What is claimed is:

1. A method for proliferating epithelial cells ex vivo, comprising:

expanding the number of cells in an originating epithelial cell population derived from differentiated tissue, embryonic stem (ES) cells, or induced pluripotent stem cells (iPSCs) under feeder-cell free expansion culture conditions, thereby generating an expanded epithelial cell population, wherein:

the expansion culture conditions comprise an agent that activates telomerase reverse transcriptase in the population and/or inhibits transforming growth factor beta (TGF-beta) signaling in the population;

the originating epithelial cell population is capable of 25 population doublings or more when cultured under the expansion culture conditions; and

the originating epithelial cell population is capable of no more than 20 population doublings when cultured under control culture conditions that do not include the agent.

2. A method for proliferating epithelial cells ex vivo, comprising:

expanding the number of cells in an originating epithelial cell population under serum-free and feeder-cell free conditions, thereby generating an expanded epithelial cell population, wherein:

the expansion culture conditions comprise an agent that activates telomerase reverse transcriptase in the population and/or inhibits transforming growth factor beta (TGF-beta) signaling in the population; and

the originating epithelial cell population comprises differentiated epithelial cells.

3. A method for proliferating epithelial cells ex vivo, comprising:

expanding the number of cells in an originating epithelial cell population under serum-free and feeder-cell free conditions, thereby generating an expanded epithelial cell population, wherein:

the expansion culture conditions comprise an agent that activates telomerase reverse transcriptase in the population and/or inhibits transforming growth factor beta (TGF-beta) signaling in the population; and

the originating epithelial cell population comprises quiescent and/or formerly quiescent epithelial cells epithelial cells.

4. A method for proliferating epithelial cells ex vivo, comprising:

expanding the number of cells in an originating epithelial cell population serum-free and feeder-cell free conditions, thereby generating an expanded epithelial cell population, wherein:

the expansion culture conditions comprise an agent that activates telomerase reverse transcriptase in the population and/or inhibits transforming growth factor beta (TGF-beta) signaling in the population; and

the originating epithelial cell population comprises lineage-committed epithelial cells.

5. The method of claim 1, wherein:

agent that activates telomerase reverse transcriptase in the population and/or inhibits transforming growth factor beta (TGF-beta) signaling in the population is a first agent,

the expansion culture conditions further comprise a second agent that modulates cytoskeletal structure in the population, and

the control culture conditions do not include the first agent and the second agent.

6. The method of claim 2, 3 or 4, wherein the expansion culture conditions further comprise an agent that modulates cytoskeletal structure in the population.

7. The method of any one of claims 1 to 6, comprising isolating the originating epithelial cell population.

8. The method of claim 7, wherein the originating epithelial cell population is isolated from differentiated tissue.

9. The method of claim 7, wherein the originating epithelial cell population is isolated from an embryo or a stem cell culture derived from an embryo.

10. The method of claim 7, wherein the originating epithelial cell population is isolated from an induced pluripotent stem cell (iPSC) culture.

~~11. The method of claim 7, wherein the originating epithelial cell population is isolated from a sample from a subject.~~

~~12. The method of claim 11, wherein the sample is selected from one or more of a tissue biopsy, blood, urine, feces, buccal swab, and amniotic fluid.~~

~~13. The method of any one of claims 1 to 12, comprising maintaining or proliferating cells of the originating epithelial cell population in cell culture after the cells are isolated and prior to contacting the originating epithelial cell population to the feeder-cell free expansion culture conditions.~~

~~14. The method of any one of claims 1 to 13, wherein the originating epithelial cell population and/or the expanded epithelial cell population contain no embryonic stem cells.~~

~~15. The method of any one of claims 1 to 14, wherein:~~

~~the originating epithelial cell population, or~~

~~the expanded epithelial cell population, or~~

~~the originating epithelial cell population and the expanded epithelial cell population,~~

~~express one or more basal epithelial cell markers.~~

~~16. The method of any one of claims 1 to 15, wherein:~~

~~the originating epithelial cell population, or~~

~~the expanded epithelial cell population, or~~

~~the originating epithelial cell population and the expanded epithelial cell population,~~

~~express one or more of ITGA6, ITGB4, KRT14, KRT15, KRT5 and TP63.~~

~~17. The method of any one of claims 1 to 16, wherein:~~

~~the originating epithelial cell population, or~~

~~the expanded epithelial cell population, or~~

~~the originating epithelial cell population and the expanded epithelial cell population,~~

do not express one or more epithelial stem cell markers.

18. The method of any one of claims 1 to 17, wherein:

the originating epithelial cell population, or

the expanded epithelial cell population, or

the originating epithelial cell population and the expanded epithelial cell population,

do not express Lgr5.

19. The method of any one of claims 1 to 18, wherein:

the originating epithelial cell population, or

the expanded epithelial cell population, or

the originating epithelial cell population and the expanded epithelial cell population,

do not express one or more pluripotent stem cell markers.

20. The method of any one of claims 1 to 19, wherein:

the originating epithelial cell population, or

the expanded epithelial cell population, or

the originating epithelial cell population and the expanded epithelial cell population,

do not express one or more of LIN28A, NANOG, POU5F1/OCT4 and SOX2.

21. The method of any one of claims 1 to 20, wherein:

the originating epithelial cell population, or

the expanded epithelial cell population, or

the originating epithelial cell population and the expanded epithelial cell population,

do not express one or more terminally differentiated epithelial cell markers.

22. The method of any one of claims 1 to 21, wherein:

the originating epithelial cell population, or

the expanded epithelial cell population, or

~~the originating epithelial cell population and the expanded epithelial cell population,~~

~~do not express one or more of CFTR, FOXJ1, IVL, KRT1, KRT10, KRT20, LOR, MUC1, MUC5AC, SCGB1A1, SFTPB and SFTPD.~~

~~23. The method of any one of claims 1 to 22, wherein:~~

~~the originating epithelial cell population, or~~

~~the expanded epithelial cell population, or~~

~~the originating epithelial cell population and the expanded epithelial cell population,~~

~~do not express one or more gastric epithelial cell markers, one or more intestinal epithelial cell markers, and/or one or more pancreatic epithelial cell markers.~~

~~24. The method of any one of claims 1 to 23, wherein:~~

~~the originating epithelial cell population, or~~

~~the expanded epithelial cell population, or~~

~~the originating epithelial cell population and the expanded epithelial cell population,~~

~~do not express one or more of CD34, HNF1A, HNF4A, IHH, KIT, LGR5, PDX1, and PROM1/CD133.~~

~~25. The method of any one of claims1 to 24, wherein:~~

~~the originating epithelial cell population, or~~

~~the expanded epithelial cell population, or~~

~~the originating epithelial cell population and the expanded epithelial cell population,~~

~~comprise quiescent and/or formerly quiescent epithelial cells.~~

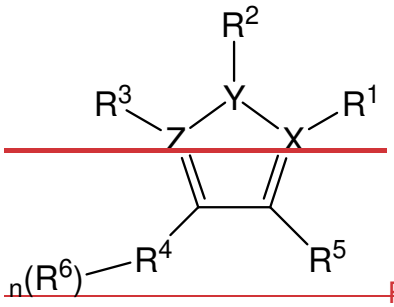
~~26. The method of any one of claims 1 to 25, wherein the agent that activates telomerase reverse transcriptase in the population and/or inhibits transforming growth factor beta (TGF-beta) signaling comprises one or more TGF-beta signaling inhibitors.~~

~~27. The method of claim 26, wherein the one or more TGF-beta signaling inhibitors comprise one or more ALK5 inhibitors.~~

28. The method of claim 27, wherein the one or more ALK5 inhibitors comprise one or more small molecule ALK5 inhibitors.

29. The method of claim 27 or 28, wherein the one or more ALK5 inhibitors comprise one or more ATP analogs.

30. The method of any one of claims 27 to 29, wherein at least one of the one or more ALK5 inhibitors comprises the structure of Formula A:



Formula A

wherein:

X, Y and Z independently are chosen from N, C and O;

R^1, R^2 and R^3 independently are chosen from hydrogen, C1-C10 alkyl, substituted C1-C10 alkyl, C3-C9 cycloalkyl, substituted C3-C9 cycloalkyl, C5-C10 aryl, substituted C5-C10 aryl, C5-C10 cycloaryl, substituted C5-C10 cycloaryl, C5-C9 heterocyclic, substituted C5-C9 heterocyclic, C5-C9 heterocycloaryl, substituted C5-C9 heterocycloaryl, -linker-(C3-C9 cycloalkyl), -linker-(substituted C3-C9 cycloalkyl), -linker-(C5-C10 aryl), -linker-(substituted C5-C10 aryl), -linker-(C5-C10 cycloaryl), -linker-(substituted C5-C10 cycloaryl), -linker-(C5-C9 heterocyclic), -linker-(substituted C5-C9 heterocyclic), -linker-(C5-C9 heterocycloaryl), -linker-(substituted C5-C9 heterocycloaryl);

n is 0 or 1;

R^4, R^5 and R^6 independently are chosen from hydrogen, C1-C10 alkyl, substituted C1-C10 alkyl, C1-C10 alkoxy, substituted C1-C10 alkoxy, C1-C6 alkanoyl, C1-C6 alkoxycarbonyl, substituted C1-C6 alkanoyl, substituted C1-C6 alkoxycarbonyl, C3-C9 cycloalkyl, substituted C3-C9 cycloalkyl, C5-C10 aryl, substituted C5-C10 aryl, C5-C10 cycloaryl, substituted C5-C10 cycloaryl, C5-C9 heterocyclic, substituted C5-C9 heterocyclic, C5-C9 heterocycloaryl, substituted C5-C9 heterocycloaryl, -linker-(C3-C9

~~cycloalkyl), -linker-(substituted C3-C9 cycloalkyl), -linker-(C5-C10 aryl), -linker-(substituted C5-C10 aryl), -linker-(C5-C10 cycloaryl), -linker-(substituted C5-C10 cycloaryl), -linker-(C5-C9 heterocyclic), -linker-(substituted C5-C9 heterocyclic), -linker-(C5-C9 hetercycloaryl), -linker-(substituted C5-C9 heterocycloaryl); and~~

~~the substituents on the substituted alkyl, alkoxy, alkanoyl, alkoxycarbonyl cycloalkyl, aryl, cycloaryl, heterocyclic or heterocycloaryl groups are hydroxyl, C1-C10 alkyl, hydroxyl C1-C10 alkylene, C1-C6 alkoxy, C3-C9 cycloalkyl, C5-C9 heterocyclic, C1-6 alkoxy C1-6 alkenyl, amino, cyano, halogen or aryl.~~

~~31. The method of any one of claims 27 to 30, wherein the one or more ALK5 inhibitors are selected from A83-01, GW788388, RepSox, and SB 431542.~~

~~32. The method of any one of claims 1 to 31, wherein the agent that modulates cytoskeletal structure comprises one or more of a Rho-associated protein kinase inhibitor, a p21-activated kinase (PAK) inhibitor, and a myosin II inhibitor.~~

~~33. The method of claim 32, wherein the Rho kinase inhibitor is selected from a Rho-associated protein kinase 1 (ROCK 1) inhibitor and a Rho-associated protein kinase 2 (ROCK 2) inhibitor.~~

~~34. The method of claim 32 or 33, wherein the Rho-associated protein kinase inhibitor comprises one or more small molecule Rho-associated protein kinase inhibitors.~~

~~35. The method of claim 34, wherein the one or more Rho-associated protein kinase inhibitors is selected from Y-27632, SR 3677, thiazovivin, HA1100 hydrochloride, HA1077 and GSK-429286.~~

~~36. The method of any one of claims 32 to 35, wherein the p21-activated kinase (PAK) inhibitor is selected from a PAK1 inhibitor, a PAK2 inhibitor, a PAK3 inhibitor and a PAK4 inhibitor.~~

~~37. The method of claim 36, wherein the p21-activated kinase (PAK) inhibitor comprises one or more small molecule PAK1 inhibitors.~~

~~38. The method of claim 37, wherein the one or more small molecule PAK1 inhibitors comprise IPA3.~~

~~39. The method of any one of claims 32 to 38, wherein the myosin II inhibitor comprises one or more non-muscle myosin II (NM II) inhibitors.~~

~~40. The method of claim 39, wherein the one or more non-muscle myosin II (NM II) inhibitors comprise one or more small molecule non-muscle myosin II (NM II) inhibitors.~~

~~41. The method of claim 40, wherein the one or more small molecule non-muscle myosin II (NM II) inhibitors comprise blebbistatin.~~

~~42. The method of any one of claims 1 to 41, wherein the expansion culture conditions further comprise an agent that increases intracellular cyclic adenosine monophosphate (cAMP) levels in the population.~~

~~43. The method of claim 42, wherein the agent that increases intracellular cyclic adenosine monophosphate (cAMP) levels comprises one or more beta-adrenergic receptor agonists.~~

~~44. The method of claim 43, wherein the one or more beta-adrenergic receptor agonists comprise isoproterenol.~~

~~45. The method of any one of claims 1 to 44, wherein the expansion culture conditions are serum-free culture conditions.~~

~~46. The method of any one of claims 1 to 45, wherein the expansion culture conditions are defined serum-free culture conditions.~~

~~47. The method of any one of claims 1 to 46, wherein the expansion culture conditions are xeno-free culture conditions.~~

~~48. The method of any one of claims 1 to 47, wherein the expansion culture conditions are defined xeno-free culture conditions.~~

~~49. The method of any one of claims 1 to 48, wherein the expansion culture conditions comprise calcium at a concentration below 300 μM.~~

~~50. The method of any one of claims 1 to 48, wherein the expansion culture conditions comprise calcium at a concentration below 100 μM.~~

~~51. The method of claim 50, wherein the calcium is present at a concentration of about 90 μM.~~

~~52. The method of any one of claims 1 to 51, wherein the expansion culture conditions comprise one or more mitogenic growth factors.~~

~~53. The method of claim 52, wherein one or more mitogenic growth factors comprise EGF, FGF, or EGF and FGF.~~

~~54. The method of any one of claims 1 to 53, wherein the expansion culture conditions comprise no extracellular matrix comprising heterogeneous components.~~

~~55. The method of any one of claims 1 to 54, wherein the originating epithelial cell population is capable of 30 population doublings or more when cultured under the expansion culture conditions.~~

~~56. The method of any one of claims 1 to 54, wherein the originating epithelial cell population is capable of 50 population doublings or more when cultured under the expansion culture conditions.~~

~~57. The method of any one of claims 1 to 54, wherein the originating epithelial cell population is capable of 80 population doublings or more when cultured under the expansion culture conditions.~~

~~58. The method of any one of claims 1 to 54, wherein the originating epithelial cell population is capable of 100 population doublings or more when cultured under the expansion culture conditions.~~

~~59. The method of any one of claims 1 to 58, wherein the method does not comprise selecting for continuously proliferating epithelial stem cells and/or an in vivo population of continuously proliferating epithelial stem cells.~~

~~60. The method of any one of claims 1 to 58, wherein the originating epithelial cell population does not comprise continuously proliferating epithelial stem cells and/or does not comprise cells derived from an in vivo population of continuously proliferating epithelial stem cells.~~

~~61. The method of any one of claims 1 to 60, further comprising isolating a population of ex vivo expanded epithelial cells.~~

~~62. The method of any one of claims 1 to 61, further comprising storing a population of ex vivo expanded epithelial cells in a cell bank.~~

~~63. A population of ex vivo expanded epithelial cells produced by a method according to any one of claims 1 to 62.~~

~~64. Use of the population of ex vivo expanded epithelial cells of claim 63 for production of genetically modified cells.~~

~~65. Use of the population of ex vivo expanded epithelial cells of claim 63 for identifying one or more candidate treatments for a subject.~~

~~66. Use of the population of ex vivo expanded epithelial cells of claim 63 for identifying one or more abnormal epithelial cells in a subject.~~

~~67. Use of the population of ex vivo expanded epithelial cells of claim 63 for monitoring the progression of a disease or treatment of a disease in a subject.~~

1. A cell culture composition for expanding a cell population comprising lineage-committed epithelial cells, comprising

a) a serum-free and feeder cell-free base medium,

b) one or more transforming growth factor beta (TGF-beta) inhibitors, and

c) one or more agents that modulate cytoskeletal structure.

2. The cell culture composition of claim 1, wherein the one or more transforming growth factor beta (TGF-beta) inhibitors comprise one or more inhibitors of ALK5, ALK4, and/or ALK7.

3. The cell culture composition of claim 2, wherein the one or more inhibitors of ALK5, ALK4, and/or ALK7 are selected from A83-01, GW788388, RepSox, and SB 431542.

4. The cell culture composition of claim 1, wherein the one or more agents that modulate cytoskeletal structure comprise one or more inhibitors selected from a Rho-associated protein kinase inhibitor, a p21-activated kinase (PAK) inhibitor, and a myosin II inhibitor.

5. The cell culture composition of claim 4, wherein the Rho-associated protein kinase inhibitor is selected from Y-27632, SR 3677, thiazovivin, HA1100 hydrochloride, HA1077 and GSK-429286; the PAK inhibitor is IPA3; and the myosin II inhibitor is blebbistatin.

6. The cell culture composition of claim 1, further comprising one or more components chosen from:

a beta-adrenergic receptor agonist,

calcium at a concentration below 100 μM, and

one or more mitogenic growth factors.

7. The cell culture composition of claim 6, wherein the beta-adrenergic receptor agonist is isoproterenol.

8. A cell culture composition for expanding a cell population comprising lineage-committed epithelial cells, comprising

a) a serum-free and feeder cell-free base medium;

b) one or more transforming growth factor beta (TGF-beta) inhibitors;

c) one or more agents that modulate cytoskeletal structure;

d) a beta-adrenergic receptor agonist;

e) one or more mitogenic growth factors; and

f) calcium at a concentration below 100 μM.

9. The cell culture composition of claim 8, wherein the one or more transforming growth factor beta (TGF-beta) inhibitors comprise one or more inhibitors of ALK5, ALK4, and/or ALK7; and the one or more agents that modulate cytoskeletal structure comprise one or more inhibitors selected from a Rho-associated protein kinase inhibitor, a p21-activated kinase (PAK) inhibitor, and a myosin II inhibitor.

10. The cell culture composition of claim 9, wherein the one or more inhibitors of ALK5, ALK4, and/or ALK7 are selected from A83-01, GW788388, RepSox, and SB 431542; the Rho-associated protein kinase inhibitor is selected from Y-27632, SR 3677, thiazovivin, HA1100 hydrochloride, HA1077 and GSK-429286; the PAK inhibitor is IPA3; and the myosin II inhibitor is blebbistatin.

11. A composition comprising

a) a cell population comprising lineage-committed epithelial cells in a serum-free and feeder cell-free base medium;

b) one or more transforming growth factor beta (TGF-beta) inhibitors; and

c) one or more agents that modulate cytoskeletal structure.

12. The composition of claim 11, wherein the one or more transforming growth factor beta (TGF-beta) inhibitors comprise one or more inhibitors of ALK5, ALK4, and/or ALK7.

13. The composition of claim 12, wherein the one or more inhibitors of ALK5, ALK4, and/or ALK7 are selected from A83-01, GW788388, RepSox, and SB 431542.

14. The composition of claim 11, wherein the one or more agents that modulate cytoskeletal structure comprise one or more inhibitors selected from a Rho-associated protein kinase inhibitor, a p21-activated kinase (PAK) inhibitor, and a myosin II inhibitor.

15. The composition of claim 14, wherein the Rho-associated protein kinase inhibitor is selected from Y-27632, SR 3677, thiazovivin, HA1100 hydrochloride, HA1077 and GSK-429286; the PAK inhibitor is IPA3; and the myosin II inhibitor is blebbistatin.

16. The composition of claim 11, further comprising one or more components chosen from:

a beta-adrenergic receptor agonist,

calcium at a concentration below 100 μM, and

one or more mitogenic growth factors.

17. The composition of claim 16, wherein the beta-adrenergic receptor agonist is isoproterenol.

18. A composition comprising

a) a cell population comprising one or more of basal epithelial cells, epithelial progenitor cells, epithelial precursor cells, differentiating epithelial cells, differentiated epithelial cells, and transit-amplifying epithelial cells;

b) one or more transforming growth factor beta (TGF-beta) inhibitors; and

c) one or more agents that modulate cytoskeletal structure.

19. The composition of claim 18, wherein the one or more transforming growth factor beta (TGF-beta) inhibitors comprise one or more inhibitors of ALK5, ALK4, and/or ALK7; and the one or more agents that modulate cytoskeletal structure comprise one or more inhibitors selected from a Rho-associated protein kinase inhibitor, a p21-activated kinase (PAK) inhibitor, and a myosin II inhibitor.

20. The composition of claim 19, wherein the one or more inhibitors of ALK5, ALK4, and/or ALK7 are selected from A83-01, GW788388, RepSox, and SB 431542; the Rho-associated protein kinase inhibitor is selected from Y-27632, SR 3677, thiazovivin, HA1100 hydrochloride, HA1077 and GSK-429286; the PAK inhibitor is IPA3; and the myosin II inhibitor is blebbistatin.