

Supplemental data for: Bile acid administration elicits an intestinal antimicrobial program and reduces the bacterial burden in two mouse models of enteric infection.

Sarah Tremblay, Guillaume Romain, Mélisange Roux, Xi-Lin Chen, Kirsty Brown, Deanna L. Gibson, Sheela Ramanathan and Alfredo Menendez.

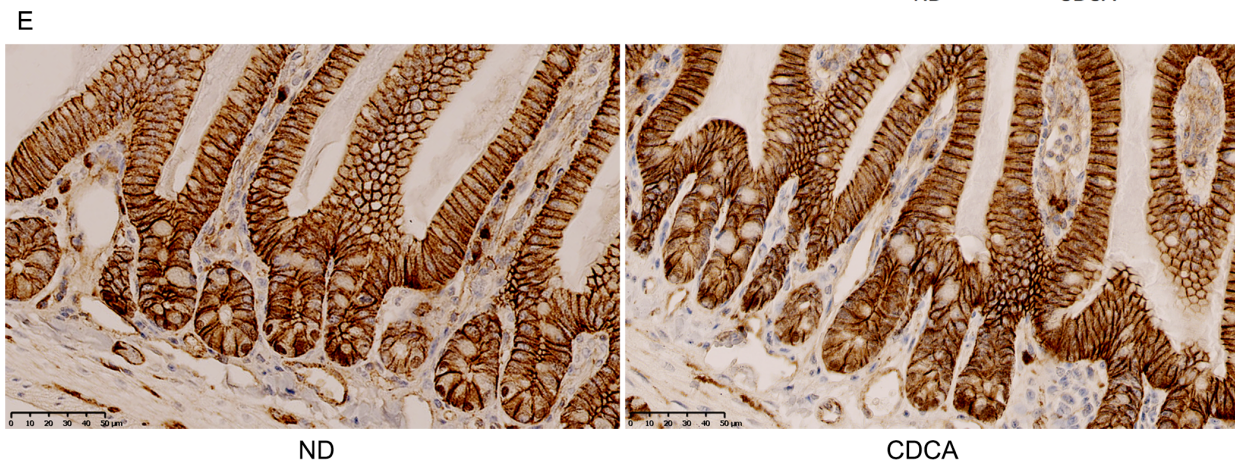
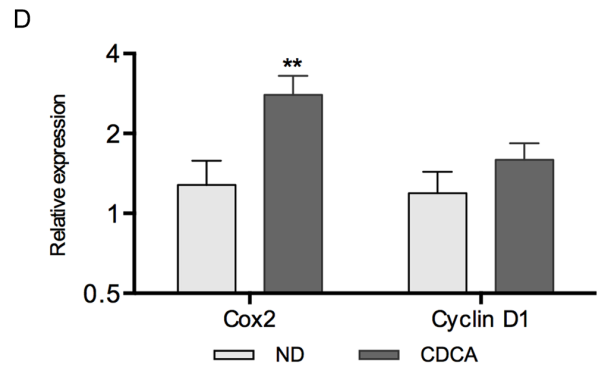
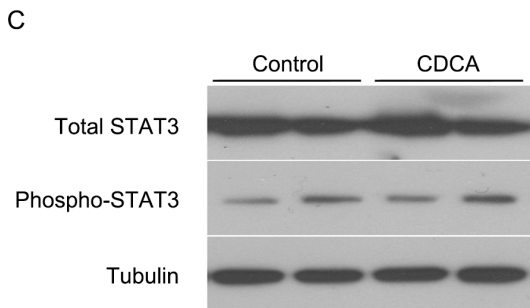
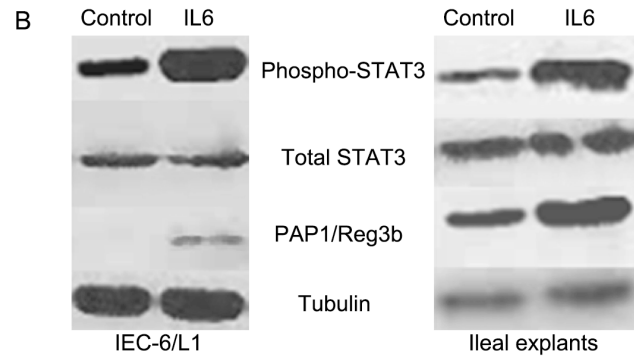
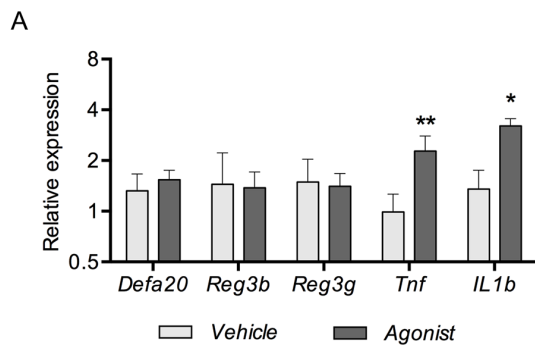


Figure S1. (A) The TGR5 agonist 3-(2-Chlorophenyl)-*N*-(4-chlorophenyl)-*N*,5-dimethyl-4-isoxazolecarboxamide (1 μ M) does not induce AMPP in ileal explants; relative transcript levels by qPCR. (B) Activation of the IL6/STAT3 pathway correlates with induction of Reg3b synthesis in cultured intestinal epithelial cells and ileal explants. Western blots of total lysates from rat intestinal epithelial cells IEC-6/L1 and mouse ileal explants, the control is PBS, two independent replicas of each treatment are shown. (C) CDCA does not activate the STAT3 pathway in IEC-6/L1. Western blot of total lysates from IEC-6/L1, control is EtOH. (D) CDCA feeding induces Cox2 but not Cyclin D1. Relative transcript levels (qPCR) of ileal *Ptgs2* and *Ccnd1* in mice fed with normal (ND) and CDCA diets, n= 9-15 mice/group, statistical significance shown by asterisks (** $p < 0.01$). (E) CDCA feeding doesn't activate beta-catenin in the ileal epithelium. Beta-catenin immunohistochemistry of ileum sections from mice fed with normal and CDCA diets. Scale bars are 50 μ m.

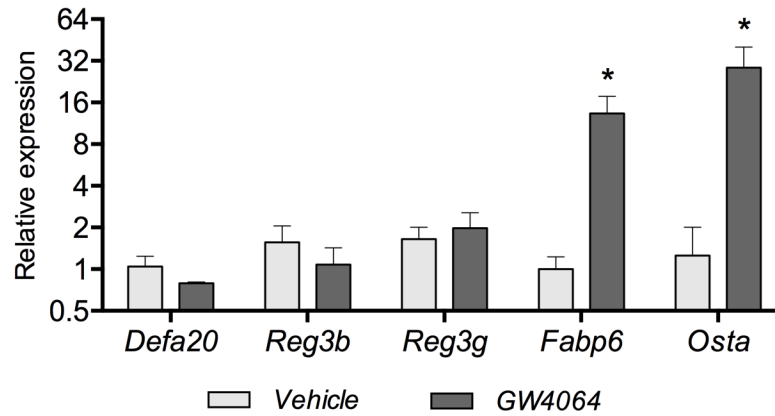


Figure S2. The FXR agonist GW4064 does not induce AMPP in ileal explants. Relative transcript levels determined by qPCR, n= 7 explants/group, statistical significance shown by asterisks (* $p < 0.05$).

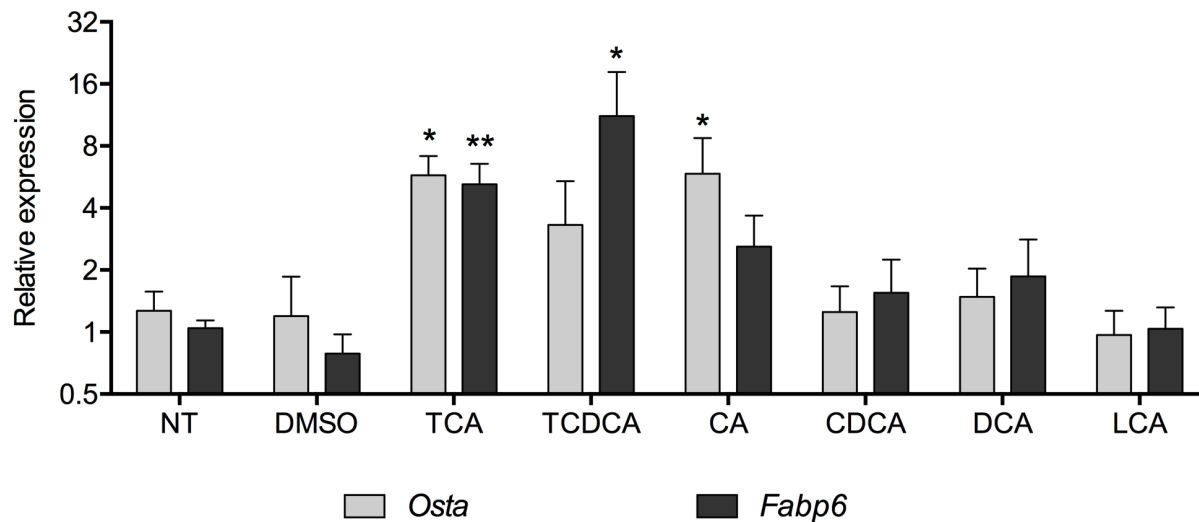


Figure S3. CDCA does not induce the expression of FXR target genes in ileal explants. Relative transcript levels of *Slc51a* (organic solute transporter alpha) and *Fabp6* (fatty acid binding

protein 6) genes in ileal explants treated with various bile acids. TCA: taurocholic acid; TCDC: taurochenodeoxycholic acid; CA: cholic acid; CDCA: chenodeoxycholic acid; DCA: deoxycholic acid; LCA: lithocholic acid. Samples are the same as in **Fig 1**, data is by qPCR, n= 6-8 samples per group. Statistically significant differences are shown by asterisks (* $p < 0.05$).

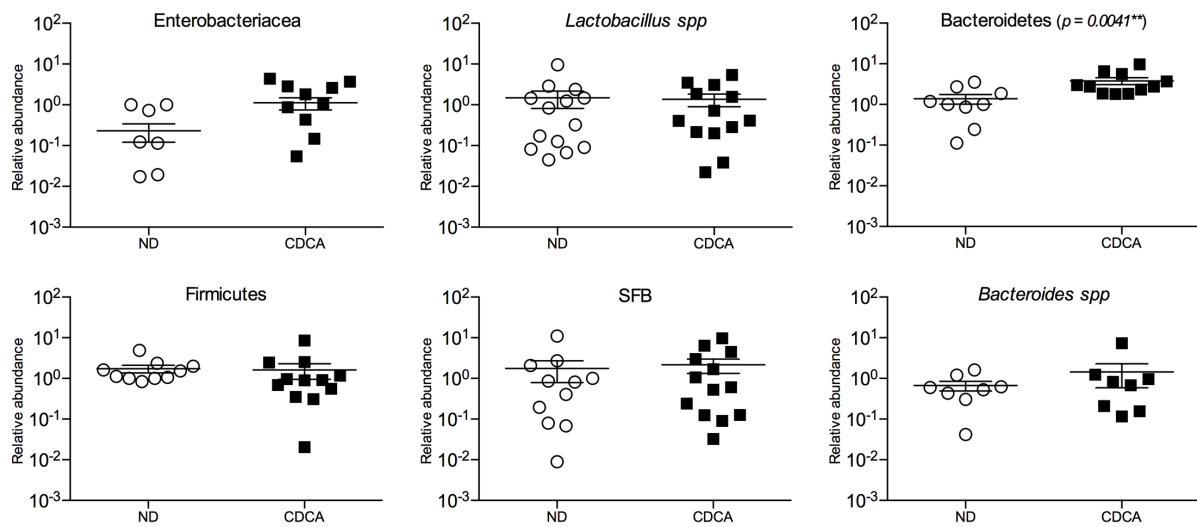


Figure S4. The relative abundance of major members of the normal ileal microbiota is unaffected by dietary CDCA. 16S qPCR using primers specific for the dominant gut phyla Firmicutes and Bacteroidetes, the family Enterobacteriaceae and the genus *Lactobacillus*, *Bacteroides* and Segmented Filamentous Bacteria. Abundance was determined relative to the universal bacteria primer set.

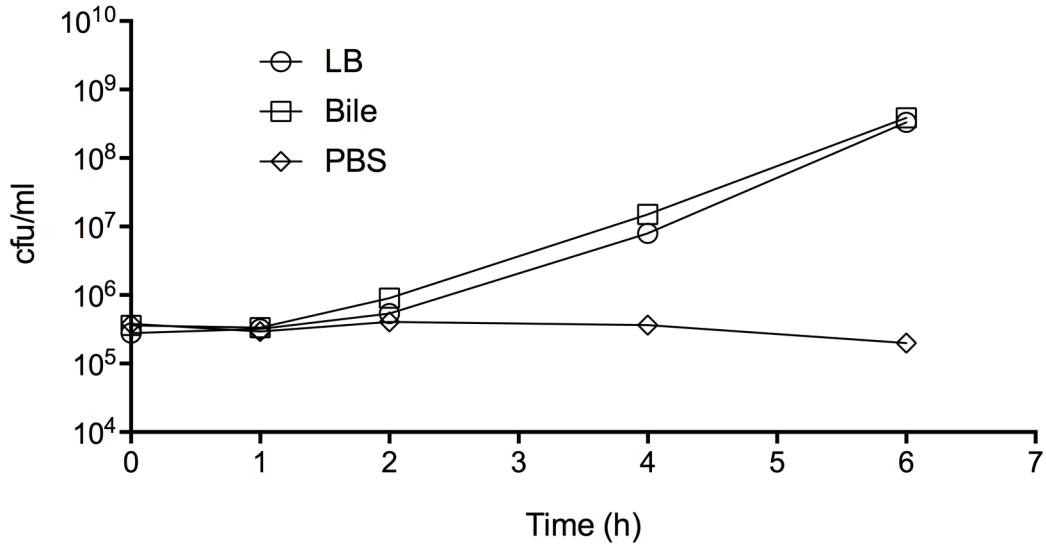


Figure S5. *Citrobacter rodentium* replicates effectively in mouse bile. Bile extracted from the gallbladder of mice was seeded with *C. rodentium* DS100 and maintained at 37 °C for the times indicated, without shaking. Bacterial growth was followed by plating.

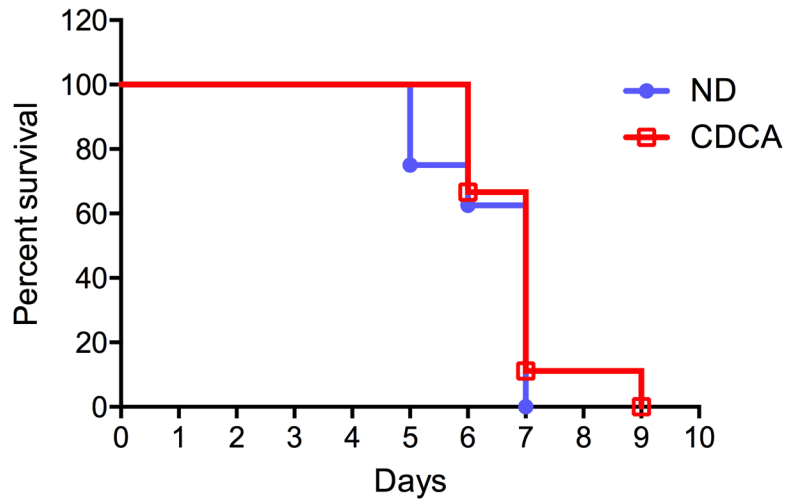


Figure S6. C57/BL6 mice survival after oral infection with *Salmonella typhimurium* SL1344.

Results are from two independent experiments with a total of 8 animals per group.