CLAIMS

What is claimed is:

1. A pluripotent stem cell (PSC) comprising: an engineered polynucleotide comprising an open reading frame encoding a protein selected from NR5A1 and a RUNX family protein.

2. The PSC of claim 1, wherein the RUNX family protein is RUNX1 or RUNX2.

3. The PSC of claim 1, wherein the PSC expresses or overexpresses: NR5A1; RUNX1; RUNX2; NR5A1 and RUNX1; NR5A1 and RUNX2; or NR5A1, RUNX1, and RUNX2.

4. The PSC of any one of claims 1 to 3, wherein PSC further comprises an engineered polynucleotide comprising an open reading frame encoding a TCF21 protein and/or an engineered polynucleotide comprising an open reading frame encoding a GATA4 protein.

5. The PSC of claim 4, wherein the PSC expresses or overexpresses TCF21 and/or GATA4.

6. The PSC of any one of claims 1 to 5, wherein the open reading frame of the engineered polynucleotide is operably linked to a heterologous promoter or an inducible promoter.

7. A pluripotent stem cell (PSC) comprising: a protein selected from NR5A1 and a RUNX family protein, wherein the protein is overexpressed.

8. The PSC of claim 7, wherein the PSC expresses or overexpresses: NR5A1; RUNX1; RUNX2; NR5A1 and RUNX1; NR5A1 and RUNX2; or NR5A1, RUNX1, and RUNX2.

9. The PSC of claim 8, wherein the PSC further comprises a TCF21 protein and/or a GATA4 protein.

10. The PSC of claim 9, wherein the PSC expresses or overexpresses TCF21 and/or GATA4.

11. The PSC of any one of claims 7 to 10, wherein the PSC is a human PSC or an induced PSC (iPSC).

12. The PSC of any one of claims 7 to 11, wherein the PSC comprises 1-20, or 8-10, copies of the engineered polynucleotide comprising the open reading frame encoding the protein selected from NR5A1 and a RUNX family protein.

13. A composition comprising: a population of the PSC of any one of claims 1 to 12; or a population comprising at least 10,000/cm^2 of the PSC of any one of claims 1 to 12.

14. A method, comprising: culturing, in culture media, a population of pluripotent stem cells (PSCs) to produce an expanded population of PSCs; and expressing in PSCs of the expanded population a protein selected from NR5A1 and a RUNX family protein to produce granulosa-like cells.

15. The method of claim 14, wherein the PSCs of the expanded population comprise an engineered polynucleotide comprising an open reading frame encoding NR5A1 and/or an engineered polynucleotide comprising an open reading frame encoding a RUNX family protein.

16. The method of claim 15, wherein the RUNX family protein is RUNX1 or RUNX2.

17. The method of any one of claims 15 to 16, wherein the PSCs of the expanded population further comprise an engineered polynucleotide comprising an open reading frame encoding a TCF21 protein and/or an engineered polynucleotide comprising an open reading frame encoding a GATA4 protein.

18. The method of any one of claims 15 to 17, wherein the open reading frame of the engineered polynucleotide is operably linked to a heterologous promoter or an inducible promoter.

19. The method of any one of claims 14 to 18, wherein the population comprises $1x10^2$ - $1x10^7$ PSCs, and/or wherein the population of PSCs is cultured for about 4-10 days or for about 6 days.

20. The method of any one of claim 14 to 19, wherein the granulosa-like cells are $AMHR2^+$, $CD82^+$, $FOXL2^+$, and/or $EPCAM^-$.

21. A method comprising:

(a) delivering to pluripotent stem cells (PSCs) an engineered polynucleotide comprising an inducible promoter operably linked to an open reading frame encoding a protein selected from NR5A1 and a RUNX family protein;

(b) culturing the PSCs in feeder-free, serum-free culture media to produce an expanded population of PSCs; and

(c) culturing PSCs of the expanded population in a series of induction media comprising an inducing agent to produce $AMHR2^+$, $CD82^+$, $FOXL2^+$, and/or EPCAM granulosa-like cells.

22. The method of claim 21, comprising delivering to PSCs (i) an engineered polynucleotide comprising an inducible promoter operably linked to an open reading frame encoding NR5A1 and (ii) an engineered polynucleotide comprising an inducible promoter operably linked to an open reading frame encoding a RUNX family protein.

23. The method of any one of claims 21 to 22, wherein the RUNX family protein is RUNX1 or RUNX2.

24. The method of any one of claims 21 to 23, wherein the engineered polynucleotide is a transposon and the delivering further comprises delivering a transposase to the PSCs.

25. The method of any one of claims 21 to 24, wherein the inducible promoter is a chemically-inducible promoter, or a doxycycline-inducible promoter.

26. The method of any one of claims 21 to 25, wherein the feeder-free, serum-free culture media of (b) comprises:

a solubilized basement membrane preparation extracted from the Engelbreth- Holm-Swarm (EHS) mouse sarcoma, and/or

growth factors selected from recombinant human basic fibroblast growth factor (rh bFGF) and recombinant human transforming growth factor β (rh TGFβ).

27. The method of claim 26, wherein the solubilized basement membrane preparation comprises extracellular matrix (ECM) proteins and growth factors.

28. The method of claim 27, wherein the ECM proteins are selected from Laminin, Collagen IV, heparan sulfate proteoglycans, and entactin/nidogen.

29. The method of any one of claims 21 to 28, wherein the culturing of (b) is for about 6-24 hours.

30. The method of any one of claims 21 to 29, wherein the culturing of (c) comprises (i) culturing the PSCs at a density of about 10,000 cells/cm^2 to about 20,000 cells/cm^2 and/or (ii) culturing the PSCs in a first induction media and culturing the PSCs in a second induction media.

31. The method of claim 23, wherein:

the first induction media comprises one or more of L-alanyl-L-glutamine, antibiotic, Dulbecco's Modified Eagle Medium (DMEM)/F-12, Advanced RPMI (Roswell Park Memorial Institute) 1640 Medium, a glycogen synthase kinase (GSK) 3 inhibitor, a small molecule or protein inhibitor of the BMP signaling pathway, a small molecule ROCK inhibitor, and an inducing agent; and/or

the second induction media comprises one or more of L-alanyl-L-glutamine, antibiotic, Advanced RPMI 1640 Medium, DMEM/F-12, and an inducing agent.

32. The method of claim 30, wherein the culturing the PSCs is a first induction media is for about 36 to about 60 hours, optionally about 48 hours.

33. The method of claim 30, wherein the culturing the PSCs in a second induction media is for about 96 to about 144 hours, or about 120 hours.

34. The method of claim 33, wherein the second induction media is removed and replaced with fresh second induction media at about 24-hour intervals.

35. A granulosa-like cell produced by the method of any one of claims 21 to 34.

36. An ovarian organoid comprising granulosa-like cells of claim 35 and human primordial germ cell-like cells (hPGCLCS).

37. The method of any one of claims 21 to 34 further comprising combining the granulosa-like cells with hPGCLCS to form an ovarian organoid.

38. The PSC of claim 3, wherein the PSC expresses or overexpresses NR5A1 and RUNX2.

39. The PSC of claim 3, wherein the PSC expresses or overexpresses NR5A1, RUNX2, and GATA4.

40. The method of claim 14, wherein the PSCs express or overexpress NR5A1 and RUNX2.

41. The method of claim 14, wherein the PSCs express or overexpress NR5A1, RUNX2, and GATA4.