

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

Sarah Tomkovich¹, Ana Taylor¹, Jacob King¹, Joanna Colovas¹, Lucas Bishop¹, Kathryn McBride¹, Sonya Royzenblat¹, Nicholas A. Lesniak¹, Ingrid L. Bergin², Patrick D. Schloss^{1†}

† To whom correspondence should be addressed: pschloss@umich.edu

1. Department of Microbiology and Immunology, University of Michigan, Ann Arbor, MI, USA
2. The Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, MI, USA

1 Abstract

2 (Modify depending on target journal, currently abstract submitted to World Microbe Forum)

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 (1). However, antibiotics are not the only types of medications that disrupt the
33 microbiota (2–4). Although, other medications have been implicated as risk factors for CDIs through
34 epidemiological studies, whether the association is due to their impact on the microbiome is still
35 unclear (5–9). Many of the non-antibiotic medications associated with CDIs are known to modulate
36 intestinal motility, which in turn also strongly impacts microbiota composition and function (10, 11).

37 Stool consistency often serves as an approximation of intestinal motility. Our group has shown that
38 when *C. difficile* negative controls are separated into two groups based on stool consistency, there
39 are microbiota features such as alpha diversity that overlap between samples from CDI patients and
40 control patients with diarrhea (12). These results led to our hypothesis that bacterial communities
41 from patients experiencing diarrhea are susceptible, but may not have been exposed to *C. difficile*
42 spores.

43 Osmotic laxatives can lead to diarrhea depending on the administered dose and temporarily disrupts
44 the intestinal microbiota in humans (13). The ubiquitous osmotic laxative, polyethylene glycol (PEG)
45 3350 is found in Miralax, Nulytely, and Golytely and is also commonly used as bowel preparation
46 for colonoscopies. Interestingly, previous studies have shown that treating mice with PEG alone
47 rendered the mice susceptible to *C. difficile* infection, altered microbiota composition, reduced
48 acetate and butyrate and altered the mucus barrier (14–17). 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). However, whether laxative results in more severe CDIs in mice is
51 unclear.

52 Beyond susceptibility, PEG is also relevant in the context of treating recurrent CDIs via fecal
53 microbiota transplant (FMT) where a healthy microbiota is administered to the patient to restore
54 colonization. For FMTs that are delivered via colonoscopy, patients typically undergo bowel
55 preparation by taking an osmotic laxative prior to the procedure. Many of the FMT studies to date
56 rationalize the use of laxatives (Ref) based on a 1996 case study with 2 pediatric patients where the
57 authors suggested the laxative may help flush *C. difficile* spores and toxins from the intestine (18).

58 In the past, our group has used C57BL6 mice to characterize how antibiotics disrupt the microbiota
59 and influence *C. difficile* susceptibility and clearance [ref]. Although, two groups have now shown
60 PEG treatment alone renders mice susceptible to *C. difficile*, these studies have raised additional
61 questions that should be addressed given their relevance to CDIs. Here, we used our C57BL/6
62 clindamycin model as a control group to characterize how long PEG-treated mice remain susceptible,
63 whether PEG treatment results in sustained *C. difficile* colonization, and if PEG treatment post-CDI
64 can promote *C. difficile* clearance.

65 **Results**

66 **Laxative treatment alone leads to prolonged *C. difficile* colonization in mice.** We compared
67 PEG-treated mice to our standard 10 mg/kg clindamycin treatment, which temporarily renders the
68 mice susceptible to *C. difficile*, with mice typically clearing *C. difficile* within 10 days post-infection
69 (9, 19). All PEG-treated mice were administered a 15% PEG solution in the drinking water for
70 5-days, one group was also treated with clindamycin, and one group was allowed to recover for 10
71 days prior to challenge (Fig. 1A). After PEG and/or antibiotic treatment all mice were challenged
72 with 10^3 *C. difficile* 630 spores.

- 73 • Figure 1. 5-day PEG treatment prolongs susceptibility and mice become persistently colonized
74 with *C. difficile*.
- 75 • Figure 2. 5-day PEG treatment disrupts the stool microbiota for a longer amount of time
76 compared to clindamycin-treated mice.
- 77 • Figure S1. 5-day PEG treatment plus 10-day recovery mice microbiota dynamics
78 post-infection.
- 79 • Figure 3. 5-day PEG treatment does not result in more severe CDIs, although mucosal
80 microbiota is altered.
- 81 • Figure 4. 1-day PEG treatment renders mice susceptible to transient *C. difficile* colonization.
- 82 • Figure 5. 1-day PEG treatment post *C. difficile* challenge prolongs colonization regardless of

83 whether an FMT is also administered.

84 • Figure 6. Specific microbiota features associated with prolonged *C. difficile* colonization in
85 PEG treated mice.

86 • Figure S2. Specific OTUs associated with clearance that are mostly absent in mice with
87 prolonged *C. difficile* colonization.

88 – Ex. *Muribaculum intestinale*.

89 • Figure 7. Schematic summarizing findings.

90 Discussion

91 • Summary of major findings

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

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

96 – Antibiotics may also impact mucus layer

97 – Strain of bacteria used

98 • Protective bacteria missing in PEG-treated mice

99 • Discuss what these findings might mean for human patients

100 – What's known regarding laxatives and susceptibility to CDIs

101 – Relevance to human FMTs? Unclear what the best administration route is because there
102 have been no studies designed to evaluate the best administration route for FMTs.

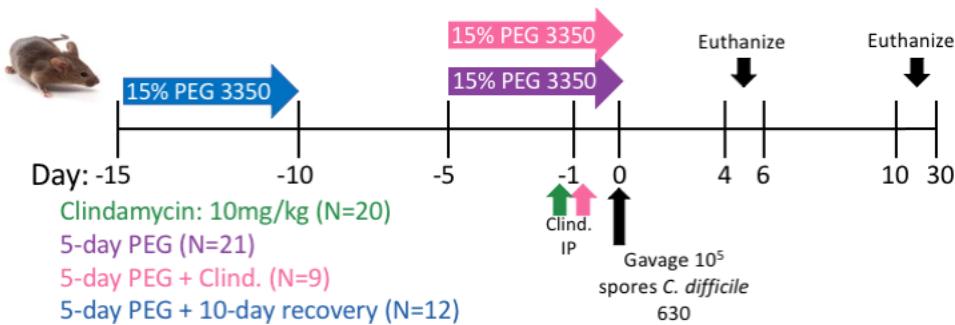
103 **Conclusions**

104 **Acknowledgements**

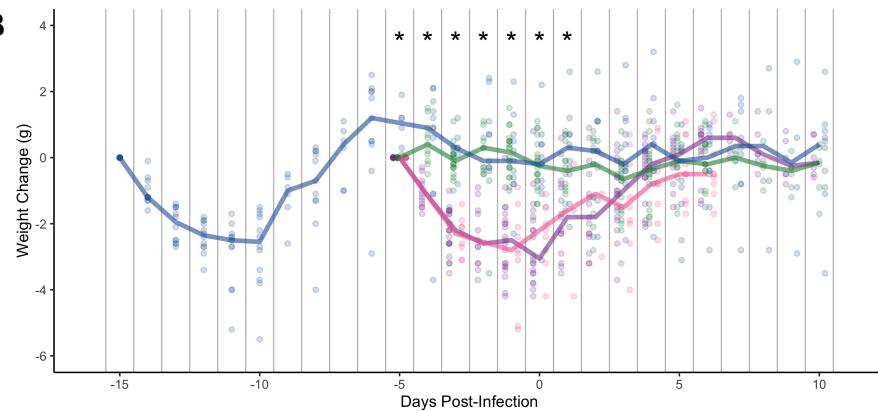
105 We thank members of the Schloss lab for feedback on planning the experiments and data
106 presentation. We also thank Andrew Henry for help with media preparation and bacterial culture.
107 We also thank the Unit for Laboratory Animal Medicine at the University of Michigan for maintaining
108 our mouse colony and providing the institutional support for our mouse experiments. Finally,
109 we thank Kwi Kim, Austin Campbell, and Kimberly Vendrov for their help in maintaining the
110 Schloss lab's anaerobic chamber. This work was supported by the National Institutes of Health
111 (U01AI124255). ST was supported by the Michigan Institute for Clinical and Health Research
112 Postdoctoral Translation Scholars Program (UL1TR002240 from the National Center for Advancing
113 Translational Sciences).

114 **Materials and Methods**

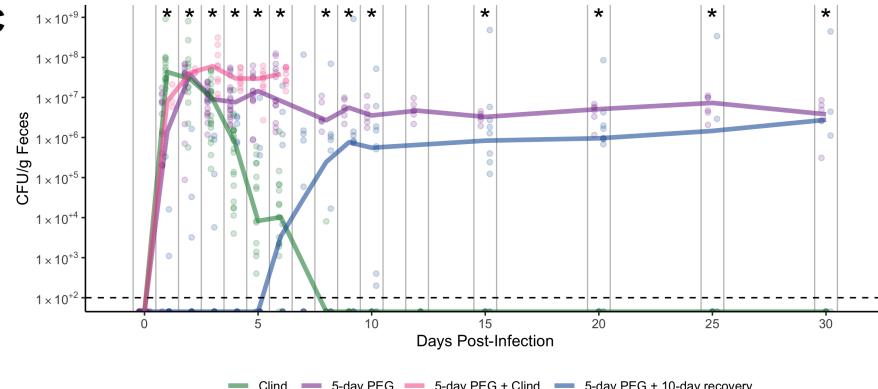
A



B



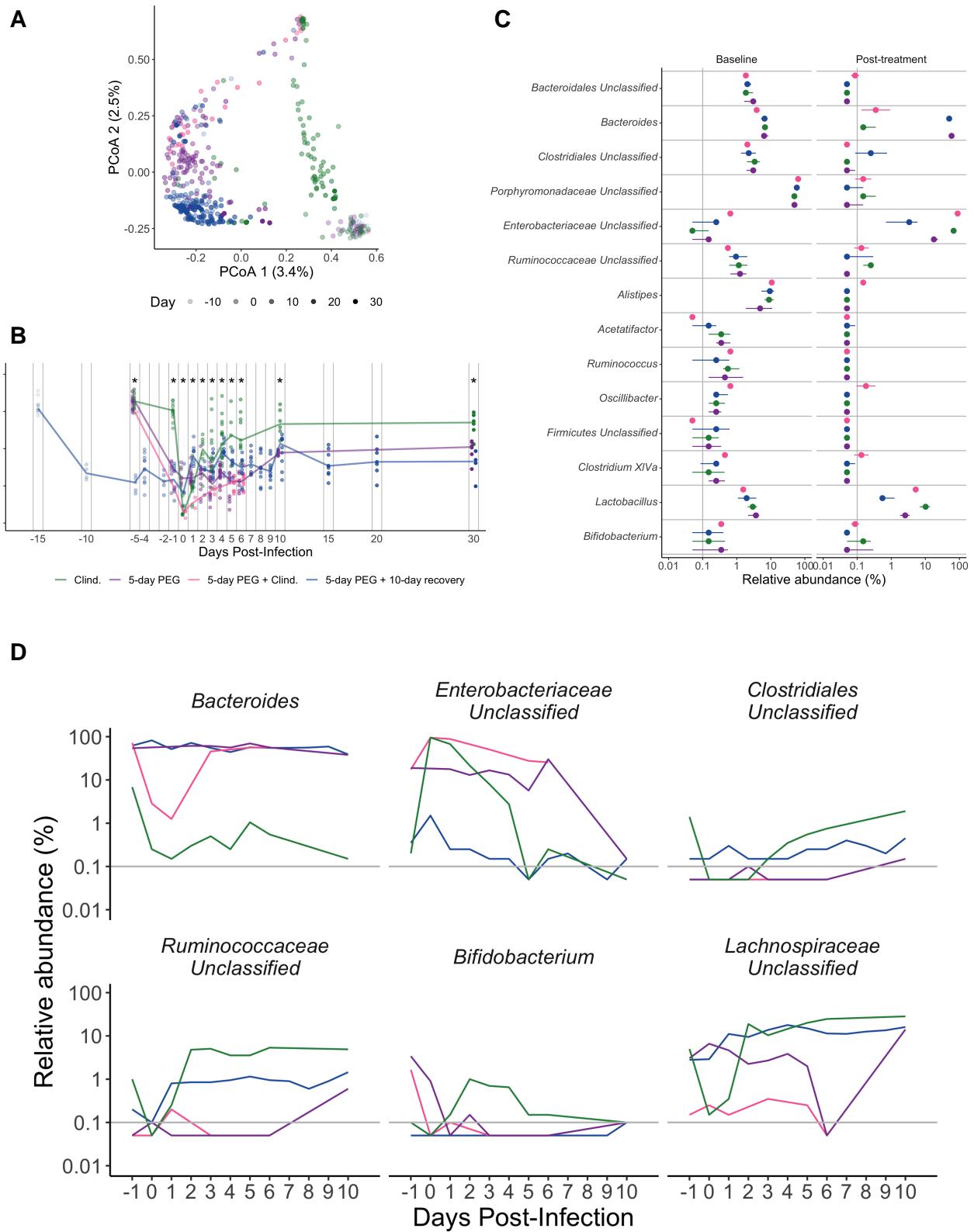
C



115

116 **Figure 1. 5-day PEG treatment prolongs susceptibility and mice become persistently**
117 **colonized with *C. difficile*.** A. Setup of the experimental timeline for subset of experiments with
118 5-day PEG treated mice. B. Weight change from baseline weight in groups after treatment with
119 PEG and/or clindamycin, followed by *C. difficile* challenge. C. *C. difficile* CFU/gram stool measured
120 over time (N = 4-(insert variable name) mice per timepoint) via serial dilutions. The black line
121 represents the limit of detection for the first serial dilution. CFU quantification data was not available
122 for each mouse due to stool sampling difficulties (particularly the day the mice came off of the

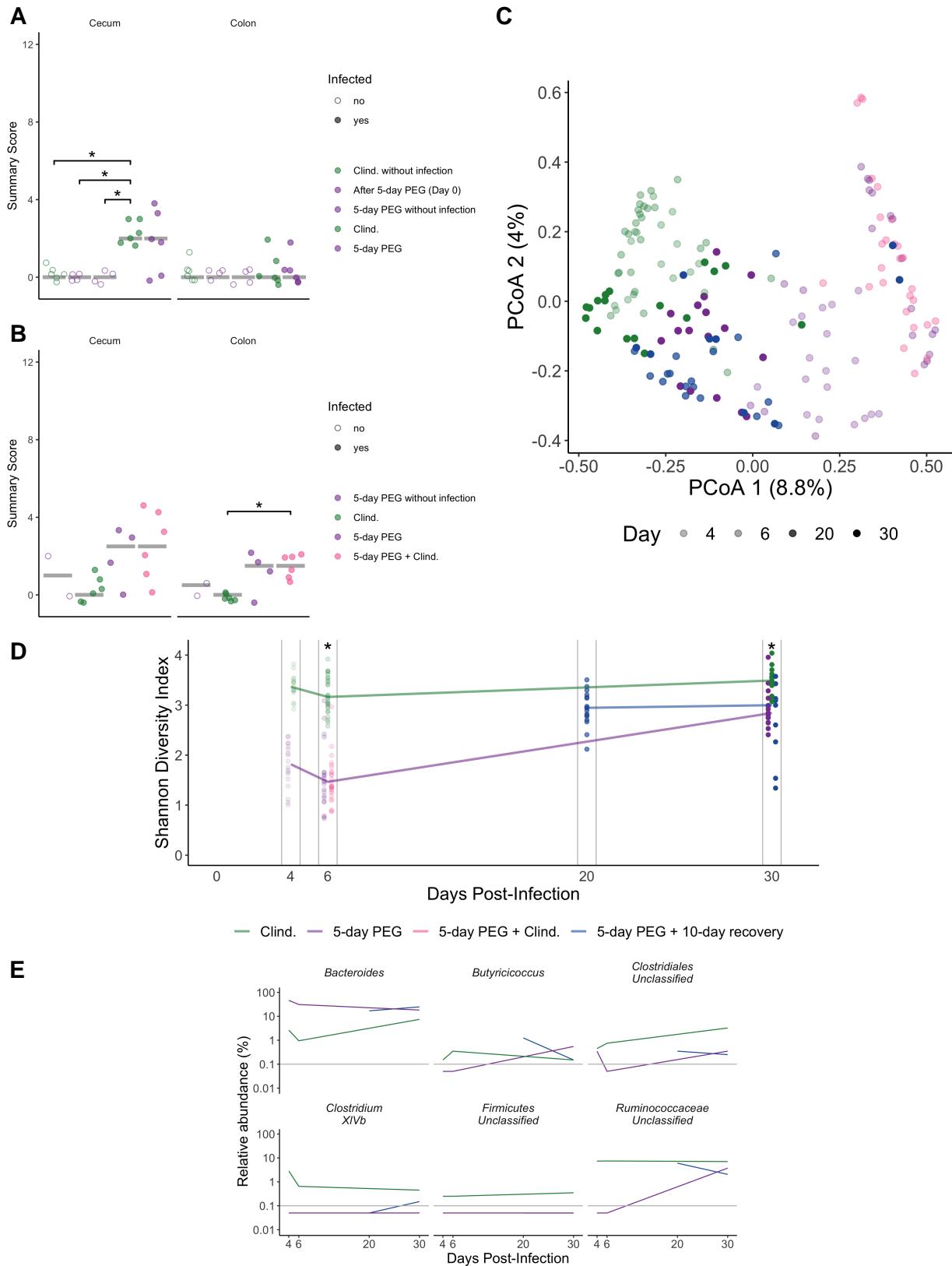
¹²³ PEG treatment) or early deaths. Lines represent the median for each source and circles represent
¹²⁴ individual mouse samples. Asterisks indicate timepoints where the weight change or CFU/g was
¹²⁵ significantly different between groups by the Kruskal-Wallis test with Benjamini-Hochberg correction
¹²⁶ for testing multiple timepoints.



127

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

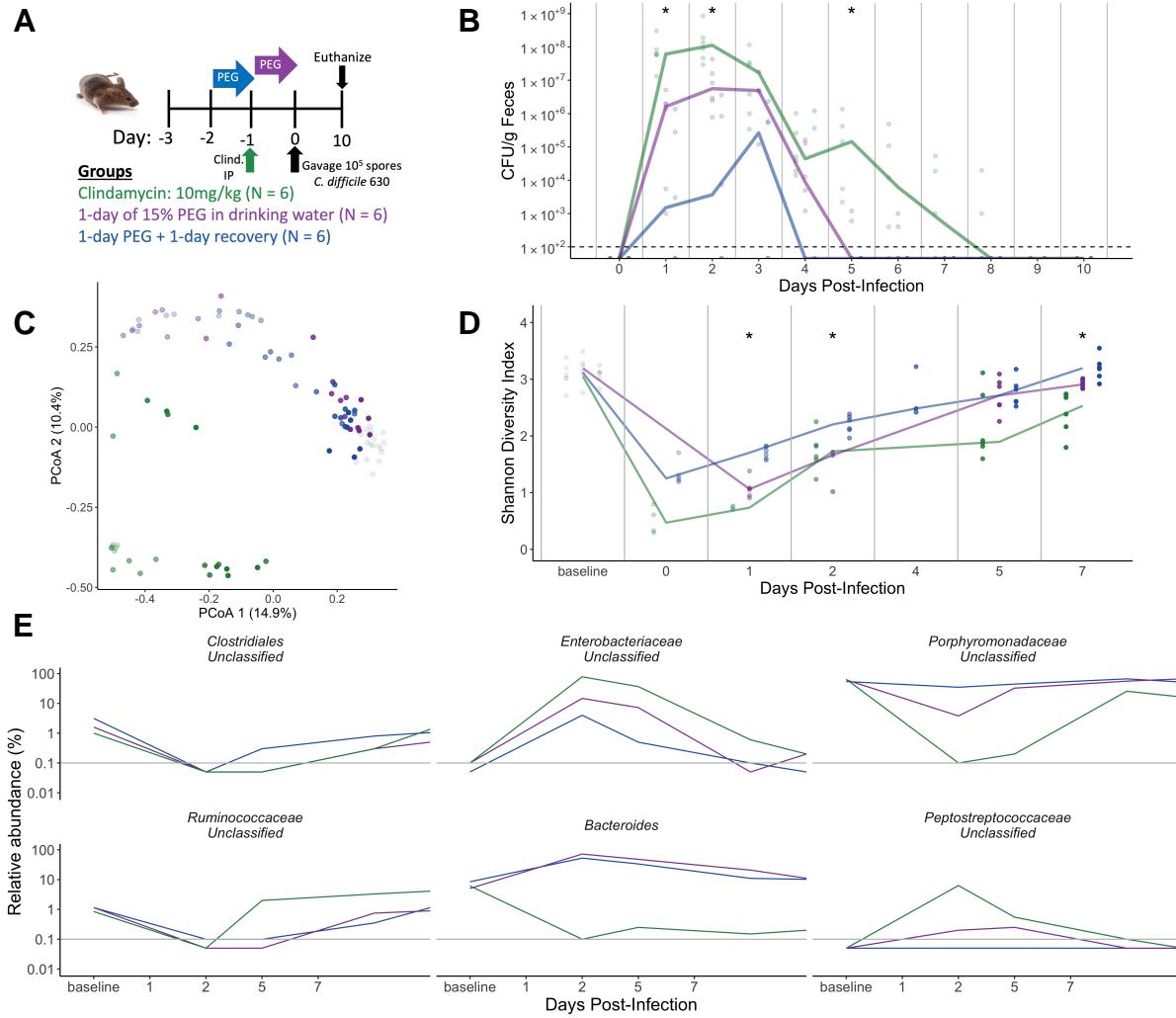
¹²⁹ compared to clindamycin-treated mice. A.



130

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

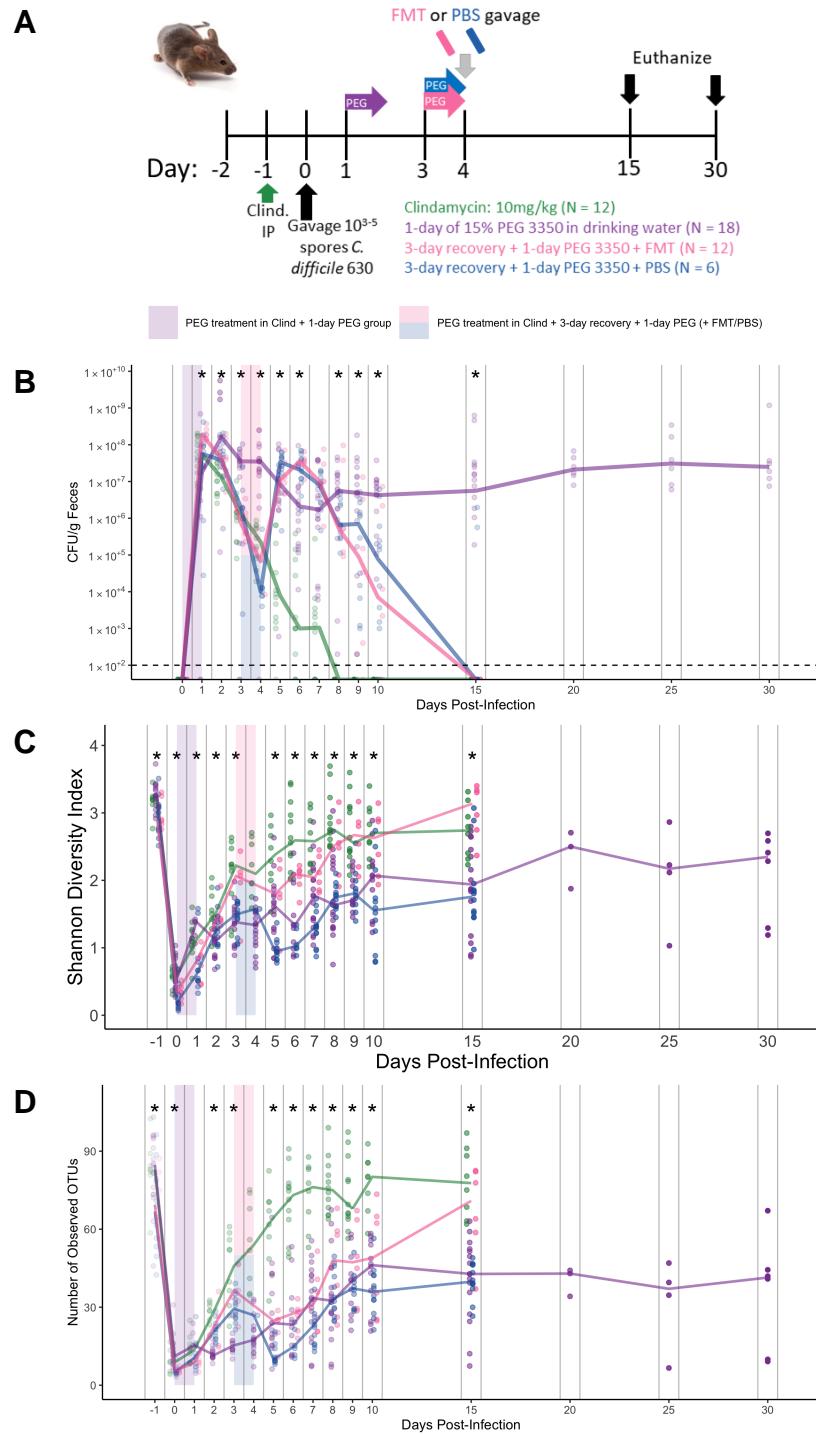
¹³² **microbiota is altered.** A.



133

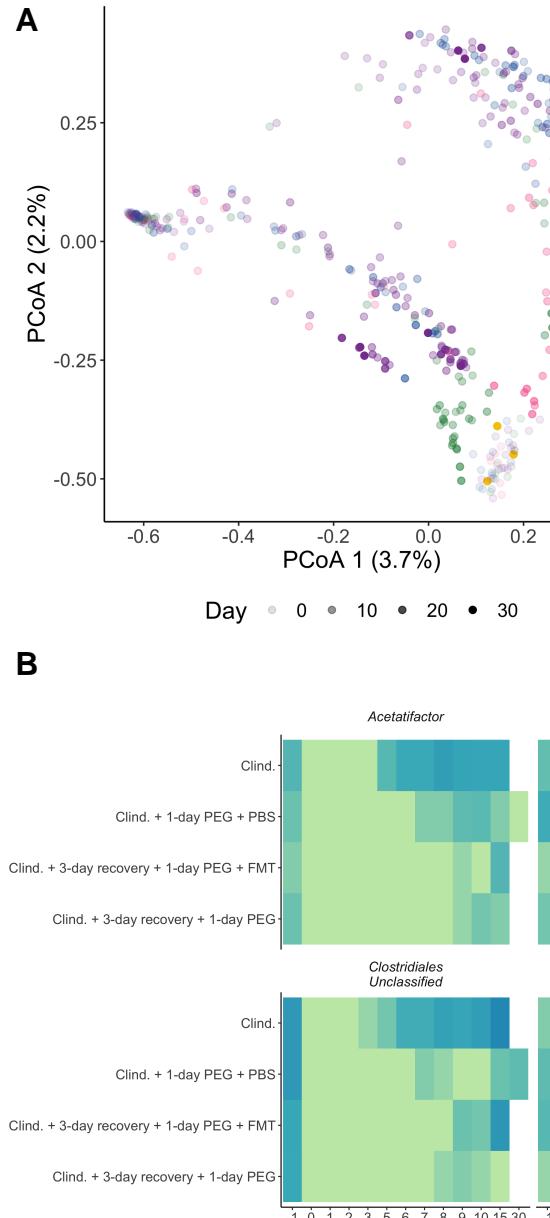
134 **Figure 4. 1-day PEG treatment renders mice susceptible to transient *C. difficile***
 135 **colonization.** A. Setup of the experimental timeline for the 1-day PEG treated subset of
 136 mice. B. CFU/gram stool measured over time (N = 6 mice per timepoint) via several dilutions.
 137 The black dotted line represents the limit of detection for the first serial dilution. Asterisks
 138 indicate timepoints where the CFU/gram was significantly different between groups using the
 139 Kruskall-Wallis test with a Benjamini-Hochberg correction for multiple timepoints. C. Principle
 140 Coordinate Analysis plot of the groups over time with the alpha representing the same time scale
 141 as in panel D (day: $R^2 = 0.43$; group: $R^2 = 0.19$). D. Shannon Diveristy Index of the groups over
 142 time. Only days with samples from all groups are shown. Samples for some mice were difficult to
 143 obtain due to the laxative treatment. The alpha scale follows accordingly with the timeline. E. Line
 144 plots of relative percent abundance of selected genera over time. Only days with samples from all

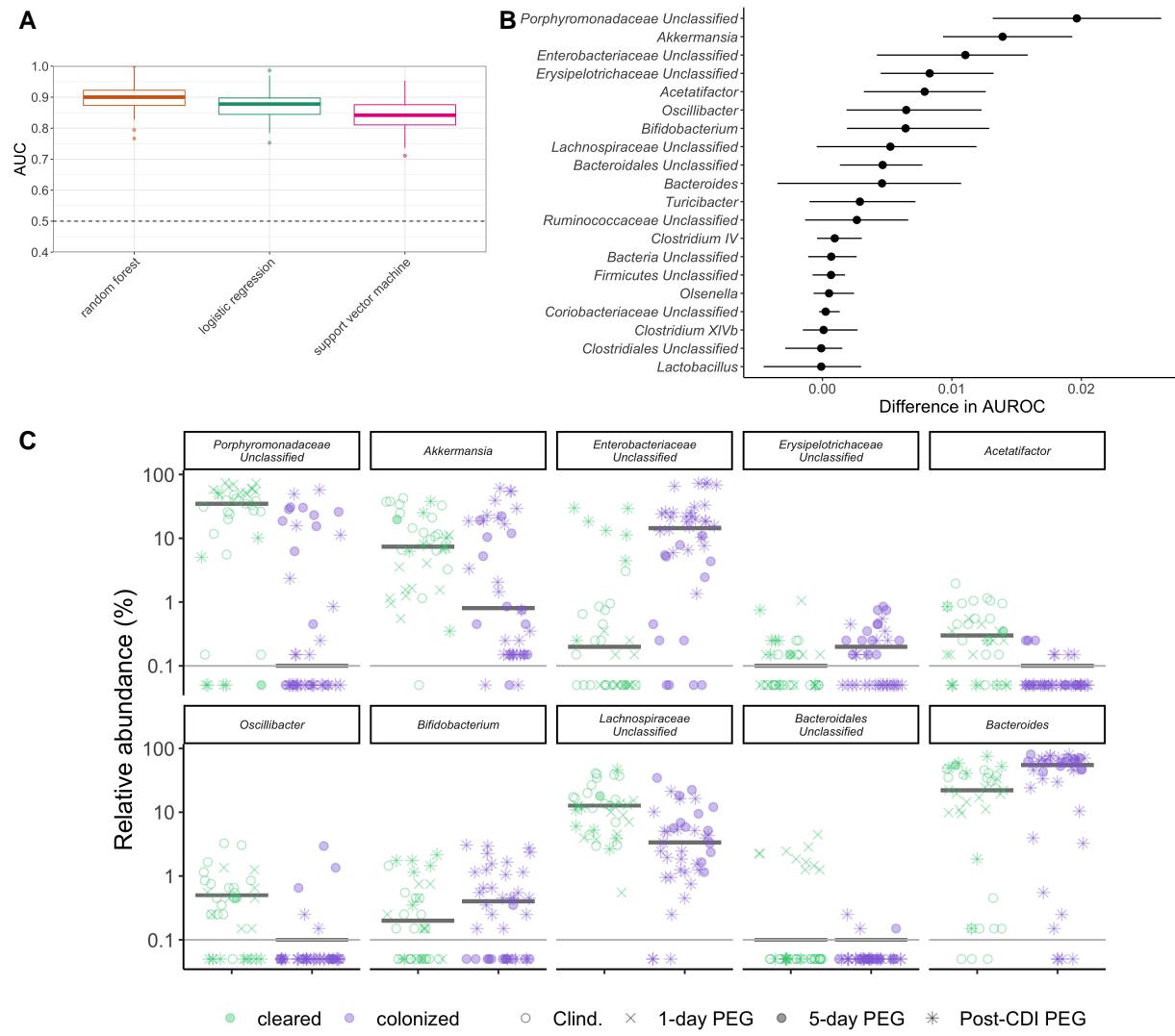
145 groups shown. The gray line represents the limit of detection.



146

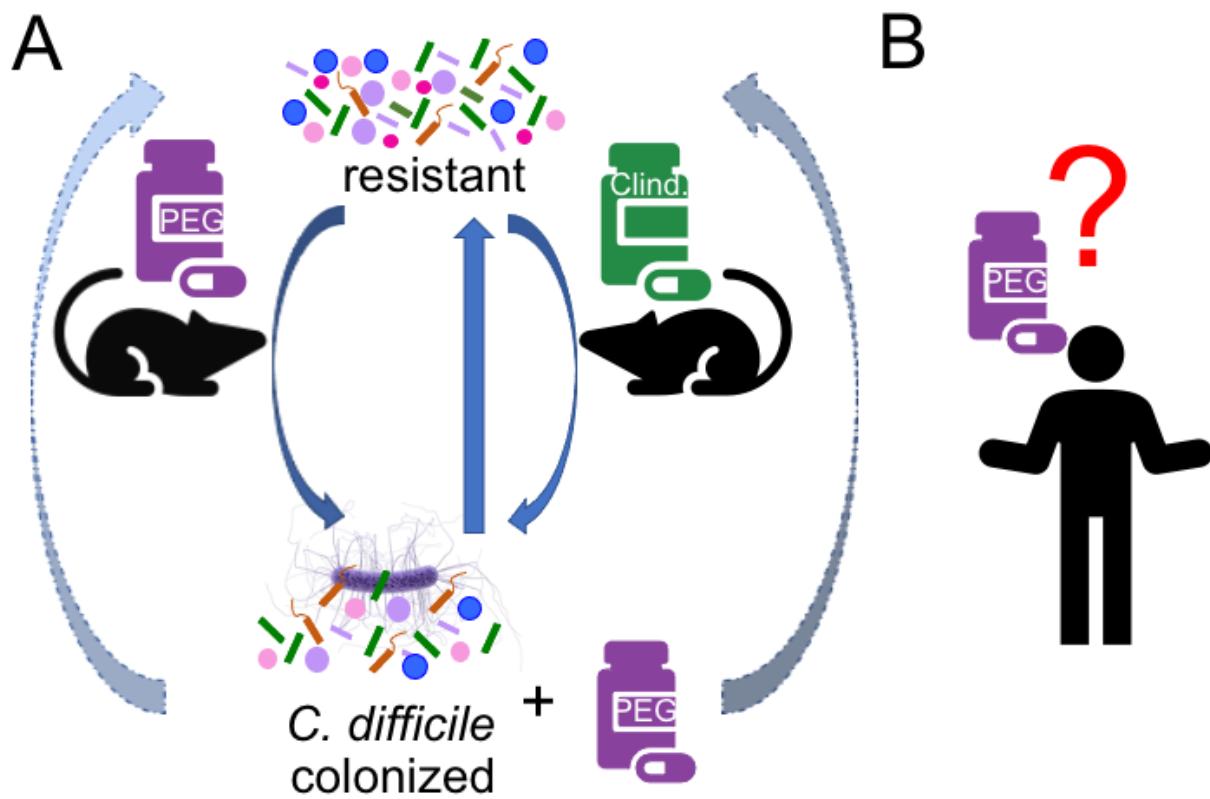
147 **Figure 5. 1-day PEG treatment post *C. difficile* challenge prolongs colonization regardless**
148 **of whether an FMT is also administered. A.**





149

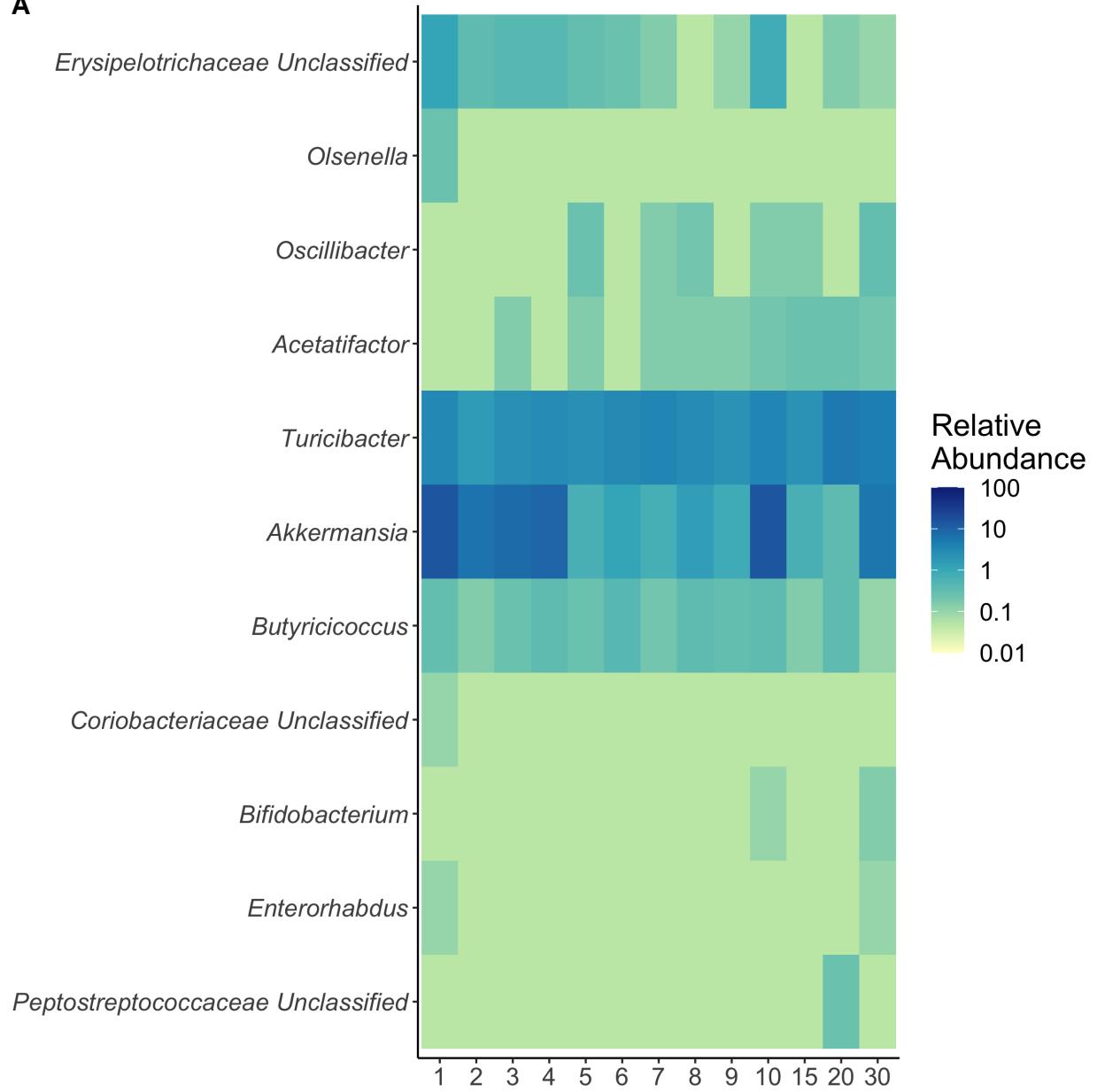
150 **Figure 6. Specific microbiota features associated with prolonged *C. difficile* colonization
151 in PEG treated mice. A.**



152

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

A



A

Potential Muribaculum OTUs Blastn Results



157

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

160 **References**

- 161 1. **Britton RA, Young VB.** 2014. Role of the intestinal microbiota in resistance to colonization by
162 clostridium difficile. *Gastroenterology* **146**:1547–1553. doi:10.1053/j.gastro.2014.01.059.
- 163 2. **Maier L, Pruteanu M, Kuhn M, Zeller G, Telzerow A, Anderson EE, Brochado AR,**
164 **Fernandez KC, Dose H, Mori H, Patil KR, Bork P, Typas A.** 2018. Extensive impact of
165 non-antibiotic drugs on human gut bacteria. *Nature* **555**:623–628. doi:10.1038/nature25979.
- 166 3. **Bastard QL, Al-Ghalith GA, Grégoire M, Chapelet G, Javaudin F, Dailly E, Batard**
167 **E, Knights D, Montassier E.** 2017. Systematic review: Human gut dysbiosis induced by
168 non-antibiotic prescription medications. *Alimentary Pharmacology & Therapeutics* **47**:332–345.
169 doi:10.1111/apt.14451.
- 170 4. **Vila AV, Collij V, Sanna S, Sinha T, Imhann F, Bourgonje AR, Mujagic Z, Jonkers DMAE,**
171 **Masclee AAM, Fu J, Kurilshikov A, Wijmenga C, Zhernakova A, Weersma RK.** 2020. Impact
172 of commonly used drugs on the composition and metabolic function of the gut microbiota. *Nature*
173 *Communications* **11**. doi:10.1038/s41467-019-14177-z.
- 174 5. **Oh J, Makar M, Fusco C, McCaffrey R, Rao K, Ryan EE, Washer L, West LR, Young VB,**
175 **Guttag J, Hooper DC, Shenoy ES, Wiens J.** 2018. A generalizable, data-driven approach to
176 predict daily risk of Clostridium difficile infection at two large academic health centers. *Infection*
177 *& Hospital Epidemiology* **39**:425–433. doi:10.1017/ice.2018.16.
- 178 6. **Mora AL, Salazar M, Pablo-Caeiro J, Frost CP, Yadav Y, DuPont HL, Garey KW.**
179 2012. Moderate to high use of opioid analgesics are associated with an increased risk of
180 clostridium difficile infection. *The American Journal of the Medical Sciences* **343**:277–280.
181 doi:10.1097/maj.0b013e31822f42eb.
- 182 7. **Nehra AK, Alexander JA, Loftus CG, Nehra V.** 2018. Proton pump inhibitors: Review of
183 emerging concerns. *Mayo Clinic Proceedings* **93**:240–246. doi:10.1016/j.mayocp.2017.10.022.
- 184 8. **Krishna SG, Zhao W, Apewokin SK, Krishna K, Chepyala P, Anaissie EJ.** 2013. Risk factors,
185 preemptive therapy, and antiperistaltic agents for Clostridium difficile infection in cancer patients.

- 186 Transplant Infectious Disease n/a–n/a. doi:10.1111/tid.12112.
- 187 9. **Tomkovich S, Lesniak NA, Li Y, Bishop L, Fitzgerald MJ, Schloss PD.** 2019. The proton
188 pump inhibitor omeprazole does not promote *Clostridioides difficile* colonization in a murine model.
189 *mSphere* **4**. doi:10.1128/msphere.00693-19.
- 190 10. **Vandeputte D, Falony G, Vieira-Silva S, Tito RY, Joossens M, Raes J.** 2015. Stool
191 consistency is strongly associated with gut microbiota richness and composition, enterotypes
192 and bacterial growth rates. *Gut* **65**:57–62. doi:10.1136/gutjnl-2015-309618.
- 193 11. **Vujkovic-Cvijin I, Sklar J, Jiang L, Natarajan L, Knight R, Belkaid Y.** 2020. Host
194 variables confound gut microbiota studies of human disease. *Nature* **587**:448–454.
195 doi:10.1038/s41586-020-2881-9.
- 196 12. **Schubert AM, Sinani H, Schloss PD.** 2015. Antibiotic-induced alterations of the murine gut
197 microbiota and subsequent effects on colonization resistance against *Clostridium difficile*. *mBio* **6**.
198 doi:10.1128/mbio.00974-15.
- 199 13. **Nagata N, Tohya M, Fukuda S, Suda W, Nishijima S, Takeuchi F, Ohsugi M, Tsujimoto
200 T, Nakamura T, Shimomura A, Yanagisawa N, Hisada Y, Watanabe K, Imbe K, Akiyama J,
201 Mizokami M, Miyoshi-Akiyama T, Uemura N, Hattori M.** 2019. Effects of bowel preparation on the
202 human gut microbiome and metabolome. *Scientific Reports* **9**. doi:10.1038/s41598-019-40182-9.
- 203 14. **Kashyap PC, Marcabal A, Ursell LK, Larauche M, Duboc H, Earle KA, Sonnenburg
204 ED, Ferreyra JA, Higginbottom SK, Million M, Tache Y, Pasricha PJ, Knight R, Farrugia
205 G, Sonnenburg JL.** 2013. Complex interactions among diet, gastrointestinal transit, and gut
206 microbiota in humanized mice. *Gastroenterology* **144**:967–977. doi:10.1053/j.gastro.2013.01.047.
- 207 15. **Ferreyra JA, Wu KJ, Hryckowian AJ, Bouley DM, Weimer BC, Sonnenburg JL.** 2014. Gut
208 microbiota-produced succinate promotes *C. difficile* infection after antibiotic treatment or motility
209 disturbance. *Cell Host & Microbe* **16**:770–777. doi:10.1016/j.chom.2014.11.003.
- 210 16. **Tropini C, Moss EL, Merrill BD, Ng KM, Higginbottom SK, Casavant EP, Gonzalez CG,
211 Fremin B, Bouley DM, Elias JE, Bhatt AS, Huang KC, Sonnenburg JL.** 2018. Transient

²¹² osmotic perturbation causes long-term alteration to the gut microbiota. *Cell* **173**:1742–1754.e17.
²¹³ doi:10.1016/j.cell.2018.05.008.

²¹⁴ 17. **VanInsberghe D, Elsherbini JA, Varian B, Poutahidis T, Erdman S, Polz MF.** 2020.
²¹⁵ Diarrhoeal events can trigger long-term clostridium difficile colonization with recurrent blooms.
²¹⁶ *Nature Microbiology* **5**:642–650. doi:10.1038/s41564-020-0668-2.

²¹⁷ 18. **Liacouras CA, Piccoli DA.** 1996. Whole-bowel irrigation as an adjunct to the treatment of
²¹⁸ chronic, relapsing clostridium difficile colitis. *Journal of Clinical Gastroenterology* **22**:186–189.
²¹⁹ doi:10.1097/00004836-199604000-00007.

²²⁰ 19. **Tomkovich S, Stough JMA, Bishop L, Schloss PD.** 2020. The initial gut microbiota and
²²¹ response to antibiotic perturbation influence clostridioides difficile clearance in mice. *mSphere* **5**.
²²² doi:10.1128/msphere.00869-20.