**Suppl. Fig. S1. m6dA is abundant in the neuronal genome and accumulates in response to neural activation.**  (A) Experimental plan to determine whether m6dA is a functionally relevant base modification in neurons. (B) Representative LC-MS/MS chromatograms: Control compound (m6dA standard) and isolated RNase-treated gDNA samples, which were extracted from primary cortical neurons, were used to directly quantify the global level of m6dA (~46 per 106 dNs) by LC-MS/MS. (C) Dpn1 enzyme cuts DNA specifically at methylated adenine in GATC linker sequences; using Dpn1 digestion reveals the abundance of m6dA in DNA derived from primary cortical neurons, but not in DNA from liver (from the left; lane 1-3: Dpn1 digested DNA from mouse primary cortical neurons, lane 4: Dpn1 digested DNA from mouse liver, lane 5: DNA ladder, lane 6: Dpn1 digested DNA *from* e. coli, lane 7: undigested DNA from *e. coli*. (D) Dot blot assay shows global accumulation of m6dA in stimulated primary cortical neurons (n=3/group, 7DIV, 20mM KCl, 7 hours, \*p<.05). (Error bars represent SEM)

**Suppl. Fig. S2. Flow cytometry scatterplots demonstrating enriched for activated neurons using Arc and NeuN as tags.** (A) Liver cells were used as a negative control to calibrate FACS sorting parameters. (B) Distinct group of neurons express both Arc and NeuN.

**Suppl. Fig. S3. Genomic distribution of DpnI-seq.** m6da accumulates in the promoter, 5’UTR and CDS.

**Suppl. Fig. S4. DpnI-seq data indicating a highly specific accumulation of m6dA at the GATC site within the BDNF P4 promoter.**

**Suppl. Fig. S5. m6dA methyltransferase N6amt1 mRNA expression is inducible and increases in cortical neurons under neuronal activation** (A) Activity-induced N6amt1 mRNA expression in primary cortical neurons, *in vitro* (\*\*p<.01). (B) No effect of neuronal stimulation on N6amt2 mRNA expression. **(C)(D) Enzymatic activity data.**

**Suppl. Fig. S6. N6amt1-mediated and m6dA-related changes in chromatin and transcriptional machinery do not occur at a distal GATC sequence in the BDNF P4 promoter.** KCl-induced depolarization did not affect (A) N6amt1 occupancy (B) the deposition of m6dA, or (C-E) the presence of H3K4me3, YY1 and RNA Pol II at the distal GATC site within the BDNF P4 promoter (n=3/group, Error bars represent SEM).

**Suppl. Fig. S7. N6amt1-mediated accumulation of m6dA is required for activity-induced bdnf exon IV mRNA expression.** N6amt1 knockdown (A-B)eliminatedthe activity-induced increase in N6amt1 occupancy (two-way ANOVA F1,8 = 22.31, p<.01; Fisher’s posthoc test; Scrambled Control KCl+ vs. N6amt1 shRNA KCl+, \*\*p<.01.), and m6dA deposition (two-way ANOVA F1,8 = 20.27, p<.005; Fisher’s posthoc test; Scrambled Control KCl+ vs. N6amt1 shRNA KCl+, \*\*p<.01), at the bdnf exon IV locus, (C) reduced H3K4me3 (two-way ANOVA F1,8 = 21.82, p<.001; Fisher’s posthoc test; Scrambled Control KCl+ vs. N6amt1 shRNA KCl+, \*\*\*\*p<.0001.), (D) decreased recruitment of YY1 (two-way ANOVA F1,8 = 5.299, p=0.0503; Fisher’s posthoc test; Scramble Control KCl+ vs. N6amt1 shRNA KCl+, \*p<.05), (E) diminished Pol II occupancy (two-way ANOVA F1,8 = 37.21, p<.001; Fisher’s posthoc test; Scramble Control KCl+ vs. N6amt1 shRNA KCl+, \*\*\*p<.001), and (F) inhibited bdnf exon IV mRNA expression (two-way ANOVA F1,8 = 17.73, p<.005; Fisher’s posthoc test; Scramble Control KCl+ vs. N6amt1 shRNA KCl+, \*\*\*p<.001) (All n=3/group, Error bars represent SEM).

**Supply. Fig. S8. N6amt1 mRNA expression is inducible and increased after fear extinction in ILPFC** (A) Extinction-learning leads to increased expression of N6amt1 in the ILPFC (\*\*p<.01). (B) No effect of learning on N6amt2 expression in the ILPFC. (B-D) No effect of cued-fear extinction learning on N6amt1 and N6amt2 expression in the hippocampus. (All n=3-4/group, Error bars represent SEM).

**Suppl. Fig. S9. N6amt1-mediated and m6dA-related changes in chromatin and transcriptional machinery do not occur at a distal GATC sequence in the BDNF P4 promoter in ILPFC after learning.** KCl-induced depolarization did not affect (A) N6amt1 occupancy (B) the deposition of m6dA, or (C-E) the presence of H3K4me3, YY1 and RNA Pol II at the distal GATC site within the BDNF P4 promoter (n=6-8/group, Error bars represent SEM).

**Suppl. Fig. S10. N6amt1 shRNA inhibits N6amt1 mRNA expression, *in vivo.*** (A) Left: representative image of cannula placement in the ILPFC, Right: transfection of N6amt1 shRNA into the ILPFC. (B) N6amt1 shRNA blocks the induction of N6amt1 mRNA expression following extinction learning (two-way ANOVA, F1,12=11.44,P<.01; Fisher’s posthoc test; Scrambled control EXT vs. N6amt1 shRNA EXT, \*\*\*p<.001, Error bars represent SEM).