CLAIMS

What is claimed is:

1. A composition for induced pluripotent stem cell (iPSC) production comprising:

(i) a TGFβ family protein,

(ii) a ROCK inhibitor, and

(iii) a MEK inhibitor and a WNT activator,

wherein the composition does not comprise a TGFβ inhibitor,

wherein the composition is effective to improve iPSC pluripotency and genomic stability in a long-term iPSC maintenance.

2. The composition of claim 1,

(a) wherein the long-term iPSC maintenance comprises one or more of stages comprising: single cell dissociation of iPSC colonies, single cell sorting of dissociated iPSCs, iPSC single cell clonal expansion, clonal iPSC master cell bank (MCB) cryopreservation, thawing of iPSC MCB, and optionally additional cryopreserve-thaw cycles of the iPSC MCB; or

(b) wherein the TGFβ family protein is optionally added to the composition at single cell dissociation of iPSC colonies, or at iPSC single cell clonal expansion, or at any stage in-between; or

(c) wherein the MEK inhibitor and/or the WNT activator is at an amount 30-60% of that used in a reprogramming composition for reprogramming a non-pluripotent cell to the iPSC.

3. The composition of claim 1,

(i) wherein the TGFβ family protein comprises at least one of Activin A, TGFβ, Nodal, and functional variants or fragments thereof; and/or

(ii) wherein the WNT activator comprises a GSK3 inhibitor.

4. The composition of claim 2,

(i) wherein the non-pluripotent cell comprises a somatic cell, a progenitor cell, or a multipotent cell; or

(ii) wherein the non-pluripotent cell comprises a T cell; or

(iii) wherein the reprogramming compisition comprises a ROCK inhibitor, a MEK inhibitor, a WNT activator, a TGFβ inhibitor, and optionally an HDAC inhibitor, wherein the TGFβ inhibitor and the HDAC inhibitor are included in the reprogramming composition at specific stages during reprogramming.

5. The composition of claim 1,

(i) wherein the improved long-term iPSC pluripotency is indicated by reduced pluripotency reversion or reduced spontaneous differentiation as compared to iPSCs without contact of the composition; and

(ii) wherein the improved genomic stability is indicated by a lower propensity for genomic abnormalities as compared to iPSCs without contact of the composition.

6. The composition of claim 5, wherein the improved genomic stability comprises reduction or prevention of trisomy or karyotype abnormality in iPSCs obtained from reprogramming a T cell.

7. The composition of claim 1, further comprising an iPSC, optionally wherein the iPSC comprises at least one genomic edit.

8. The composition of claim 2,

(i) wherein the iPSC maintenance further comprises iPSC genetic editing to obtain an engineered iPSC pool, single sell sorting of engineered iPSC pool, engineered iPSC single cell clonal expansion, clonal engineered iPSC master cell bank (MCB) cryopreservation, thawing of engineered iPSC MCB, and optionally additional cryopreserve-thaw cycles of the engineered iPSC MCB; and

(ii) wherein the engineered iPSC comprises at least one genomic edit.

9. A composition for induced pluripotent stem cell (iPSC) production comprising:

(i) a ROCK inhibitor, a MEK inhibitor, and a WNT activator;

(ii) an HDAC inhibitor; and

(iii) a TGFβ inhibitor,

wherein the composition is effective to improve reprogramming of a non-pluripotent cell to obtain iPSCs having established pluripotency and improved genomic stability, and optionally,

wherein, addition of (i), (ii) or (iii) to the composition is stage-specific during reprogramming of the non-pluripotent cell for an increased reprogramming efficiency.

10. The composition of claim 9,

(a) wherein the reprogramming of the non-pluripotent cell comprises one or more stages comprising: somatic cell transfection (day 0), exogenous gene expression, increase of heterochromatin, loss of somatic cell identity, and iPSC colony formation; or

(b) wherein the addition of the HDAC inhibitor is optionally at chromatin restructuring, or at around day 2-3 (post transfection); or

(c) wherein the addition of the TGFβ inhibitor is optionally at the loss of somatic cell identity, or at around day 6-8 (post transfection),

wherein the one or more stages in reprogramming is indicated by cell morphological change and/or marker gene profiling.

11. The composition of claim 9, wherein:

(i) the HDAC inhibitor comprises valproic acid (VPA) or a functional variant or derivative thereof;

(ii) the WNT activator comprises a GSK3 inhibitor;

(iii) the non-pluripotent cell comprises a somatic cell, a progenitor cell, or a multipotent cell;

(iv) the non-pluripotent cell comprises a T cell;

(v) the established pluripotency comprises a ground state pluripotency;

(vi) the established pluripotency is represented by increased naïve-specific gene expression comprising one or more of: MAEL, KLF4, DNMT3L, DPPA5, PRDM14, FGF4, UTF1, TFCP2L1, and TBX3;

(vii) the improved genomic stability is indicated by a lower propensity for genomic abnormalities than iPSCs obtained without contact of the composition during reprogramming; and/or

(viii) the increased reprogramming efficiency is indicated by the higher percentage of cells expressing pluripotency maker genes in an iPSC pool after reprogramming than that of the iPSC pool obtained without contact of the composition during reprogramming.

12. A method of producing induced pluripotent stem cell (iPSC), comprising a step of cryopreserving a population of iPSCs,

wherein the iPSCs are in contact with the composition of any one of claims 1-8, and

wherein pluripotency and genomic stability of the iPSCs are maintained during cryopreservation; and optionally,

wherein the population of iPSCs comprising homogeneous iPSCs is expanded from a clonal iPSC single cell.

13. The method of claim 12, further comprising a step of expanding a single cell iPSC clone to obtain the population of clonal iPSCs,

wherein the iPSCs are in contact with the composition of any one of claims 1-8, and

wherein pluripotency and genomic stability of the iPSCs are maintained during expansion.

14. The method of claim 13, further comprising a step of single cell sorting of dissociated iPSCs to obtain a single cell iPSC clone,

wherein the iPSCs are in contact with the composition of any one of claims 1-8, and

wherein pluripotency and genomic stability of the iPSCs are maintained during single cell sorting.

15. The method of claim 14, further comprising a step of dissociating iPSC colonies to single cell iPSCs,

wherein the iPSCs are in contact with the composition of any one of claims 1-8, and

wherein pluripotency and genomic stability of the iPSCs are maintained during iPSC single cell dissociation.

16. The method of claim 15, further comprising a step of obtaining at least one colony comprising iPSCs generated from reprogramming a non-pluripotent cell.

17. The method of any one of claims 12-16, wherein the iPSCs are reprogrammed from a somatic cell, a progenitor cell, or a multipotent cell, or wherein the iPSCs are reprogrammed from a T cell.

18. The method of any one of claims 12-17,

(i) wherein the pluripotency comprises a ground state pluripotency; and/or

(ii) wherein the pluripotency is represented by increased naïve-specific gene expression comprising one or more of: MAEL, KLF4, DNMT3L, DPPA5, PRDM14, FGF4, UTF1, TFCP2L1, and TBX3; and/or

(iii) wherein the genomic stability comprises a lower propensity for genomic abnormalities than iPSCs in said step without contact of the composition.

19. A method of producing induced pluripotent stem cell (iPSC), wherein the method comprises:

(i) transferring to a non-pluripotent cell one or more reprogramming factors to initiate reprogramming of the cell; and

(ii) contacting the cell after step (i) with the composition of any one of claims 9-11 for a sufficient period, thereby generating at least one colony comprising iPSCs by reprogramming the non-pluripotent cell.

20. The method of claim 19, wherein the step of transferring comprises introducing to the non-pluripotent cell:

(i) one or more first plasmids, wherein each of the first plasmids comprises a replication origin, and a polynucleotide encoding one or more reprogramming factors, but does not comprise polynucleotides encoding an EBNA or a variant thereof; wherein the one or more first plasmids collectively comprise polynucleotides encoding at least OCT4, or at least OCT4, YAP1, SOX2 and large T antigen (LTag); wherein the introduction of one or more first plasmids induces a reprogramming process; and

(ii) one of: (1) a second plasmid comprising a nucleotide sequence encoding an EBNA, wherein the second plasmid does not comprise a replication origin or polynucleotide(s) encoding reprogramming factor(s); (2) an EBNA mRNA; and (3) an EBNA protein.

21. The method of claim 19, wherein the step of contacting comprises:

(a) contacting the cell after step (i) with a combination comprising a ROCK inhibitor, a MEK inhibitor, and a WNT activator, optionally at a stage of exogenous reprogramming factor expression, or at day 1-2 post reprogramming factor transferring (day 0);

(b) contacting the cell of step (a) with an HDAC inhibitor, optionally at a stage of chromatin restructuring, or at around day 2-3 post reprogramming factor transferring; and

(c) contacting the cell of step (b) with a TGFβ inhibitor, optionally at a stage of loss of somatic cell identity, or at around day 6-8 (post transfection),

thereby generating at least one colony comprising iPSCs;

wherein said stage is indicated by cell morphological change and/or marker gene profiling; and/or

wherein the iPSCs are footprint-free, have established pluripotency and improved genomic stability, and are produced with a higher efficiency as compared to reprogramming without steps (a), (b) and (c).

22. The method of claim 19,

(i) wherein the non-pluripotent cell comprises a somatic cell, a progenitor cell, or a multipotent cell; or

(ii) wherein the non-pluripotent cell comprises a T cell.

23. A method of producing induced pluripotent stem cell (iPSC), wherein the method comprises:

(i) transferring to a non-pluripotent cell one or more reprogramming factors to initiate reprogramming of the cell;

(ii) contacting the cell after step (i) with the composition of any one of claims 9-11 for a sufficient period, thereby generating at least one colony comprising iPSCs, wherein pluripotency and genomic stability of the iPSCs are established;

(iii) dissociating the iPSC colony of step (ii) to dissociated iPSCs, wherein the iPSCs are in contact with the composition of any one of claims 1-8;

(iv) sorting dissociated iPSCs to obtain one or more single cell iPSC clones, wherein the single cell iPSC clones are in contact with the composition of any one of claims 1-8; and optionally,

(v) expanding the single cell iPSC clone to a population of clonal iPSCs, wherein the population of clonal iPSCs is in contact with the composition of any one of claims 1-8; and optionally

(vi) cryopreserving the population of clonal iPSCs, wherein the cryopreserved population is in contact with the composition of any one of claims 1-8;

wherein pluripotency and genomic stability of the iPSCs are maintained during the step of dissociating, sorting, expanding, cryopreserving, or thawing.

24. The method of claim 23, wherein the step (i) of transferring comprises introducing to the non-pluripotent cell:

(a) one or more first plasmids, wherein each of the first plasmids comprises a replication origin, and a polynucleotide encoding one or more reprogramming factors, but does not comprise polynucleotides encoding an EBNA or a variant thereof; wherein the one or more first plasmids collectively comprise polynucleotides encoding at least OCT4, or at least OCT4, YAP1, SOX2 and large T antigen (LTag); wherein the introduction of one or more first plasmids induces a reprogramming process; and

(b) one of: (1) a second plasmid comprising a nucleotide sequence encoding an EBNA, wherein the second plasmid does not comprise a replication origin or polynucleotide(s) encoding reprogramming factor(s); (2) an EBNA mRNA; and (3) an EBNA protein.

25. The method of claim 23, wherein the step (ii) of contacting further comprises:

(a) contacting the cell after step (i) with a combination comprising a ROCK inhibitor, a MEK inhibitor, and a WNT activator, optionally at a stage of exogenous reprogramming factor expression, or at day 1-2 post reprogramming factor transferring (day 0);

(b) contacting the cell of step (a) with an HDAC inhibitor, optionally at a stage of chromatin restructuring, or at around day 2-3 post reprogramming factor transferring; and

(c) contacting the cell of step (b) with a TGFβ inhibitor, optionally at a stage of loss of somatic cell identity, or at around day 6-8 (post transferring),

thereby generating at least one colony comprising iPSCs;

wherein said stage is indicated by cell morphological change and/or marker gene profiling; and/or

wherein the iPSCs have established pluripotency and improved genomic stability, and are produced with a higher efficiency as compared to reprogramming without steps (a), (b) and (c).

26. The method of claim 23, further comprising:

(1) contacting the cell of the sorting step (iv), expanding step (v), and cryopreserving step (vi), and optionally of the dissociating step (iii) with a ROCK inhibitor, a MEK inhibitor, and a WNT activator, wherein concentration of one or both of the MEK inhibitor and the WNT activator is 30%-60% of that in step (ii); and

(2) additionally contacting the cell of the expanding step (v) and cryopreserving step (vi), and optionally of the dissociating step (iii) and/or sorting step (iv) with a TGFβ family protein; and

wherein the cells in steps (iii), (iv), (v) and (vi) are not in contact with either a TGFβ inhibitor or an HDAC inhibitor.

27. The method of claim 23,

(i) wherein the non-pluripotent cell comprises a somatic cell, a progenitor cell, or a multipotent cell; or

(ii) wherein the non-pluripotent cell comprises a T cell.

28. The method of claim 23, wherein the method further comprises genetic editing of an iPSC to obtain an engineered iPSC pool, single sell sorting of engineered iPSC pool, engineered iPSC single cell clonal expansion, clonal engineered iPSC master cell bank (MCB) cryopreservation, thawing of engineered iPSC MCB, and optionally additional cryopreserve-thaw cycles of the engineered iPSC MCB; and wherein the engineered iPSC comprises at least one genomic edit.

29. A composition comprising an induced pluripotent cell (iPSC), a cell line, a clonal population or a master cell bank thereof, wherein the iPSC is contacted by a combination of a ROCK inhibitor, a MEK inhibitor, a WNT activator, and a TGFβ family protein, and wherein the iPSC comprises increased naïve-specific gene expression comprising one or more of: MAEL, KLF4, DNMT3L, DPPA5, PRDM14, FGF4, UTF1, TFCP2L1, and TBX3; and optionally the iPSC has at least one of the properties: high clonality, genetic stability, and ground state pluripotency.

30. The composition of claim 29, wherein:

(i) the TGFβ family protein comprises at least one of Activin A, TGFβ, Nodal, and functional variants or fragments thereof;

(ii)the WNT activator comprises a GSK3 inhibitor;

(iii) the iPSC is generated from reprogramming a non-pluripotent cell; optionally wherein the non-pluripotent cell comprises a somatic cell, a progenitor cell, or a multipotent cell; or wherein the non-pluripotent cell comprises a T cell; or

(iv) the composition further comprises a feeder-free medium

(v) the iPSC has at least one of the properties: high clonality, genetic stability, and ground state pluripotency.

31. An induced pluripotent cell (iPSC), a cell line, a clonal population or a master cell bank thereof produced by a method according to any one of claims 12-28.

32. The induced pluripotent cell (iPSC), a cell line, a clonal population or a master cell bank thereof of claim 31, wherein the iPSC comprises at least one genomic edit.

33. A derived non-natural cell or population thereof obtained from *in vitro* differentiation of the pluripotent cell or cell line of claim 31 or 32.

34. The derived non-natural cell or population thereof of claim 33, wherein:

(i) the cell is an immune effector cell, and optionally, the immune effector cell comprises at least a genomic edit comprised in the iPSC;

(ii) the cell comprises a CD34 cell, a hemogenic endothelium cell, a hematopoietic stem or progenitor cell, a hematopoietic multipotent progenitor cell, a T cell progenitor, an NK cell progenitor, a T cell, a NKT cell, an NK cell, a B cell, or an immune regulatory cell; or

(iii) the cell is a rejuvenated cell comprising at least one of the following properties: global increase of heterochromatin; improved mitochondrial function; increased DNA damage responses; telomere elongation and decrease of percentage of short telomere; decrease in the fraction of senescent cells; and higher potential for proliferation, survival, persistence, or memory like functions, in comparison to its natural cell counterpart.

35. An *in vitro* system for initiating reprogramming in a non-pluripotent cell, wherein the system comprises:

one or more first plasmids, wherein each of the first plasmids comprises a replication origin, and a polynucleotide encoding one or more reprogramming factors but does not encode

an EBNA or a derivative thereof; wherein the one or more first plasmids collectively comprise polynucleotides encoding OCT4, YAP1, SOX2 and LTag; and optionally one of:

(1) a second plasmid comprising a nucleotide sequence encoding an EBNA, wherein the second plasmid does not comprise a replication origin or polynucleotide(s) encoding reprogramming factor(s);

(2) an EBNA mRNA; and

(3) an EBNA protein.

36. The system of claim 35, wherein:

(i) the second plasmid has a high rate of loss; and wherein the expression of EBNA is transient and temporal;

(ii) the system does not provide EBNA replication and/or continuous expression in the nucleus;

(iii) the system enables a transient/cytoplasmic expression of EBNA for a short duration, and prior to the appearance of pluripotency cell morphology and the induced expression of endogenous pluripotency genes; and/or

(iv) the system enables a transient/cytoplasmic expression of one or more reprogramming factors comprised in the first plasmid(s) for a short duration, and prior to the appearance of pluripotency cell morphology and the induced expression of endogenous pluripotency genes.

37. The system of any one of claims 35 or 36, wherein:

(i) the replication origin is selected from the group consisting of a Polyomavirinae virus, a Papillomavirinae virus, and a Gammaherpesvirinae virus;

(ii) the replication origin is one selected from the group consisting of SV40, BK virus (BKV), bovine papilloma virus (BPV), and Epstein–Barr virus (EBV);

(iii) the replication origin corresponds to, or is derived from, the wild-type replication origin of EBV; or

(iv) the EBNA is EBV-based.

38. The system of any one of claims 35-37, wherein:

(i) the one or more first plasmids further collectively comprise polynucleotides encoding reprogramming factor(s) comprising (i) one or more of NANOG, KLF, LIN28, MYC, ECAT1,

UTF1, ESRRB, HESRG, CDH1, TDGF1, DPPA4, DNMT3B, ZIC3, and L1TD1; or (ii) MYC, LIN28, ESRRB, and ZIC3;

(ii) the system comprises two or more first plasmids, with each first plasmid comprising the same or different reprogramming factors encoded by at least one copy of the polynucleotide;

(iii) the system comprises four first plasmids, with each first plasmid comprising the same or different reprogramming factors encoded by at least one copy of the polynucleotide;

(iv) the system comprises four first plasmids, with each first plasmid comprising at least one copy of polynucleotides encoding OCT4 and YAP1, SOX2 and MYC, LIN28 and LTag, and ESRRB and ZIC3, respectively; and/or

(v) the first plasmid comprises more than one polynucleotide encoding reprogramming factors, wherein the adjacent polynucleotides are operatively connected by a linker sequence encoding a self-cleaving peptide or an IRES.

39. A kit comprising the system of any one of claims 35-38.