CLAIMS:

1. A culture medium for expanding and/or differentiating a population of adult stem cells, wherein said culture medium comprises:
   i. any one of Rspondin 1-4 and/or an Rspondin mimic; and
   ii. one or more inhibitor that directly or indirectly negatively regulates TGF-beta signalling.
2. The culture medium of claim 1, wherein the one or more inhibitor binds to and reduces the activity of one or more serine/threonine protein kinases selected from the group consisting of ALK5, ALK4, TGF-beta receptor kinase 1 and ALK7.
3. The culture medium of claim 1 or claim 2, wherein the one or more inhibitor that directly or indirectly negatively regulates TGF-beta signalling is selected from the group consisting of A83-01, SB-431542, SB-505124, SB-525334, SD-208, LY-36494 and SJN-2511.
4. The culture medium of any one of the preceding claims, wherein the culture medium further comprises an inhibitor that directly or indirectly negatively regulates p38 signalling.
5. The culture medium of claim 4, wherein the one or more inhibitor that directly or indirectly negatively regulates p38 signalling is selected from the group consisting of SB-202190, SB-203580, VX-702, VX-745, PD-169316, RO-4402257 and BIRB-796.
6. The culture medium of any one of the preceding claims, wherein the culture medium comprises A83-01 and SB-202190 or A83-01 and SB-203580.
7. The culture medium of any one of the preceding claims, wherein the inhibitor is added at a concentration of between 1nM and 100 μM, between 10 nM and 100 μM, between 100 nM and 10 μM, or approximately 1 μM, for example, wherein the total concentration of the one or more inhibitor is between 10 nM and 100 μM, between 100 nM and 10 μM, or approximately 1 μM.
8. The culture medium of any one of the preceding claims, wherein the culture medium comprises one or more additional components selected from: a BMP inhibitor, a Wnt agonist, a receptor tyrosine kinase ligand, a Rock inhibitor, nicotinamide and gastrin.
9. The culture medium of any one of the preceding claims, wherein the culture medium comprises any one of Rspondin 1-4 and/or an Rspondin mimic, a BMP inhibitor (for example, Noggin), a TGF-beta inhibitor, a receptor tyrosine kinase ligand (for example, EGF), Nicotinamide, a Wnt agonist (for example, Wnt(3a)), and optionally one or more additional components selected from: a p38 inhibitor, gastrin, FGF10, HGF and a Rock inhibitor.
10. The culture medium of claim 8 or claim 9, wherein the BMP inhibitor is selected from the group consisting of Noggin, Chordin, a chordin-like protein comprising a chordin domain, Follistatin, a follistatin-related protein comprising a follistatin domain, DAN, a DAN-like proteins comprising a DAN cystein-knot domain, sclerostin/SOST and alpha-2 macroglobulin.

11. The culture medium of claim 8 or claim 9, wherein the Wnt agonist is selected from the group consisting of Wnt-3a, a GSK-inhibitor (such as CHIR99021), Wnt 5, Wnt-6a, Norrin, and any other Wnt family protein.
12. The culture medium of claim 8 or claim 9, wherein the receptor tyrosine kinase ligand is a mitogenic growth factor, for example selected from a family of growth factors consisting of epidermal growth factor (EGF), Transforming Growth Factor-alpha (TGF-alpha), basic Fibroblast Growth Factor (bFGF), brain-derived neurotrophic factor (BDNF), Hepatocyte growth factor (HGF) and Keratinocyte Growth Factor (KGF).
13. The culture medium of claim 8 or claim 9, wherein the Rock inhibitor is selected from the group consisting of R-(+)-trans-4-(1-aminoethyl)-N-(4-Pyridyl)cyclohexanecarboxamide dihydrochloride monohydrate (Y-27632), 5-(1,4-diazepan- 1-ylsulfonyl)isoquinoline (fasudil or HA1071), and (S)-(+)-2-methyl- 1-[(4-methyl-5-isoquinolinyl)sulfonyl] -hexahydro-1H- 1,4-diazepine dihydrochloride (H-1 152).
14. The culture medium of any one of the preceding claims, wherein the culture medium additionally comprises an activator of the prostaglandin signalling pathway, for example, PGE2 and/or AA.
15. The culture medium of any one of the preceding claims, wherein the culture medium additionally comprises testosterone, for example, (dihydro)testosterone.
16. A culture medium according to any one of claims 1 to 15 for culturing intestinal cells, comprising or consisting of a basal medium, Wnt-3a, EGF, Noggin, any one of Rspondin 1-4, a TGF-beta inhibitor, nicotinamide, and preferably a p38 inhibitor.
17. A culture medium according to any one of claims 1 to 15 for culturing gastric cells, comprising or consisting of a basal medium, Wnt-3a, EGF, Noggin, any one of Rspondin 1-4, a TGF-beta inhibitor, gastrin, nicotinamide, FGF-10, and preferably a p38 inhibitor.
18. A culture medium according to any one of claims 1 to 15 for expanding liver cells, comprising or consisting of a basal medium, any one of Rspondin 1-4, Noggin, nicotinamide, EGF, FGF10, HGF, gastrin, a TGF-beta inhibitor and PGE2, and preferably Wnt-3a.
19. A culture medium according to any one of claims 1 to 15 for expanding pancreas cells, comprising or consisting of a basal medium, any one of Rspondin 1-4, Noggin, EGF, FGF10, gastrin, a TGF-beta inhibitor, and preferably exendin 4 and Wnt-3a.
20. A culture medium according to any one of claims 1 to 15 for culturing prostate cells, comprising or consisting of a basal medium, EGF, any one of Rspondin 1-4, Noggin, nicotinamide a TGF-beta inhibitor and preferably Wnt-3a, and FGF-10.
21. A culture medium for culturing prostate cells according to claim 20, wherein the culture medium further comprises testosterone, for example (dihydro)testosterone.

22. A culture medium of any one of the preceding claims for culturing cancer cells, for example cancer stem cells, such as adenocarcinoma or carcinoma cells from a tissue type of interest, wherein the culture medium comprises or consists of the components of the culture medium used for culturing the cells from the corresponding non-cancerous tissue type of interest, optionally wherein one or more of the following are excluded from the medium that is used to culture the non-cancerous cells of the tissue type of interest: Wnt-3a, EGF, Noggin, Rspondin, TGF-beta inhibitor, p38 inhibitor, nicotinamide, gastrin, FGF10 and HGF.
23. A culture medium of any one of the preceding claims for differentiating stem cells from a tissue of interest, wherein the culture medium comprises or consists of the components of the culture medium used for expanding the stem cells from the tissue type of interest but wherein one or more of the following are excluded from the medium for differentiating stem cells: Wnt, Rspondin, BMP inhibitor, TGF-beta inhibitor, receptor tyrosine kinase ligand, p38 inhibitor and nicotinamide.
24. A culture medium according to claim 23 for differentiating intestinal cells, comprising or consisting of a basal medium, EGF, Noggin, a TGF-beta inhibitor and a p38 inhibitor.
25. A culture medium according to claim 23 for differentiating liver cells comprising or consisting of a basal medium, Noggin, EGF, gastrin, a TGF-beta inhibitor, a gamma-secretase inhibitor such as DAPT or DBZ, and preferably Wnt-3a.
26. A culture medium according to claim 23 for differentiating pancreas cells comprising or consisting of a basal medium, Noggin, EGF, FGF10, gastrin, a TGF-beta inhibitor, gamma-secretase inhibitor and preferably exendin 4.
27. The culture medium according to any one of the preceding claims, wherein the culture medium is in contact with an extracellular matrix or a 3D matrix that mimics the extracellular matrix by its interaction with the cellular membrane proteins such as integrins.
28. The culture medium of claim 27, wherein the extracellular matrix is a laminin-containing extracellular matrix such as Matrigel™ (BD Biosciences).
29. A composition comprising a culture medium according to any one of claims 1 to 26 and an extracellular matrix or a 3D matrix that mimics the extracellular matrix by its interaction with the cellular membrane proteins such as integrins, for example, a laminin-containing extracellular matrix such as Matrigel™ (BD Biosciences).
30. A hermetically-sealed vessel containing a culture medium or composition according to any one of the preceding claims.
31. Use of a culture medium according to any one of claims 1 to 28 for expanding and/or differentiating a stem cell, population of stem cells, tissue fragment or organoid.
32. Use according to claim 31, wherein the stem cell, population of stem cells, tissue fragment or organoid is selected from the group consisting of one or more intestinal stem cells, small

intestinal crypts, colonic crypts, gastric stem cells, liver stem cells, pancreas stem cells and prostate stem cells.

33. Use according to claim 31 or claim 32, wherein the stem cell, population of stem cells, tissue fragment or organoid is obtainable from a normal tissue.

34. Use according to claim 31 or 32, wherein the stem cell, population of stem cells, tissue fragment or organoid is obtainable from a diseased tissue, for example from an adenoma, a carcinoma, an adenocarcinoma, an intestine of a patient having cystic fibrosis or an intestine of a patient having inflammatory bowel disease.

35. A method for expanding a single stem cell, a population of stem cells or a tissue fragment, preferably to obtain an organoid, wherein the method comprises culturing the single stem cell or population of stem cells in a culture medium according to any one of claims 1 to 28.

36. A method according to claim 35, wherein the method comprises:

providing a stem cell, a population of stem cells or an isolated tissue fragment;

providing a culture medium according to any one of claims 1 to 28;

contacting the stem cells with the culture medium;

culturing the cells under appropriate conditions.

37. A method according to claim 35, wherein the method comprises bringing the stem cell, the population of stem cells or the isolated tissue fragment and the culture medium into contact with an extracellular matrix or a 3D matrix that mimics the extracellular matrix by its interaction with the cellular membrane proteins such as integrins, for example a laminin-containing extracellular matrix such as Matrigel™ (BD Biosciences).

38. The method of claim 37, wherein the culture medium is diffused into the extracellular matrix.

39. A method according to any one of claims 35 to 38, wherein the method comprises:

culturing the stem cell, population of stem cells or tissue fragments in a first expansion medium;

continuing to culture the stem cell, population of stem cells or tissue fragments and replenishing the medium with a differentiation medium, wherein the differentiation medium does not comprise one or more of, preferably all of the factors selected from: a TGF-beta inhibitor, a p38 inhibitor, nicotinamide and Wnt.

40. A method according to any one of claims 35-38 for obtaining a small intestinal or colon organoid comprising:

expanding small intestinal or colon stem cells or tissue fragments in a culture medium according to claim 17; and optionally

differentiating the expanded small intestinal or colon stem cells or tissue fragments in a culture medium according to claim 24.

41. A method according to any one of claims 35-39 for obtaining a gastric organoid comprising culturing gastric stem cells or tissue fragments in the culture medium of claim 16.
42. A method according to any one of claims 35-39 for obtaining a liver organoid comprising:
expanding liver cells or tissue fragments in a culture medium according to claim 19; and optionally
differentiating the expanded liver cells or tissue fragments in a culture medium according to claim 25.
43. A method according to any one of claims 35-39 for obtaining a pancreatic organoid comprising
expanding pancreatic cells or tissue fragments in a culture medium according to claim 20; and optionally
differentiating the expanded pancreatic cells or tissue fragments in a culture medium according to claim 26.
44. A method according to any one of claims 35-39 for obtaining a prostate organoid comprising culturing prostate stem cells or tissue fragments in the culture medium of claim 19.
45. A method according to any one of claims 35-39 for obtaining an adenocarcinoma or carcinoma organoid comprising culturing adenocarcinoma or carcinoma stem cells or tissue fragments in the culture medium of claim 22.
46. The method of any one of claims 35-39, wherein a Rock inhibitor is added to the culture medium for the first 1, 2, 3, 4, 5, 6 or 7 days, optionally every second day.
47. The method according to any one of claims 35-39, which comprises culturing the stem cells for 3 or more months, for example 4, 5, 6, 7, 8, 9 or more months using a culture medium according to any one of claims 1 to 28.
48. An organoid or population of cells obtainable by the method of any one of claims 35 to 47.
49. An organoid or population of cells according to claim 48, which is capable of surviving in culture for at least 3 months, for example at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 9 months, or at least 12 months or more, when cultured in a culture medium according to any one of claims 1 to 28.
50. An organoid or population of cells according to claim 48, wherein the organoid or population of cells has been cultured for at least 3 months, for example at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 9 months, or at least 12 months or more.
51. An organoid or population of cells according to any one of claims 48 to 50, wherein the organoid or population of cells expands at a rate of at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold or at least 10 fold per week.
52. The organoid or population of cells of any one of claims 48 to 51, wherein the organoid or population of cells is a human organoid or a human population of cells.

53. The organoid or population of cells of any one of claims 48 to 52, wherein the organoid or population of cells is a normal organoid or population of cells or a disease organoid or population of cells, for example obtained by culturing stem cells taken from a human or animal with a disease.

54. An organoid or population of cells according to any one of claims 48 to 53 which is frozen and stored at below -5°C, below -10°C, below -20°C, below -40°C, below -60°C, below -80°C, below -100°C, or below -150°C, for example at approximately -180 °C.

55. An organoid, preferably according to any one of claims 48 to 54, which is a three-dimensional organoid comprising epithelial cells surrounding a central lumen, wherein optionally the epithelial cells exist in distinct dividing domains and differentiating domains.

56. The organoid of claim 55, which is a three-dimensional organoid comprising epithelial cells arranged in regions of monolayers, optionally folded monolayers and regions of stratified cells.

57. The organoid of any one of claims 48 to 56, wherein non-epithelial cells are absent from said organoid.

58. The organoid of any one of claims 48 to 57, wherein all differentiated cell types of the normal *in vivo* tissue are present in said organoid.

59. The organoid of any one of claims 48 to 58, wherein the organoid is a small intestine organoid, a colon organoid, a gastric organoid, a pancreatic organoid, a liver organoid or a prostatic organoid.

60. A composition comprising:

i) one or more organoids or population of cells according to any one of claims 48 to 59; and

ii) a culture medium according to any one of claims 1 to 28 and/or an extracellular matrix.

61. An organoid according to any one of claims 48 to 59 or a population of cells according to any one of claims 48 to 54 or a composition according to claim 29 or claim 60 for use in drug screening, target validation, target discovery, toxicology, toxicology screens, personalized medicine, regenerative medicine or *ex vivo* cell/organ models, for example for use as a disease model.

62. The organoid, population of cells or composition for use according to claim 61, wherein the regenerative medicine or personalized medicine comprises transplantation of said organoid, population of cells or composition into a mammal, preferably into a human.

CLAIMS:

1. A hermetically-sealed vessel containing a culture medium for expanding or differentiating a population of adult stem cells, wherein said culture medium comprises:
    i. any one of Rspondin 1-4 and/or an Rspondin mimic; and
    ii. one or more TGF-beta inhibitor, wherein the inhibitor is a TGF-beta inhibitor if it can inhibit TGF-beta signalling in a cellular assay in which cells are stably transfected with a reporter construct comprising the human PAI-1 promoter.