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frontiers in Pharmacology

ORIGINAL RESEARCH
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CrossMark

Propionate Ameliorates Dextran Sodium Sulfate-Induced Colitis by Improving Intestinal Barrier Function and Reducing Inflammation and Oxidative Stress

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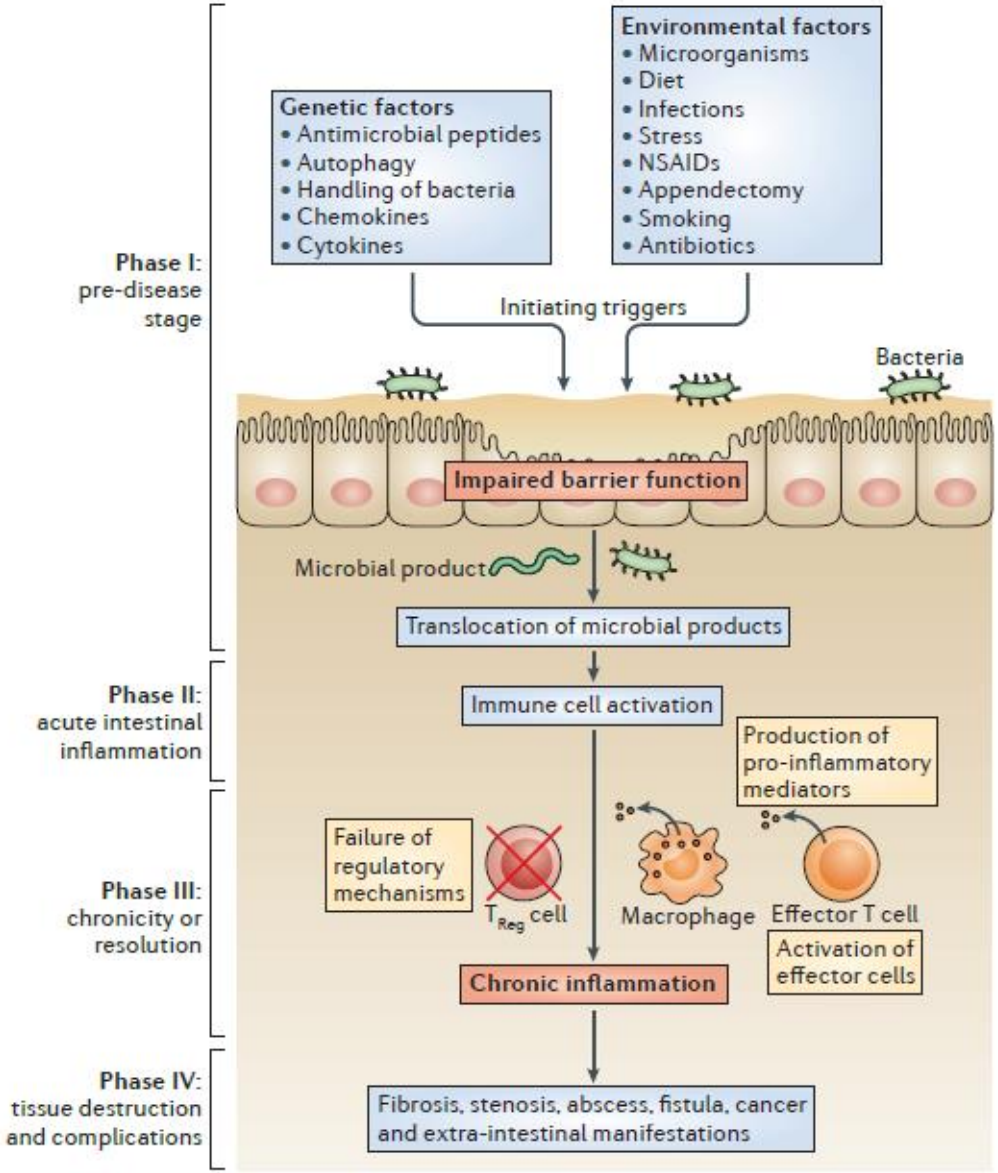
Inflammatory bowel disease (IBD)

Multifactorial disorder

- gene susceptibility, immune dysregulation, microbial flora, environmental factors

Two principal types

- Crohn’s disease (entire gastrointestinal tract)
- Ulcerative colitis (colon, rectum)



Markus F. Neurath. Cytokines in inflammatory bowel disease. *Nature Reviews Immunology* 14: 329-342 (2014)

DSS

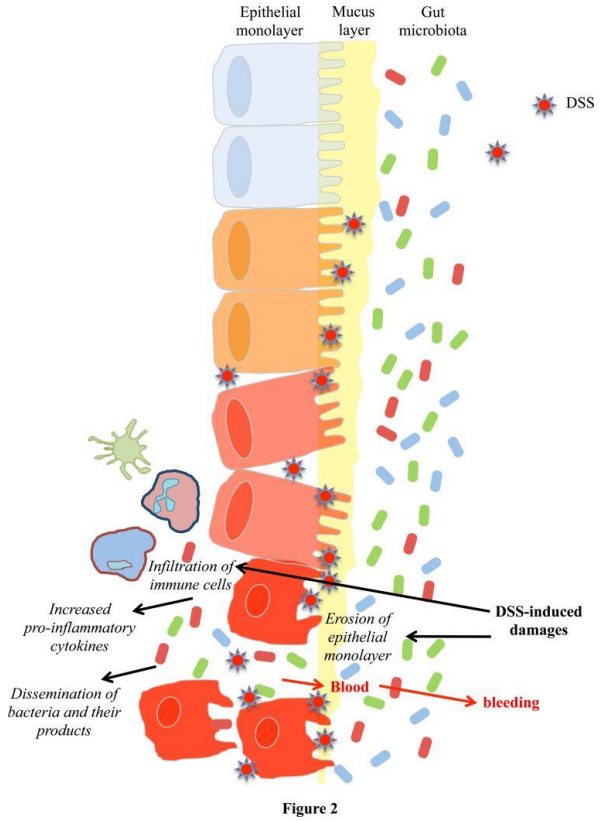


Figure 2

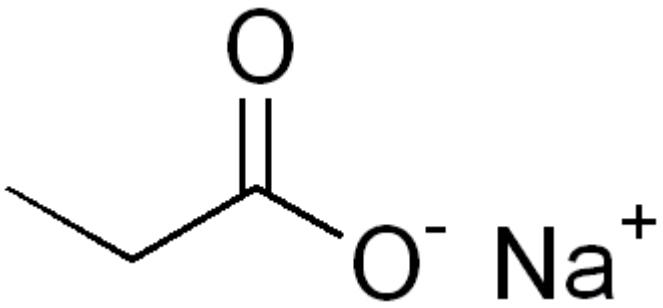
- Induction of experimental IBD with Dextran Sulfate Sodium (DSS)
- Loss of tight junction proteins → loss of colonic integrity → inflammation
- DSS binds to Medium-Chain-Length Fatty Acids forming a complex (~200nm) → able to fuse with colonocyte membranes

Chassaing B. *et al.* Dextran sulfate sodium (DSS)-induced colitis in mice. *Curr Protoc Immunol.* 104:Unit 15.25 (2014).

Common therapy

- Glucocorticoids, sulfasalazine, immunosuppressive drugs
- Clinical application of these substances is limited → adverse effects

Sodium propionate



- Short chain fatty acid
- Produced by anaerobic fermentation
- Reducing the production of pro-inflammatory cytokines
- Enhancing intestinal barrier function
- Inhibition of oxidative stress

Methods: DSS induced colitis in Animals

- C57BL/6J male mice
- 40 mice randomized to four groups:
    - Control group (drinking water for 14 days)
    - Propionate group (1% in ddH2O for 14 days)
    - DSS group (d1-d6 drinking water, d7-d14 3% DSS in ddH2O)
    - DSS/Propionate group (d1-d6 1% propionate from d7-d14 supplemented with 3% DSS)

Methods:
Histopathological assessment

- Measurement of colon length
- Paraffin embedded → cross-sectioning → HE-stain
- Histopathological evaluation
- 0: no obvious inflammatory reaction
- 1: the presence of low-level inflammatory reaction with a few scattered inflammatory cells
- 2: the presence of moderate inflammatory infiltration
- 3: the presence of severe inflammatory reaction in the colon tissue as represented by increased vascular density and thickness
- 4: the presence of large amounts of inflammation cell infiltration and rupture of goblet cell mass.

Methods:
In vivo Intestinal Permeability

- Mice were fastened o/n
- FITC-dextran delivered via gavage
- Scarification 4h after administration
- Serum levels of FITC (480 and 520nm microplate flourometer)

Methods:
RNA Isolation and Quantitative RT-PCR

- RNA extraction from colon tissue
- Inflammatory factors:
    - TNFα
    - IL-1β
    - IL-6

Methods: Immunoblotting

- Protein extraction
- Antibodies used:
    - Anti-ZO-1
    - Anti-occludin
    - Anti-E-cadherin
    - Anti-STAT3
    - Anti-p-STAT3

Methods: Measurement of Myeloperoxidase(MPO) Level in Colon and Serum

- MPO can modulate hydrogen peroxide
- Measurement of MPO activity
- MPO activity was defined as the quantity of enzyme degrading 1 mmol/ml of peroxideat 37°C

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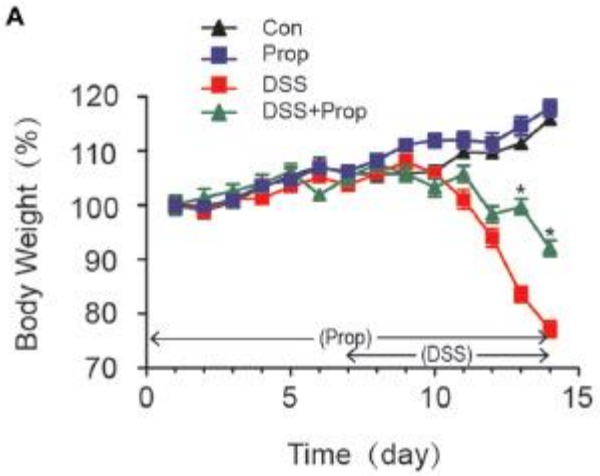
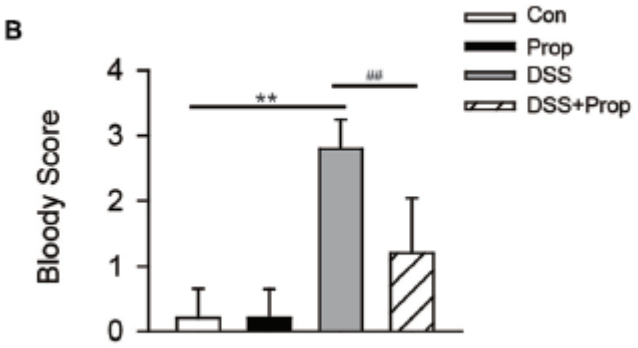
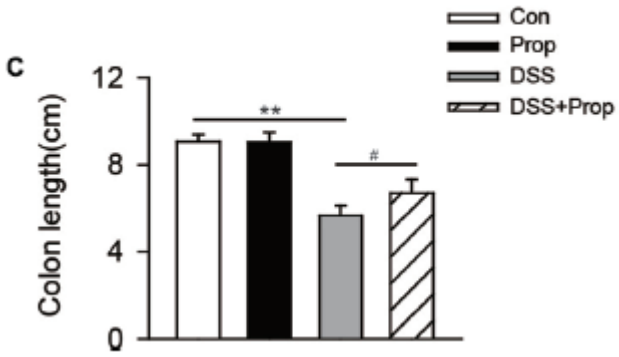
Methods: Assessment of Macrophages in Colonic Mucosa by Immunofluorescence

- Immunoflouresence of colonic tissue
- Anti-CD68-antibody

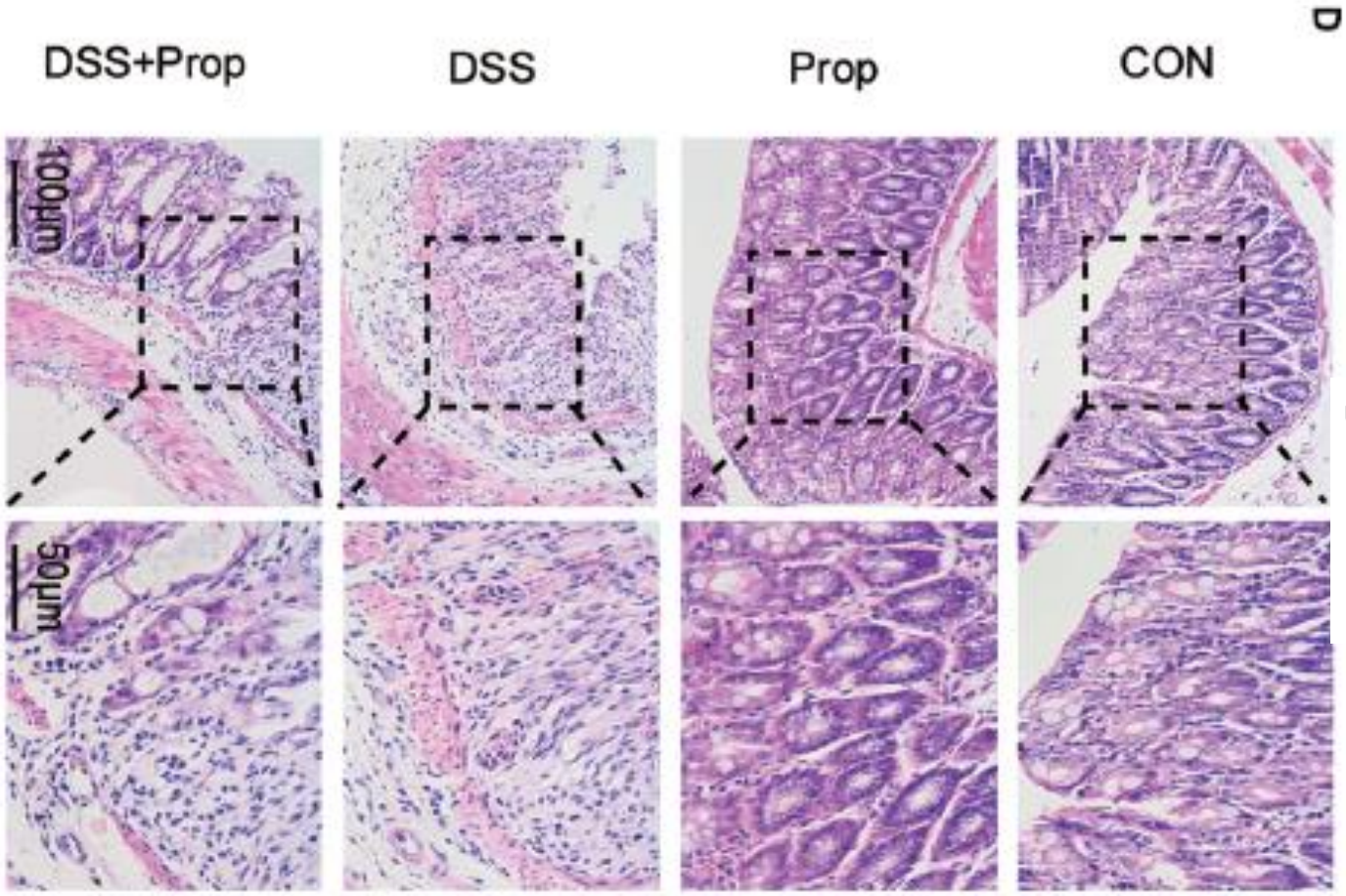
Results

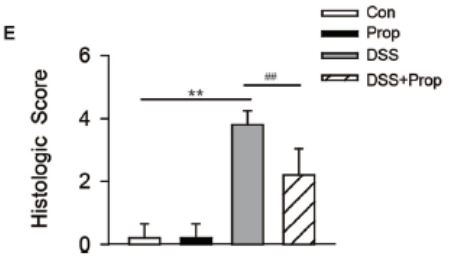

A
Con
Prop
DSS
DSS+Prop
Body Weight (%)
120
110
100
90
80
70
0
5
10
15
Time (day)
(Prop)
(DSS)
*
*


B
Con
Prop
DSS
DSS+Prop
Bloody Score
4
3
2
1
0
**
##

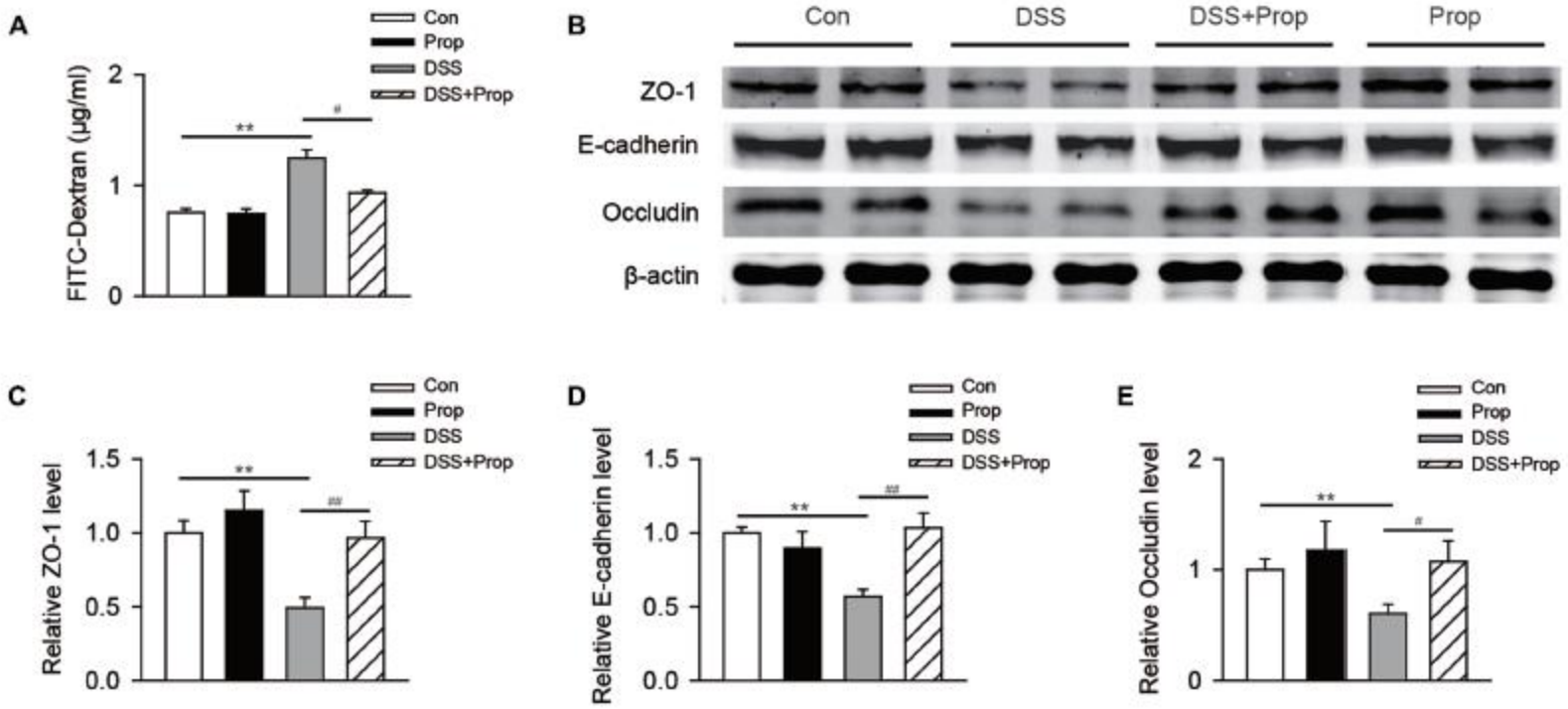

C
Con
Prop
DSS
DSS+Prop
Colon length(cm)
12
8
4
0
**
#

Results

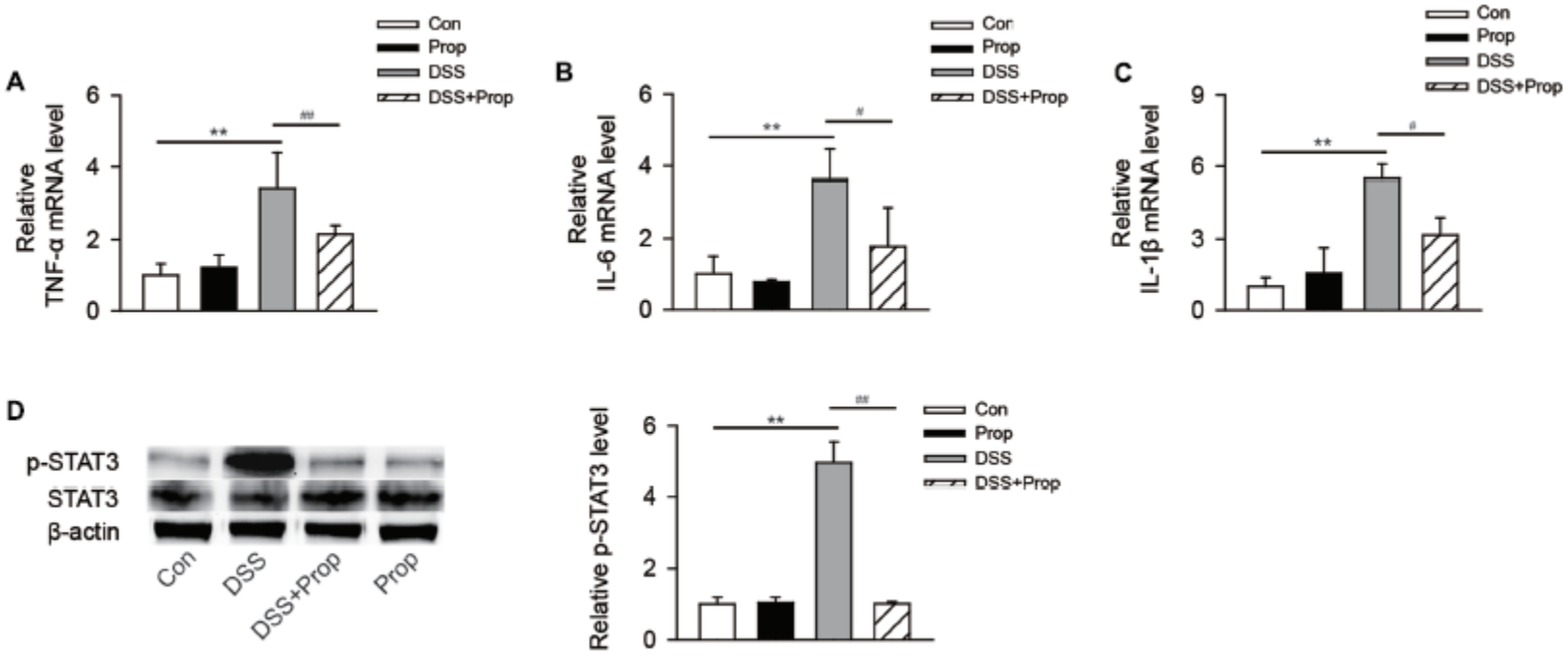

DSS+Prop
DSS
Prop
CON
100μm
50μm


E
Con
Prop
DSS
DSS+Prop
Histologic Score
6
4
2
0
**
##

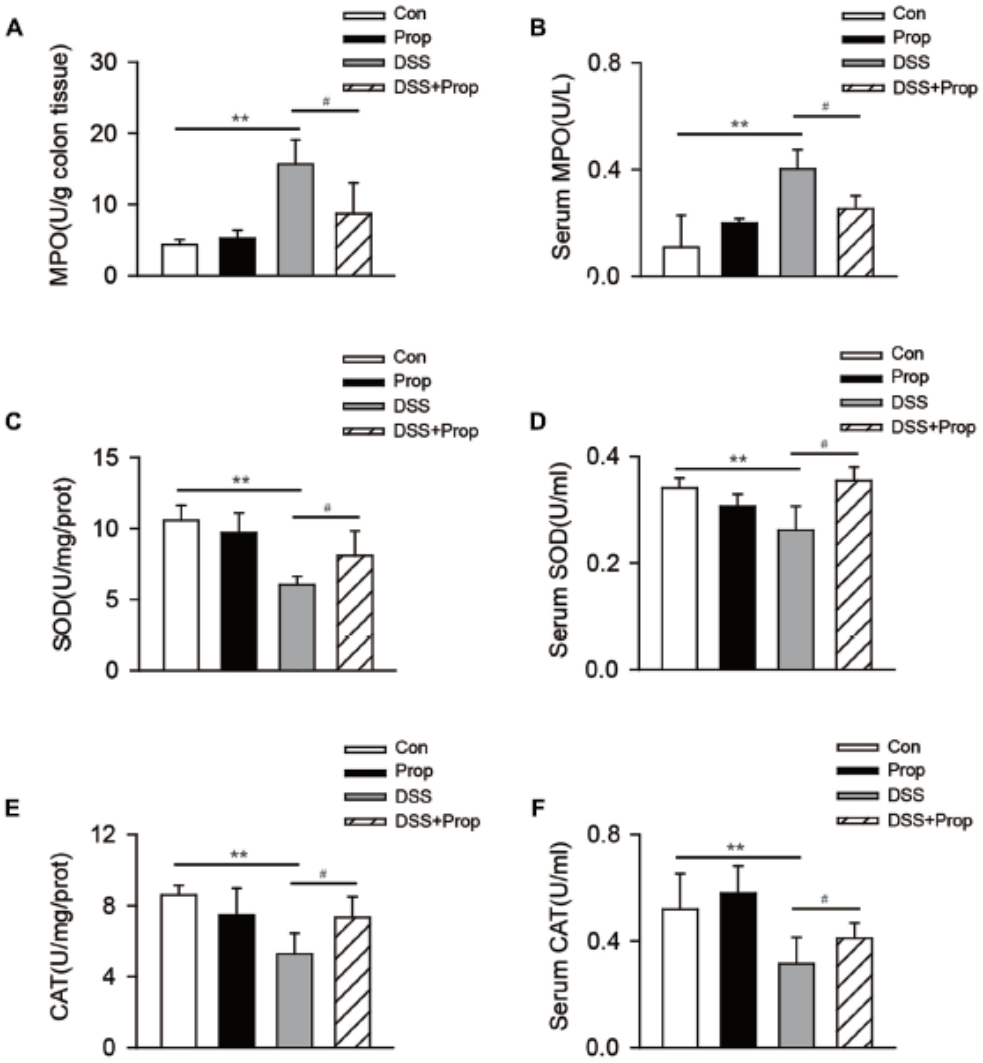
Results


A
Con
Prop
DSS
DSS+Prop
FITC-Dextran (μg/ml)
2
1
0
**
#
B
Con
DSS
DSS+Prop
Prop
ZO-1
E-cadherin
Occludin
β-actin
C
Relative ZO-1 level
1.5
1.0
0.5
0.0
**
##
D
Relative E-cadherin level
1.5
1.0
0.5
0.0
**
##
E
Relative Occludin level
2
1
0
**
#

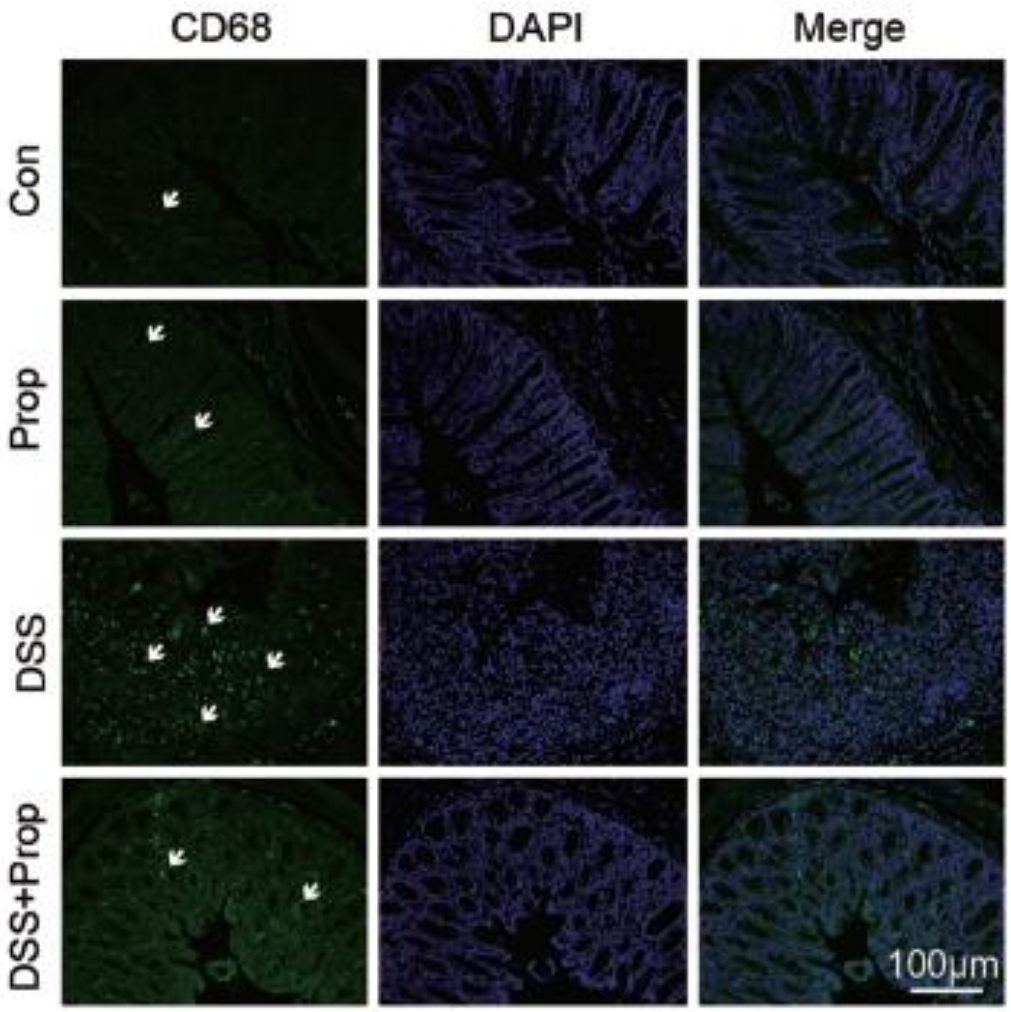
Results

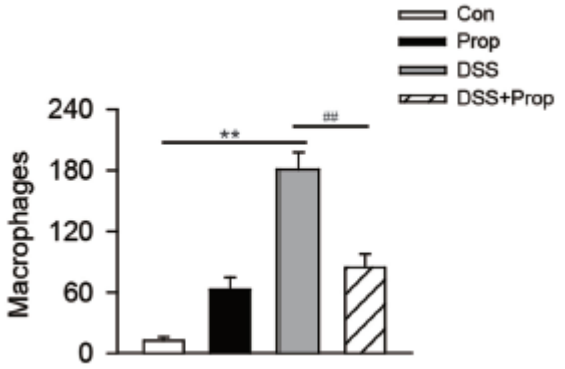

A
Relative TNF-α mRNA level
Con
Prop
DSS
DSS+Prop
B
Relative IL-6 mRNA level
C
Relative IL-1β mRNA level
D
p-STAT3
STAT3
β-actin
Con
DSS
DSS+Prop
Prop
Relative p-STAT3 level

Results


A
MPO(U/g colon tissue)
30
20
10
0
Con
Prop
DSS
DSS+Prop
**
#
B
Serum MPO(U/L)
0.8
0.4
0.0
C
SOD(U/mg/prot)
15
10
5
0
D
Serum SOD(U/ml)
0.4
0.2
0.0
E
CAT(U/mg/prot)
12
8
4
0
F
Serum CAT(U/ml)
0.8
0.4
0.0

Results


CD68
DAPI
Merge
Con
Prop
DSS
DSS+Prop
100µm


Con
Prop
DSS
DSS+Prop
Macrophages
240
180
120
60
0
**
##

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Discussion

- Sodium propionate inhibits down-regulation of ZO-1, occluding, E-cadherin
- Sodium propionate reduces the expression of pro-inflammatory cytokines: inflammatory factors TNF-α, IL-1β, and IL-6 mRNA
- Sodium propionate reduces CD68 expression in colonic tissue $\downarrow$ macrophages infiltration
- Sodium propionate inhibits oxidative stress reduces MPO activity and enhances SOD and CAT activities in serum
- Sodium propionate inhibits phosphorylation of STAT3