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9 Article type : Special Issue  
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12 LARGE-SCALE GENOME SAMPLING REVEALS UNIQUE IMMUNITY AND METABOLIC ADAPTATIONS IN BATS  
13 GENE FAMILY EVOLUTION IN BATS

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/MEC.16027](https://doi.org/10.1111/MEC.16027)

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## 40 Abstract

41 Comprising more than 1,400 species, bats possess adaptations unique among mammals including powered  
42 flight, unexpected longevity, and extraordinary immunity. Some of the molecular mechanisms underlying  
43 these unique adaptations includes DNA repair, metabolism and immunity. However, analyses have been  
44 limited to a few divergent lineages, reducing the scope of inferences on gene family evolution across the  
45 Order Chiroptera. We conducted an exhaustive comparative genomic study of 37 bat species, one generated  
46 in this study, encompassing a large number of lineages, with a particular emphasis on multi-gene family  
47 evolution across immune and metabolic genes. In agreement with previous analyses, we found lineage-  
48 specific expansions of the APOBEC3 and MHC-I gene families, and loss of the proinflammatory PYHIN gene  
49 family. We inferred more than 1,000 gene losses unique to bats, including genes involved in the regulation of  
50 inflammasome pathways such as epithelial defense receptors, the natural killer gene complex and the  
51 interferon-gamma induced pathway. Gene set enrichment analyses revealed genes lost in bats are involved in  
52 defense response against pathogen-associated molecular patterns and damage-associated molecular  
53 patterns. Gene family evolution and selection analyses indicate bats have evolved fundamental functional  
54 differences compared to other mammals in both innate and adaptive immune system, with the potential to  
55 enhance anti-viral immune response while dampening inflammatory signaling. In addition, metabolic genes  
56 have experienced repeated expansions related to convergent shifts to plant-based diets. Our analyses  
57 support the hypothesis that, in tandem with flight, ancestral bats had evolved a unique set of immune  
58 adaptations whose functional implications remain to be explored.

## 59 Key Words

60 adaptive immunity, gene family evolution, innate immunity, inflammatory pathway, metabolism, viral  
61 tolerance

## 62 Introduction

63 Comparative genomics provides a framework for identifying the molecular mechanisms underlying unique  
64 organismal adaptations, in their endless forms. To date, comparative genomic approaches have revealed the  
65 mechanisms underlying terrestrial adaptations in mudskipper fish (You et al., 2014), heat tolerance in coral  
66 (Bay, Rose, Logan, & Palumbi, 2017), cold stress tolerance in *Draba* (Nowak et al., 2020), and extreme  
67 longevity in naked mole rats (X. Zhou et al., 2020). In most cases the search for molecular adaptations has  
68 focused on orthologous single-copy genes, but gene loss and duplication can also be adaptive and are critical  
69 to understanding of how phenotypic adaptations evolve. Analyses based on highly contiguous genome  
70 assemblies have uncovered gene expansions likely associated with production of urushiol and anthocyanins in  
71 mango (P. Wang et al., 2020), the earliest events of gene duplication in cytoskeletal and membrane-trafficking  
72 families in eukaryotic cellular evolution (Vosseberg et al., 2020), pseudogenization in genes associated with  
73 testicular descent in afrotherian mammals (Sharma, Lehmann, Stuckas, Funke, & Hiller, 2018), gene losses  
74 associated with diving-related adaptations in cetaceans (Huelsmann et al., 2019), and losses associated with  
75 physiological and metabolic adaptations in fruit bats (Sharma, Hecker, Roscito, Foerster, Langer & Hiller,  
76 2018). Given the importance of gene family evolution, multiple large-scale genome sequencing consortia such  
77 as the Earth BioGenome Project (Lewin et al., 2018), the Vertebrate Genomes Project (Rhie et al., 2020), and  
78 Bat1K (Teeling et al., 2018) aim to generate high-quality genome assemblies for species spanning entire  
79 clades and even the entire phylogenetic 'Tree of Life', thereby enabling greater confidence in analyses of gene  
80 loss and gene family evolution.

81 Gene family expansions and contractions are influenced by selection, including from biological factors  
82 such as pathogens. Host-pathogen interactions are shaped by reciprocal selection, an evolutionary arms race  
83 which has forced hosts to evolve complex immune defense mechanisms (Papkou et al., 2019; Sironi, Cagliani,  
84 Forni, & Clerici, 2015). Vertebrates have two types of immune response: innate immunity, which is non-  
85 specific and acts as a first line of defense; and adaptive immunity, which is highly specific and generates  
86 immune memory (Delves, Martin, Burton, & Roitt, 2017; Janeway & Travers 2001.). Several immune-related  
87 gene families that have experienced substantial evolutionary changes during mammal evolution. While many  
88 important facets of the immune system are conserved, immune gene families have high rates of evolution

89 whether measured via substitution rate ratios or birth–death turnover (Bernatchez & Landry, 2003; Goebel et  
90 al., 2017; Minias, Pikus, Whittingham, & Dunn, 2019; Santos et al., 2016; Shultz & Sackton, 2019; Van  
91 Oosterhout, 2009). This is especially true of the Major Histocompatibility Complex (MHC), which is  
92 responsible for generating cell surface proteins that play essential functions in the adaptive immune system  
93 (Janeway & Travers 2001).

94 This combination of highly conserved, and highly variable components of the immune system, is  
95 particularly intriguing among bats. Among mammals, bat diversity is second only to that of rodents, and  
96 encompasses over 1,400 species that occupy a broad diversity of ecological niches on six continents (Fenton  
97 & Simmons, 2015; Nogueira et al., 2018). The success of bats is likely related to a suite of adaptations unique  
98 both to the clade as a whole and to various subclades within the Order Chiroptera. The most obvious of these  
99 is powered flight, allowing bats to occupy a unique aerial niche not utilized by any other mammal. While this  
100 unique niche limits body size, within that constraint bats have been exceptionally successful and have  
101 diversified in ways unparalleled among other mammals. For example, bats evolved virtually every mammalian  
102 dietary strategy (e.g., frugivory, carnivory, nectarivory, piscivory) and have done so in a relatively short  
103 evolutionary time frame (Dumont et al., 2012). Another less obvious but likely more interesting adaptation is  
104 the exceptional longevity and increased health span (the period of life during which an organism is in  
105 generally good health) exhibited by many bat species given their body size. Many species such as the  
106 Bechstein’s bat (*Myotis bechstein*) the little brown bat, Brandt’s bat (*Myotis brandtii*), greater mouse-eared  
107 bat (*Myotis myotis*) and greater horseshoe bat (*Rhinolophus ferrumequinum*) have unexpectedly long health  
108 spans, living 30 - 40 years (Fleischer, Gampe, Scheuerlein & Kerth, 2017; Foley et al., 2018; Podlustyky,  
109 Khritankov, Ovodov & Austad, 2005; Seim et al., 2013; Wilkinson & Adams, 2019). Such longevity defies the  
110 expectation that large species are longer-lived than small species; despite constrained body size, bats live  
111 longer than other mammals of similar size (Austad & Fischer, 1991; Healy et al., 2014). Bat longevity and  
112 health span may be influenced by their exposure to extrinsic mortality factors. Powered, mostly nocturnal  
113 flight may lower bats’ exposure to some sources of extrinsic mortality, including predation (Healy et al.,  
114 2014). Yet, the risk of exposure to another extrinsic source of mortality, contagious infection, increases  
115 among bat species that roost in large colonies (Brook & Dobson, 2015; H. Han et al., 2015). Thus, to achieve  
116 such longevity and decreased senescence, long-lived bat populations must overcome the burden of  
117 infectious diseases.



118 The uniqueness of bats extends to the immune repertoire. Early in the age of whole-genome  
119 analyses, it was clear that inflammation-related gene families had expanded or contracted, and certain  
120 single-copy genes associated with immunity and cell repair had experienced selection in bats (G. Zhang et al.,  
121 2013). There is still debate as to whether bats harbor a disproportionately large number of viruses, or  
122 whether viral load is simply a function of species richness (Moratelli & Calisher, 2015; Olival et al., 2017;  
123 Mollentze & Streicker, 2020). However there is no doubt that several recent viral intrusions into our own  
124 species ultimately originated from bat hosts (Drexler et al., 2012; Goldstein et al., 2018; Hu et al., 2017;  
125 Memish, Perlman, Van Kerkhove, & Zumla, 2020; Towner et al., 2007). This likely includes the current SARS-  
126 CoV-2 pandemic (Boni et al., 2020; Lau et al., 2020). Bats appear to have the ability to tolerate these viruses  
127 with few health impacts, hence recent studies have focused on bat comparative genomics (Jebb et al., 2020)  
128 and its emphasis on viral response (reviewed in: Gorbunova, Seluanov, & Kennedy, 2020; Hayman, 2019).  
129 Although little is known from this perspective, there is a growing body of functional analyses showing that  
130 bats are unusual among mammals in how they deal with viruses (Ahn et al., 2019; A. Banerjee et al., 2020;  
131 Miller et al., 2016; Schountz, Baker, Butler, & Munster, 2017; Xie et al., 2018).

132 The 'inflammosome' is typically highly conserved across mammals, but bats exhibit a reduced  
133 inflammatory response that may be tied to their ability to cope with viral infection while experiencing  
134 minimal impact (Pavlovich et al., 2018). For example, the PYHIN gene family, namely, appears to have been  
135 almost completely lost in bats (Ahn, Cui, Irving, & Wang, 2016; G. Zhang et al., 2013) while at least one PYHIN  
136 gene can be found in all other eutherians examined. Similarly, in bats, the inflammatory function of  
137 interferons (G. Zhang et al., 2013) appears distinct among bat species, where IFN contractions and  
138 constitutive expression of IFN- $\alpha$  has been observed in some bats (P. Zhou et al., 2016), and the APOBEC3  
139 repertoire, which is associated with anti-viral response, is expanded (Jebb et al., 2020; Hayward et al., 2018).  
140 All of these functional patterns suggest an overall dampened inflammatory reaction despite a robust immune  
141 response to viruses whose origins may lie in the gene repertoires available to bats (A. Banerjee, Rapin,  
142 Bollinger, & Misra, 2017; A. Banerjee et al., 2020).

143 Gene family evolution also likely plays a role in the unique dietary ecology of bats. Several studies  
144 have found a variety of mechanisms influencing dietary adaptation. For example, convergent amino acid  
145 substitutions in several lineages of frugivorous bats have occurred independently (Gutiérrez-Guerrero et al.,  
146 2020; Shen, Han, Zhang, Rossiter, & Zhang, 2012; Teeling et al., 2018; K. Wang et al., 2020), and are  
147 associated with the shift to a high-sugar diet. Another strategy has been to repurpose a given gene to

148 accommodate such dietary shifts (Shen, Han, Jones, Rossiter, & Zhang, 2013). With the exception of olfactory  
149 receptors (Hayden et al., 2014; Hughes et al., 2018; Tsagkogeorga, Müller, Dessimoz, & Rossiter, 2017), the  
150 roles of gene loss and gain in shaping dietary evolution of bats have not been comprehensively explored.

151 Here we investigate bat gene family evolution related to immunity, metabolism, and dietary  
152 adaptations, using the most extensive genomic sampling within bats to date. Despite variability in quality of  
153 assemblies, the ecological diversity of lineages for which assemblies are available allows, for the first time, an  
154 investigation of gene family evolution across 10 families, two suborders, and a complete coverage of the  
155 entire range of diets. We find two major patterns. First, system-wide gene losses related to inflammatory  
156 response and selection on genes associated with antiviral immunity appear to have influenced bat lineages.  
157 This suggests that bats— compared to other mammals such as cow, dog, horse, pig, mouse and human—  
158 have evolved complex, complementary adaptations across multiple functional pathways to simultaneously  
159 reduce inflammatory response while maintaining strong antiviral defenses, potentially underlying their  
160 suspected tolerance of viruses. Second, the move from the ancestral arthropod diet to high-sugar nectar and  
161 fruit-based diets is associated with lineage-specific gene family expansions in metabolic gene families.

## 162 **Materials and Methods**

### 163 *Whole genome sequencing*

164 We generated a whole genome assembly for a male *Phyllostomus hastatus*, PE091, collected in Jenaro  
165 Herrera, Peru. Field-collected tissues from *Phyllostomus hastatus* specimen PE091 were lawfully collected  
166 under permit #0122–2015–SERFOR–DGGSPFFS, exported under SERFOR permit #0002287, and imported  
167 under USFW 3-177 2015MI1694291.

168 Samples were preserved in RNAlater for one week before flash-freezing in a liquid nitrogen dry shipper,  
169 following previously published protocols (Yohe et al., 2019). High molecular weight genomic DNA was  
170 extracted from flash-frozen liver using the Qiamp DNA Micro Kit (Germantown, MD, USA) and sequenced on a  
171 PromethION instrument (Oxford Nanopore Technologies, New York, NY, USA) at Cold Spring Harbor  
172 Laboratory. Additionally, short-read Illumina whole genome sequencing was performed at Novogene, Inc  
173 (California, USA). Genomic DNA from lung was randomly fragmented to 350bp, end-repaired, adenylated,  
174 ligated with Illumina sequencing adapters, and further PCR-enriched. The final libraries were purified  
175 (AMPure XP system) and library quality and size verification were assessed on an Agilent 2100 Bioanalyzer  
176 (Agilent Technologies, CA, USA). Molar concentration was assessed using real-time PCR.

177 *De novo* genome assembly was performed using Flye v.2.7.1 (Kolmogorov, Yuan, Lin, & Pevzner,  
178 2019) using default *--nano-raw* parameterization. The obtained pre-assembly was polished using Illumina  
179 short-reads with POLCA tool built-in MaSuRCA genome assembly and analysis toolkit (Zimin et al., 2013).

#### 180 *Genome database construction*

181 Publicly-available genome assemblies for an additional 36 bat species (Supplementary Table 1) were  
182 downloaded from open-source databases to maximize bat taxonomic sampling (D. Dong et al., 2017; Eckalbar  
183 et al., 2016; Gutiérrez-Guerrero et al., 2020; Jebb et al., 2020; Parker et al., 2013; Seim et al., 2013; K. Wang  
184 et al., 2020; Zepeda Mendoza et al., 2018; G. Zhang et al., 2013). Assemblies were masked with  
185 RepeatMasker v.4.1.0 (Smit, Hubley, & Green, n.d.) using a custom library combining known mammalian  
186 transposable elements (TE) from Repbase (v20181026), a *de novo* mammalian TE library generated using  
187 assemblies from the Zoonomia Project (Genereux et al., 2020) and the Dfam database, and a custom bat-  
188 specific TE library generated by manual curation (Jebb et al., 2020).

189 All assemblies were annotated or re-annotated with the MAKER annotation pipeline v.2.31.10 (Holt &  
190 Yandell, 2011) to avoid bias in downstream analyses caused by differences in genome assembly annotation  
191 quality. Two iterations of MAKER were performed for each species. During the first run we provided  
192 expressed sequence tags (ESTs) and transcriptomic data as inputs (Davies et al., 2020; Potter et al., n.d.)  
193 (Supplementary Table 2). If species-specific transcriptomic data were unavailable, we used information from a  
194 related species of the same genus. We used two databases for protein homology the Uniprot/Swiss-Prot  
195 protein sequence database (Bateman, 2019) and a bat-specific protein database obtained from high-quality  
196 genome annotations for six bat species (Jebb et al., 2020). Repeat evidence was provided using the repeat  
197 annotation GFF3 file generated by RepeatMasker. Gene models generated on the first run were used for gene  
198 predictions with two gene software packages, SNAP (Korf, 2004) and Augustus (Stanke & Waack, 2003). Only  
199 gene models with an AED score < 0.25 and with more than 50 amino acids were retained. For the second run,  
200 focusing on re-annotation, the MAKER control file was edited to include the GFF3 output file from the first  
201 run gene predictions generated by SNAP and the Augustus gene prediction species model as inputs.  
202 Functional annotation was performed with BlastP (Camacho et al., 2009) using the Uniprot/Swiss-Prot  
203 database and protein domain annotation with InterProScan (Jones et al., 2014).

## 204 *Homology inference*

205 Protein homology was inferred among the proteins of 43 mammals: Including *Homo sapiens* and *Mus*  
206 *musculus*, two well-studied model organisms, and more closely related species from the superorder  
207 Laurasiatheria: *Sus scrofa*, *Bos taurus*, *Equus caballus*, *Canis lupus familiaris*, and the 37 bat species  
208 (Supplementary Table 1). Orthologous groups (orthogroups) were assigned with Orthofinder v.2.4.0 (Emms &  
209 Kelly, 2019). When no orthologs were inferred for the Chiroptera in a given orthogroup, we independently  
210 analyzed the genome data to confirm gene losses in bats (Supplementary Fig. 1). To this end, we performed a  
211 BLAST search against the 37 bat genomes using the following criteria: an e-value of 1e-6 and an identity and  
212 protein coverage greater than 80%. Then, genomic regions with a BLAST hit were extracted along with 200bp  
213 upstream and downstream. Sequences were aligned with the MAFFT aligner tool v.7.402 (Katoh & Standley,  
214 2013) and visualized using Geneious version 11.1.3 (Kearse et al., 2012) to discriminate annotation errors.  
215 Additionally, BLAST searches were also performed against transcriptomic data from 22 bat species  
216 (Supplementary Table 2) (Potter et al., n.d.). For these searches, potential matches were filtered more strictly,  
217 and those with identity and protein coverage  $\geq 90\%$  were retained. Subsequent blast hit extraction, alignment  
218 and visualization were as for the genome searches.

## 219 *Enrichment in chiropteran gene losses*

220 We conducted pathway enrichment analyses with the final list of genes missing from all bat species using two  
221 databases: BioPlanet (R. Huang et al., 2019) and DICE GOnet (Pomaznoy, Ha, & Peters, 2018). In each case, we  
222 used the list of gene symbols as input with a cutoff value of 0.05 (BioPlanet) and a similar p-value in the DICE  
223 GOnet biological process classification for the mouse model. In both cases, all genes found to be missing were  
224 used as input and compared to a reference set of genes annotated in the corresponding database.

## 225 *Inferring bat phylogeny*

226 To infer gene family evolution, we first inferred an ultrametric phylogenomic tree based on 350 single copy  
227 orthologous genes (207,551 amino acid sites). All the orthologs were concatenated into a single 207,551-  
228 amino acid “contig” and sequence alignment was performed using the MAFFT aligner tool v.7.402 (Katoh &  
229 Standley, 2013). We evaluated the best-fit models of protein evolution with ProtTest v.3 (Darriba, Taboada,  
230 Doallo, & Posada, 2011) using two criteria: the Akaike Information Criterion (AIC) and the Bayesian  
231 Information Criterion (BIC) (distribution JTT, +G +I +I +G and 80% consensus threshold). A maximum likelihood  
232 tree was inferred for the concatenated data set with RAxML v.8 (Stamatakis, 2014). Estimation of species

233 divergence times was performed with Bayesian phylogenetic methods using the MCMCtree tool in the PAML  
234 v.4.9 package (Yang, 2007). We calibrated divergence dates using six points based on fossil records: 1)  
235 *Icaronycteris*, considered as one of the oldest echolocating fossil bats, dated at 52 Mya (Gunnell & Simmons,  
236 2005; Simmons, Seymour, Habersetzer, & Gunnell, 2008); *Tachypteron*, the oldest known emballonurid fossil  
237 from the early Middle Eocene, with an age range of 48.6 to 40 Mya (Storch, Sigé, & Habersetzer, 2002);  
238 *Hipposideros africanum*, the oldest fossil record of the family Hipposideridae, its records date at 41.3 Mya  
239 (Ravel et al., 2016); Vespertilionidae indet. (41.3 Mya) (Eiting & Gunnell, 2009); Phyllostomidae indet. (30  
240 Mya) (Nicholas J Czaplewski, 2010), and *Palynephyllum* (11.8 Mya) (Nicolas J Czaplewski, Takai, Naeher, &  
241 Setoguchi, 2003; Dávalos, Velazco, Warsi, Smits, & Simmons, 2014). Additionally, we included and  
242 corroborated the molecular dates for the base of the ingroup root estimated by Teeling et al. (2005).

#### 243 *Gene family evolution*

244 While previous analyses that included bat species have analyzed signals of positive selection across bats (e.g.  
245 Parker et al., 2013), fewer have explicitly centered on gene family evolution (Jebb et al., 2020; Tsagkogeorga  
246 et al., 2017). To analyze our comprehensive bat-focused sample, we modeled gene family expansions and  
247 contractions using CAFE (Computational Analysis of Gene Family Evolution) v.4.2.1 (M. V. Han, Thomas, Lugo-  
248 Martinez, & Hahn, 2013). CAFE fits a birth and death parameter ( $\lambda$ ) to estimate the probability of gene gains  
249 or losses across a specified phylogeny (Hahn, De Bie, Stajich, Nguyen, & Cristianini, 2005), and we used the  
250 newly inferred phylogeny to this end.

251 When we included all species in the CAFE analysis, we observed a systematic bias in gene family  
252 contractions among fragmented genomes. This effect of genome quality on downstream gene predictions is  
253 well documented and leads to an overestimation of gene gains and losses (Denton et al., 2014; Tsagkogeorga  
254 et al., 2017). To mitigate the bias, only genome assemblies with BUSCO completeness scores over 80%,  
255 totaling 34 species (28 bat species and 6 outgroup mammals) were used for CAFE. This smaller subset of  
256 protein sequences was filtered, retaining only the longest isoform. Homology clustering was performed with  
257 Orthofinder v.2.4.0 (Emms & Kelly, 2019).

258 We filtered the final input for CAFE to reduce systematic bias in inferring gene family evolution. First,  
259 we retained only gene families present at the most recent common ancestor of the phylogeny, with at least  
260 one gene present in each of the four clades assigned: a) Euarchontoglires (*Homo sapiens* and *Mus musculus*),  
261 b) non-Chiroptera Laurasiatheria (*Bos taurus*, *Canis familiaris*, *Equus caballus*, *Sus scrofa*), c) Yangochiroptera  
262 and d) Yinpterochiroptera. Second, gene families missing in more than 50% of bat species were excluded.

263 Finally, families with large gene copy number variance ( $\geq 100$  gene copies) were excluded for the global birth  
264 and death ( $\lambda$ ) rate inference.

265 To analyze families with at least one gene copy across the taxa sampled, we first estimated a global  $\lambda$   
266 for all branches. The global model was compared against a three multi- $\lambda$  model that fits each lineage with its  
267 own gene family evolution rate. To test which model fits better with our dataset, we performed a likelihood  
268 ratio test for 100 gene family evolution simulations. We ran CAFE in error correction mode to account for  
269 genome assembly and annotation errors and estimate the global distribution of error with the assumption  
270 that all branches share a unique  $\lambda$  rate ( $\lambda=0.0033734$ ) as described in Han et al. (2013). Finally, we used  
271 complementary tools; the Protein Analysis Through Evolutionary Relationships (PANTHER v.15) (Mi,  
272 Muruganujan, Ebert, Huang, & Thomas, 2019) and Gene Ontology Analysis (GOnet) to annotate genes with  
273 gene ontology (GO) terms (Ashburner et al., 2000; Carbon et al., 2019) and assign them to gene families,  
274 pathways, and biological process categories.

#### 275 *Selection tests*

276 We identified genes under positive selection by evaluating 268 single-copy genes involved in immune  
277 response, based on a curated database of 1,793 genes downloaded from the IMIMPORTDB repository  
278 (Bhattacharya et al., 2014) available at <https://www.immport.org/home>. Gene alignments were built with  
279 MAFFT v.7.402 (Katoh & Standley, 2013) and manually filtered to remove sequences with less than 70% of  
280 protein coverage based on the homologous human protein. Only alignments represented by at least 30% of  
281 the species were used for downstream analysis. For each gene in the codeml analyses, we built a phylogeny  
282 with RAxML (Stamatakis, 2014) and a codon alignment for each gene with PAL2NAL (Suyama, Torrents, &  
283 Bork, 2006).

284 We tested for evidence of positive selection among sites along bat lineages using the strict branch-  
285 site model (Yang, Wong, & Nielsen, 2005; J. Zhang, Nielsen, & Yang, 2005) with maximum-likelihood  
286 estimations implemented in codeml in PAML v.4.9 (Yang, 2007). We implemented model 2 as this allows the  
287 dN/dS ratio ( $\omega$ ) to vary across branches and sites and to detect if selection differs in a few amino acid residues  
288 in specific lineages (foreground branches). We compared two hypotheses, assigning the 37 bat species as  
289 foreground branches: 1) the null hypothesis with a fixed  $\omega$  ( $\omega=1$ ) for all branches does not allow for positive  
290 selection, and 2) an alternative hypothesis assuming that the foreground branches have a greater proportion  
291 of sites under positive selection ( $\omega > 1$ ) than the background branches. The null hypothesis was tested against  
292 the alternative model with the likelihood-ratio test (LRT); the p-value was calculated under a chi-square

293 distribution with 1 degree of freedom, additionally we adjusted the p-value using the false discovery rate  
294 (FDR) correction. To detect sites under positive selection, we used the Bayes Empirical Bayes (BEB) (Yang et  
295 al., 2005) approach to calculate posterior probabilities that a site has a significant value of  $\omega > 1$ . The residues  
296 with a high posterior probability ( $P > 95\%$ ) were considered.

297 To determine how robust the signals of positive selection detected were, we used the adaptive  
298 Branch-Site Random Effects Likelihood (aBSREL) (Smith et al., 2015) model, as implemented in HyPhy  
299 (Kosakovsky Pond, Frost, & Muse, 2005). The aBSREL model explores whether a proportion of sites have  
300 evolved under positive selection in each branch of the phylogeny, and was applied to all alignments using  
301 their respective gene trees. The false discovery rate method of multiple testing correction was applied to all  
302 p-values generated for each branch and gene.

## 303 Results

### 304 *Genome sequencing*

305 The final assembly for *P. hastatus* comprised 2.1 Gb and has a N50 contig length  $>39$  Mb. Assembly quality  
306 completeness was estimated at 95.4%. These values are similar to those observed for bat assemblies inferred  
307 using similar methods (Jebb et al., 2020).

### 308 *Homology inference*

309 BUSCO analysis results indicated that the bat genome assemblies contained between 68.5 and 96.5% of the  
310 single-copy orthologs present among mammals (Figure 1). Orthologs were grouped into 42,441 groups, of  
311 which 1,193 were single copy. In total, 5,528 orthogroups had at least one representative in each of the entire  
312 set of 43 species that were analyzed. In contrast, 1,055 orthogroups were represented in at least 50% of bat  
313 species but missing from the six outgroup taxa (Supplementary table 3). To annotate diets, we used the semi-  
314 quantitative database compiled by Rojas, Ramos, Fonseca and Dávalos (2018), which focuses on neotropical  
315 noctilionoids (Yangochiroptera), supplemented with summaries from Animal Diversity Web  
316 (<https://animaldiversity.org/>).

### 317 *Enrichment in chiropteran gene losses*

318 We inferred the first densely sampled chiropteran phylogeny based on hundreds of loci (Figure 1). Our results  
319 confirmed the monophyly of the suborders Yinpterochiroptera and Yangochiroptera but the phylogeny of the  
320 neotropical leaf-nosed bats (family Phyllostomidae) differed from previous phylogenies (Dávalos, Velasco, &  
321 Rojas, 2020), in the paraphyly of plant-eating lineages. As the obtained phylogeny is the best supported by all

322 genome-scale analyses available thus far (S.J. Rossiter and M. Hiller pers. obs.), we used this phylogeny for  
323 gene family evolution analyses.

324 A total of 1,115 genes (Supplementary Table 4) were identified as missing in bats, even after filtering  
325 BLAST searches against the genomes and transcriptomes. Based on this list, we identified eight over-  
326 represented pathways in BioPlanet (Supplementary Table 5) and 63 GO terms in GOnet (Supplementary Table  
327 6). While the former included 104 genes, of which 49 were unique, the latter included 339 unique missing  
328 genes. As expected, over-represented categories included chemosensory gene losses in the categories of  
329 olfactory transduction, G-protein-coupled receptors (GPCR), and signal transduction. BioPlanet pathways  
330 were also enriched for less common categories including immune system pathways that include alpha and  
331 beta defensins, antigen process and presentation, and graft-versus-host disease (Supplementary Table 5).  
332 GOnet analyses also identified the expected enrichments in chemosensory gene losses and general response  
333 to stimuli categories, but also included many more immune categories. Of the latter, the categories  
334 comprising the most genes were defense response (58 genes), defense response to other organism (54),  
335 response to bacterium (53), innate immune response (46), defense response to bacterium (44), humoral  
336 immune response (34), adaptive immune response based on somatic recombination of immune receptors  
337 built from immunoglobulin superfamily domains (23), lymphocyte mediated immunity (23), and leukocyte  
338 mediated immunity (23). Although these categories share many genes across them, a preponderance of  
339 immune system losses is evident in Supplementary Table 6. We used BioRender to summarize the immune  
340 gene ontology categories and connections, highlighted in Figure 2.

#### 341 *Gene family evolution*

342 To determine branches and gene families with significant gene family expansions and contractions, we  
343 analyzed 14,171 orthogroups under two models: a global rate of gene family evolution, and a three multi- $\lambda$   
344 model. The three-rate model best fit the data ( $p < 0.01$ ), this analysis estimated a higher rate of gene family  
345 turnover ( $\lambda_{\text{Yangochiroptera}} = 0.0048$ ) in the ancestral Yangochiroptera lineage than in the Yinpterochiroptera  
346 ancestral lineage ( $\lambda_{\text{Yinpterochiroptera}} = 0.0024$ ), with the lowest turnover rate for outgroup lineages ( $\lambda_{\text{Outgroups}} =$   
347 0.0017).

348 With an estimated error distribution of 0.049 (i.e., 4.9% of gene families showed an error in gene size), we  
349 identified 2,555 orthogroups with significant expansions or contractions along at least one of the branches in  
350 the species tree (Supplementary Table 7). Given our focus on immune system and metabolic evolution, we  
351 extracted PANTHER annotations for the most frequent (900 orthogroups) biological process categories:



352 immune response, metabolic process, and cellular process. All GOnet annotations were used and binned into  
353 immune, metabolic, and two additional processes: response to stress (271 orthogroups) and autophagy (19).  
354 PANTHER and GOnet annotations were mostly complementary; orthogroups were often annotated in one  
355 database but not the other (1,268 orthogroups). When annotations were available from both databases,  
356 these tended to agree on both immune and metabolic categories (594 orthogroups), or to agree on one or  
357 the other (404), with only 48 orthogroups disagreeing completely in immune and metabolic annotations  
358 between the databases. The remaining 241 were not annotated in either database. Categories, locations, and  
359 size of significant gene family changes were summarized using tools in the R package ggtree (Yu, Smith, Zhu,  
360 Guan, & Lam, 2017) and are shown in Figure 3. Although several pairs of sister species showed apparently  
361 large differences along corresponding tips (e.g., *Rhinolophus*, *Miniopterus*), such variation is common in  
362 analyses that include genome assemblies of varying quality (Denton et al., 2014; Tsagkogeorga et al., 2017).  
363 Therefore, we focus our discussion on the more robust inference of gene family expansions and contractions  
364 for non-sister lineages in immunity and metabolism genes.

#### 365 *Selection tests*

366 Branch-site selection tests identified 37 of 268 single-copy genes with evidence for positive selection, of  
367 which 27 remained after false discovery rate correction (Table 1). This subset included genes involved in  
368 interferon-gamma (IFNG) signaling, inflammatory response, as well as cytokines, chemokines, and  
369 interleukins. A total of 16,979 branches across 268 genes were analysed using the aBSREL model in HyPhy.  
370 After FDR correction, 683 branches from 191 gene trees were found to be significant, 25 of which were  
371 consistent with CODEML results (Supplementary table 8).

#### 372 **Discussion**

373 Gene losses in inflammation-related gene families and positive selection in single-copy genes associated with  
374 immune and cell repair functions in mammalian models have been evident since the very first bat genome  
375 assemblies were published (G. Zhang et al., 2013). Although subsequent studies have confirmed those initial  
376 results (Ahn et al., 2016; Seim et al., 2013), confidence in assessing both gene losses and gene family  
377 expansions has strengthened only recently, with the publication of highly contiguous assemblies for a few bat  
378 species (Jebb et al., 2020; Scheben et al., 2020). Examining a comprehensive sample of bat lineages while  
379 checking against high quality genome assemblies and multi organ RNA Seq, our analyses reveal system wide  
380 gene losses with the potential to modify the sensitivity, targets, and magnitude of immune responses across  
381 all bats. These inferred losses are particularly concentrated along inflammasome activation pathways, which

382 are triggered by the innate immune recognition of pathogenic signals through both pathogen-associated  
383 molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs). In contrast with more  
384 pathogen-driven PAMPs, DAMPs result from host cellular distress signals such as mitochondrial stress and  
385 reactive oxygen species (ROS) (Zheng, Liwinski, & Elinav, 2020), which bats produce during active flight  
386 (Costantini, Lindecke, Petersons, & Voigt, 2019). Bat cells, in turn, display exceptional mechanisms of repair  
387 (Pickering, Lehr, Kohler, Han, & Miller, 2014) and resist damage (Harper, Salmon, Leiser, Galecki, & Miller,  
388 2007), connecting molecular signaling and cell processes to extreme longevity (Salmon et al., 2009; Wilkinson  
389 & Adams, 2019).

390 Based on our genomic surveys, immune-related losses can be divided into three categories: the  
391 epithelial defense receptors (defensins), the Natural Killer gene complex (NKC) and the interferon-induced  
392 pathway (IFI; HIN; PYHIN) (Figure 2). This particular combination of losses in crucial components of immune  
393 activation seems contradictory, as it would imply that these losses could lead to an ineffective immune  
394 response in bats. This contradiction notwithstanding, these results complement previous findings indicating  
395 that bats have evolved efficient mechanisms of regulation that allow them to mount a low intensity immune  
396 response to primarily intracellular pathogens. Integrating these genomic findings with published functional  
397 data suggests complex, systemic adaptation, in line with both previous analyses of bat immune system  
398 responses (A. Banerjee et al., 2020; Basler, 2020; P. Zhou, 2020) and the growing body of evidence for cellular  
399 mechanisms underlying longevity (Z. Huang, Whelan, Dechmann, & Teeling, 2020; Z. Huang et al., 2019,  
400 Kacprzyk et al., 2017). We review these losses in a stratigraphic order, from the outer cellular matrix to the  
401 inner cellular pathways, starting with the defensins.

402 While defensins are the primary barrier of the immune system, with broad antimicrobial activity that  
403 covers bacteria, fungi, and viruses (Semple & Dorin, 2012; Xu & Lu, 2020), bat defensin losses consist mainly  
404 of orthologs of genes localized to epithelial cells. Our results indicate that both  $\alpha$  and  $\beta$  defensin genes have  
405 undergone a rapid evolutionary change through either loss or positive selection (Table 1, Figure 2a,  
406 Supplementary Table 4). Rapid evolution and diversification of defensins, driven by the microbiome, varies  
407 considerably among species, even in closely related species (Tu et al., 2015). Among vertebrates, an  
408 expansion of  $\beta$  defensins occurred in mammals, with bovines having the largest number of copies (Tu et al.,  
409 2015), while  $\alpha$  defensins, exclusive from mammals (Xiao et al., 2004), are lost in bovines (Fjell et al., 2008).

410 Defensins can function as modulators of the host's cell surface receptors, and  $\alpha$  and  $\beta$  defensins  
411 genes have pleiotropic effects on the regulation of carcinogenesis and inflammation (Xu & Lu, 2020). By

412 acting as chemokines to alter the adaptive immune response, defensins also serve as a bridge between innate  
413 and adaptive immunity (Grigat, Soruri, Forssmann, Riggert, & Zwirner, 2007). In humans, defensins can elicit  
414 proinflammatory cytokine production (Niyonsaba et al., 2010; Wiens, Wilson, Lucero, & Smith, 2014), but  
415 overexpression of certain defensins can actually enhance viral infection (Rapista et al., 2011). We hypothesize  
416 that specific defensin losses in bats (Figure 2a) complement several other mechanisms (Ahn et al., 2019; A.  
417 Banerjee et al., 2017; Xie et al., 2018) contributing to a dampened inflammatory response, reduced host-  
418 driven damage from viral infections, and enhanced longevity (Baker & Schountz, 2018; Brook & Dobson,  
419 2015; Gorbunova et al., 2020). For example, modifying defensin repertoires on epithelial cells would result in  
420 fewer instances of both immune cell recruitment and initiation of inflammatory pathways known to damage  
421 healthy tissue (e.g., focal necrosis in lungs, spleen and lymph nodes during the inflammatory response during  
422 SARS-Cov2 infection (Merad & Martin, 2020)). In humans, loss of  $\beta$ -defensins prevents the inhibition of  
423 neutrophil apoptosis and thus averts the production of proinflammatory cytokines and chemokines (Nagaoka,  
424 Niyonsaba, Tsutsumi-Ishii, Tamura, & Hirata, 2008), avoiding the amplification of the immune response, and  
425 may have a similar effect in bats. Losses of some epithelial surface defensins would thus reduce inflammation  
426 without compromising responses to intracellular pathogens.

427 Another result with inferred implications for reducing proinflammatory reactions involves losses of  
428 Natural Killer (NK) receptors that play an important role in the recognition of MHC-I molecules and regulation  
429 of cytotoxic activity against virus-infected cells. While killer-cell immunoglobulin like receptors (KIR) and killer  
430 cell lectin-like receptors (KLR) receptor losses has been previously reported for *Pteropus alecto* and *Myotis*  
431 *dauidii* (Papenfuss et al., 2012; G. Zhang et al., 2013), our analyses confirm these losses across Chiroptera  
432 (Supplementary Table 4). Although the *Killer Cell Lectin Like Receptor K1* (KLRK1 or NKG2D) gene is present in  
433 bats, its ligands, gene subfamilies *RAET1* and *H60* responsible for binding and activating NKG2D receptors,  
434 recruiting natural killer cells, and stimulating them to secrete Interferon gamma (IFN- $\gamma$ ) (Zhi et al., 2010), were  
435 absent in all bat species (Figure 2b).

436 We hypothesize that these losses lead to low recruitment of proinflammatory NK cells and reduce B-  
437 cell signaling (Arapović et al., 2009; Stolberg et al., 2014; Takada et al., 2008; Wortham et al., 2012), as they  
438 do in mice and humans. Loss of this particular mechanism of activation of the MHC-I pathway prevents  
439 proliferation of immune cells, which can be cytotoxic, proinflammatory, and targets of viral infections  
440 (Djelloul, Popa, Pelletier, Raguénez, & Boucraut, 2016; Wortham et al., 2012). For example, NKG2D-deficient  
441 mice infected with influenza viruses exhibit less airway damage and reduced inflammation without

442 compromising viral clearance; similarly, knockout of NKG2D in mice and humans during cytomegalovirus  
443 infection helps to avoid the destruction of non-infected cells by NK (Muntasell et al. 2010; Slavuljica,  
444 Krmpotić, & Jonjić, 2011). NKG2D stimulation is a central pathway to tumor, stress and viral-mediated NK cell  
445 hyper responsiveness (Wortham et al. 2012) and has been shown to be involved in autoimmune disorders,  
446 such as rheumatoid arthritis, type I diabetes, and celiac disease (reviewed in Caillat-Zucman, 2006; Guerra et  
447 al. 2013), and inflammatory diseases such as Crohn's disease (Vadstrup et al. 2017), chronic respiratory  
448 diseases (Wortham et al. 2012; Guerra et al. 2013) and more recently with age-dependent COVID-19 severity  
449 (Akbar & Gilroy, 2020). During viral exposure, rarer activation of NKG2D function would therefore lead to less  
450 inflammatory exacerbation. Reducing instances of NKG2D activation might also reduce B cell signaling, as it  
451 occurs in NKG2D-deficient mice (Lenartić et al., 2017; Zafirova et al., 2009), and complements losses of  
452 immunoglobulin heavy chain variable regions IGHV1, IGVH3, and IGHV14 genes that modify the B cell  
453 receptor signaling pathway, and thus B lymphocyte differentiation (M. Banerjee, Mehr, Belevsky, Spencer,  
454 & Dunn-Walters, 2002; McHeyzer-Williams, Okitsu, Wang, & McHeyzer-Williams, 2012; Reddy et al., 2010).  
455 Based on the roles of both NKG2D and B cell activation in promoting inflammation in viral infection, and since  
456 some viral proteins have been shown to specifically target the NKG2D receptor via the RAET1 and H60 loci  
457 (Arapović et al., 2009), we propose that these losses resulted from selection during viral infections early in the  
458 evolutionary history of bats. While the functional implications for bats need to be tested, in humans, lack of  
459 specificity of the T and B cells in children results in a broader immune response to novel viruses (Pierce et al.,  
460 2020), and it may confer analogous advantages in bats.

461 Complementing losses in defensins and NK signaling, the third large group of gene losses involves the  
462 IFN- $\gamma$  pathway (Figure 2c). While representatives of the PYRIN and HIN domain (PYHIN) gene family, immune  
463 sensors of cytosolic DNA activating the inflammasome and IFN- $\gamma$ , are present in all mammals, they have not  
464 been found in any of the bat genomes analyzed thus far examined (Ahn et al., 2016; G. Zhang et al., 2013;  
465 Jebb et al., 2020). Previous genomic analyses linked losses in this inflammasome pathway not only to immune  
466 implications, but also to the unique demands of bat flight and in response to increased ROS production (G.  
467 Zhang et al., 2013). In other mammals, the presence of dsDNA, DAMPs and PAMPs, or, especially, bacteria  
468 and DNA viruses, induces the (PYHIN) AIM2 inflammasome, while the IFI16 inflammasome (Interferon-  
469 inducible protein 16, also missing in bats) recognizes viruses replicating in the nucleus (Zheng et al., 2020).  
470 Hence, these bat gene losses could undermine innate defense against viruses. We hypothesize that bats have  
471 evolved mechanisms to overcome this potential disadvantage in rapid recognition and response against

472 viruses through expansion of MHC-I class genes (Supplementary Table 7). These genes are involved in the  
473 recognition and binding of intra cellular peptides, and previous studies have described a unique 5–amino acid  
474 insertion at the exon 2 peptide binding region (PBR) on bats which may allow the host to recognize longer  
475 peptides (Ng et al., 2016; Papenfuss et al., 2012). Besides implications for immunity, IFN- $\gamma$  pathway gene  
476 losses also point to changes in autophagy. In mice, loss of the IFN- $\gamma$  inducible immunity related GTPase gene  
477 (IRGM1 and IRGM2) results in an IFN- $\gamma$  induced autophagic death program in lymphocytes (Feng et al., 2008).  
478 Along with the loss of other IFN- $\gamma$  related genes (IGTO, IIGP, TGTP2), these losses may help achieve apoptosis  
479 of infected cells without runaway inflammation.

480 While some mechanisms of activation of IFN- $\lambda$  are lost in bats, IFN- $\gamma$  itself is under positive selection  
481 within branches (Table 1, Supplementary Table 7). IFN- $\gamma$  is a crucial part for the first line of defense against  
482 viruses, helps shape adaptive immune memory (Schroder, Hertzog, Ravasi, & Hume, 2004), and its deficiency  
483 increases inflammation (Loo et al., 2017). Thus, evolutionary adaptation may have shaped bats' unique ability  
484 to induce a rapid antiviral response without triggering runaway inflammation. This fine-tuned response may  
485 be achieved by expressing high levels of IFN- $\gamma$  early on, which recruits broad-spectrum immune cells to the  
486 site of injury, while negatively regulating the IFN- $\gamma$  pathway receptors that trigger inflammation (Ahn et al.,  
487 2019; Ferber et al., 1996).

488 By generating a controlled induction of immune response, bats' unique regulatory mechanisms, have  
489 sparked an extraordinary immune tolerance against viruses, a key factor in bats as natural viral reservoirs.  
490 Evidence of this viral tolerance has been observed in bats with high viral load (reviewed in; Subudhi, Rapin, &  
491 Misra, 2019; Irving et al., 2021). In addition, *in silico* experiments have shown that a trade-off of this viral  
492 tolerance in bats is the rapid spread of viruses within the host; thus, favoring viruses to evolve adaptations  
493 that increase their replication rates (Brook et al. 2020). While this rapid transmission may not have a  
494 significant harmful effect in bats, it could be detrimental for other species, as recent spillovers have shown.

495 In contrast to a pattern of proinflammatory signal losses common to all bats, most other variation in  
496 gene families within Chiroptera corresponded to cell processes and metabolic functions with the notable  
497 exceptions of APOBEC3 and MHC-I. Besides confirming the previously reported APOBEC3 expansion in  
498 *Pteropus vampyrus* (Hayward et al. 2018), we also inferred expansions in the common ancestors of *Desmodus*  
499 and *Artibeus*, of Vespertilionids, *Myotis*, and of *M. brandtii* and *Lucifugus*, including species-specific  
500 expansions in the latter. With this denser sampling, expansions formerly traced to *Myotis myotis* and  
501 *Pipistrellus kuhlii* (Jebb et al. 2020), are instead part of broader vespertilionid dynamics especially within

502 *Myotis*. Other species-specific expansions were inferred in the phyllostomids *Tonatia saurophila* and  
503 *Desmodus rotundus*, both of which shift from an ancestral bat insectivorous diet to one including vertebrates,  
504 exclusively so for *Desmodus*. While MHC-I expansions have been highlighted in *Pteropus alecto* (Ng et al.  
505 2016) and *Rousettus aegyptiacus* (Pavlovich et al. 2018), here we find much greater expansions in neotropical  
506 noctilionoids including *Noctilio*, *Mormoops*, and especially within Phyllostomidae including *Artibeus*, *Sturnira*,  
507 *Tonatia*, *Leptonycteris*, *Musonycteris*, *Anoura*, *Desmodus*, and *Macrotus*. As with APOBEC3, MHC-I evolution  
508 in vespertilionids was found to be dynamic, with significant expansions inferred for every *Myotis* species, as  
509 well as *Pipistrellus* and *Eptesicus*. While APOBEC3 function has been examined in *Pteropus alecto* (Hayward et  
510 al. 2018), our analyses highlight the need for characterization in vespertilionids. With greater potential for  
511 ligand binding, rich MHC-I repertoires may provide both better self recognition for NK tuning and finer  
512 resolution of MHC-I pathogen mimics (Parham & Moffett, 2013), suggesting further research avenues in  
513 phyllostomids, vespertilionids, and *Miniopterus*. Our analyses overlooked both the potential for unique MHC-I  
514 features that alter antigen presentation, as in *Pteropus alecto*, and population variation, already found in the  
515 phyllostomids *Carollia perspicillata* (Qurkhuli et al. 2019), suggesting these as potential research avenues.

516 Expansions and contractions in metabolic genes were common throughout the bat phylogeny (Figure  
517 3), but many ecological differences across species (e.g., biogeography, hibernation, life history) could be  
518 driving these changes (Seim et al. 2013; Y. Han et al. 2015). Taking advantage of our relatively dense taxon  
519 sampling within bats (Figure 1), we focus on parallel adaptation to plant-rich diets across suborders  
520 Yinpterochiroptera and Yangochiroptera, a set of traits of known metabolic implications (Voigt & Speakman,  
521 2007). Shifts from the ancestral bat insectivorous diet to including nectar and fruit and the resulting  
522 mutualistic relationships between bats and plants appear to have led to elevated rates of diversification and  
523 the evolution of new morphological traits (Dumont et al., 2012; Jones, Bininda-Emonds, & Gittleman, 2005),  
524 but gene family evolution has remained underexplored. Regarding significant expansions (Supplementary  
525 Table 7), we identified few —only nine— sets of duplications independently replicated across all pteropodids  
526 and phyllostomids with convergent, plant-based diets (Figure 1). In addition to a trace amine associated  
527 receptor (TAAR) of unknown chemosensory function (Liberles & Buck, 2006) and a putative homolog of the  
528 yeast protein transport protein YIP1, two genes stand out as candidates for diet-linked adaptive gene family  
529 evolution: those encoding homologs of *inositol monophosphatase 1* (IMPA1) and *integrin alpha-D/beta-2*  
530 (ITAD). Glycolysis, the metabolic pathway that breaks down glucose to ultimately phosphorylate more ADP  
531 into ATP than the reverse, begins with the phosphorylation of glucose into D-glucose 6-phosphate (Berg,

532 Tymoczko, & Stryer, 2002). This metabolite, however, cannot diffuse through the membrane and is thus  
533 highly osmotic; its accumulation would cause cells to swell. Through the synthesis of *myo*-inositol from D-  
534 glucose 6-phosphate, IMPA1 provides one avenue to protect cells, particularly in the brain (Parthasarathy,  
535 Parthasarathy, & Vadnal, 1997), from the osmotic stress of this glucose metabolite (Rafikov et al., 2019). We  
536 found independent IMPA1 duplications in the pteropodid ancestor, *A. jamaicensis*, *A. caudifer*, *P. discolor*,  
537 and the common ancestor of phyllostomids and *Mormoops*. Except for the aerial insectivore *Mormoops*, all  
538 the lineages with IMPA1 duplications include nectar and fruit in their diet (Figure 1), are expected to at least  
539 occasionally experience high blood glucose levels (Amitai et al., 2010; Ayala & Schondube, 2011; Kelm, Simon,  
540 Kuhlow, Voigh & Ristow, 2011; Welch, Herrera & Suarez, 2008; Meng, Zhu, Huang, Irwin, & Zhang, 2016), and  
541 therefore require options for processing metabolites from glycolysis. Although beta integrins, including ITAD,  
542 are regulators of leukocyte function and therefore not annotated as directly involved in metabolism,  
543 leukocyte adhesion has been found to modulate glucose homeostasis via lipid metabolism (Meakin et al.,  
544 2015). Specifically, mice deficient in a paralogous beta-2 integrin become spontaneously obese in old age  
545 despite a normal diet (Z. Dong, Gutierrez-Ramos, Coxon, Mayadas, & Wagner, 1997), and when fed a fat rich  
546 diet show obesity, inflammation, high neutrophil activity and insulin resistance in skeletal muscle (Meakin et  
547 al., 2015). Likewise, mice deficient in this same integrin are unable to respond to fasting by increasing fat  
548 uptake and reduce insulin levels slowly compared to normal mice (Babic et al., 2004). We found single ITAD  
549 duplications in lineages that include sugar rich foods in their diet: ancestral pteropodids and phyllostomids, as  
550 well as *Leptonycteris yerbabuena*, two each in *Macroglossus*, *Anoura*, and *Tonatia*, and three in *Artibeus*  
551 *jamaicensis*. While the function of these lineage-specific bat paralogs remain unknown, their phylogenetic  
552 distribution warrants future exploration and functional analysis.

553 In summary, our results, grounded on the most comprehensive survey of bat genomes to date,  
554 suggest bats have evolved complex mechanisms of inflammasome regulation. These may have evolved to  
555 prevent uncontrolled inflammatory response against DAMPs byproducts of the high metabolic rate required  
556 for powered flight (Banerjee et al., 2017; Banerjee et al., 2020; Subudhi, Rapin & Misra, 2019; Xie et al., 2018),  
557 to better respond against intra-cellular pathogens such as viruses, or some combination of both. Regardless  
558 of the ecological origin of selection, compared to mammals such as humans or mice, bat genomes reveal  
559 systemwide immune evolution that prevents or dampens aggressive inflammatory responses. In contrast with  
560 these gene losses, we found significant expansions in gene families involved with glucose degradation,

561 coinciding with the transition from a diet based mainly on insects to a high-glucose content diet that includes  
562 fruit and nectar.

563 By undertaking large-scale comparative genomic analyses encompassing many ecologically divergent  
564 lineages, the present study demonstrates the impact of genomics in non-model organisms. Such analyses  
565 allow elucidating the broad evolutionary mechanisms in a given clade, with potential for functional  
566 implications. Yet, heterogeneity in assembly quality continues to limit the scope of inference. Hence, the need  
567 to generate high quality genomes for future studies endures.

568

## 569 Acknowledgments

570 For support in long-read Oxford Nanopore sequencing, we thank Dr. Sara Goodwin from Cold Spring Harbor  
571 Laboratories. L.M.D. was supported, in part, by NSF-DEB 1838273, NSF-DGE 1633299, with S.J.R by NSF-DEB  
572 1442142, and with A.M.B. by NSF-IOS 2032063 and 2031906. D.A.R. and D.D.M.S. were supported, in part, by  
573 NSF-DEB 1838283, and NSF-IOS 2032006. A.P.C was supported, in part, by NSF-IOS 2032011 and 2031926.  
574 T.M.L was supported by NSF-PRFB 2010853. L.R.Y. was supported by NSF-IOS 2032073 and NSF-DBI 1812035.  
575 E.C.T. was supported in part by an Irish Research Council Laureate Award IRCLA/2017/58. SCV was supported  
576 by a Max Planck Research Group awarded by the Max Planck Gesellschaft, a Human Frontiers Science  
577 Program Grant (RGP0058/2016) and a UKRI Future Leaders Fellowship (MR/T021985/1). The authors would  
578 like to thank Stony Brook Research Computing and Cyberinfrastructure, and the Institute for Advanced  
579 Computational Science at Stony Brook University for access to the high-performance SeaWulf computing  
580 system, which was made possible by a \$1.4M National Science Foundation grant (#1531492). The High-  
581 Performance Computing Center at Texas Tech University and The Scientific Computing Department at  
582 the Instituto de Ecología, Universidad Nacional Autónoma de México provided computational  
583 infrastructure and technical support throughout the work.

## 584 References

585 Ahn, M., Anderson, D. E., Zhang, Q., Tan, C. W., Lim, B. L., Luko, K., ... Wang, L. F. (2019). Dampened NLRP3-  
586 mediated inflammation in bats and implications for a special viral reservoir host. *Nature Microbiology*, 4,  
587 789-799. doi: 10.1038/s41564-019-0371-3  
588 Ahn, M., Cui, J., Irving, A. T., & Wang, L. F. (2016). Unique loss of the PYHIN gene family in bats amongst  
589 mammals: Implications for inflammasome sensing. *Scientific Reports*, 6. doi: 10.1038/srep21722



590 Amitai, O., Holtze, S., Barkan, S., Amichai, E., Korine, C., Pinshow, B., & Voigt, C. C. (2010). Fruit bats  
591 (Pteropodidae) fuel their metabolism rapidly and directly with exogenous sugars. *The Journal of*  
592 *Experimental Biology*, 213(215), 2693-2699. doi: 10.1242/jeb.043505

593 Arapović, J., Lenac, T., Antulov, R., Polić, B., Ruzsics, Z., Carayannopoulos, L. N., ... Jonjić, S. (2009). Differential  
594 Susceptibility of RAE-1 Isoforms to Mouse Cytomegalovirus. *Journal of Virology*, 83(16), 8198-8207. doi:  
595 10.1128/jvi.02549-08

596 Akbar, A. N., & Gilroy, D. W. (2020). Aging immunity may exacerbate COVID-19. *Science*, 369(6501), 256-257.  
597 doi.org/10.1126/science.abb0762

598 Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., ... Sherlock, G. (2000). Gene  
599 ontology: Tool for the unification of biology. *Nature Genetics*, 25, 25-29. doi: 10.1038/75556

600 Austad, S. N., & Fischer, K. E. (1991). Mammalian aging, metabolism, and ecology: Evidence from the bats and  
601 marsupials. *Journals of Gerontology*, 46(2), 47-53. doi: 10.1093/geronj/46.2.B47

602 Ayala-Berdon, J., & Schondube, J. E. (2011). A physiological perspective on nectar-feeding adaptation in  
603 phyllostomid bats. *Physiological and Biochemical Zoology*, 84, 458-466. doi: 10.1086/661541

604 Babic, A. M., Wang, H. W., Lai, M. J., Daniels, T. G., Felbinger, T. W., Burger, P. C., ... Wagner, D. D. (2004).  
605 ICAM-1 and  $\beta$ 2 integrin deficiency impairs fat oxidation and insulin metabolism during fasting. *Molecular*  
606 *Medicine*, 10(7-12), 72-79. doi: 10.2119/2004-00038.Wagner

607 Baker, M. L., & Schountz, T. (2018). Mammalia: Chiroptera: Immunology of Bats. In E. Cooper (Eds.), *Springer*  
608 *International Publishing* (pp. 869-832). doi.org/10.1007/978-3-319-76768-0\_23

609 Banerjee, A., Baker, M. L., Kulcsar, K., Misra, V., Plowright, R., & Mossman, K. (2020). Novel Insights Into  
610 Immune Systems of Bats. *Frontiers in Immunology*, 11, 1-26. doi: 10.3389/fimmu.2020.00026

611 Banerjee, A., Rapin, N., Bollinger, T., & Misra, V. (2017). Lack of inflammatory gene expression in bats: A  
612 unique role for a transcription repressor. *Scientific Reports* 7, 1-15. doi: 10.1038/s41598-017-01513-w

613 Banerjee, M., Mehr, R., Belelovsky, A., Spencer, J., & Dunn-Walters, D. K. (2002). Age- and tissue-specific  
614 differences in human germinal center B cell selection revealed by analysis of IgVH gene hypermutation  
615 and lineage trees. *European Journal of Immunology*, 32(7), 1947-1957. doi: 10.1002/1521-  
616 4141(200207)32:7<1947::AID-IMMU1947>3.0.CO;2-1

617 Basler, C. (2020). Innate Immunity in Bats. In Eugenia Corrales-Aguilar & M. Schwemmler (Eds.), *Bats and*  
618 *Viruses: Current Research and Future Trends* (pp. 119-134). Caister Academic Press.  
619 doi:10.21775/9781912530144.08

- 620 Bateman, A. (2019). UniProt: A worldwide hub of protein knowledge. *Nucleic Acids Research*, 47(D1), D506-  
621 D515. doi: 10.1093/nar/gky1049
- 622 Bay, R. A., Rose, N. H., Logan, C. A., & Palumbi, S. R. (2017). Genomic models predict successful coral  
623 adaptation if future ocean warming rates are reduced. *Science Advances*, 3(11). doi:  
624 10.1126/sciadv.1701413
- 625 Berg, J., Tymoczko, J., & Stryer, L. (2002). *Biochemistry* (5th ed.). New York: WH Freeman.
- 626 Bernatchez, L., & Landry, C. (2003). MHC studies in nonmodel vertebrates: What have we learned about  
627 natural selection in 15 years? *Journal of Evolutionary Biology*, 16(3), 363-377. doi: 10.1046/j.1420-  
628 9101.2003.00531.x
- 629 Bhattacharya, S., Andorf, S., Gomes, L., Dunn, P., Schaefer, H., Pontius, J., ... Butte, A. J. (2014). ImmPort:  
630 Disseminating data to the public for the future of immunology. *Immunologic Research*, 58, 234-239. doi:  
631 10.1007/s12026-014-8516-1
- 632 Boni, M. F., Lemey, P., Jiang, X., Lam, T. T. Y., Perry, B. W., Castoe, T. A., ... Robertson, D. L. (2020).  
633 Evolutionary origins of the SARS-CoV-2 sarbecovirus lineage responsible for the COVID-19 pandemic.  
634 *Nature Microbiology*, 5, 1408-1417. doi: 10.1038/s41564-020-0771-4
- 635 Brook, C. E., & Dobson, A. P. (2015). Bats as "special" reservoirs for emerging zoonotic pathogens. *Trends in*  
636 *Microbiology*, 23(3), 172-180. doi: 10.1016/j.tim.2014.12.004
- 637 Brook, C. E., Boots, M., Chandran, D., Dobson, A. P., Drosten, Ch., ...van Leeuwen, A. (2020). Accelerated viral  
638 dynamics in bat cell lines, with implications for zoonotic emergence. *eLife*, 9, e48401.  
639 doi:10.7554/eLife.48401
- 640 Caillat, S. (2006). How NKG2D ligands trigger autoimmunity? *Human Immunology*, 63(3), 204-207.  
641 doi:10.1016/j.humimm.2006.02.013
- 642 Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L. (2009). BLAST+:  
643 Architecture and applications. *BMC Bioinformatics*, 10(421). doi: 10.1186/1471-2105-10-421
- 644 Carbon, S., Douglass, E., Dunn, N., Good, B., Harris, N. L., Lewis, S. E., ... Westerfield, M. (2019). The Gene  
645 Ontology Resource: 20 years and still GOing strong. *Nucleic Acids Research*, 47(D1), D330-D338. doi:  
646 10.1093/nar/gky1055
- 647 Costantini, D., Lindecke, O., Petersons, G., & Voigt, C. C. (2019). Migratory flight imposes oxidative stress in  
648 bats. *Current Zoology*, 65(2), 147-153. doi: 10.1093/cz/zoy039
- 649 Czaplewski, N. J. (2010). Bats ( Mammalia : Chiroptera ) from Gran Barranca ( early Miocene , Colhuehuapian

650 ), Chubut Province , Argentina. In Madden, R., Carlini, A. A., Guiomar, V. M., & Kay, R. F.(Eds.), *The*  
651 *Paleontology of Gran Barranca. Evolution and environmental change through the middle Cenozoic of*  
652 *Patagonia.* (pp. 240–252). Cambridge University Press.

653 Czaplewski, Nicolas J, Takai, M., Naeher, T. M., & Setoguchi, T. (2003). Additional bats from the middle  
654 miocene la venta fauna of Colombia. *Revista de la academia colombiana de ciencias*, 27(103), 263-282.

655 Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2011). ProtTest 3: Fast selection of best-fit models of  
656 protein evolution. *Bioinformatics (Oxford, England)*. 27(8), 1164-1165. doi:  
657 10.1093/bioinformatics/btr088

658 Davalos, L. M., Velazco, P. M., & Rojas, D. (2020). Phylogenetics and Historical Biogeography. In *Phyllostomid*  
659 *bats: A Unique Mammalian Radiation* (pp. 87–103). Chicago, University of Chicago Press.

660 Dávalos, L. M., Velazco, P. M., Warsi, O. M., Smits, P. D., & Simmons, N. B. (2014). Integrating incomplete  
661 fossils by isolating conflicting signal in saturated and non-independent morphological characters.  
662 *Systematic Biology*, 63(4), 582-600. doi: 10.1093/sysbio/syu022

663 Davies, K. T. J., Yohe, L. R., Almonte, T., Sanchez, M. K. R., Rengifo, E. M., Dumont E. R., ... Rossiter, S. J. (2020).  
664 Foraging shifts and visual preadaptation in ecologically diverse bats. *Molecular Ecology*, 28(10), 1839-  
665 1859. <https://doi.org/10.1111/mec.15445>

666 Delves, P. J., Martin, S. J., Burton, D. R., & Roitt, I. M. (2017). *Roitt's Essential Immunology* (13th edition).  
667 London, UK: Wiley Blackwell.

668 Denton, J. F., Lugo-Martinez, J., Tucker, A. E., Schridder, D. R., Warren, W. C., & Hahn, M. W. (2014). Extensive  
669 Error in the Number of Genes Inferred from Draft Genome Assemblies. *PLoS Computational Biology*,  
670 10(12). doi: 10.1371/journal.pcbi.1003998

671 Djelloul, M., Popa, N., Pelletier, F., Raguénez, G., & Boucraut, J. (2016). RAE-1 expression is induced during  
672 experimental autoimmune encephalomyelitis and is correlated with microglia cell proliferation. *Brain*,  
673 *Behavior, and Immunity*, 58, 209-217. doi: 10.1016/j.bbi.2016.07.147

674 Dong, D., Lei, M., Hua, P., Pan, Y. H., Mu, S., Zheng, G., ... Zhang, S. (2017). The genomes of two bat species  
675 with long constant frequency echolocation calls. *Molecular Biology and Evolution*, 34(1), 20-34. doi:  
676 10.1093/molbev/msw231

677 Dong, Z. M., Gutierrez-Ramos, J. C., Coxon, A., Mayadas, T. N., & Wagner, D. D. (1997). A new class of obesity  
678 genes encodes leukocyte adhesion receptors. *Proceedings of the National Academy of Sciences of the*  
679 *United States of America*, 94(14), 7526-7530. doi: 10.1073/pnas.94.14.7526

680 Drexler, J. F., Corman, V. M., Müller, M. A., Maganga, G. D., Vallo, P., Binger, T., ... Drosten, C. (2012). Bats  
681 host major mammalian paramyxoviruses. *Nature Communications*, 3(796). doi: 10.1038/ncomms1796  
682 Dumont, E. R., Dávalos, L. M., Goldberg, A., Santana, S. E., Rex, K., & Voigt, C. C. (2012). Morphological  
683 innovation, diversification and invasion of a new adaptive zone. *Proceedings of the Royal Society B:  
684 Biological Sciences*, 279(1734). doi: 10.1098/rspb.2011.2005  
685 Eckalbar, W. L., Schlebusch, S. A., Mason, M. K., Gill, Z., Parker, A. V., Booker, B. M., ... Ahituv, N. (2016).  
686 Transcriptomic and epigenomic characterization of the developing bat wing. *Nature Genetics*, 48, 528-  
687 536. doi: 10.1038/ng.3537  
688 Eiting, T. P., & Gunnell, G. F. (2009). Global completeness of the bat fossil record. *Journal of Mammalian  
689 Evolution*, 16, 151-173. doi: 10.1007/s10914-009-9118-x  
690 Emms, D. M., & Kelly, S. (2019). OrthoFinder: Phylogenetic orthology inference for comparative genomics.  
691 *Genome Biology*, 20, 238. doi: 10.1186/s13059-019-1832-y  
692 Feng, C. G., Zheng, L., Jankovic, D., Báfica, A., Cannons, J. L., Watford, W. T., ... Sher, A. (2008). The immunity-  
693 related GTPase Irgm1 promotes the expansion of activated CD4+ T cell populations by preventing  
694 interferon- $\gamma$ -induced cell death. *Nature Immunology*, 9, 1279-1287. doi: 10.1038/ni.1653  
695 Fenton, M. B., & Simmons, N. B. (2015). *Bats: a world of science and mystery*. Chicago, IL: The University of  
696 Chicago Press.  
697 Ferber, I. A., Brocke, S., Taylor-Edwards, C., Ridgway, W., Dinisco, C., Steinman, L., ... Fathman, C. G. (1996).  
698 Mice with a disrupted IFN-gamma gene are susceptible to the induction of experimental autoimmune  
699 encephalomyelitis (EAE). *Journal of Immunology (Baltimore, Md. : 1950)*, 156(1), 5-7.  
700 Fjell, C. D., Jenssen, H., Fries, P., Aich, P., Griebel, P., Hilpert, K., ... , Cherkasov, A. (2008). Identification of  
701 novel host defense peptides and the absence of  $\alpha$ -defensins in the bovine genome. *Proteins*, 73, 420-  
702 430. doi:10.1002/prot.22059  
703 Fleischer, T., Gampe, J., Scheuerlein, A., & Kerth, G. (2017). Rare catastrophic events drive population  
704 dynamics in a bat species with negligible senescence. *Scientific reports*, 7, 7370. doi.org/10.1038/s41598-  
705 017-06392-9  
706 Foley, N. M., Hughes, G. M., Huang, Z., Clarke, M., Jebb, D., Whelan, C. V., ... Teeling, E. C. (2018). Growing old,  
707 yet staying young: The role of telomeres in bat's exceptional longevity. *Genetics*, 4. doi:  
708 10.1126/sciadv.aao0926  
709 Genereux, D. P., Serres, A., Armstrong, J., Johnson, J., Marinescu, V. D., Murén, E., ... Karlsson, E. K. (2020). A

710 comparative genomics multitool for scientific discovery and conservation. *Nature*, 587, 240-245. doi:  
711 10.1038/s41586-020-2876-6

712 Goebel, J., Promerová, M., Bonadonna, F., McCoy, K. D., Serbielle, C., Strandh, M., ... Fumagalli, L. (2017). 100  
713 million years of multigene family evolution: Origin and evolution of the avian MHC class IIB. *BMC*  
714 *Genomics*, 18, 460. doi: 10.1186/s12864-017-3839-7

715 Goldstein, T., Anthony, S. J., Gbakima, A., Bird, B. H., Bangura, J., Tremeau-Bravard, A., ... Mazet, J. A. K.  
716 (2018). The discovery of Bombali virus adds further support for bats as hosts of ebolaviruses. *Nature*  
717 *Microbiology*, 3, 1084-1089. doi: 10.1038/s41564-018-0227-2

718 Gorbunova, V., Seluanov, A., & Kennedy, B. K. (2020). The World Goes Bats: Living Longer and Tolerating  
719 Viruses. *Cell Metabolism*, 32(1), 31-43. doi: 10.1016/j.cmet.2020.06.013

720 Grigat, J., Soruri, A., Forssmann, U., Riggert, J., & Zwirner, J. (2007). Chemoattraction of Macrophages, T  
721 Lymphocytes, and Mast Cells Is Evolutionarily Conserved within the Human  $\alpha$ -Defensin Family. *The*  
722 *Journal of Immunology*, 179(6), 3958-3965. doi: 10.4049/jimmunol.179.6.3958

723 Guerra, N., Pestal, K., Juarez, T., Beck, J., Tkach, K., Wang, L., & Raulet, D. H. (2013). A selective role of NKG2D  
724 in inflammatory and autoimmune diseases. *Clinical immunology*, 149(3), 432-439.  
725 doi:10.1016/j.clim.2013.09.003

726 Gunnell, G. F., & Simmons, N. B. (2005). Fossil evidence and the origin of bats. *Journal of Mammalian*  
727 *Evolution*, 12, 209-246. doi: 10.1007/s10914-005-6945-2

728 Gutiérrez-Guerrero, Y. T., Ibarra-Laclette, E., Martínez Del Río, C., Barrera-Redondo, J., Rebollar, E. A., Ortega,  
729 J., ... Eguiarte, L. E. (2020). Genomic consequences of dietary diversification and parallel evolution due to  
730 nectarivory in leaf-nosed bats. *GigaScience*, 9(6). doi:10.1093/gigascience/giaa059

731 Hahn, M. W., De Bie, T., Stajich, J. E., Nguyen, C., & Cristianini, N. (2005). Estimating the tempo and mode of  
732 gene family evolution from comparative genomic data. *Genome Research*, 15(8), 1153-1160. doi:  
733 10.1101/gr.3567505

734 Han, H. J., Wen, H. ling, Zhou, C. M., Chen, F. F., Luo, L. M., Liu, J. wei, & Yu, X. J. (2015). Bats as reservoirs of  
735 severe emerging infectious diseases. *Virus Research*, 505, 1-6. doi: 10.1016/j.virusres.2015.05.006

736 Han, M. V., Thomas, G. W. C., Lugo-Martinez, J., & Hahn, M. W. (2013). Estimating gene gain and loss rates in  
737 the presence of error in genome assembly and annotation using CAFE 3. *Molecular Biology and*  
738 *Evolution*, 30(8), 1987-1997. doi: 10.1093/molbev/mst100

739 Han, Y., Zheng, G., Yang, T., Zhang, S., Dong, D., & Pan, YH. 2015. Adaptation of peroxisome proliferator-

740 activated receptor alpha to hibernation in bats. *BMC Evolutionary Biology*, 15(88). doi:10.1186/s12862-  
741 015-0373-6

742 Harper, J. M., Salmon, A. B., Leiser, S. F., Galecki, A. T., & Miller, R. A. (2007). Skin-derived fibroblasts from  
743 long-lived species are resistant to some, but not all, lethal stresses and to the mitochondrial inhibitor  
744 rotenone. *Aging Cell*, 6(1), 1-13. doi: 10.1111/j.1474-9726.2006.00255.x

745 Hayden, S., Bekaert, M., Goodbla, A., Murphy, W. J., Dávalos, L. M., & Teeling, E. C. (2014). A cluster of  
746 olfactory receptor genes linked to frugivory in bats. *Molecular Biology and Evolution*. 31(4), 917-927.  
747 doi: 10.1093/molbev/msu043

748 Hayman, D. T. S. (2019). Bat tolerance to viral infections. *Nature Microbiology*, 4, 728-729. doi:  
749 10.1038/s41564-019-0430-9

750 Hayward, J. A., Tachedjian, M., Cui, J., Cheng, A. Z., Johnson, A., Baker, M. L., ... Tachedjian, G. (2018).  
751 Differential evolution of antiretroviral restriction factors in pteropid bats as revealed by APOBEC3 gene  
752 complexity. *Molecular Biology and Evolution*, 35(7), 1626-1637. doi: 10.1093/molbev/msy048

753 Healy, K., Guillerme, T., Finlay, S., Kane, A., Kelly, S. B. A., McClean, D., ... Cooper, N. (2014). Ecology and  
754 mode-of-life explain lifespan variation in birds and mammals. *Proceedings of the Royal Society B:  
755 Biological Sciences*, 281(1784). doi: 10.1098/rspb.2014.0298

756 Holt, C., & Yandell, M. (2011). MAKER2: An annotation pipeline and genome-database management tool for  
757 second-generation genome projects. *BMC Bioinformatics*, 12, 491. doi: 10.1186/1471-2105-12-491

758 Hu, B., Zeng, L. P., Yang, X. Lou, Ge, X. Y., Zhang, W., Li, B., ... Shi, Z. L. (2017). Discovery of a rich gene pool of  
759 bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. *PLoS  
760 Pathogens*. doi: 10.1371/journal.ppat.1006698

761 Huang, R., Grishagin, I., Wang, Y., Zhao, T., Greene, J., Obenauer, J. C., ... Austin, C. P. (2019). The NCATS  
762 BioPlanet – An integrated platform for exploring the universe of cellular signaling pathways for  
763 toxicology, systems biology, and chemical genomics. *Frontiers in Pharmacology*, 10, 445. doi:  
764 10.3389/fphar.2019.00445

765 Huang, Z., Whelan, C. V., Dechmann, D., & Teeling, E. C. (2020). Genetic variation between long-lived versus  
766 short-lived bats illuminates the molecular signatures of longevity. *Aging*, 12(16), 15962-15977. doi:  
767 10.18632/aging.103725

768 Huang, Z., Whelan, C. V., Foley, N. M., Jebb, D., Touzalin, F., Petit, E. J., ... Teeling, E. C. (2019). Longitudinal  
769 comparative transcriptomics reveals unique mechanisms underlying extended healthspan in bats.  
770 *Nature Ecology and Evolution*, 3(7), 1110-1120. doi: 10.1038/s41559-019-0913-3

771 Huelsmann, M., Hecker, N., Springer, M. S., Gatesy, J., Sharma, V., & Hiller, M. (2019). Genes lost during the  
772 transition from land to water in cetaceans highlight genomic changes associated with aquatic  
773 adaptations. *Science Advances*, 5(9), eaaw6671. doi: 10.1126/sciadv.aaw6671

774 Hughes, G. M., Boston, E. S. M., Finarelli, J. A., Murphy, W. J., Higgins, D. G., & Teeling, E. C. (2018). The birth  
775 and death of olfactory receptor gene families in Mammalian niche adaptation. *Molecular Biology and*  
776 *Evolution*, 35(6), 1390-1406. doi: 10.1093/molbev/msy028

777 Irving, A. T., Ahn, M., Goh, G., Anderson, D., & Wang L. F. (2021). Lessons from the host defenses of bats, a  
778 unique viral reservoir. *Nature*, 589, 363-370. doi:10.1038/s41586-020-03128-0

779 Janeway, C. A., & Travers, M. W. (2001). The major histocompatibility complex and its functions.  
780 *Immunobiology: The Immune System in Health and Disease*. Retrieved from  
781 <https://www.ncbi.nlm.nih.gov/books/NBK10757/>

782 Jebb, D., Huang, Z., Pippel, M., Hughes, G. M., Lavrichenko, K., Devanna, P., ... Teeling, E. C. (2020). Six  
783 reference-quality genomes reveal evolution of bat adaptations. *Nature*, 538(7817), 578-584. doi:  
784 10.1038/s41586-020-2486-3

785 Jones, K. E., Bininda-Emonds, O. R. P., & Gittleman, J. L. (2005). Bats, clocks, and rocks: diversification patterns  
786 in chiroptera. *Evolution; international journal of organic evolution*, 59(10), 2243-2255. doi: 10.1554/04-  
787 635.1

788 Kacprzyk, J., Hughes, G. M., Palsson-McDermott, E., Quinn, S. R., Puechmaille, S. J., O'Neill, L. A. J. & Teeling, E.  
789 C. (2017). A potent anti-inflammatory response in bat macrophages may be linked to extended longevity  
790 and viral tolerance. *Acta Chiropterologica*. 19(2), 219-228. doi: 10.3161/15081109ACC2017.19.2.001

791 Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in  
792 performance and usability. *Molecular Biology and Evolution*, 30(4), 772-780. doi:  
793 10.1093/molbev/mst010

794 Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., ... Drummond, A. (2012). Geneious  
795 Basic: An integrated and extendable desktop software platform for the organization and analysis of  
796 sequence data. *Bioinformatics (Oxford, England)*, 28(12), 1647-1649. doi:10.1093/bioinformatics/bts199

- 797 Kelm, D.H., Simon, R., Kuhlow, D., Voigt, C. C., Ristow M. (2011). High activity enables life on a high-sugar diet:  
798 blood glucose regulation in nectar-feeding bats. *Proceedings of the Royal Society B: Biological Sciences*,  
799 278(1724),3490-3496. doi.org/10.1098/rspb.2011.0465
- 800 Kolmogorov, M., Yuan, J., Lin, Y., & Pevzner, P. A. (2019). Assembly of long, error-prone reads using repeat  
801 graphs. *Nature Biotechnology*, 37(5), 540-546. doi: 10.1038/s41587-019-0072-8
- 802 Korf, I. (2004). Gene finding in novel genomes. *BMC Bioinformatics*, 5, 59. doi: 10.1186/1471-2105-5-59
- 803 Kosakovsky Pond, S. L., Frost, S. D. W., & Muse, S. V. (2005). HyPhy: Hypothesis testing using phylogenies.  
804 *Bioinformatics (Oxford, England)*, 21(5), 676-679. doi: 10.1093/bioinformatics/bti079
- 805 Lau, S. K. P., Luk, H. K. H., Wong, A. C. P., Li, K. S. M., Zhu, L., Zirong, H., ... Woo, P. C. Y. (2020). Possible Bat  
806 Origin of Severe Acute Respiratory Syndrome Coronavirus 2. *Emerging infectious diseases*, 26(7), 1542-  
807 1547. doi: https://dx.doi.org/10.3201/eid2607.200092.
- 808 Lenartić, M., Jelenčić, V., Zafirova, B., Ožanić, M., Marečić, V., Jurković, S., ... Polić, B. (2017). NKG2D Promotes  
809 B1a Cell Development and Protection against Bacterial Infection. *The Journal of Immunology (Baltimore,*  
810 *Md. : 1950)*, 198(4), 1531-1542. doi: 10.4049/jimmunol.1600461
- 811 Lewin, H. A., Robinson, G. E., Kress, W. J., Baker, W. J., Coddington, J., Crandall, K. A., ... Zhang, G. (2018).  
812 Earth BioGenome Project: Sequencing life for the future of life. *Proceedings of the National Academy of*  
813 *Sciences of the United States of America*, 115(17), 4325-4333. doi: 10.1073/pnas.1720115115
- 814 Liberles, S. D., & Buck, L. B. (2006). A second class of chemosensory receptors in the olfactory epithelium.  
815 *Nature*, 442(7103), 645-650. doi: 10.1038/nature05066
- 816 McHeyzer-Williams, M., Okitsu, S., Wang, N., & McHeyzer-Williams, L. (2012). Molecular programming of B  
817 cell memory. *Nature Reviews Immunology*, 12(1), 24–34. doi: 10.1038/nri3128
- 818 Meakin, P. J., Morrison, V. L., Sneddon, C. C., Savinko, T., Uotila, L., Jality, S. M., ... Fagerholm, S. C. (2015).  
819 Mice lacking beta2-integrin function remain glucose tolerant in spite of insulin resistance, neutrophil  
820 infiltration and inflammation. *PLoS ONE*, 10(9), e0138872. doi: 10.1371/journal.pone.0138872
- 821 Memish, Z. A., Perlman, S., Van Kerkhove, M. D., & Zumla, A. (2020). Middle East respiratory syndrome.  
822 *Lancet (London, England)*, 395(10229), 1063-1077. doi: 10.1016/S0140-6736(19)33221-0
- 823 Meng, F., Zhu, L., Huang, W., Irwin, D. M., & Zhang, S. (2016). Bats: Body mass index, forearm mass index,  
824 blood glucose levels and SLC2A2 genes for diabetes. *Scientific Reports*, 6, 29960. doi: 10.1038/srep29960
- 825 Merad, M., & Martin, J. C. (2020). Pathological inflammation in patients with COVID-19: a key role for  
826 monocytes and macrophages. *Nature Reviews Immunology*, 20(6), 355-362. doi: 10.1038/s41577-020-



827 0331-4

828 Mollentze, N., & Streicker, D. G. (2020). Viral zoonotic risk is homogeneous among taxonomic orders of  
829 mammalian and avian reservoir hosts. *Proceedings of the National Academy of Sciences*, 117(17), 9423-  
830 9430. doi:10.1073/pnas.1919176117

831 Mi, H., Muruganujan, A., Ebert, D., Huang, X., & Thomas, P. D. (2019). PANTHER version 14: More genomes, a  
832 new PANTHER GO-slim and improvements in enrichment analysis tools. *Nucleic Acids Research*, 47(D1),  
833 D419-D426. doi: 10.1093/nar/gky1038

834 Miller, M. R., McMinn, R. J., Misra, V., Schountz, T., Müller, M. A., Kurth, A., & Munster, V. J. (2016). Broad  
835 and Temperature Independent Replication Potential of Filoviruses on Cells Derived from Old and New  
836 World Bat Species. *Journal of Infectious Diseases*, 214(suppl 3), S297-S302. doi: 10.1093/infdis/jiw199

837 Minias, P., Pikus, E., Whittingham, L. A., & Dunn, P. O. (2019). Evolution of copy number at the MHC varies  
838 across the avian tree of life. *Genome Biology and Evolution*, 11(1), 17-28. doi: 10.1093/gbe/evy253

839 Moratelli, R., & Calisher, C. H. (2015). Bats and zoonotic viruses: Can we confidently link bats with emerging  
840 deadly viruses? *Memorias Do Instituto Oswaldo Cruz*, 110(1), 1-22. doi: 10.1590/0074-02760150048

841 Muntasell, A., Magri, G., Pende, D., Angulo A., López-Botet, M. (2010). Inhibition of NKG2D expression in NK  
842 cells by cytokines secreted in response to human cytomegalovirus infection. *Blood*, 115 (25), 5170–5179.  
843 doi:10.1182/blood-2009-11-256479

844 Qurkhuli, T., Schwensow, N., Brändel, S. D., Tschapka, M., & S. Sommer. 2019. Can extreme MHC class I  
845 diversity be a feature of a wide geographic range? The example of Seba's short-tailed bat (*Carollia*  
846 *perspicillata*). *Immunogenetics*, 71,575-587. doi: 10.1007/s00251-019-01128-7

847 Nagaoka, I., Niyonsaba, F., Tsutsumi-Ishii, Y., Tamura, H., & Hirata, M. (2008). Evaluation of the effect of  
848 human  $\beta$ -defensins on neutrophil apoptosis. *International Immunology* 20(4), 543–553. doi:  
849 10.1093/intimm/dxn012

850 Ng, J. H. J., Tachedjian, M., Deakin, J., Wynne, J. W., Cui, J., Haring, V., ... Baker, M. L. (2016). Evolution and  
851 comparative analysis of the bat MHC-I region. *Scientific reports*, 6, 21256. doi: 10.1038/srep21256

852 Niyonsaba, F., Ushio, H., Hara, M., Yokoi, H., Tominaga, M., Takamori, K., ... Okumura, K. (2010). Antimicrobial  
853 Peptides Human  $\beta$ -Defensins and Cathelicidin LL-37 Induce the Secretion of a Pruritogenic Cytokine IL-31  
854 by Human Mast Cells. *The Journal of Immunology (Baltimore, Md. : 1950)*, 184(7), 3526–3534. doi:  
855 10.4049/jimmunol.0900712

856 Nogueira, R. M. R., Lima, I. P., Garbino, G. S. T., Moratelli, R., Tavares, V. C., Gregorin, R., & Peracchi, A. L.

857 (2018). No Updated checklist of Brazilian bats: version 2018. Comitê da Lista de Morcegos do Brasil-  
858 CLMB. Retrieved from Sociedade Brasileira para o Estudo de Quirópteros Retrieved from  
859 <https://www.sbeq.net/lista-de-especies>

860 Nowak, M. D., Birkeland, S., Mandáková, T., Roy Choudhury, R., Guo, X., Gustafsson, A. L. S., ... Brochmann, C.  
861 (2020). The genome of *Draba nivalis* shows signatures of adaptation to the extreme environmental  
862 stresses of the Arctic. *Molecular Ecology Resources*. doi: 10.1111/1755-0998.13280

863 Olival, K. J., Hosseini, P. R., Zambrana-Torrel, C., Ross, N., Bogich, T. L., & Daszak, P. (2017). Host and viral  
864 traits predict zoonotic spillover from mammals. *Nature*, *546*(7660), 646–650. doi: 10.1038/nature22975

865 Papenfuss, A. T., Baker, M. L., Feng, Z. P., Tachedjian, M., Crameri, G., Cowled, C., ... Wang, L. F. (2012). The  
866 immune gene repertoire of an important viral reservoir, the Australian black flying fox. *BMC Genomics*,  
867 *13*(1). doi: 10.1186/1471-2164-13-261

868 Papkou, A., Guzella, T., Yang, W., Koepper, S., Pees, B., Schalkowski, R., ... Schulenburg, H. (2019). The  
869 genomic basis of red queen dynamics during rapid reciprocal host–pathogen coevolution. *Proceedings of*  
870 *the National Academy of Sciences of the United States of America*, *116*(3), 923–928.. doi:  
871 10.1073/pnas.1810402116

872 Parham, P., & Moffett, A. (2013). Variable NK cell receptors and their MHC class I ligands in immunity,  
873 reproduction and human evolution. *Nature Reviews Immunology*, *13*, 133–144. 10.1038/nri3370.

874 Parker, J., Tsagkogeorga, G., Cotton, J. A., Liu, Y., Provero, P., Stupka, E., & Rossiter, S. J. (2013). Genome-wide  
875 signatures of convergent evolution in echolocating mammals. *Nature*, *502*(7470), 228–231. doi:  
876 10.1038/nature12511

877 Parthasarathy, R., Parthasarathy, L., & Vadnal, R. (1997). Brain inositol monophosphatase identified as a  
878 galactose 1-phosphatase. *Brain Research*, *778*(1), 99–106. doi: 10.1016/S0006-8993(97)01042-1

879 Pavlovich, S. S., Lovett, S. P., Koroleva, G., Guito, J. C., Arnold, C. E., Nagle, E. R., ... Palacios, G. (2018). The  
880 Egyptian Rousette Genome Reveals Unexpected Features of Bat Antiviral Immunity. *Cell*, *173*(5), 1098–  
881 1110.e18. doi: 10.1016/j.cell.2018.03.070

882 Pickering, A. M., Lehr, M., Kohler, W. J., Han, M. L., & Miller, R. A. (2014). Fibroblasts from longer-lived species  
883 of primates, rodents, bats, carnivores, and birds resist protein damage. *Journals of Gerontology - Series*  
884 *A Biological Sciences and Medical Sciences*, *70*(7), 791–799. doi: 10.1093/gerona/glu115

885 Pierce, C. A., Preston-Hurlburt, P., Dai, Y., Aschner, C. B., Cheshenko, N., Galen, B., ... Herold, B. C. (2020).  
886 Immune responses to SARS-CoV-2 infection in hospitalized pediatric and adult patients. *Science*

887 *Translational Medicine*, 12(564), eabd5487. doi: 10.1126/scitranslmed.abe8120

888 Podlutzky, A. J., Khritankov, A. M., Ovodov, N. D., & Austad, S. N. (2005). A new field record for bat longevity.  
889 *The journals of gerontology*, 60(11), 1366-1368. doi.org/10.1093/gerona/60.11.1366

890 Pomaznoy, M., Ha, B., & Peters, B. (2018). GOnet: A tool for interactive Gene Ontology analysis. *BMC*  
891 *Bioinformatics*, 19(1), 470. doi: 10.1186/s12859-018-2533-3

892 Potter, J., Davies, K., Yohe, L. R., Struebig, M., Warren, K., Tsagkogeorga, G., ... Rossiter, S. J. (in press). Dietary  
893 diversification and specialisation in New World bats facilitated by early molecular evolution. *Molecular*  
894 *Biology and Evolution*.

895 Rafikov, R., McBride, M. L., Zemskova, M., Kurdyukov, S., McClain, N., Niihori, M., ... Rafikova, O. (2019).  
896 Inositol monophosphatase 1 as a novel interacting partner of RAGE in pulmonary hypertension.  
897 *American Journal of Physiology - Lung Cellular and Molecular Physiology*, 316(3), L428–L444. doi:  
898 10.1152/ajplung.00393.2018

899 Rapista, A., Ding, J., Benito, B., Lo, Y. T., Neiditch, M. B., Lu, W., & Chang, T. L. (2011). Human defensins 5 and  
900 6 enhance HIV-1 infectivity through promoting HIV attachment. *Retrovirology*, 8, 45. doi: 10.1186/1742-  
901 4690-8-45

902 Ravel, A., Adaci, M., Bensalah, M., Charruault, A.-L., Essid, E. M., AMmar, H. K., ... Marivaux, L. (2016). Origine  
903 et radiation initiale des chauves-souris modernes : nouvelles découvertes dans l'Éocène d'Afrique du  
904 Nord. *Geodiversitas*, 38(3), 355-434. doi: 10.5252/g2016n3a3

905 Reddy, S. T., Ge, X., Miklos, A. E., Hughes, R. A., Kang, S. H., Hoi, K. H., ... Georgiou, G. (2010). Monoclonal  
906 antibodies isolated without screening by analyzing the variable-gene repertoire of plasma cells. *Nature*  
907 *Biotechnology*, 28(9), 965–969. doi: 10.1038/nbt.1673

908 Rhie, A., Mccarthy, S. A., Fedrigo, O., Damas, J., Formenti, G., London, S. E., ... Friedrich, S. R. (2020). Towards  
909 complete and error-free genome assemblies of all vertebrate species. *BioRxiv*, 1–56.  
910 doi:10.1101/2020.05.22.110833

911 Rojas, D., Ramos Pereira, M. J., Fonseca, C., & Dávalos, L. M. (2018). Eating down the food chain: generalism is  
912 not an evolutionary dead end for herbivores. *Ecology Letters*, 21(3), 402–410. doi: 10.1111/ele.12911

913 Salmon, A. B., Leonard, S., Masamsetti, V., Pierce, A., Podlutzky, A. J., Podlutzkaya, N., ... Chaudhuri, A. R.  
914 (2009). The long lifespan of two bat species is correlated with resistance to protein oxidation and  
915 enhanced protein homeostasis. *FASEB journal : official publication of the Federation of American*  
916 *Societies for Experimental Biology*, 23(7), 2317–2326. doi: 10.1096/fj.08-122523

- 917 Slavuljica, I., Krmpotić, A., & Jonjić, S. (2011). Manipulation of NKG2D ligands by cytomegaloviruses: impact on  
918 innate and adaptive immune response. *Frontiers in immunology*, 2, 85.  
919 doi.org/10.3389/fimmu.2011.00085
- 920 Santos, P. S. C., Courtiol, A., Heidel, A. J., Höner, O. P., Heckmann, I. Nagy, ... Sommer S. (2016). MHC-  
921 dependent mate choice is linked to a trace-amine-associated receptor gene in a mammal. *Scientific*  
922 *Reports*, 6,38490. doi.org/10.1038/srep38490
- 923 Scheben, A., Ramos, O. M., Kramer, M., Goodwin, S., Oppenheim, S., Becker, D. J., ... McCombie, R. (2020).  
924 Unraveling molecular mechanisms of immunity and cancer-resistance using the genomes of the  
925 Neotropical bats *Artibeus jamaicensis* and *Pteronotus mesoamericanus*. *BioRxiv*.  
926 doi.org/10.1101/2020.09.09.290502
- 927 Schountz, T., Baker, M. L., Butler, J., & Munster, V. (2017). Immunological control of viral infections in bats  
928 and the emergence of viruses highly pathogenic to humans. *Frontiers in Immunology*, 8, 1098. doi:  
929 10.3389/fimmu.2017.01098
- 930 Schroder, K., Hertzog, P. J., Ravasi, T., & Hume, D. A. (2004). Interferon- $\gamma$ : an overview of signals, mechanisms  
931 and functions. *Journal of Leukocyte Biology*, 75(2), 163–189. doi: 10.1189/jlb.0603252
- 932 Seim, I., Fang, X., Xiong, Z., Lobanov, A. V., Huang, Z., Ma, S., ... Gladyshev, V. N. (2013). Genome analysis  
933 reveals insights into physiology and longevity of the Brandt's bat *Myotis brandtii*. *Nature*  
934 *Communications*, 4,2212. doi: 10.1038/ncomms3212
- 935 Semple, F., & Dorin, J. R. (2012).  $\beta$ -Defensins: Multifunctional modulators of infection, inflammation and  
936 more? *Journal of Innate Immunity*, 4(4), 337–348. doi: 10.1159/000336619
- 937 Sharma, V., Hecker, N., Roscito, J. G., Foerster, L., Langer, B. E. & Hiller, M. (2018) A genomics approach  
938 reveals insights into the importance of gene losses for mammalian adaptations. *Nature communications*,  
939 9(1), 1215. doi: 10.1038/s41467-018-03667-1
- 940 Sharma, V., Lehmann, T., Stuckas, H., Funke, L., & Hiller, M. (2018). Loss of RXFP2 and INSL3 genes in  
941 Afrotheria shows that testicular descent is the ancestral condition in placental mammals. *PLoS Biology*,  
942 16(6), e2005293. doi: 10.1371/journal.pbio.2005293
- 943 Shen, B., Han, X., Jones, G., Rossiter, S. J., & Zhang, S. (2013). Adaptive Evolution of the Myo6 Gene in Old  
944 World Fruit Bats (Family: Pteropodidae). *PLoS ONE*, 8(4), e62307. doi: 10.1371/journal.pone.0062307
- 945 Shen, B., Han, X., Zhang, J., Rossiter, S. J., & Zhang, S. (2012). Adaptive evolution in the glucose transporter 4  
946 gene Slc2a4 in old world fruit bats (family: Pteropodidae). *PLoS ONE*, 7(4), e33197. doi:

947 10.1371/journal.pone.0033197

948 Shultz, A. J., & Sackton, T. B. (2019). Immune genes are hotspots of shared positive selection across birds and  
949 mammals. *ELife*, *8*, e41815. doi: 10.7554/eLife.41815

950 Simmons, N. B., Seymour, K. L., Habersetzer, J., & Gunnell, G. F. (2008). Primitive Early Eocene bat from  
951 Wyoming and the evolution of flight and echolocation. *Nature*, *451*(7180), 818-821. doi:  
952 10.1038/nature06549

953 Sironi, M., Cagliani, R., Forni, D., & Clerici, M. (2015). Evolutionary insights into host-pathogen interactions  
954 from mammalian sequence data. *Nature Reviews Genetics*, *16*(4), 224-236. doi: 10.1038/nrg3905

955 Smit, A. F. A., Hubley, R. M., & Green, P. (n.d.). RepeatMasker. Retrieved from: <http://Repeatmasker.Org>.

956 Smith, M. D., Wertheim, J. O., Weaver, S., Murrell, B., Scheffler, K., & Kosakovsky Pond, S. L. (2015). Less is  
957 more: An adaptive branch-site random effects model for efficient detection of episodic diversifying  
958 selection. *Molecular Biology and Evolution*, *32*(5), 1342-1353. doi: 10.1093/molbev/msv022

959 Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large  
960 phylogenies. *Bioinformatics (Oxford, England)*, *30*(9), 1312-1313. doi: 10.1093/bioinformatics/btu033

961 Stanke, M., & Waack, S. (2003). Gene prediction with a hidden Markov model and a new intron submodel.  
962 *Bioinformatics (Oxford, England)*, *19*, 215-225. doi: 10.1093/bioinformatics/btg1080

963 Stolberg, V. R., Martin, B., Mancuso, P., Olszewski, M. A., Freeman, C. M., Curtis, J. L., & Chensue, S. W.  
964 (2014). Role of CC chemokine receptor 4 in natural killer cell activation during acute cigarette smoke  
965 exposure. *The American Journal of Pathology*, *184*(2), 454-463. doi: 10.1016/j.ajpath.2013.10.017

966 Storch, G., Sigé, B., & Habersetzer, J. (2002). Tachypteran franzeni n. gen., n. sp., earliest emballonurid bat  
967 from the Middle Eocene of Messel (Mammalia, Chiroptera). *Paläontologische Zeitschrift*, *79*(189-199).  
968 doi: 10.1007/bf02989856

969 Subudhi, A., Rapin, N., & Misra, V. (2019). Immune system modulation and viral persistence in bats:  
970 Understanding viral spillover. *Viruses*, *11*(2), 192 .doi: 10.3390/v11020192

971 Suyama, M., Torrents, D., & Bork, P. (2006). PAL2NAL: Robust conversion of protein sequence alignments into  
972 the corresponding codon alignments. *Nucleic Acids Research*, *34*, 609-612. doi: 10.1093/nar/gkl315

973 Takada, A., Yoshida, S., Kajikawa, M., Miyatake, Y., Tomaru, U., Sakai, M., ... Kasahara, M. (2008). Two Novel  
974 NKG2D Ligands of the Mouse H60 Family with Differential Expression Patterns and Binding Affinities to  
975 NKG2D. *Journal of immunology (Baltimore, Md. : 1950)*, *180*(3), 1678-1685. doi:  
976 10.4049/jimmunol.180.3.1678

- 977 Teeling, E. C., Vernes, S. C., Dávalos, L. M., Ray, D. A., Gilbert, M. T. P., & Myers, E. (2018). Bat Biology,  
978 Genomes, and the Bat1K Project: To Generate Chromosome-Level Genomes for All Living Bat Species.  
979 *Annual Review of Animal Biosciences*, 6, 23-46. doi: 10.1146/annurev-animal-022516-022811
- 980 Towner, J. S., Pourrut, X., Albariño, C. G., Nkogue, C. N., Bird, B. H., Grard, G., ... Leroy, E. M. (2007). Marburg  
981 virus infection detected in a common African bat. *PLoS ONE*, 2(8), e764. doi:  
982 10.1371/journal.pone.0000764
- 983 Tsagkogeorga, G., Müller, S., Dessimoz, C., & Rossiter, S. J. (2017). Comparative genomics reveals contraction  
984 in olfactory receptor genes in bats. *Scientific Reports*, 7(1), 259. doi: 10.1038/s41598-017-00132-9
- 985 Tu, J., Li, D., Li, Q., Zhang, L., Zhu, Q., Gaur, U., ... Yang, M. (2015). Molecular Evolutionary Analysis of  $\beta$ -  
986 Defensin Peptides in Vertebrates. *Evolutionary bioinformatics online*, 11, 105-114.  
987 doi:10.4137/EBO.S25580
- 988 Vadstrup, K., Galsgaard, E. D., Jensen, H., Lanier, L. L., Ryan, J. C., Chen, S. Y., ... Bendtsen, F. (2017). NKG2D  
989 ligand expression in Crohn's disease and NKG2D-dependent stimulation of CD8<sup>+</sup> T cell migration.  
990 *Experimental and molecular pathology*, 103(1), 56-70. doi:10.1016/j.yexmp.2017.06.010
- 991 Van Oosterhout, C. (2009). A new theory of MHC evolution: Beyond selection on the immune genes.  
992 *Proceedings of the Royal Society B: Biological Sciences*, 276(1657), 657-665. doi:  
993 10.1098/rspb.2008.1299
- 994 Voigt, C. C., & Speakman, J. R. (2007). Nectar-feeding bats fuel their high metabolism directly with exogenous  
995 carbohydrates. *Functional Ecology*, 21,913-921. doi:10.1111/j.1365-2435.2007.01321.x
- 996 Vosseberg, J., Hoof, J. E., Marcet-Hoube, M. Vlimmeren, A., Wijk, T. G., & Snel B. (2020). Timing the origin of  
997 eukaryotic cellular complexity with ancient duplications. *Nature Ecology & Evolution*, 5, 92-100.  
998 doi:10.1038/s41559-020-01320-z
- 999 Wang, K., Tian, S., Galindo-González, J., Dávalos, L. M., Zhang, Y., & Zhao, H. (2020). Molecular adaptation and  
1000 convergent evolution of frugivory in Old World and neotropical fruit bats. *Molecular Ecology*, 29(22),  
1001 4366-4381. doi: 10.1111/mec.15542
- 1002 Wang, P., Luo, Y., Huang, J., Gao, S., Zhu, G., Dang, Z., ... Chen Y. (2020). The genome evolution and  
1003 domestication of tropical fruit mango. *Genome Biology*, 21(1), 60. doi.org/10.1186/s13059-020-01959-8
- 1004 Welch, K. C., Herrera, M. L. G., & Suarez, R. K. (2008). Dietary sugar as a direct fuel for flight in the  
1005 nectarivorous bat *Glossophaga soricina*. *Journal of Experimental Biology*, 211, 310-316. doi:  
1006 10.1242/jeb.012252

- 1007 Wiens, M. E., Wilson, S. S., Lucero, C. M., & Smith, J. G. (2014). Defensins and Viral Infection: Dispelling  
1008 Common Misconceptions. *PLoS Pathogens*, *10*(7), e1004186. doi: 10.1371/journal.ppat.1004186
- 1009 Wilkinson, G. S., & Adams, D. M. (2019). Recurrent evolution of extreme longevity in bats. *Biology Letters*,  
1010 *15*(4), 20180860. doi: 10.1098/rsbl.2018.0860
- 1011 Wortham, B. W., Eppert, B. L., Motz, G. T., Flury, J. L., Orozco-Levi, M., Hoebe, K., ... Borchers, M. T. (2012).  
1012 NKG2D Mediates NK Cell Hyperresponsiveness and Influenza-Induced Pathologies in a Mouse Model of  
1013 Chronic Obstructive Pulmonary Disease. *Journal of immunology (Baltimore, Md. : 1950)*, *188*(9), 4468–  
1014 4475. doi: 10.4049/jimmunol.1102643
- 1015 Xiao, Y., Hughes, A. L., Ando, J., Matsuda, Y., Cheng, J.F., Skinner, D., & Zhang, G. (2004). A genome-wide  
1016 screen identifies a single  $\beta$ -defensin gene cluster in the chicken: implications fo the origin and evolution  
1017 of mammalian defensins. *BMC Genomics*, *5*(1), 56. doi:10.1186/1471-2164-5-56
- 1018 Xie, J., Li, Y., Shen, X., Goh, G., Zhu, Y., Cui, J., ... Zhou, P. (2018). Dampened STING-Dependent Interferon  
1019 Activation in Bats. *Cell Host and Microbe*, *23*(3), 294-301. doi: 10.1016/j.chom.2018.01.006
- 1020 Xu, D., & Lu, W. (2020). Defensins: A Double-Edged Sword in Host Immunity. *Frontiers in Immunology*,  
1021 *11*(764), 1–9. doi: 10.3389/fimmu.2020.00764
- 1022 Yang, Z. (2007). PAML 4: Phylogenetic analysis by maximum likelihood. *Molecular Biology and Evolution*,  
1023 *24*(8), 1586–1591. doi: 10.1093/molbev/msm088
- 1024 Yang, Z., Wong, W. S. W., & Nielsen, R. (2005). Bayes empirical Bayes inference of amino acid sites under  
1025 positive selection. *Molecular Biology and Evolution*, *22*(4), 1107-1118. doi: 10.1093/molbev/msi097
- 1026 Yates, A. D., Achuthan P., Akanni, W., Allen, J., Alvares, J. J., ... Flicek, P. (2020). Ensembl 2020. *Nucleic Acids*  
1027 *Research*, *48*(D1), D682-D688. doi:10.1093/nar/gkz966
- 1028 Yohe, L. R., Devanna, P. Davies, K. T. J., Potter, J. H. T., Rossiter, S. J., Teeling, E. C., ..., Dávalos L. M. (2019).  
1029 Tissue Collection of Bats for –Omics Analyses and Primary Cell Culture. *Journal of Visualized*  
1030 *Experiments:JoVE*, (152). doi: 10.3791/59505
- 1031 You, X., Bian, C., Zan, Q., Xu, X., Liu, X., Chen, J., ... Shi, Q. (2014). Mudskipper genomes provide insights into  
1032 the terrestrial adaptation of amphibious fishes. *Nature Communications*, *5*, 5594. doi:  
1033 10.1038/ncomms6594
- 1034 Yu, G., Smith, D. K., Zhu, H., Guan, Y., & Lam, T. T. Y. (2017). ggtree: an r package for visualization and  
1035 annotation of phylogenetic trees with their covariates and other associated data. *Methods in Ecology*  
1036 *and Evolution*, *8*(1), 28-36. doi: 10.1111/2041-210X.12628

- 1037 Zafirova, B., Mandarić, S., Antulov, R., Krmpotić, A., Jonsson, H., Yokoyama, W. M., ... Polić, B. (2009). Altered  
1038 NK Cell Development and Enhanced NK Cell-Mediated Resistance to Mouse Cytomegalovirus in NKG2D-  
1039 Deficient Mice. *Immunity* 31(2), 270-282. doi: 10.1016/j.immuni.2009.06.017
- 1040 Zepeda Mendoza, M. L., Xiong, Z., Escalera-Zamudio, M., Runge, A. K., Thézé, J., Streicker, D., ... Gilbert, M. P.  
1041 T. (2018). Hologenomic adaptations underlying the evolution of sanguivory in the common vampire bat.  
1042 *Nature Ecology and Evolution*, 2(4), 659-668. doi: 10.1038/s41559-018-0476-8
- 1043 Zhang, G., Cowled, C., Shi, Z., Huang, Z., Bishop-Lilly, K. A., Fang, X., ... Wang, J. (2013). Comparative analysis of  
1044 bat genomes provides insight into the evolution of flight and immunity. *Science*, 339(6118), 456-460.  
1045 doi: 10.1126/science.1230835
- 1046 Zhang, J., Nielsen, R., & Yang, Z. (2005). Evaluation of an improved branch-site likelihood method for  
1047 detecting positive selection at the molecular level. *Molecular Biology and Evolution*, 22(12), 2472-2479.  
1048 doi: 10.1093/molbev/msi237
- 1049 Zheng, D., Liwinski, T., & Elinav, E. (2020). Inflammasome activation and regulation: toward a better  
1050 understanding of complex mechanisms. *Cell Discovery*, 6, 36. doi: 10.1038/s41421-020-0167-x
- 1051 Zhi, L., Mans, J., Paskow, M. J., Brown, P. H., Schuck, P., Jonjić, S., ... Margulies, D. H. (2010). Direct interaction  
1052 of the mouse cytomegalovirus m152/gp40 immunoevasin with RAE-1 isoforms. *Biochemistry*, 49(11),  
1053 2443-2453. doi: 10.1021/bi902130j
- 1054 Zhou, P. (2016). Contraction of the type I IFN locus and unusual constitutive expression of IFN- $\alpha$  in bats.  
1055 *Proceedings of the National Academy of Sciences of the United States of America*, 113(10), 2696-2701.  
1056 doi: 10.1073/pnas.15182401138240113
- 1057 Zhou, P. (2020). Immune (Adaptive) Response in Bats. In Corrales-Aguilar, E & Schwemmler, M. (Eds.), *Bats  
1058 and Viruses: Current Research and Future Trends* (pp. 135–148). Caister Academic Press. doi:  
1059 10.21775/9781912530144.09
- 1060 Zhou, X., Dou, Q., Fan, G., Zhang, Q., Sanderford, M., Kaya, A., ... Gladyshev, V. N. (2020). Beaver and Naked  
1061 Mole Rat Genomes Reveal Common Paths to Longevity. *Cell Reports*, 32(4), 107949. doi:  
1062 10.1016/j.celrep.2020.107949
- 1063 Zimin, A. V., Marçais, G., Puiu, D., Roberts, M., Salzberg, S. L., & Yorke, J. A. (2013). The MaSuRCA genome  
1064 assembler. *Bioinformatics (Oxford, England)*, 29(21), 2669-2677. doi: 10.1093/bioinformatics/btt476  
1065



1066 **Declarations**

1067 The authors claim no conflicts of interest.

1068 **Data Accessibility**

1069 -Final genome assembly is deposited at Genbank under BioProjectID: PRJNA733208 and accession number  
1070 JAHKBD000000000.

1071 -Scripts for genome assembly, ultrametric tree construction, gene family, and selection test are deposited in  
1072 Dryad repository <https://doi.org/10.5061/dryad.59zw3r265>.

1073

1074 **Author Contributions**

1075

1076 LMD, DAR, DDMS conceived of the study; DAR, JHP, LRY, SJR, and HZ collected samples; DAR, DDMS, ECT, GM,  
1077 JHP, KTJD, LMD, LRY, PD, SJR, SV, YTGG, and ZH generated data; AMB, APC, DDMS, GH, LMD, TML, YTGG, and  
1078 ZH analyzed data-guided, in part, by DAR and FGH; APC, DAR, DDMS, LMD, and TML wrote the manuscript. All  
1079 authors reviewed the manuscript prior to submission.

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## Tables

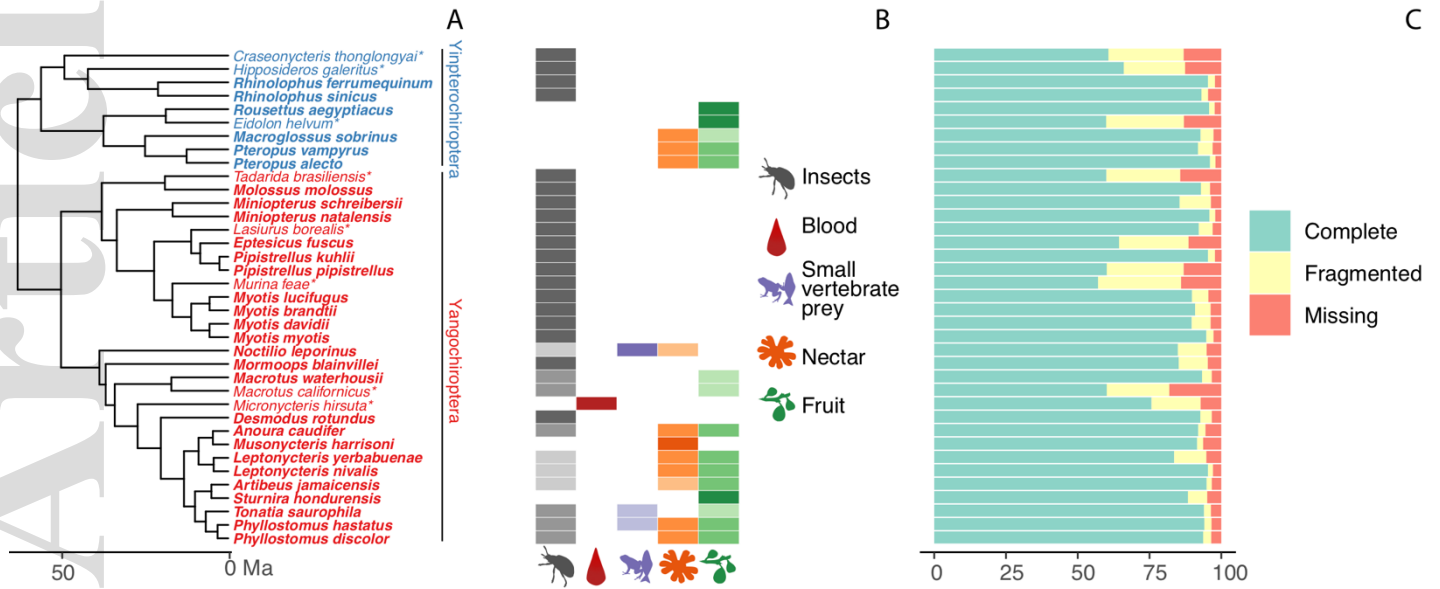
**Table 1.** Branch-site codeml results for all species on single-copy immune system genes. FDR, false discovery rate; LR, likelihood ratio; *P*, nominal *P*-value.

Symbol	Name	Category	Alt	Null	LRT	P-val	FDR
Bbc3	BCL2 Binding Component 3	Inflammatory response	-4704.07	-4724.64	41.15	0.00	0.000
BPIFB5	BPI fold containing family B member 4	Antimicrobials	-5438.63	-5448.49	19.73	0.00	0.000
CCL1	C-C motif chemokine 1	Chemokines/Cytokines/Anti microbials	-2449.96	-2454.54	9.16	0.00	0.023
CD3E	CD3e molecule	TCR signaling Pathway	-4463.25	-4485.65	44.80	0.00	0.000
CD79B	CD79b molecule	BCR Signaling Pathway	-4298.73	-4303.68	9.91	0.00	0.017
CD86	CD86 molecule	Antimicrobials	-5668.52	-5673.13	9.22	0.00	0.023
CSF2	colony stimulating factor 2	Cytokines	-1895.79	-1901.28	10.98	0.00	0.012
CXCL13	C-X-C motif chemokine 13	Chemokines/Cytokines/Anti microbials	-2446.76	-2474.82	56.11	0.00	0.000
DEFB129 †	Beta-defensin 129	Antimicrobials	-4093.98	-4100.00	12.05	0.00	0.008
DEFB133	defensin beta 133	Antimicrobials	-935.69	-944.53	17.67	0.00	0.001
F2RL1	F2R like trypsin receptor 1	Antimicrobials	-10695.69	-	91.64	0.00	0.000
				10741.51			
HRK†	Harakiri, BCL2 Interacting Protein	Inflammatory response	-1232.08	-1248.01	31.86	0.00	0.000
IFNG	interferon gamma	Antigen Processing and Presentation	-5525.65	-5538.95	26.60	0.00	0.000
IL17A	Interleukin-17A	Cytokines/Interleukins	-4495.35	-4500.65	10.60	0.00	0.014

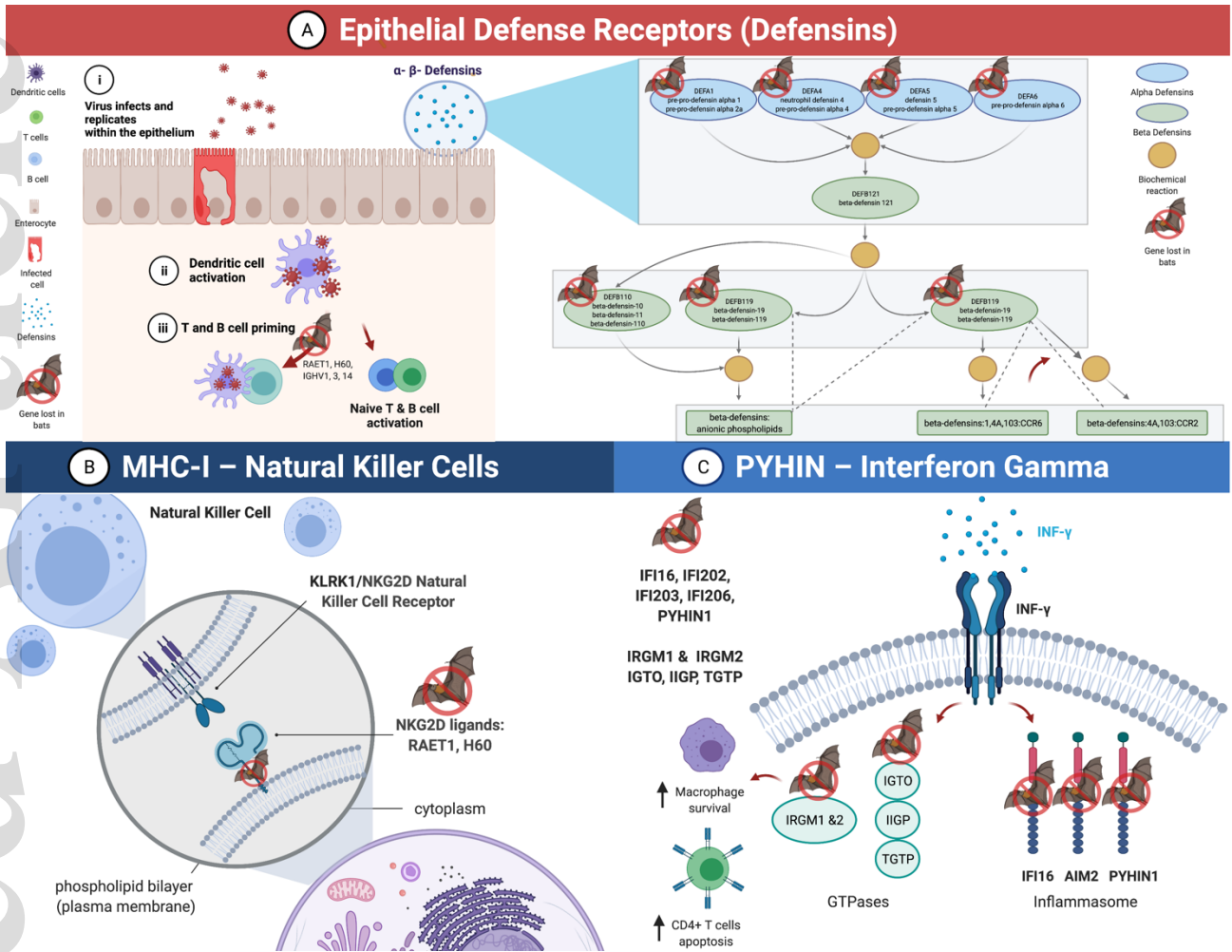
IL17RC	interleukin 17 receptor C	Cytokines	-3585.03	-3623.10	76.14	0.00	0.000
IL1A	interleukin 1 alpha	Cytokines	-6876.43	-6880.12	7.39	0.01	0.052
IL20RA	interleukin 20 receptor subunit alpha	Cytokine Receptors	-12518.47	-	7.49	0.01	0.051
				12522.21			
INHBE	Inhibin beta E chain	Cytokines/TGFb family	-8225.60	-8257.30	63.40	0.00	0.000
JUN	Jun proto-oncogene, AP-1 transcription factor subunit	BCR Signaling Pathway	-4109.81	-4141.82	64.03	0.00	0.000
MAPKBP1	Mitogen-Activated Protein Kinase Binding Protein 1	Antimicrobials/Inflammatory response	-17784.73	-	12.54	0.00	0.006
				17791.00			
NPFF	neuropeptide FF-amide peptide precursor	Cytokines	-2619.77	-2623.89	8.23	0.00	0.037
NRG1	neuregulin 1	Cytokines	-1737.10	-1741.12	8.05	0.01	0.038
TRDC	T cell receptor delta constant	TCR signaling Pathway	-4159.64	-4192.39	65.50	0.00	0.000
TRDV3	T cell receptor delta variable 3	TCR signaling Pathway	-2903.04	-2908.09	10.09	0.00	0.016
TRH	Pro-thyrotropin-releasing hormone	Cytokines	-6601.02	-6606.58	11.12	0.00	0.012
TRIML1	Tripartite Motif Family Like 1	Antimicrobials	-10302.78	-	10.27	0.00	0.015
				10307.91			
TYROBP	TYRO protein tyrosine kinase-binding protein	NaturalKiller Cell Cytotoxicity	-1824.09	-1829.22	10.27	0.00	0.015

†Genes non significant in aBSREL

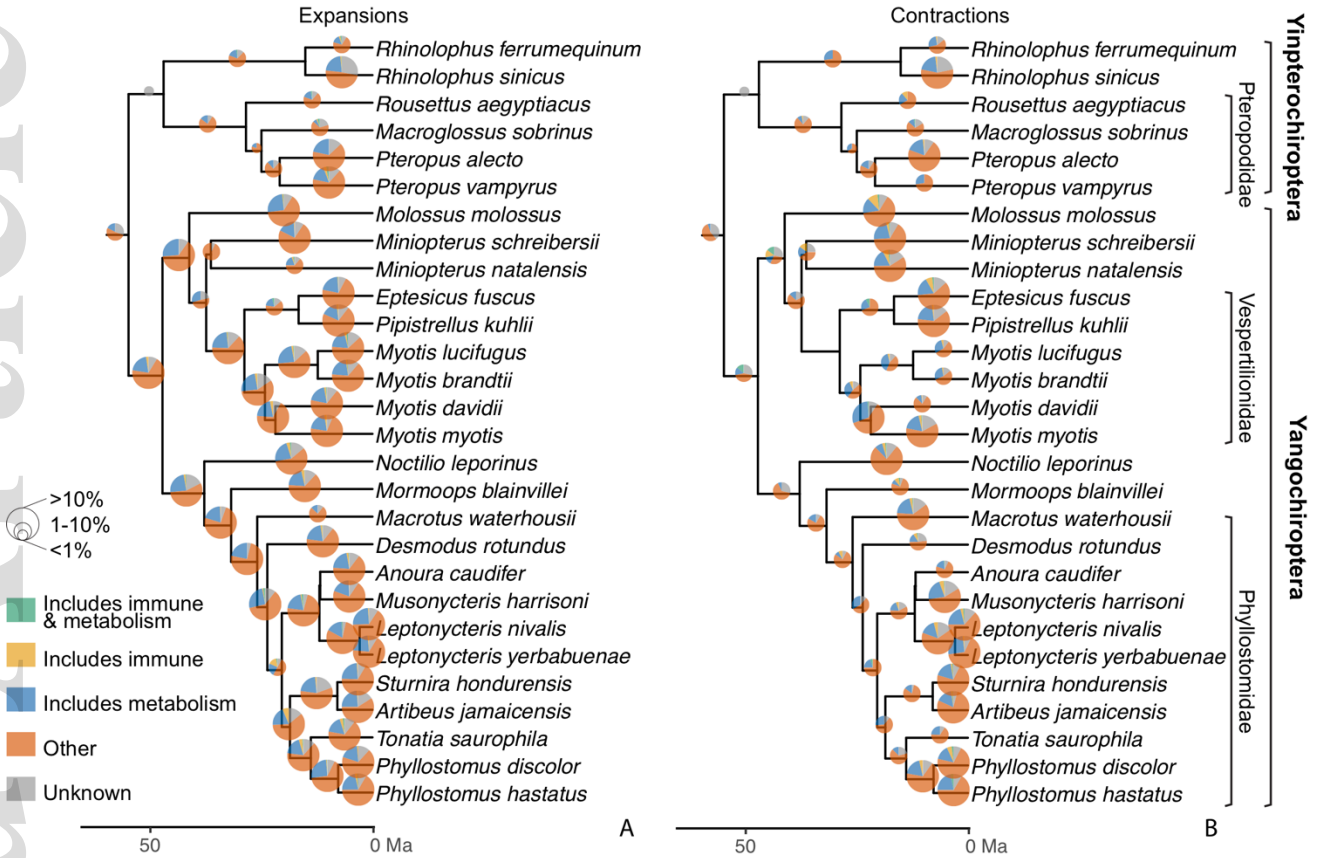
# Figures



**Figure 1.** Phylogeny, dietary diversity, and BUSCO completeness across bat genomes. A) Species tree based on >300 genome-wide loci dated using penalized likelihood smoothing. \*Genomes excluded from CAFE analyses. B) Diet composition and relative reliance indicated by color intensity (Rojas et al., 2018). C) BUSCO completeness for the corresponding genome.



**Figure 2.** Graphical summary of the cellular location and biological process categorization for genes involved in the inflammasome activation pathway found to be missing across all bats. A) Gene loss of specific epithelial  $\alpha$  and  $\beta$  defensins. B) Gene losses of NKG2D ligands RAET1 and H60, involved in recruiting NK cells and IFN- $\gamma$  stimulation. C) Losses in IFN- $\gamma$  activating PYRIN and HIN domain (PYHIN) gene family (AIM2, IFI16, PYHIN1), along with the IFN- $\gamma$  inducible related GTPase genes (IRGM1, IRGM2, IGTO, IIGP, TGTP2); loss of IRGM1 and 2 results in increase macrophage survival and CD4+ T cells apoptosis.



**Figure 3.** Gene ontology categories, phylogenetic locations, and relative size of significant gene family expansions (A) and contractions (B) inferred using CAFE. “Other” category comprises mostly Panther cellular processes, and GOnet response to stress and autophagy. Pie sizes are relative to a maximum of 594 expansions and 579 contractions.