

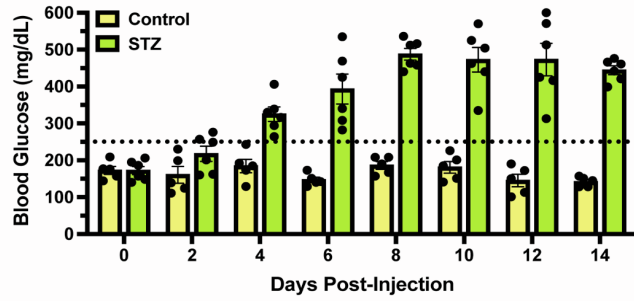
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Supplemental information

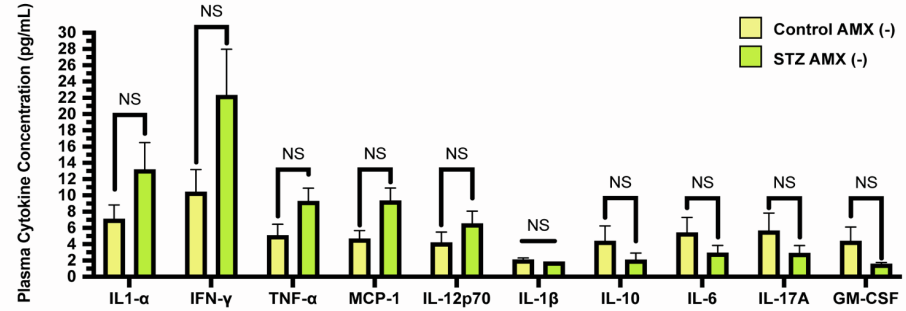
**Streptozotocin-induced hyperglycemia alters
the cecal metabolome and exacerbates
antibiotic-induced dysbiosis**

Jenna I. Wurster, Rachel L. Peterson, Claire E. Brown, Swathi Penumutchu, Douglas V. Guzior, Kerri Neugebauer, William H. Sano, Manu M. Sebastian, Robert A. Quinn, and Peter Belenky

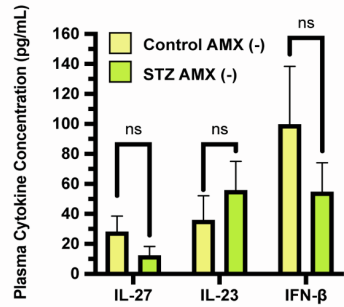
A Fasting Blood Glucose



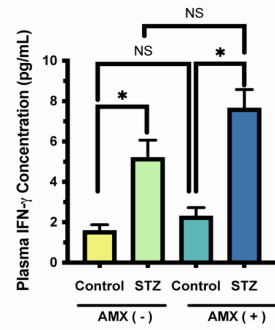
B Inflammatory Cytokines



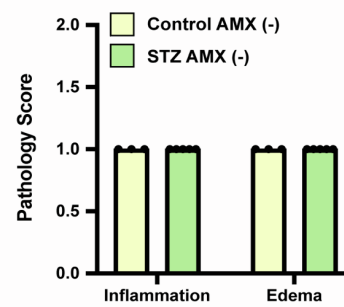
C Inflammatory Cytokines



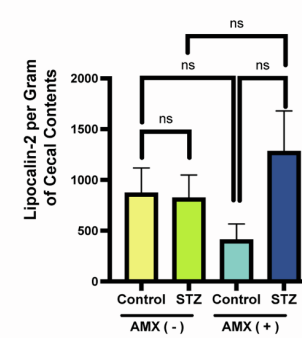
D Inflammatory Cytokines



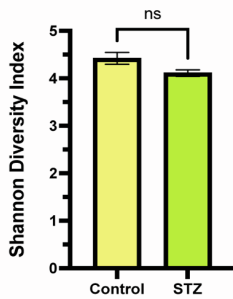
E GI Histopathology



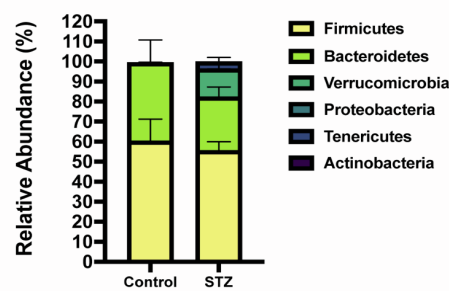
F Cecal Lipocalin-2



G 16S Alpha Diversity



H 16S Taxonomy (Phylum)



I 16S Taxonomy (Phylum)

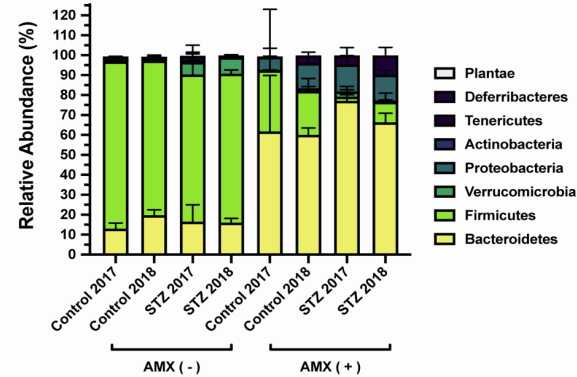


Figure S1. The impact of streptozotocin treatment on host physiology and microbiome composition without additional. Related to Figure 1.

- A. Fasting blood glucose of individual mice before STZ injection (Day 0) and on 2 days intervals for up to 14 days post-injection. The Day 14 time-point is representative of the final day of experiments described in Figure 7.
- B. Plasma cytokine concentrations in STZ-treated and control mice 3 days post-injection. Data represent averaged concentrations \pm SEM for cytokines whose concentration falls between 0 and 23 pg/mL.
- C. Plasma cytokine concentrations in STZ-treated and control mice 3 days post-injection. Data represent averaged concentrations \pm SEM for cytokines whose concentration falls between 12 and 160 pg/mL.
- D. Plasma concentration of IFN- γ in STZ-treated and control mice +/- AMX 4 days after STZ injection.
- E. Pathological assessment of fixed, H&E-stained colon sections 3 days after STZ injection.
- F. Cecal lipocalin-2 concentrations. Data represent average concentrations \pm SEM.
- G. Alpha diversity as measured by the Shannon diversity index for STZ-treated and control animals 3 days post-injection. Data represent average \pm SEM.
- H. Phylum-level taxonomic composition of the cecal microbiome 3 days post STZ-injection. Data represent average abundance + SEM.
- I. Phylum-level taxonomic composition of the cecal microbiome in STZ and control mice +/- AMX treatment. Data represent average abundance + SEM.

For A: N = 5 or 6 per group

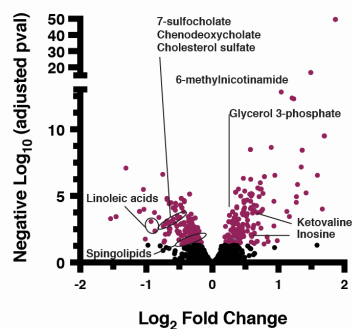
For B & C: N = 4 per group; *, P < 0.05; unpaired T-test with Welch's correction

For D & F: N = 4 or 5 per group; *, P < 0.05; Welch's ANOVA with Dunnett T3 test for multiple hypothesis testing

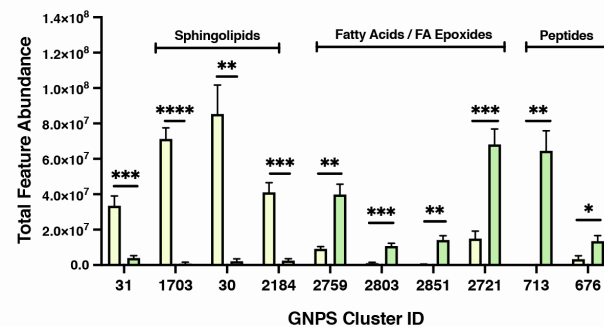
For E: N = 4 to 6 per group. Inflammation (0: absent, 1: minimal, 2: mild affecting mucosa and sub-mucosa, 3: moderate affecting mucosa, 4: severe). Edema (0: < 10%, 1: 10-25%, 2: 25%-50%, 3: 50%-75%, 4: over 75%).

For G -I: N = 3 to 5 per group; ; *, P < 0.05; unpaired T-test with Welch's correction

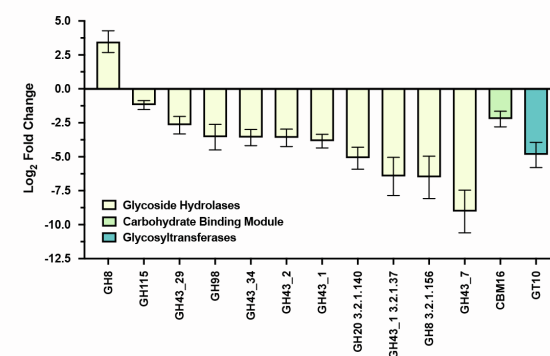
A Q-TOF-MS Feature Abundance



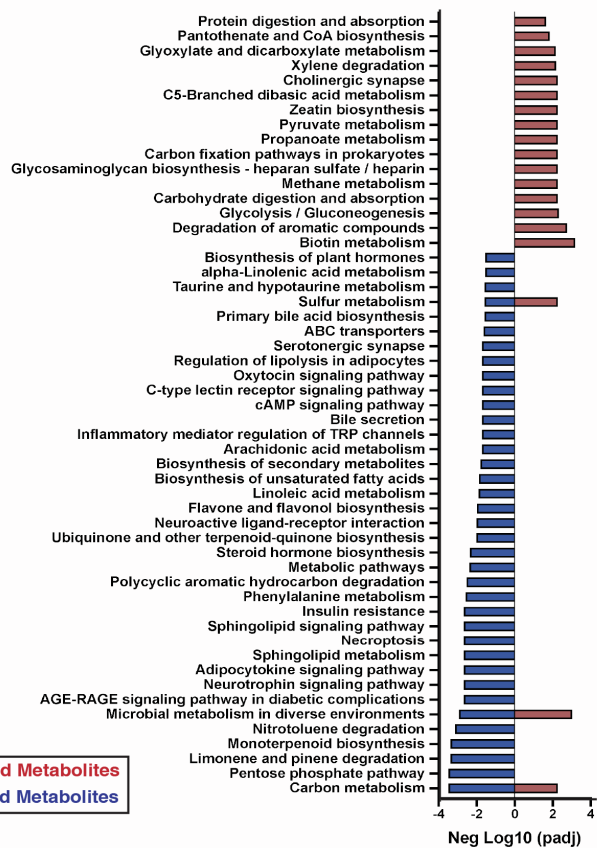
C LC-MS/MS Annotated Features



D CAZyme Transcript Abundance



B Metabolite Pathway Enrichment



E Bacteroides thetaioaomicron Gene Expression

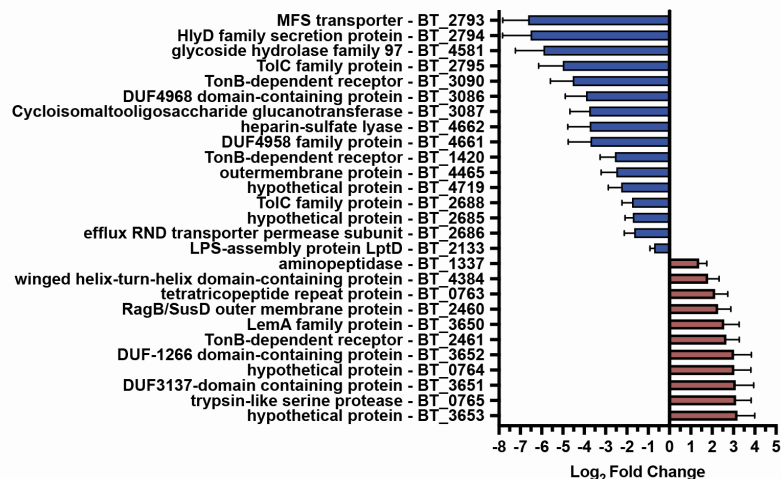


Figure S2. STZ-induced hyperglycemia modifies both the cecal metabolome and metatranscriptome. Related to Figure 2.

- A. Volcano plot of the cecal metabolome in STZ-treated mice relative to normoglycemic controls. Purple points represent differentially abundant metabolite features. Metabolites of interest are labeled. See Table S1 for full results (N = 6 per group, 2 technical replicates per mouse)
- B. KEGG pathway enrichment of differentially abundant Q-TOF-MS metabolites in STZ-treated mice compared to controls. Colors indicate whether the metabolites contributing to pathway scoring were enriched (red) or depleted (blue) in STZ-treated animals compared to controls. See Table S3 for full results.
- C. Differentially abundant GNPS-annotated clusters that contain known metabolites within the cluster. Clusters were selected from the top-50 most relevant features via Random Forest Testing. Comparison is between STZ-treated mice and controls. See Table S2 for full results.
- D. Differentially abundant CAZyme transcripts in STZ-treated mice. Data represent log₂ fold change relative to controls \pm SEM. See Table S4 for full results.
- E. Differentially abundant *B. thetaiotaomicron* transcripts after STZ treatment. Data represent log₂ fold change versus controls \pm SEM See Table S5 for full results.

For A – C: N = 6 per group, 2 technical replicates per sample

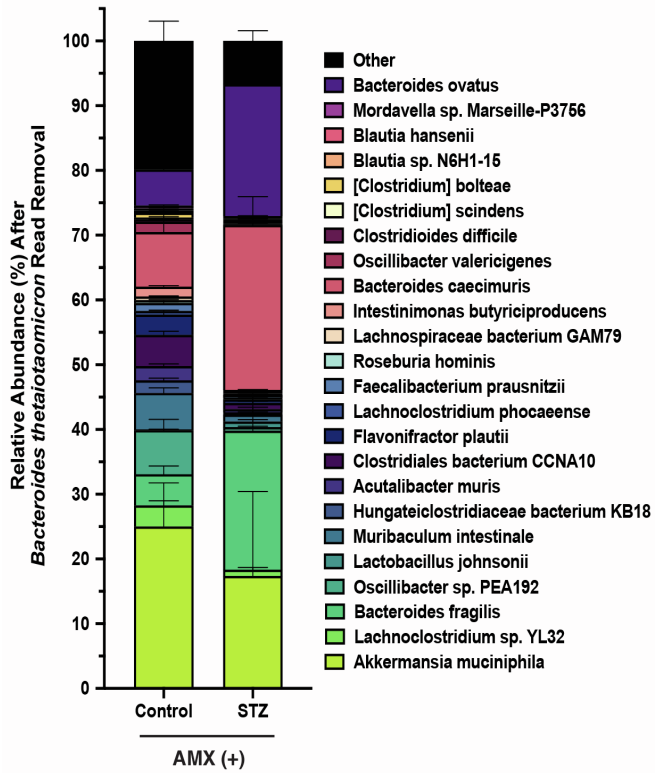
For D & E : N = 4 per group

For A, D, & E: Differentially abundant = Benjamini-Hochberg adjusted p value < 0.05

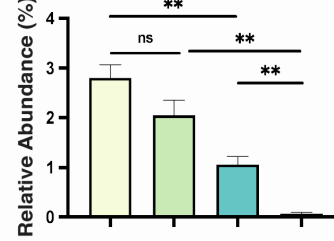
For B: Significance = unpaired T-test p value < 0.05

For C: *, P < 0.05; **, P < 0.01; ***, P < 0.001, **** P < 0.0001; unpaired T-test with Welch's correction

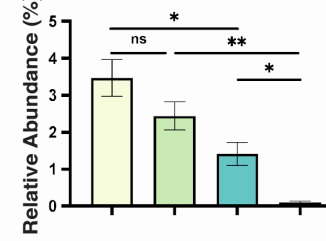
A Taxonomic Abundance (Species)



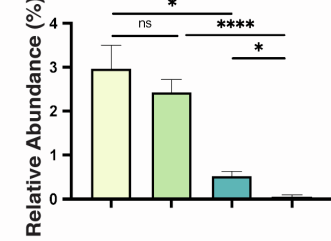
B *Clostridiales* bacterium CCNA10



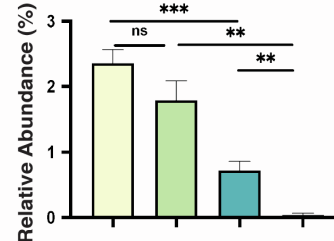
C *Muribaculum intestinale*



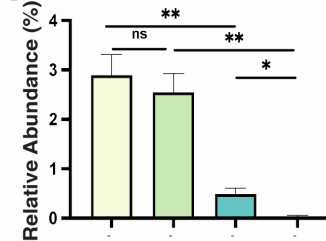
D *Acutalibacter muris*



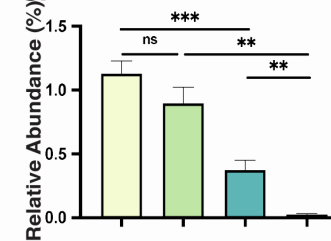
E *Flavonifractor plautii*



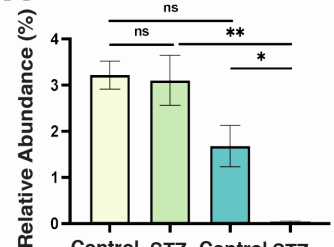
F *Hungateiclostridiaceae* KB18



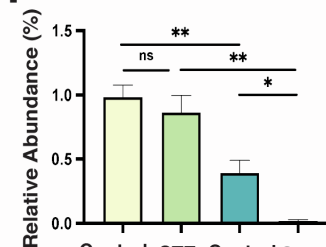
G *Intestinimonas butyriciproducens*



H *Oscillibacter* sp. PEA192



I *Oscillibacter valericigenes*



J *Akkermansia muciniphila*

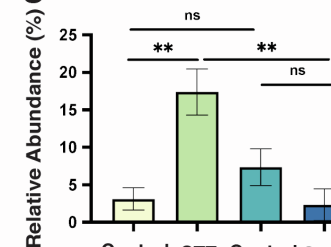


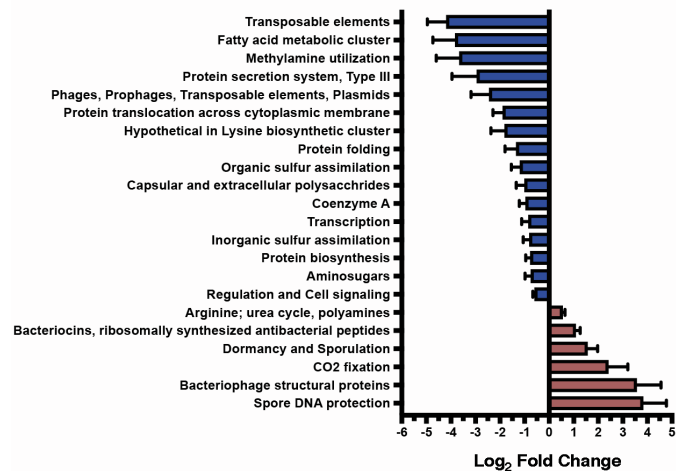
Figure S3. Streptozotocin impacts taxonomic composition after Amoxicillin treatment. Related to Figure 3.

- A. Average relative abundance of species from A after the removal of reads assigned to *B. thetaiotaomicron*. Data are represented as mean \pm SEM for each species
- B. Average relative abundance of reads assigned to *Clostridiales* bacterium CCNA10.
- C. Average relative abundance of reads assigned to *Muribaculum intestinale*.
- D. Average relative abundance of reads assigned to *Acutalibacter muris*.
- E. Average relative abundance of reads assigned to *Flavonifractor plautii*.
- F. Average relative abundance of reads assigned to *Hungateiclostrideaceae* bacterium KB18.
- G. Average relative abundance of reads assigned to *Intestinimonas butyriciproducens*.
- H. Average relative abundance of reads assigned to *Oscillibacter* species PEA192.
- I. Average relative abundance of reads assigned to *Oscillibacter valericigenes*
- J. Average relative abundance of reads assigned to *Akkermansia muciniphila*.

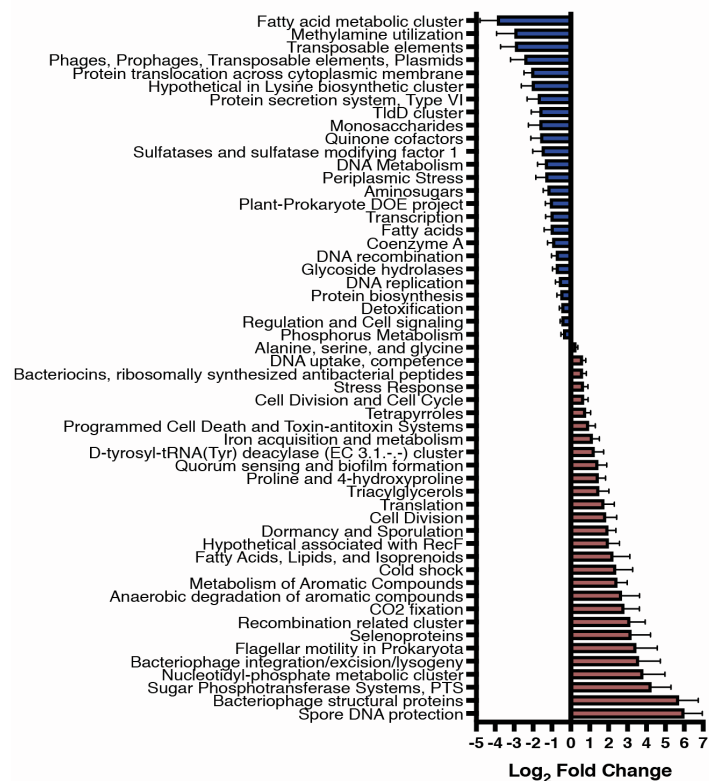
For all panels: N = 5 to 8 per group

For panels B-J, (*, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001, Welch's ANOVA with Dunnett T3 test for multiple hypothesis testing).

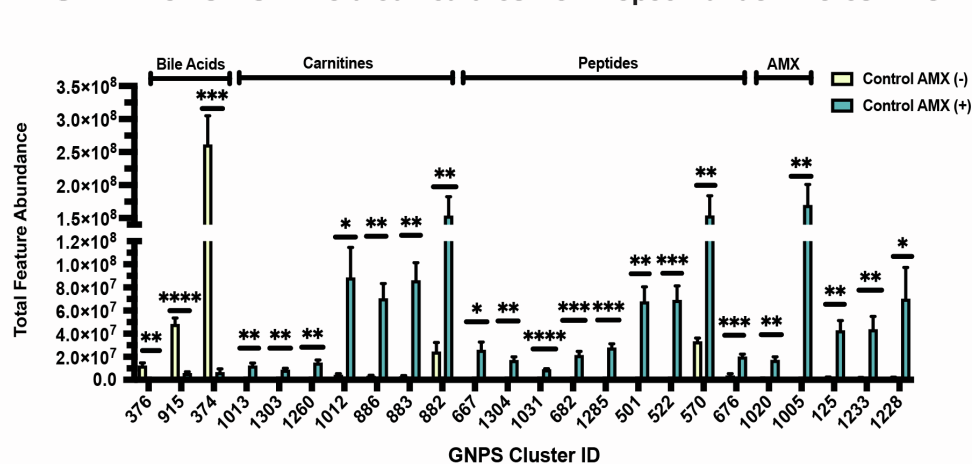
A SEED Transcript Abundance after Amoxicillin (Control)



B SEED Transcript Abundance after Amoxicillin (STZ)



C LCMS/MS Annotated Features from Top50 Random Forest Hits



D LCMS/MS Annotated Features from Top50 Random Forest Hits

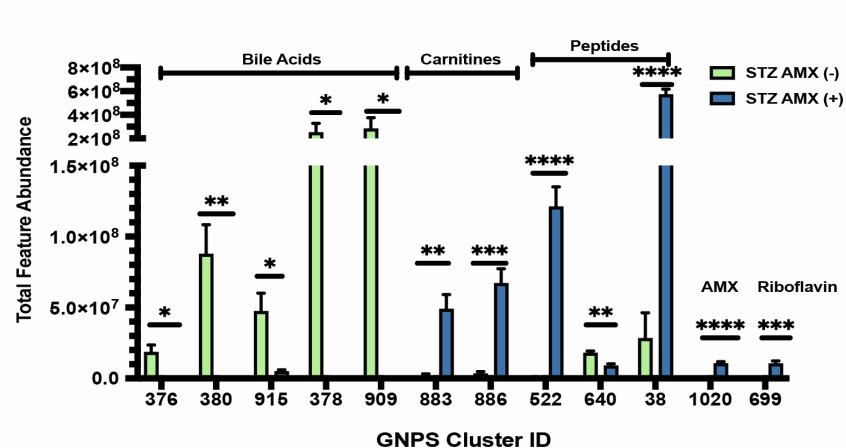


Figure S4: Streptozotocin modifies the metatranscriptomic and metabolomic responses of the gut microbiome to amoxicillin. Related to Figures 4 & 5.

- A. Differentially abundant level 2 SEED Subsystem transcripts in normoglycemic control mice after AMX treatment. Data represent \log_2 fold change relative to vehicle controls \pm SEM. See Table S7 for full results.
- B. Differentially abundant level 2 SEED Subsystem transcripts in STZ-treated mice after AMX treatment. Data represent \log_2 fold change relative to vehicle controls \pm SEM. See Table S7 for full results.
- C. Differentially abundant GNPS-annotated clusters that contain known metabolites within the cluster. Clusters were selected from the top-50 most relevant features via Random Forest Testing. Comparison is between AMX-treated mice and vehicle-treated mice for normoglycemic controls. See Table S2 for full results.
- D. Differentially abundant GNPS-annotated clusters that contain known metabolites within the cluster. Clusters were selected from the top-50 most relevant features via Random Forest Testing. Comparison is between AMX-treated mice and vehicle-treated mice for STZ-treated mice. See Table S2 for full results.

For A & B: N = 4 per group; Differentially abundant = Benjamini-Hochberg adjusted p value < 0.05

For C & D: N = 6 per group, 2 technical replicates per sample; (*, P < 0.05; **, P < 0.01; ***, P < 0.001, **** P < 0.0001); unpaired T-test with Welch's correction

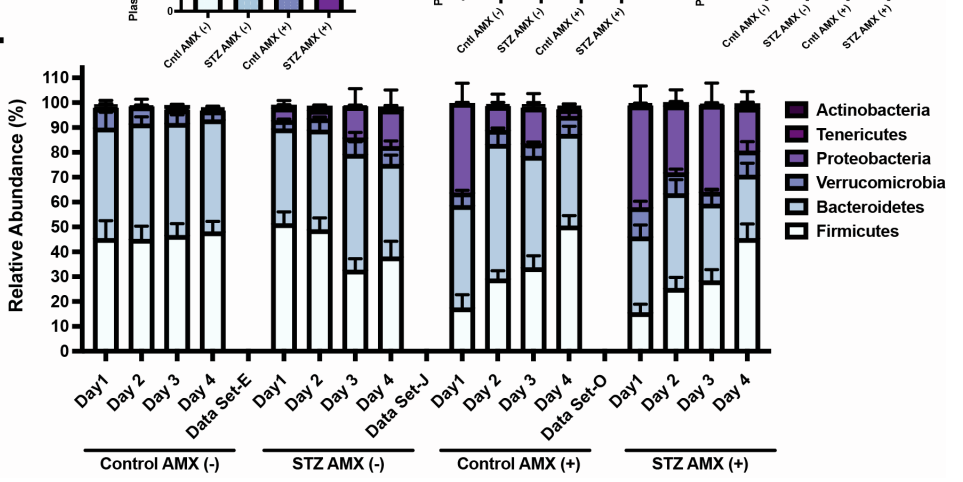
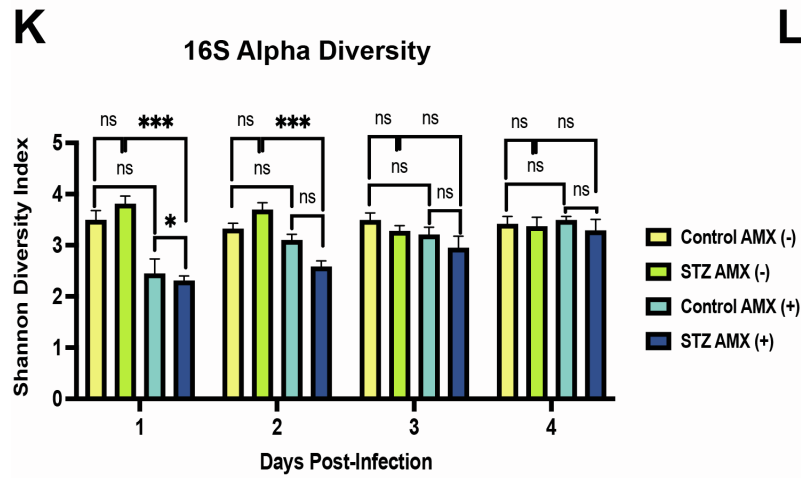
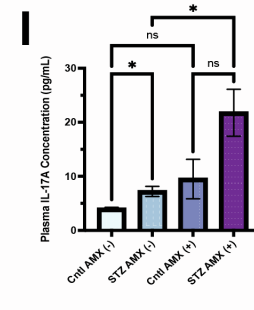
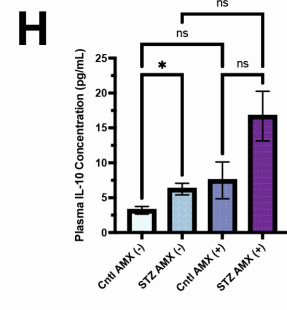
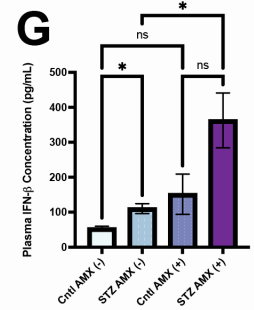
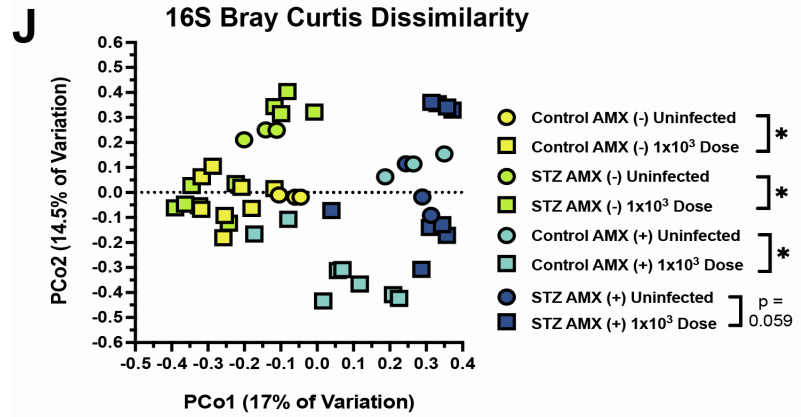
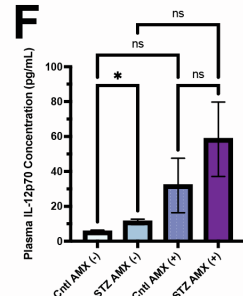
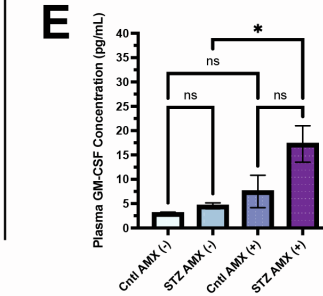
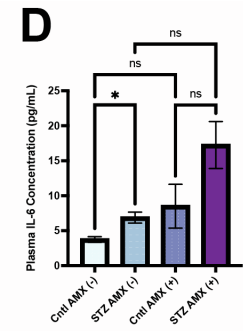
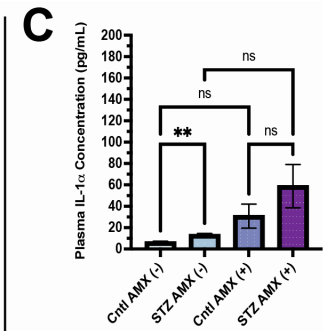
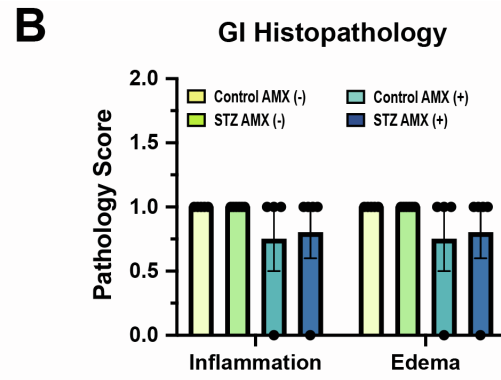
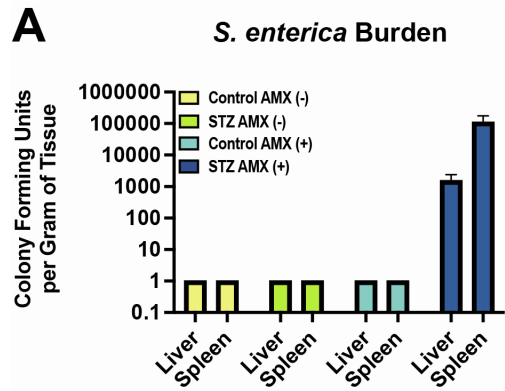


Figure S5: STZ and amoxicillin dual treatment worsens outcomes during *Salmonella enterica* infection. Related to Figure 7.

- A. *Salmonella enterica* Typhimurium colony forming units (CFU) per gram of hepatic and splenic tissue in control AMX(+/-), and hyperglycemic AMX(+/-) mice over the course of infection with an inoculum of 1×10^3 cells. Data represent mean CFU \pm SEM.
- B. Pathological assessment of fixed, H&E-stained colon sections 4 days after infection with an inoculum of 1×10^3 cells.
- C. Plasma concentration of IL-1 α in STZ-treated and control mice +/- AMX
- D. Plasma concentration of IL-6 in STZ-treated and control mice +/- AMX
- E. Plasma concentration of GM-CSF in STZ-treated and control mice +/- AMX
- F. Plasma concentration of IL-12p70 in STZ-treated and control mice +/- AMX
- G. Plasma concentration of IFN- β in STZ-treated and control mice +/- AMX
- H. Plasma concentration of IL-10 in STZ-treated and control mice +/- AMX
- I. Plasma concentration of IL-17A in STZ-treated and control mice +/- AMX
- J. Principal Coordinates Analysis of Bray-Curtis Dissimilarity between uninfected controls and mice infected with an inoculum of 1×10^3 cells 24 hours post-infection.
- K. Alpha diversity as measured by the Shannon diversity index of fecal 16S rRNA reads. Data represent average score \pm SEM during infection time course after dosage with an inoculum of 1×10^3 cells.
- L. Phylum-level taxonomic composition of the fecal microbiome during infection time course after dosage with an inoculum of 1×10^3 cells. Data represent average abundance \pm SEM.

For A - I: N = 4 to 7 per group

For J: N = 3 to 10 per group

For B: Inflammation (0: absent, 1: minimal, 2: mild affecting mucosa and sub-mucosa, 3: moderate affecting mucosa, 4: severe). Edema (0: < 10%, 1: 10-25%, 2: 25%-50%, 3: 50%-75%, 4: over 75%).

For C - I: (*, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001; Welch's ANOVA with Dunnet T3 test for multiple hypothesis testing).

For J: (*, P < 0.05; **, P < 0.01; ***, P < 0.001; permutational ANOVA)

For K: (*, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001; Welch's ANOVA with Dunnet T3 test for multiple hypothesis testing)