Supplementary information, Data S1
Supplemental Methods

Preparation of mouse MEFs and TTFs.

For MEF isolation, the head and visceral tissues were removed from embryonic day (E) 13.5 embryos. The remaining bodies were minced and cultured in DMEM containing 10% FBS. To establish TTFs, the tails of 10-week-old male mice were peeled, minced into small pieces, and cultured in DMEM containing 10% FBS. MEF and TTF cells were used at passage 3 for iPS cell generation. All mouse experiments were approved by the Animal Care Committee at the College of Life Science and Biotechnology, Korea University, and were performed in accordance with government and institutional guidelines and regulations.

AP staining and immunostaining.

AP staining was performed using the Alkaline Phosphatase Detection Kit (Chemicon/Millipore) according to the manufacturer's instructions. Immunostaining was performed as described previously [1]. The primary and secondary antibodies used are listed in Supplementary information, Table S4.

Western blot analysis.

Total protein was extracted using RIPA buffer containing protease inhibitor cocktail (Roche). The proteins were separated by SDS-PAGE on a 4–12% gradient-precast gel and transferred onto PVDF membranes (Millipore). The membrane was incubated with the indicated primary antibody (Supplementary information, Table S4) followed by HRP-conjugated secondary antibodies against mouse, rabbit, or goat IgG. The secondary antibodies were detected with the Super Signal West Pico Kit (Pierce).

RT-PCR and Real-Time PCR. RNA was prepared from samples using TRIzol (Invitrogen), and cDNA was generated using Reverse Transcriptase II (Invitrogen), both according to the manufacturer's instructions. To amplify various marker genes, 25 ng of cDNA was used with PCR primers (Bioneer) under the conditions outlined in Supplementary Table 3. Real-time RT-PCR was conducted using the iCycler iQ (Bio-Rad). Reactions were performed using SYBR Green PCR Master Mix (Bio-Rad). As an internal control, glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) levels were quantified in parallel with target genes. Normalization and fold changes were calculated using the ΔΔCt method [2].

FACS analysis.

MEFs, mES cells, and iPS cells were trypsinized and added to FACS tubes at 1×10^6 cells/tube (BD Biosciences). After three rinses with cold buffer solution, the cells were incubated at 4ºC for 1 hr with primary antibody (Supplementary information, Table S4), washed with 1% FBS in PBS, resuspended in 100 μl of secondary antibody, and incubated for an additional 1 hr at 4ºC. The cells were then washed, fixed with fixative solution, and resuspended in PBS containing 1% FBS for FACS analysis.

Genotyping of iPS cells and chimeras.

Genotyping was performed on genomic DNA isolated from iPS cells, MEFs, and mouse tails using the Genomic DNA Purification Kit (Promega). PCR was carried out with 100–300 ng of DNA template, 10 pmol of primers (Supplementary information, Table S3), and PCR premix (Bioneer) in a thermocycler T3000 (Biometra) using the following program: a 5 min denaturation at 94ºC; 30 cycles of 94ºC for 30 s, 58ºC or 62ºC for 30 s, and 72ºC for 30 s; and a final 10 min extension at 72ºC.

Karyotype analysis.

To analyze iPS cell karyotype, iPS cells were cultured in proliferation medium as described above. Cell division was blocked in metaphase by the addition of 0.05 μg/ml colcemid (Gibco/Invitrogen) for 1–2 hr. The chromosomes were then visualized by G-band staining. At least 100 cells in metaphase were analyzed, and a minimum of 10 cells per cell line were karyotyped.

References

1 Yoon, B. S. *et al.* Optimal Suppression of Protein Phosphatase 2A Activity is Critical for Maintenance of Human Embryonic Stem Cell Self-renewal. *Stem Cells* (2010).

2 Miller, G. E. & Chen, E. Life stress and diminished expression of genes encoding glucocorticoid receptor and beta2-adrenergic receptor in children with asthma. *Proc Natl Acad Sci U S A* **103**, 5496-5501 (2006).