Supplementary Information includes:

Supplementary Figure 1

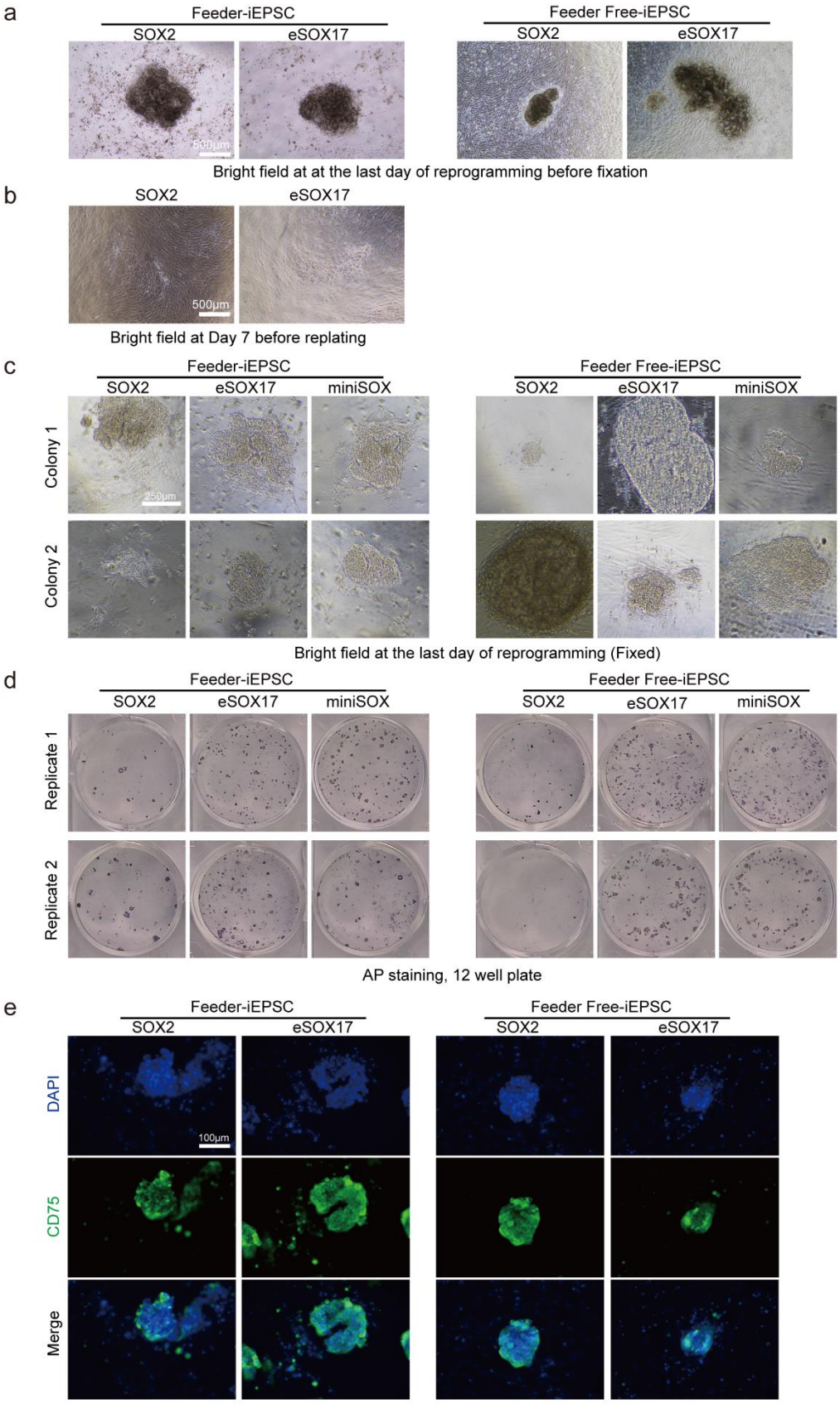
Supplementary Figure 2

Supplementary Figure 3

Supplementary Table 1

Supplementary Table 2

Supplementary Table 3


a
Feeder-iEPSC
SOX2
eSOX17
Feeder Free-iEPSC
SOX2
eSOX17
500μm
Bright field at at the last day of reprogramming before fixation
b
SOX2
eSOX17
500μm
Bright field at Day 7 before replating
c
Feeder-iEPSC
SOX2
eSOX17
miniSOX
Feeder Free-iEPSC
SOX2
eSOX17
miniSOX
Colony 1
Colony 2
250μm
Bright field at the last day of reprogramming (Fixed)
d
Feeder-iEPSC
SOX2
eSOX17
miniSOX
Feeder Free-iEPSC
SOX2
eSOX17
miniSOX
Replicate 1
Replicate 2
AP staining, 12 well plate
e
Feeder-iEPSC
SOX2
eSOX17
Feeder Free-iEPSC
SOX2
eSOX17
DAPI
CD75
Merge
100μm

Supplementary Figure 1. Engineered SOX17 variants outperforms SOX2 in iEPSC reprogramming (related to Figure 1).

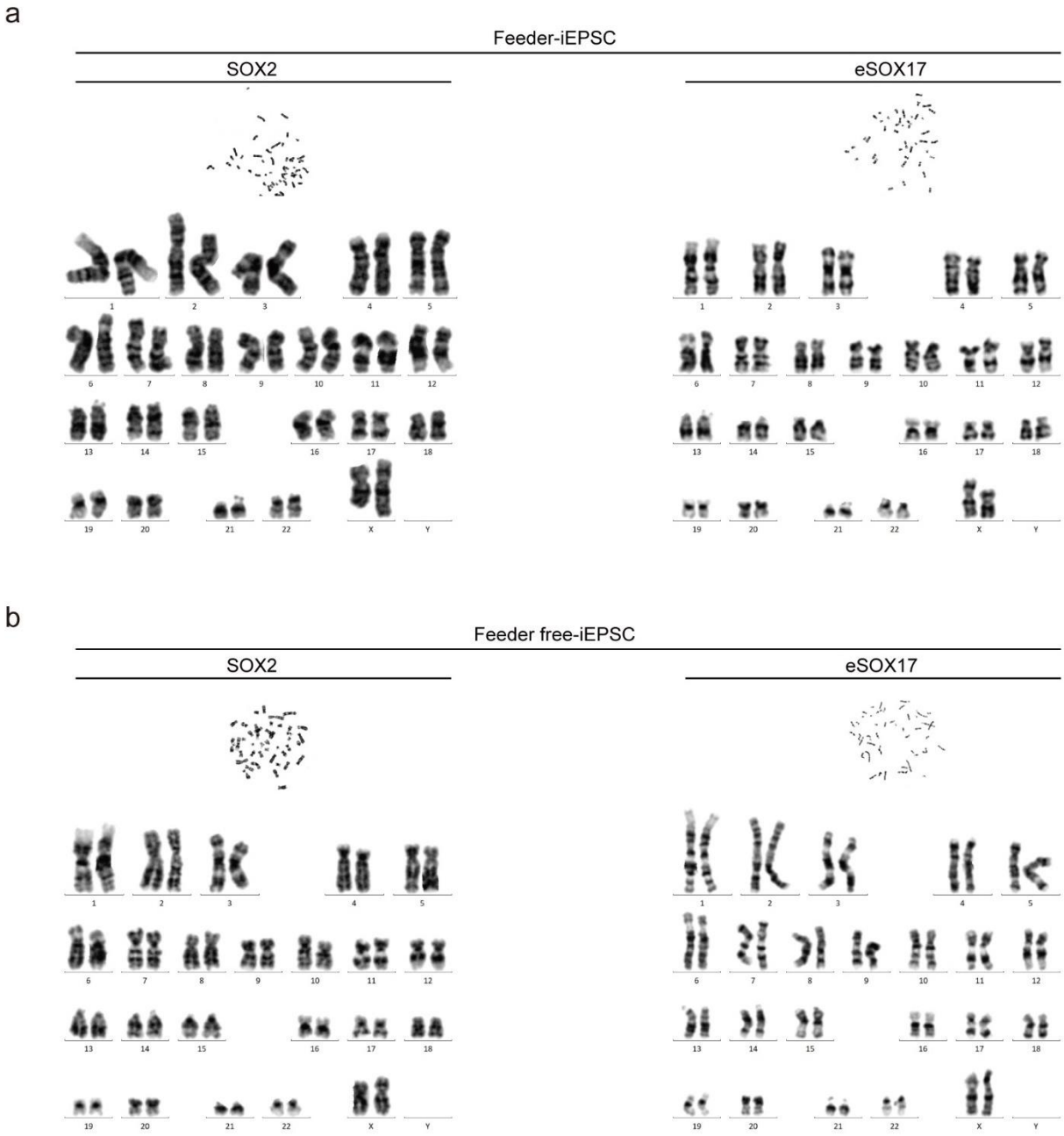
(a) Bright field images of the morphology of iEPSC colonies during reprogramming. Scale bar, 500 μm.

(b) Bright field images of the HDFa during reprogramming showing morphology change. Scale bar, 500 μm.

(c) Bright field images of the morphology of iEPSC colonies after fixation and ICC staining. Scale bar, 250 μm.

(d) Alkaline phosphatase staining of iEPSC reprogramming cultures in 12-well plates. Cells were fixed at the end day of reprogramming using 4% PFA.

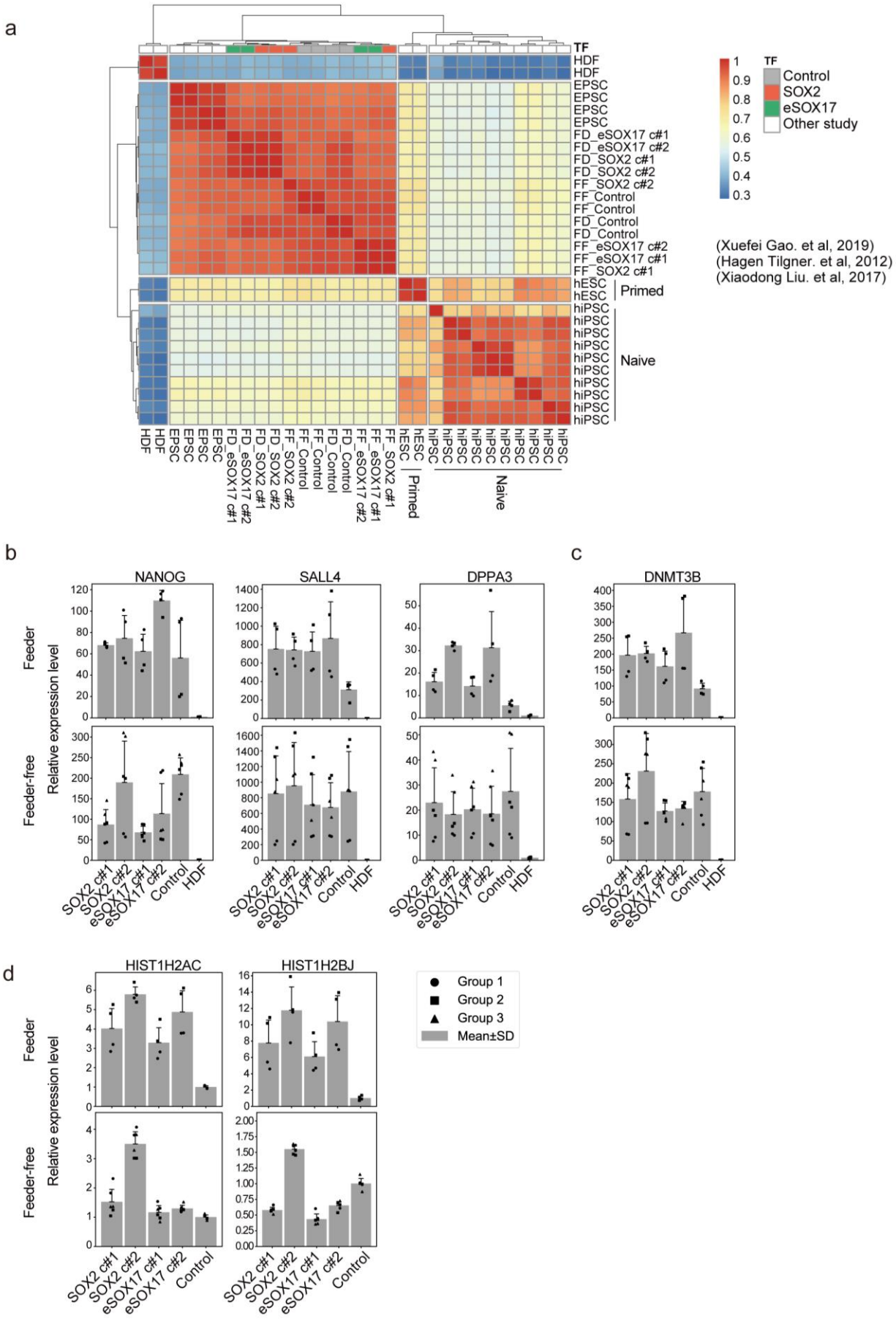
(e) Immunostaining of representative human iEPSC colonies with the naïve pluripotency marker CD75 at a higher magnification, demonstrating its cell surface localization. Scale bar, 100 μm.



Supplementary Figure 2. iEPSCs all showed correct karyotype (related to Figure 2).

(a) Karyotping results of feeder-based human iEPSC clonal cell lines.

(b) Karyotping results of feeder-free human iEPSC clonal cell lines.


a
TF
HDF
HDF
EPSC
EPSC
EPSC
EPSC
FD_eSOX17 c#1
FD_eSOX17 c#2
FD_SOX2 c#1
FD_SOX2 c#2
FF_SOX2 c#2
FF_Control
FF_Control
FD_Control
FD_Control
FF_eSOX17 c#2
FF_eSOX17 c#1
FF_SOX2 c#1
hESC
hESC
Primed
hiPSC
hiPSC
hiPSC
hiPSC
hiPSC
hiPSC
hiPSC
hiPSC
hiPSC
hiPSC
Naive
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
TF
Control
SOX2
eSOX17
Other study
(Xuefei Gao. et al, 2019)
(Hagen Tilgner. et al, 2012)
(Xiaodong Liu. et al, 2017)
b
NANOG
SALL4
DPPA3
c
DNMT3B
Feeder
Feeder-free
Relative expression level
SOX2 c#1
SOX2 c#2
eSOX17 c#1
eSOX17 c#2
Control
HDF
d
HIST1H2AC
HIST1H2BJ
Group 1
Group 2
Group 3
Mean±SD

Supplementary Figure 3. iEPSCs were transcriptionally similar to EPSCs (related to Figure 3).

(a) Correlation heatmaps of global gene expression profiles determined by bulk RNA-seq. FD: Feeder-based; FF: Feeder-free; c means colony serial number for biological replicates.

(b-d) RT-qPCR results of individual gene expression profiles. Data were shown from two (for feeder-based) or three (for feeder-free) independent replicates, each done in two technical replicates, mean ± SD. (Control: embryonic-EPSCs).

Supplementary Table 1. Lists of antibodies used in this study

This table details the names, sources, identifiers, and working concentrations of the antibodies used in this study.

Antibodies used in ICC			
Antibodies	Source	Identifier	Working concentration
Mouse anti-CD75	Biolegend	326901	1:100
Goat anti-OCT4	Santa Cruz	Sc-8628	1:500
Rabbit anti-NANOG	Novus Biologicals	NB100-58842	1:100
Mouse anti-SSEA4	Santa Cruz	Sc-21704	1:500
Mouse anti TRA-1-60	Sigma-Aldrich	MAB4360	1:500
Rabbit anti-FOXA2	Cell Signaling	8186T	1:500
Mouse anti-αSMA	Sigma-Aldrich	A2547	1:400
Mouse anti TUJ1	Biolegend	801212	10ug/mL
Mouse anti GATA3	R&D Systerms	MAB6330	10ug/mL
Goat anti GATA2	R&D Systerms	AF2046	5ug/mL
Rabbit anti-KRT7	Abcam	ab181598	1:100
Donkey anti Mouse Alexa Fluor 488	Thermo Fisher Scientific	A-21202	1:1000
Donkey anti Goat Alexa Fluor 488	Thermo Fisher Scientific	A-11055	1:1000
Donkey anti Rabbit Alexa Fluor 488	Thermo Fisher Scientific	A-21206	1:1000
Donkey anti Rabbit Alexa Fluor 594	Thermo Fisher Scientific	A32754	1:1000
Donkey anti Mouse Alexa Fluor 594	Thermo Fisher Scientific	A-21203	1:1000

Supplementary Table 2. Lists of primers and oligos used in this study

This table details the forward and reverse sequences for all qPCR primers.

Primers for qPCR			
Name	Sequence-forward (5'-3')	Sequence-reverse (5'-3')	Source for acquisition
GAPDH	CTGGGCTACACTGAGCACC	AAGTGGTCGTTGAGGGCAATG	Synthesized by BGI
NANOG	CCCCAGCCTTTACTCTTCCTA	CCAGGTTGAATTGTTCCAGGTC	Synthesized by BGI
SALL4	GAAACCACATCCTTCCAGGCAC	GGATAAACGTGGAAGGGAGACTG	Synthesized by BGI
DPPA3	CGCATGAAAGAAGACCAACAAACAA	TTAGACACGCAGAAACTGCAGGGA	Synthesized by BGI
DNMT3B	TAACAACGGCAAAGACCGAGGG	TCCTGCCACAAGACAAACAGCC	Synthesized by BGI

HIST1H2AC	GTGCCTGCCAAACTTACCAA	CCTCCTTATCTCCCCACACA	Synthesized by BGI
HIST1H2BJ	CCACAATGGCTGCATCTTCT	CTCTGGCATGGCTGTTTCTG	Synthesized by BGI
Viral_OCT4	TGAACCGTCAGATCGCCTGG	GGGCGAGAAGGCGAAATC	Synthesized by BGI

Supplementary Table 3. Statistics

For statistical analysis, data were first subjected to Shapiro-Wilk test to assess the normality of each variant's data. Levene's test was conducted to check for equal variances across groups. Due to the non-uniform normal distribution of the data, we opted for the non-parametric Mann-Whitney U test for RT-qPCR and reprogramming data. Here we provided the exact values for all tests used in the manuscript.

Feeder-based iEPSC from HDFa		
Shapiro–Wilk normality per group		
	W	P
eSOX17	0.880	0.0002342
miniSOX	0.884	0.003492
SOX2	0.875	0.0003201
Levene's test		
stat	P	
9.001	0.0002358	
Mann–Whitney U tests (two-sided)		
	U	P
eSOX17 vs SOX2	1813.000	1.403e-14
miniSOX vs SOX2	1042.000	6.86e-07
Adjusted p-values (FDR-BH)		
eSOX17	2.81e-14	
miniSOX	6.86e-07	

Feeder-free iEPSC from HDFa		
Shapiro–Wilk normality per group		
	W	P
eSOX17	0.984	0.7649
miniSOX	0.962	0.3418
SOX2	0.901	0.0018
Levene's test		
stat	P	

16.177	6.635e-07	
Mann–Whitney U tests (two-sided)		
	U	P
eSOX17 vs SOX2	1814.000	1.311e-14
miniSOX vs SOX2	1087.000	4.038e-08
Adjusted p-values (FDR-BH)		
eSOX17	2.62e-14	
miniSOX	4.04e-08	

Feeder-based iEPSC from HDFneo		
Shapiro–Wilk normality per group		
	W	P
eSOX17	0.950	0.1343
miniSOX	0.931	0.1046
SOX2	0.886	0.001692
Levene’s test		
stat	P	
20.515	4.708e-08	
Mann–Whitney U tests (two-sided)		
	U	P
eSOX17 vs SOX2	1128.000	1.481e-11
miniSOX vs SOX2	673.500	9.442e-05
Adjusted p-values (FDR-BH)		
eSOX17	2.96e-11	
miniSOX	9.44e-05	