Supporting Information

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SI Materials and Methods

Mouse Embryonic Fibroblasts Derivation. Oct4-GFP (OG2) transgenic mice harboring a GFP reporter gene under the control of the Oct4 promoter (1) were crossed with Rosa26/hprt-Cre (R26) mice (2) to get OG2/R26 mice. Mouse embryonic fibroblasts (MEFs) were derived from either OG2/R26 mice or OG2 mice for reprogramming. MEF derivation was performed according to the protocol from WiCell Research Institute. All the animal work was performed under the Institutional Animal Care and Use Committee protocol approved by the City of Hope Institutional Animal Care and Use Committee.

Retrovirus Preparation. The pMX-based retroviral vectors for mouse Oct4, Sox2, Klf4, and cMyc were obtained from Addgene. Retrovirus was produced using an established protocol (3). Specifically, 4×10^6 293T cells were seeded onto a 10-cm dish in DMEM with 10% (vol/vol) FBS 24 h before transfection. Medium was changed 5 h before transfection on the next day. At the time of transfection, cell confluency was around 80–90%. Fifteen micrograms of plasmid DNAs were introduced into 293T cells using calcium phosphate transfection method. Medium was changed the day after transfection. At 48~72 h after transfection, virus-containing supernatant was collected, filtered through 0.45-μm filter, and concentrated by ultracentrifugation.

Induced Pluripotent Stem Cell Derivation in Mouse ESC Media. OG2/R26 MEFs were seeded onto six-well plates at the density of 3×10^4 cells per well in DMEM with 10% FBS, 0.1 mM nonessential amino acids (NEAA), and 0.1 mM L-glutamine, 24 h before transduction. Freshly concentrated viruses of the four factors, Oct4, Sox2, c-Myc, and Klf4, were added to the cells. Two days after viral infection, the viral-transduced OG2/R26 MEFs were split into new six-well plates with irradiated MEF feeder cells and cultured in mouse ESC culture medium containing 15% ESC qualified FBS, 2 mM L-glutamine, 0.1 mM 2-mercaptoethanol, 0.1 mM NEAA, 1 mM sodium pyruvate, and 1,000 U/mL of leukemia inhibitory factor (LIF), and this day was defined as day 0. 1 μM of each compound (OAC1, OAC2, and OAC3) was added to the 4F-transduced cells at day 0 and treatment was continued for 1 wk. When induced pluripotent stem cells (iPSC) colonies emerged, these colonies were picked for expansion on irradiated MEF feeders in mouse ESC medium.

iPSC Derivation in iSF1 Media. OG2 MEFs were seeded onto 6-well plates at the density of 3×10^4 cells per well in DMEM with 10% FBS, 0.1 mM NEAA, and 0.1 mM L-glutamine, 24 h before transduction. Freshly prepared viruses of the 4F, Oct4, Sox2, c-Myc, and Klf4, were added to the cells. Two days after viral infection, the transduced OG2 MEFs were split into new six-well plates with irradiated MEF feeders and cultured in iSF1 reprogramming media containing high-glucose DMEM, 10% knockout serum, 0.5% N2, 5 ng/mL b-FGF, 2 mM L-glutamine, 0.1 mM 2-mercaptoethanol, 0.1 mM NEAA, and 1,000 U/mL of LIF. This day was defined as day 0. On day 1, 1 μM of each compound (OAC1, OAC2, and OAC3) was added to the 4F-transduced OG2 MEFs. Treatment was continued for 1 wk. When iPSC colonies emerged, these colonies were picked for expansion on the irradiated MEF feeders in mouse ESC medium.

In Vitro Differentiation. For embryoid body formation, iPSCs were treated with 0.25% trypsin-EDTA. The dissociated single cells were transferred into ultra-low-attachment T-25 flasks in the presence of complete growth medium without LIF. Medium was changed every 2 d. After 8 d of suspension culture, one or two embryoid bodies were transferred to 24-well plates coated with 0.1% gelatin and cultured for another 4 d. The resultant cells were stained with anti-FoxA2 (GeneTex, 1:250), anti-α smooth muscle actin (SMA) (Abcam, 1:500), and anti-βIII Tublin (Tuj1) (Covance; 1:6,000) antibodies.

Teratoma Formation. iPSCs were dissociated into single cells and resuspended at 10^7 cells/mL. 10^6 cells in a volume of 100 μL were injected subcutaneously into the dorsal flank of the immunodeficient Nude mice. Four weeks after injection, tumors were dissected from the transplanted mice, fixed in formalin, and paraffin-embedded. The tumor tissues were sectioned and stained with H&E.

Chimera Mouse Production. The 4F+OAC iPSCs derived from OG2/R26 MEFs were microinjected into albino blastocysts to allow identification of chimeras based on coat color markers. Albino blastocyst embryos were generated by injecting female mice with 5 IU of pregnant mare's serum gonadotropin, followed by injecting with 5 IU of human CG 48 h later, and mating to fertile male mice. Hormones for super ovulation were obtained from the National Hormone and Peptide program (Harbor-University of California at Los Angeles). The microinjected blastocysts were transferred surgically into 2.5 d pseudopregnant recipient females, and litters were born naturally or delivered by caesarian section.

Oct4-luc and Nanog-luc Stable Cell Line Construction. The human Oct4 and Nanog promoters were amplified by PCR with the following primers: hOct4 forward: 5′-GTG CAG AGA AGT CTA CAT TCC CAT GT-3′ and hOct4 reverse: 5′-CGA GAA GGC AAA ATC TGA AGC CAG G-3′; hNanog forward: 5′-AGA CAC CCA CCA CCA TGC GTG GCT-3′ and hNanog reverse: 5′-TCC TGG AGT CTC TAG ATT-3′ using human genomic DNA as the template. Then the promoter sequences were cloned into pGL4.4-luc-Hyg vector (Promega) to get Oct4-luc or Nanog-luc construct and the fidelity of the Oct4 and Nanog promoter DNA sequences was confirmed by bidirectional sequencing. CV1 cells were transfected with the Oct4-luc or Nanog-luc constructs and the stably-transfected cells were selected by hygromycine resistance.

Luciferase Reporter Assays. The Oct4-luc or Nanog-luc cells were treated with compound OAC1 or its structural analogs at 1 μM concentration or at indicated concentrations in triplicates. Other compounds used include 2 μM BIO, 2 μM BIX (an inhibitor of the G9a histone methyltransferase), 2 μM 5′-azacytidine (AzaC), 25 μg/mL Vitamin C (Vc), 10 nM Am580 (a retinoic acid receptor agonist), 5 μM tranylcypromine, and 0.5 mM valporic acid (VPA). Luciferase reporter assays were performed as previously described (4) 24 h after compound treatment or at indicated time points. For Topflash reporter assays, 0.2 μg β-catenin–responsive Topflash reporter gene plasmid was introduced into CV1 cells using trasfection (Bio-Rad). Compounds were added 6 h after transfection. Luciferase activity was measured 48 h after compound treatment using the Glo Luciferase Assay System (Promega).

RT-PCR Analysis. Total RNA was purified with TRIzol reagent (Invitrogen). Reverse transcription was performed with 1 μg of RNA using Omniscript Reverse Transcription Kit (Qiagen). Quantitative PCR was performed using iTaq SYBR Green Supermix with Rox (Bio-Rad) in Applied Biosystem Step one plus

real-time PCR system. The primers for RT-PCR include: exo-Oct4-forward 5′ GCT CAG TGA TGC TGT TGA TC 3′, exo-Oct4-reverse 5′ CGG CTT CGG CCA GTA AC 3′; exo-Sox2-forward 5′ ACT GCA CAT GGC CCA GCA CTA 3′, exo-Sox2-reverse 5′ CGG CTT CGG CCA GTA AC 3′; exo-Klf4-forward 5′ CGG ACC ACC TTG CCT TAC ACA 3′, exo-Klf4-reverse 5′ CGG CTT CGG CCA GTA AC 3′; exo-c-Myc-forward 5′ CGA GCA CAA GCT CAC CTC TGA 3′, exo-c-Myc-reverse 5′ CGG CTT CGG CCA GTA AC 3′; Oct4-forward 5′ GCA TAC TGT GGA CCT CAG GTT 3′, Oct4-reverse 5′ TCG AAG CGA CAG ATG GTG GT 3′; Nanog-forward 5′ CTG ACA TGA GTG TGG GTC TTC 3′, Nanog-reverse 5′ GAA TGG AGG AGA GTT CTT GCA 3′; Sox2-forward 5′ TGC ACA ACT CGG AGA TCA GCA 3′, Sox2-reverse 5′ CTC CTG CAT CAT GCT GTA GCT 3′; p53-forward 5′ TCT GGG ACA GCC AAG TCT GT 3′, p53-reverse 5′ GGA GTC TTC CAG TGT GAT GA 3′; p21-forward 5′ CGC ACA GGA GCA AAG TGT GCC GT 3′, p21-reverse 5′ TGC CCT CCA GCG GCG TCT CCG TG 3′; Tet1-forward 5′ GAG CCT GTT CCT CGA TGT GG 3′, Tet1-reverse 5′ CAA ACC CAC CTG AGG CTG TT 3′; Actin forward 5′ CCG AGC GTG GCT ACA GCT TC 3′, Actin reverse 5′ ACC TGG CCG TCA GGC AGC TC 3′.

Bisulfite Genomic Sequencing. Genomic DNA was isolated from human IMR90 fibroblasts, MEFs, mouse ESCs, 4F+OAC1 and 4F+OAC2-iPSCs by digestion with proteinase K, followed by phenol-chloroform extraction and ethanol precipitation. Bisulfite conversion of genomic DNAs was carried out using the EZ DNA Methylation-Gold kit (Zymo Research) according to the manufacturer's instruction. The bisulfite-modified DNA was then used as a template for PCR to amplify the promoter regions of Oct4 and Nanog using PCR primers for human OCT4 (5), mouse Oct4 (6), and Nanog (7). The amplified products were cloned into the pCR2.1-TOPO cloning vector (Invitrogen), and 10 randomly selected clones were sequenced with T7 or M13R primers.

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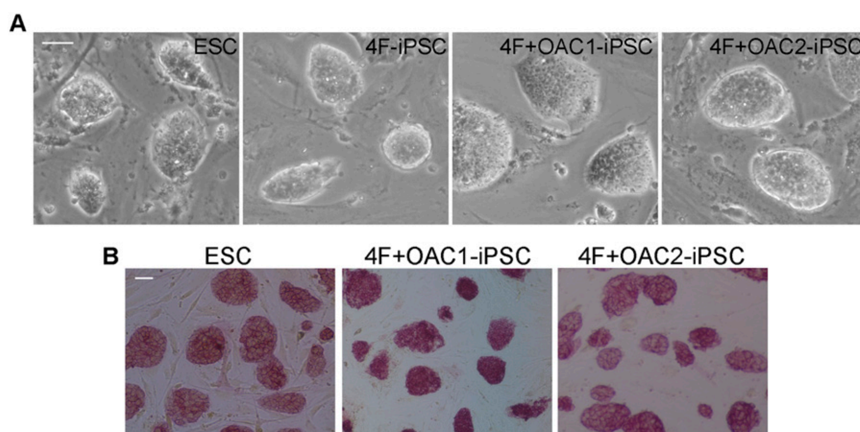
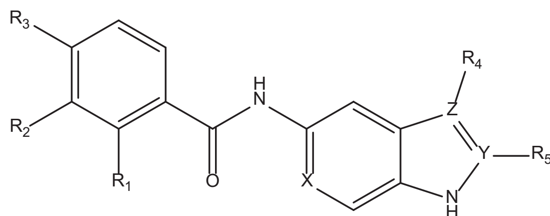


Fig. S1. Characterization of 4F+OAC1 and 4F+OAC2-iPSCs. (*A*) The morphology of mouse ESCs, 4F, 4F+OAC1 and 4F+OAC2-iPSCs. (Scale bar, 50 μm.) (*B*) AP staining of mouse ESCs, 4F+OAC1 and 4F+OAC2-iPSCs. (Scale bar, 100 μm.)

Table S1. The Oct4-activating activity of compound OAC1 and its structural analogs



Compound	X	Y	Z	R1	R2	R3	R4	R5	Oct4-luc Activity	Oct4-luc fold Induction	Nanog-luc fold Induction
OAC1	N	C	C	H	H	H	H	H	Active	5.0	4.5
OAC2	CH	C	C	H	H	H	H	H	Active	3.6	3.9
OAC3	CH	C	C	H	H	F	H	H	Active	3.7	3.5
OAC4	CH	C	C	H	H	MeO	H	H	Active	4.8	4.9
OAC5	CH	C	C	H	=*N*-O-*n* =		H	H	Active	2.9	2.4
OAC6	CH	N	C	H	H	MeO	H	—	Active	3.2	2.9
OAC7	CH	N	C	H	H	F	H	—	Active	2.2	2.0
OAC8	CH	N	C	H	H	H	H	—	Active	1.9	1.8
OAC9	CH	N	C	H	H	EtO	H	—	Active	4.4	4.4
OAC10	CH	N	C	H	H	Et	H	—	Active	4.5	4.4
OAC11	CH	N	C	H	H	iso-Pr	H	—	Active	3.8	3.9
OAC12	CH	N	C	Cl	H	Cl	H	—	Active	3.6	3.4
OAC13	CH	N	C	H	Me	Me	H	—	Active	2.8	2.9
OAC14	CH	N	C	H	EtO	H	H	—	Active	2.7	2.6
OAC15	CH	N	C	H	MeO	H	H	—	Active	1.9	1.9
C16	CH	C	C	H	H	F	N—	H	Inactive	1.0	1.1
C17	CH	C	C	H	H	Me	CN	H	Inactive	1.1	1.1
C18	CH	N	C	H	F	H	H	—	Inactive	1.3	1.2
C19	CH	N	C	H	Cl	H	H	—	Inactive	1.6	1.5
C20	CH	N	C	H	Br	H	H	—	Inactive	1.5	1.1
C21	CH	N	C	H	CF3	H	H	—	Inactive	1.2	1.2
C22	CH	N	C	F	H	H	H	—	Inactive	1.5	1.6
C23	CH	N	C	Cl	H	H	H	—	Inactive	1.7	1.6
C24	CH	N	C	Br	H	H	H	—	Inactive	1.5	1.6
C25	CH	N	C	MeO	H	H	H	—	Inactive	1.5	1.6
C26	CH	N	C	EtO	H	H	H	—	Inactive	1.2	1.2
C27	CH	N	C	MeO	MeO	H	H	—	Inactive	1.4	1.4
C28	CH	N	C	MeO	H	MeO	H	—	Inactive	1.4	1.4
C29	CH	N	C	H	MeO	MeO	H	—	Inactive	1.1	1.1
C30	CH	N	C	H	EtO	EtO	H	—	Inactive	1.0	1.1
C31	CH	N	N	H	Cl	H	—	—	Inactive	1.0	1.1
C32	CH	C	N	H	H	NO2	—	Me	Inactive	1.3	1.2

Compounds that induce Oct4-luc 1.8-fold or more were classified as “active” and named Oct4-activating compounds (OACs), and molecules that induce Oct4-luc less than 1.8-fold were indicated as “inactive” and named compounds (C).

Table S2. A list of reprogramming efficiency

Cell type	Seeding numbers	Reprogramming factors	Compound treatment	Colony numbers	Reprogramming efficiency (%)	Source
MEFs*	3×10^4	OSKM	—	200 (day 8)	0.68	Present study
	3×10^4	OSKM	OAC1	800 (day 8)	2.75	
MEFs	3×10^4	OSKM	—	56 (day 18)	0.186	
	3×10^4	OSKM	OAC1	130 (day 18)	0.43	
MEFs	8×10^5	OSKM	—	160	0.02	(1)
MEFs	2.7×10^5	OSKM	—	0 (day 8)	0	(2)
	2.7×10^5	OSKM	5-AzaC	10 (day 8)	0.004	
	2.7×10^5	OSKM	VPA	241 (day 8)	0.089	
MEFs	3.5×10^4	OSKM	—	~20	0.06	(3)
MEFs	5×10^4	OSKM	—	60 (day 25)	0.12	(4)
	5×10^4	OSKM	Kenpaullone	100 (day 25)	0.2	
MEFs	7, 500	OSKM	—	5 (day 30)	0.067	(5)
	7, 500	OSKM	RepSox2	7.5 (day 30)	0.1	

Reprogramming was performed in mouse ESC media in most cases. An asterisk signifies that the reprogramming was done in iSF1 media. The reprogramming efficiency was calculated by dividing the colony numbers with seeding cell numbers. OSKM stands for Oct4, Sox2, Klf4, and c-Myc. "—" means no compound treatment.

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