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Supplemental Data

Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

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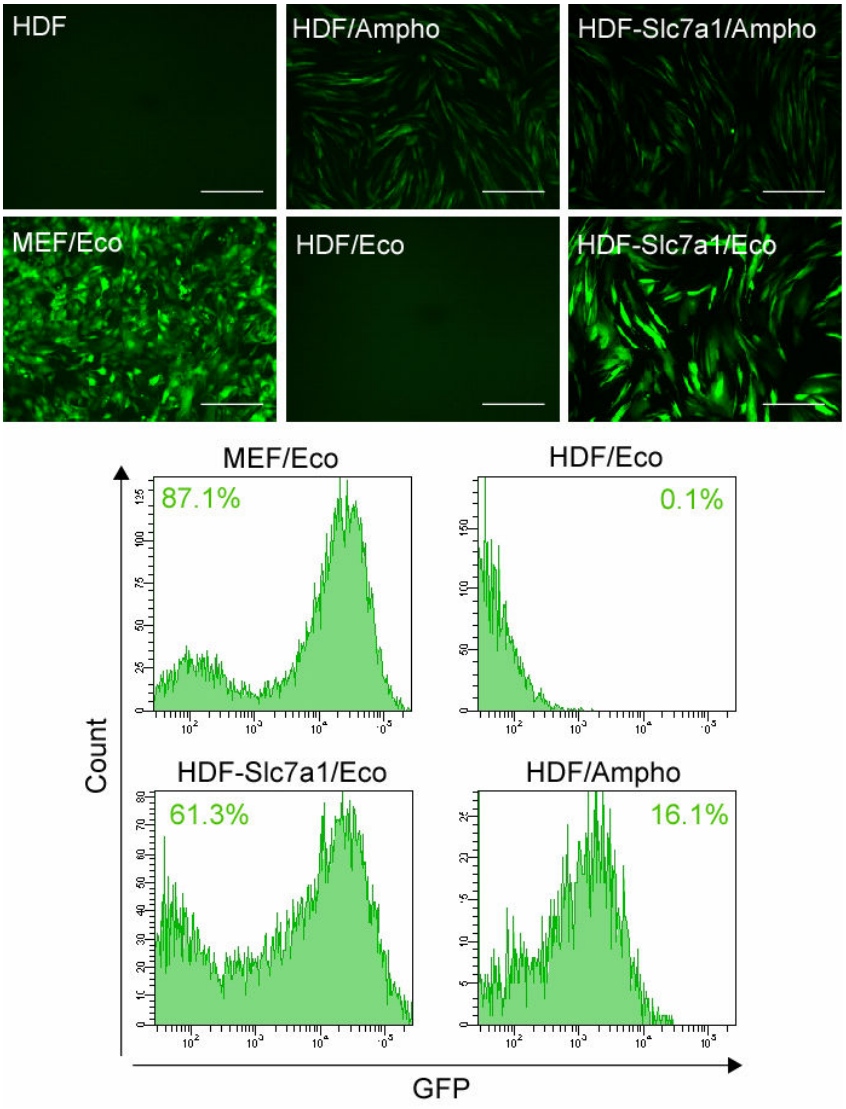

Figure S1. Improved Transduction Efficiency of Retroviruses in HDF

HDFs or HDFs expressing mouse *Slc7a1* gene (HDF-Slc7a1) were introduced with ecotropic (Eco) or amphotropic (Ampho) pMXs retroviruses containing the *GFP* cDNA. The upper panel shows the images of fluorescent microscope. Bars indicate 200 μm. The lower panel shows the results of flow cytometry. Shown are percentages of cells expressing GFP.

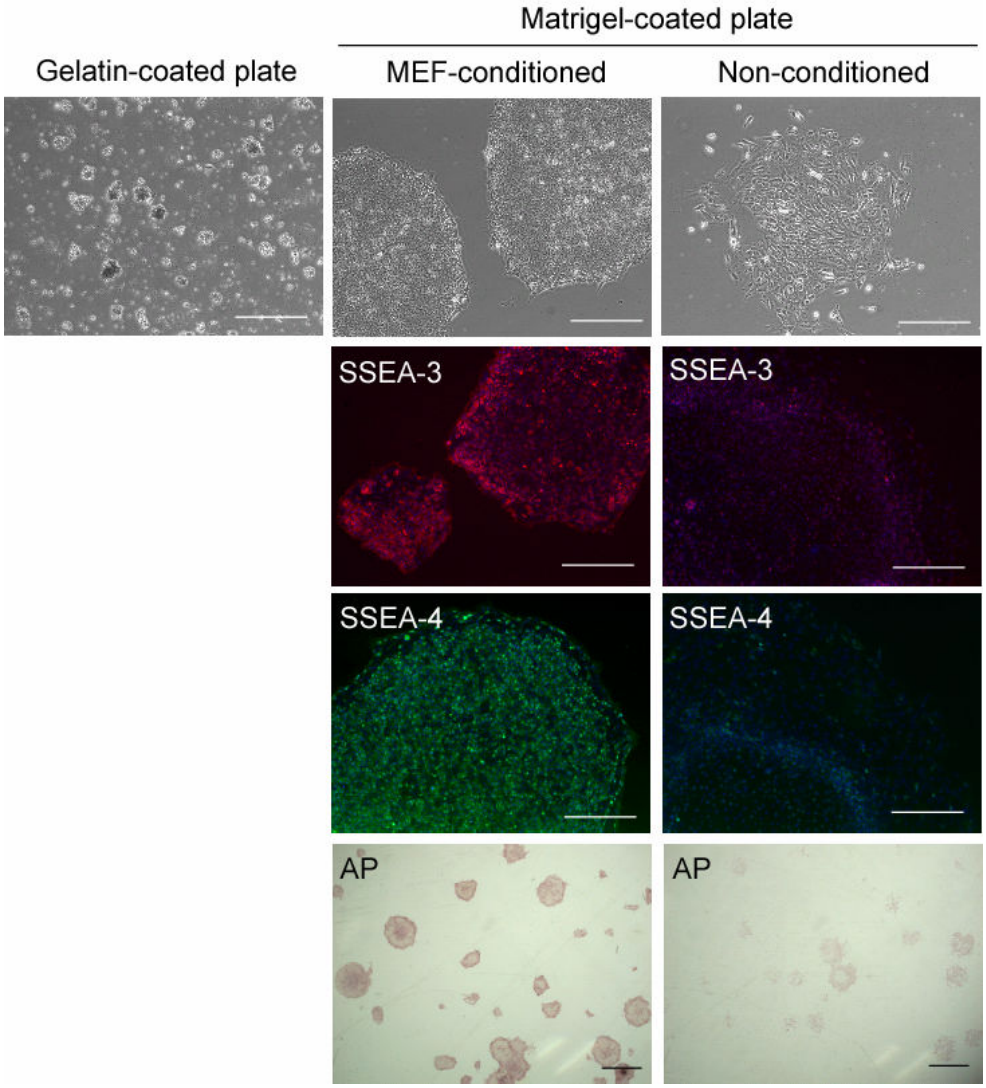

Figure S2. Feeder Dependency of Human iPS Cells

(Left) Image of iPS cells plated on gelatin-coated plate.

(Center) Images of iPS cells cultured on Matrigel-coated plate in MEF-conditioned human ES cell medium.

(Right) Images of iPS cells cultured on Matrigel-coated plates with non-conditioned medium.

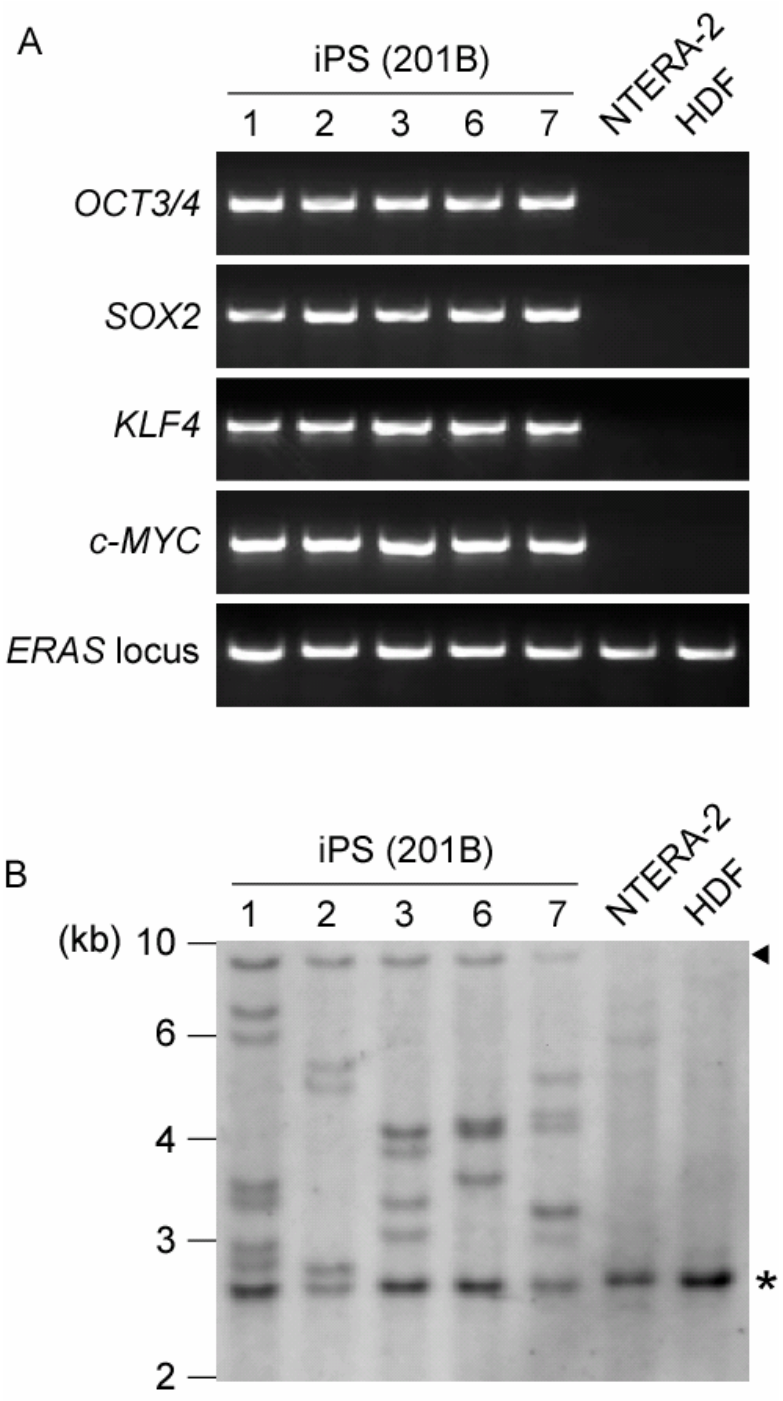

Figure S3. Genetic Analyses of Human iPS Cells

(A) Genomic PCR revealed integration of all the four retroviruses in all clones.

(B) Southern blot analyses with a *c-MYC* cDNA probe. Asterisk indicates the endogenous *c-MYC* alleles (2.7 kb). Arrowhead indicates mouse *c-Myc* alleles derived from SNL feeder cells (9.8 kb).

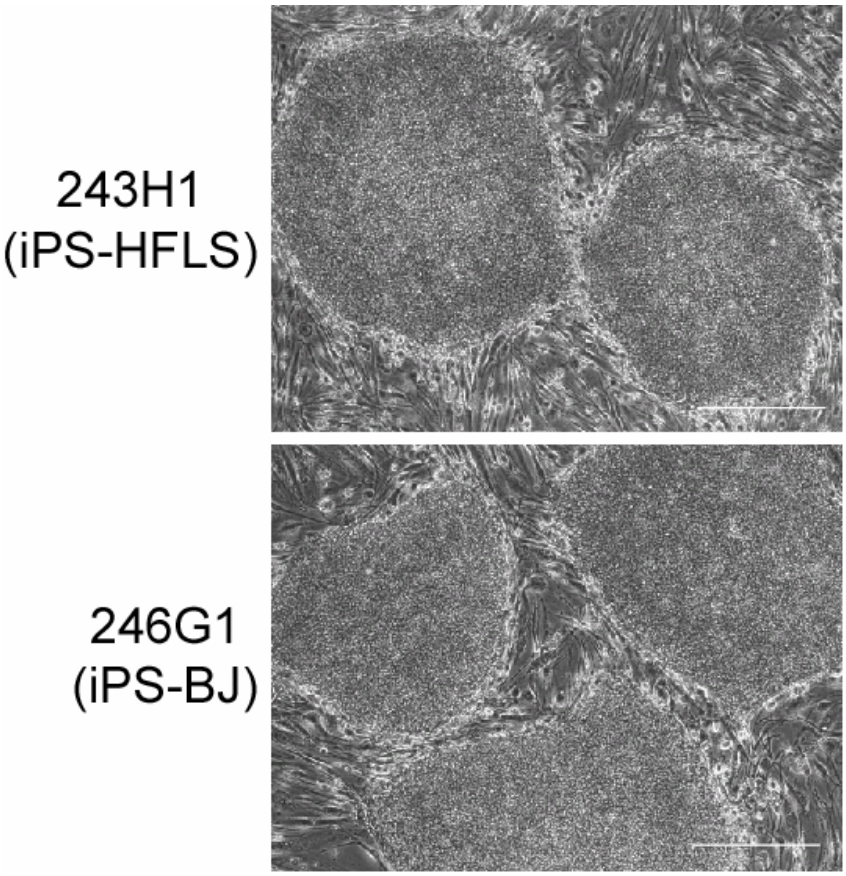

Figure S4. Human iPS Cells Derived from Fibroblast-Like Synoviocytes and BJ Fibroblasts

Phase contrast images of iPS cells derived from fibroblast-like synoviocyte (HFLS, clone 243H1) and BJ fibroblast (clone 246G1). Bars = 200 μm.

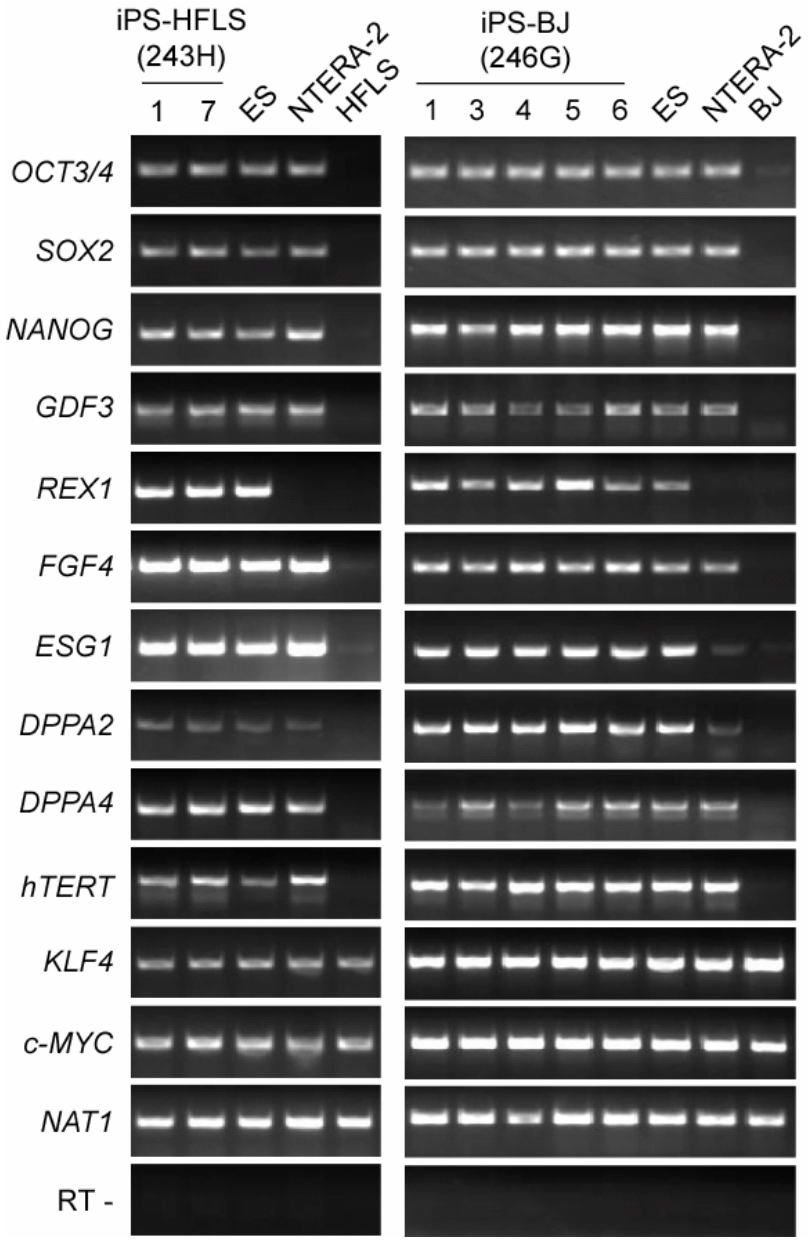

Figure S5. Expression of ES Cell Marker Genes in iPS Cells derived from HFLS and BJ Fibroblasts

Total RNA were isolated from iPS cells and analyzed with RT-PCR. Primers used for *OCT3/4*, *SOX2*, *KLF4*, and *c-MYC* specifically detect the transcripts from the endogenous genes, but not from the retroviral transgenes.

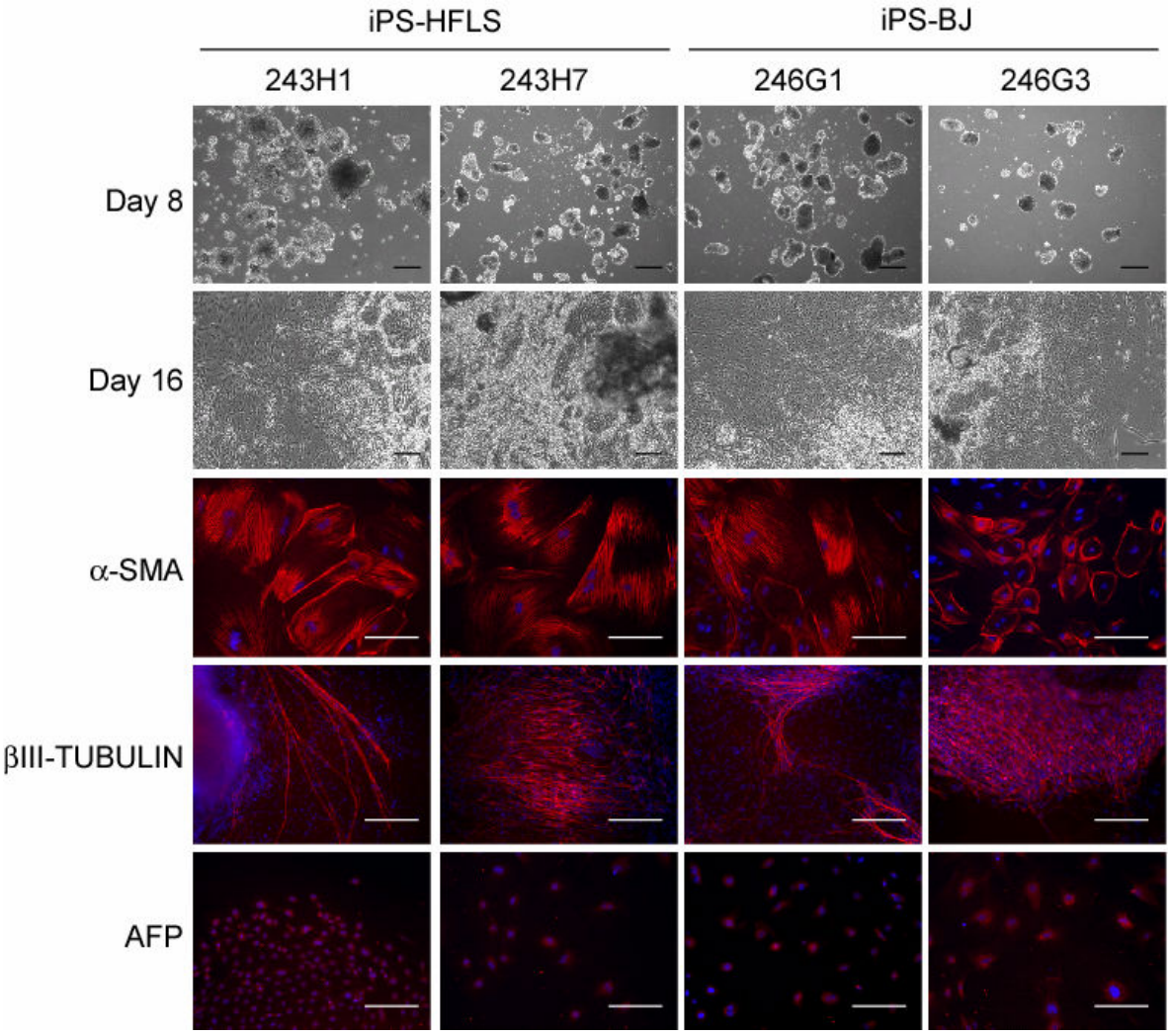

Figure S6. Embryoid Body–Mediated Differentiation of iPS Cells Derived from HFLS and BJ Fibroblasts

iPS cells were cultured as floating culture for 8 days. Images of differentiated cells were recorded at day 16. Shown are immunocytochemistry of α-smooth muscle actin (α-SMA), βIII-TUBULIN, and α-fetoprotein (AFP). Bars = 100 μm. Nucleuses were stained with Hoechst 33342 (blue).

Table S1. Summary of the iPS cell induction experiments

Exp. ID	Parental cells	Cell No. seeded at d6	No. of ES-like colony	No. of total colony	No. of picked up colony	No. of established clone
201B	HDF	50000	7	129	7	5
243H	HFLS	500000	0	> 1000		
		50000	17	679	6	2
246B	HDF	500000	0	420		
		500000	2	508		
		50000	8	92	6	6
246G	BJ	50000	7	10	6	5
		500000	86	98		
		500000	106	108		
249D	HDF	500000	0	320		
		500000	0	467		
		50000	8	179	6	4
253F	HDF	50000	5	78	3	2
		50000	6	128	3	3
		500000	10	531		
		500000	3	738		
282C	HDF	50000	11	224	3	1
282H	BJ	50000	13	15	3	2
282R	HFLS	5000	31	98	6	2

Table S2. Characterization of established clones

Clone	Source	Marker expression		Pluripotency			
		RT-PCR	IC	EB	PA6	Cardio-myocyte	Teratoma
201B1	HDF	√					
201B2		√	√	√	√	√	
201B3		√					
201B6		√	√	√	√	√	
201B7		√	√	√	√	√	√
243H1	HFLS	√		√			
243H7		√		√			
246B1	HDF	√					
246B2		√					
246B3		√					
246B4		√					
246B5		√					
246B6		√					
246G1	BJ	√		√			
246G3		√		√			
246G4		√					
246G5		√					
246G6		√					
253F1	HDF	√					
253F2		√					
253F3		√					
253F4		√					
253F5		√					

IC; immunocytochemistry, EB; embryoid body

Table S9. STR analyses of HDF-derived iPS cells

Locus／Clone	201B1	201B2	201B3	201B6	201B7	NTERA-2	HDF
D3S1358	15 17	15 17	15 17	15 17	15 17	15	15 17
TH01	5	5	5	5	5	9	5
D21S11	28	28	28	28	28	29 30	28
D18S51	14	14	14	14	14	13	14
Penta_E	7 19	7 19	7 19	7 19	7 19	5 14	7 19
D5S818	11	11	11	11	11	8 11	11
D13S317	10 14	10 14	10 14	10 14	10 14	14	10 14
D7S820	9 10	9 10	9 10	9 10	9 10	12	9 10
D16S539	11 13	11 13	11 13	11 13	11 13	11 16	11 13
CSF1PO	10	10	10	10	10	9 11	10
Penta_D	8 10	8 10	8 10	8 10	8 10	11 12	8 10
AMEL	X	X	X	X	X	X Y	X
vWA	15 18	15 18	15 18	15 18	15 18	19	15 18
D8S1179	8 10	8 10	8 10	8 10	8 10	13 15	8 10
TPOX	8 9	8 9	8 9	8 9	8 9	8	8 9
FGA	20 22	20 22	20 22	20 22	20 22	23	20 22

Table S10 STR analyses of HFLS-derived iPS cells

Locus / Clone	243H1		243H7		HFLS	
D3S1358	16	17	16	17	16	17
TH01	5	9	5	9	5	9
D21S11	28	30	28	30	28	30
D18S51	14	17	14	17	14	17
Penta_E	5	12	5	12	5	12
D5S818	10	12	10	12	10	12
D13S317	13		13		13	
D7S820	9	12	9	12	9	12
D16S539	11	13	11	13	11	13
CSF1PO	10	11	10	11	10	11
Penta_D	9	11	9	11	9	11
AMEL	X		X	Y	X	Y
vWA	17	19	17	19	17	19
D8S1179	13		13		13	
TPOX	8	11	8	11	8	11
FGA	21	22	21	22	21	22

Table S11. STR analyses of BJ-derived iPS cells

Locus / Clone	246G1		246G3		246G4		246G5		246G6		BJ	
D3S1358	13	15	13	15	13	15	13	15	13	15	13	15
TH01	6	7	6	7	6	7	6	7	6	7	6	7
D21S11	28		28		28		28		28		28	
D18S51	16	18	16	18	16	18	16	18	16	18	16	18
Penta_E	7	17	7	17	7	17	7	17	7	17	7	17
D5S818	11		11		11		11		11		11	
D13S317	9	10	9	10	9	10	9	10	9	10	9	10
D7S820	11	12	11	12	11	12	11	12	11	12	11	12
D16S539	9	13	9	13	9	13	9	13	9	13	9	13
CSF1PO	9	11	9	11	9	11	9	11	9	11	9	11
Penta_D	11	12	11	12	11	12	11	12	11	12	11	12
AMEL	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y
vWA	16	18	16	18	16	18	16	18	16	18	16	18
D8S1179	9	11	9	11	9	11	9	11	9	11	9	11
TPOX	10	11	10	11	10	11	10	11	10	11	10	11
FGA	22	23	22	23	22	23	22	23	22	23	22	23

Table S12. Primer sequences

Primer	Sequence (5' to 3')	Applications
hOCT3/4-S944	CCC CAG GGC CCC ATT TTG GTA CC	*OCT3/4* Tg genomic and RT-PCR
hSOX2-S691	GGC ACC CCT GGC ATG GCT CTT GGC TC	*SOX2* Tg genomic and RT-PCR
hKLF4-S1128	ACG ATC GTG GCC CCG GAA AAG GAC C	*KLF4* endo and Tg genomic and RT-PCR
hMYC-S1011	CAA CAA CCG AAA ATG CAC CAG CCC CAG	*c-MYC* Tg genomic and RT-PCR
pMXs-AS3200	TTA TCG TCG ACC ACT GTG CTG CTG	Tg genomic and RT-PCR
pMXs-L3205	CCC TTT TTC TGG AGA CTA AAT AAA	Tg genomic and RT-PCR
hOCT3/4-S1165	GAC AGG GGG AGG GGA GGA GCT AGG	Endo *OCT3/4* RT-PCR
hOCT3/4-AS1283	CTT CCC TCC AAC CAG TTG CCC CAA AC	
hSOX2-S1430	GGG AAA TGG GAG GGG TGC AAA AGA GG	Endo *SOX2* RT-PCR
hSOX2-AS1555	TTG CGT GAG TGT GGA TGG GAT TGG TG	
ECAT4-macaca-968S	CAG CCC CGA TTC TTC CAC CAG TCC C	*NANOG* RT-PCR
ECAT4-macaca-1334AS	CGG AAG ATT CCC AGT CGG GTT CAC C	
hGDF3-S243	CTT ATG CTA CGT AAA GGA GCT GGG	*GDF3* RT-PCR
hGDF3-AS850	GTG CCA ACC CAG GTC CCG GAA GTT	
hREX1-RT-U	CAG ATC CTA AAC AGC TCG CAG AAT	*REX1* RT-PCR
hREX1-RT-L	GCG TAC GCA AAT TAA AGT CCA GA	
hFGF4-RT-U	CTA CAA CGC CTA CGA GTC CTA CA	*FGF4* RT-PCR
hFGF4-RT-L	GTT GCA CCA GAA AAG TCA GAG TTG	
hpH34-S40	ATA TCC CGC CGT GGG TGA AAG TTC	*ESG1*/*DPPA5* RT-PCR
hpH34-AS259	ACT CAG CCA TGG ACT GGA GCA TCC	
hECAT15-1-S532	GGA GCC GCC TGC CCT GGA AAA TTC	*DPPA4* RT-PCR
hECAT15-1-AS916	TTT TTC CTG ATA TTC TAT TCC CAT	
hECAT15-2-S85	CCG TCC CCG CAA TCT CCT TCC ATC	*DPPA2* RT-PCR
hECAT15-2-AS667	ATG ATG CCA ACA TGG CTC CCG GTG	
hTERT-S3234	CCT GCT CAA GCT GAC TCG ACA CCG TG	*hTERT* RT-PCR
hTERT-AS3713	GGA AAA GCT GGC CCT GGG GTG GAG C	
hKLF4-AS1826	TGA TTG TAG TGC TTT CTG GCT GGG CTC C	Endo *KLF4* RT-PCR
hMYC-S253	GCG TCC TGG GAA GGG AGA TCC GGA GC	Endo *c-MYC* RT-PCR
hMYC-AS555	TTG AGG GGC ATC GTC GCG GGA GGC TG	
hMSX1-S665	CGA GAG GAC CCC GTG GAT GCA GAG	*MSX1* RT-PCR

hMSX1-AS938	GGC GGC CAT CTT CAG CTT CTC CAG	
hBRACHYURY-S1292	GCC CTC TCC CTC CCC TCC ACG CAC AG	*BRACHYURY*/*T* RT-PCR
hBRACHYURY-AS1540	CGG CGC CGT TGC TCA CAG ACC ACA GG	
hGFAP-S1040	GGC CCG CCA CTT GCA GGA GTA CCA GG	*GFAP* RT-PCR
hGFAP-AS1342	CTT CTG CTC GGG CCC CTC ATG AGA CG	
hPAX6-S1206	ACC CAT TAT CCA GAT GTG TTT GCC CGA G	*PAX6* RT-PCR
hPAX6-AS1497	ATG GTG AAG CTG GGC ATA GGC GGC AG	
hFOXA2-S208	TGG GAG CGG TGA AGA TGG AAG GGC AC	*FOXA2* RT-PCR
hFOXA2-AS398	TCA TGC CAG CGC CCA CGT ACG ACG AC	
hSOX17-S423	CGC TTT CAT GGT GTG GGC TAA GGA CG	*SOX17* RT-PCR
hSOX17-AS583	TAG TTG GGG TGG TCC TGC ATG TGC TG	
hAFP-S948	GAA TGC TGC AAA CTG ACC ACG CTG GAA C	*AFP* RT-PCR
hAFP-AS1201	TGG CAT TCA AGA GGG TTT TCA GTC TGG A	
hCK8-S734	CCT GGA AGG GCT GAC CGA CGA GAT CAA	*CK8* RT-PCR
hCK8-AS956	CTT CCC AGC CAG GCT CTG CAG CTC C	
hCK18-S1125	AGC TCA ACG GGA TCC TGC TGC ACC TTG	*CK18* RT-PCR
hCK18-AS1322	CAC TAT CCG GCG GGT GGT GGT CTT TTG	
hAADC-S1378	CGC CAG GAT CCC CGC TTT GAA ATC TG	*AADC* RT-PCR
hAADC-AS1594	TCG GCC GCC AGC TCT TTG ATG TGT TC	
hChAT-S1360	GGA GGC GTG GAG CTC AGC GAC ACC	*ChAT* RT-PCR
hChAT-AS1592	CGG GGA GCT CGC TGA CGG AGT CTG	
hMAP2-S5401	CAG GTG GCG GAC GTG TGA AAA TTG AGA GTG	*MAP2* RT-PCR
hMAP2-AS5587	CAC GCT GGA TCT GCC TGG GGA CTG TG	
hDAT-S1935	ACA GAG GGG AGG TGC GCC AGT TCA CG	*SLC6A3*/*DAT* RT-PCR
hDAT-AS2207	ACG GGG TGG ACC TCG CTG CAC AGA TC	
hLMX1B-S770	GGC ACC AGC AGC AGC AGG AGC AGC AG	*LMX1B* RT-PCR
hLMX1B-AS1020	CCA CGT CTG AGG AGC CGA GGA AGC AG	
hMYL2A-S258	GGG CCC CAT CAA CTT CAC CGT CTT CC	*MYL2A* RT-PCR
hMYL2A-AS468	TGT AGT CGA TGT TCC CCG CCA GGT CC	
hTnTc-S524	ATG AGC GGG AGA AGG AGC GGC AGA AC	*TnTc* RT-PCR
hTnTc-AS730	TCA ATG GCC AGC ACC TTC CTC CTC TC	
hMEF2C-S1407	TTT AAC ACC GCC AGC GCT CTT CAC CTT G	*MEF2C* RT-PCR
hMEF2C-AS1618	TCG TGG CGC GTG TGT TGT GGG TAT CTC G	
hMYHCB-S5582	CTG GAG GCC GAG CAG AAG CGC AAC G	*MYHCB* RT-PCR
hMYHCB-AS5815	GTC CGC CCG CTC CTC TGC CTC ATC C	
hDNMT3B-S2502	TGC TGC TCA CAG GGC CCG ATA CTT C	*DNMT3B* RT-PCR
hDNMT3B-S2716	TCC TTT CGA GCT CAG TGC ACC ACA AAA C	

hGABRB3-S1029	CCT TGC CCA AAA TCC CCT ATG TCA AAG C	*GABRB3* RT-PCR
hGABRB3-AS1280	GTA TCG CCA ATG CCG CCT GAG ACC TC	
hTDGF1-S490	CTG CTG CCT GAA TGG GGG AAC CTG C	*TDGF1* RT-PCR
hTDGF1-AS700	GCC ACG AGG TGC TCA TCC ATC ACA AGG	
hGAL-S415	TGC GGC CCG AAG ATG ACA TGA AAC C	*GAL* RT-PCR
hGAL-AS579	CCC AGG AGG CTC TCA GGA CCG CTC	
hLEFTB-S794	CTT GGG GAC TAT GGA GCT CAG GGC GAC	*LEFTB* RT-PCR
hLEFTB-AS1023	CAT GGG CAG CGA GTC AGT CTC CGA GG	
hIFITM1-S166	CCC CAA AGC CAG AAG ATG CAC AAG GAG	*IFITM1* RT-PCR
hIFITM1-AS368	CGT CGC CAA CCA TCT TCC TGT CCC TAG	
hNODAL-S693	GGG CAA GAG GCA CCG TCG ACA TCA	*NODAL* RT-PCR
hNODAL-AS900	GGG ACT CGG TGG GGC TGG TAA CGT TTC	
hUTF1-S832	CCG TCG CTG AAC ACC GCC CTG CTG	*UTF1* RT-PCR
hUTF1-AS979	CGC GCT GCC CAG AAT GAA GCC CAC	
hEBAF-S782	GCT GGA GCT GCA CAC CCT GGA CCT CAG	*EBAF* RT-PCR
hEBAF-AS1032	GGG CAG CGA GGC AGT CTC CGA GGC	
hGRB7-S1250	CGC CTC TTC AAG TAC GGG GTG CAG CTG T	*MYHCB* RT-PCR
hGRB7-AS1467	TGG GCA GGC TGA GGC GGT GGT TTG	
hPODXL-S1204	TCC AGC CCC ACA GCA GCA TCA ACT ACC	*GRB7* RT-PCR
hPODXL-AS1403	CCG GGT TGA AGG TGG CTT TGA CTG CTC	
hCD9-S369	GTG CAT GCT GGG ACT GTT CTT CGG CTT C	*CD9* RT-PCR
hCD9-AS564	CAC GCC CCC AGC CAA ACC ACA GCA G	
hBRIX-S596	CAC CAC GGT ATC ATC CCA AAA GCC AAC C	*BRIX* RT-PCR
hBRIX-AS798	ACG CCG ATG CAT GTT TGG TGA CTG GTA G	
hCDX2-ChIP-S1	CCC CTA GCT CGC CTC CAG TTA TGC ACG	*CDX2* ChIP
hCDX2-ChIP-AS1	CCC AAG GAA ATT ACT CGC CCT CCG CAC	
hGATA6-ChIP-S1	TGA GCG CAG TTC CGA CCC ACA GCC TG	*GATA6* ChIP
hGATA6-ChIP-AS1	GGG CGA GCG CGA GTC CGG GGT CTG	
hPAX6-ChIP-S1	TTG TGT GAG AGC GAG CGG TGC ATT TG	*PAX6* ChIP
hPAX6-ChIP-AS1	CAC CGC TCC TCA CTG GCC CAT TAG C	
hMSX2-ChIP-S1	TTC TGG CGG TAG AGG GAG AGT GGG ATG G	*MSX2* ChIP
hMSX2-ChIP-AS1	ATC ACG CCG AAA CTG AAA AGC CCG AGA C	
hOCT3/4-ChIP-S2	TTG CCA GCC ATT ATC ATT CA	*OCT3/4* ChIP
hOCT3/4-ChIP-AS2	TAT AGA GCT GCT GCG GGA TT	
hSOX2-ChIP-S1	GAG AAG GGC GTG AGA GAG TG	*SOX2* ChIP
hSOX2-ChIP-AS1	AAA CAG CCA GTG CAG GAG TT	
hNANOG-ChIP-S2	GAT TTG TGG GCC TGA AGA AA	*NANOG* ChIP
hNANOG-ChIP-AS2	GGA AAA AGG GGT TTC CAG AG	

hMYOG-ChIP-S1	GTG CCC ATG AAT GCC CAG AAT CTG AAG C	*MYOG* ChIP
hMYOG-ChIP-AS1	GGG GGA GGA GGG AAC AAG GAA GGG TAG G	
hHAND1-ChIP-S1	CCA TTG GCT CCC GGG AGA GGT TGA C	*HAND1* ChIP
hHAND1-ChIP-AS1	CCG GGC AAG GCT GAA AAT GAG ACG C	
hEIF4G2-ChIP-S1	AGG GTT CGG GGG AGG TAA GGG TGC	*NAT1* ChIP
hEIF4G2-ChIP-AS1	AGG GTT GCG TGC GTA AAG CCG GAG	
dT_{20}	TTT TTT TTT TTT TTT TTT TT	Reverse transcription
hMYC-S857	GCC ACA GCA AAC CTC CTC ACA GCC CAC	Southern blot probe
hMYC-AS1246	CTC GTC GTT TCC GCA ACA AGT CCT CTT C	
hOCT3/4-S	CAC CAT GGC GGG ACA CCT GGC TTC AG	*OCT3/4* cloning
hOCT3/4-AS	ACC TCA GTT TGA ATG CAT GGG AGA GC	
hSOX2-S	CAC CAT GTA CAA CAT GAT GGA GAC GGA GCT G	*SOX2* cloning
hSOX2-AS	TCA CAT GTG TGA GAG GGG CAG TGT GC	
hKLF4-S	CAC CAT GGC TGT CAG TGA CGC GCT GCT CCC	*KLF4* cloning
hKLF4-AS	TTA AAA ATG TCT CTT CAT GTG TAA GGC GAG	
hMYC-S	CAC CAT GCC CCT CAA CGT TAG CTT CAC CAA	*c-MYC* cloning
hMYC-AS	TCA CGC ACA AGA GTT CCG TAG CTG TTC AAG	
Slc7a1-S	CAC CAT GGG CTG CAA AAA CCT GCT CGG	Mouse *Slc7a1* cloning
Slc7a1-AS	TCA TTT GCA CTG GTC CAA GTT GCT GTC	
hREX1-pro5K-S-SalI	ATT GTC GAC GGG GAT TTG GCA GGG TCA CAG GAC	Promoter cloning
hREXx1-pro5K-AS-BglII	CCC AGA TCT CCA ATG CCA CCT CCT CCC AAA CG	
hOCT3/4-pro5K-S-XhoI	CACTCG AGG TGG AGG AGC TGA GGG CAC TGT GG	
hOCT3/4-pro5K-AS-BglII	CAC AGA TCT GAA ATG AGG GCT TGC GAA GGG AC	
mehREX1-F1-S	GGT TTA AAA GGG TAA ATG TGA TTA TAT TTA	Bisulfite sequencing
mehREX1-F1-AS	CAA ACT ACA ACC ACC CAT CAA C	
mehOCT3/4 F2-S	GAG GTT GGA GTA GAA GGA TTG TTT TGG TTT	

mehOCT3/4 F2-AS	CCC CCC TAA CCC ATC ACC TCC ACC ACC TAA	
mehNANOG-F1-S	TGG TTA GGT TGG TTT TAA ATT TTT G	
mehNANOG-F1-AS	AAC CCA CCC TTA TAA ATT CTC AAT TA	