**Supplemental information**

Tet2 regulates Sin3a recruitment at active enhancers in embryonic stem cells

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A

Sample	Reads	Trimmed Reads	Unmapped Reads	Mapped Reads	Mapping Rate
$Tet2^{+/+}$ (1)	33,987,129	33,976,925	4,720,825	29,256,100	86.11 %
$Tet2^{+/+}$ (2)	30,107,691	30,101,476	4,227,395	25,874,081	85.96 %
$Tet2^{+/+}$ (3)	24,397,386	24,391,914	3,424,944	20,966,971	85.96 %
$Tet2^{m/m}$ (1)	28,428,775	28,421,579	3,965,493	24,456,086	86.05 %
$Tet2^{m/m}$ (2)	30,763,456	30,756,818	4,293,529	26,463,290	86.04 %
$Tet2^{-/-}$ (1)	28,198,796	28,190,677	4,002,955	24,187,722	85.80 %
$Tet2^{-/-}$ (2)	27,789,579	27,782,273	3,797,631	23,984,643	86.33 %

B
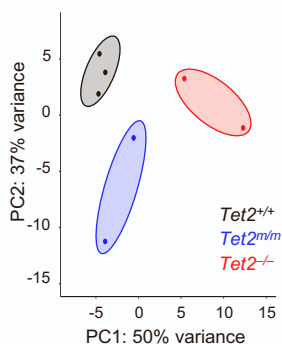
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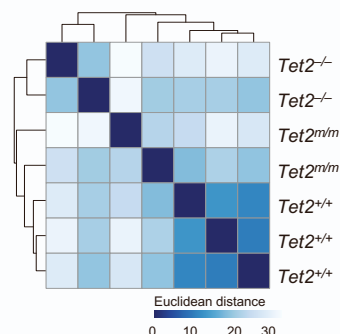
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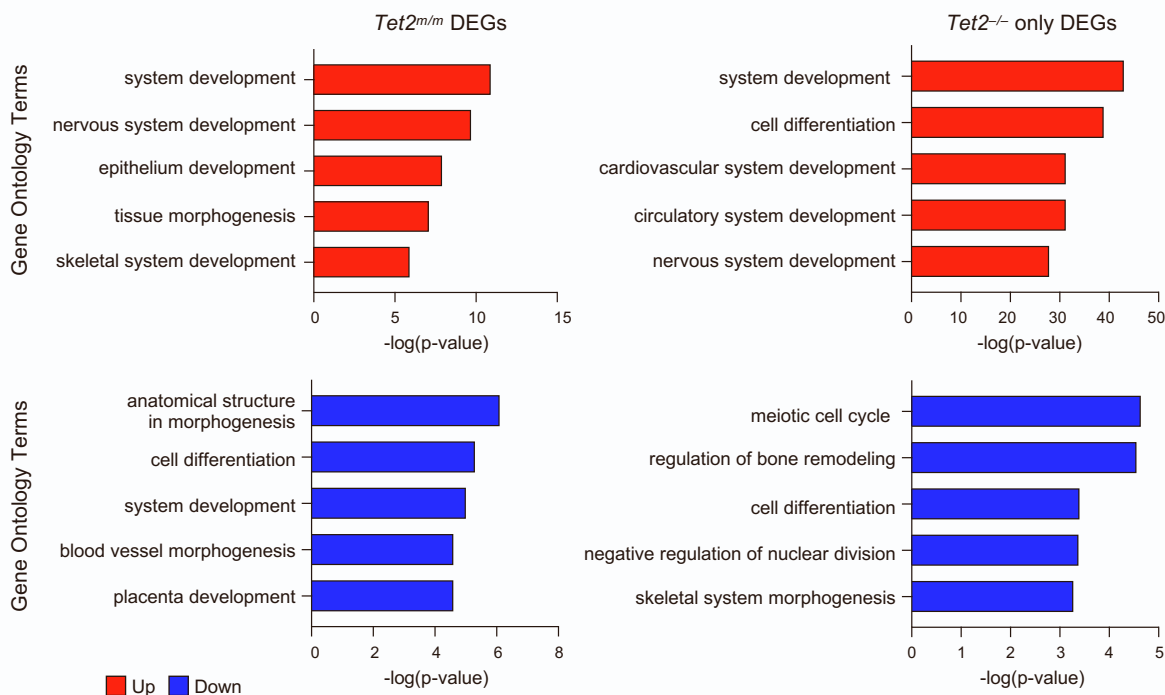
Figure S1. Gene expression profiling of $Tet2^{m/m}$ and $Tet2^{-/-}$ ESCs by RNA-seq, related to Figure 2.

A. Summary of RNA-seq raw, trimmed, and mapped reads.

B. Principal component analysis (PCA) analysis of wildtype, $Tet2^{m/m}$, and $Tet2^{-/-}$ ESCs gene expression. Note that samples of the same genotype cluster together.

C. Distance plot showing clustering of normalized expression counts in wildtype, $Tet2^{m/m}$, and $Tet2^{-/-}$ ESCs.

D. Top significantly enriched gene ontology terms of up and downregulated DEGs (–log of the p-value for each term is plotted).
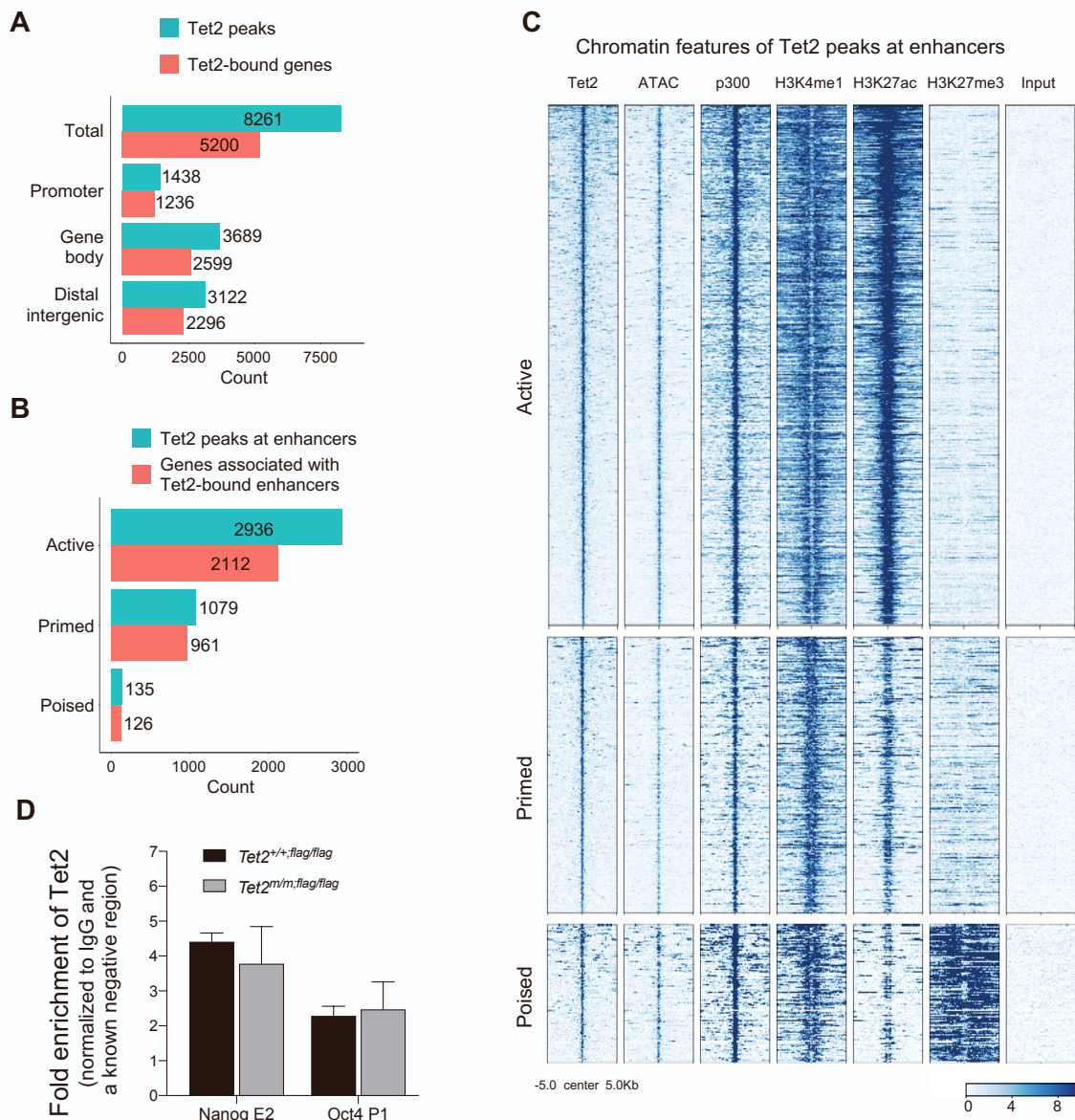
Figure S2. Tet2 occupancy at enhancers in ESCs, related to Figure 2.

A. Number of Tet2 peaks at genomic regions, and the number of genes associated with Tet2 peaks (Tet2-bound genes) (Tet2 ChIP-seq data from Rasmussen et al., 2019).

B. Number of Tet2 peaks at enhancers and the number of genes associated with them (enhancer coordinates from Cruz-Molina et al., 2017).

C. Heatmap showing chromatin accessibility, p300 occupancy, and histone mark enrichment at Tet2-bound enhancers using previously published datasets (ATAC-seq data from Chronis et al., 2017; p300, H3K4me1, H3K27ac, and H3K27me3 ChIP-seq data from Cruz-Molina et al., 2017).

D. Fold enrichment of wildtype and catalytic mutant Tet2 at *Nanog* enhancer 2 (E2) and *Oct4* promoter 1 (P1) in $Tet2^{+/+;flag/flag}$ and $Tet2^{m/m;flag/flag}$ ESCs quantified by ChIP-qPCR and normalized to IgG and a region negative for Tet2. Error bars=SD.

A

Genotype	Reads	Trimmed Reads	Unmapped Reads	Mapped Reads	Mapping Rate	Deduplicated Reads	% Duplication
Tet2+/+	558,402,736	558,401,705	192,318,778	366,082,927	65.56%	335,313,164	8.41%
Tet2m/m	551,476,891	551,475,866	191,621,744	359,854,122	65.25%	339,920,598	5.54%
Tet2−/−	478,291,311	478,290,449	122,389,133	355,901,316	74.41%	327,568,438	7.96%

B

Hyper-DMRs

Tet2m/m   *Tet2−/−*
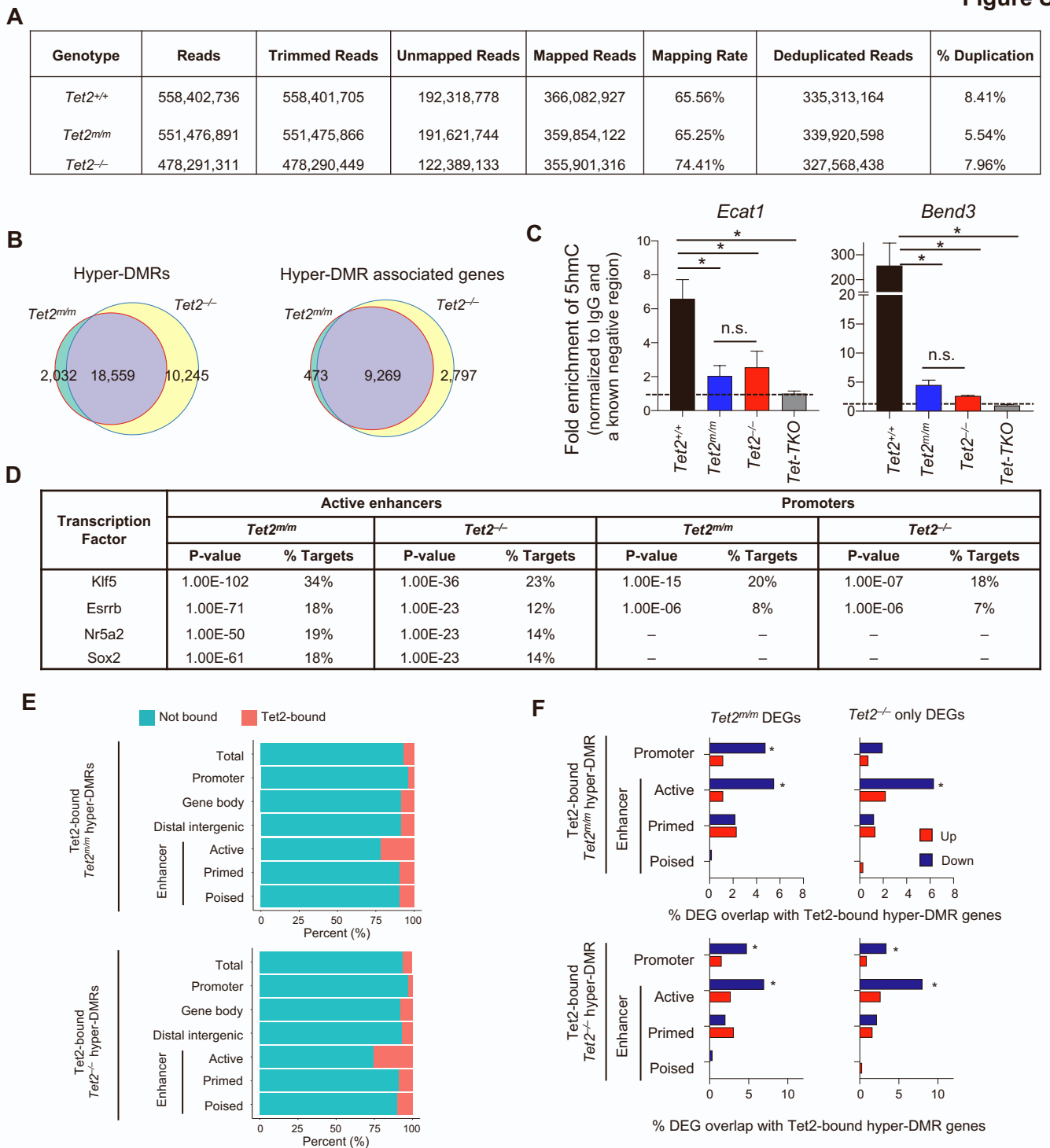
2,032   18,559   10,245

Hyper-DMR associated genes

Tet2m/m   *Tet2−/−*

473   9,269   2,797

C

Ecat1

Bend3

Fold enrichment of 5hmC (normalized to IgG and a known negative region)

D

Transcription Factor	Active enhancers				Promoters			
	Tet2m/m		*Tet2−/−*		*Tet2m/m*		*Tet2−/−*	
	P-value	% Targets	P-value	% Targets	P-value	% Targets	P-value	% Targets
Klf5	1.00E-102	34%	1.00E-36	23%	1.00E-15	20%	1.00E-07	18%
Esrrb	1.00E-71	18%	1.00E-23	12%	1.00E-06	8%	1.00E-06	7%
Nr5a2	1.00E-50	19%	1.00E-23	14%	–	–	–	–
Sox2	1.00E-61	18%	1.00E-23	14%	–	–	–	–

E

Not bound   Tet2-bound

Tet2-bound *Tet2m/m* hyper-DMRs

Total, Promoter, Gene body, Distal intergenic, Enhancer (Active, Primed, Poised)

Percent (%)

Tet2-bound *Tet2−/−* hyper-DMRs

Total, Promoter, Gene body, Distal intergenic, Enhancer (Active, Primed, Poised)

Percent (%)

F

Tet2m/m DEGs   *Tet2−/−* only DEGs

Tet2-bound *Tet2m/m* hyper-DMR

Promoter, Enhancer (Active, Primed, Poised)

Up   Down

% DEG overlap with Tet2-bound hyper-DMR genes

Tet2-bound *Tet2−/−* hyper-DMR

Promoter, Enhancer (Active, Primed, Poised)

% DEG overlap with Tet2-bound hyper-DMR genes

Figure S3. Mapping DNA methylation levels in *Tet2m/m* and *Tet2−/−* ESCs, related to Figure 3.

A. Summary of whole-genome bisulfite sequencing (WGBS) reads.

B. Venn diagram showing the overlap of hyper-DMRs and their associated genes in *Tet2m/m* and *Tet2−/−* ESCs.

C. 5hmC levels quantified at selected hyper DMRs by hMeDIP-qPCR. Data normalized to a 5hmC negative region. Tet1/2/3 TKO ESCs are used as negative control (base line). *significant than WT. ns= not significant.

D. Pluripotency transcription factor motifs enriched at promoters and active enhancers hyper-methylated in *Tet2m/m* and *Tet2−/−* ESCs.

E. Percent Tet2-bound and unbound hyper-DMRs in *Tet2m/m* and *Tet2−/−* ESCs. Note that Tet2-bound hyper-DMRs are enriched for active enhancers.

F. % DEGs bound by Tet2 and hypermethylated in *Tet2m/m* and *Tet2−/−* ESCs. Note that downregulated DEGs are significantly associated with Tet2-bound promoters & active enhancers (* p<0.05 hypergeometric test).

A

3xFlag-Tet2 allele

Tet2 $^+$

E11   TGA
255 bp

Tet2 ^{flag}
E11   3xFlag   TGA
333 bp

B

Tet2$^{flag/flag}$

Tet2:   +/+   m/m   +/+

Flag   (333 bp)
+   (255 bp)

C

Tet2$^{flag/flag}$

Tet2: +/+ –/– +/+ m/m

Flag

Tet2

Vinculin

D

Whole cell lysate

Tet2:   +/+   m/m   –/–

Sin3a
(150 kDa)

Actin
(42 kDa)
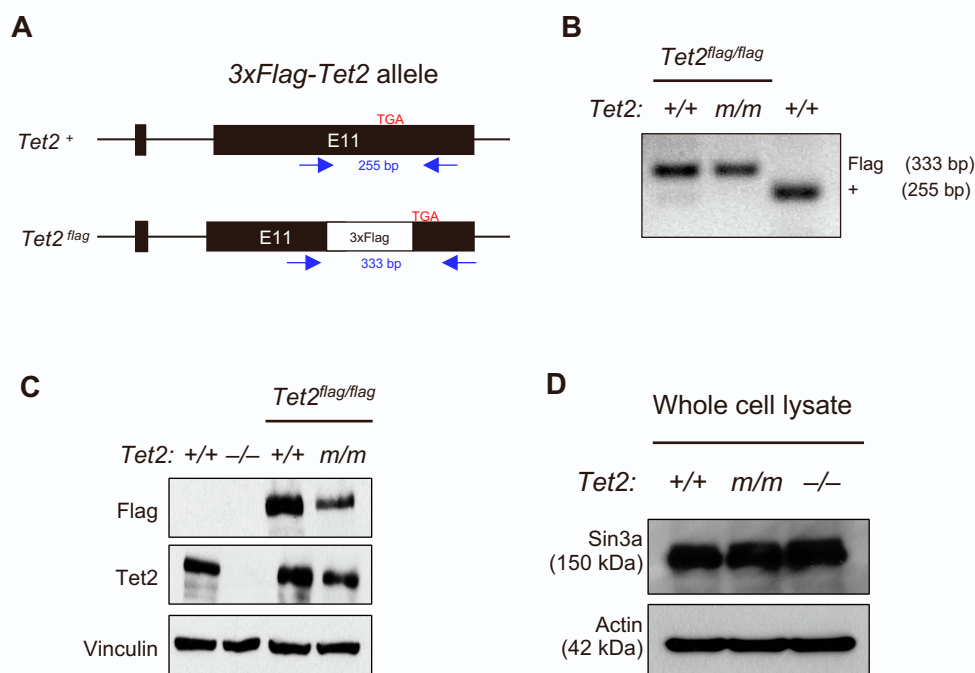
Figure S4. Generation of endogenously Tet2 Flag-tagged ESCs (*Tet2$^{+/+;flag/flag}$* and *Tet2$^{m/m;flag/flag}$* ESCs) for mapping Tet2 binding partners in ESCs, related to Figure 4.

A. Schematic of knocking in a 3xFlag tag before the stop codon of *Tet2* gene in *Tet2$^{+/+}$* and *Tet2$^{m/m}$* ESCs.

B. Genotyping of properly targeted *Tet2$^{+/+;flag/flag}$* and *Tet2$^{m/m;flag/flag}$* ESCs lines by PCR.

C. Confirmation of proper expression of Flag-tagged Tet2 protein by Western blot. Vinculin is used as a loading control.

D. Sin3a protein levels quantified by Western blot in wildtype, *Tet2$^{m/m}$*, and *Tet2$^{-/-}$* ESCs. Actin is used as loading control.

A

Sin3a CUT&Tag sequencing data summary

Sample	Reads	Mapped Reads	Mapping Rate	After Deduplication	% Duplicate	# of Sin3a peaks called
Tet2+/+	18,133,451	16,196,720	89.79%	5,795,408	56.75%	16,866
Tet2m/m	18,676,749	16,984,697	91.49%	3,767,894	73.69%	16,066
Tet2–/–	21,296,629	19,125,257	90.31%	5,941,860	62.92%	13.983
IgG	39,726,736	22,728,181	57.49%	767,145	95.49%	-

B

H3K27ac CUT&Tag sequencing summary

Samples	Reads	Mapping Rate	After Deduplication	% Duplicate
Tet2+/+	15,478,958	96.26%	10,717,725	18.12%
Tet2m/m	14,525,477	96.11%	9,470,946	23.29%
Tet2–/–	15,033,689	96.28%	10,081,492	21.62%
IgG	24,999,018	64.64%	413,205	96.05%

C

H3K27ac peak annotation

D

Figure S5. Mapping Sin3a and H3K27ac levels in *Tet2m/m* and *Tet2–/–* ESCs by CUT&Tag, related to Figure 5.

A. Summary of Sin3a CUT&Tag reads in wildtype, *Tet2m/m,* and *Tet2–/–* ESCs.

B. Summary of H3K27ac CUT&Tag reads in wildtype, *Tet2m/m,* and *Tet2–/–* ESCs.

C. Percent of H3K27ac CUT&Tag peaks at indicated genomic regions.

D. H3K27ac levels at Tet2-Sin3a bound promoters and active enhancers in wildtype, *Tet2m/m*, and *Tet2–/–* ESCs.

A   Genotyping of DMUT and DKO ESCs

RFLP

PCR

B

C   Sin3a CUT&Tag sequencing reads summary

Sample	Reads	Mapped Reads	Mapping Rate	After Deduplication	% Duplicate
WT1	19,493,785	15,672,418	81.08%	6,054,236	54.54%
WT2	23,519,181	19,021,865	81.64%	7,375,066	54.77%
DMUT1	21,763,814	18,422,812	85.44%	6,311,517	60.14%
DMUT2	16,768,006	14,085,024	84.79%	6,298,209	47.99%
DKO1	21,023,528	18,031,275	86.57%	7,049,236	54.46%
DKO2	21,807,518	18,446,226	85.33%	6,113,072	60.90%
IgG	33,727,919	14,382,486	42.97%	410,874	96.31%

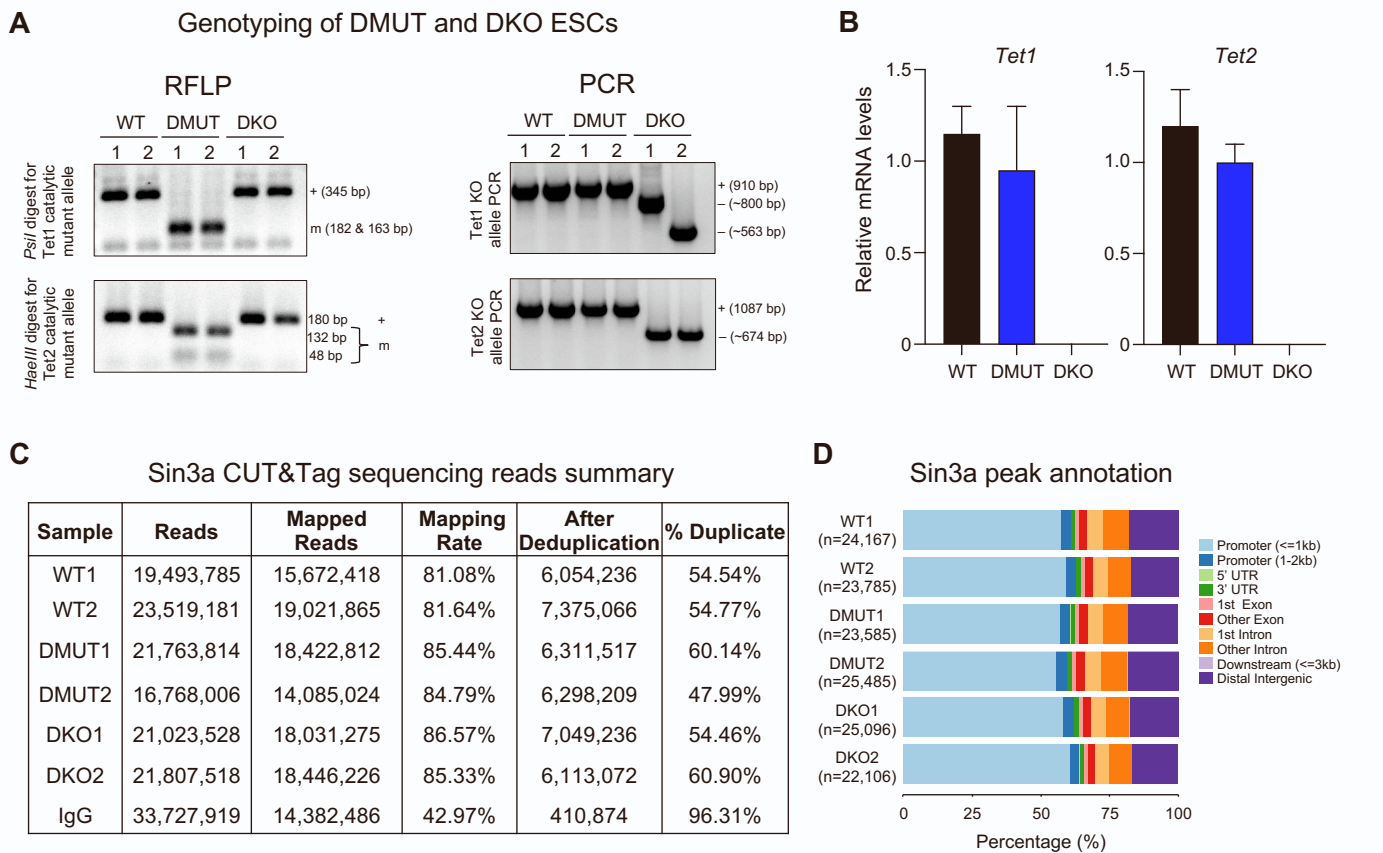**D**   Sin3a peak annotation

Figure S6. Characterization of *Tet1$^{m/m}$;Tet2$^{m/m}$* (DMUT) and *Tet1$^{-/-}$;Tet2$^{-/-}$* (DKO) ESCs, related to Figure 5.

A. RFLP and PCR showing correct targeting of Tet2 loci in published *Tet1$^{m/m}$* and *Tet1$^{-/-}$* ESCs for generating *Tet1$^{m/m}$;Tet2$^{m/m}$* (DMUT) and *Tet1$^{-/-}$;Tet2$^{-/-}$* (DKO) ESCs.

B. Expression of *Tet1* and *Tet2* in DMUT and DKO ESCs quantified by RTqPCR. Data normalized to Gapdh. Error bars = SD

C. Summary of Sin3a CUT&Tag reads in wildtype, DMUT, and DKO ESCs (n=2 of each genotype).

D. Percent of Sin3a peaks annotated to indicated genomic regions in wildtype, DMUT, and DKO ESCs.