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(54) **HUMAN BROWN ADIPOSE DERIVED STEM CELLS AND USES**

STAMMZELLEN AUS MENSCHLICHEM BRAUNEN FETTGEWEBE UND VERWENDUNGEN

CELLULES SOUCHES DÉRIVÉES DU TISSU ADIPEUX BRUN HUMAIN ET LEURS UTILISATIONS

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- **PATEL AMIT N ET AL: "Putative Population of Adipose-Derived Stem Cells Isolated From Mediastinal Tissue During Cardiac Surgery", CELL TRANSPLANTATION, vol. 22, no. 3, 2 April 2012 (2012-04-02), pages 507-511, XP002726920,**
- **ARTURO HERNANDEZ ET AL: "Dexamethasone Inhibits Growth Factor-Induced Type 3 Deiodinase Activity and mRNA Expression in a Cultured Cell Line Derived from Rat Neonatal Brown Fat Vascular-Stromal Cells", ENDOCRINOLOGY, vol. 143, no. 7, 1 July 2002 (2002-07-01), pages 2652-2658, XP055127771, ISSN: 0013-7227, DOI: 10.1210/endo.143.7.8923**
- **M. CIGOLINI ET AL: "Human brown adipose cells in culture", EXPERIMENTAL CELL RESEARCH, vol. 159, no. 1, 1 July 1985 (1985-07-01), pages 261-266, XP055040049, ISSN: 0014-4827, DOI: 10.1016/S0014-4827(85)80056-2**
- **TIM J SCHULZ ET AL: "Identification of inducible brown adipocyte progenitors residing in skeletal muscle and white fat", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, NATIONAL ACADEMY OF SCIENCES, US , vol. 108, no. 1 4 January 2011 (2011-01-04), pages 143-148, XP002675155, ISSN: 0027-8424, DOI: 10.1073/PNAS.1010929108 Retrieved from the Internet: URL:http://www.pnas.org/content/108/1/143 [retrieved on 2010-12-20]**

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(Cont. next page)

- KLEIN J ET AL: "Novel adipocyte lines from brown fat: a model system for the study of differentiation, energy metabolism, and insulin action", BIOESSAYS, JOHN WILEY & SONS LTD, GB, vol. 24, 1 January 2002 (2002-01-01), pages 382-388, XP003005168, ISSN: 0265-9247, DOI: 10.1002/BIES.10058
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Description

RELATED APPLICATIONS

[0001] This application claims priority to US Provisional applications 61/906,087 and 61/813,771 which were filed on November 19, 2013 and April 19, 2013, respectively;

FIELD OF THE INVENTION

[0002] The invention relates generally to the field of cell culture and more specifically to the field of determining cell type, and in particular to implantable constructs comprising a scaffold and a human neonatal brown adipose tissue differentiated cell derived from an isolated human neonatal brown adipose tissue stem cell or an isolated human neonatal brown adipose tissue stem cell cultured on the scaffold.

BACKGROUND OF THE INVENTION

[0003] Brown adipose tissue (BAT) plays a key role in the evolutionarily conserved mechanisms underlying energy homeostasis in mammals. It is characterized by fat vacuoles 5-10 microns in diameter and expression of uncoupling protein 1 (UCP1), central to the regulation of thermogenesis. In the human newborn, depots of BAT are typically grouped around the vasculature and solid organs. These depots maintain body temperature during cold exposure by warming the blood before its distribution to the periphery. They also ensure an optimal temperature for biochemical reactions within solid organs. BAT had been thought to involute throughout childhood and adolescence. Recent studies, however, have confirmed the presence of active brown adipose tissue in adult humans, with depots residing in cervical, supraclavicular, mediastinal, paravertebral and/or suprarenal regions, for example. Also, it has also been reported that BAT, alternatively called beige fat can be found within white adipose tissue (WAT).

PATEL AMIT N ET AL, "Putative Population of Adipose-Derived Stem cells Isolated From Mediastinal Tissue During Cardiac Surgery", CELL TRANSPLANTATION, vol. 22, no. 3, 2 April 2012 (2012-04-02), pages 507-511, describes a population of adipose derived stem cell lines isolated from mediastinal brown adipose tissue and shows that these cells express CD44, CD105, CD166 and CD90 and are negative for CD34, CD45 and HLA-DR. Cell lines are also derived by single cell plating in 96 well plates. The cells are shown to be capable of undergoing adipogenesis, osteogenesis and chondrogenesis.

ARTURO HERNANDEZ ET AL, "Dexamethasone Inhibits Growth Factor-Induced Type 3 Deiodinase Activity and mRNA Expression in a Cultured Cell Line Derived from Rat Neonatal Brown Fat Vascular-Stromal Celly", ENDOCRINOLOGY, vol. 143, no. 7, 1 July 2002 (2002-07-01), pages 2652-2658, ISSN 0013-7227, DOI 10.1210/endo. 143.7.8923", discloses a cell line derived from vascular stromal precursor cells of brown fat.

WO 2009/156413 A1 describes a population of adipose tissue derived stem cells that are able to differentiate into different lineages such as adipocytes, osteoblasts and myocytes and that can also be induced to differentiate into brown adipocytes.

M. CIGOLINI ET AL, "Human brown adipose cells in culture", EXPERIMENTAL CELL RESEARCH, vol. 159, no. 1, 1 July 1985 (1985-07-01), pages 261-266, ISSN 0014-4827, DOI 10.1016/S0014-4827(85)80056-2, describes the isolation of a brown adipose tissue cell population from human neonates.

TIM J SCHULZ ET AL, "Identification of inducible brown adipocyte progenitors residing in skeletal muscle and white fat", vol. 108, no. 1, 4 January 2011 (2011-01-04), pages 143-148, DOI 10.1073/PNAS.1010929108, describes the isolation of a population of inducible brown adipocyte progenitor cells.

KLEIN J ET AL, "Novel adipocyte lines from brown fat: a model system, for the study of differentiation, energy metabolism, and insulin action", BIOESSAYS, JOHN WILEY & SONS LTD, GB, vol. 24, 1 January 2002 (2002-01-01), pages 382-388, ISSN 0265-9247, DOI 10.1002/BIES.10058, describes an immortalized brown adipose tissue cell line derived from brown adipose tissue from neonate mice.

PONTUS BOSTRÖM ET AL, "A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis", NATURE, vol. 481, no. 7382, 1 January 2012 (2012-01-01), pages 463-468, XP055052526, ISSN 0028-0836, DOI 10.103 8/nature.10777, teaches that FNDC5 induces a brown fat gene program and results in increased expression of fat brown specific genes, such as UCP-1, and teaches adult fat tissue derived cells.

SUMMARY

[0004] The invention relates to implantable constructs as claimed in claims 1 and 5. Further embodiments are described in the following description and dependent claims. In one embodiment an isolated human brown adipose tissue stem cell line is isolated from a one day old male neonate. In one embodiment, the isolated human brown adipose tissue stem cell line expresses markers such as CREB1, DIO2, IRS1, MAPK14, NRF1, FOXC2, PPARD, PGC1-A, PGC1-B, PRDM16, SRC, UCP1, and WNT5A. In another embodiment, the cells also express higher levels of anti-white adipose tissue genes, such as GATA2, KLF2, and KLF3. In still another embodiment, the isolated human brown adipose tissue stem cell line is capable of differentiating into osteoblasts, chondrocytes, and adipocytes. In still yet another embodiment, the line is derived from a one day old male neonate.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] The patent or application file contains at least

one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee. The present teachings described herein will be more fully understood from the following description of various illustrative embodiments, when read together with the accompanying drawings. It should be understood that the drawings described below are for illustration purposes only and are not intended to limit the scope of the present teachings in anyway.

Fig. 1 is a PET scan of 18F-FDG uptake in an adult;

Fig. 2(a) is a photograph of mediastinal adipose tissue from a neonate;

Fig. 2(b) is a photograph of mediastinal adipose tissue from a 56 year old;

Fig. 2(c) is a photograph of mediastinal adipose tissue from an 80 year old;

Fig. 2(d) is a photomicrograph of a section of the tissue of Fig. 2(a);

Fig. 2(e) is a photomicrograph of a section of the tissue of Fig. 2(b);

Fig. 2(f) is a photomicrograph of a section of the tissue of Fig. 2(c);

Fig. 3(a) is a series of flowcytometry and immunocytochemistry graphs for cell surface markers on neonatal brown adipose derived stem cells;

Fig. 3(b) is a table of comparisons of expression levels for various markers for neonatal brown adipose derived stem cells and white adipose derived stem cells;

Fig. 3(c) is a gene expression profile for neonatal brown adipose derived stem cells (BADSC) and white adipose derived stem cells (WADSC);

Fig. 4(a) is a scanning electron microscopy (SEM) of brown adipose derived stem cells cultured on porous extracellular matrix scaffolds;

Fig. 4(b) is an SEM of directionally differentiated brown adipocytes on scaffolds;

Fig. 5(a) is a map of a first transformation construct used to immortalize neonatal brown adipose derived stem cells;

Fig. 5(b) is a map of a second transformation construct used to immortalize neonatal brown adipose derived stem cells;

Fig. 5(c) is a map of a third transformation construct used to immortalize neonatal brown adipose derived stem cells;

Fig. 5(d) is a map of a fourth transformation construct used to immortalize neonatal brown adipose derived stem cells;

Fig. 5(e) is a map of a fifth transformation construct used to immortalize neonatal brown adipose derived stem cells;

Fig. 6 is a graph of cell growth rate by passage;

Fig. 7 is a graph of telomerase activity for transfected and control cells;

Fig. 8 is a photomicrograph of transfected BADSC showing normal cell morphology;

Fig. 9 is a photomicrograph of BADSC having undergone white, brown adipogenesis;

Fig. 10 is a photomicrograph of BADSC having undergone osteogenesis;

Fig. 11 is a photomicrograph of BADSC having undergone chondrogenesis;

Fig. 12(a) is a graph of glucose levels of mice transplanted with BADSC/scaffolds and controls;

Fig. 12(b) is a graph of the weights of mice transplanted with BADSC/scaffolds and controls; and

Fig. 13 is a depiction of the steps for high throughput analysis of compounds.

DETAILED DESCRIPTION

[0006] A population of metabolically active brown adipose tissue stem cells has been isolated from a one day old male new born. The stem cell population, termed "neonate brown adipose derived stem cells" (neonate BADSCs) demonstrated the potential to: (1) be expanded *in vitro*; (2) exhibit multi-lineage potential; and (3) functionally differentiate into metabolically active brown adipocytes. Such a stem cell population could offer new cell-based means to restore and enhance energy homeostasis *in vivo* for the treatment of obesity and related metabolic disorders. These stem cells also are a useful tool for studying adipose tissue biology.

[0007] In order to identify a stem cell population within newborn mediastinal brown adipose depots, explants were generated and plated into tissue culture plates. Adherent cells were successfully derived from the brown adipose tissue explants, these primary cell cultures were fed every 3 days in media comprising DMEM low glucose,

1× Glutamax, 1× NEAA, and 10% platelet lysate. In order to define a clonal population of cells, cell lines were derived by single-cell plating in 96 well plates. Confluency was reached at 6 days and the cells exhibited Mesenchymal Stem Cell (MSC)-like morphology, as depicted in Fig. 8. Mediastinal brown adipose tissue from a 56 year old and an 80 year old are shown in Figs. 2b and 2c, respectively, for comparison with the newborn mediastinal brown adipose tissue, as depicted in Fig. 2a. The decrease in the brown adipose tissue is evident. Figs. 2d-2f are photomicrographs of H/E sections taken of the biopsied tissues of Figs. 2a-2c, respectively.

[0008] Growth kinetics of the clonal cell population demonstrated that the population could be propagated for greater than 90 passages. Karyotyping of the clonal cell population at passage 7 demonstrated normal diploid cells without chromosomal aberrations.

[0009] Referring to Figs. 3(a) and (3b), to further characterize these neonate BADSC, flow cytometry, and immunocytochemistry were used. The neonate BADSC were found to exhibit characteristics that are similar to other MSCs but are not identical. For instance, the neonate BADSC were positive for CD9, SSEA4, CD44, CD90, CD166, and CD73, but were negative for hematopoietic markers CD14, CD34, and CD45. Furthermore, the neonate BADSC did not express STRO-1, which has been previously found to be expressed in mesenchymal stem cells derived from various tissues.

[0010] An analysis of the gene expression profiles of passage 2 neonate BADSC demonstrate that the neonate BADSC have a distinct gene expression profile, as depicted in Fig. 3(c), in comparison with white adipose derived stem cells. The genes whose expression is enriched in BADSC include pro-brown adipose selective genes such as, for example, CREB1, DIO2, IRS1, MAPK14, NRF1, FOXC2, PPARD, PGC1-A, PGC1-B, PRDM16, SRC, UCP1, and WNT5A. The cells also express higher levels of anti-white adipose tissue genes, such as GATA2, KLF2, and KLF3. Importantly, expression of these pro-brown selective genes can distinguish brown adipose derived stem cells from stem cells derived from white adipose depots.

[0011] Passage 2 neonate BADSC were induced to differentiate into osteo, chondro, white, and brown adipogenic cell lineages to determine multi-lineage potential. After three weeks of induction the cells demonstrated the ability to differentiate into osteoblasts, chondrocytes, and adipocytes. When induced to differentiate under osteogenic promoting conditions, the cells formed a mineralized matrix, which was confirmed by alizarin red staining; immunocytochemistry staining of osteocalcin, and RT-PCR analysis for osteopontin, osteonectin, and alkaline-phosphatase, further confirmed differentiation. Chondrogenic differentiation was confirmed by alcian blue staining for sulfated proteoglycans on induced cell pellets. RT-PCR confirmed expression of collagen 2A, biglycan, and A6, which are markers of chondrogenic differentiation. White adipogenic differentiation was confirmed by Oil Red O staining of lipid droplets. Immunocytochemistry confirmed expression of FABP4, and RT PCR confirmed expression of FABP4, LPL, and PPARγ, which are markers of adipogenic differentiation.

[0012] Real-time qPCR of neonatal brown adipose-differentiated cells demonstrated upregulation of UCP1, elongation of very long chain fatty acids like-3 (ELOVL3) and peroxisome proliferator-activated receptor γ 1-α (PGC1A), a major regulator of mitochondrial biogenesis, compared to non-FNDC5 differentiated cells. Conversely, leptin--a gene associated with white fat development--is down regulated in brown-adipose differentiated cells. Higher levels of expression of these brown adipocyte marker genes are consistent with a mature brown adipocyte fate. These findings demonstrate that brown adipose depots from newborns are a source of stem cells that have unique properties than stem cells found in adult brown adipose depots, subcutaneous adipose and visceral adipose depots, and have the ability to differentiate into multiple cell types.

[0013] BADSC in general can be grown on scaffolding as, as depicted in Fig. 4(a), and thereby made more easily handled for implantation and other experimentation.

[0014] Also disclosed, but not part of the invention, various BADSC lines were immortalized by transfection. In one embodiment a BADSC150 line was used.

[0015] Five different plasmid constructs were created, as depicted in Figs. 5(a)-5(e). These were a 7286 bp construct termed Blas-T, encoding an EEF1 promoter driving TERT expression, a 4868 bp construct termed Blas-B, encoding an EEF1 promoter driving BMI-1 expression; an 8348 bp construct termed Blas-B-F-T, encoding an EEF1 promoter driving both BMI1 and TERT expression, an 8348 bp construct termed pBlas-BIT encoding an EEF1a1 promoter driving both TERT and BsrS2, and a 16,243 bp construct termed pUCP1-CP-BfT encoding an EEF1A1 promoter driving expression of BMI1 and TERT and UCP1 promoter driving expression of a reporter gene cherry picker.

[0016] In one embodiment, the transfection used Lipofectamine LTX and PLUS reagent. In a second embodiment, the transfection used Fugene HD, Xfect Adult Stem Cell Transfection Reagent and Lipofectamine LTX with PLUS reagent according to manufacturers recommended protocol. Each reagent was tested for efficiency. Fugene HD was the least toxic to the cells and resulted in the highest transfection efficiency for BADSC150 primary cells transfected with construct Blas-BFT encoding EEF1 promoter driving expression of BMI1 and TERT (BMI1-TERT FMDV2-self processing polypeptide and TERT-BMI1 FMDV2-self processing polypeptide). Because the two orientations of the self processing polypeptides will function with differing efficiencies depending upon the cell line, both forms were tested in any given experiment.

[0017] In other embodiments, which are not part of the invention the BADSC150 line was transfected with the following combination of constructs listed below. The

transfected cells with the blastocidin resistance gene were selected with a concentration of 6ug/ml. blastocidin. A stable immortalized brown adipose derived stem cell line was generated and tested for functional differentiation potential.

[0018] Under EEF1A1 control, all four resistance options were available:

pUNO-hpf Hygromycin;
pUNO-pur Puromycin;
pUNO-zeo Zeocin; and
pUNO-bla Blasticidin

[0019] The following single reporter systems were used:

*mCherry alone;
*NanoLuc (NL) alone; and
*Secreted NanoLuc (sNL) alone.

[0020] The following combination Reporter systems were also used:

CP1-NL FMDV2-self processing polypeptide;
CP1-sNL FMDV2-self processing polypeptide;
NL-CP1 FMDV2-self processing polypeptide; and
sNL-CP1 FMDV2-self processing polypeptide.

[0021] In other experiments, the EEF1 promoter was also replaced with pro-brown (i.e. PRDM16, PGC-1α, C/EBPβ, Plac8 and UCP-1) and pro-white specific genes (i.e. PPARγ, C/EBPα, and AKT-1) used with different combinations of the reporter systems. All of these constructs contain EEF1 promoter driving expression of BMI1 and TERT.

[0022] Once the BDSC150 cell line was transfected and selected, it was tested to determine the effects of extended passages. BADSC150 BMI1-TERT FMDV2-self processing polypeptide (Fig. 5(d)) and TERT-BMI1 FMDV2-self processing polypeptide were passaged to p20 (Fig. 6). Cells expressed telomerase activity (Fig. 7) which was not present in the control (non-transfected BADSC150) and retained normal cell morphology (Fig. 8).

[0023] Also disclosed but not part of the invention, Immortalized BADSC150 cells were also tested to determine their ability to functionally differentiate. Cells were induced to undergo white, brown adipogenesis (Fig. 9), osteogenesis (Fig. 10) and chondrogenesis (Fig. 11). This cell line with the different combinations of these constructs can be used to screen libraries of small molecules and brown adipose inducers (i.e. FNDC5, BMP7, retinoic acid).

[0024] In one combination, where the construct is UCP1-CherryPicker BfT, (Fig. 5(e)), EEF1A1 is driving the expression of Bmi1 and Tert1. This construct is used to immortalize the cells. UCP1 is driving the expression of CherryPicker, chimeric membrane-anchored CherryPicker fluorescent protein, which can be monitored via fluorescence microscopy and be captured on magnetic beads using a specific antibody. In this embodiment, BADSC150 cells with this construct are immortal and CherryPicker is only activated when UCP1 is induced. The induction of UCP1 can be accomplished by exposing this cell population to either naturally occurring or synthetic small molecules that up-regulate brown adipose tissue specific genes.

[0025] In another embodiment, CherryPicker is replaced with secreted nanoluc (sNL). In this construct, sNL is synthesized only when the promoter is turned "ON". In one embodiment, the promoter is UCP1. Since sNL is secreted from the cell and into the cell culture media, one is able to assay the media for levels of sNL for a quantitative analysis of how efficacious the compound is for inducing UCP1 to turn "on" and in turn produce sNL. This system allows for high throughput screening of thousands of small molecules at different concentrations at one time.

[0026] Also disclosed, but not part of the invention, all combinations of constructs also have been transfected into white adipose stem cells for the purpose of identifying molecules that convert white fat into brown or increase metabolic activity. This cell line, in tandem with its reporter systems, permits for the effective study of the kinetics of brown fat biology; specifically, the mechanism underlying UCP1 up-regulation and increased metabolic activity. This human brown fat stem cell line is valuable due to the fact that the amino acid composition of mouse and human UCP1 is less than 80%, thereby rendering mouse brown fat cell lines inadequate for identifying compounds that activate brown fat specific genes

[0027] In addition, a population of immortal implantable BADSC would provide a population of brown adipose tissue that can help regulate metabolism in humans. Referring to Figs. 12(a) and 12(b), the two graphs show the effects of BADSC on scaffolds implanted in mice. Fig. 12(a) shows that in the weeks after implantation, the basal blood glucose levels for control mice fed with a high calorie diet are significantly higher (about 66%) than mice fed with the same diet but implanted with BADSC. Similarly, in Fig. 12(b) the body weight for the control mice is about 40% higher than the body weight of the mice with an implant. This indicates that BADSC may be useful in treating metabolic disorders such as diabetes and obesity.

[0028] A cell culture plate comprises human neonatal brown adipose derived cells. The plate can be, for example, a multiwell plate. One or more wells of the cell culture plate can be seeded with human neonatal brown adipose derived cells. The cells can be differentiated brown fat cells. The cells also can be immortalized. The cell culture plates are useful for screening drug compounds by contacting the cells with a candidate drug compound and observing the effect of the drug compound on the human neonatal brown adipose derived cell.

[0029] Referring to Fig. 13, such a screening method

for various potential drugs begins by plating wells with either transfected BADSC cells (such as BADSC150FS.UCP1-sNL-BfT) or white adipose stem cells (such as WADSCFS.UCP1-sNL-Bft) (Step 1). Then the compounds of interest are added to the well (Step 2) and the wells are read in a plate reader and the media assayed for secreted nanoluc (Step 3), for example. The presence of nanoluc indicates that the drug has activated the stem cells.

[0030] It is to be understood that the figures and descriptions of the invention have been simplified to illustrate elements that are relevant for a clear understanding of the invention, while eliminating, for purposes of clarity, other elements. Those of ordinary skill in the art will recognize, however, that these and other elements may be desirable. However, because such elements are well known in the art, and because they do not facilitate a better understanding of the invention, a discussion of such elements is not provided herein. It should be appreciated that the figures are presented for illustrative purposes and not as construction drawings. Omitted details and modifications or alternative embodiments are within the purview of persons of ordinary skill in the art.

[0031] The invention may be embodied in other specific forms. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are intended to be embraced therein.

Claims

1. An implantable construct **characterized by**:

   a scaffold; and
   a human neonatal brown adipose tissue differentiated cell derived from an isolated human neonatal brown adipose tissue stem cell;
   wherein the isolated human neonatal brown adipose tissue stem cell is grown in a culture medium comprising fibronectin type III domain-containing protein 5 (FNDC5) and differentiates into the human neonatal brown adipose differentiated cell; and
   wherein UCP-1, ELOVL3, and PGC1A gene expression is upregulated in the human neonatal brown adipose tissue differentiated cell compared to non-FNDC5 differentiated cells.

2. The implantable construct of claim 1, wherein the scaffold comprises a porous extracellular matrix.

3. The implantable construct of claim 1, wherein the human neonatal brown adipose tissue differentiated cell is a human brown adipocyte.

4. The implantable construct of any of the preceding claims, for use in treating a metabolic disorder, for example obesity and/or diabetes, in a patient, wherein the implantable construct is implanted in the patient.

5. An implantable construct **characterized by**:

   a scaffold; and
   an isolated human neonatal brown adipose tissue stem cell cultured on the scaffold;
   wherein the isolated human neonatal brown adipose tissue stem cell is cultured in a culture medium comprising fibronectin type III domain-containing protein 5 (FNDC5) and differentiates into a human neonatal brown adipose tissue differentiated cell;
   wherein UCP-1, ELOVL3, and PGC1A gene expression is upregulated in the human neonatal brown adipose tissue differentiated cell compared to non-FNDC5 differentiated cells.

6. The implantable construct of claim 5, for use in treating a metabolic disorder, for example obesity and/or diabetes, in a patient, wherein the implantable construct is implanted in the patient.

Patentansprüche

1. Implantierbares Konstrukt, **gekennzeichnet durch**:

   ein Gerüst; und
   eine differenzierte Zelle des menschlichen neonatalen braunen Fettgewebes, die von einer isolierten Stammzelle des menschlichen neonatalen braunen Fettgewebes abgeleitet ist;
   wobei die isolierte Stammzelle des menschlichen neonatalen braunen Fettgewebes in einem Kulturmedium gezüchtet wird, das Fibronectin Type III Domain-Containing Protein 5 (FNDC5) umfasst, und in die differenzierte menschliche neonatale braune Fettzelle differenziert; und
   wobei eine UCP-1-, ELOVL3- und PGC1A-Genexpression in der differenzierten Zelle des menschlichen neonatalen braunen Fettgewebes im Vergleich zu nicht-FNDC5-differenzierten Zellen hochreguliert ist.

2. Implantierbares Konstrukt nach Anspruch I, wobei das Gerüst eine poröse extrazelluläre Matrix umfasst.

3. Implantierbares Konstrukt nach Anspruch I, wobei die differenzierte Zelle des menschlichen neonatalen braunen Fettgewebes ein menschlicher brauner Adipozyt ist.

4. Implantierbares Konstrukt nach einem der vorhergehenden Ansprüche zur Verwendung bei der Behandlung einer Stoffwechselstörung, beispielsweise Fettleibigkeit und/oder Diabetes, in einem Patienten, wobei das implantierbare Konstrukt in den Patienten implantiert wird.

5. Implantierbares Konstrukt, **gekennzeichnet durch**:

ein Gerüst; und
eine isolierte Stammzelle des menschlichen neonatalen braunen Fettgewebes, die auf dem Gerüst gezüchtet wird;
wobei die isolierte Stammzelle des menschlichen neonatalen braunen Fettgewebes in einem Kulturmedium gezüchtet wird, das Fibronectin Type III Domain-Containing Protein 5 (FNDC5) umfasst, und in eine differenzierte Zelle des menschlichen neonatalen braunen Fettgewebes differenziert;
wobei eine UCP-1-, ELOVL3- und PGC1A-Genexpression in der differenzierten Zelle des menschlichen neonatalen braunen Fettgewebes im Vergleich zu nicht-FNDC5-differenzierten Zellen hochreguliert ist.

6. Implantierbares Konstrukt nach Anspruch 5 zur Verwendung bei der Behandlung einer Stoffwechselstörung, beispielsweise Fettleibigkeit und/oder Diabetes, in einem Patienten, wobei das implantierbare Konstrukt in den Patienten implantiert wird.

Revendications

1. Construction implantable **caractérisée par** :

un échafaudage ; et
une cellule différenciée de tissu adipeux brun néonatale humaine dérivée d'une cellule souche de tissu adipeux brun néonatale humaine ;
dans laquelle la cellule souche de tissu adipeux brun néonatale humaine isolée est mise à croître dans un milieu de culture comprenant de la protéine 5 contenant le domaine de fibronectine type III (FNDC5) et se différencie en la cellule différenciée adipeuse brune néonatale humaine ; et
dans laquelle une expression de gène UCP-1, ELOVL3 et PGC1A est régulée à la hausse dans la cellule différenciée de tissu adipeux brun néonatale humaine en comparaison à des cellules différenciées non-FNDC5.

2. Construction implantable selon la revendication 1, dans laquelle l'échafaudage comprend une matrice extracellulaire poreuse.

3. Construction implantable selon la revendication 1, dans laquelle la cellule différenciée de tissu adipeux brun néonatale humaine est un adipocyte brun humain.

4. Construction implantable selon l'une quelconque des revendications précédentes, pour utilisation dans le traitement d'un trouble métabolique, par exemple l'obésité et/ou le diabète, chez un patient, dans laquelle la construction implantable est implantée chez le patient.

5. Construction implantable **caractérisée par** :

un échafaudage ; et
une cellule souche de tissu adipeux brun néonatale humaine isolée cultivée sur l'échafaudage ;
dans laquelle la cellule souche de tissu adipeux brun néonatale humaine isolée est cultivée dans un milieu de culture comprenant de la protéine 5 contenant le domaine de fibronectine type III (FNDC5) et se différencie en une cellule différenciée de tissu adipeux brun néonatale humaine ;
dans laquelle l'expression de gène UCP-1, ELOVL3 et PGC1A est régulée à la hausse dans la cellule différenciée de tissu adipeux brun néonatale humaine en comparaison à des cellules différenciées non-FNDC5.

6. Construction implantable selon la revendication 5, pour utilisation dans le traitement d'un trouble métabolique, par exemple l'obésité et/ou le diabète, chez un patient, dans laquelle la construction implantable est implantée chez le patient.

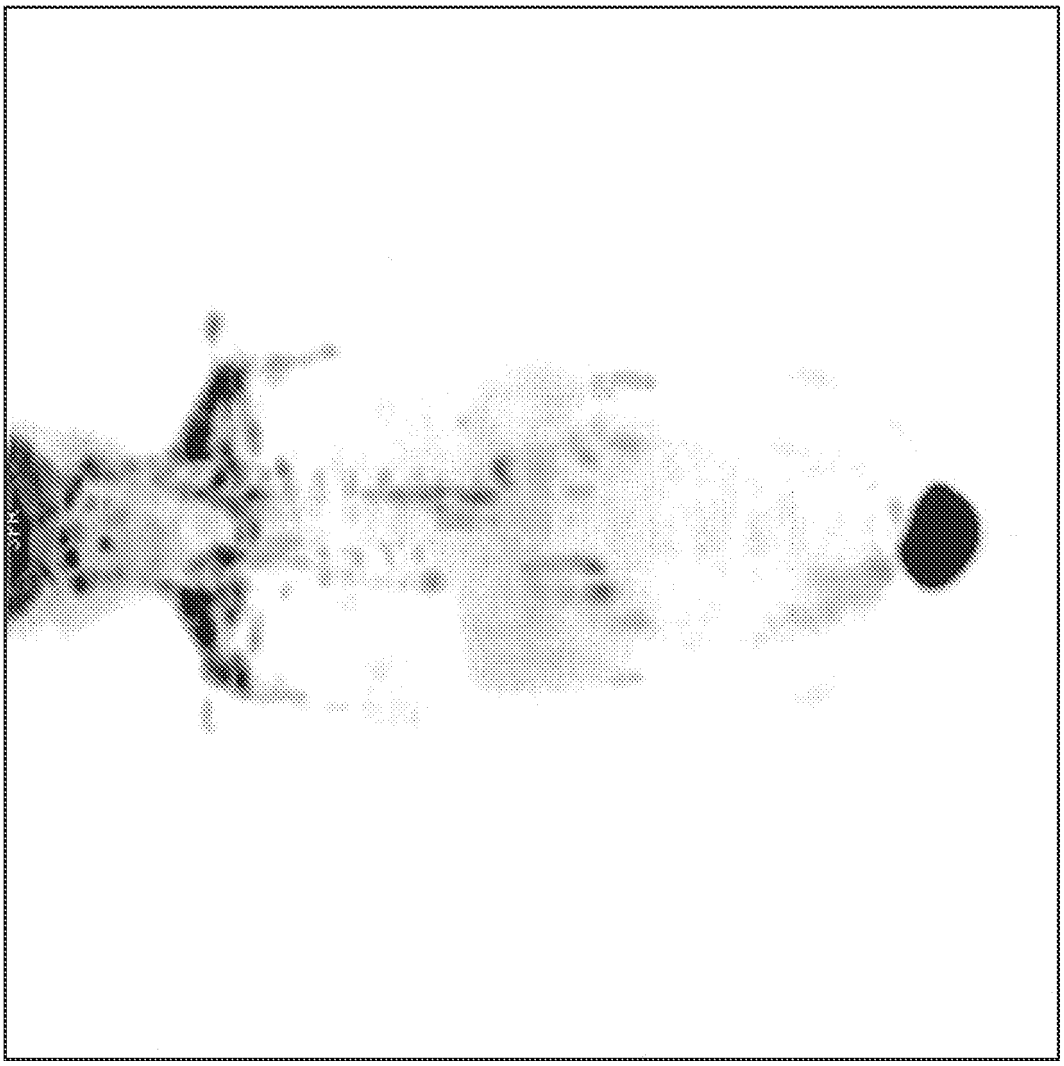

FIG. 1

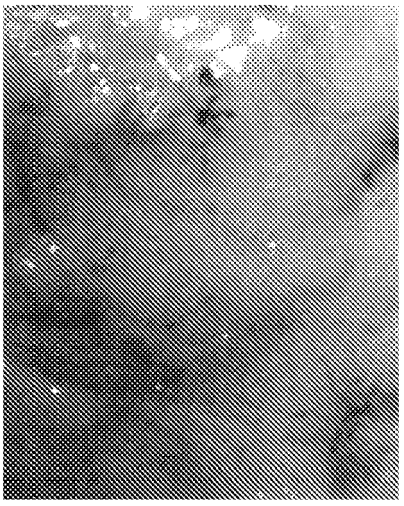

FIG. 2B

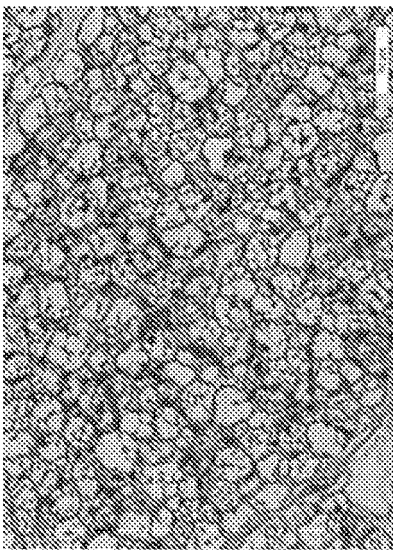

FIG. 2D

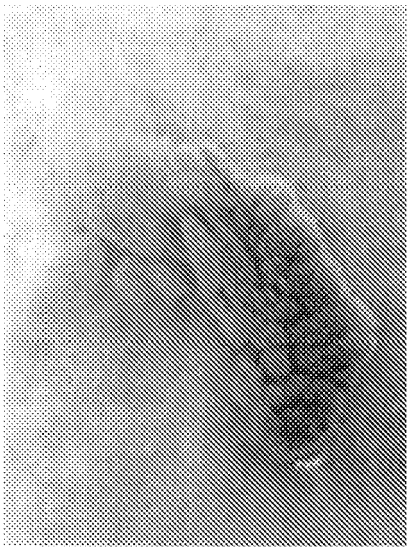

FIG. 2A

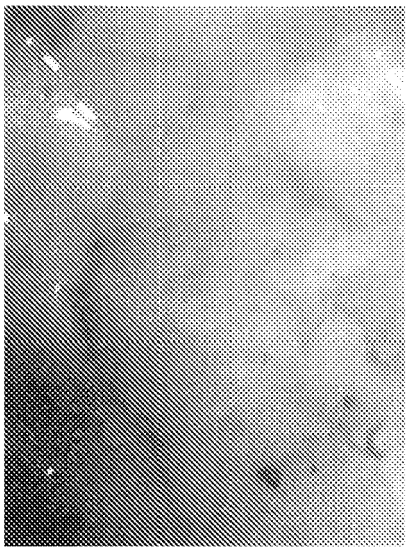

FIG. 2C

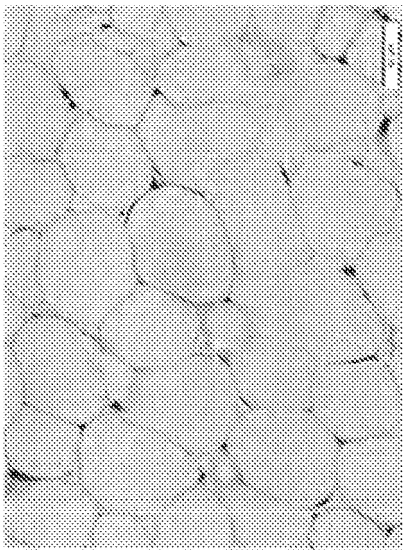

FIG. 2F

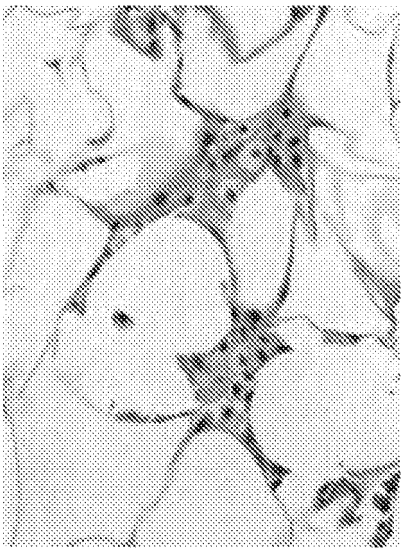

FIG. 2E

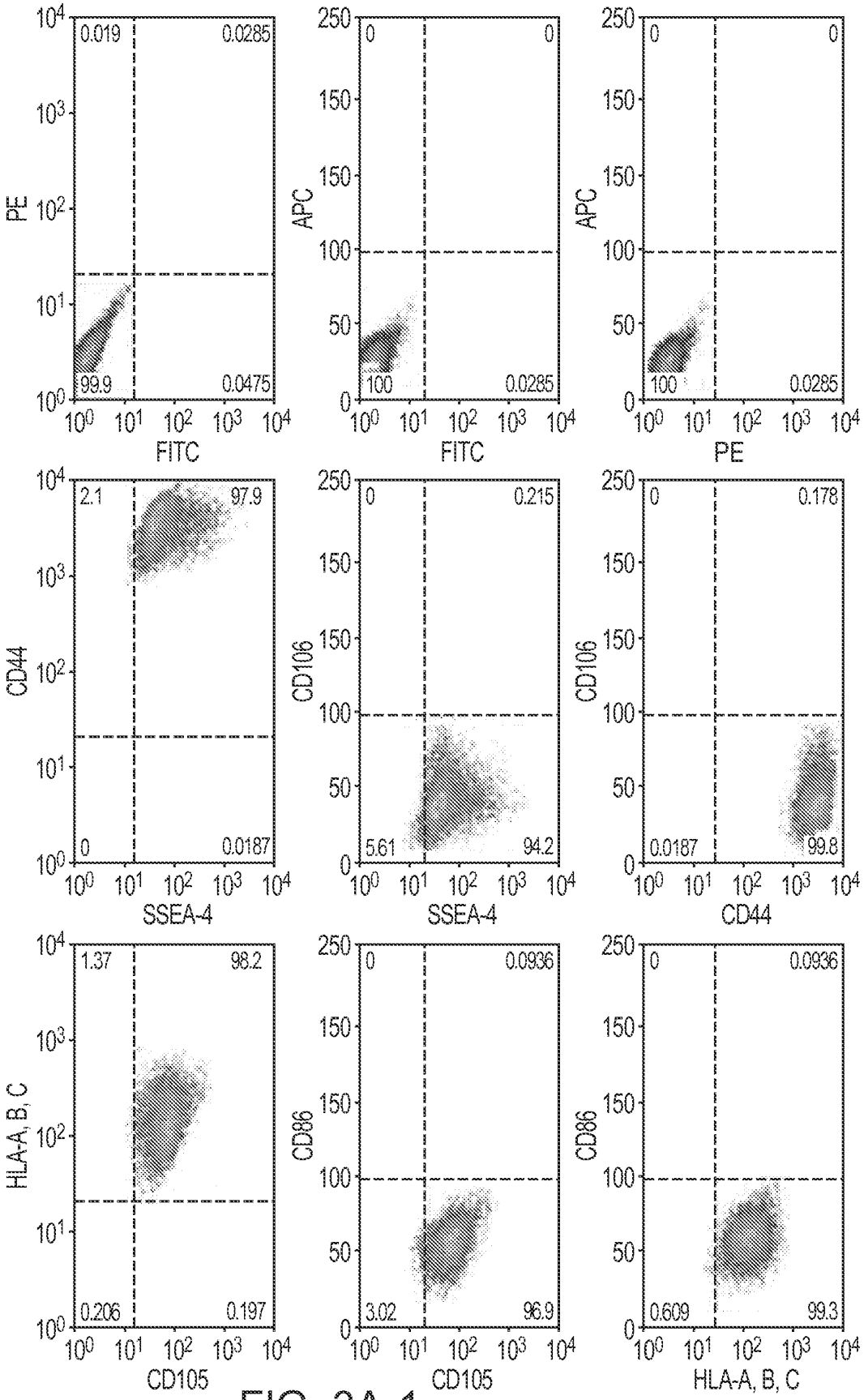
PE
FITC
0.019
0.0285
99.9
0.0475
APC
FITC
0
0
100
0.0285
APC
PE
0
0
100
0.0285
CD44
SSEA-4
2.1
97.9
0
0.0187
CD106
SSEA-4
0
0.215
5.61
94.2
CD106
CD44
0
0.178
0.0187
99.8
HLA-A, B, C
CD105
1.37
98.2
0.206
0.197
CD86
CD105
0
0.0936
3.02
96.9
CD86
HLA-A, B, C
0
0.0936
0.609
99.3

FIG. 3A-1

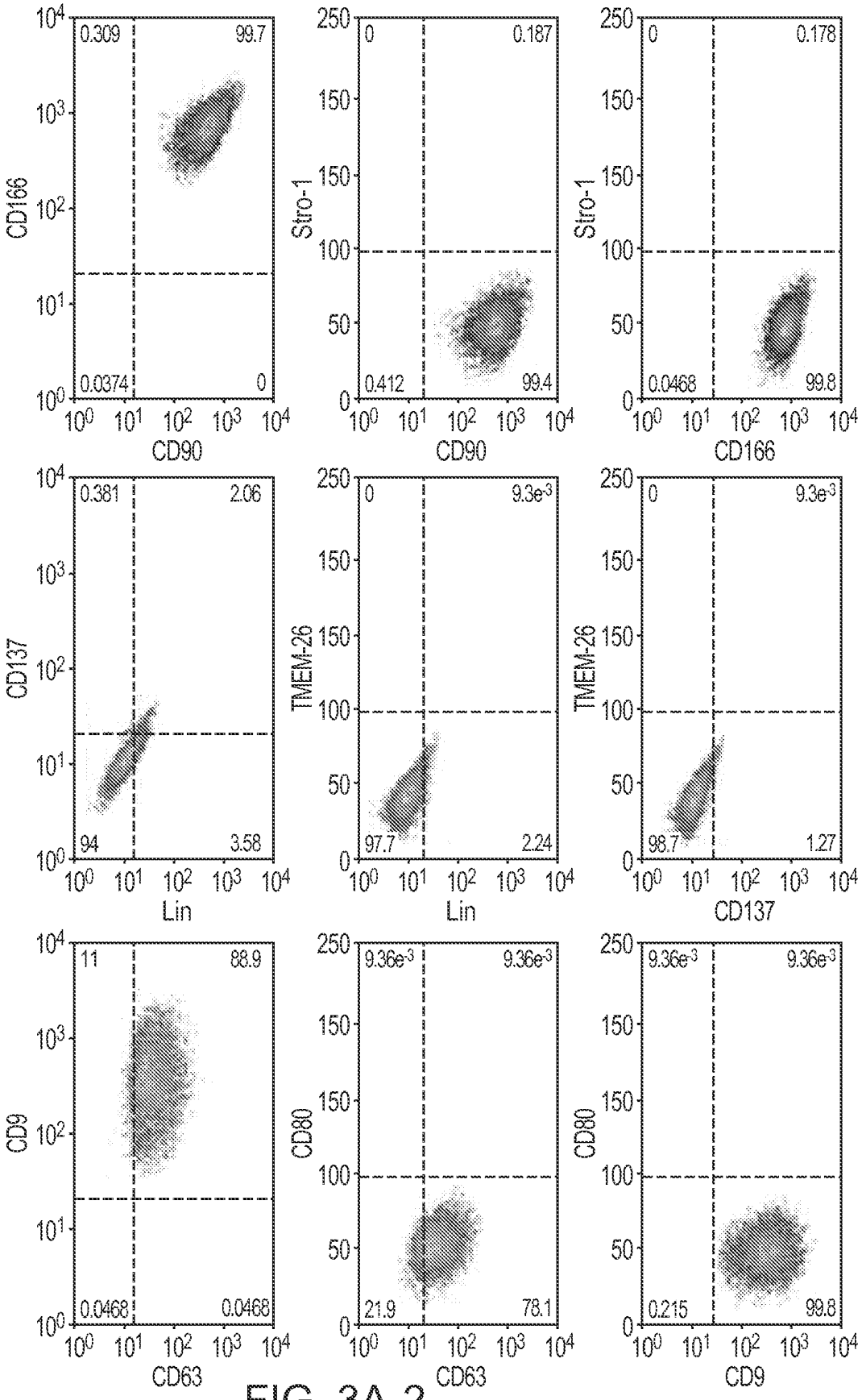
CD166
CD90
0.309
99.7
0.0374
0
Stro-1
CD90
0
0.187
0.412
99.4
Stro-1
CD166
0
0.178
0.0468
99.8
CD137
Lin
0.381
2.06
94
3.58
TMEM-26
Lin
0
9.3e-3
97.7
2.24
TMEM-26
CD137
0
9.3e-3
98.7
1.27
CD9
CD63
11
88.9
0.0468
0.0468
CD80
CD63
9.36e-3
9.36e-3
21.9
78.1
CD80
CD9
9.36e-3
9.36e-3
0.215
99.8

FIG. 3A-2

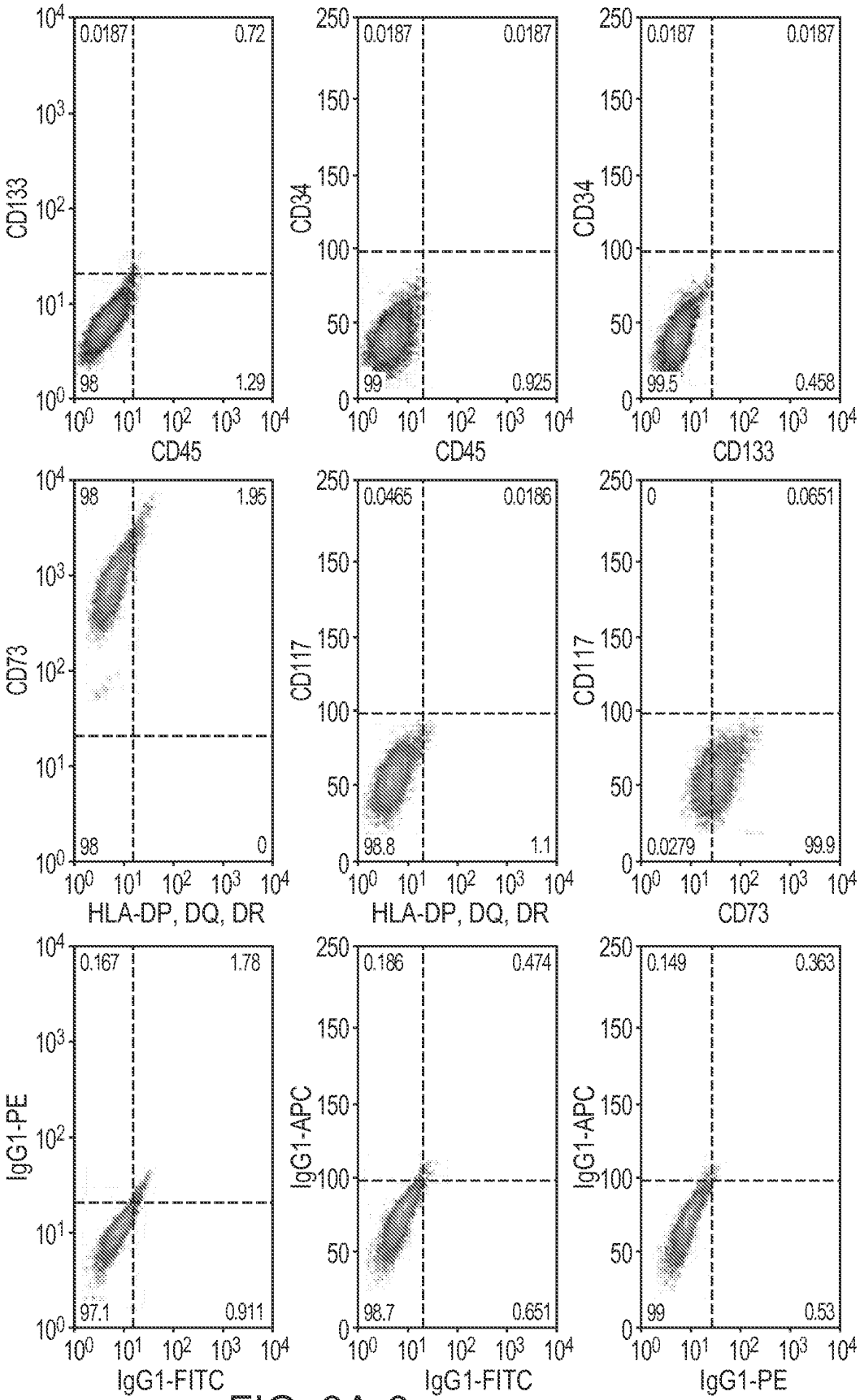
CD133
CD45
0.0187
0.72
98
1.29
CD34
CD45
0.0187
0.0187
99
0.925
CD34
CD133
0.0187
0.0187
99.5
0.458
CD73
HLA-DP, DQ, DR
98
1.95
98
0
CD117
HLA-DP, DQ, DR
0.0465
0.0186
98.8
1.1
CD117
CD73
0
0.0651
0.0279
99.9
IgG1-PE
IgG1-FITC
0.167
1.78
97.1
0.911
IgG1-APC
IgG1-FITC
0.186
0.474
98.7
0.651
IgG1-APC
IgG1-PE
0.149
0.363
99
0.53

FIG. 3A-3

	CD90	CD166	STRO-1	CD45	CD133	CD34	SSEA-4	CD44	CD106	CD80
BADSC	99.6	100	0	0	0	0	97.7	100	0	0
WADSC	98.9	98.9	1.3	1	0.7	2	8.8	100	1.6	2

	LIN	DR,DP,DQ	CD73	CD117	CD105	A,B,C	CD86	CD63	CD9
BADSC	1.4	0	100	0	98.3	100	0	86	100
WADSC	11	0.5	99	1	68.3	86	2	99	95

FIG. 3B

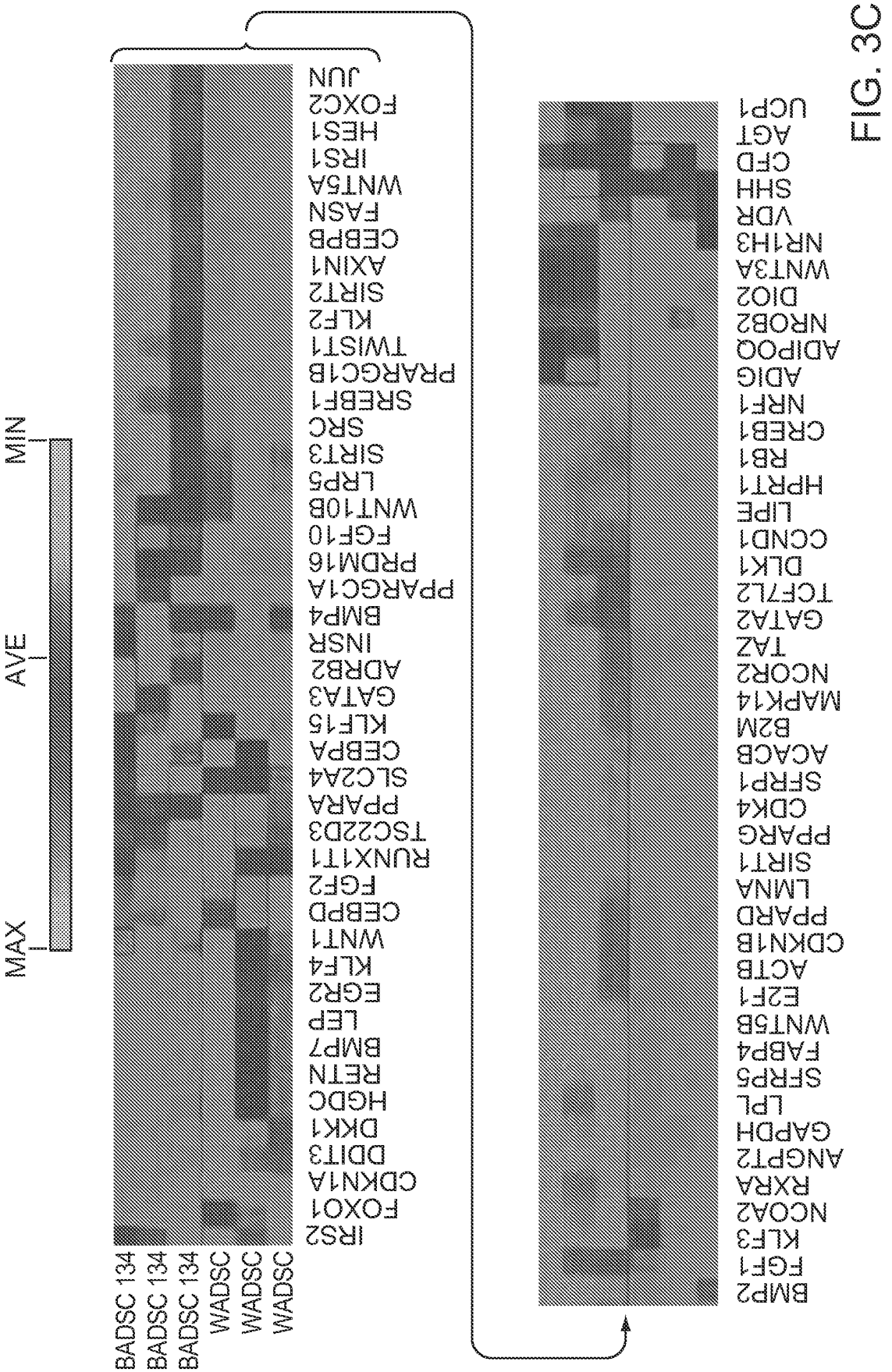
MAX
AVE
MIN
BADSC 134
BADSC 134
BADSC 134
WADSC
WADSC
WADSC
IRS2
FOXO1
CDKN1A
DDIT3
DKK1
HGDC
RETN
BMP7
LEP
EGR2
KLF4
WNT1
CEBPD
FGF2
RUNX1T1
TSC22D3
PPARA
SLC2A4
CEBPA
KLF15
GATA3
ADRB2
INSR
BMP4
PPARGC1A
PRDM16
FGF10
WNT10B
LRP5
SIRT3
SRC
SREBF1
PRARGC1B
TWIST1
KLF2
SIRT2
AXIN1
CEBPB
FASN
WNT5A
IRS1
HES1
FOXC2
JUN
BMP2
FGF1
KLF3
NCOA2
RXRA
ANGPT2
GAPDH
LPL
SFRP5
FABP4
WNT5B
E2F1
ACTB
CDKN1B
PPARD
LMNA
SIRT1
PPARG
CDK4
SFRP1
ACACB
B2M
MAPK14
NCOR2
TAZ
GATA2
TCF7L2
DLK1
CCND1
LIPE
HPRT1
RB1
CREB1
NRF1
ADIG
ADIPOQ
NROB2
DIO2
WNT3A
NR1H3
VDR
SHH
CFD
AGT
UCP1

FIG. 3C

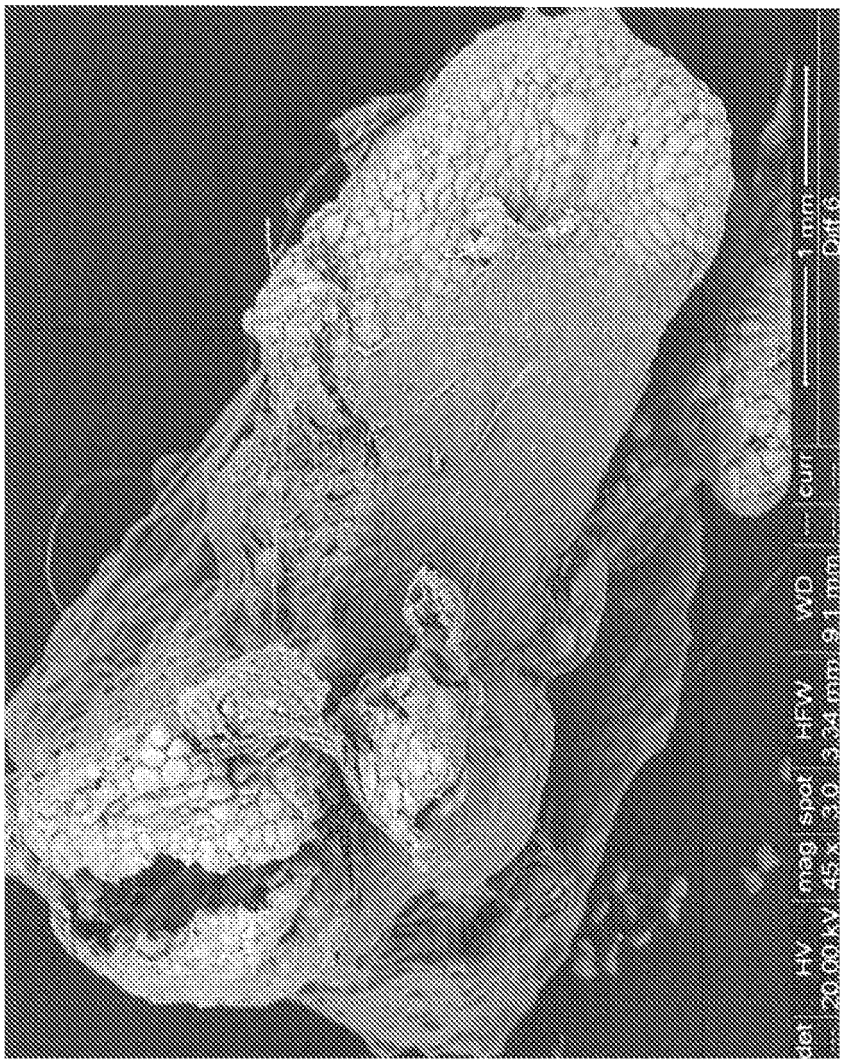

FIG. 4A

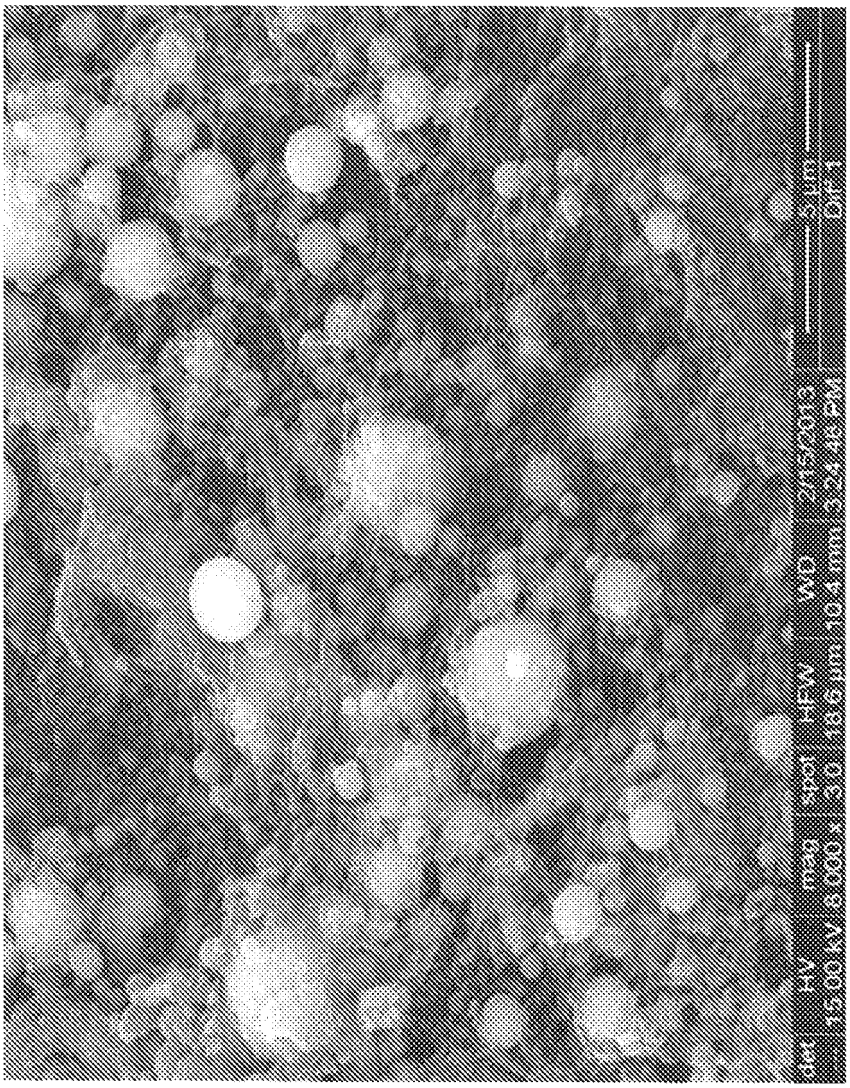

FIG. 4B

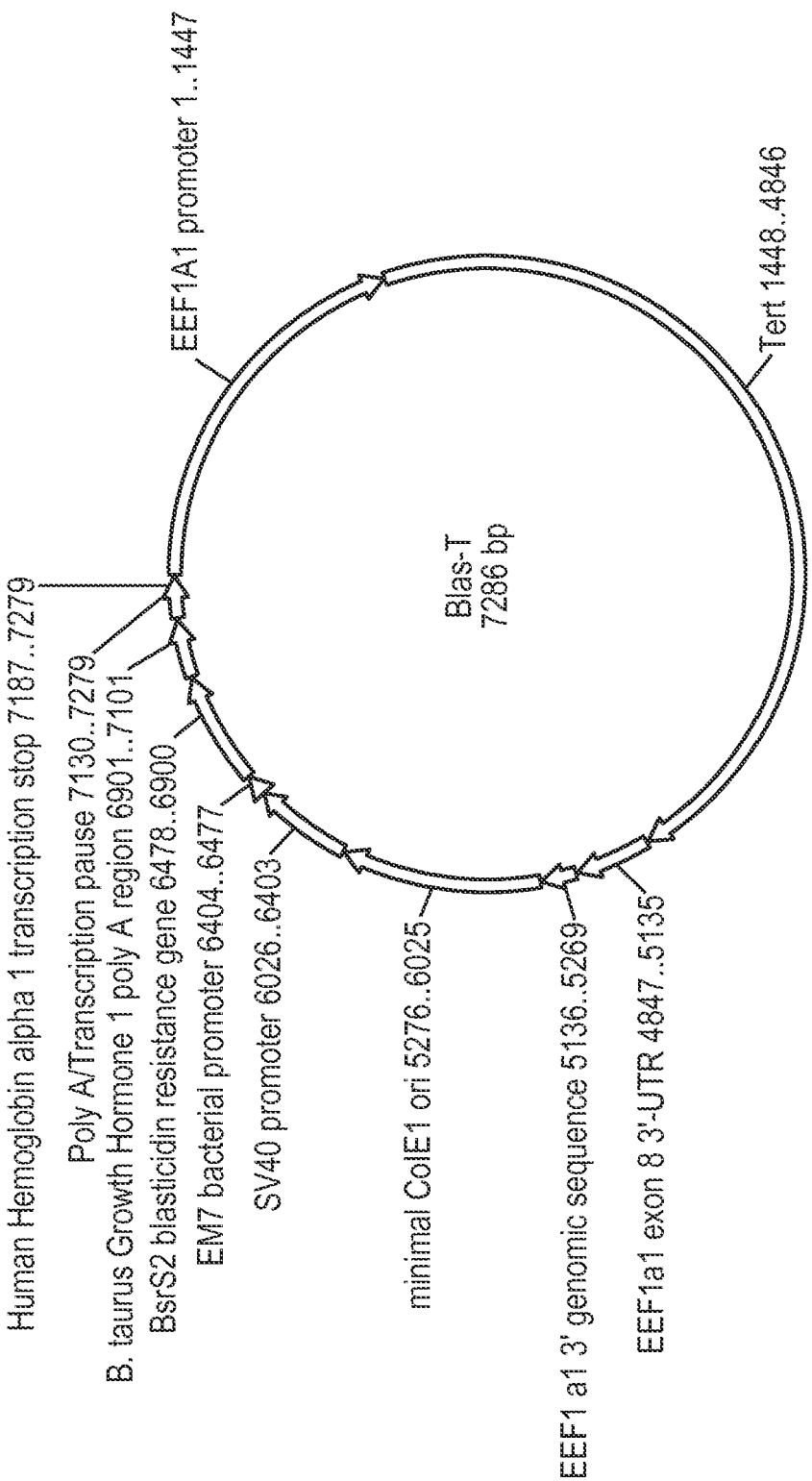
Human Hemoglobin alpha 1 transcription stop 7187..7279
Poly A/Transcription pause 7130..7279
B. taurus Growth Hormone 1 poly A region 6901..7101
BsrS2 blasticidin resistance gene 6478..6900
EM7 bacterial promoter 6404..6477
SV40 promoter 6026..6403
minimal ColE1 ori 5276..6025
EEF1 a1 3' genomic sequence 5136..5269
EEF1a1 exon 8 3'-UTR 4847..5135
Tert 1448..4846
EEF1A1 promoter 1..1447
Blas-T
7286 bp

FIG. 5A

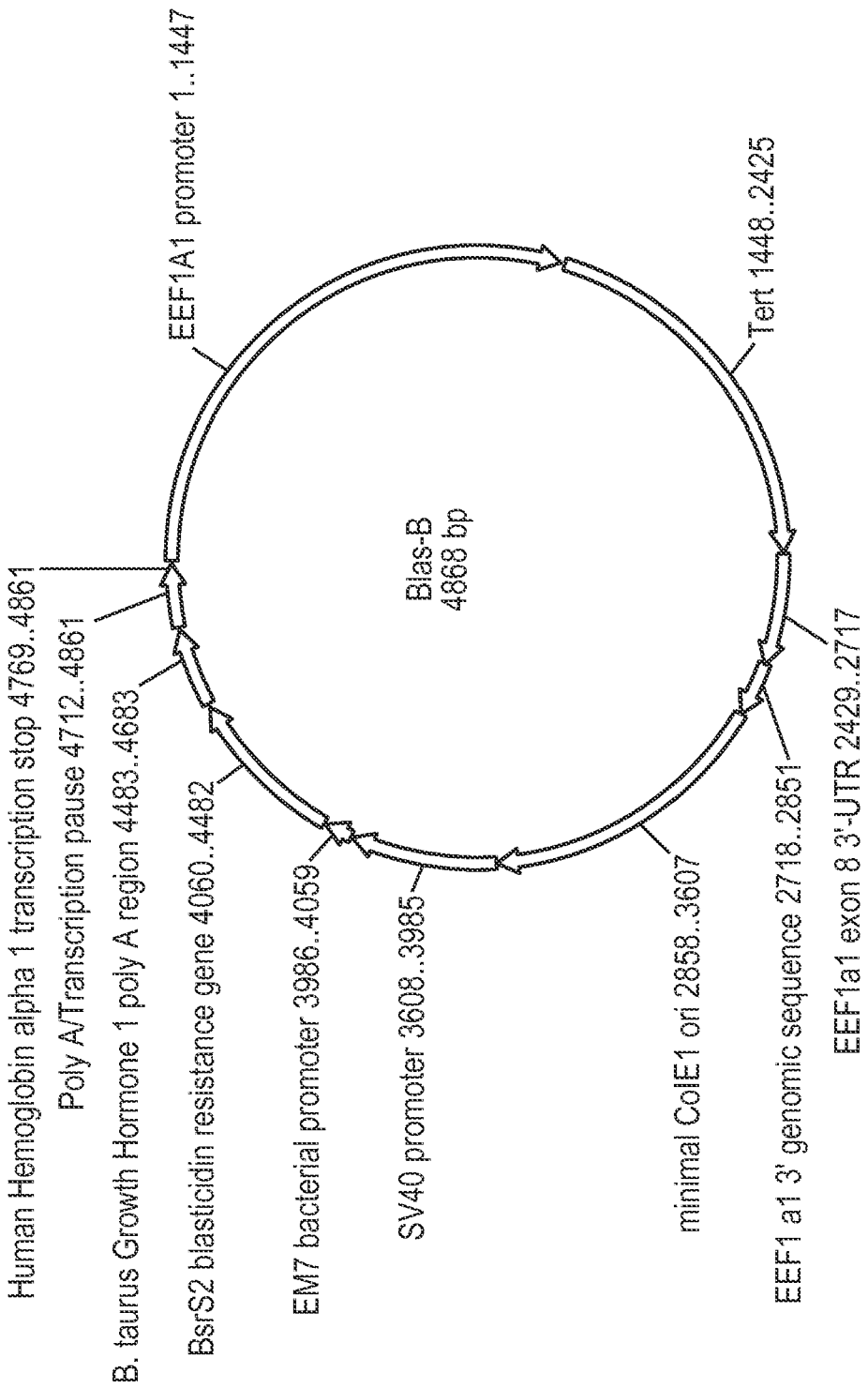
Blas-B
4868 bp
EEF1A1 promoter 1..1447
Tert 1448..2425
EEF1a1 exon 8 3'-UTR 2429..2717
EEF1 a1 3' genomic sequence 2718..2851
minimal ColE1 ori 2858..3607
SV40 promoter 3608..3985
EM7 bacterial promoter 3986..4059
BsrS2 blasticidin resistance gene 4060..4482
B. taurus Growth Hormone 1 poly A region 4483..4683
Poly A/Transcription pause 4712..4861
Human Hemoglobin alpha 1 transcription stop 4769..4861

FIG. 5B

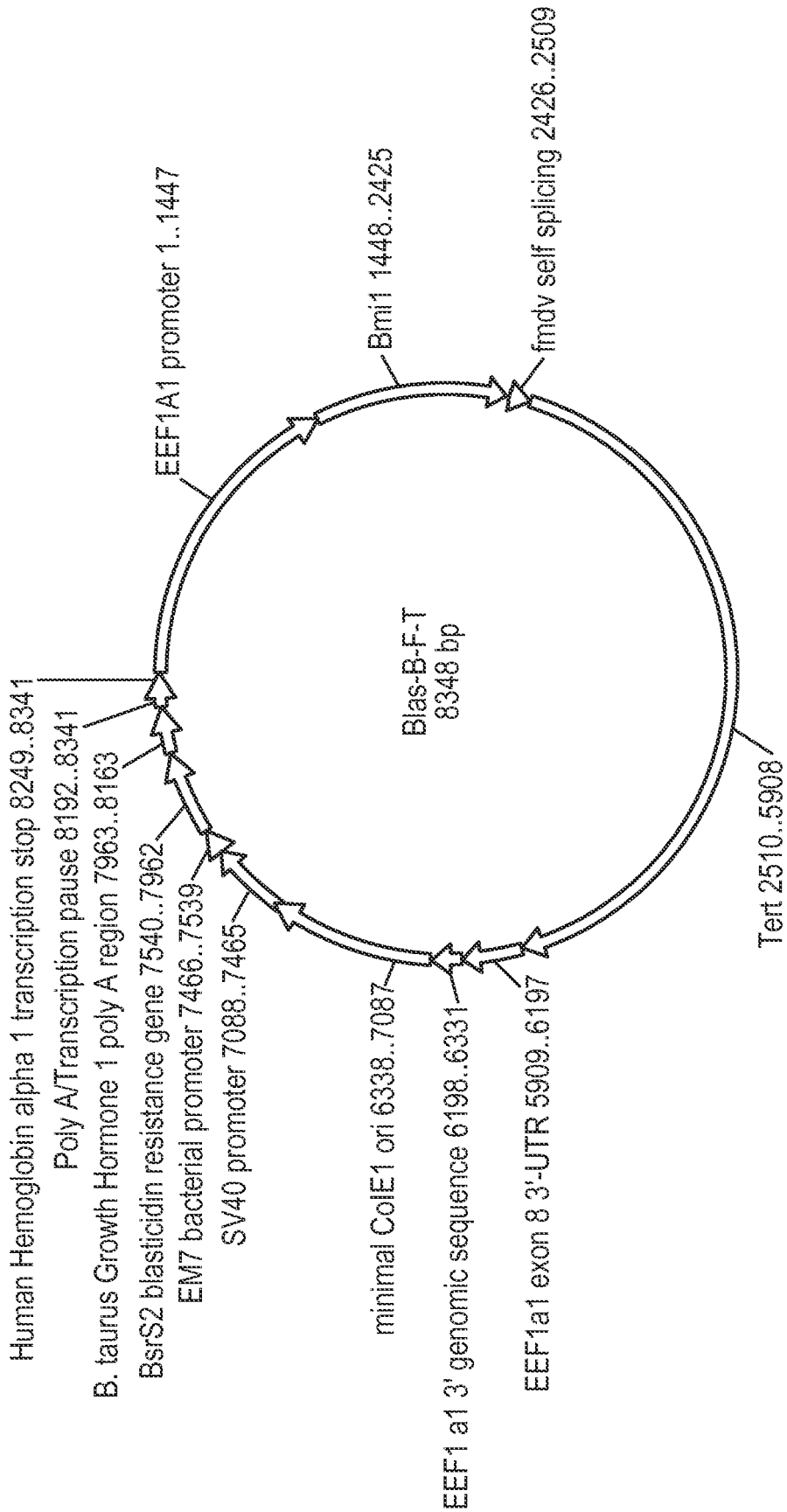
FACS DATA
Human Hemoglobin alpha 1 transcription stop 8249..8341
Poly A/Transcription pause 8192..8341
B. taurus Growth Hormone 1 poly A region 7963..8163
BsrS2 blasticidin resistance gene 7540..7962
EM7 bacterial promoter 7466..7539
SV40 promoter 7088..7465
minimal ColE1 ori 6338..7087
EEF1 a1 3' genomic sequence 6198..6331
EEF1a1 exon 8 3'-UTR 5909..6197
Tert 2510..5908
EEF1A1 promoter 1..1447
Bmi1 1448..2425
fmdv self splicing 2426..2509
Blas-B-F-T
8348 bp

FIG. 5C

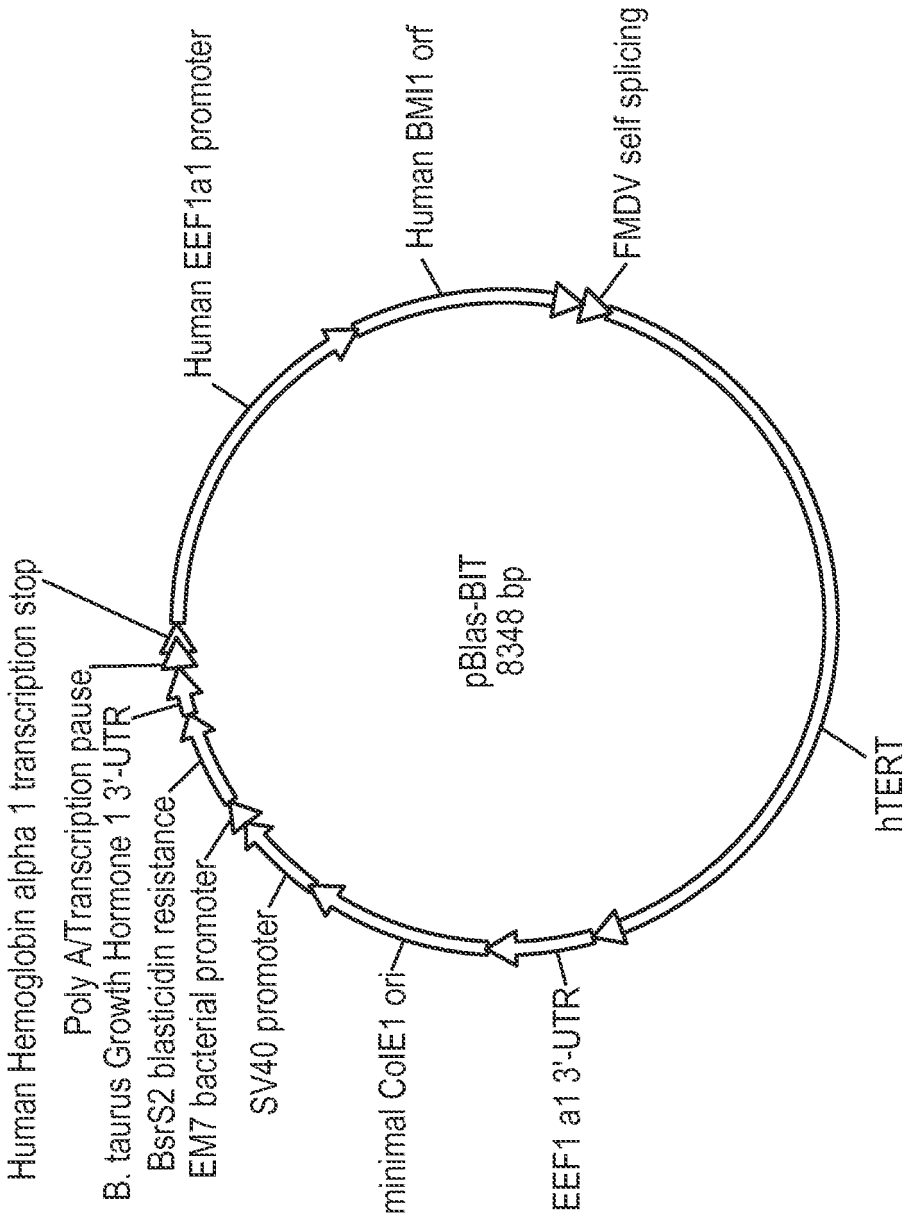
pBlas-BIT
8348 bp
Human EEF1a1 promoter
Human BMI1 orf
FMDV self splicing
hTERT
EEF1 a1 3'-UTR
minimal ColE1 ori
SV40 promoter
EM7 bacterial promoter
BsrS2 blasticidin resistance
B. taurus Growth Hormone 1 3'-UTR
Poly A/Transcription pause
Human Hemoglobin alpha 1 transcription stop

FIG. 5D

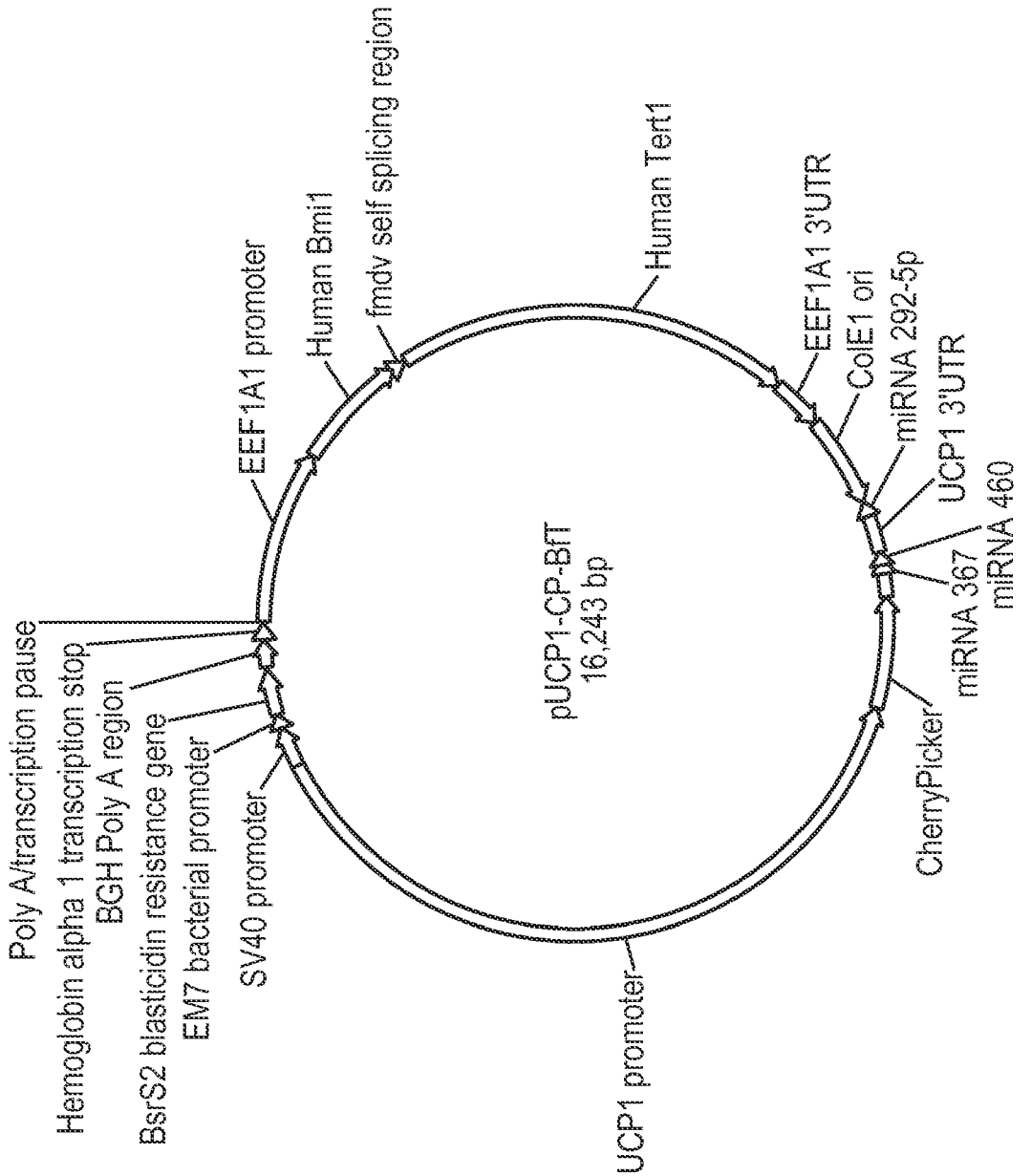
pUCP1-CP-BfT
16,243 bp
Poly A/transcription pause
Hemoglobin alpha 1 transcription stop
BGH Poly A region
BsrS2 blasticidin resistance gene
EM7 bacterial promoter
SV40 promoter
EEF1A1 promoter
Human Bmi1
fmdv self splicing region
Human Tert1
EEF1A1 3'UTR
ColE1 ori
miRNA 292-5p
UCP1 3'UTR
miRNA 460
miRNA 367
CherryPicker
UCP1 promoter

FIG. 5E

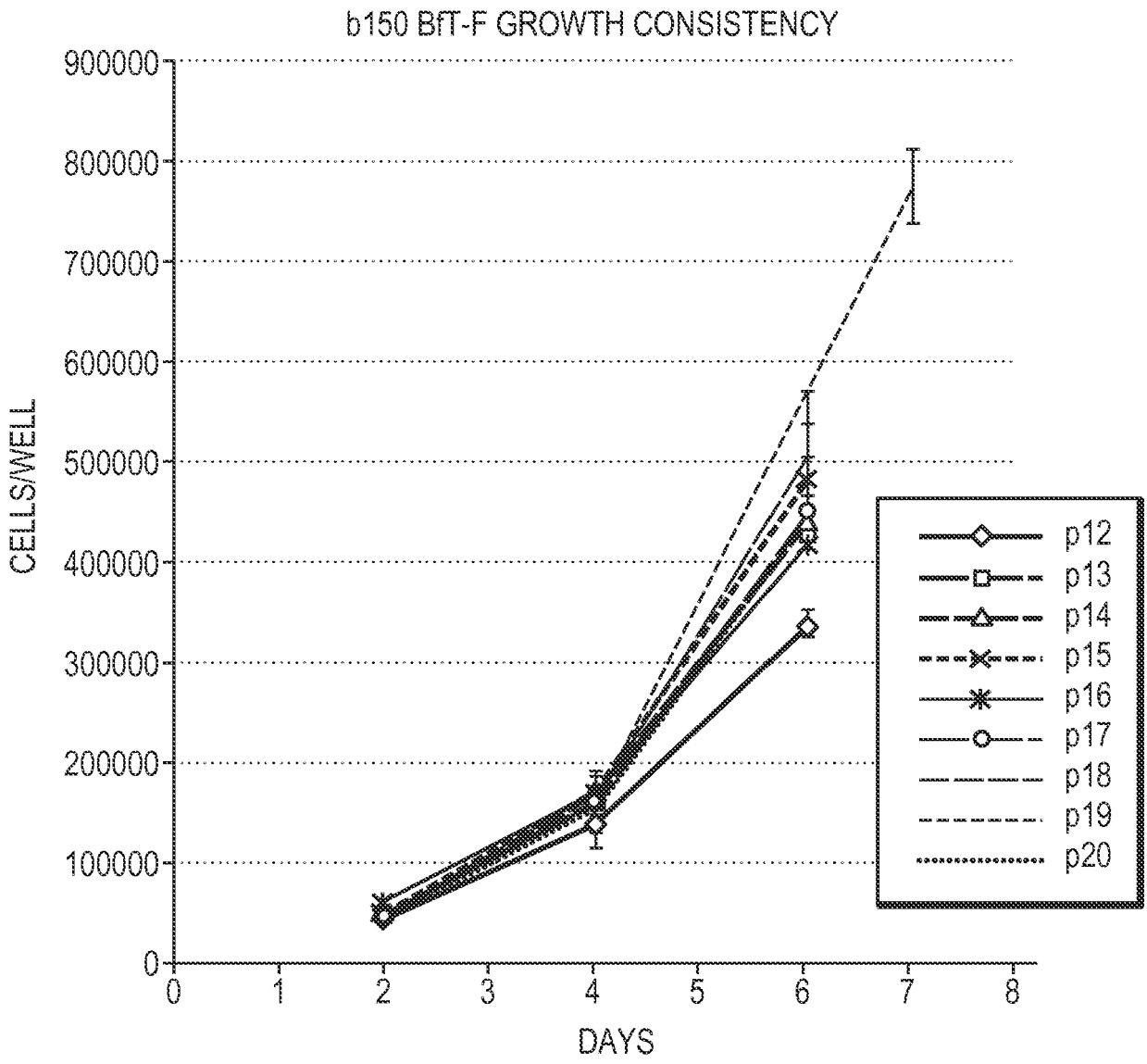
b150 BfT-F GROWTH CONSISTENCY
CELLS/WELL
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
0
1
2
3
4
5
6
7
8
DAYS
p12
p13
p14
p15
p16
p17
p18
p19
p20

FIG. 6

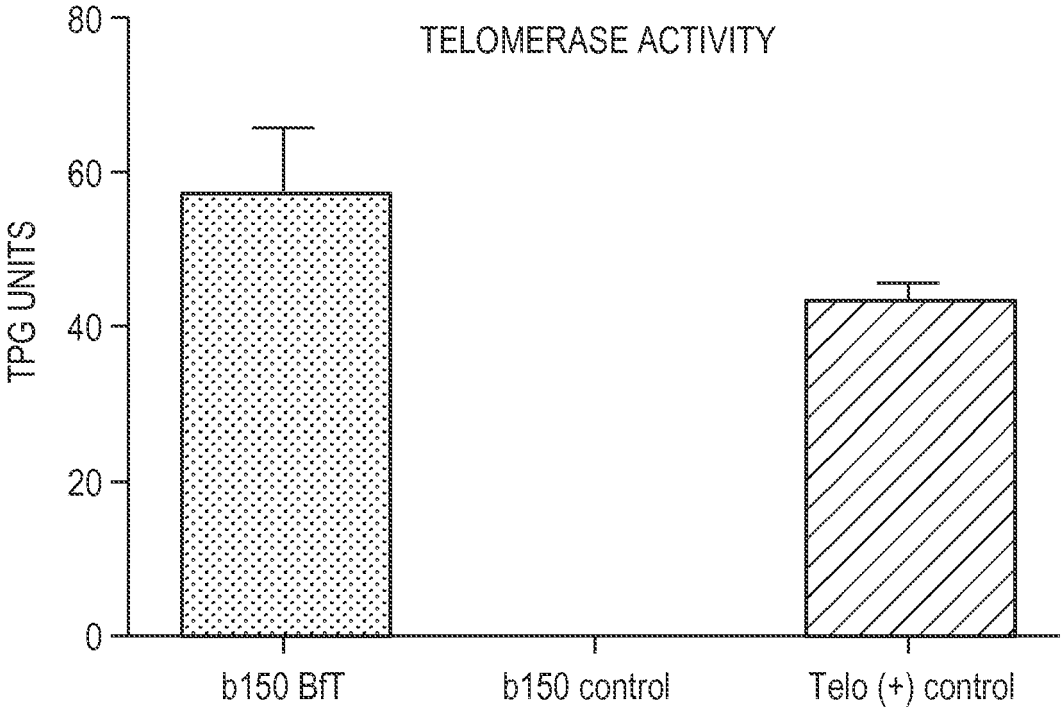
TELOMERASE ACTIVITY
TPG UNITS
80
60
40
20
0
b150 BfT
b150 control
Telo (+) control

FIG. 7

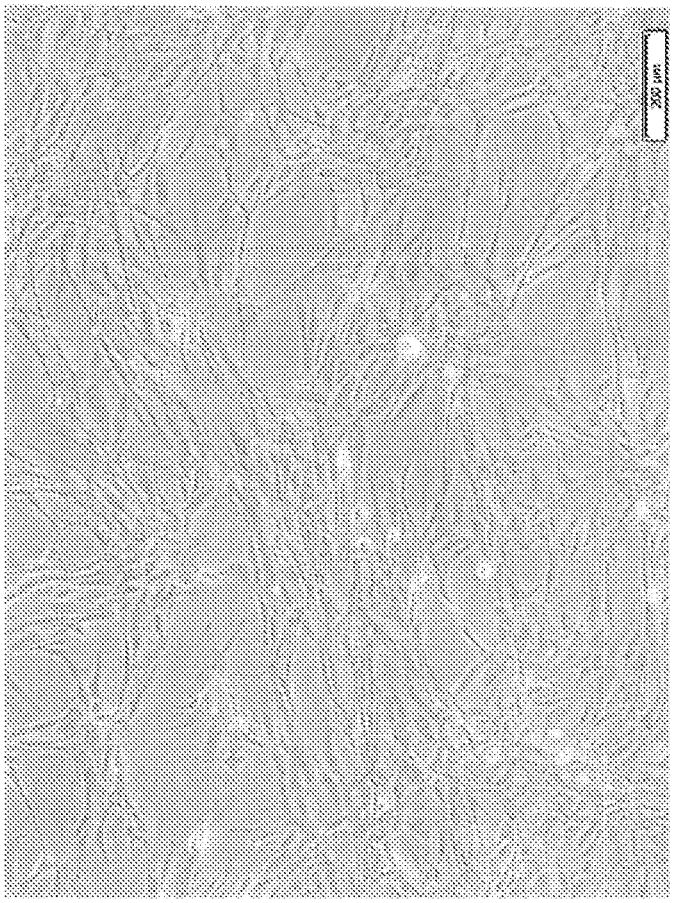

FIG. 8

FIG. 9

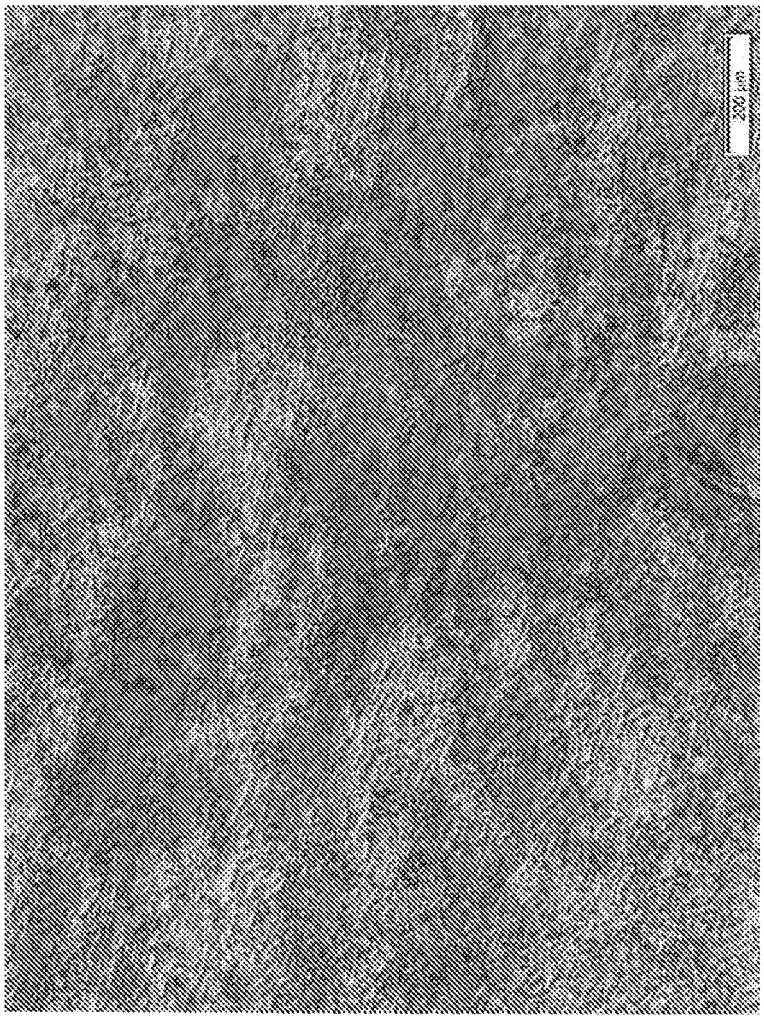

FIG. 10

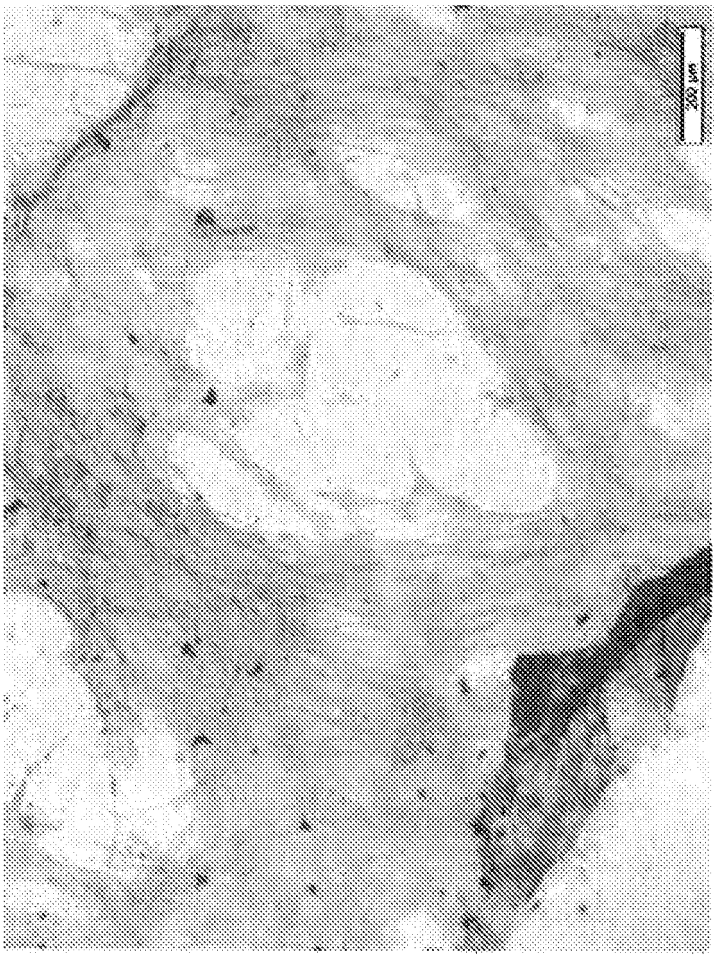

FIG. 11

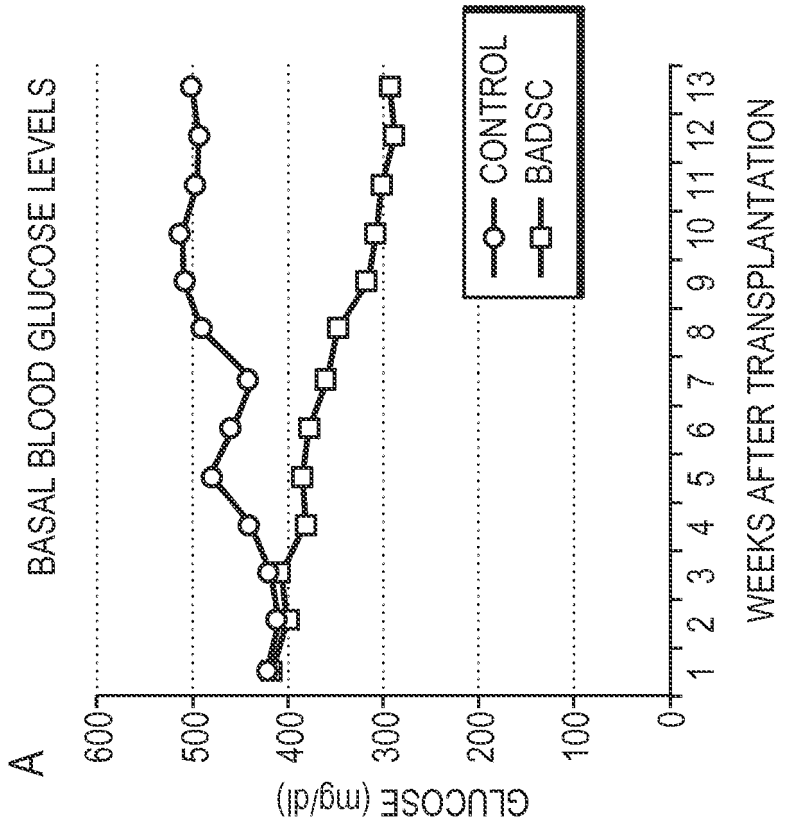

FIG. 12A

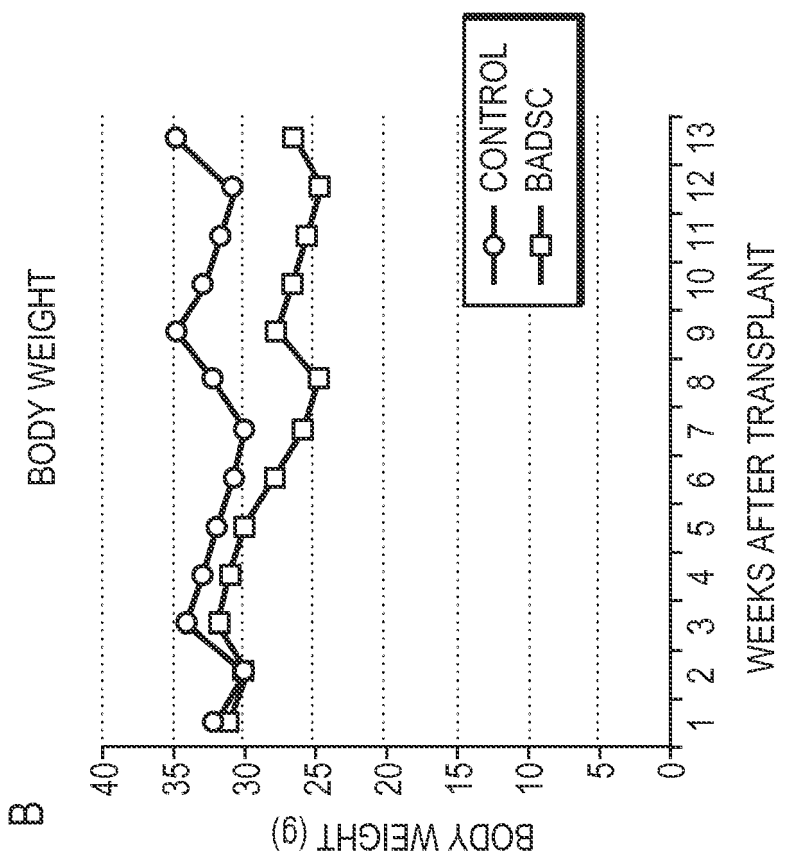

FIG. 12B

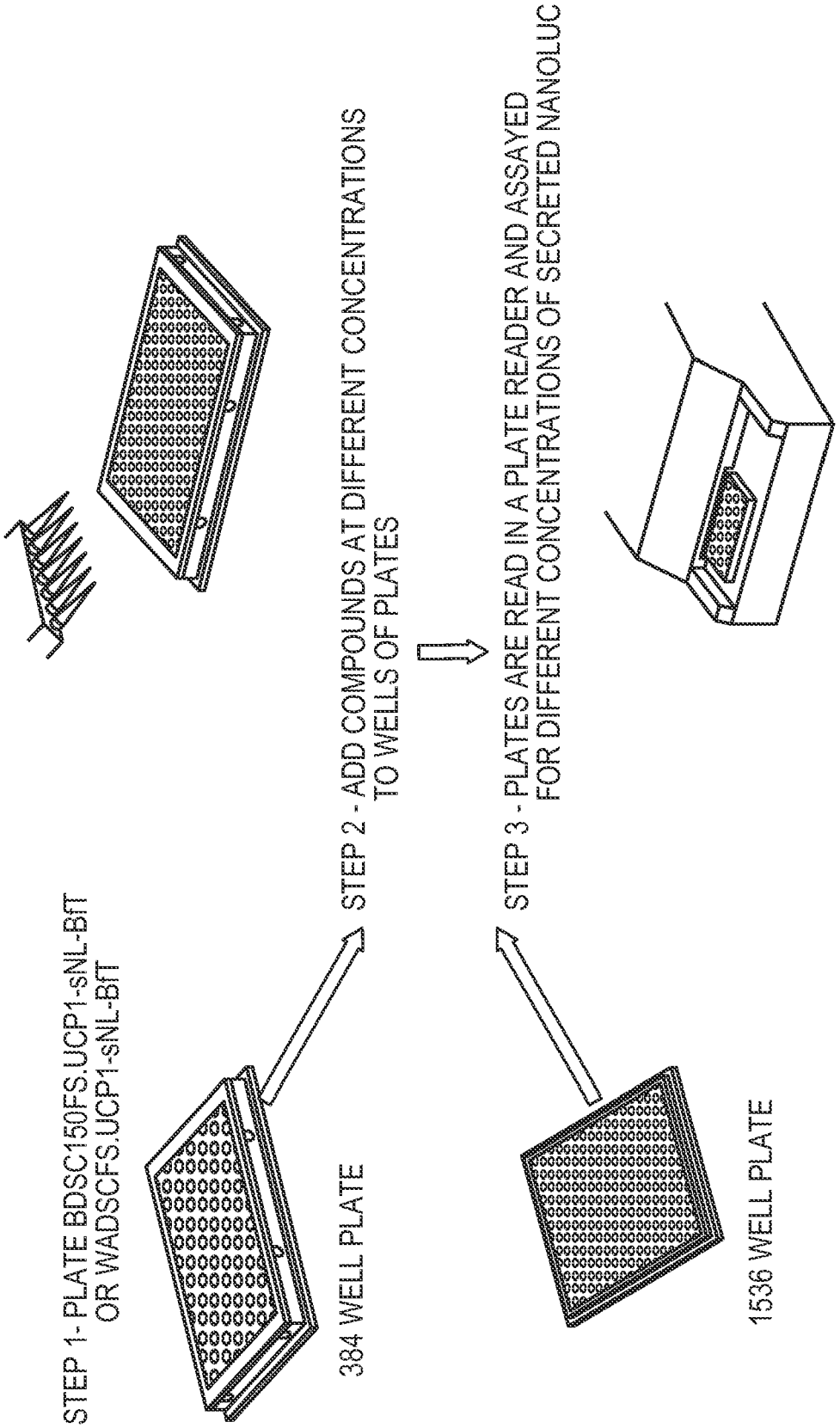
STEP 1- PLATE BDSC150FS.UCP1-sNL-BfT
OR WADSCFS.UCP1-sNL-BfT
384 WELL PLATE
STEP 2 - ADD COMPOUNDS AT DIFFERENT CONCENTRATIONS
TO WELLS OF PLATES
STEP 3 - PLATES ARE READ IN A PLATE READER AND ASSAYED
FOR DIFFERENT CONCENTRATIONS OF SECRETED NANOLUC
1536 WELL PLATE

FIG. 13

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 61906087 **[0001]**
- US 61813771 B **[0001]**
- WO 2009156413 A1 **[0003]**

Non-patent literature cited in the description

- **PATEL AMIT N et al.** Putative Population of Adipose-Derived Stem cells Isolated From Mediastinal Tissue During Cardiac Surgery. *CELL TRANSPLANTATION,* 02 April 2012, vol. 22 (3), 507-511 **[0003]**
- **ARTURO HERNANDEZ et al.** Dexamethasone Inhibits Growth Factor-Induced Type 3 Deiodinase Activity and mRNA Expression in a Cultured Cell Line Derived from Rat Neonatal Brown Fat Vascular-Stromal Celly. *ENDOCRINOLOGY,* 01 July 2002, vol. 143 (7), ISSN 0013-7227, 2652-2658 **[0003]**
- **M. CIGOLINI et al.** Human brown adipose cells in culture. *EXPERIMENTAL CELL RESEARCH,* 01 July 1985, vol. 159 (1), ISSN 0014-4827, 261-266 **[0003]**
- **TIM J SCHULZ et al.** *Identification of inducible brown adipocyte progenitors residing in skeletal muscle and white fat,* 04 January 2011, vol. 108 (1), 143-148 **[0003]**
- Novel adipocyte lines from brown fat: a model system, for the study of differentiation, energy metabolism, and insulin action. **KLEIN J et al.** BIOESSAYS. JOHN WILEY & SONS LTD, 01 January 2002, vol. 24, 382-388 **[0003]**
- **PONTUS BOSTRÖM et al.** A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *NATURE,* 01 January 2012, vol. 481 (7382), ISSN 0028-0836, 463-468 **[0003]**