

**Infection with a small intestinal helminth *Heligmosomoides polygyrus bakeri***

**consistently alters microbial communities throughout the small and large intestine.**

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## 14 **Abstract**

15 Increasing evidence suggests that intestinal helminth infection can alter intestinal microbial  
 16 communities with important impacts on the mammalian host. However, all of the studies to  
 17 date utilize different techniques to study the microbiome and access different sites of the  
 18 intestine with little consistency noted between studies. In the present study, we set out to  
 19 perform a comprehensive analysis of the impact of intestinal helminth infection on the  
 20 mammalian intestinal bacterial microbiome. For this purpose, we investigated the impact of  
 21 experimental infection using the natural murine small intestinal helminth, *Heligmosomoides*  
 22 *polygyrus bakeri* (Hpb) and examined possible alterations in both the mucous and luminal  
 23 bacterial communities along the entire small and large intestine. We also explored the  
 24 impact of common experimental variables, including the parasite batch and pre-infection  
 25 microbiome, on the outcome of helminth-bacterial interactions. This work provides  
 26 evidence that helminth infection reproducibly alters intestinal microbial communities – with  
 27 an impact of infection noted along the entire length of the intestine. Although the exact  
 28 nature of helminth-induced alterations to the intestinal microbiome differed depending on  
 29 the parasite batch and microbiome community structure present prior to infection, changes  
 30 extended well beyond the introduction of new bacterial species by the infecting larvae.  
 31 Moreover, striking similarities between different experiments were noted, including the  
 32 consistent outgrowth of a bacterium belonging to the Peptostreptococcaceae family  
 33 throughout the intestine.

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## 37 **Author Summary**

38 Increasing evidence indicates a role for interactions between intestinal helminths and the  
 39 microbiome in regulating mammalian health, and a greater understanding of helminth-  
 40 microbiota interactions may open the path for the development of novel  
 41 immunomodulatory therapies. However, such studies are hampered by the inconsistent  
 42 nature of the data reported so far. Such inconsistencies likely result from variations in the  
 43 experimental and technological methodologies employed to investigate helminth-  
 44 microbiota interactions and well has natural variation in the starting microbiome  
 45 composition and/or worm genetics. We conducted a thorough study in which the  
 46 reproducibility of helminth-induced alterations of microbial communities was determined  
 47 and impact of common experimental variables – such as the starting microbiome and  
 48 parasite batch - was determined. Our work reveals the robust ability of small intestinal  
 49 helminth infection to alter microbial communities along the entire length of the intestine  
 50 and additionally identifies a single bacterium that is strongly associated with infection  
 51 across multiple experiments.

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## 54 Introduction

55 The eradication of helminths from developed regions constitutes a major achievement  
 56 towards the improvement of human health and continued efforts to lower the burden of  
 57 infection in endemic areas remain essential. Yet intestinal helminths have inhabited the  
 58 intestine of mammals throughout evolution and are highly likely to have interacted with,  
 59 and impacted on, the complex bacterial communities present at this site (1). The  
 60 eradication of helminths is also thought to contribute to the increased incidence of immune  
 61 disorders, including allergy and autoimmunity, observed in developed societies (2,3). The  
 62 mammalian host and its common intestinal inhabitants – including helminths and bacteria -  
 63 form a complex ecosystem, and the disruption of one of these partners may have  
 64 important implications for the other players of the ecosystem and ultimately for human  
 65 health. Indeed evidence is arising indicating that interactions between helminths and  
 66 bacteria do occur and that these interactions can impact on the mammalian host to shape  
 67 the host immune response (4,5). Although the hypothesis that intestinal helminth infection  
 68 impacts on the mammalian intestinal microbiome is generally accepted, the impact of  
 69 intestinal helminth infection on the human intestinal microbiome has only been  
 70 investigated in a small number of studies, and studies using veterinary animals or  
 71 experimental models have yielded contrasting results. These differences are to be  
 72 expected as the field has been hampered by difficulties characterizing the intestinal  
 73 microbiome as a result of strong inter-individual variations not only in humans but also in  
 74 inbred mice (6). Past studies have also employed a wide array of helminth parasites, host  
 75 species, and sampling sites and utilized diverse technologies to characterize the  
 76 microbiome (1), making cross study comparisons difficult.

77  
 78 Many of the experimental studies have utilized the natural murine parasite,  
 79 *Heligmosomoides polygyrus bakeri* (Hpb), which has a strictly enteric life cycle that is  
 80 similar to the one of trichostrongyloid parasites affecting human and livestock (7–9).  
 81 Experimental infection with this parasite occurs via oral gavage of infective larvae from  
 82 laboratory cultures. Following gavage the larvae penetrate the intestinal epithelium in the  
 83 upper part of the small intestine and reside in the intestinal outer muscle layer of the  
 84 intestinal wall (10). Here the worm matures into its sexually mature form in a process that  
 85 includes two successive moults at days 3-4 and 8-9 post-infection. It finally exits the host  
 86 tissue and establishes itself as an adult worm in the lumen of the small intestine, where it  
 87 can survive for months (11,12). Hpb infection triggers a highly polarized type two immune  
 88 response characterized by the production of IL-4, IL-5 and IL-13 cytokines, proliferation of

89 IgE and IgG1 producing B cells and mucus secretion (13,14). Increased smooth muscle  
90 contractility together with increased epithelial cells secretions favor the eventual expulsion  
91 of adult worms from the intestinal lumen in a combined mechanism that is commonly  
92 referred to as the “weep and sweep” response (15)

93

94 To date seven studies have investigated the possible interaction between Hpb and the  
95 intestinal microbiome (4,5,16–20). The first of these used a method based on the  
96 generation of 16S clone libraries and Sanger sequencing to investigate the impact of  
97 infection of the bacterial communities and reported higher proportions of Lactobacillaceae  
98 in the ileum of infected mice (16). A later study, using real-time PCR and culture-based  
99 methods, reported differences between infected and non-infected mice in the bacterial  
100 communities residing in the ileum, the cecum and the colon at two weeks post infection  
101 (17). Another study using qPCR and Sanger sequencing reported the association of Hpb  
102 infection with the abundance of the bacterium *Lactobacillus taiwanensis* in the duodenum  
103 of susceptible C57BL/6 mice but not resistant Balb/c mice. These authors also reported  
104 that administration of *L. taiwanensis* to resistant Balb/c mice resulted in higher worm  
105 burdens, suggesting that *L. taiwanensis* promotes helminth infection (18). More recently,  
106 using high throughput sequencing of the bacterial 16S gene, our own group reported that  
107 Hpb infection leads to long-lasting impacts on cecal bacterial communities which are  
108 maintained following fecal transfer (4). These findings were confirmed by a later study  
109 using a similar method and fecal transfer but focusing on the colonic bacterial communities  
110 (19). Lastly, Hpb infection has been shown to provide resistance against *Bacteroides*  
111 *vulgatus* outgrowth in Nod2 deficient mice through a mechanisms involving host type two  
112 cytokine production(5). Of note, Hpb excretory/secretory products were recently described  
113 to exert anti-microbial activity, raising the possibility that direct helminth-bacterial  
114 interactions may take place (20). Taken together these observations endorse the view that  
115 intestinal helminths can and do impact on the microbiome, but indicate that the outcome of  
116 helminth-microbial-host interactions may be variable.

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118 Numerous factors might be expected to influence the impact of helminth infection on the  
119 intestinal microbiome including the genetically variability of the parasites used for infection,  
120 the pre-infection or ‘starting’ microbiome of the host, and the possible introduction of  
121 bacteria from the infective parasitic larvae. Moreover, Hpb-associated changes in the  
122 bacterial community composition may occur only within distinct sites of the intestine, and  
123 the infection may impact communities in the intestinal mucus layer differently from the

124 communities present in the intestinal lumen. We considered it particularly important  
 125 address the latter question given the hypothesis that the worm may impact on the  
 126 intestinal microbiome indirectly through the host immune response (5), and would thus be  
 127 expected to have a major impact on those bacteria living in close association with the host  
 128 intestinal epithelium. We therefore set out to determine the full extent of the impact of Hpb  
 129 infection on bacterial communities by analyzing microbiomes from the intestinal lumen and  
 130 epithelium-attached mucus at multiple points along the entire length of the small and large  
 131 intestine. We also investigated the impact of common variables, including the use of  
 132 different parasite batches or mice with distinct pre-infection microbiomes, on the ability of  
 133 Hpb to alter murine intestinal microbial communities.

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# 147 Results

148 *Small intestinal helminth infection impacts on the microbiome present at multiple sites*  
 149 *along the small and large intestine, with distinct parasite batches contributing to variability*  
 150 *in the exact bacterial community composition found at each site.*

151 Many parasitic helminths, including Hpb exhibit a sexual reproductive cycle resulting in  
 152 large genetic heterogeneity amongst individual worms. Even parasites maintained in  
 153 laboratories for use in experimental work are produced in batches that can each be  
 154 considered as different communities and may show significant differences including in  
 155 term of virulence (11,21). To test the impact of distinct parasite batches on helminth-  
 156 induced microbial changes, we assessed the impact of two distinct batches of worms on  
 157 the same microbiome by performing a large experiment in which all mice were subjected  
 158 to ‘normalization’ of the ‘starting intestinal microbiome’ by mixing beddings between cages  
 159 and randomizing mice once a week for four weeks prior to infection (Fig 1). Normalization  
 160 of the microbiome is necessary as mice bred in the same facility demonstrate inter-cage  
 161 variations (6). Mice were then sacrificed at day 40 post infection and the bacterial  
 162 communities in the duodenum, jejunum, ileum, cecum, colon, and feces evaluated by high-  
 163 throughput sequencing of the v1-v2 hyper-variable regions of the bacterial 16S rRNA gene  
 164 as described in the Materials and Methods section. Statistical evidences that infection with  
 165 Hpb affected the composition of the bacterial communities were found at multiple intestinal  
 166 sites, both in the mucus and in the luminal content (Fig 2). These evidences were  
 167 reproducibly observed using both batches of Hpb, either in the mucus or the lumen of the  
 168 duodenum, the jejunum, the cecum and the colon, but not in the ileum (Fig 2).  
 169 Interestingly, no statistical evidences were observed in the feces with neither of the two  
 170 batches of worms (Fig 2). In addition, infection with batch a larvae led to a higher diversity  
 171 in the cecum lumen while infection with batch b larvae led to a higher diversity in the  
 172 colonic lumen, as shown in term of species richness (Fig 3A) and Shannon diversity index  
 173 (Fig 3B).

174  
 175 Beyond these differences, a PCoA analysis highlighted that bacterial communities tend to  
 176 cluster according the sampling site rather than infection, with the ileum clearly separating  
 177 from the rest of the samples (Fig 3C). This indicates that although helminth infection can  
 178 alter bacterial communities, the niche provided by distinct intestinal sites plays a more  
 179 important role in determining community structure, with the ileum forming a particularly  
 180 unique environment as compared to the rest of the intestine. Differences in the proportions

181 of individual bacterial species between Hpb infected and non-infected mice at each  
 182 sampling site along the intestine notably involved members of the Lachnospiraceae,  
 183 Clostridiaceae, S24-7, Lactobacillaceae, Ruminococcaceae and Peptostreptococcaceae  
 184 families, a member of the class Clostridia as well as members of the Allobaculum,  
 185 Bifidobacterium, Ruminococcus, Sutterella and Turicibacter genera (Fig 4). Finally, the  
 186 relative abundance of some bacteria were found to be significantly ( $p < 0.05$ ) altered across  
 187 all studies in response to infection (Fig 5). Most strikingly, a member of the  
 188 Peptostreptococcaceae family was found in higher proportions in the intestinal mucus  
 189 layer of infected mice at all sampling sites along the intestine independently of the larvae  
 190 batch used to infect the mice (Fig 5A and Fig 5C). By contrast, a member of the  
 191 Bifidobacterium genus was found in lower proportions in both intestinal mucus and lumen  
 192 at all sampling sites in mice infected with larvae from batch a, but was not detected in mice  
 193 infected with batch b larvae (Fig 5A and Fig 5B). Members of the Allobaculum and  
 194 Turicibacter genus's were found in higher proportions in the intestinal lumen of all infected  
 195 mice, regardless of larval batch used (Fig 5B and Fig 5D).  
 196 Taken together these data demonstrate that infection with a small intestinal helminth, Hpb,  
 197 leads to significant changes in bacterial community composition throughout the small and  
 198 large intestine, but that parasitic variability (resulting from batch variability) can influence  
 199 the exact nature of these changes.

200

201 *The 'starting point' of the host intestinal microbiome impacts on the outcome of helminth-*  
 202 *bacterial interactions.*

203 Beyond inter-individual or inter-cage variations within a given experiment, the microbial  
 204 communities of mice bred in laboratories are well known to differ between facilities and to  
 205 change over time (22,23). For evident reasons, any impact of helminth infection on the  
 206 microbiome would first depend on its initial state. For example, outgrowth of particular  
 207 species following helminth infection could only be observed if these species were present  
 208 in the community prior to the infection. To gain further insight into the robustness of the  
 209 observed impact of Hpb infection on intestinal bacterial communities, we conducted a  
 210 second experiment (exp. 2) employing a distinct "starting point" to our first experiment  
 211 (exp. 1, reported in Figures 1-5). These different "starting points" were obtained by simply  
 212 conducting experiments at different time periods (approximately 12 months) using mice  
 213 obtained from the same provider. As for exp. 1 normalization of the starting microbiome  
 214 within mice entered into exp. 2 was achieved by employing a period where mice were

215 randomized and bedding were mixed between cages once a week for four weeks prior to  
 216 infection (Fig 6). By necessity the experiment also employed the use of a new batch of  
 217 parasite larvae (batch c) as Hpb larvae only maintain viability for a period of 8-12 weeks.  
 218 As expected major differences were found between bacterial communities from the mice  
 219 employed for exp. 1 (Hpb-1) and exp. 2 Hpb-2 (Fig 7A). Notably, 510 identified OTUs were  
 220 not shared between these two groups (Fig 7B).

221 Statistical evidences for an alteration of the microbiome were found in the lumen of the  
 222 duodenum and in both the mucus layer and the lumen of the ileum, the cecum and the  
 223 colon (Fig 8A). As seen in exp. 1, a PCoA analysis revealed that intestinal site rather than  
 224 infection status has the largest impact on bacterial communities structure, with the trend  
 225 for a clear separation of ileal samples being repeated (Fig 8B). Similarly to infection with  
 226 larvae batch a in exp. 1, a higher bacterial diversity (measured by the Shannon diversity  
 227 index) was observed in the lumen of the cecum in infected mice (Fig 8D). In contrast to  
 228 exp. 1 however, Hpb infection resulted in a lower diversity, both in terms of richness (Fig  
 229 8C) and Shannon diversity index (Fig 8D), in the ileum mucus. Differences between Hpb  
 230 infected and non-infected mice at each sampling site along the intestine notably involved  
 231 members of the Lachnospiraceae, S24-7, Rikenellaceae, Ruminococcaceae and  
 232 Peptostreptococcaceae families, two OTUs respectively assigned to orders Clostridiales  
 233 and Coriobacteriales, an OTU assigned to class Clostridia, members of the  
 234 Ruminococcus, Turicibacter, Escherichia and Sutterella genera as well as Ruminococcus  
 235 gnavus (Fig 9A). As for exp. 1, some bacteria were found significantly ( $p < 0.05$ ) and  
 236 consistently increased or decreased by the infection across all sampling sites (Fig 9B and  
 237 Fig 9C). Strikingly, the same bacterium belonging to the Peptostreptococcaceae family  
 238 that was consistently seen in higher relative abundance in the intestinal mucus layer of  
 239 infected mice from exp. 1 (Fig 5A and Fig 5C), was also seen consistently higher in both  
 240 the mucus layer (Fig 9B) and the lumen (Fig 9C) of infected mice in exp. 2, with the  
 241 exception of the ileal mucus layer where its relative abundance was lower (Fig 9A). In  
 242 addition, a member of the Clostridiaceae family was found in higher proportions in both  
 243 intestinal mucus and lumen of infected mice at all sampling sites except the ileal mucus  
 244 layer, where its relative abundance was lower (Fig 9). As seen in mice infected with larvae  
 245 from batch b in exp. 1, an OTU assigned to genus Turicibacter was observed to be  
 246 consistently less abundant along the intestinal lumen in infected mice from exp. 2 (Fig 9).  
 247 Also, a member of the Lachnospiraceae family together with a member of the genus  
 248 Sutterella were seen in higher proportions in the lumen at all sampling sites except in the  
 249 ileum (Fig 9).

250

251 *Helminth-induced alterations to the bacterial microbiome extend well beyond the*  
 252 *introduction of new bacterial species by the parasitic larvae.*

253 Nematodes are known to carry their own microbiome (24,25) and Hpb is hatched from  
 254 laboratory cultures of feces harvested from infected mice and containing parasite eggs  
 255 (11). We therefore assessed the possibility that bacteria observed in higher proportions  
 256 within the intestine of infected mice were simply carried there from infecting larvae. To  
 257 address this question we characterized the microbiome of the same batch of Hpb larvae  
 258 (batch c) used to perform the infections detailed in exp. 2. Amongst all the bacteria  
 259 significantly affected by the infection at any sampling site and either in the intestinal mucus  
 260 layer or in the intestinal lumen, only 26 were detected in Hpb batch c, including  
 261 *Turicibacter* and *Clostridiaceae* (Fig 10A-B). Of note *Peptostreptococcaceae* 259212 was  
 262 not present in the Hpb larvae batch c, showing that the effect of Hpb infection on the  
 263 abundance of this bacterium - which was noted across all experiments performed - was  
 264 not due to it's introduction into the host by the parasite.

265

## 266 **Discussion**

267 The results of this study showed that infection with a small intestinal helminth, Hpb, can  
 268 consistently and significantly alter bacterial microbial communities throughout the mucous  
 269 layer and intestinal lumen of the small and large intestine. The use of a large set of mice  
 270 with a 'normalized' microbiome and infected with two distinct batches of helminth larvae  
 271 provides unequivocal proof that helminth infection can alter the intestinal microbiome. The  
 272 finding that both the microbiome starting point and the parasite batch employed leads to  
 273 important differences in the exact nature and site of microbiome alterations is likely to  
 274 explain, at least in part, why previous studies of helminth-bacterial interactions have  
 275 reported diverse outcomes. These data highlight the need to carefully control all  
 276 experiments investigating helminth-microbiome interactions and the difficulty in comparing  
 277 data generated across different laboratories or even within the same laboratory at different  
 278 times.

279

280 The contribution of parasite batch to variability in helminth-induced microbial alterations  
 281 likely arises from genetic variations resulting from the sexual life cycle of these complex  
 282 organisms, however they may also arise from differences in the microbiome associated  
 283 with the infective larvae. Major differences were also observed in the 'starting' microbiome

284 of mice sourced at different times from the same provider, also introducing variability to the  
 285 data obtained. However, in spite of these variables we found that Hpb infection  
 286 consistently altered the community structure of bacteria found in the mucous and luminal  
 287 compartments of the cecum and colon. Alterations also occurred within the small intestine,  
 288 however the exact compartment affected varied between experimental conditions. In terms  
 289 of diversity, infection resulted in differences across all experimental conditions but these  
 290 were smaller and less consistent than alterations to community structure. Of note,  
 291 variations in bacterial communities along the intestinal tract were poorly reflected in the  
 292 feces, suggesting a greater stability of the microbial composition in the feces compared to  
 293 the intestinal tract. This observation emphasizes that a particular attention should be given  
 294 to sampling site in the study of microbiome. Based on this observation in mice, we further  
 295 hypothesize that the changes in fecal bacterial composition observed in human after  
 296 helminths clearance may reflect an even more dramatic change occurring within more  
 297 distal sites of the intestine.

298  
 299 Although we highlight that different microbiome 'starting points' can lead to different  
 300 responses, metagenomics has shown that a particular niche within the microbiome can be  
 301 occupied by several species and that communities with different species composition can  
 302 display more similarity in terms of metabolic functions (26). Therefore, the metagenomic  
 303 approach used in the present study may fail to capture a more robust impact of Hpb  
 304 infection at the functional level, a view supported by the previous finding that Hpb  
 305 consistently leads to higher levels of bacteria-derived SCFAs within the cecum (4). We  
 306 also consistently noted a higher proportion of a member of the Peptostreptococcaceae  
 307 family following Hpb infection. This was observed along the entire length of the intestine  
 308 and across varying experimental conditions. The fact that Hpb larvae did not harbor any  
 309 Peptostreptococcaceae at the time of the infection rules out the possibility of co-inoculation  
 310 and indicates that bacteria already present in the host intestine increased in abundance in  
 311 response to infection. However this observation was only possible because the bacteria in  
 312 question was already part of the microbial communities prior to infection and may not  
 313 occur in experiments using mice from other facilities. The Peptostreptococcaceae family  
 314 (order Clostridiales) includes six described genera which members were isolated from  
 315 various environments ranging from human and animal microbiome to swine manure and  
 316 deep-sea hydrothermal vents (27). Of particular interest, this family of bacteria has been  
 317 described as anaerobic and produces acetate as a product of fermentation. This raises the  
 318 possibility that the promotion of Peptostreptococcaceae species outgrowth by Hpb may

319 contribute to the previously observed high SCFAs levels during Hpb infection. The 16S  
320 rRNA based phylogeny of the Peptostreptococcaceae family has been subject to  
321 remodeling and several species of Clostridium have been included into this family in the  
322 database of the Ribosomal Database Project (RDP). Based on the alignment of the 16S  
323 rRNA gene representative sequence for the Peptostreptococcaceae seen in the present  
324 study (OTU 259212) to the NCBI 16S database with BLAST, the phylogenetically closest  
325 identified match was *Romboutsia timonensis* strain DR1 (with only 91% similarity).  
326 Altogether, the exact taxonomy of this Peptostreptococcaceae remains unclear in the  
327 current state and further characterization (first requiring isolation from the mouse gut)  
328 would be necessary in order to understand the possible role of this bacteria within the  
329 helminth-associated microbiome and to identify possible mechanisms promoting its  
330 outgrowth during Hpb infection.

331  
332 In conclusion, this work provides conclusive evidence confirming the hypothesis that  
333 intestinal helminths can impact on the mammalian intestinal microbiome. Our work also  
334 indicates that helminth-induced changes can occur at regions distal to the parasite site of  
335 infection and extend well beyond the introduction of new bacterial species carried-over by  
336 the infecting larvae. These findings provide impetus for further studies investigating the full  
337 impact of helminth-microbial interactions on host health, and determining the molecular  
338 mechanisms by which helminths alter microbial communities.

339

## 340 **Materials and Methods**

### 341 *Ethics statement*

342 All animal experiments were approved by the Service de la consommation et des affaires  
343 vétérinaires (1066 Epalinges, Switzerland) with the authorization number 2238.

### 344 *Mice*

345 Four weeks old female C57BL/6 wild-type mice were purchased from Charles River  
346 Laboratories (France), and housed in specific-pathogen-free (SPF) conditions at the EPFL  
347 Faculty of Life Sciences facility for animal housing. Mice were infected with 300 infective  
348 L3 Hpb larvae administered by oral gavage in 200ul of saline (Gibco, 10010-015). Control  
349 mice received 200ul of saline by the same route.

### 350 *Parasites*

351 Hpb L3 infective larvae were generated in the laboratory by Manuel Kulagin and Luc  
352 Lebon based on previously described methods (11) Briefly, feces from infected mice were

353 mixed with charcoal and water and incubated at 26°C for one week. The larvae were  
 354 recovered using a modified Baermann apparatus and washed with saline. Larvae were  
 355 then incubated for 4 to 6 hours in an antibiotic saline solution containing 5 mg/ml  
 356 enrofloxacin (injectable Baytril, Bayer), 2 mg/ml amoxicilin and 0.2 mg/ml clavulanic acid  
 357 (injectable Co-amoxi-Mapha 2200, Mepha Pharma). Larvae were finally washed in saline  
 358 and stored at 4°C until infection.

359

### 360 *Analysis of intestinal bacterial communities*

361 Bacterial communities were assessed by high-throughput sequencing of the v1-v2 hyper-  
 362 variable regions of the bacterial 16S rRNA gene as previously described ((28), following  
 363 the basic protocol “Bacterial 16S rRNA sequencing for bacterial communities present in  
 364 intestinal contents of mice, or from fecal samples collected from mice or humans”).  
 365 Samples were processed in nine sequencing runs on an Illumina MiSeq platform using  
 366 Paired End (PE) v2 2x250 chemistry. The sequences were processed using scripts from  
 367 the Quantitative Insight Into Microbial Ecology (Qiime) v1.9.0 pipeline (29). Briefly,  
 368 sequences were trimmed to remove bases showing a Phred quality score lower than 20  
 369 using the Seqtk software. Forward and reverse reads were then merged using the  
 370 join\_paired\_ends.py script from Qiime (which implements the fastq-join algorithm), setting  
 371 a minimal overlap of 100bp and allowing a maximal alignment mismatch of 10%. Sample  
 372 demultiplexing was done using the split\_libraries\_fastq.py script from Qiime. In addition,  
 373 reads were truncated at first 3 consecutive bases showing a Phred quality score below 20  
 374 and the resulting sequences were discarded if truncated by more than 25% of their length.  
 375 Reads containing more than 2 N (unknown) nucleotides or were discarded. Reads were  
 376 finally clustered at 97% similarity and mapped to the GreenGene (30) database as  
 377 described previously (31). The computation was performed using the clusters of the Vital-it  
 378 center for high-performance computing of the Swiss Institute of Bioinformatics. Sequences  
 379 that were not assigned to any taxonomy were discarded. Samples showing less than  
 380 5,000 observations were also discarded. The final distribution of samples among  
 381 experimental groups is describe in Table 1 below.

382

383

Table 1. Number of samples obtained per intestinal site per experimental group.

|       | Duo. <sup>m</sup> | Duo. <sup>l</sup> | Jej. <sup>m</sup> | Jej. <sup>l</sup> | Ile. <sup>m</sup> | Ile. <sup>l</sup> | Cec. <sup>m</sup> | Cec. <sup>l</sup> | Col. <sup>m</sup> | Col. <sup>l</sup> | Fec. |
|-------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------|
| Hpb-1 | 4                 | 10                | -                 | 10                | 8                 | 8                 | 10                | 10                | 10                | 10                | 9    |
| Hpb+a | 6                 | 8                 | -                 | 10                | 10                | 6                 | 10                | 10                | 9                 | 10                | 10   |
| Hpb+b | 5                 | 10                | -                 | 10                | 10                | 10                | 9                 | 10                | 10                | 10                | 9    |
| Hpb-2 | 8                 | 5                 | 10                | 12                | 14                | 15                | 15                | 14                | 15                | 15                | -    |
| Hpb+c | 12                | 10                | 14                | 13                | 15                | 15                | 14                | 15                | 15                | 14                | -    |

Duo.- duodenum; Jej. - jejunum; Ile. - ileum; Cec. - cecum; Col. - colon; Fec. - feces; <sup>m</sup> – mucus; <sup>l</sup> – lumen.

# *Data analysis*

Data analysis was performed using Genocrunch (<https://genocrunch.epfl.ch>). Differences between bacteria communities at each sampling sites in Fig. 2 and Fig. 6.A were assessed at the species level using the Adonis method based on the Jaccard index. Venn diagrams representing significant changes ( $p < 0.05$ ) in individual OTUs across sampling sites were generated in R (32). Rarefaction at the depth of 5,141 observations per sample was applied prior to all analysis with the exception of Fig. 4 and Fig. 7, where rarefaction depth was adapted individually for each sampling site to maximize discovery of bacteria affected by the worm infection (Table 2 below). Differences in individual OTUs proportions and diversity between infected and non-infected mice were assessed by ANOVA. OTUs named in Fig. 4 and Fig. 7.A were selected based on the following criterion: OTUs were first scored based on statistical differences between infected and non-infected groups across sampling sites. Briefly, for each sampling site, a score was assigned ( $p < 0.001 = 3$ ,  $0.001 < p < 0.01 = 2$ ,  $0.01 < p < 0.05 = 1$ ,  $p > 0.05 = 0$ ) and the overall score was calculated as the sum across all sampling site. OTUs showing consistency in fold-change sign at least across five sampling sites together with an overall score higher than five were selected.

406 Table 2. Number of samples obtained per intestinal site per experimental group.

|        | Duo. <sup>m</sup> | Duo. <sup>l</sup> | Jej. <sup>m</sup> | Jej. <sup>l</sup> | Ile. <sup>m</sup> | Ile. <sup>l</sup> | Cec. <sup>m</sup> | Cec. <sup>l</sup> | Col. <sup>m</sup> | Col. <sup>l</sup> | Fec.   |
|--------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|--------|
| Exp. 1 | 6,213             | 23,528            | -                 | 17,217            | 26,165            | 42,587            | 53,071            | 9,367             | 20,404            | 49,050            | 17,019 |
| Exp. 2 | 5,318             | 12,306            | 5,058             | 21,777            | 5,168             | 58,390            | 7,204             | 9,955             | 5,873             | 5,442             | -      |

407 Duo.- duodenum; Jej. - jejunum; Ile. - ileum; Cec. - cecum; Col. - colon; Fec. - feces; <sup>m</sup> –  
408 mucus; <sup>l</sup> – lumen.

409

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415

## 416 References

1. Rapin A, Harris NL. Helminth-Bacterial Interactions: Cause and Consequence. Trends Immunol. 2018 Jun 22;
2. Maizels RM. Infections and allergy - helminths, hygiene and host immune regulation. Curr Opin Immunol. 2005 Dec;17(6):656–61.
3. Cooper PJ. Interactions between helminth parasites and allergy. Curr Opin Allergy Clin Immunol. 2009 Feb;9(1):29–37.
4. Zaiss MM, Rapin A, Lebon L, Dubey LK, Mosconi I, Sarter K, et al. The Intestinal Microbiota Contributes to the Ability of Helminths to Modulate Allergic Inflammation. Immunity. 2015 Nov 17;43(5):998–1010.
5. Ramanan D, Bowcutt R, Lee SC, Tang MS, Kurtz ZD, Ding Y, et al. Helminth infection promotes colonization resistance via type 2 immunity. Science. 2016 Apr 29;352(6285):608–12.
6. Mamantopoulos M, Ronchi F, Van Hauwermeiren F, Vieira-Silva S, Yilmaz B, Martens L, et al. Nlrp6- and ASC-Dependent Inflammasomes Do Not Shape the Commensal Gut Microbiota Composition. Immunity. 2017 15;47(2):339-348.e4.

7. Bartlett A, Ball PA. *Nematospiroides dubius* in the mouse as a possible model of endemic human hookworm infection. *Ann Trop Med Parasitol*. 1972 Mar;66(1):129–34.
8. Gause WC, Urban JF, Stadecker MJ. The immune response to parasitic helminths: insights from murine models. *Trends Immunol*. 2003 May;24(5):269–77.
9. Pritchard DI, Williams DJ, Behnke JM, Lee TD. The role of IgG1 hypergammaglobulinaemia in immunity to the gastrointestinal nematode *Nematospiroides dubius*. The immunochemical purification, antigen-specificity and in vivo anti-parasite effect of IgG1 from immune serum. *Immunology*. 1983 Jun;49(2):353–65.
10. Sukhdeo MV, O'Grady RT, Hsu SC. The site selected by the larvae of *Heligmosomoides polygyrus*. *J Helminthol*. 1984 Mar;58(1):19–23.
11. Camberis M, Le Gros G, Urban J. Animal model of *Nippostrongylus brasiliensis* and *Heligmosomoides polygyrus*. *Curr Protoc Immunol*. 2003 Aug;Chapter 19:Unit 19.12.
12. Reynolds LA, Filbey KJ, Maizels RM. Immunity to the model intestinal helminth parasite *Heligmosomoides polygyrus*. *Semin Immunopathol*. 2012 Nov;34(6):829–46.
13. Hasnain SZ, Gallagher AL, Grencis RK, Thornton DJ. A new role for mucins in immunity: insights from gastrointestinal nematode infection. *Int J Biochem Cell Biol*. 2013 Feb;45(2):364–74.
14. Hasnain SZ, Evans CM, Roy M, Gallagher AL, Kindrachuk KN, Barron L, et al. Muc5ac: a critical component mediating the rejection of enteric nematodes. *J Exp Med*. 2011 May 9;208(5):893–900.
15. Shea-Donohue T, Sullivan C, Finkelman FD, Madden KB, Morris SC, Goldhill J, et al. The role of IL-4 in *Heligmosomoides polygyrus*-induced alterations in murine intestinal epithelial cell function. *J Immunol Baltim Md 1950*. 2001 Aug 15;167(4):2234–9.
16. Walk ST, Blum AM, Ewing SA-S, Weinstock JV, Young VB. Alteration of the murine gut microbiota during infection with the parasitic helminth *Heligmosomoides polygyrus*: *Inflamm Bowel Dis*. 2010 Nov;16(11):1841–9.

17. Rausch S, Held J, Fischer A, Heimesaat MM, Kühl AA, Bereswill S, et al. Small Intestinal Nematode Infection of Mice Is Associated with Increased Enterobacterial Loads alongside the Intestinal Tract. Allen IC, editor. PLoS ONE. 2013 Sep 10;8(9):e74026.
18. Reynolds LA, Smith KA, Filbey KJ, Harcus Y, Hewitson JP, Redpath SA, et al. Commensal-pathogen interactions in the intestinal tract: Lactobacilli promote infection with, and are promoted by, helminth parasites. Gut Microbes. 2014 Jul;5(4):522–32.
19. Su C, Su L, Li Y, Long SR, Chang J, Zhang W, et al. Helminth-induced alterations of the gut microbiota exacerbate bacterial colitis. Mucosal Immunol. 2018 Jan;11(1):144–57.
20. Rausch S, Midha A, Kuhring M, Affinass N, Radonic A, Kühl AA, et al. Parasitic Nematodes Exert Antimicrobial Activity and Benefit From Microbiota-Driven Support for Host Immune Regulation. Front Immunol. 2018;9:2282.
21. Bouchery T, Volpe B, Shah K, Lebon L, Filbey K, LeGros G, et al. The Study of Host Immune Responses Elicited by the Model Murine Hookworms *Nippostrongylus brasiliensis* and *Heligmosomoides polygyrus*. Curr Protoc Mouse Biol. 2017 Dec 20;7(4):236–86.
22. Rausch P, Basic M, Batra A, Bischoff SC, Blaut M, Clavel T, et al. Analysis of factors contributing to variation in the C57BL/6J fecal microbiota across German animal facilities. Int J Med Microbiol IJMM. 2016 Aug;306(5):343–55.
23. Franklin CL, Ericsson AC. Microbiota and reproducibility of rodent models. Lab Anim. 2017 Mar 22;46(4):114–22.
24. Hsu SC, Johansson KR, Donahue MJ. The bacterial flora of the intestine of *Ascaris suum* and 5-hydroxytryptamine production. J Parasitol. 1986 Aug;72(4):545–9.
25. Zhang F, Berg M, Dierking K, Félix M-A, Shapira M, Samuel BS, et al. *Caenorhabditis elegans* as a Model for Microbiome Research. Front Microbiol. 2017;8:485.
26. Franzosa EA, Morgan XC, Segata N, Waldron L, Reyes J, Earl AM, et al. Relating the metatranscriptome and metagenome of the human gut. Proc Natl Acad Sci U S A. 2014 Jun 3;111(22):E2329–2338.

27. Slobodkin A. The Family Peptostreptococcaceae. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F, editors. The Prokaryotes: Firmicutes and Tenericutes [Internet]. Berlin, Heidelberg: Springer Berlin Heidelberg; 2014 [cited 2018 Nov 27]. p. 291–302. Available from: [https://doi.org/10.1007/978-3-642-30120-9\\_217](https://doi.org/10.1007/978-3-642-30120-9_217)
28. Rapin A, Pattaroni C, Marsland BJ, Harris NL. Microbiota Analysis Using an Illumina MiSeq Platform to Sequence 16S rRNA Genes. *Curr Protoc Mouse Biol*. 2017 Jun 19;7(2):100–29.
29. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods*. 2010 May;7(5):335–6.
30. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol*. 2006 Jul;72(7):5069–72.
31. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J*. 2012 Aug;6(8):1621–4.
32. R Core Team. R: A Language and Environment for Statistical Computing [Internet]. R Foundation for Statistical Computing; 2018. Available from: <https://www.R-project.org/>

## 418 **Figure Captions**

419

420 *Fig 1. Experimental design of experiment 1 (exp. 1)*

421 Analysis of the bacterial communities in the intestinal lumen and intestinal mucus layer 40  
422 days post infection with two distinct batches of Hpb larvae.

423

424 *Fig 2. Bacterial communities are affected by Hpb infection at multiple sites along the gut*

425 Average proportions of bacterial families in non-infected (-) and infected (+) mice from exp.  
426 1. (A) Non-infected mice (Hpb-1) are compared to infected mice with infective larvae from  
427 batch a (Hpb+a). (B) Non-infected mice (Hpb-1) are compared to infected mice with  
428 infective larvae from batch b (Hpb+b). Groups were compared using the Adonis method  
429 based on the Jaccard distance ( $p < 0.05$ : \*,  $p < 0.01$ : \*\*,  $p < 0.001$ : \*\*\*,  $p > 0.05$ : ns).

430

431 *Fig 3. Hpb infection affects bacterial community diversity*

432 Bacterial communities diversity in the mucus layer and lumen of the intestinal tract and  
433 feces in terms of richness (A) and Shannon diversity index (B) for non-infected mice from  
434 exp. 1 (Hpb-1), infected mice with infective larvae from batch a (Hpb+a) and infected mice  
435 with infective larvae from batch b (Hpb+b). Groups were compared by ANOVA ( $p < 0.05$ : \*,  
436  $p < 0.01$ : \*\*,  $p < 0.001$ : \*\*\*,  $p > 0.05$ : ns). (C) Principal coordinates analysis (PCoA) based on  
437 the Jaccard distance of bacterial communities in the mucus layer along the intestinal tract  
438 for non-infected mice from exp. 1 and infected mice with infective larvae from batch a (a)  
439 or batch b (b).

440

441 *Fig 4. Select bacteria are altered by Hpb infection.*

442 Differences in proportions of selected bacterial species in the mucus layer and lumen of  
443 the intestine and feces between non-infected mice from exp. 1 (Hpb-1) and mice infected  
444 with larvae from batch a (Hpb+a) (A) or batch b (Hpb+b) (B). Differences are represented  
445 by the log value of the fold-change as described in the Materials and Methods chapter  
446 above. Species were selected based on statistical significance and consistency across  
447 multiple sampling sites as described in the Methods section above. Higher proportions in  
448 samples from Hpb-1 are represented in a negative (blue) scale while higher proportions in  
449 infected samples are represented in a positive (red) scale.

450

451 *Fig 5. A subset of bacteria are consistently associated with Hpb infection across all*  
452 *intestinal sites*

Venn diagrams highlighting overlaps between sampling sites along the intestine for all significant ( $p < 0.05$ ) differences in proportions of observed species between non-infected mice from exp. 1 (Hpb-1) and mice infected with larvae from batch a (Hpb+a) (A, B) or batch b (Hpb+b) (C, D) in the mucus layer (A, C) and the lumen (B, D).

457

*Fig 6. Experimental design of experiment 2 (exp. 2)*

Analysis of the bacterial communities in the intestinal lumen and intestinal mucus layer 40 days post infection with Hpb larvae.

461

*Fig 7. Mice from experiment 1 (exp. 1) and experiment 2 (exp. 2) harbor distinct bacterial communities*

(A) Principal coordinates analysis (PCoA) based on the Bray-Curtis dissimilarity for intestinal and fecal bacterial communities (from both mucus layer and lumen samples) in non-infected mice from exp. 1 (Hpb-1) and 2 (Hpb-2). Colors represent clusters of bacterial communities as defined by unbiased similarity network clustering based on Bray-Curtis dissimilarity. (B) Overlaps between non-infected mice from exp. 1 (Hpb-1) and 2 (Hpb-2) for observed bacterial species in intestinal and fecal sample (from the mucus layer and the lumen).

471

*Fig 8. The bacterial community is reproducibly impacted by Hbp infection at multiple sites along the gut, but nature of the impact depends on the initial state of the microbiome*

(A) Average proportions of bacterial families in non-infected (-, Hpb-2) and infected (+, Hpb+c) mice from exp. 2. Groups were compared using the Adonis method based on the Jaccard distance ( $p < 0.05$ : \*,  $p < 0.01$ : \*\*,  $p < 0.001$ : \*\*\*,  $p > 0.05$ : ns). (B) Principal coordinates analysis (PCoA) based on the Jaccard distance of bacterial communities in the mucus layer along the intestinal tract for non-infected (Hpb-2) and infected (Hpb+c) mice from exp. 2. (C, D) Bacterial communities diversity in the mucus layer and lumen of the intestinal tract and feces for exp. 2 in terms of richness (C) and Shannon diversity index (D). Groups were compared by ANOVA ( $p < 0.05$ : \*,  $p < 0.01$ : \*\*,  $p < 0.001$ : \*\*\*,  $p > 0.05$ : ns).

482

*Fig 9. A member of the Peptostreptococcaceae family is consistently associated with Hpb infection across intestinal sites and experimental conditions*

(A) As in Fig 4: Differences in proportions of selected bacterial species in the mucus layer and lumen of the intestine and feces between non-infected (Hpb-2) and infected (Hpb+c) mice from exp. 2. (B, C) Overlaps between sampling sites along the intestine for all

488 significant ( $p < 0.05$ ) differences in proportions of observed species between non-infected  
 489 (Hpb-2) and infected (Hpb+c) mice from exp. 2 in the mucus layer (B) and the lumen (C).  
 490

491 *Fig 10. Only a limited number of bacteria found affected by Hpb infection in the gut were*  
 492 *observed on Hpb infective larvae before the infection*

493 Venn diagrams highlighting overlaps between bacterial species included in Fig 9B (A) or  
 494 Fig 9C (B) and bacterial species observed in the batch of infective Hpb larvae used in exp.  
 495 2.

496

497

498

Cages  
(n=30)

Weeks

*Hpb*

Gut microbiome

Lumen

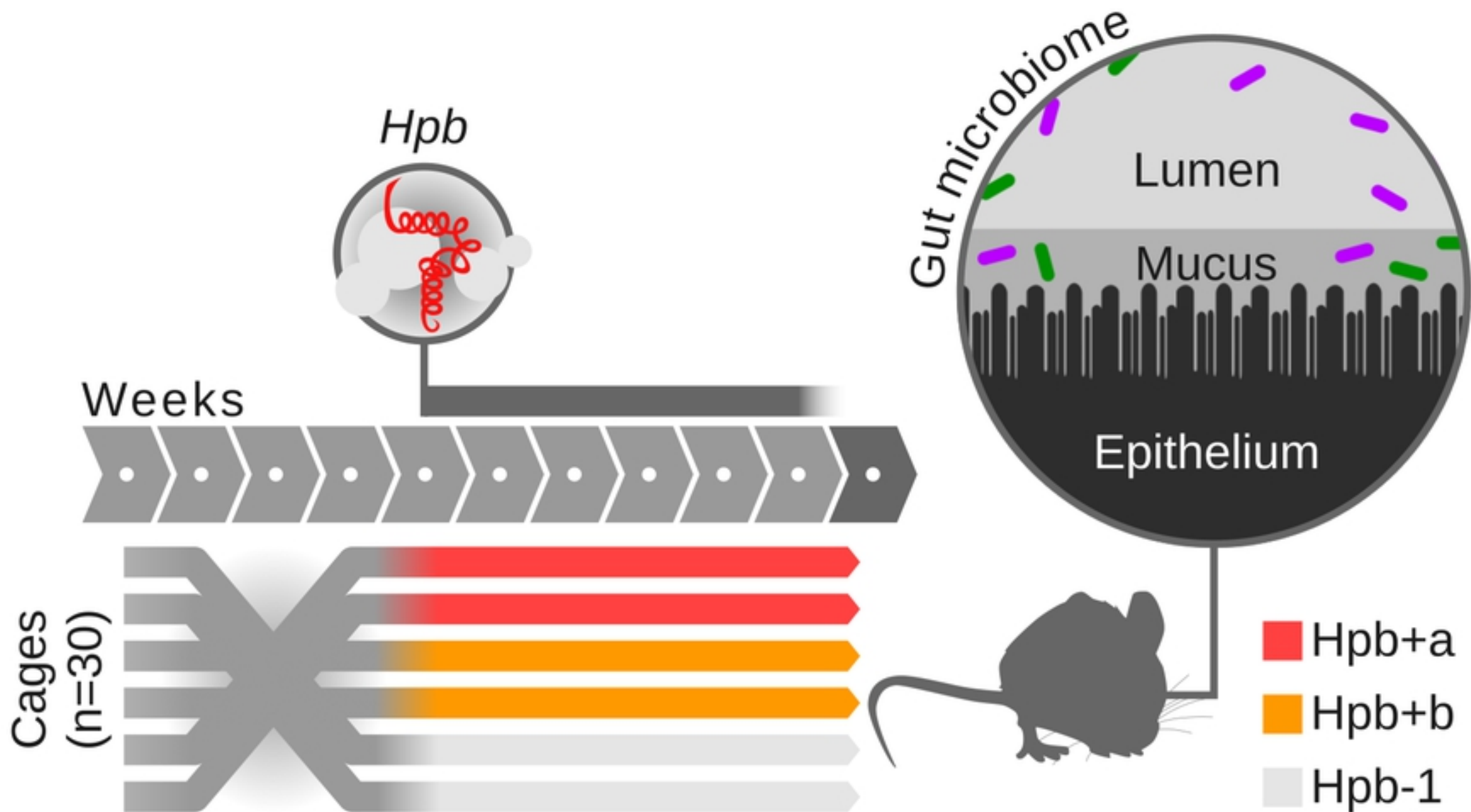
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Epithelium

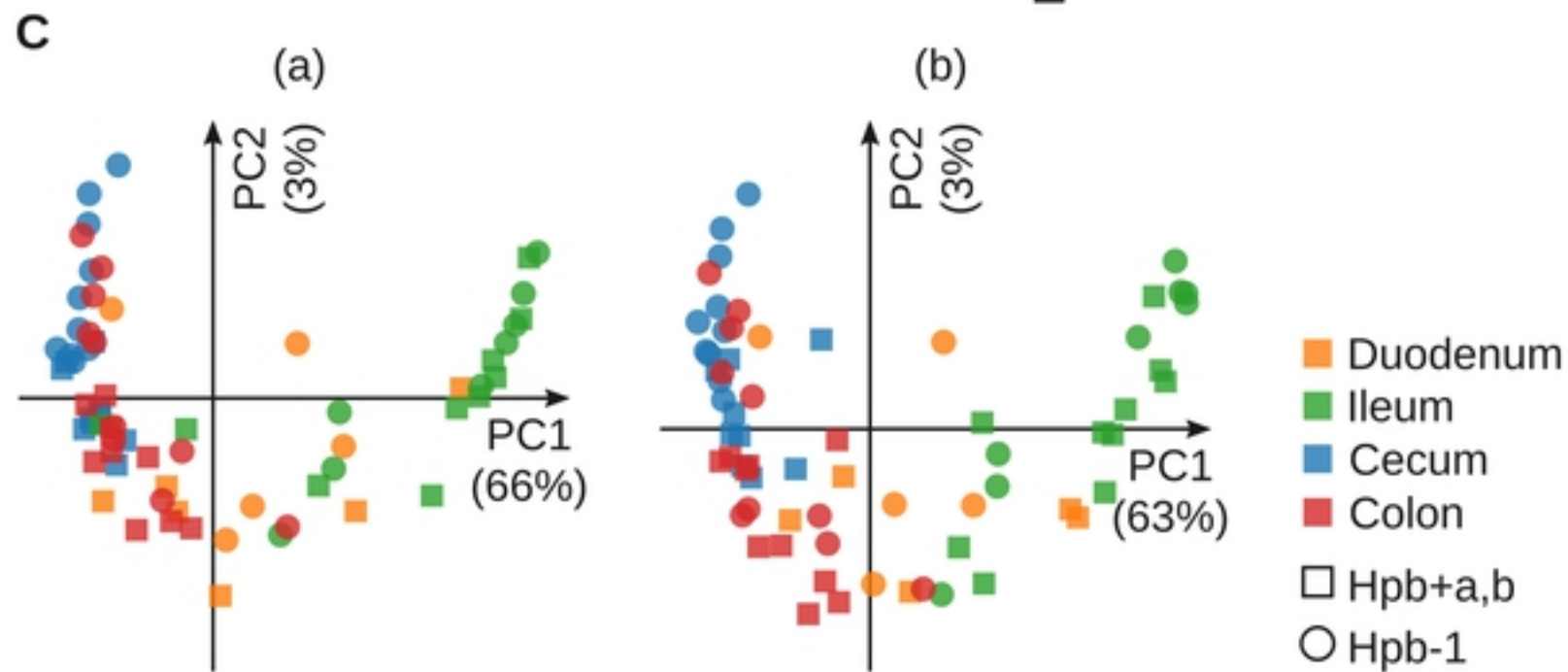
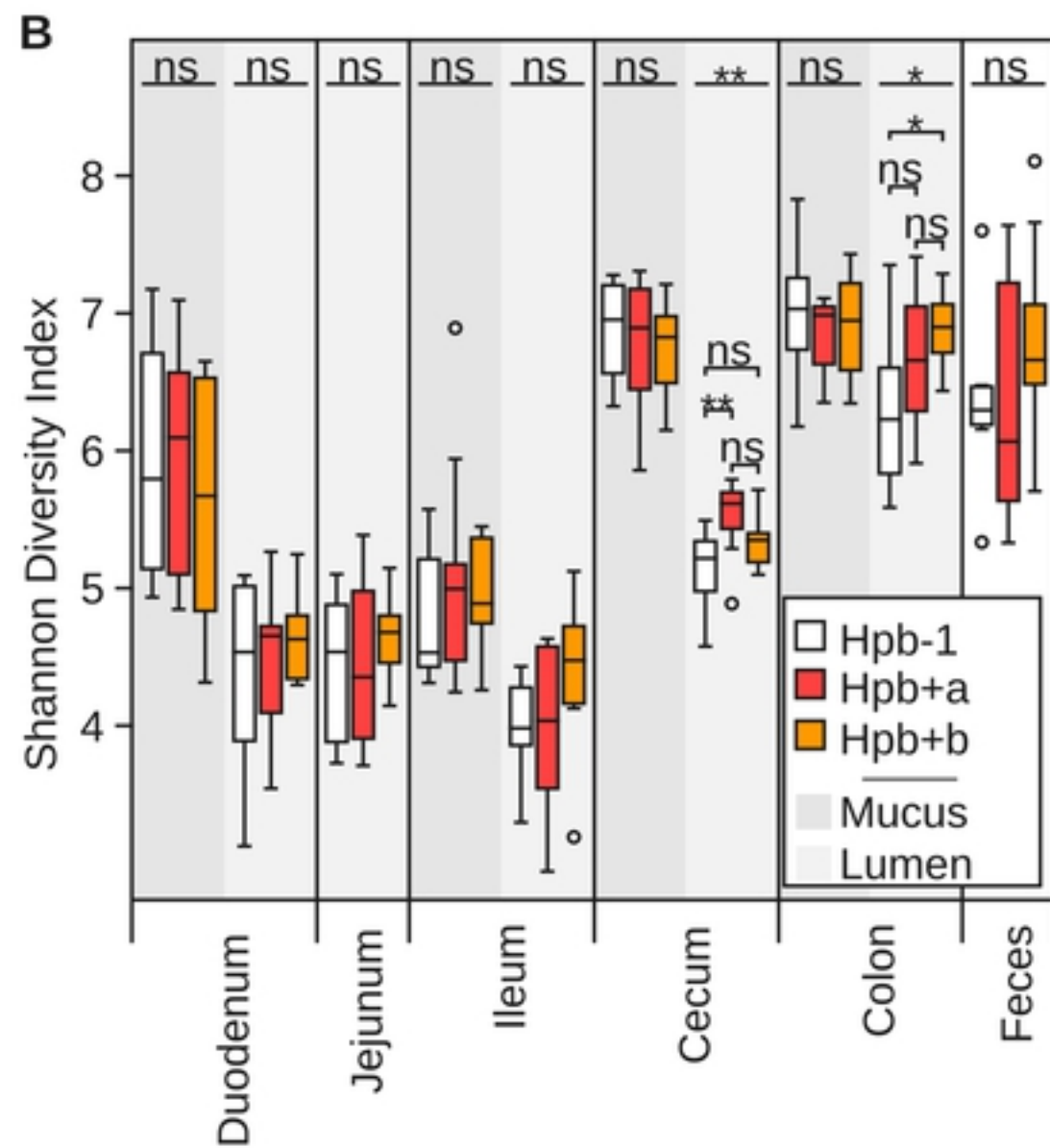
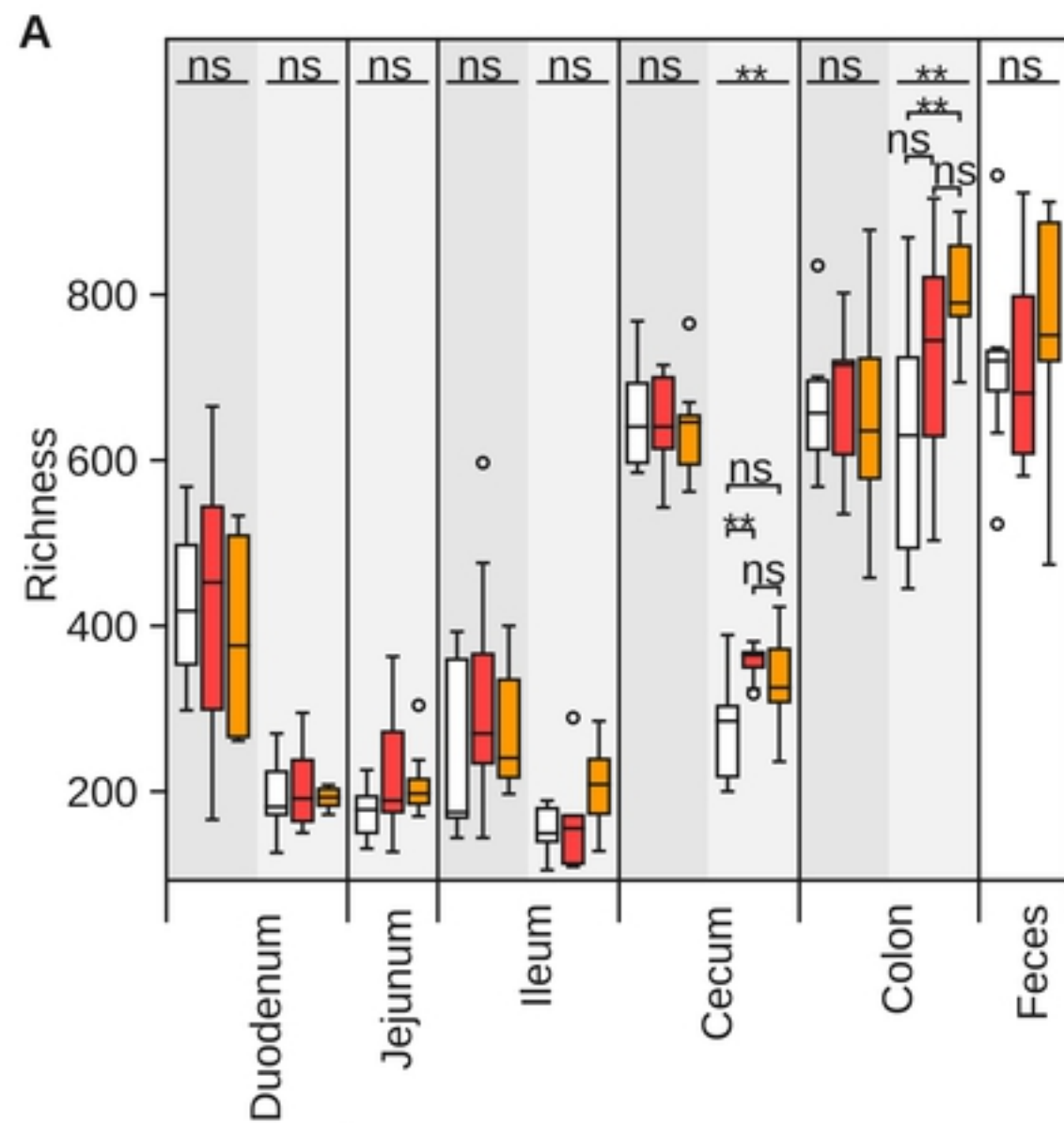
■ *Hpb+a*

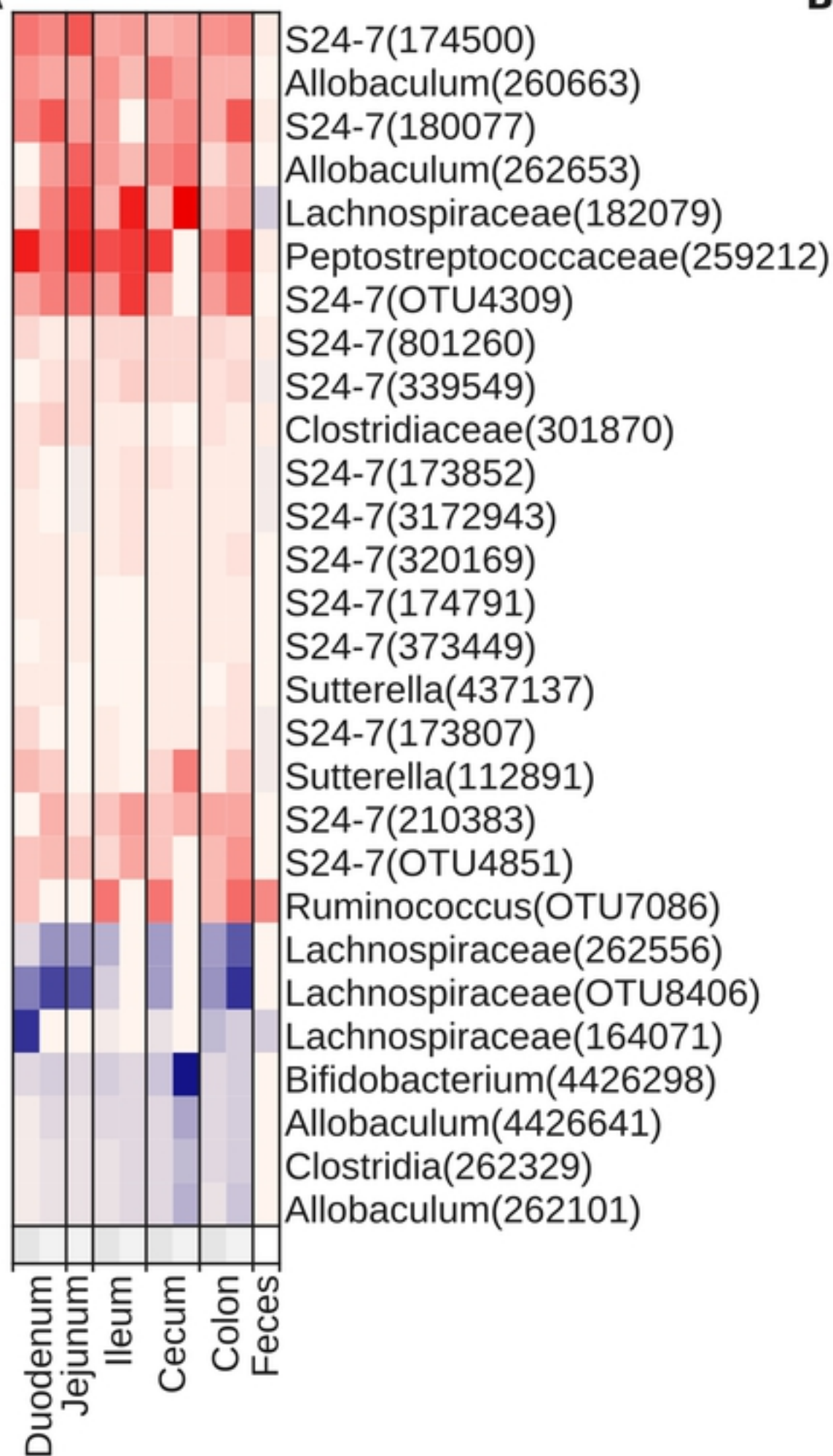
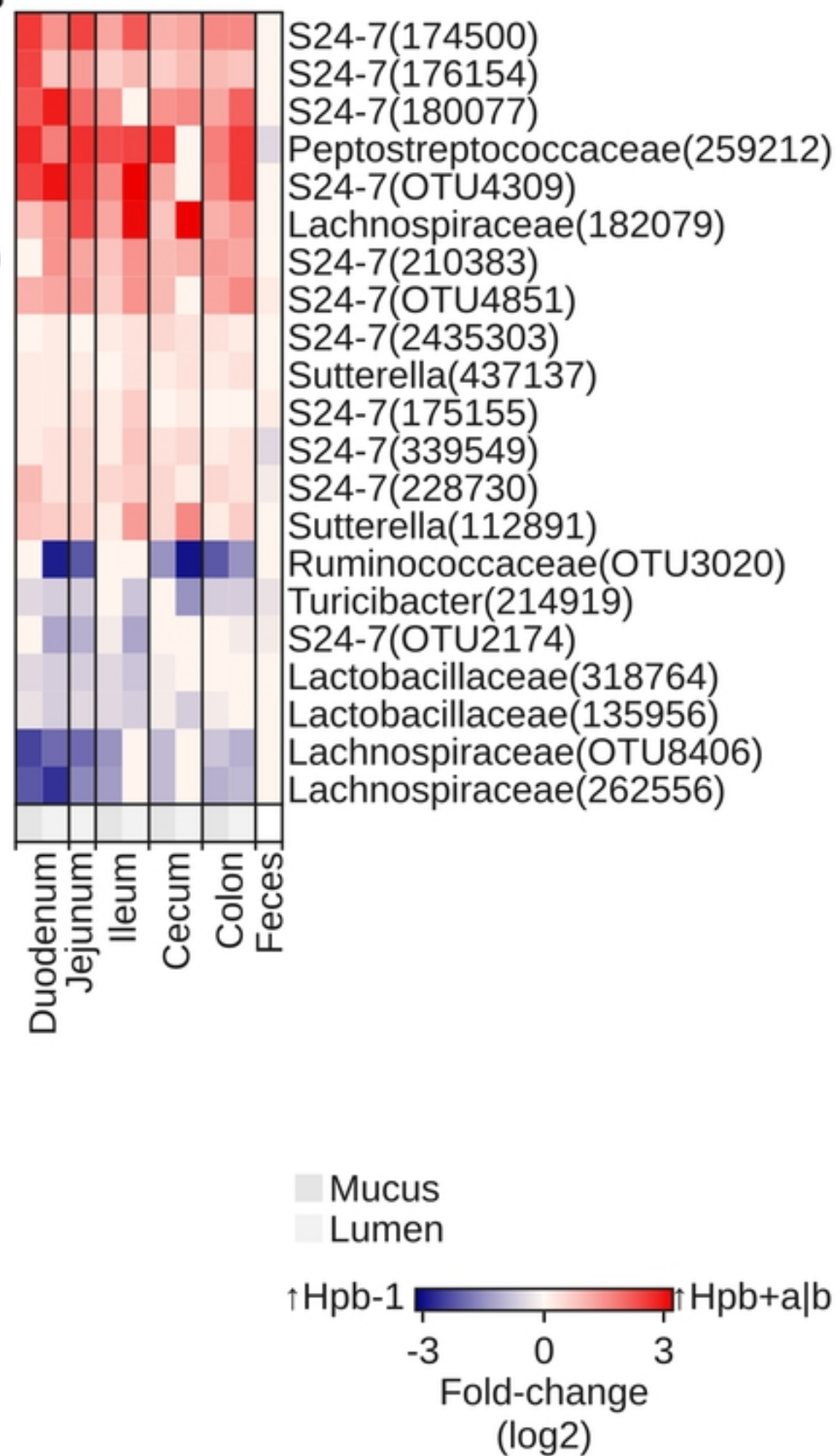
■ *Hpb+b*

■ *Hpb-1*





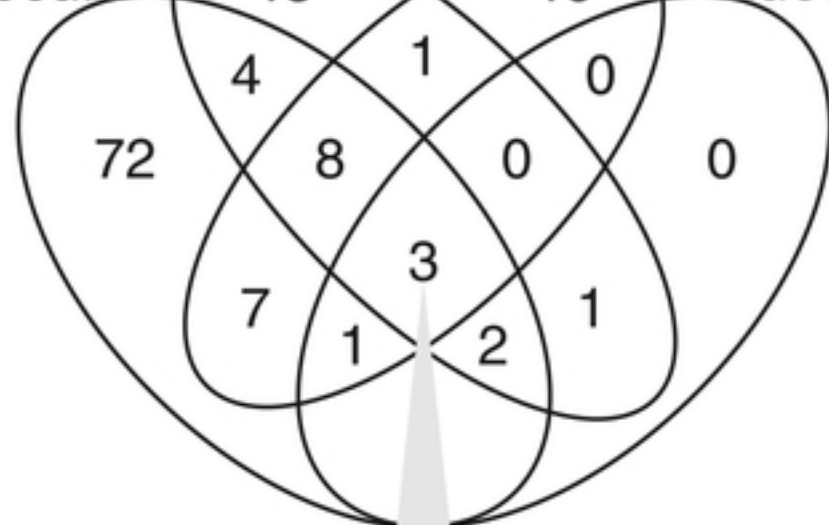


**A****B**

**A**

Ileum Cecum Colon Duodenum

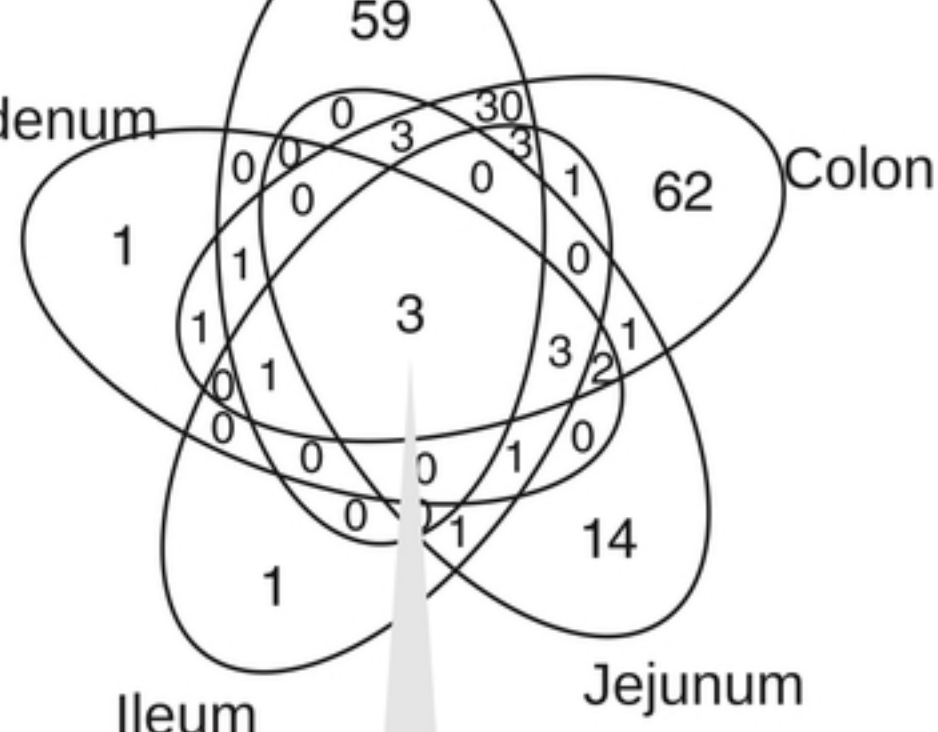
bioRxiv preprint doi: <https://doi.org/10.1101/575787>; this version posted March 12, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.



Peptostreptococcaceae(259212)  
Bifidobacterium(4426298)  
S24-7(173852)

**B**

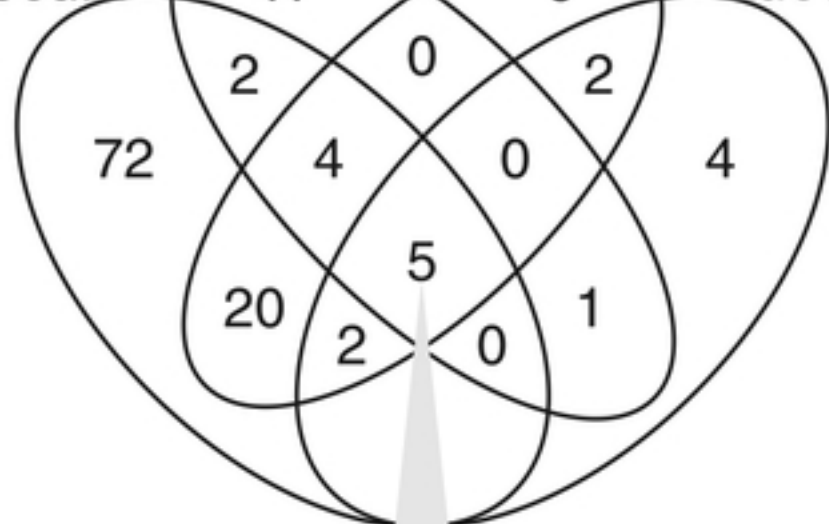
Cecum Duodenum Colon Ileum Jejunum



Bifidobacterium(4426298)  
Allobaculum(262653)  
S24-7(339549)

**C**

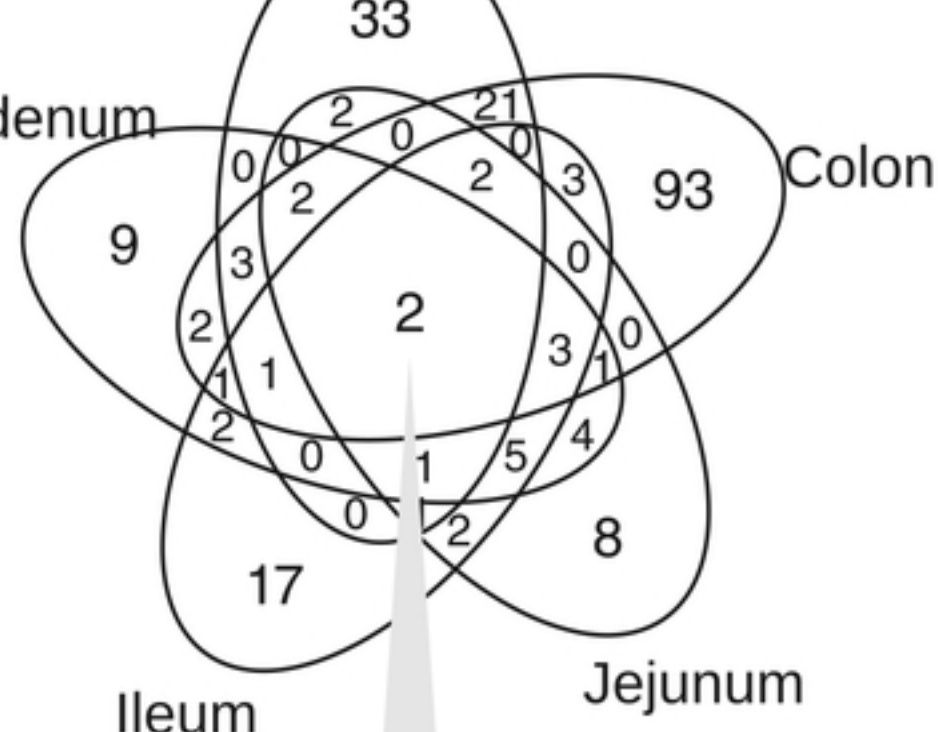
Ileum Cecum Colon Duodenum



Peptostreptococcaceae(259212)  
S24-7(174500)  
S24-7(176154)  
S24-7(OTU4309)  
S24-7(OTU4851)

**D**

Cecum Duodenum Colon Ileum Jejunum



Turicibacter(214919)  
S24-7(210383)

Cages  
(n=30)

Weeks

*Hpb*

Gut microbiome

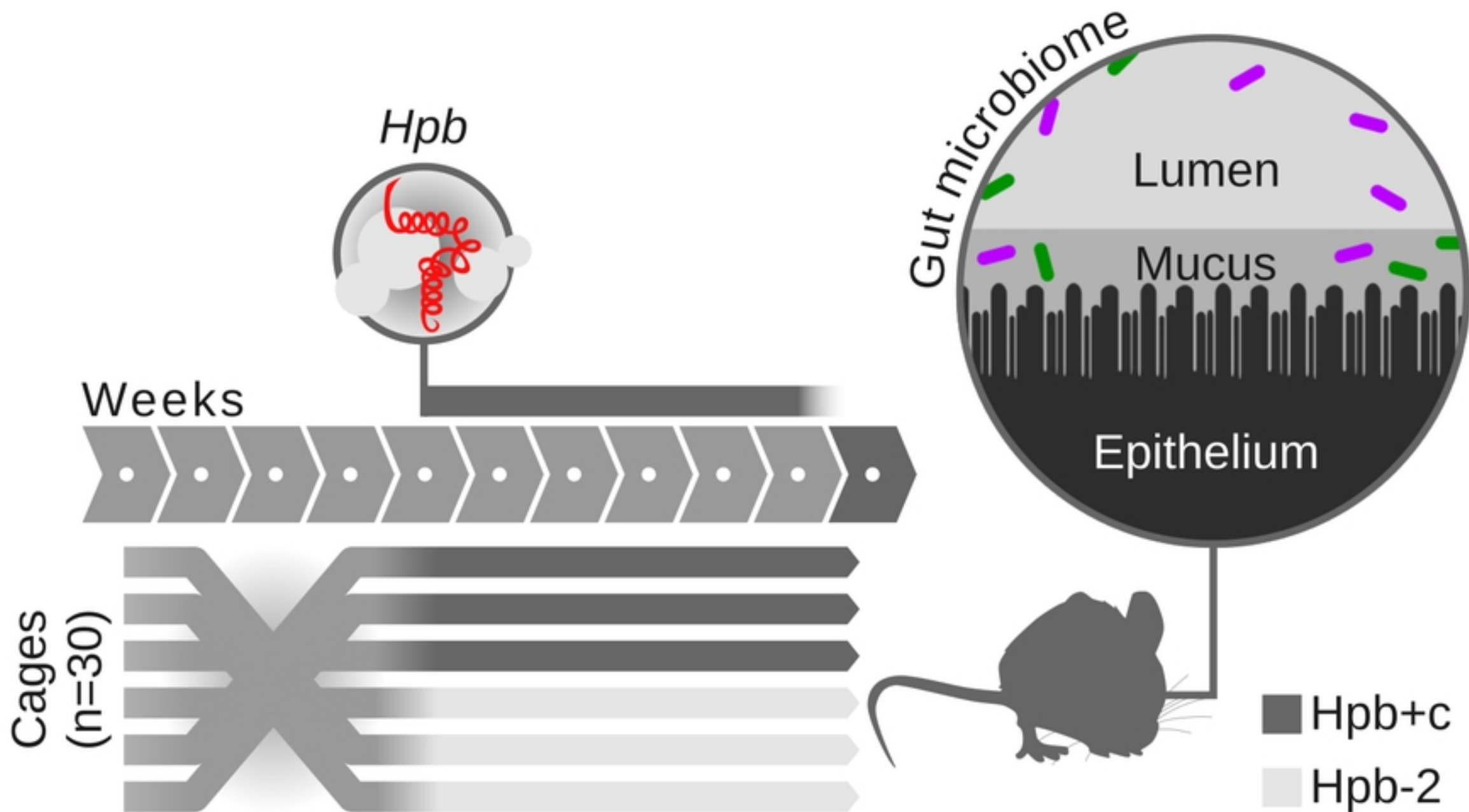
Lumen

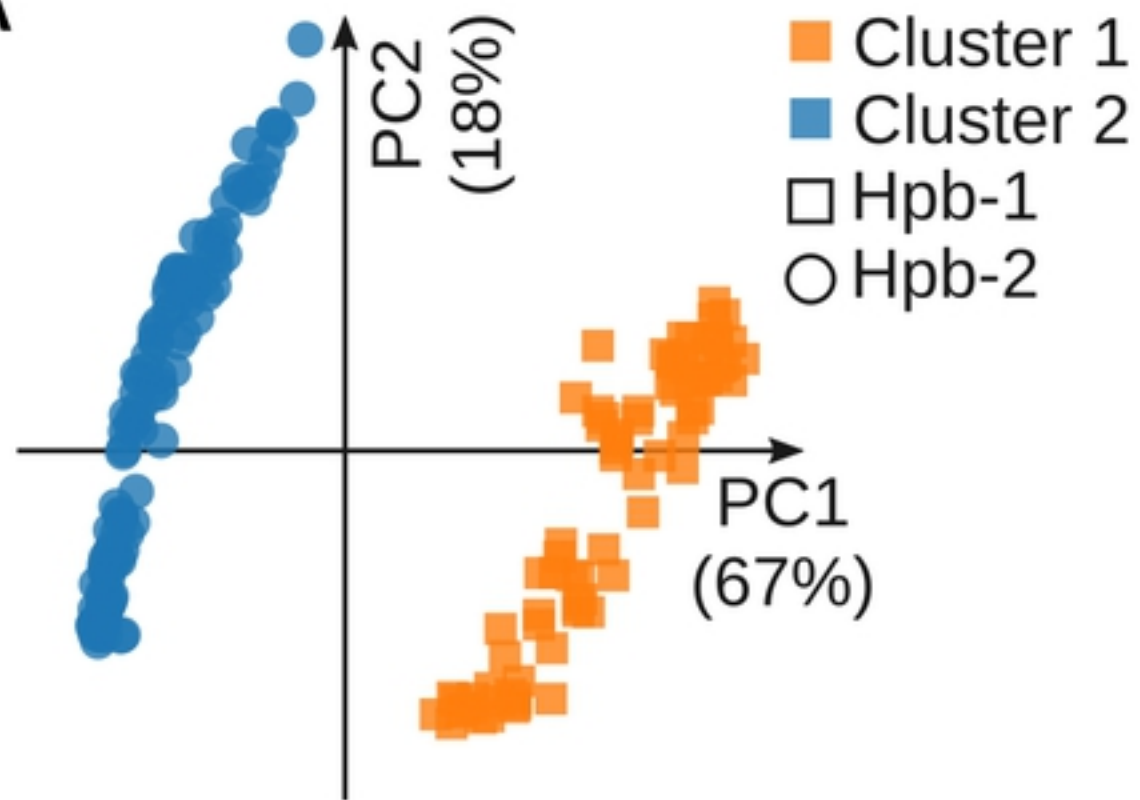
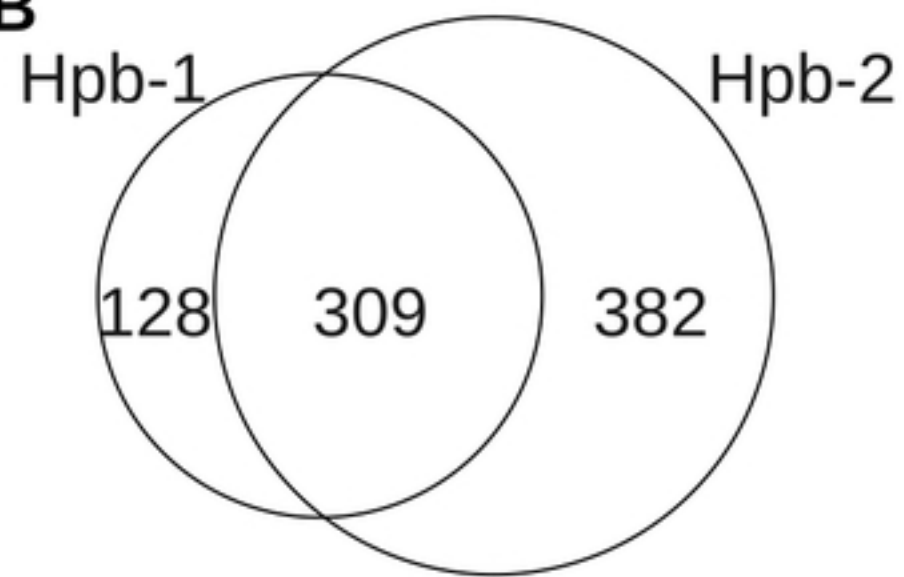
Mucus

Epithelium

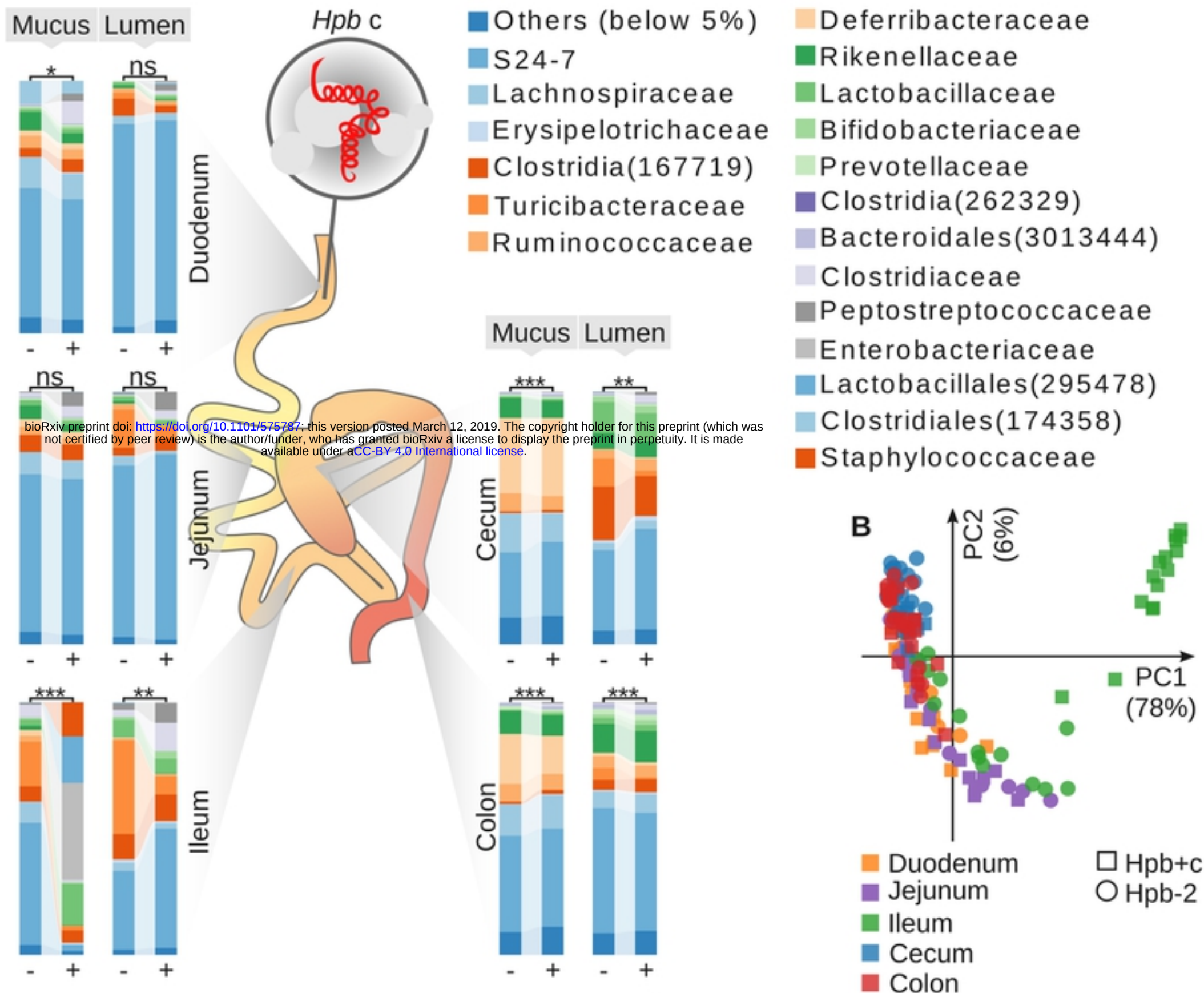
■ *Hpb+c*

■ *Hpb-2*

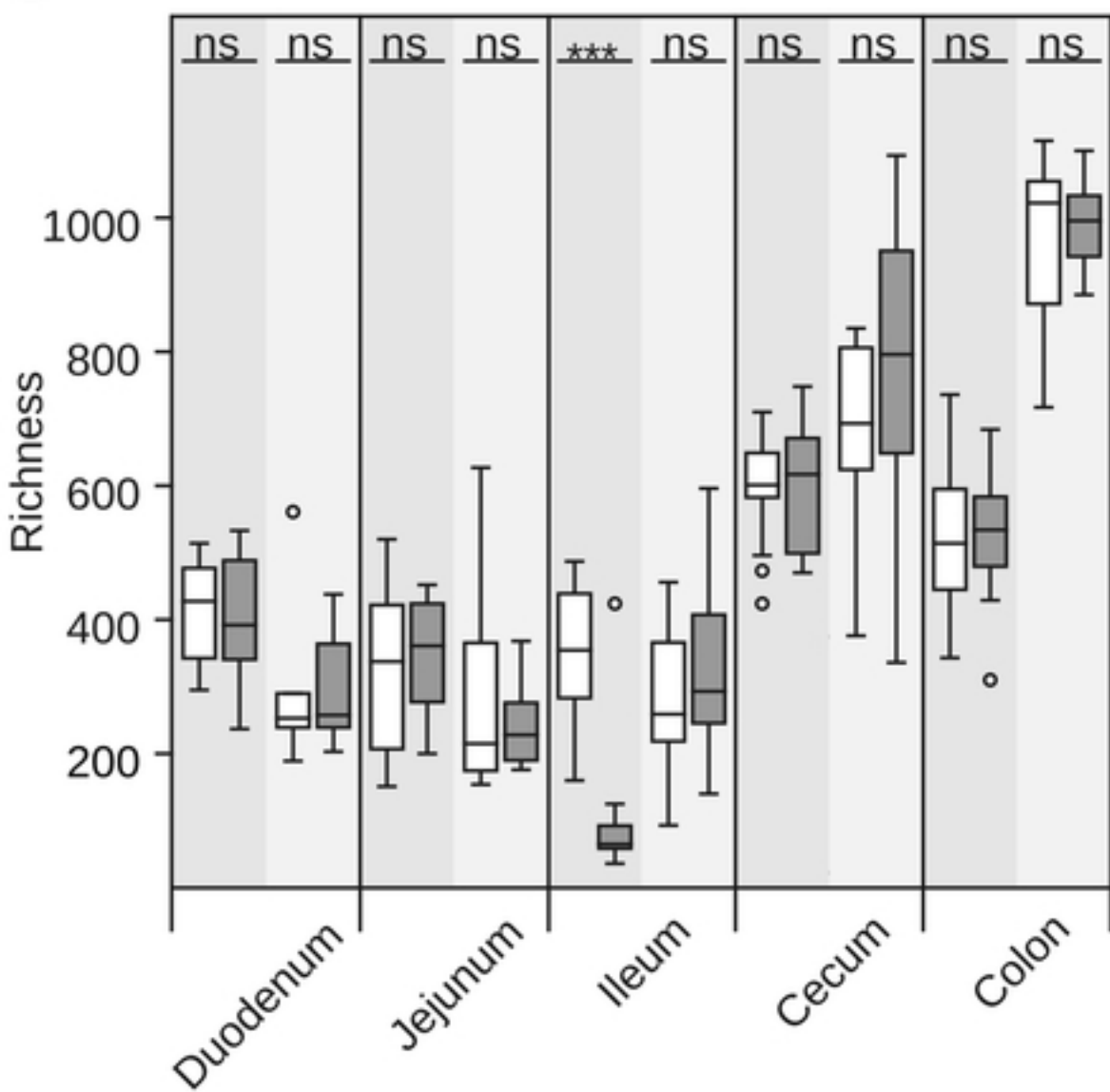


**A****B**

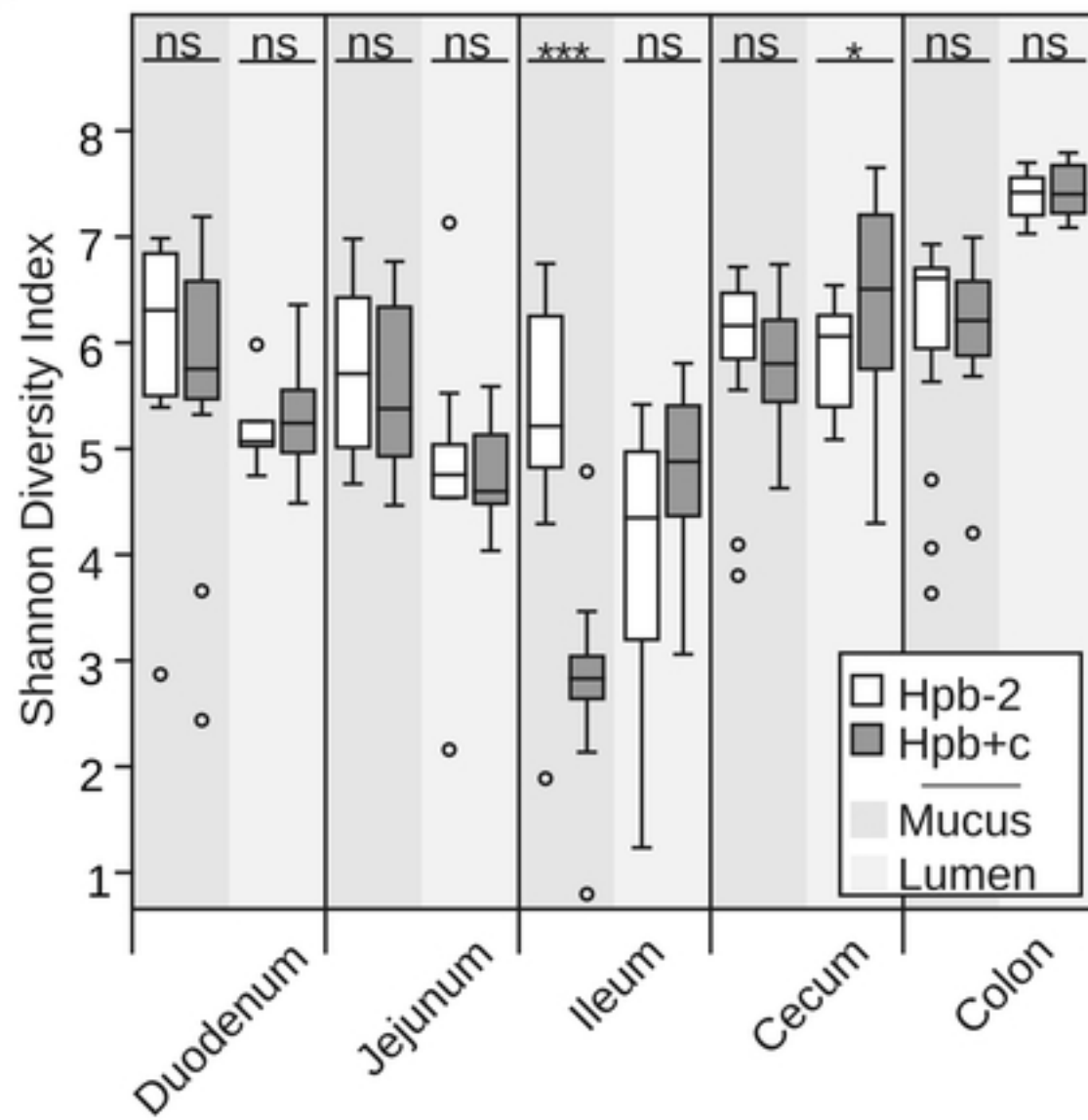
bioRxiv preprint doi: <https://doi.org/10.1101/575787>; this version posted March 12, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

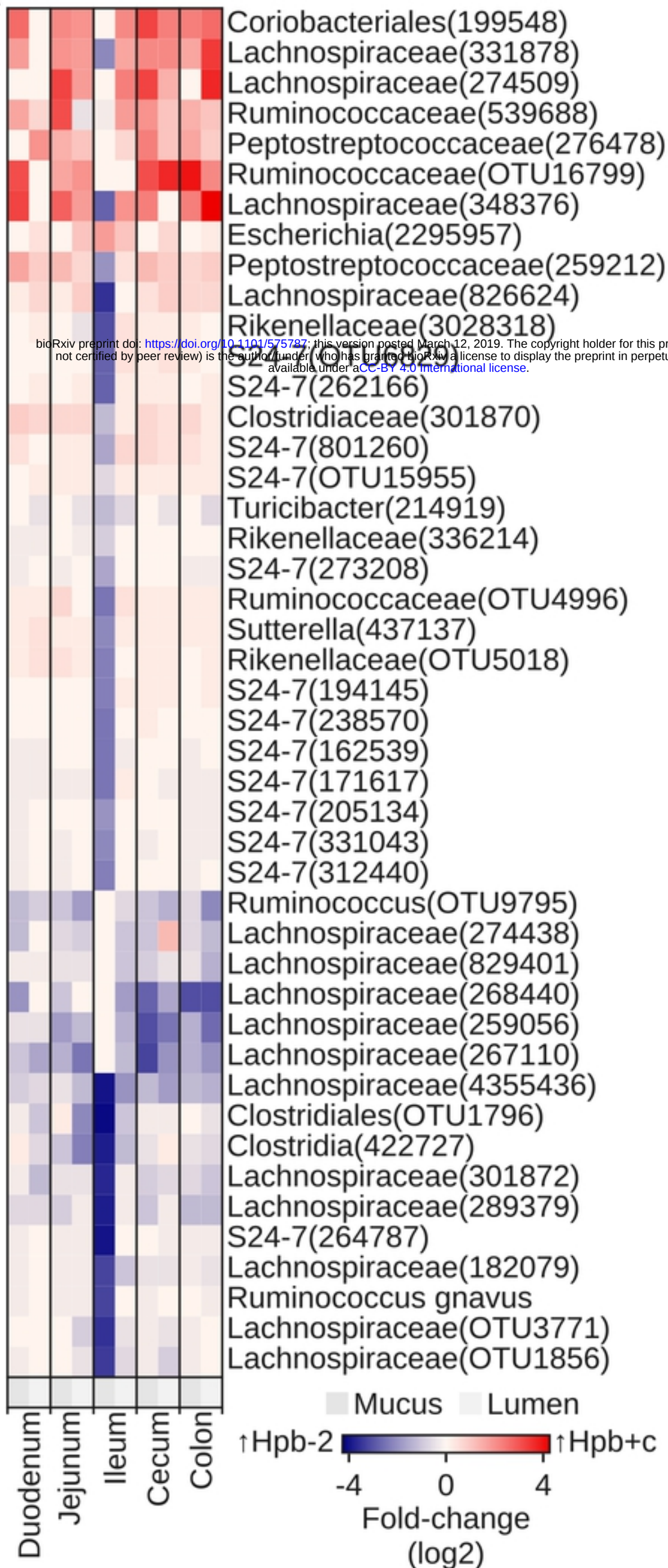
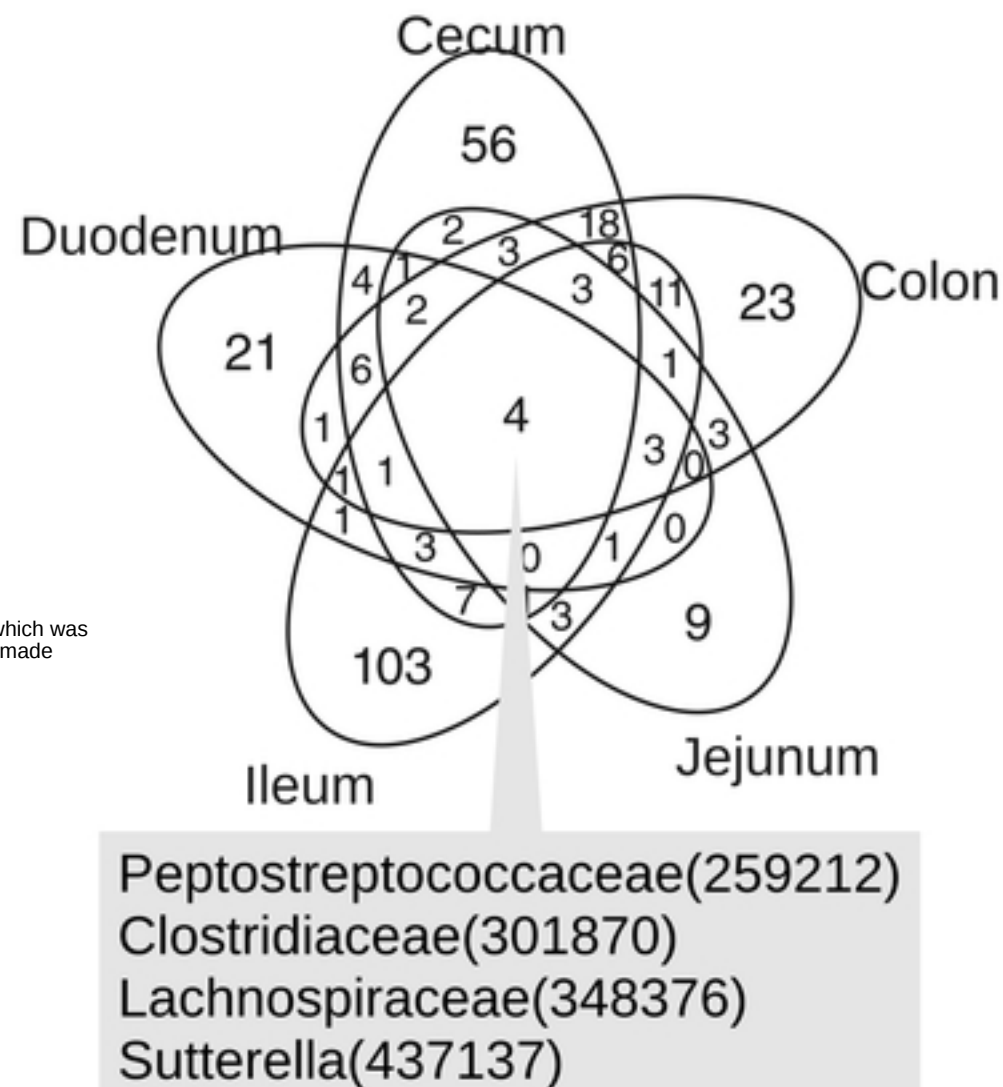
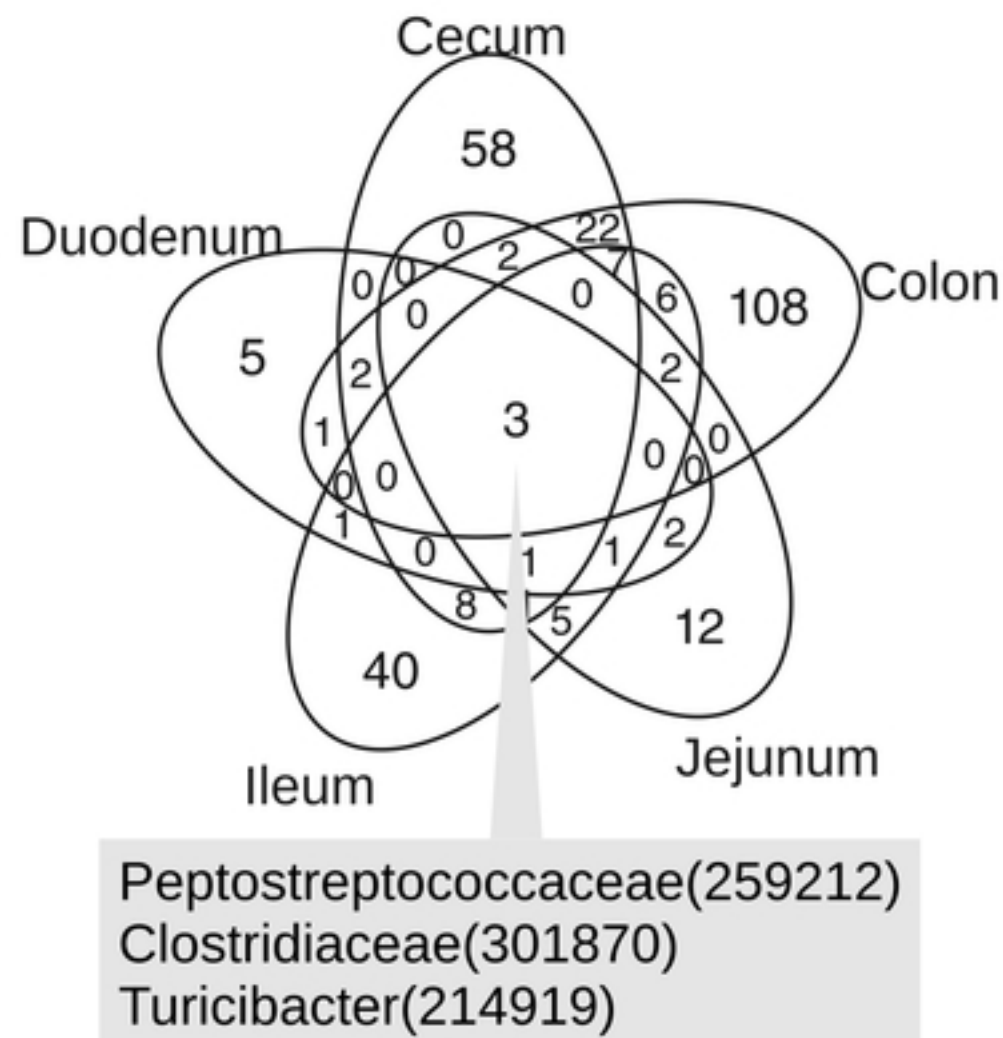


**C**



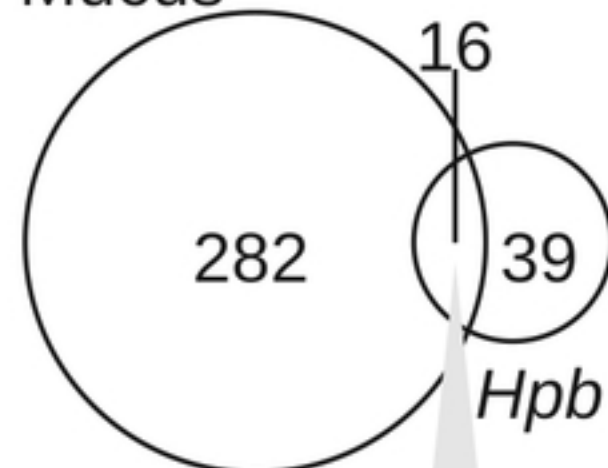
D



**A****B****C**

**A**

Mucus

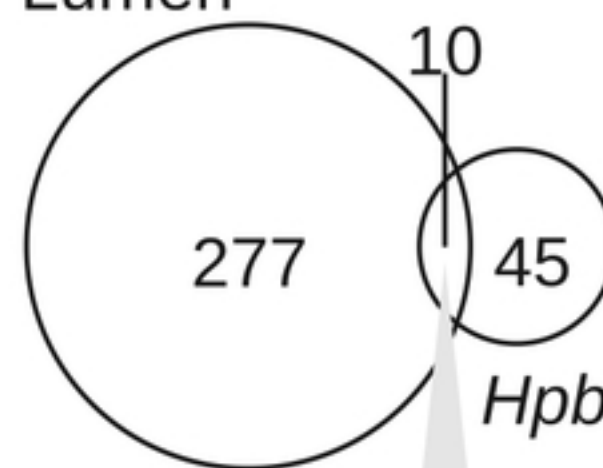


*Hpb*

Clostridiaceae(301870)  
 Turicibacter(214919)  
 Clostridia(167719)  
 Enterobacteriaceae(1141188)  
 Enterobacteriaceae(299267)  
 Enterobacteriaceae(4375000)  
 Enterobacteriaceae(4388820)  
 Escherichia(2295957)  
 S24-7(260927)  
 S24-7(262166)  
 S24-7(174791)  
 S24-7(205134)  
 Lactobacillaceae(135956)  
 Lactobacillales(295478)  
 Propionibacterium acnes  
 Bacteroides(228601)

**B**

Lumen



*Hpb*

Clostridiaceae(301870)  
 Turicibacter(214919)  
 Clostridia(167719)  
 Enterobacteriaceae(1141188)  
 Enterobacteriaceae(299267)  
 Enterobacteriaceae(4375000)  
 Enterobacteriaceae(4388820)  
 Escherichia(2295957)  
 S24-7(260927)  
 S24-7(262166)