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Supplemental Information

Excluding Oct4 from Yamanaka Cocktail Unleashes the Developmental Potential of iPSCs

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Figure S1

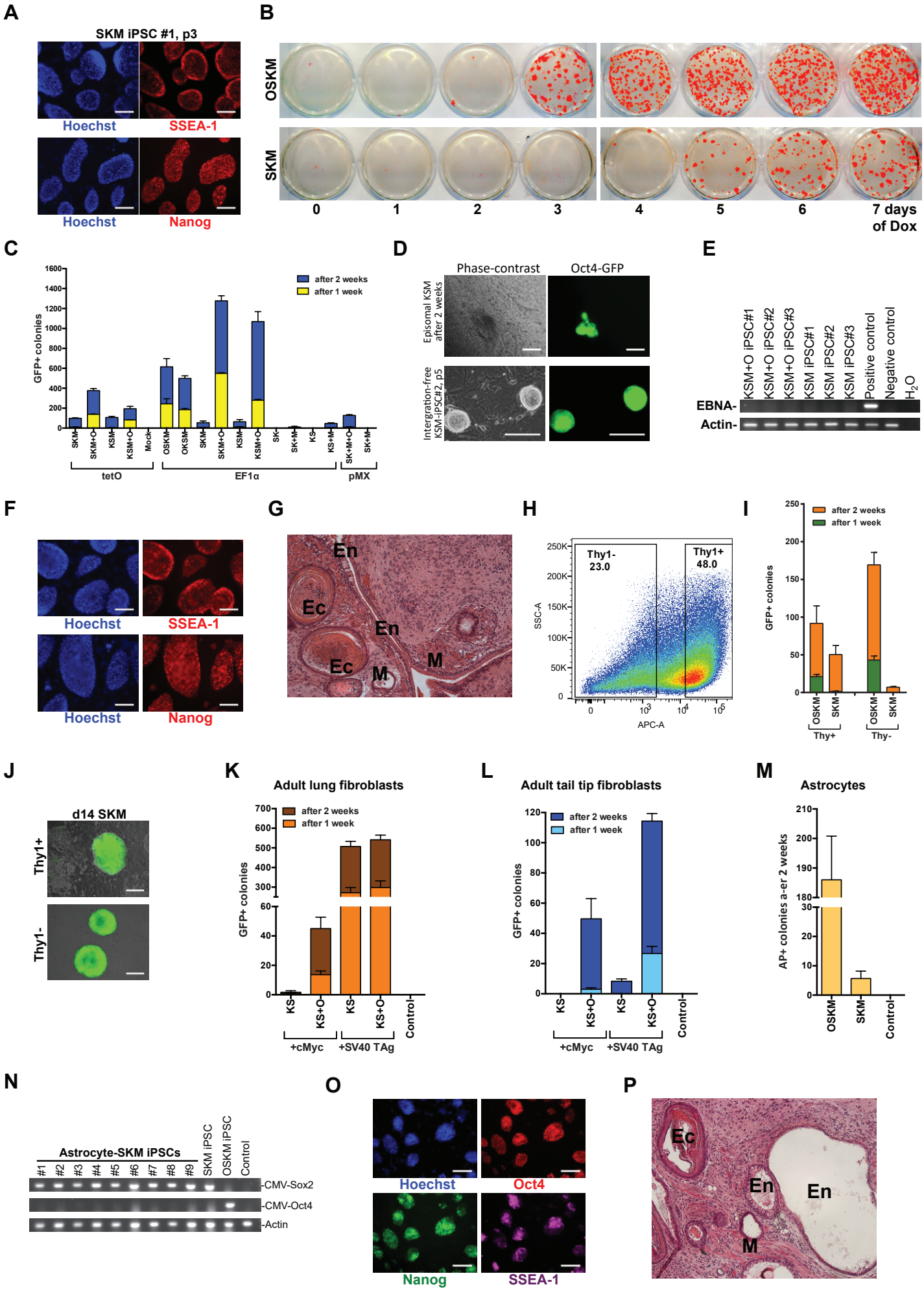

A
SKM iPSC #1, p3
Hoechst
SSEA-1
Hoechst
Nanog
B
OSKM
SKM
0
1
2
3
4
5
6
7 days of Dox
C
GFP+ colonies
after 2 weeks
after 1 week
SKM
SKM+O
KSM
KSM+O
Mock
OSKM
OKSM
SKM
SKM+O
KSM
KSM+O
SK-
SK+M
KS-
KS+M
SK+M+O
SK+M
tetO
EF1α
pMX
D
Phase-contrast
Oct4-GFP
Episomal KSM after 2 weeks
Intergration-free KSM-iPSC#2, p5
E
KSM+O iPSC#1
KSM+O iPSC#2
KSM+O iPSC#3
KSM iPSC#1
KSM iPSC#2
KSM iPSC#3
Positive control
Negative control
H2O
EBNA-
Actin-
F
Hoechst
SSEA-1
Hoechst
Nanog
G
En
Ec
En
Ec
M
M
H
Thy1- 23.0
Thy1+ 48.0
SSC-A
APC-A
I
after 2 weeks
after 1 week
GFP+ colonies
OSKM
SKM
OSKM
SKM
Thy+
Thy-
J
d14 SKM
Thy1+
Thy1-
K
Adult lung fibroblasts
after 2 weeks
after 1 week
GFP+ colonies
KS-
KS+O
KS-
KS+O
Control
+cMyc
+SV40 TAg
L
Adult tail tip fibroblasts
after 2 weeks
after 1 week
GFP+ colonies
KS-
KS+O
KS-
KS+O
Control
+cMyc
+SV40 TAg
M
Astrocytes
AP+ colonies a-er 2 weeks
OSKM
SKM
Control
N
Astrocyte-SKM iPSCs
#1
#2
#3
#4
#5
#6
#7
#8
#9
SKM iPSC
OSKM iPSC
Control
-CMV-Sox2
-CMV-Oct4
-Actin
O
Hoechst
Oct4
Nanog
SSEA-1
P
Ec
En
En
M

Figure S1. Related to Figure 1

Sox2, Klf4, and cMyc can reprogram to pluripotency without exogenous POU factor expression

(A) Nanog and SSEA1 immunofluorescence staining of SKM iPSC line at p8. Nuclei were stained with Hoechst 33342.

(B) Alkaline phosphatase (AP) staining of iPSC colonies generated from GOF18-Rosa26-rtTA MEFs by time-restricted expression of SKM or OSKM. The reprogramming experiment started with 10^3 transduced MEFs plated in each well. The colonies were imaged 10 days after induction.

(C) Reprogramming of Oct4-GFP MEFs by SKM expressed under either tet-inducible or constitutive EF1alpha promoter.

(D) Phase-contrast and Oct4-GFP images of primary colonies and established clonal lines of integration-free KSM iPSCs generated with episomal vector (scale=250 μm).

(E) PCR genotyping confirming the absence of genomic integration or episomal expression of pCXLE reprogramming vector in iPSC lines at p5.

(F) Nanog and SSEA1 immunofluorescence staining of integration-free KSM iPSC line, p8. Nuclei were stained with Hoechst 33342.

(G) Hematoxylin and eosin staining of teratoma sections with representation of all three germ layers (ectoderm: keratinizing epithelium; mesoderm: smooth muscles, connective tissue; endoderm: cuboidal and gut epithelium).

(H) FACS to sorting of Thy- and Thy+ MEF sub-populations.

(I) Reprogramming of of Thy- and Thy+ Oct4-GFP MEFs (C) by OSKM or SKM. The colonies were counted 1 and 2 weeks after infection.

(J) Representative phase-contrast and Oct4-GFP images of primary colonies from (D) (scale=250 μm).

(K), (L) Reprogramming of Oct4-GFP adult lung fibroblasts (K) and adult tail tip fibroblasts (L) by KS in combination with cMyc, Oct4, or SV40 large T Antigen (SV40LT).

(M) Reprogramming of mouse cortical astrocytes with OSKM vs SKM. The cells were fixed and stained for AP activity after 2 weeks of infection.

(N) PCR genotyping analysis of 9 clonal astrocyte-derived iPSCs confirming the integration of SKM transgene and the absence of Oct4.

(O) Oct4, Nanog, and SSEA1 immunofluorescence staining of astrocyte-derived iPSC line #4, p4. Nuclei were stained with Hoechst 33342 (blue).

(P) Hematoxylin and eosin staining of teratoma sections generated with astrocyte-derived SKM-iPSCs with representation of all three germ layers (ectoderm: keratinizing epithelium; mesoderm: smooth muscles; endoderm: cuboidal and gut epithelium).

Figure S2

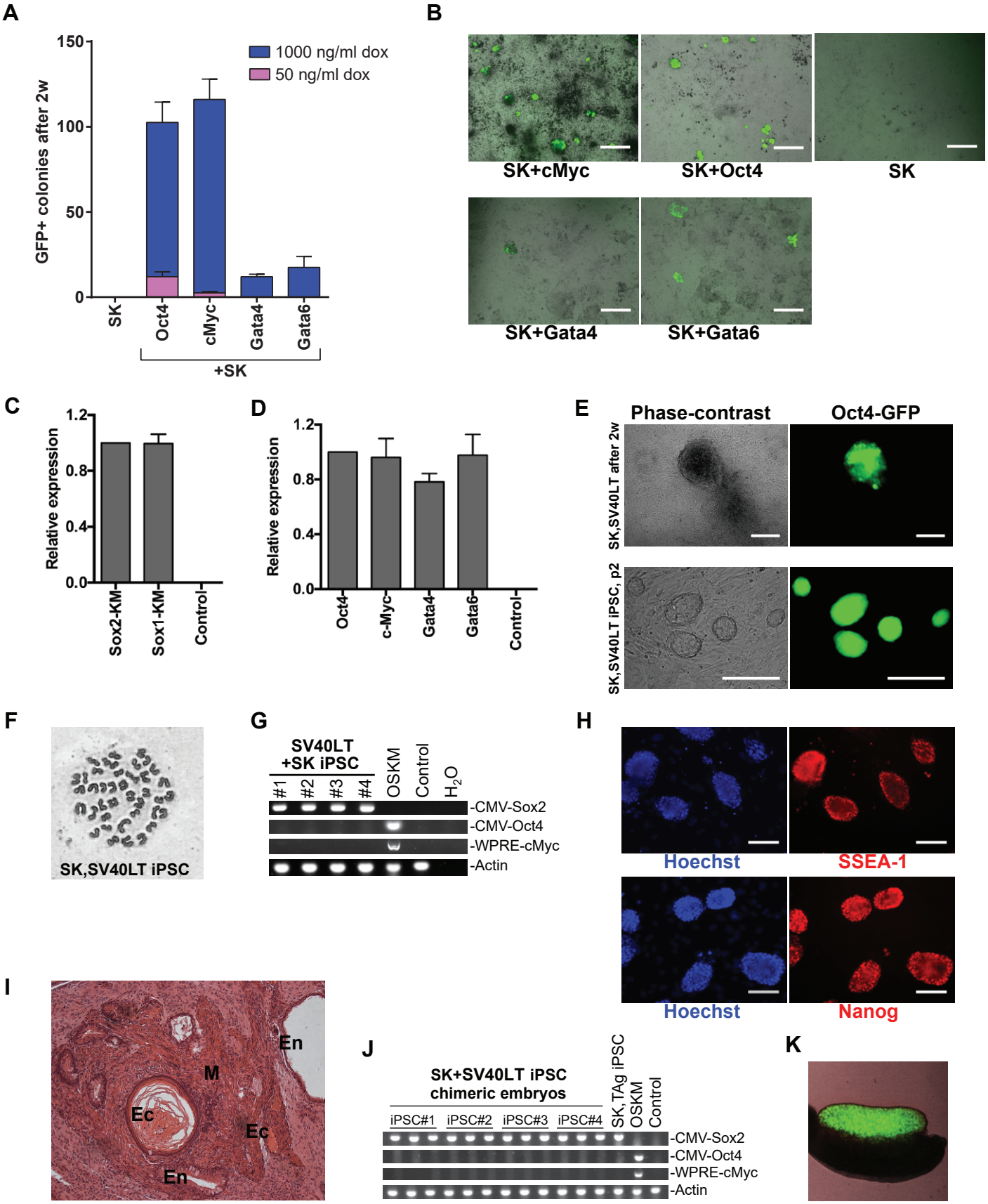

A
GFP+ colonies after 2w
150
100
50
0
1000 ng/ml dox
50 ng/ml dox
SK
Oct4
cMyc
Gata4
Gata6
+SK
B
SK+cMyc
SK+Oct4
SK
SK+Gata4
SK+Gata6
C
Relative expression
1.2
0.8
0.4
0.0
Sox2-KM
Sox1-KM
Control
D
Relative expression
1.2
0.8
0.4
0.0
Oct4
c-Myc
Gata4
Gata6
Control
E
Phase-contrast
Oct4-GFP
SK,SV40LT after 2w
SK,SV40LT iPSC, p2
F
SK,SV40LT iPSC
G
SV40LT
+SK iPSC
#1
#2
#3
#4
OSKM
Control
H2O
-CMV-Sox2
-CMV-Oct4
-WPRE-cMyc
-Actin
H
Hoechst
SSEA-1
Hoechst
Nanog
I
En
M
Ec
Ec
En
J
SK+SV40LT iPSC
chimeric embryos
iPSC#1
iPSC#2
iPSC#3
iPSC#4
SK,TAg iPSC
OSKM
Control
-CMV-Sox2
-CMV-Oct4
-WPRE-cMyc
-Actin
K

Figure S2. Related to Figure 2

Sox2 and Klf4 are sufficient for reprograming of highly-proliferative cells to iPSCs

(A) Reprogramming of Oct4-GFP MEFs by bicistronic SK in combination with Oct4, cMyc, Gata4, or Gata6. Different level of transgene expression was achieved by induction with either 1 µg/ml, 50 ng/ml of dox for 12 days. GFP^+ colonies were counted after 14 dpi.

(B) Brightfield and Oct4-GFP–merged representative overview images of reprogrammed colonies from Figure S2A (scale=1 mm).

(C), (D) qPCR titration of polycistronic lentiviral vectors using universal lentivirus 3'LTR (WPRE region) primers. *Rpl37a* and *Hprt1* were used as reference genes, the error bars represent SD between the two.

(E) Phase-contrast and Oct4-GFP images of primary colonies and passaged SK iPSCs generated from immortalized Oct4-GFP MEF (scale=250 µm).

(F) Giemsa-stained metaphase spreads of clonal SV40LT, SK iPSC#2 line.

(G) PCR genotyping verifying tet-miniCMV-SK transgene in four SV40LT, SK-generated iPSC lines while confirming the absence of Oct4 or cMyc integration.

(H) Nanog and SSEA1 immunofluorescence staining of SV40LT, SK iPSC#2 line, p7. Nuclei were stained with Hoechst 33342.

(I) Hematoxylin and eosin staining of teratoma sections with representation of all three germ layers (ectoderm: keratinizing epithelium, thyroid; mesoderm: smooth muscles; endoderm: cuboidal and gut epithelium).

(J) PCR genotyping verifying tetO-SK transgene in all the tested chimeric embryos derived from 4 lines of SV40LT, SK iPSC and absence of Oct4 or cMyc integration.

(K) Brightfield and GFP merged images of embryonic day a 13.5 gonad dissected from SV40LT, SK iPSC#4 chimeric embryo.

Figure S3

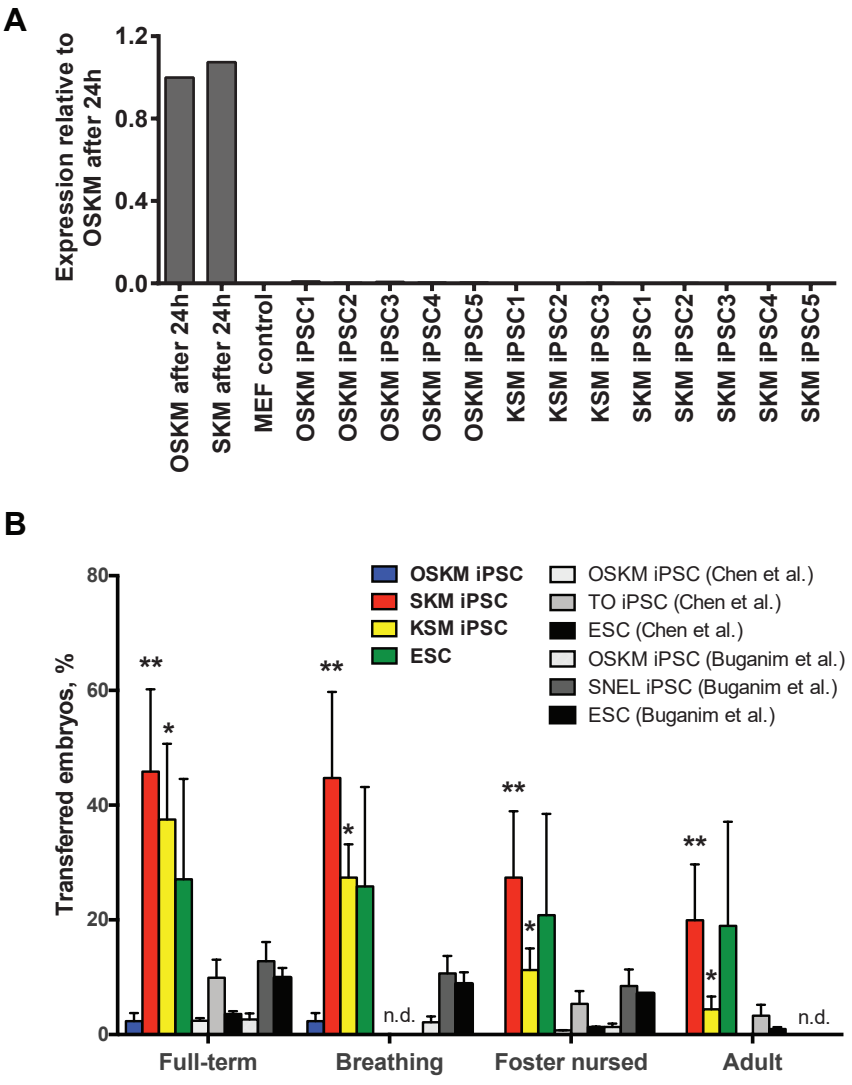


Figure S3. Related to Figure 3

SKM iPSCs hold dramatically enhanced developmental potential

(A) qPCR analysis of lentiviral transgene expression of OSKM and SKM transgenes after 24h of induction in MEFs and in the clonal iPSC lines. *Rpl37a* was used as a housekeeping gene.

(B) Percentage of 4N-aggregated embryos that gave rise to full-term pups, pups that initiated breathing, pups that survived foster-nursing (at least 48 hours), and pups that survived until adulthood (at least 3 months). The grey and black bars depict previously published data for comparison. Data are represented as the mean of all tested lines. The statistical significance was determined by Mann-Whitney test. Error bars represent SEM.

Figure S4

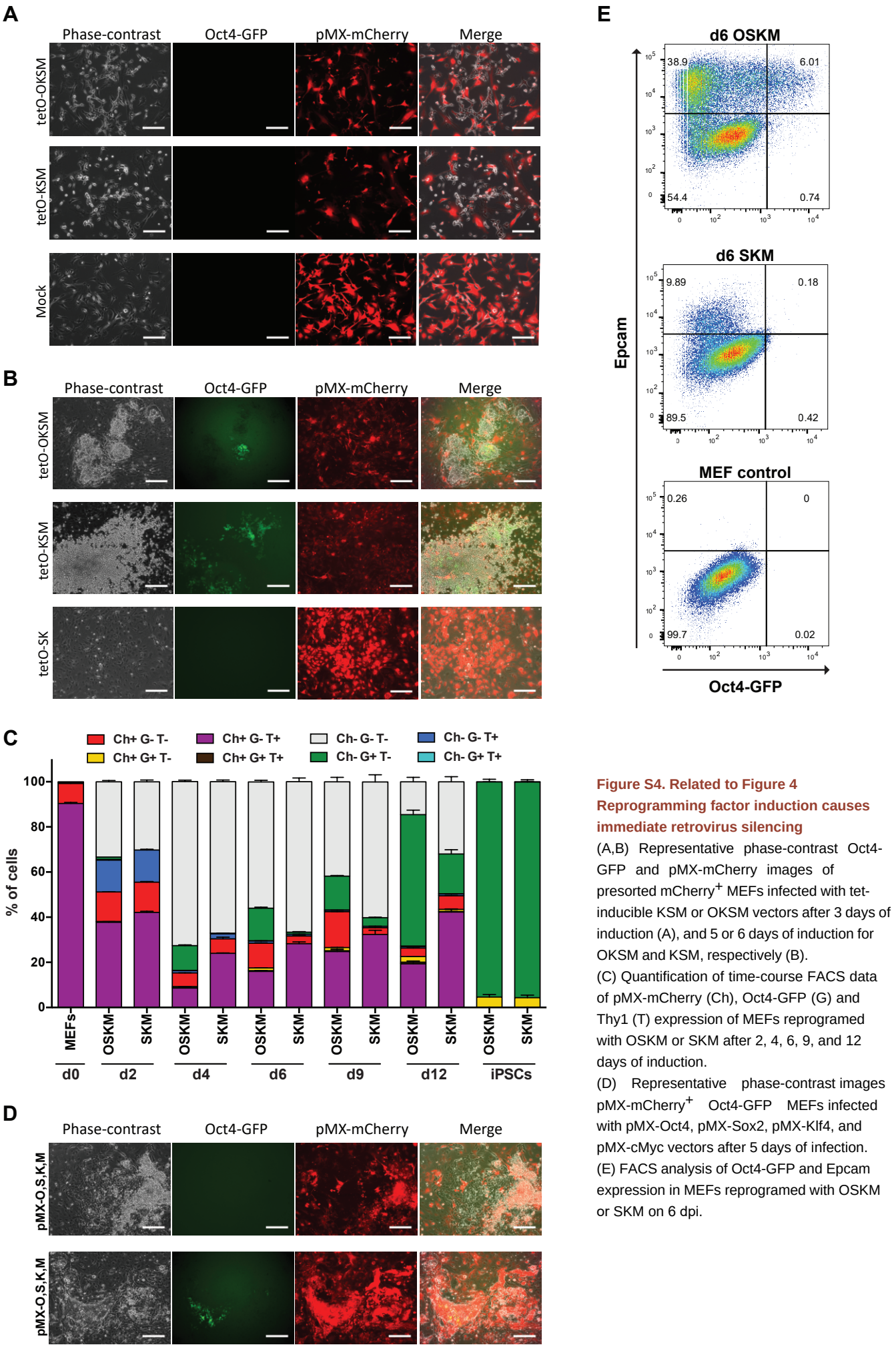


Figure S4. Related to Figure 4
Reprogramming factor induction causes immediate retrovirus silencing

(A,B) Representative phase-contrast Oct4-GFP and pMX-mCherry images of presorted mCherry^{+} MEFs infected with tet-inducible KSM or OKSM vectors after 3 days of induction (A), and 5 or 6 days of induction for OKSM and KSM, respectively (B).

(C) Quantification of time-course FACS data of pMX-mCherry (Ch), Oct4-GFP (G) and Thy1 (T) expression of MEFs reprogramed with OSKM or SKM after 2, 4, 6, 9, and 12 days of induction.

(D) Representative phase-contrast images pMX-mCherry^{+} Oct4-GFP MEFs infected with pMX-Oct4, pMX-Sox2, pMX-Klf4, and pMX-cMyc vectors after 5 days of infection.

(E) FACS analysis of Oct4-GFP and Epcam expression in MEFs reprogramed with OSKM or SKM on 6 dpi.

Figure S5

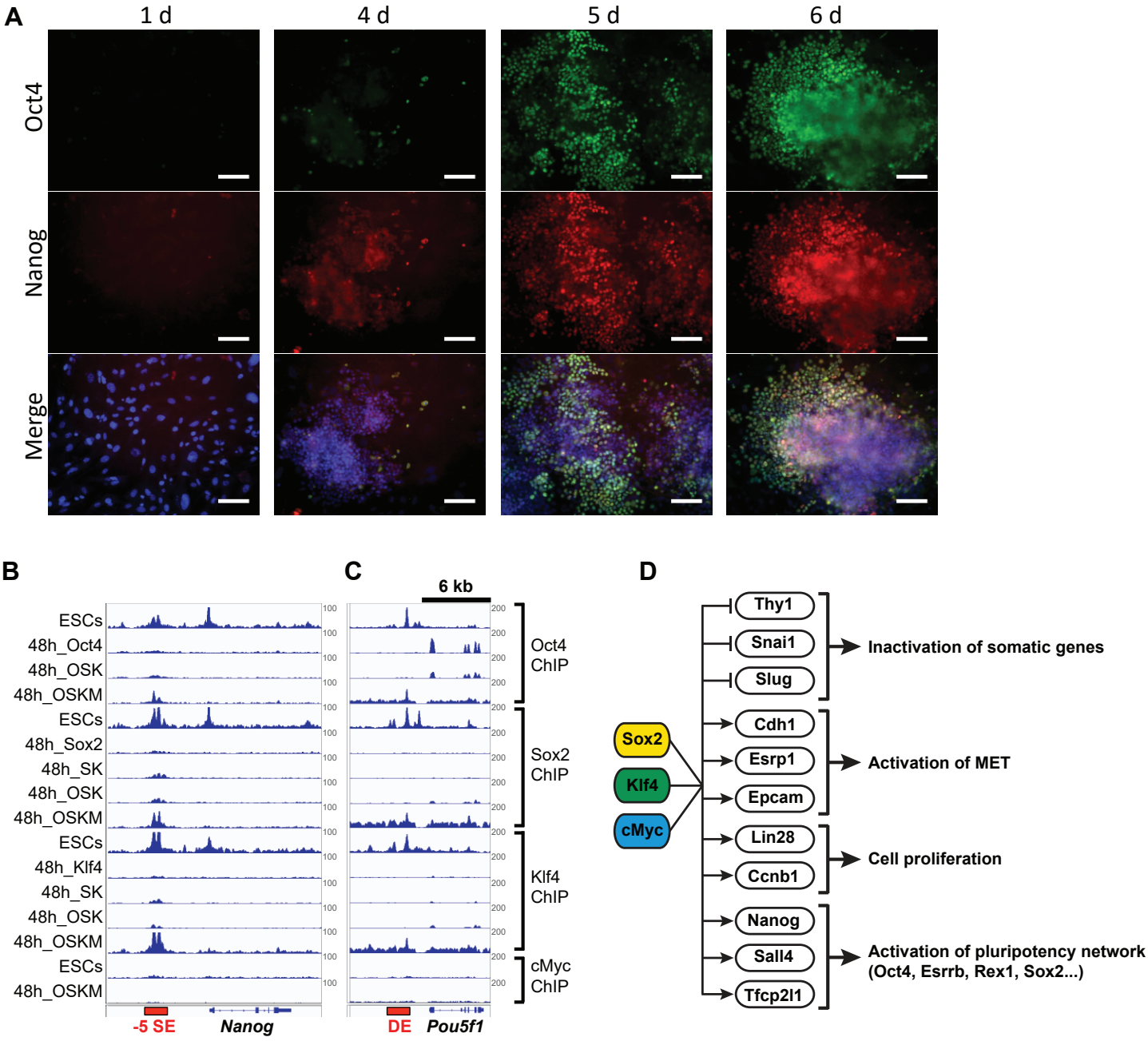


Figure S5. Related to Figure 5

Nanog is a direct target of Sox2 and Klf4

(A) Time-course immunostaining for Oct4 and Nanog of GOF18-Rosa26-rtTA MEFs induced with SKM. Nuclei were stained with Hoechst 33342.

(B,C) Genome plots of Oct4, Sox2, Klf4, and cMyc binding at *Nanog* (B) and *Pou5f1* (C) loci.

(D) Model summarizing time-course RNA-seq data showing the molecular roadmap of SKM reprogramming.

Figure S6

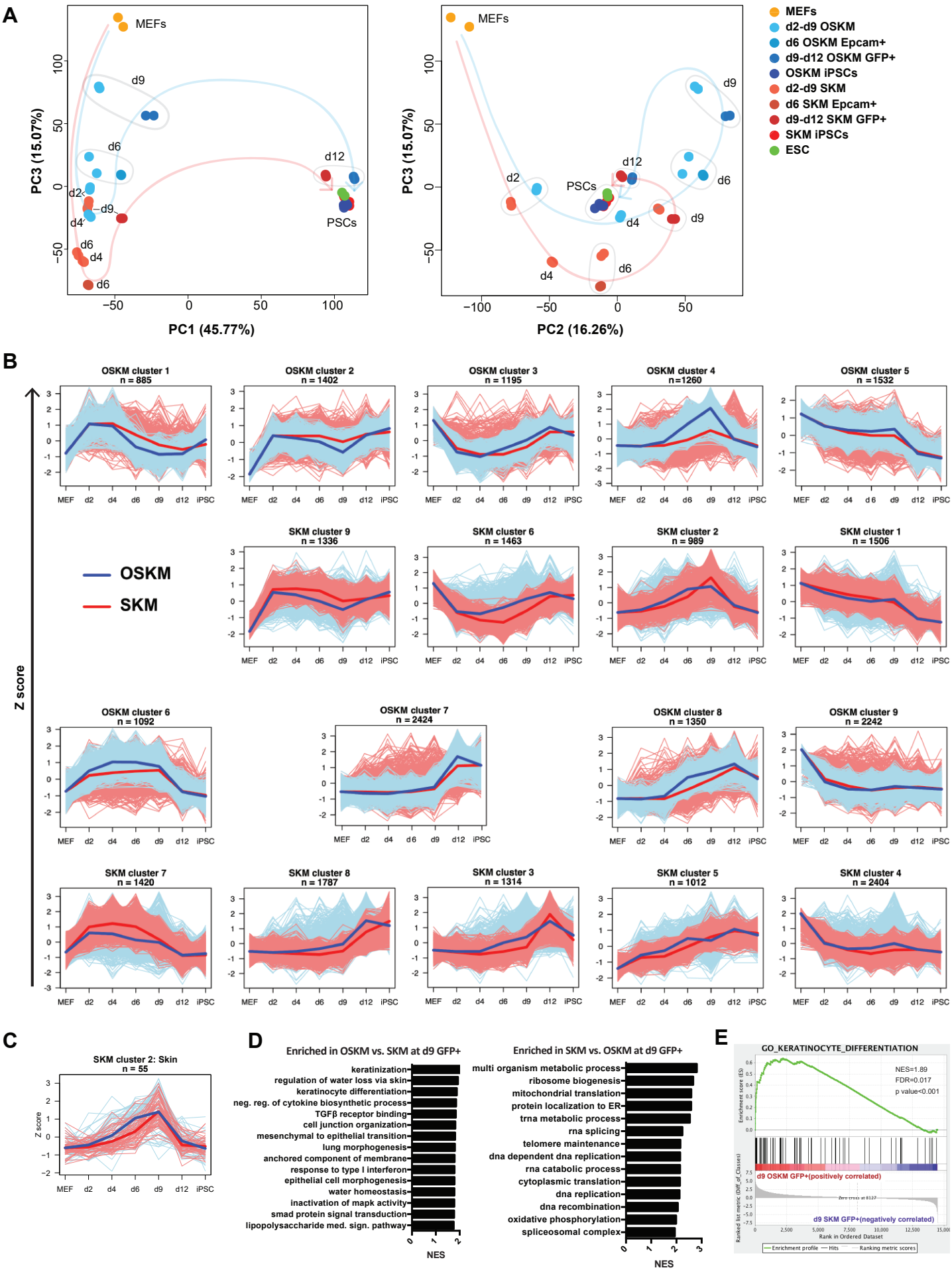

A
MEFs
d2-d9 OSKM
d6 OSKM Epcam+
d9-d12 OSKM GFP+
OSKM iPSCs
d2-d9 SKM
d6 SKM Epcam+
d9-d12 SKM GFP+
SKM iPSCs
ESC
PC1 (45.77%)
PC3 (15.07%)
PC2 (16.26%)
PSCs
B
OSKM
SKM
Z score
OSKM cluster 1 n = 885
OSKM cluster 2 n = 1402
OSKM cluster 3 n = 1195
OSKM cluster 4 n=1260
OSKM cluster 5 n = 1532
SKM cluster 9 n = 1336
SKM cluster 6 n = 1463
SKM cluster 2 n = 989
SKM cluster 1 n = 1506
OSKM cluster 6 n = 1092
OSKM cluster 7 n = 2424
OSKM cluster 8 n = 1350
OSKM cluster 9 n = 2242
SKM cluster 7 n = 1420
SKM cluster 8 n = 1787
SKM cluster 3 n = 1314
SKM cluster 5 n = 1012
SKM cluster 4 n = 2404
MEF d2 d4 d6 d9 d12 iPSC
C
SKM cluster 2: Skin n = 55
D
Enriched in OSKM vs. SKM at d9 GFP+
keratinization
regulation of water loss via skin
keratinocyte differentiation
neg. reg. of cytokine biosynthetic process
TGFβ receptor binding
cell junction organization
mesenchymal to epithelial transition
lung morphogenesis
anchored component of membrane
response to type I interferon
epithelial cell morphogenesis
water homeostasis
inactivation of mapk activity
smad protein signal transduction
lipopolysaccharide med. sign. pathway
NES
Enriched in SKM vs. OSKM at d9 GFP+
multi organism metabolic process
ribosome biogenesis
mitochondrial translation
protein localization to ER
trna metabolic process
rna splicing
telomere maintenance
dna dependent dna replication
rna catabolic process
cytoplasmic translation
dna replication
dna recombination
oxidative phosphorylation
spliceosomal complex
NES
E
GO_KERATINOCYTE_DIFFERENTIATION
NES=1.89
FDR=0.017
p value<0.001
d9 OSKM GFP+(positively correlated)
d9 SKM GFP+(negatively correlated)
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores

Figure S6. Related to Figure 6

Comparison of global gene expression changes of during OSKM and SKM reprogramming

(A) Principle component analysis (PCA) of global gene expression in time-course samples during OSKM and SKM reprogramming. PC1 vs. PC2 plot is shown in Figure 6A.

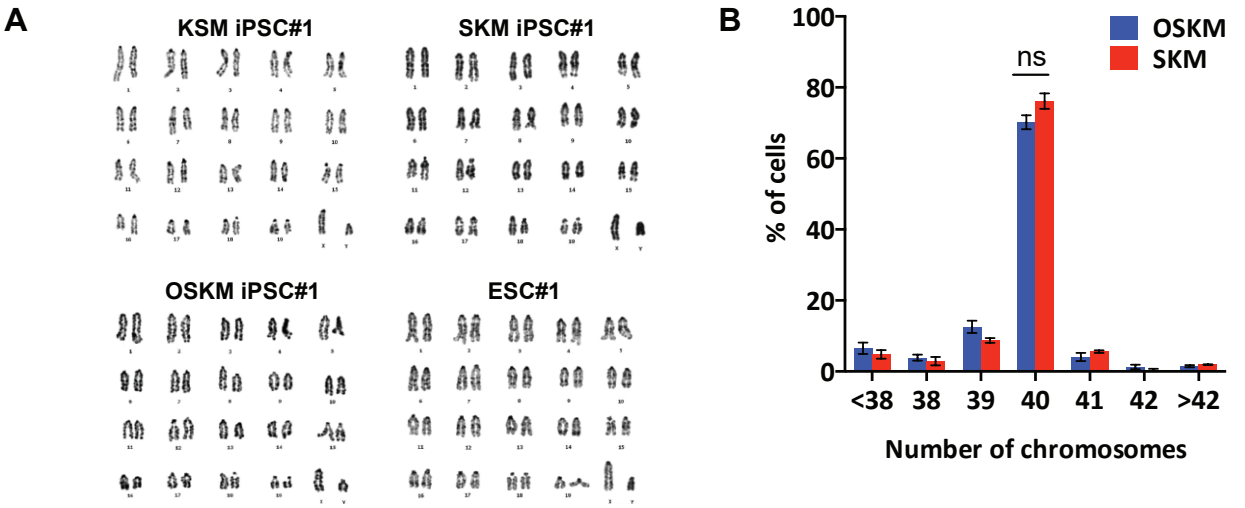
(B) Time-course gene expression of 9 OSKM and 9 SKM gene clusters exhibiting similar kinetics (downregulation, upregulation, transient upregulation, etc.), determined by mFuzz package. Z-scores of all the genes within each cluster were plotted and compared between OSKM and SKM samples. The plots of SKM clusters were placed below OSKM clusters with similar behavior for convenience. Only the genes with TPM at least 1 and FC>2 across the samples were considered.

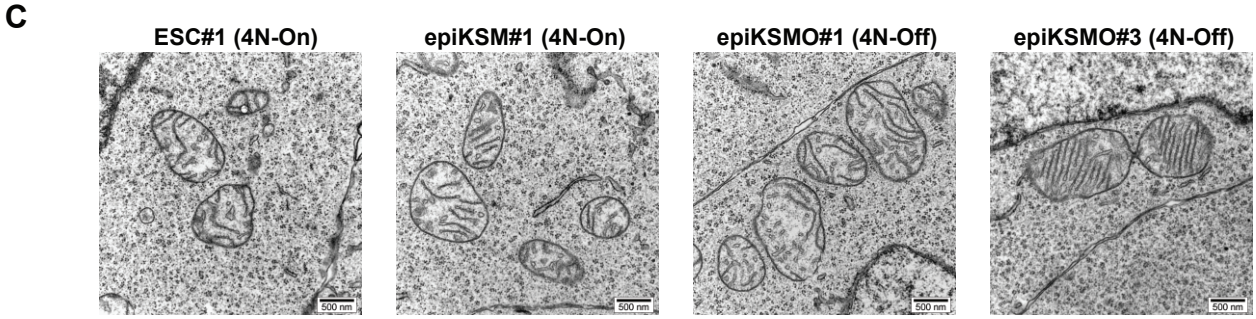
(C) Time-course plots of 'Skin' GO term genes from SKM mFuzz cluster 2 (for OSKM mFuzz cluster 4 see Figure 6D).

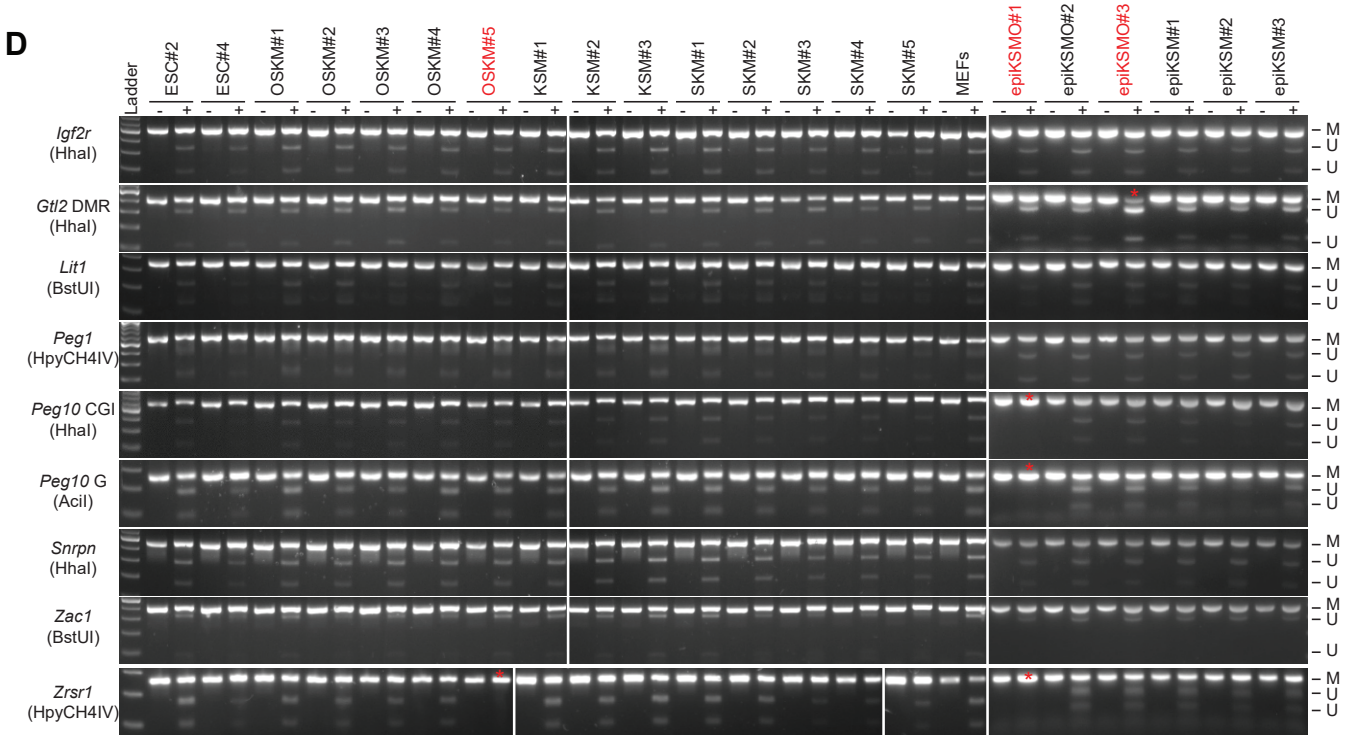
(D) Gene set enrichment analysis of d9 GFP^+ OSKM vs. SKM samples performed by GSEA3.0 package. The data are represented as normalized enrichment score (NES). Only nonredundant categories are shown ($p < 0.05$ and FDR < 0.1).

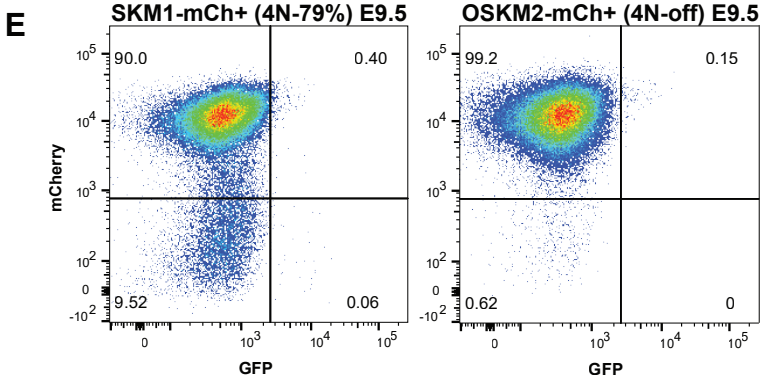
(E) A representative gene set enrichment plot of d9 GFP^+ OSKM vs. SKM samples generated by GSEA3.0 for gene ontology term 'Keratinocyte differentiation'.

Figure S7









F

Top GO cell. comp. term for epiKSMO1 vs 4N-on down (FDR<0.05, Enrichment>2):

- collagen-containing extracellular matrix
- extracellular matrix
- anchored component of presynaptic membrane
- synapse

Top GO bio term for epiKSMO1 vs 4N-on up (FDR<0.01, Enrichment>4):

- secondary alcohol biosynthetic process
- cholesterol biosynthetic process
- lipid biosynthetic process

Figure S7. Related to Figure 7

SKM iPSCs are characterized by more ESC-line gene expression

(A) Representative images of Giemsa-stained metaphase spreads of established OSKM, SKM, and KSM iPSC and ESC lines.

(B) Karyotyping analysis of bulk OSKM or SKM iPSCs on the second passage. Three bulk lines were generated with each reprogramming vector by sorting the Oct4-GFP^{+} cells 2 weeks after reprogramming. At least 100 chromosomal spreads were analyzed for each line. Error bars represent SD, n=3. Statistical significance was calculated using t-test.

(C) Representative electron microscopy images of mitochondria for 4N-on and 4N-off iPSC and ESC lines.

(D) Combined bisulfite restriction analysis (COBRA) assay of the imprinted genes that could be misregulated during reprogramming. U, non-methylated band; M, methylated band. Red font and red asterisk mark the lines exhibiting LOI.

(E) Representative FACS plots of E9.5 embryos derived from 4N aggregates with 4N-on and 4N-off iPSCs marked with mCherry. The mCherry+ cells were subsequently used for RNA-seq.

(F) Representative and the top gene onthology (GO) term enriched in epiKSMO#1 iPSC 4N-off line vs. 4N-on iPSCs (≥5 reads in any sample, FC>1.2, $p<0.05$ by DESeq2).

Supplementary Table 1. Related to Figure 1
Overview of the studies attempting to replace Oct4 in Yamanaka cocktail

Reprogramming cocktail	Somatic cell type	Vectors used	Reprogramming efficiency compared to Oct4	Negative control	Reference
KSM KSM+Oct1 KSM+Oct6	Mouse embryonic fibroblasts	Moloney murine leukemia virus (MMLV)-based, monocistronic	0%	KSM	(Nakagawa et al., 2008)
KSM + BIX-01294	Mouse fetal neural progenitor cells	Murine stem cell virus (MSCV)-based, monocistronic	~13%	KSM	(Shi et al., 2008)
KSM + Nr5a1 KSM + Nr5a2 KS + Nr5a2	Mouse embryonic fibroblasts	MMLV-based, monocistronic	n.d., >0%	KS KSM KSM + Nanog/Sall4 / Esrrb/Klf2/ Klf5/N-Myc/ Stat3/Zfx	(Heng et al., 2010)
KSM + E-cadherin	Mouse embryonic fibroblasts	MMLV-based, monocistronic	~2%	KSM	(Redmer et al., 2011)
KSM + Sall4 + Nanog	Mouse embryonic fibroblasts	Lentiviral TetO, monocistronic	n.d., >0%	n.d.	(Buganim et al., 2012)
KSM + human Oct4 KSM + Xenopus Oct91 KSM + medaka Pou2 KSM + axolotl Oct4 KSM + axolotl Pou2 KSM + zebrafish Pou2	Mouse embryonic fibroblasts, human skin fibroblast	MMLV-based, monocistronic	~100% for human Oct4 ~70% for Xenopus Oct91 ~10% for medaka Pou2 ~10% for axolotl Pou2 ~2% for axolotl Oct4 0% for zebrafish Pou2	KSM	(Tapia et al., 2012)
KSM + Gata3 KSM + Gata4 KSM + Gata6 KSM + Sox7 KSM + Pax1 KSM + CEBPa KSM + HNF4a KSM + GRB2 KM+ Sox1/Sox3 + Gata3/Gata6/Pax1	Mouse embryonic fibroblasts; for KSM + Gata3 also mouse gastric epithelial cells, mouse adult dermal fibroblast, and mouse keratinocytes	Lentiviral TetO, monocistronic	~140% for Gata6 ~115% for Gata3 ~80% for Sox7 ~50% for Pax1 ~40% for Gata4	KSM	(Shu et al., 2013)
$KS^{VP16}M$ + $Gata3^{VP16}$ $KS^{VP16}M$ + $Gata4^{VP16}$ $KS^{VP16}M$ + $Gata6^{VP16}$	Human foreskin fibroblast	MMLV-based, MSCV-based, monocistronic	~0.1% for $Gata3^{VP16}$ 0% for $Gata4^{VP16}$ 0% for $Gata6^{VP16}$	$KS^{VP16}M$	(Montserrat et al., 2013)
KSM + Tet1 KS + Tet1	Mouse embryonic fibroblasts, mouse trophoblast stem cells	Lentiviral TetO, monocistronic	~70%	KSM	(Gao et al., 2013b)
KSM + forskolin KSM + 2-Me-5HT KSM + D4476	Mouse embryonic fibroblasts	Lentiviral CMV, monocistronic	~100 and 14% for forskolin	KSM KS	(Hou et al., 2013)

KS + FSK KS + 2-Me-5HT KS + D4476			~30 and 0% for 2-Me-5HT ~30 and 0% for D4476 with and without cMyc, respectively		
KSM + TALE-based transcriptional activator of Oct4	Mouse embryonic fibroblasts	Piggyback-transposed dox-inducible polycistronic KSM + monocistronic Oct4/TALE	~160%	n.d.	(Gao et al., 2013a)
KSM + SB43 +VPA	Mouse embryonic fibroblasts	Lentiviral TetO, monocistronic	~70%	KSM	(Tan et al., 2015)
KSM + Brn4	Mouse embryonic fibroblasts	Lentiviral TetO-STEMCCA-BKSM, polycistronic	n.d., >0%	n.d.	(Bar-Nur et al., 2015)
KSM + Gata1 KSM + Gata2 KSM + Gata3 KSM + Gata4 KSM + Gata5 KSM + Gata6	Mouse embryonic fibroblasts	Lentiviral TetO, monocistronic	~190% for Gata2 ~140% for Gata6 ~130% for Gata1 ~115% for Gata3 ~80% for Gata5 ~40% for Gata4	KSM	(Shu et al., 2015)
KSM + GNAS KSM + forskolin	Mouse embryonic fibroblasts	Lentiviral TetO-STEMCCA-KSM, polycistronic + lentiviral GNAS	~10% for forskolin ~5% for GNAS	KSM*	(Fritz et al., 2015)
KSM + library of artificial TFs	Mouse embryonic fibroblasts	MMLV-based, monocistronic KSM + monocistronic lentiviral artificial TFs	n.d., >0%	KSM	(Eguchi et al., 2016)
KSM + Oct6 mutant	Mouse embryonic fibroblasts	MMLV-based, monocistronic	~3%	KSM + Oct6	(Jerabek et al., 2016)

* a few $Oct4^{+}$ colonies were generated, but not characterized

Supplementary Table 2. Related to Figure 3

Results of tetraploid complementation experiments

Lines tested	Karyotype	Aggregates transferred	Full-Term pups	Breathing	Survived after 48h	Survived after 3 months
OSKM#1	40, XY	46	1	1	0	0
OSKM#2	40, XY	42	0	0	0	0
OSKM#3	40, XY	55	1	1	0	0
OSKM#4	40, XY	52	4	4	0	0
OSKM#5	41, XY, +14	63	0	0	0	0
ESC line#1	40, XY	30	22	22	22	22
ESC line#2	40, XY	40	14	12	4	1
ESC line#3	40, X0 +19	30	0	0	0	0
ESC line#4	39, X0	30	0	0	0	0
KSM#1	40, XY	43	19	15	3	3
KSM#2	40, XY	32	18	10	6	2
KSM#3	41, XY +1	25	3	4	2	0
SKM#1	40, XY	33	26	26	23	19
SKM#2	40, XY	22	13	13	6	3
SKM#3	39, X0	22	15	15	6	4
SKM#4	40, XY	43	4	4	3	2
SKM#5	39, X0	36	5	3	2	2
epi-KSM#1	40, XY	42	19	13	12	9
epi-KSM#2	40, XY	50	15	12	4	3
epi-KSM#3	40, XY	43	24	17	11	10
epi-KSM+O#1	40, XY	31	0	0	0	0
epi-KSM+O#2	40, XY	30	19	16	8	5
epi-KSM+O#3	40, XY	29	0	0	0	0

Supplementary Table 3. Related to Key Resource Table
Primers used in the study

Primers for PCR genotyping

Gene Name	Sequence	Annealing temperature
Viral Oct4	5'-GAGACGCCATCCACGCTGT-3'	60°C
	5'-GGTGAGAAGGCGAAGTCTGAAG-3'	
Viral Brn4	5'-GAGACGCCATCCACGCTGT-3'	60°C
	5'-ATGGACAAGGGAGCTGGAAC-3'	
Viral SKM	5'-GAGACGCCATCCACGCTGT-3'	60°C
	5'-GGCTTCAGCTCCGTCTCCAT-3'	
Viral KSM	5'-GAGACGCCATCCACGCTGT-3'	60°C
	5'-GTGGAGAAGGACGGGAGCAG-3'	
Viral cMyc	5'-ACAGCTTCGAAACTCTGGTGCAT-3'	60°C
	5'-AGGAAGGTCCGCTGGATTGA-3'	
ActB	5'-ACTGCCGCATCCTCTTCCTC-3'	60°C
	5'-AGGAAGGTCCGCTGGATTGA-3'	

Primers bisulfate sequencing and COBRA

Gene Name	Sequence	Annealing temperature
Col1a1	5'-TGGTATAAAAGGGGTTTAGGTTAGT-3'	60°C
	5'-ACAATAACCCCTAAAAAAACAAAAA-3'	
Nanog	5'-TTTGTAGGTGGGATTAATTGTGAA-3'	60°C
	5'-AAAAAAACAAAACACCAACCAAAT-3'	
Pou5f1 (Oct4)	5'-GGGTTAGAGGTTAAGGTTAGAGGG-3'	60°C
	5'-CCCCCACCTAATAAAAATAAAAAAA-3'	
Viral KSM	5'-GAGACGCCATCCACGCTGT-3'	60°C
	5'-GTGGAGAAGGACGGGAGCAG-3'	
Viral cMyc	5'-ACAGCTTCGAAACTCTGGTGCAT-3'	60°C
	5'-AGGAAGGTCCGCTGGATTGA-3'	
ActB	5'-ACTGCCGCATCCTCTTCCTC-3'	60°C
	5'-AGGAAGGTCCGCTGGATTGA-3'	

Q-PCR primers

Gene Name	Sequence	Annealing temperature
WPRE	5’-TGTTGCCACCTGGATTCTGC-3’	60°C
	5’-AGGAAGGTCCGCTGGATTGA-3’	
Viral cMyc	5’-ACAGCTTCGAAACTCTGGTGCAT-3’	60°C
	5’-AGGAAGGTCCGCTGGATTGA-3’	
Rlp37a	5’- ACTTGCTCCTTCTGTGGCAAGAC -3’	60°C
	5’- TTCATGCAGGAACCACAGTGC -3’	
Hprt1	5’-CTGGTGAAAAGGACCTCTCGA-3’	60°C
	5’-CTGAAGTACTCATTATAGTCAAGGGCAT-3’	
Nr5a2	5’-AAGGGTGAGCTGCAAAGGGGA-3’	60°C
	5’-CCCAGGTTTTGGGGACGCTCG-3’	
Nanog	5’-CTTTCACCTATTAAGGTGCTTGC-3’	60°C
	5’-ATGGCATCGGTTCATCATGGTAC-3’	
Sall4	5’-TGGCAGACGAGAAGTTCTTTC-3’	60°C
	5’-TCCAACATTTATCCGAGCACAG-3’	
Thy1	5’-TTACCCTAGCCAACTTCACCACCA-3’	60°C
	5’-AAATGAAGTCCAGGGCTTGGAGGA-3’	
Slug (Snai1)	5’-CACATTCGAACCCACACATTGCCT-3’	60°C
	5’-TGTGCCCTCAGGTTTGATCTGTCT-3’	
Epcam (Cd326)	5’-GCTGGCAACAAGTTGCTCTCTGAA-3’	60°C
	5’-CGTTGCACTGCTTGGCTTTGAAGA-3’	
Cdh1(E-cad)	5’-AACAACTGCATGAAGGCGGGAATC-3’	60°C
	5’-CCTGTGCAGCTGGCTCAAATCAAA-3’	
Oct4-CDS	5’-GGCTAGAGAAGGATGTGGTTCGAG-3’	60°C
	5’-CCTGGGAAAGGTGTCCCTGTAG-3’	
Esrrb	5’-GCCTTTACTATCTGTGCCTGGT-3’	60°C
	5’-TAGTGCTTCTCTTTGGTGCTGT-3’	
Tfcp2l1	5’-AACCCGCCCAGGTAGAGGCT-3’	60°C
	5’-AGGGCAGCCACGTGGGAAGA-3’	
Rex1 (Zfp42)	5’-GGCTGCGAGAAGAGCTTTATTCA-3’	60°C
	5’-AGCATTTCTTCCCGGCCTTT-3’	
Lin28a	5’-CCGCAGTTGTAGCACCTGTCT-3’	60°C
	5’-GAAGAACATGCAGAAGCGAAGA-3’	