Supplemental Text and Methods

Random forests classifier and cross-validation estimation of accuracy

Using the random forests classifier, we determined the most discriminative genus-level taxa between wild, semi-captive, and captive NHPs. We then assessed the accuracy of the classifier using 10-fold cross-validation. In other words, we trained the classifier on 90% of the samples, and then used the discovered signatures to predict which populations the remaining 10% of samples belonged, and then repeated the process 10 times. This analysis revealed that individual primate populations have such distinct signature microbiomes that they can be identified from their microbiota with an estimated 99.6% accuracy (Figure 2). From this analysis we identified the bacterial genera most important for distinguishing the populations (random forests feature importance score ≥ 0.01). We found that wild NHPs possessed higher relative abundances of a variety of microbes, including *Collinsella*, *Tannerella*, *Oscillospira*, *Coprococcus*, etc., while captive NHPs possessed higher relative abundances of *Bacteroides*, *Prevotella*, *Parabacteroides*, *Treponema*, etc. Compared to wild and captive NHPs, our population of semi-captive NHPs possessed higher relative abundances of a variety of microbes, including *Akkermansia*, *Turicibacter*, *Methylobacterium*, and other taxa.

Importance of concordant data generation methods for meta-analysis

A major challenge in performing the meta-analyses combining microbiome data from multiple sources is the presence of batch effects due to study bias from different processing methods. To extract meaningful patterns from our comparison of NHP and human microbiome data required joint analysis of multiple wild populations of species, together with previously published human data. Previous work has characterized gut microbiome variation in a number of specific groups of primate species, such as chimpanzees, African apes, and baboons (1, 2). However, these published data are generated using varied approaches to sample storage, DNA extraction, amplification, and DNA sequencing, impeding efforts toward large-scale meta-analyses. Quantitation of microbiome data requires application of consistent, standardized methods to avoid batch effects. In this study, all NHP fecal samples were obtained by our group using the same protocols, were processed using comparable methods, and were sequenced at the same sequencing facility using the same method (i.e., EMP method; V4 region) as published human data (3), which resulted in wild, semi-captive, and captive NHP microbiome samples that were amenable to meta-analysis.

Detailed discussion of captive primate diet homogeneity

Captive NHP diets are dramatically different from those of wild or even semi-wild NHPs. Diets fed to captive NHPs are typically generalized and rarely species-specific. NHP species are regularly categorized as folivores, frugivores, and omnivores based on the dietary niche they occupy. In captive settings, this results in NHPs being given very similar, if not identical diets (4). However, even if wild primates do belong in similar feeding guilds, different feeding ecologies, morphologic and physiologic adaptations, and habitats all contribute to varied nutrient intake in the wild (5), typically rich in plant fibers. In contrast, the recommended diet of most captive NHPs is based primarily on corn and soy. It is high in fat (5%) and protein (23%) while low in fiber (14%) (6) when compared to the diet of wild leaf-eating NHPs that has approximately 0% fat, 10-13% protein and 23-54% fiber (7). Unlike corn and soy, tree leaves contain plant secondary compounds (such as alkaloids, phenolics, and cyanide) in addition to nutrients (8–12). For example the Golden Bamboo lemur consumes four times the human-lethal dose of cyanide every day (13), indicating highly specialized digestive capability across different primates. Thus, loss of dietary fiber content and fiber diversity in captive NHPs is a likely contributor to their concomitant loss of gut health and microbial diversity.

Detailed discussion of emergence of Bacteroides

One of the marked effects of captivity on the gut microbiome of NHPs, as well as Westernization on the gut microbiome of humans, is an increase in relative abundance of *Bacteroides*. Using both wild ape and human microbiomes, Moeller et al. (2014) determined that *Bacteroides* has increased in relative abundance in humans living in the USA greater than fivefold since their divergence from other human populations. The bacterial genus *Bacteroides* has a known positive association with the consumption of a diet rich in animal fat and protein (1, 14), which are major components of a

Western diet. A Western diet is considered to be a diet high in fat and animal protein (e.g., red meat), high in sugar, and low in plant-based fiber (15–17). Previous studies examining the relationship between dietary patterns and dysbiosis suggest a strong association between Western lifestyle, notably diet, and a dysbiotic gut microbiome (14, 15, 17), as the Western diet is evolutionarily discordant from the diet of ancestral humans (15, 18). Taken together, the relative abundance of *Bacteroides* in the gut appears to be strongly regulated by dietary intake.

Breakdown of Douc and Howler sample population

Doucs: Fecal samples (n = 111) were collected from captive (n = 27 samples, 9 individuals), semi-captive (n = 18 samples, 18 individuals), and wild (n = 66 samples from 7 known individuals and 39 unknown individuals) red-shanked doucs (*Pygathrix nemaeus*) between 2012-2013. One captive population was located at the Philadelphia Zoo in the USA while another was located at the Singapore Zoo in SE Asia. Doucs housed at the Endangered Primate Rescue Center in Ninh Binh, Vietnam served as the semi-captive population. Doucs inhabiting Son Tra Nature Reserve, Da Nang, Vietnam (16°06'—16°09'N, 108°13'—108°21'E) served as the wild population in this comparative study (19).

Howlers: Fecal samples (n = 56) were collected from captive (n = 5 samples, 5 individuals) and wild (n = 51 samples, 28 known individuals, 17 unknown individuals) mantled howling monkeys (*Alouatta palliata*) between July-August 2010. Howlers inhabiting the forests of Hacienda La Pacifica, which is a privately owned cattle/tilapia farm of approximately 2,000 hectares located at the base of the Cordillera de Tilaran in the Province of Guanacaste, Costa Rica (latitude 10°28'N, longitude 85°07'W), served as the wild subjects (20, 21). Howlers housed at Las Pumas Rescue Center, which is located within Hacienda La Pacifica, served as the captive subjects.

The remaining eight NHP species sampled consisted of captive individuals housed at the Como Zoo in Saint Paul, MN (Supplemental Table 2).

Supplemental Figures

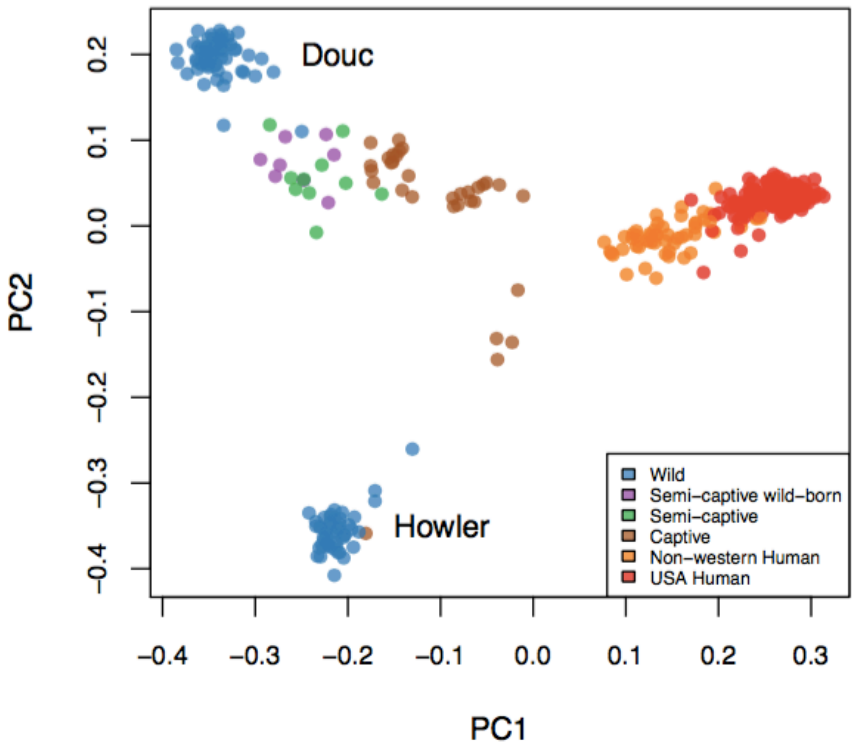

Figure S1. ***Primate microbiome clustering by captivity, location, and species does not depend on inclusion of USA zoo #2 samples.*** Principal coordinates plot of unweighted UniFrac distances between all primate and samples shown in Figure 1, excluding the 33 samples from the second USA zoo. Similar to the plot in Figure 1, this plot shows separation of the wild doucs and howlers, and convergence of the douc and howler microbiomes toward the same state in captivity. Principal coordinates analysis was performed only on the subset of samples to demonstrated that the observed clustering was not driven by the second USA zoo samples.

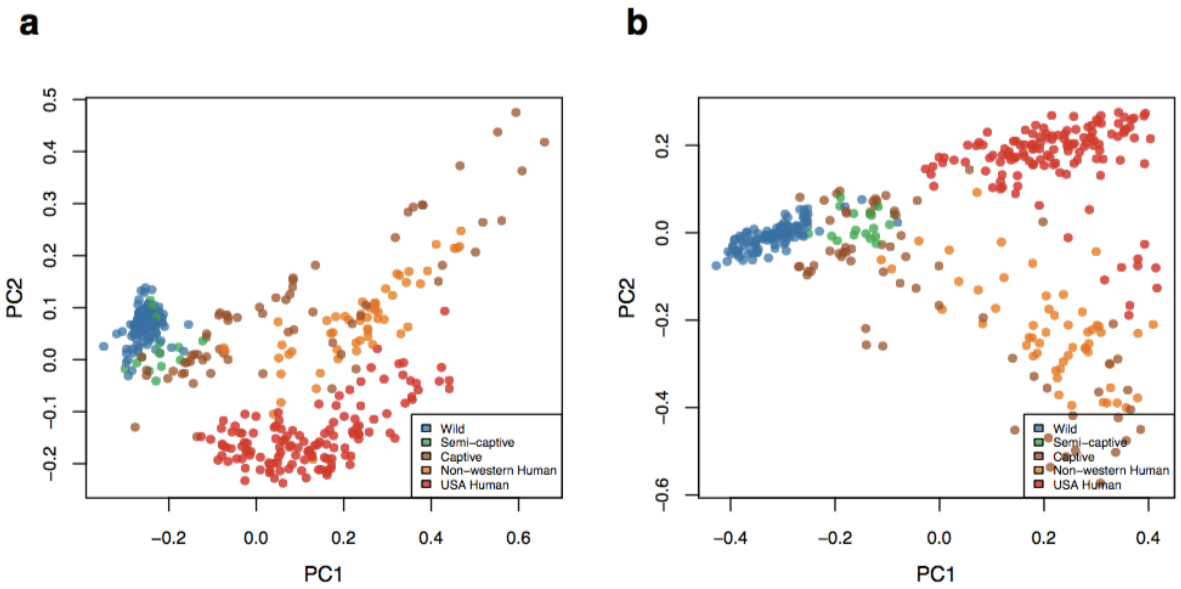

Figure S2. ***Captive primate dysbiosis converges toward the modern human microbiome.*** Principal coordinates plot of (a) weighted UniFrac and (b) Bray-Curtis distances between all samples, showing ecological distance between gut microbial communities in wild, semi-captive (from a sanctuary), and captive nonhuman primates, as well as non-westernized humans, and humans living in the USA (i.e., Westernized). Unweighted UniFrac (Figure 1) provided much stronger clustering of our experimental data by population than weighted UniFrac or Bray Curtis distances, indicating that the clustering is likely driven by presence or absence of key taxa in different populations, rather than by shifts in the ratios of dominant members of the microbiota. These distances based on relative abundance show captive primates overlapping more with the non-westernized modern humans.

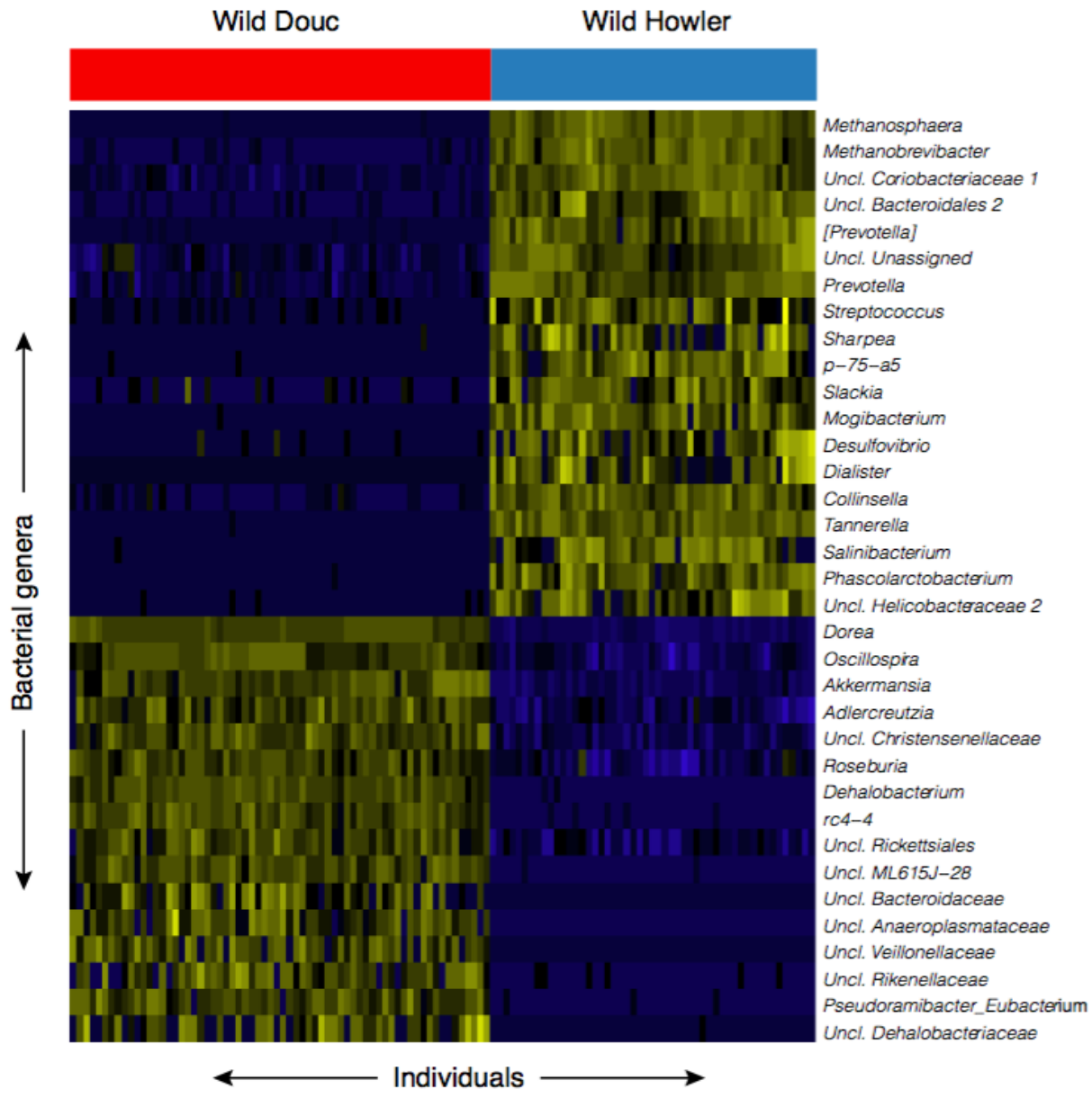

Figure S3. ***Heatmap of most predictive taxa discriminating gut microbiomes of two wild primate species.*** The transformed relative abundance of the 20 most strongly predictive bacterial genera for discriminating between two species of primates, the red-shanked douc and the mantled howling monkey, as determined by the random forests classifier. This heatmap shows very strong signature species for each of these groups.

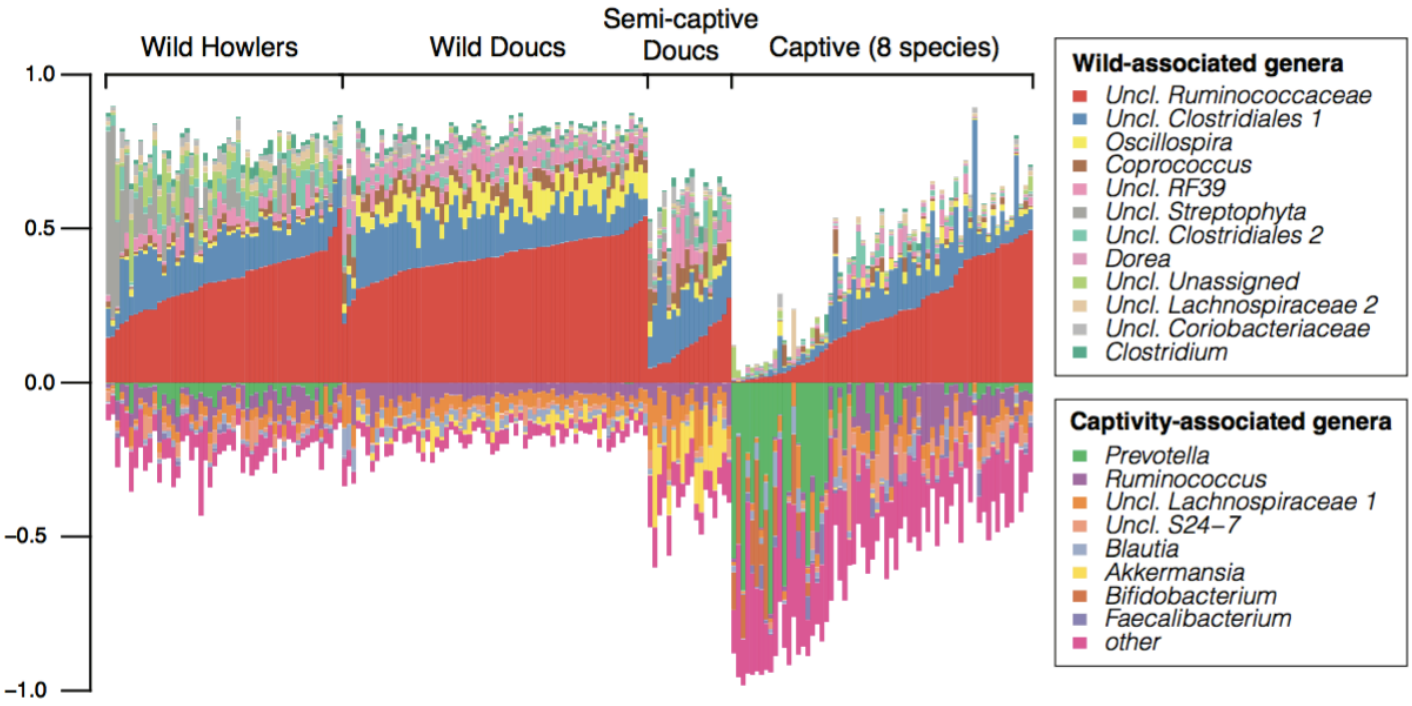

Figure S4. ***Captivity reduces native primate microbiota.*** Standard box plot of microbiome variation (unweighted UniFrac distance) explained by different experimental factors, showing that captivity in general is associated with a greater change in microbiome state than variation in host species, zoological institution, or individual. (c) Stacked bar plot of relative abundance of the 20 most abundant genera across all wild and captive douc and howler individuals. Bars above zero correspond to genera more prevalent in wild primates; bars below zero correspond to genera more common in captive primates.

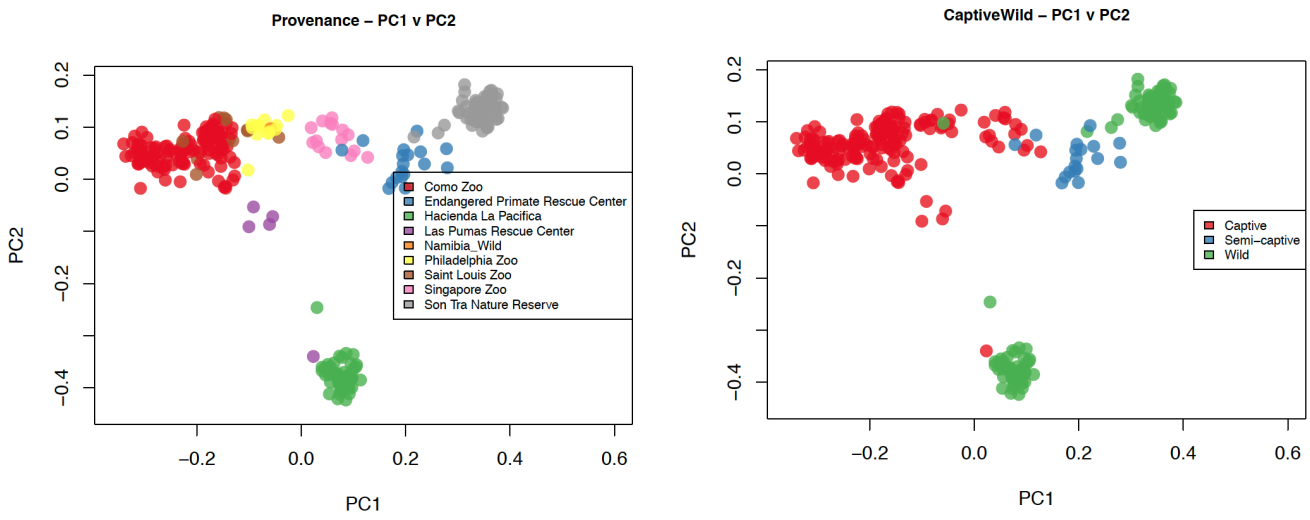

Figure S5. ***Captivity causes nonhuman primate microbiomes to converge toward the same compositional state.*** Principal coordinates plot of genus-level unweighted UniFrac distances showing ecological distance between gut microbial communities in wild, semi-captive (from a sanctuary), and captive nonhuman primates plotted by population location (left panel) and captivity status (right panel). Novel NHP microbiomes (doucs, howlers, and Como Zoo population) are based on V4 16S sequences (R1 only), and previously published NHP microbiomes (Saint Louis Zoo and Namibia_Wild) by Muegge et al. (2011) are based on V2 16S sequences. Although in wild populations the douc and howler microbiomes are highly distinctive, captivity causes them to converge toward the same composition. Semi-captive doucs (green) fall in between wild and captive doucs along the same axis of convergence. The inclusion of additional captive nonhuman primate populations, represented by 14 distinctive primate species (Como Zoo and Saint Louis Zoo), further highlights the convergence that occurs when primates are kept in captivity. We note that although location is an important driver of microbiome variation (left panel), the effect of zoo location is smaller than the overall effect of captivity. This is also shown in Figure 3b.

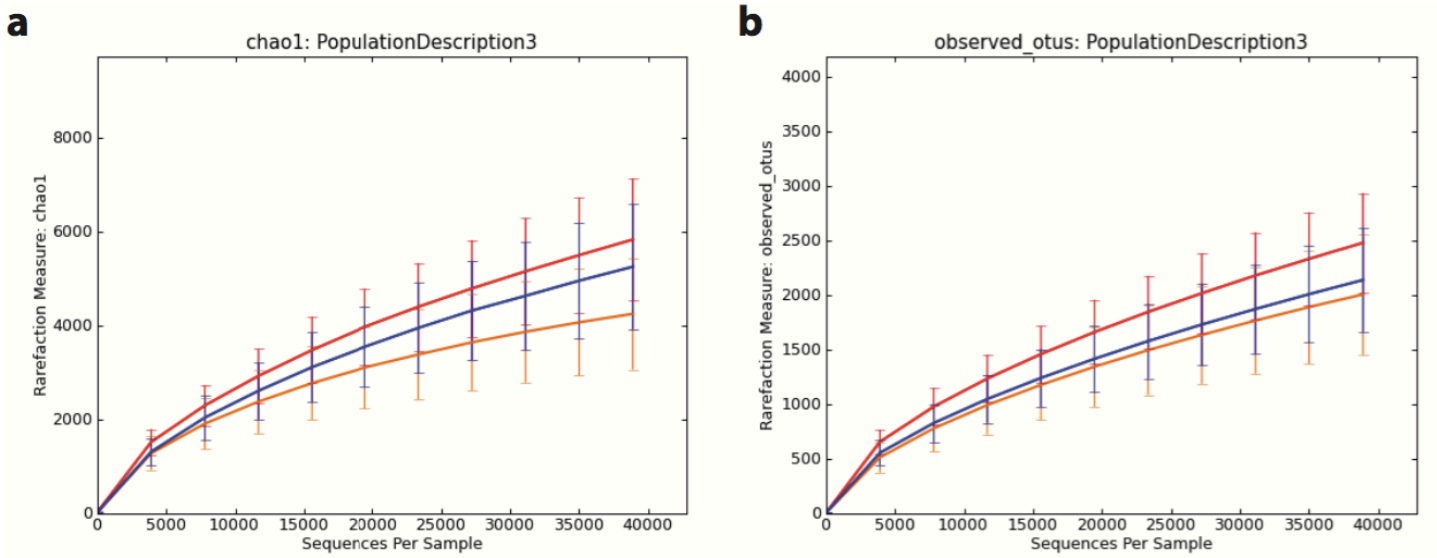

Figure S6. ***Rarefaction curves for different primate groups.*** (left) Chao-1 estimator as a measure of alpha diversity; (right) Observed number of OTUs as a measure of alpha diversity. Red: wild; blue: semi-captive; orange: captive. We also compared diversity between groups of different captivity status using data at the full rarefaction depth (14100 sequences/sample). Dropping singleton OTUs present in only one sample, the wild NHPs (2544.3 ± 390.9 OTUs) harbored the highest number of OTUs (i.e., greatest diversity), followed by the semi-captive NHPs (2141.4 ± 293.0 OTUs), and captive NHPs (1967.9 ± 538.2 OTUs). By this metric, wild NHPs had significantly higher diversity than captive or semi-captive (t-test $p = 2.1\times10^{-21}$, 1.9×10^{-5}, respectively), and captive had higher diversity than semi-captive (t-test $p = 0.040$). We repeated this analysis with the Chao1 estimator of the true number of OTUs (i.e., species richness) in our samples. Using the Chao1 estimator differences were significant between all three populations (t-test $p = 4.6\times10^{-39}$, 7.3×10^{-8}, 0.0099 for wild vs. captive, wild vs. semi-captive, and captive vs. semi-captive, respectively) (see Figure 3a).

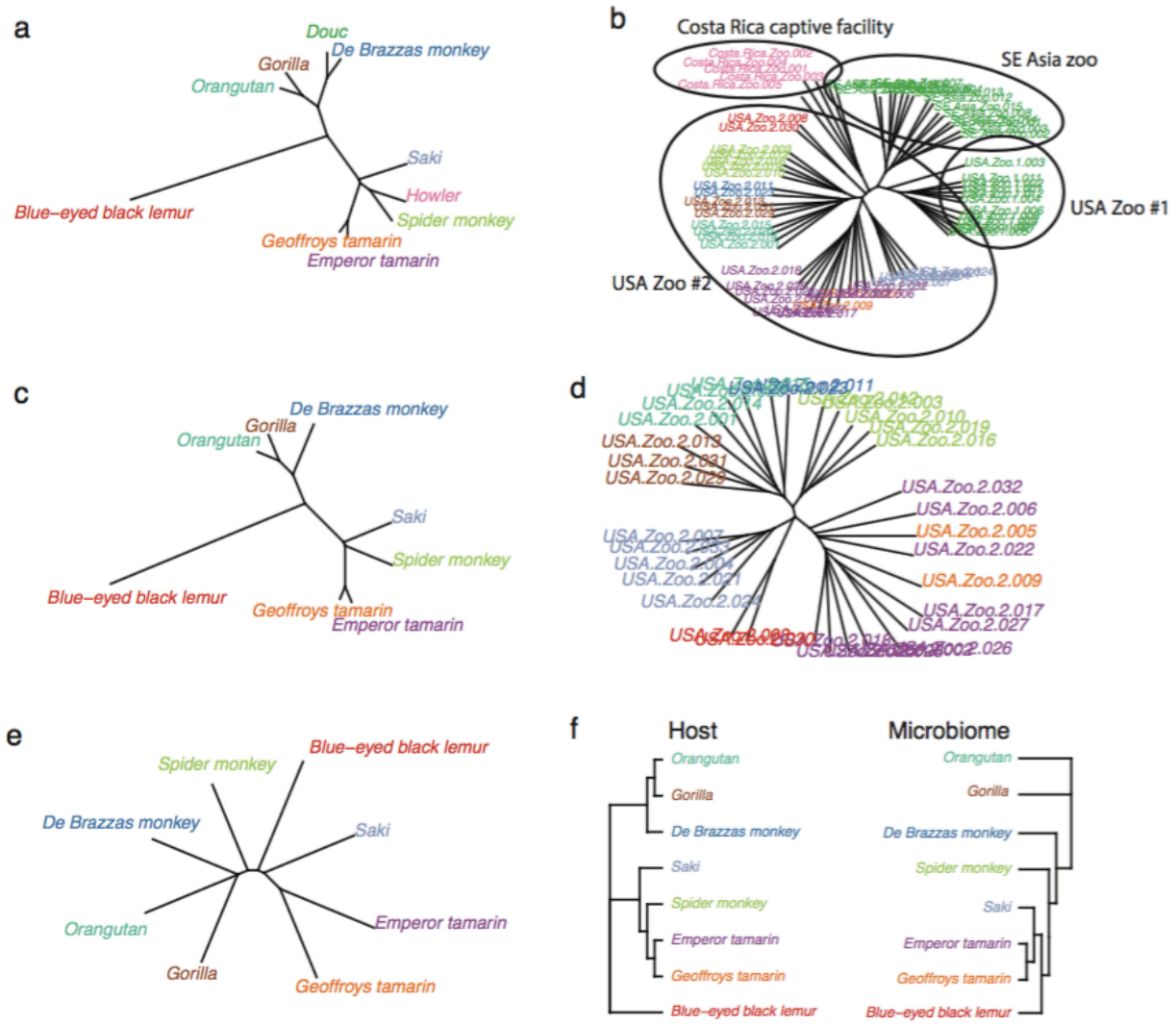

Figure S7. ***Association of microbiome and host phylogeny.*** (a) host phylogeny from Perelman *et al.* (22) for the 10 unique primate species sampled in this study. (b) microbiome phylogeny of all captive, semi-captive, and wild individuals according to unweighted UniFrac distance, using the Nei-Saitou neighbor-joining method (23). This shows major clustering by zoological institution location, and minor nested clustering by host species. (c) Host phylogeny for subset of species present in USA Zoo #2. (d) Microbiome phylogeny as in (b) for samples from USA Zoo #2. This shows some concordance with (c) but concordance is hard to assess due to multiple individuals sampled per species. (e) Microbiome phylogeny as in (d) but depicting a phylogeny built from the average distances between species, averaging over individuals, for comparison with (c). (f) Direct comparison of 8-species host phylogeny (c) with 8-species microbiome-based phylogeny (e), showing significant concordance between the two phylogenies ($p < 0.0001$, permutation test of cophenetic distance (23) when permuting species labels).

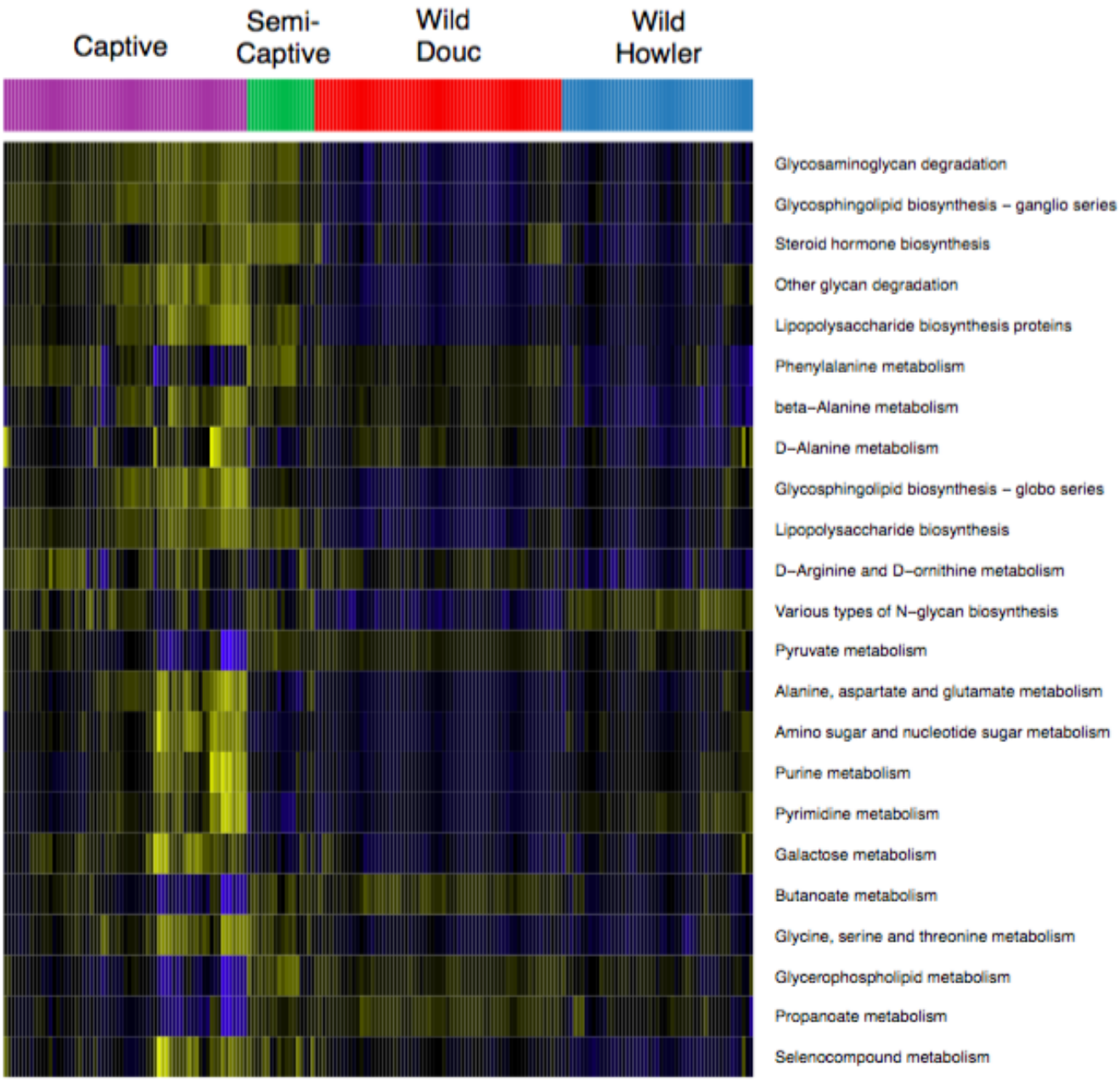

Figure S8. ***Heatmap of KEGG level 2 metabolic pathways that discriminate the four major populations.*** Only pathways with random forests feature importance > 0.01 are shown. Wild NHPs (notably doucs) possessed higher relative abundances of a variety of pathways, including pyruvate metabolism, butanoate metabolism, glycerophospholipid metabolism, and propanoate metabolism (random forests feature importance score > 0.01), consistent with increased plant fiber degradation.

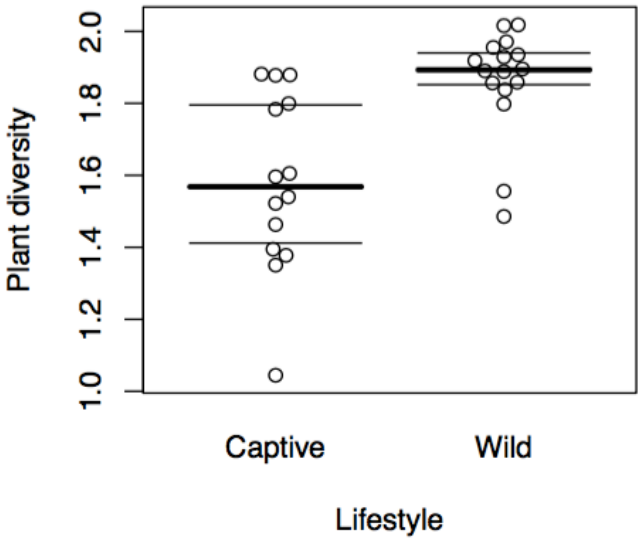

Figure S9. ***Dietary plant diversity and the non-human primate microbiome.*** Estimated dietary plant diversity (Shannon index) in captive and wild doucs and howlers based on whole-genome shotgun data aligning at 97% identity to known plant genomes. These data include 14 captive individuals (9 douc, 5 howler) and 16 wild individuals (8 douc, 8 howler). Wild individuals have higher dietary plant diversity based on plant DNA in their stool (Mann-Whitney U test, $p < 0.001$), despite the fact that whole genome reference databases only contain a small number of cultivated plant genomes.

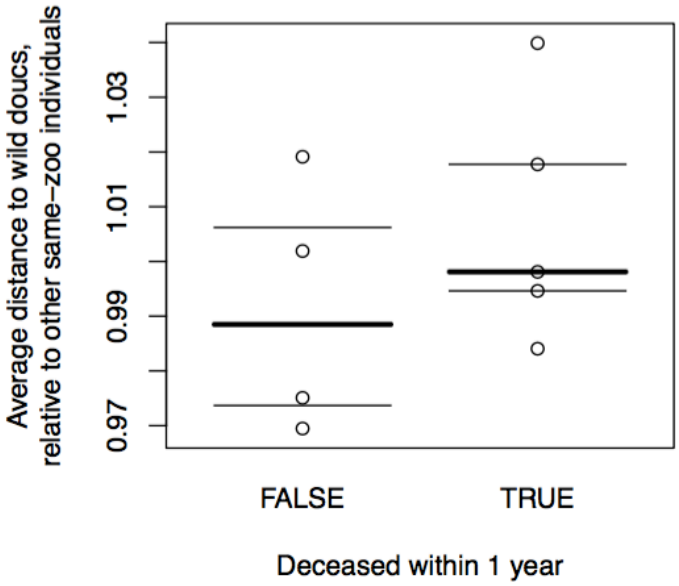

Figure S10. ***Severity of captive douc dysbiosis and risk of mortality.*** Beeswarm plot with median and upper/lower quartiles of average unweighted UniFrac distance from each captive douc to all wild douc individuals, stratified by survival status at 1 year post-sampling. Distances are normalized to the mean within each captive population in order to adjust for microbiome variation associated with sampling location. Individuals who died tended to have microbiomes more divergent from the wild douc microbiome than their captive counterparts within the same zoo, but there were only 5 deceased and 4 living individuals and the trend was not significant (t-test, $p = 0.35$).

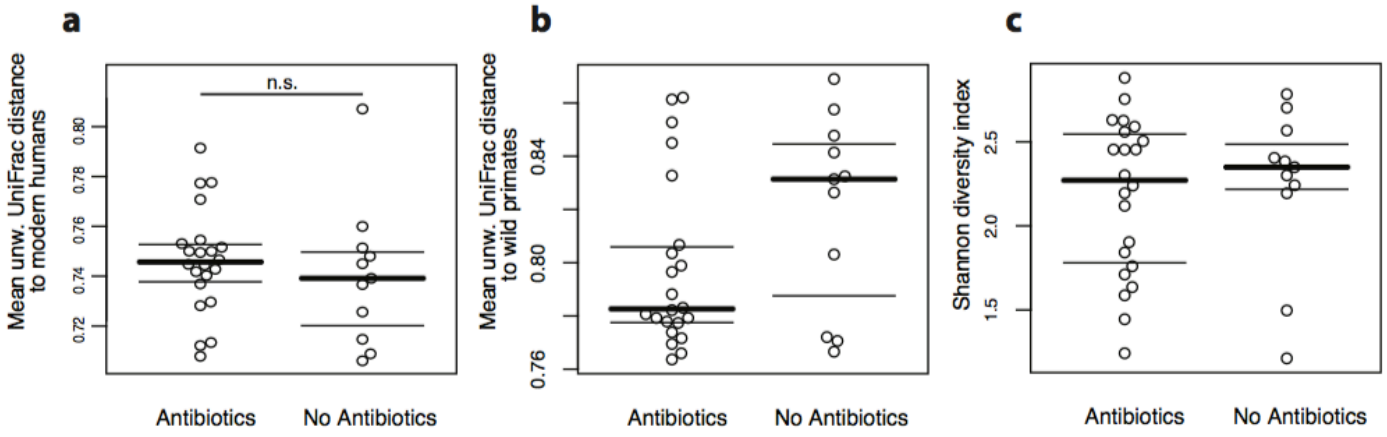

Figure S11. ***Antibiotic exposure and captive primate microbiome dysbiosis.*** (a) Beeswarm plot of mean ecological similarity of individual captive primates to all modern humans, stratified by antibiotic exposure (USA zoo #2 samples only). This shows that antibiotic exposure in captive primates was not associated with having a more human-like microbiome. 11 individual animals sampled from the second USA zoo had never taken antibiotics, while the remaining 22 individuals had received an average of 4.6 +/- 4.0 courses throughout life. (b) as in (a) but showing unweighted UniFrac distance of the individuals in the left panel to wild primates. (c) Shannon diversity of 33 captive individuals in the USA Zoo #2, 11 of whom had never had antibiotics. There is no statistical difference between the two groups, indicating that lifetime exposure to antibiotics is not related to captive primate microbiome diversity. These results demonstrate that lifetime exposure to antibiotics is not likely to be causing captive primate microbiome diversity.

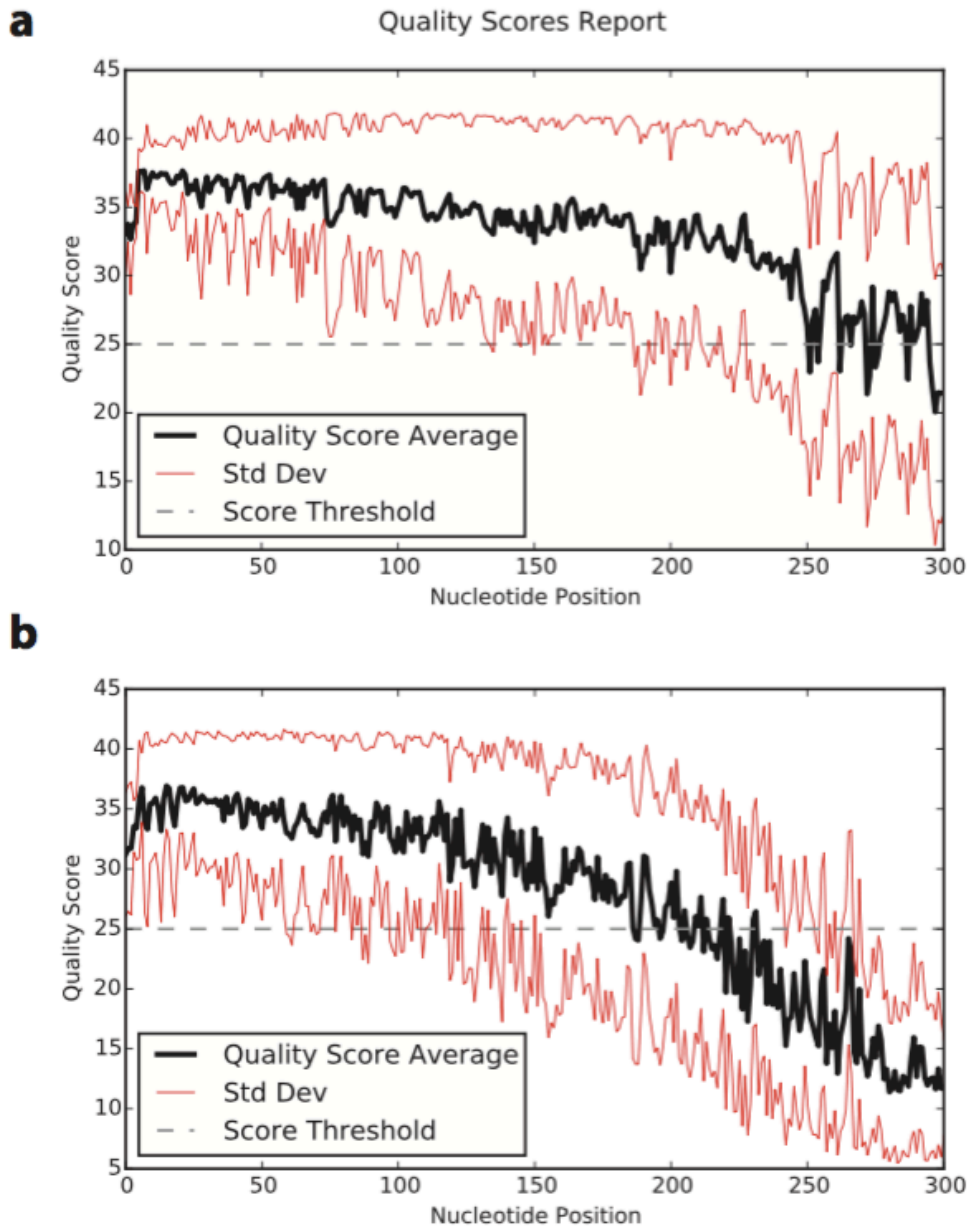

Figure S12. ***Sequencing quality scores in forward and reverse reads.*** Forward-read quality scores (a) and Reverse-read quality scores (b) plotted against nucleotide position. Forward-read score lower standard deviation drops consistently below q=25 at approximately 185 bases; reverse-read score lower standard deviation drops consistently below q=25 at approximately 100 bases.

Supplemental Tables

Supplemental Table 1. ***Nutrient content from dry matter aggregate dietary material for each population.***

Diet component	Wild	EPRC	Southeast Asia Zoo[1]	USA Zoo
Crude Protein (%)	9.46	16.52	13.37	16.7
Crude Fat (%)	*	3.23	3.12	3.71
Soluble Sugars (%)	2.7	2.28	*	7.9
Acid detergent Fiber (%)	46.76[2]	23.2[3]	23.07	8.65
Neutral detergent Fiber (%)	53.67[2]	35.6[3]	31.97	12.64
Calcium (%)	0.49	1.05	0.22	0.72
Potassium (%)	0.96	0.76	0.21	0.29
Sodium (%)	0.01	0.01	0.24	0.27
Zinc (mg/kg)	19.4	10.16	8.25	26.3
Iron (mg/kg)	26.5	33.73	20.74	64.33

[1]Southeast Asian zoo diet also included a vitamin and mineral supplement which was not included in the analysis.
[2,3]Values marked with ([2]) are from Ulibarri (2013) and those marked with ([3]) are from Otto (2005) as NDF and ADF were not available from the laboratory analyses for these diets. Data from Ulibarri (2013) were weighted according to feeding season to represent the proportion of plants part selected.
*Not detected.

Supplemental Table 2. ***Primate species and associated lifestyles included in this study.***

NHP species common name	NHP species scientific name	Lifestyle	Provenance
Red-shanked douc	*Pygathrix nemaeus*	Wild	Son Tra Nature Reserve (Da Nang, Vietnam)
		Semi-captive	Endangered Primate Rescue Center (Ninh Binh, Vietnam)
		Captive	Singapore Zoo (Singapore, Singapore)
		Captive	Philadelphia Zoo (Philadelphia, PA, USA)
Mantled howling monkey	*Alouatta palliata*	Wild	Hacienda La Pacifica (Guanacaste, Costa Rica)
		Captive	Las Pumas Rescue Center (Guanacaste, Costa Rica)
Western lowland gorilla	*Gorilla gorilla gorilla*	Captive	Como Zoo (Saint Paul, MN, USA)
Sumatran orangutan	*Pongo abelii*	Captive	Como Zoo (Saint Paul, MN, USA)
De Brazza's monkey	*Cercopithecus neglectus*	Captive	Como Zoo (Saint Paul, MN, USA)
Black-handed spider monkey	*Ateles geoffroyi*	Captive	Como Zoo (Saint Paul, MN, USA)
White-faced saki	*Pithecia pithecia*	Captive	Como Zoo (Saint Paul, MN, USA)
Blue-eyed black lemur	*Eulemur macaco flavifrons*	Captive	Como Zoo (Saint Paul, MN, USA)
Emperor tamarin	*Saguinus imperator subgrisescens*	Captive	Como Zoo (Saint Paul, MN, USA)
Geoffroy's tamarin	*Saguinus geoffroyi*	Captive	Como Zoo (Saint Paul, MN, USA)

References

1. Moeller AH, et al. (2014) Rapid changes in the gut microbiome during human evolution. *Proc Natl Acad Sci U S A* 111(46):16431–16435.
2. Tung J, Rudolph J, Altmann J, Alberts SC (2007) Parallel effects of genetic variation in ACE activity in baboons and humans. *Am J Phys Anthropol* 134(1):1–8.
3. Yatsunenko T, et al. (2012) Human gut microbiome viewed across age and geography. *Nature* 486(7402):222–227.
4. Kleiman DG, Thompson KV, Baer CK (2010) *Wild Mammals in Captivity: Principles and Techniques for Zoo Management, Second Edition* (University of Chicago Press).
5. Kaumanns W, Hampe K, Schwitzer C, Stahl D *Primate nutrition: towards an integrated approach* (Filander Verlag, Furth, The Netherlands).
6. Leaf-Eater Primate Diet - Biscuit # 5M02 - 25 lb *Mazuri Shopp Cart*. Available at: http://www.mazuri.com/mazurileaf-eaterprimatediet-1-1.aspx [Accessed May 31, 2016].
7. Glander KE (1981) Feeding patterns in mantled howling monkeys. Available at: http://dukespace.lib.duke.edu/dspace/handle/10161/7083 [Accessed May 31, 2016].
8. Ehrlich PR, Raven PH (1964) Butterflies and Plants: A Study in Coevolution. *Evolution* 18(4):586–608.
9. Feeny PP (1968) Effect of oak leaf tannins on larval growth of the winter moth Operophtera brumata. *J Insect Physiol* 14(6):805–817.
10. Janzen DH (1969) Seed-Eaters Versus Seed Size, Number, Toxicity and Dispersal. *Evolution* 23(1):1–27.
11. Rhoades DF, Cates RG (1976) Toward a General Theory of Plant Antiherbivore Chemistry. *Biochemical Interaction Between Plants and Insects*, Recent Advances in Phytochemistry., eds Wallace JW, Mansell RL (Springer US), pp 168–213.
12. Rosenthal AZ, Matson EG, Eldar A, Leadbetter JR (2011) RNA-seq reveals cooperative metabolic interactions between two termite-gut spirochete species in co-culture. *ISME J* 5(7):1133–42.
13. Consumption of cyanogenic bamboo by a newly discovered species of bamboo lemur - Glander - 2005 - American Journal of Primatology - Wiley Online Library Available at: http://onlinelibrary.wiley.com/doi/10.1002/ajp.1350190205/abstract [Accessed May 31, 2016].
14. Wu GD, et al. (2011) Linking Long-Term Dietary Patterns with Gut Microbial Enterotypes. *Science* 105(2011). doi:10.1126/science.1208344.
15. Hold GL (2014) Western lifestyle: a “master” manipulator of the intestinal microbiota? *Gut* 63(1):5–6.
16. Hawrelak JA, Myers SP (2004) The causes of intestinal dysbiosis: a review. *Altern Med Rev J Clin Ther* 9(2):180–197.
17. Martinez-Medina M, et al. (2014) Western diet induces dysbiosis with increased E coli in CEABAC10 mice, alters host barrier function favouring AIEC colonisation. *Gut* 63(1):116–124.
18. Cordain L, et al. (2005) Origins and evolution of the Western diet: health implications for the 21st century. *Am J Clin Nutr* 81(2):341–354.
19. Lippold LK, Thanh VN (2008) The Time is Now: Survival of the Douc Langurs of Son Tra, Vietnam. *Primate Conserv* 23(1):75–79.
20. Glander KE *Habitat and resource utilization: An ecological view of social organization in mantled howling monkeys*.
21. Glander K, Nisbett R (1996) Community structure and species density in tropical dry forest associations at Hacienda La Pacífica in Guanacaste province, Costa Rica.
22. Perelman P, et al. (2011) A molecular phylogeny of living primates. *PLoS Genet* 7(3):e1001342.
23. Paradis E, Claude J, Strimmer K (2004) APE: Analyses of Phylogenetics and Evolution in R language. *Bioinforma Oxf Engl* 20(2):289–290.