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## Supplemental Information

# H1foo Has a Pivotal Role in Qualifying Induced Pluripotent Stem Cells

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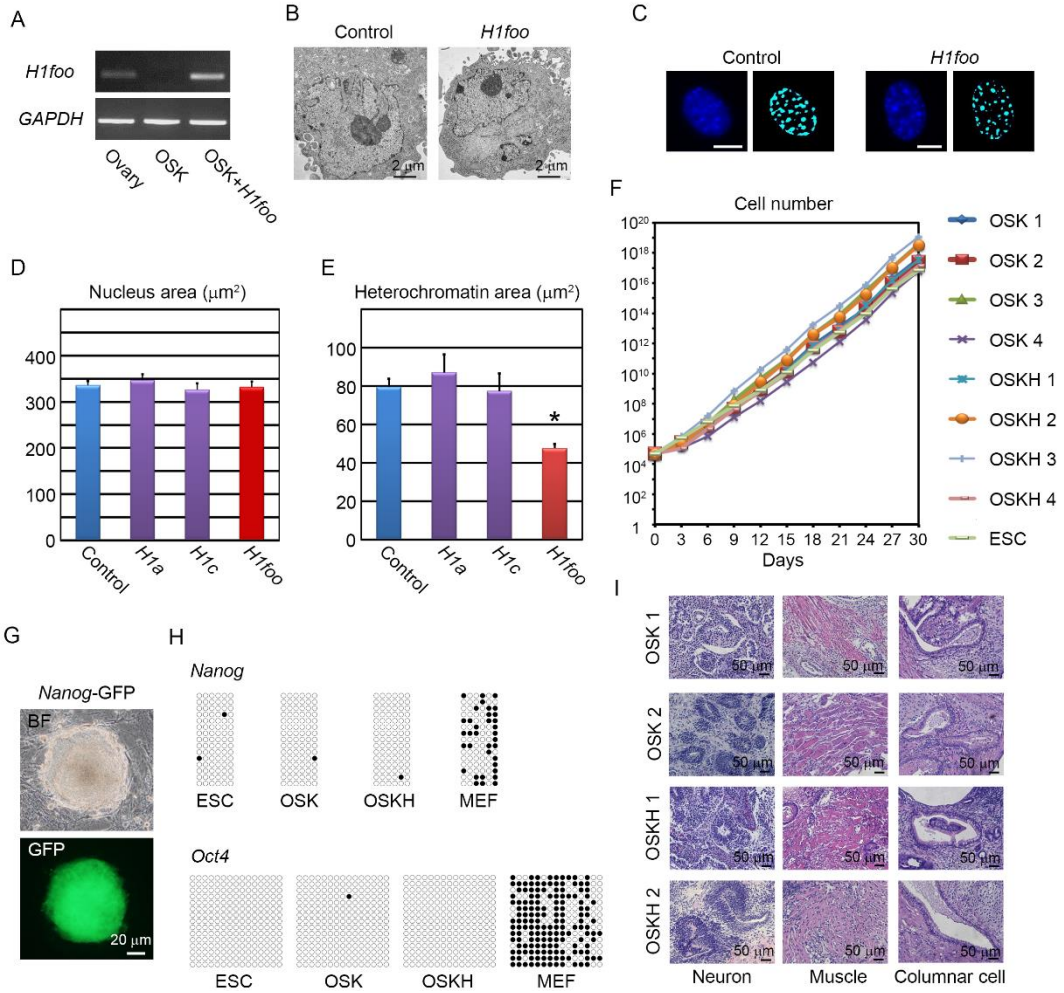
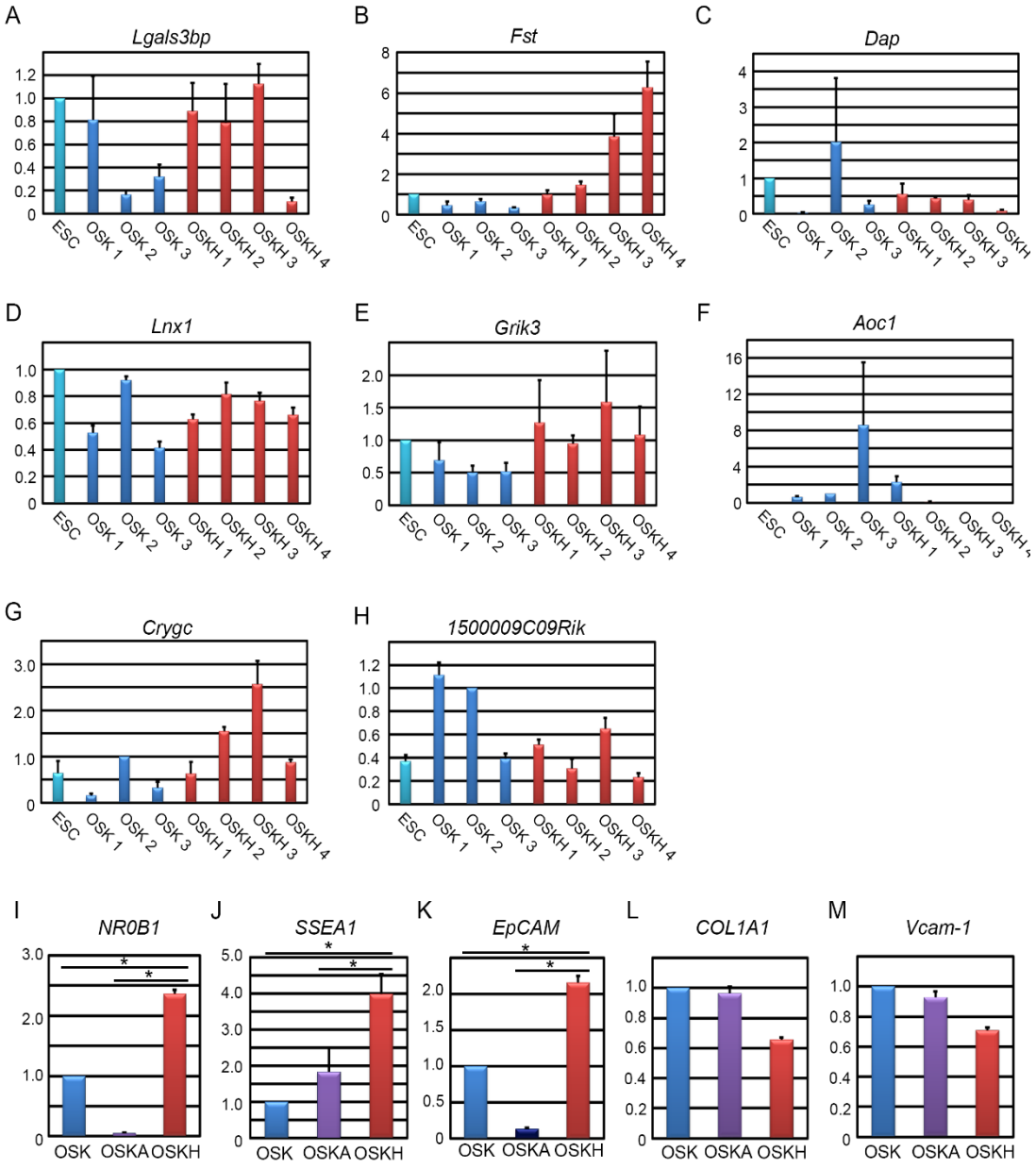
**Supplemental Figures**

**Figure S1 (Related to Figure 1). Characteristics of *H1foo* overexpressed fibroblasts and OSKH-induced iPSC generation**




(A) RT-PCR analysis of *H1foo* in the ovaries, OSK-overexpressed fibroblasts, and OSKH-overexpressed fibroblasts. (B) Electron microscope image of control and *H1foo* overexpressed-fibroblasts. Scale bar, 2 μm. (C) Nuclei stained with DAPI of control and H1foo overexpressed fibroblasts (left side), DAPI intense stained heterochromatin domains (right side). Scale bar, 50 μm. (D) Nucleus area of control, *H1a*, *H1c* and *H1foo* overexpressed fibroblasts Error bars represent the SEM (n=4 independent experiments, 25 nuclei per experiment). *P<0.05. (E) DAPI intense stained heterochromatin domains of control, *H1a*, *H1c* and *H1foo* overexpressed fibroblasts Error bars represent the SEM (n=4 independent experiments, 25 nuclei per experiment). *P<0.05. (F) Cell growth of each iPSC. (G) *Nanog*-GFP positive iPSC colony from adult mouse tale-tip fibroblasts. Scale bar, 20 μm. (H) Bisulfite genomic sequencing of the promoter regions of *Nanog* and *Oct4* in ESCs, OSK-iPSCs, OSKH-iPSCs, and MEFs. Open circles indicate unmethylated CpG dinucleotides, and filled circles indicate methylated CpG dinucleotides (n=3 independent experiments). (I) Teratoma formation from each iPSC. Scale bar, 50 μm.

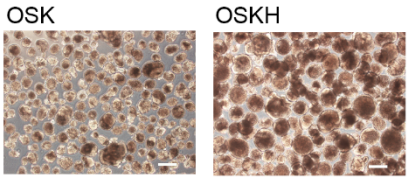
**Figure S2 (Related to Figure 2). Quantitative RT-PCR analysis of differentially expressed genes among ESCs, OSK-iPSCs and OSKH-iPSCs. Quantitative RT-PCR analysis of early reprogramming markers and source cell markers.**



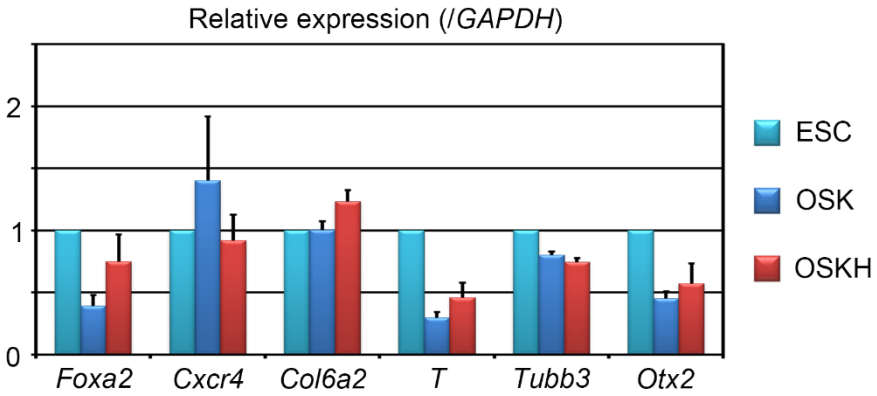

(A-H) Quantitative RT-PCR analysis of differentially expressed genes between OSK-iPSCs and OSKH-iPSCs. Error bars represent the SEM (n=3 independent experiments). *P<0.05. (I-K) Quantitative RT-PCR analysis of early reprogramming markers on day 5 after induction of reprogramming factors. Error bars represent the SEM (n=3 independent experiments). *P<0.05. (L, M) Quantitative RT-PCR analysis of fibroblast markers on day 5 after induction of reprogramming factors. Error bars represent the SEM (n=3 independent experiments).

**Figure S3 (Related to Figure 3). Characteristics of embryoid bodies generated from OSK-iPSCs and OSKH-iPSCs.**

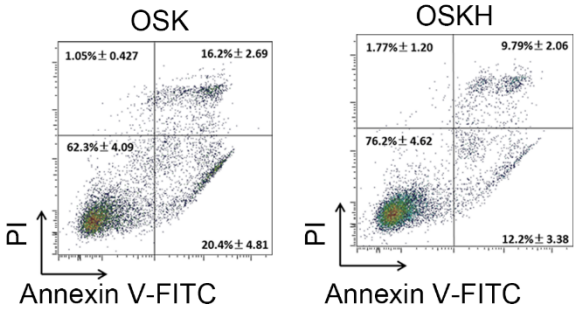
A




B




C

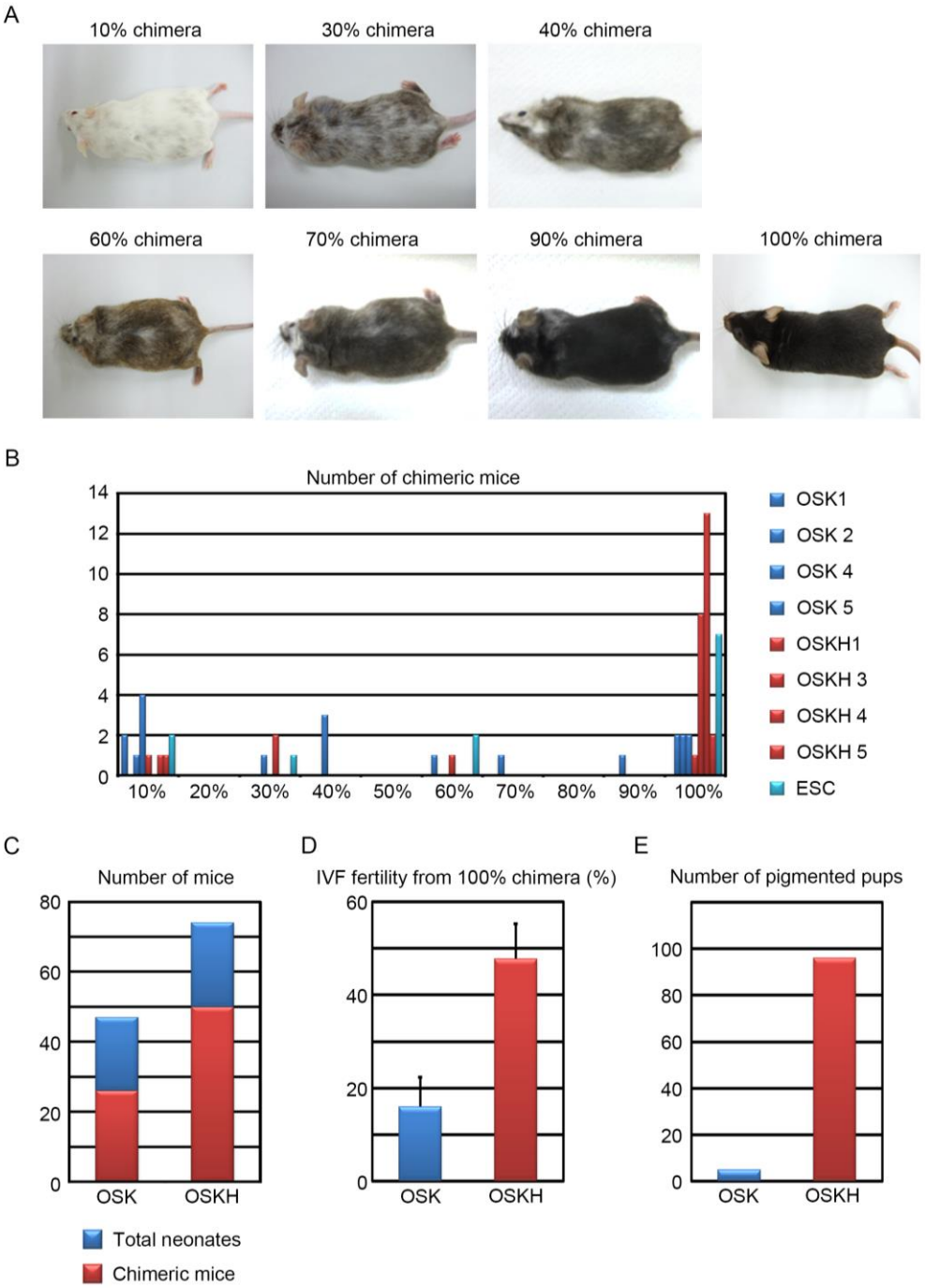



(A) EBs from each iPSC on day 5 after differentiation. Scale bar, 300 μm.

(B) The expression of endodermal markers (*Foxa2* and *Cxcr4*), mesodermal markers (*Col6a2* and *T*), and ectodermal markers (*Tubb3* and *Otx2*) was examined in ESCs and each iPSC on day 2 after differentiation. Error bars represent the SEM (n=3 independent experiments).

(C) Apoptotic cell distribution determined by FACS analysis of cells labeled with annexin V-FITC and propidium iodide (PI) on day 1 after differentiation induction (n=3 independent experiments).

**Figure S4 (Related to Figure 4). Chimeric mouse generation from ESCs and iPSCs**




(A) Images of representative chimeras with agouti coat color indicating iPSC origin. (B) Number of agouti coat colored chimeric mice derived from each iPSCs and ESCs. (C) Number of total neonates and chimeric mice from each four replicates of iPSCs. (D) IVF fertility from 100% chimeric mice derived from each four replicates of iPSCs. Error bars represent the SEM. (E) Number of pigmented pups of each coat color from 100% chimeric mice derived from each four replicates of iPSCs.

**Table S1.**

**Primers for qRT-PCR**

| | Forward | Reverse |
|---|---|---|
| *Nanog* | AGGGTCTGCTACTGAGATGCT | CAACACCTGGTTTTTCTGCCACCG |
| *Oct4* (Endogenous) | TCTTTCCACCAGGCCCCCGGCTC | TGCGGGCGGACATGGGGAGATCC |
| *Sox2* (Endogenous) | TAGAGATAGACTCCGGGCGATGA | TTGCCTTAAACAAGACCACGAAA |
| *Oct4* (Transgene) | CCCCAGGGCCCCATTTTGGTACC | CCCTTTTTCTGGAGACTAATAAA |
| *Sox2* (Transgene) | GGCACCCCTGGCATGGCTCTTGGCTC | TTATCGTCGACCACTGTGCTGCTG |
| *Rex1* | ACGAGTGGCAGTTTCTTCTTGGGA | TATGACTCACTTCCAGGGGGCACT |
| *Sall4* | CCCTGGGAACTGCGATGAAG | TCAGAGAGACTAAAGAACTCGGC |
| *H1foo* | GGCACAGGCTTTCTTTGTCT | TCCAACACAAGTACCCGACA |
| *Foxa2* | AGCACCATTACGCCTTCAAC | CCTTGAGGTCCATTTTGTGG |
| *Cxcr4* | CGGGATGAAAACGTCCATTT | ATGACCAGGATCACCAATCCA |
| *Col6a2* | CCACCACTGAAAGGAACAACAA | TCCAACACGAAATACACGTTGAC |
| *T* | TGTCCTCCCTTGTTGCCTTA | ATGTTCCAAGGGCAGAACAG |
| *β3-Tubulin* | CCCAGCGGCAACTATGTAGG | CCAGACCGAACACTGTCCA |
| *Otx2* | CCAAATCTACCCACCAAGGA | AGAGCTTCCAGAACGTCGAG |
| *Ki67* | TCTGATGTTAGGTGTTTGAG | CACTTTTCTGGTAACTTCTTG |
| *PCNA* | TAAAGAAGAGGAGGCGGTAA | TAAGTGTCCCATGTCAGCAA |
| *GAPDH* | TTCAACGGCACAGTCAAGG | CATGGACTGTGGTCATGAG |
| *Lgals3bp* | GCTGGAACTATGGCTTCTCG | GAGCCTTCAAAGCTGGTGAC |
| *FST* | GCAGCCGGAACTAGAAGTACA | ACACAGTAGGCATTATTGGTCTG |
| *Dap* | TTACCAGGCTGTGTCGCTA | TTATGGCTTTAAGGTCCCTTCCTA |
| *Lnx1* | ATGAAGGCGCTGCTGCTTCTGG | CGCTCTCA AGATGGCTGTCCTG |
| *Grik3* | TGGAACCCTACCGCTACTCG | TGCGACGCTCGCTGGTAGCA |
| *Aoc1* | GCGTGTTGCCTATGAGGTCAG | AAAGCATCCAGGAAAGTAGCG |
| *Crygc* | TGCTGCCTCATCCCCCAACA | TCGCCTAAAGAGCCAACTT |
| *1500009C09Rik* | TCATTACTTGACTTTGCACAC | GCAAAGGTGGCCATAGAACC |
| *Nr0b1* | ACCGTGCTCTTTAACCCAGA | CCGGATGTGCTCAGTAAGG |
| *SSEA1* | AGCTTTGCAGTGCACATCAC | AACCAGTCTGCCAAGTTGTG |
| *EpCAM* | AGGGGCGATCCAGAACAACG | ATGGTCGTAGGGGCTTTCT |
| *Col1a1* | GACGCCATCAAGGTCTACTG | ACGGGAATCCATCGGTCA |
| *Vcam1* | TGCCGAGCTAAATTACACATTG | CCTTGTGGAGGGATGTACAGA |

**Primers for bisulfite sequence**

| | | |
|---|---|---|
| *Oct4*-DMR | GGTTTTTTAGAGGATGGTTGAGTG | TCCAACCCTACTAACCCATCACC |
| *Nanog*-DMR | GATTTTGTAGGTGGGATTAATTGTGAATTT | ACCAAAAAAACCCACACTCATATCAATATA |

**Primers for pyrosequence**

| | | |
|---|---|---|
| *IG*-DMR | GTGGTTTGTTATGGGTAAGTTT | CCCTTCCCTCACTCCAAAAATTAA |
| *Gtl2*-DMR | AGTTATTTTTTGTTTGAAAGGATGTGTA | CTAACTTTAAAAAAAAATCCCCAACACT |

## Supplemental Experimental Procedures

### Generation of mouse iPSCs and cell culture

The generation of mouse iPSCs was based on a published protocol (Takahashi et al., 2007). We generated iPSCs from tale tip fibroblasts of C57BL/6J mouse using a pMXs retroviral vector with *Oct4*, *Sox2*, *Klf4*, and *H1foo* or *DsRed* as a control. The mouse ES cells (B6J-23$^{UTR}$) (Tanimoto et al., 2008) were obtained from the Laboratory Animal Resource Center at the University of Tsukuba and were used in accordance with the Guidelines for Derivation and Utilization of Human Embryonic Stem Cells of the Ministry of Education, Culture, Sports, Science, and Technology, Japan. These cell lines were cultured in mouse iPSC medium consisting of DMEM (Sigma-Aldrich, MO, USA) supplemented with 20% KnockOut Serum Replacement (Gibco, CA, USA), 1 mM GlutaMAX (Gibco), 1 mM Non-Essential Amino Acids (Sigma-Aldrich), 0.1 mM 2-mercaptoethanol, 50 U penicillin, 50 mg/ml streptomycin (Gibco), and mouse leukemia inhibitory factor. The iPSC and ESCs were maintained on irradiated mouse embryonic fibroblast (iMEF) feeder cells from wild-type ICR mice in mouse iPSC medium, which was changed every 2-3 days, and the cells were passaged using 0.5 mM trypsin-EDTA (Gibco) every 2-3 days.

### *Nanog*-GFP mouse

We generated iPSC from tale tip fibroblasts of *Nanog*-GFP-IRES-puro transgenic mice (Okita et al., 2007) using the same protocol as described above. The iPSCs generated with the *Nanog*-GFP transgene were positive for GFP, and we counted GFP positive ES-like colonies on day 21 after retroviral infection. Animal care was in accordance with the guidelines of Keio University for animal and recombinant DNA experiments.

### Plasmid construction

*H1foo* cDNA (Teranishi et al., 2004) was inserted into the BamH1 and Sal1 sites of the pMXs plasmid. *H1a* cDNA (Lin et al., 2013) and *H1c* cDNA (Teranishi et al., 2004) was inserted into the EcoR1 and Sal1 sites of the pMXs plasmid. The plasmids were confirmed by DNA sequencing.

### Embryoid body formation

The iPSCs and ESCs were harvested with 1 mg/ml collagenase IV, and we transferred $5\times10^4$ cells into 100 mm low attachment plates (AGC, Tokyo, JAPAN) containing differentiation medium. The differentiation medium consisted of Minimum Essential Medium Alpha Medium (Gibco) supplemented with 20% fetal bovine serum (Gibco), 2 mM GlutaMAX (Gibco), 0.1 mM Non-Essential Amino Acids (Sigma-Aldrich), 0.1 mM 2-mercaptoethanol, 50 U/ml penicillin and 50 mg/ml streptomycin (Gibco). The medium was replaced every 2-3 days. The time window of differentiation for analyzing the size and number of embryoid bodies was 5 days from the beginning of the differentiating conditions.

**Teratoma formation**

The iPSCs were injected into the testis of SCID mice (CLEA, Tokyo, JAPAN). Prior to injection, mice were anesthetized using a mixture of ketamine (50 mg/kg), xylazine (10 mg/kg), and chlorpromazine (1.25 mg/kg). Adequate anesthesia was maintained by monitoring the heart rate, muscle relaxation, and loss of sensory reflex response (i.e., no response to tail pinching) in mice. At approximately 8 weeks after injection, mice were sacrificed by cervical dislocation, and the teratomas were dissected, fixed in 10% paraformaldehyde (PFA) overnight, and embedded in paraffin. The sections were stained with hematoxylin and eosin. ~~All experiments were performed in accordance with the Keio University Animal Care Guidelines and were approved by the Ethics Committee of Keio University (20-041-4), which conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.~~

**Coculture aggregation of ESCs and iPSCs**

Two-cell embryos were collected from ICR (CD-1®) female mice that had been superovulated and mated naturally and were then cultured to the blastocyst stage. ESCs and iPSCs were dissociated with 0.25% trypsin just before coculture aggregation, and 15 to 20 cells were aggregated with 8-cell blastomeres from which the zonae pellucidae had been removed. Manipulated chimeric embryos were transferred at the blastocyst stage into the uterine horns of ICR pseudopregnant mice at 2.5 d postcoitus.

**Immunohistochemistry**

The iPSCs and fibroblasts plated on glass bottomed dishes (AGC) were washed once with PBS and fixed with 4% paraformaldehyde (MUTO Pure Chemicals, Tokyo, JAPAN) at 4°C for 15 min. After fixation, the cells were permeabilized with 0.5% Triton X-100 in PBS for 10 min at room temperature. After blocking with ImmunoBlock (DS Pharma Biomedical, Osaka, JAPAN) for 20 min, the cells were incubated at room temperature for 60 min with the primary antibodies, followed by washing with the blocking medium and incubation at room temperature for 60 min with the corresponding secondary antibodies. Immunostaining was performed with the following primary antibodies and reagents: Nanog (RCAB0001P, ReproCELL, Kanagawa, JAPAN), SSEA1 (sc-21702, Santa Cruz Biotechnology, CA, USA), H1foo (HPA037992, Sigma-Aldrich), and 6-Diamidino-2-Phenylindole (DAPI, Life Technologies, CA, USA). The secondary antibodies used included anti-rabbit IgG and anti-mouse IgG or IgM conjugated with Alexa Fluor 488 or Alexa Fluor 568 (Life Technologies). The signal was detected using a conventional fluorescence laser microscope equipped with a color charge-coupled device camera (BZ-9000 KEYENCE, Osaka, JAPAN), optical microscope (IX71, Olympus) and laser confocal microscope (LSM 510 META, Carl Zeiss, Jena, Germany).

**Electron microscopy**

Control and *H1foo* overexpressed fibroblasts were fixed with 2.5% glutaraldehyde in 60 mM HEPES, pH 7.4 for 2 hours at 4°C, and washed three times with 0.2 M Phosphate buffer. Secondary fixation was performed by 1% OsO4 in 60 mM HEPES, pH 7.4 for 120 min. at 4°C. For en bloc stain, 2% uranyl acetate was used for 30 min. at 4°C. Tissues were dehydrated with gradually increasing concentration of ethanol and embedded by plain resin, before sectioning and staining.

**Quantitative RT-PCR analysis**

Total RNA samples were isolated using the TRIZOL reagent (Life Technologies) according to the manufacturer's instructions. The concentration and purity of the RNA samples were determined with an ND-1000 spectrophotometer (Thermo Fisher Scientific, MA, USA), and cDNA was synthesized with ReverTra Ace qPCR RT Master Mix (TOYOBO, Osaka, JAPAN). Quantitative PCR (QT-PCR) was performed with a 7500 real-time PCR system (Life Technologies) with SYBR Premix ExTaq (TaKaRa, Shiga, JAPAN). The amount of mRNA was normalized to the amount of GAPDH mRNA. The primer sequences are listed in Table S1.

**Apoptosis assay**

On day 1 after initiating the differentiating conditions, embryoid bodies from iPSCs were trypsinized and suspended in annexin A5 binding buffer. Single cells were stained with annexin-A5-FITC and propidium iodide (BD, CA, USA) according to the manufacturer's protocol. Cells were filtered through 70 mm pore nylon membranes and analyzed by FACS Aria3 (BD) flow cytometry using CellQuest software (BD). Finally, the data obtained were analyzed with FlowJo software (Tree Star, CA, USA).

**DNA methylation analysis**

For the bisulfite sequence, genomic DNA was extracted from bulk cell culture samples by a SV Genomic DNA purification kit (Promega, Madison, WI, USA). Purified genomic DNA was used as the input for bisulfite conversion with an EZ DNA Methylation-Gold Kit (ZYMO RESEARCH, CA, USA) according to the manufacturer's protocol. For each bisulfite PCR, TaKaRa EpiTaq HS (TaKaRa) was used to catalyze amplification. PCR was performed with the following thermal cycling conditions: denaturation at 98°C for 10 min and 40 cycles, each cycle comprising incubation at 95°C for 20 sec, 55°C for 30 sec, and 72°C for 60 sec, followed by a final extension for 5 min at 72°C. The primers used for bisulfite PCR are listed in Table S1. For sequencing, the purified PCR products were TA-cloned into the pGEM-T vector (Promega). For pyrosequencing, genomic DNA was extracted and bisulfite converted as described above. PCR products were sequenced, and the methylation status of each locus was analyzed using PyroMark Q24 (QIAGEN, Venlo, Netherlands) following the manufacturer's instructions.

**Global gene expression analysis**

Total RNA was isolated from iPSCs and ESCs. Cyanine-labeled antisense RNA was amplified using the Quick Amp Labeling Kit (Agilent Technologies, CA, USA), hybridized with the Gene Expression Hybridization Kit onto a Whole Mouse Genome Oligo Microarray (Agilent Technologies), and analyzed using the Agilent Microarray Scanner. The data were analyzed with GeneSpring GX12.0 software (Agilent Technologies). ~~Microarray data are available through GEO accession number GSE79515.~~

**Accession number**

The accession number for the microarray data reported in this paper is GSE79515.

**Statistical analysis**

Values are reported as the means ± SEM. The data were analyzed using StatView J-4.5 software. Comparisons between two groups were performed with Student's *t*-test or Mann-Whitney test. Comparisons among groups were performed by one-way ANOVA with Bonferroni's *post hoc* test or Benjamini-Hochberg method. The probability level accepted for significance was * $P<0.05$, ** $P<0.01$.

**Supplemental References**

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