## Online Appendix

### Microbiome and Microbial-derived Product Analyses

Following amplification, purification and sequencing of the V4 hypervariable region of the 16S rRNA gene (1), data were analysed using R Statistical Programming language. OTU counts were read in using Phyloseq and were agglomerated at the taxonomic levels of Family, Phylum, Genus and Species. To compare relative abundances, we used the ALDEx R package that estimates the technical variation inherent in high-throughput sequencing by Monte-Carlo sampling from a Dirichlet distribution (2). Specifically, Anova-like t-tests were performed with 250 Monte Carlo samples used, and Benjamin Hochberg adjustment of p values. To infer abundance of functional genes, Picrust2 was used to predict functional profiles based on the abundances of 16S marker genes. The default pipeline was used. Moreover, when there was a high number of statistically significant functions (ECs, KOs or pathways), they were summarized at the level of GO terms. KO to GO term mapping was obtained through LinkDB, metacyc pathway and EC to GOterm mapping was done using Metacyc smart tables (3).

### RNA-sequencing Analyses

RNA sequencing data was pseudo-aligned to transcripts of the GRCm38.p6 genome assembly using Kallisto (4). All data filtering and quality control were performed using R 3.5.0 and the packages edgeR and limma (5-7). Data were log-transformed and filtered to exclude genes that had zero reads in more than 1 sample. PCA and density plots were used to assess data quality before and after filtering. Batches resulting from sort date were accounted for and corrected in the linear model (8, 9). Heat maps were generated using the pheatmap package with row scaling centred around mean gene expression to enable optimal insight into individual gene expression variation. Each sample gene expression colour is scaled within the row (each gene individually)

as defined by the equation: (raw value - mean)/standard deviation. Gene set enrichments were generated using the limma's camera function and GO terms mapped to mouse Entrez Gene IDs (10-14). These were then formatted into a generic table for network analysis in Cytoscape's EnrichmentMap (15-17). We employed the default EnrichmentMap parameters of a 0.1 FDR q-value node cutoff and a combined Overlap + Jaccard metric that seeks to optimize connectivity and minimize false overlap in the computation of network edges. AutoAnnotate was used to create clusters and labels with GLay community clustering and biggest four words based on a word cloud drawn from the gene sets in each community. Communities with less than 3 nodes were excluded to ensure high confidence data presentation.

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## Supplemental Figure Legends

**Supplemental Figure 1.** (**A**) Body weight gain (n=102), (**B**) visceral (n=6) and (**C**) subcutaneous (n=6) fat mass, as well as (**D**) 24-hr energy intake pattern (n=6), in C57Bl/6J mice fed either RC or WD for 16-wk. 4-Hr fasting (**E**) blood glucose, and plasma (**F**) insulin, (**G**) glucagon, (**H**) GIP and (**I**) GLP-1, measured at 6 time points throughout the light-dark cycle in RC- and WD-mice (n=20). (**J**) Individual responses of 4-hr fasted RC- and WD-fed mice to identical insulin tolerance tests throughout the 24-hr day, and (**K**) the resultant 24-hr area-under-the-curve (AUC) pattern (n=6). * $p<0.05$, ** $p<0.01$, *** $p<0.001$ (conducted using Student's t-test).

**Supplemental Figure 2.** Individual responses (**A**) and the corresponding delta area-under the curve (ΔAUC, (**B**)) in GLP-1 following OGTTs in 4-hr fasted RC- and WD-fed Gcg-Venus mice at ZT2 and ZT14 (n=12). * $p<0.05$, ** $p<0.01$, *** $p<0.001$ (conducted using Student's t-test for panel A).

**Supplemental Figure 3.** (**A**) Principle component analysis, (**B**) species diversity and top 10 (**C**) genera and (**D**) species in colonic feces from RC- and WD-fed mice at ZT2 and ZT14 (n=12).

**Supplemental Figure 4.** (**A**) Principle component analysis, (**B**) species diversity and (**C**) top 10 genera in colonic feces from AIMD RC- and WD-fed mice at ZT2 and ZT14 (n=8).

**Supplemental Figure 5**. 4-Hr fasting (**A**) plasma insulin and (**B**) blood glucose, as well as (**C**) individual responses in insulin, and blood glucose, and the corresponding ΔAUC responses of

(**D**) plasma insulin and (**E**) blood glucose following identical OGTTs conducted throughout the 24-hr day in germ-free mice (n=8).

**Supplemental Figure 6**. 4-Hr fasting (**A**) plasma insulin and (**B**) blood glucose, as well as (**C)** individual responses in insulin, and blood glucose, and the corresponding ΔAUC responses of (**D**) plasma insulin and (**E**) blood glucose following identical OGTTs at ZT2 and ZT14 in germ-free animals following RC-fecal microbiome transplantation (n=8). ). * $p<0.05$, ** $p<0.01$ (conducted using Student's t-test).