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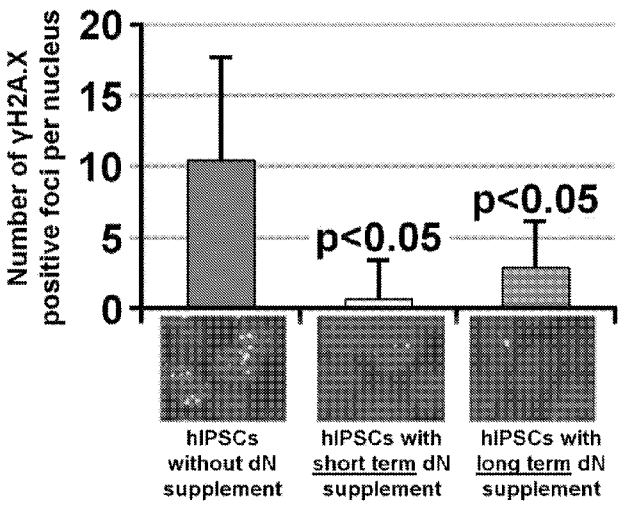

Fig. 7

(57) Abstract: In various embodiments, a cell culture medium, or a nucleoside cocktail transmission (NCT) medium for the culture of stem cells with improved genetic stability, cellular function and regeneration ability is provided. Illustrative culture media comprise a basal culture medium for stem cells, where the culture medium is supplemented with one or more nucleoside triphosphates (*e.g.*, dNTPs and/or NPs) or one or more precursors thereof. Illustrative NCT media comprise a delivery vehicle (*e.g.* skin creams or other vehicle) containing one or more nucleoside triphosphates (e.g., dNTPs and/or NPs) or one or more precursors thereof. The NCT medium provides, *inter alia*, direct delivery of nucleoside cocktails into human tissues (such as skin) for regenerative, cosmetic and/or therapeutic purposes.

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NUCLEOSIDE SUPPLEMENTATION TO PROMOTE CELLULAR FUNCTION, GENETIC STABILITY AND AUGMENT TRANSGENIC EXPRESSION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. provisional application Serial No. 62/147,936 filed April 15, 2015, which is incorporated herein by reference in its entirety.

STATEMENT OF GOVERNMENTAL SUPPORT

[Not Applicable]

BACKGROUND

Although induced pluripotent stem cells (iPSCs) share great similarities with embryonic stem cells (ESCs), the somatic cell reprogramming methods used for iPSC derivation have caused both scientific interest and concerns about the new cell type. There are important biosafety concerns about the genomic integrity and stability of iPSCs, as well as ESCs, during their derivation and prolonged culture, in addition to significant concerns regarding substandard/subphysiological cellular functions (such as suboptimal proliferation and differentiation) in suboptimally supplemented environmental conditions. The usefulness of human induced pluripotent stem cells (or other stem or somatic cells) in research, cosmetic and therapeutic/regenerative applications highly relies on their genomic integrity, stability and functionality. Certain genomic or epigenomic abnormalities may not only compromise the differentiation potential but also cause tumorigenesis in the recipients of iPSC-based therapies. Genomic abnormalities have been observed as karyotypic aberrations such as changes in chromosomal number and structures, copy number variations (CNVs) such as subkaryotypic or subchromosomal amplifications and deletions, loss of heterozygosity (LOH) due to acquired uniparental disomy, and random or site-specific integration of alien DNA into the host genome.

Studies indicate that various somatic and stem cells and essentially all IPSC clones derived using current approaches acquire genomic instability during

reprogramming and *in vitro* culture-induced stress (Martins-Taylor and Xu (2012) *Stem Cells* 30: 22-27; Lund *et al.* (2012) *Nat. Rev. Genet.,* 13: 732-744) in addition to possessing substandard physiology (such as impaired cellular proliferation). The issue of hiPSC genomic instability is one of the most important bottlenecks in advancing personalized iPSC-based regenerative therapies to the clinic (Martins-Taylor and Xu (2012) *Stem Cells* 30: 22-27; Lund *et al.* (2012) *Nat. Rev. Genet.,* 13: 732-744; Byrne (2013) *Gene Therapy and Regulation* 7: 1230002), given the established link between genomic instability and an increased risk of malignant transformation.

The causes of genomic instability and impaired cellular physiology during hiPSC reprograming and culture, and indeed for other pluripotent stem cells (such as human embryonic stem cells) and somatic stem cells (such as neural stem cells, mesenchymal stem cells and dermal stem cells) are unclear, and approaches to reduce and eventually eliminate the occurrence of this highly deleterious phenomenon have yet to be developed.

SUMMARY

It was discovered that the genomic integrity, stability and functionality, of stem cells and somatic cells can be augmented by exogenous supplementation of nucleosides (*e.g.*, deoxyribonucleosides). Such a nucleoside supplement (referred to as a nucleoside cocktail (NC) or, in certain embodiments a deoxyribonucleoside Cocktail (DC) can be used to augment cell culture media or can be combined with a delivery vehicle to produce a nucleoside cocktail transmission (NCT) medium, or in certain embodiments a deoxyriobnucleoside transmission cocktail (DCT). Such supplementation, in addition to improving genomic stability can enhance cellular function (for example, by speeding up cell proliferation, promoting differentiation into target therapeutic cell types (such as hepatocytes), and/or promoting human tissue regeneration (such as stimulating division of human skin cells *in vivo* for regenerative purposes).

Moreover it was observed that exposure of human skin fibroblasts to a nucleoside cocktail can stimulate proliferation at a significantly augmented rate, and this phenomenon, when combined with nucleoside transmission media such as a skin

cream, can prove very useful in directly regenerating/rejuvenating human skin or other tissues.

The nucleoside cocktail not only improves genomic stability, it also significantly augments proliferation rate of a wide variety of somatic and stem cells in addition to augmenting differentiation of pluripotent stem cells into target cell types (such as hepatocytes for treatment of liver disease).

Thus, some embodiments provide a basal culture medium for somatic and stem cells, where the culture medium is supplemented with one or more nucleoside triphosphates (*e.g.*, dNTPs and/or NTPs) or one or more precursors thereof. Other embodiments combine the one or more nucleoside triphosphates (*e.g.*, dNTPs and/or NTPs) or one or more precursors thereof with topical and/or delivery mechanisms *in vivo* use inclusive of, but not limited to: 1) skin creams, 2) topical cosmetics and/or 3) extrinsic derivery mechanisms (EDMs), such as injectables, ingestion, inhalation or other.

Accordingly, in various aspects, the invention(s) contemplated herein may include, but need not be limited to, any one or more of the following embodiments:

In various aspects, the invention(s) contemplated herein may include, but need not be limited to, any one or more of the following embodiments:

Embodiment 1: A cell culture medium for the culture of stem cells with improved genetic stability, said culture medium including: a basal culture medium for stem cells, where said culture medium is supplemented with one or more nucleoside triphosphates or one or more precursors thereof.

Embodiment 2: A nucleoside cocktail transmission (NCT) medium for improving somatic or stem cell genetic stability said medium including: a cosmetic or pharmaceutical vehicle; and one or more nucleoside triphosphates or precursors thereof.

Embodiment 3: The cell culture medium of embodiment 1 or nucleoside cocktail transmission medium of embodiment 2, wherein said one or more nucleoside triphosphates are independently selected from the group consisting of deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (NCTP), deoxythymidine triphosphate (dTTP)

deoxyuridine triphosphate, adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), 5-methyluridine triphosphate (m5UTP), and uridine triphosphate (UTP).

Embodiment 4: The cell culture medium of embodiment 1 or nucleoside cocktail transmission medium of embodiment 2, wherein said one or more nucleoside triphosphates are independently selected from the group consisting of deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (NCTP), deoxythymidine triphosphate (dTTP) and deoxyuridine triphosphate.

Embodiment 5: The cell culture medium of embodiment 1 or nucleoside cocktail transmission medium of embodiment 2, wherein said one or more nucleoside triphosphates are independently selected from the group consisting of adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), 5-methyluridine triphosphate (m5UTP), and uridine triphosphate (UTP).

Embodiment 6: The cell culture medium according to any one of embodiments 1 and 3-5 or nucleoside cocktail transmission medium according to any one of embodiments 2-5, wherein said culture medium is supplemented with, or said NCT medium includes, one or more precursors of nucleoside triphosphates independently selected from the group consisting of deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (NCTP), deoxythymidine triphosphate (dTTP) deoxyuridine triphosphate, adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), 5-methyluridine triphosphate (m5UTP), and uridine triphosphate (UTP).

Embodiment 7: The cell culture or NCT medium of embodiment 6, wherein said one or more precursors of nucleoside triphosphates are independently selected from the group consisting of deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (NCTP), deoxythymidine triphosphate (dTTP) and deoxyuridine triphosphate.

Embodiment 8: The cell culture or NCT medium of embodiment 6, wherein said one or more precursors of nucleoside triphosphates are independently selected from the group consisting of adenosine triphosphate (ATP), guanosine

triphosphate (GTP), cytidine triphosphate (CTP), 5-methyluridine triphosphate (m5UTP), and uridine triphosphate (UTP).

Embodiment 9: The cell culture medium according to any one of embodiments 1 and 3-8, or the NCT medium according to any one of embodiments 3-8, wherein said culture medium is supplemented with, or said NCT medium includes, deoxyadenosine triphosphate (dATP), or a precursor thereof.

Embodiment 10: The cell culture medium or NCT medium of embodiment 9, said culture medium is supplemented with, or said NCT medium includes, deoxyadenosine triphosphate.

Embodiment 11: The cell culture medium or NCT medium of embodiment 9, said culture medium is supplemented with, or said NCT medium includes, a precursor of deoxyadenosine triphosphate.

Embodiment 12: The cell culture medium or NCT medium of embodiment 11, wherein said precursor of deoxyadenosine triphosphate is selected from the group consisting of deoxyadenosine diphosphate, deoxyadenosine monophosphate, deoxyadenosine, and adenine.

Embodiment 13: The cell culture medium according to any one of embodiments 1, and 3-12, or the NCT medium according to any one of embodiments 2-12, wherein said culture medium is supplemented with, or said NCT medium includes, deoxyguanosine triphosphate (dGTP), or a precursor thereof.

Embodiment 14: The cell culture medium or NCT medium according of embodiment 13, wherein said cell culture medium is supplemented with or said NCT medium includes deoxyguanosine triphosphate.

Embodiment 15: The cell culture medium or NCT medium according of embodiment 13, wherein said cell culture medium is supplemented with or said NCT medium includes a precursor of deoxyguanosine triphosphate.

Embodiment 16: The cell culture or NCT medium of embodiment 15, wherein said precursor of deoxyguanosine triphosphate selected from the group consisting of deoxyguanosine diphosphate, deoxyguanosine monophosphate, deoxyguanosine, and guanine.

Embodiment 17: The cell culture medium according to any one of embodiments 1, or 3-16, or the NCT medium according to any one of embodiments 2-16, wherein said culture medium is supplemented with or said NCT medium includes deoxycytidine triphosphate (NCTP), or a precursor thereof.

Embodiment 18: The cell culture medium or NCT medium of embodiment 17, wherein said cell culture medium is supplemented with, or said NCT medium includes deoxycytidine triphosphate.

Embodiment 19: The cell culture medium or NCT medium of embodiment 17, wherein said cell culture medium is supplemented with, or said NCT medium includes a precursor of deoxycytidine triphosphate.

Embodiment 20: The cell culture medium or NCT medium of embodiment 19, wherein precursor of deoxycytidine is selected from the group consisting of deoxycytidine diphosphate, deoxycytidine monophosphate, deoxycytidine, and cytosine.

Embodiment 21: The cell culture medium according to any one of embodiments 1, and 3-20, or the NCT medium according to any one of embodiments 2-20, wherein said culture medium is supplemented with, or said NCT includes deoxythymidine triphosphate (dTTP), or a precursor thereof.

Embodiment 22: The cell culture medium or NCT medium of embodiment 20, wherein said culture medium is supplemented with, or said NCT medium includes deoxythymidine triphosphate.

Embodiment 23: The cell culture medium or NCT medium of embodiment 20, wherein said culture medium is supplemented with, or said NCT medium includes a precursor of deoxythymidine triphosphate.

Embodiment 24: The cell culture medium or NCT medium of embodiment 23, wherein said precursor of deoxythymidine triphosphate is selected from the group consisting of deoxythymidine diphosphate, deoxythymidine monophosphate, deoxythymidine, and thymine.

Embodiment 25: The cell culture medium according to any one of embodiments 1, and 3-24, or the NCT medium according to any one of embodiments

2-24, wherein said culture or NCT medium is supplemented with deoxyuridine triphosphate, or a precursor thereof.

Embodiment 26: The cell culture medium or NCT medium of embodiment 25, wherein said culture medium is supplemented with, or said NCT medium includes deoxyuridine triphosphate.

Embodiment 27: The cell culture medium or NCT medium of embodiment 25, wherein said culture medium is supplemented with, or said NCT medium includes a precursor of deoxyuridine triphosphate.

Embodiment 28: The cell culture medium or NCT medium of embodiment 27, wherein said precursor of deoxyuridine triphosphate is selected from the group consisting of deoxyuridine diphosphate, deoxyuridine monophosphate, deoxyuridine, and uracil.

Embodiment 29: The cell culture medium according to any one of embodiments 1, and 3-28, or the NCT medium according to any one of embodiments 2-28, wherein said culture or NCT medium is supplemented with adenosine triphosphate (ATP) or a precursor thereof.

Embodiment 30: The cell culture medium or NCT medium of embodiment 29, wherein said culture medium is supplemented with, or said NCT medium includes, adenosine triphosphate (ATP).

Embodiment 31: The cell culture medium or NCT medium of embodiment 29, wherein said culture medium is supplemented with, or said NCT medium includes, a precursor of adenosine triphosphate.

Embodiment 32: The cell culture medium or NCT medium of embodiment 31, wherein said precursor of adenosine triphosphate (ATP) is selected from the group consisting of adenosine diphosphate, adenosine monophosphate, adenosine, and adenine.

Embodiment 33: The cell culture medium according to any one of embodiments 1, and 3-32, or the NCT medium according to any one of embodiments 2-32, wherein said culture medium is supplemented with, or said NCT medium includes guanosine triphosphate (GTP) or a precursor thereof.

Embodiment 34: The cell culture medium or NCT medium of embodiment 33, wherein said culture medium is supplemented with, or said NCT medium includes, guanosine triphosphate (GTP).

Embodiment 35: The cell culture medium or NCT medium of embodiment 33, wherein said culture medium is supplemented with, or said NCT medium includes, a precursor of guanosine triphosphate.

Embodiment 36: The cell culture medium or NCT medium of embodiment 35, wherein said precursor of guanosine triphosphate is selected from the group consisting of guanosine diphosphate, guanosine monophosphate, guanosine, and guanine.

Embodiment 37: The cell culture medium according to any one of embodiments 1, and 3-36, or the NCT medium according to any one of embodiments 2-36, wherein said cell culture medium is supplemented with, or said NCT medium includes cytidine triphosphate (CTP), or a precursor thereof.

Embodiment 38: The cell culture medium or NCT medium of embodiment 37, wherein said culture medium is supplemented with, or said NCT medium includes, cytidine triphosphate.

Embodiment 39: The cell culture medium or NCT medium of embodiment 37, wherein said culture medium is supplemented with, or said NCT medium includes, a precursor of cytidine triphosphate.

Embodiment 40: The cell culture medium or NCT medium of embodiment 39, wherein said precursor of cytidine triphosphate is selected from the group consisting of cytidine diphosphate, cytidine monophosphate, cytidine, and cytosine.

Embodiment 41: The cell culture medium according to any one of embodiments 1, and 3-40, or the NCT medium according to any one of embodiments 2-40, wherein said culture or NCT medium is supplemented with 5-methyluridine triphosphate (m5UTP), or a precursor thereof.

Embodiment 42: The cell culture medium or NCT medium of embodiment 41, wherein said culture medium is supplemented with, or said NCT medium includes, 5-methyluridine triphosphate.

Embodiment 43: The cell culture medium or NCT medium of embodiment 41, wherein said culture medium is supplemented with, or said NCT medium includes, a precursor of 5-methyluridine triphosphate.

Embodiment 44: The cell culture medium or NCT medium of embodiment 43, wherein said precursor of 5-methyluridine triphosphate is selected from the group consisting of 5-methyluridine diphosphate, 5-methyluridine monophosphate, 5-methyluridine, and thymine.

Embodiment 45: The cell culture medium according to any one of embodiments 1, and 3-44, or the NCT medium according to any one of embodiments 2-44, wherein said culture medium is supplemented with, or said NCT medium includes uridine triphosphate (UTP), or a precursor thereof.

Embodiment 46: The cell culture medium or NCT medium of embodiment 45, wherein said culture medium is supplemented with, or said NCT medium includes uridine triphosphate.

Embodiment 47: The cell culture medium or NCT medium of embodiment 45, wherein said culture medium is supplemented with, or said NCT medium includes a precursor of uridine triphosphate.

Embodiment 48: The cell culture medium or NCT medium of embodiment 47, wherein precursor of uridine triphosphate is selected from the group consisting of uridine diphosphate, uridine monophosphate, uridine, and uracil.

Embodiment 49: The nucleoside cocktail transmission (NCT) medium according to any one of embodiments 2-48, wherein said NCT medium includes a vehicle formulated for administration via a route selected from the group consisting of subcutaneous administration, parenteral administration, topical administration, oral administration, nasal or inhalation administration, local administration such as by paint, aerosol, or transdermally.

Embodiment 50: The nucleoside cocktail transmission (NCT) medium according to any one of embodiments 2-48, wherein said NCT medium includes a vehicle formulated for topical administration, subdermal administration, or intradermal administration.

Embodiment 51: The nucleoside cocktail transmission (NCT) medium of embodiment 50, wherein said medium is formulated in a vehicle selected from the group consisting of a mud, an herbal mixture, a fat, an emulsions, a lotion, a cream, a gel, a biological, a solution, a spray, an ointment, a foams, a mousses, a liquid, a suspensions, a dispersion, an aerosol, a soap, a shampoo, and a conditioner.

Embodiment 52: The nucleoside cocktail transmission (NCT) medium of embodiment 50, wherein said medium is formulated as a cream (*e.g.*, a face cream).

Embodiment 53: The nucleoside cocktail transmission (NCT) medium according to any one of embodiments 56-58, wherein said medium includes a formulation selected from the group consisting of a wrinkle removing cream, a dermal filler, a scar-reducing cream, and an acne treatment.

Embodiment 54: The cell culture medium according to any one of embodiments 1, and 3-48 or the nucleoside cocktail transmission (NCT) medium according to any one of embodiments 2-53, wherein said nucleoside triphosphate or precursor thereof supplementing said culture medium, or including said NCT medium, are present at a concentration sufficient to improve the short term and/or long term genetic stability of stem cells as compared to the same cells cultured in the same medium without supplementation by a nucleoside triphosphate or precursor thereof.

Embodiment 55: The cell culture medium or the nucleoside cocktail transmission (NCT) medium of embodiment 54, wherein said stem cells are stem cells selected from the group consisting of somatic cells (*e.g.* fibroblasts) or stem cells (*e.g.*, neural stem cells, mesenchymal stem cells, hematopoietic stem cells, adipose stem cells, embryonic stem cells, cord stem cells, and induced pluripotent stem cells).

Embodiment 56: The cell culture medium according to any one of embodiments 1, 3-48, and 54-55, or the NCT medium according to any one of embodiments 2-55, wherein each nucleoside triphosphate or precursor thereof supplementing said culture medium, or including said NCT medium is present at a concentration (*e.g.*, either in transmission medium and/or resulting in such a concentration on target cells *in vivo*, such as extrinsic delivery of nucleoside cocktail into human skin using a skin cream as the NCT medium) ranging from about 1 μM up

to about 50 μM, or from about 1 μM to about 40 μM, or from about 1 μM up to about 35 μM, or from about 1 μM up to about 30 μM.

Embodiment 57: The cell culture medium according to any one of embodiments 1, 3-48, and 54-55, or the NCT medium according to any one of embodiments 2-55, wherein each nucleoside triphosphate or precursor thereof supplementing said culture medium, or included in said NCT medium, is at a concentration starting (stock) or final (extrinsic delivery) of about 50 μM or lower, or about 40 μM or lower, or about 30 μM or lower or about 25 μM or lower, or about 20 μM or lower, or about 15 μM or lower, or about 10 μM or lower, or about 5 μM or lower.

Embodiment 58: The cell culture medium, or NCT medium of embodiment 57, wherein each nucleoside triphosphate or precursor thereof supplementing said culture medium, or included in said NCT medium, is present at a starting (stock) or final (extrinsic delivery) concentration ranging from about 5 μM to about 30 μM.

Embodiment 59: The cell culture medium, or NCT medium of embodiment 57, wherein each nucleoside triphosphate or precursor thereof supplementing said culture said culture medium, or included in said NCT medium, is present at a starting (stock) or final (extrinsic delivery) concentration of about 30 μM.

Embodiment 60: The cell culture medium, or NCT medium of embodiment 57, wherein each nucleoside triphosphate or precursor thereof supplementing said culture said culture medium, or included said NCT medium, is present at a starting (stock) or final (extrinsic delivery) concentration of about 5 μM.

Embodiment 61: The cell culture medium according to any one of embodiments 1, 3-48, and 54-60, or the NCT medium according to any one of embodiments 2-60, wherein said cell culture medium, or said NCT medium is xenopathogen-free.

Embodiment 62: The cell culture medium according to any one of embodiments 1, 3-48, and 54-61, or the NCT medium according to any one of embodiments 2-61, wherein said cell culture medium, or said NCT medium is without animal or human derived serum albumin.

Embodiment 63: The cell culture medium according to any one of embodiments 1, 3-48, and 54-62, wherein the supplemented medium is selected from the group consisting of DMEM (Dulbecco's Modified Eagle's Medium), MEM (Minimal Essential Medium), BME (Basal Medium Eagle), RPMI 1640, DMEM/F-12 (Dulbecco's Modified Eagle's Medium: Nutrient Mixture F-12), DMEM/F-10 (Dulbecco's Modified Eagle's Medium: Nutrient Mixture F-10), α-MEM (α-Minimal essential Medium), G-MEM (Glasgow's Minimal Essential Medium), IMDM (Isocove's Modified Dulbecco's Medium), essential 8 (E8) medium, and KnockOut DMEM.

Embodiment 64: The cell culture medium of embodiment 63, wherein the basal medium is DMEM/F12.

Embodiment 65: The nucleoside cocktail transmission (NCT) medium of embodiment 2, wherein said NCT medium comprises said vehicle and a cell culture medium according to any one of embodiments 1, 3-48, and 55-64.

Embodiment 66: A somatic or stem cell culture or nucleoside cocktail transmission (NCT) culture, said cell culture including cells in a cell culture medium according to any one of embodiments 1, 3-48, and 55-64, or including cells in an NCT medium according to any one of embodiments 2-62, and 65.

Embodiment 67: The cell culture or NCT culture of embodiment 66, wherein said somatic or stem cells are cells selected from the group consisting of somatic cells (*e.g.*, fibroblasts) or stem cells (*e.g.*, neural stem cells, mesenchymal stem cells, hematopoietic stem cells, adipose stem cells, embryonic stem cells, cord stem cells, induced pluripotent stem cells, and the like).

Embodiment 68: The cell culture or NCT culture of embodiment 66, wherein said cells comprise stem cells.

Embodiment 69: The cell culture or NCT culture of embodiment 68, wherein said stem cells are selected from the group consisting of embryonic stem cells and adult stem cells, including, but not limited to neural stem cells, hepatic stem cells, hematopoietic stem cells, umbilical cord blood stem cells, epidermal stem cells, gastrointestinal stem cells, endothelial stem cells, muscle stem cells, mesenchymal stem cells, and pancreatic stem cells.

Embodiment 70: The cell culture or NCT culture of embodiment 68, wherein said cells are induced pluripotent stem cells (IPSCs) (*e.g.*, such as autologous hIPSCs derived from a patient).

Embodiment 71: The cell culture or NCT culture of embodiment 70, wherein said IPSCs are reprogrammed from cells selected from the group consisting of fibroblasts, neural stem cells, stomach cells, liver cells, keratinocytes, melanocytes, amniotic cells, blood cells, β-cells, and adipose cells.

Embodiment 72: The cell culture or NCT culture of embodiments 70 or 71, wherein the IPSCs comprise cells reprogramed two or more factors selected from the group consisting of KLF4 (K), LIN28 (L), c-MYC (M), NANOG (N); OCT4 (O), SOX2 (S), and valproic acid (VPA).

Embodiment 73: The cell culture or NCT culture of embodiment 72, wherein the ISPCs includes cells reprogramed using the four canonical Yamanaka factors KLF4 (K), c-MYC (M),OCT4 (O), and SOX2 (S).

Embodiment 74: The cell culture or NCT culture of embodiment 73, wherein the reprogramming factors further comprise LIN28.

Embodiment 75: The cell culture or NCT culture according to any one of embodiments 70-74, wherein said IPSCs are reprogrammed using a vector selected from the group consisting of an integrating vector, a non-integrating vector, an excisable vector, and a DNA-free vector.

Embodiment 76: A method of reducing genetic instability of stem cells said method including culturing said cells in a cell culture medium according to any one of embodiments of embodiments a culture medium according to any one of embodiments 1, 3-48, and 55-64.

Embodiment 77: A method of performing autologous stem cell transfer, said method including isolating stem cells from a subject or generating IPSCs from said subject and expanding and/or culturing said stem cells or IPSCs in a cell culture medium according to any one of embodiments 1, 3-48, and 55-64, or in an NCT medium according to any one of embodiments 2-62, and 65.

Embodiment 78: A method of promoting regeneration and/or maintenance of tissues, said method including administering to a subject, an NCT medium according to any one of embodiments 2-62, and 65.

Embodiment 79: The method of embodiment 78, wherein said NCT medium includes a vehicle formulated for administration via a route selected from the group consisting of subcutaneous administration, parenteral administration, topical administration, oral administration, nasal or inhalation administration, local administration such as by paint, aerosol, or transdermally.

Embodiment 80: The method of embodiment 78, wherein, wherein said NCT medium includes a vehicle formulated for topical administration, subdermal administration, or intradermal administration.

Embodiment 81: The method of embodiment 80, wherein said NCT medium is formulated in a vehicle selected from the group consisting of a mud, an herbal mixture, a fat, an emulsions, a lotion, a cream, a gel, a biological, a solution, a spray, an ointment, a foams, a mousses, a liquid, a suspensions, a dispersion, an aerosol, a soap, a shampoo, and a conditioner.

Embodiment 82: The method of embodiment 80, wherein said NCT medium is formulated as a cream (*e.g.*, a face cream).

Embodiment 83: The method of embodiment 80, wherein said NCT medium includes a formulation selected from the group consisting of a wrinkle removing cream, a dermal filler, a scar-reducing cream, and an acne treatment.

Embodiment 84: The method according to any one of embodiments 78-83, wherein said NCT is applied to the skin of a human.

Embodiment 85: The method of embodiment 84, wherein said NCT is applied to reduce scarring.

Embodiment 86: The method of embodiment 84, wherein said NCT is applied to reduce wrinkles.

Embodiment 87: The method according to any one of embodiments 78-83, wherein said NCT is applied intradermally or subdermally to increase tissue volume.

Embodiment 88: A method of increasing transduction of a cell with a heterologous nucleic acid construct and/or increasing transcription and/or translation of said construct, said method including transducing and/or culturing said cell in a cell culture medium according to any one of embodiments 1, 3-48, and 55-64, or in an NCT medium according to any one of embodiments 2-62, and 65.

Embodiment 89: A method of increasing or preserving genomic stability and/or reducing genomic instability of a cell, said method including transducing and/or culturing said cell in a cell culture medium according to any one of embodiments 1, 3-48, and 55-64, or in an NCT medium according to any one of embodiments 2-62, and 65 90.

Embodiment 90: The method according to any one of embodiments 88-89, wherein said cell culture medium and/or said NCT medium includes 2'-deoxycytidine (dC), 2'-deoxyadenosine monohydrate, 2'-deoxyguanosine monohydrate, and thymidine.

Embodiment 91: The method of embodiment 90, wherein said cell culture medium and/or said NCT medium further includes dimethylsulfoxide (DMSO).

Embodiment 92: The method according to any one of embodiments 90-91, wherein said cell culture medium and/or said NCT medium includes mTeSR1 basal medium

Embodiment 93: The method according to any one of embodiments 90-92, wherein said cell culture medium and/or said NCT medium includes NutriStem XF/FF culture medium.

Embodiment 94: The method according to any one of embodiments 90-93, wherein said cell culture medium and/or said NCT medium includes dC and dA at at least twice the concentration, or at least 3 times the concentration, or at least 4 times the concentration, or at least 5 times the concentration of dT and dG.

Embodiment 95: The method of embodiment 94, wherein said cell culture medium and/or said NCT medium includes 30 μM dC, 30 μM dA, 5 μM dT, and 5 μM dG.

Embodiment 96: The method according to any one of embodiments 90-95 , wherein said cell is selected from the group consisting of: 1) exocrine secretory epithelial cells (such as, salivary gland mucous cell, prostate gland cell, bulbourethral gland cell, Bartholin's gland cell, Gland of Littre cell, uterus endometrium cell, isolated goblet cell of respiratory and digestive tracts, stomach lining mucous cell, gastric gland zymogenic cell, gastric gland oxyntic cell, pancreatic acinar cell, Paneth cell of small intestine, Type II pneumocyte of lung, and Clara cell of lung); 2) hormone secreting cells (such as anterior pituitary cells, intermediate pituitary cell, magnocellular neurosecretory cells, gut and respiratory tract cells, thyroid gland cells (*e.g.*, thyroid epithelial cell and parafollicular cell), parathyroid gland cells (*e.g.*, parathyroid chief cell and oxyphil cell), adrenal gland cells (*e.g.*, chromaffin cells), Leydig cell of testes, theca interna cell of ovarian follicle, Corpus luteum cell of ruptured ovarian follicle (*e.g.*, granulosa lutein cells and theca lutein cells), juxtaglomerular cell, macula densa cell of kidney, peripolar cell of kidney, and mesangial cell of kidney); 3) cells derived primarily from ectoderm (such as keratinizing epithelial cells (*e.g.*, epidermal keratinocyte, epidermal basal cell, keratinocyte of fingernails and toenails, nail bed basal cell, medullary hair shaft cell, cortical hair shaft cell, cuticular hair shaft cell, cuticular hair root sheath cell, hair root sheath cell of Huxley's layer, hair root sheath cell of Henle's layer, external hair root sheath cell, and hair matrix cell), wet stratified barrier epithelial cells (*e.g.*, surface epithelial cell of stratified squamous epithelium of cornea, tongue, oral cavity, esophagus, anal canal, distal urethra and vagina, basal cell of epithelia of cornea, tongue, oral cavity, esophagus, anal canal, distal urethra and vagina, and urinary epithelium cells), sensory transducer cells (*e.g.*, auditory inner hair cell of organ of Corti, auditory outer hair cell of organ of Corti, basal cell of olfactory epithelium, cold-sensitive primary sensory neurons, heat-sensitive primary sensory neurons, Merkel cell of epidermis, olfactory receptor neuron, pain-sensitive primary sensory neurons, photoreceptor cells of retina in eye (such as photoreceptor rod cells, photoreceptor blue-sensitive cone cell of eye, photoreceptor green-sensitive cone cell of eye, and photoreceptor red-sensitive cone cell of eye), proprioceptive primary sensory neurons, touch-sensitive primary sensory neurons, Type I carotid body cell, Type II carotid body cell, Type I hair cell of vestibular system of ear, Type II hair cell of vestibular system of ear, and Type I taste bud cell), Autonomic neuron cells (*e.g.*, cholinergic neural cell, adrenergic neural cell, and peptidergic neural cell), sense

organ and peripheral neuron supporting cells (*e.g.*, inner pillar cell of organ of Corti, outer pillar cell of organ of Corti, inner phalangeal cell of organ of Corti, outer phalangeal cell of organ of Corti, border cell of organ of Corti, Hensen cell of organ of Corti, vestibular apparatus supporting cell, taste bud supporting cell, olfactory epithelium supporting cell, Schwann cell, satellite glial cell, and enteric glial cell), central nervous system neurons and glial cells (*e.g.*, astrocyte, neuron cells, oligodendrocyte, and spindle neuron), lens cells (*e.g.*, anterior lens epithelial cell and crystallin-containing lens fiber cell); 4) cells derived primarily from mesoderm (such as metabolism and storage cells (*e.g.*, hepatocyte, adipocyte, and liver lipocyte), barrier function cells (*e.g.*, kidney parietal cell, kidney glomerulus podocyte, kidney proximal tubule brush border cell, Loop of Henle thin segment cell, kidney distal tubule cell, kidney collecting duct cell, Type I pneumocyte, pancreatic duct cell, nonstriated duct cell (of sweat gland, salivary gland, mammary gland, *etc.* (*e.g.*, principal cell and intercalated cell), duct cell (of seminal vesicle, prostate gland, *etc.*), intestinal brush border cell, exocrine gland striated duct cell, gall bladder epithelial cell, Ductulus efferens nonciliated cell, epididymal principal cell, and epididymal basal cell), extracellular matrix cells (*e.g.*, ameloblast epithelial cell, Planum semilunatum epithelial cell of vestibular system of ear, organ of Corti interdental epithelial cell, loose connective tissue fibroblasts, corneal fibroblasts, tendon fibroblasts, bone marrow reticular tissue fibroblasts, other nonepithelial fibroblasts, pericyte, nucleus pulposus cell of intervertebral disc, cementoblast/cementocyte, odontoblast/odontocyte, hyaline cartilage chondrocyte, fibrocartilage chondrocyte, elastic cartilage chondrocyte, osteoblast/osteocyte, osteoprogenitor cell, hyalocyte of vitreous body of eye, stellate cell of perilymphatic space of ear, hepatic stellate cell (Ito cell), and pancreatic stelle cell); contractile cells (*e.g.*, red skeletal muscle cell, white skeletal muscle cell, intermediate skeletal muscle cell, nuclear bag cell of muscle spindle, nuclear chain cell of muscle spindle, satellite cell, heart muscle cells, ordinary heart muscle cell, nodal heart muscle cell, Purkinje fiber cell, smooth muscle cell, myoepithelial cell of iris, and myoepithelial cell of exocrine glands), blood and immune system cells (*e.g.*, erythrocyte, megakaryocyte, monocyte, connective tissue macrophage, epidermal Langerhans cell, osteoclast, dendritic cell, microglial cell, neutrophil granulocyte, eosinophil granulocyte, basophil granulocyte, hybridoma cell, mast cell, T cells (such as helper T cell, suppressor T cell, cytotoxic T cell, and natural killer T cell), B cell, natural killer cell, reticulocyte, and stem cells and

committed progenitors for the blood and immune system), germ cells (*e.g.*, oogonium/oocyte, spermatid, spermatocyte, spermatogonium cell, and spermatozoon), nurse cells (*e.g.*, ovarian follicle cell, Sertoli cell, and thymus epithelial cell) and interstitial cells (*e.g.*, interstitial kidney cells); or any cell line thereof.

Embodiment 97: A method of reducing aging of skin cells or tissue, said method including contacting said skin cells or tissue with a medium according to any one of embodiments 2-62, and 65.

Embodiment 98: The method of embodiment 97. wherein said reducing aging includes a decrease in the amount, size, or onset of skin wrinkles or folds, or a decrease in the amount, size, or onset of age spots, or a decrease in the reduction of skin elasticity often observed with the aging process, or maintaining or promoting skin moisture, or maintaining or promoting skin thickness.

Embodiment 99: A method of increasing or decreasing age-related markers of skin cells or tissue, said method including contacting said skin cells or tissue with a medium according to any one of embodiments 2-62, and 65.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Figure 1: Characterization of human induced pluripotent stem cells (hIPSCs). (a) Morphology and immunocytochemical detection of stem cell markers (insert is DAPI); (b) Histological characterization following H&E staining of the three germ layers observed in transgene-free hIPSC-derived teratomas, (c) Karyotype analysis of the transgene-free iPSCs before and after CC-based stress. Red arrow highlights extra chromosome 12. Scale bar equals 100 microns.

Figure 2: Significant reduction of dNTP pools following hIPSC reprogramming. *The size of the dNTP pool was standardized relative to the level observed in the original dermal fibroblasts.

Figure 3A and 3B: Visualization (Fig. 3A) and quantification (Fig. 3B) of YH2A.X positive foci. Green staining highlights yH2A.X foci, blue staining

highlights DAPI stained nuclei. HDFs were reprogrammed into hIPSCs and then cultured for four days with and without deoxyribonucleoside (dN) supplementation.

Figure 4: Single nucleotide polymorphism (SNP)-based loss of heterozygosity (LOH) analysis following "clinical-grade conversion" (CC) based stress.

Figure 5: Supplementing with 30 μM of dNs significantly increases hIPSC proliferation rate. First experiment treated cells with 0 μM, 30 μM, 100 μM, 200 μM dN over 5 day period. 200 μM was observed to be toxic (data not shown). Second experiment: treated cells with 0 μM, 30 μM, 50 μM, 75 μM, 100 μM dN over 5 day period

Figure 6 illustrates a significant reduction of dNTP pools following hIPSC reprogramming and dNTP rescue with deoxyribonucleoside (dN) culture media supplementation. The size of the dNTP pool was standardized relative to the level observed in the original skin cells.

Figure 7 shows visualization and quantification of γH2A.X positive foci (indicative of double strand breaks).

Figure 8 shows that use of deoxyribonucleoside cocktail (DC) can stimulate both skin fibroblasts and human induced pluripotent stem cells to divide more quickly. Cell counts of fibroblasts and stem cells grown with and without supplement were taken at 0 and 48 hours post-plating. For the AD1 stem cells, one subclone that had been reprogrammed with culture supplement was taken off supplement temporarily to conduct doubling rate experiments. The equation used to calculate population doubling time (PDT) 48 your post plating was $48/(\text{Log}(T_{48})-\text{Log}(T_0))$. To calculate doubling rate per 24 hours: 24/PDT.

Figure 9 shows that a nucleoside cocktail (NC) can significantly rescue dNTP pools in hIPSCs.

Figure 10 shows visualization and quantification of γH2A.X positive foci (indicative of double strand breaks) reducing in the presence of a nucleoside coctail. The incidence of DNA damage (via γH2AX expression) was decreased with culture supplement. DNA damage levels were measured via γH2AX expression (green) which is indicative of double-stranded breaks. Blind counts were conducted

to determine percent damaged nuclei. Supplement causes a decrease in damage in two separate stem cell lines.

Figure 11 shows differentiation of pluripotent stem cells into a therapeutic target cell type (in this case hepatocyte-like cells, hereby called hepatocytes).

Figure 12 shows alpha-fetoprotein expressing hepatocytes can be derived within two weeks when DC supplement used, but not without DC.

Figure 13 shows a decrease in genetic damage of fibroblast cells under supplementation conditions.

Figure 14 shows an increase in OCT4 expression in cells under supplementation conditions.

DETAILED DESCRIPTION

In various embodiments, the methods an decompositions described herein pertain to the discovery that deoxyribonucleoside (dN) and/or nucleoside (ribonucleoside) supplementation can increase genetic stability in stem cells including induced pluripotent stem cells (IPSCs) in culture.

As demonstrated herein, it was determined that deoxyribonucleotide triphosphate (dNTP) pools in rapidly dividing hIPSCs are significantly smaller in size than the dNTP pools of cells (*e.g.*, dermal fibroblasts, from which the hIPSCs are derived (*see, e.g.,* Figs. 2 and 6). It was also determined that hIPSCs demonstrate significantly more double strand breaks (DSBs) than the fibroblasts of origin (*see, e.g.,* Figs. 3A and 3B).

Data presented herein indicate that: 1) hIPSCs can utilize exogenous NTP precursors added to the hIPSC culture or DCT medium in the form of a mixture of deoxyribonucleosides (dNs) and 2) that culture medium supplementation with deoxyribonucleoside (dN) and/or nucleosides or precursors thereof can both ameliorate the hIPSC dNTP pool deficiency and significantly reduce the genomic instability experienced by these cells (*see, e.g.,* Fig. 4).

It was also discovered that exogenous NTP precursors can be incorporated into delivery vehicles (*e.g.*, pharmaceutical delivery vehicles, cosmetic delivery vehicles, cosmetics, *etc.*) to form a nucleoside cocktail transmission (NCT)

medium that can significantly stimulate proliferation of stimulate cellular proliferation of both human skin cells and human induced pluripotent stem cells (*see, e.g.*, Fig. 8). As used here, the terms nucleoside cocktail transmission (NCT) or nucleoside cocktail delivery (NCD) medium refers to a formulation comprising a vehicle supplemented with nucleosides or precursors thereof or deoxynucleosides or precursors thereof formulated for administration *in vivo* to a subject (*e.g.*, a human, a non-human mammal, *etc.*). Deoxynucleoside cocktail transmission (DCT) medium or deoxynucleoside cocktail delivery (DCD) medium refers to to a formulation comprising a vehicle supplemented with deoxynucleosides or precursors thereof formulated for administration *in vivo* to a subject (*e.g.*, a human, a non-human mammal, *etc.*).

It is believed that a nucleoside cocktail can also stimulate cellular proliferation of other cells *in vitro* as well as stimulating cellular proliferation and/or tissue rejuvenation/regeneration *in vivo* (such as following direct topical application of an NCT in the form of, for example, a skin cream).

Data presented herein indicate that nucleoside supplementation can significantly rescue dNTP pools in human induced pluripotent stem cells (*see, e.g.*, Fig. 9) and it is believed that nucleoside supplementation can also augment dNTP pool size in a wide range of other human and non-human cell types.

Data presented herein indicate that nucleoside supplementation can significantly reduce the incidence of genetic damage in human induced pluripotent stem cells, *e.g.*, as measured by gammaH2A.X immunocytochemistry (*see, e.g.,* Fig. 10) and it is believed that nucleoside supplementation can also reduce the incidence of genetic damage in a wide range of other human and non-human cell types.

Data presented herein indicate that nucleoside supplementation can significantly augment the differentiation of hIPSCs into hepatocyte-like cells (*see, e.g.*, Figs. 11 and 12) and it is believed that nucleoside DC supplementation can also augment differentiation into a wide range of other human and non-human cell types.

Mammalian cells synthesize dNTPs via two pathways: the *de novo* pathway (DNP), which uses glucose and amino acids, and the nucleoside salvage pathway (NSP), which uses preformed dNs from the extracellular environment. Without being bound to a particular theory, it is believed that: 1) insufficient

production of dNTP pools via the DNP is an important cause of replication stress, DNA damage and genomic instability in rapidly dividing hIPSCs (or other stem cells in culture) and 2) enabling the utilization by the hIPSC cells (or other stem cells) of the NSP by supplementing the culture medium with one or more deoxyribonucleotide substrates or precursors thereof will increase dNTP pools and will alleviate replication stress, DNA damage and genomic instability in stem cells (including iPSCs) in culture.

Similarly, it is believed that administration of a nucleoside cocktail transmission medium (NCT), *e.g.*, a pharmaceutical or cosmetic formulation comprising one or more deoxynucleosides or precursors thereof as described herein will alleviate replication stress, DNA damage and genomic instability in stem cells and somatic cells (*e.g.*, fibroblasts) *in vivo.*

It was discovered that hIPSCs cultured under nucleoside-free conditions demonstrate dNTP deficiency (Figs. 2 and 6), and high levels of genomic instability especially when placed under "clinical conversion" (CC)-based stress (Fig. 1). However, when standard hIPSC culture media was supplemented with different concentrations of dNs, it was found that 30 μM of each dN could increase hIPSC proliferation rate and genetic stability over 4-5 days (*see,* Example 1). It was also demonstrated that lower doses of dNs (*e.g.*, 5 μM) will ensure genetic stability of hIPSCs (or other stem cells) over longer periods of time while also still significantly reducing the incidence of genomic damage as assayed via gammaH2A.X staining of double strand breaks.

The data presented herein show that a simple and cost-effective culture media supplementation approach that can be easily implemented by members of the stem cell biology and regenerative medicine field, can increase the clinical potential of personalized hIPSC-based cellular therapeutics.

The data presented herein show that a simple and cost-effective nucleoside supplementation approach that can be easily implemented by members of the cosmetic, regenerative and therapeutic fields to directly stimulate regeneration of human tissues (such as skin). Specifically, the direct topical application of a nucleoside cocktail (and/or other *in vivo* delivery mechanisms) will induce

regeneration through the stimulation of proliferation of skin fibroblasts (Fig. 8), mesenchymal stem cells and/or other cells in the skin.

It is believed that nucleoside (*e.g.*, dN) supplemented cells have improved genetic stability and pose significantly less risk of neoplasm formation following personalized stem cell-based (*e.g.*, hIPSC-based) therapeutics, than stem cells derived, cultured and differentiated using the existing art (nucleoside-free (*e.g.*, dN-free) culture media).

Accordingly, in various embodiments, culture media is provided that enhances the short term or long term genetic stability of stem cells (*e.g.*, embryonic stem cells, adult stem cells, induced pluripotent stem cells, *etc.*) cultured in that medium. The culture media comprises essentially any culture medium utilized for the culture of stem cells (or other cells) where that medium is medium is supplemented with one or more deoxynucleoside or nucleoside (triphosphates) and/or precursors thereof.

Similarly, the nucleoside cocktail transmission (NCT) medium comprises a pharmaceutical or cosmetic formulation (*e.g.*, as described herein) containing one or more deoxynucleoside or nucleoside (triphosphates) and/or precursors thereof.

Culture media.

In various embodiments the culture media comprise essentially any culture medium utilized for the culture of stem cells, where that medium is supplemented with one or more deoxynucleoside or nucleoside (triphosphates) and/or precursors thereof. In certain embodiments the culture medium is supplemented with two different deoxynucleoside and/or nucleoside (triphosphates) and/or precursors thereof. In certain embodiments the culture medium is supplemented with three different deoxynucleoside and/or nucleoside (triphosphates) and/or precursors thereof. In certain embodiments the culture medium is supplemented with four different deoxynucleoside and/or nucleoside (triphosphates) and/or precursors thereof. In certain embodiments the culture medium is supplemented with even more different deoxynucleoside and/or nucleoside (triphosphates) and/or precursors thereof.

As indicated above, in various embodiments, the basal culture medium can be any culture medium capable of expanding, maintaining, or differentiating stem

cells including IPSCs. In certain embodiments the basal medium may be manually prepared according to conventional methods. In certain embodiments the basal medium may be a commercially available medium or a mixture thereof. For example, the basal medium may be selected from the group consisting of DMEM (Dulbecco's Modified Eagle's Medium; GIBCO), MEM (Minimal Essential Medium; GIBCO), BME (Basal Medium Eagle; GIBCO), RPMI 1640 (GIBCO), DMEM/F-12 (Dulbecco's Modified Eagle's Medium: Nutrient Mixture F-12; GIBCO), DMEM/F-10 (Dulbecco's Modified Eagle's Medium: Nutrient Mixture F-10; GIBCO), α-MEM (α-Minimal essential Medium; GIBCO), G-MEM (Glasgow's Minimal Essential Medium; GIBCO), IMDM (Isocove's Modified Dulbecco's Medium; GIBCO), and KnockOut DMEM (GIBCO), essential 8 (E8) medium, and the like.

In various embodiments, the basal medium may contain one or more supplements, which includes, but not limited to, KnockOut Serum Replacement (GIBCO), KnockOut SR XenoFree (GIBCO), KnockOut SR XenoFree Growth Factor Cocktail (GIBCO), N2 supplement (GIBCO), B27 supplement (GIBCO), and so on. In certain embodiments the basal medium is a xenopathogen-free medium (*i.e.*, xeno-free medium), in order to avoid any safety problem by materials derived from animal source. Typically such xeno-free medium does not include xenopathgen(s), such as bovine serum albumin, and recombinant proteins purified from animal cells.

As indicated above, such media can routinely be prepared in the laboratory, or can be commercially supplied. By way of non-limiting illustration, the composition of DMEM/F12 is shown in Table 1.

Table 1. Composition of DMEM/F12 basal medium.

Components	**Molecular Weight**	**Concentration (mg/L)**	**mM**
Amino acids:			
Glycine	75	18.75	0.25
L-Alanine	89	4.45	0.05
L-Arginine hydrochloride	211	147.5	0.699052
L-Asparagine-H2O	150	7.5	0.05
L-Aspartic acid	133	6.65	0.05
L-Cysteine hydrochloride-H2O	176	17.56	0.099773
L-Cystine 2HCl	313	31.29	0.099968
L-Glutamic Acid	147	7.35	0.05
L-Glutamine	146	365	2.5
L-Histidine hydrochloride-H2O	210	31.48	0.149905

L-Isoleucine	131	54.47	0.415802
L-Leucine	131	59.05	0.450763
L-Lysine hydrochloride	183	91.25	0.498634
L-Methionine	149	17.24	0.115705
L-Phenylalanine	165	35.48	0.21503
L-Proline	115	17.25	0.15
L-Serine	105	26.25	0.25
L-Threonine	119	53.45	0.44916
L-Tryptophan	204	9.02	0.044216
L-Tyrosine disodium salt dihydrate	261	55.79	0.213755
L-Valine	117	52.85	0.451709
Vitamins:			
Biotin	244	0	0.000014
Choline chloride	140	8.98	0.064143
D-Calcium pantothenate	477	2.24	0.004696
Folic Acid	441	2.65	0.006009
Niacinamide	122	2.02	0.016557
Pyridoxine hydrochloride	206	2.01	0.009772
Riboflavin	376	0.22	0.000582
Thiamine hydrochloride	337	2.17	0.006439
Vitamin B12	1,355	0.68	0.000502
i-Inositol	180	12.6	0.07
Inorganic salts:			
Calcium Chloride (CaCl2) (anhyd.)	111	116.6	1.05045
Cupric sulfate (CuSO4-5H2O)	250	0	0.000005
Ferric Nitrate (Fe(NO3)3"9H2O)	404	0.05	0.000124
Ferric sulfate (FeSO4-7H2O)	278	0.42	0.0015
Magnesium Chloride (anhydrous)	95	28.64	0.301474
Magnesium Sulfate (MgSO4) (anhyd.)	120	48.84	0.407
Potassium Chloride (KCl)	75	311.8	4.157333
Sodium Bicarbonate (NaHCO3)	84	2,438	29.02381
Sodium Chloride (NaCl)	58	6,995.5	120.61207
Sodium Phosphate dibasic (Na2HPO4) anhydrous	142	71.02	0.500141
Sodium Phosphate monobasic (NaH2PO4-H2O)	138	62.5	0.452899
Zinc sulfate (ZnSO4-7H2O)	288	0.43	0.0015
Other components:			
D-Glucose (Dextrose)	180	3,151	17.505556
Hypoxanthine Na	159	2.39	0.015031
Linoleic Acid	280	0.04	0.00015
Lipoic Acid	206	0.1	0.00051
Phenol Red	376.4	8.1	0.02152
Putrescine 2HCl	161	0.08	0.000503

Sodium Pyruvate	110	55	0.5
Thymidine	242	0.36	0.001508

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The foregoing basal culture media are intended to be illustrative and non-limiting. One of skill will readily recognize that the supplements described herein can be used with virtually any culture media or incorporated into a pharmaceutical or cosmetic to form any of a number of NCT media.

Nucleoside cocktail transmission (delivery) medium.

In certain embodiments the nucleoside cocktail transmission (NCT) medium comprises a delivery vehicle (*e.g.*, a cosmetic formulation) containing one or more deoxynucleoside or nucleoside (triphosphates) and/or precursors thereof. The NCT medium is formulated to deliver the nucleoside(s) or nucleoside precursor(s) to a desired location in or on a mammal and to provide appropriate local concentration of the nucleoside or nucleoside precursor(s).

A "delivery vehicle" refers to, for example, a diluent, adjuvant, excipient, auxiliary agent or carrier with which one or more of the nucleosides and/or nucleoside precursors described herein is administered.

The NCTs can be formulated for subcutaneous, parenteral, topical, oral, nasal (or otherwise inhaled), or formulated for local administration, such as by aerosol or transdermally, *e.g.*, to stimulate cellular proliferation of stem cells or other cells *in vitro* as well as to stimulate cellular proliferation and/or tissue rejuvenation/regeneration *in vivo*, and/or to improve cellular maintenance of introduced exogenous cells (*e.g.*, stem cells), endogenous stem cells, somatic cells, and the like in a variety of contexts. The compositions can be administered in a variety of dosage/concentration forms depending upon the method of administration. Suitable unit dosage forms, include, but are not limited to powders, tablets, pills,

capsules, lozenges, suppositories, patches, nasal sprays, injectables, implantable sustained-release formulations, lipid complexes, *etc.*

In certain embodiments, the NCTs are formulated for topical administration, subdermal administration, or intradermal administration. In certain formulation for administration to wound sites, including for example, acute wound sites, surgical sounds, burn sites, *e.g.*, to promote healing and reduce scarring is contemplated.

In certain embodiments the NCTs are formulated with one or more pharmaceutical agents. Illustrative pharmaceutical agents include, but are not limited to, agents used for the treatment of wound sites, burn sites, acne, and other topical disfigurements (*e.g.*, various kinds of dermal scarring). Illustrative agents include, but are not limited to for example, topical tamoxifen for dermal scarring, benzyol peroxide and antibiotics for acne, and the like.

Illustrative NCT vehicles, include cosmetic muds, herbal mixtures, fats (*e.g.*, animal fats), emulsions, lotions, creams, gels, biologicals, gels, solutions, sprays, ointments, foams, mousses, liquids, suspensions, dispersions, aerosols, soaps, shampoos, conditioners, cleansers, and general cosmetic products. In certain embodiments, vehicles comprising agents for the reduction of wrinkles, and/or skin discoloration, and/or dermal fillers are contemplated.

Suitable vehicles to which the nucleoside(s) and/or nucleoside precursor(s) can be added include, but are not limited to, mud, herbal mixtures, animal fats, emulsions, lotions, creams, gels, biologicals, gels, solutions, sprays, ointments, foams, mousses, liquids, suspensions, dispersions, aerosols, soaps, shampoos, conditioners, cleansers, and general cosmetic products.

In certain embodiments, suitable vehicles include any carrier or vehicle commonly used as a base for creams, lotions, sprays, foams, gels, emulsions, lotions or paints for topical administration. Examples include emulsifying agents, inert carriers including hydrocarbon bases, emulsifying bases, non-toxic solvents or water-soluble bases. Particularly suitable examples include pluronics, HPMC, CMC and other cellulose-based ingredients, lanolin, hard paraffin, liquid paraffin, soft yellow paraffin or soft white paraffin, white beeswax, yellow beeswax, cetostearyl alcohol, cetyl alcohol, dimethicones, emulsifying waxes, isopropyl myristate, microcrystalline

wax, oleyl alcohol and stearyl alcohol. Also contemplated are nonionic polyoxyethylene-polyoxypropylene copolymers, also referred to as poloxamers. One illustrative poloxamer is poloxamer 407, also known as Pluronic F-127 (BASF). Additional carriers, include, but are not limited to, alginates, polyvinyl alcohol, hydrogels, including hydrogels that contain a cellulose derivative and/or polyacrylic acid; cellulose-based carrier, including hydroxyethyl cellulose, hydroxymethyl cellulose, carboxymethyl cellulose, hydroxypropylmethyl cellulose and mixtures thereof.

In certain embodiments the vehicle comprises a lotion. Lotions can contain finely powdered substances that are insoluble in the dispersion medium through the use of suspending agents and dispersing agents. Alternatively, lotions can have as the dispersed phase liquid substances that are immiscible with the vehicle and are usually dispersed by means of emulsifying agents or other suitable stabilizers. In one embodiment, the lotion is in the form of an emulsion having a viscosity of between 100 and 1000 centistokes. The fluidity of lotions permits rapid and uniform application over a wide surface area. Lotions are typically intended to dry on the skin leaving a thin coat of their medicinal components on the skin's surface.

In certain embodiments, the vehicle comprises a topical cream. Creams may contain emulsifying agents and/or other stabilizing agents. In one embodiment, the formulation is in the form of a cream having a viscosity of greater than 1000 centistokes, typically in the range of 20,000-50,000 centistokes. Creams are often time preferred over ointments as they are generally easier to spread and easier to remove. The basic difference between a cream and a lotion is the viscosity, which is dependent on the amount/use of various oils and the percentage of water used to prepare the formulations. Creams are typically thicker than lotions, may have various uses and often one uses more varied oils/butters, depending upon the desired effect upon the skin. In a cream formulation, the water-base percentage is about 60-75 % and the oil-base is about 20-30 % of the total, with the other percentages being the emulsifier agent, preservatives and additives for a total of 100 %. Representative lotions and/or creams include, for example, La Mer regenerating serum by Estee Lauder, Ole Henriksen truth serum collagen booster by Ole Henriksen, Clarins double serum complete age control concentrate by Clarins, Lancome Genefique repair crème by Lancome, Elemis Pro-collagen marine cream by Elemis, Kiehl's powerful wrinkle

reducing cream by Kiehl's, Origins Plantscription anti-aging power serum by Origins, and L'Occitabe Divine Youth Oil by L'Occitane.

In various embodiments the vehicle comprises an ointment. Examples of suitable ointment bases include hydrocarbon bases (*e.g.*, petrolatum, white petrolatum, yellow ointment, and mineral oil); absorption bases (hydrophilic petrolatum, anhydrous lanolin, lanolin, and cold cream); water-removable bases (*e.g.*, hydrophilic ointment), and water-soluble bases (*e.g.*, polyethylene glycol ointments). Pastes typically differ from ointments in that they contain a larger percentage of solids. Pastes are typically more absorptive and less greasy that ointments prepared with the same components.

In certain embodiments the vehicle comprises a gel. Some emulsions may be gels or otherwise include a gel component. Some gels, however, are not emulsions because they do not contain a homogenized blend of immiscible components. Suitable gelling agents include, but are not limited to, modified celluloses, such as hydroxypropyl cellulose and hydroxyethyl cellulose; Carbopol homopolymers and copolymers; and combinations thereof. Suitable solvents in the liquid vehicle include, but are not limited to, diglycol monoethyl ether; alklene glycols, such as propylene glycol; dimethyl isosorbide; alcohols, such as isopropyl alcohol and ethanol. The solvents are typically selected for their ability to dissolve the drug. ther additives, which improve the skin feel and/or emolliency of the formulation, may also be incorporated. Examples of such additives include, but are not limited, isopropyl myristate, ethyl acetate, C12-C15 alkyl benzoates, mineral oil, squalane, cyclomethicone, capric/caprylic triglycerides, and combinations thereof.

In certain embodiments the vehicle comprises a foam. Foams consist of an emulsion in combination with a gaseous propellant. The gaseous propellant consists primarily of hydro fluoroalkanes (HFAs). Suitable propellants include HFAs such as 1, 1,1,2-tetrafluoroethane (HFA 134a) and 1, 1, 1,2,3,3, 3-heptafluoropropane (HFA 227), but mixtures and admixtures of these and other HFAs that are currently approved or may become approved for medical use are suitable. The propellants preferably are not hydrocarbon propellant gases which can produce flammable or explosive vapors during spraying. Furthermore, the compositions preferably contain no volatile alcohols, which can produce flammable or explosive vapors during use.

It will be recognized that, in various embodiments the vehicles can contain additional ingredients, *e.g.*, to enhance shelf-life, reduce biological contamination, improve wetting, maintain optimum pH, viscosity, maintain or improve color, smell, texture, and the like. Illustrative, additional ingredients include, but are not limited to excipients, diluents, emollients, surfactants, emulsifier, buffers, preservatives, penetration enhancer, scents, colorants, and the like.

Appropriate excipients are selected based on the type of formulation. Standard excipients include gelatin, casein, lecithin, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glyceryl monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, polyoxyethylene stearates, colloidol silicon dioxide, phosphates, sodium dodecyl sulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethycellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, polyvinylpyrrolidone, sugars, and starches.

"Diluents" may be included in the formulations to dissolve, disperse or otherwise incorporate the carrier. Examples of diluents include, but are not limited to, water, buffered aqueous solutions, organic hydrophilic diluents, such as monovalent alcohols, and low molecular weight glycols and polyols (*e.g.* propylene glycol, polypropylene glycol, glycerol, butylene glycol).

"Emollients" are an externally applied agent that softens or soothes skin and are generally known in the art and listed in compendia, such as the "Handbook of Pharmaceutical Excipients", 4th Ed., Pharmaceutical Press, 2003. These include, without limitation, almond oil, castor oil, ceratonia extract, cetostearoyl alcohol, cetyl alcohol, cetyl esters wax, cholesterol, cottonseed oil, cyclomethicone, ethylene glycol palmitostearate, glycerin, glycerin monostearate, glyceryl monooleate, isopropyl myristate, isopropyl palmitate, lanolin, lecithin, light mineral oil, medium-chain triglycerides, mineral oil and lanolin alcohols, petrolatum, petrolatum and lanolin alcohols, soybean oil, starch, stearyl alcohol, sunflower oil, xylitol and combinations thereof. In one embodiment, the emollients are ethylhexylstearate and ethylhexyl palmitate.

"Surfactants" are surface-active agents that lower surface tension and thereby increase the emulsifying, foaming, dispersing, spreading and wetting properties of a product. Suitable non-ionic surfactants include emulsifying wax, glyceryl monooleate, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polysorbate, sorbitan esters, benzyl alcohol, benzyl benzoate, cyclodextrins, glycerin monostearate, poloxamer, povidone and combinations thereof. In one embodiment, the non-ionic surfactant is stearyl alcohol.

"Emulsifiers" are surface active substances which promote the suspension of one liquid in another and promote the formation of a stable mixture, or emulsion, of oil and water. Common emulsifiers are: metallic soaps, certain animal and vegetable oils, and various polar compounds. Suitable emulsifiers include acacia, anionic emulsifying wax, calcium stearate, carbomers, cetostearyl alcohol, cetyl alcohol, cholesterol, diethanolamine, ethylene glycol palmitostearate, glycerin monostearate, glyceryl monooleate, hydroxpropyl cellulose, hypromellose, lanolin, hydrous, lanolin alcohols, lecithin, medium-chain triglycerides, methylcellulose, mineral oil and lanolin alcohols, monobasic sodium phosphate, monoethanolamine, nonionic emulsifying wax, oleic acid, poloxamer, poloxamers, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene stearates, propylene glycol alginate, self-emulsifying glyceryl monostearate, sodium citrate dehydrate, sodium lauryl sulfate, sorbitan esters, stearic acid, sunflower oil, tragacanth, triethanolamine, xanthan gum and combinations thereof. In one embodiment, the emulsifier is glycerol stearate.

"Buffers" are used to control pH of a composition. Preferably, the buffers buffer the composition from a pH of about 4 to a pH of about 7.5, more preferably from a pH of about 4 to a pH of about 7, and most preferably from a pH of about 5 to a pH of about 7. In a preferred embodiment, the buffer is triethanolamine.

"Preservatives" can be used to prevent the growth of fungi and microorganisms. Suitable antifungal and antimicrobial agents include, but are not limited to, benzoic acid, butylparaben, ethyl paraben, methyl paraben, propylparaben, sodium benzoate, sodium propionate, benzalkonium chloride, benzethonium chloride, benzyl alcohol, cetylpyridinium chloride, chlorobutanol, phenol, phenylethyl alcohol, and thimerosal.

"Penetration enhancers" are frequently used to promote transdermal delivery of drugs across the skin, in particular across the stratum corneum. A penetration enhancer may be added to enable the active agents to cross the barrier of the stratum corneum. Some penetration enhancers cause dermal irritation, dermal toxicity and dermal allergies. However, the more commonly used ones include urea, (carbonyldiamide), imidurea, N, N-diethylformamide, N-methy 1-2 -pyrrolidine, 1 -dodecal-azacyclopheptane-2-one, calcium thioglycate, 2-pyyrolidine, N,N-diethyl-m-toluamide, oleic acid and its ester derivatives, such as methyl, ethyl, propyl, isopropyl, butyl, vinyl and glycerylmonooleate, sorbitan esters, such as sorbitan monolaurate and sorbitan monooleate, other fatty acid esters such as isopropyl laurate, isopropyl myristate, isopropyl palmitate, diisopropyl adipate, propylene glycol monolaurate, propylene glycol monooleatea and non-ionic detergents such as BRIJ® 76 (stearyl poly(10 oxy ethylene ether), BRIJ® 78 (stearyl poly(20)oxyethylene ether), BRIJ® 96 (oleyl poly(10)oxy ethylene ether), and BRIJ® 721 (stearyl poly (21) oxyethylene ether) (ICI Americas Inc. Corp.).

It will be recognized that the composition of the delivery vehicle will vary with the modality of administration, site of application, and the like. Methods of preparing pharmaceutical and cosmetic vehicles are well known to those of skill in the art (*see, e.g.*, *Remington's Pharmaceutical Sciences*, 18th Ed. Mack Printing Company, 1990; Barel, *Handbook of Cosmetic Science and Technology*, 3rd Ed., CRC Press; and Rosen, *Delivery System Handbook for Personal Care and Cosmetic Products: Technology, Applications and Formulations*, Elsevier Science (2005)).

The foregoing formulations and administration methods are intended to be illustrative and not limiting. It will be appreciated that, using the teaching provided herein, other suitable formulations and modes of administration can be readily devised.

Nucleoside supplementation.

In various embodiments, the culture medium is supplemented with, or the NCT medium comprises one or more deoxynucleoside or nucleoside (triphosphates) and/or precursors thereof. Illustrative deoxyriboneucleoside triphosphates include, but need not be limited to deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (dCTP),

deoxythymidine triphosphate (dTTP), and deoxyuridine triphosphate (dUTP). Illustrative precursors of the dNTPs include, but are not limited to:

For deoxyadenosine triphosphate (dATP): deoxyadenosine diphosphate, deoxyadenosine monophosphate, deoxyadenosine, adenine, and the like. In some embodiments the precursors include one or more precursors selected from the group consisting of deoxyadenosine diphosphate, deoxyadenosine monophosphate, and deoxyadenosine, or any combination thereof.

For deoxyguanosine triphosphate (dGTP): deoxyguanosine diphosphate, deoxyguanosine monophosphate, deoxyguanosine, guanine, and the like. In some embodiments the precursors include one or more precursors selected from the group consisting of deoxyguanosine diphosphate, deoxyguanosine monophosphate, and deoxyguanosine, or any combination thereof.

For deoxycytidine triphosphate (dCTP): deoxycytidine diphosphate, deoxycytidine monophosphate, deoxycytidine, cytosine, and the like. In some embodiments the precursors include one or more precursors selected from the group consisting of deoxycytidine diphosphate, deoxycytidine monophosphate, and deoxycytidine, or any combination thereof.

For deoxythymidine triphosphate (dTTP): deoxythymidine diphosphate, deoxythymidine monophosphate, deoxythymidine, thymine, and the like. In some embodiments the precursors include one or more precursors selected from the group consisting of deoxythymidine diphosphate, deoxythymidine monophosphate, and deoxythymidine, or any combination thereof.

For deoxyuridine triphosphate deoxyuridine diphosphate, deoxyuridine monophosphate, deoxyuridine, uracil, and the like. In some embodiments the precursors include one or more precursors selected from the group consisting of deoxyuridine diphosphate, deoxyuridine monophosphate, and deoxyuridine, or any combination thereof.

In various embodiments, the basal culture medium is additionally or alternatively supplemented with, or the NCT comprises one or more nucleoside triphosphates and/or precursors thereof. Illustrative nucleoside triphosphates include, but need not be limited to adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), 5-methyluridine triphosphate (m5UTP), uridine

triphosphate (UTP), and the like. In certain embodiments when ATP is present, at least one other nucleoside and/or deoxynucleoside triphosphate or precursor thereof is also present as a supplement. Illustrative precursors of the NTPs include, but are not limited to:

For adenosine triphosphate (ATP): adenosine diphosphate, adenosine monophosphate, adenosine, adenine, and the like, or any combination thereof. In some embodiments the precursors include one or more precursors selected from the group consisting of adenosine diphosphate, adenosine monophosphate, and adenosine.

For guanosine triphosphate (GTP): guanosine diphosphate, guanosine monophosphate, guanosine, guanine, and the like, or any combination thereof. In some embodiments the precursors include one or more precursors selected from the group consisting of guanosine diphosphate, guanosine monophosphate, and guanosine.

For cytidine triphosphate (CTP): cytidine diphosphate, cytidine monophosphate, cytidine, cytosine, and the like, or any combination thereof. In some embodiments the precursors include one or more precursors selected from the group consisting of cytidine diphosphate, cytidine monophosphate, and cytidine.

For 5-methyluridine triphosphate (m5UTP): 5-methyluridine diphosphate, 5-methyluridine monophosphate, 5-methyluridine, thymine, and the like, or any combination thereof. In some embodiments the precursors include one or more precursors selected from the group consisting of 5-methyluridine diphosphate, 5-methyluridine monophosphate, and 5-methyluridine.

For uridine triphosphate (UTP): uridine diphosphate, uridine monophosphate, uridine, uracil, and the like, or any combination thereof. In some embodiments the precursors include one or more precursors selected from the group consisting of uridine diphosphate, uridine monophosphate, and uridine.

In certain embodiments the precursor does not include the base alone.

In certain embodiments the culture medium is supplemented with, or the NCT medium comprises two different deoxynucleoside or nucleoside (triphosphates) and/or precursors thereof, or with three different deoxynucleoside or nucleoside (triphosphates) and/or precursors thereof, or with four (or more) different deoxynucleoside or nucleoside (triphosphates) and/or precursors. In certain

embodiments, the culture medium is supplemented with, or the DCT medium comprises, at least, a purine and a pyramidine (or precursors thereof). In certain embodiments the culture medium is supplemented with, or the DCT medium comprises, at least, two purines (or precursors thereof). In certain embodiments the culture medium is supplemented with, or the NCT medium comprises, at least, two pyramidines (or precursors thereof). In certain embodiments the culture medium is supplemented with, or the NCT medium comprises, at least, two purines (or precursors thereof) and at least a pyramidine (or precursor thereof). In certain embodiments the culture medium is supplemented with, or the NCT medium comprises, at least, two pyramidines (or precursors thereof) and a purine (or precursor thereof). In certain embodiments the culture medium is supplemented with, or the NCT medium comprises, at least, two purines (or precursors thereof)and two pyramidines (or precursors thereof).

By way of illustration, in some embodiments the supplement (*e.g.*, incorporated the culture medium or comprising the NCT medium) comprises or consists of one or more of the following:

1) dATP (or a precursor thereof);
2) dGTP (or a precursor thereof);
3) dCTP (or a precursor thereof);
4) dTTP (or a precursor thereof);
5) dUTP (or a precursor thereof) ;
6) dATP (or a precursor thereof) and dGTP (or a precursor thereof);
7) dATP (or a precursor thereof) and dCTP (or a precursor thereof);
8) dATP (or a precursor thereof) and dTTP (or a precursor thereof);
9) dATP (or a precursor thereof) and dUTP (or a precursor thereof);
10) dGTP (or a precursor thereof) and dCTP (or a precursor thereof);
11) dGTP (or a precursor thereof) and dTTP (or a precursor thereof);
12) dGTP (or a precursor thereof) and dUTP (or a precursor thereof);
13) dCTP (or a precursor thereof) and dTTP (or a precursor thereof);
14) dCTP (or a precursor thereof) and dUTP (or a precursor thereof);
15) dTTP (or a precursor thereof) and dUTP (or a precursor thereof);
16) dATP (or a precursor thereof), dGTP (or a precursor thereof), and dCTP (or a precursor thereof) ;

17) dATP (or a precursor thereof), dGTP (or a precursor thereof), and dTTP (or a precursor thereof);

18) dATP (or a precursor thereof), dCTP (or a precursor thereof), and dTTP (or a precursor thereof);

19) dGTP (or a precursor thereof), dCTP (or a precursor thereof), and dTTP (or a precursor thereof);

20) dATP (or a precursor thereof), dGTP (or a precursor thereof), and dCTP (or a precursor thereof) ;

21) dATP (or a precursor thereof), dGTP (or a precursor thereof), and dUTP (or a precursor thereof);

22) dATP (or a precursor thereof), dCTP (or a precursor thereof), and dUTP (or a precursor thereof);

23) dGTP (or a precursor thereof), dCTP (or a precursor thereof), and dUTP (or a precursor thereof);

24) dATP (or a precursor thereof), dGTP (or a precursor thereof), dCTP (or a precursor thereof), and dTTP (or a precursor thereof);

25) dATP (or a precursor thereof), dGTP (or a precursor thereof), dCTP (or a precursor thereof), and dUTP (or a precursor thereof);

It will be recognized that in any of the foregoing supplements, the deoxynucleoside can be a nucleoside or a precursor thereof. These supplements are intended to be illustrative and non-limiting.

Typically, the dNTPs and/or NTPs are present in the culture or NCT medium in an amount sufficient to improve the short term and/or long term genetic stability of stem cells as compared to the same cells cultured in the same culture medium or exposed to the NCT medium without supplementation by a nucleoside triphosphate or precursor thereof and/or to improve proliferation rate, and/or to improve viability.

In certain embodiments the NTPs and/or dNTPs or precursor(s) thereof supplementing the culture or DCT medium are each, independently, present at a concentration ranging from about 1 μM up to about 50 μM, or from about 1 μM to about 40 μM, or from about 1 μM up to about 35 μM, or from about 1 μM up to about 30 μM. In certain embodiments the NTPs and/or dNTPs or precursor(s) thereof supplementing the culture or DCT medium are each, independently, present at a

concentration of about 50 μM or lower, or about 40 μM or lower, or about 35 μM or lower, or about 30 μM or lower or about 25 μM or lower, or about 20 μM or lower, or about 15 μM or lower, or about 10 μM or lower, or about 5 μM or lower (with the understanding that at least 0.1 μM of at least one nucleoside triphosphate or precursor thereof is present such that "lower" is not meant to be construed as an absence of every nucleoside triphosphate or precursor thereof)..

In certain embodiments the NTPs and/or dNTPs or precursor(s) thereof supplementing the culture medium or comprising the NCT medium are each independently present at a concentration ranging from about 5 μM to about 30 μM.

In certain embodiments the NTPs and/or dNTPs or precursor(s) thereof supplementing the culture medium or comprising the NCT medium are each independently present at a concentration of about 1 μM, or about 2 μM, or about 3 μM, or about 4 μM, or about 5 μM, or about 6 μM, or about 7 μM, or about 8 μM, or about 9 μM, or about 10 μM, or about 11 μM, or about 12 μM, or about 13 μM, or about 14 μM, or about 15 μM, or about 16 μM, or about 17 μM, or about 18 μM, or about 19 μM, or about 20 μM, or about 21 μM, or about 22 μM, or about 23 μM, or about 24 μM, or about 25 μM, or about 26 μM, or about 27 μM, or about 28 μM, or about 29 μM, or about 30 μM, or about 31 μM, or about 32 μM, or about 33 μM, or about 34 μM, or about 35 μM, or about 36 μM, or about 37 μM, or about 38 μM, or about 39 μM, or about 40 μM, or about 41 μM, or about 42 μM, or about 43 μM, or about 44 μM, or about 45 μM, or about 46 μM, or about 47 μM, or about 48 μM, or about 49 μM, or about 50 μM.

In certain embodiments the NTPs and/or dNTPs or precursor(s) thereof supplementing the culture medium or comprising the NCT medium are each present at a concentration ranging from about μ 1 μM, or about 2 μM, or about 3 μM, or about 4 μM, or about 5 μM up to about 50 μM, or up to about 40 μM, or up to about 30 μM, or up to about 25 μM, or up to about 20 μM, or up to about 15 μM.

In certain embodiments the total NTPs and/or dNTPs or precursor(s) thereof supplementing the culture medium or comprising the NCT medium is present at a concentration ranging from about 1 μM, or about 2 μM, or about 3 μM, or about 4 μM, or about 5 μM, or about 6 μM, or about 7 μM, or about 8 μM, or about 9 μM, or about 10 μM, or about 11 μM, or about 12 μM, or about 13 μM, or about 14 μM,

or about 15 μM, or about 16 μM, or about 17 μM, or about 18 μM, or about 19 μM, or about 20 μM up to about 200 μM, or up to about 180 μM, or up to about 150 μM, or up to about 145 μM, or up to about 140 μM, or up to about 135 μM, or up to about 130 μM, or up to about 125 μM, or up to about 120 μM, or up to about 115 μM, or up to about 110 μM, or up to about 105 μM, or up to about 100 μM.

In certain embodiments, a stem cell culture is also provided herein, where the stem cell culture comprises stem cells in a culture medium supplemented with one or more dNTPs and/or NTPs as described herein. In certain embodiments the stem cells can be *in vivo* and exposed to an NCT as described herein. In various embodiments, the stem cells may be embryonic stem cells or adult stem cells including, but not limited to neural stem cells, hepatic stem cells, hematopoietic stem cells, umbilical cord blood stem cells, epidermal stem cells, gastrointestinal stem cells, endothelial stem cells, muscle stem cells, mesenchymal stem cells, pancreatic stem cells, and the like. In certain embodiments the stem cells include induced pluripotent stem cells, especially human IPSCs. The stem cells (including IPSCs) can be non-human animal stem cells or human stem cells.

In certain embodiments the stem cells are induced pluripotent stem cells, especially human IPSCs. Illustrative stem cells include, but are not limited to, stem cells are selected from the group consisting of embryonic stem cells and adult stem cells, including, but not limited to neural stem cells, hepatic stem cells, hematopoietic stem cells, umbilical cord blood stem cells, epidermal stem cells, gastrointestinal stem cells, endothelial stem cells, muscle stem cells, mesenchymal stem cells, and pancreatic stem cells.

In certain embodiments, where the stem cells are IPSCs the IPSCs are reprogrammed from cells selected from the group consisting of fibroblasts, neural stem cells, stomach cells, liver cells, keratinocytes, melanocytes, amniotic cells, blood cells, β-cells, and adipose cells. In certain embodiments the IPSCs comprise cells reprogramed two or more factors selected from the group consisting of KLF4 (K), LIN28 (L), c-MYC (M), NANOG (N); OCT4 (O), SOX2 (S), and valproic acid (VPA). In certain embodiments the ISPCs comprises cells reprogramed using the four canonical Yamanaka factors KLF4 (K), c-MYC (M),OCT4 (O), and SOX2 (S).

Methods of reprogramming cells to produce IPSCs well known to those of skill in the art. In certain embodiments reprogramming can be accomplished using for example integrating vectors (*e.g.*, lent viral vectors, inducible lentiviral vectors, and the like), excisable vectors (*e.g.*, transposon vectors, *loxP*-flanked lentiviral vectors, and the like), non-integrating vectors (*e.g.*, adenoviral vectors, plasmid vectors, *etc.*), DNA free vectors (*e.g.*, Sendai virus, protein vectors, modified mRNA vectors, microRNA vectors, and the like). It is noted that a number of reprogramming kits are commercially available (*e.g.*, (Epi5™ Episomal iPSC Reprogramming Kit and the CytoTune®-iPS Sendai Reprogramming Kit from Life Technologies, and the like).

In various embodiments, methods of reducing the genetic instability of induced stem cells (including pluripotent stem cells) are provided where the method comprises culturing the cells in a cell culture medium supplemented with NTPs and/or dNTPs and/or precursor(s) thereof as described herein or contacting the cells, *e.g.*, *in vivo*, with a NCT medium comprising one or more NTPs and/or dNTPs and/or precursor(s) thereof as described herein.

In various embodiments a stem cell (*e.g.*, an induced pluripotent stem cell) present in a cell culture medium supplemented with, or contacted with a NCT medium comprising, NTPs and/or dNTPs and/or precursor(s) thereof as described herein is provided.

Also provided are methods of providing autologous stem cell transfer. The methods typically involve providing stem cells isolated from a subject or IPSCs generated from a subject and expanding and/or culturing said stem cells or IPSCs in a cell culture medium or an NCT medium supplemented with NTPs and/or dNTPs and/or precursor(s) thereof as described herein.

In each of the embodiments described herein, the subject matter (*i.e.*, cell culture, DCT medium, cells, methods, *etc.*) may be free of any NTPs and/or dNTPs and/or precursor(s) thereof that have been radiolabeled. Thus, in some embodiments, the NTPs and/or dNTPs and/or precursor(s) thereof used in such embodiments have not or are not linked or incorporating any radiolabel (such as, for example, ^{3}H, ^{51}Cr, or ^{32}P, and the like). Thus, the embodiments can be free of, for example, ^{3}H-dTTP or ^{3}H-TTP or any precursor thereof.

Any supplementation composition described herein containing any one or more NTPs or dNTPS, in any combination, can also be used to supplement a wide variety of cells including, but not limited to, 1) exocrine secretory epithelial cells (such as, salivary gland mucous cell, prostate gland cell, bulbourethral gland cell, Bartholin's gland cell, Gland of Littre cell, uterus endometrium cell, isolated goblet cell of respiratory and digestive tracts, stomach lining mucous cell, gastric gland zymogenic cell, gastric gland oxyntic cell, pancreatic acinar cell, Paneth cell of small intestine, Type II pneumocyte of lung, and Clara cell of lung); 2) hormone secreting cells (such as anterior pituitary cells, intermediate pituitary cell, magnocellular neurosecretory cells, gut and respiratory tract cells, thyroid gland cells (*e.g.*, thyroid epithelial cell and parafollicular cell), parathyroid gland cells (*e.g.*, parathyroid chief cell and oxyphil cell), adrenal gland cells (*e.g.*, chromaffin cells), Leydig cell of testes, theca interna cell of ovarian follicle, Corpus luteum cell of ruptured ovarian follicle (*e.g.*, granulosa lutein cells and theca lutein cells), juxtaglomerular cell, macula densa cell of kidney, peripolar cell of kidney, and mesangial cell of kidney); 3) cells derived primarily from ectoderm (such as keratinizing epithelial cells (*e.g.*, epidermal keratinocyte, epidermal basal cell, keratinocyte of fingernails and toenails, nail bed basal cell, medullary hair shaft cell, cortical hair shaft cell, cuticular hair shaft cell, cuticular hair root sheath cell, hair root sheath cell of Huxley's layer, hair root sheath cell of Henle's layer, external hair root sheath cell, and hair matrix cell), wet stratified barrier epithelial cells (*e.g.*, surface epithelial cell of stratified squamous epithelium of cornea, tongue, oral cavity, esophagus, anal canal, distal urethra and vagina, basal cell of epithelia of cornea, tongue, oral cavity, esophagus, anal canal, distal urethra and vagina, and urinary epithelium cells), sensory transducer cells (*e.g.*, auditory inner hair cell of organ of Corti, auditory outer hair cell of organ of Corti, basal cell of olfactory epithelium, cold-sensitive primary sensory neurons, heat-sensitive primary sensory neurons, Merkel cell of epidermis, olfactory receptor neuron, pain-sensitive primary sensory neurons, photoreceptor cells of retina in eye (such as photoreceptor rod cells, photoreceptor blue-sensitive cone cell of eye, photoreceptor green-sensitive cone cell of eye, and photoreceptor red-sensitive cone cell of eye), proprioceptive primary sensory neurons, touch-sensitive primary sensory neurons, Type I carotid body cell, Type II carotid body cell, Type I hair cell of vestibular system of ear, Type II hair cell of vestibular system of ear, and Type I taste bud cell), Autonomic neuron cells (*e.g.*, cholinergic neural cell, adrenergic neural cell,

and peptidergic neural cell), sense organ and peripheral neuron supporting cells (*e.g.*, inner pillar cell of organ of Corti, outer pillar cell of organ of Corti, inner phalangeal cell of organ of Corti, outer phalangeal cell of organ of Corti, border cell of organ of Corti, Hensen cell of organ of Corti, vestibular apparatus supporting cell, taste bud supporting cell, olfactory epithelium supporting cell, Schwann cell, satellite glial cell, and enteric glial cell), central nervous system neurons and glial cells (*e.g.*, astrocyte, neuron cells, oligodendrocyte, and spindle neuron), lens cells (*e.g.*, anterior lens epithelial cell and crystallin-containing lens fiber cell); 4) cells derived primarily from mesoderm (such as metabolism and storage cells (*e.g.*, hepatocyte, adipocyte, and liver lipocyte), barrier function cells (*e.g.*, kidney parietal cell, kidney glomerulus podocyte, kidney proximal tubule brush border cell, Loop of Henle thin segment cell, kidney distal tubule cell, kidney collecting duct cell, Type I pneumocyte, pancreatic duct cell, nonstriated duct cell (of sweat gland, salivary gland, mammary gland, *etc.* (*e.g.*, principal cell and intercalated cell), duct cell (of seminal vesicle, prostate gland, *etc.*), intestinal brush border cell, exocrine gland striated duct cell, gall bladder epithelial cell, Ductulus efferens nonciliated cell, epididymal principal cell, and epididymal basal cell), extracellular matrix cells (*e.g.*, ameloblast epithelial cell, Planum semilunatum epithelial cell of vestibular system of ear, organ of Corti interdental epithelial cell, loose connective tissue fibroblasts, corneal fibroblasts, tendon fibroblasts, bone marrow reticular tissue fibroblasts, other nonepithelial fibroblasts, pericyte, nucleus pulposus cell of intervertebral disc, cementoblast/cementocyte, odontoblast/odontocyte, hyaline cartilage chondrocyte, fibrocartilage chondrocyte, elastic cartilage chondrocyte, osteoblast/osteocyte, osteoprogenitor cell, hyalocyte of vitreous body of eye, stellate cell of perilymphatic space of ear, hepatic stellate cell (Ito cell), and pancreatic stelle cell); contractile cells (*e.g.*, red skeletal muscle cell, white skeletal muscle cell, intermediate skeletal muscle cell, nuclear bag cell of muscle spindle, nuclear chain cell of muscle spindle, satellite cell, heart muscle cells, ordinary heart muscle cell, nodal heart muscle cell, Purkinje fiber cell, smooth muscle cell, myoepithelial cell of iris, and myoepithelial cell of exocrine glands), blood and immune system cells (*e.g.*, erythrocyte, megakaryocyte, monocyte, connective tissue macrophage, epidermal Langerhans cell, osteoclast, dendritic cell, microglial cell, neutrophil granulocyte, eosinophil granulocyte, basophil granulocyte, hybridoma cell, mast cell, T cells (such as helper T cell, suppressor T cell, cytotoxic T cell, and natural killer T cell), B cell, natural killer cell,

reticulocyte, and stem cells and committed progenitors for the blood and immune system), germ cells (*e.g.*, oogonium/oocyte, spermatid, spermatocyte, spermatogonium cell, and spermatozoon), nurse cells (*e.g.*, ovarian follicle cell, Sertoli cell, and thymus epithelial cell) and interstitial cells (*e.g.*, interstitial kidney cells); or any cell line thereof.

Any supplementation composition described herein containing any one or more NTPs or dNTPS, in any combination, can also be used to: 1) increase transduction of any cell described herein, and/or increase transcription and/or translation within any cell described herein; and 2) increase or preserve genomic stability and/or reduce genomic instability of any cell described herein.

In various embodiments, methods of reducing aging of skin tissue are provided in which a subject is administered any of the nucleoside cocktails described herein. In various embodiments, the nucleoside cocktails may comprise NTPs and/or dNTPs and/or precursor(s) thereof as described herein. In various embodiments, the skin of a subject is contacted with a cream or lotion comprising one or more NTPs and/or dNTPs and/or precursor(s) thereof as described herein.

In various embodiments, the anti-aging effects can be a decrease in the amount, size, or onset of skin wrinkles or folds. In various embodiments, the anti-aging effects can be a decrease in the amount, size, or onset of age spots. In various embodiments, the anti-aging effects can be maintaining or promoting skin moisture, or maintaining or promoting skin thickness. In various embodiments, the anti-aging effects can be a decrease in the reduction of skin elasticity often observed with the aging process. That is, the methods described herein can promote skin elasticity or slow the rate of skin inelasticity.

In various embodiments, the methods disclosed herein can be used to increase or decrease age-related markers. For example, senescence-associated β-galactosidase accumulates during aging in the lysosomes, cellular compartments responsible for cleavage of defective biomolecules. An increased level of senescence-associated β-galactozidase (SA-β-gal) is traditionally believed to be an aging marker. SA-β-gal activity is increased in old cells. SA-β-gal is an enzyme that cleaves a sugar from sugar-containing molecules. Thus, in various embodiments, the methods described herein can be used to decrease the level of SA-β-gal in skin cells.

Cells with unusual shape of the nuclei accumulate during aging. Instead of being round, these nuclei have bulges and bubble-like structures in their nuclear membrane.

The number of such cells may serve as an indicator of the amount of old cells in the population. Nuclear lamina is a fibrillary net with rigid structure and forms a basis of the nuclear membrane. Thus, in various embodiments, the methods described herein can be used to decrease the rate of nuclear membrane bulges in skin cells.

During experiments by others on cell lines from Hutchinson-Gilford progeria patients and also cell lines from elderly donors, structural conformations of nuclear

lamina were shown, which result from accumulation of progerin. Progerin is a protein, defective variant of the nuclear lamina A gene, LMNA. Thus, in various embodiments, the methods described herein can be used to decrease the rate of accumulation of LMNA in skin cells.

Telomere regions of DNA shorten each time the cell undergoes division. According to the telomere theory of aging, due to this molecular mechanism the overall number of cell divisions lacking telomerase activity cannot exceed a certain limit – Hayflick limit, and depletion of proliferative potential of cells in particular tissues may be sufficient for incurrence of aging-associated diseases. Thus, in various embodiments, the methods described herein can be used to decrease the rate of telomere shortening.

The number of cell divisions depends on telomerase activity. It has been observed that activation of telomerase in various murine tissues leads to life extension by 10%. It has also been shown that telomerase activation together with enhanced tumor suppressor genes activity leads to systemic slowing down of aging processes and median lifespan extension. Thus, in various embodiments, the methods described herein can be used to increase telomerase activity in skin cells.

γ-H2AX is a marker of double strand DNA brakes and severe conformational chromatin changes. Accumulation of γ-H2AX foci can be seen in nuclei of aging cells in culture, in cells taken from old donors, and in cells taken from progeria patients. Accumulation of cells in culture, in which γ-H2AX foci are emerging without any influence from external damaging agents, can be one of the objective aging criteria on the cellular level. γ-H2AX is the histone protein H2AX phosphorylated on lysine at the 139 position. Histone H2A is one of the 5 proteins

responsible for the chromatin structure, and is part of the nucleosome, the core around which DNA molecule is coiled. Thus, in various embodiments, the methods described herein can be used to decrease the rate of γ-H2AX accumulation in skin cells.

As a result of the cell cycle arrest, a cell can either become senescent (a state of cell cycle arrest), or transform into a cancer cell, or "commit suicide" via apoptosis. P16 protein is a marker of p53-independent apoptosis. P16 protein plays a crucial role in the cell cycle regulation. Thus, in various embodiments, the methods described herein can be used to modify the level of P16 in skin cells.

P21 is an effector of functions of p53 protein that promote senescence. P21 suppression is associated with increased regenerative capacity in mice. P21 protein is the inhibitor of the cyclin-dependent kinase, and plays a critical role in DNA damage response of the cell and performs cell cycle control functions. Thus, in various embodiments, the methods described herein can be used to increase P21 suppression in skin cells.

Usually in normal fibroblasts, p53 cannot be detected using the method of indirect immunofluorescence, however, it stabilizes and becomes visible in those cells that are undergoing global cellular damage response, or in aging cells. P53 protein is a transcription factor regulating the cell cycle. It is a very potent tumor suppressor. P53 plays a role in DNA damage recognition and in the subsequent DNA repair process. Thus, in various embodiments, the methods described herein can be used to increase the level of P53 in skin cells.

The presence of P53-binding protein 1 (53BP1) foci is a reliable marker of aging cells. ATM phosphorylates 53BP1 on serine at the 1219 position (S1219) in the presence of DNA damage resulting from ionizing radiation. This protein also plays an important role in the damage response of the cell. 53BP1 binds to p53 protein. Thus, in various embodiments, the methods described herein can be used to modify the level of 53BP1 in skin cells.

DNA methylation levels in aging cells are significantly reduced. A form of histone H3 that is trimethylated on lysine at the 27 position (H3K27me3) is a specific marker of facultative heterochromatin, which is densely packed regions of DNA, but capable of becoming transcriptionally active. It was shown that levels of H3K27me3 decreases with age or in the process of accelerated aging. This decline may be a reliable marker of aging cells. Thus, in various embodiments, the methods described herein can be used to modify the level of H3K27me3 in skin cells.

Another form of histone H3 that is trimethylated on lysine at the 9 position (H3K9me3) is a specific marker of facultative heterochromatin, which is densely packed regions of DNA, but capable of becoming transcriptionally active. It was shown that levels of H3K9me3 decreases with age or in the process of accelerated aging. This decline may be a reliable marker of aging cells. Thus, in various embodiments, the methods described herein can be used to modify the level of H3K9me3 in skin cells.

Aging-associated heterochromatin foci (SAHF) are special structures of condensed chromatin, which make packaged genes inactive. SAHF formation occurs spontaneously with aging. Each chromosome produces only one such heterochromatin focus. The occurrence of SAHF is associated with the onset of irreversible shutdown of cell proliferation by suppressing gene expression, triggering it, such as cyclin A and others, that transcriptionally depend on E2F. It is assumed that the mechanism of SAHF formation defines the irreversibility of cell cycle arrest with aging. Thus, in various embodiments, the methods described herein can be used to decrease the number or rate of formation of SAHF in skin cells.

The embodiments described herein are intended to be illustrative and non-limiting. Using the teachings provided herein, numerous variations of the compositions and methods described herein will be available to one of skill in the art.

EXAMPLES

The following examples are offered to illustrate, but not to limit the claimed invention.

Example 1

Nucleoside Supplementation In hIPSC Culture Or In Nucleoside Cocktail Transmission (NCT) Medium To Promote Genetic Stability, Enhance Cellular Function and Enhance Regeneration

Significance

Human induced pluripotent stem cells (hIPSCs) have significant therapeutic potential (Byrne (2008) *Human Molecular Genetics* 17: R37-41). However, our findings (Fig. 1, Preliminary Data), and those of others (Martins-Taylor and Xu (2012) *Stem Cells* 30: 22-27; Lund *et al.* (2012) *Nat. Rev. Genet.,* 13: 732-744), indicate that essentially all iPSC clones derived using current approaches

acquire genomic instability during reprogramming and *in vitro* culture-induced stress. The issue of hIPSC genomic instability is one of the most important bottlenecks in advancing personalized iPSC-based regenerative therapies to the clinic (Martins-Taylor and Xu (2012) *Stem Cells* 30: 22-27; Lund *et al.* (2012) *Nat. Rev. Genet.*, 13: 732-744; Byrne (2013) *Gene Therapy and Regulation* 7: 1230002), given the established link between genomic instability and an increased risk of malignant transformation.

The causes of genomic instability during hIPSC reprograming and culture remain unknown, and approaches to reduce and eventually eliminate the occurrence of this highly deleterious phenomenon have yet to be developed. In ongoing experiments, we find that deoxyribonucleotide triphosphate (dNTP) pools in rapidly dividing hIPSCs are significantly smaller in size than the dNTP pools of dermal fibroblasts, from which the hIPSCs are derived (Fig. 2). We also find that hIPSCs demonstrate significantly more double strand breaks (DSBs) than the fibroblasts of origin (Figs. 3A and 3B). Our observations of decreased dNTP pools and increased DNA damage in hIPSCs may provide new mechanistic insight into the etiology of genomic instability of hIPSCs, and present a method for preventing this phenomenon. Thus, our data indicate that: 1) hIPSCs can utilize exogenous dNTP precursors added to the hIPSC culture medium in the form of a mixture of deoxyribonucleosides (dNs) and 2) that culture medium supplementation with dNs can both ameliorate the hIPSC dNTP pool deficiency and significantly reduce the genomic instability experienced by these cells (Fig. 4).

Hypotheses and Rationale.

Mammalian cells synthesize dNTPs via two pathways: the de novo pathway (DNP), which uses glucose and amino acids, and the nucleoside salvage pathway (NSP), which uses preformed dNs from the extracellular environment. Without being bound by a particular theory, we hypothesize that: 1) insufficient production of dNTP pools via the DNP is an important cause of replication stress, DNA damage and genomic instability in rapidly dividing hIPSCs and 2) enabling the utilization by the hIPSC cells of the NSP by supplementing the culture medium with a defined mixture of dN substrates of the NSP will increase dNTP pools and will alleviate replication stress, DNA damage and genomic instability.

Without being bound by a particular theory, it was hypothesized that: 1) insufficient production of dNTP pools from glucose and amino acids via the *be novo* pathway (DNP), is an important cause of replication stress, DNA damage and genomic instability in hIPSCs and 2) enabling the hIPSCs to also use a second dNTP biosynthetic pathway, the nucleoside salvage pathway (NSP) in addition to the DNP will alleviate replication stress, DNA damage, and genomic instability. In an illustrative embodiment, enabling the use of the NSP was accomplished by supplementing the hIPSC culture medium with a mixture of deoxyribonucleoside (dNs) substrates of the NSP.

In summary, a simple, effective, generally applicable, and cost-efficient solution to the problem of genomic instability is provided that is useful for hIPSCs produced using the current methodology. Specifically, it is shown that the occurrence of genomic instability in stem cells and hIPSCs can be prevented by enabling these cells to synthesize dNTPs via the NSP. This can be achieved by supplementing the culture media with an optimized mixture of dN substrates of the NSP

Derivation and Characterization of Human Induced Pluripotent Stem Cells

We have reprogrammed adult human dermal fibroblasts (HDFs) into human induced pluripotent stem cells (hIPSCs) using either a LoxP-flanked polycistronic reprogramming vector (which was then removed from the genome using Cre recombinase (Sommer *et al.* (2010) *Stem Cells* 28: 64-74)), or by using a synthetic mRNA based approach (Warren *et al.* (2010) *Cell Stem Cell,* 7: 618-630). All reprogramming and subsequent culture were performed in standard hIPSC culture medium, which, according to the current standard practice, was not supplemented with any nucleosides (www.stemgent.com/products/show/69. NUTRISTEM™ XF/FF Culture Medium). Both transgene-free hIPSC lines demonstrated standard characterization parameters, as previously described (Byrne *et al.* (2009) *PloS One* 4: e7118). These parameters included embryonic stem cell (ESC)- like morphology, significant expression of the key pluripotency markers (NANOG, OCT4, SSEA3, SSEA4, Tra-1-60 and Tra-1-81), which were not detected in the original skin fibroblasts (Fig. 1, panel A), and differentiation into representatives of all three germ layers following teratoma formation in severe combined immunodeficiency (SCID) mice (Fig. 1, panel B). We then converted our transgene-free hIPSC lines from

research-grade conditions (containing animal-derived epitopes: Matrigel substrate and Knockout Serum Replacement, KSR, containing culture medium) into putative clinical-grade animal epitope-free "xeno-free" defined conditions (CellStart substrate and an optimized xeno-free media, 50% mTeSRI, Stemcell Technologies, and 50% NutriStem, Stemgent), as previously described (Karumbayaram *et al.* (2012) *Stem Cells Trans. Med.* 1: 36-43). Both hIPSC lines were karyotyped, as we have previously described (Byrne *et al.* (2009) *PloS One* 4: e7118) both before and after "clinical-grade conversion" (CC).

Following CC-induced stress and expansion both lines went from being karyotypically normal (46XY) to possessing an extra chromosome 12 (Fig. 1, panel C). The observed genomic instability correlates closely with several published studies showing that hIPSCs are genetically unstable, particularly when placed under conditions of extrinsic stress (Nagaria *et al.* (2013) *Biochimica et Biophysica Acta,* 1830: 2345-2353), and preferentially duplicate chromosome 12 (Draper *et al.* (2004) *Nat. Biotechnol.* 22: 53-54). It has previously been suggested that all chromosomes in hIPSCs have the same initial incidence of chromosomal translocation and duplication, but that duplication of chromosome 12, when it occurs, results in a preferential expansion throughout the hIPSC population following extended *in vitro* culture through a selection process (*Id.*). It should be noted that we consider CC-based stress to be a useful model for longer term culture (>6-12 month) induced stress, which also tends to result in hIPSCs and hESCs accumulating karyotypic abnormalities, especially chromosome 12, as we observed following CC-based stress over a 2 month period.

Smaller dNTP Pools in hIPSCs vs. HDFs of origin

We quantified deoxyribonucleotide triphosphate (dNTP) pools in the original HDFs and the early passage (none-CC-stressed) hIPSCs as described previously (Austin *et al.* (2012) *J. Exp. Med.,* 209: 2215-2228). We observed a significant drop (between 32-52%) in levels of all four dNTPs after hIPSC reprogramming (Fig. 2).

Genes involved in dNTP Biosynthesis are Expressed at Similar Levels in Rapidly Dividing hIPSCs and in HDFs with a Slower Rate of Cell Division

An Affymetrix global transcriptional analysis on HDFs and derived early passage hIPSCs (performed as previously described (Awe *et al.* (2013) *Stem Cell Res. & Theap.*, 4: 15)) showed significant upregulation of key pluripotency-specific markers; the expression of key fibroblast-specific markers was decreased following hIPSC reprogramming (Table 2). We also observed upregulation of genes previously linked to the induction of genomic instability (CCNE1, E2F2, E2F35). However, we did not observe any significant differences in the expression of key dNTP biosynthetic genes (Table 2). As hIPSCs have a faster average population doubling time (PDT) than HDFs (18 hrs vs. 48 hrs), these data support our hypothesis that the small dNTP pools in hIPSCs may result from 1) a faster rate of cell proliferation which increases the usage of dNTPs for DNA replication, and 2) an inability to upregulate the expression of genes involved in dNTP biosynthesis.

Table 2. Change in gene expression following hIPSC reprogramming.

Gene name	Gene ID	Type	Fold change	p-Value
POU5F1	Hs.450254	Pluripotency specific	40 fold increase	0.01
NANOG	Hs.661360	Pluripotency specific	100 fold increase	0.01
SOX2	Hs.518438	Pluripotency specific	30 fold increase	0.004
COL1A1	Hs. 172928	Fibroblast specific	20 fold decrease	0.03
COL3A1	Hs.443625	Fibroblast specific	170 fold decrease	0.001
COL6A3	Hs.233240	Fibroblast specific	40 fold decrease	0.02
CCNE1	Hs.244723	Genomic instability	7 fold increase	0.03
E2F2	Hs. 194333	Genomic instability	8 fold increase	0.02
E2F3	Hs.269408	Genomic instability	3 fold increase	0.01
dCK	Hs.709	Nucleotide metabolism	No difference	N/A
TK1	Hs.515122	Nucleotide metabolism	No difference	N/A
UPP1	Hs.488240	Nucleotide metabolism	No difference	N/A
RRM1	Hs.445705	Nucleotide metabolism	No difference	N/A
RRM2	Hs.226390	Nucleotide metabolism	No difference	N/A

Elevated levels of DNA damage in hIPSCs vs. HDFs: dN supplementation reduces DNA damage in hIPSCs

We utilized gamma-histone 2A.X (γH2A.X) staining, as previously described (Bester *et al.* (2011) *Cell,* 145: 435-446), in order to estimate the number of double strand breaks (DSBs) in HDFs and early passage hIPSCs. Significantly more γH2A.X positive foci were detected in hIPSCs than in the HDFs of origin. We also observed significantly less γH2A.X positive foci following 4 days of dN supplementation (Fig. 3A, compare middle and lower panels). This finding provides additional support for the hypothesis that hIPSCs demonstrate higher levels of genomic instability than dermal fibroblasts and genomic instability could be prevented with dN supplementation of the cell culture medium. We also observed significant heterogeneity in the number of γH2A.X positive foci within the hIPSC population, supporting, our hypothesis that the incidence of genomic instability is non-uniform across the hIPSC population, and that a subpopulation of hIPSCs are more likely to incur additional genomic DNA damage, as assayed by γH2A.X staining, and thus represent the cells most susceptible to developing karyotypic abnormalities.

dN supplementation promotes hIPSC genomic stability following CC-based stress

We cultured our hIPSCs in conditions with and without dNs in order to investigate whether dN supplementation could prevent genomic instability observed during CC-based stress. It should be noted that, while hIPSCs have been demonstrated to accumulate genomic damage during reprogramming and extended culture (Ben-David *et al.* (2010) *Cell Cycle (Georgetown, Tex.)* 9: 4603-4604), we observed the most extreme genomic damage (karyotypic abnormalities), in two independent hIPSC lines, when we stressed the cells by performing CC. This observation provides a rapid and consistent experimental assay to induce severe genomic damage over a relatively short period of time (2 months). Human iPSCs were first cultured on Matrigel, in a combination of mTeSRI (Stemcell Technologies) and NutriStem (Stemgent). They were then divided into two groups grown in the presence or absence of exogenously supplied dNs in the cell culture medium (30 μM of each dN). After one week of supplementation, we began the conversion process to xeno-free conditions, which entailed switching to CellStart as a basement membrane

and NutriStem medium only. After two weeks of culture, genomic DNA (gDNA) was extracted for single nucleotide polymorphism (SNP)-based loss-of-heterozygosity (LOH) analysis, which was previously shown to be an accurate measure of DSB-based genomic instability (Bester *et al.* (2011) *Cell,* 145: 435-446). To determine levels of LOH between the two conditions (+/- dNs), analysis was performed using Affymetrix's SNP 6.0 array as previously described (*Id.*). Supplementation with dNs reduced the incidence of genomic instability, as assayed by LOH, to 20% of that observed in unsupplemented medium (Fig. 4). Together with the significant decrease in yH2A.X positive foci following 4 days of dN supplementation (Figs. 3A and 3B), the reduction in LOH provides evidence in support of our hypothesis that dN supplementation reduces replication stress and the associated genomic instability in hIPSC reprogramming and culture.

It is important to note that previous research has correlated the incidence of DSB with LOH in human cancer cells (*Id.*), highlighting the value of SNP-based LOH measurements as a quantifiable method for assaying genomic instability. In our study of CC-stressed hIPSCs with and without dN supplementation, we observed a five-fold increase in genomic stability, as assayed by the reductions in LOH, following CC-induced stress. This can be observed in Fig. 4, where 12 LOH chromosomal loci were identified following CC-based stress. Five times as many LOH loci, which are indicative of genomic damage, were observed in the untreated hIPSCs (identified by green arrows), than were observed in the dN-treated hIPSCs (identified by the red arrow). This provides strong evidence in support of our hypothesis that dN supplementation can ameliorate CC-induced genomic instability in hIPSCs. However, it is important to note that half of the LOH loci (as identified by the blue arrows) were still unstable in both dN treated and untreated samples.

While efficacy has been demonstrated, it is believed the dN supplementation procedure can be further optimized. Data generated thus far support the hypothesis that dNTP pool deficiency plays a critical role in the genomic instability we and others have observed in hIPSCs following general culture and induced stress.

Moreover, our preliminary data also suggest a simple and generally applicable culture medium deoxyribonucleoside supplementation procedure to enhance the genomic stability of clinical grade hIPSCs and their derivatives.

In summary, we propose that establishing such an optimized media system capable of maintaining genetically stable stem cells and hIPSC and derivatives will be instrumental towards safely unlocking the future promise of personalized pluripotent stem cell-based therapeutics for patients.

Example 2

Obtaining Stable Stem dN Concentration

We have obtained multiple different STABLESTEM™ concentrations, one for short term culture (30μM of each dN), one for long term culture (5μM of each dN) as well as a suitable 30:30:5:5 cocktail that has proven robust in enhancing genomic stability, increasing cellular function and improving differentiation potential across multiple cell types (*see, e.g.* Table 3).

Table 3. dN concentrations.

Culture supplement	Dose of each dN	3-5 day cell culture	3+ weeks cell culture
Short term	30μM	Good	Not good
Long term	5μM	Good	Good

The long term STABLESTEM™ culture media supplement allows human iPS cells to proliferate for at least 3 weeks, while dramatically reducing the amount of genomic damage the cells are exposed to (Figure 7); as measured by γH2A.X-positive double strand breaks. The causes of genomic instability during hIPSC reprograming and culture have been unclear. However, we recently discovered that deoxyribonucleotide triphosphate (dNTP) pools in rapidly dividing hIPSCs are significantly smaller in size than the dNTP pools of the skin cells from which the hIPSCs are derived. In addition, we discovered that supplementing the stem cell culture media with deoxyribonucleosides (dNs) can significantly rescue the dNTP pools (Figure 6).

Example 3

Increasing Transgenic Expression and Decreasing in Genetic Damage

A nucleoside composition comprising 2'-deoxycytidine (Sigma D3897; dC), 2'-deoxyadenosine monohydrate (Sigma D8668; dA), 2'-deoxyguanosine monohydrate (Sigma D0901; dG), thymidine (Sigma T9250; dT), dimethylsulfoxide (DMSO), UltraPure distilled water (Life 10977-015), mTeSR1

basal medium (StemCell Technologies 05850), and NutriStem XF/FF culture medium (Stemgent 01-0005) was prepared. Briefly, a 100 mM stock supplement for long-term storage was prepared by: 1) making a 100 mM stock solutions of all four deoxyribonucleotides by weighing appropriate amounts of each nucleotide and resuspending in 1 mL of either filtered DMSO or UltraPure Water, and then resuspending dC and dT in water and dA and dG in DMSO; 2) 100 uL aliquots were prepared and stored in -80°C for long-term storage. To prepare 100 uM working concentrations for culture, the following was carried out: 1) from the 100 mM stocks, 100 uM aliquots (working stocks) were prepared for each nucleotide (100 µL of stock in 9.9 mL of stem cell media (50:50 blend of mTeSR1 and Nutristem)); and 2) from the 100 uM working stocks, the final supplemented culture media for stem cell application was prepared such that the final stem cell culture media contained 30 µM dC, 30 µM dA, 5 µM dT, and 5 µM dG. Fresh supplemented stem cell media was prepared weekly. The 100 µM stocks can be made with any media, depending on specific desired culture conditions.

We have recently discovered that nucleoside supplementation can promote expression of transgenic expression in a cellular population. This increased transgenic expression (see, Fig. 14) is correlated with a decrease in genetic damage as assayed via gamma H2AX staining (see, Fig. 13). Adult skin fibroblasts were supplemented with nucleosides before and during the reprogramming process. Such supplementation resulted in a decrease in DNA damage in cells 24 hours and 6 days after lentiviral transduction of reprogramming factors. DNA damage was quantified by γ-H2AX foci counts and quantified in a blinded manner. In addition, nucleoside supplementation also resulted in increased protein expression, as shown in Fig. 14. Strongly OCT4 expressing cells were observed to be increased under supplementation conditions at 24 hours and 6 days post lentiviral transduction. These results demonstrate for the first time that reduction in replicative stress can increase the expression of a desired transgenic product. This methodology has applications across a wide range of fields and can be used to reduce genetic damage and promote transgenic expression for essentially any *in vitro* application.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the

spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

CLAIMS

What is claimed is:

1. A cell culture medium for the culture of stem cells with improved genetic stability, said culture medium comprising:

a basal culture medium for stem cells, where said culture medium is supplemented with one or more nucleoside triphosphates or one or more precursors thereof.

2. A nucleoside cocktail transmission (NCT) medium for improving somatic or stem cell genetic stability said medium comprising:

a cosmetic or pharmaceutical delivery vehicle; and

one or more nucleoside triphosphates or precursors thereof.

3. The cell culture medium of claim 1 or nucleoside cocktail transmission medium of claim 2, wherein said one or more nucleoside triphosphates are independently selected from the group consisting of deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (NCTP), deoxythymidine triphosphate (dTTP) deoxyuridine triphosphate, adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), 5-methyluridine triphosphate (m5UTP), and uridine triphosphate (UTP).

4. The cell culture medium of claim 1 or nucleoside cocktail transmission medium of claim 2, wherein said one or more nucleoside triphosphates are independently selected from the group consisting of deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (NCTP), deoxythymidine triphosphate (dTTP) and deoxyuridine triphosphate.

5. The cell culture medium of claim 1 or nucleoside cocktail transmission medium of claim 2, wherein said one or more nucleoside triphosphates are independently selected from the group consisting of adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), 5-methyluridine tiphosphate (m5UTP), and uridine triphosphate (UTP).

6. The cell culture medium according to any one of claims 1 and 3-5 or nucleoside cocktail transmission medium according to any one of claims 2-5, wherein said culture medium is supplemented with, or said NCT medium comprises, one or more precursors of nucleoside triphosphates independently selected from the group consisting of deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (NCTP), deoxythymidine triphosphate (dTTP) deoxyuridine triphosphate, adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), 5-methyluridine triphosphate (m5UTP), and uridine triphosphate (UTP).

7. The cell culture or NCT medium of claim 6, wherein said one or more precursors of nucleoside triphosphates are independently selected from the group consisting of deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (NCTP), deoxythymidine triphosphate (dTTP) and deoxyuridine triphosphate.

8. The cell culture or NCT medium of claim 6, wherein said one or more precursors of nucleoside triphosphates are independently selected from the group consisting of adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), 5-methyluridine triphosphate (m5UTP), and uridine triphosphate (UTP).

9. The cell culture medium according to any one of claims 1 and 3-8, or the NCT medium according to any one of claims 3-8, wherein said culture medium is supplemented with, or said NCT medium comprises, deoxyadenosine triphosphate (dATP), or a precursor thereof.

10. The cell culture medium or NCT medium of claim 9, said culture medium is supplemented with, or said NCT medium comprises, deoxyadenosine triphosphate.

11. The cell culture medium or NCT medium of claim 9, said culture medium is supplemented with, or said NCT medium comprises, a precursor of deoxyadenosine triphosphate.

12. The cell culture medium or NCT medium of claim 11, wherein said precursor of deoxyadenosine triphosphate is selected from the group consisting of deoxyadenosine diphosphate, deoxyadenosine monophosphate, deoxyadenosine, and adenine.

13. The cell culture medium according to any one of claims 1, and 3-12, or the NCT medium according to any one of claims 2-12, wherein said culture medium is supplemented with, or said NCT medium comprises, deoxyguanosine triphosphate (dGTP), or a precursor thereof.

14. The cell culture medium or NCT medium according of claim 13, wherein said cell culture medium is supplemented with or said NCT medium comprises deoxyguanosine triphosphate.

15. The cell culture medium or NCT medium according of claim 13, wherein said cell culture medium is supplemented with or said NCT medium comprises a precursor of deoxyguanosine triphosphate.

16. The cell culture or NCT medium of claim 15, wherein said precursor of deoxyguanosine triphosphate selected from the group consisting of deoxyguanosine diphosphate, deoxyguanosine monophosphate, deoxyguanosine, and guanine.

17. The cell culture medium according to any one of claims 1, or 3-16, or the NCT medium according to any one of claims 2-16, wherein said culture medium is supplemented with or said NCT medium comprises deoxycytidine triphosphate (NCTP), or a precursor thereof.

18. The cell culture medium or NCT medium of claim 17, wherein said cell culture medium is supplemented with, or said NCT medium comprises deoxycytidine triphosphate.

19. The cell culture medium or NCT medium of claim 17, wherein said cell culture medium is supplemented with, or said NCT medium comprises a precursor of deoxycytidine triphosphate.

20. The cell culture medium or NCT medium of claim 19, wherein precursor of deoxycytidine is selected from the group consisting of deoxycytidine diphosphate, deoxycytidine monophosphate, deoxycytidine, and cytosine.

21. The cell culture medium according to any one of claims 1, and 3-20, or the NCT medium according to any one of claims 2-20, wherein said culture medium is supplemented with, or said NCT comprises deoxythymidine triphosphate (dTTP), or a precursor thereof.

22. The cell culture medium or NCT medium of claim 20, wherein said culture medium is supplemented with, or said NCT medium comprises deoxythymidine triphosphate.

23. The cell culture medium or NCT medium of claim 20, wherein said culture medium is supplemented with, or said NCT medium comprises a precursor of deoxythymidine triphosphate.

24. The cell culture medium or NCT medium of claim 23, wherein said precursor of deoxythymidine triphosphate is selected from the group consisting of deoxythymidine diphosphate, deoxythymidine monophosphate, deoxythymidine, and thymine.

25. The cell culture medium according to any one of claims 1, and 3-24, or the NCT medium according to any one of claims 2-24, wherein said culture or NCT medium is supplemented with deoxyuridine triphosphate, or a precursor thereof.

26. The cell culture medium or NCT medium of claim 25, wherein said culture medium is supplemented with, or said NCT medium comprises deoxyuridine triphosphate.

27. The cell culture medium or NCT medium of claim 25, wherein said culture medium is supplemented with, or said NCT medium comprises a precursor of deoxyuridine triphosphate.

28. The cell culture medium or NCT medium of claim 27, wherein said precursor of deoxyuridine triphosphate is selected from the group consisting of deoxyuridine diphosphate, deoxyuridine monophosphate, deoxyuridine, and uracil.

29. The cell culture medium according to any one of claims 1, and 3-28, or the NCT medium according to any one of claims 2-28, wherein said culture or NCT medium is supplemented with adenosine triphosphate (ATP) or a precursor thereof.

30. The cell culture medium or NCT medium of claim 29, wherein said culture medium is supplemented with, or said NCT medium comprises, adenosine triphosphate (ATP).

31. The cell culture medium or NCT medium of claim 29, wherein said culture medium is supplemented with, or said NCT medium comprises, a precursor of adenosine triphosphate.

32. The cell culture medium or NCT medium of claim 31, wherein said precursor of adenosine triphosphate (ATP) is selected from the group consisting of adenosine diphosphate, adenosine monophosphate, adenosine, and adenine.

33. The cell culture medium according to any one of claims 1, and 3-32, or the NCT medium according to any one of claims 2-32, wherein said culture medium is supplemented with, or said NCT medium comprises guanosine triphosphate (GTP) or a precursor thereof.

34. The cell culture medium or NCT medium of claim 33, wherein said culture medium is supplemented with, or said NCT medium comprises, guanosine triphosphate (GTP).

35. The cell culture medium or NCT medium of claim 33, wherein said culture medium is supplemented with, or said NCT medium comprises, a precursor of guanosine triphosphate.

36. The cell culture medium or NCT medium of claim 35, wherein said precursor of guanosine triphosphate is selected from the group consisting of guanosine diphosphate, guanosine monophosphate, guanosine, and guanine.

37. The cell culture medium according to any one of claims 1, and 3-36, or the NCT medium according to any one of claims 2-36, wherein said cell culture medium is supplemented with, or said NCT medium comprises cytidine triphosphate (CTP), or a precursor thereof.

38. The cell culture medium or NCT medium of claim 37, wherein said culture medium is supplemented with, or said NCT medium comprises, cytidine triphosphate.

39. The cell culture medium or NCT medium of claim 37, wherein said culture medium is supplemented with, or said NCT medium comprises, a precursor of cytidine triphosphate.

40. The cell culture medium or NCT medium of claim 39, wherein said precursor of cytidine triphosphate is selected from the group consisting of cytidine diphosphate, cytidine monophosphate, cytidine, and cytosine.

41. The cell culture medium according to any one of claims 1, and 3-40, or the NCT medium according to any one of claims 2-40, wherein said culture or NCT medium is supplemented with 5-methyluridine triphosphate (m5UTP), or a precursor thereof.

42. The cell culture medium or NCT medium of claim 41, wherein said culture medium is supplemented with, or said NCT medium comprises, 5-methyluridine triphosphate.

43. The cell culture medium or NCT medium of claim 41, wherein said culture medium is supplemented with, or said NCT medium comprises, a precursor of 5-methyluridine triphosphate.

44. The cell culture medium or NCT medium of claim 43, wherein said precursor of 5-methyluridine triphosphate is selected from the group consisting of 5-methyluridine diphosphate, 5-methyluridine monophosphate, 5-methyluridine, and thymine.

45. The cell culture medium according to any one of claims 1, and 3-44, or the NCT medium according to any one of claims 2-44, wherein said culture

medium is supplemented with, or said NCT medium comprises uridine triphosphate (UTP), or a precursor thereof.

46. The cell culture medium or NCT medium of claim 45, wherein said culture medium is supplemented with, or said NCT medium comprises uridine triphosphate.

47. The cell culture medium or NCT medium of claim 45, wherein said culture medium is supplemented with, or said NCT medium comprises a precursor of uridine triphosphate.

48. The cell culture medium or NCT medium of claim 47, wherein precursor of uridine triphosphate is selected from the group consisting of uridine diphosphate, uridine monophosphate, uridine, and uracil.

49. The nucleoside cocktail transmission (NCT) medium according to any one of claims 2-48, wherein said NCT medium comprises a vehicle formulated for administration via a route selected from the group consisting of subcutaneous administration, parenteral administration, topical administration, oral administration, nasal or inhalation administration, local administration such as by paint, aerosol, or transdermally.

50. The nucleoside cocktail transmission (NCT) medium according to any one of claims 2-48, wherein said NCT medium comprises a vehicle formulated for topical administration, subdermal administration, or intradermal administration.

51. The nucleoside cocktail transmission (NCT) medium of claim 50, wherein said medium is formulated in a vehicle selected from the group consisting of a mud, an herbal mixture, a fat, an emulsions, a lotion, a cream, a gel, a biological, a solution, a spray, an ointment, a foams, a mousses, a liquid, a suspensions, a dispersion, an aerosol, a soap, a shampoo, and a conditioner.

52. The nucleoside cocktail transmission (NCT) medium of claim 50, wherein said medium is formulated as a cream (*e.g.*, a face cream).

53. The nucleoside cocktail transmission (NCT) medium according to any one of claims 56-58, wherein said medium comprises a formulation selected

from the group consisting of a wrinkle removing cream, a dermal filler, a scar-reducing cream, and an acne treatment.

54. The cell culture medium according to any one of claims 1, and 3-48 or the nucleoside cocktail transmission (NCT) medium according to any one of claims 2-53, wherein said nucleoside triphosphate or precursor thereof supplementing said culture medium, or comprising said NCT medium, are present at a concentration sufficient to improve the short term and/or long term genetic stability of stem cells as compared to the same cells cultured in the same medium without supplementation by a nucleoside triphosphate or precursor thereof.

55. The cell culture medium or the nucleoside cocktail transmission (NCT) medium of claim 54, wherein said stem cells are stem cells selected from the group consisting of somatic cells.

56. The cell culture medium according to any one of claims 1, 3-48, and 54-55, or the NCT medium according to any one of claims 2-55, wherein each nucleoside triphosphate or precursor thereof supplementing said culture medium, or comprising said NCT medium is present at a concentration ranging from about 1 μM up to about 50 μM, or from about 1 μM to about 40 μM, or from about 1 μM up to about 35 μM, or from about 1 μM up to about 30 μM.

57. The cell culture medium according to any one of claims 1, 3-48, and 54-55, or the NCT medium according to any one of claims 2-55, wherein each nucleoside triphosphate or precursor thereof supplementing said culture medium, or comprising said NCT medium, is at a concentration starting (stock) or final (extrinsic delivery) of about 50 μM or lower, or about 40 μM or lower, or about 30 μM or lower or about 25 μM or lower, or about 20 μM or lower, or about 15 μM or lower, or about 10 μM or lower, or about 5 μM or lower.

58. The cell culture medium, or NCT medium of claim 57, wherein each nucleoside triphosphate or precursor thereof supplementing said culture medium, or comprising said NCT medium, is present at a starting (stock) or final (extrinsic delivery) concentration ranging from about 5 μM to about 30 μM.

59. The cell culture medium, or NCT medium of claim 57, wherein each nucleoside triphosphate or precursor thereof supplementing said culture said culture medium, or comprising said NCT medium, is present at a starting (stock) or final (extrinsic delivery) concentration of about 30 μM.

60. The cell culture medium, or NCT medium of claim 57, wherein each nucleoside triphosphate or precursor thereof supplementing said culture said culture medium, or comprising said NCT medium, is present at a starting (stock) or final (extrinsic delivery) concentration of about 5 μM.

61. The cell culture medium according to any one of claims 1, 3-48, and 54-60, or the NCT medium according to any one of claims 2-60, wherein said cell culture medium, or said NCT medium is xenopathogen-free.

62. The cell culture medium according to any one of claims 1, 3-48, and 54-61, or the NCT medium according to any one of claims 2-61, wherein said cell culture medium, or said NCT medium is without animal or human derived serum albumin.

63. The cell culture medium according to any one of claims 1, 3-48, and 54-62, wherein the supplemented medium is selected from the group consisting of DMEM (Dulbecco's Modified Eagle's Medium), MEM (Minimal Essential Medium), BME (Basal Medium Eagle), RPMI 1640, DMEM/F-12 (Dulbecco's Modified Eagle's Medium: Nutrient Mixture F-12), DMEM/F-10 (Dulbecco's Modified Eagle's Medium: Nutrient Mixture F-10), α-MEM (α-Minimal essential Medium), G-MEM (Glasgow's Minimal Essential Medium), IMDM (Isocove's Modified Dulbecco's Medium), essential 8 (E8) medium, and KnockOut DMEM.

64. The cell culture medium of claim 63, wherein the basal medium is DMEM/F12.

65. The nucleoside cocktail transmission (NCT) medium of claim 2, wherein said NCT comprises said vehicle and a cell culture medium according to any one of claims 1, 3-48, and 55-64.

66. A somatic or stem cell culture or nucleoside cocktail transmission (NCT) culture, said cell culture comprising cells in a cell culture medium according to any one of claims 1, 3-48, and 55-64, or comprising cells in an NCT medium according to any one of claims 2-62, and 65.

67. The cell culture or NCT culture of claim 66, wherein said somatic or stem cells are cells selected from the group consisting of somatic cells or stem cells.

68. The cell culture or NCT culture of claim 66, wherein said cells comprise stem cells.

69. The cell culture or NCT culture of claim 68, wherein said stem cells are selected from the group consisting of embryonic stem cells and adult stem cells, including, but not limited to neural stem cells, hepatic stem cells, hematopoietic stem cells, umbilical cord blood stem cells, epidermal stem cells, gastrointestinal stem cells, endothelial stem cells, muscle stem cells, mesenchymal stem cells, and pancreatic stem cells.

70. The cell culture or NCT culture of claim 68, wherein said cells are induced pluripotent stem cells (IPSCs).

71. The cell culture or NCT culture of claim 70, wherein said IPSCs are reprogrammed from cells selected from the group consisting of fibroblasts, neural stem cells, stomach cells, liver cells, keratinocytes, melanocytes, amniotic cells, blood cells, β-cells, and adipose cells.

72. The cell culture or NCT culture of claims 70 or 71, wherein the IPSCs comprise cells reprogramed two or more factors selected from the group consisting of KLF4 (K), LIN28 (L), c-MYC (M), NANOG (N); OCT4 (O), SOX2 (S), and valproic acid (VPA).

73. The cell culture or NCT culture of claim 72, wherein the ISPCs comprises cells reprogramed using the four canonical Yamanaka factors KLF4 (K), c-MYC (M),OCT4 (O), and SOX2 (S).

74. The cell culture or NCT culture of claim 73, wherein the reprogramming factors further comprise LIN28.

75. The cell culture or NCT culture according to any one of claims 70-74, wherein said IPSCs are reprogrammed using a vector selected from the group consisting of an integrating vector, a non-integrating vector, an excisable vector, and a DNA-free vector.

76. A method of reducing genetic instability of stem cells said method comprising culturing said cells in a cell culture medium according to any one of claims of claims a culture medium according to any one of claims 1, 3-48, and 55-64.

77. A method of performing autologous stem cell transfer, said method comprising isolating stem cells from a subject or generating IPSCs from said subject and expanding and/or culturing said stem cells or IPSCs in a cell culture medium according to any one of claims 1, 3-48, and 55-64, or in an NCT medium according to any one of claims 2-62, and 65.

78. A method of promoting regeneration and/or maintenance of tissues, said method comprising administering to a subject, an NCT medium according to any one of claims 2-62, and 65.

79. The method of claim 78, wherein said NCT medium comprises a vehicle formulated for administration via a route selected from the group consisting of subcutaneous administration, parenteral administration, topical administration, oral administration, nasal or inhalation administration, local administration such as by paint, aerosol, or transdermally.

80. The method of claim 78, wherein, wherein said NCT medium comprises a vehicle formulated for topical administration, subdermal administration, or intradermal administration.

81. The method of claim 80, wherein said NCT medium is formulated in a vehicle selected from the group consisting of a mud, an herbal mixture, a fat, an emulsions, a lotion, a cream, a gel, a biological, a solution, a spray,

an ointment, a foams, a mousses, a liquid, a suspensions, a dispersion, an aerosol, a soap, a shampoo, and a conditioner.

82. The method of claim 80, wherein said NCT medium is formulated as a cream (*e.g.*, a face cream).

83. The method of claim 80, wherein said NCT medium comprises a formulation selected from the group consisting of a wrinkle removing cream, a dermal filler, a scar-reducing cream, and an acne treatment.

84. The method according to any one of claims 78-83, wherein said NCT is applied to the skin of a human.

85. The method of claim 84, wherein said NCT is applied to reduce scarring.

86. The method of claim 84, wherein said NCT is applied to reduce wrinkles.

87. The method according to any one of claims 78-83, wherein said NCT is applied intradermally or subdermally to increase tissue volume.

88. A method of increasing transduction of a cell with a heterologous nucleic acid construct and/or increasing transcription and/or translation of said construct, said method comprising transducing and/or culturing said cell in a cell culture medium according to any one of claims 1, 3-48, and 55-64, or in an NCT medium according to any one of claims 2-62, and 65.

89. A method of increasing or preserving genomic stability and/or reducing genomic instability of a cell, said method comprising transducing and/or culturing said cell in a cell culture medium according to any one of claims 1, 3-48, and 55-64, or in an NCT medium according to any one of claims 2-62, and 65.

90. The method according to any one of claims 88-89, wherein said cell culture medium and/or said NCT medium comprises 2'-deoxycytidine (dC), 2'-deoxyadenosine monohydrate, 2'-deoxyguanosine monohydrate, and thymidine.

91. The method of claim 90, wherein said cell culture medium and/or said NCT medium further comprises dimethylsulfoxide (DMSO).

92. The method according to any one of claims 90-91, wherein said cell culture medium and/or said NCT medium comprises mTeSR1 basal medium.

93. The method according to any one of claims 90-92, wherein said cell culture medium and/or said NCT medium comprises NutriStem XF/FF culture medium.

94. The method according to any one of claims 90-93, wherein said cell culture medium and/or said NCT medium comprises dC and dA at at least twice the concentration, or at least 3 times the concentration, or at least 4 times the concentration, or at least 5 times the concentration of dT and dG.

95. The method of claim 94, wherein said cell culture medium and/or said NCT medium comprises 30 μM dC, 30 μM dA, 5 μM dT, and 5 μM dG.

96. The method according to any one of claims 90-95 , wherein said cell is selected from the group consisting of: 1) exocrine secretory epithelial cells (such as, salivary gland mucous cell, prostate gland cell, bulbourethral gland cell, Bartholin's gland cell, Gland of Littre cell, uterus endometrium cell, isolated goblet cell of respiratory and digestive tracts, stomach lining mucous cell, gastric gland zymogenic cell, gastric gland oxyntic cell, pancreatic acinar cell, Paneth cell of small intestine, Type II pneumocyte of lung, and Clara cell of lung); 2) hormone secreting cells (such as anterior pituitary cells, intermediate pituitary cell, magnocellular neurosecretory cells, gut and respiratory tract cells, thyroid gland cells (*e.g.*, thyroid epithelial cell and parafollicular cell), parathyroid gland cells (*e.g.*, parathyroid chief cell and oxyphil cell), adrenal gland cells (*e.g.*, chromaffin cells), Leydig cell of testes, theca interna cell of ovarian follicle, Corpus luteum cell of ruptured ovarian follicle (*e.g.*, granulosa lutein cells and theca lutein cells), juxtaglomerular cell, macula densa cell of kidney, peripolar cell of kidney, and mesangial cell of kidney); 3) cells derived primarily from ectoderm (such as keratinizing epithelial cells (*e.g.*, epidermal keratinocyte, epidermal basal cell, keratinocyte of fingernails and toenails, nail bed basal cell, medullary hair shaft cell, cortical hair shaft cell, cuticular hair shaft cell, cuticular hair root sheath cell, hair root sheath cell of Huxley's layer, hair

root sheath cell of Henle's layer, external hair root sheath cell, and hair matrix cell), wet stratified barrier epithelial cells (*e.g.*, surface epithelial cell of stratified squamous epithelium of cornea, tongue, oral cavity, esophagus, anal canal, distal urethra and vagina, basal cell of epithelia of cornea, tongue, oral cavity, esophagus, anal canal, distal urethra and vagina, and urinary epithelium cells), sensory transducer cells (*e.g.*, auditory inner hair cell of organ of Corti, auditory outer hair cell of organ of Corti, basal cell of olfactory epithelium, cold-sensitive primary sensory neurons, heat-sensitive primary sensory neurons, Merkel cell of epidermis, olfactory receptor neuron, pain-sensitive primary sensory neurons, photoreceptor cells of retina in eye (such as photoreceptor rod cells, photoreceptor blue-sensitive cone cell of eye, photoreceptor green-sensitive cone cell of eye, and photoreceptor red-sensitive cone cell of eye), proprioceptive primary sensory neurons, touch-sensitive primary sensory neurons, Type I carotid body cell, Type II carotid body cell, Type I hair cell of vestibular system of ear, Type II hair cell of vestibular system of ear, and Type I taste bud cell), Autonomic neuron cells (*e.g.*, cholinergic neural cell, adrenergic neural cell, and peptidergic neural cell), sense organ and peripheral neuron supporting cells (*e.g.*, inner pillar cell of organ of Corti, outer pillar cell of organ of Corti, inner phalangeal cell of organ of Corti, outer phalangeal cell of organ of Corti, border cell of organ of Corti, Hensen cell of organ of Corti, vestibular apparatus supporting cell, taste bud supporting cell, olfactory epithelium supporting cell, Schwann cell, satellite glial cell, and enteric glial cell), central nervous system neurons and glial cells (*e.g.*, astrocyte, neuron cells, oligodendrocyte, and spindle neuron), lens cells (*e.g.*, anterior lens epithelial cell and crystallin-containing lens fiber cell); 4) cells derived primarily from mesoderm (such as metabolism and storage cells (*e.g.*, hepatocyte, adipocyte, and liver lipocyte), barrier function cells (*e.g.*, kidney parietal cell, kidney glomerulus podocyte, kidney proximal tubule brush border cell, Loop of Henle thin segment cell, kidney distal tubule cell, kidney collecting duct cell, Type I pneumocyte, pancreatic duct cell, nonstriated duct cell (of sweat gland, salivary gland, mammary gland, *etc.* (*e.g.*, principal cell and intercalated cell), duct cell (of seminal vesicle, prostate gland, *etc.*), intestinal brush border cell, exocrine gland striated duct cell, gall bladder epithelial cell, Ductulus efferens nonciliated cell, epididymal principal cell, and epididymal basal cell), extracellular matrix cells (*e.g.*, ameloblast epithelial cell, Planum semilunatum epithelial cell of vestibular system of ear, organ of Corti interdental epithelial cell, loose connective tissue fibroblasts, corneal fibroblasts,

tendon fibroblasts, bone marrow reticular tissue fibroblasts, other nonepithelial fibroblasts, pericyte, nucleus pulposus cell of intervertebral disc, cementoblast/cementocyte, odontoblast/odontocyte, hyaline cartilage chondrocyte, fibrocartilage chondrocyte, elastic cartilage chondrocyte, osteoblast/osteocyte, osteoprogenitor cell, hyalocyte of vitreous body of eye, stellate cell of perilymphatic space of ear, hepatic stellate cell (Ito cell), and pancreatic stelle cell); contractile cells (*e.g.*, red skeletal muscle cell, white skeletal muscle cell, intermediate skeletal muscle cell, nuclear bag cell of muscle spindle, nuclear chain cell of muscle spindle, satellite cell, heart muscle cells, ordinary heart muscle cell, nodal heart muscle cell, Purkinje fiber cell, smooth muscle cell, myoepithelial cell of iris, and myoepithelial cell of exocrine glands), blood and immune system cells (*e.g.*, erythrocyte, megakaryocyte, monocyte, connective tissue macrophage, epidermal Langerhans cell, osteoclast, dendritic cell, microglial cell, neutrophil granulocyte, eosinophil granulocyte, basophil granulocyte, hybridoma cell, mast cell, T cells (such as helper T cell, suppressor T cell, cytotoxic T cell, and natural killer T cell), B cell, natural killer cell, reticulocyte, and stem cells and committed progenitors for the blood and immune system), germ cells (*e.g.*, oogonium/oocyte, spermatid, spermatocyte, spermatogonium cell, and spermatozoon), nurse cells (*e.g.*, ovarian follicle cell, Sertoli cell, and thymus epithelial cell) and interstitial cells (*e.g.*, interstitial kidney cells); or any cell line thereof.

97. A method of reducing aging of skin cells or tissue, said method comprising contacting said skin cells or tissue with a medium according to any one of claims 2-62, and 65.

98. The method of claim 97. wherein said reducing aging comprises a decrease in the amount, size, or onset of skin wrinkles or folds, or a decrease in the amount, size, or onset of age spots, or a decrease in the reduction of skin elasticity often observed with the aging process, or maintaining or promoting skin moisture, or maintaining or promoting skin thickness.

99. A method of increasing or decreasing age-related markers of skin cells or tissue, said method comprising contacting said skin cells or tissue with a medium according to any one of claims 2-62, and 65.

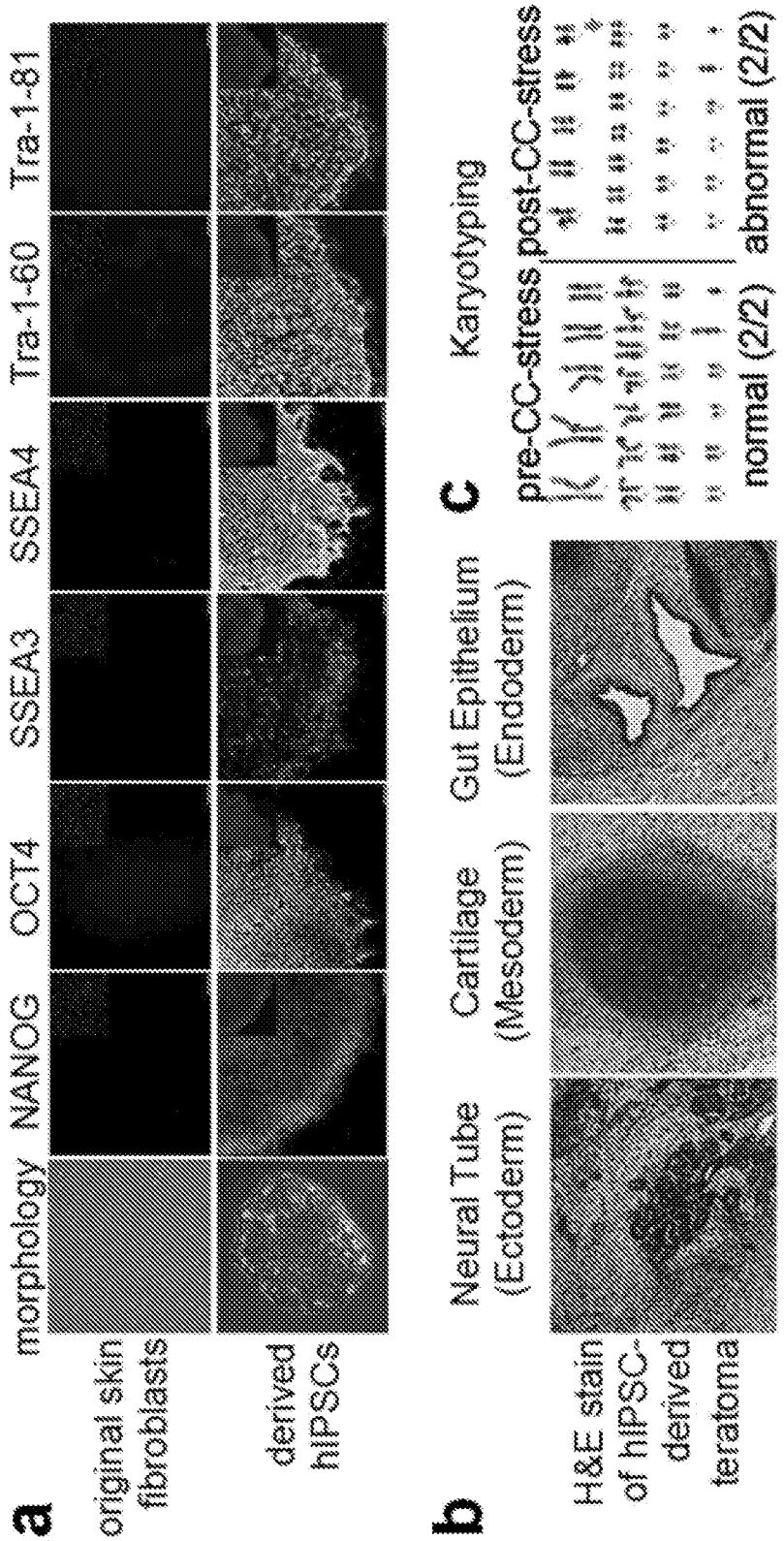
a
morphology
NANOG
OCT4
SSEA3
SSEA4
Tra-1-60
Tra-1-81
original skin fibroblasts
derived hIPSCs
b
Neural Tube (Ectoderm)
Cartilage (Mesoderm)
Gut Epithelium (Endoderm)
H&E stain of hIPSC-derived teratoma
c
Karyotyping
pre-CC-stress
post-CC-stress
normal (2/2)
abnormal (2/2)

Fig. 1

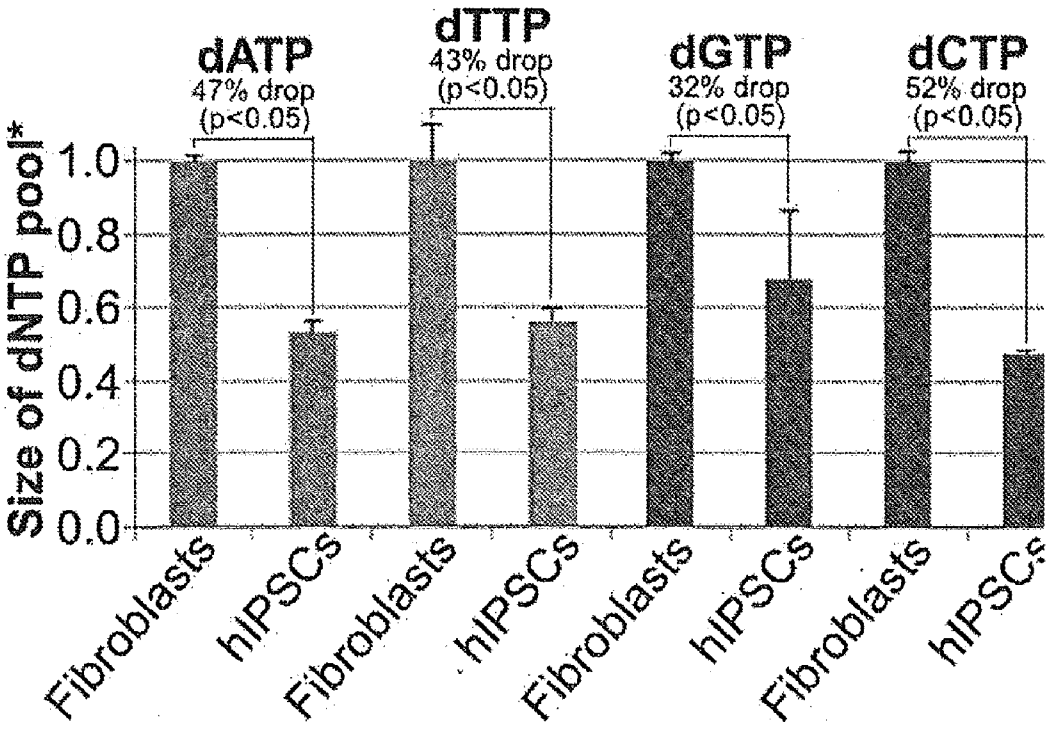
dATP
47% drop
(p<0.05)
dTTP
43% drop
(p<0.05)
dGTP
32% drop
(p<0.05)
dCTP
52% drop
(p<0.05)
Size of dNTP pool*
1.0
0.8
0.6
0.4
0.2
0.0
Fibroblasts
hIPSCs
Fibroblasts
hIPSCs
Fibroblasts
hIPSCs
Fibroblasts
hIPSCs

Fig. 2

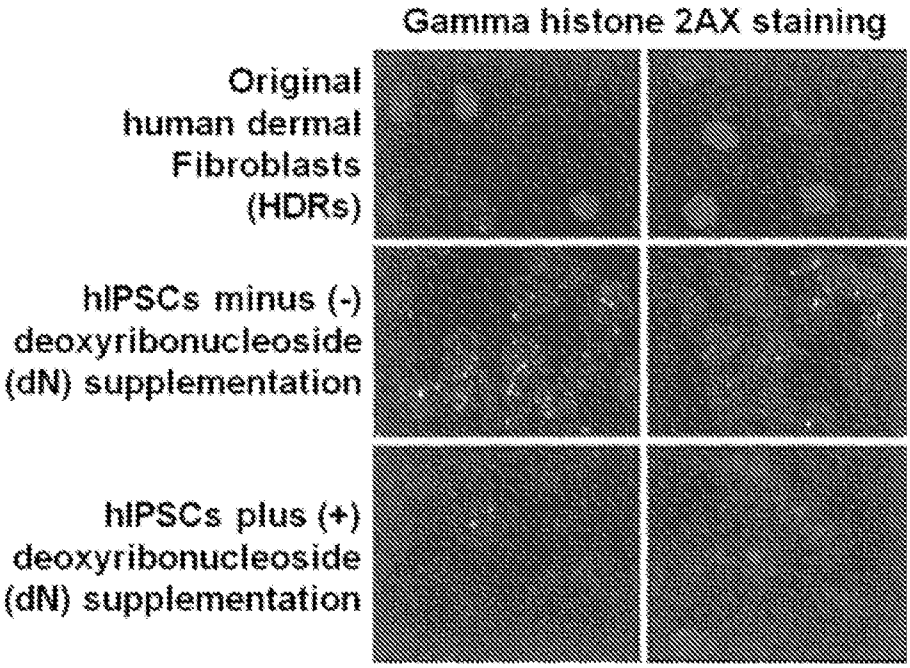
Gamma histone 2AX staining
Original human dermal Fibroblasts (HDRs)
hIPSCs minus (-) deoxyribonucleoside (dN) supplementation
hIPSCs plus (+) deoxyribonucleoside (dN) supplementation

Fig. 3A

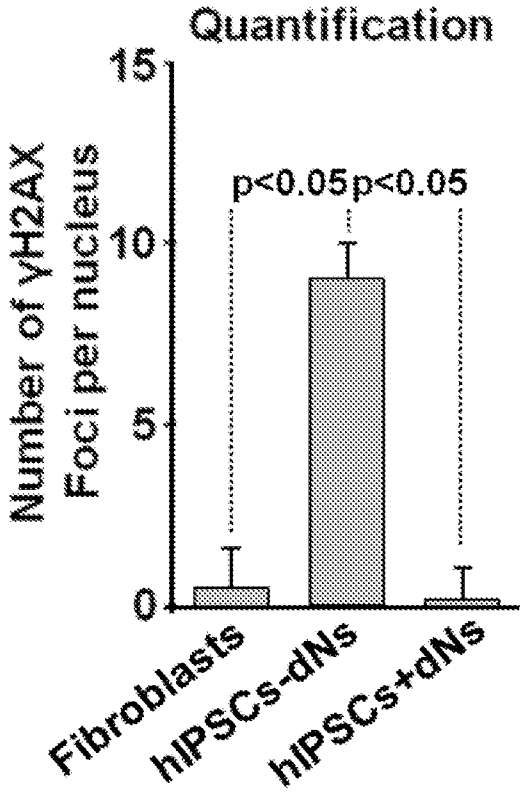
Quantification
Number of γH2AX Foci per nucleus
15
10
5
0
p<0.05
p<0.05
Fibroblasts
hIPSCs-dNs
hIPSCs+dNs

Fig. 3B

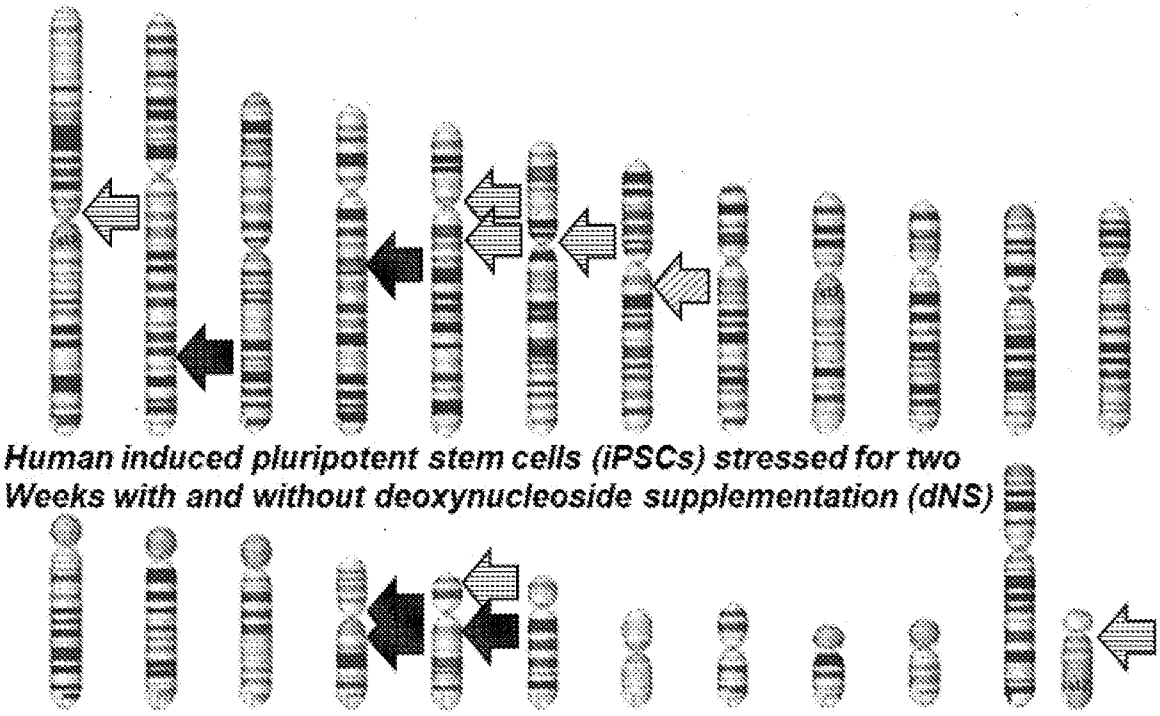
Human induced pluripotent stem cells (iPSCs) stressed for two
Weeks with and without deoxynucleoside supplementation (dNS)
Arrows highlight chromosomal location of novel SNP-based LOH:
Arrows highlight LOH in both dNS treated and dNS untreated (6/12)
Arrows highlight LOH only in dNS untreated (5/12)
Arrows highlight LOH only in dNS treated (1/12)

Fig. 4

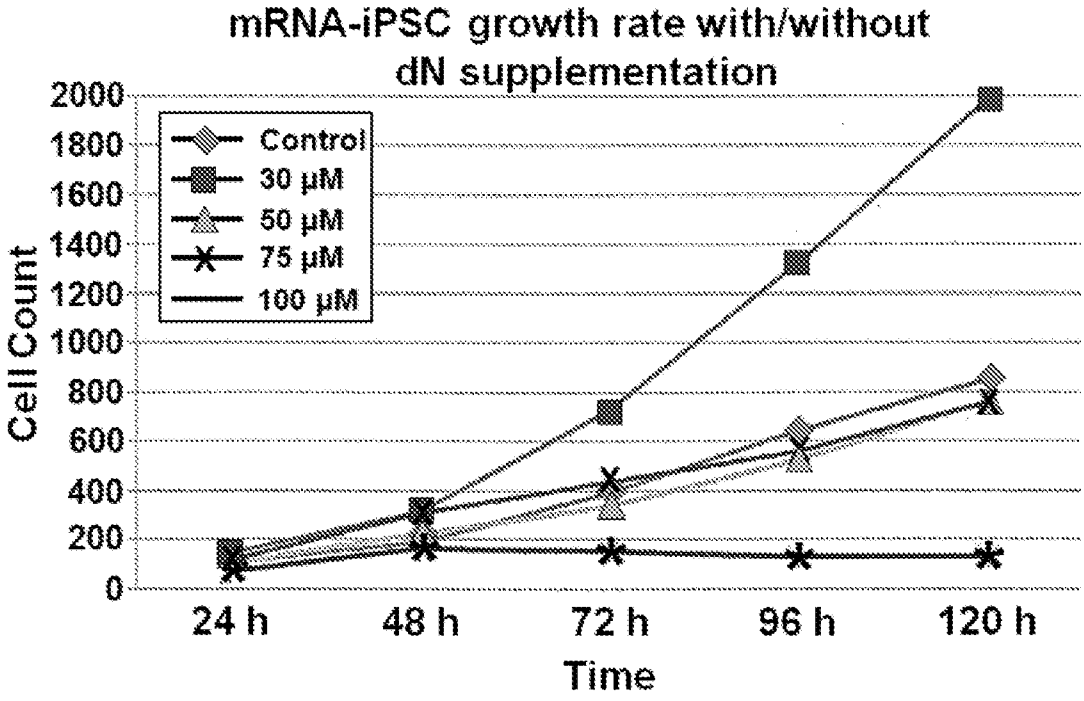
mRNA-iPSC growth rate with/without dN supplementation
Cell Count
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Control
30 µM
50 µM
75 µM
100 µM
24 h
48 h
72 h
96 h
120 h
Time

Fig. 5

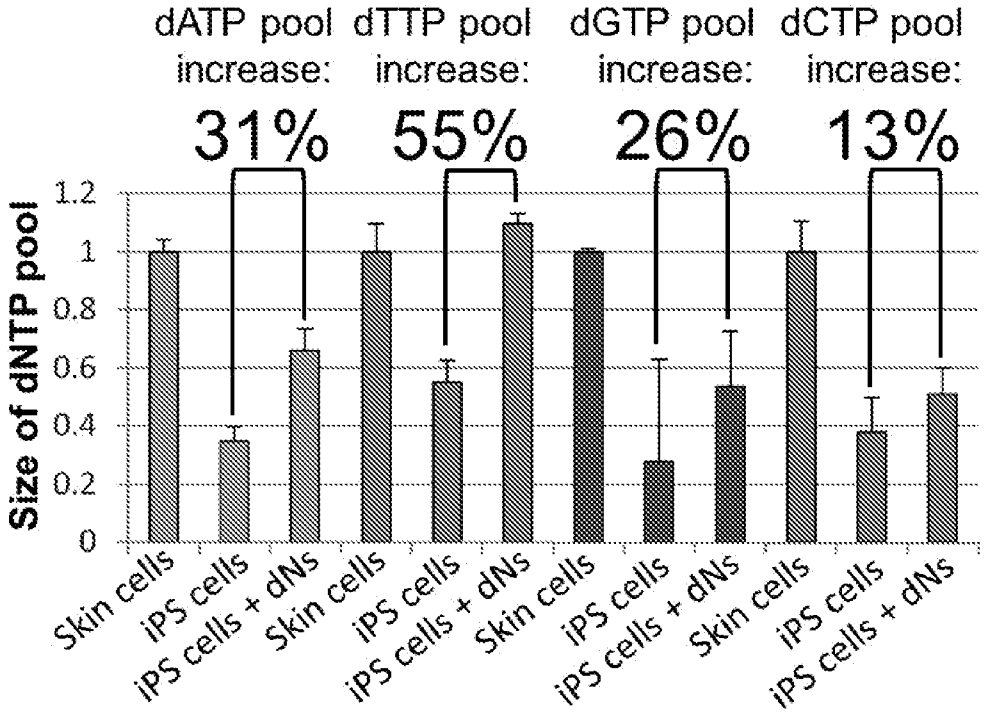
dATP pool increase:
31%
dTTP pool increase:
55%
dGTP pool increase:
26%
dCTP pool increase:
13%
Size of dNTP pool
1.2
1
0.8
0.6
0.4
0.2
0
Skin cells
iPS cells
iPS cells + dNs

Fig. 6

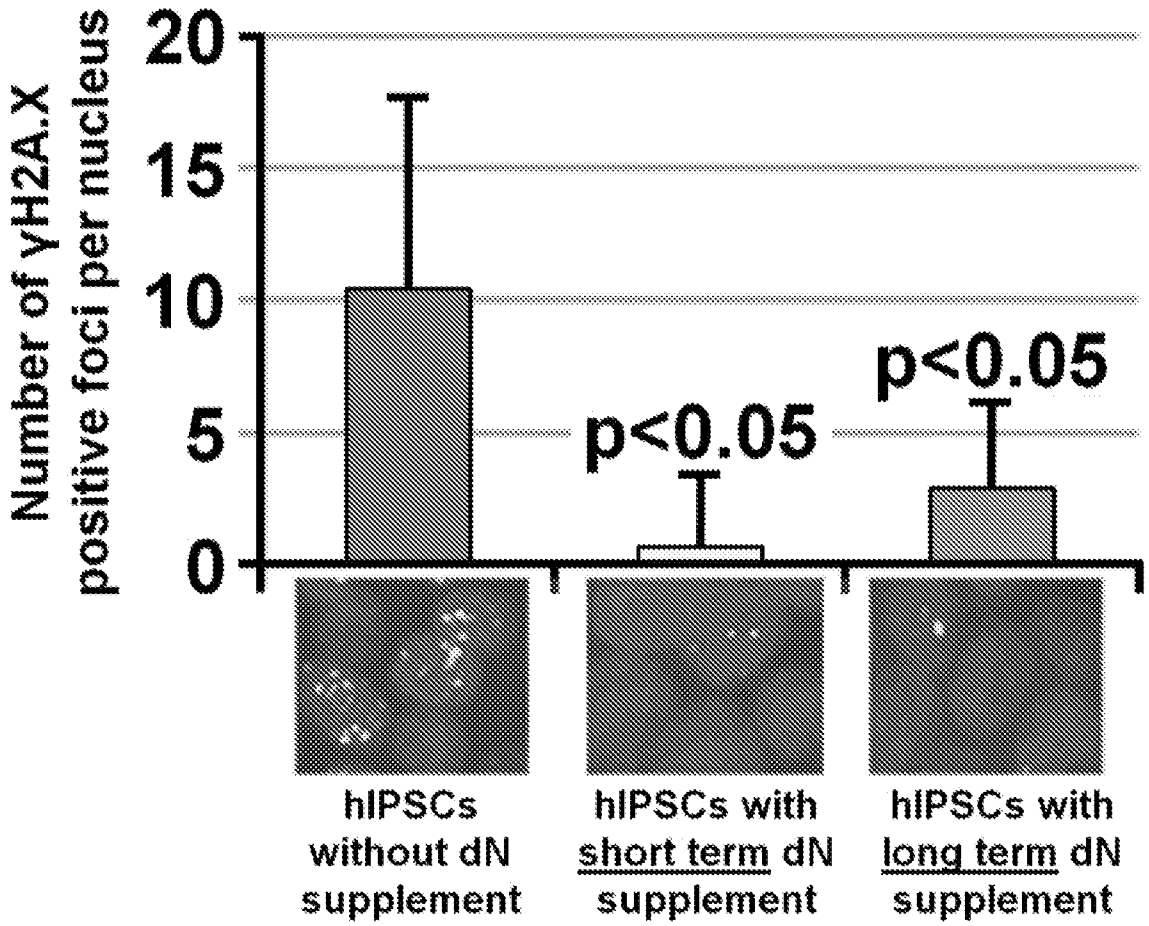
Number of γH2A.X positive foci per nucleus
20
15
10
5
0
p<0.05
p<0.05
hIPSCs without dN supplement
hIPSCs with short term dN supplement
hIPSCs with long term dN supplement

Fig. 7

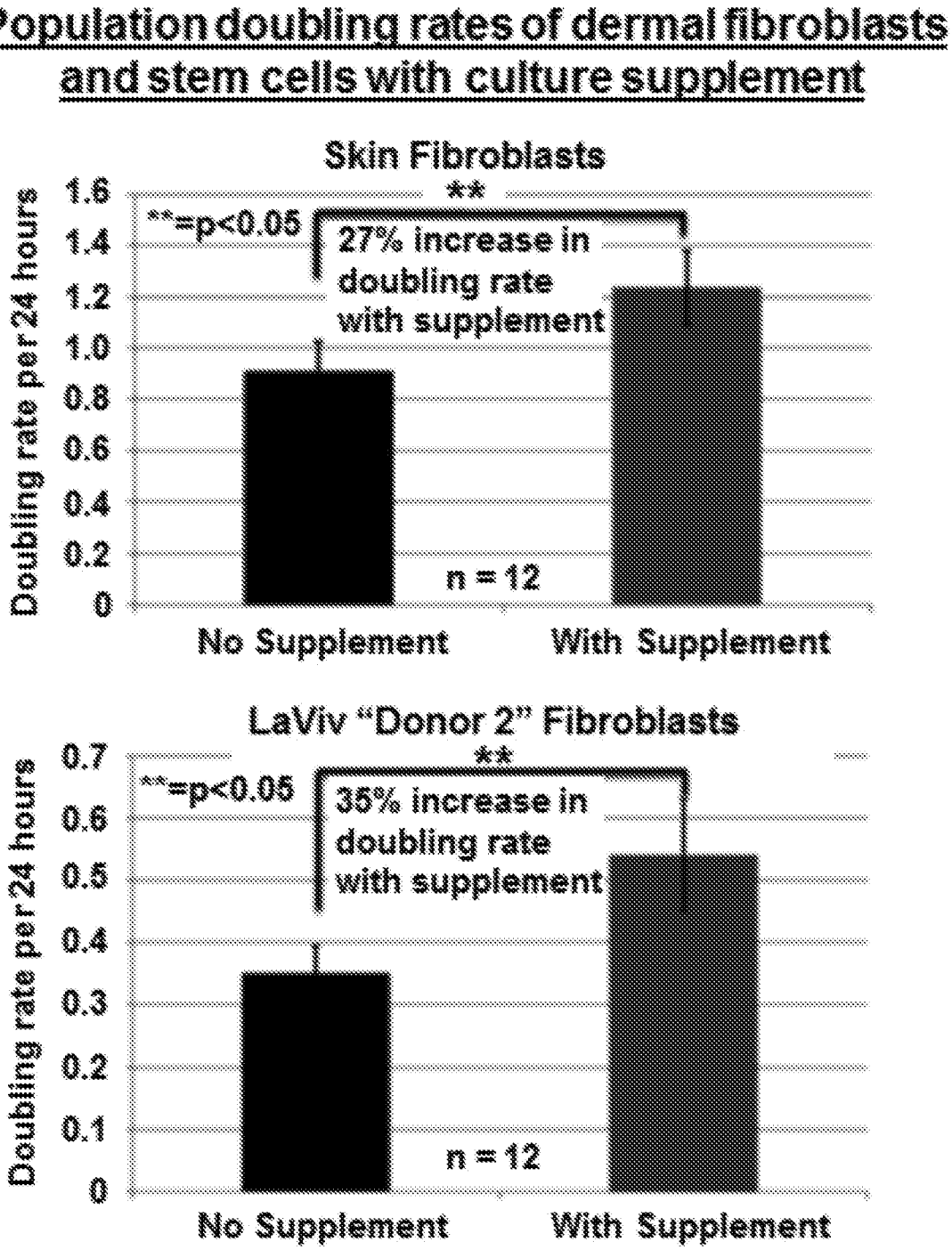
Population doubling rates of dermal fibroblasts and stem cells with culture supplement
Skin Fibroblasts
Doubling rate per 24 hours
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
**
**=p<0.05
27% increase in doubling rate with supplement
n = 12
No Supplement
With Supplement
LaViv "Donor 2" Fibroblasts
Doubling rate per 24 hours
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
**
**=p<0.05
35% increase in doubling rate with supplement
n = 12
No Supplement
With Supplement

Fig. 8

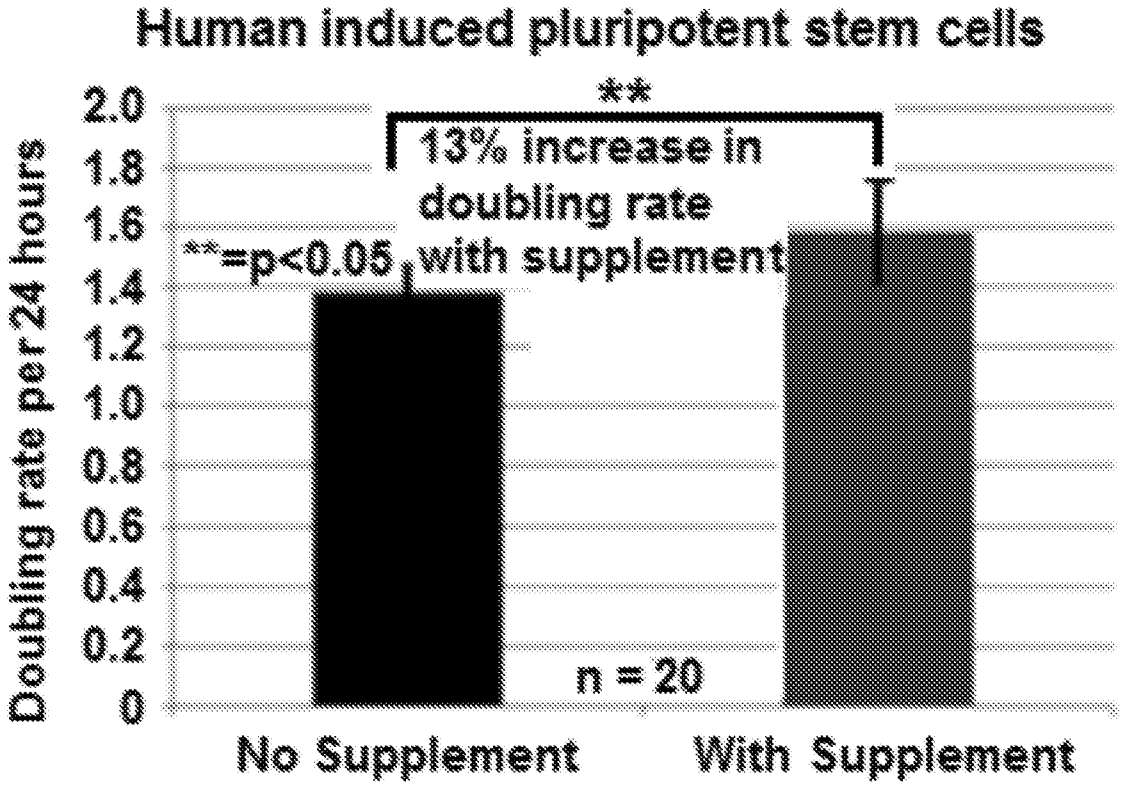
Human induced pluripotent stem cells
Doubling rate per 24 hours
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
**
13% increase in doubling rate with supplement
**=p<0.05
n = 20
No Supplement
With Supplement

Fig. 8, cont'd

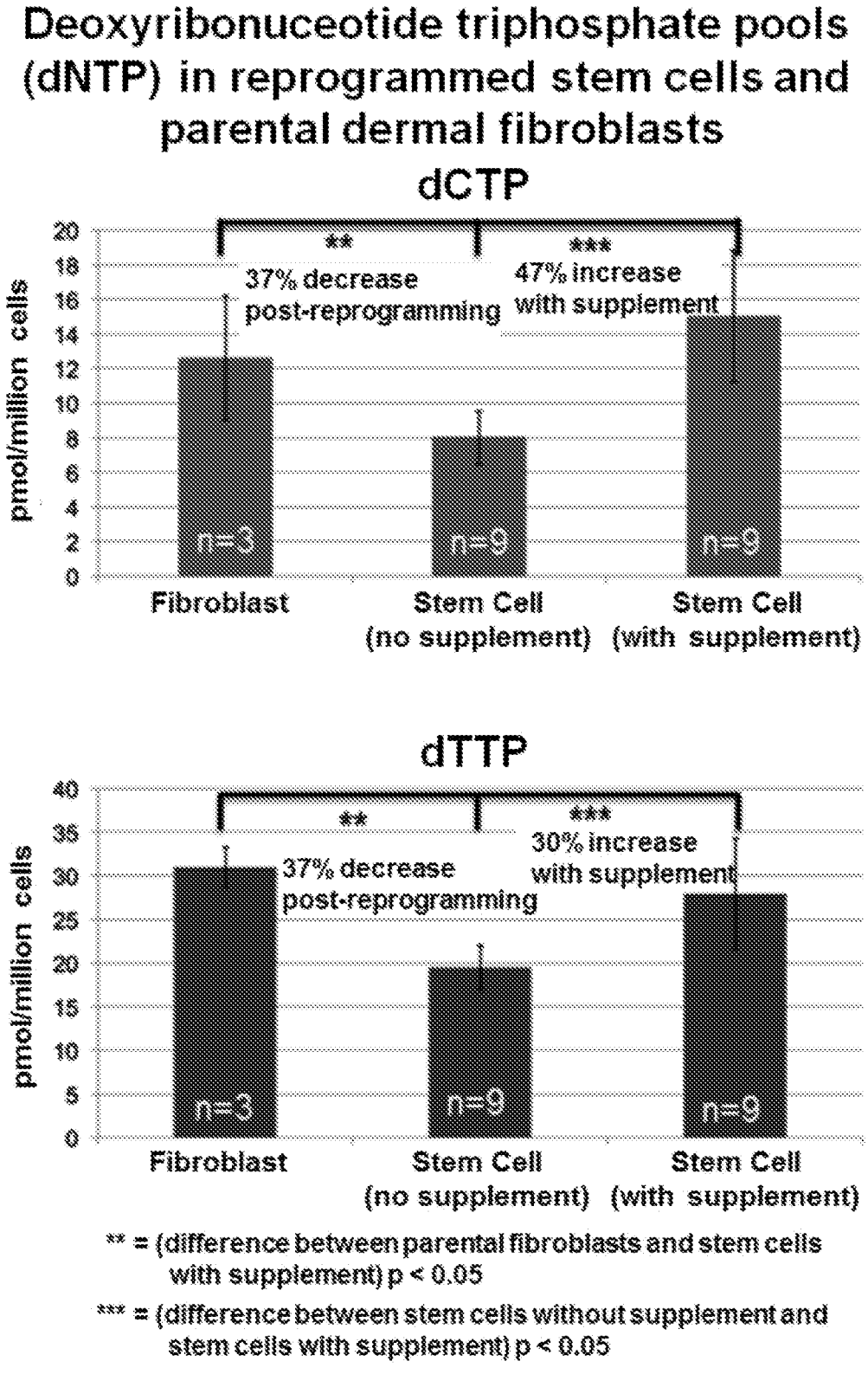
Deoxyribonuceotide triphosphate pools (dNTP) in reprogrammed stem cells and parental dermal fibroblasts
dCTP
pmol/million cells
20
18
16
14
12
10
8
6
4
2
0
**
***
37% decrease post-reprogramming
47% increase with supplement
n=3
n=9
n=9
Fibroblast
Stem Cell (no supplement)
Stem Cell (with supplement)
dTTP
pmol/million cells
40
35
30
25
20
15
10
5
0
**
***
37% decrease post-reprogramming
30% increase with supplement
n=3
n=9
n=9
Fibroblast
Stem Cell (no supplement)
Stem Cell (with supplement)
** = (difference between parental fibroblasts and stem cells with supplement) p < 0.05
*** = (difference between stem cells without supplement and stem cells with supplement) p < 0.05

Fig. 9

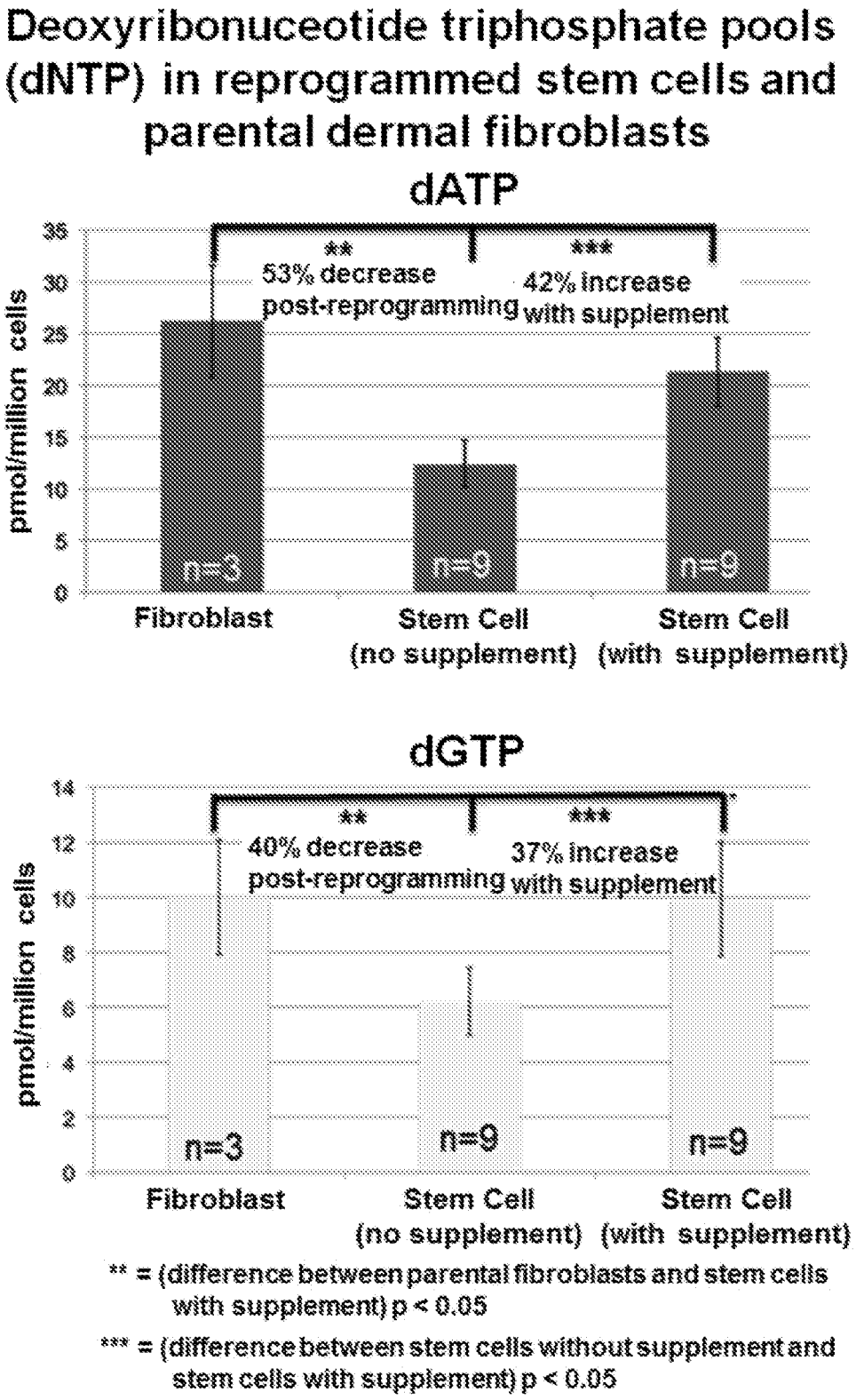
Deoxyribonuceotide triphosphate pools (dNTP) in reprogrammed stem cells and parental dermal fibroblasts
dATP
pmol/million cells
**
53% decrease post-reprogramming
***
42% increase with supplement
n=3
n=9
n=9
Fibroblast
Stem Cell (no supplement)
Stem Cell (with supplement)
dGTP
pmol/million cells
**
40% decrease post-reprogramming
***
37% increase with supplement
n=3
n=9
n=9
Fibroblast
Stem Cell (no supplement)
Stem Cell (with supplement)
** = (difference between parental fibroblasts and stem cells with supplement) p < 0.05
*** = (difference between stem cells without supplement and stem cells with supplement) p < 0.05

Fig.9, cont'd.

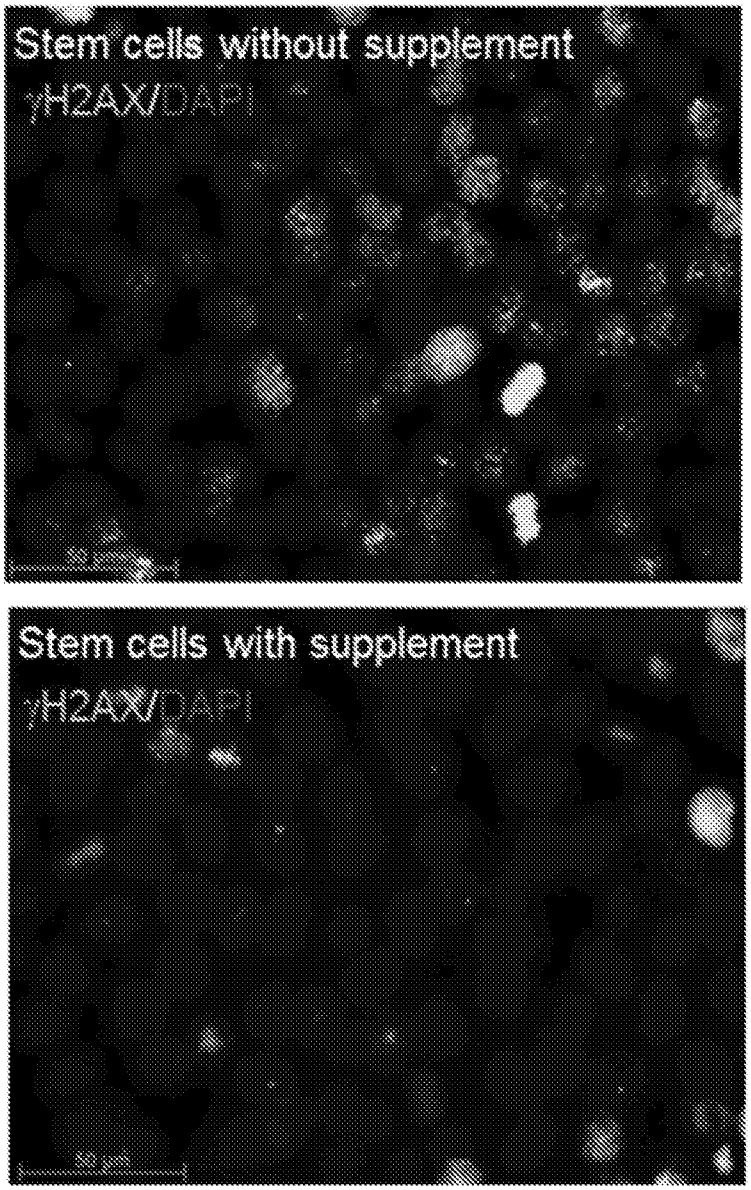
Stem cells without supplement
γH2AX/DAPI
Stem cells with supplement
γH2AX/DAPI

Fig. 10

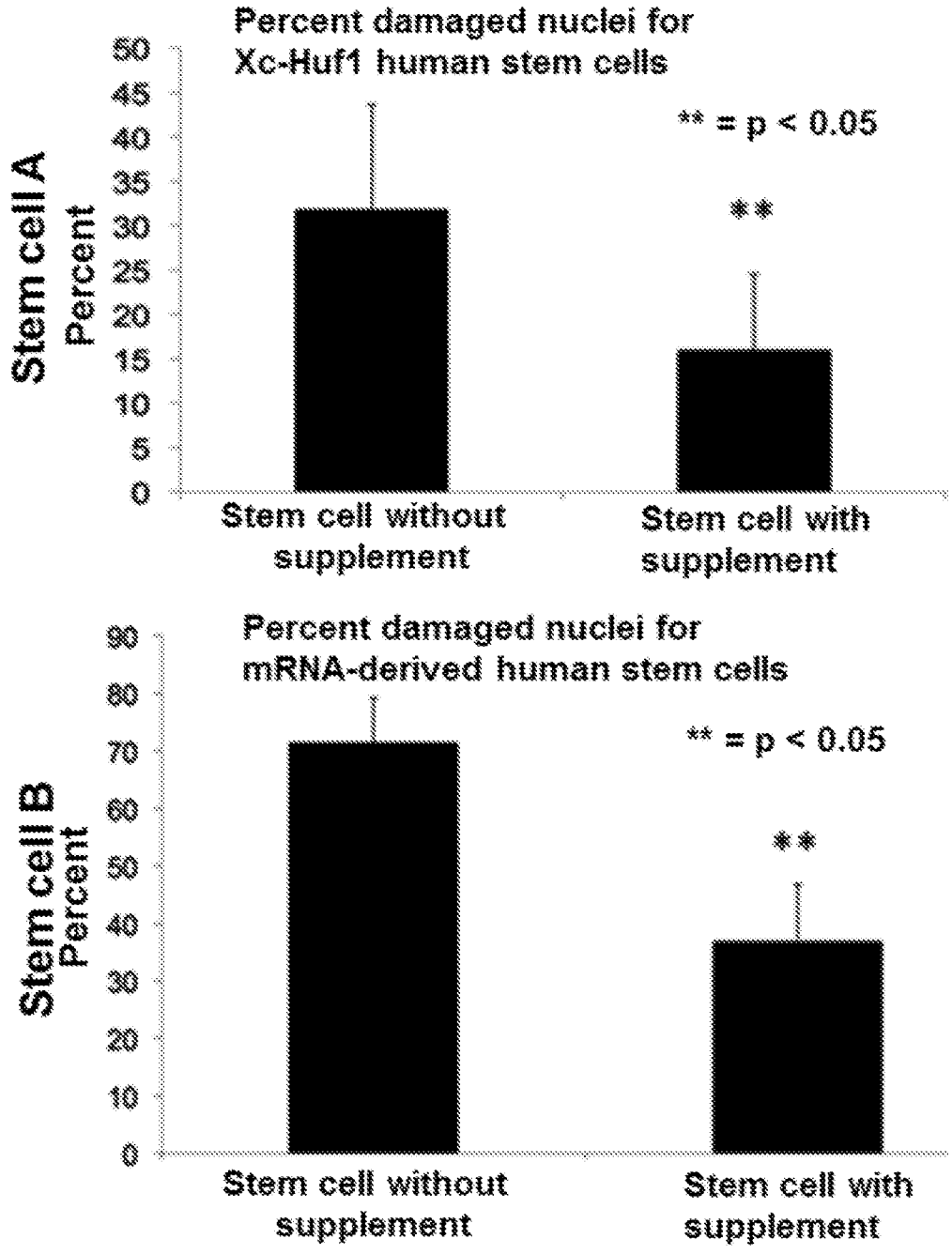
Percent damaged nuclei for
Xc-Huf1 human stem cells
** = p < 0.05
**
Stem cell A
Percent
50
45
40
35
30
25
20
15
10
5
0
Stem cell without supplement
Stem cell with supplement
Percent damaged nuclei for
mRNA-derived human stem cells
** = p < 0.05
**
Stem cell B
Percent
90
80
70
60
50
40
30
20
10
0
Stem cell without supplement
Stem cell with supplement

Fig.10, cont'd.

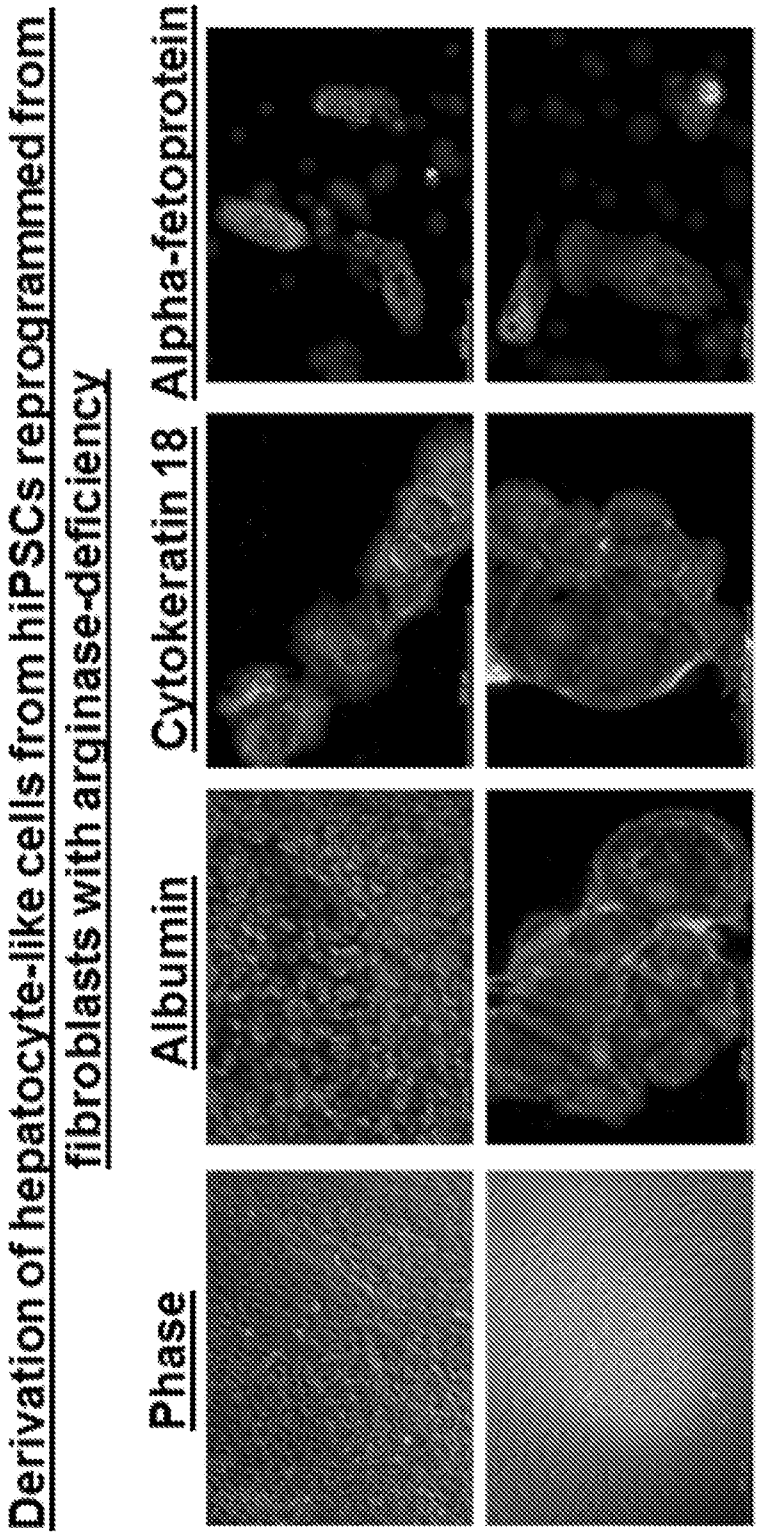
Derivation of hepatocyte-like cells from hiPSCs reprogrammed from fibroblasts with arginase-deficiency
Phase
Albumin
Cytokeratin 18
Alpha-fetoprotein

Fig. 11

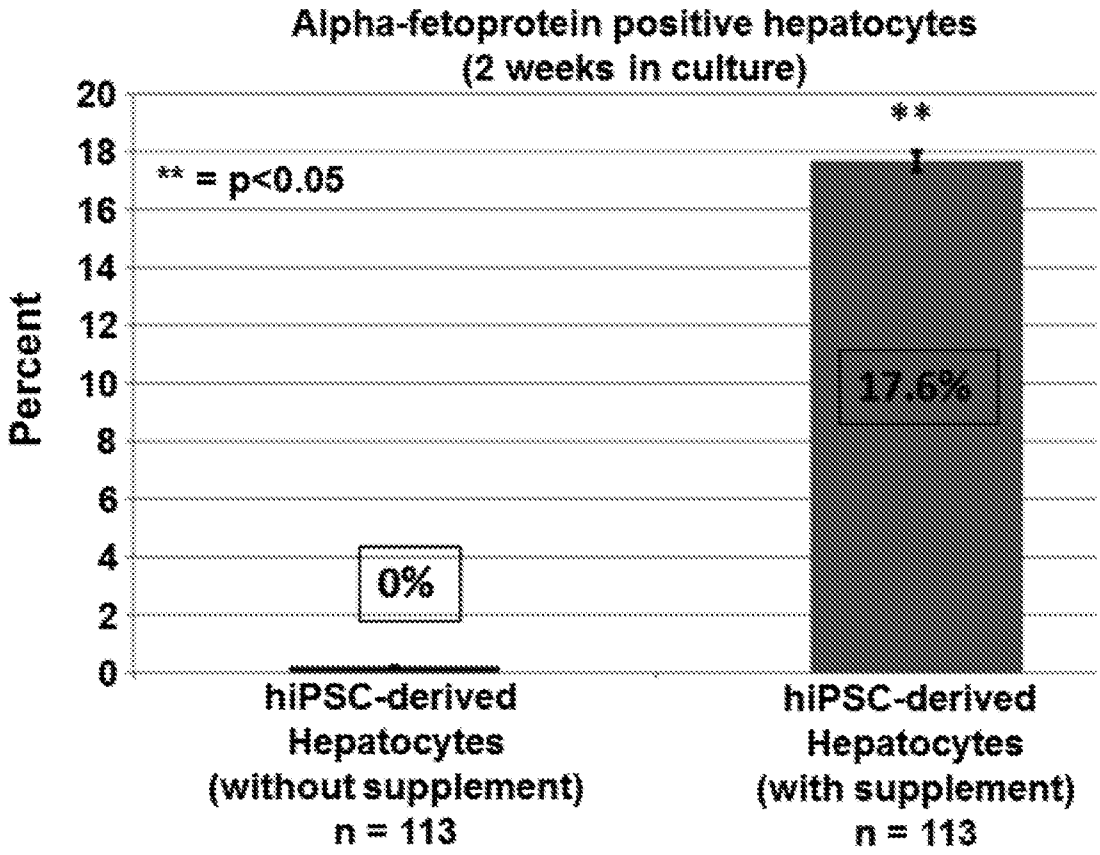
Alpha-fetoprotein positive hepatocytes
(2 weeks in culture)
** = p<0.05
**
Percent
20
18
16
14
12
10
8
6
4
2
0
17.6%
0%
hiPSC-derived Hepatocytes (without supplement) n = 113
hiPSC-derived Hepatocytes (with supplement) n = 113

Fig. 12

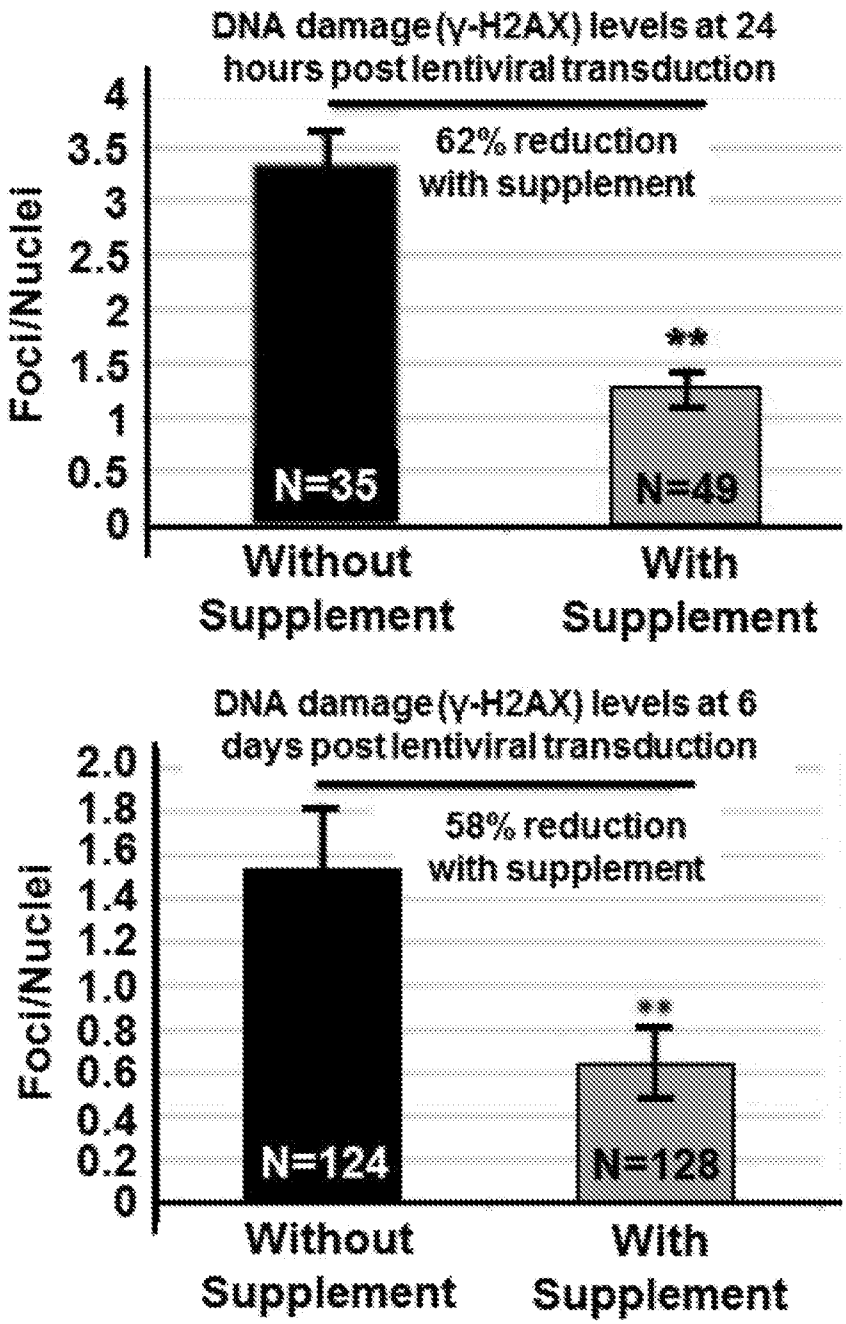
DNA damage (γ-H2AX) levels at 24 hours post lentiviral transduction
62% reduction with supplement
Foci/Nuclei
4
3.5
3
2.5
2
1.5
1
0.5
0
**
N=35
N=49
Without Supplement
With Supplement
DNA damage (γ-H2AX) levels at 6 days post lentiviral transduction
58% reduction with supplement
Foci/Nuclei
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
**
N=124
N=128
Without Supplement
With Supplement

Fig. 13

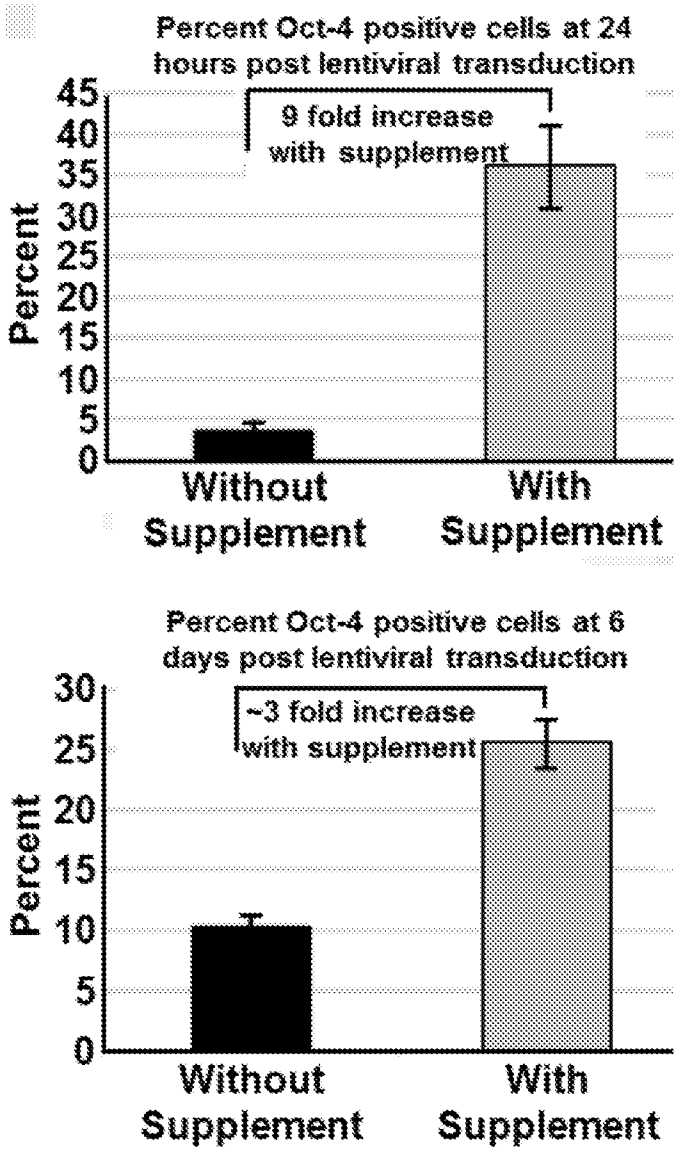
Percent Oct-4 positive cells at 24 hours post lentiviral transduction
9 fold increase with supplement
Percent
45
40
35
30
25
20
15
10
5
0
Without Supplement
With Supplement
Percent Oct-4 positive cells at 6 days post lentiviral transduction
~3 fold increase with supplement
Percent
30
25
20
15
10
5
0
Without Supplement
With Supplement

Fig. 14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/027940

A. CLASSIFICATION OF SUBJECT MATTER

C12N 5/02(2006.01)i, C12N 5/07(2010.01)i, C07H 19/02(2006.01)i, A61K 8/98(2006.01)i, A61K 35/12(2006.01)i, A61Q 19/00(2006.01)i, A61P 17/00(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N 5/02; C12N 5/06; C12N 5/074; A61K 31/7064; C07H 19/02; A61K 8/98; A61K 35/12; A61Q 19/00; A61P 17/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: cell culture medium, stem cell, genetic stability, nucleoside triphosphates

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TRUONG, BRIAN et al., 'Investigating the role of nucleotide deficiency and oxidative stress in maintaining genomic instability in human induced pluripotent stem cells', In: Science poster day, 17th annual, University of California, Los Angeles, 13 May 2014, p. 103 See page 103.	1-5
A	MATHEWS, CHRISTOPHER K., 'DNA precursor metabolism and genomic stability', The FASEB Journal, July 2006, Vol. 20, No. 9, pp. 1300-1314 See the whole document.	1-5
A	BYRNE, JAMES A, 'Nuclear reprogramming and the current challenges in advancing personalized pluripotent stem cell-based therapies',Gene Therapy and Regulation, December 2012, Vol. 7, No. 1, 1230002 (1-24 pages) See the whole document.	1-5
A	KE, PO-YUAN et al., 'Control of dTTP pool size by anaphase promoting complex/cyclosome is essential for the maintenance of genetic stability', Genes & Development, 15 August 2005, Vol. 19, No. 16, pp. 1920-1933 See the whole document.	1-5
A	US 2006-0286668 A1 (PRICE, PAUL et al.) 21 December 2006 See abstract; claims 29 and 32.	1-5

☒ 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	Date of mailing of the international search report
25 July 2016 (25.07.2016)	**26 July 2016 (26.07.2016)**

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International Application Division
Korean Intellectual Property Office
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/027940

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.: 77-87
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 77-87 pertain to a method for treatment of the human body by therapy or surgery, and thus relate to a subject matter which this International Searching Authority is not required, under PCT Article 17(2)(a)(i) and PCT Rule 39.1(iv), to search.

2. ☒ Claims Nos.: see extra sheet
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:
Claims 7-8, 10-12, 14-16, 18-20, 22-24, 26-28, 30-32, 34-36, 38-40, 42-44, 46-48, 51-52, 55, 58-60, 64, 67-71, 73-74, 79-83, 85-86, 91, 95 and 98 are unclear since they are referring to the multiple dependent claims which do not comply with PCT Rule 6.4(a).

3. ☒ Claims Nos.: see extra sheet
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:

1. ☐ As all required addtional 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 any 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.:

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/US2016/027940

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	US 2015-0105343 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 16 April 2015 See abstract; claims 1-4 and 6-7.	1-5

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2016/027940

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2006-0286668 A1	21/12/2006	US 2004-0171152 A1	02/09/2004
		US 2009-0061516 A1	05/03/2009
		US 2011-0039330 A1	17/02/2011
		US 2013-0084640 A1	04/04/2013
		US 2014-0273205 A1	18/09/2014
		US 6103529 A	15/08/2000
US 2015-0105343 A1	16/04/2015	CA 2927355 A1	23/04/2015
		WO 2015-057997 A1	23/04/2015

Continuation of Box No. II:

- Item (2):

  Claim Nos.: 7-8, 10-12, 14-16, 18-20, 22-24, 26-28, 30-32, 34-36, 38-40, 42-44, 46-48, 51-52, 55, 58-60, 64, 67-71, 73-74, 79-83, 85-86, 91, 95 and 98

- Item (3):

  Claim Nos.: 6, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, 49-50, 53-54, 56-57, 61-63, 65-66, 72, 75-78, 84, 87-90, 92-94, 96-97 and 99