Supplemental Methods

In vitro development of mouse pre-implantation embryos

Embryos collected 2.5 days post-coitum in Medium 2 [Millipore] from oviduct and uterus of C57BL/6 female mice mated with DBA/2 male mice were cultured in KSOM [Millipore] for 1 DIV, permitting the embryos to develop into blastocysts. The cells to be assayed were injected into morula or blastocyst embryos; differences in results between the two were not appreciated. Blastocyst embryos were plated onto mitomycin C-treated mouse embryonic fibroblast (MEF)-coated dishes or Matrigel [Becton Dickinson]-coated dishes and were cultured in Connaught Medical Research Laboratories (CMRL) 1066-based medium supplemented with 1% non-essential amino acids (NEAA), 0.1mM β-mercaptoethanol, and 2mM Glutamax [all Life Technologies] (CMRL-based medium) and with sera. CMRL-based medium supplemented with 10% fetal bovine serum (FBS) was used from 1 DIV to 3 DIV. An equal volume of CMRL-based medium containing 30% FBS was added at 3 DIV to raise the final FBS concentration to 20%. After attachment at 4 DIV, medium was replaced with CMRL medium supplemented with 10% FBS and 30% rat IC (immediately centrifuged) serum [Charles River Laboratories Japan, Yokohama]. CMRL medium supplemented with 50% rat IC serum was used from 5 DIV and was exchanged daily. All media were placed in an incubator for 1-2 hours before feeding to adjust temperature and pH. Rat IC serum was supplemented with 2 mg/ml glucose. Stirred culture supported normal development beyond egg-cylinder stage.

Preparation of rodent ESC / iPSC lines

A *DsRed*-expressing mouse ESC line (EB3DR), a *tdTomato*-expressing rat ESC line (BLK-RT2) (Kobayashi et al., 2012), and a rat iPSC line (T1-3) (Hamanaka et al., 2011) were assayed. T1-3 cells were labeled with tdTomato as were hiPSCs. These rodent PSC lines were cultured in mouse ESC medium (DMEM / F12: Neurobasal medium supplemented with 1% volume of fraction V bovine serum albumin, 2 mM Glutamax, 1% NEAA, 0.5% N2 supplement, and 1% B27 supplement [all Life Technologies], and 1,000 U/ml of mouse LIF [Millipore, MA], 1 μM of CHIR99021, and 1 μM of PD0325901. Rat ESC and iPSC lines were maintained on mitomycin-C-treated MEF-coated dishes. Mouse ESCs were maintained on 0.1% gelatin-coated dishes.

Preparation of rat EpiSC lines

The rat EpiSC line was derived from a BLK-RT2-injected interspecies chimera developed *in vivo*, and was established from a BLK-RT2-injected interspecies chimeric E6.5 embryo. Rat EpiSCs that expressed tdTomato were purified by cell sorting. Rat EpiSCs were maintained in hESC medium on mitomycin-C-treated MEF feeder cells.

Preparation of nonhuman primate ESC lines

We used an established CMK6 cynomolgus monkey ESC line (Suemori et al., 2001). Cell culture and labeling with *tdTomato* were performed as with hiPSCs.

Immunohistochemistry for primordial germ cells

Frozen sections were made from genital ridges obtained from E13.5 EpiSC-sub cells derived chimeric embryos. The sections were incubated with anti-Mvh antibody (ab13840; 1:200 dilution; Abcam, Cambridge, UK), were washed with PBS, and then were stained with Alexa Fluor 647 conjugated anti-rabbit IgG antibody (Life Technologies) to mark primordial germ cells. Nuclei were stained with DAPI (Wako Pure Chemicals). Fluorescence microscopy was performed using a BZ-9000 microscope (Keyence, Osaka, Japan).

Flow-cytometry analysis

For flow-cytometry analysis, cells were dissociated into single cells using Accutase (Innovative Cell Technologies, San Diego, CA), and then stained with APC-conjugated anti-mouse CD31 antibody (390; BioLegend, San Diego, CA), isotype control (R35-95; BD Biosciences, San Jose, CA), or anti-SSEA1 antibody (MC-480; eBioscience, San Diego, CA) antibody. The stained cells were analyzed using a FACSCanto flow cytometer (BD Biosciences).

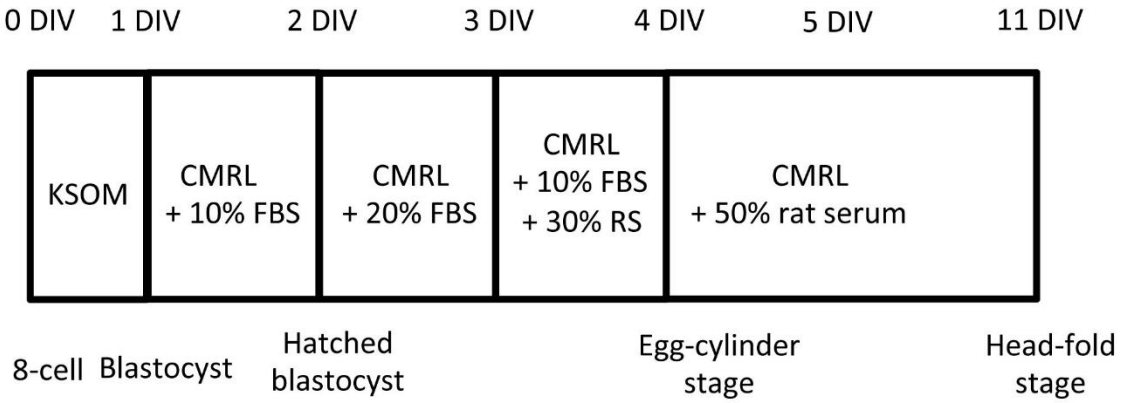

Fig. S1. Stepwise culture conditions for *in vitro* development of mouse pre-implantation embryos

The figure illustrates culture media and expected developmental stages at each culture day.

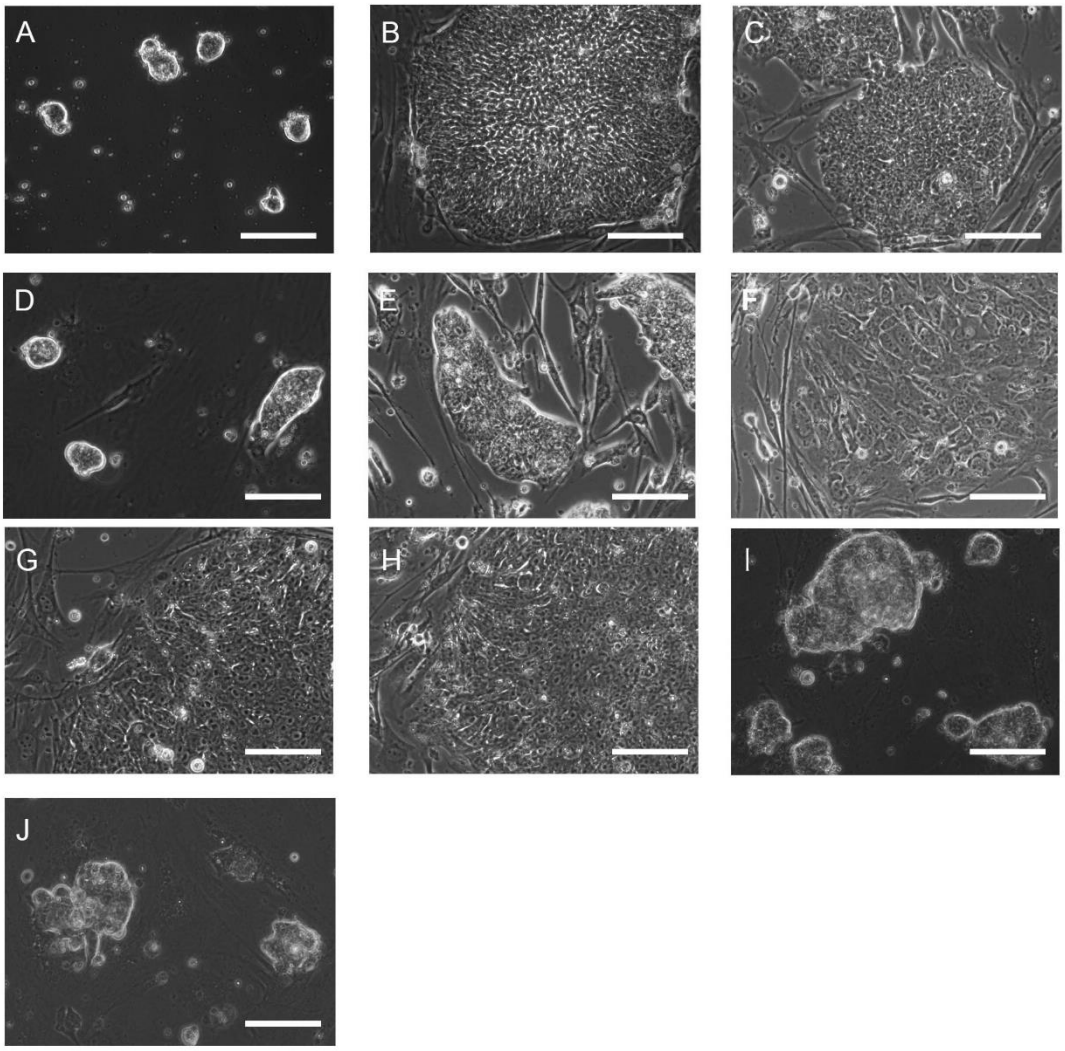

Fig. S2. Appearances of engrafted cell lines

Brightfield images of the lines used in the assay. (A) EB3DR mouse ESC. (B) Parental mouse EpiSC of EpiSC-sub line. (C) EpiSC-sub line. (D) BLK-RT2 rat ESC. (E) BLK-RT2 EpiSC. (F) CMK6 monkey ESC. (G) Parental human iPSC of DR-hiPSC. (H) DR-hiPSC. (I-J) Human naïve-like PSCs cultured with 2iL and Dox (I), or with 2iL and PKCi 2 weeks after withdrawal of Dox (J). Note that EpiSC-sub cells and DR-hiPSCs are morphologically similar to their respective parental cells. Scale bar indicates 100 μm.

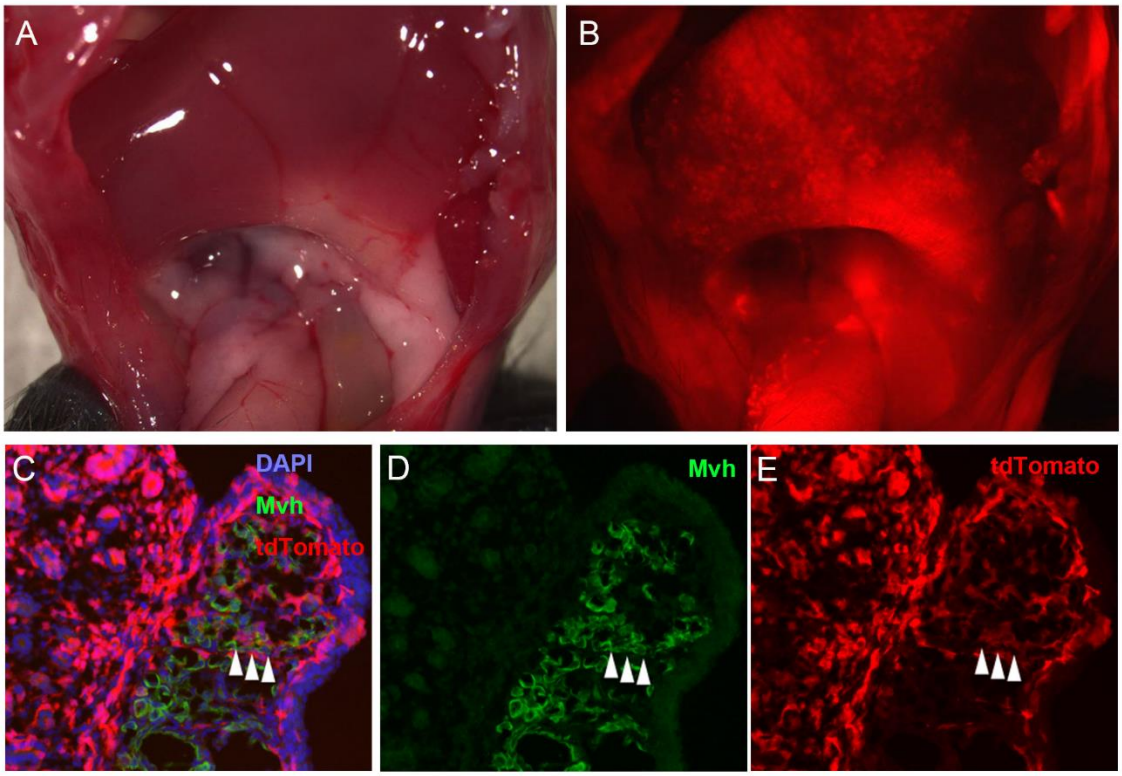

Fig. S3. Distribution of EpiSC-sub cells-derived progeny

(A, B) Chimeric offspring were sacrificed at postnatal day 14 and analyzed for the distribution of tdTomato-labeled descendants of EpiSC-sub cells. Fluorescence images indicate that EpiSC-sub cells contributed to bone, skeletal muscle, liver, pancreas, and most observed organs (B). (C-E) To clarify germline contribution, frozen sections of genital ridges obtained from E13.5 chimeric embryos were stained using antibody against Mvh, a PGC marker. (C) Overlaid image of DAPI staining, Mvh antibody staining (D), and tdTomato expression (E). Arrowheads indicate co-localization of tdTomato expression and Mvh staining.

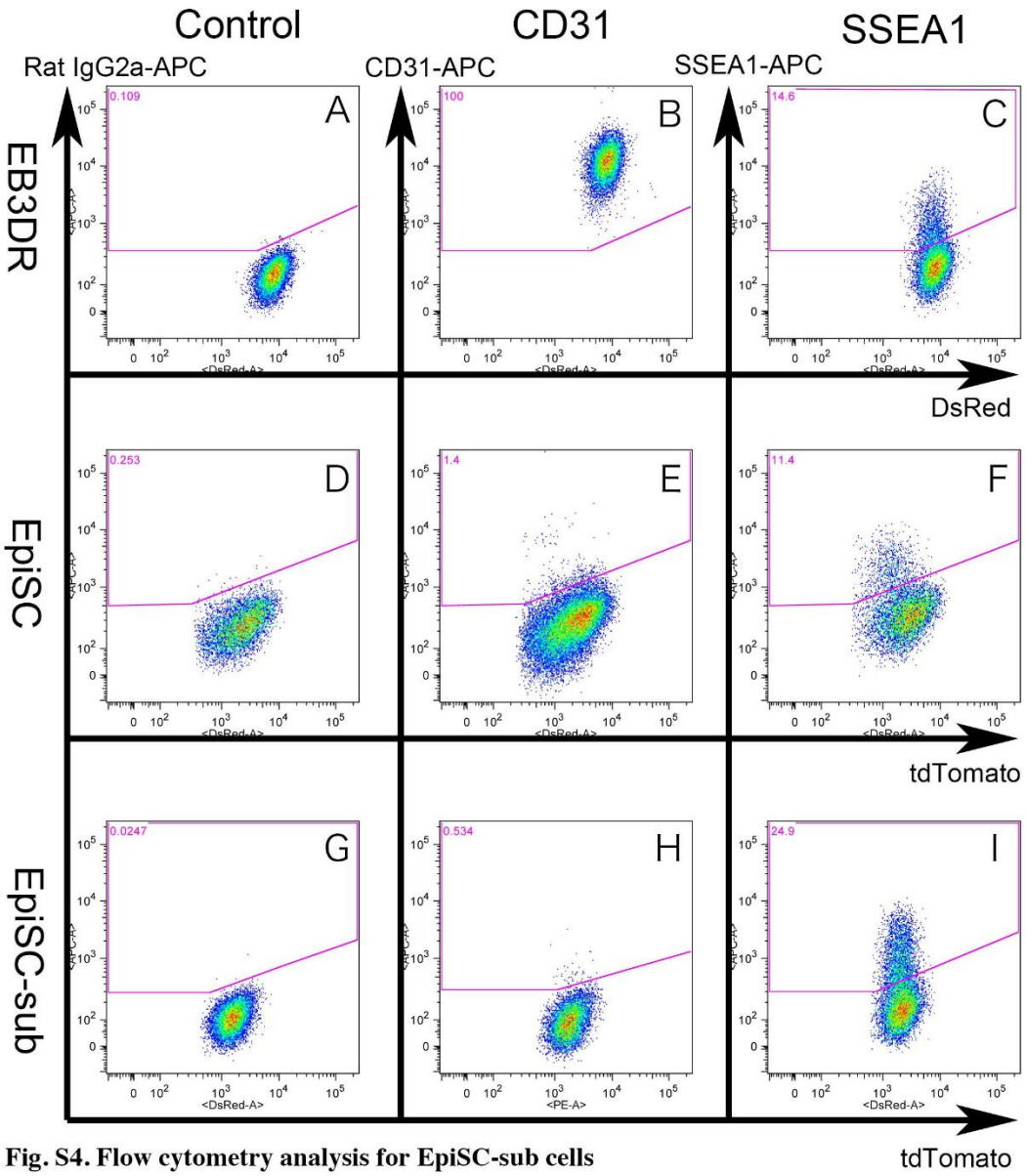

Fig. S4. Flow cytometry analysis for EpiSC-sub cells

EB3DR mouse ESCs (A-C), parental EpiSCs (D-E), and EpiSC-sub cells were stained with APC-conjugated anti-mouse CD31 antibody (B, E, H) or anti-SSEA1 antibody (C, F, I). Indicated controls (A, D, G) were stained with APC-conjugated isotype control antibody for anti-mouse CD31 antibody.

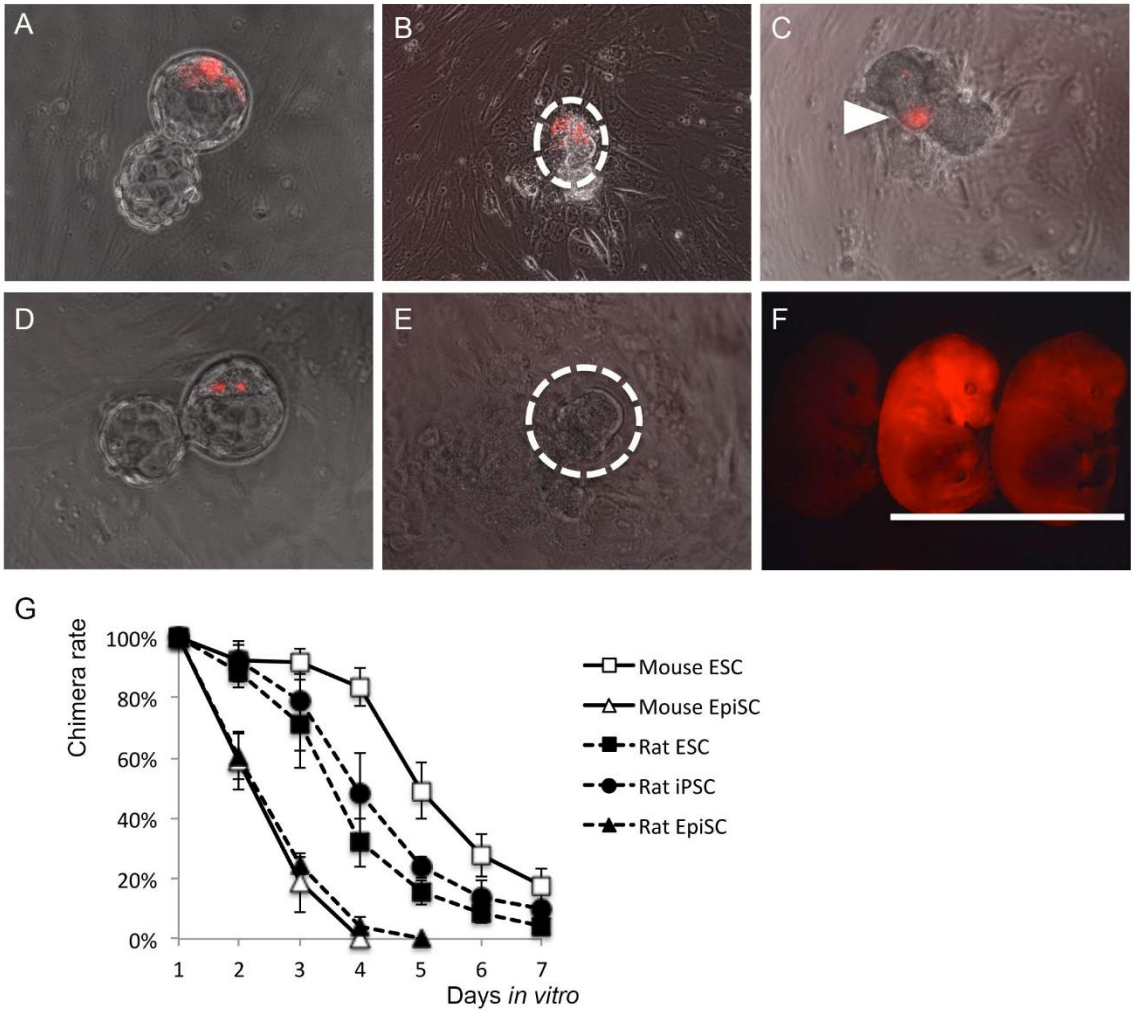

Fig. S5. Interspecies in vitro chimera assay: Rat PSCs.

To clarify whether our in vitro chimera assay results held for interspecies chimeras, rat ESCs, iPSCs, and EpiSCs were tested for chimera formation ability in vitro. (A-E) Overlaid images show the distribution of engrafted rat ESC- (A-C) or EpiSC (D, E) -derived cells in red at 2 DIV (A, D), 4 DIV (B, E), and 7 DIV (F). (F) Fluorescence-microscopy image of rat ESC-derived interspecies chimera developed in vivo. (G) Plots indicate proportion of rat ESCs-, iPSCs-, or EpiSCs-injected chimeric embryos compared with mouse ESC- or EpiSC-injected embryos at each culture day.

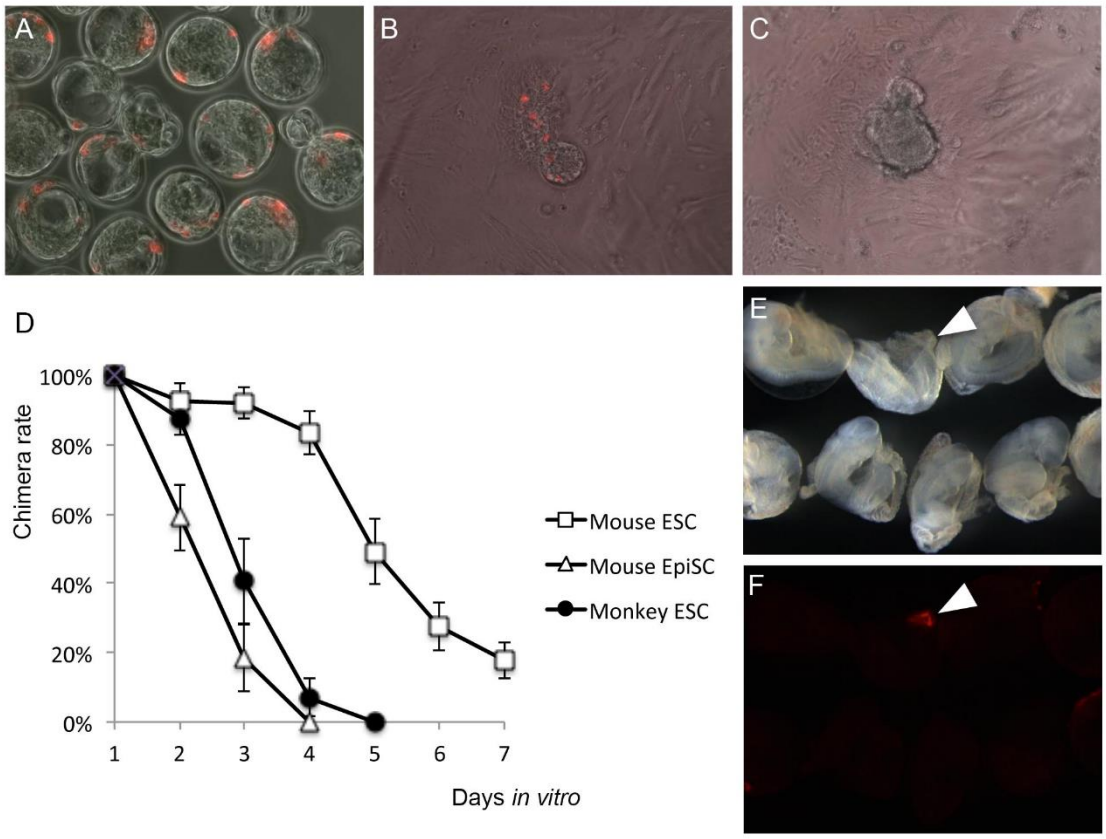

Fig. S6. Interspecies in vitro chimera assay: Monkey PSCs.

To clarify whether interspecies-in vitro chimera assay results held for primates, monkey ESC-line CMK6 cellslabeled with tdTomato were subjected to interspecies-in vitro chimera assay. (A-C) Overlaid images show the distribution of monkey ESC-derived cells in red at 2 DIV (A), 4 DIV (B), and 6 DIV (C). (D) Plots indicate proportion of CMK6-derived chimeric embryos among started embryos at each culture day. (E, F) In vivo chimera assay was also performed for CMK6 cells. E8.5 embryos were observed by brightfield (E) and fluorescence (F) microscopy. Arrowhead indicates autofluorescent placental tissue.

Table S1. Results of in vitro chimera assay with mouse embryos

Mouse ESC

	No. of chimera	Chimera rate	No. of chimera	Chimera rate	No. of chimera	Chimera rate	No. of chimera	Chimera rate
1DIV	50	100%	21	100%	20	100%	30	100%
2DIV	49	98%	20	95%	18	90%	26	87%
3DIV	48	96%	20	95%	18	90%	26	87%
4DIV	45	90%	18	86%	15	75%	25	83%
5DIV	31	62%	10	48%	8	40%	14	47%
6DIV	18	36%	5	24%	6	30%	6	20%
7DIV	11	22%	4	19%	4	20%	3	10%

Mouse EpiSC

	No. of chimera	Chimera rate	No. of chimera	Chimera rate	No. of chimera	Chimera rate	No. of chimera	Chimera rate	No. of chimera	Chimera rate
1DIV	40	100%	30	100%	31	100%	28	100%	33	100%
2DIV	23	58%	15	50%	17	55%	21	75%	19	58%
3DIV	5	13%	4	13%	5	16%	10	36%	5	15%
4DIV	0	0%	0	0%	0	0%	0	0%	0	0%
5DIV	0	0%	0	0%	0	0%	0	0%	0	0%
6DIV	0	0%	0	0%	0	0%	0	0%	0	0%
7DIV	0	0%	0	0%	0	0%	0	0%	0	0%

EpiSC-sub

	No. of chimera	Chimera rate	No. of chimera	Chimera rate	No. of chimera	Chimera rate
1DIV	30	100%	30	100%	33	100%
2DIV	25	83%	27	90%	24	73%
3DIV	20	67%	21	70%	24	73%
4DIV	15	50%	13	43%	21	64%
5DIV	8	27%	7	23%	12	36%
6DIV	3	10%	4	13%	5	15%
7DIV	2	7%	3	10%	4	12%

Rat ESC

	No. of chimera	Chimera rate	No. of chimera	Chimera rate	No. of chimera	Chimera rate	No. of chimera	Chimera rate	No. of chimera	Chimera rate
1DIV	36	100%	30	100%	27	100%	40	100%	24	100%
2DIV	31	92%	28	93%	24	89%	36	90%	19	79%
3DIV	27	75%	25	83%	21	78%	30	75%	11	46%
4DIV	8	22%	11	37%	11	41%	14	35%	6	25%
5DIV	5	8%	5	17%	5	19%	7	18%	4	17%
6DIV	2	6%	3	10%	3	11%	5	13%	1	4%
7DIV	2	6%	1	3%	2	7%	2	5%	0	0%

Rat iPSC

	No. of chimera	Chimera rate	No. of chimera	Chimera rate	No. of chimera	Chimera rate	No. of chimera	Chimera rate
1DIV	50	100%	50	100%	24	100%	27	100%
2DIV	47	94%	41	82%	23	96%	26	96%
3DIV	37	74%	29	58%	23	96%	24	89%
4DIV	21	42%	16	32%	15	63%	15	56%
5DIV	11	22%	11	22%	7	29%	6	22%
6DIV	4	8%	8	16%	5	21%	3	11%
7DIV	2	4%	6	12%	4	17%	2	7%

Rat EpiSC

	No. of chimera	Chimera rate	No. of chimera	Chimera rate	No. of chimera	Chimera rate
1DIV	23	100%	27	100%	24	100%
2DIV	12	52%	18	67%	15	63%
3DIV	6	26%	7	26%	5	21%
4DIV	0	0%	2	7%	1	4%
5DIV	0	0%	0	0%	0	0%
6DIV	0	0%	0	0%	0	0%
7DIV	0	0%	0	0%	0	0%

Monkey ESC

	No. of chimera	Chimera rate	No. of chimera	Chimera rate	No. of chimera	Chimera rate	No. of chimera	Chimera rate
1DIV	36	100%	40	100%	40	100%	40	100%
2DIV	32	89%	33	83%	34	85%	31	78%
3DIV	11	31%	13	33%	19	48%	12	30%
4DIV	2	6%	0	0%	4	10%	2	5%
5DIV	0	0%	0	0%	0	0%	0	0%
6DIV	0	0%	0	0%	0	0%	0	0%
7DIV	0	0%	0	0%	0	0%	0	0%

Human iPSC

	No. of chimera	Chimera rate	No. of chimera	Chimera rate	No. of chimera	Chimera rate	No. of chimera	Chimera rate	No. of chimera	Chimera rate
1DIV	40	100%	34	100%	50	100%	36	100%	54	100%
2DIV	33	83%	29	85%	43	86%	32	89%	44	81%
3DIV	11	28%	16	47%	12	24%	13	36%	13	24%
4DIV	5	13%	7	21%	3	6%	5	14%	3	6%
5DIV	0	0%	0	0%	0	0%	0	0%	0	0%
6DIV	0	0%	0	0%	0	0%	0	0%	0	0%
7DIV	0	0%	0	0%	0	0%	0	0%	0	0%
	No. of chimera	Chimera rate	No. of chimera	Chimera rate	No. of chimera	Chimera rate	No. of chimera	Chimera rate	No. of chimera	Chimera rate
1DIV	35	100%	30	100%	26	100%	20	100%	20	100%
2DIV	31	89%	19	63%	19	73%	13	65%	14	70%
3DIV	23	66%	7	23%	9	35%	3	15%	6	30%
4DIV	3	9%	0	0%	4	15%	0	0%	0	0%
5DIV	0	0%	0	0%	0	0%	0	0%	0	0%
6DIV	0	0%	0	0%	0	0%	0	0%	0	0%
7DIV	0	0%	0	0%	0	0%	0	0%	0	0%

DR-hiPSC

	No. of chimera	Chimera rate	No. of chimera	Chimera rate	No. of chimera	Chimera rate	No. of chimera	Chimera rate	No. of chimera	Chimera rate
1DIV	50	100%	28	100%	30	100%	26	100%	26	100%
2DIV	40	80%	21	75%	25	83%	19	73%	19	73%
3DIV	18	36%	11	39%	13	43%	15	58%	9	35%
4DIV	6	12%	4	14%	2	7%	11	42%	4	15%
5DIV	3	6%	1	4%	0	0%	6	23%	0	0%
6DIV	0	0%	0	0%	0	0%	0	0%	0	0%
7DIV	0	0%	0	0%	0	0%	0	0%	0	0%

Reset cell

	No. of chimera	Chimera rate	No. of chimera	Chimera rate	No. of chimera	Chimera rate
1DIV	40	100%	40	100%	41	100%
2DIV	33	83%	31	78%	31	76%
3DIV	13	33%	16	40%	18	44%
4DIV	4	10%	5	13%	2	5%
5DIV	0	0%	0	0%	0	0%
6DIV	0	0%	0	0%	0	0%
7DIV	0	0%	0	0%	0	0%

Table S2. Results of *in vivo* chimera assay with mouse embryos

Graft cell type	Grafted cell line	Host embryos	Recipients	Analyzed stage	Pups (Fetus)	Chimera	Chimera rate
mouse ESC	EB3DR-P25	60	3	Neonate	13	10	77%
	EB3DR-P27	20	1	E9.5	11	8	73%
	EB3DR-P27	20	1	E13.5	9	6	67%
mouse iPSC	GT3.2-P18	40	2	E13.5	21	16	76%
	GT3.2-P19	80	4	Neonate	22	12	55%
	GT3.2-P19	80	4	Neonate	26	14	54%
	GT3.2-P21	80	4	Neonate	23	16	70%
	GT3.2-P21	80	4	Neonate	21	13	62%
mouse iPSC	EB3DR-EpiSC-P8+3	40	2	E9.5	26	0	0%
	BDF1-EpiSC-tdT-P5+2	20	1	E9.5	11	0	0%
	BDF1-EpiSC-tdT-P5+4	20	1	E7.5	15	0	0%
	BDF1-EpiSC-tdT-P5+4	20	1	E6.5	10	0	0%
	BDF1-EpiSC-tdT-P5+7	20	1	E9.5	12	0	0%
	EpiSC-tdT-P35+15	40	2	E9.5	21	0	0%
mouse EpiSC subclone	EpiSC-sub-P35+7	20	1	E9.5	17	4	24%
	EpiSC-sub-P35+8	20	1	E9.5	20	7	35%
	EpiSC-sub-P35+8	20	1	Neonate	8	3	38%
	EpiSC-sub-P35+15	40	2	E9.5	21	8	38%
rat ESC	BLK-RT2-P24	80	4	Neonate	15	7	47%
	BLK-RT2-P25	20	1	E9.5	12	4	33%
rat iPSC	riPSC-WI-T1-3-P24	80	4	Neonate	17	7	41%
rat EpiSC	BLK-RT2-EpiSC-P6	40	2	E9.5	25	0	0%
	BLK-RT2-EpiSC-P7	17	1	E9.5	12	0	0%
	BLK-RT2-EpiSC-P8	32	2	E9.5	22	0	0%