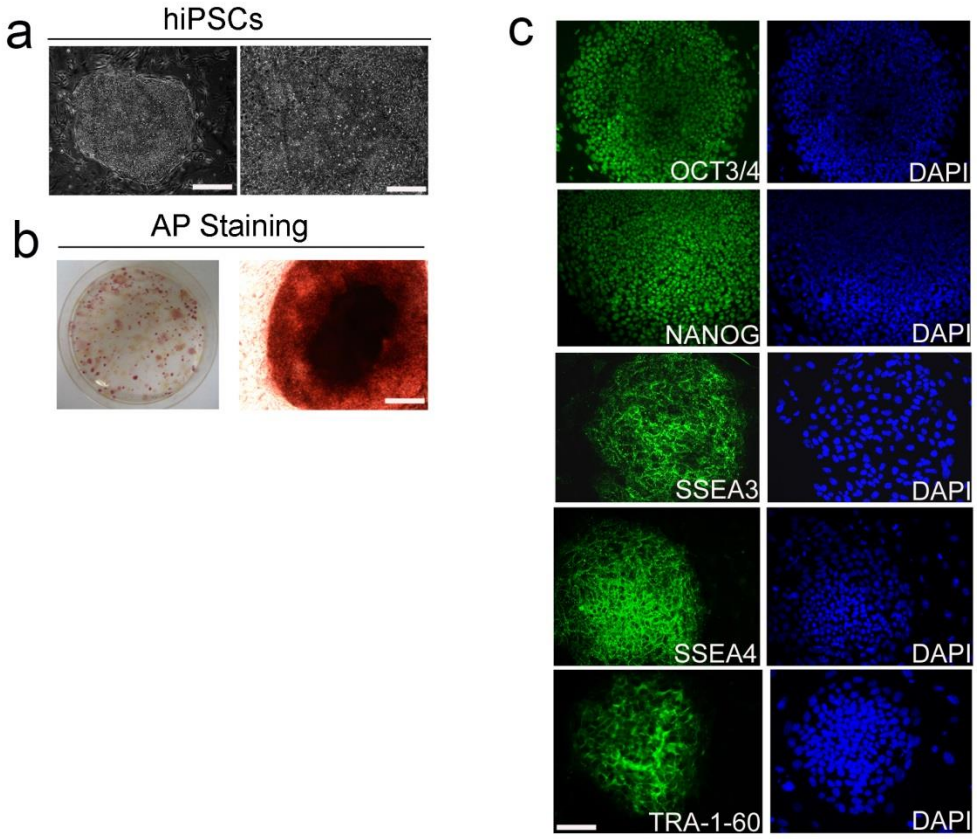
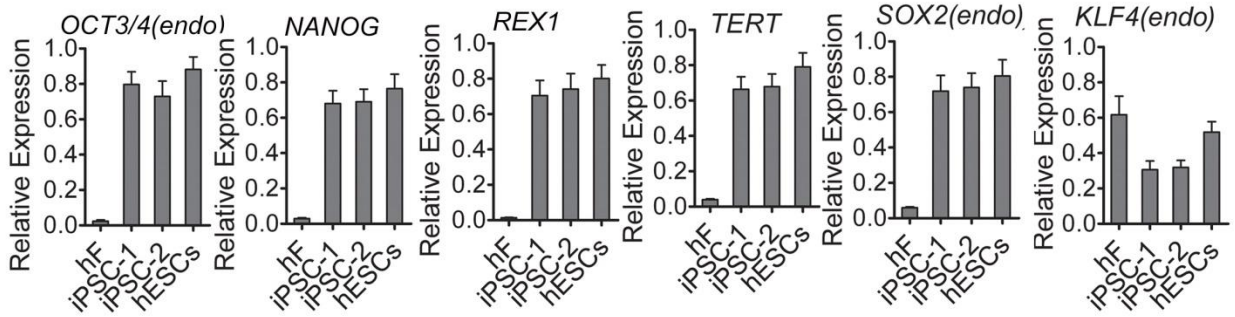
Supplementary Information

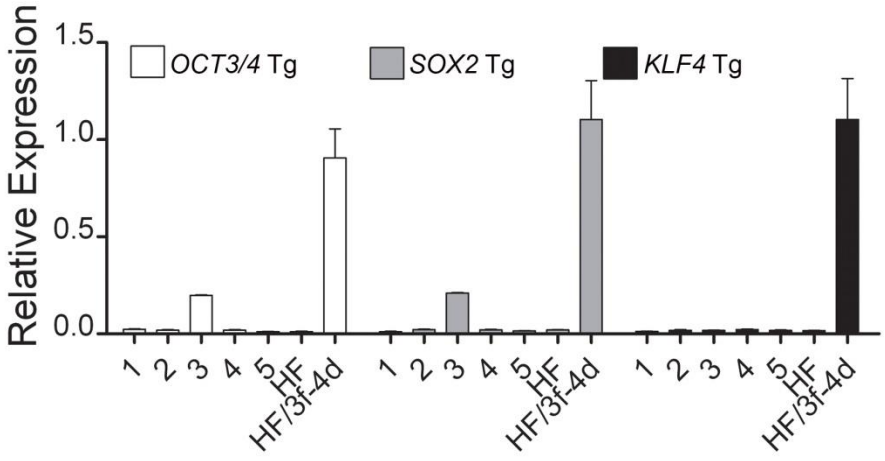
Supplementary Figures



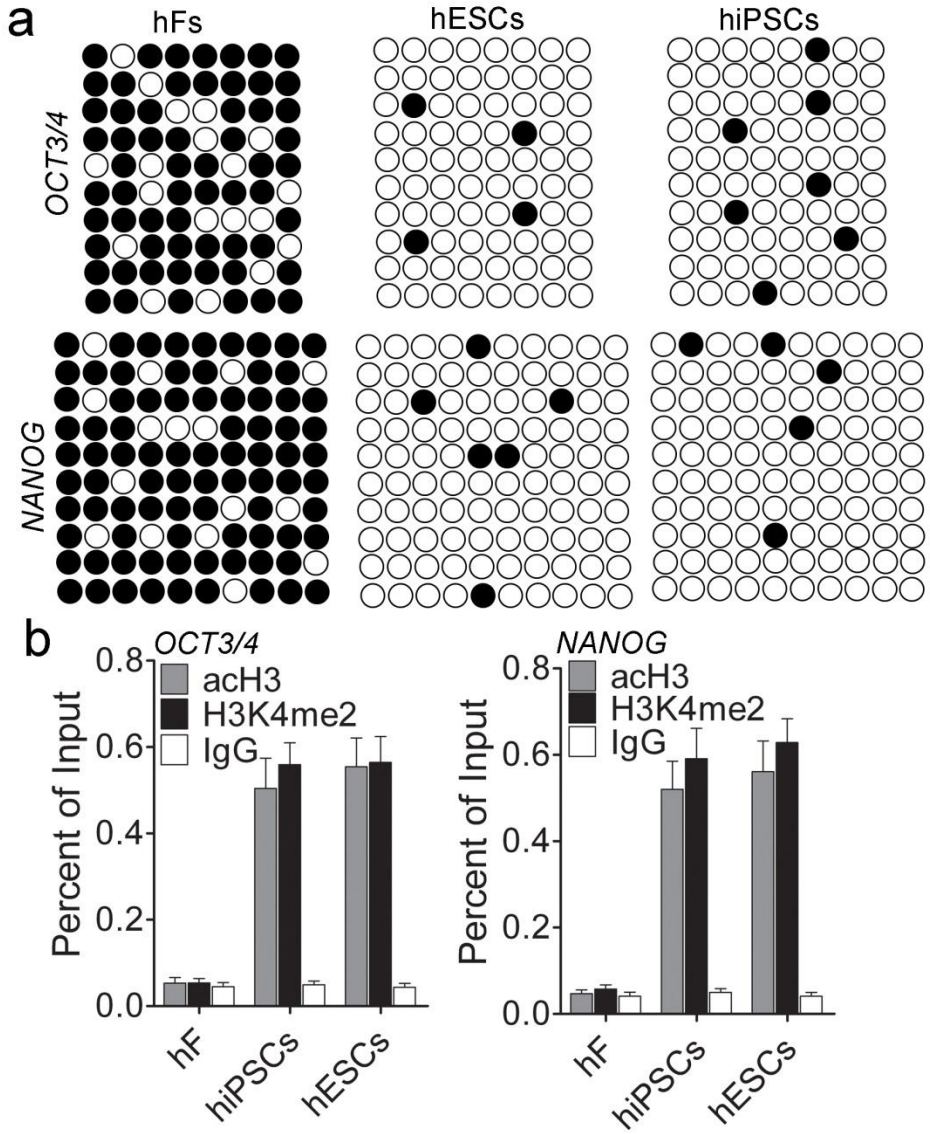
Supplementary Figure 1. Characterization of hiPSCs derived from primary human fibroblasts. a,b. Morphology of hiPSCs. hiPSCs exhibit hESC-like morphology in co-culture with mouse embryonic feeder fibroblasts (Scale bars: left panel, 200 μm; right panel, 50 μm) (a), AP staining of hiPSCs (Scale bars of left panel, 200 μm) (b). c. As shown via immunostaining, hiPSC clones express markers common to pluripotent cells, including OCT3/4, NANOG, SSEA3, SSEA4 and TRA-1-60. 4, 6-Diamidino-2-phenylindole (DAPI) staining indicates cell nuclear. Scale bar, 30 μm.



Supplementary Figure 2. Gene expression in hiPSCs is similar to hESCs.
qPCR assay for expression of *OCT3/4*(endo), *SOX2*(endo), *NANOG*, *TERT*, *KLF4*(endo) and *REX1* in hESCs, hiPSCs and parental fibroblasts (hFs). Individual PCR reactions were normalized against internal controls (β-actin). Data shown are mean ± SD of the expression from three different experiments.

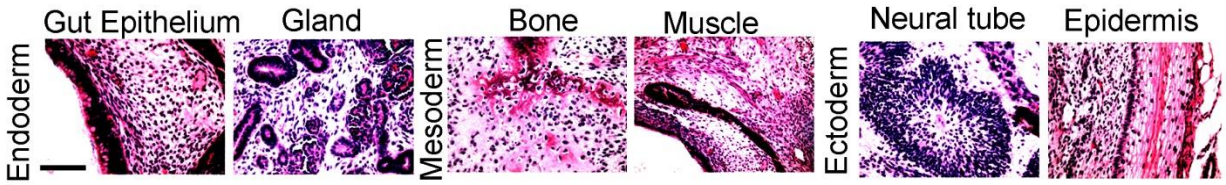


Supplementary Figure 3. qPCR analysis of retroviral transgene expression in the hiPSC clones. Transgene-specific PCR primers permit determination of the relative retrovirally expressed (transgene) genes (*OCT3/4*, *SOX2* and *KLF4*) via qPCR. Tg represents transgene. Five different hiPSC clones were tested. hF stands for human fibroblasts and hF/3f-4d stands for human fibroblasts 4 days after virally infection with OCT3/4, SOX2 and KLF4. Data shown are mean ± SD of the expression from three different experiments.

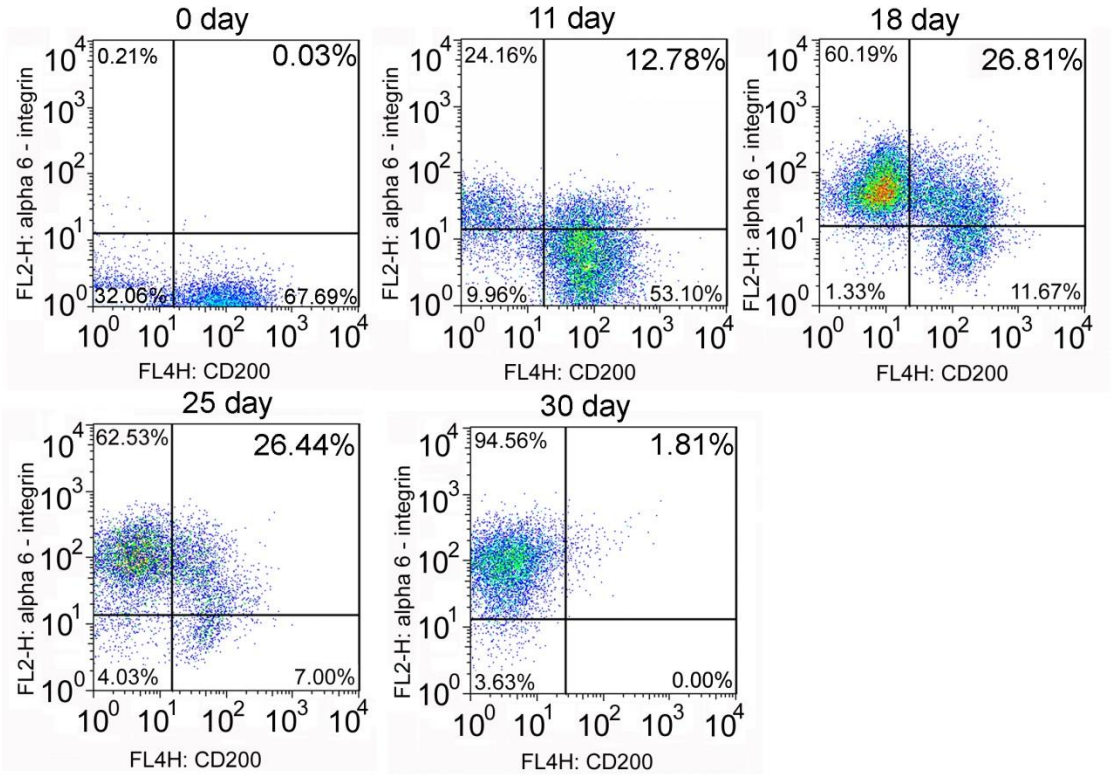


Supplementary Figure 4. Analysis of DNA methylation and histone modification in the *OCT3/4* and *NANOG* promoters in the hiPSCs, hESCs and hFs.

a. Bisulfite genomic sequencing of the promoter regions of *OCT3/4* and *NANOG* in 10 randomly selected hiPSC and hESC clones, as well as human fibroblasts (hFs). Open circles indicate unmethylated CpG dinucleotides, whereas closed circles indicate methylated CpGs. b. Chromatin immunoprecipitation was performed using antibodies against dimethylated histone H3K4 (H3K4me2) and H3 acetylation (acH3). *OCT3/4* and *NANOG* promoters showed enrichment for the active (H3K4me2 and acH3) mark in hiPSCs, similar to hESCs. In hFs, *OCT3/4* and *NANOG* promoters appeared in the inactive state. Data shown are mean ± SD of histone modification from three independent experiments.

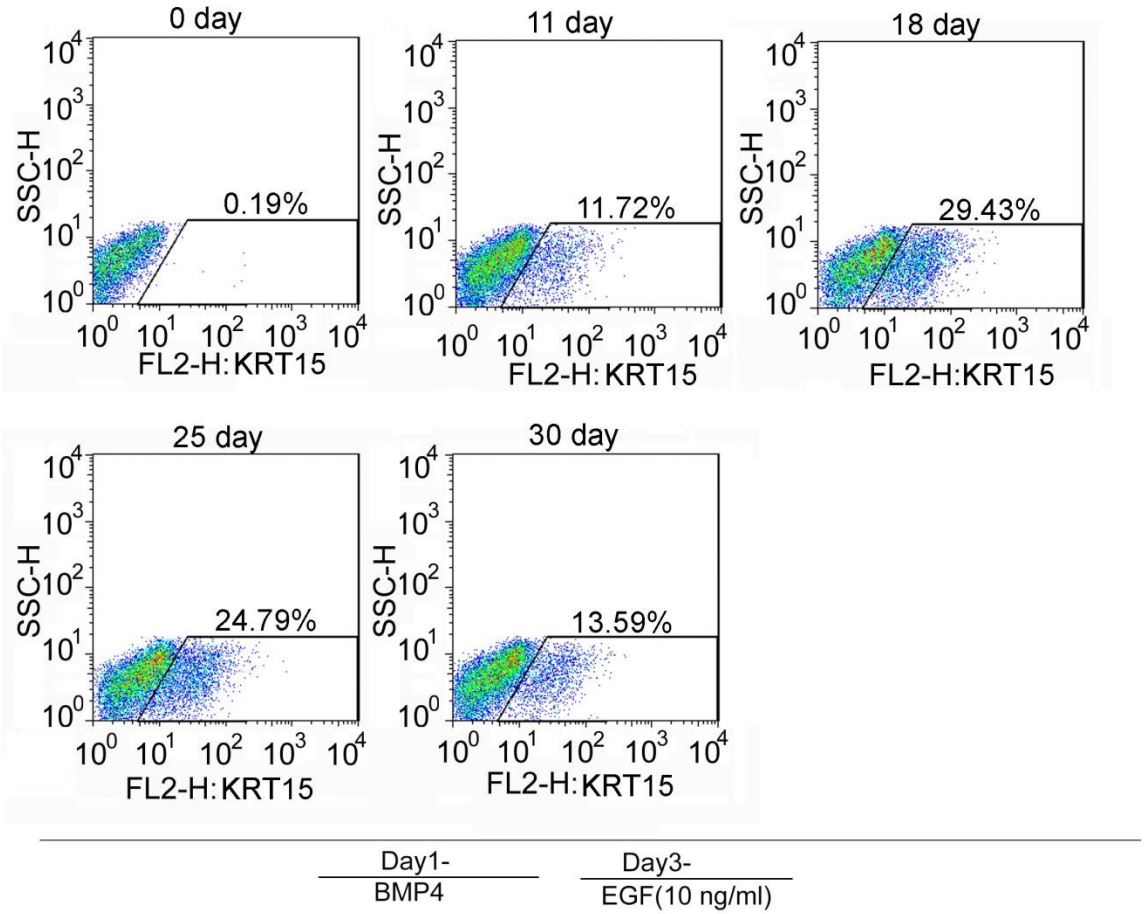


Supplementary Figure 5. Pluripotent characterization of hiPSCs.
hiPSCs were injected into immunodeficient mouse and generated well-differentiated teratoma-like masses containing all three embryonic germ layers (endoderm, mesoderm and ectoderm). Scale bar, 50 μm.

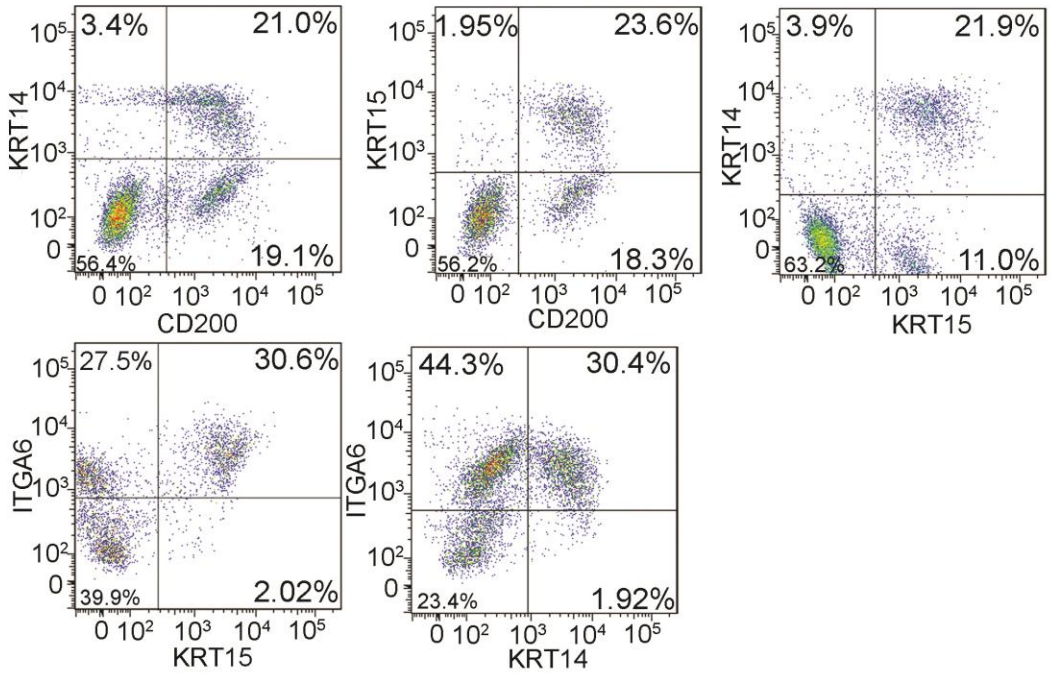


Supplementary Figure 6. Flow cytometric analysis of positive cells for CD200 and ITGA6 during epithelial cell differentiation from hiPSCs.

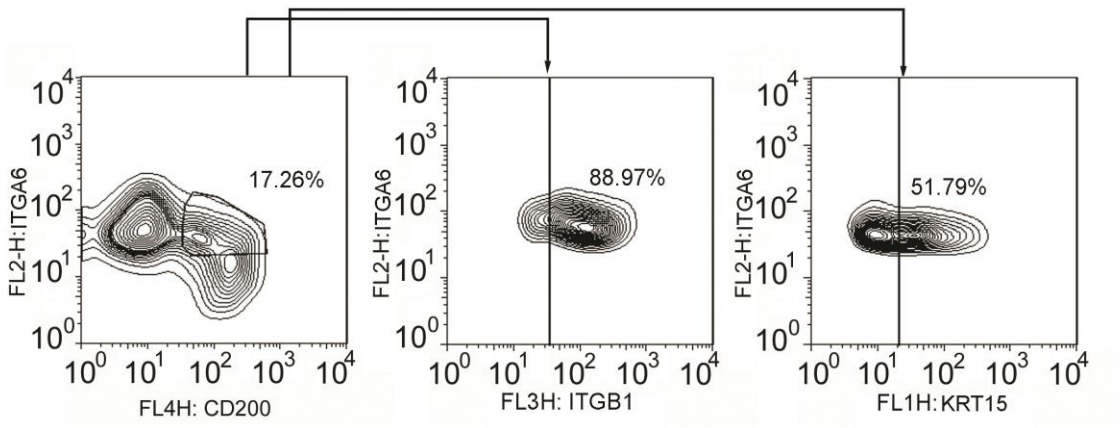
Flow cytometric analysis was performed using antibodies specific for CD200 and ITGA6. $CD200^{+}/ITGA6^{+}$ cell percentage was monitored at 0, 11, 18, 25 and 30 days respectively.



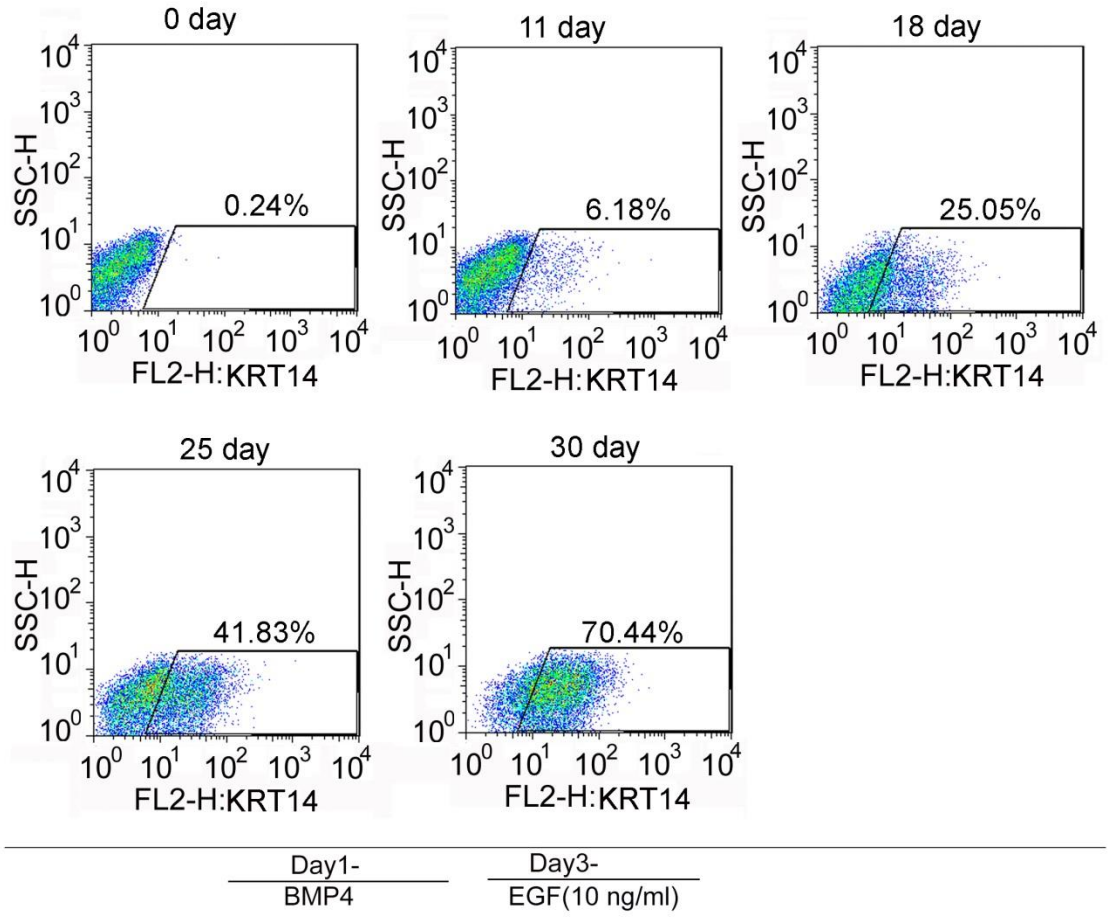
Supplementary Figure 7. Flow cytometric analysis of positive cells for KRT15 during epithelial cell differentiation from hiPSCs.
Flow cytometric analysis was performed using antibody specific for KRT15 and KRT15^{+} cell percentage was monitored at 0, 11, 18, 25 and 30 days respectively.



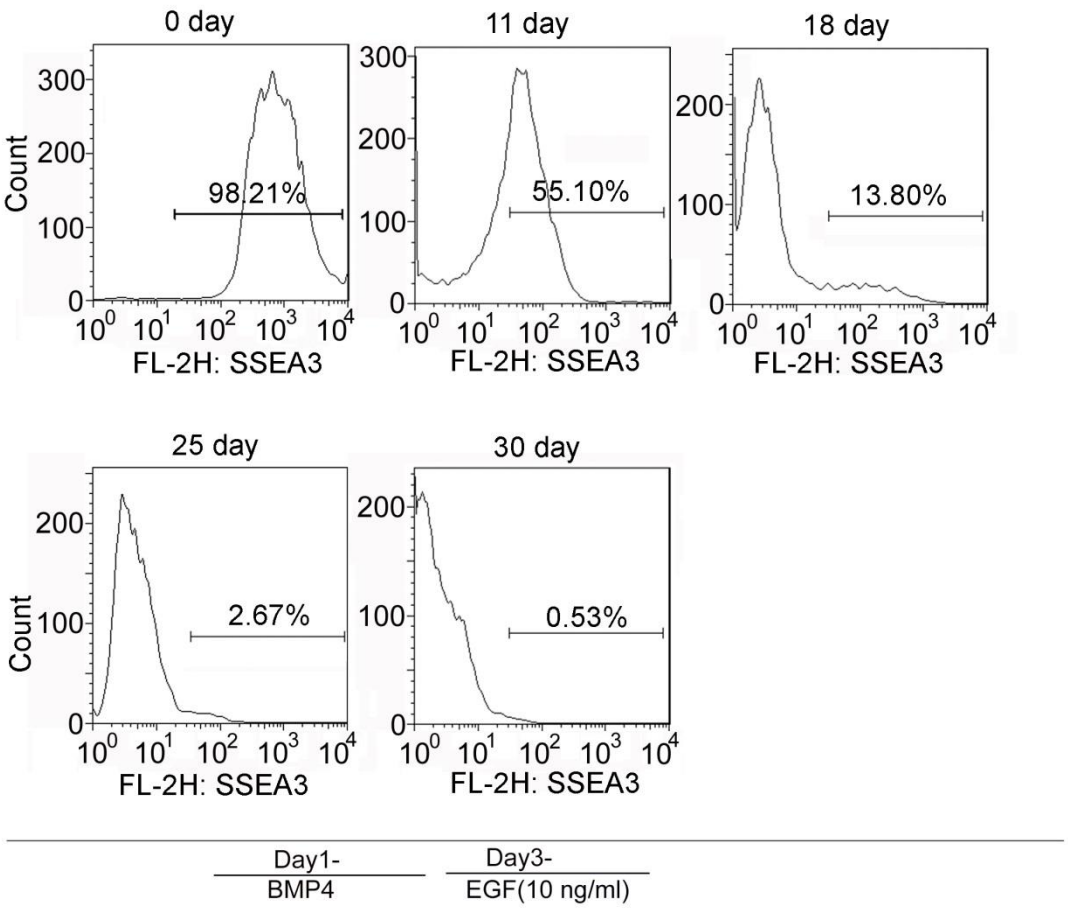
Supplementary Figure 8. Flow cytometric analysis of the percentage of $CD200^{+}/KRT15^{+}$, $CD200^{+}/KRT14^{+}$, $ITGA6^{+}/KRT15^{+}$, $ITGA6^{+}/KRT14^{+}$ and $KRT14^{+}/KRT15^{+}$ cell populations at day 18 druing the epithelial cell differentiation.



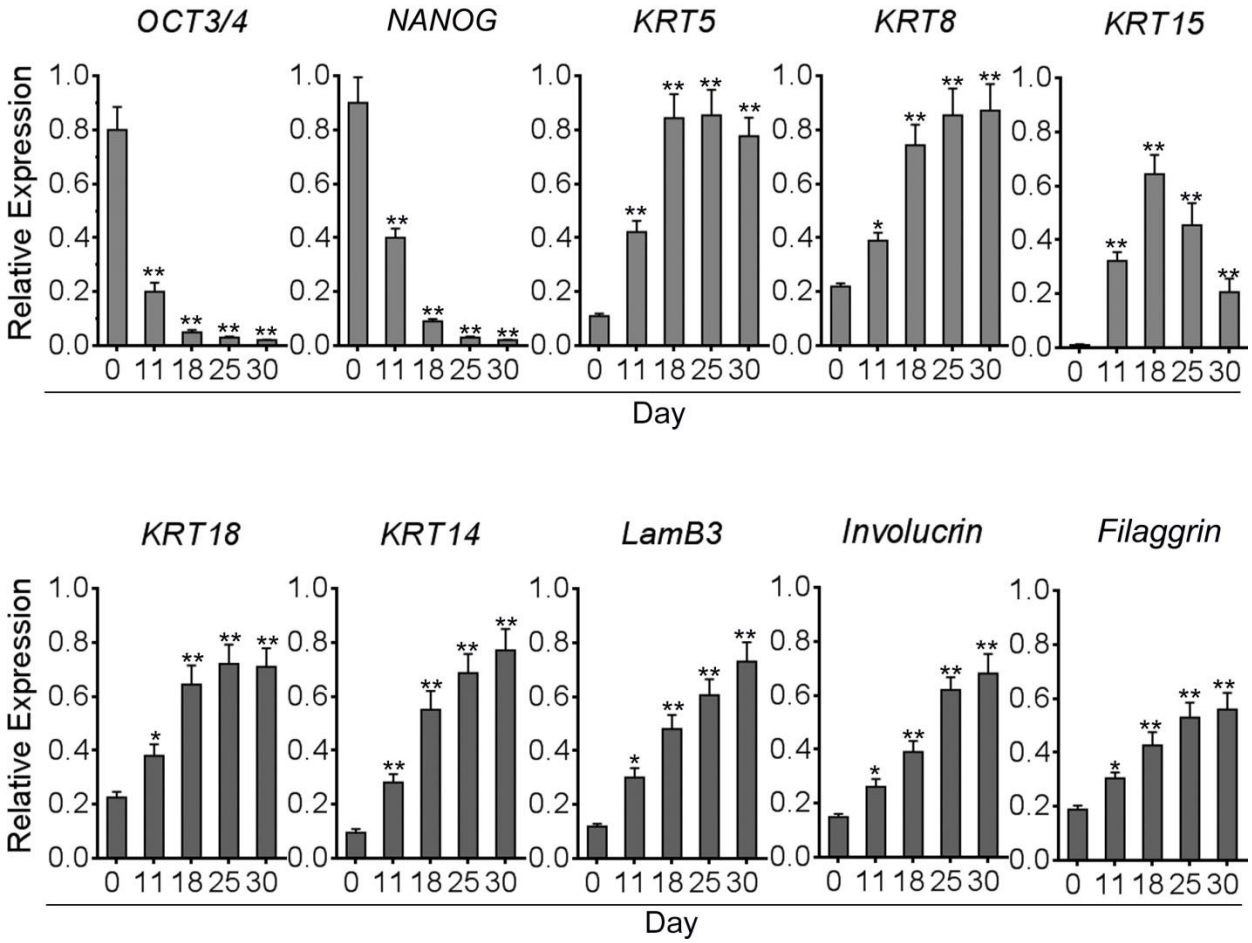
Supplementary Figure 9. Flow cytometric analysis of ITGB1$^+$ or KRT5$^+$ cell percentage in CD200$^+$/ITGA6$^+$ cells.
CD200$^+$/ITGA6$^+$ cell population was gated out. Almost all the cells expressed ITGB1 and only about 50% cells expressed KRT15.



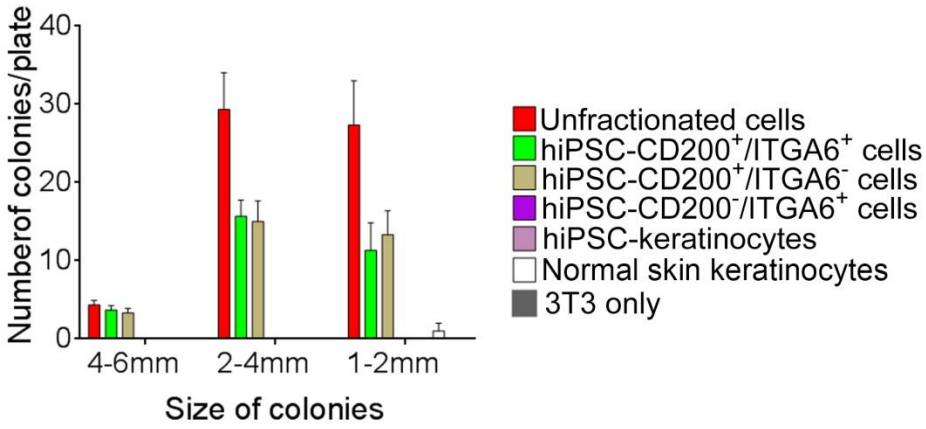
Supplementary Figure 10. Flow cytometric analysis of positive cells for KRT14 during epithelial cell differentiation from hiPSCs.
Flow cytometric analysis was performed using antibody against KRT14 and $KRT14^{+}$ cell percentage was monitored at 0, 11, 18, 25 and 30 days respectively.



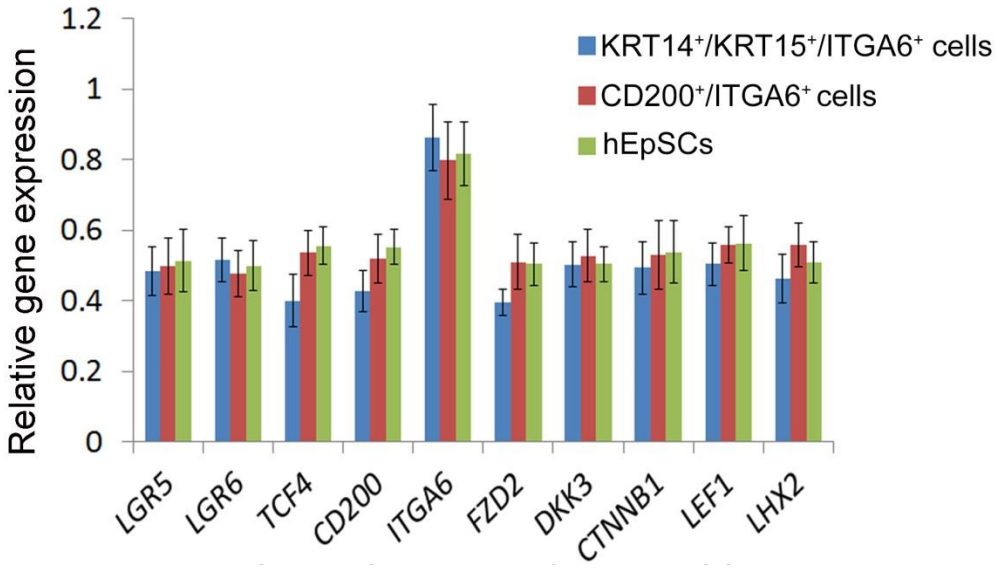
Supplementary Figure 11. Flow cytometric analysis of positive cells for SSEA3 during epithelial cell differentiation from hiPSCs.
Flow cytometric analysis was performed using antibody specific for SSEA3 and $SSEA3^{+}$ cell percentage was monitored at 0, 11, 18, 25 and 30 days respectively.



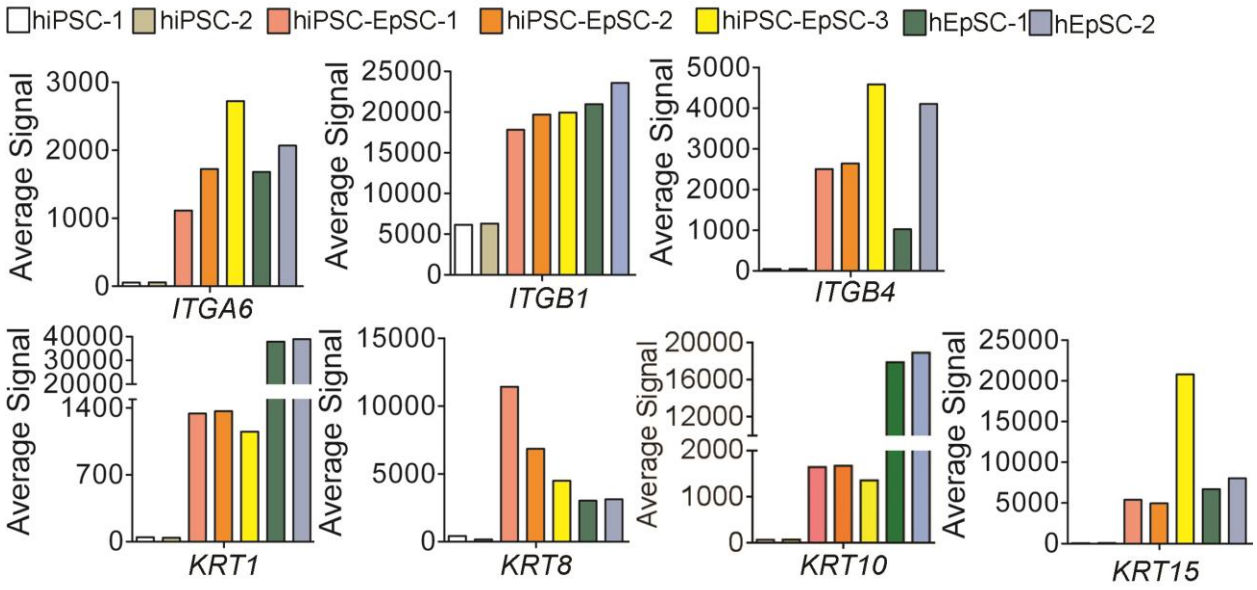
Supplementary Figure 12. qPCR analysis of *OCT3/4*, *NANOG*, *KRT5*, *KRT8*, *KRT14*, *KRT15*, *LamB3*, *Involucrin* and *Filaggrin* in hiPSC-derived cells at different stages of differentiation. Data shown are mean ± SD of gene expression from three independent experiments. * indicates $p<0.05$ and ** indicates $p<0.01$. One-Way ANOVA was used to calculate the p values. The expression of *OCT3/4* and *NANOG* decreased significantly at day 11, 18, 25 and 30 compared with the expression at day 0. Reversely, the expression of *KRT5*, *KRT8*, *KRT15*, *KRT18*, *KRT14*, *LamB3*, *involucrin* and *filaggrin* increased significantly at day 11, 18, 25 and 30 compared with the expression at day 0. The expression of *KRT15* decreased significantly at day 30 compared with the expression at day 18 ($P<0.01$). Student's Test was used to calculate the p value.



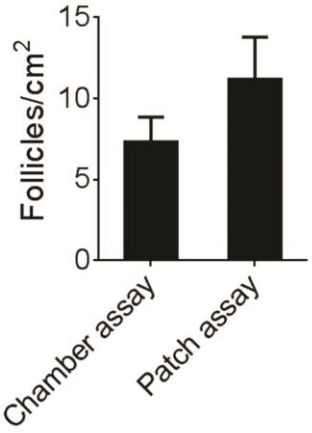
Supplementary Figure 13.Quantification of size and number of colonies from unfractionated cells, $CD200^{+}/ITGA6^{+}$ cells (hiPSC-$CD200^{+}/ITGA6^{+}$ cells), $CD200^{+}/ITGA6^{-}$ cells (hiPSC-$CD200^{+}/ITGA6^{-}$ cells) and $CD200^{-}/ITGA6^{+}$ cells (hiPSC-$CD200^{-}/ITGA6^{+}$cells) derived from hiPSCs at day 18 differentiation; mature keratinocytes derived from hiPSC (hiPSC-keratinocytes)and normal skin keratinocytes (passage 3). Data shown are mean ± SD of the colony numbers from three independent experiments. 3T3 feeder cells used as a negative control.



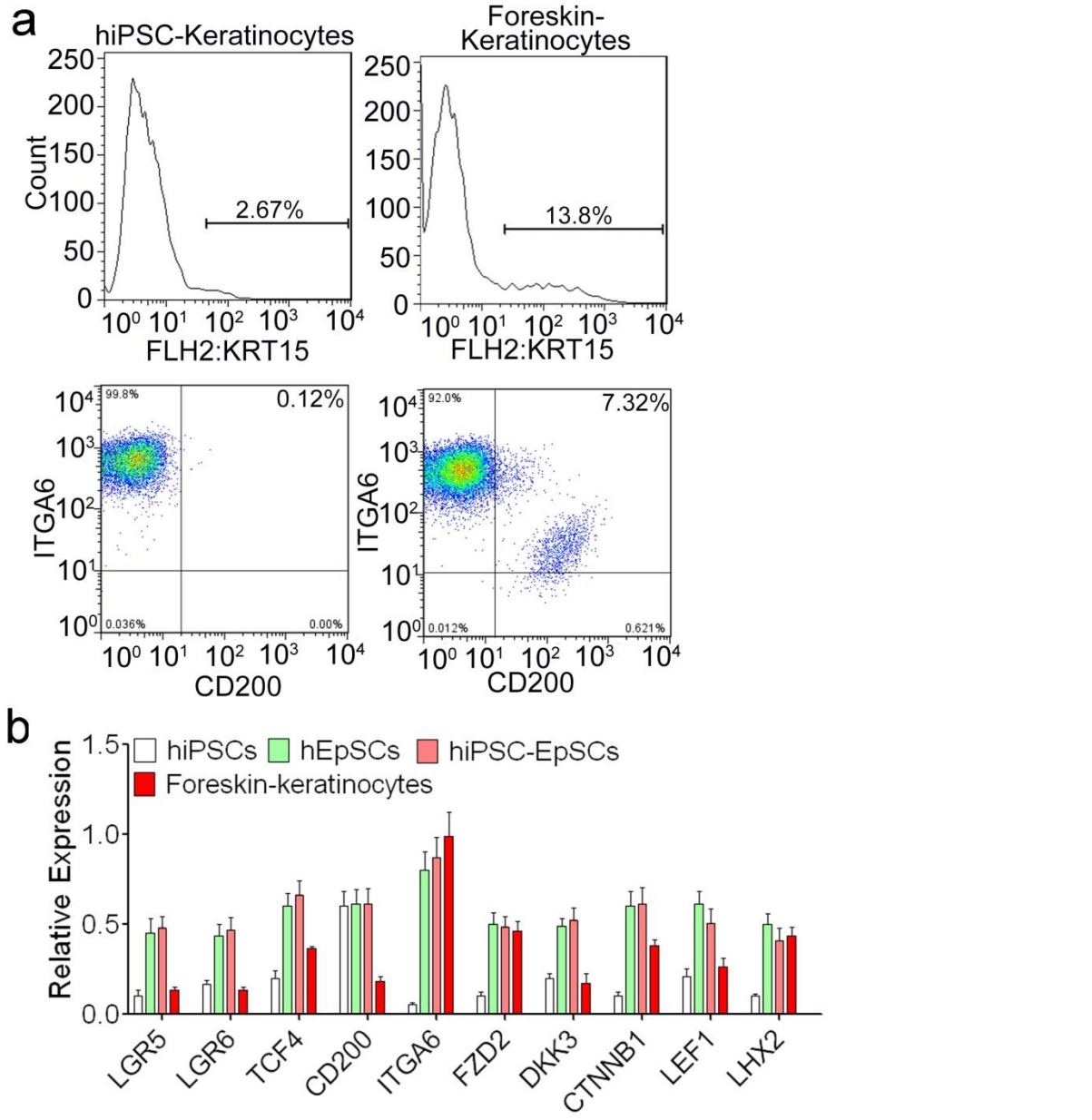
Supplementary Figure 14. qPCR analysis of known EpSC markers, including *LGR5*, *LGR6*, *CD200*, *KRT15*, *ITGA6*, *TCF4*, *FZD2*, *DKK3*, *CTNNB1*, *LEF1* and *LHX2* in KRT14^{+}/KRT15^{+}/ ITGA6^{+} cells compared with control CD200^{+}/ITGA6^{+} cells and hEpSCs. Data shown are mean ± SD of the expression from three independent experiments.



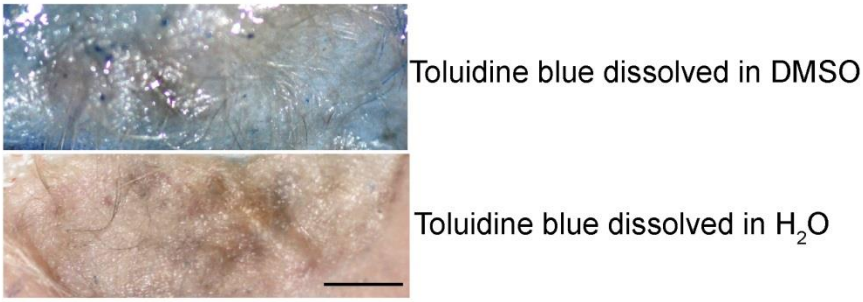
Supplementary Figure 15. Microarray analysis of the expression of *KRT1*, *KRT8*, *KRT10*, *KRT15*, *ITGA6*, *ITGB1* and *ITGB4* in hiPSCs, hiPSC-derived EpSCs and hEpSCs from hair follicles. *KRT1*, *KRT8*, *KRT10*, *KRT15*, *ITGA6*, *ITGB1* and *ITGB4* were selected and the expression data were shown from raw data of microarray.



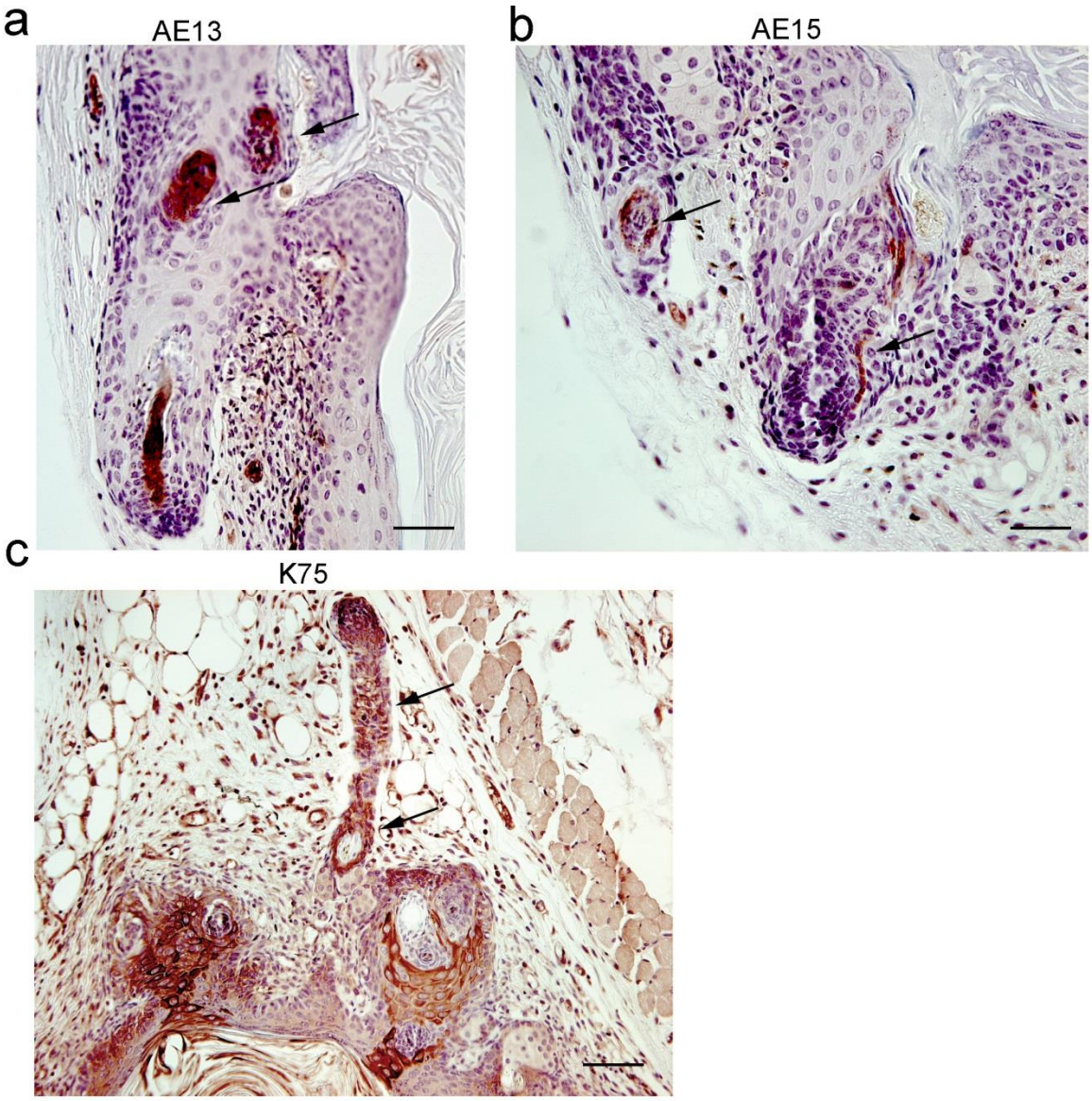
Supplementary Figure 16. Statistical analysis of the densities of hair follicles produced by patch assay and chamber assay using EpSCs derived from hiPSCs and neonatal mouse dermal fibroblasts. Data shown are mean ± SD of the hair follicle numbers from three independent experiments in the chamber assay. Data shown are mean ± SD of the hair follicle numbers from seven independent experiments in the patch assay.



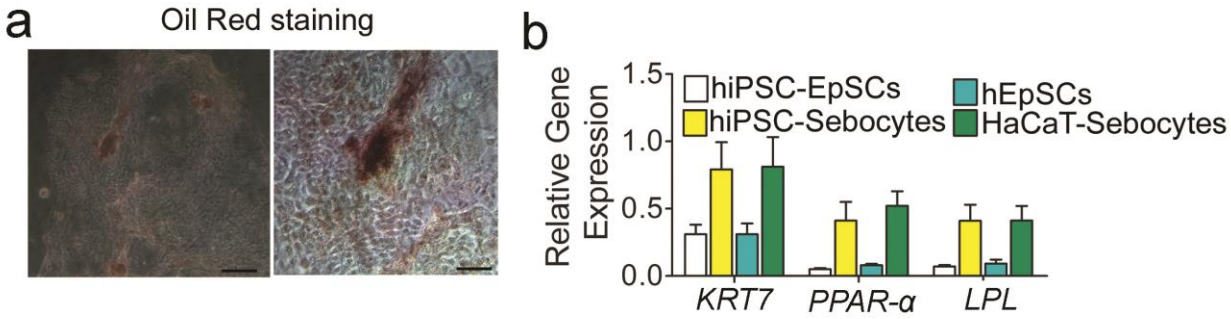
Supplementary Figure 17. a. Flow cytometric analysis of the percentage of $CD200^+$ and $KRT15^+$ cells in the neonatal foreskin-derived keratinocytes and hiPSC-derived keratinocytes. b. qPCR analysis of the expression of epithelial marekers among hiPSCs, hEpSCs, hiPSC-EpSCs and neonatal foreskin-derived keratinocytes (Foreskin-keratinocytes). Data shown are mean ± SD of the expression from three independent experiments.



Supplementary Figure 18. Skin permeability assay
Human-like skin in the chamber graft was treated with toluidine blue dissolved in DMSO (up panel) and H_2O (down panel) respectively. Human-like skin was impermeable to toluidine blue dissolved in H_2O. Toluidine blue dissolved DMSO permeated into skin and skin was dyed in blue. Scale bar indicates 2mm.

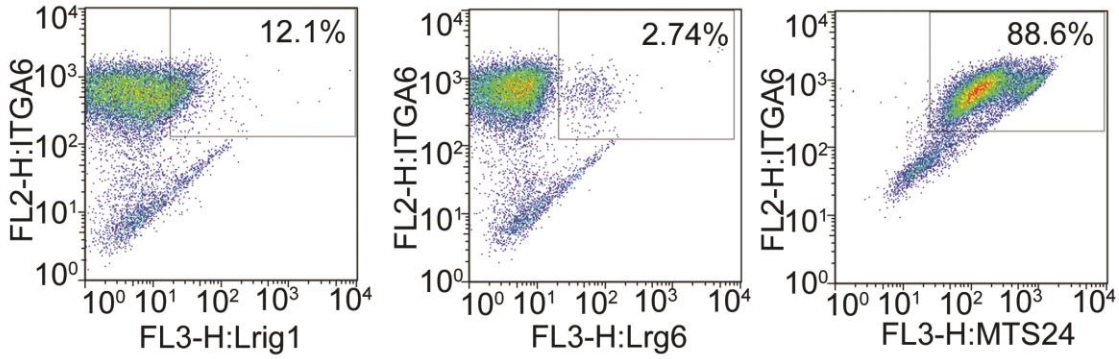


Supplementary Figure 19. Original immunostaing results of AE13 (a), AE15 (b) and K75 (c) in the hair follicle derived from hiPSCs, which are illustrated in Figure 4c, 4d and 4e. Scale bar indicates 50 um (a and b) and 200 um(c).

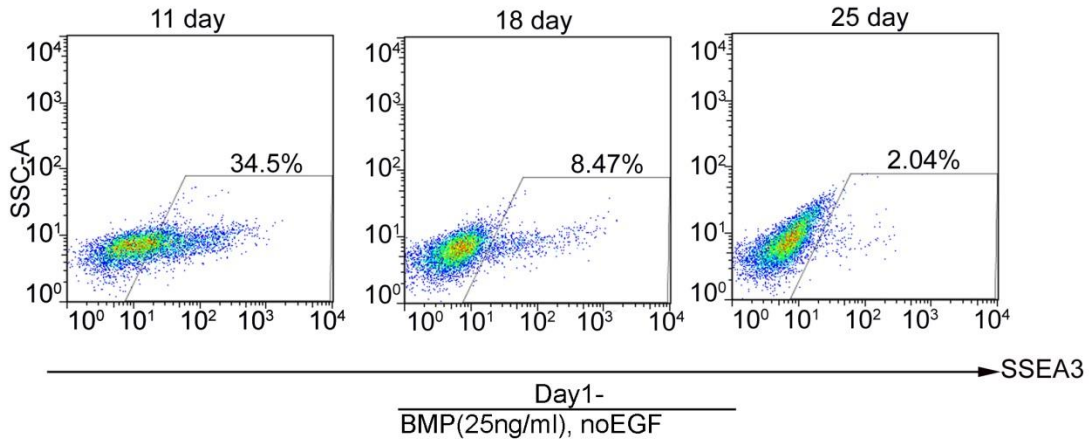


Supplementary Figure 20. Oil red staining of sebocyte and qPCR analysis of sebocyte markers.

a. Sebocytes derived from iPSC-EpSCs were stained with Oil and showed Oil red staining. Scale bar: left panel, 100 um; right panel, 20 um. b. qPCR analysis of sebocyte markers, including *KRT7*, *PPAR-α* and *LPL* in hiPSC-EpSCs, hEpSCs, hiPSC-Sebocytes and HaCaT-Sebocytes. Data shown are mean ± SD of the expression from three independent experiments.



Supplementary Figure 21. Flow cytometric analysis of cells positive for Lrig1^{+}/ITGA6^{+}, Lrg6^{+}/ITGA6^{+} or MTS24^{+}/ITGA6^{+} during epithelial cell differentiation from hiPSCs at day 18. Flow cytometric analysis was performed using antibodies specific for Lrig1, Lrg6, MTS24 and ITGA6. Positive cell percentage was monitored at day 18.



Supplementary Figure 22. Flow cytometric analysis of positive cells for SSEA3 when only BMP4 was added during the differentiation.
Flow cytometric analysis was performed using antibody specific for SSEA3. $SSEA^{+}$ cell percentage was monitored at 11, 18 and 25 days respectively

Supplementary Tables

Supplementary Table 1 Primer sequences

Gene	Forward sequence	Reverse sequence	Applications
ACTB	TGAAGTGTGACGTGGACATC	GGAGGAGCAATGATCTTGAT	RT-PCR
ITGB4	CTGTACCCGTATTGCGACT	AGGCCATAGCAGACCTCGTA	RT-PCR
ITGA6	GCTGGTTATAATCCTTCAATATCAATTGT	TTGGGCTCAGAACCTTGGTTT	RT-PCR
COL7A1	GATGACCCACGGACAGAGTT	ACTTCCCGTCTGTGATCAGG	RT-PCR
LAMB3	GACAGGACTGGAGAAGCGTGTG	CCATTGGCTCAGGCTCAGCT	RT-PCR
KRT5	ATCTCTGAGATGAACCGGATGATC	CAGATTGGCGCACTGTTTCTT	RT-PCR
KRT14	GGCCTGCTGAGATCAAAGACTAC	CACTGTGGCTGTGAGAATCTTGTT	RT-PCR
KRT8	GATCGCCACCTACAGGAAGCT	ACTCATGTTCTGCATCCCAGACT	RT-PCR
KRT18	GAGTATGAGGCCCTGCTGAACATCA	GCGGGTGGTGGTCTTTTGGAT	RT-PCR
OCT4(endo)	CCTCACTTCACTGCACTGTA	CAGGTTTTCTTTCCFTAGCT	RT-PCR
NANOG	TGAACCTCAGCTACAAACAG	TGGTGGTAGGAAGAGTAAAG	RT-PCR
KLF4(endo)	GATGAACTGACCAGGCACTA	GTGGGTCATATCCACTGTCT	RT-PCR
SOX2(endo)	CCCAGCAGACTTCACATGT	CCTCCCATTTCCCTCGTTTT	RT-PCR
REX1	TCGCTGAGCTGAAACAAATG	CCCTTCTTGAAGGTTTACAC	RT-PCR
hTERT	TGTGCACCAACATCTACAAG	GCGTTCTTGGCTTTCAGGAT	RT-PCR
OCT4 transgene	CCCCAGGGCCCCATTTTGGTACC	CAACAACCGAAAATGCACCAGCCCCAG	RT-PCR
KLF4 transgene	ACGATCGTGGCCCCGGAAAAGGACC	CAACAACCGAAAATGCACCAGCCCCAG	RT-PCR
SOX2 transgene	GGCACCCCTGGCATGGCTCTTGGCTC	CAACAACCGAAAATGCACCAGCCCCAG	RT-PCR
Involucrin	CTCCTCCAGTCAATACCCATCAG	ACATTCTTGCTCAGGCAGTCC	RT-PCR
Flaggrin	TTCGGCAAATCCTGAAGAATC	CTTGAGCCAACTTGAATACCATC	RT-PCR
LGR5	CCTTCCAACCTCAGCGTCTTC	GGGAATGTATGTCAGAGCGTTTC	RT-PCR
LGR6	CACACCCCAGTGTCCAGTGTAG	CCACAGGAAATGCCAGTCAAG	RT-PCR
TCF4	CAGCAAGCACTGCCGACTAC	CCAGGCTGATTCATCCCACT	RT-PCR
CD200	TGACTCTGTCTCACCCAAATG	GCTTAGCAATAGCGGAACTG	RT-PCR
FZD2	GGACATCGCCTACAACCAGAC	GCGTACATGGAGCACAGGAAG	RT-PCR
DKK3	ACCGAGAAATTCACAAGATAACC	CTGGCAGGTGTACTGGAAGC	RT-PCR
LEF1	CCATCACGGGTGGATTCAG	TCTGTTCATGCTGAGGCTTCAC	RT-PCR
LHX2	ATACCCGAGCAGCCAGAAGAC	GGAGGACCCGCTTGGTGAG	RT-PCR
KRT7	GGACATCGAGATCGCCACCT	TGCCACCGCCACTGCTACT	RT-PCR
KRT19	AACCAAGTTTGAGACGGAACAG	GAGCGGAATCCACCTCCAC	RT-PCR
LPL	TCATTCCCGGAGTAGCAGAGT	GGCCACAAGTTTTGGCACC	RT-PCR
PPARA	TTCGCAATCCATCGGCGAG	CCACAGGATAAGTCACCGAGG	RT-PCR
meOCT4	GAGGTTGGAGTAGAAGGATTGTTTTGGTTT	CCCCCCTAACCCATCACCTCCACCACCTAA	Bisulfite sequencing
meNANOG	TGGTTAGGTTGGTTTTAAATTTTT G	AACCCACCCTTATAAATTCTCAATTA	Bisulfite sequencing

Supplementary Table 2 Antibody Information

Antibody Name	**Applications**	**Dilution**	**Company**
CD200	Flow Cytomentry	1:20	Ebioscience
ITGA6	Flow Cytomentry	1:100	Ebioscience
ITGB4	Flow Cytomentry	1:100	Ebioscience
ITGB1	Flow Cytomentry	1:100	Ebioscience
ITGB4	Immunofluorescence	1:200	Ebioscience
ITGB1	Immunofluorescence	1:200	Ebioscience
Krt14	Immunofluorescence	1:1000	Lab Vision
p67	Immunofluorescence	1:500	Lab Vision
Krt15	Immunofluorescence	1:500	Lab Vision
E-CAD	Immunofluorescence	1:40	Dako
Cytokeratin	Immunofluorescence	1:150	Dako
SSEA3	Immunofluorescence	1:20	DSHB
SSEA4	Immunofluorescence	1:20	DSHB
TR-1-60	Immunofluorescence	1:100	Santa Cruz
OCT4t	Immunofluorescence	1:100	Santa Cruz
Nanog	Immunofluorescence	1:100	Millipore
AE13	Immunochemistry	1:100	Abcam
AE15	Immunochemistry	1:100	Abcam
K75	Immunochemistry	1:100	Sigma-Aldrich