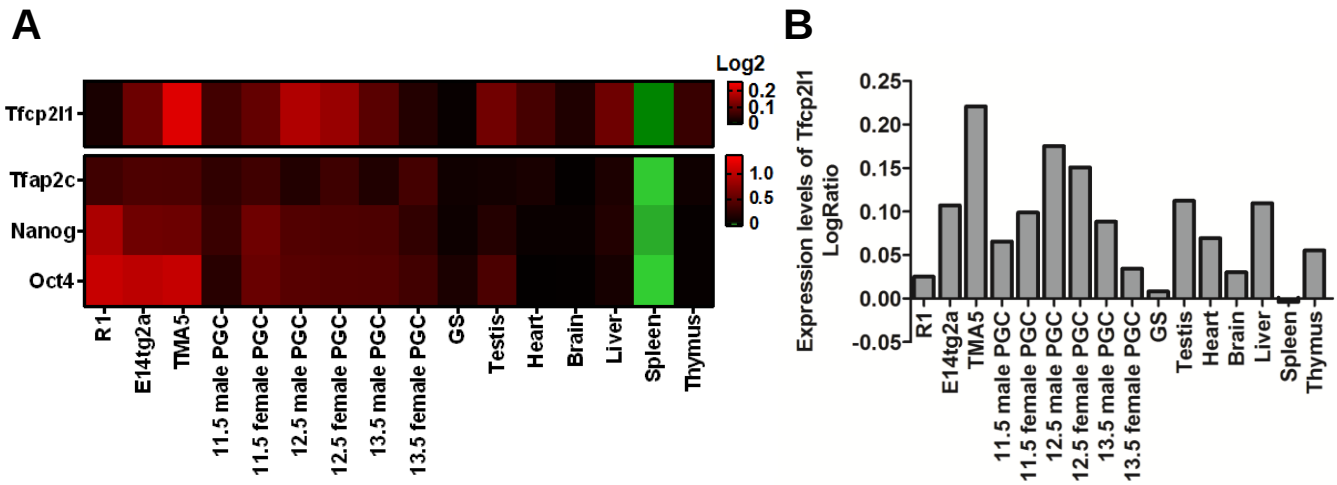
Supplementary Information

Figure S1



C

Expression levels of Tfcp2l1

Cell type	LogRatio	P-value
R1	0.025005486	0.078561383
E14tg2a	0.107196589	0.028020273
TMA5	0.220784314	0.01600607
11.5 male PGC	0.065645616	0.05851093
11.5 female PGC	0.098944786	0.154311874
12.5 male PGC	0.175269916	0.024131606
12.5 female PGC	0.150989943	0.023442215
13.5 male PGC	0.088334016	0.020987634
13.5 female PGC	0.034481705	0.027288662
GS	0.008159588	0.312230001
Testis	0.112393396	0.03231936
Heart	0.069472401	0.038303264
Brain	0.03040561	0.21359739
Liver	0.109530409	0.047332975
Spleen	-0.0039475	0.259002609
Thymus	0.055328578	0.077085245

Figure S1. Tfcp2l1 expression in different tissue samples

A. Heatmap showing the expression of Tfcp2l1, Tfap2c, Nanog and Oct4 in mouse ESCs, PGCs and different adult tissues. R1, E14tg2a and TMA5 are mouse ESC lines.

B and C. Quantification of Tfcp2l1 expression in mouse ESCs, PGCs and different adult tissues.

Figure S2

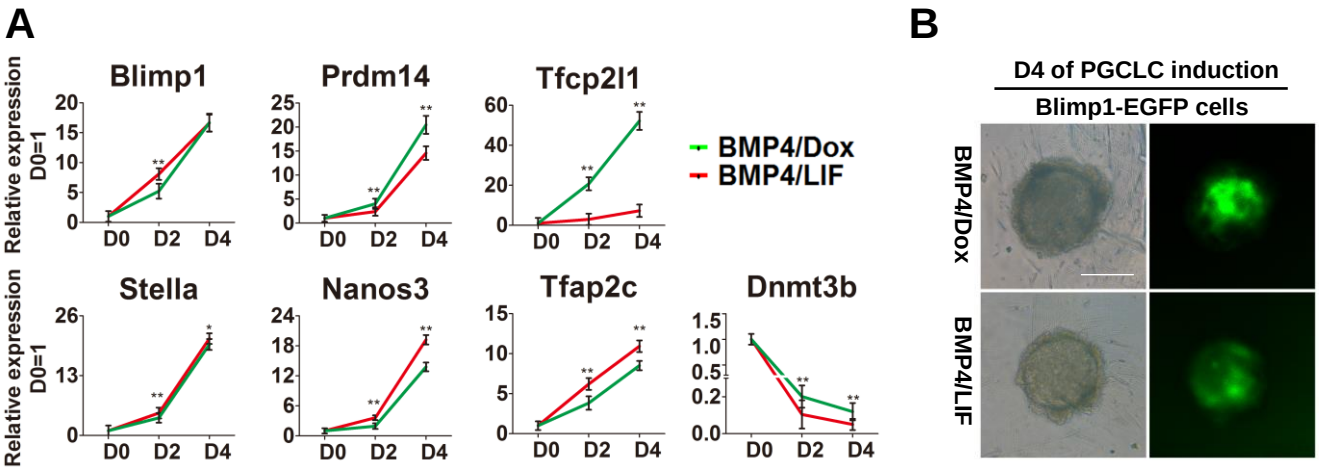


Figure S2. LIF induced higher levels of PGC markers than Tfcp2l1

(A) qRT–PCR analysis of the expression levels of PGC genes in i-Tfcp2l1 ESCs differentiated into EpiLCs and then cultured in GMEM/KSR/BMP4 conditions supplemented with LIF or Dox for 4 days. Data are the mean±s.d. (three independent experiments). *P<0.05, **P<0.01 versus BMP4/LIF.

(B) Fluorescence intensity induced in Blimp1-EGFP i-Tfcp2l1 cells treated with BMP4/Dox or BMP4/LIF. Bar, 100 μm.

Figure S3

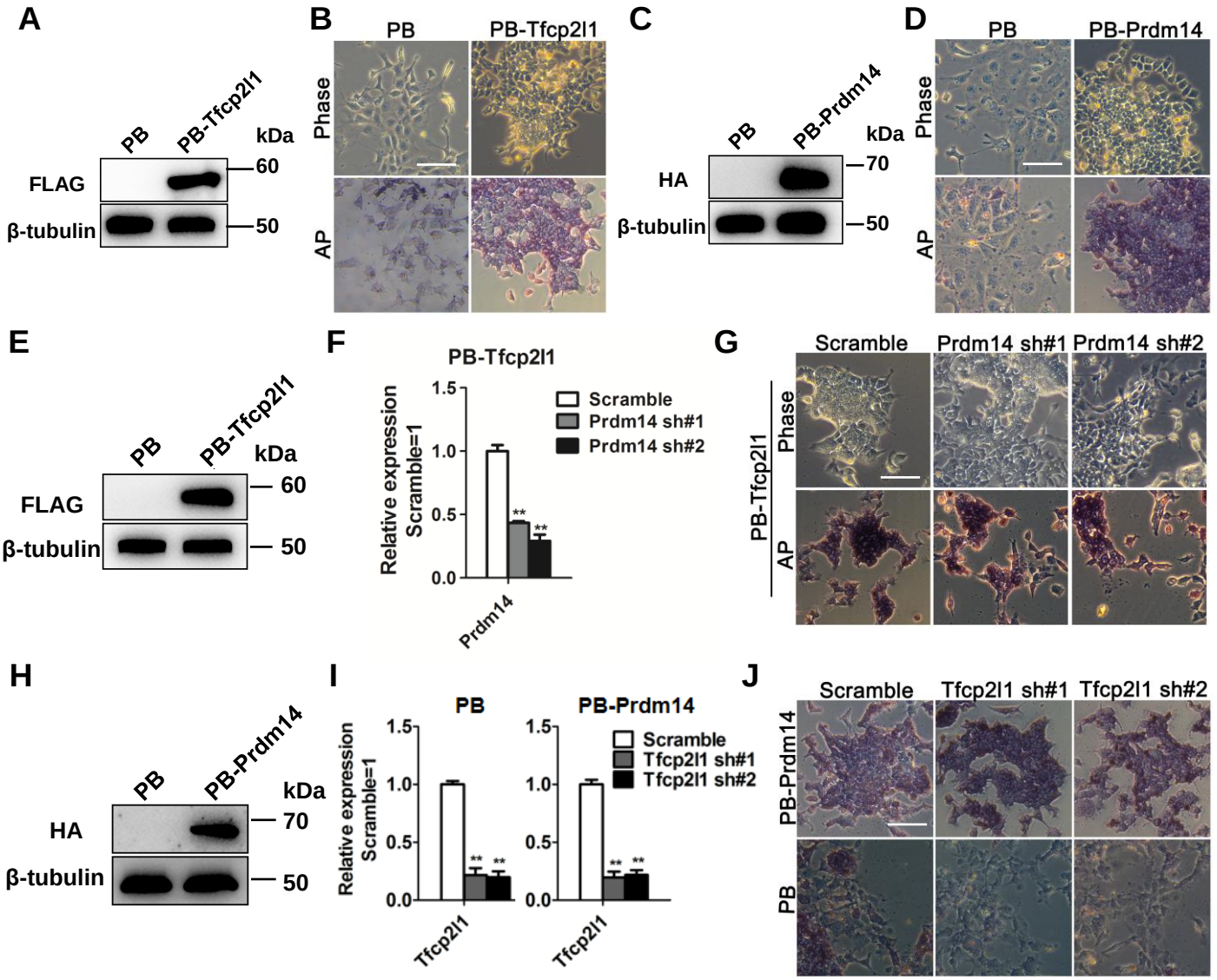

A
PB
PB-Tfcp2l1
kDa
FLAG
β-tubulin
60
50
B
PB
PB-Tfcp2l1
Phase
AP
C
PB
PB-Prdm14
kDa
HA
β-tubulin
70
50
D
PB
PB-Prdm14
Phase
AP
E
PB
PB-Tfcp2l1
kDa
FLAG
β-tubulin
60
50
F
PB-Tfcp2l1
Relative expression
Scramble=1
Scramble
Prdm14 sh#1
Prdm14 sh#2
Prdm14
G
Scramble
Prdm14 sh#1
Prdm14 sh#2
PB-Tfcp2l1
Phase
AP
H
PB
PB-Prdm14
kDa
HA
β-tubulin
70
50
I
PB
PB-Prdm14
Relative expression
Scramble=1
Scramble
Tfcp2l1 sh#1
Tfcp2l1 sh#2
Tfcp2l1
J
Scramble
Tfcp2l1 sh#1
Tfcp2l1 sh#2
PB-Prdm14
PB

Figure S3. Tfcp2l1 promotes mouse ESC self-renewal partially through Prdm14 induction

(A) Western blot analysis of FLAG in 46C mouse ESCs overexpressing empty vector (PB) or FLAG-tagged Tfcp2l1(PB-Tfcp2l1).

(B) Phase contrast images and AP staining of PB and PB-Tfcp2l1 mouse ESCs cultured in serum containing medium without LIF for 7 days. Bar, 100 μm.

(C) Western blot analysis of HA in 46C mouse ESCs overexpressing PB or the HA-tagged Prdm14 gene.

(D) Phase contrast images and AP staining of PB and PB-Prdm14 mouse ESCs cultured in serum medium in the absence of LIF for 7 days. Bar, 100 μm.

(E) Western blot analysis of FLAG in PB and PB-Tfcp2l1 mouse ESCs.

(F) qRT–PCR analysis of Prdm14 in PB-Tfcp2l1 mouse ESCs infected with Scramble or mouse Prdm14 shRNA lentiviruses (Prdm14 sh#1, Prdm14 sh#2). Data are the mean±s.d. (three independent experiments). **P<0.01 versus Scramble.

(G) Phenotypic and AP staining of Scramble control and Prdm14 shRNA mESCs overexpressing PB-Tfcp2l1 and cultured in serum-containing condition without LIF for 7 days. Bar, 100 μm.

(H) Western blot analysis of HA in PB and PB-Prdm14 in 46C mouse ESCs.

(I) qRT–PCR analysis of Tfcp2l1 in PB and PB-Prdm14 mouse ESCs infected with Scramble or mouse Tfcp2l1 shRNA lentiviruses (Tfcp2l1 sh#1, Tfcp2l1 sh#2). Data are the mean±s.d. (three independent experiments). **P<0.01 versus Scramble.

(J) AP staining of PB and PB-Prdm14 mouse ESCs infected with Scramble or Tfcp2l1 shRNA lentiviruses, and cultured in serum-containing medium without LIF for 7 days. Bar, 100 μm.

Figure S4

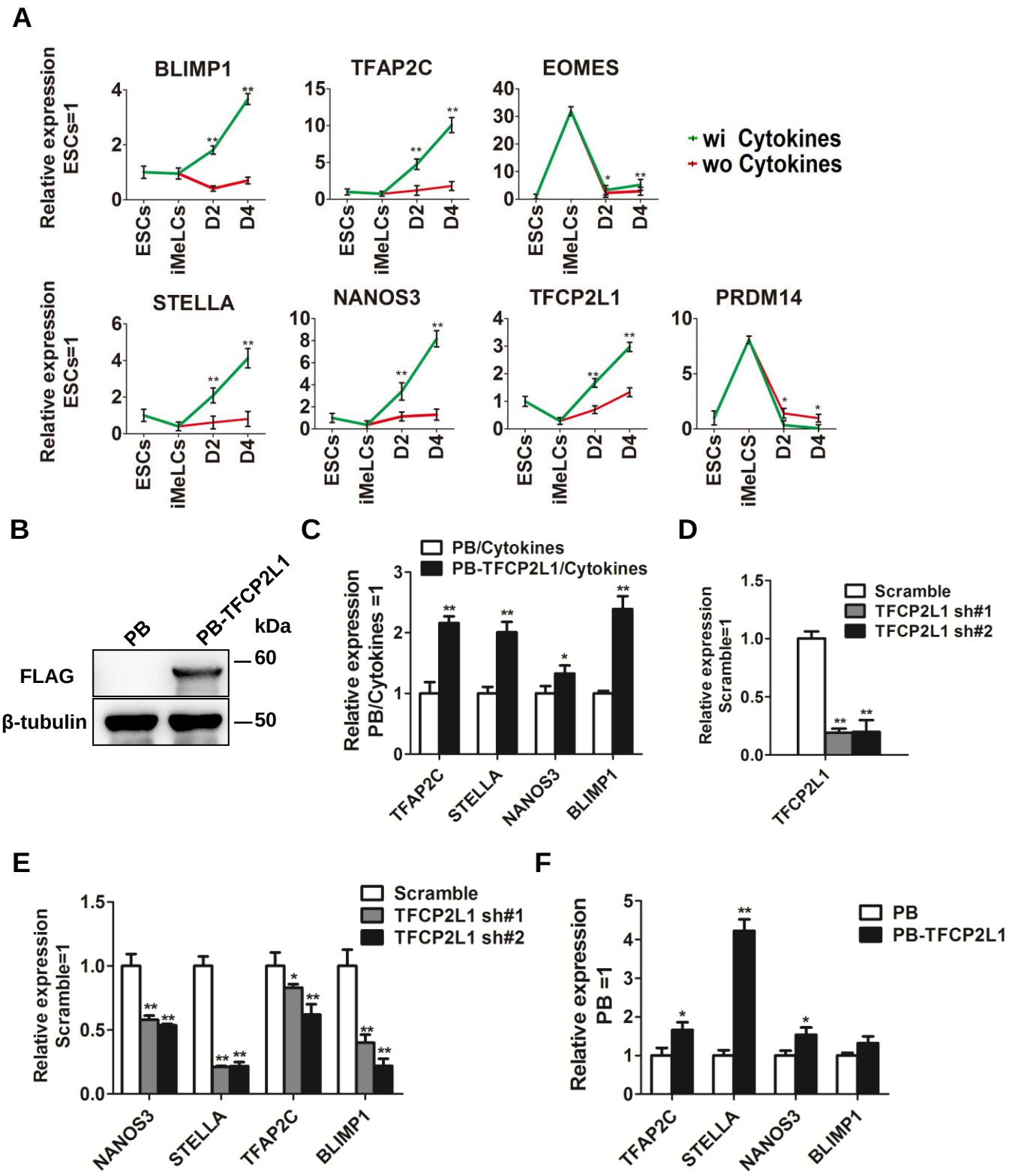

A
BLIMP1
TFAP2C
EOMES
STELLA
NANOS3
TFCP2L1
PRDM14
Relative expression ESCs=1
ESCs
iMeLCs
D2
D4
wi Cytokines
wo Cytokines
B
PB
PB-TFCP2L1
kDa
FLAG
β-tubulin
60
50
C
PB/Cytokines
PB-TFCP2L1/Cytokines
Relative expression PB/Cytokines =1
TFAP2C
STELLA
NANOS3
BLIMP1
D
Scramble
TFCP2L1 sh#1
TFCP2L1 sh#2
Relative expression Scramble=1
TFCP2L1
E
Scramble
TFCP2L1 sh#1
TFCP2L1 sh#2
Relative expression Scramble=1
NANOS3
STELLA
TFAP2C
BLIMP1
F
PB
PB-TFCP2L1
Relative expression PB =1
TFAP2C
STELLA
NANOS3
BLIMP1

Figure S4. Tfcp2l1 favors human PGCLC specification in H9 ESCs

(A) qRT–PCR analysis of the expression levels of TFCP2L1 and PGC markers during human PGCLC generation from H9 human ESCs. Data are the mean±s.d. (three independent experiments). *P<0.05, **P<0.01 versus wo Cytokines.

(B) Western blot analysis of FLAG in human ESCs overexpressing empty vector (PB) and FLAG-tagged human TFCP2L1 gene (PB-TFCP2L1).

(C) qRT–PCR analysis of the expression levels of PGC genes in PB and PB-TFCPL21 human ESCs exposed to cytokines, including BMP2, LIF, SCF and EGF, for 4 days. Data are the mean±s.d. (three independent experiments). *P<0.05, **P<0.01 versus PB/Cytokines.

(D) qRT–PCR analysis of human TFCP2L1 expression in H9 cells infected with Scramble or TFCP2L1 shRNA lentiviruses. Data are mean±s.d. (N=3 biological replicates). **P<0.01 versus Scramble.

(E) qRT–PCR analysis of the PGC markers in H9 cells infected with Scramble or human TFCP2L1 shRNA lentiviruses and induced into PGCLCs under PGCLC-inductive cytokines for 4 days. Data are the mean±s.d. (N=3 biological replicates). *P<0.05,**P<0.01 versus Scramble.

(F) qRT–PCR analysis of the expression levels of PGC genes in PB and PB-TFCP2L1 in H9 cells transformed into iMeLCs and then cultured in GK15 medium for 4 days. Data are the mean±s.d. (N=3 biological replicates).*P<0.05,**P<0.01 versus PB.

Table S1. Primer sequence for gene overexpression experiment

Symbol	Forward sequence (5'-3')	Reverse sequence (5'-3')
	Mus musculus	
Tfcp2l1	CGGGATCCATGCTGTTCTGGCACACGCAG	CCGCTCGAGTCAGAGTCCACACTTCAGGATGATG
Prdm14	GAAGATCTATGGCCTTACCGCCCTCTGG	CCGCTCGAGCTAGCAGGTTTTATGAAGCCTCATG
	Homo sapiens	
TFCP2L1	GGATGATCAATGCTCTTCTGGCACACGCAGCCCG	CCGCTCGAGTCAGAGTCCACATTTCAGGATGATG

Table S2. Primer sequence for gene knockdown experiment

Symbol	Forward sequence (5'-3')
	Mus musculus
Tfcp2l1 sh#1	CGGCTCAAGAGAAGGAGAAAT
Tfcp2l1 sh#2	CGGCTCAAGAGAAGGAGAAAT
Prdm14 sh#1	ACCTTGAATTACAGGATTAAG
Prdm14 sh#1	TTAAGTCGTCCCAGTCAATAT
	Homo sapiens
TFCP2L1 sh#1	CGAGTCCAGATTGACACGTTT
TFCP2L1 sh#2	CCGAGATGATTTGGTCCAGAT

Table S3. Primer sequence for qRT-PCR analysis

Symbol	Forward sequence (5'-3')	Reverse sequence (5'-3')
	Mus musculus	
Rpl19	GACGGAAGGGCAGGCATATG	TGTGGATGTGCTCCATGAGG
Tfcp2l1	AGGTGCTGACCTCCTGAAGA	CAGGCTGTTATCCCCACTGT
Prdm14	AAGCCTTTGCATCTCATGCT	AGGAAGCCTTTCCCACAAAT
Blimp1	AGCATGACCTGACATTGACACC	CTCAACACTCTCATGTAAGAGGC
Tfap2c	GGGCTTTTCTCTCTTGGCTGGT	TCCACACGTCACCCACACAA
Stella	AGGCTCGAAGGAAATGAGTTTG	TCCTAATTCTTCCCGATTTTCG
Nanos3	CACTACGGCCTAGGAGCTTGG	TGATCGCTGACAAGACTGTGG
Nanog	TACCTCAGCCTCCAGCAGA	CCTCCAAGTCACTGGCAG
Dnmt3b	CTCGCAAGGTGTGGGCTTTTGTAAC	CTGGGCATCTGTCATCTTTGCACC
	Homo sapiens	
β-ACTIN	ATAGCAACGTACATGGCTGG	CACCTTCTACAATGAGCTGC
TFCP2L1	ATGTGAGGCCAAAGATGACC	CAGACAGGTTGCTGTCTCCA
BLIMP1	CGGGGAGAATGTGGACTGGGTAGAG	CTGGAGTTACACTTGGGGGCAGC
TFAP2C	CGCTCATGTGACTCTCCTGACATCC	TGGGCCGCCAATAGCATGTTCT
EOMES	CTGGCTTCCGTGCCCACGTC	CATGCGCCTGCCCTGTTTCG
NANOS3	CCCGAAACTCGGCAGGCAAGA	AAGGCTCAGACTTCCCGGCAC
STELLA	ACGCCGATGGACCCATCACAGTTT	TCTCGGAGGAGATTTGAGAGGCCC
PRDM14	CTACCGAGCCCGAGTGGCCTAC	TAGAGCCATCCCGGGACCGCA

Table S4.ChIP-qPCR primers used to identify different binding motifs of Tfcp2l1 in the promoter of Prdm14

Location From UCSC	Primer sequence for CHIP-qPCR(5'-3')	
-2042~-1868	CCCAAAGAGGAACTGGAATG	TGTGCTCAACGACTGCATTT
-2745~-2603	TGTGTGGTGTGTGCATGTGT	GTTGTTGTTGTTGTTGTTGATTCAG