Supplement Information

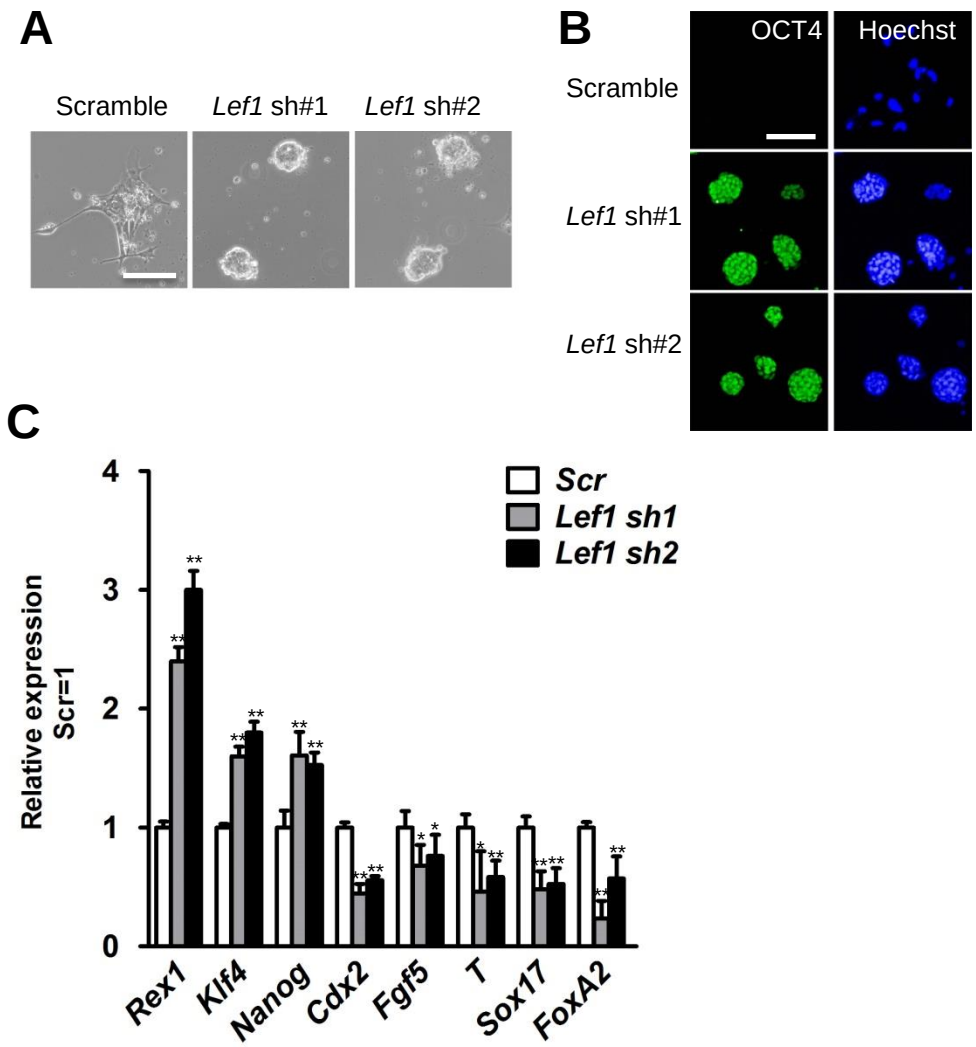

Fig. S1. Knockdown of *Lef1* promotes R1 ESC self-renewal.

(A) Morphology of scramble control and *Lef1* knockdown R1 ESCs cultured in N2B27/CHIR condition for five passages. Scar bar, 100 μm.

(B) Immunostaining of scramble control and *Lef1* knockdown R1 ESCs for OCT4. Scar bar, 100 μm.

(C) qRT–PCR analysis of *Klf4*, *Nanog*, *Rex1*, *Cdx2*, *Fgf5*, *T*, *Sox17* and *FoxA2* expression in Scr and Lef1 shRNA mESCs cultured in N2B27/CHIR condition. Data represent mean±s.d. of three biological replicates. *P<0.05, **P<0.01 vs Scr.

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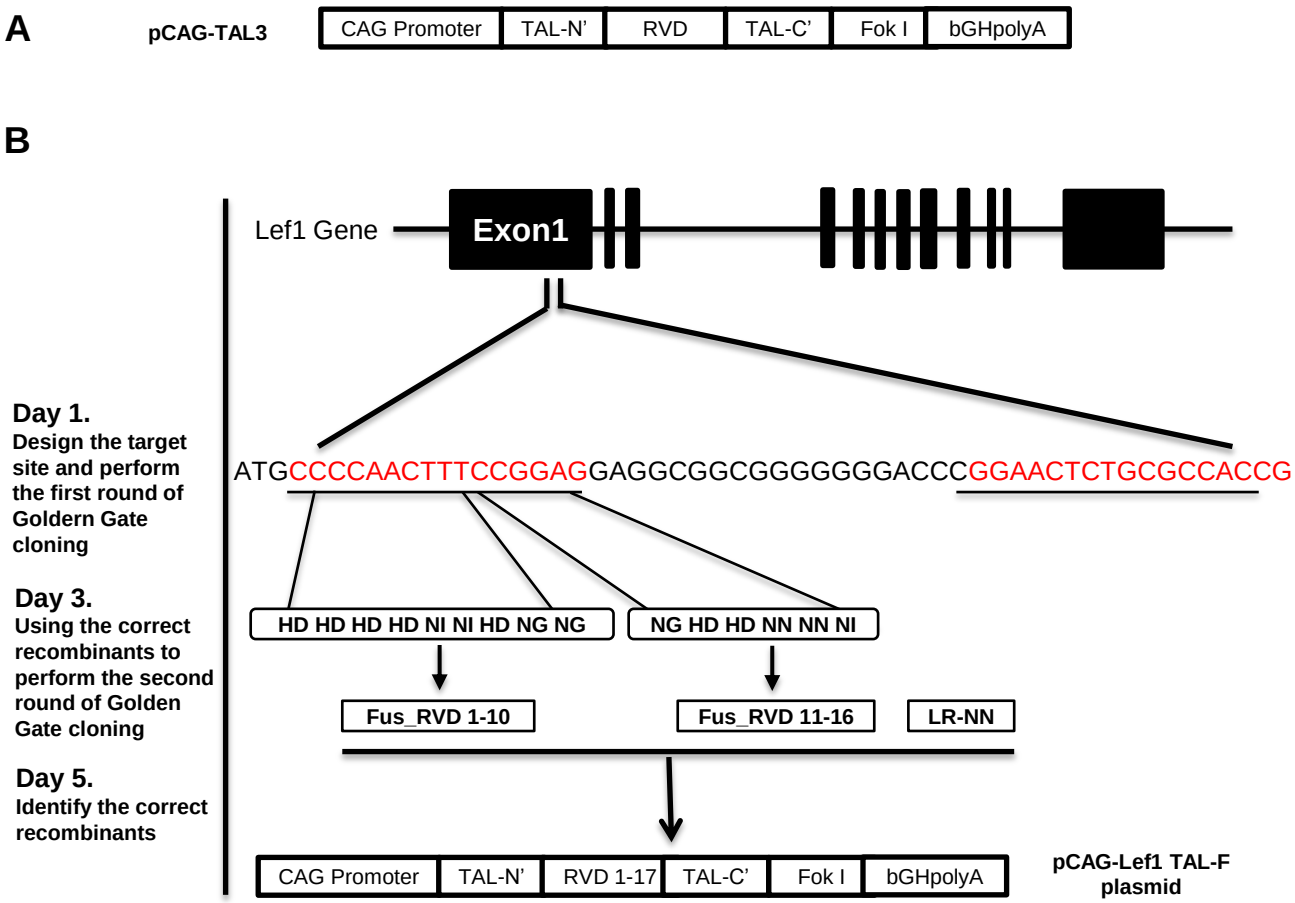

C Lef1 mutant: ATGCCACAGCTATCGGGTGGAGGCGGCGGGGGGGACCCAGAGCTATGTGCTACGG

Fig. S2. The strategy for constructing *Lef1* targeting TALENs

(A) The structure of plasmid pCAG-TAL3 (Addgene. http://www.addgene.org/TALeffector/goldengateV2/).

(B) The TALEN target is located in exon 1 of *Lef1*. The left and right TALENs binding sequences are underlined. The pCAG-*Lef1*-TAL-F was constructed using two rounds of Golden Gate cloning method.

(C) Blue sequences were synonymous mutation corresponding to wild type sequences of *Lef1*. Overlapping PCR was used to generate the *Lef1* mutations

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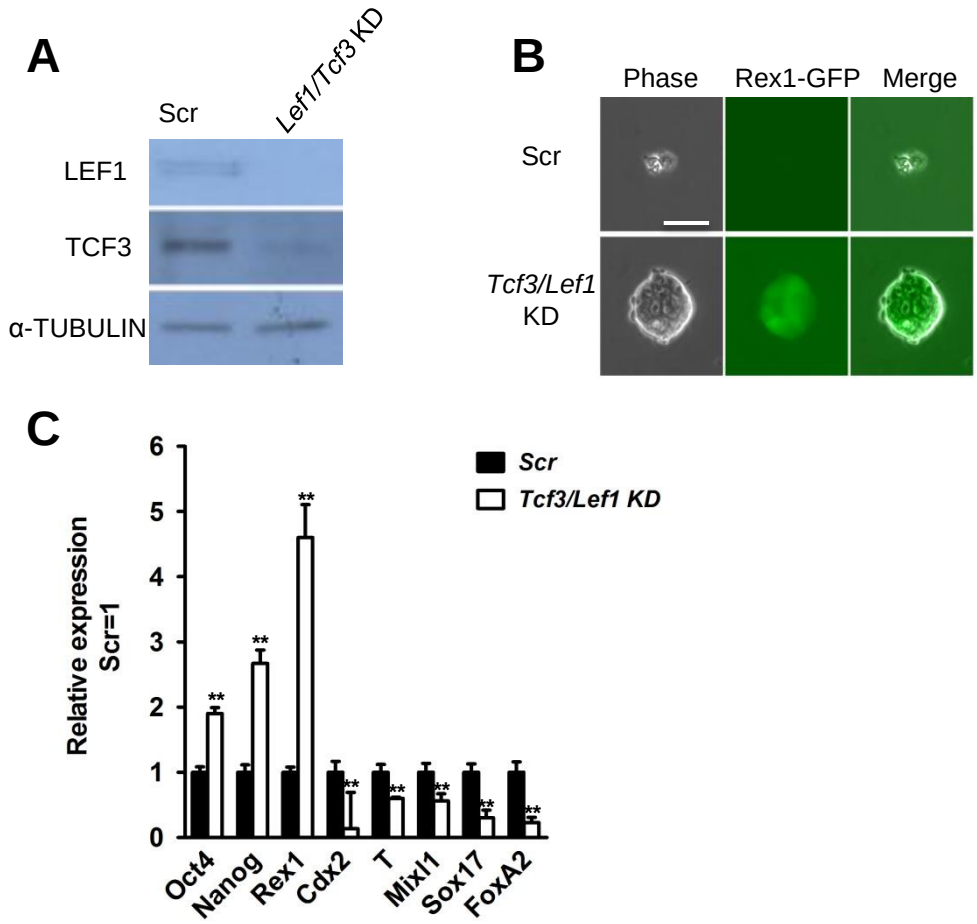

Fig. S3. Double knockdown *Tcf3* and *Lef1* delayed differentiation of Rex1-GFP mouse ESCs.

(A) Western blot analysis of LEF1 and TCF3 proteins in *Lef1* and *Tcf3* double knockdown (KD) and Scramble (Scr) control mouse ESCs.

(B) Morphology of scramble control and double knockdown of *Tcf3* and *Lef1* mouse ESCs cultured in N2B27 condition for five passages. Scar bar, 100 μm.

(C) qRT–PCR analysis of *Oct4*, *Nanog*, *Rex1*, *Cdx2*, *T*, *Mixl1*, *Sox17*, and *FoxA2* expression in Scr and *Tcf3/Lef1* dKD mESCs cultured in condition. Data represent mean±s.d. of three biological replicates. **P<0.01 vs Scr.

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Table S1. List of primers used for qRT-PCR analysis.

Gene	Forward sequence (5'-3')	Reverse sequence (5'-3')
Gapdh	TGTGAGGGAGATGCTCAGTG	TGTTCCTACCCCCAATGTGT
Sox17	AGCCATTTCCTCCGTGGTGT	AACACTGCTTCTGGCCCTCAG
Oct4	GAAGCAGAAGAGGATCACCTTG	TTCTTAAGGCTGAGCTGCAAG
Nanog	TCCAGAAGAGGGCGTCAGAT	CAAATCCCAGCAACCACATG
Rex1	TCACTGTGCTGCCTCCAAGT	GGGCACTGATCCGCAAAC
Klf2	AGGCCTGTGGGTTCGCTATAAA	GGCAAATTATGGCTCAAAGTAGCAG
Egr1	CCACAACAACAGGGAGACCT	ACTGAGTGGCGAAGGCTTTA
Isx	AGAGGACTCCAGGCAGACAA	AGCTGCTCTGTGGTGAAGGT
Lef1	TCACTGTCAGGCGACACTTC	TGAGGCTTCACGTGCATTAG
Tcf3	AGTTCAACCCCCTCTGTCCT	TGCCTGCCACTCTGATACTG
Esrrb	TTTCTGGAACCCATGGAGAG	AGCCAGCACCTCCTTCTACA
Gata4	TCTCACTATGGGCACAGCAG	GCGATGTCTGAGTGACAGGA
Gata6	TCCTCCCCTGCCGAAGTC	AGGGCCAGAGCACACCAA
Fgf5	GCAGCCCACGGGTCAA	CGGTTGCTCGGACTGCTT

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