Materials and Methods

Mice: 9-11 week-old C57BL/6 male mice were housed four per cage, maintained on a 12hr light/dark schedule, and allowed free access to food and water. All testing was conducted during the light phase in red-light-illuminated testing rooms following protocols approved by the Animal Ethics Committee of the University of Queensland.

DNA/RNA Extraction: lLPFC tissue from naïve and trained mice was prepared by dounce homogenization in 500µl PBS. 400µl of homogenate was used for DNA extraction, and 100µl was used for RNA extraction. Preparation of DNA was carried out using the DNeasy Blood & Tissue Kit (Qiagen), and RNA was extracted using Trizol reagent (Invitrogen). Both extraction protocols were followed according to the manufacturer's instructions.

qRT-PCR: Total 500ng RNA was used for cDNA synthesis using the Quantitect Reverse Transcription Kit (Qiagen). Quantitative PCR was performed on a RotorGeneQ (Qiagen) real-time PCR cycler with SYBR-Green Master mix (Qiagen), using primers for target genes and for PGK as an internal control (supplementary table 1). The threshold cycle for each sample was chosen from the linear range and converted to a starting quantity by interpolation from a standard curve run on the same plate for each set of primers. All mRNA levels were normalized for each well relative to PGK using the $_{\Delta\Delta}$CT method, and each PCR reaction was run in duplicate for each sample and repeated at least twice.

5-hmC and 5-mC capture: Genomic DNA was sheared (Covaris) with an average size of 300bp prior to capture. 5hmC/5-mC enrichment was performed using an hMeDIP kit (Active Motif) for enrichment of 5-hmC, or a MeDIP kit (Active Motif) for 5-mC. The procedures were followed according to manufacturer's instructions.

High-throughput DNA sequencing on 5-hmC-enriched samples: Sequencing libraries were generated for each of 8 mice in each group by the Illumina TrueSeq Prep kit (Illumina) as per the manufacturer's protocol without the final PCR amplification step. Briefly, 100ng of DNA was used for library preparation and a normalized pooling step was performed on indexed genomic DNA from each individual animal prior to 5-hmC capture. Following this step, final PCR amplification using the Illumina TrueSeq Prep Kit. and PCR-amplified DNA libraries were quantified on an Agilent 2100 Bioanalyzer. Cluster densities were created and each library, at a final concentration of 8pM, was used for subsequent cluster generation using the version 3 flow cell. The Illumina Hiseq2000 was then used for paired-end (100bp) sequencing. Image processing and sequence extraction were achieved using the standard Illumina Genome Analyzer software and pipelines developed in-house at the Queensland Brain Institute, The University of Queensland.

CASAVA software (v1.8.2) was used to demultiplex the samples and generate raw reads for each sample in fastq format.

Bioinformatics: Alignment of short reads to reference genome: Paired-end reads were aligned to the mouse reference genome (mm9) using BWA (v0.6.2). Samtools (v0.1.17) and Picard (v1.72) software packages were then used to convert aligned ".sam" files to ".bam" files, sort the ".bam" and remove duplicates. Reads with low mapping quality (<20) were excluded from the downstream analysis. Normalized depth of coverage for plotting peaks: Based on the outcomes from "Align short reads to reference" (quality filtered bam files), for each position (at single base pair resolution) of the peak and nearby region, we counted the number of DNA fragment that can cover this position. "DNA fragment" here means the one in the sequencing library, with both ends being sequenced in the paired-end sequencing strategy (For example, read1 is aligned to chr1:201-301, and read2 is aligned to chr1:310-410, then the DNA fragment of this read-pair would be chr1:201-410). The number of DNA fragments in each sample was normalized to 10 million total DNA fragments (equal to 20 million total reads) and normalized to the corresponding "input" sample. The mean normalized values of 8 biological replicates from each condition were used to plot the depth of coverage. A custom PERL script that integrates Samtools and R codes was applied to do this work mentioned above. Peak calling: MACS (v1.4.2) was used to call peaks for each sample using a parameter setting "-f BAM --keep-dup=all -g mm --nomodel --shiftsize 100" and applying two criteria for *P*-value cutoff: "1e-2" and "1e-5". Peaks identified in all samples with *P*-value cutoff "1e-2" were then integrated based on pairwise comparison using custom PERL scripts with the following criteria: if the distance between two peak summits is equal or less than 300 bp, then these two peaks were considered as a common peak and grouped together. For each peak group, we counted the number of biological replicates in each condition, and this number represents the intensity of each peak in each condition illustrated in Figure 1a. In total, we have four conditions: namely CXT, FC, FC-No EXT and EXT, with 8 biological replicates in each condition, so in theory the maximal number of samples in all 4 conditions to support a peak will be 4×8=32. In Figure 1a, we only plotted peaks with the supported number larger than 4 in all conditions to eliminate most of the noises, and ensure that we wouldn't miss any potential interesting peaks that only exist in one condition (e.g. 0 in CXT, FC and FC-No EXT conditions, while 5 in EXT condition). Functional annotation clustering: Peaks supported by at least 5 of 8 biological replicates in extinction training group but not in other groups were considered as 5-hmC peaks induced by extinction training. In total, 143 5-hmC peaks were parsed. Genes located within 50 kb distance from these peaks were considered, and there are 233 genes included. These genes were then applied for functional annotation clustering using DAVID 6.7 web-based functional annotation tool suites. Analysis of dinucleotide combinations in peak regions: Both core regions and summit regions of peaks identified in each biological replicate were picked up to calculate the proportions of "CA", "CC", "CG" and "CT" dinucleotide combinations using custom PERL scripts. Here, the core region is defined as 100bp extension from each peak summit in both 5' and 3' directions. The summit region is defined as the interval that was covered by

the highest number of fragments in a peak region. Analysis of peak locations: All peaks identified in eight biological replicates were included to analyze their genomic locations according to the gene coordinates defined in the GTF file for *Mus musculus* (mm9) from Illumina iGenomes database (http://cufflinks.cbcb.umd.edu/igenomes.html). Six categories including promoter, 5' UTR, 3' UTR, CDS, intron and intergenic regions were considered. Promoter region was defined as 2000 bp upstream from the first exon of each transcript. If peaks locate in several different categories, we put in the order of priority: Promoter, 5'-UTR, 3'-UTR, CDS, Intron and Intergenic regions.

Tet3/Tet1 knockdown lentiviral constructs: Lentiviral plasmids were generated by inserting either Tet1 or Tet3 shRNA using the following sequences: shRNA-Tet1: GCAGATGGCCGTGACACAAAT; shRNA-Tet3: GCCTGTTAGGCAGATTGTTCT (the Tet3 shRNA sequence was provided by Dr. Yi Zhang, Harvard) immediately downstream of the human H1 promoter in a modified FG12 vector (FG12H1, derived from the FG12 vector originally provided by David Baltimore, CalTech). Lentivirus was prepared and maintained according to previously published protocols[20].

Lentiviral surgery: Double cannulae (PlasticsOne) were implanted in the anterior posterior plane, along the midline, into the infralimbic prefrontal cortex (ILPFC), a minimum of 3 days prior to viral infusion. The injection locations were centered at +1.78 mm in the anterior-posterior plane (AP), and -2.5 mm in the dorsal-ventral plane (DV). A total volume of 4.0µl of lentivirus was introduced via 2 injections delivered within 48 hours. For both knockdown experiments, mice were first fear conditioned, followed by 2 lentiviral infusions 24 hours post-fear conditioning, and, after a one-week of incubation, the mice were extinction trained.

Behavioral Tests: Two contexts (A and B) were used for all behavioral fear testing. Both conditioning chambers (Coulbourn Instruments) had two transparent walls and two stainless steel walls with a steel grid floors (3.2 mm in diameter, 8 mm centers); however, the grid floors in context B were covered by flat white plastic transparent surface to minimize context generalization. Individual digital cameras were mounted in the ceiling of each chamber and connected via a quad processor for automated scoring of freezing measurement program (FreezeFrame). Fear conditioning was performed in context A with spray of lemon-alcohol (5% lemon and 10% alcohol). Then, actual fear condition protocol was starting with 120 sec pre-fear conditioning incubation; then, followed by three pairing of a 120 sec, 80dB, white noise conditioned stimulus (CS) co-terminating with a 1 sec (2 min intervals), 0.7 mA foot shock (US). Mice were matched into equivalent treatment groups based on freezing during the third training CS. For extinction, mice were exposed in context B with a stimulus light on and spray of vinegar. Mice allowed to be acclimated for 2 min, and then, extinction training comprised 30 non-reinforced 120 sec CS presentations (5-sec intervals). For the behavior control experiments, context exposure was performed for both fear condition and fear extinction training. Animal, inside 3CS-US or 30CS treatment, only exposed into either context A or B with

equal times of mice spend there by fear condition or extinguished mice but were not exposed to any 3CS-US or 30 CS. For the retention test, all mice were returned to context B and following a 2 min acclimation (used to minimize context generalization), freezing was assessed during two 120sec CS presentations (120 sec intertribal interval). Memory was calculated as the percentage of time spent freezing during the tests.

Behavioral Training (for tissue collection): Naïve animals remained in their home cage until sacrifice. For the other groups, fear conditioning consisted of three pairing (120sec iner-trial interval ITI) of a 120sec, 80dB, white noise conditioned stimulus (CS) Co-terminating with a 2s, 0.7mA foot shock in context A. Mice were matched into equivalent treatment groups based on freezing during the third training CS. Context A exposure group spent an equivalent amount of time in context A without any CS and US. Tissue for Fear conditioned mice and context A exposure group was collected 26hr after the context A training session. One day later, the fear-conditioned mice were brought to context B, where the extinction group (EXT) was presented with 30CS presentations (5s ITI). The fear-conditioned without extinction (FC No-EXT) group spent an equivalent amount of time in context B without any CS presentations. Tissue was collected from both of these groups 2hr and 24hr after the end of their context B session.

Immunohistochemistry: Mice were euthanized with 100mg/Kg ketamine, after which 50ml of 1:100000 sodium nitrite, in PBS, was pumped through the circulatory system serving as a vasodilator. To fix the tissue, 4% paraformaldehyde in PBS was used. Following extraction, the brains were stored in 0.05% sodium azide. The brains were placed in 30% sucrose for a minimum 24hr prior to cryostat slicing. Sectioning at 14um was performed using Zeiss Microm HM560 crystat, and sections were mounted on Menzel-Glaser Superfrost Plus microscope slides. Briefly, the sections were incubated 1-2hr in blocking buffer, after which primary antibodies (Tet3, GFP or NeuN) were added and the slides incubated at 4°C overnight. The slides were then washed 3 times with PBS containing 0.02% Tween 20 (PBS-T), after which secondary antibodies were added (Dylight 488-conjugated AffiniPure sheep anti-mouse IgG or Dylight 549-conjugated AffiniPure goat anti-rabbit IgG, Jackson ImmunoReasearch Laboratories). The slides were then incubated at room temperature for 2hr, washed 3 times with PBS or PBST, and cover-slipped.

Chromatin immunoprecipitation: Chromatin immunoprecipitation (ChIP) was performed following modification of the Invitrogen ChIP kit protocol. Tissue was fixed in 1% formaldehyde and cross-linked cell lysates were sheared by Covaris in 1% SDS lysis buffer to generate chromatin fragments with an average length of 300bp. The chromatin was then immunoprecipitated using the specific antibody to each targets (list of antibodies in supplementary Table 2) or equivalent amount of control normal rabbit IgG (Santa Cruz) overnight at 4°C. Protein-DNA-antibody complexes were precipitated with protein G-magnetic beads for 1hr at 4°C, followed by three washes in low salt buffer, and three washes in high salt buffer. The precipitated protein-DNA complexes were eluted from the antibody with 1% SDS and 0.1M $NaHCO_3$, then incubated

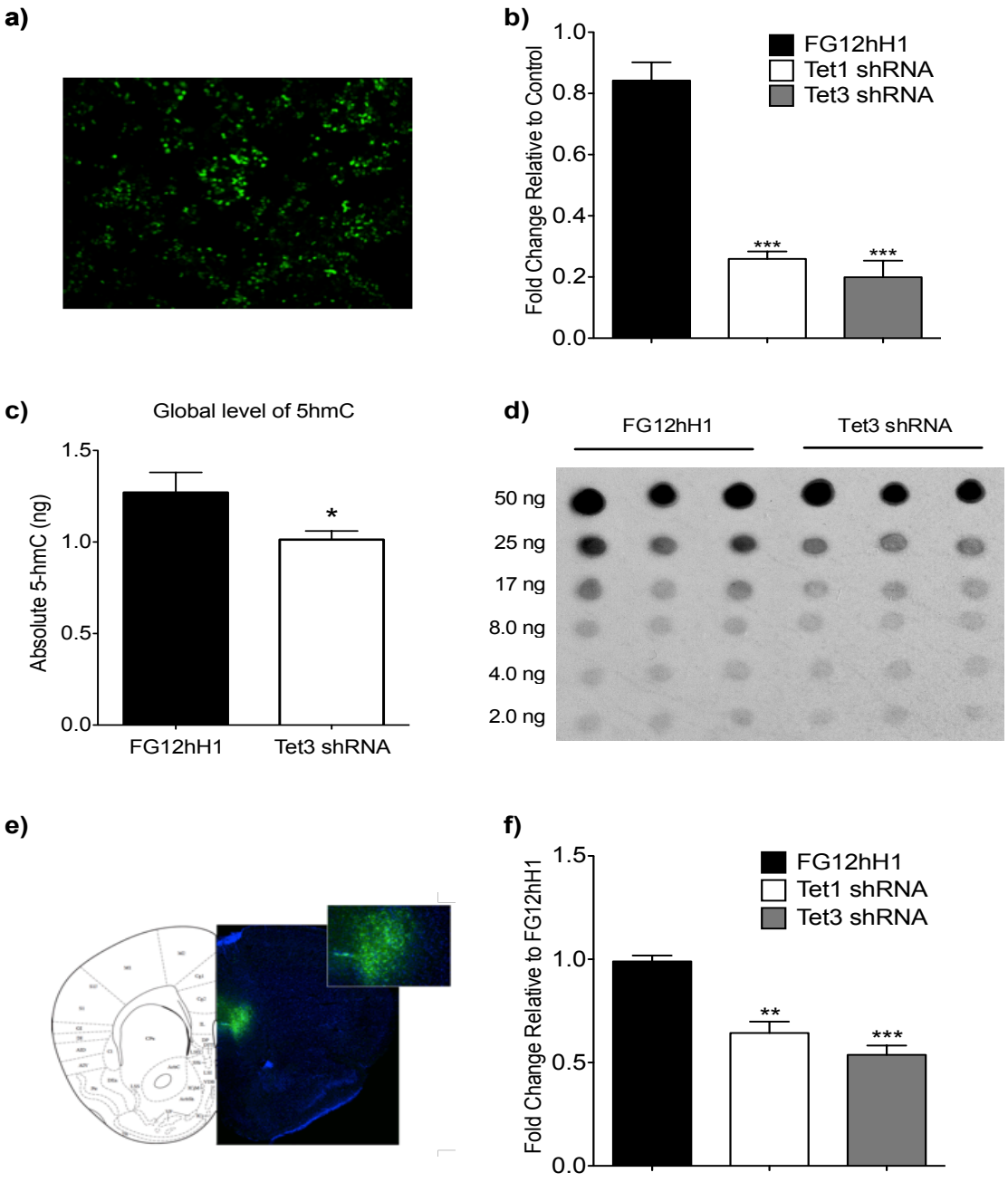
overnight at 65C in 200mM NaCl to reverse formaldehyde cross-link. Following proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation, samples were subjected to qPCR using primers specific for 200bp segments corresponding to the target regions.

Primary cortical neuron and N2a cell culture: Cortical tissue was isolated from E18 mouse embryos in a sterile atmosphere. To dissociate the tissue it was finely chopped followed by digestion in 0.125% Trypsin (GIBCO 25200) for 12 mins. To prevent clumping of cells due to DNA from dead cells, tissue was treated with 2 unit/µl of DNase I. Cells finally went through the 40µm cell strainer (BD Falcon 352340) and were plated onto 6 well plate coated with Poly-L- Ornithine (Sigma P2533) and Fibronectin (GIBCO 33016-015) at a density of $1x10^6$ cells per well. The medium used was Neurobasal medium (GIBCO 21103) containing 5% serum, B27 supplement (GIBCO 17504-044) and 0.5-1% Pen/Strep (GIBCO 15140). N2a cell was maintained in medium contains half DMEM, high glucose (GIBCO 11965-092), half OptiMEM 1 (Gibco 26140-079) with 5% serum and 1% Pen/Strep. KCl treatment: final concentration of 25mM of KCl was used for KCl stimulation on primary cortical neurons.

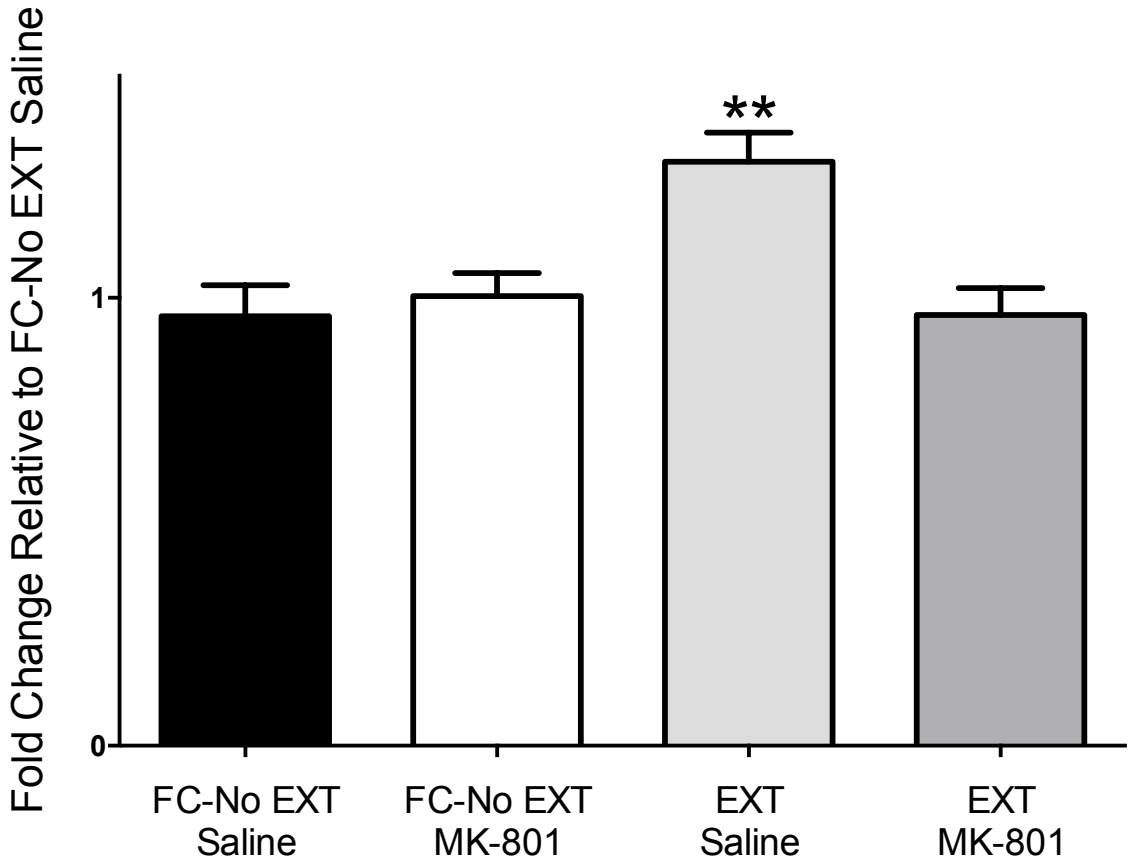
5-hmC Dot blot: Primary cortical neurons were cultured for 2weeks prior to lentiviral treatment. 5µl of wither Fg12hH1 or Tet3 shRNA lentivirus added into cell culture. After a 7-day incubation, KCl treatment was applied to the infected neurons. 7hr after, genomic DNA was collected from cell culture. Then, genomic DNA were denatured with 0.1 N NaOH and spotted on nitrocellulose blotting membranes (PALL Life Sciences). The membrane was baked at 80 °C and then blocked in 5% skimmed milk in TBS containing 0.1% Tween 20 (TBST) for 1 h at room temperature. The membranes were then incubated with 1:10000 dilution of 5h-mC (Active Motif) overnight at 4 °C. After three washes with TBST, membranes were incubated with 1:2000 dilution of HRP-conjugated anti-rabbit. The membranes were then washed with TBST followed by TBS, and then treated with ECL.

Quantification of 5-hmC: Genomic DNA was extracted from lentiviral infected primary cortical neurons after KCl treatment. The global level of 5-hmC was assessed using a MethylFlash Hydroxymethylated DNA Quantification Kit (Epigentek). The procedure was followed according to the manufacturer's instructions.

Supplemental Data



Supplenmental Figure 1. Tet1 and Tet3 validation. a) Representative image of viral expression in N2A cells (6 days in vitro) after transfection with Tet1 and Tet3 shRNA. b) We achieved 70% knockdown of Tet1 mRNA after transfection of N2A cells, and 80% knockdown of Tet3 mRNA after transfection of N2A cells with either Tet1 or Tet3 shRNA, respectively (n=3 per group, $F_{2,8} = 53.52$, $P<.0001$, Tukey's posthoc test FG12hH1 vs Tet1 shRNA, ***$p<0.0001$; FG12hH1 vs Tet3 shRNA ***$p<0.0001$). c) There was a 20% reduction in global 5-hmC in primary cortical neurons after Tet3 shRNA transfection. (n=3, $t_4=2.155$ $p<.05$). d) Representative image of global decrease in 5-hmC, in primary cortical neurons, by Tet3 knockdown. e) Representative image of Tet1 and Tet3 shRNA viral expression, in vivo, after 7 days transfection. f) We acheived 40% knockdown of Tet1 mRNA and 50% knockdown of Tet3 mRNA after either Tet1 or Tet3 shRNA lentiviral transfection in vivo (n=3, per group, $F_{2,8} = 28.80$, $P<.0008$, Tukey's post-hoc test FG12hH1 vs Tet1 shRNA, **$p<0.005$; FG12hH1 vs Tet3 shRNA ***$p<0.0001$).



Supplemental Figure 2. In the presence of the NMDA antagonist MK-801, which impairs the acquisition of fear extinction memory, extinction training does not lead to increased Tet3 mRNA expression (n=4 per group, $F_{3, 15}$=7.243, p<.005; Dunnett's posthoc test FC-No EXT Saline vs. all other groups, Error bars represent S.E.M. **P<.01)

Sample ID	Total reads	Properly aligned reads		Reads with high mapping-quality	
	(n)	(n)	(%)	(n)	(%)
Biological replicates and input for CXT samples:					
S1_M1	82813682	76849866	92.80%	66661234	80.50%
S1_M2	112054006	104318108	93.10%	90976550	81.19%
S1_M3	59739794	56023256	93.78%	49547718	82.94%
S1_M4	54605460	51178904	93.72%	45163600	82.71%
S1_M5	48934858	45994296	93.99%	40684262	83.14%
S1_M6	63559930	59940010	94.30%	53261106	83.80%
S1_M7	66977022	60779026	90.75%	53414666	79.75%
S1_M8	114348778	104280004	91.19%	92573542	80.96%
S1_input	52949598	49040590	92.62%	43874848	82.86%
Biological replicates and input for FC samples:					
S2_M1	122457440	114445032	93.46%	99948412	81.62%
S2_M2	45702306	43048230	94.19%	38428530	84.08%
S2_M3	64711214	61466604	94.99%	55153470	85.23%
S2_M4	63585774	60068268	94.47%	53036188	83.41%
S2_M5	83060502	78451488	94.45%	69840072	84.08%
S2_M6	30503326	28245458	92.60%	24977124	81.88%
S2_M7	103345940	93884592	90.84%	83713524	81.00%
S2_M8	54905478	50835630	92.59%	45207068	82.34%
S2_input	57599848	54385364	94.42%	48429596	84.08%
Biological replicates and input for FC-No EXT samples:					
S3_M1	100762130	93229072	92.52%	80232808	79.63%
S3_M2	97076064	91527160	94.28%	81443088	83.90%
S3_M3	42275882	39912288	94.41%	35273374	83.44%
S3_M4	41424246	39248234	94.75%	34851572	84.13%
S3_M5	58448422	55488390	94.94%	49565766	84.80%
S3_M6	59368876	55705588	93.83%	49861462	83.99%
S3_M7	66571246	61070398	91.74%	53965576	81.06%
S3_M8	76244754	70190044	92.06%	61900114	81.19%
S3_input	67750382	63051834	93.06%	56247886	83.02%
Biological replicates and input for EXT samples:					
S4_M1	62836406	58995126	93.89%	51822052	82.47%
S4_M2	74263672	69772052	93.95%	61443168	82.74%
S4_M3	83581306	79208682	94.77%	70545828	84.40%
S4_M4	76287798	71795594	94.11%	63422870	83.14%
S4_M5	61068024	57100826	93.50%	51253448	83.93%
S4_M6	73549352	68575804	93.24%	60444720	82.18%
S4_M7	86609390	81127560	93.67%	72318390	83.50%
S4_M8	82921966	77294708	93.21%	69141852	83.38%
S4_input	45113746	41243620	91.42%	36548098	81.01%

Supplementary Table 1. The sequencing statistics for 8 biological replicates and input DNA in each condition. Shown are the number of raw sequencing reads and the number of successfully aligned reads, with the read length of 101bp. “Properly aligned reads” represents the paired-end reads are mapped to the reference genome in a proper pair, and “reads with high mapping-quality” denotes paired-end reads are mapped in a proper pair with at least Q20 of the mapping quality.

Categories	Peaks before normalisation		Peaks after normalisation	
	(n)	(%)	(n)	(%)
Peaks identified in CXT samples:				
Promoter	1928	0.91%	4140	16.05%
5' UTR	114	0.05%	2021	7.84%
3' UTR	627	0.30%	2834	10.99%
CDS	377	0.18%	1157	4.49%
Intron	57812	27.23%	6513	25.26%
Intergenic	151453	71.34%	9121	35.37%
Peaks identified in FC samples:				
Promoter	1173	0.86%	2518	14.74%
5' UTR	81	0.06%	1436	8.41%
3' UTR	478	0.35%	2161	12.65%
CDS	301	0.22%	923	5.41%
Intron	37951	27.95%	4275	25.03%
Intergenic	95798	70.55%	5769	33.77%
Peaks identified in FC-No EXT samples:				
Promoter	2214	0.77%	4754	13.61%
5' UTR	175	0.06%	3103	8.89%
3' UTR	978	0.34%	4421	12.66%
CDS	463	0.16%	1421	4.07%
Intron	77253	26.74%	8703	24.92%
Intergenic	207861	71.94%	12518	35.85%
Peaks identified in EXT samples:				
Promoter	1493	6.53%	3206	23.64%
5' UTR	172	0.75%	3049	22.49%
3' UTR	452	1.98%	2043	15.07%
CDS	1159	5.07%	3557	26.24%
Intron	9989	43.72%	1125	8.30%
Intergenic	9584	41.94%	577	4.26%

Supplementary Table 2. Shown is the absolute amount as well as the proportion of peaks in each genomic category for each condition. To observe the unbiased distribution of peaks in each genomic category, we normalised the total length of each genomic category to the length of 100 Mbp and the number of peaks was proportionally scaled in each category. Since the genomic categories is mainly based on the known gene models, only peaks within chromosome 1 ~ 19, X and Y were considered for this analysis.

	Total peaks (n)	Peaks overlap with DNase1 hypersensitive regions (n)	(%)
CXT	215170	11810	5.49%
FC	137116	6898	5.03%
FC-No EXT	292064	13885	4.75%
EXT	23084	6278	27.20%

Supplementary Table 3. Shown are total numbers of peaks overlapping with DNase1 hypersensitive regions in each condition.

CXT		
	Number	%
Total peaks	215170	
Peaks overlap with Dnase1	11810	5.49%
Individual Samples		
s1M1	4485	5.00%
s1M2	3685	5.00%
s1M3	189	10.00%
s1M4	702	12.00%
s1M5	780	12.00%
s1M6	193	11.00%
s1M7	1462	4.00%
s1M8	314	4.00%

FC		
	Number	%
Total peaks	137116	
Peaks overlap with Dnase1	6898	5.03%
Individual Samples		
s2M1	1888	4.00%
s2M2	811	9.00%
s2M3	151	8.00%
s2M4	208	7.00%
s2M5	47	7.00%
s2M6	2161	6.00%
s2M7	35	5.00%
s2M8	1597	4.00%

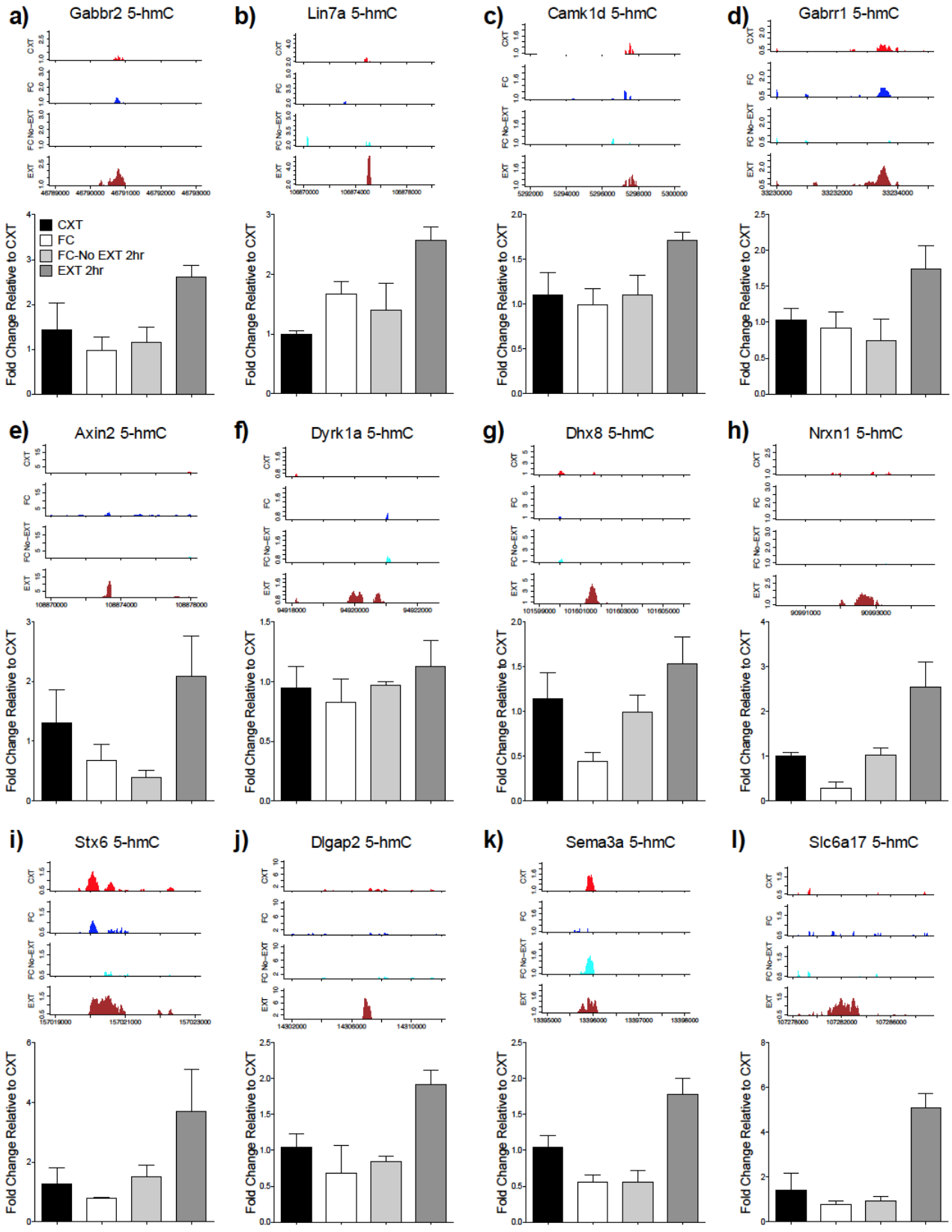
FC-No EXT		
	Number	%
Total peaks	292064	
Peaks overlap with Dnase1	13885	4.75%
Individual Samples		
s3M1	4616	5.00%
s3M2	2761	5.00%
s3M3	873	8.00%
s3M4	898	9.00%
s3M5	413	9.00%
s3M6	341	7.00%
s3M7	2481	4.00%
s3M8	1502	3.00%

EXT		
	Number	%
Total peaks	23084	
Peaks overlap with Dnase1	6278	27.20%
Individual Samples		
s4M1	184	12.00%
s4M2	259	7.00%
s4M3	78	15.00%
s4M4	130	15.00%
s4M5	4894	40.00%
s4M6	409	20.00%
s4M7	137	18.00%
s4M8	187	18.00%

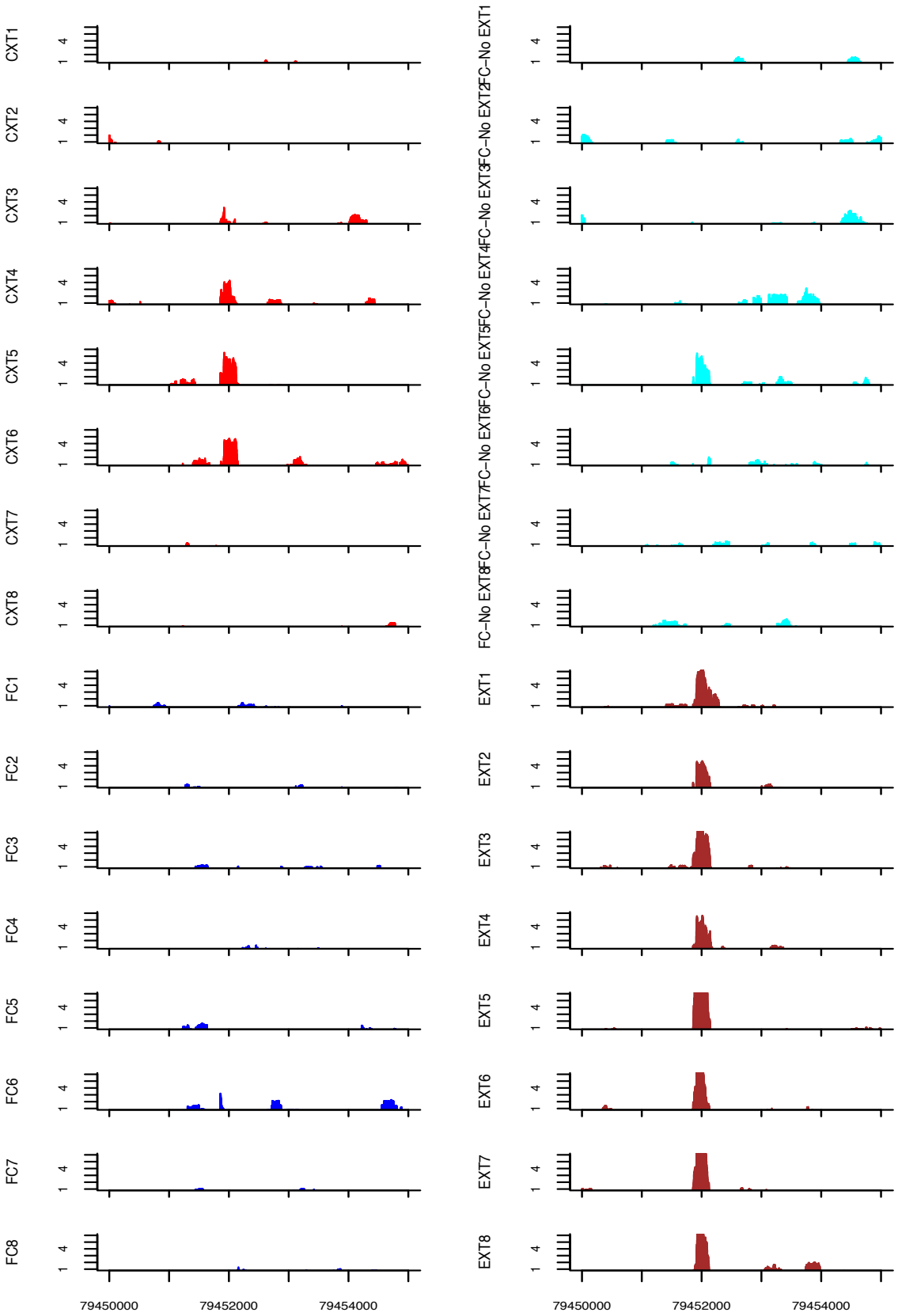
Supplemental Table 4. Absolute number of peaks, as well as the proportion of peaks overlapping with DNase1-hypersensitive regions, in each individual sample.

Chromosome	Coordinates	Accession	Gene	Locus	Function
Chr 10	106874444 - 106875435	NM_001033223	Lin7a	Distal promoter	Ensures the proper localization of GRIN2B (subunit 2B of the NMDA receptor) to neuronal postsynaptic density and may function in localizing synaptic vesicles at synapses where it is recruited by beta-catenin and cadherin
Chr 12	79451546 - 79452206	NM_172952	Gphn	Intronic region	Anchors inhibitory neurotransmitter receptors to the postsynaptic cytoskeleton via high affinity binding to receptor subunit domain and tubulin dimers
Chr 4	46790275 - 46791304	NM_001081141	Gabbr2	Intronic region	Inhibits neuronal activity through G protein-coupled second-messenger systems, which regulate the release of neurotransmitters, and the activity of ion channels and adenylyl cyclase
Chr 4	33232743 - 33234064	NM_008075	Gabrr1	Intronic region	Variation in Gabrr1 is associated with bipolar disorder and schizophrenia
Chr 3	107280962 -107282861	NM_172271	Slc6a17	Intronic region	Acts as a transporter for neurotransmitters
Chr 8	14306397 - 14307611	NM_172910	Dlgap2	Intronic region	Plays a role in the molecular organization of synapses and in neuronal cell signalling. Also, this gene is biallelically expressed in the brain
Chr 1	157019773 - 157021196	NM_021433	Stx6	Intronic region	Involved in intracellular vesicle trafficking
Chr 2	5297035 - 5298239	NM_177343	Camk1d	Intronic region	Regulates basal dendritic growth of neurons
Chr 16	94919771 - 94921197	NM_001113389	Dyrk1a	Intragenic region	A strong candidate gene for learning defects associated with Down syndrome
Chr 11	101600970 - 101602074	NM_144831	Dhx8	Intronic region	This protein facilitates nuclear export of spliced mRNA by releasing the RNA from the spliceosome
Chr 17	90991625 - 90993660	NM_177284	Nrxn1	Intronic region	Neurexin1 is a neuronal membrane bound protein that is involved in cell recognition and cell adhesion. Also, it may play a role in the formation and maintenance of synaptic junctions
Chr 11	108872621-108873849	NM_015732	Axin2	Intragenic region	Plays an important role in the regulation of beta-catenin stability within the Wnt signaling pathway
Chr 5	13395512- 13396352	NM_001243072	Sema3a	Proximal promoter	Stimulates the growth of apical dendrites

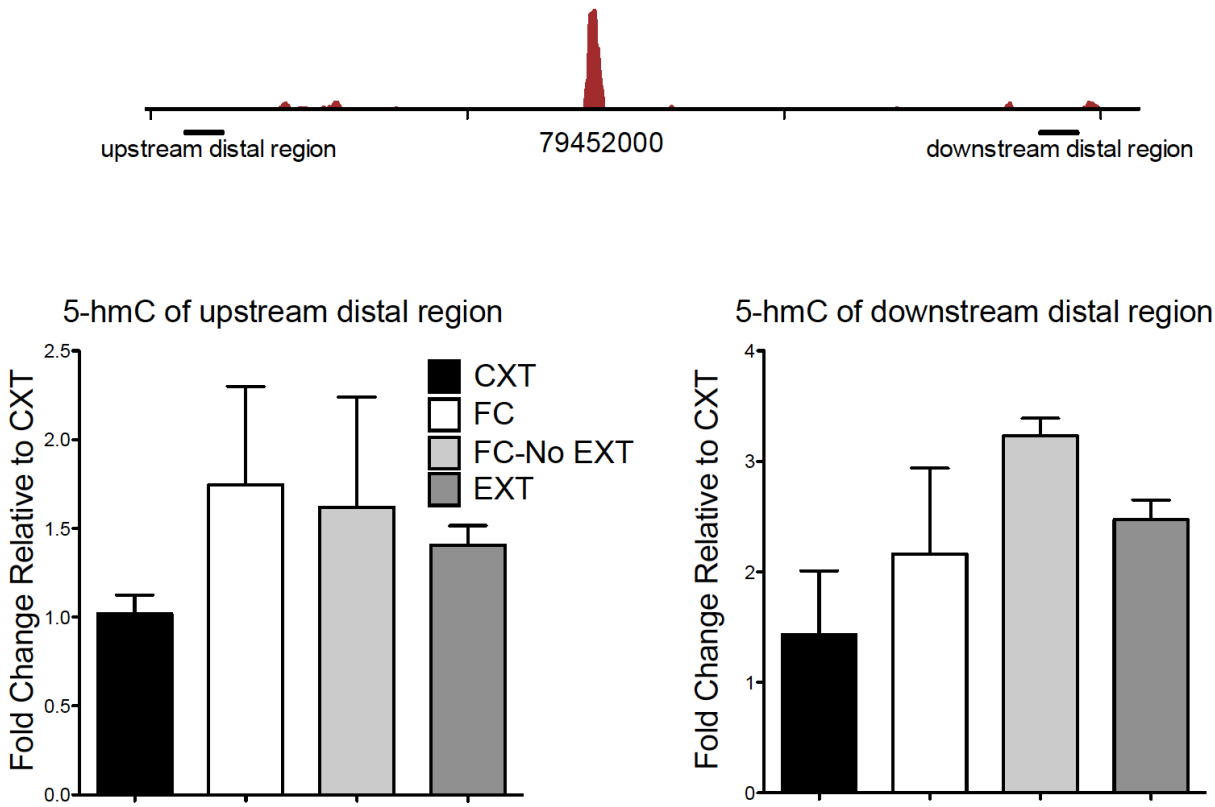
Supplemental Table 5. Candidate gene list selected based on proximity to 5-hmC peaks occurring after extinction training.



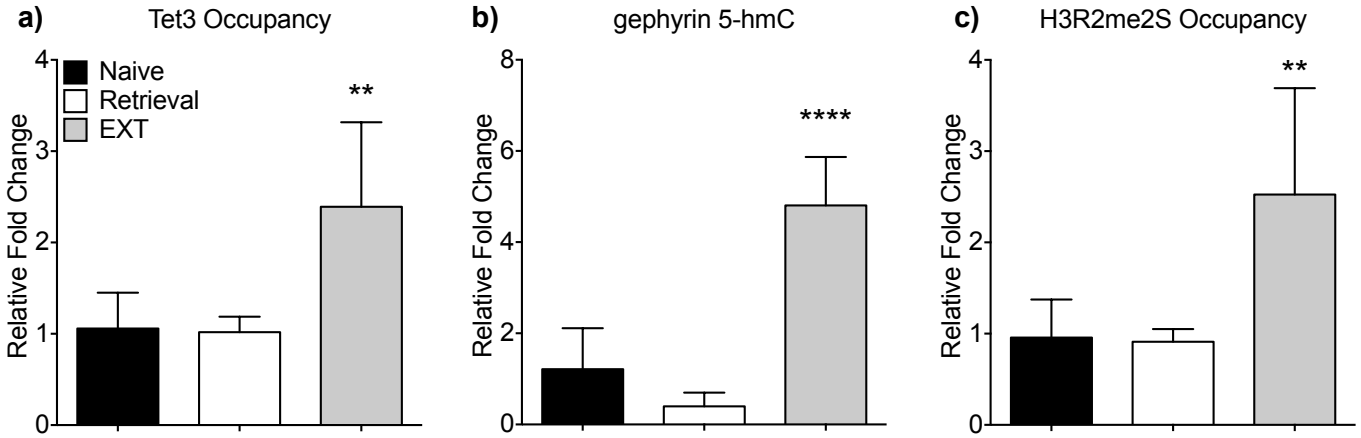
Supplemental Figure 3. Validation of twelve candidate genes associated with 5-hmC enrichment by hMeDIP-qPCR. For each panel, top: plots for normalized base counts from sequencing data; bottom: hMeDIP-qPCR result.



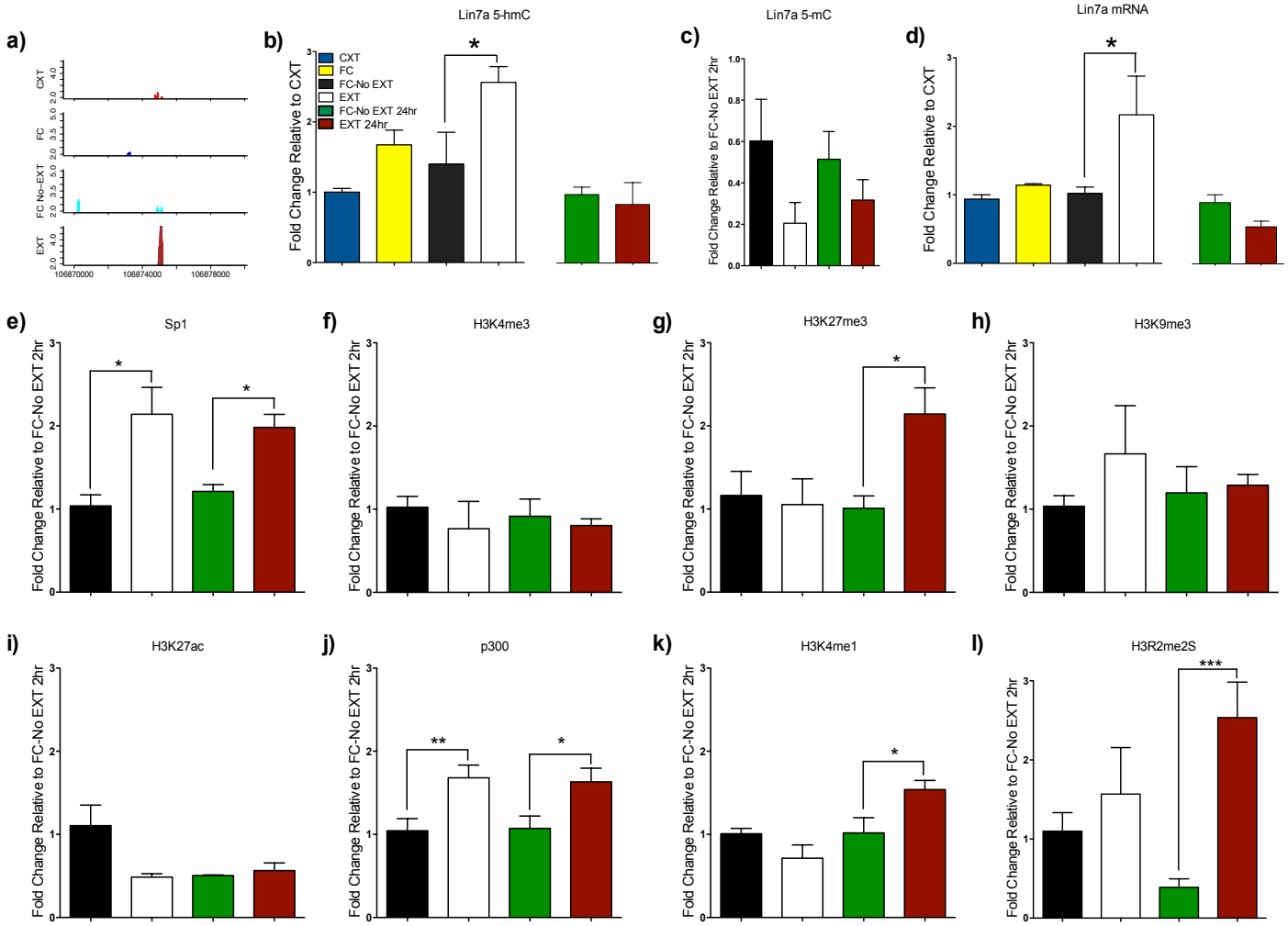
Supplenmental Figure 4. Plots for normalized base counts of gephyrin genomic region from each individual sample.



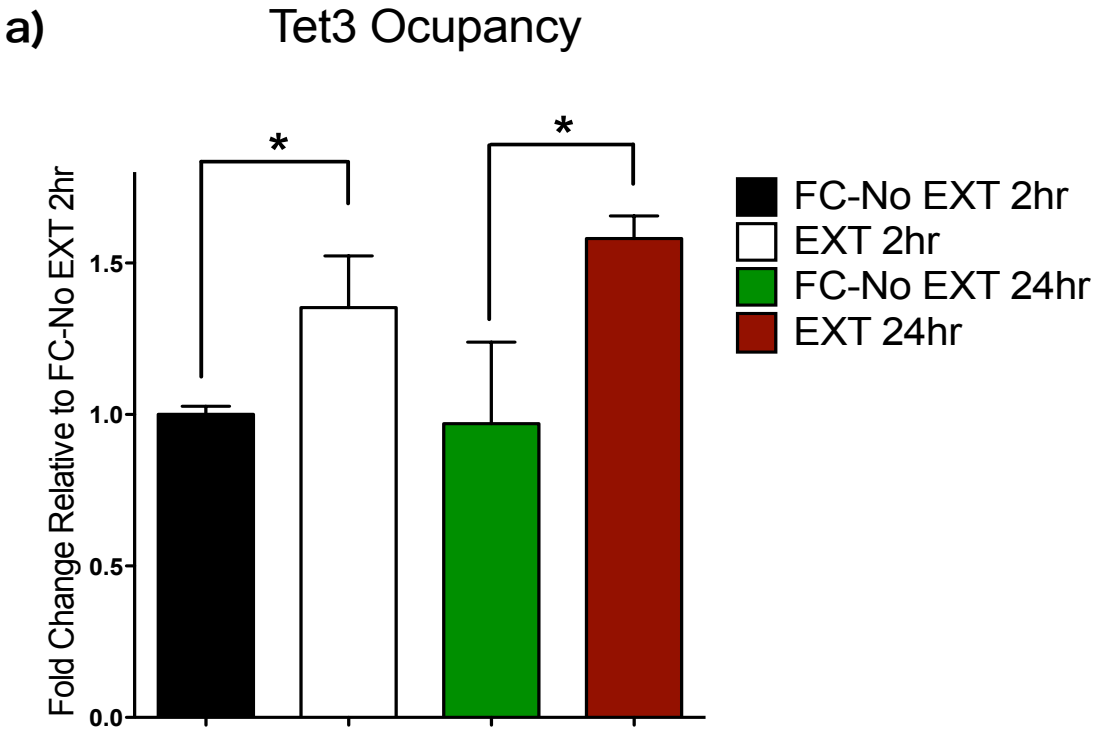
Supplemental Figure 5. There is no accumulation of 5-hmC within regions upstream or downstream of the gephyrin intronic locus.

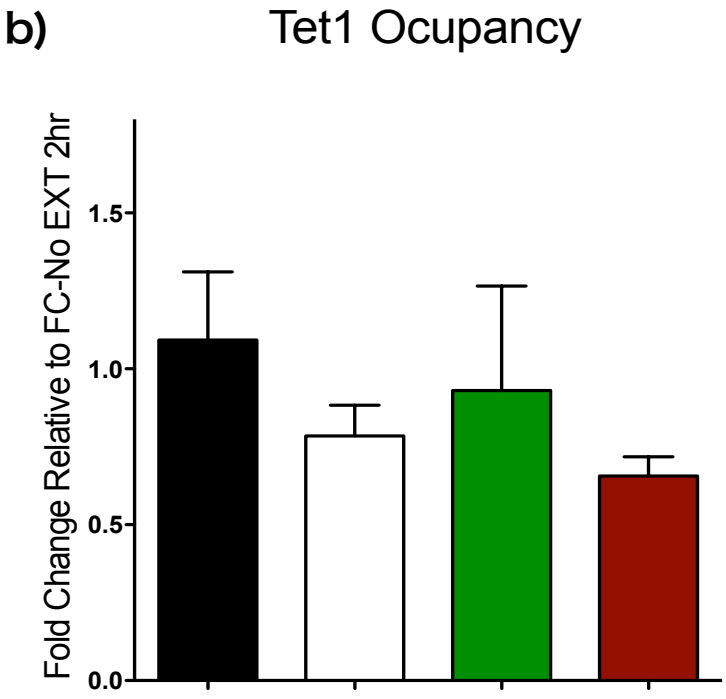


Supplemental Figure 6. A 30 CS fear extinction training protocol induces epigenetic modifications that are distinct from a single CS evoked retrieval- or reconsolidation-induced epigenetic state. Relative to naïve or 1 CS exposure, extinction-trained mice exhibit a significant increase in a) Tet3 occupancy at the gephrin locus (n=6/group $F_{2,15}$=10.54, p<.01, Tukey's post-hoc analysis naïve and retrieval vs EXT, **p<.01), b) the accumulation of 5-hmC at the gephyrin locus (n=6/group $F_{2,15}$=48.74, p<.0001, Tukey's post-hoc analysis naïve and retrieval vs EXT, ****p<.0001), and c) H3R2me2S occupancy at the gephryin locus (n=5-6/group $F_{2,13}$=8.82, p<.01, Tukey's post-hoc analysis naïve and retrieval vs EXT, **p<.01).



Supplemental Figure 7. Fear extinction learning-mediated accumulation of 5-hmC within the distal promoter region of Lin7a is associated with altered chromatin environment. a) Shown is the normalized plot of coverage for the 5-hmC peak in the distal promoter region of Lin7a. b) Significant enrichment of 5-hmC within the distal promoter region of Lin7a occurs after fear extinction training (n = 3-4 per group, 2 hrs $F_{3,14}$ = 5.04, p<.01; Tukey's post-hoc analysis FC-No EXT 2hr vs EXT 2 hr, *p<.02). c) No significant change in methylation accumulation around the distal promoter region of Lin7a after training. Fear extinction led to a transient increase in gephyrin mRNA expression (d; n = 5-6 per group, $F_{3,19}$ = 4.67, p<.05; Tukey's post-hoc analysis FC-No EXT 2hr vs EXT 2hr, *p<.05), a persistent increase in the occupancy of Sp1 (e; n = 4-5 per group, $F_{3,16}$ = 5.88, p<.05; Tukey's post-hoc analysis FC-No EXT 2hr vs EXT 2hr, *p<.05; FC-No EXT 24hr vs EXT 24hr, *p<.05), no effect on $H3K4^{me3}$(f), a transient increase of $H3K27^{me3}$ 24hr post-extinction training (g; t_6 = 3.269 p<.05), no effect on heterochromatin mark $H3K9^{me3}$ (h), a decrease of $H3K27^{ac}$ occupancy after fear condition and fear extinction training (i), a persistent increase in presence of the enhancer-related elements p300 (j; 2 hrs, t_8 = 3.019 p<.001; 24 hrs, t_8 = 2.54 p<.05), an increase of $H3K4^{me1}$ 24hrs post-extinction training (k; t_8 = 2.454 p<.05), and an increase in $H3R2^{me2s}$ (l; t_4 = 4.643 p<.0001) within the infralimbic prefrontal cortex. (Error bars represent S.E.M.)





Supplemental Figure 8. Extinction learning leads to an increase in Tet3 occupancy at the Lin7a promoter. a) Fear extinction learning leads to a persistent increase in Tet3 occupancy in the distal promoter of Lin7a (2 hrs, $t_6 = 2.03$ $p<.05$; 24 hrs, $t_8 = 2.18$ $p<.05$). b) There is no significant change in Tet1 occupancy in the Lin7a distal promoter region.

Antibodies

Name	Host	cat#	Company
H3K4Me3	rabbit	39915	active motif
H3K27Me3	rabbit	39155	active motif
H3K4Me1	rabbit	39297	active motif
H3R2Me2s	rabbit	39703	active motif
H2A.Z	rabbit	ab4147	Abcam
Sp1	rabbit	07-645	Millipore
Tet1	rabbit	09-872	Millipore
Tet3-chIP	rabbit	SC-139186	Santa Cruz
Tet3-Immuno	rabbit	ab139311	Abcam
NeuN	mouse	MAB377	Mellipore

Primers

qPCR Primer	
Gphn LP	CAACCACGACCATCAAATCCG
Gphn RP	CCAACAAAGAAGGATCTTGGACA
Pgk LP	TGCACGCTTCAAAAGCGCACG
Pgk RP	AAGTCCACCCTCATCACGACCC
Tet1 LP	GAGCCTGTTCCTCGATGTGG
Tet1 RP	CAAACCCACCTGAGGCTGTT
Tet3 LP	TCCGGATTGAGAAGGTCATC
Tet3 RP	CCAGGCCAGGATCAAGATAA
Gabbr2-hmeDIP-LP	TCCAGGAAGGTGACTACAGTG
Gabbr2-hmeDIP-RP	GCACCCAGTCCTGGATACAT
Dlgap2-hmeDIP-LP	TGATCGTGAGAGGCAAGATG
Dlgap2-hmeDIP-RP	AATCTCCTCTCTGCCCACTG
Camk1d-hmeDIP-LP	AGTTTGGGAGAGGGGAAAAA
Camk1d-hmeDIP-RP	AGGAGTCAGCCACCAACACT
stx6-hmeDIP-LP	AGGGATGTGAAGGACCCTCT
stx6-hmeDIP-RP	TTCCCAGCAGATGCAGAAC
AXIN2-hmeDIP-LP	CTGGATTTGGGGATCTGAAC
AXIN2-hmeDIP-RP	GAACTTTGAAGGCCGAAGTG
Sema3a-hmeDIP-LP	TTTTGGAGGAGGGAGTTTGA
Sema3a-hmeDIP-RP	GGCAGATTGGTCATTCAGTG
Gabrr1-hmeDIP-LP	CTTTCCTGCCAAGCTCATTC
Gabrr1-hmeDIP-RP	CACGTCTCTGTGAGGAGCAC
Lin7a-hmeDIP-LP	ATTCGCAATGCACACACTTT
Lin7a-hmeDIP-RP	TGGACAGGACGGTGTGAATA
Slc6A-hmeDIP17 LP	GGGGTATGGCTGGACTGATG
Slc6A17-hmeDIP RP	CCACTGTGTCTGCATCACCT
Dhx8-hmeDIP LP1	TTTCTCTCAGGAAGCGGTGG
Dhx8-hmeDIP RP1	GCTGTGGACACAGACAGACA
Nrxn1-hmeDIP LP1	AACAAGTGAGGTTGGTGGCT
Nrxn1-hmeDIP RP1	CTCAAACTGAAATTGACCCCACA
Gphn-hmeDIP LP	TAGAACACTGCCTTGTCCCC
Gphn-hmeDIP RP	TCACCATAGCAACCCGTCTT
Dyrk1a-hmeDIP LP	ATGGGAGGCACTTTGTGAGG
Dyrk1a-hmeDIP RP	CTACAGCTGATACCCAGCCAG