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Supplemental Information

2i Maintains a Naive Ground State in ESCs through Two Distinct Epigenetic Mechanisms

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Supplemental information

Supplemental Materials and Methods

Cell culture and inhibitors

The mouse ESC lines J1 (ATCC SCRC-1010), CCE, and *Jmjd2c* KO were cultured on mitomycin C-treated CF1 MEF feeder cells or 0.1% gelatin-coated plates at 37°C with 5% CO_2 in Dulbecco's modified Eagle medium (DMEM; Hyclone) supplemented with 15% fetal bovine serum (FBS; Hyclone), 0.1 mM β-mercaptoethanol (Gibco), 100× penicillin/streptomycin (Gibco), 2 mM GlutaMAX (Gibco), 0.1 mM NEAA, and 1000 U/ml LIF (Millipore). We passaged the ESCs at a ratio of 1:20 every 3 days. We cultured 293T cells in DMEM supplemented with 10% FBS, 100× penicillin/streptomycin (Gibco), 2 mM GlutaMAX (Gibco), and 0.1 mM NEAA at 37°C with 5% CO_2. For the 2i culture condition, we cultured ESCs on 0.1% coated gelatin plates using the same ESC culture medium supplemented with Mek1 and Gsk3 inhibitors without LIF. We used the following inhibitors in our study: 1 μM PD0325901 (Stemgent, 04-0006-02), 3 μM CHIR99021 (Stemgent, 04-0004-02), 5 μM FR180204 (Millipore, 328010), 10 μM SU5402 (Abmole, M2194), 5 μM MG132 (Merck, 474790), 5 μM 5-Carboxyl-8-HQ (Merck, 420201), and 1–10 μM UNC0638 (Sigma-Aldrich, U4885).

Dot blot

We isolated genomic DNA (gDNA) using the Qiagen DNeasy Blood & Tissue Kit according to the manufacturer's protocol. We denatured the isolated gDNA in 0.8 M NaOH/20 mM EDTA for 10 min at 95°C. We then neutralized the samples with 2 M ammonium acetate (pH7.0) and serially diluted them twofold. We spotted the samples on a nitrocellulose membrane using a Bio-Dot apparatus (Bio-Rad). We washed the blotted membrane in 2×SSC buffer and exposed it to UV light for 2 min. After blocking the membrane with 5% skim milk for 1 hour, we incubated it overnight at 4°C with primary antibodies diluted in blocking solution. The primary antibodies were mouse anti-5-methylcytosine antibody (Abcam 1:500) and rabbit anti-5-hydroxymethylcytosine polyclonal antibody (Active Motif, 1:2000).

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

We extracted total RNA with TRIzol reagent (Invitrogen) according to the manufacturer's protocol. We dissolved the RNA pellets in nuclease-free water and determined the RNA concentration of each sample with a NanoDrop. We reverse-transcribed 1 μg RNA into cDNA using SuperScript III Reverse Transcriptase (Invitrogen). We amplified the cDNA using IQ

SYBR Green Supermix (Bio-Rad) with appropriate PCR primer sets (Table S2). We performed real-time PCR assays on a CFX96 Touch real-time PCR detection system.

Table S2. qPCR primers used in the study

	Forward	**Reverse**
L32	CGCAAGTTCCTGGTCCACA	TGCTGCTCTTTCTACAATGGCT
Jmjd2c	ATGGATCGCAGATTGCAATGA	TTCCTCTCCCCTTGGATTACAT
Oct4	TAGCATTGAGAACCGTGTGAG	ACTTGATCTTTTGCCCTTCTGG
Sox2	TTTGCAAGCAACTTTTGTACAGTA	TCCTTCCTTGTTTGTAACGGTC
Dnmt3a	CCATTACCACCAGGTCAAACTCTA	AAGCGGCTCATGTTGGAGAC
Dnmt3b	ACAACCGTCCATTCTTCTGGAT	TTCATTCCGGGTAGGTTACC
Dnmt3l	AGGATGTCCGTGGCAGAGACTA	AGCTTGCTCCTGCTTCTGACTT
Tet1	CTTTCTCTGGTGTCACCTGTTG	TATAGTGGCAGGACGTGGAGTT
Tet2	ATTCTCAGGAGTCACTGCATGTT	TACATAGGCAGCACGTGGAACT
Prdm14	TTCTTCACGTCCATGAGAGGC	TCTGATGTGTGTTCGGAGTATGCT
G9a	AAGATCTACGGTTCCCACGCATT	TTGCACTTCTCAGAGCCACACT

Generation of mouse *Jmjd2c* KO ESCs

We transfected cells with pSpCas9(BB)-2A-GFP (PX458) or pSpCas9(BB)-2A-Puro (PX459) (Addgene #48138, 48139) to clone a *Jmjd2c*-guide sequence. We performed an insert/deletion assay using T7 endonuclease I (NEB). To generate *Jmjd2c* KO ESCs, we transfected CCE cells with pSpCas9 (BB) containing *Jmjd2c*-guide RNA and homologous recombination (HR) donor with a puromycin selection cassette. We incubated the cells with 3 μg/ml puromycin to isolate puromycin-resistant colonies. We confirmed HR events by gDNA-mediated PCR to detect HR events in selected colonies. Finally, we confirmed the *Jmjd2c* KO cells by PCR, sequencing, and Western blot.

Western blot

To perform Western blot analysis, we washed the cells with PBS and lysed them with RIPA buffer (50 mM Tris-HCl pH 7.4, 150 mM of NaCl, 1% sodium deoxycholate, 1% Triton X-100, and 0.1% SDS). We used the cell lysates for Western blots and other experiments. We subjected 20 μg lysate protein to SDS-polyacrylamide gel electrophoresis, transferred it to a PVDF membrane, and blotted it with appropriate antibodies. We detected proteins using HRP-conjugated secondary antibodies and ECL reagents. We visualized the bands using the ChemiDoc MP System (Bio-Rad).

GST pull down, *in vitro* kinase assay, ubiquitination assay, and *in vitro* methylation assay

For the pull-down analysis, we generated a mammalian GST expression vector system. We transfected GST-transgenes into 293T cells using a PEI transfection method. Two days after transfection, we washed cells with PBS and lysed them with RIPA buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% sodium deoxycholate, 1% Triton X-100, and 0.1% SDS). After clarifying cell lysates in a microcentrifuge, we added Glutathione Sepharose 4B and incubated them at 4°C overnight. We then washed the beads with RIPA buffer three times, added 1×SDS sample buffer, and performed Western blots.

We conducted an *in vitro* Mek1 kinase assay as follows. We transfected GST-JMJD2C and GST-MEK1 into 293T cells, isolated the proteins individually using glutathione beads, mixed them together, and incubated them in kinase buffer (25 mM Tris-HCl pH 7.5, 150 mM NaCl, 10 mM $MgCl_2$, 20 mM β-GP, 100 μM Na_3O_4V, 400 μM ATP, and 2 mM DTT) for 6 h at 37°C. We stopped the reaction by adding 2×SDS sample buffer and then performed Western blots. For ubiquitination assays, we transfected GST-JMJD2C and 5Myc-MEK1 with HA-Ub separately into 293T cells. We detected JMJD2C ubiquitination using anti-HA antibody after the pull-down assay. After washing the beads three times with RIPA buffer, we added 1×SDS sample buffer and performed Western blots.

We transfected GST-PRDM14, GST-DNMT1, GST-DNMT3A, and/or DNMT3B into 293T cells, individually isolated the proteins using glutathione beads, mixed them together, and incubated them in methylation buffer (50 mM Tris-HCl pH 8.8, 5 mM $MgCl_2$, and 4 mM DTT) with S-adenosyl-l-[methyl-^{3}H] methionine (85 Ci/mmol from a 0.5 mCi/ml stock solution, Perkin-Elmer) for 6 hours at RT. We stopped the reaction by adding 2×SDS sample buffer and performed Western blots. We used the following antibodies: anti-JMJD2C (Abcam, ab85454 or Bethyl, A300-885A), anti-GAPDH (Cell signaling, 2118), anti-H3K4me3 (Millipore, 07-473), anti-H3K4me2 (Millipore, 07-030), anti-H3K9me3 (Abcam, ab8898), anti-H3K9me2 (Abcam, ab1220), anti-H3K27me3 (Millipore, 07-449), anti-H3K27me2 (Millipore, 07-451), anti-H3K36me3 (Abcam, ab9050), anti-H3K36me2 (Millipore, 07-369), anti-Histone3 (Abcam, ab1791), anti-OCT4 (Santa, sc-5279), anti-SOX2 (Abcam, ab97959), anti-Phospho-ERK1/2 (Cell Signaling, 9106), anti-ERK1/2 (Cell Signaling 9102), anti-4G10 (Millipore, 05-321), anti-GST (Abfrontier, LF-PA0189), anti-HA (Santa, sc-7392), anti-Myc (Santa, sc-40), anti-GFP (Santa, sc-9996), anti-DNMT3A (Novus, IMG-268A), anti-DNMT3B (Abcam, ab2851), anti-FLAG (Sigma, F3165), anti-TET1 (Abcam, ab156993), and anti-PRDM14 (Abcam, ab187881).

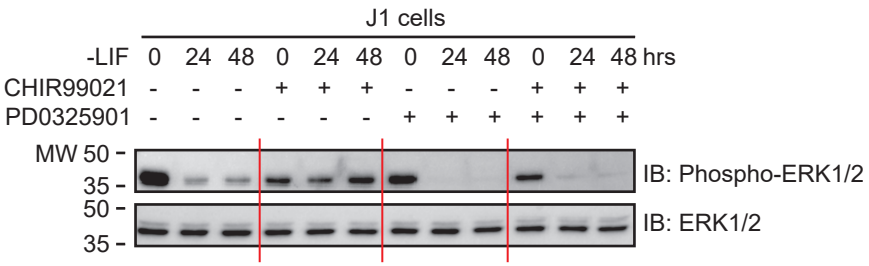
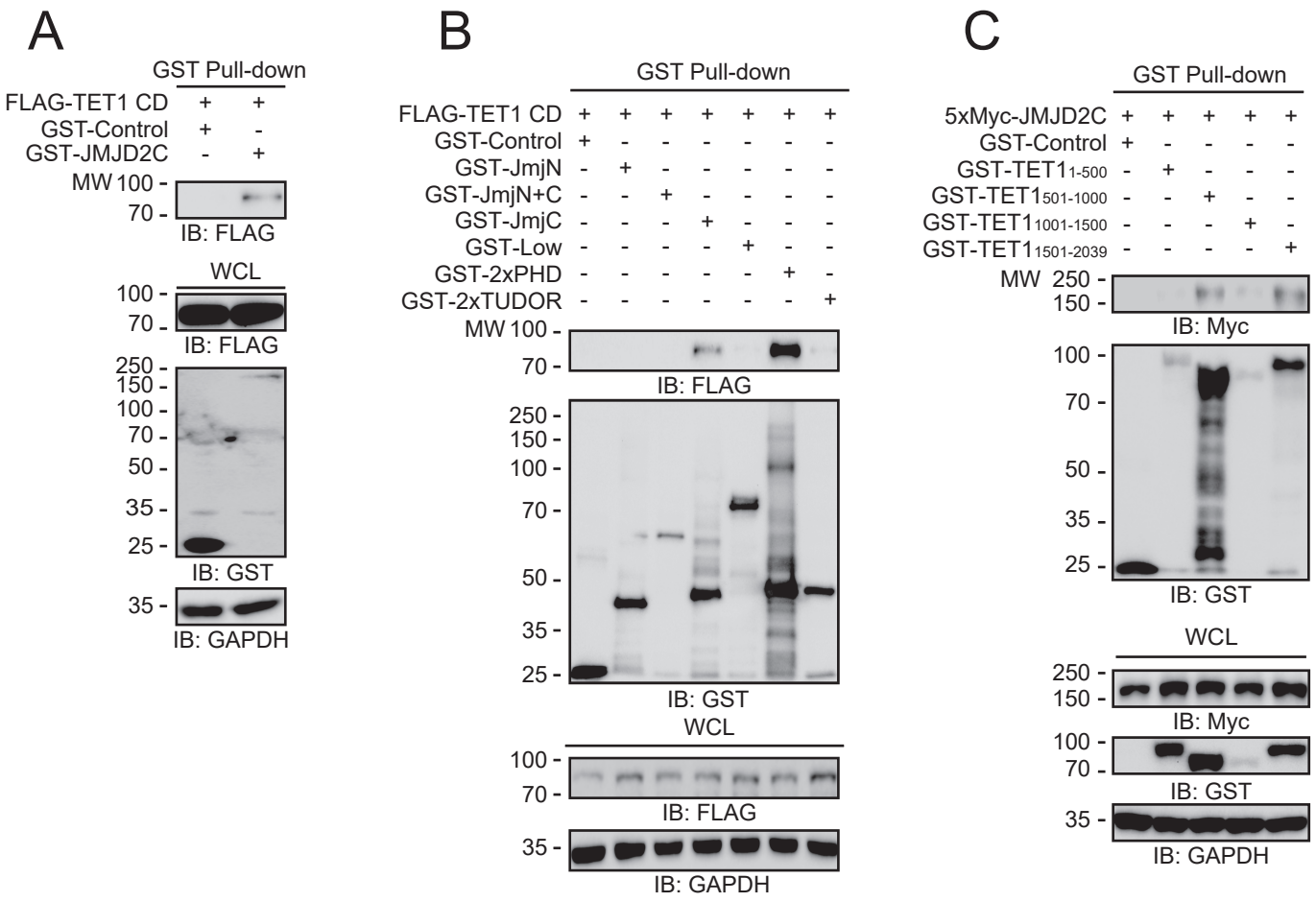

J1 cells
-LIF 0 24 48 0 24 48 0 24 48 0 24 48 hrs
CHIR99021 - - - + + + - - - + + +
PD0325901 - - - - - - + + + + + +
MW 50
35
50
35
IB: Phospho-ERK1/2
IB: ERK1/2

Figure S1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FLAG-TET1 CD + +
GST-Control + -
GST-JMJD2C - +
MW 100 -
70 -
IB: FLAG
WCL
100 -
70 -
IB: FLAG
250 -
150 -
100 -
70 -
50 -
35 -
25 -
IB: GST
35 -
IB: GAPDH
B
GST Pull-down
FLAG-TET1 CD + + + + + + +
GST-Control + - - - - - -
GST-JmjN - + - - - - -
GST-JmjN+C - - + - - - -
GST-JmjC - - - + - - -
GST-Low - - - - + - -
GST-2xPHD - - - - - + -
GST-2xTUDOR - - - - - - +
MW 100 -
70 -
IB: FLAG
250 -
150 -
100 -
70 -
50 -
35 -
25 -
IB: GST
WCL
100 -
70 -
IB: FLAG
35 -
IB: GAPDH
C
GST Pull-down
5xMyc-JMJD2C + + + + +
GST-Control + - - - -
GST-TET1 1-500 - + - - -
GST-TET1 501-1000 - - + - -
GST-TET1 1001-1500 - - - + -
GST-TET1 1501-2039 - - - - +
MW 250 -
150 -
IB: Myc
100 -
70 -
50 -
35 -
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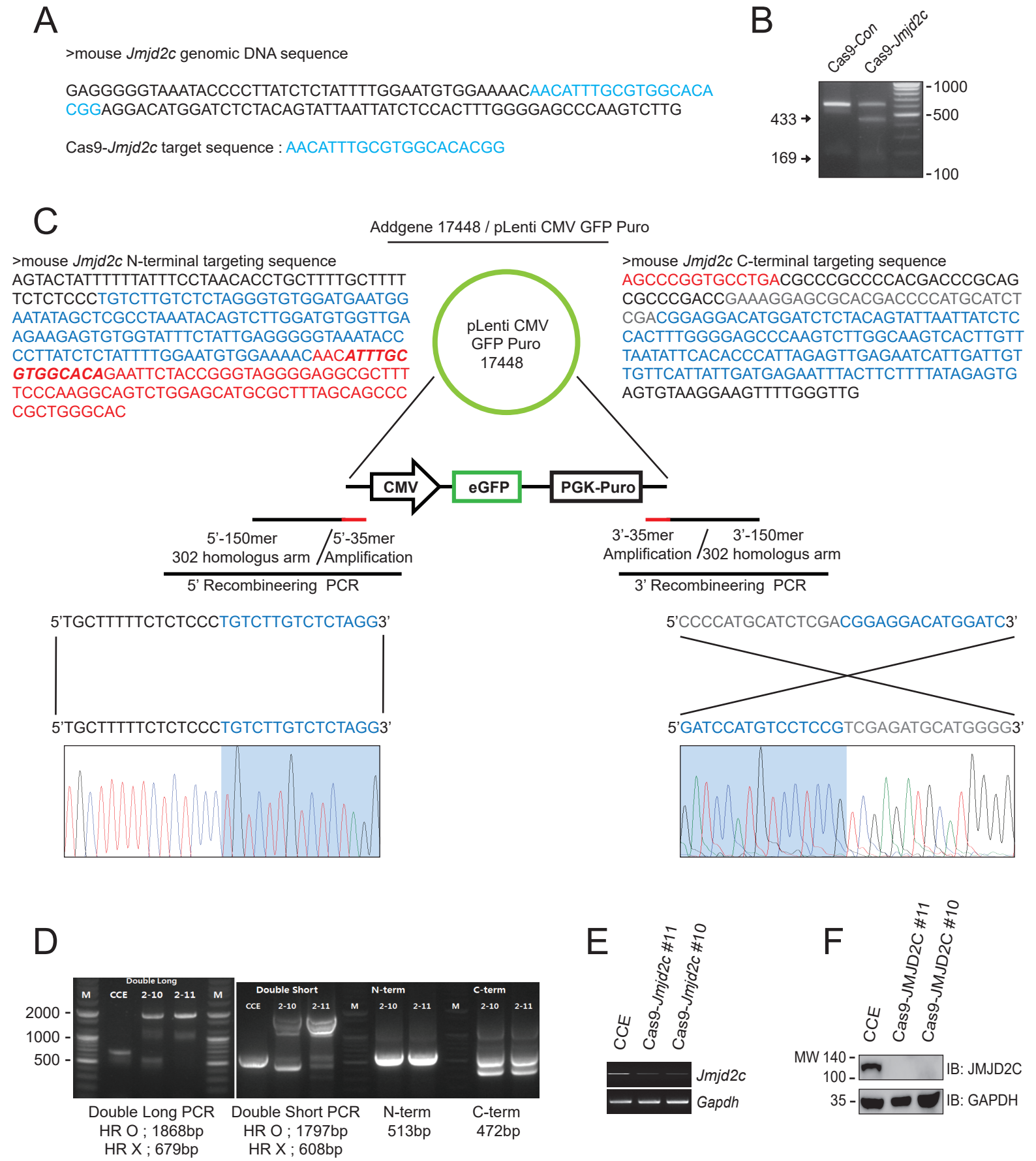
IB: GST
WCL
250 -
150 -
IB: Myc
100 -
70 -
IB: GST
35 -
IB: GAPDH

Figure S2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Cas9-Jmjd2c target sequence : AACATTTGCGTGGCACACGG
B
Cas9-Con
Cas9-Jmjd2c
433
169
1000
500
100
C
Addgene 17448 / pLenti CMV GFP Puro
>mouse Jmjd2c N-terminal targeting sequence
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AGAAGAGTGTGGTATTTCTATTGAGGGGGTAAATACC
CCTTATCTCTATTTTGGAATGTGGAAAACAAC***ATTTGC***
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CGCTGGGCAC
>mouse Jmjd2c C-terminal targeting sequence
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CGACGGAGGACATGGATCTCTACAGTATTAATTATCTC
CACTTTGGGGAGCCCAAGTCTTGGCAAGTCACTTGTT
TAATATTCACACCCATTAGAGTTGAGAATCATTGATTGT
TGTTCATTATTGATGAGAATTTACTTCTTTTATAGAGTG
AGTGTAAGGAAGTTTTGGGTTG
pLenti CMV GFP Puro 17448
CMV
eGFP
PGK-Puro
5'-150mer 302 homologus arm
5'-35mer Amplification
5' Recombineering PCR
3'-35mer Amplification
3'-150mer 302 homologus arm
3' Recombineering PCR
5'TGCTTTTTCTCTCCCTGTCTTGTCTCTAGG3'
5'TGCTTTTTCTCTCCCTGTCTTGTCTCTAGG3'
5'CCCCATGCATCTCGACGGAGGACATGGATC3'
5'GATCCATGTCCTCCGTCGAGATGCATGGGG3'
D
Double Long
M
CCE
2-10
2-11
M
Double Short
N-term
C-term
CCE
2-10
2-11
M
2-10
2-11
M
2-10
2-11
2000
1000
500
Double Long PCR
HR O ; 1868bp
HR X ; 679bp
Double Short PCR
HR O ; 1797bp
HR X ; 608bp
N-term
513bp
C-term
472bp
E
CCE
Cas9-Jmjd2c #11
Cas9-Jmjd2c #10
Jmjd2c
Gapdh
F
CCE
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Cas9-JMJD2C #10
MW 140
100
35
IB: JMJD2C
IB: GAPDH

Figure S3

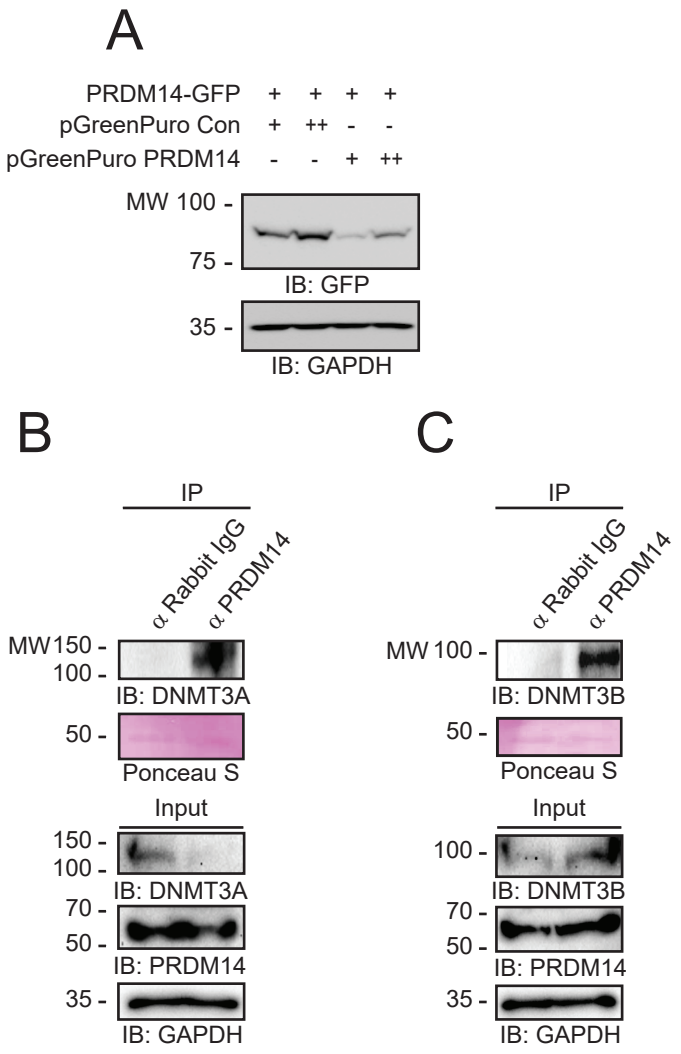
A
PRDM14-GFP + + + +
pGreenPuro Con + ++ - -
pGreenPuro PRDM14 - - + ++
MW 100 -
75 -
IB: GFP
35 -
IB: GAPDH
B
IP
α Rabbit IgG
α PRDM14
MW150 -
100 -
IB: DNMT3A
50 -
Ponceau S
Input
150 -
100 -
IB: DNMT3A
70 -
50 -
IB: PRDM14
35 -
IB: GAPDH
C
IP
α Rabbit IgG
α PRDM14
MW 100 -
IB: DNMT3B
50 -
Ponceau S
Input
100 -
IB: DNMT3B
70 -
50 -
IB: PRDM14
35 -
IB: GAPDH

Figure S4

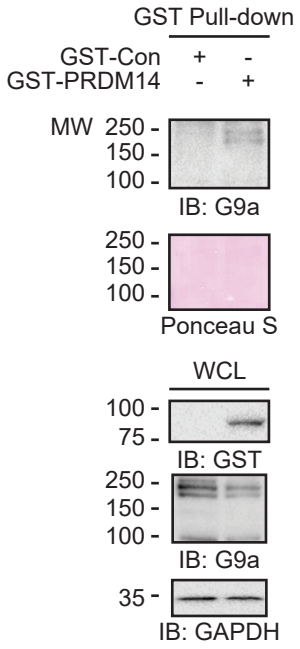
GST Pull-down
GST-Con + -
GST-PRDM14 - +
MW 250 -
150 -
100 -
IB: G9a
250 -
150 -
100 -
Ponceau S
WCL
100 -
75 -
IB: GST
250 -
150 -
100 -
IB: G9a
35 -
IB: GAPDH

Figure S5

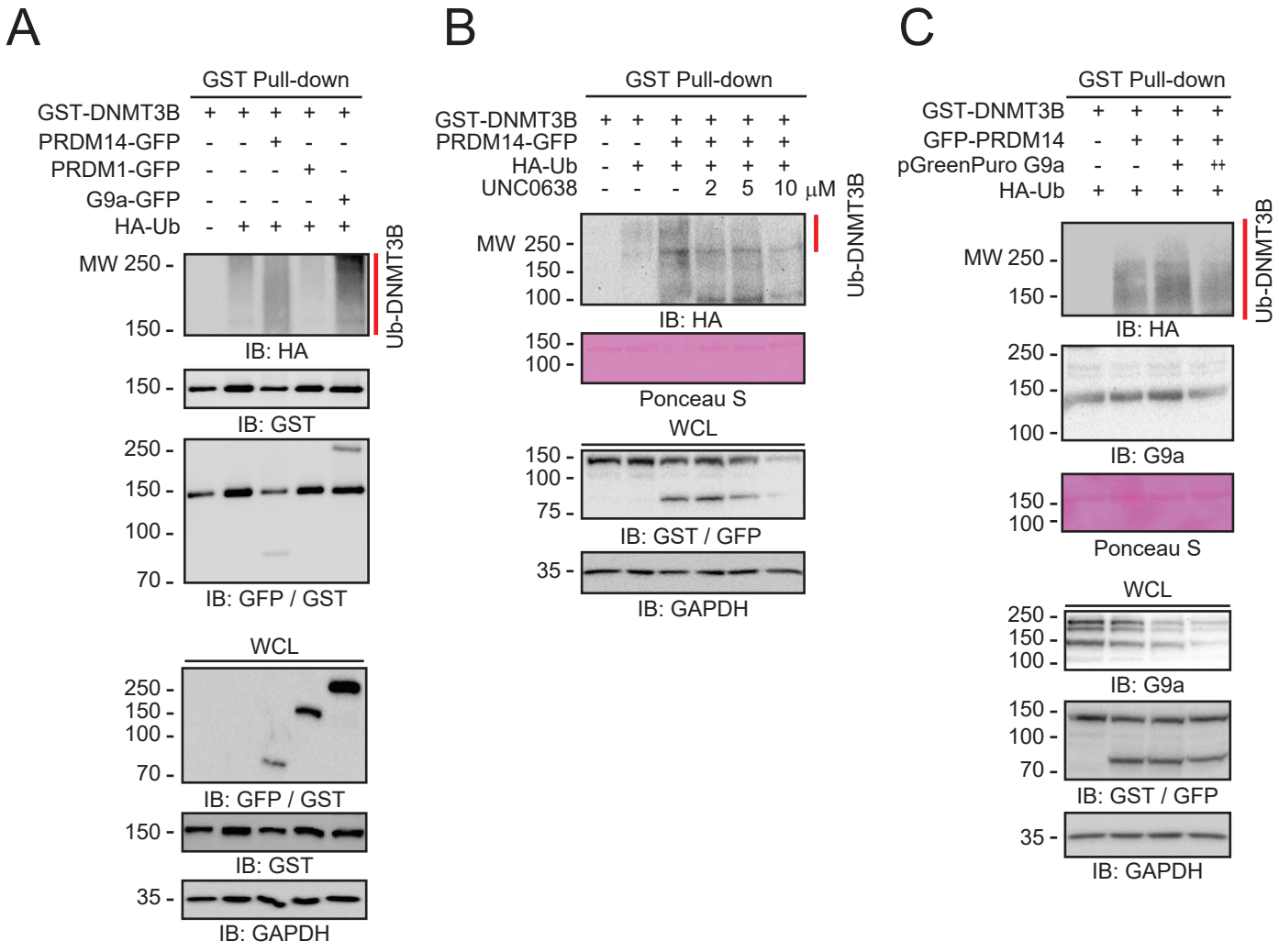
A
GST Pull-down
GST-DNMT3B + + + + +
PRDM14-GFP - - + - -
PRDM1-GFP - - - + -
G9a-GFP - - - - +
HA-Ub - + + + +
MW 250 -
150 -
Ub-DNMT3B
IB: HA
150 -
IB: GST
250 -
150 -
100 -
70 -
IB: GFP / GST
WCL
250 -
150 -
100 -
70 -
IB: GFP / GST
150 -
IB: GST
35 -
IB: GAPDH
B
GST Pull-down
GST-DNMT3B + + + + + +
PRDM14-GFP - - + + + +
HA-Ub - + + + + +
UNC0638 - - - 2 5 10 μM
MW 250 -
150 -
100 -
Ub-DNMT3B
IB: HA
150 -
100 -
Ponceau S
WCL
150 -
100 -
75 -
IB: GST / GFP
35 -
IB: GAPDH
C
GST Pull-down
GST-DNMT3B + + + +
GFP-PRDM14 - + + +
pGreenPuro G9a - - + ++
HA-Ub + + + +
MW 250 -
150 -
Ub-DNMT3B
IB: HA
250 -
150 -
100 -
IB: G9a
150 -
100 -
Ponceau S
WCL
250 -
150 -
100 -
IB: G9a
150 -
100 -
70 -
IB: GST / GFP
35 -
IB: GAPDH

Figure S6

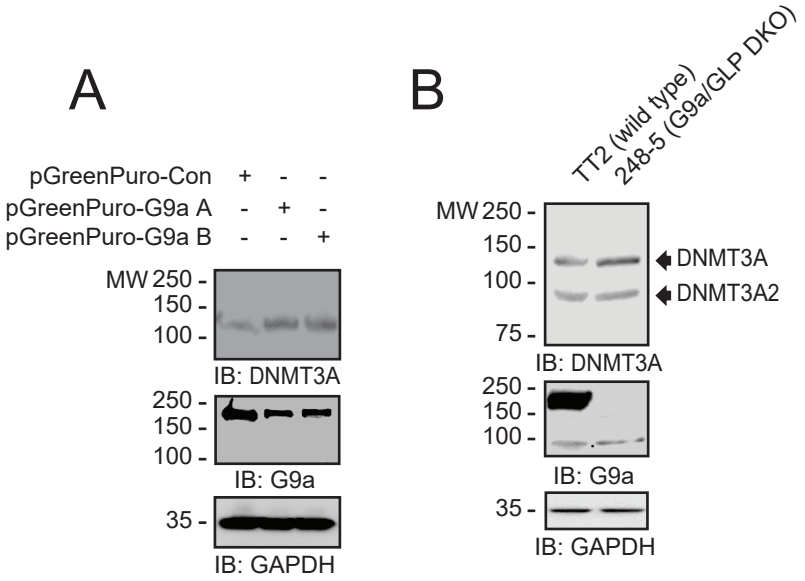
A
pGreenPuro-Con + - -
pGreenPuro-G9a A - + -
pGreenPuro-G9a B - - +
MW 250 -
150 -
100 -
IB: DNMT3A
250 -
150 -
100 -
IB: G9a
35 -
IB: GAPDH
B
TT2 (wild type)
248-5 (G9a/GLP DKO)
MW 250 -
150 -
100 -
75 -
DNMT3A
DNMT3A2
IB: DNMT3A
250 -
150 -
100 -
IB: G9a
35 -
IB: GAPDH

Figure S7

Supplemental figure legends

Figure S1 (related to Figure 1)

PD0325901, but not CHIR99021, specifically decreases phospho-ERK1/2 levels.

Figure S2 (related to Figure 3)

TET1 associates with JMJD2C.

(A) A pull-down assay shows that TET1 CD, a TET1 catalytic mutant, associates with JMJD2C. (B) A pull-down assay shows that TET1 CD binds to the JmjC and 2×PHD domains in JMJD2C. (C) A pull-down assay shows that JMJD2C associates with the CXXC and CD domains of TET1.

Figure S3 (related to Figure 3)

***Jmjd2c* KO strategy using CRISPR/Cas9 technology.**

(A) Mouse *Jmjd2c* genomic DNA sequences and a *Jmjd2c*-specific 20-bp guide RNA sequence. (B) T7 Endonuclease I assay shows that a *Jmjd2c*-specific guide RNA effectively targets *Jmjd2c* genomic DNA. (C) To disrupt genomic regions of the *Jmjd2c* gene through homologous recombination with an antibiotic puromycin cassette, we designed the primers containing the gene-specific homologous sequences (150 bp) with amplifying gene sequences (30 bp) for the PGK-Puro cassette using pLenti GFP Puro (Addgene Plasmid #17448) as a template. (D) Puromycin-resistant ESC colonies are determined by increasing PCR size due to the insertion of HR donor into *Jmjd2c* genomic DNA. To identify HR donor insertion by homologous recombination, 5’ and 3’ gene-specific primers were chosen from the adjacent upstream and downstream regions of the HR destination site in *Jmjd2c*. (E) *Jmjd2c*-KO clones have reduced *Jmjd2c* mRNA levels compared with control CCE cells, determined using RT-PCR assay. (F) *Jmjd2c*-KO clones do not express JMJD2C proteins as revealed by a Western blot.

Figure S4 (related to Figure 4)

PRDM14 associates with DNMT3A and DNMT3B.

(A) pGreenPuro *Prdm14*, a *Prdm14* shRNA, decreases PRDM14 expression

(B) Endogenous PRDM14 binds to DNMT3A in an immunoprecipitation assay. (C) Endogenous DNMT3B associates with PRMD14 in an immunoprecipitation assay.

Figure S5 (related to Figure 5)

GST-PRDM14 associates with endogenous G9a in a pull-down assay.

Figure S6 (related to Figure 5)

PRDM14-mediated G9a promotes DNMT3B ubiquitination.

(A) DNMT3B ubiquitination is increased in proportion to the expression of G9a > PRDM14 > Control > PRDM1 in a GST pull-down assay. (B) A GST pull-down assay shows that DNMT3B ubiquitination is increased by PRDM14, whereas UNC0638 inhibits a PRDM14-mediated DNMT3B degradation in a dose-dependent manner. (C) Silencing of endogenous *G9a* using pGreenPuro *G9a* reduces DNMT3B ubiquitination.

Figure S7 (related to Figure 7)

G9a/GLP deficiency causes an increase of DNMT3A protein levels.

(A) Western blots show that knockdown of G9a expression by pGreenPuro *G9a-A* and *-B* increases endogenous DNMT3A expression. (B) Western blots reveal that deficiency of *G9a* and *GLP* increases DNMT3A protein levels in the 248-5 ESC cell line compared with wild-type TT2 cells.

Supplemental Table S1

		DEGs (fold-change threshold: 99% of null)				P-value				log2-fold-change																				
Gene ID	Gene Symbol	2iLIF/DMSO	2i/DMSO	CHIR/DMSO	PD/DMSO	2iLIF/DMSO	2i/DMSO	CHIR/DMSO	PD/DMSO	2iLIF/DMSO	2i/DMSO	CHIR/DMSO	PD/DMSO	GSM1062296 2iLIF_1	GSM1062297 2iLIF_2	GSM1062298 2iLIF_3	GSM1062299 2i_1	GSM1062300 2i_2	GSM1062301 2i_3	GSM1062302 CHIR_1	GSM1062303 CHIR_2	GSM1062304 CHIR_3	GSM1062305 PD03_1	GSM1062306 PD03_2	GSM1062307 PD03_3	GSM1062308 DMSO_1	GSM1062309 DMSO_2	GSM1062310 DMSO_3
NM_001081209	Prdm14	up	up	up	up	2.08E-05	1.78E-05	4.43E-05	1.82E-06	2.6937435	2.34664416	1.390618992	1.936412179	9.734662919	9.759732764	9.728707596	9.240583411	9.387563579	9.448215371	8.36141719	8.431538411	8.514229286	8.964565943	8.977331598	8.977331598	7.040919419	7.113215776	7.020838302
NM_007548	Prdm1	up	up	--	up	0.015374885	0.018305151	0.889130277	0.000498108	0.581183883	0.421455258	0.02536966	0.885518698	7.676576678	7.750157138	7.809001314	7.590428513	7.677852968	7.499011318	7.089715969	7.194342915	7.237305238	8.110252936	7.956157	8.054491953	7.312161899	7.168973265	7.112545712
NM_011535	Tbx3	up	up	up	up	0.000188878	0.000122543	0.000238796	0.001625501	2.581117932	1.923297366	1.355536961	0.84483231	10.40761161	10.32354924	10.2811795	9.640098166	9.722249172	9.665718674	9.062702788	9.172724328	9.097968269	8.480920935	8.621280203	8.587263618	8.008584586	7.626197293	7.742431308
NM_177099	Lefty2	up	up	up	up	5.30E-06	5.17E-07	8.61E-06	6.53E-07	4.31458919	3.934158218	1.993614377	2.859179016	9.894282973	9.925499612	9.768624961	9.363892349	9.513852001	9.643134575	7.57330816	7.47760483	7.633268096	8.438872799	8.387664087	8.551826999	5.478257139	5.579693783	5.619299399
NM_010094	Lefty1	up	up	up	up	0.000169583	3.07E-06	0.000177938	8.30E-07	3.175251135	3.96176601	1.839246142	3.78969569	9.628162157	9.745735212	9.690076275	10.39588146	10.47659115	10.54332629	8.298337367	8.354071282	8.425055434	10.21191	10.30452083	10.40687749	5.967479632	6.744165359	6.51482514
NM_026796	Smyd2	up	up	up	up	0.001394222	0.000458713	0.000641199	0.001161743	1.494694834	1.438612042	1.004273727	0.801399211	9.30780558	9.46350715	9.389697544	9.160576543	9.333614752	9.352680818	8.81566046	8.911742177	8.899276437	8.736603081	8.656722313	8.696401921	7.89500271	7.808687401	8.114987414
NM_013611	Nodal	up	up	up	up	0.000367443	4.67E-05	1.70E-05	2.44E-05	1.529936354	1.615492264	1.257028321	1.12330889	10.26603101	10.36085282	10.23243931	10.35158692	10.24952867	10.35496544	9.983344185	9.993122977	10.01512809	9.850712405	9.859403546	9.907517928	8.736094656	8.75469834	8.687766487
NM_008816	Pecam1	up	up	up	up	0.010766991	0.004162959	0.008929888	0.008131249	0.853203199	0.791584336	0.568051215	0.529394893	9.617938224	9.749584955	9.720535044	9.648232494	9.700653777	9.668916181	9.350158745	9.491353159	9.43538306	9.396726738	9.375189418	9.458740539	9.154002822	8.867331845	8.823570533
NM_001159500	Esrrb	up	up	up	up	0.003316319	0.001722777	0.00342007	0.002628875	1.19294666	1.00258396	0.69134219	0.70113917	11.66703712	11.70981212	11.6542869	11.47471628	11.47665442	11.51315757	11.16541265	11.16100748	11.21460694	11.17969821	11.1709077	11.17520963	10.73581495	10.47407046	10.38108872
NM_008235	Hes1	up	up	up	up	0.000243889	0.000172928	0.00122072	0.002357167	1.828768241	1.220376893	0.726373658	0.742781808	9.377064875	9.310411597	9.248213301	8.752585227	8.681640153	8.702002249	8.208017014	8.329053562	8.115068294	8.292115473	7.951240993	8.224425164	7.553524088	7.481643356	7.423561014
NM_009694	Apobec2	up	up	up	up	0.00073187	0.000230407	0.00100059	0.000142323	1.897923915	1.691566139	0.639195488	1.384445177	9.595004172	9.663694771	9.784248094	9.457336995	9.375916202	9.464262913	8.205240129	8.579430112	8.404966344	9.135297842	9.203014836	9.150216033	8.055604884	7.703379932	7.765770856
NM_008452	Klf2	up	up	up	up	0.000386747	0.000109329	9.27E-05	2.92E-05	1.38690784	1.31390931	0.988623174	1.21855983	10.12457281	10.08904652	10.0939834	10.02098487	10.00554933	10.08050307	9.695698734	9.674610688	9.704146094	9.92563539	9.956417994	9.898291188	8.791942629	8.70707556	8.690499
NM_010637	Klf4	up	up	up	up	0.000140419	0.000427339	0.000297603	0.007711892	2.685471163	1.304755636	1.163306683	0.544261596	10.06780809	9.853522256	9.819504993	8.422381824	8.472836729	8.510343403	8.393148032	8.331441776	8.243525405	7.946282585	7.570614741	7.712342689	7.266383853	6.971213521	7.168081093
NM_173780	Klf8	up	up	up	up	0.001833927	0.001262853	0.002995577	0.000918119	1.443620126	1.127134742	0.743156225	0.947144003	10.22436429	10.23641037	10.17911043	9.861520042	9.907878906	9.973130342	9.468154669	9.523900389	9.544040012	9.727888167	9.637016966	9.754951787	9.055430513	8.692796398	8.780744164
NM_028016	Nanog	up	up	up	up	0.002646581	0.000709352	0.000874425	0.000378383	1.00864412	0.97985524	0.73748891	0.85312098	12.71561246	12.76219024	12.67611748	12.66457041	12.68682358	12.71849913	12.42537538	12.44445725	12.44945098	12.56008932	12.53749764	12.5954956	11.85690756	11.70696834	11.66723663
NM_001038695	Kdm3a	up	up	up	up	0.002362949	0.00039174	0.000585021	0.000244472	0.72941103	0.62571509	0.55650105	0.59326283	11.15419626	11.24088232	11.16668202	11.09236587	11.06288608	11.09263587	10.94699935	11.03953382	10.99377204	11.03053382	10.9813431	11.03821171	10.44660054	10.41496279	10.43727099
NM_011567	Tead4	up	up	up	up	0.00096028	0.00025724	0.000809718	0.002993825	1.255557287	0.880686081	0.525141108	0.398197823	10.33780188	10.37898115	10.22344488	9.934756294	9.962930674	9.971883822	9.653896468	9.607385701	9.590313758	9.480442416	9.59507105	9.480202752	9.099589833	9.026808214	9.082244593
NM_001166584	Tead1	up	up	up	up	0.012334144	0.003018747	0.004875627	0.003787883	0.612960083	0.684819563	0.544222183	0.500647833	10.33239521	10.40377108	10.32981643	10.40425469	10.42212806	10.38425853	10.35477357	10.26365731	10.18181986	10.22008296	10.25664611	10.19310094	9.884421547	9.673273905	9.719435127
NM_001122733	Kit	up	up	up	up	0.000565536	0.000510548	0.002016518	0.006722219	2.127678311	1.550875491	0.92702609	0.652810213	10.63535073	10.70207044	10.62866825	10.05854791	10.11315151	10.01755924	9.434698509	9.530332563	9.396293103	9.147440186	9.303593616	9.160482632	8.897316678	8.507672419	8.472143779
NM_013633	Pou5f1	--	--	--	--	0.752532776	0.554494344	0.444950151	0.66441425	-0.03193208	-0.03193208	-0.04637732	-0.01963887	12.55592081	12.51253421	12.47263575	12.51253421	12.4720509	12.52785514	12.49808897	12.44931726	12.53041096	12.5339524	12.50332011	12.52482742	12.52332201	12.54446629	12.56011014
NM_011443	Sox2	--	--	--	--	0.230457536	0.255813153	0.323908414	0.066274529	0.16835954	0.13320697	0.1041358	0.19516181	11.53896461	11.53473488	11.51163415	11.53642189	11.49858231	11.48750535	11.46860448	11.56831524	11.46951114	11.60285996	11.56053715	11.5472621	11.51163415	11.35333728	11.36537534
NM_019827	Gsk3b	--	--	--	--	0.857189906	0.917417208	0.874287682	0.041931898	0.031215567	-0.00258941	0.012135553	0.14678535	10.08194985	10.06724024	9.988520194	10.07981837	10.0272999	10.03304116	10.0481661	10.06990867	10.01340809	10.22310225	10.13625458	10.18281592	10.03602057	10.00791245	10.04291419
NM_008927	Map2k1	--	--	--	--	0.707975641	0.859487444	0.928780391	0.786366271	0.06484592	0.05841333	0.01128136	0.0381458	10.90221148	10.95420631	10.98437426	10.87400416	10.95072213	10.94777372	10.88650378	10.90064175	10.92789463	10.92750619	10.94763697	10.92693714	10.97705799	10.88936039	10.88759514
NM_011952	Mapk3	down	--	--	--	0.008021259	0.012377608	0.024736711	0.448122734	-0.4409217	-0.27141057	-0.16186575	-0.03555903	10.75867544	10.7388364	10.68403822	10.95248658	10.87025653	10.908347	11.01789182	11.02585883	10.96409268	11.14619854	11.15866212	11.09137072	11.17975757	11.21192671	11.17006183
NM_008413	Jak2	--	--	--	--	0.18452948	0.113128249	0.085783974	0.124461246	0.363497588	0.315661558	0.329140933	0.279811678	7.389392089	7.577141169	7.689115479	7.529305139	7.77156398	7.495695582	7.473609452	7.710385966	7.542784514	7.493455259	7.417586559	7.621553086	7.568945327	7.0591505	7.213643581
NM_146145	Jak1	--	--	--	--	0.344951099	0.239696979	0.188233158	0.66079261	-0.113625033	-0.122180614	-0.109323749	0.0539118	9.922462231	9.923316127	9.997779761	9.902889512	9.998210354	9.914760546	9.927617411	9.965740174	9.895724335	10.1173677	10.07450217	10.09085296	10.14911841	9.985655615	10.03694116
NM_133872	Kdm1a	--	--	--	--	0.098244884	0.491351679	0.508656687	0.076034216	-0.13449044	-0.02954174	-0.0258367	0.11583082	11.29406189	11.28856278	11.324372	11.39901059	11.40973993	11.38290161	11.40271563	11.365013	11.41113083	11.54438315	11.51409714	11.54916463	11.42480865	11.42855233	11.47688573
NM_007971	Ezh2	--	--	--	--	0.954825324	0.520541013	0.952501056	0.988816343	0	-0.02035724	0.01417589	0.00739475	12.13368336	12.15222567	12.16144658	12.13186843	12.13728129	12.10926723	12.16940156	12.16890615	12.15523734	12.15962042	12.1711899	12.15222567	12.15222567	12.15227353	12.17995759
NM_001040400	Tet2	--	--	--	--	0.039743399	0.03808757	0.16460603	0.085863196	0.33152927	0.17416958	0.11812634	0.11460853	10.66612501	10.49800662	10.48743803	10.34124693	10.31256837	10.35798179	10.31856089	10.28520369	10.2116253	10.28168588	10.35434522	10.28041874	10.15633078	10.21274353	10.16707735
NM_007626	Cbx5	--	--	--	--	0.104771008	0.448512498	0.234366588	0.378675847	-0.1130471	-0.03324619	-0.0403085	0.04003962	11.9467603	11.98404002	11.96189243	12.04388512	11.99567834	12.04169334	12.03788678	12.03463103	12.02985775	12.08957936	12.1281104	12.11497915	12.07493953	12.05996135	12.08896336
NM_007622	Cbx1	--	--	--	--	0.029503499	0.031523059	0.09084253	0.242738549	-0.36432701	-0.22477881	-0.13733489	-0.0588725	10.50529175	10.56805632	10.66250848	10.70760452	10.72856585	10.61287766	10.75142734	10.79504844	10.86726985	10.87351075	10.83427461	10.88334215	10.93238333	10.92568575	11.00416614
NM_007624	Cbx3	--	--	--	--	0.125400264	0.212025786	0.050707961	0.578932935	0.14377367	0.082500588	0.175570951	0.059109856	9.369629651	9.26609133	9.268912178	9.224143305	9.15902597	9.207639096	9.315945681	9.300709459	9.207639096	9.184248364	9.088749562	9.193745451	9.10153102	9.125138508	9.137930853
NM_007872	Dnmt3a	down	down	down	down	5.79E-05	0.000156539	0.000394534	0.000423353	-2.614256669	-1.416622014	-0.89621702	-0.78195397	8.517299868	8.595657624	8.540243521	9.737878176	9.692769845	9.790956263	10.2811659	10.17247751	10.25828317	10.37254622	10.36961084	10.40209056	11.02348414	11.15450019	11.18834663
NM_001003961	Dnmt3b	down	down	down	down	8.89E-05	0.000407288	0.001073072	0.001584939	-2.38162021	-1.3013442	-0.67309286	-0.59547939	9.475157644	9.49111621	9.552179138	10.57139222	10.48226958	10.70994357	11.19964356	11.11466499	11.20354583	11.32857493	11.198151	11.27725703	11.78645283	11.87273642	11.9638793
NM_019448	Dnmt3l	down	down	--	--	7.20E-05	0.001784253	0.009081909	0.024567144	-2.140145475	-0.93206149	-0.32303863	-0.21060058	9.68469214	9.718660685	9.741563683	10.88282404	10.92674467	11.15320308	11.53576753	11.47950544	11.58499331	11.6861369	11.64820558	11.62409026	11.78159166	11.85889016	11.88153958
NM_027384	Tet1	down	down	down	--	0.012043531	0.001155823	0.003071994	0.188987465	-0.42177471	-0.52307951	-0.46890966	-0.0869057	11.83198186	11.78612132	11.73438113	11.68481652	11.64049752	11.68559214	11.85201809	11.73898637	11.7103168	12.16724092	12.12099033	12.09557375	12.21380166	12.17114233	12.20789603
NM_008709	Mycn	down	down	down	--	0.013255175	0.017733096	0.008417377	0.768439968	-0.6957288	-0.50192142	-0.51756819	-0.0490553	10.31278258	10.30103231	10.26090287	10.45473712	10.49483969	10.58456398	10.53634327	10.42601119	10.47919292	10.93964712	10.94770581	10.96206391	10.74352251	10.99955502	10.99676111
NM_010849	Myc	down	down	--	down	0.013617308	0.00289638	0.051284719	0.00095902	-0.889698966	-1.182810231	-0.414287531	-1.115077446	8.948131986	8.937675203	8.995396738	8.533296375	8.655020721	8.940751119	9.561489576	9.117559098	9.425543421	8.902701586	8.706861089	8.727253506	9.492794828	9.837630962	9.994381558
NM_183199	Usp44	down	down	down	down	0.000779259	0.000674732	0.003011611	0.000925336	-1.947622374	-1.108267546	-0.545201009	-0.696340342	6.994547718	7.16628917	7.424994803	8.005643998	8.054480223	7.882985553	8.568710535	8.486495177	8.598122343	8.417571202	8.409248914	8.456448046	9.113911544	9.075069593	9.290357453
BC150968	Pten	down	down	down	down	0.004347225	0.006175368	0.001199066	0.003978405	-0.935188344	-0.570273462	-0.528000839	-0.475490823	5.578639849	7.811929349	7.839265746	8.176844231	8.207157566	7.950639526	8.219116854	8.28933393	8.187012248	8.27096065	8.27162687	8.437611888	8.704290873	8.754209157	8.747117693
NM_011207	Ptpn3	down	down	down	down	0.004440574	0.001948325	0.00888838	0.001706495	-0.913158601	-0.804734379	-0.431924736	-0.604328065	8.325787749	8.435461897	8.365109234	8.473533456	8.528471108	8.428626633	8.718789125	8.904097049	8.846343099	8.67393977	8.67393977	8.660783444	9.276267835	9.216358731	9.456847074
NM_010203	Fgf5	down	down	down	down	0.003484177	0.001632991	0.003482057	0.00102103	-1.617623133	-1.493532399	-0.99527515	-1.257445917	7.11848357	7.127738512	7.103914189	7.475628506	7.204091533	7.242574304	7.843069138	7.566585779	7.740831553	7.471326069	7.478660786	7.56497826	8.337056459	8.736106703	9.140624536
NM_019479	Hes6	down	down	down	down	0.00186274	0.000984241	0.000628476	0.001928117	-1.02709816	-0.778450329	-0.683468263	-0.463202073	9.113122189	9.22012992	9.264193154	9.468777751	9.369580878	9.487614841	9.513085207	9.563759817	9.623797577	9.783694537	9.867355544	9.784026007	10.24722808	10.21997049	10.31767539
NM_023794	Etv5	down	down	down	down	0.00151885	0.000348124	0.00229291	0.000170104	-1.05308862	-1.28758063	-0.52562959	-0.72042855	10.25418848	10.3336498	10.19790214	9.989238588	10.01969647	10.2084079	10.88920744	10.73603722	10.78164751	10.58684855	10.60035282	10.57206562	11.37769272	11.3072771	11.29448691
NM_144763	Wt1	down	down	down	down	0.003550673	0.002019742	0.000958958	0.00038444	-1.440428912	-1.271910697	-1.246769169	-1.396640088	6.712424404	6.596103021	6.7446734	7.013497549	6.710421334	6.880760619	7.059348405	6.809774561	6.903902147	6.727946747	6.880760619	6.756031228	7.693281143	8.190188229	8.152671316
NM_175136	Rnf122	down	down	down	down	0.001033724	0.000640698	0.00047229	0.004211157	-1.406982756	-0.874992466	-0.800619676	-0.404030457	8.109772499	8.301907523	8.166331442	8.665307542	8.699863842	8.698321732	8.776723631	8.673796677	8.772694522	9.169283741	9.230628473	9.134220074	9.70048347	9.573314198	9.557400412
NM_020510	Frzd2	down	down	down	down	0.0011768	0.000624916	0.000618187	0.001556291	-1.477551854	-0.87747113	-0.737054155	-0.504505036	7.49228724	7.242459746	7.294703168	7.947765894	7.891149748	7.894783892	8.055733162	8.035200867	7.949156109	8.267749986	8.309992516	8.266084794	8.66412532	8.772255022	8.77786016

Supplemental table legend

Supplemental Table S1 (related to Figure 1E)

Quantitative gene expression data induced by combinatorial 2i treatments in ESCs

Bioinformatics data come from the GSE43597 in GEO DataSet.