Supplementary Table 1 Primary antibody used for immunocytochemistry

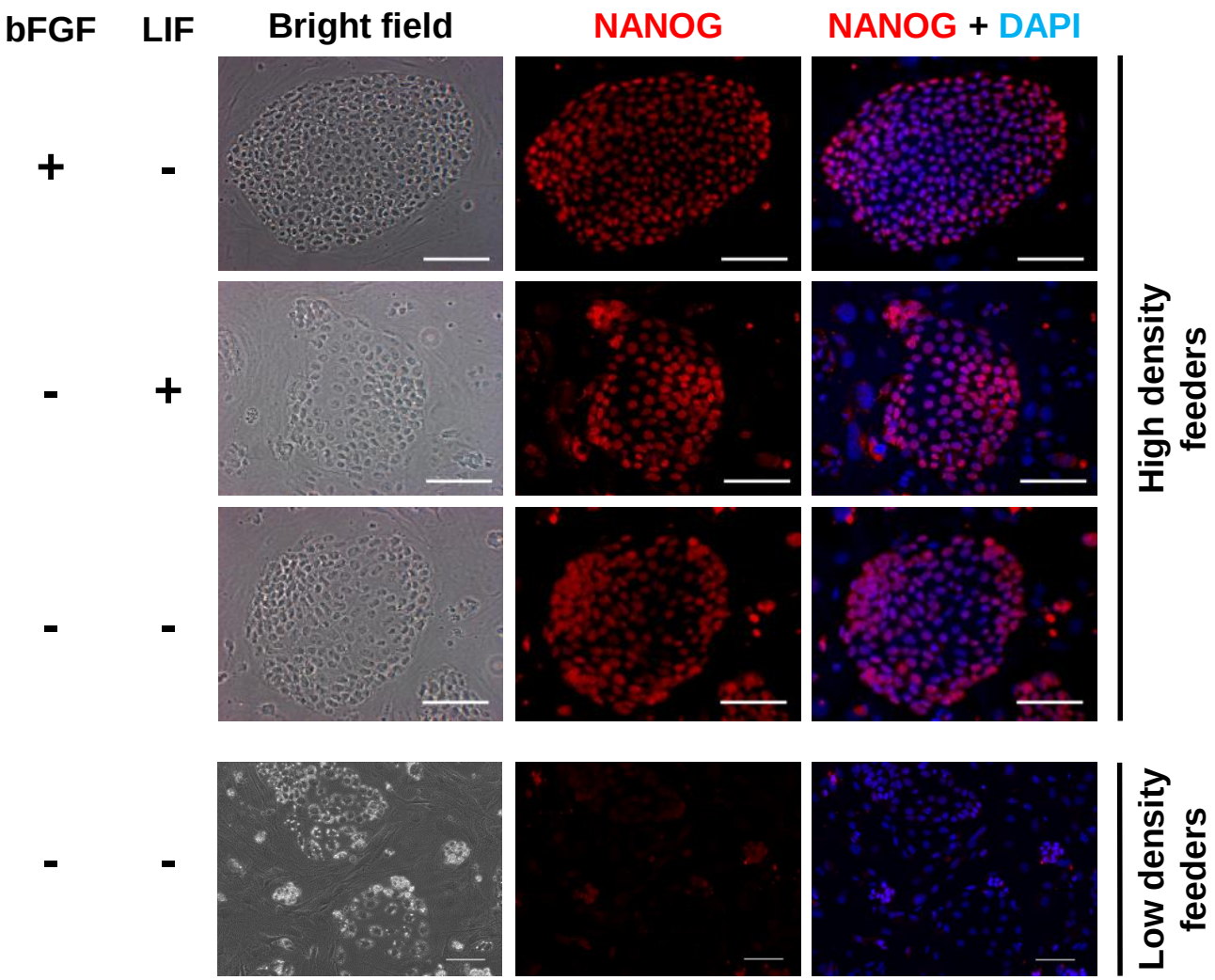
Antibody name	Company, catalog number
Human Nanog Affinity Purified Polyclonal Ab, Goat IgG	R&D Systems, AF1977
Oct-3/4 Antibody (C-10)	Santa Cruz, sc-5279
Oct-3/4 Antibody (N-19)	Santa Cruz, sc-8628
Sox-2 Antibody (H-65)	Santa Cruz, sc-20088
SSEA-4 Antibody (813-70)	Santa Cruz, sc-21704
TRA-1-60 Antibody (TRA-1-60)	Santa Cruz, sc-21705
Anti-TRA-1-81 Antibody, clone TRA-1-81	Millipore, MAB4381
Anti-Pax6 Antibody	Millipore, MAB5552
Polyclonal Rabbit Anti-Human Albumin/FITC	Dako, F0117

Supplementary Table 2 Primer sequences used to detect mRNA expression

Primer name	Primer sequence
FGFR1 sense	GAAGTTCAAATGCCCTTCCA
FGFR1 antisense	TCGATGTGCTTTAGCCACTG
FGFR2 sense	CCAAGAAGCCAGACTTCAGC
FGFR2 antisense	AACTCCCATTTTGGGTCCTC
FGFR3 sense	GTCCATGAGCTCCAACACG
FGFR3 antisense	CAGCATCTTCACAGCCACAG
FGFR4 sense	GGCCCCTGTATGTGATCGTA
FGFR4 antisense	GCCCAAAGTCAGCAATCTTC
LIFR sense	AGAGCAAGTTGTTGGGATGG
LIFR antisense	CACAGGAATGTCTTCCCATTT
gp130 sense	AAGCCCAATCCACCACATAA
gp130 antisense	TGATCCCACTTGCTTCTTCA
NANOG sense	TTCCTTCCTCCATGGATCTG
NANOG antisense	TCCTTGGCCAGTTGTTTTTC
T sense	GCCCTACCCCAGCCCCTACACTC
T antisense	CTCATGGGGAGCATGCTGGGATG
SOX17 sense	GGGGACATGAAGGTGAAGGG
SOX17 antisense	GCCGGTACTTGTAGTTGGGG
FOXA2 sense	TGCACTCGGCTTCCAGTATG
FOXA2 antisense	ACGACATGTTCATGGAGCCC
EOMES sense	TGAACGAGCCCTCAAAGACC
EOMES antisense	AGCGGGGTTGAGGTAAAGTG
GATA6 sense	AGGAATTCAAACCAGGAAACGA
GATA6 antisense	GGATTAGTGCTCTCTCCCGC
CER1 sense	GACCCAGTCCTTCATCCAGC
CER1 antisense	CATCCCAGCATGCTCCCCATGAG
ZNF521 sense	CTGCCAAACTTCAGTGCCAC
ZNF521 antisense	GCACTAACTGCTGTGTTGGG
MIXL1 sense	CTGCTGGAGCTCGTCTTTCG
MIXL1 antisense	CAGTTCCAGGAGCAGAGTGG
β-actin sense	CCAACCGTGAGAAGATGACC
β-actin antisense	CAGAGGCGTACAAGGAAAGC

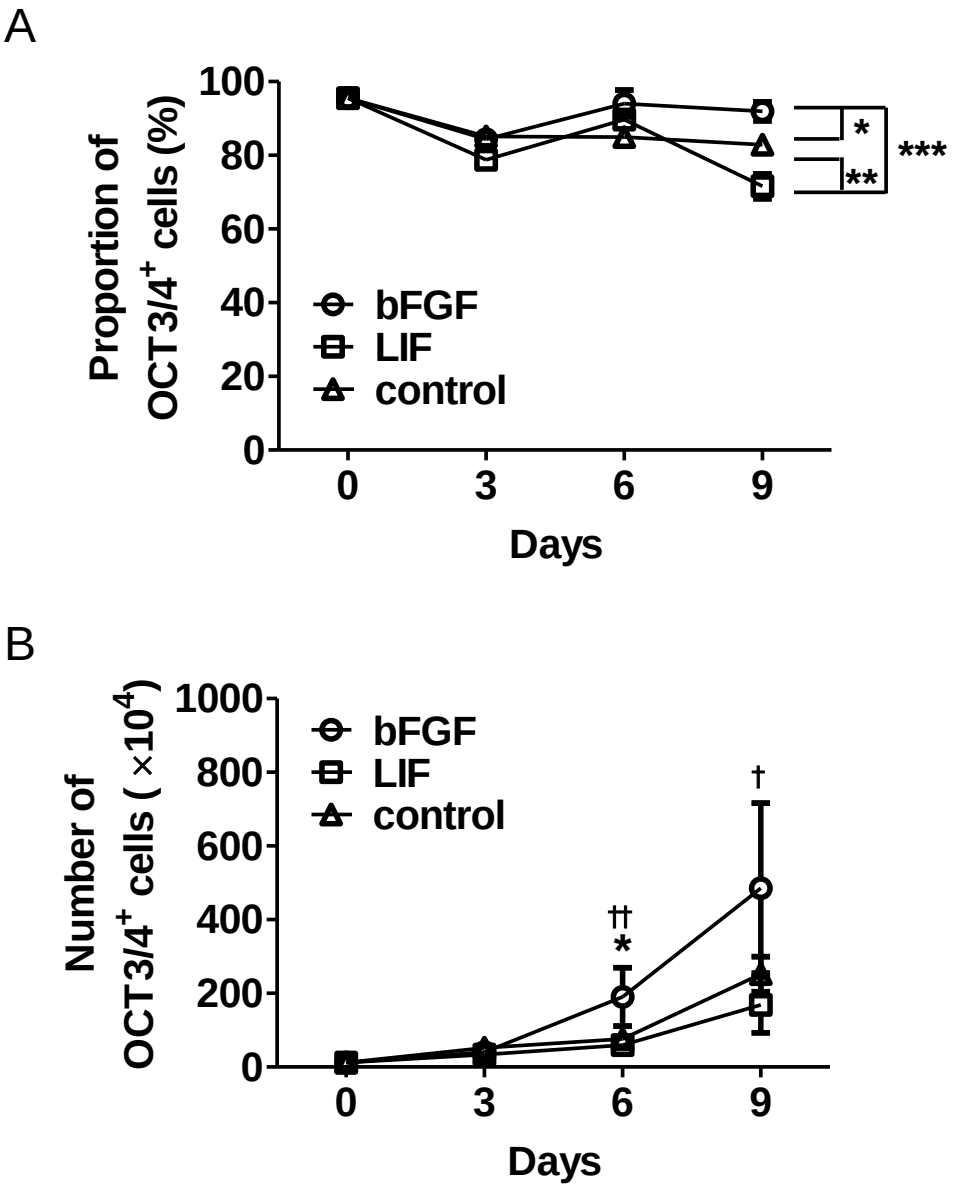
Supplementary Table 3 Primary antibody used for Western blotting analysis

Antibody name	Company, catalog number
Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb	Cell Signaling Technology, #4060
Akt (pan) (40D4) Mouse mAb	Cell Signaling Technology, #2920
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb	Cell Signaling Technology, #4370
p44/42 MAPK (Erk1/2) (L34F12) Mouse mAb	Cell Signaling Technology, #4696
p-Smad2/3 Antibody (Ser 423/425)	Santa Cruz, sc-11769
Smad2/3 (D7G7) XP® Rabbit mAb	Cell Signaling Technology, #8685
Phospho-Stat3 (Tyr705) (D3A7) XP® Rabbit mAb	Cell Signaling Technology, #9145
Stat3 (124H6) Mouse mAb	Cell Signaling Technology, #9139
Monoclonal Anti-α-Tubulin antibody produced in mouse	SIGMA-ALDRICH, T5168



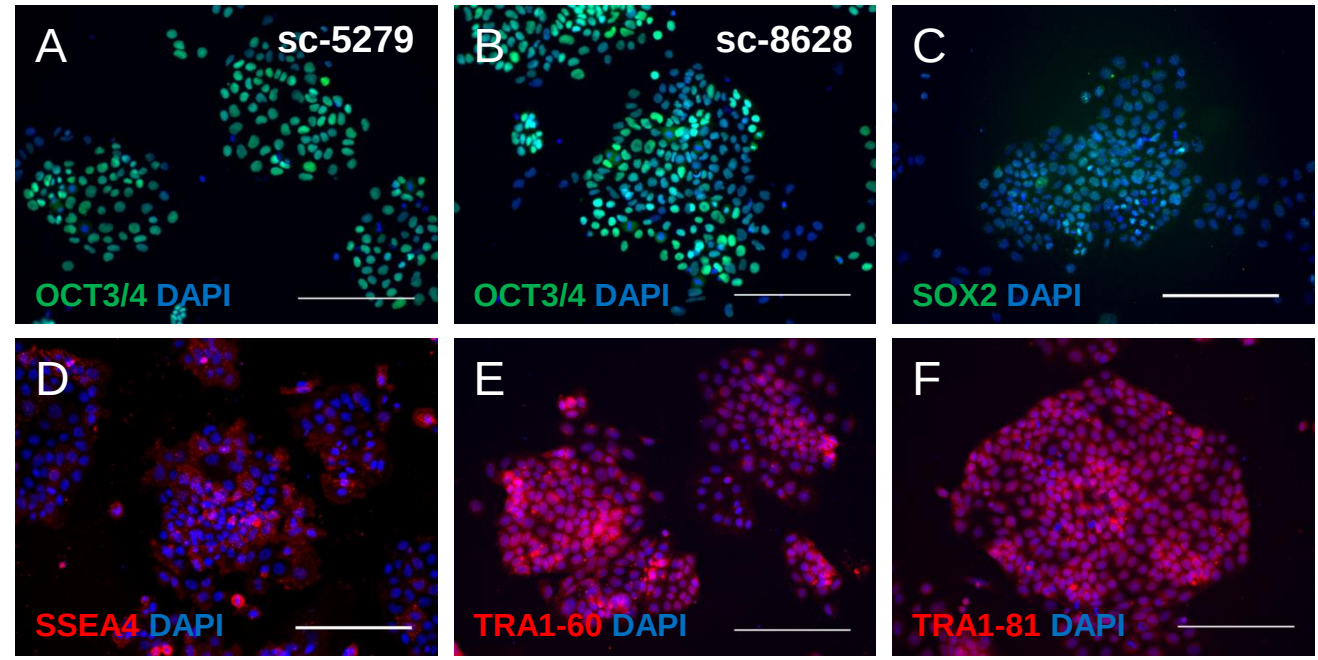
Supplementary Fig. S1 Morphological and immunocytochemical analyses.
CM ESCs (CM40) were cultured in the presence of the indicated growth factor (bFGF or LIF) on high-density feeder cells (3.5×10^4 cells/cm^2) for more than ten passages or on low-density feeder cells (1.0×10^4 cells/cm^2) for four passages. The cells were then fixed, stained, and analyzed by fluorescence microscopy. Bright field images (left), NANOG (red, middle), and merged images with DAPI staining (blue, right) are shown. Scale bars represent 100 μm. Note that CM ESCs were maintained on high-density feeder cells in the absence of bFGF or LIF even after ten passages, and it was lost on low-density feeders after four passages.

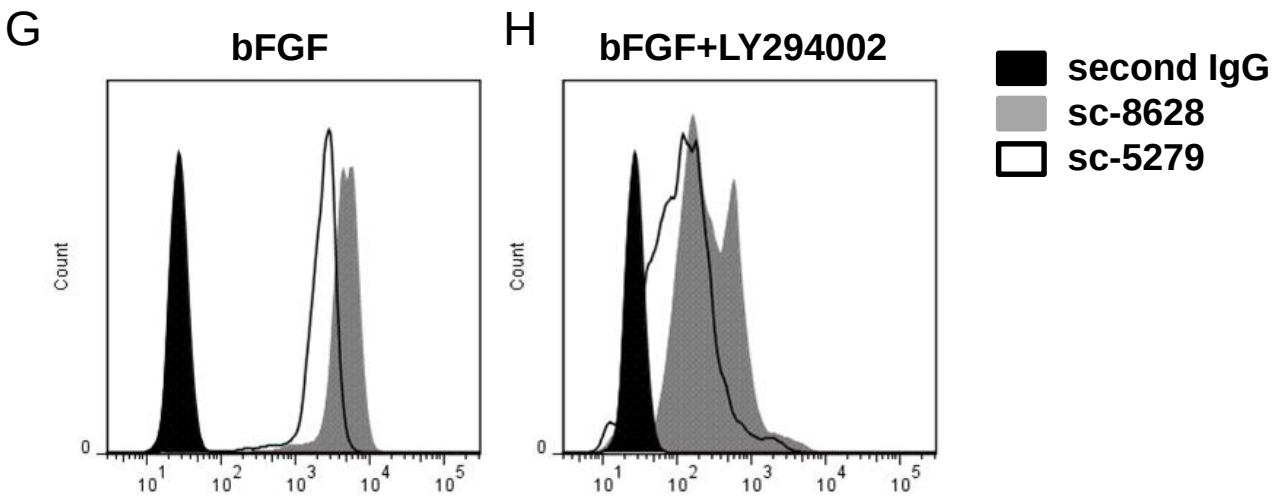
Supplementary Figure S1



Supplementary Fig. S2 bFGF promotes self-renewal of CM ESCs on feeder cells.
Cj11 cells (1.5×10^5) were seeded on MMC-treated MEFs and cultured with LIF (open square), bFGF (open circle), or without growth factors (open triangle). (A) The percentage of OCT3/4$^+$ cells was determined by FCM analysis. Data are shown as the mean±SD (n=4). One-way ANOVA followed by the Tukey's post-hoc test were used to test inter-group differences. $^*P<0.05$, $^{**}P<0.01$ and $^{***}P<0.005$. (B) Growth curves of OCT3/4$^+$ cells were generated by multiplying the number of live cells by the percentage of OCT3/4$^+$ cells and the passage ratio together. Data are shown as the mean±SD (n=4). One-way ANOVA followed by the Tukey's post-hoc test were used to test inter-group differences. $^*P<0.05$ (bFGF vs. control), $^†P<0.05$ (bFGF vs. LIF) and $^{††}P<0.01$ (bFGF vs. LIF).

Supplementary Figure S2

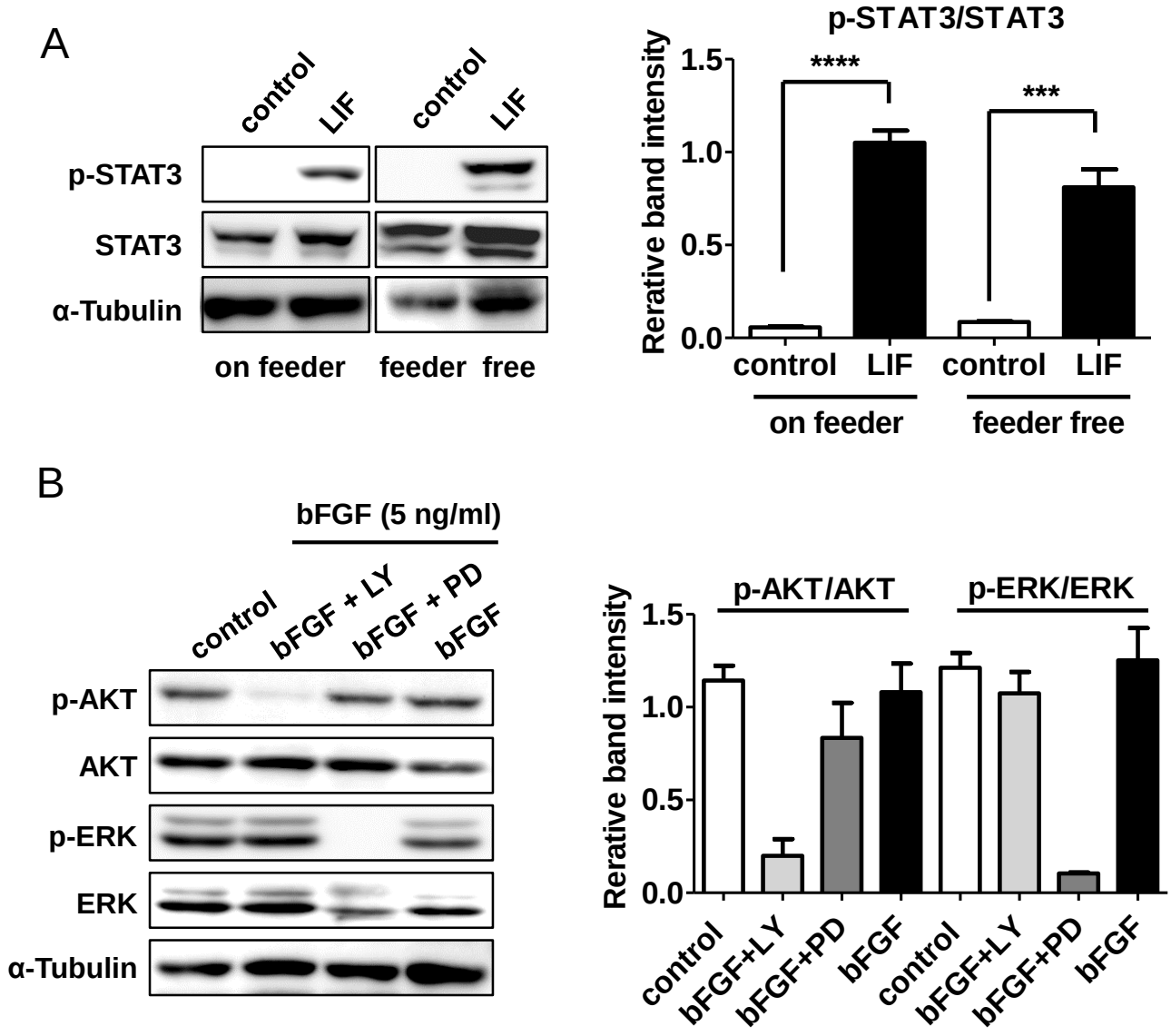




Supplementary Fig. S3 CM ESCs cultured under feeder free condition expressed pluripotent stem cell markers.

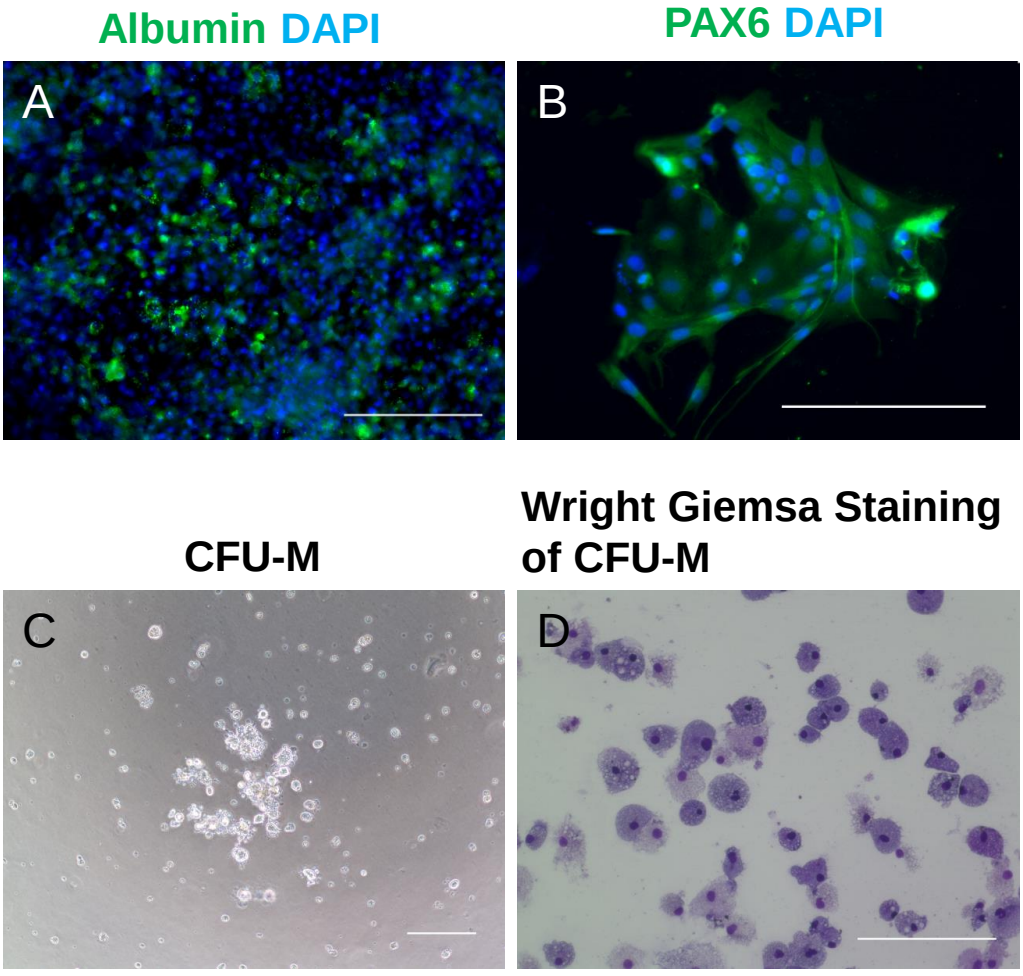
CM40 cells were cultured under feeder free condition for 4 days, and then cells were fixed and stained with OCT3/4 (A: sc-5279, B: sc-8628), SOX2 (C), SSEA4 (D), TRA1-60 (E) and TRA1-81 antibody (F). Scale bars represent 200 μm. (G and H) CM40 cells were seeded on MMC-treated MEFs and cultured with bFGF (G) or bFGF+LY294002 (H) for 9 days. The percentage of $OCT3/4^+$ cells was determined by FCM analysis. sc-5279: bFGF, 99.13±0.15%; bFGF+LY294002, 46.87±2.78%. sc-8682: bFGF, 99.03±0.31%; bFGF+LY294002, 50.90±2.26%. mean±SD (n=3). Note that sc-5279 and sc-8628 were raised against amino acids 1-134 and 1-19 of human OCT3/4, respectively.

Supplementary Figure S3



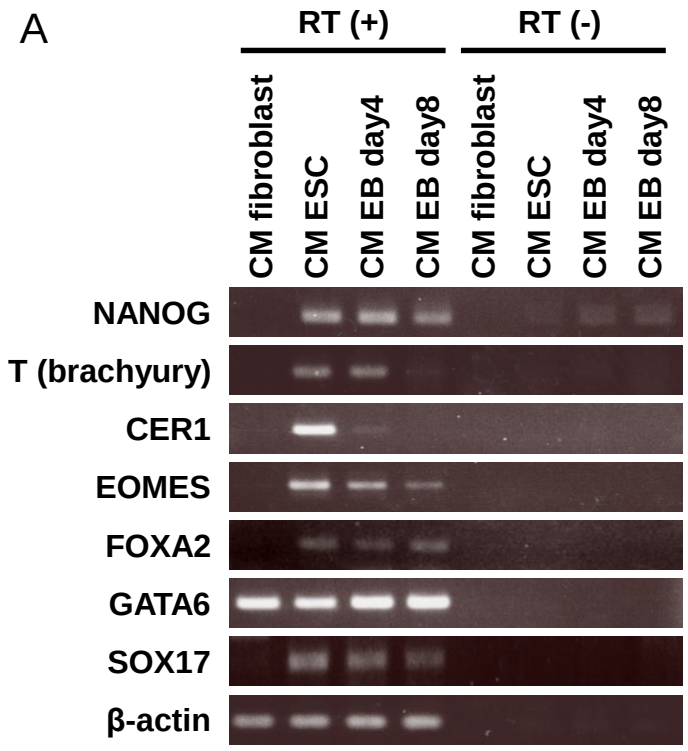
Supplementary Fig. S4 LIF activates the JAK-STAT3 pathway in CM ESCs.
Western blot showing the activation of STAT3 (A) , AKT and ERK1/2(B). (A) CM40 cells cultured on MEF feeders or under feeder free condition were starved of KSR overnight, and stimulated with LIF for 30 min. (B) CM40 cells cultured under feeder free condition were starved of KSR overnight, and stimulated with 5 ng/ml of bFGF for 30 min. The cells were pre-treated with LY294002 or PD0325901for 1 h before stimulation with bFGF. Equal amounts of whole cell extracts were analyzed by Western blotting. STAT3, AKT, ERK1/2 and α-Tubulin were used as loading controls. Band intensities were measured by ImageJ software. The relative band intensity of p-STAT3/STAT3, p-AKT/AKT and p-ERK/ERK are shown in right panels. Data are shown as the mean±SD (n=3). The Student's t-test was used to test inter-group differences of (A). $^{***}P<0.005$, $^{****}P<0.001$. One-way ANOVA followed by the Tukey's post-hoc test was used to test inter-group differences of (B). $^{**}P<0.01$ and $^{***}P<0.005$.

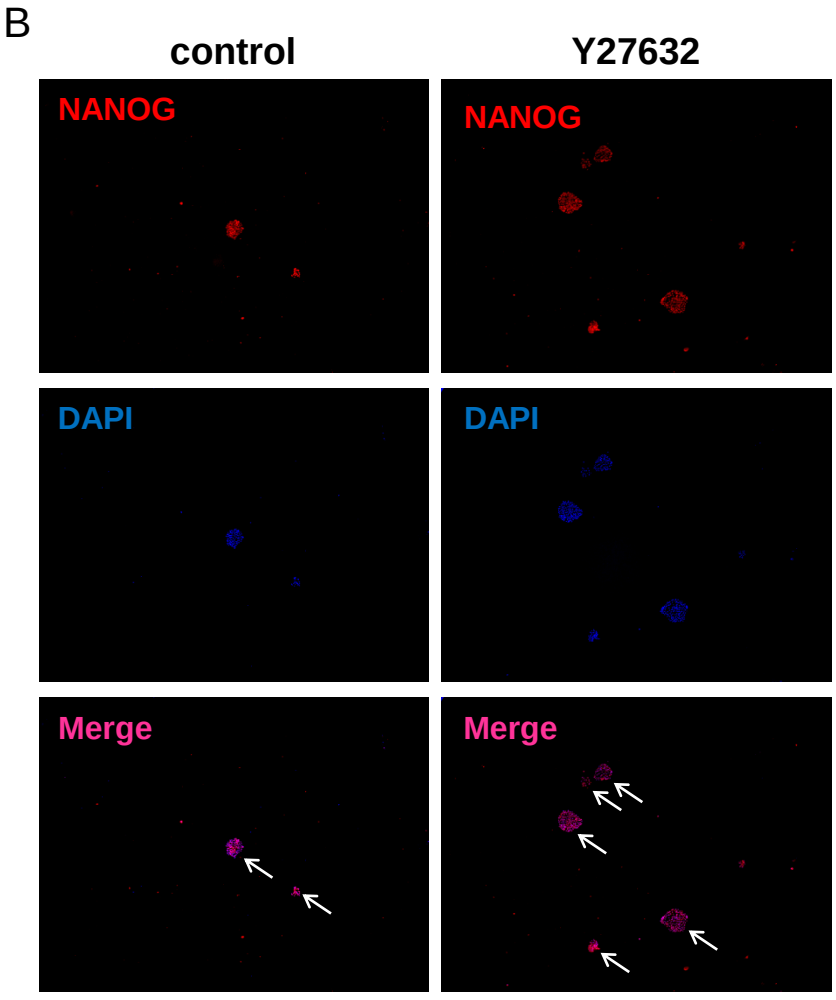
Supplementary Figure S4

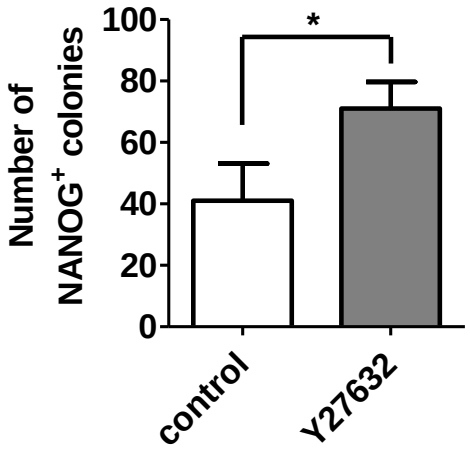


Supplementary Fig. S5 CM ESCs cultured under feeder free condition maintained pluripotency.
CM40 cells were cultured in CM ESC medium without bFGF on MEF feeders for more than ten passages, and then cultured under feeder free condition for four days. Then differentiation of the cells into three lineages was induced as described in Supplementary Materials and Methods. Then cells were stained with differentiation markers such as Albumin (A, hepatic cell marker, endoderm) and PAX6 (B, neuroepithelial cell marker, ectoderm). (C) Cells differentiated into hematopoietic cells showed the formation of colony forming unit-macrophage (CFU-M). (D) Wright Giemsa Staining of CFU-M. Scale bars represent 200 μm.

Supplementary Figure S5



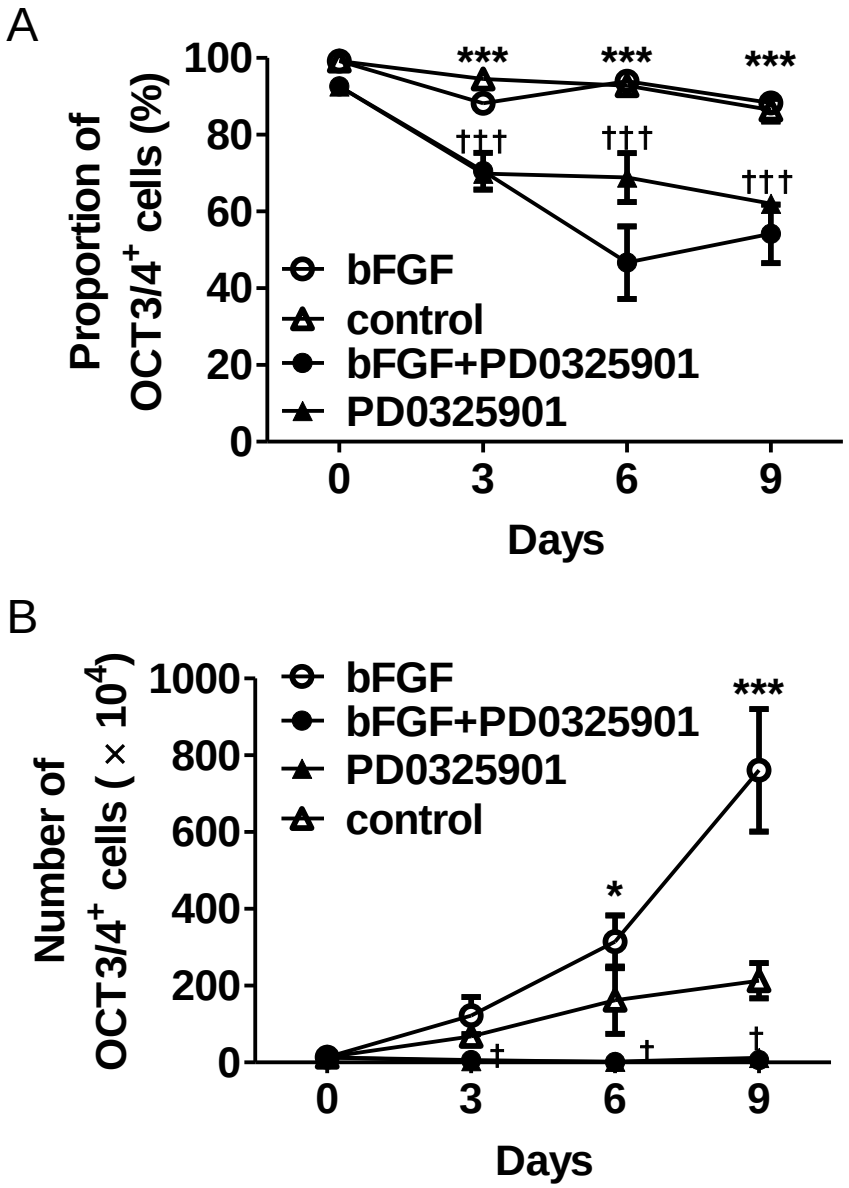




Supplementary Figure S6

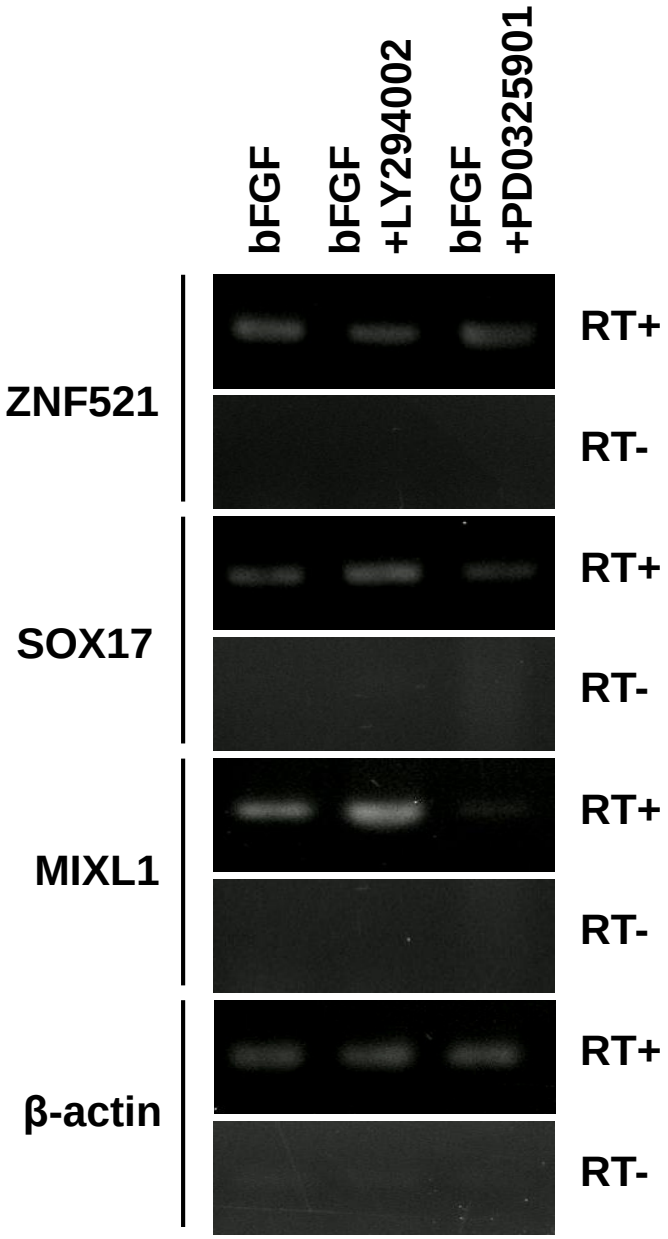
Supplementary Fig. S6 CM ESCs are phenotypically similar to human ESCs and mouse EpiSCs
(A) Semi-quantitative RT-PCR analysis showing the expression of markers for human ESCs and mouse EpiSCs in CM fibroblasts, CM ESCs and CM ESC derived embryoid bodies (CM EBs). CM fibroblasts and CM EBs were used as controls. (B and C) Treatment with the ROCK inhibitor Y27632 significantly inhibited dissociation-induced apoptosis of CM ESCs. CM ESCs (CM40; 300 cells) cultured on feeder cells were trypsinized, and then CM ESC colonies were isolated by passing the cell suspension through a 40 μm filter. CM ESCs were then completely dissociated by extensive pipetting, and the resulting single cell suspension was seeded on Matrigel-coated dishes and cultured in Essential 8 medium with or without 10 μM Y27632 for 6 days. Live colonies were then fixed and stained for NANOG (red) and with DAPI (blue). White arrows indicate $NANOG^{+}$ colonies. Note that almost all of the colonies were positive for NANOG in Y27632-treated cultures and in the controls. Data are shown as the mean±SD. The Student's t-test was used to test inter-group differences. $^{*}P<0.05$.

Supplementary Figure S6



Supplementary Fig. S7 MEK-ERK pathway is required for self-renewal of CM ESCs. (A and B) CM40 cells (1.4×10^5) were seeded on MMC-treated MEFs and cultured in medium containing bFGF with or without a MEK inhibitor (PD0325901) (bFGF, open circle; control, open square; bFGF+PD0325901, closed circle; PD0325901, closed square). (A) The percentage of OCT3/4$^+$ cells was determined by FCM at the indicated days. Data are shown as the mean±SD. One-way ANOVA followed by the Tukey's post-hoc test was used to test inter-group differences. $^{***}P < 0.005$, bFGF vs. control;$^{†††}P < 0.005$, bFGF+ PD0325901 or PD0325901 vs. control. (B) The number of live cells was counted by trypan blue exclusion. Growth curves were generated by multiplying the number of live cells by the percentage of OCT3/4$^+$ cells and the passage ratio together. Data are shown as the mean±SD. One-way ANOVA followed by the Tukey's post-hoc test was used to test inter-group differences. $^{*}P < 0.05$, $^{***}P < 0.005$, bFGF vs. control;$^{†}P < 0.05$, bFGF+ PD0325901 or PD0325901 vs. control.

Supplementary Figure S7



Supplementary Fig. S8 Inhibition of PI3K or MEK affected the expression of germ layer specific genes in CM ESCs.
CM40 cells were seeded on MMC-treated MEFs and cultured in medium containing bFGF with or without LY294002 or PD0325901 for 6 days. Then RNAs were extracted from the cells, and RT-PCR was performed using the primers for ZNF521 (ectoderm), SOX17 (endoderm) and MIXL1(mesoderm). Two independent experiments showed the similar results. β-actin was used as control.

Supplementary Figure S8