Supporting Information

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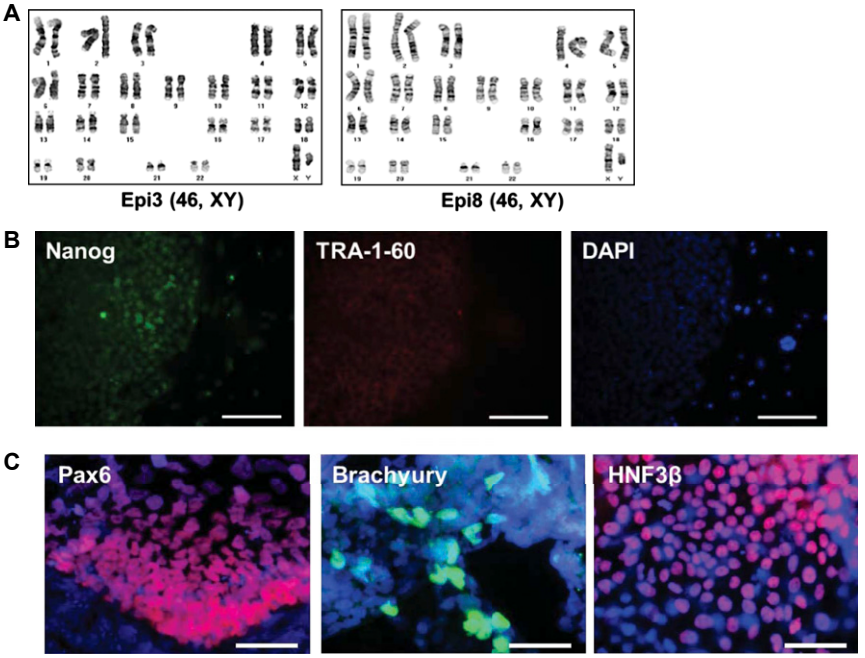

Fig. S1. Characterization of human induced pluripotent stem cells (iPSCs) generated by episomal reprogramming vectors. (*A*) Karyotype analyses were performed on chromosomes from WT-iPSC lines at passages 10 (Epi3) and 12 (Epi8). (*B*) Expression of Nanog and TRA-1-60, which are human embryonic stem cell (ESC)-specific surface markers, was detected by immunocytochemistry. DAPI signals indicate the total cell presence in the image. (Scale bars, 100 μm.) (*C*) The expression of marker proteins representing ectoderm (Pax6), mesoderm (Brachyury), and endoderm [hepatocyte nuclear factor 3-β (HNF3β)]. (Scale bars, 50 μm.)

B

	Amount of genomic DNA (Copy number per half genome)							Frequency (%)		
	10 ng (3000)	3 ng (1000)	1 ng (300)	300 pg (100)	100 pg (30)	33 pg (10)	10 pg (3)	Estimate	Upper and Lower limits	p value (Fit)
Z10	16/16	16/16	6/16	4/16	0/1	0/1	0/1	0.25	(0.2-0.4)	0.25
TALEN 01		16/16	16/16	11/16	8/16	7/16	0/16	1.9	(1.2-2.8)	0.11

Fig. S2. Frequencies of targeted inversions. (*A*) The frequency of targeted inversions was estimated by digital PCR. Genomic DNA samples isolated from cells transfected with transcription activator-like effector nuclease (TALEN)-encoding plasmids were serially diluted and subjected to digital PCR analysis. (*B*) Estimated frequencies of targeted chromosomal inversions created via zinc-finger nucleases (ZFNs) or TALENs. Z10 is a ZFN pair targeting the intron1 homolog of the *F8* gene. The frequency of 140-kbp inversion events was measured by digital PCR analysis. Upper and lower limits indicate 95% confidence intervals.

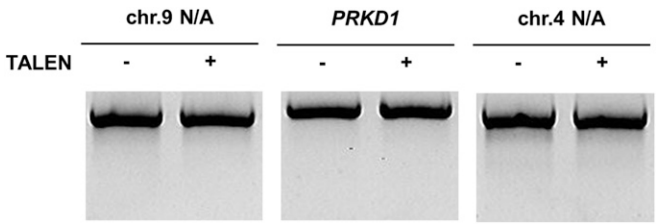

Fig. S3. Analysis of TALEN off-target effects. Potential off-target sites of TALENs designed for this study were searched in silico. The three potential off-target sites most similar to the TALEN target site were selected and subjected to T7E1 analysis to confirm the off-target cleavage activities at these sites.

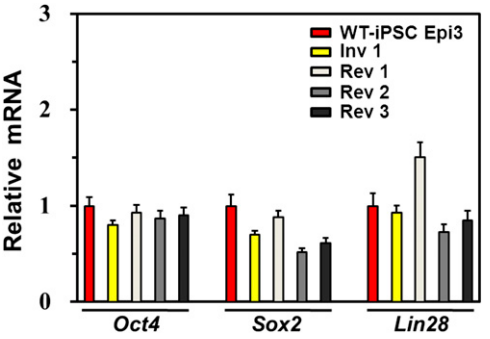

Fig. S4. Expression of human ES markers from inverted and reverted clones. *Oct4*, *Sox2*, and *Lin28* mRNA levels from wild-type iPSC line (WT-iPSCEpi3), inversion clone (Inv 1), and reverted clones (Rev 1, 2, and 3) were quantified by quantitative PCR (qPCR). *GAPDH* mRNA levels were used for normalization.

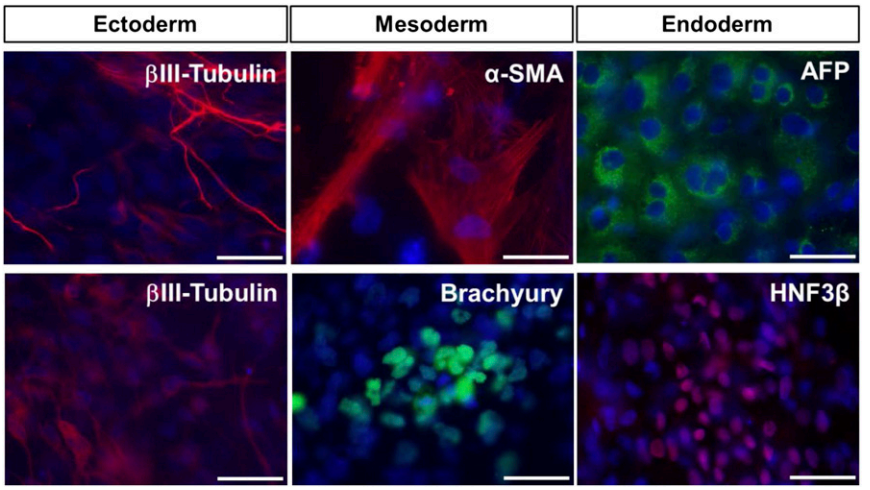

Fig. S5. In vitro differentiation of inverted and reverted clones. The expression of marker proteins representing ectoderm (βIII-Tubulin), mesoderm [α-smooth muscle actin (α-SMA) and Brachyury], and endoderm [α-fetoprotein (AFP) and HNF3β] in inversion clone 1 (*Upper*) and reverted clone 1 (*Lower*). (Scale bars, 50 μm.)

Table S1. Short tandem repeat (STR) analyses of iPS cell lines

Locus/lines	HDF		Epi3		Epi4		Epi8	
D8S1179	11	15	11	15	11	15	11	15
D21S11	29	30	29	30	29	30	29	30
D7S820	10	11	10	11	10	11	10	11
CSF1PO	11	13	11	13	11	13	11	13
D3S1358	16	18	16	18	16	18	16	18
TH01	8	9	8	9	8	9	8	9
D13S317	8	10	8	10	8	10	8	10
D16S539	9	13	9	13	9	13	9	13
D2S1338	20	23	20	23	20	23	20	23
D19S433	13	14	13	14	13	14	13	14
vWA	14	18	14	18	14	18	14	18
TPOX	8	11	8	11	8	11	8	11
D18S51	14	24	14	24	14	24	14	24
D5S818	12	12	12	12	12	12	12	12
FGA	23	26	23	26	23	26	23	26

Table S2. Potential off-target sites of TALEN 01

Chromosome no.	Gene name	Left-half site (5′ to 3′)	Spacer, bp	Right-half site (5′ to 3′)
9	N/A	TATAGATTtGCCAtTtTCTC	13	TAAAaTATAAaGAAAAgTtT
14	*PRKD1*	TgTAGATTGGtCAGTgTCTC	12	aAAAGcAaAcTcAAAACTGT
4	N/A	TtTtGATTGGCCAGcCTCTC	12	aAAAGaAaAcTGAAAACaGa

Bioinformatic analysis was performed to search for potential off-target sites that are most similar to the TALEN 01 target site. We defined potential off-target sites as any heterodimeric half-sites separated by 12- to 14-bp spacers. The three most likely potential off-target sites are listed. Mismatched bases are shown in lowercase letters.

Table S3. Primer pairs used in this study

Primer name	Sequence (5′ to 3′)	Used for the experiment of
Homolog 1-1F	AAATCACCCAAGGAAGCACA	Inversion and reversion
Homolog 1-1R	TGGCATTAACGTATTACTTGGAGA	Inversion and reversion
Homolog 2-2F	GGCAGGGATCTTGTTGGTAAA	Inversion and reversion
Homolog 2-2R	TGCTGAGCTAGCAGGTTTAATG	Inversion and reversion
GAPDH-F	CCCCTCAAGGGCATCCTGGGCTA	qPCR and RT-PCR
GAPDH-R	GAGGTCCACCACCCTGTTGCTGTA	qPCR and RT-PCR
Oct4-F	CCTCACTTCACTGCACTGTA	qPCR
Oct4-R	CAGGTTTTCTTTCCCTAGCT	qPCR
Sox2-F	CCCAGCAGACTTCACATGT	qPCR
Sox2-R	CCTCCCATTTCCCTCGTTTT	qPCR
Lin28-F	AGCCAAGCCACTACATTC	qPCR
Lin28-R	AGATACGTCATTCGCACA	qPCR
Nanog-F	TGAACCTCAGCTACAAACAG	qPCR
Nanog-R	TGGTGGTAGGAAGAGTAAAG	qPCR
F8-F	CTGCTTTAGTGCCACCAGAAGA	RT-PCR
F8-R	GACTGACAGGATGGGAAGCC	RT-PCR
FOXA2-F	CTACGCCAACATGAACTCCA	RT-PCR
FOXA2-R	AAGGGGAAGAGGTCCATGAT	RT-PCR
Sox17-F	AGCGCCCTTCACGTGTACTA	RT-PCR
Sox17-R	CTTGCACACGAAGTGCAGAT	RT-PCR
GAPDH-F	GAACATCATCCCTGCCTCTACTG	iPS generation (PCR)
GAPDH-R	CAGGAAATGAGCTTGACAAAGTGG	iPS generation (PCR)
EBNA-1-F	ATGGACGAGGACGGGGAAGA	iPS generation (PCR)
EBNA-1-R	GCCAATGCAACTTGGACGTT	iPS generation (PCR)
293-F	GAGCAGGGAGGCAAGAATTA	TALENs activity screening
293-R	TGAGGGAAAACGCATCTAGG	TALENs activity screening