Fasting-Mimicking Diet Modulates Microbiota and Promotes Intestinal Regeneration to Reduce Inflammatory Bowel Disease Pathology

Priya Rangan et.al.

Published in Cell Reports 03/2019

Impact Factor: 7,815 (2018/19)

Presented by: Dragan Copic, PhD student

Content

- Introduction/Background Information
- Materials and Methods
- Results
- Conclusion

Inflammatory Bowel Disease (IBD)

Ulcerative Colitis	Crohn's Disease
Restricted to Rectum/Colon	Whole GIT can be affected
Primary manifestation: rectum	Primary manifestation: Ileum/Caecum
Continuous spread	Discontinuous spread
Inflammation is restricted to mucosa/submucosa	Transmural inflammation
Rarely stenosis	Stenosis
Rarely Fistula	Fistula

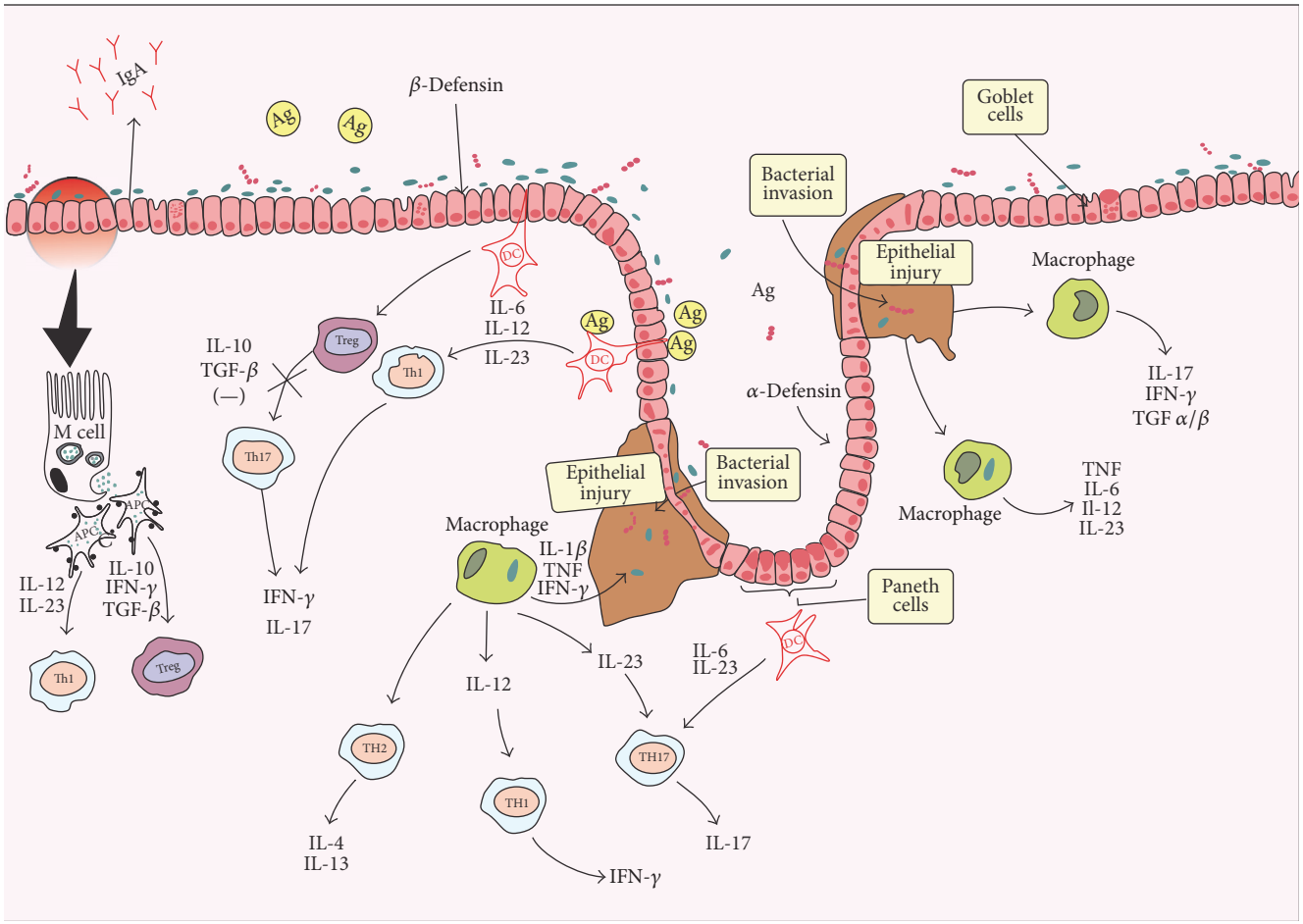
Inflammatory Bowel Disease

- Considered multifactorial diseases (non-dietaray and dietary risk factors)
- Characterized by chronic intestinal inflammation
- Prevalence of IBD is highest in the second to third decade of life
- clinical characteristics of IBD are
  -hemorrhagic diarrhea
  -tenesmus
  - abdominal pain
  -weight loss, anorexia
- Extraintestinal manifestations involve: Arthritis, uveitis, fever, erythema nodosum

Inflammatory Bowel Disease

- IBD is an immune-mediated disease
- The food and microbial flora within the intestine represent a enormous antigenic load
- Normal flora influences the maintenance of intestinal immunological homeostasis
- Microbial flora affects immune processes
  - secretion of antimicrobial peptides
  - regulatory and effector immune cells
- An altered balance of commensal pathogenic microbiota could lead to a pro-inflammatory milieu that exacerbates intestinal inflammation

Immunological Basis of IBD



Francesca A. et al. The Immunological Basis of Inflammatory Bowel Disease, Gastroenterology Research and Practic , 2016

Materials and Methods

- **Chronic dextran sodium sulfate (DSS)-induced mouse model**
- C57BL/6J (8 weeks old)
- 1DSS cycle = 5 consecutive days of 2% w/w Dextran sulfate sodium salt followed by 9 days of purified water
- After 33 days random-assignement to experimental groups → single-housing for the remainder of the experiment

  - two 2-day water only fasts or two 4-day FMD fasting cycles

- After the respective fasting cycles mice were fed with standard rodent chow

Disease Acticity Index (DAI) Scoring

Score	Body weight loss	Stool consistency	Rectal bleeding (Hemoccult)
0	no weight loss	solid pellets	No sign
1	1%–5%	soft but adherent in pellet shape	Hemoccult positive
2	5%–10%;	loose stool but with some solidity	Hemocult positive with visible pellet bleeding
3	10%–20%	loose stool with signs of liquid consistency	Hemoccult positive with visual pellet and rectal bleeding
4	greater than 20%	diarrhea	Hemocult positive with gross visual pellet and rectal bleeding

Mouse fasting mimicking diet

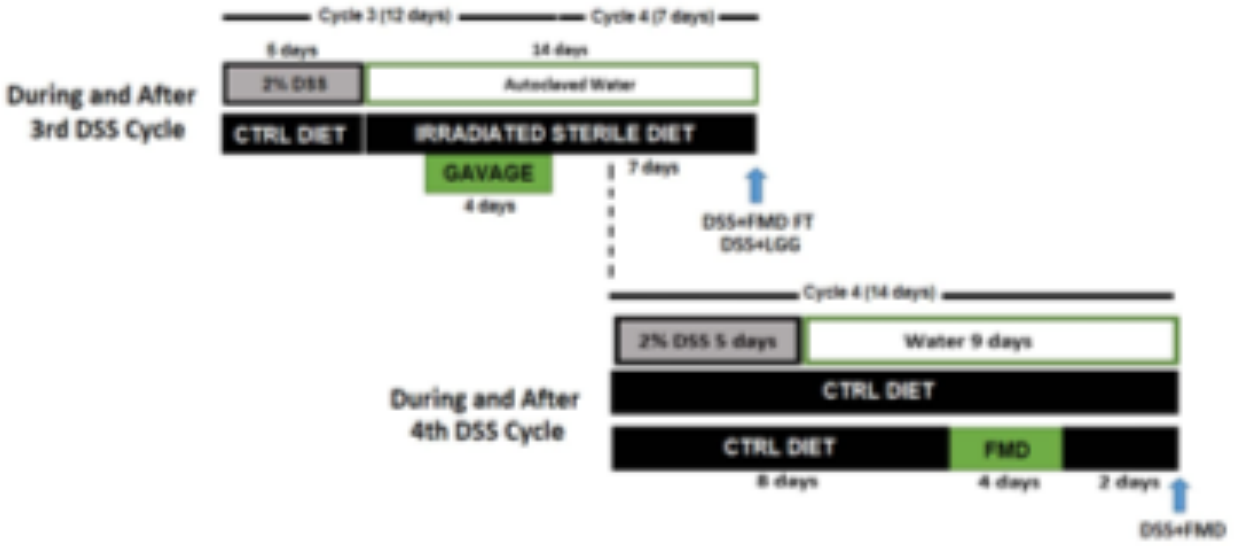
- first day of FMD, mice consumed 50% of their normal caloric intake (8.08 kJ/g; 0.56 kJ fat, 0.68 kJ carbohydrates, 0.11 kJ protein)

- From the second through fourth days of FMD, mice consumed 10% of their normal caloric intake (1.10 kJ/g; 0.27 kJ carbohydrates)

- flavored broth mixes, extra virgin olive oil (EVOO), essential fatty acids, vegetable powders vitamins, and minerals were thoroughly mixed and bound together with heated hydrogel

Fecal transplant and Lactobacillus transplant models

- ceca contents were removed from **naive** and chronic **DSS-induced mice** (**with or without FMD treatment**)
  → aseptically flushed into a sterile 50% glycerol/PBS solution
- **Lactobacillus rhamnosus GG** 5x10^7 cfu/mouse/day



Human FMD Trial

- 100 participants (generally healthy adult volunteers and 18 to 70 years of age; BMI, 18.5 and up) without a diagnosed medical condition in the previous 6 months were enrolled
- Instructed to consume the FMD for 5 continuous days and to return to their normal diet until the next cycle that was initiated approximately 25 days later
- Participants completed three cycles of this 5-day FMD
- Blood drawn at baseline (A), end of the first FMD (B) and after 5 to 7 days of normal caloric intake after the third FMD cycle (C)
- WBC and lymphocyte data was stratified post hoc with C-reactive protein levels < 1 mg/L (normal risk group) versus subjects with > 1 mg/L CRP (elevated risk group) at baseline.

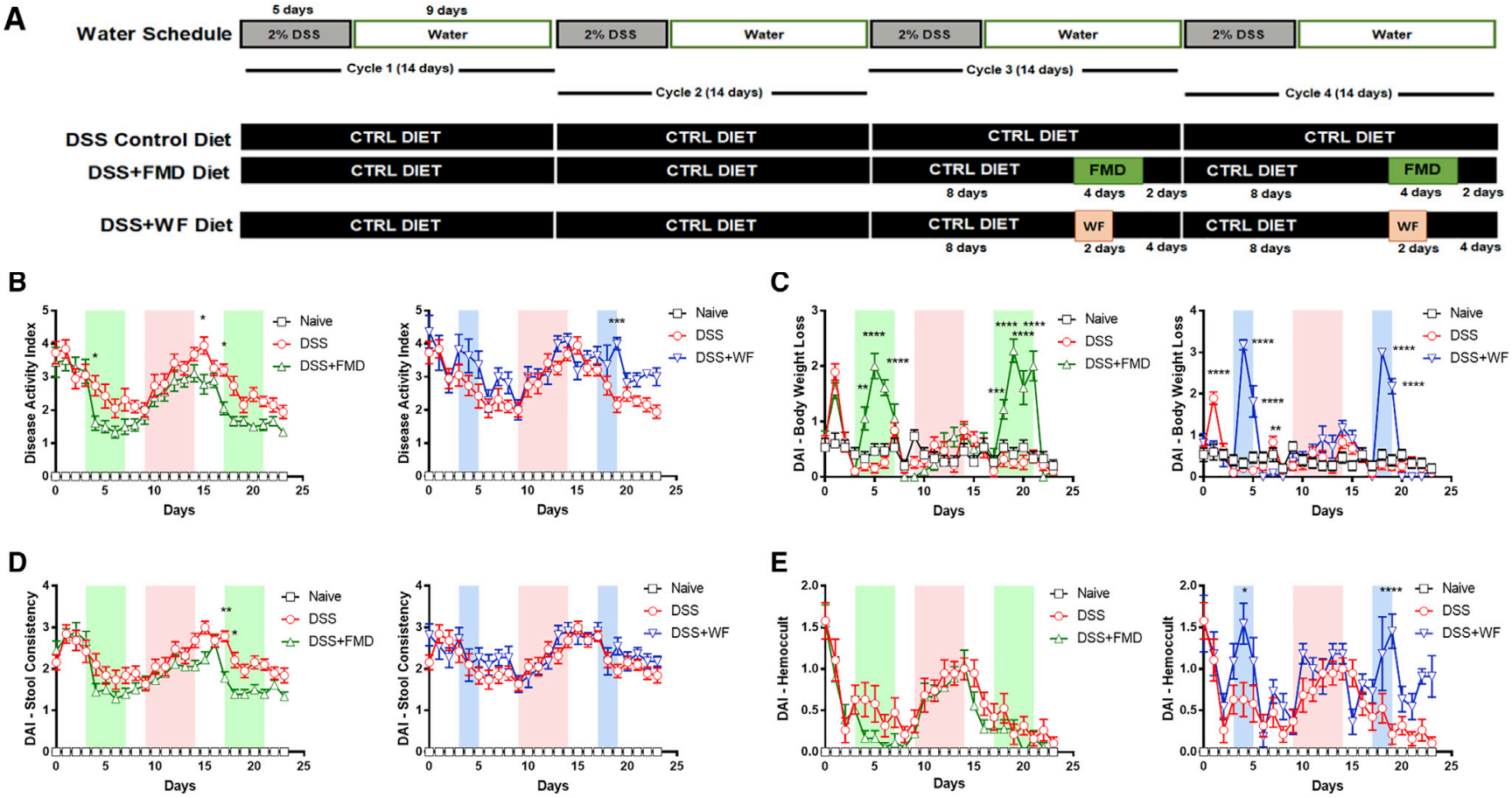
Further Examinations

- Colon Inflammation scoring in H&E tissue sections
- Immunohistochemystry, Immunofluorescence
- FITC Dextran permeability
- FACS analyses for different immune cell populations
- Cytokines profiling
  Serum, colonic supernatant, and colonic tissue homogenate
- Microbiome sequencing

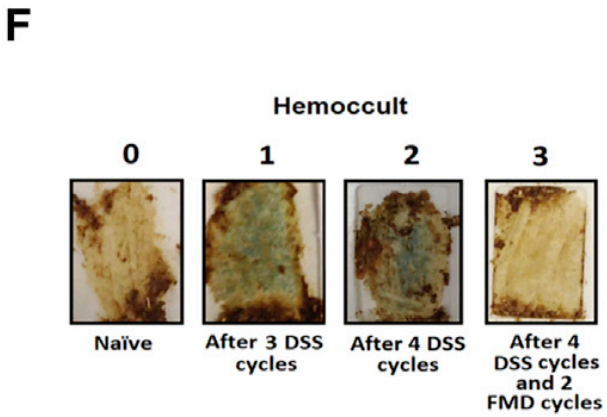
Results

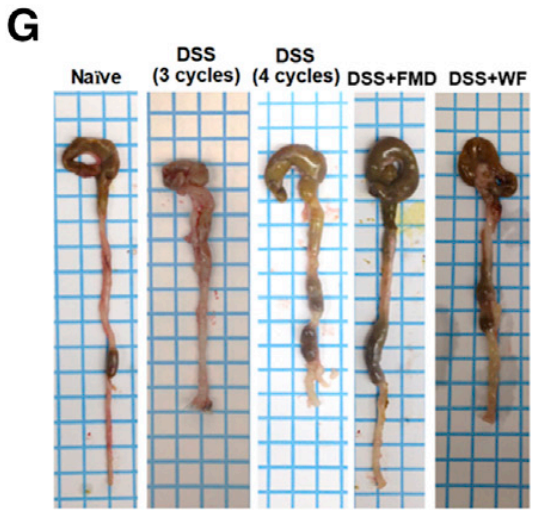
- FMD Cycles Ameliorate IBD-Associated Phenotypes
- FMD Cycles Alter Immune Cell Profile to Reduce Intestinal Inflammation
- FMD Stimulates an Increase in Microbial Strains Known to be Associated with T-Cell Regulation and Gut Regeneration
- Fecal Transplant from FMD-Treated Mice Promotes Positive Changes in IBD-Associated Symptoms
- FMD Cycles Reduce IBD-Associated Inflammation in Humans and Mice, in Part, by Modulating White Blood Cell Counts

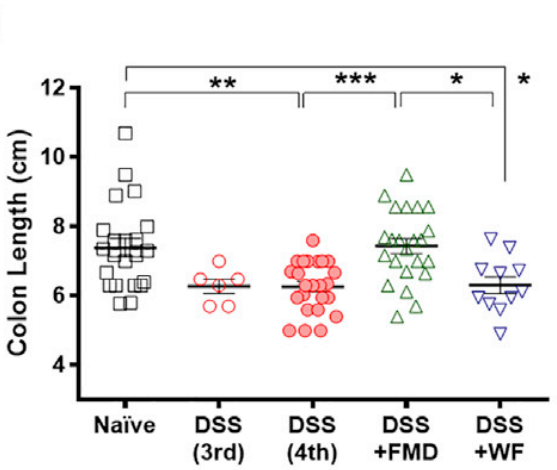
FMD Cycles Ameliorate IBD-Associated Phenotypes I

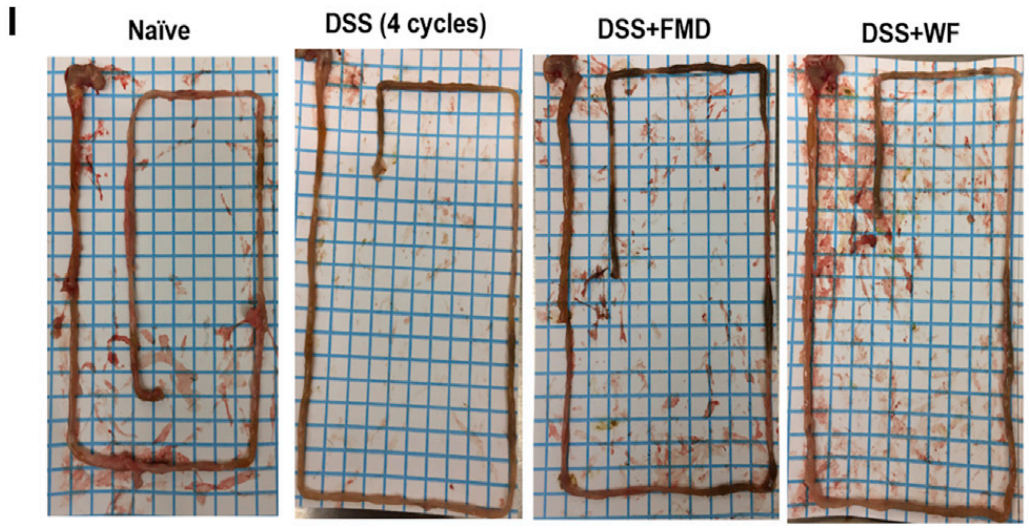


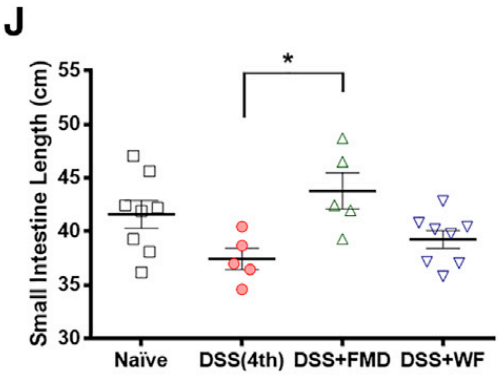
FMD Cycles Ameliorate IBD-Associated Phenotypes II



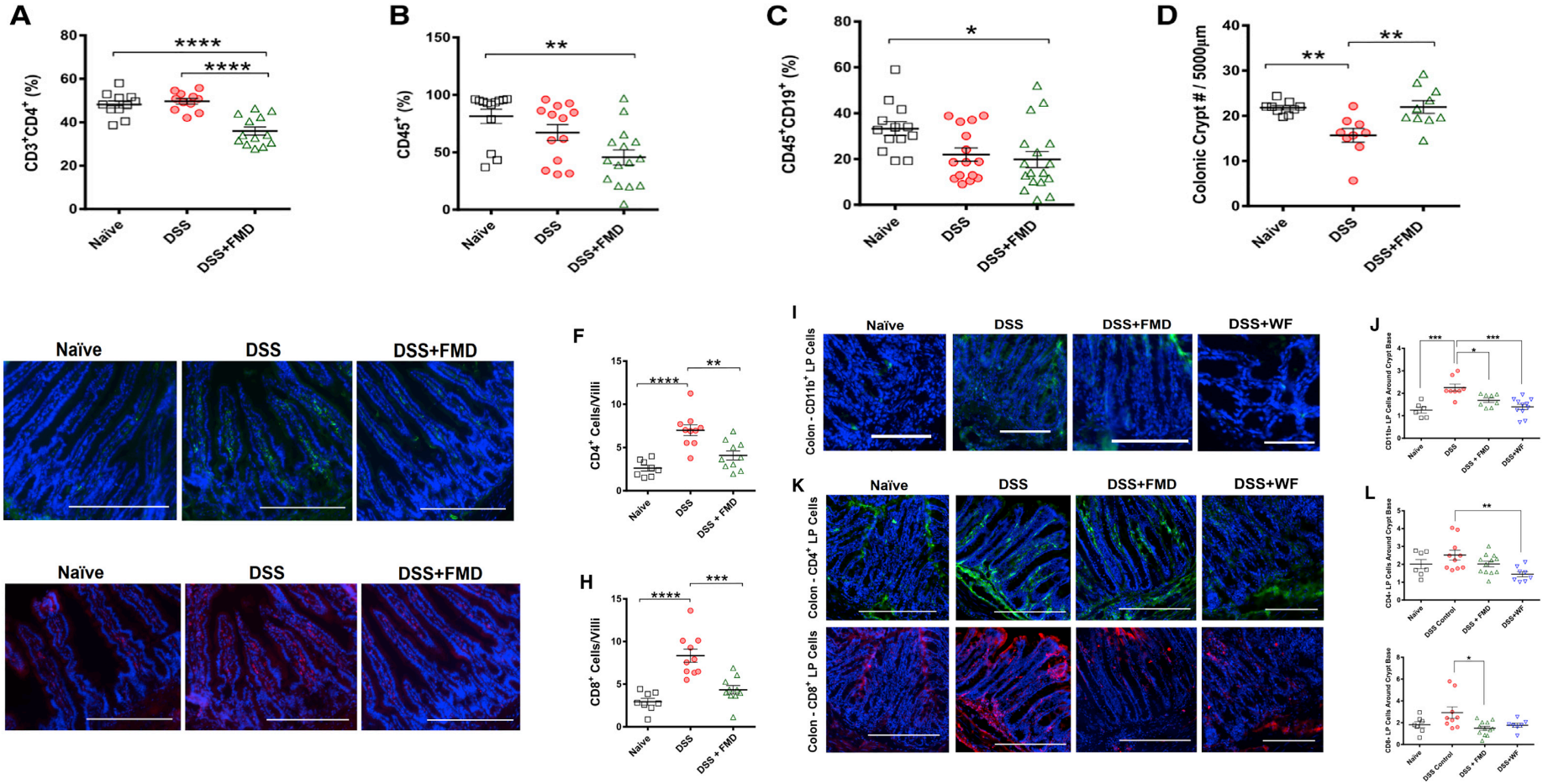




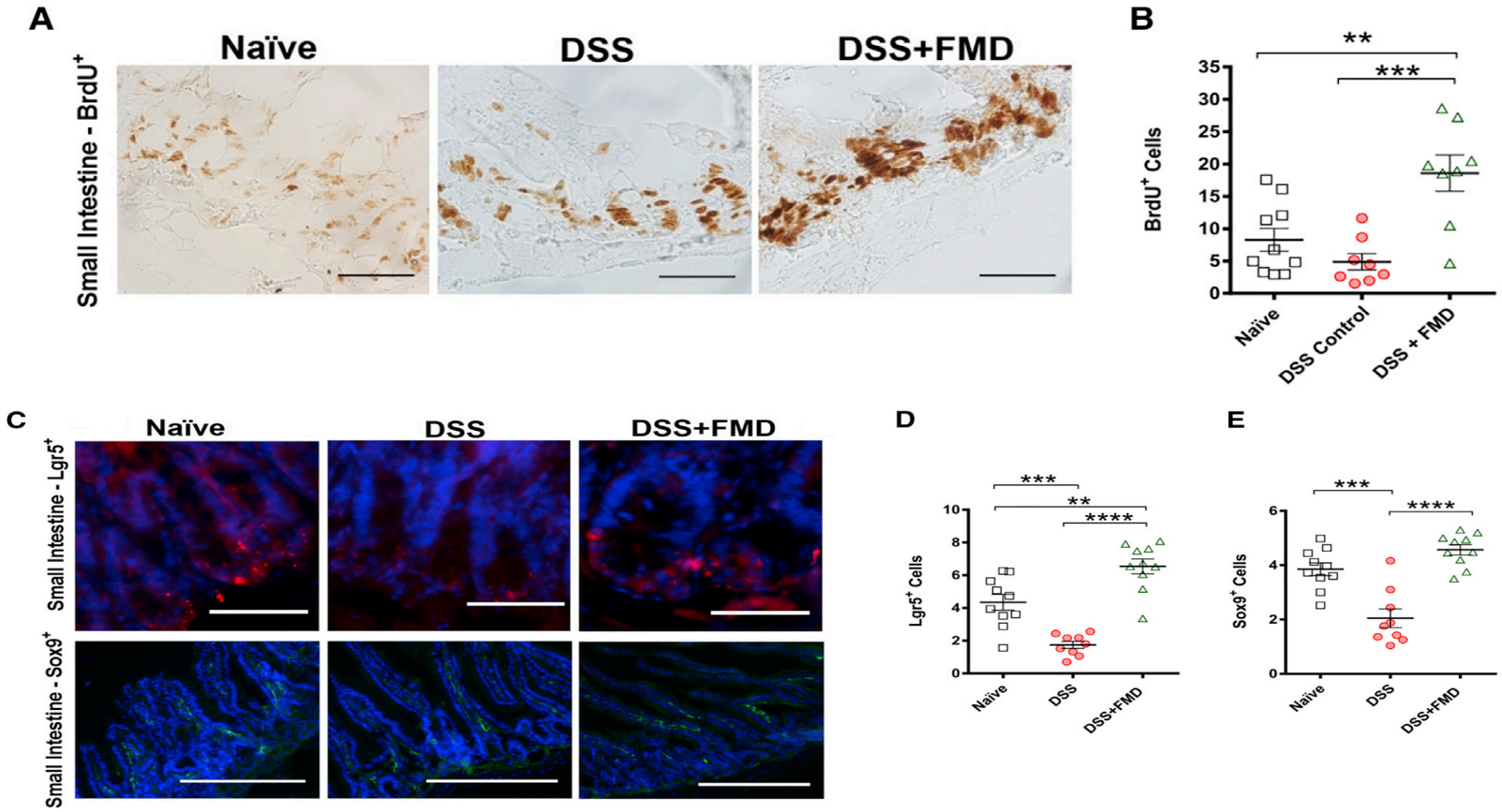




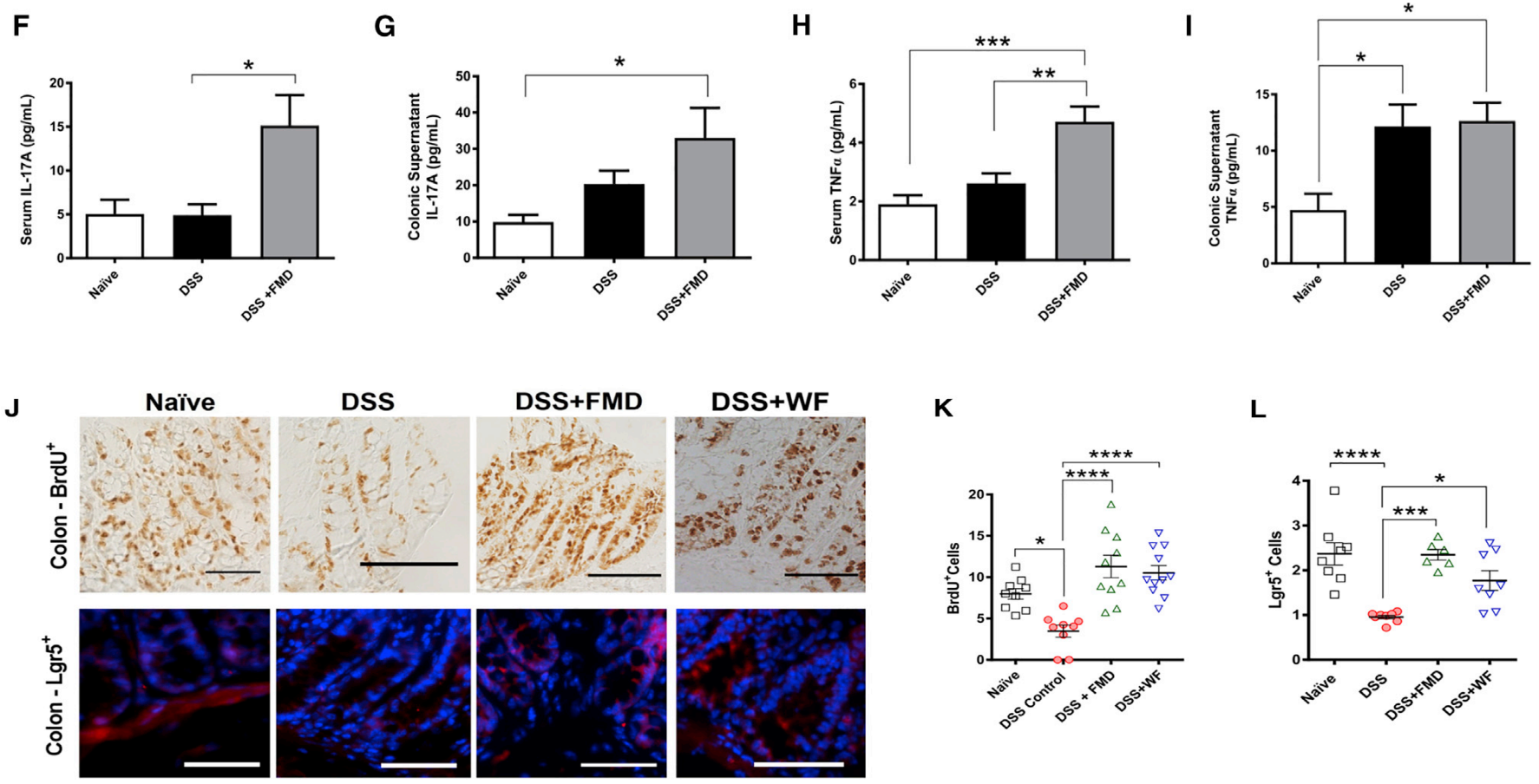
FMD Cycles Alter Immune Cell Profile to Reduce Intestinal Inflammation



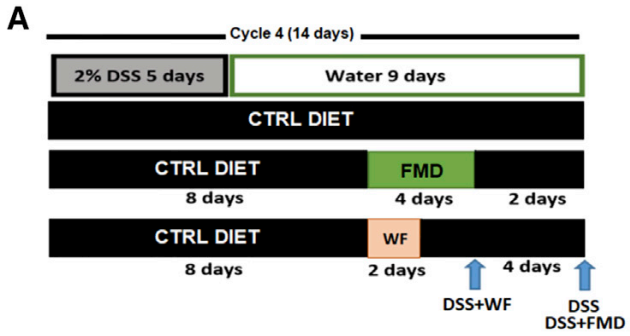
FMD Promotes Intestinal Regeneration I



FMD Promotes Intestinal Regeneration II



FMD Stimulates an Increase in Microbial Strains known to be Associated with T-Cell Regulation and Gut Regeneration



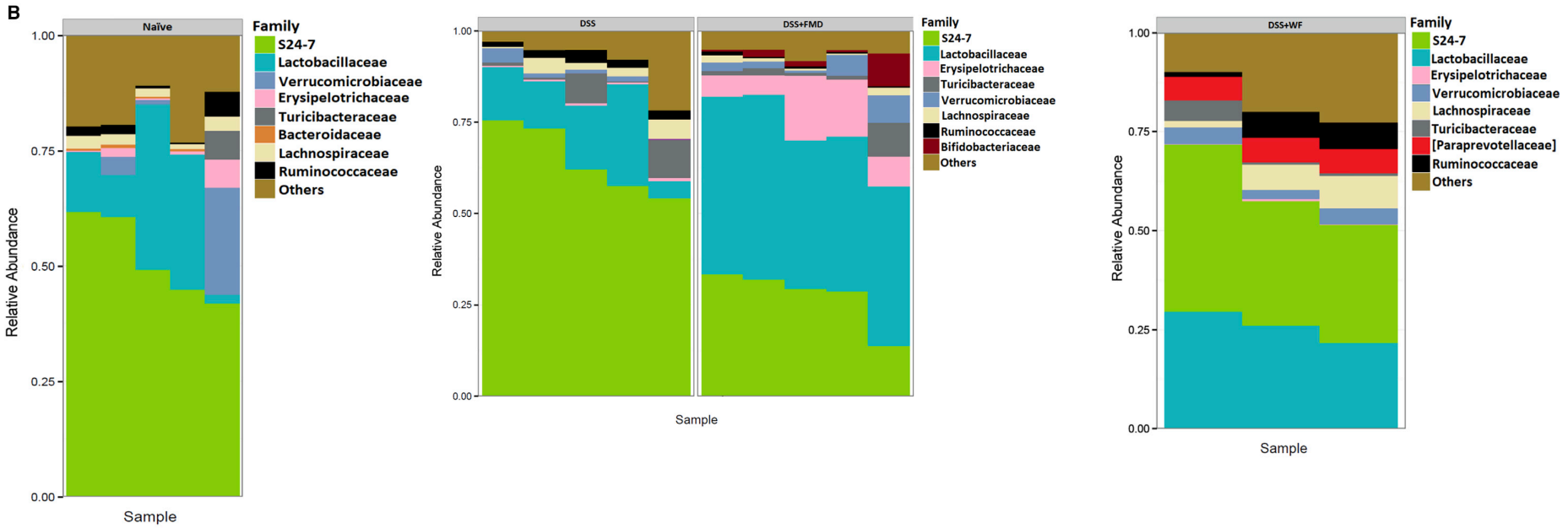


Table 1. Top 8 Most Abundant Families among the Naive, DSS, DSS+FMD, and DSS+WF Groups

Family	Naive Mean (SD)	DSS Mean (SD)	DSS+FMD Mean (SD)	DSS+WF Mean (SD)
S24-7	51.6 (9.08)	64.6 (9.46)	27.5 (7.9)	34.5 (6.72)
Lactobacillaceae	17.8 (14.2)	15.5 (8.36)	45.2 (4.2)	25.8 (3.97)
Erysipelotrichaceae	1.87 (2.41)	0.565 (0.226)	10.5 (5.71)	0.286 (0.184)
Turicibacteraeae	1.25 (2.78)	4.1 (4.83)	2.84 (3.63)	2.17 (2.59)
Verrucomicrobiaceae	5.65 (9.98)	1.57 (1.37)	3.65 (2.88)	3.5 (1.05)
Lachnospiraceae	2.18 (0.801)	2.83 (1.96)	1.16 (0.756)	5.42 (3.32)
Ruminococcaceae	2.09 (1.97)	2.36 (0.783)	0.568 (0.308)	4.81 (3.09)
Bacteroidaceae[a]	0.409 (0.257)	–	–	–
Bifidobacteriaceae[b]	–	0.0346 (0.0384)	2.67 (3.56)	–
[*Paraprevotellaceae*][c]	–	–	–	6.13 (0.148)

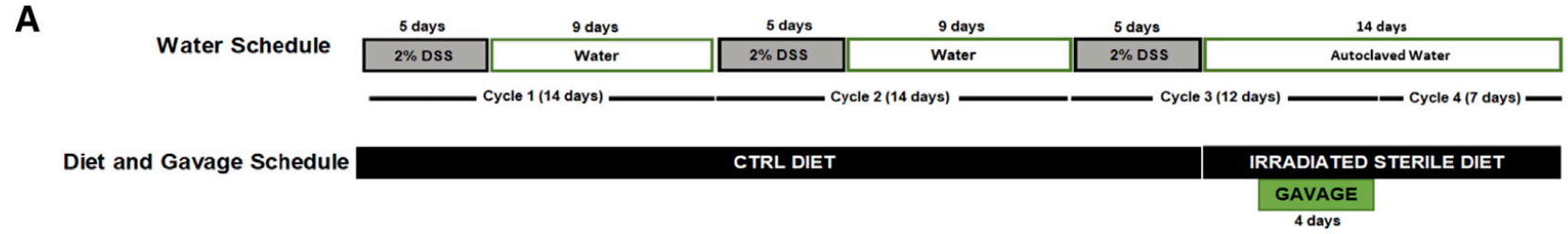
Related to Figures 4 and S3 and Tables S1–S6.

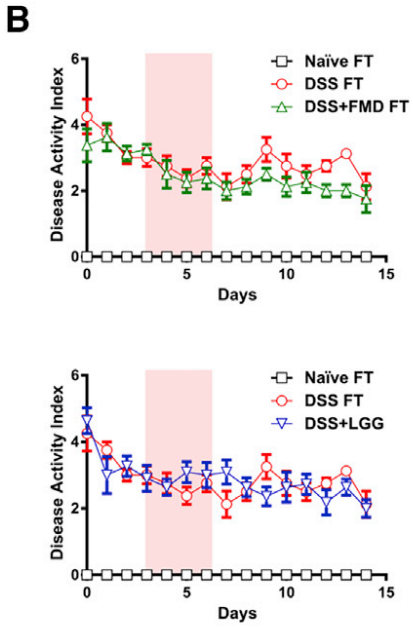
[a]Not ranked in top 8 most abundant families for DSS, DSS+FMD, and DSS+WF groups.

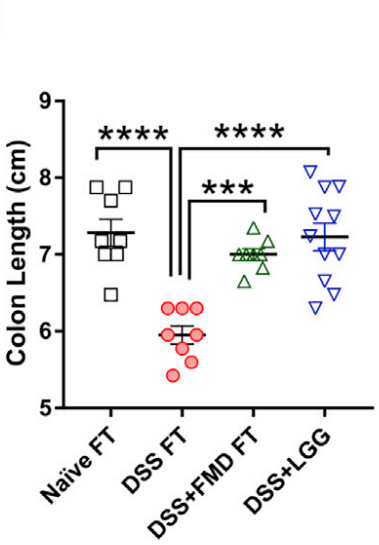
[b]Not ranked in top 8 most abundant families for Naive and DSS+WF group.

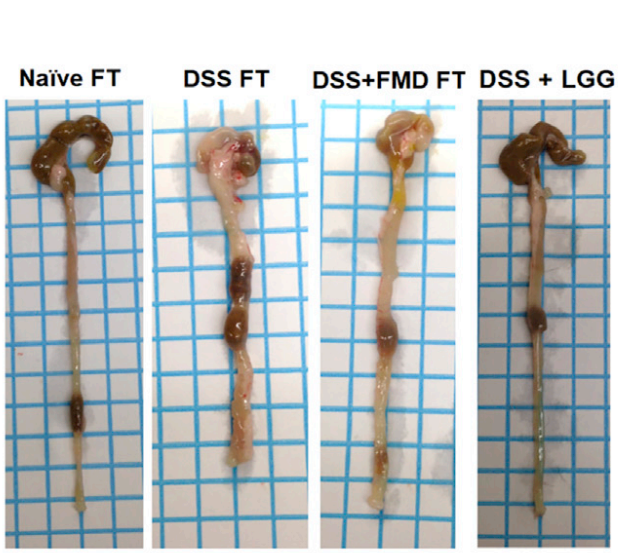
[c]Not ranked in top 8 most abundant families for Naive, DSS, and DSS+FMD groups.

Fecal Transplant from FMD-Treated Mice Improves IBD-Associated Phenotypes

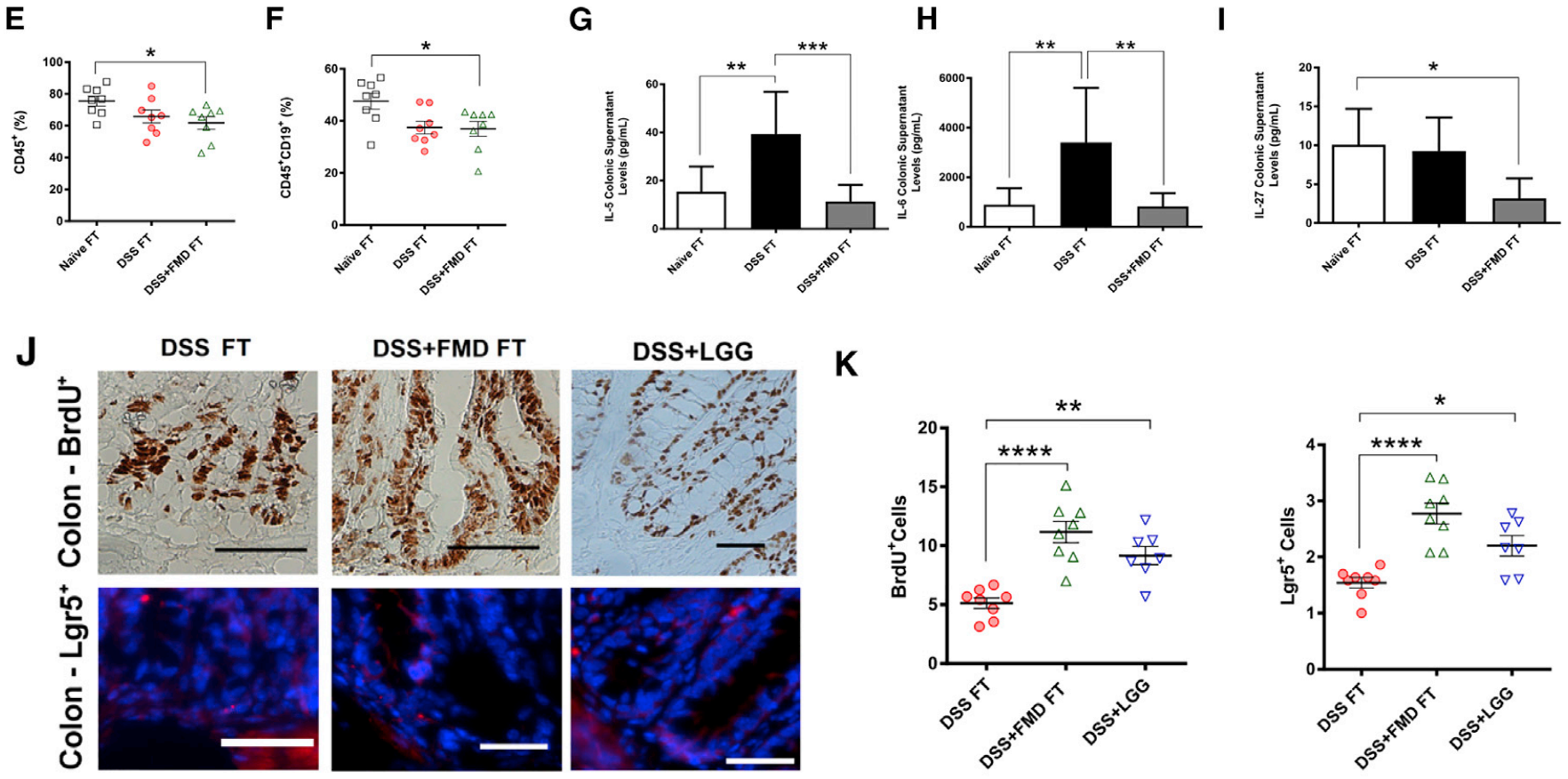




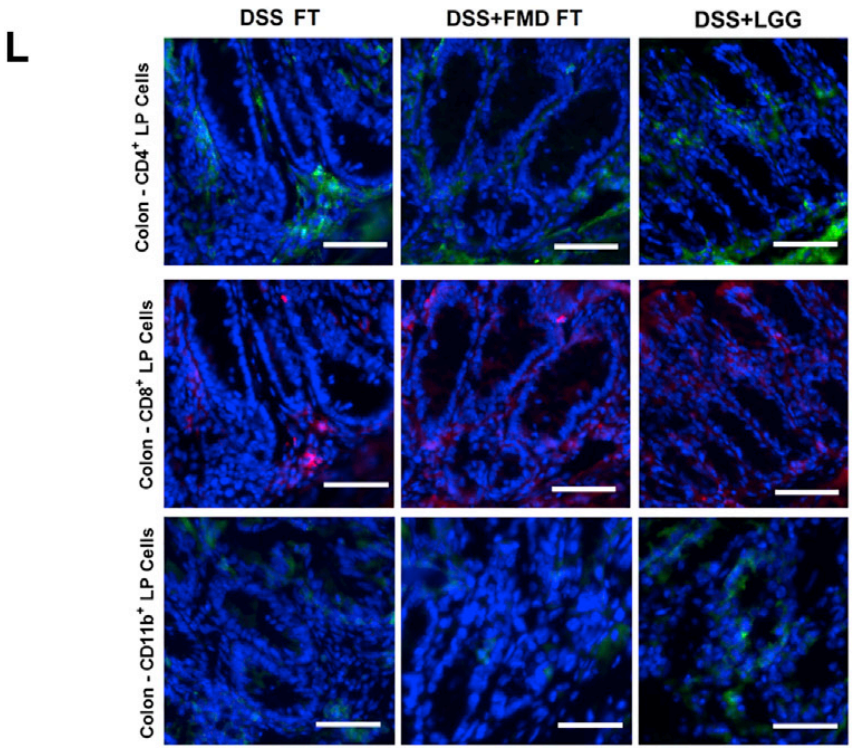


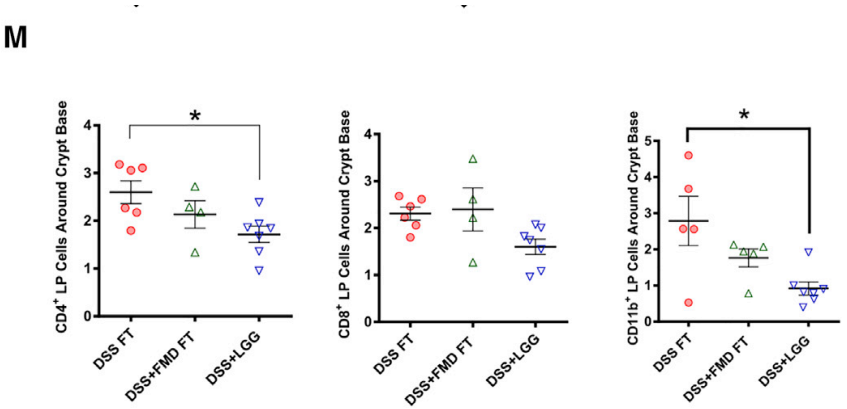


Fecal Transplant from FMD-Treated Mice Alters Immune Cell Profile and stimulates Regeneration in the Colon I

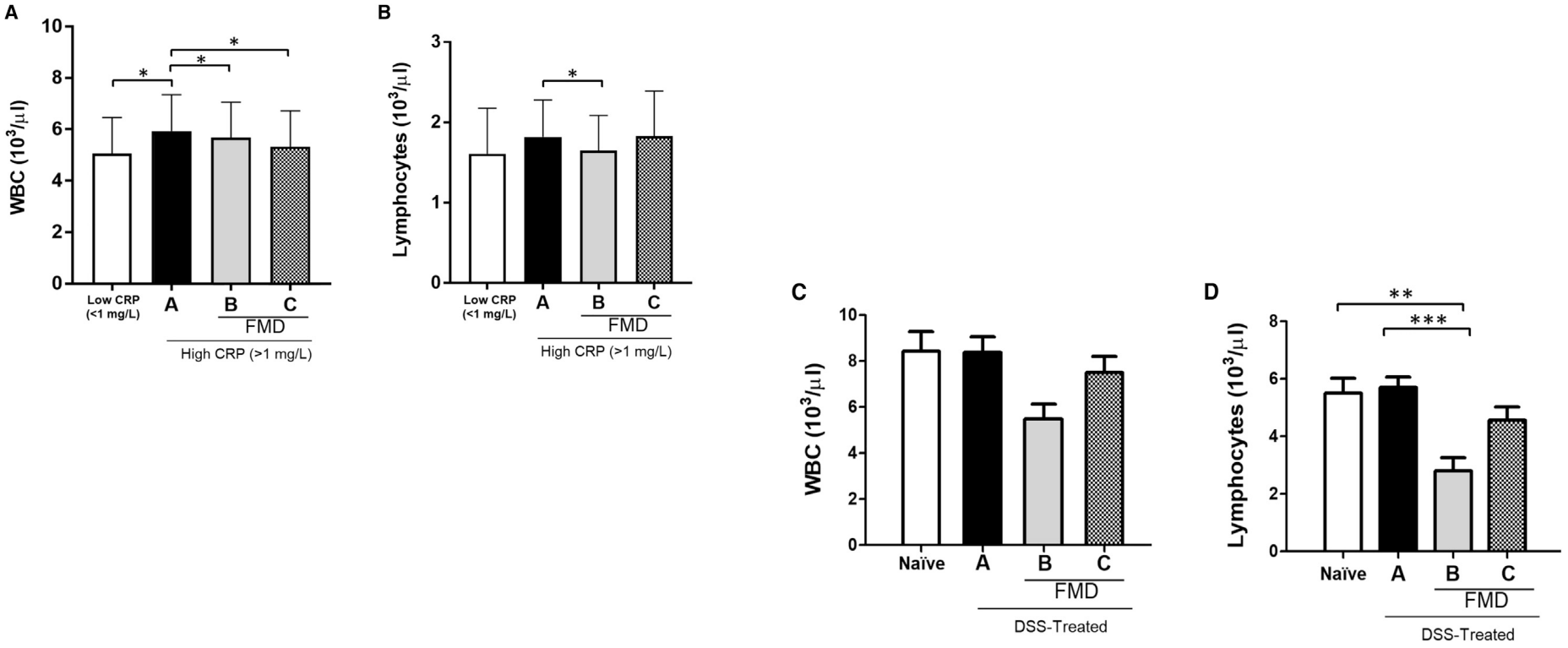


Fecal Transplant from FMD-Treated Mice Alters Immune Cell Profile and stimulates Regeneration in the Colon II





White Blood Cell (WBC) and Lymphocyte Counts in Humans and Mice with Systemic Inflammation



Discussion

- Dietary interventions have high potential to ameliorate and possibly reverse CD and ulcerative colitis
- Two cycles of a 4-day FMD followed by a normal diet are sufficient to mitigate some, and reverse other, IBD-associated pathologies or symptoms through modulation of the gut microbiome
- Certain nutrients in the FMD contribute to the microbial and anti-inflammatory changes necessary to maximize the effects of the fasting regimen
- fasting alone is not sufficient to reverse the pathology associated with IBD, but it is its combination with certain ingredients that is effective

Discussion

- growth and replacement of damaged intestinal tissues occur strongly during the re-feeding post-FMD
- They hypothesize that FMD cycles can first reduce the inflammation associated with IBD and subsequently promote regeneration during the refeeding stage

Thank you for your attention