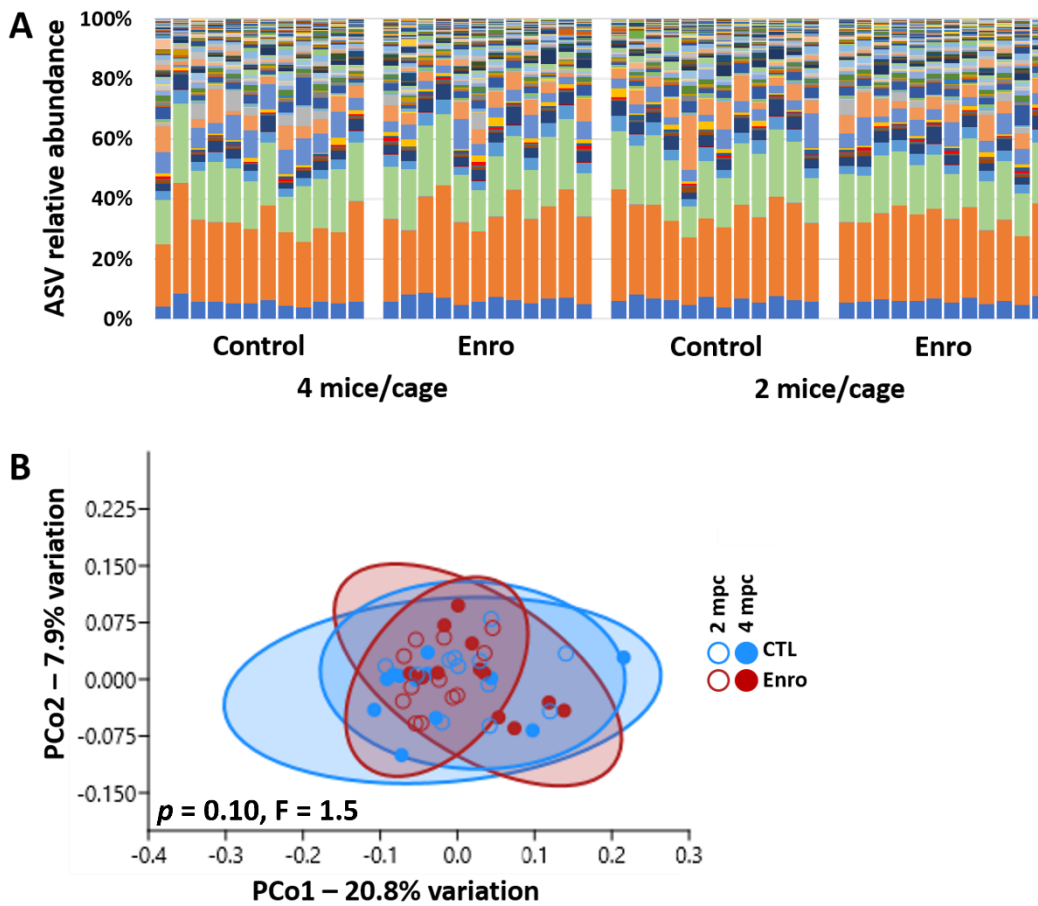


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Supplemental information

**Reduced housing density improves statistical
power of murine gut microbiota studies**

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2 **Figure S1. No differences between groups in GM composition at baseline in Cohort 1, related to Figure 1. (A)**

3 Stacked bar chart showing the amplicon sequence variant (ASV) relative abundance, upon arrival and prior to

4 treatment, in feces from mice assigned to housing at 4 or 2 mpc, and to receive either sham (control) or enrofloxacin

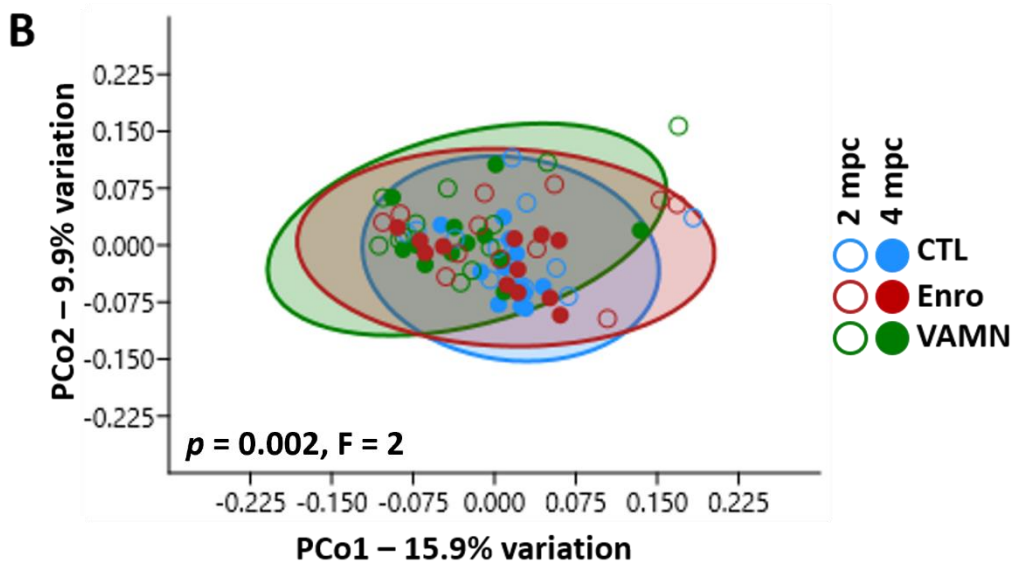
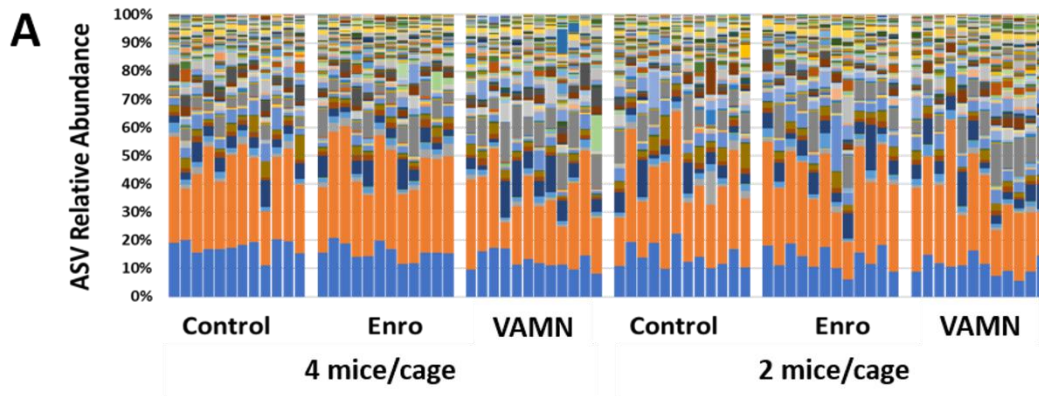
5 (Enro) in their drinking water. (B) Principal coordinate analysis of the samples shown in A, legend at right; p and F

6 value denotes results of PERMANOVA between all four groups.

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11 **Figure S2. No differences between groups in GM composition at baseline in Cohort 2, related to Figure 1. (A)**

12 Stacked bar chart showing the amplicon sequence variant (ASV) relative abundance, upon arrival and prior to

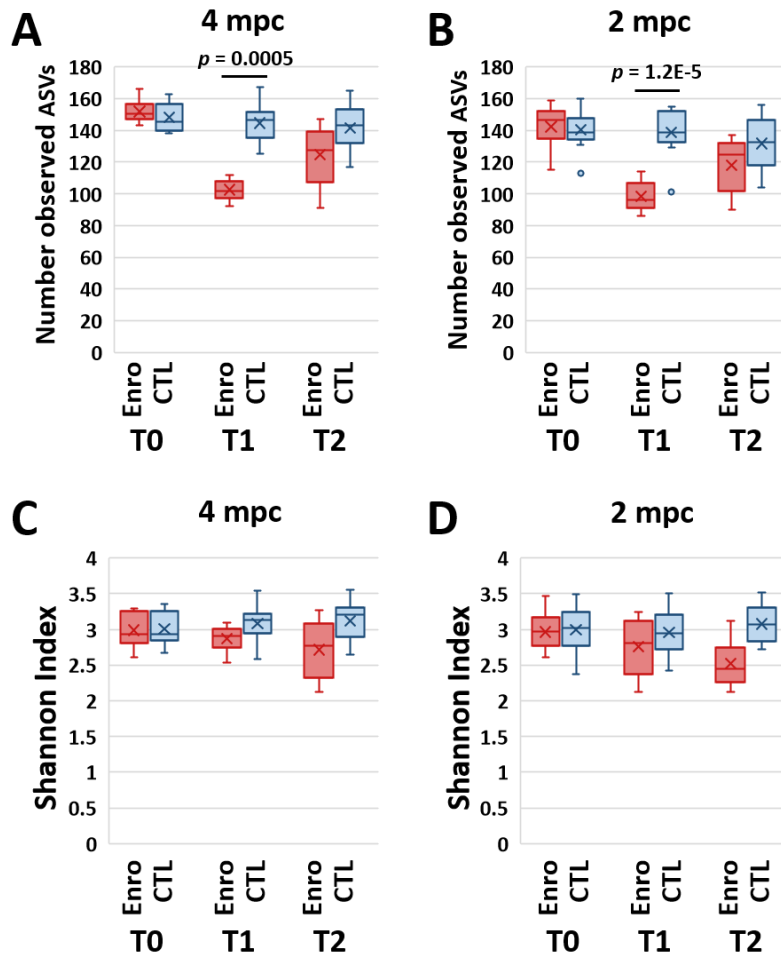
13 treatment, in feces from mice assigned to housing at 4 or 2 mpc, and to receive either sham (control), enrofloxacin

14 (Enro), or broad-range (VAMN) antibiotics in their drinking water. (B) Principal coordinate analysis of the samples

15 shown in A, legend at right; p and F values denote results of PERMANOVA between all four groups

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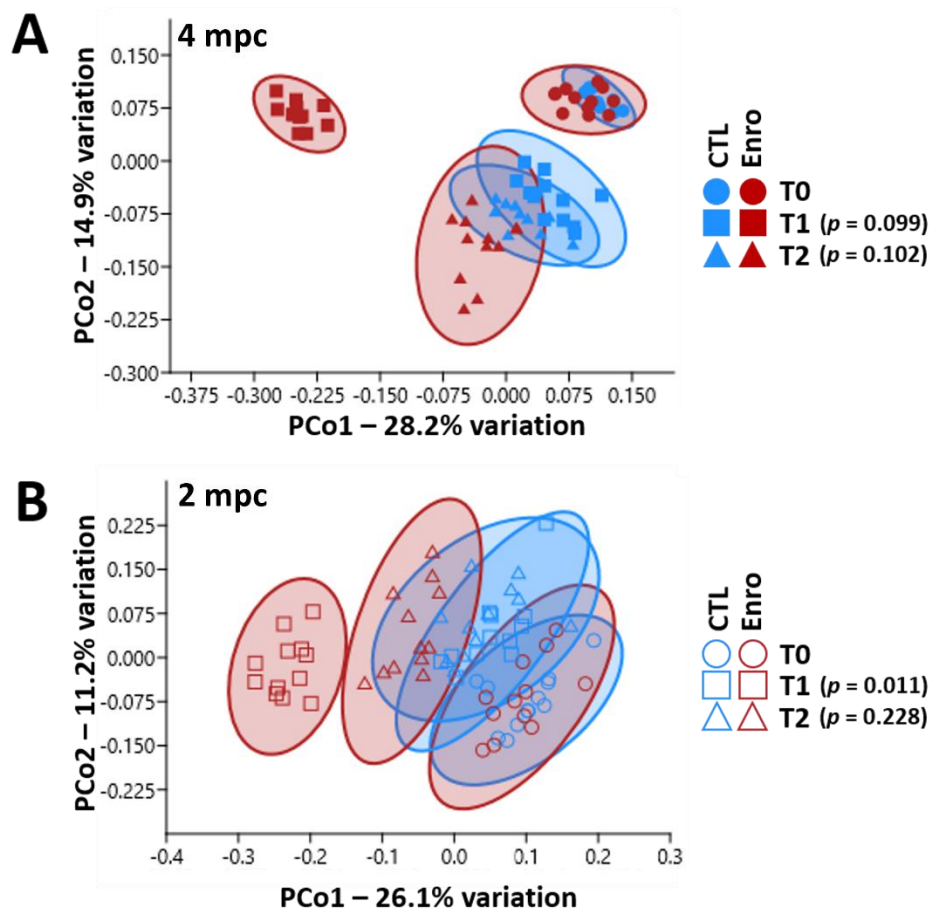
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19 **Figure S3. Effects of enrofloxacin on richness and alpha-diversity in Cohort 2, related to Figure 2.** Richness as
 20 represented by the number of distinct observed amplicon sequence variants (ASVs) in mice housed four per cage
 21 (A) or two per cage (B) upon arrival (T0), immediately after one week of exposure to antibiotics (or control) (T1),
 22 and three weeks after cessation of exposure (T2). Alpha diversity as estimated by the Shannon diversity index in
 23 mice housed four per cage (C) or two per cage (D). *p*-values were obtained from the mixed effect models.

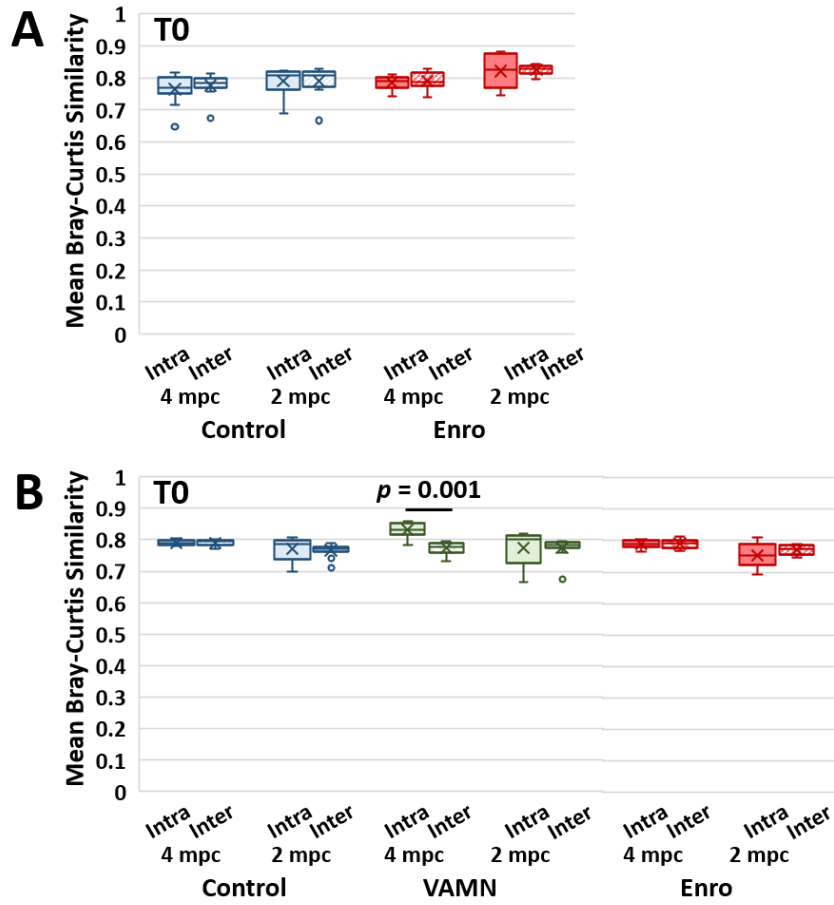
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26 **Figure S4. Enhanced effects of enrofloxacin on beta-diversity in mice housed 2 mpc, related to Figure 3.** Principal
 27 coordinates analysis of fecal samples from cohort 2 mice housed four mice per cage (A) or two mice per cage (B)
 28 and exposed to enrofloxacin (Enro) or control (CTL), collected upon arrival from the supplier (T0), immediately after
 29 one week of exposure to enrofloxacin or control (T1), and three weeks after cessation of exposure (T2). p -values
 30 were obtained from the nested PERMANOVA.

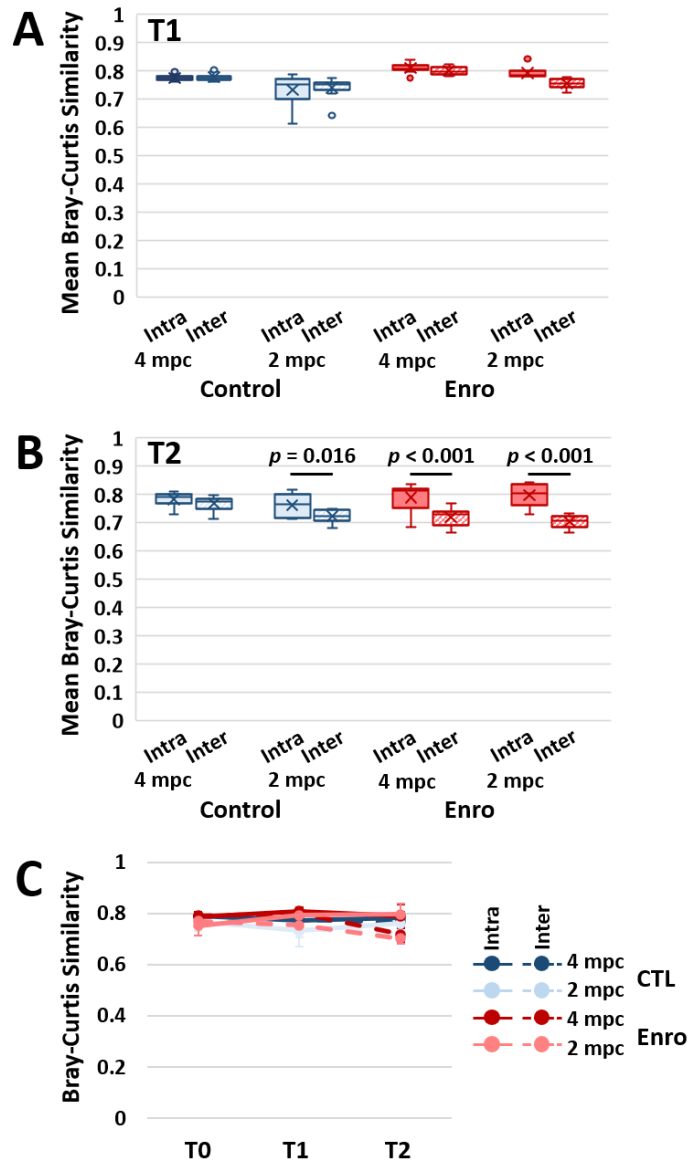
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33 **Figure S5. Few significant cage effects at baseline, related to Figure 4.** Tukey box plots showing mean intra-cage
 34 and inter-cage similarity between all possible sample pairs within a given treatment group and housing density
 35 immediately upon arrival at our institution, in Cohort 1 (**A**) and Cohort 2 (**B**)

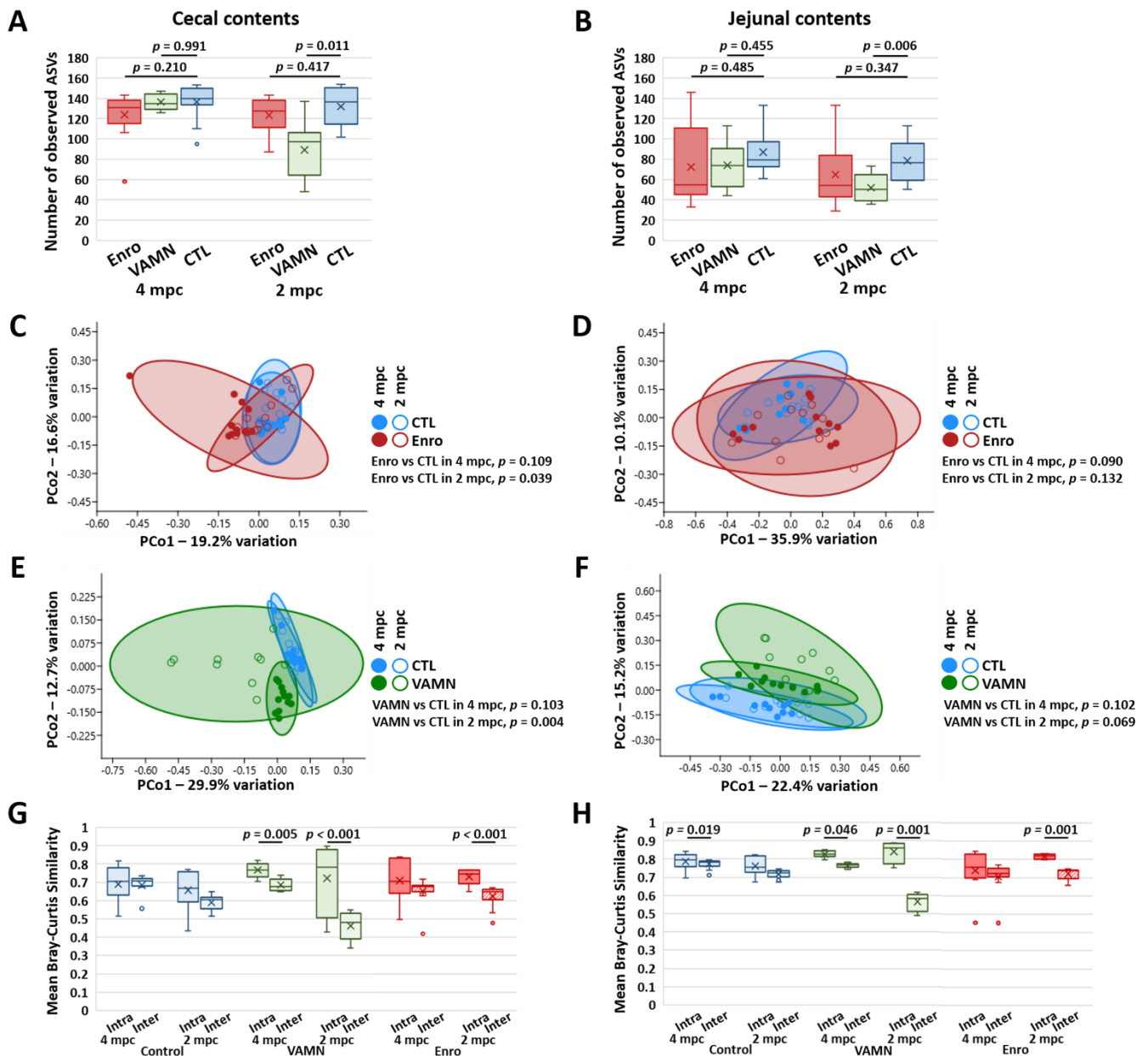
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38 **Figure S6. Housing density-mediated effects on cage effects, related to Figure 4.** Tukey box plots showing mean
 39 intra-cage and inter-cage similarity between all possible sample pairs within a given treatment group and housing
 40 density immediately after one week of exposure (**A**, T1) to enrofloxacin (Enro) or control (CTL), or after three weeks
 41 of recovery (**B**, T2) in cohort 2. Line chart for enrofloxacin and control groups in cohort 2 (**C**) showing the mean \pm
 42 SD intra- and inter-cage Bray-Curtis similarity upon arrival from the supplier (T0), T1, and T2. Legend at right.

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45 **Figure S7. Effects of housing density on antibiotic-induced changes in cecal and jejunal richness, alpha- and beta-**
 46 **diversity (Cohort 2), related to Figure 5.** Richness as represented by the number of observed amplicon sequence
 47 variants (ASVs) in the cecum (A) and jejunum (B) of mice housed four per cage or two per cage at three weeks after
 48 cessation of enrofloxacin (Enro), broad-spectrum antibiotics (VAMN), or sham (CTL) treatment; p values denote
 49 ABX-associated effects, based on mixed effect model with cage as a random effect. Principal coordinate analysis
 50 plots of Bray-Curtis similarities of cecal (C) and jejunal (D) microbiota of enrofloxacin and control groups, and cecal

51 (E) and jejunal (F) microbiota of VAMN and control groups, in Cohort 2; *p* values denote treatment-associated
52 effects based on PERMANOVA. Tukey box plots showing mean intra-cage and inter-cage similarity between all
53 possible sample pairs within a given treatment group and housing density, three weeks after cessation of treatment,
54 in the cecal (G) and jejunal (H) microbiota.

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