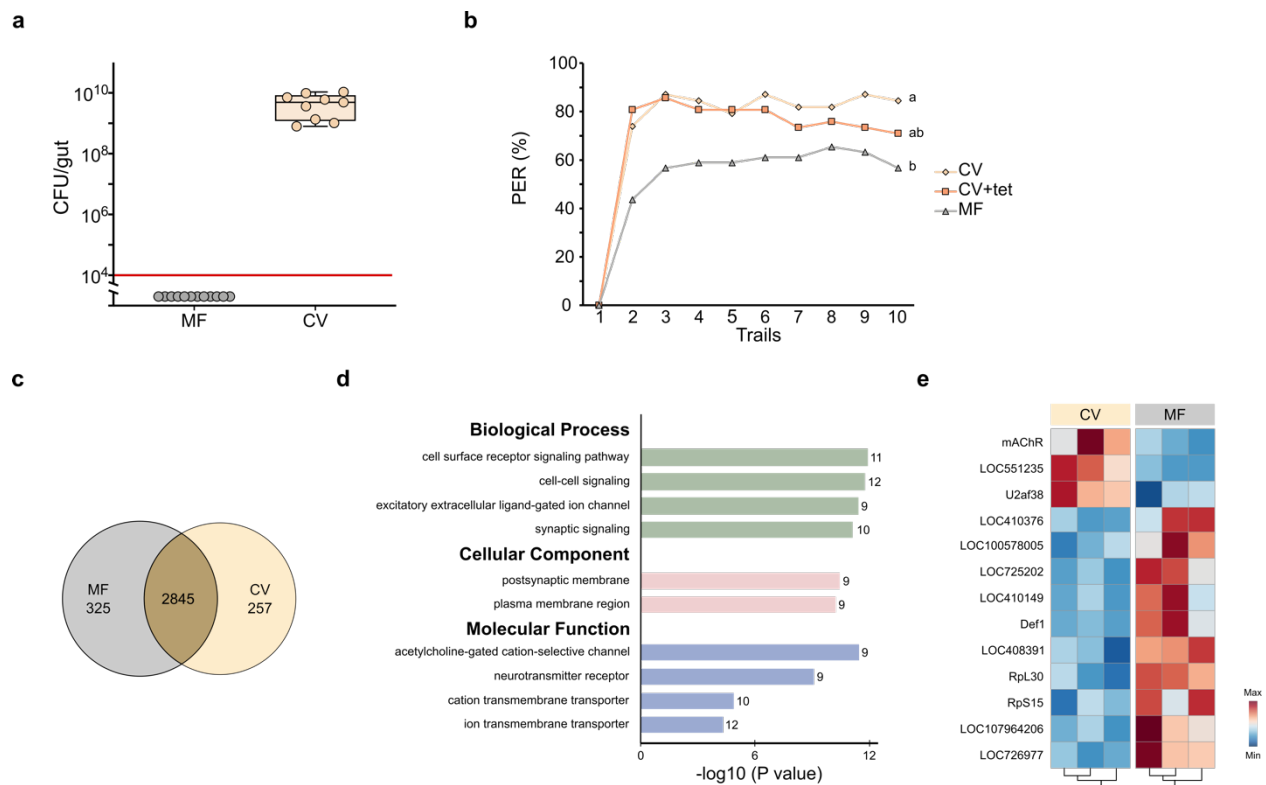
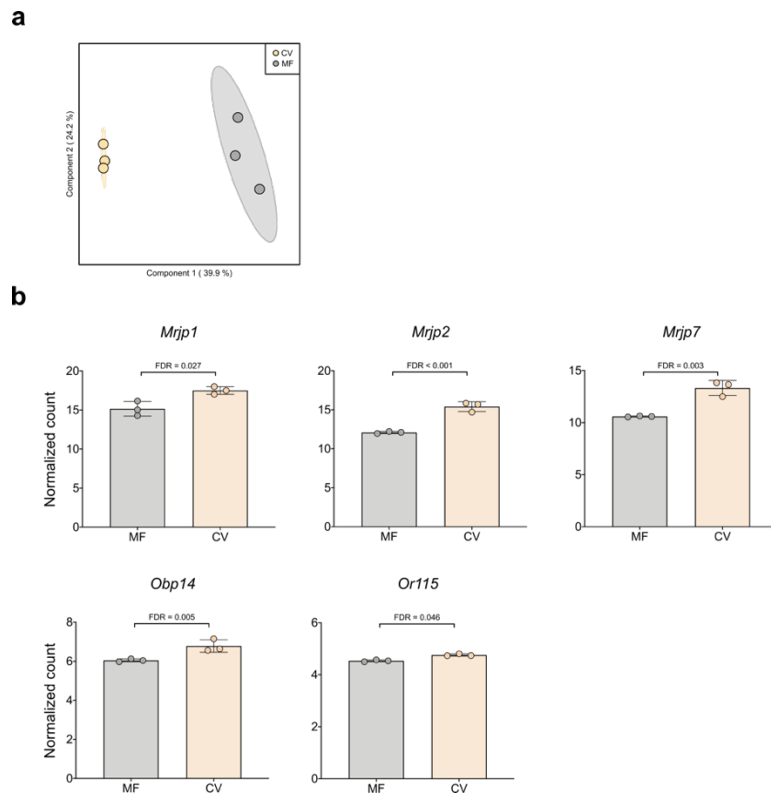


**Supplementary Information: Honeybee gut *Lactobacillus*
modulates host learning and memory behaviors via regulating
tryptophan metabolism**

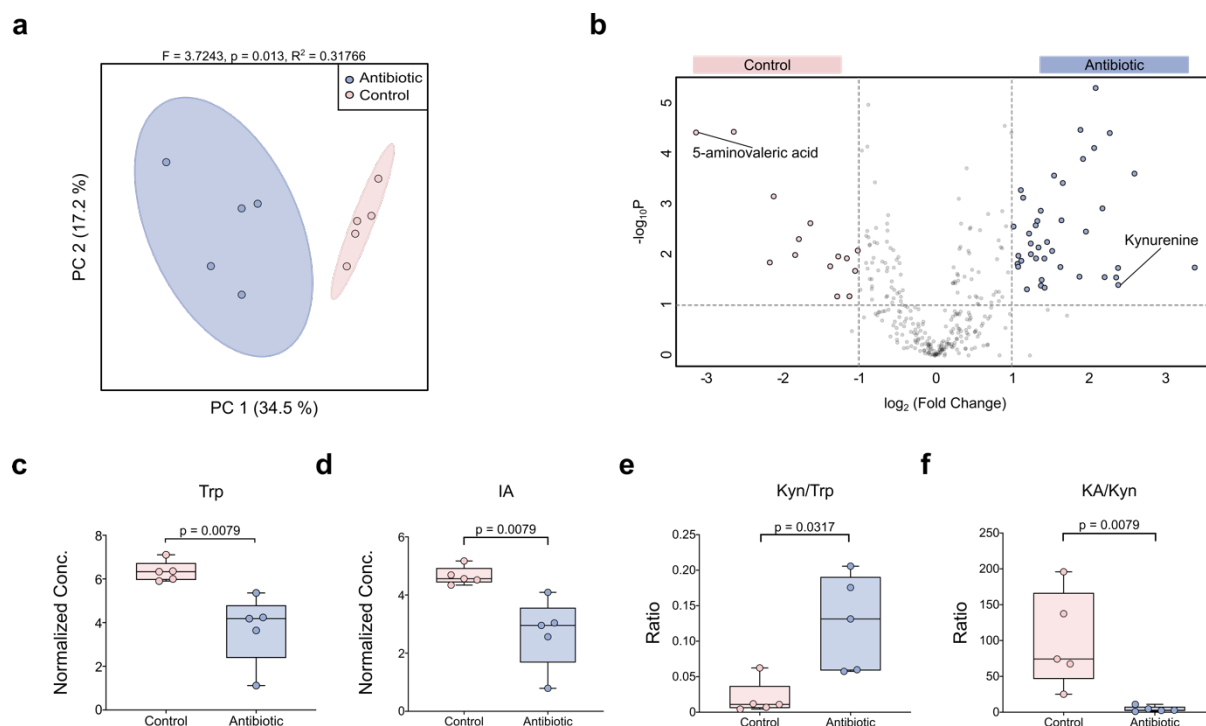
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Supplementary Fig. 2. Gut microbiota impacts learning performance and the proteomic profiling in the honeybee brain. **a** Boxplots of the total CFU per gut estimated by bacteria culture for MF bees ($n = 12$), or by qPCR for the CV group ($n = 9$). Error bars represent min and max. **b** Acquisition curves (% of PER to the nonanol odor) of bees in CV ($n = 38$), CV+tet ($n = 41$), and MF groups ($n = 46$) during 10 training trials. Different letters (a, b, c) stand for statistical differences between groups (two-sided Fisher's exact test: $p = 0.0088$ between MF and CV groups; $p = 0.1862$ between CV+tet and CV groups). **c** Venn diagrams indicating the numbers of shared and unique proteins identified in the brains of CV and MF bees. **d** The most significantly enriched GO terms of identified proteins unique to the CV group ($p < 0.0001$, one-way ANOVA test). The number of genes involved in each GO term is displayed next to the bars. **e** Heatmap of differentially expressed proteins detected in the brains of CV and MF bees. Source data are provided as a Source Data file.

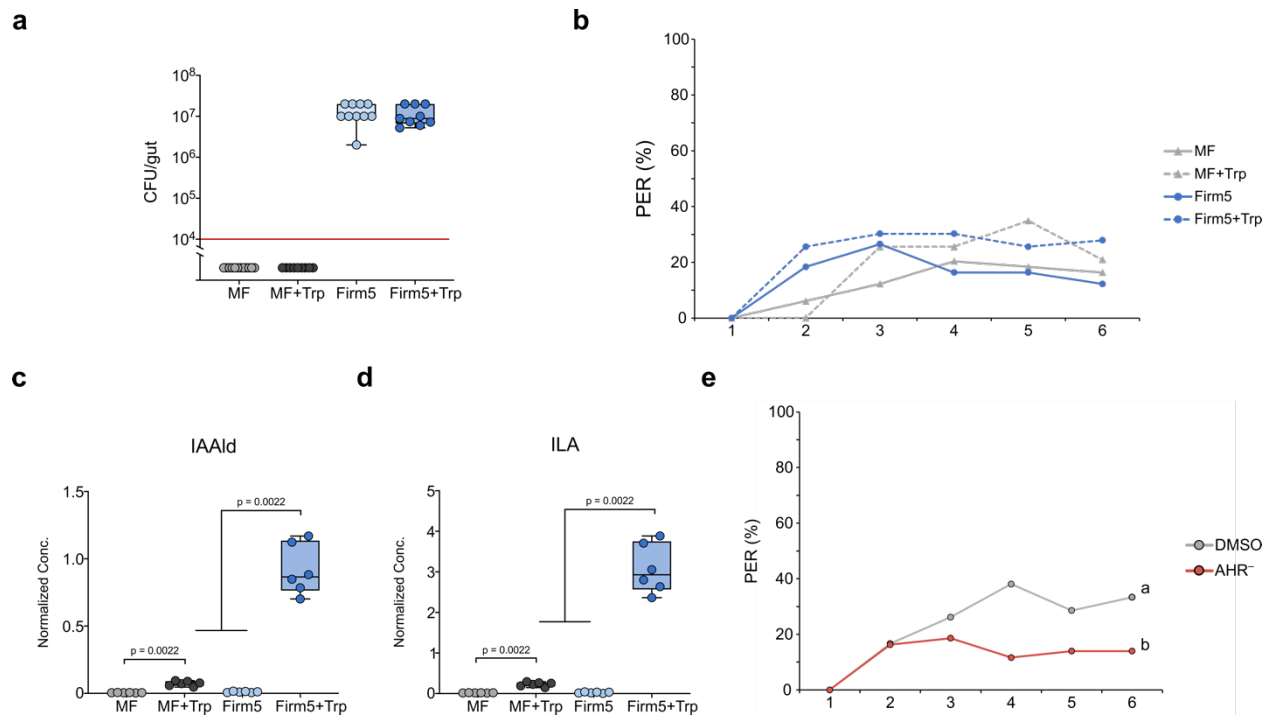


Supplementary Fig. 3. Gut microbiota impacts transcriptomic profile in the honeybee brain. **a** Sparse PLS-DA based on normalized gene expression in the brain of MF and CV bees. **b** Relative expression levels of differentially expressed genes in the brains of MF and CV groups ($n = 3$ bees for both groups). Differences between CV bees and the MF group were tested by Wald test with Benjamini-Hochberg correction. Data are presented as mean $\pm$ SD. Source data are provided as a Source Data file.



Supplementary Fig. 4. Antibiotic treatment affects gut Trp metabolism under field

conditions. **a** Principal coordinate analysis based on all metabolites detected in guts of control and antibiotic-treated bees. Group differences were tested by permutational multivariate ANOVA (PERMANOVA). **b** Volcano plot showing the differentially regulated metabolites. Metabolites significantly enriched in control bees are shown in pink, and those enriched in antibiotic-treated bees are in blue. **c-f** Boxplots of **(c)** the normalized concentration of Trp and **(d)** IA, **(e)** the Kyn/Trp ratio, and **(f)** the KA/Kyn ratio in the guts of control and antibiotic-treated bees ($n = 5$ bees for both groups). Group differences were tested by two-sided Mann-Whitney u test. Error bars represent min and max. Source data are provided as a Source Data file.



Supplementary Fig. 5. *Lactobacillus apis* modulates honeybee learning performance

via the activation of AhR. **a** Boxplots of the total CFU per gut estimated by bacteria culture for MF ($n = 12$), MF+Trp ($n = 9$), Firm5 ($n = 10$), and Firm5+Trp ($n = 9$) bees. Error bars represent min and max. **b** Acquisition curves (% of PER to the nonanol odor) of bees in MF ($n = 49$, grey triangles), MF+Trp ($n = 43$, grey triangles with dotted line), Firm5 ($n = 49$, blue circles), and Firm5+Trp groups ($n = 43$, blue circles with dotted line) during 6 training trials. Different letters (a and b) stand for statistical differences between groups (Chi-square test, $p = 0.2617$). **c-d** Boxplots of the normalized concentration of (c) indole-3-acetaldehyde (IAAld) and (d) indole-3-lactic acid (ILA) in the gut of MF, MF+Trp, Firm5, and Firm5+Trp bees ($n = 5$ bees for all groups). Group differences were tested by two-sided Mann-Whitney u test. Error bars represent min and max. **e** Acquisition curves (% of PER to the nonanol odor) of bees in DMSO ($n = 42$, grey circles) and AHR⁻ ($n = 43$, red circles) during 6 training trials. Different letters (a and b) stand for statistical differences between groups (two-sided Fisher's exact test: $p = 0.0431$). Source data are provided as a Source Data file.