Supporting Information

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SI Methods

Derivation of hASCs. hASCs were obtained by lipoaspiration after acquiring informed consent from patients, in accordance with Stanford University human IRB guidelines. All suction assisted lipoaspiration procedures were performed using the VASER Lipo System (Sound Surgical Technologies). hASCs were harvested from the adipose tissue of male or female patients between the ages of 40 and 65 undergoing elective lipoaspiration. Participating patients had no prior knowledge or evidence of ongoing systemic disease at the time of operation. All specimens were immediately placed on ice and were washed sequentially in serial dilutions of dilute Betadine, followed by two PBS washes of equal volume. Adipose tissues were subsequently digested with an equal volume of 0.075% (wt/vol) Type II collagenase in Hank's balanced salt solution at 37 °C in water bath with agitation at 125 rpm for 30 min. After inactivation of collagenase with serum, the stromal vascular fraction was pelleted via centrifugation at 1,200 × *g* for 5 min. The cell pellet was resuspended and filtered through a 100-μm cell strainer, and the collected cells were plated within 15-cm dishes for further expansion.

Construction of Lentiviral Plasmids. Human Oct3/4, Sox2, Klf4, and c-MYC cDNAs were amplified by PCR using the high fidelity PfuUltra II fusion HS DNA polymerase (Agilent Technologies) and subcloned into modified pLL3.7 plasmids under the cytomegalovirus (CMV) promoter (Fig. S1). The sequence of each construct was confirmed as accurate by automated DNA sequencing at Geneway Incorporation. Protein expression of each factor was confirmed positive by western blotting analyses of transfected 293FT cells.

Reprogramming hASCs with Individual Lentiviruses. Reprogramming of hASCs was carried out either on MEF feeder cells or on Matrigel-coated feeder-free surfaces. For reprogramming on feeder cells, 2×10^5 hASCs were seeded in six-well tissue culture dish and maintained with hASC growth medium. The second day, cells were transduced with individual lentiviruses containing human Oct4, Sox2, Klf4, and c-MYC at a 1:1:1:1 ratio plus 5 μg/mL polybrene (Sigma). The day of this first time transduction was considered as day 0. Transduction was repeated on day 2 using the same batch of all four lentiviruses. On day 3, cells were digested off the culture dish with 0.05% trypsin-EDTA (Gibco) and counted with a hemocytometer. Cells (≈50,000) were then transferred onto mouse embryonic fibroblast (MEF) feeder layer in a gelatin-coated 10-cm culture dish and cultured with human embryonic stem (hES) cell growth medium mTeSR-1. The old medium was aspirated, and the cells were refreshed with new mTeSR-1 medium everyday. Background non-ES-like colonies usually appeared from day 5–6, while ES-like colonies with distinct light refractive property appeared as early as on day 12–13. On day 16–20, the living ES-like colonies were immunostained with TRA-1–60 mAb (Millipore) and Alexafluor488 secondary antibody (Invitrogen). Positive colonies with ES morphologies were picked out with a glass needle and seeded on Matrigel surface in a new culture dish. Each single picked colony was then maintained and expanded following routine ES cell passaging and culturing protocols and established as one individual hASC-iPS cell line. For feeder-free reprogramming, 2×10^5 hASCs were seeded in a well of six-well tissue culture dish previously coated with hES qualified matrigel (BD Biosciences). Cells were transduced twice with the four individual lentiviruses on day 0 and day 2. On day 4–5, the medium was switched from hASC growth medium to mTeSR-1. Cells were refreshed with mTeSR-1 everyday. ES-like colonies appeared as early as on day 13–14. On day 18–20, positive colonies with ES morphologies and TRA-1–60 expression were picked out and expanded in feeder-free condition.

Karyotype Analysis. Karyotype analyses of hASCs and hASC-iPS cells were carried out at the Cytogenetics Laboratory at Stanford Hospital and Clinics, Department of Pathology. Cells were treated with 0.1 mg/mL colcemid for induction of mitotic arrest and were subsequently harvested by trypsin dispersal, hypotonic shock, and fixation with 3:1 methanol:acetic acid. For each cell line, 20 metaphases were analyzed by the standard G-banding method.

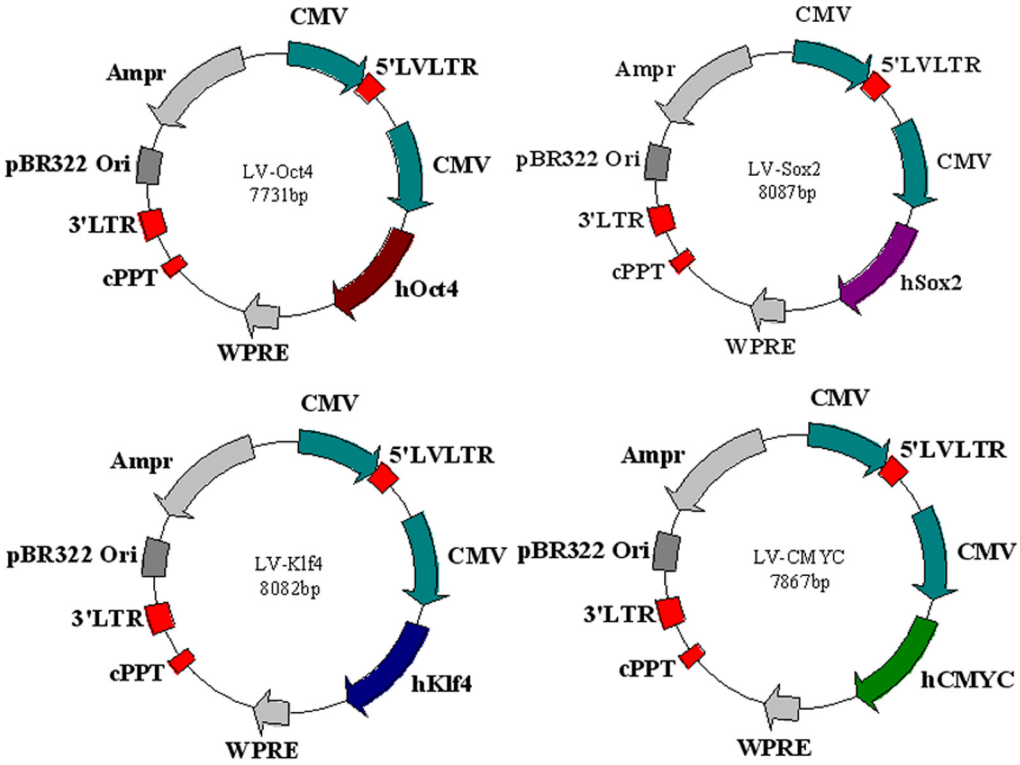

Fig. S1. Diagram showing the lentiviral constructs containing human Oct4, Sox2, Klf4, and c-MYC genes used for lentiviruses production. The pLL3.7 lentiviral plasmid was modified and used as the backbone for cloning human Oct4, Sox2, Klf4, or c-MYC driven under the CMV promoter.

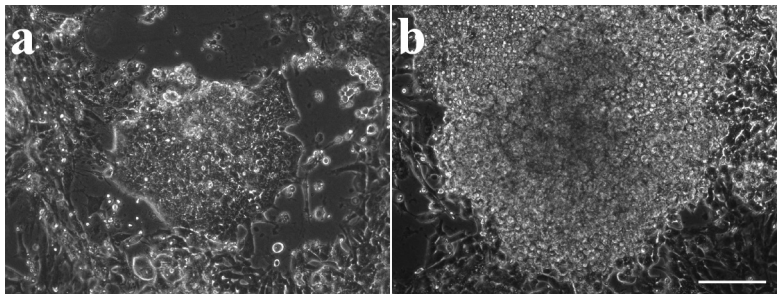

Fig. S2. (*a*) A typical ES cell-like colony and (*b*) non-ES cell-like colony derived from reprogramming hASCs on day 12 after transduction. (Scale bar, 100 μm.)

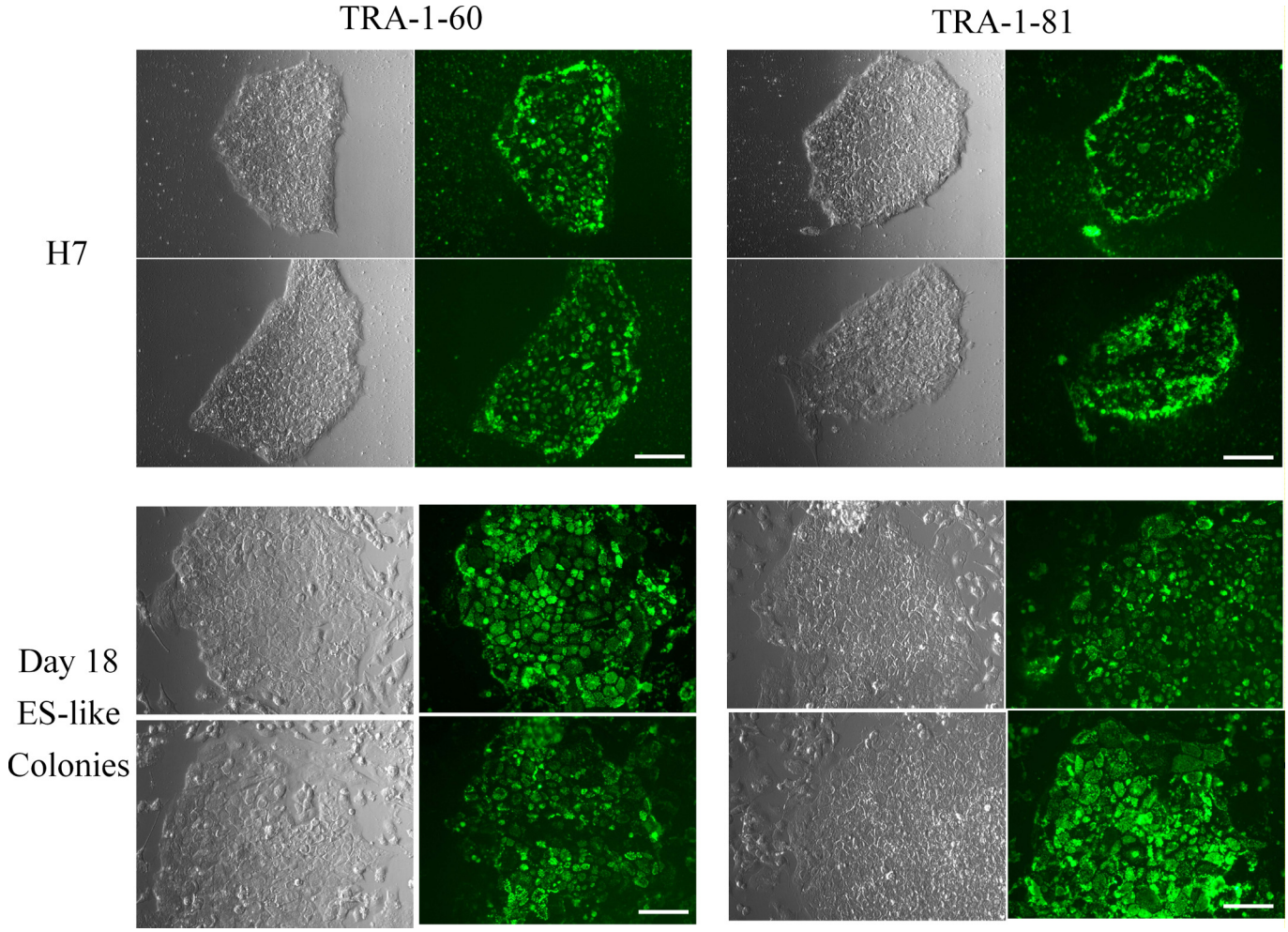

Fig. S3. The staining patterns of TRA-1–60 on hES cells and the ES cell-like putative hASC-iPS colonies were comparable with those of TRA-1–81 immunostaining. Left column, TRA-1–60 immunostaining (green, right panel) of H7 hES cells and the ES cell-like colonies of hASC reprogramming (day 18 after transduction) on Matrigel. Two representative colonies were shown for each cell type, and the left panels were phase images of the colonies. Right column, TRA-1–81 immunostaining (green, right panel) of H7 hES cells and the ES cell-like colonies of hASC reprogramming (day 18 after transduction) on Matrigel. Note that both TRA-1–60 and TRA-1–81 staining were specific for the hES cells or the reprogrammed ES cell-like hASC-iPS colonies and exhibited similar pattern of small dense dots spread on the surface of the cells. The overall appearances of TRA-1–60 and TRA-1–81 immunostaining were also similar for the entire hES cells or the reprogrammed ES cell-like colonies.

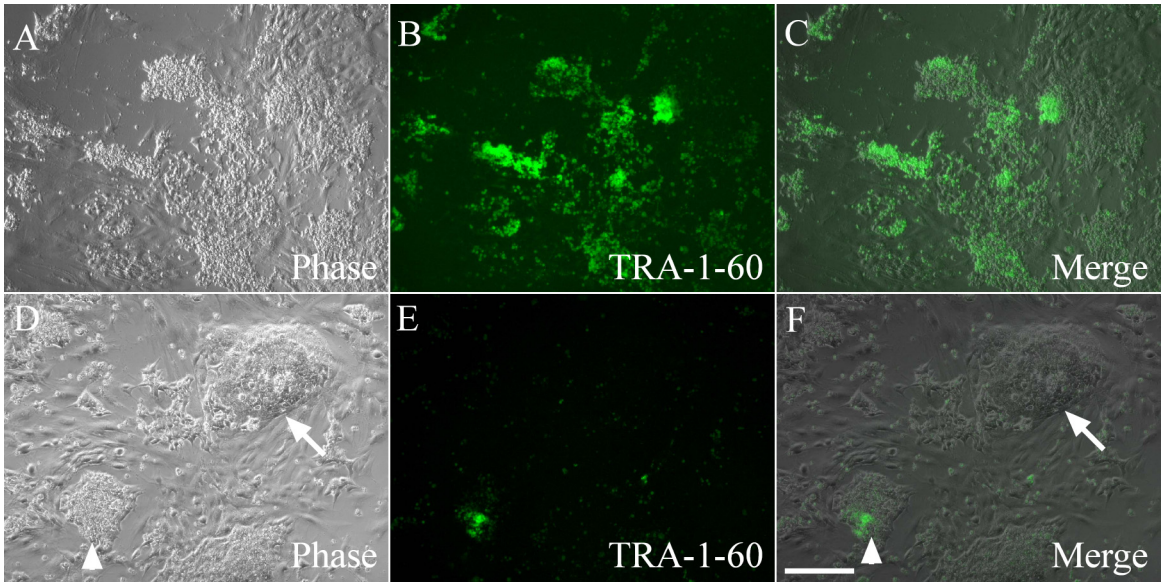

Fig. S4. Non-ES cell-like colonies with background nonspecific TRA-1–60 immunofluorecence and ES cell-like colonies negative for TRA-1–60. (*A–C*), Representative non-ES cell-like colonies with background nonspecific TRA-1–60 immunofluorescnence. (*D–F*), An ES cell-like colony (arrow) negative for TRA-1–60 immunofluorescence and a non-ES cell-like colony (arrow head) with partial background nonspecific TRA-1–60 immunofluorescence. (Scale bar, 100 μm.)

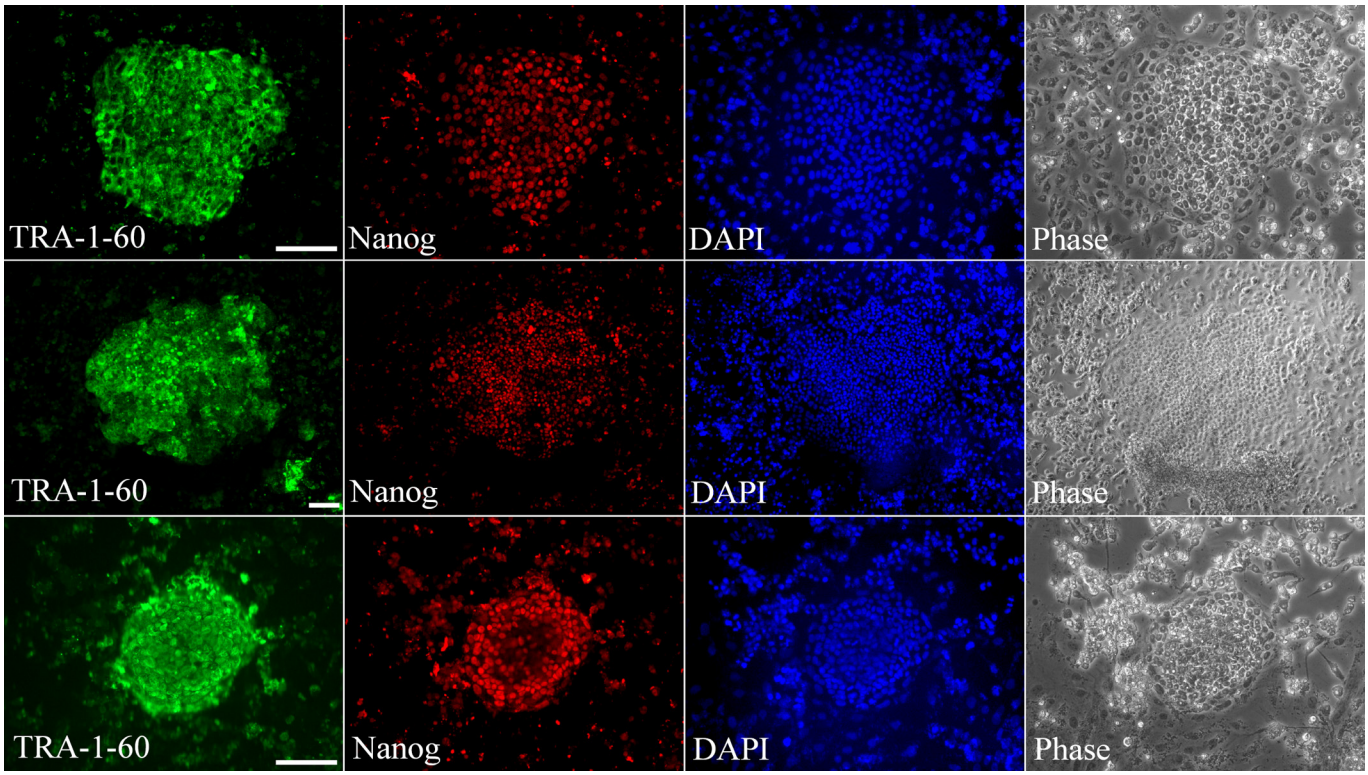

Fig. S5. Immunostaining of TRA-1–60 and endogenous Nanog expression of the ES cell-like colonies appeared day 16–18 posttransduction. Shown are three representative ES cell-like colonies that were positive for TRA-1–60 (green) and Nanog (red). Nuclei were stained blue with DAPI. Note that the phase images of the ES cell-like colonies lost the typical light refractive properties after fixation for immunostaining. (Scale bars, 50 μm.)

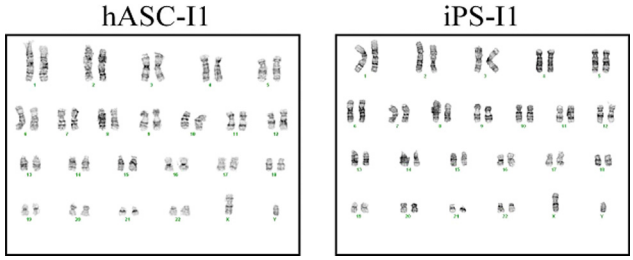

Fig. S6. hASC-iPS cells have a normal karyotype. Representative images showing iPS cells derived from hASCs of individual 1 (I1) have the same normal 46,XY karyotype.

	hASC-1	hASC-2	hASC-iPSC-1	hASC-iPSC-2	hESC-1	hESC-2
hASC-1	1.00					
hASC-2	0.97	1.00				
hASC-iPSC-1	0.12	0.12	1.00			
hASC-iPSC-2	0.12	0.12	0.98	1.00		
hESC-1	-0.03	-0.02	0.75	0.75	1.00	
hESC-2	-0.03	-0.02	0.73	0.73	0.98	1.00

Fig. S7. Pearson correlation values of the microarray data shown in Fig. 2*D*.

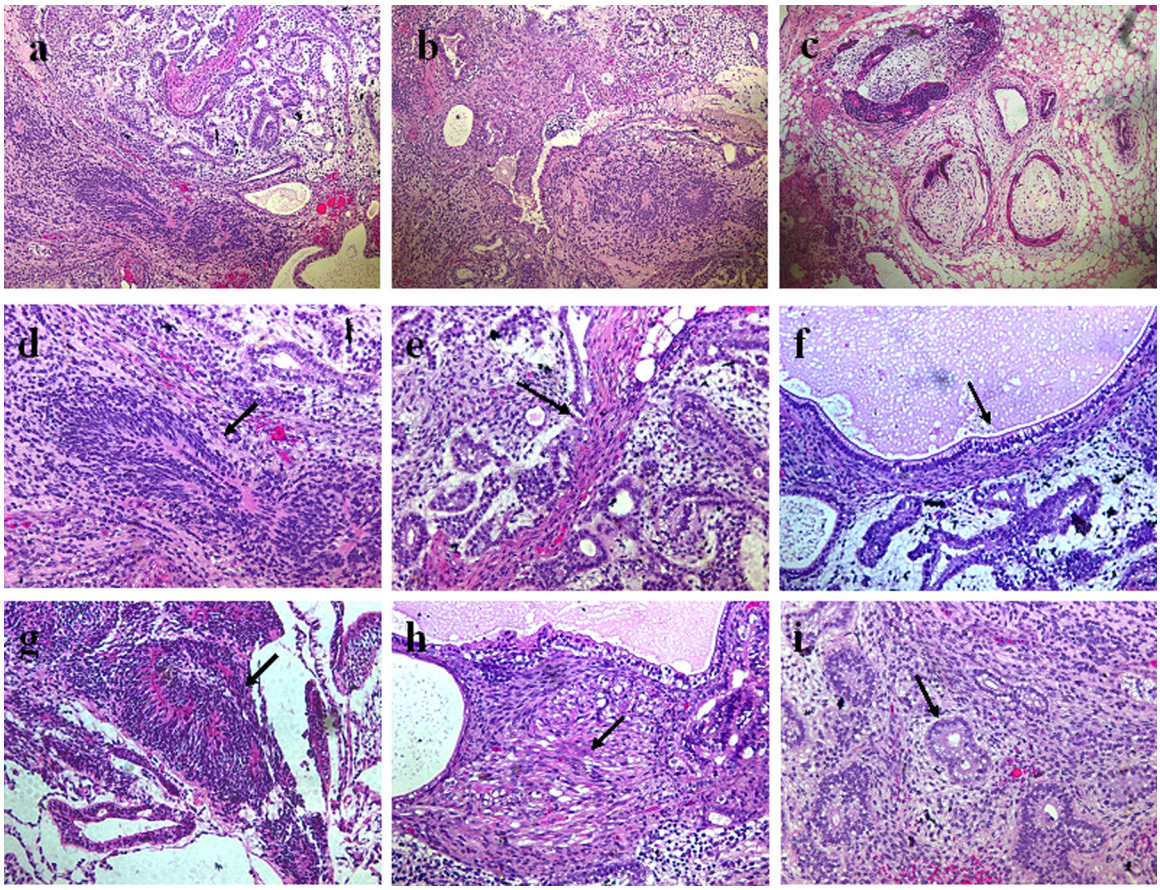

Fig. S8. Teratomas containing cells from three germ layers developed from iPS cell lines injected into the dorsal flank of nude mice. (*a*–*c*) Representative images of three teratomas developed from three different feeder-free derived hASC-iPS cell lines. (*d* and *g*) Neuroectoderm (arrows). (*e* and *h*) Muscle tissue (mesoderm) (arrows). (*f*) Gut epithelium (endoderm) (arrow). (*i*) Respiratory epithelium (endoderm) (arrow).

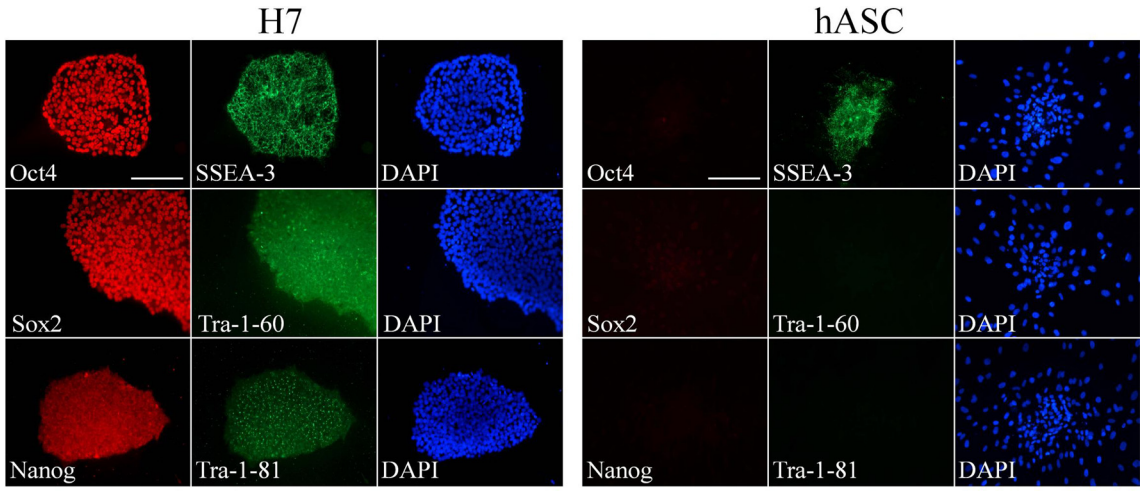

Fig. S9. Immunostaining of ES cell marker expression within H7 hES cells and within hASCs. Note that hASCs shown here formed the colony forming unit-fibroblast (CFU-F) in the center and expressed SSEA-3 within the CFU-F but not in surrounding individual cells. hASCs did not express other ES cell markers that were positive in H7 cells. (Scale bars, 100 μm.)

Table S1. Derivation of hASC-iPS cell lines

	Individual	Colonies picked	Cell line established	Teratoma tested
Feeder-Free	1	8	6	2
	2	6	6	2
	3	6	5	2
	4	6	6	2
With Feeder	1	4	4	N
	2	4	4	N
	3	4	4	N
	4	4	3	N

N = None tested