CLAIMS

1. A method of inducing differentiation of pluripotent stem cells into thymic epithelial cells (TECs) or thymic epithelial cell progenitors (TEPs) comprising the steps of

a. differentiating the pluripotent stem cells into definitive endoderm cells;

b. culturing the definitive endoderm cells and differentiating the definitive endoderm cells into anterior foregut cells by contacting or incubating the definitive endoderm cells with an agent which inhibits Bone Morphogenic Protein (BMP) and an agent which inhibits TGFβ signaling, and further contacting or incubating the definitive endoderm cells with an agent which stimulates the expression of *HOXA3* and an agent which stimulates the expression of *TBX1*;

c. culturing the anterior foregut cells and differentiating the anterior foregut cells into pharyngeal endoderm cells by contacting or incubating the anterior foregut cells with an agent which stimulates the expression of *HOXA3*, an agent which stimulates the expression of *TBX1* and an agent which stimulates the expression of *PAX9* and *PAX1*;

d. culturing the pharyngeal endoderm cells and differentiating the pharyngeal endoderm cells into distal pharyngeal pouch (PP) specification cells, TECs or TEPs by contacting or incubating the pharyngeal endoderm cells with an agent which inhibits BMP; and

e. culturing the pharyngeal endoderm cells from step c. or step d. and differentiating the pharyngeal endoderm cells into distal pharyngeal pouch (PP) specification cells, TECs or TEPs by contacting or incubating the pharyngeal endoderm cells with BMP

wherein the agent which inhibits BMP is Noggin or dorsomorphin,
wherein the agent which inhibits TGFβ signaling is SB431542,
wherein the agent which stimulates the expression of HOXA3 is retinoic acid,
wherein the agent which stimulates the expression of TBX1 is FGF8b, and
wherein the agent which stimulates the expression of PAX9 and PAX1 is Sonic Hedgehog (Shh).

2. The method of claim 1, wherein step a. is performed for ~~about~~ one to ~~about~~ six days.

3. The method of claim 1, wherein in step a., the pluripotent stem cells are cultured in serum-free differentiation medium and contacted or incubated with BMP in an amount of about 0.5 ng/ml, human b Fibroblast Growth Factor (bFGF) in an amount of about 2.5 ng/ml and human Activin A in an amount of about 100 ng/ml.

4. The method of claim 1, wherein step b. is performed starting at ~~about~~ day 3 to ~~about~~ day 5 for ~~about~~ 2 days to ~~about~~ 3 days.

~~5. The method of claim 1, wherein in step b. the agent which inhibits BMP is selected from the group consisting of Noggin and dorsomorphin, the agent which inhibits TGF signaling is SB431542, the~~

~~agent which stimulates the expression of *HOXA3* is retinoic acid, and the agent which stimulate the expression of *TBX1* is FGF8b.~~

~~6~~5. The method of claim 1, wherein step c. is performed starting at ~~about~~ day 5 to ~~about~~ day 8 for ~~about~~ 6 days to ~~about~~ 10 days.

~~7. The method of claim 1, wherein in step c. the agent which stimulates the expression of *HOXA3* is retinoic acid, the agent which stimulates expression of *TBX1* is FGF8b, and the agent which stimulates the expression of *PAX9* and *PAX1* is Sonic Hedgehog (Shh).~~

~~6~~8. The method of claim ~~7~~1, wherein in step c. at about 24 hours FGF8b is used in an amount of about 50 ng/ml and retinoic acid is used in an amount of about 0.25 µM and at 48 hours FGF8b is used in an amount of about 50 ng/mL and Shh in an amount of about 100 ng/ml.

~~9~~7. The method of claim 1, wherein step d. is performed starting at ~~about~~ day 12 to ~~about~~ day 18 for ~~about~~ 4 days to ~~about~~ 7 days.

~~10. The method of claim 1, wherein in step d. the agent which inhibits BMP is selected from the group consisting of Noggin and dorsomorphin~~.

~~11~~8. The method of claim 1, wherein step e. is performed starting at ~~about~~ day 19 to ~~about~~ day 25 for ~~about~~ 5 days to ~~about~~ 15 days.

~~12~~9. The method of claim 1, wherein in step e. BMP is used in an amount of about 50 ng/ml.

~~13~~10. The method of claim 1, further comprising a step of contacting or incubating the TECs or TEPs at the end of the method with a survivin inhibitor, optionally wherein the survivin inhibitor is YM155.

~~14~~11. A method for inducing differentiation of pluripotent stem cells into thymic epithelial cells (TECs), or thymic epithelial cell progenitors (TEPs) comprising the steps of:

a. differentiating the pluripotent stem cells into definitive endoderm cells by culturing the pluripotent stem cells in a serum-free differentiation medium and contacting or incubating the pluripotent stem cells with human Bone Morphogenic Protein (BMP), human basic Fibroblast Growth Factor (bFGF) and human Activin A;

b. differentiating the definitive endoderm cells from step a. into anterior foregut cells by culturing the definitive endoderm cells in the serum-free differentiation medium and contacting or incubating the definitive endoderm cells with Noggin, and SB431542, optionally further comprising retinoic acid or FGF8b;

c. differentiating the anterior foregut cells from step b. into pharyngeal endoderm cells by culturing the anterior foregut cells in the serum-free differentiation medium, and

contacting or incubating the anterior foregut cells with FGF8b and retinoic acid followed by FGF8b and Sonic Hedgehog (Shh);

d. differentiating the pharyngeal endoderm cells from step c. into 3rd pharyngeal pouch specification cells, TECs or TEPs by culturing the pharyngeal endoderm cells in the serum-free differentiation medium and contacting or incubating the cells with Noggin; and

e. optionally further differentiating the pharyngeal endoderm cells from step c. or step d. into 3rd pharyngeal pouch specification cells, TECs or TEPs by culturing the pharyngeal endoderm cells in the serum-free differentiation medium and contacting or incubating the pharyngeal endoderm cells with BMP.

~~15~~12. The method of claim ~~14~~11, wherein the pluripotent stem cell is an embryonic stem cell or an induced pluripotent stem cell.

~~16~~13. ~~.~~ The method of claim ~~14~~11, wherein step a. performed for ~~about~~ one to ~~about~~ six days.

~~17~~14. The method of claim ~~14~~ 11 wherein in step a., the BMP is used in an amount of about 0.5 ng/ml, the human b Fibroblast Growth Factor is used in an amount of about 2.5 ng/ml and the human Activin A is used in an amount of about 100 ng/ml.

~~18~~15. The method of claim ~~14~~ 11 wherein step b. is performed starting at ~~about~~ day 3 to ~~about~~ day 5 for ~~about~~ 2 days to ~~about~~ 3 days.

~~19~~16. The method of claim ~~14~~11, wherein in step b., the Noggin is used in an amount of about 200 ng/ml, the SB431542 is used in an amount of about 10 µM, the retinoic acid is used in an amount of about 0.25 µM, and the FGF8b is used in an amount of about 50 ng/mL.

~~20~~17. The method of claim ~~14~~11, wherein step c. is performed starting at ~~about~~ day 5 to ~~about~~ day 8 for ~~about~~ 6 days to ~~about~~ 10 days.

~~21~~18. The method of claim ~~14~~11, wherein in step c. at about 24 hours from the start of step c., FGF8b is used in an amount of about 50 ng/mL and Retinoic Acid is used in an amount of about 0.25 µM.

~~22~~19. The method of claim ~~14~~11, wherein in step c. at about 48 hours from the start of step c., FGF8b is used in an amount of about 50 ng/mL and Shh is used in an amount of about 100 ng/ml.

~~23~~20. The method of claim ~~14~~11, wherein step d. is performed starting at ~~about~~ day 12 to ~~about~~ day 18 for ~~about~~ 4 days to ~~about~~ 7 days.

~~24~~21. The method of claim ~~14~~11, wherein in step d. Noggin is used in an amount of about 100 ng/ml.

~~25~~22. The method of claim ~~14~~11, wherein step e. is performed starting at ~~about~~ day 19 to ~~about~~ day 25 for ~~about~~ 5 days to ~~about~~ 15 days.

~~26~~23. The method of claim ~~14~~11, wherein in step e. BMP is used in an amount of about 50 ng/ml.

~~27~~24. The method of claim ~~14~~11, further comprising a step of contacting or incubating the TECs or TEPs at the end of the method with a surviving inhibitor, optionally wherein the survivin inhibitor is YM155.

2825. An isolated population of thymic epithelial cells (TECs) or a thymic epithelial cell progenitors (TEPs) obtained by the method comprising the steps of.

a. differentiating pluripotent stem cells into definitive endoderm cells by culturing the pluripotent stem cells in a serum-free differentiation medium and contacting or incubating the pluripotent stem cells with human Bone Morphogenic Protein (BMP), human basic Fibroblast Growth Factor (bFGF) and human Activin A;

b. differentiating the definitive endoderm cells from step a. into anterior foregut cells by culturing the definitive endoderm cells in the serum-free differentiation medium and contacting or incubating the definitive endoderm cells with Noggin, and SB431542, optionally further comprising retinoic acid or FGF8b;

c. differentiating the anterior foregut cells from step b. into pharyngeal endoderm cells by culturing the anterior foregut cells in the serum-free differentiation medium, and contacting or incubating the anterior foregut cells with FGF8b and retinoic acid followed by FGF8b and Sonic Hedgehog (Shh);

d. differentiating the pharyngeal endoderm cells from step c. into 3rd pharyngeal pouch specification cells, thymic epithelial cells, or thymic epithelial cell progenitor cells by culturing the pharyngeal endoderm cells in the serum-free differentiation medium and contacting or incubating the cells with Noggin; and

e. optionally, further differentiating the pharyngeal endoderm cells from step c. or step d. into 3rd pharyngeal pouch specification cells, thymic epithelial cells or thymic epithelial cell progenitor cells by culturing the pharyngeal endoderm cells in the serum-free differentiation medium and contacting or incubating the pharyngeal endoderm cells with BMP.

2926. Use of a therapeutically effective amount of the isolated population of TECs or TEPs ~~thymic epithelial cell or thymic epithelial cell progenitor~~ of claim ~~30~~ 25 in the manufacture of a medicament for preventing and/or treating a disease of the thymus, optionally wherein the disease is an autoimmune disease.

3027. Use of the isolated population of TECs or TEPs ~~thymic epithelial cell or thymic epithelial cell progenitor~~ of claim ~~30~~25 in the manufacture of a medicament for recovering or restoring the impaired function of the thymus, optionally wherein the impaired function of the thymus is due to injury, aging or congenital abnormality.