Supporting Information

The Anti-Oxidant Drug Tempol Promotes Functional Metabolic Changes in the Gut Microbiota

Jingwei Cai, Limin Zhang, Richard A. Jones, Jared B. Correll, Emmanuel Hatzakis, Philip B. Smith, Frank J. Gonzalez, Andrew D. Patterson

Figure S1 - Two dimensional ^{1}H-^{1}H total correlation spectroscopy (TOCSY) NMR for SCFAs identification.

Figure S2 - Tempol-associated weight loss is not associated with any gross histological changes in the liver.

Figure S3 - Tempol causes pronounced changes in bacteria fermentation.

Figure S4 - Tempol causes pronounced changes in cecal and fecal metabolites determined by 1H NMR

Figure S5 - Quantification of gross heat of feces by bomb calorimetry.

Figure S6 - Tempol modulates bacterial community quantitatively and compositionally

Table S1 - Primer sequences for qPCR analysis of genes associated with glucose and lipid metabolic pathway, related to Figure 4.

Table S2 - Bacterial primer sequences, related to Figure S6.

Figure S1

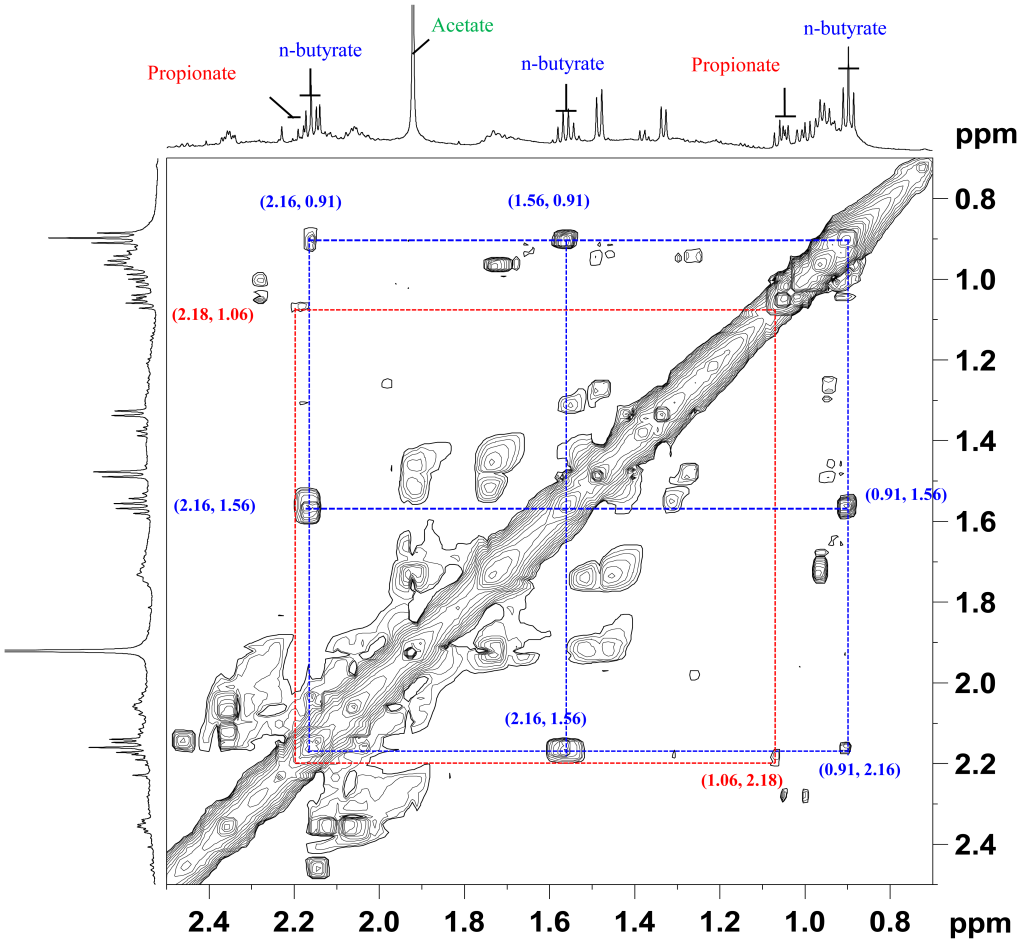

Figure S1. Two dimensional ^{1}H-^{1}H total correlation spectroscopy (TOCSY) NMR for SCFAs identification. The cross peaks of propionate and n-butyrate are highlighted with red and blue dotted lines, respectively.

Figure S2

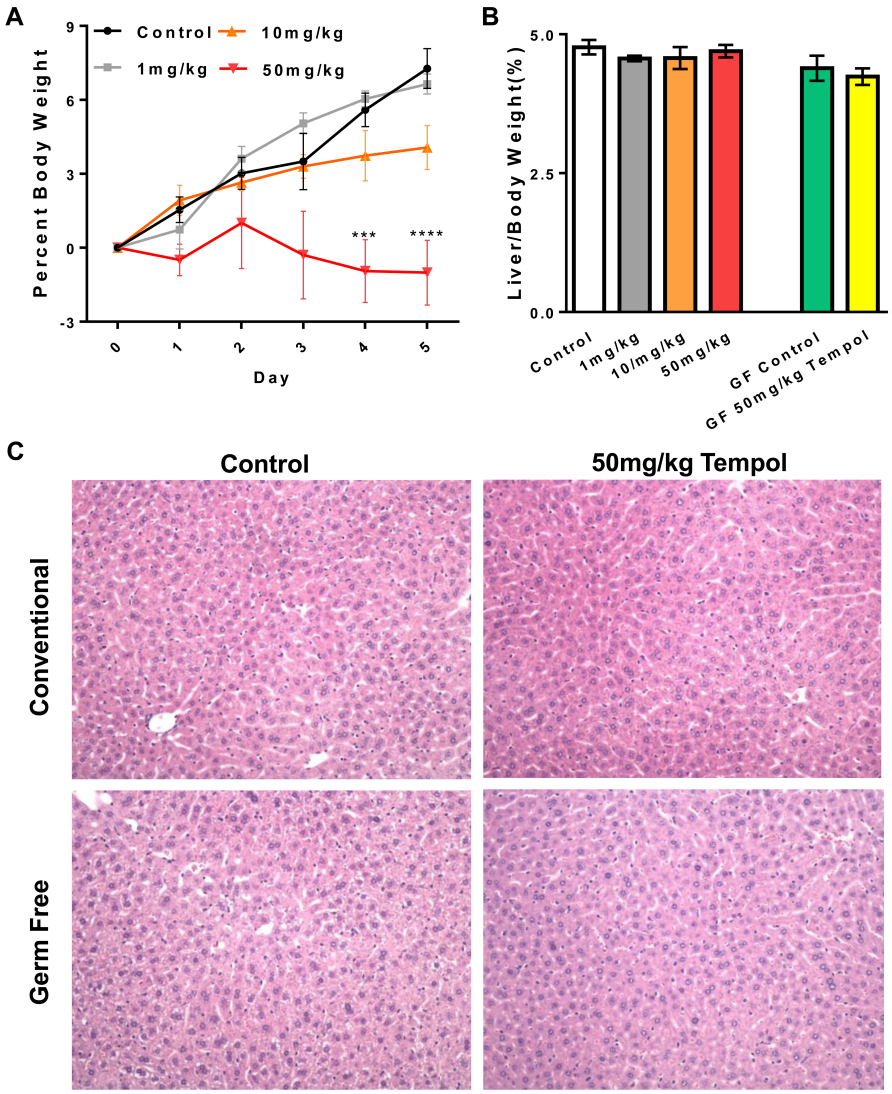

Figure S2. Tempol-associated weight loss is not associated with any gross histological changes in the liver. (A) Body weight change over 5 day tempol treatment (0.9% saline, 1 mg/kg, 10 mg/kg, 50 mg/kg) on a normal chow diet (n=5 mice per group). (B) Liver to body weight ratios in mice after gavage with tempol (n=5 mice per group). (C) Hepatic histology of representative hematoxylin and eosin-stained liver sections. All data are presented as mean ± SEM and analyzed using one-way ANOVA with Tukey's correction or two-tailed student's t-test. ***p<0.001, ****p<0.0001.

Figure S3

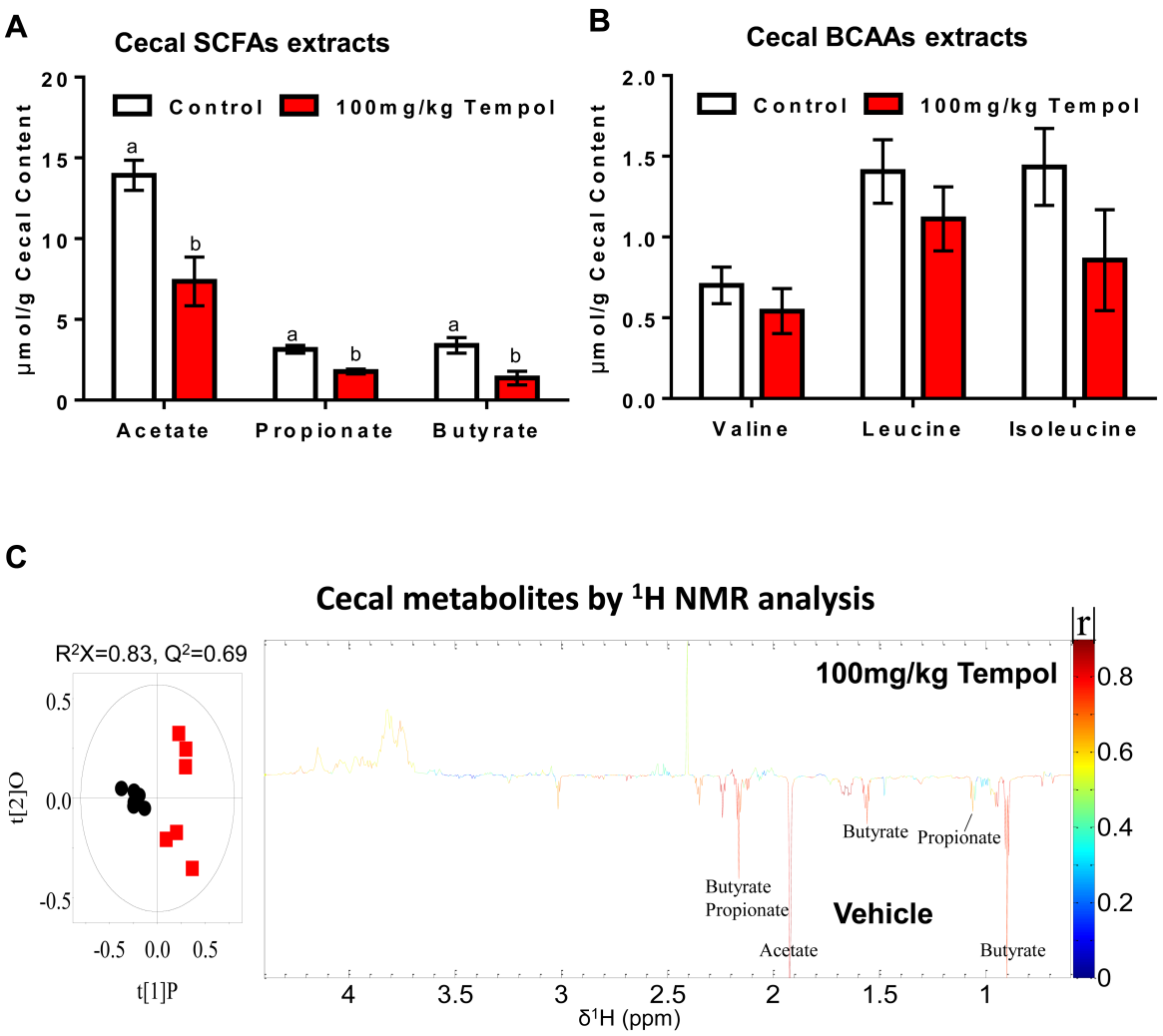

Figure S3. Tempol causes pronounced changes in bacteria fermentation. (A-B) GC-MS Quantification of cecal SCFAs and BCAAs in mice gavaged with 100 mg/kg tempol for 5 days (n=6 mice per group). Mann-Whitney test. Groups with different letters are significantly different ($p<0.05$). All data are presented as mean ± SEM. (C) Cecal metabolites change determined by ^{1}H NMR. O-PLS-DA scores (left) represent indicative power of models and correlation coefficient-coded loadings plots for the models (right) from NMR spectra displaying changes and significance (n=6 mice per group).

Figure S4

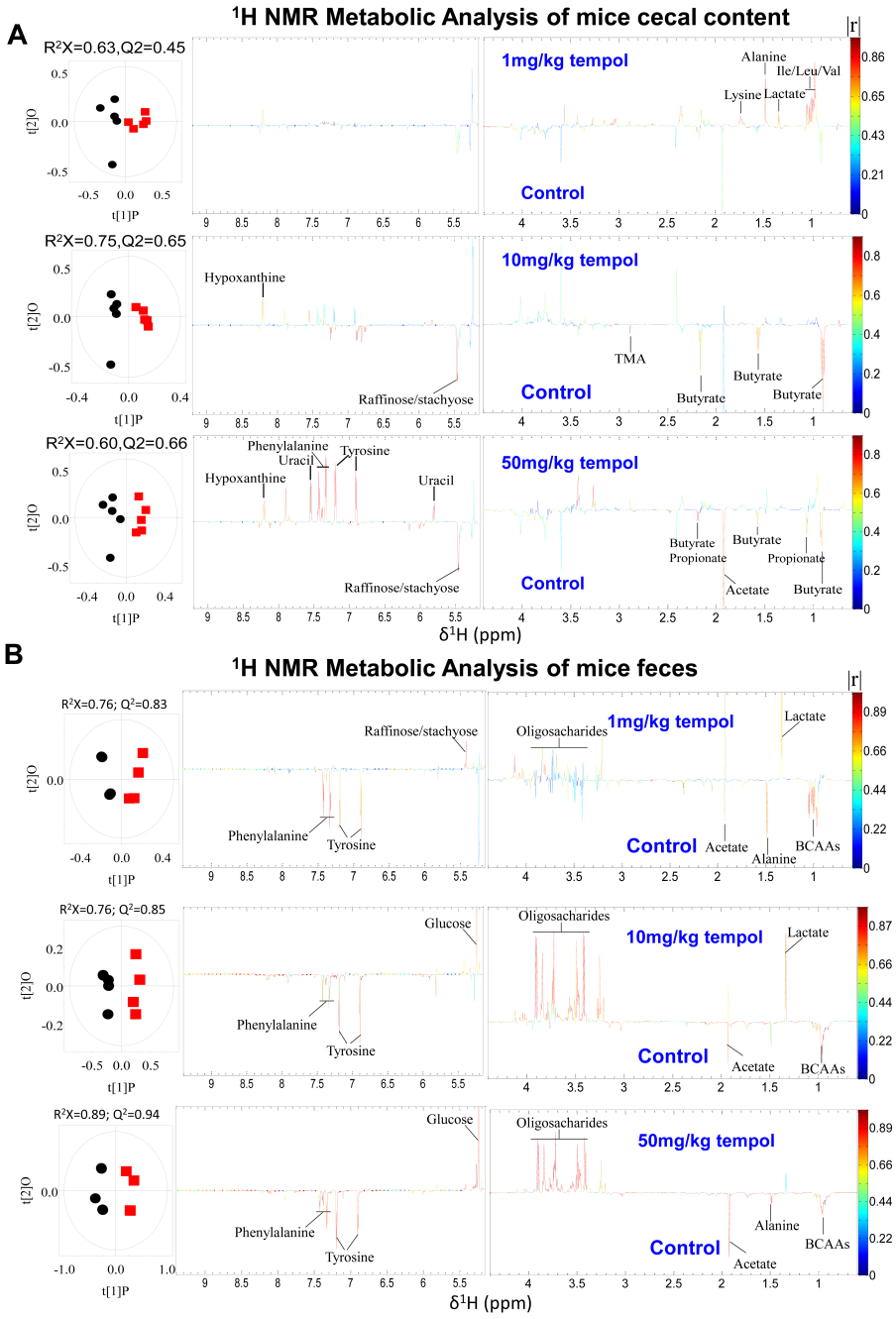

Figure S4. Tempol causes pronounced changes in cecal and fecal metabolites determined by 1H NMR. O-PLS-DA scores (left) and correlation coefficient-coded loadings plots for the models (right) from NMR spectra of (A) cecal content, (B) feces obtained from different dose of tempol treated mice, displaying changes of metabolites between control group (black circles) and tempol treated group (red squares). (n=5 mice per group).

Figure S5

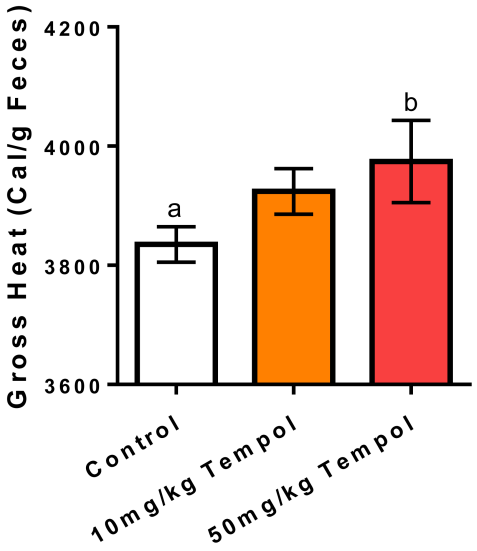

Figure S5. Quantification of gross heat of feces by bomb calorimetry. Each Gross heat was averaged from duplications (n=5 mice per group). Groups with different letters are significantly different ($p<0.05$). Groups without labels are not significantly different from the other groups. One-way ANOVA with Tukey's correction. All Data are presented as mean ± SEM.

Figure S6

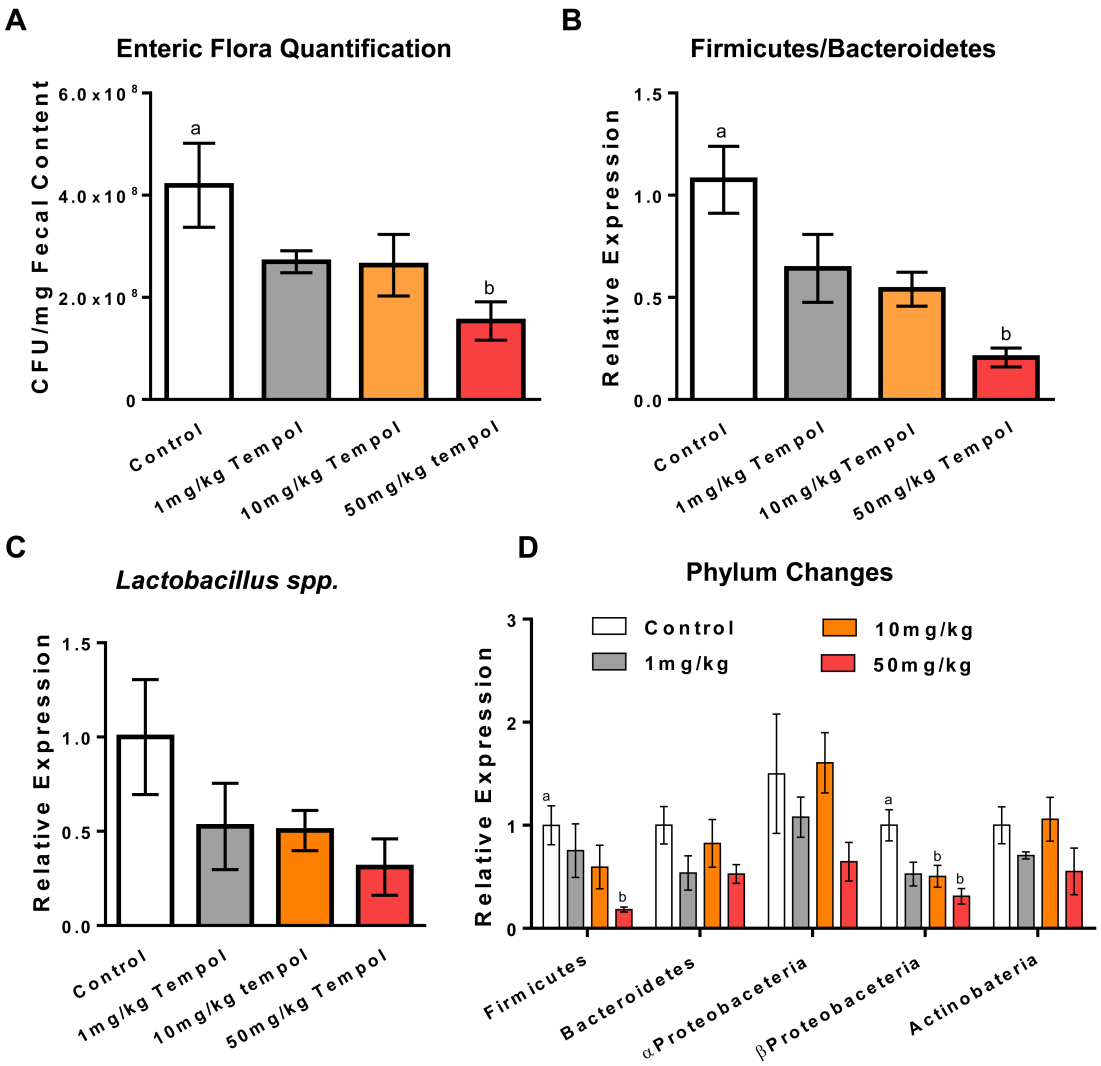

Figure S6. Tempol modulates bacterial community quantitatively and compositionally (A) Quantitative PCR analysis of the fecal microbiome using universal 16S rRNA gene primers after 5-day tempol treatment (n=5 per group). Groups with different superscript letters are statistically different ($p<0.05$). One-way ANOVA with Tukey's correction. All data are presented as mean ± SEM. (B-D) QPCR quantification of targeted 16S rRNA genes using genus and phyla specific primers after 5-day gradient tempol treatment (n=5 per group). Groups with different superscript letters are statistically different ($p<0.05$). Groups without labels are not significantly different from other groups. One-way ANOVA with Tukey's correction. All data are presented as mean ± SEM.

Table S1. Primer sequences for qPCR analysis of genes associated with glucose and lipid metabolic pathway, related to Figure 4.

species	gene name	sequence 5'-3'	sequence 3'-5'
mouse	*GADPH*	CCTCGTCCCGTAGACAAAATG	TGAAGGGGTCGTTGATGGC
mouse	*G6Pase*	CCATGCAAAGGACTAGGAACAA	TACCAGGGCCGATGTCAAC
mouse	*Pepck*	CCACAGCTGGTGCAGAACA	GAAGGGTCGATGGCAAA
mouse	*Glut2*	GTCCAGAAAGCCCCAGATACC	GTGACATCCTCAGTTCCTCTTAG
mouse	*Hnf4a*	TGAGCACCTGCTGCTTGGA	TCGAGGATGCGAATGGACAC
mouse	*ChREBP*	CTGGGGACCTAAACAGGAGC	GAAGCCACCCTATAGCTCCC
mouse	*Fabp1*	TCAAGCTGGAAGGTGACAATAA	GTCTCCATTGAGTTCAGTCACG
mouse	*Fabp2*	TCGGTTCCTGAGGATACAAGAT	TTTGATGACTGTGGGATTGAAG
mouse	*Fabp5*	ACAGGGTTTTTGCATTCCTG	TTGGTTCTTTCGAACCTTG
mouse	*Cd36*	TGGCCTTACTTGGGATTGG	CCAGTGTATATGTAGGCTCATCCA

Table S2. Bacterial primer sequences, related to Figure S6.

target group	forward primer	reverse primer
All bacteria	AGAGTTTGATCCTGGCTCAG	CTGCTGCCTCCCGTAGGAGT
Lactobacillus.spp	AGCAGTAGGGAATCTTCCA	CACCGCTACACATGGAG
Firmicutes	GCAGTAGGGAATCTTCCG	ATTACCGCGGCTGCTGG
Bacteroidetes	GTACTGAGACACGGACCA	ATTACCGCGGCTGCTGG
αProteobacteria	ACTCCTACGGGAGGCAGCAG	TCTACGRATTTCACCYCTAC
βProteobacteria	ACTCCTACGGGAGGCAGCAG	TCACTGCTACACGYG
Actinobacteria	CGCGGCCTATCAGCTTGTTG	ATTACCGCGGCTGCTGG