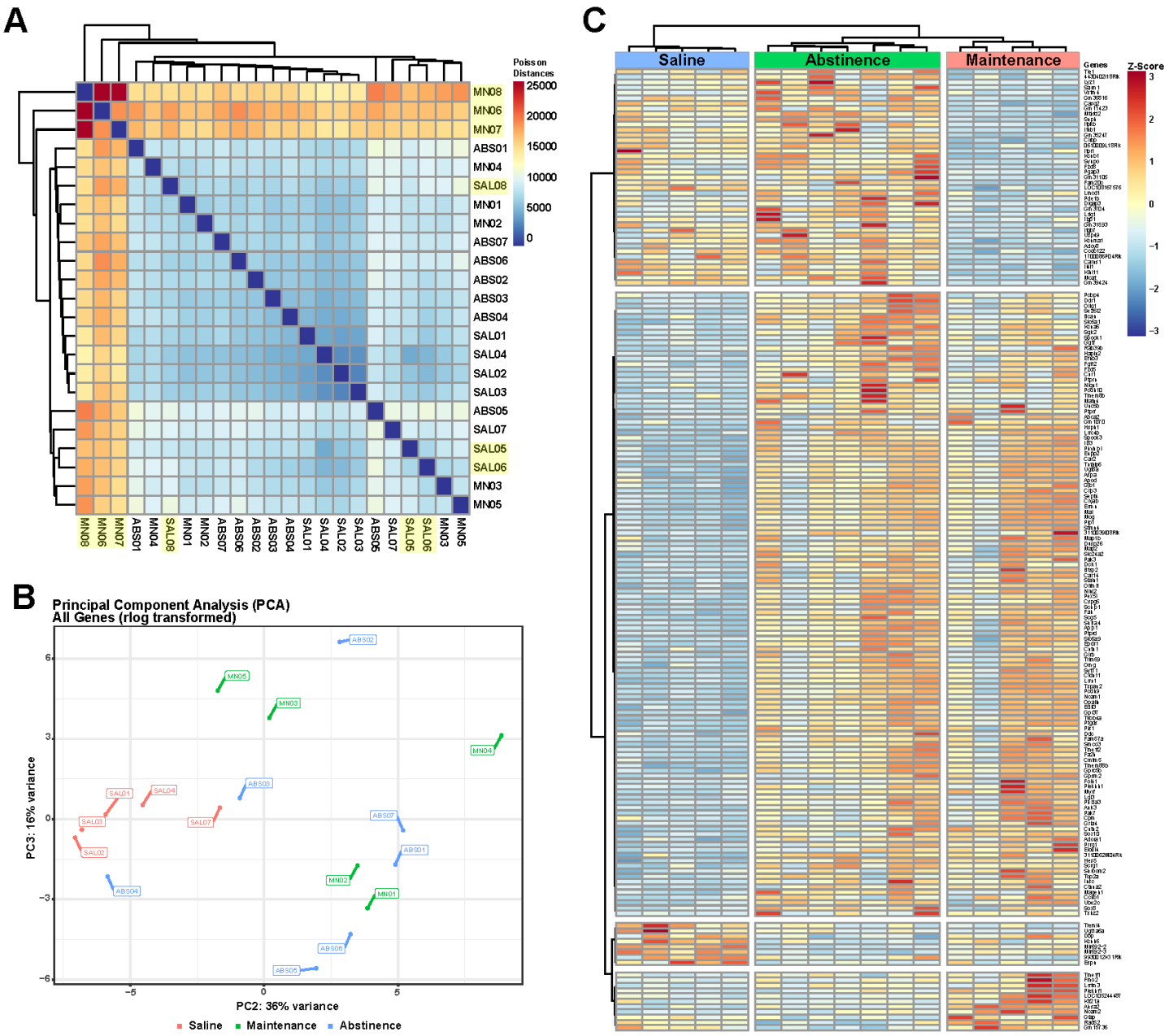
Supplementary Methods and Results

RNA-sequencing analysis of isolated striatal microglia.

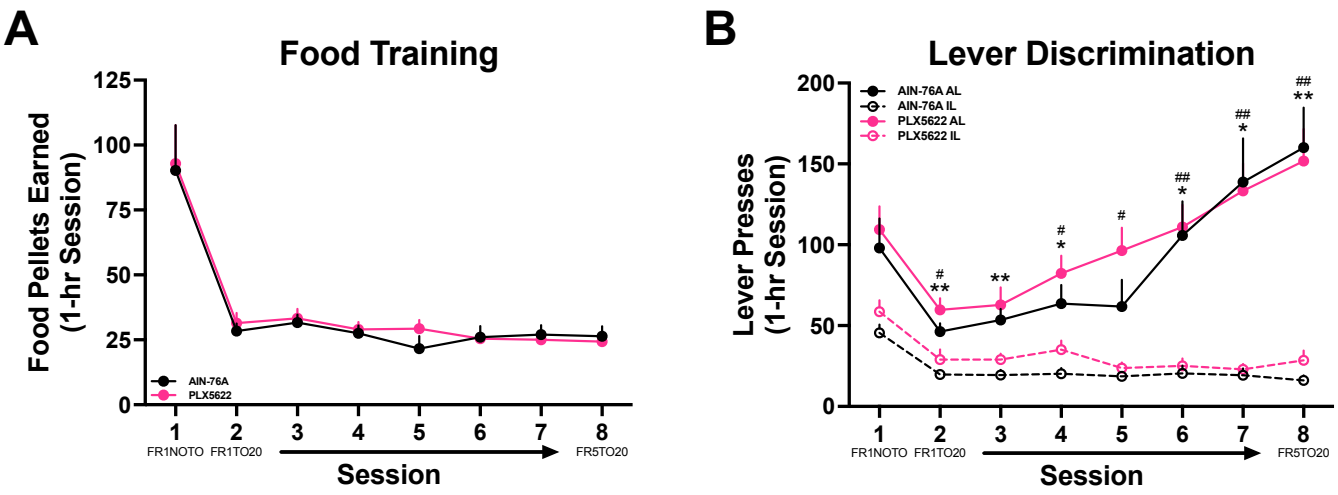
Biological replicates determined to be outliers were removed for differential gene expression analysis (**Supplementary Fig. 1A**). Principal component analysis (PCA) (**Supplementary Fig. 1B**) and heatmap of hierarchical clustering of conditions based on gene expression (**Supplementary Fig. 1C**) shows high similarity of samples within condition, and that animals exposed to methamphetamine (Maintenance and Abstinence) cluster more closely than to Saline.

Microglia are not required for natural food reinforcement.

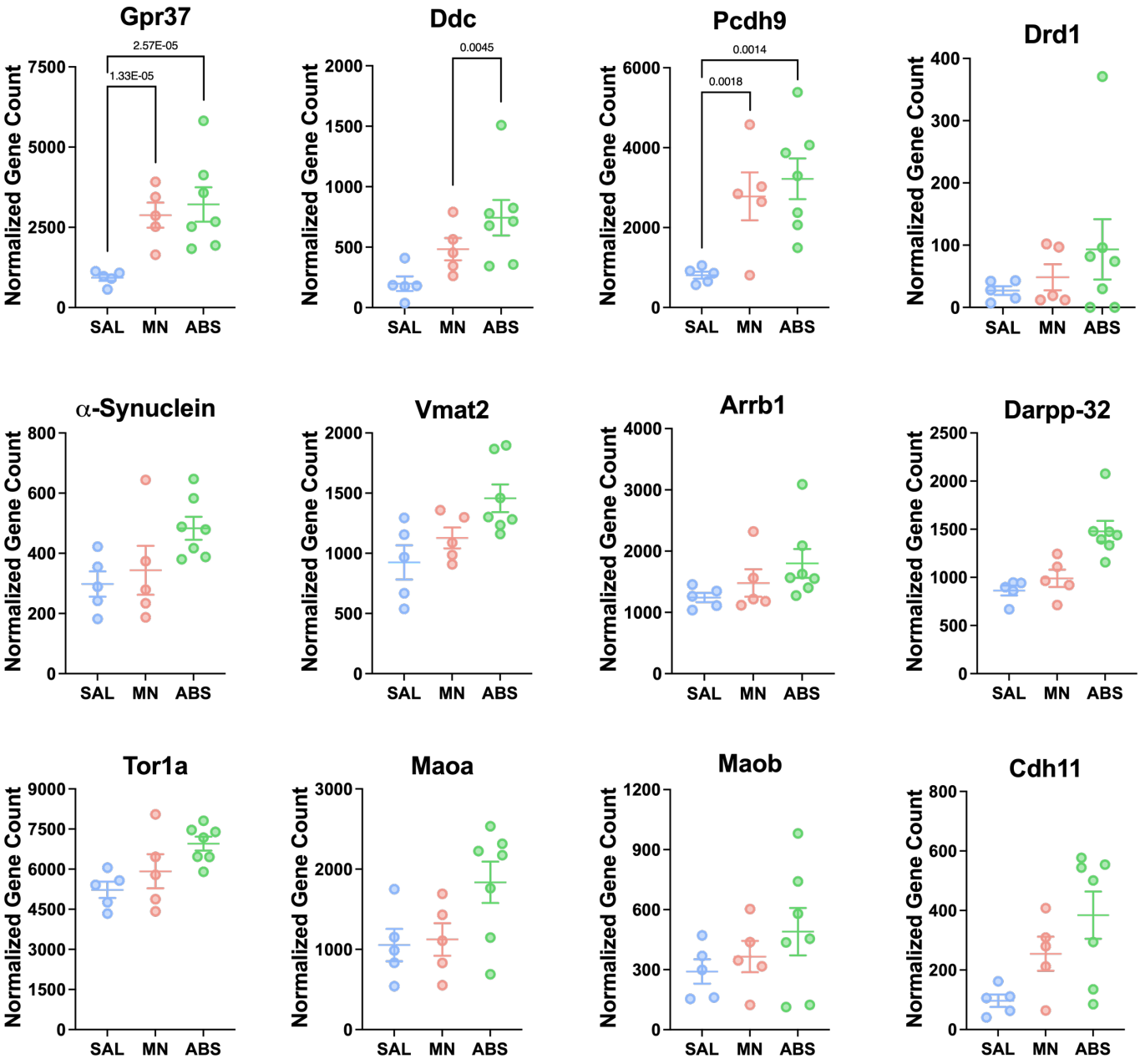
To test if microglia are necessary for learned operant behavior, we food-trained mice up to FR5 for 8 consecutive days (**Supplementary Fig. 2**). Mice were treated with PLX5622 (1200 ppm in AIN-76A chow) for the duration of the experiment. Microglial ablation does not affect natural food reinforcement in number of rewards earned (**Supplementary Fig. 2A**) (Two-way RM ANOVA; AIN-76A vs PLX5622, $F(1, 13) = 0.07321$, $p=0.7910$) or lever discrimination (**Supplementary Fig. 2B**) (Two-way RM ANOVA; Active vs Inactive Lever, $F(3, 26) = 24.38$, $p<0.0001$) and time to acquire operant lever pressing behavior (**Supplementary Fig. 2B**) (Two-way RM ANOVA; AIN-76A vs PLX5622, $F(1, 13) = 0.3855$, $p=0.5454$).



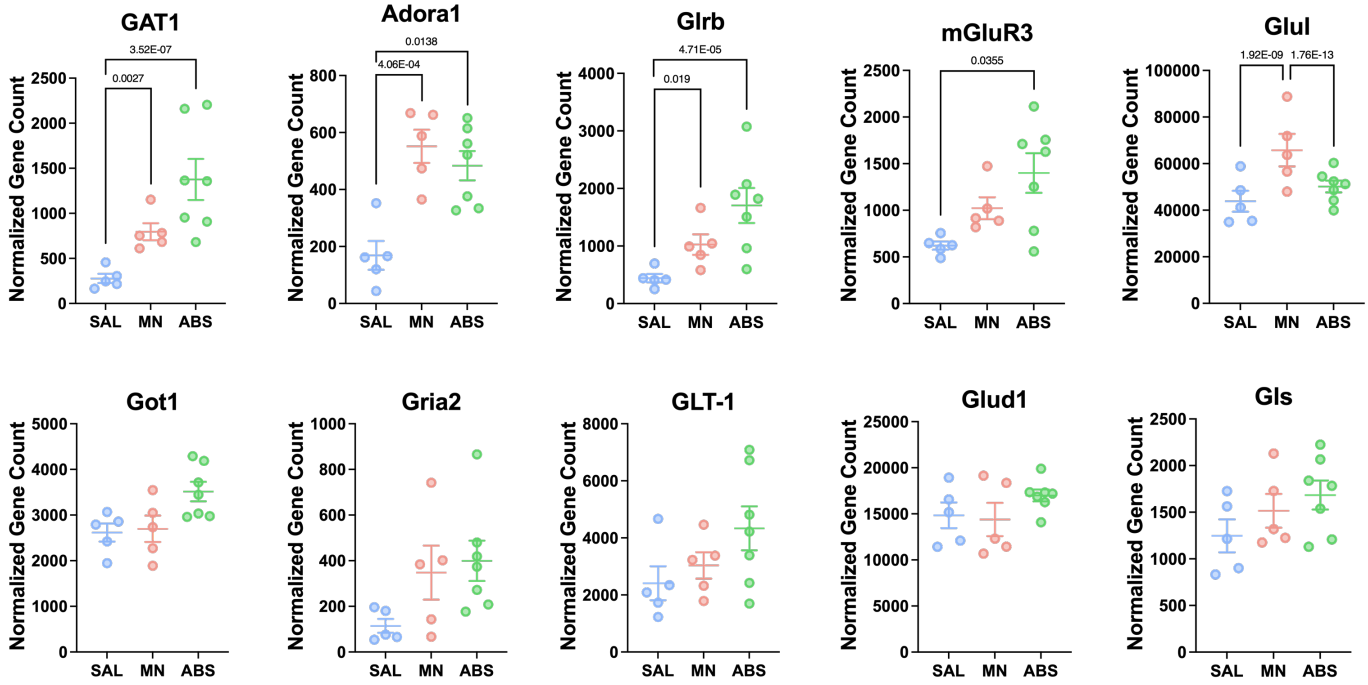
Supplementary Figure 1. RNA-sequencing of isolated striatal microglia from METH IVSA. A) Hierarchical clustering heatmap of expression profiles for samples (n=23) based on Poisson distance. Highlighted samples were determined to be outliers and were removed from analyses. **B)** PCA plot for samples (n=17) following removal of outliers. **C)** Heatmap showing unsupervised clustering of samples based on gene expression.



Supplementary Figure 2. Pharmacological ablation of microglia does not affect operant responding. **A)** Number of food rewards earned during 8 daily 1-hr sessions. **(B)** Active vs inactive lever presses during 8 daily 1-hr sessions (Two-way RM ANOVA with Bonferroni post-hoc test; AIN-76A Active vs Inactive Lever, *p < 0.05, **p < 0.01; PLX5622 Active vs Inactive Lever, $^{\#}$p < 0.05, $^{\#\#}$p < 0.01). AIN-76A (n=8), PLX5622 (n=7). Data are represented as mean ± SEM.



Supplementary Figure 3. Dopamine signaling-related genes. Normalized counts of DE genes with adjusted p-value for each comparison.



Supplementary Figure 4. GABA, glutamate and adenosine signaling-related genes. Normalized counts of DE genes related to GABA, glutamate, and adenosine signaling with adjusted p-value for each comparison.