Supporting Information

Flannigan et al. 10.1073/pnas.1413390111

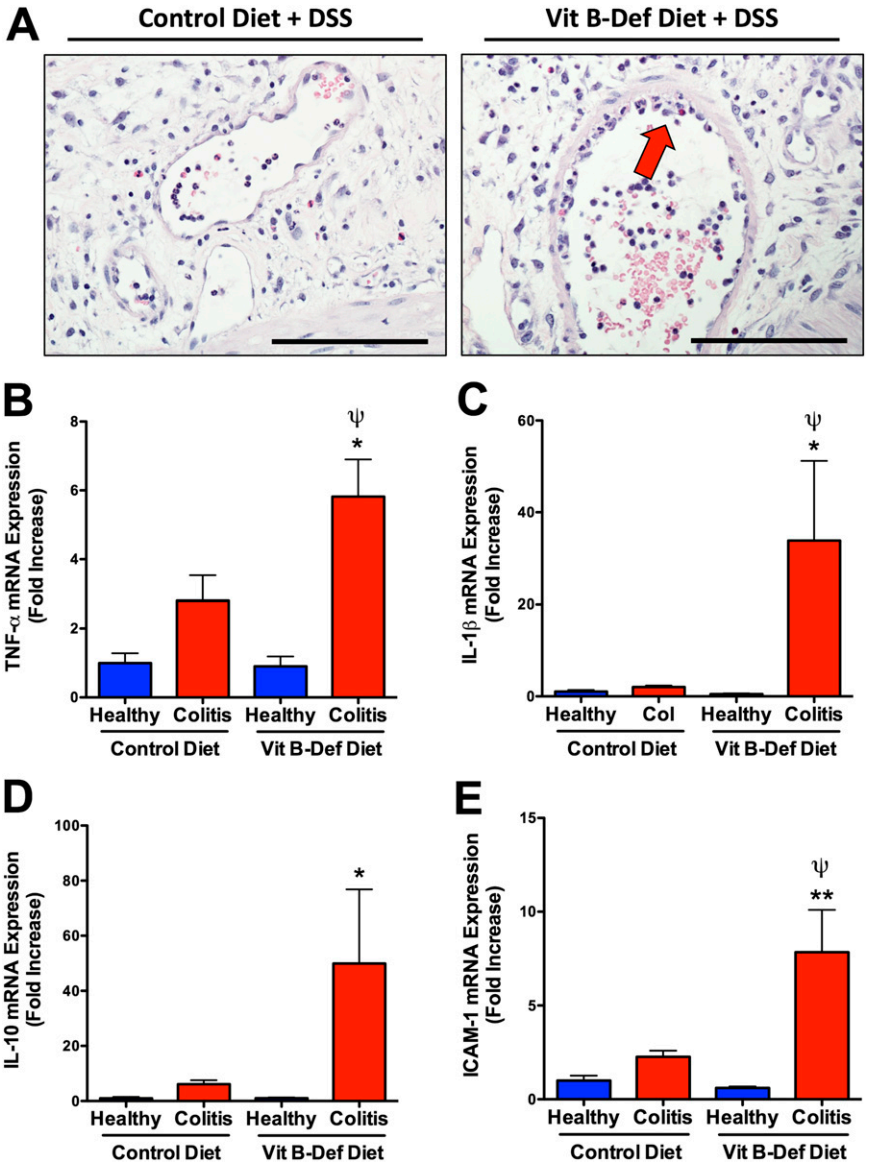

Fig. S1. Colitis induced by dextran sodium sulfate (DSS) was more severe in rats fed a vitamin B-deficient (B-Def) diet than in rats fed a control diet. In the rats on the B-Def diet, there was markedly increased leukocyte margination in blood vessels (*A*, arrow) than in rats on the control diet. (Scale bars, 100 μm.) The more severe colitis in rats on the B-Def diet was accompanied by markedly increased expression of TNF-α, IL-1β, IL-10, and intercellular adhesion molecule (ICAM) 1 (*B–E*, respectively). Results are the mean ± SEM of 5–10 rats per group (*$P < 0.05$, **$P < 0.01$ versus the corresponding healthy group; $^{\Psi}P < 0.05$ versus the corresponding control diet colitis group; ANOVA and Dunnett's multiple comparison test).

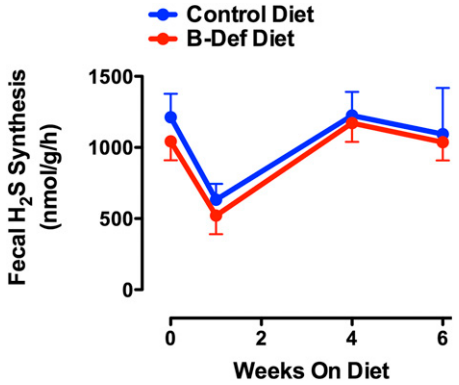

Fig. S2. Production of hydrogen sulfide (H_2S) by fecal samples from rats provided the vitamin B-deficient diet or control diet for 6 wk. Fecal H_2S synthesis is derived largely from enteric bacteria (1). There were no significant differences between the two groups at any time point. Under the conditions of this assay, there is negligible H_2S production from prokaryotic cells in feces. Results are expressed as mean ± SEM for 10 rats per group.

1. Flannigan KL, McCoy KD, Wallace JL (2011) Eukaryotic and prokaryotic contributions to colonic hydrogen sulfide synthesis. *Am J Physiol Gastrointest Liver Physiol* 301(1):G188–G193.

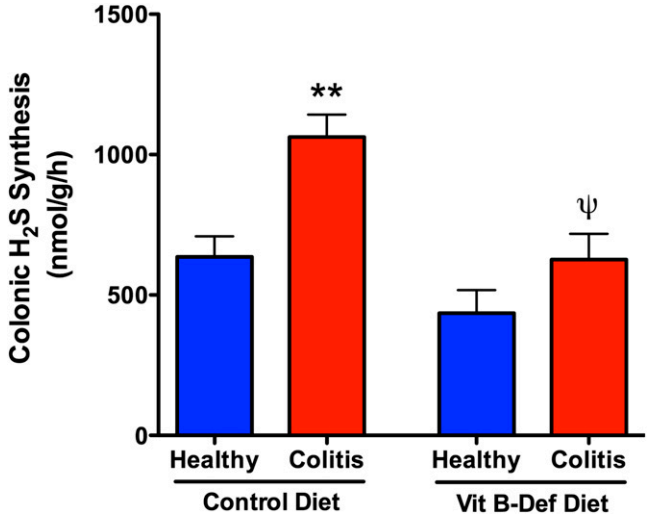

Fig. S3. Colitis induced by DSS resulted in a significant increase in colonic H_2S synthesis via the 3-mercaptopyruvate pathway in rats on the control diet, but not in rats on the vitamin B-deficient diet, despite more severe colitis. Results are expressed as mean ± SEM for 5–10 rats per group (**$P < 0.01$ versus healthy rats on the same diet; $^{\psi}P < 0.05$ versus DSS-treated rats on the control diet; ANOVA and Dunnett's multiple comparison test).

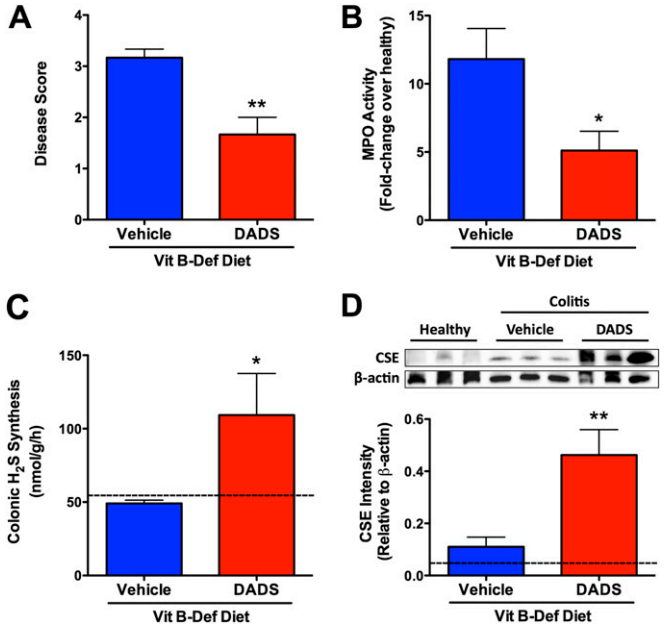

Fig. S4. Treatment with an H_2S donor reversed the effects of the vitamin B-deficient diet in rats with colitis. Twice-daily treatment of rats with diallyl disulfide (DADS; 30 μmol/kg intracolonically) significantly reduced the severity of colitis as measured by the blindly evaluated "disease score" (*A*) and colonic myeloperoxidase (MPO) activity (*B*). Colitis was induced by providing the rats with drinking water supplemented with DSS (administration of DADS had no effect on the amount of water consumed by the rats). DADS administration also significantly increased colonic H_2S synthesis (*C*) and expression of cystathionine γ-lyase (CSE), a key enzyme for H_2S synthesis. The dashed lines in *C* and *D* represent the mean levels of H_2S synthesis and CSE expression, respectively, for healthy rats fed the control diet. Mean ± SEM for six rats per group (*$P < 0.05$, **$P < 0.01$ versus the corresponding vehicle-treated group; Student *t* test).

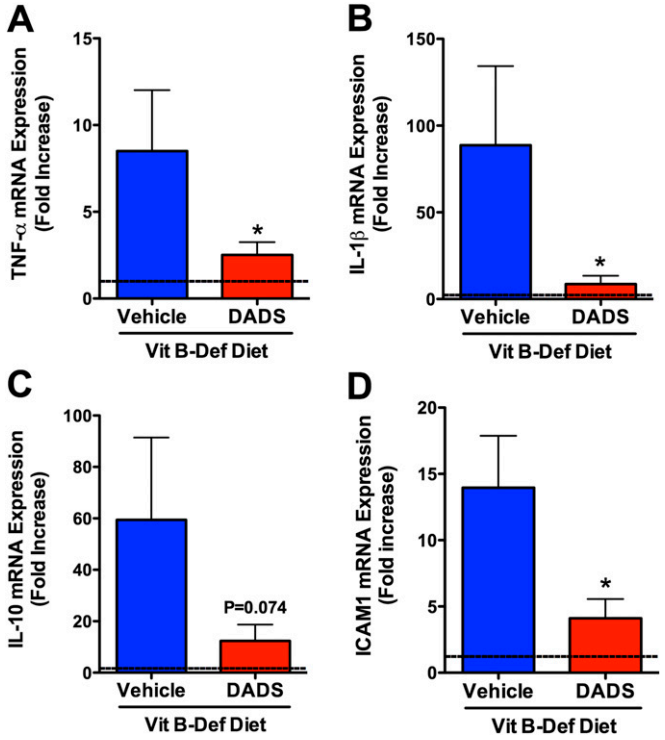

Fig. S5. Daily treatment with an H_2S donor, diallyl disulfide, significantly reduced colonic expression of TNF-α, IL-1β, and ICAM-1 in rats with colitis induced by DSS who had been on a vitamin B-deficient diet for the previous 6 wk. The decrease in expression of IL-10 did not achieve statistical significance. The horizontal line on each graph represents the mean expression in healthy rats on the control diet. Each bar represents the mean ± SEM for 6–10 rats per group (*$P < 0.05$; Student's *t* test).

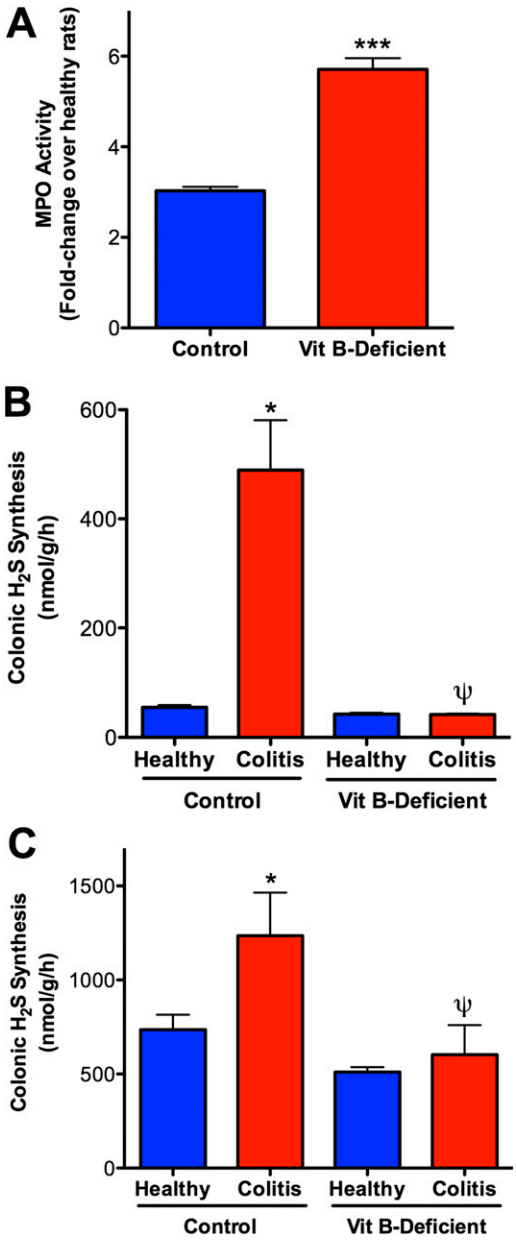

Fig. S6. Colitis induced by dinitrobenzene sulfonic acid is significantly more severe and is accompanied by impaired H_2S synthesis. Colonic MPO activity, a marker of granulocyte infiltration, was elevated in rats with colitis, but the increase was significantly greater (***$P < 0.001$) in rats that were fed the vitamin B-deficient diet (*A*). In rats with colitis, colonic H_2S synthesis was markedly increased (*$P < 0.05$ versus the corresponding healthy group) via both the pyroxidal–5′-phosphate–dependent (*B*) and –independent (*C*) pathways. However, in rats on the vitamin B-deficient diet, induction of colitis was not accompanied by a significant increase in colonic H_2S synthesis via either pathway ($^{\Psi}P < 0.05$ versus the control group with colitis). Results are expressed as mean ± SEM for 5–10 rats per group (ANOVA and Dunnett's multiple comparison test).