B. Alkaline phosphatase staining of reprogrammable cells grown in the absence or presence of doxycycline. Doxycycline was withdrawn at day 21, and colonies were stained and counted at day 30.
C. Morphology and expression of OCT4 and Tra-1-81 in doxycycline-independent secondary hiPS cells.
D. Microarray analysis of gene expression between BJ fibroblasts, HUES8 hES cells, primary fibroblast-derived hiPS cells, and a resulting secondary hiPS clone. Shown are genes with >2-fold expression value between BJ fibroblasts and HUES8 hES cells.
E. *In vitro* differentiation of secondary hiPS cells into lineages from all three germ layers. Immunostaining for i) Tuj1, ii) cardiac troponin T, and iii) alpha-fetoprotein.
F. Temporal requirement of factor expression in hiPS-derived fibroblast-like cells. 10^4 cells were plated per time-point and doxycycline was withdrawn daily from days 4 through 19. The number of hES-like colonies that expressed Tra-1-81 were counted at day 25.

Supplementary Data

Supplementary Figure 1: Molecular characterization of hiPS cells.

A. Quantitative RT-PCR analysis for total, endogenous, and viral gene expression of the reprogramming factors. WA09 hES cells, uninfected keratinocytes (K0) and BJ fibroblasts, and 5-factor-infected BJ fibroblasts (3 days +dox) served as controls. Values were standardized to GAPDH, then normalized to WA09 hESC (total and endogenous) or infected BJs (viral).
B. Bisulfite sequencing of the NANOG and the OCT4 promoter regions in BJ fibroblasts, BJ fibroblast-derived hiPS, and WA09 hES cells. Promoter regions containing differentially methylated CpGs are shown. Open circles represent unmethylated CpGs; closed circles denote methylated CpGs.
C. Temporal requirement of factor expression in keratinocytes. Cells infected with five factors were seeded at $7.5x10^4$ cells per time point and doxycycline was withdrawn

every four days from days 6 through 18. The number of hES-like colonies was counted at day 30. All colonies could be expanded in the absence of doxycycline.

Supplementary Figure 2: Characterization of hiPS-derived fibroblast-like cells.

A. Quantitative RT-PCR analysis of reprogramming factor and pluripotency gene expression in hiPS-derived fibroblasts, WA09 hES cells, and BJ fibroblasts. Values were standardized to GAPDH.
B. Quantitative RT-PCR analysis for viral transgene reactivation in hiPS-derived fibroblast-like cells from four BJ-hiPS clones. Cells were analyzed two days after doxycycline administration. Clones #5 and #8 were generated with four factors; clones #11 and #12 with five factors. Values were standardized to GAPDH, then calculated as the ratio of expression between +/- doxycycline.

Supplementary Figure 3: Visual tracking of hiPS colony formation. hiPS-derived fibroblast-like cells were induced to form secondary hiPS cells, and individual colonies were tracked during the reprogramming process. Doxycycline was withdrawn at various time points.

A. Representative colony that failed to reprogram.
B. Representative colony that underwent successful reprogramming.
C. Representative colonies that gave rise to a hiPS clone; two adjacent colonies were tracked. By day 15, these colonies had regressed and an hES-like colony began to form between them (arrows), which continued to develop as hiPS cells.

Supplementary Table 1: Summary of expanded hiPS cell lines.

Supplementary Table 2: Primers used in this study.

Figure 1.

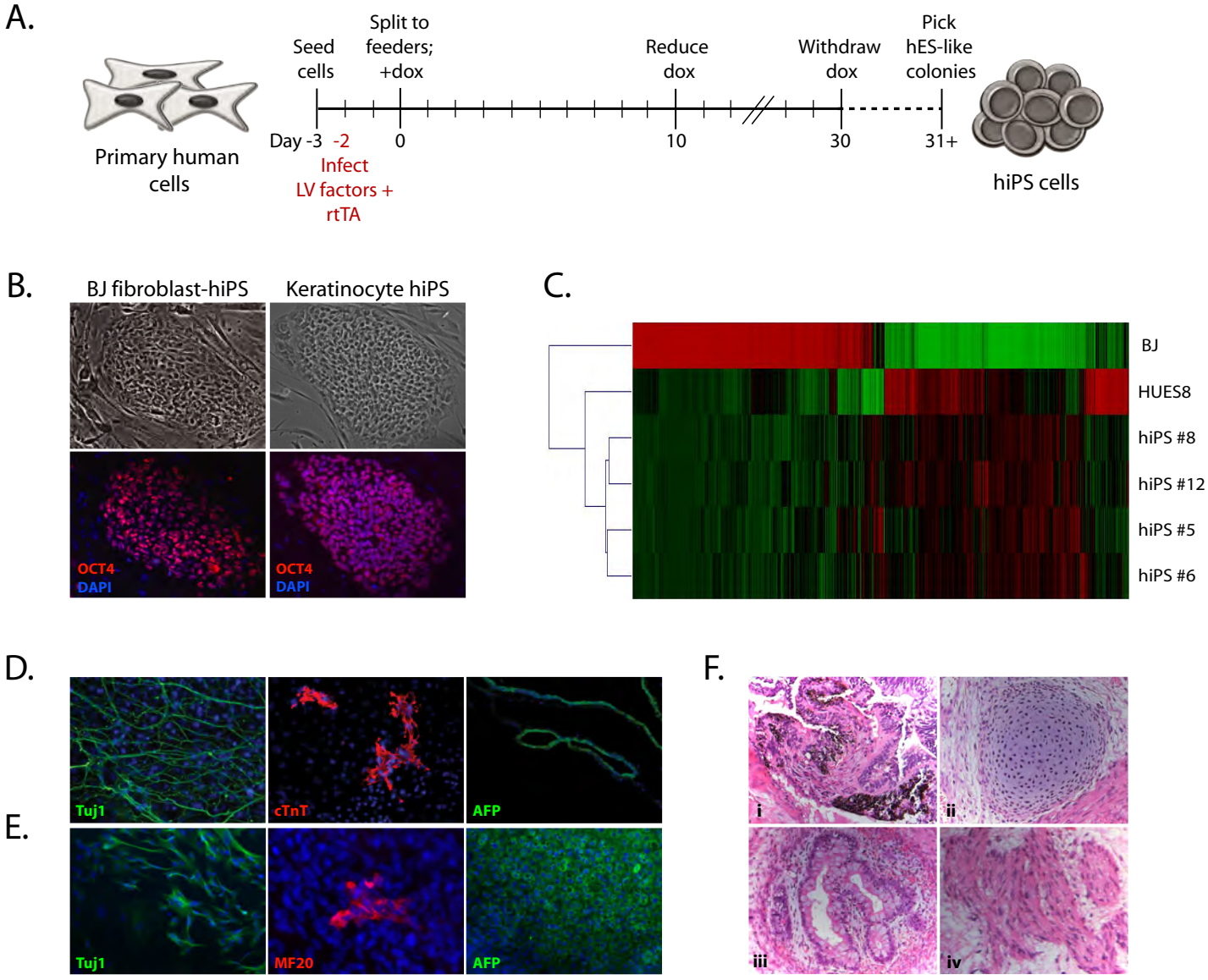
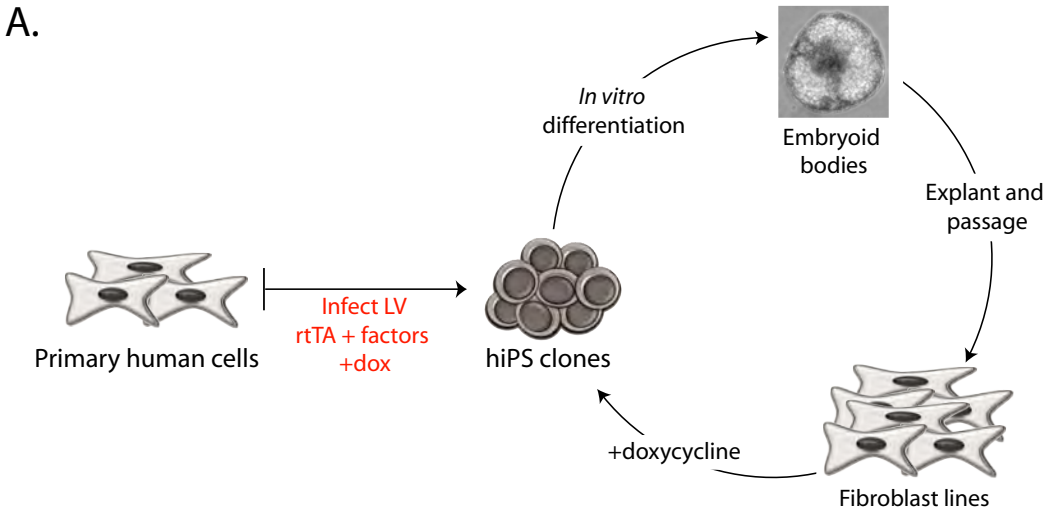
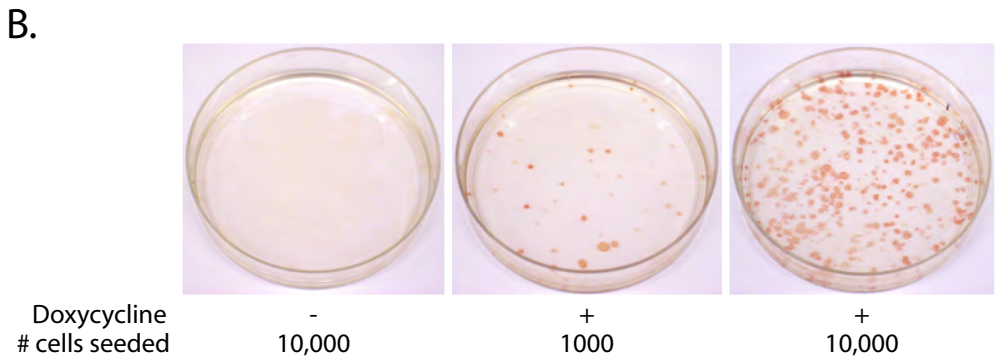
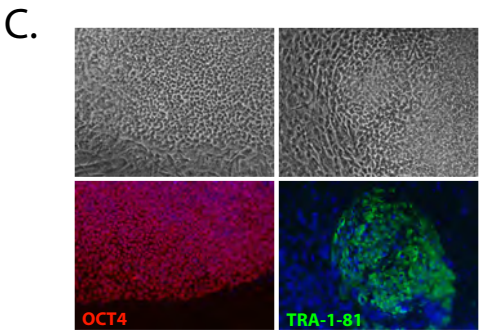
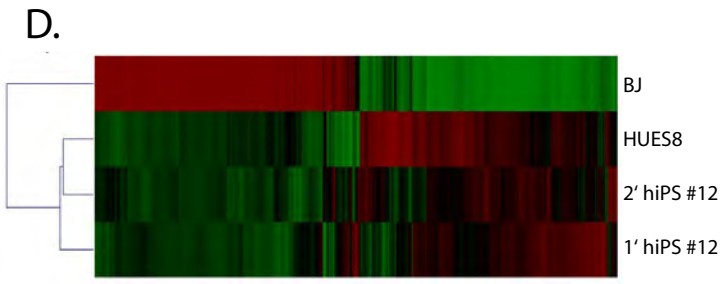

A.
Primary human cells
Seed cells
Split to feeders; +dox
Reduce dox
Withdraw dox
Pick hES-like colonies
Day -3
-2
0
10
30
31+
Infect LV factors + rtTA
hiPS cells
B.
BJ fibroblast-hiPS
Keratinocyte hiPS
OCT4
DAPI
OCT4
DAPI
C.
BJ
HUES8
hiPS #8
hiPS #12
hiPS #5
hiPS #6
D.
Tuj1
cTnT
AFP
E.
Tuj1
MF20
AFP
F.
i
ii
iii
iv

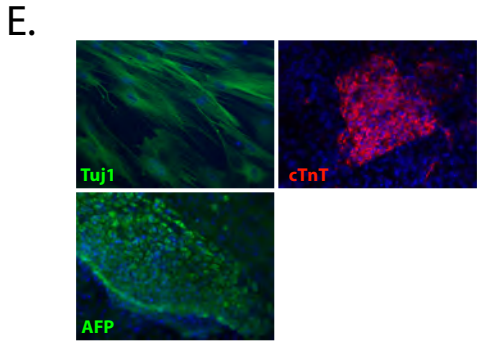
Figure 2.

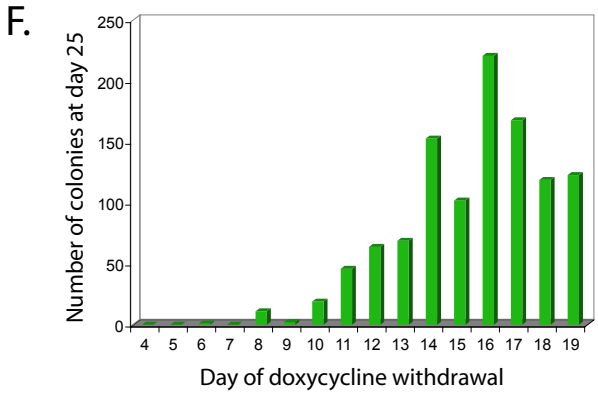

A.
In vitro
differentiation
Embryoid
bodies
Explant and
passage
Primary human cells
Infect LV
rtTA + factors
+dox
hiPS clones
+doxycycline
Fibroblast lines


B.
Doxycycline
cells seeded
-
10,000
+
1000
+
10,000

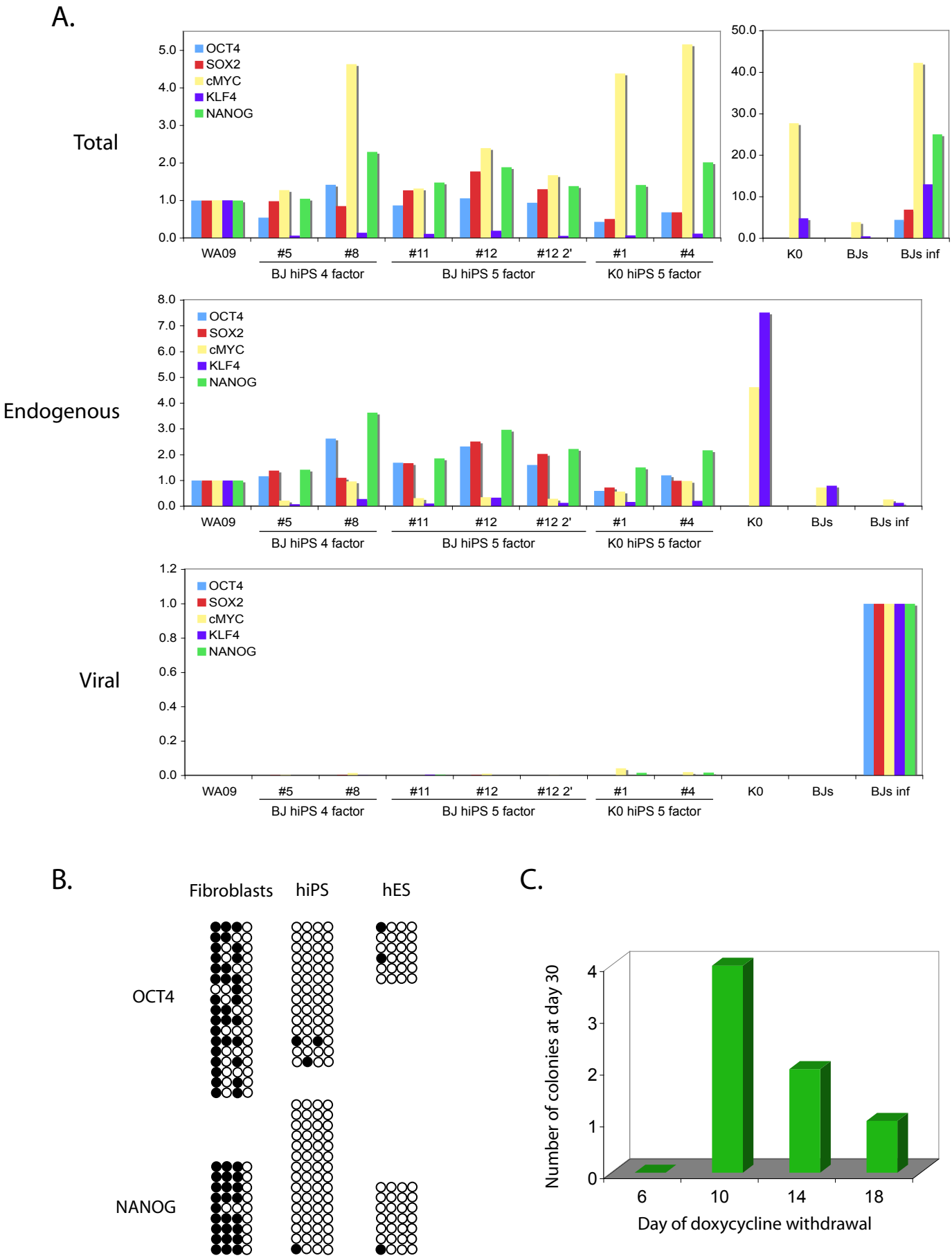

C.
OCT4
TRA-1-81


D.
BJ
HUES8
2' hiPS #12
1' hiPS #12


E.
Tuj1
cTnT
AFP

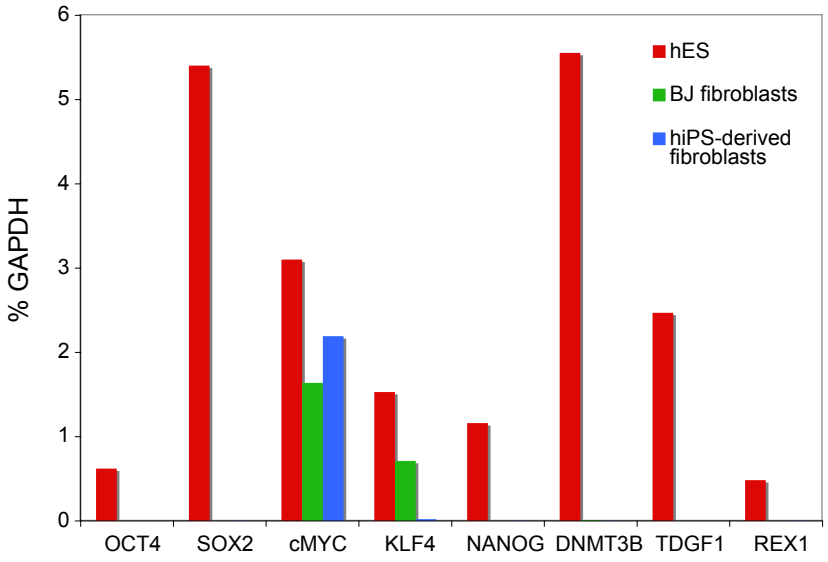

F.
Number of colonies at day 25
250
200
150
100
50
0
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Day of doxycycline withdrawal

Supplementary Figure 1.

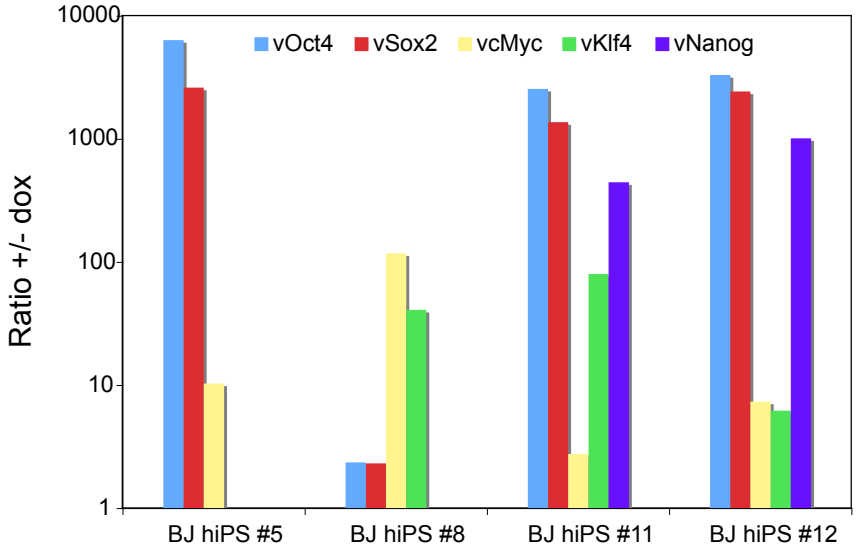


Supplementary Figure 2.

A.



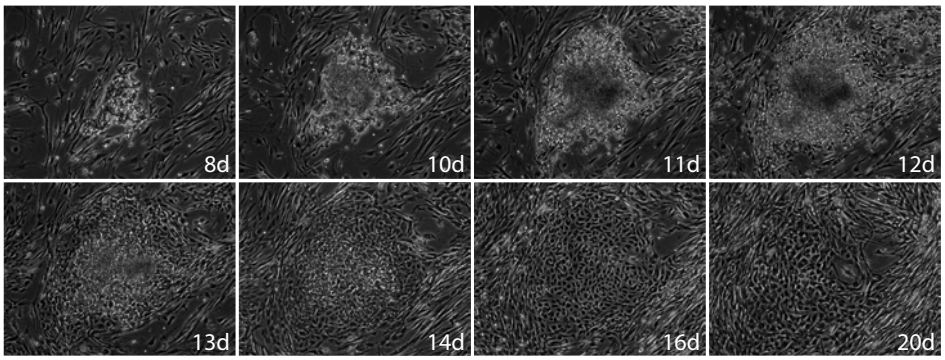
B.



Supplementary Figure 3.

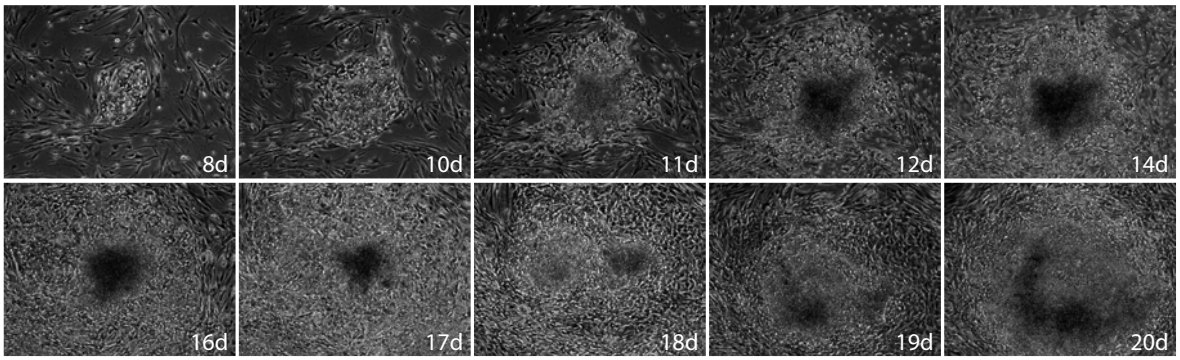
A.

Day 10 dox withdrawal



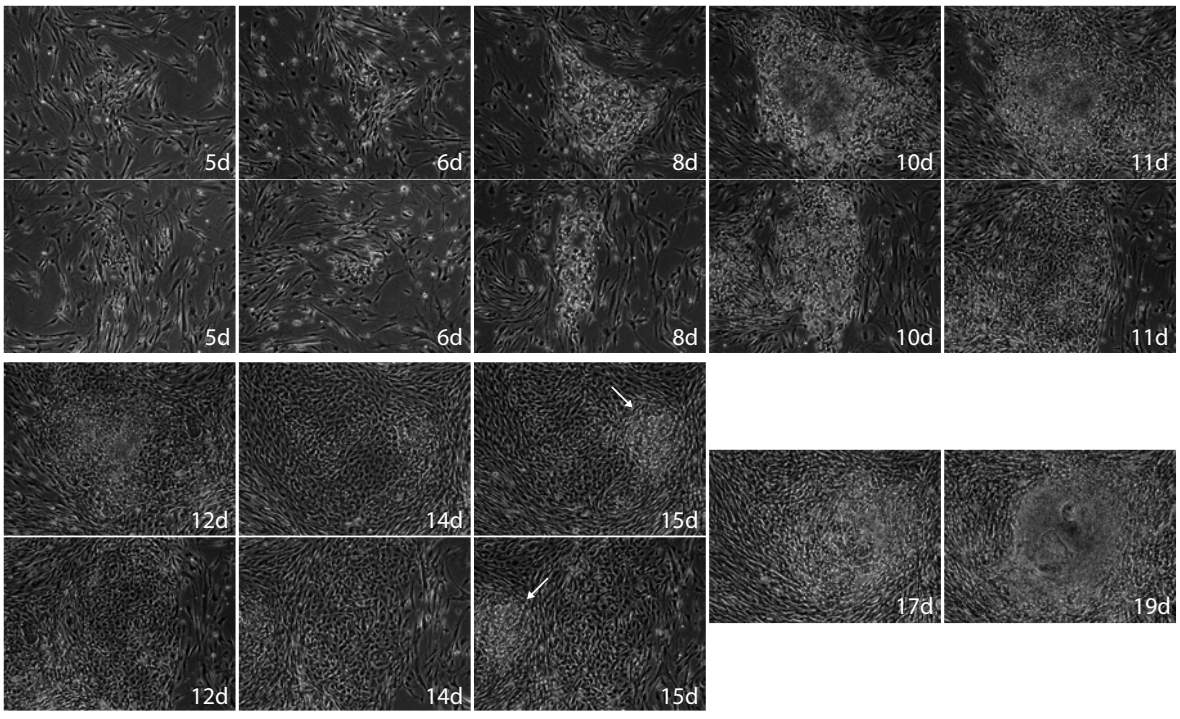
B.

Day 14 dox withdrawal



C.

Day 9 dox withdrawal



Supplementary Table 1. Summary of expanded hiPS cell lines.

Parental cell	Factors	Clones derived	Current passage (July 2008)	Karyotype
BJ fibroblasts (neonatal foreskin)	4 + GFP	#5	30	46, XY
		#6	20	47,XY,+mar [2] 48,XY,+5,+13 [1] 46,XY[17]
		#8	35	46, XY
	4 + NANOG	#11	28	46, XY
		#12	30	46, XY
BJ hiPS #12-derived fibroblast-like cells	(4 + NANOG)	#12 secondary	20	46, XY
Epidermal keratinocytes (neonatal foreskin)	4 + NANOG	#1	7	ND
		#2	5	ND
		#4	6	ND

4 factors = OCT4, SOX2, cMYC, KLF4
ND = not determined

Supplementary Table 2: Primers used in this study

Gene name	Forward primer sequence	Reverse primer sequence
Total OCT4	GAG GAG TCC CAG GAC ATC AA	TGG CTG AAT ACC TTC CCA AA
Total SOX2	AGC TAC AGC ATG ATG CAG GA	GGT CAT GGA GTT GTA CTG CA
Total cMYC	ACT CTG AGG AGG AAC AAG AA	TGG AGA CGT GGC ACC TCT T
Total KLF4	CCC AAT TAC CCA TCC TTC CT	ACG ATC GTC TTC CCC TCT TT
Total NANOG	TAC CTC AGC CTC CAG CAG AT	CCT TCT GCG TCA CAC CAT T
Lentiviral OCT4	CCC CTG TCT CTG TCA CCA CT	CCA CAT AGC GTA AAA GGA GCA
Lentiviral SOX2	ACA CTG CCC CTC TCA CAC AT	CAT AGC GTA AAA GGA GCA ACA
Lentiviral cMYC	AAG AGG ACT TGT TGC GGA AA	TTG TAA TCC AGA GGT TGA TTA TCG
Lentiviral KLF4	GAC CAC CTC GCC TTA CAC AT	CAT AGC GTA AAA GGA GCA ACA
Lentiviral NANOG	ACA TGC AAC CTG AAG ACG TG	CAC ATA GCG TAA AAG GAG CAA
Endogenous OCT4	TGT ACT CCT CGG TCC CTT TC	TCC AGG TTT TCT TTC CCT AGC
Endogenous SOX2	GCT AGT CTC CAA GCG ACG AA	GCA AGA AGC CTC TCC TTG AA
Endogenous cMYC	CGG AAC TCT TGT GCG TAA GG	CTC AGC CAA GGT TGT GAG GT
Endogenous KLF4	TAT GAC CCA CAC TGC CAG AA	TGG GAA CTT GAC CAT GAT TG
Endogenous NANOG	CAG TCT GGA CAC TGG CTG AA	CTC GCT GAT TAG GCT CCA AC
OCT4 promoter	AAG TTT TTG TGG GGG ATT TGT AT	CCA CCC ACT AAC CTT AAC CTC TA
NANOG promoter	TTA ATT TAT TGG GAT TAT AGG GGT G	AAA CCT AAA AAC AAA CCC AAC AAC
DNMT3B	CCA ATC CTG GAG GCT ATC CG	ACT GGG GTG TCA GAG CCA T
TDGF1 (CRIPTO)	AAG ATG GCC CGC TTC TCT TAC	AGA TGG ACG AGC AAA TTC CTG
ZFP42 (REX1)	AAC GGG CAA AGA CAA GAC AC	GCT GAC AGG TTC TAT TTC CGC
GAPDH	TGT TGC CAT CAA TGA CCC CTT	CTC CAC GAC GTA CTC AGC G