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Supplementary Materials for

Vancomycin relieves mycophenolate mofetil–induced gastrointestinal toxicity by eliminating gut bacterial β-glucuronidase activity

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This PDF file includes:

Supplementary Materials

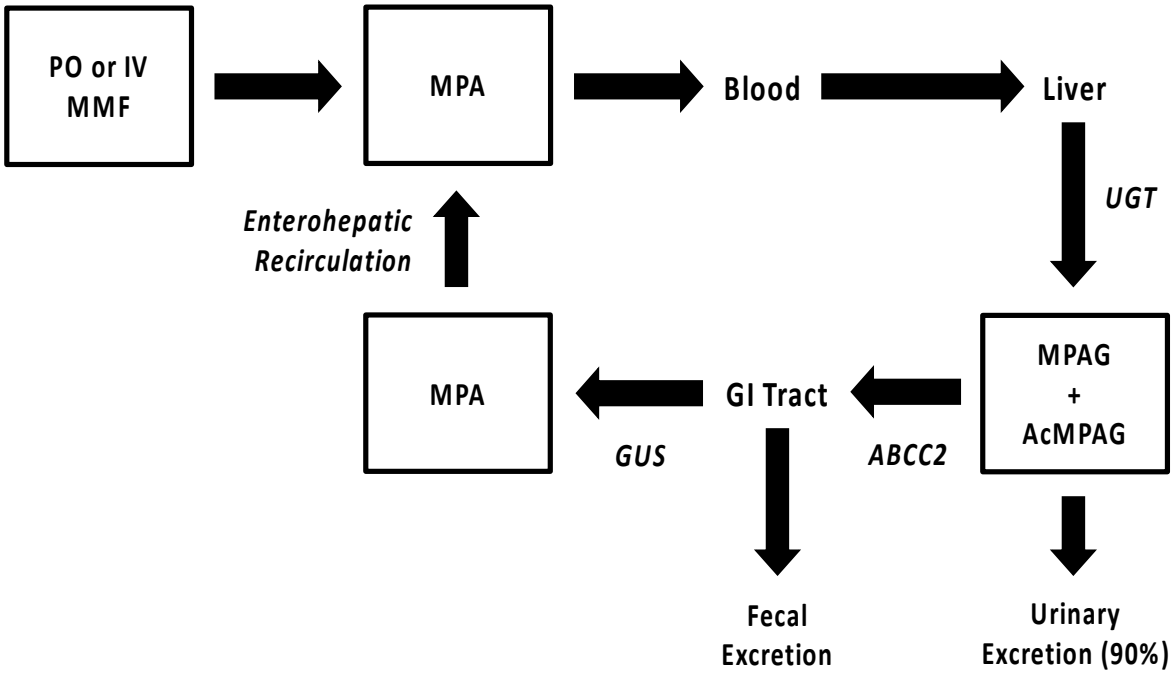

Fig. S1. MMF metabolism. MMF consumed either orally (PO) or intravenously (IV) is converted to active MPA in the stomach and circulation and then glucuronidated by hepatic UGT enzymes which create inactive MPAG and AcMPAG. MPAG and AcMPAG are mostly excreted in the urine but ~10% is transported by ABCC2 into the GI tract where bacterial GUS enzymes remove the GA moiety to produce free GA and MPA which undergoes enterohepatic recirculation.

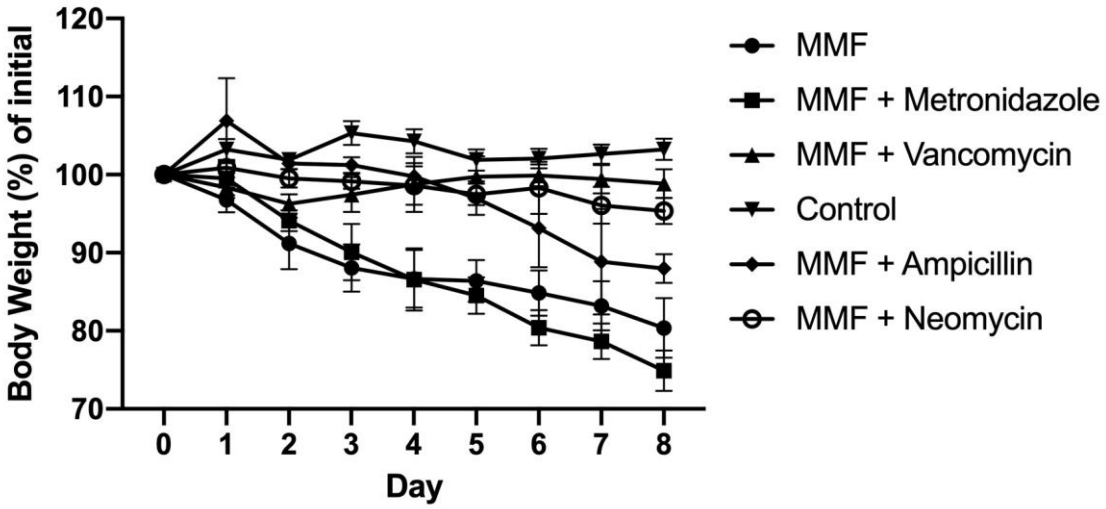

Fig. S2. Effect of individual antibiotics (ampicillin, neomycin, metronidazole or vancomycin) on mouse body weight in the presence of MMF. Control animals received normal chow and water. Data are shown as mean and standard deviation for each day.

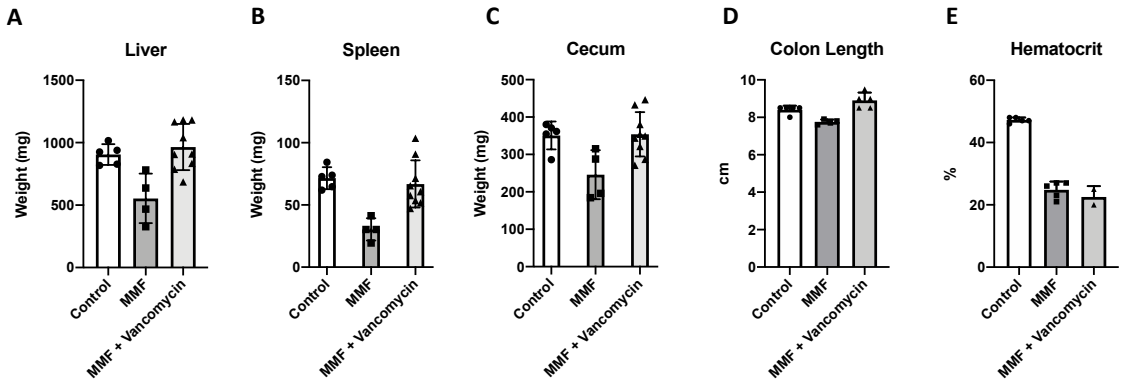

Fig. S3. Tissue weights. Effect of 8 days of exposure to MMF alone or MMF with vancomycin on weight of (**A**) liver, (**B**) spleen and (**C**) cecum. Effect of MMF and MMF with vancomycin after 8 days on (**D**) colon length and (**E**) hematocrit. Control animals received unmedicated chow and plain water. Data are shown as mean and standard deviation.

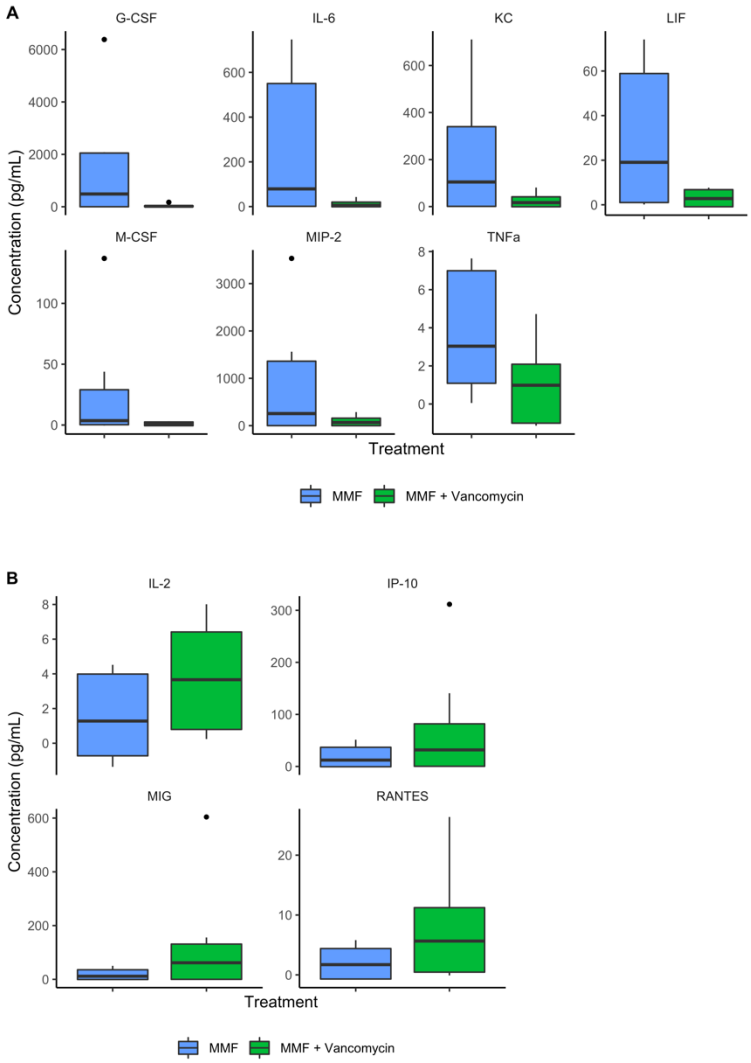

Fig. S4. Effect of 8 days of exposure to MMF alone or MMF with vancomycin on individual cytokines and chemokines. (**A**) Vancomycin induced a significant decrease in seven cytokines. (**B**) Vancomycin resulted in a significant increase in four cytokines.

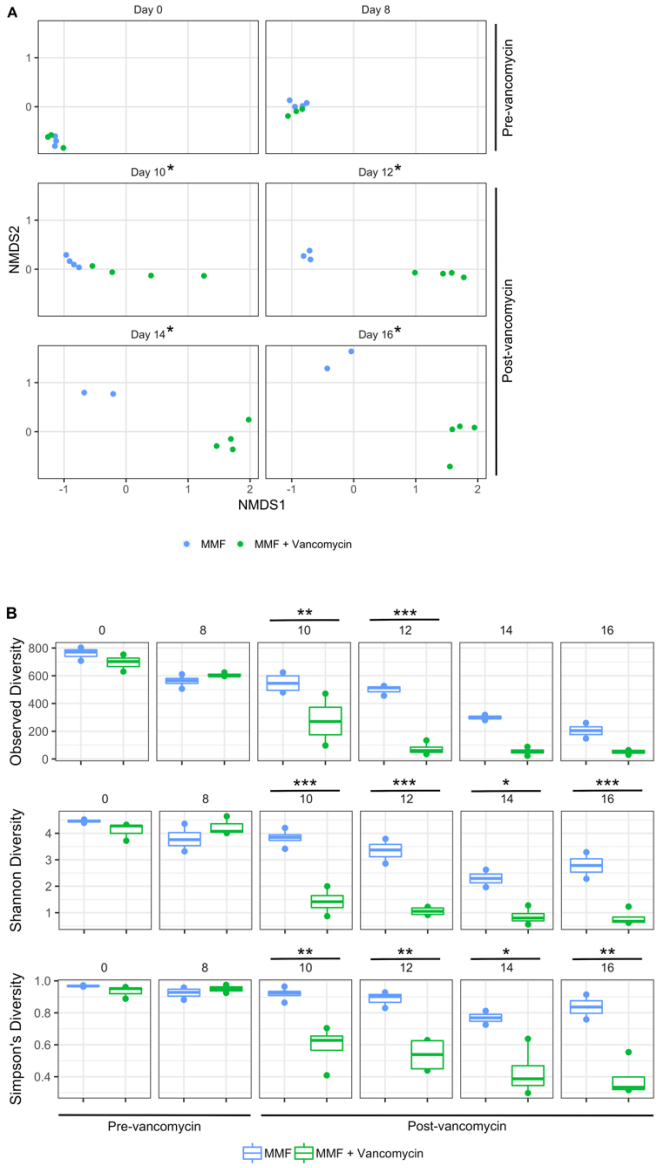

Fig. S5. Changes in bacterial diversity. (**A**) Non-metric multidimensional scaling (NMDS) plots for β-diversity (Bray-Curtis dissimilarity) for bacteria in fecal pellets collected from mice exposed to MMF only (n=2-4) or MMF with vancomycin (n=4 individual animals). Community composition was determined using 16S rRNA amplicon sequencing of bacterial DNA extracted from fecal pellets. Each data point represents an individual sample. Intergroup comparisons were performed for each time point using Adonis with *p <0.1. (**B**) Effect of MMF alone and MMF with vancomycin exposure on bacterial α-diversity for three different indices (Observed, Shannon and Simpson's). Community composition was determined using 16S rRNA amplicon sequencing from mouse fecal pellets collected from 2-4 individual animals. Significant differences in diversity between groups was determined using a two-way ANOVA with *p <0.05, **p < 0.001, ***p < 0.0005.

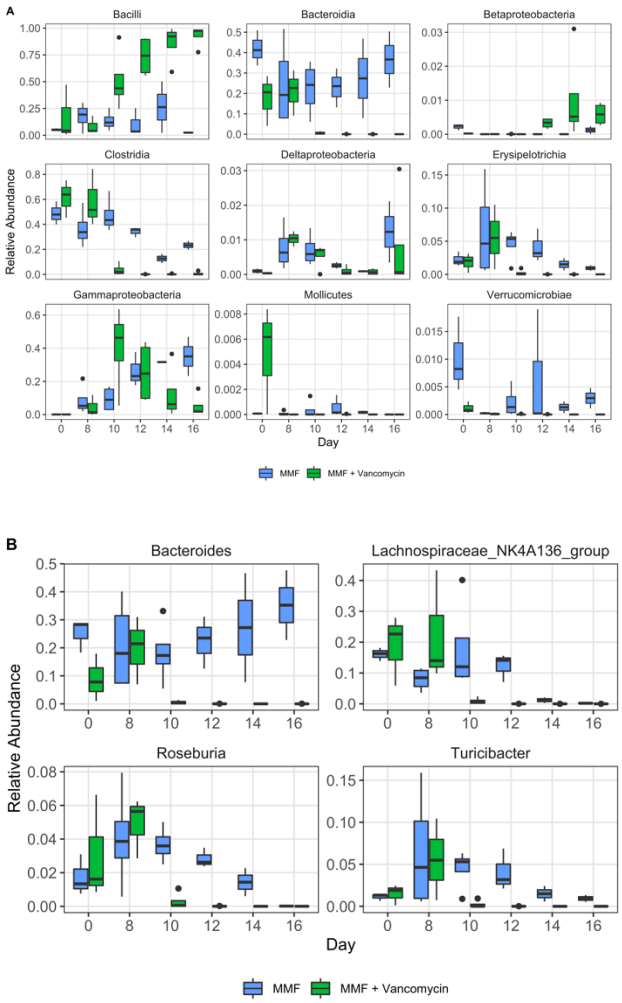

Fig. S6. Changes in bacterial abundance. (**A**) Effect of MMF and vancomycin (introduced on day 8) on relative abundances of bacterial classes in mouse fecal pellets. (**B**) Changes in relative abundance of bacterial genera that showed differential abundances in the absence and presence of vancomycin.

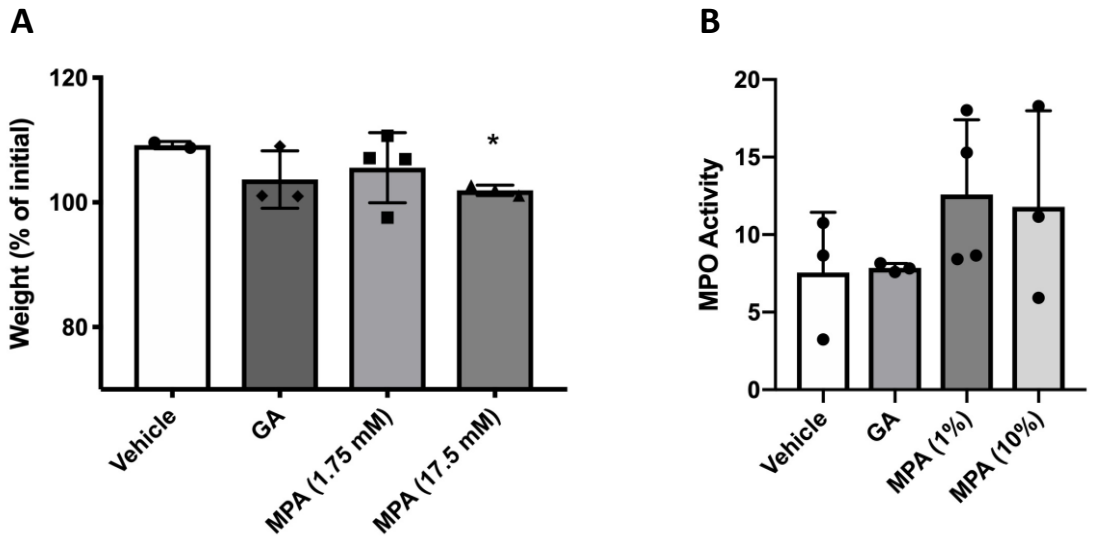

Fig. S7. Intrarectal metabolite effects. (**A**) Effect of daily intrarectal administration of MPA (1.75 mM and 17.5 mM concentrations) and GA (17.5 mM) on mouse body weight after 8 days. Only 17.5 mM MPA exposure resulted in significant weight loss (*p = 0.002). (**B**) Effect of daily intrarectal administration of MPA (1% and 10% concentrations) and GA on MPO activity in whole colonic homogenates. There was a positive trend towards increased MPO activity (units/mg tissue) in mice treated with MPA but not GA. Data are shown as mean and standard deviation.