iScience, Volume 24

# **Supplemental information**

# Effects of early-life penicillin exposure

#### on the gut microbiome and frontal

#### cortex and amygdala gene expression

Angelina Volkova, Kelly Ruggles, Anjelique Schulfer, Zhan Gao, Stephen D. Ginsberg, and Martin J. Blaser

# Supplemental Figures

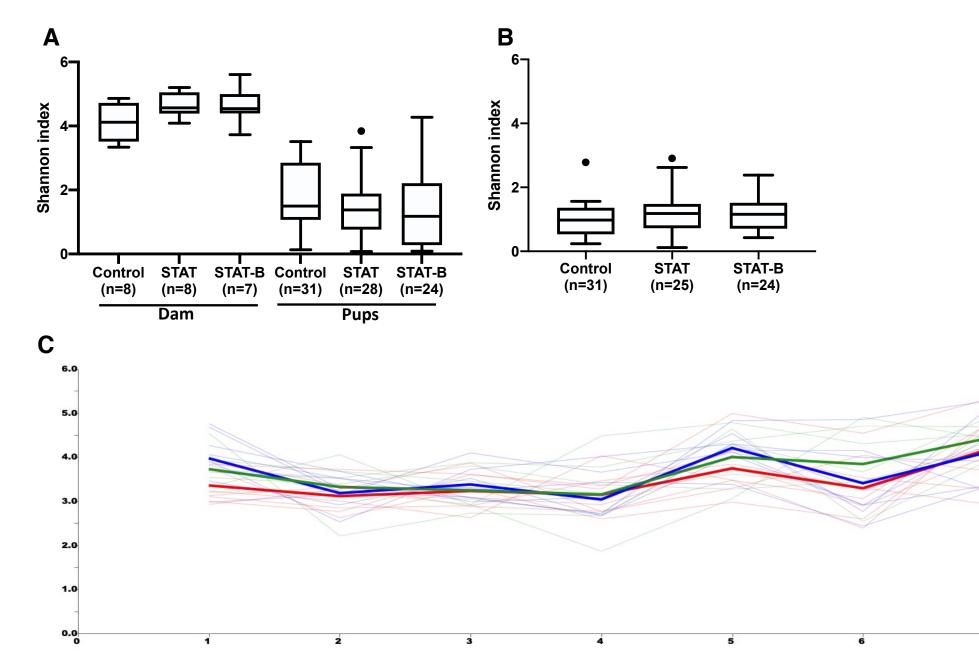
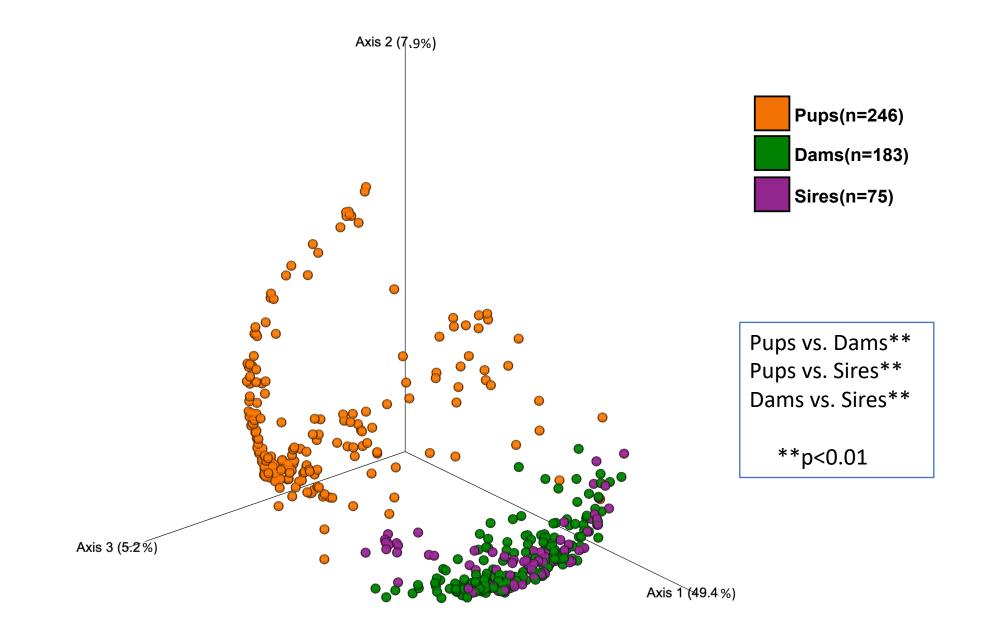


Figure S1. Alpha diversity, represented by Shannon index, with reference to STAR Methods.

7

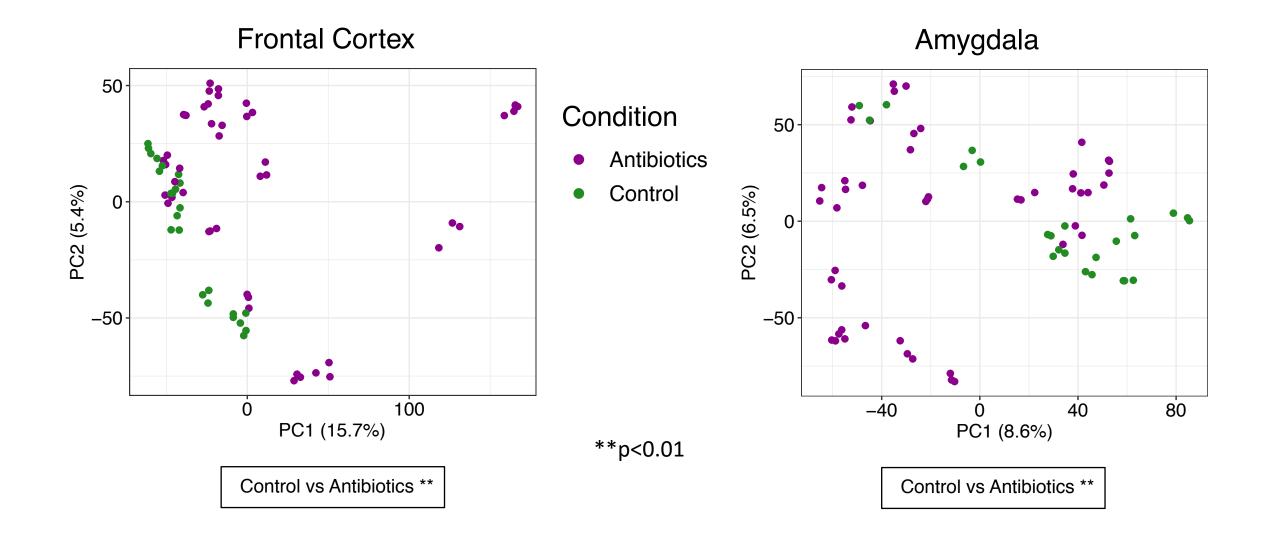
**Figure S1. Alpha diversity, represented by Shannon index, with reference to STAR Methods. Panels: A.** Cecal content from dams and pups; **B.** Small intestinal content from pups. No significant differences were found between different treatment groups by Kruskal-Wallis test (p>0.05); **C.** Volatility of Shannon index in fecal samples from dams. Colors are: control (Red), STAT (blue), STAT-Birth (green). Time points indicate: 1. Start of whittening; 2. End of mating; 3. Post-mating 1; 4. Pregnancy check; 5. Post-mating 2; 6. Pup observation; 7. Sacrifice. No significant differences were found among the three treatment groups at the different timepoints by one-way ANOVA test (p>0.05).



**Figure S2.** Beta-diversity of pup, mother, and sire, according to Bray-Curtis distance matrix analysis of 504 samples, as shown by PCoA, with reference to STAR Methods.

Figure S2. Beta-diversity of pup, mother, and sire, according to Bray-Curtis distance matrix analysis of 504 samples, as shown by PCoA, with reference to STAR Methods. All three groups differ significantly in composition, as determined by Pairwise Permanova test (\*\*p < 0.01).

**Figure S3.** PCA based on relative expression of all genes in the frontal cortex and amygdala in the two-group comparison, in relation to Figure 4.

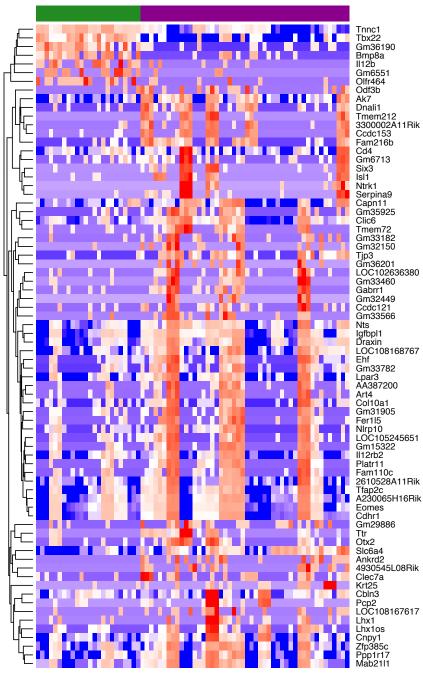


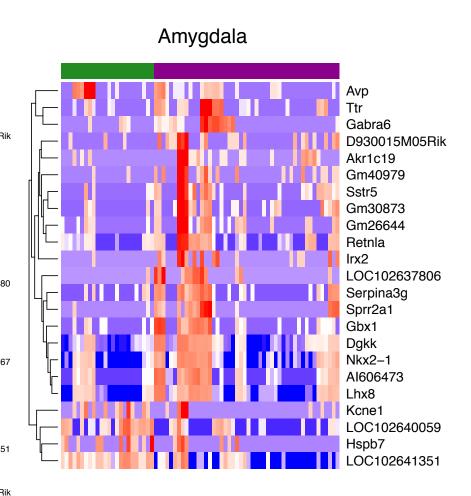
# Figure S3. PCA based on relative expression of all genes in the frontal cortex and amygdala in the two-group comparison,

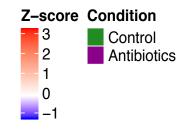
in relation to Figure 4. Comparisons are between the control mice and the mice in the two antibiotic exposure groups (STAT and STAT-Birth) combined into a single group. Global expression data for each mouse in each anatomic site are represented by

PCA. The significance of differences was tested by pairwise Wann-Whitney test based on PC1 (\*\*p<0.01).

### Frontal Cortex







**Figure S4.** Heat map of genes in the frontal cortex and amygdala that are differentially expressed in the two-group comparison (combining the STAT and the STAT-Birth groups) compared to controls, in relation to Figure 6.

Figure S4. Heat map of genes in the frontal cortex and amygdala that are differentially expressed in the two-group comparison (combining the STAT and the STAT-Birth groups) compared to controls, in relation to Figure 6. Only

genes that are significantly significant (p < 0.01 with log fold-change > 1.5) are shown.