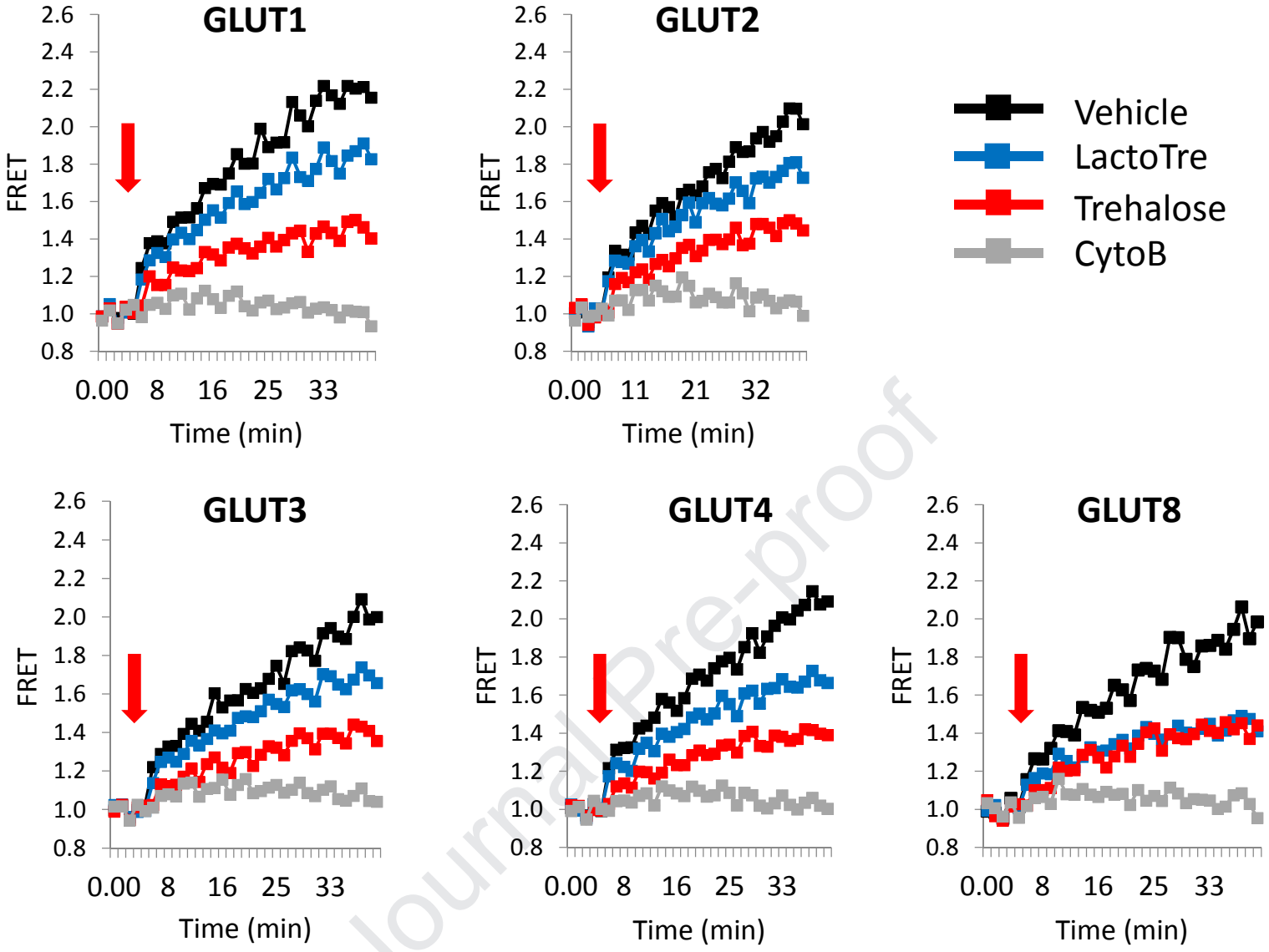
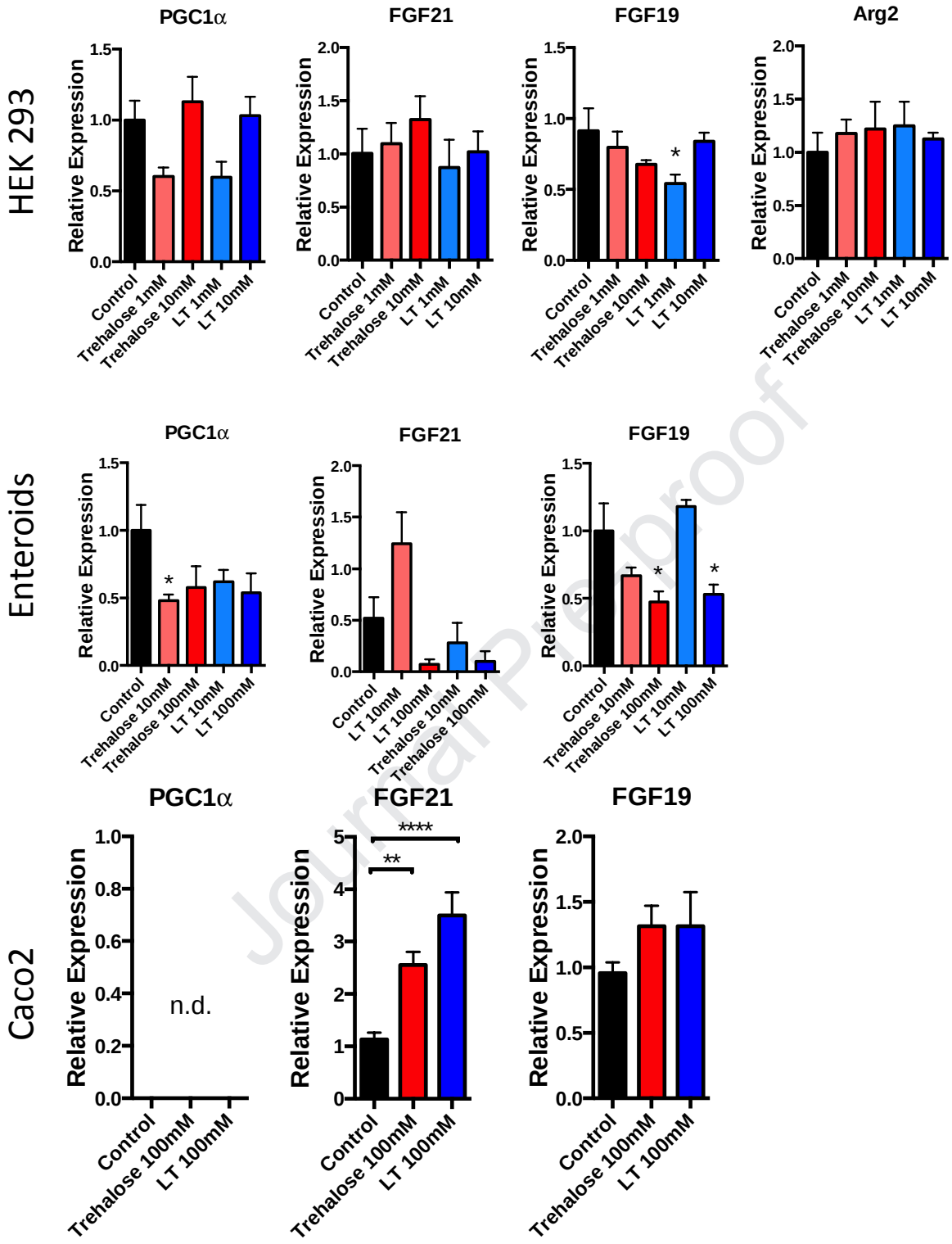
Supplemental Figure 1



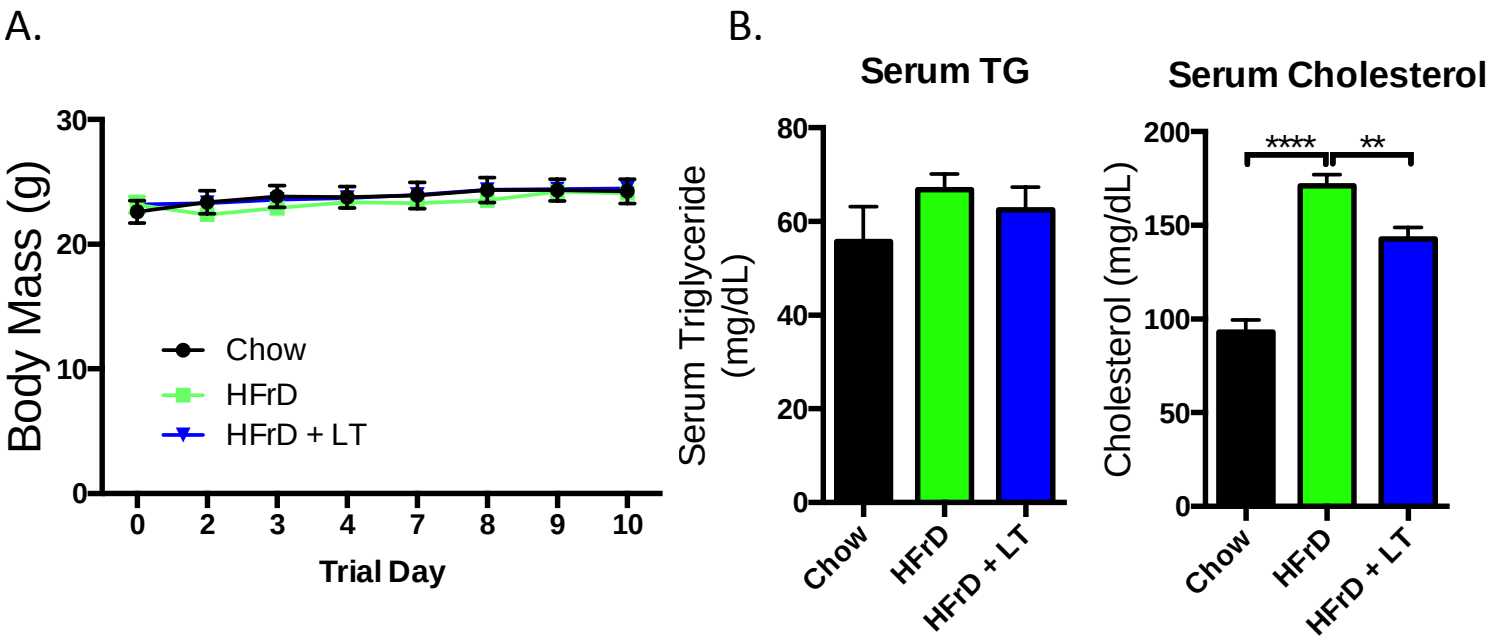
Supplemental Figure 1. LT inhibits GLUT-mediated glucose uptake. FRET analysis demonstrating effects of trehalose and LT on glucose uptake in 293T cells overexpressing each of the class I GLUTs, or GLUT8. 200 µM Cytochalasin B was administered prior to the addition of glucose in the group labled, "CytoB". Red arrow: glucose added into culture media. Values are depicted as cyan fluorescence:yellow fluorescence ratio (e.g. FRET donor:FRET acceptor).

Supplemental Figure 2



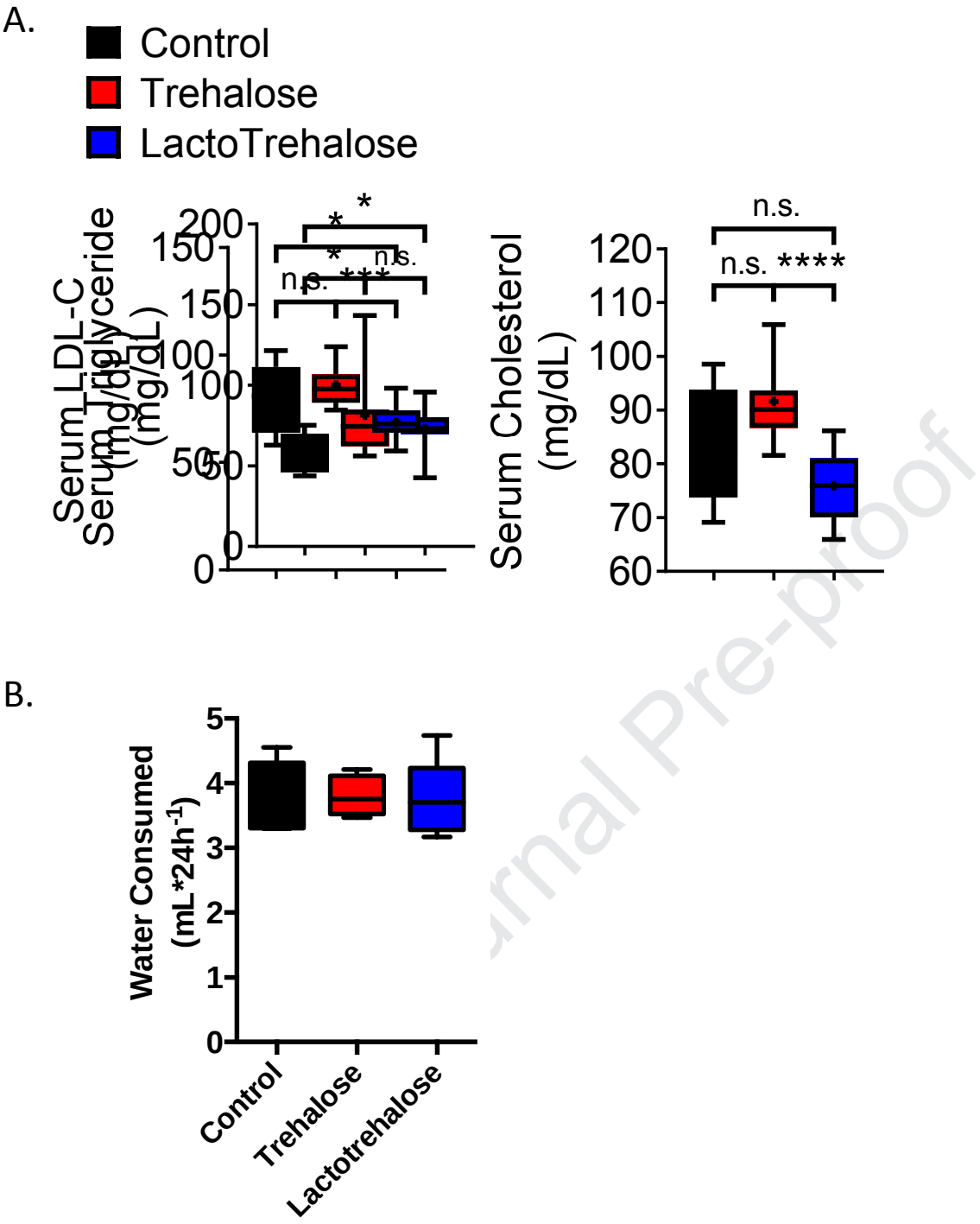
Supplemental Figure 2. Fasting pathway gene expression in extrahepatic cell types. (Top) qRT-PCR probing fasting-response genes in human embryonic kidney (HEK293) cells treated with 1-10mM trehalose or LT. (Middle). qRT-PCR probing fasting-response genes PGC1α and FGF21, or the post-prandial hormone FGF19 in human small bowel enteroids treated with 10-100mM trehalose or LT. (Bottom) qRT-PCR probing fasting-response genes PGC1α and FGF21, or the post-prandial hormone FGF19 in Caco2 colonic epithelial cultures treated with 10-100mM trehalose or LT. n.d., transcript not detected. *, **, ****, $P < 0.05$, 0.01, or 0.0001 versus comparison group by two-tailed t-test with Bonferroni-Dunn post hoc correction for multiple comparisons.

Supplemental Figure 3



Supplemental Figure 3. LT reduces serum cholesterol in HFrD-fed mice. A. Body weight over time in mice fed chow or HFrD with or without concomitant LT treatment (3% ad libitum in drinking water, initiated 2 days prior to HFrD feeding). B. Serum TG and cholesterol in mice fed chow or HFrD with or without concomitant LT treatment (3% ad libitum in drinking water, initiated 2 days prior to HFrD feeding). **, ****, $P < 0.01$, or < 0.0001 versus comparison group by two-tailed t-test with Bonferroni-Dunn post hoc correction for multiple comparisons.

Supplemental Figure 4



Supplemental Figure 4. LT reduces LDL-C in healthy, chow-fed mice without altering water consumption. A. Serum LDL-C and total cholesterol analysis in mice treated with or without trehalose or LT (3% in water ad libitum, 5 days). B. Water consumption per mouse per 24h as determined by indirect calorimeter water bottle weight change per unit time. *, ***, ****, $P < 0.05$, 0.001, or 0.0001 versus comparison group by two-tailed t-test with Bonferroni-Dunn post hoc correction for multiple comparisons. In box-whisker plots: middle bar represents the dataset median; boxes represent 25%ile and 75%ile lines; whiskers represent maximum and minimum values in the dataset.

Supplemental Figure 5

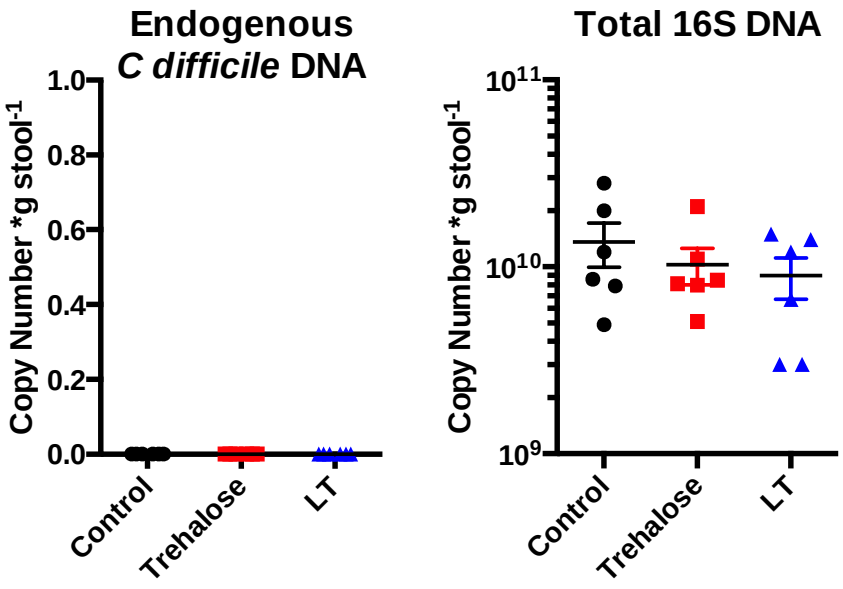
Genus

DAME

variable	Cont	LactoTre	Tre	P
Chao1	28.75 (1.164)	27.617 (0.757)	28.833 (0.477)	0.44993
Observed	28.25 (0.854)	27.5 (0.764)	28.667 (0.494)	0.46757
Shannon	1.51 (0.095)	1.903 (0.03)	1.808 (0.036)	0.00051
Simpson	0.639 (0.041)	0.798 (0.006)	0.765 (0.014)	0.00041

Supplemental Figure 5. Rapid shifts in microbial diversity after trehalose or LT feeding in vivo. Shown are genus-level diversity indices in stool bacterial microbiota of mice fed water, trehalose, or LT (3% in water, fed ad libitum, 5 days).

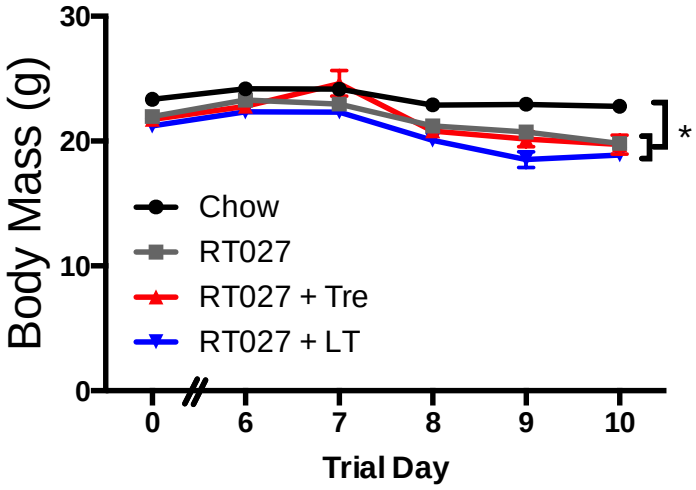
Supplemental Figure 6



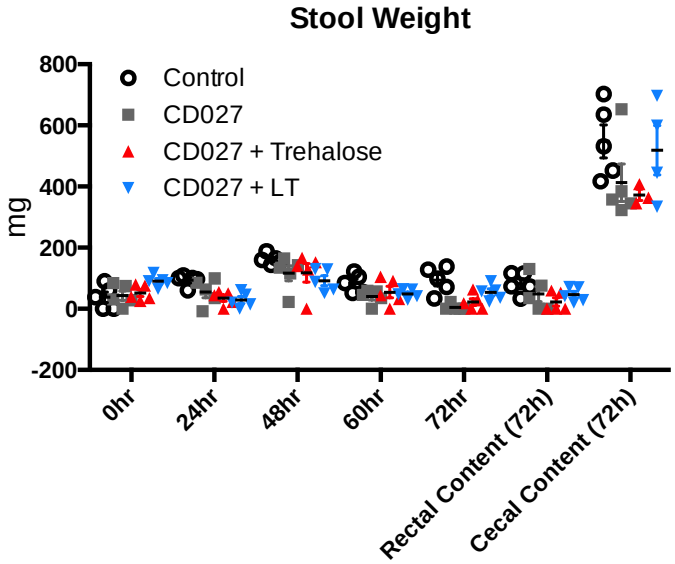
Supplemental Figure 6. Long-term trehalose and LT feeding do not alter indigenous CD populations. Shown is qRT-PCR quantification of CD-specific DNA and of total 16S ribosomal DNA in stool from mice fed trehalose or LT (3% in drinking water, *ad libitum*).

Supplemental Figure 7

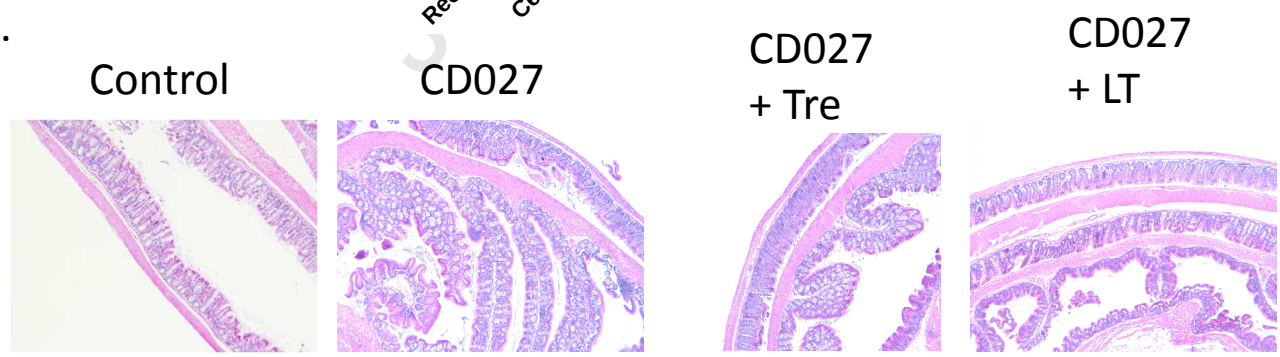
A.



B.

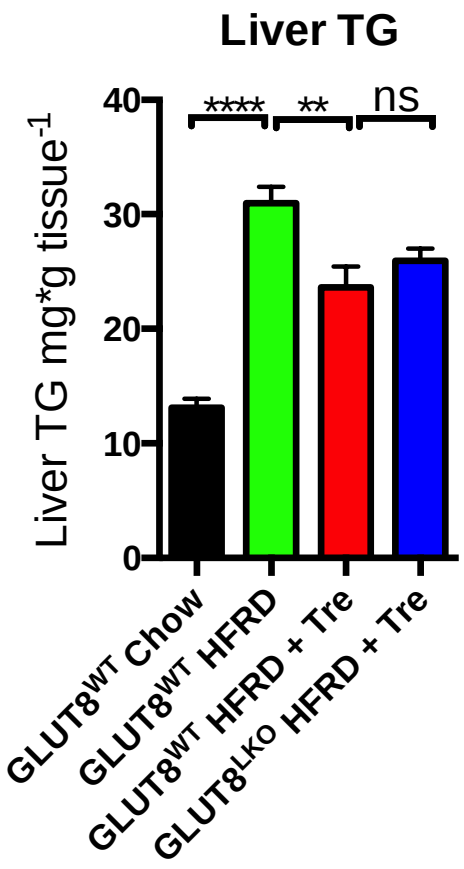


C.



Supplemental Figure 7. **Trehalose and LT do not affect body weight or rectal histology after CD027 infection**. A. Body weight in mice before and after CD027 infection in the presence or absence of prior trehalose or LT treatment (3% in water, *ad libitum* administered 2d prior to infection). B. Stool, rectal content and cecal content mass in mice before and after CD027 infection. Stool was collected at regular intervals and weighed. Cecal and rectal contents were retrieved at sacrifice and weighed. No significant differences were detected for any group at any timepoint (one-way ANOVA). C. Colonic tissue analysis by H&E staining in colons obtained from mice 72 h after CD027 infection in the presence or absence of prior trehalose or LT treatment (3% in water, *ad libitum* administered 2d prior to infection).

Supplemental Figure 8



Supplemental Figure 8. No additional therapeutic effect of trehalose treatment (3% in water, *ad libitum* administered 2d prior to infection) on HFrD-induced liver TG accumulation. Wild-type littermates were randomized and fed native trehalose 2 days prior to HFrD initiation. 10 days after HFrD initiation, liver chloroform:MeOH extracts were analysed in each group for quantitative TG content by enzymatic colorimetric assay.

Supplemental Figure 9

Murine Tissue cDNA Primers	Forward (5' - 3')	Reverse (5' - 3')
Acc1	TGT CCG CAC TGA CTG TAA CCA	TGC TCC GCA CAG ATT CTT CA
Aloxe3	ATG GCA GTA TAT CGG CTG TGT	GCT TCT GCT TAG GGC TTT CAC
Arg2	AGG AGT GGA ATA TGG TCC AGC	AGG GAT CAT CTT GTG GGA CAT T
Atf4	AGC AAA ACA AGA CAG CAG CC	ACT CTC TTC TTC CCC CTT GC
Fgf21	CTG CTG GGG GTC TAC CAA G	CTG CGC CTA CCA CTG TTC C
Grp78	GAA AGG ATG GTT AAT GAT GCT GAG	GTC TTC AAT GTC CGC ATC CTG
Il-1β	GCA ACT GTT CCT GAA CTC AAC T	ATC TTT TGG GGT CCG TCA ACT
Il-6	CTG CAA GAG ACT TCC ATC CAG	AGT GGT ATA GAC AGG TCT GTT GG
Kc	ACC GAA GTC ATA GCC ACA CTC AAG	ACT TGG GGA CAC CTT TTA GCA TC
Pgc1α	ACA CCG CAA TTC TCC CTT GT	CGG CGC TCT TCA ATT GCT TT
S100a8	AAA TCA CCA TGC CCT CTA CAA G	CCC ACT TTT ATC ACC ATC GCA A
S100a9	ATA CTC TAG GAA GGA AGG ACA CC	TCC ATG ATG TCA TTT ATG AGG GC
Tnfα	CAG GCG GTG CCT ATG TCT C	CGA TCA CCC CGA AGT TCA GTA G
Ucp1	ACA CCG CAA TTC TCC CTT GT	CGG CGC TCT TCA ATT GCT TT
C. difficile DNA Primers	Forward (5' - 3')	Reverse (5' - 3')
16S	TTG AGC GAT TTA CTT CGG TAA AGA	CCA TCC TGT ACT GGC TCA CCT
treA	TAC GCT GAT GGT CCT CGT AT	CGC CTC CTT TAT AAT CTG TTT TC

Supplemental Figure 9. Primers used in qRT-PCR analysis.

SUPPLEMENTAL METHODS:

Cell Line Experiments

HEK293 and Caco2 cells were purchased directly from the American Type Culture Center (ATCC), and subcultured precisely per ATCC protocol as we reported[4, 13]. Cells were grown in 6-well plates in regular growth media to 80% confluence prior to treatment with or without trehalose or LT (1-100mM, 24h). RNA was isolated using Trizol reagent prior to qRT-PCR, described below.

Human Enteroids

De-identified intestinal tissue was obtained with a waiver of parental consent with approval from the Washington University in St. Louis Institutional Review Board (IRB# 201804040). The de-identified small bowel sample was obtained from a term neonate with a diagnosis of intestinal obstruction requiring exploratory laparotomy and small bowel resection. The tissue sample was digested in 2 mg*ml^{-1} collagenase I (Invitrogen) for 10 min at 37 ℃ followed by mechanical dissociation. The resulting crypts were filtered through a 70 µm cell strainer and washed in DMEM supplemented with 10% FBS. The crypts were centrifuged at 2000 rpm for 5 minutes, resuspended in growth factor-reduced Matrigel (Becton Dickinson) and seeded into culture plates. Organoids were grown in 50% L-WRN conditioned medium supplemented with 10µM of Y-27632 and SB 202190 (R&D Systems).

Metabolic Cages

Indirect calorimetry experiments were performed in an 8-module indirect calorimeter (TSE Systems) as described previously[12, 14, 15, 33-35]. Mice were acclimatized for 6 hours prior to data collection, and were provided *ad libitum* access to food and water with or without 3% trehalose or LT. Mice were maintained on a 12 hr light-dark cycle within a specific pathogen-free facility.

GC-MS

GC-MS was performed as previously reported with minor modifications[4]. Derivatized samples were analyzed on an Agilent 7890A gas chromatograph interfaced to an Agilent 5975C mass spectrometer. The GC column used for the study was a HP-5MS (30 m, 0.25mm internal diameter, 0.25um film coating P.J. Cobert St. Louis, MO). A linear temperature gradient was used. The initial temperature of 80° was held for 2 minute and increased to 300 at 10°/minute. The temperature was held at 300° for 2 minutes. The samples were run by electron ionization (EI) and the source temperature, electron energy and emission current were 200°C, 70eV and

300uA, respectively. The injector and transfer line temperatures were 250°C. $^{13}C_{12}$ trehalose (Omicron Biochemical, South Bend IN) was used as the internal standard. Samples were run in the SIM mode monitoring 361 and 367 m/z.

RNAseq

RNAseq was performed by the Washington University Genome Technology Access Center (GTAC) as described[14, 15]. Briefly, library preparation was performed with 10ug of total RNA with a Bioanalyzer RIN score greater than 8.0. Ribosomal RNA was removed by poly-A selection using Oligo-dT beads (mRNA Direct kit, Life Technologies). mRNA was fragmented prior to the RT reaction using SuperScript III RT enzyme (Life Technologies, per manufacturer's instructions) and random hexamers. A second strand reaction was performed to yield ds-cDNA. cDNA was blunt ended, had an A base added to the 3' ends, and then had Illumina-ligated fragments were amplified using primers incorporating unique index tags. Fragments were sequenced on an Illumina HiSeq-3000 using single reads extending 50 bases. Reads were aligned to the Ensembl release 76 top-level assembly with STAR version 2.0.4b. Gene counts were derived from the number of uniquely aligned unambiguous reads by htseq-count version version 0.6.1. Genes with reads greater than 1 read per 2 million aligned reads (0.5 rpm), in at least 75% of the samples in any treatment group, were accepted for further analysis. Counts from these 13768 genes were imported into the R/Bioconductor package edgeR and TMM normalization size factors were calculated to adjust for samples for differences in library size. The TMM size factors and the matrix of counts were then imported into R/Bioconductor package Limma and weighted likelihoods based on the observed mean-variance relationship of every gene and sample were then calculated for all samples with the *Voom With Quality Weights* function. Generalized linear models were then created to test for gene differential expression. Functional grouping of genes with FDR < 0.05 in any pairwise comparison was achieved using the R/Bioconductor packages GAGE and Pathview. These packages assess functional similarity of input gene lists by assignment to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.

Microbial analysis was performed precisely as in prior reports[68]**.** Briefly, Bacterial DNA was isolated from cecal contents using the QIAamp Fast DNA stool mini kit (Qiagen) including a bead-beating step (Precellys bead-beating homogenizer). Fifty ng of genomic DNA was utilized for amplification of the V4 variable region of the 16S rRNA gene using 515 F/806 R primers. Forward and reverse primers were dual-indexed to accommodate multiplexing of up

to 384 samples per run. Paired-end sequencing (2 × 250 bp) of pooled amplicons was carried out on an Illumina MiSeq with ~30% PhiX DNA.

Processing and quality filtering of reads was performed by using scripts in QIIME (v1.9.1) and other in-house scripts. Paired reads were stitched with PEAR and assembled reads were further filtered based on Phred quality scores and for chimeric reads using USEARCH61. Filtered reads (mean counts per sample = 37,971) were demultiplexed within QIIME and samples with less than 5000 reads were excluded from further analysis. UCLUST was used to cluster sequences into operational taxonomical units (OTUs based on >97% identity). OTU picking was performed using open-reference method, which encompasses clustering of reads against a reference sequence collection and also performs *de novo* OTU picking on the reads that fail to align to any known reference sequence in the database. To eliminate erroneous mislabeling, the resulting OTU tables were checked for mislabeling sequences. Representative sequences were further aligned using PyNAST with the Greengenes core-set alignment template. Construction of the phylogenetic tree was performed using the default (FASTTREE) method in QIIME. Further analysis of ecological diversity measures and group differences in bacterial taxonomic abundance is described in the statistical analyses section. PICRUSt was used to identify differences in predictive metagenome function. OTUs were normalized by the predicted 16S copy number, and functions were predicted with the use of GreenGenes 13_5 database for KEGG Orthologs. Linear Discriminant Analysis (LDA) Effect Size (LEfSe) was used to assess differences in microbial communities. For LEfSe analysis the α-value for the factorial Kruskal-Wallis test among classes was set at 0.05 and for the pairwise Wilcoxon test between subclasses at 0.05.