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Supplemental Information

Stat3 Activation Is Limiting for Reprogramming to Ground State Pluripotency

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INVENTORY OF SUPPLEMENTAL INFORMATION

Figure S1. *Tbx3* is not expressed in EpiSCs and is not induced by Lif signalling in either EpiSCs or ES cells

Additional information for Figure 1 relating to *Tbx3*.

Figure S2. Epi-iPS cell derivation from GY118F EpiSCs using Gcsf in serum free medium without 2i

Additional information for Figure 2.

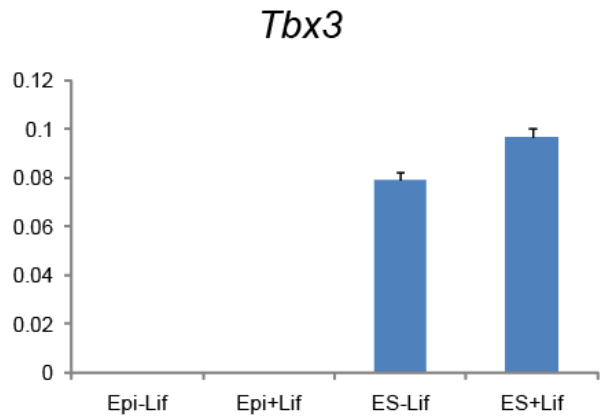
Figure S3. Characterisation of tatCre excision of transgene in *PB*-GY118F Epi-iPS cells.

Additional information for Figure 2.

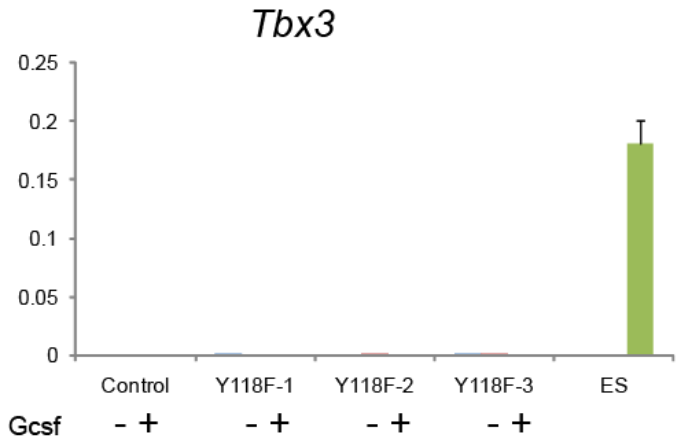
List of PCR Primers and qPCR Taqman Assays

Figure S1. *Tbx3* is not expressed in EpiSCs and is not induced by LIF in either EpiSCs or ES cells

A



B



Taqman analyses were performed on total RNA preparations.

(A) EpiSCs or ES Cells were cultured in unsupplemented medium for 4 hours then stimulated with Lif for 1 hour.

(B) Empty vector or GY118F transfected EpiSCs maintained in Activin and Fgf2 were unstimulated or treated with Gcsf for 1 hour.

Error bars are standard deviations from the mean of triplicate determinations.

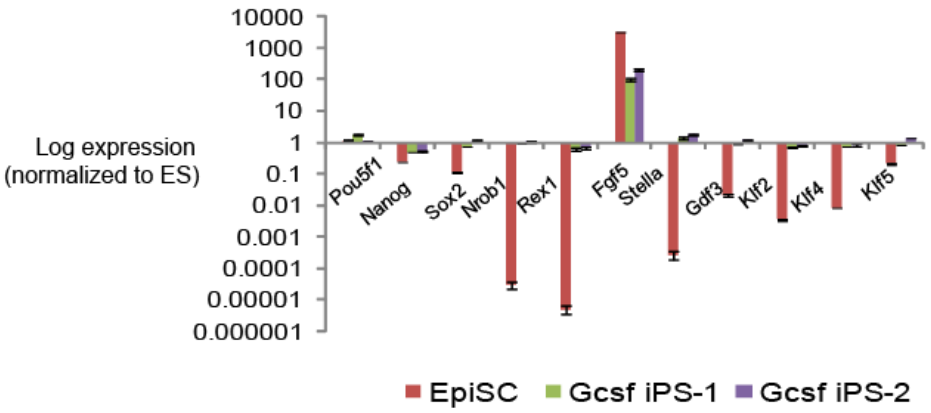
Figure S2 Epi-iPS cell derivation from GY118F EpiSCs using Gcsf in serum free medium without 2i

A

Numbers of Oct4GFP+ colonies per well after 8 days

Transgenic Lines	N2B27	N2B27/Gcsf
Vector	0	0
GY118F-1	0	8; 5; 10
GY118F-2	0	10; 3; 2
GY118F-3	0	15; 17; 22

B



(A) Numbers of Oct4GFP positive colonies generated from three independent GY118F EpiSC clones by treatment with Gcsf in serum-free medium. *PB*-GY118F O4G EpiSCs were plated in triplicate at $2x10^4$ cells per well in Activin and Fgf2 for 24 hours then transferred into serum-free N2B27 medium supplemented with Gcsf for 8 days. Oct4-GFP positive colonies were then counted.

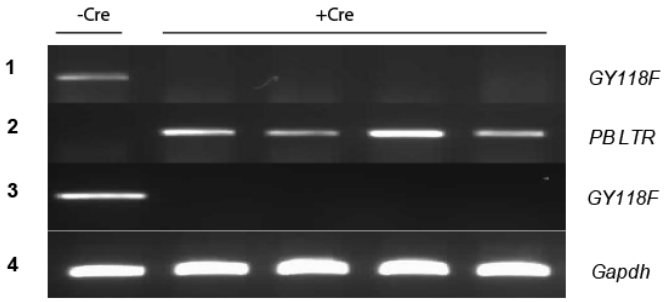
(B) qRT-PCR analysis of ground state and EpiSC markers. Oct4-GFP positive colonies were expanded in N2B27 plus Gcsf in the presence of puromycin (1μg/ml) to eliminate differentiated cells. Expression is relative to *Gapdh* and normalized to ES cells. The increased expression of *Fgf5* relative to ES cells in 2i is expected for cultures with active Fgf/Erk signaling.

Error bars are standard deviations from the mean of triplicate determinations.

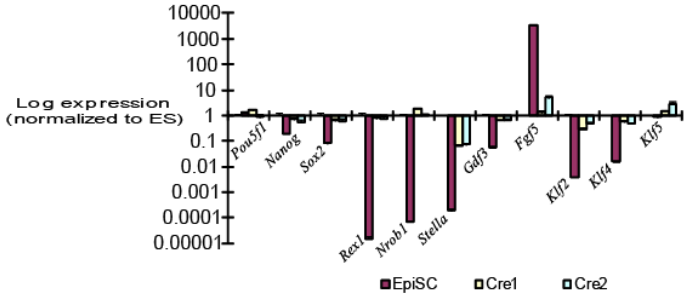
Figure S3 Characterisation of tatCre excision of transgene in *PB*-GY118F Epi-iPS cells.

A

B



C



(A) Oct4 positive Epi-iPS colony obtained after tatCre protein transduction. Colony is dsRed negative, indicative of transgene deletion.

(B) Untreated and four tatCre-treated GY118F Epi-iPS clones. Genomic PCR analysis of transgene (panel 1, 2); RT-PCR analysis of transgene expression (panel 3); *Gapdh* expression as loading control (panel 4).

(C) qRT-PCR analysis of marker gene expression in Cre excised Epi-iPS cells. Gene expression level is relative to *Gapdh* and normalized to ES cells.

Error bars are standard deviations from the mean of triplicate determinations.

Supplemental Information on PCR and qPCR assays

List of primers

GY118F (gPCR and RT-PCR):

Forward cagcatgtctatgcctactc; Reverse accactgggcaatatgactc

PBLTR (gPCR)

Forward ccctagaaagataatcatattgtgac; Reverse ccctagaaagatagtctgcgtaaaat

STAT3ER (gPCR and RT-PCR):

Forward tagtgctgccccgtacctgaagac; Reverse cttcactgaagggtctggtaggat

Gapdh (RT-PCR):

Forward cccactaacatcaaatgggg; Reverse ccttccacaatgccaaagtt

Taqman assays

Il6st(gp130), Mm00439668_m1*; Lifr,* Mm00432942_m1*; Socs3,* Mm01249143_g1*; Stat3,* Mm01219775_m1*; Pou5f1 (Oct4)*, Mm00658129_gH; Sox2, Mm03053810_s1; *Klf4*, Mm00516104_m1; *Klf2*, Mm01244979_g1; *Klf5*, Mm00456521_m1; *Nanog*, Mm02384862_g1; *Rex1*,Mm03053975_g; *Fgf5*, Mm00438919_m1; *Gdf3,* Mm00433563_m1; *Nr0b1*, Mm00431729_m1; *Stella* (*Dppa3*), Mm00836373_g1; *T (brachyury),* Mm01318252_m1; *Lefty*, Mm00438615_m1; *Tbx3*, Mm00809779_s1; c-*myc*, Mm00487804_m1; *Snai2*, Mm00441531_m1; *Zeb2*, Mm00497193_m1*; Gapdh*, 4352339E

Expression of retroviral transgenes was determined using Custom TaqMan Gene Expression Assays (Applied Biosystems):
pMXs-Oct4 PF: 5'- TGGTACGGGAAATCACAAGTTTGTA, PR: 5'-GGTGAGAAGGCGAAGTCTGAAG, probe: 5'- FAM-CACCTTCCCCATGGCTG-MGB. pMXs-Klf4 PF: 5'-TGGTACGGGAAATCACAAGTTTGTA, PR: 5'-GAGCAGAGCGTCGCTGA, probe: 5'- FAM-CCCCTTCACCATGGCTG-MGB. pMXs-cMyc PF: 5'- TGGTACGGGAAATCACAAGTTTGTA, PR: 5'-GGTCATAGTTCCTGTTGGTGAAGTT, probe: 5'-FAM-CCCTTCACCATGCCCC-