Supplementary Information

JAK/STAT3 signalling is sufficient and dominant over antagonistic cues for the establishment of naïve pluripotency

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Supplementary Information includes:

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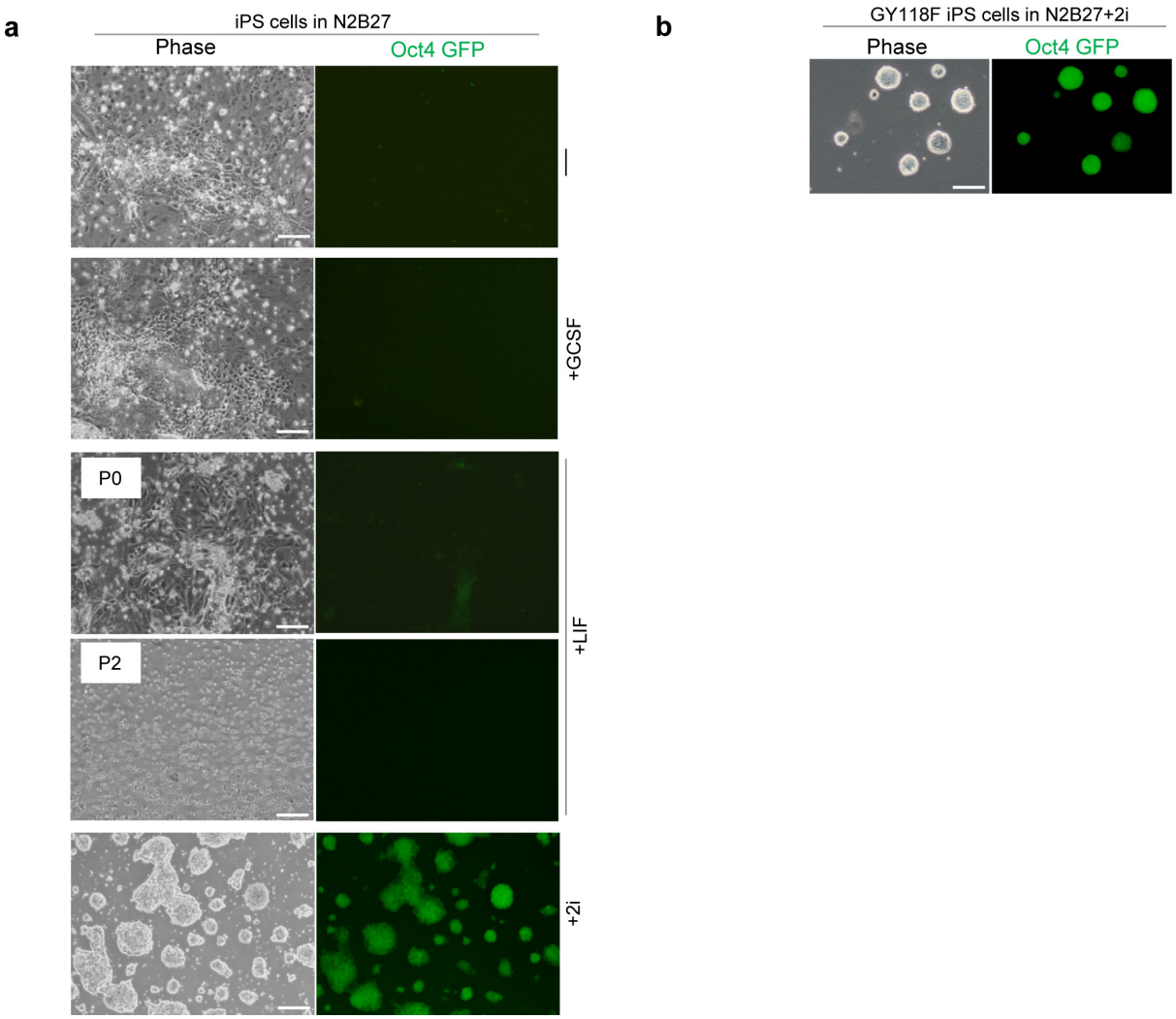
Supplementary Figures

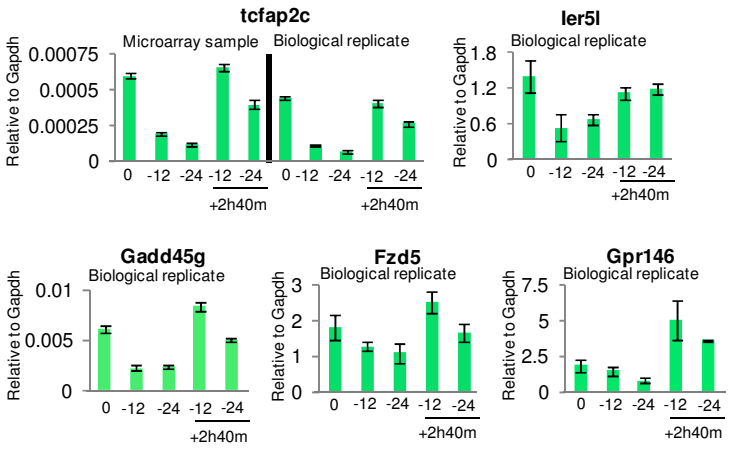


Supplementary Figure S1 N2B27 plus GCSF or LIF does not support self-renewal of pluripotent cells

(**a**) Phase and fluorescent images of wild type (WT) iPS cells after medium switch to N2B27 supplemented with either GCSF or LIF. N2B27 plus 2i was used as a control culture condition that supports self-renewal. White bars correspond to 119.4 µm. (**b**) Phase and fluorescent images of GY118F iPS cells derived and maintained in N2B27 plus GCSF switched to 2i culture conditions. White bars correspond to 161.4 µm.

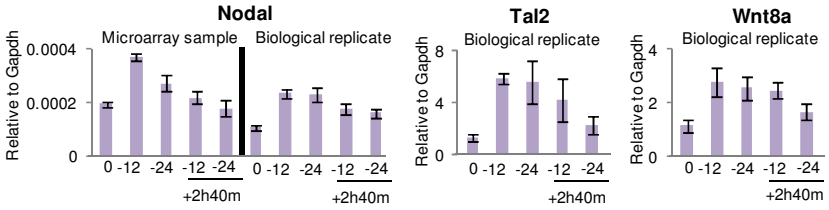
a

Gene	
Serpina3h	N.V.
Tcfap2c	pattern confirmed p=0.001
LOC381140	N.V.
LOC100047268	N.V.
Ier5l	pattern confirmed p=0.05
Sbno2	trend not confirmed
Gadd45g	pattern confirmed p=0.002
Gadd45b	trend not confirmed
Fzd5	pattern confirmed p=0.1
Gpr146	upregulated upon readdition
Cish	trend not confirmed

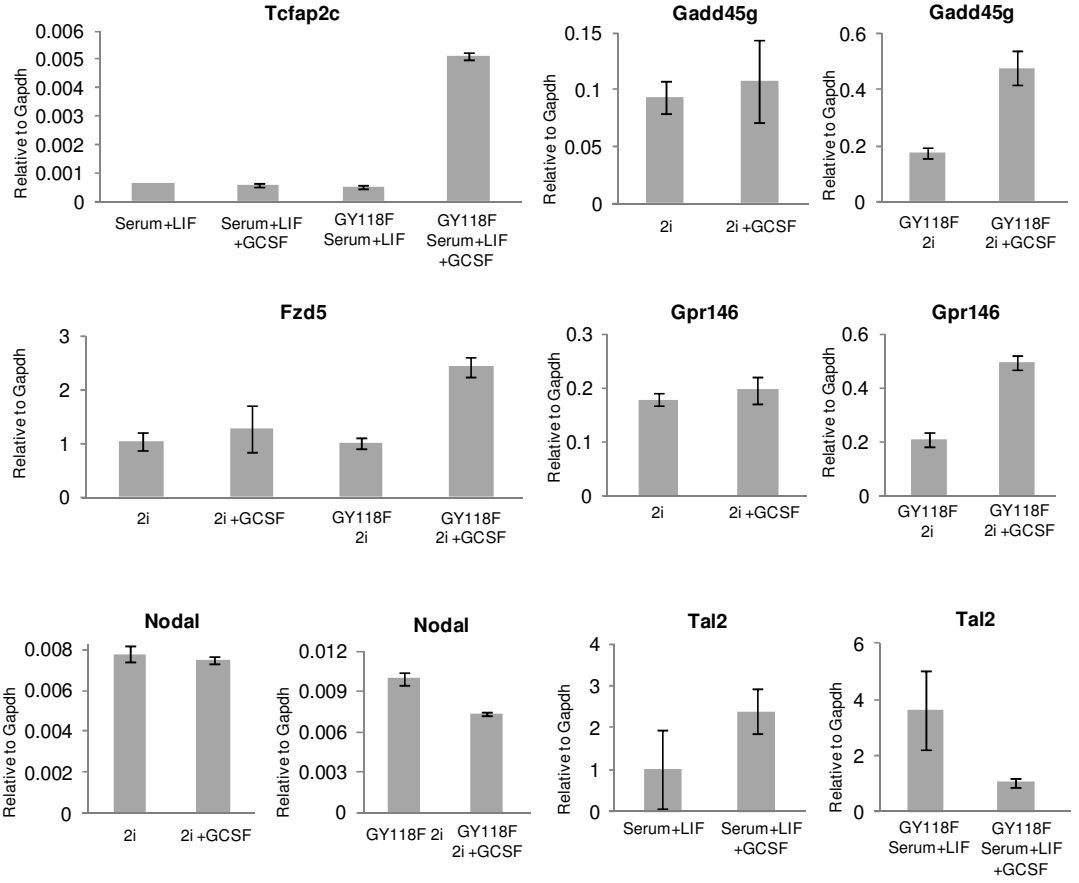


b

Gene	
Wnt8a	trend
Prickle1	trend not confirmed
Tal2	trend
Nodal	pattern confirmed p=0.05

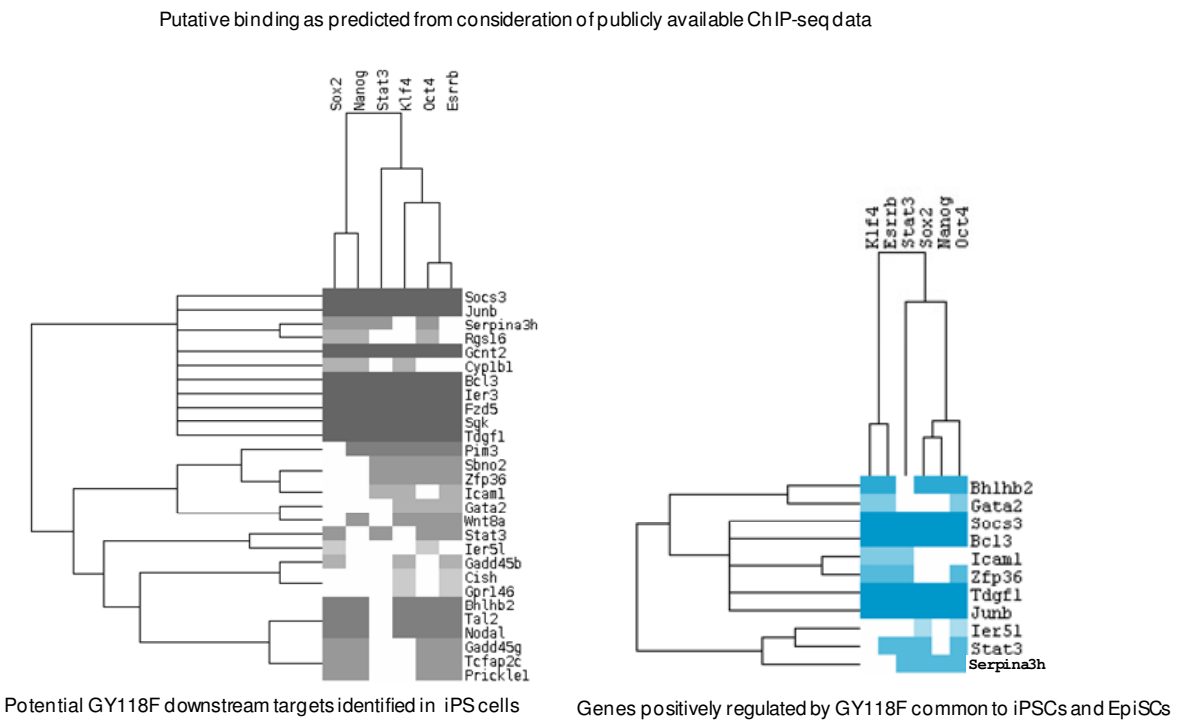


c



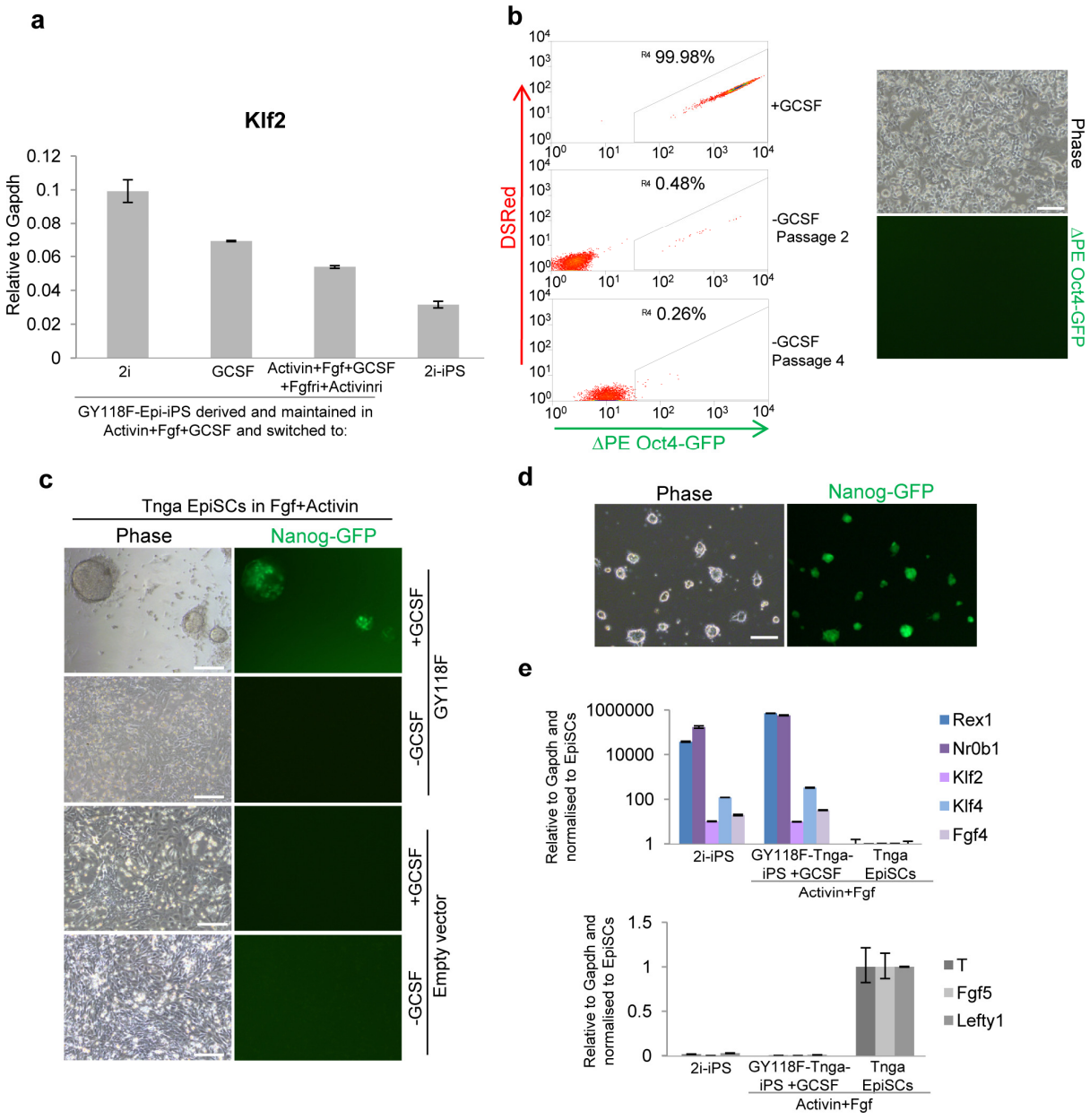
Supplementary Figure S2 GCSF induced transcriptional activity is mediated by GY118F

(**a**) List of genes not previously reported as LIF/STAT3 targets that were found to be downregulated upon GCSF withdrawal and upregulated upon its re-addition (≥1.4 fold change threshold). The cells in green represent array hits that have been validated by qRT-PCR in a biological replicate as depicted by respective graphs. Significance has been determined by T testing. Array hits in white cells have not been verified (N.V.). Array hits in red cells did not have pattern validated by qRT-PCR. Error bars indicate ± 1 standard deviation (s.d.) (n=3). (**b**) List of genes that were found to be upregulated upon GCSF withdrawal and that were downregulated upon readdition of GCSF (≥1.4 fold change threshold). The cells in purple correspond to array hits for which gene expression trend was confirmed by qRT-PCR in a biological replicate as depicted by respective graphs. Where significance is stated this has been determined by T-testing. Prickle1 did not show array pattern by qRT-PCR for a biological replicate. Error bars indicate ± 1 s.d. (n=3). (**c**) As pluripotent cells cannot self-renew in N2B27 only we could not control for putative GCSF mediated responses independent of the GY118F transgene in this culture condition. To address this we analysed the response of ES cells to GCSF stimulation in 2i or serum plus LIF culture conditions. ES cell lines transfected with the GY118F or control vector transgene were subjected to GCSF stimulation for 2 hours and 40 minutes. Results show that response to GCSF for Tcfap2c, Gadd45g, Fzd5, Gpr146, Nodal and Tal2 is mediated by GY118F. No conclusive response to GCSF stimulation was observed for Ier5l and Wnt8a suggesting that this may be specific to the N2B27 only. Error bars indicate ± 1 s.d. (n=3). The GCSF receptor is not expressed in iPS cells.



Supplementary Figure S3 STAT3 and pluripotency transcription factor binding to putative GY118F downstream targets

Consideration of publicly available ChIP-seq data to predict putative binding of candidate GY118F downstream targets by STAT3 and pluripotency transcription factors Oct4, Sox2, Nanog, Klf4 and Esrrb. Transcription factor binding profiles of the genes were clustered using the Pearson correlation coefficient as a metric and complete linkage clustering. Gray or blue shading represents the fraction of all considered transcription factors that bind to the gene.



Supplementary Figure S4 GY118F induces naïve pluripotency despite a culture environment that instructs and maintains a primed state

(**a**) GY118F iPS cells derived and maintained in Activin plus Fgf plus GCSF cultured in N2B27 plus 2i, N2B27 plus GCSF or in the presence of inhibitors for Activin and Fgf signalling maintain pluripotency as confirmed by the continued expressing of Klf2. Error bars indicate ± 1 s.d. (n=3). (**b**) Flow cytometry analysis and phase and fluorescent images showing loss of ΔPE-Oct4 GFP expression for GY118F iPS cells that were passaged in

N2B27 plus Activin plus FGF in the absence of GCSF. The white bar corresponds to 129µm. **(c)** Phase and fluorescent images showing the emergence of Nanog-GFP positive colonies from GY118F-tnga EpiSCs cultured in N2B27 plus Activin plus FGF plus GCSF. White bars correspond to 161.4µm. (**d**) Nanog-GFP positive cells isolated and maintained in EpiSC culture conditions supplemented with GCSF. (**e**) qRT-PCR analysis for naïve pluripotency and EpiSC marker genes. Gene expression and associated error bars representing s.d. (n=3) were normalised to EpiSCs. The white bar corresponds to 129µm.

Supplementary Table S1 Applied Biosystems taqman probes:

Gene name:	Probe ID:
GAPD	4352339E
mSocs3	Mm01249143_g1
FGF4	Mm00438917_m1
Nr0b1	Mm00431729_m1
Nanog	Mm02384862_g1
Rex1	Mm03053975_g1
Klf4	Mm00516104_m1
Klf2	Mm01244979_g1
FGF5	Mm00438919_m1
Lefty1	Mm00438615_m1
Lefty2	Mm00774547_m1
T	Mm01318252_m1
Tcfap2c	Mm00493473_m1
Nodal	Mm00443040_m1
Gadd45g	Mm00442225_m1
Gadd45b	Mm00435123_m1

Supplementary Table S2 Applied Biosystems custom taqman probes:

Name:	Sequence:
Retroviral Oct4-F	TGGTACGGGAAATCACAAGTTTGTA
Retroviral Oct4-R	GGTGAGAAGGCGAAGTCTGAAG
Retroviral Oct4-probe	FAM-CACCTTCCCCATGGCTG-MGB
Retroviral Klf4-F	TGGTACGGGAAATCACAAGTTTGTA
Retroviral Klf4-R	GAGCAGAGCGTCGCTGA
Retroviral Klf4-probe	FAM-CCCCTTCACCATGGCTG-MGB
Retroviral c-Myc-F	TGGTACGGGAAATCACAAGTTTGTA
Retroviral c-Myc-R	GGTCATAGTTCCTGTTGGTGAAGTT
Retroviral c-Myc-probe	FAM-CCCTTCACCATGCCCC-MGB
Retroviral Sox2-F	TGGTACGGGAAATCACAAGTTTGTA
Retroviral Sox2-R	GCCCGGCGGCTTCA
Retroviral Sox2-probe	FAM-CTCCGTCTCCATCATGTTAT-MGB
Endogenous Oct4-F	TTCCACCAGGCCCCC
Endogenous Oct4-R	GGTGAGAAGGCGAAGTCTGAAG
Endogenous Oct4-probe	FAM-CCCACCTTCCCCATGGCT-MGB

Supplementary Table S3 Primers used with SYBR green:

Gene name:	Primer sequence:
GAPD-F	cccactaacatcaaatgggg
GAPD-R	ccttccacaatgccaaagtt
Stat3-F	ggaaataacggtgaaggtgct
Stat3-R	catgtcaaacgtgagcgact
Ier5l-F	catccactggcttctaccg
Ier5l-R	cgtgagtgtccaggtcca
Gadd45g-F	ccgtggccaggatacagttc
Gadd45g-R	tcgttgaagctgcggctctc
Fzd5-F	cagcaggatcctccgaga
Fzd5-R	cagcactcagttccacacca
Gpr146-F	agagaaagcattcggcaaag
Gpr146-R	ccagacacacagatgaacgtc

Wnt8a-F	actgcggctgtgacgagt
Wnt8a-R	cccgaactccacgttgtc
Tal2-F	cgctgcgacagctacctt
Tal2-R	tggtcatgtccaggttcctt
Sbno2-F	gggacttccctccacatga
Sbno2-R	agtgcaggagtcggttctgt
Cish-F	gacatggtcctttgcgtaca
Cish-R	atgccccagtgggtaagg
Prickle1-F	tgctcaggagatccaagtcc
Prickle1-R	ctctcttcaaagtgatacgc
Dusp4-F	gtacctcccagcaccaatga
Dusp4-R	gaggaaagggaggatttcca