

Supplementary Material

Chih-I Tai et al. doi: 10.1242/bio.20149514

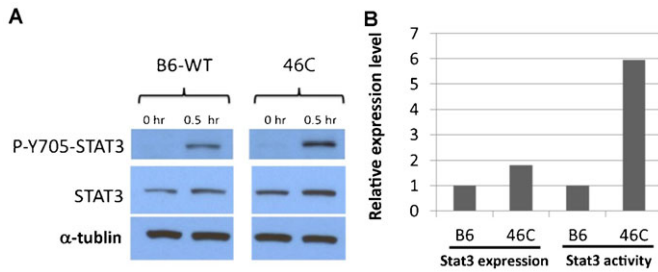


Fig. S1. Total and phosphorylated Stat3 levels in B6 and 46C mESCs. (A) Western blot analysis of phospho-Stat3 (Tyr705) and total Stat3 expression levels in B6-WT and 46C mESCs treated with or without LIF for 30 mins. B6-WT and 46C mESCs were starved in serum free medium for overnight before the treatment. (B) Relative expression levels of total and phosphorylated Stat3 in B6-WT and 46C mESCs treated with LIF for 30 mins. The Stat3 expression levels were normalized to α -tubulin.

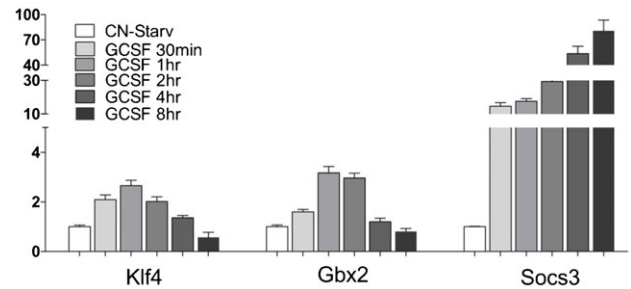


Fig. S3. qPCR analysis of the expression of Stat3 target genes in B6-S3Y118F mESCs treated with GCSF for the indicated times.

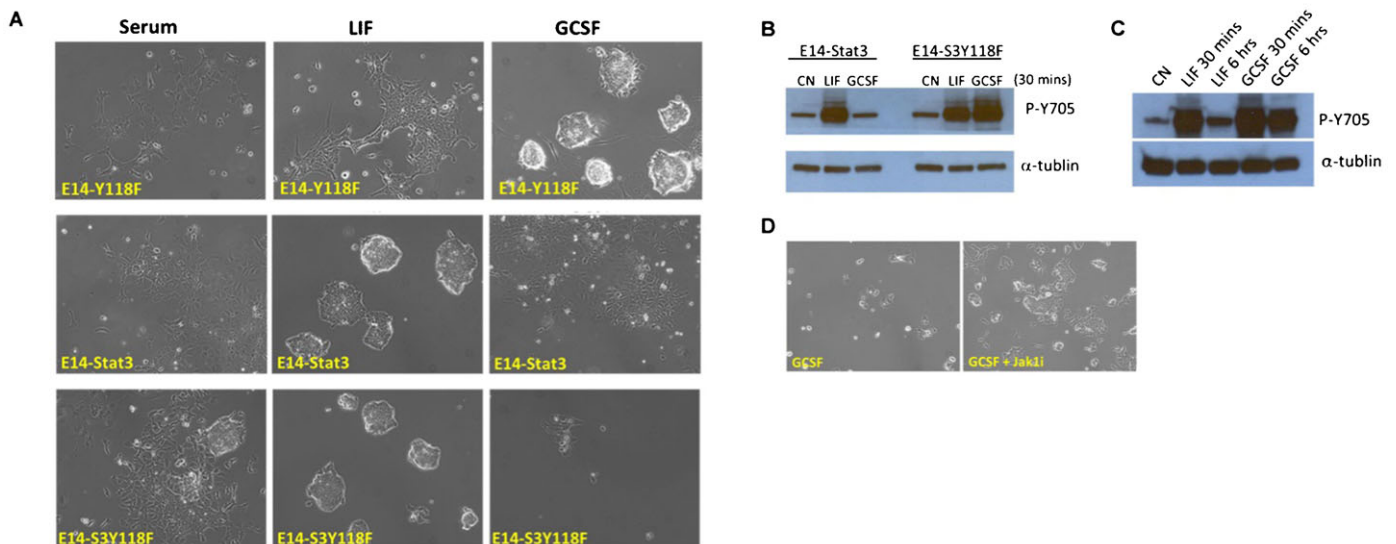


Fig. S2. Hyperactivation of Stat3 induces differentiation of E14-S3Y118F mESCs. (A) Phase contrast images of E14-Y118F, E14-Stat3, and E14-S3Y118F mESCs cultured in mESC medium only or mESC medium supplemented with LIF or GCSF. Images were taken 6 days after plating. (B) Western blot analysis of phospho-Stat3 (Tyr705) levels in E14-Stat3 and E14-S3Y118F mESCs treated with LIF or GCSF for 30 mins. Both E14-Stat3 and E14-S3Y118F mESCs were starved in the serum free medium for overnight before the treatment. (C) Western blot analysis of phospho-Stat3 (Tyr705) levels in E14-S3Y118F ESCs treated with LIF or GCSF for 30 mins or 6 hours. The mESCs were starved in the serum free medium for overnight before the treatment. (D) Phase contrast images of E14-S3Y118F ESCs cultured in GCSF or GCSF plus JAK1i for 3 days.

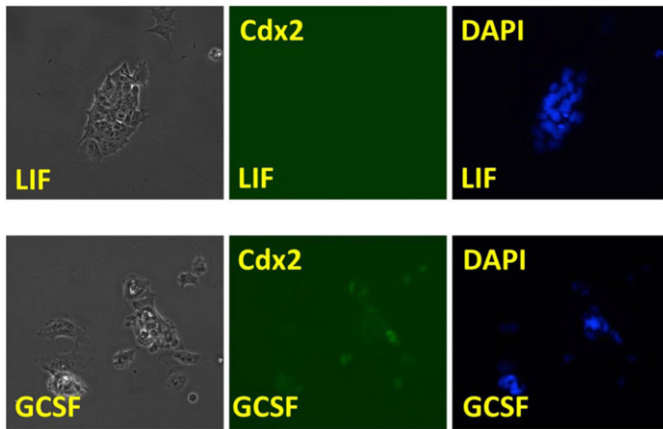


Fig. S4. Immunofluorescence staining of Cdx2 in B6-S3Y118F mESCs cultured in the presence of LIF or GCSF for 48 h.

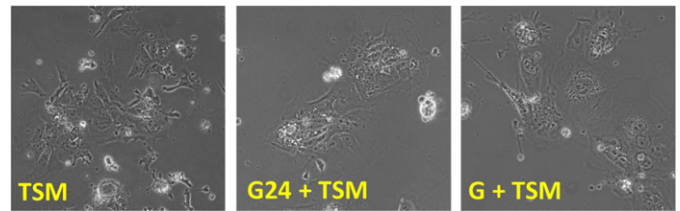


Fig. S5. GCSF induces B6-S3Y118F mESC differentiation towards terminally differentiated TE cells. Left panel: phase contrast image of B6-S3Y118F mESCs cultured in trophoblast stem cell medium (TSM) for 5 days; middle panel: phase contrast image of B6-S3Y118F mESCs treated with GCSF for 24 h and then cultured in TSM for 5 days; right panel: phase contrast image of B6-S3Y118F mESCs cultured in TSM supplemented with GCSF for 5 days.

Table S1. The list of primers used for qRT-PCR analysis

Symbol	Primer sequence for RT-PCR (5'→3')	Symbol	Primer sequence for RT-PCR (5'→3')
Gbx2	F: TCGCTGCTCGCTTTCTCT R: GGGTCATCTTCCACCTTTGA	Nestin	F: CTCGAGCAGGAAGTGGTAGG R: TTGGGACCAGGGACTGTTAG
Klf4	F: CGAACTCACACAGGCGAGAA R: CGGAGCGGGCGAATTT	Sox1	F: GGCCGAGTGAAGGTCATGT R: TCCGGGTTCCTTCATGTG
Esrrb	F: AACAGCCCCCTACCTGAACCT R: CTCATCTGGTCCCCAAGTGT	Mixl1	F: TTGAATTGAACCCTGTTGTCCC R: GAAACCCGTTCTCCCATCCACC
Oct4	F: GAAGCAGAAGAGGATCACCTTG R: TTCTTAAGGCTGAGCTGCAAG	T	F: CCGGTGCTGAAGGTAAATGT R: CCTCCATTGAGCTTGTTGGT
Sox2	F: ATGGGCTCTGTGGTCAAGTC R: CCCTCCAATTCCTTGTAT	Eomes	F: GGCAAAGCGGACAATAACAT R: AGCCTCGTTGGTATTTGTG
Gata4	F: TCTCACTATGGGCACAGCAG R: GCGATGTCTGAGTGACAGGA	Cdx2	F: ACCGGAATTGTTTGCTGCTGT R: TCCCGACTTCCCTTACCACAT
Gata6	F: TCCTCCCCTGCCGAAGTC R: AGGGCCAGAGCACACCAA	Dlx3	F: TACTCGCCCAAGTCGGAATA R: AGTAGATCGTTCGCGGCTTT
FoxA2	F: CCTCAAGGGAGCAGTCTCAC R: TTTCTCCTGGTCCGGTACAC	Esx1	F: GAGCTGGAGGCCTTTTTCCA R: ACACCCACAGGGGGACTCAT
Sox17	F: AGCCATTTCTCCGTGGTGT R: AACACTGCTTCTGGCCCTCAG	Gata3	F: GGGCTACGGTGACAGAGGTAT R: TGGATGGACGTCTTGGAGAA
Psx1	F: CGATGGATGGGTGTGGATGA R: TGACAGGGCTGGCACTCAAG	Tfap2c	F: ATCCCTCACCTCTCCTCTCC R: CCAGATGCGAGTAATGGTCCG
Gapdh	F: TGTGAGGGAGATGCTCAGTG R: TGTTCTACCCCAATGTGT		