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Supplementary information

Permissive epigenomes endow reprogramming competence to transcriptional regulators

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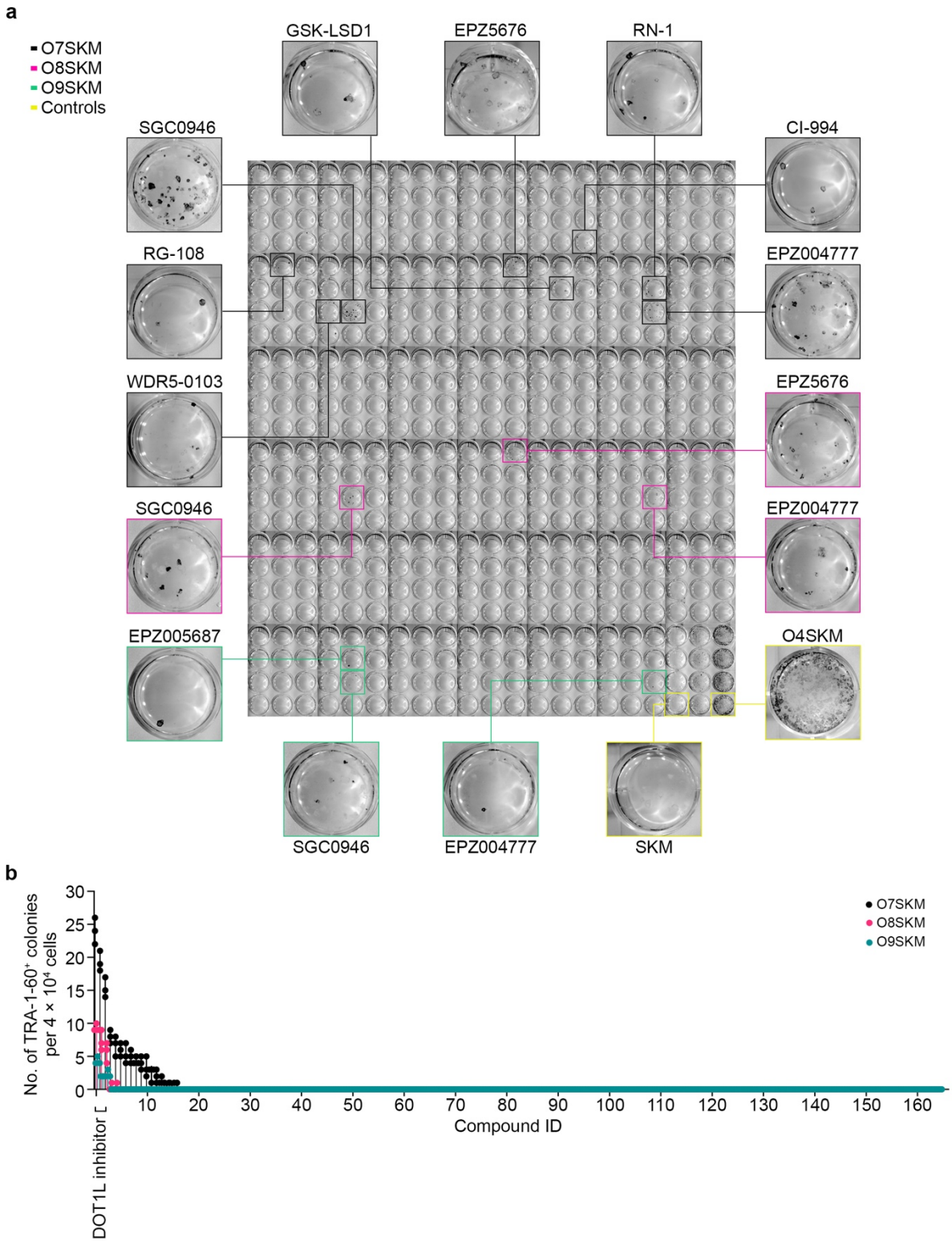
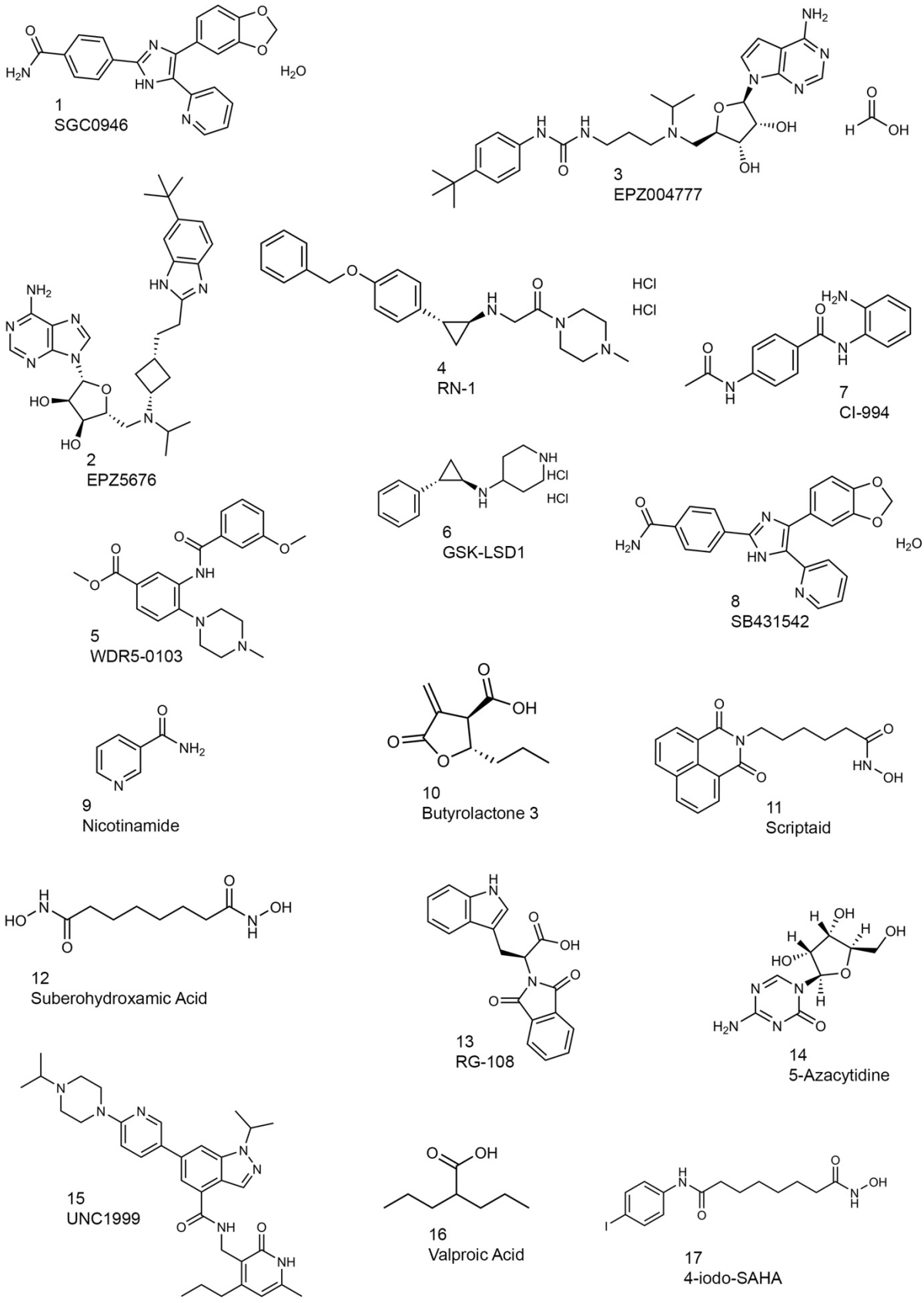
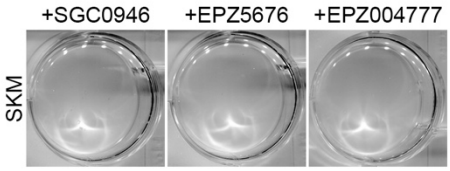


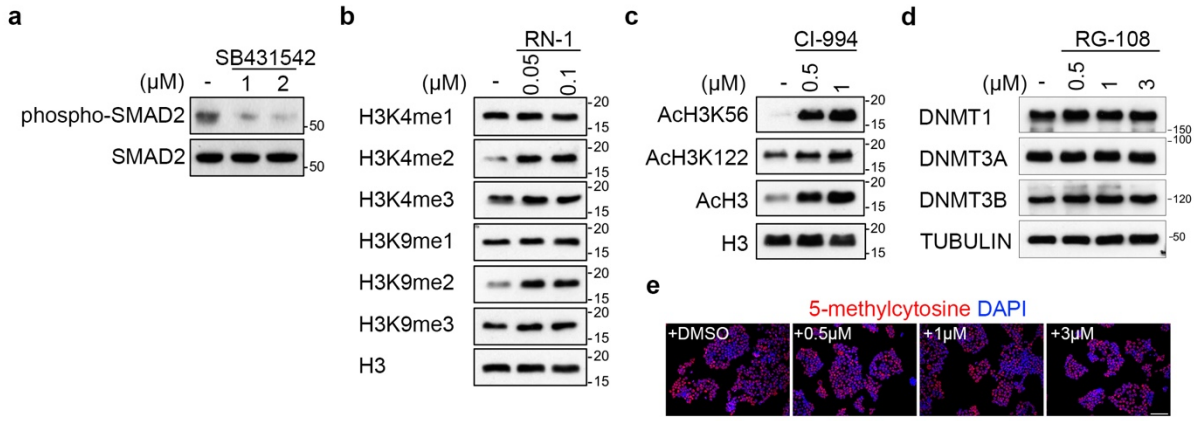
Supplementary Fig. 1. Chemical screening. **a**, A screen of 165 epigenetic compounds (Supplementary Dataset 1). O7SKM-, O8SKM- and O9SKM-transduced cells were cultured with individual compounds, and the effect of the compounds in reprogramming was determined by TRA-1-60 staining. **b**, Quantification of TRA-1-60$^+$ colonies that had emerged from the screening plates depicted in **a**. Compound ID can be found in Supplementary Dataset 4. Data are presented as mean values ± s.d. (**b**, n=3 biologically independent samples).



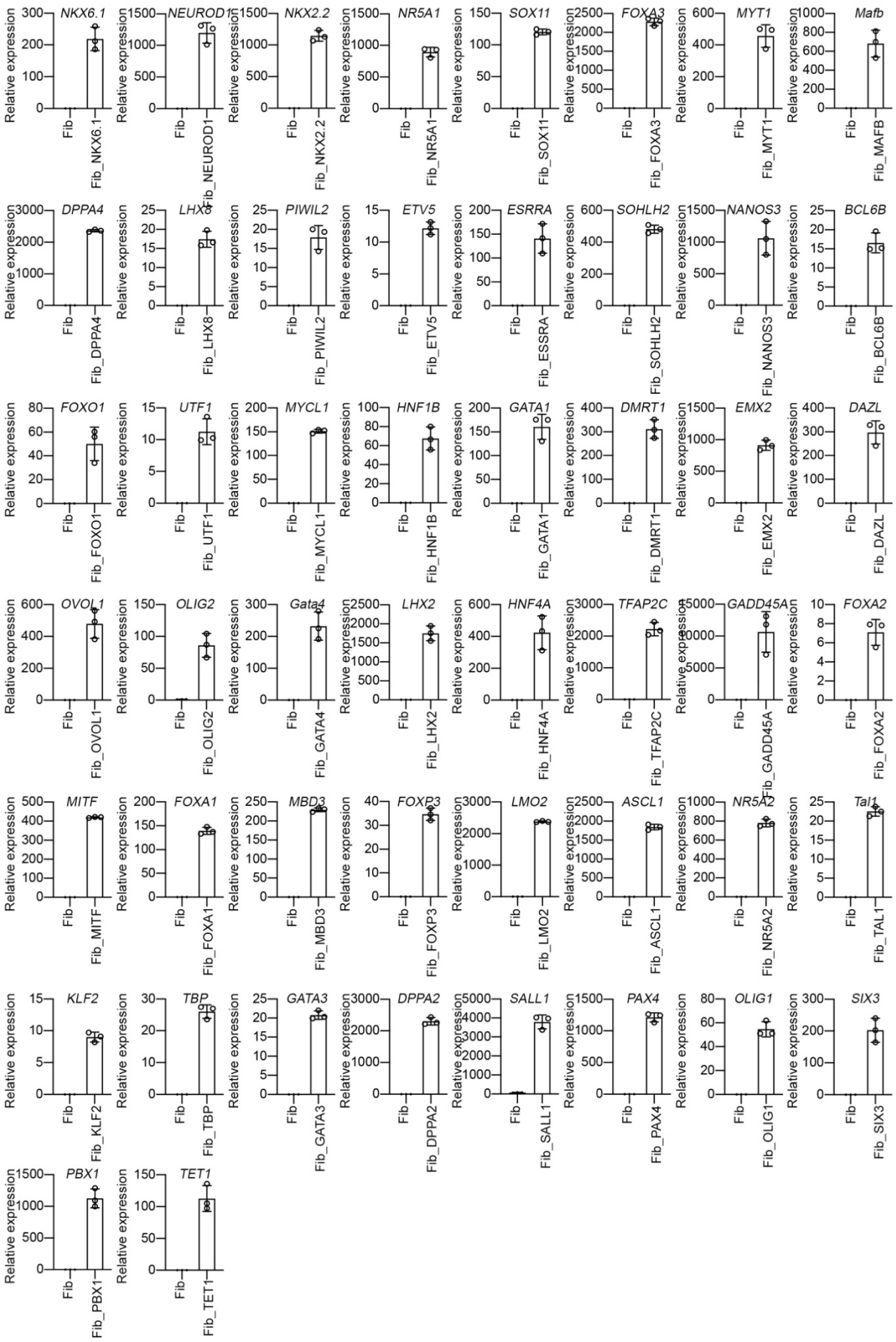
Supplementary Fig. 2. Structures of 17 compounds. Structures of 17 compounds which were identified through the screen.



Supplementary Fig. 3. SKM alone failed to induce pluripotency upon DOT1L inhibition. Fibroblasts transduced with SKM alone and subsequently cultured with DOT1L inhibitors did not yield any iPSC colonies. The experiments were repeated independently three times with similar results and representative images are shown.



Supplementary Fig. 4. Functional consequences of compound treatments were evaluated by western blot and Immunofluorescence. a, SB431542 treatment reduced the level of phospho-SMAD2. **b**, RN-1 treatment increased the levels of H3K4me2 and H3K9me2. **c**, CI-994 treatment led to a significant increase in levels of acetyl-H3K56, acetyl-H3K122 and acetyl-H3. **d**, RG-108 treatment did not alter expression levels of DNMTs. **e**, RG-108 treatment did not alter the level of 5-methylcytocine. DAPI was used as a nuclear counterstain. Scale bar, 100 μm. The experiments were repeated independently three times with similar results and representative images are shown. See Source Data for uncropped blot images.


NKX6.1
NEUROD1
NKX2.2
NR5A1
SOX11
FOXA3
MYT1
Mafb
DPPA4
LHX8
PIWIL2
ETV5
ESRRA
SOHLH2
NANOS3
BCL6B
FOXO1
UTF1
MYCL1
HNF1B
GATA1
DMRT1
EMX2
DAZL
OVOL1
OLIG2
Gata4
LHX2
HNF4A
TFAP2C
GADD45A
FOXA2
MITF
FOXA1
MBD3
FOXP3
LMO2
ASCL1
NR5A2
Tal1
KLF2
TBP
GATA3
DPPA2
SALL1
PAX4
OLIG1
SIX3
PBX1
TET1
Relative expression
Fib
Fib_NKX6.1
Fib_NEUROD1
Fib_NKX2.2
Fib_NR5A1
Fib_SOX11
Fib_FOXA3
Fib_MYT1
Fib_MAFB
Fib_DPPA4
Fib_LHX8
Fib_PIWIL2
Fib_ETV5
Fib_ESSRA
Fib_SOHLH2
Fib_NANOS3
Fib_BCL6B
Fib_FOXO1
Fib_UTF1
Fib_MYCL1
Fib_HNF1B
Fib_GATA1
Fib_DMRT1
Fib_EMX2
Fib_DAZL
Fib_OVOL1
Fib_OLIG2
Fib_GATA4
Fib_LHX2
Fib_HNF4A
Fib_TFAP2C
Fib_GADD45A
Fib_FOXA2
Fib_MITF
Fib_FOXA1
Fib_MBD3
Fib_FOXP3
Fib_LMO2
Fib_ASCL1
Fib_NR5A2
Fib_TAL1
Fib_KLF2
Fib_TBP
Fib_GATA3
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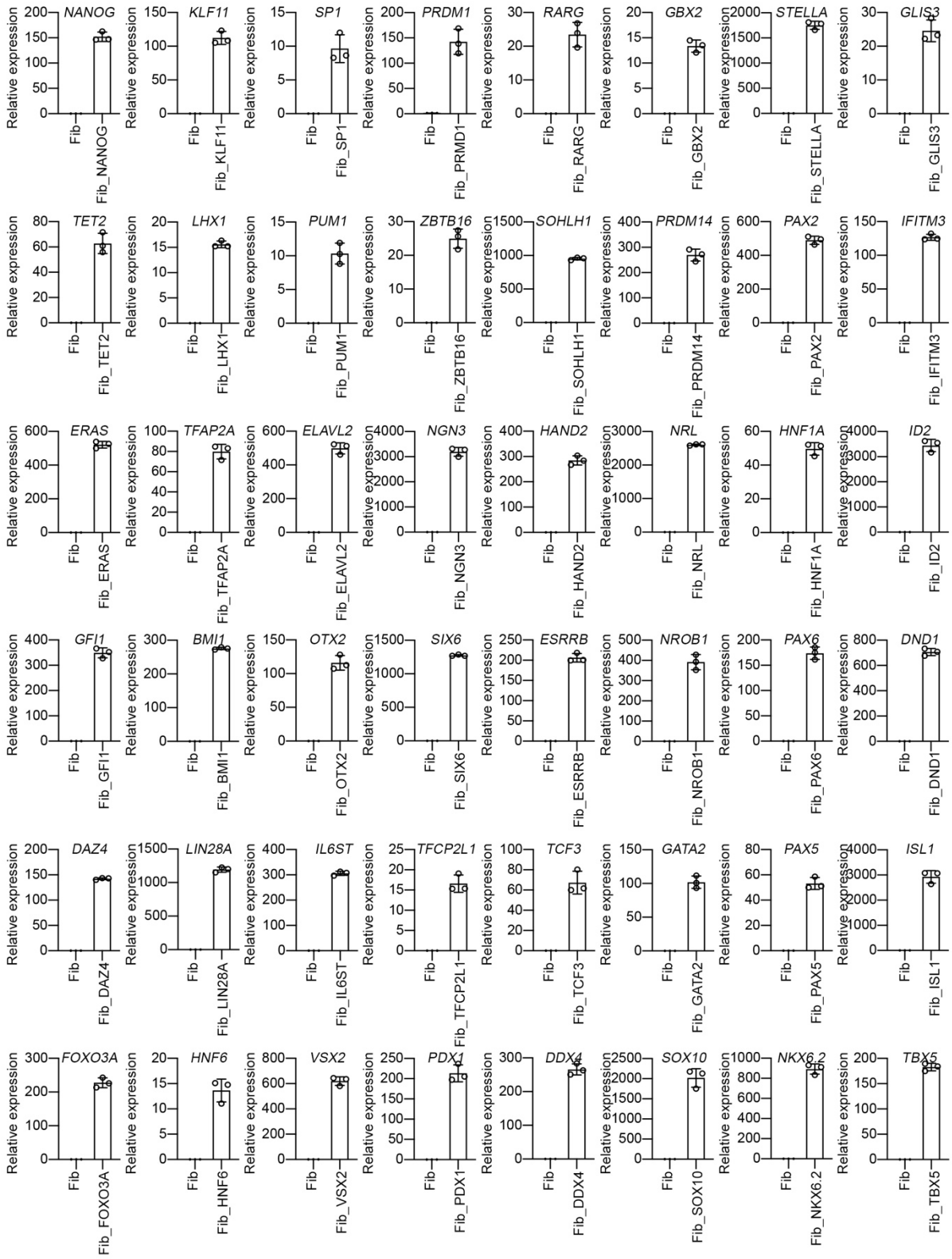
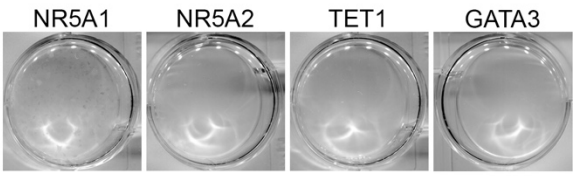
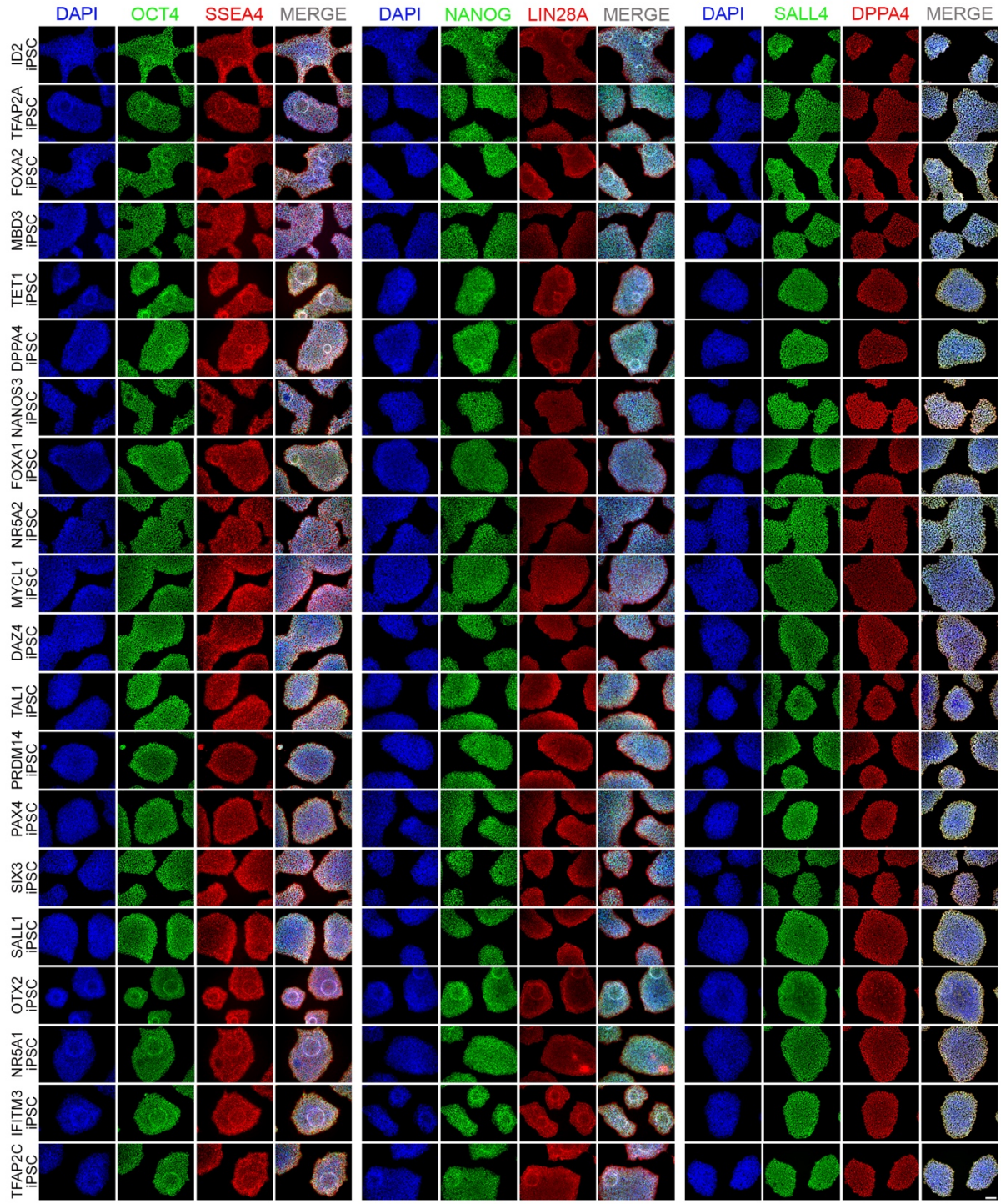
Fib_PBX1
Fib_TET1



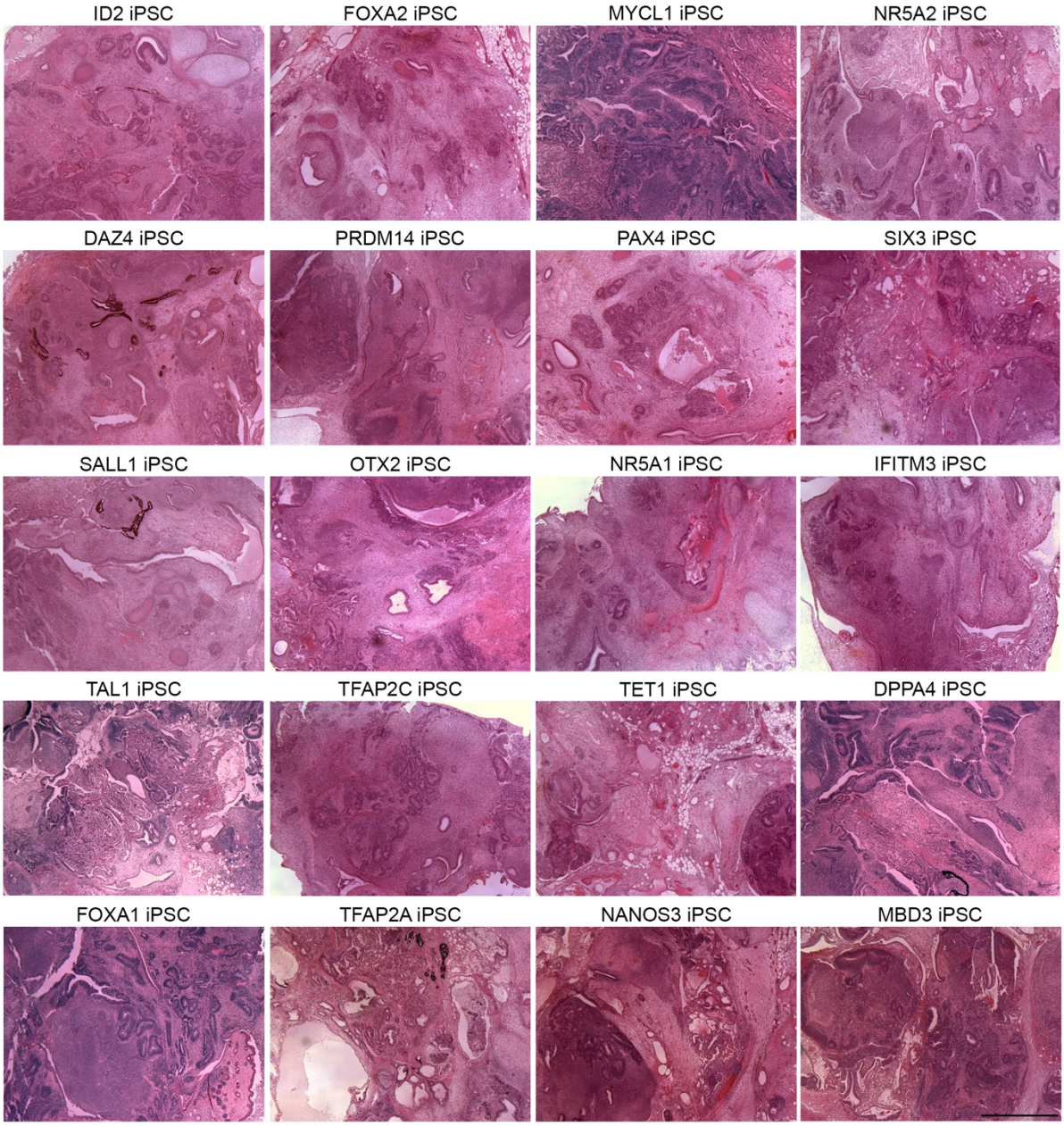
Supplementary Fig. 5. Transgene expression was evaluated by qPCR. Relative expression levels of the indicated transgenes. Expression values of these genes were normalized by expression value of *RPL37A*. Data are presented as mean values ± s.d. (n=3 biologically independent samples).



Supplementary Fig. 6. Reprogramming competence of NR5A1, NR5A2, TET1 and GATA3 in humans. No TRA-1-60^{+} colonies were found in fibroblasts that were transduced with the indicated factors along with SKM. The experiments were repeated independently three times with similar results and representative images are shown.



Supplementary Fig. 7. Immunofluorescent images of human iPSC lines generated with various transcriptional regulators. iPSC lines that were generated by various transcriptional regulators were stained positive for OCT4, SSEA4, NANOG, LIN28A, SALL4 and DPPA4. DAPI was used as a nuclear counterstain. Scale bar, 100 μm. The experiments were repeated independently three times with similar results and representative images are shown.



Supplementary Fig. 8. Teratoma assay of human iPSC lines generated by various transcriptional regulators. iPSC lines that were generated by various transcriptional regulators formed teratomas containing all three germ layers. Scale bar, 1mm. The experiments were repeated independently three times with similar results and representative images are shown.

Supplementary Table 1. Small molecule screening data

Category	Parameter	Description
Assay	Type of assay	*In vitro* reprogramming efficiency assessment of human fibroblasts
	Target	TRA-1-60 antigen
	Primary measurement	Detection of TRA-1-60 positive iPSC colony
	Key reagents	TRA-1-60 antibody–conjugated with HRP, 3,3'-diaminobenzidine (DAB) peroxidase staining kit
	Assay protocol	See Methods section (chemical screening, DAB staining)
	Additional comments	
Library	Library size	165 compounds
	Library composition	Epigenetics screening library
	Source	Cayman (item no. 11076)
	Additional comments	
Screen	Format	12-well plate
	Concentration(s) tested	3 μM
	Plate controls	DMSO treated well
	Reagent/ compound dispensing system	Handled manually
	Detection instrument and software	Epson perfection V370 photo scanner
	Assay validation/QC	Secondary assay with the compounds from different vendors
	Correction factors	None
	Normalization	Not applicable
	Additional comments	
Post-HTS analysis	Hit criteria	the emergence of at least one putative TRA-1-60 positive iPSC colonies in the well
	Hit rate	7.88% (13/165)
	Additional assay(s)	None
	Confirmation of hit purity and structure	Purchase from different vendors with QC data (purity >95%)
	Additional comments	