

SUPPLEMENTARY INFORMATION

Methamphetamine-induced regional-specific transcriptomic and epigenetic changes in the brain of male rats

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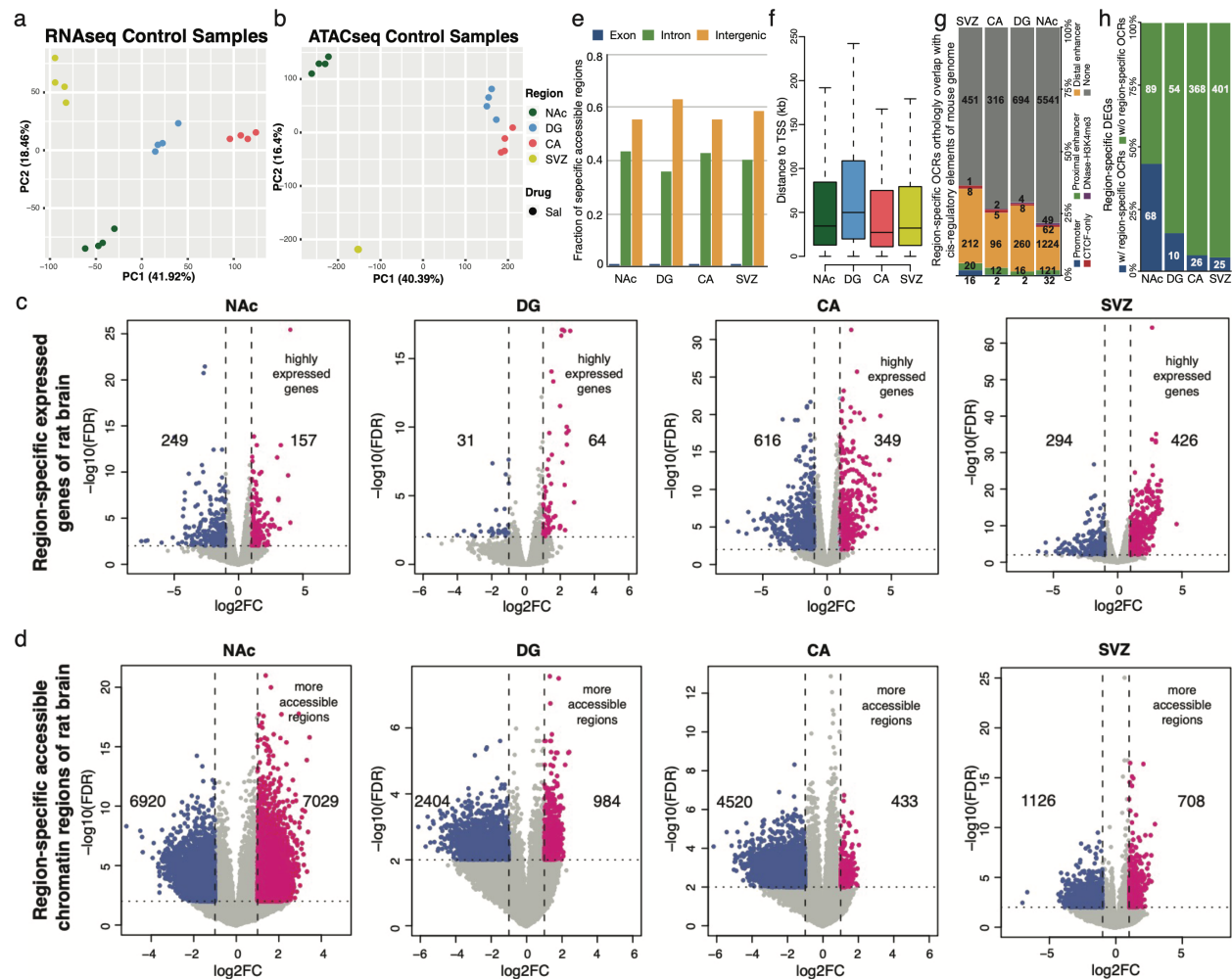
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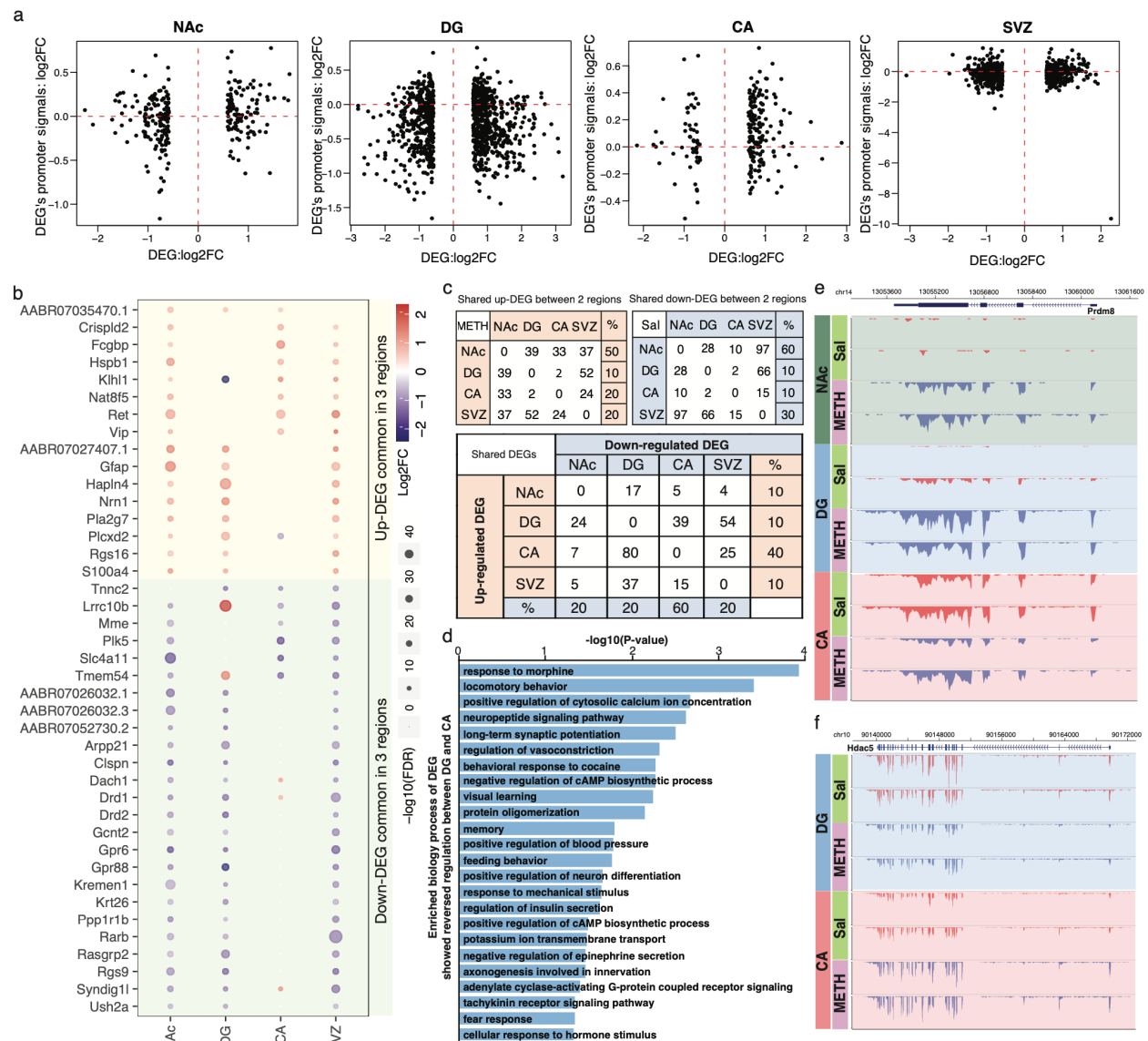
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Supplementary figures: 6

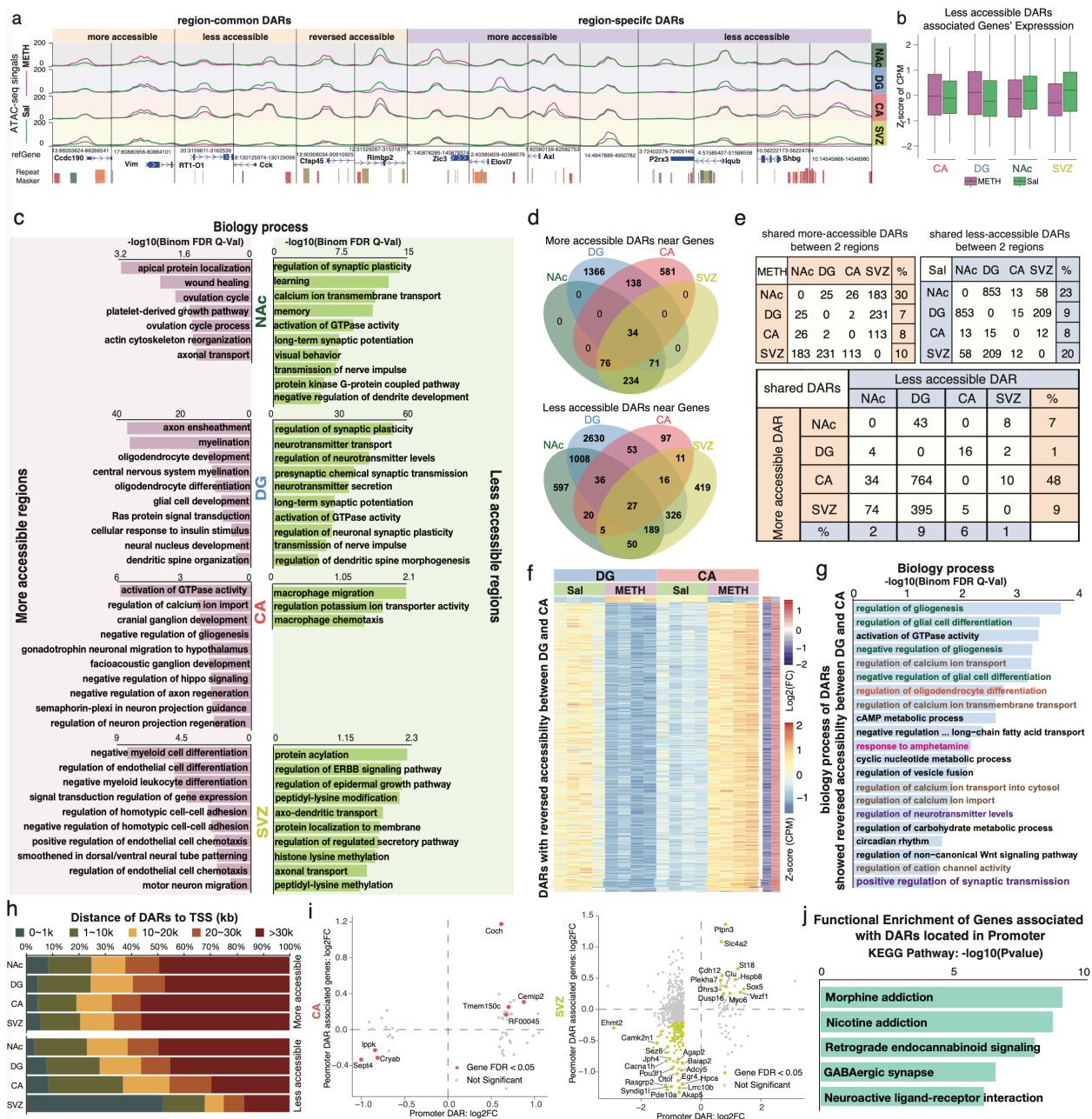
Supplementary Figures



Supplementary Figure 1. RNA-seq and ATAC-seq data of 4 brain regions from control and binge METH exposure rat samples. The principal analysis (PCA) of RNA-seq data (**a**) and ATAC-seq data (**b**) from 4 normal rat brain regions. **c-d**, The volcano plots of region-specific expressed genes (**c**) and accessible chromatin regions (**d**) in 4 rat brain regions. Red dots represented the specific genes and accessible regions in each brain region. Log2FC: log2 value of fold changes with one normal region against other regions; -log10(FDR): -log10 value of false discover rate (FDR). **e**, Genomic distribution of 4 rat brain region-specific accessible regions in rat genome: exon, intron, and intergenic regions. Large fraction of those specific accessible regions located in intergenic and intronic regions. **f**, Boxplot about the distance to TSS of region-specific accessible regions in 4 rat brain regions. The boxplots show the median, upper and lower quartiles, maximums, and minimums. **g**, Distribution of rat-mouse ortholog region-specific OCRs in cis-regulatory elements of the mouse genome (mm10), including CTCF-only, DNase-H3K4me3, promoter, proximal enhancer and distal enhancer. **h**, The percentage of region-specific DEGs around region-specific OCRs in 4 brain regions.

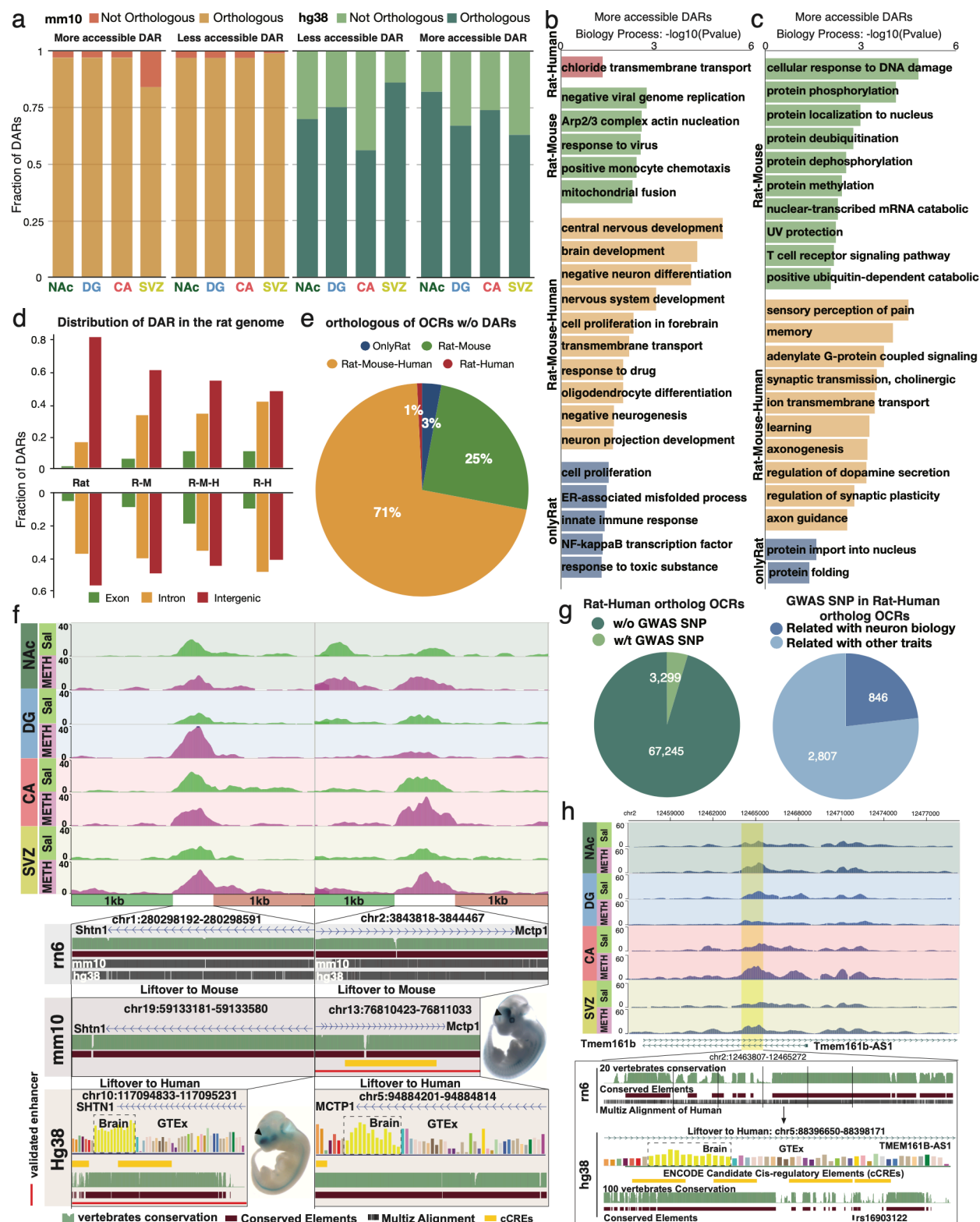


Supplementary Figure 2. The differential expressed genes (DEGs) identified in 4 rat brain regions responding to binge METH exposure. **a**, Correlation between ATAC-seq signal on promoters and expression of METH-induced DEGs in all four brain regions. **b**, Up and down-regulated DEGs were common in at least 3 brain regions. **c**, Number of common genes between 2 regions separately for up and down-regulated DEGs, and the number of common DEGs between DG and CA showed reversed regulation pattern. Large percent of up and down-regulated DEGs in NAc were also identified in another one region. And large percent of DEG in CA regions showed reversed regulation pattern in another one region, especially in DG region. **d**, Biology processes of GO term enriched in the DEGs that showed reversed regulation between DG and CA regions. **e-f**, Examples of *Prdm8* and *Hdac5* genes showing differential expression in brain regions visualized by WashU Epigenome Browser.



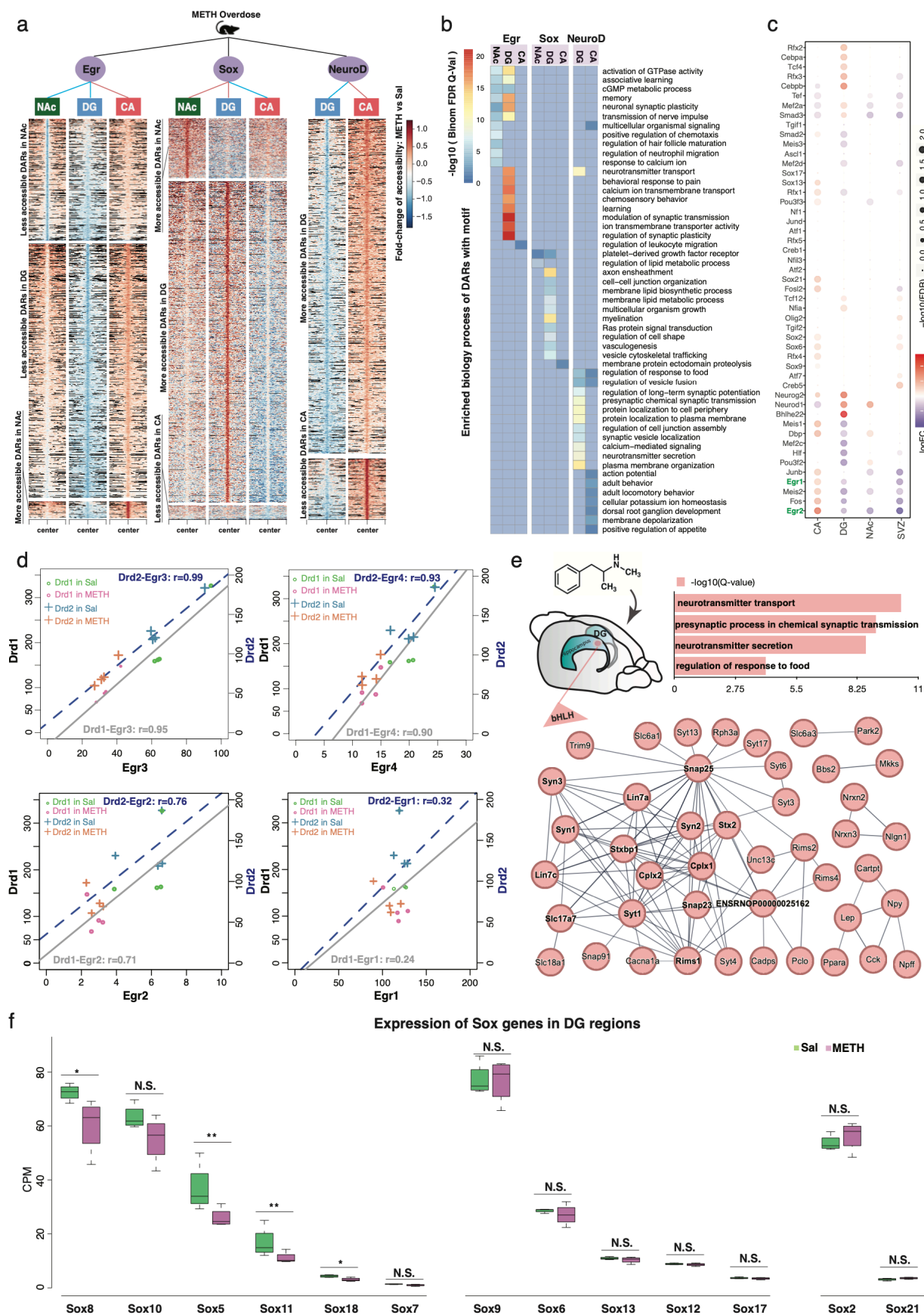
Supplementary Figure 3. The differential accessible regions (DARs) in rat brain regions after binge METH exposure. **a**, Examples of DARs showed common and specific accessibility in 4 rat brain regions. **b**, Expression (Z-score) of nearest genes assigned to less accessible DARs. The boxplots show the median, upper and lower quartiles, maximums, and minimums. **c**, Enriched biology process of more and less accessible DARs in 4 rat brain regions. **d**, Shared number of genes associated with more and less accessible DARs across 4 rat brain regions. **e**, Number of common DARs between 2 regions separately for more and less accessible DARs, and the number of common DARs between DG and CA showed reversed accessibility. Large percent of more accessible DARs in CA region showed reversed accessibility in DG

region. **f**, Heatmap about Z-score of DARs that displayed reversed accessibility between DG and CA. **g**, Biology processes significantly enriched in DARs showing reversed accessibility between DG and CA. **h**, Distance distribution of DARs to the transcript start sites. **i**, The log2-foldchange distribution of promoter-DARs and those DARs associated genes that assigned the DARs to the nearest genes. **j**, Enriched KEGG pathways based on associated genes of DARs located in the promoter regions.



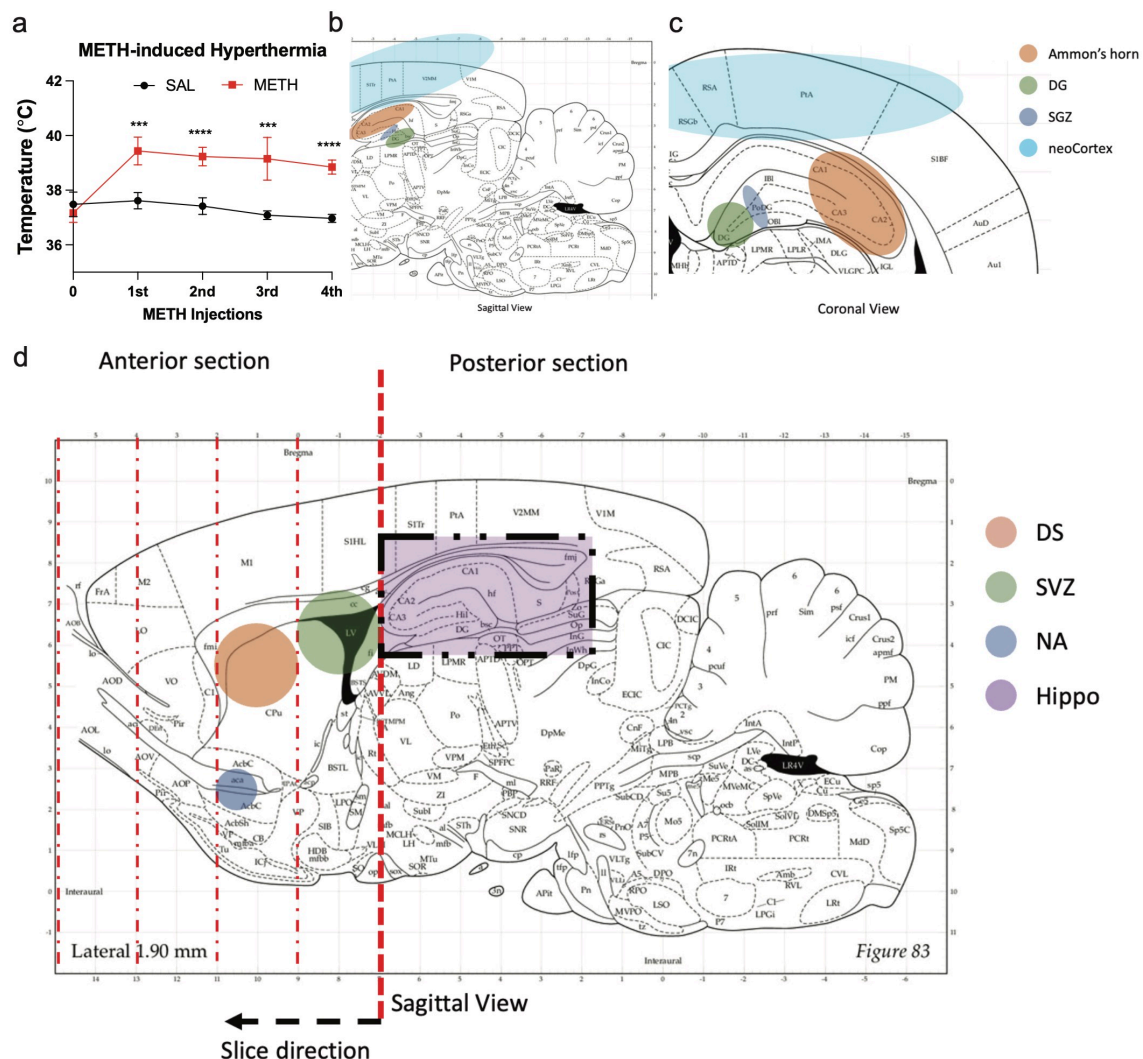
Supplementary Figure 4. Evolutionary conservation of Rat brain DARs responding to binge METH exposure. a, Percent of more and less accessible DARs had orthologous counterparts in mouse and

human genome (mm10 and hg38). **b-c**, Biology processes enriched in genes around (within 20kb) DARs separately for more and less accessible DARs with different conservation status: rat-mouse-human (R-M-H), rat-human (R-H), rat-mouse (R-M) and rat-only (Rat). **d**, Genomic distribution of DARs from 4 different conservation status: exon, intron, and intergenic regions. Large fraction of DARs located in intergenic regions for all 4 groups. **e**, Percent of open chromatin regions (OCRs) without DARs from different conservation status. More than 70% of those OCRs were ortholog in rat, mouse, and human genome. **f**, Two examples of DARs with orthologous interactions with human and mouse validated enhancers. **g**, Number of Rat-Human ortholog OCRs with variants from genome-wide association studies (GWAS SNP) and number of those GWAS SNP associated with neuron biology. **h**, Example of rat-human ortholog DAR overlap with GWAS SNPs associated with neuron biology.



Supplementary Figure 5. METH exposure induced DARS-associated regulatory networks in rat brain.

a, Heatmap of more and less accessible DARS contained the Egr, Sox and NeuroD binding motifs in different brain regions. **b**, Enriched biology processes based on DARS with Egr, Sox and NeuroD binding motifs. **c**, The binge METH induced expression changes of transcription factors (TFs) whose binding motifs enriched in DARS. **d**, The correlation of expression changes between Drd1/2 genes and Egr family genes in rat NAc region with Meth stimulus. **e**, Gene regulatory network and enriched biology process built by genes around DARS with bHLH binding motif in DG. **f**, Expression changes of Sox genes in DG region responding to binge METH exposure. The boxplots show the median, upper and lower quartiles, maximums, and minimums.



Supplementary Figure 6. Experiment of 4 Rat brain regions. **a**, The temperature (°C) of Rat after the METH injections. The error bars are standard errors of the mean. **b-d**, different sections of rat brain regions. The original section plots of brain regions from online tool developed by Gaidica Lab (<http://labs.gaidi.ca/rat-brain-atlas/>).