

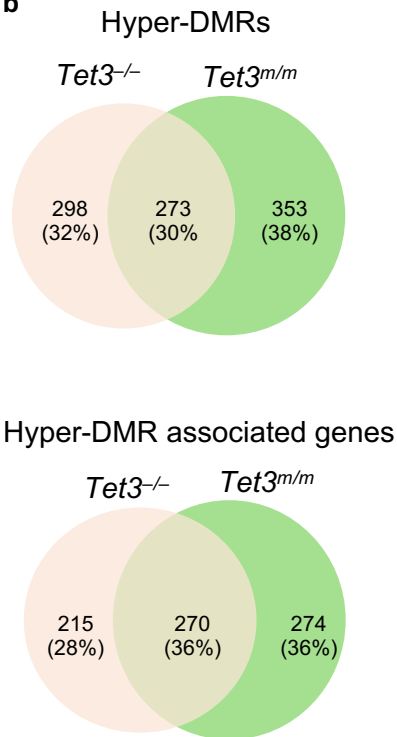
Supplementary Figure 1: RNA-seq analysis of *Tet3^{m/m}* and *Tet3^{-/-}* neuroectoderm cells

- Summary of total, trimmed and mapped reads, and mapping rate per sample.
- Sample distribution plot showing that samples of the same genotype cluster together.
- Principle component analysis (PCA) of RNA-seq data showing that samples of the same genotype cluster together.
- RNA-seq normalized counts of *Tet1* and *Tet2*. Note, neither *Tet1* nor *Tet2* are deregulated. Error bars represent standard deviation.
- Plot of RNA-seq normalized counts of selected neural (top) and mesodermal (bottom) genes. Neural genes are downregulated in both *Tet3^{m/m}* and *Tet3^{-/-}* cell lines while mesodermal genes are uniquely upregulated in *Tet3^{-/-}* cells. Error bars represent standard deviation.
- Plot of RNA-seq normalized counts of cyclin dependent kinase inhibitor (Cdkn) genes. Note *p15*, *p16*, and *p21* are uniquely upregulated in *Tet3^{-/-}* cells. Error bars represent standard deviation.
- Heatmap of up and downregulated genes in *Tet3^{m/m}* and *Tet3^{-/-}* cells that are ameliorated by re-expression of TET3 catalytic domain. Empty vector (EV) is used as control.

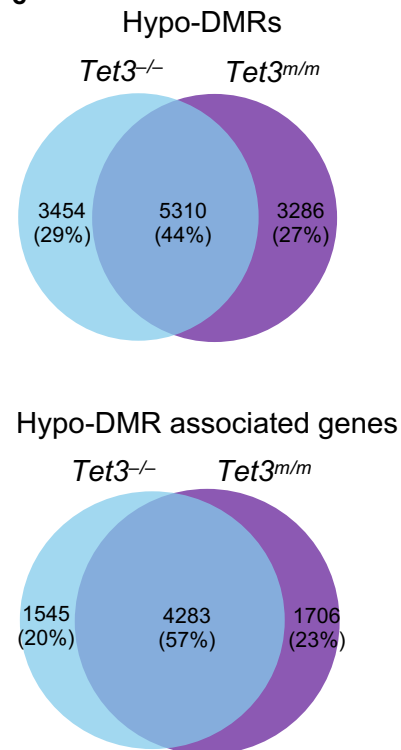
a

Sample Name	Reads	Trimmed Reads	Mapped Reads	% Mapping Rate	After Deduplicate	% Duplicate
<i>Tet3^{+/+}</i>	514,498,240	514,497,215	378646820	73.60%	341,578,304	9.79%
<i>Tet3^{m/m}</i>	540,538,184	540,537,178	394296833	72.95%	355,789,910	9.77%
<i>Tet3^{-/-}</i>	473,848,041	473,847,181	334638862	70.62%	303,987,670	9.16%

b

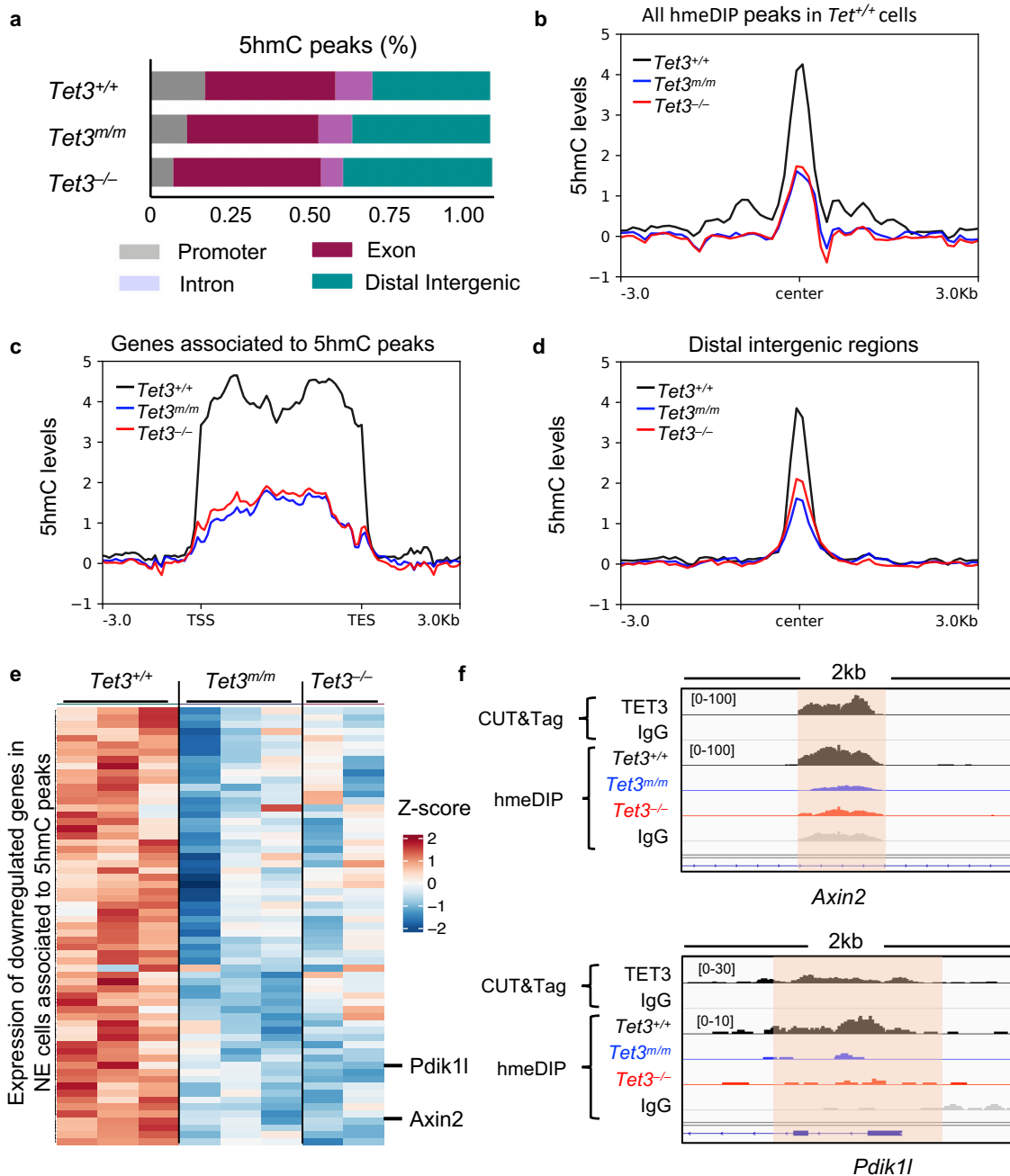


c



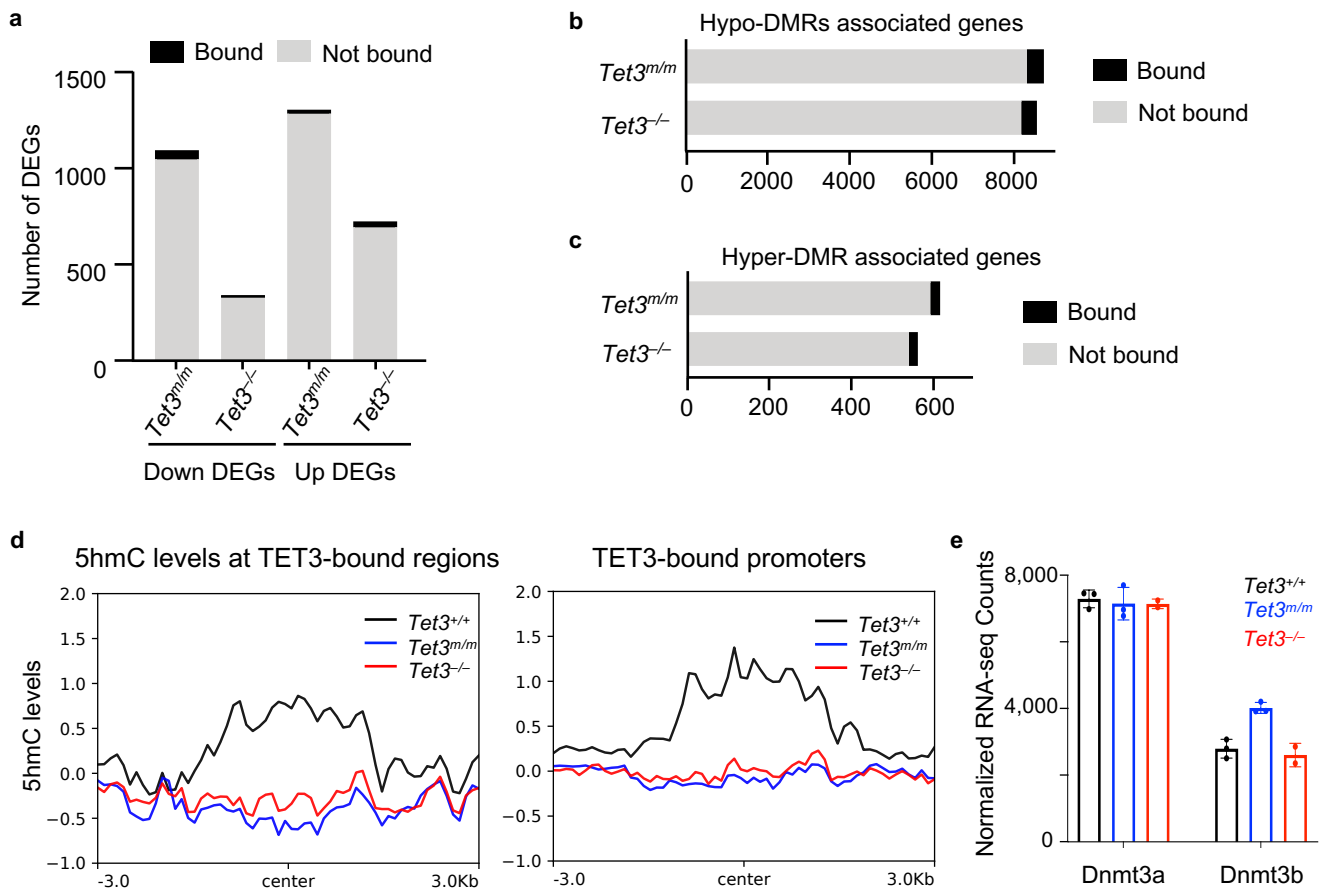
Supplementary Figure 2: Genome wide mapping of DNA methylation in *Tet3^{m/m}* and *Tet3^{-/-}* neuroectoderm cells by WGBS.

- Summary of total, trimmed and mapped reads, mapping rate, and duplication per sample.
- Overlap of hyper-DMRs (top) and hyper-DMR-associated genes (bottom) identified using methpipe.
- Overlap of hypo-DMRs(top) and hypo-DMR-associated genes (bottom).



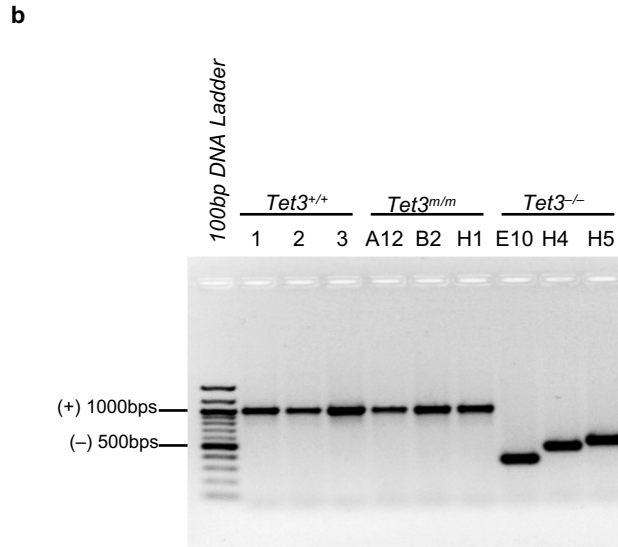
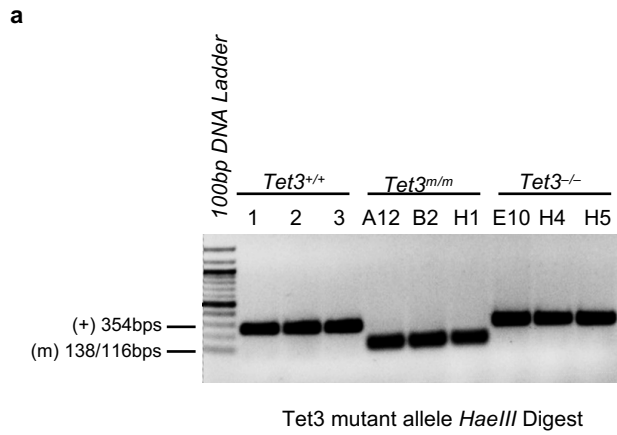
Supplementary Figure 3: Analysis 5hmC by hmeDIP in *Tet3^{+/+}*, *Tet3^{m/m}* and *Tet3^{-/-}* NE cells

- Genomic distribution of 5hmC peaks in *Tet3^{+/+}*, *Tet3^{m/m}* and *Tet3^{-/-}* cells.
- 5hmC levels at all hmeDIP peaks in *Tet3^{+/+}* cells. Note that 5hmC levels at these peaks are reduced in *Tet3^{m/m}* and *Tet3^{-/-}* cells.
- 5hmC levels across transcription start site (TSS) to transcription end site (TES) of genes.
- 5hmC levels across distal intergenic regions.
- Heatmap of genes associated to 5hmC peaks in wild type NE cell. Note that these genes are downregulated in *Tet3^{m/m}* and *Tet3^{-/-}* cells.
- Genome browser tracks showing 5hmC levels at TET3-enriched regions at representative loci.



Supplementary Figure 4: Summary of TET3 CUT&Tag in *Tet3^{+/+}* neuroectoderm cells

- Number of DEGs bound by TET3.
- Number of hypo-DMR associated genes bound by TET3.
- Number of hyper-DMR associated genes bound by TET3.
- 5hmC levels at all TET3 bound regions (left) and at TET3 bound promoters (right).
- RNA-seq normalized counts of *Dnmt3a* and *Dnmt3b* in NE cells. Count numbers are not significant between genotypes based on DESeq2. Error bars represent standard deviation.



Supplementary Figure 5: Uncropped images of gels shown in Fig. 1b

- a. Genotyping of *Tet3* mutant cells by RFLP with *HaeIII*.
- b. Genotyping of *Tet3* knockout cells by PCR with primers flanking exon 4.

Supplementary Table 1: List of oligonucleotides used in this study

Name	Sequence	Purpose	Source
Tet3 Mut exon 9 gRNA For	CCTAAATGTTCTCATCCTGG	Mutating Tet3 exon 9	This paper
Tet3 Mut exon 9 gRNA Rev	CCAGGATGAGAACATTTAGG	Mutating Tet3 exon 9	This paper
Tet3 Mut gene block sequence	5'agctatcgGAATTCTGT...500bp....CATGGACTTCTGT GCCACAGCCT TCAAGGCC CAACATAACCTCTACAA TGGGTGCACTGTGGTA...410bp.....Ctcgatcgt3'	Introducing HKD to YKA mutation the catalytic pocket of Tet3 in exon 9	This paper
Tet3 Mut Genotyping For	ATTCACACAGGTGACCAATGAG	Genotyping Tet3 Mut allele	This paper
Tet3 Mut Genotyping Rev	CCTAGGTAGTGTGGCTTAGAT	Genotyping Tet3 Mut allele	This paper
Tet3 KO exon 4 left gRNA For	TCCTAAATGTTCTCATCTGG	Deletion of Tet3 Exon 4	This paper
Tet3 KO exon 4 left gRNA Rev	CCAGATGAGAACATTTAGGA	Deletion of Tet3 Exon 4	This paper
Tet3 KO exon 4 right gRNA For	TTCCACAGCCTGGTTTTCT	Deletion of Tet3 Exon 4	This paper
Tet3 KO exon 4 right gRNA Rev	AGAAAACCAGGCTGTGGGAA	Deletion of Tet3 Exon 4	This paper
Tet3 KO Genotyping For	CTTAAGCAAAGCTCCCATGC	Genotyping Tet3 KO allele	This paper
Tet3 KO Genotyping Rev	GACTCTGGGTATTTGGGGT	Genotyping Tet3 KO allele	This paper
Tet3 exon 3 RTqPCR For	CCGTGACTGTGCTCTCAACT	Real time qPCR	Reference #15
Tet3 exon 4 RTqPCR Rev	CCATGAGTTCCCGGATAGAA	Real time qPCR	Reference #15
Tet2 exon 3 RTqPCR For	CAAGCAACCAAAAGCACAGA	Real time qPCR	Reference #14
Tet2 exon 4 RTqPCR Rev	GGACCAGCTCCTAGATGGGTA	Real time qPCR	Reference #14
Tet1 RTqPCR For	TGCACCTACTGCAAGAATCG	Real time qPCR	Reference #13
Tet1 RTqPCR Rev	AAATTGGCATCACAGCTTCC	Real time qPCR	Reference #13
Dnmt1 RTqPCR For	GCTACCAGTGCACCTTTGGT	Real time qPCR	Reference #46
Dnmt1 RTqPCR Rev	CAGAGGCAGCTTTTCTCCTG	Real time qPCR	Reference #46
Gapdh RTqPCR For	ACATCTCACTCAAGATTGTCAGCA	Real time qPCR	Reference #10
Gapdh RTqPCR Rev	ATGGCATGGACTGTGGTCAT	Real time qPCR	Reference #10

Supplementary Table 2: List of reagents and resources used in this study

Reagent/Resource	Source	Identifier
Antibodies		
Anti-Tet3	Millipore	ABE290
Rabbit IgG isotype control DA1E	CST	3900
Guinea pig anti-rabbit	Antibodies Online	ABIN101961
Kits		
Qiagen RNeasy Mini kit	Qiagen	74104
Quick-DNA miniprep kit	Zymo	D3024
Superscript IV first strand	Invitrogen	18091050
XtremeGene 9 DNA transfection reagent	Roche	06365787001
Click-It 647 EdU	Thermo Fisher	C10424
MinElute PCR Purification Kit	Qiagen	28004
hmeDIP kit	Active Motif	55010
Primers/Oligos		
RT-qPCR primers	Table S1	N.A.
Genotyping Primers	Table S1	N.A.
gRNAs for gene targeting	Table S1	N.A.
Plasmids		
Topo pcr2.1 Tet3 mutant donor vector	This paper	N.A.
pX330-Tet3-gRNA	This paper	N.A.
pCAG-Myc-IRESblast	From Taiping Chen Lab	N.A.
pCAG-Myc-Dnm1-IRESblast	From Taiping Chen Lab	N.A.
FUW-HA-2A-TdTomato	Reference #46	N.A.
FUW-HA-Tet3CD-2A-TdTomato	This Paper	N.A.
Software		
Trim galore v0.6.7	Babraham Institute	https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/
STAR v2.7.9a	Reference #48	https://github.com/alexdobin/STAR
DESeq2 v1.40.2	Reference #49	http://www.bioconductor.org/packages/release/bioc/html/DESeq2.html
DAVID 6.8	Reference #50	https://david.ncicrf.gov
Bismark v0.23.1	Reference #51	https://github.com/FelixKrueger/Bismark
Methpipe v5.0.1	Reference #52	http://smithlabresearch.org/software/methpipe/
ChIPseeker v1.36.0	Github	https://guangchuangyu.github.io/software/ChIPseeker/
Bedtools v2.30.0	Reference #54	https://bedtools.readthedocs.io/en/latest/
deepTools v3.5.1	Reference #53	https://deeptools.readthedocs.io/en/develop/index.html
Bowtie2 v2.4.5	Reference #55	http://bowtie-bio.sourceforge.net/bowtie2/index.shtml
Integrative Genomics Viewer v2.14.1	Broad Institute	http://software.broadinstitute.org/software/igv/
MACS v2.2.7.1	Reference #56	https://github.com/macs3-project/MACS
GraphPad Prism 8 v8.4.2	GraphPad	https://www.graphpad.com/