

1 **Herpes Simplex Virus Infection, Acyclovir and IVIG Treatment All Independently Cause Gut**  
2 **Dysbiosis.**

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30

31 **Abstract.**

32

33 Herpes simplex virus 1 (HSV) is a ubiquitous human virus resident in a majority of the global  
34 population as a latent infection. Acyclovir (ACV), is the standard of care drug used to treat primary  
35 and recurrent infections, supplemented in some patients with intravenous immunoglobulin (IVIG)  
36 treatment to suppress deleterious inflammatory responses. We found that HSV, ACV and IVIG  
37 can all independently disrupt the gut bacterial community in a sex biased manner when given to  
38 uninfected mice. Treatment of HSV infected mice with ACV or IVIG alone or together revealed  
39 complex interactions between these drugs and infection that caused pronounced sex biased  
40 dysbiosis. ACV reduced *Bacteroidetes* levels in male but not female mice, while levels of the  
41 Anti-inflammatory Clostridia (AIC) were reduced in female but not male mice, which is significant  
42 as these taxa are associated with protection against the development of GVHD in hematopoietic  
43 stem cell transplant (HSCT) patients. Gut barrier dysfunction is associated with GVHD in HSCT  
44 patients and ACV also decreased *Akkermansia muciniphila*, which is important for maintaining  
45 gut barrier functionality. Cumulatively, our data suggest that long-term prophylactic ACV treatment  
46 of HSCT patients may contribute to GVHD and potentially impact immune reconstitution. These  
47 data have important implications for other clinical settings, including HSV eye disease and genital  
48 infections, where ACV is given long-term.

49

50 **Author Summary.**

51

52 Primary and reactivated HSV and VZV infections are treated with Acyclovir (ACV), an  
53 antiviral drug that blocks viral DNA synthesis. In some patients IVIG is used as adjunctive therapy  
54 to block deleterious inflammation. Long term preventative treatment of patients who receive stem  
55 transplants for various blood cancers has been successful in preventing life threatening  
56 reactivated HSV and VZV infections, but GVHD remains a major factor limiting transplant

57 success. Studies reported here reveal that HSV infection, ACV and IVIG given alone can all  
58 disrupt the gut microbiota and that complex interactions between these drugs and infection results  
59 in even more pronounced sex biased changes in the gut bacteria community structure.  
60 Importantly, ACV treatment decreased the levels of specific bacterial taxa, including the anti-  
61 inflammatory *Clostridia* and *Bacteroidetes* that have been shown to protect against development  
62 of GVHD in stem cell transplant patients. These data suggest that long term preventative  
63 treatment of patients with ACV may contribute to GVHD in transplant patients and have negative  
64 consequences in other HSV induced diseases treated long term with ACV. The health effects of  
65 long term ACV and IVIG treatments warrant further clinical studies.

66

## 67 **Introduction.**

68

69 Herpes Simplex Virus type 1 (HSV), a ubiquitous human virus is the major cause of HSV  
70 encephalitis (HSE), the most prevalent sporadic encephalitis resulting from either primary  
71 infection or reactivation of latent virus. However, despite improved diagnostic procedures and  
72 effective antiviral therapies, most HSE survivors have persistent neurological impairments,  
73 including memory and behavior disturbances, dysphasia and seizures, and only 50-65% of these  
74 survivors return to independent living [1, 2]. A delay in initiating Acyclovir (ACV) treatment past  
75 the second hospital day is associated with poor neurological outcomes [3, 4]. Recent clinical trials  
76 evaluating prolonged oral ACV/valaciclovir (VACV) treatment following standard 14-day  
77 intravenous ACV treatment reported improved neurocognitive outcomes in neonates but not  
78 adults for reasons that are obscure [5, 6]. Although, it is generally accepted that replication  
79 induced pathology underlies HSV related neurological dysfunction, supporting experimental or  
80 clinical evidence is lacking. Overwhelming evidence has linked inflammation to the development  
81 of various neurological disorders and neuropsychiatric diseases, including Alzheimer's disease

82 (AD), schizophrenia, autism spectrum disorder (ASD), multiple sclerosis (MS), Parkinson's  
83 disease (PD), depression and anxiety [7-9].

84

85 Having unequivocally established that HSE arises from exaggerated CNS inflammatory  
86 responses and that the immunomodulatory activities of intravenous immunoglobulins (IVIG) can  
87 prevent HSE in a mouse model [10], we tested the hypothesis that persistent inflammation, which  
88 is documented in humans and mice after HSE [11-14], causes neurobehavioral impairments in  
89 survivors, that should be impeded by IVIG's anti-inflammatory activity [10]. Compared to treatment  
90 of HSV infected mice with ACV or PBS alone, treatment with ACV+IVIG from day 4 pi reduced  
91 CNS inflammation and anxiety, consistent with our hypothesis. Strikingly, development of learning  
92 and memory (LM) deficits that were evident only in female PBS treated mice, were inhibited by  
93 ACV treatment and counterintuitively, aggravated by ACV+IVIG treatment. Treatment of infected  
94 male mice with ACV+IVIG also impaired LM compared to ACV or PBS alone, revealing that IVIG  
95 antagonized the beneficial effects of ACV [15]. Intriguingly, the differential antagonistic effects of  
96 ACV+IVIG on cognitive behavior in HSV infected mice, compared to ACV and PBS treatment  
97 alone, were reflected in differential serum proteomic profiles [15]. These reported antagonistic  
98 effects of ACV and IVIG on LM present a conundrum, since they are at odds with the known  
99 mechanisms of action of these drugs.

100

101 Rapidly accumulating evidence is revealing the critical role of the microbiome in regulating  
102 brain homeostasis and function such that perturbation of the gut bacteria community structure  
103 and function is increasingly being implicated in a variety of neurodegenerative and  
104 neuropsychiatric diseases. In an effort to gain insight into how HSV induces LM impairment and  
105 the paradoxical effects of ACV and IVIG, we investigated a role for the gut microbiota. HSV  
106 infection, ACV and IVIG were all associated with significant disruption of the gut bacterial  
107 community structure that was sex biased. Furthermore, treating HSV infected mice with either

108 ACV or IVIG alone or both drugs together resulted in more pronounced sex-biased shifts in the  
109 gut bacterial community structure compared to uninfected mice. These results have significant  
110 clinical implications, particularly when patients receive prolonged ACV or IVIG treatment.

111

## 112 **Results.**

113

114 Equal numbers (n=8) of female and male C57BL/6 mice were bilaterally inoculated with  
115 virulent HSV1 strain 17+ ( $1 \times 10^5$  PFU/eye) by corneal scarification as previously described [15].  
116 At day 4 post infection (pi), ACV was administered at 1.25 mg / mouse by intraperitoneal injection  
117 (ip) daily for 3 days, while IVIG was given as single dose of 25 mg/mouse by ip injection on day  
118 4pi [15]. Fresh fecal pellets (n=1-2/ mouse) were collected on day 7 pi and stored at  $-80^{\circ}\text{C}$  until  
119 processed for Illumina 16S rRNA gene sequencing to determine the effects of infection and drug  
120 treatment on the gut microbiome. Normal male and female mice differed in gut bacteria  
121 composition and unexpectedly, HSV ocular infection caused further shifts in the gut bacteria  
122 community and amplified this sex difference, as shown in a PCoA plot of Hellinger beta diversity  
123 distance values for infected compared to uninfected male and female mice (**Figure 1A**;  $P < 0.05$ ,  
124 Adonis Tests). In addition, HSV infection had a greater effect on gut bacterial communities in  
125 males ( $P = 0.003$ ) compared to females ( $P = 0.011$ ) (**Figure 1A**). Significant differences were  
126 observed at the phyla level, particularly for firmicutes (**Figure 1B**) with more marked differences  
127 evident at the species level for *Clostridium aerotolerans* and other clostridial species, for example  
128 *Clostridium XIVa* that ferment carbohydrates in the gut resulting in production of short chain fatty  
129 acids (SFCs) that contribute to barrier integrity and also exhibit anti-inflammatory properties  
130 (**Figure 1C**). A notable difference was also observed for *Akkermansia muciniphila* that has many  
131 health promoting activities, including maintaining gut barrier health (**Figure 1C**).

132

133 Treating HSV infected mice with ACV from day 4 pi for three days resulted in even more  
134 drastic shifts in the gut bacteria composition and exaggerated sex differences (**Figure 2A**), than  
135 for infection alone. Considerable abundance changes were evident at the Phyla level for  
136 *Bacteroidetes*, *Firmicutes* and *Verrucomicrobia* (**Figure 2B**) and at the species level (**Figure 2C**).  
137 Notably, whereas HSV infection reduced the abundance of *Firmicutes* significantly in male but  
138 not female mice (**Figure 1B**), ACV reversed this effect restoring the abundance to the level in  
139 uninfected male mice, while also increasing the abundance in female mice (**Figure 2B** and **Figure**  
140 **1B**). Notable abundance changes at the species level included drastic suppression of *Clostridium*  
141 *aerotolerans* in infected male mice compared to increased abundance in females (**Figure 1C**),  
142 while ACV treatment further increased this abundance only in females (**Figure 2C**). *Akkermansia*  
143 *muciniphila* abundance was increased by infection in male mice but reduced in females (**Figure**  
144 **1C**), while ACV treatment resulted in total suppression of this species in female mice compared  
145 to a marked reduction in male mice (**Figure 2C**). There are many other similar changes in species  
146 abundance that are differentially impacted by ACV treatment in a sex-biased manner, indicative  
147 of complex interactions between infection, ACV effects on infected host cells, and bacteria, as  
148 well as metabolites produced by bacterial metabolism of ACV.

149  
150 Treatment of uninfected mice with IVIG alone also shifted the gut bacteria community  
151 composition with a notable marked sex effect as determined by a beta diversity analysis (**Figure**  
152 **3**). Males and females showed a major reduction in *A. muciniphila*, and a lesser reduction of  
153 *Verrucomicrobia* in males, compared to females that showed increased abundance of this phylum  
154 in response to IVIG treatment (**Figure 4**). The abundance of many other bacterial species was  
155 differentially altered by IVIG treatment of males and females, for example, *Clostridium*  
156 *aerotolerans*, *Bacteroides acidifaciens* and *Porphyromonadaceae* (**Figure 4B**). The response to  
157 IVIG was distinct in HSV infected mice, and the complex interactions between infection, ACV and  
158 IVIG were also evident at the phyla and species levels and were strongly sex biased as well

159 **(Figure 4A and 4B)**. IVIG treatment decreased *A. muciniphila* abundance markedly in infected  
160 males and females as did ACV, whereas in contrast, treatment with ACV+IVIG caused a notable  
161 increase in its abundance, indicative of antagonistic effects of these two drugs in the context of  
162 infection **(Figure 4B)** In a similar vein, *C. aerotolerans* abundance increased markedly in males,  
163 but was unchanged in females treated with IVIG, while in contrast, it was strongly decreased in  
164 males but slightly increased in females treated with ACV alone. In contrast, treatment with  
165 ACV+IVIG suppressed an IVIG-induced increase in males and an ACV-induced increase in  
166 females, revealing antagonism between ACV and IVIG in the context of HSV infection **(Figure**  
167 **4B)**.

168  
169 Patients with hematologic and other malignancies have benefited immensely from  
170 allogeneic hematopoietic stem cell transplantation (allo-HSCT or HSCT), which can be a potent  
171 curative immunotherapy. However, life threatening complications such as graft-versus-host  
172 disease (GVHD), relapse, and infections that include reactivated HSV and VZV limit its application  
173 [16]. HSV and varicella zoster (VZV) reactivation has been successfully suppressed by  
174 prophylactic ACV treatment, though ACV-resistant (ACVr) HSV is an emerging problem [17, 18].  
175 Long term ACV prophylactic treatment is now routine for HSCT patients, because it was found to  
176 correlate with reduced HSV and ACVr HSV disease in those treated for longer than 1 year [19].

177  
178 Given this routine clinical practice, we evaluated the effects of ACV on fecal bacteria,  
179 because gut microbes have been implicated in GVHD pathophysiology and because we posit that  
180 ACV contributes to the development of GVHD by changing the gut microbiota. First, we identified  
181 gut bacterial changes in humans with GVHD [20-30]. Next, we determined whether the ACV-  
182 induced changes that we detected in this mouse study matched those GVHD-associated  
183 changes. Whenever we identified taxa that were altered in both types of studies, the direction of  
184 the change was the same, and it was consistent with our hypothesis that ACV contributes to the

185 development of human GVHD by changing the gut microbiota. In the following, we describe these  
186 results, and we note that these ACV-induced changes were only observed in the HSV-infected  
187 mice and not in the uninfected mice.

188

189 Reduced levels of several taxa belonging to the phylum *Bacteroidetes* have been shown  
190 to be associated with GVHD, indicating that these gut bacteria may play a protective role. In a  
191 pediatric study, GVHD patients had lower levels of the family *Bacteroidaceae* and the genus  
192 *Parabacteroides* [30]. In a longitudinal study, pediatric patients that had lower levels of  
193 *Bacteroidetes* prior to HSCT were more likely to develop GVHD [24]. In our study, all three of these  
194 taxa were reduced by ACV treatment in male but not female mice (**Figure 5A**).

195

196 Reduced levels of Anti-Inflammatory Clostridia (AIC) have also been detected in human  
197 GVHD patients [20, 23-25, 27-30], indicating that these gut bacteria may play a protective role.  
198 This terminology was first introduced by Piper et al. [31] in the context of short bowel syndrome,  
199 and then introduced to the GVHD literature by Simms-Waldrip et al. [30]. AIC taxa include  
200 members of the families *Clostridiaceae*, *Erysipelotrichaceae*, *Eubacteriaceae*, *Lachnospiraceae*  
201 and *Ruminococcaceae*. In a pediatric study, decreases in *Blautia* and *Clostridium bolteae* were  
202 associated with the development of GVHD [30]. In an adult study, lower levels of *Blautia*, *Blautia*  
203 *hansenii*, and *Blautia stercoris* were associated with the development of GVHD [28]. In a  
204 longitudinal study, reduced levels of the *Blautia* before HSCT was shown to be a predictive marker  
205 for the development of GVHD [27]. In our study, all of these taxa were reduced by ACV treatment  
206 in female but not male mice (**Figure 5B**).

207

208 In a more detailed analysis of AIC bacteria, we observed that while HSV infection  
209 increased the abundance of *Blautia hansenii* only in males, ACV treatment reduced its abundance  
210 in females but had no effect on its abundance in males (**Supplemental Figure 1**). Remarkably,



211 a dramatic increase in *B. hansenii* in uninfected females was observed after IVIG treatment, and  
212 this increase was abrogated by ACV (compare NoHSV\_F, NoHSV\_IVIG\_F and  
213 NoHSV\_ACVplusIVIG\_F) (**Supplemental Figure 1**), a result that supports sex-based differential  
214 effects of these drugs. However, during HSV infection, both IVIG and ACV reduced *B. hansenii*  
215 in females, whereas only IVIG reduced abundance in males. Interestingly, HSV infection  
216 significantly increased the abundance of the AIC genera *Blautia*, *Allobaculum*, and *Clostridium*  
217 XVIII but not *Turicibacter* in both males and females (**Supplemental Figure 2**). ACV treatment of  
218 HSV infected female mice resulted in significant decreases in the abundances of 4 AIC genera:  
219 *Blautia*, *Allobaculum*, *Clostridium* XVIII and *Turicibacter*, whereas in infected males, ACV  
220 decreased the abundance of *Marvinbryantia* and *Oscillibacter* (**Supplemental Figure 2**). In  
221 addition, ACV increased the abundance of *Turicibacter* in uninfected females but not males.

222

223 Finally, the two most abundant operational taxonomic units (OTUs), which exhibited a  
224 change in their relative abundances due to ACV treatment, were assigned to the family  
225 *Porphyromonadaceae* and the species *A. muciniphila* (**Figure 5C**). While we did not find these  
226 taxa associated with GVHD in prior human studies, GVHD has been associated with intestinal  
227 barrier dysfunction [32-36]. Supporting our hypothesis that ACV contributes to the development  
228 of GVHD by changing the gut microbiota, members of the *Porphyromonadaceae* have been  
229 shown to cause gut barrier dysfunction [37, 38], and our *Porphyromonadaceae* OTU was  
230 increased in its abundance by ACV. In addition, *A. muciniphila* was decreased by ACV treatment  
231 in our study, and it has been shown to strengthen gut barrier functioning [39-41].

232

## 233 **Discussion.**

234

235 Our intention in this brief report is to alert the scientific community and especially clinicians  
236 to the fact that HSV infection, the antiviral drug ACV, and the immunomodulatory biological, IVIG,

237 can all independently result in significant perturbations of the gut bacterial communities. Our data  
238 reveal complex interactions between HSV infection and ACV or/and IVIG treatment that result in  
239 marked alterations to gut bacterial communities. Although the clinical consequences of these  
240 changes have not yet been elucidated, they could have profound implications in several settings  
241 including HSCT-associated GVHD.

242

243         Though the mechanisms by which ocular HSV infection causes gut dysbiosis are unclear,  
244 neuroinflammatory mechanisms and effects on the enteric nervous system via connected  
245 brainstem neuronal circuits can be envisaged [15, 42]. Indeed, recent paradigm-shifting reports  
246 reveal that peripheral neurons, including nociceptive and sensory neurons, can directly sense and  
247 respond to environmental alarms by releasing neuropeptides that can regulate immune responses  
248 in target organs including the gut [43, 44]. Persistence of gut dysbiosis was not evaluated here,  
249 but results from a behavioral study alluded to earlier suggest long-term effects of infection and  
250 drug treatment on gut bacterial ecology should be investigated [15]. Sex biased effects on HSV  
251 induced dysbiosis merit further study, as these may involve microglial responses to HSV infection  
252 and the microglial compartment is known to be regulated by the microbiota in a sex biased manner  
253 [45-47].

254

255         The mechanism by which ACV, the standard antiviral for HSV infections, changes the gut  
256 microbiota likely involves its uptake into bacteria. ACV is preferentially phosphorylated by the viral  
257 encoded thymidine kinase (Tk) resulting in cell retention and eventual incorporation into viral DNA  
258 resulting in inhibition of viral replication via DNA chain termination. Because Tk is conserved in  
259 numerous bacterial species, ACV can be taken up and incorporated into DNA, resulting in  
260 bactericidal effects [48-51]. Indeed, early studies on DNA replication mechanisms relied on  
261 labeling bacterial DNA with tritiated thymidine and many bacterial taxa can be imaged using  
262 nucleoside analogues such as 1-(2\_-deoxy-2\_-fluoro-\_-D-arabinofuranosyl)-5-[125I] iodouracil

263 ([125I]FIAU) that are substrates for HSV Tk [52-55]. Incorporation of [*methyl*-<sup>3</sup>H]thymidine into  
264 DNA has been unequivocally demonstrated for members of the *Clostridium* genus [56] and our  
265 data show ACV reduced the abundance of the *Blautia* genus (order *Clostridiales*; [57]) *Blautia*  
266 *hansenii*, *Blautia stercoris*, and *Clostridium bolteae* in females but not males. Additionally,  
267 interrogating the NCBI reference genome sequence for *Blautia hansenii* confirmed the presence  
268 of a thymidine kinase enzyme. Our data are therefore consistent with ACV causing dysbiosis by,  
269 at least in part, inhibiting the growth of various bacteria taxa via the Tk mechanism, though other  
270 mechanisms involving bacterial metabolism of ACV cannot be excluded. Clearly, the mechanisms  
271 by which ACV affects gut bacterial ecology are complex, which is further supported by the sex-  
272 biased effects.

273

274         We also explored the effects of IVIG treatment alone and in combination with ACV in HSV-  
275 infected and uninfected mice, because IVIG has been used to treat HSV encephalitis (HSE) and  
276 is also a frontline therapy for autoimmune encephalitis, which is triggered by HSE and other insults  
277 [58-60]. Moreover, IVIG is being evaluated in a randomized control trial for children with all-cause  
278 encephalitis to determine whether neurological outcomes are improved compared to standard  
279 antiviral therapy alone, which is similar to our behavioral study that generated paradoxical results  
280 [15, 61]. Reports that IVIG's antigenic repertoire includes reactivities to a variety of gut commensal  
281 antigens and metabolites have increased recently [62-64], which is consistent with a report that  
282 gut commensals can somehow trigger systemic IgG responses under homeostatic conditions that  
283 protect against systemic infection [65, 66]. We speculate that by neutralizing bacterial/host  
284 antigens/metabolites, IVIG is able to influence host immunity, the nervous system, and other  
285 physiological processes, resulting in perturbation of gut bacteria ecology. We speculate that the  
286 disparate and complex effects of ACV and IVIG alone and in combination on the gut bacteria  
287 ecology likely account for their antagonistic effects on cognitive behavior in mice latently infected  
288 with HSV that we alluded to earlier [15].

289

290           This study has several limitations. Being exploratory in nature, analyses of the gut bacteria  
291 were done at a single time point immediately after infection or drug treatment, rather than as a  
292 longitudinal study that would have provided information on the persistence of the dysbiotic state  
293 as well as mechanistic insights as to how HSV, ACV and IVIG provoke dysbiosis. Ideally, the  
294 effects of ACV should be tested in latently infected mice, since virtually all HSCT patients harbor  
295 latent HSV. However, because HSV infection alone disrupts the gut bacterial community,  
296 assessing the effects of ACV on the gut bacteria community structure in the latently infected mice  
297 would likely be difficult. Because ACV was given ip to mice but usually orally to HSCT patients  
298 [67], its effects on the gut bacteria community maybe underestimated in our study.

299

300           Notwithstanding these caveats, our finding that ACV treatment of HSV infected mice  
301 decreased the relative abundances of several bacterial taxa is important because these bacteria  
302 have been negatively correlated with the induction of and mortality from GVHD in HSCT patients  
303 [24, 27, 28, 30]. These results are also consistent with our hypothesis that ACV contributes to the  
304 development of GVHD by changing the gut microbiota. In the context of allo-HSCT, GVHD occurs  
305 when donor immune cells recognize recipient tissues as foreign, leading to immune-mediated  
306 damage to several organs and tissues including the gastrointestinal tract. This has led  
307 researchers to posit that the reduction of anti-inflammatory bacteria such as AIC contribute to  
308 GVHD pathology [30]. The results from our study extend this hypothesis to include ACV treatment  
309 as a putative contributor to GVHD, because ACV reduced AIC bacteria in the gut. ACV treatment  
310 also decreased the relative abundances of several members of the *Bacteroidetes*, some of which  
311 have been shown to exhibit anti-inflammatory properties [68-71]. More relevantly, the capsular  
312 polysaccharide A (PSA) from *Bacteroides fragilis* reduced HSV-associated mortality in mice by  
313 dramatically reducing immune-mediated inflammation [72]. In addition, the two most abundant  
314 OTUs identified in our study, whose relative abundances were positively (*Porphyromonadaceae*)

315 and negatively (*A. muciniphila*) correlated with ACV treatment, have been shown to weaken [37,  
316 38] and strengthen [39-41] gut barrier function, respectively. These results provide an additional  
317 link between ACV treatment and GVHD, because barrier dysfunction, which can cause systemic  
318 inflammation, is a hallmark of GVHD [32-36]. Finally, long-term ACV prophylaxis initiated early  
319 after HSCT might also impair immune reconstitution based on results from a study of antibiotic  
320 depletion of gut bacteria in a murine model of syngeneic bone marrow transplantation [73]. These  
321 tantalizing results warrant independent validation and further detailed studies using a murine  
322 autologous BMT model to more rigorously evaluate the impact of long-term ACV prophylaxis on  
323 GVHD and engraftment, because results from such studies might eventually lead to improved  
324 outcomes for HSCT patients. Ideally, such future studies should be performed with mice harboring  
325 wild microbiota, because several recent reports show that immune responses in mice with wild  
326 microbiomes model human immune responses more closely than conventional mice with SPF  
327 microbiota [74-76].

328

## 329 **Materials and Methods.**

330

### 331 **Ethics Statement**

332 All animal procedures were performed with prior approval of the City of Hope Institutional Animal  
333 Care and Use Committee (IACUC) under protocol # 07043 and within the framework of the Guide  
334 for the Care and Use of Laboratory Animals. C57BL6/J (B6) were bred in the vivarium at City of  
335 Hope.

336

### 337 **Mouse Studies**

338

339 Master stocks of HSV1 strain 17 composed of only of cell-released virus were prepared in  
340 and their titers determined on mycoplasma-free CV-1 cell monolayers. Single use aliquots of virus

341 in Hanks balanced salt solution supplemented with 2% fetal bovine serum were stored at -80°C.  
342 Male and female mice, 6–8 weeks of age, were infected with HSV1 17<sup>+</sup>, a virulent strain. Mice  
343 were sedated with ketamine (60 mg/kg) and xylazine (5 mg/kg) prior to HSV inoculation by corneal  
344 scarification. B6 mice were bilaterally inoculated with 1x 10<sup>5</sup> PFU per eye and monitored daily as  
345 previously described [15, 77].

346

#### 347 **Administration of Acyclovir and Intravenous Immunoglobulins.**

348

349 ACV obtained from (APP Pharmaceuticals, Schaumburg, IL) was given at 50 mg/kg of  
350 body weight by intraperitoneal (ip) injection daily for 3 days starting on day 4 pi and PBS was  
351 given according to the same schedule to control mice. IVIG (Carimune, NF) obtained from CSL  
352 Behring (King of Prussia, PA, USA) was given ip as a single 0.5 ml dose (25 mg/mouse) on day  
353 4 pi or it was given in combination with a 3 day course of ACV.

354

#### 355 **Illumina Bacterial 16S rRNA gene sequencing.**

356

357 Illumina bacterial 16S rRNA gene libraries were constructed as follows. PCRs were  
358 performed in an MJ Research PTC-200 thermal cycler (Bio-Rad Inc., Hercules, CA, USA) as 25  
359 µl reactions containing: 50 mM Tris (pH 8.3), 500 µg/ml bovine serum albumin (BSA), 2.5 mM  
360 MgCl<sub>2</sub>, 250 µM of each deoxynucleotide triphosphate (dNTP), 400 nM of the forward PCR primer,  
361 200 nM of each reverse PCR primer, 1 µl of DNA template, and 0.25 units JumpStart Taq DNA  
362 polymerase (Sigma-Aldrich, St. Louis, MO, USA). PCR primers 515F  
363 (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) were used to  
364 targeted the 16S rRNA gene containing portions of the hypervariable regions V4 and V5, with the  
365 reverse primers including a 12-bp barcode [78]. Thermal cycling parameters were 94°C for 5 min;  
366 35 cycles of 94°C for 20 s, 50°C for 20 s, and 72°C for 30 s, and followed by 72°C for 5 min. PCR

367 products were purified using the MinElute 96 UF PCR Purification Kit (Qiagen, Valencia, CA,  
368 USA).

369

### 370 **16S rRNA gene data processing.**

371

372 We used the UPARSE pipeline for de-multiplexing, length trimming, quality filtering and  
373 operational taxonomic units (OTU) picking using default parameters or recommended guidelines  
374 that were initially described in [79] and which have been updated at  
375 [https://www.drive5.com/usearch/manual/uparse\\_pipeline.html](https://www.drive5.com/usearch/manual/uparse_pipeline.html). Briefly, after demultiplexing,  
376 sequences were trimmed to a uniform length of 249 bp, then filtered at the recommended 1.0  
377 expected error threshold. Sequences were then dereplicated and clustered into zero-radius OTUs  
378 using the UNOISE3 algorithm [80], which also detects and removes chimeric sequences; this  
379 method is based on making OTUs at 100% identity. An OTU table was then generated using the  
380 otutab command. OTUs having non-bacterial DNA were identified by performing a local BLAST  
381 search [81] of their seed sequences against the nt database. OTUs were removed if any of their  
382 highest-scoring BLAST hits contained taxonomic IDs within Rodentia, Viridiplantae, Fungi, or  
383 PhiX. Taxonomic assignments to the OTUs were performed with SINTAX [82] using RDP  
384 Classifier 16S training set number 16 [83] as the reference database.

385

### 386 **16S rRNA gene data analyses.**

387 Beta diversity was measured using QIIME 1.9.1 [84] to calculate a Hellinger beta diversity  
388 distance matrix, which was depicted using principle coordinates analysis (PCoA), and statistically  
389 assessed by performing Adonis tests. Statistical differences among the taxa were determined  
390 using edgeR [85, 86]. Taxa relative abundance figures were made using Prism (GraphPad, La  
391 Jolla, CA). Comparative analyses of the bacterial taxa between human GVHD studies and our  
392 mouse study excluded sequence-selective qPCR, because the selectivity of such assays is

393 questionable given the conserved nature of the 16S rRNA gene, and because the results of such  
394 studies are not typically validated by sequence analyses. The bacterial sequences have been  
395 deposited in the National Center for Biotechnology Information (NCBI)'s Sequence Read Archive  
396 (SRA) under the BioProject Accession Number PRJNA549765.

397

398



399 **References.**

400

- 401 1. McGrath N, Anderson NE, Croxson MC, Powell KF. Herpes simplex encephalitis treated  
402 with acyclovir: diagnosis and long term outcome. *Journal of Neurology, Neurosurgery and*  
403 *Psychiatry.* 1997;63(3):321-6. doi: 10.1136/jnnp.63.3.321.
- 404 2. Raschilas F, Wolff M, Delatour Fdr, Chaffaut C, De Broucker T, Chevret S, et al. Outcome  
405 of and Prognostic Factors for Herpes Simplex Encephalitis in Adult Patients: Results of a  
406 Multicenter Study. *Clin Infect Dis.* 2002;35(3):254-60. doi: 10.1086/341405.
- 407 3. Kennedy PGE, Steiner I. Recent issues in herpes simplex encephalitis. *Journal of*  
408 *NeuroVirology.* 2013;19(4):346-50. doi: 10.1007/s13365-013-0178-6.
- 409 4. Bradshaw MJ, Venkatesan A. Herpes Simplex Virus-1 Encephalitis in Adults:  
410 Pathophysiology, Diagnosis, and Management. *Neurotherapeutics.* 2016;13(3):493-508. doi:  
411 10.1007/s13311-016-0433-7.
- 412 5. Gnann JW, Sköldenberg B, Hart J, Aurelius E, Schliamser S, Studahl M, et al. Herpes  
413 Simplex Encephalitis: Lack of Clinical Benefit of Long-term Valacyclovir Therapy. *Clin Infect Dis.*  
414 2015;61(5):683-91. doi: 10.1093/cid/civ369.
- 415 6. Kimberlin DW, Whitley RJ, Wan W, Powell DA, Storch G, Ahmed A, et al. Oral Acyclovir  
416 Suppression and Neurodevelopment after Neonatal Herpes. *New England Journal of Medicine.*  
417 2011;365(14):1284-92. doi: doi:10.1056/NEJMoa1003509.
- 418 7. Chiara G, Marcocci M, Sgarbanti R, Civitelli L, Ripoli C, Piacentini R, et al. Infectious  
419 Agents and Neurodegeneration. *Molecular Neurobiology.* 2012;46(3):614-38. doi:  
420 10.1007/s12035-012-8320-7.
- 421 8. Hope S, Hoseth E, Dieset I, Mørch RH, Aas M, Aukrust P, et al. Inflammatory markers are  
422 associated with general cognitive abilities in schizophrenia and bipolar disorder patients and  
423 healthy controls. *Schizophrenia Research.* 2015;165(2-3):188-94. doi:  
424 <http://dx.doi.org/10.1016/j.schres.20151654.04.004>.
- 425 9. Kuntz T, Gilbert J. Does the brain listen to the gut? *eLife.* 2016;5:e17052. doi:  
426 10.7554/eLife.17052.
- 427 10. Ramakrishna C, Newo ANS, Shen Y-W, Cantin E. Passively Administered Pooled Human  
428 Immunoglobulins Exert IL-10 Dependent Anti-Inflammatory Effects that Protect against Fatal HSV  
429 Encephalitis. *PLoS Pathog.* 2011;7(6):e1002071. doi: 10.1371/journal.ppat.1002071.
- 430 11. Cantin EM, Hinton DR, Chen J, Openshaw H. Gamma interferon expression during acute  
431 and latent nervous system infection by herpes simplex virus type 1. *J Virol.* 1995;69(8):4898-905.
- 432 12. Liu T, Tang Q, Hendricks RL. Inflammatory infiltration of the trigeminal ganglion after  
433 herpes simplex virus type 1 corneal infection. *J Virol.* 1996;70(1):264-71.
- 434 13. Khanna KM, Lepisto AJ, Decman V, Hendricks RL. Immune control of herpes simplex  
435 virus during latency. *Current Opinion in Immunology.* 2004;16(4):463-9.
- 436 14. Aurelius E, Forsgren M, Skoldenberg B, Strannegard O. Persistent intrathecal immune  
437 activation in patients with herpes simplex encephalitis. *J Infect Dis.* 1993;168(5):1248-52.
- 438 15. Ramakrishna C, Golub MS, Chiang A, Hong T, Kalkum M, Cantin EM. Effects of Acyclovir  
439 and IVIG on Behavioral Outcomes after HSV1 CNS Infection. *Behavioural Neurology.*  
440 2017;2017:14. doi: 10.1155/2017/5238402.
- 441 16. Shono Y, van den Brink MRM. Gut microbiota injury in allogeneic haematopoietic stem  
442 cell transplantation. *Nature Reviews Cancer.* 2018. doi: 10.1038/nrc.2018.10.
- 443 17. Frobert E, Burrel S, Ducastelle-Lepretre S, Billaud G, Ader F, Casalegno J-S, et al.  
444 Resistance of herpes simplex viruses to acyclovir: An update from a ten-year survey in France.  
445 *Antiviral Research.* 2014;111(0):36-41. doi: <http://dx.doi.org/10.1016/j.antiviral.2014.08.013>.
- 446 18. Baumrin E, Cheng MP, Kanjilal S, Ho VT, Issa NC, Baden LR. Severe Herpes Zoster  
447 Requiring Intravenous Antiviral Treatment in Allogeneic Hematopoietic Cell Transplantation

- 448 Recipients on Standard Acyclovir Prophylaxis. *Biology of Blood and Marrow Transplantation*.  
449 2019. doi: <https://doi.org/10.1016/j.bbmt.2019.04.015>.
- 450 19. Dadwal SS. Herpes Virus Infections Other than Cytomegalovirus in the Recipients of  
451 Hematopoietic Stem Cell Transplantation. *Infect Dis Clin North Am*. 2019;33(2):467-84. doi:  
452 <https://doi.org/10.1016/j.idc.2019.02.012>.
- 453 20. Taur Y, Jenq RR, Perales MA, Littmann ER, Morjaria S, Ling L, et al. The effects of  
454 intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell  
455 transplantation. *Blood*. 2014;124(7):1174-82. Epub 2014/06/19. doi: 10.1182/blood-2014-02-  
456 554725. PubMed PMID: 24939656; PubMed Central PMCID: PMC4133489.
- 457 21. Weber D, Oefner PJ, Dettmer K, Hiergeist A, Koestler J, Gessner A, et al. Rifaximin  
458 preserves intestinal microbiota balance in patients undergoing allogeneic stem cell  
459 transplantation. *Bone Marrow Transplant*. 2016;51(8):1087-92. Epub 2016/03/22. doi:  
460 10.1038/bmt.2016.66. PubMed PMID: 26999466.
- 461 22. Shono Y, Docampo MD, Peled JU, Perobelli SM, Velardi E, Tsai JJ, et al. Increased  
462 GVHD-related mortality with broad-spectrum antibiotic use after allogeneic hematopoietic stem  
463 cell transplantation in human patients and mice. *Sci Transl Med*. 2016;8(339):339ra71. Epub  
464 2016/05/20. doi: 10.1126/scitranslmed.aaf2311. PubMed PMID: 27194729; PubMed Central  
465 PMCID: PMC4991773.
- 466 23. Peled JU, Devlin SM, Staffas A, Lumish M, Khanin R, Littmann ER, et al. Intestinal  
467 Microbiota and Relapse After Hematopoietic-Cell Transplantation. *J Clin Oncol*.  
468 2017;35(15):1650-9. Epub 2017/03/16. doi: 10.1200/jco.2016.70.3348. PubMed PMID:  
469 28296584; PubMed Central PMCID: PMC455763.
- 470 24. Biagi E, Zama D, Nastasi C, Consolandi C, Fiori J, Rampelli S, et al. Gut microbiota  
471 trajectory in pediatric patients undergoing hematopoietic SCT. *Bone Marrow Transplant*.  
472 2015;50(7):992-8. doi: 10.1038/bmt.2015.16.
- 473 25. Jenq RR, Ubeda C, Taur Y, Menezes CC, Khanin R, Dudakov JA, et al. Regulation of  
474 intestinal inflammation by microbiota following allogeneic bone marrow transplantation. *J Exp*  
475 *Med*. 2012;209(5):903-11. Epub 2012/05/02. doi: 10.1084/jem.20112408. PubMed PMID:  
476 22547653; PubMed Central PMCID: PMC3348096.
- 477 26. Holler E, Butzhammer P, Schmid K, Hundsrucker C, Koestler J, Peter K, et al.  
478 Metagenomic analysis of the stool microbiome in patients receiving allogeneic stem cell  
479 transplantation: loss of diversity is associated with use of systemic antibiotics and more  
480 pronounced in gastrointestinal graft-versus-host disease. *Biol Blood Marrow Transplant*.  
481 2014;20(5):640-5. Epub 2014/02/05. doi: 10.1016/j.bbmt.2014.01.030. PubMed PMID:  
482 24492144; PubMed Central PMCID: PMC4973578.
- 483 27. Biagi E, Zama D, Rampelli S, Turrone S, Brigidi P, Consolandi C, et al. Early gut microbiota  
484 signature of aGvHD in children given allogeneic hematopoietic cell transplantation for  
485 hematological disorders. *BMC Med Genomics*. 2019;12(1):49. Epub 2019/03/09. doi:  
486 10.1186/s12920-019-0494-7. PubMed PMID: 30845942; PubMed Central PMCID:  
487 PMC6404274.
- 488 28. Jenq RR, Taur Y, Devlin SM, Ponce DM, Goldberg JD, Ahr KF, et al. Intestinal *Blautia* Is  
489 Associated with Reduced Death from Graft-versus-Host Disease. *Biol Blood Marrow Transplant*.  
490 2015;21(8):1373-83. Epub 2015/05/16. doi: 10.1016/j.bbmt.2015.04.016. PubMed PMID:  
491 25977230; PubMed Central PMCID: PMC4516127.
- 492 29. Weber D, Jenq RR, Peled JU, Taur Y, Hiergeist A, Koestler J, et al. Microbiota Disruption  
493 Induced by Early Use of Broad-Spectrum Antibiotics Is an Independent Risk Factor of Outcome  
494 after Allogeneic Stem Cell Transplantation. *Biol Blood Marrow Transplant*. 2017;23(5):845-52.  
495 Epub 2017/02/25. doi: 10.1016/j.bbmt.2017.02.006. PubMed PMID: 28232086; PubMed Central  
496 PMCID: PMC5546237.
- 497 30. Simms-Waldrup TR, Sunkersett G, Coughlin LA, Savani MR, Arana C, Kim J, et al.  
498 Antibiotic-Induced Depletion of Anti-inflammatory Clostridia Is Associated with the Development

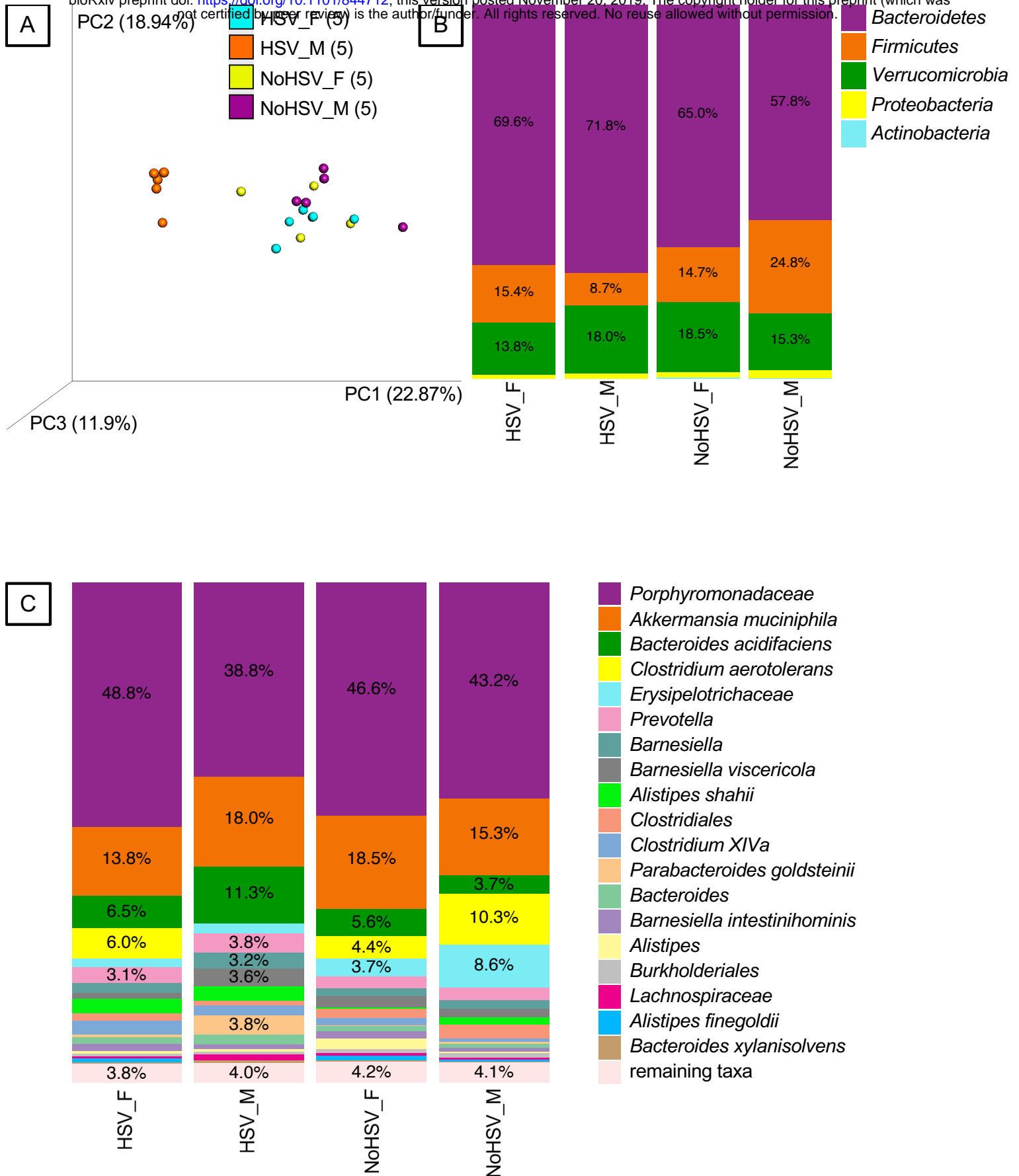
- 499 of Graft-versus-Host Disease in Pediatric Stem Cell Transplantation Patients. *Biol Blood Marrow*  
500 *Transplant.* 2017;23(5):820-9. Epub 2017/02/14. doi: 10.1016/j.bbmt.2017.02.004. PubMed  
501 PMID: 28192251.
- 502 31. Piper HG, Fan D, Coughlin LA, Ho EX, McDaniel MM, Channabasappa N, et al. Severe  
503 Gut Microbiota Dysbiosis Is Associated With Poor Growth in Patients With Short Bowel  
504 Syndrome. *JPEN J Parenter Enteral Nutr.* 2017;41(7):1202-12. Epub 2016/07/14. doi:  
505 10.1177/0148607116658762. PubMed PMID: 27406942.
- 506 32. Melson J, Jakate S, Fung H, Arai S, Keshavarzian A. Crypt loss is a marker of clinical  
507 severity of acute gastrointestinal graft-versus-host disease. *Am J Hematol.* 2007;82(10):881-6.  
508 Epub 2007/06/16. doi: 10.1002/ajh.20976. PubMed PMID: 17570511.
- 509 33. Spencer GD, Shulman HM, Myerson D, Thomas ED, McDonald GB. Diffuse intestinal  
510 ulceration after marrow transplantation: a clinicopathologic study of 13 patients. *Hum Pathol.*  
511 1986;17(6):621-33. Epub 1986/06/01. doi: 10.1016/s0046-8177(86)80135-6. PubMed PMID:  
512 3011641.
- 513 34. Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA, et al. Clinical  
514 manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched  
515 sibling donors. *Transplantation.* 1974;18(4):295-304. Epub 1974/10/01. doi: 10.1097/00007890-  
516 197410000-00001. PubMed PMID: 4153799.
- 517 35. Ponc R, Hackman RC, McDonald GB. Endoscopic and histologic diagnosis of intestinal  
518 graft-versus-host disease after marrow transplantation. *Gastrointest Endosc.* 1999;49(5):612-21.  
519 Epub 1999/05/06. doi: 10.1016/s0016-5107(99)70390-1. PubMed PMID: 10228260.
- 520 36. Sale GE, Shulman HM, McDonald GB, Thomas ED. Gastrointestinal graft-versus-host  
521 disease in man. A clinicopathologic study of the rectal biopsy. *Am J Surg Pathol.* 1979;3(4):291-  
522 9. Epub 1979/08/01. PubMed PMID: 44107.
- 523 37. Nakajima M, Arimatsu K, Kato T, Matsuda Y, Minagawa T, Takahashi N, et al. Oral  
524 Administration of *P. gingivalis* Induces Dysbiosis of Gut Microbiota and Impaired Barrier Function  
525 Leading to Dissemination of Enterobacteria to the Liver. *PLoS One.* 2015;10(7):e0134234. Epub  
526 2015/07/29. doi: 10.1371/journal.pone.0134234. PubMed PMID: 26218067; PubMed Central  
527 PMCID: PMC4517782.
- 528 38. Flak MB, Colas RA, Munoz-Atienza E, Curtis MA, Dalli J, Pitzalis C. Inflammatory arthritis  
529 disrupts gut resolution mechanisms, promoting barrier breakdown by *Porphyromonas gingivalis*.  
530 *JCI Insight.* 2019;4(13). Epub 2019/07/12. doi: 10.1172/jci.insight.125191. PubMed PMID:  
531 31292292; PubMed Central PMCID: PMC6629160.
- 532 39. van der Lugt B, van Beek AA, Aalvink S, Meijer B, Sovran B, Vermeij WP, et al.  
533 *Akkermansia muciniphila* ameliorates the age-related decline in colonic mucus thickness and  
534 attenuates immune activation in accelerated aging *Ercc1 (-/Delta7)* mice. *Immun Ageing.*  
535 2019;16:6. Epub 2019/03/23. doi: 10.1186/s12979-019-0145-z. PubMed PMID: 30899315;  
536 PubMed Central PMCID: PMC6408808.
- 537 40. Grander C, Adolph TE, Wieser V, Lowe P, Wrzosek L, Gyongyosi B, et al. Recovery of  
538 ethanol-induced *Akkermansia muciniphila* depletion ameliorates alcoholic liver disease. *Gut.*  
539 2018;67(5):891-901. Epub 2017/05/28. doi: 10.1136/gutjnl-2016-313432. PubMed PMID:  
540 28550049.
- 541 41. Wu W, Lv L, Shi D, Ye J, Fang D, Guo F, et al. Protective Effect of *Akkermansia*  
542 *muciniphila* against Immune-Mediated Liver Injury in a Mouse Model. *Front Microbiol.*  
543 2017;8:1804. Epub 2017/10/17. doi: 10.3389/fmicb.2017.01804. PubMed PMID: 29033903;  
544 PubMed Central PMCID: PMC5626943.
- 545 42. Gesser RM, Koo SC. Oral inoculation with herpes simplex virus type 1 infects enteric  
546 neuron and mucosal nerve fibers within the gastrointestinal tract in mice. *Journal of virology.*  
547 1996;70(6):4097-102. PubMed PMID: 8648749.

- 548 43. Basso L, Serhan N, Tauber M, Gaudenzio N. Peripheral neurons: Master regulators of  
549 skin and mucosal immune response. *European Journal of Immunology*. 2019;0(0). doi:  
550 10.1002/eji.201848027.
- 551 44. Pavlov VA, Tracey KJ. Neural regulation of immunity: molecular mechanisms and clinical  
552 translation. *Nat Neurosci*. 2017;20(2):156-66. doi: 10.1038/nn.4477.
- 553 45. Lokensgard JR, Cheeran MC, Hu S, Gekker G, Peterson PK. Glial cell responses to  
554 herpesvirus infections: role in defense and immunopathogenesis. *J Infect Dis*. 2002;186 Suppl  
555 2:S171-9. doi: 10.1086/344272. PubMed PMID: 22311297.
- 556 46. Marques CP, Cheeran MCJ, Palmquist JM, Hu S, Urban SL, Lokensgard JR. Prolonged  
557 Microglial Cell Activation and Lymphocyte Infiltration following Experimental Herpes Encephalitis.  
558 *J Immunol*. 2008;181(9):6417-26.
- 559 47. Thion MS, Low D, Silvín A, Chen J, Grisel P, Schulte-Schrepping J, et al. Microbiome  
560 Influences Prenatal and Adult Microglia in a Sex-Specific Manner. *Cell*. 2018;172:1-17. doi:  
561 10.1016/j.cell.2017.11.042.
- 562 48. Black ME, Hruby DE. Nucleotide sequence of the *Escherichia coli* thymidine kinase gene  
563 provides evidence for conservation of functional domains and quaternary structure. *Molecular*  
564 *Microbiology*. 1991;5(2):373-9. doi: 10.1111/j.1365-2958.1991.tb02119.x.
- 565 49. Jeffrey WH, Paul JH. Thymidine uptake, thymidine incorporation, and thymidine kinase  
566 activity in marine bacterium isolates. *Applied and Environmental Microbiology*. 1990;56(5):1367-  
567 72.
- 568 50. Konrad A, Yarunova E, Tinta T, Piškur J, Liberles DA. The global distribution and evolution  
569 of deoxyribonucleoside kinases in bacteria. *Gene*. 2012;492(1):117-20. doi:  
570 <https://doi.org/10.1016/j.gene.2011.10.039>.
- 571 51. Lönnqvist B, Palmblad J, Ljungman P, Grimfors G, Järnmark M, Lerner R, et al. Oral  
572 acyclovir as prophylaxis for bacterial infections during induction therapy for acute leukaemia in  
573 adults. *Support Care Cancer*. 1993;1(3):139-44. doi: 10.1007/bf00366060.
- 574 52. Brewin N, Cairns J. State of the DNA replication fork during thymine deprivation of  
575 *Escherichia coli*, as observed by pulse-labelling with [3H]thymidine. *Journal of Molecular Biology*.  
576 1977;111(3):353-63. doi: [https://doi.org/10.1016/S0022-2836\(77\)80057-0](https://doi.org/10.1016/S0022-2836(77)80057-0).
- 577 53. Bettogowda C, Foss CA, Cheong I, Wang Y, Diaz L, Agrawal N, et al. Imaging bacterial  
578 infections with radiolabeled 1-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-5-iodouracil. *Proceedings*  
579 *of the National Academy of Sciences of the United States of America*. 2005;102(4):1145-50. doi:  
580 10.1073/pnas.0408861102.
- 581 54. Davis SL, Be NA, Lamichhane G, Nimmagadda S, Pomper MG, Bishai WR, et al. Bacterial  
582 Thymidine Kinase as a Non-Invasive Imaging Reporter for *Mycobacterium*  
583 *tuberculosis* in Live Animals. *PLoS ONE*. 2009;4(7):e6297. doi:  
584 10.1371/journal.pone.0006297.
- 585 55. Peterson KL, Reid WC, Freeman AF, Holland SM, Pettigrew RI, Gharib AM, et al. The use  
586 of 14C-FIAU to predict bacterial thymidine kinase presence: Implications for radiolabeled FIAU  
587 bacterial imaging. *Nuclear Medicine and Biology*. 2013;40(5):638-42. doi:  
588 <http://dx.doi.org/10.1016/j.nucmedbio.2013.01.005>.
- 589 56. Wellsbury P, Herbert RA, John Parkes R. Incorporation of [methyl-3H]thymidine by  
590 obligate and facultative anaerobic bacteria when grown under defined culture conditions. *FEMS*  
591 *Microbiology Ecology*. 1993;12(2):87-95. doi: 10.1111/j.1574-6941.1993.tb00020.x.
- 592 57. Jenq RR, Taur Y, Devlin SM, Ponce DM, Goldberg JD, Ahr KF, et al. Intestinal *Blautia* Is  
593 Associated with Reduced Death from Graft-versus-Host Disease. *Biology of Blood and Marrow*  
594 *Transplantation*. 2015;21(8):1373-83. doi: 10.1016/j.bbmt.2015.04.016.
- 595 58. Stingl C, Cardinale K, Van Mater H. An Update on the Treatment of Pediatric Autoimmune  
596 Encephalitis. *Current Treatment Options in Rheumatology*. 2018;4(1):14-28. doi:  
597 10.1007/s40674-018-0089-z.

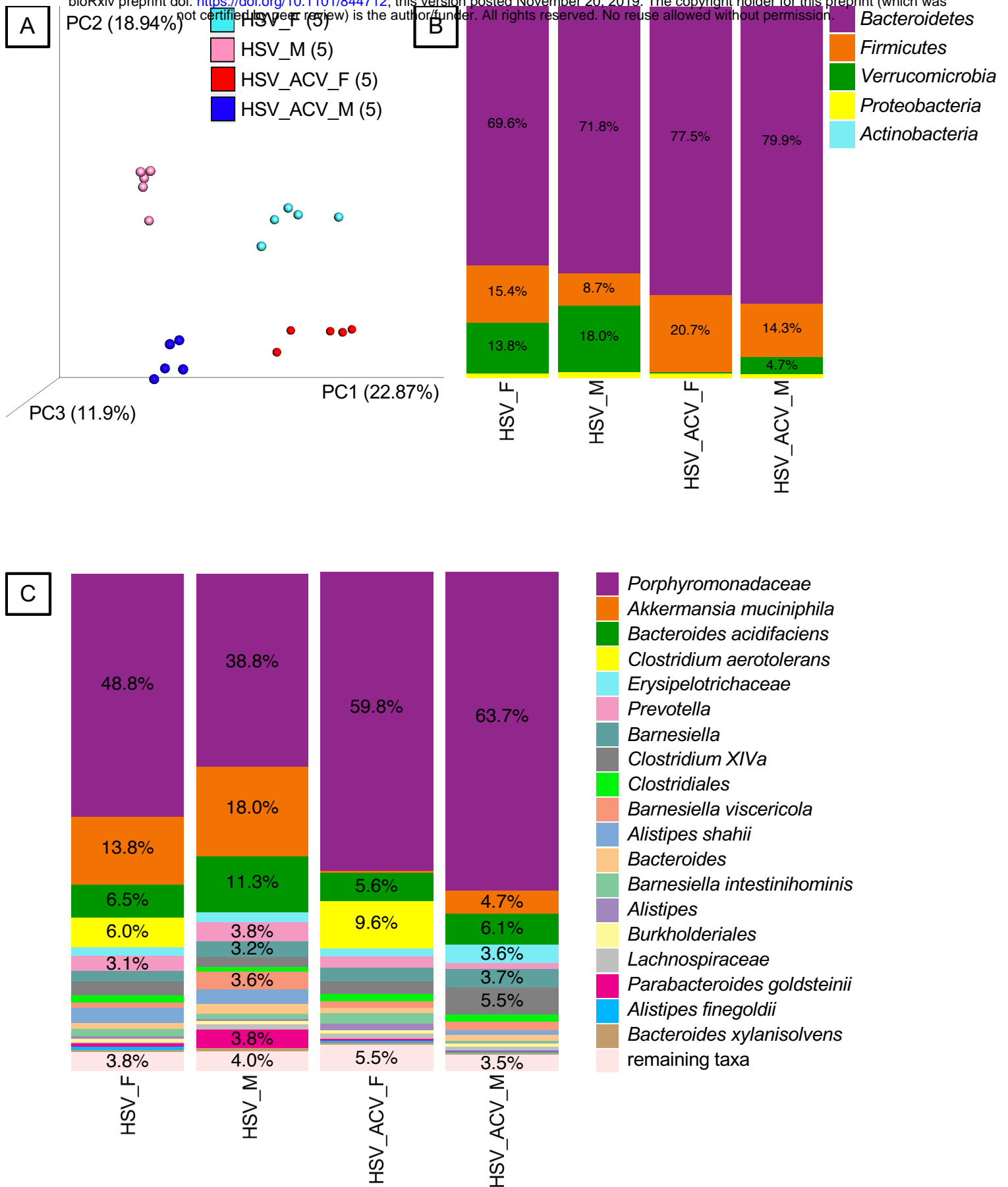


- 598 59. Armangue T, Spatola M, Vlasea A, Mattozzi S, Cárceles-Cordon M, Martinez-Heras E, et  
599 al. Frequency, symptoms, risk factors, and outcomes of autoimmune encephalitis after herpes  
600 simplex encephalitis: a prospective observational study and retrospective analysis. *The Lancet*  
601 *Neurology*. 2018;17(9):760-72. doi: [https://doi.org/10.1016/S1474-4422\(18\)30244-8](https://doi.org/10.1016/S1474-4422(18)30244-8).
- 602 60. Wekerle H. Brain Autoimmunity and Intestinal Microbiota: 100 Trillion Game Changers.  
603 *Trends in Immunology*. 2017;38(7):483-97. doi: <https://doi.org/10.1016/j.it.2017.03.008>.
- 604 61. Iro MA, Sadarangani M, Absoud M, Chong WK, Clark CA, Easton A, et al. Immunoglobulin  
605 in the Treatment of Encephalitis (IgNiTE): protocol for a multicentre randomised controlled trial.  
606 *BMJ Open*. 2016;6(11). doi: 10.1136/bmjopen-2016-012356.
- 607 62. Ye SL, Lei M, Jiang P, Liu FJ, Wang ZK, Cao HJ, et al. Demonstration of the IgG antibody  
608 repertoire against the bacteria *Escherichia coli* in Chinese intravenous immunoglobulins. *Journal*  
609 *of Pharmaceutical and Biomedical Analysis*. 2017;133:8-14. doi: 10.1016/j.jpba.2016.10.018.  
610 PubMed PMID: WOS:000392894900002.
- 611 63. Uchimura Y, Fuhrer T, Li H, Lawson MA, Zimmermann M, Yilmaz B, et al. Antibodies Set  
612 Boundaries Limiting Microbial Metabolite Penetration and the Resultant Mammalian Host  
613 Response. *Immunity*. 2018;49(3):545-59.e5. doi: <https://doi.org/10.1016/j.immuni.2018.08.004>.
- 614 64. Schneider C, Smith DF, Cummings RD, Boligan KF, Hamilton RG, Bochner BS, et al. The  
615 human IgG anti-carbohydrate repertoire exhibits a universal architecture and contains specificity  
616 for microbial attachment sites. *Science Translational Medicine*. 2015;7(269):269ra1-ra1. doi:  
617 10.1126/scitranslmed.3010524.
- 618 65. Zeng Melody Y, Cisalpino D, Varadarajan S, Hellman J, Warren HS, Cascalho M, et al.  
619 Gut Microbiota-Induced Immunoglobulin G Controls Systemic Infection by Symbiotic Bacteria and  
620 Pathogens. *Immunity*. 2016;44(1-12). doi: <http://dx.doi.org/10.1016/j.immuni.2016.02.006>.
- 621 66. Negm OH, MacKenzie B, Hamed MR, Ahmad OAJ, Shone CC, Humphreys DP, et al.  
622 Protective antibodies against *Clostridium difficile* are present in intravenous immunoglobulin and  
623 are retained in humans following its administration. *Clinical & Experimental Immunology*.  
624 2017;n/a-n/a. doi: 10.1111/cei.12946.
- 625 67. Kakiuchi S, Tsuji M, Nishimura H, Yoshikawa T, Wang L, Takayama-Ito M, et al.  
626 Association of the Emergence of Acyclovir-Resistant Herpes Simplex Virus Type 1 With  
627 Prognosis in Hematopoietic Stem Cell Transplantation Patients. *The Journal of Infectious*  
628 *Diseases*. 2017;215(6):865-73. doi: 10.1093/infdis/jix042.
- 629 68. Shen Y, Giardino Torchia ML, Lawson GW, Karp CL, Ashwell JD, Mazmanian SK. Outer  
630 membrane vesicles of a human commensal mediate immune regulation and disease protection.  
631 *Cell Host Microbe*. 2012;12(4):509-20. Epub 2012/09/25. doi: 10.1016/j.chom.2012.08.004.  
632 PubMed PMID: 22999859; PubMed Central PMCID: PMC3895402.
- 633 69. Round JL, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a  
634 commensal bacterium of the intestinal microbiota. *Proc Natl Acad Sci U S A*. 2010;107(27):12204-  
635 9. Epub 2010/06/23. doi: 10.1073/pnas.0909122107. PubMed PMID: 20566854; PubMed Central  
636 PMCID: PMC2901479.
- 637 70. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal  
638 inflammatory disease. *Nature*. 2008;453(7195):620-5. Epub 2008/05/30. doi:  
639 10.1038/nature07008. PubMed PMID: 18509436.
- 640 71. Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of  
641 symbiotic bacteria directs maturation of the host immune system. *Cell*. 2005;122(1):107-18. Epub  
642 2005/07/13. doi: 10.1016/j.cell.2005.05.007. PubMed PMID: 16009137.
- 643 72. Ramakrishna C, Kujawski M, Chu H, Li L, Mazmanian SK, Cantin EM. *Bacteroides fragilis*  
644 polysaccharide A induces IL-10 secreting B and T cells that prevent viral encephalitis. *Nat*  
645 *Commun*. 2019;10(1):2153. Epub 2019/05/16. doi: 10.1038/s41467-019-09884-6. PubMed  
646 PMID: 31089128; PubMed Central PMCID: PMC6517419.
- 647 73. Staffas A, Burgos da Silva M, Slingerland AE, Lazrak A, Bare CJ, Holman CD, et al.  
648 Nutritional Support from the Intestinal Microbiota Improves Hematopoietic Reconstitution after

- 649 Bone Marrow Transplantation in Mice. *Cell Host & Microbe*. 2018;23(4):447-57. doi:  
650 10.1016/j.chom.2018.03.002.
- 651 74. Rosshart SP, Herz J, Vassallo BG, Hunter A, Wall MK, Badger JH, et al. Laboratory mice  
652 born to wild mice have natural microbiota and model human immune responses. *Science*.  
653 2019;365(6452):eaaw4361. doi: 10.1126/science.aaw4361.
- 654 75. Rosshart SP, Vassallo BG, Angeletti D, Hutchinson DS, Morgan AP, Takeda K, et al. Wild  
655 Mouse Gut Microbiota Promotes Host Fitness and Improves Disease Resistance. *Cell*.  
656 2017;171(5):1015-28.e13  
657 . doi: 10.1016/j.cell.2017.09.016.
- 658 76. Viney M, Riley EM. The Immunology of Wild Rodents: Current Status and Future  
659 Prospects. *Frontiers in Immunology*. 2017;8(1481). doi: 10.3389/fimmu.2017.01481.
- 660 77. Lundberg P, Ramakrishna C, Brown J, Tyszka JM, Hamamura M, Hinton DR, et al. The  
661 immune response to herpes simplex virus type 1 infection in susceptible mice is a major cause of  
662 central nervous system pathology resulting in fatal encephalitis. *J Virol*. 2008;82(14):7078-88. doi:  
663 10.1128/JVI.00619-08. PubMed PMID: 18480436; PubMed Central PMCID: PMC2446972.
- 664 78. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, et  
665 al. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc*  
666 *Natl Acad Sci U S A*. 2011;108. doi: 10.1073/pnas.1000080107.
- 667 79. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat*  
668 *Methods*. 2013;10. doi: 10.1038/nmeth.2604.
- 669 80. Edgar RC. UNOISE2: Improved error-correction for Illumina 16S and ITS amplicon reads.  
670 bioRxiv. 2016. doi: <http://dx.doi.org/10.1101/081257>doi:<http://dx.doi.org/10.1101/081257>.
- 671 81. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool.  
672 *J Mol Biol*. 1990;215(3):403-10. Epub 1990/10/05. doi: 10.1016/s0022-2836(05)80360-2.  
673 PubMed PMID: 2231712.
- 674 82. Edgar RC. Edgar RC. 2016. SINTAX, a simple non-Bayesian taxonomy classifier for 16S  
675 and ITS sequences. bioRxiv. 2016. doi:  
676 <http://dx.doi.org/10.1101/074161>doi:<http://dx.doi.org/10.1101/074161>.
- 677 83. Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y, et al. Ribosomal Database  
678 Project: data and tools for high throughput rRNA analysis. *Nucleic Acids Res*. 2014;42(Database  
679 issue):D633-42. Epub 2013/11/30. doi: 10.1093/nar/gkt1244. PubMed PMID: 24288368; PubMed  
680 Central PMCID: PMC3965039.
- 681 84. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al.  
682 QIIME allows analysis of high-throughput community sequencing data. *Nature methods*.  
683 2010;7(5):335-6.
- 684 85. McCarthy DJ, Chen Y, Smyth GK. Differential expression analysis of multifactor RNA-Seq  
685 experiments with respect to biological variation. *Nucleic Acids Res*. 2012;40(10):4288-97. Epub  
686 2012/01/31. doi: 10.1093/nar/gks042. PubMed PMID: 22287627; PubMed Central PMCID:  
687 PMC3378882.
- 688 86. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential  
689 expression analysis of digital gene expression data. *Bioinformatics*. 2010;26(1):139-40. doi:  
690 10.1093/bioinformatics/btp616.  
691

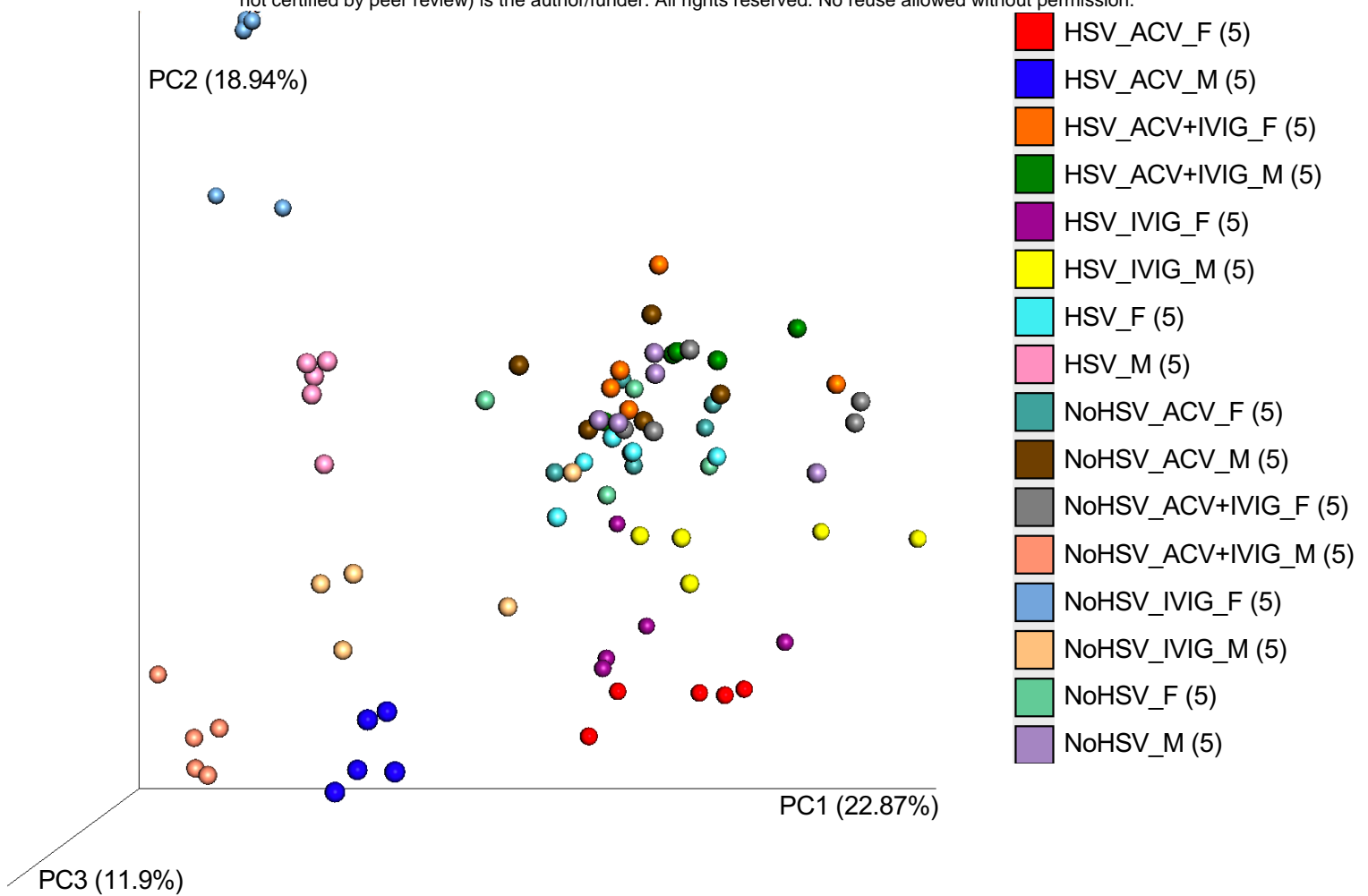


**Figure 1. Fecal Bacteria from HSV-Infected and Uninfected Mice.** A. Principal-coordinates analysis (PCoA) of Hellinger beta diversity distance values generated from 16S rRNA gene sequences. All four groups were different ( $P < 0.05$ , Adonis Tests). The number of mice ( $n$ ) in each genotype-microbiota group are shown in parentheses. B. Bacteria phyla associated with HSV-infected and uninfected mice. C. Bacterial species (or higher taxa) associated with HSV-infected and uninfected mice. Females = \_F and Males = \_M.



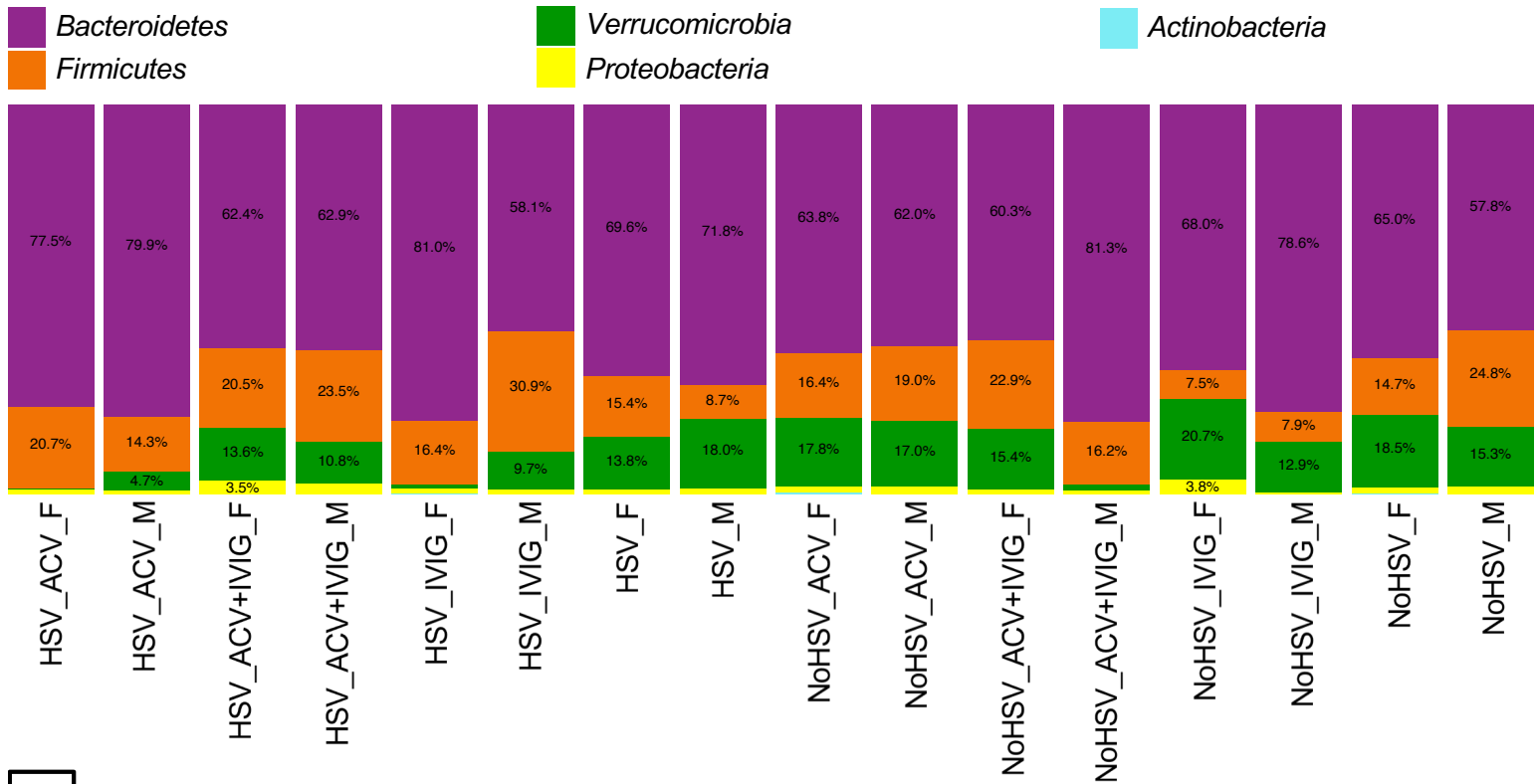
**Figure 2. Fecal Bacteria from HSV-Infected Mice Treated and Not Treated with ACV.** A. Principal-coordinates analysis (PCoA) of Hellinger beta diversity distance values generated from 16S rRNA gene sequences. All four groups were different ( $P < 0.05$ , Adonis Tests). The number of mice ( $n$ ) in each genotype-microbiota group are shown in parentheses. B and C. Bacteria phyla and species (or higher taxa), respectively, associated with HSV-infected mice treated and not treated with ACV. Females = \_F and Males = \_M.



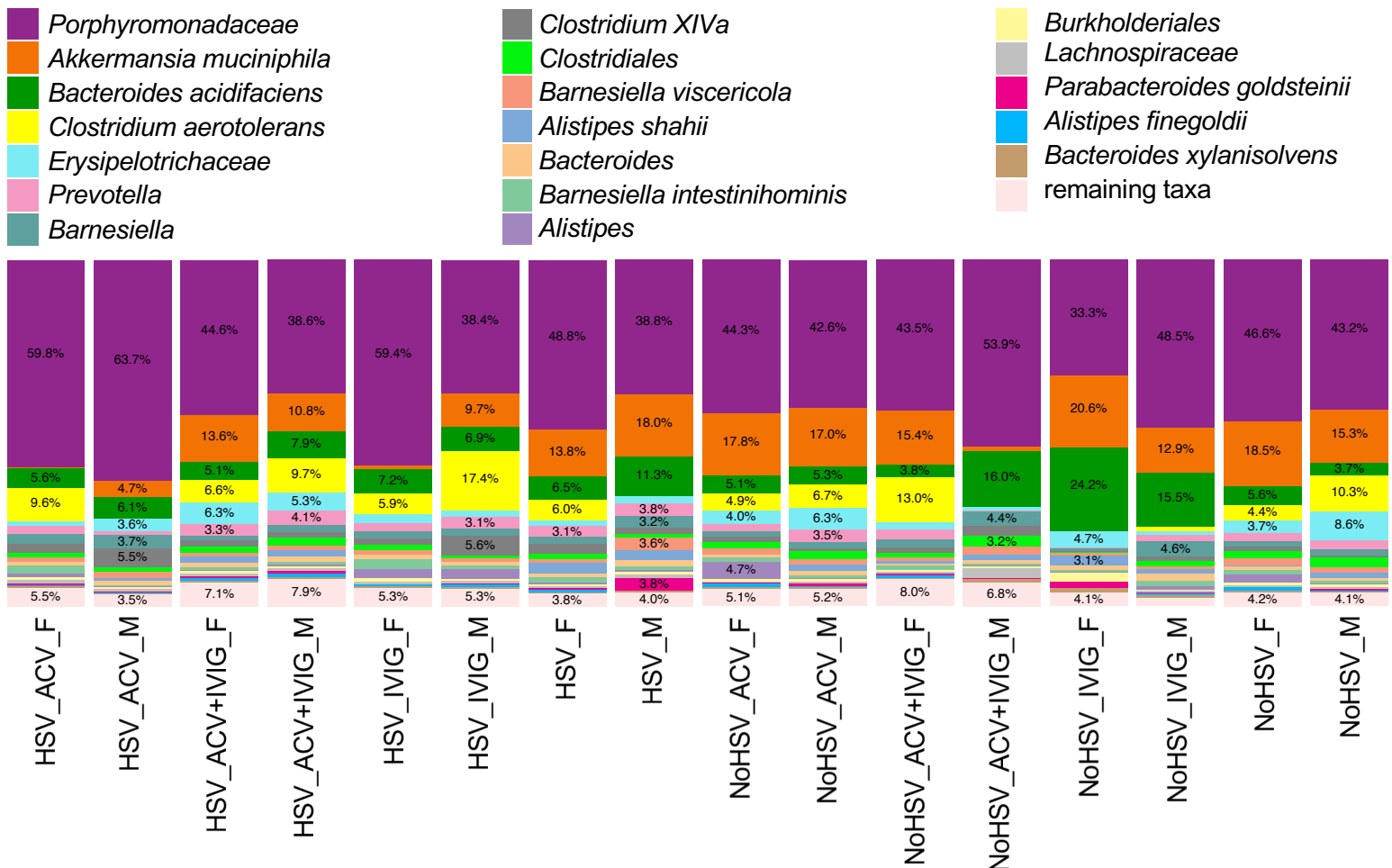


**Figure 3. Beta Diversity Analysis of Fecal Bacteria from HSV-Infected and Uninfected Mice Treated and Not Treated with ACV and/or IVIG.** Principal-coordinates analysis (PCoA) of Hellinger beta diversity distance values generated from 16S rRNA gene sequences. The number of mice (n) in each genotype-microbiota group are shown in parentheses. Females = \_F and Males = \_M.

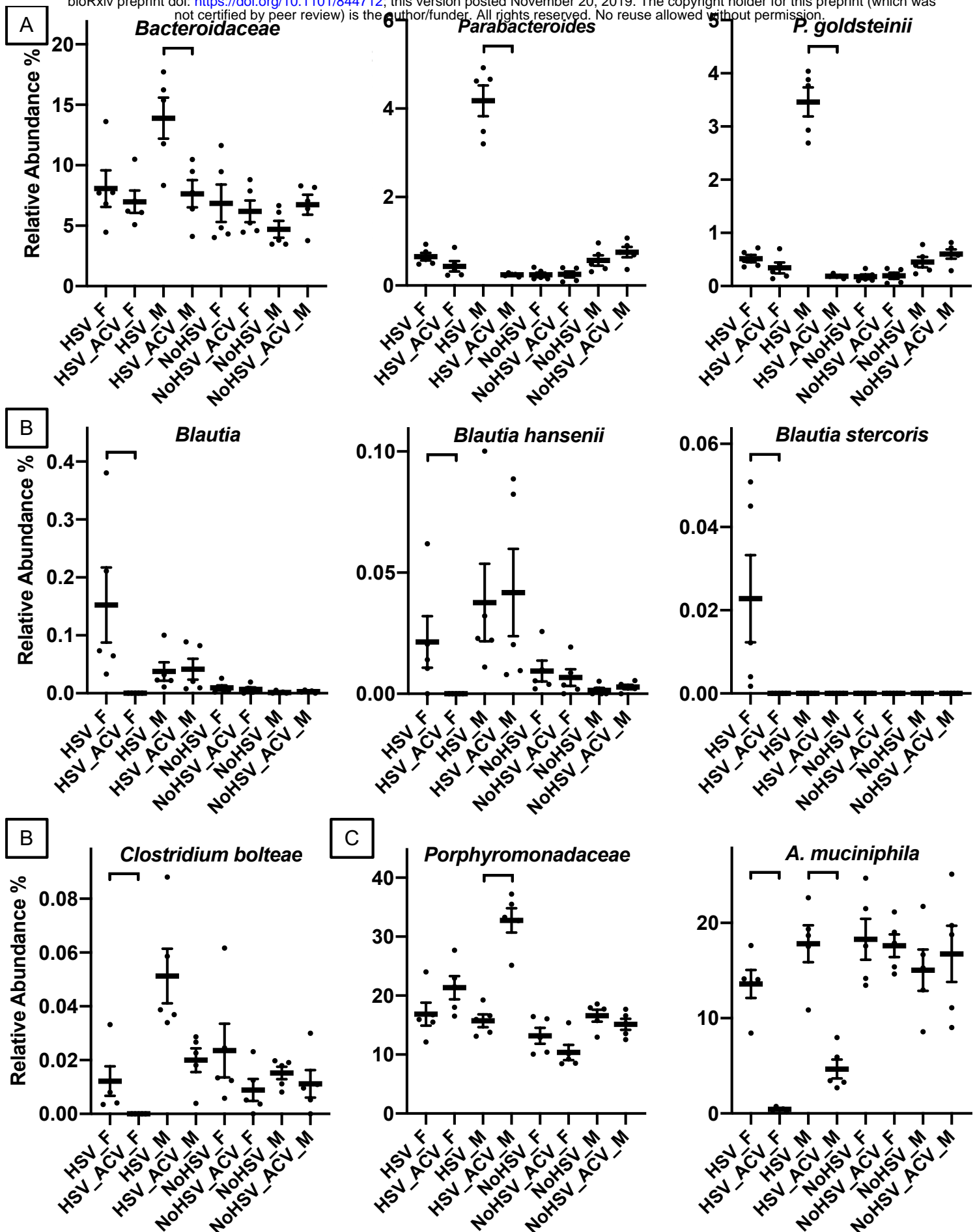
A



B



**Figure 4. Fecal Bacterial from HSV-Infected and Uninfected Mice Treated and Not Treated with ACV and/or IVIG.** A and B. Bacteria phyla and species (or higher taxa), respectively, associated with HSV-infected and uninfected mice treated and not treated with ACV, IVIG, or ACV+IVIG. Females = \_F and Males = \_M.



**Figure 5. Fecal Bacterial from HSV-Infected and Uninfected Mice Treated and Not Treated with ACV.** A and B. Fecal bacterial taxa that were changed in both human GVHD studies and by ACV in this study. A and B. Members of the *Bacteroidetes* and AIC, respectively. C. The two most abundant bacterial OTUs. The only pairwise differences shown are between ACV treated and untreated mice for each sex (FDR-adjusted P values < 0.05). Bars = standard error. Females = \_F and Males = \_M.