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(54) **Title:** METHODS FOR REPROGRAMMING A SOMATIC CELL

(57) **Abstract:** The invention provides methods for enhancing reprogramming in a somatic cell comprising the step of exposing the cell to a miRNA. The invention also provides methods for reprogramming a somatic cell comprising the step of exposing the cell to a miRNA and one, two or more reprogramming factors.



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METHODS FOR REPROGRAMMING A SOMATIC CELL

FIELD OF THE INVENTION

The invention relates to methods for enhancing reprogramming in a somatic cell comprising the step of exposing the cell to a miRNA. The invention also relates to
5 methods for reprogramming a somatic cell comprising the step of exposing the cell to a miRNA and one, two or more reprogramming factors.

BACKGROUND OF THE INVENTION

Reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) can be achieved by the expression of the transcription factors OCT4, SOX2, KLF4, and cMYC
10 (Takahashi and Yamanaka, 2006, *Cell* 126(4): 663-76). Reprogramming strategies based on doxycycline-inducible lentivirus, which facilitate temporal control in the expression of the reprogramming factors, have been recently developed in the art. These reprogramming systems, based on doxycycline-inducible lentivirus expressing the reprogramming factors, represented a step toward further understanding the mechanisms
15 governing cell reprogramming (Brambrink *et al.*, 2008, *Cell Stem Cell* 2(2): 151-59; Stadtfeld *et al.*, 2008, *Cell Stem Cell* 2(3): 230-40; Stadtfeld *et al.*, 2010, *Nat. Methods* 7(1): 53-55; Wernig *et al.*, 2008, *Nat. Biotechnol.* 26(8): 916-24; Markoulaki *et al.*, 2009, *Nat. Biotechnol.* 27(2): 169-71; Hockemeyer *et al.*, 2008, *Cell Stem Cell* 3(3): 346-53; Maherali *et al.*, 2008, *Cell Stem Cell* 3(3): 340-45). These reprogramming systems have
20 been used as powerful tools for screening in mouse cells.

Mouse induced pluripotent stem cells (miPSCs) colonies were initially selected based on the neomycin resistance provided by the reactivation of the Fbx15 promoter (Takahashi and Yamanaka). Although these miPSCs contributed to all three germ layers after injection into blastocysts, no live chimeric mice were obtained, probably due to the
25 incomplete reprogramming of the miPSCs (Takahashi and Yamanaka). Later reports showed that selection based on the promoter reactivation of alternative stem cell markers, such as OCT4 or NANOG, rendered miPSCs capable of generating germline-competent live chimeric mice (Maherali *et al.*, 2007, *Cell Stem Cell* 1(1): 55-70; Okita *et al.*, 2007, *Nature* 448(7151): 313-17; Wernig *et al.*, 2007, *Nature* 448(7151): 318-24) and even all-
30 miPSC mice (Zhao *et al.*, 2009, *Nature* 461(7260): 86-90; Boland *et al.*, 2009, *Nature* 461(7260): 91-94; Kang *et al.*, 2009, *Cell Stem Cell* 5(2): 135-38) demonstrating the full pluripotency of these cells. These reports highlighted the importance of using selective

stem cell markers to identify fully reprogrammed iPSC colonies. The system based on doxycycline-inducible lentivirus expressing the reprogramming factors was used to generate chimeric mice with doxycycline-inducible miPSCs or engineered mouse embryonic stem cells (mESCs) with a reprogramming cassette that facilitated the generation of "secondary" miPSCs from a wide variety of somatic tissues (Stadtfeld *et al.*; Wernig *et al.*). Somatic cells from these mice can be reprogrammed by the addition of doxycycline only, and are easily traceable by the re-expression of a green fluorescent protein (GFP) gene driven by the NANOG or OCT4 gene promoters, yielding reprogramming efficiencies 15 to 50-fold greater than those observed in traditional protocols (Wernig *et al.*, 2008, *Nat. Biotechnol.* 26: 916-24). Generation of transgenic mice with defined doxycycline-inducible subsets of the four reprogramming factors has also recently been reported (Markoulaki *et al.*). Mouse embryonic fibroblasts (MEFs) isolated from these transgenic mice could generate "secondary" GFP-positive miPSC only when the missing factor was re-introduced. *Id.* Mouse induced pluripotent stem cells (miPSCs) colonies were initially selected based on the neomycin resistance provided by the reactivation of the Fbx15 promoter (Takahashi and Yamanaka). These systems have greatly facilitated the characterization of the reprogramming process and have provided an invaluable tool for genetic or chemical screenings for functional substitutes of reprogramming factors with traceable fluorescent markers. However, similar reporter systems in human cells have not been generated due to the challenge in modifying genetically human pluripotent cells.

The inventors previously developed a human reporter system based on the expression of a GFP gene driven by the endogenous OCT4 promoter to follow early events of cell reprogramming (Ruiz *et al.*, 2011, *Curr. Biol.* 21(1): 45-52). The inventors also reported a differentiated knock-in OCT4^{GFP} human H1 embryonic stem cell line (Zwaka and Thomson, 2003, *Nat. Biotechnol.* 21(3): 319-21) into a fibroblast-like population of cells (dFib-OCT4^{GFP}) (Ruiz *et al.*). The inventors verified that the population of dFib-OCT4^{GFP} cells displayed the expected morphology and expressed fibroblast markers at a similar level to what is observed in human fibroblasts (Ruiz *et al.*). Moreover, it was determined that these cells no longer expressed GFP, due to silencing by methylation of the OCT4 promoter, or several pluripotent markers detected in the H1-OCT4^{GFP} cell line. After reprogramming these derived fibroblasts by transduction with a combination of retroviruses encoding for OCT4, sex determining region Y-box 2 (SOX2), Krueppel-like factor 4 (KLF4), and/or cMYC (OSKC), the inventors observed the reactivation of the endogenous OCT4 locus which correlated with the appearance of GFP. *Id.* However, the inventors were not able to reprogram dFib-OCT4^{GFP} cells by transduction of only OCT4 or

OCT4/SOX2. Generation of drug-inducible reprogramming systems in human cells with higher efficiencies compared to retroviral-based protocols have recently been described in the art (Hockemeyer *et al.*; Maherali *et al.*, 2008). Although these cellular systems can be used to dissect the underlying molecular and epigenetic events occurring during the reprogramming of human cells, the technical challenges in engineering pluripotent human cells that allow for the identification of bona-fide hiPSC colonies based on the reactivation of endogenous stem cell promoters have precluded their use for screening purposes. Therefore, the inventors generated a drug inducible reprogramming system using the human dFib-Oct4GFP cells. They obtained different cells with an inducible expression of different subsets of reprogramming factors.

The description provides new insights into microRNA regulation of cell reprogramming. This was accomplished through analysis of the effect on the reprogramming efficiency of pluripotent-enriched miRNAs. miRNAs are 22 nucleotide non-coding RNAs that regulate the expression of downstream targets by mRNA destabilization and translational inhibition (Bartel, 2009, *Cell* 136(2): 215-33). Most mRNA-miRNA targeting occurs through incomplete nucleotide complementation between a short sequence located in the 5' region of the miRNA (the "seed sequence") and its mRNA target. A single miRNA can target hundreds of different mRNAs and multiple pathways that make them powerful regulators of cell function. miRNAs have emerged recently in the art as critical factors in regulating cell fate, as well as in the maintenance and acquisition of pluripotency during cell reprogramming (Mallanna *et al.*, 2010, *Dev. Biol.* 344(1): 16-25). Introduction of mouse embryonic stem cell-specific miRNAs, such as members of the miR-290 cluster (mmu-miR-291, mmu-miR-294 or mmu-miR295), the miR-106b~25 cluster (mmu-miR93 and mmu-miR106b) or depletion of fibroblast-specific miRNAs such as mmu-miR-21 or mmu-miR-29a, promoted the formation of miPSCs in the absence of cMYC (Judson *et al.*, 2009, *Nat. Biotechnol.* 27(5): 459-61; Li *et al.*, 2011, *EMBO J.* 30(5): 823-34; Yang *et al.*, 2011, *RNA* 17(8): 1451-60). It has also been suggested that the expression of specific subsets of different miRNAs could be sufficient to reprogram somatic cells (Anokye-Danso *et al.*, 2011, *Cell Stem Cell* 8(4): 376-88; Miyoshi *et al.*, 2011, *Cell Stem Cell* 8(6): 633-38). Although extensive analysis for the role of miRNAs in self-renewal and pluripotency or during the generation of iPSCs from mouse cells has been reported, little is known about their role in a human context. Thus, it would be desirable to identify miRNAs able to induce or enhance reprogramming of human somatic cells.

SUMMARY OF THE INVENTION

The invention provides a method for enhancing reprogramming in a somatic cell comprising the step of exposing the cell to a miRNA.

The invention also provides a method for reprogramming a somatic cell comprising the step of exposing the cell to a miRNA and one, two or more reprogramming factors.

- 5 The invention further provides a method for reprogramming or enhancing reprogramming in a somatic cell comprising the step of exposing the cell to hsa-miR-519a and the reprogramming factors OCT4, SOX2, and KLF4.

10 In a first aspect, the present invention relates to an *in vitro* method for enhancing reprogramming in a somatic cell wherein said method comprises the step of exposing the cell to a miRNA.

In a first embodiment, said miRNA comprises a seed sequence selected from the group consisting of SEQ ID NO: 28, SEQ ID NO: 27 and SEQ ID NO: 29-33. In another embodiment, said miRNA is selected from the group consisting of hsa-miR-519a (SEQ ID NO: 3), hsa-miR-429 (SEQ ID NO: 9), hsa-miR-367 (SEQ ID NO: 19), hsa-miR-372 (SEQ ID NO: 17), hsa-miR-373 (SEQ ID NO: 16), hsa-miR-200b (SEQ ID NO: 12), hsa-miR-520c-3p (SEQ ID NO: 26), hsa-miR-371-3p (SEQ ID NO: 18), hsa-miR-512-3p (SEQ ID NO: 8), and hsa-miR-20b (SEQ ID NO: 10). In a preferred embodiment, said miRNA is hsa-miR-519a (SEQ ID NO: 3) or hsa-miR-429 (SEQ ID NO: 9).

20 In another embodiment of this first aspect of the invention, said cell of the present method is exposed to the miRNA by contacting the cell with the miRNA. In another embodiment, said cell is exposed to the miRNA by infecting the cell with a viral vector encoding the miRNA. In another embodiment, said viral vector is a retrovirus or a lentivirus.

In another embodiment, said method further comprises exposing the cell to a second miRNA. In another embodiment, said second miRNA is selected from the group consisting of hsa-miR-519a (SEQ ID NO: 3), hsa-miR-429 (SEQ ID NO: 9), hsa-miR-367 (SEQ ID NO: 19), hsa-miR-372 (SEQ ID NO: 17), hsa-miR-373 (SEQ ID NO: 16), hsa-miR-200b (SEQ ID NO: 12), hsa-miR-520c-3p (SEQ ID NO: 26), hsa-miR-371-3p (SEQ ID NO: 18), hsa-miR-512-3p (SEQ ID NO: 8), and hsa-miR-20b (SEQ ID NO: 10). In another embodiment of the present method, said cell is exposed to the second miRNA by contacting the cell with the second miRNA. In another embodiment, said cell is exposed to the second miRNA by infecting the cell with a viral vector encoding the second miRNA. In another embodiment, said viral vector is a retrovirus or a lentivirus.

In a second aspect, the present invention relates to an *in vitro* method for reprogramming a somatic cell wherein said method comprises the steps of exposing the cell to (i) a miRNA and (ii) one, two or more reprogramming factors.

In a first embodiment, said miRNA comprises a seed sequence selected from the group consisting of SEQ ID NO: 28, SEQ ID NO: 27 and SEQ ID NO: 29-33. In another embodiment, said miRNA is selected from the group consisting of hsa-miR-519a (SEQ ID NO: 3), hsa-miR-429 (SEQ ID NO: 9), hsa-miR-367 (SEQ ID NO: 19), hsa-miR-372 (SEQ ID NO: 17), hsa-miR-373 (SEQ ID NO: 16), hsa-miR-200b (SEQ ID NO: 12), hsa-miR-520c-3p (SEQ ID NO: 26), hsa-miR-371-3p (SEQ ID NO: 18), hsa-miR-512-3p (SEQ ID NO: 8), and hsa-miR-20b (SEQ ID NO: 10). In a preferred embodiment, said miRNA is hsa-miR-519a (SEQ ID NO: 3) or hsa-miR-429 (SEQ ID NO: 9).

In another embodiment of this aspect of the present invention said cell of the present method is exposed to the miRNA by contacting the cell with the miRNA. In another embodiment, said cell is exposed to the miRNA by infecting the cell with a viral vector encoding the miRNA. In another embodiment, said viral vector is a retrovirus or a lentivirus.

In another embodiment of the present invention, at least one of the reprogramming factors of the present method is selected from the group consisting of OCT4, SOX2, KLF4, and cMYC. In another embodiment, the one, two or more reprogramming factors of the present method are selected from the group consisting of OCT4, SOX2, KLF4, and cMYC. In another embodiment of this aspect of the present invention, said cell of the present method is exposed to the reprogramming factor(s) by contacting the cell with the reprogramming factor(s). In another embodiment, said cell of the present method is exposed to the reprogramming factor(s) by infecting the cell with a viral vector encoding the reprogramming factor(s). In another embodiment, said viral vector is a retrovirus or a lentivirus.

In another embodiment of this aspect of the invention, said method further comprises exposing the cell to a second miRNA. In another embodiment, said second miRNA is selected from the group consisting of hsa-miR-519a (SEQ ID NO: 3), hsa-miR-429 (SEQ ID NO: 9), hsa-miR-367 (SEQ ID NO: 19), hsa-miR-372 (SEQ ID NO: 17), hsa-miR-373 (SEQ ID NO: 16), hsa-miR-200b (SEQ ID NO: 12), hsa-miR-520c-3p (SEQ ID NO: 26), hsa-miR-371-3p (SEQ ID NO: 18), hsa-miR-512-3p (SEQ ID NO: 8), and hsa-miR-20b (SEQ ID NO: 10). In another embodiment of the present method, said cell is exposed to the second miRNA by contacting the cell with the second miRNA. In another embodiment, said cell is exposed to the second miRNA by infecting the cell with a viral vector encoding the second miRNA. In another embodiment, said viral vector is a retrovirus or a lentivirus.

In a particular aspect, the present invention relates to an *in vitro* method for reprogramming or enhancing reprogramming in a somatic cell wherein said method comprises the steps of exposing the cell to (i) a miRNA comprising a seed sequence of SEQ ID NO: 28 and (ii) the reprogramming factors OCT4, SOX2, and KLF4.

In a first embodiment, said miRNA is hsa-miR-519a (SEQ ID NO: 3). In another embodiment, said cell of the present method is exposed to hsa-miR-519a (SEQ ID NO: 3) by contacting the cell with hsa-miR-519a. In another embodiment said cell is exposed to hsa-miR-519a (SEQ ID NO: 3) by infecting the cell with a viral vector encoding hsa-miR-519a (SEQ ID NO: 3). In another embodiment, said viral vector is a retrovirus or a lentivirus.

In another embodiment of this aspect of the present invention, said cell of the present method is exposed to the reprogramming factor(s) by contacting the cell with the reprogramming factor(s). In another embodiment, said cell of the present method is exposed to the reprogramming factor(s) by infecting the cell with a viral vector encoding the reprogramming factor(s). In another embodiment, said viral vector is a retrovirus or a lentivirus.

In another embodiment of this aspect of the invention, said method further comprises exposing the cell to a second miRNA. In another embodiment, said second miRNA is selected from the group consisting of hsa-miR-519a (SEQ ID NO: 3), hsa-miR-429 (SEQ ID NO: 9), hsa-miR-367 (SEQ ID NO: 19), hsa-miR-372 (SEQ ID NO: 17), hsa-miR-373 (SEQ ID NO: 16), hsa-miR-200b (SEQ ID NO: 12), hsa-miR-520c-3p (SEQ ID NO: 26), hsa-miR-371-3p (SEQ ID NO: 18), hsa-miR-512-3p (SEQ ID NO: 8), and hsa-miR-20b (SEQ ID NO: 10). In another embodiment of the present method, said cell is exposed to the second miRNA by contacting the cell with the second miRNA. In another embodiment, said cell is exposed to the second miRNA by infecting the cell with a viral vector encoding the second miRNA. In another embodiment, said viral vector is a retrovirus or a lentivirus.

In another embodiment of this aspect of the present invention, said method further comprises exposing the cell to an additional reprogramming factor, wherein the additional reprogramming factor is a reprogramming factor other than OCT4, SOX2, or KLF4. In another embodiment, said cell is exposed to the additional reprogramming factor by contacting the cell with the additional reprogramming factor. In another embodiment, said cell is exposed to the additional reprogramming factor by infecting the cell with a viral vector encoding the additional reprogramming factor. In another embodiment, said viral vector is a retrovirus or lentivirus.

Additional features and advantages are described herein, and will be apparent from the following Detailed Description, Drawings and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

- 5 **Figure 1** shows the generation of hiPSC lines with doxycycline-inducible expression of different subsets of reprogramming factors. **Figure 1A** shows a schematic representation of the rationale followed to obtain dFib-OCT4^{GFP}-ind fibroblast-like cells expressing different subsets of reprogramming factors. The right side shows different combinations of retroviruses and lentiviruses used to express the reprogramming factors. **Figure 1B**
- 10 shows the results of real time PCR analysis performed for the pluripotent markers OCT4, SOX2, NANOG, the reprogramming factors KLF4 and cMYC, the fibroblast marker COL1A1 and for GFP. Data are shown as relative averages $\pm$ SD of two biological replicates analyzed in triplicate.
- Figure 2** shows generation of dFib-OCT4^{GFP}-ind lines with doxycycline-inducible expression of different subsets of reprogramming factors. **Figure 2A** shows the results of real time PCR analysis performed for the pluripotent markers OCT4, SOX2, NANOG, the reprogramming factors KLF4 and cMYC, the fibroblast marker COL1A1 and for GFP. Data are shown as relative averages $\pm$ SD of two biological replicates analyzed in triplicate.
- 20 **Figure 2B** shows the results of real time PCR analyses performed on dFib-OCT4^{GFP}-indSKC, dFib-OCT4^{GFP}-ind-OKC and dFib-OCT4^{GFP}-ind-OSK cells infected with retroviruses encoding OCT4, SOX2 and cMYC respectively, and either untreated or treated with 100 ng/ml of doxycycline for 24 hours to detect the transcripts corresponding to the four reprogramming factors. No expression from the factors was delivered by
- 25 retrovirus to generate originally the primary hiPSC lines. Data are shown as relative averages $\pm$ SD of two biological replicates analyzed in triplicate. **Figure 2C** shows cell cultures of the indicated dFib-OCT4^{GFP}-ind cells which were infected with retroviruses encoding the missing reprogramming factor and treated with different doses of doxycycline for 18 days (upper wells). Uninfected cells either untreated or treated with
- 30 doxycycline were used as negative controls of the experiment (lower wells). hiPSC colonies were detected by alkaline phosphatase staining.
- Figure 3** shows miR-519a enhancement of reprogramming efficiency through TGF β RII downregulation. **Figure 3A** shows pictures of cell cultures of dFib-OCT4^{GFP}-indOSK-11 infected with lentiviruses encoding the indicated miRNAs and treated with doxycycline.
- 35 Relative reprogramming efficiency normalized to the efficiency observed in pmiR-000-

infected (a plasmid expressing a scrambled miRNA, taken as a control) cells is shown with the fold changes indicated in a second graph. Uninfected cells were used as a negative control for all experiments. n=number of independent experiments. All error bars depict the SD. A representative example of the colonies formed is shown (upper panel) (*=p<0.05; **=p<0.01). **Figures 3B and 3C** show a similar experiment as described for Figure 3A but BJ fibroblasts (CRL-2522, ATCC), infected with retroviruses encoding the three factors (B, OSK) or the four factors (C, OSKC) plus lentiviruses encoding the indicated miRNAs, were used instead (*=p<0.05; **=p<0.01). **Figure 3D and 3E** show cultures of BJ fibroblasts (D) or dFib-OCT4^{GFP}-ind cells (E) infected with retroviruses encoding the three factors (OSK) and transfected at day 0 and 5 with 30nM of the indicated miRNA mimics. Relative reprogramming efficiency normalized to the efficiency observed in mimic-control cells, is shown with the fold changes indicated. n=number of independent experiments. All error bars depict the SD (*=p<0.05; **=p<0.01). **Figure 3F** shows a schematic representation of the putative target sites of miR-519a for the TGFβRII mRNA. **Figure 3G** shows real time PCR analysis used to detect the transcripts of TGFβRII in BJ fibroblasts that were either uninfected or infected with the indicated lentiviruses. Data are shown as relative averages ± SD of two biological replicates analyzed in triplicate.

Figure 4 shows identification of miRNAs strongly expressed in pluripotent cells or downregulated in somatic cell types. Samples plotted along the first and second principal components. Pluripotent cells notably group together.

DETAILED DESCRIPTION

The disclosure describes a human drug-inducible reporter system utilizing, by way of example, dFib-OCT4^{GFP}-ind cells. This system presents a reliable, genetically homogenous and simple system that can be used for high-throughput screening of functional substitutes for reprogramming factors or modifiers of the reprogramming efficiency in manner. The reprogramming of these cells can easily be tracked by the reactivation of the endogenous OCT4 promoter through the appearance of GFP and by the only addition of doxycycline without the need of additional viral infections.

The disclosure also describes an example of this system for use in screening of pluripotent-enriched miRNAs, which uncovered new players of the reprogramming process. This screening of a pluripotent-enriched miRNA library identified unreported inducers of the reprogramming efficiency.

The data presented herein describe the existence of human pluripotent-specific miRNAs with the ability to increase the reprogramming efficiency. Of those, hsa-miR-519a (SEQ ID NO: 3) emerged as a potent inducer of pluripotency likely through the downregulation of TGF β RII.

- 5 The section headings are used herein for organizational purposes only, and are not to be construed as in any way limiting the subject matter described. The invention as disclosed herein is not limited to the particular methodology, protocols, cell lines, vectors, or reagents described herein because they may vary without departing from the spirit and scope of the invention.
- 10 Conventional and standard techniques may be used for recombinant DNA molecule, protein, and antibody production, as well as for tissue culture and cell transformation. Enzymatic reactions and purification techniques are typically performed according to the manufacturer's specifications or as commonly accomplished in the art using conventional procedures known in the art, or as described herein. Unless specific definitions are
- 15 provided, the nomenclature utilized in connection with, and the laboratory procedures and techniques of analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques may be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.
- 20 Further, the terminology used herein is for the purpose of exemplifying particular embodiments only and is not intended to limit the scope of the invention as disclosed herein. Any method and material similar or equivalent to those described herein can be used in the practice of the invention as disclosed herein and only exemplary methods, devices, and materials are described herein.
- 25 All patents and publications mentioned herein are incorporated by reference in their entirety for the purpose of describing and disclosing the proteins, enzymes, vectors, host cells, and methodologies reported therein that might be used with and in the invention as disclosed herein. However, nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.
- 30 As used herein, "NANOG" is human NANOG protein, which is a 305 amino acid protein with a conserved homeodomain motif that is localized to the nuclear component of cells. The NANOG homeodomain region facilitates DNA binding.
- As used herein, the term "TRA-1-60" means a keratan sulfate antigen found on the surface of stem cells.
- 35 The terms "cell," "cell line," and "cell culture" include progeny thereof. It is also understood that all progeny may not be precisely identical, such as in DNA content, due

to deliberate or inadvertent mutation. Variant progeny that have the same function or biological property of interest, as screened for in the original cell, are included. In certain embodiments, the cell is a somatic cell or a pluripotent cell. In certain embodiments, the cell is a human somatic cell.

5 Provided herein is a method for enhancing reprogramming in a somatic cell comprising the step of exposing the cell to a miRNA. As used herein, "reprogramming" means the process of changing or inducing a cell from a more differentiated state into a less differentiated state. Changing or inducing a differentiated somatic cell to de-differentiate into a pluripotent cell or induced pluripotent cell (iPSC), is accomplished through the
10 process of reprogramming, as non-limiting examples. "Enhancing reprogramming" as used herein, means improving the ability of a cell to change its type.

In a certain embodiment, the cell is a somatic cell that is to be changed into a pluripotent stem cell. As used herein, a "somatic cell" is any biological cell other than a gamete, germ cell, gametocyte or undifferentiated stem cell and refers to differentiated
15 body cells. Induced pluripotent stem cells (iPS cells or iPSCs) are a type of pluripotent stem cell artificially derived from a non-pluripotent cell, typically an adult somatic cell, by the induction of expression of certain embryonic genes or "reprogramming factors" as used herein. Induced pluripotent stem cells are similar to natural pluripotent stem cells, such as embryonic stem (ES) cells, in many aspects, such as the expression of certain
20 stem cell genes and proteins, chromatin methylation patterns, doubling time, embryoid body formation, teratoma formation, viable chimera formation, and potency and differentiability.

"Differentiation" as used herein, refers to process through which a stem cell loses its capacity for self-renewal and becomes a mature and definitive cell-type *i.e.*, a
25 differentiated cell, thereby acquiring the features of a specialized cell.

As used herein, "exposing" encompasses any means by which a miRNA or reprogramming factor becomes associated with a cell or with cell function. In certain embodiments, the cell is exposed to a miRNA or reprogramming factor by "contacting" the cell with the miRNA or reprogramming factor. Contacting can be achieved, for example,
30 by adding a reprogramming factor to the cell media. In another embodiment, the cell is exposed to a miRNA or reprogramming factor by "infection." In specific embodiments, cell infection is accomplished via infection by retrovirus or lentivirus. Are also encompassed in the notion of "contacting" or "exposing" a cell to miRNAs or reprogramming factors in the present invention, numerous transfection methods evident for the skilled in the art which
35 actively favors the contact between a cell and miRNAs and/or reprogramming factors by their intracellular delivery.

As used herein "miRNA(s)" refers to 20-24 nucleotide RNAs that are 22 nucleotide non-coding RNAs that regulate eukaryotic gene expression post-transcriptionally by the degradation or translational inhibition of their target messenger RNAs (mRNAs) (Bartel, 2009, *Cell* 136(2): 215-33). miRNAs are initially transcribed as primary microRNAs (pri-miRNAs) followed by a two step processing into precursor miRNAs (pre-miRNAs) then mature microRNAs and incorporation into the RNA-induced silencing complex (RISC). Mature microRNAs downregulate their target-mRNAs by sequence-specific base-pairing with their 3'-untranslated regions (3'-UTRs) and act as key regulatory molecules in various cellular processes like proliferation, differentiation, apoptosis and metabolism.

Most mRNA-miRNA targeting occurs through incomplete nucleotide complementation between a short sequence located in the 5' region of the miRNA (the "seed sequence") and the 3' UTR of its mRNA target. As an illustrative example, the seed sequence of has-miR-519a (SEQ ID NO: 3) is AAGUGCA (SEQ ID NO: 28). A single miRNA can target hundreds of different mRNAs and multiple pathways that make them powerful regulators of cell function. Under a standard nomenclature system, the double-stranded precursor miRNA is noted "pre-mir-" or "mir-" and the single-stranded mature miRNA is noted "miR-", all followed by a number naming said miRNA. Species of origin is designated with a three-letter prefix, e.g., has-miR-429 (SEQ ID NO: 9) is a human (*Homo sapiens*) miRNA and oar-miR-429 is a sheep (*Ovis aries*) miRNA. miRNAs with nearly identical sequences except for one or two identical nucleotides are annotated with an additional lower case letter. For example, hsa-miR-517c (SEQ ID NO: 6) differs from has-miR-517a (SEQ ID NO: 7) by only one nucleotide. Pre-miRNAs that lead to 100% identical mature miRNAs but that are located at different places in the genome are indicated with an additional dash-number suffix. For example, has-mir-519a-1 and has-mir-519a-2 are distinct pre-miRNAs that generate the same mature miRNA: has-miR-519a (SEQ ID NO: 3). When two mature miRNAs originate from opposite arms of the same pre-miRNA, they are denoted with a -3p or -5p suffix as for example for has-miR-512-3p (SEQ ID NO: 8).

Also provided herein is a method for reprogramming a somatic cell comprising the step of exposing the cell to (i) a miRNA and (ii) one, two or more reprogramming factors. As for the method provided herein for enhancing reprogramming in a somatic cell comprising the step of exposing the cell to a miRNA, in certain embodiments, the miRNA is selected from the group consisting of hsa-miR-302a (SEQ ID NO: 23), hsa-miR-302b (SEQ ID NO: 22), hsa-miR-302d (SEQ ID NO: 20), hsa-miR-302c (SEQ ID NO: 21), hsa-miR-372 (SEQ ID NO: 17), hsa-miR-520c-3p (SEQ ID NO: 26), hsa-miR-373 (SEQ ID NO: 16), hsa-miR-367 (SEQ ID NO: 19), hsa-miR-363 (SEQ ID NO: 14), hsa-miR-371-3p (SEQ ID NO: 18), hsa-miR-200c (SEQ ID NO: 13), hsa-miR-200b (SEQ ID NO: 12), hsa-miR-429 (SEQ ID NO:

9), hsa-miR-141 (SEQ ID NO: 15), hsa-miR-519a (SEQ ID NO: 3), hsa-miR-519b-3p (SEQ ID NO: 2), hsa-miR-512-3p (SEQ ID NO: 8), hsa-miR-517a (SEQ ID NO: 7), hsa-miR-517c (SEQ ID NO: 6), hsa-miR-518b (SEQ ID NO: 5), hsa-miR-518e (SEQ ID NO: 4), hsa-miR-520h (SEQ ID NO: 24), hsa-miR-520g (SEQ ID NO: 25), hsa-miR-519d (SEQ ID NO: 1), hsa-miR-20b (SEQ ID NO: 10) and hsa-miR-205 (SEQ ID NO: 11). In other embodiments, the miRNA is selected from the group consisting of hsa-miR-363 (SEQ ID NO: 14), hsa-miR-200c (SEQ ID NO: 13), hsa-miR-200b (SEQ ID NO: 12), hsa-miR-429 (SEQ ID NO: 9), hsa-miR-141 (SEQ ID NO: 15), hsa-miR-519a (SEQ ID NO: 3), hsa-miR-519b-3p (SEQ ID NO: 2), hsa-miR-512-3p (SEQ ID NO: 8), hsa-miR-517a (SEQ ID NO: 7), hsa-miR-517c (SEQ ID NO: 6), hsa-miR-518b (SEQ ID NO: 5), hsa-miR-518e (SEQ ID NO: 4), hsa-miR-520h (SEQ ID NO: 24), hsa-miR-520g (SEQ ID NO: 25), hsa-miR-519d (SEQ ID NO: 1), hsa-miR-20b (SEQ ID NO: 10) and hsa-miR-205 (SEQ ID NO: 11). In a specific embodiment, the miRNA is selected from the group consisting of hsa-miR-367 (SEQ ID NO: 19), hsa-miR-372 (SEQ ID NO: 17), hsa-miR-373 (SEQ ID NO: 16), hsa-miR-429 (SEQ ID NO: 9), hsa-miR-519a (SEQ ID NO: 3), hsa-miR-200b (SEQ ID NO: 12), hsa-miR-520c-3p (SEQ ID NO: 26), hsa-miR-371-3p (SEQ ID NO: 18), hsa-miR-512-3p (SEQ ID NO: 8), and hsa-miR-20b (SEQ ID NO: 10). In another specific embodiment, the miRNA is hsa-miR-429 (SEQ ID NO: 9) or hsa-miR-519a (SEQ ID NO: 3). In another specific embodiment, provided herein is a method for enhancing reprogramming in a somatic cell comprising the step of exposing the cell to hsa-miR-519a (SEQ ID NO: 3). In another specific embodiment, provided herein is a method for reprogramming a somatic cell comprising the step of exposing the cell to (i) hsa-miR-519a (SEQ ID NO: 3) and (ii) one, two or more reprogramming factors. In other embodiments, the cell is exposed to the miRNA by contacting the cell with the miRNA or by expressing it after the delivery inside cell of a vector encoding said miRNA. As immediately apparent to the skilled in the art, throughout the present invention, miRNAs may be replaced with primary miRNAs transcripts or precursors miRNA (pre-miRNAs) leading to them upon maturation, or chemically synthesized molecules equivalent to these molecular species; as an illustrative example, miRNAs of the present invention may be replaced by miRNA mimics such as miScript miRNA Mimics (Qiagen) which are chemically synthesized, double-stranded RNAs which mimic mature endogenous miRNAs after transfection into cells. miRNAs of the present invention may originate from numerous organisms; as a non-limiting example, miRNAs of the present invention may originate from mammals. As another non-limiting example, miRNAs may originate from human, i.e. being a human miRNA. miRNAs, as used herein in the methods of the present invention, also encompass miRNAs which share the same seed sequence than selected miRNAs. As a first non-limiting example, is

provided herein a method for enhancing reprogramming in a somatic cell comprising the step of exposing the cell to a miRNA, wherein said miRNA shares the same seed sequences than miRNAs selected from the group consisting of hsa-miR-367 (SEQ ID NO: 19), hsa-miR-372 (SEQ ID NO: 17), hsa-miR-373 (SEQ ID NO: 16), hsa-miR-429 (SEQ ID NO: 9), hsa-miR-519a (SEQ ID NO: 3), hsa-miR-200b (SEQ ID NO: 12), hsa-miR-520c-3p (SEQ ID NO: 26), hsa-miR-371-3p (SEQ ID NO: 18), hsa-miR-512-3p (SEQ ID NO: 8), and hsa-miR-20b (SEQ ID NO: 10). In other words, a method is provided herein for enhancing reprogramming in a somatic cell comprising the step of exposing the cell to a miRNA, wherein said miRNA comprises a seed sequence selected from the group of SEQ ID NO: 27-33. As a second non-limiting example, a method is provided herein for reprogramming a somatic cell comprising the step of exposing the cell to (i) a miRNA which shares the same seed sequence than hsa-miR-519a (SEQ ID NO: 3) and (ii) one, two or more reprogramming factors. In other words, a method is provided herein for reprogramming a somatic cell comprising the step of exposing the cell to (i) a miRNA wherein said miRNA comprises a seed sequence of SEQ ID NO: 28 and (ii) one, two or more reprogramming factors. It is intended that the methods of the present invention can notably be carried out *in vitro*.

Are encompassed in the scope of the present invention, miRNAs according to the present invention for enhancing the reprogramming of a somatic cell. Are also encompassed in the scope of the present invention, miRNAs according to the present invention for reprogramming a somatic cell by contacting said cell with at least said miRNAs. Are also encompassed in the present invention, use of miRNAs according to the present invention for enhancing the reprogramming of a somatic cell.

The term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. A "vector" in the present invention includes, but is not limited to, a viral vector, a plasmid, a RNA vector or a linear or circular DNA or RNA molecule which may consist of a chromosomal, non-chromosomal, semi-synthetic or synthetic nucleic acids. In specific embodiments, vectors are those capable of autonomous replication (episomal vector) and/or expression of nucleic acids to which they are linked (expression vectors). Large numbers of suitable vectors are known to those of skill in the art and commercially available. In another embodiment, the cell is exposed to the miRNA by infecting the cell with a viral vector encoding the miRNA.

Viral vectors include retrovirus, adenovirus, parvovirus (e.g., adenoassociated viruses), coronavirus, negative strand RNA viruses such as orthomyxovirus (e.g., influenza virus), rhabdovirus (e.g., rabies and vesicular stomatitis virus), paramyxovirus (e.g., measles and Sendai), positive strand RNA viruses such as picornavirus and alphavirus, and double-

stranded DNA viruses including adenovirus, herpesvirus (e.g., Herpes Simplex virus types 1 and 2, Epstein-Barr virus, cytomegalovirus), and poxvirus (e.g., vaccinia, fowlpox and canarypox). In one embodiment, the viral vector is a retrovirus or lentivirus. As used herein, the term "retrovirus" means an RNA virus that is duplicated in a host cell using the reverse transcriptase enzyme to produce DNA from its RNA genome. The DNA is then incorporated into the host's genome by an integrase enzyme. The virus thereafter replicates as part of the host cell's DNA. Retroviruses are enveloped viruses that belong to the viral family *Retroviridae*. As used herein, the term "lentivirus" means a genus of viruses of the *Retroviridae* family, characterized by a long incubation period. Lentiviruses can deliver a significant amount of viral RNA into the DNA of the host cell. In certain embodiments a lentivirus is HIV, SIV, BIV, Equine Infectious Anemia Virus, Maedi-visna Virus or Caprine Encephalitis Arthritis Virus as illustrating examples. Lentiviral vectors derived from such lentivirus can be integrative or non-integrative.

In another embodiment, the method herein further comprises exposing the cell to a second miRNA. In certain embodiments, the miRNA is selected from the group consisting of hsa-miR-302a (SEQ ID NO: 23), hsa-miR-302b (SEQ ID NO: 22), hsa-miR-302d (SEQ ID NO: 20), hsa-miR-302c (SEQ ID NO: 21), hsa-miR-372 (SEQ ID NO: 17), hsa-miR-520c-3p (SEQ ID NO: 26), hsa-miR-373 (SEQ ID NO: 16), hsa-miR-367 (SEQ ID NO: 19), hsa-miR-363 (SEQ ID NO: 14), hsa-miR-371-3p (SEQ ID NO: 18), hsa-miR-200c (SEQ ID NO: 13), hsa-miR-200b (SEQ ID NO: 12), hsa-miR-429 (SEQ ID NO: 9), hsa-miR-141 (SEQ ID NO: 15), hsa-miR-519a (SEQ ID NO: 3), hsa-miR-519b-3p (SEQ ID NO: 2), hsa-miR-512-3p (SEQ ID NO: 8), hsa-miR-517a (SEQ ID NO: 7), hsa-miR-517c (SEQ ID NO: 6), hsa-miR-518b (SEQ ID NO: 5), hsa-miR-518e (SEQ ID NO: 4), hsa-miR-520h (SEQ ID NO: 24), hsa-miR-520g (SEQ ID NO: 25), hsa-miR-519d (SEQ ID NO: 1), hsa-miR-20b (SEQ ID NO: 10) and hsa-miR-205 (SEQ ID NO: 11).. In other embodiments, the miRNA is selected from the group consisting of hsa-miR-363 (SEQ ID NO: 14), hsa-miR-200c (SEQ ID NO: 13), hsa-miR-200b (SEQ ID NO: 12), hsa-miR-429 (SEQ ID NO: 9), hsa-miR-141 (SEQ ID NO: 15), hsa-miR-519a (SEQ ID NO: 3), hsa-miR-519b-3p (SEQ ID NO: 2), hsa-miR-512-3p (SEQ ID NO: 8), hsa-miR-517a (SEQ ID NO: 7), hsa-miR-517c (SEQ ID NO: 6), hsa-miR-518b (SEQ ID NO: 5), hsa-miR-518e (SEQ ID NO: 4), hsa-miR-520h (SEQ ID NO: 24), hsa-miR-520g (SEQ ID NO: 25), hsa-miR-519d (SEQ ID NO: 1), hsa-miR-20b (SEQ ID NO: 10) and hsa-miR-205 (SEQ ID NO: 11). In a specific embodiment, the second miRNA is selected from the group consisting of hsa-miR-367 (SEQ ID NO: 19), hsa-miR-372 (SEQ ID NO: 17), hsa-miR-373 (SEQ ID NO: 16), hsa-miR-429 (SEQ ID NO: 9), hsa-miR-519a (SEQ ID NO: 3), hsa-miR-200b (SEQ ID NO: 12), hsa-miR-520c-3p (SEQ ID NO: 26), hsa-miR-371-3p (SEQ ID NO: 18), hsa-miR-512-

3p (SEQ ID NO: 8), and hsa-miR-20b (SEQ ID NO: 10). In another specific embodiment, the cell is exposed to the second miRNA by contacting the cell as explained above with the second miRNA or infecting the cell with a viral vector encoding the second miRNA. In a specific embodiment, the viral vector is a retrovirus or lentivirus. In another
5 embodiment, the method for enhancing reprogramming in a somatic cell herein comprises the step of exposing the cell to more than one miRNA, *i.e.*, exposing the cell to successive miRNAs or to a combination of two, three, four or five miRNAs selected from the groups mentioned above.

As used herein, "reprogramming factors" include proteins, small molecules, and miRNAs
10 capable of inducing reprogramming, or nucleic acid encoding for such proteins or miRNAs. Examples of reprogramming factors include but are not limited to OCT4, SOX2, KLF4, and cMYC. Additional reprogramming factors may be C/EBP α , Lin28, Orphan nuclear receptors Esrrb and Nr5a2, Sox1, Sox3, Klf2, Klf5, N-Myc, L-Myc, Tert, Sall4, Tbx3 Wnt3a, Utf1, SV40LT and Glis1 (as reviewed in Stadtfeld and Hochedlinger, 2010,
15 *Genes Dev.* 24(20): 2239-63, Nakhaei-Rad et al, 2012 *Biochem. Cell. Biol* 90: 115-123 and in Mochiduki and Okita, 2012 *Biotechnol. J.* 7: 789-797) or small molecules e.g. those described in International Publication No. WO2010/068955 such as 6-bromoindirubin-3'-oxime (BIO), Indirubin-5-nitro-3'-oxime (INO), Valproic acid, 2-(3-(6-Methylpyridin-2-yl)-1H-pyrazol-4-yl)-1,5-naphthyridine, 1-(4-Methylphenyl)-2-(4,5,6,7-tetrahydro-2-imino-
20 3(2H)-benzothiazolyl)ethanone HBr, Pifithrin-alpha, Prostaglandin J2 and Prostaglandin E2, as non-limiting examples.

As used herein, "Oct-4" is a transcription factor that is initially active as a maternal factor in the oocyte but remains active in embryos throughout the pre-implantation period. Oct-4 expression is associated with an undifferentiated phenotype and tumors. Gene
25 knockdown of Oct-4 promotes differentiation, thereby demonstrating a role for these factors in human embryonic stem cell self-renewal. Oct-4 can form a heterodimer with Sox2, allowing these two proteins to bind DNA together. It has been shown that reactivation of the OCT4 promoter is a very reliable marker to identify fully reprogrammed mouse cells for screening purposes (Shi *et al.*, 2008, *Cell Stem Cell* 3(5): 568-74; Ichida
30 *et al.*, 2009, *Cell Stem Cell* 5(5): 491-503).

As used herein SRY (sex determining region Y)-box 2, also known as "SOX2," is a transcription factor that is essential for maintaining self-renewal, or pluripotency, of undifferentiated embryonic stem cells. Sox2 is a member of the Sox family of transcription factors, which have been shown to play key roles in many stages of
35 mammalian development. This protein family shares highly conserved DNA binding domains known as HMG (High-mobility group) box domains containing approximately 80

amino acids. Sox2 has a critical role in maintenance of embryonic and neural stem cells (Avilion *et al.*, 2003, Genes and Development 17: 126-140).

As used herein, Krueppel-like factor 4 or "KLF4" is a protein that in humans is encoded by the KLF4 gene. In embryonic stem cells (ESCs), KLF4 has been demonstrated to be a
5 good indicator of stem-like capacity. As used herein, "cMyc" is a regulator gene that codes for a transcription factor that is known to be involved with certain cancers.

In a certain embodiment, the cell is exposed to the reprogramming factors by contacting the cell with the reprogramming factors. In a specific embodiment, the cell is exposed to the reprogramming factors following transformation by one or several vectors expressing
10 said reprogramming factors. In a specific embodiment, the cell is exposed to the reprogramming factors by infecting the cell with one or more viral vectors encoding the reprogramming factors. In one embodiment, at least one of the viral vectors is a retrovirus or lentivirus.

Provided herein is a method for reprogramming a somatic cell comprising the step of
15 exposing the cell to at least (i) a miRNA and (ii) one, two or more reprogramming factors said one, two or more reprogramming factors being selected from the group of proteins consisting of OCT4, SOX2, KLF4, cMYC, C/EBP α , Lin28, Orphan nuclear receptors Esrrb and Nr5a2, Sox1, Sox3, Klf2, Klf5, N-Myc, L-Myc, Tert, Sall4, Tbx3 Wnt3a, Utf1, SV40LT and Glis1 and/or from the group of small molecules selected from the group consisting of
20 6-bromoindirubin-3'-oxime (BIO), Indirubin-5-nitro-3'-oxime (INO), Valproic acid, 2-(3-(6-Methylpyridin-2-yl)-1H-pyrazol-4-yl)-1,5-naphthyridine, 1-(4-Methylphenyl)-2-(4,5,6,7-tetrahydro-2-imino-3(2H)-benzothiazolyl)ethanone HBr, Pifithrin-alpha, Prostaglandin J2 and Prostaglandin E2.

In a specific embodiment, provided herein is a method for reprogramming somatic cell
25 comprising the step of exposing the cell to (i) a miRNA and (ii) two reprogramming factors wherein said two reprogramming factors are selected from the group of proteins consisting of OCT4, SOX2, KLF4, cMYC, C/EBP α , Lin28, Orphan nuclear receptors Esrrb and Nr5a2, Sox1, Sox3, Klf2, Klf5, N-Myc, L-Myc, Tert, Sall4, Tbx3 Wnt3a, Utf1, SV40LT and Glis1.

In another specific embodiment, provided herein is a method for reprogramming somatic cell comprising the step of exposing the cell to (i) a miRNA and (ii) two reprogramming factors wherein said two reprogramming factors are selected from the group of small molecules consisting of 6-bromoindirubin-3'-oxime (BIO), Indirubin-5-nitro-3'-oxime (INO), Valproic acid, 2-(3-(6-Methylpyridin-2-yl)-1H-pyrazol-4-yl)-1,5-naphthyridine, 1-(4-
35 Methylphenyl)-2-(4,5,6,7-tetrahydro-2-imino-3(2H)-benzothiazolyl)ethanone HBr, Pifithrin-alpha, Prostaglandin J2 and Prostaglandin E2.

In another specific embodiment, the method for reprogramming somatic cell provided herein comprises the step of exposing the cell to (i) a miRNA and (ii) two reprogramming factors wherein one of said reprogramming factors is selected from the group of proteins consisting of OCT4, SOX2, KLF4, cMYC, C/EBP α , Lin28, Orphan nuclear receptors Esrrb and Nr5a2, Sox1, Sox3, Klf2, Klf5, N-Myc, L-Myc, Tert, Sall4, Tbx3 Wnt3a, Utf1, SV40LT and Glis1 and the other one is selected from the group of small molecules consisting of 6-bromoindirubin-3'-oxime (BIO), Indirubin-5-nitro-3'-oxime (INO), Valproic acid, 2-(3-(6-Methylpyridin-2-yl)-1H-pyrazol-4-yl)-1,5-naphthyridine, 1-(4-Methylphenyl)-2-(4,5,6,7-tetrahydro-2-imino-3(2H)-benzothiazolyl)ethanone HBr, Pifithrin-alpha, Prostaglandin J2 and Prostaglandin E2.

In another embodiment, the method for reprogramming somatic cell provided herein comprises the step of exposing the cell to (i) a miRNA and (ii) more than two reprogramming factors, *i.e.*, three, four, five or six reprogramming factors. In this embodiment, said three, four, five or six reprogramming factors can be selected from the group of proteins consisting of OCT4, SOX2, KLF4, cMYC, C/EBP α , Lin28, Orphan nuclear receptors Esrrb and Nr5a2, Sox1, Sox3, Klf2, Klf5, N-Myc, L-Myc, Tert, Sall4, Tbx3 Wnt3a, Utf1, SV40LT and Glis1 and/or can be selected from the group of small molecules consisting of 6-bromoindirubin-3'-oxime (BIO), Indirubin-5-nitro-3'-oxime (INO), Valproic acid, 2-(3-(6-Methylpyridin-2-yl)-1H-pyrazol-4-yl)-1,5-naphthyridine, 1-(4-Methylphenyl)-2-(4,5,6,7-tetrahydro-2-imino-3(2H)-benzothiazolyl)ethanone HBr, Pifithrin-alpha, Prostaglandin J2 and Prostaglandin E2. It is well understood in this case that all combinations of reprogramming factors selected from both proteins and small molecules categories of reprogramming factors are encompassed in the scope of the method provided herein.

In a specific embodiment, the method for reprogramming somatic cell provided herein comprises the step of exposing the cell to (i) a miRNA and (ii) OCT4, SOX2 and KLF4 reprogramming factors. In another specific embodiment, the method for reprogramming somatic cell provided herein comprises the step of exposing the cell to (i) hsa-miR-519a (SEQ ID NO: 3) and (ii) OCT4, SOX2 and KLF4 reprogramming factors. In another specific embodiment, the method for reprogramming somatic cell provided herein comprises the step of exposing the cell to (i) a miRNA wherein said miRNA comprises a seed sequence of SEQ ID NO: 28 and (ii) OCT4, SOX2 and KLF4 reprogramming factors. In another specific embodiment, the method for reprogramming somatic cell provided herein comprises the step of exposing the cell to (i) a miRNA and (ii) OCT4, SOX2, KLF4 reprogramming factors and a fourth reprogramming factor selected from the group of small molecules consisting of 6-bromoindirubin-3'-oxime (BIO), Indirubin-5-nitro-3'-oxime

(INO), Valproic acid, 2-(3-(6-Methylpyridin-2-yl)-1H-pyrazol-4-yl)-1,5-naphthyridine, 1-(4-Methylphenyl)-2-(4,5,6,7-tetrahydro-2-imino-3(2H)-benzothiazolyl)ethanone HBr, Pifithrin-alpha, Prostaglandin J2 and Prostaglandin E2.

In another specific embodiment, the method for reprogramming somatic cell provided herein comprises the step of exposing the cell to (i) hsa-miR-519a and (ii) OCT4, SOX2, KLF4 reprogramming factors and a fourth reprogramming factor selected from the group of small molecules consisting of 6-bromoindirubin-3'-oxime (BIO), Indirubin-5-nitro-3'-oxime (INO), Valproic acid, 2-(3-(6-Methylpyridin-2-yl)-1H-pyrazol-4-yl)-1,5-naphthyridine, 1-(4-Methylphenyl)-2-(4,5,6,7-tetrahydro-2-imino-3(2H)-benzothiazolyl)ethanone HBr, Pifithrin-alpha, Prostaglandin J2 and Prostaglandin E2. In another specific embodiment, the method for reprogramming somatic cell provided herein comprises the step of exposing the cell to (i) a miRNA wherein said miRNA comprises a seed sequence of SEQ ID NO: 28 and (ii) OCT4, SOX2, KLF4 reprogramming factors and a fourth reprogramming factor selected from the group of small molecules consisting of 6-bromoindirubin-3'-oxime (BIO), Indirubin-5-nitro-3'-oxime (INO), Valproic acid, 2-(3-(6-Methylpyridin-2-yl)-1H-pyrazol-4-yl)-1,5-naphthyridine, 1-(4-Methylphenyl)-2-(4,5,6,7-tetrahydro-2-imino-3(2H)-benzothiazolyl)ethanone HBr, Pifithrin-alpha, Prostaglandin J2 and Prostaglandin E2.

In another specific embodiment, the method for reprogramming somatic cell provided herein comprises the step of exposing the cell to (i) a miRNA and (ii) OCT4, SOX2, cMYC and KLF4 reprogramming factors.

In another specific embodiment, the method for reprogramming somatic cell provided herein comprises the step of exposing the cell to (i) hsa-miR-519a and (ii) OCT4, SOX2, cMYC and KLF4 reprogramming factors.

In another specific embodiment, the method for reprogramming somatic cell provided herein comprises the step of exposing the cell to (i) a miRNA and (ii) OCT4, SOX2, cMYC, KLF4 reprogramming factors and a fourth reprogramming factor selected from the group of small molecules consisting of 6-bromoindirubin-3'-oxime (BIO), Indirubin-5-nitro-3'-oxime (INO), Valproic acid, 2-(3-(6-Methylpyridin-2-yl)-1H-pyrazol-4-yl)-1,5-naphthyridine, 1-(4-Methylphenyl)-2-(4,5,6,7-tetrahydro-2-imino-3(2H)-benzothiazolyl)ethanone HBr, Pifithrin-alpha, Prostaglandin J2 and Prostaglandin E2. In another specific embodiment, the method for reprogramming somatic cell provided herein comprises the step of exposing the cell to (i) hsa-miR-519a and (ii) OCT4, SOX2, cMYC, KLF4 reprogramming factors and a fourth reprogramming factor selected from the group of small molecules consisting of 6-bromoindirubin-3'-oxime (BIO), Indirubin-5-nitro-3'-oxime (INO), Valproic acid, 2-(3-(6-Methylpyridin-2-yl)-1H-pyrazol-4-yl)-1,5-naphthyridine, 1-(4-

Methylphenyl)-2-(4,5,6,7-tetrahydro-2-imino-3(2H)-benzothiazolyl)ethanone HBr, Pifithrin-alpha, Prostaglandin J2 and Prostaglandin E2.

In another specific embodiment, the method for reprogramming somatic cell provided herein comprises the step of exposing the cell to (i) a miRNA and (ii) at least one reprogramming factor or a combination of reprogramming factors as listed in Table 2 of Stadtfeld and Hochedlinger and / or in Tables 1 of respectively Nakhaei-Rad et al, 2012 and Mochiduki and Okita, 2012, all incorporated here by reference.

In another specific embodiment, the method for reprogramming somatic cell provided herein comprises the step of exposing the cell to (i) hsa-miR-519a (SEQ ID NO: 3) and (ii) at least one reprogramming factor or a combination of reprogramming factors as listed in Table 2 of Stadtfeld and Hochedlinger and / or in Tables 1 of respectively Nakhaei-Rad et al, 2012 and Mochiduki and Okita, 2012.

In one embodiment, the method as described herein further comprises exposing the cell to a second miRNA. In certain embodiments, the miRNA is selected from the group consisting of hsa-miR-302a (SEQ ID NO: 23), hsa-miR-302b (SEQ ID NO: 22), hsa-miR-302d (SEQ ID NO: 20), hsa-miR-302c (SEQ ID NO: 21), hsa-miR-372 (SEQ ID NO: 17), hsa-miR-520c-3p (SEQ ID NO: 26), hsa-miR-373 (SEQ ID NO: 16), hsa-miR-367 (SEQ ID NO: 19), hsa-miR-363 (SEQ ID NO: 14), hsa-miR-371-3p (SEQ ID NO: 18), hsa-miR-200c (SEQ ID NO: 13), hsa-miR-200b (SEQ ID NO: 12), hsa-miR-429 (SEQ ID NO: 9), hsa-miR-141 (SEQ ID NO: 15), hsa-miR-519a (SEQ ID NO: 3), hsa-miR-519b-3p (SEQ ID NO: 2), hsa-miR-512-3p (SEQ ID NO: 8), hsa-miR-517a (SEQ ID NO: 7), hsa-miR-517c (SEQ ID NO: 6), hsa-miR-518b (SEQ ID NO: 5), hsa-miR-518e (SEQ ID NO: 4), hsa-miR-520h (SEQ ID NO: 24), hsa-miR-520g (SEQ ID NO: 25), hsa-miR-519d (SEQ ID NO: 1), hsa-miR-20b (SEQ ID NO: 10) and hsa-miR-205 (SEQ ID NO: 11). In other embodiments, the miRNA is selected from the group consisting of hsa-miR-363 (SEQ ID NO: 14), hsa-miR-200c (SEQ ID NO: 13), hsa-miR-200b (SEQ ID NO: 12), hsa-miR-429 (SEQ ID NO: 9), hsa-miR-141 (SEQ ID NO: 15), hsa-miR-519a (SEQ ID NO: 3), hsa-miR-519b-3p (SEQ ID NO: 2), hsa-miR-512-3p (SEQ ID NO: 8), hsa-miR-517a (SEQ ID NO: 7), hsa-miR-517c (SEQ ID NO: 6), hsa-miR-518b (SEQ ID NO: 5), hsa-miR-518e (SEQ ID NO: 4), hsa-miR-520h (SEQ ID NO: 24), hsa-miR-520g (SEQ ID NO: 25), hsa-miR-519d (SEQ ID NO: 1), hsa-miR-20b (SEQ ID NO: 10) and hsa-miR-205 (SEQ ID NO: 11). In a specific embodiment, the second miR is selected from the group consisting of hsa-miR-367 (SEQ ID NO: 19), hsa-miR-372 (SEQ ID NO: 17), hsa-miR-373 (SEQ ID NO: 16), hsa-miR-429 (SEQ ID NO: 9), hsa-miR-519a (SEQ ID NO: 3), hsa-miR-200b (SEQ ID NO: 12), hsa-miR-520c-3p (SEQ ID NO: 26), hsa-miR-371-3p (SEQ ID NO: 18), hsa-miR-512-3p (SEQ ID NO: 8), and hsa-miR-20b (SEQ ID NO: 10). In a specific embodiment, the cell is

exposed to the second miRNA by contacting the cell with the second miRNA or by infecting the cell with a viral vector encoding the second miRNA. In one embodiment the viral vector is a retrovirus or lentivirus.

Provided herein is a method for reprogramming or enhancing reprogramming in a somatic cell comprising the step of exposing the cell to (i) hsa-miR-519a and (ii) the reprogramming factors OCT4, SOX2, and KLF4. In an embodiment, the cell is exposed to hsa-miR-519a (SEQ ID NO: 3) by contacting the cell with hsa-miR-519a (SEQ ID NO: 3). In another specific embodiment the cell is exposed to hsa-miR-519a (SEQ ID NO: 3) by infecting the cell with a viral vector encoding hsa-miR-519a (SEQ ID NO: 3). In another embodiment the viral vector is a retrovirus or lentivirus.

In one embodiment, the cell is exposed to the reprogramming factors by contacting the cell with the reprogramming factors. In certain embodiments, the cell is exposed to the reprogramming factors by infecting the cell with one or more viral vectors encoding the reprogramming factors. In other embodiments, at least one of the viral vectors is a retrovirus or lentivirus.

Provided herein is a method for reprogramming or enhancing reprogramming in a somatic cell comprising the step as described throughout the present specification wherein the somatic cell is a human cell. In certain embodiments, the cell is a cell line derived from human cells. As non-limiting examples, cell lines may be 293T cells or BJ fibroblasts.

In one embodiment the method as disclosed herein comprises exposing the cell to an additional reprogramming factor, wherein the additional reprogramming factor is a reprogramming factor other than OCT4, SOX2, or KLF4. In a specific embodiment, the cell is exposed to the additional reprogramming factor by contacting the cell with the additional reprogramming factor. In a certain embodiment the cell is exposed to the additional reprogramming factor by infecting the cell with a viral vector encoding the additional reprogramming factor, in one embodiment the viral vector is a retrovirus or lentivirus.

Another object of the present invention is a composition which comprises, as active principle, a miRNA according to the present invention. This composition comprises an effective dose of said miRNA and at least one suitable excipient appropriate with the use of said composition.

Are also encompassed in the scope of the present invention, kits to implement the methods of the present invention, comprising at least one miRNA according to the present invention and instructions to use it in order to reprogram or enhance the reprogramming of a somatic cell.

Materials and Methods

1. hES cell culture and differentiation

H1 (WA01), H7 (WA07), H9 (WA09) and H1-OCT4^{GFP} embryonic stem cell lines were obtained from WiCell Research Institute and maintained on mouse embryonic fibroblasts (MEFs) or Matrigel (BD Biosciences) using mTeSR1 medium (Stem Cell Technologies). hESC colonies were split using a solution of dispase (2 mg/ml) or collagenase (1 mg/ml) and scraping the colonies with a glass pipette. Derived hiPSCs were cultured similarly as described above for hESCs. 293T cells and dFib-OCT4^{GFP} fibroblast-like cells and BJ human fibroblasts (ATCC, CRL-2522) were cultured in DMEM (Invitrogen) supplemented with 10% FBS and 0.1 mM non-essential amino acids. Commercial primary cells obtained from ATCC, Lonza and Promocell (see table 1 below) were cultured according to the recommendations of the supplier.

2. Human hiPSC generation

For the generation of human primary hiPSCs derived from dFib-OCT4^{GFP} cells, a mix of retroviruses plus lentiviruses was used to infect the fibroblast-like cells by spinfection at 1850 rpm for 1 hour at room temperature in the presence of polybrene (4µg/ml). As an example, for the generation of hiPSC-OCT4^{GFP}-indSKC, the ratio of viruses used was 0.5:0.05:0.05:0.05:0.15 (pMX-OCT4:pLVFUtetO-SOX2:pLVFUtetO-KLF4:pLVFUtetO-cMYC:FUdeltaGW-rtTA). Similarly, the remaining hiPSC lines were obtained by using different combinations of retroviruses and lentiviruses. After two serial infections, cells were plated onto fresh MEFs with DMEM (Invitrogen), 10% FBS and 0.1 mM non-essential amino acids supplemented with 100ng/ml (unless otherwise specified) of doxycycline. The following day, cells were switched to hESC medium:DMEM/F12 (Invitrogen) supplemented with 20% Knockout Serum Replacement (KOSR, Invitrogen), 1 mM L-glutamine, 0.1 mM non-essential amino acids, 55 µM β-mercaptoethanol, 10 ng/ml bFGF (Joint Protein Central) and 100ng/ml doxycycline. For the derivation of the hiPSC lines, GFP-positive colonies were manually picked and maintained on fresh MEF feeder layers for five passages before growth in Matrigel/mTesR1 conditions. For the generation of secondary hiPSC lines, the corresponding dFib-OCT4^{GFP} fibroblast-like cells were serially infected twice with retroviruses encoding the missing reprogramming factor or with miRNA-encoding lentiviruses. Immediately after the second infection, cells were incubated with DMEM (Invitrogen), 10% FBS and 0.1 mM non-essential amino acids supplemented with 100ng/ml (unless other specified) of doxycycline and two days later plated onto fresh MEFs or Matrigel. The following day, cells were switched to hESC medium supplemented with doxycycline until colonies appeared in the well. In all cases,

hiPSC colonies were stained for either alkaline phosphatase (AP) or NANOG expression, or used to establish independent cell lines. hiPSC43A2, hiPSC43B2, hiPSC43D6 and hiPSC57A5, hiPSC57A7, hiPSC57B7 lines were generated from human fibroblasts CRL-2429 and CRL-2522 using the commercial polycistronic lentivirus STEMCCA encoding the four reprogramming factors (Millipore, SCR510). F1hiPSC4F line was generated from F1 fibroblasts using the polycistronic lentivirus STEMCCA. CBhiPSC2F3, CBhiPSC3F12 and CBhiPSC4F3 lines were generated from human cord blood samples by retroviral infection. These hiPSC lines were maintained on MEFs or human feeder fibroblasts using KO DMEM medium (Life Technologies) in the presence of KOSR (Life Technologies) supplemented with 0.1 mM non-essential amino acids, 0.1mM mercaptoethanol, 1x glutamax and 10 ng/ml bFGF (Life Technologies).

3. Evaluation of reprogramming efficiency

To calculate the efficiency of reprogramming, the same number of dFib-OCT4^{GFP} fibroblast-like cells was plated on MEFs after the infection. The relative percentage of AP or NANOG⁺ colonies is shown as fold change relative to the value of the number of colonies generated with dFib-OCT4^{GFP} fibroblast-like cells infected with pmiR-000 lentiviruses (which encoded for a control miRNA). At least two independent experiments were performed by triplicate for verification.

4. Plasmids

pMX-OCT4, pMX-SOX2, pMX-KLF4, pMX-cMyc, FUDeltaGW-rtTA, pLVFUtetO-OCT4, pLVFUtetO-SOX2, pLVFUtetO-KLF4 and pLVFUtetO-cMYC were obtained from Addgene (plasmids 17217, 17218, 17219, 17220, 19780, 19778, 19779, 19777 and 19775 respectively). The lentiviral vectors encoding specific miRNAs (pMIRNA1) were purchased to SBY, System Biosciences.

5. Retroviral and lentiviral production

Moloney-based retroviruses were generated as described. Third generation lentiviral vectors (pLV-FU-tetO and pMIRNA) were co-transfected with packaging plasmids (pMDL, REV and VSVg, kindly provided by Dr. Oded Singer, Laboratory of Genetics Lab, The SALK Institute, La Jolla, CA) in 293T cells using Lipofectamine (Invitrogen). Supernatants were collected and passed through a 0.45 µM filter to remove cellular debris.

6. Immunostainings

Immunodetection of pluripotent markers in hiPSCs or of differentiation-associated markers in embryoid bodies or teratomas was performed as described in Ruiz *et al.*, 2011. Cells were fixed with 4% paraformaldehyde in PBS for 15 minutes, washed in PBS and incubated with 0.5% Triton-X100 in PBS for 10 minutes and blocked with 5 % normal donkey serum in 1% PBS-BSA for 1 hour at RT. Antibodies against NANOG (Abcam, Ab21624), TRA-1-60 (Chemicon, MAB4360), AFP (DAKO, A0008), FOXA2 (R&D, AF2400), TUJ1 (Covance, MMS-435-P), Alpha-smooth muscle actin (ASMA) (Sigma, A5228), Alpha-sarcomeric actin (ASA) (Sigma, A2172) and GFAP (Dako, AB1980) diluted in 1% PBS-BSA were used for overnight incubation at 4°C. After extensive washes with PBS, cells were incubated with secondary biotin-conjugated anti-rabbit antibody, AlexaFluor 488 or 568 (Invitrogen) anti-mouse, rabbit or goat antibodies where correspond for an additional 2 hours at room temperature. For the immunodetection of NANOG, cells were afterwards incubated with streptavidin-HRP (Vector) and a DAB substrate kit for peroxidase (Vector, SK-4100) was used to develop the staining. DAPI was used to visualize nuclei at a concentration of 10µg/ml in PBS.

7. RNA isolation and real time-PCR analysis

Total RNA was obtained using Trizol Reagent (Invitrogen) according the manufacturer's recommendations. The reverse transcription (RT) step was performed with 2µg of total RNA using the RT Supermix M-MuLV kit (BioPioneer). 0.25 µl of the final reaction was used to quantify gene expression by real time PCR using the SYBR-Green PCR Master mix (Applied Biosystems) in the ViiA 7 Real Time PCR System (Applied Biosystems). Values of gene expression were normalized using GAPDH expression and data is shown as fold change relative to the value of the sample control. All reactions were done in triplicate. Lists of the primers used for real time-PCR experiments are available in Panopoulos *et al.*, 2011, *PLoS One* 6(5): e19743, and in Maherali *et al.*, 2008, *Cell Stem Cell* 3: 340-45, each incorporated herein by reference. For the miRNA arrays, total RNA (including small RNA) from the samples indicated in the table below was extracted from 3·10⁶ cells by using RNeasy® Mini Kit (Qiagen) according to manufacturer's protocols. RNA was quantified on a NanoDrop 8000 spectrophotometer (Thermo Scientific). For microarray analysis, RNA quality was determined on a Bioanalyser 2100 (Agilent) and only RNA samples with an RNA integrity number (RIN) between 8 and 10 were used.

Table 1: Samples profiled on miRNA arrays.

<i>Sample name</i>	<i>Cell type</i>	<i>Origin</i>
CRL-2429	Neonatal fibroblasts	ATCC
CRL-2522	BJ foreskin fibroblasts	ATCC
CC-2511	Adult fibroblasts	Lonza
CC-2509	Neonatal fibroblasts	Lonza
CDMPRO	Melanocytes	Promocell
WA01	ESC H1 cell line	Wicell
WA07	ESC H7 cell line	Wicell
WA09	ESC H9 cell line	Wicell
hiPSC43A2	CRL-2429 fibroblasts reprogrammed with lentivirus STEMCCA	
hiPSC43B2	CRL-2429 fibroblasts reprogrammed with lentivirus STEMCCA	
hiPSC43ID6	CRL-2429 fibroblasts reprogrammed with lentivirus STEMCCA	
hiPSC57A5	CRL-2522 fibroblasts reprogrammed with lentivirus STEMCCA	
hiPSC57A7	CRL-2522 fibroblasts reprogrammed with lentivirus STEMCCA	
hiPSC57B7	CRL-2522 fibroblasts reprogrammed with lentivirus STEMCCA	
F1hiPSC4F	F1 fibroblasts reprogrammed with lentivirus STEMCCA	
CBhiPSC4F3	umbilical cord blood cells reprogrammed with retroviruses encoding OCT4, SOX2, KLF4 and cMYC	
CBhiPSC3F12	umbilical cord blood cells reprogrammed with retroviruses encoding OCT4, SOX2 and KLF4	
CBhiPSC2F3	umbilical cord blood cells reprogrammed with retroviruses encoding OCT4 and SOX2	

8. miRNA quantitation and data normalization

Megaplex profiling using human TaqMan Low Density miRNA Arrays (TLDA) (Applied Biosystems) was used to evaluate the expression of 667 miRNAs as described by the manufacturer. 600 ng of total RNA were used in two Megaplex reverse transcription (RT) reactions containing each one, a pool of Megaplex RT primers designed to detect and quantify up to 380 miRNAs and controls in a single reaction. No prior miRNA pre-amplification step was needed. Each reverse transcribed product was mixed with 2X TaqMan Universal PCR Master Mix, without AmpErase UNG (Applied Biosystems) and loaded onto a TLDA v2.0 containing 380 TaqMa miRNAs assays. TLDA were run on a 7900HT Thermocycler (Applied Biosystems) using Sequence Detection Systems (SDS) software version 2.3. Data analysis was performed using SDS RQ manager v1.2 (Applied Biosystems) which utilizes the delta-delta CT method. The threshold cycle (CT) is defined as the fractional cycle number at which the fluorescence exceeds the fixed threshold of 0.1. For each sample, the median of the CT values from detected signals was used to normalize the RNA input. A total of 26 miRNAs were identified as uniquely or strongly expressed in pluripotent cells compared to somatic cells (see Table 2 for list of miRNAs).

9. Derivation and validation of dFib-OCT4^{GFP} cells

For differentiation of pluripotent cells into fibroblast-like cells by forming embryoid bodies (EBs), pluripotent cell colonies growing on Matrigel were loosely detached by dispase treatment, resuspended in DMEM/F12 supplemented with 10% FBS (Atlanta Biologicals), 0.5 mM L-glutamine, 0.1 mM non-essential amino acids and 55 μ M β -mercaptoethanol and maintained on low attachment plates with daily media changes. After four days in suspension, EBs were plated onto gelatin-coated tissue culture plates and maintained in EB media for two additional days followed by their maintenance in DMEM, 10% FBS and 1% NEAA, until cells showed fibroblast morphology. Derived fibroblasts-like cells were serially passaged by using Tryple (Invitrogen) and tested for loss of pluripotent marker as well as GFP expression, and gain of expression of fibroblast markers.

10. AP-staining

For AP staining, cells were fixed in a solution of 4% paraformaldehyde in PBS for 20 minutes. After extensive washes in PBS, cells were incubated in NTMT solution (10 mM NaCl, 100 mM Tris-HCl (pH 9), 50 mM MgCl₂ and 0.1% Tween-20) for 5 minutes and then in NTMT solution supplemented with NBT (Nitro-Blue Tetrazolium Chloride) and

BCIP (5-Bromo-4-Chloro-3'-Indolylphosphate p-Toluidine Salt) in the dark until the staining was developed.

11. Reprogramming with miRNA-mimics

5 For the evaluation of the reprogramming efficiency after transfection with miRNA-mimics, BJ fibroblasts were infected only once by spinfection with a mix of retroviruses encoding OCT4, SOX2 and cMYC (day 0). At days 0 and 5 after initial infection, cells were transfected with miRNA-mimics (obtained from Qiagen) at 30 nM final concentration using Lipofectamine (Invitrogen) following manufacturer's recommendations. At day 6, cells
10 were transferred onto fresh MEFs with DMEM (Invitrogen), 10% FBS and 0.1 mM non-essential aminoacids. The following day, cells were switched to hESC medium (see above in section 2) until hiPSC colonies developed.

12. Statistical analyses

15 To obtain the list of miRNAs enriched in pluripotent cells, the results of three statistical procedures we accounted for. Pearson correlation (GeneSpring version 11.5.1 software, Agilent) was employed to identify and rank miRNAs with expression profiles similar to that of hsa-miR-302a, which miRNA is reportedly associated with pluripotency. Then, hypothesis-free hierarchical clustering (Ward method) and a principal components
20 analysis (SPSS Statistics® version 19 software, IBM) were used. Briefly, the samples were first classified into two main clusters: pluripotent samples and somatic cells. A two-tailed Student's-T test was used for evaluating the significance of the difference in expression levels of each miRNA between the two groups. The 177 major miRNAs which presented a significant ($p < 0.05$) difference between those two groups were then used in
25 the principal components analysis. The first principal component separated the hiPSC lines from the differentiated cells and was, therefore, interpreted as the pluripotency component (see Figure 4). The loading coefficients of principal component 1 ranked the miRNAs as predictors of pluripotency. Lastly, the expression levels of the miRNAs in each sample-group were binned into four categories: +++ (high); ++ (medium); + (low); - (no
30 expression). On the basis of the ranks of each miRNA resulting from the above statistical tests and its binned expression levels, 26 miRNAs were selected. The chromosome-19 cluster of miRNA genes is referred to as C19MC. TargetSca miRNA families are as described in <http://www.targetscan.org>, whereas hairpin families (mir-#) are as described in <http://www.mirbase.org/ftp.shtml> (miRBase file: hairpin.fa). Gene expression levels are

reported as mean $\pm$ SD. The number of biological replicates, independent experiments and technical replicates are given in the Brief Description of the Drawings section.

EXAMPLES

5

The invention now will be exemplified for the benefit of the artisan by the following non-limiting examples that depict some of the embodiments by and in which the invention can be practiced.

10 Example 1: Human inducible system for reprogramming of cells

Cell reprogramming was facilitated by the fact that retroviral expression of exogenous factors is epigenetically silenced after the reactivation of the pluripotent endogenous network, whereas the expression of the reprogramming factors delivered by lentiviruses can be still modulated by doxycycline independently of the somatic or pluripotent cell state. Takahashi and Yamanaka, 2006, *Cell* 26: 663-76. Therefore, infecting dFib-OCT4^{GFP} cells with a combination of retrovirus expressing one factor and a mix of inducible lentiviruses expressing the remaining three factors allowed for generation of primary hiPSCs (hiPSC-OCT4^{GFP}) (see Figure 1A). These cells can then be differentiated towards fibroblasts-like cells which retain the ability to re-express the three lentiviral-delivered reprogramming factors after the addition of doxycycline while the remaining retroviral-delivered factor will not be expressed. By infecting dFib-OCT4^{GFP} cells with retroviruses expressing OCT4 (pMX-OCT4) and lentiviruses expressing SOX2, KLF4 and cMYC (pLVFUtetO-SOX2, KLF4 and cMYC) hiPSCs-OCT4^{GFP}-indSKC were generated that could be further differentiated towards fibroblast-like cells (dFib-OCT4^{GFP}-indSKC) (Figures 1A). dFib-OCT4^{GFP}-indSKC cells express SOX2, KLF4 and cMYC after the addition of doxycycline but not OCT4. Under the proper culture conditions these cells do not reprogram unless exogenous OCT4 or a functional OCT4 substitute is supplied to the cells and detection of GFP can be used to identify fully reprogrammed cells. This cellular system represents a useful tool for screen functional substitutes of the reprogramming factors or new players in the reprogramming process.

dFib-OCT4^{GFP} cells were infected with different combinations of retroviruses and drug-inducible lentiviruses. This led to the successful generation of primary hiPSC-OCT4^{GFP} colonies (Figures 1A). Four hiPSC-OCT4^{GFP}-SKC, four hiPSC-OCT4^{GFP}-OKC, and nine hiPSC-OCT4^{GFP}-OSK lines were isolated and expanded in the absence of doxycycline.

35 Based on the expression of the reprogramming factors after the addition of doxycycline in

these hiPSC-OCT4^{GFP} lines, the hiPSC-OCT4^{GFP}-SKC-2 and 4, hiPSC-OCT4^{GFP}-OKC-2 and hiPSC-OCT4^{GFP}-OSK-10 and 11 lines were selected for further analysis. These reprogrammed cells were first verified for regained expression of GFP, expression of endogenous pluripotent markers, and downregulation of fibroblast markers (Figure 1B).

5 The pluripotency of these hiPSC lines was confirmed as they differentiated *in vitro* and contributed *in vivo* to the three embryonic germ layers.

The hiPSC-OCT4^{GFP} lines were then differentiated using an embryoid body protocol to obtain morphologically fibroblast-like cells (dFib-OCT4^{GFP}-ind). It was next determined that the different dFib-OCT4^{GFP}-ind fibroblast-like cells regained the expression of fibroblasts markers, downregulated the expression of pluripotent markers and lost the expression of GFP (Figure 2A). It was also observed that treatment of dFib-OCT4^{GFP}-ind cells with doxycycline induced the expression of the lentiviral-delivered reprogramming factors, but not the expression of the factor initially supplied by retroviral infection (Figure 2A).

15 **Example 2: Functional validation of the human reprogramming system**

Populations of dFib-OCT4^{GFP}-ind cells were validated for having the ability to reprogram back into pluripotency by the addition of doxycycline and supplying of a missing reprogramming factor. To accomplish this, dFib-OCT4^{GFP}-indSKC, dFib-OCT4^{GFP}-indOKC, and dFib-OCT4^{GFP}-indOSK cells were infected with retroviruses encoding OCT4, SOX2, and cMYC, respectively, and incubated with or without doxycycline in the culture media. They were then compared to untreated cells to evaluate their reprogramming abilities. First determined was the optimal concentration of doxycycline to reprogram the dFib-OCT4^{GFP}-ind cells (it has been reported that the level of expression of reprogramming factors can influence the efficiency of the reprogramming process) (Figure 2A). It was observed that in the case of dFib-OCT4^{GFP}-indSKC and dFib-OCT4^{GFP}-indOKC cells, reprogrammed colonies were only obtained when doxycycline and the missing factor were supplied to the cells. However, dFib-OCT4^{GFP}-indOSK could reprogram, although with much lower reprogramming efficiency, by the addition of doxycycline only. The appearance of reprogrammed colonies from dFib-OCT4^{GFP}-ind cells correlated with the expression of GFP. Moreover, GFP-positive hiPSCs also expressed pluripotent markers such as NANOG or TRA-1-60. These hiPSC colonies could be isolated and expanded as independent hiPSC cell lines which showed GFP expression, regained the expression of endogenous pluripotent markers and downregulated fibroblast markers (Figure 2A). These data demonstrate that an easy and reliable system which provides a GFP readout can be used to screen substitutes for the

functions of reprogramming factors or find new players in the reprogramming process in a high-throughput manner through addition of doxycycline only, and without any further viral manipulation.

5 **Example 3: miRNA expression increases the reprogramming efficiency**

A miRNA population of 18 samples comprising different subsets of pluripotent and somatic cells was profiled (see Table 1). Statistical analysis based on similarity with the hsa-miR-302a expression profile and principal component analysis combined with expression level comparisons led to the identification of 26 miRNAs specifically expressed
 10 in pluripotent cells or strongly downregulated in somatic cells (Figure 8A; Tables 2 and 3). Various miRNAs belonging to clusters were identified, such as hsa-miR-302, hsa-miR-372, and hsa-miR-373 (human orthologous to mouse mmu-miR-290), hsa-miR-200, or hsa-miR-520, which have been described as having a role in stem cell maintenance or participate in cell reprogramming in mouse cells (Judson *et al.*; Yang *et al.*; Subramanyam
 15 *et al.*, 2011, *Nat. Biotechnol.* 29(5): 443-48; Wang *et al.*, 2008, *Nat. Genet.* 40(12): 1478-83). Other identified miRNAs had never been associated with pluripotency. Whether the expression of these miRNAs influence the reprogramming efficiency in the absence of cMYC in human fibroblasts was then investigated.
 dFib-OCT4^{GFP}-indOSK-11 cells were infected with lentiviruses encoding each of the 26
 20 miRNAs selected for analysis (see Table 2 below), adding doxycycline to the media and followed the formation of hiPSC colonies.

Table 2: selected miRNAs

Sequence Name	Sequence	SEQ ID NO
hsa-miR-519d MIMAT0002853 Homo sapiens miR-519d	CAAAGUGCCUCCCUUUAGAGU	1
hsa-miR-519b-3p MIMAT0002837 Homo sapiens miR-519b-3p	AAAGUGCAUCCUUUUAGAGGUU	2
hsa-miR-519a MIMAT0002869 Homo sapiens miR-519a	AAAGUGCAUCCUUUUAGAGUGU	3
hsa-miR-518e MIMAT0002861 Homo	AAAGCGCUUCCCUUCAGAGUG	4

sapiens miR-518e		
hsa-miR-518b MIMAT0002844 Homo sapiens miR-518b	CAAAGCGCUCCCCUUUAGAGGU	5
hsa-miR-517c MIMAT0002866 Homo sapiens miR-517c	AUCGUGCAUCCUUUUAGAGUGU	6
hsa-miR-517a MIMAT0002852 Homo sapiens miR-517a	AUCGUGCAUCCCUUUAGAGUGU	7
hsa-miR-512-3p MIMAT0002823 Homo sapiens miR-512-3p	AAGUGCUGUCAUAGCUGAGGUC	8
hsa-miR-429 MIMAT0001536 Homo sapiens miR-429	UAAUACUGUCUGGUAAAACCGU	9
hsa-miR-20b MIMAT0001413 Homo sapiens miR-20b	CAAAGUGCUCAUAGUGCAGGUAG	10
hsa-miR-205 MIMAT0000266 Homo sapiens miR-205	UCCUUCAUUCCACCGGAGUCUG	11
hsa-miR-200b MIMAT0000318 Homo sapiens miR-200b	UAAUACUGCCUGGUAAUGAUGA	12
hsa-miR-200c MIMAT0000617 Homo sapiens miR-200c	UAAUACUGCCGGGUAAUGAUGGA	13
hsa-miR-363 MIMAT0000707 Homo sapiens miR-363	AAUUGCACGGUAUCCAUCUGUA	14
hsa-miR-141 MIMAT0000432 Homo sapiens miR-141	UAACACUGUCUGGUAAAGAUGG	15
hsa-miR-373 MIMAT0000726 Homo sapiens miR-373	GAAGUGCUUCGAUUUUGGGGUGU	16
hsa-miR-372 MIMAT0000724 Homo sapiens miR-372	AAAGUGCUGCGACAUUUGAGCGU	17
hsa-miR-371-3p MIMAT0000723 Homo	AAGUGCCGCCAUCUUUUGAGUGU	18

sapiens miR-371-3p		
hsa-miR-367 MIMAT0000719 Homo sapiens miR-367	AAUUGCACUUUAGCAAUGGUGA	19
hsa-miR-302d MIMAT0000718 Homo sapiens miR-302d	UAAGUGCUUCCAUGUUUGAGUGU	20
hsa-miR-302c MIMAT0000717 Homo sapiens miR-302c	UAAGUGCUUCCAUGUUUCAGUGG	21
hsa-miR-302b MIMAT0000715 Homo sapiens miR-302b	UAAGUGCUUCCAUGUUUUAGUAG	22
hsa-miR-302a MIMAT0000684 Homo sapiens miR-302a	UAAGUGCUUCCAUGUUUUGGUGA	23
hsa-miR-520h MIMAT0002867 Homo sapiens miR-520h	ACAAAGUGCUUCCCUUUAGAGU	24
hsa-miR-520g MIMAT0002858 Homo sapiens miR-520g	ACAAAGUGCUUCCCUUUAGAGUGU	25
hsa-miR-520c-3p MIMAT0002846 Homo sapiens miR-520c-3p	AAAGUGCUUCCUUUUAGAGGGU	26

Table 3 : selected miRNA and data for their selection

miRNA	PC1+	Expr. level	Cluster chr (cluster)	miR family TargetScan	Gene family miRBase
hsa-miR-302a	0.936	-/+++	chr4 (302)	miR-106/302	mir-302 (hsa-mir-302b/c/d/f)
hsa-miR-302b	0.935	-/++	chr4 (302)	miR-106/302	
hsa-miR-302d	0.878	-/+++	chr4 (302)	miR-106/302	
hsa-miR-302c	0.841	-/+++	chr4 (302)	miR-106/302	
hsa-miR-372	0.869	-/++	chr19 (371/372/373)	miR-106/302	mir-290 (hsa-mir-371/371b/372)
hsa-miR-520c-3p	0.861	-/++	chr19 (C19MC)	miR-106/302	mir-515
hsa-miR-373	0.839	-/++	chr19 (371/372/373)	miR-106/302	mir-373
hsa-miR-367	0.928	-/+++	chr4 (302)	miR-25/32/92ab/363/367	mir-367 (hsa-mir367)
hsa-miR-363	0.796	-/++	chrX (363/92a-2/19b-2/20b/18b/106a)	miR-25/32/92ab/363/367	mir-363 (hsa-mir-363)
hsa-miR-371-3p	0.821	-/++	chr19 (371/372/373)	miR-371-3p	mir-290 (hsa-mir-371/371b/372)
hsa-miR-200c	0.810	-/+++	chr12 (200c/141)	miR-200bc/429	mir-8 (hsa-mir-141/-200a/-200b/-200c/-429)
hsa-miR-200b	0.767	-/++	chr1 (200b/200a/429)	miR-200bc/429	
hsa-miR-429	0.747	-/++	chr1 (200b/200a/429)	miR-200bc/429	
hsa-miR-141	0.844	-/++	chr12 (200c/141)	miR-141/200a	
hsa-miR-519a	0.914	-/+	chr19 (C19MC)	miR-291b-3p/519a/519b-3p/519c-3p	mir-515 (hsa-mir-515/-515/-516a/-516b/-517a/-517b/-517c/-518a/-518b/-518c/-518d/-518e/-518f/-518g/-519a/-519b/-519c/-519d/-519e/-520a/-520b/-520c/-520d/-520e/-520f/-520g/-520h/-521/-522/-523/-524/-525/-526a/-526b/-527/-1283)
hsa-miR-519b-3p	0.690	-/+	chr19 (C19MC)	miR-291b-3p/519a/519b-3p/519c-3p	
hsa-miR-512-3p	0.944	-/++	chr19 (C19MC) (512-1; 512-2)	miR-512-3p/1186	mir-506 (hsa-mir-509-1/-509-2/-509-3/-510/-511/-512/-513/-514/-515/-516/-517/-518/-519/-520/-521/-522/-523/-524/-525/-526a/-526b/-527/-1283)
hsa-miR-517a	0.962	-/++	chr19 (C19MC)	miR-517ac	mir-515
hsa-miR-517c	0.960	-/++	chr19 (C19MC)	miR-517ac	mir-515
hsa-miR-518b	0.906	-/+	chr19 (C19MC)	miR-518a-3p/518b/518c/518d-3p	mir-515
hsa-miR-518e	0.869	-/+	chr19 (C19MC)	miR-518e	mir-515
hsa-miR-520h	0.921	-/++	chr19 (C19MC)	miR-520gh	mir-515
hsa-miR-520g	0.912	-/++	chr19 (C19MC)	miR-520gh	mir-515
hsa-miR-519d	0.941	-/++	chr19 (C19MC)	miR-17-5p/20/93.mr/106/519.d	mir-515
hsa-miR-20b	0.830	+ /+++	chrX (363/92a-2/19b-2/20b/18b/106a)	miR-17-5p/20/93.mr/106/519.d	mir-17 (hsa-mir-17/-18a/-18b/-20a/-20b/-93/-106a/-106b)
hsa-miR-205	0.792	-/++	chr1 (no)	miR-205	mir-205 (hsa-mir-205)

It was observed that, although the expression of hsa-miR-200b (SEQ ID NO: 12), -520c-3p (SEQ ID NO: 26), -371-3p (SEQ ID NO: 18), -512-3p (SEQ ID NO: 8), or -20b (SEQ ID NO: 10) promoted the formation of hiPSC colonies to some extent, the expression of hsa-miR-367 (SEQ ID NO: 19), -372 (SEQ ID NO: 17), -373 (SEQ ID NO: 16), -429 (SEQ ID NO: 9), and -519a (SEQ ID NO: 3) greatly improved the reprogramming efficiency in the absence of cMYC (Figure 3A). To validate these effects, a similar reprogramming experiment was performed using BJ fibroblasts (Figure 3B). Similar results were observed and it was determined that expression of stem cell-specific miRNAs with a shared seed sequence, as reported for mouse cells, have a profound impact in reprogramming efficiency (Wang *et al.*). Due to its great effect in reprogramming efficiency, hsa-miR-519a (SEQ ID NO: 3) was investigated further. Whether the effect of hsa-miR-519a (SEQ ID NO: 3) was dependent on the expression of cMYC was first analyzed, as it has been shown that expression of some miRNAs, such as hsa-miR-429 (SEQ ID NO: 9) or members of the hsa-miR-290 cluster, are directly regulated by the binding of cMYC to their promoters. Expression of hsa-miR-291, -294, and -295 do not have a positive further effect in the formation of hiPSC colonies when cMYC is included in the reprogramming experiments (Figure 3C) (Judson *et al.*). However, it was observed that the effect of hsa-miR-519a (SEQ ID NO: 3) seemed to be independent of cMYC because the reprogramming efficiency remained increased in experiments where cMYC was also expressed (Figure 3C). Furthermore, reprogramming efficiency was also increased after transfection of hsa-miR-519a (SEQ ID NO: 3) mimics in BJ fibroblasts or dFib-OCT4^{GFP} cells (Figures 3D and 3E).

To get insights into the mechanisms by which hsa-miR-519a (SEQ ID NO: 3) positively affects cell reprogramming, cellular targets of this miRNA were investigated based on bioinformatic predictions from DIANA-MICROT, MICRORNA.org, MIRDB, RNA22-HSA, TARGETMINER and TARGETSCAN-VERT according to links found in miRNA database. Among all the predicted targets, TGF β RII was focused on because it has been already demonstrated to be a target of several miRNAs implicated in enhancing the reprogramming efficiency (Li *et al.*; Subramanyam *et al.*). The 3' UTR region of the TGF β RII mRNA contains two well conserved recognition sequences for hsa-miR-519a (SEQ ID NO: 3) and its expression downregulates the level of TGF β RII mRNA in fibroblasts (Figures 3F and 3G).

[0001] It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

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CLAIMS

1. An *in vitro* method for reprogramming a somatic cell comprising the step of
5 exposing the cell to (i) a miRNA and (ii) one, two or more reprogramming factors.
2. The method of claim 1 wherein the miRNA comprises a seed sequence selected from the group consisting of SEQ ID NO: 28, SEQ ID NO: 27 and SEQ ID NO: 29-33.
- 10 3. The method of claim 2, wherein the miRNA is selected from the group consisting of hsa-miR-519a (SEQ ID NO: 3), hsa-miR-429 (SEQ ID NO: 9), hsa-miR-367 (SEQ ID NO: 19), hsa-miR-372 (SEQ ID NO: 17), hsa-miR-373 (SEQ ID NO: 16), hsa-miR-200b (SEQ ID NO: 12), hsa-miR-520c-3p (SEQ ID NO: 26), hsa-miR-371-3p (SEQ ID NO: 18), hsa-miR-512-3p (SEQ ID NO: 8), and hsa-miR-20b (SEQ ID NO: 10).
- 15 4. The method of claim 3, wherein the miRNA is hsa-miR-519a (SEQ ID NO: 3) or hsa-miR-429 (SEQ ID NO: 9).
5. The method of claims 1-4, wherein the cell is exposed to the miRNA by contacting
20 the cell with the miRNA.
6. The method of claims 1-5, wherein the cell is exposed to the miRNA by infecting the cell with a viral vector encoding the miRNA.
- 25 7. The method of claim 6, wherein the viral vector is a retrovirus or lentivirus.
8. The method of claims 1-7, wherein at least one of the reprogramming factors is selected from the group consisting of OCT4, SOX2, KLF4, and cMYC.
- 30 9. The method of claims 1-8, wherein the cell is exposed to the reprogramming factor by contacting the cell with the reprogramming factor.
10. The method of claims 1-9, wherein the cell is exposed to the reprogramming factor by infecting the cell with a viral vector encoding the reprogramming factor.

11. The method of claim 10, wherein the viral vector is a retrovirus or lentivirus.

12. The method of claims 1-11, wherein the one, two or more reprogramming factors are selected from the group consisting of OCT4, SOX2, KLF4, and cMYC.

5

13. The method of claim 12, wherein the cell is exposed to the reprogramming factors by contacting the cell with the reprogramming factors.

14. The method of claims 12-13, wherein the cell is exposed to the reprogramming factors by infecting the cell with one or more viral vectors encoding the reprogramming factors.

10

15. The method of claim 14, wherein at least one of the viral vectors is a retrovirus or lentivirus.

15

16. The method of claims 1-15, further comprising exposing the cell to a second miRNA.

17. The method of claim 16, wherein the second miRNA is selected from the group consisting of hsa-miR-519a (SEQ ID NO: 3), hsa-miR-429 (SEQ ID NO: 9), hsa-miR-367 (SEQ ID NO: 19), hsa-miR-372 (SEQ ID NO: 17), hsa-miR-373 (SEQ ID NO: 16), hsa-miR-200b (SEQ ID NO: 12), hsa-miR-520c-3p (SEQ ID NO: 26), hsa-miR-371-3p (SEQ ID NO: 18), hsa-miR-512-3p (SEQ ID NO: 8), and hsa-miR-20b (SEQ ID NO: 10).

20

18. The method of claims 16-17, wherein the cell is exposed to the second miRNA by contacting the cell with the second miRNA.

19. The method of claims 16-18, wherein the cell is exposed to the second miRNA by infecting the cell with a viral vector encoding the second miRNA.

30

20. The method of claim 19, wherein the viral vector is a retrovirus or lentivirus.

21. An *in vitro* method for reprogramming or enhancing reprogramming in a somatic cell comprising the step of exposing the cell to (i) a miRNA comprising a seed sequence of SEQ ID NO: 28 and (ii) the reprogramming factors OCT4, SOX2, and KLF4.

35

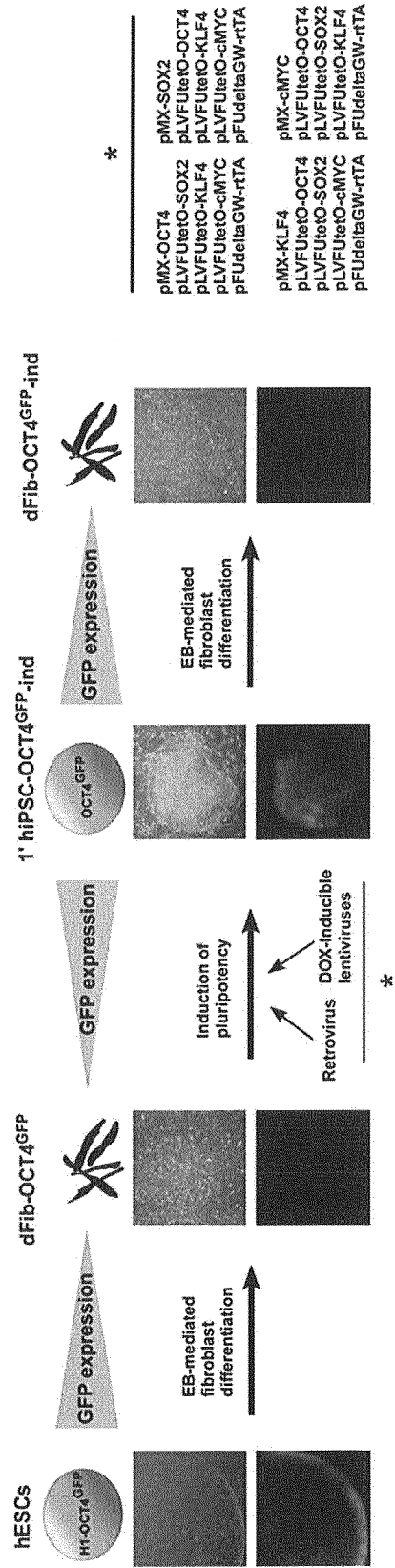
22. The method of claim 21 wherein the miRNA is hsa-miR-519a (SEQ ID NO: 3).

23. *In vitro* use of a miRNA for enhancing reprogramming in a somatic cell.

5

24. Use according to claim 23, wherein said miRNA is a miRNA as defined in anyone of claims 1 to 4.

Fig 1A



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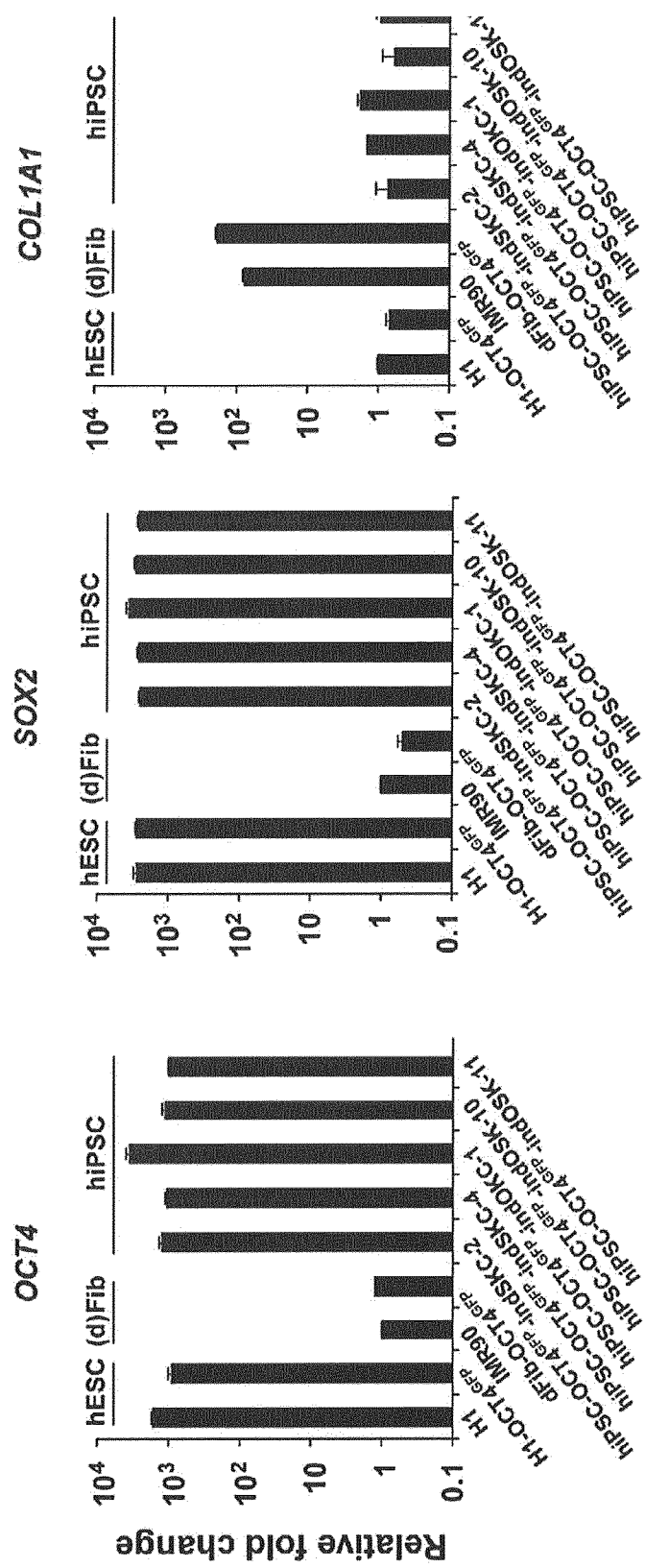


Fig 1B

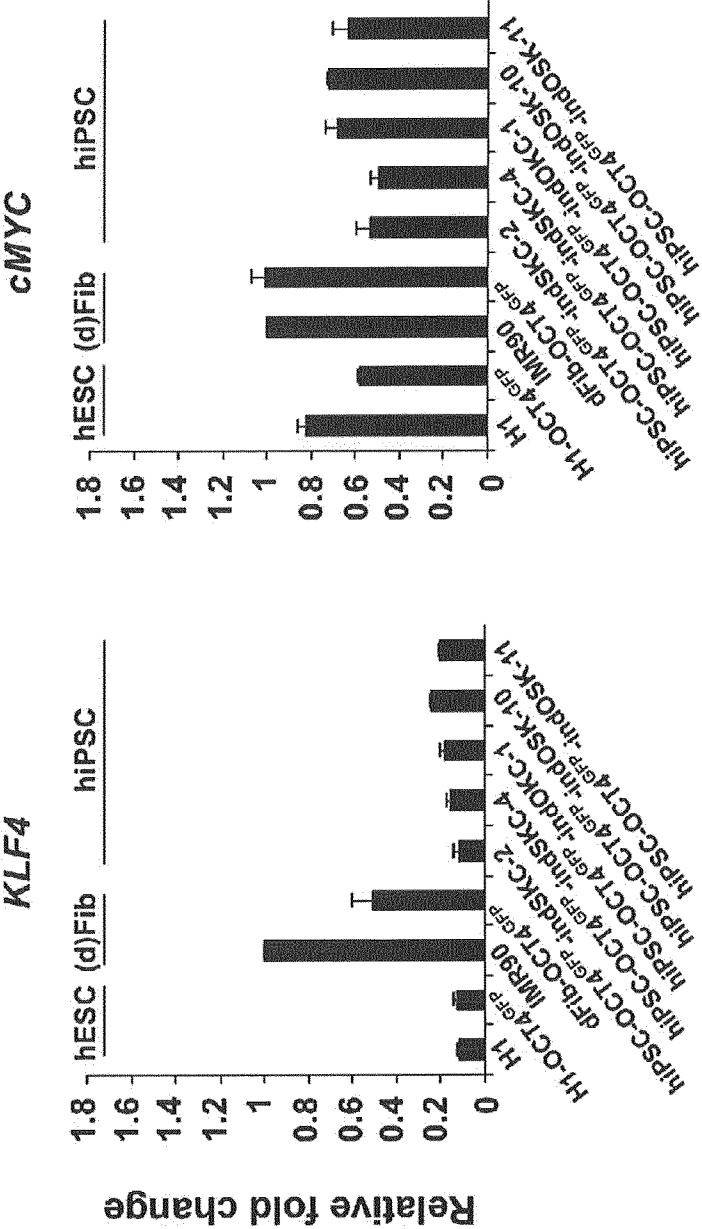


Fig 1B

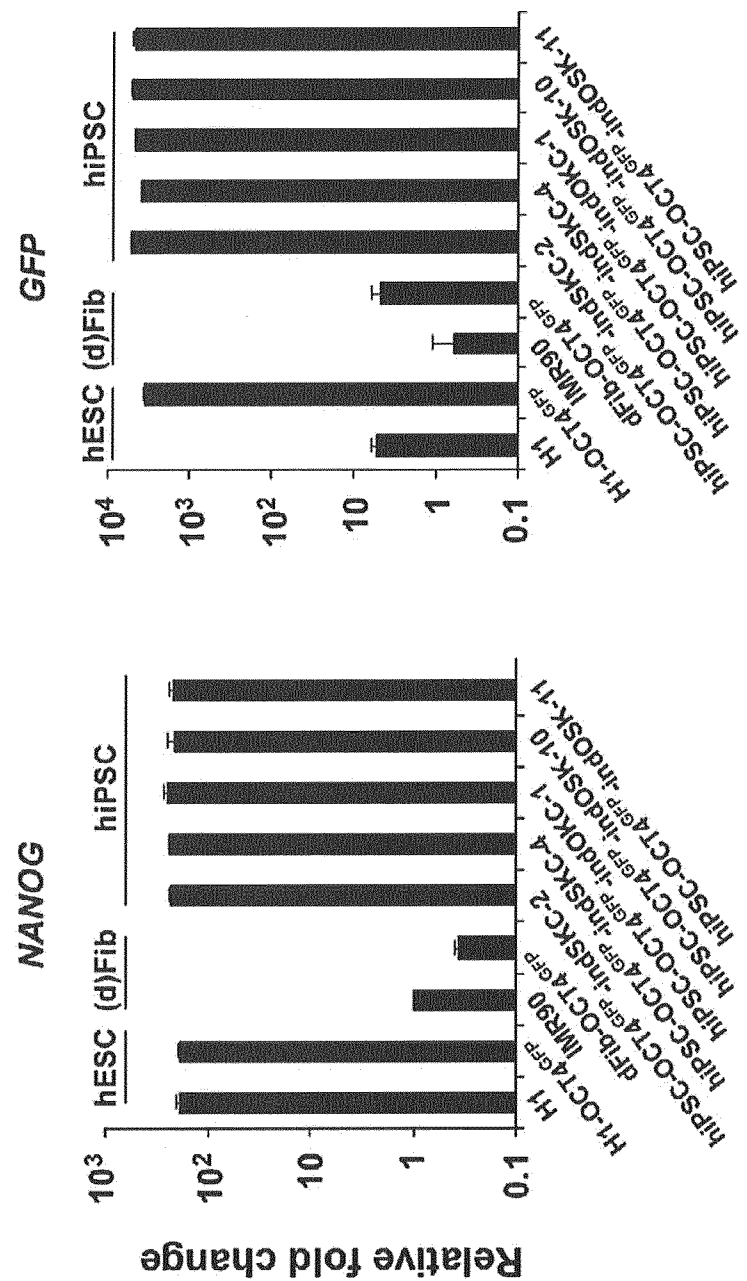
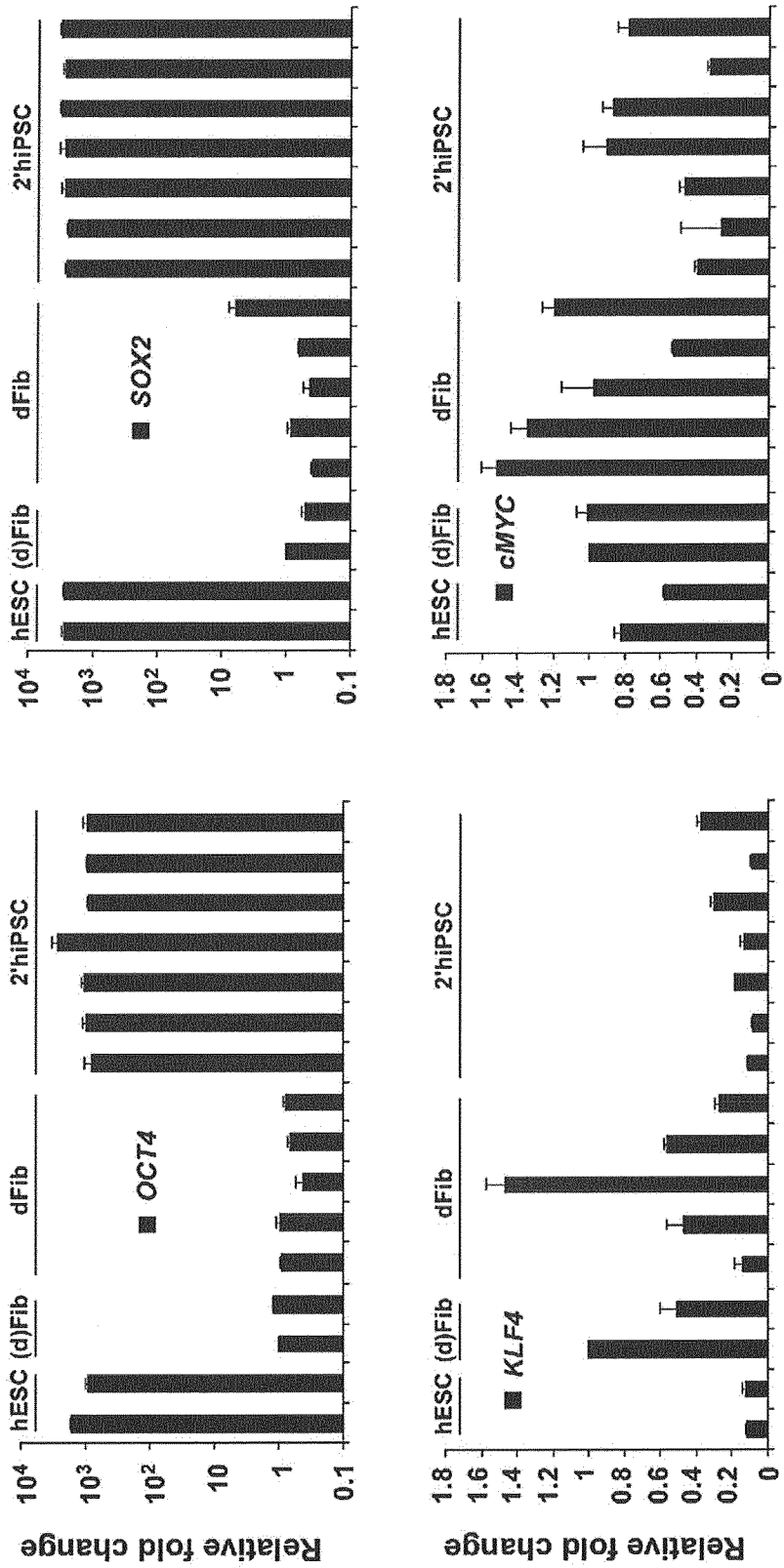


Fig 2A



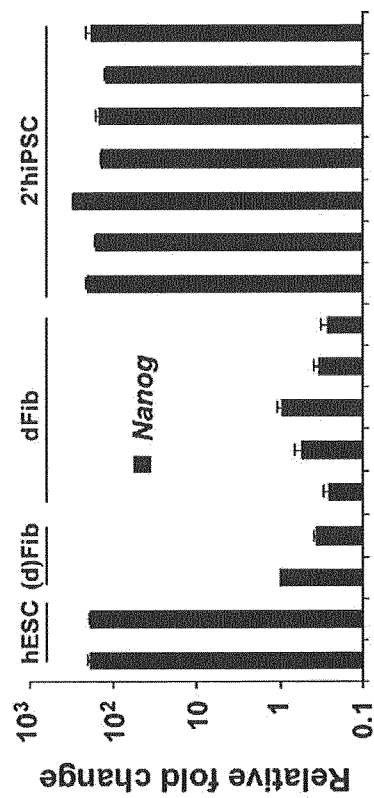
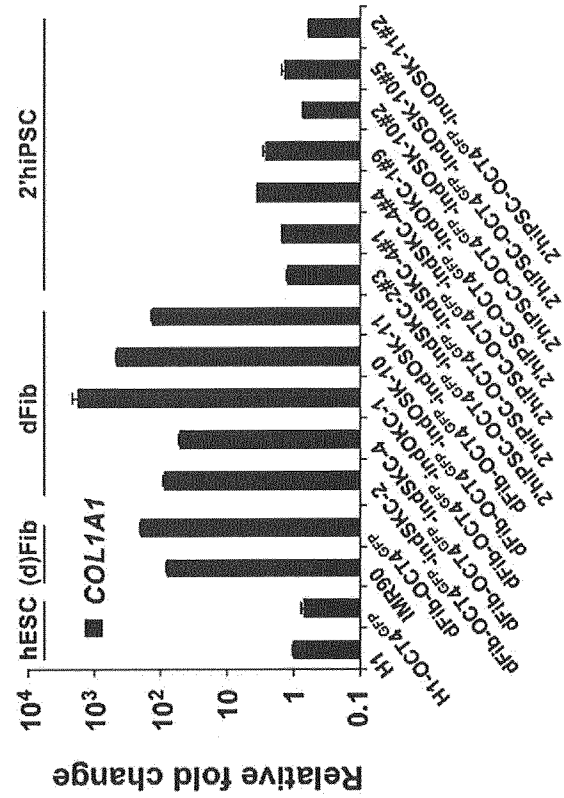
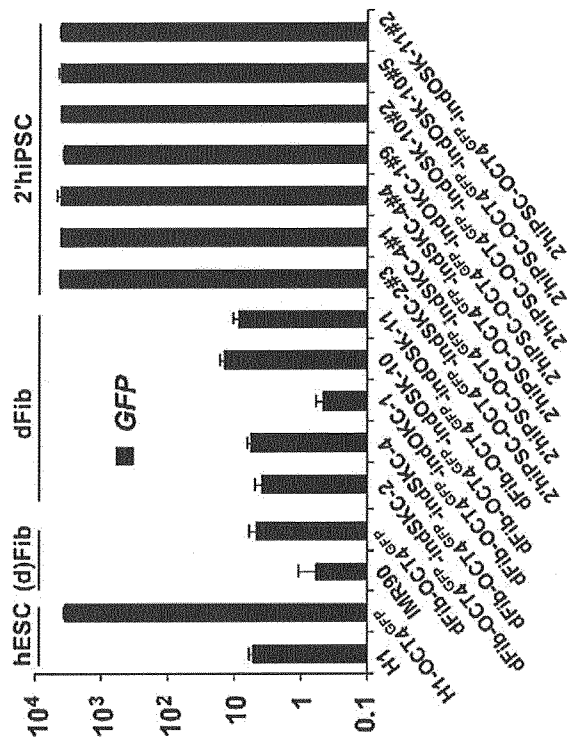


Fig 2B

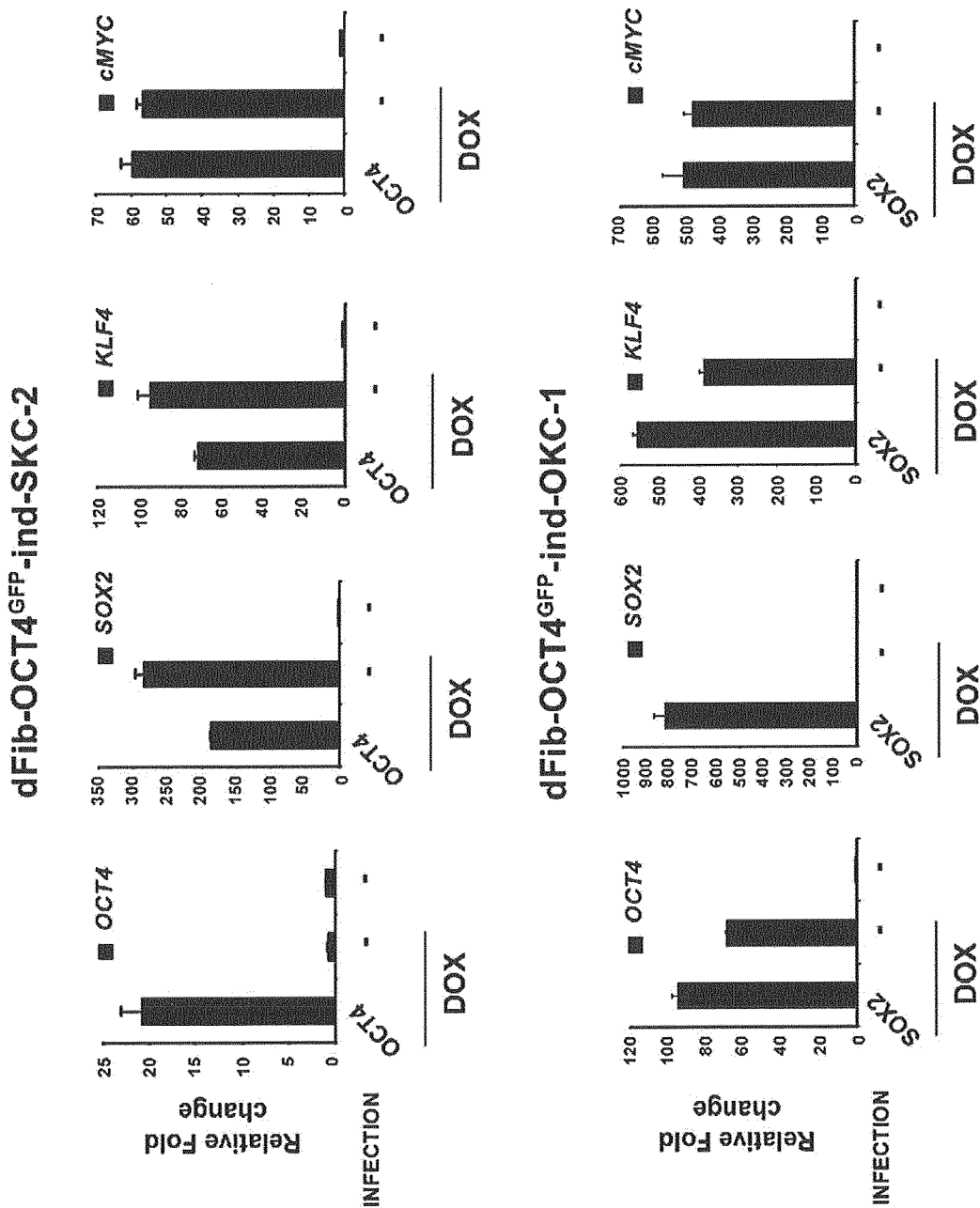


Fig 2B

dFib-OCT4^{GFP}-ind-OSK-11

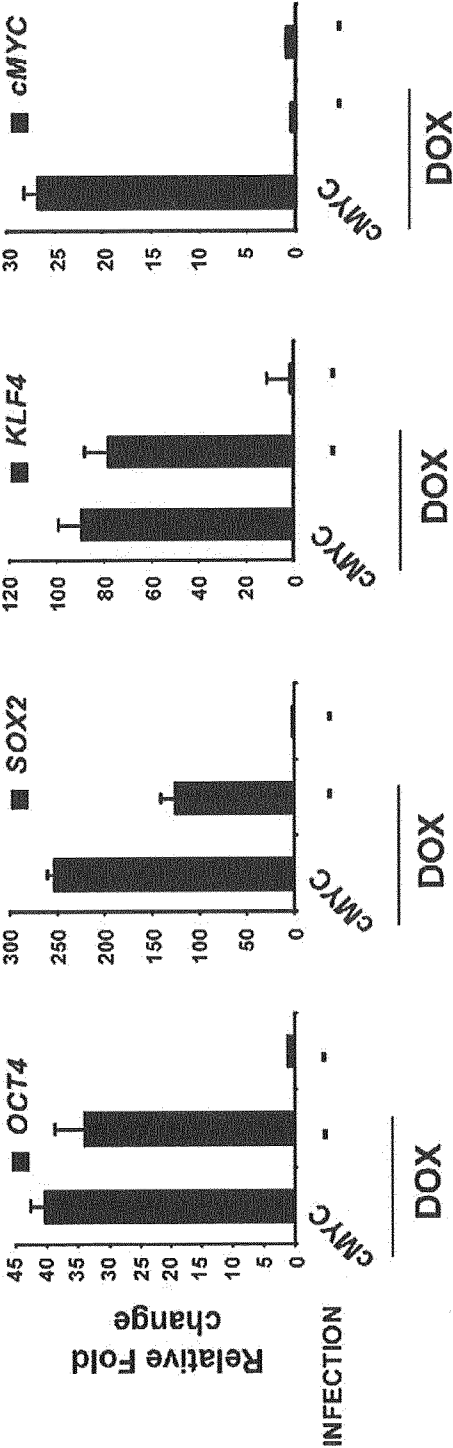
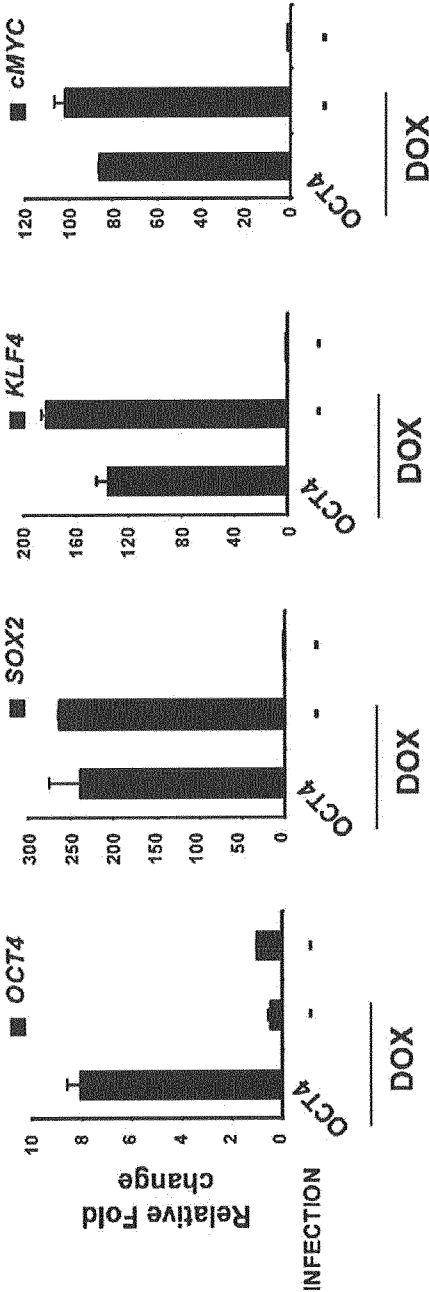


Fig 2B

dFib-OCT4^{GFP}-ind-SKC-4



dFib-OCT4^{GFP}-ind-OSK-10

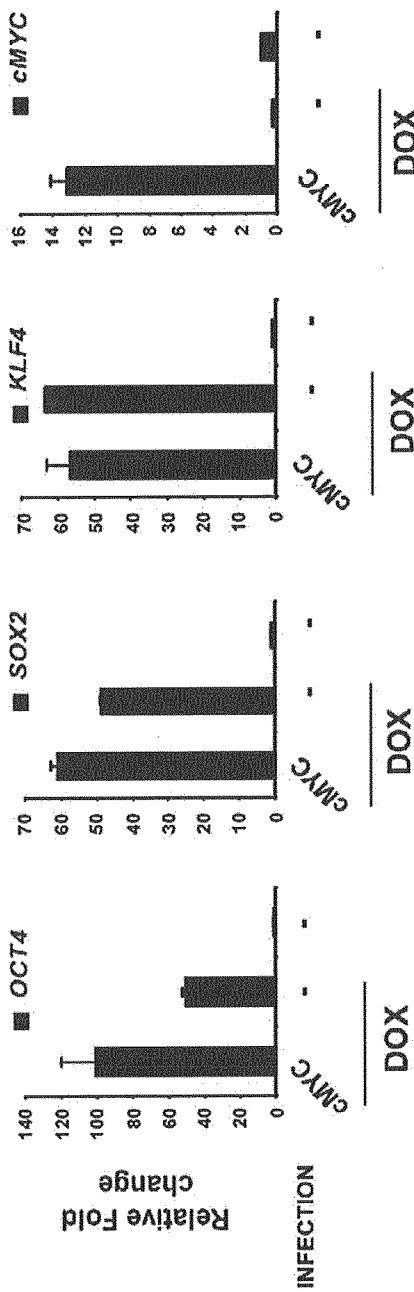
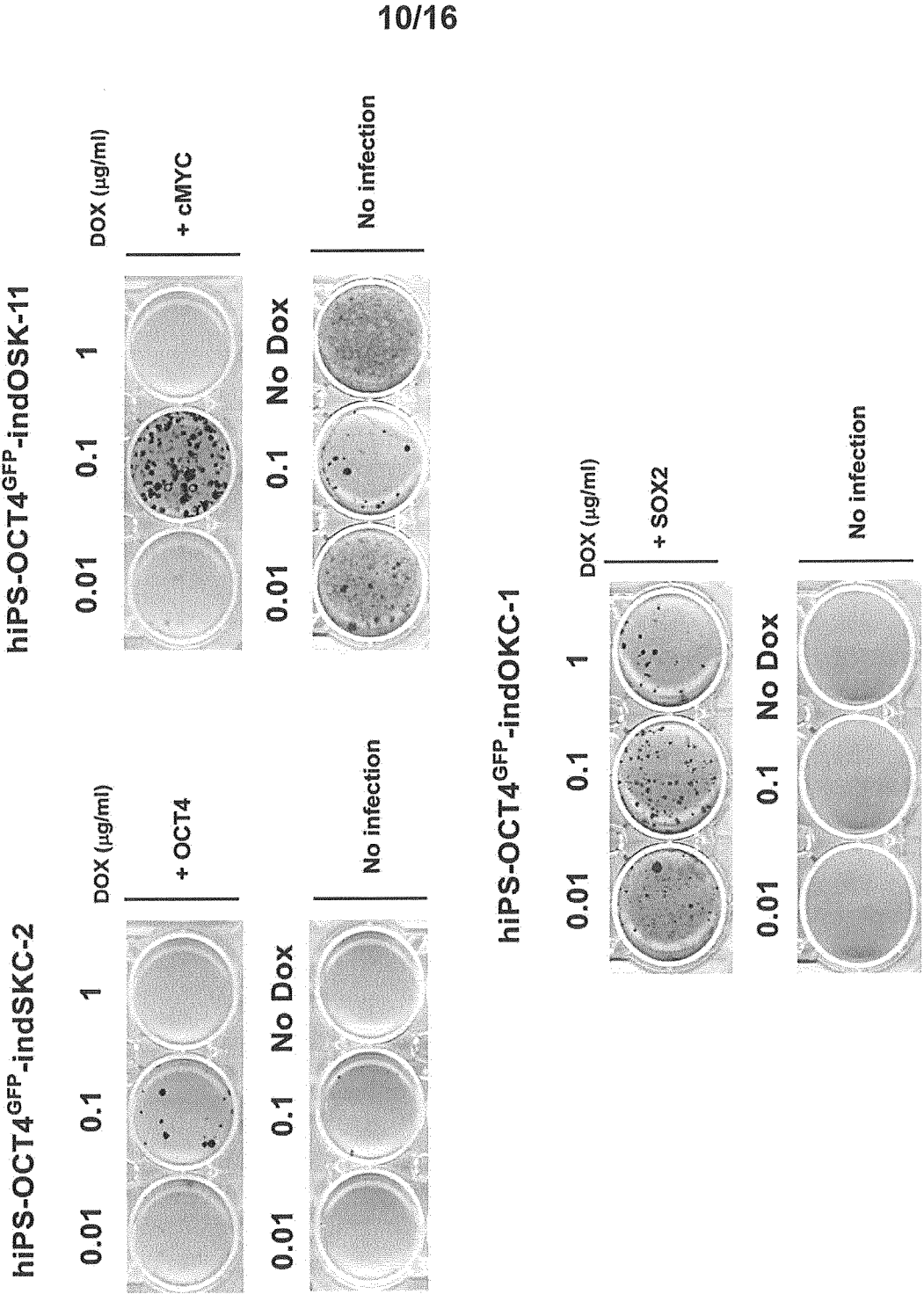


Fig 2C



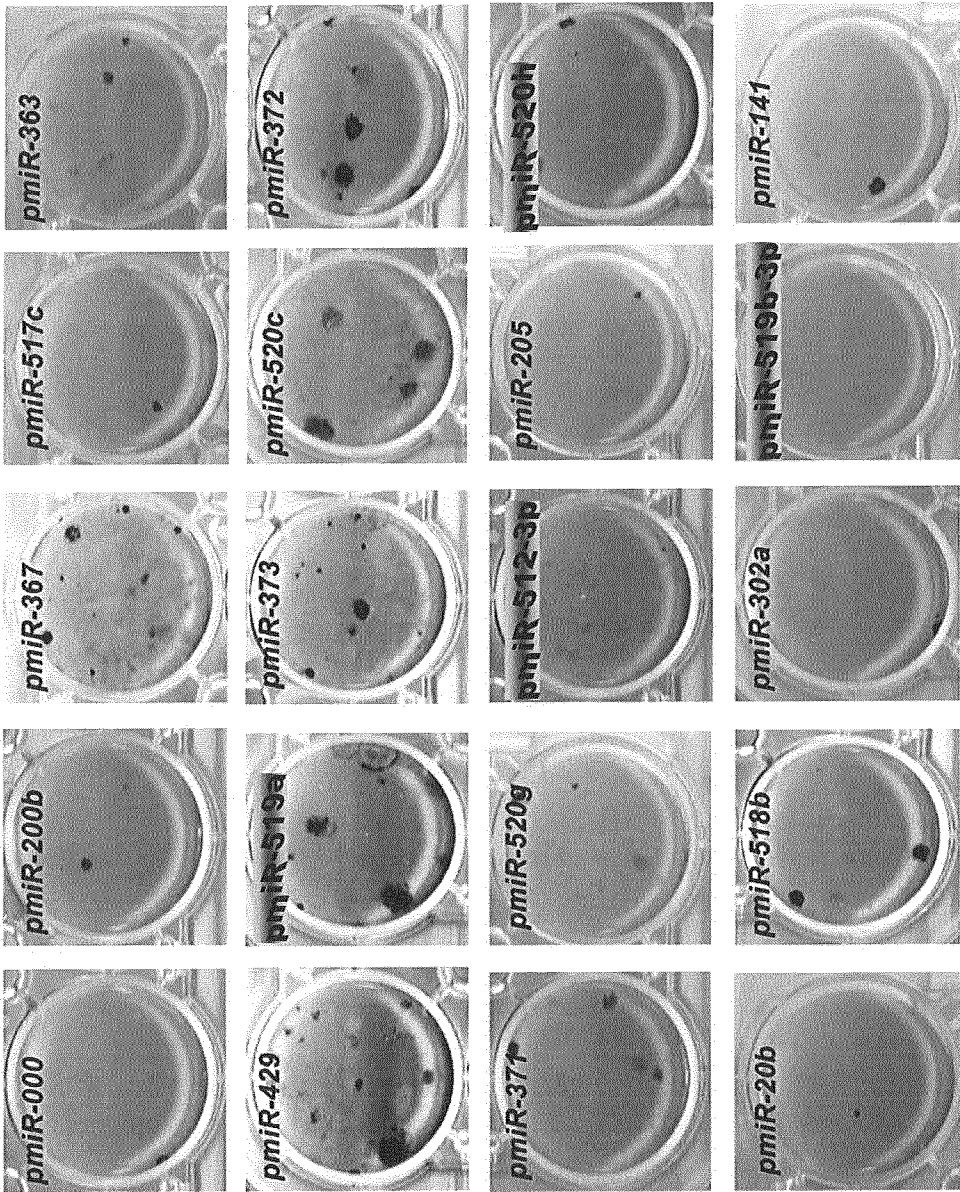
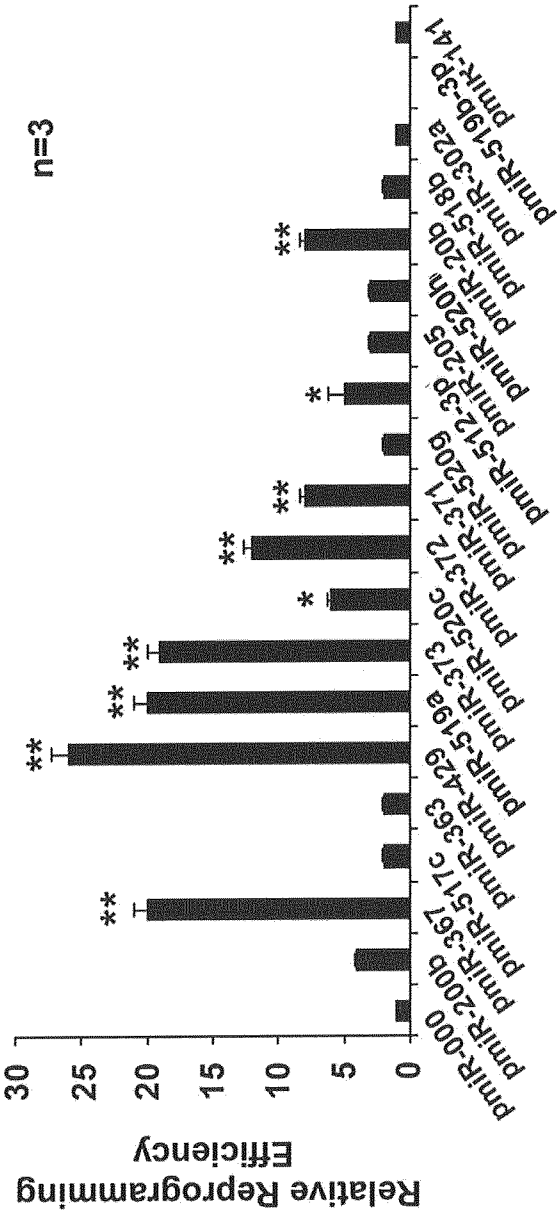


Fig 3A

Fig 3A



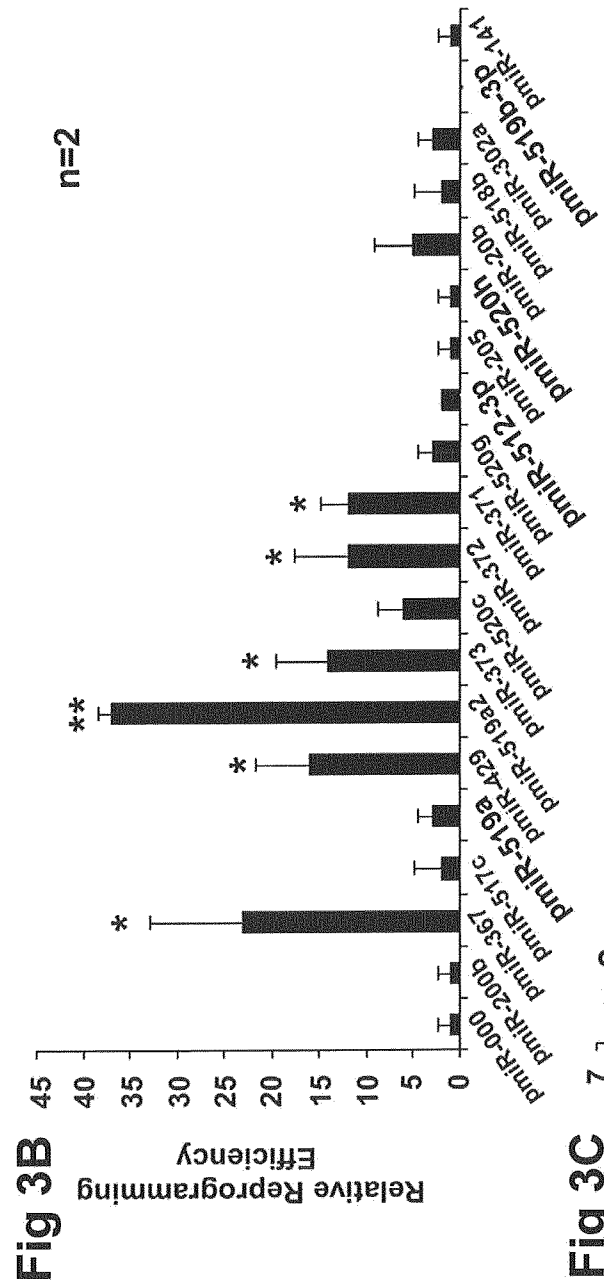
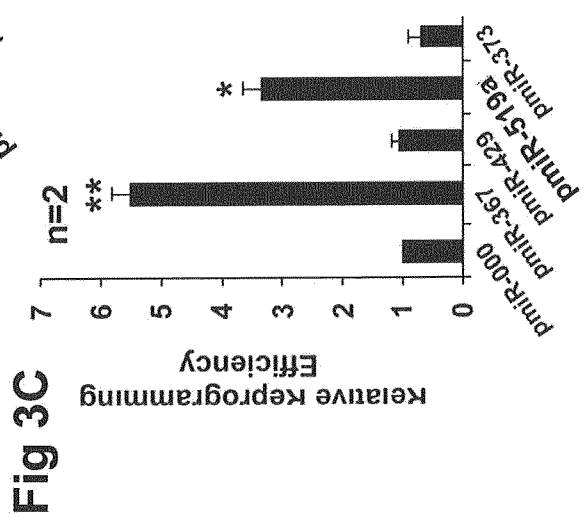


Fig 3E

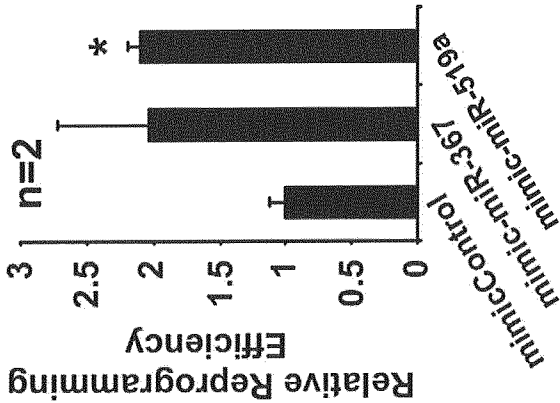
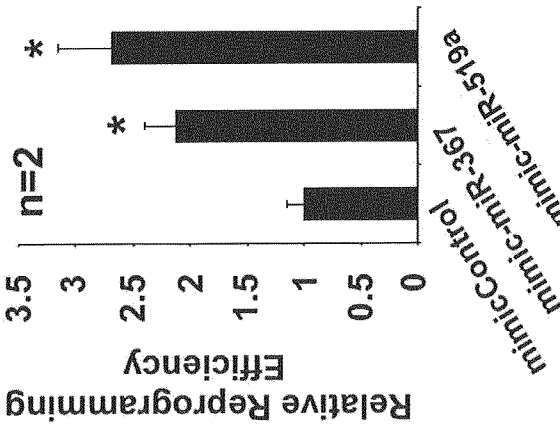
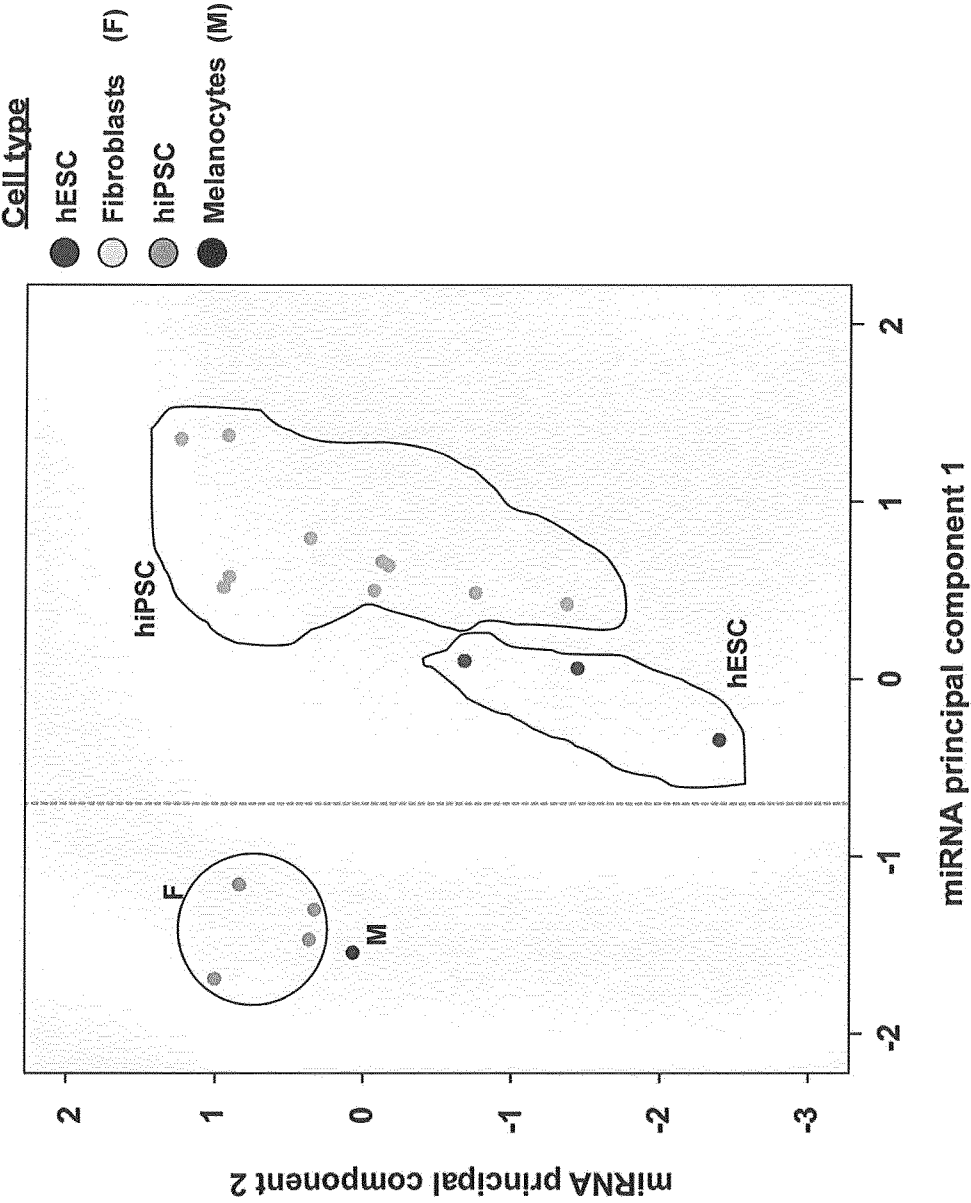


Fig 3D





INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2013/068678

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-24(partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2013/068678

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N15/113 C07K14/47
ADD.

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 C07K

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

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

EPO-Internal, EMBASE, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/023413 A1 (ALCEDO BIOTECH GMBH [DE]; BULLERDIEK JOERN [DE]; RIPPE VOLKHARD [DE]) 3 March 2011 (2011-03-03)	23,24
Y	claim 16	1-22
Y	----- US 2010/075421 A1 (YAMANAKA SHINYA [JP] ET AL) 25 March 2010 (2010-03-25)	1-22
A	claims 1, 7, 8, 12, 20, 23; table 2	23,24
X,P	----- WO 2012/151309 A1 (SANFORD BURNHAM MED RES INST [US]; RANA TARIQ M [US]) 8 November 2012 (2012-11-08)	1-24
	claims 1, 8, 9, 39, 44 ----- -/-	



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

8 November 2013

Date of mailing of the international search report

22/01/2014

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Faber, Cerstin

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2013/068678

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEN JIE ET AL: "Synergetic cooperation of microRNAs with transcription factors in iPS cell generation.", PLOS ONE 2012, vol. 7, no. 7, E40849, 13 July 2012 (2012-07-13), pages 1-13, XP002702926, ISSN: 1932-6203 the whole document -----	1-24
A	CHEN JING ET AL: "Overexpression of miR-429 induces mesenchymal-to-epithelial transition (MET) in metastatic ovarian cancer cells.", GYNECOLOGIC ONCOLOGY, vol. 121, no. 1, April 2011 (2011-04), pages 200-205, XP002702927, ISSN: 1095-6859 the whole document -----	1-24
A	WO 2011/037270 A1 (UNIV KYOTO [JP]; YAMANAKA SHINYA [JP]; TANABE KOJI [JP]) 31 March 2011 (2011-03-31) claims 7, 8 -----	1-24
A	US 2008/293143 A1 (LIN SHI-LUNG [US] ET AL) 27 November 2008 (2008-11-27) the whole document -----	1-24
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A	WELSTEAD G G ET AL: "The reprogramming language of pluripotency", CURRENT OPINION IN GENETICS & DEVELOPMENT, vol. 18, no. 2, 1 April 2008 (2008-04-01), pages 123-129, XP022698490, CURRENT BIOLOGY LTD ISSN: 0959-437X, DOI: 10.1016/J.GDE.2008.01.013 [retrieved on 2008-03-20] the whole document -----	1-24
A	LAURENT LOUISE C ET AL: "Comprehensive microRNA profiling reveals a unique human embryonic stem cell signature dominated by a single seed sequence", STEM CELLS, vol. 26, no. 6, 1 June 2008 (2008-06-01), pages 1506-1516, XP002577532, ALPHAMED PRESS, INC, UNITED STATES ISSN: 1549-4918, DOI: 10.1634/STEMCELLS.2007-1081 [retrieved on 2008-04-10] the whole document -----	1-24

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-24(partially)

An in vitro method for reprogramming a somatic cell comprising the steps of exposing the cell to (i) hsa-miR-519a (SEQ ID NO: 3) and (ii) one, two or more reprogramming factors; an in vitro method for reprogramming or enhancing reprogramming in a somatic cell comprising the step of exposing the cell to (i) hsa-miR-519a (SEQ ID NO: 3) comprising a seed sequence of SEQ ID NO: 28 and (ii) the reprogramming factors OCT4, SOX2 and KLF4; in vitro use of hsa-miR-519a (SEQ ID NO: 3) for enhancing reprogramming in a somatic cell.

2. claims: 1-24(partially)

An in vitro method for reprogramming a somatic cell comprising the steps of exposing the cell to (i) hsa-miR-429 (SEQ ID NO: 9) and (ii) one, two or more reprogramming factors; an in vitro method for reprogramming or enhancing reprogramming in a somatic cell comprising the step of exposing the cell to (i) hsa-miR-429 (SEQ ID NO: 9) comprising a seed sequence of SEQ ID NO: 28 and (ii) the reprogramming factors OCT4, SOX2 and KLF4; in vitro use of hsa-miR-429 (SEQ ID NO: 9) for enhancing reprogramming in a somatic cell.

3. claims: 1-24(partially)

An in vitro method for reprogramming a somatic cell comprising the steps of exposing the cell to (i) hsa-miR-367 (SEQ ID NO: 19) and (ii) one, two or more reprogramming factors; an in vitro method for reprogramming or enhancing reprogramming in a somatic cell comprising the step of exposing the cell to (i) hsa-miR-367 (SEQ ID NO: 19) comprising a seed sequence of SEQ ID NO: 28 and (ii) the reprogramming factors OCT4, SOX2 and KLF4; in vitro use of hsa-miR-367 (SEQ ID NO: 19) for enhancing reprogramming in a somatic cell.

4. claims: 1-24(partially)

An in vitro method for reprogramming a somatic cell comprising the steps of exposing the cell to (i) hsa-miR-372 (SEQ ID NO: 17) and (ii) one, two or more reprogramming factors; an in vitro method for reprogramming or enhancing reprogramming in a somatic cell comprising the step of exposing the cell to (i) hsa-miR-372 (SEQ ID NO: 17) comprising a seed sequence of SEQ ID NO: 28 and (ii) the reprogramming factors OCT4, SOX2 and KLF4; in vitro use of

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hsa-miR-372 (SEQ ID NO: 17) for enhancing reprogramming in a somatic cell.

5. claims: 1-24(partially)

An in vitro method for reprogramming a somatic cell comprising the steps of exposing the cell to (i) hsa-miR-373 (SEQ ID NO: 16) and (ii) one, two or more reprogramming factors; an in vitro method for reprogramming or enhancing reprogramming in a somatic cell comprising the step of exposing the cell to (i) hsa-miR-373 (SEQ ID NO: 16) comprising a seed sequence of SEQ ID NO: 28 and (ii) the reprogramming factors OCT4, SOX2 and KLF4; in vitro use of hsa-miR-373 (SEQ ID NO: 16) for enhancing reprogramming in a somatic cell.

6. claims: 1-24(partially)

An in vitro method for reprogramming a somatic cell comprising the steps of exposing the cell to (i) hsa-miR-200b (SEQ ID NO: 12) and (ii) one, two or more reprogramming factors; an in vitro method for reprogramming or enhancing reprogramming in a somatic cell comprising the step of exposing the cell to (i) hsa-miR-200b (SEQ ID NO: 12) comprising a seed sequence of SEQ ID NO: 28 and (ii) the reprogramming factors OCT4, SOX2 and KLF4; in vitro use of hsa-miR-200b (SEQ ID NO: 12) for enhancing reprogramming in a somatic cell.

7. claims: 1-24(partially)

An in vitro method for reprogramming a somatic cell comprising the steps of exposing the cell to (i) hsa-miR-520c-3p (SEQ ID NO: 26) and (ii) one, two or more reprogramming factors; an in vitro method for reprogramming or enhancing reprogramming in a somatic cell comprising the step of exposing the cell to (i) hsa-miR-520c-3p (SEQ ID NO: 26) comprising a seed sequence of SEQ ID NO: 28 and (ii) the reprogramming factors OCT4, SOX2 and KLF4; in vitro use of hsa-miR-520c-3p (SEQ ID NO: 26) for enhancing reprogramming in a somatic cell

8. claims: 1-24(partially)

An in vitro method for reprogramming a somatic cell comprising the steps of exposing the cell to (i) hsa-miR-371-3p (SEQ ID NO: 18) and (ii) one, two or more reprogramming factors; an in vitro method for reprogramming or enhancing reprogramming in a somatic cell comprising the step of exposing the cell to (i) hsa-miR-371-3p (SEQ ID NO: 18) comprising a seed sequence of SEQ ID NO: 28 and (ii) the

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reprogramming factors OCT4, SOX2 and KLF4; in vitro use of hsa-miR-371-3p (SEQ ID NO: 18) for enhancing reprogramming in a somatic cell.

9. claims: 1-24(partially)

An in vitro method for reprogramming a somatic cell comprising the steps of exposing the cell to (i) hsa-miR-512-3p (SEQ ID NO: 8) and (ii) one, two or more reprogramming factors; an in vitro method for reprogramming or enhancing reprogramming in a somatic cell comprising the step of exposing the cell to (i) hsa-miR-512-3p (SEQ ID NO: 8) comprising a seed sequence of SEQ ID NO: 28 and (ii) the reprogramming factors OCT4, SOX2 and KLF4; in vitro use of hsa-miR-512-3p (SEQ ID NO: 8) for enhancing reprogramming in a somatic cell.

10. claims: 1-24(partially)

An in vitro method for reprogramming a somatic cell comprising the steps of exposing the cell to (i) hsa-miR-20b (SEQ ID NO: 10) and (ii) one, two or more reprogramming factors; an in vitro method for reprogramming or enhancing reprogramming in a somatic cell comprising the step of exposing the cell to (i) hsa-miR-20b (SEQ ID NO: 10) comprising a seed sequence of SEQ ID NO: 28 and (ii) the reprogramming factors OCT4, SOX2 and KLF4; in vitro use of hsa-miR-20b (SEQ ID NO: 10) for enhancing reprogramming in a somatic cell.
