SUPPLEMENTAL MATERIALS

Epigenetic Alterations in Cytochrome P450 Oxidoreductase (*Por*) in Sperm of Rats Exposed to Tetrahydrocannabinol (THC)

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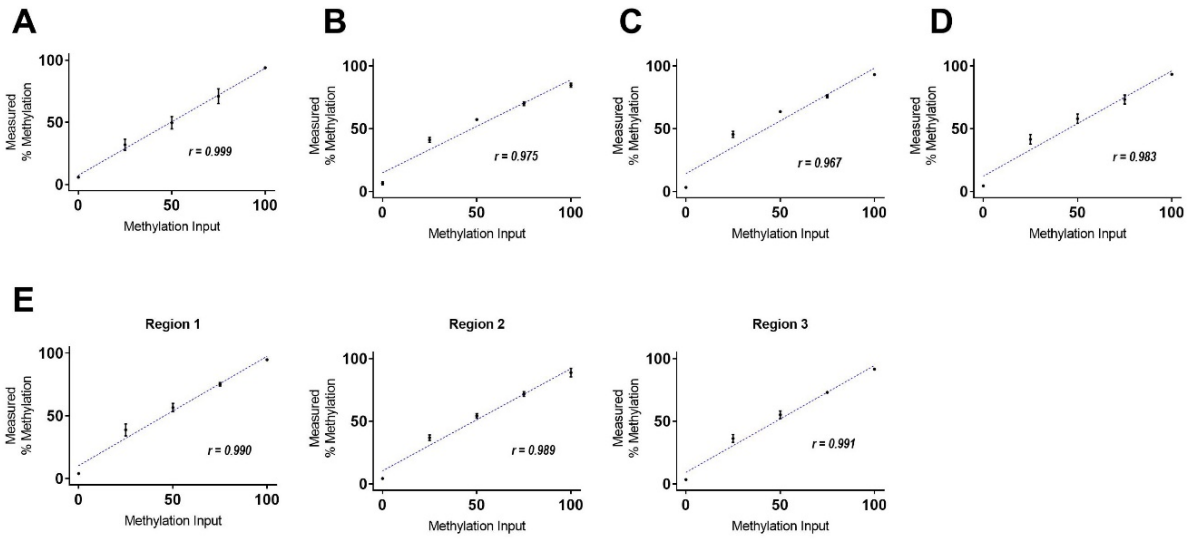

Supplemental Figure 1. Validation curves, showing the mean +/- SD for triplicate measures, for bisulfite pyrosequencing assays of *Adora2a* (A), *Cbx6* (B), *Hipk4* (C), *Mag* (D), and *Por* (three regions) (E). Graphs compare expected DNA methylation on the x axis (based on known methylation percentage of commercially-available unmethylated and methylated rat DNAs) and observed DNA methylation on the Y axis.

Supplemental Table 1: PCR and Pyrosequencing Conditions

Gene	Primer Sequences	PCR Amplicon	PCR Conditions (touchdown annealing temperatures, in ◦C)
Adora2a	F: (Btn)GGTTGATATTGTAGTGGGTGT R: TCCCAAAACCCTACCCTCTATAC S: AATCTAAACCTTATACAC	250 bp	64-61-58
Cbx6	F: GGGAAGTTTTGAGGTTTTATTT R: (Btn)CAAACCAAAATTACCCCCTTATA S: GTTTTTAGTATTAGTGATGT	232 bp	65-62-59
Hipk4	F: AAGGTTTTAGGAATGTAGAGAGT R: (Btn)AATAACTACTTTAAATAAAACCAACATA S: ATTTGAGTTTGTAGGAGGT	132 bp	61-59-56
Mag	F: (Btn)TTGTTTTTATAATTTTTTTGGAAT R: AATAAAACTTAAACCTAACCCC S: CTAAATACACAAACTCCCTC	220 bp	60-58-56
Por	F: GTATTGGGATTGTTTTTTTTATGG R: (Btn)ACCTCACCTTATAAACCTACTCCC S1: TTTTTTTTATGGGTTTTATT S2: GTTTAGGTAAGGAGGTGG S3: GTTAGGATGAGGATTATTTG	293 bp	68-65-62

F = forward primer, R = reverse primer, S = sequencing primer, Btn = Biotin label, bp = base pairs.