

**Supplementary Information for**  
**Environmental enrichment increases transcriptional and epigenetic**  
**differentiation between mouse dorsal and ventral dentate gyrus**

Tie-Yuan Zhang<sup>1,2,3,†,\*</sup>, Christopher L Keown<sup>4,†</sup>, Xianglan Wen<sup>1,2,3</sup>, Junhao Li<sup>4</sup>, Dulcie A. Vousden<sup>5</sup>, Christoph Anacker<sup>1,2,3</sup>, Urvashi Bhattacharyya<sup>4</sup>, Richard Ryan<sup>1,2,3</sup>, Josie Diorio<sup>1,2,3</sup>, Nicholas O'Toole<sup>1,2,3</sup>, Jason P. Lerch<sup>5</sup>, Eran A. Mukamel<sup>4,\*</sup>, Michael J. Meaney<sup>1,2,3,6</sup>

\*Correspondence to: Tie-Yuan Zhang (tieyuan.zhang@mcgill.ca) and Eran A. Mukamel (emukamel@ucsd.edu).

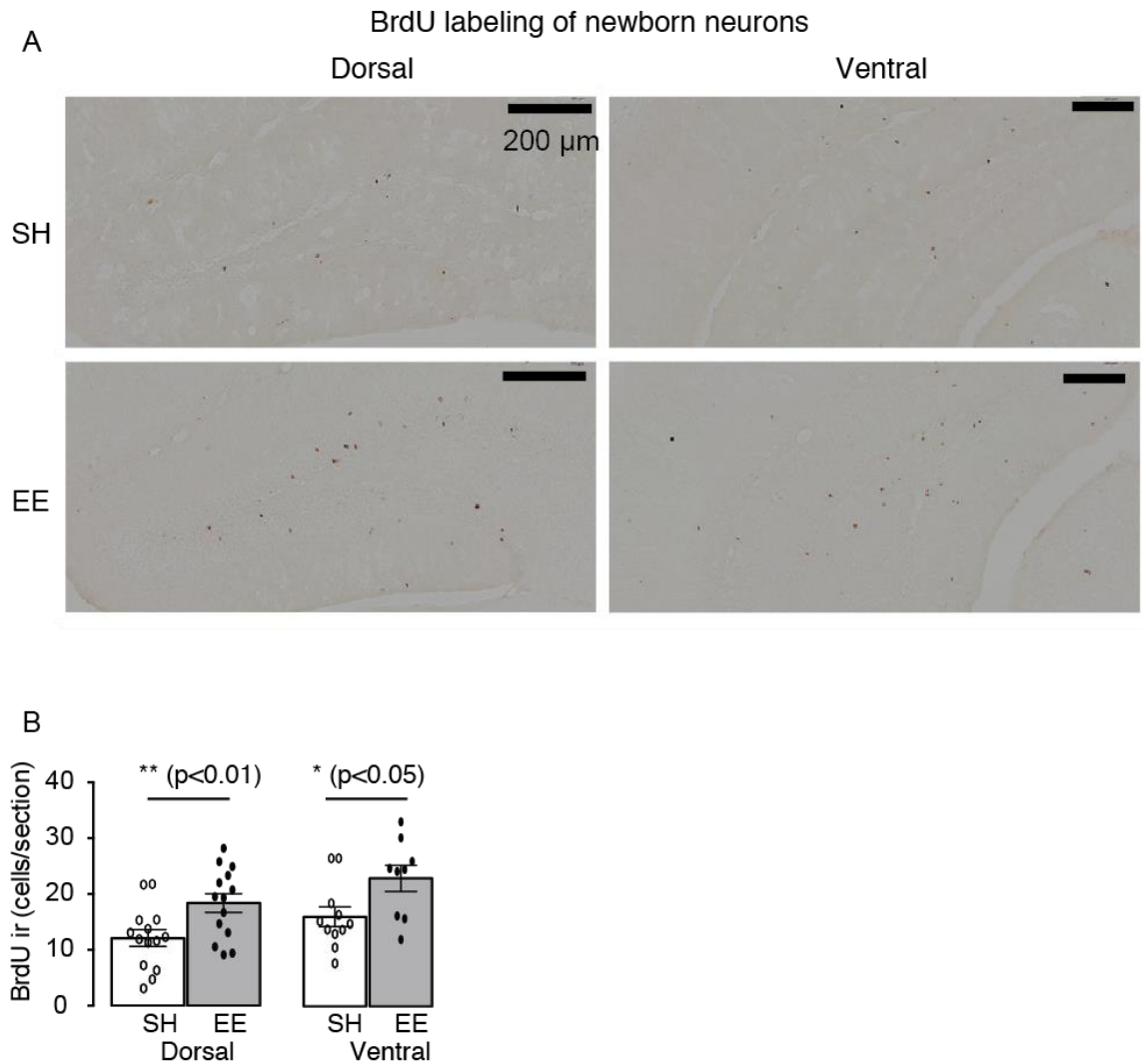
†These authors contributed equally

**This PDF file includes:**

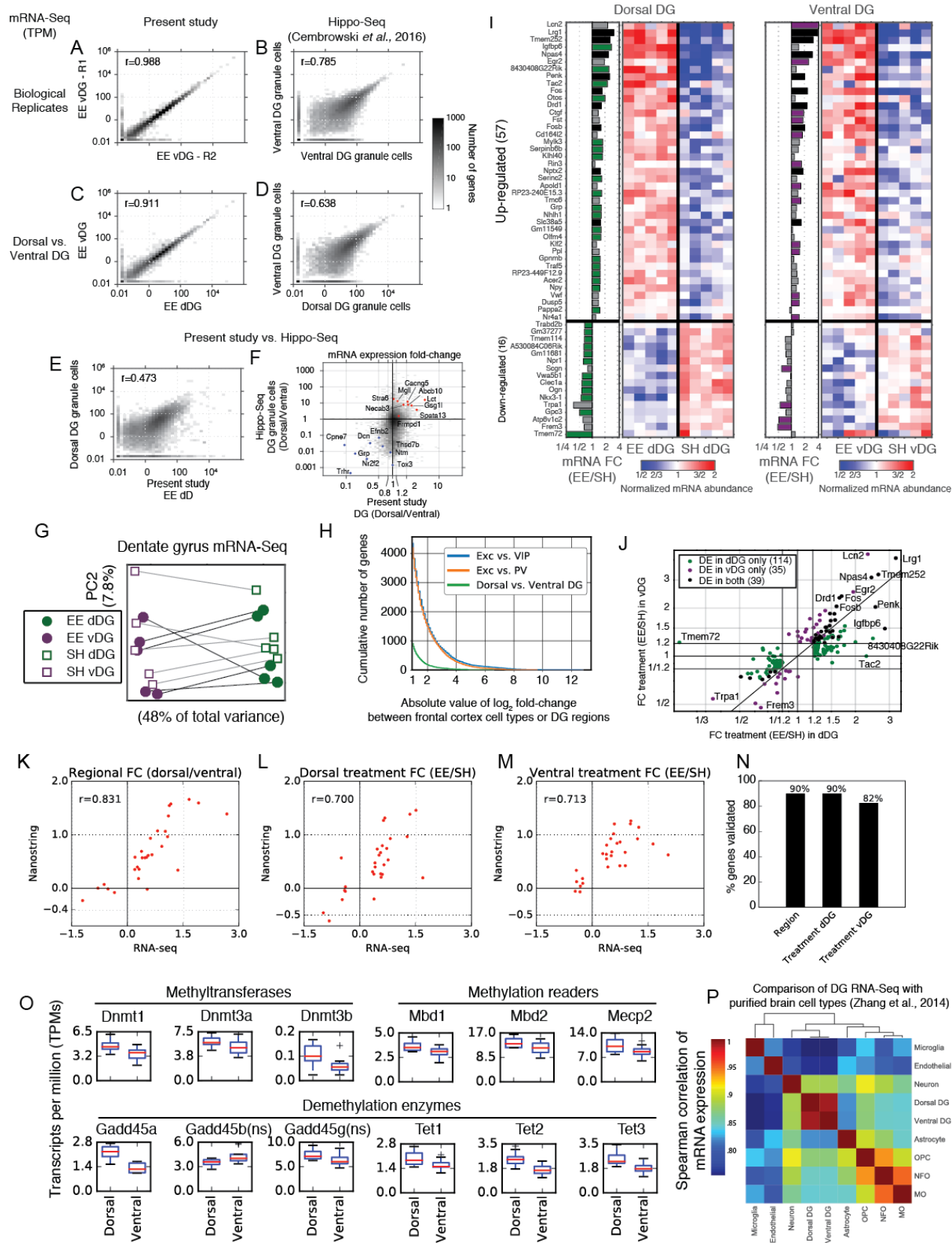
Supplementary Figures 1-5

**Other Supplementary Materials for this manuscript include:**

Supplementary Data 1

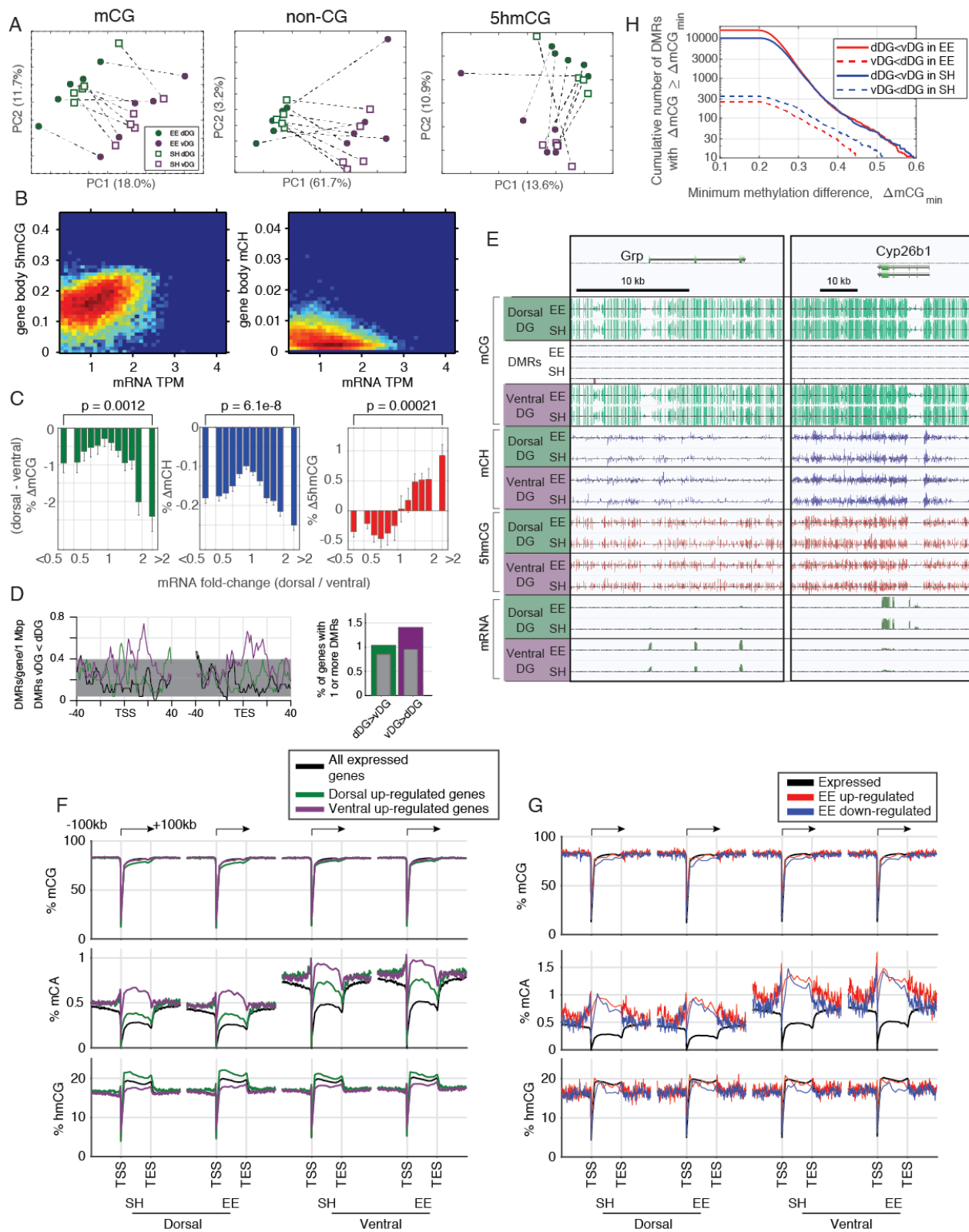


**Supplementary Fig. 1 : Increased adult neurogenesis in dorsal and ventral DG following environmental enrichment.** (A) BrdU immunoreactive cells in coronal section of dorsal and ventral dentate gyrus of mice. (B) Mean  $\pm$  SEM BrdU-positive cells in dorsal and ventral dentate gyrus of mice (n = 9-14 per group, F (1,44) = 13.33, p = 0.0007).

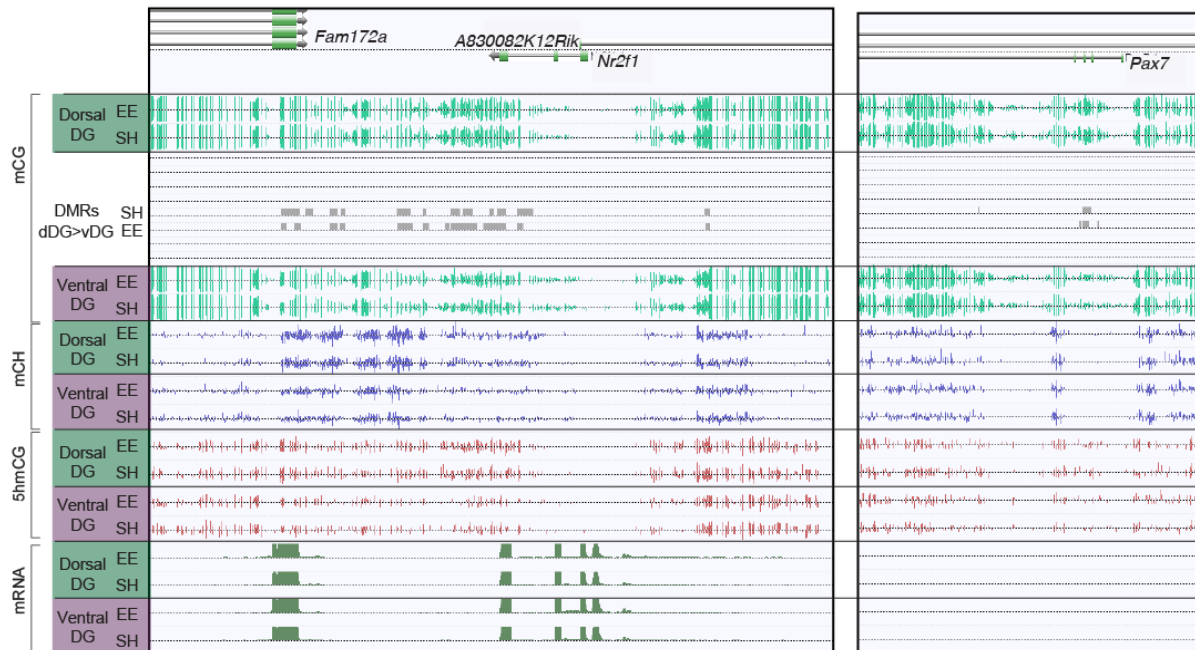


**Supplementary Fig. 2: Transcriptome analysis of dorsal and ventral DG in enriched environment.**

(A,B) Comparison of biological replicates shows the consistency of gene expression estimates (TPM) in ventral DG in the present study (A) and for ventral DG granule cells in Hippo-Seq<sup>2</sup> (B). (C,D) Dorsal vs. ventral expression estimates for the present study (C) and hippo-seq (D). (E) Direct comparison of individual replicates from the present study with hippo-seq granule cells. (F) Comparison of regional differences in expression between the present data set (DG tissue) and purified granule cells from Hippo-Seq (Cembrowski et al. 2016) shows highly consistent differential expression for markers of dorsal and ventral DG granule cells. (G) Effects of EE on gene expression are larger in dDG compared with vDG. (H) Dorsal and ventral DG expression differences are ~4-fold smaller than the differences between distinct cortical cell types. (I) The top DE genes include immediate early genes (*Fos*, *Egr2*, *Npas4*). (J) More genes are upregulated (EE>SH) than downregulated (EE<SH). (K-N) Nanostring digital quantification validates RNA-Seq results. (O) Expression of genes associated with (de)methylation and methylation readers in dorsal and ventral DG. All genes are significantly upregulated in dorsal DG over ventral (FDR < .05) except *Gadd45b*, which is significantly upregulated in ventral DG, and *Gadd45g*, which is not differentially expressed. *Not significant (ns)*. (P) Correlation of expression levels between DG tissue and purified brain cell types<sup>16</sup> reveals expression in DG is most strongly correlated with neurons. *Oligodendrocyte progenitor cells (OPC)*, *newly formed oligodendrocytes (NFO)*, *mature oligodendrocytes (MO)*.

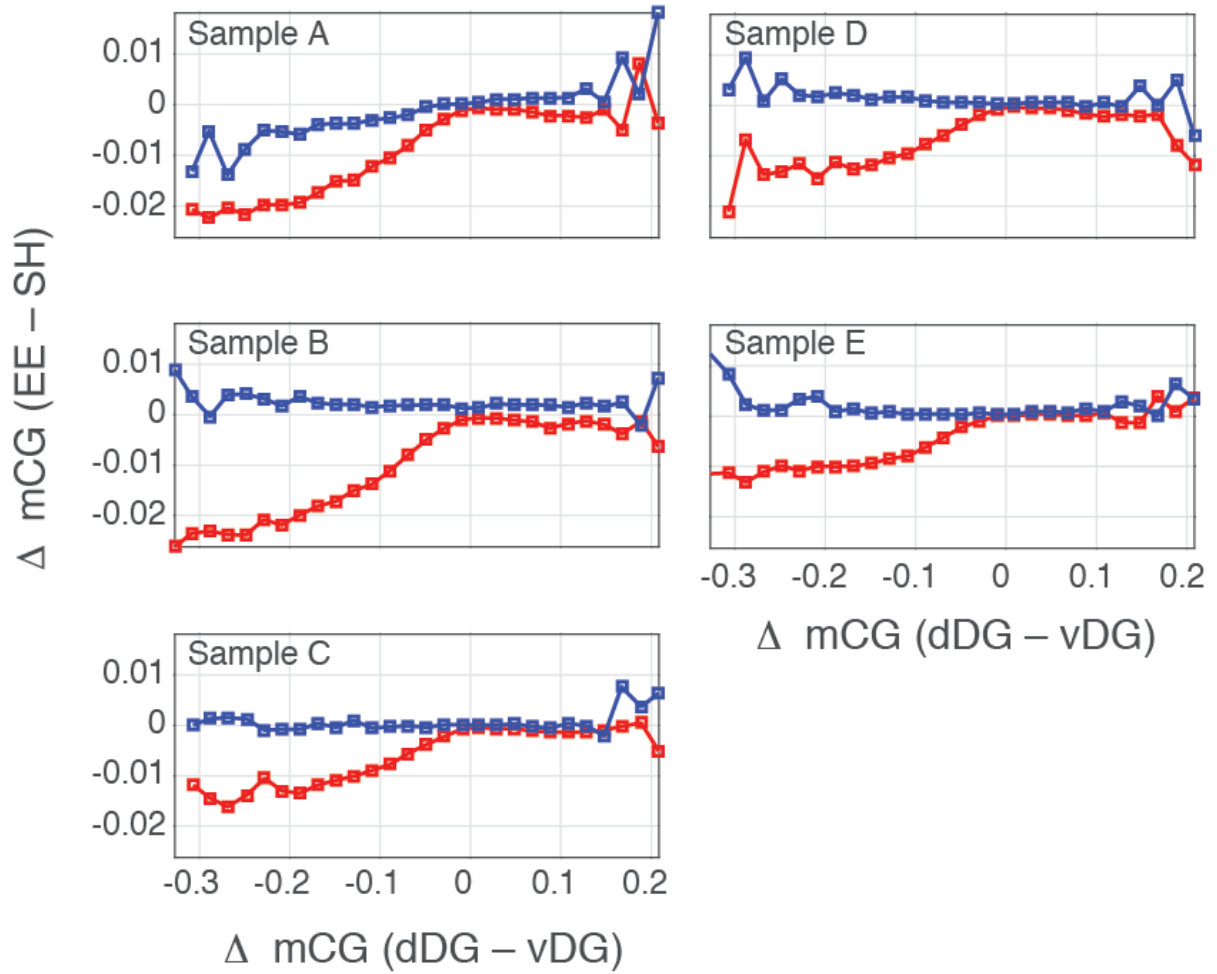


**Supplementary Fig. 3.** (A) Transcriptome principal components separate dorsal and ventral samples using mCG, mCH or 5hmCG. Dashed lines connect dorsal and ventral DG from the same group of mice. (B) Gene body mCH is associated with transcriptional repression, whereas 5hmCG is positively correlated with expression<sup>21,25</sup>. (C) Genes up-regulated in dorsal vs. ventral DG have lower promoter mCG, gene body mCH, and higher gene body 5hmCG compared with down-regulated genes. (D) Ventral hypo-methylated DMRs are enriched within the gene body of genes up-regulated in ventral DG. (E) Browser shots showing examples of genes up-regulated in ventral (*Grp*) or dorsal (*Cyp26b1*) DG, but which lack DMRs. (F,G) Mean mCG, mCA and hmCG levels within gene bodies  $\pm 100$  kb are shown for all expressed genes, as well as genes differentially expressed in dorsal or ventral DG. mCA is depleted, and hmCG is enriched, within the gene bodies of dorsal expressed genes (green lines); the opposite pattern prevails within the bodies of ventral expressed genes (purple lines). (H) Number of hypomethylated DMRs in dorsal (solid lines) and ventral (dashed lines) DG as a function of the methylation difference threshold.



**Supplementary Fig 4.** Browser views of examples of genes hypomethylated in ventral DG.

(Left) *Nr2f1* is differentially expressed in adult ventral DG, consistent with the lower mCG level in the gene body and surrounding region. (Right) *Pax7* is not expressed in the adult DG, but is expressed during development and helps to define dorsal fate<sup>37</sup>. Interestingly, we find that *Pax7* is more methylated in the dorsal DG, which may be a vestigial signature of its early developmental activity in the dorsal DG similar to vestigial DMRs observed in other neuronal cell types<sup>9</sup>.



**Supplementary Fig. 5.** Cross-validated analysis of the correlation of region- and treatment-based effects on DNA methylation. Each panel shows the result of analyzing regional DNA methylation in one of the five biological replicates (samples A-E, x-axis) vs. the treatment-based effects estimated using the remaining four samples. DNA methylation was estimated within 1kb bins across all autosomes; we excluded bins with <10 CG basecalls in any sample, or <100 basecalls after summing all samples. Binned mCG levels were used to compute the dorsal-ventral and EE-SH difference in methylation for each bin. We then grouped all bins according to their level of dorsal-ventral difference in mCG, and computed the median EE-SH difference.