Chromatin lncRNA *Platr10* controls stem cell pluripotency by coordinating an intrachromosomal regulatory network

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SUPPLEMENTAL TABLES

Table S1. Oligonucleotide primers used for PCR

ID	Oligo Name	Oligo sequence	Product size
RT-PCR			
Platr10	SJ229	CACTGCTGGTTTGGAGCTCCAT	121bp
	SJ230	TGGGACAGTCTCTGGATGGCCT	
Oct4	JH116	CAATGCCGTGAAGTTGGAGAAG	179bp
	JH117	GGCTGAACACCTTTCCAAAGAGA	
Sox2	JH118	GGTTACCTCTTCCTCCCACTCCAG	193bp
	JH119	TCACATGTGCGACAGGGGCAG	
Nanog	JH120	TCTCCTCCATTCTGAACCTGAGC	150bp
	JH121	TGCTGGGATACTCCACTGGTGCT	
Palr34	JH4413	GATTGACTAACCAGGGTGGAC	132bp
	JH4414	CACTCGAGGGAGTCAATGCAG	
Palr33	JH4119	GAGATGGTGGCTAAACCAGG	103bp
	JH4120	GAGGCACTGGAGACCATGATG	
Palr35	JH4123	CAAGATGGGCAGCCTAGTTC	123bp
	JH4124	GGCATTTGGGGTGAGCTAGGGC	
β-Actin	J880	CAGGTCATCACCATTGGCAATGAGC	135bp
	J881	CGGATGTCCACGTCACACTTCATGA	
U2	JH1055	ATCTGTTCTTATCAGTTTAATATCTG	151bp
	JH1056	GGGTGCACCGTTCCTGGAGGTAC	
Tet1	JH6031	GAACAGCCAYCAGATCTGTAAG	170bp
	JH6032	CTGAYTTGGGGCCATTTACTG	
Tet2	JH6033	GTCCTYATGTGGCAGCTATTAG	129bp
	JH6034	TAGCAATAGGACATCCCTGAG	
Tet3	JH6035	CYAAGAGTCTGCTGGACACAC	154bp
	JH6036	TCCTCCATGAGTTCCCGGATA	
RAT Oct4 binding			
5'-CT	SJ476-JH4348	CTGAGTCCTCTGCAAGATGC	137bp
	SJ477-JH4349	CCAAGGCACCTGCCTAGGATT	
pOct4	SJ484-J648	CAGAGGATGGCTGAGTGGGCTGTA	123bp
	SJ485-JH4354	CACCCCTGCCTTGGGTCACCG	
Off-target	JH5877	AGCCATCCTGTCCTCCGCCTG	128bp
	JH5878	CTGCACGGAAGGTCACGATG	

Platr10 shRNAs

1	TTCTGTGTATCTGTTGAGCCAGGCA
2	CCTGCTGCCTGTCAATCCAAATGTA
3	CTGCCAGCATCTGACTAAGATAGAT
4	TAGTATGGCTGTCCTCGGAGAGGCT
Control (shCT)	GCAGCAACTGGACACGTGATCTTAA TGAAATGTACTGCGCGTGGAGACTA

CRIST Cas9 gRNA

Oct4-1	GAACATTCAATGGATGTTTT
Oct4-2	GTGTGAGGGGATTGGGGCTC
Sox2-1	GGGGTTGAGGACACGTGCTG
Sox2-2	GAGCCAATATTCCGTAGCAT
Control (gCT)	GAAGTGGGATGATCCTCTGA

CRIST PCR

SJ516	CCGCATCTGGCTTCCCAGGATAC
SJ517	CGTTAACTTGCCACAAACCACCTG
SJ484	CAGAGGATGGCTGAGTGGGCTGTA
SJ485	CACCCCTGCCTTGGGTCACCG
JH5683	CAGCTCTCAGGAGGTTCCAG
JH5684	CACTTCCACCAACTAGGAACT

3C primers

Oct4 functional loop

SJ518	GCAGACAGGCACTCTGAGGGC
SJ536	TGCAGGTCTTGGGCACCCCTG
SJ515	ACCATCTCTGGCTGGGGACGTG
SJ530	AGGCTCCGGGTGGAGAAAGCTG
SJ534	GTCTCATGCAGCCCAGGCTGAC
SJ525	ACTGACTGCTCTGCCAGAGGTC
SJ520	GGACACCTGGCTTCAGACTTCG

Igf2/H19 positive control

SJ1175	GAAAGCTTCCTTGGTCAGGCTA
SJ1202	TCTGGAAGGGGTGGCAGGGCT
SJ1196	ACCAGGTGAGACCAGCATGAT

Ercc3 ligation control

JH6286	TGGAGCAGTGGAAAGCCCAGT
JH6282	CTTCATAAGTGTCTTAGCAGAGCT

RAT primers

Platr10	
JH4397R	CATGATGCTGGAGAGGTAGCT
JH4398R	AGAGGGAATCTAGGCAGGTAG
JH4023R	TGGGACAGTCTCTGGATGGCCT
SJ225R	GACATGATGCTGGAGAGGTAGC
Palr34	
JH4122R	GCCTCAGAAGCTTAAAAGCC
JH4164R	GTAGATTTATCAGGGCAGCACAAG
JH4414R	CACTCGAGGGAGTCAATGCAG
Palr35	
JH4124R	GGCATTTGGGGTGAGCTAGGGC
JH4175R	GTTAACGCTGGTGCCGAGTG
JH4176R	GGTACTGCTCCATTTTGCGCATC
JH4177R	CATGCACCCAGGTTAGTTGAC
RAT control	
JH5849	ATGGACTGATGATCTTATGC
JH5850	TACATAGTAGATCAGATACT

TBE mapping

F1 JH6155	CACGTGGTCACCTGAAGCTG
JH6156	TCATGTCCGGGTTCGAGCCT
F2 JH6157	TCCGAGATGCCTCCCAGGCT
JH6158	ATGGAGCTCCAAACCAGCAGTGT
F3 JH4022	CACTGCTGGTTTGGAGCTCCAT
JH4023	TGGGACAGTCTCTGGATGGCCT
F4 JH6159	TCCAGAGACTGTCCCACCTTG
JH6160	CGAGGACAGCCATACTAGGCT
F5 JH4082	CTCGGAGAGGCTCTGCCAGCAT
JH4083	AGCTGTCAGAACATGCATGTC
F6 JH6161	GACATGCATGTTCTGACAGCT
JH6162	CAGCTGCCTGGCTCAACAG
F7 JH6163	TGAGCCAGGCAGCTGCCCTGT
JH6164	GTGACACACTTCCTCCAGCT
F8 JH6165	CTCATTTGGTGTGGCCAAGCT
JH6166	ACTTGGCTTGGCTTGGCTTGAG
F9 JH6167	CTGCTGCCTGTCAATCCAAATG
JH6168	TGGGCTGGCTTCACATTTCAGA

DNA methylation

Platr10 promoter		
SJ1131	TAATGATTTTTGTATATGTTATAGGTG	
SJ1132	TAATCTTACTTCTTTTACATATCTATA	
SJ1133	TGGGAGTTTGTTTAGTTATTTTGGAGT	
SJ1134	TTTGAGTAGATTAGTTTGTTTTGGTTT	
Oct4 promoter		
SJ629	GGATAGGTCGAGAGGGTGTAGTGTT	
SJ630	CACCCTCTAACCTTAACCTCTAAC	
SJ631	GTGGGATTGGGGAGGGAGAGGTGAA	
SJ632	TCCAAACCCACCTAAAAACCCTTAA	
SJ633	TTTGTTTGTTAAAGGTGGTGATGGT	
SJ634	ATAACCTACATAACAAAAACCAAAC	
Platr10 CRISPR Cas9 knockout		
Platr10 SpCas9 gRNA		
gRNA1	GCTCTCAGCTGTATCAGAGT	
gRNA2	GGTGAACTCTAACCTGGAGT	
Platr10 NmCas9 gRNA		
gRNA1	TGTCAGTAGAGCCTAGAAGAAAGT	
gRNA2	CCTGATAGTTCGGACCCATTGTAT	
Cas9 control gRNA		
shCT	GAAGTGGGATGATCCTCTGA	
Platr10 arm primers		
ARM1-F (SJ2150)	AGTGCATAACTGGTTAATACTTGCT	
ARM1-R (SJ2152)	CAGCCCGTGAAATAATGCTGCA	
ARM2-F (SJ2153)	CCATCTCTTTATTCCCAAGGGTTC	
ARM2-R (SJ2154)	CTCAGGAGGAACACAGGTACTGTC	
Recombination PCR/sequencing		
SJ2155	CTGAGAAATTTCAAGCTGTCTGC	888bp
SJ1667	CCATAAGGTCATGTACTGGGCA	

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Mapping of the *Oct4* interacting lncRNAs by chromatin-RNA *in situ* reverse transcription trap sequencing (CRIST-Seq).

A. CRIST-seq assay. The CRIST assay combines the specificity of Cas9 gene targeting with the simplicity of biotin-lncRNA labeling. Cells carrying the CRISPR Cas9 Oct4-gRNA cassette were first treated with formaldehyde to fix the Cas9 gRNA-Oct4 promoter chromatin complex structure. The promoter-interacting RNAs were *in situ* reverse transcribed into biotin-cDNAs (cDNAs) in the isolated nuclei with biotin-dCTP. The promoter chromatin-lncDNA complex was then isolated by Cas9 immunoprecipitation and was subsequently purified form genomic DNA by biotin-streptavidin bead purification. The CRIST-captured cDNAs were used for library construction. Illumina sequencing was used to identify lncRNAs that interact with the *Oct4* promoter.
B. CRIST targeting and control vectors. pEF1: EF1-alpha promoter; dCas9: the catalytically inactive CRISPR Cas9; gRNA1-2: Cas9 guiding RNAs that target the *Oct4* promoter; U6: RNA polymerase III U6 promoter; H1: human H1 RNA polymerase III promoter; gCT: a random gRNA control. Two Cas9 gRNAs are transcribed by U6 and H1 promoters, respectively, and guides Cas9 to the *Oct4* promoter. Cas9 gCT and empty vector were used as the CRIST controls.
C. The targeting and control sites in the *Oct4* promoter. pOct4: Oct4 promoter where the Cas9 gRNAs are designed; 5'-Ct: the 5'- 3C negative control site that is 13.9 kb away from the Cas9 gRNA targeting site.

D. Off-target control. A 5'-upstream site of the GAPDH house keeping gene was used as the off-target control site.

E. Specificity of the CRIST targeting. The CRIST signals were detected by qPCR at the 5'-Ct, pOct4 targeting site and the off-target site. Vector: cells that were treated with the Cas9 control vector that lacks the gRNAs; gRNA: cells that were targeted by both Cas9 and *Oct4* gRNAs; gCT: cells that were treated with the random control gRNA vector. Throughout the manuscript, the data are presented as the mean ± SD from three independent experiments unless they are specifically defined. ** $p<0.01$ as compared with Vector and gCT controls.

Figure S2. Location of the Cas9-Oct4 gRNAs in the *Oct4* promoter.

A. Diagram of the CRISPR Cas9-guided chromatin immunoprecipitation (Cas9-IP) vector. Cas9: CRISPR Cas9; gRNA: Cas9 guiding RNAs that target the *Oct4* promoter (sequences under the diagram); pEF1: the human EF-1a promoter; pH1: human H1 promoter; pU6: U6 promoter; T5: the TTTTT termination signal of RNA polymerase III.

B. Location of the two *Oct4* promoter Cas9 gRNAs. *Oct4* mRNA is shown in green and the coding sequences in red. Two Cas9 gRNAs are highlighted in yellow and PAM sequences in red in the sequence of the *Oct4* promoter region. gRNA1 is located immediately the upstream of the TF binding site.

Figure S3. CRIST targeting of the *Sox2* promoter.

A. Location of the two Cas9 gRNAs in the *Sox2* promoter. Two *Sox2* Cas9 gRNAs are highlighted in yellow and PAM sequences in red in front of the *Sox2* transcription initiation site (TS+1).

B. CRISPR Cas9 *Sox2*-gRNA vector and control vectors. dCas9: defective CRISPR Cas9; gRNA1, 2: two Cas9 guiding RNAs that target the *Sox2* promoter.

C. Specific targeting of the *Sox2* promoter. pSox2: the Cas9 gRNA targeting site in the *Sox2* promoter; 5'-Ct: a control site that is 14.6 kb away from the pSox2 target site; Off-target: a CRIST control site that is 33.8 kb upstream of the housekeeping gene GAPDH. Cas9 enrichment signals were quantitated by qPCR using specific primers derived from the pSox2 targeting site, 5'-Ct control site and off-target site. All data shown are mean±SEM from three independent experiments after normalization over the IgG control. ** $p<0.01$ as compared with the Cas9 Vector and Cas9-gCT controls.

Figure S4. Gene structure of *Platr10*.

A. The IGV plot of *Platr10* from RNA-seq data of iPSCs and fibroblasts. *Paltr10* (Gene ID ENSMUSG00000099370) is located at chromosome 3: (chr3:75,647,445-75,655,731). Both the iPSC and fibroblast RNA-seq Bam data were analyzed by IGV, and the Sashimi plot was acquired for *Platr10*. Note differential expression of *Platr10* in two cell populations.

B. The location and exons of *Platr10*.

Figure S5. Characterization of *Platr10* variants.

A. The 5'- and 3'-RACE of *Platr10* lncRNA. After racing, the PCR bands were cut and cloned in pJet vector for sequencing.
B. Diagram of the two alternatively spliced variants.
C. The sequences of NONMMUT043505 variant 1 (V1). The underlying capital letters in red are the sites of intron splicing. V1 contains an extra exon as compared with variant 2 (V2).
D. The sequences of NONMMUT043505 variant 2 (V2).

Figure S6. Expression abundance of *Platr10* and promoter DNA demethylation in reprogramming.

A. The CpG islands in the *Platr10* promoter. E1-E4: exons 1-4 of the *Platr10* gene. Two CpG islands in the *Platr10* promoter were selected for the measurement of DNA methylation
B. Sodium bisulfite sequencing. Cells were collected during the process of reprogramming. Genomic DNA was isolated and treated with sodium bisulfite to convert the unmethylated cytosines into uracils, while the methylated cytosines were not affected. The treated DNA was amplified, cloned into pJet vector, and sequenced. Open circle: unmethylated CpG; solid circle: methylated CpG. The *Platr10* promoter DNA becomes demethylated in iPSCs, which is associated with the activation of *Platr10* in reprogramming.

Figure S7. Functional studies of *Platr10* lncRNA.

A. *Platr10* shRNA knockdown vectors. pH1: human H1 promoter; pU6: U6 promoter; T5: the TTTTT termination signal of RNA polymerase III. Two lentiviral shRNA vectors (shPlatr10-1 and shPlatr10-2) are constructed to knockdown *Platr10*. Each lentiviral vector carries two shRNAs under the control of pH1 and pU6 promoters to target *Platr10* lncRNA.
B. Knockdown of *Platr10* by shRNA in E14 cells. shPlatr10-1, -2: shRNA lentiviruses. shCT: random shRNA control; Vector: lentiviral empty vector control. The abundance of *Platr10* was measured by qPCR and the value of the vector control was set as 1. ** $p < 0.01$ as compared with the Vector and shCT controls.
C. *Platr10* enhances cell reprogramming. MEF cells were transfected with the lentiviruses carrying the empty vector (Vector), lncRNA control (CTL), and *Platr10*. Doxycycline (DOX) was added in the medium to initiate reprogramming. iPSC colonies were immunostained using anti NANOG antibody (green).

Figure S8. Genomic deletion of *Platr10* by CRISPR Cas9 targeting.

A. Schematic diagram of *Platr10* targeting. SpCas9: Streptococcus pyogenes Cas9; NmCas9: Neisseria meningitides Cas9; gRNA: Cas9 guiding RNA; pCMV: CMV promoter; pU6: human U6 promoter; pH1: RNA polymerase III H1 promoter; GPF: copGFP; Puro: puromycin; Arm1, Arm2: two *Platr10* genomic sequences used for Cas9-mediated recombination. Under the guidance of gRNA, Cas9 mediated genomic recombination at the *Platr10* locus, resulting in the

deletion of the region covering the *Platr10* promoter and coding region. Cells were treated with puromycin for positive selection and ganciclovir for negative selection. The copGFP-positive cells were selected and validated by PCR DNA sequencing.

B. Cas9-targeted E14 cells. *Platr10*-deleted cells were copGFP-positive (red arrow). Cell morphology was altered in the *Platr10*-gRNA group, but not in the gRNA control group (while arrow).

C. Loss of the pluripotent marker NANOG in *Platr10*-deleted cells. Expression of the pluripotent maker NANOG was detected by immunostaining. Note the loss of the pluripotent NANOG marker in *Platr10*-deleted cells (red arrow), but not in control cells (white arrow).

Figure S9. Characterization of *Platr10* Oct4 binding element.

A. The sequence of Oct4 binding element (OBE) of *Platr10* lncRNA. In the IGV program, each sequencing read from the peak region was downloaded and a 50 bp consensus sequence was taken for the OBE of *Platr10*.

B. The predicted structure of the *Platr10* OBE. The structure of OBE was obtained by submitting the OBE sequence to the RNA structure prediction website:https://rna.urmc.rochester.edu/RNAstructureWeb/Servers/Predict1/ResultsPages/20180916.191929-72078a8d/Results.html.

Figure S10. Nuclear localization of *Platr10*.

A. Subcellular localization of *Platr10* by RT-PCR. Cytoplasmic and nuclear RNAs were isolated and used for RT-PCR of cellular *Platr10* on agarose gel. Nuclear

U2 was used as the positive control.

B. Quantitation of subcellular *Platr10* by qPCR. β-Actin was used as the cytoplasmic control and U6 was used as the nuclear control.

C. Chromatin-bound *Platr10*. Chromatin and nucleoplasm fractions were isolated and RNAs in each fraction were purified for qPCR. Note the primary location of *Platr10* in the chromatin fraction.

D. *Platr10* RNA FISH. *Platr10* lncRNA in E14 cells was detected with Ribo lncRNA FISH Probe Mix (red, Guangzhou RiboBio, China). The nuclear DNA was stained with DAPI (blue). shCT: E14 cells that were transfected with shRNA random control; shPaltr10-1: cells that were treated with shPlatr10-1 shRNA.

Figure S11. Specific interaction of *Platr10* with multiple stemness genes.

The RAT-seq bam data were converted to coverage and uploaded to the UCSC browser. The IGV plot shows the specific binding of *Platr10* lncRNA to *Oct4* (A), *Sox2* (B), *Klf4* (C), and *c-Myc* (D). RAT-CT: RAT-seq control, in which the RAT library was constructed using two random oligonucleotides, instead of specific antisense oligonucleotides from *Platr10*. *Palr35*: the RAT-seq lncRNA control. LncRNA *Palr35* is also differentially expressed in correlation with reprogramming. Like *Platr10*, it is silenced in fibroblasts and becomes activated in iPSCs. However, unlike *Platr10*, it does not bind to the stemness gene loci (top panel). These data suggest that the binding of *Platr10* to the stemness gene loci is lncRNA-specific.

Figure S12. Confirmation of the *Platr10-Oct4* interaction by RAT and ChIRP assays.

A. *Platr10-Oct4* interaction by RAT-qPCR. The RAT samples were used to quantitate the binding intensity of *Platr10* lncRNA to the *Oct4* promoter. The results were normalized to the value of the RAT control that was constructed using random oligo primers. 5'-CT: the 5'-upstream control site; pOct4: Oct4 promoter; shPlatr10-1, 2: two shRNAs used to knockdown *Platr10*; shCT: random shRNA control; *Palr35*: control RAT using primers from lncRNA *Palr35* that is differentially expressed in reprogramming, but it is not in the *Oct4* CRIST-seq list. ** $p < 0.01$ as compared with shRNA-treated groups and control lncRNA group.

B. Validation of the *Platr10-Oct4* interaction by the ChIRP assay (Chromatin Isolation by RNA Purification). After cell lysis and sonication, the *Platr10* chromatin complex was hybridized to biotinylated oligonucleotide probes and was pulled down with streptavidin beads for quantitation of the *Platr10*-interacting target signal. Random: the ChIRP control using random biotinylated oligonucleotide DNA probes; *Platr10*: *Platr10* biotinylated oligonucleotide DNA probes. pOct4: the *Oct4* promoter; 5'-CT: the 5' upstream control region of *Oct4*. For comparison, the Random control group was set as 1. ** $p<0.01$ as compared with the 5'-CT site.

Figure S13. Confirmation of the *Oct4* 3C loop products by DNA sequencing.

The *Oct4* intrachromosomal loop was examined by 3C PCR. The 3C products were sequenced. Blue line on the top of the sequence: the 3C ligation product between the

ligated BamH1 and Bgl2 sites.

Figure S14. Confirmation of the TET1-*Platr10* interaction by CLIP and RNA pulldown MS sequencing.

A. *Platr10* RNA pulldown MS sequencing. The biotin-labeled *Platr10* sense lncRNA was used to pull down its binding proteins in E14 cells. The *Platr10* antisense RNA was used as the control. MS fragment sequences were analyzed using PEAKS 7 with a false discovery rate (FDR) threshold <5%. *Platr10*-bindnig proteins were calculated as the protein enrichment ratio (the PEAKS score, −10logP) after adjusting over that of the antisense control.
B. The TET1 and *Platr10* interaction by CLIP. E14 cells were UV cross-linked in a CL-1000 UVP UV cross-linker oven and were lysed with the lysis buffer. After sonication, immunoprecipitation was performed with anti-TET1 antibody. The precipitated RNAs were used for qPCR to determine the TET1-*Platr10* interaction. IgG: the CLIP control. For comparison, the IgG group was set as 1. ** $p<0.01$ as compared with control groups.

Figure S15. Confirmation of the TET1-*Oct4* interaction by ChIP.

A. Location of ChIP primers. pOct4: the *Oct4* promoter; 5'-CT: the 5'- control region.
B. TET1-Oct4 interaction by ChIP. The ChIP signal was adjusted over the IgG group and set the 5'-CT as 1. ** $p < 0.01$ as compared with shPlatr10-treated groups.

Figure S16. Mutation of *Platr10* OBE and TBE elements.

A. Sequences and locations of OBE and TBE in *Platr10* lncRNA. E1-E4; *Platr10* exons; OBE50: 50 bp Oct4 binding element; TBE58: consensus TET1 binding element.

B. *Platr10* mutation constructs. F947: the full length *Platr10* lncRNA; OBE50D: *Platr10* lncRNA that lacks the 50 bp Oct4 binding element; TBE58D: *Platr10* lncRNA that lacks the 58 bp TET1 binding element; TBE58M: *Platr10* lncRNA that has the replacement of 58 bp TET1 binding element with a random 58 bp DNA sequence.

C. *Oct4* promoter-luciferase assay. The *Oct4* 3.9K promoter-luciferase vector was co-transfected into 293T cells with the vectors that carry the full length *Platr10* (1041bp), OBE50D that lacks the 50 bp *Oct4* binding element (OBE), TBE58D that lacks the 58 bp consensus TET1 binding element (TBE), and TBE58M that contains a mutant TBE. Forty-eight hours after co-transfection, cells were collected for the measurement of luciferase activity. The data are the mean ± SD from three independent experiments. * $p < 0.01$ as compared with the Vector control; ** $p < 0.01$ as compared with the full length *Platr10*. Deletion of the 50 bp *Oct4* binding element abolishes the function of *Platr10* in the activation of the *Oct4* promoter-luciferase reporter vector.

Figure S17. *Platr10* partially corrects LIF withdrawal-induced defects in E14 cells.

A. Diagram of the LIF-withdrawal model. E14 cells were treated three times with lentiviruses carrying *Platr10* and its mutants. After puromycin selection, cells

were reseeded at very low density in DMEM medium that lacks LIF. Stem cell spheroid formation and expression of pluripotent marker *Nanog* were measured.

B. Formation of stem cell spheroids. Images were taken 5 days after LIF withdrawal. After LIF withdrawal, E14 cells at low density failed to thrive to form stem cell spheres. However, the wild type *Platr10* but not its mutants was able to partially correct this defect.

C. Expression of pluripotent marker gene *Nanog*. Cells were collected for qPCR quantitation of *Nanog*. The wild type *Platr10* reduced the loss of *Nanog* induced by LIF withdrawal. ** $p < 0.01$ as compared with LIF- and mutant groups.

Figure S18. *Platr10* regulates DNA demethylation in the *Oct4* promoter

A. CpG islands in the *Oct4* promoter. 5'-Enh: the 5'-enhancer; pOct4: *Oct4* promoter; E1-E5: *Oct4* exons; CpGs: CG dinucleotides.

B. Knockdown of *Platr10* induces CpG demethylation in the *Oct4* promoter. shCT: random shRNA control; Vector: empty vector with shRNA; shPlatr10-1, -2: shRNAs targeting *Platr10*. Solid circle: methylated CpG. After shRNA knockdown, E14 cells were collected and genomic DNAs were extracted and treated by sodium bisulfite. The *Oct4* promoter DNAs were amplified with *Oct4*-specific primers and cloned into the pJet vector for sequencing. Each line represents sequencing from one clone.

C. *Platr10* partially corrects the altered epigenotype induced by LIF-withdrawal. E14 cells were transfected with *Platr10* and cultured at very low density in the LIF^- medium to induce differentiation. Sodium bisulfite sequencing was used to compare the status of DNA methylation in the *Oct4* promoter. After LIF withdrawal, the *Oct4* promoter became methylated. Overexpression of *Platr10*,

but not its mutants, partially corrected the LIF withdrawal-induced defect. F1041: full length *Platr10*; D58: the 3'-end deleted *Platr10*; M58: the 3'-end mutated *Platr10*; Vector: lentiviral expression vector control.

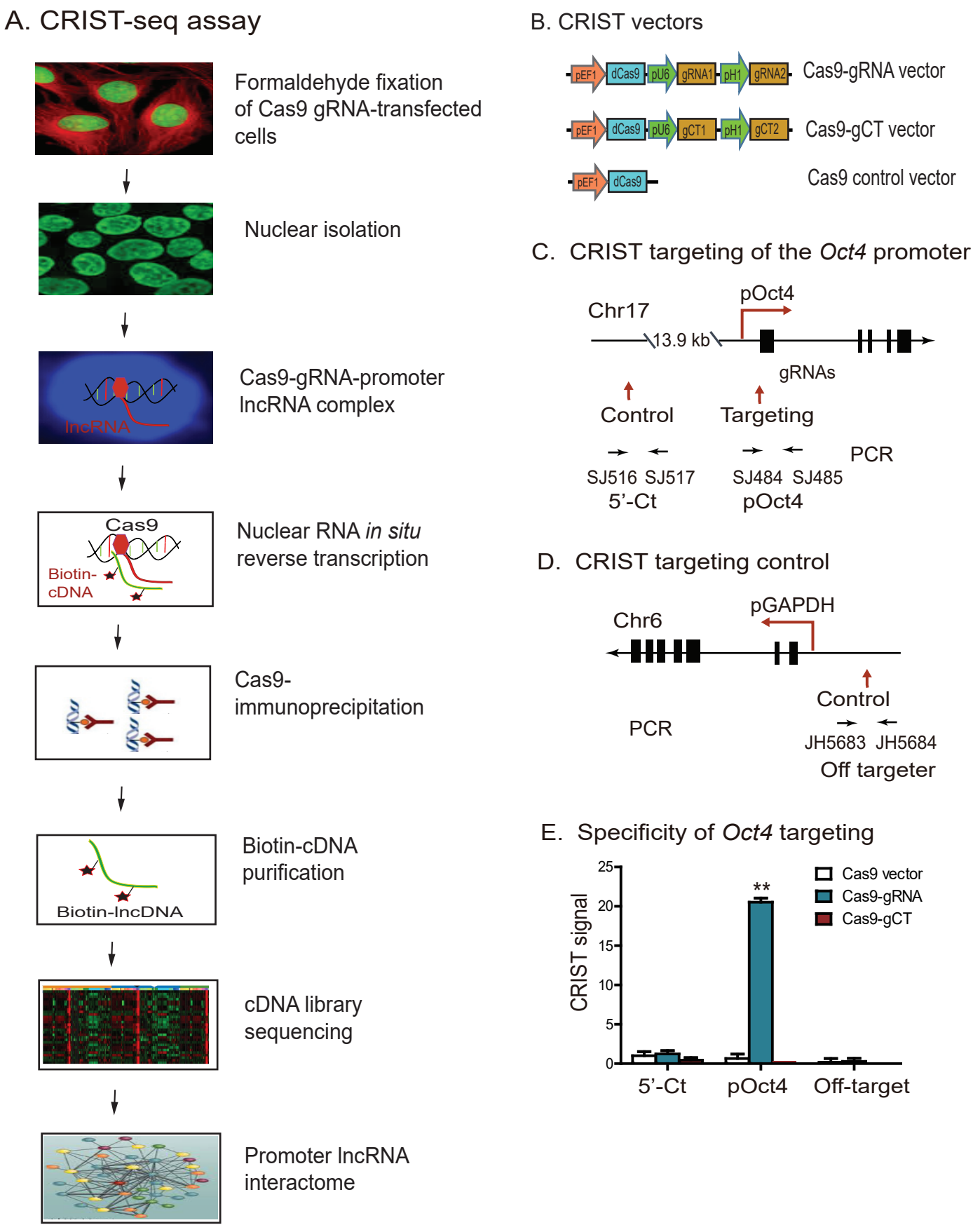
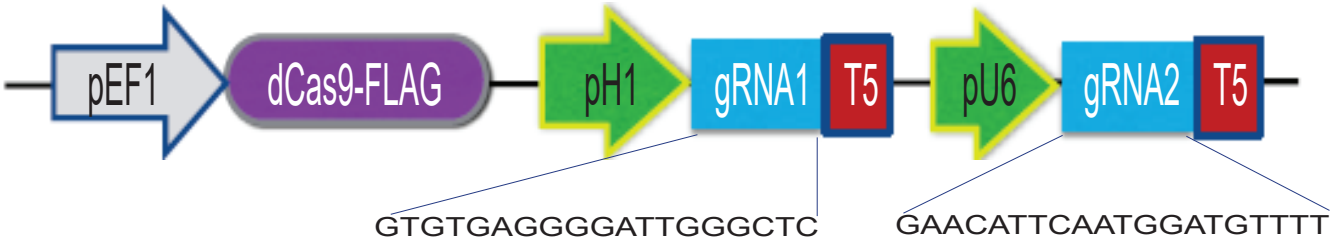
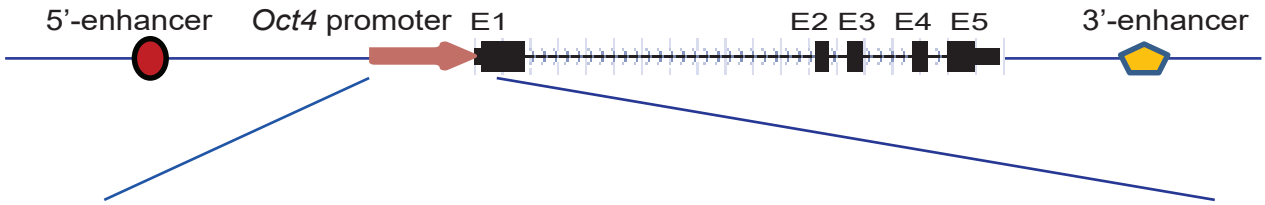


Figure S1. CRIST-Seq to map the *Oct4* promoter RNA interactome

A. CRIST-seq vector



B. Location of CRIST Cas9 gRNAs

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43021 ctttgaggag aggtggagag ctggggaagt cttGTGTGAG GGGATTGGGG CTCAGGaggg  gRNA1-PAM
43081 ggttggggag caggaagttg tccccagggg agccatcctg gcccattcaa gggttgagta  TF binding
43141 cttgtttagg gttagagctg ccccctctgg ggaccaggat tgtccagcca aggccattgt  site
43201 cctgcccccc tcccccagtc cctcccaggc ccctttgaac ctgaagtcag atatttcttc
43261 tctctaccca cctcccaccc gttgggtttc tccacccagg aactaggctg gaagcctggg
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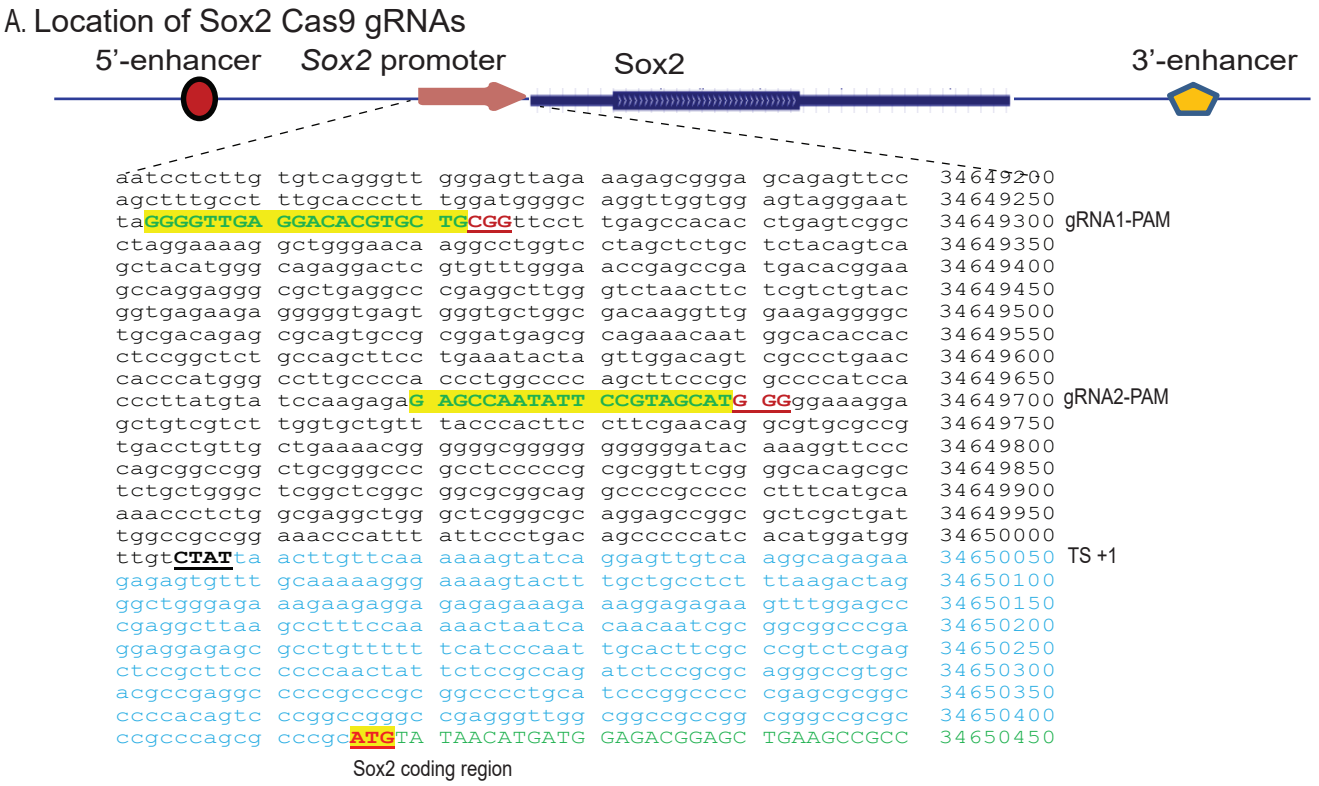
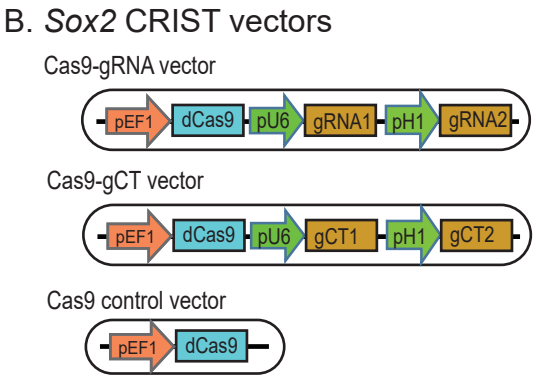
43621 tactgggttc acccatatct agaactctag acggtgggt aagcaagaac tgaggagtgg
43681 ccccagaaat aattggcaca tGAACATTCA ATGGATGTTT TAGGctctcc agaggatggc  gRNA2-PAM
43741 tgagtgggct gtaaggacag gccgagaggg tgcagtgcca agaggccttg tggtgcgatg
43801 gggcatccga gcaactggtt tgtgaggtgt ccggtgaccc aaggcagggg tgagaggacc
43861 ttgagggttg aaaatgaagg cctcctgggg tccgtcctа agggttgtcc tgtccagacg
43921 tccccaacct ccgtctggaa gacacaggca gatagcgctc gcctcagttt ctcccacccc
43981 cacagctctg ctcctccacc cacccagggg gcgggccag aggtcaaggc tagagggtgg
44041 gattggggag ggagaggtga AACCGTCCCT AGGTGAGCCG TCTTTCCACC AGGCCCCGGG  TS +1
44101 CTCGGGGTGC CCACCTTCCC CATGGCTGGA CACCTGGCTT CAGACTTCGC CTTCTCACCC
```

Oct4 coding region

Figure S2. Location and sequnce of *Oct4* Cas9 gRNAs





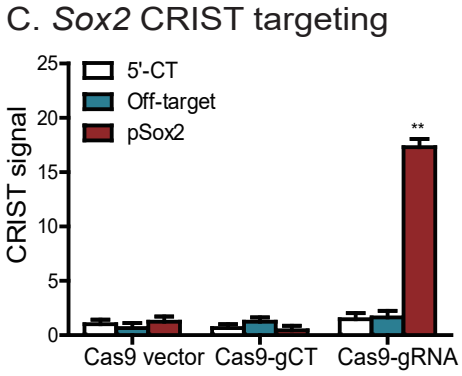
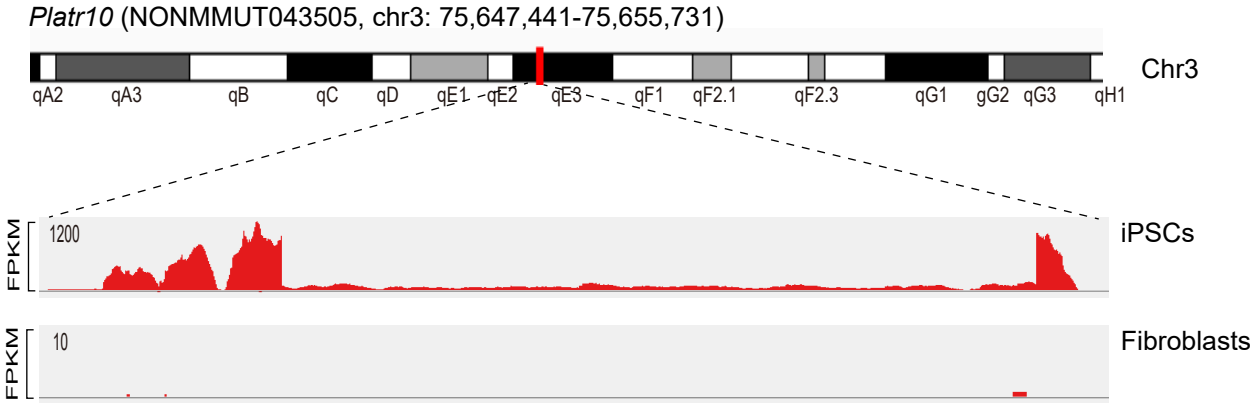


Figure S3. *Sox2* CRIST-seq targeting

A. RNA-Seq data



B. Location of *Platr10* exons

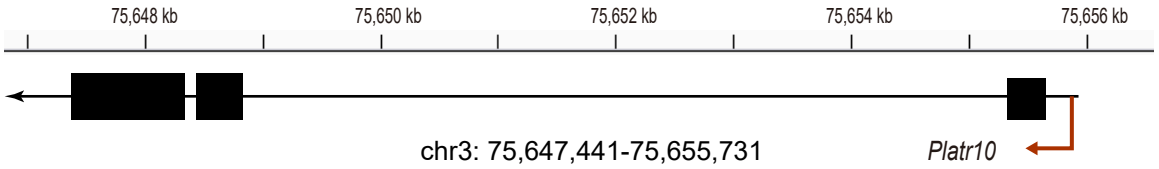
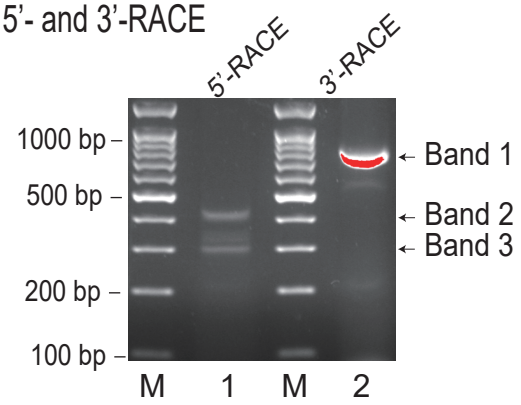
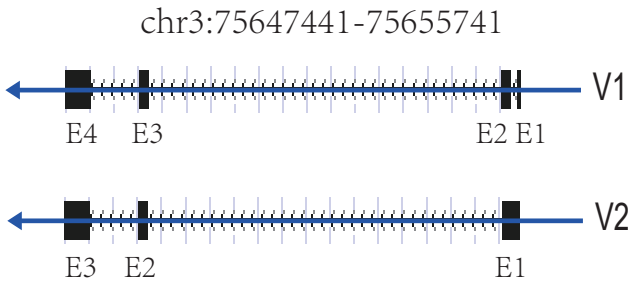


Figure S4. *Platr10* RNA-seq IGV data

A. 5'- and 3'-RACE



B. LncRNA (NONMMUT043505)



C. NONMMUT043505 variant 1

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D. NONMMUT043505 variant 2

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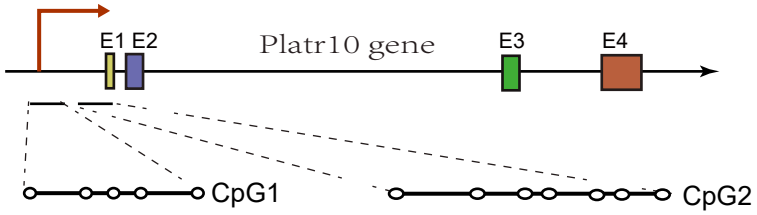
Figure S5. Alternative splicing of lncRNA NONMMUT043505 (*Platr10*)

A. CpG islands in the *Platr10* promoter



B. CpG demethylation in reprogramming

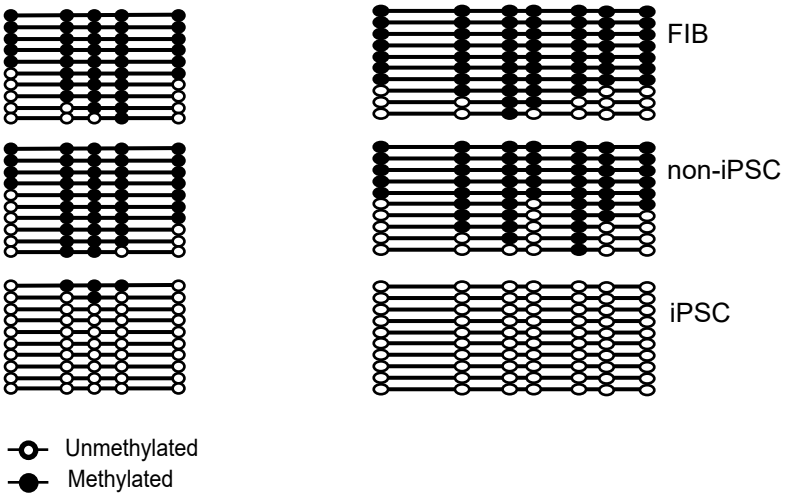
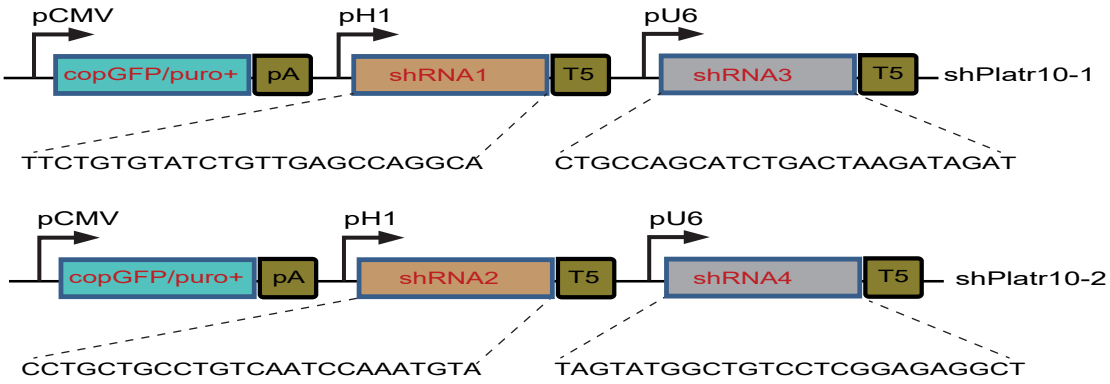
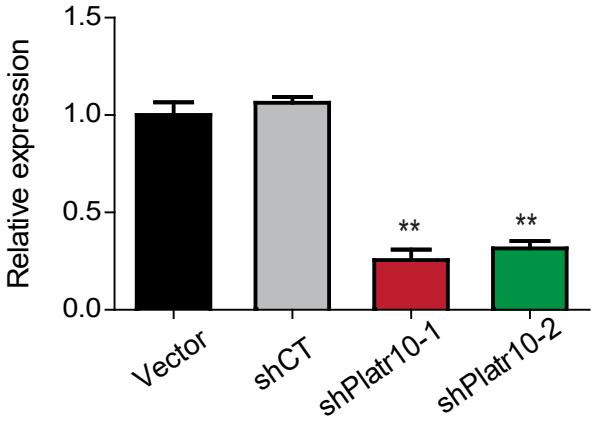


Figure S6. *Platr10* promoter DNA methylation

A. *Platr10* knockdown vectors



B. *Platr10* knockdown



C. *Platr10* promotes reprogramming

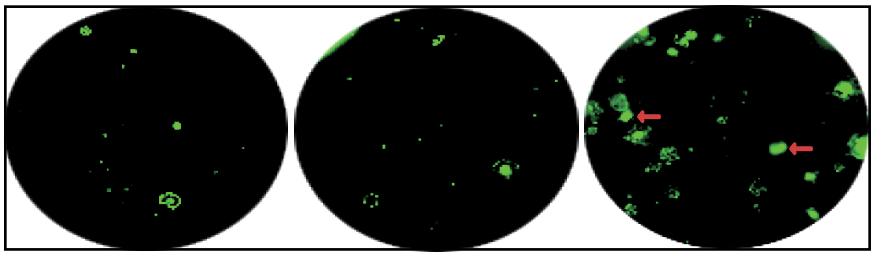
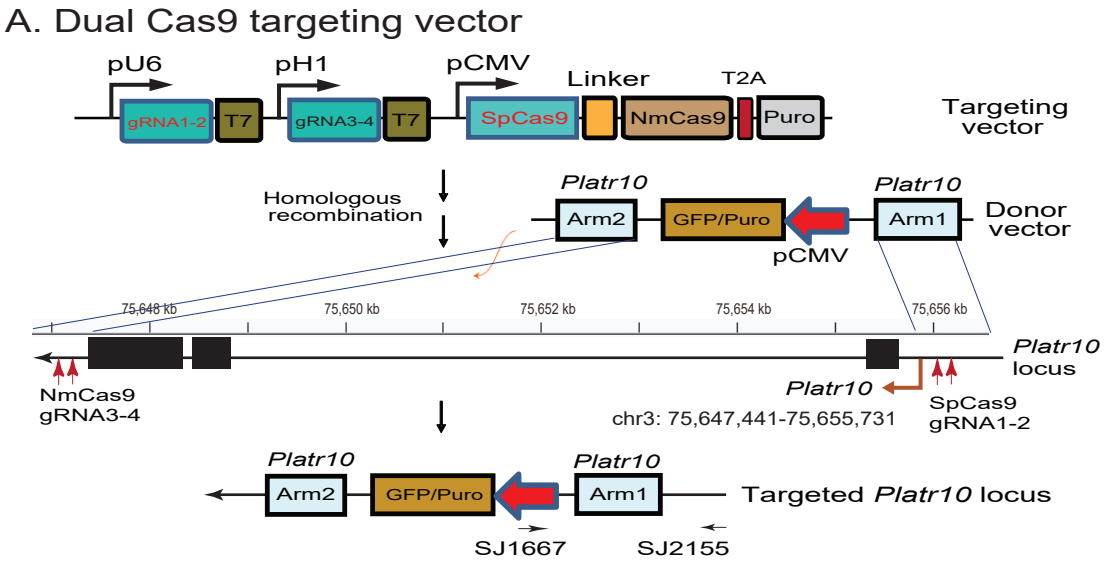
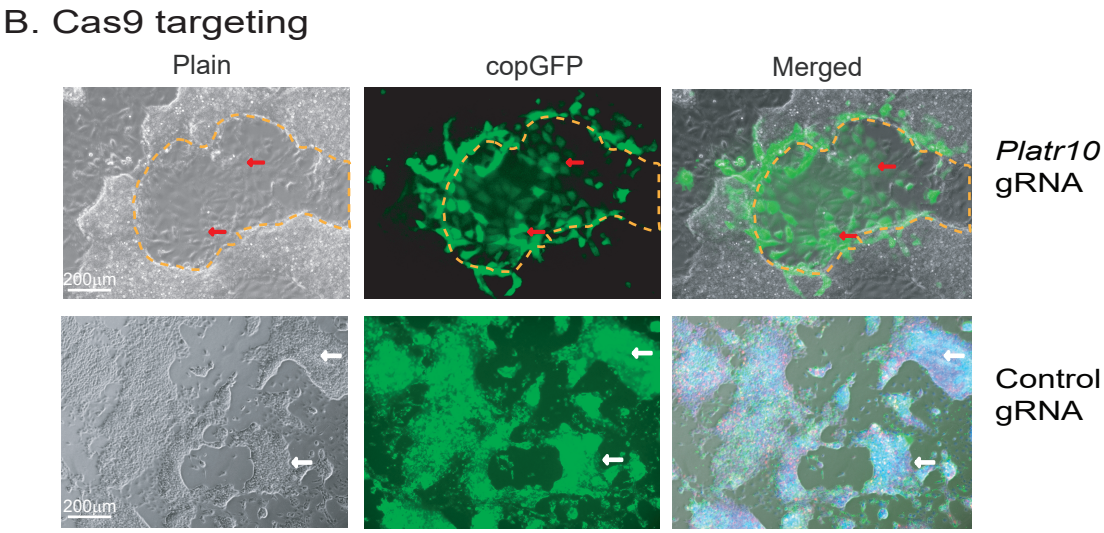


Figure S7. Functional studies of *Platr10*





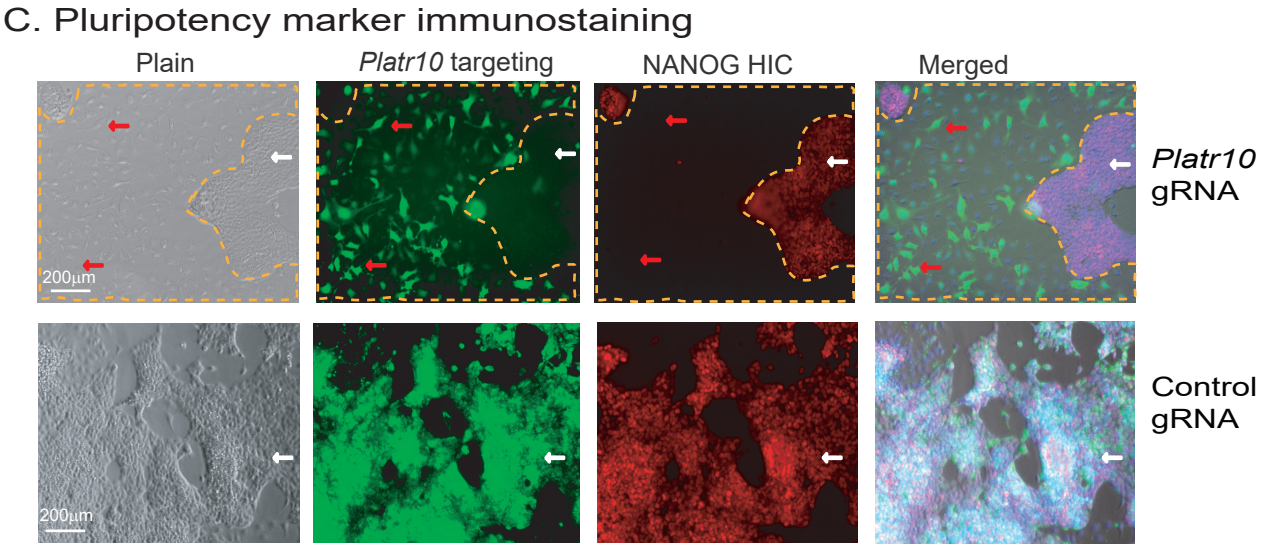
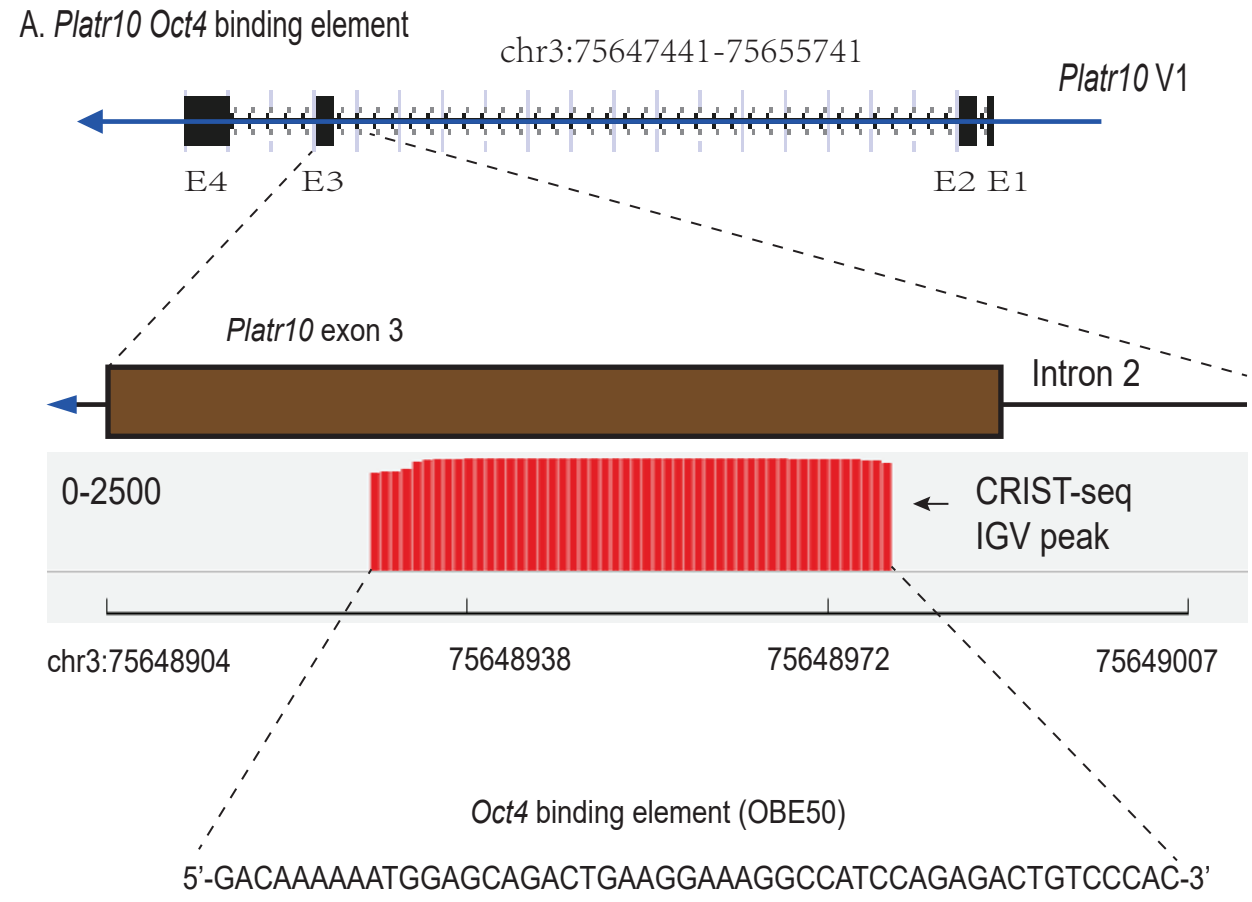


Figure S8. Dual Cas9 targeting of *Platr10* in E14 cells



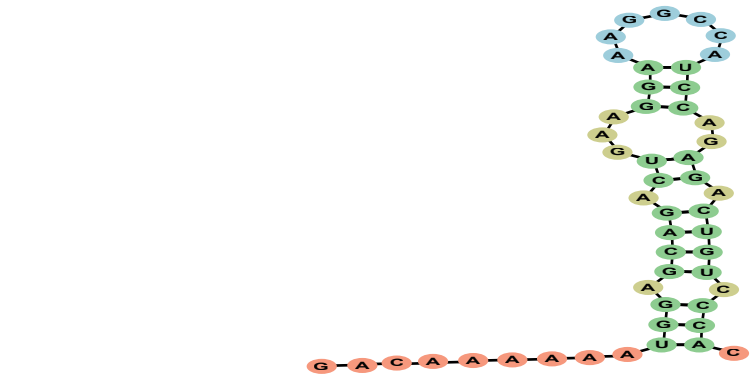


Figure S9. *Platr10 Oct4* binding element (OBE50) by CRIST-seq IGV

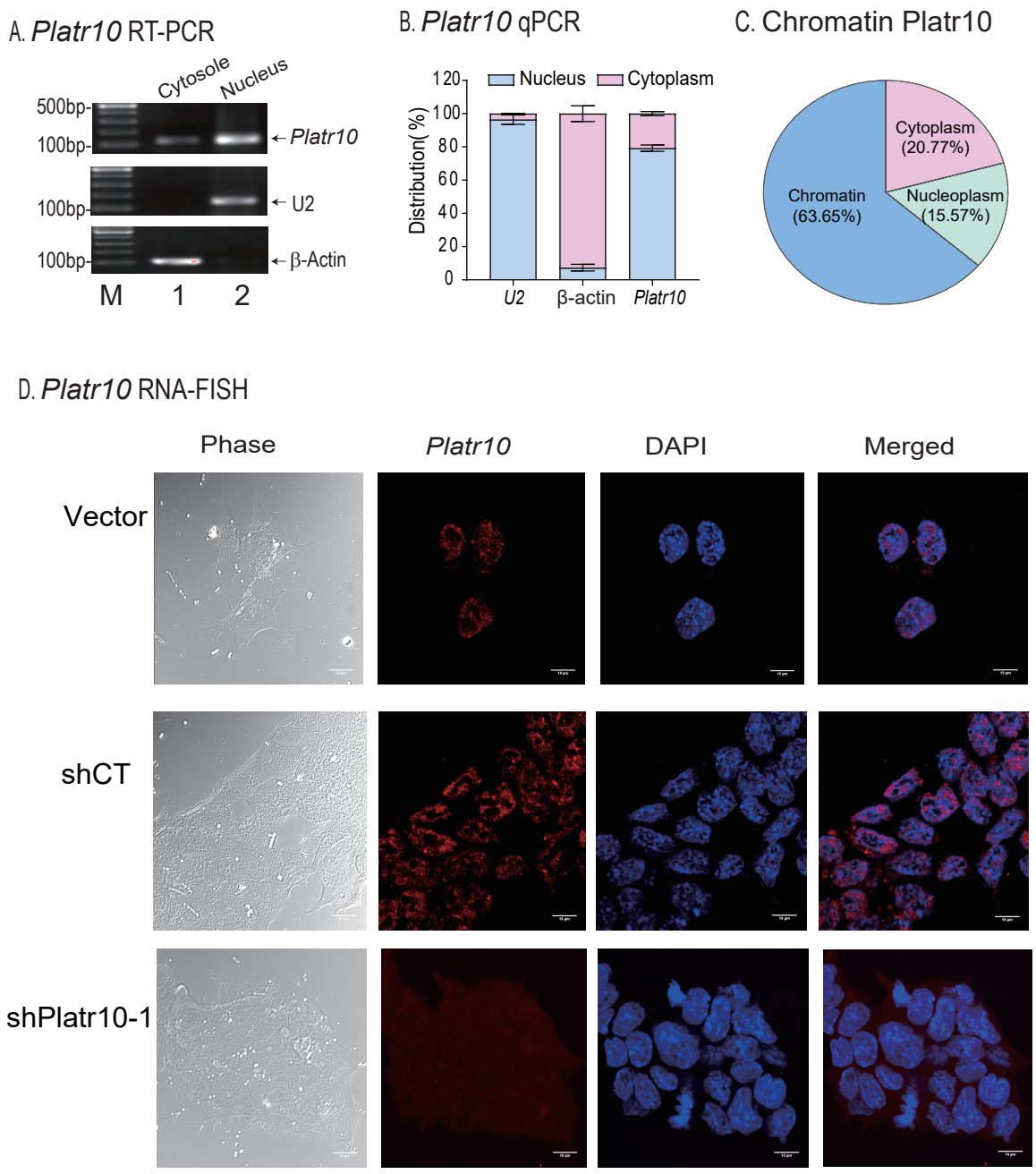


Figure S10. Nuclear localization of *Platr10*

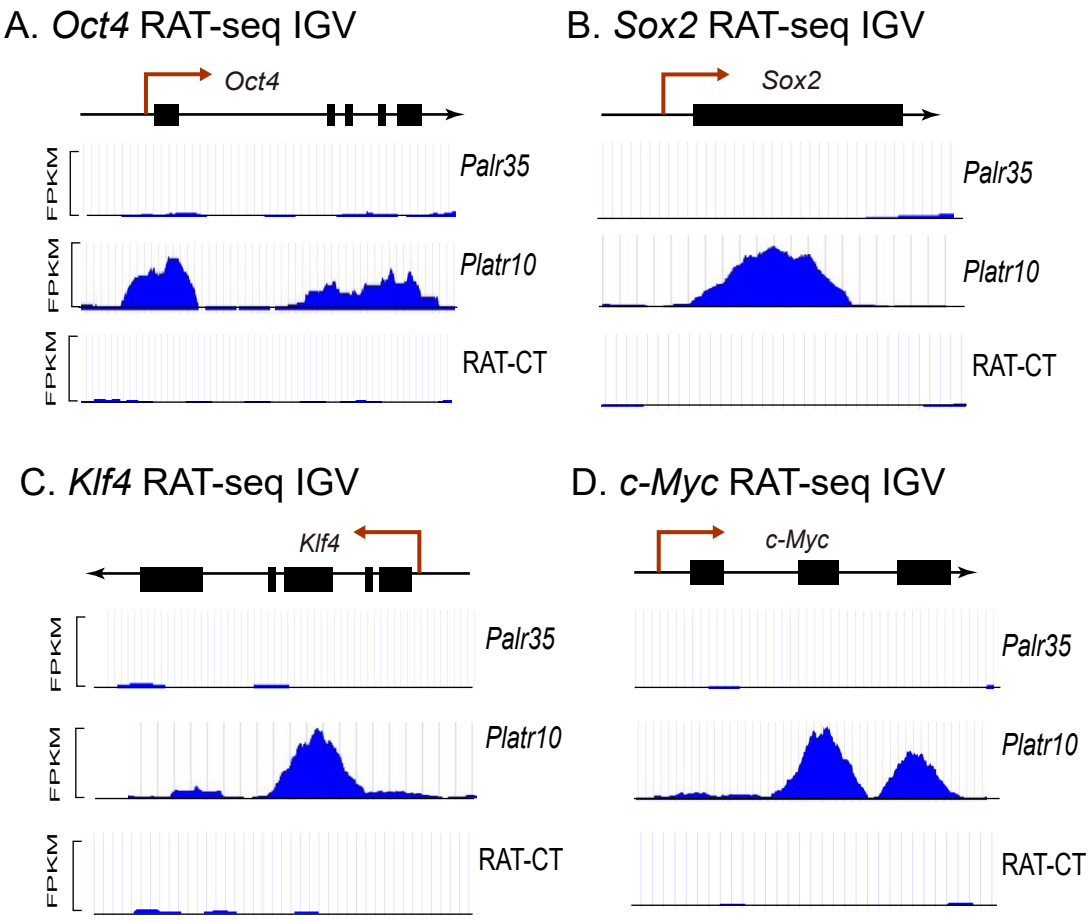


Figure S11. Specfiic binding of *Platr10* to stemness genes

A. *Platr10-Oct4* binding by RAT B. *Platr10-Oct4* binding by ChIRP

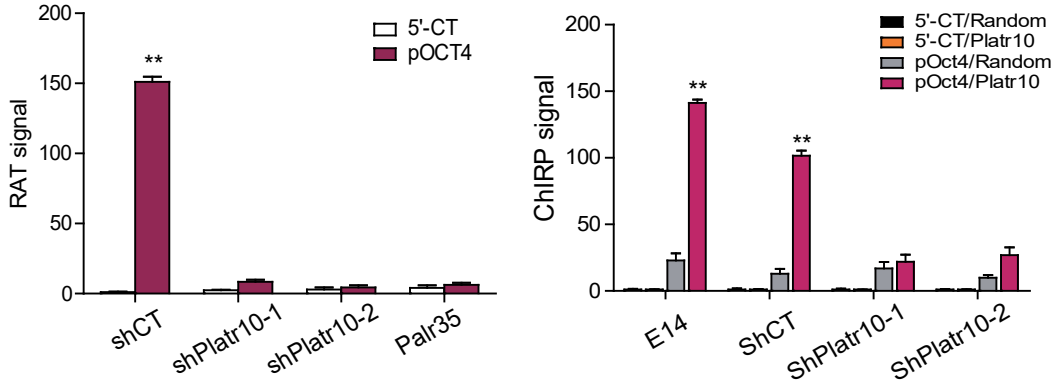


Figure S12. The *Platr10-Oct4* interaction

A. *Oct4* 3C sequencing

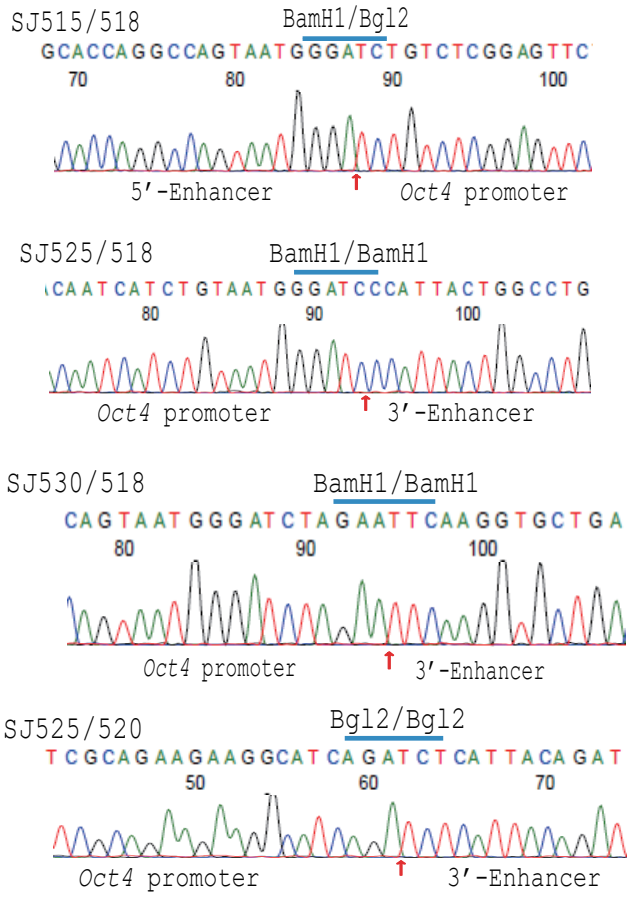


Figure S13. The 3C loop at the *Oct4* locus by DNA sequencing

A. *Platr10* RNA pulldown-MS protein targets

GO Biological pathway	Proteins	Gene name	-10lgP
DNA methylation and demethylation (5)			
	Methylcytosine dioxygenase TET1	Tet1	220.6
	Histone-lysine N-methyltransferase EZH2	Ezh2	162.3
	Transcriptional repressor CTCF	Ctcf	170.0
	Methylcytosine dioxygenase TET2	Tet2	160.1
	DNA (cytosine-5)-methyltransferase 1	Dnmt1	231.5
Stem cell population maintenance (3)			
	Catenin beta-1	Ctnnb1	131.2
	Transcription factor SOX-2	Sox2	111.6
	NF-kappa-B-activating protein	Nkap	93.2
Covalent chromatin modification (7)			
	Actin-like protein 6A	Actl6a	122.9
	Histone acetyltransferase KAT6B	Kat6b	114.5
	Bromodomain-containing protein 3	Brd3	98.4
	Histone acetyltransferase KAT7	Kat7	92.1
	Lysine-specific demethylase 3B	Kdm3b	68.9
	Histone chaperone ASF1A	Asf1a	61.3
	Histone acetyltransferase KAT8	Kat8	34.8
Chromatin remodeling and assembly (6)			
	Actin-like protein 6A	Actl6a	122.9
	AT-rich interactive domain-containing protein 1B	Arid1b	96.6
	Death domain-associated protein 6	Daxx	89.5
	Chromatin-remodeling ATPase INO80	Ino80	77.9
	Actin-related protein 8	Actr8	50.1
	Cellular tumor antigen p53	Tp53	47.2
Sister chromatid cohesion (3)			
	Sister chromatid cohesion protein PDS5 homolog A	Pds5a	192.7
	Structural maintenance of chromosomes protein 1A	Smc1a	176.9
	Cohesin subunit SA-2	Stag2	56.4

B. TET1-*Platr10* interaction by CLIP

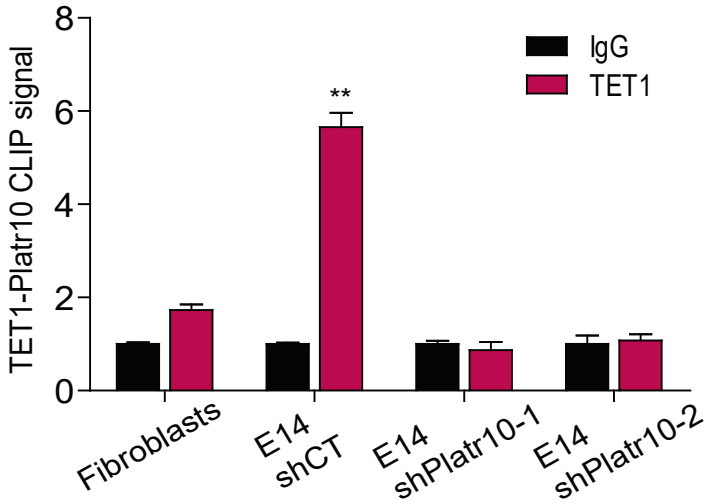


Figure S14. *Platr10*-TET1 interaction by CLIP and RNA pulldown-MS sequencing

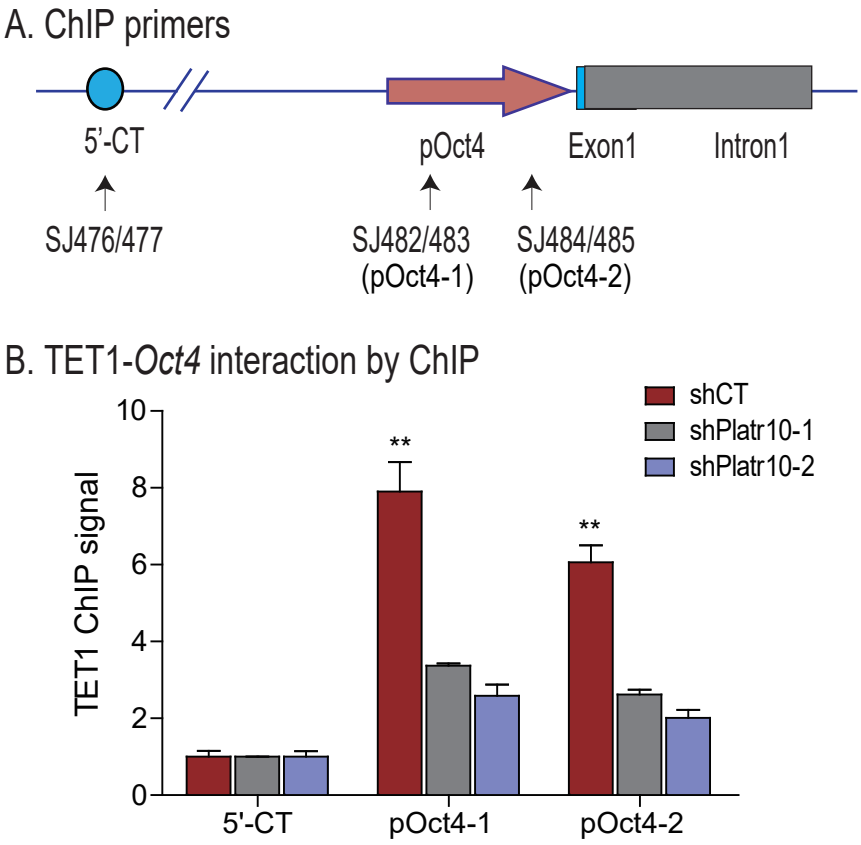
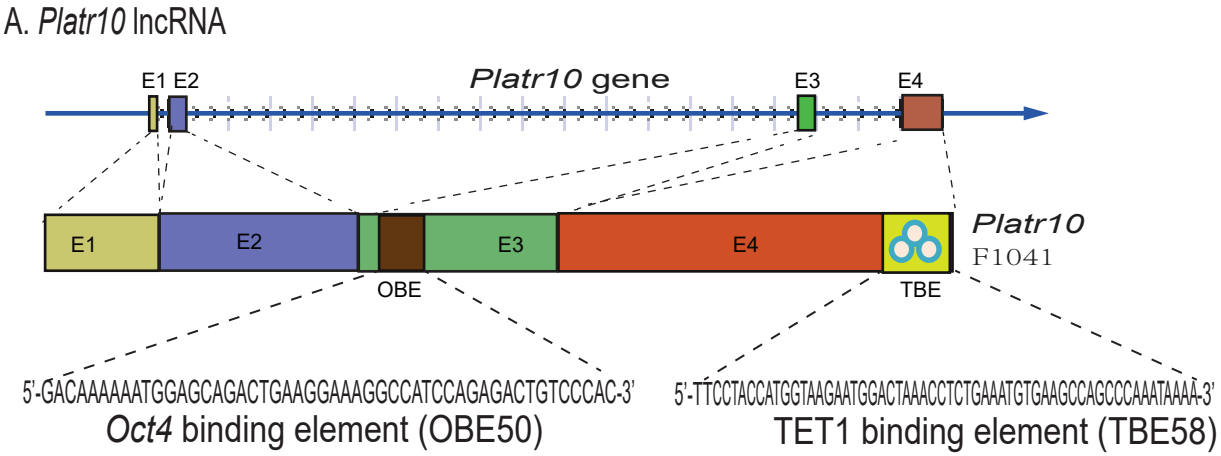
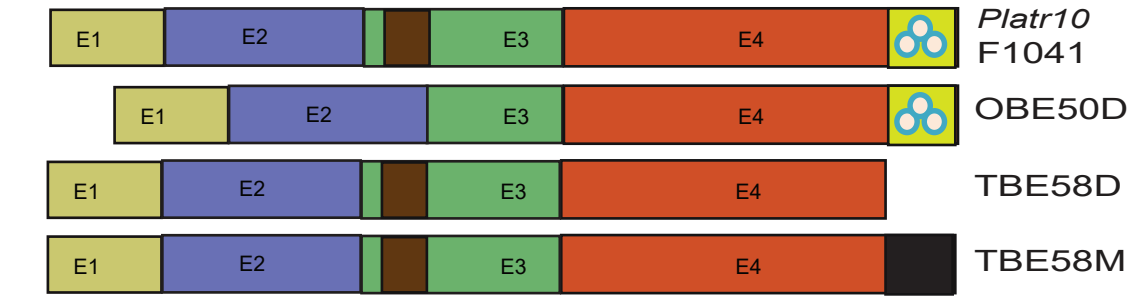


Figure S15. Interaction of TET1 with the *Oct4* promoter.





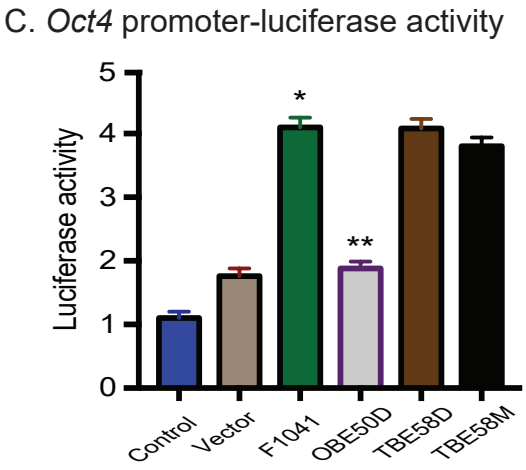


Figure S16. Mutation of *Platr10-OcT4* binding element and TET1 binding element

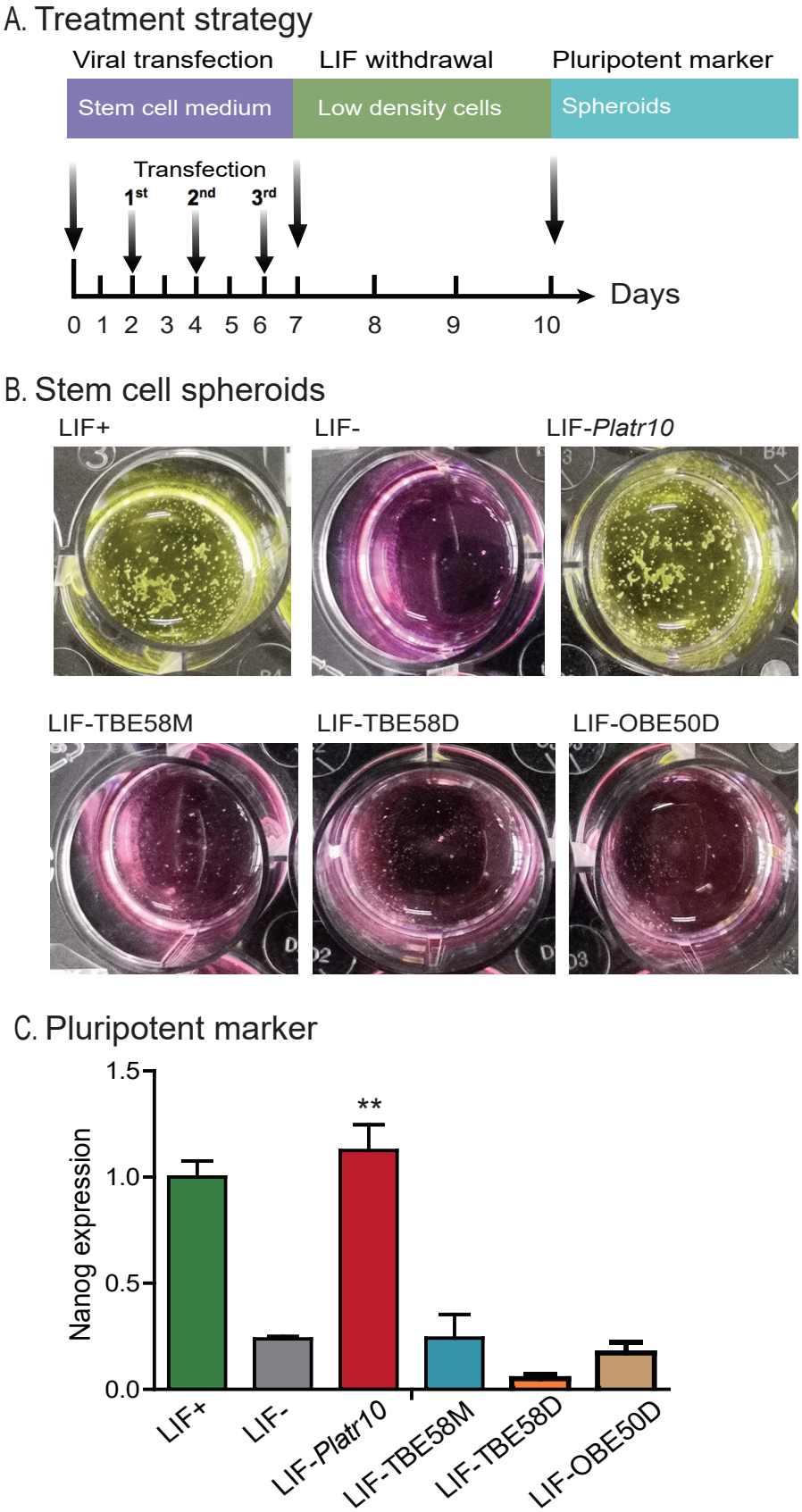


Figure S17. *Platr10* partially corrects the LIF withdrawal-induced defects

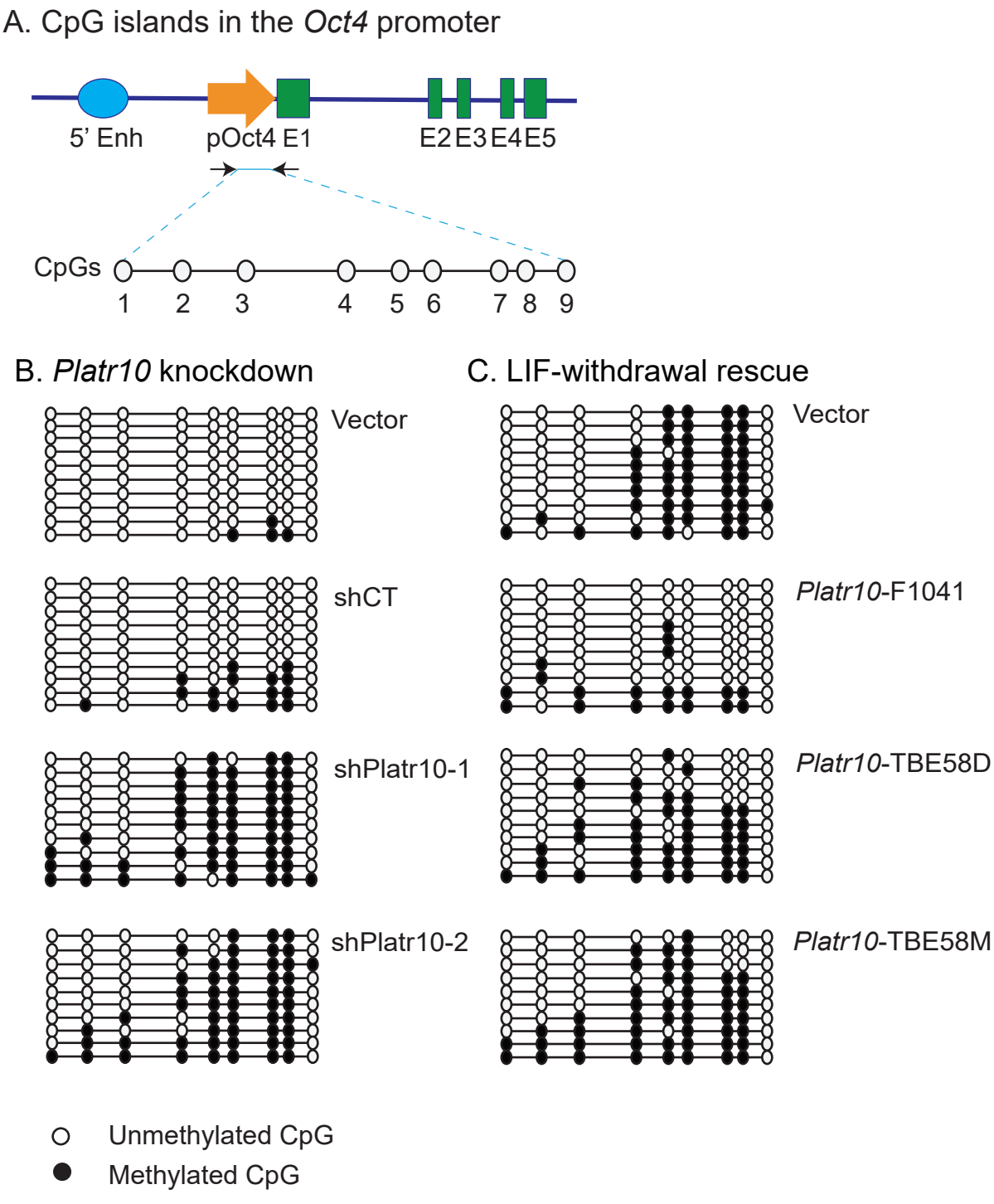


Figure S18. The role of *Platr10* and its mutants in *Oct4* DNA methylation