**Fig. 1. 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) Dpn1 enzyme cuts DNA specifically at methylated adenine in GATC linker sequences. (C) 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) 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. (E) 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)

**Fig. 2.** **Activity-induced N6amt1 occupancy and the accumulation of m6dA are associated with increased bdnf exon IV mRNA expression.** KCl-induced depolarization leads to (A) an increase in N6amt1 occupancy (\*p<.05), but not N6amt2 (B), (C) increased deposition of m6dA (\*\*p<.01), (D) an increase in m6dA at a GATC site adjacent to the consensus sequence for YY1 (\*p<.05), (E) increased occupancy of the chromatin mark H3K4me3 (\*p<.05), (F) increased recruitment of YY1 proximal to the m6dA modified adenine (\*\*p<.01), (G) a concomitant increase in the presence of Pol II (\*\*p<.01), (H) a correlated increase in the induction of bdnf exon IV mRNA expression (\*\*\*p<.001). (I) A schematic of activity-dependent deposition of m6dA by N6amt1, which occurs in a tightly regulated, and spatiotemporally controlled manner through locus-specific chromatin modification and the recruitment of activating transcription machinery. (All n=3-4/group, Error bars represent SEM)

**Fig. 3. Extinction** **learning-induced accumulation of m6dA is associated with bdnf exon IV mRNA expression.** Fear extinction learning (EXT), relative to mice fear conditioned and exposed to a novel context (FC-No EXT), led to (A) a selective increase in N6amt1 occupancy (\*p<.05), (B) increased m6dA at the previously identified GATC site (\*p<.05), (C) a significant increase in H3K4me3 occupancy (\*p<.05), (D) an increase in the recruitment of YY1 (\*p<.05), (E) an increase in Pol II occupancy (\*\*p<.01). (F) a significant increase in bdnf exon IV mRNA expression within the ILPFC (\*\*p<.01). (All n=6-8/group, Error bars represent SEM)

**Fig. 4. N6amt1-mediated accumulation of m6dA is required for fear extinction memory and for learning-induced bdnf exon IV mRNA expression in the ILPFC.** (A) Schematic of the behavioral protocol used to test effect of lentiviral-mediated knockdown of N6amt1 in the ILPFC on fear extinction memory. (B-C) There was no effect of N6amt1 shRNA on within-session performance during the first 10 conditioned stimulus exposures during fear extinction training. (D) Although there was no effect of N6amt1 shRNA on fear expression in mice that had been fear conditioned and exposed to a novel context without extinction training, N6amt1 knockdown led to a significant impairment in fear extinction memory (\*p<.05). (E-G) N6amt2 knockdown in the ILPFC had no effect on the formation of fear extinction memory (\*p<.05). (All n=8/group, Error bars represent SEM).

**Fig. 5.** **N6amt1 knockdown prevents the learning-induced accumulation of m6dA and related changes in chromatin and transcriptional landscape associated with the BDNF P4 promoter**. Following ILPFC infection with N6amt1 shRNA, N6amt1 shRNA blocked (A) increase of N6amt1 occupancy (\*\*p<.01) and (B) the deposition of m6dA (\*p<.05), or (C) accumulation of H3K4me3 (\*\*p<.01), (D) YY1 (\*\*p<.01) and (E) RNA Pol II (\*\*p<.01) occupancy at the proximal GATC site within the BDNF P4 promoter (F) N6amt1 shRNA inhibited bdnf exon IV expression (\*\*p<.01) (All n=4/group, Error bars represent SEM).

**Suppl. Fig. S1. N6amt1 mRNA expression is inducible and increases in the prefrontal cortex in response to extinction learning.** (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) Extinction-learning leads to increased expression of N6amt1 in the ILPFC (\*\*p<.01). (D) No effect of learning on N6amt2 expression in the ILPFC. (All n=3-4/group, Error bars represent SEM).

**Suppl. Fig. S2. Overexpression of N6amt1 promotes the global accumulation of m6A.** (A) Validation of N6amt1ox protein expression by flow cytometry (one way ANOVA F2,9 =2.925, p<.05, Tukey’s posthoc test; scrambled control vs. N6amt1ox, \*p<.05). (B) Validation of n6amt1 overexpression by western blot and (C) showed a more than 2 fold increase in protein level (\*\*\*\*p<.0001). (D) Overexpression of N6amt1 in HEK cells induced the global level of m6dA. (E) Co-transfected overexpression and shRNA of N6amt1 blocked the global induction of m6dA in PCN. (F) At baseline, overexpression of N6amt1 did not promote the accumulation of m6dA at the GATC site within BDNF P4 locus; however, co-transfection of N6amt1ox and N6amt1 shRNA blocked the KCl-induced accumulation of m6dA (\*p<.05; \*\*p<0.01. All n=3 per/group, Error bars represent SEM).

**Suppl. Fig. S3. 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. S4. N6amt1 shRNA inhibits N6amt1 mRNA expression *in vitro*.** Reduced N6amt1 mRNA expression following lentiviral-mediated knockdown of N6amt1 (\*\*\*\*p< .0001, n=3/group, Error bars represent SEM).

**Suppl. Fig. S5. N6amt1-mediated accumulation of m6dA is required for activity-induced bdnf exon IV mRNA expression.** (A-B)N6amt1 knockdown 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 = 39.50, p<.001; Fisher’s posthoc test; Scrambled Control KCl+ vs. N6amt1 shRNA KCl+, \*p<.05), 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<.01.), (D) decreased the recruitment of YY1 (two-way ANOVA F1,8 = 5.299, p<.05; Fisher’s posthoc test; Scrambled 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; Scrambled Control KCl+ vs. N6amt1 shRNA KCl+, \*\*\*p<.001), and (F) inhibited bdnf exon IV mRNA expression (two-way ANOVA F1,8 = 17.73, p<.01; Fisher’s posthoc test; Scrambled Control KCl+ vs. N6amt1 shRNA KCl+, \*\*\*p<.001) (All n=3/group, Error bars represent SEM).

**Suppl. Fig. S6. 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 (n=4/group, \*p<.05, Error bars represent SEM).

**Suppl. Fig. S7. N6amt1 knockdown in the PLPFC does not affect the formation of fear extinction memory.** (A) Schematic of the behavioral protocol used to test effect of lentiviral-mediated knockdown of N6amt1 in the PLPFC on fear extinction memory. (B-C) There was no effect of N6amt1 shRNA on within-session performance during the first 10 conditioned stimulus exposures during fear extinction training. (D) There was no effect of N6amt1 shRNA on fear expression in mice that had been fear conditioned and exposed to a novel context without extinction training. Relative to scrambled control FC-NO EXT trained mice, N6amt1 knockdown mice showed normal extinction memory (\*p<.05) (All n=4-6 per group. Error bars represent SEM).

**Suppl. Fig. S8. Learning induced N6amt1-mediated and m6dA-related changes in chromatin and transcriptional machinery do not occur at a distal GATC sequence in the BDNF P4 promoter.** Fear extinction 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) in ILPFC.