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Table S1. Primers used in this study.

Gene	Forward Primer	Reverse Primer
oct4	AGTTGGCGTGGAGACTTTGC	CAGGGCTTTCATGTCCTGG
nanog	TTGCTTACAAGGGTCTGCTACT	ACTGGTAGAAGAATCAGGGCT
sox2	GCTCGCAGACCTACATGAAC	GCC TCGGACTTGACCACAG
socs3	CAAGAACCTACGCATCCAGTG	CCAGCTTGAGTACACAGTCGAA
stat3	TGGCACCTTGGATTGAGAGTC	GCAGGAATCGGCTATATTGCT
gp130	ATCAGAGTGGGCAACAGAGAA	TGAGGAGACCTTCCCAAGGG
lif-r	ACCTCCTCCTTACTACTGAAGTG	TTCCGTCCTTGGATTCTGTAGA
klf2	CTCAGCGAGCCTATCTTGCC	CACGTTGTTTAGGTCCTCATCC
klf4	TCCTTTCCAACTCGCTAACCC	CGGATCGGATAGCTGAAGCTG
pecam1	ACGCTGGTGCTCTATGCAAG	TCAGTTGCTGCCCATTCATCA
e-cadherin	CAGGTCTCCTCATGGCTTTGC	CTTCCGAAAAGAAGGCTGTCC
tbx3	AGGAGCGTGTCTGTCAGGTT	GCCATTACCTCCCCAATTTT
rex1	CCCTCGACAGACTGACCCTAA	TCGGGGCTAATCTCACTTTCAT
fgf5	TGTGTCTCAGGGGATTGTAGG	AGCTGTTTTCTTGGAATCTCTCC
alp	CAGTATGAATTGAATCGGAACAACC	CAGCAAGAAGAAGCCTTTGAGG
dppa3/stella	GACCCAATGAAGGACCCTGAA	GCTTGACACCGGGGTTTAG
dazl	ATGTCTGCCACAACTTCTGAG	CTGATTTCGGTTTCATCCATCCT
stra8	ACAACCTAAGGAAGGCAGTTTAC	GACCTCCTCTAAGCTGTTGGG
nr0b1/dax1	GGGCAGCATCTTATACAGCTTG	CACTCTGGGTACAGTAGGACAG
fbxo15	TCGTGGGACTGAGCACAACTA	TGACAGATGAGCCTCTAACAAAC
piwil2	TTGGCCTCAAGCTCCTAGAC	GAACATGGACACCAAACCTACA
gbx2	CAACTTCGACAAAGCCGAGG	ACTCGTCTTTCCCTTGCCCT
gdf3	ATGCAGCCTTATCAACGGCTT	AGGCGCTTTCTCTAATCCCAG
tdgf1	CAGTGCGTTTGAATTTGGACC	GCACGAACTGGAAAGACCGA
c-myc	TCTCCATCCTATGTTGCGGTC	TCCAAGTAACTCGGTCATCATCT
nodal	GTGACCGGACAGAACTGGAC	GGAAAATGTCAATGGTGAGTGGG
acvr2b	ACCCCCAGGTGTACTTCTG	CATGGCCGTAGGGAGGTTTC
gal	GGCAGCGTTATCCTGCTAGG	CTGTTCAGGGTCCAACCTCT
cldn6	ATGGCCTCTACTGGTCTGCAA	GCCAACAGTGAGTCATACACCTT
otx2	TATCTAAAGCAACCGCCTTACG	AAGTCCATACCCGAAGTGGTC
ebaf	CCAACCGCACTGCCCTTAT	CGCGAAACGAACCAACTTGT
lefty2	CAGCCAGAATTTTCGAGAGGT	CAGTGCGATTGGAGCCATC
foxa2	CCCTACGCCAACATGAACTCG	GTTCTGCCGGTAGAAAGGGA
eomes	GGCCCCTATGGCTCAAATTCC	CCTGCCCTGTTTGGTGATG
dkk1	CTCATCAATTCCAACGCGATCA	GCCCTCATAGAGAACTCCCG
gata6	GGCAGTGTGAGTGGAGGTG	TGGTACGTTCCGTTCAGCG
sox17	GATGCGGGATACGCCAGTG	CCACCACCTCGCCTTTCAC
cer1	CTCTGGGGAAGGCAGACCTAT	CCACAAACAGATCCGGCTT
pitx2	GCAGCCGTTGAATGTCTCTTC	GTCCGTGAACTCGACCTTTTT
acta2	GTCCCAGACATCAGGGAGTAA	TCGGATACTTCAGCGTCAGGA

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egr1	TCGGCTCCTTTCCTCACTCA	CTCATAGGGTTGTTCGCTCGG
gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
b-actin	GAAATCGTGCGTGACATCAAAG	TGTAGTTTCATGGATGCCACAG

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Table S2

Colony counts of ALP-positive CDEs (cells seeded at 10^5 unless otherwise noted)

CDE	CDE-μP	CDE-μP_{LIF}
0	11	28
3	24	32
6 (seeded @$7.5x10^4$)	26	44
7	28	59
8		70

Colony counts of ALP-positive 129S2C1a (cells seeded at 10^5 unless otherwise noted)

129S2C1a	129S2C1a-μP	129S2C1a-μP_{LIF}
0	57	301
1	166	433
8	176	455
		108 (seeded @ $5x10^4$)

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Figure S1

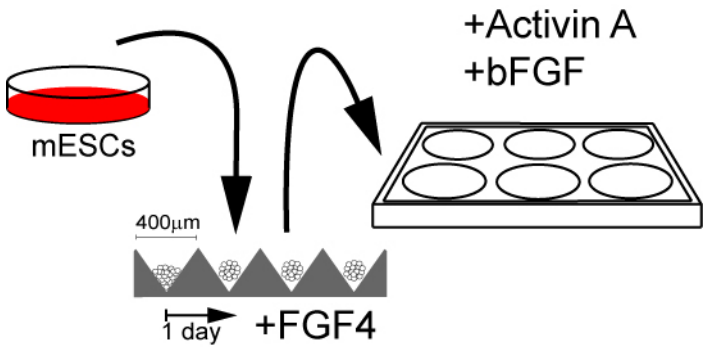

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Figure S2

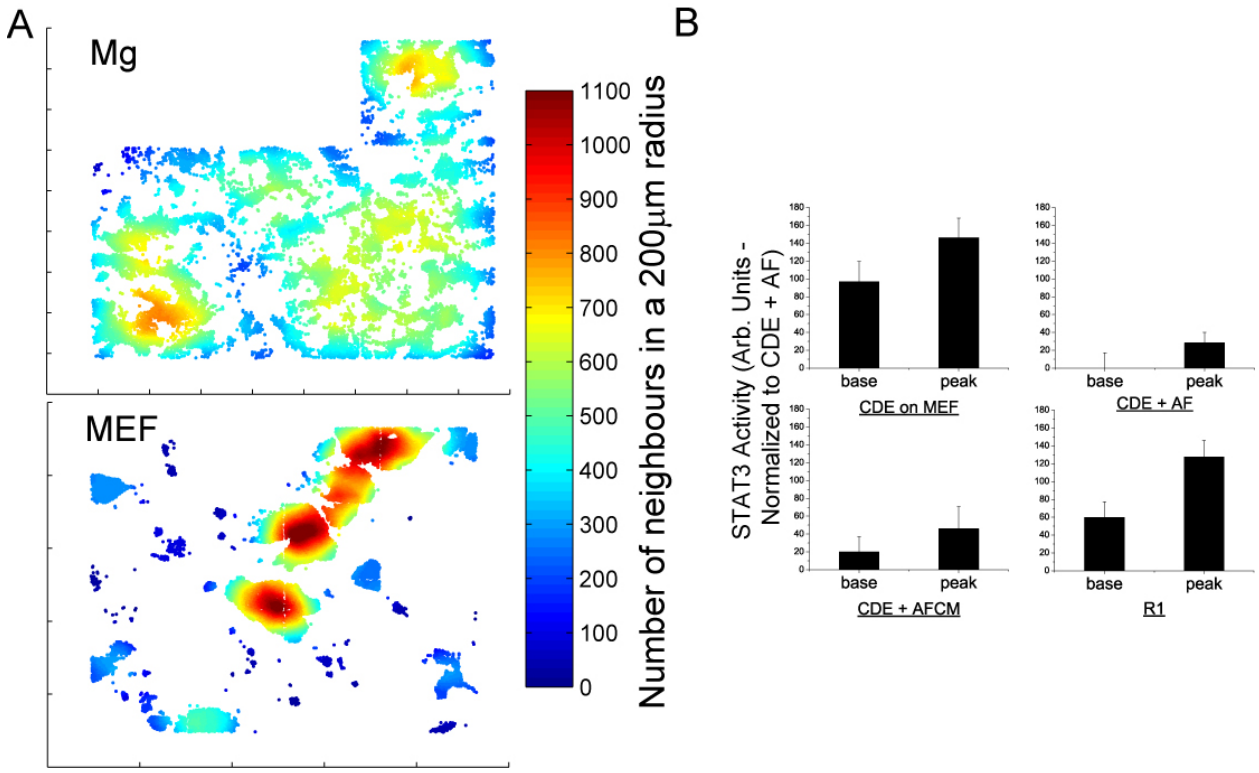

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Figure S3

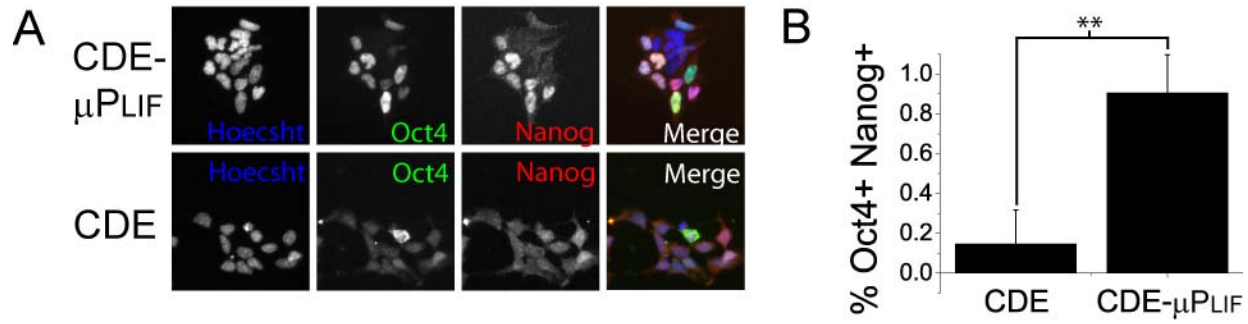

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Figure S4

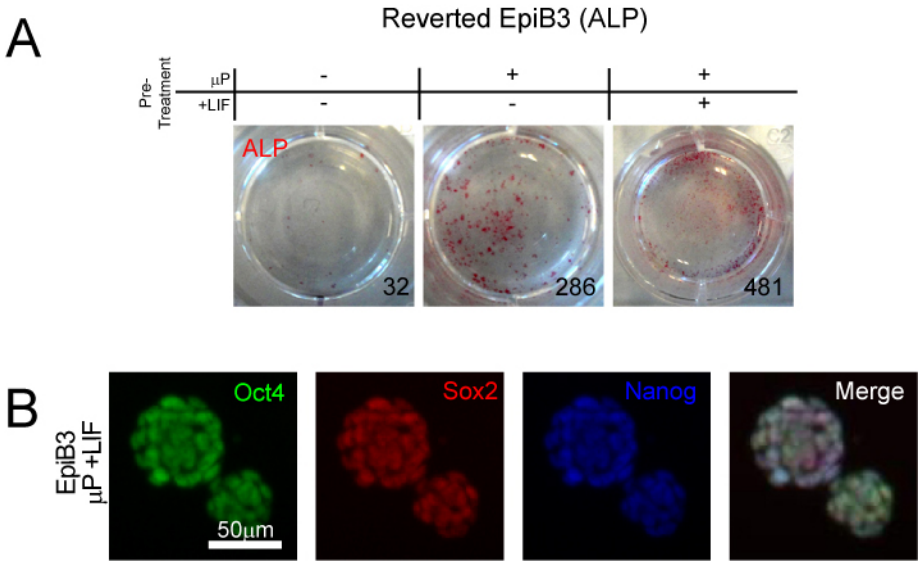

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Figure S1. Protocol for generation of CDEs. CDEs were derived from mESCs by size-controlled aggregation using microwells followed by culture in the microwells in the presence of 10ng/ml FGF4 for 1 day and then seeding onto Matrigel-coated plates in 20ng/ml Activin A and 20ng/ml bFGF.

Figure S2. CDEs cultured on MEFs demonstrate an increase in local cell density and increase in LIF responsiveness. A) Choropleth map of the number of neighbours in 200mm radius in Matrigel (Mg)-coated plates(top panel) vs. MEF co-culture (bottom panel) B) Quantification of pSTAT3 staining in CDEs cultured on MEFs (MEF), CDEs cultured on Mg in AF media alone (AF), CDEs cultured on Mg in MEF-conditioned AF media (AF_{CM}), and R1 mESCs (R1). Data represented as mean ± S.D. (n=4).\

Figure S3. Staining for Oct4 and Nanog predicts reversion frequency at 2 days following seeding. A) Micropatterned CDEs treated with LIF were plated into 2iL media (CDE-μP_{LIF}) and stained for Oct4 and Nanog after 2 days B) Quantification of Oct4/Nanog double positive cells. Data represented as mean ± S.D. (n=4) **p<0.01 as determined by two-sample t-test assuming equal variance.

Figure S4. Reversion of another embryo-derived EpiSC line, EpiB3. A) ALP staining of embryo-derived EpiB3 cells in 2iL following treatment with (+) or without (-) micropatterning (μP) and LIF. Colony counts listed in inset. B) Staining of revertant cells generated by μP_{LIF}-treated EpiB3s demonstrates robust expression of Oct4, Sox2 and Nanog.

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Supplementary Experimental Procedures

qRT-PCR

RNA was isolated using the RNeasy RNA isolation kit (Qiagen). Complimentary DNA (cDNA) was obtained from the RNA using SuperScript™ III Reverse Transcriptase (Invitrogen). cDNA was then loaded into 384-well plates (Applied Biosystems) along with primers (Supp. Table 1), and 2x Fast start SYBR green master mix (Roche). PCR was run for 40 cycles at 92°C, 60°C, and 72°C for 30s, 30s, and 15s, respectively on the ABI 7900HT Fast System (Applied Biosystems). Primer sequences were obtained from Primer Bank[1] or designed with Primer 3[2]. Data was normalized to a β-actin or GAPDH housekeeping control.

Immunocytochemistry

Prior to imaging, cells were fixed in 3.7% formaldehyde for 10 minutes at 37°C, permeabilized in methanol for 2 minutes at room temperature, and blocked in 10% fetal bovine serum (FBS) in PBS overnight at 4°C. Primary antibodies were then added and left overnight at 4°C. Antibodies for phospho-STAT3 (Cell Signal Technologies), Oct3/4 (BD biosciences), Nanog (eBiosciences), and Sox2 (R&D), and H3K27me3 (Millipore) were all used at a 1:200 dilution. Alexa-Fluor™ (Invitrogen) secondary antibodies and Hoecsht dye were added for 1 hour at room temperature diluted at 1:200 and 1:100, respectively. Stained cells were then imaged and fluorescence intensity was quantified using the Cellomics ArrayScan VTI high content screening platform (Thermo Fisher). Normalization was performed by first taking data from single-stained (e.g. Oct4 only) cells and measuring a bleed-through curve to determine the extent of background in the

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channel of interest (e.g. pSTAT3). Compensation was then performed to eliminate the background from other channels.

Alkaline Phosphatase Staining

To identify alkaline phosphatase (ALP) positive colonies, cells were fixed in 10% formaldehyde for 45m at room temperature then washed 3 times in PBS. Fixed cells were then incubated in a mix of Fast Red Violet LB salt (Sigma), Napthol AS-MX (Sigma), N,N-dimethyformamide (Sigma), and 0.1mM pH=8.3 Tris-HCl Buffer for 45m at room temperature in the dark. Cells were washed with PBS and kept in PBS for imaging or colony counting. Alternatively, following fixation, the Vector® Red Alkaline Phosphatase Substrate Kit I (VectorLabs) was used as described in the kit protocol.

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References

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2. S. Rozen and H. Skaletsky, *Methods Mol Biol,* 2000, *132*. 365-86.