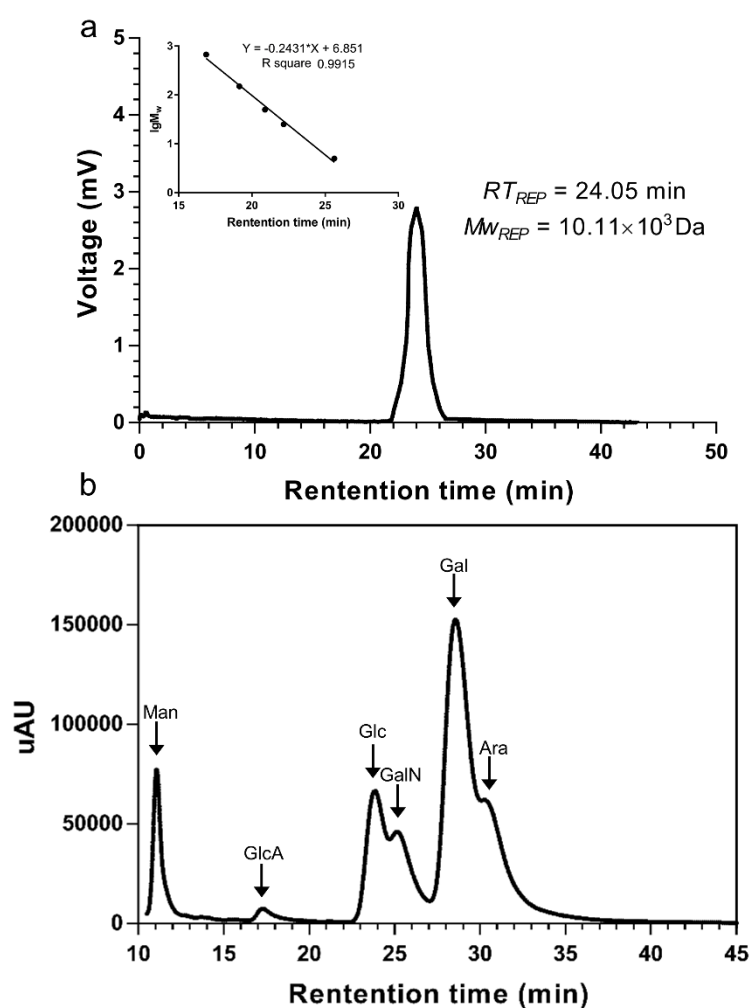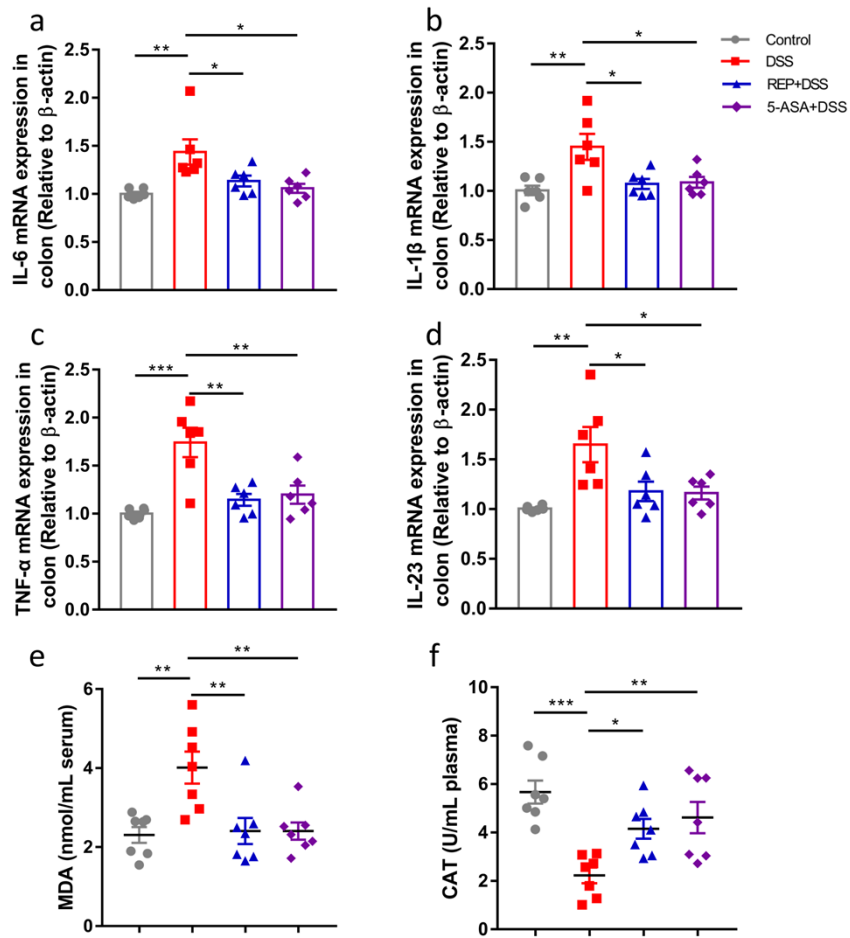# Supplementary Materials

Gut microbiota mediates anti-colitis effects of polysaccharides derived from

Rhopilema esculentum Kishinouye in mice
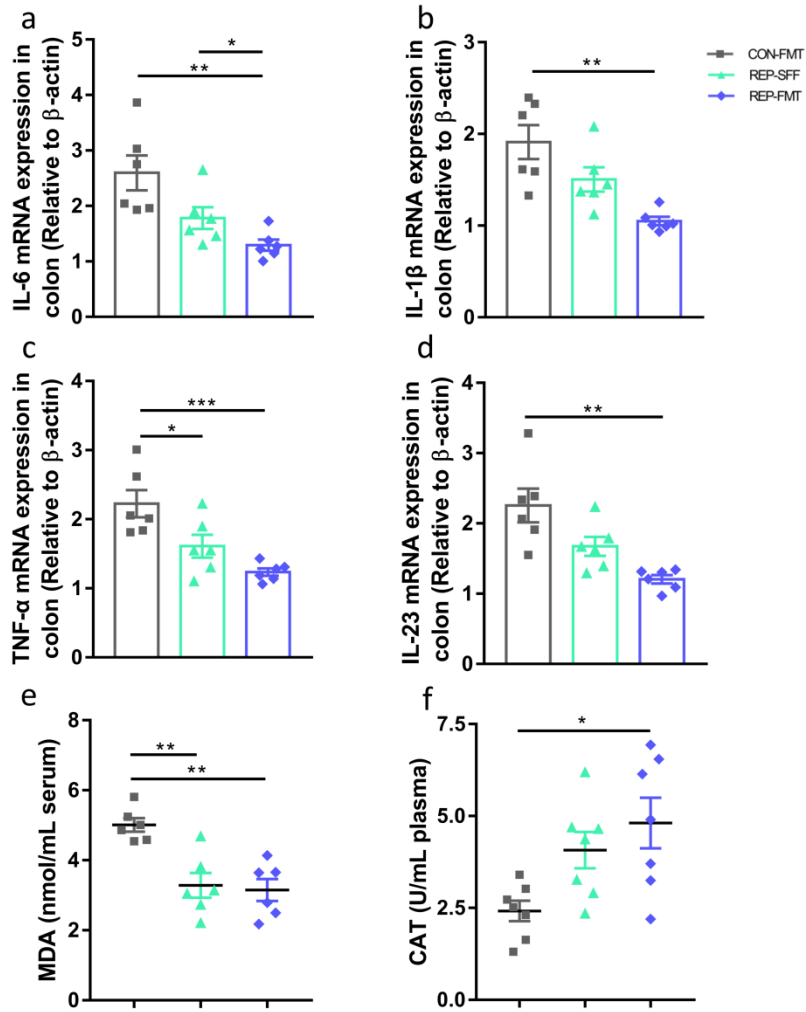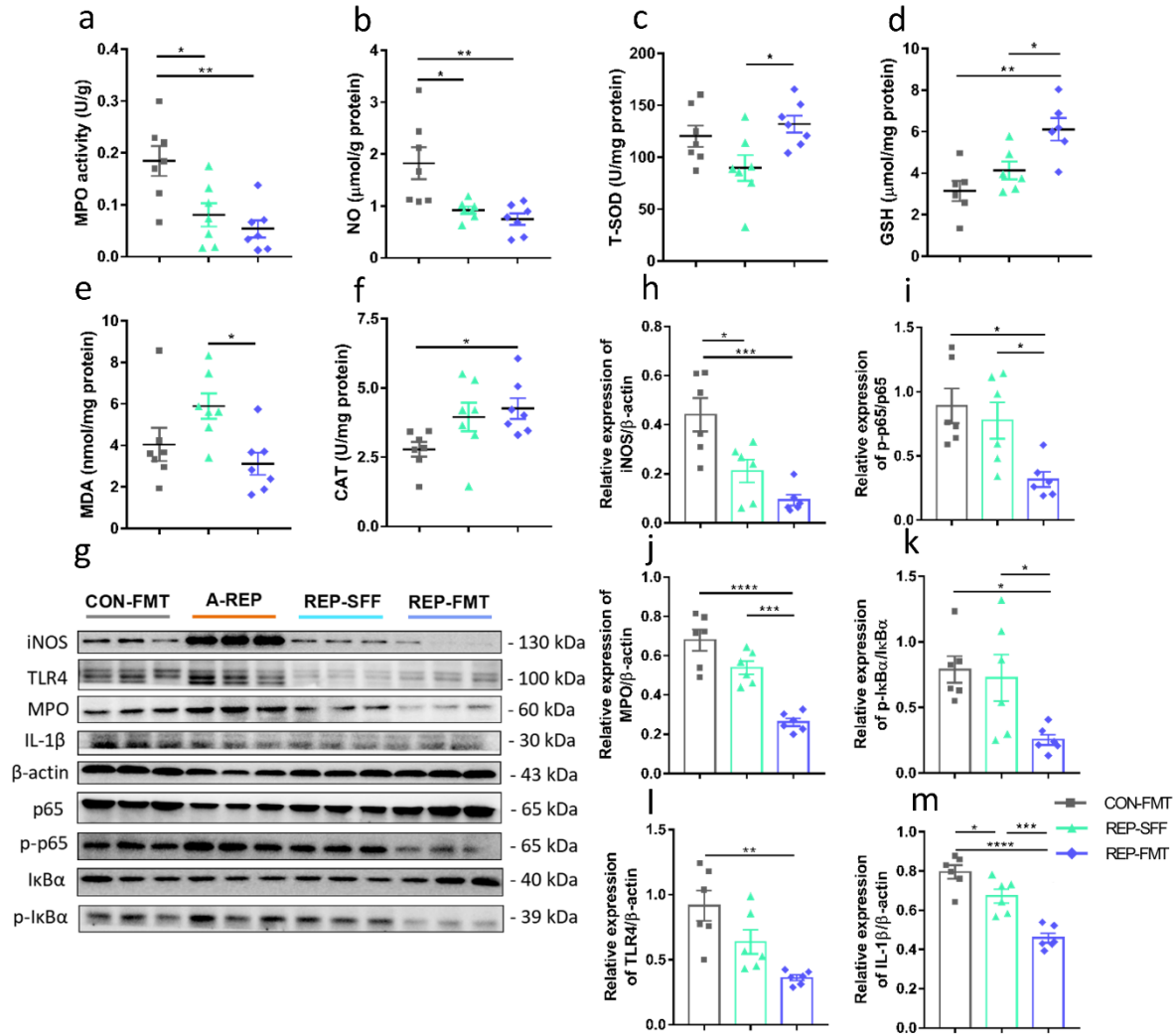
Supplementary Figures

Fig. S1. The characterization results of *Rhopilema esculentum* polysaccharide (REP).

(a) The HPGPC profile of molecular weight of REP. (b) HPLC-MS chromatograms for monosaccharide composition analysis of REP.
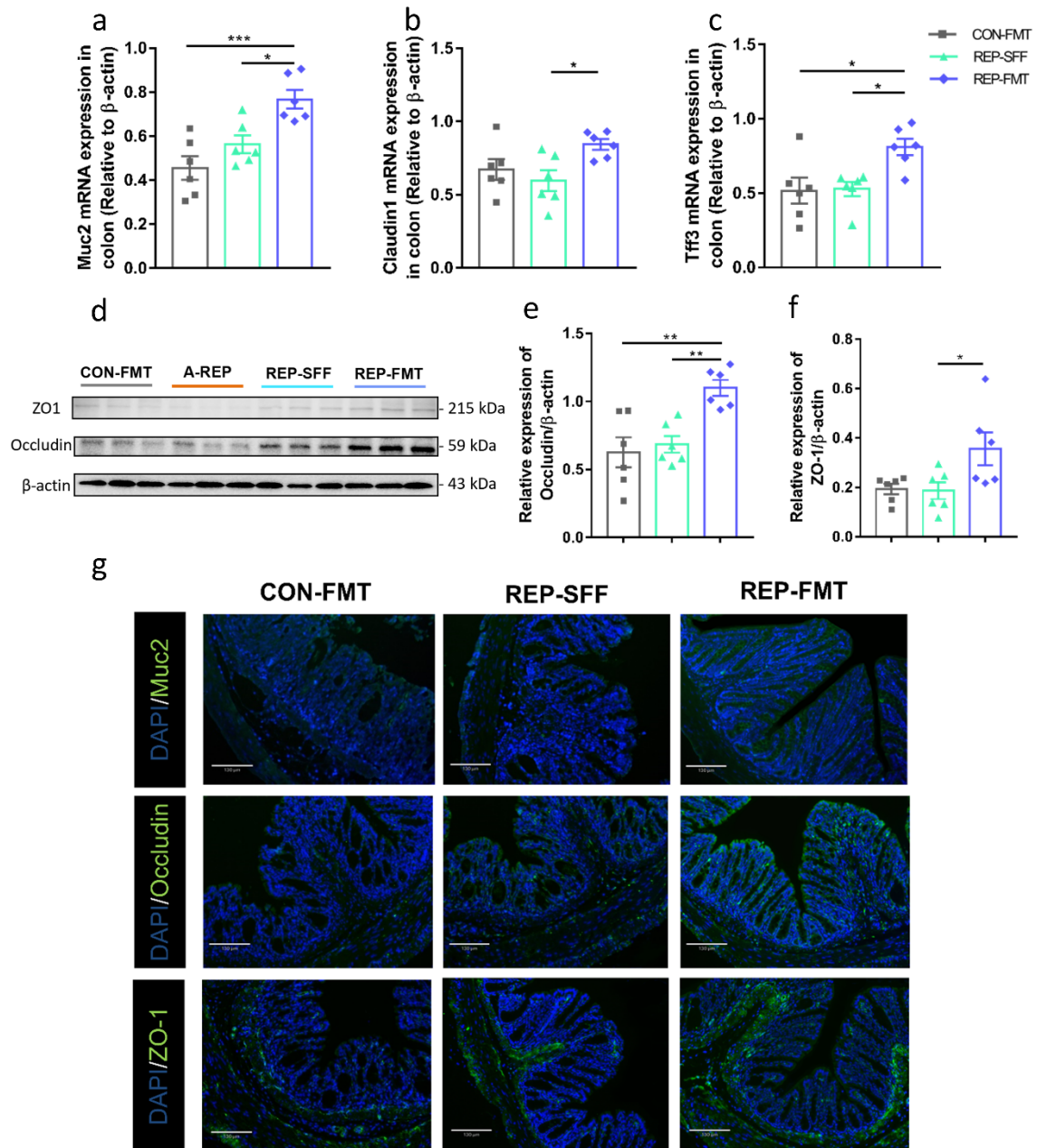
Fig. S2. REP modulated inflammatory cytokines in colonic tissues and regulated oxidative stress in serum and plasma. Relative mRNA expression of (a) IL-6, (b) IL-1β, (c) TNF-α, (d) IL-23 in the colonic tissue (n=6). Concentration of MDA (e) in serum and CAT (f) in the plasma (n=7).

Fig. S3. REP-FMT contributed more to modulate inflammatory cytokines in colonic tissues and oxidative stress in serum and plasma. Relative mRNA expression of (a) IL-6, (b) IL-1β, (c) TNF-α, (d) IL-23 in the colonic tissue (n=6). Concentration of MDA (e) in serum and CAT (f) in the plasma (n=6).

Fig. S4. REP-FMT attenuated the oxidative stress and regulated inflammatory responses compared to CON-FMT and REP-SFF. Concentrations of MPO activity (a), NO (b), T-SOD (c), GSH (d), MDA (e) and CAT (f) in the colon (n=6-7). (g) Western blot images of iNOS, p65, p-p65, TLR4, MPO, IL-1β, β-actin, IκB-α and p-IκB-α. The relative expression of respective protein (h-m) was quantified via Image J software (n=6). Results were presented as mean± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Fig. S5. REP-FMT improved colonic epithelial integrity and mucosal barrier function compared to CON-FMT and REP-SFF. Relative mRNA expression of Muc2, Claudin1 and Tff3 (a-c) in the colonic tissue (n=6). (d) Expression of ZO-1 and Occludin in the colon was measured by western blot, and the relative intensity of ZO-1 (e) and Occludin (f) proteins was analyzed by Image J software (n=6). (g) Immunofluorescence image of expression of Muc2 and Tjs. Magnification:×100. Results were displayed as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Supplementary Tables

Table S1: List of ELISA kits

ELISA kits	Catalog Number	Manufacturer
IL-6	ml002293-C	
IL-1β	ml063132-C	
TNF-α	ml002095-C	
IL-8	ml028580	Shanghai Enzyme-linked Biotechnology Co., Ltd
IL-23	ml001919	
IFN-γ	ml002277	
LPS	ml037221-J	

Table S2: Primer sequences used for RT-PCR experiments

Genes	Gene ID	Forward primer	Reverse primer
β-actin	11461	CCTGAACCCTAAGGCCAACC	CGGAGTCCATCACAATGCCT
IL-6	16193	CTCAGCGCTGAGTTG	CCTGTAGCCCACGTCGTAGC
TNF-α	21926	ACTGAACTTCGGGGTGATCG	GGCTACAGGCTTGTCACTCG
IL-1β	16176	GAGCACCTTCTTTTCCTTCATCTT	TCACACACCAGCAGGTTATCATC
IL-23R	209590	ACTATGCCATCAAAGTCAAGGT	CCAAGAGTTCAGCCATCCTC
Occludin	18260	GGCAAGCGATCATACCCAGAG	AGGCTGCCTGAAGTCATCCAC
ZO-1	21872	GACCAATAGCTGATGTTGCCAGAG	TATGAAGGCGAATGATGCCAGA
Muc2	17831	TCATGACGTCTGGTGGAATGG	AGCTTGTCGTGGCAGCTTGA
E-cadherin	12550	GCCAATCCTGATGAAATTGGAAAC	CCACTGCCCTCGTAATCGAAC
Tff3	21786	TGCTGCCATGGAGACCAGAG	GACATTTGCCGGCACCATAC

Table S3: List of antibodies

Name of Antibody	Catalog number	Concentration	Company
NF-κB/p65	#8242	1:1000	Cell Signaling Technology
p-NF-κB/p65	#3033	1:1000	Cell Signaling Technology
IκBα	#4814	1:1000	Cell Signaling Technology
p-IκBα	#2859	1:1000	Cell Signaling Technology
iNOS	A3774	1:1000	ABclonal Technology
MPO	A1374	1:1000	ABclonal Technology
TLR4	A5258	1:1000	ABclonal Technology
IL-1β	A16288	1:1000	ABclonal Technology
β-actin	4970	WB: 1:1000	Cell Signaling Technology
Occludin	27260-1-AP	IF: 1:300 WB: 1:1000	Proteintech Group
ZO-1	A0659	WB: 1:500	ABclonal Technology
Muc2	27675-1-AP	IF: 1:1000	Proteintech Group
ZO-1	Ab221547	IF: 1:100	Abcam plc
Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488)	Ab150077	IF: 1:400	Abcam plc