Adenocarcinoma, Adenoid cystic carcinoma, Adenoma, Adenomatoid odontogenic tumor, Adrenocortical carcinoma, Adult T-cell leukemia, Aggressive NK-cell leukemia, AIDS-Related Cancers, AIDS-related lymphoma, Alveolar soft part sarcoma, Ameloblastic fibroma, Anal cancer, Anaplastic large cell lymphoma, Anaplastic thyroid cancer, Angioimmunoblastic T-cell lymphoma, Angiomyolipoma, Angiosarcoma, Appendix cancer, Astrocytoma, Atypical teratoid rhabdoid tumor, Basal cell carcinoma, Basal-like carcinoma, B-cell leukemia, B-cell lymphoma, Bellini duct carcinoma, Biliary tract cancer, Bladder cancer, Blastoma, Bone Cancer, Bone tumor, Brain Stem Glioma, Brain Tumor, Breast Cancer, Brenner tumor, Bronchial Tumor, Bronchioloalveolar carcinoma, Brown tumor, Burkitt's lymphoma, Cancer of Unknown Primary Site, Carcinoid Tumor, Carcinoma, Carcinoma in situ, Carcinoma of the penis, Carcinoma of Unknown Primary Site, Carcinosarcoma, Castleman's Disease, Central Nervous System Embryonal Tumor, Cerebellar Astrocytoma, Cerebral Astrocytoma,

Cervical Cancer, Cholangiocarcinoma, Chondroma, Chondrosarcoma, Chordoma, Choriocarcinoma, Choroid plexus papilloma, Chronic Lymphocytic Leukemia, Chronic monocytic leukemia, Chronic myelogenous leukemia, Chronic Myeloproliferative Disorder, Chronic neutrophilic leukemia, Clear-cell tumor, Colon Cancer, Colorectal cancer, Craniopharyngioma, Cutaneous T-cell lymphoma, Degos disease, Dermatofibrosarcoma protuberans, Dermoid cyst, Desmoplastic small round cell tumor, Diffuse large B cell lymphoma, Dysembryoplastic neuroepithelial tumor, Embryonal carcinoma, Endodermal sinus tumor, Endometrial cancer, Endometrial Uterine Cancer, Endometrioid tumor, Enteropathy-associated T-cell lymphoma, Ependymoblastoma, Ependymoma, Epithelioid sarcoma, Erythroleukemia, Esophageal cancer, Esthesioneuroblastoma, Ewing Family of Tumor, Ewing Family Sarcoma, Ewing's sarcoma, Extracranial Germ Cell Tumor, Extragonadal Germ Cell Tumor, Extrahepatic Bile Duct Cancer, Extramammary Paget's disease, Fallopian tube cancer, Fetus in fetu, Fibroma, Fibrosarcoma, Follicular lymphoma, Follicular thyroid cancer, Gallbladder Cancer, Gallbladder cancer, Ganglioglioma, Ganglioneuroma, Gastric Cancer, Gastric lymphoma, Gastrointestinal cancer, Gastrointestinal Carcinoid Tumor, Gastrointestinal Stromal Tumor, Gastrointestinal stromal tumor, Germ cell tumor, Germinoma, Gestational choriocarcinoma, Gestational Trophoblastic Tumor, Giant cell tumor of bone, Glioblastoma multiforme, Glioma, Gliomatosis cerebri, Glomus tumor, Glucagonoma, Gonadoblastoma, Granulosa cell tumor, Hairy Cell Leukemia, Hairy cell leukemia, Head and Neck Cancer, Head and neck cancer, Heart cancer, Hemangioblastoma, Hemangiopericytoma, Hemangiosarcoma, Hematological malignancy, Hepatocellular carcinoma, Hepatosplenic T-cell lymphoma, Hereditary breast-ovarian cancer syndrome, Hodgkin Lymphoma, Hodgkin's lymphoma, Hypopharyngeal Cancer, Hypothalamic Glioma, Inflammatory breast cancer, Intraocular Melanoma, Islet cell carcinoma, Islet Cell Tumor, Juvenile myelomonocytic leukemia, Kaposi Sarcoma, Kaposi's sarcoma, Kidney Cancer, Klatskin tumor, Krukenberg tumor, Laryngeal Cancer, Laryngeal cancer, Lentigo maligna melanoma, Leukemia, Leukemia, Lip and Oral Cavity Cancer, Liposarcoma, Lung cancer, Luteoma, Lymphangioma, Lymphangiosarcoma, Lymphoepithelioma, Lymphoid leukemia, Lymphoma, Macroglobulinemia, Malignant Fibrous Histiocytoma, Malignant fibrous histiocytoma, Malignant Fibrous Histiocytoma of Bone, Malignant Glioma, Malignant Mesothelioma, Malignant peripheral nerve sheath tumor, Malignant rhabdoid tumor, Malignant triton tumor, MALT lymphoma, Mantle cell lymphoma, Mast cell leukemia, Mediastinal germ cell tumor, Mediastinal tumor, Medullary thyroid cancer, Medulloblastoma, Medulloblastoma, Medulloepithelioma, Melanoma, Melanoma, Meningioma, Merkel Cell Carcinoma, Mesothelioma, Mesothelioma, Metastatic Squamous Neck Cancer with Occult Primary, Metastatic urothelial carcinoma, Mixed Mullerian tumor, Monocytic leukemia, Mouth Cancer, Mucinous tumor, Multiple Endocrine Neoplasia Syndrome, Multiple Myeloma, Multiple myeloma, Mycosis Fungoides, Mycosis fungoides, Myelodysplastic Disease, Myelodysplastic Syndromes, Myeloid leukemia, Myeloid sarcoma, Myeloproliferative Disease, Myxoma, Nasal Cavity Cancer, Nasopharyngeal Cancer, Nasopharyngeal carcinoma, Neoplasm, Neurinoma, Neuroblastoma, Neuroblastoma, Neurofibroma, Neuroma, Nodular melanoma, Non-Hodgkin Lymphoma, Non-Hodgkin lymphoma, Nonmelanoma Skin

Cancer, Non-Small Cell Lung Cancer, Ocular oncology, Oligoastrocytoma, Oligodendroglioma, Oncocytoma, Optic nerve sheath meningioma, Oral Cancer, Oral cancer, Oropharyngeal Cancer, Osteosarcoma, Osteosarcoma, Ovarian Cancer, Ovarian cancer, Ovarian Epithelial Cancer, Ovarian Germ Cell Tumor, Ovarian Low Malignant Potential Tumor, Paget's disease of the breast, Pancoast tumor, Pancreatic Cancer, Pancreatic cancer, Papillary thyroid cancer, Papillomatosis, Paraganglioma, Paranasal Sinus Cancer, Parathyroid Cancer, Penile Cancer, Perivascular epithelioid cell tumor, Pharyngeal Cancer, Pheochromocytoma, Pineal Parenchymal Tumor of Intermediate Differentiation, Pineoblastoma, Pituicytoma, Pituitary adenoma, Pituitary tumor, Plasma Cell Neoplasm, Pleuropulmonary blastoma, Polyembryoma, Precursor T-lymphoblastic lymphoma, Primary central nervous system lymphoma, Primary effusion lymphoma, Primary Hepatocellular Cancer, Primary Liver Cancer, Primary peritoneal cancer, Primitive neuroectodermal tumor, Prostate cancer, Pseudomyxoma peritonei, Rectal Cancer, Renal cell carcinoma, Respiratory Tract Carcinoma Involving the NUT Gene on Chromosome 15, Retinoblastoma, Rhabdomyoma, Rhabdomyosarcoma, Richter's transformation, Sacrococcygeal teratoma, Salivary Gland Cancer, Sarcoma, Schwannomatosis, Sebaceous gland carcinoma, Secondary neoplasm, Seminoma, Serous tumor, Sertoli-Leydig cell tumor, Sex cord-stromal tumor, Sezary Syndrome, Signet ring cell carcinoma, Skin Cancer, Small blue round cell tumor, Small cell carcinoma, Small Cell Lung Cancer, Small cell lymphoma, Small intestine cancer, Soft tissue sarcoma, Somatostatinoma, Soot wart, Spinal Cord Tumor, Spinal tumor, Splenic marginal zone lymphoma, Squamous cell carcinoma, Stomach cancer, Superficial spreading melanoma, Supratentorial Primitive Neuroectodermal Tumor, Surface epithelial-stromal tumor, Synovial sarcoma, T-cell acute lymphoblastic leukemia, T-cell large granular lymphocyte leukemia, T-cell leukemia, T-cell lymphoma, T-cell prolymphocytic leukemia, Teratoma, Terminal lymphatic cancer, Testicular cancer, Thecoma, Throat Cancer, Thymic Carcinoma, Thymoma, Thyroid cancer, Transitional Cell Cancer of Renal Pelvis and Ureter, Transitional cell carcinoma, Urachal cancer, Urethral cancer, Urogenital neoplasm, Uterine sarcoma, Uveal melanoma, Vaginal Cancer, Verner Morrison syndrome, Verrucous carcinoma, Visual Pathway Glioma, Vulvar Cancer, Waldenstrom's macroglobulinemia, Warthin's tumor, Wilms' tumor, and combinations thereof.

EXAMPLES

[0347] Example 1: Enhanced differentiation of stem cells

[0348] For enhanced tissue repair or regeneration, stem cells (e.g., iPSCs or MSCs) can be modified or engineered by treating with a heterologous polypeptide comprising an actuator moiety, as disclosed herein, to promote differentiation of the stem cells towards a desired or targeted lineage. The actuator moiety can be fused with an epigenetic modifier, such that the epigenetic modifier can induce epigenetic modification in a target polynucleotide of the cell. The target polynucleotide can be operatively coupled to a gene encoding a specific transcription factor of the cell, such that the epigenetic modification of the target polynucleotide modifies expression profile of the specific transcription factor, thereby promoting the differentiation of the stem cells. In some cases, the methods and systems for epigenetic modification

as disclosed herein can reduce or even eliminate the need for the use of ectopic or exogenous agents (e.g., small molecules or growth factors) typically needed for promoting such differentiation.

[0349] *Muscle repair/regeneration:*

[0350] Stem cells (e.g, iPSCs, MSCs, MuSCs) are contacted by at least one heterologous polypeptide comprising an actuator moiety linked to an epigenetic modifier, as disclosed herein, to be differentiated to a skeletal muscle phenotype. The epigenetic modifier can induce an epigenetic modification of a target polynucleotide, to enhance (e.g., permanently or transiently) an expression level of a terminal transcription factor MyoD. In some examples, the epigenetic modification can be sufficient to induce differentiation into a skeletal muscle phenotype without one or more of the growth factors comprising LIF; Bmp; Fgf; Activin; or TGF.

[0351] *Spinal cord repair/regeneration:*

[0352] Stem cells (e.g., iPSCs, MSCs) are contacted by at least one heterologous polypeptide comprising an actuator moiety linked to an epigenetic modifier, as disclosed herein, to be differentiated to a spinal motor neuron phenotype. The epigenetic modifier can induce an epigenetic modification of a target polynucleotide, to enhance (e.g., permanently or transiently) an expression level of a terminal transcription factor comprising Ngn2; Isll; or Lhx3. In some examples, the epigenetic modification can be sufficient to induce differentiation into a spinal motor neuron phenotype without one or more of the ectopic agents comprising LIF; BMP; FGF; Activin; or TGF.

[0353] *Heart tissue repair/regeneration:*

[0354] Stem cells (e.g., iPSCs, MSCs) are contacted by at least one heterologous polypeptide comprising an actuator moiety linked to an epigenetic modifier, as disclosed herein, to be differentiated to a cardiomyocyte phenotype. The epigenetic modifier can induce an epigenetic modification of a target polynucleotide, to enhance (e.g., permanently or transiently) an expression level of a terminal transcription factor Gata5. In some examples, the epigenetic modification can be sufficient to induce differentiation into a cardiomyocyte phenotype without one or more of the ectopic agents comprising LIF; BMP; FGF; Activin; or TGF.

[0355] *Liver repair/regeneration:*

[0356] Stem cells (e.g., iPSCs, MSCs) are contacted by at least one heterologous polypeptide comprising an actuator moiety linked to an epigenetic modifier, as disclosed herein, to be differentiated to a hepatocyte or hepatoblast phenotype. The epigenetic modifier can induce an epigenetic modification of a target polynucleotide, to enhance (e.g., permanently or transiently) an expression level of a terminal transcription factor comprising Hnf1α, Hnf4α, Hnf1β, FoxA2, or LRH-1. In some examples, the epigenetic modification can be sufficient to induce differentiation into a hepatocyte or hepatoblast phenotype without one or more of the ectopic agents comprising HGF; LIF; BMP; FGF; Activin; or TGF.

[0357] *Cartilage repair/regeneration:*

[0358] Stem cells (e.g., iPSCs, MSCs) are contacted by at least one heterologous polypeptide comprising an actuator moiety linked to an epigenetic modifier, as disclosed herein, to be differentiated to

a chondrocyte phenotype. The epigenetic modifier can induce an epigenetic modification of a target polynucleotide, to enhance (e.g., permanently or transiently) an expression level of a terminal transcription factor Sox9. In some examples, the epigenetic modification can be sufficient to induce differentiation into a chondrocyte phenotype without one or more of the ectopic agents comprising TGFβ1, TGFβ3, or dexamethasone.

[0359] *Bone repair/regeneration:*

[0360] Stem cells (e.g., iPSCs, MSCs) are contacted by at least one heterologous polypeptide comprising an actuator moiety linked to an epigenetic modifier, as disclosed herein, to be differentiated to an osteoblast phenotype. The epigenetic modifier can induce an epigenetic modification of a target polynucleotide, to enhance (e.g., permanently or transiently) an expression level of a terminal transcription factor Runx2. In some examples, the epigenetic modification can be sufficient to induce differentiation into an osteoblast phenotype without one or more of the growth factors comprising BMP-2, BMP-4, TGFβ1, TGFβ3, or dexamethasone.

[0361] *Tooth repair/regeneration:*

[0362] Stem cells (e.g., MSCs, dental pulp stem cells) can be contacted by at least one heterologous polypeptide comprising an actuator moiety linked to an epigenetic modifier, as disclosed herein, to be differentiated to an odontoblast phenotype. The epigenetic modifier can induce an epigenetic modification of a target polynucleotide, to enhance (e.g., permanently or transiently) an expression level of a terminal transcription factor comprising Sox2 or Klf5. In some examples, the epigenetic modification can be sufficient to induce differentiation into an odontoblast phenotype without one or more of the ectopic agents comprising GDF11 or NFG.

[0363] **Example 2: Skeletal repair**

[0364] *Modification of MSCs:*

[0365] An isolated adult-derived stem cell (e.g., MSC) can be treated ex vivo with a heterologous polypeptide comprising an actuator moiety fused with an epigenetic modifier, then administered or transplanted to a subject to promote enhanced skeletal tissue regeneration (e.g., bone, cartilage, ligament, muscles, etc.).

[0366] In some cases, (i) mRNA encoding dCas9-epigenetic modifier fusion protein or (ii) recombinant form of the dCas9-epigenetic modifier fusion protein, each together with synthetic sgRNA can be delivered into primary MSCs using cationic lipid nanoparticles (LNP). In an example, as illustrated in FIG. 2, MSCs are extracted and isolated from the fat tissue of the patients within about 4 to about 30 minutes of extraction of the fat tissue from the patients. Subsequently, the dCas9-epigenetic modifier fusion protein mRNA or the recombinant form of the dCas9-epigenetic modifier fusion protein, each with synthetic sgRNA, are transfected into the isolated MSCs using cationic LNP (e.g., within about 10 minutes of isolation of the MSCs). Following an incubation period (e.g., about 2-4 hours), the modified MSCs are injected back into the patient for tissue repair (e.g., bone damage repair, meniscus repair, etc.).

[0367] Alternatively, the dCas9-epigenetic modifier fusion protein mRNA or the recombinant form of the dCas9-epigenetic modifier fusion protein, each with synthetic sgRNA, are administered to a target bodily tissue of the subject (e.g., damaged/diseased bone, damaged/diseased cartilage, etc.) to interact with MSCs in vivo.

[0368] *Bone repair or regeneration:*

[0369] FIG. 3A illustrates Runx2 signaling pathway that is turned on during osteogenesis. Runx2 is a key transcription factor in osteogenesis. FIG. 3B illustrates role of Runx2 in promoting osteoblast/osteocyte differentiation from various mesenchymal stem cells. By transiently upregulating Runx2 (e.g., via use of the dCas9-epigenetic modifier fusion protein in accordance with any of the methods disclosed herein), differentiation of adipose-derived MSCs and/or bone marrow MSC into osteoblast/osteocytes can be induced, either ex vivo or in vivo.

[0370] In some cases, the dCas9-epigenetic modifier fusion protein and the sgRNA can be designed to activate positive endogenous regulators of osteogenesis, such as SP7/OSX, DLX5, BMPR1B, BMPR2, MSX2, DLX2, DLX3, DLX6, MSX1, ATF4, VDR, PDLIM7, ETS1, FOSL1, WNT9A, BMP2, BMP4, BMP6, BMP7, SMAD1, SMAD5, SMAD8, and/or IGF. In some cases, the dCas9-epigenetic modifier fusion protein and the sgRNA can be designed to suppress negative endogenous regulators of osteogenesis, such as PPARg, LIPE, DKK1, DKK2, TWIST1, SMURF1, TGFBR1, TGFBR2.

[0371] *In vitro osteoblast differentiation of MSCs:*

[0372] Primary MSCs were transfected, in vitro, with (i) mRNA encoding the dCas9-epigenetic modifier fusion protein in combination with (ii) sgRNA using cationic lipid nanoparticles (LNP), a resulting RNP complex capable of activating Runx2 expression. The employed sgRNA was designed to target the endogenous Runx2. As a control, a separate population of primary MSCs were transfected with exogenous mRNA encoding Runx2. Subsequently (e.g., after about 12 to about 48 hours), the transfection media was replenished with osteogenic medium (e.g., comprising ascorbic acid, β-glycerophosphate, and dexamethasone). The modified MSCs were further cultured for proliferation, matrix maturation, and mineralization (e.g., total of about 14 days), as illustrated in FIG. 4A. the resulting cell culture was imaged by alizarin red staining imaging (FIG. 4B) and bright field imaging (FIG. 4C), to visualize a presence of mineral forms in culture. A presence of minerals can be an indication of osteogenesis in culture. As shown in FIG. 4B, contacting the cell with the dCas9-epigenetic modifier fusion protein (e.g., dCas9-VPR-p300, dCas9-TET1, dCas9-VPR-P300 + TET1) complexed with the sgRNA induced comparable mineral formation as compared to the expression of heterologous Runx2 (Runx2 mRNA) control (FIG. 4B). The "+" in "dCas9-VPR-P300 + TET1" indicates that the corresponding cell(s) were co-transfected with two constructs: one beingVPR-P300, and the other being dCas9-TET1. Without wishing to be bound by a particular theory, fusing the P300 acetyltransferase and the VPR tripartite activator together allowed alteration of both epigenetics and transcription with enhanced activation and long-term control. Under brightfield imaging, a "white film" indicating mineral formation (or calcified extracellular matrix (ECM)) is present for the MSCs treated with the dCas9-

epigenetic modifier fusion protein (e.g., dCas9-VPR-p300, dCas9-TET1, dCas9-VPR-P300 + TET1) complexed with the sgRNA or the heterologous Runx2 (FIG. 4C).

[0373] *Runx2 mRNA level in MSCs:*

[0374] MSCs were transfected with (i) sgRNA and mRNA encoding the dCas9-epigenetic modifier fusion protein or (ii) Runx2 mRNA using LNPs, and the mRNA level of Runx2 in the MSCs were measured over time, e.g., via PCR. Treatment with epigenetic modification of Runx2 using the sgRNA-dCas-epigenetic modifier complex induced a transient modification of the endogenous Runx2 mRNA level in the MSCs (FIG. 5A). The modified expression profile of Runx2 mRNA exhibited a local maximum at about 24 hours, with about at least 12-fold increase in the Runx2 mRNA expression as compared to without the treatment. The peak expression of the Runx2 mRNA was subsequently reversed in part over the course of 3 days. In contrast, MSCs transfected with heterologous Runx2 mRNA exhibited about 800-fold increase in the expression level of Runx2 mRNA that did not include the heterologous Runx2 mRNA that was added to the cells. The PCR primers employed in FIG. 5B did not capture the heterologous Runx2 mRNA that the cells were transfected with. Even at 4 days post-transfection, the Runx2 mRNA expression level of the treated MSCs was higher than 800-fold as compared to control, without any hint of reversal of the modified expression profile. Without wishing to be bound by theory, the transient modification of expression profile of endogenous Runx2 that is induced by epigenetic modification as disclosed herein (as shown in FIG. 5A) can better mimic the natural expression profile of Runx2 during osteogenesis, as compared to that shown in FIG. 5B.

[0375] *Scaffold preparation*

[0376] The modified MSCs, as abovementioned, are combined with a porous collagen sponge/scaffold as a delivery vehicle. As illustrated in FIG. 6, the modified MSCs are grated onto the collagen sponge and cultured in vitro in appropriate media (e.g., growth media or osteogenic media) overnight. Subsequently, the collagen scaffold comprising the modified MSCs are implanted into the animal.

[0377] *In vivo bone regeneration:*

[0378] The modified MSCs are added to a collagen scaffold, as abovementioned, then administered to a site of interest to promote bone repair or regeneration (e.g., from trauma) or new bone formation (e.g., for spinal fusion).

[0379] In some cases, rat critical size femoral defect model is used to assess the ability of the modified MSCs to heat a bridge a defect gap, which would not be able to bridge without human intervention. Once under anesthesia, the animal's femur is exposed, and a 5 mm segmental defect is created using a high-speed oscillating saw. Subsequently, the proximal and distal segments of the femur is fixed by a titanium bone plate with screws. After flushing the fracture site with normal saline solution, a scaffold carrying the modified MSCs as disclosed herein is inserted in the femur defect to fill the gap. After closing the wound, progress of healing of the critical size femoral defect in the animals is imaged live (e.g., X-ray or CT scan) or after sacrifice (e.g., quantitative analysis based on micro-computed

tomography (μCT), histology).

[0380] In some cases, rat posterolateral lumbar intertransverse spinal fusion model is used to assess the ability of the modified MSCs to promote bilateral spinal fusion between L4 and L5 transverse processes, which would not occur without human intervention. Once under anesthesia, the animal's L4 and L5 transverse processes are exposed, followed by irrigation with sterile gentamicin/saline solution, and decortication of the superficial cortical layer with a high-speed burr. Subsequently, the collagen scaffold carrying the modified MSCs as disclosed herein is implanted bilaterally in the paraspinal musculature between the transverse processes. After closing the wound, progress of bilateral spinal fusion in the animals is imaged live (e.g., X-ray or CT scan) or after sacrifice (e.g., quantitative analysis based on μCT, histology). In addition, progress of bilateral spinal fusion in the animals can be assessed overtime via manual palpation and an established scoring system while the animals are alive.

[0381] **Example 3: Increased Expression of BMP6 in AD-MSCs**

[0382] In this example, CRISPRa was used to treat human AD-MSCs with a heterologous polypeptide comprising an actuator moiety as disclosed herein, to increase transcription of human bone morphogenic protein 6 (BMP6). Bone morphogenic proteins induced the growth of bone and cartilage. FIG. 7 shows qRT-PCR quantification of these stem cells over the seven days post-reprogramming that they were harvested. BMP6 transcription was greatly increased in reprogrammed AD-MSCs as compared to control AD-MSCs. This experiment showed that epigenetic modification of target polynucleotides can modify the expression profile of the corresponding target transcription factor, thereby promoting differentiation of stem cells.

[0383] **Example 4: Stem Cell Reprogramming of Osteoblasts *in vitro***

[0384] In this example, MSCs were reprogrammed by transient delivery of CRISPRa. After the cells differentiated into osteocyte-like cells, they were transplanted into mice in a spheroid format (FIG. 8).

[0385] After the MSCs were reprogrammed using RunX2, Osx, and DLx5 (ROD)-CRISPR reprogramming, osteogenesis was observed at 7 and 14 days (FIG. 9A-B). Calcium deposit accumulation over both one and two weeks increased by about four-fold and by about eight-fold, respectively, in reprogrammed MSCs as compared to the control. qRT-PCR relative quantification analysis of *COL11a1* and *SPP1* in micropheroid cultures harvested at 7 and 10 days post-reprogramming confirmed increased transcripts of those genes (FIG. 9C-D). Reprogrammed spheroids showed a six-fold increase in the level of *COL11a1* transcripts after one week, and reprogrammed shperoids showed a sustained 20-fold increase in the level of *SPP1* after both one and two weeks. These experiments showed that epigenetic modification of target polynucleotides can modify the expression profile of osteocyte-related genes to form osteocyte-like cells *in vivo*.

[0386] **Example 5: Stem Cell Reprogramming of Osteoblasts *in vivo***

[0387] In this example, MSCs were reprogrammed using RunX2, Osx, andDLx5(ROD)-CRISPRa. FIG. 10A-B show the measured radiance of a firefly luciferase gene reporter 7 days post transplantation of the primary osteoblasts into 5mm Calvarial defects generated in nude rats. Measurements taking one

month post-transplantation showed increased bone regrowth over the control in which no cells were transplanted (FIG. 10C-D).

[0388] **Example 6: Stem Cell Reprogramming of Chondrocytes *in vivo***

[0389] In this example, AD-MSCs were reprogrammed using BMP6 and TGFB3(BP)-CRISPRa. FIG 11A-B show the measured qRT-PCR relative quantification of *Col2a1* and *ACAN* in the resulting microspheroids harvested 14 days following reprogramming. FIG 11B shows the average measured radiance of a firefly luciferase gene reporter 1 motnh post-transplantation into a mouse model of MIA-induced osteoarthritis. FIG. 11D-G show improvement in gait phenotype analysis tests at different timepoints in mice who received reprogrammed MSCs over the gait phenotype analysis tests of mice who received control cells.

[0390] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. It is not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described with reference to the aforementioned specification, the descriptions and illustrations of the embodiments herein are not meant to be construed in a limiting sense. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is therefore contemplated that the invention shall also cover any such alternatives, modifications, variations or equivalents. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A system for inducing differentiation of an adult-derived stem cell, the system comprising:

a first heterologous polypeptide comprising an actuator moiety configured to form a first complex with a first target gene, to modify an expression profile of the first target gene; and

a second heterologous polypeptide comprising an actuator moiety configured to form a second complex with a second target gene, to modify an expression profile of the second target gene,

wherein the modification of the expression profile of the first target gene and the expression profile of the second target gene effects differentiation of the adult-derived stem cell into a target cell type,

wherein the modification comprises either (i) enhancing the expression profile of the first target gene and the expression profile of the second target gene or (ii) decreasing the expression profile of the first target gene and the expression profile of the second target gene.

2. The system of claim 1, wherein the modification of the expression profile of the target gene effects differentiation of the adult-derived stem cell into a specific cell type.

3. The system of claim 2, wherein the specific cell type is derived from osteogenesis.

4. The system of claim 3, wherein the specific cell type derived from osteogenesis is effected by activating BMP6 and TGFB3.

5. The system of claim 2, wherein the specific cell type is derived from chondrogenesis.

6. The system of claim 5, wherein the specific cell type derived from chondrogenesis is effected by activating RUNX2, OSX, and DLX5.

7. The system of claim 1, wherein the target gene comprises a positive regulator of differentiation selected from the group consisting of: Runx2, SP7/OSX, DLX5, BMPR1B, BMPR2, MSX2, DLX2, DLX3, DLX6, MSX1, ATF4, VDR, PDLIM7, ETS1, FOSL1, WNT9A, BMP2, BMP4, BMP6, BMP7, SMAD1, SMAD5, SMAD8, IGF, Pax7, and MyoD.

8. The system of claim 1, wherein the target gene comprises a negative regulator of the differentiation selected from the group consisting of: PPARg, LIPE, DKK1, DKK2, TWIST1, SMURF1, TGFBR1, TGFBR2.

9. The system of claim 1, wherein the adult-derived stem cell comprises a mesenchymal stem cell (MSC) or an induced pluripotent stem cell (iPSC).

10. The system of claim 1, wherein the adult-derived stem cell exhibits a maximum change within the modified expression profile of the target gene in less than about 3 days.

11. The system of claim 1, wherein the modification is transient.

12. A system for inducing differentiation of an adult-derived stem cell, the system comprising:

a first heterologous polypeptide comprising an actuator moiety configured to form a first complex with a first target gene, to modify an expression profile of the first target gene; and

a first heterologous polypeptide comprising an actuator moiety configured to form a second complex with a second target gene, to modify an expression profile of the second target gene,

wherein the modification of the expression profile of the first target gene and the expression profile of the second target gene effects differentiation of the adult-derived stem cell into a target cell type,

wherein the first target gene and the second target gene are different, and wherein the first target gene or and/or the second target gene is not (i) Sox9 or (ii) PPARγ.

13. The system of claim 12, wherein the modification of the expression profile of the target gene effects differentiation of the adult-derived stem cell into a specific cell type.

14. The system of claim 13, wherein the specific cell type is derived from osteogenesis.

15. The system of claim 14, wherein the specific cell type derived from osteogenesis is effected by activating BMP6 and TGFB3.

16. The system of claim 12, wherein the specific cell type is derived from chondrogenesis.

17. The system of claim 16, wherein the specific cell type derived from chondrogenesis is effected by activating RUNX2, OSX, and DLX5.

18. The system of claim 12, wherein the target gene comprises a positive regulator of the differentiation selected form the group consisting of: Runx2, SP7/OSX, DLX5, BMPR1B, BMPR2, MSX2, DLX2, DLX3, DLX6, MSX1, ATF4, VDR, PDLIM7, ETS1, FOSL1, WNT9A, BMP2, BMP4, BMP6, BMP7, SMAD1, SMAD5, SMAD8, IGF, Pax7, and MyoD.

19. The system of claim 12, wherein the target gene comprises a negative regulator of the differentiation selected from the group consisting of: PPARg, LIPE, DKK1, DKK2, TWIST1, SMURF1, TGFBR1, TGFBR2.

20. The system of claim 12, wherein the adult-derived stem cell comprises a mesenchymal stem cell (MSC) or an induced pluripotent stem cell (iPSC).

21. The system of claim 12, wherein the adult-derived stem cell exhibits a maximum change within the modified expression profile of the target gene in less than about 3 days.

22. The system of claim 12, wherein the modification is transient.

23. A system for inducing differentiation of an adult-derived stem cell, the system comprising:

a heterologous polypeptide comprising an actuator moiety configured to form a complex with a target polynucleotide in the adult-derived stem cell, wherein the actuator moiety is operatively linked to an epigenetic modifier to modify an expression profile of a target gene in the adult-derived stem cell, and

wherein the modification of the expression profile of the target gene induced by the formation of the complex sufficiently effects chondrogenic differentiation of the adult-derived stem cell.

24. The system of claim 23, wherein the modification of the expression profile of the target gene induced by the formation of the complex sufficiently induces expression of one or more chondrogenic differentiation markers.

25. The system of claim 23, wherein the system occurs in an environment that is substantially free of

an additional chondrogenic factor in medium.

26. The system of claim 23, wherein chondrogenic differentiation is effected by activating RUNX2, OSX, and DLX5.

27. The system of claim 23, wherein the target gene comprises a positive regulator of the differentiation selected form the group consisting of: Runx2, SP7/OSX, DLX5, BMPR1B, BMPR2, MSX2, DLX2, DLX3, DLX6, MSX1, ATF4, VDR, PDLIM7, ETS1, FOSL1, WNT9A, BMP2, BMP4, BMP6, BMP7, SMAD1, SMAD5, SMAD8, IGF, Pax7, and MyoD.

28. The system of claim 23, wherein the target gene comprises a negative regulator of the differentiation selected from the group consisting of: PPARg, LIPE, DKK1, DKK2, TWIST1, SMURF1, TGFBR1, TGFBR2.

29. The system of claim 23, wherein the adult-derived stem cell comprises a mesenchymal stem cell (MSC) or an induced pluripotent stem cell (iPSC).

30. The system of claim 23, wherein the adult-derived stem cell exhibits a maximum change within the modified expression profile of the target gene in less than about 3 days.

31. The system of claim 23, wherein the modification is transient.

32. A system for inducing osteogenic differentiation of an adult-derived stem cell, the system comprising:

a heterologous polypeptide comprising an actuator moiety configured to form a complex with a target polynucleotide in the adult-derived stem cell, wherein the actuator moiety is operatively linked to an epigenetic modifier to modify an expression profile of a target gene in the adult-derived stem cell, and

wherein the modification of the expression profile of the target gene induced by the formation of the complex effects osteogenic differentiation of the adult-derived stem cell.

33. The system of claim 32, wherein the modification of the expression profile of the target gene induced by the formation of the complex sufficiently induces osteogenic differentiation of the adult-derived stem cell.

34. The system of claim 32, wherein the modification of the expression profile of the target gene induced by the formation of the complex sufficiently induces expression of one or more osteogenic differentiation markers.

35. The system of claim 32, wherein the system occurs in an environment that is substantially free of dexamethasone in medium.

36. The system of claim 32, wherein the osteogenic differentiation is effected by activating BMP6 and TGFB3.

37. The system of claim 32, wherein the target gene comprises a positive regulator of the differentiation selected form the group consisting of: Runx2, SP7/OSX, DLX5, BMPR1B, BMPR2, MSX2, DLX2, DLX3, DLX6, MSX1, ATF4, VDR, PDLIM7, ETS1, FOSL1, WNT9A, BMP2, BMP4, BMP6, BMP7, SMAD1, SMAD5, SMAD8, IGF, Pax7, and MyoD.

38. The system of claim 32, wherein the target gene comprises a negative regulator of the

differentiation selected from the group consisting of: PPARg, LIPE, DKK1, DKK2, TWIST1, SMURF1, TGFBR1, TGFBR2.

39. The system of claim 32, wherein the adult-derived stem cell comprises a mesenchymal stem cell (MSC) or an induced pluripotent stem cell (iPSC).

40. The system of claim 32, wherein the adult-derived stem cell exhibits a maximum change within the modified expression profile of the target gene in less than about 3 days.

41. The system of claim 32, wherein the modification is transient.

42. A method for inducing differentiation of an adult-derived stem cell, the method comprising:

contacting a cell with a first heterologous polypeptide comprising an actuator moiety configured to form a first complex with a first target gene, to modify an expression profile of the first target gene; and

contacting a cell with a second heterologous polypeptide comprising an actuator moiety configured to form a second complex with a second target gene, to modify an expression profile of the second target gene,

wherein the modification of the expression profile of the first target gene and the expression profile of the second target gene effects differentiation of the adult-derived stem cell into a target cell type,

wherein the modification comprises either (i) enhancing the expression profile of the first target gene and the expression profile of the second target gene OR (ii) decreasing the expression profile of the first target gene and the expression profile of the second target gene.

43. A method for inducing differentiation of an adult-derived stem cell, the method comprising:

contacting a cell with a first heterologous polypeptide comprising an actuator moiety configured to form a first complex with a first target gene, to modify an expression profile of the first target gene; and

contacting a cell with a second heterologous polypeptide comprising an actuator moiety configured to form a second complex with a second target gene, to modify an expression profile of the second target gene,

wherein the modification of the expression profile of the first target gene and the expression profile of the second target gene effects differentiation of the adult-derived stem cell into a target cell type,

wherein the first target gene and the second target gene are different, and wherein the first target gene or and/or the second target gene is not (i) Sox9 or (ii) PPARγ.

44. A method for inducing differentiation of an adult-derived stem cell, the method comprising:

contacting a cell with a heterologous polypeptide comprising an actuator moiety configured to form a complex with a target polynucleotide in the adult-derived stem cell, wherein the actuator moiety is operatively linked to an epigenetic modifier to modify an expression profile of a target gene in the adult-derived stem cell, and

wherein the modification of the expression profile of the target gene induced by the formation of the complex sufficiently effects chondrogenic differentiation of the adult-derived stem cell.

45. A method for inducing osteogenic differentiation of an adult-derived stem cell, the method comprising:

contacting a cell with a heterologous polypeptide comprising an actuator moiety configured to form a complex with a target polynucleotide in the adult-derived stem cell, wherein the actuator moiety is operatively linked to an epigenetic modifier to modify an expression profile of a target gene in the adult-derived stem cell, and

wherein the modification of the expression profile of the target gene induced by the formation of the complex effects osteogenic differentiation of the adult-derived stem cell.

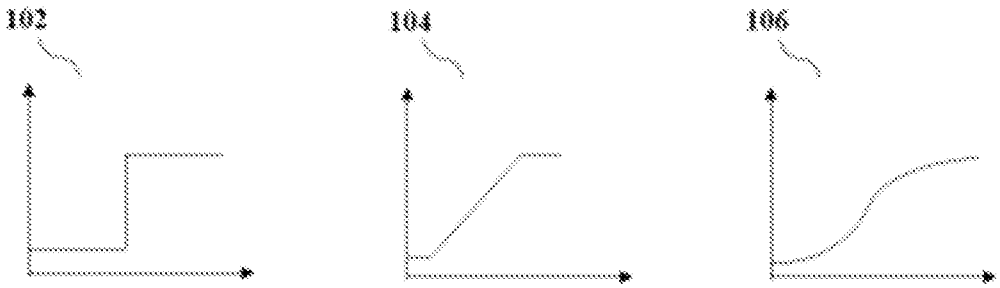


FIG. 1A

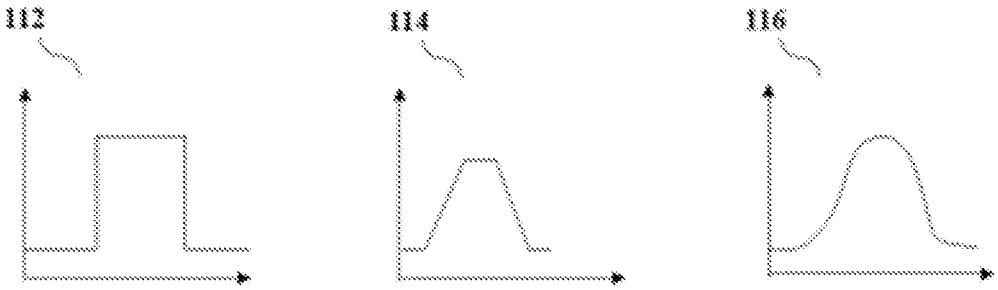


FIG. 1B

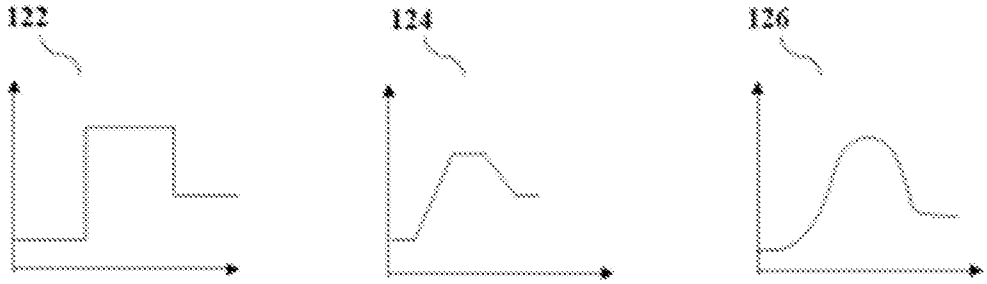


FIG. 1C

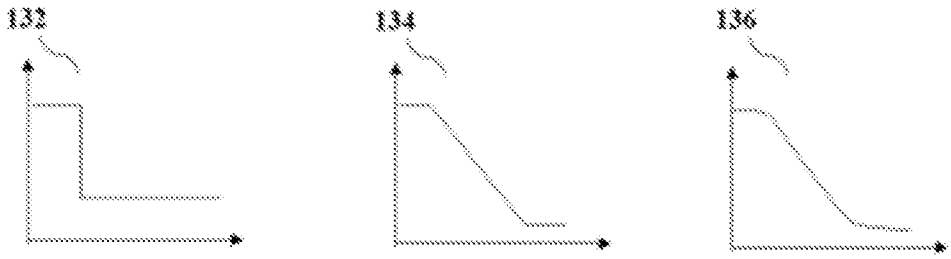


FIG. 1D

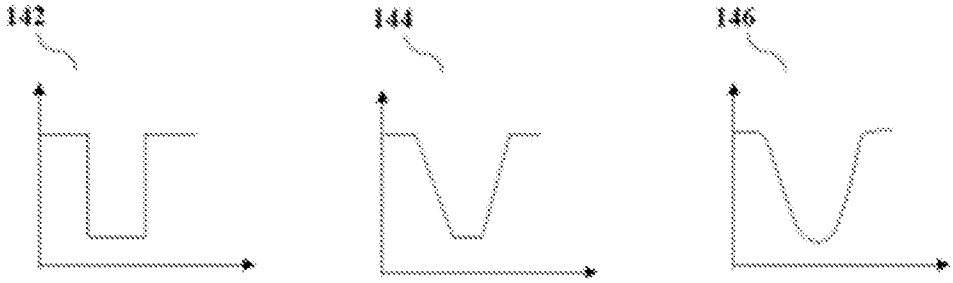


FIG. 1E

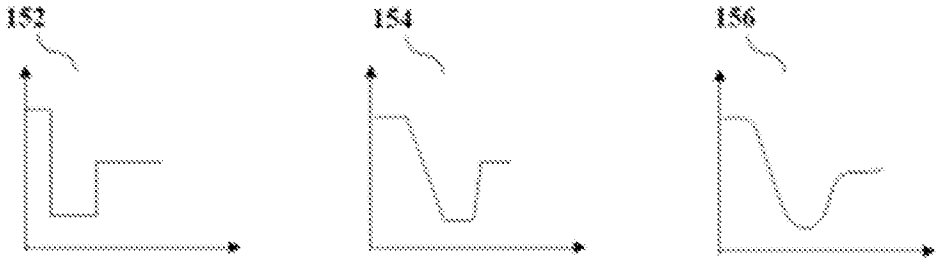


FIG. 1F

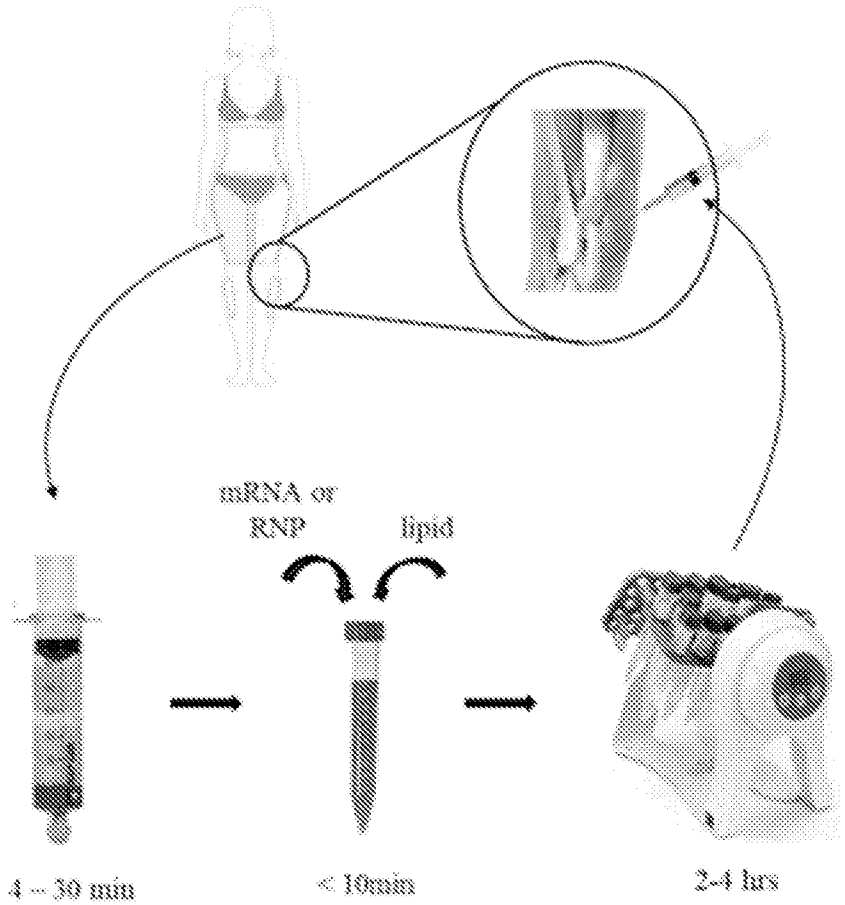


FIG. 2

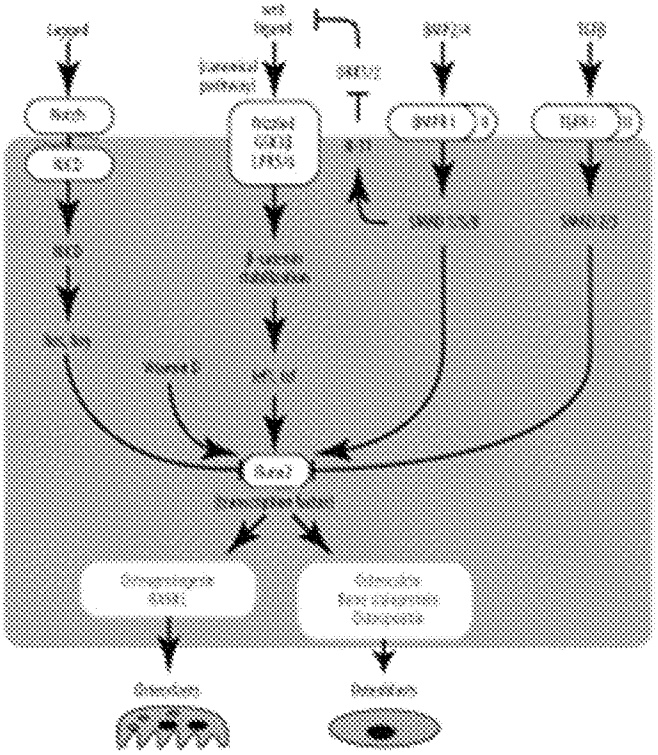


FIG. 3A

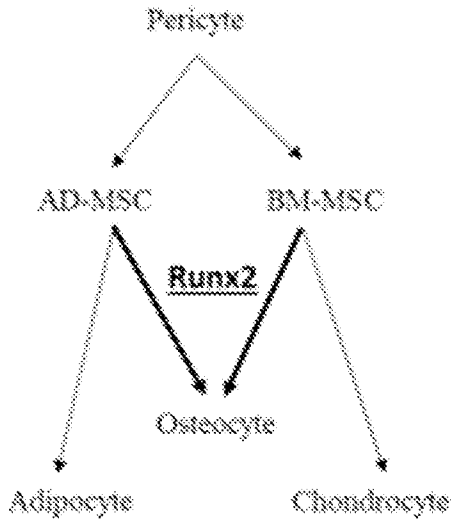

Pericyte
AD-MSC
BM-MSC
Runx2
Osteocyte
Adipocyte
Chondrocyte

FIG. 3B

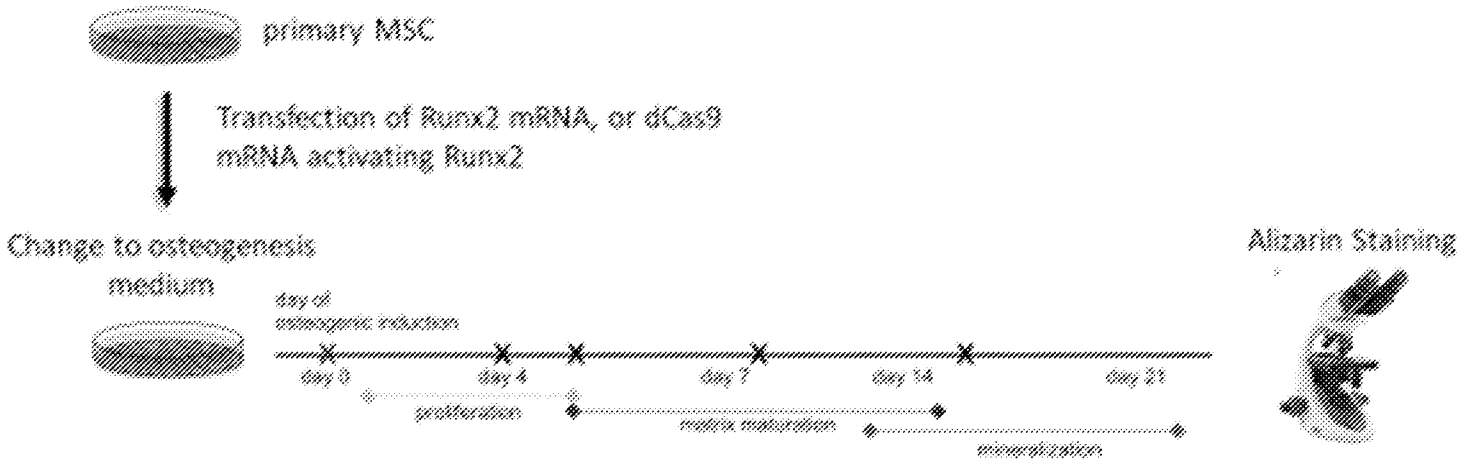


FIG. 4A

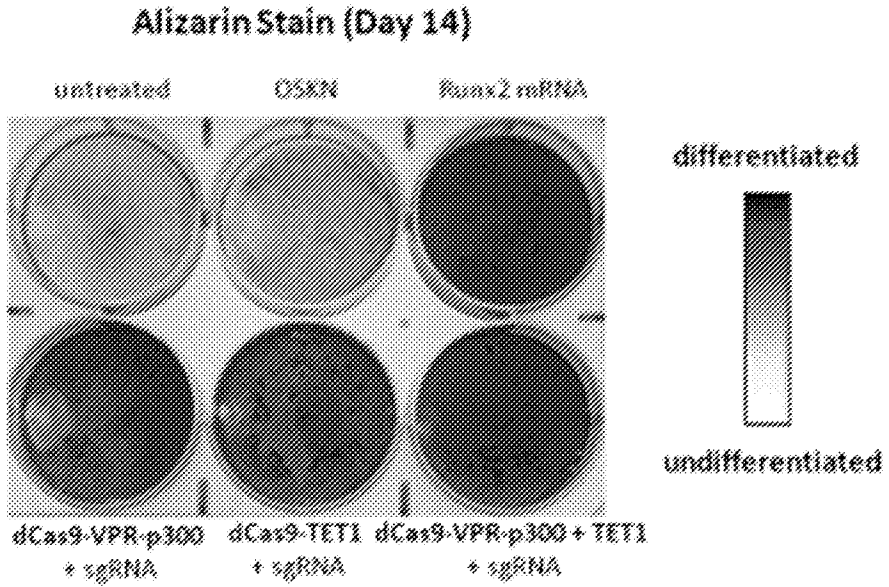


FIG. 4B

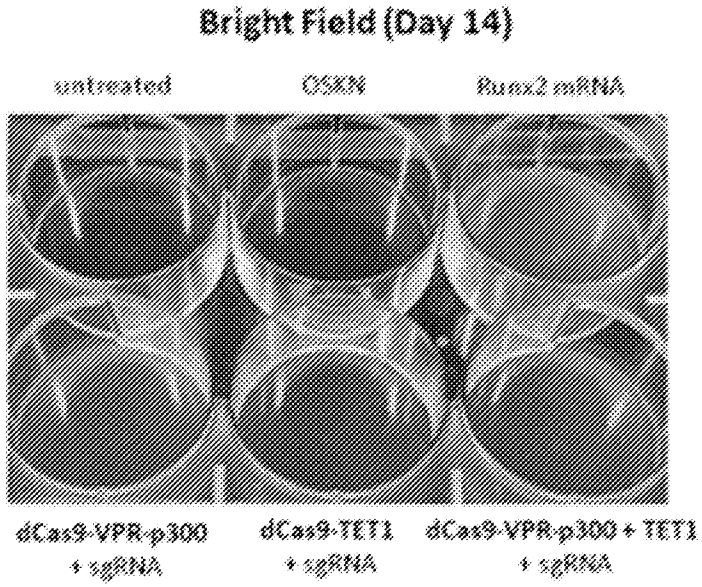


FIG. 4C

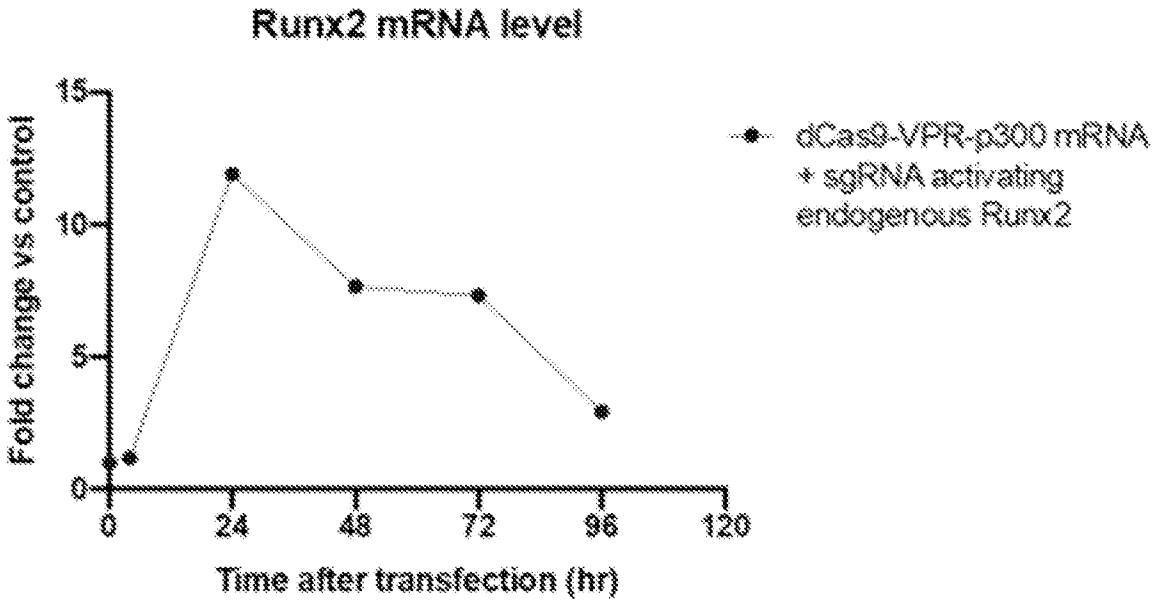

Runx2 mRNA level
Fold change vs control
15
10
5
0
0
24
48
72
96
120
Time after transfection (hr)
dCas9-VPR-p300 mRNA + sgRNA activating endogenous Runx2

FIG. 5A

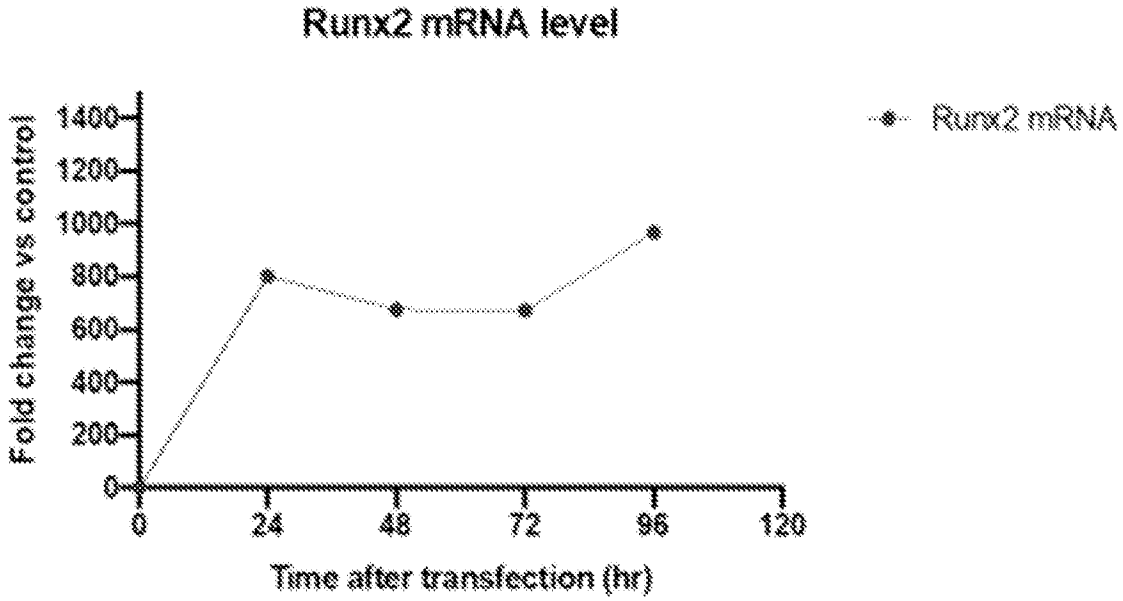

Runx2 mRNA level
Fold change vs control
1400
1200
1000
800
600
400
200
0
0
24
48
72
96
120
Time after transfection (hr)
Runx2 mRNA

FIG. 5B

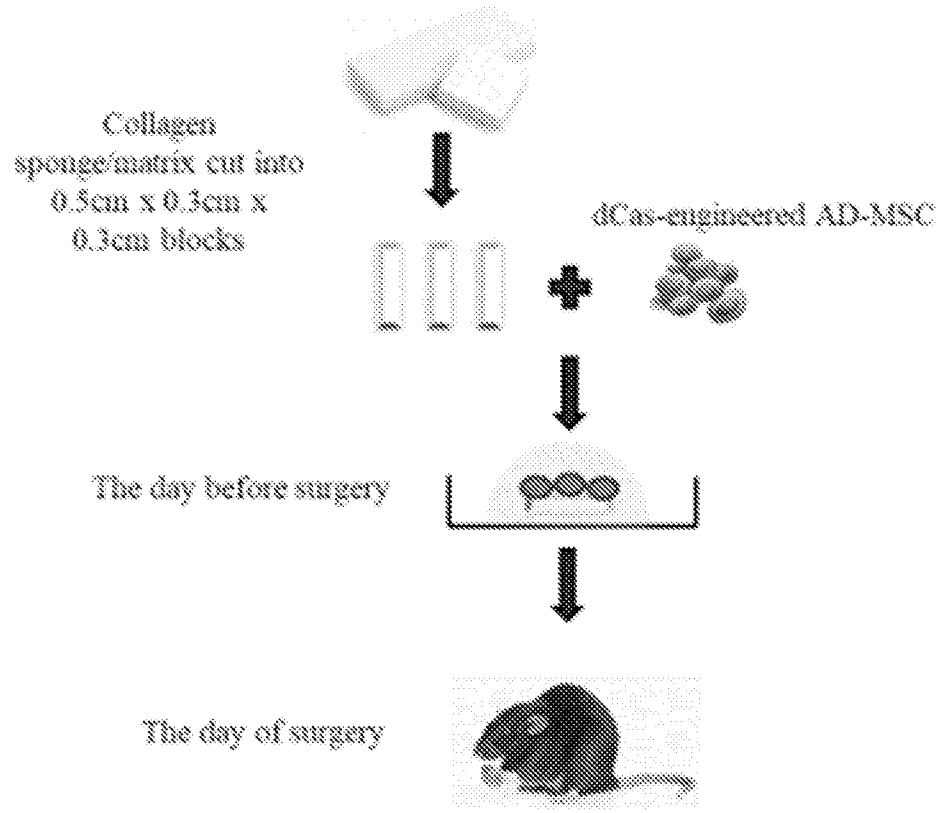

Collagen sponge/matrix cut into 0.5cm x 0.3cm x 0.3cm blocks
dCas-engineered AD-MSC
The day before surgery
The day of surgery

FIG. 6

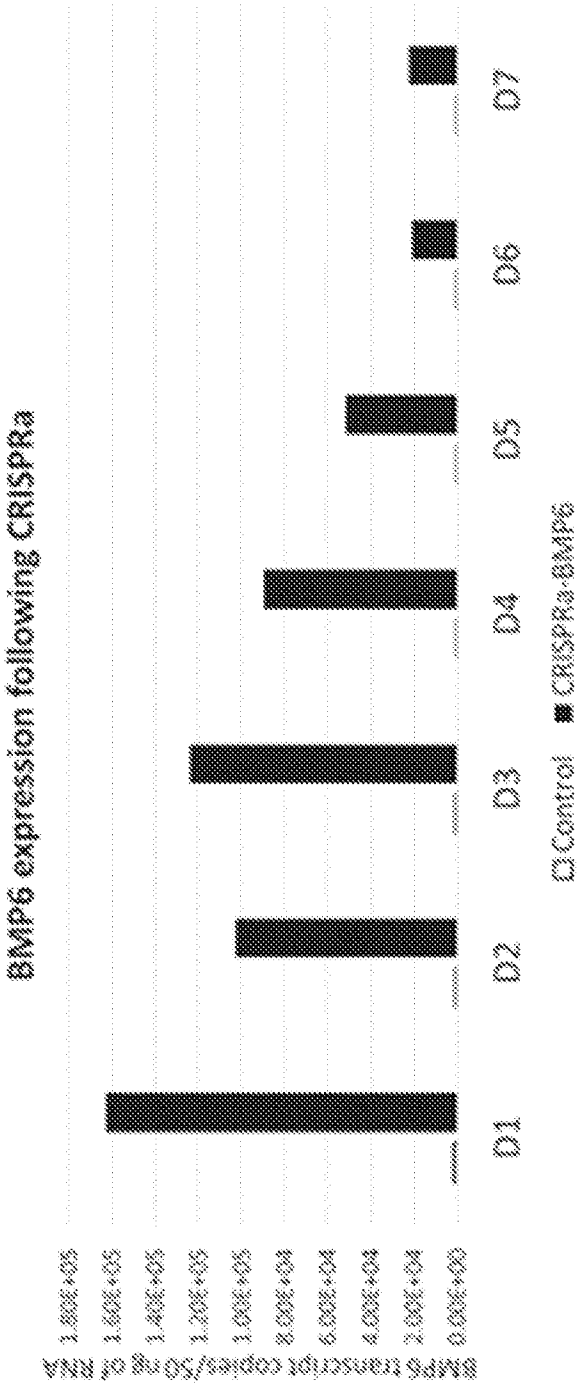

BMP6 expression following CRISPRa
BMP6 transcript copies/50 ng of RNA
1.60E+05
1.40E+05
1.20E+05
1.00E+05
8.00E+04
6.00E+04
4.00E+04
2.00E+04
0.00E+00
D1
D2
D3
D4
D5
D6
D7
Control
CRISPRa-BMP6

FIG. 7

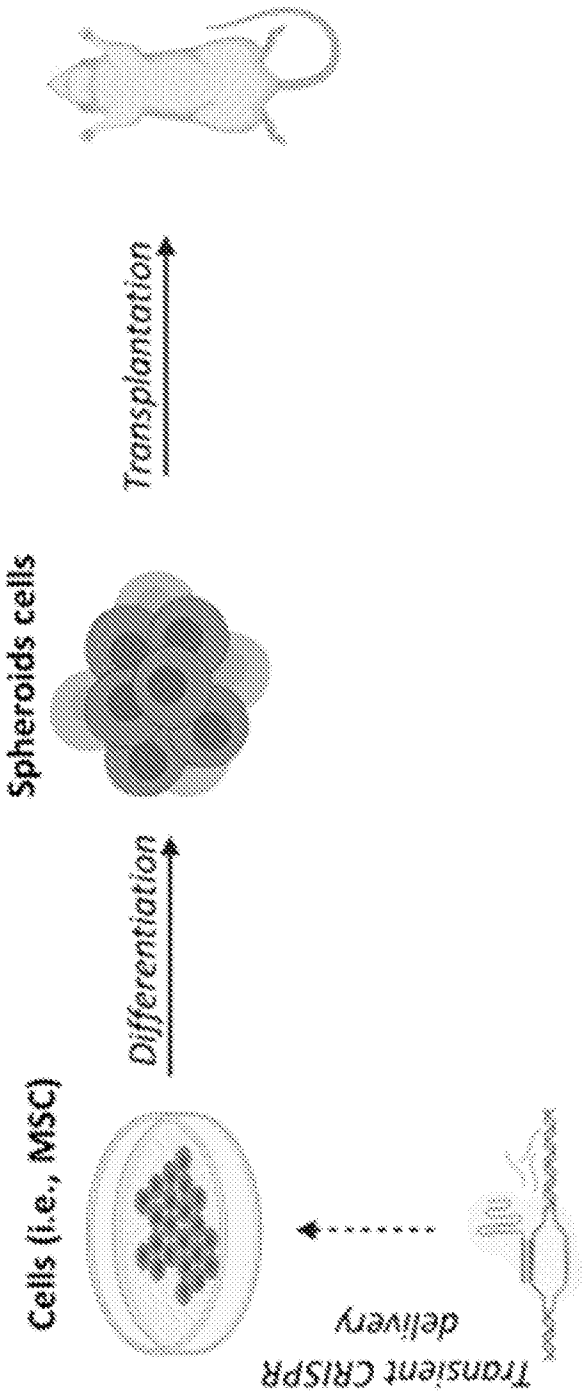

Transient CRISPR
delivery
Cells (i.e., MSC)
Differentiation
Spheroids cells
Transplantation

FIG. 8

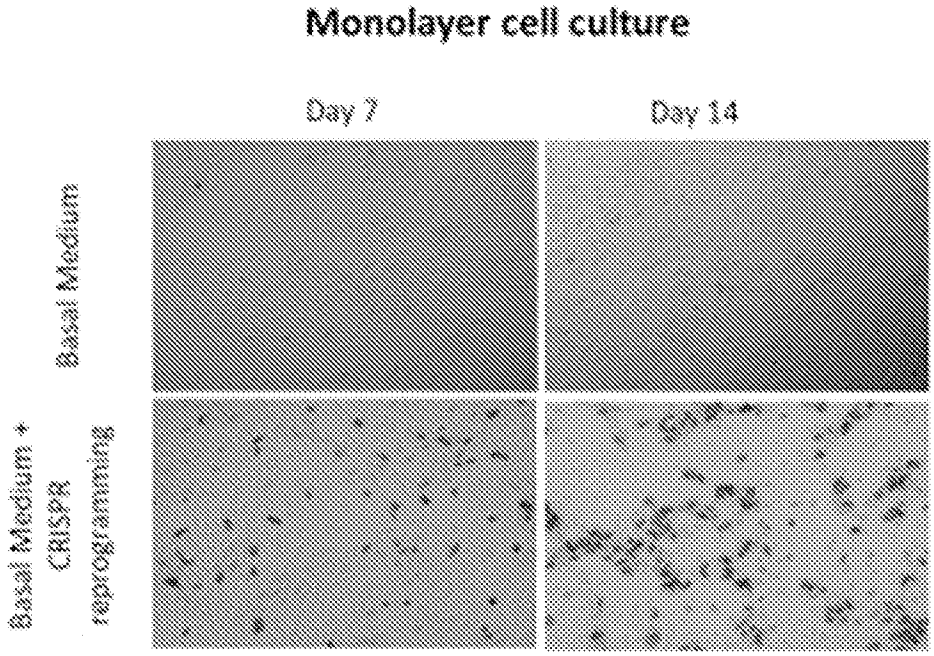

Monolayer cell culture
Day 7
Day 14
Basal Medium
Basal Medium + CRISPR reprogramming

FIG. 9A

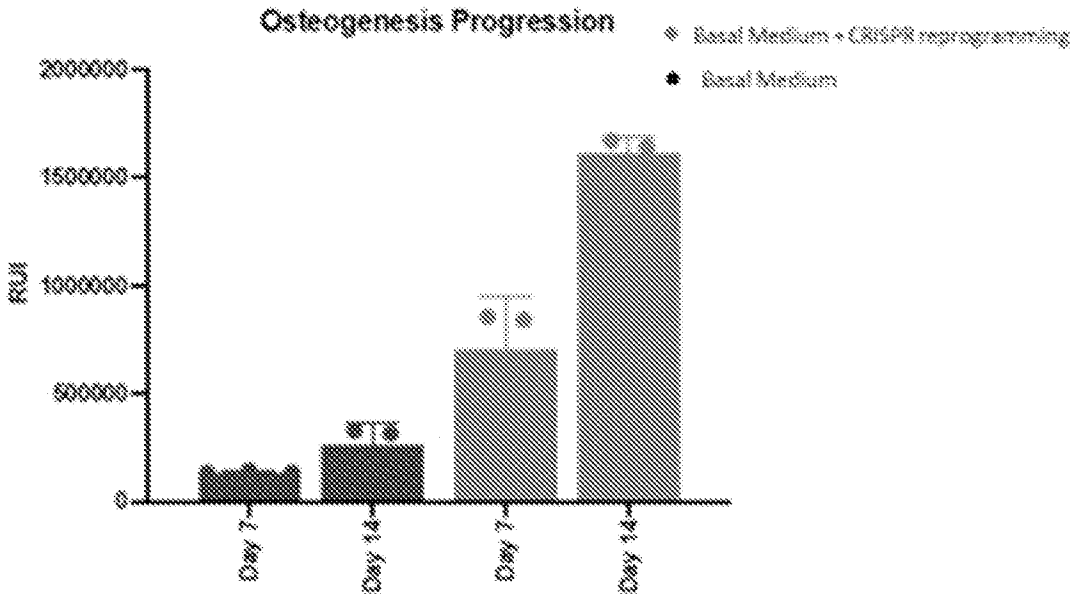

Osteogenesis Progression
Basal Medium + CRISPR reprogramming
Basal Medium
RUI
2000000
1500000
1000000
500000
0
Day 7
Day 14
Day 7
Day 14

FIG. 9B

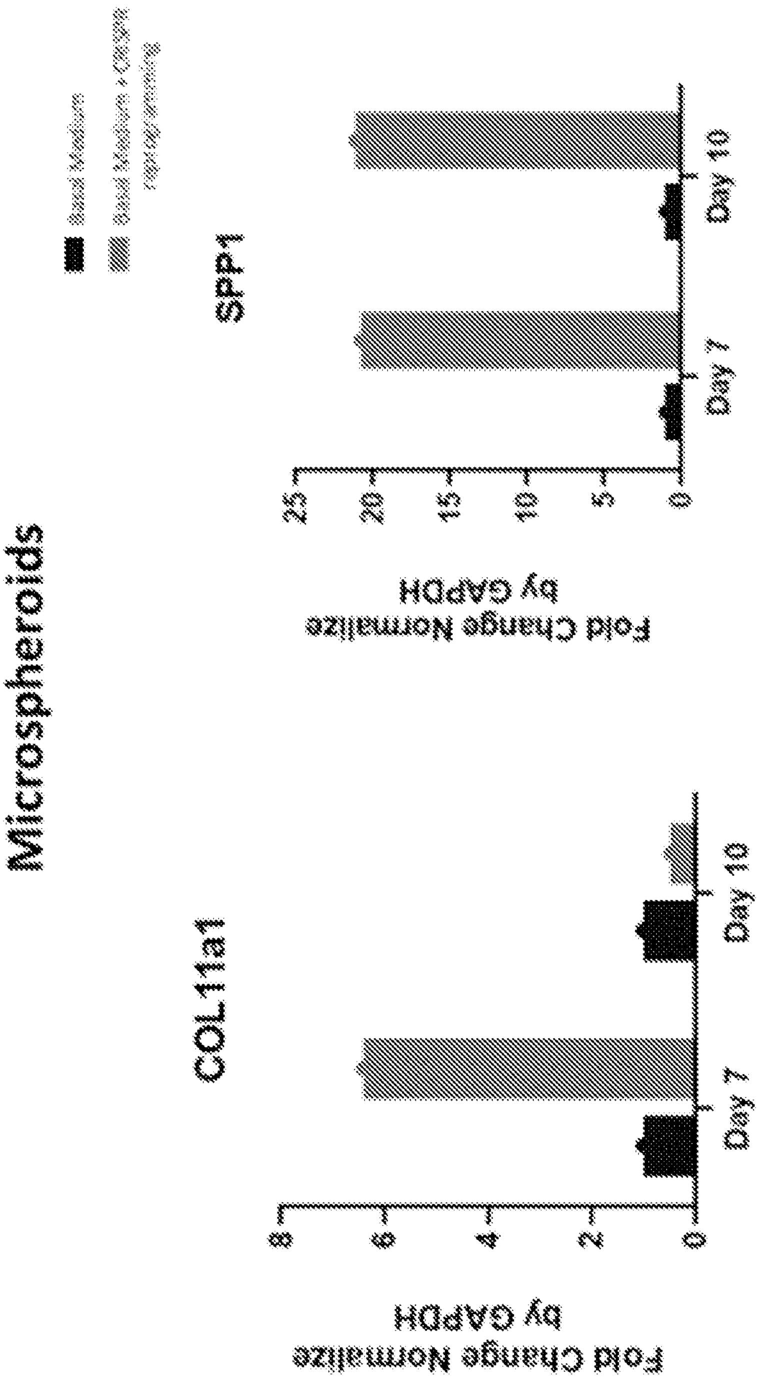

Microspheroids
SPP1
COL11a1
Fold Change Normalize by GAPDH
Day 7
Day 10
25
20
15
10
5
0
8
6
4
2
0

FIG. 9D

FIG. 9C

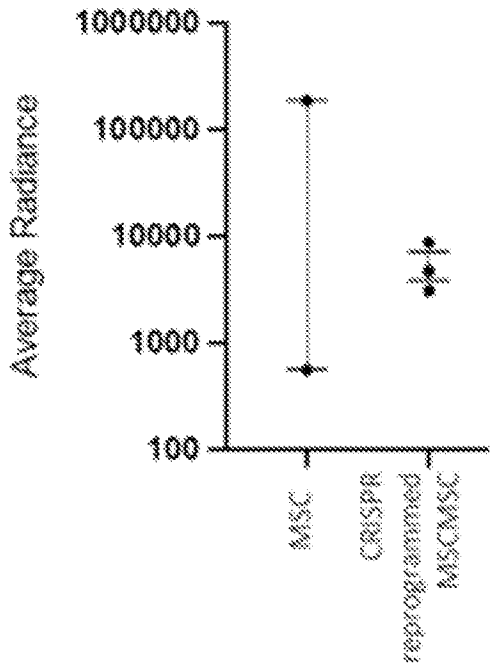


FIG. 10A

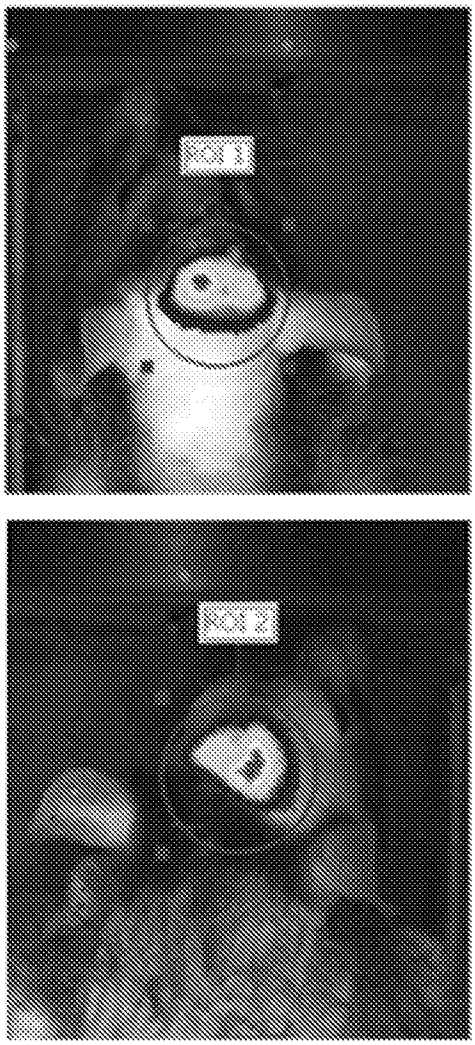
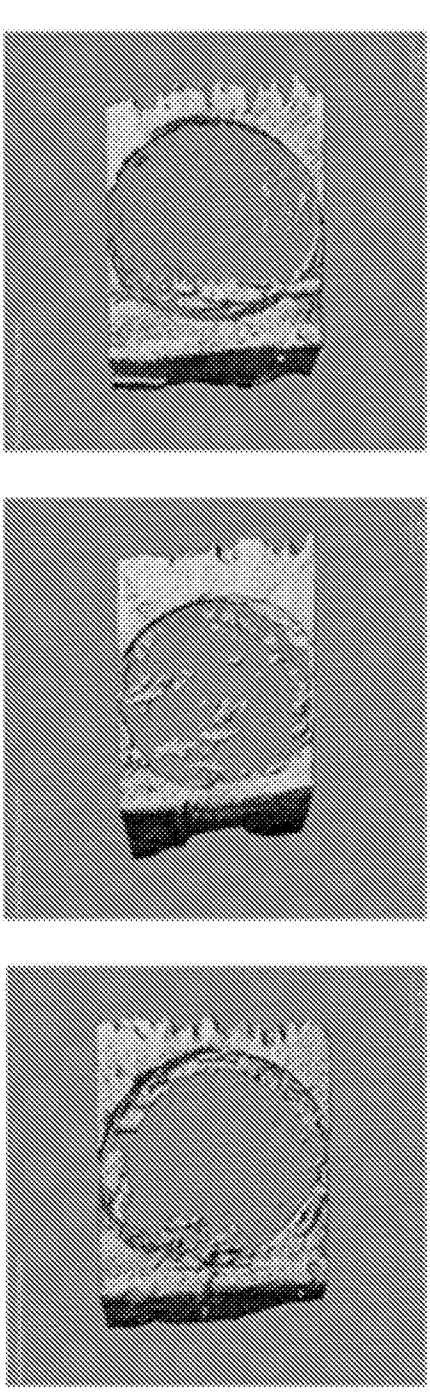


FIG. 10B



Left Bone Voxel Volume		
No Cells	150K Cells	600K Cells
10.8434	12.8437	18.2266

FIG. 10C

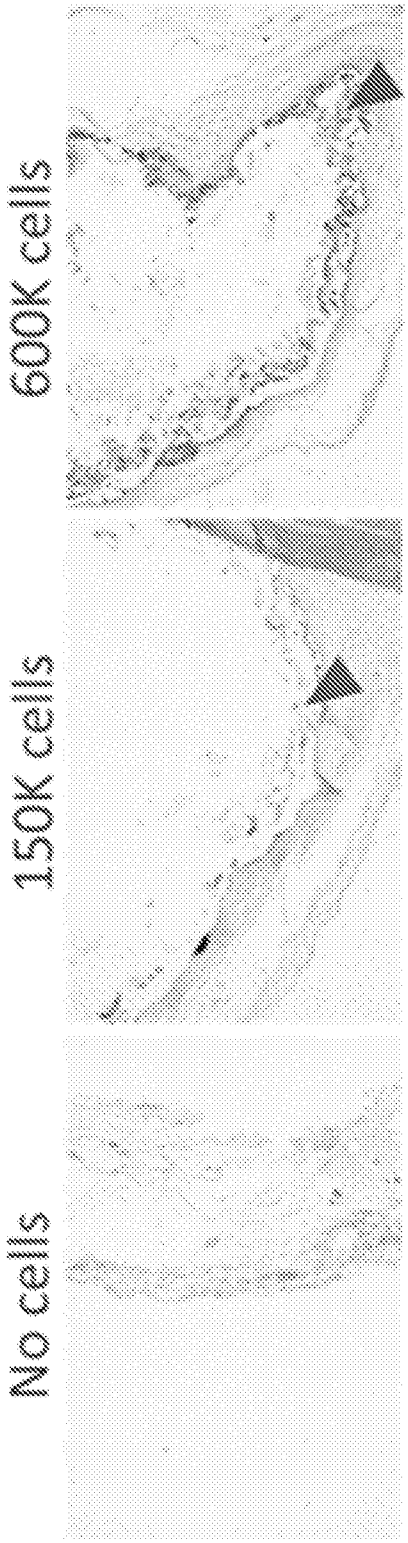


FIG. 10D

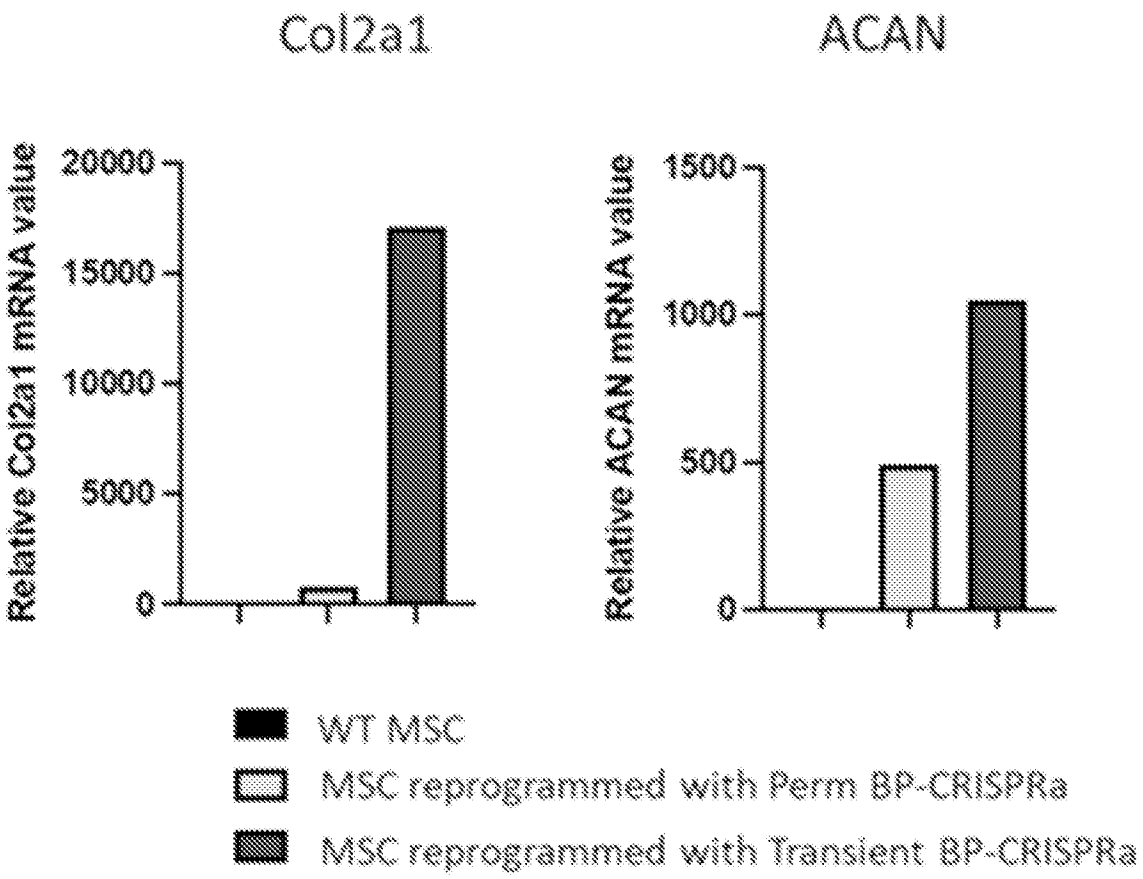


FIG. 11A *FIG. 11B*

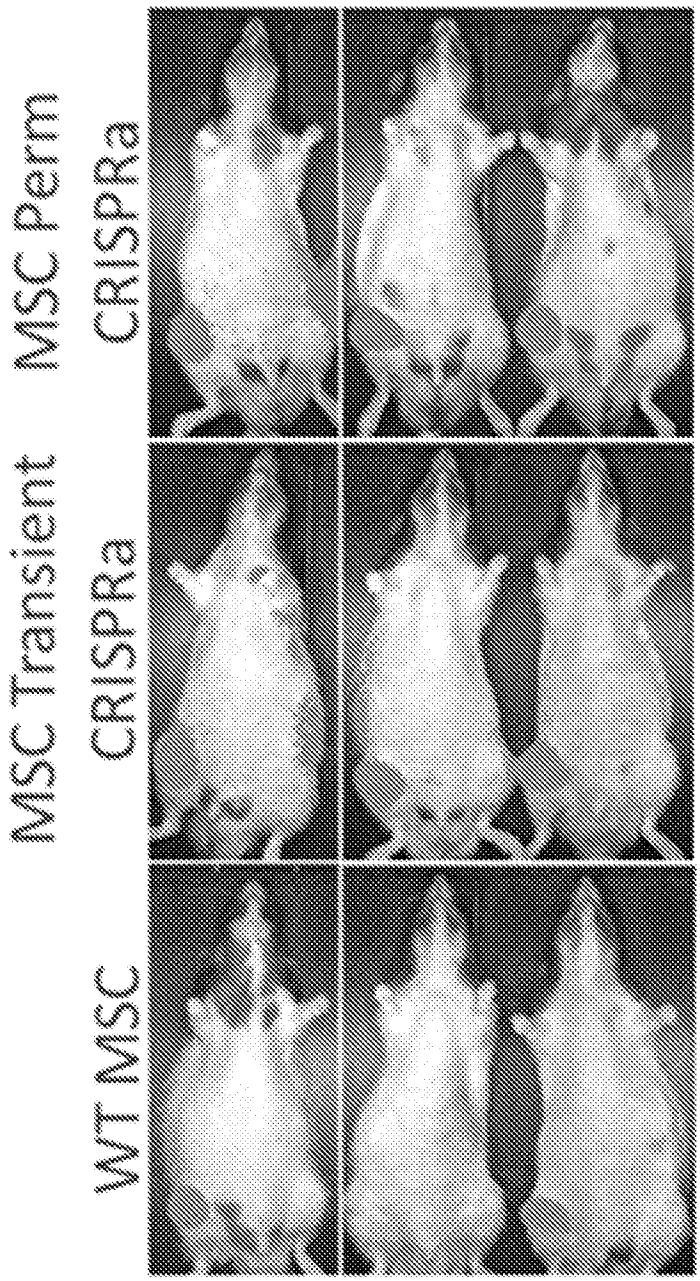


FIG. 11C

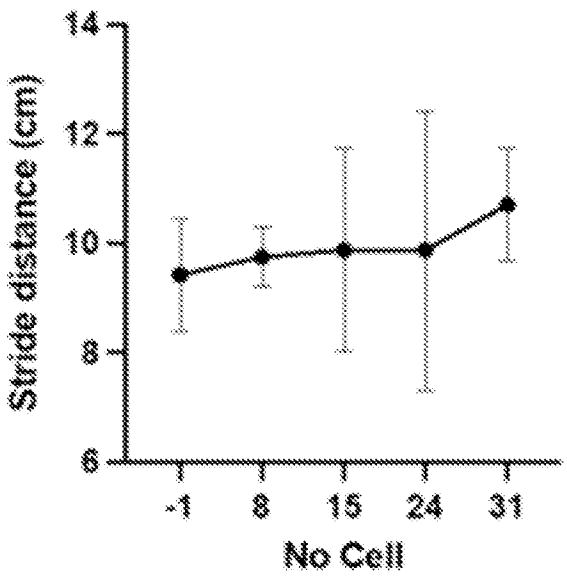


FIG. 11D

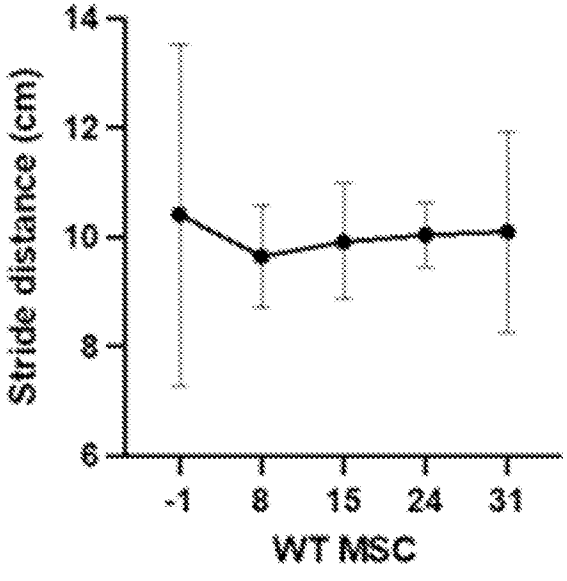


FIG. 11E

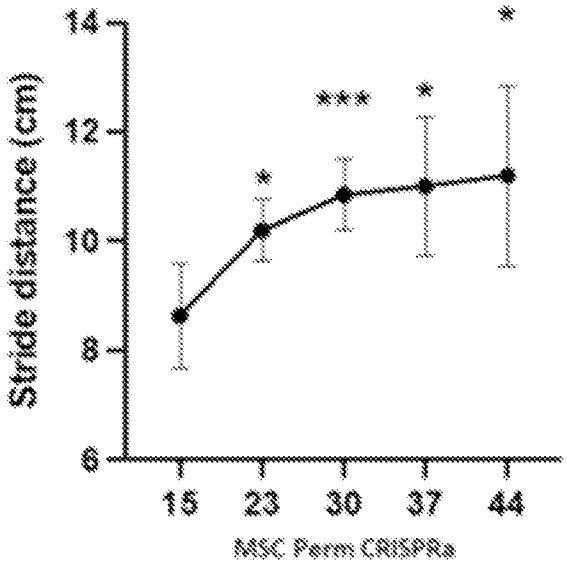


FIG. 11F

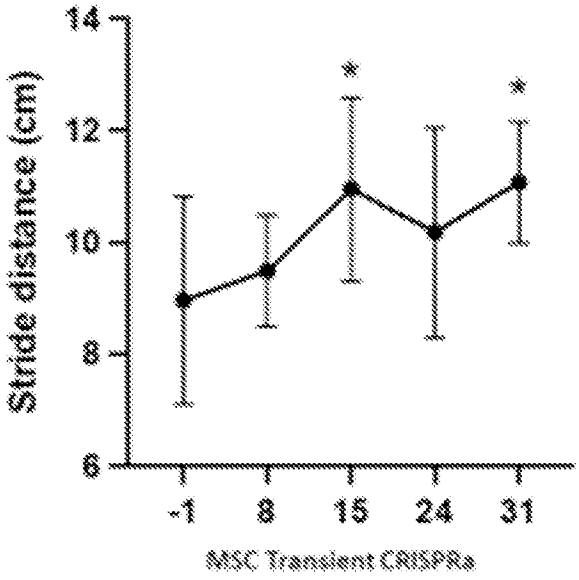


FIG. 11G

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/63758

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 5/074, A61K 35/28, C12N 5/02, C12N 15/06, C12N 5/0789 (2022.01)

CPC - A61K 35/28, C12N 5/16, C12N 15/06, C12N 5/0611, C12N 2506/04, C12N 2506/00, C12N 2501/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2017/0224741 A1 (WOLFFE et al.) 10 August 2017 (10.08.2017) para [0013], [0026], [0029], [0031]-[0032], [0041], [0064], [0088], [0090], [0109], [0143], [0145], [0147], [0157], [0158], [0167]-[0168], [0175]-[0176], [0180], [0186], [0193]-[0195], [0220], [0251], [0256]-[0257], [0269] -[0272], Claim 1, Table 1, Abstract	1-3, 9-14, 20-22, 32-35, 39-41 ---------- 4, 7, 15, 18, 36-37
Y	LU et al., Regenerating cartilages by engineered ASCs: prolonged TGF-B3/BMP-6 expression improved articular cartilage formation and restored zonal structure, Molecular Therapy, January 2014, Vol 22, No 1, Pages 186-195, Especially pg 186 col 2 para 2, pg 187 col 1 para 3, pg 187 col 1 para 5, pg 193 col 2 para 2, Figure 1, Abstract	4, 7, 15, 18, 36-37
A	US 2018/0161373 A1 (AELAN CELL TECHNOLOGIES, INC) 14 June 2018 (14.06.2018) para [0074], [0076], [0085], [0089], Claim 6, Claim 20	1

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 February 2022	MAY 16 2022
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Kari Rodriquez Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/63758

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

----------Please see continuation in first extra sheet----------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-4, 7, 9-15, 18, 20-22, 32-37, 39-41, limited to BMP6 and TGFB3

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Continuation of Box No. III. Observations where unity of invention is lacking

Group I+, claims 1-41, directed to a system. The system will be searched to the extent that the system encompasses wherein the specific/target cell type is derived from osteogenesis, the first and second target genes encompass BMP6 and TGFB3, and wherein the modification of the expression profile encompasses enhancing the expression profile of (activating) BMP6 and TGFB3. It is believed that claims 1-4, 7, 9-15, 18, 20-22, 32-37, 39-41 encompass this first named invention, and thus these claims will be searched without fee to the extent that the specific/target cell type is derived from osteogenesis, the first and second target genes encompass BMP6 and TGFB3, and the modification of the expression profile encompasses enhancing the expression profile of (activating) BMP6 and TGFB3. Additional specific/target cell type(s), and/or a set of target genes modification(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected specific/target cell type(s), and/or a set of target genes modification(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be where the specific/target cell type is derived from chondrogenesis, the first and second target genes encompass two or more of RUNX2, OSX, and/or DLX5, and the modification of the expression profile encompasses enhancing the expression profile of (activating) RUNX2, OSX, and DLX5 (claims 1-2, 5-7, 9-13, 16-18, 20-27, 29-31).

Group II, claims 42-45, directed to a method.

The inventions listed as Groups I+ and II do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features

Group I+ has the special technical feature of a system comprising heterologous polypeptides, that is not required by Group II.

Group II has the special technical feature of a method comprising contacting a cell with a first heterologous polypeptide, that is not required by Group I+.

The inventions of Group I+ each include the special technical feature of a different specific/target cell type, and/or a different set of target genes modified, and is considered a distinct technical feature.

Common technical features

The inventions of Group I+ and Group II share the common technical feature of inducing differentiation of an adult-derived stem cell, comprising:
a first heterologous polypeptide comprising an actuator moiety configured to form a first complex with a first target gene, to modify an expression profile of the first target gene; and
a second heterologous polypeptide comprising an actuator moiety configured to form a second complex with a second target gene, to modify an expression profile of the second target gene,
wherein the modification of the expression profile of the first target gene and the expression profile of the second target gene effects differentiation of the adult-derived stem cell into a target cell type,
wherein the modification comprises either (i) enhancing the expression profile of the first target gene and the expression profile of the second target gene or (ii) decreasing the expression profile of the first target gene and the expression profile of the second target gene; and
sufficiently effecting (osteogenic or chondrogenic) differentiation of the adult-derived stem cell.

The feature shared by Groups I+ and II and the feature shared by the inventions listed as Group I+ are disclosed by US 2017/0224741 A1 to Wolfe et al (hereinafter 'Wolfe').

Wolfe discloses a system for inducing differentiation of an adult-derived stem cell (Abstract - "Methods and compositions for modifying stem cells using one or more ZFPs are disclosed. Such methods and compositions are useful for facilitating processes such as...differentiating stem cells into the desired phenotype"; para [0026] - "compositions and methods useful for differentiating stem cells into a desired differentiated state are provided."; Claim 1), the system comprising:
a first heterologous polypeptide comprising an actuator moiety configured to form a first complex with a first target gene (para [0145] - "formation of a fusion protein...between a zinc finger binding domain and a functional domain...Essentially any molecule capable of recruiting a repressive complex and/or repressive activity (such as, for example, histone deacetylation) to the target gene"; para [0195] - "the differentiated phenotype is fixed. Such a mechanism might be exploited by permanently switching on or off an endogenous gene that regulates differentiation, for example by use of a factor (e.g., ZFP) that will bind to and specifically modify the specified genes."; Note Instant Applicant Specification para [0307] - "The actuator moiety as disclosed herein can comprise...zinc finger"),

---continued in next Box----

Continuation of previous box

Box No. III. Observations where unity of invention is lacking

to modify an expression profile of the first target gene (para [0029] - "The ZFPs can be engineered for their ability to regulate gene expression, for example by activating and/or inhibiting genes involved in differentiation. The disclosure also contemplates the use of combinations of ZFPs that modulate expression of one or more genes involved in propagation, development and differentiation."; para [0195] - "the differentiated phenotype is fixed. Such a mechanism might be exploited by permanently switching on or off an endogenous gene that regulates differentiation, for example by use of a factor (e.g., ZFP) that will bind to and specifically modify the specified genes."); and
a second heterologous polypeptide comprising an actuator moiety configured to form a second complex with a second target gene (para [0029] - "ZFPs that modulate expression of one or more genes involved in propagation, development and differentiation."; para [0013] - "one or more of the ZFPs modulate expression of genes involved in growth or differentiation"; para [0195] - "by use of a factor (e.g., ZFP) that will bind to and specifically modify the specified genes."), to modify an expression profile of the second target gene (para [0029] - "ZFPs that modulate expression of one or more genes"; para [0013] - "one or more of the ZFPs modulate expression of genes"; para [0195] - "by permanently switching on or off an endogenous gene that regulates differentiation, for example by use of a factor (e.g., ZFP) that will bind to and specifically modify the specified genes."),
wherein the modification of the expression profile of the first target gene and the expression profile of the second target gene effects differentiation of the adult-derived stem cell into a target cell type (para [0029] - "ZFPs that modulate expression of one or more genes involved in propagation, development and differentiation."; para [0013] - "one or more of the ZFPs modulate expression of genes involved in growth or differentiation"; para [0031] - "ZFP(s) can be used facilitate the regulation of many processes involved in development and differentiation...differentiation to a desired specialized cell type; and cloning."; para [0032] - "Advantages of the presently-disclosed methods and compositions include, but are not limited to, (i) the ability to directly and specifically control core processes that direct stem cell differentiation (e.g., modulate expression of one or more genes, either by activating or repressing genes); (ii) the ability to reprogram stem cells ex vivo; (iii) the ability to generate all functional splice variants of the target protein; (iv) the ability to limit or eliminate uncontrolled massive overexpression of a target protein to toxic levels; (v) the ability to direct stem cell differentiation or dedifferentiation through epigenetic mechanisms;"; para [0167]-[0168]; para [0251]),
wherein the modification comprises either (i) enhancing the expression profile of the first target gene and the expression profile of the second target gene (para [0186] - "regulation of genes involved in differentiation by zinc finger proteins is used to obtain populations of differentiated cells. The populations of cells so obtained can be fully differentiated (i.e., terminally differentiated) or partially differentiated (i.e, multipotent but lineage-restricted). For example, up-regulation of a gene that drives differentiation") or (ii) decreasing the expression profile of the first target gene and the expression profile of the second target gene (para [0186] - "regulation of genes involved in differentiation by zinc finger proteins is used to obtain populations of differentiated cells...or down-regulation of a gene which drives stem cell proliferation and/or self-renewal, can be used to move a cell toward a more differentiated state."; para [0195] - "by permanently switching...off an endogenous gene that regulates differentiation, for example by use of a factor (e.g., ZFP) that will bind to and specifically modify the specified genes."; para [0256]-[0257]).

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I+ and II inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.