

An osmotic laxative renders mice susceptible to prolonged *Clostridioides difficile* colonization and hinders clearance

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1 Abstract

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3 Antibiotics are a major risk factor for *Clostridioides difficile* infections (CDIs) because of their impact

4 on the intestinal microbiome. However, non-antibiotic medications such as the ubiquitous osmotic

5 laxative polyethylene glycol (PEG) 3350, also alter the microbiota, but whether PEG impacts CDI

6 susceptibility and clearance is unclear. To examine how PEG impacts susceptibility, we treated

7 C57Bl/6 mice with 5-day and 1-day doses of 15% PEG in the drinking water and then challenged

8 the mice with *C. difficile* 630 spores. We used clindamycin-treated mice as a control because they

9 consistently clear *C. difficile* within 10 days post-infection (dpi). To examine how PEG treatment

10 impacts clearance, we administered PEG for 1 day to clindamycin-treated, *C. difficile*-challenged

11 mice either immediately following challenge or 3 dpi. We collected longitudinal stool samples

12 to examine *C. difficile* levels in the stool via anaerobic culture and profiled the microbiota by

13 16S rRNA sequencing. PEG treatment alone was sufficient to render mice susceptible to CDI

14 and 5-day PEG-treated mice remain colonized for up to 30 dpi. Additionally, 5-day PEG treated

15 mice remained susceptible to CDI 10-days post treatment. In contrast, 1-day PEG treated mice

16 were transiently colonized, clearing *C. difficile* within 7 dpi. Although 5-day PEG-treated mice

17 exhibited prolonged *C. difficile* colonization, we saw no difference in histological inflammation

18 between PEG- and clindamycin-treated mice. Additionally, administering PEG to mice after *C.*

19 *difficile* challenge prolonged colonization up to 30 dpi in mice that received PEG immediately after

20 challenge and 15 dpi in mice that received PEG 3 dpi. When we examined microbiota composition

21 across our different treatment groups, we found decreased richness in the PEG-treated mice that

22 exhibited prolonged *C. difficile* colonization. Importantly, there were increased Bacteroides and

23 Enterobacteriaceae and decreased Lachnospiraceae and Oscillibacter in most of the PEG-treated

24 mice with prolonged *C. difficile* colonization. Our findings suggest the osmotic laxative PEG 3350

25 alters the mouse microbiota and disrupts colonization resistance to *C. difficile*, as well as clearance

26 in mice with a CDI. Considering that most hospitals recommend not performing *C. difficile* testing

27 on patients taking laxatives and laxatives are used when administering fecal microbiota transplants

28 via colonoscopy to patients with recurrent CDIs, further studies are needed to evaluate if laxatives

29 impact human microbiota colonization resistance.

30 **Introduction**

31 Antibiotics are a major risk factor for *Clostridioides difficile* infections (CDIs) because of their impact
32 on the microbiota. However, antibiotics are not the only types of medications that disrupt the
33 microbiota. Although, other medications such as proton pump inhibitors have been implicated as
34 risk factors for CDIs through epidemiological studies, whether the association is due to their impact
35 on the microbiome is still unclear (1). Many of the non-antibiotic medications associated with CDIs
36 are known to modulate intestinal motility, which in turn also strongly impacts microbiota composition
37 and function.

38 Interestingly our group has previously demonstrated that there are similar microbiota features in
39 stool samples from CDI patients and patients with diarrhea that tested negative for *C. difficile* with
40 both groups having lower alpha diversity than non-diarrheal control samples (2). These results
41 led to our hypothesis that bacterial communities from patients experiencing diarrheal controls
42 are susceptible, but have not been exposed to *C. difficile* spores. To examine if diarrhea alone
43 is sufficient to disrupt microbiota colonization resistance, we turned to osmotic laxatives, which
44 increase intestinal motility and can lead to diarrhea. The ubiquitous osmotic laxative, polyethylene
45 glycol (PEG) 3350 is found in Miralax, Nulytely, and Golytely and is also commonly used as bowel
46 preparation for colonoscopies. Interestingly, previous studies has shown that treating mice with
47 PEG alone rendered the mice susceptible to *C. difficile* infection, altered microbiota composition,
48 reduced acetate and butyrate and altered the mucus barrier (3–6). The mucus barrier is thought to
49 mediate protection from *C. difficile* infections by protecting intestinal epithelial cells from the toxins
50 produced by *C. difficile* (Ref). PEG is also relevant in the context of treating recurrent CDIs via
51 fecal microbiota transplant (FMT). For FMTs that are delivered via colonoscopy, patients typically
52 undergo bowel preparation by taking an osmotic laxative prior to the procedure. Many of the FMT
53 studies to date rationalize the use of laxatives (Ref) based on a case study with 2 pediatric patients
54 where the authors suggested the laxative may help flush *C. difficile* spores and toxins from the
55 intestine (7).

- 56 • 2 main questions:
- 57 – Does PEG 3350 prolong colonization and result in more severe *C. difficile* infections?
- 58 – How does PEG 3350 influence *C. difficile* clearance and fecal microbiota transplant

59 (FMT) in mice?

60 **Results**

61 **Laxative treatment alone leads to prolonged *C. difficile* colonization in mice.** We compared
62 PEG-treated mice to our standard 10 mg/kg clindamycin treatment, which temporarily renders the
63 mice susceptible to *C. difficile*, with mice typically clearing *C. difficile* within 10 days post-infection
64 (1, 8). All PEG-treated mice were administered a 15% PEG solution in the drinking water for 5-days,
65 one group was also treated with clindamycin, and one group was allowed to recover for 10 days
66 prior to challenge (Fig. 1A). After PEG and/or antibiotic treatment all mice were challenged with
67 10^3 *C. difficile* 630 spores.

- 68 • Figure 1. 5-day PEG treatment prolongs susceptibility and mice become persistently colonized
69 with *C. difficile*.
- 70 • Figure 2. 5-day PEG treatment disrupts the stool microbiota for a longer amount of time
71 compared to clindamycin-treated mice.
- 72 • Figure S1. 5-day PEG treatment plus 10-day recovery mice microbiota dynamics
73 post-infection.
- 74 • Figure 3. 5-day PEG treatment does not result in more severe CDIs, although mucosal
75 microbiota is altered.
- 76 • Figure 4. 1-day PEG treatment renders mice susceptible to transient *C. difficile* colonization.
- 77 • Figure 5. 1-day PEG treatment post *C. difficile* challenge prolongs colonization regardless of
78 whether an FMT is also administered.
- 79 • Figure 6. Specific microbiota features associated with prolonged *C. difficile* colonization in
80 PEG treated mice.
- 81 • Figure S2. Specific OTUs associated with clearance that are mostly absent in mice with
82 prolonged *C. difficile* colonization.

83 – Ex. *Muribaculum intestinale*.

84 • Figure 7. Schematic summarizing findings.

85 Discussion

86 • Summary of major findings

87 • Discussion of prolonged persistence. C. difficile sequences detected in tissue samples.
88 Association with mucin-degrading bacteria suggested by recent papers.

89 • Discuss why we might not have observed more severe histology in PEG mice relative to
90 clindamycin-treated mice

91 – Antibiotics may also impact mucus layer
92 – Strain of bacteria used

93 • Protective bacteria missing in PEG-treated mice

94 • Discuss what these findings might mean for human patients

95 – What's known regarding laxatives and susceptibility to CDIs
96 – Relevance to human FMTs? Unclear what the best administration route is because there
97 have been no studies designed to evaluate the best administration route for FMTs.

98 Conclusions

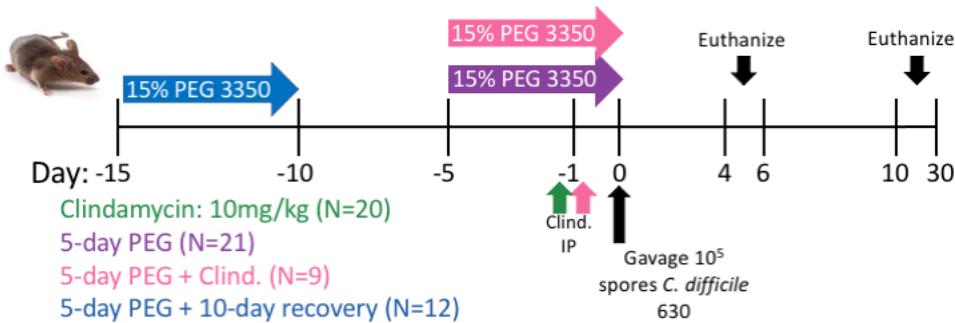
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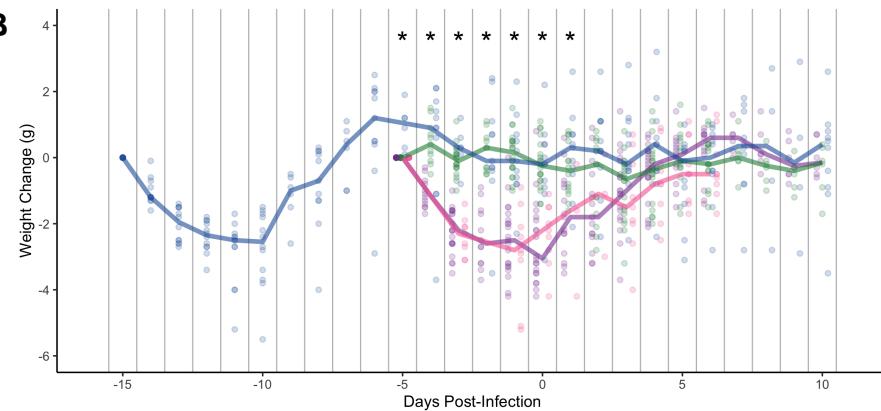
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¹⁰⁸ Translational Sciences).

¹⁰⁹ **Materials and Methods**

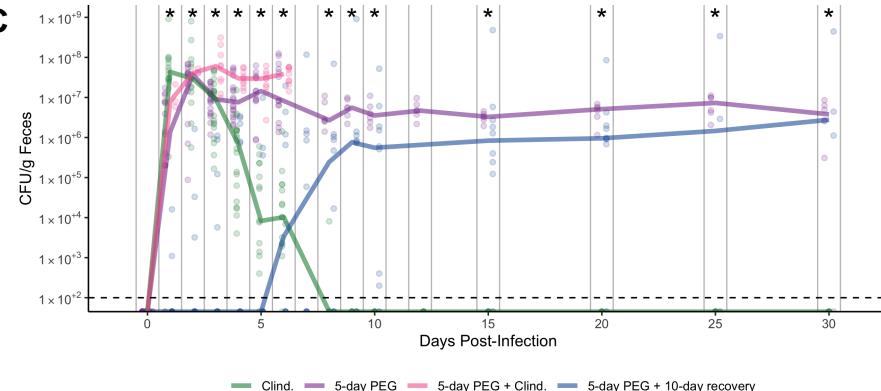
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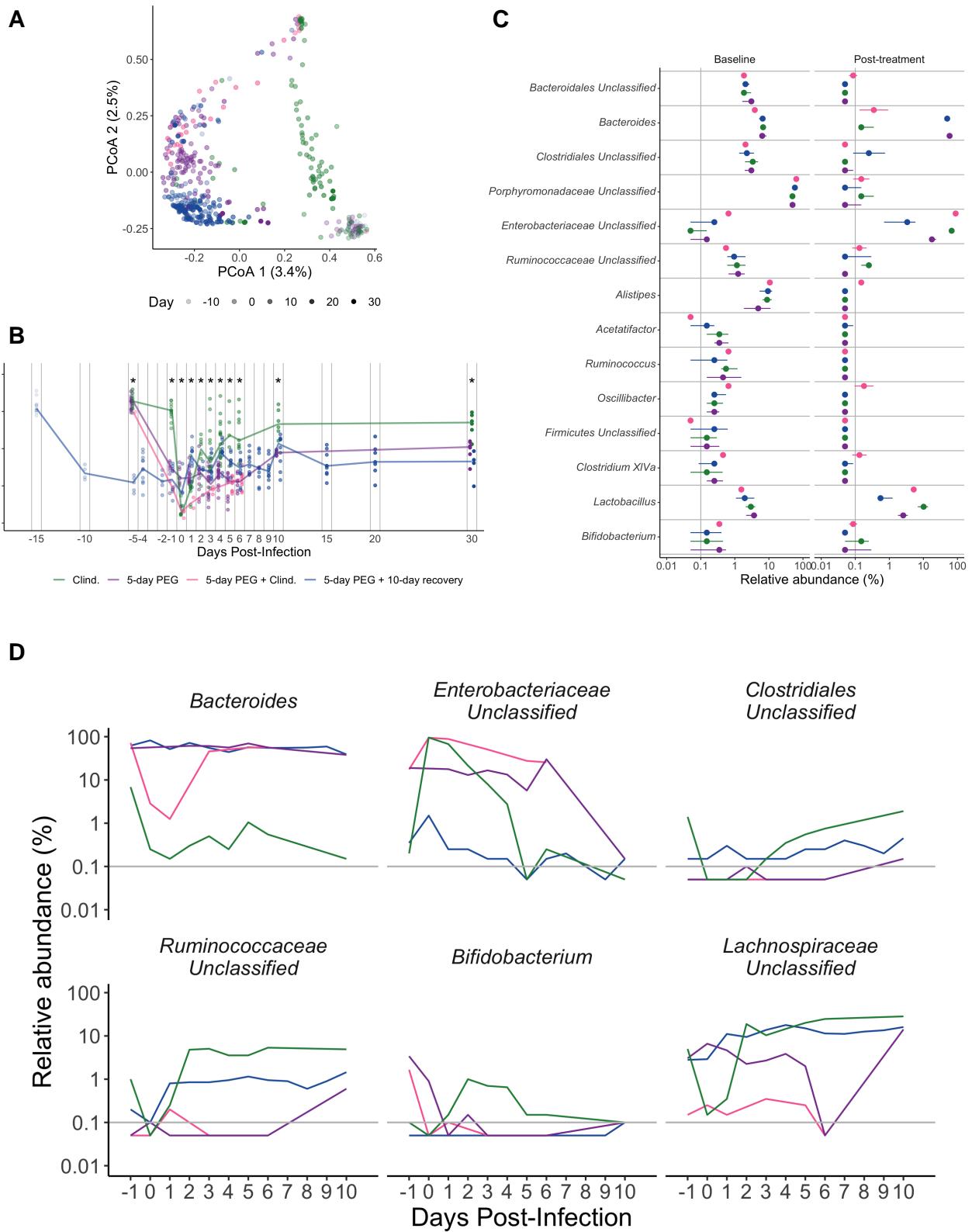
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111 **Figure 1. 5-day PEG treatment prolongs susceptibility and mice become persistently**
112 **colonized with *C. difficile*.** A. Setup of the experimental timeline for subset of experiments
113 with 5-day PEG treated mice. B. Weight change from baseline weight in groups after treatment with
114 PEG and/or clindamycin, followed by *C. difficile* challenge. C. *C. difficile* CFU/gram stool measured
115 over time ($N = 4$ - $(\text{insert variable name})$ mice per timepoint) via serial dilutions. The black line
116 represents the limit of detection for the first serial dilution. CFU quantification data was not available
117 for each mouse due to stool sampling difficulties (particularly the day the mice came off of the

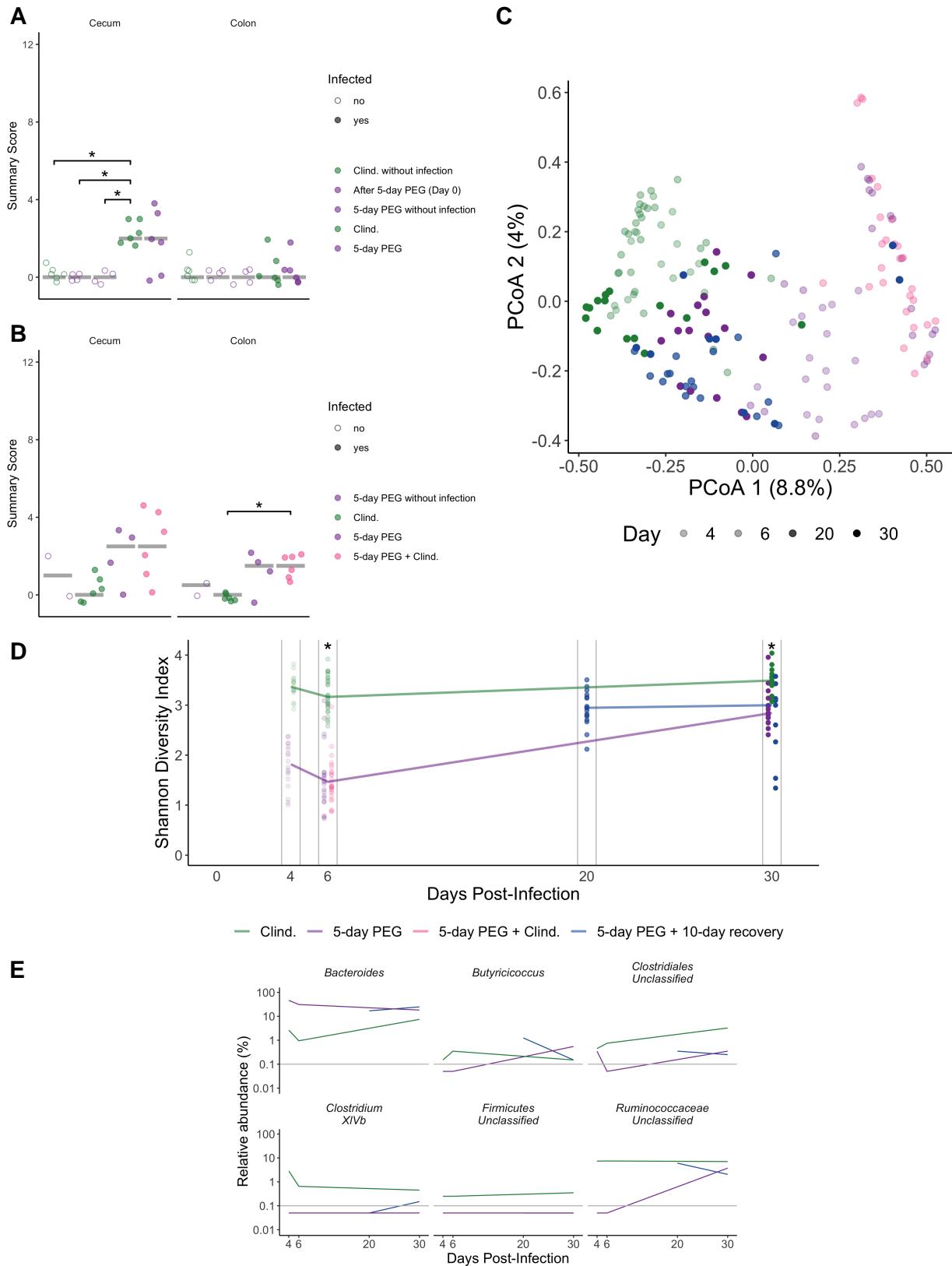
¹¹⁸ PEG treatment) or early deaths. Lines represent the median for each source and circles represent
¹¹⁹ individual mouse samples. Asterisks indicate timepoints where the weight change or CFU/g was
¹²⁰ significantly different between groups by the Kruskal-Wallis test with Benjamini-Hochberg correction
¹²¹ for testing multiple timepoints.



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123 **Figure 2. 5-day PEG treatment disrupts the stool microbiota for a longer amount of time**

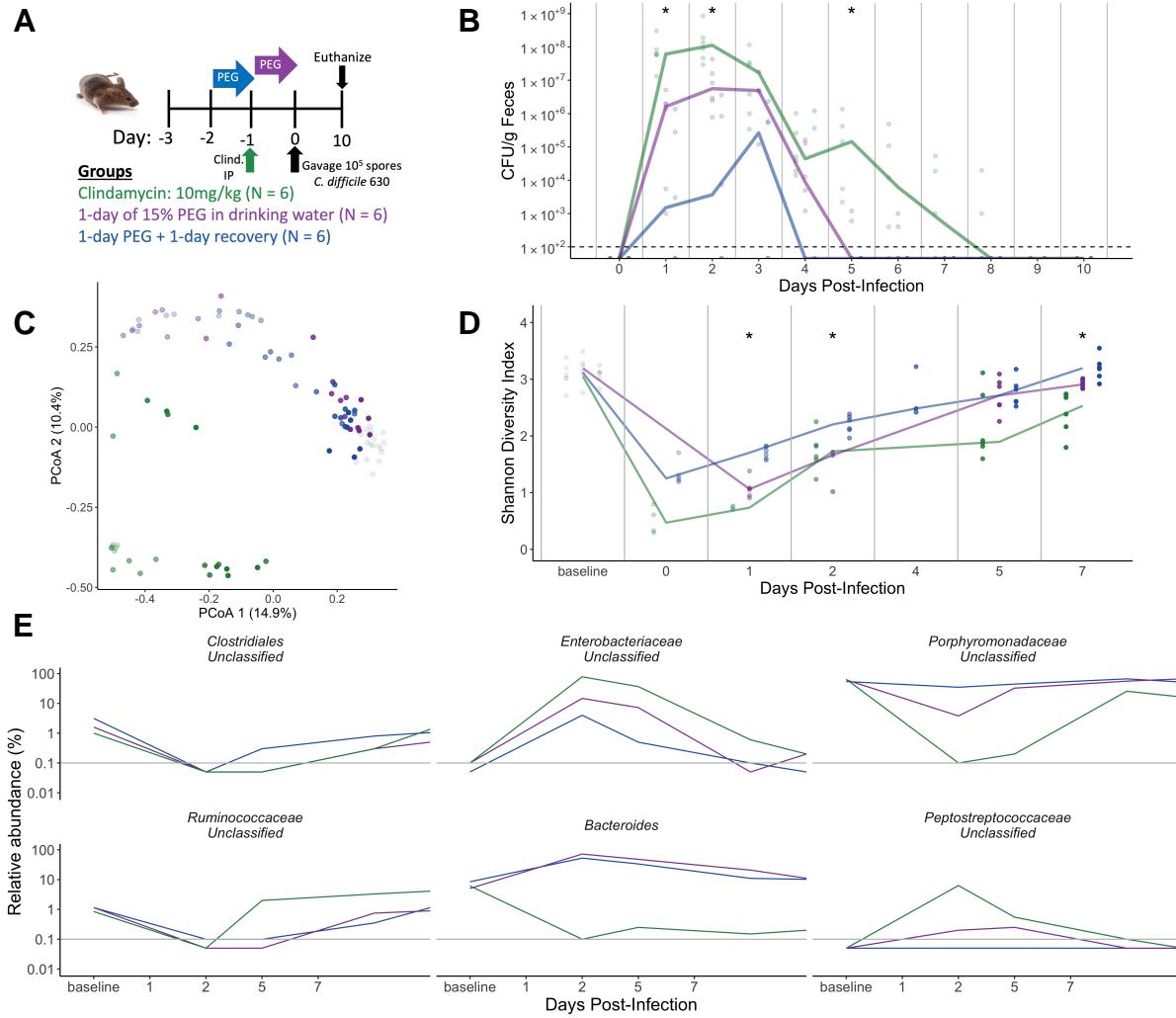
¹²⁴ compared to clindamycin-treated mice. A.



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Figure 3. 5-day PEG treatment does not result in more severe CDIs, although mucosal

¹²⁷ **microbiota is altered.** A.

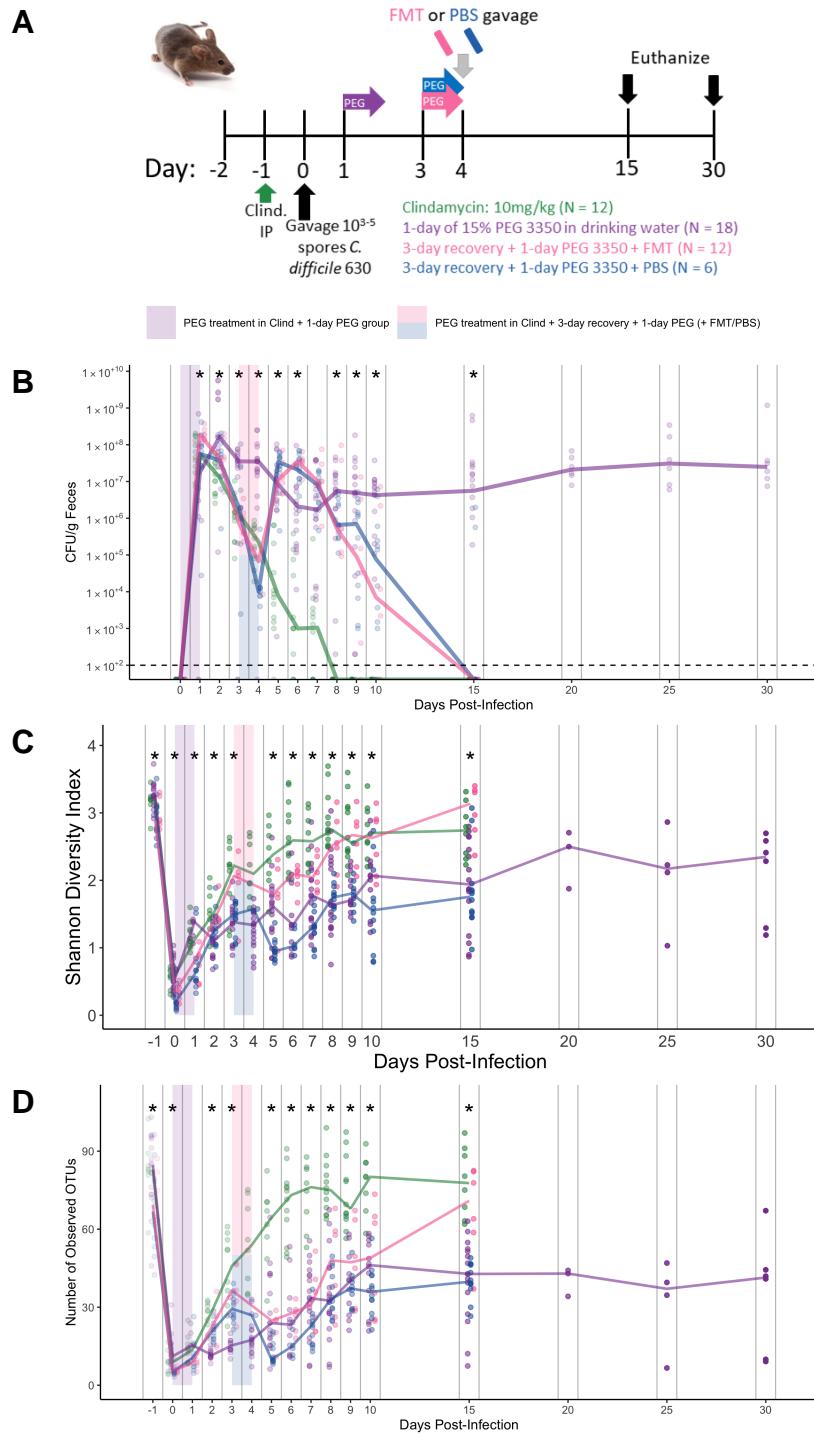


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129 **Figure 4. 1-day PEG treatment renders mice susceptible to transient *C. difficile* colonization.**

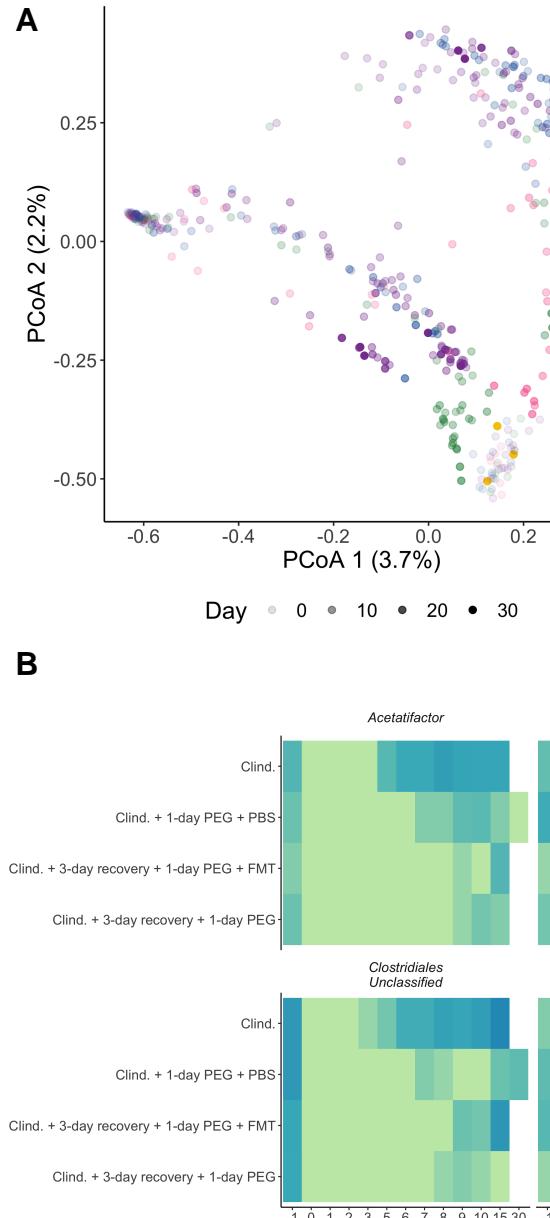
130 A. Setup of the experimental timeline for the 1-day PEG treated subset of mice. B. CFU/gram
131 stool measured over time (N = 6 mice per timepoint) via several dilutions. The black dotted line
132 represents the limit of detection for the first serial dilution. Asterisks indicate timepoints where
133 the CFU/gram was significantly different between groups using the Kruskall-Wallis test with a
134 Benjamini-Hochberg correction for multiple timepoints. C. Principle Coordinate Analysis plot of
135 the groups over time with the alpha representing the same time scale as in panel D (day: R^2
136 = 0.43; group: R^2 = 0.19). D. Shannon Diveristy Index of the groups over time. Only days with
137 samples from all groups are shown. Samples for some mice were difficult to obtain due to the
138 laxative treatment. The alpha scale follows accordingly with the timeline. E. Line plots of relative
139 percent abundance of selected genera over time. Only days with samples from all groups shown.

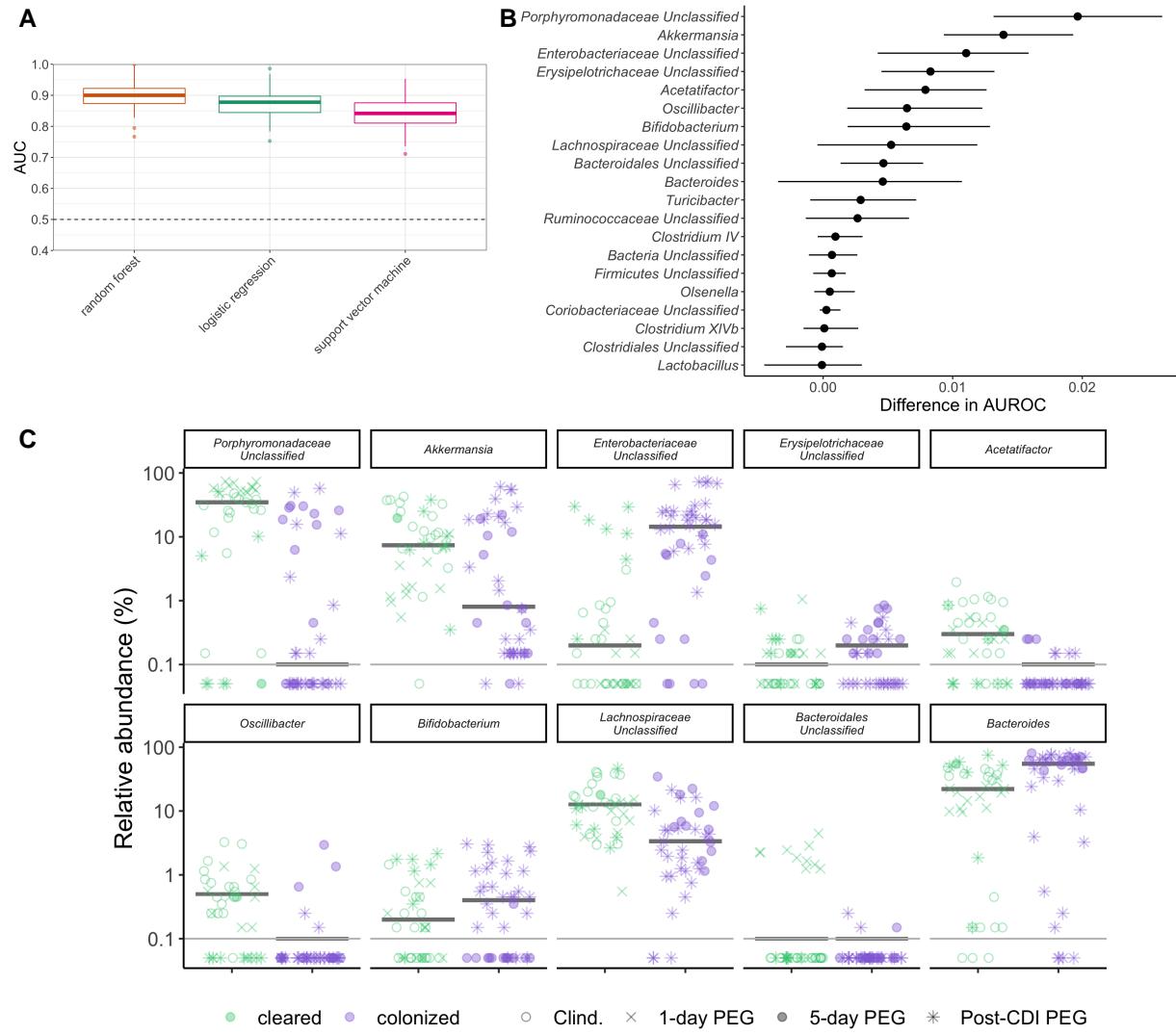
140 The gray line represents the limit of detection.



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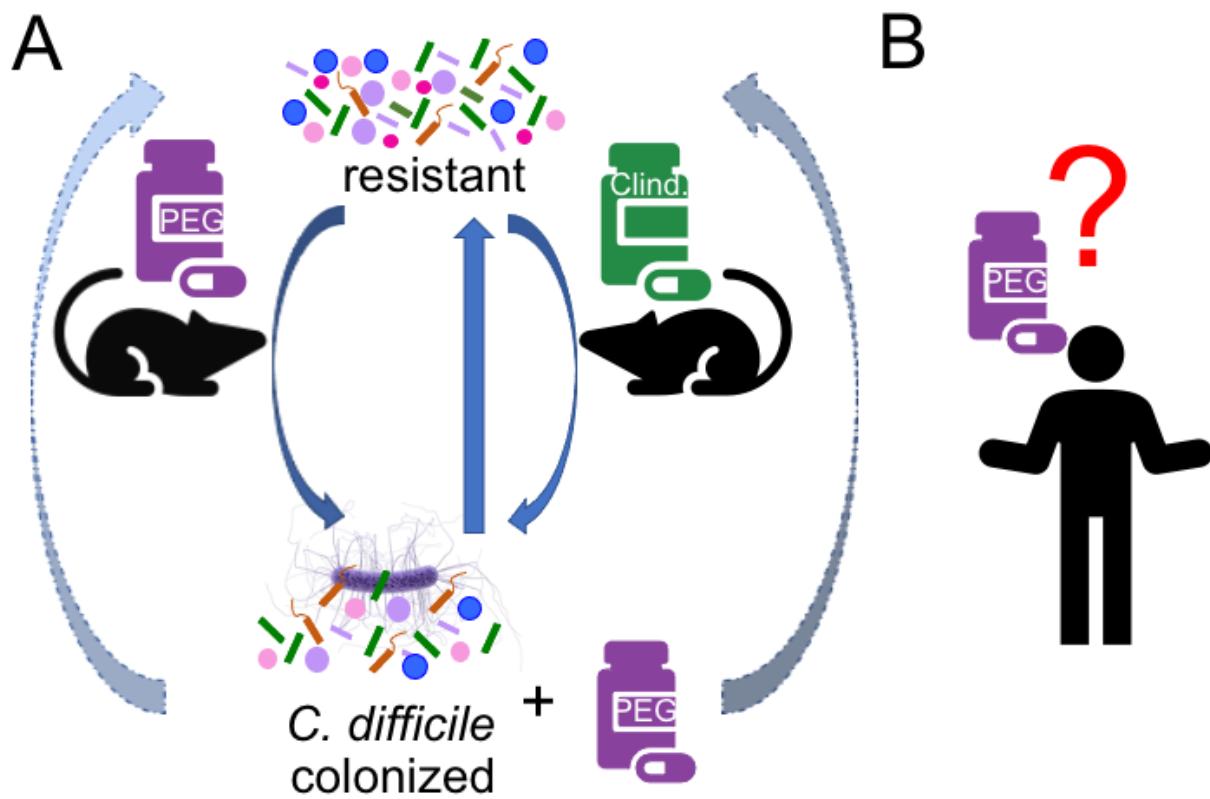
142 **Figure 5. 1-day PEG treatment post *C. difficile* challenge prolongs colonization regardless**
143 **of whether an FMT is also administered. A.**





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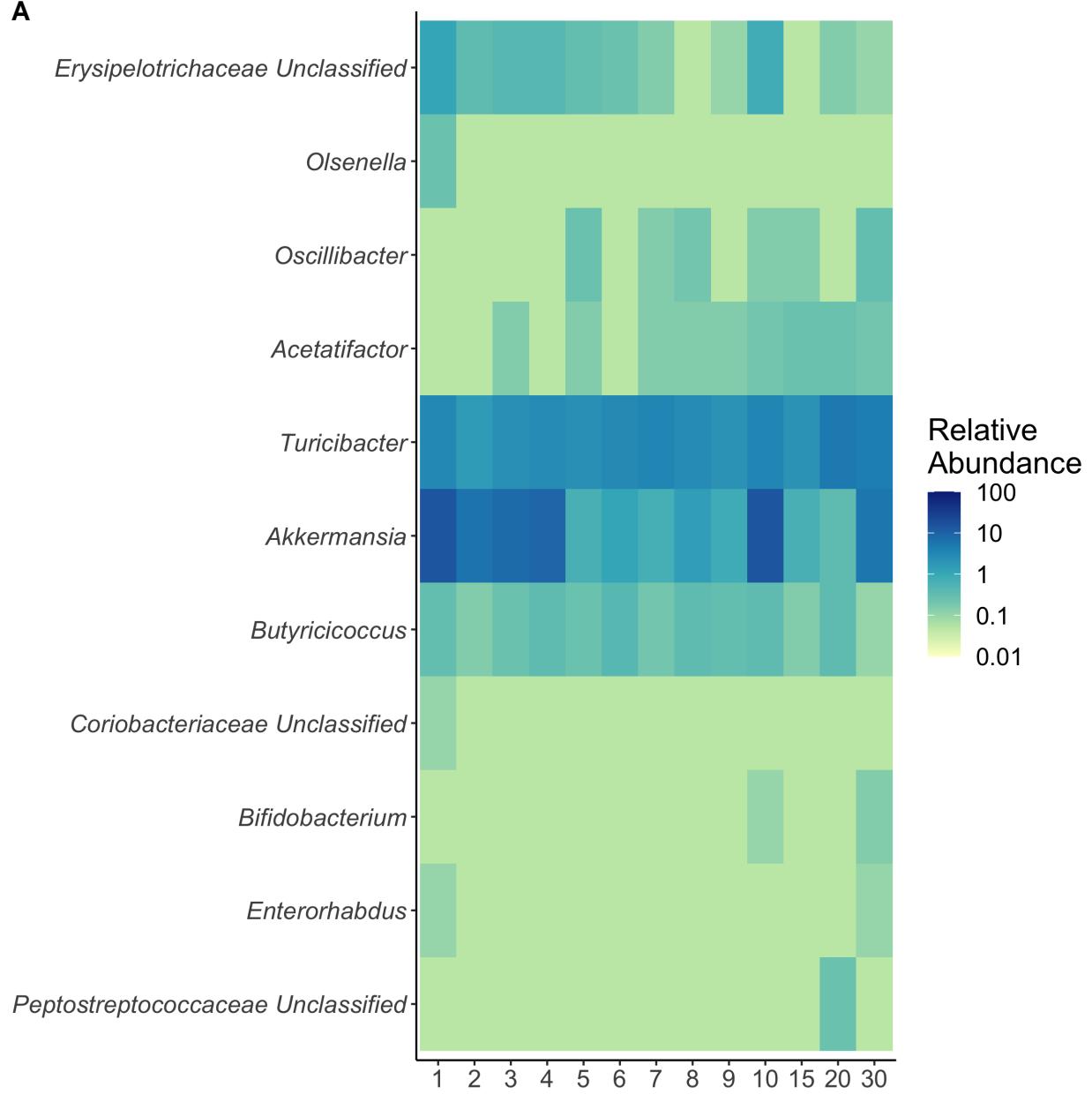
145 **Figure 6. Specific microbiota features associated with prolonged *C. difficile* colonization in
146 PEG treated mice. A.**



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148 **Figure 7. Schematic summarizing findings. A.**

A



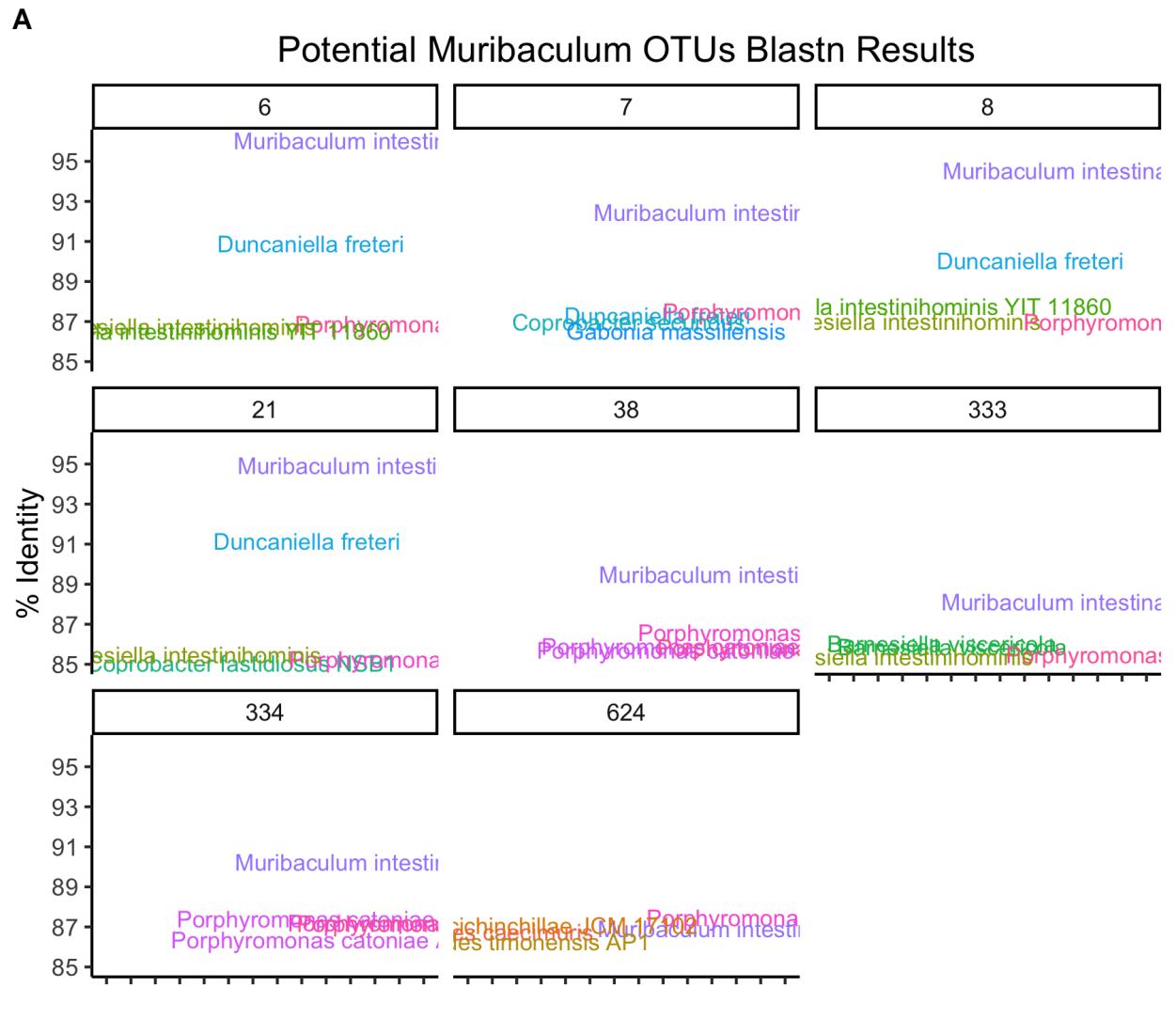


Figure S2. Specific OTUs associated with clearance that are mostly absent in mice with prolonged *C. difficile* colonization. Ex. *Muribaculum intestinale*. A.

155 **References**

- 156 1. **Tomkovich S, Lesniak NA, Li Y, Bishop L, Fitzgerald MJ, Schloss PD.** 2019. The proton
157 pump inhibitor omeprazole does not promote *Clostridioides difficile* colonization in a murine model.
158 mSphere **4**. doi:10.1128/msphere.00693-19.
- 159 2. **Schubert AM, Sinani H, Schloss PD.** 2015. Antibiotic-induced alterations of the murine gut
160 microbiota and subsequent effects on colonization resistance against *Clostridium difficile*. mBio **6**.
161 doi:10.1128/mbio.00974-15.
- 162 3. **Kashyap PC, Marcabal A, Ursell LK, Larauche M, Duboc H, Earle KA, Sonnenburg
163 ED, Ferreyra JA, Higginbottom SK, Million M, Tache Y, Pasricha PJ, Knight R, Farrugia
164 G, Sonnenburg JL.** 2013. Complex interactions among diet, gastrointestinal transit, and gut
165 microbiota in humanized mice. Gastroenterology **144**:967–977. doi:10.1053/j.gastro.2013.01.047.
- 166 4. **Ferreyra JA, Wu KJ, Hryckowian AJ, Bouley DM, Weimer BC, Sonnenburg JL.** 2014. Gut
167 microbiota-produced succinate promotes c. difficile infection after antibiotic treatment or motility
168 disturbance. Cell Host & Microbe **16**:770–777. doi:10.1016/j.chom.2014.11.003.
- 169 5. **Tropini C, Moss EL, Merrill BD, Ng KM, Higginbottom SK, Casavant EP, Gonzalez CG,
170 Fremin B, Bouley DM, Elias JE, Bhatt AS, Huang KC, Sonnenburg JL.** 2018. Transient
171 osmotic perturbation causes long-term alteration to the gut microbiota. Cell **173**:1742–1754.e17.
172 doi:10.1016/j.cell.2018.05.008.
- 173 6. **VanInsberghe D, Elsherbini JA, Varian B, Poutahidis T, Erdman S, Polz MF.** 2020. Diarrhoeal
174 events can trigger long-term clostridium difficile colonization with recurrent blooms. Nature
175 Microbiology **5**:642–650. doi:10.1038/s41564-020-0668-2.
- 176 7. **Liacouras CA, Piccoli DA.** 1996. Whole-bowel irrigation as an adjunct to the treatment of
177 chronic, relapsing clostridium difficile colitis. Journal of Clinical Gastroenterology **22**:186–189.
178 doi:10.1097/00004836-199604000-00007.
- 179 8. **Tomkovich S, Stough JMA, Bishop L, Schloss PD.** 2020. The initial gut microbiota and
180 response to antibiotic perturbation influence clostridioides difficile clearance in mice. mSphere **5**.

