**Reprogramming of mouse and human somatic cells by high performance engineered factors**

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# SUPPLEMENTARY INFORMATION

## Methods

**Plasmid construction.** Full-length cDNAs of murine and human Oct4, Sox2 and Nanog genes were ligated with cDNA encoding the VP16 activation domain (amino acids 446-490; from MLGDG to DEYGG) with a region encoding a glycine-rich linker (TSGLGGGSGGGGSGGGGSG, for Oct4 and Sox2) or without the linker (Nanog). Fusion genes were cloned into retroviral vector pMXs (Takahashi and Yamanaka, 2006) and inducible lentiviral vector pLV-TRE-EF1a-GFP (Wu et al., 2009). For episomal vector construction, coding sequences for OCT4-VP16 (X), KLF4, SOX2-VP16 (Y) and NANOG-VP16 (Z) were linked in this order with 2A elements (Okita et al., 2008) and subcloned into pCEP4 (Invitrogen).

**Cell culture.** Mouse ES cells and iPS cells were maintained on feeder layers of mitomycin C-treated mouse embryonic fibrobla 4) were used for sts (MEFs) in DMEM (Invitrogen) supplemented with 15% heat-inactivated fetal bovine serum (FBS, Invitrogen), 2 mM L-glutamine, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, 0.1 mM β-mercaptoethanol (Sigma), 1000 units/ml leukemia inhibiting factor (LIF, Chemicon) and 50 units/50 mg/ml penicillin/streptomycin. *Oct4*-GFP MEFs were prepared from E13.5 embryos obtained from the intercross between male TgOG2 transgenic mice (Szabo et al., 2002) or *Oct4*-GFP knock-in mice (Lengner et al., 2007) and female wild-type C57BL/6 mice. MEFs were grown in DMEM supplemented with 10% FBS (Hyclone), 2 mM L-glutamine, 0.1 mM non-essential amino acids, and 100 units/100 mg/ml penicillin/streptomycin. MEFs in early passages (up to passage generation of iPS cells.

Human ES and iPS cells were maintained on feeder layers in DMEM supplemented with 20% Knockout Serum Replacement (KSR, Invitrogen), 2 mM L-glutamine, 0.1 mM

non-essential amino acids, 0.1 mM β-mercaptoethanol, 4 ng/ml basic-FGF (Invitrogen) and 100 units/100 mg/ml penicillin/streptomycin. Human foreskin fibroblasts were from a 25-yr-old normal male and cultured in DMEM with 10% FBS and 100 units/100 mg/ml penicillin/streptomycin.

**Retroviral production and mouse iPS induction.** Retroviral production and infection followed the previously published protocol (Takahashi et al., 2007). Plat-E cells were seeded at $7\times10^6$ cells per 100-mm dish. On the next day, 9 μg pMXs-based retroviral vectors were transfected into Plat-E cells using Lipofectamine 2000 reagents (Invitrogen) according to the manufacturer's recommendations. After overnight transfection, the medium was replaced. Another 48 h later, virus-containing supernatants were collected and filtered through a 0.45 μm PVDF filter (Millipore), supplemented with 4 μg/ml polybrene (Sigma). *Oct4*–GFP MEF cells (seeded at $5\times10^4$ cells per well in 6-well plate) were incubated with virus-containing supernatants for 12 h. Two days after infection, the medium was changed to mouse ES medium. Eight days after infection, the transduced *Oct4*–GFP MEF cells were re-plated onto mitomycin-C-treated MEF feeder layers at $5\times10^4$ cells per well in 6-well plate. About seven days after re-plating, numbers of GFP positive and alkaline phosphatase positive colonies were scored. Alkaline phosphatase staining was performed with NBT/BCIP (Roche) according to manufacturer's instructions. For the generation of single-factor mouse iPS cells, optimized medium was used as described (Chen et al., 2010).

**Generation of iPS cells from MEFs with episomal vector.** For reprogramming with oriP/EBNA1-based episomal vector, 5 μg of episomal plasmid pCEP4-XKYZ were transfected into $1\times10^6$ MEFs via nucleofection (Amaxa). Transfected MEFs were directly

plated on 2×10-cm feeder-seeded dishes with no drug selection. On day 2 posttransfection, the culture medium was replaced with an optimized culture medium (Chen et al., 2010). Culture medium was changed every other day with no drug selection. GFP-positive colonies with morphology similar to ES were visible on day 18 post-transfection and picked for characterization.

**Lentiviral production and human iPS induction**. Lentiviral vector (10 μg) together with PAX2 (7.5 μg) and VSV-G (2.5 μg), were co-transfected into 293T cells with the $Ca_3(PO_4)_2$-method in 10-cm dishes and incubated overnight. 12 hours later, the medium was changed and virus was collected after subsequent 36 hours cultivation. Viral supernatant was filtered through a 0.45 μm PVDF filter (Millipore), and supplemented with 4 μg/ml polybrene (Sigma). $1 \times 10^5$ human foreskin fibroblasts (HFFs) seeded in a 6-cm dish were infected with lentiviral supernatants overnight and then cultured in HFF medium supplemented with doxycycline (Sigma) to 1 μg/μl. Three days post infection transduced HFFs were re-plated onto mitomycin-C-treated MEF feeder layers at 1:3 ratio and were changed to human ES medium supplemented with 1 μg/μl doxycycline. Two weeks post infection, doxycycline was withdrawn. Around three weeks after infection, iPS colonies were picked and alkaline phosphatase positive, hES-like colonies were scored. The experiments were repeated more than 3 times.

**Immunofluorescence analysis.** Cells were fixed with 4% paraformaldehyde for 30 min and then permeabilized with 0.2% Triton X-100 for 45 min followed by blocking with 2% BSA (Sigma). Cells were incubated in primary antibody overnight at 4°C and secondary antibody at room temperature for 1 h. The following antibodies were used: SSEA1 (Santa Cruz), SSEA4 (R&D), Nanog (Chemicon), Oct4 (Santa Cruz), SOX2

(R&D), TRA-1-60 (Chemicon), TRA-1-81 (Chemicon), FOXA2 (abcam), SOX17 (Santa Cruz), SMA (AbboMax), BRACHYURY (abcam), GFAP (Dako), β-TUBULIN (Covance). Alkaline phosphatase staining was performed with the Vector Red substrate kit (Vector Laboratories).

**Generation of chimeras and tetraploid embryo complementation.** To generate chimeras, iPS cells were injected into ICR E3.5 blastocysts. To generate mice by tetraploid embryo complementation, two-cell embryos were collected from oviducts of ICR females, and electro-fused to produce one-cell tetraploid embryos that were then cultured in KSOM medium. About ten to fifteen iPS cells were injected into the tetraploid blastocyst cavity. The blastocysts were kept in KSOM with amino acids until embryo transfer. Fifteen to twenty injected blastocysts were transferred to uterine horn of 2.5-days-postcoitum pseudopregnant ICR females. Embryos derived from tetraploid blastocyst injection (4N) were dissected at E13.5. One iPS cell line (XSKZ #4) was tested for tetraploid complementation. From 40 tetraploid blastocysts injected with the iPS cells, we obtained one live E13.5 embryo but no live-born mice.

**Karyotype analysis of human iPS cells.** Human iPS cells were treated with 0.1 μg/ml colcemid (Invitrogen) for 3 hours at 37°C and then were trypsinized and resuspended in 0.075 M of KCl for 20 minutes. The hypotonic solution-treated cells were then fixed in methanol: acetic acid (3:1) for 30 minutes at room temperature. The cells were then placed onto pre-cleaned slides and stained with Giemsa. Metaphase spreads were counted and calculated.

**In vitro differentiation of human iPS cells.** For EB formation, human iPS cells (lines #6) were harvested by treating with collagenase IV. The clumps of the cells were

transferred to low attachment dish in DMEM/F12 containing 20% Knockout Serum Replacement, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 0.1 mM β-mercaptoethanol. The medium was changed every other day. After 8 days in a floating culture, EBs were transferred to gelatin-coated plate and cultured in the same medium for another 8 days.

**Teratoma formation of human iPS cells.** For teratoma formation, human iPS cells (lines #3 and #6) were harvested by collagenase IV treatment, collected into tubes, and the pellets were resuspended in DMEM/F12. The cells from a confluent 60 mm dish were injected subcutaneously to dorsal flank of a NOD-SCID mouse. Eight weeks after injection, tumors were dissected and fixed with PBS containing 4% paraformaldehyde. Paraffin-embedded tissue was sliced and stained with hematoxylin and eosin.

**Western analysis.** MEFs were infected with reprogramming factor : pMIG retroviruses at 2:1, and cell lysates were collected three days after infection. Primary antibodies included anti-Oct4 (Santa Cruz), Nanog (Chemicon), Sox2 (Chemicon), Flag (Sigma), VP16 (Clontech), GFP (Santa Cruz), p53 (Santa Cruz), p21 (Santa Cruz), p16 (Santa Cruz) and β-actin (Sigma).

**RT–PCR.** Total RNA was isolated using TRIZOL (Invitrogen) and 1 μg was used to synthesize cDNA using the ReverTra Ace First-Strand cDNA synthesis kit (Toyobo) according to manufacturer's protocols. PCR was performed with primer sets corresponding to Table S3. For quantitative RT–PCR, EvaGreen (Stratagene) was used.

**DNA microarray.** Total RNA from *Oct4-GFP* MEFs, J1 ES cells and iPS cells (clone XSKZ #4) were labeled with Phycoerythrin. Samples were hybridized to a Mouse Genome 430 2.0 Array (Affymetrix) according to the manufacturer's protocol. Arrays

were scanned with a Gene array Scanner 3000 (Affymetrix). Data were analyzed using Affymetrix GCOS1.2 software.

**Bisulfite genomic sequencing.** Genomic DNA was treated with sodium bisulfite and subjected to nested PCR as previously described(Li et al., 2007). For sequencing analysis, the PCR products were cloned into T-vectors (Takara) and individual clones were sequenced.

**Flow Cytometry.** Cultures were harvested and single-cell suspensions were obtained by repetitive pipetting and transferring through a 40 μm cell strainer. Cells were incubated with Alexa Fluor® 647 anti-mouse SSEA-1 (BioLegend) and sorted/analyzed on a FACSAria (BD Biosciences). Data were analyzed with FlowJo software (Tree Star).

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**Supplementary Table S1.** Summary of synthetic factors tested for reprogramming of OG2/Oct4-GFP reporter MEFs.

| Fusion moieties | | | | Effect on iPS colony formation |
|---|---|---|---|---|
| **Transcription factor** | **Linker Sequence** | **Regulator and their location** | **Flag location** | |
| GCNF (1-266) | RS | VP16 AD (446-490) in C-terminal | C | no |
| RARα (1-167) | | | | no |
| PPARγ (31-183) | | | | no |
| SF-1 (1-96) | | | | no |
| LRH-1 (1-129) | | | | no |
| Nanog | | | | + |
| Oct4 | $G(SGGGG)_2SG$ GGLGSTEF | VP16 AD (446-490) in N-terminal | N | ++ |
| Sox2 | | | | + |
| Nanog | | | | + |
| Oct4 | RSTSGLGGGS $(GGGGS)_2G$ | VP16 AD (446-490) in C-terminal | C | ++ |
| Sox2 | | | | + |
| Nanog | | | | + |
| Klf4 | | | | no |
| Oct4 | QLTSGLGGGS $(GGGGS)_2G$ | Three tandem VP16 AD (446-490) in C-terminal | N | +++ |
| Klf4 | | Engrailed repressor (2-298) in C-terminal | | – |
| Oct4 | | | | – |

"+" denotes stimulative effect; "−" denotes inhibitive effect.

For transcription factors (TFs) GCNF, RARα, PPARγ, SF-1 and LRH-1 that reportedly regulate the *Oct4* promoter, only their DNA binding domains were used to fuse with VP16. The effect of their addition was determined in the MEF reprogramming assay based on Oct4, Klf4 and Sox2. For other TFs, full-length ORFs were used to fuse with an activation or a repressor domain. Their effect was determined in comparison to their respective unmodified factor in the OKS three-factor reprogramming assay (for Oct4, Klf4 and Sox2 fusions) or in the OKSN four-factor assay (for the Nanog fusions) in which the unmodified factor was replaced. All fusions were cloned into the retroviral vector pMXs and verified by sequencing. Protein expression of each fusion in transduced MEFs was confirmed by Western analysis using antibody against the Flag epitope and at least one other antibody against a fusion partner (also refer to the Supplementary Fig S1 and Methods). All transcription factors were from mouse except RARα from human. There was no significant difference when VP16 was fused to the N- or C-terminal of Oct4, Sox2, and Nanog. The requirement for linker sequence between the transcription factor and the regulatory moiety in each fusion remains undetermined.

**Supplementary Table S2. Summary of chimera formation and germline transmission from injections of XYKZ iPS cells into diploid blastocysts.**

| cell lines | genetic background | blastocysts injected | mice born | live chimeras | chimerism | | germline transmission (number*) |
|---|---|---|---|---|---|---|---|
| | | | | | >50% | <50% | |
| XYKZ #1 | OG2 with Oct4-GFP transgene (C57/CBA) | 57 | 41 | 29 | 16 | 13 | no** |
| XYKZ #2 | | 31 | 24 | 6 | 4 | 2 | no |
| XYKZ #3 | | 47 | 9 | 5 | 3 | 2 | no |
| XYKZ #4 | | 41 | 8 | 5 | 2 | 3 | yes (1) |
| XYKZ #5 | | 44 | 6 | 5 | 2 | 3 | no |
| XYKZ #6 | | 53 | 15 | 5 | 1 | 4 | no |
| XYKZ #7 | *Oct4-GFP* Knock-in (C57/129) | 60 | 20 | 17 | 15 | 2 | yes (4) |
| XYKZ #8 | | 61 | 16 | 9 | 5 | 4 | yes (2) |

* "number" in bracket indicates the number of chimeric mice that showed germline transmission.

** "no" indicates there was no germline transmission from the cell line until submission of the manuscript.

**Supplementary Table S3.** Primers for PCR reactions.

| Gene name | Forward primer | Reverse primer |
|---|---|---|
| For mouse iPS RT-PCR | | |
| Endo-*Oct4* | TCTTTCCACCAGGCCCCCGGCTC | TGCGGGCGGACATGGGGAGATCC |
| Endo-*Sox2* | TAGAGCTAGACTCCGGGCGATGA | TTGCCTTAAACAAGACCACGAAA |
| Endo-*Nanog* | TAGGCTGATTTGGTTGGTGTCTTG | AGTGTGATGGCGAGGGAAGG |
| Oct4-GFP | AGAAGAACGGCATCAAGG | GCTCAGGTAGTGGTTGTC |
| *Esg1* | GAAGTCTGGTTCCTTGGCAGGATG | ACTCGATACACTGGCCTAGC |
| *Dax1* | TGCTGCGGTCCAGGCCATCAAGAG | GGGCACTGTTCAGTTCAGCGGATC |
| *eRas* | ACTGCCCCTCATCAGACTGCTACT | CACTGCCTTGTACTCGGGTAGCTG |
| *Rex1* | ACGAGTGGCAGTTTCTTCTTGGGA | TATGACTCACTTCCAGGGGGCACT |
| *Zfp296* | CCATTAGGGGCCATCATCGCTTTC | CACTGCTCACTGGAGGGGGCTTGC |
| *Ecat1* | TGTGGGGCCCTGAAAGGCGAGCTGAGAT | ATGGGCCGCCATACGACGACGCTCAACT |
| *Thy1* | AGAAGGTGACCAGCCTGACA | GTTCTGAACCAGCAGGCTTA |
| *Dnmt3a2* | CTCACACCTGAGCTGTACTGCAGAG | CTCCACCTTCTGAGACTCTCCAGAG |
| *Dnmt3b* | TTCAGTGACCAGTCCTCAGACACGAA | TCAGAAGGCTGGAGACCTCCCTCTT |
| *Dnmt3L* | GTGCGGGTACTGAGCCTTTTTAGA | CGACATTTGTGACATCTTCCACGTA |
| *Ink4a* | GTGTGCATGACGTGCGGG | GCAGTTCGAATCTGCACCGTAG |
| *Arf* | GCTCTGGCTTTCGTGAACATG | TCGAATCTGCACCGTAGTTGAG |
| *Gapdh* | AGTCAAGGCCGAGAATGGGAAG | AAGCAGTTGGTGGTGCAGGATG |
| For Quantitative PCR | | |
| Viral-*X* | TCTCCCATGCATTCAAACTG | CTTTTATTTTATCGTCGACC |
| Viral-*Y* | CTGCCCCTGTCGCACATGTG | CTTTTATTTTATCGTCGACC |
| Viral-*Z* | CATCGCAGCTTGGATACAC | GCATTGATGAGGCGTTCC |
| Viral-*Klf4* | CCTTACACATGAAGAGGCAC | CTTTTATTTTATCGTCGACC |
| *Oct4* | TCTTTCCACCAGGCCCCCGGCTC | TGCGGGCGGACATGGGGAGATCC |
| *Sox2* | TAGAGCTAGACTCCGGGCGATGA | TTGCCTTAAACAAGACCACGAAA |
| *Nanog* | CTCAAGTCCTGAGGCTGACA | TGAAACCTGTCCTTGAGTGC |
| *Dppa3* | TGTGGAGAACAAGAGTGA | CTCAATCCGAACAAGTCTT |
| *Rex1* | CCCTCGACAGACTGACCCTAA | TCGGGGCTAATCTCACTTTCAT |
| *Dnmt3L* | GTGCGGGTACTGAGCCTTTTTAGA | CGACATTTGTGACATCTTCCACGTA |
| *Gtl2* | TCCTCACCTCCAATTTCCCCT | GAGCGAGAGCCGTTCGATG |
| *β-actin* | GAAATCGTGCGTGACATCAAAG | TGTAGTTTCATGGATGCCACAG |
| For bisulfite PCR | | |
| *Oct4*-outside | GAGGATTGGAGGTGTAATGGTTGTT | CTACTAACCCATCACCCCCACCTA |
| *Oct4*-inside | CAAGCTTTGGGTTGAAATATTGGGTTTATTT | CGGATCCCTAAAACCAAATATCCAACCATA |
| *Nanog*-outside | AAGTATGGATTAATTTATTAAGGTAGTT | AAAAAACCCACACTCATATCAATATA |
| *Nanog*-inside | AAGTATGGATTAATTTATTAAGGTAGTT | CAACCAAATCAACCTATCTAAAAA |
| For human iPS RT-PCR | | |
| Endo-*OCT4* | GACAGGGGGAGGGGAGGAGCTAGG | CTTCCCTCCAACCAGTTGCCCCAAAC |
| Endo-*SOX2* | GGGAAATGGGAGGGGTGCAAAAGAGG | TTGCGTGAGTGTGGATGGGATTGGTG |
| Endo-*NANOG* | CAGCCCCGATTCTTCCACCAGTCCC | CGGAAGATTCCCAGTCGGGTTCACC |
| *Rex1* | CAGATCCTAAACAGCTCGCAGAAT | GCGTACGCAAATTAAAGTCCAGA |
| *DPPA5* | ATATCCCGCCGTGGGTGAAAGTTC | ACTCAGCCATGGACTGGAGCATCC |
| *GDF3* | CTTATGCTACGTAAAGGAGCTGGG | GTGCCAACCCAGGTCCCGGAAGTT |
| *ECAT15-1* | GGAGCCGCCTGCCCTGGAAAATTC | TTTTTCCTGATATTCTATTCCCAT |
| *ECAT15-2* | CCGTCCCCGCAATCTCCTTCCATC | ATGATGCCAACATGGCTCCCGGTG |
| *GAPDH* | TGTTGCCATCAATGACCCCTT | CTCCACGACGTACTCAGCG |

| For DNA integration screen | | |
|---|---|---|
| *X* (360 bp) * | AGCGACTATGCACAACGAGAGGA | GGCCATATCCAGAGCGCCGT |
| *Y* (395 bp) | GCTCTTGGCTCCATGGGTTCGG | TCGGCCATATCCAGAGCGCCG |
| *NANOG* (462 bp) | CTGCTGAGATGCCTCACACG | ACATTGGAAGGTTCCCAGTCG |
| *VP16* (135 bp) | CGGGATCCATGTTGGGGGACGGG | CGGGAATTCCCCACCG |
| *KLF4* (300 bp) | GTCTCTTCGTGCACCCACTT | ACGATCGTCTTCCCCTCTTT |
| *EBNA-1* (300 bp) | CACCCTCATCTCCATCACCT | AGTCGTCTCCCCTTTGGAAT |
| *Hygromycin* (322 bp) | GTGTCACGTTGCAAGACCTG | ACATTGTTGGAGCCGAAATC |

* The number in bracket indicates the size of the PCR fragment that primers would amplify.

**Supplementary figure legends:**

**Fig S1.** Protein expression of synthetic transcription factors in mouse embryonic fibroblasts. Individual synthetic factors on retroviral vectors were transduced alone (**A**) or in combination together with Klf4 (**B**) into MEFs as indicated. Protein expression was analyzed by western blotting with indicated antibodies. Co-transduced GFP served as a control.

**Fig S2.** Comparison of reprogramming kinetics and efficiency between the synthetic and native factors.

**(A)** Kinetics of reactivation of SSEA-1 and Oct4-GFP in MEFs during reprogramming. OG2 MEFs transduced with two different sets of reprogramming factors (OSKN or XYKZ) were collected at day 6, 9, 12, immunostained with anti-SSEA-1, and analyzed by FACS. MEFs untransduced and iPS cells of an established line were used for comparison. The number in quadrants indicates the percentage of each subpopulation. (**B**) OG2-MEFs were infected by XYKZ (synthetic factor combination) or OSKN (combination of native factors) together with a DsRed construct (for labeling infected cells and monitoring retroviral silencing). 2 days later DsRed positive MEFs were sorted into 96-well plates (one cell per well) coated with feeder cells. For each factor combination, ten 96-well plates were seeded. At day 10 and day 20 after seeding, GFP+/DsRed− and GFP+/DsRed+ iPS colonies were counted. 'GFP+' reflected reactivation of the endogenous *Oct4* while 'DsRed−' reflected the silencing of retroviral constructs.

**Fig S3.** Characterization of iPS cells generated with synthetic factors.

(**A**) iPS cells generated with Klf4 and synthetic factors X, Y and Z exhibited typical ES cell morphology by phase contrast, expressed *Oct4*-GFP homogeneously and were positive for AP staining. DAPI is used as a nuclear counterstain. Scale bars, 200 μm.

(**B**) Immunofluorescence staining of XYKZ iPS cells for the pluripotency markers SSEA-1 and Nanog. Scale bars, 200 μm.

(**C**) RT-PCR analysis of key ES cell marker genes in XYKZ iPS cell lines, ES cells and MEFs. *GAPDH* was used as a loading control.

(**D**) Quantitative RT-PCR specific for viral transcripts in MEFs 4 d after viral infection and in six established XYKZ iPS cell lines ($n$=3). *Actin* was used as input control. Data were normalized to the expression levels in MEFs.

(**E**) DNA methylation analysis by bisulfite sequencing in the promoter regions of *Oct4* and *Nanog* in an iPS cell line (XYKZ #1), ES cells and MEFs. Open circles indicate unmethylated CpG dinucleotides, while black circles indicate methylated CpGs.

(**F-H**) Scatter plots comparing global gene expression profiles.

(**F**) iPS cells vs MEFs; (**G**) iPS cells vs ES cells; (**H**) ES cells vs MEFs. Positions of Oct4, Sox2, Nanog, Klf4, c-myc and Lin28 are marked with arrows. All comparisons are using a synthetic factor-derived MEF iPS cell line (XSKZ #4). Numbers along axes represent folds of changes in gene expression. Parallel lines indicate the linear equivalent (center) and ±two fold changes in gene expression levels between the samples.

**Fig S4.** Characterization of episome-based mouse iPS cells.

(**A**) Southern blotting analysis of plasmid integrations in episome-based mouse iPS cells. Genomic DNA (15 μg) extracted from episomal iPS (lines #1-5, 11 and 14) and OG2-MEF cells was digested with *Eco*RV for blotting with transgene-specific probes corresponding to each human full-length gene or mouse *Klf4*. Episomal vector pCEP4-XKYZ diluted to the equivalents of 0.2 and 1-copy integration per genome was used as positive controls (0.2× and 1×). Note that the weak band of 8.3 kb (open arrow) is likely to be derived from cross-hybridization of the human *OCT4* probe to the mouse homologous gene, expected to be generated by *Eco*RV digestion, as it is present in MEFs. The detection of the endogenous mouse *Klf4* (a 10.5-kb band indicated with a black arrow) served as a positive control for the Southern experiment.

(**B**) Immunostaining of Oct4, Nanog and SSEA-1 in episomal iPS cells (upper panels). Lower panels show DAPI counterstaining. Scale bars, 100 μm.

(**C**) Quantitative RT-PCR analysis of pluripotent marker gene expression in episomal iPS cell lines compared to MEFs and ES (J1 and MPI-II). Expression values were relative to that in ES-J1. $n = 3$.

(**D, E**) Comparisons of global gene expression profile of episome-based mouse iPS cells (line #2) with those of MEFs and ES cells. (**D**) iPS cells vs MEFs; (**E**) iPS cells vs ES cells.

(**F**) Normal karyotype of episome-based mouse iPS cells from line #2.

**Fig S5.** Generation of iPS cells with a single synthetic factor, Oct4-VP16.

(**A**) Kinetics of reprogramming induced by Oct4-VP16 and Oct4-3×VP16. MEFs transduced with Oct4 and Oct4-VP16 fusions were cultured in an optimized

medium(Chen et al., 2010). GFP- positive colonies were scored from D9 to D17 (n=3). Error bars indicate s.d.

(**B**) Morphology representative of primary iPS colonies (P0) induced by Oct4-VP16 and the iPS cell lines (P3) derived from these colonies. Scale bars, 250 μm.

(**C**) Immunostaining of Oct4, Nanog and SSEA-1 expression in Oct4-VP16 iPS cells (upper panels). Lower panels show DAPI counterstaining. Scale bars, 100 μm.

(**D**) Quantitative RT-PCR analysis of pluripotent marker gene expression in Oct4-VP16 iPS cells compared to MEFs and an ES cell line (R1). Expression values were relative to that in MEFs. $n = 3$.

(**E**) Confirmation of the derivation of Oct4-VP16 iPS cell lines (O1-1, O1-2 and O2-1). The presence of the retroviral *Oct4* transgene as indicated on the left was examined by genomic PCR. Two 2-factor lines (OK-4 and OS-1) with their control PCRs (*Sox2* and *Klf4*) are also shown for comparison.

(**F**) Oct4-VP16 iPS cells contribute to chimera (black arrow) that exhibits germline transmission (white arrow). The agouti and black coat colors were derived from iPS cells (O2-1) with a mixed genetic background (C57 1/4, CBA 1/4 and 129 1/2) injected into a recipient embryo of ICR background.

**Fig S6.** Normal karyotype of XYKZ-based human iPS cells generated from HFFs. Two hiPS cell lines (XYKZ #3 and #6) were examined by karyotyping. One representative metaphase chromosome spread of undifferentiated iPS cells with a normal set of 46 human chromosomes is shown.

**Fig S7.** Ectopic expression of the synthetic factors did not affect p53 and p21 levels. Western analysis was performed on protein extracts from MEFs three days after retroviral transduction. Factor M is c-Myc.

Figure S1

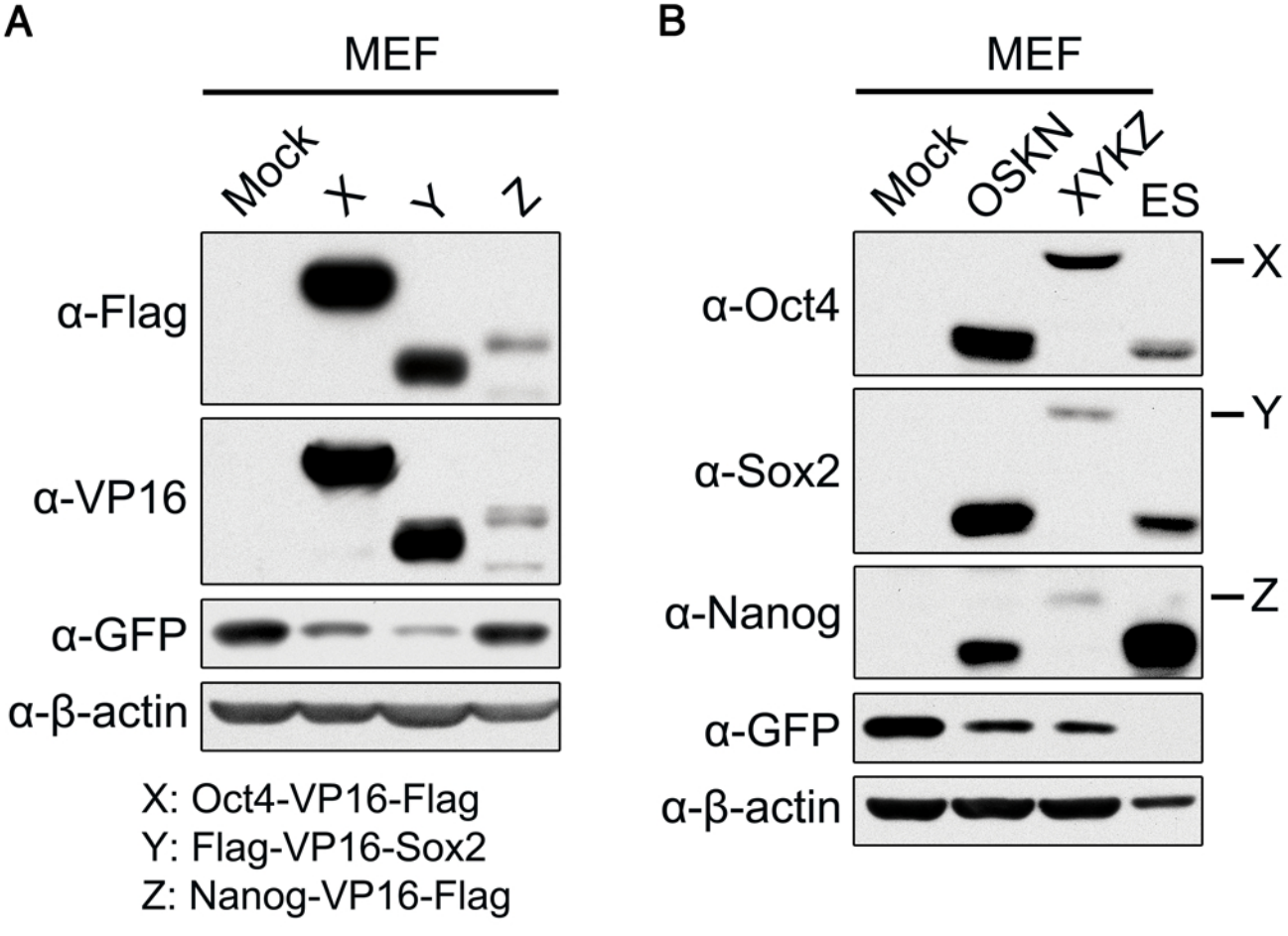
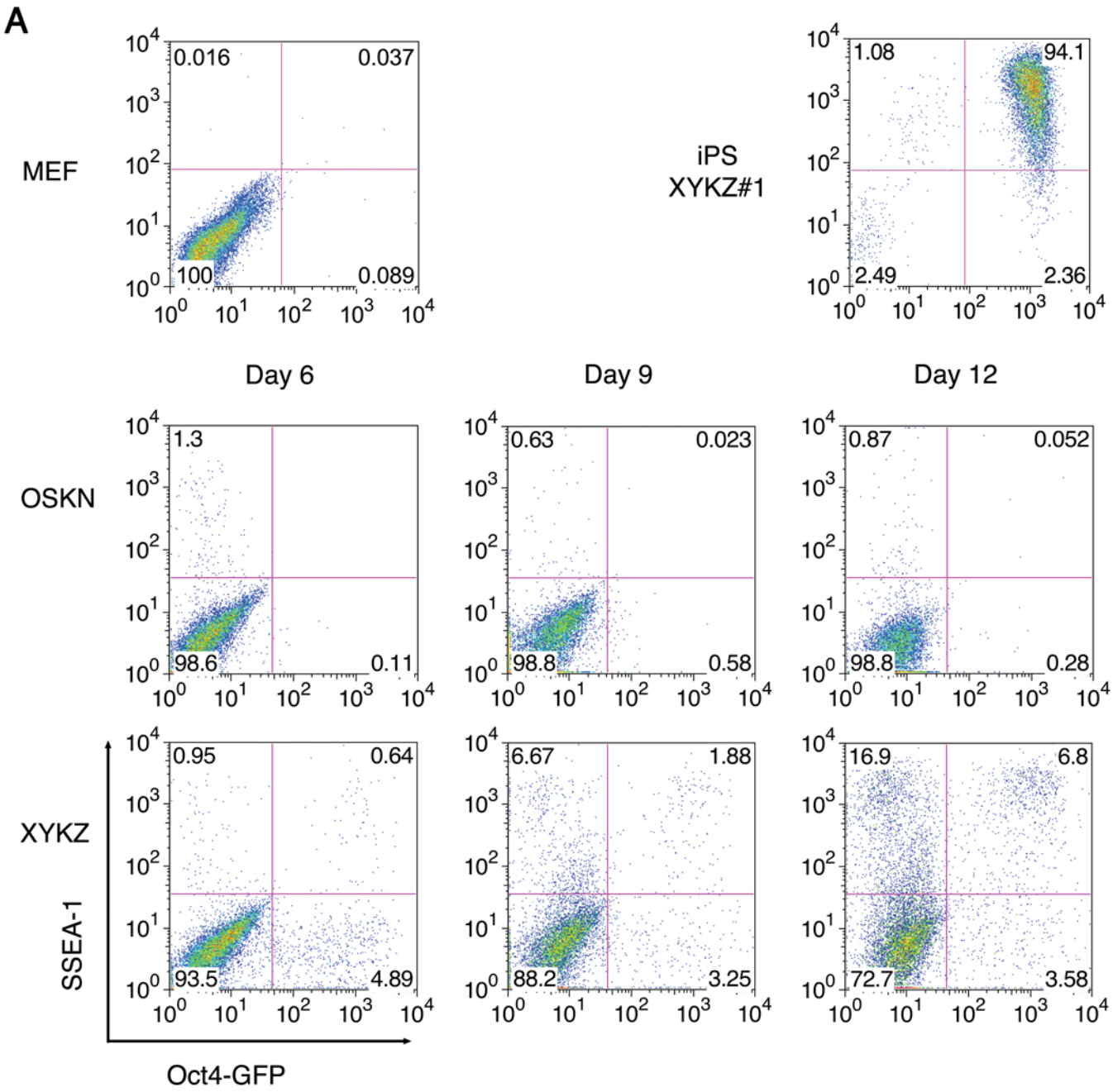

A
MEF
Mock
X
Y
Z
α-Flag
α-VP16
α-GFP
α-β-actin
X: Oct4-VP16-Flag
Y: Flag-VP16-Sox2
Z: Nanog-VP16-Flag
B
MEF
Mock
OSKN
XYKZ
ES
α-Oct4
α-Sox2
α-Nanog
α-GFP
α-β-actin
X
Y
Z

Figure S2


A
MEF
0.016
0.037
100
0.089
iPS
XYKZ#1
1.08
94.1
2.49
2.36
Day 6
Day 9
Day 12
OSKN
1.3
98.6
0.11
0.63
0.023
98.8
0.58
0.87
0.052
98.8
0.28
XYKZ
0.95
0.64
93.5
4.89
6.67
1.88
88.2
3.25
16.9
6.8
72.7
3.58
SSEA-1
Oct4-GFP

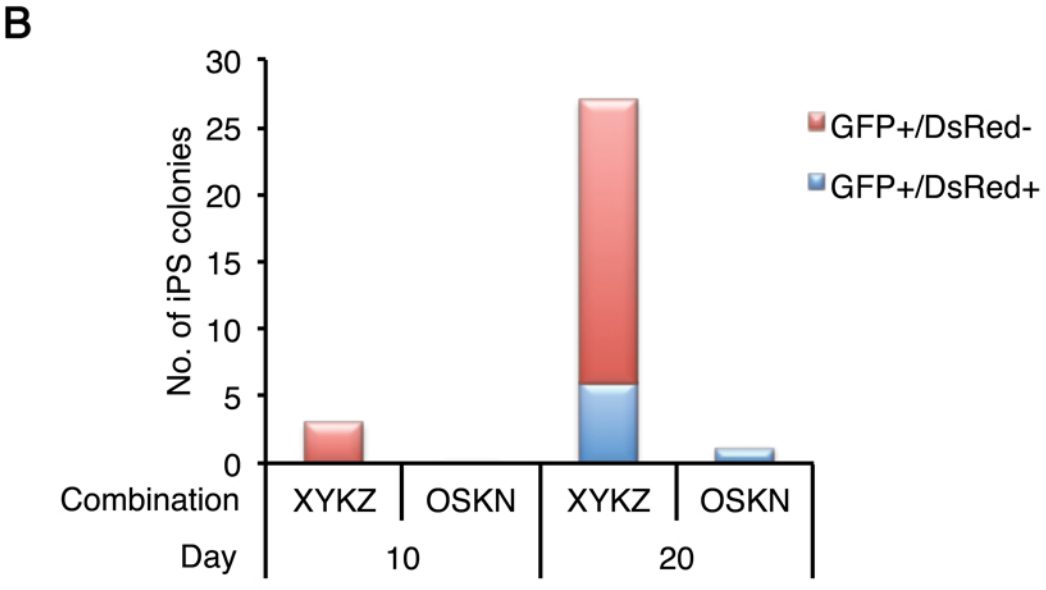

B
No. of iPS colonies
30
25
20
15
10
5
0
GFP+/DsRed-
GFP+/DsRed+
Combination
XYKZ
OSKN
XYKZ
OSKN
Day
10
20

Figure S3

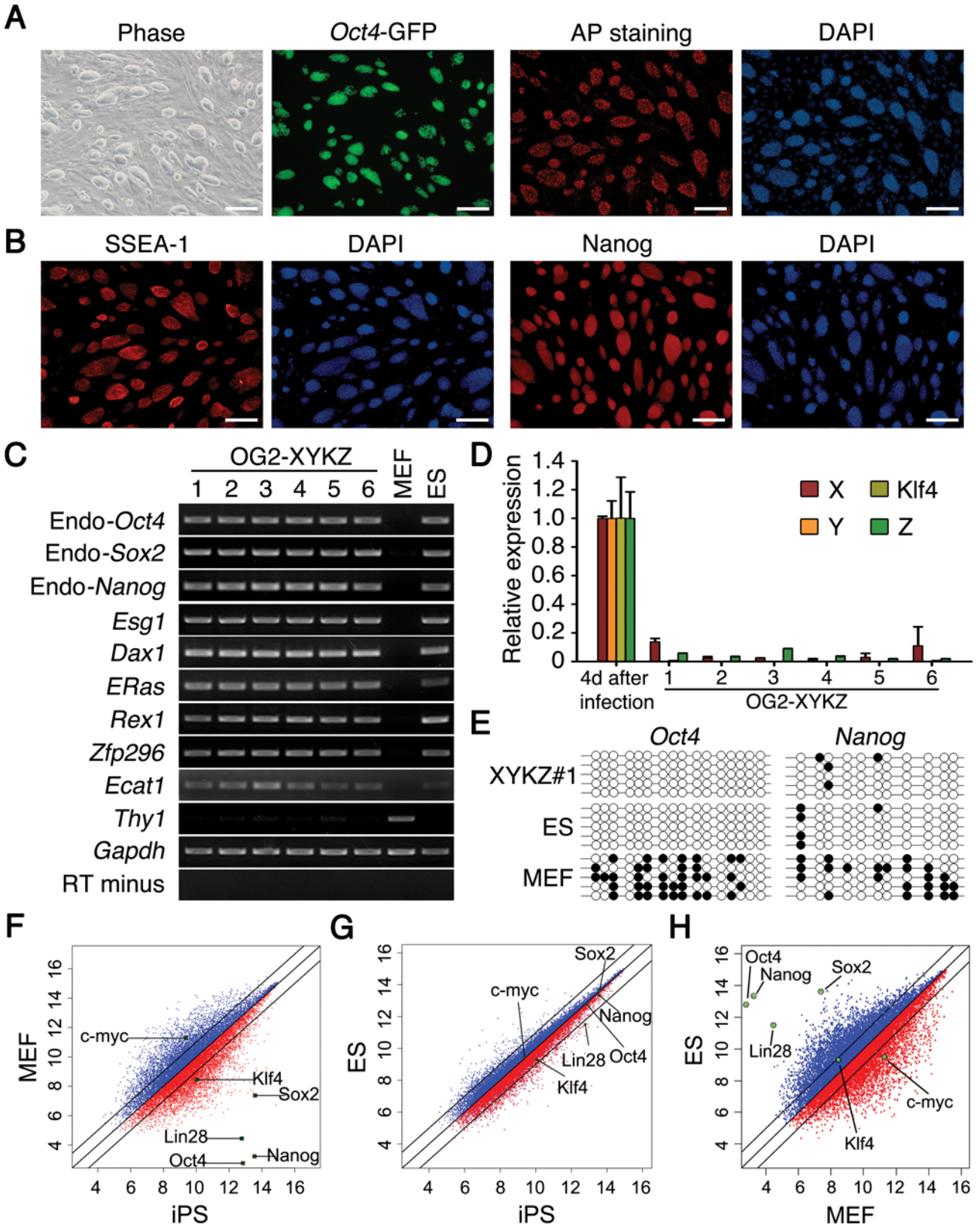

A
Phase
Oct4-GFP
AP staining
DAPI
B
SSEA-1
DAPI
Nanog
DAPI
C
OG2-XYKZ
1 2 3 4 5 6
MEF
ES
Endo-Oct4
Endo-Sox2
Endo-Nanog
Esg1
Dax1
ERas
Rex1
Zfp296
Ecat1
Thy1
Gapdh
RT minus
D
Relative expression
X
Klf4
Y
Z
4d after infection
OG2-XYKZ
E
Oct4
Nanog
XYKZ#1
ES
MEF
F
MEF
iPS
c-myc
Klf4
Sox2
Lin28
Oct4
Nanog
G
ES
iPS
Sox2
c-myc
Nanog
Lin28
Oct4
Klf4
H
ES
MEF
Oct4
Nanog
Sox2
Lin28
c-myc
Klf4

Figure S4

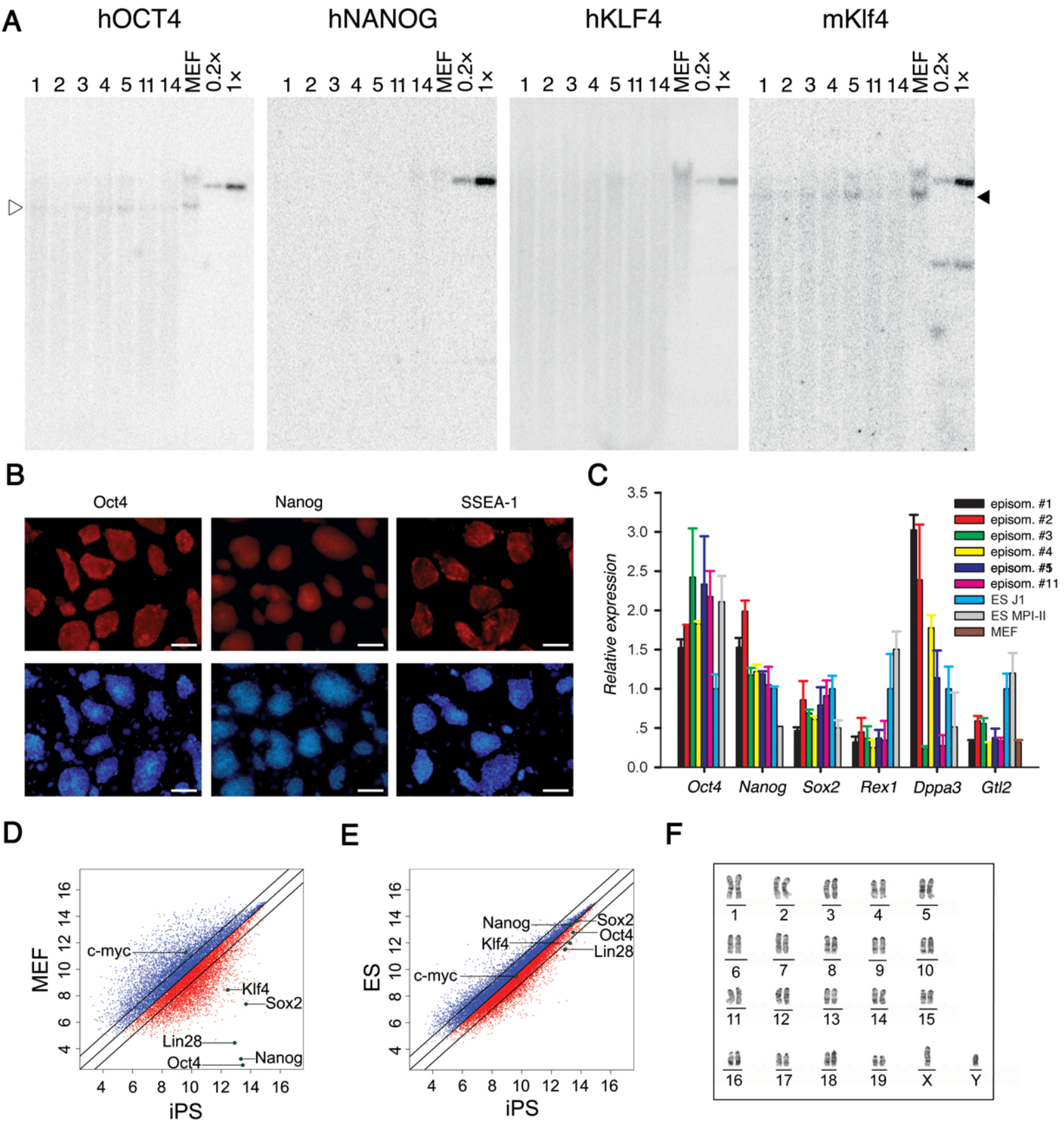
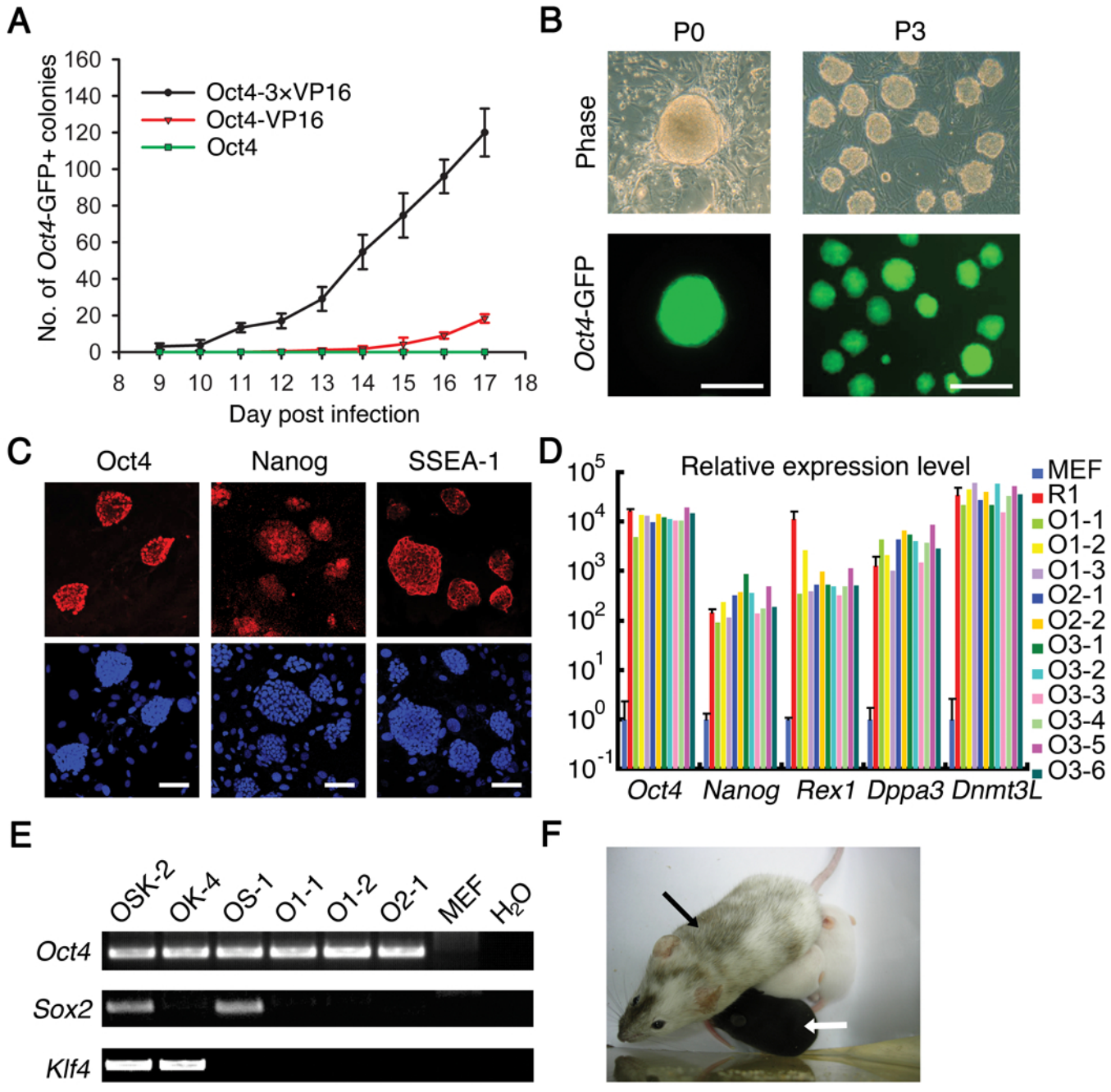

A
hOCT4
hNANOG
hKLF4
mKlf4
1 2 3 4 5 11 14 MEF 0.2× 1×
B
Oct4
Nanog
SSEA-1
C
Relative expression
episom. #1
episom. #2
episom. #3
episom. #4
episom. #5
episom. #11
ES J1
ES MPI-II
MEF
Oct4 Nanog Sox2 Rex1 Dppa3 Gtl2
D
MEF
iPS
c-myc
Klf4
Sox2
Lin28
Oct4
Nanog
E
ES
iPS
Nanog
Sox2
Klf4
Oct4
Lin28
c-myc
F

**Figure S5**


A
No. of Oct4-GFP+ colonies
160
140
120
100
80
60
40
20
0
Oct4-3×VP16
Oct4-VP16
Oct4
8 9 10 11 12 13 14 15 16 17 18
Day post infection
B
P0
P3
Phase
Oct4-GFP
C
Oct4
Nanog
SSEA-1
D
Relative expression level
$10^5$
$10^4$
$10^3$
$10^2$
$10^1$
$10^0$
$10^{-1}$
MEF
R1
O1-1
O1-2
O1-3
O2-1
O2-2
O3-1
O3-2
O3-3
O3-4
O3-5
O3-6
Oct4 Nanog Rex1 Dppa3 Dnmt3L
E
OSK-2
OK-4
OS-1
O1-1
O1-2
O2-1
MEF
$H_2O$
Oct4
Sox2
Klf4
F

# Figure S6

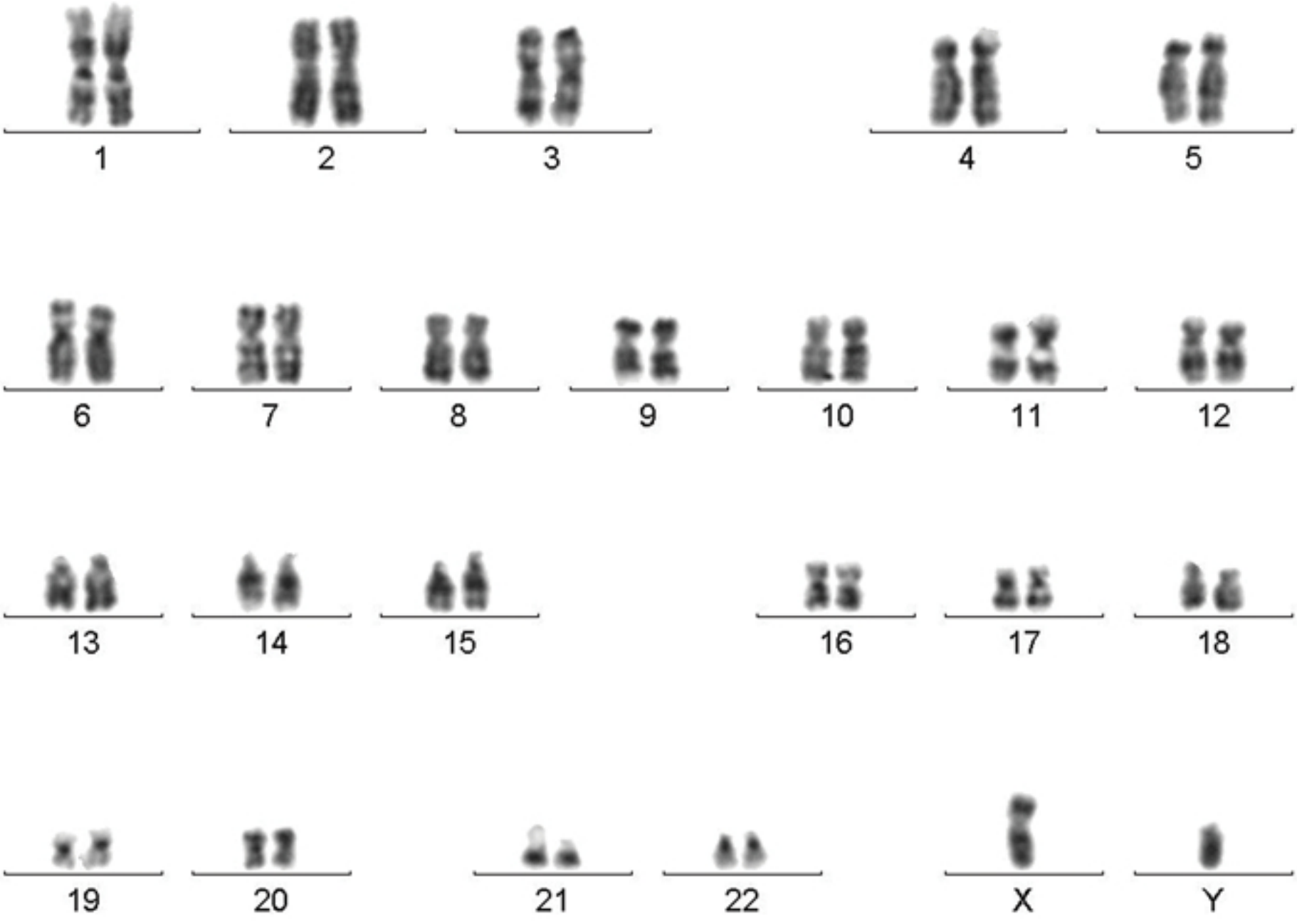


**Figure S7**