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Supplementary Materials for

An engineered Sox17 induces somatic to neural stem cell fate transitions independently from pluripotency reprogramming

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

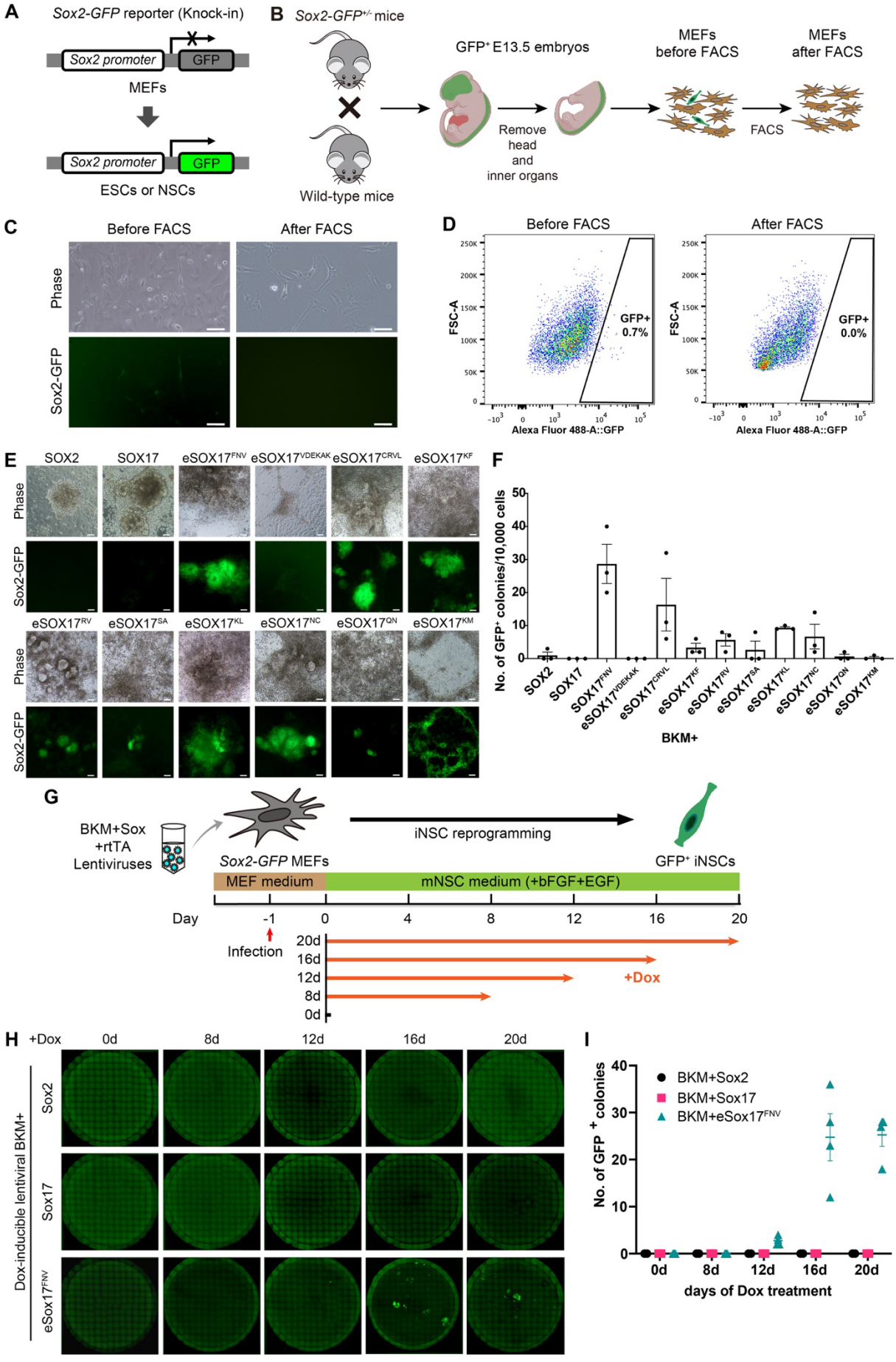
A
Sox2-GFP reporter (Knock-in)
Sox2 promoter
GFP
MEFs
Sox2 promoter
GFP
ESCs or NSCs
B
Sox2-GFP+/- mice
Wild-type mice
GFP+ E13.5 embryos
Remove head and inner organs
MEFs before FACS
FACS
MEFs after FACS
C
Before FACS
After FACS
Phase
Sox2-GFP
D
Before FACS
After FACS
FSC-A
GFP+ 0.7%
GFP+ 0.0%
Alexa Fluor 488-A::GFP
E
SOX2
SOX17
eSOX17FNV
eSOX17VDEKAK
eSOX17CRVL
eSOX17KF
eSOX17RV
eSOX17SA
eSOX17KL
eSOX17NC
eSOX17QN
eSOX17KM
Phase
Sox2-GFP
F
No. of GFP+ colonies/10,000 cells
BKM+
G
BKM+Sox +rtTA Lentiviruses
Sox2-GFP MEFs
iNSC reprogramming
GFP+ iNSCs
MEF medium
mNSC medium (+bFGF+EGF)
Day
Infection
+Dox
20d
16d
12d
8d
0d
H
+Dox
0d
8d
12d
16d
20d
Dox-inducible lentiviral BKM+
Sox2
Sox17
eSox17FNV
I
BKM+Sox2
BKM+Sox17
BKM+eSox17FNV
No. of GFP+ colonies
days of Dox treatment

Fig. S1. Generation of Sox2-positive cells from Sox2-GFP MEFs with eSox17^{FNV}, related to Fig. 1. (**A**) Illustration of *Sox2-GFP* reporter. (**B**) Workflow of MEF preparation. (**C-D**) Purification of *Sox2-GFP* MEFs by FACS. Microscope images (C) and FACS plots (D) of *Sox2-GFP* MEFs before and after cell sorting by FACS. (**E**) Representative microscope images of miNSC reprogramming with BKM+eSOX17 variants 4-factor combinations. Images were took at day 20 of reprogramming. Scale bar, 80 μm. (**F**) Number of Sox2-GFP^{+} colonies at day 20 of reprogramming in indicated BKM+eSox17 variants 4-factor conditions (n=3, mean ± SEM). (**G**) Scheme of reprogramming using Sox2-GFP MEFs and Dox-inducible lentiviral vectors. Transduced cells were treated with Dox for 8, 12, 16 or 20 days and were cultured in mNSC medium for up to 20 days. (**H**) Whole-well scan images of iNSC reprogramming in 12-well plates at day 20 with indicated lentiviral cocktails and Dox induction time periods. (**I**) Number of Sox2-GFP^{+} colonies counted at day 20 of reprogramming with indicated lentiviral cocktails and Dox induction time periods (n=4, mean ± SEM).

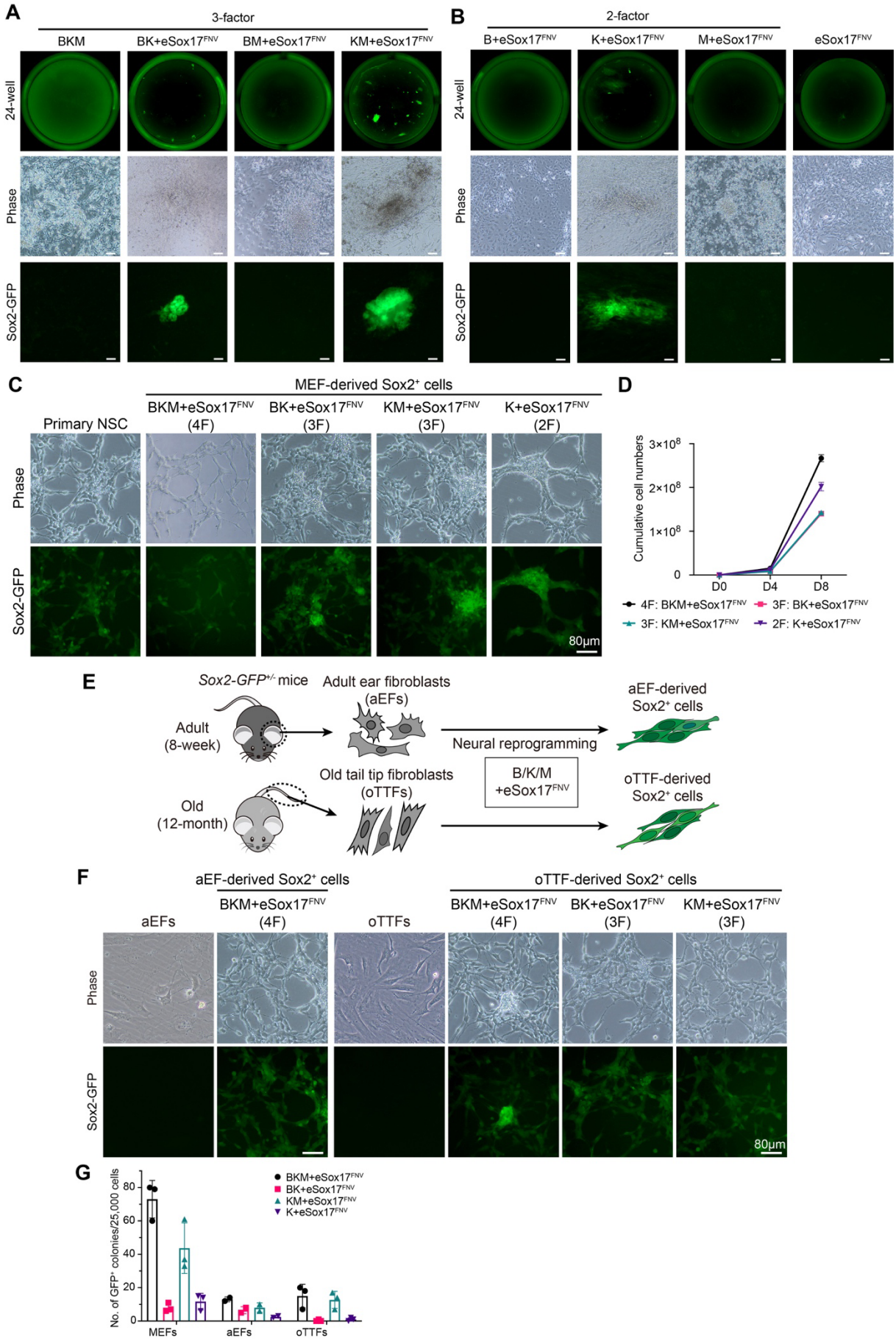
A
3-factor
BKM
BK+eSox17FNV
BM+eSox17FNV
KM+eSox17FNV
24-well
Phase
Sox2-GFP
B
2-factor
B+eSox17FNV
K+eSox17FNV
M+eSox17FNV
eSox17FNV
C
MEF-derived Sox2+ cells
Primary NSC
BKM+eSox17FNV (4F)
BK+eSox17FNV (3F)
KM+eSox17FNV (3F)
K+eSox17FNV (2F)
80μm
D
Cumulative cell numbers
3×10^8
2×10^8
1×10^8
0
D0
D4
D8
4F: BKM+eSox17FNV
3F: BK+eSox17FNV
3F: KM+eSox17FNV
2F: K+eSox17FNV
E
Sox2-GFP+/- mice
Adult (8-week)
Adult ear fibroblasts (aEFs)
Old (12-month)
Old tail tip fibroblasts (oTTFs)
Neural reprogramming
B/K/M +eSox17FNV
aEF-derived Sox2+ cells
oTTF-derived Sox2+ cells
F
aEF-derived Sox2+ cells
oTTF-derived Sox2+ cells
aEFs
BKM+eSox17FNV (4F)
oTTFs
BKM+eSox17FNV (4F)
BK+eSox17FNV (3F)
KM+eSox17FNV (3F)
80μm
G
No. of GFP+ colonies/25,000 cells
80
60
40
20
0
MEFs
aEFs
oTTFs
BKM+eSox17FNV
BK+eSox17FNV
KM+eSox17FNV
K+eSox17FNV

Fig. S2. Generation of Sox2-positive cells from fetal, adult and aged fibroblasts with eSox17^{FNV}, related to Fig. 1. (**A-B**) Whole-well scans (upper panels) of 24-well plates using a GFP channel, representative phase contrast (middle panel) and corresponding GFP fluorescence (lower panel) images of cells reprogrammed from Sox2-GFP MEFs for indicated 3-factor (A), 2-factor/1-factor (B) conditions at day 20. Scale bar, 80 μm. (**C**) Representative microscope images of primary mNSCs, and Sox2^{+} generated from MEFs with indicated 4F, 3F and 2F cocktails. Scale bar, 80 μm. (**D**) Proliferation rate of Sox2^{+} cells. Cells were passaged and 50,000 cells were plated onto Matrigel-coated 24-well plates every 4 days. Cells corresponding to passages 19-21 (n=3, mean ± SEM). (**E**) Schematic illustration of isolation of adult ear fibroblasts (aEFs) and old tail tip fibroblasts (oTTFs) from Sox2-GFP mice for reprogramming experiments. (**F**) Representative microscope images of adult ear fibroblasts (aEFs), old tail-tip fibroblasts (oTTFs) and Sox2^{+} cells generated from aEFs and oTTFs with indicated 4F and 3F cocktails. Scale bar, 80 μm. (**G**) Counts of GFP^{+} colonies at day 20 of miNSC reprogramming from MEFs, aEFs and oTTFs in indicated conditions (n=3, mean ± SEM).

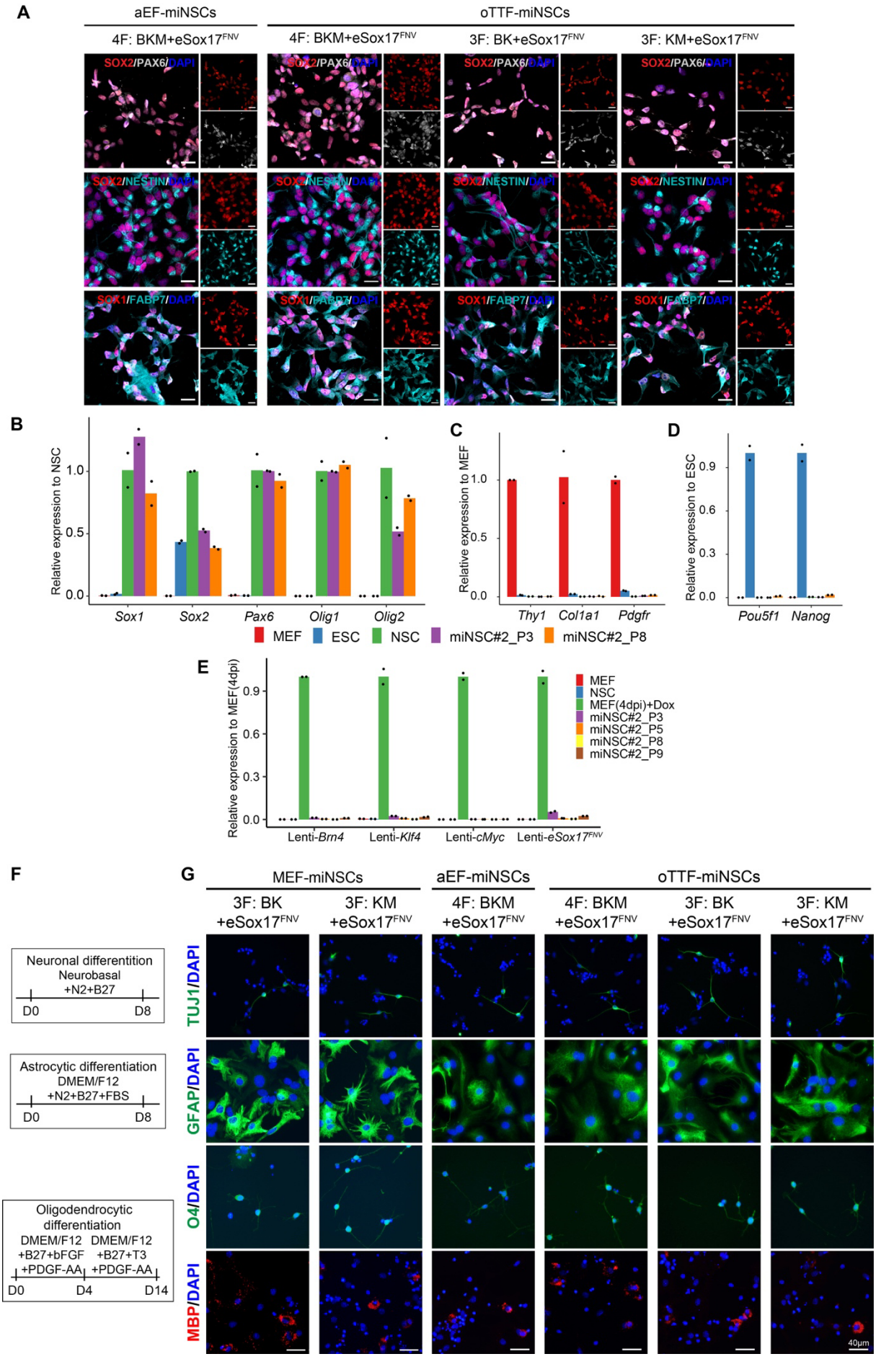
A
aEF-miNSCs
oTTF-miNSCs
4F: BKM+eSox17FNV
4F: BKM+eSox17FNV
3F: BK+eSox17FNV
3F: KM+eSox17FNV
SOX2/PAX6/DAPI
SOX2/NESTIN/DAPI
SOX1/FABP7/DAPI
B
Relative expression to NSC
1.0
0.5
0.0
Sox1
Sox2
Pax6
Olig1
Olig2
C
Relative expression to MEF
1.0
0.5
0.0
Thy1
Col1a1
Pdgfr
D
Relative expression to ESC
0.9
0.6
0.3
0.0
Pou5f1
Nanog
MEF
ESC
NSC
miNSC#2_P3
miNSC#2_P8
E
Relative expression to MEF(4dpi)
0.9
0.6
0.3
0.0
Lenti-Brn4
Lenti-Klf4
Lenti-cMyc
Lenti-eSox17FNV
MEF
NSC
MEF(4dpi)+Dox
miNSC#2_P3
miNSC#2_P5
miNSC#2_P8
miNSC#2_P9
F
Neuronal differentition
Neurobasal
+N2+B27
D0
D8
Astrocytic differentiation
DMEM/F12
+N2+B27+FBS
D0
D8
Oligodendrocytic differentiation
DMEM/F12 +B27+bFGF +PDGF-AA
DMEM/F12 +B27+T3 +PDGF-AA
D0
D4
D14
G
MEF-miNSCs
aEF-miNSCs
oTTF-miNSCs
3F: BK +eSox17FNV
3F: KM +eSox17FNV
4F: BKM +eSox17FNV
4F: BKM +eSox17FNV
3F: BK +eSox17FNV
3F: KM +eSox17FNV
TUJ1/DAPI
GFAP/DAPI
O4/DAPI
MBP/DAPI
40μm

Fig. S3. Characterization of miNSCs generated with eSox17^{FNV} from MEFs, aEF and oTTF in vitro, related to Fig. 2. (**A**) Immunocytochemistry of aEF and oTTF-derived miNSCs using antibodies against NESTIN, SOX1, SOX2, PAX6 and FABP7. Scale bar 20 µm. (**B-D**) Gene expression of MEF-derived B/K/M/eSox17^{FNV} 4F-miNSCs clone #2 at passage 3 (P3) and passage 8 (P8) detected by qRT-PCR. MEFs, ESCs and primary NSCs were used as controls. Expression of NSC genes was normalized to primary NSCs (B), fibroblast genes was normalized to MEFs (C), and pluripotency genes was normalized to ESCs (D). P, passage. n = 2, mean. (**E**) Expression of lentiviral transgenes *Brn4*, *Klf*, *c-Myc* and *eSox17^{FNV}* in 4F-miNSC clone #2 at indicated passage normalized to MEFs 4 days post lentiviral infection (4dpi) cultured in NSC medium containing Dox. (**F**) Schematic representation for differentiation of miNSCs to neurons, astrocytes and oligodendrocytes. (**G**) Differentiation of indicated miNSCs into Tju1^{+} neurons, GFAP^{+} astrocytes, O4^{+} or MBP^{+} oligodendrocytes determined by immunocytochemistry. Scale bar, 40 µm.

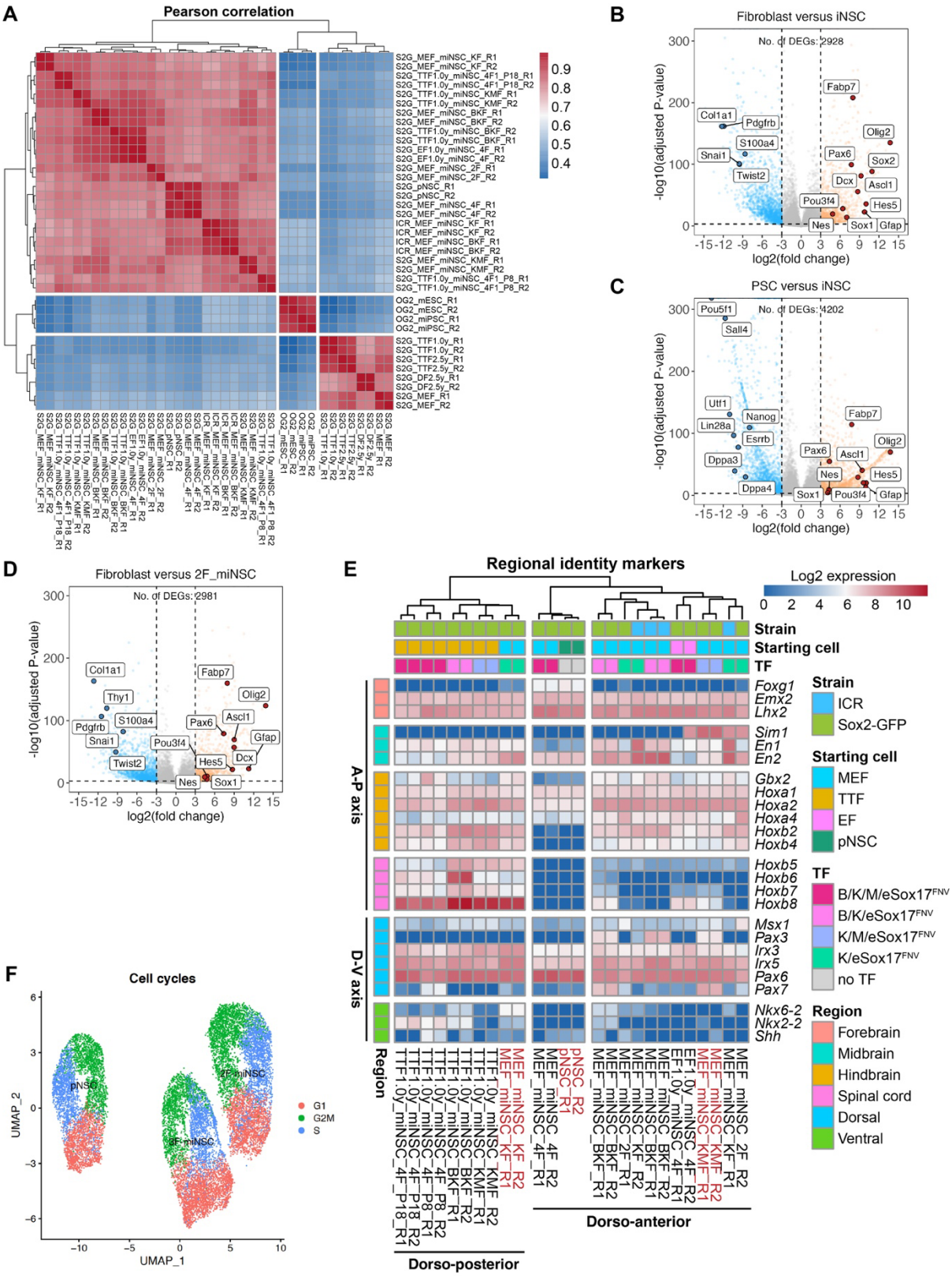
A
Pearson correlation
B
Fibroblast versus iNSC
No. of DEGs: 2928
-log10(adjusted P-value)
log2(fold change)
C
PSC versus iNSC
No. of DEGs: 4202
D
Fibroblast versus 2F_miNSC
No. of DEGs: 2981
E
Regional identity markers
Log2 expression
Strain
Starting cell
TF
A-P axis
D-V axis
Region
Dorso-posterior
Dorso-anterior
F
Cell cycles
UMAP_1
UMAP_2
G1
G2M
S

Fig. S4. Regional identify of miNSCs, related to Fig. 3. (**A**) Hierarchically clustered heatmap based on Pearson correlation coefficients (r^2) using normalized read counts of bulk RNA-seq with two technical replicates as input. (**B-D**) Volcano plots of differentially expressed genes (DEGs) in all miNSCs versus the parental fibroblasts (B), all miNSCs versus pluripotent stem cells (PSC) (C) and 2F-miNSCs versus parental fibroblasts (D). Selected differentially expressed fibroblast, NSC and PSC genes are labelled. (**E**) Expression of selected forebrain, midbrain, hindbrain, spinal cord, dorsal and ventral regional identity genes in bulk RNA-seq data represented as log2 transformed read counts. (**F**) Cell cycle analysis of scRNA-seq data.

A

Cell line	Starting cell	Reprogramming factors	Cell type	Passage number	Chronological age (months)	Biological age (months)
young NSC#1	pNSC	-	pNSC	P4	0	4.5
young NSC#2	pNSC	-	pNSC	P4	0	4.5
MEF#1	MEF	-	Fibroblast	P1	0	2.4
MEF#2	MEF	-	Fibroblast	P1	0	1.8
old NSC#1	pNSC	-	pNSC	P5	30	34.3
old NSC#2	pNSC	-	pNSC	P5	30	42.1
old miNSC#1	oTTF	B/K/M/eSox17^{FNV}	miNSC	P6	30	27.9
old miNSC#2	oTTF	B/K/M/ eSox17^{FNV}	miNSC	P6	30	13.4
old miNSC#3	oTTF	K/M/ eSox17^{FNV}	miNSC	P4	30	6.9
old miNSC#4	oTTF	K/M/ eSox17^{FNV}	miNSC	P6	30	11.3
old miNSC#5	oTTF	B/K/ eSox17^{FNV}	miNSC	P5	30	22.6

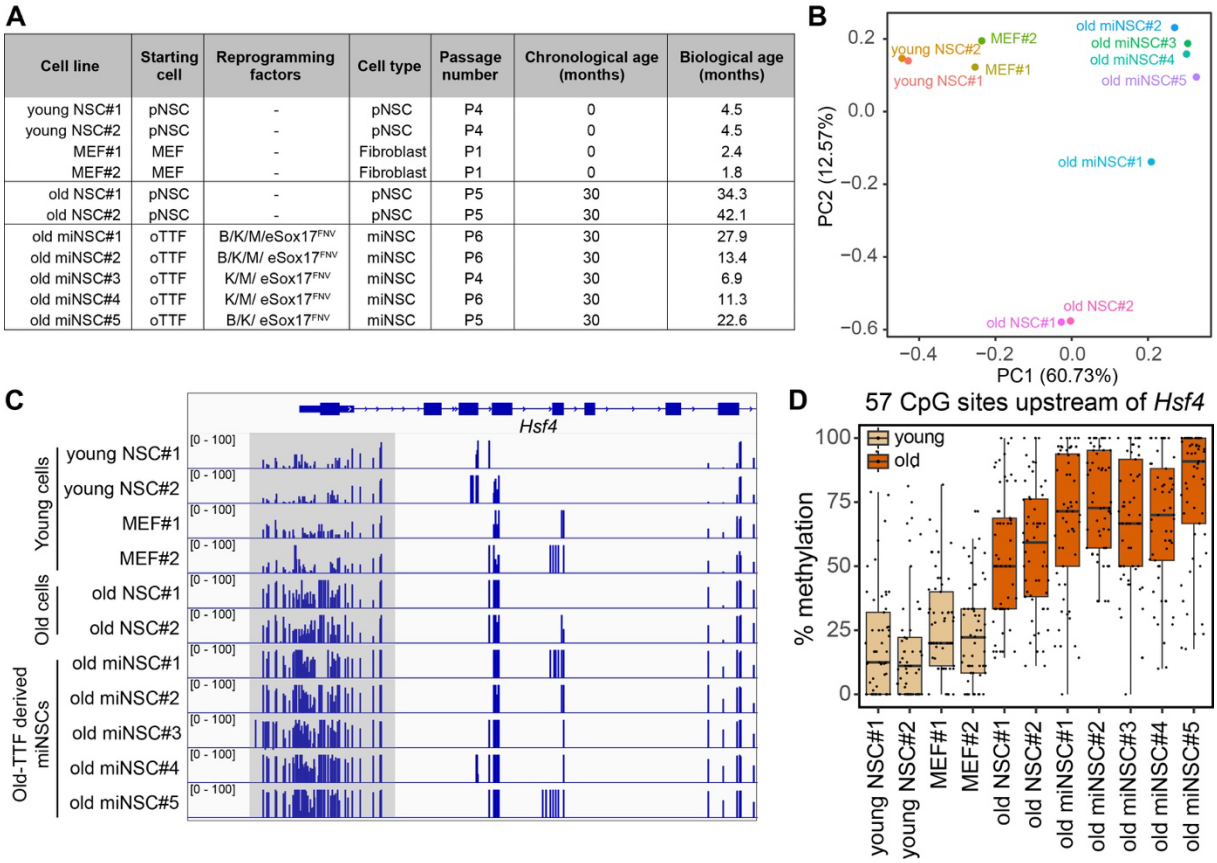

Fig. S5. Methylation age of miNSCs predicted using RRBS data, related to Fig. 3. (**A**) Mouse cell lines for RRBS including young NSCs and MEFs from embryos, old NSCs from 2.5-year-old brains and old miNSCs reprogrammed from fibroblasts of aged mice. Methylation age of the cells are predicted using 329 CpG sites in Mouse Epigenetic Clock (*41*) based on RRBS data. (**B**) PC analysis of 404 CpG sites in 20 aging-related hypermethylated regions (*42*) in RRBS data. (**C**) Selected genomic views of the methylation levels at selected CpG sites. Genome browser tracks from Integrative Genomics Viewer (IGV) using BigWig files as input were shown for *Hsf4* loci (chr8:105,269,017-105,272,689). Differentially methylated (DM) promoter loci is marked with a gray box. Number on the left side of each track indicates the percentage of methylation at individual CpG sites (0 - 100). (**D**) Boxplot of methylation levels of 57 CpG sites in the DM promoter loci of *Hsf4* in (C).

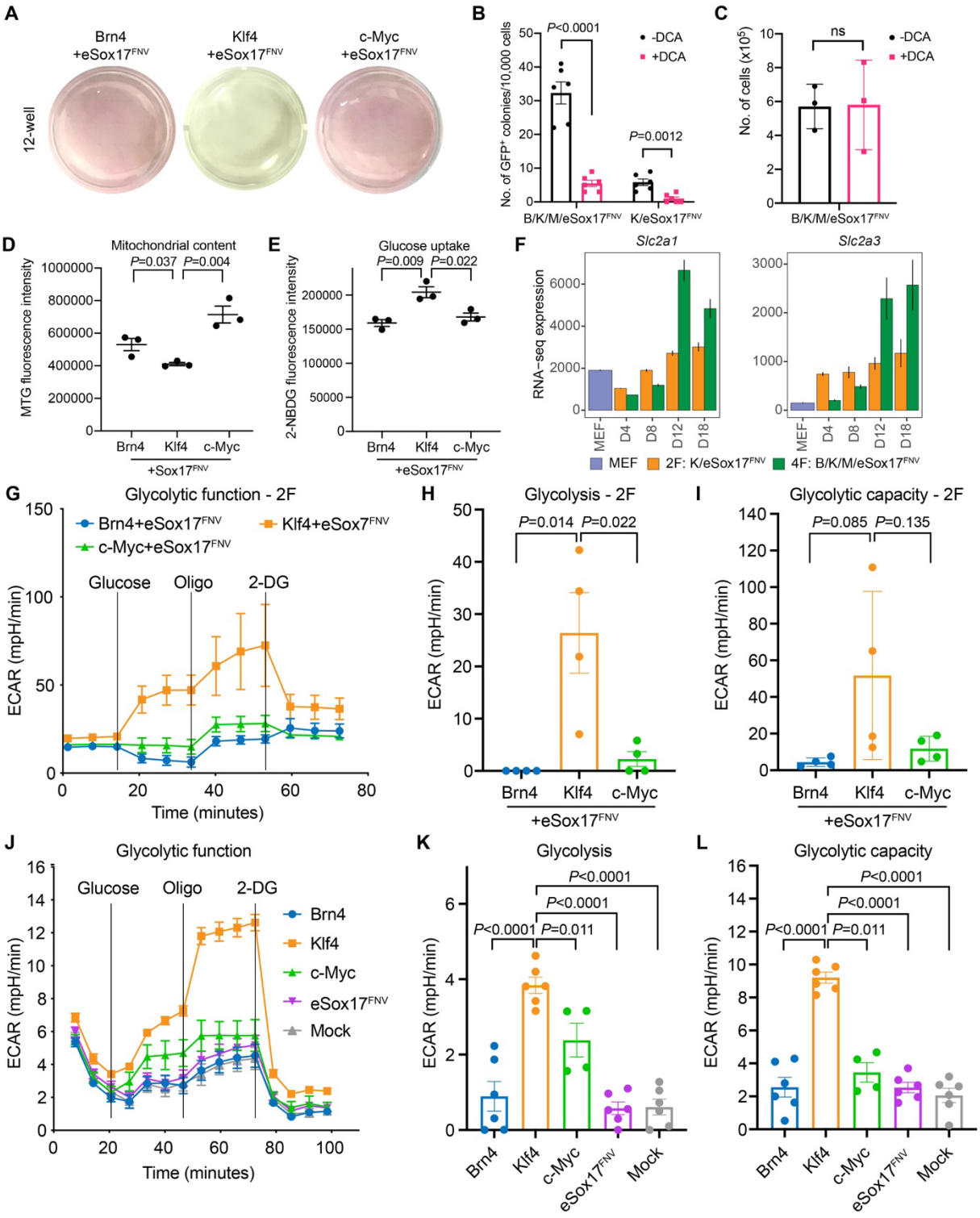
A
Brn4 +eSox17FNV
Klf4 +eSox17FNV
c-Myc +eSox17FNV
12-well
B
P<0.0001
-DCA
+DCA
P=0.0012
No. of GFP+ colonies/10,000 cells
B/K/M/eSox17FNV
K/eSox17FNV
C
ns
-DCA
+DCA
No. of cells (x10^5)
B/K/M/eSox17FNV
D
Mitochondrial content
P=0.037 P=0.004
MTG fluorescence intensity
Brn4 Klf4 c-Myc
+Sox17FNV
E
Glucose uptake
P=0.009 P=0.022
2-NBDG fluorescence intensity
Brn4 Klf4 c-Myc
+eSox17FNV
F
Slc2a1
Slc2a3
RNA−seq expression
MEF D4 D8 D12 D18
MEF
2F: K/eSox17FNV
4F: B/K/M/eSox17FNV
G
Glycolytic function - 2F
Brn4+eSox17FNV
Klf4+eSox7FNV
c-Myc+eSox17FNV
Glucose Oligo 2-DG
ECAR (mpH/min)
Time (minutes)
H
Glycolysis - 2F
P=0.014 P=0.022
ECAR (mpH/min)
Brn4 Klf4 c-Myc
+eSox17FNV
I
Glycolytic capacity - 2F
P=0.085 P=0.135
ECAR (mpH/min)
Brn4 Klf4 c-Myc
+eSox17FNV
J
Glycolytic function
Glucose Oligo 2-DG
Brn4
Klf4
c-Myc
eSox17FNV
Mock
ECAR (mpH/min)
Time (minutes)
K
Glycolysis
P<0.0001
P<0.0001
P<0.0001 P=0.011
ECAR (mpH/min)
Brn4 Klf4 c-Myc eSox17FNV Mock
L
Glycolytic capacity
P<0.0001
P<0.0001
P<0.0001 P=0.011
ECAR (mpH/min)
Brn4 Klf4 c-Myc eSox17FNV Mock

Fig. S6. Klf4 directs a metabolic switch during miNSC reprogramming. (**A**) Images of wells with cell culture media of cells overexpressing indicated factors at reprogramming day 10 showing that Klf4 induces a colour change. Cells were fed with fresh mNSC medium every two days. (**B**) Counts of Sox2-GFP^{+} miNSC colonies at day 20 of reprogramming with B/K/M/eSox17^{FNV} (4F) or K/eSox17^{FNV} (2F) cocktail in mNSC medium with or without 3.5 mM of the glycolysis inhibitor DCA (n=6, mean ± SEM). (**C**) Total live cell number on each well of 24-well plate after 10 days of reprogramming with the 4F cocktail in mNSC medium with or without 3.5 mM DCA (n=3, mean ± SEM). (**D-E**) Mean fluorescence intensities (MFI) of cells stained with MitoTracker Green (MTG) (D) and 2-NBDG (E) at day 5 of reprogramming. One out of two representative experiments is shown (n = 3, mean ± SEM). (**F**) Mean RNA-seq expression of glucose transporter genes *Slc2a1* and *Slc2a3* at day 4, 8, 12 and 18 of iNSC reprogramming with B/K/M/eSox17^{FNV} (4F) or K/eSox17^{FNV} (2F) cocktail. (**G**) Representative extracellular acidification rate (ECAR) at day 10 of reprogramming with indicated single factors (n = 4-6 for each group, mean ± SEM). (**H-I**) Glycolysis calculated (h) and glycolytic capacity (I) from ECAR values from the glycolysis-stress test in (G) (n = 4-6, mean ± SEM). Student's t-test (two-tailed, unpaired, with assumption of the same SD), P-values are indicated. (**J**) Representative extracellular acidification rate (ECAR) at day 10 of reprogramming with indicated two 2F conditions (n = 4, mean ± SEM). (**K-L**) Glycolysis calculated (J) and glycolytic capacity (L) from ECAR values from the glycolysis-stress test in (J) (n = 4, mean ± SEM). Student's t-test (two-tailed, unpaired, with assumption of equal variance), P-values are indicated.

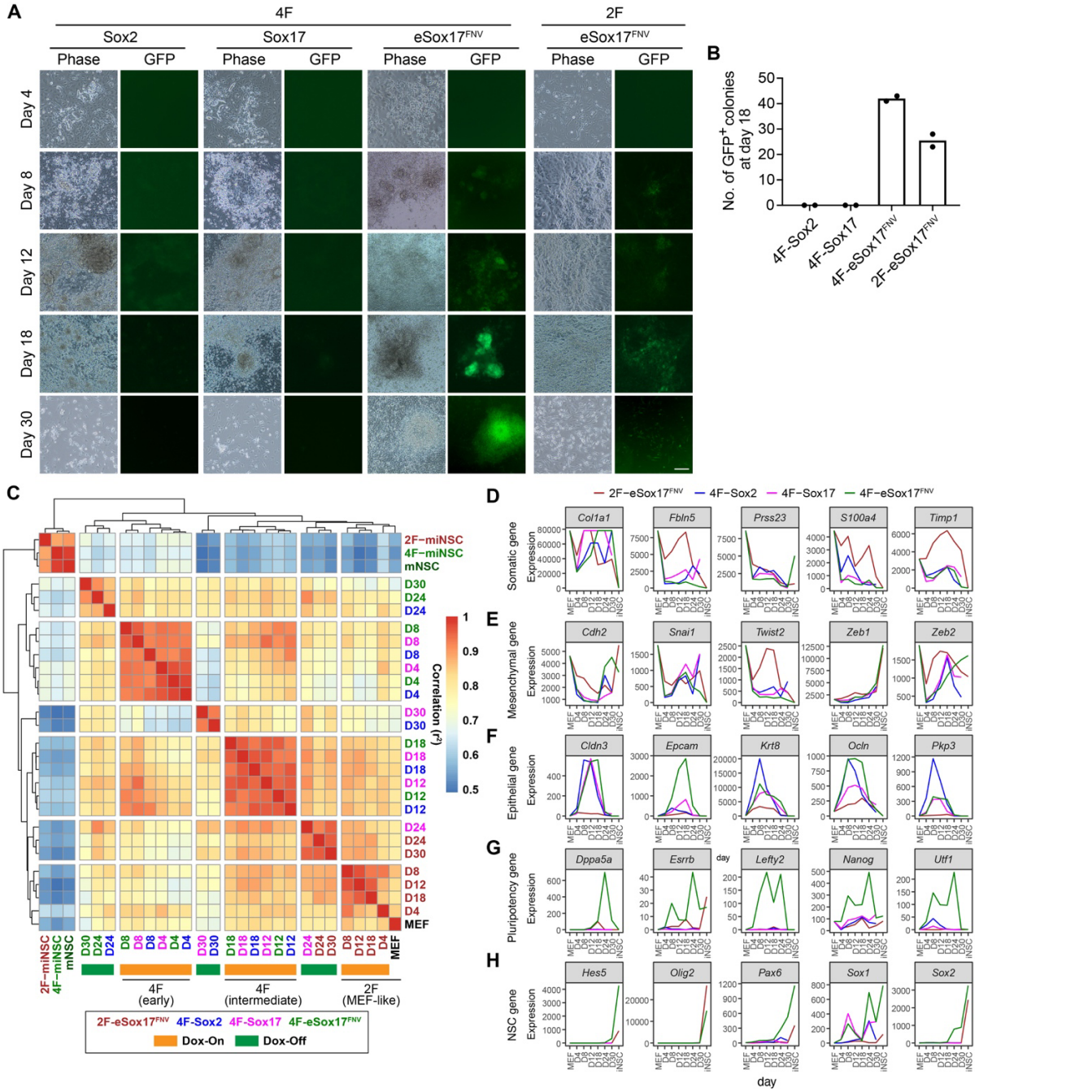
A
4F
2F
Sox2
Sox17
eSox17FNV
eSox17FNV
Phase
GFP
Day 4
Day 8
Day 12
Day 18
Day 30
B
No. of GFP+ colonies at day 18
4F-Sox2
4F-Sox17
4F-eSox17FNV
2F-eSox17FNV
C
2F−miNSC
4F−miNSC
mNSC
MEF
Correlation (r²)
4F (early)
4F (intermediate)
2F (MEF-like)
2F-eSox17FNV 4F-Sox2 4F-Sox17 4F-eSox17FNV
Dox-On
Dox-Off
D
2F−eSox17FNV
4F−Sox2
4F−Sox17
4F−eSox17FNV
Somatic gene
Col1a1
Fbln5
Prss23
S100a4
Timp1
E
Mesenchymal gene
Cdh2
Snai1
Twist2
Zeb1
Zeb2
F
Epithelial gene
Cldn3
Epcam
Krt8
Ocln
Pkp3
G
Pluripotency gene
Dppa5a
Esrrb
Lefty2
Nanog
Utf1
H
NSC gene
Hes5
Olig2
Pax6
Sox1
Sox2
Expression
day
I
4F-Sox2_D30 versus 4F-eSox17FNV_D30
No. of DEGs: 1678
4F-Sox17_D30 versus 4F-eSox17FNV_D30
No. of DEGs: 1752
−log10(adjusted P-value)
log2(fold change)
Dcx
Nes
Ascl1
Olig2
Hes5
Fabp7
Sox1
Gfap
Pax6
Twist2
Snai1
S100a4
J
PC1 Top 200 genes
gliogenesis
regulation of neurogenesis
forebrain development
glial cell differentiation
synapse organization
neural precursor cell proliferation
negative regulation of neuron differentiation
glial cell development
oligodendrocyte differentiation
astrocyte differentiation
GeneRatio
Count
pvalue

Fig. S7. Time-course RNA-seq of miNSC reprogramming experiment, related to Fig. 4. (**A**) Representative microscopy images of cells reprogrammed with indicated conditions on indicated days of reprogramming. Scale bar, 160 µm. (**B**) Count of Sox2-GFP^{+} colonies in indicated conditions at reprogramming day 18 (n = 2, mean ± SEM). (**C**) Hierarchically clustered heatmap based on Pearson correlation coefficients (r^2) using RNA-seq mean read counts of duplicates as input. (**D-H**) RNA-seq expression of somatic (D), mesenchymal (E), epithelia (F), pluripotency (G) and NSC genes (H) in indicated conditions at day 4, 8, 12, 18, 24 and 30 of miNSC reprogramming. (**I**) Volcano plots of differentially expressed genes (DEGs) in 4F-Sox2 reprogrammed cells versus the 4F-eSox17^{FNV} reprogrammed cells (left) and 4F-Sox17 reprogrammed cells versus the 4F-eSox17^{FNV} reprogrammed cells (right) at day30 (D30) of miNSC reprogramming. Selected differentially expressed fibroblast and NSC genes are labelled. (**J**) Gene ontology (GO) analysis performed using top 200 genes showing the highest contribution for PC1 in the PC analysis in Fig. 4B.

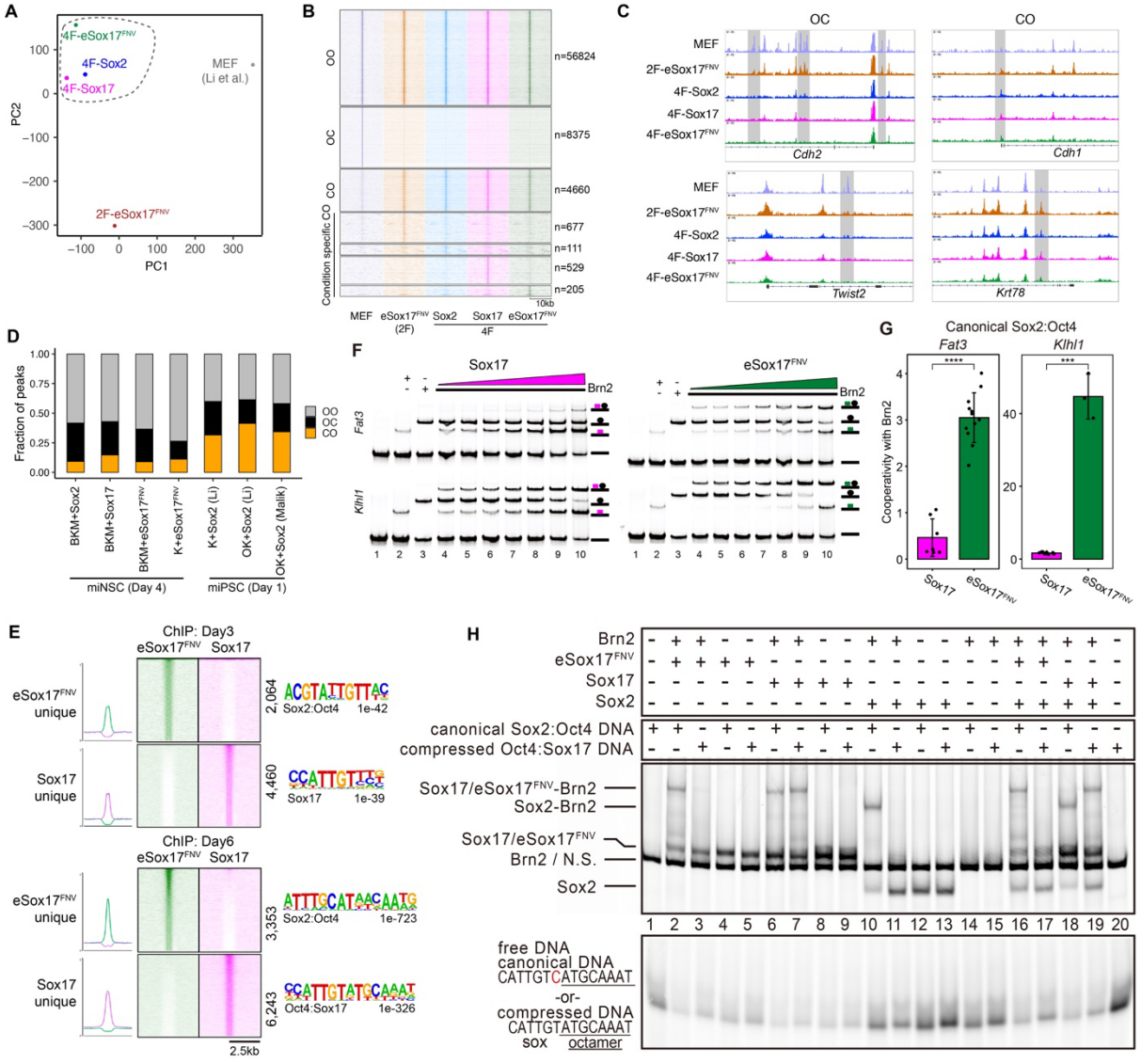
A
4F-eSox17FNV
4F-Sox2
4F-Sox17
MEF
(Li et al.)
2F-eSox17FNV
PC1
PC2
B
OO
OC
CO
Condition specific CO
n=56824
n=8375
n=4660
n=677
n=111
n=529
n=205
10kb
MEF
eSox17FNV (2F)
Sox2
Sox17
eSox17FNV
4F
C
OC
CO
MEF
2F-eSox17FNV
4F-Sox2
4F-Sox17
4F-eSox17FNV
Cdh2
Cdh1
Twist2
Krt78
D
Fraction of peaks
OO
OC
CO
BKM+Sox2
BKM+Sox17
BKM+eSox17FNV
K+eSox17FNV
K+Sox2 (Li)
OK+Sox2 (Li)
OK+Sox2 (Malik)
miNSC (Day 4)
miPSC (Day 1)
F
Sox17
eSox17FNV
Brn2
Fat3
Klhl1
G
Canonical Sox2:Oct4
Fat3
Klhl1
Cooperativity with Brn2
Sox17
eSox17FNV
E
ChIP: Day3
eSox17FNV
Sox17
eSox17FNV unique
Sox17 unique
2,064
Sox2:Oct4
1e-42
4,460
Sox17
1e-39
ChIP: Day6
3,353
Sox2:Oct4
1e-723
6,243
Oct4:Sox17
1e-326
2.5kb
H
Brn2
eSox17FNV
Sox17
Sox2
canonical Sox2:Oct4 DNA
compressed Oct4:Sox17 DNA
Sox17/eSox17FNV-Brn2
Sox2-Brn2
Sox17/eSox17FNV
Brn2 / N.S.
Sox2
free DNA
canonical DNA
CATTGTCATGCAAAT
-or-
compressed DNA
CATTGTATGCAAAT
sox
octamer

Fig. S8. Chromatin opening by eSox17^{FNV}, related to Fig. 5. (**A**) PC analysis of ATAC-seq signals from 4F conditions containing Sox2, Sox17 or eSox17^{FNV} and eSox17^{FNV} 2F condition as well as publicly available ATAC-seq data for MEFs (GSE93026). Duplicates were merged for analysis. (**B**) Heatmap of ATAC-seq signals around ATAC-seq peaks grouped using glbase3 (*72*). ATAC-seq peaks were redefined using *redefine_peaks* function and subsequent 101,731 redefined peaks from all of the samples were grouped by *chip_seq_cluster* function from *glbase3* packages. Concurrent and unique peak groups were selected and visualized using EaSeq. Changes in chromatin states from non-transduced MEFs to cells at day 4 of reprogramming are indicated. CO: closed in MEFs but open in reprogrammed cells; OC: open in MEFs but closed in reprogrammed cells. The number of peaks defined in each group is indicated in the middle gray boxes. (**C**) Selected genomic views of the ATAC-seq signals for the indicated groups. Genome browser tracks from Integrative Genomics Viewer (IGV) using BigWig files as input were shown for representative genomic loci: Cdh2 (chr18:16,679,575-16,844,074), Twist2 (chr1:91,790,669-91,841,242), Cdh1 (chr8:106,581,620-106,640,290), Krt78 (chr15:101,960,722-102,007,895). OC/CO loci are marked with a gray box. Number on the left side of each track indicates the vertical scale of genome views (0 - 30 or 0 - 60). (**D**) Fraction of OO, OC and CO peaks in reprogramming cells compared with MEFs in indicated reprogramming conditions. Data for miPSC reprogramming day 1 of K/Sox2 (Li) and O/K/Sox2 (Li) are from GSE93026, and O/K/Sox2 (Malik) from GSE103980. (**E**) ChIP-seq signals from GSE107987 around unique peaks defined by MAnorm between eSox17^{FNV} (green) and Sox17 (purple) in O/K/M/S 4-F iPSC reprogramming conditions at day 6, with a peak number of each cluster. Top known motifs discovered in each cluster are shown as sequence logos with P values. (**F**) Heterodimer EMSAs with 50nM Cy5 labeled DNA probes for genomic sequences with the Sox2:Oct4 DNA motif (*Fat3* and *Klhl1*) to monitor the complex formation of Brn2 (black circle) with Sox17 (magenta square) and eSox17^{FNV} (green square). (**G**) Quantifications of heterodimer EMSAs and calculation of cooperativity factors (*68*) from (F). Data are shown as mean ± sd (n=3-9). *** and **** for P-value < 0.001 and 0.0001 from an unpaired t-test. (**H**) Whole-cell extract EMSA of full-length eSox17^{FNV}, Sox17, and Sox2 with or without Brn2 on 280nM Cy5 labelled DNA of canonical or compressed SoxOct motifs using HEK293T cell lysates.

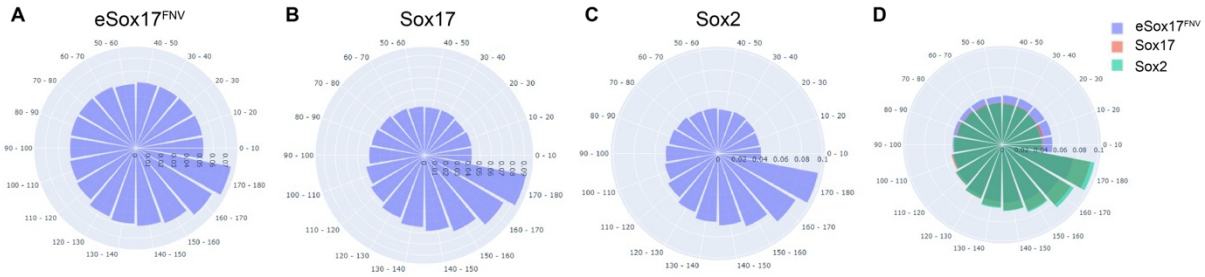

Fig. S9. Angle analysis of Sox factors, related to Fig. 5. (**A-D**) Individual and combined distribution of angles in polar coordinates formed from two consecutive frames found from SMT trajectory analysis.

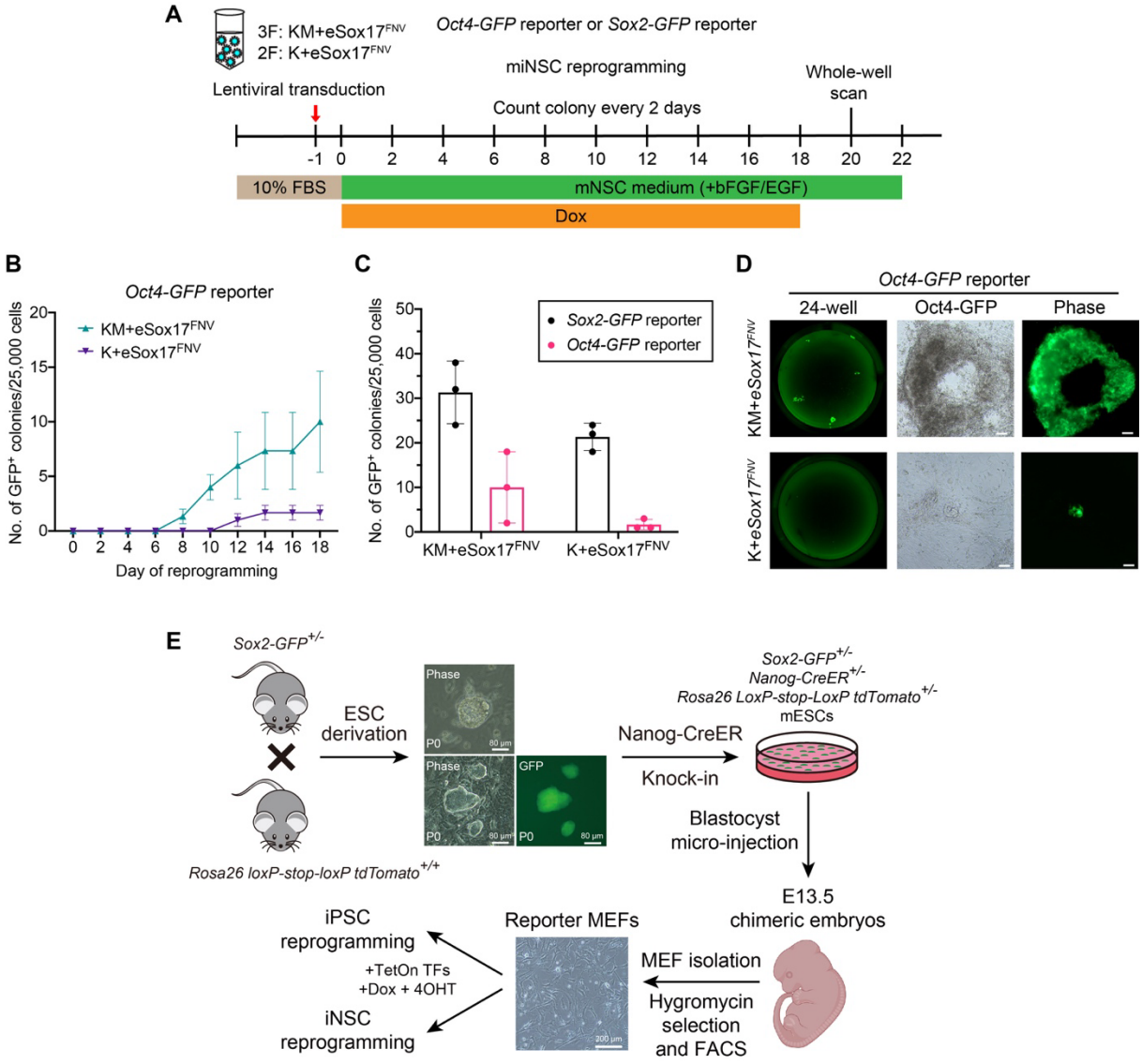

Fig. S10. miPSC and miNSC reprogramming with Oct4-GFP reporter, related to Fig. 6. (**A**) Schematic illustration of miNSC reprogramming using *Oct-GFP* MEFs and *Sox2-GFP* MEFs as starting cells and indicated TF combinations containing eSox17^{FNV}. (**B**) Number of Oct4-GFP^{+} colonies in indicated conditions from day 0 to day 18 of neural reprogramming using OG2 reporter (n=3, mean ± SEM). (**C**) Counts of GFP^{+} colonies at day 18 of reprogramming in indicated conditions. One out of three representative experiments is shown (n=3, mean ± SEM). (**D**) Whole-well scan of 24-well plates at day 20 of neural reprogramming using *Oct4-GFP* reporter (left), representative microscope phase-contrast (middle) and fluorescence (right) images of Oct4-GFP^{+} colonies in indicated conditions at day 20. Scale bar, 80 µm. (**E**) Scheme illustrating generation of the dual reporter in MEFs used for either miPSC or miNSC reprogramming.

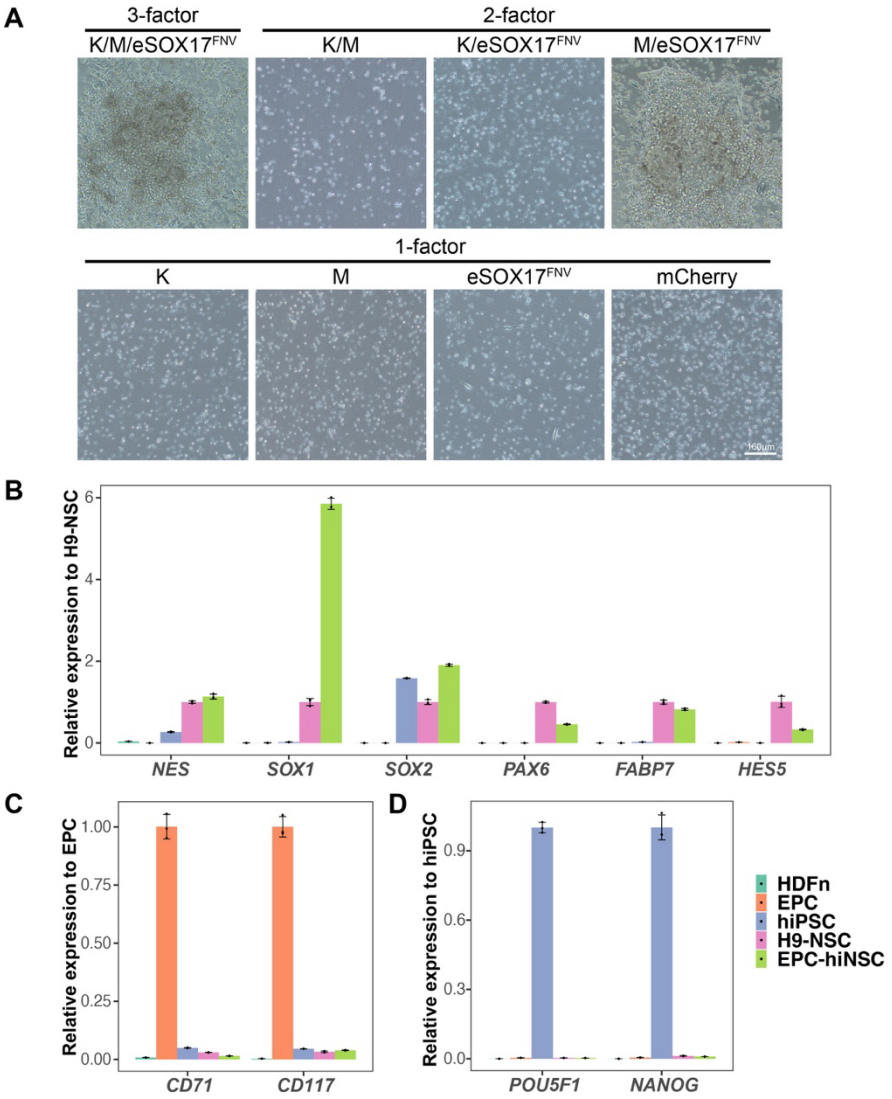

Fig. S11. eSOX17^{FNV} and C-MYC are sufficient to reprogram human blood cells into iNSCs, related to Fig. 7. (**A**) Representative phase-contrast microscope images of hiNSC reprogramming from human erythroid progenitor cells (EPCs) with indicated TFs at day 20. hiNSC-like colonies were only formed in 3-factor K/M/eSOX17^{FNV} and 2-factor M/eSOX17^{FNV} conditions. Scale bar, 160 μm. (**B-D**) Gene expression of M/eSOX17^{FNV} reprogrammed 2F-hiNSCs detected by qRT-PCR. Neonatal human dermal fibroblasts (HDFn), EPCs, hiPSCs and H9-NSCs were used as controls. Expression of NSC genes was normalized to H9-NSCs (B), EPC genes was normalized to EPCs (C), and pluripotency genes was normalized to hiPSCs (D). n = 3, mean ± sd.

Table S1. Primers used in this study.

qRT-PCR primers			
Name	**Species**	**Sequence-forward (5'-3')**	**Sequence-reverse (5'-3')**
Gapdh	Mouse	CCAATGTGTCCGTCGTGGAT	TGCCTGCTTCACCACCTTCT
Sox1	Mouse	GGCCGAGTGGAAGGTCATGT	TCCGGGTGTTCCTTCATGTG
Sox2	Mouse	ACGGCCATTAACGGCACACT	TTTTGCACCCCTCCCAATTC
Olig1	Mouse	AGGGTTTCCGAGCTGGATGTT	AGGAACCTCTCCACTTCGCATC
Olig2	Mouse	ACCACCACGTGTCGGCTATG	TGGTCCAGCTCCCCTTCTTG
Pax6	Mouse	CAAGTTCCCGGGAGTGAACC	TCCACATAGTCATTGGCAGA
Thy1	Mouse	TTCCCTCTCCCTCCTCCAAGC	TCGAGGGCTCCTGTTTCTCCTT
Pdgfr	Mouse	CAGGACCTCTGGCTGAAGCA	TCTGGGAGGCAGAAGGGAGAT
Col1a1	Mouse	CCCTGCCTGCTTCGTGTAAA	TCGTCTGTTTCCAGGGTTGG
Nanog	Mouse	CTCAAGTCCTGAGGCTGACA	TGAAACCTGTCCTTGAGTGC
Pou5f1	Mouse	TAGGTGAGCCGTCTTTCCAC	GCTTAGCCAGGTTCGAGGAT
Lentiviral *Brn4*	Mouse	TGAACCGTCAGATCGCCTGG	ATGGACAAGGGAGCTGGAAC
Lentiviral *Klf4*	Mouse	TGAACCGTCAGATCGCCTGG	GTGGAGAAGGACGGGAGCAG
Lentiviral *cMyc*	Mouse	TGAACCGTCAGATCGCCTGG	TCGAGGTCATAGTTCCTGTTGGTG
Lentiviral *Sox2*	Mouse	TGAACCGTCAGATCGCCTGG	GGCTTCAGCTCCGTCTCCAT
Lentiviral *Sox17*	Mouse	TGAACCGTCAGATCGCCTGG	TCACTGGCGTATCCCGCATC
NESTIN	Human	CTGTAGGCCCTGTTTCTCCTGCT	CTGCGGGCTACTGAAAAGTTCCA
SOX1	Human	CAGCAGTGTCGCTCCAATTCA	GCCAAGCACCGAATTCACAG
SOX2	Human	TGGCGAACCATCTCTGTGGT	CCAACGGTGTCAACCTGCAT
PAX6	Human	CCAGAAAGGATGCCTCATAAA	TCTGCGCGCCCCTAGTTA
FABP7	Human	GGCTTTGCCACTAGGCAGG	TGACCACTTTGTCTCCTTCTTGA
HES5	Human	TCCTGGAGATGGCTGTCAGCTA	CGTGGAGCGTCAGGAACTGCA
CD71	Human	ACCATTGTCATATACCCGGTTCA	CAATAGCCCAAGTAGCCAATCAT
CD117	Human	CGTTCTGCTCCTACTGCTTCG	CCCACGCGGACTATTAAGTCT
POU5F1	Human	GGTGGAGGAAGCTGACAACA	ATCTGCTGCAGTGTGGGTTT
NANOG	Human	CCCCAGCCTTTACTCTTCCTA	CCAGGTTGAATTGTTCCAGGTC
GAPDH	Human	CTGGGCTACACTGAGCACC	AAGTGGTCGTTGAGGGCAATG
Genotyping PCR primers			
Name	**Species**	**Sequence (5'-3')**	
Sox2-EGFP-mu-F	Mouse	AAGTTCATCTGCACCACCG	
Sox2-EGFP-mu-R	Mouse	TGCTCAGGTAGTGGTTGTCG	
Nanog-common-F	Mouse	AGTGGTAGCAACGGTGGTAGTG	
Nanog-wt-R	Mouse	GTTTGGGGTTTGGAAGGAGCAC	
Nanog-CreER-mu-R	Mouse	GTCCATCAGGTTCTTGCGAACCTC	
Rosa26-wt-F	Mouse	AAGGGAGCTGCAGTGGAGTA	
Rosa26-wt-R	Mouse	CCGAAAATCTGTGGGAAGTC	

Rosa26-tdT-mu-F	Mouse	CTGTTCCTGTACGGCATGG
Rosa26-tdT-mu-R	Mouse	GGCATTAAAGCAGCGTATCC

Table S2. Antibodies used in this study.

Antibodies	**Source**	**Identifier**
Rabbit anti-Sox1	Novus Biologicals	NBP2-24486
Mouse anti-Sox2	Santa Cruz	sc-365823
Rabbit anti-Sox2	Stemgent	09-0024
Rabbit anti-Pax6	GeneTex	GTX113241
Mouse anti-Nestin	Sigma-Aldrich	MAB353
Mouse anti-Fabp7	Santa Cruz	sc-374588
Mouse anti-Tubb3 (Tuj1)	BioLegend	801202
Mouse anti-NF	BioLegend	801701
Mouse anti-MAP2	Sigma-Aldrich	M4403
Rabbit anti-MAP2	Cell Signaling	4542S
Rabbit anti-TH	Novus Biologicals	NB300-109
Mouse anti-vGlut2	Novus Biologicals	NBP2-59330
Mouse anti-HB9	Santa Cruz	sc-515769
Rabbit anti-Isl1	Abcam	ab20670
Rabbit anti-SYN1	Novus Biologicals	NB300-104
Rabbit anti-PSD95	Thermo Scientific	51-6900
Rabbit anti-GFAP	Agilent Dako	Z0334
Mouse anti-O4	R&D Systems	MAB1326
Rabbit anti-Olig2	Millipore	AB9610
Donkey anti-Mouse, Alexa Fluor 488	Invitrogen	A-21202
Goat anti-Rabbit, Alexa Fluor 488	Invitrogen	A-11008
Donkey anti-Mouse, Alexa Fluor 594	Invitrogen	A-21203
Donkey anti-Rabbit, Alexa Fluor 594	Invitrogen	A-21207
Donkey anti-Mouse, Alexa Fluor 647	Invitrogen	A-31571
Donkey anti-Rabbit, Alexa Fluor 647	Invitrogen	A-31573
Nucblue Fixed Cell Stain (DAPI)	Invitrogen	R37606

Movie S1.
Fluorescence live-cell imaging of Fluo-4 shows dynamic changes in intracellular calcium in hiNSC-derived neurons. Calcium sparks represent spontaneous activity of neurons, related to Fig. 7H.

Data S1.
Data of colony count of GFP positive colonies in miNSC reprogramming, qRT-PCR and EMSA.

Data S2.
Marker genes for each clusters identified in scRNA-seq data, related to Fig. 3F.