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(54) Title: GENERATING INDUCED PLURIPOTENT STEM CELLS AND PROGENITOR CELLS FROM FIBROBLASTS

(57) Abstract: The present disclosure provides a method of generating progenitor cells, such as hematopoietic or neural progenitor cells, from fibroblasts, such as dermal fibroblasts, comprising providing fibroblasts that express or are treated with a POU domain containing gene or protein and culturing the cells under conditions that allow production of progenitor cells, without traversing the pluripotent state. Also provided is a method of isolating a subpopulation of fibroblasts with reprogramming potential comprising providing fibroblasts that express an Oct-4-reporter and isolating cells that are positive for the reporter. Further provided is a method of generating reprogrammed fibroblast-derived induced pluripotent stem cells. Also provided are uses and assays of the cells produced by the methods of the disclosure.

Figure 1

(Continued on next page)

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Title: GENERATING INDUCED PLURIPOTENT STEM CELLS AND PROGENITOR CELLS FROM FIBROBLASTS

Cross Reference to Related Applications

[0001] This application claims the benefit of priority of copending U.S. provisional application 61/256,170 filed on October 29, 2009, the contents of which are incorporated herein by reference in their entirety.

Field of the disclosure

[0002] The disclosure relates to reprogramming of fibroblasts. In particular, the disclosure relates to methods of generating progenitor cells and induced pluripotent stems cells derived from fibroblasts and the cells produced by the methods.

Background of the disclosure

[0003] Several groups have demonstrated the ability to reprogram human fibroblasts to induced pluripotent stem cells (iPSCs) following transduction with Oct-4 together with other factors (Takahashi et al., 2007; Takahashi and Yamanaka, 2006; Yu et al., 2007). For example, dermal fibroblasts can be reprogrammed to a pluripotent state by ectopic expression of a cocktail of pluripotent factors including Oct-4 (POU5F1), Sox-2, Klf-4, c-Myc, Nanog, and Lin28 (Takahashi et al., 2007; Yu et al., 2007). With the exception of Oct4, further studies indicated that the majority of these factors could be eliminated by use of unique stem/progenitor cells (Heng et al.; Aasen et al. 2008; Eminli et al. 2008; Eminli et al. 2009; Kim et al. 2009) or, alternatively, by addition of chemicals targeting the epigenome of dermal fibroblast sources (Shi et al. 2008; Lyssiotis et al. 2009). These studies demonstrate there are several approaches and methods for generation of iPSCs, however, the cellular and molecular mechanisms underlying reprogramming to the pluripotent state remain largely unknown (Jaenisch and Young, 2008). Although iPSCs can be differentiated towards the blood fate, the resulting hematopoietic cells preferentially generate primitive blood cells that utilize embryonic programs. Moreover, the methods remain inefficient, making it difficult to contemplate transplantation or modeling hematological
diseases (Lengerke and Daley, 2010). Characterization of these processes is further complicated by cellular intermediates that fail to establish a stable pluripotent state, potentially due to the inability to achieve the correct combination, stoichiometry, or expression levels of reprogramming factors ideal for complete pluripotency induction (Chan et al., 2009; Kanawaty and Henderson, 2009; Lin et al., 2009; Mikkelsen et al., 2008). Consistent with this idea, intermediate cells derived from fibroblasts have been shown to co-express genes associated with several differentiated lineages (neurons, epidermis, and mesoderm) (Kanawaty and Henderson, 2009; Mikkelsen et al., 2008), nevertheless the exact identity and differentiation potential of these cell types remain elusive. This creates the possibility that under unique conditions the fibroblasts expressing a small subset of transcription factors can be induced to differentiate towards specified lineages without achieving pluripotency, as recently been demonstrated by converting fibroblasts into specific cell types such as neurons, cardiomyocytes, and macrophage-like cells (Feng et al., 2008; leda et al., 2010; Vierbuchen et al., 2010). While these studies have examined fibroblast conversion in the murine model, this concept remains to be extrapolated for human applications.

[0004] Previous studies have shown that proteins containing POU domains, such as Oct-4, along with Oct-2 (POU2F2) and Oct-1 (POU2F1) bind similar DNA target motifs (Kang et al., 2009). Whilst both Oct-2 and Oct-1 play a role in hematopoietic development (Brunner et al., 2003; Emslie et al., 2008; Pfisterer et al., 1996), Oct-4 is yet to be implicated in this process. Nonetheless, recent studies have predicted that Oct-4 possesses the capacity to bind to the promoters of the hematopoietic genes Runx1 and CD45, thus potentially regulating their expression (Kwon et al., 2006; Sridharan et al., 2009). Despite the similarities in binding and regulation, the exact functional role of individual Oct family members appears to be cell context specific (Kang et al., 2009; Pardo et al., 2010).

[0005] The ability to generate pluripotent stem cells from human dermal fibroblasts allows for generation of complex genetic disease models, and
provides an unprecedented source for autologous transplantation without concern of immune rejection (Takahashi and Yamanaka 2006; Hanna et al. 2007; Yu et al. 2007; Okita et al. 2008; Park et al. 2008; Park et al. 2008b; Soldner et al. 2009).

Although a variety of somatic cell types can be reprogrammed, the vast majority of studies aimed at characterizing the mechanisms that govern the reprogramming process utilize fibroblasts (Takahashi and Yamanaka 2006; Takahashi et al. 2007; Wernig et al. 2007; Yu et al. 2007; Aoi et al. 2008; Brambrink et al. 2008; Eminli et al. 2008; Hanna et al. 2008; Huangfu et al. 2008; Lowry et al. 2008; Stadtfeld et al. 2008; Zhou et al. 2008; Carey et al. 2009; Feng et al. 2009; Gonzalez et al. 2009; Guo et al. 2009; Kaji et al. 2009; Utikal et al. 2009; Woltjen et al. 2009; Yusa et al. 2009; Zhou et al. 2009). As such, the current understanding of the molecular mechanisms and cellular nature of reprogramming is nearly exclusively derived from fibroblast-based reprogramming. Fibroblasts can be generated from multiple tissue sites including dermal skin, however, little is known about the origins and composition of fibroblasts used experimentally.

Cellular reprogramming to the pluripotent state was originally demonstrated using in vitro cultured mammalian fibroblasts (Takahashi and Yamanaka 2006). To date, iPSCs have been derived from a number of other tissue-derived cells including liver, pancreas, intestine, stomach, adipose, melanocytes, and hematopoietic sources (Aoi et al. 2008; Hanna et al. 2008; Zhou et al. 2008; Eminli et al. 2009; Sun et al. 2009; Utikal et al. 2009) using a variety of transcription factors including the oncogenes c-myc and klf4 (Takahashi and Yamanaka 2006; Takahashi et al. 2007; Aasen et al. 2008; Hanna et al. 2008; Park et al. 2008; Eminli et al. 2009; Hanna et al. 2009; Woltjen et al. 2009; Zhao et al. 2009). To date, the reprogramming process remains inefficient, but can be enhanced by utilization of initial cell types that already possess stem/progenitor proliferative capacity (Kim et al. 2009; Eminli et al. 2008; Eminli et al. 2009), or by enhancing cell cycle state by knocking down inhibitors of cell cycle progression such as p53/p21 (Kawamura et al.
2009; Li et al. 2009; Utikal et al. 2009). However, altering cell cycle regulators or introduction of oncogenes increases the risk of uncontrolled growth and tumor formation and thus raises potential safety concerns for future human therapeutic applications (Lebofsky and Walter 2007; Okita et al. 2007; Nakagawa et al. 2008; Markoulaki et al. 2009).

Summary of the disclosure

The present inventors used human dermal fibroblasts to investigate direct conversion of the fibroblasts into hematopoietic cells (CD45+ cells) and to investigate reprogramming fibroblasts to induced pluripotent stem cells.

Accordingly, in one embodiment, the disclosure provides a method of generating progenitor cells from fibroblasts comprising:

a) providing fibroblasts that express or are treated with POU domain containing gene or protein; and

b) culturing the cells of step (a) under conditions to allow production of progenitor cells without traversing the pluripotent state.

In one embodiment, fibroblasts that express a gene or protein containing a POU domain include overexpression of an endogenous gene or protein containing a POU domain or ectopic expression of a gene or protein containing a POU domain. In an embodiment, the fibroblasts do not additionally overexpress or ectopically express or are not treated with Nanog or Sox-2. In another embodiment, fibroblasts that express a POU domain containing gene or protein are produced by transfecting or transducing the fibroblasts with a vector comprising the POU domain. In an embodiment, fibroblasts that express a gene or protein containing a POU domain are produced by lentiviral transduction. In an embodiment, the POU domain containing gene or protein is Oct-1, -2, -4 or -11. In another embodiment, the POU domain containing gene or protein is Oct-4. The POU domain containing gene or protein includes, without limitation, functional variants and fragments thereof as well as small molecule mimetics.
Conditions that allow production of progenitor cells are known in the art and include, without limitation, colony forming assays for a culture period from 15-25 days, optionally 21 days. In another embodiment, the fibroblasts are dermal fibroblasts. In yet another embodiment, the progenitor cells are hematopoietic progenitor cells and the conditions are hematopoietic conditions. In a further embodiment, the progenitor cells are neural progenitor cells and the conditions are neural conditions.

In another embodiment, the method further comprises culturing the cells produced in step (b) in differentiation medium under conditions that allow production of differentiated cells. Such conditions include culturing the cells in medium for a culture period from 10 to 21 days, optionally 16 days. In one embodiment, the differentiation medium is hematopoietic medium comprising a hematopoietic cytokine, such as, Flt3 ligand, SCF and/or EPO. In an embodiment, the differentiated hematopoietic cells are of the myeloblast lineage, such as monocytes or granulocytes. In another embodiment, the differentiated hematopoietic cells are of the erythroid or megakaryocytic lineage. In another embodiment, the differentiation medium is neural medium comprising neural basal media supplemented with fibroblast growth factor and epidermal growth factor. In an embodiment, the differentiated neural cells are neurons and/or glial cells including oligodendrocytes and or astrocytes.

Also provided herein are the isolated progenitor and differentiated cells generated by the methods described herein and uses of the cells for engraftment and transplantation. The hematopoietic progenitor cells are also useful as a source of blood, cellular and acellular blood components, blood products and hematopoietic cells.

Further provided herein is a screening assay comprising

a) preparing a culture of progenitor cells or cells derived therefrom by the methods described herein;

b) treating the cells of a) with a test agent or agents; and
c) subjecting the treated progenitor cells or cells derived therefrom to analysis.

[0015] In one embodiment, the progenitor cells are differentiated prior to treating with the test agent.

5 [0016] In another aspect, there is provided a method of isolating a subpopulation of fibroblasts with increased reprogramming potential comprising

a) providing fibroblasts that express an Oct-4-reporter; and

b) isolating cells positive for the reporter.

10 [0017] In one embodiment, the reporter gene comprises a fluorescent protein (such as GFP) and the cells are isolated in step (b) by detection of the fluorescence. In another embodiment, the reporter gene encodes a gene conferring antibiotic resistance, such as to puromycin, and the cells are isolated by survival in the presence of the antibiotic. In an embodiment, the fibroblasts that express an Oct-4-reporter gene are produced by lentiviral transduction. In an embodiment, the fibroblasts are dermal fibroblasts. In another embodiment the fibroblasts are foreskin fibroblasts.

[0018] The disclosure further provides a method of generating reprogrammed fibroblast-derived induced pluripotent stem (iPS) cells at higher efficiency comprising

a) providing (i) a population of fibroblasts with increased expression of Oct-4 and (ii) a mixed population of fibroblasts or a population of Oct-4 negative fibroblasts;

b) treating the cells of a) with Oct-4, Nanog, Sox2 and Lin28;

25 and

c) culturing the treated cells of b) under conditions that allow the production of iPS cells.

[0019] The method optionally further comprises analyzing and selecting cells that express a marker of undifferentiated stem cells, such as TRA-1-60, SSEA-3, Sox2, Nanog, SSEA4, TRA-1-81, IGF1 receptor, connexin 43, E-
cadherin, Alkaline phosphatase, REX1, CRIPTO, CD24, CD90, CD29, CD9 and CD49f. In a particular embodiment cells are selected for expression of TRA-1-60 and/or SSEA-3.

[0020] In one embodiment, the population of fibroblasts with increased expression of Oct-4 are produced by the method of isolating a subpopulation of fibroblasts with reprogramming potential as described herein. In an embodiment, the ratio of the cells of i) to the cells of ii) in (a) is 50:50, 25:75 or 10:90. In an embodiment, the fibroblasts are dermal fibroblasts.

[0021] Also provided herein are isolated induced pluripotent stem cells generated by the method described herein and cells differentiated therefrom and uses of the cells for engraftment, transplantation and as a source of induced pluripotent stem cells.

[0022] Further provided herein is a screening assay comprising

a) preparing a culture of induced pluripotent stem cells by the methods described herein or cells differentiated therefrom;

b) treating the cells with a test agent or agents; and

c) subjecting the treated cells to analysis.

[0023] Other features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the disclosure are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

25 Brief description of the drawings

[0024] The disclosure will now be described in relation to the drawings in which:

[0025] Figure 1 shows Oct-4 transduced human adult dermal fibroblasts and fetal fibroblasts give rise to CD45+ve colonies.
Representative bright field images of untransduced (Fibs) and Oct-4+ (Fibs^{Oct-4}), Sox-2- (Fibs^{Sox-2}) or Nanog- (Fibs^{Nanog}) transduced Fibs, untransduced fetal fibroblasts (Fetal Fibs) and Oct-4 transduced fetal Fibs (Fetal Fibs^{Oct-4}) at day 21 post-transduction (D21) (colonies- dashed lines and arrows) (n=6). b. Relative gene expression of Sox-2, Nanog and Oct-4 in Fibs, Fetal Fibs, Fibs^{Oct-4}, Fibs^{Sox-2} and Fibs^{Nanog}, Fetal Fibs^{Oct-4} in comparison with the expression of these genes in established iPSCs (n=3, *p<0.001). c. Representative FACS plots of the CD45 levels in Fibs^{Oct-4} and Fetal Fibs^{Oct-4} compared with untransduced Fibs or -Fetal Fibs, Fibs^{Sox-2} or Fibs^{Nanog} (n=6).

[0026] Figure 2 shows a. Global gene analysis based on fibroblasts specific marker expression over the course of CD45+ve cell emergence in Fibs and sorted CD45+ Fibs^{Oct-4}. b. Representative bright field images of Fibs, Fetal Fibs, and Fibs^{Oct-4} or Fetal Fibs^{Oct-4} plus/minus Flt3 and SCF at day 21 (D21) (n=6). c. Enumeration of colonies in Fibs, Fetal Fibs, and Fibs^{Oct-4} or Fetal Fibs^{Oct-4} plus/minus SCF and Flt3 at day 21 (D21) (colonies-white arrows; n=6; *p<0.001). d. Pluripotency gene signature of Fibs and sorted CD45+ Fibs^{Oct-4}.

[0027] Figure 3 shows Oct-4 transduced human dermal fibroblasts bypass the pluripotency. a. Quantitative analysis of SSEA3 levels, and b. Tra-1-60 levels over the 31-day timeline of hiPSC derivation in Fibs and Fibs^{Oct-4} plus/minus SCF and Flt-3 and Fibs transduced with Oct-4, Sox-2, Nanog and Lin-28 (n=3). c. Teratomas derived from hiPSCs showing mesoderm, endoderm and ectoderm and testicular sections representing the lack of teratomas from Fibs and Fibs^{Oct-4} plus/minus Flt3 and SCF (Control-saline injected).

[0028] Figure 4 shows in vitro reconstitution of the myeloid lineage by hematopoietic cytokine treated Oct-4 transduced CD45 positive Fibs. a. Schema presenting Oct-4 transduced CD45+ve Fibs (CD45+Fibs^{Oct-4}) isolation and subsequent hematopoietic cytokine treatment, followed by in vitro and in vivo analysis. In vitro analysis includes colony forming unit (CFU) assay and FACS analysis; and the in vivo analysis is the hematopoietic reconstitution
assay using NOD/SCID IL2Ryc null (NSG) mice. b. FACS analysis of myeloid cells (CD45+CD13+ and CD13+CD33+ cells) derived from CD45+Fibs0ct-4 (n=6). c. Representative FACS plots of monocytes (CD45+CD14+ cells) and d. corresponding Giemsa-Wright images of monocytes with distinguishing nuclear morphology (white arrow) derived from CD45+Fibs0ct-4 (n=6). e. Representative FACS plots of FITC-labeled latex-bead uptake indicating the presence of macrophages in the CD45+Fibs0ct-4 population versus Fibs (no beads). Upper panel graph shows quantitative analysis of FITC-labeled latex-bead uptake by CD45+Fibs0ct-4 and Fibs (n=3). f. Representative Giemsa Wright stained image of a macrophage and immunofluorescence image of FITC-beads (white arrow) taken up by macrophages. g. Representative FACS plot of granulocytes (CD45+CD15+ cells) derived from CD45+Fibs0ct-4 (n=6). h. Giemsa Wright stained CD45+CD15+ granulocytes containing neutrophils, eosinophils and basophils (characteristic nuclear morphology-white arrows) (n=6). i. CD45+Fibs0ct-4 hematopoietic cytokine treated cells give rise to hematopoietic progenitors (CD45+CD34+ cells) (n=9). j. Representative images of granulocytic (CFU-G), monocytic (CFU-M) colony forming units (CFU) derived from adult dermal or fetal foreskin CD45+ve cells (20x). k. Quantitation of granulocytic (CFU-G), monocytic (CFU-M) and mixed granulocytic and monocytic (CFU-GM) CFU formation from 1000 sorted CD45+CD34+ cells derived from adult dermal Fibs, fetal foreskin Fibs and umbilical cord blood (UCB) (n=3). l. CFU formation frequency in adult and fetal CD45+Fibs0ct-4 cells and UCB derived hematopoietic progenitors (n=3; *p<0.001).

Figure 5 shows in vivo reconstitution capacity of CD45+Fibs0ct-4 cells. a. Schematic representation of the xenograft models used for primary and secondary injection of CD45+ve cells into adult NOD/SCID IL2Ryc null mice and subsequent analysis of the engrafted cells. b. Graph representing human chimerism at week 10 following intrafemoral injection of CD45+Fibs0ct-4 cells treated with cytokines (n=12). c. Representative FACS histograms of engrafted CD45+Fibs0ct-4 cells (HLA A/B/C+ve cells) showing the presence of
CD45+ve and CD14+ve population (n=12) in engrafted mice versus saline injected mice. d. Graph representing human chimerism at week 10 following intrafemoral injection of cord blood (UCB) derived progenitors and mobilized-peripheral blood (M-PB) cells (n=4). e. Representative FACS histograms of engrafted UCB and M-PB cells showing the presence of CD45+ve and CD14+ve population (n=4). f. Colony formation capacity per 1000 mouse cell depleted CD45+ve cells derived from engrafted CD45+Fibs^{0,ct-4} cells versus UCB (n=3).

Figure 6 shows CD45+Fibs^{0,ct-4} cells are able to reconstitute the erythroid and megakaryocytic lineages following EPO treatment. a. Representative FACS histograms of erythroblast marker, CD71, levels in Fibs, CD45+Fibs^{0,ct-4} cells and CD45+Fibs^{0,ct-4} cells treated with EPO (n=3). b. Representative FACS histograms of Glycophorin A (red blood cell marker) levels in Fibs, CD45+Fibs^{0,ct-4} and CD45+Fibs^{0,ct-4} cells treated with EPO (n=3). c. Representative FACS histograms of adult globin, beta-globin, levels in Fibs, CD45+Fibs^{0,ct-4} and CD45+Fibs^{0,ct-4} cells treated with EPO (upper panel is FACS analysis of differentiated human pluripotent stem cells (hPSCs)) (n=3). d. Relative mRNA expression of the embryonic globin (zeta), fetal-globin (epsilon) and adult globin (beta) in Fibs, CD45+Fibs^{0,ct-4} cells and CD45+Fibs^{0,ct-4} cells treated with EPO (n=3; *p<0.001). e. Giemsa Wright stained EPO treated CD45+Fibs^{0,ct-4} cells showing primitive (black arrow) and mature (white arrow) erythrocyte morphologies. f. Representative CFU images of EPO treated adult and fetal fibroblast derived CD45+Fibs^{0,ct-4} cells (20x; n=3). (Erythroid blast forming units- BFU-E; granulocyte colony forming units- CFU-G; monocyte colony forming units- CFU-M; Colony forming units containing all lineages- CFU-Mix) g. Quantitative analysis of CFU formation in adult and fetal Fibs, CD45+Fibs^{0,ct-4} and CD45+Fibs^{0,ct-4} cells treated with or without EPO versus UCB (n=3). h. Representative megakaryocytic CFU (CFU-Mk) images (CD41+ve cells) (20x) derived from Fibs (left panel), CD45+Fibs^{0,ct-4} cells treated with (right panel) or without EPO (left panel) (n=3). i. Quantitative representation of megakaryocytic CFU formation (right panel, n=3; *p<0.001).
Figure 7 shows Oct-4 transduction results in hematopoietic program activation in human dermal fibroblasts.

a. Proposed model for hematopoietic fate conversion following transduction of Fibs with Oct-4 alone over the time course of CD45<sup>+</sup> cells emergence (day 0 (DO), 4 (D4), 21 (D21) and 37 (D37)) versus hematopoietic differentiation from human iPSCs. Global gene culturing based on fibroblasts specific marker expression (b), pluripotency signature (c), hematopoietic cytokines (d) and hematopoietic transcription factors (e) over the course of CD45<sup>+</sup> cell emergence; i.e. in Fibs (DO), puromycin selected Day 4 Fibs<sup>0</sup>Oct<sup>-4</sup> (D4) and sorted CD45<sup>+</sup>Fibs<sup>0</sup>Oct<sup>-4</sup> (D21).

f. Relative mRNA expression analysis of mesodermal genes (GATA2, Brachyury), hematopoietic specific genes (SCL, MixL1, Runxl, GATA1, PU.1 and C/EBPa) and pluripotency genes (Oct-4, Sox-2 and Nanog) in Fibs (DO) versus sorted CD45<sup>+</sup>Fibs<sup>0</sup>Oct<sup>-4</sup> cells at D21 and hematopoietic cytokine treated sorted CD45<sup>+</sup>Fibs<sup>0</sup>Oct<sup>-4</sup> cells at D37 (n=4, *p<0.001).

g. Gene expression profile of POU family of genes (including Oct-4 - POU5F1) over the time course of CD45<sup>+</sup> cell emergence; i.e. in Fibs (DO), puromycin selected Day 4 Fibs<sup>0</sup>Oct<sup>-4</sup> (D4), sorted CD45<sup>+</sup>Fibs<sup>0</sup>Oct<sup>-4</sup> cells (D21) and hematopoietic cytokine treated sorted CD45<sup>+</sup>Fibs<sup>0</sup>Oct<sup>-4</sup> cells (D37).

h. Schematic representation of the known native (SEQ ID NO:1) and predicted octamer (SEQ ID NO:2) (POU domain) binding sequences that Oct-4, Oct-1 and Oct-2 can occupy (N- can be any nucleotide (A, T, C or G); starred and underlined region represent the core conserved octamer binding region).

i. Right panel - Relative Oct-4 occupancy of hematopoietic specific gene SCL, Runxl, CD45, GATA1, PU.1, Oct-2 and C/EBPa promoter or enhancer regions over the course of CD45<sup>+</sup> cell emergence compared to hFib control (n=3; *p<0.001).

j. Right panel - Relative Oct-4 occupancy of non-hematopoietic gene Gadd45a, Pol2ra, Myf5 and Nkx2.5 over the course of CD45<sup>+</sup> cell emergence compared to hFib control (n=3; *p<0.001).

k. Right panel - Relative Oct-4 occupancy of pluripotency gene Oct-4, Sox-2, Tbx3 and c-Myc promoter regions over the course of CD45<sup>+</sup> cell emergence compared to hFib control (n=3; *p<0.001). i-k. Left panel - proximity of primer designed at a resolution 500-1000 bp (arrows) relative to the native or predicted octamer-binding region (Black box).
Pluripotent stem cells - hPSCs; Fibroblasts - Fibs (DO); Puromycin selected Day 4 Oct-4 transduced hFibs - Day 4 Fibs₀<sup>Oct-4</sup> (D4) and Oct-4 transduced CD45<sup>+</sup> cells - CD45<sup>+</sup> Fibs₀<sup>Oct-4</sup> (D21).

[0032] Figure 8 shows characterization of the iPSC derived from human dermal fibroblasts (Fibs) transduced with Oct-4, Nanog, Sox-2 and Lin-28. a. Representative images of iPSC colonies (dashed lines and arrows) derived from human Fibs transduced with Oct-4, Nanog, Sox-2 and Lin-28 (20x). b. Representative FACS plots of pluripotency markers, SSEA-3, Tra1-60, Oct-4 and SSEA-4, in iPSCs (n=4). c. Intratesticular injection of iPSCs into immunodeficient mice (NOD/SCID) resulted in teratoma formation containing all 3 germ layers: ectoderm (skin), endoderm (lumen with goblet cells) and mesoderm (cartilage) (20x; n=6).

[0033] Figure 9 shows intermediate colonies derived during iPSC derivation have a hematopoietic phenotype. a. Intermediate colonies (arrows) possessing a hematopoietic cellular morphology (rounded cells) were present in four different iPSC lines (1-4). b. Live staining for CD45 positive hematopoietic cells (green) and Tra-1-60 positive iPSC colonies (red) showing that CD45 is exclusive to intermediate colonies, while Tra-1-60 is present only in iPSCs (20x; n=4). c. Representative FACS histogram of CD45 levels in four independent iPSC lines (n=4). d. Relative Oct-4, Sox-2 and Nanog mRNA expression: 1, in sorted CD45<sup>+</sup>ve cells derived from the 4 different iPSC lines; 2, in manually isolated iPSC colonies and 3, Fibs (n=4; *p<0.001). Levels were normalized to human embryonic stem cells (hESCs). e. Lentiviral integration of Oct-4, Sox-2, Nanog and Lin-28 in Fibs (untransduced), iPSCs and sorted CD45<sup>+</sup>ve iPSC (n=4).

[0034] Figure 10 shows a schematic representation of CD45 positive cell derivation from human dermal fibroblasts (Fibs). a. Human dermal Fibs were transduced with Oct-4 lentivirus on matrigel. On day 3-post transduction the cells were transferred onto matrigel coated dishes containing either 1. F12 medium supplemented with IGFII and bFGF or 2. F12 medium supplemented with IGFII, bFGF, Flt3 and SCF. Hematopoietic CD45 positive colonies were
enumerated between days 14 and 21 post Oct-4 transduction. b. Representative blot showing integration of Oct-4 (Fibs\textsuperscript{0,ctd-4}), Sox-2 (Fibs\textsuperscript{Sox-2}) and Nanog (Fibs\textsuperscript{Nanog})-lentivectors; human iPSCs derived from dermal Fibs transduced with Oct-4, Sox-2, Lin-28 and Nanog were used as the positive control (lane 1) and untransduced Fibs or Fetal Fibs were used as a negative control (lane 2 and 6). (n=6). c. Global Oct-4 gene expression (POU5F1 probe sets) following Oct-4 transduction over the course of CD45\textsuperscript{+ve} cell emergence from Fibs (Day 0 - DO and Day 21 (D21) - CD45\textsuperscript{+} Fibs\textsuperscript{0,ctd-4}). POU5F1 (Oct-4) specific probe sets increase upon Oct-4 transduction over the time line of CD45\textsuperscript{+ve} cell emergence from hFibs irrespective of the probe set used for detection.

[0035] Figure 11 shows CD45 positive colonies emerged from Oct-4 transduced cell between day 14-to-21 post transduction. a. Representative bright field images of human Fibs and hFibs\textsuperscript{Oet-4} plus/minus SCF and Flt3 over the time line of colony emergence (white arrows) (day 0-21) (n=6). Enlarged box represents live CD45 stained colonies at day 21.

[0036] Figure 12 shows Oct-4 transduced CD45 positive colonies do not acquire a pluripotent phenotype. a. Gene expression profile of Oct (POU) family members differentially regulated. Oct-4 (POU5F1) was the only POU family member differentially regulated over the time course of CD45\textsuperscript{+ve} cell emergence; i.e. in Fibs (DO), Day 4 Fibs\textsuperscript{Oet-4} (D4) and CD45\textsuperscript{+} Fibs\textsuperscript{0,ctd-4} (D21). b. Gene expression profile of POU family of genes that were not differentially regulated (excluding Oct-4) over the time course of CD45\textsuperscript{+ve} cell emergence; i.e. in Fibs (DO), Day 4 Fibs\textsuperscript{0,ctd-4} (D4) and CD45\textsuperscript{+} Fibs\textsuperscript{0,ctd-4} (D21). c. Representative FACS histogram of SSEA3 positive population frequency in untransduced Fibs and Fibs\textsuperscript{Oet-4} plus/minus SCF and Flt3 and iPSC (n=6). d. Representative FACS histogram of Tra-1-60 positive population frequency in untransduced Fibs, Fibs\textsuperscript{0,ctd-4} plus/minus SCF and Flt3 and iPSC (n=6). e. Live staining for Tra-1-60 positive colonies (arrows and dashed lines) in untransduced-Fibs, Fibs\textsuperscript{0,ctd-4} plus SCF and Flt3 and iPSCs.
Figure 13 shows growth dynamics and c-Myc expression over the time course of CD45^{+} cell emergence. 

a. Growth/expansion dynamics of Fibs, CD45^{+} Fibs^{Oct-4} cells and human iPSCs (hiPSC) over 7 passages (n=9).

b. Gene expression profile of c-Myc over the time course of CD45^{+} cell emergence; i.e. in Fibs versus CD45^{+} Fibs^{Oct-4} (day 21 - D21).

Figure 14 shows global gene signatures cluster mononuclear cells with Oct4 positive CD45^{+}ve cells and cord blood derived progenitors with day 4 Oct-4 transduced Fibs. 

a. Global gene cluster analysis of mononuclear cells (MNC), cord blood derived hematopoietic progenitors (UCB), Fibs, osteoblasts, Day 4 Fibs^{Oct-4} and CD45^{+} Fibs^{Oct-4}. 

b. Hematopoietic gene analysis of MNCs, UCB cells, Fibs, osteoblasts, Day 4 Fibs^{Oct-4} and CD45^{+} Fibs^{Oct-4}. 

c. CD45^{+}Fibs^{Oct-4} treated with hematopoietic cytokines over an additional 16 days (day 37 - D37) had enhanced proliferation capacity versus CD45^{+}Fibs^{Oct-4} before cytokine treatment (day 21 - D21) and untreated CD45^{+}Fibs^{Oct-4} at day 37 (D37) (n=6; *p<0.001). 

d. Cell viability of CD45^{+}Fibs^{Oct-4} cells with and without hematopoietic cytokine treatment at day 37 (D37) and CD45^{+}Fibs^{Oct-4} cells at day 21 (D21) (n=6).

Figure 15 shows in vitro reconstitution of the myeloid cells by hematopoietic cytokine treated Oct-4 transduced CD45 positive fetal foreskin derived Fibs at day 37. 

FACS analysis of myeloid cells (CD45^{+}CD13^{+} and CD1^{+}CD33^{+} cells) derived from Fetal CD45^{+}Fibs^{Oct-4} at day 37 (D37) (n=3).

Figure 16 shows in vitro reconstitution of the monocyte lineage by hematopoietic cytokine treated Oct-4 transduced CD45 positive Fetal and adult Fibs at day 37. 

a. Representative FACS plots of monocytes at day 37 (D37) (CD45^{+}CD14^{+} cells; n=3). 

b. FITC-labeled beads uptake by CD45^{+}Fibs^{Oct-4} derived macrophages (40X) compared with untransduced Fibs (white arrows-cells containing beads).

Figure 17 shows in vitro reconstitution of the myeloid lineage by hematopoietic cytokine treated Oct-4 transduced CD45 positive Fibs at day 37. 

a. Representative FACS analysis of CD45^{+} Fibs^{Oct-4} cells triple-stained with CD45, CD14 and CD15, showing lack of CD14 and CD15 co-expression
at day 37 (D37) (n=3). b. Representative FACS plot of granulocytes (CD45^+CD1^+ cells) derived from Fetal CD45^+Fibs^0^Oct-4 at day 37 (D37) (n=3).

c. Representative bulk images of Giemsa Wright stained CD45^+ Fibs^0^ct-4 cells treated with cytokines at day 37 (D37) (20X and 40X; n=6) (arrows-hematopoietic cells).

[0042] Figure 18 shows in vitro reconstitution of the myeloid lineage in the absence of hematopoietic cytokine treatment in Oct-4 transduced CD45 positive Fibs at day 37. a. FACS analysis of myeloid cells (CD45^+CD1^+ and CD13^+CD33^+ cells) in the absence of hematopoietic cytokine in CD45^+Fibs^0^ct-4 at day 37 (D37) (n=6). b. Representative FACS plots of monocytes (CD45^+CD14^+ cells) and granulocytes (CD45^+CD15^+ cells) in the absence of hematopoietic cytokine in CD45^+Fibs^Oct-4 at day 37 (D37) (n=6).

[0043] Figure 19 shows in vitro reconstitution of the myeloid lineage by hematopoietic cytokine treated Oct-4 transduced CD45 positive fetal foreskin derived Fibs. Hematopoietic cytokine treated Fetal CD45^+Fibs^0^ct-4 cells give rise to hematopoietic progenitors (CD45^+CD34^+ cells) at day 37 (D37) (n=3).

[0044] Figure 20 shows colony forming units derived from immunodeficient mice engrafted CD45^+Fibs^Oct-4 cells maintained CD45 expression. a. Bright field image of CFUs derived from engrafted CD45^+Fibs^0^ct-4 cells (n=3). b. Representative FACS histogram indicating CD45 expression in CFUs derived from engrafted CD45^+Fibs^Oct-4 (n=3). c. Representative FACS plots indicating CD45 versus CD14 expression in CFUs derived from engrafted CD45^+Fibs^Oct-4 (n=3). d. Representative FACS plots indicating CD45 versus CD15 expression in CFUs derived from engrafted CD45^+Fibs^Oct-4 (n=3).

[0045] Figure 21 shows in vivo reconstitution of the Oct-4 transduced CD45 positive cells derived Fibs. a. Representative FACS plot showing engraftment of CD45^+Fibs^0^ct-4 (D37) cells in contralateral bone of injected immunodeficient (NSG) mice compared with saline injected counterparts (n=8; p<0.01) b. Primary and secondary reconstitution capacity of the engrafted CD45^+Fibs^Oct-4 cells. Human chimerism in bone and spleen of
recipient NSG mice was analyzed via the presence of human chromosome 17. Positive control - mobilized peripheral blood (M-PB); Negative control- spleen and bones from saline injected mice; Control- no genomic DNA- no gDNA.

[0046] Figure 22 shows EPO treatment resulted in erythroid colony forming units formation. a. Quantification of colony forming units derived from Fibs, CD45+ Fibs^{Oct-4} with or without EPO treatment (n=3; *p<0.001). b. Bar graph representing the frequency of colony (CFU) formation per 5,000 cells plated (n=3; *p<0.001). (monocytic - CFU-M; granulocytic - CFU-G, erythroid - BFU-E; mixed colonies containing erythroid, granulocytic, monocytic- CFU-Mix).

[0047] Figure 23 shows Oct-4 induced changes over the time course of CD45^{+ve} cell emergence. a. Fatigo analysis of molecular/functional pathways in adult dermal Fibs versus Fibs at day 4 post Oct-4 transduction (threshold set at 2-fold; p<0.001). b. Gene expression profile of hematopoietic genes showing significant transcriptional regulation (p<0.001) and c. showing the absence of transcriptional regulation over the time course of CD45^{+ve} cell emergence; i.e. in Fibs (day 0 - D0), puromycin selected Day 4 Fibs^{Oct-4} (day 4 - D4) and sorted CD45^{+} Fibs^{Oct-4} (day 21 - D21).

[0048] Figure 24 shows hematopoietic gene expression during maturation of Oct4 transduced CD45^{+ve} cells. a. Schematic representation of the hematopoietic genes shown to be involved in hematopoietic specification (Runx1, SCL) and maturation (PU.1, Runx1, C/EBPa and GATA1). b. Relative mRNA expression analysis of mesodermal genes (GATA2, Brachyury), hematopoietic specific genes (SCL, MixL1, Runx1, GATA1, PU.1 and C/EBPa) and pluripotency genes (Oct-4, Sox-2 and Nanog) in CD45^{+} Fibs^{Oct-4} with or without hematopoietic cytokine cocktail treatment (Flt-3, G-CSF, SCF, IL6, IL3, BMP-4) at day 37 (D37) (n=3, *p<0.001). c. Adult hemoglobin (beta, alpha and delta) expression at day 21 (D21) and day 37 (D37) following Oct-4 transduction in CD45^{+} Fibs^{Oct-4} cells. (HBB- β-hemoglobin; HBA- α-hemoglobin; HBD- δ-hemoglobin).
Figure 25 shows Oct enhancer driven GFP expression in unique subset of cells derived from fibroblast cultures. (a) Schematic representation of PGK-EGFP (positive control), promoter-less EGFP (negative control) vector, C3+EOS EGFP IRES Puro vector. (b) Representative phase and fluorescence microscopy images of hESC and human dermal adult fibroblasts (hFibs) transduced with PGK-EGFP, negative control and C3+ EOS GFP IRES Puro vectors. GFP positive (GFP\textsuperscript{+ve}) from C3+EOS vector are indicated with arrows. (c) Representative FACS plots of GFP\textsuperscript{+ve} cell frequency upon C3+EOS transduction in breast derived hFibs and positive control hESC. (d) Representative phase and fluorescence microscopy images of foreskin and lung derived fibroblasts (hFibs) transduced with C3+ EOS GFP vector. GFP positive (GFP\textsuperscript{+ve}) from C3+EOS vector are indicated with arrows. (e) Frequency of GFP\textsuperscript{+ve} cells in Breast (n=5), foreskin (n=3) and lung derived fibroblasts (n=3) upon C3+EOS transduction was studied using flow cytometry. (f) Schematic representation of the strategy used for sorting GFP\textsuperscript{+ve} and GFP\textsuperscript{-ve} hFibs from total hFibs transduced with C3+ EOS lentivirus and subsequent analysis of the sorted subfractions. (g) Representative provirus integration and GFP expression profile was studied in sorted cells. (h) Phase and fluorescence microscopy images of GFP\textsuperscript{-ve} fibroblast fraction that was transduced with pSIN Oct4 lentivirus. Arrows indicate GFP cells that are observed after Oct4 overexpression.

Figure 26 shows EOS\textsuperscript{+ve} fibroblasts cells express pluripotency genes. (a) Schematic representation of Oct4 locus and primers spanning various exons on the loci. (b and c) Expression of Oct4 isoforms in semi quantitative and quantitative PCR analysis. (d) Relative expression of key pluripotency genes Nanog, Sox2 and Brachyury (BrachT) in various subfractions of fibroblasts. (e) Expression of Oct4, Nanog, and Sox2 from GFP\textsuperscript{+ve} hFjbs was compared to hESCs by quantitative RT-PCR analysis, (f) Hierarchical clustering of total hFibs, NOS\textsuperscript{+exp} fibroblasts, NOS\textsuperscript{-exp} fibroblasts, hESC, iPSC NOS\textsuperscript{+exp} fibroblasts and iPS from public data sets (Fib-BJ1, iPS BJ1 ans iPS BJ2). Expression profiles are based on genes enriched in mouse ESCs (Takahashi et al. 2007), human ESCs and adult fibroblast markers (Yu
et al. 2007). (g) Representative images (10X) and enlarged images for immunostaining of Oct4 in control hESC and GFP+ve cells using specific antibody. (h) Expression of Oct4, Nanog, and Sox2 in total hFibs, GFP+ve hFibs, 293, 293 overexpressing Oct4 and control hESCs by western blotting. (i) Occupancy of Oct4, Nanog, and Sox2 on Oct4 Enhancer (CR4) of C3+ EOS GFP IRES Puro vector was studied using ChIP assay. (j) Epigenetic state of Oct4, Nanog, and Sox2 loci in control hESC, NOS+exp, NOS*exp and total hFib cells was analyzed using ChIP to identify H3K4Me3 (black bars) and H3K27Me3 (white bars) marks.

[0051] Figure 27 shows NOS+exp cells separated from total fibroblast cultures exhibit reduced reprogramming efficiency. (a) Schematic representation of protocol used for reprogramming human Fibs and its subfraction NOS+exp on matrigel. (b) iPSC derivation from 10,000 total fibroblasts or NOS+exp cells on matrigel. Reprogramming of total fibroblast was performed 9 times and NOS+exp for n=6, using three different viral titers. (c) Representative phase images of iPSC and non-iPSC like colonies derived total fibroblasts. Fluorescence microscopy images show live staining of Tra 1-60 in both the colonies. (d) Expression of pluripotency markers SSEA3, Tra 1-60 and Oct4 staining was verified in iPSC and non-iPSC like colonies by flow cytometry. (e) Average number of Tra 1-60+ve colonies derived from total hFibs. (f) Semiquantitative PCRs showing expression of key ES specific markers in iPSC and non-iPSC colonies obtained from total hFib reprogramming. (g) Hematoxylin and eosin staining of teratoma derived from iPSC cells showing mesoderm, endoderm, and ectoderm differentiation. (h) NOS+exp hFibs were mixed with total hFibs in the indicated ratio, Lentivirus encoding Oct4, Sox2, Nanog, and Lin28 were transduced 24hrs post plating. Graph represents quantification of number of colonies three-week post transduction. Data represented is from three biological replicates performed in duplicates using three different viral titers. (i) Representative phase and fluorescence images 1K +9K mixtures (1:9), GFP+ve colonies were contributed by NOS+exp (EOS+ve) cells which was further confirmed by EOS provirus integration. EOS+ve colonies were contributed by total hFibs. To differentiate
between fully versus partially reprogrammed colonies Tra 1-60 live staining was performed. (j) Semi quantitative PCRs of pluripotency genes to study reactivation of ES specific genes in reprogrammed colonies from mixture experiments, (asterisks indicate these colonies were selected for further flow
cytometry analysis). (k) Flow cytometry analysis for reprogrammed colonies derived from NOS\textsuperscript{exp} and total hFibs in mixture experiments. (i) NOS\textsuperscript{exp} hFib derived iPSC cells was injected into mouse testicle for teratoma formation. Hematoxylin and eosin staining of teratoma showing differentiation of all three germ layers (mesoderm, endoderm, and ectoderm).

Figure 28 shows NOS\textsuperscript{exp} cells are predisposed for reprogramming. (a) hFibs were transduced with C3+ EOS GFP vector, NOS\textsuperscript{exp} cells were sorted on matrigel and combined with total fibroblast in 1:9 ratio (1000 NOS\textsuperscript{exp} cells plus 9000 total hFibs) or 10000 total fibroblasts cells and plated on matrigel. Lentivirus encoding Oct4, Sox2, Nanog, and Lin28 were transduced 24hrs post plating. Right panel represents quantification of colony number derived from 1K to 9K mixture experiments. Left panel represents colony contribution from EOS\textsuperscript{-ve} and EOS\textsuperscript{+ve} cells. (b) Tra 1-60 live staining was performed to study the complete reprogramming in colonies derived from NOS\textsuperscript{exp} (EOS\textsuperscript{+ve} derived from 1K) or total hFibs (EOS\textsuperscript{-ve} derived from 9K) in mixture experiment. (c) Frequency of complete reprogramming (Tra 1-60\textsuperscript{+ve} colonies) was studied in a mixture experiment by dividing number of Tra 1-60\textsuperscript{+ve} colonies from each compartment to its input cell count [no.of Tra 1-60\textsuperscript{+ve} (EOS\textsuperscript{+ve})/9000 or no.of Tra 1-60\textsuperscript{+ve} (EOS\textsuperscript{-ve})/1000].

Figure 29 shows molecular state of NOS\textsuperscript{exp} can be regulated by microenvironment for cellular reprogramming competency. (a) Ten thousand cells containing indicated densities of the NOS\textsuperscript{exp} were seeded on matrigel coated plates. Lentivirus encoding Oct4, Sox2, Nanog, and Lin28 were transduced 24hrs post plating. Number of colonies were counted three weeks post transduction and average numbers of colonies are represented. (b-c) Total hFibs and de novo isolated NOS\textsuperscript{exp} hFibs were analyzed by ChIP assays to assess the endogenous chromatin state followed by gene
expression analysis of key pluripotency genes. (d) Representative phase and fluorescence images of NOS+exp hFibs cultured alone, or co-cultured with total hFibs and MEFs from left to right respectively. (e) ChIP assay performed in cultured NOS+exp hFibs indicated bivalency at Oct4 loci while Nanog and Sox2 promoter loci were repressed. Quantitative PCR analysis indicated reduced expression of Oct4 in cultured NOS+exp compared to de novo isolated cells. (f) NOS+exp hFibs were cocultured with total hFibs or mouse embryonic fibroblasts in 50-50 ratios. Cocultured NOS+exp (GFP+ve) were isolated directly from co-cultures purified population analyzed for histone modifications and gene expression. ChIP assay from co-cultured NOS+exp on total hFibs indicated active marks on endogenous pluripotency genes. Quantitative PCR of cocultured NOS+exp analysis indicated regained expression of Nanog, Oct4 and Sox2 compared to that of cultured NOS+exp. (g) Reprogramming potential of NOS+exp and NOS+exp cells was tested on MEFs. Left panel- Phase and fluorescence images of NOS+exp/+/exp cells on MEFs or without MEFs (matrigel). Right panel- Phase and fluorescence images of three- five week post reprogramming. Induced pluripotent colonies were only observed when NOS+exp cells were reprogrammed on MEFs. Observed colonies were GFP+ve and Tra 1-60+ve.

[0054] Figure 30 shows unique NOS+exp population identified in hFibs exhibits distinct molecular state and cell cycle properties. (a) Hierarchical clustering of total hFibs, NOS+exp hFibs, NOS−exp hFibs, Skin derived precursors (SKPs), keratinocytes and bulge stem cells (BSC) gene expression signature of fibroblasts and molecular markers specific to individual skin stem/progenitor. (b) Global Cluster analysis of adult stem/progenitor cells. (c) Pie chart for the genes differentially upregulated in NOS+exp over total population. Genes were filtered based on 3-fold cutoff and were 100% present across NOS+exp replicate samples. (d) Hierarchical clustering of gene expression profiles based on cell cycle pathway (http://www.genome.ip/keqq/) expression in control hESC, Total hFibs, NOS+exp, NOS−exp, iPS NOS+exp cells and public data set Fib BJ1, iPS BJ1.2. Featured cell cycle genes are upregulated/downregulated in NOS+exp
fibroblasts/iPS cells compared to total hFibs are indicated. (e) HMMR (CD168) staining was performed in hFibs transduced with EOS vector. HMMR localization was observed in the nuclei of dividing NOS+exp hFibs. (f) hFibs were transduced with EOS vector and growth of NOS+exp and total fibroblasts were measured at every passage by flow cytometry.

Figure 31 shows a proposed model for the role of predisposed NOS+exp hFibs towards pluripotent reprogramming.

Figure 32 shows GFP+ve cells are unique in total hFib cultures. (a) Total hFibs were transduced with pGK-EGFP and number of GFP+ve cells were estimated by flow cytometry analysis. (b) Transduction of C3+ EOS lentivirus in control hESC followed by flow cytometry demonstrated consistent increase in GFP+ve cells. (c) Representative phase and immunofluorescence and 3D Z-stack images of hFibs transduced with EOS vector. Arrows indicate EOS transduced GFP+ve cells in the different plane than total hFibs. (d-f) Total hFibs were transduced with C3+ EOS lentivirus. Percentage of GFP+ve cells and mean florescence intensities calculated by flow cytometry analysis indicated constant 3-4 % GFP+ve cells upon EOS C3+ transduction irrespective of viral dilution suggesting these cells are not the artifact due to high copy viral integration. (g) To demonstrate the GFP+ve cells are not the artifacts of high copy viral integration GFP+ve cells were sorted from total hFibs and secondary EOS C3+ transfections were performed. GFP+ve cells from secondary infections were further sorted to perform tertiary infections. An increase in GFP+ve cells was not observed with tertiary and quaternary infections due to the increasing viral copy number. (h) Emergence of green cells upon PGK-EGFP transduction in quaternary-infected cells suggested lack of GFP+ve cells upon EOS C3+ transduction was not due to problems associated with viral uptake.

Figure 33 shows unique cells in fibroblasts cultures express pluripotency gene Oct4. (a) Immunostaining of Oct4 in total hFib cultures, 293 cells and 293 cells overexpressing Oct4 transgene. Arrows indicate Oct4 staining colocalizing with DAPI in the nucleus. (b) MeDIP ChIP was performed
in total hFib cultures, 293 cells, hESC, NOS\textsuperscript{+exp} (GFP \textsuperscript{+ve}) cells. The graph shows specific enrichment of Oct4 promoter methylation in 293 and total fibroblasts compared to that hESC and NOS\textsuperscript{+exp} (GFP \textsuperscript{+ve}) cells.

[0058] Figure 34 shows isotype staining for the reprogrammed colonies from total Fibroblast cells. (a) Flow cytometry analysis for surface isotype staining (control for SSEA3 surface staining) and internal isotype staining (control for Oct4 staining) in iPS like colony derived from total hFibs (b) Flow cytometry analysis for surface isotype staining (control for SSEA3 surface staining) and internal isotype staining (control for Oct4 staining) in non-iPSC colony derived from total hFibs.

[0059] Figure 35 shows induced Pluripotent cells generated from NOS\textsuperscript{+exp} cells can be differentiated into various lineages. (a) \textit{In vitro} EB differentiation of human ES and iPS cells derived from NOS\textsuperscript{+exp} Fibs towards the hematopoietic lineage as shown by CD45 pan hematopoietic factor staining. (b) Human ES cells and iPSC cells derived from NOS\textsuperscript{+exp} hFibs differentiate towards the neuronal lineage as shown by A2B5 staining.

[0060] Figure 36 shows NOS\textsuperscript{-exp} hFibs are slow growing and do not contribute to reprogramming. (a) Purified NOS\textsuperscript{-exp} hFibs were transduced with lentivirus containing Oct4, Nanog, Sox2, and Lin28. Cultures were monitored for 6 weeks, NOS\textsuperscript{-exp} hFibs did not generate iPSC colonies. (b) NOS\textsuperscript{-exp} were mixed with heterogeneous hFibs at a ratio indicated, and transduced with lentivirus containing Oct4, Nanog, Sox2, and Lin28. Between 2-6 weeks, only one colony was detected in any experiment. (c) Fifty thousand NOS\textsuperscript{-exp} were seeded and growth rate was monitored by cell counting over serial passages indicated.

[0061] Figure 37 shows hFibs were transduced with C3+ EOS Lentivirus and NOS\textsuperscript{+exp} hFibs were sorted and maintained for indicated passages. At every passage GFP expression was measured by flow cytometry.
[0062] Figure 38 shows Oct-4 transduced human fibroblasts give rise to astrocytes, oligodendrocytes and neurons. a. Schema presenting neural lineage specification time line upon Oct-4 transduction. b. Representative bright field images of untransduced fibroblasts and Oct-4-transduced fibroblast that gave rise to astrocytes, oligodendrocytes and neurons (n=3). c. Representative immunofluorescence image of astrocytes stained with GFAP (n=3). d. Representative FACS plot of GFAP levels in fibroblasts (Fibs) and Oct-4 transduced fibroblasts (Fibs$^{Oct-4}$) (n=3; p<0.01). e. Representative immunofluorescence image of neurons stained with beta-Tubulin III (n=3). f. Representative FACS plot of beta-Tubulin III levels in fibroblasts (Fibs) and Oct-4 transduced fibroblasts (Fibs$^{Oct-4}$) (n=3; p<0.01). g. Representative immunofluorescence image of oligodendrocytes stained with Olig-4 (n=3). h. Representative FACS plot of Olig-4 levels in fibroblasts (Fibs) and Oct-4 transduced fibroblasts (Fibs$^{Oct-4}$) (n=3; p<0.01). i. Frequency of GFAP, Olig-4 and beta-Tubulin III levels in Oct-4 transduced fibroblasts (n=3).

[0063] Figure 39 shows Oct-4 transduced human fibroblasts give rise to mature neurons with a dopaminergic phenotype. Schema presenting dopamnergic neuron derivation time line (right panel) and dopaminergic neural immunofluorescence staining beta-Tubulin III and Tyrosine Hydroxylase.

[0064] Figure 40 shows transduction of fibroblasts with Oct4 induces the expression of genes associated with neural progenitor development. Gene expression patterns obtained by affymetrix array hybridization of samples derived from fibroblasts and fibroblasts transduced with Oct4 after 4 days. Gene expression patterns were compared in silico and are differences are depicted as fold change of fibroblasts+OCT4/fibroblasts. Statistical significance testing was performed using Student's t-test.

**Detailed description of the disclosure**

A. Direct Conversion of Fibroblasts to Progenitor and Differentiated Cells
The present inventors have shown that Oct-4 transduced dermal fibroblasts give rise to hematopoietic and neural progenitor cells. The inventors further showed that the hematopoietic progenitor cells had the capacity to fully reconstitute the myeloid lineage.

Accordingly, the present disclosure provides a method of generating progenitor cells from fibroblasts comprising:

a) providing fibroblasts that express or are treated with the POU domain containing gene or protein; and

b) culturing the cells of step (a) under conditions to allow production of progenitor cells without traversing the pluripotent state.

The term "POU domain containing gene or protein" as used herein refers to a gene or protein containing a POU domain that binds to Octamer DNA binding sequences as shown in Figure 7 or SEQ ID NOs:1 or 2. In one embodiment, the POU domain containing gene or protein is an Oct gene or protein, including without limitation, the Oct-1, -2, -4, or -11. In a particular embodiment, the Oct gene or protein is Oct-4.

The term "progenitor cell" as used herein refers to a less specialized cell that has the ability to differentiate into a more specialized cell. Types of progenitor cells include, without limitation, cells that give rise to neural and hematopoietic lineages. In one embodiment, the progenitor cell is a hematopoietic progenitor cell. In another embodiment, the progenitor cell is a neural progenitor cell.

The phrase "without traversing the pluripotent state" as used herein refers to the direct conversion of the fibroblast to the progenitor cell, for example, the produced cells lack pluripotent stem cell properties, such as Tra-1-60 or SSEA3.

The term "hematopoietic progenitor cell" as used herein refers to a cell that gives rise to blood cells and includes, without limitation, CD45+ cells. Accordingly, in an embodiment, the cells of (b) are sorted to purify CD34 or CD45 positive cells.
The term "neural progenitor cell" as used herein refers to a cell that gives rise to cells of the neural lineage, including, without limitation, neurons and glial cells, for example, astrocytes and oligodendrocytes. Neural progenitor markers include, without limitation, A2B5, nestin, GFAP, betta tubulin III, oligo-4 and tyrosin Hydroxylase. In an optional embodiment, the neural cells are sorted using these markers.

The term "fibroblast" as used herein refers to a type of cell encountered in many tissues of the body including connective tissue and that can be derived using standard cell culture methods. For example, fibroblasts can be generated from adult and fetal tissues including blood, bone marrow, cord blood and placenta. In one embodiment, the fibroblast is a dermal fibroblast. The term "dermal fibroblast" as used herein refers to fibroblasts isolated from skin of any animal, such as a human. In one embodiment, the animal is an adult. In another embodiment, the fibroblast has been cryopreserved. In an alternative embodiment, cells expressing POU domain containing genes other than fibroblasts can be used in step (a).

The term "Oct-4" as used herein refers to the gene product of the Oct-4 gene and includes Oct-4 from any species or source and includes analogs and fragments or portions of Oct-4 that retain enhancing activity. The Oct-4 protein may have any of the known published sequences for Oct-4 which can be obtained from public sources such as Genbank. An example of such a sequence includes, but is not limited to, NM_002701. OCT-4 also referred to as POU5-F1 or MGC22487 or OCT3 or OCT4 or OTF3 or OTF4.

The term "Oct-1" as used herein refers to the gene product of the Oct-1 gene and includes Oct-1 from any species or source and includes analogs and fragments or portions of Oct-1 that retain enhancing activity. The Oct-1 protein may have any of the known published sequences for Oct-1 which can be obtained from public sources such as Genbank. An example of such a sequence includes, but is not limited to, NM_002697.2. Oct-1 also referred to as POU2-F1 or OCT1 or OTF1.
The term "Oct-2" as used herein refers to the gene product of the Oct-2 gene and includes Oct-2 from any species or source and includes analogs and fragments or portions of Oct-2 that retain enhancing activity. The Oct-2 protein may have any of the known published sequences for Oct-2 which can be obtained from public sources such as Genbank. An example of such a sequence includes, but is not limited to, NM_002698.2. Oct-2 is also referred to as POU2-F2 or OTF2.

The term "Oct-11" as used herein refers to the gene product of the Oct-11 gene and includes Oct-11 from any species or source and includes analogs and fragments or portions of Oct-11 that retain enhancing activity. The Oct-11 protein may have any of the known published sequences for Oct-11 which can be obtained from public sources such as Genbank. An example of such a sequence includes, but is not limited to, NM_014352.2. Oct-11 is also referred to as POU2F3.

In one embodiment, fibroblasts that express a POU domain containing gene or protein, such as Oct-1, -2, -4 or -11, include overexpression of the endogenous POU domain containing gene or ectopic expression of the POU domain containing gene or protein. In an embodiment, the fibroblasts do not additionally overexpress or ectopically express or are not treated with Nanog or Sox-2.

Fibroblasts that express a POU domain containing protein or gene, such as Oct-1, -2, -4 or -11, can be obtained by various methods known in the art, including, without limitation, by overexpressing endogenous POU domain containing gene, or by introducing a POU domain containing protein or gene into the cells to produce transformed, transfected or transduced cells. The terms "transformed", "transfected" or "transduced" are intended to encompass introduction of a nucleic acid (e.g. a vector) into a cell by one of many possible techniques known in the art. For example, nucleic acid can be introduced into mammalian cells via conventional techniques such as calcium phosphate or calcium chloride co-precipitation, DEAE-dextran mediated transfection, lipofectamine, electroporation or microinjection or via viral
transduction or transfection. Suitable methods for transforming, transducing and transfecting cells can be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual, 3rd Edition, Cold Spring Harbor Laboratory Press, 2001), and other laboratory textbooks. Suitable expression vectors for directing expression in mammalian cells generally include a promoter (e.g., derived from viral material such as polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40), as well as other transcriptional and translational control sequences. Examples of mammalian expression vectors include pCDM8 (Seed, B., Nature 329:840 (1987)) and pMT2PC (Kaufman et al., EMBO J. 6:187-195 (1987)).

[0079] In one embodiment, fibroblasts that express a POL domain containing gene or protein are produced by lentiviral transduction. In another embodiment, the fibroblasts that are treated with a POU domain containing gene or protein include addition of exogenous POU domain containing protein or functional variants or fragments thereof or peptide mimetics thereof. In another embodiment, the fibroblasts that are treated with a POU domain containing gene or protein include addition of a chemical replacer that can be used that induces a POU domain containing gene or protein expression.

[0080] The POU domain containing proteins may also contain or be used to obtain or design "peptide mimetics". For example, a peptide mimic may be made to mimic the function of a POU domain containing protein. "Peptide mimetics" are structures which serve as substitutes for peptides in interactions between molecules (See Morgan et al (1989), Ann. Reports Med. Chem. 24:243-252 for a review). Peptide mimetics include synthetic structures which may or may not contain amino acids and/or peptide bonds but retain the structural and functional features. Peptide mimetics also include molecules incorporating peptides into larger molecules with other functional elements (e.g., as described in WO 99/25044). Peptide mimetics also include peptoids, oligopeptoids (Simon et al (1972) Proc. Natl. Acad. Sci USA 89:9367) and peptide libraries containing peptides of a designed length.
representing all possible sequences of amino acids corresponding to a POU domain containing peptide.

[0081] Peptide mimetics may be designed based on information obtained by systematic replacement of L-amino acids by D-amino acids, replacement of side chains with groups having different electronic properties, and by systematic replacement of peptide bonds with amide bond replacements. Local conformational constraints can also be introduced to determine conformational requirements for activity of a candidate peptide mimetic. The mimetics may include isosteric amide bonds, or D-amino acids to stabilize or promote reverse turn conformations and to help stabilize the molecule. Cyclic amino acid analogues may be used to constrain amino acid residues to particular conformational states. The mimetics can also include mimics of the secondary structures of the proteins described herein. These structures can model the 3-dimensional orientation of amino acid residues into the known secondary conformations of proteins. Peptoids may also be used which are oligomers of N-substituted amino acids and can be used as motifs for the generation of chemically diverse libraries of novel molecules.

[0082] The term "variant" as used herein includes modifications, substitutions, additions, derivatives, analogs, fragments or chemical equivalents of the POU domain containing proteins that perform substantially the same function in substantially the same way. For instance, the variants of the POU domain containing proteins would have the same function of being useful in binding the Octamer sequences shown in Figure 7.

[0083] Conditions that allow production of progenitor cells are readily known in the art. For example, colony formation is a standard method known in the art for culturing progenitor cells. The cell culture medium can be any medium that can support the growth of cells including, without limitation, a semi-solid medium. In one embodiment, the conditions comprise a culture period from 14-31 days, optionally 21 days.

[0084] In another embodiment, the cells are cultured in any medium that can support the growth of cells and then, for example, after at least 3
days, are placed in differentiation media, such as hematopoietic medium, or
neural medium, under conditions to allow production of differentiated cells,
such as hematopoietic and neural cells.

[0085] The term "differentiation" or "differentiated" as used herein refers
to the process by which a less specialized cell, such as a stem cell, becomes
a more specialized cell type, such that it is committed to a specific lineage.

[0086] The term "hematopoietic medium" as used herein refers to cell
culture media that supports growth and/or differentiation of hematopoietic
cells. In one embodiment, the hematopoietic medium comprises at least one
hematopoietic cytokine, such as Flt3, SCF or EPO. In an embodiment, the
cytokine is Flt3 or SCF. In one embodiment, the differentiated hematopoietic
cell is of the myeloblast lineage, such as a monocyte or granulocyte. In
another embodiment, the hematopoietic cytokine is EPO and the
differentiated hematopoietic cell is of the erythroid or megakaryocytic lineage.

[0087] The term "neural medium" as used herein refers to cell culture
media that supports growth and/or differentiation of neural cells. In one
embodiment, the neural medium comprises neural basal media supplemented
with fibroblast growth factor, epidermal growth factor or bone morphogenetic
factor 4 (BMP-4), bFGF (10ng/ml), the N-terminal active fragment of human
SHH (200 ng/ml), FGF8 (100 ng/ml; R&D), GDNF (20 ng/ml), BDNF (20
ng/ml) and/or fetal bovine serum. In an embodiment, the differentiated neural
cell is a neuron or a glial cell such as an astrocyte, and/or oligodendrocyte.

[0088] In another aspect, the present disclosure provides isolated
progenitor or differentiated cells generated by the methods described herein.
Such cells do not express a number of pluripotency markers, such as TRA-1-
60 or SSEA-3. In addition, during development such cells lose the expression
of the Oct-4 pluripotency marker and thus represent a new source of safe
alternatives for progenitor cells.

[0089] In yet another aspect, the disclosure provides use of the cells
described herein for engraftment or cell replacement. In another embodiment,
the disclosure provides the cells described herein for use in engraftment or cell replacement. Further provided herein is use of the cells described herein in the manufacture of a medicament for engraftment or cell replacement. "Engraftment" as used herein refers to the transfer of the hematopoietic cells produced by the methods described herein to a subject in need thereof. The graft may be allogeneic, where the cells from one subject are transferred to another subject; xenogeneic, where the cells from a foreign species are transferred to a subject; syngeneic, where the cells are from a genetically identical donor or an autograft, where the cells are transferred from one site to another site on the same subject. Accordingly, also provided herein is a method of engraftment or cell replacement comprising transferring the cells described herein to a subject in need thereof. The term "cell replacement" as used herein refers to replacing cells of a subject, such as red blood cells or platelets, or neurons or glial cells or hematopoietic progenitors. In yet another embodiment, cells for engraftment or cell replacement may be modified genetically or otherwise for the correction of disease. Fibroblasts before or after transfection or transduction with a POU domain containing gene may be genetically modified to overexpress a gene of interest capable of correcting an abnormal phenotype, cells would be then selected and transplanted into a subject. In another aspect, fibroblasts or POU domain containing gene-expressing fibroblasts overexpressing or lacking complete expression of a gene that is characteristic of a certain disease would produce progenitor or differentiated cells for disease modeling, for example drug screening.

[0090] The term "subject" includes all members of the animal kingdom, including human. In one embodiment, the subject is an animal. In another embodiment, the subject is a human.

[0091] In one embodiment, the engraftment or cell replacement described herein is for autologous or non-autologous transplantation. The term "autologous transplantation" as used herein refers to providing fibroblasts from a subject, generating progenitor or differentiated cells from the isolated fibroblasts by the methods described herein and transferring the
generated progenitor or differentiated cells back into the same subject. The term "non-autologous transplantation" refers to providing fibroblasts from a subject, generating progenitor or differentiated cells from the isolated fibroblasts by the methods described herein and transferring the generated progenitor or differentiated cells back into a different subject.

[0092] In yet another aspect, the disclosure provides use of the cells described herein as a source of blood, cellular or acellular blood components, blood products, hematopoietic stem cells and neural cells. Such sources can be used for replacement, research and/or drug discovery.

[0093] The methods and cells described herein may be used for the study of the cellular and molecular biology of progenitor cell development, for the discovery of genes, growth factors, and differentiation factors that play a role in differentiation and for drug discovery. Accordingly, in another aspect, the disclosure provides a method of screening progenitor or differentiated cells comprising

a) preparing a culture of progenitor or differentiated cells by the methods described herein;

b) treating the progenitor or differentiated cells with a test agent or agents; and

c) subjecting the treated progenitor or differentiated cells to analysis.

[0094] In one embodiment, the test agent is a chemical or other substance, such as a drug, being tested for its effect on the differentiation of the cells into specific cell types. In such an embodiment, the analysis may comprise detecting markers of differentiated cell types. For example: CD45, CD13, CD33, CD14, CD15, CD71, CD235a (Glycophorin A), CD133, CD38, CD127, CD41a, beta-globin, HLA-DR, HLA-A,B,C, CD34, A2B5, nestin, GFAP, beta tubulin III, oligo-4 and tyrosin Hydroxylase. In another embodiment, the test agent is a chemical or drug and the screening is used as a primary or secondary screen to assess the efficacy and safety of the
agent. Such analysis can include measuring cell proliferation or death or cellular specific features such as mast cell degranulation, phagocytosis, oxygen exchange, neural signaling, presence of action potential, secretion of certain proteins, activation of specific genes or proteins, activation or inhibition of certain signaling cascades.

B. Reprogramming Fibroblasts into Induced Pluripotent Stem Cells

[0095] Given the unknown origins of human fibroblasts that form the foundation for cellular reprogramming toward human iPSCs, the present inventors sought to characterize adult dermal fibroblasts in the context of the cellular reprogramming process. The present inventors have identified and characterized a subpopulation of adult human dermal fibroblasts responsible for the generation of reprogrammed cells.

[0096] Accordingly, the present disclosure provides a method of isolating a subpopulation of fibroblasts with increased reprogramming potential comprising

a) providing fibroblasts that express an Oct-4-reporter; and
b) isolating cells positive for the reporter.

[0097] Definitions from part A that are relevant to this section apply to this section as well.

[0098] Fibroblasts that express an Oct-4-reporter can be produced by various methods known in the art, including, without limitation, introduction of a nucleic acid construct or vector by transformation, transfection or transduction as herein defined. In one embodiment, the Oct-4-reporter gene is introduced by lentiviral transduction.

[0099] The term "reprogramming potential" as used herein refers to the potential of the cells to regain progenitor or stem cell capacity or pluripotent state. The term "increased reprogramming potential" as used herein means that the reprogramming potential is greater than the potential for a mixed population of fibroblasts that have not been selected or isolated.
The term "Oct-4-reporter" as used herein refers to DNA sequences that are bound by Oct-4 upstream of a reporter that allow or enhance transcription of the downstream sequences of the reporter. Oct-4 reporters are known in the art. For example, an Oct-4 reporter is described in Hotta et al. 2009 and Okumura-Nakanishi et al. 2005 incorporated herein by reference in its entirety.

The term "reporter gene" and "reporter" as used herein refers to any gene that encodes a protein that is identifiable. Reporter genes and reporter products are readily identified by a skilled person. In an embodiment, more than one reporter gene/reporter is used. In one embodiment, the reporter gene comprises a fluorescent protein (such as green fluorescent protein, GFP) and the cells are isolated in step (b) by detection of the fluorescent protein under fluorescence. In another embodiment, the reporter gene encodes a gene conferring antibiotic resistance, such as to puromycin, and the cells are isolated by survival in the presence of the antibiotic. In one embodiment, the fibroblasts are dermal fibroblasts. The reporter gene could also encode a tag and the cells can be isolated based on immuno separation (http://www.miltenyibiotech.com/en/PG_167_501_MACSelect_Vectors_and_Taq_Vector_Sets.aspx).

The disclosure also provides a method of generating reprogrammed fibroblast-derived induced pluripotent stem (iPS) cells comprising

a) providing (i) a population of fibroblasts with increased expression of Oct-4 and (ii) a mixed population of fibroblasts or a population of Oct-4 negative fibroblasts;

b) treating the fibroblasts of a) with Oct-4, Sox-2, Nanog and Lin-28; and

c) culturing the cells of (b) under conditions that allow the production of iPS cells.
In one embodiment, the fibroblasts in b) are treated with Oct-4, Sox-2, Nanog and Lin-28 by introducing the respective genes by viral transduction, such as lentiviral transduction.

The term "stem cell" as used herein refers to a cell that has the ability for self-renewal. In one embodiment, the stem cell is a pluripotent stem cell. The term "pluripotent" as used herein refers to an undifferentiated cell that maintains the ability to allow differentiation into various cell types. The term "induced pluripotent stem cell" refers to a pluripotent stem cell that has been artificially derived from a non-pluripotent stem cell.

The term "Sox-2" as used herein refers to the gene product of the Sox-2 gene and includes Sox-2 from any species or source and includes variants, analogs and fragments or portion of Sox-2 that retain activity. The Sox-2 protein may have any of the known published sequences for Sox-2, which can be obtained from public sources such as GenBank. An example of such a sequence includes, but is not limited to, NM_003106.

The term "Nanog" as used herein refers to the gene product of the Nanog gene and includes Nanog from any species or source and includes variants, analogs and fragments or portion of Nanog that retain activity. The Nanog protein may have any of the known published sequences for Nanog, which can be obtained from public sources such as GenBank. An example of such a sequence includes, but is not limited to, NM_024865.

The term "Lin-28" as used herein refers to the gene product of the Lin-28 gene and includes Lin-28 from any species or source and includes variants, analogs and fragments or portions of Lin-28 that retain activity. The Lin-28 protein may have any of the known published sequences for Lin 28, which can be obtained from public sources such as GenBank. An example of such a sequence includes, but is not limited to, BC028566.2. Lin-28 also called CSDD1 or ZCCHC1 or Lin28A.

The term "mixed population" as used herein refers to a mixed population of fibroblasts derived from an animal as opposed to a selected
subpopulation. The term bulk population may also be used interchangeably in this disclosure. The mixed population contains cells that express varying levels of Oct-4, Sox-2 and/or Nanog.

[00109] In another embodiment, the method further comprises analyzing and selecting cells that express a marker of undifferentiated stem cells, such as TRA-1-60, SSEA-3, Sox2, Nanog, SSEA4, TRA-1-81, IGF1 receptor, connexin 43, E-cadherin, Alkaline phosphatase, REX1, CRIPTO, CD24, CD90, CD29, CD9 and CD49f. In one embodiment, the cells are selected for expression of TRA-1-60 and/or SSEA-3.

[00110] The term "TRA-1-60" as used herein refers to the gene product of the TRA-1-60 gene and includes TRA-1-60 from any species or source and includes analogs and fragments or portion of TRA-1-60 that retain activity. The TRA-1-60 protein may have any of the known published sequences for TRA-1-60, which can be obtained from public sources such as GenBank. Examples of such sequences include, but are not limited to, NM_0010181 and NM_005397.

[00111] The term "SSEA-3" as used herein refers to the gene product of the SSEA-3 gene and includes SSEA-3 from any species or source and includes analogs and fragments or portion of SSEA-3 that retain activity. The SSEA-3 protein may have any of the known published sequences for SSEA-3 which can be obtained from public sources such as GenBank. Examples of such sequences include, but are not limited to NM_00122993.

[00112] In an embodiment, the population of fibroblasts with increased expression of Oct-4 are produced by the method described herein for isolating a subpopulation of fibroblasts with reprogramming potential. In an embodiment, the population of fibroblasts expressing Oct-4 comprise expression of Oct-4 or its isoform B1 but not its cytoplasmic isoform Oct4B.

[00113] In one embodiment, the fibroblasts are dermal fibroblasts. Dermal fibroblasts may be derived, for example, from the skin of an animal.
In one embodiment, the ratio of cells in step (a) (i) to cells in step (a) (ii) is 50:50 to 10:90. In an embodiment, the ratio of cells in step (a) (i) to cells in step (a) (ii) is 50:50. In another embodiment, the ratio of cells in step (a) (i) to cells in step (a) (ii) is 10:90. In yet another embodiment, the ratio of cells in step (a) (i) to cells in step (a) (ii) is 25:75.

Conditions that allow the production of iPS cells are readily known in the art and include, without limitation, colony forming assays for a culture period from 2 to 3 weeks.

The present disclosure further provides isolated induced pluripotent stem (iPS) cells generated by the method described herein and cells differentiated therefrom.

In yet another aspect, the disclosure provides use of the iPS cells described herein or cells differentiated therefrom for engraftment. The disclosure also provides the iPS cells described herein or cells differentiated therefrom for use in engraftment. Further provided is the use of the iPS cells described herein in the preparation of a medicament for engraftment. "Engraftment" as used herein refers to the transfer of the cells produced by the methods described herein to a subject in need thereof. The graft may be allogeneic, where the cells from one subject are transferred to another subject; xenogeneic, where the cells from a foreign species are transferred to a subject; syngeneic, where the cells are from a genetically identical donor or an autograft, where the cells are transferred from one site to another site on the same subject. Accordingly, also provided herein is a method of engraftment comprising transferring the iPS cells described herein or cells differentiated therefrom to a subject in need thereof.

The term "subject" includes all members of the animal kingdom, including human. In one embodiment, the subject is an animal. In another embodiment, the subject is a human.

In one embodiment, the engraftment described herein is for autologous or non-autologous transplantation. The term "autologous
transplantation" as used herein refers to providing fibroblasts from a subject, generating iPS cells from the isolated fibroblasts by the methods described herein and transferring the generated iPS cells or cells differentiated therefrom back into the same subject. The term "non-autologous transplantation" refers to providing fibroblasts from a subject, generating iPS cells from the isolated fibroblasts by the methods described herein and transferring the generated iPS cells or cells differentiated therefrom back into a different subject. For cells differentiated from the iPS cells, the iPS cells are first differentiated \textit{in vitro} and then transferred into the subject.

In yet another aspect, the disclosure provides use of the cells described herein as a source of iPS cells or differentiated cells therefrom.

The methods and cells described herein may be used for the study of the cellular and molecular biology of stem cell development, for the discovery of genes, growth factors, and differentiation factors that play a role in stem cell differentiation and for drug discovery. Accordingly, in another aspect, the disclosure provides a method of screening iPS cells or cells differentiated therefrom comprising

a) preparing a culture of iPS cells by the methods described herein or cells differentiated therefrom;

b) treating the cells with a test agent or agents; and

c) subjecting the treated cells to analysis.

In one embodiment, the test agent is a chemical or other substance, such as a drug, being tested for its effect on the differentiation of the iPS cells into specific cell types. In such an embodiment, the analysis may comprise detecting markers of differentiated cell types. For example, CD45, CD13, CD33, CD14, CD15, CD71, CD235a (Glycophorin A), CD133, CD38, CD127, CD41a, beta-globin, HLA-DR, HLA-A,B,C, and CD34, A2B5, nestin, GFAP, beta tubulin III, oligo-4 and tyrosin Hydroxylase. In another embodiment, the test agent is a chemical or drug and the screening is used as a primary or secondary screen to assess the efficacy and safety of the
agent. In an embodiment, the analysis comprises analyzing cell proliferation or cell death or cell differentiation, or generation of progenitors or differentiated cells of interest.

[00123] The above disclosure generally describes the present disclosure. A more complete understanding can be obtained by reference to the following specific examples. These examples are described solely for the purpose of illustration and are not intended to limit the scope of the disclosure. Changes in form and substitution of equivalents are contemplated as circumstances might suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitation.

[00124] The following non-limiting examples are illustrative of the present disclosure:

Examples

EXAMPLE 1: Direct Conversion from Dermal Fibroblasts to Blood

Results

Emergence of a CD45^+ population from hFibs transduced with Oct-4

[00125] Reprogramming towards pluripotency requires a cascade of events that encompasses generation of various intermediate cells among a rare subset of stable induced pluripotent stem cells (iPSCs) capable of teratoma formation (Takahashi et al., 2007; Takahashi and Yamanaka, 2006) (Figs. 8a-c). A portion of these intermediates form colonies that possess round cellular morphology resembling hematopoietic cells (Fig. 9a) and express the human pan-hematopoietic marker CD45 (CD45^+), but lack co-expression of the pluripotency marker Tra-1-60 (Chan et al., 2009), indicative of iPSCs (Figs. 9b-c). These human fibroblast (hFib)-derived CD45^+ cells could be isolated by FACS and shown to preferentially express ectopic Oct-4 whilst demonstrating low levels of Sox-2 and Nanog (Figs. 9d-e). These findings indicate that, unlike the fully reprogrammed iPSCs, hFib-derived
intermediates could acquire distinct lineage-specific phenotype, as exemplified by the acquisition of the human hematopoietic marker CD45.

[00126] Based on the acquisition of CD45, together with higher levels of Oct-4 vs Nanog or Sox-2, the role of Oct-4 during colony emergence from two independent sources of adult dermal and fetal (foreskin) hFibs was compared with that of Nanog or Sox-2 alone (Fig. 1a). Transduced and untransduced hFibs were examined for colony formation between 14-21 days post-transduction (illustrated in Fig. 10). Unlike untransduced, or hFibs transduced with Sox-2 (hFibs$^{\text{Sox-2}}$) or Nanog (hFibs$^{\text{Nanog}}$), hFibs expressing Oct-4 (hFib$^{\text{Oct-4}}$) gave rise to colonies (Fig. 1a and Fig. 10b) and exhibited Oct-4 expression similar to the levels detected in established iPSCs (Fig. 1b). Only the hFibs$^{\text{Oct-4}}$ gave rise to hematopoietic-like CD45+$^{\text{ve}}$ cells (Fig. 1c; adult hFibs -38%; fetal hFibs -24%). Furthermore, CD45+$^{\text{ve}}$ cells (CD45+hFibs$^{\text{Oct-4}}$ (day 21)) showed an increase in Oct-4 expression using multiple probe sets (Fig. 9c) with a concomitant decrease in the fibroblast specific gene expression (Yu et al., 2007) (Fig. 2a), demonstrating the acquisition of a distinct gene signature. Approximately 1000 genes were downregulated and an equal number upregulated by day 4 post-transduction resulting in the observed shift from a fibroblast phenotype towards a CD45+$^{\text{ve}}$ phenotype (Table 3). Collectively, these data indicate that Oct-4 is uniquely sufficient to initiate the CD45+$^{\text{ve}}$ cell emergence from multiple sources of human dermal fibroblasts.

[00127] To better characterize emerging CD45+$^{\text{ve}}$ hFibs, it was aimed to enhance CD45+$^{\text{ve}}$ colony formation using Flt3 (FMS-like tyrosine kinase 3) ligand and SCF (stem cell factor), representing inductive growth factors essential for early hematopoiesis (Gabbianelli et al., 1995; Hassan and Zander, 1996; Lyman et al., 1993). Treatment with Flt3L and SCF increased the frequency of CD45+$^{\text{ve}}$ colony emergence from both fetal and adult hFibs$^{\text{Oct-4}}$ by 4- and 6-fold respectively, compared with untreated hFib$^{\text{Oct-4}}$ counterparts (Figs. 2b-c), while no effect was detectable in the control hFibs (Figs. 2b-c and Fig. 11). These results indicated that CD45+$^{\text{ve}}$ cells derived
from Oct-4-transduced hFibs are responsive to early hematopoietic growth factors.

**CD45^{+ve} cell derivation from hFibs does not traverse the pluripotent state**

Ectopic expression of Oct-4 alone has been shown to result in pluripotent reprogramming of neural progenitors that endogenously express Sox-2 (Kim et al., 2009). Accordingly, the expression of a panel of genes known to be essential for induction and maintenance of pluripotency (Takahashi and Yamanaka, 2006) was examined during Oct-4-induced emergence of CD45^{+ve} hFibs. Apart from upregulation of Oct-4 (POU5F1) (Fig. 12a), Oct-4 transduction did not alter the pluripotency gene expression profile of the hFibs (Figs. 2d). Furthermore, related POU family members Oct-2 (POU2F2) and Oct-1 (POU2F1) remained unaffected (Fig. 12b). In addition, established markers of fully reprogrammed iPSCs such as SSEA3 and Tra-1-60 levels were examined during Oct-4-induced CD45^{+ve} colony emergence vs iPSC derivation from hFibs transduced with complete set of reprogramming factors (Oct-4, Sox-2, Nanog, and Lin-28) (Yu et al., 2007). Upon ectopic expression of Oct-4 alone, neither SSEA3 nor Tra-1-60 was detectable between days 0 to 31 in hFibs^{0.ctl-4}, whereas SSEA3 and Tra-1-60 levels gradually increased during establishment of pluripotent iPSCs (Figs. 3a-b, Figs. 12c-e). Unlike the fully reprogrammed iPSCs that are able to give rise to endoderm, mesoderm, and ectoderm germ layers, injection of an equal number of Oct-4-transduced hFibs into immunodeficient mice failed to give rise to teratomas (Fig. 2c and Table 1). Unlike iPSCs, neither hFibs nor CD45^{+ve}hFibs^{0.ctl-4} were immortalized, but could be maintained for approximately 7 passages (Fig. 13a), without elevation of oncogenic-transforming factor c-Myc (Lebofsky and Walter, 2007) (Fig. 13b). Accordingly, the results indicate that the hFib^{0.ctl-4} cells manifest a cell fate decision conducive to hematopoietic fate selection without the detectible phenotype or functional properties of transformed or pluripotent cells.

**CD45^{+ve}hFibs^{0.ctl-4} possess in vitro and in vivo hematopoietic progenitor**
Global gene expression analysis indicated that the CD45+ve hFibs0.0t4 cluster with mononuclear cells (MNCs) derived from mobilized peripheral blood (M-PB) and umbilical cord blood (UCB)-derived hematopoietic progenitors (CD34+ve cells) (Figs. 14a-b). This suggests that CD45+ve hFibs0.0t4 may possess functional hematopoietic potential of multiple blood cell types. To define functional human hematopoietic capacity, both adult and fetal CD45+ve hFibs0.0c4 were physically isolated and subsequently cultured with a cytokine cocktail known to support human adult hematopoietic progenitor development (Wang et al., 2005) (Fig. 4a), which allowed subsequent expansion of CD45+ve hFibs0.0ct4 (Figs. 14c-d). The resulting progeny retained CD45 expression and acquired myeloid-specific markers CD33 and CD13 (Fig. 4b and Fig. 15). A subfraction of CD45+ve hFibs0.0c4 progeny included monocytes expressing CD14 (Figs. 4c-d and Fig. 16a) that could be further stimulated by responsiveness to M-CSF and IL-4 to functionally mature into macrophages capable of phagocytosis (Silverstein et al., 1977). CD45+ve hFibs0.0c4-derived monocytes were able to engulf FITC-labeled latex beads, as indicated by FACS (Fig. 4e) and immunofluorescence analysis (Fig. 4f and Fig. 16b), while untransduced cytokine treated hFibs were devoid of this unique property (Fig. 4e). Hematopoietic cytokine-treated CD45+ve hFibs0.0ct4 derived from multiple sources of hFibs (adult and fetal) could also give rise to granulocytic cell types distinct from the monocytic cells (Fig. 17a), as indicated by expression of granulocyte marker CD15 (Fig. 4g and Fig. 17b) and by characteristic cellular and polynuclear morphologies that are associated with granulocytic subtypes including neutrophils, eosinophils, and basophils (Fig. 4h and Fig. 17c). Without cytokines, CD45+ve hFibs0.0c4 cells retained CD45 expression, however, myeloid-specific markers were significantly reduced and monocytic and granulocytic lineages were absent (Figs. 18a-b). These results indicate that cytokine stimulation is necessary for hematopoietic expansion and maturation from CD45+ve hFibs0.0ct4.

Approximately ¼ of the cytokine-stimulated CD45+ve hFibs0.0c4 from either adult or fetal dermal sources co-expressed CD34 and CD45,
suggestive of hematopoietic progenitor potential (Fig. 4i and Fig. 19). Clonal proliferative and developmental potential to the granulocytic and monocytic hematopoietic lineages were measured by standard colony forming unit (CFU) assays (Figs. 4j-k). Similar to somatic UCB-derived hematopoietic progenitors, CD45^{+ve}hFibs^{Oct-4} were able to give rise to CFUs at relatively equal capacity (Figs. 4k-l). Collectively, the data indicates that the CD45^{+ve}hFibs^{Oct-4} have the ability to give rise to functional hematopoietic progenitor-like cells that are able to mature into human myeloid lineages in vitro.

[00131] Based on the primitive myeloid capacity detected in vitro, CD45^{+ve}hFibs^{Oct-4} progeny (day 37 post transduction) were transplanted into immunodeficient NOD/SCID IL2Ryc-null (NSG) mice by intrafemoral injection to characterize their in vivo reconstitution potential (Fig. 5a). CD45^{+ve}hFibs^{Oct-4}-derived cells engrafted all transplanted NSG recipients up to levels of 20%, as indicated by presence of human cells (HLA-A/B/C^{+ve}) (Fig. 5b), while injection of adult hFibs or saline did not give rise to a graft in NSG mice (Fig. 5c). The levels of engraftment of CD45^{+ve}hFibs^{Oct-4} were comparable to those observed for the engrafted UCB-derived progenitors and M-PB (Fig. 5d). The cells that primarily reconstituted the NSG recipients exhibited a predominantly myeloid phenotype (-41% - CD45^{+ve}CD14^{+}) (Fig. 5c), compared with UCB- and M-PB-derived engrafted cells (Fig. 5e). After 10 weeks of in vivo hematopoietic engraftment (Fig. 5a), the human cells were isolated from recipients and analyzed for their ability to form CFUs in vitro as a measure of sustained progenitor capacity. A proportion of the engrafted cells retained CFU initiation potential similar to hematopoietic engrafted cells derived from human UCB (Fig. 5f), which was verified by flow cytometry analysis (Figs. 20a-d). The ability to generate human hematopoietic progenitors after 10 weeks of engraftment and the presence of engraftment, albeit at low levels, in the contralateral bones of the primary NSG recipients (Fig. 21a) further supports the in vivo functional capacity of CD45^{+ve}hFibs^{Oct-4}-derived cells. Engrafted CD45^{+ve} cells possessed limited secondary grafts in recipient NSG
mice, (Fig. 21b) indicating they do not possess transformed leukemic stem cell properties (Hope et al., 2004), thus representing safer alternatives as hematopoietic transplantation products in comparison to hPSC-derived cells that retain tumor potential (Amariglio et al., 2009; Roy et al., 2006).

5 **EPO induces erythroid and megakaryocyte capacity from CD45^ve hFibs**

Despite the ability to derive all myeloid lineages from CD45^ve hFibs^0cf^4, erythroid cells were not detected. Erythropoietin (EPO) has been shown to induce early erythroid differentiation (Fried, 2009), thus it was chosen to induce erythroid cell derivation from CD45^ve hFibs^0cf^4. Upon Oct-4 transduction, hFibs expressed the erythroblast marker CD71 at a frequency of nearly 40% (Fig. 6a), which increased 2-fold following EPO induction. In addition, expressions of Glycophorin-A (critical membrane protein required for erythrocyte function) (Fig. 6b), and expression of human adult β-globin protein (uniquely required for oxygen transport by red blood cells) (Fig. 6c), were also induced upon EPO treatment. Untransduced hFibs (Fig. 6c) and hematopoietic cells derived from hPSCs (Fig. 6c inset) lacked β-globin protein levels. In the absence of EPO, only β-globin transcript was expressed in the CD45^ve hFibs^0cf^4 (Fig. 5d), while β-globin protein was undetectable (Fig. 6c).

In contrast, and unlike hematopoietic cells derived from hPSCs (Cerdan et al., 2004; Perlingeiro et al., 2001), hematopoietic cells derived from CD45^ve hFibs^0cf^4 lacked embryonic (zeta) globin expression and only expressed modest levels of fetal (epsilon) globin (Fig. 6d). EPO-treated CD45^ve hFibs^0cf^4 exhibited both primitive and mature erythrocyte (enucleated) morphologies (Fig. 6e) and allowed for erythroid progenitor emergence, detected by colony formation (BFU-E) and CFU-Mixed colonies (CFU-Mix; dual myeloid and erythroid capacity), similar to that observed for UCB, without reduction in monocytic or granulocytic progenitor capacity (Figs. 6f-g, Figs. 22a-b). Based on BFU-E potential and the presence of both adult β-globin protein and enucleated red cells, EPO-treated CD45^ve hFibs^0cf^4 may utilize definitive (adult) and not primitive (embryonic) hematopoietic programs (Orkin and Zon, 2002) during conversion of hFibs to hematopoietic fate.
Studies have indicated that erythroid and megakaryocytic lineage commitment occurs together and potentially arises from a common precursor population (Debili et al., 1996; Klimchenko et al., 2009). Accordingly, the emergence of megakaryocytic lineage following EPO stimulation of CD45^+ve^hFibs^0^ct^-^4 was tested using an in vitro assay available for detection of Megakaryocytic (Mk)-CFUs that serves as a surrogate measure for predicting megakaryocytic recovery in patients (Strodtbeck et al., 2005). Treatment of the CD45^+ve^hFibs^{Oct^-^4} with EPO resulted in the emergence of megakaryocytes (CFU-Mk), as indicated by the presence of Mk-specific antigen GPIIb/IIIa (CD41) positive colonies (Fig. 6h-right panel and Fig. 6i), while this hematopoietic progenitor type was absent (non-CFU-Mk devoid of GPIIb/IIIa ) in CD45^+ve^hFibs^{0^ct^-^4} not stimulated with EPO (Fig. 6h-middle panel, Fig. 6i) or control hFibs (Fig. 6h-left panel and Fig. 6i). These data indicate that CD45^+ve^hFibs^{Oct^-^4} possess both erythroid and megakaryocytic potential. Based on the ability of EPO to reveal additional hematopoietic lineage capacities, CD45^+ve^hFibs^{Oct^-^4} may possess physiological competency and responsiveness to growth factors similar to hematopoietic progenitors derived from the human adult bone marrow compartment (Wojchowski et al., 2006).

**Role of Oct-4 during hematopoietic program activation in hFibs**

To develop a broader understanding of the role of POU domain containing protein Oct-4 during hematopoietic conversion of hFibs, gene expression profiles and Oct-4 promoter occupancy of hematopoietic, non-hematopoietic and pluripotency factors were examined over the time course of CD45^+ve^ cell emergence and maturation (Fig. 7a). Global gene expression analysis indicated several changes in both transcriptional activation and repression. As early as day 4 post Oct-4 transduction, significant changes occur in numerous molecular pathways including metabolic and developmental processes (Fig. 23a). Furthermore, global gene expression of the hFibs taken at three time points over the course of CD45^+ve^ cell emergence (hFibs (day 0), CD45^+ve^hFibs^{0^ct^-^4} (day 4) and CD45^+ve^hFibs^{0^ct^-^4} (day 21)) indicated a decrease in fibroblast-specific gene expression (Yu et
al., 2007) (Fig. 7b), without pluripotency gene induction, excluding the predictable increase in Oct-4 (POU5F1-specific probe sets) (Fig. 7c). Oct-4-transduced hFibs immediately demonstrated an upregulation of a number of hematopoietic cytokine receptors required for responsiveness to cytokines, including Flt3 and c-kit receptors of FLT3L and SCF respectively (Fig. 7d). In addition, transcription factors associated with early human hematopoietic development were also upregulated (Fig. 7e and Figs. 23b-c). These data indicate that Oct-4 induces a cascade of molecular changes in hFibs that orchestrate the hematopoietic fate conversion.

[00135] Ground state bulk populations of hFibs possess nearly undetectable levels of genes associated with pluripotency, such as Nanog and Sox-2, or hematopoietic specification, such as SCL/Tal-1 (T-cell acute lymphocytic leukemia protein 1), Runxl (Runt-related transcription factor 1), C/EBPa (CCAAT/enhancer-binding protein alpha), GATA1 (GATA binding factor 1) or PU.1/Spi-1 (Feng et al., 2008; Friedman, 2007; Ichikawa et al., 2004; Shivdasani et al., 1995) (Fig. 7f and Fig. 24a). However, transduction with Oct-4 was accompanied by a substantial increase of specific hematopoietic genes including SCL, C/EBPa, GATA1, and Runxl (Fig. 7f). Interestingly, hematopoietic-associated genes PU.1 and MixL1, which were previously shown to regulate primitive blood development (Feng et al., 2008; Koschmieder et al., 2005; Ng et al., 2005), were not differentially regulated (Figs. 7e-f and Figs. 23 and 24b-c), suggesting these genes may not be essential for the conversion to blood fate from hFibs. Expression of genes associated with mesodermal transition from the pluripotent state, such as Brachyury and GATA2, were absent in both untransduced hFibs and CD45<sup>+</sup>hFibs<sup>Oct-4</sup> (Fig. 7f), indicating that hematopoietic specification from hFibs does not involve embryonic programs akin to mesodermal specification from hPSCs (Tsai et al., 1994; Vijayaragavan et al., 2009). Molecular analysis of CD45<sup>+</sup>hFibs<sup>0cd-4</sup> following cytokine treatment (D37) that resulted in hematopoietic maturation also reduced Oct-4 levels, but maintained levels of Runxl, SCL, and C/EBPa (Fig. 24b), whereas expression of all adult globins
was induced, including hemoglobin-alpha, beta, and delta (Fig. 24c and Fig. 6d).

Similar to Oct-4, POU domain containing proteins Oct-1 and -2 are also able to regulate hematopoietic-specific genes implicated in specification and maturation of blood cells (Table 2) (Boyer et al., 2005; Ghozi et al., 1996; Kistler et al., 1995; Rodda et al., 2005; Sridharan et al., 2009). Accordingly, gene expression profile of POU domain containing proteins was evaluated in emergence of CD45\textsuperscript{+ve} hFibs. While the expression of Oct-4 (POU5F1) increased during CD45\textsuperscript{+ve} cell emergence, followed by a significant reduction upon cytokine treatment, the expression levels of Oct-2 (POU2F2) and Oct-1 (POU2F1) remained unchanged (Fig. 7g), suggesting that Oct-4 does not target other Oct family members. Nevertheless, Oct-1, -2 and -4 have the potential to bind the same octamer (POU) binding sequences in a cell context specific manner, thereby raising the possibility that Oct-4 has the capacity to bind and potentially regulate similar gene targets of Oct-1 and -2 (Boyer et al., 2005; Kistler et al., 1995; Rodda et al., 2005; Sridharan et al., 2009) (Fig. 7h and Table 2). Thus, to obtain more insight into the possible mechanism by which Oct-4 induces hematopoietic conversion, Oct-4 occupancy of hematopoietic, non-hematopoietic, and pluripotency genes that contain shared Oct 1, 2 or 4 binding sequences in their putative promoters/enhancers was examined (Fig. 7h, Table 2). Consistent with changes in gene expression (Fig. 7f), Runxl, SCL, and GATA1 displayed substantial Oct-4 occupancy (Fig. 7i), a phenomenon previously reported in partially reprogrammed mouse iPSCs and in mouse fibroblasts expressing Oct-4 alone (Sridharan et al., 2009). In addition, the CD45\textsuperscript{+ve}hFibs\textsuperscript{Oct-4} also showed an increase in Oct-4 occupancy at the CD45 promoter (Fig. 7i). To assess the specificity of Oct-4 occupancy of hematopoietic targets during CD45\textsuperscript{+ve} cells emergence, non-hematopoietic associated promoters previously shown to bind Oct-1 or -2, thus possessing the capacity to bind Oct-4 were also examined. Consistent with global gene expression data (Fig. 14a), housekeeping genes Gadd45a and Pol2ra exhibited an increase in Oct-4 occupancy at their respective promoters, while non-hematopoietic genes
Myf5 and Nkx2.5, associated with mesodermal development did not demonstrate significant Oct-4 occupancy in either Oct-4 transduced hFibs or CD45⁺ cells (Fig. 7j). However, Oct-4 uniquely occupied a network of promoters in human pluripotent stem cells (hPSCs) such as Nanog, c-Myc, and Tbx3 (Fig. 7k), which were not bound by Oct-4 in the CD45⁺ hFibs0 ct-4, further supporting the idea that Oct-4 DNA occupancy is cell context-dependent. While Oct-4 binds its own promoter (Fig. 7k), it does not bind the Oct-2 promoter (Fig. 7i), consistent with the gene expression profile of Oct-2 (Figs. 12a-b). Despite these analyses, due to the conserved octamer binding sequences among Oct-1, -2 and -4 (Table 2), it remains plausible that ectopic expression of Oct-4 could act as a surrogate for Oct-1 or -2 during this process. Collectively, temporal gene expression analyses along with Oct-4 occupancy studies shown here demonstrate that ectopic Oct-4 expression results in induction of a hematopoietic program in hFibs that supports blood fate conversion.

Discussion

The present Example demonstrates the ability of human adult dermal and fetal foreskin fibroblasts to be directly converted to multipotent hematopoietic cells of the myeloid, erythroid, and megakaryocytic blood fates via Oct-4-dependent cellular programming without traversing the pluripotent state or activation of mesodermal pathways (Tsai et al., 1994; Vijayaragavan et al., 2009). Furthermore, given that transition from primitive to definitive hematopoiesis is delineated by the shift from embryonic to adult hemoglobin expression (Orkin and Zon, 2002), it is demonstrated that CD45⁺ fibroblasts, unlike hPSC-derived hematopoietic cells (Chang et al., 2006), acquire an exclusive adult-globin protein and hematopoietic gene profile which indicates that definitive hematopoietic programs were being recruited during this conversion process.

Although recent reports demonstrate conversion of mouse fibroblasts to neural, cardiac, and macrophage-like cells from mouse fibroblasts (Feng et al., 2008; leda et al., 2010; Vierbuchen et al., 2010), the
present Example uniquely demonstrates the ability to generate multipotent vs unipotent cell types from human fibroblasts, hence establishing a future clinical application for these multipotent blood cells. Clinical transplantation studies have estimated that a minimum of 1.5 x 10^6 CD34+ve blood cells (enriched for hematopoietic progenitors) are required to achieve rapid engraftment in an average 60kg patient for recovery of neutrophils, red blood cells, and megakaryocyte after myeloablative therapies (Bender et al., 1992; Feugier et al., 2003). Taking into account the yield, expansion capacity and clinical feasibility using this direct conversion approach to hematopoietic fate (Table 4), the present method could provide a reasonable basis for autologous cell replacement therapies.

[00139] The present Example reveals a previously unknown role for Oct-4 that permitted the fibroblasts to acquire a hematopoietic phenotype via upregulation of hematopoiesis-specific cytokine receptors and transcription factors. The acquisition of this phenotype is linked to the direct binding of Oct-4 to the regulatory loci of hematopoietic-specific genes (i.e. SCL, Runxl, CD45, and GATA1) (Boyer et al., 2005; Ghozi et al., 1996; Kwon et al., 2006; Sridharan et al., 2009). While Oct-1 and Oct-2 have been shown to play a role in adult lymphopoiesis (Brunner et al., 2003; Emslie et al., 2008; Pfisterer et al., 1996), Oct-4 has not been previously implicated in blood development. Given the high conservation between the native or predicted octamer binding sequences among Oct-1, -2 and -4, it is predicted that POU domains shared among Oct proteins have a redundant role in human fibroblast conversion to hematopoietic fate. However, while Oct-4 converts fibroblasts to myeloid and erythroid progenitors, lymphoid hematopoietic fate was absent. Nonetheless, it is predicted that ectopic expression of Oct-4, -1 and -2, coupled with specific culture conditions that support B-cell and T-cell development, may support lymphoid conversion from fibroblasts.

[00140] Thus, the present inventors have demonstrated that adult human dermal fibroblasts can be directly converted into CD45+ hematopoietic cells by transduction with Oct-4 alone that have hematopoietic reconstitution
capacity. The CD45+ Oct-4 transduced cells under the right stimuli are able to give rise to hematopoietic progenitors as well as mature blood cells, such as macrophages, basophils, neutrophils, eosinophils, megakaryocytes and erythroid cells, without traversing the pluripotent or mesodermal progenitor state. Furthermore, the presence of beta-globin in EPO treated CD45+ Oct-4 transduced cells provide the hallmark that the cells are utilizing definitive hematopoiesis versus primitive hematopoiesis that is observed for iPSCs and hESC. The present study uncovers a novel method for derivation of hematopoietic cells. Such cells can provide a quicker, cheaper and safer alternative for example, for autologous transplantation, due to both their in vitro and in vivo competence.

**Methods**

[00141] **Cell Culture** - Primary human dermal adult fibroblasts were derived from breast dermal tissue and the fetal fibroblasts were derived from foreskin tissue and were initially maintained in fibroblast medium (DMEM (Gibco) supplemented with 10% v/v FBS (Fetal Bovine Serum, HyClone), 1mM L-glutamine (Gibco), 1% v/v non essential amino acids (NEAA; Gibco) before transduction with Oct-4 lentivirus-vector. Human dermal fibroblasts transduced with Oct-4 were maintained on matrigel-coated dishes in complete F12 media (F12 DMEM; Gibco) supplemented with 10% knockout serum replacement (Gibco), 1% nonessential amino acids (Gibco), 1 mM L-glutamine (Gibco), and 0.1 mM β-mercaptoethanol) containing 16 ng/ml bFGF (BD Biosciences) and 30 ng/ml IGFII (Millipore) or complete F12 medium containing 16 ng/ml bFGF and 30 ng/ml IGFII and supplemented with 300 ng/ml Flt-3 (R&D Systems) and 300 ng/ml stem cell factor (SCF; R&D Systems) for 21 days. The arising CD45+ Oct-transduced cells were transferred onto low attachment 24-well plates in hematopoietic medium consisting of 80% knockout DMEM (KO-DMEM) (Gibco), 20% v/v non-heat inactivated fetal calf serum (FCS) (HyClone), 1% v/v nonessential amino acids, 1 mM L-glutamine, and 0.1 mM β-mercaptoethanol (Sigma) for 16
days. Cultures were replaced with hematopoietic differentiation medium with cytokines (SCF, G-CSF, Flt3, IL-3, IL-6 and BMP-4; R&D Systems) or for erythroid/megakaryocytic differentiation the media was supplemented with hematopoietic cytokines plus 3 U/ml EPO and changed every 4 days, followed by collection for molecular and functional analysis.

[00142] **Lentivirus Production** - Lentiviral vectors (pSIN) containing cDNAs of Oct-4, Nanog, Sox-2 and Lin-28 were obtained from Addgene. These vectors were transfected with virapower in 293-FT packaging cells line. Viral supernatants were harvested 48h post transfection and ultracentrifuged to concentrate the virus. Equal amount of each virus was used for fibroblast transduction in presence of 8 μg/ml polybrene.

[00143] **Lentivirus transduction** - For generation of cells containing single transcription factors, human adult dermal fibroblasts (Fibs) (derived from breast skin; age between 30-40 yrs.) or fetal foreskin Fibs were seeded at the density of 10,000 cells/well on matrigel coated 12-well plates. Twenty-four hours post seeding, Fibs were infected with lentivirus expressing either Oct-4 or Nanog or Sox-2 (Nanog and Sox-2 transduction was only performed for adult dermal Fibs). Transduced fibroblasts were then grown in complete F12 medium media containing 16 ng/ml bFGF and 30 ng/ml IGFII supplemented with 300 ng/ml Flt-3 and 300 ng/ml SCF or complete F12 media containing 16 ng/ml bFGF and 30 ng/ml IGFII alone for up to 21 days. Emerging CD45⁺ ve colonies were counted 14 to 21 days post infections. Colonies were picked manually and maintained on matrigel-coated wells. Molecular analysis was done on purified untransduced Fibs (DO), Oct-4 transduced Fibs at day 4 (D4), CD45⁺ ve Fibs at day 21 (D21) and hematopoietic cytokine treated or untreated CD45⁺ ve Fibs at day 37 (D37). Day 4 post Oct-4 transduction was chosen as the early event time point based on a number of criteria: a, optimal time for recovery following transduction; b, visible morphological changes within the culture; and c, resumption of normal cell cycle kinetics. The day 4 Oct-4 transduced Fibs (D4) were isolated by
puromycin selection overnight (Oct-4 vector contains puromycin resistance cassette), purity of sample was validated by staining for Oct-4 followed by Oct-4 expression analysis using flow cytometry; samples used for molecular analysis exhibited 99% Oct-4 levels. The day 21 (D21) and day 37 (D37) CD45+ Fibs were isolated based on their CD45 expression. D21 and D37 cells were stained with CD45-APC antibody (BD Biosciences) and sorted using FACSARia II (Becton-Dickinson); samples used for molecular analysis exhibited 99% CD45 levels.

[00144] **Induction of Reprogramming** - For generation of reprogrammed cells from fibroblasts; cells were seeded at the density of 10,000 cells/well on matrigel coated 12-well plates. Twenty-four hours post seeding, fibroblasts were transduced with lentivirus expressing Oct-4/Nanog/Sox-2/Lin-28 (Yu et al. 2007). Transduced fibroblasts were then grown in F12 media supplemented with 30 ng/ml IGFII and 16 ng/ml bFGF. Reprogrammed iPSC colonies were counted four weeks post infections. Colonies were picked manually and maintained on matrigel-coated wells.

[00145] **Live Staining** - For live staining sterile Tra-1-60 antibody (Millipore) was preconjugated with sterile Alexa Fluor-647 at room temperature. Reprogrammed colonies were washed once with F12 medium and incubated with Tra-1-60-Alexa 647 antibodies for 30 mins. at room temperature. Cultures were then washed twice to remove unbound antibody. Cells were visualized by Olympus 1X81 fluorescence microscope.

[00146] **Flow Cytometry** - For pluripotency marker expression, cells were treated with collagenase IV, and then placed in cell dissociation buffer for 10 minutes at 37°C (Gibco). Cell suspensions were stained with SSEA3 antibody (1:100) (Developmental Studies Hybridoma Bank, mAB clone MC-631, University of Iowa, Iowa City, IA) or Tra-1-60-PE (1:100) antibody (BD Biosciences). For SSEA3 staining Alexa Fluor-647 goat anti-rat IgM (1:1000) (Molecular Probes, Invitrogen) was used as the secondary antibody. Live cells were identified by 7-Amino Actinomycin (7AAD) exclusion and then analyzed.
for cell surface marker expression using the FACSCalibur (Becton-Dickinson). Collected events were analyzed using FlowJo 8.8.6 Software (Tree Star Inc.).

[00147] Cells from the hematopoietic differentiation medium were disassociated with TrypLE (Gibco) at day 16 and analyzed for expression of hematopoietic progenitor and mature hematopoietic markers. Hematopoietic cells were identified by staining single cells with fluorochrome-conjugated monoclonal antibodies (mAb): CD34-FITC and APC- or FITC-labelled anti-human CD45 (BD Biosciences), FITC-anti-CD33 (BD Pharmingen), PE-anti-CD13 (BD Pharmingen), PE- or FITC-anti-CD71 (BD Pharmingen), FITC-anti-HLA-A/B/C (BD Pharmingen), PE-anti-CD15 (BD Pharmingen), PE-anti-CD1 5 (BD Pharmingen); PE anti-CD14 (BD Pharmingen), FITC- or PE-anti-GlyA (BD Pharmingen), and APC- or PE-anti-beta-globin (SantaCruz Biotech). The mAb and their corresponding isotypes were used at 1-2 mg/ml, optimal working dilutions were determined for individual antibodies. Frequencies of cells possessing the hemogenic and hematopoietic phenotypes were determined on live cells by 7AAD (Immunotech) exclusion, using FACSCalibur (Beckman Coulter), and analysis was performed using the FlowJo 8.8.6 Software.

[00148] RT-PCRs and q-PCRs - Total RNA was isolated using Norgen RNA isolation kit. RNA was then subjected to cDNA synthesis using superscript III (Invitrogen). Quantitative PCR (qPCR) was performed using Platinum SYBR Green-UDP mix (Invitrogen). For the analysis of the sample, the threshold was set to the detection of Gus-B (beta-glucuronidase) (Oschima et al. 1987) and then normalized to internal control GAPDH. The base line for the experiment was set to the gene expression levels observed in fibroblasts. Given the expression of some of the genes within this starting population of fibroblasts, the gene expression pattern for these cells was included. Hence, the data is represented as delta cycle threshold (AC(t)) versus delta AC(t) (AAC(t)). (qPCR primer sequences are provided in Table 5).
Genomic DNA was isolated using ALL IN ONE isolation kit (Norgen). For integration studies 150 ng genomic DNA was used per PCR reaction. PCR reactions were performed using 2X PCR Master Mix (Fermentas).

**Affymetrix Analysis** - Total RNA was extracted from human dermal fibroblasts (2 replicates), puromycin selected day 4 Oct-4 transduced fibroblasts (2 replicates) and sorted CD45+ve cells (2 replicates) using the Total RNA Purification Kit (Norgen). RNA integrity was assessed using the Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Sample labeling and hybridization to Human Gene 1.0 ST arrays (Affymetrix) were performed by the Ottawa Health Research Institute Microarray Core Facility (OHRI; Ottawa, Canada). Affymetrix data were extracted, normalized, and summarized with the robust multi-average (RMA) method implemented in the Affymetrix Expression Console. CEL files were imported into dChIP software (Li and Wong 2001) for data normalization, extraction of signal intensities and probe-level analysis.

**Chromatin Immunoprecipitation** - ChIP was performed as described previously (Rampalli et al. 2007). Briefly, human pluripotent cells (H9 and iPSC1.2), human dermal fibroblast cells, puromycin selected day 4 Oct-4 transduced cells, sorted day 21 CD45+ve cells were cross-linked using 1% formaldehyde. Chromatin was digested in buffer containing 0.1% SDS to obtain fragments of approximately of 1000 bp length. Sonicated DNA was subjected to immunoprecipitation using anti-Oct4 (ChIP quality antibody; Cell Signaling Technology) and anti rabbit IgG antibodies (Santacruz Biotechnology). Immunoprecipitated DNA was further reverse cross-linked, purified and subjected to qPCR analysis using UDG-Platinium Syber Green mix (Invitrogen). The promoter specific ChIP primers are listed in Table 6. To calculate the relative enrichment, signals observed in control antibody were subtracted from signals detected from the specific antibody; the resulting differences were divided by signals observed from 1/50th ChIP input material.
[00152] **Megakaryocyte assay** - To detect human megakaryocytes, MegaCult™-C Complete Kit with Cytokines (Stem Cell Technologies) was used. The derivation of megakaryocytes was done according to instruction included with the kit. The kit includes pre-screened components for optimal growth of megakaryocyte CFUs, such as thrombopoietin (TPO), Interleukin-3 (IL-3), IL-6, IL-11 and SCF, chamber slides for growth and antibodies for subsequent immunocytochemical staining. In short 10,000 CD45^+^ EPO treated cells were plated in the MegaCult medium containing cocktail of growth factors stated above. The human CFU-Mks were detectable by day 10 to 15 and were subsequently fixed and stained according to protocol. Mk-specific antigen GPIIb/IIIa (CD41) linked to a secondary biotinylated antibody-alkaline phosphatase avidin conjugated detection system was used, where Mk-CFUs were red/pink in colour.

[00153] **Cytospin** - 1000 CD45^+^ Oct-4 transduced cells were washed twice in cold 2% FBS in PBS and dilute in 500 µl of cold 1% FBS in PBS. The samples were loaded into the appropriate wells of the Cytospin. The samples were spun at 500 rpm for 5 minutes to allow adherence to the slides. The slides were fixed with methanol for 1 min. and allowed to dry for 30 min. Then slides were stained with Giemsa-Wright stain for 3 min followed by 10 min. in PBS and a quick wash in distilled water. The slides were allowed to dry overnight and mounted with mounting medium (Dako). Slides were viewed by Olympus 1x81 microscope.

[00154] **Macrophage phagocytosis assay** - Fluorescein (FITC) conjugated-latex beads (Sigma) were used as particle tracers to analyze phagocytosis by monocytes derived from CD45^+^Fibs^Oct-4^ cells treated with IL-4 and M-CSF. To measure phagocytosis, 10 µl of packed beads suspended in 3% FBS in PBS was added to 10^6^ cells in Teflon tubes. After incubation for 90 min at either 37°C, cells were washed three times with cold PBS containing 3% FBS and 0.1% EDTA to remove free beads. The cells were then labeled to detect expression of CD45 (APC-conjugated CD45 mAb) together with FITC-bead uptake, and analyzed by flow cytometry using FACSCalibur (BD).
or visualized by cytospinning 1000 cells onto tissue culture quality slides (VWR) and viewed by Olympus 1X81 fluorescence microscope.

[00155] **Methylcellulose colony-forming assay** - Cells were plated at 1,000 FACS™ II sorted (Becton-Dickinson) CD45 +CD34 + cells or 5000 total cell (EPO treatment) in 1 ml Methocult GF H4434 (Stem Cell Technologies, Vancouver, BC). Colonies were scored after 14 days of culture using standard morphological criteria and analyzed using the FACSCalibur (Becton-Dickinson) for hematopoietic surface markers. Collected events were analyzed using FlowJo 8.8.6 Software (Tree Star Inc.). For colony derivation from xenotransplant derived engrafted cells, the cells were first sorted based on HLA-A/B/C (BD Biosciences) followed by CD45 expression using a human specific anti-CD45 (BD Biosciences). The HLA-A/B/C and CD45 double positive cells were then plated at a density of 1000 cell/ml in Methocult GF H4434. The colonies derived from engrafted cells were further analyzed for hematopoietic surface markers using FACS™ II (Becton-Dickinson). Collected events were analyzed using FlowJo 8.8.6 Software (Tree Star Inc.).

[00156] **Xenotransplant assays** - NOD/SCID IL2Ryc null adult mice (NSG) were sublethally irradiated with 325 rads 24 hours before transplantation. 5.0 x 10^5 CD45 ^+ve Oct-transduced (D37) or human dermal fibroblasts or human mobilized peripheral blood or human umbilical cord blood lineage depleted cells were transplanted by intrafemoral injection. After 10 weeks, animals were culled, and bone marrow (BM) from injected femur, contralateral bones and spleen were analyzed for the presence of human cells by flow cytometry (FACSCalibur, Becton-Dickinson), followed by data analysis using FlowJo 8.8.6 Software (Tree Star Inc.). Cells positive for HLA-A/B/C and CD45 were analyzed for the expression of hematopoietic lineage specific markers such as CD14. For secondary transplants, total engrafted bone marrow cells were transplanted intravenously (IV injection) in adult irradiated NSG mice as described for primary transplants. Genomic DNA from engrafted cells were then analyzed using conventional PCR by primers specific for a-satellite of human chromosome 17: forward- 5'-
GGGATAATTTCAGCTGACTAAACAG-3' (SEQ ID NO:3) and reverse- 5'-TTCCGTTTAGTTAGGTGCAGTTATC-3' (SEQ ID NO:4).

[00157] Teratoma Assay - The McMaster University Animal Care Council approved all procedures and protocols. Adult dermal fibroblasts, fetal dermal (foreskin) fibroblasts, CD45+ve Oct-4 transduced adult dermal fibroblasts, CD45+ve Oct-4 transduced fetal fibroblasts and iPSC 1.1 to 1.4 were treated with collagenase IV for 5-10 min followed by collection and washing 2X with saline and resuspended in saline. 500,000 cells per sample were injected intratesticularly into male NOD-SCID mice. Mice were killed 10-12 weeks after initial injection. Teratomas were extracted, embedded in paraffin and sectioned in 5 µm intervals followed by deparaffinization in xylene and processing through a graded series of alcohol concentrations. Samples were stained with Hematoxylin and eosin or Oct4 followed by dehydration and xylene treatment. Slides were mounted using Permount and imaged by scanning slides using Aperio Scan Scope and images were captured using Image Scope v9.0.19.1516. software. Tissue was also collected from a variety of organs including lung, spleen, liver, brain and kidney to investigate the presence of metastatic cells. Tissue typing was performed based on stringent histological and morphological criteria specific for each germ layer subtype. Mesoderm lineages, such as bone were identified using presence of osteocytes and bone spicules; cartilage was identified by the presence of chondrocytes and specific staining of the extra cellular matrix. Endoderm lineages, such as intestinal lumens were identified by the presence of goblet cells in the lumen epithelium. Ectoderm lineages, such as skin were identified based on distinguishing cell layer morphologies (i.e. stratified); brain or neural tube was identified based on specific histological criteria. The presence of the germ layers and tissue typing was confirmed by McMaster Pathology.

[00158] Statistical analysis - All tests were performed using InStat Version 3.0a statistical software (GraphPad Software). Descriptive statistics including mean and s.e.m. along with one-way ANOVAs, independent sample
two-tailed t-tests were used to determine significant differences. \( p < 0.01 \) was considered significant.

**EXAMPLE 2:** Reprogramming Dermal Fibroblasts into Induced Pluripotent Stem Cells

5 Results

Human dermal fibroblasts contain a rare subpopulation

Transcription factors Oct4 and Sox2 share common DNA binding motifs, and regulate enhancer and promoter regions of genes implicated in the pluripotency network (Loh et al. 2006; Kim et al. 2008).

Human embryonic stem cells (hESCs) and human fibroblasts (hFibs) were transduced with a recently reported EOS lentiviral vector containing trimerized (C3+) Oct4 enhancer elements, (Fig 25a) (Hotta et al. 2009). Using positive control vector where GFP expression is controlled by the pGK promoter, GFP expressing (GFP\(^{+}\)) cells were readily detectable by microscopy (Fig 25b) and had an overall lentiviral transduction efficiency into hFibs or hESCs of 50-60%, quantitated by flow cytometry (Fig 25c and Fig 32a). Using a negative control vector devoid of promoter elements, GFP expression was not detectable in either hFibs or hESCs using microscopy (Fig 25b) or flow cytometric analysis (Fig 25c). As expected, a high frequency of GFP\(^{+}\) hESCs transduced with C3+EOS vector was observed (Fig 25b,c and Fig 32b). However, C3+EOS transduction into adult breast-derived hFibs revealed a rare population of dermal fibroblasts expressing GFP (Fig 25b,c). Confocal Z-stack imaging of hFib cultures transduced with C3+EOS indicated rare GFP\(^{+}\) hFibs are morphologically and spatially distinct from other hFibs residing in the culture (Fig 32c). Detection of this subpopulation of hFibs was not due to high copy integrations in individual cells, as transduction of hFibs using different concentrations of EOS lentivirus did not alter frequencies of GFP\(^{+}\) hFibs, whereas individual GFP\(^{+}\) hFibs expressed an indistinguishable level of GFP on a per cell basis irrespective of viral concentration (Fig 32d-f). Given that the composition of *in vitro* cultured fibroblasts varies depending on the tissue from which they are derived, unique
sources of human neonatal foreskin- and adult lung-derived fibroblasts, both devoid of hair follicles that contain tissue-specific dermal stem cell populations, were examined (Terunuma et al. 2008). Similar to adult breast-derived dermal hFibs, foreskin and lung fibroblasts transduced with C3+EOS vector contained GFP^+ve subsets at a frequency of 0.5-4%, indicating that the presence of the GFP^+ve subset is not dependent on human fibroblast source (Fig 25d,e).

[00160] To rule out the potential bias in viral uptake among hFibs, hFibs were serially infected with C3+EOS lentivirus starting with primary GFP^ve hFib subfraction (Fig 32g). As a result of secondary EOS lentivirus transduction of primary GFP^ve hFibs, 1.34% GFP^+ve emerging was observed (Fig 32g), indicating a small frequency of these unique cells was not detected due to initial limits of 50% overall transduction efficiency (Fig 25c). FACS isolation of secondary GFP^ve hFibs, followed by subsequent transduction with C3+EOS lentivirus, demonstrated a frequency of <0.1% GFP^+ve cells, whereas tertiary and quaternary transductions with C3+EOS lentivirus were unable to show any increase in GFP Oct reporter expression (Fig 32g); indicating all hFibs competent for C3+EOS expression had been saturated. To ensure sequentially transduced GFP^ve hFibs were not simply resistant to lentiviral infection, quaternary GFP^ve hFibs were transduced with positive control vector pGK-EGFP that gave rise to robust GFP^+ve hFibs, thus confirming these cells were competent for lentiviral infection (Fig 32h). These studies demonstrate that observed GFP^+ve subpopulation of hFibs was not due to high copy integrations in individual cells, or to differences in infection rate between subpopulations.

[00161] To molecularly validate C3+EOS reporter expression and presence of integrated provirus, GFP^+ve and GFP^ve hFibs transduced with EOS vector were prospectively isolated at 99.99% purity (Fig 25f). Using isolated populations, pro-virus was shown to be present in both fractions, whereas GFP transcript expression was present only in GFP^+ve hFibs, and absent in GFP^ve hFibs (Fig 25g). To ensure GFP^ve hFibs containing
integrated C3+EOS vector were not silenced, these hFibs with Oct4 expressing lentivirus were transduced. Upon ectopic expression of Oct4, GFP\(^{+}\) cells could be induced to express GFP (Fig 25h), demonstrating the integrated proviral vector was functional in these cells.

Collectively, these results indicate that human fibroblasts cultured in vitro are heterogeneous by revealing a unique subset that permits expression of the Oct4 reporter EOS vector independent of ontogenic source or anatomical location from which hFibs were derived.

**Rare subset of hFibs possesses molecular features of pluripotent cells**

Aside from pluripotent stem cells (PSCs), Oct4 expression has also been reported in multiple somatic tissues including dermis, multipotent stem cells, and cancer cells (Li et al.; Jiang et al. 2002; Goolsby et al. 2003; Dyce et al. 2004; Johnson et al. 2005; Moriscot et al. 2005; Zhang et al. 2005; D'Ippolito et al. 2006; Dyce et al. 2006; Izadpanah et al. 2006; Nayernia et al. 2006; Ren et al. 2006; Yu et al. 2006; Izadpanah et al. 2008), while the surrogacy and function of Oct4 in non-PSCs remains elusive (Lengner et al. 2007; Lengner et al. 2008). Given that activation of the C3+EOS vector is based on the presence of Oct4, the expression of Oct4 was carefully examined in total hFibs and GFP\(^{+}\) vs. GFP\(^{-}\) hFib subsets. Several Oct4 isoforms and pseudogenes have sequence similarity (Atlasi et al. 2008), making interpretation of transcript detection complex and potentially leading to false positives. As such, Oct4 transcripts were identified in hFibs using multiple primer sets recently characterized (Atlasi et al. 2008) that faithfully recognize: 1. Oct4; 2. Oct4B1 embryonic-specific Oct4 isoforms; and 3. Oct4B cytoplasmic variants (Fig 26a). GFP\(^{+}\) hFibs were enriched for expression of Oct4 and its isoform B1, but lacked expression of cytoplasmic isoform Oct4B similar to that of hESC controls (Fig 26b,c), whereas total and GFP\(^{-}\) hFibs did not express any form of Oct transcript (Fig 26b,c). The mesodermal gene Brachyury used as a control for lineage-specific gene expression was not differentially expressed (Fig 26c). The possibility that GFP\(^{+}\) hFibs expressed other genes associated with Oct4 and pluripotency was next examined. In
addition to Oct4 (Fig 26b,c), quantitative gene expression analysis demonstrated expression of Nanog and Sox2 in GFP+ve hFibs (Fig 26d), albeit at lower levels compared to hESCs (Fig 25e). Whole genome expression profiles from total hFibs vs. GFP+ve and GFP-ve hFibs were compared to hESC, iPSC lines using 3' oligonucleotide arrays and evaluated for expression of genes specific to human and mouse ESCs vs. genes associated with heterogeneous fibroblast cultures (Takahashi et al. 2007; Yu et al. 2007). GFP-ve hFibs strongly clustered with multiple sources of heterogeneous human dermal fibroblasts, whereas GFP+ve hFibs did not cluster with total hFibs from which they were derived, but instead with pluripotent hESC and iPSC lines (Fig 26f).

[00164] As transcript expression is not a determinant of protein, protein expression of Oct4 was examined using Oct4-specific antibodies. Oct4 intracellular localization was examined using immunofluorescent staining analysis. In GFP+ve hFibs, Oct4 co-localized with DAPI stained nuclei similar to that observed in hESCs that served as a positive control (Fig 26g), and 293 cells transduced with Oct4 transgene (Fig 33a). Oct4 staining was not detected in untransduced 293 cells that served as a negative control, while rare Oct4 positive cells were seen in total hFib cultures, consistent with the frequency of GFP+ve cells detected by the EOS vector (0.5-4%) (Fig 33a). Using western analysis, in addition to Oct4, protein levels of both Nanog and Sox2 were differentially expressed in GFP+ve hFibs (Fig 26h,i), where hESCs and Oct4-transduced and untransduced 293 cells served as positive and negative controls (Fig 26h). Based on these analyses, these subsets of hFibs were termed as Nanog, Oct4, and Sox2 expressing hFibs, or NOS+exp hFibs, vs. majority of hFibs that were GFP-ve hFibs, and as such termed NOS-ve hFibs.

[00165] To better understand the molecular nature of rare NOS+exp hFibs, chromatin precipitation (ChIP) was performed using specific antibodies against Oct4, Nanog, Sox2, and Brachyury proteins in hESCs, total hFibs, NOS+exp and NOS-ve hFib subsets for binding to CR4 enhancer motifs within
the EOS vector. Occupancy of these pluripotent factors to CR4 motif was highly enriched in NOS+exp hFibs, whereas negative control Brachyury was not bound (Fig 26j). Comparison of active (H3K4Me3) and repressive (H3K27Me3) histone modification marks at the endogenous promotor loci revealed that NOS+exp hFibs possessed active marks for Oct4, Nanog, and Sox2 similar to that of hESC positive controls, whereas total unselected hFibs and NOS−exp loci were repressed (Fig 26k). As demethylation of Oct4 loci associated with gene activation is extensively studied in PSCs (Simonsson and Gurdon 2004), MeDIP ChiP assays were performed. Reduced methylation of Oct4 promoter was similarly detected in hESCs and NOS+exp hFibs, in contrast to 293 cells and NOS−exp hFibs (Fig 33b). These results further confirm Oct4 loci is activated in NOS+exp hFibs.

[00166] The role of Oct4 and other pluripotent-associated factors in somatic compartment has been met with skepticism due to inappropriate controls for transcript and protein expression detection, and absence of any functional evidence for the role of these cells or factors expressed (Lengner et al. 2007). The present results have extended characterization of such cells beyond simple PCR transcript detection of a single gene such as Oct4, and has analyzed chromatin, protein, subcellular localization, and global gene expression cluster analysis for Oct4, Nanog, and Sox2, together with positive (hESCs) and negative controls (293 cells) for each factor. Collectively, these data provide the foundation for the existence of NOS+exp hFibs that represent a unique and rare subset within human fibroblasts that shares common molecular features with human PSCs. The ability to isolate these subsets of NOS+exp and NOS−exp hFibs provides the unprecedented opportunity to perform functional analysis and define the biological significance of these shared features with PSCs.

Heterogeneous total hFibs, in contrast to purified NOS+exp subsets, can be reprogrammed under feeder-free conditions

[00167] Since NOS+exp hFibs share hallmark features of gene expression with fully reprogrammed iPSCs, their capacity for functional
reprogramming compared to total hFibs was examined. The majority of iPSC lines are derived using heterogeneous hFibs and use mouse embryonic fibroblast (MEF) feeder layers to support iPSC generation (Park et al. 2008). However, clinical applications of iPSCs will require xeno-free conditions and methods that allow for rapid and simple isolation and separation of human iPSCs from supportive cells such as MEFs. To specifically address this application-based limitation, human iPSCs were derived on matrigel using feeder-free conditions as schematically illustrated in Fig 27a. Total and NOS+exp hFibs were transduced with previously defined reprogramming factors (Hotta et al. 2009), and cultures were examined by both phase contrast and live fluorescence microscopy to characterize morphological changes, identify colony formation, and identify subfraction of colonies expressing Tra1-60. Consistent with previously reported frequencies for human iPSC generation (Utikal et al. 2009; Aasen et al. 2008; Meissner et al. 2007), total hFibs exhibited approximately 0.9% colony formation efficiency (per 10,000 input cells), whereas the same number of highly purified NOS+exp hFibs isolated failed to generate any colonies (Fig 27b). This result was consistently observed in 6 independent experimental replicates, indicating that from over 60,000 NOS+exp hFibs (6 x 10,000) analyzed, formation of proliferating colonies towards iPSC generation could not be derived using this purified subset.

Although colony formation is the initial requirement for iPSC generation, colony formation alone does not denote fully reprogrammed cells. As such, Tra1-60 expression colonies were quantitatively identified using recently established live staining methods (Fig 27c) that faithfully identify reprogrammed iPSCs from non-iPSC-like colonies (Chan et al. 2009) using total hFibs that generated colonies in the absence of feeders (Fig 27b). In addition, these colonies were also assessed for expression of SSEA3 and Oct4 by flow cytometry and compared to Tra1-60 acquisition. Both Tra1-60+ve and Tra1-60-ve colonies expressed high levels of Oct4, but only Tra1-60+ve colonies expressed pluripotency marker SSEA3, while Tra1-60-ve colonies lacked SSEA3 expression (Fig 27d). Overall, the Tra1-60+ve colonies
represented 50% of total number of colonies generated (Fig 27e). These two
types of colonies (Tra1-60^{+ve} and Tra1-60^{-ve}) were further examined using a
subset of genes strongly associated with pluripotency (Rex1, Tbx3, TcF3, and
Dppa4), and indicated that only Tra1-60^{+ve} colony acquired a pluripotent gene
expression signature (Fig 27f). Finally, in vivo differentiation potential of Tra1-
60^{+ve} colonies was tested by teratoma formation assay demonstrating that
these colonies have potential to give rise to all three germ layers (Fig 27g).
Using these collective criteria, starting with colony formation and subsequent
Tra1-60, Oct4 and SSEA3 expression analysis, together with pluripotent gene
expression analysis and ability to form pluripotent teratomas, independent
measures are provided to define iPSC generation, thereby establishing that, in
contrast to purified NOS^{+exp} hFibs, total heterogeneous hFibs can be
reprogrammed under feeder-free conditions.

**NOS^{+exp} hFibs represent the major contributor to pluripotent
reprogramming**

[00169] Purified NOS^{+exp} hFibs failed to generate reprogrammed
colonies upon isolation from heterogeneous cultures of total hFibs (Fig 27b).
Given the well established effects of the niche on the regulation of stem cell
properties (Bendall et al. 2007), it was hypothesized that the reprogramming
potential of the NOS^{+exp} subpopulation may be dependent on complex
microenvironmental cues that prevented iPSC emergence from highly purified
NOS^{+exp} hFibs. Since NOS^{+exp} hFibs are transduced with the EOS vector,
GFP and provirus integration provide a fluorescent and molecular marker of
NOS^{+exp} cells that can be used to distinguish the contribution of NOS^{+exp} hFibs
upon co-culture with heterogeneous fibroblasts. NOS^{+exp} hFibs were mixed
with total hFibs in a ratio of 1:9 in a competitive assay to measure
reprogramming ability and contribution to iPSC generation using established
criteria (Fig 27b-g).

[00170] Consistent with previous observations (Yamanaka 2009), total
hFibs (10,000 input cells) exhibited an expected low frequency of colony
formation, while co-cultures of NOS^{+exp} hFibs (total input of 10,000 cells
comprising 1,000 NOS+exp hFibs together with 9,000 total hFibs=1:9 ratio) remarkably garnered a 14-fold increase in colony formation (Fig 27h). To quantitatively assess the contribution of NOS+exp vs. total hFibs towards reprogramming, colonies identified for iPSC-like morphology by phase contrast were enumerated and further scrutinized by live fluorescence microscopy for Tra1-60 expression, and the presence or absence of GFP expression and EOS proviral integration. A representative experiment using this approach is shown in Fig 27i displaying detailed analysis on individual colonies identified. Combined results from 6 independent mixture experiments demonstrated that 90% of the Tra1-60+ve colonies were positive for GFP and EOS provirus, while the remaining 10% were contributed by total hFibs (Fig 27i). Tra1-60+ve and -ve colonies derived from NOS+exp and total hFibs were isolated and examined for activation of pluripotency factors and SSEA3 expression to ascertain and quantitate the number of complete reprogrammed iPSCs. Representative analysis from EOS+ve colonies that could only be derived from NOS+exp hFibs (C2 and C9) that were positive (C2) and negative (C9) for Tra1-60 expression vs. EOS-ve colonies (C1 1 and C12) positive (C1 1) and negative (C12) for Tra1-60 expression are shown (Fig 27j,k). Tra1-60 provided a strong surrogate marker for colonies capable of pluripotent gene activation (Fig 27j) and SSEA3 expression (Fig 27k), independent of NOS+exp or total hFib origins. Fully reprogrammed colonies derived from NOS+exp hFibs were capable of teratoma formation comprising all three germ layers (Fig 27i), and possessed in vitro differentiation capacity towards the mesodermal (hematopoietic, Fig 35a) and ectodermal (neuronal, Fig 35b) lineages similar to pluripotent hESCs shown as a positive control for lineage development (Fig 35a-b). Since NOS+exp hFibs were capable of reprogramming and generating iPSCs upon co-culture, reprogramming potential of remaining NOS-exp hFibs derived from heterogeneous hFib cultures were similarly examined. Direct analysis for reprogramming ability demonstrated that highly purified NOS-exp hFibs cultured in feeder-free conditions were completely devoid of colony generation (Fig 29a), and co-culture of NOS- exp hFibs with total hFibs resulted in a biologically insignificant colony frequency of <0.01% (Fig 36a-b).
represents a single colony per 10,000 input cells in 3 independent experiments (Fig 36b) that is likely derived from total hFibs that do contain N0 S,exp hFibs unmarked by C3+EOS transduction.

[00171] To quantitatively determine the precise contribution of NOS+exp hFibs to generation of iPSCs in co-cultures with total hFibs, the overall data set from 6 independent mixture experiments was analyzed. Firstly, identification of iPSC-like colony formation enumerated by microscopy indicated an average of 12 colonies could be generated from an input of 10,000 cells comprising 9K of total hFibs and 1K of NOS+exp hFibs (Fig 28a). Despite a 9-fold greater proportion of total hFib input cells (GFP-ve, EOS-ve), the contribution of NOS+exp hFibs (GFP+ve, EOS+ve) colony formation was 4-fold higher, based on definitive criteria of GFP expression, and the presence of EOS proviral integration (Fig 28a). Quantitative analysis of Tra1-60 expression among EOS-ve colonies (derived from 9,000 total hFibs) vs. EOS+ve colonies (derived from 1,000 NOS+exp hFibs) indicated that an equal proportion of Tra+ve vs. Tra-ve colonies arise from total hFibs, whereas colonies derived from NOS+exp hFibs enriches for Tra1-60+ve fully reprogrammed colonies (Fig 28b). On a per 10,000 cell input basis, direct comparative analysis indicates that the overall reprogramming efficiency of unselected total hFibs was 0.18 vs. an average of 7.6 arising from NOS+exp hFibs (Fig 28c). Accounting for the 9-fold difference in the input cells, these results demonstrate a 42-fold increase in reprogramming efficiency using NOS+exp hFib isolation and enrichment (n=6, Fig 28c).

[00172] Although NOS+exp hFibs are incapable of cell-autonomous reprogramming in purified cultures, these results reveal that this unique, but rare subset of hFibs is the major contributor of cells to reprogrammed iPSCs, but requires co-culture with heterogeneous hFibs. These functional studies suggest that NOS+exp hFibs possess a predisposition to cellular reprogramming induction due to their unique molecular and epigenetic state (Fig 36) that is already akin to pluripotent cells prior to induced reprogramming.
Molecular state of NOS\textsuperscript{+exp} hFibs can be modulated by microenvironment for pluripotent reprogramming competency

In the presence of microenvironment provided by total heterogeneous hFibs, purified NOS\textsuperscript{+exp} hFibs generated iPSC colonies in co-cultures containing 10% NOS\textsuperscript{+exp} hFibs and 90% total hFibs (Fig 28). Accordingly, whether microenvironment composition could influence the reprogramming frequency of predisposed population as a product of NOS\textsuperscript{+exp} hFib relative cellular densities was explored. Using a range of relative enrichment densities of NOS\textsuperscript{+exp} hFibs vs. total hFibs, reprogramming capacity was examined by colony formation and NOS\textsuperscript{+exp} hFib contribution was distinguished by GFP expression. Increase in the densities of NOS\textsuperscript{+exp} hFibs towards 50% demonstrated a plateau for reprogramming efficiency (Fig 29a). Beyond this plateau, the reprogramming capacity of NOS\textsuperscript{+exp} hFibs decreased as supportive total hFib proportion decreased, eventually demonstrating the complete absence of colony formation once supportive total hFibs were absent (Fig 29a). Decreasing the density of NOS\textsuperscript{+exp} hFibs to <2.5% in the mixtures resulted in reduced colony generation (Fig 30a), reminiscent of the low frequency of iPSC generation derived from total hFibs (Fig 27a-g) These results suggested that the reprogramming capacity of NOS\textsuperscript{+exp} hFibs is dependent on specific densities relative to microenvironment or supportive niche cells.

To better understand the molecular basis for the requirement of supportive heterogenous hFibs to NOS\textsuperscript{+exp} hFib reprogramming, gene expression and epigenetic status of total hFibs and purified NOS\textsuperscript{+exp} hFibs before (\textit{de novo} isolated) and after co-culture were evaluated. In contrast to total hFibs (Fig 29b), \textit{de novo} prospectively isolated NOS\textsuperscript{+exp} hFibs demonstrated detectable expression of pluripotent factors and active marks on gene loci (Fig 29c). \textit{De novo} isolated NOS\textsuperscript{+ exp} hFibs cultured multiple passages retained stable GFP expression (Fig 37). Next, chromatin state at the endogenous loci of Oct4, Nanog, and Sox2, and transcript expression for these genes in NOS\textsuperscript{+exp} hFibs cultured alone, and then in the presence of hFibs or co-cultured with MEFs was compared (Fig 29d). Cultured NOS\textsuperscript{+exp}
hFibs alone induced a bivalent state at Oct4 loci and a loss of active histone marks for Nanog and Sox2 loci (Fig 30f) that were corroborated with reduced gene expression for Oct4 and complete absence of Nanog and Sox2 transcripts (Fig 30e). These molecular changes correlated to the inability to reprogram NOS+exp hFibs cultured alone in feeder-free conditions (Fig 27b). However, NOS+exp hFibs subsequently co-cultured with total hFibs or with MEFs were able to re-acquire active chromatin marks on Oct4, Nanog, and Sox2 loci (Fig 30g), and gene expression upon co-culturing with total hFibs or with MEFs (Fig 30g).

Role of microenvironment in derivation and maintenance of pluripotent stem cells has been reported (Schnerch et al.; Bendall et al. 2007; Stewart et al. 2008), and is consistent with the inferred requirement of MEFs as iPSC derivation protocols include the use of MEF feeders (Takahashi and Yamanaka 2006; Takahashi et al. 2007; Wernig et al. 2007; Yu et al. 2007; Aasen et al. 2008; Hanna et al. 2008; Lowry et al. 2008; Park et al. 2008; Woltjen et al. 2009). To determine whether co-culture-induced modulation of epigenetic state of NOS+exp hFibs affects reprogramming competency, NOS+exp hFibs and NOS-exp hFibs were cultured in the presence or absence of MEFs and hFib fractions were exposed to lentivirus-expressing reprogramming factors. A total of 10,000 NOS+exp or NOS-exp hFibs were transduced with reprogramming factors, and cultures were examined 3 and 6 weeks post-infection for colony formation, GFP expression, and colonies expressing Tra1-60. Consistent with the previous results (Fig 27b and Fig 36a-b), NOS+exp or NOS-exp hFibs did not generate colonies in the absence of co-cultured cells at either 3-week or extended 6-week cultures (Fig 29h). Similarly iPSC generation was not detectable from the NOS-exp hFibs, even upon co-culture with MEFs (Fig 29h). However, NOS+exp hFibs co-cultured on MEFs produced detectable colonies at 3-weeks post transduction and continued to demonstrate complete reprogrammed iPSCs at 6-weeks of MEF co-culture (Fig 29h). Colonies generated expressed GFP and the pluripotency marker Tra1-60, indicative of complete reprogramming (Chan et al. 2009) (Fig 29h).
Collectively, comparative molecular analysis of de novo isolated NOS\textsuperscript{+exp} hFibs vs. absence and presence of co-cultured heterogeneous hFibs or MEFS revealed that NOS\textsuperscript{+exp} hFibs respond and modulate their epigenetic state and gene expression through currently unknown signaling mechanisms that are provided by microenvironmental cues. Molecular changes induced by co-culture microenvironment are restricted to NOS\textsuperscript{+exp} hFib subfraction, and are required to maintain predisposed state and competency for pluripotent reprogramming.

**NOS\textsuperscript{+exp} hFibs are molecularly exclusive from other human stem/progenitor cells and possess unique cell cycle properties**

Previous studies have demonstrated isolation of multipotent stem cells from various regions of the skin including bulge region of hair follicle (Bulge Stem Cells), interfollicular epidermis (IFE stem cells), and the dermal papillae (SKPs) (Manabu Ohyama 2006; Biernaskie et al. 2009; Jensen et al. 2009). In order to assess the potential similarity between NOS\textsuperscript{+exp} hFibs and previously described multipotent stem/progenitor cells isolated from skin, this unique population of hFibs was further examined based on global genome expression profiles. Hierarchical clustering of total hFibs, NOS\textsuperscript{+exp} hFibs, and NOS\textsuperscript{-exp} hFibs; compared to Bulge Stem Cells, Keratinocytes, and SKPs (Toma et al. 2005; Manabu Ohyama 2006; Jensen et al. 2009), using fibroblast gene signature and molecular markers specific to individual skin stem/progenitors (Fig 30a) revealed that NOS\textsuperscript{+exp} hFibs are distinct from pre-existing skin stem/progenitors and are further distinguished by their expression of the pluripotency transcriptional network that includes Nanog, Oct4, and Sox2 (Fig 30a). In addition to dermal-derived stem/progenitor cells, neural, hematopoietic, and keratinocyte progenitors have been shown to possess enhanced reprogramming capacities (Aasen et al. 2008; Eminli et al. 2009). As such, the global molecular phenotype of NOS\textsuperscript{+exp} hFibs to these lineage-specific adult stem cells was compared which indicated that NOS\textsuperscript{+exp} hFibs did not cluster with these stem cell types (Fig 29b). Collectively, these analyses indicate that NOS\textsuperscript{+exp} hFibs are distinct from stem/progenitor cells previously associated with dermal skin derivatives or
tissue-specific progenitors reported to undergo enhanced reprogramming (Fig 29b).

[00178] Next, global gene expression differences between NOS$$^{+\exp}$$ hFibs and total hFibs were evaluated towards identification of additional features, other than those shared with human PSCs that may distinguish NOS$$^{+\exp}$$ hFibs from bulk total hFibs. Gene ontology analysis of the list of differentially expressed genes revealed several categories that were enriched in NOS$$^{+\exp}$$ hFibs vs. total heterogeneous hFib cultures. These predominantly included gene products involved in development, cell cycle, and cell division (Fig 30c). Of these ontologies, genes involved in cell cycle progression were most prevalently differentially expressed (17.48%, p<0.000003). Further in-depth analysis of cell cycle-associated genes revealed higher expression of genes associated with replication and mitotic processing in NOS$$^{+\exp}$$ hFibs that were also co-expressed uniquely in hESCs and fibroblast-derived iPSCs (Fig 30d). Of these genes, a non-integral cell surface receptor CD168 [also called Hyaluronan-mediated motility receptor (HMMR)] found in the nucleus and associated with cells in the developing human embryo (Choudhary et al. 2007; Manning and Compton 2008) was co-expressed with GFP expressing NOS$$^{+\exp}$$ hFibs amongst heterogeneous hFibs transduced with EOS vector (Fig 30e). Consistent with unique cell cycle regulation of NOS$$^{+\exp}$$ hFibs, direct comparison of growth rates between NOS$$^{+\exp}$$ hFibs to total hFibs indicated NOS$$^{+\exp}$$ hFibs proliferate at a higher rate (Fig 30f), thereby functionally validating the unique proliferative properties of NOS$$^{+\exp}$$ hFibs.

[00179] Taken together, these data provide further comparative characterization of these previously unidentified NOS$$^{+\exp}$$ hFibs predisposed for cellular reprogramming that is best defined by unprecedented expression of genes associated with pluripotency and proliferation.

Discussion

[00180] Using human dermal fibroblasts as a clinically relevant model system for understanding and enhancing pluripotent reprogramming, evidence is provided for the existence of a predisposed cell population with
molecular similarities to pluripotent cells and inherent cell cycle status that is conducive towards pluripotent reprogramming and without wishing to be bound by theory, a model is proposed for the role of these cells in the reprogramming process (Fig 31). These predisposed human dermal fibroblasts possess unique cell cycle properties including enhanced expression of cell cycle activators (such as CCNB1/2, PCNA, MCM 2-7, and ANAPC1) and are identified and distinguished by expression of Nanog, Oct4, and Sox2, therefore termed NOS⁺exp hFibs (Fig 31). The unique molecular and epigenetic ground state of NOS⁺exp hFibs are distinct from heterogeneous cultures of fibroblasts or previously reported stem/progenitor populations capable of enhanced reprogramming, and are similar to human iPSCs and ESCs. The remaining hFibs (NOS⁻exp) do not participate in reprogramming to iPSCs, despite co-culture with supportive niche, or prolonged culture periods (Fig 31). Nevertheless, the possibility that some unique conditions may allow induced pluripotency, such as introduction of oncogenes or perturbation of cell cycle regulators is not excluded (Fig 31). Since both stem/progenitor and enhanced proliferation state positively influence iPSC generation, a cell type similar to NOS⁺exp hFibs identified here that has intrinsic cell cycle properties is amenable for efficient and enhanced reprogramming. Consistent with this notion, a recent study published by Smith et al (Smith et al. 2010) provides evidence that the small and fast-dividing subfraction of MEFs contributes to iPSC colony formation, however, no further characterization has been done, likely due to the current inability to define and isolate these unique cell types among MEFs. Since reprogramming is thought to remove existing epigenetic states or cellular "memory" of target cells required to establish a new pluripotent state, given the similar molecular phenotype and epigenetic status of NOS⁺exp hFibs to hPSCs the extent to which reprogramming converts terminally differentiated fibroblasts vs. overcoming limiting commitment steps essential to achieve pluripotency warrants further conceptual and experimental examination. These results support an elite stochastic model to describe reprogramming induction at the cellular level, where an elite subset
of predisposed cells is uniquely capable of responding to inductive molecular
changes required to establish pluripotent state.

[00181] These results identify a predisposed cell population that
exclusively contributes to reprogramming in a niche-dependent manner that
can be supplied by either heterogeneous hFibs or MEFs (Fig 31). As such, a
previously unappreciated role of microenvironment in iPSC derivation from
human dermal fibroblasts has been uncovered. Although the results indicate
that the fibroblasts are not equipotent for reprogramming, it does not discount
the possibility that other subfractions among heterogeneous human
fibroblasts could be induced to reprogram under uniquely designed
conditions. This is similar to recent reports that suggest that the
reprogramming of mouse B-cells might be a stochastic event by
demonstrating that almost every donor cell can be reprogrammed to the
pluripotent state by continuous and prolonged expression of reprogramming
factors for extended periods (Hanna et al. 2009). Interestingly, 3-5% of
colonies emerged after only two weeks of reprogramming and may represent
predisposed cell types similar to NOS⁺exp hFibs identified here, while the
appearance of remaining iPSC colonies emerging over the subsequent 4-5
months with continued doxycyclin induction of reprogramming factors may be
a result of the specific selective conditions utilized that include the use of
drug-induced gene expression involving the oncogene c-myc. Such
experiments in non-predisposed fractions of human hFibs (NOS⁻exp cells)
could provide more insights into prerequisite of oncogenic processes for
pluripotent reprogramming.

[00182] Similar to all current reports of pluripotent reprogramming, the
relevance of predisposed NOS⁺exp hFibs pertains to in vitro processes of
somatic cell reprogramming and the use of derived cells once reprogrammed
in vitro. To date, no reports have indicated that cells can be reprogrammed to
the pluripotent state in vivo. While plasticity of fibroblast cells in invertebrates
has recently been documented (Kragl et al. 2009), such plasticity is not fully
explored in mammals (Sanchez Alvarado 2009), thus existence of
predisposed hFibs and its in vivo function is intriguing, but its role in normal in vivo physiology is merely speculative at this point, and is likely limited to in vitro phenomenon. Nevertheless, NOS+exp hFibs can easily be derived from a variety of human tissue, and represent the most rapid and robust contributor to human iPSC generation reported to date. These properties underscore the clinical utility of NOS+exp hFibs where immediate isolation and characterization of fully reprogrammed iPSCs from patients is required for rapid drug and genetic screening or cell transplantation upon differentiation induction.

**Methods**

[00183] **Cell Culture** - Adult Human dermal fibroblasts were derived from breast skin (obtained passage 1; recommended expansion- 15 population doubling), neonatal dermal fibroblasts derived from foreskin (obtained passage 1; recommended expansion- 15 population doubling), and lung fibroblasts were derived from lung tissue (obtained passage 10; recommended expansion- 24 population doubling) [Sciencell] and maintained in fibroblast medium (DMEM (Gibco) supplemented with 10% FBS (HyClone), L-glutamine (Gibco), nonessential amino acids (NEAA; Gibco). All the experiments were conducted using breast derived dermal fibroblasts unless mentioned otherwise. Human iPS cells were maintained on matrigel-coated dishes in iPS media (F12 DMEM (Gibco) supplemented with 20% knockout serum replacement (Gibco), L-glutamine (Gibco), NEAA, betamercaptoethanol supplemented with 16 ng/ml bFGF (BD Biosciences). hESCs were maintained on matrigel coated dishes in MEF-condition media supplemented with 8ng/ml bFGF. Pluripotent cells were transduced with different concentrations of EOS C3+ lentivirus on day 2 following passage. 293 cells were cultured in DMEM containing 10% fetal bovine serum, essential amino acids and L-glutamine. 293 cells were seeded in chamber slides prior to transfection. One microgram pSIN-Oct4 vector was transfected using lipofectamine 2000 reagent (Invitrogen). Experiments were performed 36 hr post transfection. For generation of cultured NOS+exp hFibs, adult dermal fibroblast cells were transduced with EOS C3+ Oct4 lentiviral vector,
NOS-^exp_ cells were sorted and cultured for at least 5 passages in fibroblasts media.

**Lentivirus Production** - Lentiviral pSIN-EGFP, pSIN-PGK-EGFP and pSIN-C3+EOS vectors were synthesized and described by Hotta et al 2008. Lentiviral vectors (pSIN) containing cDNAs of Oct4, Nanog, Sox2, and Lin28 were obtained from Addgene. These vectors were co-transfected with virapower in 293-FT packaging cells line. Viral supernatants were harvested 48h post transfection and ultracentrifuged to concentrate the virus. To confirm the transduction efficiency of positive control pGK EGFP lentivirus was transduced in fibroblasts at indicated dilution. Equal amount of each virus was used for fibroblast transduction in presence of 8 ng/ml polybrene.

**Human Adult Fibroblast sorting** - Fibroblast cells were transduced at passage three with C3+ EOS vector and maintained for three passages. Cells were trypsinized and live cells were identified using 7AAD exclusion. Fibroblasts were sorted based on GFP expression on FACS Ariall (BD). For qRT-PCR assays and chromatin immunoprecipitation (ChIP) assays 50,000 GFP^+ve (NOS^+exp) and GFP^ve (NOS^-exp) cells were sorted into the tubes containing 0.5% FBS in PBS (v/v). Cells were either collected by centrifugation for RNA extraction or cross-linked using 1% Formaldehyde for ChIP studies.

**Induction of Reprogramming** - On matrigel: For generation of reprogrammed cells from total hFibs, GFP^+ve cells (referred to as 10,000 NOS^+exp cells) and 10,000 GFP^-ve cells (referred to as 10,000 NOS^-exp cells), cells were seeded at the density of 10,000 cells/well on matrigel coated 12-well plates. For mixture experiments NOS^-exp cultured cells were mixed in 1:9 ratio (1000 NOS^-exp cultured + 9000 total hFibs) or in 1:1 ratio (5000 NOS^-exp cultured + 5000 total hFibs). For the experiments pertaining to demonstration of predisposition, 1000 NOS^-exp cells were sorted from total hFib cultures and combined with 9000 total hFibs onto matrigel-coated dishes. 24 hrs post seeding, fibroblasts were transduced with lentiviruses expressing Oct4, Nanog, Sox2, and Lin28. Transduced fibroblasts were then grown in iPSC
media. Reprogrammed colonies were counted three to 6 weeks post infections. Colonies were picked manually and maintained on matrigel-coated wells. **On MEFs:** 10,000 NOS<sup>exp</sup> or NOS<sup>-exp</sup> cells were seeded in 12-well dish in triplicates. 24 hrs post seeding, hFibs were transduced with lentiviruses expressing Oct4, Nanog, Sox2, and Lin28. 36 hrs post transduction, hFibs were collected by trypsinization and transferred on to plates containing irradiated MEFs. Reprogrammed colonies were counted 3 to 6 weeks post infections. Colonies were picked manually and maintained on MEFs.

### [00187] Hematopoietic and Neuronal differentiation assays - Human

ES cells or iPSC cells derived on matrigel were grown until 80% confluence and EBs were made as described previously (Chadwick et al. 2003). Cells were transferred to low attachment 6-well plates in differentiation medium consisting of 80% knockout DMEM (KO-DMEM) (Gibco), 20% non-heat inactivated fetal Bovine serum (FBS) (HyClone), 1% nonessential amino acids, 1 mM L-glutamine, and 0.1 mM β-mercaptoethanol. Cultures were replaced with fresh differentiation medium or medium supplemented with 50 ng/ml BMP-4 (R&D Systems), 300ng/ml stem cell factor (SCF) (Amgen), and 300 ng/ml Flt-3 ligand (R&D Systems). EBs were maintained for 15 days, and medium was changed every 4 days. For neural precursor differentiation, EBs were cultured in EB medium alone for 4 days. After the initial 4 days the EBs were transferred to 12-well plates coated with poly-L-lysine/fibronectin and maintained in neural proliferation medium consisting of DMEM/F12 with B27 and N2 supplements (Gibco), 10ng/ml bFGF, 10ng/ml human epidermal growth factor (hEGF), 1ng/ml human platelet derived growth factor-AA (PDGF-AA) (R&D Systems), and 1 ng/ml human insulin-like growth factor-1 (hIGF-1) (R&D systems). Cultures were allowed to adhere to the plates and expand as a monolayer over 4 days.

### [00188] RT-PCRs and PCRs - Total RNA was isolated using Norgen total RNA isolation kit. RNA was then subjected to cDNA synthesis using superscript III (Invitrogen). Quantitative PCRs were performed using Platinium SYBR Green -UDP mix (Invitrogen). Genomic DNA was isolated using ALL
IN ONE isolation kit (Norgen). For EOS provirus integration studies 150 ng genomic DNA was used for amplification of GFP in PCR reactions. PCR reactions were performed using 2X PCR Master Mix (Fermentas). Products were resolved on 1.2 % agarose gels. Primer sequences are provided in Table 7.

[00189] Western blotting - Cell extracts were prepared in lysis buffer [50 mM Tris (pH 8.0), 150 mM NaCl, 1% (v/v) Nonidet P-40, 0.1% (w/v) SDS, 0.5% (v/v) sodium deoxycholate and Complete protease inhibitors (GE Healthcare)] from hESC, total fibroblasts, 293, 293 overexpressing Oct4, GFP+ve (NOS+exp) and total hFibs. Approximately 60 μg of protein was loaded for western blotting with indicated antibodies.

[00190] Chromatin Immunoprecipitations - Chromatin IPs were performed as described previously (Rampalli et al. 2007). In brief cells were crosslinked using 1% formaldehyde and chromatin was digested in buffer containing 0.1 % SDS to obtain fragments of approximately 400 bp length. Sonicated DNA was subjected to immunoprecipitation using ChIP grade antibodies (anti-trimethyl H3K4 (Abeam), anti trimethyl-H3K27 (Abeam), anti Oct4 (Cell Signaling), anti Nanog (Cell Signaling), anti Sox2 (Cell Signaling), anti BrachuryT (Abeam), anti rabbit IgG and anti mouse IgG antibodies). Immunoprecipitated DNA was further reverse crosslinked, purified and subjected to qPCR analysis using Platinium Syber Green-UDP mix. To calculate relative enrichment, control -IP signals were subtracted from specific ones and the resulting difference was divided by signal observed from 1/50th of input material.

[00191] MeDip ChIP assay was performed as described previously. Briefly genomic DNA was extracted from 293, hESC, total hFibs and NOS+exp (GFP+ve) cells by overnight Proteinase K treatment, phenol-chloroform extraction, ethanol precipitation and RNase digestion. Before carrying out MeDIP, genomic DNA was sonicated to produce random fragments ranging in size from 300 to 1,000 bp. Immunopurified DNA was subjected to qPCR analysis using Platinium Sybr Green-UDP mix. To calculate relative
enrichment, control -IP signals were subtracted from specific ones and the resulting difference was divided by signal of input material. Primers for quantitative PCR analysis are provided in Table 7.

[00192] Live Staining - For live staining sterile Tra-1-60 antibody (Millipore) was preconjugated with sterile Alexa Fluor 647 goat anti-mouse IgM (Molecular Probes, Invitrogen) at room temperature. Reprogrammed colonies were washed once with iPSC medium and incubated with Tra-1-60-Alexa 647 antibodies for 30 mins at room temperature. Cultures were then washed twice to remove unbound antibody. Cells were visualized using the Olympus fluorescence microscope.

[00193] Flow Cytometry - Induced pluripotent cells were treated with collagenase IV (Gibco), and then placed in cell dissociation buffer (Gibco) for 10 minutes at 37°C. Cell suspensions were stained with SSEA-3 (Developmental Studies Hybridoma Bank, mAB clone MC-631, University of Iowa, Iowa City, IA). Cells were visualized with Alexa Fluor 647 goat anti-rat IgM (Molecular Probes, Invitrogen). Appropriate negative controls were utilized. Live cells were identified by 7-Amino Actinomycin (7AAD) exclusion and then analyzed for cell surface marker expression using the FACS Calibur (BDIS). Collected events were analyzed using FlowJo 6.4.1 Software (Tree Star Inc.). EBs generated from iPSC cells were disassociated with 0.4 U/ml Collagenase B (Roche Diagnostics, Laval, QC, Canada) at day 15 and analyzed for expression of hemogenic and hematopoietic markers. Hematopoietic cells (CD45+) were identified by staining single cells (2-5x 10^5 cells/ml) with fluorochrome-conjugated monoclonal antibodies (mAb) pan-leukocyte marker CD45-APC (Milteny Biotech, Germany). The mAb and their corresponding isotype was used at 1-2 mg/ml. Frequencies of cells possessing the hematopoietic phenotypes were determined on live cells by 7AAD (Immunotech) exclusion, using FACS Calibur, and analysis was performed using the FlowJo software (Tree Star). EBs in neural proliferation medium were trypsinized after 4 d in culture and stained with the cell surface marker A2B5 (R&D Systems). Cells were visualized using Alexa Fluor 647
goat-anti-mouse IgM (Molecular Probes, Invitrogen). Frequencies of cells expressing A2B5 were determined on live cells by 7AAD (Immunotech) exclusion, using FACS Calibur, and analysis was performed using the FlowJo software (Tree Star).

5 **Immunocytochemistry** - Total fibroblasts, 293, 293 transfected with pSIN-Oct4 vector and sorted NOS+exp cells were seeded on chamber slides. hESC transduced with EOS C3+ were grown on matrigel coated 12-well dishes. Cells fixed in paraformaldehyde and permeabilized in Triton X-100 prior to staining for human Oct4 (Rat anti-Human Oct3/4 monoclonal antibody clone 240408) (R&D systems). Cells were then stained with secondary antibody Alexa Fluor 647 anti-Rat IgG (Molecular Probes). Chamber slides were mounted and counterstained with Vectashield Mounting Medium containing DAPI (Vector Labs). For HMMR staining adult dermal fibroblast cells were transduced with EOS C3+ lentivirus and CD168 (HMMR) (ab 67003) staining was performed as described above. Cells were visualized using the Olympus 1X81 fluorescence microscope.

[00195] **Teratoma Assay** - The McMaster University Animal Care Council approved all procedures and protocols. Induced pluripotent stem cell cultures were treated with collagenase IV for 5-10 min followed by collection and washing 2X with saline and resuspended in saline. 500,000 cells per sample were injected intratesticularly into male NOD-SCID mice. Mice were killed 10-12 weeks after initial injection. Teratomas were extracted, embedded in paraffin and sectioned in 5 µm intervals followed by deparaffinization in xylene and processing through a graded series of alcohol concentrations. Samples were stained with hematoxylin and eosin or Oct4 followed by dehydration and xylene treatment. Slides were mounted using Permount and imaged by scanning slides using Aperio Scan Scope and images were captured using Image Scope v9.0.19.1516. software. Tissue typing was performed based on stringent histological and morphological criteria specific for each germ layer subtype. Mesoderm lineages, such as bone were identified using presence of osteocytes and bone spicules; cartilage was
identified by the presence chondrocytes and specific staining of the extra cellular matrix. Endoderm lineages, such as intestinal lumens were identified by the presence of goblet cells in the lumen epithelium. Ectoderm lineages, such as skin were identified based on distinguishing cell layer morphologies (i.e. stratified); brain or neural tube was identified based on specific histological criteria. The presence of the germ layers and tissue typing was confirmed by McMaster Pathology.

**[00196] 3-D reconstitution/Z-stacking** - Adult dermal fibroblasts transduced with EOS vector were seeded in chamber slides. Cells were washed with PBS and fixed with 4% paraformaldehyde/PBS for 10 minutes, followed by permeabilization in Triton X-100. Slides were mounted and counterstained using VECTASHIELD HardSet Mounting Medium with DAPI (Vector Labs). Cells were visualized using the Olympus 1X81 microscope and z-stacks (30 sections per field) were captured with a Photometrix Cool Snap HQ2 camera using In Vivo version 3.1.2 (Photometrix) software. Z-sections/image stacks were pseudo-coloured and 3-D mapped using ImageJ software.

**[00197] Microarray Analysis** - Total RNA was isolated from adult dermal fibroblasts (total), NOS +exp (GFP +ve), NOS -exp (GFP -ve) cells, iPSC NOS +ve and hESC using total RNA purification kit (Norgen) according to the manufacturer's instructions. RNA amplification, GeneChip 3' oligonucleotide microarray hybridization and processing was performed by the OGIC, Ottawa Health Research Institute, Ottawa, Ontario according to the manufacturer's protocols (Affymetrix). For each sample, 200 ng of single-stranded DNA was labeled and hybridized to the Affymetrix HG-U133 Plus 2.0 chips. Expression signals were scanned on an Affymetrix GeneChip Scanner and data extraction was performed using Affymetrix AGCC software. Data normalization and analysis was performed using Dchip software (Li and Wong 2001 PNAS). Hierarchical clustering using Pearson correlation coefficients was performed on the normalized data. Differentially upregulated genes were
analyzed using D-ChIP. Gene Ontology (GO) analysis was performed using FATIGO (http://babelomics.bioinfo.cipf.es).

**EXAMPLE 3: Direct Neural Conversion from Human Dermal Fibroblasts**

**Results and discussion**

Oct-4 (POU5F1) together with neuronal cytokines (bFGF, EGF) was used to promote neuronal conversion from human dermal fibroblasts. While, Vierbuchen and colleagues (Vierbuchen et al., 2010) have shown mouse fibroblast conversion to single neuronal cell-type, namely neurons, the present example demonstrates the conversion of human fibroblasts to oligodendrocytes, astrocytes and neurons while bypassing a pluripotent state (Fig 38). Human dermal fibroblasts transduced with POL domain binding protein Oct-4 were plated for standard neural, oligodendrocyte and astrocyte differentiation assays used in the field using human laminin coated dishes and cultured in neural/oligodendrocyte or astrocyte differentiation medium supplemented with bFGF, EGF and BMP-4 (Fig 38a). Unlike untransduced/control fibroblasts, human dermal fibroblasts transduced with Oct-4 gave rise to all three neural lineages (neurons, astrocytes and oligodendrocyte), as demonstrated by acquisition of neural lineage specific morphologies (Fig 38b). The Oct-4 transduced fibroblasts were further analyzed for expression of neural lineage specific marker expression, such as astrocyte specific marker GFAP (Glial fibrillary acidic protein), oligodendrocyte specific marker Olig-4 (oligodendrocyte transcription factor 4) and neuron specific marker (TUBB3) beta-Tubulin III. Human dermal fibroblasts transduced with Oct-4 expressed GFAP, TUBB3 and Olig-4, as demonstrated by immunofluorescence imaging (Fig 38c, e, g) and FACS analysis (Fig 38d, f, h, i) indicative of astrocyte, neuron and oligodendrocyte emergence.

To demonstrate that the neurons are able to give rise to mature and functional dopaminergic neurons, the human dermal fibroblasts were further differentiated as described by Roy and colleagues (2006). The human dermal fibroblasts transduced with Oct-4 gave rise to dopaminergic neurons as indicated by co-expression of TUBB3 and Tyrosine Hydroxylase (markers...
of dopaminergic neurons) (Fig 39). Collectively, these results indicate that human dermal fibroblasts ectopically expressing Oct-4 in conjunction with neural lineage inductive conditions are able to give rise to astrocyte, neuron and oligodendrocyte, as well as functional and mature neurons with dopaminergic phenotypes.

[00200] Further gene expression comparisons between untransduced and Oct4 transduced fibroblasts at day 4 after treatment evidenced a significant increase in the expression of certain genes associated with neural development such as BMI1, POU3F2, and NEFL between 1.6 and 1.8 fold (p<0.009; Figure 40). These data support the activation of neural differentiation programs in progenitors derived from dermal fibroblasts transduced with Oct-4.

Methods

[00201] Neural Precursor Differentiation - Adapted from (Pollard et al., 2009; Reubinoff et al., 2001; Roy et al., 2006). Adult dermal and fetal dermal fibroblasts were cultured in F12-DMEM media supplemented with 20% FBS, IGF 1 and bFGF. Fibroblasts were transduced with Oct-4 lentivirus and cultured in the media described above. Further neuronal differentiation was carried out in neural precursor medium consisting of DMEM/F12 with B27 and N2 supplements (Gibco), 20ng/ml bFGF and 20ng/ml human epidermal growth factor (hEGF), (R&D systems) (Carpenter et al., 2001). Cells were allowed to adhere to the plates and expand as a monolayer over 14 days. Medium was replaced every 3 days, and cells were passed on day 7 by dissociation into a single cell suspension using Accutase (Sigma) for 5 minutes.

[00202] Dopaminergic progenitor induction: For dopaminergic progenitor differentiation cultures were prepared as previously described (Roy et al., 2006), Briefly, neural precursor cultures were dissociated in Accutase for 5 minutes, and then transferred to new laminin-coated plates (BD Biosciences) in midbrain neuron media consisting of DMEM/F12 supplemented with N2 (Gibco), bFGF (10ng/ml), the N-terminal active
fragment of human SHH (200 ng/ml), and FGF8 (100 ng/ml; R&D). Medium was replaced every 3 days. After 7 days, dopaminergic neuron differentiation was induced by withdrawing SHH and the FGFs, and replacing with DMEM/F12 media supplemented with N2, GDNF (20 ng/ml), BDNF (20 ng/ml) and 0.5% FBS. Cultures were maintained for 14 days in these conditions and then fixed for staining (ie. Tyrosine Hydroxylase, βIII Tubulin for dopaminergic neurons).

[00203] While the present disclosure has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the disclosure is not limited to the disclosed examples. To the contrary, the disclosure is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

[00204] All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.
TABLES

**TABLE 1:**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Biological Replicates (n)</th>
<th>Mesoderm (layer/biological replicate)</th>
<th>Ectoderm (layer/biological replicate)</th>
<th>Endoderm (layer/biological replicate)</th>
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5 **TABLE 2:**

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<tr>
<td>GATA1</td>
<td>5' side</td>
<td>ATGCANNN</td>
<td>Oct-1/Oct-4</td>
<td>(Sridharan et al 2009, Cell)</td>
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<td>Oct-2</td>
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<td>ND</td>
<td>(Boyer et al 2005, Cell)</td>
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<td>Nanog</td>
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<td>(Boyer et al 2005, Cell)  (Rodda et al, 2005 JBC)</td>
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<td>Oct-2</td>
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Table 3. Global gene expression profile of hFibs vs. Oct-4 transduced hFibs at Day 4

<table>
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<tr>
<th>GENES</th>
<th>Accession</th>
<th>Fold change</th>
<th>p-value</th>
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<tbody>
<tr>
<td>ILIA: interleukin 1, alpha</td>
<td>NM_000575</td>
<td>25.87</td>
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<td>BIRC3: baculoviral IAP repeat-containing 3</td>
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<td>TNFAIP3: tumor necrosis factor, alpha-induced protein 3</td>
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<td>BHLHB3: basic helix-loop-helix domain containing, class B, 3</td>
<td>NM_030762</td>
<td>18.02</td>
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<td>HDAC9: histone deacetylase 9</td>
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<td>RRA9D: Ras-related associated with diabetes</td>
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<td>BC027999</td>
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<td>CYP1A1: cytochrome P450, family 1, subfamily A, polypeptide 1</td>
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<tr>
<td>EGR2: early growth response 2 (Krox-20 homolog, Drosophila)</td>
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<td>DDT3 : DNA-damage-inducible transcript 3</td>
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<td>ATF3: activating transcription factor 3</td>
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<td>ZNF844: zinc finger protein 844</td>
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<td>NUA1K: NUA1 family, SNFI-like kinase, 1</td>
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<td>HLF: hepatic leukemia factor</td>
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<td>POU5F1: POU class 5 homeobox 1</td>
<td>NM_002701</td>
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<td>SLC2A5A25 : solute carrier family 25</td>
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<td>LIF: leukemia inhibitory factor (cholinergic differentiation</td>
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HOXB9: homeobox B9  
NR1D1: nuclear receptor subfamily 1, group D, member 1  
RIT1: Ras-like without CAAX  
HIST1H4H: histone cluster 1, H4h  
EFCAB7: EF-hand calcium binding domain 7  
ZNF596: zinc finger protein 596  
NFKBIA  
DKFZp686024166: hypothetical protein DKFZp686024166  
ZNF441: zinc finger protein 441  
EGR1: early growth response 1  
PPP1R15A: protein phosphatase 1, regulatory subunit 15A  
LOC253724: hypothetical LOC253724  
GNPDA1: glucosamine-6-phosphate deaminase 1  
HSPC159: galectin-related protein  
PMAIP1: phorbol-1 2-myristate-13-acetate-induced protein 1  
SETDB2: SET domain, bifurcated 2  
ZBTB2: zinc finger and BTB domain containing 2  
FOSB: FosB murine osteosarcoma viral oncogene homolog B  
PLD6: phospholipase D family, member 6  
DLX2: distal-less homeobox 2  
HEY1: hairy/enhancer-of-split related with YRPW motif 1  
HIST2H2BE: histone cluster 2, H2be  
HLA3: HERV-H LTR-associating 3  
ZNF331: zinc finger protein 331  
SYT14: synaptotagmin XIV  
CLCN6: chloride channel 6  
TNFSF4: tumor necrosis factor (ligand) superfamily, member 4  
PMEPA1: prostate transmembrane protein, androgen induced 1  
BAMBI: BMP and activin membrane-bound inhibitor homolog  
ZC3H12C: zinc finger CCCH-type containing 12C  
ADNP2: ADNP homeobox 2  
DRAM: damage-regulated autophagy modulator  
CHRM4: cholinergic receptor, muscarinic 4  
GCNT4: glucosaminyl (N-acetyl) transferase 4, core 2  
GDF15: growth differentiation factor 15  
HSD17B14: hydroxysteroid (17-beta) dehydrogenase 14  
MAFF: v-maf musculoaponeurotic fibrosarcoma oncogene F  
POU5F1P1: POU class 5 homeobox 1 pseudogene 1  
BCOR: BCL6 co-repressor  
RBM24: RNA binding motif protein 24  
MAFG: v-maf musculoaponeurotic fibrosarcoma oncogene G  
SCG2: secretogranin II (chromogranin C)  
IL8: interleukin 8  
CPEB4: cytoplasmic polyadenylation element binding protein 4  
KCNH1: potassium voltage-gated channel, subfamily H member 1
SCYL1BP1: SCY1-like 1 binding protein 1
ZNF317: zinc finger protein 317
TIFA: TRAF-interacting protein with forhead-associated domain
ZNF79: zinc finger protein 79
C3orf34: chromosome 3 open reading frame 34
ZNF155: zinc finger protein 155
HIST1H2BK: histone cluster 1, H2bk
ZNF14: zinc finger protein 14
CCL2: chemokine (C-C motif) ligand 2
TP53INP1: tumor protein p53 inducible nuclear protein 1
C8orf46: chromosome 8 open reading frame 46
VDR: vitamin D (1,25- dihydroxyvitamin D3) receptor
ZNF461: zinc finger protein 461
HEI1: hairy and enhancer of split 1, (Drosophila)
CDKN1A: cyclin-dependent kinase inhibitor 1A (p21, Cipl)
HIST3H2A: histone cluster 3, H2a
TXNIP: thioredoxin interacting protein
RAB9A: RAB9A, member RAS oncogene family
KCNCl: potassium voltage-gated channel 1
C21orf91: chromosome 21 open reading frame 91
KCTD11: potassium channel tetramerisation domain containing 11
ZNF436: zinc finger protein 436
KLFI: Knuppl-like factor 11
ZNF790: zinc finger protein 790
PERI: period homolog 1 (Drosophila)
ZHX2: zinc fingers and homeoboxes 2
SPATA18: spermatogenesis associated 18 homolog (rat)
NR4A3: nuclear receptor subfamily 4, group A, member 3
CYP1B1: cytochrome P450, family 1, subfamily B, polypeptide 1
TSC22D3: TSC22 domain family, member 3
ZBTB1: zinc finger and BTB domain containing 1
ZNF442: zinc finger protein 442
CDKN2AIP: CDKN2A interacting protein
TNFRSF9: tumor necrosis factor receptor superfamily, member 9
RASL11B: RAS-like, family 11, member B
KIAA1370: KIAA1370
LYPLA1: lysophospholipase-like 1
STX3: syntaxin 3
CPEB2: cytoplasmic polyadenylation element binding protein 2
FAM83G: family with sequence similarity 83, member G
PLK2: polo-like kinase 2 (Drosophila)
HSPA4L: heat shock 70kDa protein 4-like
ZNF585A: zinc finger protein 585A
HS3ST2: heparan sulfate (glucosamine) 3-O-sulfotransferase 2
MAFG: v-maf musculoaponeurotic fibrosarcoma oncogene G
TNF: tumor necrosis factor (TNF superfamily, member 2)
TNF: tumor necrosis factor (TNF superfamily, member 2)
TNF: tumor necrosis factor (TNF superfamily, member 2)  NM_000594  4.95  0.012786232
ZNF433: zinc finger protein 433  NM_00108041 1  4.91  0.008982418
HBEGF: heparin-binding EGF-like growth factor  NM_001945  4.88  0.008424841
ZNF354A: zinc finger protein 354A  NM_005649  4.87  0.023629804
ZNF425: zinc finger protein 425  NM_001001661  4.86  0.024180798
JUNB: jun B proto-oncogene  NM_002229  4.86  0.021099531
HIVEP1:  NM_002114  4.83  0.004781069
GEM: GTP binding protein overexpressed in skeletal muscle  NM_005261  4.83  0.016619793
SOD2: superoxide dismutase 2, mitochondrial  NM_001024465  4.82  0.004130069
EGR3: early growth response 3  NM_004430  4.81  0.01683548
ZNF44: zinc finger protein 44  NM_016264  4.78  0.028861556
TLE4: transducin-like enhancer of split 4  NM_007005  4.77  0.033194396
FU27255: hypothetical LOC401281  AK130765  4.76  0.04371683
ZNF383: zinc finger protein 383  NM_152604  4.75  0.046317321
IFIT3: interferon-induced protein with tetratricopeptide repeats 3  NM_001031683  4.75  0.019581114
NFKBIE:  NM_004556  4.74  0.029597747
SMCR8: Smith-Magenis syndrome chromosome region, candidate 8  NM_144775  4.72  0.0131253
PLA2G4C: phospholipase A2, group IVC  NM_003706  4.7  0.002438767
EDA2R: ectodysplasin A2 receptor  NM_021783  4.69  0.008321597
TGFBI2: transforming growth factor, beta 2  NM_003238  4.68  0.040921831
RASSF9: Ras association (RalGDS/AF-6) domain family member 9  NM_005447  4.63  0.00865133
LRRC32: leucine rich repeat containing 32  NM_001128922  4.62  0.012920213
NPy1R: neuropeptide Y receptor Y1  NM_000909  4.61  0.029391154
IL6: interleukin 6 (interferon, beta 2)  NM_000600  4.6  0.005204195
TNFRSF10B: tumor necrosis factor receptor 10b  NM_003842  4.59  0.021271781
FICD: FIC domain containing  NM_007076  4.57  0.005031673
SC5DL: sterol-C5-desaturase-like  NM_006918  4.57  0.023382119
RGS5: regulator of G-protein signaling 5  NM_003617  4.57  0.003674002
AEN: apoptosis enhancing nuclease  NM_002276  4.56  0.009974851
ZNF627: zinc finger protein 627  NM_145295  4.54  0.010467624
MARCH3: membrane-associated ring finger (C3HC4) 3  NM_178450  4.53  0.023826065
BEST3: bestrophin 3  NM_032735  4.47  0.010248807
KCDT1: potassium channel tetramerisation domain containing 11  NM_001002914  4.45  0.036728406
LOC729127: hypothetical protein LOC729127  AK092418  4.44  0.036166195
DKK2: dickkopf homolog 2 (Xenopus laevis)  NM_014421  4.43  0.036152444
ZNF222: zinc finger protein 222  NM_013360  4.43  0.023199269
FRS2: fibroblast growth factor receptor substrate 2  NM_006654  4.4  0.009574668
ZNF214: zinc finger protein 214  NM_013249  4.39  0.005325101
CDC14A: CDC14 cell division cycle 14 homolog A (S. cerevisiae)  NM_003672  4.38  0.024062032
USP38: ubiquitin specific peptidase 38  NM_032557  4.37  0.026290174
PPP1R3B: protein phosphatase 1, regulatory (inhibitor) subunit 3B  NM_024607  4.35  0.023942719
OSGIN1: oxidative stress induced growth inhibitor 1  NM_013370  4.35  0.005808394
ZNF542: zinc finger protein 542  NR_003137  4.32  0.005936010
NUAK2: NUAK family, SNF1-like kinase, 2 LOC440350: similar to nuclear pore complex interacting protein  NM_001018  4.31  0.036134208
ZIMF10: zinc finger protein 10  NM_015394  4.31  0.037020672
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SCAND3: SCAN domain containing 3
ZNF462: zinc finger protein 462
STX11: syntaxin 11
GBAP: glucosidase, beta; acid, pseudogene
C10orf26: chromosome 10 open reading frame 26
DBD2: damage-specific DNA binding protein 2, 48kDa
ALKBH1: alkB, alkylation repair homolog 1 (E. coli)
ARRDC4: arrestin domain containing 4
ZBTB6: zinc finger and BTB domain containing 6
ATXN7L1: ataxin 7-like 1
RASSF2: Ras association (RalGDS/AF-6) domain family member 2
ZNF563: zinc finger protein 563
C4orf18: chromosome 4 open reading frame 18
TET3: ten-eleven translocation 3, ten-eleven translocation 3
MGC42105: hypothetical protein MGC42105
BLOC1S2: biogenesis of lysosomal organelles complex-1, subunit 2
PELI1: pellino homolog 1 (Drosophila)
ZNF160: zinc finger protein 160
ZSWIM6: zinc finger, SWIM-type containing 6
C3orf93: chromosome 3 open reading frame 59
HIST1H2AI: histone cluster 1, H2aI
BCL6: B-cell CLL/lymphoma 6
ZNF669: zinc finger protein 669
C20orf11: chromosome 20 open reading frame 111
THAP6: THAP domain containing 6
THNSL1: threonine synthase-like 1 (S. cerevisiae)
ZNF175: zinc finger protein 175
NFKB2
ZNF772: zinc finger protein 772
BHLHB2: basic helix-loop-helix domain containing, class B, 2
C6orf58: chromosome 6 open reading frame 58
ZNF211: zinc finger protein 211
C2orf67: chromosome 2 open reading frame 67
ERRFI1: ERBB receptor feedback inhibitor 1
HIST1H2AC: histone cluster 1, H2ac
TMEM555B: transmembrane protein 55B
ZNF438: zinc finger protein 438
UAP1L1: UDP-N-acetylglucosamine pyrophosphorylase 1-like 1
ZNF506: zinc finger protein 506
mAMLL2: mastermind-like 2 (Drosophila)
IKZF3: IKAROS family zinc finger 3 (Aylos)
C3AR1: complement component 3a receptor 1
SCL9A8: solute carrier family 9, member 8
DCUN1D: DCUN1D, defective in cullin neddylation 1
TNFRSF10C
EAF1: ELL associated factor 1
TGFB3: transforming growth factor, beta 3
PLEKHO2: pleckstrin homology domain containing, family O 2
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NM_007147 3.78 0.01460430
NM_002502 3.77 0.000151
NM_001024596 3.77 0.03826615
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AK303850 3.77 0.04692392
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NM_003512 3.73 0.02590260
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NM_001099269 3.7 0.01991840
NM_032427 3.66 0.00401896
NM_012481 3.65 0.04945401
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NM_173475 3.63 0.02018538
NM_003841 3.63 0.02279261
NM_033083 3.62 0.04151828
NM_003239 3.61 0.04246798
NM_JD25201 3.61 0.00613281
THAP2: THAP domain containing, apoptosis associated protein 2

CHMP2B: chromatin modifying protein 2B

IFRD1: interferon-related developmental regulator 1

ACYP2: acylphosphatase 2, muscle type

GAD1: glutamate decarboxylase 1 (brain, 67kDa)

ASH1L: ashl (absent, small, or homeotic)-like (Drosophila)

ZNF616: zinc finger protein 616

LUZP1: leucine zipper protein 1

NFKBIZ

TRIM22: tripartite motif-containing 22

ZNF267: zinc finger protein 267

EXPH5: exophilin 5

ZNF226: zinc finger protein 226

LOC400657: hypothetical LOC400657

SLC7A8: solute carrier family 7, member 8

THUMPD2: THUMP domain containing 2

TLR4: toll-like receptor 4

C3orf38: chromosome 3 open reading frame 38

FU317: 15: hypothetical protein FU31715

RNF6: ring finger protein (C3H2C3 type) 6

ZSCAN12: zinc finger and SCAN domain containing 12

MFAP4: microfibrillar-associated protein 4

CLEC28B: C-type lectin domain family 28, member B

PPM1D: protein phosphatase 1D magnesium-dependent delta isoform

IL1B: interleukin 1, beta

ZNF284: zinc finger protein 284

ZNF557: zinc finger protein 557

ZFP3: zinc finger protein 3 homolog (mouse)

URG4: up-regulated gene 4

AVP1: arginine vasopressin-induced 1

DUSP14: dual specificity phosphatase 14

FSTL3: follistatin-like 3 (secreted glycoprotein)

FNIP1: 1: fascin-like protein 1

ZNF416: zinc finger protein 416

LOC492311: similar to bovine IgA regulatory protein

RND1: Rho family GTPase 1

ZC3H6: zinc finger CCCH-type containing 6

TNFAIP6: tumor necrosis factor, alpha-induced protein 6

ZNF721: zinc finger protein 721

SLC16A8: solute carrier family 16, member 6

ZNF223: zinc finger protein 223

ZIMF701: zinc finger protein 701

IL32: interleukin 32

HIST2H2BF: histone cluster 2, H2bf

DBP: D site of albumin promoter (albumin D-box) binding protein

TGIF2: TGFB-induced factor homeobox 2

ZNF597: zinc finger protein 597

PAN2: PAN2 polyA specific ribonuclease subunit homolog
FLRT2: fibronectin leucine rich transmembrane protein 2
BAZ2B: bromodomain adjacent to zinc finger domain, 2B
FLCN: folliculin
SLC30A1: solute carrier family 30 (zinc transporter), member 1
CSF1: colony stimulating factor 1 (macrophage)
PRDM2: PR domain containing 2, with ZNF domain
REPIN1: replication initiator 1
ENC1: ectodermal-neural cortex (with BTB-like domain)
RPS27L: ribosomal protein S27-like
IMPK3B: MAP1LC3B: microtubule-associated protein 1 light chain 3 beta 2
MD1: MAX dimerization protein 1
CAMKK1: calcium/calmodulin-dependent protein kinase kinase 1A
FU4267: hypothetical LOC645644
ZNF292: zinc finger protein 292
PFKB4: 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4
RRAGD: Ras-related GTP binding D
ZNF654: zinc finger protein 654
C2orf60: chromosome 2 open reading frame 60
CAP2: CAP, adenylate cyclase-associated protein, 2 (yeast)
UVRA: UV radiation resistance associated gene
ZNF136: zinc finger protein 136
ZNF329: zinc finger protein 329
CABLES1: Cdk5 and Abl enzyme substrate 1
ZFP37: zinc finger protein 37 homolog (mouse)
GLIS2: GLIS family zinc finger 2
CLorf03: chromosome 1 open reading frame 103
CBLL1: 1-Cas-Br-Mectropic retroviral transforming sequence-like 1
RNASET7: ribonuclease, RNase A family, 7
C13orf3: chromosome 13 open reading frame 31
NSUN6: NOL1/NOP2/Sun domain family, member 6
EPC1: enhancer of polycomb homolog 1 (Drosophila)
RNF185: ring finger protein 185
KCNQ1: potassium channel regulator
FAM179B: family with sequence similarity 179, member B
HRH1: histamine receptor HI
ZNF630: zinc finger protein 630
TOPORS: topoisomerase I binding, arginine-serine-rich
ZNF23: zinc finger protein 23 (KOX 16)
MOSPD1: motile sperm domain containing 1
AMY2B: amylase, alpha 2B (pancreatic)
HMGCCL: 3-hydroxy)methyl-3-methylglutaryl-Coenzyme A lyase
FIGN: fidgetin
TRAF1: Traf receptor-associated factor 1
ANKRA2: ankyrin repeat, family A (RFXANK-like), 2
CCDC126: coiled-coil domain containing 126

NM_013231 3.38 0.020458934
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NM_015920 3.36 0.013107 109
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NM_018293 3.3 0.000395798
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NM_003369 3.26 0.010565 147
NM_003437 3.26 0.035578432
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NM_023039 3.18 0.004596453
NM_138771 3.17 0.042950357
ZNF304: zinc finger protein 304
DUSP3: dual specificity phosphatase 3
FU32065: hypothetical protein FU32065
GABARAPL2: GABA(A) receptor-associated protein-like 2
LOC441734: similar to hypothetical protein
DKFZp434I1020
C1orf51: chromosome 1 open reading frame 51
KBTBD8: kelch repeat and BTB (POZ) domain containing 8
H1F0: HI histone family, member 0
CRYM: crystallin, m u
C6orf145: chromosome 6 open reading frame 145
ZNF408: zinc finger protein 408
BHLHB9: basic helix-loop-helix domain containing, class B, 9
ZNF555: zinc finger protein 555
YTHDF3: YTH domain family, member 3
SH3BGR2L2: SH3 domain binding glutamic acid-rich protein like 2
DNAH12L: dynein, axonemal, heavy chain 12-like
CTSS: cathepsin S
Jj4JD1C: jumonji domain containing 1C
PIWIL4: piwi-like 4 (Drosophila)
SLC4A5: solute carrier family 4, sodium bicarbonate cotransporter 5
Clorfi63: chromosome 1 open reading frame 63
MFAP3L: microfibrillar-associated protein 3-like
TMEM159: transmembrane protein 159
GABARAPL1: GABA(A) receptor-associated protein like 1
ZNF776: zinc finger protein 776
HIST1H2BG: histone cluster 1, H2bg
FGF1: fibroblast growth factor 1 (acidic)
BDNF: brain-derived neurotrophic factor
LACTB2: lactamase, beta 2
LCP1: lymphocyte cytosolic protein 1 (L-plastin)
MAFG: v-maf musculoaponeurotic fibrosarcoma oncogene G
ZNF440: zinc finger protein 440
SLC31A2: solute carrier family 31 (copper transporters), member 2
CREB5: cAMP responsive element binding protein 5
PDRG1: p53 and DNA damage regulated 1
ERF: Ets2 repressor factor
C5orf51: chromosome 5 open reading frame 51
ZNF137: zinc finger protein 137
RRAGC: Ras-related GTP binding C
STAT2: signal transducer and activator of transcription 2, 113kDa
ZNF644: zinc finger protein 644
CAPS2: calycaphosine 2
ZNF546: zinc finger protein 546
TME69: transmembrane protein 69
HCCS: holocytochrome c synthase (cytochrome c heme-lyase)
WBP2: WW domain binding protein 2

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NM_016486 3.02 0.021783875
NM_005333 3.01 0.019281 137
NM_012478 3.01 0.046388537
PIM3: pim-3 oncogene
EGFR: early growth response 4
PFKB2: 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2
ZNF235: zinc finger protein 235
ZNF658: zinc finger protein 658
LOC440348: similar to nuclear pore complex interacting protein
SOX4: SRY (sex determining region Y)-box 4
TRIM13: tripartite motif-containing 13
ID1: isopentenyl-diphosphate delta isomerase 1
ZNF658: zinc finger protein 658
CHIC2: cysteine-rich hydrophobic domain 2
MST3H2BB: histone cluster 3, H2bb
CCR4: chemokine (C-C motif) receptor 4
ANKRD10: ankyrin repeat domain 10
ZNF132: zinc finger protein 132
PPIF: peptidylprolyl isomerase F (cyclophilin F)
HELI308: DNA helicase HEL308
PAG1: phosphoprotein associated glycosphingolipid microdomains 1
LRRCC7B: leucine rich repeat containing 37B
TSC22D2: TSC22 domain family, member 2
MITD1: MIT domain containing 1
KCNAB1: potassium voltage-gated channel, beta member 1
ZNF523: zinc finger protein 253
AOC2: amine oxidase, copper containing 2 (retina-specific)
ZNF503: zinc finger protein 503
LHX4: LIM homeobox 4
ZNF26: zinc finger protein 26
ZNF502: zinc finger protein 502
CHMP1B: chromatin modifying protein 1B
RUNX1: runt-related transcription factor 1
H2AFJ: H2A histone family, member J
ATP6V1G1: ATPase, H+ transporting, V1 subunit G1
GZF1: GDNF-inducible zinc finger protein 1
CCDC122: coiled-coil domain containing 122
FAMS3C: family with sequence similarity 53, member C
HSD17B7: hydroxysteroid (17-beta) dehydrogenase 7
KLF7: Kruppel-like factor 7 (ubiquitous)
C9orf85: chromosome 9 open reading frame 85
ABHD4: abhydrolase domain containing 4
ZNF330: zinc finger protein 330
KIAA0415: KIAA0415
GCA: gracilacin, EF-hand calcium binding protein
SGIP1: SH3-domain GRB2-like (endophilin) interacting protein 1
TMEM144: transmembrane protein 144
FBXW7: F-box and WD repeat domain containing 7
RAB7L1: RAB7, member RAS oncogene family-like 1
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SNORD74: small nucleolar RNA, C/D box 74
MRC1: mannose receptor, C type 1
MRC1: mannose receptor, C type 1
TCEAL1: transcription elongation factor A (Sil)-like 1
ATXN7L1: ataxin 7-like 1
FRMPD4: FERM and PDZ domain containing 4
NEK10: NIMA (never in mitosis gene a)- related kinase 10
SERINC4: serine incorporator 4
LYRM1: LYR motif containing 1
BBS12: Bardet-Biedl syndrome 12
ZNF682: zinc finger protein 682
ZNF134: zinc finger protein 134
PLEKHM1: pleckstrin homology domain containing, family M 1
ZNF778: zinc finger protein 778
PARP6: poly (ADP-ribose) polymerase family, member 6
Clorf71 : chromosome 1 open reading frame 71
CTGLF1: centaurin, gamma-like family, member 1
NKIRAS1: NFKB inhibitor interacting Ras-like 1
LIN52: lin-52 homolog (C. elegans)
ZNF570: zinc finger protein 570
RUSC2: RUN and SH3 domain containing 2
SOCS2: suppressor of cytokine signaling 2
SECTM1: secreted and transmembrane 1
ZNF700: zinc finger protein 700
SPRY1: sprouty homolog 1, antagonist of FGF signaling (Drosophila)
SOX30: SRY (sex determining region Y)-box 30
IPMK: inositol polyphosphate multikinase
CFLAR: CASP8 and FADD-like apoptosis regulator
ZNF521: zinc finger protein 521
AKAP5: A kinase (PRKA) anchor protein 5
ZNF782: zinc finger protein 782
PGBD4: piggyBac transposable element derived 4
ZNF606: zinc finger protein 606
ZIK: zinc finger protein interacting with K protein 1
homolog
CYP2U1: cytochrome P450, family 2, subfamily U, polyepide 1
ZFP82: zinc finger protein B2 homolog (mouse)
RAPH1: Ras association and pleckstrin homology domains 1
SERTAD1: SERTA domain containing 1
ZFAND3: zinc finger, A/NL-type domain 3
IL23A: interleukin 23, alpha subunit pl9
HSPBAP1: HSPB (heat shock 27kDa) associated protein 1
GPR175: G-protein-coupled receptor 175
SULF2: sulfatase 2
LRP12: low density lipoprotein-related protein 12
CCNT1: cyclin T1
SH2D5: SH2 domain containing 5
ZRSR1: zinc finger, RNA-binding motif and serine/arginine rich 1
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ZBTB38: zinc finger and BTB domain containing 38
SPHK1: sphingosine kinase 1
TAF7: TAF7 RNA polymerase II
OSTM1: osteopetrosis associated transmembrane protein 1
RBM18: RNA binding motif protein 18
JMJD3: jumonji domain containing 3, histone lysine demethylase
REST: REI-silencing transcription factor
RP4-692D3.1: hypothetical protein LOC728621
ZNF124: zinc finger protein 124
GRI3A: glutamate receptor, ionotrophic, AMPA 3
FAM117A: family with sequence similarity 117, member A
XG: Xg blood group
YTHDF1: YTH domain family, member 1
CTGLF1: centaurin, gamma-like family, member 1
TIMM8B: translocase of inner mitochondrial membrane 8 homolog B
BTLA: B and T lymphocyte associated
IERS: immediate early response 5
CBX4: chromobox homolog 4 (Pc class homolog, Drosophila)
Cl4orf4: chromosome 14 open reading frame 4
Clorf56: chromosome 1 open reading frame 156
ZNF182: zinc finger protein 182
MRFAP1L1: Mrf4 family associated protein 1-like 1
ZNF562: zinc finger protein 562
SIAH1: seven in absentia homolog 1 (Drosophila)
SAMD4A: sterile alpha motif domain containing 4A
LYSMD3: LysM, putative peptidoglycan-binding, domain containing 3
RAB32: RAB32, member RAS oncogene family
ADHFE1: alcohol dehydrogenase, iron containing, 1
ZFX: zinc finger protein, X-linked
DKFz547E087: hypothetical gene LOC283846
FBX028: F-box protein 28
DKFz547E087: hypothetical gene LOC283846
ZFHX4: zinc finger homeobox 4
SAT2: spermidine/spermine N1-acetyltransferase family member 2
ZEB2: zinc finger E-box binding homeobox 2
F2RL2: coagulation factor II (thrombin) receptor-like 2
GABARAPL3: GABA(A) receptors associated protein like 3
DKFz547E087: hypothetical gene LOC283846
NFKB1
MOCS3: molybdenum cofactor synthesis 3
ZNF641: zinc finger protein 641
RCHY1: ring finger and CHY zinc finger domain containing 1
DKFz547E087: hypothetical gene LOC283846
PEA15: phosphoprotein enriched in astrocytes 15
PLCXD2
ZNF592: zinc finger protein 592
HECW2: HECT, C2 and WW domain containing E3 ubiquitin
ligase 2
VAMP2: vesicle-associated membrane protein 2
(synaptobrevin 2)
C1GALT1
C17orf48: chromosome 17 open reading frame 48
GTPBP5: GTP binding protein 5 (putative)
CTGLF1: centaurin, gamma-like family, member 1
SLC28A3: solute carrier family 28, member 3
ARMCX5: armadillo repeat containing, X-linked 5
SGK269: NKF3 kinase family member
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SLC3A2: solute carrier family 3, member 2
C2orf44: chromosome 2 open reading frame 44
EFNB2: ephrin-B2
ZNF620: zinc finger protein 620
CTNS: cystinosin, nephropathic
PEX12: peroxisomal biogenesis factor 12
UTP3: UTP3, small subunit (SSU) processome component, homolog
ZNF480: zinc finger protein 480
NSAP1: nervous system abundant protein 11
PRRX2: paired related homeobox 2
NC1: NCK adaptor protein 1
SNORD13: small nucleolar RNA, C/D box 13
SLC7A2: solute carrier family 7, member 2
ZNF221: zinc finger protein 221
JUB: jux, alba homolog (Xenopus laevis)
DNAJB2: DNAj (Hsp40) homolog, subfamily B, member 2
CTGLF1: centaurin, gamma-like family, member 1
CD163L1: CD163 molecule-like 1
SELPLG: selectin P ligand
IL10RA: interleukin 10 receptor, alpha
CTTNBP2NL: CTTNB2 P2 N-terminal like
LST1: leukocyte specific transcript 1
LST1: leukocyte specific transcript 1
LST1: leukocyte specific transcript 1
TBC1D3B: TBC1 domain family, member 3B
PIK3CD: phosphoinositide-3-kinase, catalytic, delta polypeptide
ZNF420: zinc finger protein 420
NEU1: sialidase 1 (lysozyme sialidase)
NEU1: sialidase 1 (lysozyme sialidase)
CCDC51: coiled-coil domain containing 51
MAD2L1BP: MAD2L1 binding protein
NDFIP2: Nedd4 family interacting protein 2
ITGB7: integrin, beta 7
ZNF419: zinc finger protein 419
ARL8B: ADP-ribosylation factor-like 8B
TBC1D3G: TBC1 domain family, member 3G
TBC1D3G: TBC1 domain family, member 3G
TBC1D3G: TBC1 domain family, member 3G
TBC1D3G: TBC1 domain family, member 3B
RIOK3: RIO kinase 3 (yeast)
NEK6: NIMA (never in mitosis gene a)-related kinase 6
OVGPI: ovudctal glycoprotein 1, 120kDa
TRAPPC6B: trafficking protein particle complex 6B
LIG4: ligase IV, DNA, ATP-dependent
RBM7: RNA binding motif protein 7
MGAM: maltase-glucosaminidase (alpha-glucosidase)
CTGLF1: centaurin, gamma-like family, member 1
CTGLF1: centaurin, gamma-like family, member 1
CTGLF1: centaurin, gamma-like family, member 1
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ZNF121: zinc finger protein 121
ZNF239: zinc finger protein 239
ZMYM5: zinc finger, MYM-type 5
MAP1LC3B: microtubule-associated protein 1 light chain 3
TBC1D3C: TBC1 domain family, member 3C
MCL1: myeloid cell leukemia sequence 1 (BCL2-related)
MAP3K7IP3
MGA: MAX gene associated
MUL1: mitochondrial ubiquitin ligase activator of NFKB 1
SNORD13: small nucleolar RNA, C/D box 13
TMEM128: transmembrane protein 128
C14orf129: chromosome 14 open reading frame 129
CCDC144A: coiled-coil domain containing 144A
STARID4: STAR-related lipid transfer (START) domain containing 4
CD72: CD72 molecule
HOXD10: homeobox D10
ARMCX1: armadillo repeat containing, X-linked 1
RRAGB: Ras-related GTP binding B
ADO: 2-aminoethanol (cysteamine) dioxygenase
ZNF585B: zinc finger protein 585B
ZNF619: zinc finger protein 619
IFIT5: interferon-induced protein with tetratricopeptide repeats 5
NOTUM: notum pectinacetylersterase homolog (Drosophila)
CTGLF3: centaurin, gamma-like family, member 3
ZNF251: zinc finger protein 251
LPCAT2: lysophosphatidylcholine acyltransferase 2
PARP8: poly (ADP-ribose) polymerase family, member 8
PHYH: phytanoyl-CoA 2-hydroxylase
ZNF354B: zinc finger protein 354B
ZIC5: Zic family member 5 (odd-paired homolog, Drosophila)
ZNF233: zinc finger protein 233
ATP6V1C1: ATPase, H+ transporting, VI subunit CI
ORAI3: ORAI calcium release-activated calcium modulator 3
CDC42EP1: CDC42 effector protein (Rho GTPase binding) 1
GATA1: GATA binding protein 1
NAPIL5: nucleosome assembly protein 1-like 5
ATP6V1G2: ATPase, H+ transporting, VI subunit G2
ATP6V1G2: ATPase, H+ transporting, VI subunit G2
GOSR1: golgi SNAP receptor complex member 1
SNF1LK: SNF1-like kinase
ASTE1: astereid homolog 1 (Drosophila)
ANKRD46: ankyrin repeat domain 46
CCDC148: coiled-coil domain containing 148
COOIOB: coenzyme Q10 homolog B (S. cerevisiae)
TANK: TRAF family member-associated NFKB activator
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IHPK2: inositol hexaphosphate kinase 2
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MRIRF: mitochondrial ribosome recycling factor
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ARHGAP5: Rho GTPase activating protein 5
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RPP38: ribonuclease P/MRP 38kDa subunit
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ZFNT37: zinc finger protein 737
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SLFN5: Schlafen family member 5
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ZFP106: zinc finger protein 106 homolog (mouse)
NM_022473 2.05 0.01549153

CLCN7: chloride channel 7
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USP36: ubiquitin specific peptidase 36
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TAF13: TAF13 RNA polymerase II
NM_005645 2.05 0.015411463

MCC: mutated in colorectal cancers
NM_010803577 2.05 0.010015918

SLC36A1: solute carrier family 36, member 1
NM_078483 2.05 0.037843932

ITPRIP: inositol 1,4,5-trisphosphate receptor interacting protein
NM_033397 2.05 0.009383002

TAF9: TAF9 RNA polymerase II
NM_003187 2.05 0.028770138

STYX1L: serine/threonine/tyrosine interacting-like 1
NM_016086 2.05 0.020194897

NKX3-1: NK3 homeobox 1
NM_006167 2.05 0.242E-05

WDR78: WD repeat domain 78
NM_024763 2.05 0.009053537

LOC440093: histone H3-like
NM_001013699 2.05 0.023051881

C21orf34: chromosome 21 open reading frame 34
NM_001005732 2.05 0.007370088

MTUS1: mitochondrial tumor suppressor 1
NM_001001924 2.05 0.013708432

PELO: pelota homolog (Drosophila)
NM_015946 2.04 0.00777595

IKZF2: IKAROS family zinc finger 2 (Helios)
NM_016260 2.04 0.044649477

ZNF268: zinc finger protein 268
NM_003415 2.04 0.035073367

CSTF2T: cleavage stimulation factor, 3' pre-RIMA, subunit 2
NM_015235 2.04 0.032105107

LOC349196: hypothetical LOC349196
BC093747 2.04 0.001722355

CLIP4: CAP-GLY domain containing linker protein family 4
NM_024692 2.04 0.031769127

NHLRC1: NHL repeat containing 1
NM_198586 2.04 0.034828117

ZNF764: zinc finger protein 764
NM_033410 2.04 0.030932324

Clorlf29: chromosome 1 open reading frame 129
NM_025063 2.04 0.02969779

WTAP: Wilms tumor 1 associated protein
NM_152857 2.03 0.027683281

C2CD3: calcium-dependent domain containing 3
NM_015531 2.03 0.013489428

HLA-B: major histocompatibility complex, class I, B
NM_005514 2.03 0.041877044

HMG20A: high-mobility group 20A
NM_018200 2.03 0.031159656

OPN3: opsin 3
NM_014322 2.03 0.013455

ZNF711: zinc finger protein 711
NM_021998 2.03 0.012443614

NOX4: NADPH oxidase 4
NM_016931 2.03 0.043475629

ZNF184: zinc finger protein 184
NM_007149 2.03 0.003981512

IF16: interferon, alpha-inducible protein 6
NM_002038 2.03 0.0038703

DAZAP2: DAZ associated protein 2
NM_014764 2.02 0.000783543

LOC100132426: similar to hCG1742442
ENST00000377415 2.02 0.022409773

PPM2C: protein phosphatase 2C
NM_018444 2.02 0.041825476

NPI1: nuclear pore complex interacting protein
AK294177 2.02 0.008576323

TRAF3: TNF receptor-associated factor 3
NM_145725 2.02 0.024933609

KIAA1975: KIAA1975 protein similar to MRIP2
NM_133447 2.02 0.005431778

CDC42EP3: CDC42 effector protein (Rho GTPase binding)
NM_006449 2.02 0.018466992

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BC093747 2.02 0.00404514
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AMOTL2: angiomotin like 2   NM_016201  2.02  0.007030751
CELSR3: cadherin, EGF LAG seven-pass G-type receptor 3   NM_001407  2.02  0.044323431
TLR1: toll-like receptor 1   NM_003623  2.02  0.019328967
GNA13: guanine nucleotide binding protein (G protein), alpha 13   NM_006572  2.02  0.000922653
ANKRD12: ankyrin repeat domain 12   NM_015208  2.02  0.17191634
C1orf54: chromosome 1 open reading frame 54   NM_198476  2.02  0.001 180486
SYT12: synaptotagmin-like 2   NM_206927  2.02  0.017931379
TERF2IP: telomeric repeat binding factor 2, interacting protein   NM_018975  2.02  0.01 1559857
CCDC93: coiled-coil domain containing 93   NM_019044  2.01  0.043888648
NPIP: nuclear pore complex interacting protein   AK294177  2.01  0.01874462
MST131: MSTP131   AF176921  2.01  0.030853785
KPN5A: karyopherin alpha 5 (importin alpha 6)   NM_002269  2.01  0.017091 137
A2C1: actin, alpha, cardiac muscle 1   NM_005159  2.01  0.034275937
CLCN4: chloride channel 4   NM_001830  2.01  0.023253416
SOCS4: suppressor of cytokine signaling 4   NM_199421  2.01  0.024790224
PDPK1: 3-phosphoinositide dependent protein kinase-1   AK293900  2.01  0.029054845
FU33996: hypothetical protein FU33996   AK091315  2.01  0.015553873
RNFI13A: ring finger protein 113A   NM_006978  2.01  0.041655818
SCG5: secretogranin V (7B2 protein)   NM_003020  2.01  0.031769366
FU46841: FLJ46841 protein   BX648674  2.01  0.033589672
LRRC37A2: leucine rich repeat containing 37, member A2   NM_01006607  2  0.009852389
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FNIP2: folliculin interacting protein 2   NM_020840  2  0.007031707
WARS2: tryptophanyl tRNA synthetase 2, mitochondrial   NM_201263  2  0.0065903 14
RARA: retinoic acid receptor, alpha   NM_000964  2  0.044455247
CYB5D1: cytochrome b5 domain containing 1   NM_144607  2  0.019193343
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GPRASP2: G protein-coupled receptor associated sorting protein 2   IMMM_001004051  2  0.038898801
ASA12B: N-acetylphosphoglycan amidohydrolase 2B   NM_00179516  2  0.028160 166
LOC100132346: similar to chaperonin 10   ENST000004069977  2  0.036218222
LIG3: ligase III, DNA, ATP-dependent   NM_013975  2  0.019468221
TSG14: testis specific, 14   NM_018718  2  0.023783473
HK2: hexokinase 2   NM_000189  2  0.018617371
IPF1: interferon epsilon 1   NM_176891  2  0.023047364
GTF2H2: general transcription factor IIH, polypeptide 2, 44kDa   NM_001515  2  0.00501888
GTF2H2: general transcription factor IIH, polypeptide 2, 44kDa   NM_001515  2  0.00501888
IARS2: isoleucyl-tRNA synthetase 2, mitochondrial   NM_018060  2  0.048624055
GTF2H2: general transcription factor IIH, polypeptide 2, 44kDa   NM_001515  2  0.016689034
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ANG: angiogenin, ribonuclease, RNase A family, 5
DDX3X: DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, X-linked
IPO1: importin 11
NT5DC2: 5′-nucleotidase domain containing 2
ERL1: ER lipid raft associated 1
ACTN1: actinin, alpha 1
MED17: mediator complex subunit 17
DNAJC3: DnaJ (Hsp40) homolog, subfamily C, member 3
STX2: syntaxin 2
XP04: exportin 4
NT5DC2: 5′-nucleotidase domain containing 2
DDX3X: DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, X-linked
IPO1: importin 11
ANG: angiogenin, ribonuclease, RNase A family, 5
ERL1: ER lipid raft associated 1
ACTN1: actinin, alpha 1
MED17: mediator complex subunit 17
DNAJC3: DnaJ (Hsp40) homolog, subfamily C, member 3
STX2: syntaxin 2
XP04: exportin 4
GEMIN4: gem (nuclear organelle) associated protein 4
ANAPC1: anaphase promoting complex subunit 1
AHSA1: AHA1, activator of heat shock protein ATPase homolog 1
LDVL: LTVI homolog (S. cerevisiae)
RPN1: ribophorin I
DNM1L: dynamin 1-like
QSER1: glutamine and serine rich 1
ACOT9: acyl-CoA thioesterase 9
ALG9: asparagine-linked glycosylation 9 homolog
RNASEH2A: ribonuclease H2, subunit A
BLID: BH3-like motif containing, cell death inducer
EVIS: ectropic viral integration site 5
NPAT: nuclear protein, ataxia-telangiectasia locus
KIAA1715: KIAA1715
FAM122B: family with sequence similarity 122B
THAP4: THAP domain containing 4
THAP4: THAP domain containing 4
DLAT: dihydrolipoamide S-acetyltransferase
FBXO11: F-box protein 11
SDF2L1: stromal cell-derived factor 2-like 1
CTGF: connective tissue growth factor
FBXO3: F-box protein 3
AGPAT5: l-acylglycerol-3-phosphate O-acyltransferase 5
RNASEN: ribonuclease type III, nuclear
2ADH2: zinc binding alcohol dehydrogenase domain containing 2
TUBGCP3: tubulin, gamma complex associated protein 3
RELN: reelin
FBL: fibrillarin
PTPRJ: protein tyrosine phosphatase, receptor type, J
TPN3: transporters 3
PHIP: pleckstrin homology domain interacting protein
HLTF: helicase-like transcription factor
STX2: syntaxin 2
XP04: exportin 4
CLPX: ClpX caseinolytic peptidase X homolog (E. coli)  NM_006660 -2.13 0.0186105
MBTPSl: membrane-bound transcription factor peptidase, site 1  NM_003791 -2.13 0.045645561
HNRPA1L2: heterogeneous nuclear ribonucleoprotein Al  NR_002944 -2.13 0.048491581
ABC2: ATP-binding cassette, sub-family F (GCN20), member 2  NM_007189 -2.14 0.008323273
SNX9: sorting nexin 9  NM_016224 -2.14 0.02029193
POLR1E: polymerase (RNA) I polypeptide E, 53kDa  NM_0022490 -2.14 0.041025013
IP08: importin 8  NM_006390 -2.14 0.037105295
POLE: polymerase (DNA directed), epsilon  NM_006231 -2.14 0.037359493
GLUL: glutamate-ammonia ligase (glutamine synthetase)  AY513283 -2.15 0.032958771
FHOD1: formin homology 2 domain containing 1  NM_013241 -2.15 0.040570594
LAPTM4B: lysosomal protein transmembrane 4 beta  NM_018407 -2.15 0.030389137
MCM10: minichromosome maintenance complex component 10  NM_1220751 -2.15 0.044316968
IL1RL1: interleukin 1 receptor-like 1  NM_016232 -2.15 0.03462345
NAPL3: NIPA-like domain containing 3  NM_020448 -2.15 0.036362629
ITGA3: integrin, alpha 3  NM_002204 -2.15 0.014046025
JMJD2A: jumonji domain containing 2A  NM_014663 -2.15 0.024979543
QARS: glutaminyl-tRNA synthetase  NM_005051 -2.15 0.026387003
DDX11: DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11  NM_006053 -2.15 0.01752887
GEMIN5: gem (nuclear organelle) associated protein 5  NM_015465 -2.15 0.029127837
ARSK: arylsulfatase family, member K  NM_198150 -2.16 0.04639001
ANTXR2: anthrax toxin receptor 2  NM_058172 -2.16 0.025952245
EPS15: epidermal growth factor receptor pathway substrate 15  NM_001981 -2.16 0.045957677
NAT10: N-acetyltransferase 10  NM_026662 -2.16 0.00756751
AHCTF1: AT hook containing transcription factor 1  NM_015446 -2.16 0.009160004
HHIP: hedgehog interacting protein  NM_002247 -2.17 0.00400885
SLC35C1: solute carrier family 35, member Cl  NM_018389 -2.17 0.03775651
ENDOD1: endonuclease domain containing 1  NM_015036 -2.17 0.036021
C1orf217: chromosome 1 open reading frame 217  BC009988 -2.17 0.019285607
POLDI: polymerase (DNA directed), delta 1  NM_002691 -2.17 0.035966763
C16orf88: chromosome 16 open reading frame 88  BC175626 -2.17 0.004926732
COBRAI: cofactor of BRCA1  NM_015456 -2.17 0.001775062
ANP32A: acidic nuclear phosphoprotein 32 family, member A  ENST00000267918 -2.18 0.031801866
SNORD4A: small nucleolar RNA, C/D box 4A  NR_000010 -2.18 0.012684585
ORC6L: origin recognition complex, subunit 6 like (yeast)  NM_014321 -2.18 0.042147413
PCYOX1: prenylcysteine oxidase 1  NM_016297 -2.18 0.024513574
DNMT1: DNA (cytosine-5-)-methyltransferase 1  NM_001379 -2.18 0.035942748
HIST1H2BI: histone cluster 1, H2b  NM_003525 -2.19 0.026472498
TNFRSF1A: tumor necrosis factor receptor, member 1A  NM_001065 -2.19 0.00120709
DUS1: dihydouridine synthase 1-like (S. cerevisiae)  NM_0022156 -2.19 0.003838316
PCDH18: protocadherin 18  NM_019035 -2.19 0.014273704
CCDC111: coiled-coil domain containing 111  NM_152683 -2.19 0.033015758
HIATL1: hippocampus abundant transcript-like 1  NM_032558 -2.19 0.001849756
MLH1: mutL homolog 1, colon cancer, nonpolyposis type 2  NM_000249 -2.19 0.004129459
TPFI: tissue factor pathway inhibitor  NM_006287 -2.2 0.021208595
ENTPD6: ectonucleoside triphosphate diphosphohydrolase 6  NM_031247 -2.2 0.011633209
EIF5B: eukaryotic translation initiation factor 5B  NM_015904 -2.2 0.010190315
HERC4: hect domain and RLD 4  NM_0022079 -2.2 0.015934335
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JARID2: jumonji, AT rich interactive domain 2
AOF2: amine oxidase (flavin containing) domain 2
LOC644037: hypothetical LOC644037
RPL22L1: ribosomal protein L22-like 1
DNAJC9: DnaJ (Hsp40) homolog, subfamily C, member 9
SH3BP5L: SH3-binding domain protein 5-like
FAR2: fatty acyl CoA reductase 2
CAMKK2: calcium/calmodulin-dependent protein kinase 2
ADAM10: ADAM metallopeptidase domain 10
TUBGCP4: tubulin, gamma complex associated protein 4
GOLGA5: golgi autoantigen, golgin subfamily a, 5
EZH2: enhancer of zeste homolog 2 (Drosophila)
SFXN1: sideroflexin 1
TTC14: tetratricopeptide repeat domain 14
SMURF2: SMAD specific E3 ubiquitin protein ligase 2
GALNT5
GEN1: Gen homolog 1, endonuclease (Drosophila)
FU42986: FLJ42986 protein
THOC3: THO complex 3
CCDC138: coiled-coil domain containing 138
RAD54B: RAD54 homolog B (S. cerevisiae)
SNORA29: small nucleolar RNA, H/ACA box 29
NUP133: nucleoporin 133kDa
CDCA7L: cell division cycle associated 7-like
CANT1: calcium activated nucleotidase 1
CDCA4: cell division cycle associated 4
NDST2: N-deacetylase/N-sulfotransferase 2
NCALD: neurocalcin delta
PRPF4: PRP4 pre-mRNA processing factor 4 homolog (yeast)
ATP1A1: ATPase, Na+/K+ transporting, alpha 1 polypeptide
EXOSC2: exosome component 2
ABCE1: ATP-binding cassette, sub-family E (OABP), member 1
PLAUR: plasminogen activator, urokinase receptor
SAAL1: serum amyloid A-like 1
DOCK5: dedicator of cytokinesis 5
LAMC1: laminin, gamma 1 (formerly LAMB2)
FANCL: Fanconi anemia, complementation group L
PNPT1: polynucleotide nucleotidytransferase 1
GNL3: guanine nucleotide binding protein-like 3 (nucleolar)
ATPB8: ATPase, class I, type 8B, member 1
ZYG11B: zyg-11 homolog B (C. elegans)
SRGAP2: SLIT-ROBO Rho GTPase activating protein 2
SEL1L: sel-1 suppressor of lin-12-like (C. elegans)
LNPEP: leucyl/cystylin aminopeptidase
ST3GAL4: ST3 beta-galactoside alpha-2,3-sialyltransferase 4
ELAC2: elaC homolog 2 (E. coli)
THEX1: three prime histone mRNA exonuclease 1
H2AFZ: H2A histone family, member Z

CPSF3: cleavage and polyadenylation specific factor 3, 73kDa

HDAC2: histone deacetylase 2

EPHB4: EPH receptor B4

VDAC3: voltage-dependent anion channel 3

ANKRD32: ankyrin repeat domain 32

GNL3L: guanine nucleotide binding protein-like 3 (nucleolar)-like

THOC5: THO complex 5

FUT11: fucosyltransferase 11 (alpha (1,3) fucosyl transferase)

WDR3: WD repeat domain 3

GINS3: GINS complex subunit 3 (PsF3 homolog)

ATXN10: ataxin 10

NNT: nicotinamide nucleotide transhydrogenase

LMNB2: lamin B2

LYCAT: lysocardiolipin acyltransferase

PPT1: palmitoyl-protein thioesterase 1

PAQR5: progestin and adipoQ receptor family member V

FAM102B: family with sequence similarity 102, member B

CCBE1: collagen and calcium binding EGF domains 1

CTPS2: CTP synthase II

HISPPD1: histidine acid phosphatase domain containing 1

GPD2: glycerol-3-phosphate dehydrogenase 2 (mitochondrial)

TMEM106C: transmembrane protein 106C

DARS2: aspartyl-tRNA synthetase 2, mitochondrial

MTHFD2: methyleneetetrahydrofolate dehydrogenase 2

EIF3A: eukaryotic translation initiation factor 3, subunit A

SMC3: structural maintenance of chromosomes 3

IP07: importin 7

PCNT: pericentrin

TMPO: thymopoeitin

ALG10: asparagine-linked glycosylation 10 homolog

ETFHD1: electron-transferring-flavoprotein dehydrogenase

UACA

DCBLD1: discoidin, CUB and LCCL domain containing 1

SEPH51: selenophosphate synthetase 1

MCM6: minichromosome maintenance complex component 6

MYBL1: v-mybl myeloblastosis viral oncogene homolog-like 1

MGAT5

HEATR1: HEAT repeat containing 1

METTL7A: methyltransferase like 7A

TNFRSF11B: tumor necrosis factor receptor I lb

DGCR8: DiGeorge syndrome critical region gene 8

SLC1A5: solute carrier family 1, member 5

CYR61: cysteine-rich, angiogenic inducer, 61

VANGL1: vang-like 1 (van gogh, Drosophila)

WIP2: WD repeat domain, phosphoinositide interacting 2

SNORD4B: small nucleolar RNA, C/D box 4B
C18orf55: chromosome 18 open reading frame 55  
RBM25: RNA binding motif protein 25  
SLC1A1: solute carrier family 1, member 1  
PTTG2: pituitary tumor-transforming 2  
SLC27A4: solute carrier family 27, member 4  
TSR1: TSR1, 20S rRNA accumulation, homolog (S. cerevisiae)  
TMED5  
CHMP7: CHMP family, member 7  
MCM2: minichromosome maintenance complex component 2  
EIF4A3: eukaryotic translation initiation factor 4A, isoform 3  
C8orf32: chromosome 8 open reading frame 32  
NAP1L4: nucleosome assembly protein 1-like 4  
RASA1: RAS p21 protein activator (GTPase activating protein) 1  
SNORD5: small nucleolar RNA, C/D box 5  
WNT5B: wingless-type MMTV integration site family, member 5B  
GOLIM4: golgi integral membrane protein 4  
AHCTF1: AT hook containing transcription factor 1  
ANKRD36: ankyrin repeat domain 36B  
ALG8: asparagine-linked glycosylation 8 homolog  
HOOK3: hook homolog 3 (Drosophila)  
STC1: stanniocalcin 1  
RCC2: regulator of chromosome condensation 2  
TMEM109: transmembrane protein 109  
FAM72A: family with sequence similarity 72, member A  
DDX11: DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11  
TGFBR2: transforming growth factor, beta receptor II (70/80kDa)  
ANKRD36B: ankyrin repeat domain 36B  
RPA1: replication protein A, 70kDa  
GLCE: glucuronic acid epimerase  
HMGB2: high-mobility group box 2  
SNORD31: small nucleolar RNA, C/D box 31  
EMP1: epithelial membrane protein 1  
RPN2: ribophorin II  
FAM72A: family with sequence similarity 72, member A  
PKMYT1: protein kinase, membrane associated tyrosine/threonine 1  
TMEM107: transmembrane protein 107  
XYL12: XYL12  
FAM72A: family with sequence similarity 72, member A  
ZC3H13: zinc finger CCCH-type containing 13  
WDH6: WD repeat and HMGB-box DNA binding protein 1  
SNORD73A: small nucleolar RNA, C/D box 73A  
CSE1: CSE1 chromosome segregation 1-like (yeast)  
PRPS2: phosphoribosyl pyrophosphate synthetase 2  
PPP2R3A: protein phosphatase 2, regulatory subunit B*, alpha  
INTS10: integrator complex subunit 10  
NUP88: nucleoporin 88kDa  

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SLC9A6: solute carrier family 9, member 6
LPHN2: latrophilin 2
NUP160: nucleoporin 160kDa
CLC1: chloride channel CLIC-like 1
RFC2: replication factor C (activator 1) 2, 40kDa
FANCG: Fanconi anemia, complementation group G
KIF22: kinesin family member 22
METTL3: methyltransferase like 3
TMTC3: transmembrane and tetratricopeptide repeat containing 3
EFEMP1: EGF-containing fibulin-like extracellular matrix protein 1
ARHGAP19: Rho GTPase activating protein 19
GTF3C2: general transcription factor IIIC, polypeptide 2, beta
ANP32B: acidic nuclear phosphoprotein 32 family, member B
KIF22: kinesin family member 22
OIP5: Opa interacting protein 5
GOLM1: golgi membrane protein 1
POLD3: polymerase (DNA-directed), delta 3, accessory subunit
PD55B: PDS5, regulator of cohesion maintenance, homolog B
GCS1: glucosidase I
SESTD1: SEC14 and spectrin domains 1
SMC6: structural maintenance of chromosomes 6
ITGB3: integrin, beta 3 (platelet glycoprotein IIia, antigen CD61)
LRIG3: leucine-rich repeats and immunoglobulin-like domains 3
AGPAT6: l-acylglycerol-3-phosphate O-acyltransferase 6
STIL: SCL/TAL1 interrupting locus
LOC91431: prematurely terminated mRNA decay factor-like
SERPINH1: serpin peptidase inhibitor, clade H, member 1
MMP3: matrix metallopeptidase 3 (stromelysin 1, progelatinase)
TMEM19: transmembrane protein 19
CPS1: carbamoyl-phosphate synthetase 1, mitochondrial
SNORD113: small nucleolar RNA, C/D box 113-3
ATAD5: ATPase family, AAA domain containing 5
CASP2: caspase 2, apoptosis-related cysteine peptidase
POLR2B: polymerase (RNA) II (DNA directed) polypeptide B
ADPGK: ADP-dependent glucokinase
BZW2: basic leucine zipper and W2 domains 2
SNORA6: small nucleolar RNA, H/ACA box 6
NIN: ninein (GSK3B interacting protein)
KCTD9: potassium channel tetramerisation domain containing 3
MASTL: microtubule associated serine/threonine kinase-like
PLOD2: procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2
BARD1: BRCA1 associated RING domain 1
FANCA: Fanconi anemia, complementation group A
PSMC3IP: PSMC3 interacting protein
CENPM: centromere protein M
ARPC1A: actin related protein 2/3 complex, subunit 1A, 41kDa
TROAP: trophinin associated protein (lastin)
DDX23: DEAD (Asp-Glu-Ala-Asp) box polypeptide 23
AKR1C3: aldo-keto reductase family 1, member C3
HmGCR: 3-hydroxy-3-methylglutaryl-Coenzyme A reductase
ASFI1B: ASF1 anti-silencing function 1 homolog B (S. cerevisiae)
PSRC1: proline-serine-rich coiled-coil 1
ZDHHC6: zinc finger, DHHC-type containing 6
LOXL2: lysyl oxidase-like 2
DPYS2L: dihydropyrimidinase-like 2
TM4SF1: transmembrane 4 L six family member 1
SPTLC2: serine palmitoyltransferase, long chain base subunit 2
MMP14: matrix metalloproteinase 14 (membrane-inserted)
DKK1: dickkopf homolog 1 (Xenopus laevis)
EME1: essential meiotic endonuclease 1 homolog 1 (S. pombe)
NUP155: nucleoporin 155kDa
WDR40A: WD repeat domain 40A
ABCD3: ATP-binding cassette, sub-family D (ALD), member 3
ITGA4: integrin, alpha 4
CENPO: centromere protein Q
APAF1: apoptotic peptidase activating factor 1
RALGPS2: Ral GEF with PH domain and SH3 binding motif 2
WDR4: WD repeat domain 4
PECI: peroxisomal D3,D2-enoyl-CoA isomerase
LEPRE1: leucine proline-enriched proteoglycan (leprecan) 1
C5orf34: chromosome 5 open reading frame 34
FUT8: fucosyltransferase 8 (alpha 1,6 fucosyltransferase)
MCM5: minichromosome maintenance complex component 5
POLR3B: polymerase (RNA) III (DNA directed) polypeptide B
PRG4: proteoglycan 4
CCDC77: coiled-coil domain containing 77
DDX46: DEAD (Asp-Glu-Ala-Asp) box polypeptide 46
FAM20B: family with sequence similarity 20, member B
COL6A3: collagen, type VI, alpha 3
MAD2L1: MAD2 mitotic arrest deficient-like 1 (yeast)
SLC38A1: solute carrier family 38, member 1
CTR9: Ctr9.Paf1/RNA polymerase II complex component
FAM72A: family with sequence similarity 72, member A
CUL3: cullin 3
SMARCC1
MCM4: minichromosome maintenance complex component 4
CCNF: cyclin F
MTHFD1: methylenetetrahydrofolate dehydrogenase 1
DDIT4: DNA-damage-inducible transcript 4
TTF2: transcription termination factor, RNA polymerase II
GINS1: GINS complex subunit 1 (Pcfl homolog)
TFDP1: transcription factor Dp-1
MTAP: methylthioadenosine phosphorylase
SNORD58A: small nucleolar RNA, C/D box 58A
NRP1: neuropilin 1
PTTG1: pituitary tumor transforming 1
ORC1L: origin recognition complex, subunit 1-like (yeast)
NAE1: NEDD8 activating enzyme E1 subunit 1
SNORD96A: small nucleolar RNA, C/D box 96A
SPCS3: signal peptidase complex subunit 3 homolog
CKAP5: cytoskeleton associated protein 5
CSGLCA-T: cholesterol sulfate glucuronyltransferase
ZAK: sterile alpha motif and leucine zipper containing kinase AZK
APOBEC3B
AP1M1: adaptor-related protein complex 1, mu 1 subunit
TIMELESS: timeless homolog (Drosophila)
PDCD4: programmed cell death 4
MAN1A1: mannosidase, alpha, class 1A, member 1
SASS6: spindle assembly 6 homolog (C. elegans)
NUP205: nucleoporin 205kDa
ANKRD36B: ankyrin repeat domain 36B
RAD18: RAD18 homolog (S. cerevisiae)
POPI: processing of precursor 1, ribonuclease P/MRP subunit
TYMS: thymidylate synthetase
SNORA13: small nucleolar RNA, H/ACA box 13
FANCM: Fanconi anemia, complementation group M
DHFR: dihydrofolate reductase
RTTN: rotatin
SUV39H1: suppressor of variegation 3-9 homolog 1 (Drosophila)
DHFR: dihydrofolate reductase
VCP: valosin-containing protein
AKR1C4: aldo-keto reductase family 1, member C4
HAT1: histone acetyltransferase 1
STARD7: STAR-related lipid transfer (START) domain containing 7
EMP2: epithelial membrane protein 2
CHEK1: CHK1 checkpoint homolog (S. pombe)
CNPY4: canopy 4 homolog (zebrafish)
RAD21: RAD21 homolog (S. pombe)
CTSL3: cathepsin L family member 3
MYBL2: v-myb myeloblastosis viral oncogene homolog - like 2
WDR51A: WD repeat domain 51A
GLT8D1: glycosyltransferase 8 domain containing 1
APPL1
ATL3: atlastin 3
BICD2: bicaudal D homolog 2 (Drosophila)  NM_001003800  -3.38  0.016766277
THBD: thrombomodulin  NM_000361  -3.4  0.005386297
C15orf42: chromosome 15 open reading frame 42  NM_152259  -3.4  0.044035721
NAPD3: non-SMC condensin II complex, subunit D3  NM_015261  -3.4  0.000839888
NUP107: nucleoporin 107kDa  NM_020401  -3.41  0.027814664
NEDD1:  NM_152905  -3.41  0.022874072
XRCC2: X-ray repair complementing defective repair cells 2  NM_005431  -3.42  0.04409417
HTATSF1: HIV-1 Tat specific factor 1  NM_014500  -3.42  0.019185925
CDC6: cell division cycle 6 homolog (S. cerevisiae)  NM_001254  -3.42  0.08710068
GSTC2: glutathione S-transferase, C-terminal domain containing  NM_001031720  -3.43  0.025170077
STMN1: stathmin 1/oncoprotein 18  NM_203401  -3.43  0.02312021
CCDC99: coiled-coil domain containing 99  NM_017785  -3.43  0.037055021
LSM2: LSM2 homolog, U6 small nuclear RNA associated  NM_021177  -3.45  0.023713986
LSM2: LSM2 homolog, U6 small nuclear RNA associated  NM_021177  -3.45  0.023713986
LSM2: LSM2 homolog, U6 small nuclear RNA associated  NM_021177  -3.45  0.023713986
NASP: nuclear autoantigenic sperm protein (histone-binding)  NM_172164  -3.46  0.021212208
C6orf73 : chromosome 6 open reading frame 173  NM_001012507  -3.46  0.013358256
HYOU1: hypoxia up-regulated 1  NM_006389  -3.47  0.04139999
GNE: glucosamine (UDP-N-acetyl)-2-epimerase  NM_005476  -3.48  0.0007743
IAKR: aldo-keto reductase, truncated  AB037902  -3.49  0.015434682
MYC: v-myc myelocytomatosis viral oncogene homolog (avian)  NM_002467  -3.49  0.004252963
CDK2: cyclin-dependent kinase 2  NM_001798  -3.49  0.02613663
SUPT16H: suppressor of Ty 16 homolog (S. cerevisiae)  NM_007192  -3.5  0.011056257
LBR: lamin B receptor  NM_002296  -3.51  0.004957337
MRE11A: MRE11 meiotic recombination 11 homolog A  NM_005591  -3.52  0.008653988
RFC5: replication factor C (activator 1) 5, 36.5kDa  NM_007370  -3.52  0.02187458
POLA1: polymerase (DNA directed), alpha 1, catalytic subunit  NM_016937  -3.52  0.003953822
NY-SAR-48: sarcoma antigen NY-SAR-48  NM_033417  -3.54  0.044014998
KATNAL1: katinin p60 subunit A-like 1  NM_01014380  -3.54  0.095783  192
RFWD3: ring finger and WD repeat domain 3  NM_018124  -3.55  0.011324117
CEP78: centrosomal protein 78kDa  NM_001098802  -3.57  0.0016975
MSN: moesin  NM_002444  -3.57  0.009076697
CENPN: centromere protein N  NM_001100624  -3.58  0.033516
DCLRE1B: DNA cross-link repair 1B (PS02 homolog, S. cerevisiae)  NM_022836  -3.59  0.010521683
WHSC1: Wolf-Hirschhorn syndrome candidate 1  NM_133330  -3.6  0.002776674
RGMB: RGM domain family, member B  NM_001012761  -3.61  0.037945236
BIRC5: baculoviral IAP repeat-containing 5  NM_001168  -3.62  0.01082439
DNAE1L3: deoxyribonuclease I-like 3  NM_004944  -3.65  0.00118208
LRG4: leucine-rich repeat-containing G protein-coupled receptor 4  NM_018490  -3.65  0.007489299
C6orf67: chromosome 6 open reading frame 167  NM_198468  -3.65  0.014707124
PENK: proenkephalin  NM_000621  -3.66  0.036826888
FBN1: fibrillin 1  NM_000138  -3.66  0.040182037
CDC55: cell division cycle associated 5  NM_0080668  -3.67  0.006490483
IL13RA2: interleukin 13 receptor, alpha 2  NM_000640  -3.68  0.033161  163
SGK1: serum/glucocorticoid regulated kinase 1  NM_005627  -3.69  0.015425347
CENPJ: centromere protein J  NM_018451  -3.7  0.042173402
TPR: translocated promoter region (to activated MET  NM_003292  -3.7  0.027668604
oncogene)
AKR1C1: aldo-keto reductase family 1, member C1
-3.71 0.02354857
RBBP8: retinoblastoma binding protein 8
-3.73 0.027806512
CEP97: centrosomal protein 97kDa
-3.73 0.011707226
ZC3HAV1: zinc finger CCCH-type, antiviral 1
-3.74 0.043626293
CDCA7: cell division cycle associated 7
-3.75 0.016859814
KIF2A: kinesin heavy chain member 2A
-3.75 0.013671485
ITGB3BP: integrin beta 3 binding protein (beta3-endonexin)
-3.75 0.014900666
FAM29A: family with sequence similarity 29, member A
-3.76 0.006894399
EROIL: EROI-like (S. cerevisiae)
-3.77 0.004412076
ATL3: atlastin 3
AK090822 -3.77 0.026740981
GLB1: galactosidase, beta 1
NM_000404 -3.77 0.037346081
UBASH3B: ubiquitin associated and SH3 domain containing, B
NM_001 102398 -3.78 0.021443634
HIST2H2AB: histone cluster 2, H2ab
NM_175065 -3.8 0.015412 178
KCTD20: potassium channel tetramerisation domain containing 20
NM_173562 -3.81 0.009173 158
GINS4: GINS complex subunit 4 (Sld5 homolog)
NM_002336 -3.81 0.009179253
RRM1: ribonucleotide reductase M1
NM_001033 -3.82 0.010380785
KPNB1: karyopherin (importin) beta 1
NM_002265 -3.82 0.031057362
DIAPH3: diaphanous homolog 3 (Drosophila)
NM_001042517 -3.82 0.005890863
DTL: denticless homolog (Drosophila)
NM_016448 -3.83 0.017101 198
IMCSTN: nicastrin
NM_015331 -3.84 0.000433316
DOCK10: dedicator of cytokinesis 10
NM_014689 -3.85 0.008213513
ANP3E2: acidic nuclear phosphoprotein 32 family, member E
NM_030920 -3.86 0.032209315
CKAP2L: cytoskeleton associated protein 2-like
NM_152515 -3.87 0.02341 1908
BRCA2: breast cancer 2, early onset
NM_000059 -3.87 0.002128925
CDCC5B: cell division cycle 25 homolog B (S. pombe)
NM_021873 -3.9 0.00307721 1
DDIT4L: DNA-damage-inducible transcript 4-like
NM_145244 -3.94 0.019049984
OLFML2B: olfactomedin-like 2B
NM_015441 -3.94 0.003377518
CD9: CD9 molecule
NM_001769 -3.95 0.008545553
COSAB1: CDC28 protein kinase regulatory subunit IB
NM_01826 3.95 0.006037425
CCDC80: coiled-coil domain containing 80
NM_19951 1 -3.95 0.006608983
K51B: CDC28 protein kinase regulatory subunit IB
NM_01826 -3.96 0.005028013
DKC1: dyskeratosis congenita 1, dyskerin
NM_001363 -3.99 0.027694764
MMP1: matrix metalloproteinase 1 (interstitial collagenase)
NM_002241 -4 0.014418235
FAM54A: family with sequence similarity 54, member A
NM_001099826 -4.03 0.006364463
FAM29A: family with sequence similarity 29, member A
NM_017645 -4.05 0.014763219
VRK1: vaccinia related kinase 1
NM_003384 -4.05 0.022044795
TDP1: tyrosyl-DNA phosphodiesterase 1
NM_018319 -4.05 0.025305827
HERPUD1
NM_014685 -4.07 0.013120239
CDCC2E: cell division cycle 25 homolog C (S. pombe)
NM_001790 -4.08 0.024886353
WEE1: WEE1 homolog (S. pombe)
NM_003390 -4.09 0.014041834
MCM7: minichromosome maintenance complex component 7
NM_005916 -4.13 0.024734886
C10orf54: chromosome 10 open reading frame 54
NM_173529 -4.16 0.036459394
IQGAP3: IQ motif containing GTPase activating protein 3
NM_178229 -4.18 0.000379041
KRT34: keratin 34
NM_021013 -4.18 0.014644898
CHAF1B: chromatin assembly factor 1, subunit B (p60)
NM_005441 -4.19 0.005348677
ZWILCH: Zwilch, kinetochore associated, homolog
NR_003105 -4.19 0.003365255
RFC4: replication factor C (activator 1) 4, 37kDa  
WEE1: WEE1 homolog (S. pombe)  
POLA2: polymerase (DNA directed), alpha 2 (70kD subunit)  
CYP2A41: cytochrome P450, family 24, subfamily A, polypeptide 1  
CEP55: centromere protein 0  
SLC7A11: solute carrier family 7  
SPATA5: spermatogenesis associated 5  
ARHGAP11B: Rho GTPase activating protein 11B  
GTSE1: G-2 and S-phase expressed 1  
MCM3: minichromosome maintenance complex component 3  
MSH2: mutS homolog 2, colon cancer, nonpolyposis type 1  
WEE1: WEE1 homolog (S. pombe)  
C4orf21: chromosome 4 open reading frame 21  
FST: follistatin  
MALL: mal, T-cell differentiation protein-like  
MLF1P1: MLF1 interacting protein  
MATN2: matrin 2  
RECK: reversion-inducing-cysteine-rich protein with kazal motifs  
SKP2: S-phase kinase-associated protein 2 (p45)  
REEP4: receptor accessory protein 4  
NID2: nidogen 2 (osteonidogen)  
ECT2: epithelial cell transforming sequence 2 oncogene  
HIST1H3B: histone cluster 1, H3b  
CDC8: cell division cycle associated 8  
NUP93: nucleoporin 93kDa  
AURKB: aurora kinase B  
TEK: TEK tyrosine kinase, endothelial  
HSP/A8: heat shock 70kDa protein 8  
ISLR: immunoglobulin superfamily containing leucine-rich repeat  
CSC45L: CDC45 cell division cycle 45-like (S. cerevisiae)  
BRIP1: BRCA1 interacting protein C-terminal helicase 1  
SLC38A2: solute carrier family 38, member 2  
C15orf23: chromosome 15 open reading frame 23  
UBE2C: ubiquitin-conjugating enzyme E2C  
SHCBP1: SHC SH2-domain binding protein 1  
MPOPHOSPH9: M-phase phosphoprotein 9  
CTSL1: cathepsin Li  
MAT2A: methionine adenosyltransferase II, alpha  
GALNT2: UDP-N-acetyl-alpha-D-galactosamine  
HSP54: heat shock 70kDa protein 5  
ALG6: asparagine-linked glycosylation 6 homolog  
TNC: tenascin C  
KNTC1: kinetochore associated 1  
POLQ: polymerase (DNA directed), theta  
ADAMTS1: ADAM metalloproteinase with thrombospondin type 1  
FANCI: Fanconi anemia, complementation group I
PDIA5: protein disulfide isomerase family A, member 5
TCF19: transcription factor 19 (SC1)
PARP1: poly (ADP-ribose) polymerase 1
ANPEP: aminopeptidase
CENPK: centromere protein K
TCF19: transcription factor 19 (SC1)
TCF19: transcription factor 19 (SC1)
NEIL3: nei endonuclease VHI-like 3 (E. coli)
ITGA5: integrin, alpha 5 (fibronectin receptor, alpha polypeptide)
KDEL1: KDEL (Lys-Asp-Glu-Leu) containing 1
FIBIN: fin bud initiation factor
ZWINT: ZW10 interactor
HIST1H1A: histone cluster 1, H1a
ATAD2: ATPase family, AAA domain containing 2
TOPBP1: topoisomerase (DNA) II binding protein 1
TMEM48: transmembrane protein 48
NUP85: nucleoporin 85kDa
IMTSE: 5'-nucleotidase, ecto (CD73)
ERCC6L: excision repair cross-complementing repair deficiency
KIF15: kinesin family member 15
SEMA7A: semaphorin 7A, GPI anchor protein
SMC1A: structural maintenance of chromosomes 1A
BUBIB: budding uninhibited by benzimidazoles 1 homolog beta
PODXL: podocalyxin-like
RACGAP1: Rac GTPase activating protein 1
CDC7: cell division cycle 7 homolog (S. cerevisiae) protein
SNORD45C: small nuclear RNA, C/D box 45C
AmD1: adenosylmethionine decarboxylase 1
RADS1A: RAD51 associated protein 1
KIF18A: kinesin family member 18A
PDGFRB: platelet-derived growth factor receptor beta
CKAP2: cytoskeleton associated protein 2
FANCD2: Fanconi anemia, complementation group D2
RBL1: retinoblastoma-like 1 (p107)
DEPDC1B: DEP domain containing 1B
MCM8: minichromosome maintenance complex component 8
KIF2C: kinesin family member 2C
UBE2T: ubiquitin-conjugating enzyme E2T (putative)
BRCA1: breast cancer 1, early onset
CDC3: cell division cycle associated 3
PLAT: plasminogen activator, tissue
SMC2: structural maintenance of chromosomes 2
KIAA1524: KIAA1524
NCAPD2: non-SMC condensin I complex, subunit D2
GPSM2: G-protein signaling modulator 2 (AGS3-like, C. elegans)
CLSPN: claspin homolog (Xenopus laevis)
C13orf3: chromosome 13 open reading frame 3
RFC3: replication factor C (activator 1) 3, 38kDa
C18orf24: chromosome 18 open reading frame 24
NM_001039535 -6.19 0.039810882

TACC3: transforming, acidic coiled-coil containing protein 3
NM_006342 -6.19 0.017952851

C14orf145: chromosome 14 open reading frame 145
NM_152446 -6.22 0.007797356

NEK2: NIMA (never in mitosis gene a)-related kinase 2
NM_002497 -6.33 0.002681177

NUSAP1: nucleolar and spindle associated protein 1
NM_016359 -6.33 0.049437931

HIST1H2BM: histone cluster 1, H2bm
NM_003521 -6.4 0.029910293

HIST1H1E: histone cluster 1, Hie
NM_005321 -6.41 0.004360528

PRIM1: primase, DNA, polypeptide 1 (49kDa)
NM_000946 -6.42 0.002907531

AURKA: aurora kinase A
NM_198433 -6.49 0.033564734

HJURP: Holliday junction recognition protein
NM_018410 -6.66 0.000926767

CEP55: centrosomal protein 55kDa
NM_018131 -6.68 0.032269904

DBX2: developing brain homeobox 2
ENST00000332700 -6.84 0.047533804

KIF4B: kinesin family member 4B
NM_001099293 -6.85 0.02993245

CDC20: cell division cycle 20 homolog (S. cerevisiae)
NM_001255 -6.89 0.021541425

HELLS: helicase, lymphoid-specific
NM_018063 -6.91 0.028245951

KIFC1: kinesin family member C1
NM_002263 -6.98 0.033788145

NCAPH: non-SMC condensin I complex, subunit H
NM_015341 -7.09 0.025734154

TRIP13: thyroid hormone receptor interactor 13
NM_004237 -7.1 0.002615203

RAD51: RAD51 homolog (RecA homolog, E. coli) (S. cerevisiae)
NM_002875 -7.11 0.023887145

KIF11: kinesin family member 11
NM_004523 -7.15 0.031473957

PLK4: polo-like kinase 4 (Drosophila)
NM_014264 -7.15 0.046052773

KIFC1: kinesin family member C1
NM_002263 -7.2 0.033701678

SPAG5: sperm associated antigen 5
NM_006461 -7.32 0.021107196

RNU5F: RNA, USF small nuclear
M77840 -7.33 0.032317221

ARS1: arylsulfatase family, member 1
NM_001012301 -7.39 0.024370272

PRC1: protein regulator of cytokinesis 1
NM_003981 -7.48 0.007423876

RRM2: ribonucleotide reductase M2 polypeptide
NM_001034 -7.58 0.000735057

CCNA2: cyclin A2
NM_001237 -7.72 0.020045463

KIF20B: kinesin family member 20B
NM_016195 -7.73 0.042973972

CENPE: centromere protein E, 312kDa
NM_001813 -7.74 0.045689356

CDCA2: cell division cycle associated 2
NM_152562 -7.78 0.002339029

HIST1H1B: histone cluster 1, H1b
NM_005322 -7.82 0.01239215

KIAA0101: KIAA0101
NM_014736 -7.95 0.004181814

KIF14: kinesin family member 14
NM_014875 -8.25 0.008367918

NCAPG2: non-SMC condensin II complex, subunit G2
NM_017760 -8.36 0.018978836

ITGA2: integrin, alpha 2 (CD49B)
NM_002203 -8.43 0.013749967

PRR11: proline rich 11
NM_018304 -8.47 0.007284343

KPN2A: karyopherin alpha 2 (RAG cohort 1, importin alpha 1)
NM_002266 -8.87 0.006498917

KPN2A: karyopherin alpha 2 (RAG cohort 1, importin alpha 1)
NM_002266 -9.02 0.006274906

NCAP: non-SMC condensin I complex, subunit G
NM_002234 -9.23 0.008471132

CDC2: cell division cycle 2, GI to S and G2 to M
NM_001786 -9.27 0.047076112

NDC80: NDC80 homolog, kinesinchore complex component
NM_006101 -9.28 0.038380426

EXOI: exonuclease 1
NM_130398 -9.52 0.003215089

LMNB1: lamin B1
NM_005573 -9.53 0.006541627

KIF23: kinesin family member 23
NM_138555 -9.57 0.013547107

CASC5: cancer susceptibility candidate 5
NM_170589 -9.8 0.002491149

CCNB2: cyclin B2
NM_004701 -9.81 0.034290952

KIF4A: kinesin family member 4A
NM_012310 -9.84 0.001147293
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TABLE 4

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* Calculation of value: (Total cell number X frequency of CD45+ cells)
** Calculation of value: (Total cell number X frequency of CD34+CD45+ cells)

Calculation of dermal patch size required to achieve full hematopoietic reconstitution:
- A 60 kg individual will need $1.5 \times 10^8$ CD34+ve cells
- Skin puncture of 6 mm in diameter has $1.0 \times 10^7$ cells
- Per 10,000 Fibs initially plated there are ~21,000 CD34+CD45+ cells
- Therefore, number of Fibs needed to get $1.5 \times 10^8$ CD34+ve cells
  \[ = \frac{(10,000 \times 1.5 \times 10^8)}{(21,000)} \]
  \[ = 7.14 \times 10^7 \text{ Fibs} \]
- Thus, 7.1 skin punctures are needed equaling to 42.84 mm (7.14 x 6 mm) diameter skin patch.
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<td>GAGAAAGCGAGAGCTCTCGCISEQ (SEQ ID NO:38)</td>
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<td>CACACTTGGTGGTGGTGGTSEQ (SEQ ID NO:54)</td>
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<td>PU.1 Promoter</td>
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<td>Myf5 Promoter</td>
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<td>ACAACCTTTGTGGTGGTSEQ (SEQ ID NO:58)</td>
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<td>Pol2ra Promoter</td>
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<td>CCCGGGAAAGGGCGGTGSEQ (SEQ ID NO:60)</td>
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<td>Gadd45a Promoter</td>
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<td>TTATCGTCAGGAAGGCGGSEQ (SEQ ID NO:62)</td>
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<tr>
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<td>S: ACGATTTCAGTTAAATCCTGGCCT (SEQ ID NO:73)</td>
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<tr>
<td>A: GACCACATCCTTCTCGAGCC (SEQ ID NO:6)</td>
<td>A: ATAGTATGGGCAAGCAGGGAGCTA (SEQ ID NO:74)</td>
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<td><strong>NANOG</strong></td>
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<td>S: CGAAGAATAGCAATGGGCTGACG (SEQ ID NO:7)</td>
<td>S: TTAGAAGGCAGATAGAGCGACCTGACC (SEQ ID NO:35)</td>
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<tr>
<td>A: TTCCAAAGCAGCCTTCAAGTC (SEQ ID NO:8)</td>
<td>A: TGCCTGTCTTGTGAGGGATGATGTT (SEQ ID NO:36)</td>
</tr>
<tr>
<td><strong>Sox2</strong></td>
<td><strong>Nanog</strong></td>
</tr>
<tr>
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<td>S: GACTGAGCTGTTGCGCTCAT (SEQ ID NO:75)</td>
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<tr>
<td>A: CGGATATAACATGGCAATCAAATG (SEQ ID NO:10)</td>
<td>A: GGCAGCTTTAAGACTTTTCTTGG (SEQ ID NO:76)</td>
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<td><strong>Tbx3</strong></td>
<td><strong>Sox2</strong></td>
</tr>
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<tr>
<td>A: CGCTGGGACATAAAATCTTGA (SEQ ID NO:66)</td>
<td>A: CAAAGTTTTTCTTTATTGTCATGTCGACG (SEQ ID NO:78)</td>
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<td><strong>Dppa4</strong></td>
<td><strong>Brachury</strong></td>
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<td>A: AAGGCACACAGCGGCTTA (SEQ ID NO:68)</td>
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<tr>
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<td>A: TCCTCGTTTCTGAAGACGTCA (SEQ ID NO:24)</td>
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References:


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fibroblasts to induced pluripotent stem cells with chemical
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Claims:

1. A method of generating progenitor cells from fibroblasts comprising:
   a) providing fibroblasts that express or are treated with a POU domain containing gene or protein; and
   b) culturing the cells of step (a) under conditions that allow production of progenitor cells without traversing the pluripotent state.

2. The method of claim 1, wherein fibroblasts that express a POU domain containing gene or protein in step (a) are produced by lentiviral transduction.

3. The method of claim 1, wherein fibroblasts treated with a POU domain containing gene or protein comprises providing an exogenous POU domain containing gene or protein.

4. The method of any one of claims 1 to 3, wherein the POU domain containing gene or protein is an Oct gene or protein.

5. The method of claim 4, wherein the Oct gene or protein is Oct-1, -2, -4 or -11.

6. The method of claim 5, wherein the Oct gene or protein is Oct-4.

7. The method of any one of claims 1 to 6, wherein the conditions that allow production of progenitor cells comprise a colony forming assay for a culture period of up to 25 days.

8. The method of any one of claims 1-7, further comprising culturing the cells produced in step (b) in differentiation medium under conditions that allow production of differentiated cells.

9. The method of claim 8, wherein the differentiation medium comprises hematopoietic medium comprising at least one hematopoietic cytokine.

10. The method of claim 9, wherein the at least one hematopoietic cytokine is Flt3 ligand and/or SCF.
11. The method of claim 10, wherein the differentiated hematopoietic cells are of the myeloblast lineage.

12. The method of claim 11, wherein the myeloblast lineage cells are monocytes or granulocytes.

13. The method of claim 10 or 11, wherein the at least one hematopoietic cytokine is EPO.

14. The method of claim 13, wherein the differentiated hematopoietic cells are of the erythroid or megakaryocytic lineage.

15. The method of claim 8, wherein the differentiation medium comprises neural medium comprising fibroblast growth factor, epidermal growth factor, insulin growth factor II, bone morphogenetic factor 4, bFGF, the N-terminal active fragment of human SHH, FGF8, GDNF, BDNF and/or fetal bovine serum.

16. The method of claim 15, wherein the differentiated neural cells are neurons or glial cells.

17. The method of claim 16, wherein the neurons are dopaminergic neurons.

18. The method of claim 16, wherein the glial cells are astrocytes or oligodendrocytes.

19. The method of any one of claims 1 to 18, wherein the fibroblasts are dermal fibroblasts.

20. Isolated progenitor or differentiated cells generated by the method of any one of claims 1-19.

21. A use of the cells according to claim 20 for engraftment or cell replacement in a subject in need thereof.
22. The use of claim 21 for autologous or non-autologous transplantation in a subject in need thereof.

23. The use of claim 21 or 22, wherein the subject is a human.

24. A use of the hematopoietic cells according to claim 20 as a source of blood, cellular and acellular blood components, blood products or hematopoietic stem cells.

25. A method of screening progenitor or cells derived therefrom comprising
   a) preparing a culture of progenitor or differentiated cells by the method of any one of claims 1 to 19;
   b) treating the cells with a test agent or agents; and
   c) subjecting the cells to analysis.

26. A method of isolating a subpopulation of fibroblasts with increased reprogramming potential comprising
   a) providing fibroblasts that express an Oct-4-reporter; and
   b) isolating cells positive for the reporter.

27. The method of claim 26, wherein the fibroblasts that express the Oct-4-reporter are produced by lentiviral transduction.

28. The method of claim 26 or 27, wherein the reporter gene comprises a fluorescent protein and the cells are isolated in step (b) by detection of the protein under fluorescence.

29. The method of claim 26 or 27, wherein the reporter gene encodes a gene conferring antibiotic resistance and the cells are isolated by survival in the presence of antibiotic.

30. The method of any one of claims 26 to 29, wherein the fibroblasts are dermal fibroblasts.
31. A method of generating reprogrammed fibroblast-derived induced pluripotent stem cells comprising
   a) providing (i) a population of fibroblasts with increased expression Oct-4 and (ii) a mixed population or OCT-4 negative population of fibroblasts;
   b) treating the fibroblasts of a) with Oct-4, Sox-2, Nanog and Lin-28;
   c) culturing the fibroblasts of b) under conditions that allow production of iPS cells.

32. The method of claim 31, wherein the fibroblasts of b) are treated by introducing Oct-4, Sox-2, Nanog and Lin-28 genes via lentiviral transduction.

33. The method of claim 31 or 32, further comprising analyzing and selecting cells that express TRA-1-60 and/or SSEA-3 and or any other pluripotency marker.

34. The method of any one of claims 31 to 33, wherein the population of fibroblasts with increased expression of Oct-4 in a) i) are produced by the method of any one of claims 26-30.

35. The method of any one of claims 31 to 34, wherein the ratio of cells in a) i) to a) ii) is 50:50.

36. The method of any one of claims 31 to 34, wherein the ratio of cells in a) i) to a) ii) is 10:90.

37. The method of any one of claims 31 to 36 wherein the fibroblasts are dermal fibroblasts.

38. Isolated induced pluripotent stem cells generated by the method of any one of claims 31 to 37 or cells differentiated therefrom.

39. A use of the cells according to claim 38 for engraftment in a subject in need thereof.
40. The use of claim 39 for autologous or non-autologous transplantation in a subject in need thereof.

41. The use of claim 39 or 40, wherein the subject is a human.

42. A use of the cells according to claim 38 as a source of induced pluripotent stem cells or cells differentiated therefrom.

43. A method of screening induced pluripotent stem cells or cells differentiated therefrom comprising
   a) preparing a culture of induced pluripotent stem cells by the method of any one of claims 31 to 37 or cells differentiated therefrom;
   b) treating the cells with a test agent or agents; and
   c) subjecting the treated cells to analysis.
Figure 2

(a) Fibroblast specific marker expression

(b) Adult vs Fetal

(c) Total Colony Number

(d) Pluripotency signature

CD45^+Fibs Oct-4 1.1
CD45^+Fibs Oct-4 1.2
Fibs 1.1
Fibs 1.2
Figure 6 cont'd
Figure 7 cont'd

![Bar charts and graphs illustrating gene expression and binding sequences]

**Octomer (POU domain) binding sequences**

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<th>Predicted</th>
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**Hematopoietic**

- ATGCAAN
- Runx1
- NTGCAAN
- SCL
- ATGCAAN
- PU.1
- MixL1
- ATGCAAN
- GATA1
- TTGCAAT
- CD45

**Non-hematopoietic**

- Fbs (D0)
- Day 4 Fbs Oct-4 (D4)
- CD45+ Fbs Oct-4 (D21)
- iPSC
- Gadd45
- NTGCANAT
- Pol2a
- MyoD
- NTGCANAT
- Nkx2.5

**Pluripotency**

- Fbs (D0)
- Day 4 Fbs Oct-4 (D4)
- CD45+ Fbs Oct-4 (D21)
- iPSC
- Oct4
- NTGCANAT
- Nanog
- NTGCANAT
- Tbx3
- c-Myc

**Relative Oct-4 occupancy**

- 0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0

---

**Relative Signal Intensity**

- 10^0
- 10^1
- 10^2
- 10^3
- 10^4
Figure 9

a

hiPSC1  hiPSC2  hiPSC3  hiPSC4

b

colony

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Log scale:

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d

Relative expression

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e

Adult Fibs  CD45+

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Figure 10

a

Lentivirus transduction

fibroblasts (Adult/Fetal)

Oct-4
Nanog
Sox-2

fibroblasts

1. F12 medium +IGFII+bFGF

2. F12 medium +IGFII+bFGF+SCF+Flt3

hematopoietic (CD45+) colonies

Day 0

Day 3

Day 10

Day 21
(Day 14 to 21)

b

hiPSC control
Fibs control
FibsOct-4
FibsNanog
FibsSox2
Fibs
FibsOct-4

Adult
Fetal

656 bp
Provirus

10^4
Relative Signal Intensity

10^3

732 bp
Nanog
Provirus

10^2

467 bp
Sox-2
Provirus

10^1

1

Fibs (D0)

CD45+ FibsOct-4 (D21)

POUSF1P4
POUSF1P3
POUSF1
POUSF1P1
POUSF1
POUSF1
Figure 12

(a) Relative Signal Intensity

(b) Relative Signal Intensity

(c) Fibrils (Fibs), Day 4 (FibsOct-4), CD45+ (FibsOct-4 (21))

(d) Fibrils (Fibs), Day 4 (FibsOct-4), CD45+ (FibsOct-4 (21))

(e) SSEA3

Tra-1-60
Figure 14

a) Global gene analysis

b) Hematopoietic genes

c) Relative Growth Rate of CD45*Fibs^Oct-4

- CD45*Fibs^Oct-4 (D21)
- CD45*Fibs^Oct-4 + hematopoietic cytokines (D37)
- CD45*Fibs^Oct-4 + hematopoietic cytokines (D37)

D21 D37

Days 21 37

Cell Viability (% of TAD x 100)

- CD45*Fibs^Oct-4 (D21)
- CD45*Fibs^Oct-4 - hematopoietic cytokines (D37)
- CD45*Fibs^Oct-4 + hematopoietic cytokines (D37)
Figure 15

18/46
Figure 17

(a) Adult CD45^+ FibsOct-4 (D37) vs. CD45 and CD14

(b) Fetal CD45^+ FibsOct-4 (D37) vs. SSC and CD15

(c) CD45^+ FibsOct-4 (D37) vs. SSC and CD15

Granulocytes

n=3

46.9±11.72

17.82±6.28

20X

40X

40X

20/46
Figure 18

a  Myeloid Cells

b  Monocytic Lineage

CD45^+ Fibs^Oct-4 - hematopoietic cytokines (D37)
Figure 19

Fetal CD45+Fibroblast Oct-4 (D37)
Hematopoietic Progenitors

n=3

22.2±8.21

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Figure 20

a) CFU

b) Unstained engrafted cells

Δ CFUs from engrafted cells

41.3 ± 2.96

CD45

CD14

CD15

CFUs from engrafted CD45+FibsOct-4 (D37)
Figure 24

a

b

CD45^+ Fibs^Oct-4 Day 16 post isolation (D37)

Meso  Hematopoietic  Pluripotency

C/EBP^+  Sox2  Nur77

Pluripotency

CD45^+ Fibs^Oct-4

D21  D37

HBB  HBD  HBA

Relative expression
Figure 25

a

Positive control vector (pGK-EGFP)

Negative control vector

C3+ EOS (Trimer CR4 En-EGFP)

b

hESC

Adult derived hFibs-(breast)

hESC

C3+ EOS

hFibs-(breast)

pGK-EGFP

Control Vector

C3+EOS

3.6

30.1

57.4

c

d

Transduced with C3+ EOS

Neonatal derived hFibs-(Foreskin)

Adult derived hFibs-(Lung)

f

Total hFibs

% C3+ EOS

GFP+ve cells

4

3

2

1

0

e

GFP+ve hFibs

Transduced with C3+EOS

Phase

GFP

Phase

GFP

Phase

GFP

Phase

GFP

g

mRNA

100 bp

GFP Expression

GAPDH Expression

h

Oct4 Transduction

GFP

GFP
Figure 29

(a) Average no. of colonies per 10^4 cell input

(b) Total hFibs

(c) De Novo Isolated NOS^{exp} hFibs

(d) Cultured NOS^{exp} hFibs

Phase | GFP | Phase | GFP | Phase | GFP

Alone | + Total hFibs | + MEFs
Figure 29 cont'd

e) Cultured NOS+exp hFibs

f) Cultured NOS+exp hFibs

+ Total hFibs + MEFs

Relative Histone Modifications

Nanog Odt4 Sox2 Nanog Odt4 Sox2 Nanog Odt4 Sox2

Relative Gene Expression

H3K4Me3 H3K27Me3 H3K4Me3 H3K27Me3 H3K4Me3 H3K27Me3

- MEFs - MEFs - MEFs - MEFs

Wks 0 - 3 Wks 0 - 3 Wks 0 - 3 Wks 0 - 3

Wks 3 - 6 Wks 3 - 6 Wks 3 - 6 Wks 3 - 6

iPS colonies iPS colonies

NOS+exp

NOS-exp

+ MEFs + MEFs + MEFs + MEFs

Wks 0 - 3 Wks 0 - 3 Wks 0 - 3 Wks 0 - 3

Wks 3 - 6 Wks 3 - 6 Wks 3 - 6 Wks 3 - 6

iPS colonies iPS colonies

- MEFs - MEFs - MEFs - MEFs

Wks 0 - 3 Wks 0 - 3 Wks 0 - 3 Wks 0 - 3

Wks 3 - 6 Wks 3 - 6 Wks 3 - 6 Wks 3 - 6

iPS colonies iPS colonies
Figure 30

(a) Schematic representation of the gene expression profiles in different cell types.

(b) Gene expression heatmap showing the expression levels of various genes in different cell lines.

(c) Gene expression bar chart indicating the cellular metabolic process.

(d) Gene expression heatmap showing cell cycle and differentiation.

(e) Gene expression heatmap showing the regulation of biological processes.

(f) Gene expression heatmap showing the percentage of growth rate.

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Figure 32

a) pGK Control
b) C3+ EOS
c) Graphical representation

d) C3+ EOS

e) Graphical representation

f) Graphical representation

g) Graphical representation

h) Graphical representation

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Figure 33

(a) 

Phase
Total hFibs
Phase
293 (negative control)
Phase
293+Oct4 (positive control)

(b) 

MeDip PCR-Oct4 Promoter

Relative DNA enrichment

293
Med
NOS-exp
NOS-exp
a Hematopoietic EB differentiation

Undifferentiated Control

hESC

iPSC

Pan hematopoietic marker (CD45)

b Neural EB differentiation

hESC

iPSC

Neuronal marker (A2B5)
Figure 36
Figure 38

a) Lentivirus transduction of OCT4 into fibroblasts (adult/fetal) results in neural differentiation media leading to astrocytes and oligodendrocytes. Oligodendrocytes are observed at day 10 to 14.

b) Neural differentiation media with BMP4 can convert fibroblasts into astrocytes and oligodendrocytes/neurons.

c) Immunofluorescence staining for GFAP and DAPI.

d) Quantification of GFAP expression, showing fibroblasts and fibroblasts/OCT4 with a value of 24.6±10.47.

efgh) Immunofluorescence staining for β-Tubulin III and DAPI.

ig) Immunofluorescence staining for Olig-2 and DAPI.
Figure 39

Tyrosin Hydroxylase (TH)

DAPI

β-Tubulin III

TH/β-Tubulin III/DAPI

- Oct-4 Lentivirus transduction
- Neural Precursor media
- Dopaminergic differentiation media

Day 0
Fibs (Adult/Fetal)

Day 3
Fibs

Day 10 to 14
Neuron

Day 21

Day 35
DOPA neurons
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC: C12N 5/071 (2010.01), A61K 35/12 (2006.01), C12N 15/00 (2006.01), C12Q 1/02 (2006.01), C12Q 1/68 (2006.01), C12N 15/867 (2006.01)

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)
C12N 5/071 (2010.01), A61K 35/12 (2006.01), C12N 15/00 (2006.01), C12Q 1/02 (2006.01), C12Q 1/68 (2006.01), C12N 15/867 (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Canadian Patent Database, TotalPatent, Scopus, NCBI PubMed database, Esp@cenet, Google Patents. Key Words: POU domain; OCT gene; pluripotent; progenitor cells; differentiation; fibroblast; hematopoietic; reprogramming; transplantation, and combinations thereof.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tbody>
<tr>
<td>A</td>
<td>PATEL, M. et al. Advances in reprogramming somatic cells to induced pluripotent stem cells. Stem Cell Reviews. September 2010 (09-2010); Volume 6 (3), pages 367-380. ISSN: 1550-8943.</td>
<td>1-43</td>
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[X] See patent family annex.

Date of actual completion of the international search: 10 February 2011 (10-02-2011)

Date of mailing of the international search report: 4 March 2011 (04-03-2011)

Adnan Ali, Ph.D. (819) 934-7930

Form PCT/ISA/210 (second sheet) (July 2009)
**Box No. I  Nucleotide and/or amino acid sequence(s) (Continuation of item l.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)
      
      [X ] on paper
      [X ] in electronic form

   b. (time)
      
      [X ] in the international application as filed
      [X ] together with the international application in electronic form
      [ ] subsequently to this Authority for the purposes of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:  

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