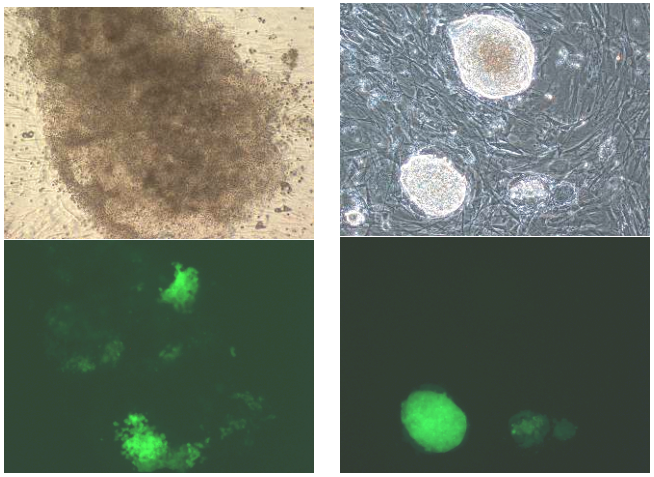
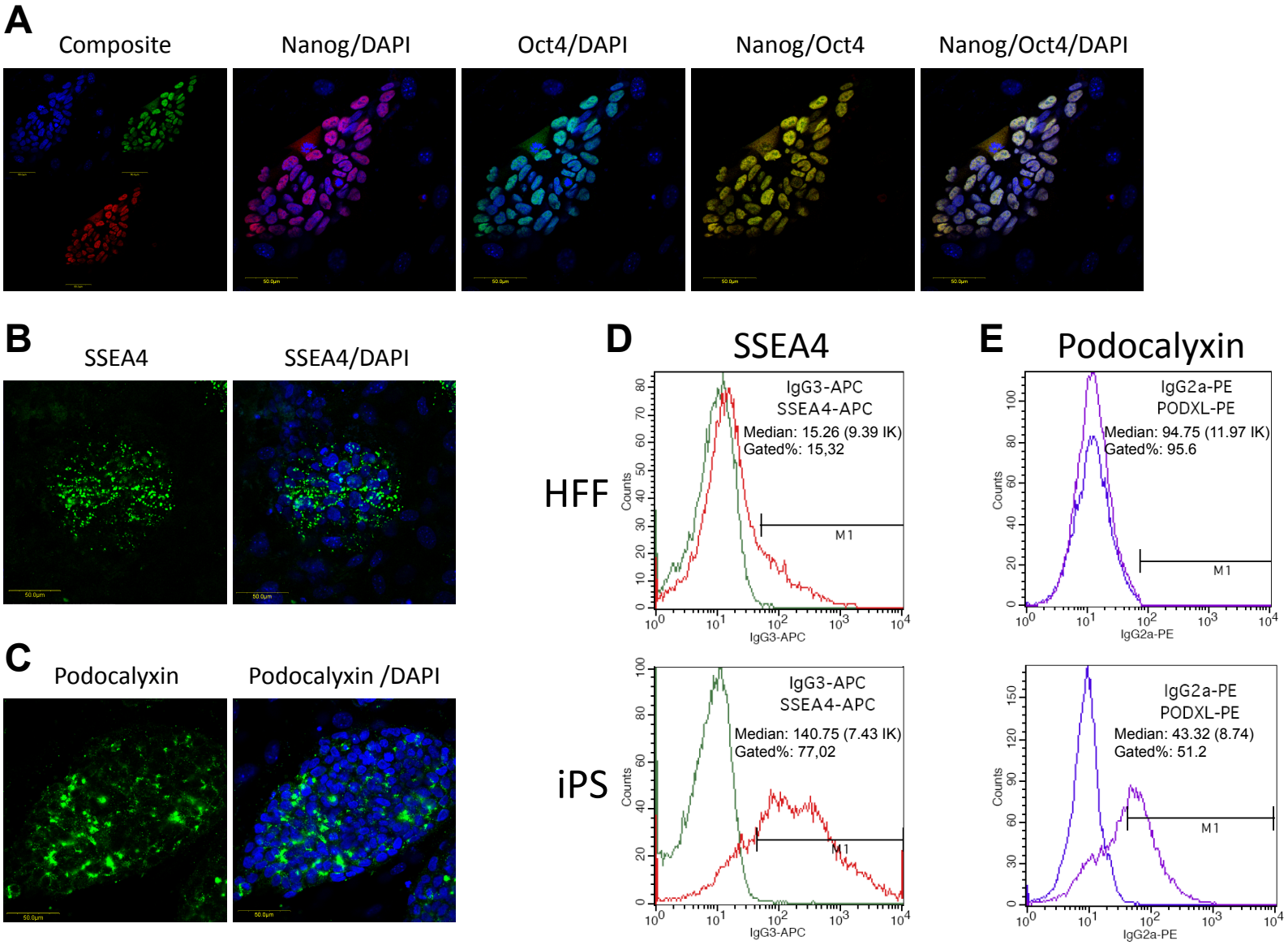
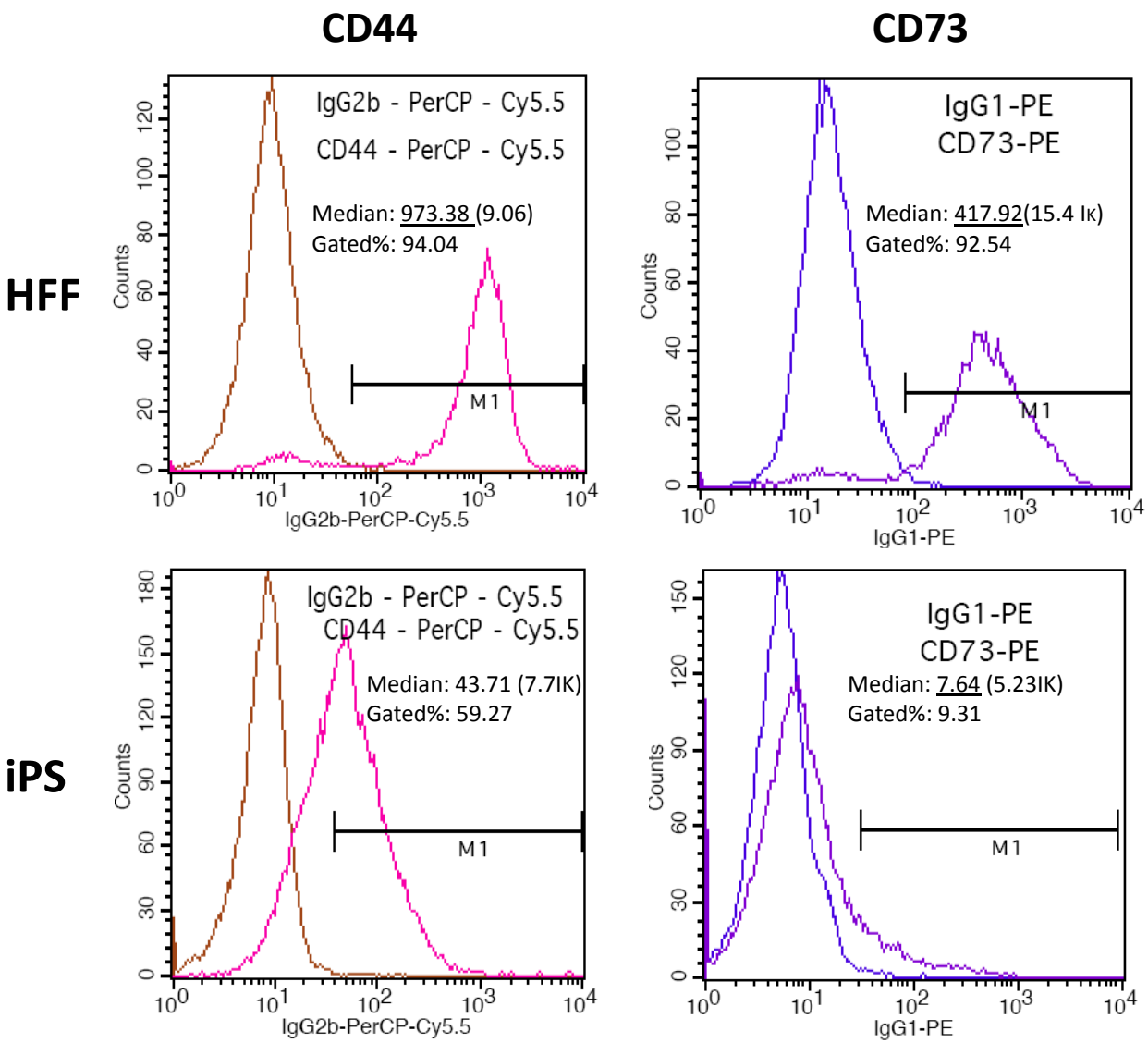


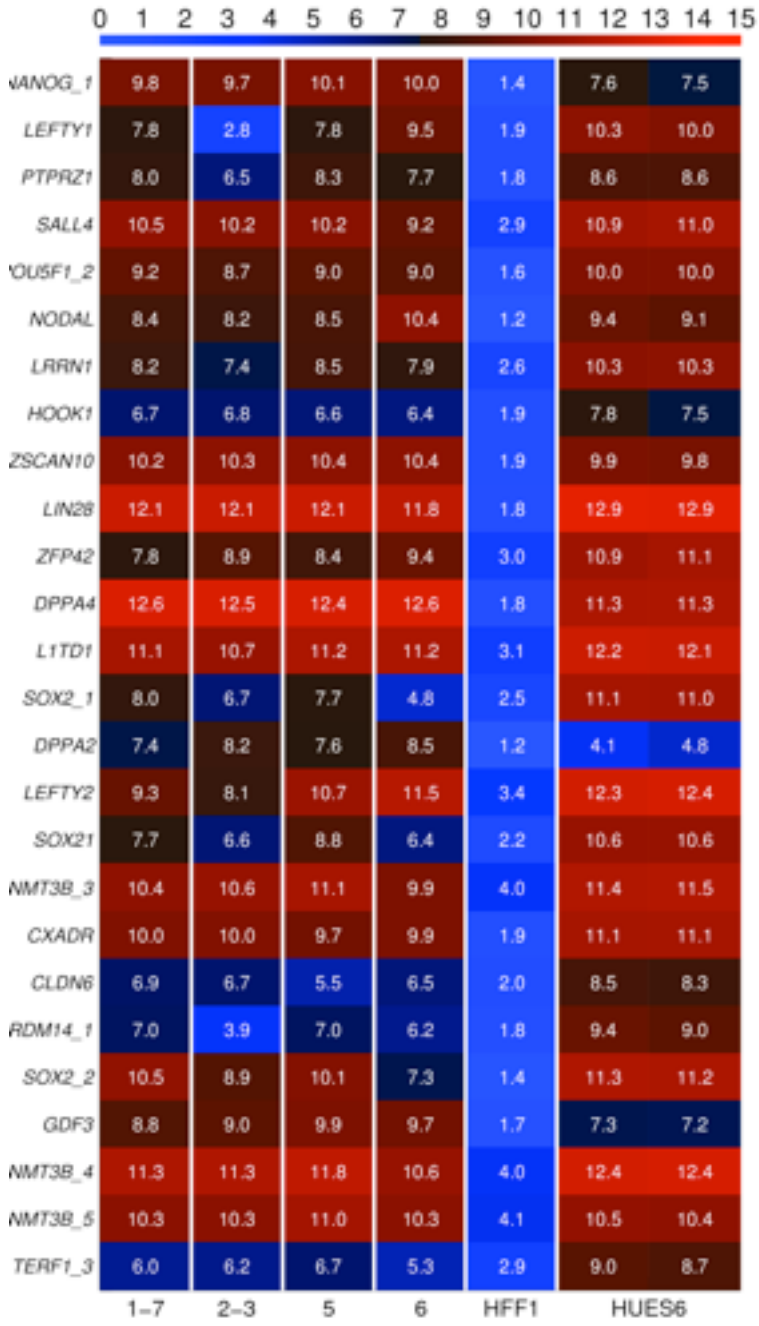
Supplementary Figure 1. Mouse iPS cell colonies generated in the absence of VPA before (left) and after (right) trypsinization and replating the cells. Top, brightfield; bottom, GFP fluorescence.



Supplementary Figure 2. Immunocytochemical characterization of human iPS cells generated by *Sleeping Beauty* transposon-mediated reprogramming. A typical iPS clump was examined by confocal microscopy after staining with antibodies against pluriopotency markers: Oct4 and Nanog (DAPI- blue, Oct4- green, Nanog- red) **(A)**, SSEA4 (**B**) and podocalyxin (**C**). FACS analyses showed that iPS cells are positive for SSEA4 (**D**) and podocalyxin (**E**). For FACS images, median values and gated percentages are presented. IK in parantheses stands for the median isotype control.

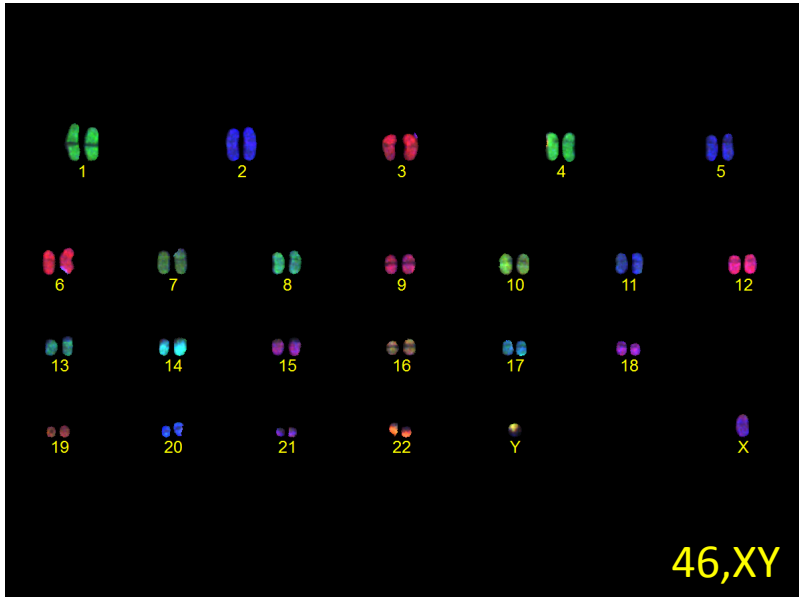


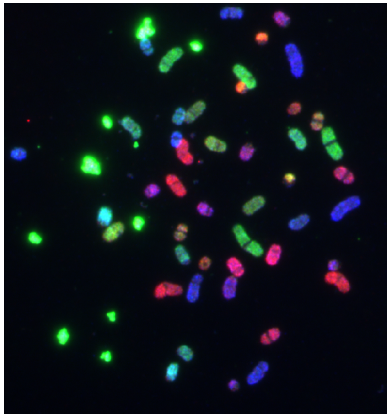
Supplementary Figure 3. Immunocytochemical characterization of human iPS cells generated by *Sleeping Beauty* transposon-mediated reprogramming. In comparison with HFF cells, human iPS cells showed decreased expression of CD44 and CD73 markers that are characteristic to more differentiated cellular phenotypes. Median values and gated percentages are presented. IK in parantheses stands for the median isotype control.



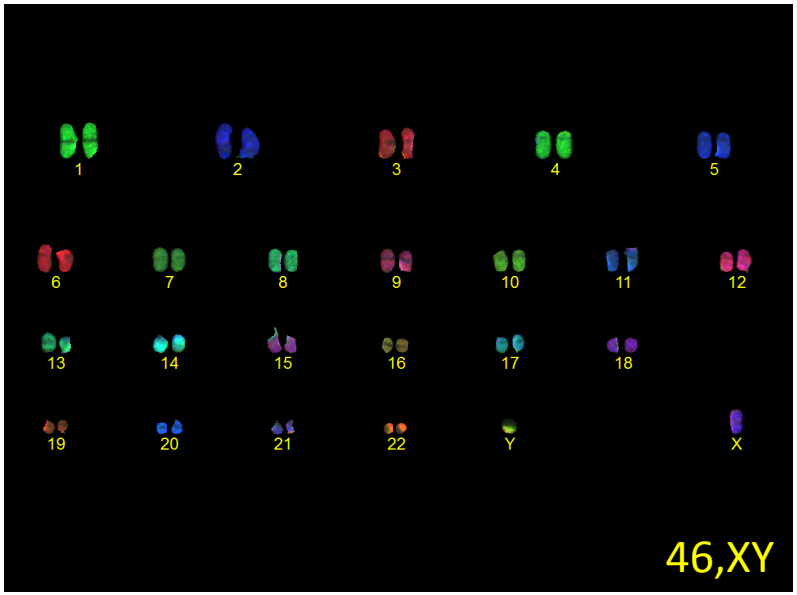
Supplementary Figure 4. Heatmap of expression profiles of pluripotency-associated genes in four different human iPS cell clones, in the human ES cell line HUES6 and in HFFs.

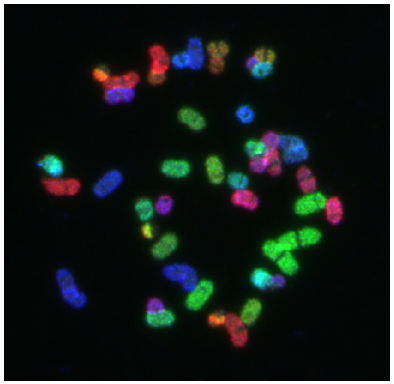
HFF



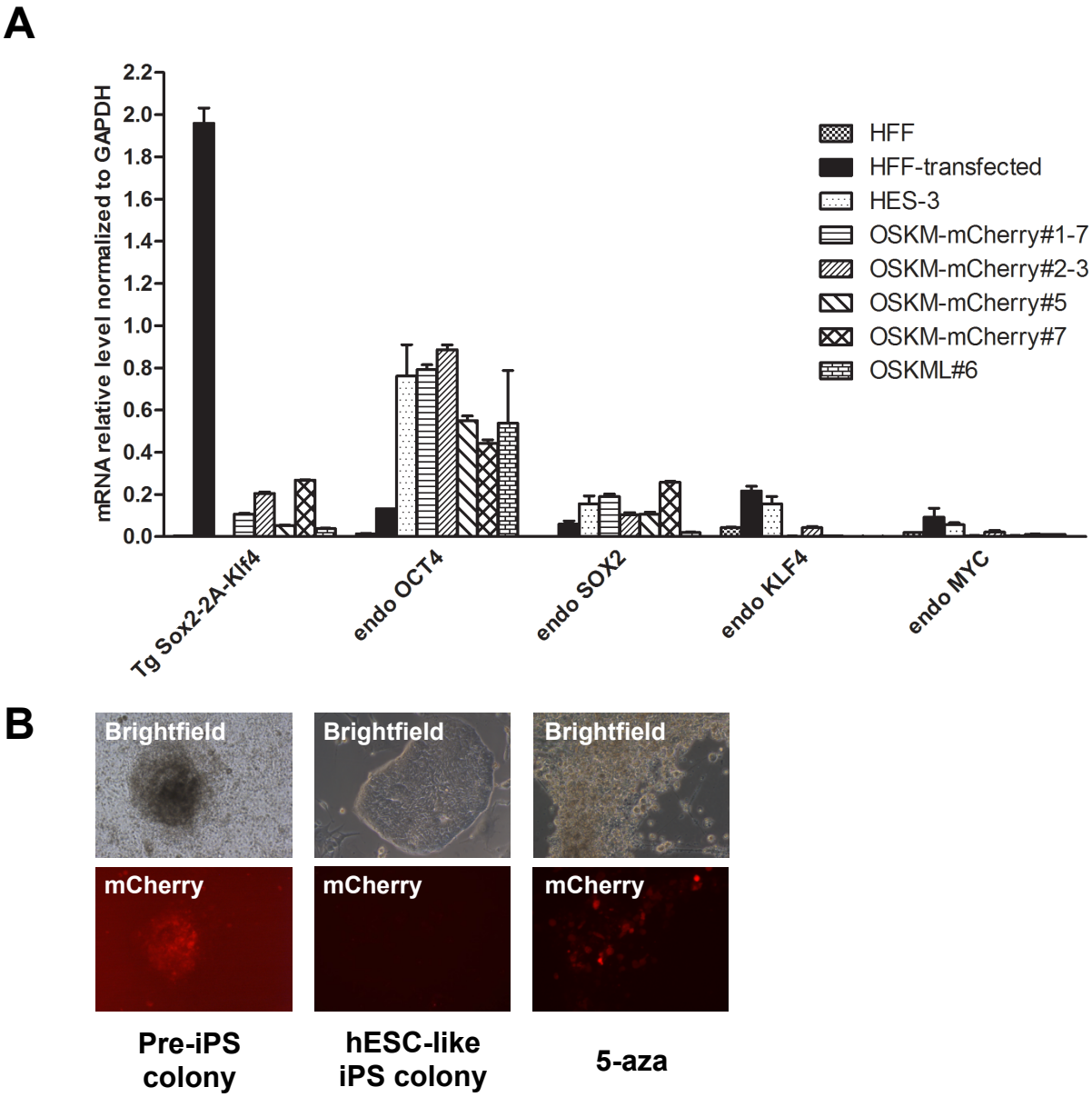


hiPSC

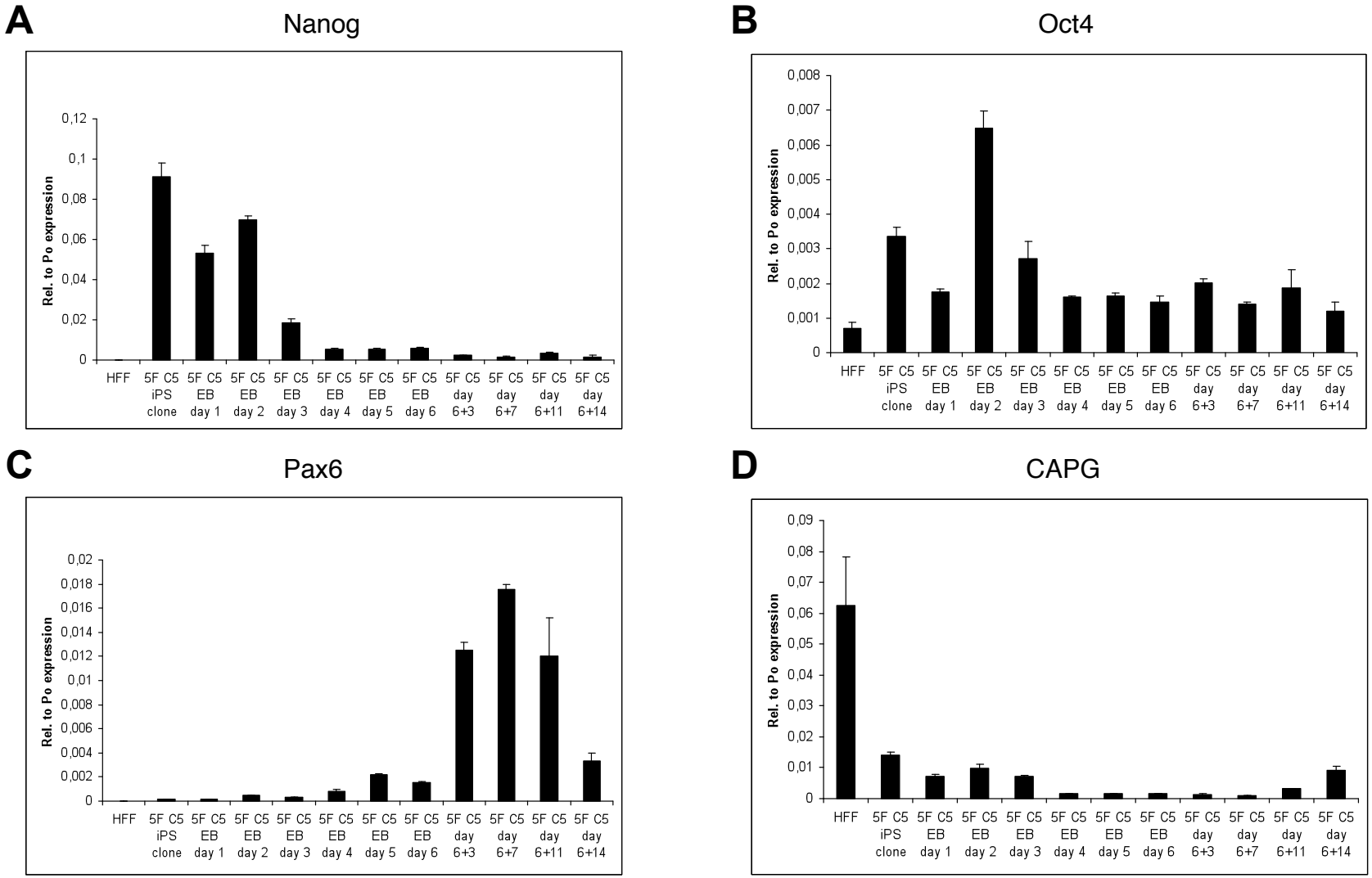




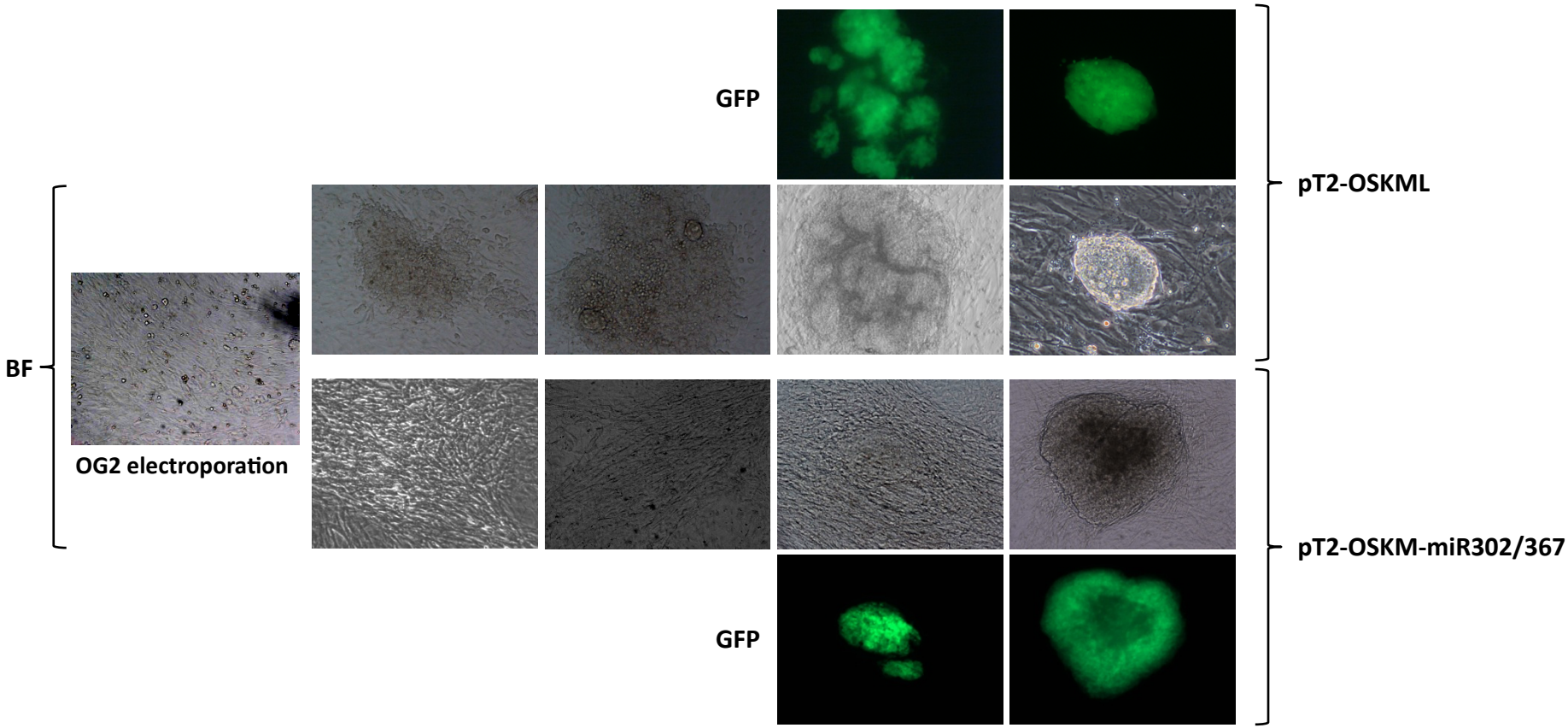
Supplementary Figure 5. COBRA-FISH analysis of human iPS and parental HFF cells. Slides with metaphase chromosomes were hybridized using multicolor COBRA-FISH. 48-color FISH staining every chromosome arm in a different color combination, digital imaging and analysis was performed as previously described (Szuhai & Tanke, Nature Protocols, 2006).



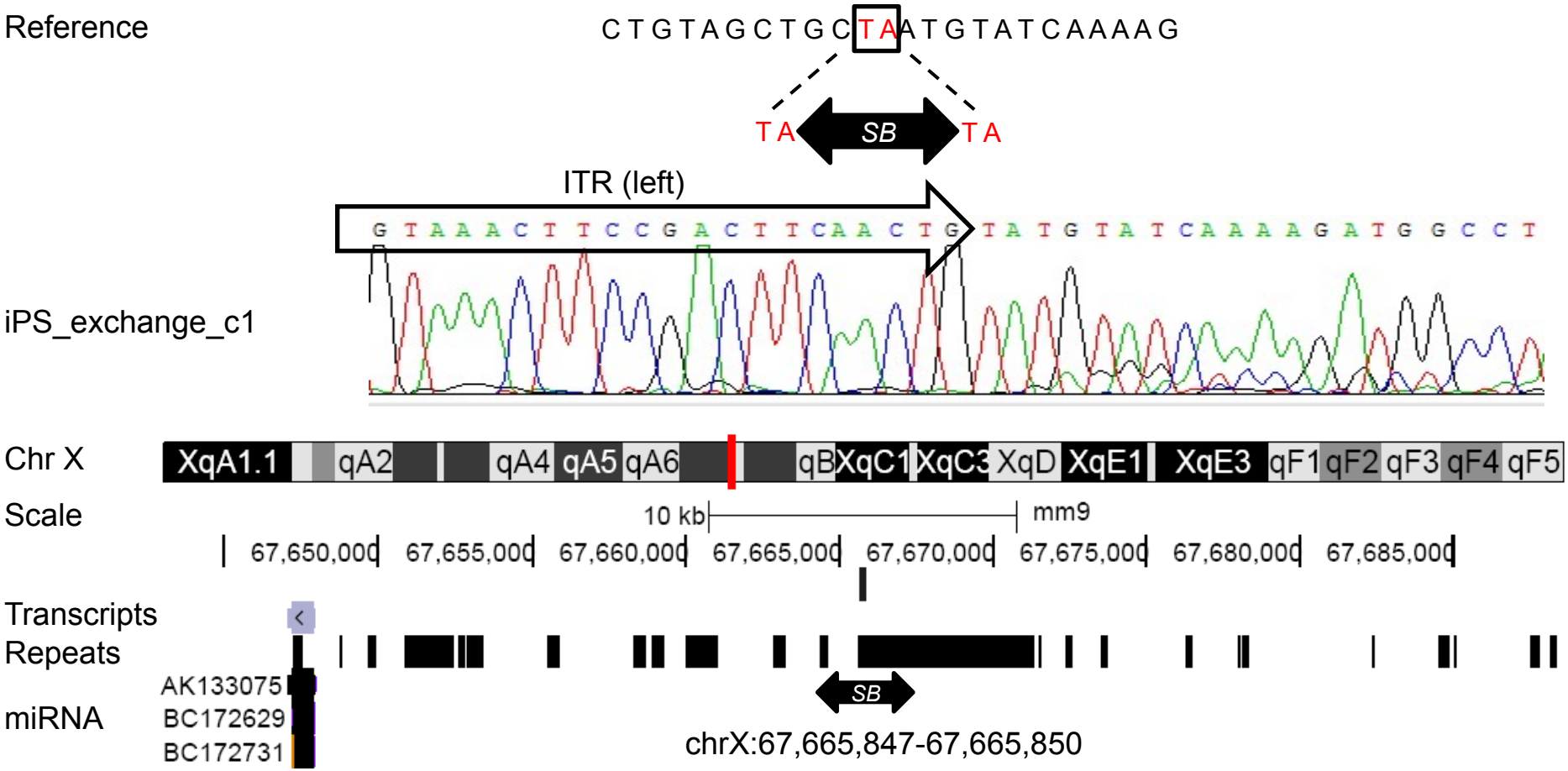
Supplementary Figure 6. Silencing of transgenes in stable human iPS cells. (A) Transgene-specific PCR primers permit determination of relative expression levels between endogenous (endo) OCT4, SOX2, MYC and KLF4 genes and *SB*-expressed (Tg Sox2-2A-Klf4) transgenes via qRT-PCR. HFF cells transfected with the *SB*-based reprogramming vector (HFF-transfected) served as an early time-point sample. **(B)** The SB integration locus is transcriptionally active in pre-iPS colonies, and express *mCherry*. In stable human iPS cell lines, *mCherry* is silenced, but can be reactivated by 5-Aza-2'-Deoxycytidine (5-Aza).



Supplementary Figure 7. mRNA expression analysis of specific differentiation markers. Differentiation assays were carried out by generating EBs for 6 days (EB day 1-6). Six days later EBs were placed onto gelatin-coated plates. The attached cells differentiated spontaneously, the differentiation process being followed for up to 14 additional days, with mRNA harvests at days 3, 7, 11, 14 (day 6+3, day 6+7, day 6+11, day 6+14). For gene expression analysis quantitative real-time PCR was carried out on cDNAs obtained after total RNA extraction and reverse transcription. HFFs were negative for Nanog (**A**), Oct4 (**B**) and Pax6 (**C**) and positive for CAPG (**D**). After reprogramming, iPS cells (clone 5, obtained with 5 factors, 5F C5) expressed Oct4 and Nanog, whose expression declined during differentiation. Differentiating cells acquired Pax6 expression during the late stages of differentiation, probably in parallel with the appearance of neuron-like cells. CAPG expression characteristic to skin cells showed a high expression level in the initial HFFs, and its expression declined in iPS or differentiated cells.



Supplementary Figure 8. Generation of mouse iPS cells with pT2-OSKML and pT2-OSKM-mir302/367 vectors. 5X10^5 mouse OG2 cells were electroporated with 500ng of either pT2-OSKML or pT2-OSKM-miR302/367 transposon- and 50ng of SB100X transposase vectors. After electroporation, cells were seeded to one geltrex-coated well of a 6-well plate and grown in mouse iPS medium in the absence of valproic acid. OG2 cells electroporated with the pT2-OSKML vector formed typical pre-iPS colonies described before and, at a later stage, typical mouse ES-like iPS colonies, which started to express GFP fluorescence together with the appearence of clump-like patches in the pre-iPS colonies. In contrast, OG2 cells electroporated with pT2-OSKM-miR302/367 retained a “flat” morphology all the way through the reprogramming process, generating distinctive iPS “nests” and pre-iPS colonies, which at a later stage formed GFP positive clones with a “flat” morphology reminescent of epiblast-derived stem cells (EpiSCs).



Supplementary Figure 9. Chromosomal mapping of *Sleeping Beauty* transposon insertion in the mouse genome. The *SB* transposon was integrated into a TA site on chromosome X, and there are no known protein coding and miRNA genes in a window of ±20 kb around the transposon insertion site.

Supplementary Methods

Primers used in this study

	Gene	Primer	Sequence	Amplicon length
Mouse iPS cells	Splinkerette linker	BfaI linker (+)	GTAATACGACTCACTATAGGGCTCCGCTTAAGGGAC	
		MboI linker (-)	p-GATCGTCCCTTAAGCGGAG-amino	
	Nested PCR	linker	GTAATACGACTCACTATAGGGC	
		Tbal rev3	CATGACATCATTTTCTGGA ATT	
		Tbal	CTTGTGTCATGCACAAAGTAGATGTCC	
		nested	AGGGCTCCGCTTAAGGGAC	
	Sox2-Klf4 junction	forward	GCA ACGGCAGCTACAGCATGATGCAG	800 bp
		reverse	CAGGAGGTCGTTGAACTCCTCGGTCTC	
	SB-mCherry junction	forward	TGACTGTGCCTTTAAACAGCTTGGA	980 bp
		reverse	GCGTGGTGACCGTGACCCAG	
	Sox2-2A-Klf4	forward	CCCGCTGCGCCCAGTAGAC	202 bp
	Sox2-2A-Klf4	reverse	AGAAGGACGGGAGCAGAGCGT	
	Oct4	forward	GGATGCTGTGAGCCAAGG	175 bp
		reverse	GAACAAAATGATGAGTGACAGACAG	
	Nanog	forward	TTCTTGCTTACAAGGGTCTGC	95 bp
		reverse	CAGGGCTGCCTTGAAGAG	
	Sox2	forward	GGCAGAGAAGAGAGTGTTTGC	76 bp
		reverse	TCTTCTTTCTCCCAGCCCTA	
	Rex1	forward	TCTTCTCTCAATAGAGTGAGTGTGC	60 bp
		reverse	GCTTTCTTCTGTGTGCAGGA	
	Dnmt3b	forward	TCGACTTGGTGATTGGTGGAA	62 bp
		reverse	TTTGCGGGCAGGATTGAC	
	Gdf3	forward	GGGTGTTCGTGGGAACCT	78 bp
		reverse	CCATCTTGGAAAGGTTTCTGTG	
	Fgf4	forward	CCAACAACTACAACGCCTACGA	64 bp
		reverse	TTCTTACTGAGGGCCATGAACA	
	Ncam1	forward	CACTTTGTGTTCAGGACCTCAG	92 bp
		reverse	AAAAGCAATGAGACCAAGGTG	
	Sox1	forward	TTTCTTTCCTGTGGTTCTGC	227 bp
		reverse	GACTCTGTGGTGGTGAGGTC	
	Pax6	forward	ACAGAGTTCTTCGCAACCTG	236 bp
		reverse	CATCTGAGCTTCATCCGAGT	
	Zic1	forward	AGCACAGTCTCTTCGCTGCT	96 bp
		reverse	GCTCGTGAAGCCCAGAAA	

	Afp	forward	TGCTGCAAATTACCCATGAT	192 bp
		reverse	AAGGTTGGGGTGAGTTCTTG	
	Gata6	forward	GGTCTCTACAGCAAGATGAATGG	94 bp
		reverse	TGGCACAGGACAGTCCAAG	
	Sox17	forward	CACAACGCAGAGCTAAGCAA	65 bp
		reverse	CGCTTCTCTGCCAAGGTC	
	Foxa2	forward	AAATAATGTAAGAGTCTGGTGTACCG	68 bp
		reverse	CCATGTCCAGAATGGGATGT	
	Eomes	forward	ACCGGCACCAAACTGAGAT	64 bp
		reverse	AAGCTCAAGAAAGGAAACATGC	
	Lmo2	forward	CGAAAGGAAGAGCCTGGAC	107 bp
		reverse	AGCGGTCCCCTATGTTCTG	
	Cd34	forward	AAGGCTGGGTGAAGACCCTTA	157 bp
		reverse	TGAATGGCCGTTTCTGGAAGT	
	T	forward	CAGCCCACCTACTGGCTCTA	72 bp
		reverse	GAGCCTGGGGTGATGGTA	
	Gapdh	forward	GTGTTCCTACCCCCAATGTGT	248 bp
		reverse	ATTGTCATACCAGGAAATGAGCTT	
Human iPS cells	OCT4	forward	CGACCATCTGCCGCTTTG	60 bp
		reverse	GCCGCAGCTTACACATGTTCT	
	SOX2	forward	ACAGCA AATGACAGCTGCAAA	68 bp
		reverse	TCGGCATCGCGGTTTTT	
	NANOG	forward	CCA AAGGCA AACAACCCACTT	62 bp
		reverse	CGGGACCTTGTCTTCCTTTTT	
	FGF4	forward	AGTACCCCGGCATGTTCATC	58 bp
		reverse	CGGTTCCCCTTCTTGGTCTT	
	KLF4	forward	GGGAGAAGACACTGCGTCA	88 bp
		reverse	GGAAGCACTGGGGGAAGT	
	MYC	forward	CACCAGCAGCGACTCTGA	102 bp
		reverse	GATCCAGACTCTGACCTTTTGC	
	TERT	forward	CGGAGACCACGTTTCAAA AGA	66 bp
		reverse	TTTGCAACTTGCTCCAGACAC T	
	REX1	forward	TCGCTGAGCTGAAACAAATG	170 bp
		reverse	CCCTTCTTGAAGGTTTACAC	
	DNMT3B	forward	CGGTGTTTCTGTGTGGAGTG	146 bp
		reverse	CGCACGTTCCAGTCCTTC	
	DPPA2	forward	GGTGCCAGTTAAAGATGACGC	188 bp
		reverse	GAGGCAAAATGGTCGGCAAG	
	DPPA4	forward	GACCTCCACAGAGAAGTCGAG	145 bp
		reverse	TGCCTTTTTCTTAGGGCAGAG	

	SALL4	forward	AGCACATCAACTCGGAGGAG	129 bp
		reverse	CATTCCCTGGGTGGTTCACTG	
	NCAM	forward	ATGGAAACTCTATTAAAGTGAACCTG	178 bp
		reverse	TAGACCTCATACTCAGCATTCCAGT	
	PAX6	forward	GTCCATCTTTGCTTGGGAAA	110 bp
		reverse	TAGCCAGGTTGCGAAGAACT	
	SOX1	forward	CTGGCTGTGGCAAGGTCTTC	97 bp
		reverse	CAGCCCTCAAACTCGCACTT	
	TUBB3	forward	GGCCAAGTTCTGGGAAGTCA	70 bp
		reverse	CGAGTCGCCCACGTAGTTG	
	ZIC1	forward	CTGGCTGTGGCAAGGTCTTC	97 bp
		reverse	CAGCCCTCAAACTCGCACTT	
	AFP	forward	AGCTTGGTGGTGGATGAAAC	182 bp
		reverse	TCTGCAATGACAGCCTCAAG	
	GATA4	forward	CGAGGAGATGCGTCCCATCAAGAC	203 bp
		reverse	AGTCCTGCTTGGAGCTGGTCTGTG	
	GATA6	forward	GAGGGTGAACCCGTGTGCAATG	178 bp
		reverse	TGGAAGTTGGAGTCATGGGAATGG	
	FOXA2	forward	TGGGAGCGGTGAAGATGGAAGG	201 bp
		reverse	CGTACGACGACATGTTCATGGAGC	
	CD34	forward	GCGCTTTGCTTGCTGAGT	67 bp
		reverse	GGGTAGCAGTACCGTTGTTGT	
	EOMES	forward	AGAGGGCTGTGCCTTCCGTTTC	213 bp
		reverse	AGCACACAGCAGAGGCCTAGCAAG	
	LMO2	forward	ACTTCCTGAAGGCCATCGACCAG	209 bp
		reverse	CACCCGCATTGTCATCTCATAGGC	
	T	forward	ACCCAGTTCATAGCGGTGAC	165 bp
		reverse	CCATTGGGAGTACCCAGGTT	
	HAND1	forward	ATGGACGTGCTGGCCAAGGATG	202 bp
		reverse	TTAACTCCAGCGCCCAGACTTGC	
	GAPDH	forward	ATGGAA ATCCCATCACCATCTT	60 bp
		reverse	CGCCCCACTTGATTTTGG	
bisulfite sequencing	Nanog_meth	forward	GATTTTGTAGGTGGGATTAATTGTGAATTT	367 bp
		reverse	ACCAAAAAAACCCACACTCATATCAATATA	
	OCT4_meth	forward	GGATGTTATTAAGATGAAGATAGTTGG	406 bp
		reverse	CCTAAACTCCCCTTCAAAATCTATT	
	NANOG_meth	forward	TTATATTTTTGATTTAAAAGTTGGAAA	306 bp
		reverse	TAACATAAAACAACCAACTCAATCC	

Determination of transgene silencing in stable human iPS cells. First, transgene-specific primers against mouse Sox2 and Klf4 were designed to determine the expression of reprogramming transgenes in human stable iPS cells by qRT-PCR as well as HFFs transfected with SB-based reprogramming vectors. Data was normalized to GAPDH expression. Secondly, stable human iPS cells was treated with 5-Aza-2'-Deoxycytidine (5-Aza) for two days followed by checking mCherry expression under fluorescence microscope.