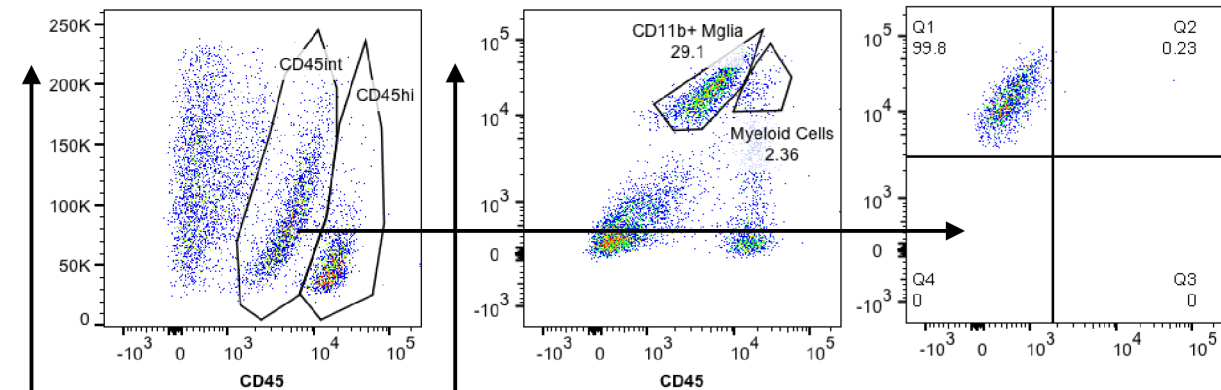
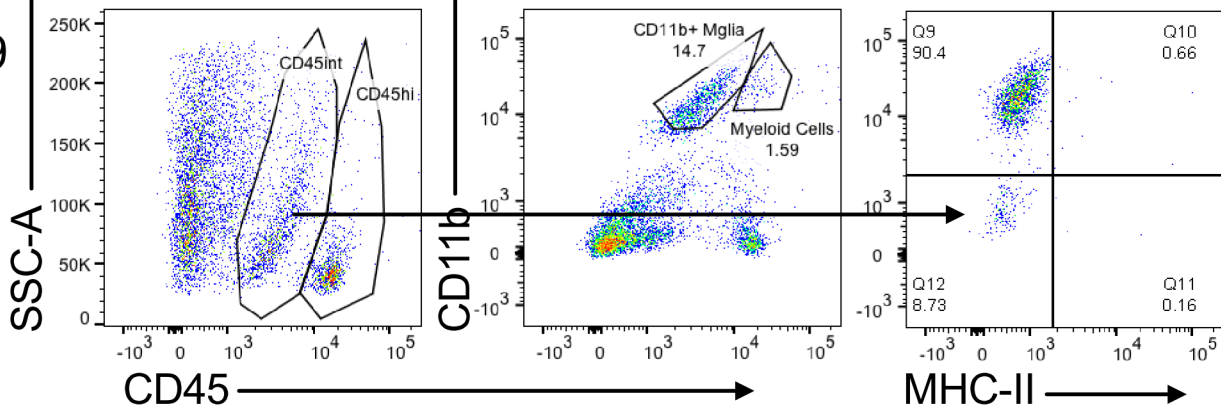
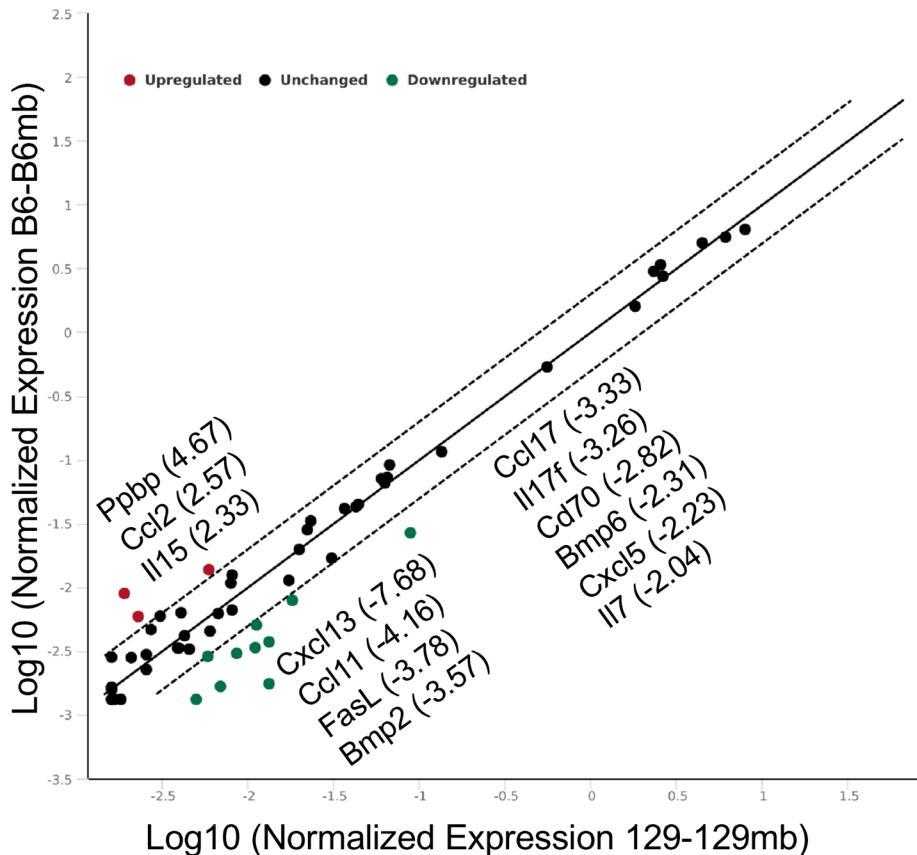


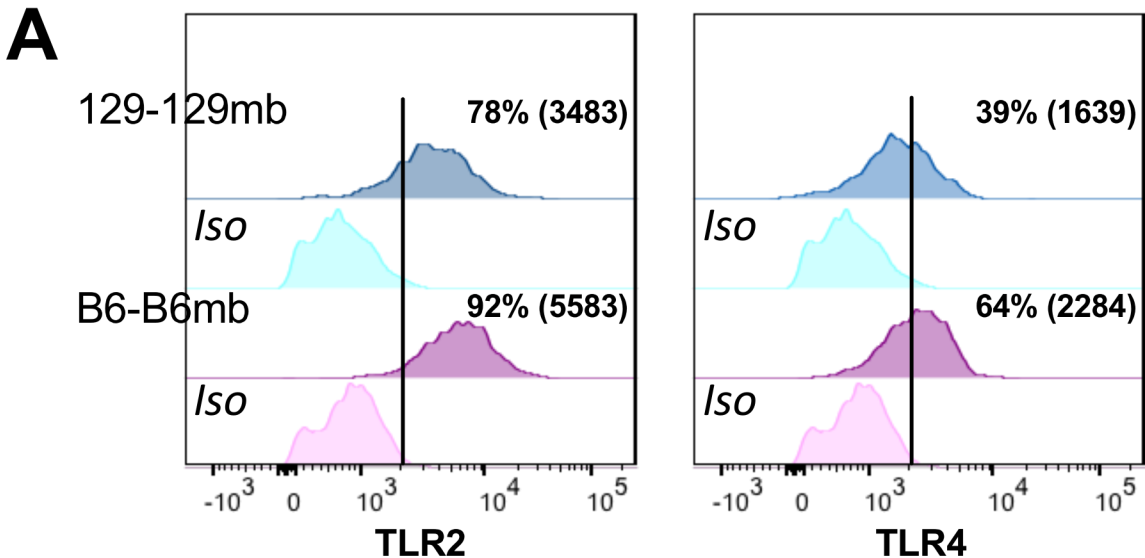
Supplementary Data.

Manuscript Title: Dominant Role of the Gut Microbiota in Chemotherapy Induced Neuropathic Pain.

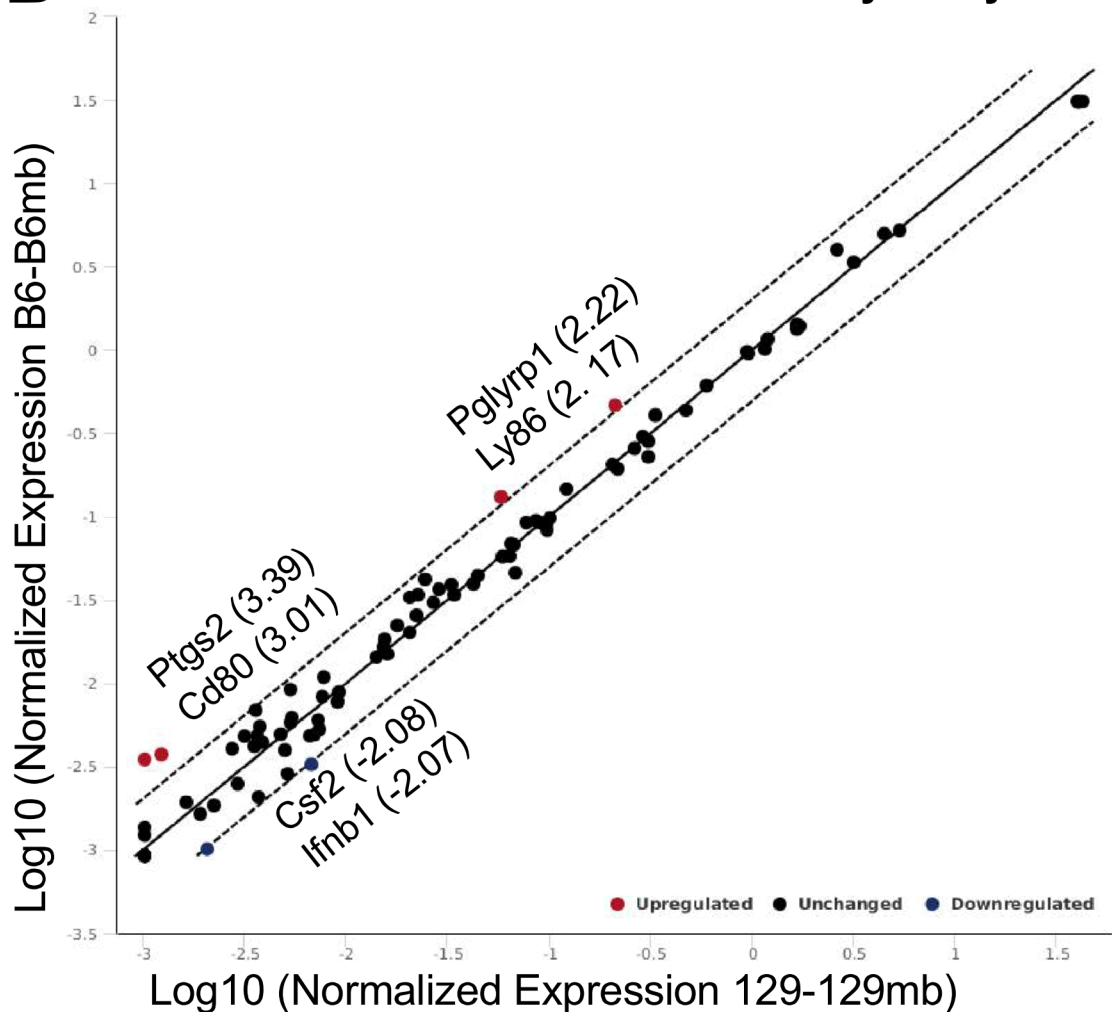
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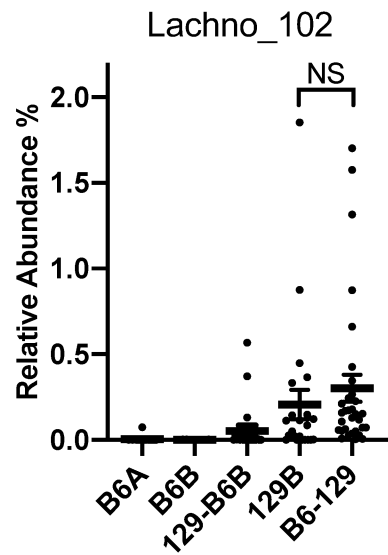
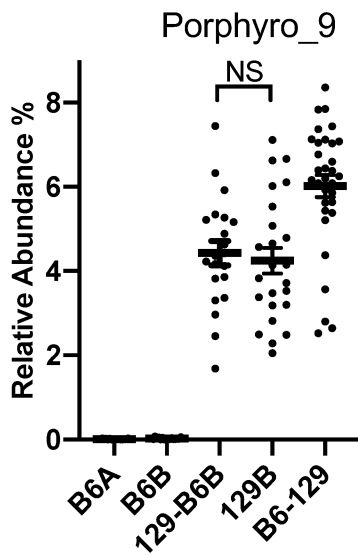
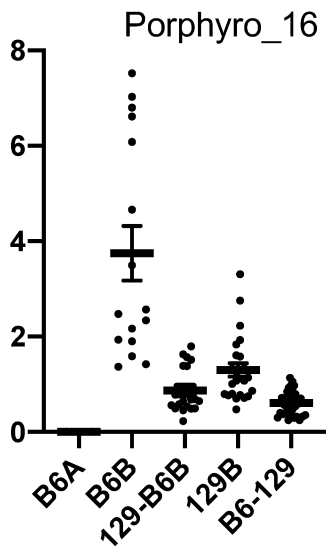
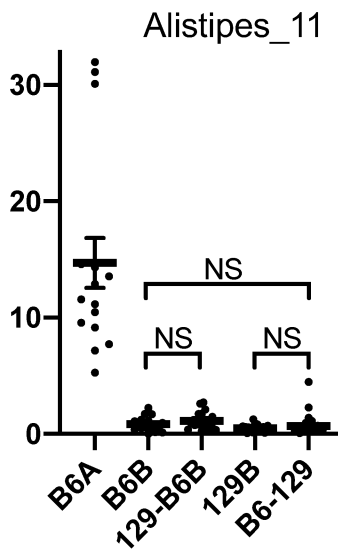
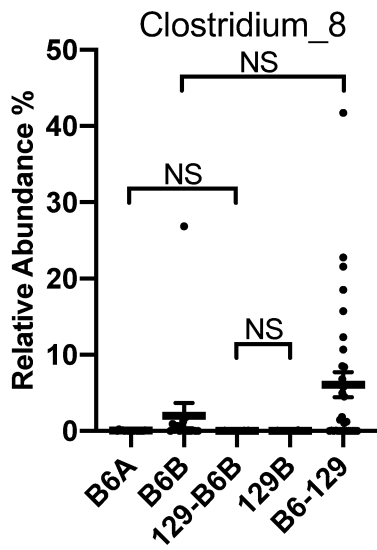
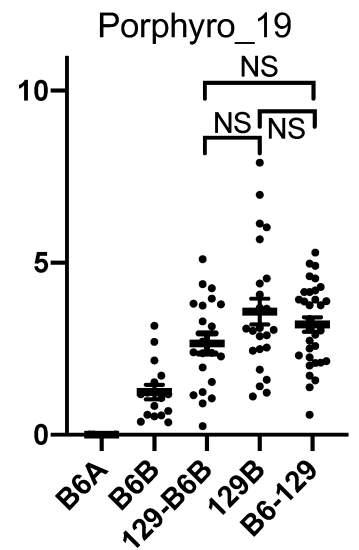
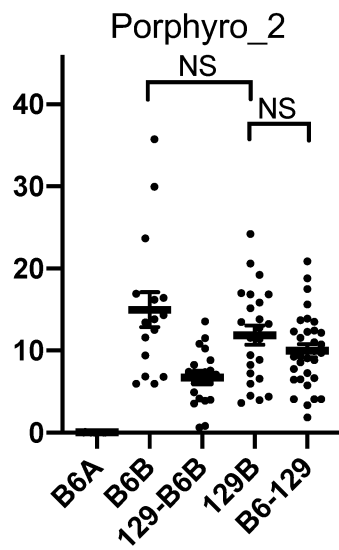
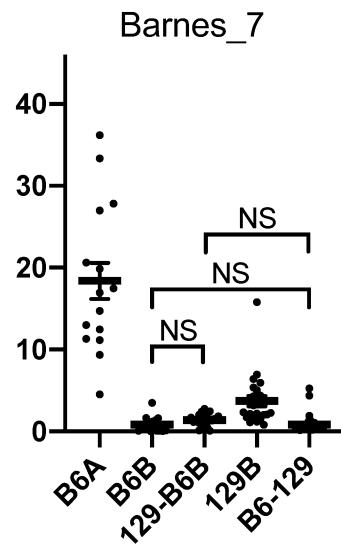
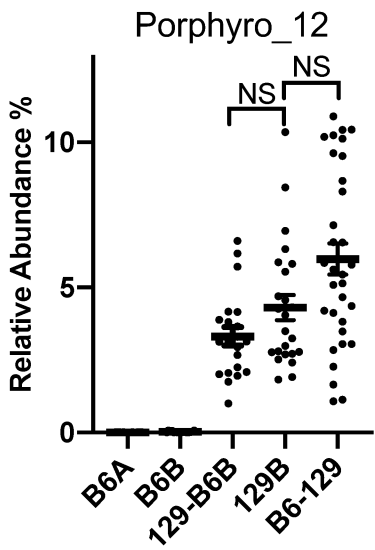
B6**129**

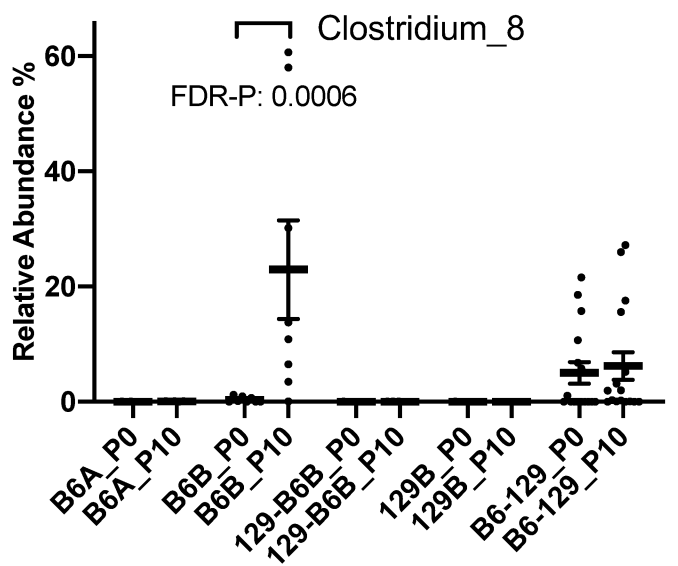
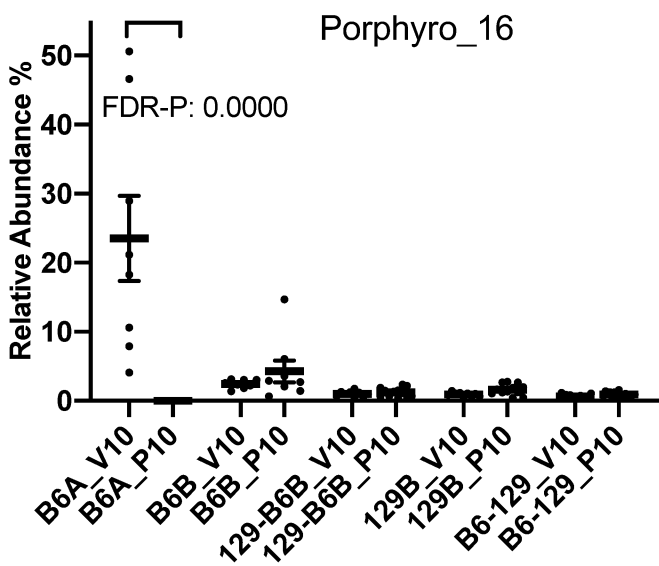
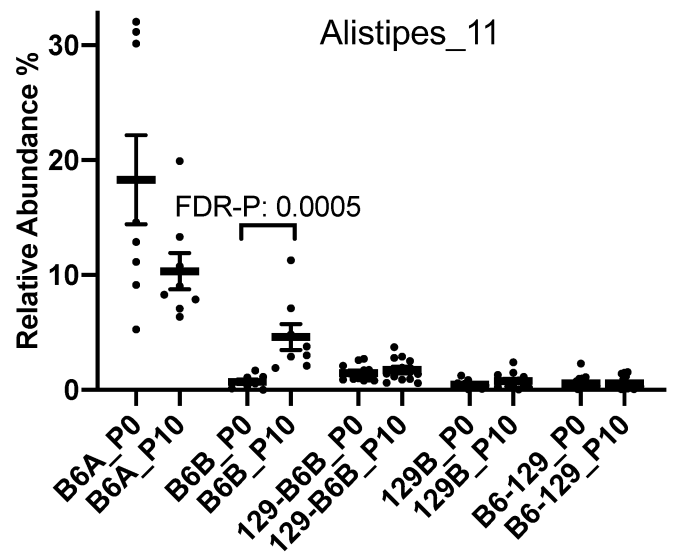
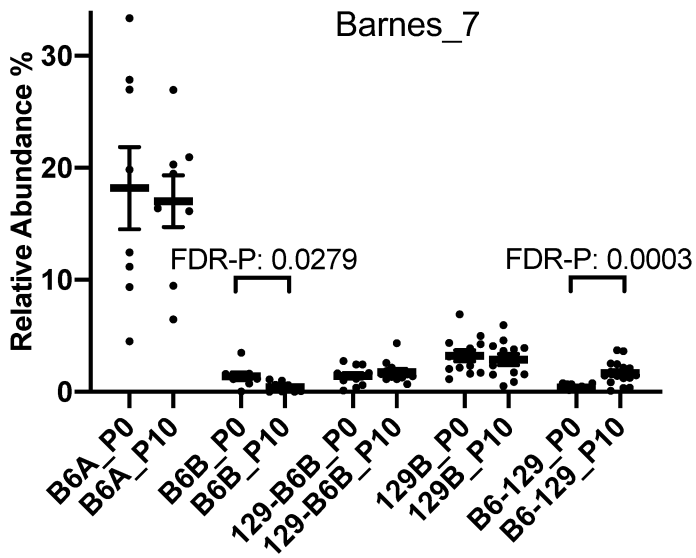
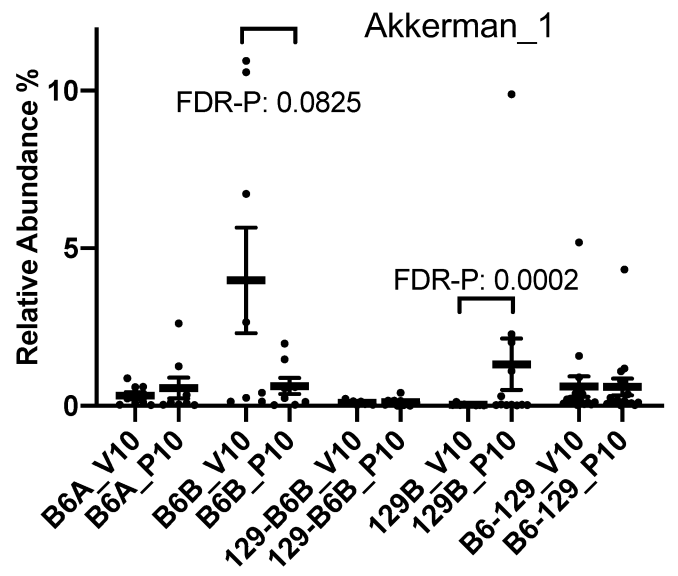
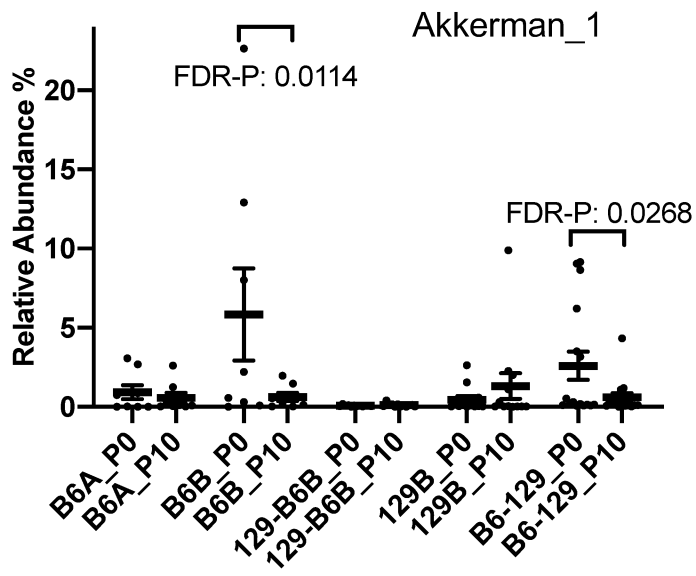


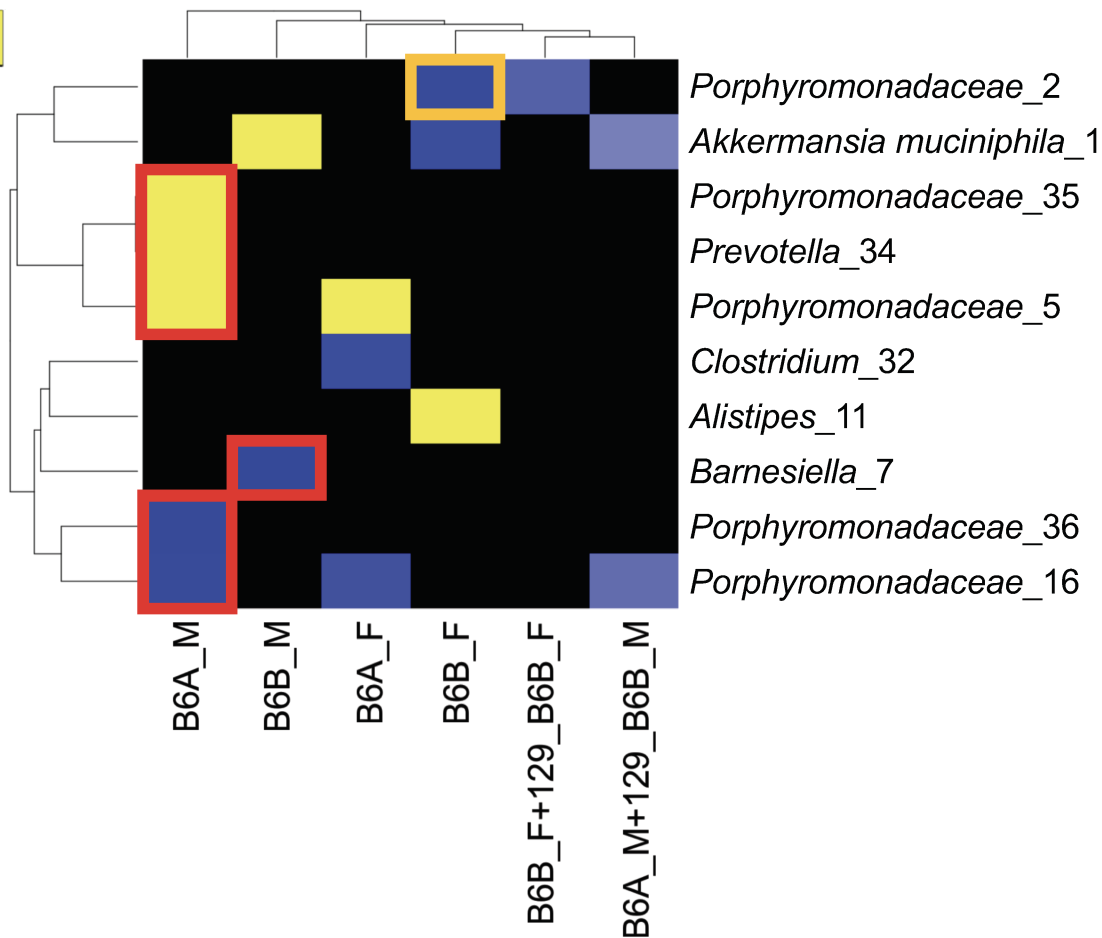
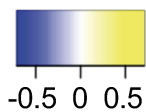
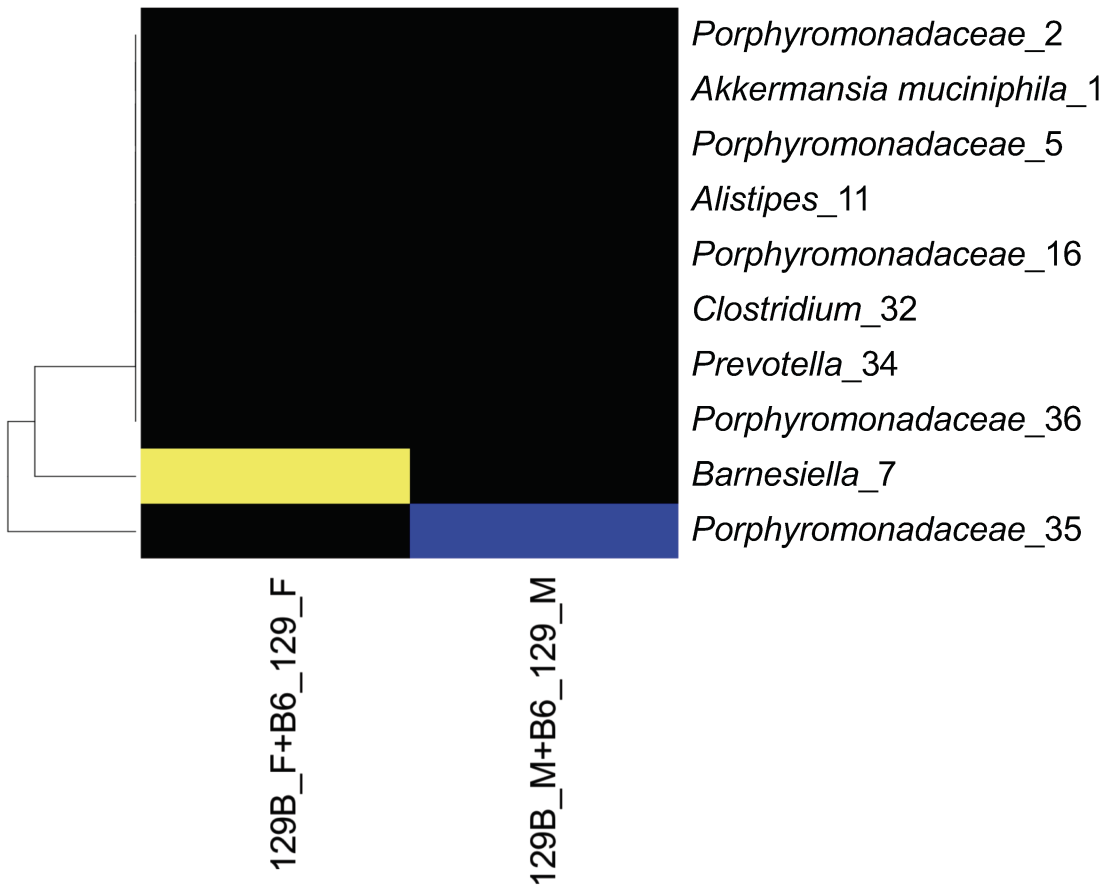
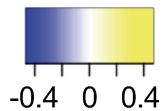


B B6-B6mb Vs 129-129mb: TLR Pathway Analysis







A**B**

1 **Supplementary Figure Legends:**

2

3 **Supplementary Figure 1: Gating Strategy for analysis of cells in the brainstem (BS) and**

4 **spinal cords (SC).** Mononuclear cells (singlets) isolated from the SC of WT B6 (top row) and

5 129 (bottom row) mice at day 5 post treatment were analyzed for CD45 expression and side

6 scatter (left plot) or CD11b expression (middle plot); CD45^{low} gated microglial (MG) cells were

7 analyzed for MHC II and CD11b (right plot).

8

9 **Supplementary Figure 2: Minor differences in inflammatory cytokine and chemokine**

10 **gene expression in SC of mice with B6 or 129 microbiome.** Plot depicting inflammatory

11 cytokine and chemokine genes upregulated in B6-B6mb mice (y-axis) or 129-129mb mice (x-

12 axis); the cutoff value to determine upregulation was +2. Data taken from 3 mice / group.

13

14 **Supplementary Figure 3: TLR expression in microglia in B6mb and 129mb mice. (A)** Flow

15 cytometry plots depicting expression of TLR2 (left plot) and TLR4 (right plot) on CD45^{low} MG of

16 B6-B6mb and 129-129mb mice at day 5 post treatment (n=4 mice / group). Iso: Isotype.

17 Numbers indicate % positive for TLR expression and numbers in parenthesis depict mean

18 fluorescence intensity. **(B)** TLR pathway analysis was performed to determine genes that were

19 different in the SC of B6-B6mb and 129-129mb at day 5 post treatment. Y-axis: B6-B6mb mice,

20 X-axis: 129-129mb mice. A cutoff value of +2 was considered as significant for upregulation.

21 Data compiled from n=3 mice / group.

22

23 **Supplementary Figure 4. OTUs Whose Abundances Were Different Among the Genotype-**

24 **Microbiota Groups at Day 0.** In each of these figures, all possible pairwise comparisons of the

25 genotype-microbiota groups are different (FDR-adjusted P value < 0.05) unless otherwise

26 indicated by NS (not significant). These rules only apply to those pairs indicated by the vertical

27 lines of the brackets; for example, for Porphyro_9, the only pair that is not statistically significant
28 is 129-B6B and 129B. Bacterial taxa abbreviations are: Alistipes, Alistipes onderdonkii; Barnes,
29 Barnesiella; Clostridium, Clostridium aerotolerans; Lachno, Lachnospiraceae; Porphyro,
30 Porphyromonadaceae; and the numbers following the underscores indicate the OTU numbers.
31 Genotype-microbiota abbreviations are: B6-129, B6-129mb; 129-B6, 129-B6mbB. Means are
32 the thick horizontal lines, error bars are standard error, and the replicate samples are the dots.
33

34 **Supplementary Figure 5. Bacterial OTUs Whose Abundances Were Different Among the**
35 **Genotype-Microbiota and Treatment Groups Due to Pac.** The statistically significant
36 comparisons are shown by brackets. Bacterial taxa abbreviations are: Akkerman, Akkermansia
37 muciniphila; Porphyro, Porphyromonadaceae; Barnes, Barnesiella; Clostridium, Clostridium
38 aerotolerans; Alistipes, Alistipes onderdonkii; and the numbers following the underscores
39 indicate the OTU numbers. Genotype-microbiota and treatments abbreviations are: B6-129, B6-
40 129mb; 129-B6, 129-B6mbB; P0, Pac at Day 0; P10, Pac at Day 10; V0, Veh at Day 0; V10,
41 Veh at Day 10. Means are the thick horizontal lines, error bars are standard error, and the
42 replicate samples are the dots.
43

44 **Supplementary Figure 6. Correlations Between Bacteria and Microglia.** Correlations
45 between abundant fecal bacterial OTUs at Day 10 (Pac & Veh) in (A) B6mb groups or (B)
46 129mb groups and the microglia index. Values in cells are Pearson correlation coefficients;
47 those shown have P-values < 0.05; those outlined in red or orange have FDR-adjusted Pvalues
48 < 0.05 or equal to 0.127, respectively. Genotype-microbiota groups not shown had no
49 correlations. Heat maps were clustered on both axes. Females: _F; Males _M. Numbers
50 following the taxa indicate OTU numbers.
51