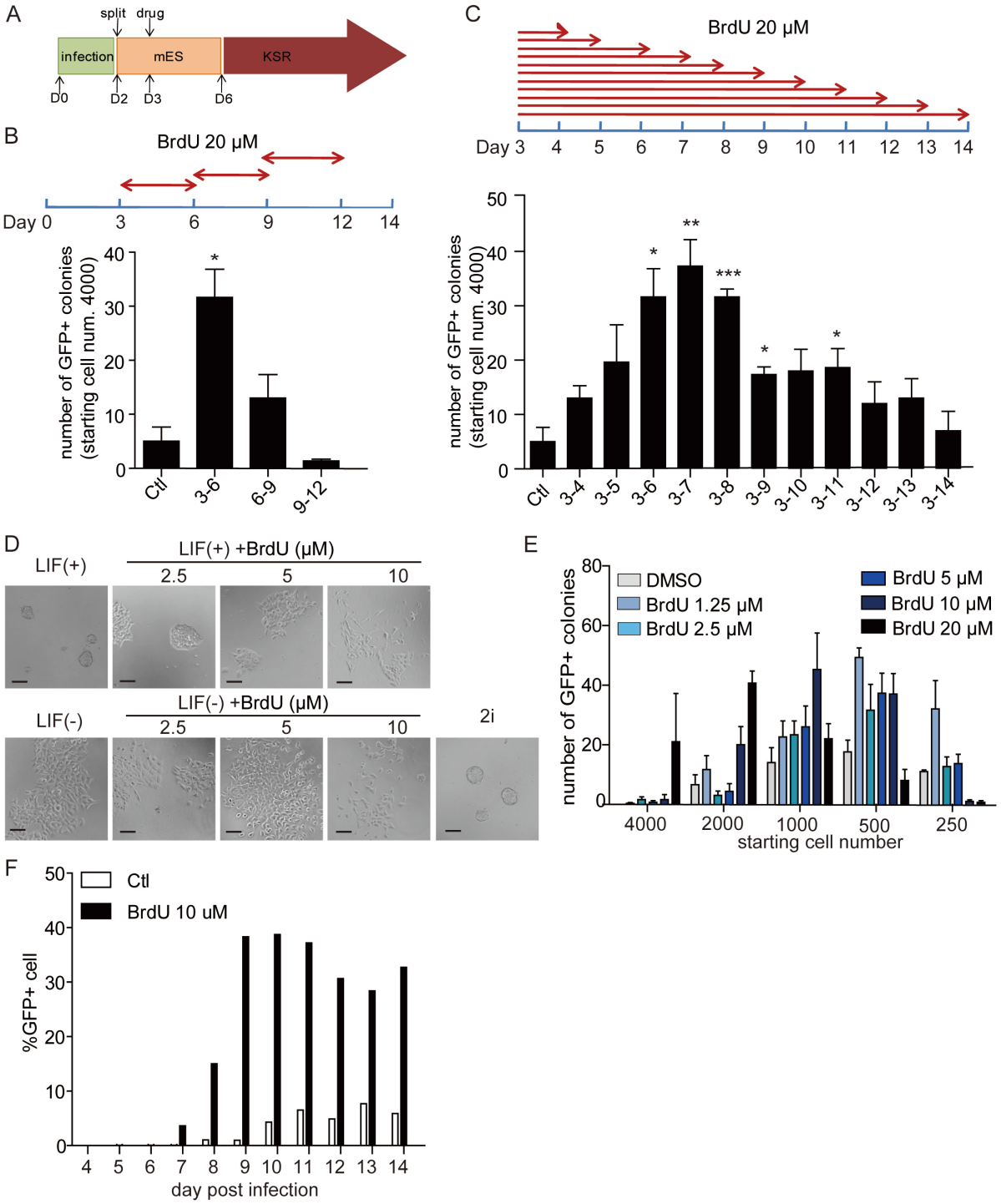
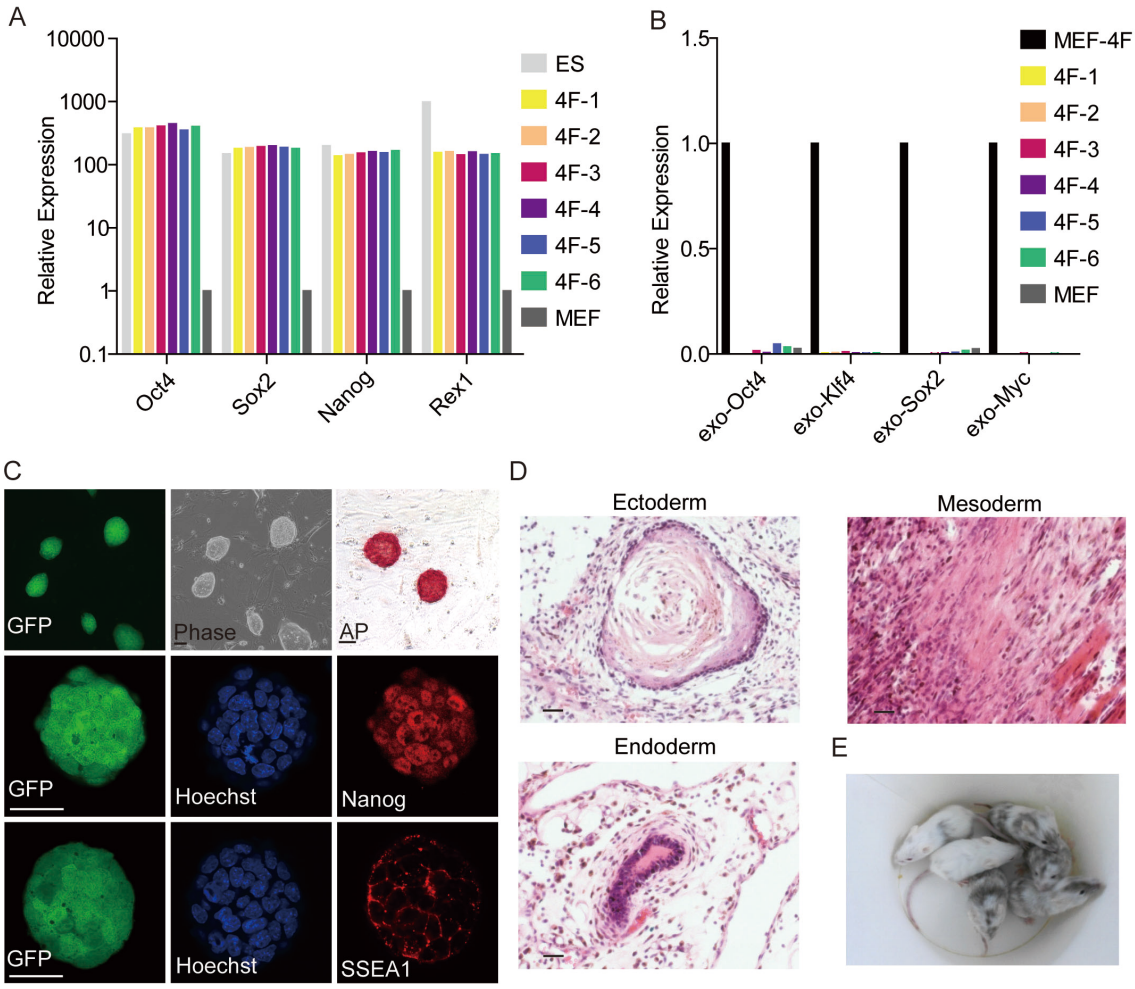
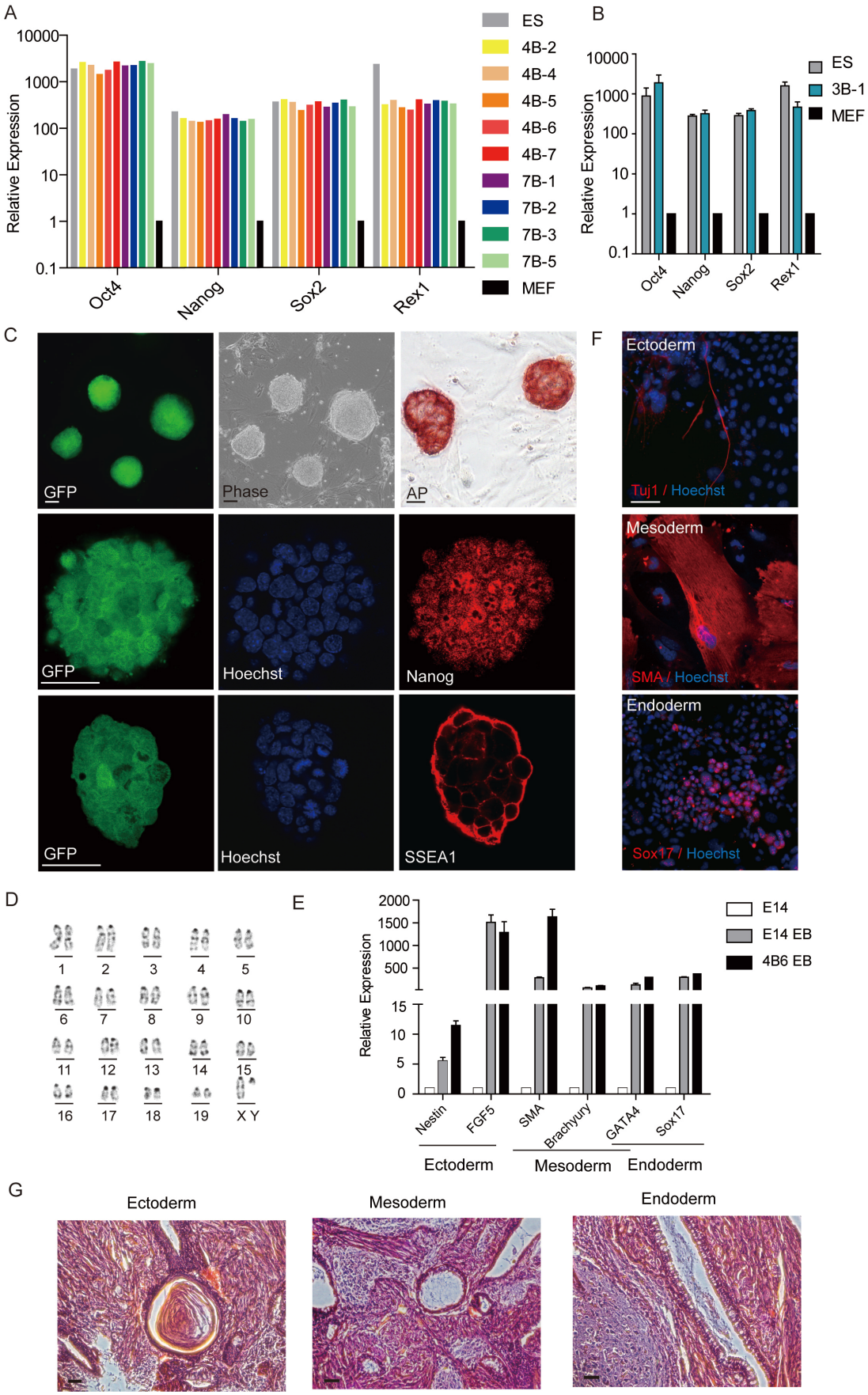


Supplementary information, Figure S1 Optimization of reprogramming conditions. (A) Schematic representation of iPSC induction protocol with Yamanaka factors and chemicals. (B) BrdU (20 μM) was added for 3 days starting from day 3, 6, 9 and 12 in OSKM (4F)-infected MEFs and GFP^{+} colonies were counted at day 14. Means ±

SEM of a representative experiment (n=3). (C) 4F-infected MEFs were treated with BrdU (20 μM) for various durations. Means ± SEM of a representative experiment (n=3). (D) Morphology of E14 cells cultured in mES medium containing BrdU (2.5, 5 and 10 μM) for 48 hours. Top: supplemented with 1,000 U/mL LIF. Bottom: without LIF. 2i (3 μM PD0325901 and 3 μM CHIR99021) was used as a control. Scale bar, 50 μm. (E) 4F-infected MEFs in various densities were treated with various concentrations of BrdU. GFP^+ colonies were counted at day 14. Means ± SEM of a representative experiment (n=3). (F) Percentage of GFP^+ cells at various time point after 4F induction and BrdU (10 μM) treatment. Starting cell density was1,000 MEFs/well in 96-well plates.



Supplementary information, Figure S2 Characterization of OSKM (4F)-iPSCs generated with BrdU. (A) qPCR analysis of endogenous pluripotency genes in 4F-iPSC clones generated with BrdU. mESC E14 and MEF were used as controls. (B) qPCR analysis of exogenous transgenes in 4F-iPSC clones generated with BrdU. MEF and MEF infected with 4F for 4 days (MEF-4F) were used as controls. (C) GFP expression, morphology, AP staining, immunofluorescent staining of pluripotency marker Nanog and SSEA-1 in iPSC clone generated with 4F and BrdU (#4F-1). Scale bar: 50 μm. (D) HE-stained sections of teratoma formed with iPSC clone 4F-1. Scale bar: 50 μm. (E) Chimeric mice produced with iPSC clone 4F-1.



Supplementary information, Figure S3 Characterization of CiPSCs. (A) qPCR

analysis of endogenous pluripotency genes in CiPSC clones generated with BrdU and other small molecules (VC6TFZP (7B) or C6FZ (4B)). E14 and MEF were used as controls. (B) qPCR analysis of endogenous pluripotency genes in CiPSCs generated with BrdU and C6F (3B-CiPSC-1). E14 and MEF were used as controls. (C) GFP expression, morphology, AP staining, immunofluorescent staining of pluripotency markers Nanog and SSEA-1 in 3B-CiPSC-1. Scale bar: 50 μm. (D) Karyotype analysis of 4B-CiPSC-6. (E) qPCR analysis of lineage-specific genes (ectoderm: *Nestin* and *FGF5*; mesoderm: *SMA* and *Brachyury*; endoderm: *GATA4* and *Sox17*) in embryoid bodies (EBs, day 6) derived from 4B-CiPSC-6. E14 and E14-EBs were used as controls. (F) Immunofluorescent staining of lineage-specific markers (ectoderm: Tuj1, mesoderm: SMA, endoderm: Sox17) in spontaneously differentiated cells from EBs (day 12) derived from 4B-CiPSC-6. Nuclei were stained with Hoechst 33342. Scale bar: 50 μm. (G) HE-stained sections of teratoma formed with 3B-CiPSC-1. Scale bar: 50 μm.

Supplementary information, Table S1 Effects of BrdU in TK gene-mutation assay

Treatment (μM)	RSG	PE0 （%）	RS0 （%）	PE3 （%）	RS3 （%）	RTG （%）	N-MF （$\times 10^{-6}$）	S-MF （$\times 10^{-6}$）	T-MF （$\times 10^{-6}$）
Ctl	1.00	52.06	100.00	88.02	100.00	100.00	2.16	1.42	3.70
25	0.65	52.06	100.00	101.96	115.85	74.91	2.38	1.37	3.89
50	0.38	35.66	68.50	56.93	64.68	24.26	11.07	6.14	19.14
100	0.18	26.72	51.32	46.10	52.38	9.52	35.85	12.54	63.55
200	0.14	15.32	29.43	14.46	16.43	2.22	135.58	22.02	218.51
MMS	0.56	35.66	68.50	76.62	87.05	48.67	93.33	14.22	144.09

RSG: Relative Suspension Growth; PE0: Plating Efficiency on day0; PE3: Plating Efficiency on day3; RS0: Relative Survival on day0; RS3: Relative Survival on day3; RTG: Relative Total Growth; N-MF: Normally Growing Mutant-Mutation Frequency; S-MF: Slowly Growing Mutant-Mutation Frequency; T-MF: Total-Mutation Frequency. MMS: methylmethansulfonate, 1 μg/mL

Supplementary information, Materials and Methods

Derivation of MEFs and cell culture

OG2 mice, which carry a transgenic Oct4 promoter driving GFP expression, were mated with C57 mice and MEF cells were isolated from E12.5 embryos heterozygous for the *Oct4*-GFP transgenic allele. Internal organs and gonads were removed before processing for isolation of MEF cells. MEFs were grown in DMEM supplemented with 10% FBS, 2 mM L-glutamax, 0.1 mM nonessential amino acids (NEAA), 100 units/ml penicillin and 100 μg/ml streptomycin. Isolated MEF cells in early passages (up to passage 3) were used for further experiments.

Transcription factor-mediated mouse iPSC generation

Retrovirus were produced by transfection of plat-E cells with pMXs retroviral vectors containing the coding sequences of mouse *Oct4*, *Sox2*, *Klf4* and *c-Myc* (obtained from Addgene). MEFs (passage 1-3) were seeded at a density of 150,000 cells per well in 6-well- plate 18-24 h before infection. Virus containing supernatants, supplemented with 8 μg/ml polybrene, were added onto the plates of MEF cell cultures and centrifuged at 2,500 rpm for 90 min to ensure the infection. Medium was changed immediately after virus transduction and this day is counted as day 0. Two days post virus infection, MEFs were trypsinized into single cells and reseeded at a density of 4,000 cells per well on 96-well-plates supplemented with mES medium (DMEM supplemented with 15% FBS, 2 mM L-glutamax, 0.1 mM nonessential amino acids (NEAA), 0.1 mM β-mercaptoethanol 1,000 U/ml LIF, 100 units/ml penicillin and 100 μg/ml streptomycin). At day 6, culture medium was replaced with

KSR medium (knockout-DMEM supplemented with 15% knockout serum replacement, 2 mM L-glutamax, 0.1 mM NEAA, 0.1 mM β-mercaptoethanol, 1,000 U/ ml LIF, 100 units/ml penicillin and 100 μg/ml streptomycin). BrdU at various concentrations was added at day 3 with various durations. GFP^+ colonies were scanned and counted using Acumen eX3 microplate reader (TTP Labtech) at various time points. Cells were also trypsinized and the percentage of GFP^+ cells was analyzed using a FACS Calibur (BD Biosciences). GFP^+ cells were gated with a control signal from the PE channel and a minimum of 50,000 events was recorded.

Full chemical-mediated mouse iPSC generation

MEFs were seeded at a density of 50,000 cells per well on 6-well-plate. On the next day (day 0), the MEFs medium was replaced with chemical inducing medium (knockout-DMEM supplemented with 10% knockout serum replacement, 10% FBS, 2 mM L-glutamax, 0.1 mM NEAA, 0.1 mM β-mercaptoethanol, 50 μg/ml bFGF, 100 units/ml penicillin and 100 μg/ml streptomycin) containing the small-molecule cocktails (VPA (0.5 mM), CHIR99021 (10 μM), RepSox (10 μM), parnate (2 μM), Forskolin (10 μM), TTNPB (50 nM), and BrdU (0-10 μM). The medium was changed every 4 days. DZNep (1 μM) was added to the cell cultures on day 16. On day 32, the chemical inducing medium containing small-molecule cocktails were replaced with 2i-medium (knockout-DMEM supplemented with 10% knockout serum replacement, 10% FBS, 2 mM L-glutamax, 0.1 mM NEAA, 0.1 mM β-mercaptoethanol, 1000 U/ ml LIF, 100 units/ml penicillin and 100 μg/ml streptomycin, 3 μM CHIR99021 and 1 μM PD0325901). GFP^+ colonies could be observed at day 45 to day 50 and were

counted at day 55 to day 60.

mESC culture

E14 cells were maintained feeder free on gelatin-coated plastics in mES medium supplemented with 1,000 U/ml LIF and passaged every 3 days. In testing conditions, ESCs were cultured in mES media with or without LIF in the presence of various concentrations of BrdU (2.5, 5, 10 μM) for 48 hours.

AP and immunofluorescent staining

For AP staining, iPSCs were fixed with 4% paraformaldehyde (PFA) in PBS for 45 s, washed once with PBS and detection was performed using a leukocyte AP kit (Sigma, catalog No 85L3R) according to the manufacturer's protocol. For immunofluorescent staining, cells were fixed with 4% PFA and incubated with primary antibodies against mSSEA-1 (Santa Cruz, sc-21702) and mNanog (Millipore, AB5731), followed by the appropriate secondary antibodies conjugated to Alexa Fluor 555 (Invitrogen). Nuclei were counterstained with Hoechst 33342 (Sigma). Images were taken with an Olympus IX51 inverted fluorescent microscope or an Olympus FV10i confocal microscope.

Real-time PCR

Total mRNA was isolated using TRIzol (Invitrogen) and 1 μg RNA were used to synthesize cDNA using the PrimeScript^{TM} RT reagent kit (Takara, DRR037A) according to the manufacturer's protocol. Real-time PCR was performed using FastStart Universal Probe Master Mix (Roche) and analyzed with a Stratagene Mx 3000P thermal cycler. Primer sequences for endogenous Oct4, Sox2, Nanog, Rex1

and viral-specific Oct4, Sox2, Klf4, and c-Myc are as previously previously described[1]. Gene expression was normalized to GAPDH.

Bisulfite sequencing

Bisulfite sequencing was used to detect DNA methylation in the promoter regions of *Oct4* and *Nanog*. Genomic DNA was isolated and bisulfite conversion was performed in agarose beads[2]. Nested PCR was performed to amplify the promoter regions after bisulfite conversion and the PCR products were cloned into pMD19-T vectors (Takara). Ten randomly selected clones from each sample were sequenced and analyzed. PCR primers are as previously described[1].

Embryonic bodies (EBs) formation and *in vitro* differentiation assay

For EB formation, 5×10^5 CiPSCs (4B-1) or E14 cells were harvested by trypsinization and re-seeded in 10 mL LIF-free mES medium in 100 mm bacterial culture dishes to generate EBs. At day 6, total RNA was extracted from EBs to detect differentiation gene expression by qRT-PCR. For immunostaining of lineage markers in spontaneously differentiated cells, cells were trypsinized into single cell suspension and EBs were formed with the hanging drop method. For each drop, 20 μL medium containing 1×10^3 CiPSCs were used. After cultured in hanging drops for 2 day, EBs were reseeded in gelatin-coated 24 well plates for another 10 days and then collected for immunostaining.

Teratoma formation and chimera production

About 1×10^6 mESCs or iPSCs were suspended in 200 μL mES medium and subcutaneously injected into the NOD-SCID mice. The animals were checked 2–3

times per week. Four weeks after injection, teratomas were harvested, fixed overnight with 4% PFA, embedded in paraffin, and sectioned. Sections of the teratomas were stained with hematoxylin and eosin and analyzed histologically.

Prepubertal (4-5weeks of age) donor ICR female mice were superovulated, mated overnight to intact ICR stud males and euthanized by cervical dislocation on day 3.5. Uteri were collected after euthanasia and flushed with FHM medium (Millipore) for the collection of blastocysts. Twelve-fifteen iPSCs were injected into the blastocoel of each blastocyst. After injection, the blastocysts were surgically transferred into recipient female ICR mouse that were pseudopregnant by mating with vasectomized males. Recipient females carried the pups to term and nursed until weaning at three weeks. Chimeric mice were identified by coat color.

TK gene-mutation assay

The human lymphoblastoid cell line TK6 is heterozygous at the thymidine kinase locus ($TK^{+/-}$) and thus the remaining wild type allele serves as the target fora mutation. Mutation of this allele leads to survival of the cells in trifluorothymidine (TFT) containing medium medium[3, 4]. TK6 cells (10^7cells in 20 mL RPMI-1640 medium containing 10% horse serum) were transferred into a 75-cm^2 flask and treated with negative (vehicle) or positive (methylmethansulfonate, MMS, 1 μg/mL) controls or various concentrations of BrdU (25, 50, 100 and 200 μM) for 24 hours at 37°C in an atmosphere of 5% CO_2. For the measurement of plating efficiency on day 0 (PE0) after exposure, the TK6 cells were transferred onto 96-well-plate at a density of 1.6 cells/well/0.2 mL medium. The numbers of colony-containing wells were counted on

day 14. For the determination of plating efficiency on day 3 (PE3) and mutation frequency (MF) ($TK^{-/-}$mutants per 10^6 cells) after exposure, the TK6 cells were transferred into the 75-cm^2 flasks and cultured for 3 days to permit the expression of the TK-deficient phenotype. The density of cells was recorded everyday and cell suspensions were diluted to keep a density below 2×10^5 cells/ml in case of overgrowth. For the measurement of plating efficiency on day 3 (PE3), the cells were seeded at a density of 1.6 cells/well onto 96-well plates and the numbers of colony-containing wells were counted on day 14. To determine the mutation frequency (MF), the cells were seeded onto 96-well plates at 40,000 cells/well in the presence of the selective agent TFT (3 μg/ml). The numbers of wells containing normally growing TK mutants were counted on day 14 and N-MF (normally growing mutant-Mutation Frequency) was calculated. The plates were then re-fed with TFT and were incubated for another 14 days. Then the numbers of wells containing newborn colonies (slowly growing TK mutants) were counted and S-MF (slowly growing mutant-Mutation Frequency) was calculated. All wells containing colonies were also counted and T-MF (Total-Mutation Frequency) was calculated. Cytotoxicity for the TK6 cells was evaluated as relative survival (RS) calculated from PE and relative suspension growth (RSG), derived from cell growth-rate during a 3-day expression period.

These parameters were calculated as previous reported[3, 4]. Briefly, The plating efficiency (PE) = (–ln (empty well number/total well number))/(cells plated/well). Relative survival (RS (%))=($PE_{test}/PE_{control}$)×100%. The relative suspension growth

(RSG)=$(DCG1\times DCG2\times DCG3)_{test}$ / $(DCG1\times DCG2\times DCG3)_{control}$. DCG is the growth rate between days 0 and 1 (DCG1) or between days 1 and 2 (DCG2) or between days 2 and 3 (DCG3). RTG (%)=RSG×RS3 (%). Mutation frequency (MF ($\times 10^{-6}$))=(PE_{mutant}/PE3).

References

1. Xu X, Wang Q, Long Y *et al.* Stress-mediated p38 activation promotes somatic cell reprogramming. *Cell research* 2013; **23**:131-141.
2. Hajkova P, el-Maarri O, Engemann S, Oswald J, Olek A, Walter J. DNA-methylation analysis by the bisulfite-assisted genomic sequencing method. *Methods in molecular biology* 2002; **200**:143-154.
3. Honma M, Hayashi M, Shimada H *et al.* Evaluation of the mouse lymphoma tk assay (microwell method) as an alternative to the in vitro chromosomal aberration test. *Mutagenesis* 1999; **14**:5-22.
4. Liber HL, Thilly WG. Mutation assay at the thymidine kinase locus in diploid human lymphoblasts. *Mutation research* 1982; **94**:467-485.