Supplemental Data

The transcription factor Gbx2 induces expression of Kruppel-like factor 4 to maintain and induce naïve pluripotency of embryonic stem cells

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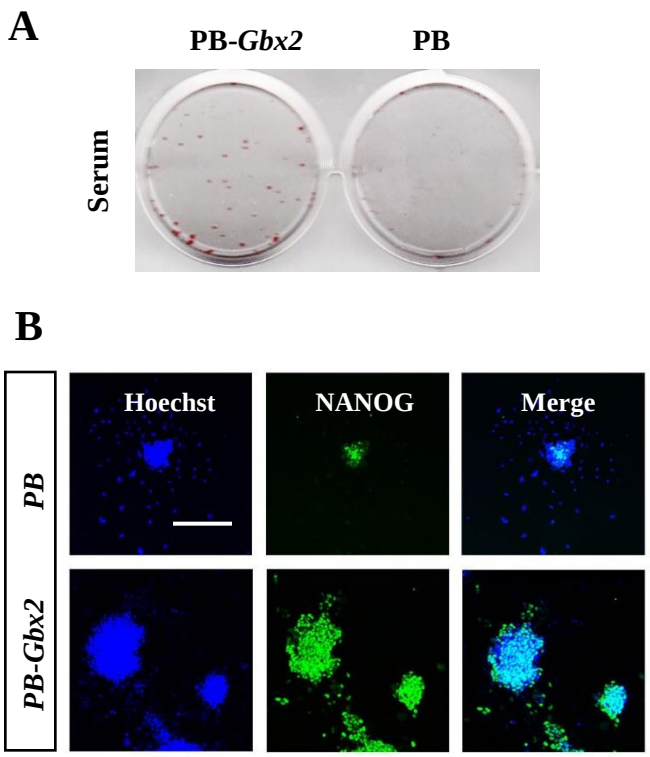

Figure S1. Overexpression of *Gbx2* is able to promote mESCs self-renewal.

A. AP staining of 46C mESCs overexpressing *Gbx2* cultured in the absence of LIF for 8 days.

B. Immunofluorescence staining of NANOG in 46C mESCs overexpressing PB or *HA* tagged *Gbx2* cultured in the absence of LIF for 8 days. Scale bar: 100 μm.

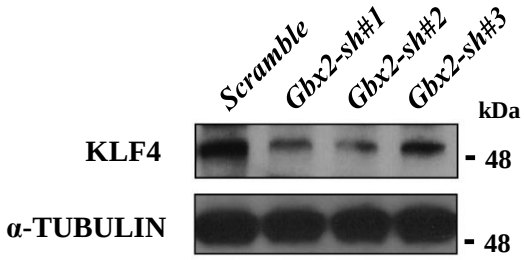

Figure S2. Knockdown of *Gbx2* reduced the KLF4 protein levels .

Western blot analysis of KLF4 in *scramble* and *Gbx2* knockdown 46C mESCs cultured in LIF/serum codition. α-TUBULIN is used as a loading control.

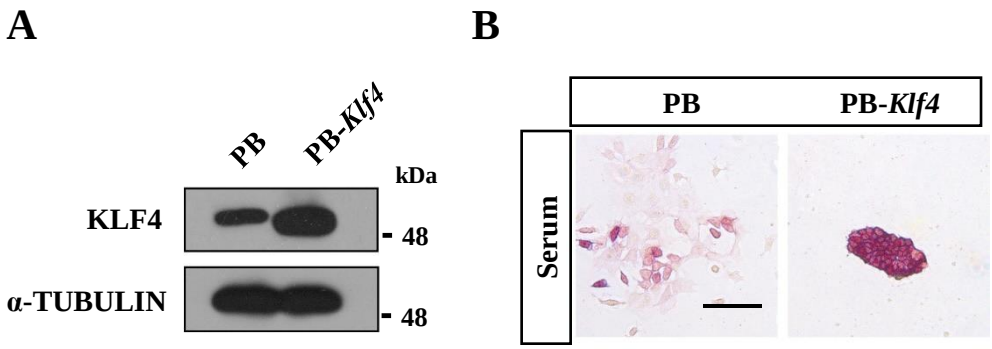

Figure S3. Elevated expression of *Klf4* supports 46C mESC self-renewal

A. Western blot analysis of KLF4 in PB and PB-*Klf4* transfected 46C mESCs. α-TUBULIN is used as a loading control.

B. AP staining of PB and PB-*Klf4* 46C mESCs cultured in the absence of LIF for 8 days. Scale bar: 100 μm.

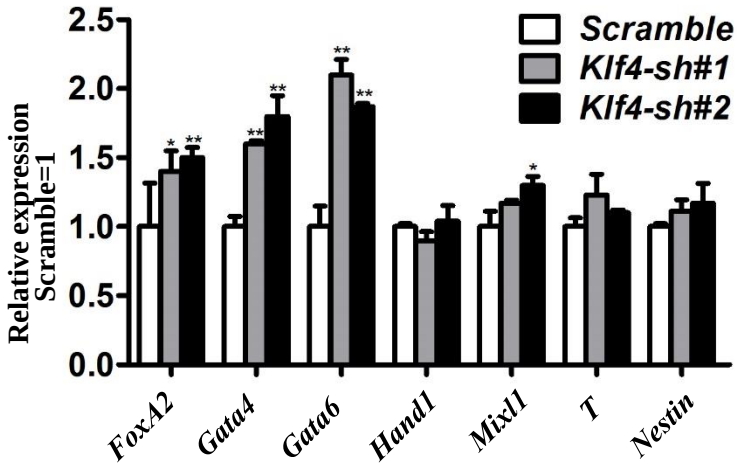

Figure S4. Knockdown of *Klf4* induces PB-*Gbx2* mESC differentiation.

qRT-PCR analysis of the expression levels of *FoxA2*, *Gata4*, *Gata6*, *Hand1*, *Mixl1*, *T* and *Nestin* in the PB-*Gbx2* cells, transfected with *scramble* or *Klf4* shRNA lentivirus, cultured in the absence of LIF. Data are presented as the mean ± s.d. of three independent experiments. *P<0.05, **P<0.01 versus *sramble*.

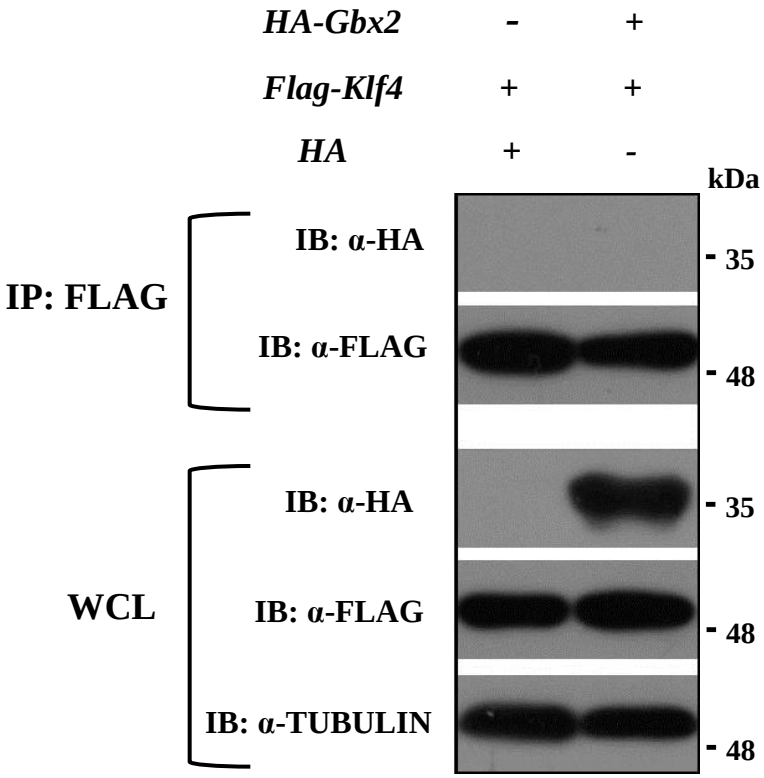

Figure S5. GBX2 does not interact with KLF4 during the mEpiSC reprogramming. *Flag*-tagged *Klf4* overexpressed CD1 mEpiSCs, cultured in Activin A, bFGF and IWR1 conditions, were transfected with *HA* and *HA*-tagged *Gbx2*, respectively. Cells were then transferred into LIF/2i culture conditions for 2 days. Co-IP of transfected *HA* or *HA*-tagged *Gbx2* were performed using *Flag* tagged *Klf4*. WCL: whole cell lysate.

Table S1. List of shRNA sequences used for Knockdown

Symbol	shRNA Sequence (5'-3')
Gbx2-sh#1	GCAAGGGAAAGACGAGTCAAA
Gbx2-sh#2	GAGAGCGATGTGGATTACA
Gbx2-sh#3	GCCTGGTCAGACTGCTCATAA
Klf4-sh#1	GCGCTACAATCATGGTCAAGT
Klf4-sh#2	GTCAGCTTGTGAATGGATAAT

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

Symbol	Forward sequence (5'-3')	Reverse sequence (5'-3')
Gbx2	F:GCAAGGGAAAGACGAGAA	R:GCCGGGGTCTTCTTCCTTAT
Klf4	F: GAAGACCAGGATTCCCTTGA	R: CCAAGCACCATCATTTAGGC
Nanog	F: TACCTCAGCCTCCAGCAGA	R: CCTCCAAGTCACTGGCAG
Tbx3	F: AGGAGCGTGTCTGTCAGGTT	R: GCCATTACCTCCCCAATTTT
Rex1	F: TCACTGTGCTGCCTCCAAGT	R: GGGCACTGATCCGCAAAC
Stella	F: TTCCGAGCTAGCTTTTGAGG	R: ACACCGGGGTTTAGGGTTAG
Fgf5	F: GCAGCCCACGGGTCAA	R: CGGTTGCTCGGACTGCTT
Klf2	F: AGGCCTGTGGGTTCGCTATAAA	R: GGCAAATTATGGCTCAAAGTAGCAG
FoxA2	F:CCTCAAGGGAGCAGTCTCAC	R:TTTCTCCTGGTCCGGTACAC
Gata6	F:GAGCTGGTGC TACCAAGAGG	R:TGCAAAAGCC CATCTCTTCT
Gata4	F:TCTCACTATG GGCACAGCAG	R:GCGATGTCTG AGTGACAGGA
Hand1	F: CGCCTGGCTACCAGTTACAT	R: GGCCTGGTCTCACTGGTTTA
Mixl1	F:GGAGCTGATC TCCTGACTGG	R:TGAGTCAAGC CGAAGGTTCT
T	F: CCGGTGCTGAAGGTAAATT	R: CTCCATTGAGCTTGTTGGT
Nestin	F:CTCGAGCAGG AAGTGGTAGG	R:TTGGGACCAG GGACTGTTAG

Table S3. List of ChIP-qRT-PCR primers for assay different promoter regions of Klf4 .

Location From UCSC	Forward sequence (5'-3')	Reverse sequence (5'-3')
-4100~-3791	F:CCGTGAACACATGCATGCCTG	R:TTCCAGCCACTCACTTAGGACCTGC
-3789~-3592	F:ACGCTACAGGGGATAAAGTGATG	R:GAACTCACTTTGCCCGTTTTCTC

Table S4. The PCR primer sequences for amplifying the different promoter regions of Klf4 to construct *Klf4* promoter-luciferase reporter.

Location From UCSC	Primer sequence for PCR (5'-3')
-4100~-3701	F:GGGGTACCCTAAATCAAGGTAGCCAGGACAG
	R:CCCAAGCTTTCAGGACTTAATAGGTTTTAATATC