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Supporting Information for

Highly efficient reprogrammable mouse lines with integrated reporters to track the route to pluripotency

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Supporting Information Text

Detailed Methods

Reagents. Dulbecco's Modified Eagle Medium (DMEM) and GlutaMAX™ were purchased from Thermo Fisher Scientific (Cat #11960069 and # 35050079, respectively). Fetal bovine serum (FBS) tested to support generation of germline chimeras, antibiotics (penicillin streptomycin), MEM non-essential amino acids (NEAA), sodium pyruvate, and 0.25 % Trypsin EDTA were obtained from Gibco by Thermo Fisher Scientific (Cat #12483-020, #15140122, #11140050, #11360070, and #25200072, respectively). Doxycycline hyclate, 2-mercaptoethanol, and mitomycin C were purchased from MilliporeSigma (Cat #D9891, # M6250, and #MO-503, respectively) and Accutase from StemCell Tech (Cat #07920). Insulin and KnockOut™ Serum Replacement (SR) were purchased from Thermo Fisher Scientific (Cat #12585014 and #10828-028, respectively) while mitogen-activated protein kinase inhibitor PD0325901 and glycogen synthase kinase-3 inhibitor CHIR99021 from Selleck Chemicals (Cat #S1036 and #S2924, respectively. The AP staining kit was purchased from Vector Laboratories (Cat #SK-5100).

The 1º antibodies were purchased as follows: NANOG from ReproCell (Cat #RCAB002P-F, lot #20100713), OCT4 from Santa Cruz (Cat #sc-5279, lot #J0703), SOX2 from R&D Systems (Cat #MAB2018, lot # KGQ0207101 and KLF4 from MiliporeSigma (Cat #09-821, lot #HG1940987). Dox pellet food was from Envigo (Cat # TD.120769).

Flow cytometry antibodies were purchased as follows: CD16/32 unconjugated FcBlock (BD 553141, lot # 5191710), Lineage panel (CD3/11b/45R/B220/Ter119/Ly-6G/C) +isotype control-PerCP-Cy5.5 (BD 561317), CD150-PE (BD 562651, lot #6273514), c-kit-BV421 (BD 566290), CD48-PC7 (BD 560731), SCA-1-V500 (BD 561228, lot # 5267707, CD-45-APC (BD Biosciences; clone 30-F11, cat# 559864) or CD-45 PerCP-Cy5.5 (eBiosciences; clone 30-F11; cat# 45-0451-82). Pre-conjugated isotype control antibodies for the cell surface markers were purchased from BD. The fixable viability dye (eFluor 455 UV) was purchased from eBiosciences (cat # 65-0868-14, lot # E19318-106).

iPSC and ESC culture. iPSCs and ROSA26-rtTA ESCs were grown at 37°C in 95% air 5% CO_2 on mitomycin C inactivated MEF obtained from TgN(DR4)1Jae/J mice (stock No. 003208) at all times, except one passage on gelatinized tissue culture plates before aggregation, based on standard protocols (1). Cells were fed three times a week and passaged when they reached 70-80% confluency. Trypsin-EDTA (0.25%) was used for the passage of cells grown in ESC medium that consisted of DMEM (high glucose) supplemented with 15% FBS, 2 mM GlutaMAX™, 1mM sodium pyruvate, 0.1mM NEAA, 50 U/ml penicillin and streptomycin, 0.1mM 2-mercaptoethanol and 1000 U/ml LIF. Reprogramming medium consisted of ESC medium as above, supplemented with dox (2.0µg/mL for Col1a1-OSKM cells and 1.5µg/mL for all other reprogramming). Accutase was used for passaging iPSCs grown in SR +2i medium [high glucose DMEM supplemented with 15% KnockOut™ Serum Replacement, 1mM sodium pyruvate, 0.1mM non-essential amino acids, 0.1mM 2-mercaptoethanol, 2 mM GlutaMAX™, 50 U/ml penicillin/streptomycin, 1000 U/ml LIF, 5µg/ml Insulin, 1µM of PD0325901 and 3µM of CHIR99021]. KnockOut™ Serum Replacement (SR) +2i medium was exclusively used for 2-4 passages prior to generation of chimeras (2).

Mice. Crl:CD-1 (ICR) and 129S2/SvPasCrl (129) mice were purchased from Charles River Laboratories. C57BL/6J (B6) and Col1a1-OSKM ($R26^{rtTA*M2}$;$Col1a1^{4F2A}$) (3) mice (stock No. 011004) were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). *Oct4-GFP* (Tg(Pou5f1-EGFP)1Nagy) (4) and ROSA26-rtTA ($Gt(ROSA)26Sor^{tm1.1(rtTA,EGFP)Nagy}$) (5) mouse lines were established in Nagy lab. *Oct4-GFP* mice were generated from R1 ES cells and maintained on outbred CD-1 background. CD-1 x 129 MEFs were derived from the cross of these animals with 129S2. *Oct4-GFP* animals were backcrossed to 129S2 and C57BL/6J for 7 generations prior to derivation of 129 and B6 MEFs described here. Animals were housed in a 12h light/dark cycle and provided with food and water ad libitum in individually ventilated units (Techniplast) in the specific-pathogen free facility at The Centre for Phenogenomics (TCP). All animal work was carried out

following Canadian Council on Animal Care guidelines for Use of Animals in Research and Laboratory Animal Care under protocols approved by the TCP Animal Care Committee.

Availability of the iRep mouse lines and their variants from the Jackson's lab.
JAX#031011 iRep1 (or Oct4-GFP; ROSA26-rtTA(Δneo)),
<https://www.jax.org/strain/031011>
(also called Oct4-GFP; ROSA26-rtTA(Δneo); OKMSCh250)
iRep1 (inducible reprogrammable mouse 1; Line 250): The mice carry the Oct4-GFP transgene and express rtTA from the widely expressed Rosa26 locus since the loxP flanked neomycin resistance gene separating the promoter from the rtTA was removed. These mice allow doxycycline-inducible expression of the polycistronic OKMS cassette encoding the four reprogramming factors ((Oct4 [Pou5f1], Klf4, c-Myc [Myc] and Sox2)) linked to mCherry from the Tet-O-OKMS-mCherry transgene, integrated into chromosome 13. GFP is expressed when endogenous OCT4 is activated.

JAX#031013 iRep1 (or ROSA26-rtTA(Δneo) - without Oct4-GFP),
<https://www.jax.org/strain/031013>
(also called ROSA26-rtTA(Δneo); OKMSCh250 without Oct4-GFP)
iRep1 variant: The mice do not carry the Oct4-GFP transgene. Otherwise, they are the same as JAX#031011.

JAX#031010 Cre recombinase conditional iRep1 (with ROSA26-rtTA(neo) - with Oct4-GFP),
<https://www.jax.org/strain/031010>
(also called Oct4-GFP; ROSA26-rtTA(neo); OKMSCh250)
Cre conditional iRep1 variant: The mice express rtTA only if a Cre recombinase expressing transgene is introduced to the system and removes the neomycin resistance gene. It could be cell type-specific, depending on the Cre expression. Otherwise, the mice are the same as JAX#031011.

JAX#031012 Cre-inducible iRep1 (ROSA26-rtTA(neo) - without Oct4-GFP),
<https://www.jax.org/strain/031012>
(also called ROSA26-rtTA(neo); OKMSCh250, without Oct4-GFP)
Cre conditional iRep1 variant: The mice are the same as JAX#031010, except they do not carry the Oct4-GFP transgene.

JAX#031009 iRep2 (or Oct4-GFP; ROSA26-rtTA(Δneo))
<https://www.jax.org/strain/031009>
(also called Oct4-GFP; ROSA26-rtTA(Δneo); OKMSCh72)
iRep2 (inducible reprogrammable mouse 2; Line 72): The mice are the same as JAX#031011 but the OKMS transgene integrated into chromosome 14.

Cell isolation
Generation of MEFs. Embryos were collected at Embryonic Day (E)12.5 or E13.5, where E0.5 is the day when a vaginal plug was observed. Embryos were decapitated, eviscerated, and placed in a drop of 500µl of trypsin in which they were minced with scalpels. After 5 min at 37ºC, 1ml of trypsin was added and single cells were obtained by repetitive pipetting. They were seeded in MEF medium (that consisted of DMEM (high glucose) supplemented with 10% FBS, 2 mM GlutaMAX™, 1 mM sodium pyruvate, 0.1 mM NEAA, 50 U/ml penicillin and streptomycin, and 0.1 mM 2-mercaptoethanol), at a density of one embryo per 10cm dish. Medium was changed the next day and each dish was passaged to 15cm dishes the day after. They were frozen or single cell plated two days later. This procedure was applied for B6, 129, and CD-1x129 embryos as well as chimeric and reprogrammable embryos.

TF: Tails were harvested, cut in small pieces and plated in a 10 cm dish with 5 ml of MEF medium. Medium was changed gently every three days. They were passaged after 14 days to a 6 cm dish.

Fetal liver cells: E13.5 embryos were harvested and the fetal liver was removed and stored in 500µL ice cold FACs buffer (PBS without Ca/Mg, 3% FBS, 1mM HEPES pH7.5). A single-cell suspension was produced by trituration into a 1mL syringe and 23-gauge needle.

Bone marrow: Adult mice were sacrificed by cervical dislocation followed by removal of tibias and femurs and flushing the bone cavity with ice cold FACs buffer, followed by trituration to produce a single-cell suspension.

Neurosphere (NS) Culture. E13.5 embryos or adult mice were decapitated. The ventricular zone (VZ) /subventricular zone (SVZ) was dissected as previously described (6–8). The tissue was mechanically and enzymatically dissociated (1.33 mg/ml trypsin (Sigma), 0.67 mg/ml hyaluronidase (Sigma), and 0.2 mg/ml kynurenic acid (Sigma)) into a single-cell suspension. Cells were plated at clonal density (10 cells/µL) in neurosphere medium (Neurobasal (Thermo Fisher Scientific) containing L-glutamine (2 mM, Thermo Fisher Scientific), penicillin/streptavidin (100 U/0.1 mg/ml (1x), Thermo Fisher Scientific), B27 (1:50, Thermo Fisher Scientific), epidermal growth factor (MilliporeSigma; 20ng/ml), fibroblast growth factor (MilliporeSigma; 10ng/ml), and heparin (2000ng/ml). NSs were passaged on Day 7 after culture initiation by mechanical dissociation into a single-cell suspension, and replated under the same conditions as the 1º culture. Secondary neurospheres were mechanically dissociated into a single-cell suspension, and live single cells were sorted by flow cytometry.

Transfection and 1º reprogramming. MEFs derived from *Oct4-GFP* transgenic mice (4) were cultured in MEF medium for three days, at which point they were transfected with three plasmids: TetO-OKMS-mCherry (1µg) (9), the reverse tetracycline transactivator (rtTA) transposon plasmid (PB-CAG-rtTA) (0.3µg) (9), and PB transposase expression plasmid (pCMV-hyPBase) (0.5µg) (Sanger, UK). Fugene HD transfection reagent (Promega; Cat #E2311) was used according to the manufacturer's protocol (1.8-2µg of DNA per 8µl of transfection agent). The reagent was washed away 24h after transfection and the cells were subsequently cultured in Reprogramming Media, which was changed every 2-3 days. Individual colonies were picked at day 11 and each placed for 10 minutes in a well of a 96-well plate containing 15µl of trypsin. After disaggregation by pipetting, they were seeded in a new 96-well plate of inactivated feeders and cultured in medium with or without dox. Once ESC-like colonies appeared, they were trypsinized and seeded in 24-well plates in Reprogramming Media. Confluent wells were passaged to 12-well plates, which were further tested for dox-independence by passaging a fraction of the culture to dox-free ESC medium. Once the cells grew in the absence of dox, they were passaged to 6-well plates and then to 10 cm dishes at which point they were frozen (usually at passage 7) in ESC medium:FBS:DMSO at the ratio of 5:4:1. The plasmids used are available for access on Addgene.

Generation of chimeras. CD-1 outbred albino females were used as embryo donors for aggregation with iPSCs and as pseudopregnant recipients. Details of morula aggregation and embryo transfer can be found in (1). Briefly, embryos were collected at day E2.5 from superovulated CD-1 mice. Zonae pellucidae of embryos were removed by treatment with acid Tyrode's solution (SigmaT1788). iPSCs colonies were briefly treated with Accutase to form loosely connected clumps of 7-10 cells. Each zona-free embryo was aggregated with a clump of 7-10 cells inside depression wells made in the plastic dish with an aggregation needle (BLS Ltd, Hungary) and cultured overnight in microdrops of KSOM medium with amino acids (Zenith Biotech/IVFonlineZEKS-50) under embryo tested mineral oil at 37°C in 94% air/6% CO_2. The next morning, morulae and blastocysts were transferred into the uteri of pseudopregnant CD-1 females previously mated with vasectomized males. Chimeric embryos were dissected at mid-gestation or left to term. Chimeras were identified at birth by the presence of black eyes and later by coat pigmentation. Chimeras with more than 50% iPSC contribution to the coat colour were bred with B6 mates and germline

transmission was determined by genotyping. Chimeric-transmitters were further bred with C57BL/6J to establish the following lines:
iRep1: TgTn(pb-tetO-OKMS,-mCherry)250Nagy with the synonym TgTn(pb-tetO-Pou5f1,-Klf4,-Myc,-Sox2,-mCherry)250Nagy (JAX#031011)
iRep2: TgTn(pb-tetO-OKMS,-mCherry)72Nagy with the synonym TgTn(pb-tetO-Pou5f1,-Klf4,-Myc,-Sox2,-mCherry)72Nagy (JAX#031009)

Cell sorting and single cell reprogramming. MEFs were harvested by trypsinization (Trypsin-EDTA (0.05%)) and were resuspended in sorting buffer (HBSS, 1% FBS, 25mM HEPES, pH 7.2) with DAPI (0.4 µg/mL). BM was flushed from femurs and tibias of adult mice in FACs staining buffer (PBS without Ca/Mg +3%FBS and 1mM HEPES pH 7.5), and filtered through a cell strainer. BM cells were stained on ice for 10 minutes with anti-CD16/32 antibody followed by 15 minutes on ice with directly conjugated antibodies (see Reagents for details). Using a MoFlo Astrios EQ cell sorter (Beckman Coulter, Miami, FL, USA) equipped with 355nm, 488nm, 561nm and 640 nm lasers, viable cells were sorted under low pressure conditions (25 psi, 100 micron nozzle tip). For some experiments, cells were sorted directly into 96-well plates, pre-plated with fibroblast feeder cells and reprogramming medium. BM and FL cells were sorted following antibody staining, and committed blood lineage (CD45+) cells were plated into a 96 well plate (one cell/well) on top of a layer of inactivated MEF feeders, in dox-containing Reprogramming Media. In contrast, MEFs, TFs, and NSs were allowed to proliferate before they were single-cell sorted and induced with dox (see Methods). 1B tetraploid MEF cells (mCherry-) (10) were used as the gating control for background mCherry fluorescence. The system was calibrated to ensure that no more than a single viable cell was plated in each well and confirmed by visual inspection using an inverted fluorescence microscope. Sorted or unsorted cells used for bulk culture reprogramming were counted using viability marker under a hemocytometer, and serially diluted to appropriate cell numbers for plating.

Immunofluorescent staining. Cultured cells were rinsed once with phosphate buffered saline and fixed with 4% paraformaldehyde solution at room temperature for 15 minutes. Intracellular epitopes were stained after 30 min permeabilization with 0.3% Triton X100 in PBS with 5% fetal bovine serum and 1% bovine serum albumin. The cells were then incubated overnight at 4ºC with 1º antibodies, extensively washed, and further incubated with the secondary antibodies for 1 h. They were then incubated in Hoechst stain for 10 min, washed, and mounted onto glass slides. For negative controls, cells were incubated without 1º antibodies and MEFs were stained in parallel to iPSCs. The cells were visualized using a confocal microscope (Zeiss). The images were processed using Zeiss's ZEN software 2011.

qRT-PCR. Total RNA was isolated by Qiagen RNAeasy Mini Kit (Cat #74104) according to instructions of the manufacturer. One microgram of total RNA was used for cDNA synthesis using the Qiagen QuantiTect Reverse Transcription Kit (Cat #205313). For quantitative RT–PCR we used LuminoCt SYBR Green qPCR ReadyMix (MilliporeSigma, L6544-2000RXN). The JANUS automated liquid handling robot (Perkin- Elmer) was used to load 384-well plates which were run on a CFX384 (Bio-Rad) with an annealing temperature of 58º C for all primers. Primer pairs were all optimized for efficiency and melt curves. All PCR reactions were performed in triplicate. Primer sequences are listed in Table S1. The colour scale is reporting the log10 value of gene expression normalized to ESC control and *EEF2* housekeeping gene.

iRep1 and iRep2 mouse genotyping. Primer sequences for PCR based genotyping are provided in Table S2. The strategy for detecting the PB:TetO-OKMS-mCherry transgenes in iRep1 and iRep2 mice are described in Fig. S2.

Time-lapse video microscopy. For live imaging, 2º MEFs were plated at a confluency of 80,000 cells per 6-well densities for iRep1, iRep2, and Col1a1. Reprogramming cells were sampled at the reported time points of reprogramming and seeded in 0.2% gelatin-coated 6-wells (Corning Inc). Cells were incubated in a humidified 5% (v/v) CO2 air environment at 37 °C in an on-stage incubation system (Zeiss). Live imaging was conducted at 10x objective with brightfield images

collected 10 min^{-1} and fluorescence 1 hr^{-1} for 72 hours (Zeiss Axio Observer). Images were analyzed using ZEN Blue software 2012 v1.1.2.0 (Zeiss).

Statistical Analyses. The survival plots in Fig. 4D were obtained by Kaplan-Meier estimates and the test of significance was performed with the log-rank test (11). The association between the time the clones reached dox-independence and the reprogramming interruption treatment (Fig. S1E) was analysed with parametric survival analysis models assuming a Weibull distribution (11). The association between efficiency of reprogramming and the interruption treatment (Fig. 3B, 4B, 4C, Fig. S1D) was performed with the beta regression (12), which models variables that assume values in the standard unit interval (0, 1). Beta regression assumes that the dependent variable is beta-distributed, and that its mean is related to a set of regressors through a linear predictor with unknown coefficients and a link function. The analysis of mCherry fluorescence intensity versus the percentage of AP+ wells or the reprogramming interruption treatment (Fig. S1F and S1G, respectively) was based on linear regression and one-sided t test. Raw correlations between the three variables: efficiency of reprogramming, days of dox dependence (log transformed) and mCherry intensity was based on Pearson correlation coefficient (Fig. S1F and S1G). Two-sided Student t-test was used in Fig. 3B. All statistical analyses, except student t-test analysis, were performed with the statistical software *R* http://www.R-project.org.

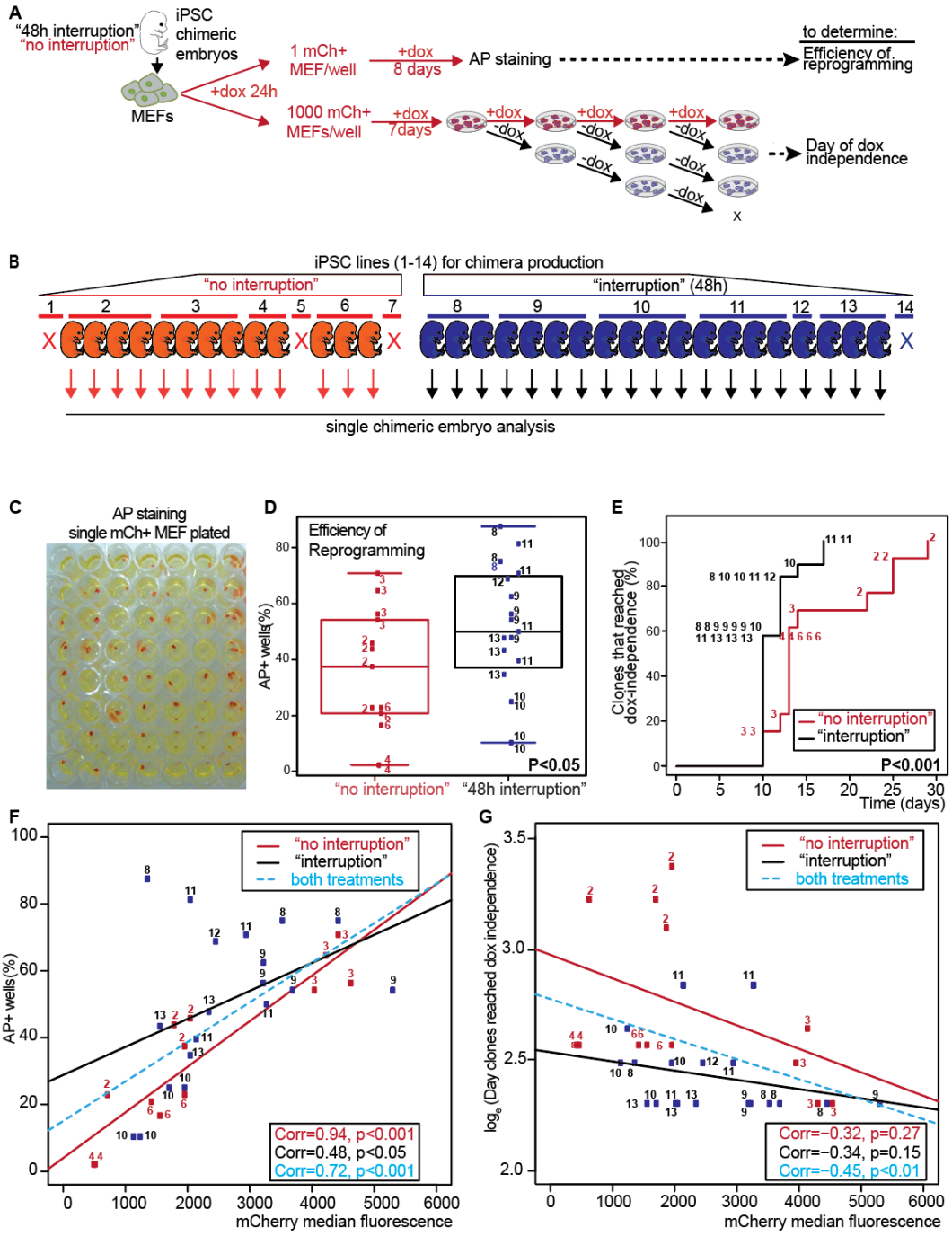

Fig. S1. Comparison of 2º systems derived from 1º iPSC clones generated using different dox treatments.

A. Experimental design for comparison of 2º MEFs derived from E12.5 embryo chimeras to determine the efficiency of reprogramming and the day of becoming exogenous reprogramming factor (doxycycline) independent pluripotent stage.

B. The iPSC lines were tested for chimera production and the number of chimeras produced and used for analysis from each line are shown. For each aggregation performed with the iPSC liens (numbered 1 to 14), we indicated the number of embryos obtained.

C. Representative AP staining. 24 hours following dox induction, 48 MEFs were single-cell sorted onto individual wells containing feeders and AP stained after 8 days of growth in reprogramming medium.

D. Efficiency of AP+ colony formation from 48 single 2º MEFs seeded. Each dot on the plot represents a single cell line (in %) obtained from n=48 cell plated individually, one cell per well. The experiment was repeated three times.
The rectangle height represents the interquartile range, the bar inside the rectangle the median and the length of the two whiskers the 5% and 95% quantiles, respectively.

E. Earliest day that 2º MEFs undergoing reprogramming became dox-independent. Cells were passaged twice a week and a subculture was assessed for its ability to grow without dox. If ESC-like colonies appeared, the day of dox-independence was designated the day that dox was removed. Each dot on the plot represents a single cell line obtained from a sample of 1000 mCh+ cells plated in a 35mm well. The experiment was repeated three times.

F. Correlation between efficiency of reprogramming and transgene expression. Transgene expression was measured by mCherry fluorescence intensity of the sorted cells, 24h after dox induction.

G. Correlation between the earliest day of dox-independence and transgene expression. The Y axis is the natural logarithm (ln) of the “Day clones reached independence”.

For Fig. S1D-G each number represents a distinct 1º iPSC clone (from which embryos were generated following diploid aggregation), and each dot represents a single embryo.

AP: alkaline phosphatase; mCh+: mCherry positive cell

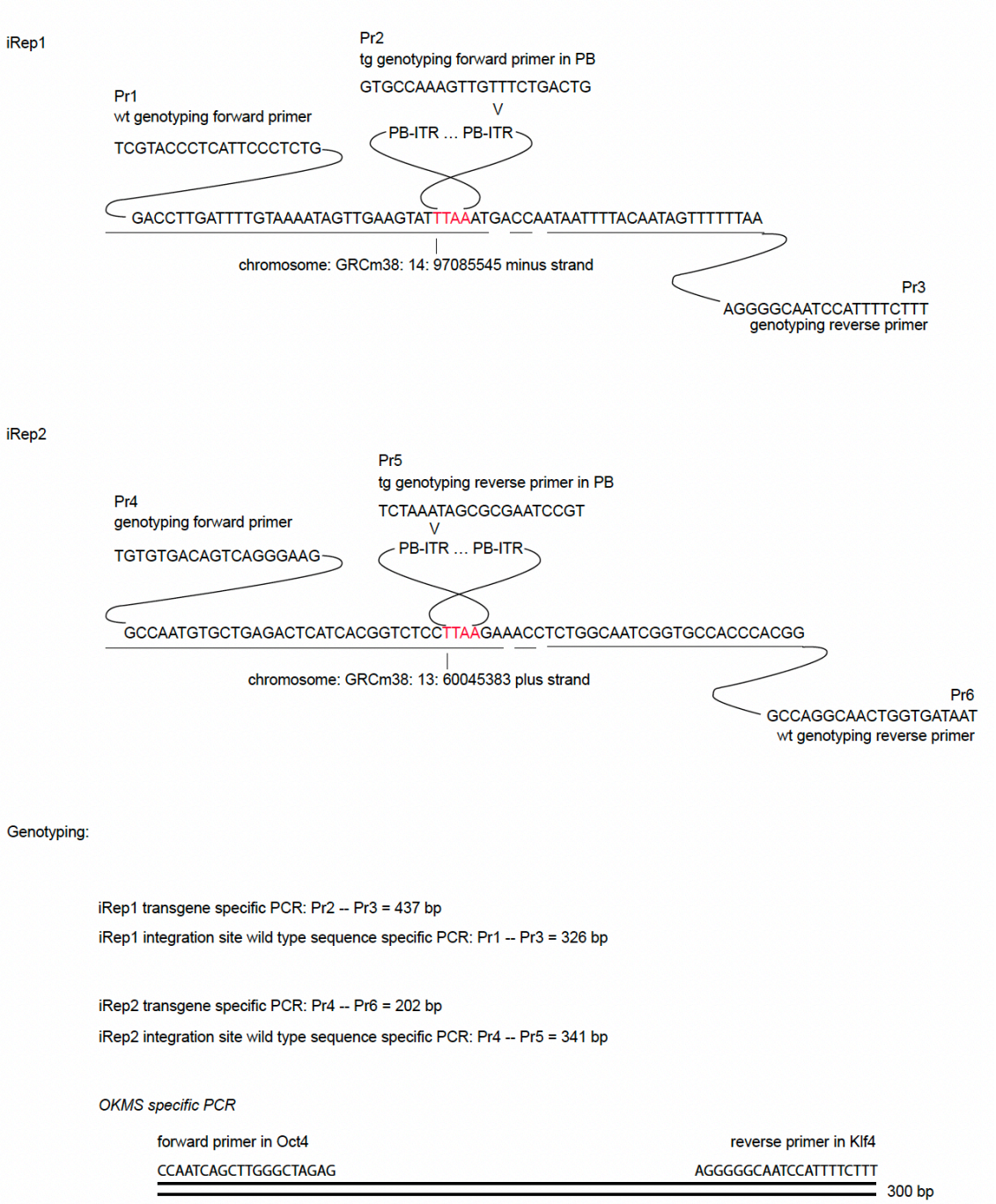

Fig. S2. Genome location of TTAA genomic insertion site for **(A)** iRep1 chr14: 97485545-97485549 and **(B)** iRep2 chr13: 60045383-60045387 mapped to mm9 genome using sequences determined from splinkerette PCR (see Methods). iRep2 inserted in the minus strand and a transcriptionally reverse orientation into a region containing an uncharacterized long non-coding RNA gene; Gm35193. The PCR primers, their position and length of the amplicons for genotyping are also indicated.

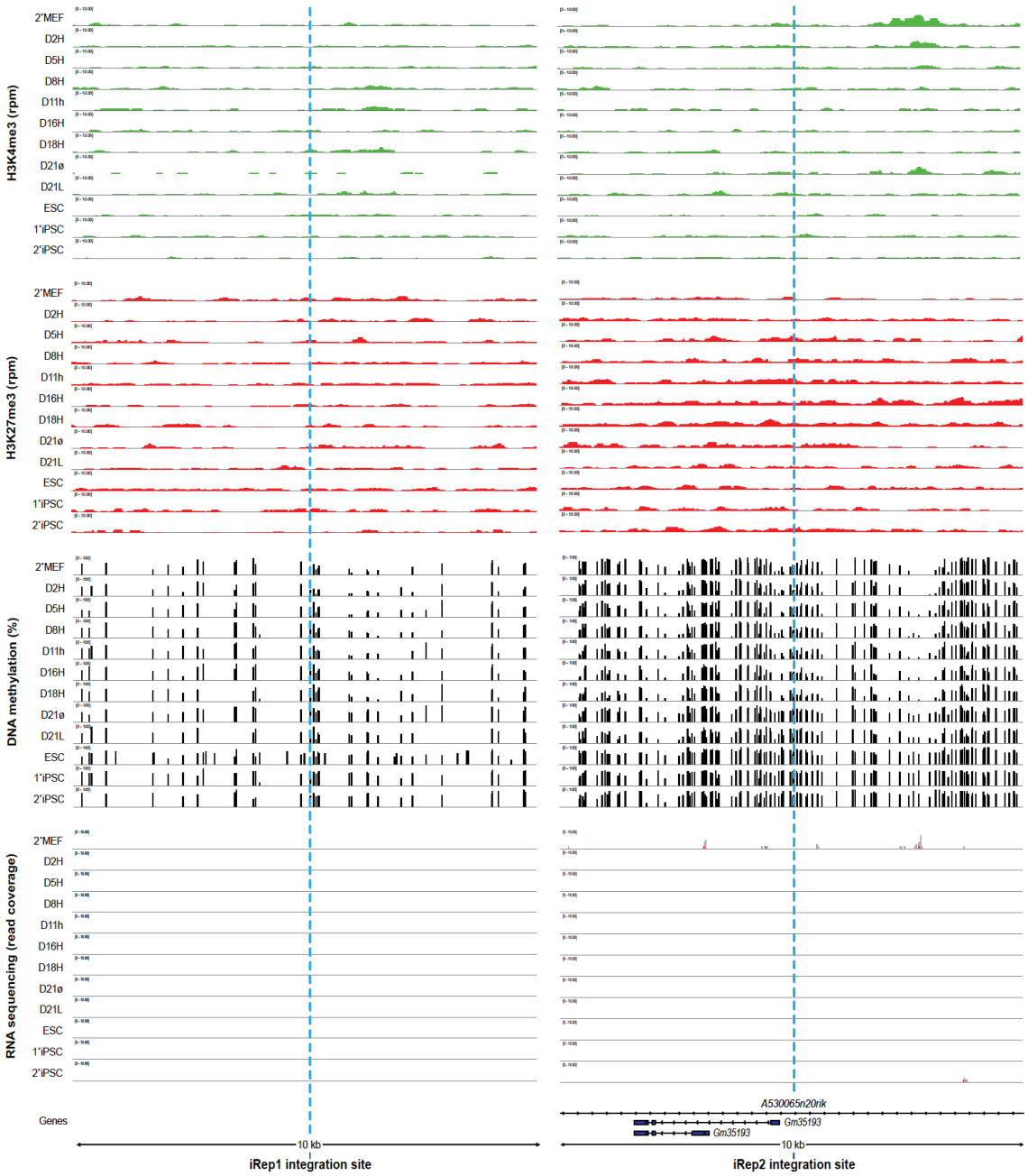

Fig. S3. Integration site-specific 'omics data originating from Hussein *et al.*, 2014 (10). Histograms represent epigenetic and gene expression changes +/- 5kb of iRep transgene integration sites during reprogramming of secondary MEFs towards iPSCs. The H3K4me3 (green) and H3K27me3 (red) epigenetic marks are in reads per million (rpm) (scale: 0 to 10 rpm); DNA methylation is represented at a scale of 0 to 100%, and RNA sequencing is represented as read coverage at a scale of 0 to 10 reads.

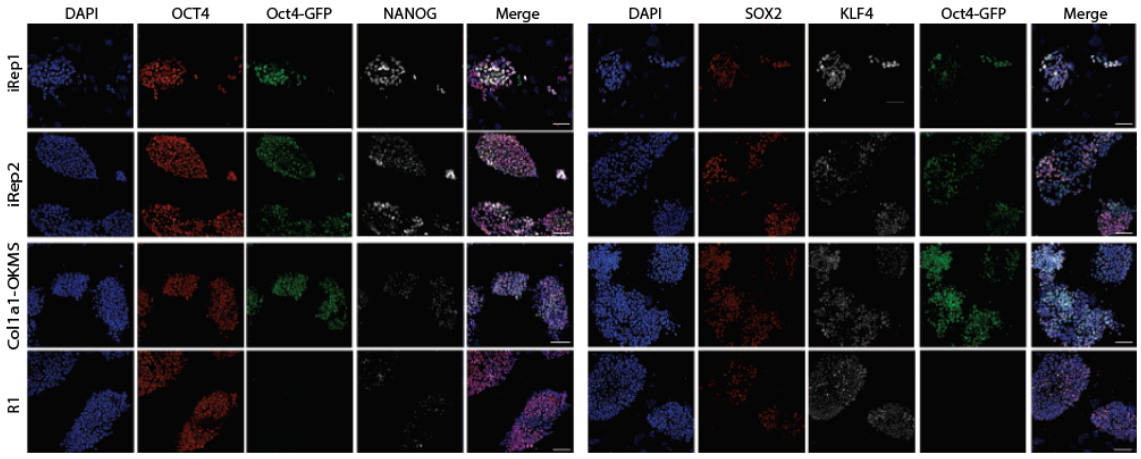

Fig. S4. iRep1, iRep2, Col1a1-OSKM and 1B 2º iPSCs imaged after immunostaining for OCT4, NANOG, SOX2, and KLF4 expression (scale bar=50μm).

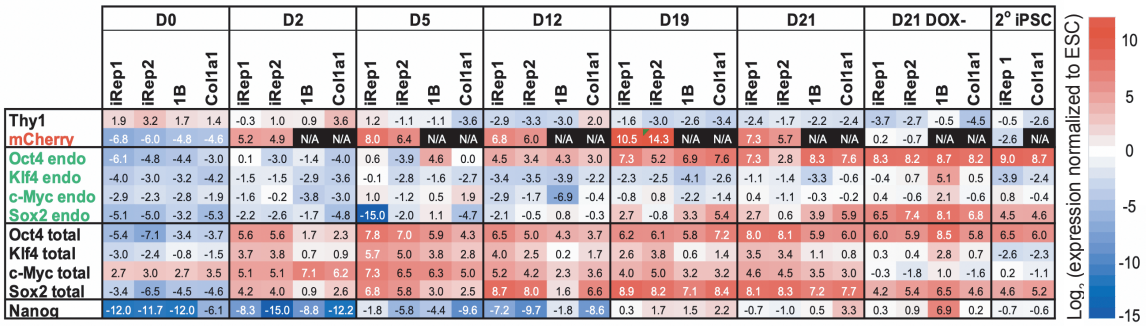

Fig. S5. Heat map showing relative gene expression levels of endogenous (E) and total (endogenous+transgene) (T) Yamanaka factors, the linked mCherry fluorescence tag, *Thy-1* and *Nanog* during reprogramming of iRep1, iRep2, 1B, and Col1a1 reprogrammable 2º MEFs. Expression levels were normalized to the housekeeping gene *mEEF2* and are shown relative to ROSA26-rtTA-ESCs. The scale is $\log_2$ of the gene expression normalized to ESCs.

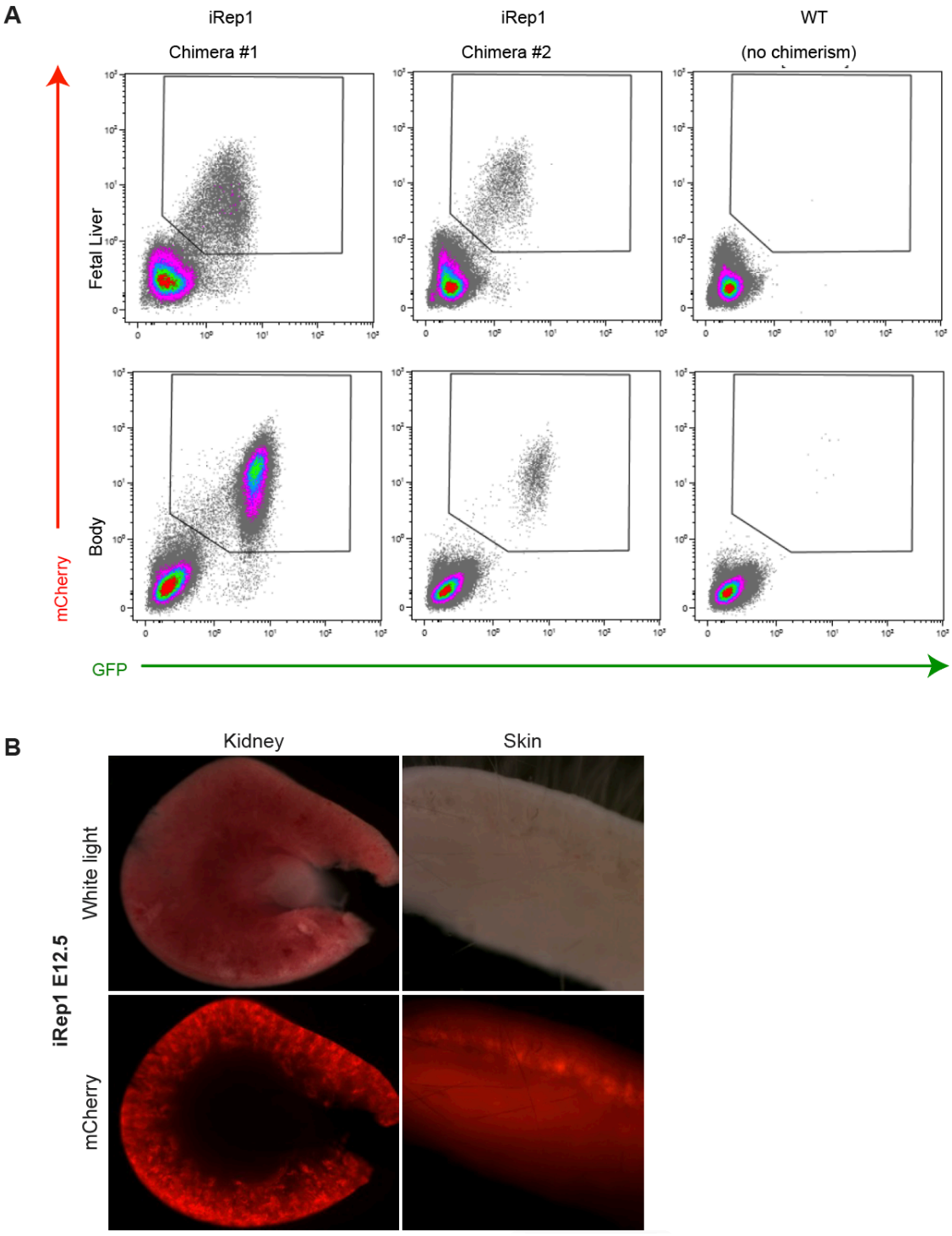

Fig. S6. Characterizing somatic tissues from iRep1 embryos and mice.

A. Flow cytometry analysis of mCherry and GFP expression of fetal liver cells and embryo bodies for two E13.5 chimeric embryos and a E13.5 non-chimeric control embryo exposed to dox in utero for 24h. Weakly positive GFP expression is attributed to the ROSA26-rtTA locus (5).

B. Whole mount white light and red fluorescence (mCherry) images of iRep1 E12.5 transgenic tissues from animals exposed to dox (drinking water, 1.5µg/ml, and dox pellets) for 24h. The experiment was repeated three times.

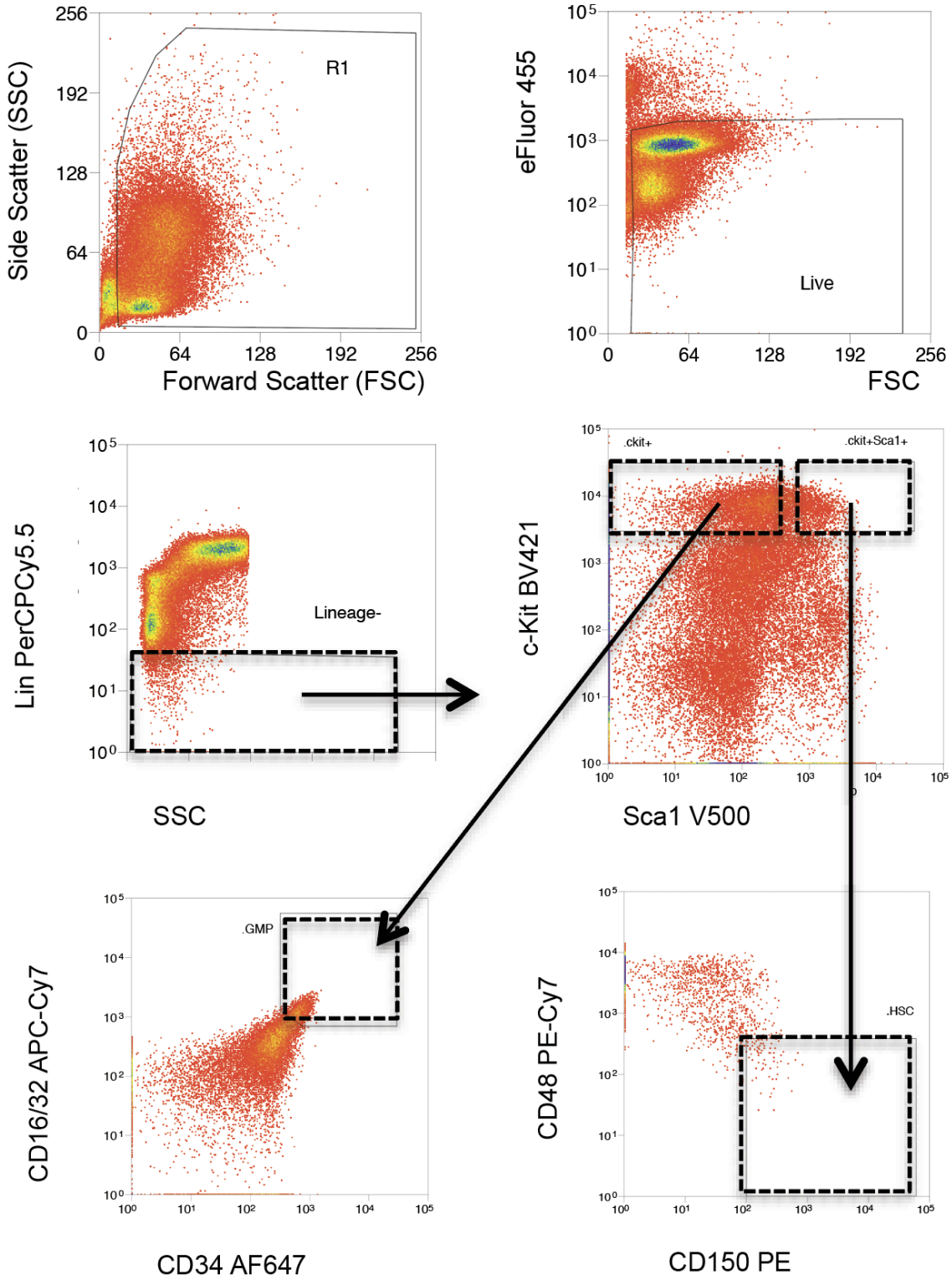

Fig. S7. Isolation of hematopoietic stem cells (HSCs) and granulocyte-macrophage progenitor cells (GMPs) by cell sorting. The gating strategy used to sort HSCs (Lin-cKit+Sca1+CD150+CD48-) and GMPs (Lin-cKit+Sca1-CD34+CD16/32+) is shown. HSCs: hematopoietic stem cells, GMPs: granulocyte-macrophage progenitors.

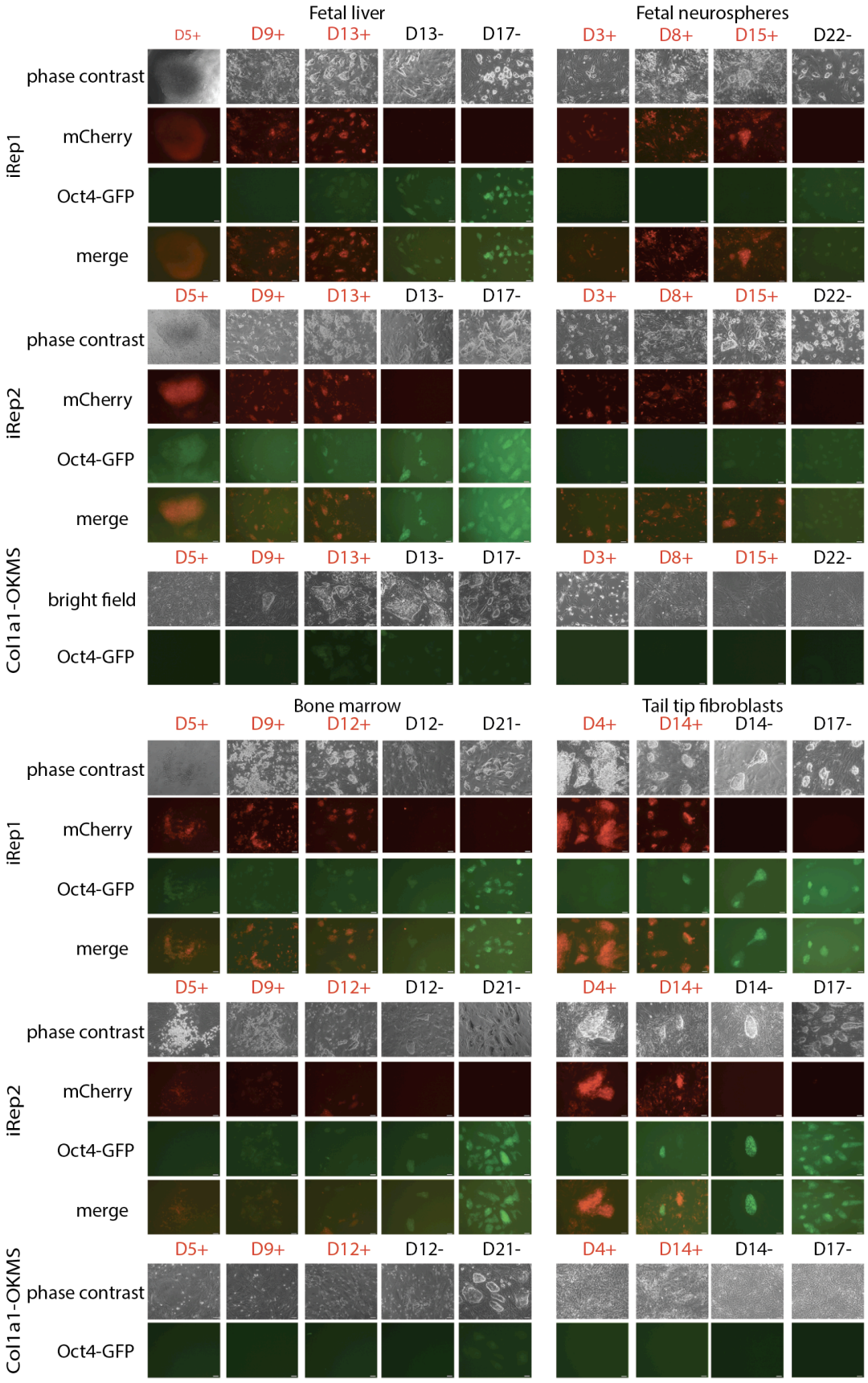
Fetal liver
Fetal neurospheres
D5+
D9+
D13+
D13-
D17-
D3+
D8+
D15+
D22-
phase contrast
mCherry
Oct4-GFP
merge
iRep1
iRep2
bright field
Col1a1-OKMS
Bone marrow
Tail tip fibroblasts
D12+
D12-
D21-
D4+
D14+
D14-

Fig. S8. Comparison of iPSC colonies derived from iRep1, iRep2 and Col1a1-OSKM 2º cells from various somatic cell types. iRep1, iRep2 and Col1a1-OSKM 2º reprogramming for fetal (liver and neurosphere) cells and adult (bone marrow and tail tip fibroblast) tissues imaged in phase contrast and fluorescence for OKMS-mCherry and OCT4-GFP (scale bar=20μm).

Table S1. qPCR primer sequences.

Gene	Forward primer (5'<3')	Reverse primer (5'<3')
Sox2 (total)	cgtgaaccagcgcatggacag	cgtaggtgcatcggttgc
Sox2 (endogenous)	tagactgcacatggcccagcac	cccctcccaattcccttgtatctc
mCherry	caagttccacatcacctcccac	acttgtacagctcgtccatgc
MEEF2	atagaagcggcctttgtcag	gtattaagagctgcgacccc
Nanog	ttgcttacaagggtctgctact	actggtagaagaatcagggct
c-Myc (total)	ctagtgctgcatgaggagacaccg	gcctcttctccacagacaccacatc
c-Myc (endogenous)	gcacaagctcacctctgaaaaggac	ctcacgagagattccagctcctcc
Klf4 (total)	agttctcatctcaaggcacacctgc	aatttccacccacagccgtcc
Klf4 (endogenous)	tctcttcttcggactccggaggac	tggacgcagtgtcttctcccttc
Oct4 (total)	cacgagtggaaagcaactca	ttcatgtcctgggactcctc
Oct4 (endogenous)	ttccctctgttcccgtcactgc	ttgtctacctcccttgccttggc

Table S2. Genotyping sequences for PCR.

Transgene/Locus name	Primer name	F/R	Sequence (5'→3')	Band sizes (bp)	Annealing temperature (°C)
Col1a1-Tet-O-OSKM (Jaenisch)	Col1a1-OSKM-1F	F	ccctccagttgtgaccaagc	WT: 331 MUT:550	60
	Col1a1-OSKM-2R	R	gcacagcattgcggacatg		
	Col1a1-OSKM-3R	R	ttgctcagcggtgctgtcca		
Rosa-rtTA	Rosa-rtTA-F	F	aaagtcgctctgagttgttat	WT: 650 MUT: 340	56
	Rosa-rtTA-R	R	ggagcgggagaaatggatatg		
	Rosa-rtTA-mutR	R	gcgaagagtttgtcctcaacc		
Oct4-GFP (Nagy)	Oct4-GFP-F	F	ctaggtgagccgtctttcca	TG: 250	58
	Oct4-GFP-R	R	tggtgcagatgaacttcagg		
iRep1	iRep1-WTF	F	tcgtaccctcattccctctg	TG:300 WT:300*	58
	iRep1-TgF	F	gtgccaaagttgttctgactg		
	iRep1-UniR	R	agggggcaatccattttcttt		
iRep2	iRep2-WTF	F	tgtgtgacagtcagggaagc	TG:300 WT:300*	58
	iRep2-TgF	F	ccgataaaacacatgcgtcaa		
	iRep2-UniR	R	gccaggcaactggtgataat		

Movie S1 (separate file). iRep1 MEFs were seeded in 0.2% gelatin-coated 6-wells and dox-induced for live imaging. Brightfield images were collected every 10 min from the time of adding dox to the media, and fluorescence images were captured every hour for a total of 48-96h. mCherry was rapidly induced in both iRep transgenic mice and clusters of mCherry positive cells started to form within 48h in iRep1 and after 72h in iRep2.

Movie S2 (separate file). iRep2 MEFs were seeded in 0.2% gelatin-coated 6-wells and dox-induced for live imaging. Brightfield images were collected every 10 min from the time of adding dox to the media, and fluorescence images were captured every hour for a total of 48-96h. mCherry was rapidly induced in both iRep transgenic mice and clusters of mCherry positive cells started to form within 48h in iRep1 and after 72h in iRep2.

SI References

1. R. Behringer, M. Gertsenstein, K. Nagy, A. Nagy, *Manipulating the Mouse Embryo: A Laboratory Manual*, 4th Ed. (2014).
2. M. Gertsenstein, *et al.*, Efficient Generation of Germ Line Transmitting Chimeras from C57BL/6N ES Cells by Aggregation with Outbred Host Embryos. *Plos One* 5, e11260 (2010).
3. B. W. Carey, *et al.*, Reprogramming factor stoichiometry influences the epigenetic state and biological properties of induced pluripotent stem cells. *Cell Stem Cell* 9, 588–598 (2011).
4. S. Viswanathan, *et al.*, Supplementation-dependent differences in the rates of embryonic stem cell self-renewal, differentiation, and apoptosis. *Biotechnol Bioeng* 84, 505–517 (2003).
5. G. Belteki, *et al.*, Conditional and inducible transgene expression in mice through the combinatorial use of Cre-mediated recombination and tetracycline induction. *Nucleic Acids Res* 33, e51–e51 (2005).
6. D. J. Martens, V. Tropepe, D. van der Kooy, Separate Proliferation Kinetics of Fibroblast Growth Factor-Responsive and Epidermal Growth Factor-Responsive Neural Stem Cells within the Embryonic Forebrain Germinal Zone. *J Neurosci* 20, 1085–1095 (2000).
7. B. L. K. Coles-Takabe, *et al.*, Don't Look: Growing Clonal Versus Nonclonal Neural Stem Cell Colonies. *Stem Cells* 26, 2938–2944 (2008).
8. V. Tropepe, C. G. Craig, C. M. Morshead, D. van der Kooy, Transforming growth factor-alpha null and senescent mice show decreased neural progenitor cell proliferation in the forebrain subependyma. *J Neurosci Official J Soc Neurosci* 17, 7850–9 (1997).
9. K. Woltjen, S.-I. Kim, A. Nagy, The piggyBac Transposon as a Platform Technology for Somatic Cell Reprogramming Studies in Mouse. *Methods Mol Biology Clifton N J* 1357, 1–22 (2016).
10. S. M. I. Hussein, *et al.*, Genome-wide characterization of the routes to pluripotency. *Nature* 516, 198–206 (2014).
11. J. F. Lawless, Statistical Models and Methods for Lifetime Data. *Wiley Ser Probab Statistics* (2002) https:/doi.org/10.1002/9781118033005.
12. S. Ferrari, F. Cribari-Neto, Beta Regression for Modelling Rates and Proportions. *J Appl Stat* 31, 799–815 (2004).