Supplementary Information for:

Prenatal and postnatal effects of gestational immune activation on synaptic and neurodevelopmental pathways via epigenetic mechanisms

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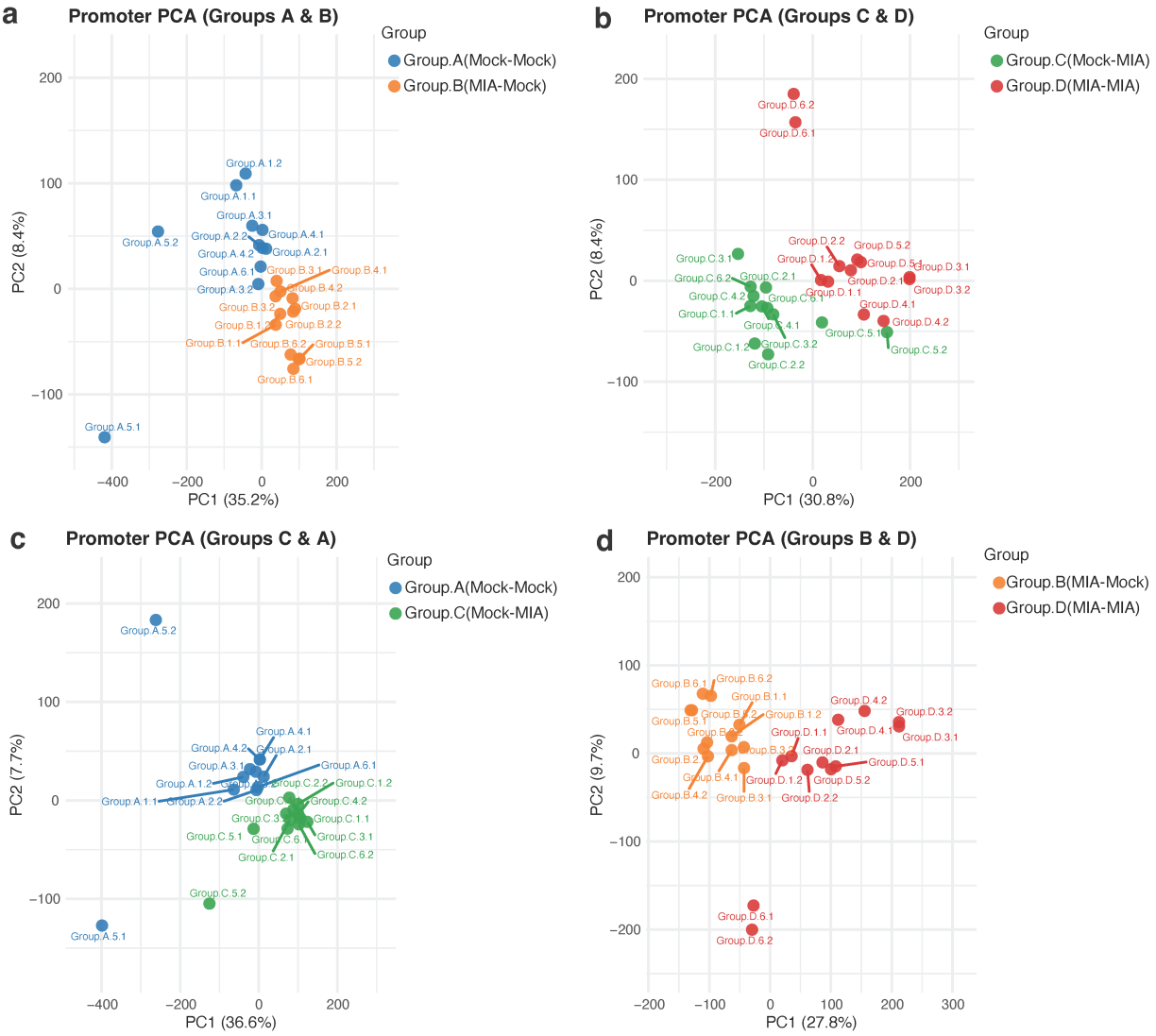
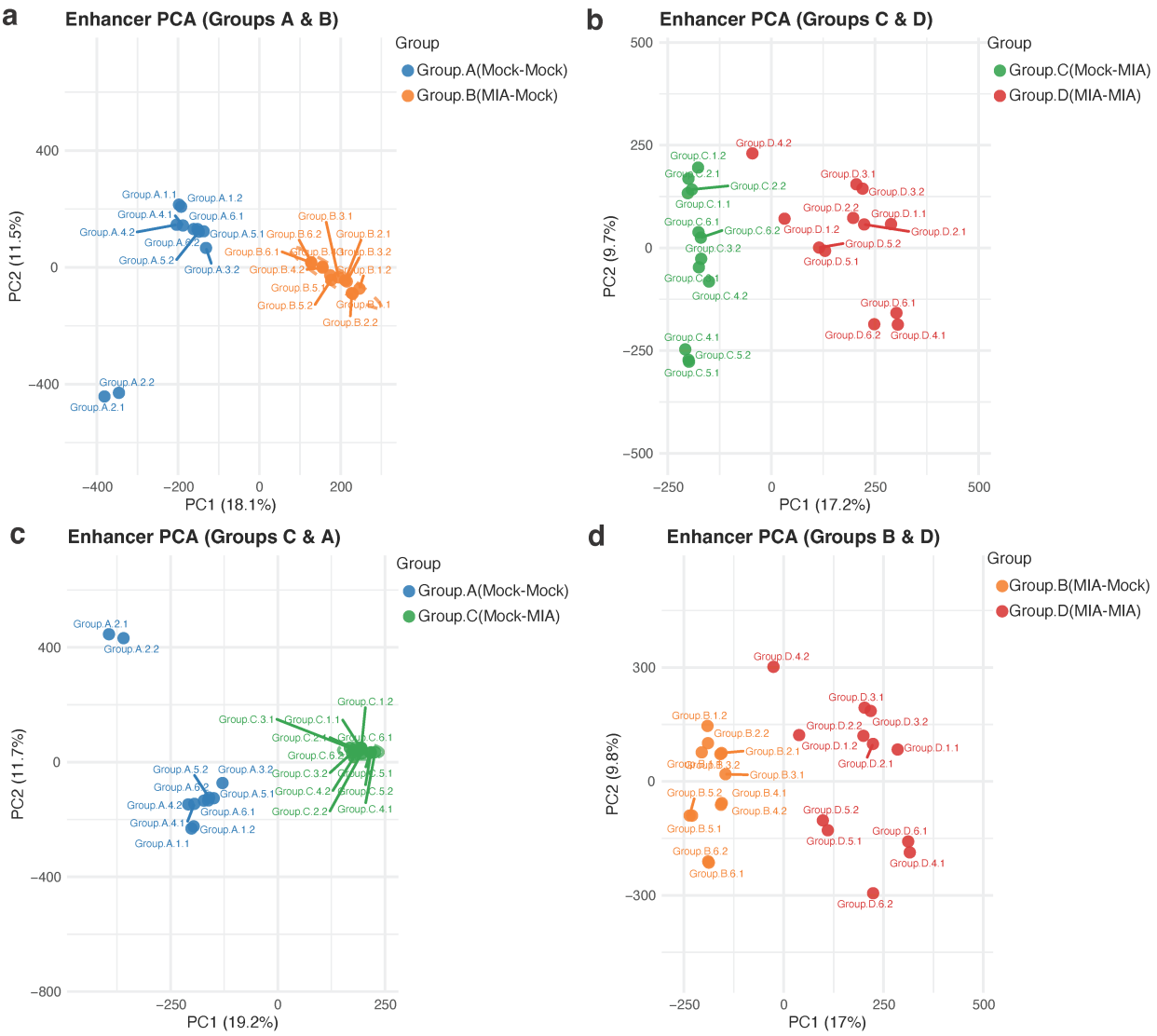
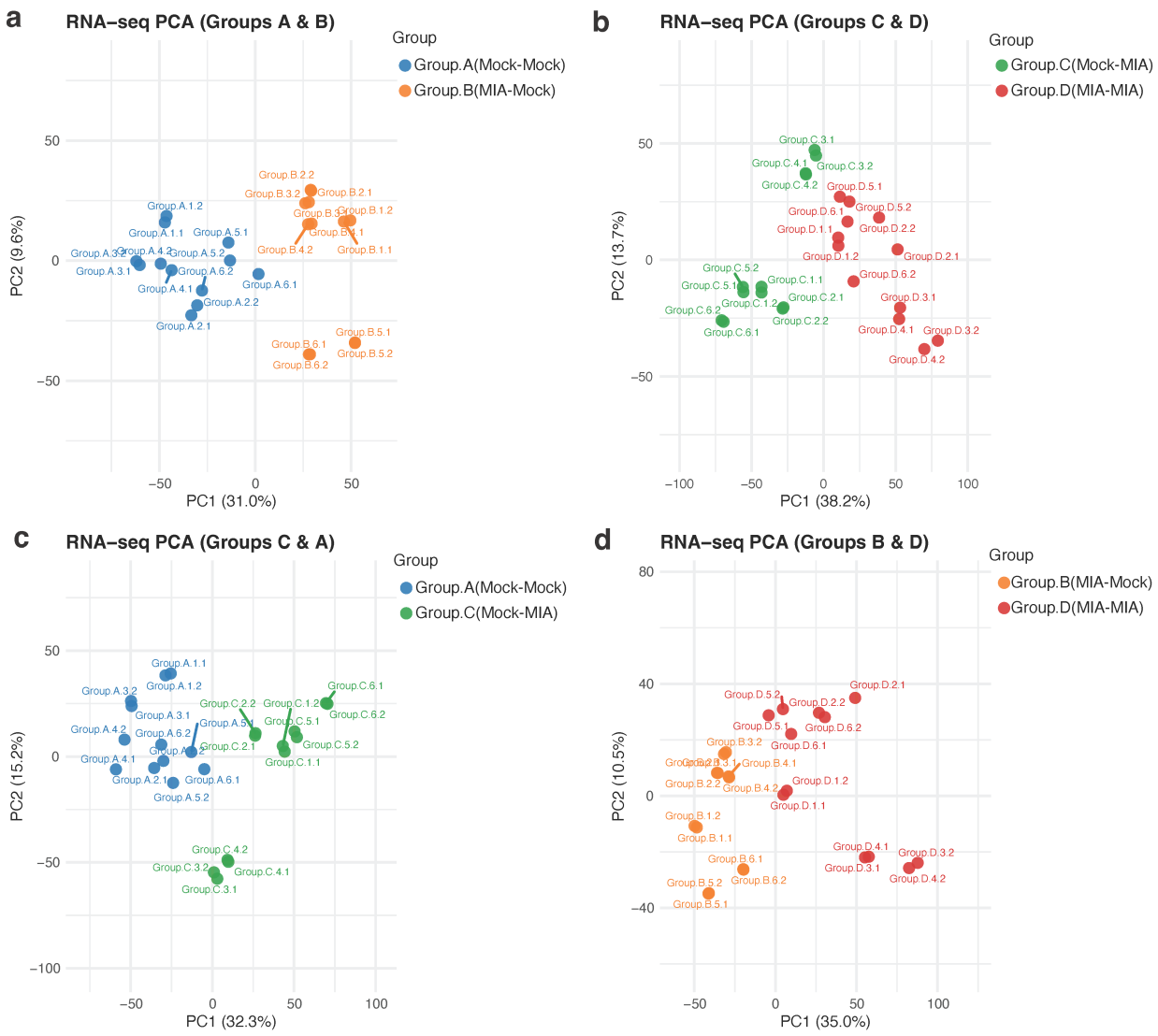
Supplementary Figures



Supplementary Fig. 1 Principal component analysis (PCA) of promoter profiles across experimental groups.
(a-d) PCA plots generated from promoter H3K4me3 signal matrices showing how samples distribute along the major axes of variance according to treatment condition. Each panel displays a pairwise comparison between two groups: (a) A (Mock–Mock) vs. B (MIA–Mock); (b) C (Mock–MIA) vs. D (MIA–MIA); (c) C (Mock–MIA) vs. A (Mock–Mock); and (d) B (MIA–Mock) vs. D (MIA–MIA).

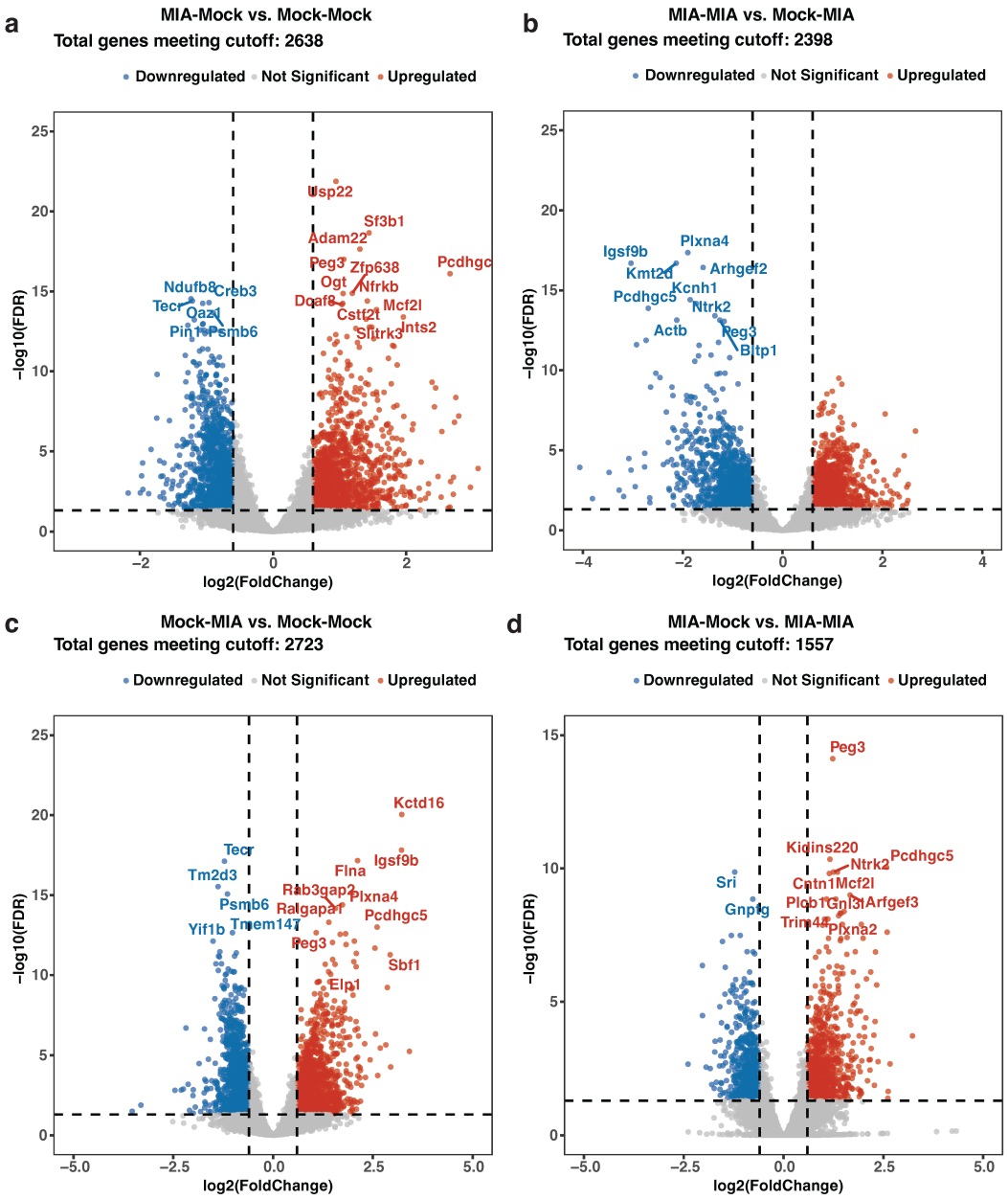


Supplementary Fig. 2 Principal component analysis (PCA) of enhancer profiles across experimental groups.
(a-d) PCA plots generated from enhancer H3K27ac signal matrices showing how samples distribute along the major axes of variance according to treatment condition. Each panel displays a pairwise comparison between two groups: (a) A (Mock–Mock) vs. B (MIA–Mock); (b) C (Mock–MIA) vs. D (MIA–MIA); (c) C (Mock–MIA) vs. A (Mock–Mock); and (d) B (MIA–Mock) vs. D (MIA–MIA).



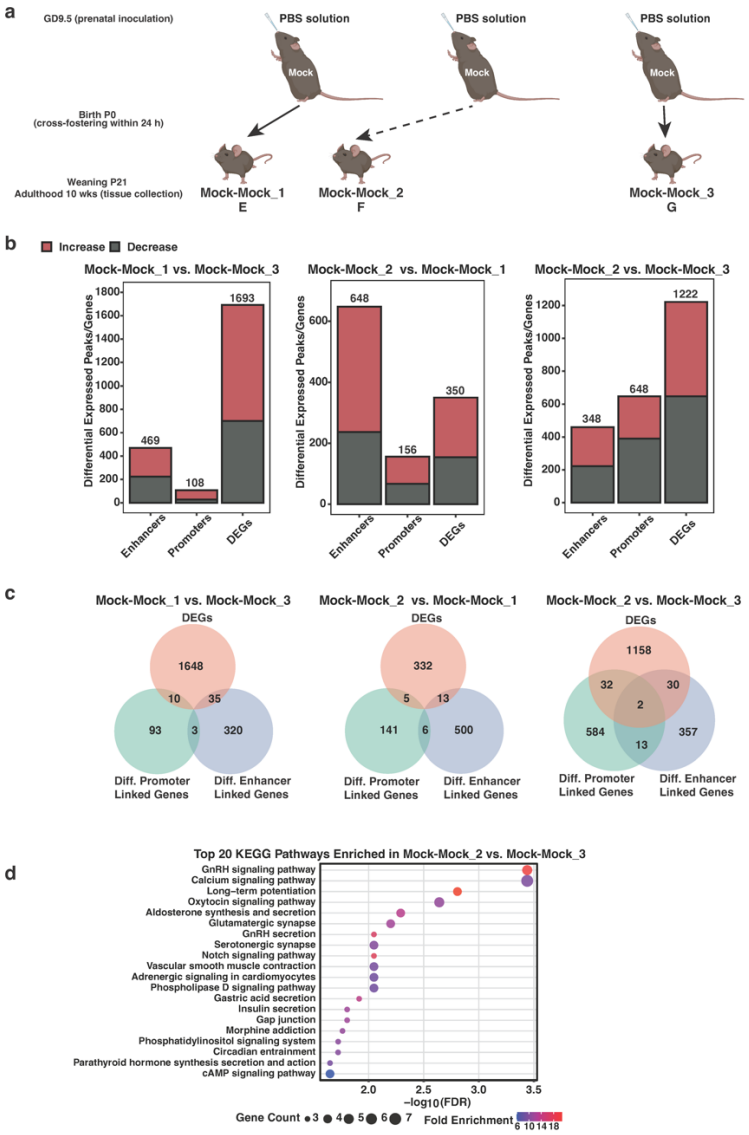
Supplementary Fig. 3 Principal component analysis (PCA) of RNA-seq profiles across experimental groups.

(a-d) PCA plots generated from gene-level RNA-seq expression matrices showing how samples distribute along the major axes of variance according to treatment condition. Each panel displays a pairwise comparison between two groups: (a) A (Mock–Mock) vs. B (MIA–Mock); (b) C (Mock–MIA) vs. D (MIA–MIA); (c) C (Mock–MIA) vs. A (Mock–Mock); and (d) B (MIA–Mock) vs. D (MIA–MIA).



Supplementary Fig. 4 Transcriptomic changes in offspring exposed to prenatal and postnatal MIA.

a-d, Volcano plots showing differential gene expression in four pairwise comparisons: (a), MIA-Mock vs. Mock-Mock; (b), MIA-MIA vs. Mock-MIA; (c), Mock-MIA vs. Mock-Mock; (d), MIA-Mock vs. MIA-MIA. Each dot represents one gene; significantly upregulated genes are shown in red, downregulated genes in blue, and non-significant genes in gray. Horizontal dashed line indicates the FDR cutoff (FDR < 0.05); vertical dashed lines indicate ±1.5-fold change thresholds. Selected genes of interest are labeled. Total number of DEGs (meeting cutoff) is indicated above each plot.



Supplementary Fig. 5 Epigenetic and transcriptomic variability in cross-fostered control mock-treated offspring.

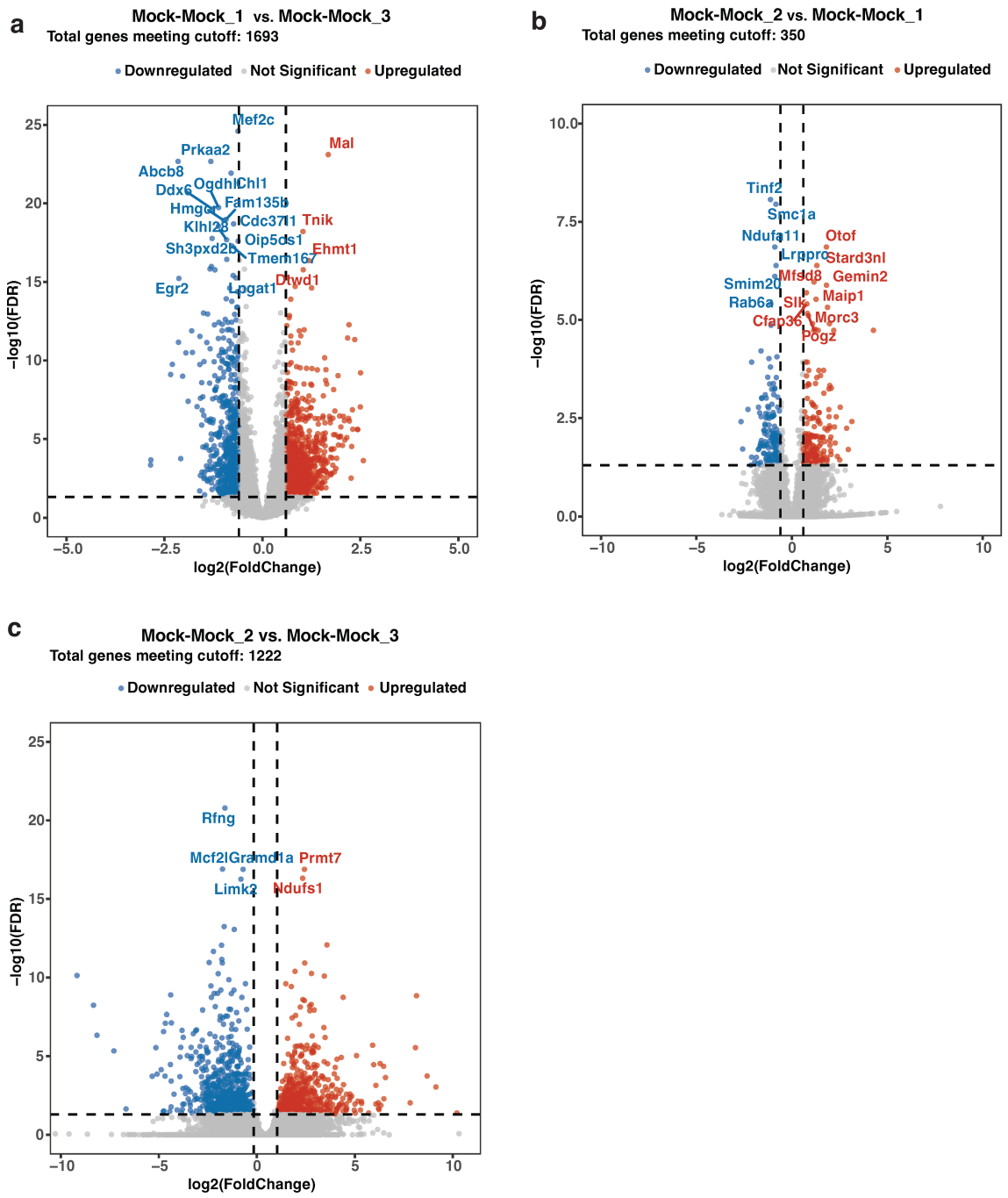
a, Schematic of the cross-fostering design used for control animals. All pregnant dams received PBS (Mock), and offspring were assigned to different postnatal environments to generate three independent comparisons: Mock-Mock_1, Mock-Mock_2, and Mock-Mock_3. N = 6 mice per group; Created with BioRender.com.

b, Bar plots showing the numbers of differential enhancers/promoters and DEGs with increased (red) or decreased (gray) intensity in Mock-Mock_1 vs. Mock-Mock_3 (left), Mock-Mock_2 vs. Mock-Mock_1 (middle), and Mock-Mock_2 vs. Mock-Mock_3 (right) comparisons.

c, Venn diagrams showing the overlap between DEGs, differential promoter-linked genes, and differential enhancer-linked genes in each comparison.

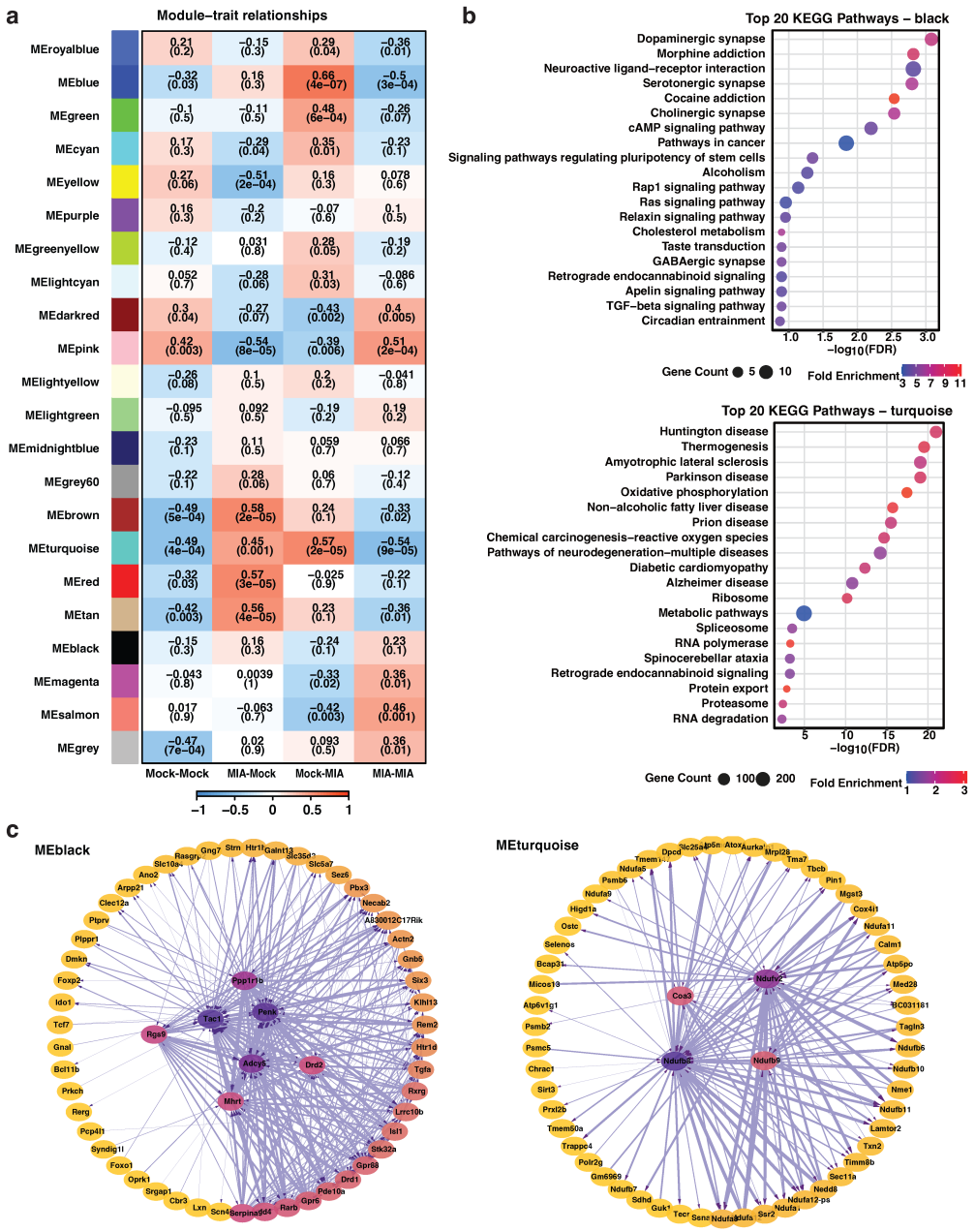
d, Top 20 KEGG pathways enriched among genes involving both transcriptomic and epigenomic (either in promoter or enhancer) changes in the Mock-Mock_2 vs. Mock-Mock_3 comparison.

Dot size reflects the number of genes per pathway; color scale indicates fold enrichment; significance is shown as $-\log_{10}$(FDR) with FDR < 0.05 used as the cutoff.



Supplementary Fig. 6 Transcriptomic changes in offspring with no exposure to MIA. a-c, Volcano plots showing differential gene expression between individual Mock-Mock comparisons: (a), Mock-Mock_1 vs. Mock-Mock_3; (b), Mock-Mock_2 vs. Mock-Mock_1; (c), Mock-Mock_2 vs. Mock-Mock_3. Each dot represents one gene; significantly upregulated genes are shown in red, downregulated genes in blue, and non-significant genes in gray. Horizontal dashed line indicates the FDR threshold (FDR < 0.05); vertical dashed lines indicate ±1.5-fold change thresholds. Selected DEGs are labeled, and the number of DEGs meeting significance cutoff is noted above each plot.

Supplementary Fig. 7 Clustering of enhancer activity across experimental groups with associated pathway and transcription factor motif enrichment.
Heatmap showing normalized signal intensity of differentially active enhancers across experimental conditions: Mock-Mock, MIA-Mock, Mock-MIA, and MIA-MIA. Enhancers were grouped into four clusters based on activity patterns across groups. For each cluster, the top enriched KEGG pathways (left) and the most significantly enriched transcription factor binding motifs (right) are shown. FDR values represent pathway enrichment significance. Motif logos and corresponding transcription factors are ranked by statistical significance (P < 1e-12), with selected known regulators indicated. Signal intensity scale reflects normalized enhancer signal strength.

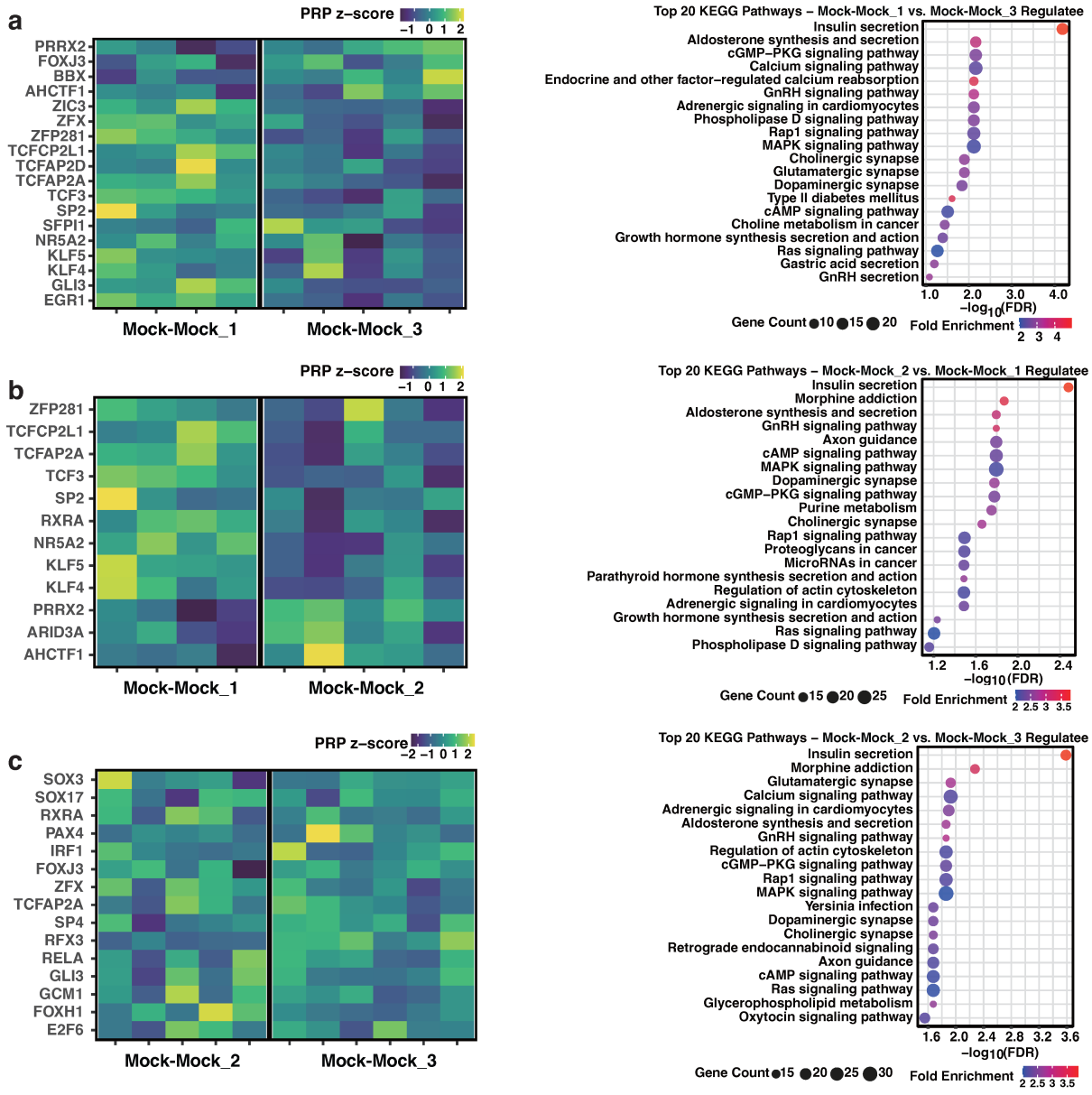


Supplementary Fig. 8 Gene co-expression network analysis identifies trait-associated modules enriched for neurodevelopmental pathways.

a, Module–trait relationship heatmap showing correlations between WGCNA modules (rows) and experimental groups (columns). Each cell displays the Pearson correlation coefficient and associated P-value.

b, KEGG pathway enrichment for genes in the two most strongly trait-associated modules: the black module (top) and the turquoise module (bottom). Dot size reflects the number of genes per pathway; color indicates fold enrichment; significance is shown as -log$_{10}$(FDR), with FDR < 0.05 used as the cutoff.

c, Network plots showing hub gene connectivity within the black (left) and turquoise (right) modules. Hub genes (purple) are shown with their first-degree connections to other genes (yellow) based on intramodular connectivity.



Supplementary Fig. 9 Differentially regulated transcription factor activity and target gene pathways across mock-treated offspring.

a, Heatmap showing transcription factors differentially regulated between Mock-Mock_1 and Mock-Mock_3, along with the top 20 KEGG pathways enriched among the regulated genes of these transcription factors.

b, Heatmap showing transcription factors differentially regulated between Mock-Mock_2 and Mock-Mock_1, along with the top 20 KEGG pathways enriched among the regulated genes of these transcription factors.

c, Heatmap showing transcription factors differentially regulated between Mock-Mock_2 and Mock-Mock_3, along with the top 20 KEGG pathways enriched among the regulated genes of these transcription factors.

Heatmap values represent z-scores of PRP-normalized transcription factor (TF) activity. Dot size reflects the number of regulated genes per pathway; color scale indicates fold enrichment; significance is shown as $-\log_{10}(\text{FDR})$, with FDR < 0.05 used as the cutoff.

Supplementary Table

Supplementary Table 1. Summary of bioinformatic tools

Category	Tool/Resource	General use	How used here
ChIP-seq preprocessing	Trim Galore! (Babraham)	Adapter and quality trimming for short reads	Trimmed reads with default settings prior to alignment
ChIP-seq preprocessing	Bowtie2	Align short reads to a reference genome	Aligned trimmed reads to mm10
ChIP-seq preprocessing	MACS2	Peak calling for ChIP-seq (enrichment over background)	Called peaks for H3K4me3 and H3K27ac
ChIP-seq preprocessing	SAMtools	Manipulate/Filter BAM/SAM files	Applied filtering to remove ENCODE-blacklisted regions; general BAM ops
ChIP-seq preprocessing	BEDtools	Genomic interval arithmetic and coverage	Extended mapped reads ±100 bp (200 bp total) and computed normalized signal
Visualization	IGV (Broad Institute)	Genome browser for track visualization	Visualized signal tracks genome-wide
Visualization/Format	bigWig	Binary track format for dense, continuous genome tracks	Signal calculated in 100-bp windows and exported to bigWig
RNA-seq preprocessing	Trim Galore! (Babraham)	Adapter and quality trimming for RNA-seq	Trimmed reads with default settings
RNA-seq alignment	HISAT2	Splice-aware alignment of RNA-seq reads	Mapped reads to GRCm38
RNA-seq QC/Exploration	SeqMonk v1.47.1 (Babraham)	Interactive QC, visualization, and quantitation from BAMs	Imported BAMs; filtered low-quality datasets by exon alignment
Quantification	featureCounts	Assign reads to genomic features (genes/exons)	Counted reads per gene for DE analysis
Differential expression	DESeq2 (R)	Model-based differential expression; normalization and testing	Identified DEGs and performed DE on ChIP peak counts (via DiffBind)
ChIP-seq differential	DiffBind (R)	Consensus peak sets, counting, normalization, differential binding	Built majority-rule consensus per condition; combined to master set; counted reads; TMM normalization; DE with DESeq2
Co-expression networks	WGCNA (R)	Weighted gene co-expression network analysis and module detection	Constructed networks using log2(FPKM); picked β by ~scale-free topology; TOM and blockwiseModules; module eigengenes; softConnectivity for hubness
TF network inference	Taiji pipeline	Integrate multi-omics to rank TFs driving condition-specific regulation	Defined active REs; linked distal enhancers to promoters (EpiTensor); built TF→gene networks; ranked TFs by network topology
GWAS heritability	LDSC v1.0.1	LD score regression for partitioned heritability	Tested enrichment of GWAS traits in differential enhancers/promoters

Supplementary Data

Supplementary Data 1. Metadata of epigenomic and transcriptomic datasets

Supplementary Data 2. Differential expressed gene list for all comparison groups

Supplementary Data 3. Overlapped genes between enhancer with DEG and promoter with DEG for all comparison groups