Nature Methods

A simple method to generate integration-free human iPS cells

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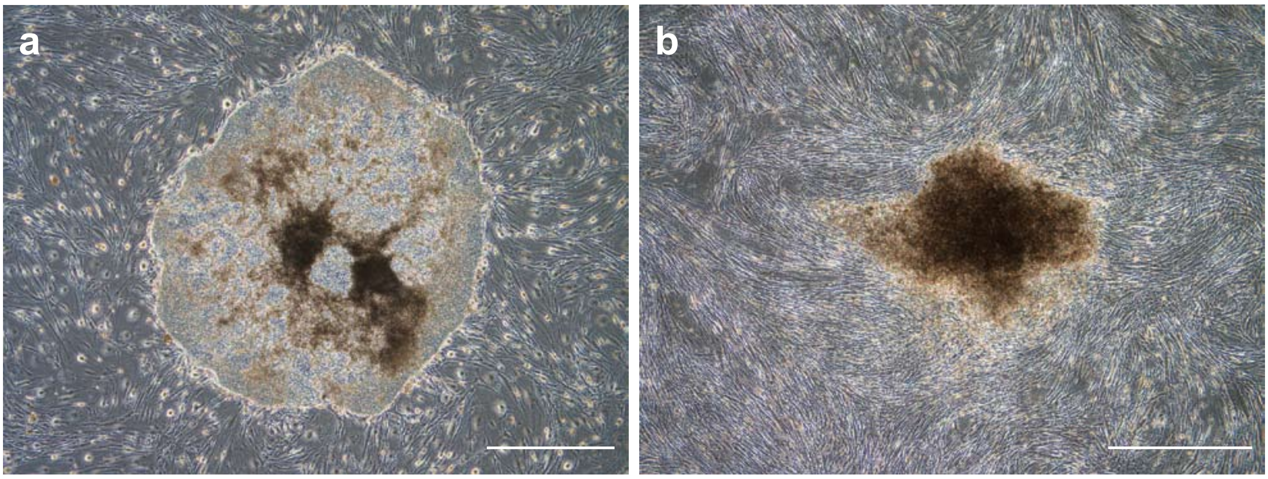
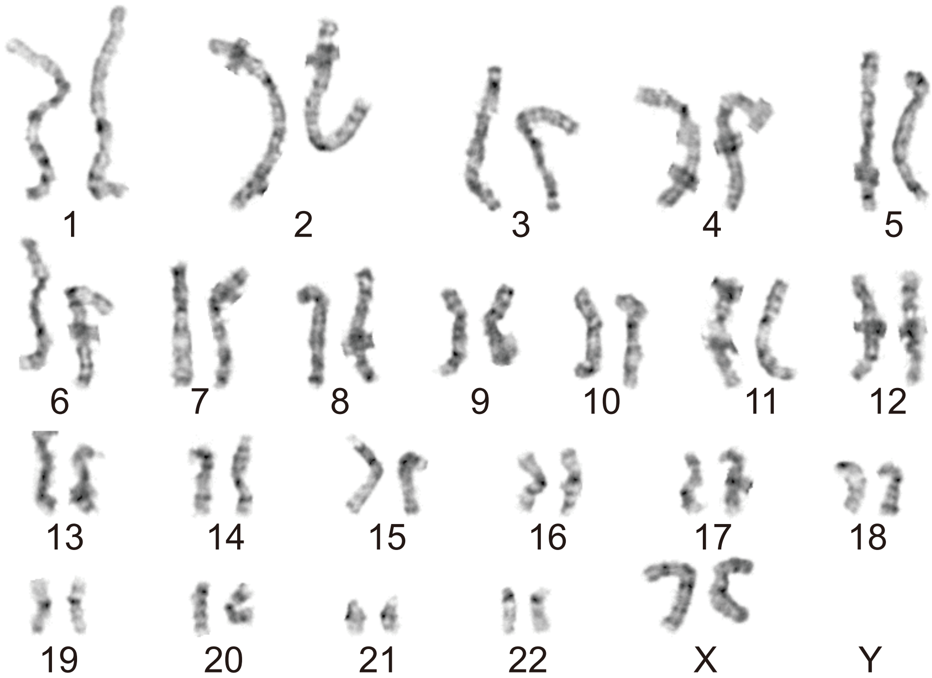
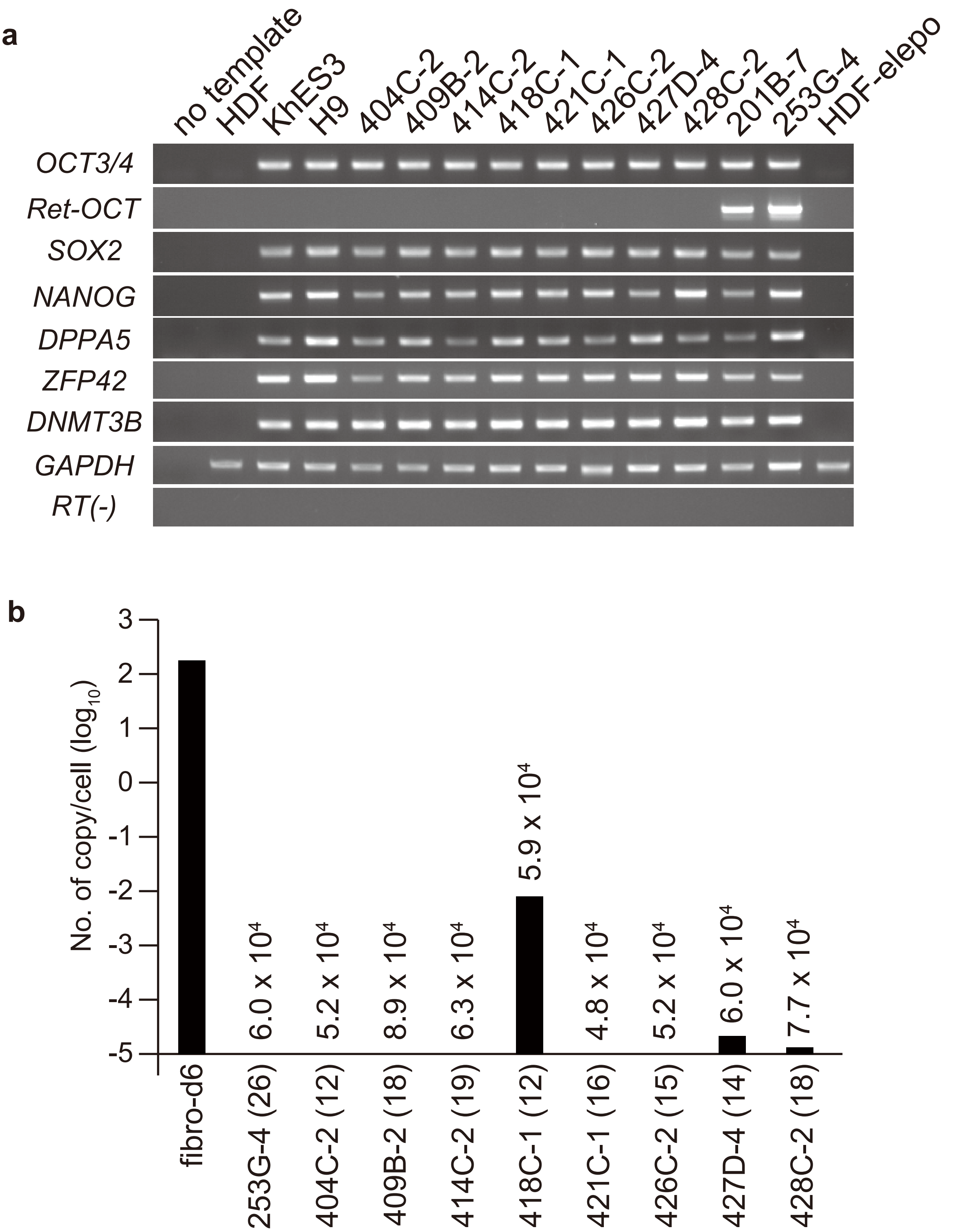


Supplementary Figure 1. Morphology of induced colonies.

(**a**) Morphology of an ESC-like colony. Bar = 1 mm. (**b**) Morphology of a non-ESC-like colony. Bar = 1 mm.

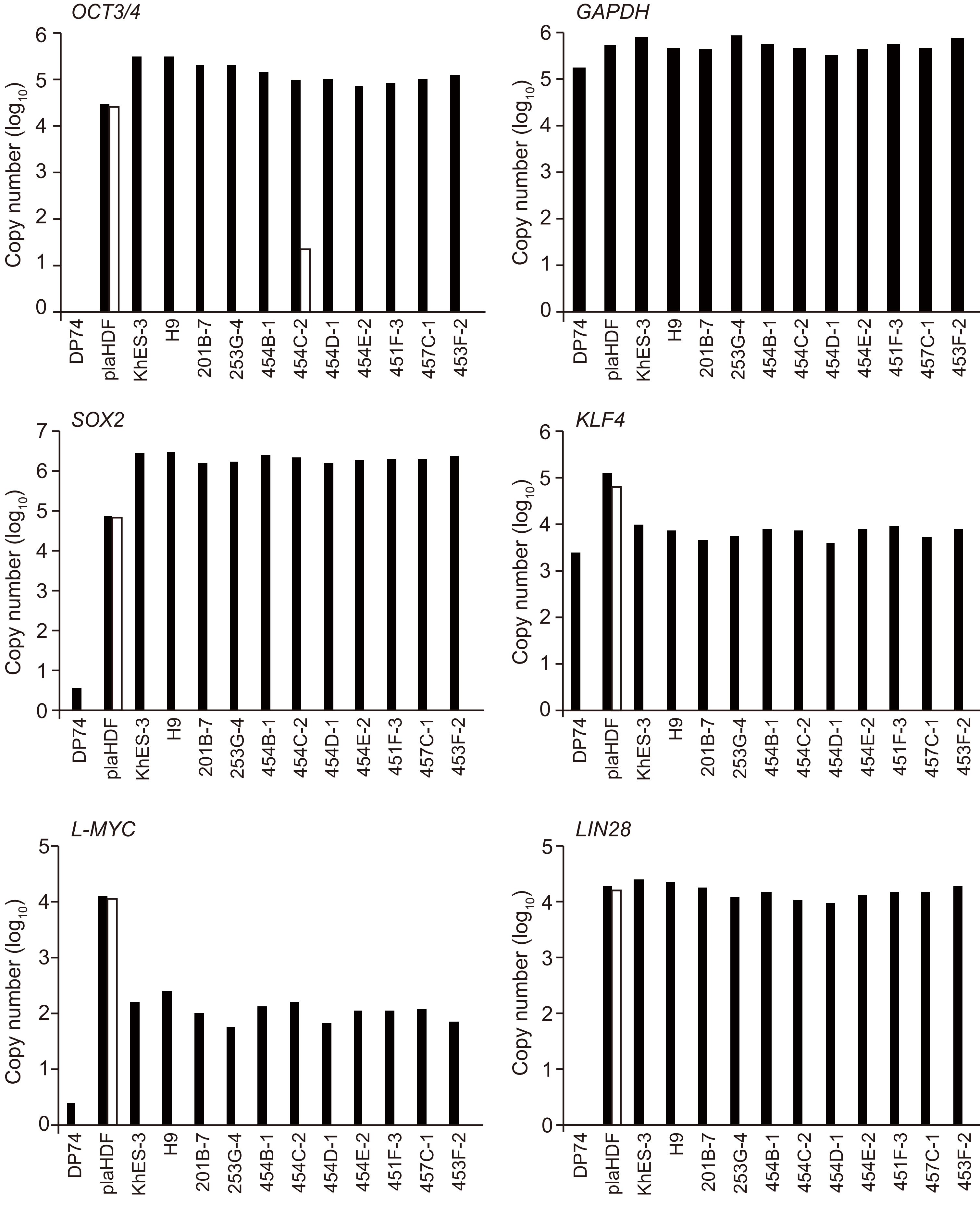


Supplementary Figure 2. G-band staining of a pla-iPSC clone (409B-2).



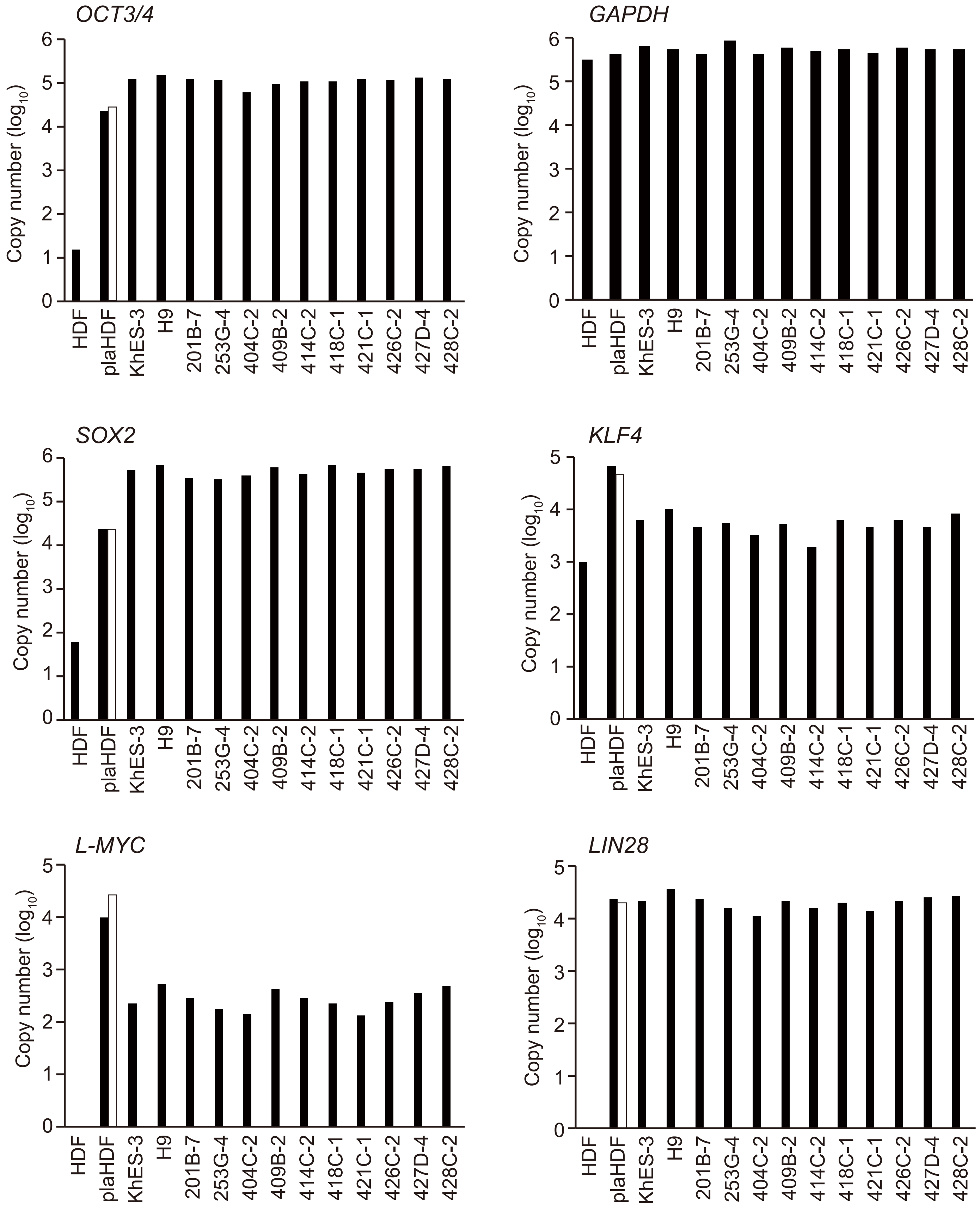
Supplementary Figure 3.
Gene expression and copy number of the episomal plasmids in fibroblast-derived pla-iPSCs.

(**a**) Expressions of pluripotent cell marker genes by an RT-PCR analysis. Pla-iPSCs established with the Y4 mix from various fibroblasts (404C-2, 409B-2, 414C-2, 418C-1, 421C-1, 426C-2, 427D-4, and 428C-2), retrovirus-derived iPSCs (201B-7 and 253G-4), and ESCs (KhES-3 and H9) were used for the analyses. Endogenous *OCT3/4* (*OCT3/4*), retrovirus-derived *OCT3/4* (*Ret-OCT*), and endogenous *SOX2* (*SOX2*) were examined. *GAPDH* was examined as a loading control. For the control, fibroblasts were examined 4 days after electroporation of Y4 mixture (HDF-elepo). (**b**) Copy numbers of episomal vectors that remained in the Pla-iPSC clones. Established clones were harvested, lysed, and used for the quantitative PCR analyses. In several clones, including 404C-2 and 409B-2, we were unable to detect any episomal vectors. The numbers in parentheses indicate the passage number. The examined cell numbers are also indicated. For the control, retrovirus-derived iPSC clone (253G-4) and fibroblasts at 6 days after electroporation of Y4 mixture (fibro-d6) were analyzed.



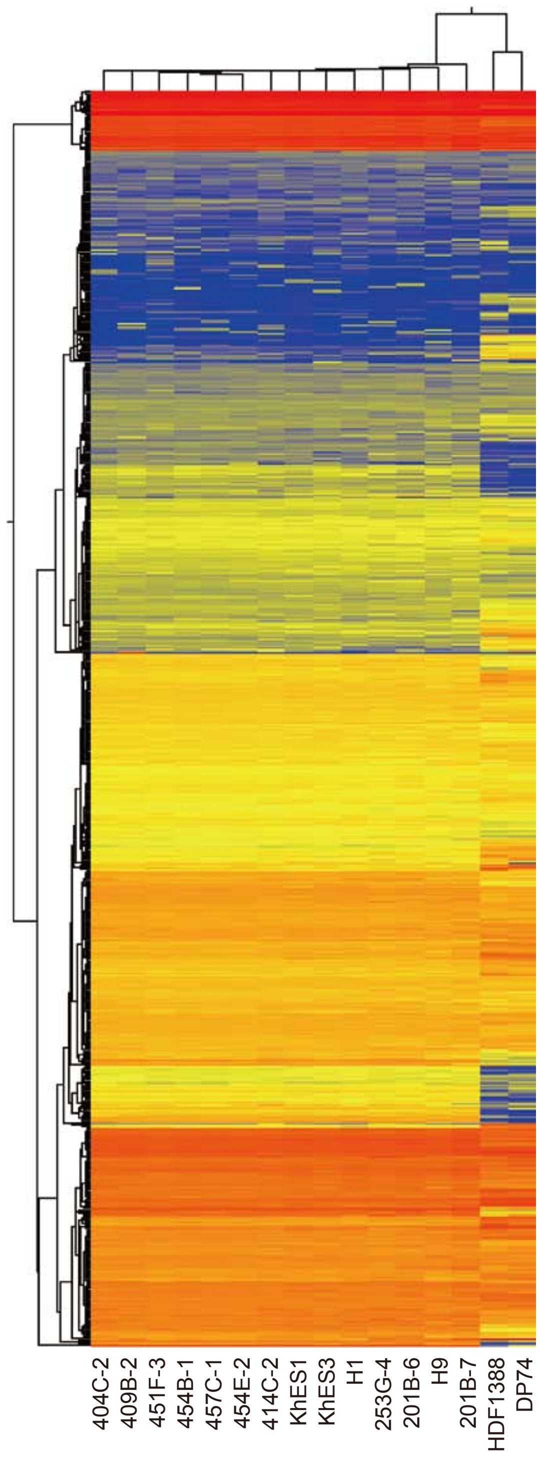
Supplementary Figure 4. Quantitative PCR for detection of transgenic expression in DP-derived pla-PSCs.

Total RNA was isolated from ESCs (KhES-3 and H9), retrovirus-derived iPSCs (201B-7 and 253G-4), and pla-iPSCs (454B-1 established by Y1 mix, 454C-2 by Y2, 454D-1 by Y3, 454E-2, 451F-3, 457C-1, and 453F-2 by Y4), and were used for the RT-PCR analyses. White columns show the copy numbers obtained with specific primers to the episomal vector of the indicated genes. Black columns show the copy numbers obtained with primers for the coding sequence, which could detect both endogenous and episomal vector-derived transcripts. For the controls, fibroblasts at 4 days after electroporation of Y4 mixture were analyzed (plaHDF).



Supplementary Figure 5. Quantitative PCR for the detection of transgene expression in fibroblast-derived pla-iPSCs.

Total RNA was extracted from ESCs (KhES-3 and H9), retrovirus-derived iPSCs (201B-7 and 253G-4), and pla-iPSCs (404C-2, 409B-2, 414C-2, 418C-1, 421C-1, 426C-2, 427D-4, and 428C-2), and were used for the RT-PCR analyses. White columns show the copy numbers obtained with specific primers against the episomal vector of the indicated genes. Black columns show the copy numbers obtained with primers for the coding sequence, which could detect both endogenous and episomal vector-derived transcripts. As a control, fibroblasts at 4 days after electroporation of Y4 mixture were analyzed (plaHDF).



Supplementary Figure 6. Comparison of the global gene expression profiles.

Unsupervised clustering was performed using microarray data from ESC clones (KhES1, KhES3, H1, and H9), pla-iPSC clones (404C-2, 409B-2, 414C-2, 451F-3, 454B-1, 454E-2, and 457C-1), retrovirus-derived iPSC clones (201B-6, 201B-7, and 253G-4), fibroblasts (HDF1388), and DP cells (DP74).

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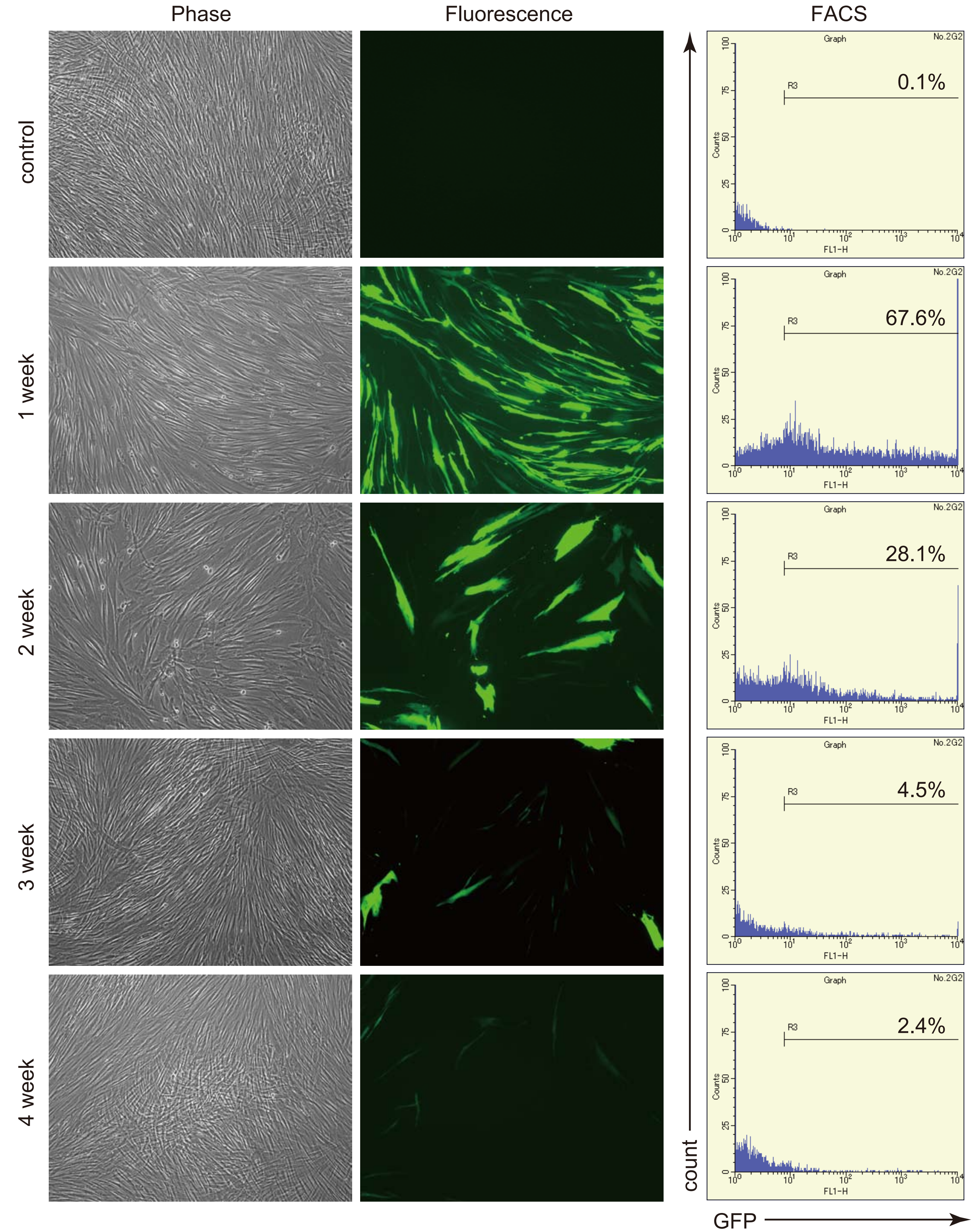
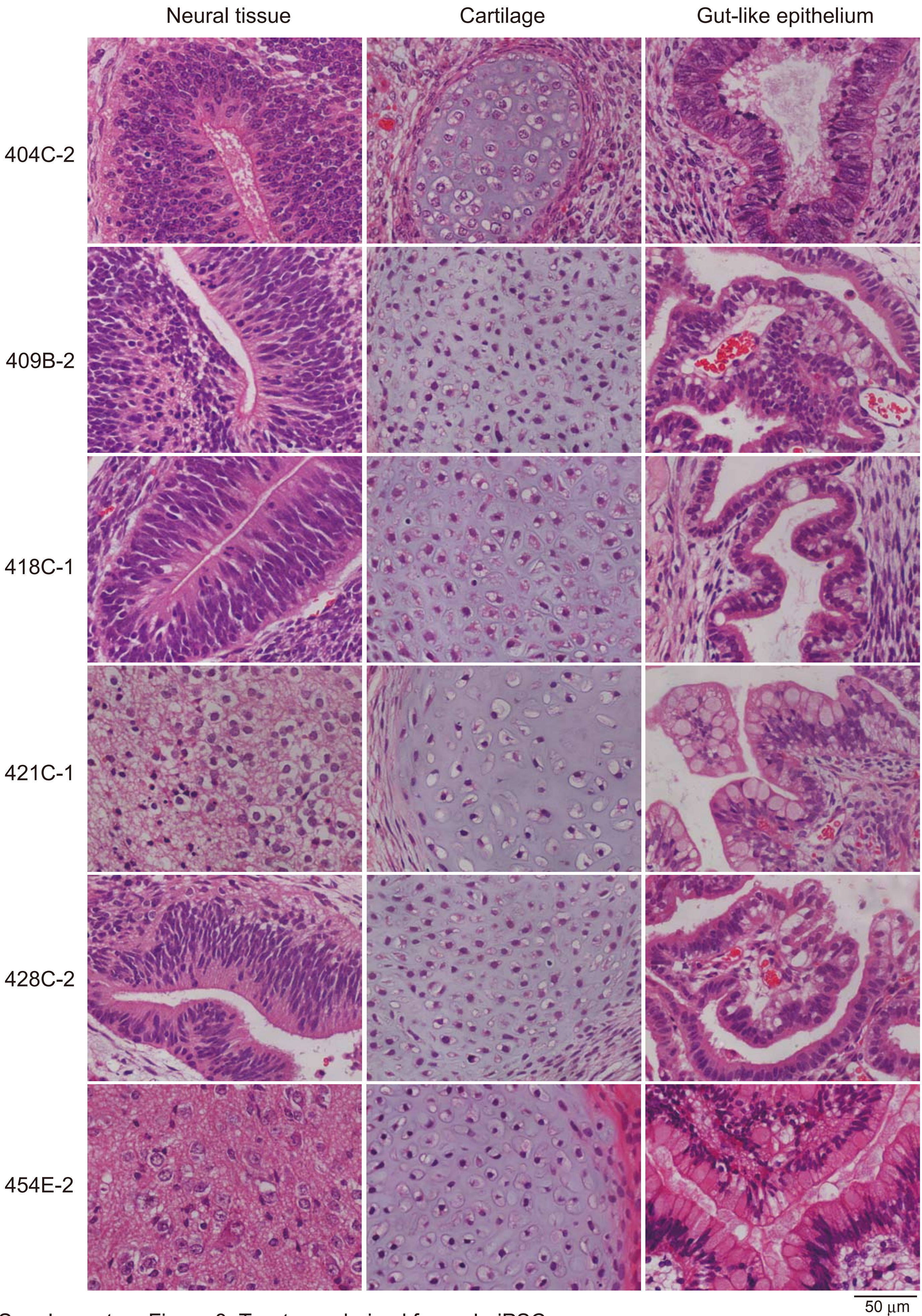


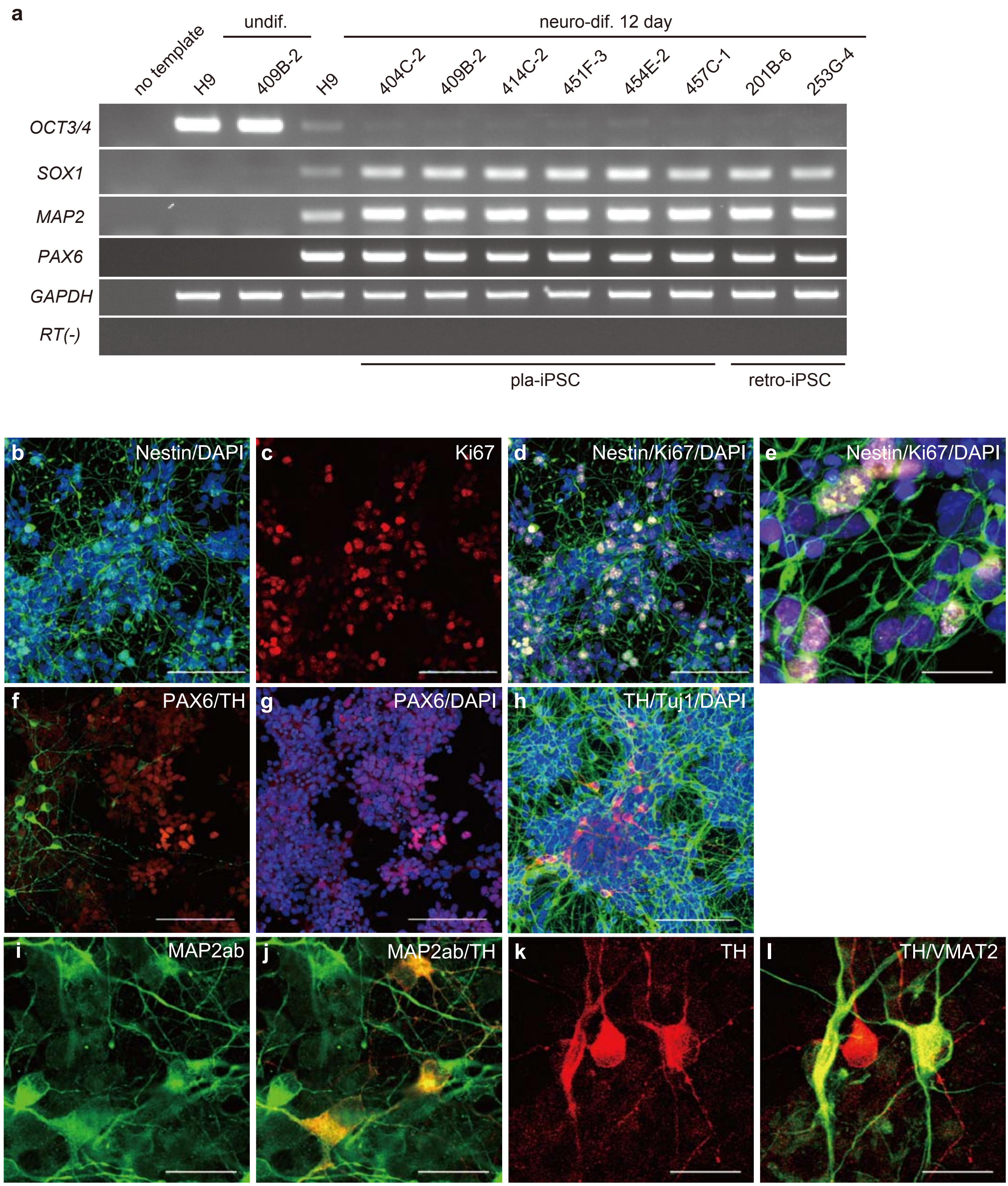
Supplementary Figure 7. Expression of GFP fluorescence from episomal vector.

Human fibroblasts were transduced with an episomal vector encoding GFP via electroporation. The cells were photographed and analyzed by flow cytometry at 1, 2, 3, and 4 weeks after transduction.



Supplementary Figure 8. Teratoma derived from pla-iPSCs.

Hematoxylin and eosin staining of teratoma derived from the indicated iPSC clones.



Supplementary Figure 9. Differentiation of pla-iPSCs into dopamine neurons.

(**a**) Expression of neural marker genes (*SOX1*, *MAP2*, and *PAX6*) detected by RT-PCR analyse. Total RNA was isolated from H9 ESCs, pla-iPSCs, and retro-iPSCs under undifferentiation condition (undif.) or 12 days after neural differentiation (neuro-dif.). (**b-l**) Twenty-nine days after induction using serum-free culture of embryoid body-like aggregates (SFEB) combined with double SMAD inhibition by a BMP antagonist and an Activin/Nodal inhibitor, the pla-iPSC clone, 454E-2, had differentiated into neurons. (**b-d**) Double immunostaining for Nestin (green) and Ki67 (red) with DAPI nuclear staining (blue). A high magnification image is shown in (**e**). (**f** and **g**) Double immunostaining for PAX6 (red) and TH (green) with DAPI nuclear staining (blue) in a same visual field. (**h**) Double immunostaining for TH (red) and TuJ1 (green) with DAPI. (**i** and **j**) Double immunostaining for TH (red) and MAP2ab (green). (**k** and **l**) Double immunostaining for TH (Red) and the vesicular monoamine transporter 2 (VMAT2, green). Scale bars: 100 μm in (**b-e**, **f-h**); 20 μm in (**e**, **i-l**).

Supplementary Table 1. Comparison of integration-free methods of human iPSC generation.

Method	Efficiency[a]	iPSC generation from adult cells	Preparation of materials	Delivery procedure	Removal of exogenous factors	References
Adenovirus	+ (2×10^{-6})[b]	Not tested	+[h]	++ (2 infections)	++[l]	[n]
Sendai virus	+++ (1×10^{-2})[c]	++ (1 HDF, 1 T cell)	+[h]	+++ (1 infection)	+[m]	[o, p]
Episomal plasmid	++ (3.9×10^{-4})[d]	+++ (8 HDF, 2 DP)	+++	+++ (1 transfection)	++[l]	
Minicircle DNA	+ (5×10^{-5})[e]	++ (3 ASC)	+[i]	+ (3 transfections, FACS sorting)	++[l]	[q]
RNA	+++ (4.4×10^{-2})[f]	+ (1 HDF)	+[j]	+ (16 daily transfections)	+++	[r]
Protein	+ (2.6×10^{-5})[g]	Not tested	+[k]	+ (6 cycle treatments)	+++	[s]

[a]The highest figure reported in each study.

[b]IMR90 embryonic fibroblasts.

[c]BJ neonate fibroblasts.

[d]Adult fibroblasts.

[e]Adult adipose stem cells (ASC), after sorting of transfected cells.

[f]Fibroblasts produced by directed differentiation of H1 ESC.

[g]Newborn fibroblasts.

[h]Production of virus in packaging cells.

[i]Construction of minicircle vectors.

[j]*in vitro* transcription with modified nucleotide.

[k]Production of proteins in HEK293 cells.

[l]Rare integration events need to be ruled out.

[m]Sendai virus tend to replicate in cytoplasm.

[n]Zhou, W. & Freed, C.R. Adenoviral gene delivery can reprogram human fibroblasts to induced pluripotent stem cells. *Stem Cells* **27**, 2667-2674 (2009).

[o]Fusaki, N., Ban, H., Nishiyama, A., Saeki, K. & Hasegawa, M. Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. *Proc Jpn Acad Ser B Phys Biol Sci* **85**, 348-362 (2009).

[p]Seki, T.*, et al.* Generation of induced pluripotent stem cells from human terminally differentiated circulating T cells. *Cell Stem Cell* **7**, 11-14 (2010).

[q]Jia, F.*, et al.* A nonviral minicircle vector for deriving human iPS cells. *Nat Methods* **7**, 197-199 (2010).

[r]Warren, L.*, et al.* Highly Efficient Reprogramming to Pluripotency and Directed Differentiation of Human Cells with Synthetic Modified mRNA. *Cell Stem Cell* **7**, 618-30 (2010).

[s]Kim, D.H.*, et al.* Generation of Human Induced Pluripotent Stem Cells by Direct Delivery of Reprogramming Proteins. *Cell Stem Cell* **4**, 472-476 (2009).

Supplementary Table 2. Summary of plasmid mixtures.

Mixture name	Plasmid name	Amount (μg)	Genes
Y1	pCXLE-hOCT3/4	1	*OCT3/4*
	pCXLE-hSK	1	*SOX2*, *KLF4*
	pCXLE-hMLN	1	*c-MYC*, *LIN28*, *NANOG*
Y2	pCXLE-hOCT3/4-shp53	1	*OCT3/4*, *p53* shRNA
	pCXLE-hSK	1	*SOX2*, *KLF4*
	pCXLE-hMLN	1	*c-MYC*, *LIN28*, *NANOG*
Y3	pCXLE-hOCT3/4	1	*OCT3/4*
	pCXLE-hSK	1	*SOX2*, *KLF4*
	pCXLE-hUL	1	*L-MYC*, *LIN28*
Y4	pCXLE-hOCT3/4-shp53	1	*OCT3/4*, *p53* shRNA
	pCXLE-hSK	1	*SOX2*, *KLF4*
	pCXLE-hUL	1	*L-MYC*, *LIN28*
T1	pEP4EO2SET2K	1.125	*OCT3/4*, *SOX2*, *SV40LT*, *KLF4*
	pEP4EO2SEN2K	1.125	*OCT3/4*, *SOX2*, *NANOG*, *KLF4*
	pCEP4-M2L	0.75	*c-MYC*, *LIN28*
T2	pEP4EO2SET2K	0.91	*OCT3/4*, *SOX2*, *SV40LT*, *KLF4*
	pEP4EO2SCK2MEN2L	2.09	*OCT3/4*, *SOX2*, *KLF4*, *c-MYC*, *NANOG*, *LIN28*
T3	pEP4EO2SET2K	0.8	*OCT3/4*, *SOX2*, *SV40LT*, *KLF4*
	pEP4EO2SEN2L	1.05	*OCT3/4*, *SOX2*, *NANOG*, *LIN28*
	pEP4EO2SEM2K	1.15	*OCT3/4*, *SOX2*, *c-MYC*, *KLF4*

Supplementary Table 3. Summary of the iPSC induction experiments.

			MEF feeder		SNL feeder	
Parental cells	Exp. ID	Factors	non ES-like	ES-like	non ES-like	ES-like
HDF1419	438	Y1	1	0	0	1
		Y2	24	17	5	17
		Y3	18	8	5	11
		Y4	81	60	66	108
		control	0	0	0	0
	449	Y4	47	134	43	150
		control	0	0	0	0
TIG121	380	Y1	2	3	0	0
		Y2	11	10	1	4
		Y3	9	23	0	0
		Y4	24	97	5	11
		control	0	0	0	0
TIG120	380	Y1	1	3	0	0
		Y2	7	11	1	2
		Y3	4	16	0	4
		Y4	37	71	17	25
		control	0	0	0	0
	428	Y1	0	1	0	0
		Y2	5	10	3	0
		Y3	-	-	2	1
		Y4	20	61	3	15
		control	0	0	0	0
	448	Y1	0	3	0	0
		Y2	2	12	0	4
		Y3	4	30	1	1
		Y4	23	92	6	19
		T1	7	0	14	0
		T2	8	0	1	0
		T3	20	0	5	0
		control	0	0	0	0
	455	Y1	3	5	0	0
		Y2	5	24	1	8
		Y3	9	45	2	9
		Y4	37	154	10	67
		T1	15	0	8	0
		T2	11	0	1	1
		T3	48	0	6	1
		control	0	0	0	0
	522	Y1	1	1	0	0
		Y2	4	4	2	2
		Y3	2	0	0	0
		Y4	5	16	2	3
		T1	4	0	0	0
		T2	1	0	0	0
		T3	6	0	0	0
		control	0	0	0	0
HDF1388	404	Y1	0	0	0	0
		Y2	0	1	0	0
		Y3	1	2	0	0
		Y4	1	5	0	2

		control	0	0	0	0
	409	Y1	0	0	0	0
		Y2	0	0	0	0
		Y3	0	2	0	0
		Y4	2	12	0	1
		T1	9	0	5	0
		T2	14	4	3	5
		T3	56	2	36	1
		control	0	0	0	0
	414	Y1	0	0	0	0
		Y2	4	1	1	0
		Y3	0	1	0	0
		Y4	3	9	0	2
		T1	5	0	3	0
		T2	30	0	1	6
		T3	18	0	5	0
		control	0	0	0	0
	415	Y1	0	0	0	0
		Y2	0	0	1	0
		Y3	0	0	0	0
		Y4	1	5	2	1
		T1	13	0	3	0
		T2	28	0	0	2
		T3	26	0	12	0
		control	0	0	0	0
	417	Y1	0	0	0	0
		Y2	103	0	0	0
		Y3	0	1	0	0
		Y4	1	4	0	3
		control	0	0	0	0
TIG114	418	Y1	0	0	0	0
		Y2	1	0	1	0
		Y3	2	0	0	0
		Y4	12	4	0	1
		control	0	0	0	0
HDF1377	436	Y1	3	1	0	0
		Y2	78	2	3	1
		Y3	7	8	1	1
		Y4	27	26	8	11
		T2	16	0	2	0
		control	0	0	0	0
HDF1429	437	Y4	34	50	4	24
		T2	22	0	1	0
		control	0	0	0	0
HDF1437	427[a]	Y1	5	0	0	1
		Y2	9	1	0	1
		Y3	11	2	3	5
		Y4	27	4	2	12
		control	0	0	0	0
	460	Y1	0	0	0	0
		Y2	6	0	1	3
		Y3	15	2	0	1
		Y4	43	11	19	29
		T1	9	0	10	0

		T2	18	0	8	1
		T3	25	0	19	0
		control	0	0	0	0
	466	Y1	0	0	0	0
		Y2	21	0	4	13
		Y3	22	0	0	8
		Y4	174	44	81	100
		T1	13	0	8	0
		T2	10	0	3	0
		T3	38	0	40	0
		control	0	0	0	0
	521	Y1	0	0	0	0
		Y2	5	1	0	3
		Y3	1	0	0	0
		Y4	17	5	6	11
		T1	14	0	2	0
		T2	5	0	3	0
		T3	32	0	19	0
		control	0	0	0	0
HDF1554	426	Y1	0	0	0	0
		Y2	1	0	0	0
		Y3	2	0	0	0
		Y4	6	0	1	2
		control	0	0	0	0
	465	Y1	0	0	0	1
		Y2	6	0	1	2
		Y3	4	0	0	0
		Y4	35	0	8	22
		control	0	0	0	0
	468	Y1	0	0	0	0
		Y2	7	0	1	2
		Y3	2	0	1	2
		Y4	11	5	11	25
		control	0	0	0	0
TIG107	421	Y1	1	0	-	-
		Y2	9	0	-	-
		Y3	0	0	-	-
		Y4	3	4	-	-
		control	0	0	-	-
	478	Y1	0	0	0	0
		Y2	0	0	0	0
		Y3	0	0	0	0
		Y4	2	5	0	0
		Y4	0	2	0	0
		control	0	0	0	0
DP74	451	Y1	0	0	0	0
		Y2	5	8	3	13
		Y3	2	5	3	7
		Y4	7	27	10	83
		T2	1	0	1	4
		T3	0	0	3	0
		control	0	0	0	0
	454	Y1	0	5	0	3
		Y2	8	12	2	11

		Y3	4	8	2	11
		Y4	10	20	10	27
		T1	0	0	0	0
		T2	0	0	1	4
		T3	0	0	0	1
		control	0	0	0	0
	457	Y1	1	0	0	0
		Y2	2	0	0	3
		Y3	2	2	0	1
		Y4	2	7	2	13
		T1	0	0	0	0
		T2	0	0	2	0
		T3	1	0	6	0
		control	0	0	0	0
	469	Y1	2	0	1	1
		Y2	4	6	4	14
		Y3	8	11	4	17
		Y4	28	23	18	97
		T1	0	0	0	0
		T2	2	0	1	2
		T3	0	0	4	4
		control	0	0	0	0
DP94	446	Y1	2	2	5	5
		Y2	17	4	19	21
		Y3	14	3	11	7
		Y4	27	10	31	30
		T1	0	0	3	1
		T2	0	0	0	0
		T3	0	0	0	0
		control	0	0	0	0
	450	Y1	5	0	2	0
		Y2	8	0	11	19
		Y3	9	2	10	8
		Y4	24	2	21	35
		T1	0	0	2	1
		T2	0	0	0	2
		T3	0	0	3	1
		control	0	0	0	0
	453	Y1	5	1	1	3
		Y2	12	3	9	12
		Y3	9	5	7	7
		Y4	36	22	30	24
		T1	0	0	1	0
		T2	1	0	0	0
		T3	0	0	0	0
		control	0	0	0	0

[a]In the exp. 427, only 0.8×10^5 cells were plated onto SNL feeder cells.

*Exp. ID 448, 455, 522, 409, 414, 415, 460, 466, 521, 454, 457, 469, 446, 450, and 453 were used for Fig. 1d.

Supplementary Table 4. Karyotype of episomal vector-derived iPSCs.

Clone	passage	Karyotype	origin	Age	Sex[a]	Race[b]	factors
			fibroblasts				
409B-2	p19	46,XX [20]	HDF1388	36	F	C	Y4
414C-2	p19	46,XX [20]	HDF1388	36	F	C	Y4
418C-1	p19	46,XY [20]	TIG114	36	M	J	Y4
421C-1	p19	46,XX [20]	TIG107	81	F	J	Y4
426C-2	p14	46,XX [20]	HDF1554	77	F	C	Y4
427D-4	p13	46,XY, add(3)(q13.2) [20]	HDF1437	56	M	C	Y4
428C-2	p20	46,XX [20]	TIG120	6	F	J	Y4
			DP cells				
451F-3	p9	46,XX [20]	DP74	16	F	J	Y4
453F-2	p12	46,XX [20]	DP94	16	F	J	Y4
454B-1	p12	46,XX [20]	DP74	16	F	J	Y1
454C-2	p10	46,XX [20]	DP74	16	F	J	Y2
454D-1	p11	46,XX [20]	DP74	16	F	J	Y3
454E-2	p12	46,XX [20]	DP74	16	F	J	Y4
454F-1	p13	46,XX,add(17)(q25)[15] / 46,XX[5]	DP74	16	F	J	T2
457C-1	p12	46,XX [20]	DP74	16	F	J	Y4

[a]M, male; F, female

[b]J, Japanese; C, Caucasian.

SupplementaryTable 5. STR analyses of pla-iPSCs.

Locus/clone	HDF1388		409B-2		414C-2		DP74		454E-2		457C-1	
D3S1358	16	18	16	18	16	18	17		17		17	
TH01	6		6		6		7		7		7	
D21S11	29		29		29		29	30	29	30	29	30
D18S51	15		15		15		13	19	13	19	13	19
Penta_E	7	19	7	19	7	19	5	15	5	15	5	15
D5S818	12		12		12		12	13	12	13	12	13
D13S317	9	13	9	13	9	13	10	14	10	14	10	14
D7S820	9	10	9	10	9	10	11		11		11	
D16S539	11	13	11	13	11	13	9	10	9	10	9	10
CSF1PO	11		11		11		12	14	12	14	12	14
Penta_D	9	11	9	11	9	11	10	13	10	13	10	13
AMEL	X		X		X		X		X		X	
vWA	15	18	15	18	15	18	17	21	17	21	17	21
D8S1179	8	10	8	10	8	10	11	12	11	12	11	12
TPOX	8	9	8	9	8	9	8		8		8	
FGA	20	22	20	22	20	22	21	24	21	24	21	24

Supplementary Table 6. Pearson correlation coefficient table.

Cells	404C-2	409B-2	414C-2	451F-3	454B-1	454E-2	457C-1	201B-6	201B-7	253G-4	H1	H9	KhES1	KhES3	HDF1388	DP74	CellType
404C-2	1	0.99134	0.99538	0.99044	0.99031	0.99198	0.98828	0.99044	0.97643	0.99336	0.99492	0.97962	0.99175	0.98902	0.82664	0.85313	Pla-iPSCs
409B-2	0.99134	1	0.99169	0.99285	0.99592	0.99557	0.99460	0.98239	0.98645	0.98373	0.99082	0.99221	0.98944	0.99396	0.82676	0.85663	Pla-iPSCs
414C-2	0.99538	0.99169	1	0.99241	0.99252	0.99232	0.99139	0.99200	0.97867	0.99466	0.99332	0.98108	0.99549	0.98914	0.83475	0.86055	Pla-iPSCs
451F-3	0.99044	0.99285	0.99241	1	0.99668	0.99784	0.99786	0.98416	0.98480	0.98417	0.99305	0.98925	0.99432	0.99540	0.83299	0.86326	Pla-iPSCs
454B-1	0.99031	0.99592	0.99252	0.99668	1	0.99840	0.99856	0.98459	0.98912	0.98475	0.99225	0.99269	0.99324	0.99471	0.83082	0.86259	Pla-iPSCs
454E-2	0.99198	0.99557	0.99232	0.99784	0.99840	1	0.99809	0.98424	0.98611	0.98469	0.99286	0.99199	0.99286	0.99596	0.83134	0.86163	Pla-iPSCs
457C-1	0.98828	0.99460	0.99139	0.99786	0.99856	0.99809	1	0.98275	0.98878	0.98256	0.99213	0.99286	0.99335	0.99577	0.83051	0.86185	Pla-iPSCs
201B-6	0.99044	0.98239	0.99200	0.98416	0.98459	0.98424	0.98275	1	0.97506	0.99221	0.98866	0.97083	0.99045	0.97956	0.82601	0.85383	Retro-iPSCs
201B-7	0.97643	0.98645	0.97867	0.98480	0.98912	0.98611	0.98878	0.97506	1	0.97174	0.98394	0.98785	0.98483	0.98621	0.81791	0.85376	Retro-iPSCs
253G-4	0.99336	0.98373	0.99466	0.98417	0.98475	0.98469	0.98256	0.99221	0.97174	1	0.99009	0.97106	0.99112	0.98138	0.82652	0.85200	Retro-iPSCs
H1	0.99492	0.99082	0.99332	0.99305	0.99225	0.99286	0.99213	0.98866	0.98394	0.99009	1	0.98488	0.99498	0.99393	0.82599	0.85782	ESCs
H9	0.97962	0.99221	0.98108	0.98925	0.99269	0.99199	0.99286	0.97083	0.98785	0.97106	0.98488	1	0.98325	0.99272	0.81722	0.85115	ESCs
KhES1	0.99175	0.98944	0.99549	0.99432	0.99324	0.99286	0.99335	0.99045	0.98483	0.99112	0.99498	0.98325	1	0.99191	0.83019	0.85962	ESCs
KhES3	0.98902	0.99396	0.98914	0.99540	0.99471	0.99596	0.99577	0.97956	0.98621	0.98138	0.99393	0.99272	0.99191	1	0.82553	0.85750	ESCs
HDF1388	0.82664	0.82676	0.83475	0.83299	0.83082	0.83134	0.83051	0.82601	0.81791	0.82652	0.82599	0.81722	0.83019	0.82553	1	0.96628	Fibroblasts
DP74	0.85313	0.85663	0.86055	0.86326	0.86259	0.86163	0.86185	0.85383	0.85376	0.85200	0.85782	0.85115	0.85962	0.85750	0.96628	1	DP cells

Supplementary Table 7. HLA Type of 107 Individuals.

No.	Cell line	HLA-A		HLA-B		HLA-DRB1		Match
1	DP1	*24	*33	*40	*44	*04	*13	
2	DP4	*03	*24	*07	*13	*01	*07	
3	DP6	*02	*02	*35	*40	*04	*09	
4	DP7	*03	*24	*44	*52	*09	*15	DP74
5	DP10	*24	*26	*13	*52	*12	*15	DP74
6	DP12	*24	*31	*07	*56	*01	*14	
7	DP13	*31	-	*40	*51	*08	*09	
8	DP14	*24	*31	*15	*52	*09	*15	DP74
9	DP15	*02	*02	*15	*46	*08	*14	
10	DP17	*24	*26	*52	*54	*09	*15	DP74
11	DP20	*26	*33	*44	*46	*04	*08	
12	DP25	*02	*24	*35	*51	*04	*08	
13	DP26	*02	*02	*15	*35	*04	*12	
14	DP28	*02	*31	*35	*46	*04	*08	
15	DP30	*24	*31	*15	*52	*09	*15	DP74
16	DP31	*11	*31	*48	*55	*09	*11	
17	DP32	*24	*26	*40	*40	*04	*08	
18	DP33	*24	*26	*52	*54	*14	*15	DP74
19	DP35	*33	-	*44	-	*08	*13	
20	DP38	*11	*31	*40	*51	*04	*14	
21	DP39	*11	*24	*15	*40	*04	*15	DP94
22	DP40	*24	*26	*15	*52	*14	*15	DP74
23	DP41	*24	*26	*54	*54	*01	*04	
24	DP42	*24	*24	*07	*51	*01	*08	
25	DP44	*24	*24	*40	*52	*12	*15	DP74
26	DP46	*11	*24	*15	*51	*04	*14	DP94
27	DP48	*24	*26	*15	*52	*14	*15	DP74
28	DP49	*24	*33	*07	*44	*01	*08	
29	DP52	*11	*26	*55	*67	*08	*16	
30	DP53	*24	*31	*07	*54	*01	*04	
31	DP54	*02	*26	*40	*46	*08	*09	
32	DP56	*24	*24	*40	*52	*09	*15	DP74
33	DP57	*24	*33	*44	*54	*04	*13	
34	DP58	*24	*26	*40	*40	*11	*09	
35	DP59	*24	*24	*07	*15	*01	*14	
36	DP60	*02	*24	*46	*52	*08	*15	DP74
37	DP62	*24	*24	*48	*59	*04	*09	
38	DP64	*02	*02	*40	*46	*08	*14	
39	DP65	*24	*24	*40	*40	*09	*14	
40	DP66	*24	*24	*40	*51	*08	*11	
41	DP68	*24	*33	*15	*55	*04	*04	
42	DP69	*02	*24	*46	*52	*09	*15	DP74
43	DP72	*02	*11	*40	*46	*08	*15	
44	DP73	*02	*31	*15	*40	*08	*09	
45	DP74[a]	*24	-	*52	-	*15	-	DP74
46	DP75	*02	*24	*15	*51	*08	-	
47	DP80	*31	*33	*44	*51	*09	*13	
48	DP81	*24	*24	*07	*52	*01	*15	DP74
49	DP83	*02	*26	*15	*40	*08	*15	
50	DP86	*24	*31	*51	*52	*09	*15	DP74
51	DP87	*24	*33	*51	*52	*14	*15	DP74
52	DP92	*02	*24	*15	*35	*04	*15	
53	DP94[a]	*11	-	*15	-	*04	-	DP94
54	DP95	*24	*24	*35	*40	*04	*09	
55	DP96	*11	*24	*54	*58	*08	*13	
56	DP97	*24	*24	*52	*54	*04	*15	DP74
57	DP98	*02	*24	*48	*54	*04	-	
58	DP99	*24	*31	*15	*52	*08	*15	DP74

59	DP100	*02	*33	*35	*51	*04	*04	
60	DP101	*33	-	*44	-	*04	*13	
61	DP105	*02	*24	*35	*40	*04	*04	
62	DP106	*24	*26	*15	*40	*04	*13	
63	DP109	*24	*24	*46	*54	*04	*15	
64	DP111	*33	-	*44	-	*09	*13	
65	DP112	*26	*33	*44	*55	*13	*15	
66	DP113	*11	*24	*51	*54	*04	*08	
67	DP115	*02	*24	*51	*40	*09	*14	
68	DP128	*24	*24	*15	*37	*09	*10	
69	DP129	*02	*11	*39	*67	*04	*15	
70	DP134	*31	*33	*44	*51	*04	*13	
71	DP135	*33	-	*15	*44	*13	*14	
72	DP136	*11	*24	*52	*54	*08	*15	DP74
73	DP138	*02	*24	*40	*46	*08	*12	
74	DP140	*31	*33	*15	*39	*09	*15	
75	DP141	*02	*24	*40	*52	*15	*15	DP74
76	DP142	*02	*33	*15	*44	*09	*15	
77	DP143	*24	*24	*54	*52	*14	*15	DP74
78	DP144	*02	*33	*44	-	*13	-	
79	DP147	*24	*24	*51	*52	*04	*15	DP74
80	DP153	*02	*02	*15	*55	*04	-	
81	DP154	*02	*24	*15	*52	*15	*15	DP74
82	DP157	*02	*33	*07	*44	*01	*04	
83	DP158	*24	*33	*51	*52	*09	*15	DP74
84	DP159	*02	*24	*15	*46	*08	*15	
85	DP160	*02	*24	*40	*54	*04	*13	
86	DP163	*24	*26	*40	*46	*04	*09	
87	DP164	*02	*24	*39	*40	*12	-	
88	DP165	*24	*24	*07	*15	*01	*09	
89	DP166	*24	*33	*40	*44	*04	*16	
90	DP167	*24	*24	*40	*52	*04	*15	DP74
91	DP169	*02	*26	*40	*51	*08	*15	
92	DP170	*02	*24	*51	*54	*04	*09	
93	DP172	*02	*24	*40	*52	*04	*15	DP74
94	DP173	*01	*33	*37	*44	*10	*14	
95	DP174	*02	*24	*44	*48	*04	*13	
96	DP175	*02	*33	*07	*44	*01	*04	
97	DP176	*24	*24	*15	*40	*04	*09	
98	DP177	*24	*33	*40	*44	*13	*14	
99	DP178	*24	*24	*40	*52	*12	*15	DP74
100	DP179	*02	*24	*39	*51	*08	*15	
101	DP181	*02	*26	*46	*48	*08	*09	
102	DP182	*24	*31	*27	*59	*13	*15	
103	DP184	*24	*33	*44	*52	*13	*15	DP74
104	DP185	*02	*24	*39	*51	*15	-	
105	DP186	*02	*11	*54	-	*04	*14	
106	DP187	*02	*02	*13	*46	*08	*12	
107	DP193	*24	*26	*39	*52	*08	*15	DP74

[a]DP lines used for induction of pla-iPS cells in this study.
This data was based on the results of 4-digit PCR-rSSOP.

DP74 covers 29, and DP94 covers 3 lines.
Total coverage is 29.9% (32/107).

Supplementary Table 9. Pla-iPSC clone list.

	Source					Gene expression							Differentiation		
Clone	Origin	Age	Sex	Race	Factors	RT-PCR	Microarray	Bisulfite	Copy number analysis	Karyotyping	HLA	STR	Teratoma	Dopa	RPE
404C-2	HDF1388	36	F	C	Y4	√	√		√				√	√	√
409B-2	HDF1388	36	F	C	Y4	√	√	√	√	√		√	√	√	√
414C-2	HDF1388	36	F	C	Y4	√	√	√	√	√		√	√	√	√
418C-1	TIG114	36	M	J	Y4	√			√	√			√		
421C-1	TIG107	81	F	J	Y4	√			√	√			√		
426C-2	HDF1554	77	F	C	Y4	√			√	√					
427D-4	HDF1437	56	M	C	Y4	√			√	√			√		
428C-2	TIG120	6	F	J	Y4	√			√	√			√		
451F-3	DP74	16	F	J	Y4	√	√		√	√			√	√	√
453F-2	DP94	16	F	J	Y4	√			√	√	√		√		
454B-1	DP74	16	F	J	Y1	√	√		√	√			√		
454C-2	DP74	16	F	J	Y2	√			√	√			√		
454D-1	DP74	16	F	J	Y3	√			√	√			√		
454E-2	DP74	16	F	J	Y4	√	√	√	√	√	√	√	√	√	√
454F-1	DP74	16	F	J	T2					√					
457C-1	DP74	16	F	J	Y4	√	√		√	√	√	√	√	√	ND

ND, we could not detect RPE differentiation in our differentiation protocol (*n* = 1).

Supplementary Table 10. Primer list for quantitative PCR.

OCT3/4 (CDS)	CCC CAG GGC CCC ATT TTG GTA CC
	ACC TCA GTT TGA ATG CAT GGG AGA GC
OCT3/4 (pla)	CAT TCA AAC TGA GGT AAG GG
	TAG CGT AAA AGG AGC AAC ATA G
KLF4 (CDS)	ACC CAT CCT TCC TGC CCG ATC AGA
	TTG GTA ATG GAG CGG CGG GAC TTG
KLF4 (pla)	CCA CCT CGC CTT ACA CAT GAA GA
	TAG CGT AAA AGG AGC AAC ATA G
SOX2 (CDS)	TTC ACA TGT CCC AGC ACT ACC AGA
	TCA CAT GTG TGA GAG GGG CAG TGT GC
SOX2 (pla)	TTC ACA TGT CCC AGC ACT ACC AGA
	TTT GTT TGA CAG GAG CGA CAA T
L-MYC (CDS)	GCG AAC CCA AGA CCC AGG CCT GCT CC
	CAG GGG GTC TGC TCG CAC CGT GAT G
L-MYC (pla)	GGC TGA GAA GAG GAT GGC TAC
	TTT GTT TGA CAG GAG CGA CAA T
LIN28 (CDS)	AGC CAT ATG GTA GCC TCA TGT CCG C
	TCA ATT CTG TGC CTC CGG GAG CAG GGT AGG
LIN28 (pla)	AGC CAT ATG GTA GCC TCA TGT CCG C
	TAG CGT AAA AGG AGC AAC ATA G
GAPDH	ACC ACA GTC CAT GCC ATC AC
	TCC ACC ACC CTG TTG CTG TA
EBNA-1	ATC AGG GCC AAG ACA TAG AGA TG
	GCC AAT GCA ACT TGG ACG TT
FBXO15	GCC AGG AGG TCT TCG CTG TA
	AAT GCA CGG CTA GGG TCA AA

CDS; for detection of cording sequence.
pla; for detection of plasmid vector-derived expression.