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Supplemental information

Clostridioides difficile infection induces a rapid influx of bile acids into the gut during colonization of the host

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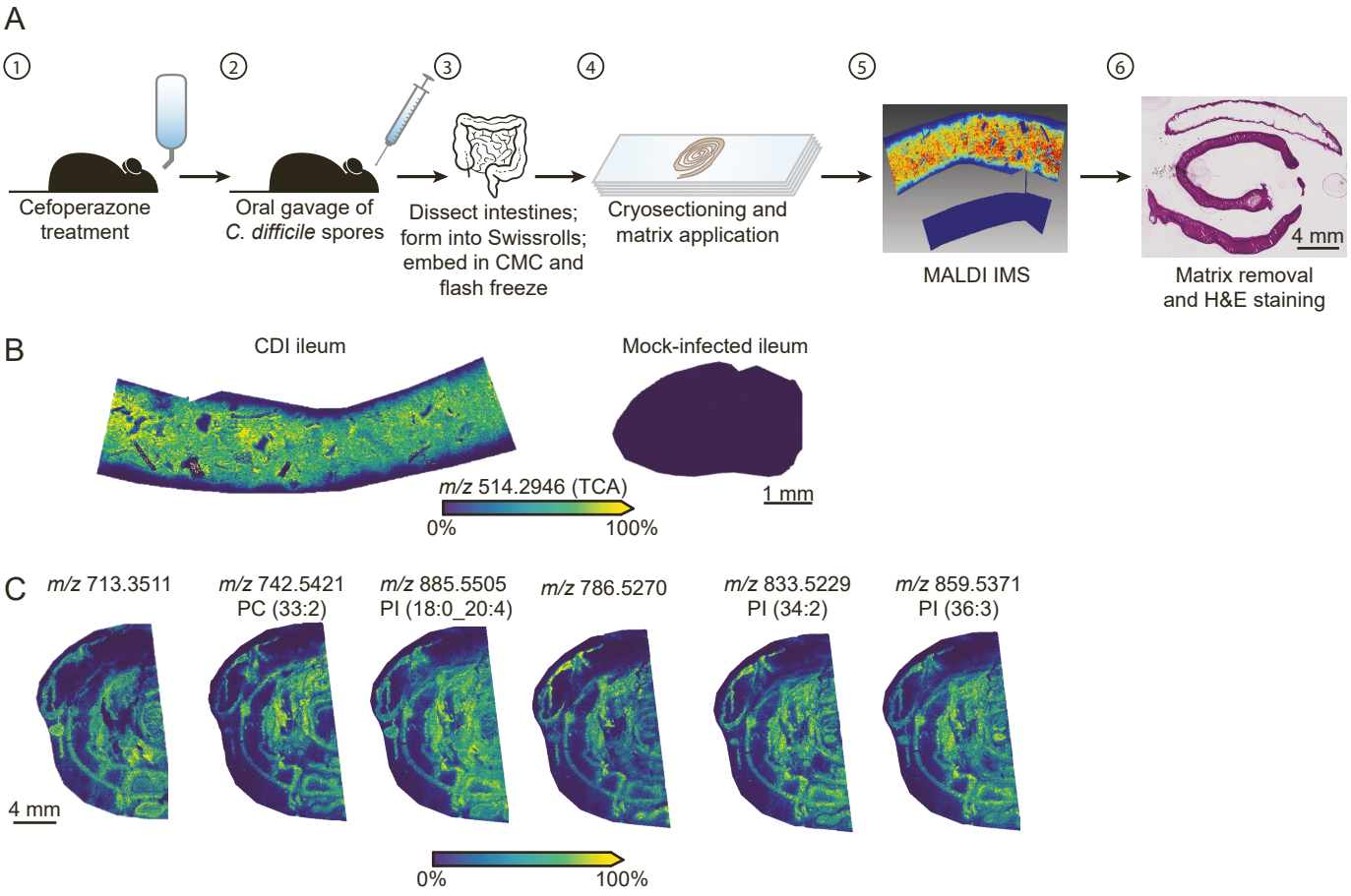
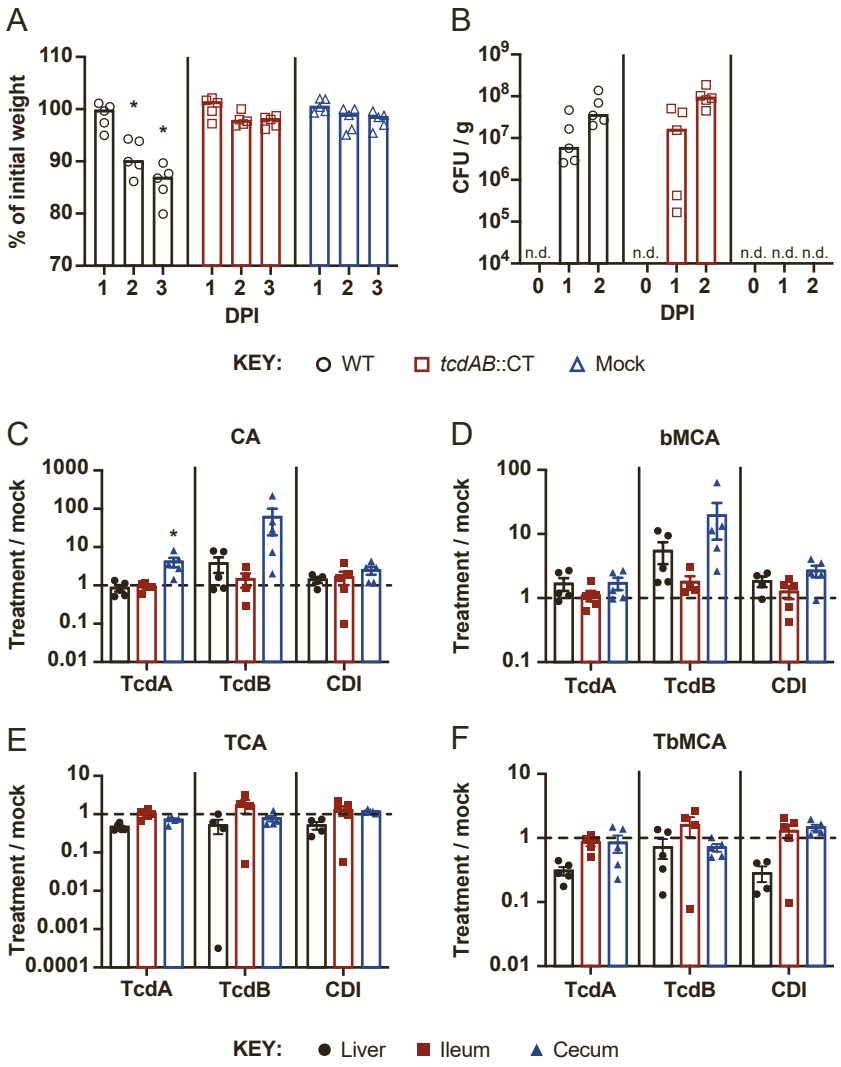
Table S1. Bacterial strains and oligonucleotide primers used in this study.

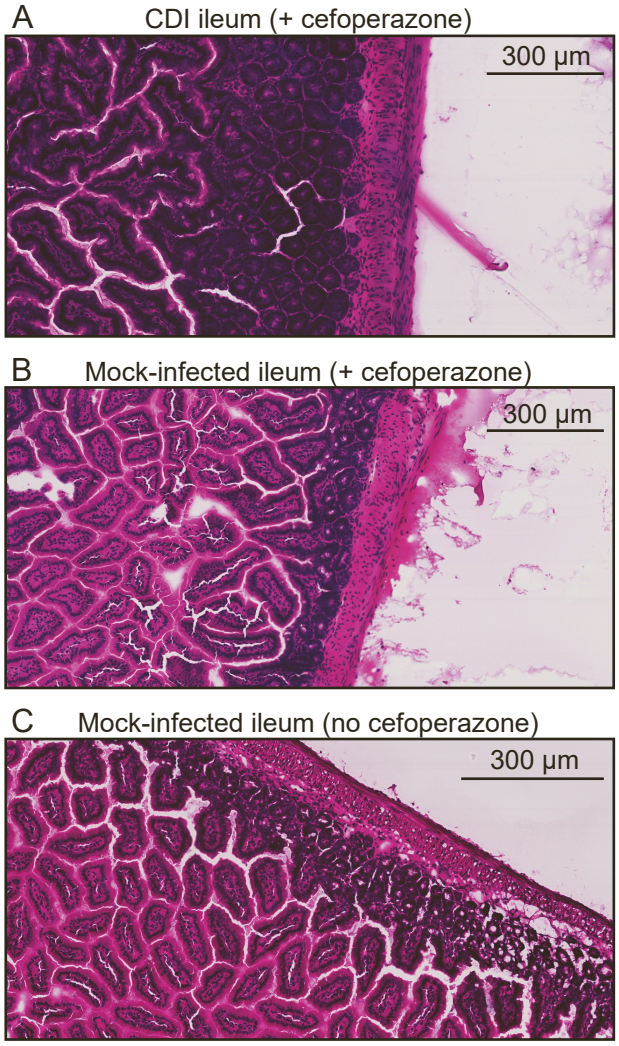
Strain or Primer name	Note / sequence (5'-3')	Reference / Source
Bacterial species and strains		
C. difficile R20291	wild-type	S. Kuehnes
C. difficile R20291 *tcdAB*::CT	*tcdA*::CT *tcdB*::CT	S. Kuehnes
Primers for qRT-PCR		
Cyp7A1_qPCR_F	TTCTTTGATCTGGGGGATTG	PMID_23752203
Cyp7A1_qPCR_R	ATTTCCCCATCAGTTTGCAG	PMID_23752203
Cyp8b1_qPCR_F	ACAAGCAGCAAGACCTGGAT	PMID_18587407
Cyp8b1_qPCR_R	ATGGAAGAGACGCTGCAACT	PMID_18587407
Baat_qPCR_F	CACCTGATTGAGCCTCCCTA	This study
Baat_qPCR_R	GGAAGGAGATGCTGCTTGAG	This study
b-actin_qPCR_F	GATCATTGCTCCTCCTGAGC	PMID_24524627
b-actin_qPCR_R	AGTCCGCCTAGAAGCACTTG	PMID_24524627



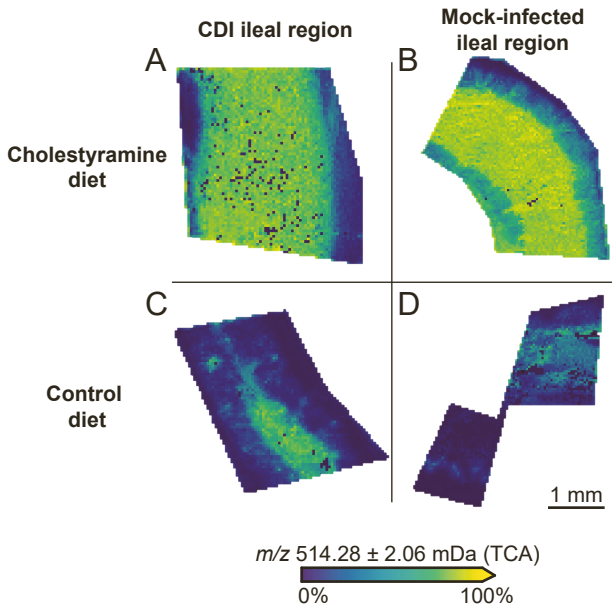
Supplemental Figure 1. MALDI IMS efficiently ionizes a variety of species from the intestinal tract, including consistent intensity differences in TCA between infected and mock-infected samples. Related to Figure 1. (A) Schematic showing key steps of our sample preparation method for subjecting fresh-frozen intestinal tissues and lumen in the Swissroll conformation to MALDI IMS and H&E staining. **(B)** TCA is consistently more highly present in the lumen of CDI tissue compared to mock-infected tissue. **(C)** The Swissroll conformation of intestinal tissue allows for the detection of a variety of lipid species with clear localization to the host epithelium



Supplemental Figure 2. C. difficile toxins are required for full pathogenicity in mice, but are dispensable for colonization and are insufficient to induce a spike in bile acid levels. Related to Figure 2. (A) Pathogenicity of indicated C. difficile strains determined as a function of percent of initial mouse weight at day 0. **(B)** Colonization levels of indicated strains as determined by CFU per gram of feces. Ratios of unconjugated **(C, D)** and taurine-conjugated **(E, F)** bile acids in the liver, ileum, and cecum of mice 8-hours after rectal infusion with 50 μg TcdA or TcdB, or oral gavage with 10^5 wild-type C. difficile R20291 spores, relative to mice rectally infused with PBS. n.d., not detected; *P<0.05, significance determined by unpaired two-sided t-tests; n=5 mice/group.



Supplemental Figure 3. Histology reveals no signs of ileal inflammation in CDI mice. Related to Figure 2. Histological analysis of H&E-stained intestinal tissues shows no significant inflammation or damage in either CDI **(A)** or mock-infected **(B)** mouse tissue at 3 DPI, and no signs of inflammation or tissue damage in non-antibiotic treated, mock-infected mouse tissue **(C)**.



Supplemental Figure 4. Cholestyramine increases the abundance of luminal TCA in the ilea of mock-infected mice. Related to Figure 4. MALDI IMS reveals elevations in ileal lumen TCA abundance in CDI mice receiving a cholestyramine diet **(A)**, mock-infected mice receiving a cholestyramine diet **(B)**, and CDI mice receiving a control diet **(C)**, relative to mock-infected mice receiving a control diet **(D)**.