Extended discussion on some of the most relevant identifications

Comparative genomics

Glucose and lipids

We identified various genes involved in digestion and glucose and lipid metabolism that evolved more rapidly or slowly compared to other genomes at a statistically significant rate. In particular, the free fatty acid receptor 1 (*FFAR1*) gene was under significant higher rate of evolution (*dN/dS* one ratio model (ω_{null})=0.159, *dN/dS* of the *D. rotundus* branch (ω_{Alt})=0.436, *P*=0.0000368, FDR=0.00241). This gene is related to the energy reserve metabolic process, it is a G-protein coupled receptor for medium and long chain saturated and unsaturated fatty acids that plays an important role in glucose homeostasis, fatty acid binding to increase glucose-stimulated insulin secretion, and may also enhance the secretion of glucagon-like peptide 1[1]. A relevant protein identified to have a frameshift mutation is melanocortin 3 receptor (*MC3R*, scaffold41:4551544-4552483:-), which is involved in maintenance of internal body temperature[2,3]. Melanocortin is a neurotransmitter that also inhibits ingestion of food[3,4].

The gene regenerating islet-derived protein 4 (*REG4*) (ω_{null}=0.340, ω_{Alt}=1.739, *P*=0.000643) was identified as having a higher rate of evolution in *D. rotundus* compared to the other bats. This gene is highly expressed in the gastrointestinal tract, and is known to maintain carbohydrate recognition activity in an acidic environment; it may be involved in inflammatory and metaplastic responses of the gastrointestinal epithelium[5,6]. Regarding other nutrients such as vitamins, the gene PDZ domain containing 11 (*PDZD11*, ω_{null}=0.271, ω_{Alt}=1.19, FDR=4.51E-8) was identified as having a higher rate of evolution in the common vampire bat compared to the other bats. This gene is involved in pantothenate (vitamin B_5) metabolism and water-soluble vitamin metabolic processes[7].

The low abundance of nutrients found in blood could have had an impact on the common vampire bat response to starvation metabolism. We identified the late endosomal/lysosomal adaptor MAPK and MTOR activator 5 gene having a higher rate of evolution in *D. rotundus* compared to the other bats (*LAMTOR5*, ω_{null}=0.239, ω_{Alt}=1.454, *P*=1.05E-9, FDR=2.02E-7). This gene is involved in macroautophagy, feature of nutrient starvation[8].

Iron

Iron is one of the most relevant aspects for the blood composition that may confer dietary challenges to a sanguivorous species. The gene polyadenylate-binding protein 1 (*PABPC1*) was identified as having a significant PROVEAN score (-6.745, G139D). This protein participates in the L13a-mediated translational silencing of ceruloplasmin expression[9]. The RAB15 effector protein coding gen (*REP15*, ω_{null}=0.264, ω_{Alt}=0.774, *P*=0.000329, FDR=0.014) regulates recycling from the endocytic recycling compartment of transferrin receptor, transferrin is the main protein in the blood that binds to iron and transports it throughout the body[10]. We also identified the plasminogen activator gene (*PLAT*, ω_{null}=0.327, ω_{Alt}=0.677, *P*=1.19E-6, FDR=0.000125)[11] under higher rate of evolution. This gene is involved in blood coagulation.

Gastro-intestine

Regarding development, the morphogenesis of the stomach and intestine in the common vampire bat has also undergone adaptations possibly linked to its diet, because absorption of the ingested blood plasma begins in the lining of the stomach. The stomach of the common vampire bat is much thinner, longer, and more muscular than those of other mammals[12]. We identified a higher rate of evolution in the gene *RAB1B* (ω_{null}=0.0718, ω_{Alt}=0.473, *P*=0.0005, FDR=0.033), which is involved in the establishment of the endothelial intestinal barrier, which exerts specific and selective control over the passage of water and solutes[13].

Urinary apparatus

Blood pressure and renal excretion are a key aspect of the common vampire bat adaptation to its diet. Given the high protein content in its diet, thus the high glomerular filtration rate and large amounts of excreted urea, the common vampire bat is considered a model for studying chronic renal failure[14]. It is also known that *D. rotundus* consumes about 20-30% of its body weight in blood, and has to stay on the ground resting and urinating for some time, they may also start urinating while eating[15]. Thus, we examined genes related to the urinary apparatus. The urinary apparatus plays key roles in the regulation of the pH, volume, pressure and composition of the blood by excreting body wastes and producing hormones. Furthermore, the kidney participates in the synthesis of glucose and vitamin D[16].

We identified under higher rate of evolution the gene coding for the proteasome subunit alpha 3 (*PSMA3*, ω_{null}=0.107, ω_{Alt}=0.675, *P*=2.08E-7, FDR=2.75E-5). This gene participates in the regulation of ornithine decarboxylase[17,18], which catalyzes the decarboxylation of ornithine (a product of the urea cycle that allows for the disposal of excess nitrogen) to form putrescine[19,20]. The solute carrier family 13 member 1 gene (*SLC13A1*, ω_{null}=0.336, ω_{Alt}=1.213, *P*=0.00655, FDR=0.109), which mediates sulfate reabsorption in the kidney[21] was found to have a higher rate of evolution in *D. rotundus* compared to the other bats. The last step of the urinary process involves the storage in and excretion of urine from the bladder. In this regard, we identified under a slower rate of evolution the gap junction protein alpha 1 coding gene (*GJA1*, ω_{null}=0.0418, ω_{Alt}=0.216, *P*=4.08E-8, FDR=6.77E-6), which acts as a regulator of bladder capacity[22,23].

Regarding the abundance of salt solutes in blood, cellular homeostasis is another challenge faced by the vampire bats. Interestingly, we identified the natriuretic peptide receptor 1 gene (*NRP1)* under expansion in *D. rotundus* (Viterbi corrected *P*=0.01466). This gene is involved in the excretion of sodium in urine[24].

Sensory apparatus

Various olfactory receptors were identified as lost in *D. rotundus* (Supplementary Information 7). However, it was not possible to validate the loss of all the chosen representative tested genes (Extended Data Fig. 12C), as distinction of the olfactory genes from the various paralogues is a complicated issue that could be discerned in future studies. This potential loss could be related to the vampires not relying on smell for identifying their prey. Instead, they use echolocation and infrared sensing. Infrared sensing is one of the traits with the most direct relation to the adaptation of the vampire bat to its sanguivorous diet. With the use of specialized pit organs around the nose, vampire bats can locate hot spots on the prey where blood flows close to the skin to bite and feed from. These pit organs receive input from low-threshold temperature heat-sensitive spinal nerve fibers[25–27]. However, the evolution of infrared sensing is *per se* a major evolutionary change. The only other vertebrates that can detect infrared radiation are three families of snakes (boas, pythons, and pit vipers). Snakes evolved this characteristic by adapting a previously heat insensitive ion channel into an infrared radiation sensor[28]. The molecular mechanism evolved in the vampire bats consists of alternative splicing of a heat sensitive channel gene called transient receptor potential cation channel subfamily V member 1 (*TRPV1*). In mammals, *TRPV1* is usually activated at 43°C

to trigger a painful sensation, while in the vampire bat it reacts at 30°C[29] from a distance of 20 cm[26]. However, previous studies to uncover the evolution of this particular capacity have been done with RNA-seq and proteomics, thus our genome wide selection analyses allowed us to identify other genes related to *TRPV1* and to the processing of heat sensations in the brain.

Specifically, we identified the protein kinase D1 gene (*PRKD1*) under higher rate of evolution in the branch-site and branch tests in *D. rotundus* (branch-site test FDR=8.01E-8, branch test FDR=0.000143, ω_{null}=0.091, ω_{Alt}=0.408). This gene has been suggested to be involved in pain transmission by directly modulating the *TRPV1* receptor[30,31]. Furthermore, *TRPV1* plays a role in consolidation of fear memories[32], which are processed in the area of the brain related to learning. Interestingly, we identified genes with functions related to learning, fear and pain, and genes related to synapses and neurons which may play a role in the processing of thermic stimulus for infrared sensing. One gene related to facial nerve structural organization was also identified to be under higher rate of evolution in the common vampire bat compared to the other bats, plexin A4[33] (*PLXNA4*, ω_{null}=0.019, ω_{Alt}=0.04, *P*=0.000826, FDR=0.027). It is also likely that many of such neuronal related genes play roles in digestion. The enteric nervous system and the autonomous nervous system regulate the gastrointestinal tract. Discovery and deeper understanding of genes potentially related to this capacity have medical implications regarding the design of drugs that act on similar heat sensors in humans or the design of drugs that suppress the activity of related ion channels, such as those involved in causing inflammatory pain[34].

Vampire bats, as any nocturnal bat, have also evolved special sight capacities. In this regard, we identified the ribosomal protein L24 gene (*Rpl24*) under expansion in *D. rotundus* (Viterbi *P*=0.0037). This gene has been linked to the development of the retina[35].

Immune system

Given that pathogenic bacteria and viruses can be transmitted by blood, one of the most important traits in the common vampire bat is its immune capacity. For instance, we identified under faster rate of evolution the genes toll-like receptor 4 (*TLR4*, ω_{null}=0.389, ω_{Alt}=0.716, *P*=0.0022, FDR=0.0545), and *TLR9* (branch-site test *P*=0.00295, branch-site test FDR=0.0916), which are key components of innate and adaptive immunity[36]. We also identified other various genes related to the

more general immune system, such as the gene tripartite motif containing 5 (*TRIM5*, Viterbi corrected *P*=0.00088), which prevents infection by non-host-adapted retroviruses[37].

Reproduction

It is known that *D. rotundus* breeds throughout the year, in contrast to other Microchiroptera bats. In this regard, we identified the ADAM metallopeptidase domain 29 gene with a significant PROVEAN score (*ADAM29*, PROVEAN score=-6.905 at position Y377L); this gene is related to spermatogenesis[38]. The neuropeptide Y receptor Y5 gene (*NPY5R*) was also identified under a lower rate of evolution in the common vampire bat compared to the other bats (ω_{null}=0.116, ω_{Alt}=0.0001, *P*=0.0083). The gene is involved in the ovulation cycle as well as the regulation of acute inflammatory responses. Importantly, variants of this gene are associated with nutrient-specific food intake in European human populations[39].

Positive selected sites and protein modeling

Proteins with a statistically significant ω and with sites with a significant PROVEAN score within a functional site in the protein structure as annotated in Uniprot, we followed in greater detail, including: FFAR1 (which plays an important role in glucose homeostasis[40]), PLXA4 (involved in facial nerve structural organization[33]), REG4 (highly expressed in the gastrointestinal tract[41]), RNAS7 (which demonstrates antimicrobial activity against many pathogenic microorganisms, remarkably potent against vancomycin resistant *Enterococcus faecium*[42]), and TA2R3 (involved in perception of bitter compounds in the oral cavity and the gastrointestinal tract[43]).

We identified evidence for positive selection in contrast to relaxation of selection pressure in *FFAR1* (lnL M1=2162.9, lnL M2=2157.82, ω_{null}=0.15, ω_{Alt}=0.35, *P*=0.001) and *PLXN4* (lnL M1=8166.58, lnL M2=7985.28, *P*=0, ω_{null}=0.02, ω_{Alt}=0.04). *FFAR1* has been implicated in type 2 diabetes and is a drug target because of its role in insulin release[44]. Previous studies of site-directed mutagenesis have identified R183 from the binding site (R-182 in the common vampire bat sequence) as an important residue for the function of FFAR1[1]. Importantly, we identified an R182H substitution (PROVEAN score=-4.88) in *D. rotundus*, and three other positions falling in its topological domains (Extended Data Table 4).

Our results are consistent with previous studies showing that vampire bats have a reduction of bitter taste receptor genes[45]. However, TA2Rs participate in functions other than taste perception, such as regulation of glucose homeostasis[46], and delay of gastric emptying[47]. In order to examine the functional relevance of the retention of TA2Rs in a species that does not rely on them for their food intake, we further analyzed TA2R3, which has been found pseudogenized in *Diphylla ecaudata* while functional in *D. rotundus*[45]. We identified *TAS2R3* to have evolved under episodic positive selection in *D. rotundus* under branch-site model A (null lnL=2959.6, BSA lnL=2953.64, proportion of sites having ω>1 in the foreground branch=0.034, background ω=0.10, foreground ω=21.28, *P*=0.001). Of the three identified PSSs, one is part of the topological domain (L/T/S/V177A) (Extended Data Table 4), and one site with a significant PROVEAN score of -4.4 is part of another topological domain (H293Q).

REG4, an important serum biomarker for gastric cancer that can inhibit apoptosis induced by the chemotherapeutic agent 5-fluorouracil[48], was also identified as evolving under ongoing positive selection in *D. rotundus*. *REG4* shows high binding response to mannan and heparin[5]. Importantly, mannan intake has been suggested to modify intestinal bacterial populations (regarding *Salmonella* and *Lactobacillus*) and improve nutrient digestibility[49,50], while heparin is a well-known anticoagulant also thought to have defense roles against invading bacteria[51]. Two of the sites with a significant PROVEAN score, R46Q and Q103R (-2.8 and -3.24, respectively) (Extended Data Table 4), are implicated in the binding ability of this protein, one in each of the identified two regions related to carbohydrate binding[5].

Antimicrobial RNases have abundant positively charged residues on the enzyme surface, and an abundance of the cationic residues K and R[52]. The cationic residues are thought to facilitate interaction with the negatively charged components of the microbial surface while the hydrophobic residues may allow for incorporation into the microbial membrane[52]. Bacterial RNases such as *RNASE7* have the potential to be new therapeutic agents against bacterial infection since they may not face the rapid bacterial drug resistance adaptations. Interestingly, *RNASE7* in *D. rotundus* contains amino acid substitutions that may increase its bactericidal capacity, T/N104K, and P113R, as well as H42E, which corresponds to the proton acceptor active site (Extended Data Table 4).

Microbial taxonomic and functional metagenomics comparisons

Microbial taxonomy

It was clear that *D. rotundus* microbial taxonomic composition is different from the other species by looking at the principal component analyses (PCAs) and the cladograms (Extended Data Figs. 6, 8, 19, Extended Data Table 6). Among the most significantly abundant taxa that contribute to the variation in the principal components obtained from the rotation matrix, we identified various taxa relevant to sanguivory (Extended Data Table 5, Supplementary Information 19).

The bacterial composition (including the identified plasmids) separates the common vampire bat samples from the samples of the other three species (*Rhinolophus ferrumequinum*, *Macroderma gigas*, and *Rousettus aegyptiacus*). The bacteria are unique both at the species and genus level (Extended Data Fig. 6), with *D. rotundus* separating from the other 3 species. The separation is particularly notable when looking at the presence/absence profile compared to that obtained from the counts, with *D. rotundus* being closer to *R. ferrumequinum* and *M. gigas*. However, when looking at the genus level, all four species are more mixed, with more samples from *R. ferrumequinum* and *M. gigas* being mixed among the *D. rotundus* samples, while most the *R. aegyptiacus* samples are still outside of the main *D. rotundus* clades. Among the most significantly abundant bacterial taxa ($P<0.05$) that contribute to most of the variation between *D. rotundus* and the other three bat species, it is interesting to note the identification of those with roles in immunity, blood digestion, and with adaptive traits to blood, fat, and nutrition (Supplementary Information 19).

The fact that most of these taxa are associated with immunity may be connected to the presence of many pathogens in the *D. rotundus* microbiome. For example, among the most abundant genera that play a role in the uniqueness of the *D. rotundus* microbiome we identified the pathogenic *Bartonella*, which are transmitted by hematophagous vectors such as ticks, fleas, sand flies, and mosquitoes[53–55]. We identified 5,246 DNA reads mapping to the genome of *Vibrio anguillarum*[56], which is a deadly hemorrhagic pathogens with very potent hemolysins, suggesting that *D. rotundus* has adapted to them and may aid in digesting the blood *D. rotundus* ingests. This bacterium is a classic fish pathogen, thus its identification suggests it is a related bacteria. The most relevant bacteria identified in *D. rotundus* that could have a protective role against potentially pathogenic

bacteria include *Actinoplanes friuliensis* and *Amycolatopsis mediterranei. A. friuliensis* produces the antibiotic friulimycin, which is active against a broad range of gram-positive pathogens such as methicillin-resistant *Staphylococcus* (MRSA, MRSE) and *Enterococcus* (MRE) strains[57]. *A. mediterranei* produces ansamycin compounds that demonstrate antiviral activity towards bacteriophages and poxviruses[58]. The genus *Amylocolatopsis* was also among the most abundant and relevant genera for the uniqueness of the *D. rotundus* microbiome. The probiotic *Lactobacillus rhamnosus*[59] was also identified. *L. rhamnosus* is involved in the increase of the re-epithelization of keratinocytes during wound healing in the digestive tract[60]. It also protects against respiratory and gastrointestinal viruses[61,62]; has been used to treat renal patients with vancomycin-resistant enterococci[63], can induce and maintain remission of Crohn's disease[64] and is used in treating patients with uropathogenic bacteria infections[65]. Furthermore, it also plays a role in lipid digestion[66].

Among other bacteria identified as significantly abundant and contributing significantly to *D. rotundus* variation and that seem to be specially adapted to survive in such nutrient poor environment is *Amphibacillus xylanus*. This is a facultative anaerobic organism which can grow in several different environments, whose success stems from its multiple metabolic pathways, each with high ATP yield, allowing it to adapt to various conditions[67,68]. The genus identified as the top most abundant and contributing to the microbiome variation of *D. rotundus* compared to that of the other bats is *Wolinella*. *W. succinogenes*, is known to have the largest density of two component signal transduction proteins, which allow it to reside in a wide variety of growth conditions[69], suggesting that this genus has been able to adapt to the unique medium in the *D. rotundus* intestine, similar to *A. xylanus*. It is also important to note the relevance in the uniqueness of *D. rotundus* of the bacteria *Clostridium acetobutylicum*, which is a commercially valuable bacterium able to produce concomitantly acetone, ethanol, and butanol from starch[70]. This suggests that this bacterium may help *D. rotundus* produce various metabolically important compounds from the low amounts of starch in the ingested blood.

The plasmid profile of *D. rotundus* clearly separates it from the other three bat species analyzed, both at the bacterial species and genera level (Extended Data Fig. 19). Interestingly, when looking at the presence/absence of the plasmids, *D. rotundus* forms a separate cluster. The only *D. rotundus* sample outside of this main *D. rotundus* cluster lies in the *R. ferrumequinum* cluster. However,

when looking at the genus level (Extended Data Fig. 19B), it is closer to *M. gigas* and *R. ferrumequinum*. Furthermore, when looking at the abundance of the plasmids, two of the *D. rotundus* samples are closer to the main *R. aegyptiacus* clade, and *R. ferrumequinum* and *M. gigas* are more mixed.

Looking at the viral presence/absence identifications and their abundance, *D. rotundus* does not distinguish itself from the other analyzed bats. The dendrogram and PCA of the taxonomic abundance at the species and genus level do not show the *D. rotundus* samples as a single separated cluster, rather *D. rotundus* seems to share identity with the three other tested species (Extended Data Fig. 8C). However, when only looking at the presence/absence of viruses, *D. rotundus* is included in the cluster containing *M. gigas* and *R. ferrumequinum*, with *R. aegyptiacus* being the most distant to the other three species. In summary, the viral content in *D. rotundus* is closer to that of its closest phylogenetic relatives. Although very few fungal and protozoan sequences were recovered, those profiles did not appear to show any unique placement of *D. rotundus* in relation to the other three bat species. However, more viral and protozoan data would be required to confirm these findings, as neither was abundant in the sequenced libraries.

Gut microbiome functional profile

When considering the statistical significance of the distributions of counts of the different microbial metabolic pathways classes, *D. rotundus* is statistically dissimilar to *M. gigas* in the glycan biosynthesis and metabolism (Wilcoxon P=0.058), and to *R. ferrumequinum* in lipid metabolism (Wilcoxon P=0.002) and xenobiotics biodegradation and metabolism (Wilcoxon P=0.05). There is no pathway subclass where *D. rotundus* is statistically closer to only one of the species and dissimilar to the other two. However, when removing the four deepest sequenced samples, *D. rotundus* is statistically closer to *R. ferrumequinum* in glycan biosynthesis and metabolism (*M. gigas* Wilcoxon P=0.05, *R. aegyptiacus* Wilcoxon P=0.08, *R. ferrumequinum* Wilcoxon P=0.8).

The PCA for the general metabolism (Extended Data Fig. 9) and for each of the metabolic classes (Extended Data Fig. 10), rotation matrix (Extended Data Table 6), and cladograms (Extended Data Table 7, Fig. 3B), show that the general *D. rotundus* metabolism is unique and distinguishes it from the other compared bats. We found that the most significant pathways to cause variation between *D.*

rotundus and the other bat species analyzed are pathways related to lipid, immunity, and blood digestion (Supplementary Information 19).

Lipid related traits include the biosynthesis of unsaturated fatty acids, fatty acid elongation, primary bile acid biosynthesis (which promotes digestion and absorption of dietary fat), and biosynthesis of cutin, suberin, and wax (wax esters are an alternative form of energy reserve to triacylglycerol). Another relevant lipid related pathway is the linoleic acid metabolism. Conjugated linoleic acids produced by gut bacteria appear to modulate the host's fatty acid composition in the liver and adipose tissue[71]. These observations suggest microbial adaptation to the common vampire bat diet, with particular regards to the low amounts of fat in blood.

Immunity related pathways include styrene and fluorobenzoate degradation. Components of the fluorobenzoate degradation pathway have been associated with the disease severity of New-Onset Crohn's disease[72], a type of inflammatory bowel disease, and intermediaries of the benzoate metabolic pathway have been shown to promote virulence in *Enterobacteriaceae*[73]. Another immunity related feature is the lower abundance of enzymes for the degradation of geraniol, which inhibits the growth of pathogenic bacteria from the human colon[74]. We also identified a pathogen related pathway for the biosynthesis of aflatoxins, which are mycotoxins produced by *Aspergillus flavus*[75].

The two most abundant pathways driving variation between the common vampire bat and the other bat species are the biosynthesis of siderophore group nonribosomal peptides, and of ubiquinone and other terpenoid-quinones. These two pathways are directly related to the capabilities required for blood digestion. Sideropores are iron chelating compounds, they are among the strongest soluble Fe^{3+} binding agents known[76], and ubiquinone can affect blood clotting[77].

When examining each metabolic class separately instead of all the metabolic pathways together, we also observed *D. rotundus* separating from the other species at various degrees, depending on the metabolic class (Extended Data Fig. 10). The metabolic categories in which *D. rotundus* is clearly separated are: amino acid metabolism, carbohydrate metabolism, energy metabolism, metabolism of cofactors and vitamins, biosynthesis of secondary metabolites, and metabolism of other amino acids. The other categories in which the separation is not as pronounced are: glycan biosynthesis

metabolism, lipid metabolism, metabolism of terpenoids and polyketides, and xenobiotics biodegradation and metabolism. Using the annotations from the DIAMOND results (the reads that did not map to the DNA whole genome databases that were searched against uniprot), *D. rotundus* is distinct from the other species only in the next metabolic classes: carbohydrate metabolism, energy metabolism, metabolism of cofactors and vitamins.

In summary, although *D. rotundus* possesses a unique microbiome at both the taxonomic and functional level, the greater level of taxonomic relatedness between the gut microbiome of *D. rotundus* with those of its phylogenetic relatives *R. ferrumequinum* and *M. gigas,* than to the more distant *R. aegyptiacus*, reflects the host phylogenetic influence. However, the functional profile differs from the taxonomic profile. All three species (meat, insect and fruit eaters) are in mixed clades and *D. rotundus* is almost completely separated from them in its own clade. This is of even greater relevance when taking into account that the examined frugivorous bat (*R. aegyptiacus*) is not from the *Phyllostomidae* family, but from a basal clade to the phyllostomids, suggesting that the functional profile is not influenced by host phylogeny as is the taxonomic profile.

When examining the significantly enriched genes in *D. rotundus* compared to the other bats, we also identified genes related to addressing the challenges of sanguivory, for example, genes involved in the urea cycle (Acetylglutamate kinase *P*=2.86E-7, Argininosuccinate synthase *P*=2.86E-7, Arginine dihydrolase *P*=0.014, Acetylornithine aminotransferase *P*=4.42E-6). Regarding the high protein levels in the blood, we identified an enrichment in the gene Saccharopine dehydrogenase (*P*=1.74E-5). Deficiencies in this gene are associated with hyperlysinemia, a metabolic disorder characterized by an abnormal increase of lysine in the blood[78]. Furthermore, the microbial gene GTP cyclohydrolase I is enriched in the common vampire bat. Mutations in this gene are associated with malignant phenylketonuria (PKU), hyperphenylalaninemia (HPA), and GTP cyclohydrolase I deficiency.

Genes involved in the reductive carboxylate cycle (CO_2 fixation), such as Alanine dehydrogenase (*P*=1.124E-6) were enriched in the common vampire bat fecal microbiome. Furthermore, we also identified enriched genes related to addressing the challenge of the abundance of iron in the blood. For example, we identified various genes involved in the synthesis of heme, such as Pre-uroporphyrinogen synthase (*P*=1.88E-6), Uroporphyrinogen decarboxylase (*P*=0.019), and

Protoheme ferro-lyase (*P*=3.67E-4). Furthermore, we identified an enrichment of genes derived from *Sulfurospirillum deleyianum*, which is a ferric iron-reducing bacteria used for the study of ferric iron reduction via sulfur cycling[79]. The microbial gene for Orotate phosphoribosyltransferase was also enriched (*P*=0.0018). In mammals, the catalytic function of this enzyme is carried out by UMP synthase, and deficiencies in this gene are associated with hypochromic anemia, in which red blood cells are paler due to a reduction in hemoglobin[80].

With regards to the low levels of glycogen in the blood, the enolase gene was enriched in the common vampire bat microbiome (*P*=0.011). This enzyme is involved in glycolysis. In humans there are three subunits of enolase, and deficiency of ENO1 is linked to hereditary hemolytic anemia[81] while ENO3 deficiency is linked to glycogen storage disease XIII[82]. We also identified other enriched glycogen related genes, such as bacterial glycogen synthase (*P*=5.59E-4). We also identified enrichment of genes for the metabolism of vitamins, such as Alpha-keto-beta-hydroxylacyl reductoisomerase (*P*=1.124E-6).

With regards to the regulation of osmolarity, the gene Trehalose-6-phosphate hydrolase was enriched. Trehalose acts as an osmoprotectant (at high osmolarity) and as a carbon source (at both high and low osmolality)[83]. Adenine phosphoribosyltransferase was also enriched (*P*=0.00639). Deficiency of this gene contributes to the formation of kidney stones and potentially contributes to kidney failure[84]. Finally, we also identified enriched genes annotated as derived from pathogenic bacteria, such as *Campylobacter fetus* (pathogenic to cattle and sheep) and *Mycoplasma bovis*, possibly derived from their prey.

References

1. Kang, Y. *et al.* Crystal structure of rhodopsin bound to arrestin by femtosecond X-ray laser. *Nature* **523,** 561–7 (2015).
2. Sutton, G. M. *et al.* The melanocortin-3 receptor is required for entrainment to meal intake. *J. Neurosci.* **28,** 12946–55 (2008).
3. Chen, A. S. *et al.* Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean body mass. *Nat. Genet.* **26,** 97–102 (2000).
4. Coll, A. P., Farooqi, I. S. & O'Rahilly, S. The hormonal control of food intake. *Cell* **129,**

251–62 (2007).

5. Ho, M.-R. *et al.* Human RegIV Protein Adopts a Typical C-Type Lectin Fold but Binds Mannan with Two Calcium-Independent Sites. *J. Mol. Biol.* **402,** 682–695 (2010).
6. Kämäräinen, M. *et al.* RELP, a novel human REG-like protein with up-regulated expression in inflammatory and metaplastic gastrointestinal mucosa. *Am. J. Pathol.* **163,** 11–20 (2003).
7. Nabokina, S. M., Subramanian, V. S. & Said, H. M. Association of PDZ-containing protein PDZD11 with the human sodium-dependent multivitamin transporter. *Am. J. Physiol. Gastrointest. Liver Physiol.* **300,** G561-7 (2011).
8. Bar-Peled, L., Schweitzer, L. D., Zoncu, R. & Sabatini, D. M. Ragulator Is a GEF for the Rag GTPases that Signal Amino Acid Levels to mTORC1. *Cell* **150,** 1196–1208 (2012).
9. Mazumder, B., Seshadri, V., Imataka, H., Sonenberg, N. & Fox, P. L. Translational silencing of ceruloplasmin requires the essential elements of mRNA circularization: poly(A) tail, poly(A)-binding protein, and eukaryotic translation initiation factor 4G. *Mol. Cell. Biol.* **21,** 6440–9 (2001).
10. Strick, D. J. & Elferink, L. A. Rab15 effector protein: a novel protein for receptor recycling from the endocytic recycling compartment. *Mol. Biol. Cell* **16,** 5699–709 (2005).
11. Pennica, D. *et al.* Cloning and expression of human tissue-type plasminogen activator cDNA in E. coli. *Nature* **301,** 214–21 (1983).
12. Breidenstein, C. P. Digestion and Assimilation of Bovine Blood by a Vampire Bat (Desmodus rotundus). *J. Mammal.* **63,** 482–484 (1982).
13. Monteiro, A. C. *et al.* JAM-A associates with ZO-2, afadin, and PDZ-GEF1 to activate Rap2c and regulate epithelial barrier function. *Mol. Biol. Cell* **24,** 2849–2860 (2013).
14. Singer, M. A. Vampire bat, shrew, and bear: comparative physiology and chronic renal failure. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **282,** R1583-92 (2002).
15. Taschek, K. *Hanging with Bats: Ecobats, Vampires, and Movie Stars*. (Albuquerque, NM: University of New Mexico Press, 2008).
16. Tortora, G. J. & Derrickson, B. H. *Principles of Anatomy and Physiology*. (2008).
17. Touitou, R. *et al.* A degradation signal located in the C-terminus of p21WAF1/CIP1 is a binding site for the C8 alpha-subunit of the 20S proteasome. *EMBO J.* **20,** 2367–75 (2001).
18. Pegg, A. E. Regulation of ornithine decarboxylase. *J. Biol. Chem.* **281,** 14529–32 (2006).
19. Iwami, K., Wang, J. Y., Jain, R., McCormack, S. & Johnson, L. R. Intestinal ornithine decarboxylase: half-life and regulation by putrescine. *Am. J. Physiol.* **258,** G308-15 (1990).

20. Henley, C. M., Muszynski, C., Cherian, L. & Robertson, C. S. Activation of ornithine decarboxylase and accumulation of putrescine after traumatic brain injury. *J. Neurotrauma* **13,** 487–96 (1996).
21. Lee, A., Beck, L. & Markovich, D. The human renal sodium sulfate cotransporter (SLC13A1; hNaSi-1) cDNA and gene: organization, chromosomal localization, and functional characterization. *Genomics* **70,** 354–63 (2000).
22. Negoro, H. *et al.* Involvement of urinary bladder Connexin43 and the circadian clock in coordination of diurnal micturition rhythm. *Nat. Commun.* **3,** 809 (2012).
23. Imamura, M. *et al.* Myocardin and microRNA-1 modulate bladder activity through connexin 43 expression during post-natal development. *J. Cell. Physiol.* **228,** 1819–26 (2013).
24. Koller, K. J. *et al.* Selective activation of the B natriuretic peptide receptor by C-type natriuretic peptide (CNP). *Science* **252,** 120–3 (1991).
25. K**?**rten, L. & Schmidt, U. Thermoperception in the common vampire bat (Desmodus rotundus). *J. Comp. Physiol. ? A* **146,** 223–228 (1982).
26. Kürten, L., Schmidt, U. & Schäfer, K. Warm and cold receptors in the nose of the vampire bat Desmodus rotundus. *Naturwissenschaften* **71,** 327–8 (1984).
27. Kishida, R., Goris, R. C., Terashima, S. & Dubbeldam, J. L. A suspected infrared-recipient nucleus in the brainstem of the vampire bat, Desmodus rotundus. *Brain Res.* **322,** 351–5 (1984).
28. Gracheva, E. O. *et al.* Molecular basis of infrared detection by snakes. *Nature* **464,** 1006–11 (2010).
29. Gracheva, E. O. *et al.* Ganglion-specific splicing of TRPV1 underlies infrared sensation in vampire bats. *Nature* **476,** 88–91 (2011).
30. Wang, Y. The functional regulation of TRPV1 and its role in pain sensitization. *Neurochem. Res.* **33,** 2008–12 (2008).
31. Zhu, H. *et al.* Interaction between protein kinase D1 and transient receptor potential V1 in primary sensory neurons is involved in heat hypersensitivity. *Pain* **137,** 574–88 (2008).
32. Genro, B. P., de Oliveira Alvares, L. & Quillfeldt, J. A. Role of TRPV1 in consolidation of fear memories depends on the averseness of the conditioning procedure. *Neurobiol. Learn. Mem.* **97,** 355–60 (2012).
33. Schwarz, Q. *et al.* Plexin A3 and plexin A4 convey semaphorin signals during facial nerve development. *Dev. Biol.* **324,** 1–9 (2008).

34. Wigmore, G. Vampire bats turn down the heat sensors to hunt. *Nature* (2011). doi:10.1038/news.2011.454
35. Oliver, E. R., Saunders, T. L., Tarlé, S. A. & Glaser, T. Ribosomal protein L24 defect in belly spot and tail (Bst), a mouse Minute. *Development* **131,** 3907–20 (2004).
36. Doyle, S. L., Jefferies, C. A., Feighery, C. & O'Neill, L. A. J. Signaling by Toll-like receptors 8 and 9 requires Bruton's tyrosine kinase. *J. Biol. Chem.* **282,** 36953–60 (2007).
37. Pertel, T. *et al.* TRIM5 is an innate immune sensor for the retrovirus capsid lattice. *Nature* **472,** 361–5 (2011).
38. Cerretti, D. P., DuBose, R. F., Black, R. A. & Nelson, N. Isolation of two novel metalloproteinase-disintegrin (ADAM) cDNAs that show testis-specific gene expression. *Biochem. Biophys. Res. Commun.* **263,** 810–5 (1999).
39. Elbers, C. C. *et al.* Variants in neuropeptide Y receptor 1 and 5 are associated with nutrient-specific food intake and are under recent selection in Europeans. *PLoS One* **4,** e7070 (2009).
40. Joyal, J.-S. *et al.* Retinal lipid and glucose metabolism dictates angiogenesis through the lipid sensor Ffar1. *Nat. Med.* **22,** 439–45 (2016).
41. Kawasaki, Y. *et al.* REG4 is a transcriptional target of GATA6 and is essential for colorectal tumorigenesis. *Sci. Rep.* **5,** 14291 (2015).
42. Harder, J. & Schroder, J.-M. RNase 7, a novel innate immune defense antimicrobial protein of healthy human skin. *J. Biol. Chem.* **277,** 46779–84 (2002).
43. Chandrashekar, J. *et al.* T2Rs function as bitter taste receptors. *Cell* **100,** 703–11 (2000).
44. Itoh, Y. *et al.* Free fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. *Nature* **422,** 173–6 (2003).
45. Hong, W. & Zhao, H. Vampire bats exhibit evolutionary reduction of bitter taste receptor genes common to other bats. *Proc. R. Soc. B Biol. Sci.* **281,** 20141079–20141079 (2014).
46. Dotson, C. D. *et al.* Bitter taste receptors influence glucose homeostasis. *PLoS One* **3,** e3974 (2008).
47. Glendinning, J. I., Yiin, Y.-M., Ackroff, K. & Sclafani, A. Intragastric infusion of denatonium conditions flavor aversions and delays gastric emptying in rodents. *Physiol. Behav.* **93,** 757–65 (2008).
48. Mitani, Y. *et al.* Reg IV is a serum biomarker for gastric cancer patients and predicts response to 5-fluorouracil-based chemotherapy. *Oncogene* **26,** 4383–93 (2007).
49. Jahanian, R. & Ashnagar, M. Effect of dietary supplementation of mannan-oligosaccharides

on performance, blood metabolites, ileal nutrient digestibility, and gut microflora in Escherichia coli-challenged laying hens. *Poult. Sci.* **94,** 2165–72 (2015).

50. Baurhoo, B., Phillip, L. & Ruiz-Feria, C. A. Effects of Purified Lignin and Mannan Oligosaccharides on Intestinal Integrity and Microbial Populations in the Ceca and Litter of Broiler Chickens. *Poult. Sci.* **86,** 1070–1078 (2007).
51. WARREN, J. R. & GRAHAM, F. The effect of heparin on the growth of bacteria and yeasts. *J. Bacteriol.* **60,** 171–4 (1950).
52. Huang, Y.-C. *et al.* The flexible and clustered lysine residues of human ribonuclease 7 are critical for membrane permeability and antimicrobial activity. *J. Biol. Chem.* **282,** 4626–33 (2007).
53. Morick, D. *et al.* Effects of Bartonella spp. on flea feeding and reproductive performance. *Appl. Environ. Microbiol.* **79,** 3438–43 (2013).
54. Janecek, E., Mietze, A., Goethe, R., Schnieder, T. & Strube, C. Bartonella spp. infection rate and B. grahamii in ticks. *Emerg. Infect. Dis.* **18,** 1689–90 (2012).
55. Maggi, R. G. *et al.* Bartonella spp. bacteremia and rheumatic symptoms in patients from Lyme disease-endemic region. *Emerg. Infect. Dis.* **18,** 783–91 (2012).
56. Frans, I. *et al.* Vibrio anguillarum as a fish pathogen: virulence factors, diagnosis and prevention. *J. Fish Dis.* **34,** 643–61 (2011).
57. Rückert, C. *et al.* Complete genome sequence of the actinobacterium Actinoplanes friuliensis HAG 010964, producer of the lipopeptide antibiotic friulimycin. *J. Biotechnol.* **178,** 41–2 (2014).
58. August, P. R. *et al.* Biosynthesis of the ansamycin antibiotic rifamycin: deductions from the molecular analysis of the rif biosynthetic gene cluster of Amycolatopsis mediterranei S699. *Chem. Biol.* **5,** 69–79 (1998).
59. Lehtoranta, L. Probiotics and virus infections : The effects of Lactobacillus rhamnosus GG on respiratory and gastrointestinal virus infections. (2012).
60. Mohammedsaeed, W., Cruickshank, S., McBain, A. J. & O'Neill, C. A. Lactobacillus rhamnosus GG Lysate Increases Re-Epithelialization of Keratinocyte Scratch Assays by Promoting Migration. *Sci. Rep.* **5,** 16147 (2015).
61. Lee, Y.-N. *et al.* Sublingual administration of Lactobacillus rhamnosus affects respiratory immune responses and facilitates protection against influenza virus infection in mice. *Antiviral Res.* **98,** 284–90 (2013).

62. Vanderhoof, J. A. *et al.* Lactobacillus GG in the prevention of antibiotic-associated diarrhea in children. *J. Pediatr.* **135,** 564–568 (1999).
63. Manley, K. J., Fraenkel, M. B., Mayall, B. C. & Power, D. A. Probiotic treatment of vancomycin-resistant enterococci: a randomised controlled trial. *Med. J. Aust.* **186,** 454–7 (2007).
64. Schultz, M. *et al.* Lactobacillus GG in inducing and maintaining remission of Crohn's disease. *BMC Gastroenterol.* **4,** 5 (2004).
65. Karlsson, M., Scherbak, N., Reid, G. & Jass, J. Lactobacillus rhamnosus GR-1 enhances NF-kappaB activation in Escherichia coli-stimulated urinary bladder cells through TLR4. *BMC Microbiol.* **12,** 15 (2012).
66. Falcinelli, S. *et al.* Lactobacillus rhamnosus lowers zebrafish lipid content by changing gut microbiota and host transcription of genes involved in lipid metabolism. *Sci. Rep.* **5,** 9336 (2015).
67. Koyama, N., Niimura, Y. & Kozaki, M. Bioenergetic properties of a facultatively anaerobic alkalophile. *FEMS Microbiol. Lett.* **49,** 123–126 (1988).
68. Niimura, Y. *et al.* Amphibacillus xylanus gen. nov., sp. nov., a Facultatively Anaerobic Sporeforming Xylan-Digesting Bacterium Which Lacks Cytochrome, Quinone, and Catalase. *Int. J. Syst. Bacteriol.* **40,** 297–301 (1990).
69. Baar, C. *et al.* Complete genome sequence and analysis of Wolinella succinogenes. *Proc. Natl. Acad. Sci. U. S. A.* **100,** 11690–5 (2003).
70. Nölling, J. *et al.* Genome sequence and comparative analysis of the solvent-producing bacterium Clostridium acetobutylicum. *J. Bacteriol.* **183,** 4823–38 (2001).
71. Rosberg-Cody, E. *et al.* Recombinant lactobacilli expressing linoleic acid isomerase can modulate the fatty acid composition of host adipose tissue in mice. *Microbiology* **157,** 609–15 (2011).
72. Gevers, D. *et al.* The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host Microbe* **15,** 382–92 (2014).
73. Freestone, P. P. E., Walton, N. J., Haigh, R. D. & Lyte, M. Influence of dietary catechols on the growth of enteropathogenic bacteria. *Int. J. Food Microbiol.* **119,** 159–69 (2007).
74. Thapa, D., Losa, R., Zweifel, B. & Wallace, R. J. Sensitivity of pathogenic and commensal bacteria from the human colon to essential oils. *Microbiology* **158,** 2870–7 (2012).
75. Varga, J., Frisvad, J. C. & Samson, R. A. Two new aflatoxin producing species, and an

overview of Aspergillus section Flavi. *Stud. Mycol.* **69,** 57–80 (2011).

76. Neilands, J. B. Siderophores: Structure and Function of Microbial Iron Transport Compounds. *J. Biol. Chem.* **270,** 26723–26726 (1995).
77. Mousa, S. A. Antithrombotic effects of naturally derived products on coagulation and platelet function. *Methods Mol. Biol.* **663,** 229–40 (2010).
78. Cederbaum, S. D., Shaw, K. N., Dancis, J., Hutzler, J. & Blaskovics, J. C. Hyperlysinemia with saccharopinuria due to combined lysine-ketoglutarate reductase and saccharopine dehydrogenase deficiencies presenting as cystinuria. *J. Pediatr*. **95,** 234–8 (1979).
79. Straub, K. L. & Schink, B. Ferrihydrite-dependent growth of Sulfurospirillum deleyianum through electron transfer via sulfur cycling. *Appl. Environ. Microbiol.* **70,** 5744–9 (2004).
80. Musick, W. D. L. & Nyhan, W. L. Structural Features of the Phosphoribosyl-Transferases and Their Relationship to the Human Deficiency Disorders of Purine and Pyrimidine Metabolis. *Crit. Rev. Biochem.* **11,** 1–34 (1981).
81. Lachant, N., Jennings, M. & Tanaka, K. Partial erythrocyte enolase deficiency: a hereditary disorder with variable clinical expression. *Blood* **68,** 55a (1986).
82. Comi, G. P. *et al.* Beta-enolase deficiency, a new metabolic myopathy of distal glycolysis. *Ann. Neurol.* **50,** 202–7 (2001).
83. Rimmele, M. & Boos, W. Trehalose-6-phosphate hydrolase of Escherichia coli. *J. Bacteriol.* **176,** 5654–64 (1994).
84. Terai, C. *et al.* Adenine phosphoribosyltransferase deficiency identified by urinary sediment analysis: cellular and molecular confirmation. *Clin. Genet.* **48,** 246–250 (2008).