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Supplemental Information

Distinct Enhancer Activity of *Oct4* in Naive and Primed Mouse Pluripotency

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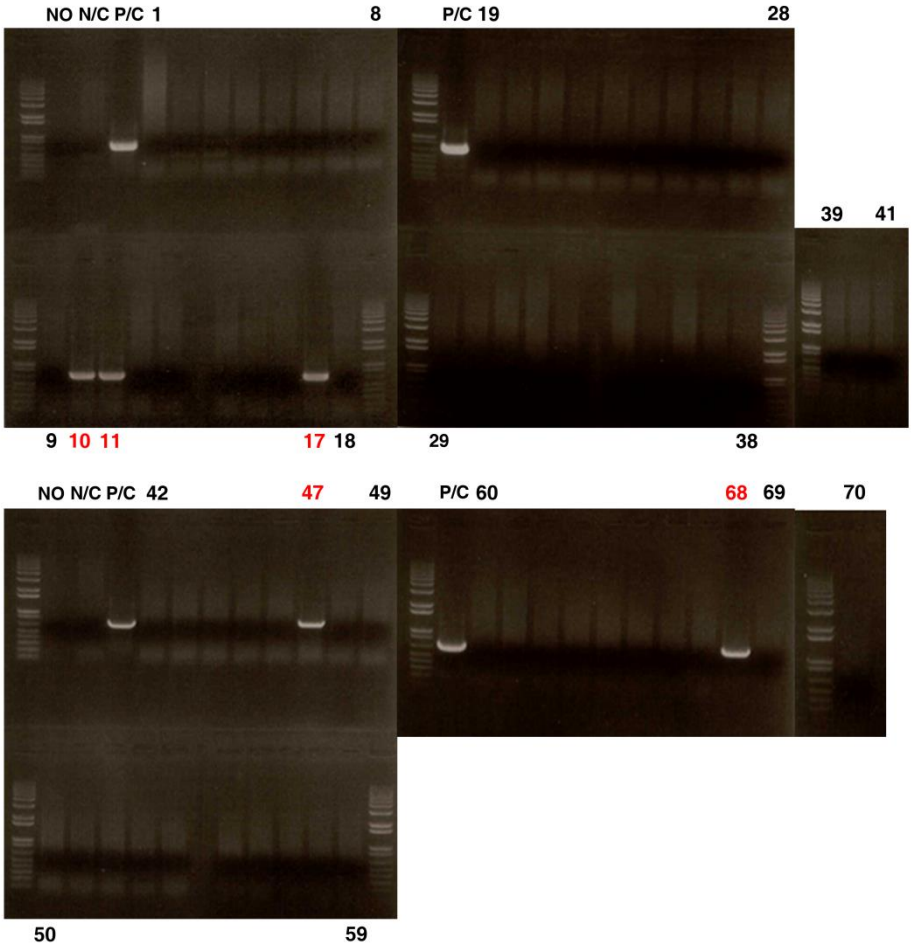

Figure S1. Genotyping of *Oct4*-ΔDE-tdTomato mice by using tdTomato specific primer. (related to Figure 1). We obtained five *Oct4*-ΔDE-tdTomato founder transgenic mice (5/70). NO: no template, N/C: normal mouse genomic DNA, P/C: positive (plasmid DNA) control.

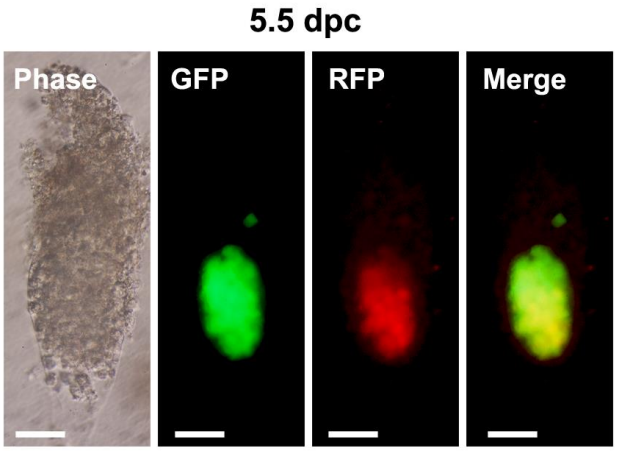

Figure S2. Oct4 enhancer activity in early post-implantation embryo (5.5 dpc) Phase and flurescence images of 5.5 dpc embryo, Scale bar = 100 μm, The epiblast of 5.5 dpc embryo expressed both O4-DE-GFP and O4-PE-RFP. However, proximal epiblast cells weakly expressed O4-PE-RFP than distal epiblast cells.

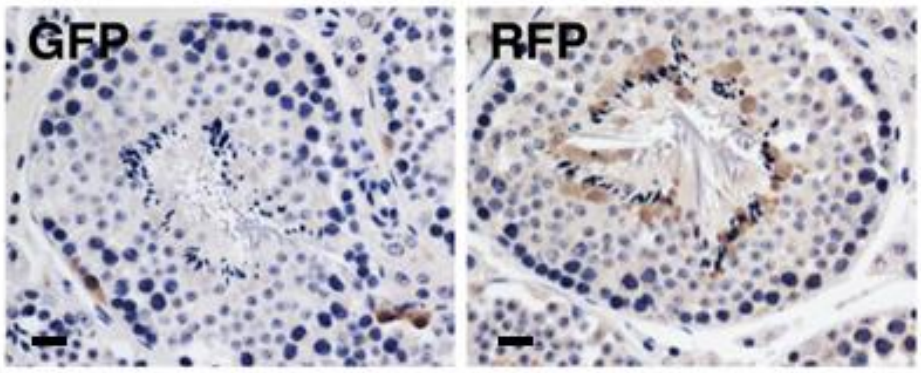

Figure S3. Immunohistochemistry analysis in testis of adult double transgenic mouse (4 weeks). (related Figure 1). GFP (brown) were positive in spermatogonia at periphery (nearby basement membrane) of seminiferous tubules in testis of adult RFP (brown) were positive from the middle to the center of seminiferous tubules. scale bar = 50 μm.

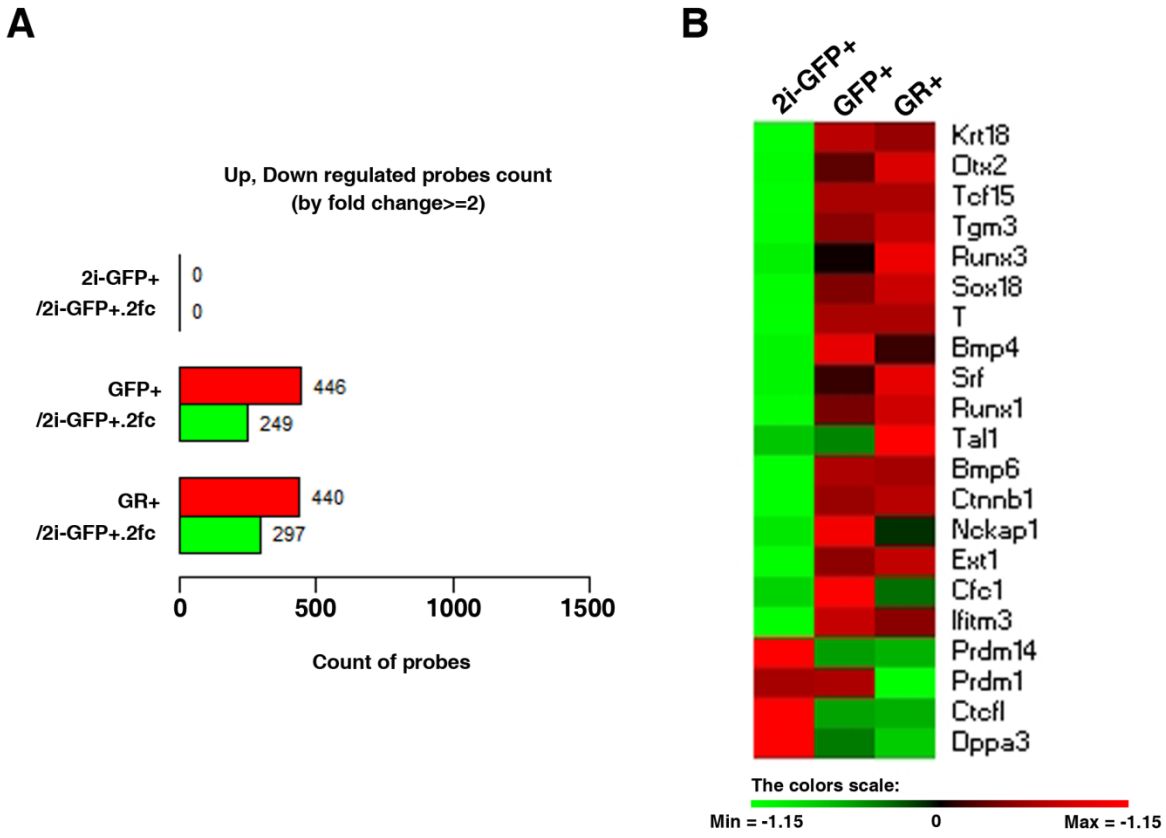

Figure S4. Different gene expression 2i-GFP$^+$ compared with GFP$^+$ or GR$^+$ cells. (related Figure 3). (A) Counting of up and down regulated probes in 2i-GFP$^+$ compared with GFP$^+$ or GR$^+$ cells. (B) Development related genes were up-regulated in GFP$^+$ and GR$^+$ cells in serum+LIF medium as ectoderm (*Krk18*, *Otx2*, *Tcf15*, and *Tgm3*), mesoderm (*Bmp4*, *Srf*, *Runx1*, *Tal1*, and *Bmp6*), and endoderm (*Ctnnb1*, *Ext1*,and *Cfc1*).

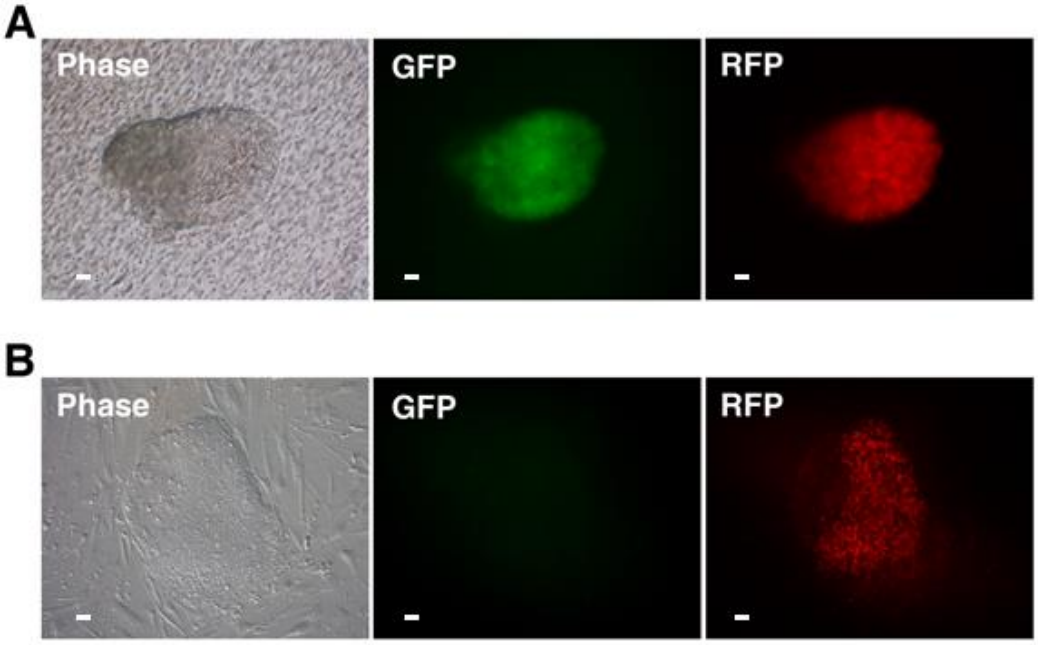

Figure S5. Derivation of EpiSCs from double transgenic embryo. (related Figure 4). **(**A) Isolated epiblast from 6.5 dpc double transgenic embryo expressed *Oct4*-ΔPE-GFP and *Oct4*-ΔDE-tdTomato (See also Figure 1C). scale bar = 50 μm. (B) Only *Oct4*-ΔDE-tdTomato positive cells were expanded in EpiSCs medium on feeder at day 1. scale bar = 50 μm.

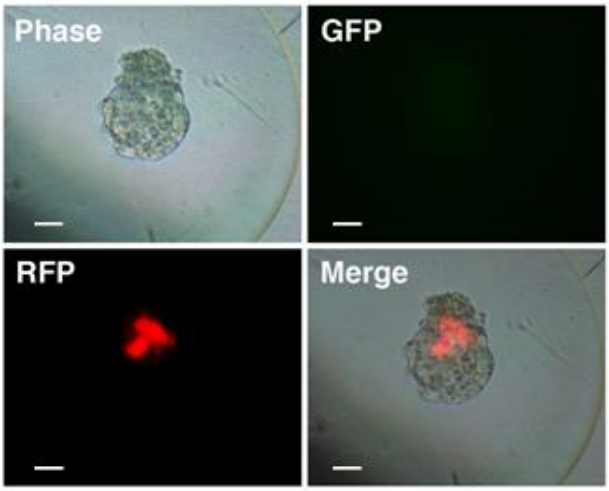

Figure S6. Only *Oct4*-ΔDE-RFP positive cells did not contribute ICM. (related Figure 5). One embryo (1/27) was aggregated with *Oct4*-ΔDE-RFP positive cells. However, *Oct4*-ΔDE-RFP positive cells did not incorporate into ICM (See also Figure 5A and B). scale bar = 50 μm.

Table S1. Gene Ontology (GO) analysis of up-regulated gene in 2i-GFP+cells

Annotation Cluster 1	Enrichment Score: 3.29			
GO Term	**Count**	**Genes**	**P_Value**	**Benjamini**
sterol biosynthetic process	6	MVD, DHCR7, INSIG1, FDPS, SC4MOL, NSDHL	1.30E-05	1.70E-02
sterol metabolic process	7	MVD, DHCR7, INSIG1, FDPS, PCSK9, SC4MOL, NSDHL	1.50E-04	6.20E-02
cholesterol metabolic process	6	MVD, DHCR7, INSIG1, FDPS, PCSK9, NSDHL	8.10E-04	2.30E-01
cholesterol biosynthetic process	5	MVD, DHCR7, INSIG1, FDPS, NSDHL	8.70E-05	5.30E-02
lipid biosynthetic process	9	SCD1, SCD2, A4GALT, MVD, DHCR7, INSIG1, FDPS, SC4MOL, NSDHL	1.00E-02	6.90E-01

Table S2. Gene Ontology (GO) analysis of up-regulated gene in GFP or GR positive cells

Annotation Cluster 1	Enrichment Score: 3.47			
GO Term	**Count**	**Genes**	**P_Value**	**Benjamini**
gland morphogenesis	12	BMP4, EGFR, WNT5A, DDR1, CAV1, FGF7, RARG, CD44, CSF1, TNC, AREG, ETV4	8.90E-07	1.50E-03
epithelium development	20	COL18A1, EGFR, WNT5A, BMP4, CEBPB, RARG, CSF1, LMO4, TNC, GJA1, GREM1, SPRR2K, DDR1, LOC100048295, CD44, ID1, POU3F1, AREG, CAR2, ETV4	2.40E-06	2.00E-03
mammary gland morphogenesis	7	BMP4, WNT5A, DDR1, CAV1, CSF1, AREG, ETV4	4.40E-05	2.40E-02
gland development	15	EGFR, WNT5A, BMP4, CAV1, CEBPB, FGF7, RARG, SOCS2, CSF1, TNC, BCL2L11, DDR1, CD44, AREG, ETV4	4.50E-05	1.90E-02
mammary gland development	10	BMP4, WNT5A, DDR1, CAV1, CEBPB, SOCS2, CSF1, AREG, BCL2L11, ETV4	5.60E-05	1.80E-02
morphogenesis of an epithelium	13	WNT5A, BMP4, EGFR, RARG, CSF1, LMO4, TNC, GREM1, DDR1, CD44, AREG, ETV4, CAR2	2.00E-04	4.00E-02
tissue morphogenesis	15	WNT5A, BMP4, EGFR, FGF7, RARG, CSF1, LMO4, TNC, GREM1, TPM1, DDR1, CD44, AREG, CAR2, ETV4	3.30E-04	5.90E-02
branching involved in mammary gland duct morphogenesis	5	WNT5A, DDR1, CSF1, AREG, ETV4	4.90E-04	7.80E-02
tube morphogenesis	12	BMP4, WNT5A, DDR1, CD44, LMO4, CSF1, GJA1, AREG, GREM1, ZIC3, BCL2L11, ETV4	6.80E-04	8.40E-02
tube development	15	WNT5A, BMP4, FGF7, CSF1, LMO4, GJA1, GREM1, FOXP1, BCL2L11, ZIC3, DDR1, CD44, ID1, AREG, ETV4	9.30E-04	1.00E-01
mammary gland duct morphogenesis	5	WNT5A, DDR1, CSF1, AREG, ETV4	1.70E-03	1.40E-01
epithelial tube morphogenesis	9	BMP4, WNT5A, DDR1, CD44, LMO4, CSF1, AREG, GREM1, ETV4	1.80E-03	1.40E-01
branching morphogenesis of a tube	8	BMP4, WNT5A, DDR1, CD44, CSF1, AREG, GREM1, ETV4	2.70E-03	1.80E-01
developmental growth involved in morphogenesis	4	BMP4, WNT5A, CSF1, AREG	3.70E-03	2.20E-01
morphogenesis of a branching structure	9	BMP4, WNT5A, DDR1, FGF7, CD44, CSF1, AREG, GREM1, ETV4	3.70E-03	2.10E-01
developmental growth	6	BMP4, WNT5A, CSF1, GJA1, HBEGF, AREG	5.10E-02	6.40E-01

Annotation Cluster 2	Enrichment Score: 2.89			
GO Term	**Count**	**Genes**	**P_Value**	**Benjamini**
vasculature development	16	BMP4, COL18A1, CAV1, SPHK1, EFNB2, GJA1, ELK3, THY1, ANXA2, LOC100048295, ID1, ROBO4, HBEGF, PLCD1, PPAP2B, TNFAIP2	1.60E-04	3.80E-02
blood vessel development	14	BMP4, COL18A1, CAV1, SPHK1, GJA1, ELK3, THY1, ANXA2, LOC100048295, ROBO4, HBEGF, PLCD1, TNFAIP2, PPAP2B	1.30E-03	1.40E-01
blood vessel morphogenesis	12	BMP4, COL18A1, CAV1, LOC100048295, ROBO4, GJA1, HBEGF, PLCD1, ELK3, TNFAIP2, ANXA2, THY1	2.20E-03	1.60E-01
angiogenesis	9	BMP4, COL18A1, ROBO4, HBEGF, PLCD1, ELK3, TNFAIP2, ANXA2, THY1	5.40E-03	2.60E-01

Annotation Cluster 3	Enrichment Score: 2.67			
GO Term	**Count**	**Genes**	**P_Value**	**Benjamini**
mammary gland morphogenesis	7	BMP4, WNT5A, DDR1, CAV1, CSF1, AREG, ETV4	4.40E-05	2.40E-02
regulation of morphogenesis of a branching structure	4	BMP4, WNT5A, FGF7, ETV4	1.30E-02	4.00E-01
negative regulation of epithelial cell proliferation	4	BMP4, WNT5A, SERPINF1, ETV4	1.60E-02	4.30E-01

Annotation Cluster 4	Enrichment Score: 2.31			
GO Term	**Count**		**P_Value**	**Benjamini**
negative regulation of protein kinase activity	7	CAV1, TRIB3, SPRED1, GADD45A, PDCD4, SPRY4, THY1	5.30E-04	7.60E-02
negative regulation of kinase activity	7	CAV1, TRIB3, SPRED1, GADD45A, PDCD4, SPRY4, THY1	5.30E-04	7.60E-02
negative regulation of transferase activity	7	CAV1, TRIB3, SPRED1, GADD45A, PDCD4, SPRY4, THY1	6.50E-04	8.60E-02
negative regulation of molecular function	10	CLN3, CAV1, TRIB3, SPRED1, GADD45A, MYC, PDCD4, SPRY4, DDIT3, THY1	1.40E-03	1.30E-01
negative regulation of catalytic activity	8	CLN3, CAV1, TRIB3, SPRED1, GADD45A, PDCD4, SPRY4, THY1	4.20E-03	2.20E-01
regulation of phosphorylation	14	BMP4, EGFR, CAV1, CSF1, SPHK1, TRIB3, PDCD4, SPRY4, IL11, THY1, CDKN1C, SPRED1, GADD45A, VLDLR	5.90E-03	2.60E-01
negative regulation of MAP kinase activity	4	CAV1, SPRED1, PDCD4, SPRY4	7.10E-03	3.00E-01
regulation of phosphate metabolic process	14	BMP4, EGFR, CAV1, CSF1, SPHK1, TRIB3, PDCD4, SPRY4, IL11, THY1, CDKN1C, SPRED1, GADD45A, VLDLR	8.00E-03	3.20E-01
regulation of phosphorus metabolic process	14	MP4, EGFR, CAV1, CSF1, SPHK1, TRIB3, PDCD4, SPRY4, IL11, THY1, CDKN1C, SPRED1, GADD45A, VLDLR	8.00E-03	3.20E-01
regulation of protein kinase activity	10	CAV1, CSF1, SPHK1, TRIB3, SPRED1, GADD45A, PDCD4, SPRY4, VLDLR, THY1	1.30E-02	4.10E-01
regulation of kinase activity	10	CAV1, CSF1, SPHK1, TRIB3, SPRED1, GADD45A, PDCD4, SPRY4, VLDLR, THY1	1.60E-02	4.30E-01
regulation of transferase activity	10	CAV1, CSF1, SPHK1, TRIB3, SPRED1, GADD45A, PDCD4, SPRY4, VLDLR, THY1	1.90E-02	4.80E-01
regulation of MAP kinase activity	5	CAV1, TRIB3, SPRED1, PDCD4, SPRY4	8.90E-02	7.60E-01

Annotation Cluster 5	Enrichment Score: 1.89			
GO Term	**Count**	**Genes**	**P_Value**	**Benjamini**
mammary gland morphogenesis	7	BMP4, WNT5A, DDR1, CAV1, CSF1, AREG, ETV4	4.40E-05	2.40E-02
developmental growth involved in morphogenesis	4	BMP4, WNT5A, CSF1, AREG	3.70E-03	2.20E-01
mammary gland epithelial cell proliferation	3	WNT5A, CEBPB, AREG	1.30E-02	4.10E-01
epithelial cell proliferation	4	BMP4, WNT5A, CEBPB, AREG	1.50E-02	4.20E-01
developmental growth	6	BMP4, WNT5A, CSF1, GJA1, HBEGF, AREG	5.10E-02	6.40E-01
growth	8	BMP4, WNT5A, INHBA, CSF1, GJA1, HBEGF, AREG, EMP1	9.60E-02	7.60E-01
cell proliferation	7	BMP4, WNT5A, SATB1, KLK8, CEBPB, AREG, PES1	3.80E-01	9.60E-01

Annotation Cluster 6	Enrichment Score: 1.83			
GO Term	**Count**	**Genes**	**P_Value**	**Benjamini**
negative regulation of multicellular organismal process	8	INHBA, BBS2, KLK8, THBD, SOCS2, ADORA2B, ANXA5, ANXA2	4.00E-03	2.20E-01
negative regulation of coagulation	3	THBD, ANXA5, ANXA2	1.30E-02	4.10E-01
regulation of coagulation	3	THBD, ANXA5, ANXA2	6.00E-02	6.80E-01

Annotation Cluster 7	Enrichment Score: 1.68			
GO Term	**Count**	**Genes**	**P_Value**	**Benjamini**
collagen metabolic process	4	MMP10, ID1, MMP3, MMP13	1.10E-02	3.70E-01
multicellular organismal macromolecule metabolic process	4	MMP10, ID1, MMP3, MMP13	1.20E-02	4.00E-01
multicellular organismal metabolic process	4	MMP10, ID1, MMP3, MMP13	1.50E-02	4.20E-01
collagen catabolic process	3	MMP10, MMP3, MMP13	4.50E-02	6.40E-01
multicellular organismal catabolic process	3	MMP10, MMP3, MMP13	5.00E-02	6.50E-01

Annotation Cluster 8	Enrichment Score: 1.6			
GO Term	**Count**	**Genes**	**P_Value**	**Benjamini**
membrane organization	13	CLN3, CAV1, ITGA3, PMAIP1, ELMO1, SYP, DAB2, SH3KBP1, CAP1, BIN1, STEAP2, UPK2, VLDLR	9.00E-03	3.40E-01
membrane invagination	10	SYP, CLN3, DAB2, CAV1, SH3KBP1, CAP1, STEAP2, BIN1, VLDLR, ELMO1	1.40E-02	4.10E-01
endocytosis	10	SYP, CLN3, DAB2, CAV1, SH3KBP1, CAP1, STEAP2, BIN1, VLDLR, ELMO1	1.40E-02	4.10E-01
vesicle-mediated transport	13	CLN3, CAV1, AP1M2, SYT4, ELMO1, SYP, DAB2, TRIM36, SH3KBP1, CAP1, BIN1, STEAP2, VLDLR	2.30E-01	8.90E-01

Annotation Cluster 9	Enrichment Score: 1.57			
GO Term	**Count**	**Genes**	**P_Value**	**Benjamini**
prostate gland epithelium morphogenesis	4	BMP4, RARG, CD44, TNC	1.50E-02	4.20E-01
prostate gland morphogenesis	4	BMP4, RARG, CD44, TNC	1.60E-02	4.30E-01
urogenital system development	8	MP4, WNT5A, LOC100048295, RARG, CD44, TNC, GREM1, BCL2L11	2.80E-02	5.50E-01
prostate gland development	4	BMP4, RARG, CD44, TNC	4.30E-02	6.30E-01
reproductive structure development	7	BMP4, WNT5A, LOC100048295, RARG, CD44, TNC, BCL2L11	4.70E-02	6.40E-01

Annotation Cluster 10	Enrichment Score: 1.46			
GO Term	**Count**	**Genes**	**P_Value**	**Benjamini**
in utero embryonic development	12	EGFR, VCAM1, DAB2, WNT7B, CEBPB, DNMT3L, GJA1, HBEGF, PLCD1, CAPN2, TPM1, BCL2L11	2.00E-02	4.80E-01
chordate embryonic development	15	BMP4, EGFR, CEBPB, LMO4, GJA1, CAPN2, TPM1, BCL2L11, VCAM1, DAB2, WNT7B, DNMT3L, HBEGF, PLCD1, TCF15	4.50E-02	6.40E-01
embryonic development ending in birth or egg hatching	15	BMP4, EGFR, CEBPB, LMO4, GJA1, CAPN2, TPM1, BCL2L11, VCAM1, DAB2, WNT7B, DNMT3L, HBEGF, PLCD1, TCF15	4.80E-02	6.50E-01

Annotation Cluster 11	Enrichment Score: 1.43			
GO Term	**Count**	**Genes**	**P_Value**	**Benjamini**
reproductive developmental process	14	WNT5A, BMP4, RARG, TDRD7, CSF1, TNC, BCL2L11, ZFP37, TAF7L, BBS2, LOC100048295, CD44, DDX25, AREG	2.70E-03	1.70E-01
reproductive cellular process	10	BMP4, BBS2, LOC100048295, TRIM36, TDRD7, DDX25, TNC, PIWIL2, TAF7L, ZFP37	8.40E-03	3.30E-01
germ cell development	7	BMP4, BBS2, LOC100048295, TDRD7, DDX25, TAF7L, ZFP37	1.60E-02	4.30E-01
sexual reproduction	14	BMP4, NANOS3, TDRD7, ITGA3, BCL2L11, ZFP37, TAF7L, BBS2, TRIM36, LOC100048295, TCFL5, DDX25, OVOL1, PIWIL2	4.90E-02	6.50E-01
multicellular organism reproduction	14	BMP4, NANOS3, CAV1, SOCS2, TDRD7, BCL2L11, ZFP37, TAF7L, BBS2, LOC100048295, TCFL5, DDX25, OVOL1, PIWIL2	6.90E-02	7.10E-01
reproductive process in a multicellular organism	14	BMP4, NANOS3, CAV1, SOCS2, TDRD7, BCL2L11, ZFP37, TAF7L, BBS2, LOC100048295, TCFL5, DDX25, OVOL1, PIWIL2	6.90E-02	7.10E-01
gamete generation	12	BMP4, NANOS3, BBS2, LOC100048295, TCFL5, TDRD7, DDX25, OVOL1, PIWIL2, BCL2L11, TAF7L, ZFP37	7.10E-02	7.20E-01
male gamete generation	9	NANOS3, BBS2, TCFL5, DDX25, OVOL1, PIWIL2, BCL2L11, TAF7L, ZFP37	1.40E-01	8.00E-01
spermatogenesis	9	NANOS3, BBS2, TCFL5, DDX25, OVOL1, PIWIL2, BCL2L11, TAF7L, ZFP37	1.40E-01	8.00E-01

Annotation Cluster 12	Enrichment Score: 1.42			
GO Term	**Count**	**Genes**	**P_Value**	**Benjamini**
regulation of epithelial cell proliferation	7	BMP4, EGFR, WNT5A, FGF7, SERPINF1, FOXP1, ETV4	1.60E-03	1.50E-01
regulation of morphogenesis of a branching structure	4	BMP4, WNT5A, FGF7, ETV4	1.30E-02	4.00E-01
respiratory system development	6	BMP4, WNT5A, FGF7, RARG, ID1, FOXP1	1.00E-01	7.60E-01
lung development	5	BMP4, WNT5A, FGF7, ID1, FOXP1	1.80E-01	8.50E-01
respiratory tube development	5	BMP4, WNT5A, FGF7, ID1, FOXP1	1.90E-01	8.60E-01

EXTENDED EXPERIMENTAL PROCEDURES

RNA isolation and qRT-PCR analysis

Total RNA was isolated using the RNeasy Mini Kit (QIAGEN) and was treated with DNase to remove genomic DNA contamination. One microgram of total RNA was reverse-transcribed with SuperScript III Reverse Transcriptase Kit (Invitrogen) and oligo(dT) primer (Invitrogen) according to the manufacturer's instructions. Quantitative polymerase chain reaction (PCR) reactions were set up in duplicate with the Power SYBR Green Master Mix (Takara) and analyzed with the Roche LightCycler 5480 (Roche). The primers for qRT-PCR used were as follows: *Oct4* sense 5'-GATGCTGTGAGCCAAGGCAAG-3', *Oct4* antisense 5'-GGCTCCTGATCAACAGCATCAC-3'; *Nanog* sense 5'-CTTTCACCTATTAAGGTGCTTGC-3', *Nanog* antisense 5'-TGGCATCGGTTCATCATGGTAC-3'; *Sox2* sense 5'-CATGAGAGCAAGTACTGGCAAG-3', *Sox2* antisense 5'- CCAACGATATCAACCTGCATGG-3'; *Rex1* sense 5'-TCCATGGCATAGTTCCAACAG-3', *Rex1* antisense 5'-TAACTGATTTTCTGCCGTATGC-3'; *Esrrb* sense 5'-CAGGCAAGGATGACAGACG-3', *Esrrb* antisense 5'-GAGACAGCACGAAGGACTGC-3'; *Klf2* sense 5'-TCGAGGCTAGATGCCTTGTGA-3', *Klf2* antisense 5'-AAACGAAGCAGGCGGCAGA-3'; *Klf4* sense 5'-AGGAGCCCAAGCCAAAGAGG-3', *Klf4* antisense 5'-CGCAGGTGTGCCTTGAGATG-3'; *Tcl1* sense 5'-TGGCCTCACTAGAACAAGAGG-3', *Tcl1* antisense 5'-CTCGGTCAAGGATGGAAGC-3'; *Tbx3* sense 5'-TTATTTCCAGGTCAGGAGATGGC-3', *Tbx3* antisense 5'-GGTCGTTTGAACCAAGTCCCTC-3'; *Prdm14* sense 5'-ACAGCCAAGCAATTTGCACTAC-3', *Prdm14* antisense 5'-TTACCTGGCATTTTCATTGCTC-3'; *Dnmt3a* sense 5'-GACTCGCGTGCAATAACCTTAG -3', *Dnmt3a* antisense 5'-GGTCACTTTCCCTCACTCTGG -3', *Dnmt3b* sense 5'-CTCGCAAGGTGTGGGCTTTTGTAAC-3', and *Dnmt3b* antisense 5'-CTGGGCATCTGTCATCTTTGCACC-3', *Dnmt3l* sense 5'-CCAGGGCAGATTTCTTCCTAAGGTC-3', and *Dnmt3l* antisense 5'-TGAGCTGCACAGAGGCATCC-3', *T*/*Brachyury* sense 5'-ATCAGAGTCCTTTGCTAGGTAG-3', and *T*/*Brachyury* antisense 5'-GTTACAATCTTCTGGCTATGC-3', *Fgf5* sense 5'-AAAACCTGGTGCACCCTAGAAG-3', and *Fgf5* antisense 5'-GCTAAACCGTCTGTGGTTTCTG-3', *Fgfr1* sense 5'-CTACCAACCCTGTCCCCAGT-3', and *Fgfr1* antisense 5'-CACAGGAAGGCCTCAGTCAG-3', *Fgfr2* sense 5'-CAAGGAGCTCTTGTTCTTCAGG-3', and *Fgfr2* antisense 5'-TAACACTGCCGTTTATGTGTGG-3'.

Bisulfite genomic sequencing

To differentiate between methylated and unmethylated CG dinucleotides, genomic DNA was treated with sodium bisulfite to convert all unmethylated cytosine residues into uracil residues using the EpiTect Bisulfite Kit (QIAGEN) according to the manufacturer's protocol. Briefly, purified genomic DNA (0.5–1 μg) was denatured at 99℃ and then incubated at 60℃. After desulfonation, neutralization, and desalting, the modified DNA was diluted in 20 μl of distilled water. Subsequently, bisulfite PCR (BS-PCR) amplification was carried out using 1- to 2-μl aliquots of modified DNA for each PCR reaction. The primers used for BS-PCR were as follows: *Oct4*-DE sense 5'- TTTAGGTTTTAGAGGTTGGTTTTG-3', *Oct4*-DE antisense 5'- CCAATTTCTATACATTCATTATAAAACAAT-3'; *Oct4*-PE first sense 5'- GGTTTTTTGAGGTTGTGTGATTTAT-3', *Oct4*-PE first antisense 5'- CTCCCCTAAAAACAACTTCCTACTC-3'; *Oct4*-PE second sense 5'- GGGATTTTTAGATTGGGTTTAGAAAA-3', *Oct4*-PE second antisense 5'- CTCCTCAAAAACAAAACCTCAAATA-3', *Oct4*-PP first sense 5'- TTTGTTTTTTTATTTATTTAGGGGG-3', *Oct4*-PP first antisense 5'- ATCCCCAATACCTCTAAACCTAATC-3'; *Oct4*-PP second sense 5'- GGGTTAGAGGTTAAGGTTAGAGGG-3', *Oct4*-PP second antisense 5'- CCCCCACCTAATAAAAATAAAAAAA-3'; *Nanog* first sense 5'- TTTGTAGGTGGGATTAATTGTGAA-3', *Nanog* first antisense 5'- AAAAAATTTTAAACAACAACCAAAAA-3', *Nanog* second sense 5'- TTTGTAGGTTGGGATTAATTGTGAA-3', *Nanog* second antisense 5'- AAAAAAACAAAACACCAACCAAAT-3'; *Stella* first sense 5'- TTTTTTTATTTTGTGATTAGGGTTG-3', *Stella* first antisense 5'- CTTCACCTAAACTACACCTTTAAAC-3'; *Stella* second sense 5'- TTTGTTTTAGTTTTTTTGGAATTGG-3', *Stella* second antisense 5'- CTTCACCTAAACTACACCTTTAAAC-3', *Dppa5* first sense 5'- GGTTTGTTTTAGTTTTTTTAGGGGTATA-3', *Dppa5* first antisense 5'- CCACAACTCCAAATTCAAAAAAT-3'; *Dppa5* second sense 5'- TTTAGTTTTTTTAGGGGTATAGTTTG-3', *Dppa5* second antisense 5'- CACAACTCCAAATTCAAAAAATTTTA-3', *LINE* sense 5'- TCAAACACTATATTACTTTAACAATTCCCA-3', *LINE* antisense 5'-CCCCCACCTAATAAAAATAAAAAAA-3'; *IAP* first sense 5'- TTGATAGTTGTGTTTTAAGTGGTAAATAAA-3', *IAP* first antisense 5'- AAAACACCACAAACCAAAATCTTCTAC-3', *IAP* second sense 5'- TTGTGTTTTAAGTGGTAAATAAATAATTTG-3', and *IAP* second antisense 5'- CAAAAAAAACACACAAACCAAAAT -3'.

Briefly, the amplified products were verified by electrophoresis on a 1% agarose gel. The desired PCR products

were used for subcloning using the TA cloning vector (pGEM-T Easy Vector, Promega). The reconstructed plasmids were purified, and individual clones were sequenced (Solgent Corporation).

Luciferase Assay

For quantifying the relative Oct4 enhancer activity, an *Oct4* upstream sequence ~2 kb (Oct4-2kb) containing distal enhancer (DE) and proximal enhancer (PE), and either ΔDE, or ΔPE was cloned into pGL3 basic vector (Promega, USA). The Oct4 upstream sequence (~2 kb) containing distal enhancer (DE) and proximal enhancer (PE) was derived from pOct4-GFP plasmid which was digested and ligated to the *Kpn*I/*Bgl*II sites of the pGL3 basic vector. The pGL3-Oct4/ΔDE or pGL3-Oct4/ΔPE reporter constructs were prepared in two steps. First, a fragment of DE 5′ or PE 5′ was PCR-amplified from pOct4-GFP plasmid using specific primer pairs, digested with *Kpn*I and *Mlu*I restriction enzymes and cloned into pGL3 basic vector to obtain pGL3-DE 5′ or pGL3-PE 5′ plasmids, respectively. Subsequently, a fragment of DE 3′ or PE 3′ was PCR amplified from pOct4-GFP plasmid using primer pairs carrying *Mlu*I and *Bgl*II restriction sites, respectively. The amplified fragment was digested and ligated to *Mlu*I/*Bgl*II sites of either pGL3-DE 5′ or pGL3-PE 5′ plasmids. Luciferase assays were performed by using the Dual-Luciferase Reporter Assay System (Promega, USA). For the reporter assay of Oct4 enhancer activity, the pGL3-Oct4-2 kb vectors, pGL3-Oct4-ΔDE, or pGL3-Oct4/ΔPE vectors (for firefly luciferase activity) and pRL-TK vector (for *Renilla* luciferase activity) were transfected individually into respectively cells. After 48 h of transfection, growth medium was removed and cells were rinsed in 1× PBS. Subsequently, the cells were lysed using 1× passive lysis buffer (PLB) and incubated for 10 min at room temperature with shaking. The cell lysate was then transferred to a 1.5 ml new tube and centrifuged at 10, 000 rpm for 5 min at 4℃. Ten microliters of the supernatant was transferred to a 96-well plate and then analyzed for luciferase expression by luminometry. Each experiment was performed in triplicate and the values obtained were recorded as relative light units (RLU).

Chromatin Immunoprecipitation (ChIP)

Cultured cells were cross-linked with 1% formaldehyde and then washed with PBS containing protease inhibitors. Genomic DNA extraction and chromatin immunoprecipitation (ChIP) were performed using SimpleChIP Plus Enzymatic Chromatin IP kit (Cell Signaling) according to the manufacturer's instructions. The antibodies used were Nanog (Bethyl, A300-397A), H3K27ac (Abcam, ab729), H3K27me3 (Cell signaling,

#9733), and H3K9me3 (Abcam, ab8898). The primers used for ChIP-qPCR were as follows: *Oct4*-DE sense 5'-GGCTGCAGGCATACTTGAAC-3', *Oct4*-DE antisense 5'-AGGGCAGAGCTATCATGCAC-3'; *Oct4*-PE sense 5'-TCCTCCTAATCCCGTCTCCT-3', and *Oct4*-PE antisense 5'-GGACTCCGGTGTTCATCCT-3'.

Microarray-based analysis

Total RNA was isolated with the RNeasy Mini Kit (Qiagen) and digested with DNase I (RNase-free DNase, Qiagen) according to the manufacturer's instructions. Total RNA was amplified, biotinylated, and purified using the Ambion Illumina RNA amplification kit (Ambion) according to the manufacturer's instructions. Labeled cRNA samples (750 ng) were hybridized to each MouseRef-8 v2 Expression BeadChip. Signal detection was performed with Amersham Fluorolink Streptavidin-Cy3 (GE Healthcare Bio-Science) according to the bead array manual. Arrays were scanned with an Illumina Bead Array Reader according to the manufacturer's instructions.

Raw data were extracted using the software provided by the manufacturer (Illumina GenomeStudio v2011.1, Gene Expression Module v1.9.0). Array data were filtered by detection p-value < 0.05 in at least 50% samples. Selected probe signal was log-transformed and normalized by the quantile method. Comparative analysis was performed using LPE test and fold-change. False discovery rate (FDR) was controlled by adjusting the p-value with the Benjamini-Hochberg algorithm. Hierarchical clustering was performed using complete linkage and Pearson distance as a measure of similarity.

Aggregation with normal embryo

The ESCs or EpiLCs were aggregated with denuded post-compacted eight-cell-stage embryos to obtain an aggregate chimera. Eight-cell embryos flushed from 2.5-dpc B6D2F1 female mice were cultured in microdrops of embryo culture medium under mineral oil. The clumps of ESCs or EpiLCs (4–10 cells) were selected and transferred into microdrops containing zona-free eight-cell embryos. Morula-stage embryos aggregated with ESCs were cultured overnight at 37 ℃ under 5% CO_2.

Accession Numbers

Microarray data for each gene are available at the Gene Expression Omibus under accession number GSE67031.