SUPPLEMENTARY INFORMATION

KDM4B-mediated reduction of H3K9me3 and H3K36me3 levels improves somatic cell reprogramming into pluripotency

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SUPPLEMENTAL MATERIALS AND METHODS

Teratomas

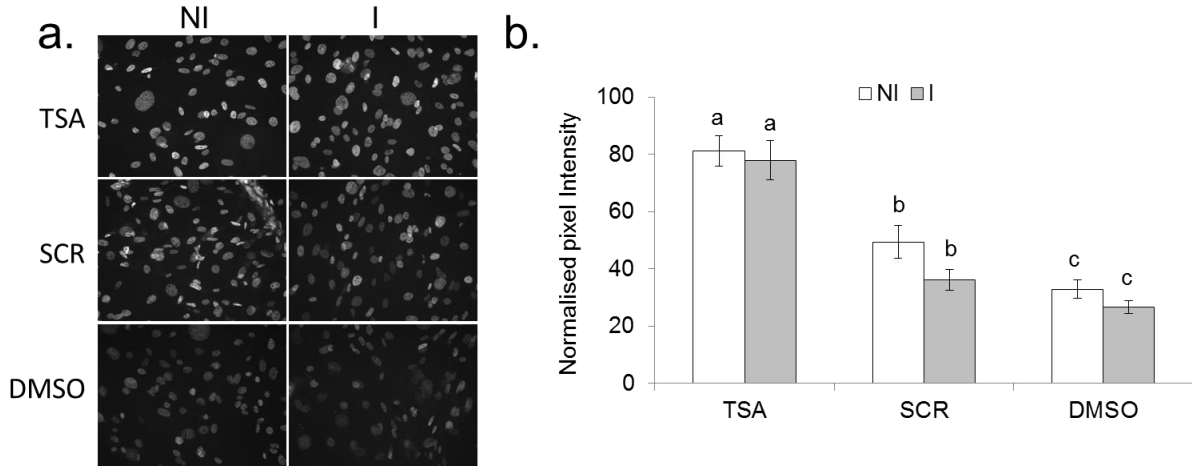
iPSC lines at passage 3-6 were harvested with a cell scraper, centrifuged and re-suspended in PBS + 1% PVA (10-30k). Using a 23G needle, 100 µl of cell suspension (approx. 1 - 5 x 10^6 cells per site) was injected intramuscularly into the quadriceps of adult immune-deficient mice. After 5–7 weeks, tumors were dissected, fixed overnight in Davidson's fixative, embedded in paraffin, sectioned, haematoxylin–eosin stained and analyzed by a pathologist service (NZVP, Hamilton, NZ).

Chimeras

iPSC lines were either aggregated with morulae or injected into host blastocysts (Swiss or FVB/NJArc) and transferred into the oviduct of day 0.5 pseudo-pregnant females. Coat-colour chimeras were bred with Swiss females for germline transmission.

SUPPLEMENTAL FIGURES

Figure S1



SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Effect of HDACi on F-*Kdm4b*-EGFP expressing MEFs (a) Immunofluorescence of H3K9ac in 48 h non-induced (NI) vs induced (I) F-*Kdm4b* MEFs. **(b)** Quantification of immunofluorescence analysis in (A). Values represent normalized pixel intensity (ROI/area) ± SEM. Asterisks indicate significant differences between induced (I) vs non-induced (NI) MEFs and their corresponding HDACi treatments (N=20 nuclei quantified per treatment, n=4 replicates). Within each group (NI vs I), bars with different superscripts differ $^{ab}P<0.005$ by two-tailed unpaired t-test.

SUPPLEMENTAL TABLES

Supplemental Table 3. Functional annotation of differently expressed genes upon *Kdm4b*-induction

Top 3 Molecular and Cellular Functions*	***P-value***
Cellular Compromise	5.60E-03 - 1.65E-04
Cellular Function and Maintenance	5.60E-03 - 1.65E-04
Cell Death and Survival	3.74E-03 - 6.24E-04
Top 3 Canonical Pathways	***P-value***
Oncostatin M Signaling	2.10E-02
Inhibition of Matrix Metalloproteases	2.41E-02
Transcriptional Regulatory Network in Embryonic Stem Cells	2.47E-02
Top 3 Associated Network Functions	***Score***
Cell Cycle	27
Immunological Disease, Molecular Transport	3
Carbohydrate Metabolism, Skeletal and Muscular System Development and Function, Small Molecule Biochemistry	3

*Gene Ontology was determined by QIAGEN's Ingenuity® Pathway Analysis.

Supplemental Table 4: In vitro development of F-*Kdm4b* NT reconstructs treated ± HDACi

Induction[a]	HDACi[b]	n	nNT[c]	Morulae (% ± SEM)	Blastocysts (% ± SEM)
NI	-	5	130	1 (1 ± 0.2%)[a]	1 (1 ± 1%)[a]
I	-	12	230	16 (7 ± 1%)[b]	11 (5 ± 2%)
NI	SCR	2	59	4 (7 ± 1%)	9 (15 ± 2%)[b]
I	SCR	4	86	6 (7 ± 2%)	9 (11 ± 2%)
NI	TSA	3	81	4 (5 ± 1%)	3 (4 ± 1%)
I	TSA	9	155	12 (8 ± 1%)	7 (5 ± 1%)

[a]MEFs (#1, 3, 9) were either Dox-induced (I) or non-induced (NI) for 48 hours: [b]NT reconstructs were treated with scriptaid (SCR), trichostatin (TSA) or DMSO; [c]number of NT reconstructs that extruded a single polar body and were cultured until day 4. Significance was determined via Fisher 2x2 test; [ab]Cells with different superscripts within a column differ with $P<0.01$

Supplemental Table 5. Teratoma formation from *Kdm4b*-EGFP iPSC lines

Animal ID	Cell line	Dox	Tumor diameter (mm)	Ectoderm*	Mesoderm*	Endoderm*
1	#1 iPSC	_	20 20	√ ND	√ ND	√ ND
2	#1 iPSC	+	19 10	√ ND	√ ND	√ ND
3	#3 iPSC	_	No tumor	-	-	-
4	#7 iPSC	_	13	-	√	√
5	v6.5 ESC	_	15 13	√ ND	√ ND	√ ND

*Germ layer identity was determined on histological sections (Fig. 6D). ID=unique animal identifier; ND=not determined

Supplemental Table 6. Chimera formation from *Kdm4b*-EGFP iPSC lines

iPSC line	sex	No. of born mice	Chimaeras*	% of Chimaeras*
#1 Col1a1$^{4F2a/F\text{-}Kdm4b}$	♀	18	3	17%
#7 Col1a1$^{4F2a/F\text{-}Kdm4b}$	♂	2	1	50%

*Chimerism was determined by coat colour (Fig. 6E).

Supplemental Table 7: Primer sequences used for qPCR

Primer		Sequence (5'- 3')	Size (bp)	Reference
2810474019Rik	F:	TTTCTGTCTCGGTCTTCCGC	181	This paper
	R:	CACGCCTCTAATTCTGTTTTTCCA		
Actb	F:	CAGAAGGACTCCTATGTGGG	200	[1]
	R:	TTGGCCTTAGGGTTCAGGG		
Transgene Kdm4b	F:	AGAAGACACCGGGACCGATC	190	[2]
	R:	TGAATTCATCCATGGTGGGG		
C430002N11Rik	F:	AGCCCCTTCGGCTGTTTTTA	136	This paper
	R:	GGGCTGAACCTCCTTCTGTC		
Chst13	F:	ATGGGAAGACGCTCCTGTTG	98	This paper
	R:	TTTTCAAATGCGGGACGCAG		
Cola1 (Wild-type)	F:	GCTCGCACGTACTTCATTCC	400	This paper
	R:	GAGAGTTCCTTGAGGGCTGG		
Endo Kdm4B	F:	AGAAGCCTTCCTGTTCTCAG	189	[2]
	R:	TGTACTGACTGGCTGTAGGG		
Ephx2	F:	GGGAAAGGAATTTACCCCGCT	72	This paper
	R:	GAGTGGTACCCACTGGGCTG		
Gapdh	F:	TGCACCACCAACTGCTTAG	176	[1]
	R:	GATGCAGGGATGATGTTC		
Glipr1	F:	CGTGCAGTGATTGCCCAAAA	90	This paper
	R:	CGACAGAGTAGTAACGTGAGACC		
Gpr56	F:	TTTTCTCTGGTGCAAGGTGC	192	This paper
	R:	GAAGCGGGGAATATCTGGGG		
Gusb	F:	ATAAGACGCATCAGAAGCCG	96	[3]
	R:	ACTCCTCACTGAACATGCGA		
Hprt	F:	GAAATGTCAGTTGCTGCGTC	332	[4]
	R:	GCCAACACTGCTGAAACATG		
Lrrn1	F:	TCCTCATCCTCCGGCTAGTG	93	This paper
	R:	GAGACGTGTCCTAAGGCTGG		
Ly6a	F:	GGAGCTGCTAGGTTTTATCTGTGC	178	This paper

	R:	GGGCAGGTAATTGATGGGCA		
M2-rtTA	F:	GAGAGTTCCTTGAGGGCTGG	407	This paper
	R:	CAATTGCTTGTCTCAGAAGTGG		
Mmp13	F:	GGAGCCCTGATGTTTCCCAT	108	This paper
	R:	GTCTTCATCGCCTGGACCATA		
Nnat	F:	CCACCCACTTTCGGAACCAT	104	This paper
	R:	ACTGGGGACAGGGTCTGC		
Prss42	F:	GAAGAGGGGAAGTGGCCTTG	130	This paper
	R:	CGTTGTACTGGATTCGGCTGTA		
Rosa26 (Wild-type)	F:	GCTGTTTTGGAGGCAGGAAG	700	This paper
	R:	TGCCAATGCTCTGTCTAGGG		
Serpina3g	F:	ACAGAGGCTGAAAAGGAGCAG	139	This paper
	R:	AGAGGCTGAAGGCAAAGTCA		
Slc29a3	F:	CTTTGAGAGCTACCTGGCAGT	91	This paper
	R:	GTGCACCTGGACCCTGTTGA		
Sry	F:	TGGGACTGGTGACAATTGTC	402	[5]
	R:	GAGTACAGGTGTGCACCTCT		
Trim24	F:	GCCATTCGCCACCCAAGT	150	This paper
	R:	CAGCTTGTACATACCTGATTAGACT		
Zfp37	F:	GGACCTGACAAAGCCAGAGG	198	This paper
	R:	TGATTTCCCATGGAGGCTGG		

Supplemental Table 8: Overview of MEF lines used for the various assays

Assay	**Induction**	**MEF lines**													
		F-Kdm4b							**M-*Kdm4b***	***F-Kdm4b/iPS***			***M-Kdm4b/iPS***		***WT/iPS***
	Dox (days)	#1 (♂)	#3 (♀)	#4 (♀)	#5 (♀)	#6 (♀)	#9 (♂)	#11 (♀)	#13 (♀)	#1 (♀)	#2 (♂)	#7 (♂)	#1 (♀)	#3 (♀)	#1 (♂)
Flow cytometry	1-2	√	-	√	√	√	√	√	√	-	-	-	-	-	-
qPCR	2	-	-	√	√	√	-	√	-	-	-	-	-	-	-
Microarray	2	-	-	√	√	√	-	-	-	-	-	-	-	-	-
mRNA-seq	2	-	-	√	√	√	-	-	-	-	-	-	-	-	-
Immunofluorescence	2	√	√	√	√	-	-	√	-	-	-	-	-	-	-
Western blot	2	-	-	-	√	-	-	√	-	-	-	-	-	-	-
Demethylase activity	2	-	-	-	√	√	-	-	-	-	-	-	-	-	-
NT	1	-	-	√	√	√	-	√	√	-	-	-	-	-	-
NT ± HDACi	1	√	√	-	-	-	√	-	-	-	-	-	-	-	-
iPS colony formation	21	-	-	-	-	-	-	-	-	√	√	√	√	√	√
Teratoma or chimera	21	-	-	-	-	-	-	-	-	√	√	√	-	-	-

√=tested for this assay; -=not determined; NT=Nuclear transfer, WT=wild-type

Supplemental Table 9: Primary and secondary antibodies

Antibody	*Dilution*		*Manufacturer (Cat.-no.)*
	IF	WB	
Alexa Fluor donkey anti mouse 488	1:1000	NA	Thermo Fisher (Cat-A11008)
Alexa Fluor goat anti rabbit 568	1:1000	NA	Thermo Fisher (Cat-A11011)
Goat anti rabbit HRP	NA	1:10000	Thermo Fisher (Cat-G21234)
Mouse anti GFP	1:1000	NA	Thermo Fisher (Cat-A11120)
Rabbit anti H3K9Ac	1:250	NA	Millipore (Cat-04-1003)
Rabbit anti H3K9me1	1:1000	1:6000	Gift by T Jenuwein (Freiburg, Germany)
Rabbit anti H3K9me2	1:1000	1:3000	Gift by T Jenuwein (Freiburg, Germany)
Rabbit anti H3K9me3	1:1000	1:3000	Gift by T Jenuwein (Freiburg, Germany)
Rabbit anti H3K27me3	1:1000	1:3000	Gift by T Jenuwein (Freiburg, Germany)
Rabbit anti H3K36me3	1:500	1:1000	Cell Signaling (#9763)
Rabbit anti NANOG	1:100	NA	Abcam (ab80892)

NA=not applied

SUPPLMENTAL REFERENCES

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