genomic region proximal to the DMR of interest. (a) There was a trend towards the overall decreased expression of *Cdh13*, $F_{3,26}$ =2.53, *p*=0.07. (b) However, this is likely due to the regulation of non-coding transcripts of *Cdh13*, as the expression of the protein-coding transcript was not significantly altered following cocaine self-administration; $F_{3,26}$ = 1.35, not significant. (c). Subsequent to self-administration, overall *Cpeb4* expression increased in all treatment groups; $F_{3,26}$ = 5.96, *p*<0.01. (d) Nevertheless, the expression of the common isoform, *Cpeb4-001*, displayed a different pattern of expression relative to that in naïve animals, with the sole significant difference in expression being between animals subject to relapse testing at 21 days of abstinence compared to those that were not ($F_{3,26}$ = 4.32, *p*<0.01, IVSA 21 R vs. IVSA 21 NR, p<0.01, Holm-Sidak *post hoc* test). (e) When the collective expression of all protein-coding isoforms of *Mctp1* was explored, the sole significant difference was again between animals subject to relapse testing at 21 days and those that were not ($F_{3,26}$ = 3.62, *p*<0.05, Holm-Sidak *post hoc*, IVSA 21 R vs. IVSA 21 NR, *p*<0.05). (f) However, when explored individually, the expression of one protein-coding isoform (*Mctp1-001*, Ensembl 37) was persistently decreased at all time points relative to naïve animals; $F_{3,26}$= 4.07, *p*<0.05. Data are displayed as mean ± SEM; all p-values are derived from Holm-Sidak *post hoc* tests relative to naïve animals except where indicated, * p<0.05, ** p<0.01.

**Supplemental Figure 1. Mean normalized reads at validated DMRs.**

Plots of mean normalized read counts from sequencing surrounding DMRs that have been validated by sequencing. (a) A plot of the normalized mean read counts surrounding a persistently methylated DMR located within an intron of *Kctd16* (potassium channel tetramerization domain containing 16), which is centered on chr 18: 4046790 (mm9 reference genome) (b) A plot of the normalized mean read counts surrounding a persistently demethylated DMR located within an exon of *Golgb1* (golgin b1) and centered on chr 16: 36915248 (c) A plot of the normalized mean read counts surrounding a persistently demethylated intergenic DMR located proximal to *Snw1* (snw1 domain containing gene) and centered on chr 12: 88821123 (d) A plot of the normalized mean read counts surrounding an abstinence-associated DMR that was located within an intron of *Glra1* (glycine receptor subunit alpha 1) and demethylated during abstinence. P-values are relative to naïve animals and are FDR-corrected p-values derived from a Benjamini-Hochberg correction following

Student's t-tests. Y-axis values are the mean number of aligned reads for each treatment group; X-axis values are location within the chromosome.

**Supplemental Figure 2. CDH13 methylation and expression**

Some DMRs that did not pass the FDR-based criterion were nevertheless validated, including an increase in methylation at the locus within *Cdh13* (cadherin 13); $F_{3,16} = 6.13$, $p<0.01$. (j) There was a trend towards the reduction of *Cdh13* expression; $F_{3,26} = 2.53$, $p=0.07$. All data are displayed as mean ± SEM, and p-values are derived from Holm-Sidak *post hoc* tests relative to naïve animals, except where specifically indicated. * $p<0.05$, ** $p<0.01$.