Supplementary Table 1. Reprogramming efficiency in mouse fibroblasts

Reprogramming efficiency

3F +	p53 shRNA	↑↑	0.421 ± 0.170%	vs 0.114 ± 0.054%
	p53 KO	↑↑↑	2.278 ± 0.438%	vs 0.197 ± 0.060%
	p21 shRNA	↑↑	0.359 ± 0.144%	vs 0.114 ± 0.054%
	Arf shRNA	↑	0.163 ± 0.013%	vs 0.085 ± 0.083%
	Arf/Ink4a shRNA	↑↑	0.366 ± 0.144%	vs 0.114 ± 0.054%
	Mdm2 inhibition	↓↓↓	0.007 ± 0.008%	vs 0.114 ± 0.054%
	Mdmx mutant(3SA)	↑↑	0.259 ± 0.167%	vs 0.041 ± 0.028%
	Bcl-2 expression	↑↑	0.268 ± 0.117%	vs 0.100 ± 0.062%
	c-Myc (4 factors)	↑↑	0.517 ± 0.264%	vs 0.114 ± 0.054%
2F +	p53 shRNA	↑	0.003 ± 0.002%	vs 0.000%

Summary of reprogramming efficiency. The number of Nanog-positive colonies was calculated after immunostaining at d12-14. The total number of Nanog-positive colonies was divided by the number of infected cells. Error numbers indicate s.d. Student's t-tests were performed for statistical analysis. p=0.007 (p53-shRNA vs mock, n=4 in each), p=0.0006 (p53-/- vs WT, n=3 in each), p=0.009 (p21-shRNA vs mock, n=4 in each), p=0.0005 (Arf-shRNA vs mock, n=3 in each), p=0.028 (Arf/Ink4a-shRNA vs mock, n=3 in each), p=0.010 (Ctl(solvent) vs Nutlin-3(10μM), n=3 in each), p=0.045 (3SA/3SA vs WT, n=3 in each), p=0.022 (Bcl-2 vs mock, n=4 in each), and p=0.012 (c-Myc vs mock, n=4 in each).

Supplementary Table 2. Reprogramming efficiency in human embryonic fibroblasts (IMR90)

	3-F		4-F	
	mock	p53shRNA	mock	p53shRNA
Efficiency	0%	**0.027% ***	0%	**0.153 ± 0.188%**
				0.208 ± 0.240% **by Tra1-81**

* Average of 2 independent experiments

Actual number of Nanog-positive colonies

3-F		4-F	
Trial 1	**0 colonies / 3x10⁴ cells (mock)**	**Trial 1**	**0 colonies / 1x10⁴ cells (mock)**
	10 colonies / 3x10⁴ cells (p53shRNA)		**86 colonies / 2x10⁴ cells (p53shRNA)**
Trial 2	**0 colonies / 2x10⁴ cells (mock)**	**Trial 2**	**0 colonies / 3x10⁴ cells (mock)**
	2 colonies / 1x10⁴ cells (p53shRNA)		**13 colonies / 2x10⁴ cells (p53shRNA)**
		Trial 3	**0 colonies / 2x10⁴ cells (mock)**
			19 colonies / 2x10⁴ cells (p53shRNA)
		Trial 4	**0 colonies / 2x10⁴ cells (mock)**
			4 colonies / 2x10⁴ cells (p53shRNA)

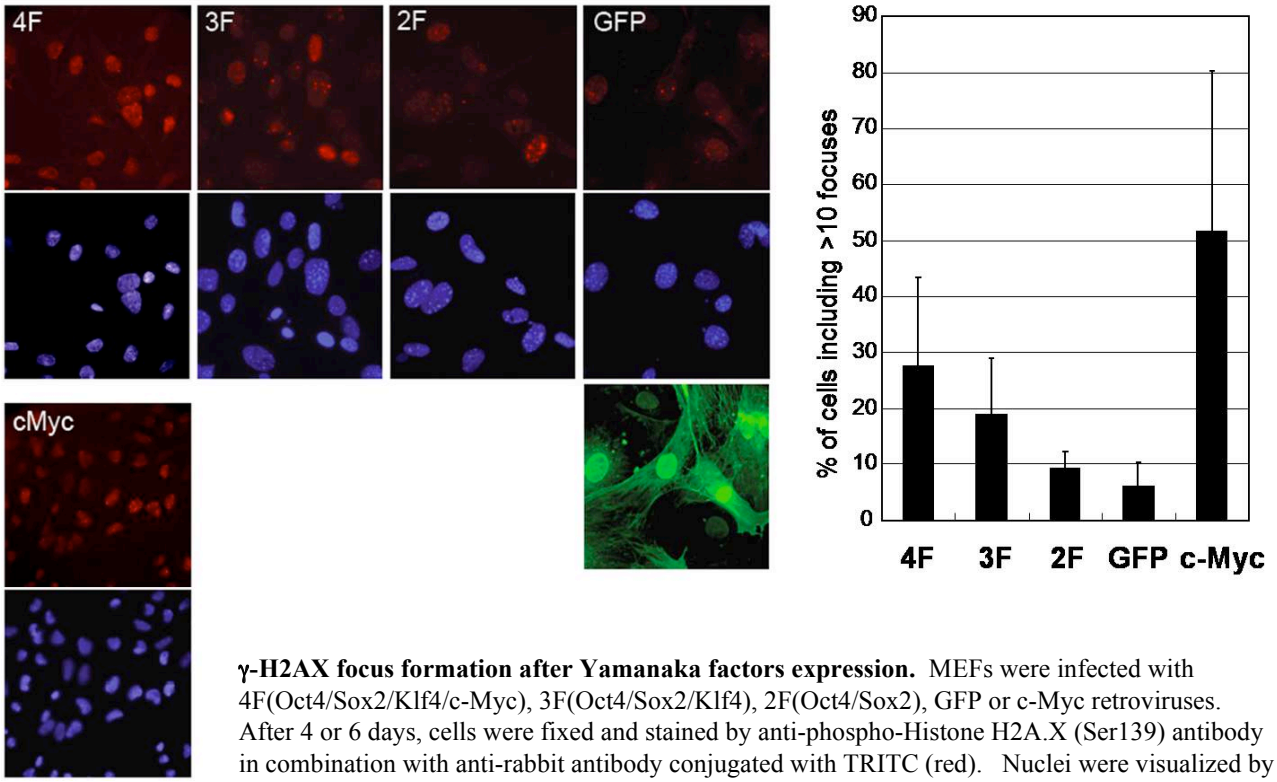
Summary of reprogramming efficiency of human embryonic fibroblasts (HEFs). The number of Nanog- or Tra1-81-positive colonies was calculated after immunostaining at d18-27. No colonies were observed from mock + 4-F or 3-F cells in all trials. The total number of colonies was divided by the number of infected cells. Error numbers indicate s.d (n=4).

Supplementary Table 3. shRNA sequences

Gene	Sequence	Reference
GFP(control)	GAAGCAGCACGACTTCTTC	1
mouse p53#1	GACTCCAGTGGGAACCTTC	2
mouse p53#2	GTACATGTGTAATAGCTCC	3
p21	TTAGGACTCAACCGTAATA	4
Arf	CACCGGAATCCTGGACCAG	5
Arf/Ink4a	AATGGCTGGATTGTTTAAA	4
human p53#1	GACTCCAGTGGTAATCTAC	2

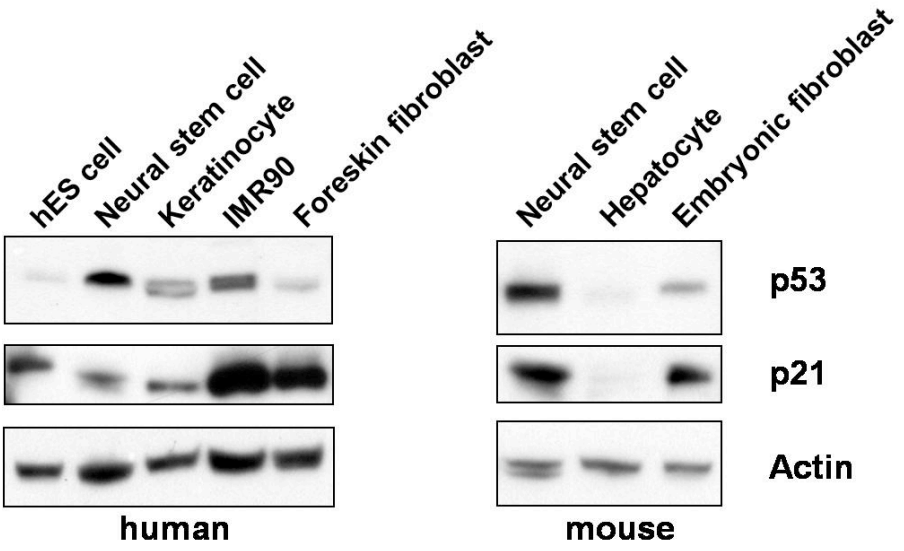
1. Takehara A, Hosokawa M, Eguchi H, Ohigashi H, Ishikawa O, Nakamura Y, and Nakagawa H. (2007). Gamma-aminobutyric acid (GABA) stimulates pancreatic cancer growth through overexpressing GABAA receptor pi subunit. Cancer Res. 67, 9704-12.
2. Brummelkamp TR, Bernards R, and Agami R. (2002). A system for stable expression of short interfering RNAs in mammalian cells. Science 296, 550-553.
3. Tiscornia G, Tergaonkar V, Galimi F, and Verma IM. (2004). CRE recombinase-inducible RNA interference mediated by lentiviral vectors. Proc Natl Acad Sci U S A. 101, 7347-51.
4. Fasano CA, Dimos JT, Ivanova NB, Lowry N, Lemischka IR, and Temple S. (2007). shRNA knockdown of Bmi-1 reveals a critical role for p21-Rb pathway in NSC self-renewal during development. Cell Stem Cell. 1, 87-99.
5. Sage J, Miller AL, Pérez-Mancera PA, Wysocki JM, and Jacks T. (2003). Acute mutation of retinoblastoma gene function is sufficient for cell cycle re-entry. Nature. 424, 223-8.

Supplementary Figure 1



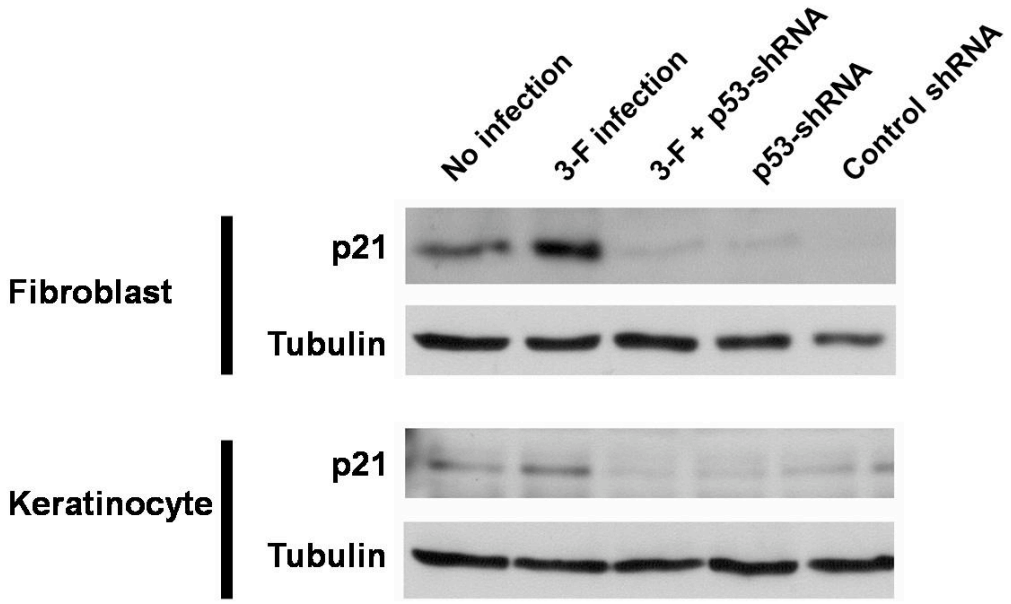
γ-H2AX focus formation after Yamanaka factors expression. MEFs were infected with 4F(Oct4/Sox2/Klf4/c-Myc), 3F(Oct4/Sox2/Klf4), 2F(Oct4/Sox2), GFP or c-Myc retroviruses. After 4 or 6 days, cells were fixed and stained by anti-phospho-Histone H2A.X (Ser139) antibody in combination with anti-rabbit antibody conjugated with TRITC (red). Nuclei were visualized by DAPI (blue). The number of cells containing >10 g-H2AX focuses in nuclei were counted under the microscope. Data was shown as % of cells including >10 g-H2AX focus in nuclei vs total cells (n=3).

Supplementary Figure 2



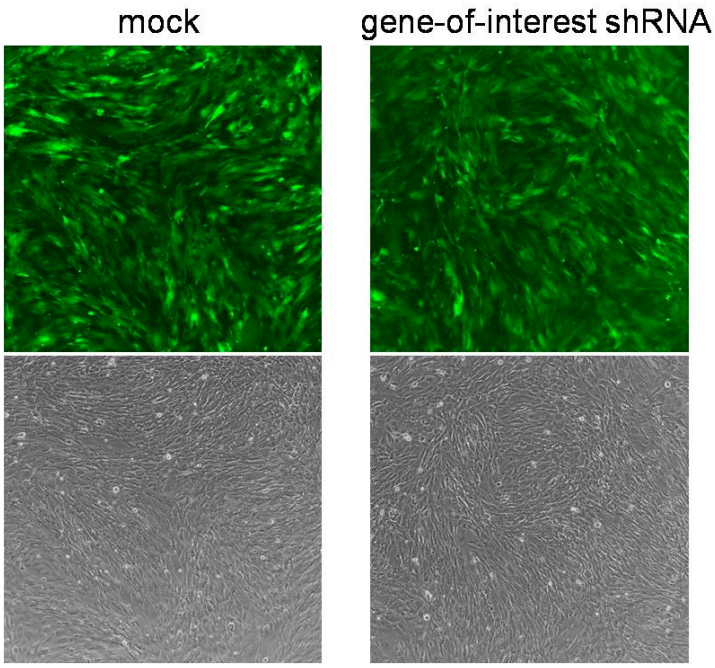
The level of p53 and p21 protein in human and mouse cells previously used for reprogramming. Equal amount of proteins (30μg) were subjected to SDS-PAGE and each protein level was analyzed by western blotting using each antibody. Mouse embryonic fibroblasts were analyzed at passage 3 of derivation. Mouse hepatocytes were isolated by by two-steps collagenase perfusion and cultured in the presence of EGF. Mouse neural stem cells are neurospheres from embryonic forebrain (E12.5). Human foreskin fibroblasts and IMR90 were analyzed at passage 6 of derivation. Human keratinocytes were isolated as previously described (Aasen, T. et al. (2008). Nat Biotechnol. 26, 1276-84). Human neural progenitors were differentiated from human ES cells (HUES6). Human ES cells are HUES9.

Supplementary Figure 3



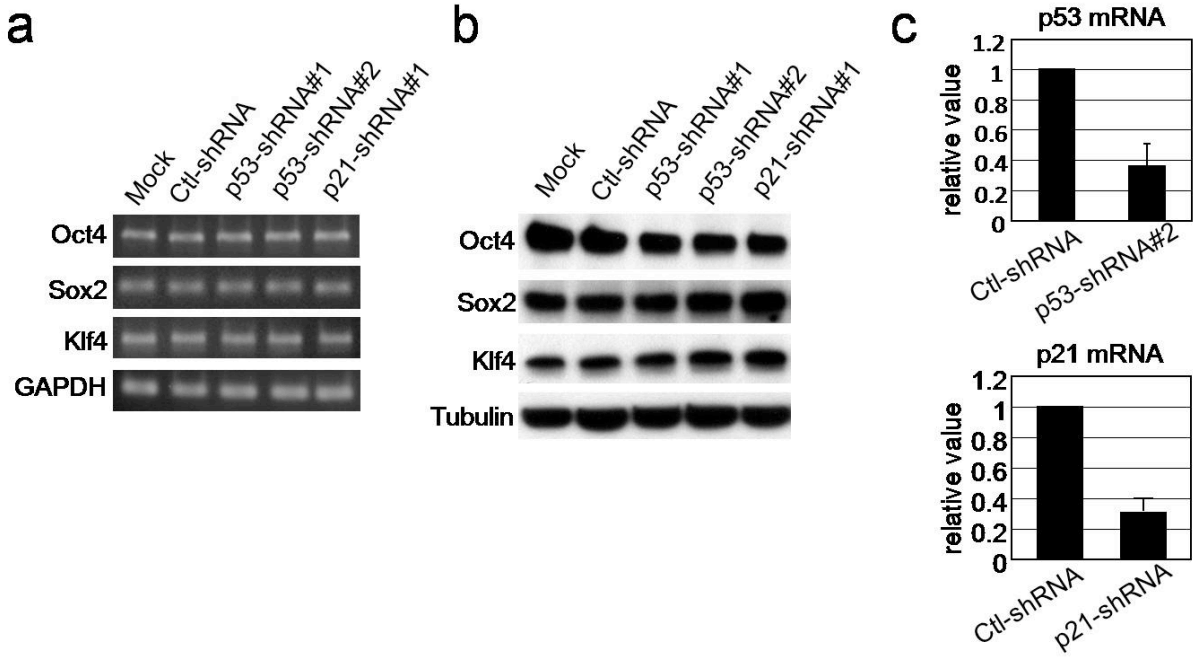
Human p53/p21 level after 3-F infection with p53shRNA in keratinocyte and fibroblast. Human keratinocytes or foreskin fibroblasts were infected (day 0) with retroviruses for 3-F (Oct4, Sox-2 and KLF4) and/or lentiviruses encoding a p53 shRNA or and empty vector control as indicated. For the non-infected control, cells were grown in exactly the same conditions. Protein samples were collected at day five; the protein concentration was carefully standardized and the samples were analyzed by western-blot as indicated in the materials and methods section. Membranes were stained using antibodies for human p21 and tubulin as a loading control.

Supplementary Figure 4



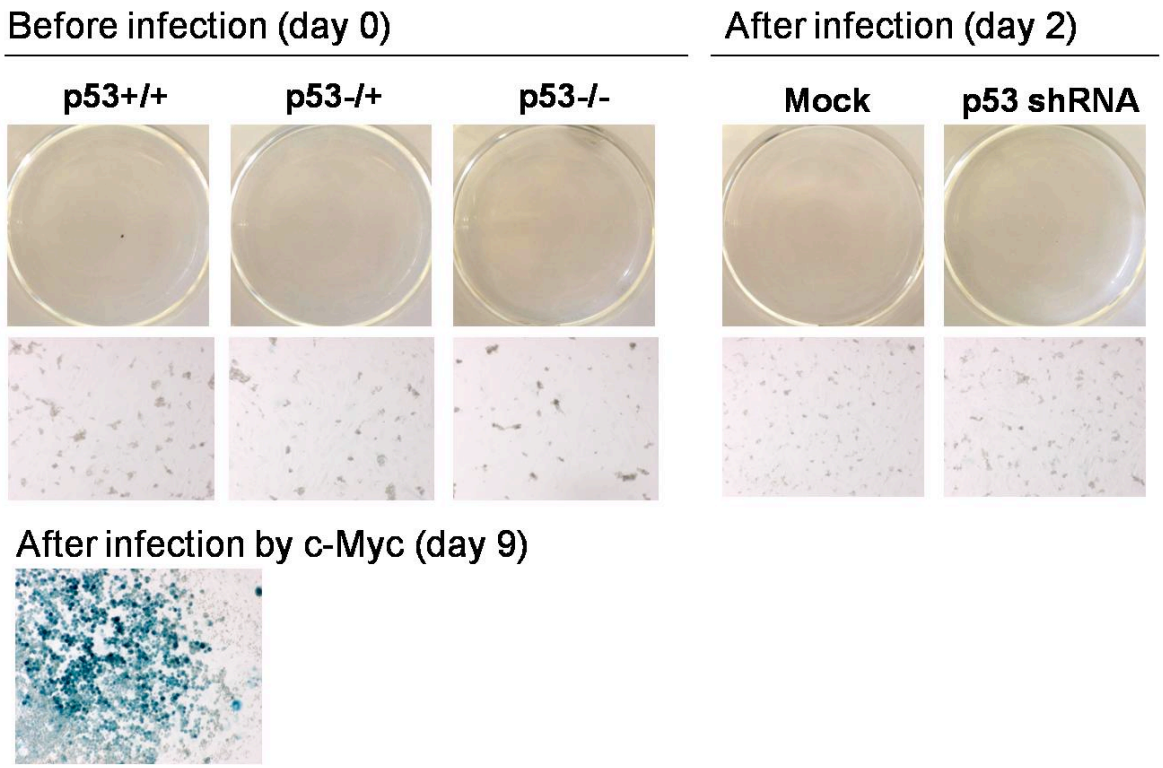
Highly efficient lentivirus-mediated shRNA expression in MEFs. pLVTHM-short hairpin RNA (shRNA) expressing lentivirus vector harbors GFP as a reporter under elongation factor promoter. After infection of this lentivirus together with retrovirus of three factors (Oct4/Sox2/Klf4), the majority of cells became positive for GFP. Cells were infected by retrovirus and lentivirus in the following proportion: pMX-Oct4:pMX-Sox2:pMX-Klf4:pLVTHM-shRNA = 1:1:1:4.

Supplementary Figure 5



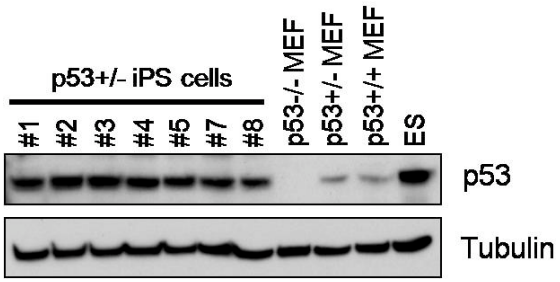
RT-PCR and QPCR analysis of three transgenes (Oct4/Sox2/Klf4), p53, and p21 four days after infection. (a) mRNA expression of three transgenes in MEFs infected with 3 factors (Oct4, Sox2, and Klf4) by retrovirus was analyzed by RT-PCR (n=3 or 4). The mRNA levels of the three factors were similar in all samples. GAPDH was used as an experimental control. (b) Western blotting shows that the protein levels of the three transgenes were similar among all the groups. (c) Decrease in p53 and p21 mRNA levels by each shRNA was shown by QPCR analysis. Data was normalized by GAPDH levels.

Supplementary Figure 6



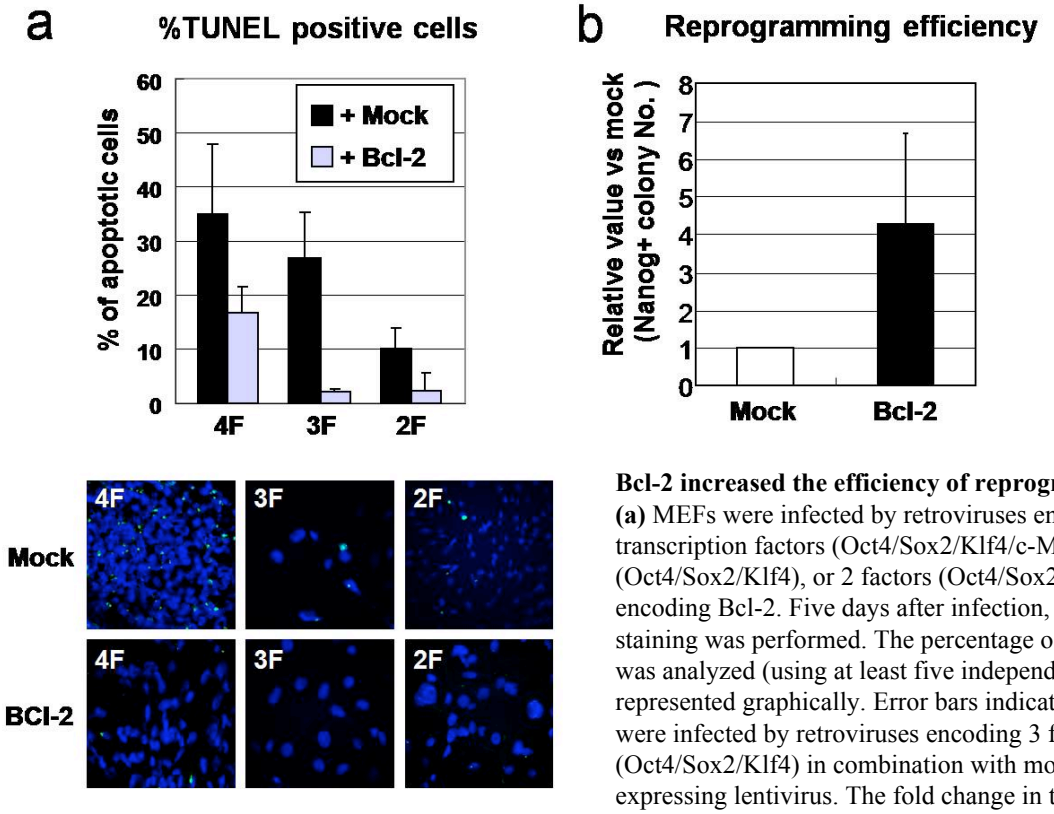
Senescence-associated β-galactosidase. Senescence-associated β-gal activity was histochemically detected as previously described (Dimri GP et al. (1995). Proc Natl Acad Sci U S A. 92, 9363–67.) P53+/+, p53-/+ and p53-/- MEFs were analyzed just before virus infection at passage 3 after derivation. MEFs were infected by mock or p53shRNA#2 together with Oct4, Sox2 and Klf4, and senescence-associated β-gal activity was analyzed 2 days after infection. No significant difference among these cells in the intensity of Senescent-associated β-gal staining. For a positive control of staining, MEFs were infected by c-Myc and cultured for 9 days after infection.

Supplementary Figure 7



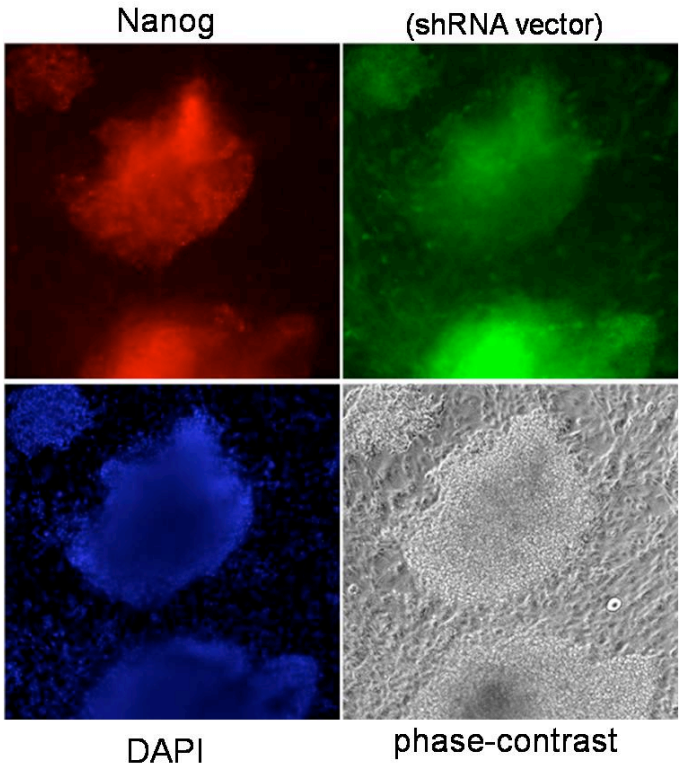
No loss of heterogeneity in iPS cell lines derived from p53+/- MEF. p53+/- MEFs were infected with 3 factors (Oct4, Sox2, and Klf4) by retrovirus and established iPS cell lines. Seven independent lines of iPS cells were analyzed for p53 protein level by western blotting. For control, p53-/-, p53-/+, p53+/+ MEFs and mouse ES cells were analyzed at the same time. Each lane was loaded 35μg of total protein. α-Tubulin was utilized for loading control. All 7 independent iPS cell lines expressed p53 protein.

Supplementary Figure 8



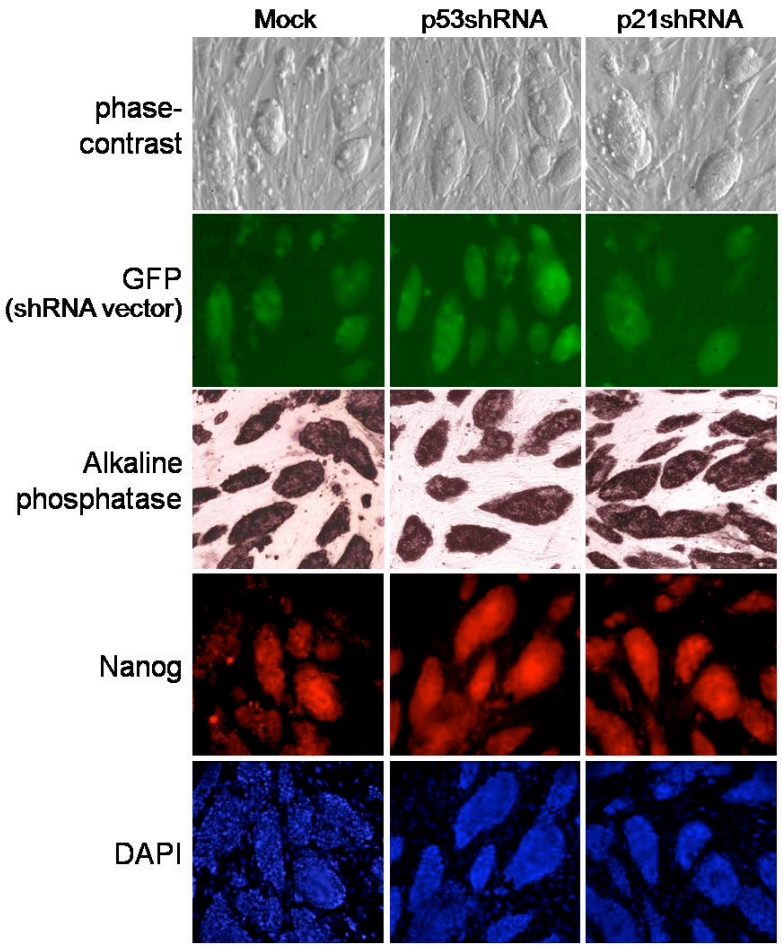
Bcl-2 increased the efficiency of reprogramming. **(a)** MEFs were infected by retroviruses encoding 4 transcription factors (Oct4/Sox2/Klf4/c-Myc), 3 factors (Oct4/Sox2/Klf4), or 2 factors (Oct4/Sox2) with lentivirus encoding Bcl-2. Five days after infection, TUNEL-staining was performed. The percentage of apoptotic cells was analyzed (using at least five independent panels) and represented graphically. Error bars indicate *s.d.* **(b)** MEFs were infected by retroviruses encoding 3 factors (Oct4/Sox2/Klf4) in combination with mock or Bcl-2 expressing lentivirus. The fold change in the number of Nanog-positive colonies compared to mock (n=3) is shown. Error bars indicate *s.d* (n=3).

Supplementary Figure 9



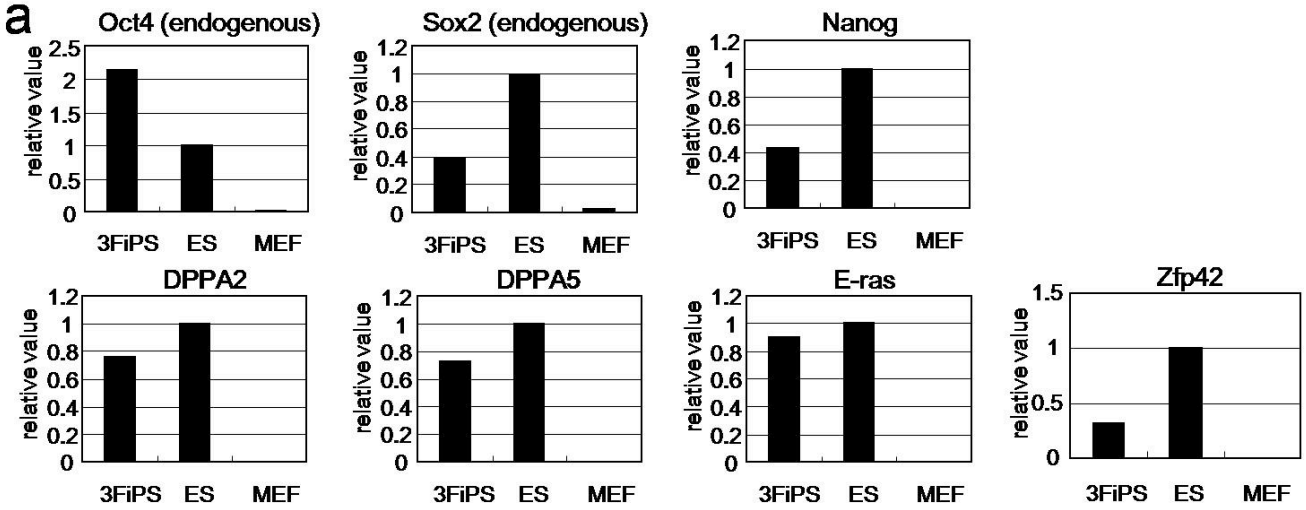
Morphology and Nanog expression in mouse iPS colonies induced by ectopic expression of 3 factors (retrovirus) plus shRNA (lentivirus). Nanog expression was detected by anti-Nanog antibody with a secondary antibody conjugated with TRITC. Incorporation of lentivirus was confirmed by GFP fluorescence. Nuclei were visualized by DAPI.

Supplementary Figure 10



Characterization of mouse 3F iPS clones. First row panels show the morphology of 3F iPS clones expressing shRNAs by lentiviral infection (left: mock, middle: p53shRNA, right: p21shRNA). Second row panels show GFP fluorescence by shRNA-encoding lentiviruses. Third row panels show alkaline phosphatase staining. Fourth row panels show Nanog immunoreactivity. Fifth row panels show nuclei staining by DAPI.

Supplementary Figure 11



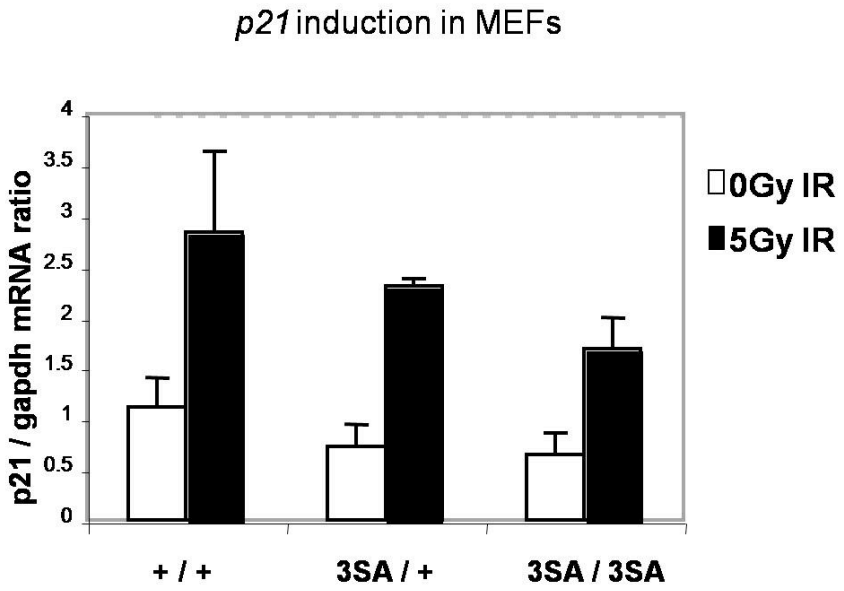
b

Endoderm		Mesoderm		Ectoderm	
AFP	112±18	GATA4	442±103	Cdx2	28±5
HNF1β	426±87	αCardiac actin	575±179	Mfap2	174±126
GATA6	289±92	SM actin	29±2	Nestin	66±34

Relative values vs undifferentiated ES cells

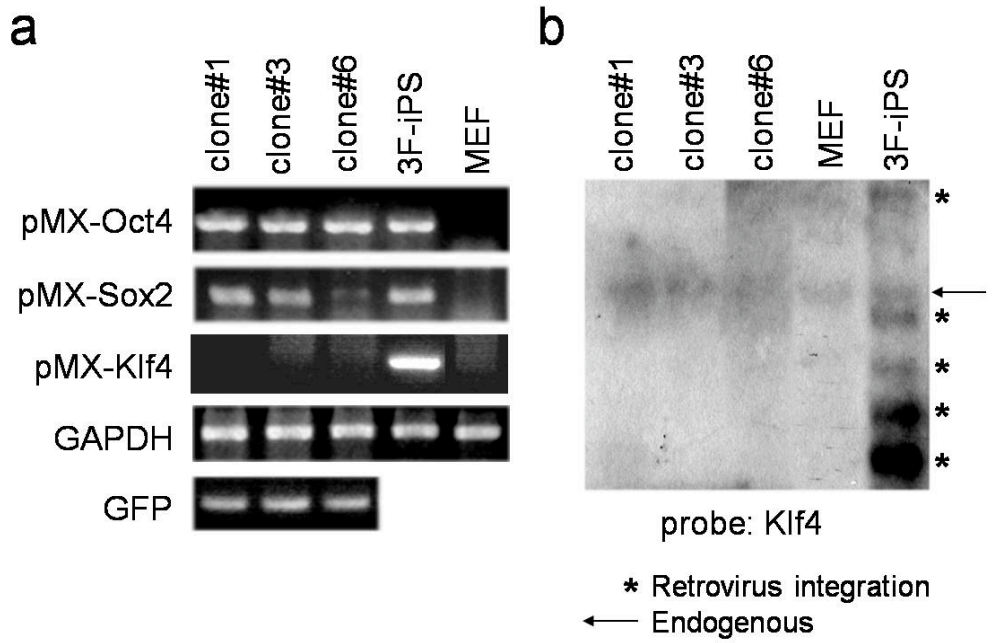
Pluripotent markers and *in vitro* differentiation of mouse 3F iPS clones. (a) Analysis of pluripotent markers and **(b)** differentiation markers in established 3FiPS clones. For pluripotent markers, data from one representative clone are shown. For differentiation markers, data from 3 clones are shown. Cells were differentiated by embryoid bodies for 14 days. For ectodermal induction, EBs were treated with retinoic acid. The relative amounts of Oct4(end), Sox2(end), c-Myc, Nanog, DPPA2, DPPA5, E-ras, Zfp42, AFP, HNF1β, GATA6, GATA4, αCardiac actin, SM actin, Cdx2, Mfap2 and Nestin mRNA were quantified by QPCR. Values were standardized to GAPDH and normalized to ES cells (n=3, ±s.e.).

Supplementary Figure 12



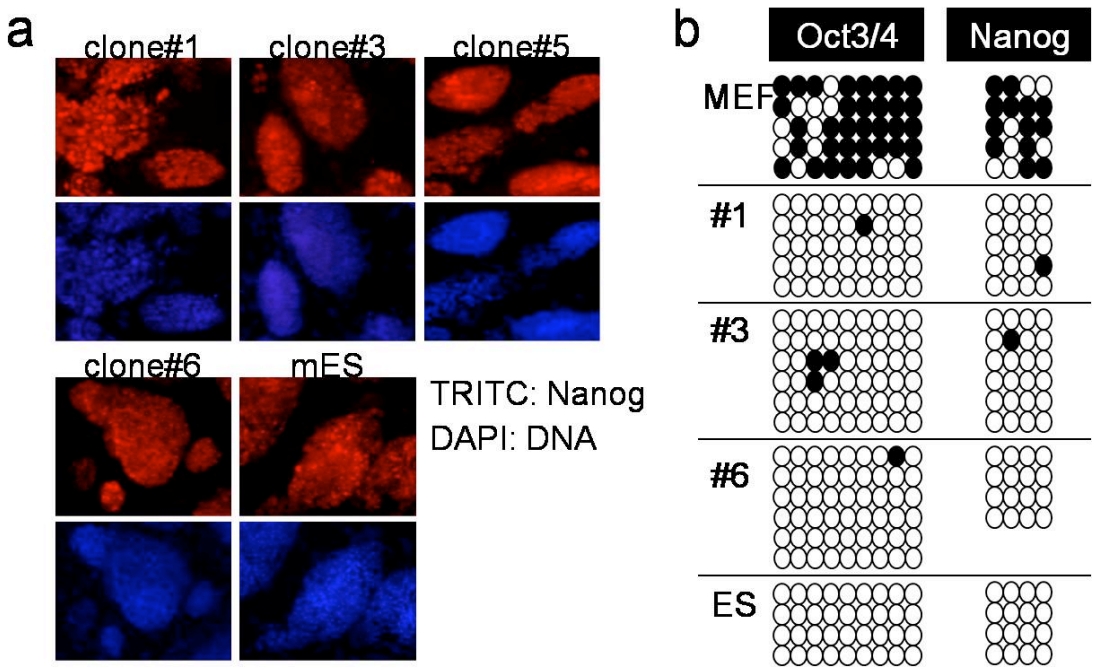
P21 induction of Mdmx-mutant MEFs in response to irradiation. MEFs isolated from wild type (+/+), Mdmx-3SA heterozygous (3SA/+) and homozygous (3SA/3SA) mice were exposed to 5Gy of gamma-irradiation for 4 hours. p21 mRNA levels were analyzed by QPCR. Results were normalized to untreated WT samples for fold induction. Error bars represent 3 PCR experiments.

Supplementary Figure 13



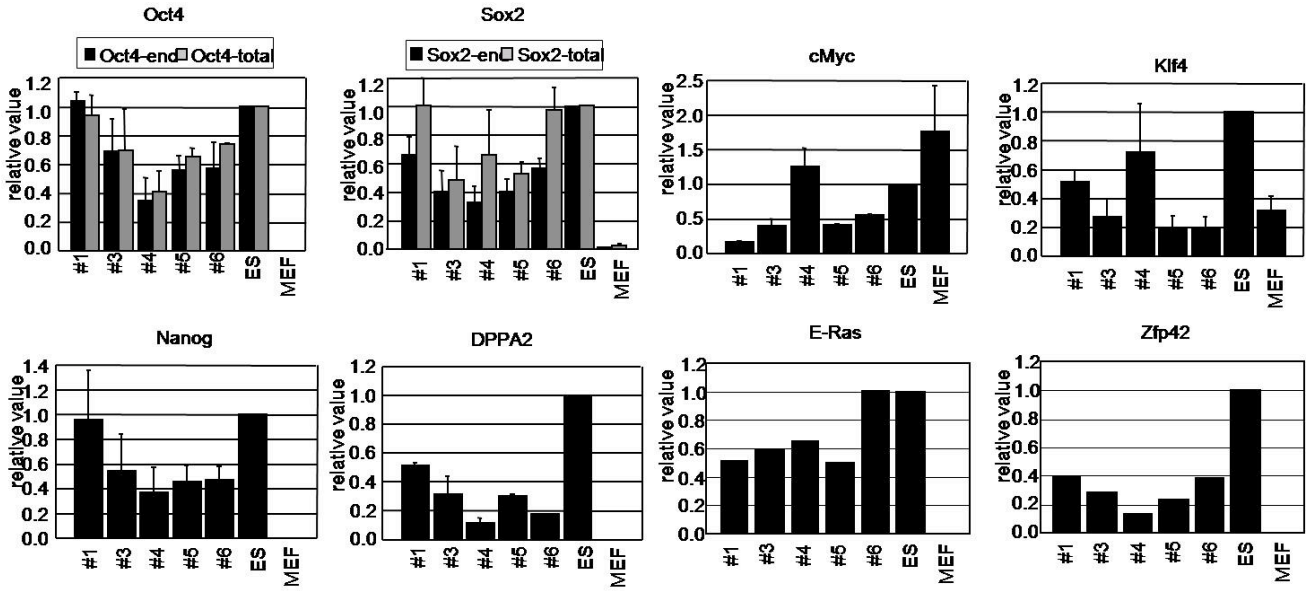
Genotyping of 2F-p53KD-iPS cell lines using PCR and Southern blotting. **(a)** Viral integration of Oct4, Sox2, and Klf4 were amplified from genomic DNA. Only virus-derived Oct4 and Sox2 were amplified (no viral Klf4) in 2F-p53KD-iPS cell clones, while viral Klf4 was amplified only from 3F-iPS cells. GFP was amplified from all three 2F-p53KD-iPS cell clones, indicating the integration of p53-shRNA lentivirus. GAPDH was used as an experimental control for PCR. **(b)** Genomic DNA in each clone was digested by BamHI and EcoRI and subjected to Southern blot analysis with a Klf4 cDNA probe, showing no viral integration of Klf4. Genomic DNA from 3F-iPS and MEFs (no virus infection) was used as positive and negative controls for Southern blot analysis.

Supplementary Figure 14



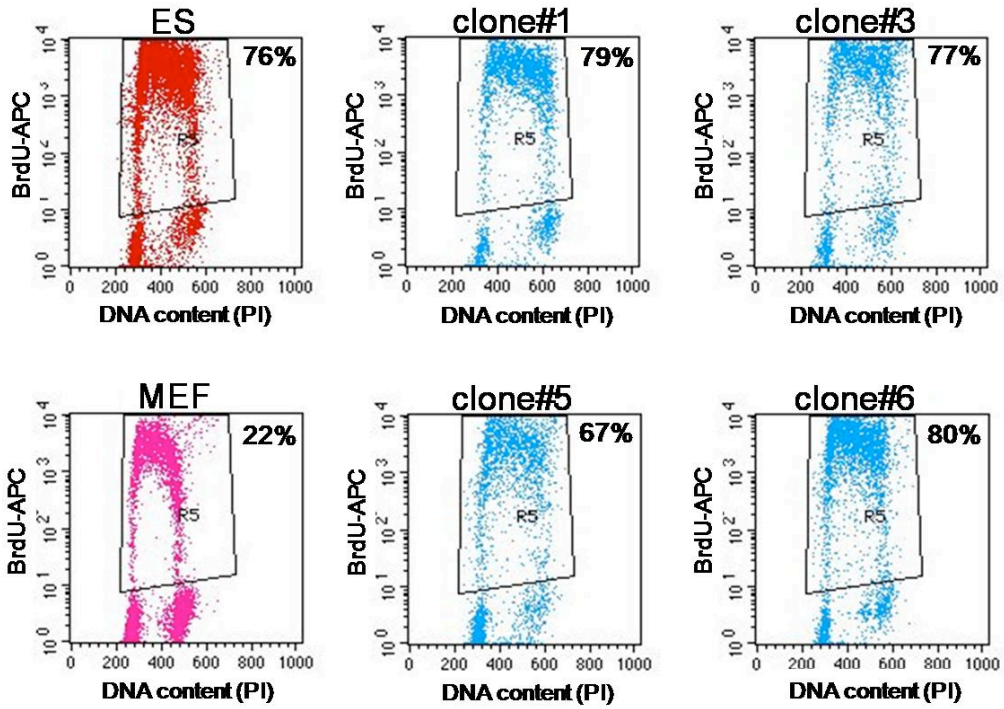
Characterization of 2F-p53KD-iPS cell lines. **(a)** Nanog-immunostaining of 2F-p53KD-iPS cell lines. DAPI was used to visualize cell nuclei. **(b)** Methylation analysis of Oct4 and Nanog promoters in 2F-p53KD-iPS cells, MEFs and ES cells. Black circles indicate methylated CpGs and white circles indicate unmethylated CpGs.

Supplementary Figure 15



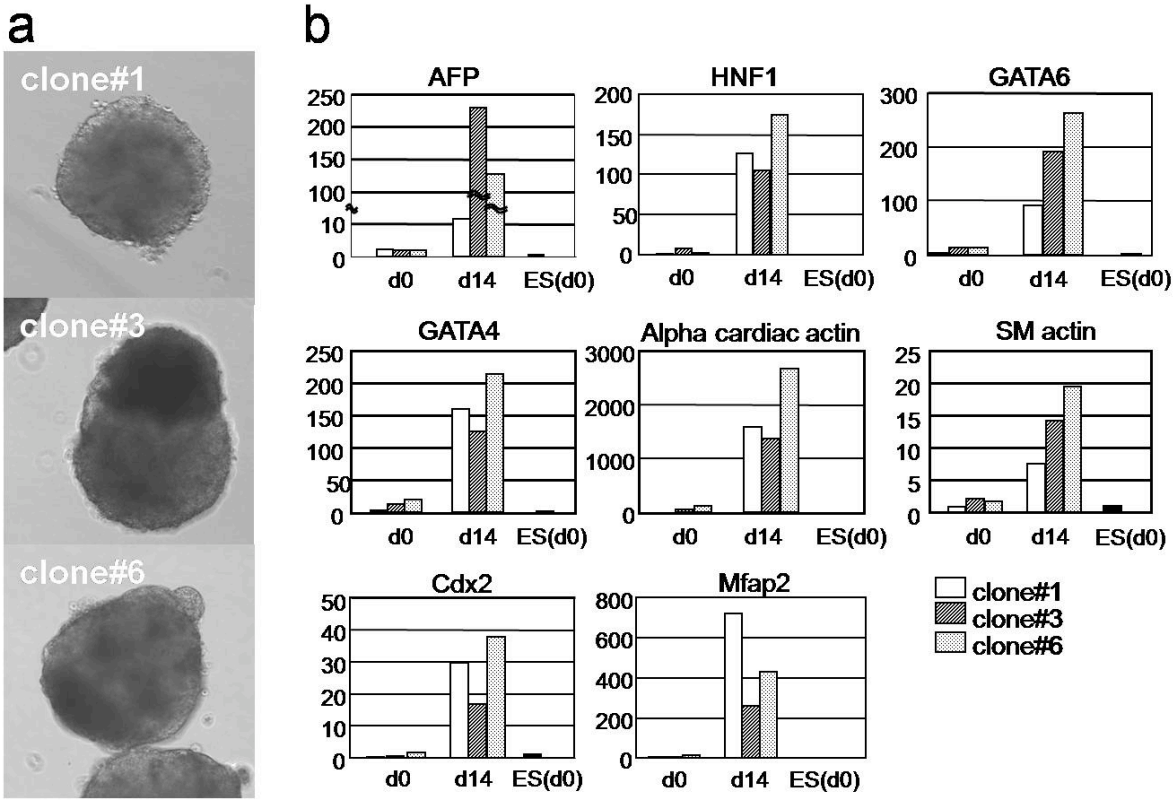
Characterization of pluripotent marker expressions in 2F-p53KD-iPS cell lines. Quantitative PCR analysis of ES cell marker genes. To investigate endogenous expression of Oct4 and Sox2,, primer sets that specifically amplify endogenous or total Oct4 or Sox2 were used. Values were standardized to GAPDH, and normalized to ES cells. Bars represent the range of values obtained from two measurements from one experiment.

Supplementary Figure 16



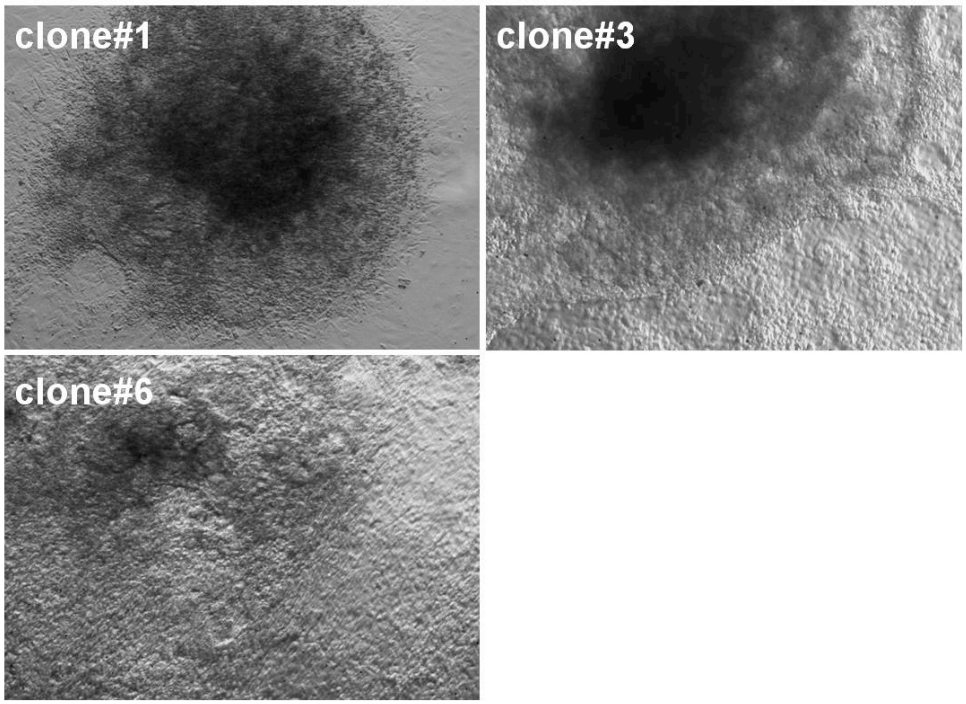
Analysis of the cell cycle profile of 2F-p53KD-iPS cell clones. A majority of the cells in 2F-p53KD-iPS cell clones and ES cells were in S-phase (clone1 = 79%, clone3 = 77%, clone5 = 67%, clone6 = 80%, ES = 76%), while only 22% of MEFs were in S phase. Four independent clones of 2F-p53KD-iPS cells, MEFs and ES cells were treated with BrdU and fixed. Cells were treated with anti-BrdU-biotin followed by avidin-APC, and incorporated BrdU was analyzed by FACS. Staining intensity for PI (*x*-axis) is plotted versus that for *anti*-BrdU-*A*PC (y- axis). The S-phase population was calculated.

Supplementary Figure 17



Embryoid body formation and characterization of differentiation marker expressions in 2F-p53KD-iPS cell lines. **(a)** Embryoid bodies (EBs) of 2F-p53KD-iPS cell clones on day 6 of differentiation. **(b)** QPCR analysis for differentiation marker genes of the three germ layers (AFP, HNF1 and GATA6 for endoderm, GATA4, alpha cardiac actin, and smooth muscle (SM) actin for mesoderm, Cdx2 and Mfap2 for ectoderm) in 2F-p53KD-iPS cells on d14 of differentiation.

Supplementary Figure 18



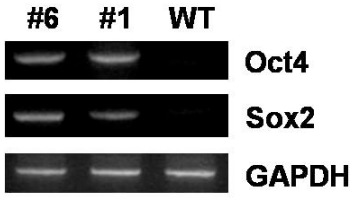
Successful formation of beating embryoid bodies attached to gelatinized dishes from three independent 2F-p53KD-iPS cell clones. Pictures were taken at day10 (Clone#1) day 14 (clone#3), and day 15 (clone#6).

Supplementary Figure 19

GeneChip expression analysis Total RNA was isolated from MEF, Total RNA was isolated from MEF, 2F-p53KD-iPS cell clones(#1 and #6) and mouse ES cells using the Trizol reagent according to the manufacturer's instructions. The GeneChip microarray processing was performed by the Salk institute microarray platform according to the manufacturer's protocols (Affymetrix, Santa Clara, CA). The data extraction was done by the Affymetrix GCOS software v.1.4.
The statistical analysis of the data was performed using R. First, the raw data was normalized using the gcRMA algorithm implemented in R[1], and a hierarchical clustering using pearson correlation coefficients was performed on the normalized data (see table X for the correlation coefficient table and figure X for the dendrogram). gcRMA normalized data were clustered using the hierarchical clustering tool in GenePattern[2] with default parameters (pearson correlation distance measure and pairwise complete linkage clustering on rows and columns), and viewed using the HierarchicalClusteringViewer applet with a relative coloring by rows. The heatmap and dendrogram represent only the genes showing an intensity value fold change of at least 2 (4) between ES and MEF.

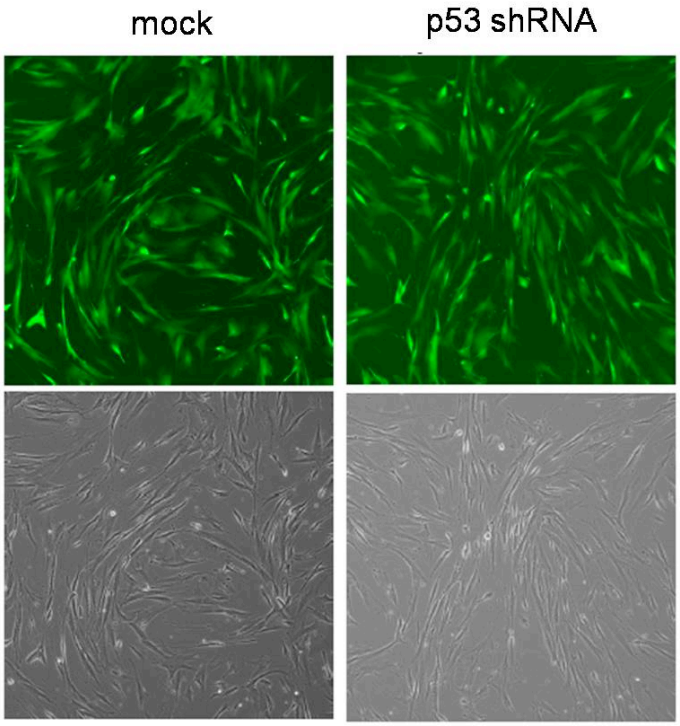
1. Wu, Z., et al., A model-based background adjustment for oligonucleotide expression arrays. Journal of the American Statistical Association, (2004). **99**: p909.
2. Reich, M., et al., GenePattern 2.0. Nat Genet. (2006). **38**: p500-1.

Supplementary Figure 20



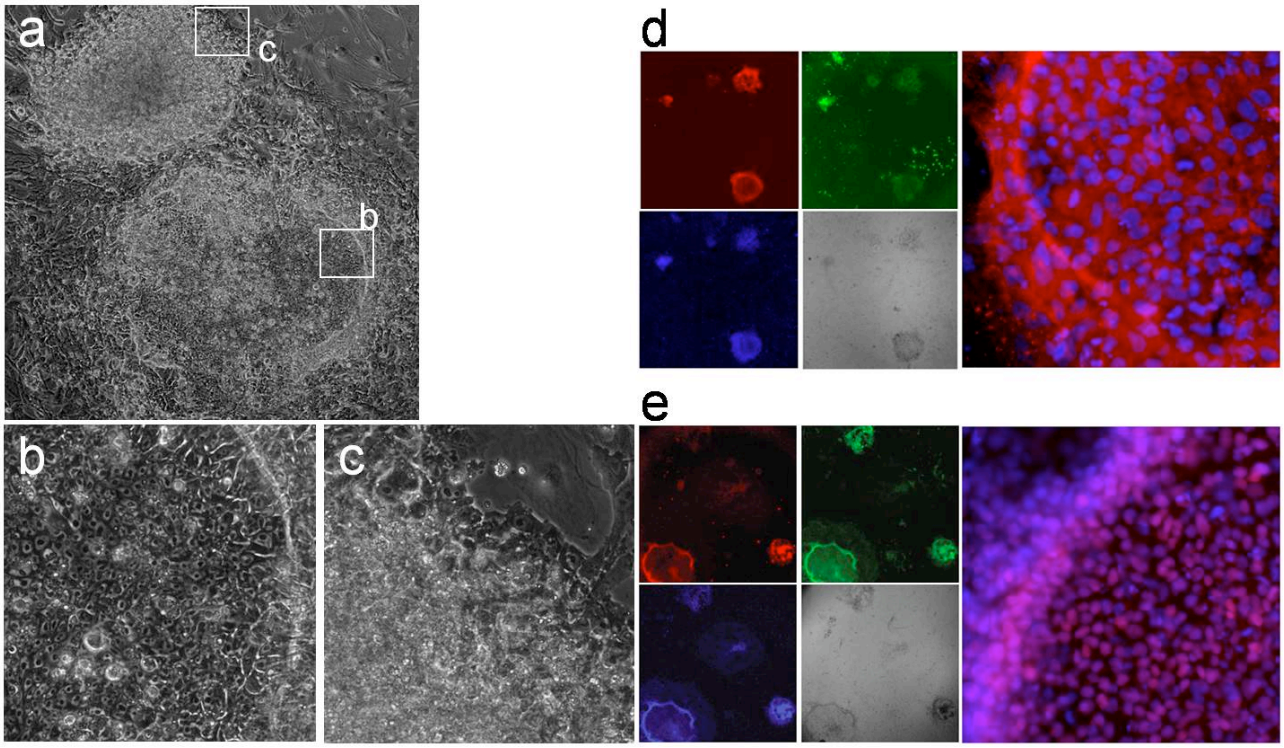
Genotyping of chimeric mice derived from 2F-iPS cells. Genotyping of chimeric mice from 2F-p53KD-iPS cell clones(#1 and #6) and wild type (WT) mouse was performed using primers specific for viral Oct4 and Sox2 genes. GAPDH was utilized for control of PCR reaction.

Supplementary Figure 21



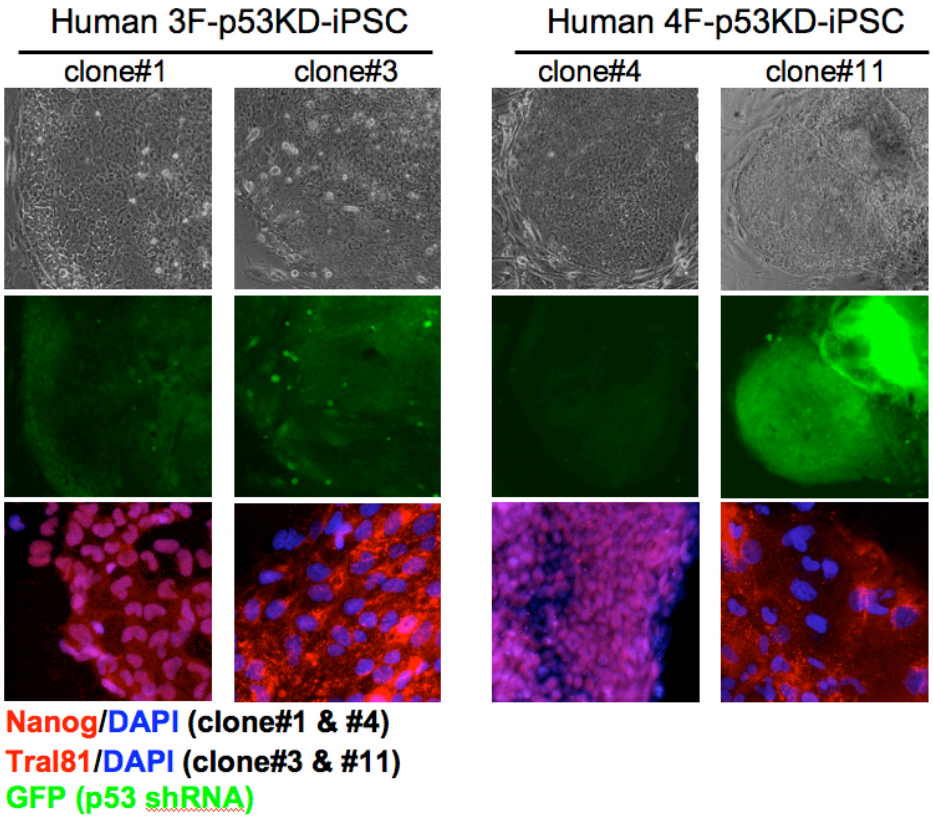
High-efficient lentivirus-mediated shRNA expression in HEFs. pLVTHM-short hairpin RNA (shRNA) expressing lentivirus vector harbors GFP as a reporter under elongation factor promoter. After infection of this lentivirus together with retrovirus of three or four factors (Oct4/Sox2/Klf4 with or without c-Myc), the majority of cells became positive for GFP. Cells were infected by retrovirus and lentivirus in the following proportion: pMSCV-Oct4:pMSCV-Sox2:pMSCV-Klf4(:pMSCV-cMyc):pLVTHM-shRNA = 1:1:1(:1):3.

Supplementary Figure 22



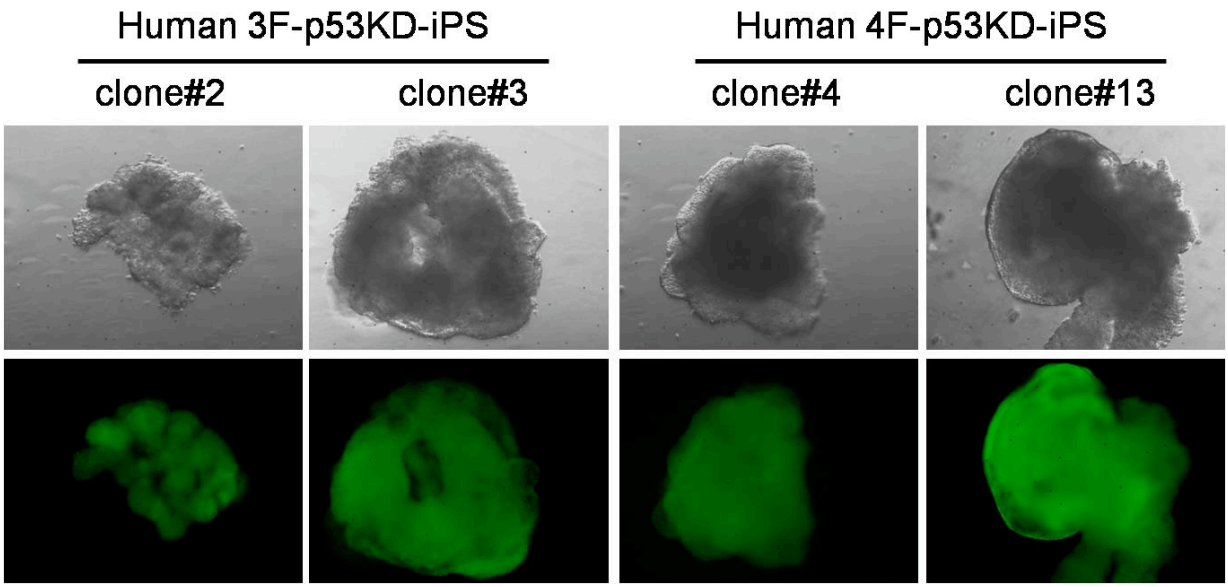
Morphology and ES marker expression in human iPS cell colonies induced by ectopic expression of 4 factors (retrovirus) plus p53 shRNA (lentivirus) in HEFs. (**a-c**), Phase-contrast images of human iPS cell colony (**b**) and non-iPS (granulated) cell colony (**c**) are shown. (**d, e**) Nanog or TraI81 expression was detected by anti-Nanog (**d**) or anti-TraI81 (**e**) antibody with a secondary antibody conjugated with TRITC (red). Incorporation of lentivirus was confirmed by GFP fluorescence (green). Nuclei were visualized by DAPI (blue).

Supplementary Figure 23



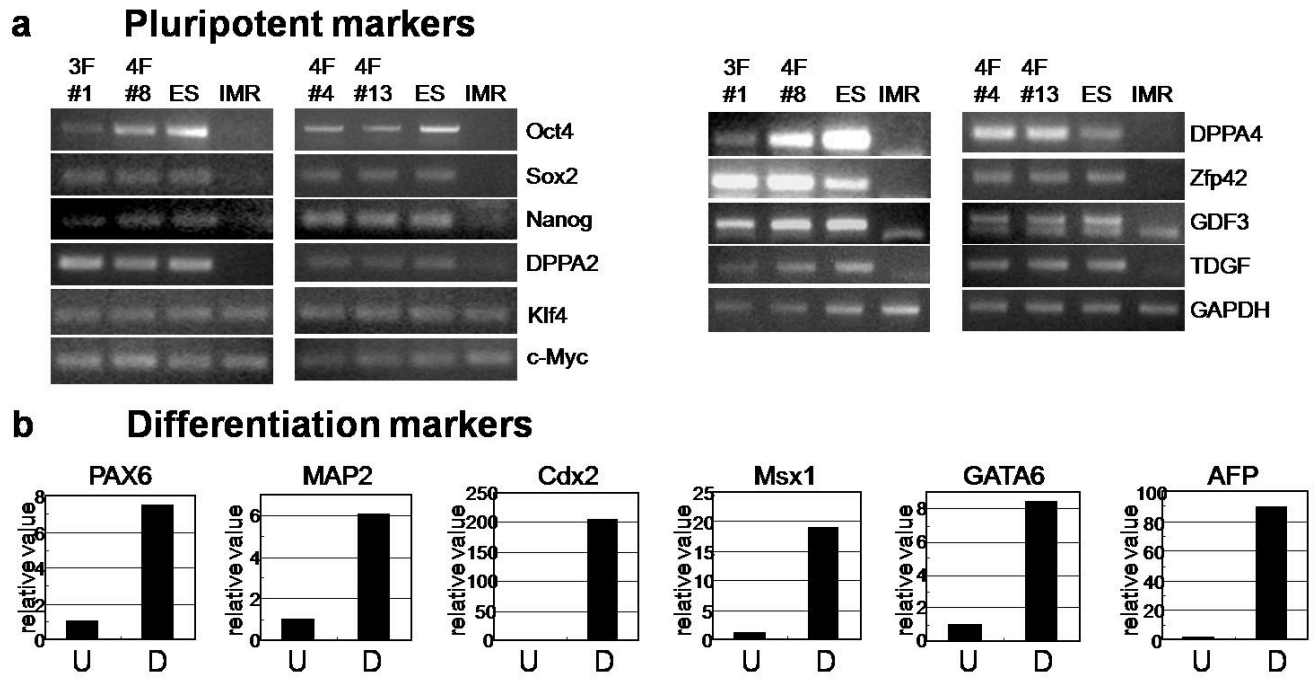
Characterization of human iPS cell lines derived from HEFs. First row panels show the morphology of 3-F or 4-F human iPS cell clones expressing p53 shRNAs by lentiviral infection. Second row panels show GFP fluorescence by shRNA-encoding lentiviruses. Third row panels show Nanog-immunoreactivity merged with nuclei staining by DAPI.

Supplementary Figure 24



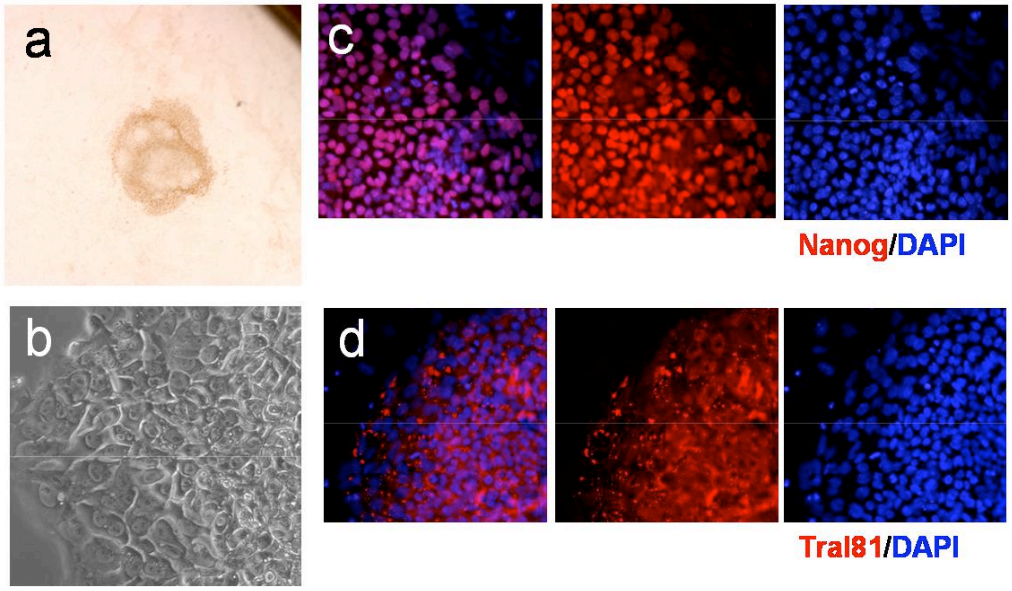
Embryoid body formation of human iPS cell derived from HEFs. Phase contrast images and GFP-fluorescence of Embryoid bodies from established human iPS clones (2 representative lines of Human 3F-p53KD-iPS cell and 2 representative lines of Human 4F-p53KD-iPS cell) are shown.

Supplementary Figure 25



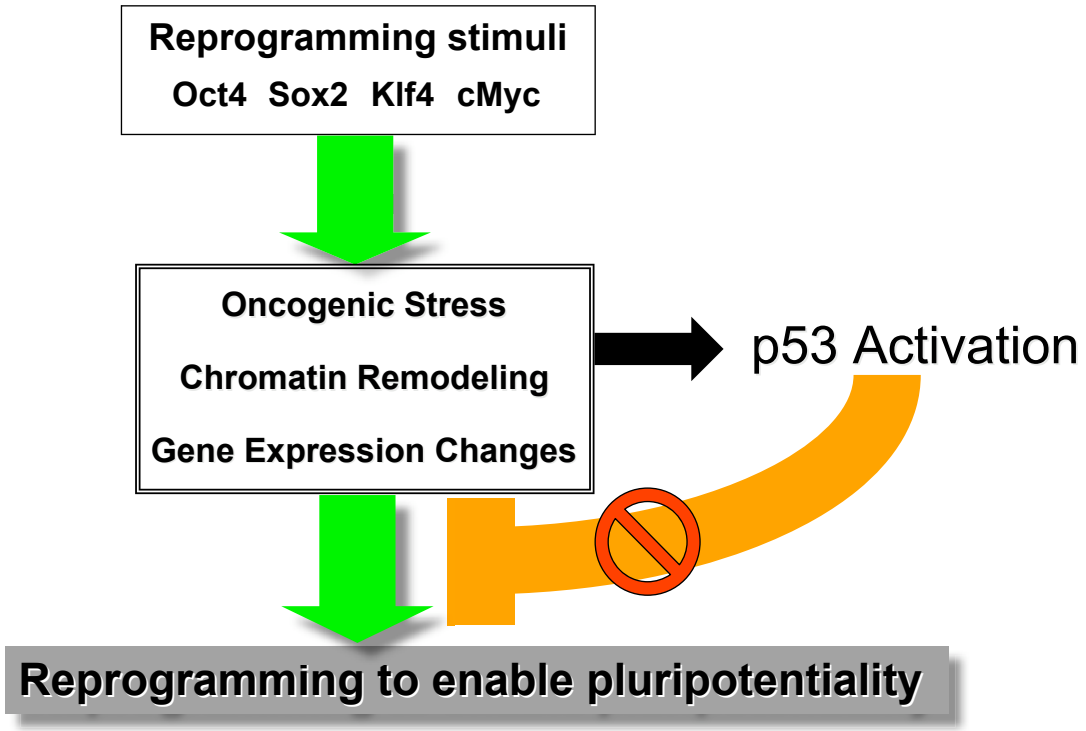
Pluripotent markers and *in vitro* differentiation of human iPS cell lines derived from HEFs. (**a**) PCR Analysis of pluripotent markers in established human iPS cell lines. Representative human iPS cell lines are 3F#1 established by 3-F infection and 4F#8, #4, #13 established by 4-F infection. For pluripotent markers, Oct4, Sox2, Nanog, DPPA2, DPPA4, Zafp42, GDF3, TDGF were analyzed. For the control of PCR reaction, GAPDH was utilized. **(b)** For differentiation markers, data from clone 4F#4 is shown. Cells were differentiated (D) by embryoid bodies for 8-10 days. The relative amounts of PAX6, MAP2, Cdx2 (ectoderm), Msx1 (mesoderm), GATA6 and AFP (endoderm) mRNA were quantified by QPCR. Values were standardized to GAPDH and normalized to undifferentiated (U) cells.

Supplementary Figure 26



Morphology and ES marker expression in human iPS cell colonies induced by ectopic expression of 2 factors (retrovirus Oct4 and Sox2) plus p53 shRNA (lentivirus) in HEFs. (**a**) Nanog-positive colony after virus transduction. (**b**) Phase-contrast images of cloned human 2F-iPS cells. (**c, d**) Nanog or TraI81 expression was detected by anti-Nanog (**c**) or anti-TraI81 (**d**) antibody with a secondary antibody conjugated with TRITC (red). Nuclei were visualized by DAPI (blue).

Supplementary Figure 27



The p53 pathway limits reprogramming efficiency. Reprogramming factors (3-F or 4-F) produce a "reprogamming stress" in somatic cells that activates the p53 pathway. This stress is caused by proto-oncogene over-expression. While the exact causes of p53 activation remain to be determined, chromatin remodeling and DNA damage are likely contributors. p53 pathway activation reduces reprogramming efficiency by activating cell cycle arrest and apoptotic responses to prevent cell division. Consequently, eliminating p53 significantly increases reprogramming. Transient p53 inhibition could facilitate development of therapeutically beneficial reprogramming applications.