Supplemental Materials

A lipidomics investigation into the intervention of celastrol on experimental colitis

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Materials and methods

Serum sample preparation for GC-MS analysis

20µl serum was mixed with 500µl methanol/chloroform (2:1 v/v) solution with 10µl heptadecanoic acid methyl ester (10mM) as an IS. The mixed solution was vortexed for 3 min and then centrifuged at 10,000 rpm for 15 min. Supernatant was collected and 250µl 0.9% NaCl solution was added in the supernatant. The mixed solution was vortexed for 5 min and then centrifuged at 10,000 rpm for 15min. The lower phase was collected and evaporated to dryness under N2 gas. Then, 500µl methanol/HCl (41.5/9.7) was added, and was then incubated at 60°C for 12h. Next, 2.5 ml hexane and 2.5ml 0.9% NaCl solution was mixed with the above solution, which was then vortexed for 1 min. Finally, hexane phase was collected and evaporated to dryness under N2 gas. The residue was suspended with hexane and mixed for 1 min prior to GC/MS analysis.

GC-MS analysis

Gas chromatography-Mass spectrometer (Agilent Technologies 7890B/5977A) was used for fatty acid analysis with a GL-5MS column (30 m×0.25 mm i.d.; film thickness 0.25 mm) (GL Sciences, Inc. Japan). GC conditions: the initial oven temperature was set at 80°C and held for 1 min, ramped to 185°C by 15°C min^{-1}, then risen from185°C to 200°C by 1°C min^{-1}, finally ramped to 280°C by 20°C min^{-1} and held for 2 min. Injection volume was 0.5µl in splitless mode, and helium was the carrier gas at a flow of 1ml min^{-1}. The inlet temperature was set at 250°C. MS conditions: ion source temperature was kept at 220°C, and the electron energy was 70eV. Full scan mode from 50 to 1000 m/z was used to analysis, and the solvent delay was set at 3 min.

For identification of the fatty acids, their mass spectra were compared with those of the available authentic standards. Concentrations of the metabolites in the samples were determined based on standard curves using EIC peak area of authentic standards.

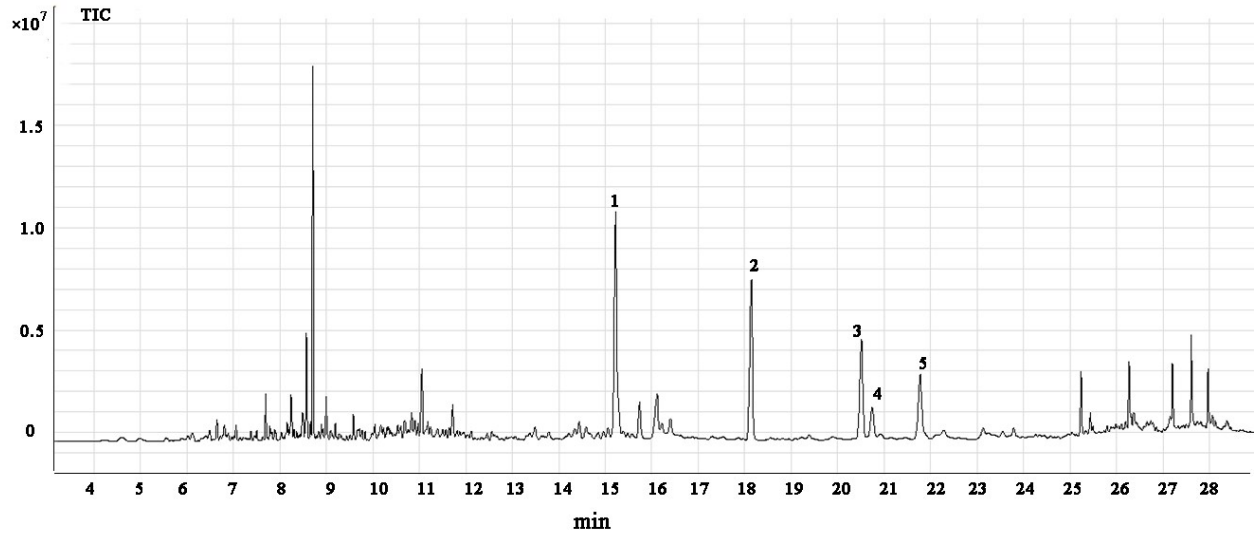

Figure S1. Typical GC-MS chromatogram of a serum sample of colitis mice. Fatty acids were methylated as described in the Materials and Methods. (1.Fatty acid C16:0; 2. Fatty acid C17:0 (IS); 3. Linoleic acid C18:2; 4. Oleic acid C18:1; 5. Stearic acid C18:0)

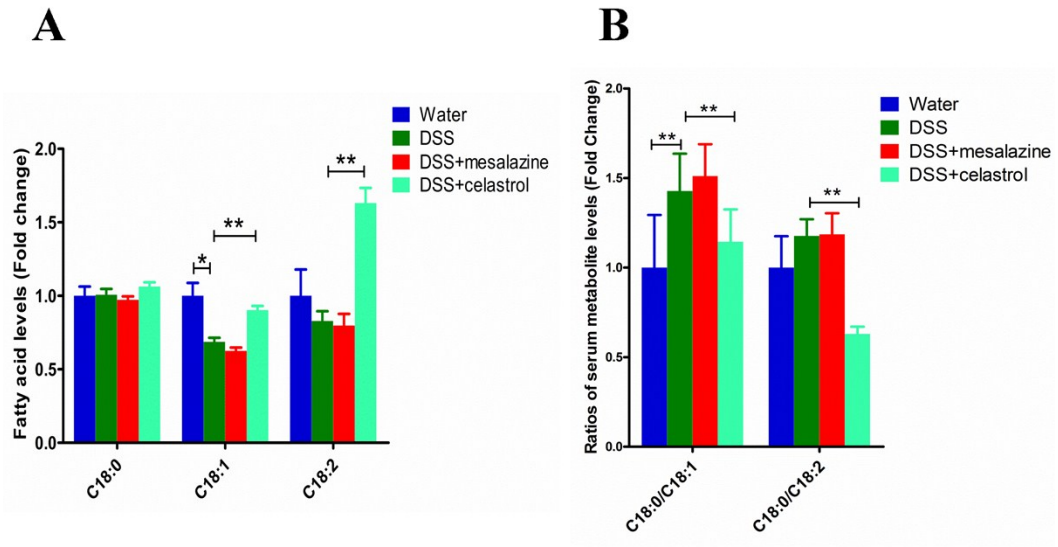

Figure S2. (A) Serum fatty acid levels in the four groups, *i.e.*control, colitis, mesalazine-treated and celastrol-treated colitis groups; (B) Serum C18:0/C18:1 and C18:0/C18:2 ratios in the four groups. Data were expressed as mean ± SD. (*P <0.05 and **P<0.01)