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2 microbiome structure during coral evolution

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30 ***Abstract***

31

32 Tropical reef-building corals exist in intimate symbiosis with diverse microbes and  
33 viruses. Coral microbiomes are generally much less diverse than their environment, but  
34 across studied corals, the biodiversity of these microbiomes varies greatly. It has  
35 previously been hypothesized that differences in coral innate immunity in general, and

36 the copy number of TIR-domain containing innate immune genes in particular, may drive  
37 interspecific differences in microbiome structure. Despite many existing studies of coral  
38 microbiomes, this hypothesis has previously been difficult to test due to a lack of  
39 consistently collected cross-species data on coral microbiomes. In this manuscript, we  
40 reannotate TIR-domain containing genes across diverse coral genomes, and use  
41 phylogenetic comparative methods to compare these innate immune gene copy numbers  
42 against 16S rRNA marker gene data on coral mucus, tissue, and skeleton microbiomes  
43 from the Global Coral Microbiome Project (GCMP). The copy number of Toll-like  
44 receptor (TLRs) and Interleukin-1 receptor (IL-1Rs) gene families, as well as the total  
45 genomic count of their constituent domains (LRR and TIR domains; and Ig and TIR  
46 domains, respectively), explained most interspecific differences in microbiome richness  
47 and beta-diversity among corals with sequenced genomes. We find that these correlations  
48 are also anatomically specific, with an especially strong correlation between IL-1R gene  
49 copy numbers and microbiome richness in the coral's endolithic skeleton. Together, these  
50 results suggest innate immunity may play a key role in sculpting microbiome structure in  
51 corals.

52

### 53 ***Introduction***

54

55 The 1681 described species of scleractinian corals<sup>1</sup> are environmentally critical  
56 ecosystem engineers that underpin many tropical reef ecosystems. Microbiomes are  
57 important contributors to the health of these tropical corals, with competing and  
58 cooperating microbes influencing animal health<sup>2</sup>. Therefore, evaluating how corals  
59 regulate this microbiome is of great importance. Numerous studies have uncovered  
60 important features of coral microbiomes, including the relative influence of differences  
61 across host anatomy<sup>3,4</sup>, between species<sup>4,5</sup>, among reefs<sup>6-8</sup>, and along environmental  
62 gradients<sup>8,9</sup>. This literature has also extensively documented coral microbiome responses  
63 to various stressors, including heat<sup>10,11</sup>, bleaching<sup>12,13</sup>, sedimentation<sup>14,15</sup>, nutrient  
64 pollution<sup>10,14,16</sup>, predation<sup>16</sup>, plastic pollution<sup>17</sup>, turf or macro-algal competition<sup>16,18,19</sup>,  
65 etc. Genetic studies within coral species have further found genotypic differences that  
66 correlate with microbiome composition<sup>20,21</sup>. Building on these ecological and population-  
67 genetic comparisons, specific coral microorganisms have been linked to important host  
68 health outcomes, such as protection against pathogens or susceptibility to them<sup>22</sup>. Recent  
69 microbiome manipulation experiments have even begun to establish the causal role of  
70 specific coral-associated bacteria in influencing key host traits like heat resistance<sup>23</sup>.  
71 Despite this thriving literature on coral microbiomes, the broader scale patterns of how  
72 modern coral microbiomes have evolved, and which host traits, if any, drive the large  
73 differences in microbiome structure and function seen between modern corals is not yet  
74 clear.

75

76 Increased attention to the question of how host traits have sculpted coral microbiomes  
77 over evolution is important. Comparative studies of coral microbiome evolution may  
78 identify host traits that have regulated coral microbiomes up to the present day.  
79 Comparative studies of coral microbiome evolution will also clarify a key part of the  
80 broader story of animal microbiome evolution. While vertebrate gut microbiomes are

81 structured by both host phylogenetic relatedness and convergently evolved host traits like  
82 diet or flight<sup>24,25</sup>, the traits shaping the microbiome evolution of basally divergent animal  
83 taxa are less clear.

84  
85 Among the many coral traits that could influence microbiome structure, differences in  
86 innate immunity are promising candidates, since they have the potential to directly  
87 regulate the microbiome by activating pathways that preferentially target particular  
88 groups of microbes. The copy number of gene families containing Toll/Interleukin  
89 Repeat (TIR) domains in particular, have received special attention as a possible  
90 influence on microbiome structure<sup>26</sup>. TIR domains are a key intracellular signaling  
91 domain, found in multiple innate immune gene families, such as Toll-like Receptors  
92 (TLR), Interleukin-1 Receptors (IL-1R), coral-specific TIR-only genes of unknown  
93 function, and Myeloid Differentiation Factor 88 (myD88). Collectively, these genes are  
94 known as TIR-domain containing genes<sup>27</sup>. The genomic copy number of some gene  
95 families of TIR-domain containing genes is known to vary greatly between coral species,  
96 and on this basis these genes have been hypothesized to influence cross-species  
97 differences in coral microbiome structure<sup>26</sup>. However, a lack of consistently collected  
98 cross-species microbiome data has so far precluded empirical testing of this intriguing  
99 idea.

100  
101 In this study we tested whether dramatic changes in the copy number of coral TLR or IL-  
102 1R gene families have driven corresponding changes in microbiome richness, evenness,  
103 or composition during more than 250 million years of scleractinian coral evolution. Our  
104 analysis combined genomic analysis of all major lineages of scleractinian coral for which  
105 genomes are publicly available with data on coral mucus, tissue, and endolithic skeleton  
106 microbiomes from the Global Coral Microbiome Project (GCMP) dataset<sup>3</sup> — a collection  
107 of more than 1440 16S rRNA libraries from diverse coral species. We then used  
108 established phylogenetic comparative methods to test whether coral species' TLR or IL-  
109 1R gene families correlated with their microbiome structure or composition. The results  
110 identify potential drivers of microbiome structure among sequenced corals, and highlight  
111 the value of comparative analysis for supporting or refuting whether specific host traits  
112 underlie differences in microbiome structure between animal species. They further  
113 support the idea that different regions of coral anatomy differ not just in microbiome  
114 composition, but also in responsiveness to host traits — with the parts of the coral that  
115 are most strongly linked to host traits sometimes being a surprise.

## 116 **Methods**

### 117 **Genomic Data Acquisition**

118 Reference coral genomes were selected based on completeness and overlap with the  
119 Global Coral Microbiome Project dataset. Twelve coral genomes were downloaded from  
120 NCBI (<https://www.ncbi.nlm.nih.gov/>) and Reef Genomics (<http://reefgenomics.org>). A  
121 list of urls for the genomes can be found in **Table S1A**.

122

### 123 **Domain Annotation**

124 The downloaded coral genomes were used to locate TIR, leucine rich repeats (LRR), and  
125 immunoglobulin (Ig) containing genes using a custom pipeline found on github

126 ([https://github.com/zaneveld/GCMP\\_genomics](https://github.com/zaneveld/GCMP_genomics)). Genome analysis occurred in two steps.  
127 First, genomes were analyzed using TransDecoder (v5.5.0)  
128 (<https://github.com/TransDecoder/TransDecoder/wiki>) to identify candidate coding  
129 regions within the genome files. During this process, TransDecoder converts the  
130 nucleotide sequences in the genome file to possible amino acid sequences using different  
131 open reading frames needed to conduct this translation. The peptide file generated from  
132 TransDecoder was next searched with HMMER ([hmmer.org](http://hmmer.org), HMMER 3.3.2 (November  
133 2020)) to locate putative TIR domains as well as known TIR-associated domains. The  
134 TIR, LRR, and Ig associated peptide alignment files used in this search were downloaded  
135 from the Pfam website (<http://pfam.xfam.org/>). These 14 alignment files used are found  
136 in **Table S2B, C**. The Pfam domain alignment files were used to build profiles in  
137 HMMER using hmmbuild by reading in the alignment file and creating a new Hidden  
138 Markov Model (HMM) profile. The HMM profiles were then used to search the  
139 TransDecoder peptide files. The output file from hmmscan contained significant matches  
140 between the HMM profile and the transdecoder peptide file. The resultant hmmscan file  
141 was used to count the number of TIR-domain containing sequences found for each  
142 organism.

143  
144 TIR-domain containing genes were further subdivided by scanning each for LRR or Ig  
145 domains using the same procedure. TIR-domain-containing genes were then subdivided  
146 into categories based on their domains: TIR only (defined by TIR domain and no LRR or  
147 Ig domains), TLR (TIR and one or more LRR domains), and IL-1R (TLR and one or  
148 more Ig domains) genes. Numbers of each type of gene, and average numbers of Ig or  
149 LRR domains within each type of gene were used in further analysis.

150  
151 **Coral sampling and microbiome analysis**  
152 The Global Coral Microbiome Project (GCMP) coral mucus, tissue and skeleton samples  
153 reanalyzed here were originally collected and processed following the methods outlined  
154 in Pollock *et al.*, 2018<sup>3</sup>. Those methods are briefly restated here. All coral samples were  
155 collected by AAUS-certified scientific divers, in accordance with local regulations. These  
156 plus additional international samples were then resequenced with protocols standard for  
157 the Earth Microbiome Project<sup>28</sup>. Bacterial and archaeal DNA were extracted using the  
158 PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA; now Qiagen, Venlo,  
159 Netherlands). To select for the 16S rRNA V4 gene region, polymerase chain reaction  
160 (PCR) was performed using the following primers with illumina adapter sequences  
161 (underlined) at the 5' ends: 515F<sup>29</sup> 5'- TCG TCG GCA GCG TCA GAT GTG TAT  
162 AAG AGA CAG GTG YCA GCM GCC GCG GTA A -3' and 806R<sup>30</sup> 5'- GTC TCG  
163 TGG GCT CGG AGA TGT GTA TAA GAG ACA GGG ACT CAN VGG GTW TCT  
164 AAT -3'. PCR, library preparation and sequencing on an Illumina HiSeq (2x125bp) was  
165 performed by the EMP<sup>28,31</sup>. The resulting 16S rRNA amplicon sequences from the  
166 GCMP are available in the Qiita database Qiita (CRC32 id: 8817b8b8 and CRC32 id:  
167 ac925c85). Metadata for all GCMP samples are available in **Table S1B**.

168  
169 **16S library preparation, sequencing, and initial quality control**  
170 16S rRNA sequencing data were processed in Qiita<sup>32</sup> using the standard EMP workflow.  
171 Briefly, sequences were demultiplexed based on 12bp Golay barcodes using

172 “split\_libraries” with QIIME 1.9.1 default parameters<sup>33</sup> and trimmed to 100bp to remove  
173 low quality base pairs. Quality control (e.g., denoising, de-replication and chimera  
174 filtering) and identification amplicon sequence variants (ASVs) were performed using  
175 deblur 1.1.0<sup>34</sup> with default parameters. The resulting biom and taxonomy tables were  
176 obtained from Qiita (CRC32 id: 8817b8b8 and CRC32 id: ac925c85) and processed using  
177 a customized QIIME2 v. 2020.8.0<sup>35</sup> pipeline in python  
178 (github.com/zaneveld/GCMP\_global\_disease).

179

#### 180 **Mitochondrial annotation and quality control**

181 Taxonomic assignment of ASVs was performed using vsearch<sup>36</sup> using a modified version  
182 of the SILVA v. 138<sup>37</sup> taxonomic reference. The sole change to the SILVA138 reference  
183 was supplementation with additional coral mitochondrial reads obtained from metaxa2<sup>38</sup>.  
184 In benchmarks, this change greatly improves annotation of coral mitochondrial rRNAs,  
185 without increasing false positive taxonomic assignments<sup>39</sup>. This expanded taxonomy is  
186 referred to as “silva\_metaxa2” in code. After taxonomic assignment, all mitochondrial  
187 and chloroplast reads were removed (**Table S3**).

188 The bacterial phylogenetic tree was built using the SATé-enabled phylogenetic placement  
189 (SEPP) insertion technique with the q2-fragment-insertion plugin<sup>40</sup>, again using the  
190 SILVA v. 138<sup>37</sup> database as reference taxonomy. The final output from this pipeline  
191 consisted of a taxonomy table, ASV feature table and phylogenetic tree that were used for  
192 downstream analyses.

193

194 Phylogenetic comparison of innate immune gene repertoire and microbiome richness  
195 Genome TIR domains were compared to the microbiome data collected from the GCMP.  
196 The  $\alpha$  and  $\beta$ -diversity of the microbial community were compared to the TIR, TLR, and  
197 IL-R domain copy numbers using phylogenetically independent contrasts (PICs) with the  
198 phytools package in R. PICs use the coral species phylogenetic information in  
199 comparison with the TIR-associated domains and ASV and Gini Index information to  
200 assess the relatedness for  $\alpha$ -diversity. PIC analysis for phylogenetic  $\beta$ -diversity metrics  
201 (Weighted and Unweighted UniFrac) were created from three PC axes (PC1, PC2, and  
202 PC3) of a PCoA ordination. Analyses were conducted on all three axes. The PIC method  
203 takes into account a priori the importance of phylogenetic history on trait variation when  
204 extracting information in relation to the common ancestor. Code used for this analysis is  
205 found on github.

206

#### 207 **Microbial taxonomic analysis**

208 Microbial taxonomic analysis was conducted using the ANCOM-BC package in phyloseq  
209 in R<sup>41</sup>. Analyses were conducted at the class and family level with relation to IL-1R and  
210 TLR copy numbers. Lists of significant microbes were generated for mucus, tissue,  
211 skeleton, and all compartments. Heatmaps were generated to visualize positive and  
212 negative correlation of the microbial community.

213

214

215 **Results**

216

217 Sequenced coral genomes vary greatly in TIR-domain containing gene copy number.  
218 Previous analyses have reported significant variation in the copy number of TIR-domain  
219 containing genes among coral genomes. We annotated the genomic copy number of TIR-  
220 domain containing genes in 11 coral genomes that were also represented in microbiome  
221 data from the Global Coral Microbiome Project (GCMP) (**Table S1**). While some prior  
222 studies have analyzed both genomes and transcriptomes in order to maximize discovery  
223 of new TLR or IL-1R homologs, we chose to exclude transcriptomes from our analysis in  
224 order to prevent any potential confounding effects of some innate immune genes not  
225 being expressed in transcriptomes. As a result of these annotations (Methods), we  
226 identified numerous TLR, IL-1R and TIR-only genes (**Table S2, 4**). Despite these  
227 methodological differences, our annotations mostly agree in trend and rank with prior  
228 studies of coral innate immune repertoires<sup>26,42</sup>.

229

230 Many innate immune genes have modular structures based on the domains that they  
231 contain, and TIR domains commonly co-occur with several other domains within innate  
232 immune genes. We further subdivided TIR-domain containing genes based on the other  
233 domains present: genes with both TIR and immunoglobulin (Ig) domains were annotated  
234 as interleukin-1 receptors (IL-1R) while genes with both TIR domains and leucine rich  
235 repeat (LRR) domains were annotated as toll-like receptors (TLR) (**Fig. 1**). We also  
236 analyzed the total count of TIR domains, regardless of which gene they were part of and  
237 the presence or absence of other co-associated domains.

238

239 In keeping with past work<sup>26,42</sup>, we find that both the copy number of TLR, IL-1R and  
240 TIR-only genes, and the total abundance of their component TIR, LRR and Ig domains  
241 varies greatly across coral genomes (**Table 1**).

242

243 **Coral IL-1R and TLR gene family copy number correlate with overall microbiome  
244 richness and evenness.**

245 In order to determine whether TLR or IL-1R might regulate microbiome biodiversity, we  
246 used phylogenetic independent contrasts (PIC) analysis to correlate changes in the  
247 genomic copy number of IL-1R and TLR against changes in microbiome richness or  
248 evenness during coral evolution.

249

250 We measured microbiome richness as the natural log of the observed number of amplicon  
251 sequence variants (ASVs) per 1000 sequences (**Table S5A**) (see Methods). Microbiome  
252 richness was significantly reduced by increases in the copy number of IL-1R (PIC  $R^2 =$   
253 0.835,  $q_{FDR} = 0.00055$ ; **Fig. 2B, D; Fig. S1C, D; Table S6A**), or TLR (PIC  $R^2 = 0.707$ ,  
254  $q_{FDR} = 0.0076$ ; **Fig. 2 C, D; Fig. S1E, F; Table S6A**). Thus, corals that harbor more TLR  
255 or IL-1R gene copies tend to have less diverse microbiomes, and vice versa. In manual  
256 inspection of the data, there were several striking examples of this statistical trend (**Table**

257 **S7).** In the genus *Porites*, *P. lutea* had more than twice as many IL-1R and TLR gene  
258 copies as the closely related *P. rus* (14 IL-1R copies and eight TLR gene copies in *P.*  
259 *lutea* vs. six IL-1R copies and two TLR copies in *P. rus*; **Table 1**). Consistent with the  
260 idea that *P. lutea*'s expanded gene repertoire may play a role in microbiome filtering, *P.*  
261 *lutea*'s microbiome was roughly half as diverse as that of *P. rus* (56 ASVs/1000 reads vs.  
262 114 ASVs/1000 reads, across all sample types).

263  
264 We also quantified the microbiome evenness of each coral species in the analysis using  
265 the Gini Index (**Table S5B**), which takes on its highest value when ecological  
266 communities are least even. Gini index scores were strongly and significantly negatively  
267 correlated with the genomic copy number of IL-1R genes (PIC  $R^2 = 0.876$ , qFDR =  
268 0.000357; **Fig. S5C, D; Table S6A**) and TLR genes (PIC  $R^2 = 0.805$ , qFDR = 0.0014; **Fig.**  
269 **S5E, F; Table S6A**), indicating that corals with more IL-1R or TLR gene copies have  
270 higher evenness.

271  
272 These results support the prior hypothesis that IL-1R and TLR gene family expansions  
273 may influence coral microbiome structure. The magnitude of the effect was, however,  
274 quite surprising, with IL-1R gene copy number explaining ~83% of the variation in  
275 microbiome richness, and ~88% of the variation in microbiome evenness, among coral  
276 species in the analysis. Although necessarily limited to coral species for which genomes  
277 are available, these correlations are much stronger than the effects of several biotic and  
278 abiotic factors in the same GCMP dataset, including depth, temperature, and turf-algal  
279 contact<sup>3</sup>.

280  
281 **Endolithic skeleton microbiomes drive correlations between TLR and IL-1R gene**  
282 **family expansion and microbiome structure.**

283 To this point, all our results were conducted by correlating genomic features of corals  
284 against the overall microbiome diversity of all available microbiome samples from each  
285 species in the GCMP dataset. However, microbiome richness has been shown to vary  
286 between coral compartments<sup>3</sup>. We expected that host tissues — where the immune  
287 system could most obviously act — would have microbiomes that most closely correlate  
288 with the innate immune gene repertoire of the host.

289  
290 To test this idea, we separated coral microbiome samples into those that derive from  
291 mucus, tissue or endolithic skeleton, and repeated tests for correlations between coral  
292 innate immune repertoires and microbiome richness and evenness within each of those  
293 regions of anatomy.

294  
295 Contrary to our hypothesis, coral endolithic skeleton — not tissue or mucus — was the  
296 sole driver of correlations between coral innate immune repertoire and microbiome  
297 richness (**Fig. 2E-G; Fig. S1-4; Table S6, 8**) and evenness (**Fig. S5-8; Table S6, 8**).  
298 Microbiome richness in coral endolithic skeleton was significantly correlated with IL-1R  
299 ( $R^2 = 0.944$ , qFDR =  $2.05 \times 10^{-5}$ ; **Fig. 2F, Fig. S4C, D; Table S6D**), and TLR ( $R^2 =$   
300  $0.90798$ , qFDR =  $9.36 \times 10^{-5}$ ; **Fig. 2G; Fig. S4E, F; Table S6D**) gene copies, whereas  
301 tissue (**Fig. S3; Table S5C**) and mucus (**Fig. S2; Table S6B**) microbiome richness was  
302 not. Microbiome evenness showed similar patterns (**Fig. S6-8; Table S6, 8**).

303 Previous studies have reported that coral endolithic skeleton microbiomes are more  
304 species-rich than mucus or tissue, and show both species-specificity and signals of  
305 phylosymbiosis with their coral hosts<sup>3</sup>. Our results further suggest that endolithic skeleton  
306 microbiome diversity has tracked gene family expansions or contractions of coral TLR  
307 and IL-1R genes over evolution.

308

309

310 **Microbiome composition varies with IL-R and TLR gene copy numbers.**

311 In addition to regulating microbiome richness and evenness, coral innate immune systems  
312 may also influence coral microbiome composition. If so, we might expect microbiome  
313 composition to correlate with the repertoire of innate immune proteins encoded in coral  
314 genomes. To test this, we compared differences in overall microbiome composition for  
315 each pair of coral species using two phylogenetic beta diversity metrics: Weighted  
316 UniFrac and Unweighted UniFrac. Using these beta-diversity distance metrics, we  
317 conducted principal coordinates analysis (PCoA), using the first three PC axes (PC1, PC2,  
318 and PC3) of the PCoA ordination (Methods). We correlated the microbiome PC  
319 coordinates against the number of predicted TLR or IL-1R gene copies (**Fig. 3; Table**  
320 **S9**).

321

322 Innate immune gene copy numbers strongly and significantly correlated with microbiome  
323 composition. When all samples were considered together (irrespective of anatomical  
324 compartment) Weighted UniFrac PC1 correlated strongly with the genomic copy number  
325 of IL-1R genes (PIC Weighted UniFrac PC1  $R^2 = 0.917$ ,  $q_{FDR} = 0.00012$ ; **Fig. 3A, C, D;**  
**Table S9A**) and TLR genes (PIC Weighted UniFrac PC1  $R^2 = 0.77$ ,  $q_{FDR} = 0.0028$ ; **Fig.**  
326 **3B, E, F; Table S9A**). Unweighted UniFrac PC1 showed similar trends (**Table S9A**).  
327 These results indicate that gene family expansions or contractions of the TLR and IL-1R  
328 gene families over evolution corresponded to dramatic changes in overall microbiome  
329 composition.  
330

331 We repeated the above protocol separately on the mucus, tissue, and skeleton  
332 compartments separately (**Table S9B-D**). In coral mucus, tissue and skeleton, IL-1R gene  
333 copy number significantly correlated with Weighted UniFrac PC1 of the microbiome  
334 ( $PIC R^2 = 0.72-0.81$ ,  $FDR q < 0.01$ ; **Table S9A-C**). TLR gene copy number significantly  
335 correlated with Weighted UniFrac PC1 in coral mucus and tissue ( $PIC R^2 = 0.73$ ,  $q_{FDR} =$   
336  $0.004$ ; **Table S9B, C**) but not endolithic skeleton (**Table S9D**). In qualitative  
337 (presence/absence) analysis of microbiome  $\beta$ -diversity, IL-1R gene copy number  
338 correlated with Unweighted UniFrac PC1 in coral tissue and skeleton compartments, but  
339 not mucus (**Table S9B-D**), while TLR gene copy number correlated with Unweighted  
340 UniFrac PC1 in coral tissue but not mucus or skeleton (**Table S9B-D**).

341

342 These  $\beta$ -diversity results were more complex than the straightforward pattern of  
343 associations between innate immune gene copy number and microbiome richness. In  
344 coral tissue both IL-1R gene copy number and TLR gene copy number correlated with  
345 key aspects of quantitative (Weighted UniFrac PC1) and qualitative (Unweighted  
346 UniFrac PC1) microbiome  $\beta$ -diversity, in keeping with our original expectation that  
347 immunity should act strongly on the tissue-associated microbiome. In mucus, both TLR  
348 and IL-1R gene copy numbers appeared correlated with microbiome  $\beta$ -diversity when

349 using quantitative but not qualitative metrics, suggesting immunity might influence  
350 microbial relative abundance more strongly than microbial presence/absence. In skeleton,  
351 IL-1R appeared correlated with IL-1R gene copy number, but not TLR gene copy number  
352 regardless of  $\beta$ -diversity metric. Together, these results suggest anatomically specific  
353 correlations between gene family expansion of some key innate immune genes and  
354 microbiome  $\beta$ -diversity.

355

356 **IL-1R and TLR copy number is associated with differential abundance of key  
357 microbes.**

358 We sought to identify microbial taxa that may be influenced by IL-1R or TLR gene copy  
359 number. This analysis could be confounded by the compositional nature of coral  
360 microbiome data. Therefore ANCOM-BC<sup>41</sup>, which accounts for compositionality, was  
361 used for the analysis. We first tested whether IL-1R copy number correlated with  
362 microbial differential abundance in all samples regardless of tissue compartment (i.e.  
363 ‘all’). In this overall analysis, IL-1R copy number correlated with the differential  
364 abundance of 102 families of bacteria and archaea, while TLR copy number correlated  
365 with 88 families (**Table S10A**).

366 Correlations between microbiome composition and IL-1R or TLR copy number varied  
367 with anatomy. The abundance of 38 bacterial families were significantly correlated with  
368 TLR copy number (ANCOM-BC qFDR < 0.05 in mucus, tissue, and skeleton; **Tables**  
369 **S10B-D; Fig. S11-S13**) and 38 bacterial families were also significantly and consistently  
370 correlated with IL-1R copy number (ANCOM-BC qFDR < 0.05 in mucus, tissue, and  
371 skeleton; **Table S10B-D**; raw heatmaps in **Fig. S14-S16**), both regardless of  
372 compartment. In contrast to the bacterial families with consistent interrelationships, some  
373 were specific to one or more compartments. One example are Rickettsiaceae, which live  
374 intracellularly in host cells. Rickettsiaceae relative abundance in tissue decreased with IL-  
375 1R (ANCOM-BC coef = -0.011, W = 0.33, qFDR = 0; **Fig. 4, Fig. S15; Table S10C**) and  
376 TLR (ANCOM-BC coef = -0.028, W = 1.36, qFDR = 0; **Fig. 4, Fig S12, Table S10C**), but  
377 this taxon was not present in mucus (**Fig. S11, Fig S14**) or skeleton (**Fig. S13, Fig. S16**).  
378 *Nitrosopumiliaceae* archaea were notable for correlating with IL-1R and TLR gene copy  
379 number across all compartments (all ANCOM-BC qFDR < 0.05; **Fig S11-S16**).  
380

381

382 **The domain architecture of TLR but not IL-1R genes is associated with microbiome  
richness.**

383 IL-1R and TLR genes are known to vary in the domain architecture of their extracellular  
384 regions, with variable numbers of Ig or LRR sensing domains, respectively. We reasoned  
385 that if higher copy numbers of TLR or IL-1R genes are associated with microbiome  
386 richness because they act as filters on the microbiome, the same selective pressures (for  
387 greater specificity in microbial associates) might also influence the domain architecture  
388 of extracellular sensing components within TLR or IL-1R genes. To test this, we  
389 analyzed the average number of LRR and Ig domain copy numbers associated with each  
390 TLR or IL-1R gene in each coral species.  
391

392

393 Interestingly, we find that there is a significant positive correlation between LRR domain  
394 copy number per TLR genes and TLR copies ( $R^2 = 0.437$ ,  $p = 0.0159$ ; **Fig. 5A, B; Fig.**  
**S9A**) but not Ig copy number with IL-1R ( $R^2 = 0.00067$ ,  $p = 0.345$ ; **Fig. 5C, D; Fig.**

395 **S9B**). In other words, corals with more TLR genes also have TLR genes with more LRR  
396 domains each.

397

398 Furthermore, we find that microbiome richness is correlated negatively with the average  
399 number of LRR domain copies ( $\text{PIC } R^2 = 0.748$ ,  $p = 0.00059$ ; **Fig 5B**) but not Ig domain  
400 copies ( $\text{PIC } R^2 = 0.341$ ,  $p = 0.059$ ; Fig 5D). That is, corals whose TLR genes typically  
401 have more elaborate domain architectures also tend to have simpler (or more specific)  
402 microbial associations. Importantly, the genomic copy number of LRR or Ig domains in  
403 total (rather than per gene) did not correlate with microbiome richness and beta diversity  
404 (**Table S8, S11**), so this finding does not simply recapitulate our previous findings  
405 regarding gene copy number.

406

#### 407 **Responses to IL-1R and TLR are correlated between bacterial families.**

408 The microbe associated molecular patterns (MAMPs) detected by species-specific  
409 members of the TLR and IL-1R gene families could in principle be the same or  
410 independent. If independent, the correlation between any given microorganism and TLR  
411 gene family expansions would be independent of IL-1R gene family expansions. To test  
412 this, we analyze whether the correlation coefficients between each microbial family's  
413 relative abundance with TLR or IL-1R copy number were themselves correlated with one  
414 another. Microbial families' responses to IL-1R and TLR gene family copy numbers  
415 were themselves very strongly correlated ( $R^2 = 0.9062$ ; **Fig S11**). Thus, microbes that are  
416 in high abundance in corals with many IL-1R gene copies also tend to be in high  
417 abundance in corals with many TLR gene copies. This suggests that TLR and IL-1R gene  
418 family expansions sculpt coral microbiomes in similar, rather than contrasting ways.

## 419 **Discussion**

420 By combining coral microbiome and genomic data in a comparative framework, we  
421 demonstrate that TIR-domain containing innate immune gene repertoires strongly  
422 correlate with microbiome structure and diversity. These results suggest that gene family  
423 expansions of innate immune genes may have contributed to differences in the structure  
424 of coral microbiomes across millions of years of evolution. Those differences in  
425 immunity and microbiome structure, in turn, may influence the ability of modern  
426 scleractinian corals to survive escalating challenges from disease and climate change.  
427

#### 428 **Coral innate immune systems may be filtering microbiome membership.**

429 A key finding of the Earth Microbiome Project was that the diverse communities of  
430 microbes associated with animals and plants are nonetheless much less diverse than most  
431 environmental microbiomes<sup>28</sup>. Indeed, these animal-associated microbiomes were found  
432 to show high nestedness relative to the environment, indicating that what lives on animals  
433 is mostly — though certainly not entirely — a subset of environmental microbes<sup>28</sup>. This  
434 suggests that filtering of environmental microbes is one key process shaping animal and  
435 plant microbiomes. That filtering is likely to be genetically encoded, since more closely  
436 related animals tend to have more similar microbiomes ('phylosymbiosis'<sup>3,43–45</sup>),  
437 excepting important deviations from this trend such as those driven by the evolution of

438 specialized diets<sup>46,47</sup> or flight<sup>48</sup>. Could evolutionary changes in host immunity drive this  
439 trend, by altering which environmental microbes are excluded?  
440  
441 Our results in corals suggest that, for this group at least, immunity plays an important role  
442 in overall microbiome richness and evenness. Both coral microbiome composition<sup>3</sup>, and  
443 coral innate immune responses<sup>49,50</sup> have long been known to vary between species.  
444 Within coral populations, there is evidence that variation in immune activity between  
445 coral fragments correlates with their microbiome structure. For example, in *Montipora*  
446 *capitata*, increases in phenoloxidase activity correlated with decreased microbiome  
447 richness<sup>51</sup>. If such correlations extended across species, it could begin to explain why  
448 coral microbiomes differ so greatly in bacterial and archaeal biodiversity.  
449 TIR-domain containing genes have undergone large gene-family expansions in some  
450 lineages of corals, which has been proposed as a driver of differences in coral  
451 microbiome richness<sup>26</sup>. Our findings support this hypothesis, and suggest that expansions  
452 of key innate immune genes reduce microbiome richness by allowing for sensing and  
453 exclusion of more diverse groups of microbes.  
454

455 **Microbiome composition changes with TLR and IL-1R copy number**  
456 We find a relationship between microbiome  $\beta$ -diversity and the copy number of TIR-  
457 containing innate immune genes. Immune genes of animals and plants have in the past  
458 been reported to alter the  $\beta$ -diversity of symbiotic microbes, including commensals. For  
459 example, using Toll-like receptor (TLR) 5 deficient compared to wild type mice, TLR  
460 genes have been correlated with membership and host-microbial interactions with  
461 specific members of the mouse gut microbiome, although the overall effect of TLR on  
462 gut microbiome  $\beta$ -diversity remains controversial<sup>52</sup>. Similarly, *Arabidopsis* with loss of  
463 function mutations in the pattern recognition receptor (PRR) gene FLS2, have altered  
464 rhizosphere microbiome  $\beta$ -diversity relative to wild type controls<sup>53</sup>. Our findings extend  
465 these observations to coral microbiomes, and suggest that differences in innate immunity  
466 may explain substantial portions of the known differences in microbiome structure  
467 between coral species.  
468  
469 In our results, the relative abundances of diverse microbial taxa correlated with TLR or  
470 IL-1R gene copy number. Interestingly, these effects were not independent: microbes that  
471 correlated with TLR copy number also tended to be correlated with IL-1R copy number.  
472 This might reflect either that TLR and IL-1R gene family expansions are driven by  
473 similar selective pressures and therefore tend to co-occur, or that both types of gene  
474 family expansion influence the microbiome in similar ways.

475  
476 **The relationship between innate immune gene repertoire and the microbiome is**  
477 **anatomically-specific**  
478 While there are clear reasons to expect coral innate immune gene repertoire to affect  
479 tissue-associated coral microbiomes, our results suggest that these gene family  
480 expansions have even more clear-cut effects on coral's endolithic skeleton microbiomes.  
481 Corals show compartmentalized differences in molecular function<sup>54</sup> and microbial  
482 richness and composition across anatomy<sup>3</sup>. Perhaps surprisingly, the microbiome of coral  
483 endolithic skeleton has been shown to be far more diverse than coral tissues or mucus<sup>3</sup>. In

484 our results, endolithic skeleton microbiomes were more strongly correlated with host  
485 innate immunity than either mucus or tissue.

486  
487 These results suggest that coral immunity strongly influences endolithic microbiomes.  
488 They also underline the importance of efforts to reevaluate how we conceptualize coral  
489 immunity and calcification. While coral calcification and immunity are typically thought  
490 of separately, recent work synthesizes these two fields<sup>55</sup>, noting that skeleton is a key  
491 barrier against pathogens, and in many species contains diverse defensive chemical  
492 compounds and enzymes. These notably include melanin, which is a key aspect of  
493 immune defense in many non-vertebrate lineages.

494  
495 **Disease-susceptible corals have more, not fewer, TIR-domain containing genes.**  
496 One counter-intuitive aspect of our results is that the coral taxa with the largest numbers  
497 of TIR-containing innate immune genes tend to be those, such as *Acropora*, regarded as  
498 more susceptible to disease<sup>56</sup>. This is surprising, because we might expect more disease-  
499 susceptible coral species to have less diverse innate immune gene repertoires. How gene-  
500 family expansion, the commensal microbiome, and coral disease interact is a rich topic  
501 for future investigation. Our results here suggest several hypotheses that could be  
502 explored. It could be that more diverse microbial ecosystems are less susceptible to  
503 invasion by pathogens, as per Elton's 1958 biotic resistance hypothesis<sup>57,58</sup>. Alternatively,  
504 less rich microbiomes may be less functionally redundant, increasing the risk that loss of  
505 any particular beneficial microbe may degrade host health<sup>59,60</sup>. Finally, the damage  
506 threshold hypothesis<sup>61</sup> proposes that corals differ in immune strategy, with many slow-  
507 growing corals constitutively expressing a variety of innate immune genes, while other  
508 fast-growing corals adopt a reactive strategy, which avoids expending resources on  
509 baseline expression of many innate immune genes in order to maximize growth or  
510 fecundity. If so, then reactive, fast-growing corals may experience stronger selective  
511 pressures to diversify sensors that can detect pathogens or cellular damage, since their  
512 baseline level of protection is low. TIR-domain containing gene family expansions in  
513 these reactive corals may enable more sensitive and/or specific responses to pathogen  
514 exposure.

515  
516 **Conclusions**  
517

518 We find that among sequenced coral genomes, gene family expansions of TLR and IL-1R  
519 genes are correlated with alterations in microbiome structure, and reductions in  
520 microbiome richness — with this apparent interplay between innate immunity and the  
521 microbiome most noticeable in coral endolithic skeleton. These findings are consistent  
522 with the idea that animal immunity sculpts microbiome structure and composition in part  
523 by sensing and filtering out many environmental microbes. This interpretation of the  
524 correlations we found between gene copy number and the microbiome is reinforced by  
525 the correlation between microbiome richness and the domain architecture within TLR  
526 genes, wherein there are more sensing (i.e. LRR) domains per TLR gene in corals that  
527 have lower microbiome richness. Our results further underscore the importance of  
528 distinguishing coral microbiomes across anatomy, and of exploring how coral innate  
529 immunity regulates corals' diverse endolithic microbiomes. Finally, these results

530 emphasize that integrating expanding coral genome and microbiome datasets in  
531 comparative frameworks is a promising approach that will help to uncover the  
532 interactions between immunity, the microbiome, and reef health.

### 533 **Data Availability**

534 Biom and taxonomy tables are available on Qiita (Study ID 10895). Analysis code is  
535 available on GitHub: [https://github.com/zaneveld/GCMP\\_Genomics/](https://github.com/zaneveld/GCMP_Genomics/)

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### 562 **Author Contributions**

563 TB, DS, JZ analyzed the data; TB, JZ, DS wrote the manuscript; RM, JFP, MM, and JZ  
564 collected GCMP data and metadata; all authors edited the manuscript.

### 565 **Conflict of Interest Statement**

566 The authors declare no conflict of interest.

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## 571 Tables

572

573 **Table 1.** TIR domain containing elements and their associated numbers per species

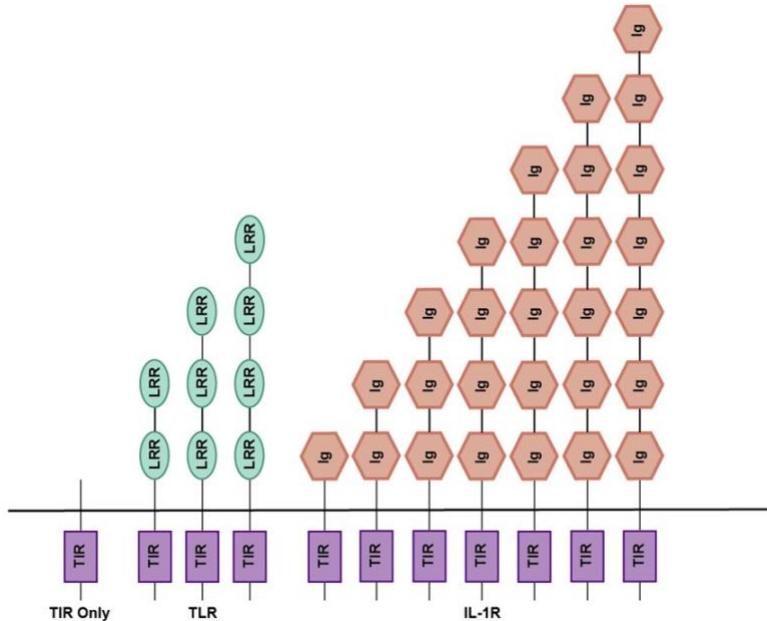
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Coral Species	TIR Isoforms	IL-1R Copies	TLR Copies	Richness (ln ASVs /1000 reads)	Genome Sequence Reference
<i>Acropora hyacinthus</i>	37	11	8	3.788	Sinzato <i>et al</i> 2020
<i>Acropora cytherea</i>	33	10	7	3.619	Sinzato <i>et al</i> 2020
<i>Pocillopora damicornis</i>	24	6	5	4.368	Cunning <i>et al</i> 2018
<i>Pocillopora verrucosa</i>	28	7	7	4.369	Buitrago-Lopez <i>et al</i> 2020
<i>Orbicella faveolata</i>	27	7	6	4.484	Prada <i>et al</i> 2016
<i>Stylophora</i>	23	8	6	4.236	Chen <i>et al</i> 2008
<i>Galaxea fascicularis</i>	19	3	1	5.266	Ying <i>et al</i> 2018
<i>Porites lutea</i>	36	14	8	4.016	Robbins <i>et al</i> 2019
<i>Fungia fungites</i>	32	7	8	4.3	Ying <i>et al</i> 2018
<i>Montipora capitata</i>	27	5	1	4.034	Helmkampf <i>et al</i> 2019
<i>Porites rus</i>	29	6	2	4.739	Celis <i>et al</i> 2018

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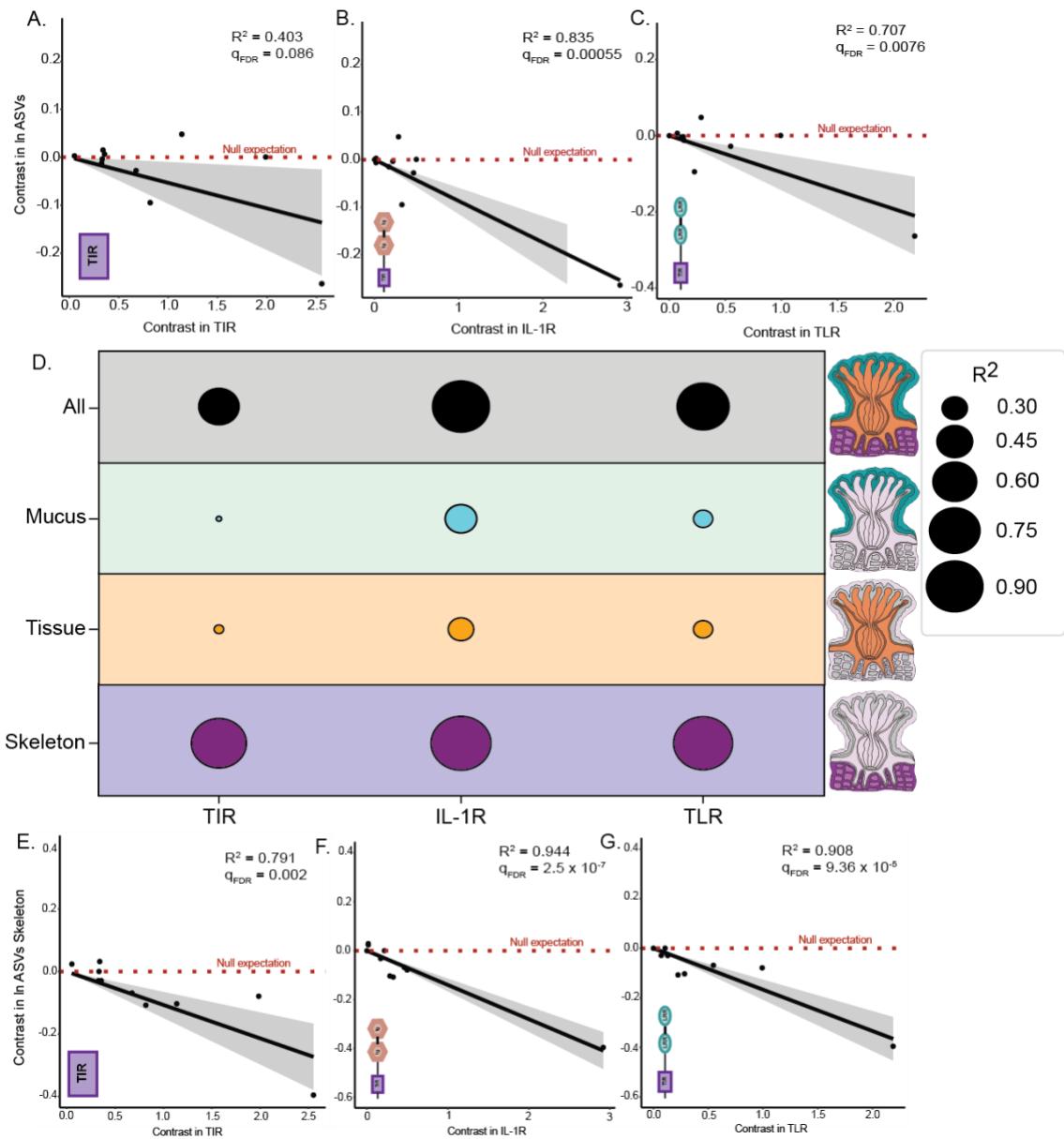
579 **Figures and Figure Legends**

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581

582 **Fig. 1.** TIR containing gene architecture in coral genomes. Diagram depicts all domains  
 583 present in TIR only, TLR, or IL-1R genes identified in this study. TIR: Toll-Interleukin  
 584 Repeat; Ig: Immunoglobulin; LRR: Leucine Rich Repeat; TLR: Toll/Interleukin-like  
 585 Receptor; IL-1R: Interleukin-1 Receptor.

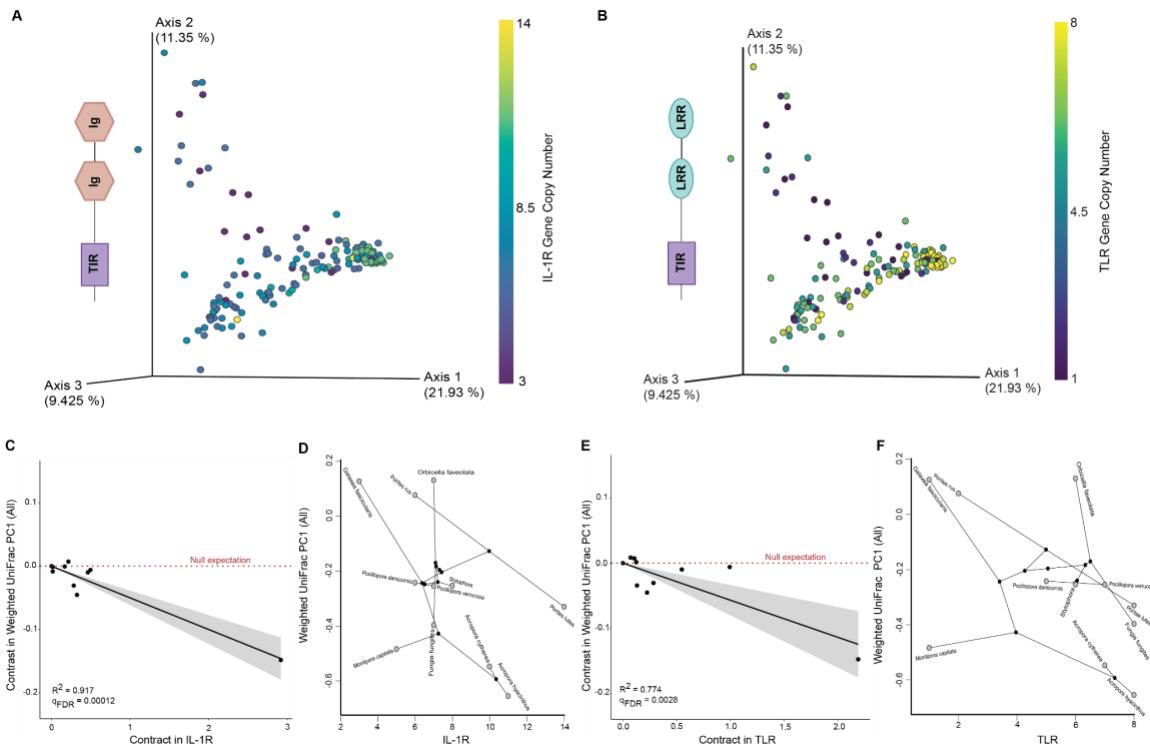
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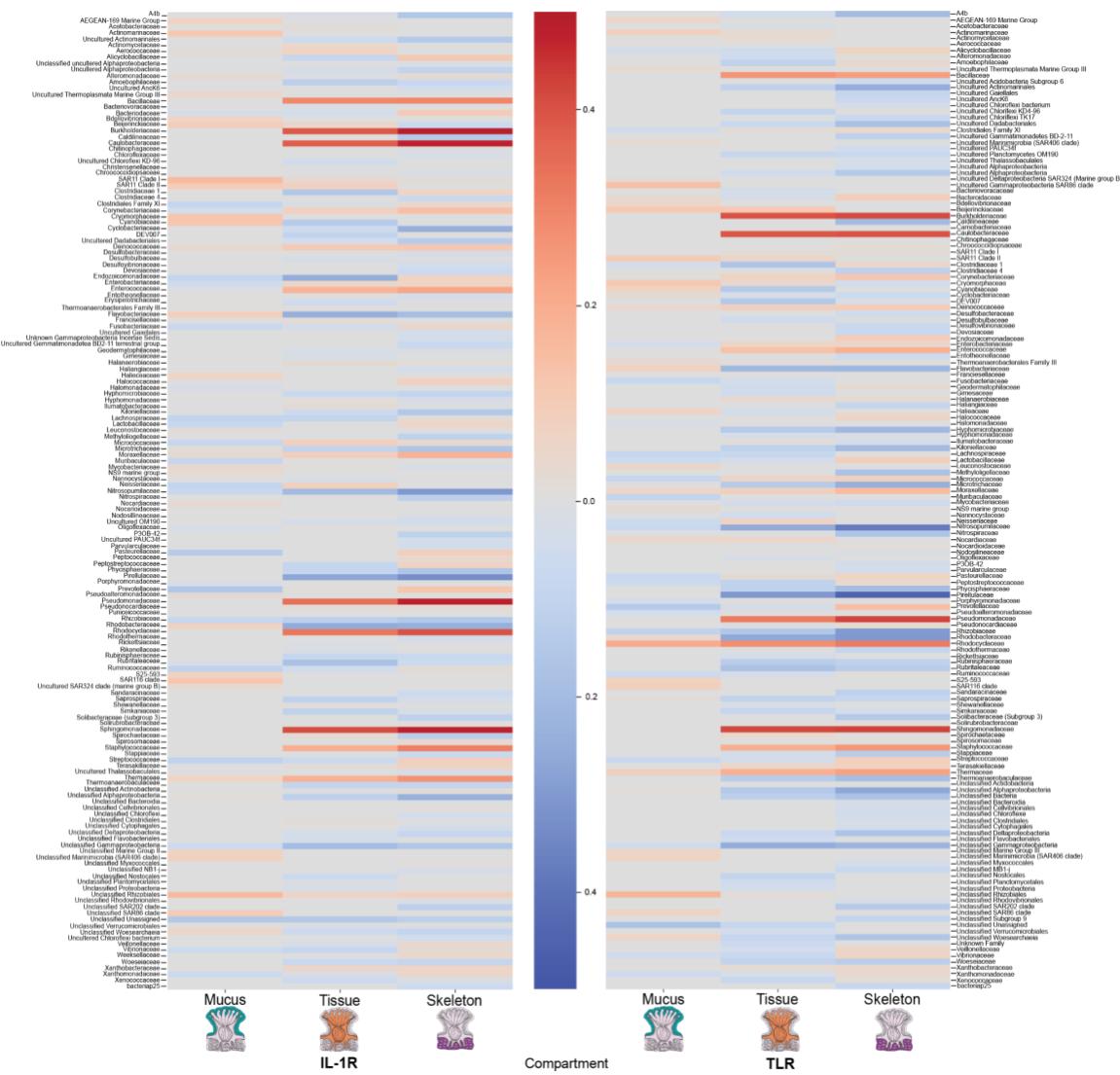
589 **Fig. 2.** Rows show phylogenetic independent contrast analysis of genomic copy number  
590 of innate immune components against log microbiome richness (ln ASVs), for all **A.** TIR  
591 domains ( $R^2 = 0.403$ ,  $q_{FDR} = 0.086$ ,  $p = 0.021$ ); **B.** IL-1R genes ( $R^2 = 0.835$ ,  $q_{FDR} =$   
592  $0.00055$ ,  $p = 5.13 \times 10^{-15}$ ); **C.** TLR genes ( $R^2 = 0.707$ ,  $q_{FDR} = 0.0076$ ,  $p = 0.0012$ ).  
593 Shading in phylogenetic independent contrasts analysis indicates the 95% confidence  
594 interval of the mean. **D.**  $R^2$  values from correlations of the genomic copy number of  
595 innate immune components against microbiome richness, organized by compartment.  
596 The bottom row uses microbiome data from coral skeleton only to show phylogenetic  
597 independent contrasts of innate immune components vs. log microbiome richness for: **E.**  
598 TIR-only genes ( $R^2 = 0.791$ ,  $q_{FDR} = 0.002$ ,  $p = 0.000252$ ); **F.** IL-1R genes ( $R^2 = 0.944$ ,  
599  $q_{FDR} = 2.15 \times 10^{-5}$ ,  $p = 6.39 \times 10^{-7}$ ); **G.** TLR genes ( $R^2 = 0.90798$ ,  $q_{FDR} = 9.36 \times 10^{-6}$ ,  
600  $p = 5.85 \times 10^{-6}$ ). No correlations had outliers, as defined by studentized residuals of  $>3$  in  
601 absolute value.

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**Fig. 3.** Comparison of innate immune gene repertoire and microbiome composition. **A.** Principle Coordinates Analysis (PCoA) of Weighted UniFrac Beta diversity distances for samples from all coral compartments, colored by genomic IL-1R copy number in the coral host. **B.** PCoA ordination of Weighted UniFrac distances for all compartments colored by genomic copy number for TLR genes. **C.** Phylogenetic independent contrasts regression of IL-1R copy number vs. Weighted UniFrac PC1 ( $R^2 = 0.917$ ,  $q_{\text{FDR}} = 0.00012$ ). **D.** Phylogenomorphospace of IL-1R copy number vs. Weighted UniFrac PC1. **E.** Phylogenetic independent contrasts regression of TLR copy number vs. Weighted UniFrac PC1 ( $R^2 = 0.774$ ,  $q_{\text{FDR}} = 0.0028$ ). **F.** Phylogenomorphospace of IL-1R copy number vs. Weighted UniFrac PC1. No correlations had outliers, as defined by studentized residuals of  $>3$  in absolute value.



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**Fig. 4.** Heatmap showing microbial families significantly correlated with IL-1R and TLR copy numbers. Columns show correlations between the relative abundance of microbial families and IL-1R and TLR copy numbers in the mucus, tissue, and skeleton, respectively.

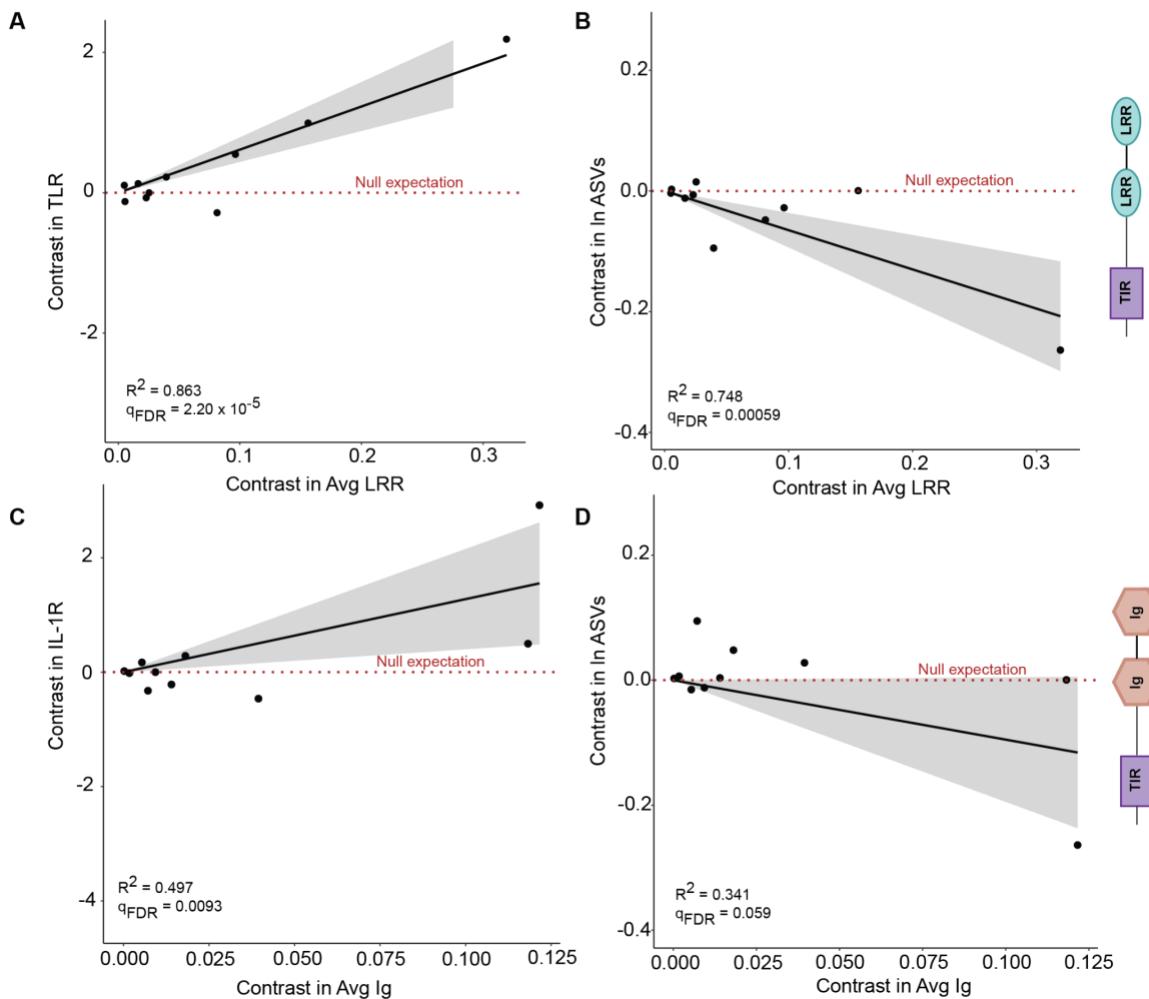
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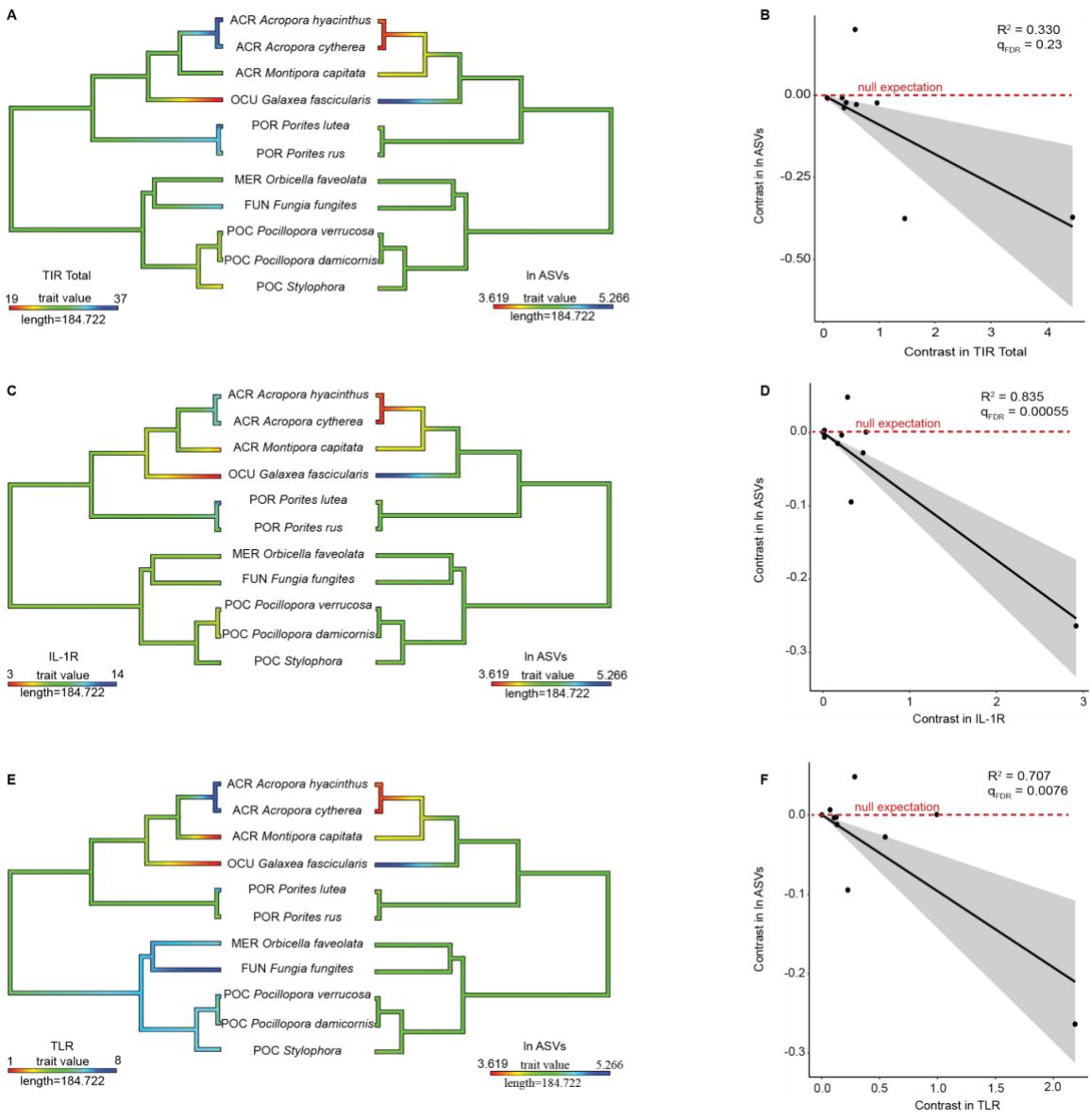
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**Fig. 5.** Domain copy numbers influence microbiome richness. Phylogenetic contrasts comparing **A.** average LRR domain copies per TLR vs TLR domain copy number ( $R^2 = 0.863$ ,  $q_{FDR} = 2.20 \times 10^{-5}$ ), **B.** average LRR domain copies per TLR vs ln ASVs ( $R^2 = 0.748$ ,  $q_{FDR} = 0.00059$ ), **C.** average Ig domain copies per IL-1R vs IL-1R copy number ( $R^2 = 0.497$ ,  $q_{FDR} = 0.0093$ ), and **D.** average Ig domain copies per IL-1R vs ln ASVs per genome ( $R^2 = 0.341$ ,  $q_{FDR} = 0.059$ ).

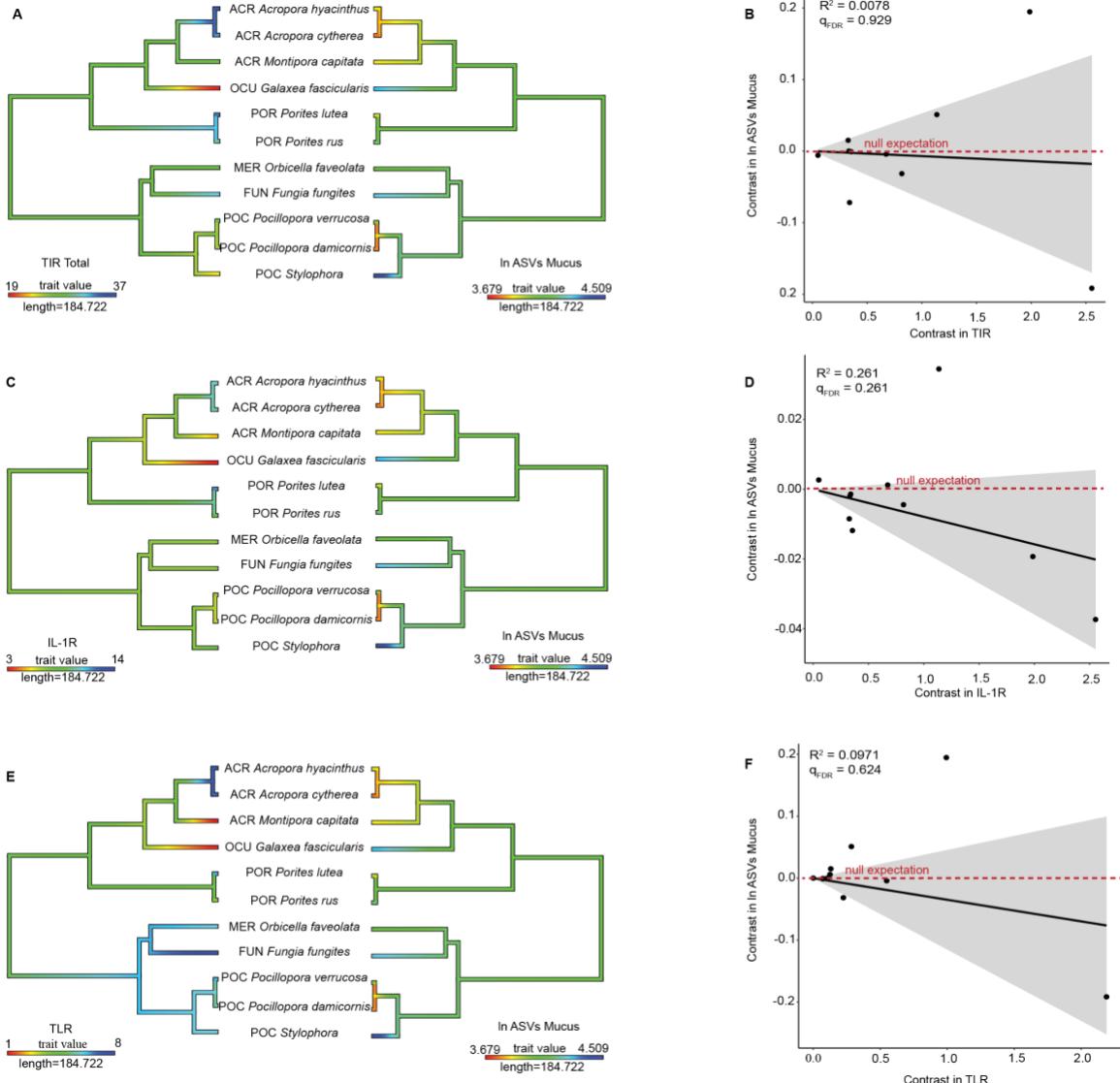
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637 **Supplementary Figures**  
638  
639 Supplementary Figures 1-4: Richness vs. gene copy number for 1) All compartments 2)  
640 mucus, 3 tissue, 4 skeleton  
641  
642 Supplementary Figures 5-8: Evenness vs. gene copy number for 1) All compartments 2)  
643 mucus, 3 tissue, 4 skeleton  
644  
645 Supplementary Figure 9: Comparison of domain copy number within genes and gene  
646 copies within genomes.  
647  
648 Supplementary Figure 10: Correlated responses of microbial families to TLR and IL-1R  
649 gene copy number  
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651 Supplementary Figures 11-16. Relative abundance of microbial classes in mucus 11,  
652 tissue 12 and skeleton 13 samples sorted by TLR copy number or IL-1R copy number  
653 (14,15,16).  
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**Fig. S1. Phylogenetic comparison of coral microbiome richness and innate immune repertoire for all compartments combined.** Rows show ancestral state reconstructions (left column) of innate immune gene copy number and microbiome richness, as well as phylogenetic independent contrast analysis for all predicted isoforms (right column) of **A,B** TIR-only genes ( $R^2 = 0.330$ ,  $q_{FDR} = 0.23$ ,  $p = 0.065$ ); **C,D** IL-1R genes ( $R^2 = 0.834$ ,  $q_{FDR} = 0.00055$ ,  $p = 5.14 \times 10^{-5}$ ); **E,F** TLR genes ( $R^2 = 0.707$ ,  $q_{FDR} = 0.0076$ ,  $p = 0.0012$ ) compared against log microbiome richness (ln ASVs). Shading in phylogenetic independent contrasts analysis indicates the 95% confidence interval of the mean.

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676 **Fig. S2. Phylogenetic comparison of coral microbiome richness and innate immune**  
 677 **repertoire for the mucus compartment.** Rows show ancestral state reconstructions (left

678 column) of innate immune gene copy number and microbiome richness, as well as

679 phylogenetic independent contrast analysis for all predicted isoforms (right column) of

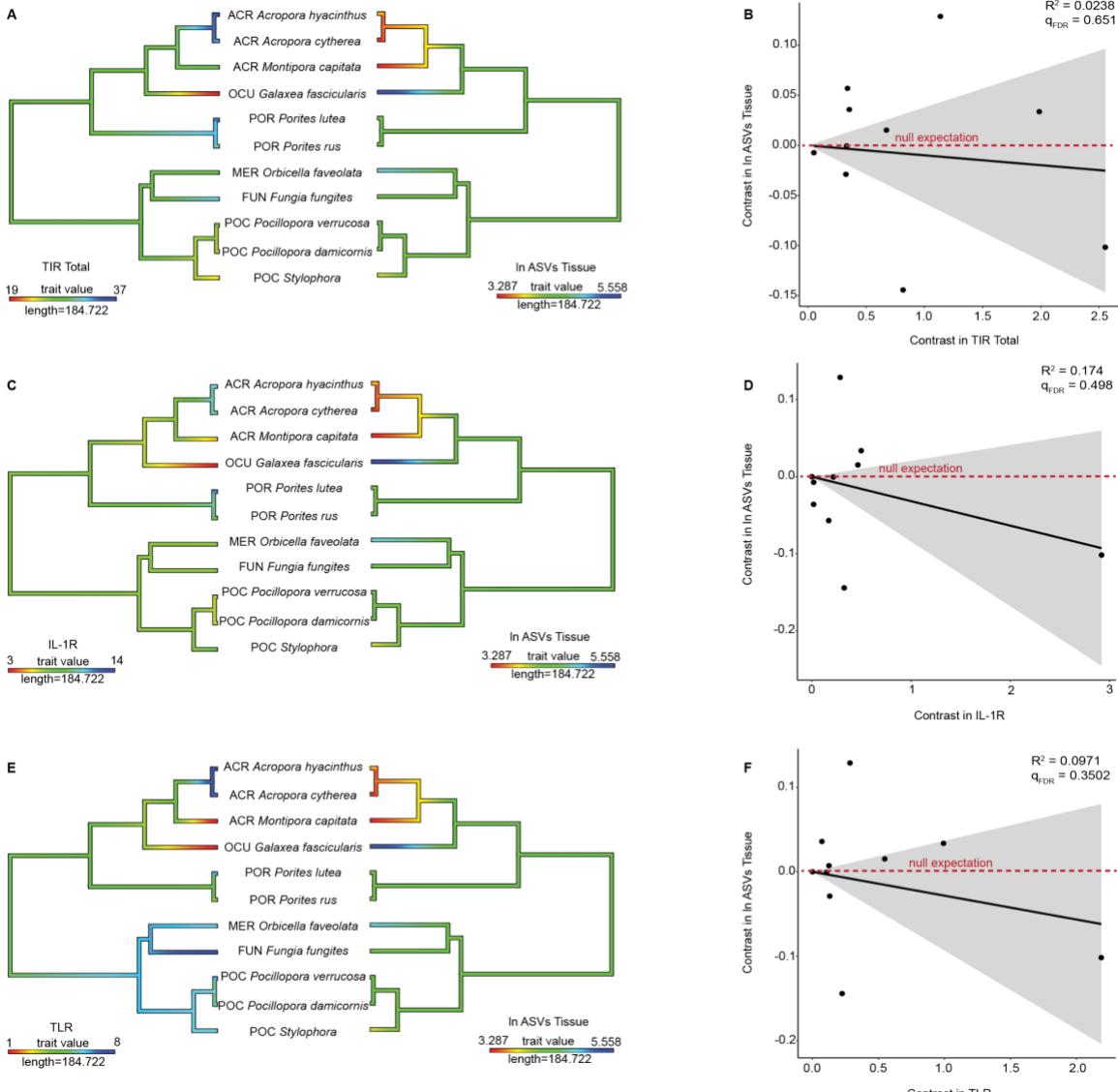
680 **A,B** TIR-only genes ( $R^2 = 0.0078$ ,  $q_{FDR} = 0.929$ ,  $p = 0.796$ ); **C,D** IL-1R genes ( $R^2 = 0.261$ ,

681  $q_{FDR} = 0.261$ ,  $p = 0.109$ ); **E,F** TLR genes ( $R^2 = 0.0971$ ,  $q_{FDR} = 0.624$ ,  $p = 0.351$ ) compared

682 against log microbiome richness (ln ASVs). Shading in phylogenetic independent

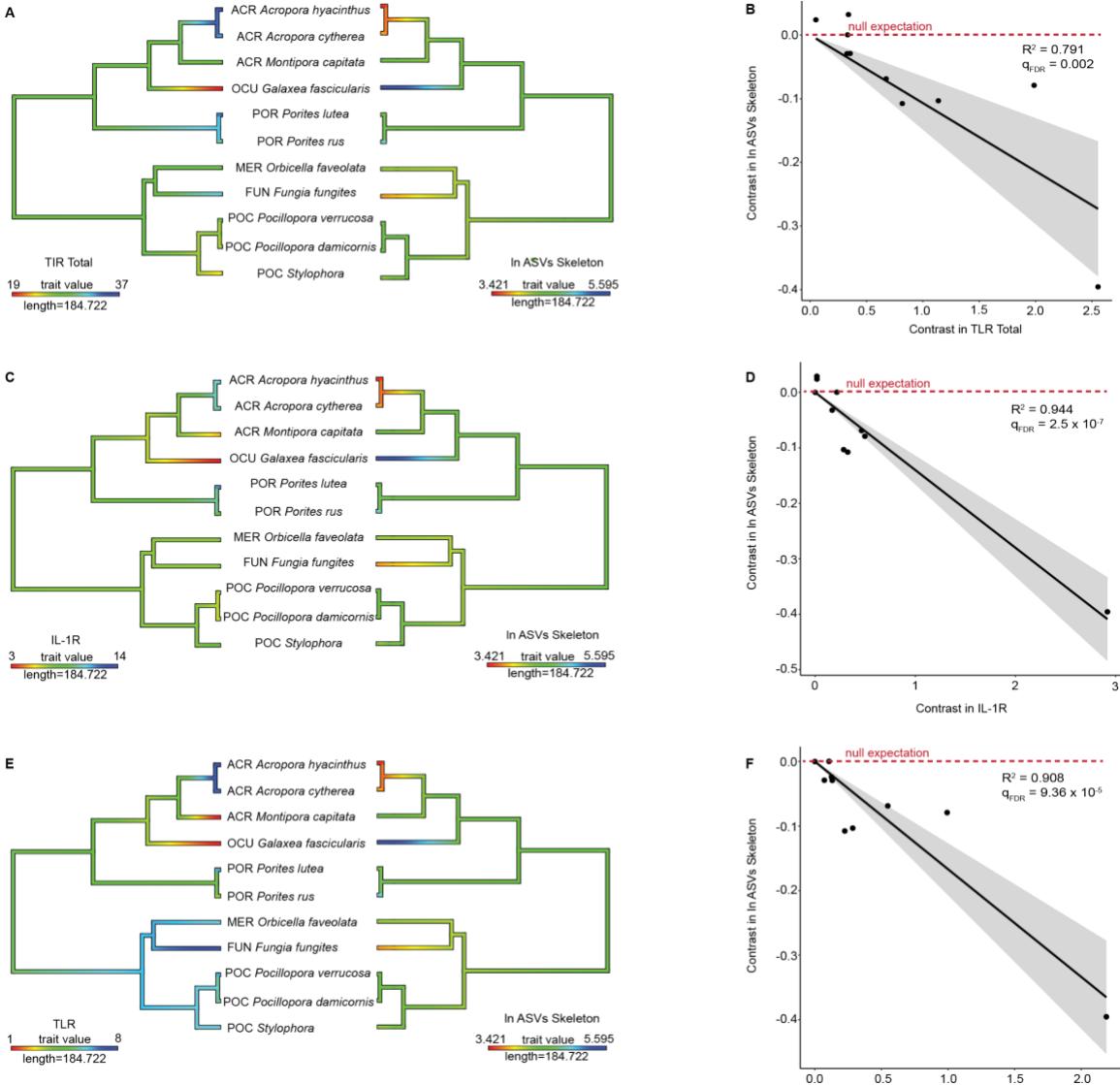
683 contrasts analysis indicates the 95% confidence interval of the mean.

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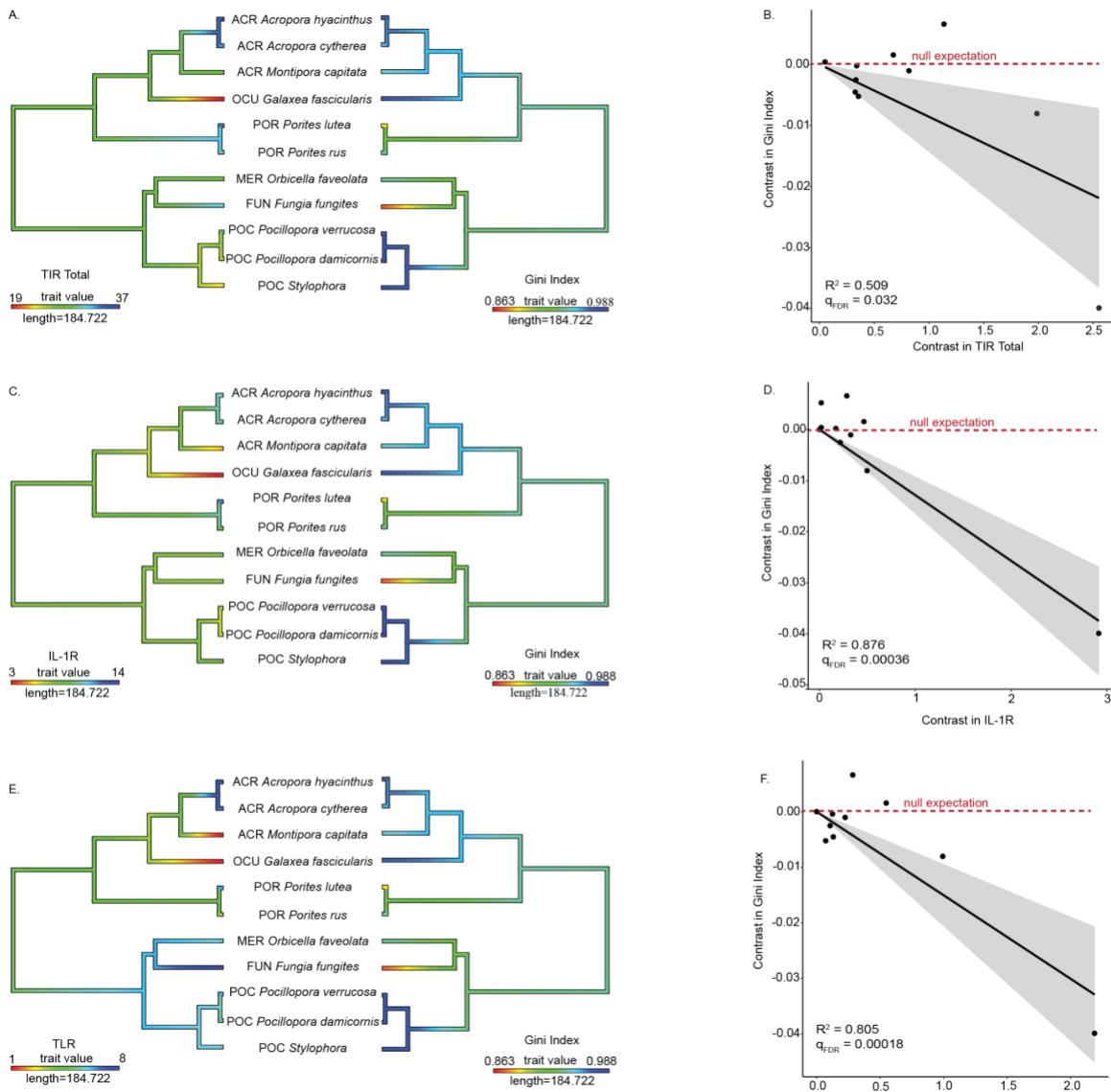
**Fig. S3. Phylogenetic comparison of coral microbiome richness and innate immune repertoire for the tissue compartment.** Rows show ancestral state reconstructions (left column) of innate immune gene copy number and microbiome richness, as well as phylogenetic independent contrast analysis for all predicted isoforms (right column) of **A,B** TIR-only genes ( $R^2 = 0.0238$ ,  $q_{FDR}=0.929$ ,  $p = 0.651$ ); **C,D** IL-1R genes ( $R^2 = 0.174$ ,  $q_{FDR}=0.498$ ,  $p = 0.202$ ); **E,F** TLR genes ( $R^2 = 0.0974$ ,  $q_{FDR}=0.624$ ,  $p = 0.3502$ ) compared against log microbiome richness (ln ASVs). Shading in phylogenetic independent contrasts analysis indicates the 95% confidence interval of the mean.



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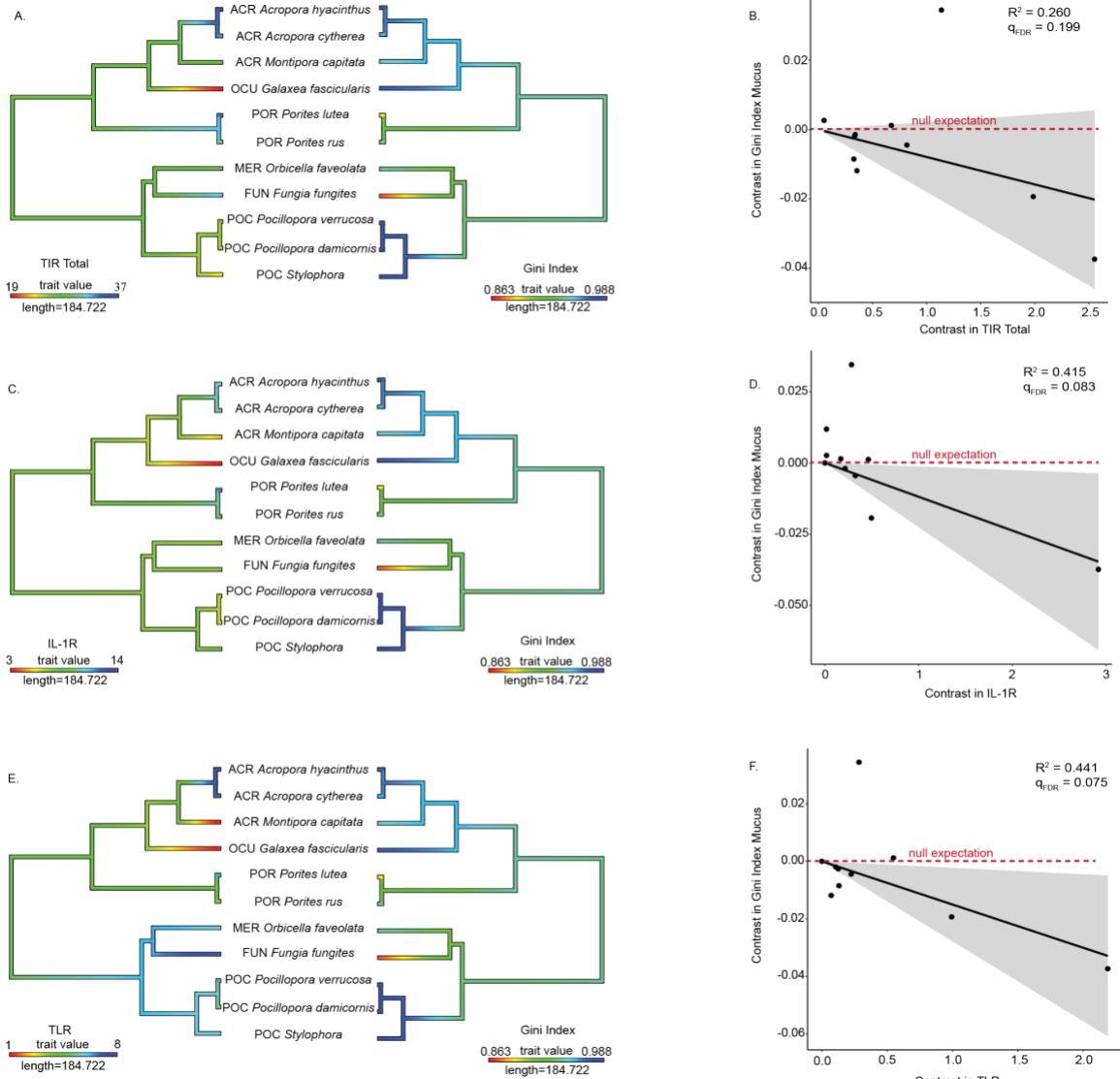
**Fig. S4. Phylogenetic comparison of coral microbiome richness and innate immune repertoire for the skeletal compartment.** Rows show ancestral state reconstructions (left column) of innate immune gene copy number and microbiome richness, as well as phylogenetic independent contrast analysis for all predicted isoforms (right column) of **A,B** TIR-only genes ( $R^2 = 0.791$ ,  $q_{FDR} = 0.002$ ,  $p = 0.000252$ ); **C,D** IL-1R genes ( $R^2 = 0.944$ ,  $q_{FDR} = 2.15 \times 10^{-5}$ ,  $p = 6.39 \times 10^{-7}$ ); **E,F** TLR genes ( $R^2 = 0.90798$ ,  $q_{FDR} = 9.36 \times 10^{-5}$ ,  $p = 5.85 \times 10^{-6}$ ) compared against log microbiome richness (ln ASVs). Shading in phylogenetic independent contrasts analysis indicates the 95% confidence interval of the mean.

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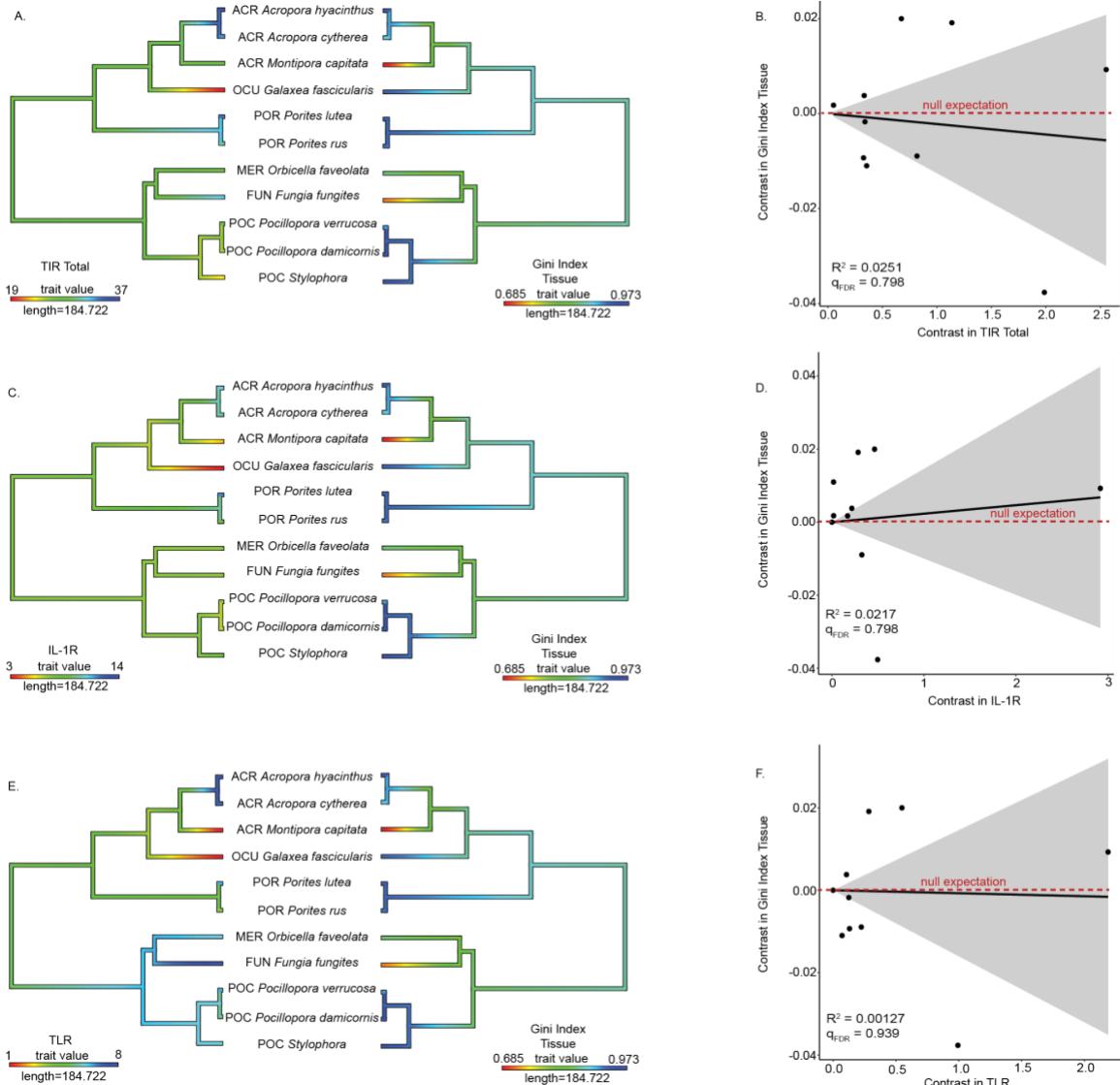
**Fig. S5. Phylogenetic comparison of coral microbiome evenness and innate immune repertoire in all compartments combined.** Rows show ancestral state reconstructions (left column) of innate immune gene copy number and microbiome evenness (Gini index), as well as phylogenetic independent contrast analysis for all predicted isoforms (right column) of **A,B** TIR-only genes ( $R^2 = 0.509$ ,  $q_{FDR} = 0.032$ ,  $p = 0.0083$ ); **C,D** IL-1R genes ( $R^2 = 0.876$ ,  $q_{FDR} = 0.00036$ ,  $p = 2.30 \times 10^{-5}$ ); **E,F** TLR genes ( $R^2 = 0.805$ ,  $q_{FDR} = 0.0014$ ,  $p = 0.00018$ ) compared against microbiome evenness (Gini index). Shading in phylogenetic independent contrasts analysis indicates the 95% confidence interval of the mean.

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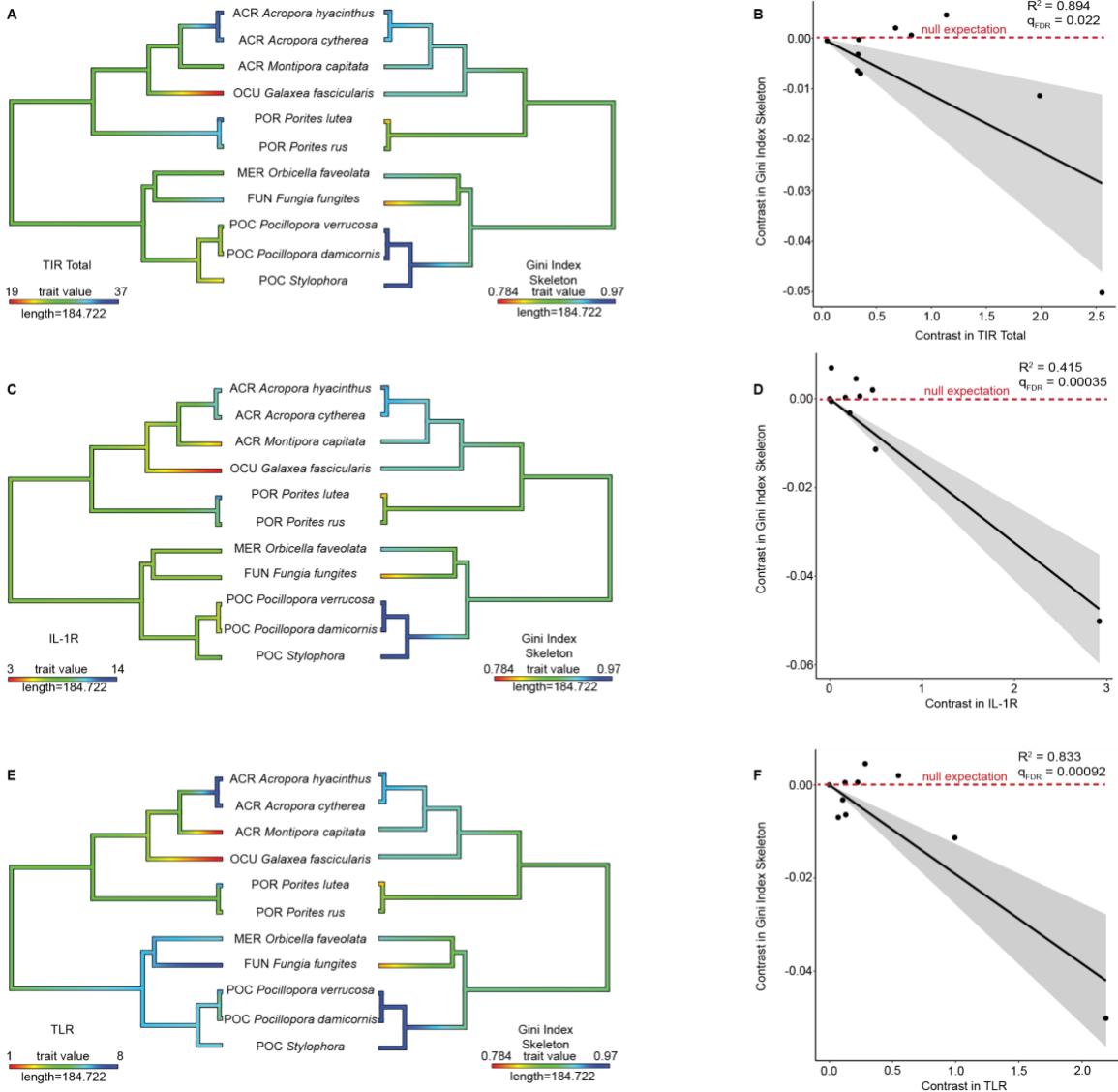
**Fig. S6. Phylogenetic comparison of coral microbiome evenness and innate immune repertoire in the mucus compartment.** Rows show ancestral state reconstructions (left column) of innate immune gene copy number and microbiome evenness (Gini index), as well as phylogenetic independent contrast analysis for all predicted isoforms (right column) of **A,B** TIR-only genes ( $R^2 = 0.260$ ,  $q_{FDR}=0.199$ ,  $p = 0.109$ ); **C,D** IL-1R genes ( $R^2 = 0.415$ ,  $q_{FDR}=0.083$ ,  $p = 0.0323$ ); **E,F** TLR genes ( $R^2 = 0.441$ ,  $q_{FDR}= 0.075$ ,  $p = 0.0258$ ) compared against microbiome evenness (Gini index). Shading in phylogenetic independent contrasts analysis indicates the 95% confidence interval of the mean.

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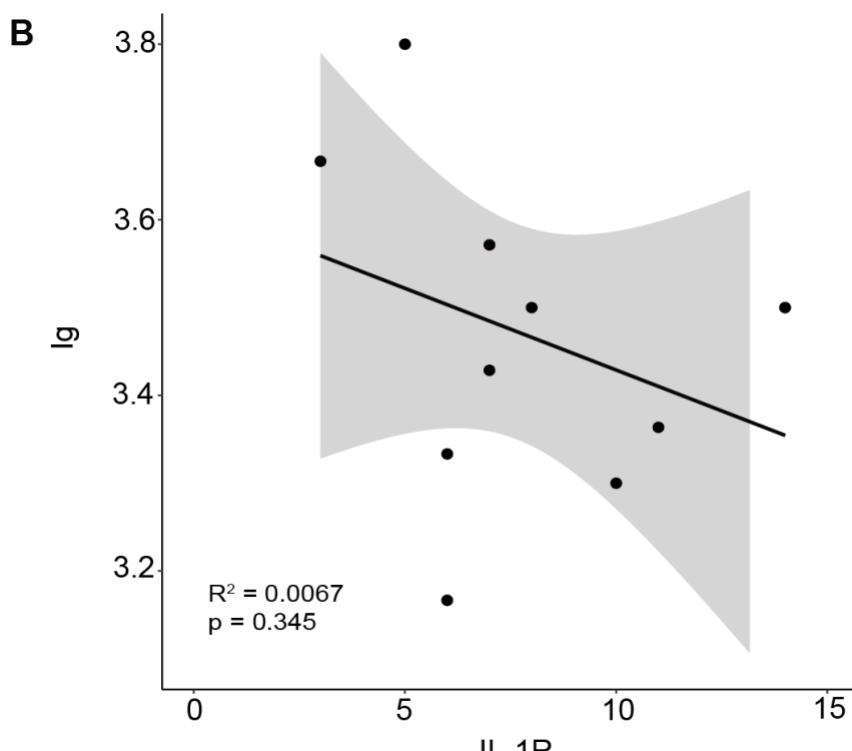
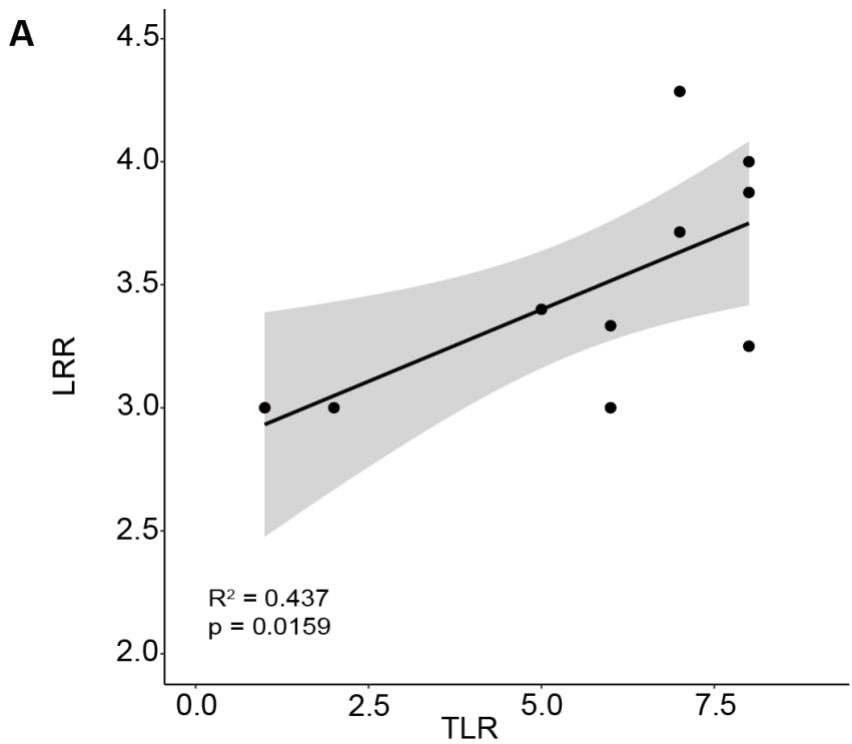


**Fig. S7. Phylogenetic comparison of coral microbiome evenness and innate immune repertoire in the tissue compartment.** Rows show ancestral state reconstructions (left column) of innate immune gene copy number and microbiome evenness (Gini index), as well as phylogenetic independent contrast analysis for all predicted isoforms (right column) of **A,B** TIR-only genes ( $R^2 = 0.0251$ ,  $q_{FDR} = 0.798$ ,  $p = 0.6421$ ); **C,D** IL-1R genes ( $R^2 = 0.0201$ ,  $q_{FDR} = 0.798$ ,  $p = 0.678$ ); **E,F** TLR genes ( $R^2 = 0.00127$ ,  $q_{FDR} = 0.939$ ,  $p = 0.917$ ) compared against microbiome evenness (Gini index). Shading in phylogenetic independent contrasts analysis indicates the 95% confidence interval of the mean.

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**Fig. S8. Phylogenetic comparison of coral microbiome evenness and innate immune repertoire in the skeleton compartment.** Rows show ancestral state reconstructions (left column) of innate immune gene copy number and microbiome evenness (Gini index), as well as phylogenetic independent contrast analysis for all predicted isoforms (right column) of **A,B** TIR-only genes ( $R^2 = 0.894$ ,  $q_{FDR}=0.022$ ,  $p = 1.12 \times 10^{-5}$ ); **C,D** IL-1R genes ( $R^2 = 0.415$ ,  $q_{FDR}=0.00035$ ,  $p = 0.0323$ ); **E,F** TLR genes ( $R^2 = 0.833$ ,  $q_{FDR}=0.00092$ ,  $p = 8.86 \times 10^{-5}$ ) compared against microbiome evenness (Gini index). Shading in phylogenetic independent contrasts analysis indicates the 95% confidence interval of the mean.

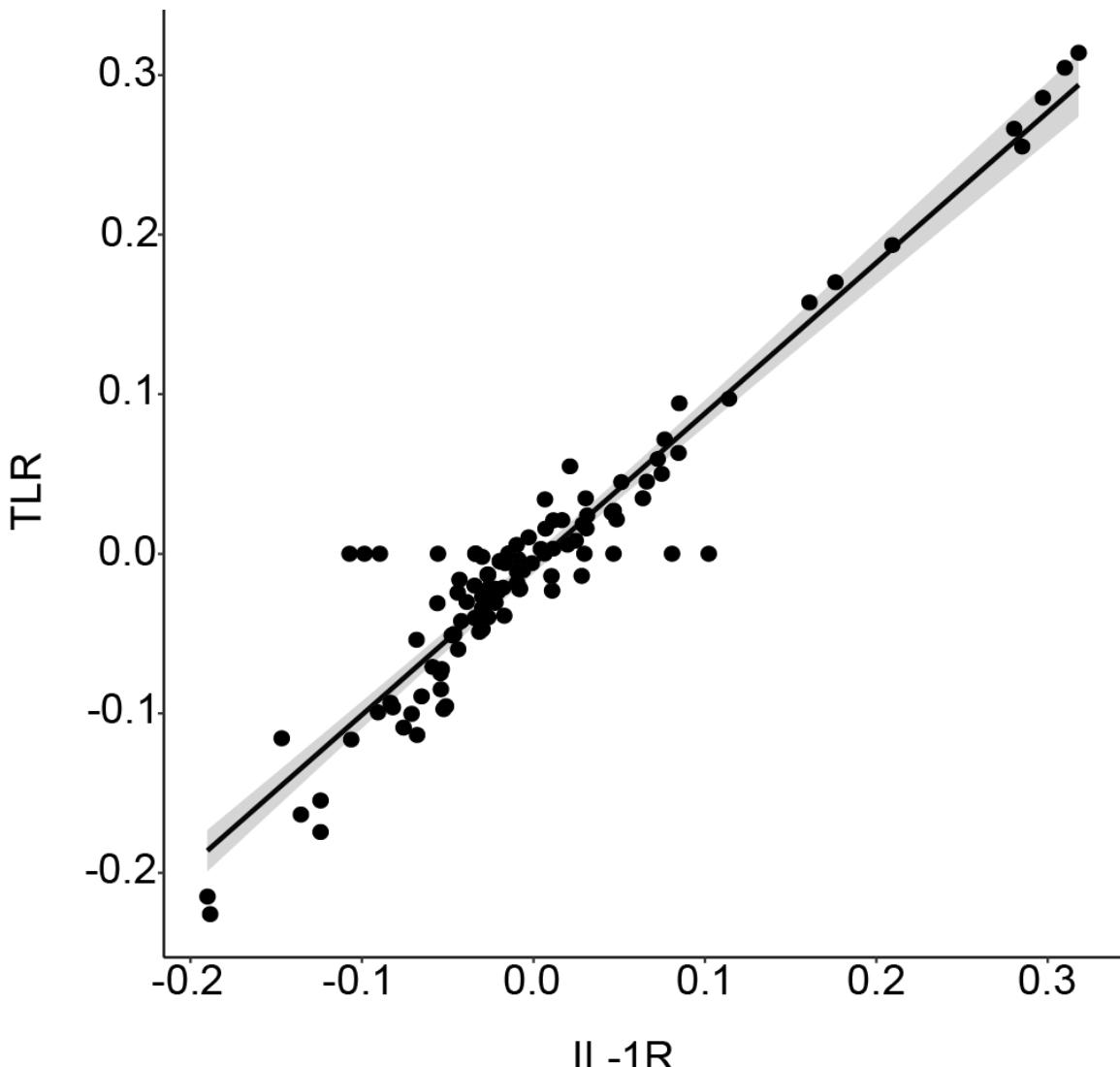


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756 **Fig. S9. Comparison of domain copy number within genes and gene copies within**  
757 **genomes. A LRR domains vs TLR and B Ig vs IL-1R plots.**

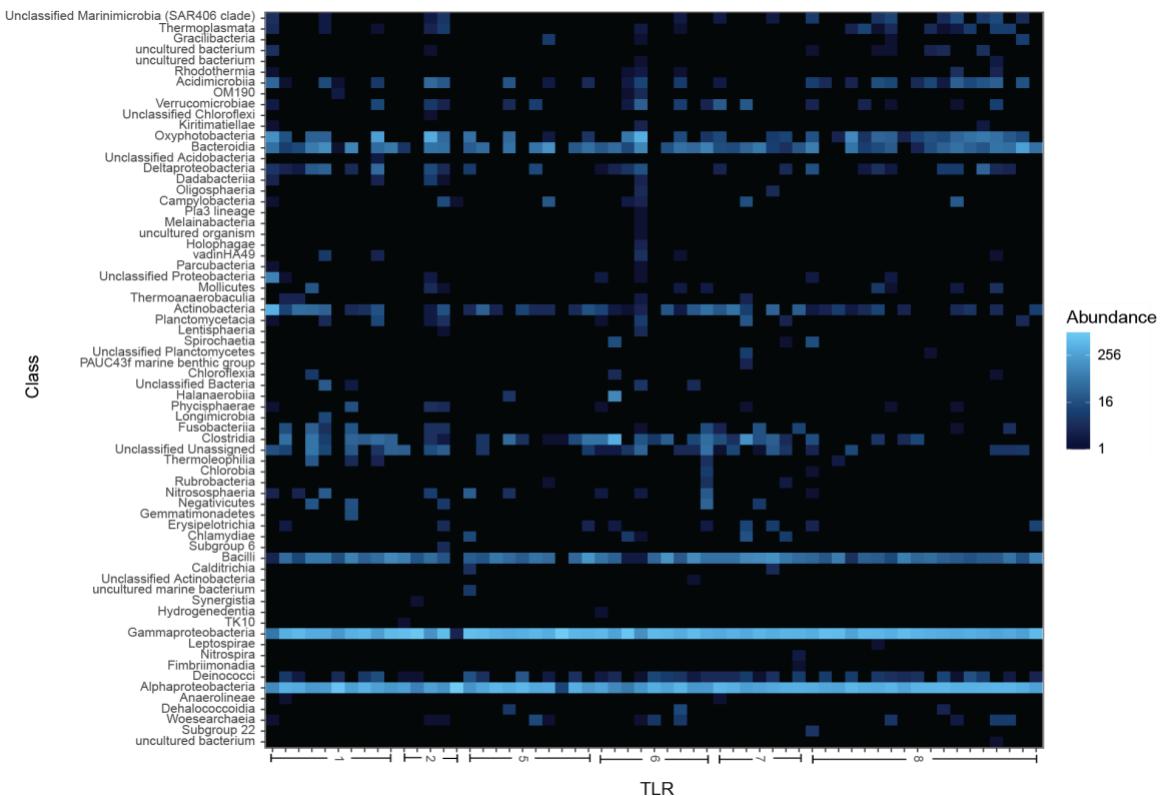
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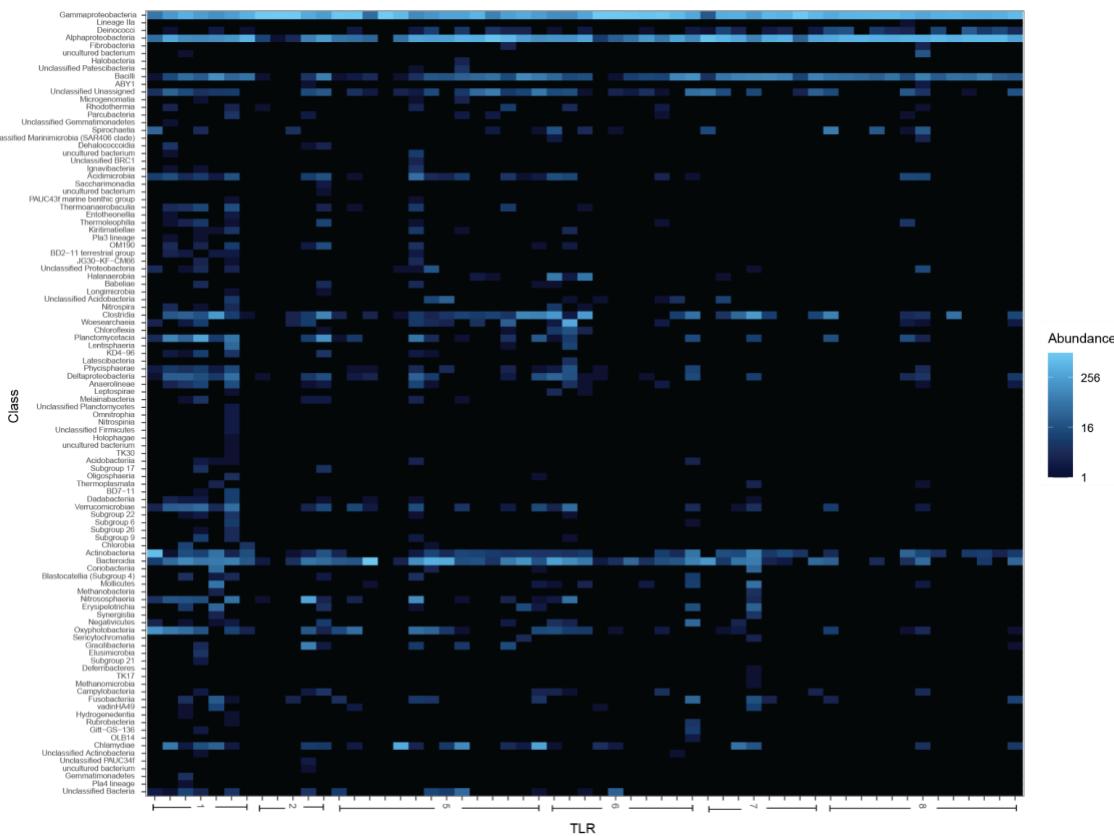
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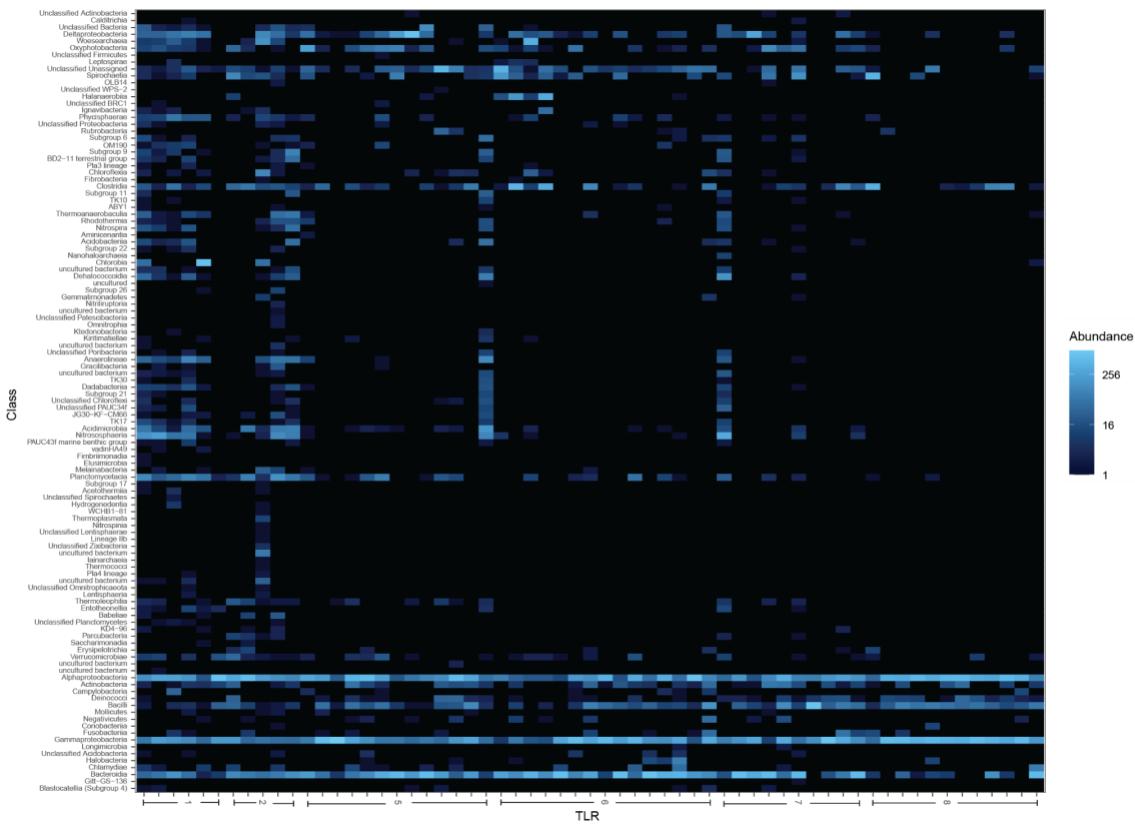
761 **Fig. S10. Correlated response of microbial families to IL-1R or TLR gene copy**  
762 **number over evolution.** Scatterplot shows contrasts in the correlation between microbial  
763 family relative abundance and IL-1R gene copy number (x-axis) and TLR gene copy  
764 number (y-axis). Each point represents one microbial family. Microbial families whose  
765 relative abundance is equally correlated with IL-1R and TLR gene copy number therefore  
766 fall near the diagonal.  
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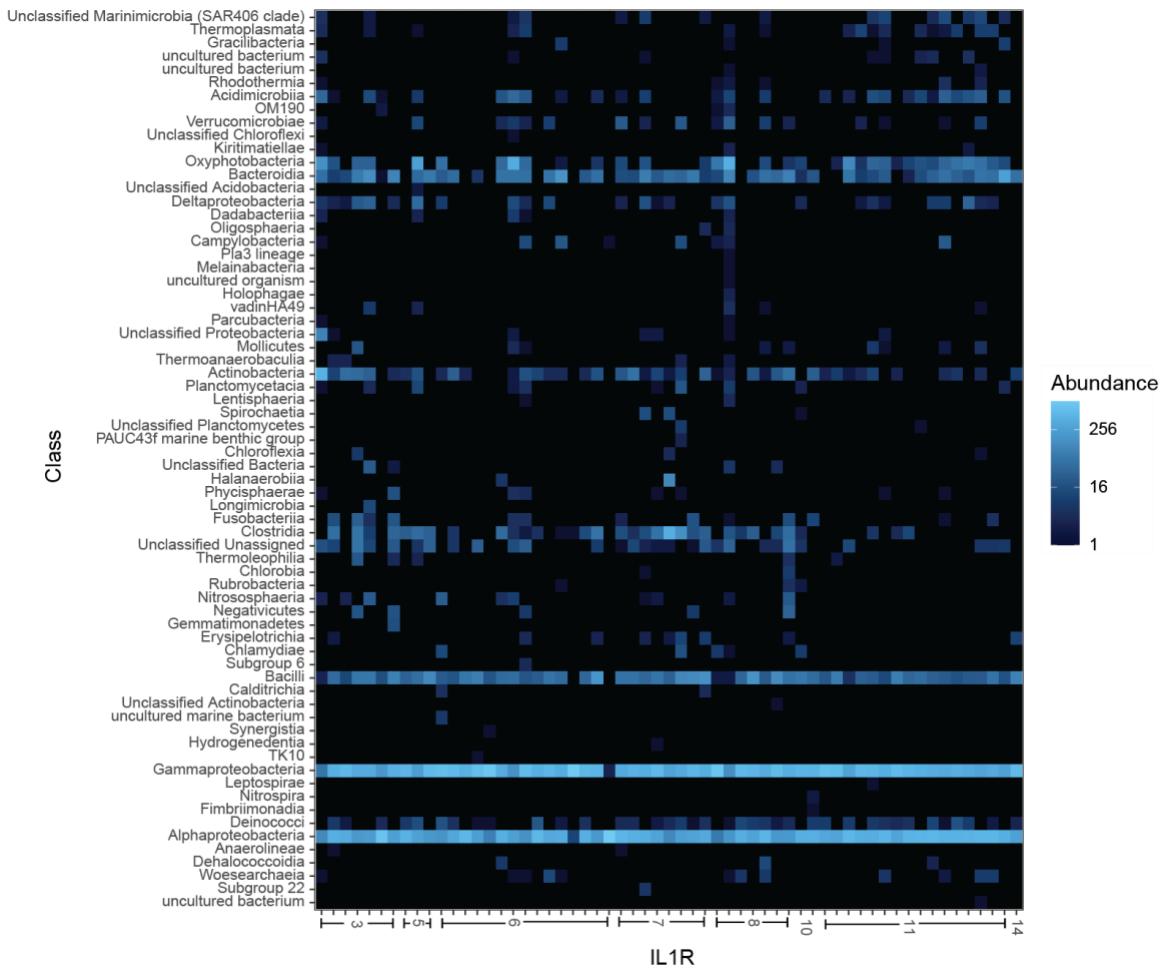
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770 **Fig. S11. Heatmap of bacterial class abundance in mucus vs. TLR gene copy**  
771 **number.** Heatmap colors depict the relative abundance of bacterial classes in coral  
772 mucus (y-axis), in samples sorted by TLR gene copy number (x-axis). Colors reflect  
773 abundance per 1000 16S rRNA gene amplicon reads.  
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777 **Fig. S12. Heatmap of bacterial class relative abundance in tissue vs. TLR gene copy**  
778 **number.** Heatmap colors depicts the relative abundance of bacterial classes in coral  
779 tissue (y-axis), in samples sorted by TLR gene copy number (x-axis). Colors reflect  
780 abundance per 1000 16S rRNA gene amplicon reads.  
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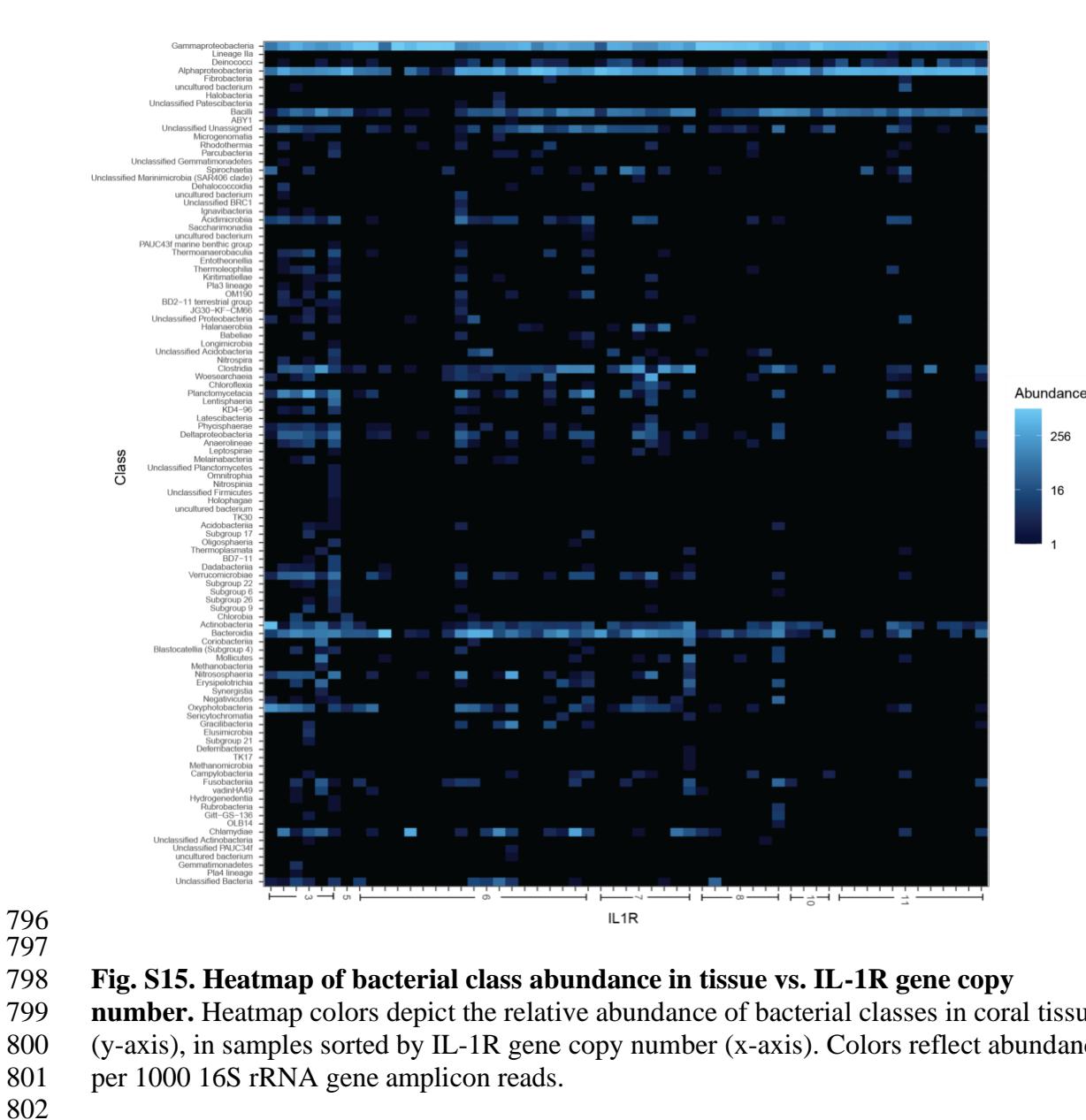
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784 **Fig. S13. Heatmap of bacterial class relative abundance in skeleton vs. TLR gene**  
785 **copy number.** Heatmap colors depict the relative abundance of bacterial classes in coral  
786 skeleton (y-axis), in samples sorted by TLR gene copy number (x-axis). Colors reflect  
787 abundance per 1000 16S rRNA gene amplicon reads.  
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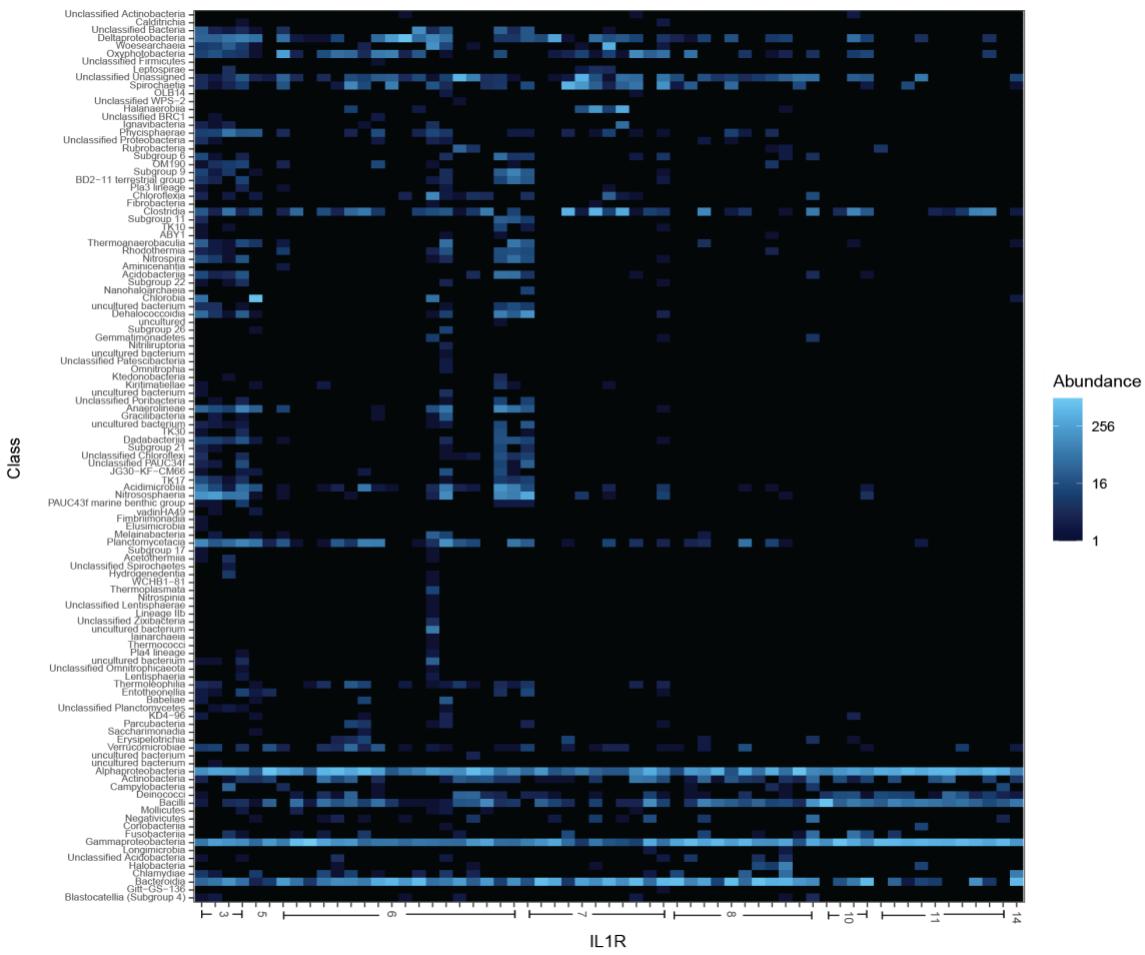


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**Fig. S14. Heatmap of bacterial class abundance in mucus vs. IL-1R gene copy number.** Heatmap colors depict the relative abundance of bacterial classes in coral mucus (y-axis), in samples sorted by IL-1R gene copy number (x-axis). Colors reflect abundance per 1000 16S rRNA gene amplicon reads.

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**Fig. S16. Heatmap of bacterial class abundance in skeleton vs. IL-1R gene copy number.** Heatmap colors depict the relative abundance of bacterial classes in coral skeleton (y-axis), in samples sorted by IL-1R gene copy number (x-axis). Colors reflect abundance per 1000 16S rRNA gene amplicon reads.

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823 **Supplementary Data Tables**  
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825 **Supplementary Table 1.** Sample and Genomic metadata. (A) Genomes used in the  
826 analysis (B) Per sample metadata for the GCMP data (C) Mapping file for the GCMP  
827 data.

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829 **Supplementary Table 2.** Annotations of TIR-domain containing gene families in coral  
830 genomes for (A) Total number of IL-1R and TLR genes, (B) Domain make up of IL-1R  
831 genes, (C) Domain make up of TLR genes.

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833 **Supplementary Table 3.** Quality control information for the sequencing depth of  
834 microbiome samples in the dataset.

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836 **Supplementary Table 4.** Distribution of TIR, LRR, and Ig domains across unique  
837 predicted protein isoforms in sequenced coral genomes.

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839 **Supplementary Table 5.** Microbiome (A) richness and (B) evenness across samples in  
840 the analysis.

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842 **Supplementary Table 6.** Phylogenetic comparison of IL-1R and TLR gene family copy  
843 number vs. microbiome alpha diversity in (A) All Samples (B) Mucus (C) Tissue, (D)  
844 Skeleton.

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846 **Supplementary Table 7.** Trait table of the genomic and microbiome traits of coral  
847 species in the analysis.

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849 **Supplementary Table 8.** Phylogenetic comparison of TIR LRR and Ig domains vs.  
850 microbiome alpha diversity in A) All Samples B) Mucus c) Tissue, d) Skeleton.

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852 **Supplementary Table 9.** Phylogenetic comparison of IL-1R and TLR gene family copy  
853 number vs. PC axes from beta-diversity PCoA analysis in (A) All Samples (B) Mucus  
854 (C) Tissue, (D) Skeleton.

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856 **Supplementary Table 10.** ANCOMBC comparison of relative abundance of microbial  
857 taxa vs. IL-1R and TLR gene family copy number in (A) All Samples (B) Mucus (C)  
858 Tissue, (D) Skeleton.

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860 **Supplementary Table 11.** Phylogenetic comparison of TIR LRR and Ig domains vs.  
861 microbiome PC axes from beta-diversity PCoA analysis in (A) All Samples (B) Mucus  
862 (C) Tissue, (D) Skeleton.

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