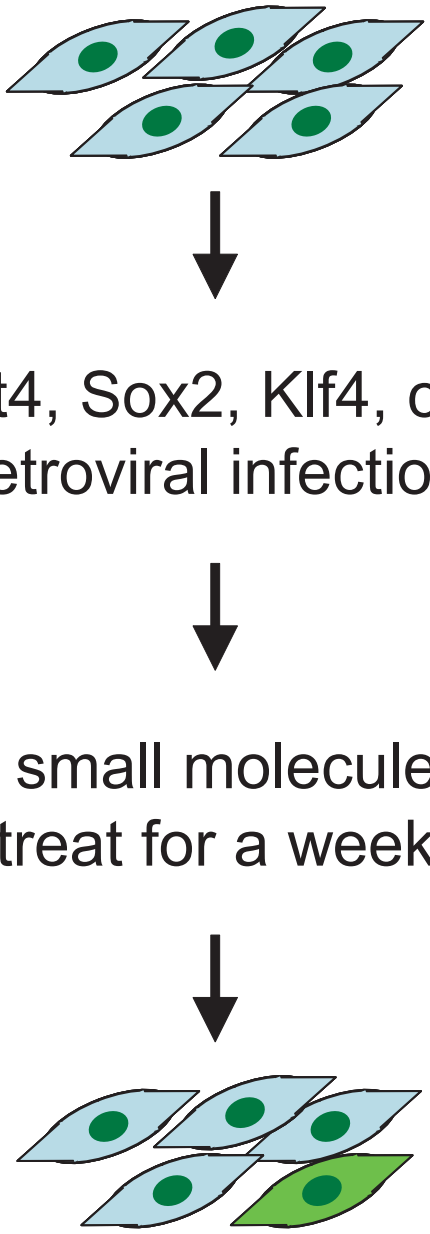
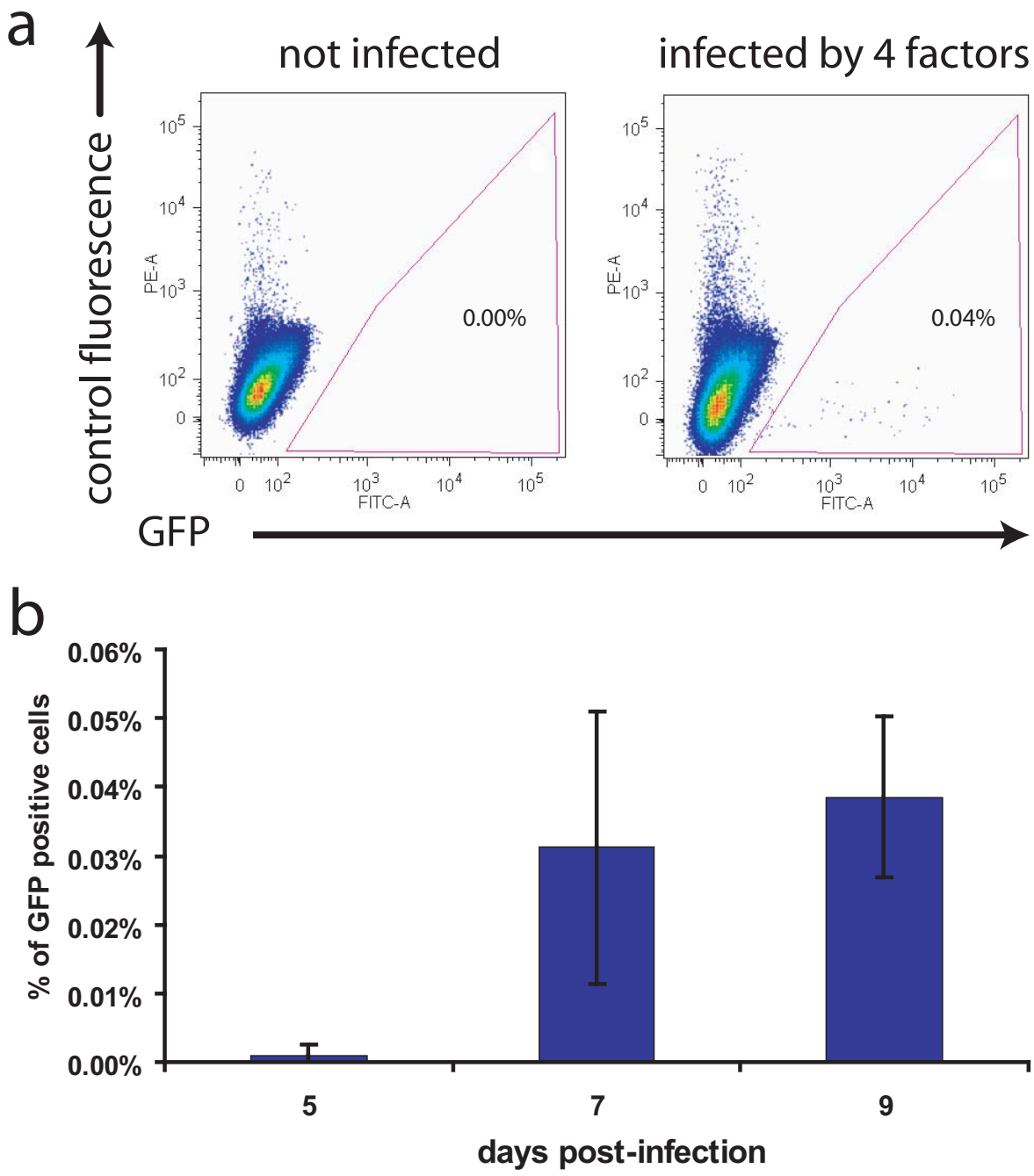
Supplementary Figure 1



Supplementary Figure 1. Schematic of the screen.

4-factor (*Oct4*, *Sox2*, *Klf4* and *c-Myc*) infected *Oct4-GFP/+* MEFs were treated with small molecules or growth factors for a week starting from 1 or 2 days post-infection. Percentages of GFP^+ cells were assessed by FACS analysis typically between 9 and 11 days post-infection.

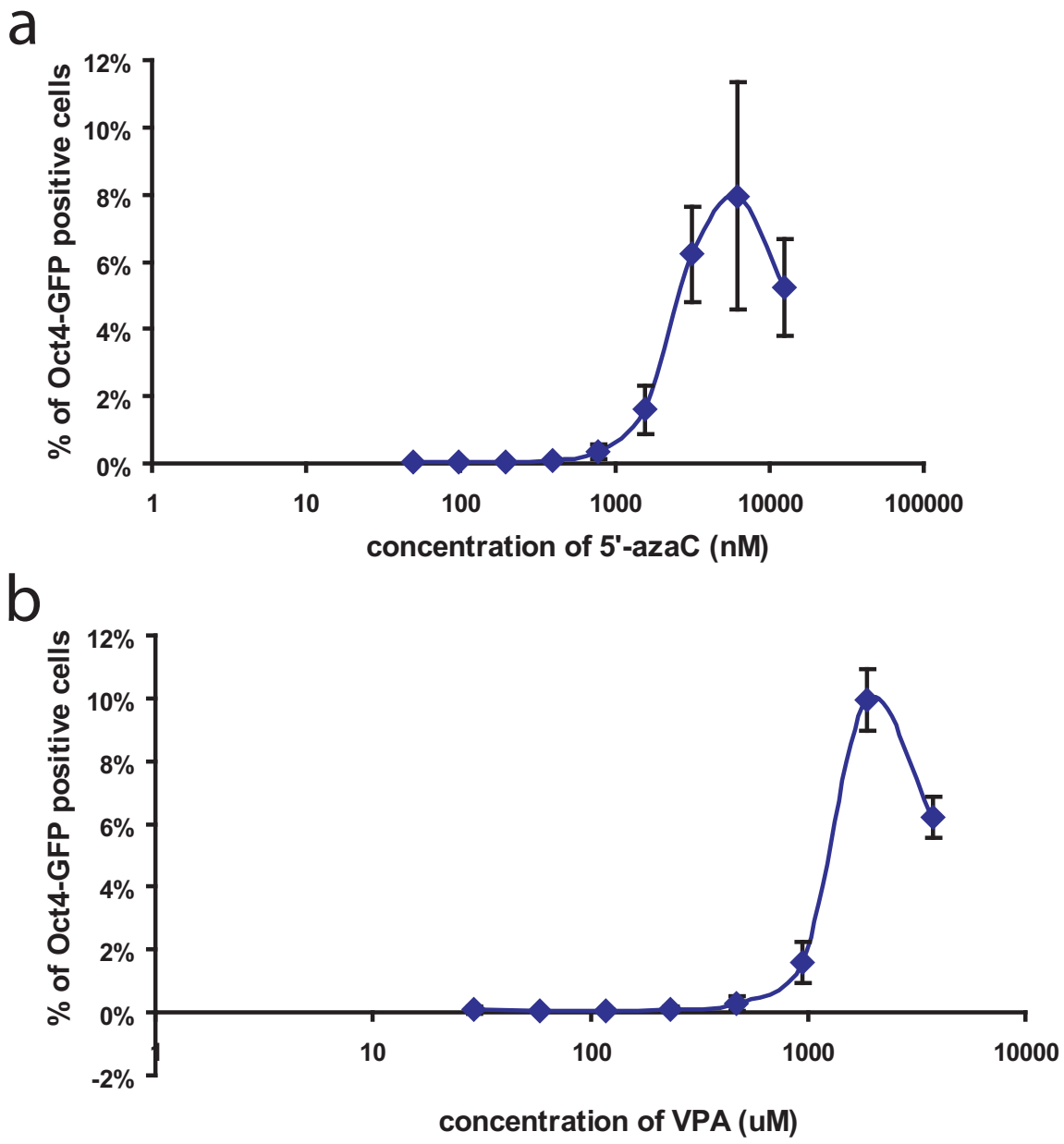
Supplementary Figure 2



Supplementary Figure 2. Reprogramming of MEF by *Oct4*, *Sox2*, *Klf4* and *c-Myc*.

Oct4-GFP/+ MEFs infected by the four factors (*Oct4*, *Sox2*, *Klf4* and *c-Myc*) were examined by FACS analysis. Around 0.04% GFP^+ cells were induced at 7 days post-infection (a), and the percentage of GFP^+ cells remained at around the same level at 9 days post-infection (b); while no GFP^+ cells were induced in the non-infected control. n=4 for each time point. Error bars indicate standard deviation.

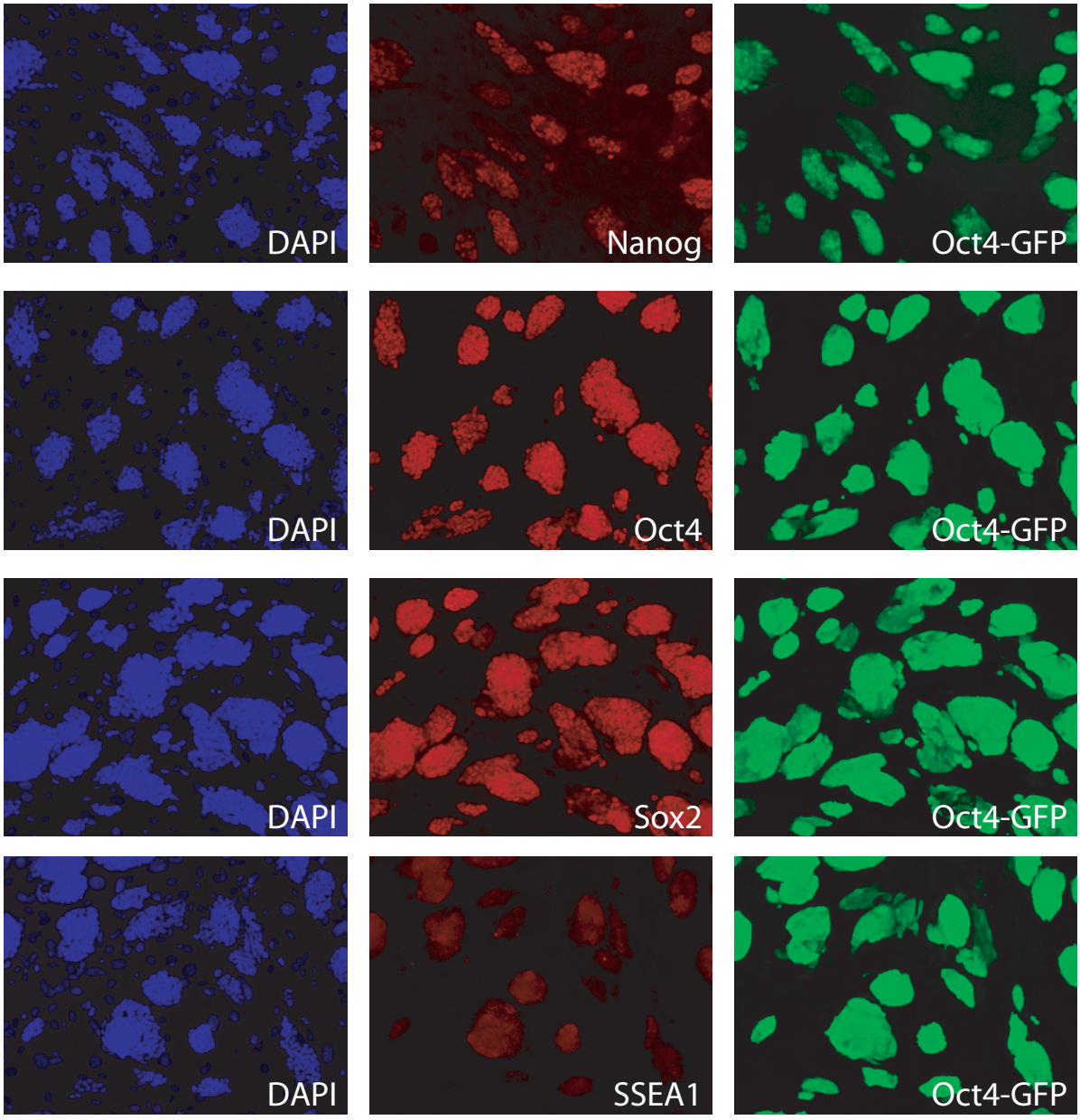
Supplementary Figure 3



Supplementary Figure 3. Dose-dependant effects on reprogramming efficiency by 5'-azaC and VPA

Dose response curves of the effects of 5'-azaC (a) and VPA (b) on reprogramming efficiency measured by the percentage of Oct4-GFP^{+} cells induced. n=4 for each concentration. Error bars indicate standard deviation.

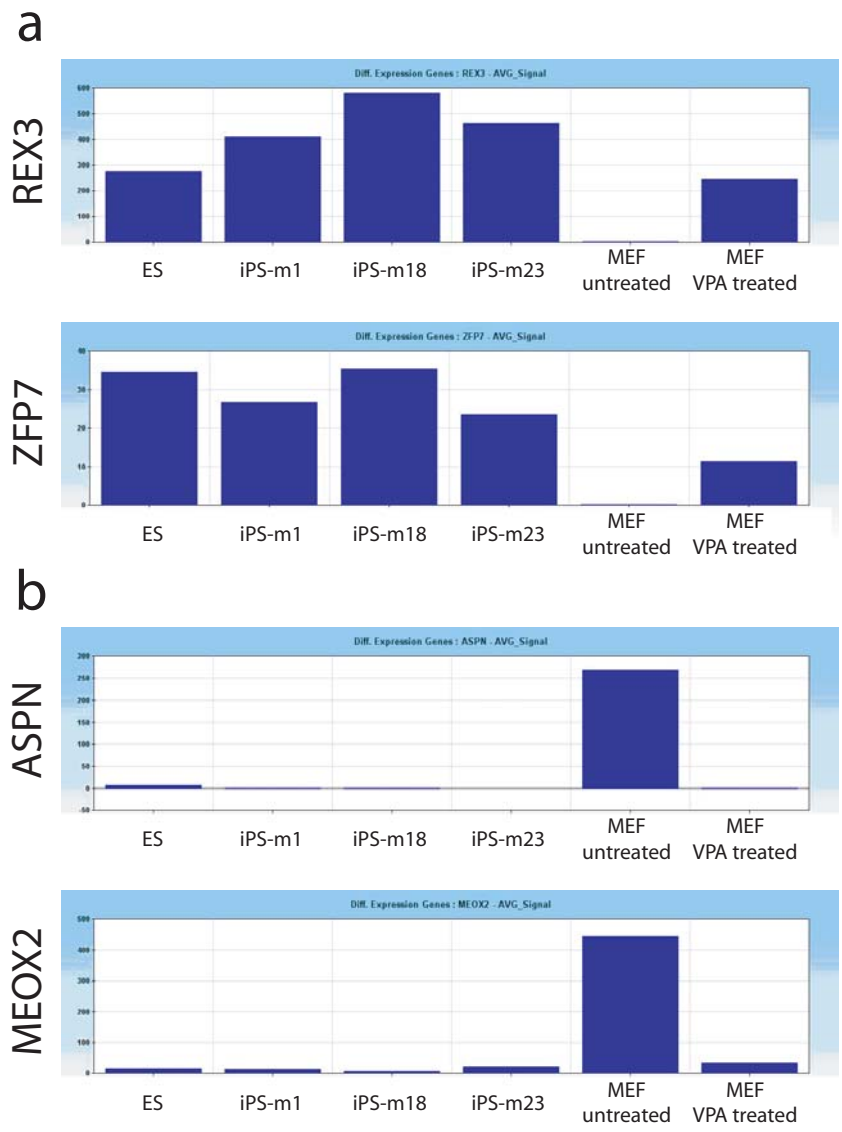
Supplementary Figure 4



Supplementary Figure 4. Characterization of c-Myc-free iPS cells induced by VPA treatment.

Immunofluorescence staining showed co-expression of pluripotent markers, Nanog, Oct4, Sox2 and SSEA1, with Oct4-GFP in c-Myc-free iPS cells induced by VPA treatment.

Supplementary Figure 5



Supplementary Figure 5. The effect of VPA treatment on uninfected MEF.

(a) Examples of the relative levels of expression of ES cell-specific transcripts (*Rex3* and *Zfp7*) in ES cells, iPS cells, untreated MEFs and MEFs treated with VPA.

(b) Examples of the relative levels of expression of MEF-specific transcripts (*Aspn* and *Meox2*) in ES cells, iPS cells, untreated MEFs and MEFs treated with VPA.

Supplementary Table 1. Transcriptional array analysis on iPS cell lines.

Global correlation of the transcriptional profiles between ES cells (AV3), MEF, and six lines of iPS cells was determined by the r^2 values. All six iPS lines were induced by retroviral infection of *Oct4*, *Sox2* and *Klf4*. All but one (iPS-m1) iPS lines were induced with VPA treatment.

	AV3	**MEF**	**iPS m1**	**iPS m18**	**iPS m23**	**iPS m82**	**iPS m83**	**iPS m84**
AV3	1.00	0.62	0.94	0.94	0.94	0.97	0.96	0.97
MEF		1.00	0.62	0.63	0.62	0.64	0.64	0.66
iPS m1			1.00	0.99	0.99	0.93	0.93	0.93
iPS m18				1.00	0.99	0.93	0.93	0.94
iPS m23					1.00	0.94	0.94	0.94
iPS m82						1.00	0.99	0.98
iPS m83							1.00	0.98
iPS m84								1.00

Supplementary Table 2. Karyotype analysis on iPS cell lines induced by VPA treatment.

Cell line	Karyotype (20 cells analyzed per cell line)
iPS-m81	**40,XY[18]/80,XXYY[2]**
iPS-m82	**40,XY[20]**
iPS-m83	**40,XY[15]/43,XY,+7,+8,+12[1]/80,XXYY[3]**
iPS-m84	**40,XY[19]**

Supplementary Methods

Derivation of MEFs and cell culture

MEFs were derived from e13.5 embryos hemizygous for the *Oct4-GFP* transgenic allele. Embryos were sexed by inspecting gonads for the pattern of Oct4-GFP expression. Gonads and internal organs were removed before processing the embryos for MEF isolation. To generate iPS cells that can be identified in mouse chimeras after blastocyst injection, we derived MEFs from e13.5 embryos that are hemizygous for *Oct4-GFP* and heterozygous for the *Rosa26-lacZ* reporter allele. MEFs were grown in DMEM supplemented with 10% FBS, L-glutamine, penicillin/streptomycin, nonessential amino acids, and sodium pyruvate. MEFs in early passages (up to passage 5) were used for generation of iPS cells. *Oct4-GFP* and *Rosa26-lacZ* mice were both obtained from the Jackson Laboratory. All animal experiments described in this article have been approved by Harvard University's Institutional Animal Care and Use Committee.

Retrovirus production and small molecule screening

Moloney-based retroviral vectors (pMXs) containing the murine complementary DNAs of *Oct4*, *Sox2*, *c-Myc*, and *Klf4*[1] were obtained from Addgene. These plasmids were co-transfected into 293T cells with packaging vectors (pUMVC and pCMV-VSVG), and viral supernatants were collected 48 hours post-transfection to infect MEFs. Two to three rounds of infection were performed during a 48 hour period. The day that viral supernatants were removed was defined as 0 day post-infection. Infected MEFs were subsequently cultured in mouse ES cell media (Knockout DMEM supplemented with 15% Hyclone FBS, L-glutamine, penicillin/streptomycin, nonessential amino acids, β-mercaptoethanol, and with 1000 U/ml LIF), and treated with small molecules or growth factors for a week starting from 1 or 2 days post-infection. After the treatment, cells were cultured in mouse ES cell media, and collected for FACS analysis typically between 9 and 11 days post-infection. All conditions were tested in quadruplicates.

Generation of iPS cells

For the generation of mouse iPS cells, infected MEFs were cultured in mouse ES cell media until iPS colonies were ready to be picked. Treatment with chemicals started typically at 1 or 2 days post-infection and lasted for a week. iPS colonies were picked between 9-21 days post-infection based on GFP expression and colony morphology. All quantifications of the effects of chemicals were done using mouse ES cell media, although some iPS cells were picked in mouse ES cell media containing knockout serum replacement instead of Hyclone FBS. The picked colonies were subsequently expanded and maintained on irradiated MEF feeder layers in mouse ES cell media. Karyotyping of the iPS cell lines was performed by the Clinical & Research Cytogenetics Laboratories at the Oregon Health & Sciences University.

Quantification of reprogramming efficiency

Two methods were used to quantify reprogramming efficiency. We used FACS analysis to quantify the induction of Oct4-GFP$^+$ cells. We also counted the number of Oct4-GFP$^+$ colonies induced at different time points. At about a week post-infection, colony numbers were counted directly under a fluorescent microscope. At about two weeks post-infection, due to the large number of colonies induced with VPA treatment, colony numbers were counted using pictures taken under a fluorescent dissection microscope.

Generation of teratoma and chimeras

Teratomas were produced by injecting ~1 million cells subcutaneously into NOD-SCID mice. Tumor samples were collected in 5 weeks, fixed in 4% paraformaldehyde and processed for paraffin embedding and hematoxylin and eosin staining following standard procedures.

Blastocysts were obtained through mating of hormone primed female BDF1 and male BDF1 or C57BL/6J mice. Chimeras were produced by injecting iPS cells

into blastocysts, followed by implantation into pseudopregnant ICR mice. Chimeric embryos were dissected 8 days after injection (about e10.5) and analyzed for β-galactosidase activity following standard protocols. Stained embryos were then fixed and embedded in paraffin, and sections were counterstained with nuclear fast red.

Use of chemicals and growth factors

The following chemicals were used: 5'-azaC from Sigma–Aldrich, SAHA from Biomol International, dexamethasone, TSA, and VPA from EMD Biosciences. Stock solutions of 5'-azaC and VPA were made in PBS or media. Stock solutions of other chemicals were made in DMSO.

Alkaline phosphatase and immunofluorescence staining

Alkaline phosphatase staining was performed with the Vector Red substrate kit from Vector Laboratories. Immunofluorescence staining was performed using the following primary antibodies: rabbit anti-GFP (Molecular Probes), rabbit anti-Nanog (Cosmobio), mouse anti-Oct4 (Santa Cruz Biotechnology), goat anti-Sox2 (Santa Cruz Biotechnology), mouse anti-SSEA1 (Developmental Studies Hybridoma Bank).

Whole-genome expression analysis

For transcriptional analysis, total RNA was isolated from cells cultured in 6 well dishes using RNeasy Mini Kit and QIAshredder from Qiagen. Biotinylated antisense RNA were amplified using Illumina Total Prep RNA amplification Kit from Ambion, hybridized to Illumina Whole-Genome Expression BeadChips (MouseRef-8) and analyzed by Illumina Beadstation 500. All samples were prepared in two to three biological repeats. Data were analyzed using the Beadstudio software provided by Illumina.