Supporting Information

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SI Methods

Plasmid Vector Construction. To make PB-TRE, PB-MSCV, and PB-CAG vectors, the TRE was amplified from pTight (Clontech), the MSCV LTR was amplified from pMSCV-Neo (Clontech), and the CAGG promoter was amplified from a pBluescript-CAG vector, and cloned into a PB-bpA vector. cDNAs of the four mouse and human Yamanaka factors were amplified (primers in Table S3) from original retroviral vectors (Addgene) and cloned into the PB-TRE, PB-MSCV, and PB-CAG transposon vectors, respectively. Mouse and human Rarg, Lrh1 and Sf1 were amplified from IMAGE clones (Geneservice) and cloned into transposon vectors.

Preparation of MEF Cells and HDFn Cells for Reprogramming. MEFs were prepared from 12.5 d postcoitum Oct4-IRES-Puro-Egfp embryos. To minimize variation among embryos, MEFs from several embryos with the same genotype were mixed together for expansion in M10 media. MEFs were passaged once before they were counted, divided into aliquots, and frozen down. Before electroporation, 1×10^6 MEFs were plated onto one gelatinized 15-cm tissue culture plate. When MEFs were 70% to 80% confluent, they were trypsinized and collected for electroporation. M10 was knockout DMEM, 10% FBS (HyClone), 1× glutamine-penicillin-streptomycin (Invitrogen), and 1× NEAA (Invitrogen).

HDFn cells (neonatal, lot nos. 709590 and 2007100654) and HDFa cells (adult, lot nos. 439656 and 617769) were purchased from Invitrogen and maintained in media 106 supplemented with low serum growth supplement (Invitrogen). The primary HDFn or HDFa culture was passaged once before being counted, divided into aliquots, and frozen. Before electroporation, 5×10^5 HDFn or HDFa cells were plated onto three T75 tissue culture flasks. When HDFn or HDFa cells were 70% to 80% confluent, they were trypsinized and collected for electroporation.

Transfection and Cell Culture. MEF transfection was performed using an Amaxa machine (Lonza) according to the manufacturer's protocol (program A-023). One million MEFs were usually transfected. After electroporation, MEFs were seeded in M15 plus LIF on STO feeders. For Tet-On experiments, M15 containing Dox (1.0 μg/mL) was added after transfection and was changed every other day. iPSC colonies were usually picked at day 7 to day 10; 96-well plates and cells were expanded according to standard mouse ES cell culture conditions.

Transfection of HDFn and HDFa cells was achieved using an Amaxa machine according to the manufacturer's protocol (program U-020 for HDFn and program P-022 for HDFa). One million HDFn or HDFa cells were usually used in a transfection. After electroporation, HDFn or HDFa cells were seeded in M15 plus LIF on STO feeders. For Tet-On experiments, PB-TRE-cDNA transposons and PB-CAG-rtTA and PBase plasmids were transfected into HDFs, which were plated onto STO feeders. M15 containing Dox (1.0 μg/mL) was added 24 h after transfection and was changed every other day. Dox induction usually lasted for 10 to 20 d. Human iPSC colonies reprogrammed by PB-CAG vectors were usually picked at day 10, and dissociated with trypsin to single-cell suspensions before seeding into in 24-well formats. Human iPSC colonies reprogrammed by PB-TRE vectors were usually picked at day 20 to 30, and dissociated with Accutase to single-cell suspensions before seeding in 24-well plates. Stable lines were established from secondary colonies and maintained according to standard mouse ES cell culture conditions.

Mouse and human iPSC colonies were visualized by AP staining using the Leukocyte Alkaline Phosphatase kit (Sigma).

Cell Proliferation Assay. Cell proliferation assay was performed using Click-iT EdU Flow Cytometry Assay Kit (Invitrogen) according to the manufacturer's recommendations. Briefly, MEFs transfected with different combinations of reprogramming factors on the PB were stained with 10 μM EdU for 1 h after transfection. EdU-incorporated cells were stained with Alexa Fluor 647 dye and analyzed by flow cytometry.

Bisulfite Genomic Sequencing. Bisulfite treatment was performed by using the EpiTect Bisulfite Kit (Qiagen) according to the manufacturer's recommendations. PCR primers are listed in Table S3. Amplified products were cloned into pGEM-T-easy (Promega). Randomly selected clones were sequenced with the M13 forward and M13 reverse primers for each promoters.

RT-PCR. RNA was isolated using the RNeasy Mini Kit (Qiagen). The samples were subsequently quantified and treated with gDNA WipeOut. First-strand cDNA was prepared by using the QuantiTect Reverse Transcription Kit (Qiagen). For each RT-PCR, we used 50 to 100 ng of cDNA and primers listed in Table S3. Standard PCR conditions were: 94 °C for 30 s, 60 °C for 30 s, and 68 °C for 30 s for 30 cycles. For real-time PCR, we used TaqMan Gene Expression Assays. TaqMan probes were purchased from Applied Biosciences or custom-designed and synthesized by Applied Biosciences (Table S4). All quantitative PCR was performed in a 9700HT Fast Real-Time PCR System (Applied Biosciences). X-linked human gene expression was determined using SYBR Green RT-PCR kit (Applied Biosciences). Primers are listed in Table S3. Mouse gene expression was determined relative to mouse β-actin using the ΔCt method. Human gene expression was determined relative to human GADPH gene using the ΔCt method.

Luciferase Assay. *Oct4*-luc2 plasmid (1.0 μg) and 100 ng of hRluc/TK (Promega) were transfected into MEFs, together with different combinations of reprogramming factors. Forty-eight hours after transfection, cells were lysed with 1× passive lysis buffer (Promega). Luciferase activities were measured with a Dual-Luciferase reporter assay system (Promega) according to the manufacturer's protocol.

In Vitro Differentiation of iPSCs. For monolayer differentiation, mouse iPSCs were harvested by trypsinization and transferred to six wells at adensity of 1 to $1.5 \times 10^4/cm^2$ in N2B27. Medium was changed every other day. On day 6, cells were fixed for immunostaining of Tuj1. For EB differentiation, mouse iPSCs were harvested and transferred to a 6-cm Petri dish at density of $1.5 \times 10^4/cm^2$ in M15 medium without LIF. After 3 d, the aggregated cells were harvested and plated onto a gelatin-coated six-well plate for another 3 d, then proceed to immunostaining of α-smooth muscle actin (α-SMA) and α-fetoprotein (AFP).

For EB differentiation, human iPSCs were harvested by trypsinization and transferred to a 6-cm Petri dish at density of $1.5 \times 10^4/cm^2$ in mouse ES medium without LIF. Medium was changed every other day. After 8 d, the aggregated cells were harvested and plated onto gelatin-coated six wells for another 8 d. Cells were fixed for immunostaining of Tuj1, α-SMA, and AFP.

Immunostaining. For dual staining of SSEA-1 and Nanog, mouse iPSCs were fixed in 4% PFA/PBS solution, blocked in PBS solution with 3% goat serum and 1% BSA, incubated with anti–SSEA-1 antibody (gift from Peter W. Andrews, the Centre for Stem Cell Biology, University of Sheffield, Sheffield, United Kingdom) at 4 °C overnight. Cells were then rinsed with PBS solution, incubated with Alexa 488-conjugated goat anti-mouse IgM (Invitrogen) for 1 h at room temperature. After permeabilization with PBST (PBS solution with 0.3% Triton), cells were incubated with anti-Nanog antibody (Abcam) at 4 °C for overnight. The third day, cells were rinsed with PBST, incubated with Alexa 594-conjugated goat anti-rabbit IgG (Invitrogen) for 1 h at room temperature, and counterstained with DAPI.

For dual staining of H3K27 and Oct4, cells were plated at 2×10^3 onto laminin-coated slides in M15 medium with or without LIF (differentiated and undifferentiated mouse iPSCs). After 5 d, cells were fixed and incubated with anti-H3K27 (Cell Signaling) and anti-Oct4 (Santa Cruz) antibodies at 4 °C for overnight. The next day, cells were rinsed with PBS solution and incubated with Alexa 594-conjugated goat anti-rabbit IgG and Alexa 488-conjugated goat anti-mouse IgG (Invitrogen) for 1 h at room temperature and counterstained with DAPI.

For Tuj1, α-SMA, and AFP immunostaining, differentiated moue iPSCs were fixed and incubated with anti-Tju1, α-SMA, or AFP antibody (R&D Systems), respectively, at 4 °C overnight. Cells were rinsed with PBS solution and incubated with Alexa 488-conjugated goat anti-mouse IgG, and counterstained with DAPI.

For human pluripotency markers immunostaining, human iPSCs were fixed in 4% PFA/PBS solution, blocked in PBS solution with 3% goat serum and 1% BSA (for cell surface markers) or PBS solution with 3% goat serum, 1% BSA and 0.1% Triton (for intracellular markers), incubated with cell surface antibodies, SSEA-1, SSEA-3, SSEA-4, Tra-1–60, Tra-1–81 (gifts from Peter W. Andrews, the Centre for Stem Cell Biology, University of Sheffield, Sheffield, United Kingdom) or intracellular antibodies, Oct4, Nanog, Tuj1, α-SMA, and AFP at 4 °C for overnight. Cells were rinsed and incubated with Alexa 488-conjugated goat anti-mouse, rat IgM, goat anti-mouse IgG or Alexa 594-conjugated goat anti-mouse, rabbit IgG, and counterstained with DAPI.

Flow Cytometry. Mouse iPSCs growing in 96-well feeder plates were trypsinized and resuspended in M15. iPSCs were centrifuged at $200 \times g$ (Eppendorf centrifuge 5702R, A-4-38 rotor) for 3 min, and the medium was removed by putting the plate upside down on tissue. iPSCs were resuspended in PBS solution and analyzed by Cytomics FC-500 (Beckman Coulter).

Human iPSCs growing in six-well plates were trypsinized and resuspended in M15. iPSCs were subsequently washed once with PBS solution, centrifuged, and incubated with SSEA-4-FITC, TRA-1–60-PE, or TRA-1–81-FITC antibodies (BD Bioscience) for 1 h. iPSCs were then washed and resuspended in PBS solution and analyzed by Cytomics FC-500 (Beckman Coulter).

Teratoma Formation. Mouse iPSCs were suspended in M10, and 1×10^6 cells were injected s.c. into both dorsal flanks of F1 (129S5/C57B6) hybrid mice. Teratomas were dissected, fixed overnight in 10% buffered formalin phosphate, and embedded in paraffin before sectioning. Human iPSCs (1×10^6) were injected s.c. into both dorsal flanks of NSG mice (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ; Jackson Laboratory). Teratomas were harvested 8 wk after injection for fixation and sectioning. Sections were stained with H&E. All animal experiments were performed in accordance with the United Kingdom 1986 Animals Scientific Procedure Act and local institute ethics committee regulations.

Microarray Analysis. Total RNA from human ES cells, human iPSCs cultured in 2i/LIF medium, and FGF-cultured HiPSCs were hybridized onto human HT-12 v3 and v4 Expression BeadChip (Illumina) according to manufacturer instructions. Arrays were then scanned using the BeadXpress Reader (Illumina). Raw expression files were exported directly from BeadStudio and loaded into R/Bioconductor. Sample data were normalized, and then the mean value was calculated for each transcript from samples in the same group. Pearson correlation analysis of global gene expression (47,232 transcripts) was performed on these arrays.

Statistical Analyses. Data are shown as mean and SD. All statistical analyses were done with Excel 2008 (Microsoft) or Prism (GraphPad).

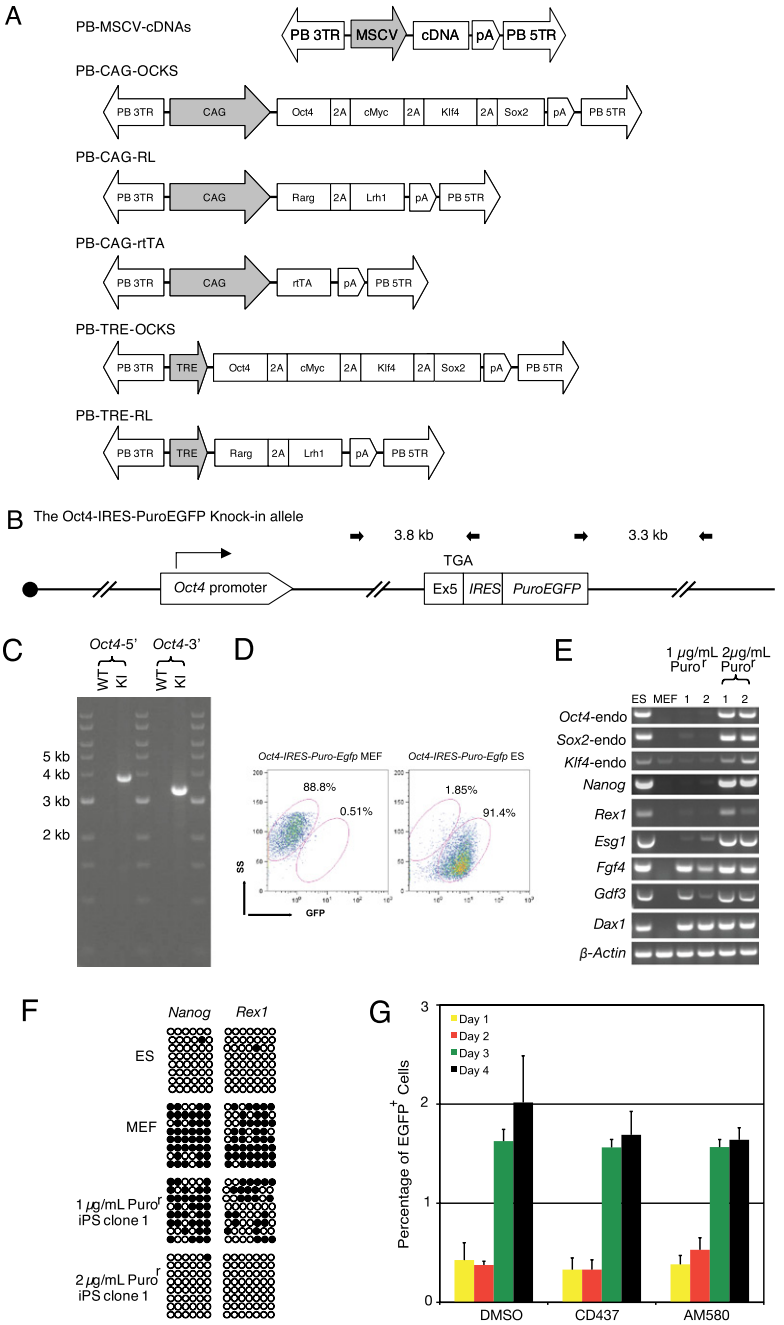

Fig. S1. (*A*) PB transposons carrying various cDNAs. TR, terminal repeats of the PB; MSCV, LTR of MSCV; CAG, CAG promoter; pA, polyadenylation signaling sequence; 2A, foot-and-mouth disease virus 2A self-cleaving peptide; rtTA, reverse tetracycline response transcriptional activator. (*B*) *Oct4-IRES-Puro-Egfp* knock-in allele. The *IRES-Puro-Egfp* cassette was targeted to the 3′UTR of the *Oct4* locus to monitor and select for activation of the locus. Arrows indicate primers used to genotype the knock-in alleles. External primers were located outside of the genomic regions used to make the targeting vector. (*C*) Long-range PCR genotyping of the *Oct4-IRES-Puro-Egfp* knock-in allele. Arrows represent primer position. Primers sequences are listed in Table S3. (*D*) Flow cytometric analysis of the *Oct4-IRES-Puro-Egfp* knock-in cells for GFP expression. ES cells are GFP^+ whereas MEFs are GFP^-. (*E* and *F*) Most AP^+ cells reprogrammed by expressing 4F or 4F+Rarg were not fully reprogrammed and resistant only to 1.0 μg/mL puromycin. Those few colonies resistant to 2.0 μg/mL or higher concentrations of puromycin appeared to be fully reprogrammed iPSCs based on expression of pluripotency genes (*E*) and on DNA methylation at the *Nanog* and *Rex1* loci (*F*). ES, *Oct4-IRES-Puro-Egfp* knock-in ES cells; MEF, *Oct4-IRES-Puro-Egfp* reporter MEF cells. (*G*) Treatment using RAR agonists CD437 or AM580 on the quality of partially reprogrammed iPS cells (4F) based on *Oct4* expression levels. Activation of the endogenous *Oct4* locus was measured by GFP expression in flow cytometry. No obvious difference was observed between cells treated with CD437 or AM580, and the control (DMSO).

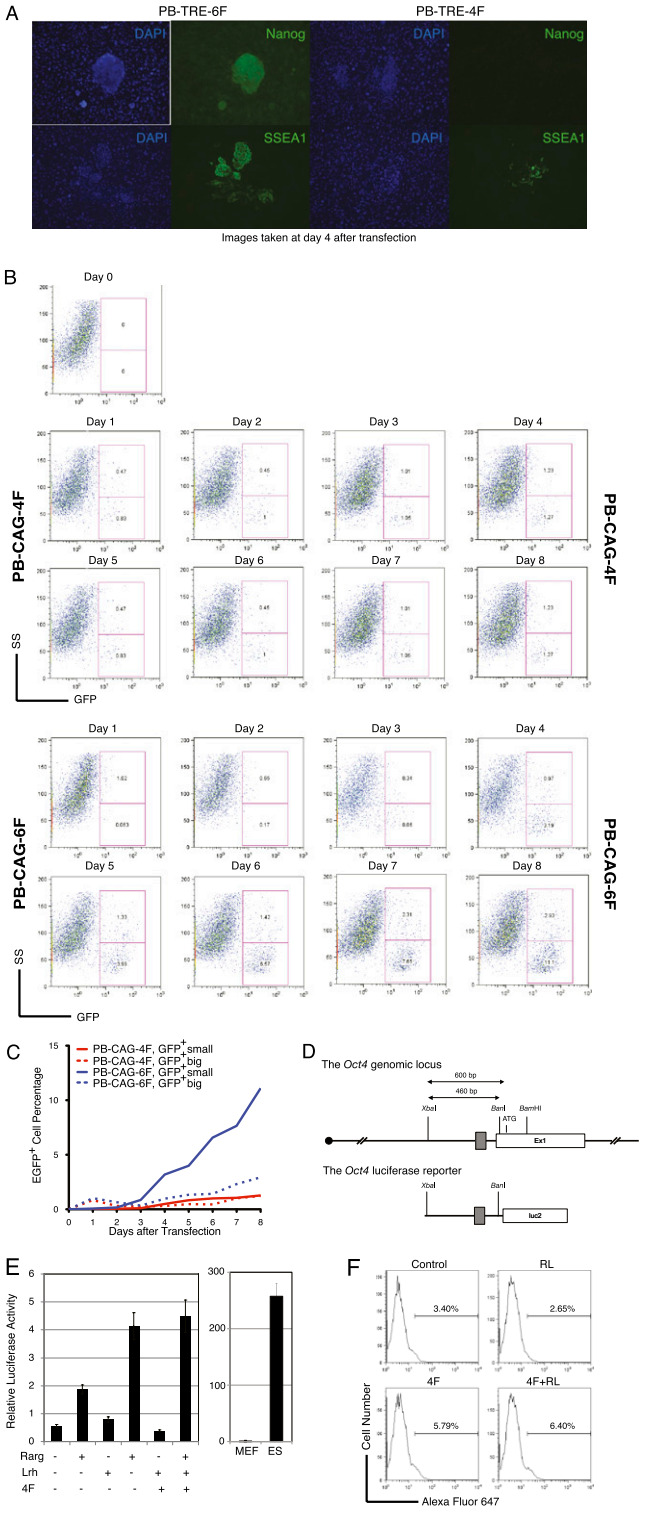

Fig. S2. Rarg and Lrh-1 synergistically promote reprogramming. (*A*) Immunostaining of small colonies induced by 4-d expression of the exogenous factors. Nanog and SSEA-1 were expressed by most if not all of the cells in the colonies by 6F, whereas in 4F colonies, Nanog was not detectable and SSEA-1 expression was only in some cells. (*B*) Activation of the endogenous *Oct4* locus measured by GFP expression in flow cytometry. *Oct4-GFP* reporter MEFs transfected with the 4F or the 6F were harvested for FACS analysis. The number of small GFP$^+$ cells increased rapidly by 6F expression. There were two types of GFP$^+$ cells: small and large. (*C*) The rapidly increased GFP$^+$ cells were those smaller ones that eventually became iPSCs. (*D*) Diagram of the reporter construct for the luciferase reporter assay. A 460-bp *Oct4* promoter DNA fragment flanking the RAREoct site was cloned in front of the luc2 coding sequence. (*E*) Synergistic interaction of Rarg and Lrh-1 to activate the *Oct4* promoter in MEFs in the luciferase reporter assay (*Left*). The reporter constructs were also tested in mouse ES cells (*Right*). Bars are mean ± SD. (*F*) No obvious effect of Rarg and Lrh-1 on cell proliferation. pBluescript plasmid was used as the control.

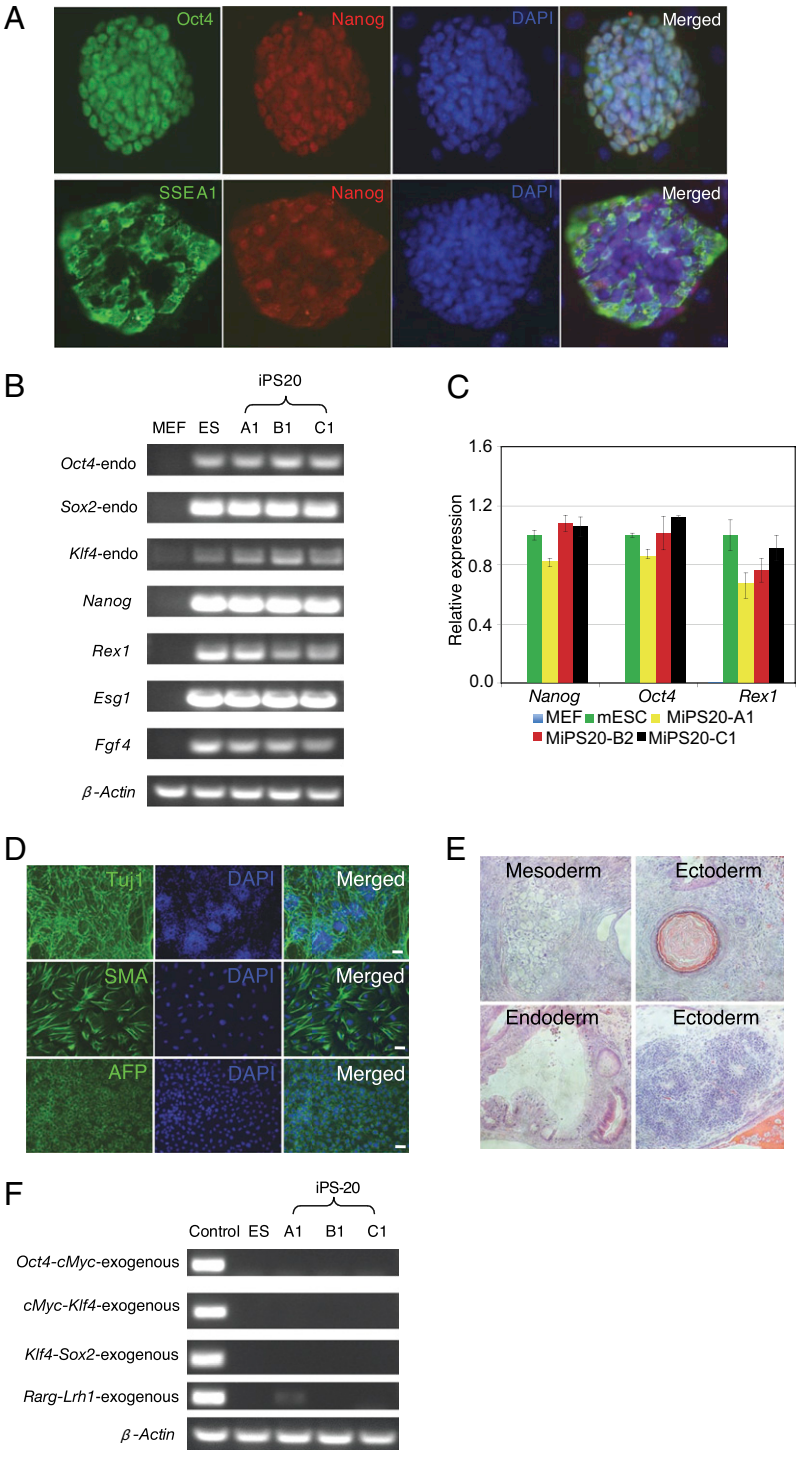

Fig. S3. Characterization of mouse iPSCs from expressing 6F. (*A*) Immunostaining of iPSCs (passage 5) to detect Oct4, Nanog, and SSEA1. (Scale bars: 20.0 μm.) (*B*) Expression of endogenous pluripotency genes in mouse iPSCs by RT-PCR with *β-actin* as the PCR control. MEF, *Oct4-IRES-Puro-Egfp* knock-in MEFs; ES, *Oct4-IRES-Puro-Egfp* knock-in ES cells. (*C*) qRT-PCR analysis of expression of *Nanog*, *Oct4,* and *Rex1*. Expression was relative to *Gapdh* and normalized against gene expression in parental *Oct4-GFP* reporter mouse ES cells. MEF, *Oct4-GFP* reporter MEF cells. (*D*) In vitro differentiation of iPSCs to cell types representing the three germ layers detected by immunostaining. Antibodies: Tuj, neuron-specific class III β-tublin; SMA, smooth muscle α-actin; AFP, α-fetoprotein. (Scale bars: 10.0 μm.) (*E*) Teratomas derived from the iPSCs contained cells types of all three germ layers. Panels show chondrocytes (*Upper Left*), keratinocytes (*Upper Right*), gut-like epithelial cells (*Lower Left*), and neuronal cells (*Lower Right*). All photographs were taken at the same magnification (200×). (*F*) RT-PCR analysis of expression of the exogenous reprogramming factors in mouse iPSC lines. Most lines were free of expression of these factors. Control, mouse iPSCs growing in the presence of Dox to induce exogenous factor expression; ES, parental *Oct4-IRES-Puro-Egfp* knock-in ES cells. Three primer pairs—*Oct4-cMyc*-exogenous, *cMyc-Klf4*-exogenous, *Klf4-Sox2*-exogenous—were used to detect junction fragments between cDNAs in PB-TRE-OCKS construct. Expression of RL from PB-TRE-RL construct used a pair of primers (*Rarg-Lrh-1*-exogenous) to detect a junction fragment between R and L. Most iPSC lines did not express the exogenous factors.

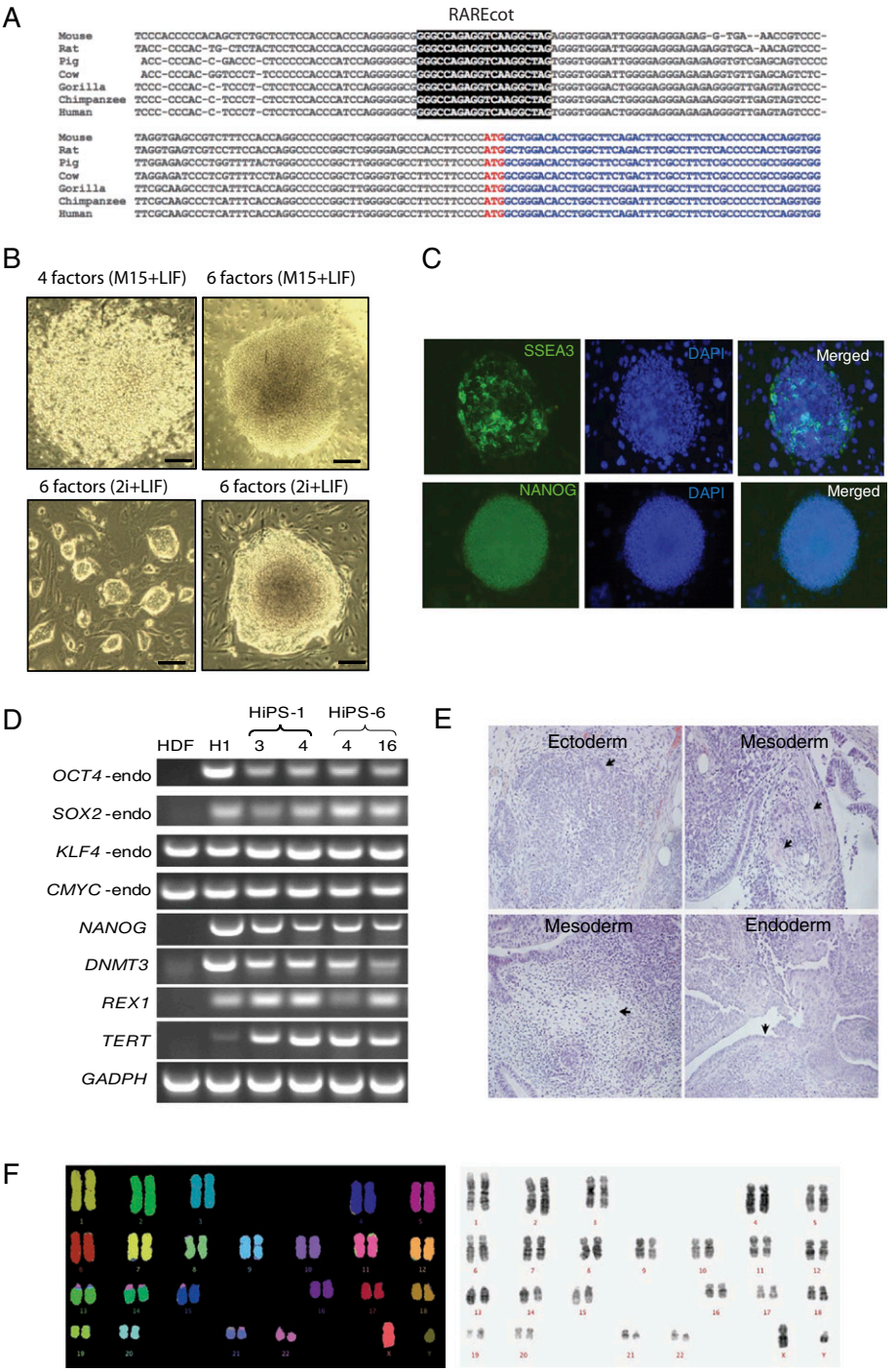

Fig. S4. Production of human iPSCs cells using 6F (CAG promoter version). (*A*) Conservation of RAREoct sequence in several mammalian species. (*B*) Human iPSCs colonies formed in M15 plus LIF media. *Upper Left*: Typical colony reprogrammed with the four Yamanaka factors. *Upper Right*: Typical iPSC colony reprogrammed using 6F. Images were taken 10 d after transfection. *Bottom Left*: iPSC colonies after the primary colonies were dissociated and replated onto feeder cells; *Lower Right*: Typical iPSC colony after subcloning at the single cell density. (*C*) Expression of endogenous pluripotency protein in human iPSCs detected by immunostaining. (*D*) Expression of pluripotency genes in human iPSCs detected by RT-PCR. HiPS1-3 and 4, HiPS6-4 and 16 were four independent iPSC lines. HDF, human dermal fibroblast cells; hESC, H1 human ESCs. (*E*) Differentiation of human iPSCs to cell types of the three germ layers in teratomas. *Upper Left*: Neural tissue with occasional rosettes (arrow). *Upper Right*: Fibromuscular fibers (arrows). *Lower Left*: Loose mesenchyme (arrow). *Lower Right*: Ciliated glandular epithelia (arrow). All photographs were taken at the same magnification (×200). (*F*) Normal karyotype in the human iPSCs after extensive passaging (>20 passages). *Upper*: DAPI. *Lower*: multicolor FISH.

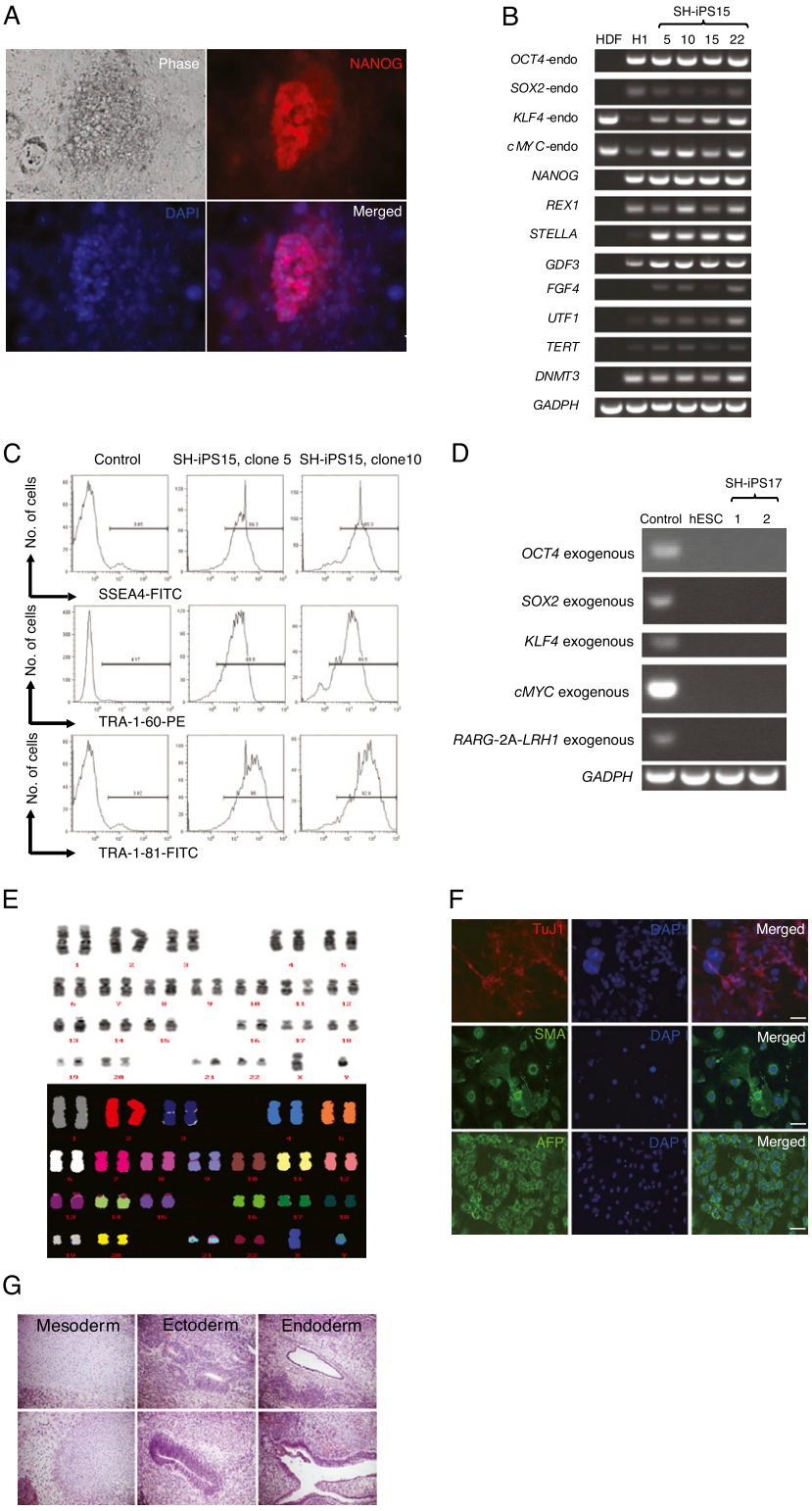

Fig. S5. Characterization of the Dox-independent human iPSCs from neonatal fibroblast cells. (*A*) NANOG expression in primary iPSC colonies (8 d of Dox induction) detected by immunostaining. (Scale bars: 20.0 μm.) (*B*) RT-PCR analysis of gene expression in human iPSCs with *GADPH* as the control. HDF, human neonatal dermal fibroblast cells; hESC, H1 human ES cells. (*C*) FACS analysis of human iPSCs for SSEA-4, Tra-1–60, and TRA-1–81 expression. *Left*: HDF control, which did not express SSEA-4, TRA-1–60, or TRA-1–81. (*D*) No exogenous factor expression was seen in human iPSCs in RT-PCR analysis. Control, expression of the exogenous factors induced by Dox; hESC, H1 human ES cells. (*E*) Normal karyotype in human iPSCs after extensive passaging (>20 passages). *Upper*: DAPI. *Lower*: MFISH. (*F*) In vitro differentiation of human iPSCs. Antibodies: Tuj, neuron-specific class III β-tublin; SMA, smooth muscle α-actin; AFP, α-fetoprotein (scale bars: 10.0 m). (*G*) Teratomas differentiated from human iPSCs. (*Left*) cartilage (mesoderm); (*Center*) neural tissues (ectoderm); ciliated glandular epithelia (endoderm). All photographs were taken at the same magnification (×200).

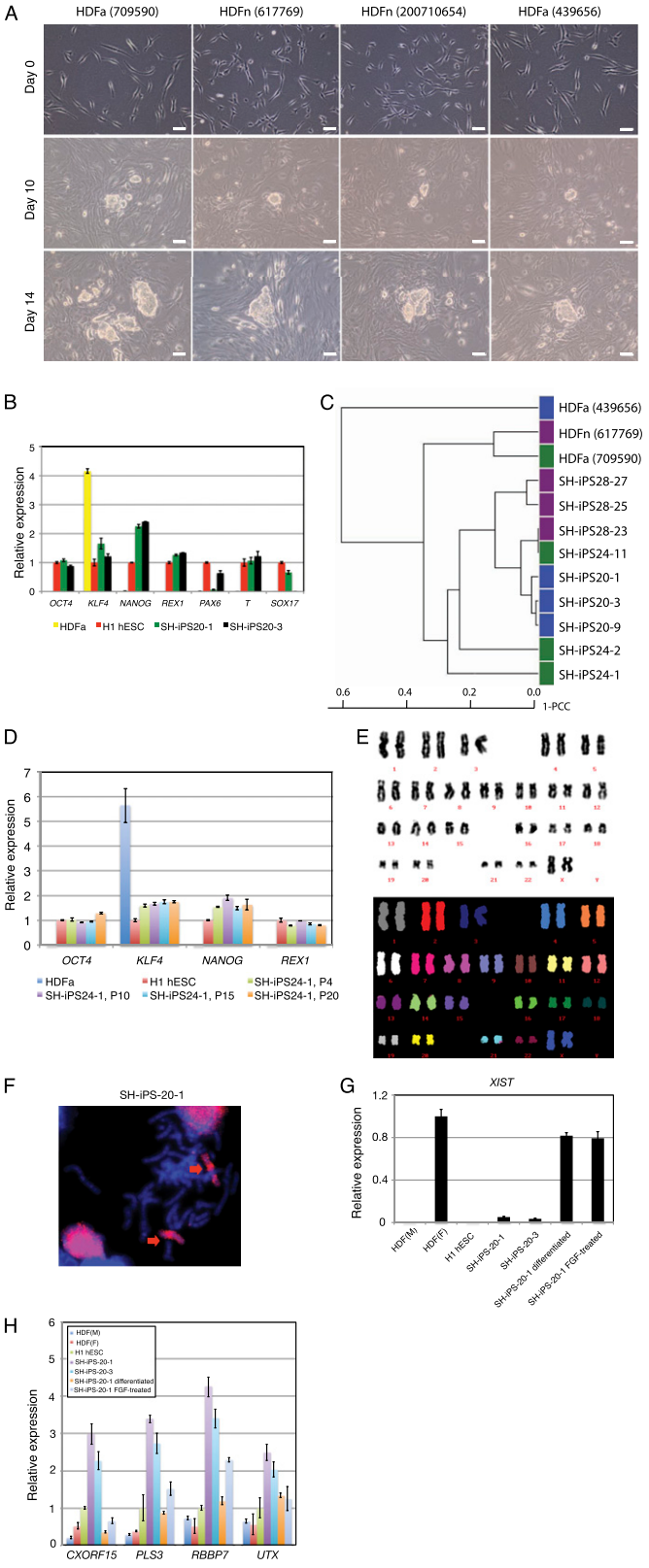

Fig. S6. Dox-independent human iPSCs from adult fibroblast cells. (*A*) Human iPSC colonies formed at two time points (day 10 and day 14 of Dox induction) during reprogramming. HDFa (709590) and HDFa (439656) are two adult primary fibroblast cell lines. HDFn (617769) and HDFn (200710654) are two neonatal primary fibroblast cell lines. *Top*: Parental HDF cell lines used for reprogramming at the day for transfection. These iPSC colonies were growing in M15 plus LIF medium staining. (Scale bars: 10.0 μm.) (*B*) qRT-PCR analysis of key genes in parental HDFa, human iPSCs (passage 10), and H1 hESC cells. SH-iPS20-1 and -3 are two independent iPSC lines derived from a female adult dermal fibroblast line (439656). Expression was relative to GAPDH and normalized against gene expression in H1 hESCs growing in KSR/FGF medium. Error bars are mean ± SD. (*C*) Global gene expression analysis of nine independent SH-iPSC lines derived

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from three individuals (two adults and one neonatal). SH-iPS20-1, -3, and -9 lines were derived from HDFa (439656); SH-iPS24-1, -2, and -11 lines were derived from HDFa (709590); and SH-iPS28-23, -25, and -27 lines were derived from HDFn (617769). 1-PCC, Pearson correlation coefficient. (*D*) qRT-PCR analysis of key pluripotency genes during establishment and passaging of SH-iPS24-1, which was derived from adult fibroblast cells. HDFa, parental fibroblast cells. P4, P10, P15, and P20 are passaging numbers. Expression was relative to *GAPDH* and normalized against gene expression in H1 hESCs growing in KSR/FGF medium. Error bars are mean ± SD. (*E*) Normal karyotype of SH-iPS24-1 cells (female) at passage 20. *Top*: DAPI staining. *Bottom*: Spectral karyotype. (*F*) Detection of two X chromosomes in SH-iPS20-1 cells (red arrows) in FISH analysis using chromosome painting. (*G*) qRT-PCR analysis of *XIST* in SH-iPSCs. *XIST* expression in male dermal fiborblast cells [HDF(M)], female dermal fibroblast cells [HDF(F)], and H1 hES cells (male) were used as the controls. Compared with the parental female fibroblast cells, SH-iPS20-1 and SH-iPS20-3 lines had little *XIST* expression. When the SH-iPSCs were differentiated or grew in FGF-medium for two passages, the levels of *XIST* expression were comparable to that of the parental female dermal fibroblast cells. Error bars are mean ± SD. (*H*) Expression of a subset of X-linked genes in SH-iPSCs and other cells by qRT-PCR. Error bars are mean ± SD.

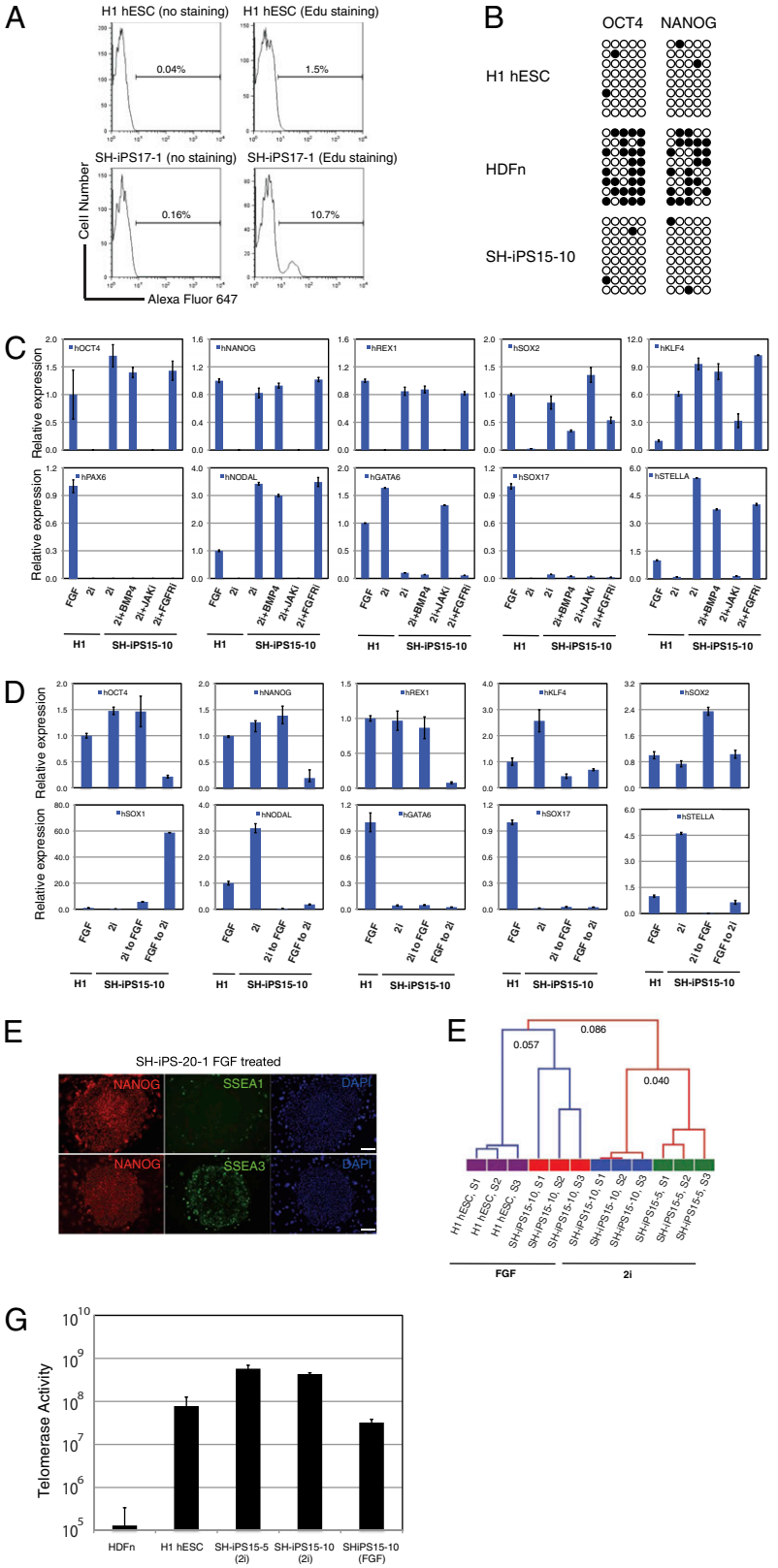

Fig. S7. Signaling dependency and gene expression analysis of human iPSCs growing in various conditions. (*A*) SH-iPSCs (passage 20) maintained in KSR/2i/LIF medium had more dividing cells than H1 hESCs cultured in KSR/FGF (10.7% vs. 1.5%). (*B*) Nearly complete demethylation in the promoters of *OCT4* and *NANOG* in human iPSCs (passage 20). (*C*) Robust expression of pluripotency genes (qRT-PCR) in human iPSCs in the presence of FGFRi, but not if a JAKi was added. H1 human ESCs growing in KSR/FGF or in KSR/2i/LIF medium were used as the controls. H1 human ESCs were differentiated in the KSR/2i/LIF medium. (*D*) Gene expression in the human iPSCs that were cultured in KSR/FGF medium but then switched to KSR/2i/LIF medium. Expression in H1 hESCs growing in KSR/FGF medium was used as the control. Bars are mean ± SD. (*E*) Immunostaining of SH-iPSCs cultured in FGF medium shows that they are SSEA-1–negative (top three

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panels) but SSEA-3–positive (bottom three panels). NANOG staining was also performed to detect pluripotent cells. (Scale bars: 20.0 μm.) (*F*) Global gene expression analysis of SH-iPSCs (passage 40, KSR/2i/LIF), human ESCs, and SH-iPSCs that were cultured first in KSR/2i/LIF medium but then in KSR/FGF. Expression profiles are clustered based on correlation. Three RNA samples (S1–S3) from each growth condition or cell type were used in the array analysis. The numbers represent the correlation value of three levels. (*G*) Telomerase activity in human ESCs, SH-iPSCs (passage 40) cultured in KSR/2i/LIF medium (2i), and the FGF-cultured human iPSCs (FGF). The arbitrary telomerase units of each sample were calculated based on TSR8 control template amplification.

Table S1. Y chromosome polymorphisms in HDFn and human iPSCs

YSTRs	HDFn	HiPS1, 3–1	HiPS1, 3–5	HiPS1, 4–3	HiPS1, 4–5
B_DYS456	14	14	14	14	14
B-DYS389I	12	12	12	12	12
B_DYS390	23	23	23	23	23
B_DYS389II	28	28	28	28	28
G_DYS458	15	15	15	15	15
G_DYS19	15	15	15	15	15
G_DYS385	13, 14	13, 14	13, 14	13, 14	13, 14
Y_DYS393	12	12	12	12	12
Y_DYS391	11	11	11	11	11
Y_DYS439	8	8	8	8	8
Y_DYS635	23	23	23	23	23
Y_DYS392	11	11	11	11	11
R_Y_GATA	11	11	11	11	11
R_DYS437	16	16	16	16	16
R_DYS438	11	11	11	11	11
R_DYS448	20	20	20	20	20

Sixteen human Y chromosome markers were used for the genotyping (copy numbers in parentheses): B_DYS456 (14), B_DYS389I (12), B_DYS390 (23), B_DYS389II (28), G_DYS458 (15), G_DYS19 (15), G_DYS385 (13,14), Y_DYS393 (12), Y_DYS391 (11), Y_DYS439 (8), Y_DYS635 (23), Y_DYS392 (11), R_Y_GATA (11) R_DYS437 (16), R_DYS438 (11), R_DYS448 (20).

Table S2. Summary of experimental data on reprogramming human fibroblast cells

Electroporation	Parental cells	Cell no. seeded per 10-cm plate	DOX induction time, d	No. of DOX-free iPS colonies	No. of picked colonies	No. of established colonies
15–1	HDFn (2007100654)	250,000	16	93	12	8
19–1	HDFn (709590)	250,000	8	2	0	0
19–2	HDFn (709590)	250,000	10	24	0	0
19–3	HDFn (709590)	250,000	12	39	0	0
19–4	HDFn (709590)	250,000	14	56	0	0
19–5	HDFn (709590)	250,000	16	76	0	0
19–6	HDFn (709590)	250,000	18	102	0	0
20–3	HDFa (439656)	250,000	20	204	15	4
21–1	HDFa (439656)	250,000	16	125	9	2
24–2	HDFa (617769)	250,000	14	196	24	6
24–2	HDFa (617769)	250,000	20	148	0	0
28–1	HDFn (709590)	250,000	10	175	18	7
28–1	HDFn (709590)	250,000	16	213	24	12

Table S3. Primers used in cDNA cloning, RT-PCR, and DMR analysis

Primer name	Primer sequence	Purpose
Mouse/human cDNA cloning primers		
mOct4-F-BglII	GAAGATCTCTCCACCTTCCCCATGGCTGGACACC	mOct4 ORF cloning
mOct4-R-EcoRI	CGGAATTCTTGATCAACAGCATCACTGAGCTTC	
mSox2-F-BglII	GAAGATCTCTTGTATAACATGATGGAGACGG	mSox2 ORF cloning
mSox2-R-EcoRI	CGGAATTCTTTCACATGTGCGACAGGGGCAGT	
mKlf4-F-BglII	GAAGATCTCACCATGGCTGTCAGCGACGCTCTGCTC	mKlf4 ORF cloning
mKlf4-R-EcoRI	CGGAATTCACATCCACTACGTGGGATTTAAAA	
mcMyc-F-BglII	GAAGATCTCACCATGCCCCTCAACGTGAACTTCACC	mcMyc ORF cloning
mcMyc-R-EcoRI	CGGAATTCTTATGCACCAGAGTTTCGAAGCTGTTCG	
mRarg-FL/DN-F-EcoRI	GGGAATTCCCGCAGCTACCATGGCCACC	mRarg-FL/DN cloning
mRarg-DN-R-XbaI	GCTCTAGATCATCATCATCCCTTAGTGCTGATGC	mRarg-DN cloning
mRarg-FL-R-XbaI	GCTCTAGAGCCCAACCCCACAACGGGG	mRarg-FL cloning
mRara-FL/DN-F-EcoRI	GGGAATTCGCTGCTTGGCATGGCCAGCA	mRara-FL/DN cloning
mRara-DN-R-XbaI	GCTCTAGATCATCATCATGGGATCTCCATCTTCAA	mRara-DN cloning
mRara-FL-R-XbaI	GCTCTAGAGTGTCGAGGTGGTCATGGGGAT	mRara-FL cloning
mSf1-F-EcoRI	GGGAATTCCCGCGGGCATGGACTATTCGTACG	mSf1 ORF cloning
mSf1-R-XbaI	GCTCTAGAGGCACCCAGGCTCAAGTCTGCTTG	
mLrh1-F-EcoRI	GGGAATTCTTTCGCTAAGAATGTCTGCTAGTTTGG	mLrh1 ORF cloning
mLrh1-R-XbaI	GCTCTAGAGGGGACTTAGGCTCTTTTGGCATGC	
hOCT4-F-EcoRI	GGGAATTCCACCATGGCGGGACACCTGGCTTCAG	hOCT4 ORF cloning
hOCT4-R-XbaI	GCTCTAGAACCTCAGTTTGAATGCATGGGAGAGC	
hSOX2-F-EcoRI	GGGAATTCCACCATGTACAACATGATGGAGACGGAGCTG	hSOX2 ORF cloning
hSOX2-R-XbaI	GCTCTAGATCACATGTGTGAGAGGGGCAGTGTGC	
hKLF4-F-EcoRI	GGGAATTCCACCATGGCTGTCAGTGACGCGCTGCTCCC	hKLF4 ORF cloning
hKLF4-R-XbaI	GCTCTAGATTAAAAATGTCTCTTCATGTGTAAGGCGAG	
hCMYC-F-EcoRI	GGGAATTCCACCATGCCCCTCAACGTTAGCTTCACCAA	hCMYC ORF cloning
hCMYC-R-XbaI	GCTCTAGATCACGCACAAGAGTTCCGTAGCTGTTCAAG	
hLRH1-F-EcoRI	GGGAATTCATGTCTTCTAATTCAGATACTGGGG	hLRH1 ORF cloning
hLRH1-R-XbaI	GCTCTAGATTATGCTCTTTTGGCATGCAAC	
hRARG-F-EcoRI	GGGAATTCATGGCCACCAATAAGGAGCG	hRARG ORF cloning
hRARG-R-XbaI	GCTCTAGATCAGGCTGGGGACTTCAGGCC	
Mouse/human RT-PCR primers		
mOct4-RT-En-F	TCTTTCCACCAGGCCCCCGGCTC	RT-PCR for endogenous mOct3/4
mOct4-RT-En-R	TGCGGGCGGACATGGGGAGATCC	
mSox2-RT-En-F	TTGCCTTAAACAAGACCACGAAA	RT-PCR for endogenous mSox2
mSox2-RT-En-R	TAGAGCTAGACTCCGGGCGATGA	
mKlf4-RT-En-F	GCGAACTCACACAGGCGAGAAACC	RT-PCR for endogenous mKlf4
mKlf4-RT-En-R	TCGCTTCCTCTTCCTCCGACACA	
mNanog-RT-En-F	CAGGTGTTTGAGGGTAGCTC	RT-PCR for mNanog
mNanog-RT-En-R	CGGTTCATCATGGTACAGTC	
mRex1-RT-F	ACGAGTGGCAGTTTCTTCTTGGGA	RT-PCR for mRex1
mRex1-RT-R	TATGACTCACTTCCAGGGGGCACT	
mEsg1-RT-F	GAAGTCTGGTTCCTTGGCAGGATG	RT-PCR for mEsg1
mEsg1-RT-R	ACTCGATACACTGGCCTAGC	
mFgf4-RT-F	CGTGGTGAGCATCTTCGGAGTGG	RT-PCR for mFgf4
mFgf4-RT-R	CCTTCTTGGTCCGCCCGTTCTTA	
mDax1-RT-F	TGCTGCGGTCCAGGCCATCAAGAG	RT-PCR for mDax1
mDax1-RT-R	GGGCACTGTTCAGTTCAGCGGATC	
mGdf3-RT-F	GTTCCAACCTGTGCCTCGCGTCTT	RT-PCR for mGdf3
mGdf3-RT-R	AGCGAGGCATGGAGAGAGCGGAGCAG	
mUtf1-RT-F	GGATGTCCCGGTGACTACGTCTG	RT-PCR for mUtf1
mUtf1-RT-R	GGCGGATCTGGTTATCGAAGGGT	
mOct4-DMR-F	TGGGTTGAAATATTGGGTTTATTT	DMR primers for mOct4 promoter
mOct4-DMR-R	CTAAAACCAAATATCCAACCATA	
mNanog-DMR-F	GATTTTGTAGGTGGGATTAATTGTGAATTT	DMR primers for mNanog promoter
mNanog-DMR-R	ACCAAAAAAAACCCACACTCATATCAATATA	
mRex1-DMR-F	TATATTAATGTTGGAAAAAGTTTAGGTAAT	DMR primers for mRex1 promoter
mRex1-DMR-R	AACTCCTTAAACCCCTCCCTTTTTAAATAA	
hOCT4-RT-En-F	GACAGGGGGAGGGGAGGAGCTAGG	RT-PCR for endogenous hOCT3/4
hOCT4-RT-En-R	CTTCCCTCCAACCAGTTGCCCCAAAC	

Table S3. Cont.

Primer name	Primer sequence	Purpose
hSOX2-RT-En-F	GGGAAATGGGAGGGGTGCAAAAGAGG	RT-PCR for endogenous hSOX2
hSOX2-RT-En-R	TTGCGTGAGTGTGGATGGGATTGGTG	
hKLF4-RT-En-F	ACGATCGTGGCCCCGGAAAAGGACC	RT-PCR for endogenous hKLF4
hKLF4-RT-En-R	GCGTCCTGGGAAGGGAGATCCGGAGC	
hCMYC-RT-En-F	GCGTCCTGGGAAGGGAGATCCGGAGC	RT-PCR for endogenous hCMYC
hCMYC-RT-En-R	TTGAGGGGCATCGTCGCGGGAGGCTG	
hNANOG-RT-F	CAGCCCCGATTCTTCCACCAGTCCC	RT-PCR for hNANOG
hNANOG-RT-R	CGGAAGATTCCCAGTCGGGTTCACC	
hREX1-RT-F	CAGATCCTAAACAGCTCGCAGAAT	RT-PCR for hREX1
hREX1-RT-R	GCGTACGCAAATTAAAGTCCAGA	
hTERT-RT-F	CCTGCTCAAGCTGACTCGACACCGTG	RT-PCR for hTERT
hTERT-RT-R	GGAAAAGCTGGCCCTGGGGTGGAGC	
hDNMT3B-RT-F	TGCTGCTCACAGGGCCCGATACTTC	RT-PCR for hDNMT3
hDNMT3B-RT-R	TCCTTTCGAGCTCAGTGCACCACAAAAC	
hUTX-RT-F	ATTCATAGCAGCGAACAGCC	qRT-PCR for hUTX by SYBR
hUTX-RT-R	CTGGACAGCCGCCTCTT	
hCXORF15-RT-F	TAGCCCACAAATTCCAGCTT	qRT-PCR for hCXORF15 by SYBR
hCXORF15-RT-R	GCCGAAGAGGCGACTGAG	
hRBBP7-RT-F	CCACCAGATGATTCTGCTCA	qRT-PCR for hRBBP7 by SYBR
hRBBP7-RT-R	CTTACCGTTCAGTGGCTTCC	
hPLS3-RT-F	TTGCAAAGGCCTCTTTGAGT	qRT-PCR for hPLS3 by SYBR
hPLS3-RT-R	CCCAGGACTCTGCGACTTTA	

Table S4. Applied Bioscience predesigned and custom-designed TaqMan probes for real-time RT-PCR of mouse and human genes

Assay ID	Target	Applied Bioscience gene name
Predesigned qPCR assays		
Mm00658129_gH	mOct4	POU domain, class 5, homeobox 1
Mm00516104_m1	mKlf4	Kruppel-like factor 4
Mm02384862_g1	mNanog	Nanog homeobox
Mm03053975_g1	mRex1	Zinc finger protein 42
4352341E	β-actin	Mouse ACTB Endogenous Control
Hs00999632_g1	hOCT4	POU class 5 homeobox 1
Hs01053049_s1	hSOX2	SRY (sex determining region Y)-box 2
Hs02387400_g1	hNANOG	Nanog homeobox
Hs00358836_m1	hKLF4	Kruppel-like factor 4
Hs01931905_g1	hSTELLA	Developmental pluripotency associated 3
Hs00415443_m1	hNODAL	Nodal homologue
Hs00232018_m1	hGATA6	GATA binding protein 6
Hs00751752_s1	hSOX17	SRY (sex determining region Y)-box 17
Hs01057642_s1	hSOX1	SRY (sex determining region Y)-box 1
Hs01088114_m1	hPAX6	Paired box 6
Hs01079824_m1	hXIST	X (inactive)-specific transcript
4326317E	GAPDH	Human GADPH endogenous control
Custom qPCR assays		
Assay ID	Primer/probe name	Primer/probe sequence
hCMYC-endo	hCMYC-taq-endo-F	CCTGAGCAATCACCTATGAACTTG
	hCMYC-taq-endo-R	TTATGCCCAAAGTCCAATTTGA
	hCMYC-taq-endo-probe	CAAATGCAACCTCACAACCTTGGCTG
hKLF4-endo	hKLF4-taq-endo-F	TTTCACACTGTCTTCCCGATGA
	hKLF4-taq-endo-R	TCCTGATTATCCACTCACAAGATGA
	hKLF4-taq-endo-probe	CCAGCCAGAAAGCACTACAATCATGGTCAA
hOCT4-endo	hOCT4-taq-endo-F	CACTGTACTCCTCGGTCCCTTTC
	hOCT4-taq-endo-R	CAACCAGTTGCCCCAAACTC
	hOCT4-taq-endo-probe	CTTTCCCCCTGTCTCCGTCACCACTC
hSOX2-endo	hSOX2-taq-endo-F	ACAGCAAATGACAGCTGCAAA
	hSOX2-taq-endo-R	AAGTCCAGGATCTCTCTCATAAAAGTTT
	hSOX2-taq-endo-probe	ATCCACACTCACGCAAAAACCGCG
hLRH1-endo	hLRH1-taq-endo-F	AAAGCTGAACTGAAACAATTCTCAAG
	hLRH1-taq-endo-R	GCTCGGGCCTTCAAAGGA
	hLRH1-taq-endo-probe	TGCATCAGCTGTACCTACAATAGCCCCTCC
hRARG-endo	hRARG-taq-endo-F	GAGCCTGGGTTTGGACTCTAAA
	hRARG-taq-endo-R	CCTCTTGCCCTGGGAAGTCT
	hRARG-taq-endo-probe	CTCAGCACTGCCCCATGGGTCC

Dataset S1. A list of mouse gene promoters that contain the putative RAREoct element

Dataset S1 (XLS)

Dataset S2. A list of human gene promoters that contain the putative RAREoct element

Dataset S2 (XLS)

Dataset S3. Global gene expression analysis of iPSC lines from human neonatal and adult fibroblast cells

Dataset S3 (TXT)

Dataset S4. Microarray analysis of gene expression in human ES cells, FGF-cultured human iPSCs and human iPSCs growing in 2i/LIF medium

Dataset S4 (TXT)