Figure S1. Graphic illustration of the experimental design.

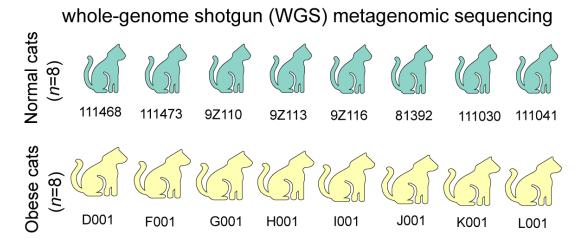
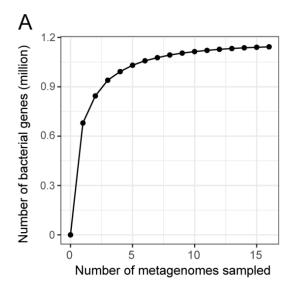
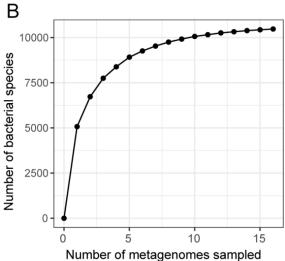


Figure S2. Rarefaction analyses to assess species and gene richness from the results of sampling.

- (A) Rarefaction curve based on bacterial gene profiles of 16 samples.
- (B) Rarefaction curve based on taxonomy profiles at the species level of 16 samples.





## Figure S3. Principal Coordinates Analysis (PCoA) plots of beta diversity between rectum microbiota of cats of different sex or age using Bray-Curtis distance.

- (A) The Principal Coordinates Analysis (PCoA) plot of Bray-Curtis distance of relative abundance of microbial species between male cats and female cats. Statistical significance was assessed using permutational multivariate analysis of variance (PERMANOVA).
- (**B**) The Principal Coordinates Analysis (PCoA) plot of Bray-Curtis distance of relative abundance of microbial species between cats at 4 months to 8 months and cats at 6 years. Statistical significance was assessed using permutational multivariate analysis of variance (PERMANOVA).

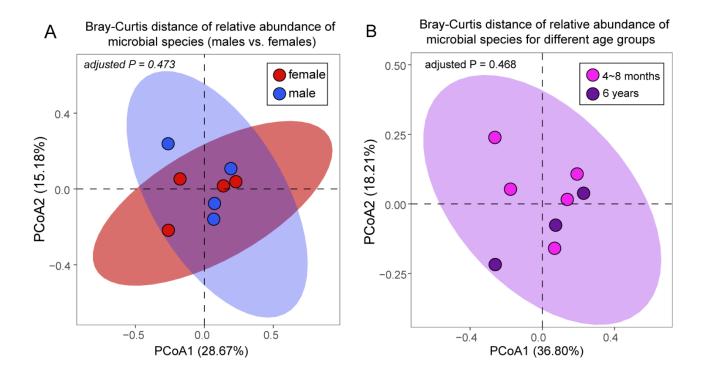
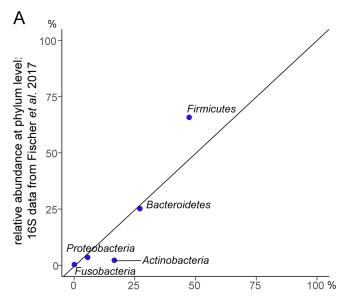
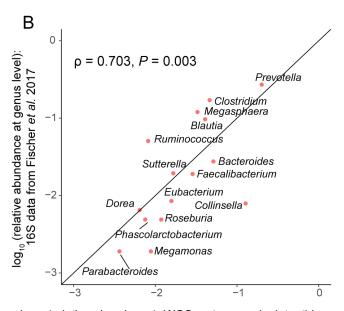


Figure S4. Bacterial relative abundance correlations between WGS metagenomic data in this research and 16S rDNA ampliconic sequencing data in Fisher *et al.* 2017 at phylum and genus levels.

- (A) Scatterplot of relative frequency of the top 5 most abundant phyla from cat intestinal WGS metagenomic data in this research and the 16S ampliconic sequencing data in Fisher *et al.* 2017.
- (**B**) Scatterplot of relative frequency at log<sub>10</sub> scale of the 15 most abundant genera from normal cat intestinal WGS metagenomic data in this research and lean cat 16S ampliconic sequencing data in Fisher *et al.* 2017.



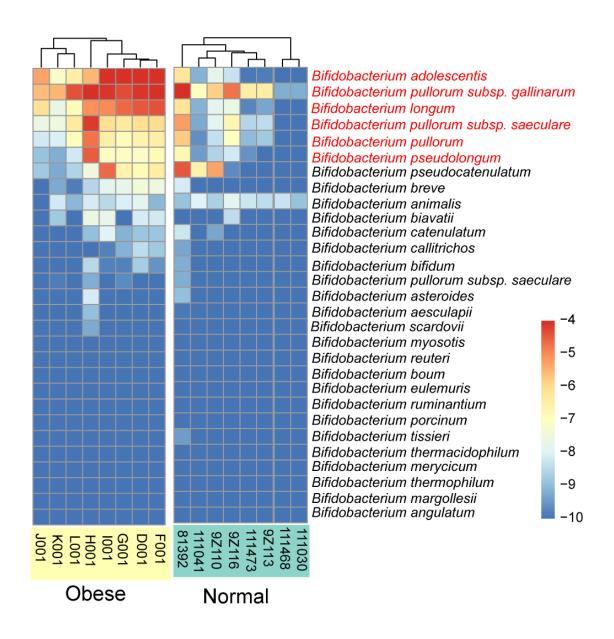
relative abundance: WGS metagenomic data, this research



log<sub>10</sub> (relative abundance): WGS metagenomic data, this research

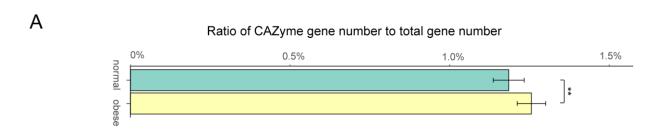
Figure S5. Heatmap of species abundance from the *Bifidobacterium* genus in normal vs. obese cat rectum microbiome.

Heatmap of relative frequency at  $\log_2$  scale for all *Bifidobacterium* species detected in the cat rectum microbiome. Six significantly overrepresented species in the obese gut microbiome ( $\log_2FC>1.5$ , q<0.10), including *B. adolescentis*, *B. longum*, *B. pseudolongum*, *B. pullorum*, *B. pullorum subsp. Gallinarum*, and *B. pullorum subsp. Saeculare*, were denoted in red.



## Figure S6. Numbers of CAZyme (Carbohydrate-Active Enzymes) genes identified in normal and obese cat gut microbiomes.

- (A) Percentage of CAZyme genes identified in normal (*green*) and obese (*yellow*) cat gut microbiomes.
- (**B**) Barplot of numbers of CAZyme encoding genes in top 20 most abundant genera in normal (*green*) and obese (*yellow*) cat gut microbiomes.



B Top genera containing the highest amount of CAZyme genes in normal and obese cat gut microbiome

