

Supplementary Data

Tfcp2l1 Induces Distinct Target Gene Expression to Promote Self-renewal of Mouse and Human Embryonic Stem Cells

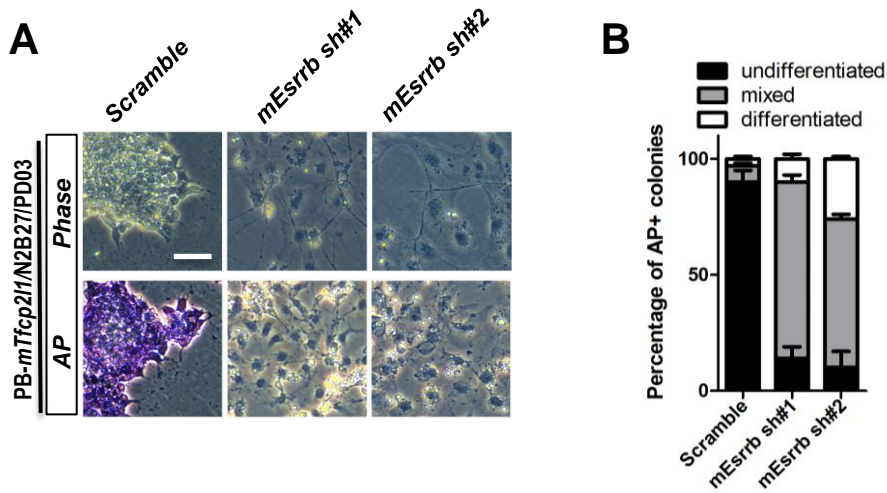


Figure S1. Knockdown of *Esrrb* impairs the function of *Tfcp2l1*

(A) Phase-contrast images and AP staining of the scramble control and *mEsrrb* shRNA mESCs overexpressing PB-*mTfcp2l1* and cultured in N2B27/PD0325901 (PD03) after three passages. Bar, 100 μ m.

(B) Quantification of AP positive colonies in Figure S1A. The data are presented as the mean \pm S.D. of three biological replicates.

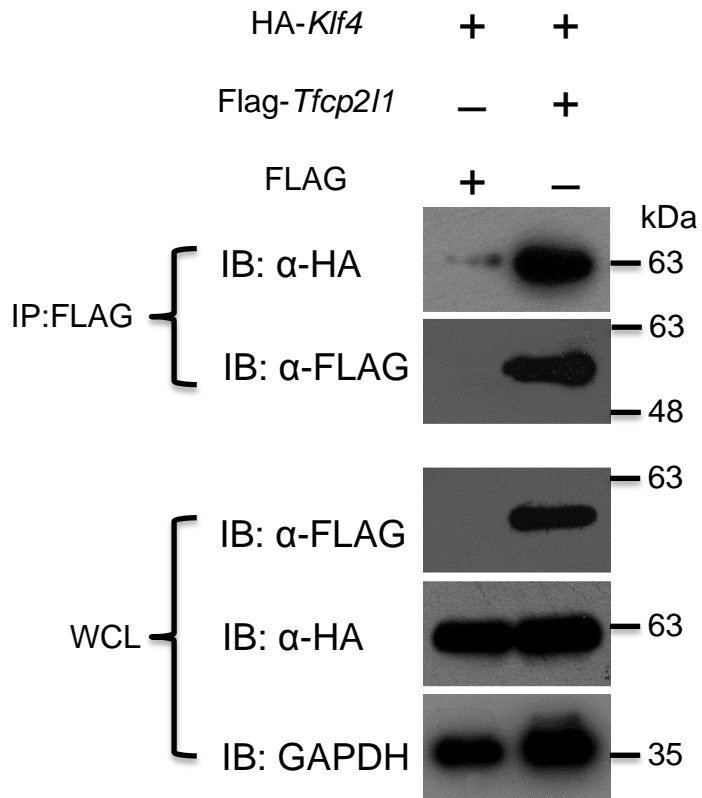


Figure S2. TFCP2L1 interacts with KLF4 in hESCs.

FLAG-tagged *Tfcp2l1* and HA-tagged *Klf4* were co-overexpressed in hESCs. Co-IP of the transfected HA *KLF4* protein using anti-FLAG affinity gel. WCL: whole cell lysate.

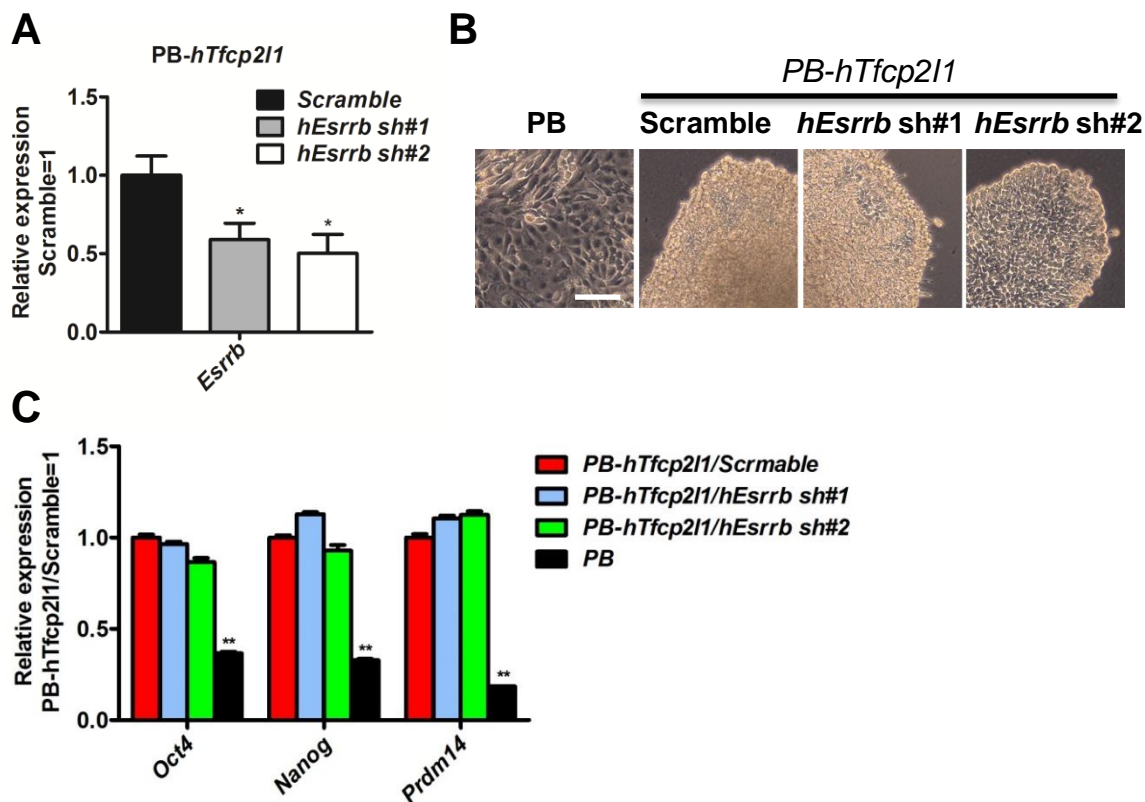


Figure S3. Knockdown of *Esrrb* can not impair the self-renewal-promoting effects of human *Tfcp2l1* in HES2 hESCs

- (A) qRT-PCR analysis of *Esrrb* expression in PB-*hTfcp2l1* hESCs infected with the scramble or human *Esrrb* shRNA lentivirus (*hEsrrb* sh#1 and *hEsrrb* sh#2). The data are presented as the mean \pm S.D. of three biological replicates. * $p < 0.05$, ** $p < 0.01$ vs Scramble.
- (B) Phase-contrast images of PB-*hTfcp2l1* mESCs infected with scramble or *hEsrrb* shRNA lentivirus and cultured in basal medium for two passages. Bar, 100 μ m.
- (C) qRT-PCR analysis of the pluripotency genes *Oct4*, *Nanog* and *Prdm14* in the indicated cells cultured in basal medium for two passages. The data are presented as the mean \pm S.D. of three biological replicates. ** $p < 0.01$ vs PB-*hTfcp2l1*/Scramble.

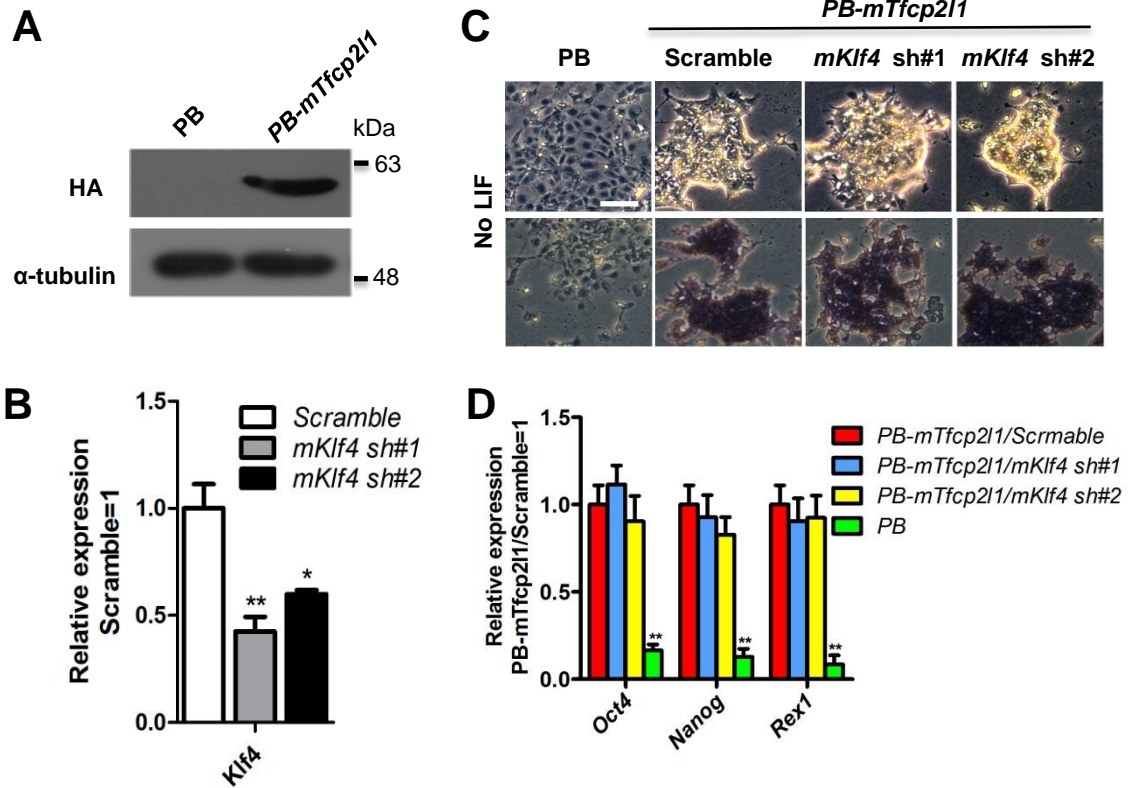


Figure S4. Knockdown of *Klf4* can not impair the self-renewal-promoting effects of mouse *Tfcp2l1* in 46C mESCs

- (A) Western blot analysis of the FLAG protein in Flag-tagged mouse *Tfcp2l1* (PB-*mTfcp2l1*) mESCs. α -tubulin was used as a loading control.
- (B) qRT-PCR analysis of *Klf4* expression in PB-*mTfcp2l1* mESCs infected with scramble or mouse *Klf4* shRNA lentivirus (*mKlf4* sh#1 and *mKlf4* sh#2). The data are presented as the mean \pm S.D. of three biological replicates. *p < 0.05, **p < 0.01 vs Scramble.
- (C) Phase-contrast images and AP staining of PB-*mTfcp2l1* mESCs cultured in infected with scramble or *mKlf4* shRNA lentivirus and cultured in basal media for five passages. Bar, 100 μ m.
- (D) qRT-PCR analysis of the pluripotency genes *Oct4*, *Nanog* and *Rex1* in PB-*mTfcp2l1* mESCs infected with scramble or *mKlf4* shRNA lentivirus and cultured in basal medium condition for five passages. The data are presented as the mean \pm S.D. of three biological replicates. **p < 0.01 vs PB-*mTfcp2l1*/Scramble.

Table S1. List of shRNA sequence used for gene knockdown

Symbol (Mouse)	shRNA sequence (5'-3')
<i>Klf4-sh#1</i>	GCGCTACAATCATGGTCAAGT
<i>Klf4-sh#2</i>	GTCAGCTTGTGAATGGATAAT
<i>Tfcp2l1-sh#1</i>	ACCTTAACATACCTCAATCAA
<i>Tfcp2l1-sh#2</i>	CGGCTCAAGAGAAGGAGAAAT
<i>Tfcp2l1-sh#3</i>	CCCGCCTTTGAATCACTAATT
<i>Esrrb-sh#1</i>	CGATTCATGAAATGCCTCAA
<i>Esrrb-sh#2</i>	GCCGAGGACTATATCATGGAT

Symbol (Human)	shRNA sequence (5'-3')
<i>Klf4-sh#1</i>	GCCAGAATTGGACCCGGTGTA
<i>Klf4-sh#2</i>	GCCTTACACATGAAGAGGCAT
<i>Esrrb-sh#1</i>	GCACAAACTCTTCCTGGAGAT
<i>Esrrb-sh#2</i>	GCTGAGGACTACATCATGGAT

Table S2. List of primers used for qRT-PCR analysis

Symbol (Mouse)	Forward sequence (5'-3')	Reverse sequence (5'-3')
<i>Tfcp2l1</i>	AGGTGCTGACCTCCTGAAGA	CAGGCTGTTATCCCCACTGT
<i>β-actin</i>	TATCGCTGCGCTGGTTCG	CCCACGATGGAGGGGAATAC
<i>Sall4</i>	CAGCCTTATGCCCTTGGATA	AGGGGTTGGAGGCATACTCT
<i>Esrrb</i>	ATGAATGTGAGATCACCAAACG	G TTCAGGTAGGGGCTGTTCTC
<i>Stella</i>	TTCCGAGCTAGCTTTTGAGG	ACACCGGGGTTTAGGGTTAG
<i>Fgf5</i>	GCAGCCCACGGGTCAA	CGGTTGCTCGGACTGCTT
<i>Rex1</i>	TCACTGTGCTGCCTCCAAGT	GGGCACTGATCCGCAAAC
<i>Nr0b1</i>	GGACCGTGCTCTTTAACCCA	TCCATGCTGACTGCACCAAT
<i>Nanog</i>	TACCTCAGCCTCCAGCAGA	CCTCCAAGTCACTGGCAG

Symbol (Human)	Forward sequence (5'-3')	Reverse sequence (5'-3')
<i>Gata4</i>	CCAATCTCGATATGTTTGACGA	TTGATGCCGTTTCATCTTGTG
<i>Gata6</i>	AACTTCCACCTCTTCTAACTCAG	CATCTTGACCCGAATACTTGAG
<i>Nanog</i>	CAAAGGCAAACAACCCACTT	TCTGCTGGAGGCTGAGGTAT
<i>Oct4</i>	GGGAAGGTATTCAGCCAAACG	GGTTCGCTTTCTCTTTTCGGG
<i>Foxa2</i>	GACAAGTGAGAGAGCAAGTG	ACAGTAGTGGAAACCGGAG
<i>Tfcp2l1</i>	CTGTCTGTGTACCACGCCAT	GTTCTGCACCATCTCGTTGC
<i>Klf4</i>	ACCTTCTTCACCCCTAGAGC	GGTCAGTTCATCTGAGCGGG
<i>Klf5</i>	GCTCACCTGAGGACTCACAC	CTTCATATGCAGGGCCAGGT
<i>β-actin</i>	GATGAGATTGGCTGGCTTT	CACCTTCACCGTTCCAGTTT

Table S3. List of Chip- qRT-PCR primers for assay different promoter regions of Esrrb

Location From UCSC	Forward sequence (5'-3')	Reverse sequence (5'-3')
-1450~-1201	TGGAGGTCTGTTGATAGGGC	TAGCCACTGTGGAGGGACTTA
-750~-501	CACCCTGCATCAAGCCACAT	GAGACAGATTCCCCTTCCCC

Table S4. List of Chip- qRT-PCR primers for assay different promoter regions of Klf4

Location From UCSC	Forward sequence (5'-3')	Reverse sequence (5'-3')
-2050~-1851	TTCCTAGGTTGACACCAGCC	GTTCGGCGCGGGACTC
-1700~-1050	TCCAGGGAAGTCCCTTTAG	TGGCCAGTAAAGGTCAAAGGA
-1450~-1251	TATTATTCTACAGTGCAATTTAGTC	CTCAAGTGATCAGCCTGC
-1250~-1051	TACTCGGGAGGCTGACGC	CGCTGTGTCGCCCAAGC
-820~-1052	TTGAGGCTCCCAGTTCACG	CCTTCTGCTGAGGGGTGG
-620~-428	CTCCAGCGCGCAGACA	CGAAGACTGGTGGGGTCAG
+1~+200	CGGCAGCCAGTCTCACCT	TCCCCACCACTGTCG

Table S5.The PCR primer sequences for amplifying the different promoter regions of Esrrb to construct promoter-luciferase reporter.

Location From USCS	Primer sequence for PCR (5'-3')
-1450~-1201	F:CGGGGTACCGTTTTGTTTTAATGATGCAGGTCTG
	R:CCCAAGCTTGCGGGTGCTGAGAGCCTCAG

Table S6.The PCR primer sequences for amplifying the different promoter regions of Klf4 to construct promoter-luciferase reporter.

Location From USCS	Primer sequence for PCR (5'-3')
-1250~-1051	F:CGGGGTACCGTCAGGTGTTTCGAGACCTGC
	R:CCCAAGCTTCGAGAAAGGGAGTGAAAGGGC