# Pretreatment with yeast-derived complex dietary polysaccharides suppresses gut inflammation, alters the microbiota composition, and increases immune regulatory short-chain fatty acid production in C57BL/6 mice

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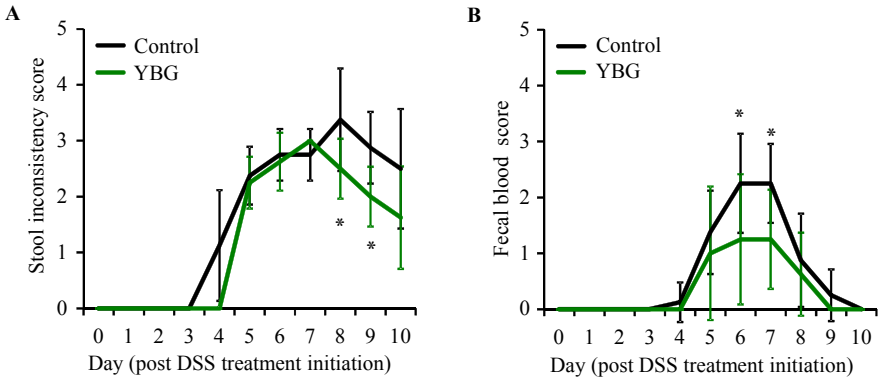
Online Supplementary Material

Supplemental Table 1:

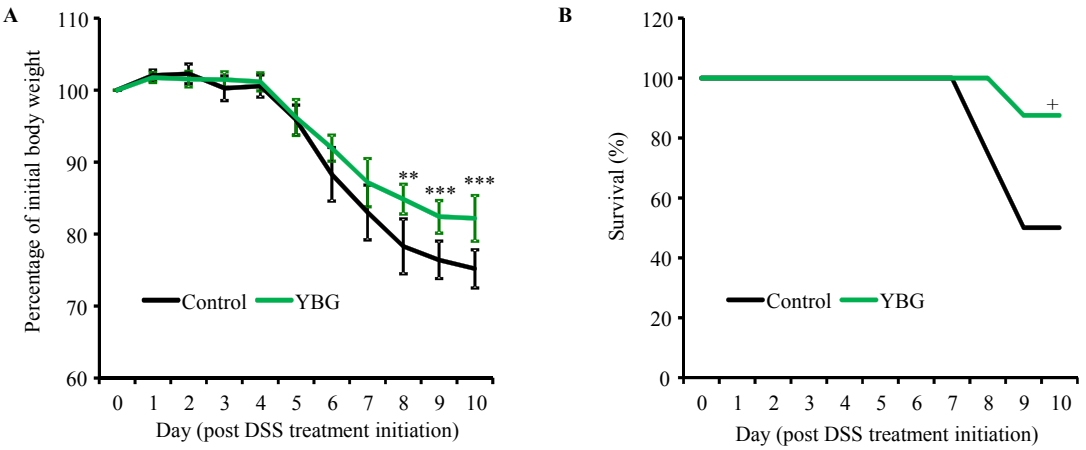
| Item | Vendor | Catalog # |
|---|---|---|
| DSS | MP Biomedicals, Irvine, CA | MFCD00081551 |
| CYBRgreen qPCR mix | Bio-Rad laboratories, Hercules, CA | 1725122 |
| Anti-mouse CD4-PEcy5 | BD Biosciences, San Jose, CA, USA | 553050 |
| Anti-mouse CD4 –PE-PETR | Invitrogen, Carlsbad, CA, USA | MCD0417 |
| Anti-mouse CD25-Alexa488 | Invitrogen, Carlsbad, CA, USA | RM6004 |
| Anti-mouse IFN-γ-PE | eBiosciencs/Affymetrix, Santa Clara, CA | 12-7311-82 |
| Anti-mouse IL-17A-PE | eBiosciencs/Affymetrix, Santa Clara, CA | 12-7177-81 |
| Anti-mouse IL-22 PE | eBiosciencs/Affymetrix, Santa Clara, CA | 12-7221-82 |
| Anti-Mouse IL-10-PE | eBiosciencs/Affymetrix, Santa Clara, CA | 12-7101-82 |
| Anti-Mouse IL-4-PE | eBiosciencs/Affymetrix, Santa Clara, CA | 53-7101-82 |
| Anti-mouse IL-9-PE | eBiosciencs/Affymetrix, Santa Clara, CA | 12-7098-42 |
| Mouse cytokine multiplex Assay | Invitrogen, Carlsbad, CA, USA | EPXR360-26092-901 |
| Teklad Global 18% Protein Rodent diet | Harlan Laboratories, Indianapolis, IN | 2018 |
| Mouse IL-4 | Invitrogen, Carlsbad, CA, USA | PMC0046 |
| Glucan from Baker's yeast | Sigma-Aldrich, St. Louis, MO, USA | G5011 |
| Percoll | Sigma-Aldrich, St. Louis, MO, USA | P1644 |
| PMA | Sigma-Aldrich, St. Louis, MO, USA | P8139 |
| Ionomycin | Sigma-Aldrich, St. Louis, MO, USA | I0634 |
| BHI agar | Sigma-Aldrich, St. Louis, MO, USA | 70138 |
| Ampicillin | Sigma-Aldrich, St. Louis, MO, USA | A1593 |
| Vancomycin | Sigma-Aldrich, St. Louis, MO, USA | 1709007 |
| Neomycin | Sigma-Aldrich, St. Louis, MO, USA | N1876 |
| Metronidazole | Sigma-Aldrich, St. Louis, MO, USA | M3761 |
| Brefeldin A | BD Biosciences, San Jose, CA, USA | 555029 |
| 96 Well MaxiSorp plate (Nunc) | Thermo-Fisher, Waltham, MA | 44-2404-21 |
| *Tnfa* 5'oligo-TTCTGTCTACTGAACTTC | Invitrogen, Carlsbad, CA, USA | Custom synthesis |
| *Tnfa* 3'oligo-CCATAGAACTGATGAGAG | Invitrogen, Carlsbad, CA, USA | Custom synthesis |
| *Il10* 5'oligo-AGCAGGTGAAGAGTGATT | Invitrogen, Carlsbad, CA, USA | Custom synthesis |
| *Il10* 3' oligo-GCAGTTGATGAAGATGTCA | Invitrogen, Carlsbad, CA, USA | Custom synthesis |
| *Raldh1a2* 5' oligo-TCACATCGGCATAGACAA | Invitrogen, Carlsbad, CA, USA | Custom Synthesis |
| *Raldh1a2* 3' oligo-TCTTCAGGTTACTTCTTCCA | Invitrogen, Carlsbad, CA, USA | Custom Synthesis |
| *Actin* 5'oligo AACTACATTCAATTCCATCA | Invitrogen, Carlsbad, CA, USA | Custom Synthesis |
| *Actin* 3-oligo GAGCAGTAATCTCCTTCT | Invitrogen, Carlsbad, CA, USA | Custom Synthesis |
| MiSeq Reagent Kit v3 | Illumina, Inc, San Diego, CA 92122 USA | MS-102-3003 |

Supplemental Fig. 1



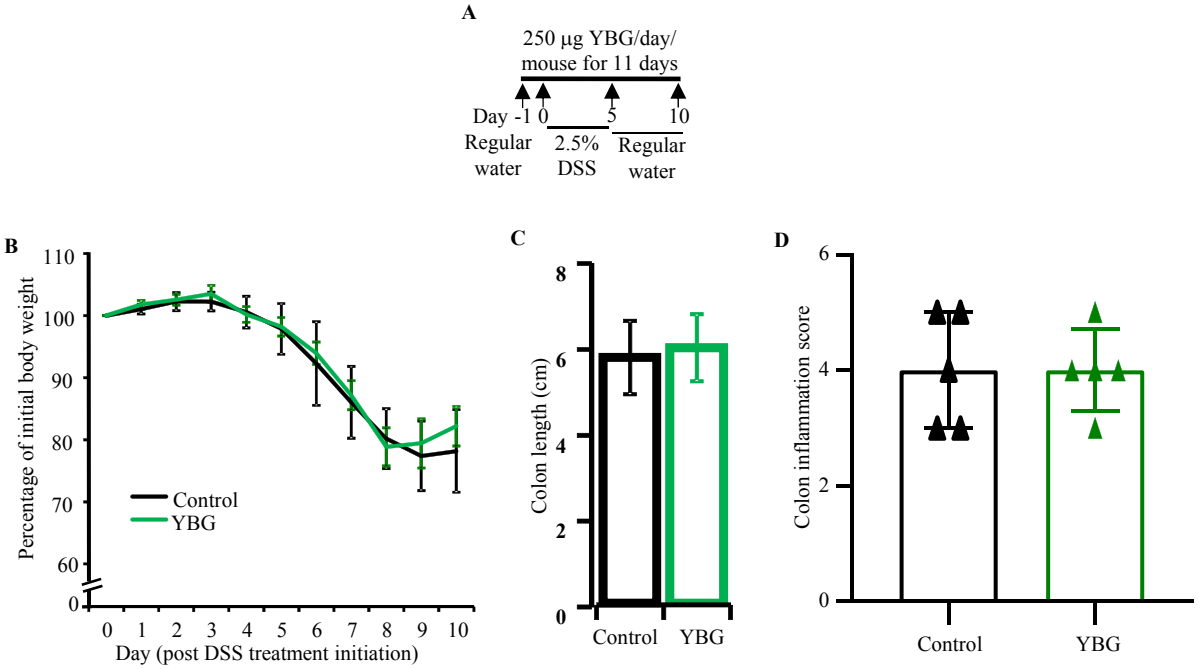

Supplemental Fig 1: Impact of pre-treatment with YBG on DSS induced colitis associated stool softening (A) and fecal blood levels (B). Stool consistency and blood content in stool of mice described in Fig. 1B were monitored daily starting at day 30 and scored. Mean±SDs (*n*=8) are shown for all panels. *Different from control, P<0.05.

Supplemental Fig. 2



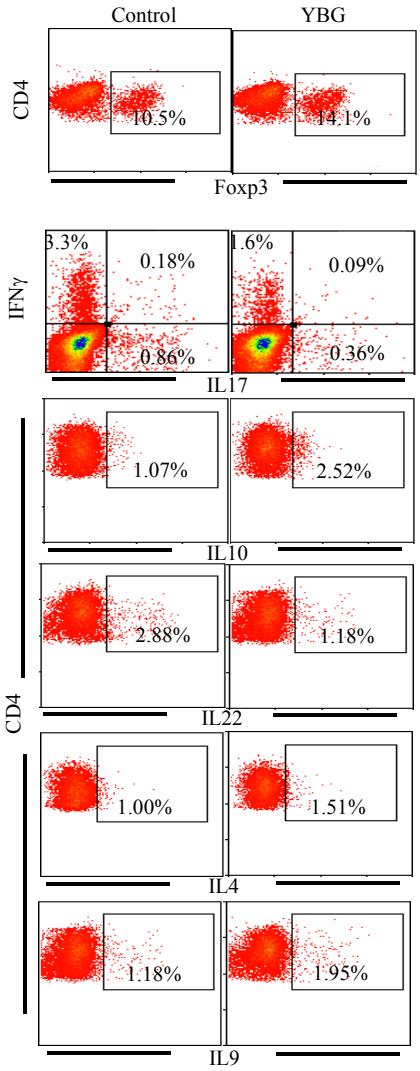

Supplemental Fig 2: Impact of pre-treatment with YBG (as shown in Fig. 1A) on DSS induced colitis associated weight loss (A) and mortality (B) in male B6 mice (Expt 1). (A) Body weights of individual animals were measured every day and changes in percentage of body weights, relative to initial body weight, are shown. (B) Percentage of total mice survived at different time-points are shown. Mean±SDs ($n$=8) are shown for panel A. $n$=8 for panel B. *,**,***Different from control; $P$<0.05, P<0.01, P<0.001 respectively. +Different from control; $P$=0.1; not significant.

Supplemental Fig. 3



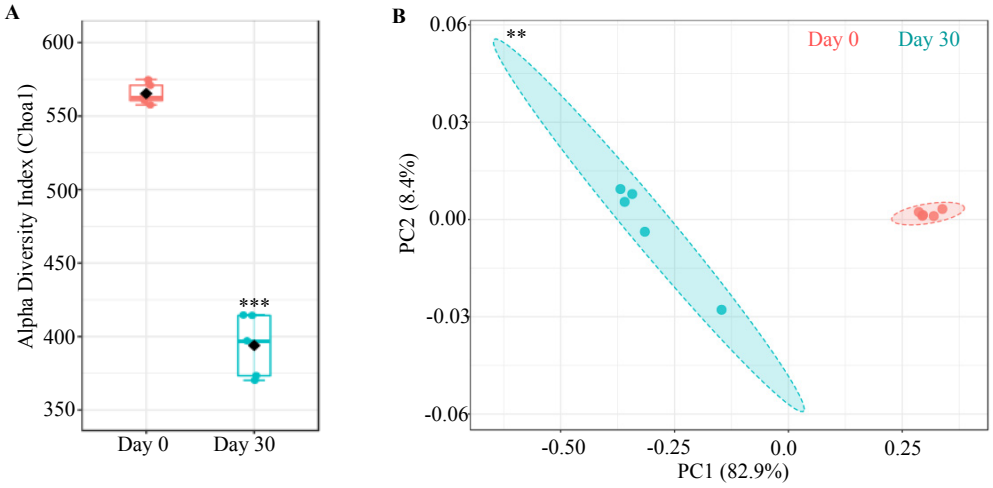

Supplemental Fig 3: Impact of YBG treatment initiation during DSS treatment (A) on colitis associated weight loss (B), shortening of colon (C) and colon inflammation (D) in B6 mice (Expt 1). (A) Cartoon depicting experimental design is shown. B) Body weights of individual animals were measured every day and changes in percentage of body weights, relative to initial body weight, are shown. (C) Colon length (right panel) of euthanized mice are shown. (D) H&E stained distal colon sections were evaluated for inflammation and severity scores are shown. (B, right panels of C, D) Mean±SDs ($n$=5).

Supplemental Fig. 4



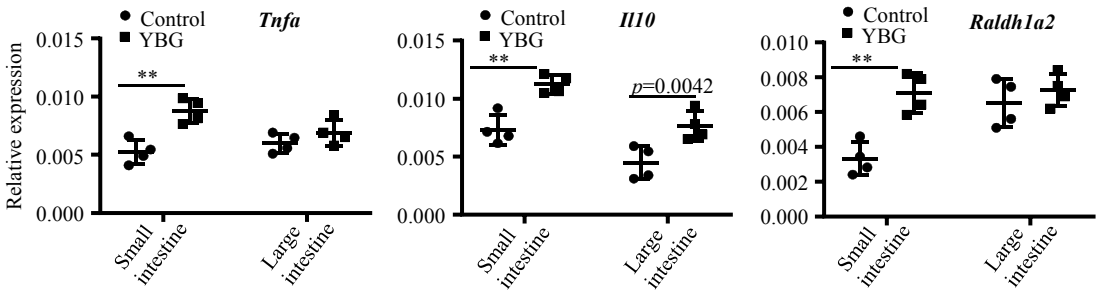

Supplemental Fig 4: Representative FACS plots showing frequencies of cells positive for indicated specific factors (Expt 1). Mean±SD values are shown in Fig. 2; panels A and B.

Supplemental Fig. 5



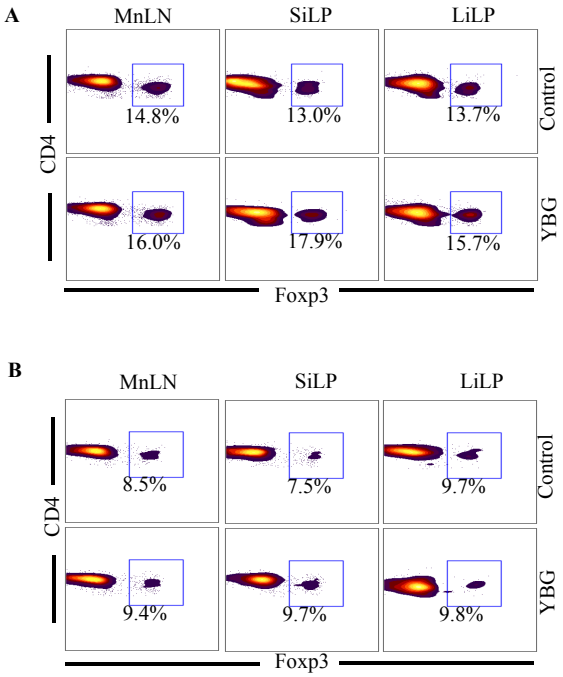

Supplemental Fig. 5: Impact of oral administration of YBG on fecal microbiota α-diversity (A) and β-diversity (B) measures in B6 mice (Expt 2). (A) (B) OTU data of samples collected on days 0 and 30 from YBG treated mice (described in Fig. 3) was examined for microbiota α-diversity/species richness and β-diversity measures. *n*=5 for all panels. *,**,*** Different from control; *P*<0.01, P<0.001 respectively.

Supplemental fig. 6



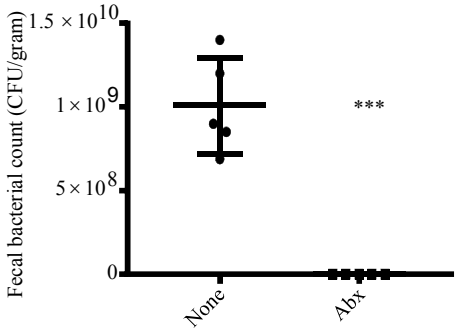

Supplemental Fig. 6: Effect of oral administration of YBG on cytokine expression in the intestine of B6 mice (Expt 2). cDNA prepared from the distal ileum and distal colons of YBG treated and control mice were subjected to qPCR and the expression levels of cytokines and non-cytokine factors relative to β-actin expression levels were compared. Mean±SDs ($n$=4) are shown. ** Different from control; $P$<0.01 respectively.

Supplemental Fig. 7




Supplemental Fig 7: Representative FACS plots showing frequencies of Foxp3+ cells in control and YBD treated B6 mice with intact (A) and depleted (B) gut microbiota (Expt 4). Mean±SD values are shown in Fig. 4 A and B, left panels.

Supplemental Fig. 8




Supplemental Fig. 8: Impact of treatment with antibiotic cocktail (Abx) on fecal microbes in B6 mice (Expt 3). Fecal pellets collected from individual mice were suspended in sterile PBS, plated onto brain heart infusion plates under anaerobic and aerobic conditions for up to 72 h and the total number of colonies (colony forming units; CFU) were determined. Mean±SDs ($n$=5) are shown. *** Different from control; $P$<0.001 respectively.