SUPPLEMENTARY INFORMATION

SUPPLEMENTARY MATERIALS AND METHODS

Fibroblasts and keratinocytes

The skin biopsy was cut into 2×2 mm pieces. The dermis was peeled off and trapped under a sterile cover slip. Human fibroblast media consisted of DMEM (Invitrogen, Carlsbad, CA), 10% fetal bovine serum (Invitrogen), and 1% penicillin/streptomycin (Invitrogen). Medium was changed every 2 days until the culture became 90% confluent, at which point it was passaged using 0.25% trypsin EDTA. Human keratinocytes were grown in EpiLife medium (Cascade Biologics, Portland, OR) supplemented with 0.06 mM Ca^{2+}, 1% EpiLife defined growth supplement, and 1% penicillin/streptomycin (Invitrogen). Cultures were maintained at 37°C in a humidified room atmosphere with 5% CO_2.

Induced pluripotent stem cells

About 50,000 keratinocytes or 100,000 fibroblasts were seeded per well of a 6-well plate and infected with a 1:1:1:1 mix of retroviral supernatants of *pMIG* containing *OCT4*, *SOX2*, *KLF4,* and *c-MYC* in the presence of 5 μg ml^{-1} protamine sulfate. Infection consisted of a 45-minute spin-infection at 1800 rpm at room temperature, after which supernatants were left in contact with the cells for 24 hours (h) at 37°C in 5% CO_2. The next day, cells were spin-infected for a second time using the same procedure. After five days, cells were trypsinized and seeded onto feeder layers of irradiated CF1 murine embryonic fibroblasts in the same culture medium. After 24 h, the medium was changed to human ES cell medium, consisting of DMEM/F12 (Invitrogen) supplemented with 10% KO-Serum Replacement (Invitrogen), 2 mM GlutaMAX (Invitrogen), 50 μM 2-mercaptoethanol (Invitrogen), 1x non-essential amino acids (Invitrogen), 50 U ml^{-1} penicillin, 50 mg ml^{-1} streptomycin, and 10 ng ml^{-1} bFGF (R&D systems, Minneapolis, MN). Cultures were maintained at 37°C, 5% CO_2, with media changes daily. Colonies were picked based on morphology 3 weeks after the initial infection.

Transcription expression profile

RNA was isolated with a GenElute Mammalian Total RNA Miniprep Kit (Sigma-Aldrich, St. Louis, MO) and treated with TURBO DNA-free (Ambion, Austin, TX) to remove genomic DNA. First-strand cDNA was synthesized with a Superscript III First Strand Synthesis SuperMix for quantitative reverse transcription PCR (Invitrogen). RT-PCR was performed with TaqMan Gene Expression Assays (list follows) and TaqMan Universal PCR Master Mix, No AmpErase UNG (Applied Biosystems, Carlsbad, CA) per the manufacturer's protocol. TaqMan Gene Expression Assays were as follows: GAPDH Hs99999905_m1; POU5F1 00999634_gH; SOX2 00602736_s1; NANOG 02387400_g1; KLF4 00358836_m1; MYC 00153408_m1; LIN28 00702808_s1; REX01 00810654_m1; ABCG2 01053790_m1; DNMT3 01003405_m1 with GAPDH 99999905_m1 used

as an endogenous control. Exogenous and total levels of *OCT4* and *SOX2* were determined with the use of the following primers: *OCT4* Endo Forward, CCTCACTTCACTGCACTGTA; *OCT4* Endo Reverse, CAGGTTTTCTTTCCCTAGCT; *OCT4* Total Forward, AGCGAACCAGTATCGAGAAC; *OCT4* Total Reverse, TTACAGAACCACACTCGGAC; *SOX2* Endo Forward, CCCAGCAGACTTCACATGT; *SOX2* Endo Reverse, CCTCCCATTTCCCTCGTTTT; *SOX2* Total Forward, AGCTACAGCATGATGCAGGA; and *SOX2* Total Reverse, GGTCATGGAGTTGTACTGCA. TaqMan gene expression assays used for iPSC-derived keratinocytes were collagen type VII (Hs01574733_g1), collagen type XVII (Hs00166711_m1), keratin 1 (Hs00196158_m1), and keratin 5 (Hs00361185_m1), with GAPDH (Hs99999905_m1) used as an endogenous control. Expression levels were measured in duplicate. For genes with expression below the CT fluorescence threshold, the CT was set to 40 to calculate the relative expression. Analysis and subsequent calculations were done with the use of an ABI PRISM 7500 sequence detection system (Applied Biosystems).

Protein expression profile

For detection of embryonic cell expression profile in live iPSCs, TRA-1-60 antibody (Millipore, 1:400) and secondary antibody Alexa 488-conjugated anti-mouse IgM (Invitrogen, 1:400) were diluted in hES medium and added to the culture. The plate was incubated in 37°C for 1 h before medium was changed to fresh conditioned medium. TRA-1-60^{+} colonies were identified under a fluorescence microscope. To comprehensively examine embryonic protein expression profiles, iPSCs were fixed with 4% paraformaldehyde for 15 min. If nuclear permeation was needed, cells were treated with 0.2% TritonX (Sigma) in PBS for 0.5 h, and blocked in 3% BSA in PBS for 2 h, and cells were incubated with primary antibody overnight at 4°C. Antibodies recognizing the following antigens were used: TRA1-60 (MAB4360, 1:400), TRA1-81 (MAB4381, 1:400), SSEA4 (MAB4304, 1:100), and SSEA3 (MAB-4303, 1:100) from Chemicon (Billerica, MA); NANOG (EB06860, 1:100) from Everest Biotech (Oxfordshire, United Kingdom); and OCT3/4(AB27985 , 1:200) from ABCAM (Cambridge, MA). Secondary antibodies incubated for 1 h at room temperature were all the Alexa Fluor Series from Invitrogen (all at 1:500). Images were taken using Olympus BX61 FV500 Confocal Microscope (Olympus, Center Valley, PA). Alkaline phosphatase staining was done per the manufacturer's recommendations (Millipore, Billerica, MA).

Bisulfite genomic sequencing

Genomic DNA was isolated with a PureLink Genomic DNA Mini Kit (Invitrogen). Bisulfite treatment was done with an EpiTect Bisulfite kit (QIAGEN, Germantown, MD). Converted DNA was PCR-amplified with *OCT4*-specific primer sets and *NANOG*-specific primer sets(Chan *et al.*, 2009). PCR products were gel purified with a PureLink Quick Gel Extraction and PCR Purification Combo Kit (Invitrogen) and cloned into bacteria with the use of the TOPO TACloning Kit for Sequencing (Invitrogen).

In vivo iPSC differentiation

For teratoma formation, immunodeficient NOD/IL-2Rγc/Rag$^{-/-}$ (NOG) young adult mice were injected with 1 million cells resuspended in mixture of DMEM/F12, Matrigel (BD Biosciences, Sparks, MD), and collagen (ratio, 2:1:1; 40 μL per mouse) into the right quadriceps muscle with the use of a 29-gauge needle attached to a 0.3-mL insulin syringe. Tumors were harvested in 6-8 weeks, sectioned, and stained with hematoxylin-eosin.

Histology

Skin biopsies, as well as teratomas, were frozen in optimal cutting temperature (OCT, Sakura Finetek USA, Torrance, CA) and cut at 6 μm sections on a cryostat. Sections were fixed at room temperature acetone for 5 minutes, rehydrated with 1x PBS and blocked with 10% normal serum (Jackson Immunoresearch, West Grove, PA). Primary antibodies used targeted L332 (1:50, Abd Serotec, Raleigh, NC; 1:50, GB3 clone from Santa Cruz Biotechnology, Santa Cruz, CA), cytokeratin 5 (1:600, Covance, Princeton, NJ), and Collagen type VII (1:250, BD Biosciences, San Jose, CA). Secondary antibodies used were donkey anti-mouse cy3 (1:500, Jackson Immunoresearch, West Grove, PA), and Alexa Fluor 488 goat anti-rabbit (1:600, Molecular Probes, Eugene, OR). Slides were coverslipped with DAPI (4,6-diamidino-2-phenylindole, Vector Labs, Burlingame, CA). Immunofluorescent images were obtained using an Olympus BX61 FV500 confocal microscope (Olympus) at 40x/1.30 magnification. Argon, green HeNe, and blue diode lasers were used to acquire the images in Olympus FluoView software version 4.3. All images were taken at room temperature. Images of hematoxylin eosin stained sections were taken with an Olympus BX51 Microscope, Spot RT software v3.2, and Spot RT camera (Spot Imaging Systems, Sterling Heights, MI) at 10x/0.40 magnification.

Correction of JEB-H iPSC-derived keratinocytes

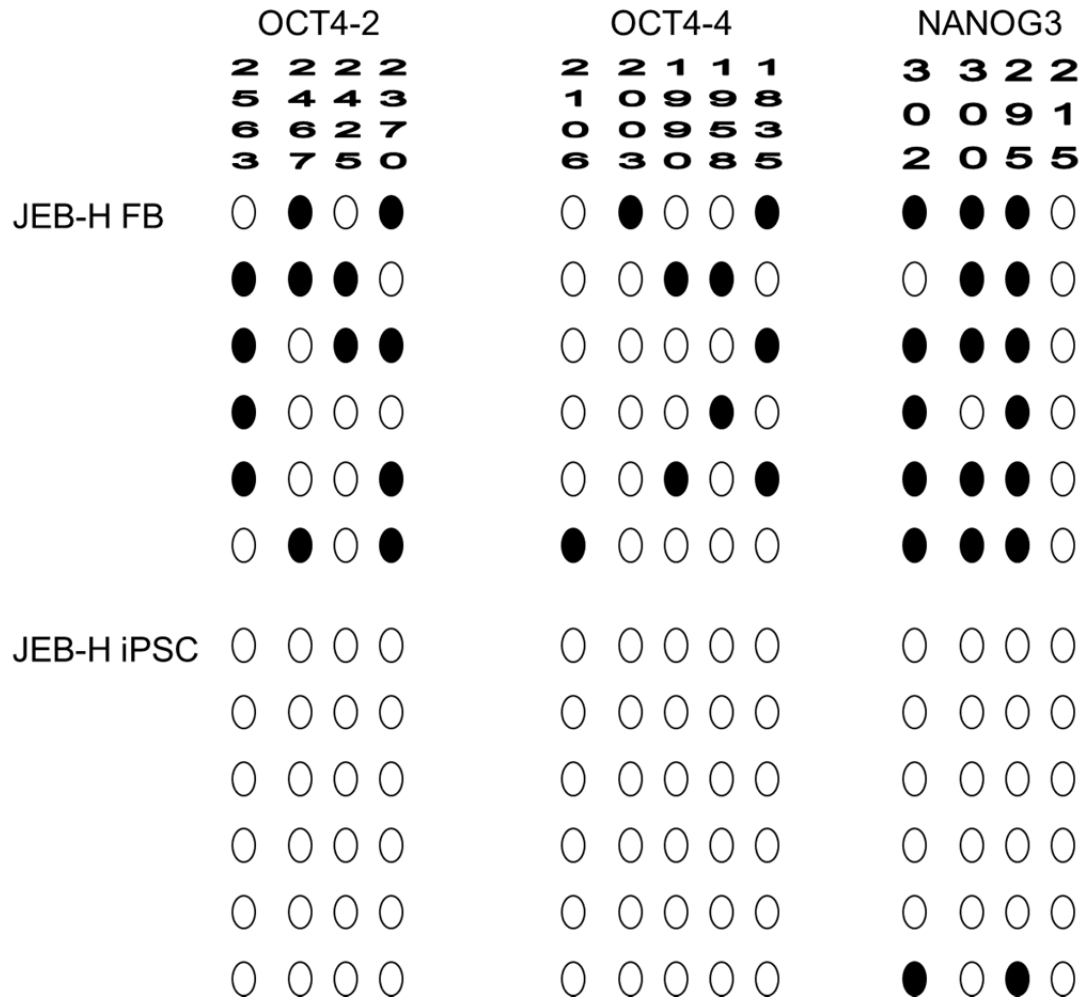
iPSC-derived embryoid bodies produced by confluent cultures were harvested by enzymatic dissociation (Accutase, Invitrogen) to single cells. Cell suspension was added to AggreWell plate (STEMCELL Technologies, Vancouver, Canada), differentiated in basic embryoid body medium containing knockout Dulbecco's Modified Eagle Medium (Invitrogen) supplemented with 20% fetal calf serum, 0.1 mM nonessential amino acids (Invitrogen), 0.1 mM ß-mercaptoethanol (Sigma-Aldrich), 1 mM L-glutamine (Invitrogen), 50 μg/mL ascorbic acid (Sigma-Aldrich), and 200 μg/mL human holo-transferrin (Sigma-Aldrich). After 24 h, embryoid bodies were moved to low-attachment dishes with medium enriched in 1μM all-trans retinoic acid and 25ng/mL BMP-4. Medium was changed daily. After 4 days colonies were resuspended in defined keratinocyte serum-free medium (Gibco, Invitrogen) and plated in gelatin-coated dishes. After 3-4 weeks, colonies of keratinocytes developed.

The human *LAMB3* cDNA (was cloned into the pLL3.7 lentiviral vector) and JEB-H iPSC-derived keratinocytes were transduced in the presence of 5μg ml^{-1} protamine sulfate. Infection consisted of a 45 min spin infection at 1800 rpm at room temperature, after which supernatants were left in contact with the cells for 24 h at 37°C and 5% CO_2. The next day, cells were spin infected for the second time using the same procedure. 72 h after beginning the last round of infection, cells were fixed with 4% paraformaldehyde for 15 min, blocked in 2% BSA in PBS for 1 h, and incubated with primary antibody (anti-L332, 1:50, Abd Serotec) for 3 h. Secondary antibody used was Cy3 (1:500, Invitrogen) for 1 h at room temperature. Images were taken using the Olympus BX61 FV500 confocal microscope.

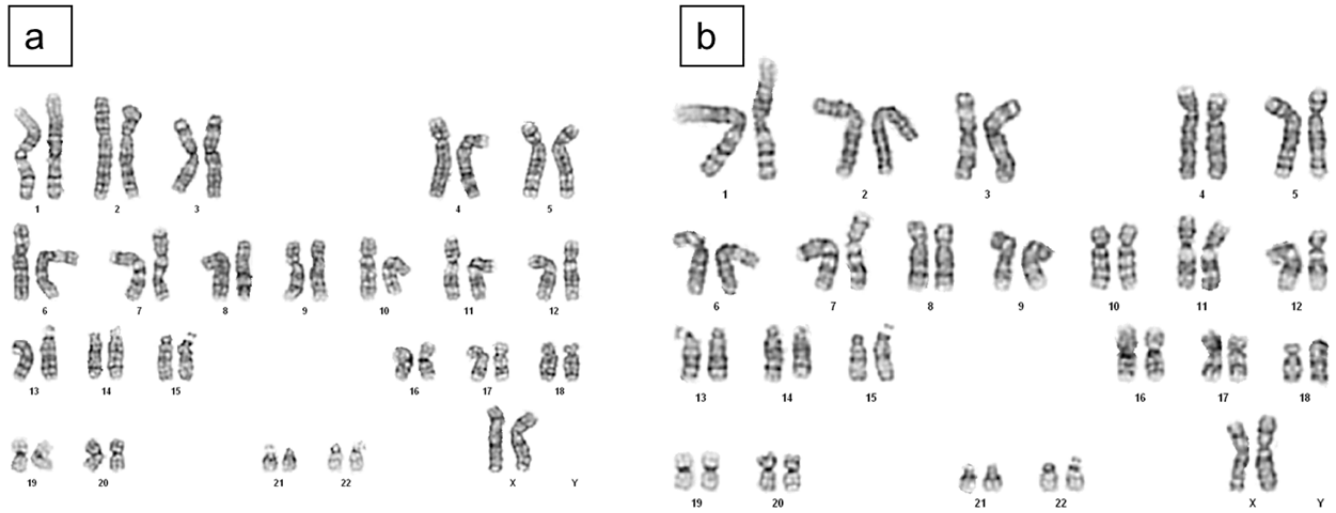
Data analysis

Differences between measurements were evaluated with the Student *t* test, with *P* values < 0.05 considered significant.

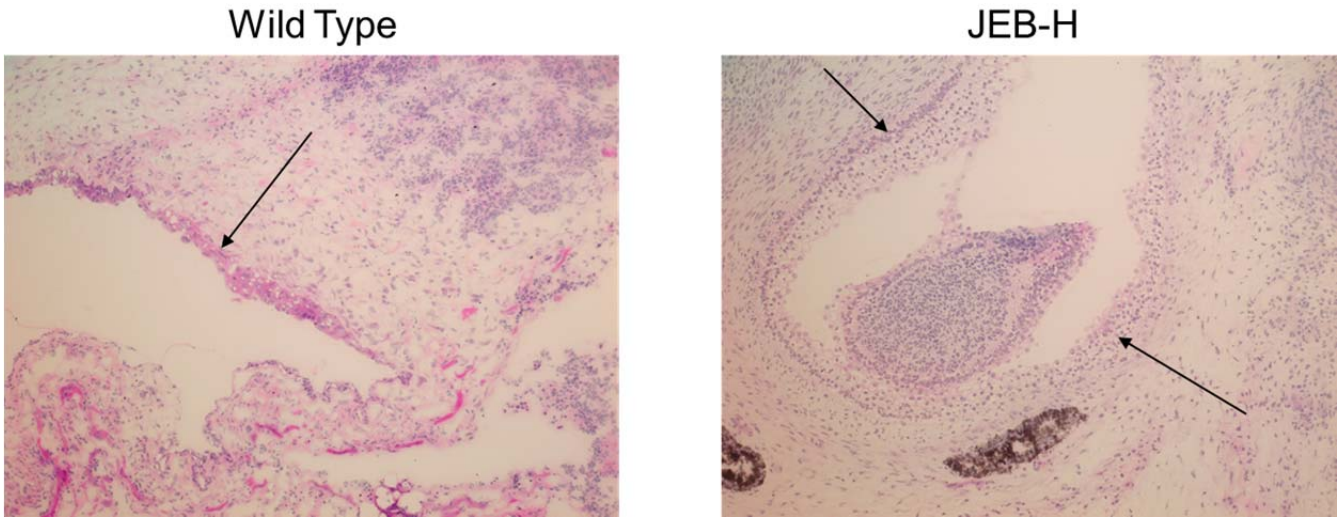
SUPPLEMENTARY FIGURES



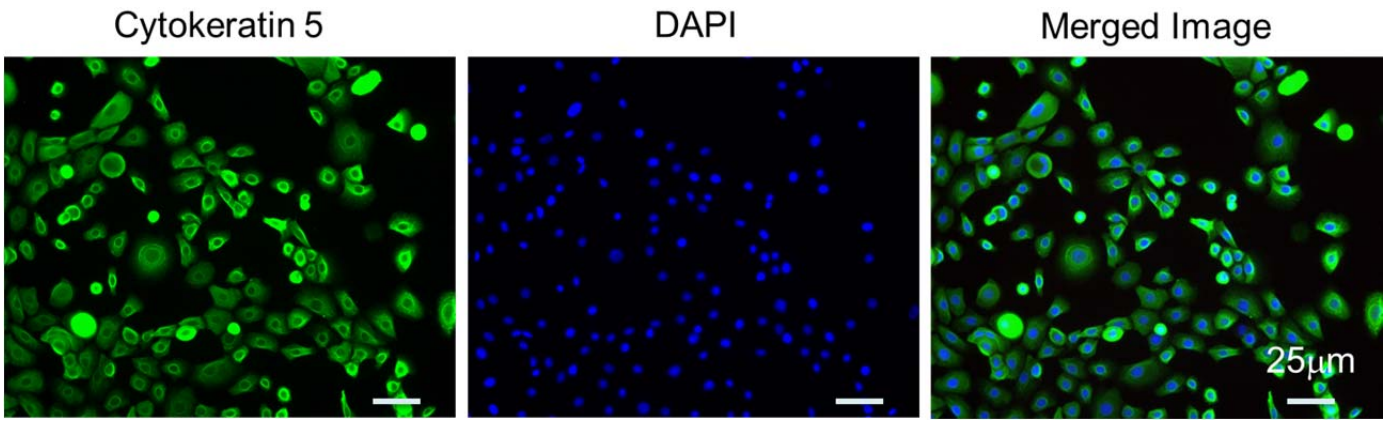
<u>Supplementary Figure 1.</u> Epigenetic modifications in JEB-iPSCs.
Bisulfite sequencing of the *OCT4* and *NANOG* promoters in parental JEB-H fibroblasts (FB) and P1 JEB-H iPSCs skin cells. Sequencing reactions of specific amplicons are represented by each row of circles(Chan *et al.*, 2009). Open circles denote unmethylated CpGs, and filled circles represent methylated CpGs. CpG position relative to the downstream transcriptional start site is shown above each column.



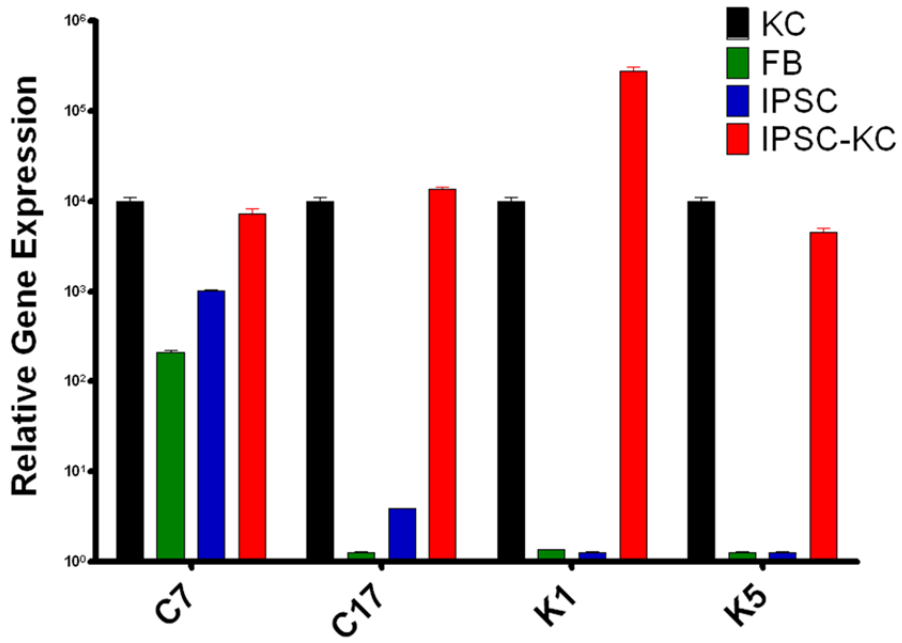
Supplementary Figure 2. Cytogenetic analysis of JEB-iPSCs.
JEB-H iPSCs (P1) were examined by high-resolution G banding at passage 7 (**a**) and passage 21 (**b**), respectively. Both karyotypes were euploid and female.



Supplementary Figure 3. JEB-iPSC-derived skin-like structures.
Hematoxylin-eosin staining of wild-type and P1 JEB-H iPSC-derived teratomas showed skin-like structures (arrows). Magnification 40x.



Supplementary Figure 4. JEB-iPSC-derived keratinocytes.
JEB iPSC-derived keratinocytes expressed cytokeratin 5 (green). Nuclei are stained DAPI (blue)



Supplementary Figure 5. Expression profile of JEB-H iPSC-derived keratinocytes. Quantitative RT-PCR analysis of collagen type VII (C7), collagen type XVII (C17), keratin 1 (K1), and keratin 5 (K5) in keratinocytes (KC, black bars); fibroblasts (FB, green bars); induced pluripotent stem cells (iPSC, blue bars); and iPSC-derived KC (iPSC-KC, red bars). iPSC-KC express collagens and keratins characteristic of KC. Expression levels of C7, C17, K1, and K5 in KC versus FB and KC versus iPSC were statistically different (P-values < 0.05), while approximately same levels of these collagens and keratins were expressed in KC and iPSC-KC.