Supplementary information, Data S1 Methods

Human ESC Culture

Human ESC lines HUES8 (passage 40-60) and H9 (passage 35-50) were used in this study. Routinely, H9 was cultured on mitomycin C-inactivated mouse embryonic fibroblasts (MEFs) in human ESC medium (DMEM/F12, 20% (vol/vol) Knockout Serum Replacement, 1% (vol/vol) non-essential amino acids, 1% (vol/vol) GlutaMax, 1% (vol/vol) penicillin-streptomycin, 55 μM β-mercaptoethanol supplemented with 8 ng/ml FGF2 (PeproTech)), whereas HUES8 was cultured in Matrigel-coated dishes with mTeSR1 medium according to the manual (STEMCELL Technologies). All cell culture media and supplements in this study were purchased from Gibco/Invitrogen, unless specifically stated.

Immunofluorescence staining

Culturing cells in chambered slides (Millipore) were fixed by 4% PFA for 15 minutes and blocked with 10% (vol/vol) donkey serum plus 0.2% Triton X-100 in PBS for 2 hours. Then samples were incubated at 4 °C for overnight with primary antibody or irrelevant IgG and then incubated at room temperature for 1 hour with appropriate secondary antibody (donkey anti-mouse IgG Tritc or donkey anti-goat IgG Alexa-488 or donkey anti-rabbit Tritc) in blocking buffer. Cells were counterstained with 4'-6-diamidino-2-phenylindole (DAPI) for 5 min before mounting with Vectorshield (Vector Laboratories). The used primary antibodies and dilutions were listed as follows: Goat anti-human FOXA2 (R&D, 1:500), Goat anti-human SOX17 (R&D, 1:500), Goat anti-human NANOG (R&D, 1:400), Rabbit anti-SOX2 (Millipore, 1:400), Mouse anti-human SSEA4 (Millipore, 1:200).

Cell Proliferation Assays

For MTT assay, the same amount cells (2000-5000 cells) were seeded in each well of Matrigel-coated 96-well plate with mTeSR1 medium and assayed at different day. Before the assay, cells were washed with PBS and then cultured with 100 μl Pheno Red-free medium. 10 μl of MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide, 5 mg/ml, Invitrogen) solution was added to each well containing cells and incubated for four hours at 37C. After the removal of medium, 50 μl of dimethyl sulfoxide (DMSO) was added to dissolve the particles and absorbance was measured at 540 nm using spectrophotometer FLUOstar Omega (BMG Labtech).

Data analysis

All data are representative of three or more experiments. Errors are standard error values of averaged results. A two-sided Student's t-test was performed and values of $p<0.05$ were taken as a significant difference between means.