Sanguisorba officinalis L. derived from herbal medicine prevents intestinal inflammation by inducing autophagy in macrophages

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Disturbed activation of autophagy is implicated in the pathogenesis of inflammatory bowel disease. Accordingly, several autophagy-related genes have been identified as Crohn’s disease susceptibility genes. We screened the autophagy activators from a library including 3,922 natural extracts using a high-throughput assay system. The extracts identified as autophagy activators were administered to mice with 2% dextran sodium sulfate (DSS). Among the autophagy inducers, Sanguisorba officinalis L. (SO) suppressed DSS-induced colitis. To identify the mechanism by which SO ameliorates colitis, epithelial cell and innate myeloid cells-specific \( \text{Atg7} \)-deficient mice (\( \text{Villin-cre}; \text{Atg7}\text{f/f} \) and \( \text{LysM-cre}; \text{Atg7}\text{f/f} \) mice, respectively) were analyzed. SO-mediated inhibition of colitis was observed in \( \text{Villin-cre}; \text{Atg7}\text{f/f} \) mice. However, SO and a mixture of its components including catechin acid, ellagic acid, gallic acid, and ziyuglycoside II (Mix4) did not suppressed colitis in \( \text{LysM-cre}; \text{Atg7}\text{f/f} \) mice. In large intestinal macrophages (M\( \phi \)) of \( \text{Atg7}\text{f/f} \) mice, SO and Mix4 upregulated the expression of marker genes of anti-inflammatory M\( \phi \) including \( \text{Arg1} \), \( \text{Cd206} \), and \( \text{Relma} \). However, these alterations were not induced in \( \text{LysM-cre}; \text{Atg7}\text{f/f} \) mice. These findings indicate that SO and its active components ameliorate DSS-induced colitis by providing intestinal M\( \phi \) with anti-inflammatory profiles via promotion of \( \text{Atg7} \)-dependent autophagy.

Inflammatory bowel diseases (IBD), including Crohn’s disease (CD) and ulcerative colitis (UC), are chronic relapsing-remitting disorders of the gastrointestinal tract. The numbers of patients with CD and UC in Japan were 70,700 and 219,685, respectively, and still increasing according to an analysis of a nationwide survey1. IBD patients are on chronic medication, such as 5-aminosalicylates, corticosteroids, and biopharmaceuticals including anti-tumor necrosis factor (TNF) and anti-interleukin (IL)-12/23p40 antibodies2. Therapeutic reagents, such as biological drugs and corticosteroids provide therapeutic benefit in the acute inflammation phase, and dramatically suppressed active inflammation. However, in the chronic phase, corticosteroids are ineffective for maintaining remission status. Therefore, development of a cure for IBD that is safe and tolerable in the long-term is needed.
Recent studies have demonstrated that several herbal medicines are beneficial for suppressing intestinal inflammation. A murine study has reported that indigo naturalis, a traditional herbal formulation, ameliorates intestinal inflammation by promoting production of IL-10 and IL-22 through activation of aryl hydrocarbon receptor. In addition, its anti-inflammatory effect was exerted in human intestine. Among herbal medicines, Sanguisorba officinalis L. (SO) has been reported to have several effects, such as anti-oxidative, anti-inflammatory, anti-tumor, anti-allergic, and anti-infection. So far, 129 components of SO were identified. Among them, tannins including catechin acid (CA), ellagic acid (EA), and gallic acid (GA) and saponins including ziyuglycoside II (ZY) have been identified as major active components of SO with anti-inflammatory capacities.

Autophagy plays an important role in the degradation of unnecessary and/or dysfunctional components in lysosome. Microtubule-associated protein light chain 3 (LC3), a mammalian homolog of fungal Atg8, is commonly used to survey autophagy state. LC3-I is converted to LC3-II that is essential for initiation of autophagy. Autophagy related 7 (Atg7), an E1-like enzyme, is a essential molecule for progression of autophagy, because it activates LC3 in an ATP-dependent manner during the autophagosome formation. Recent studies have shown that adequate activation of autophagy prevents intestinal inflammation associated with IBD. Furthermore, dysregulated activation of autophagy is implicated in the development and/or pathogenesis of autoimmune disorders, including systemic lupus erythematosus and rheumatoid arthritis. Autophagy-related genes, such as Atg16L1 and NOD2, have been reported to be associated with the pathogenesis of CD. Moreover, LRKK2 has been identified as a CD-susceptibility gene, especially in Japanese cohorts. These genes are essential for appropriate production of cytokines in macrophages (Mφ) and dendritic cells (DCs) instruct the adaptive immune system by producing pro- and anti-inflammatory cytokines, disrupted innate immune responses lead to inadequate activation of helper T (Th) cells, such as IFN-γ-producing CD4+ (Th1) cells and IL-17-producing CD4+ (Th17) cells. Accordingly, IBD patients show dysregulated Th1 and Th17 cell responses accompanied with increased number of inflammatory Mφ and DCs with promoted antigen presentation and enhanced production of pro-inflammatory cytokines in the intestinal mucosa. In the steady state, anti-inflammatory CX3CR1-high Mφ are abundantly present in murine intestine, whereas inflammatory DCs including CX3CR1-intermediate DCs increase under the inflammatory condition. To maintain intestinal mucosal tolerance, development and genes expression of Mφ and DCs are tightly regulated by various mechanisms in the intestine. For instance, anti-inflammatory cytokine IL-10 produced by Mφ and Foxp3+ regulatory T (Treg) cells negatively regulates expression of pro-inflammatory cytokines including Il23a, Il6, Il12b, Tnf in intestinal Mφ and DCs.

In the present study, we screened for potential autophagy activators contributing to the prevention of intestinal inflammation. As a result, we identified that SO inhibits intestinal inflammation through facilitating Atg7-dependent Mφ autophagy.

Results

Administration of SO ameliorates dextran sodium sulfate (DSS)-induced colitis. To identify autophagy activator that control intestinal inflammation, we first performed a high-throughput assay (Supplementary Fig. S1). Among 3922 natural extracts including several herbal medicines, we identified 33 as inducers of autophagy. To determine effects of these products on the regulation of intestinal inflammation, mice were administered DSS in the presence or absence of these autophagy activators. Among them, Sanguisorba officinalis L. (SO) at a dose of 1 mg/ml, but not 0.1 and 0.01 mg/ml, markedly suppressed weight loss and colon shortening during DSS-induced colitis without tissue damage in the liver, heart, and kidney (Supplementary Fig. S2a–c): hereafter, we administered 1 mg/ml of SO to mice. Seven days after starting DSS administration, the severe weight loss and colon shortening were inhibited by co-administration of SO (Fig. 1a,b), which was associated with less colonic histopathology including mild inflammatory cell infiltration (Fig. 1c). Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining showed that the epithelial cell apoptosis observed in mice administered DSS was greatly suppressed in the presence of SO (Fig. 1d). In this context, SO administration led to significantly less translocation of FITC-dextran into the blood (Fig. 1e). Posttreatment with SO led to promotion of tissue recovery in mice suffered from DSS-induced colitis. (Supplementary Fig. S3a). However, pretreatment with SO did not affect initiation of inflammation and tissue repair in the intestine (Supplementary Fig. S3b). These results demonstrate that co-administration of SO with DSS lead to inhibition of intestinal inflammation.

Activation of Atg7-dependent epithelial autophagy by SO linked to partial suppression of DSS-induced colitis. Previous studies have demonstrated that autophagy is involved in intestinal epithelial homeostasis. Therefore, we analyzed whether SO-induced autophagy controls epithelial barrier integrity. To examine whether SO-mediated autophagy is occurred in intestinal epithelial cells in the steady state, we administered SO to mice and analyzed induction of epithelial autophagy. SO administration did not alter the ratio of LC3-II to LC3-I in large intestinal epithelial cells, indicating that epithelial autophagy is not enhanced in mice in the steady state condition (Fig. 2a). In accordance, the expression of genes encoding anti-microbial peptides, mucins, and epithelial tight junction-related molecules was not changed by SO administration in the steady state (Fig. 2b,c). In contrast, co-administering of SO with DSS led to markedly increased ratio of LC3-II to LC3-I and promoted expression of LC3B in epithelial cells of the colon (Fig. 2d,e). To elucidate whether SO-mediated
activations of epithelial autophagy during DSS administration is responsible for preventing intestinal inflammation, epithelial cell-specific Atg7 deficient mice (villin-cre; Atg7<sup>−/−</sup>; hereafter called Atg7<sup>ΔIEC</sup> mice) were analyzed (Fig. 3a–c). Similar to Atg7<sup>−/−</sup> mice, Atg7<sup>ΔIEC</sup> mice suffered from severe weight loss with shortened colon length,
Figure 2. SO did not induce autophagy in large intestinal epithelial cells in the steady state. (a) Activation of autophagy by SO in colonic epithelial cells was analyzed by measuring the ratio of LC3-II to LC3-I that detected by western blot analysis using the indicated antibodies. Conversion of LC3 in colonic epithelial cells from C57BL/6J mice administered SO in drinking water for 7 days. The ratio of LC3-II to LC3-I was measured with Image studio lite ver. 5.2 (Li-Cor Bioscience, Nebraska, USA). Full-length blots are shown in Supplementary Fig. S10a. (b,c) Expression levels of the indicated genes in large intestinal epithelial cells from mice treated or untreated with SO in the steady state. Data are mean ± SD from three independent experiments. Data were analyzed by unpaired t-test. n.s., not significant. (d) Conversion of LC3 in colonic epithelial cells from C57BL/6J mice administered DSS with or without SO in drinking water for 7 days. *P < 0.05. Full-length blots are shown in Supplementary Fig. S10b. All data are representative of three independent experiments. (e) Expression of LC3B in the colon of C57BL/6J mice administered 2% DSS with or without SO in drinking water for 7 days were analyzed with immunohistochemistry using anti-LC3B antibody (upper). Representative images of H&E staining (bottom). Data are representative of three independent experiments. Bars; 100 μm.
which was associated with worsened histopathology including increased removal of epithelial cells and infiltration of inflammatory cells following DSS administration. SO administration decreased all of clinical parameters with a remarkable resolution of intestinal pathology in \( \text{Atg7}^{\text{f/f}} \) mice. In \( \text{Atg7}^{\Delta \text{IEC}} \) mice, SO treatment linked to reduced intestinal histopathology and modest improvement of weight loss and colon shortening. These findings demonstrate that activation of Atg7-dependent epithelia autophagy is partially implicated in SO-mediated suppression of intestinal inflammation.

SO activates autophagy in intestinal M\( \phi \) during DSS-induced colitis. Under the steady state conditions, SO administration led to reduced expression of \( \text{Il6} \), but not \( \text{Il1b}, \text{Il10}, \text{Il12b}, \text{Il17a}, \text{Il23a}, \text{Ifng} \) and \( \text{Tnf} \), in cells from the colonic lamina propria (Fig. 4a). Several studies have demonstrated that autophagy in M\( \phi \) is essential for controlling inflammatory responses. In the intestine, M\( \phi \) is a major source of IL-6. To define whether SO induces autophagy in M\( \phi \), bone marrow derived M\( \phi \) (BMM\( \phi \)) were cultured in the presence or absence of SO and stained with CYTO-ID, which labels autophagic compartments. CYTO-ID-labeled BMM\( \phi \) were observed after SO treatment (Fig. 4b). In accordance, the ratio of LC3-II to LC3-I was increased in BMM\( \phi \) treated with SO in a dose-dependent manner (Fig. 4c), indicating that SO activates autophagy in BMM\( \phi \). We further analyzed SO-dependent autophagy in several subsets of innate myeloid cells of the colon. SO administration resulted in increased CYTO-ID-labeled cells in a subset of CD11b\(^+\) CX\(_3\)CR1\(^{\text{high}}\) M\( \phi \), but not neutrophils, eosinophils, and...
CD11b⁺ DCs, from the colon of mice administered DSS (Fig. 4d and Supplementary Fig. S4a–d). These results demonstrate that SO promotes autophagy in large intestinal Mϕ.

SO prevents intestinal inflammation by regulating autophagy in intestinal Mϕ. To determine whether autophagy induced by SO in intestinal Mϕ is responsible for prevention of intestinal inflammation, we analyzed innate myeloid cell-specific Atg7⁻/⁻ deficient mice (LysM-cre; Atg7⁻/⁻ mice; hereafter called Atg7⁻/⁻ LysM-cre mice). During DSS administration, Atg7⁻/⁻ mice exhibited weight loss and shortened colon length, and severe intestinal pathology to similar levels to that in Atg7⁻/⁻ LysM-cre mice (Fig. 5a–c). In Atg7⁻/⁻ mice, co-administration

Figure 4. SO induced autophagy in bone marrow-derived Mϕ (BMMϕ) and intestinal Mϕ. (a) Expression of the indicated genes in colonic lamina propria cells from mice treated with or without SO in the steady state. Data are mean ± SD of three independent experiments. Data were analyzed by unpaired t-test, *p < 0.05. n.s., not significant. (b) CYTO-ID staining in BMMϕ treated with or without SO for 24 hours. (c) BMMϕ were cultured in the presence or absence of SO for 24 hours. The immunoblotting was performed with the indicated antibodies. The graphs show the ratio of LC3-II to LC3-I. Full-length blots are shown in Supplementary Fig. S10c. (d) C57BL/6J mice administered 2% DSS with or without SO in drinking water for 7 days and colonic lamina propria cells were isolated. The autophagosome formation stained by CYTO-ID in macrophages, eosinophils, dendric cells and neutrophils were analyzed with flow cytometry. Data are representative of two independent experiments.
of SO inhibited the weight loss, colon length shortening, epithelial disruption, and inflammatory cell infiltration. However, SO did not ameliorate DSS-induced colitis in Atg7f/f and Atg7LysM-cre mice. We further analyzed gene expression patterns in CD11b+ cells including Mϕ isolated from the colons of Atg7f/f and Atg7LysM-cre mice administered DSS with or without SO. In large intestinal CD11b+ cells of Atg7f/f mice, SO decreased the expression of pro-inflammatory cytokine including Il6 and Il23a, whereas enhanced expression of an anti-inflammatory cytokine Il10 (Fig. 6a,b). However, these alterations were not observed in Atg7LysM-cre mice. In addition, CD11b+ cells from the colon of Atg7f/f mice highly expressed Relma, Cd206, and Arg1, which are maker genes of M2Mϕ, a subset of anti-inflammatory Mϕ, compared to those in Atg7LysM-cre mice (Fig. 6c). Moreover, we observed that SO treatment induced the expression of Il10, Relma, Cd206, and Arg1 in BMMϕ (Supplementary Fig. S5). We further analyzed contribution of SO to development of tissue-resident CX3CR1high Mϕ possessing anti-inflammatory properties. There were no differences in the numbers of CX3CR1high CD11b+ Mϕ and their precursor Ly6C+ CD11b+ monocytes between Atg7f/f and Atg7LysM-cre mice (Supplementary Fig. S6a,b), suggesting that SO did not affect differentiation of CX3CR1high CD11b+ Mϕ during DSS-induced colitis. These findings demonstrate that SO ameliorates intestinal inflammation by providing anti-inflammatory profiles to Mϕ via an autophagy-dependent mechanism.

Figure 5. SO did not prevent intestinal inflammation in Atg7LysM-cre mice. Atg7f/f and Atg7LysM-cre mice were administered 2% DSS with or without SO for 7 days. (a–c) Weight changes (a), colon length (b), representative colon sections (upper), and histological score of the colon (bottom) (c) (mean values ± SD). *p < 0.05. n.s, not significant. n = 6 in each group. Bars; 100 μm. Data are of two independent experiments. Data were analyzed by un-paired t-test.
Tannins attenuate colitis by inducing Atg7-dependent Mϕ autophagy. Among SO components, tannins including catechin acid (CA), ellagic acid (EA), and gallic acid (GA) and saponins including ziyuglycoside II (ZY) have been demonstrated to suppress inflammation in several tissues10–12. To determine whether these compounds prevent intestinal inflammation, mice were administered CA, EA, GA, and ZY equivalent to amounts found in SO during DSS-induced colitis. Single administration of each compound did not suppress large intestinal inflammation (Supplementary Fig. 7). However, a mixture of the four compounds (Mix4) led to reduced intestinal pathology to similar levels to that by SO.

To determine whether induction of Atg7-dependent Mϕ autophagy is involved in the prevention of intestinal inflammation by Mix4 shown in Supplementary Fig. S7, Atg7f/f and Atg7LysM-cre mice were administered Mix4 during DSS-induced colitis. In Atg7f/f mice, treatment with Mix4 diminished all clinical parameters including weight loss and colon shortening accompanied with less intestinal histopathology (Fig. 7a–c). In contrast, Atg7LysM-cre mice suffered from severe weight loss and colon shortening with profound infiltration of inflammatory cells and epithelial disruption even after Mix4 treatment. In this context, expression of marker genes of anti-inflammatory Mϕ including Relma, Cd206, and Arg1, but not Il10, was upregulated in CD11b+ cells from the colon of Atg7f/f mice following Mix4 administration, whereas expression of these genes was not altered in Atg7LysM-cre mice (Supplementary Fig. S8). BMMϕ prepared from Atg7f/f and Atg7LysM-cre mice were cultured in the presence of SO, CA, EA, GA, and ZY for 24 hours and stained with CYTO-ID (Supplementary Fig. S9). Similar to SO, CA, EA, and GA, but not ZY, promoted autophagy in Mϕ prepared from Atg7f/f mice, while it was not induced in Atg7 deficient Mϕ. These findings demonstrate that tannins contained in SO suppress intestinal inflammation coordinately through providing Mϕ with anti-inflammatory profiles by inducing Atg7-dependent Mϕ autophagy.

Discussion
In this study, we highlighted that a herbal medicine SO induce Atg7-dependent autophagy, which providing Mϕ with anti-inflammatory profiles, and thereby suppressing intestinal inflammation.

A previous study has defined that epithelial Atg7-dependent autophagy is dispensable for inhibition of DSS-induced colitis45. In accordance, we found that lack of Atg7 in intestinal epithelial cells did not affect the intestinal pathology during DSS-induced colitis without SO. In contrast, a study has demonstrated that epithelial cell-specific Atg7 deficiency leads to dysbiosis and thereby associating with development of more severe...
DSS-induced colitis. This discrepancy seems to be explained by a differential composition of commensal bacteria between the studies, because more severe mucosal inflammation in the colon of epithelial cell-specific Atg7 mice was abrogated by antibiotics treatment.

SO promoted epithelial autophagy in the large intestine during DSS-induced colitis. In Atg7ΔIEC mice, mild colon shortening was observed even after SO treatment, although infiltration of inflammatory cells drastically reduced. A study has recently demonstrated that inappropriate activation of mesenchymal stromal cells contributes to the pathogenesis of DSS-induced colitis and human IBD. Thus, there is a possibility that SO-mediated reinforcement of mucosal barrier integrity through activation of Atg7-dependent epithelial autophagy links to regulation of activation of non-hematopoietic lamina propria cells via an unknown mechanism, which may associate with less clinical parameters including colon shortening during DSS-induced colitis. In addition to the Atg5/Atg7-conventional pathway, autophagy occurs via the Atg5/Atg7-independent alternative pathway, which is regulated by Ulk1 and beclin 1. We cannot exclude the possibility that SO suppresses intestinal inflammation by promoting epithelial autophagy through activation of the Atg5/Atg7-independent alternative pathway. Thus, it would be interesting to identify whether SO activates Ulk1/beclin 1-mediated autophagy in inflammatory conditions and alleviate intestinal inflammation.

A previous report has demonstrated that there is no difference in reduction of body weight during DSS administration between Atg7f/f and Atg7LysM-cre mice. In Atg7f/f mice, body weight gain was seen within 3 days following the completion of DSS administration, whereas Atg7LysM-cre mice kept their weight loss. These findings suggest that Atg7-dependent autophagy in Mφ may be essential for promotion of tissue repair and regeneration in the intestine. As previously reported, we found that there was no difference in the severity of intestinal inflammation

**Figure 7.** Minimal therapeutic effect of the mixture of SO components including CA, GA, EA, ZY (Mix4) on suppression of DSS-induced colitis in Atg7LysM-cre mice. Atg7f/f and Atg7LysM-cre mice were administered 2% DSS with or without Mix4 for 7 days. (a) Weight changes, (b) colon length, (c) representative colon sections (upper), and histological score of the colon (bottom). Data are mean ± SD of five mice in each group. *p < 0.05, n.s., not significant. Bars; 100 μm. Data are of two independent experiments.
between Atg7<sup>−/−</sup> and Atg7<sup>LysM-cre</sup> mice during DSS administration. In this context, SO-mediated enhancement of Atg7<sup>−/−</sup>-dependent autophagy in Mϕ led to suppression of intestinal pathology. In the current study, intestinal Mϕ and BMMϕ treated with SO acquired anti-inflammatory profiles that are similar to M2Mϕ, such as upregulated the expression of Arg1, CD206, and Relma. M2Mϕ are known to have ability to inhibit inflammation and encourage tissue repair. Previous studies have reported that lack of Atg5/Atg7-dependent autophagy in Mϕ results in reduction of M2Mϕ polarization in the liver and fat tissue. In addition, a study has shown that CD5L, a secreted glycoprotein, induce M2Mϕ through Atg7-dependent induction of Id3 expression. Therefore, SO may prevent intestinal inflammation by accelerating M2Mϕ polarization by facilitating Atg7-dependent autophagy. In the current study, co-administration of SO led to amelioration of DSS-induced intestinal inflammation, whereas pre-treatment with SO was ineffective. In the intestine, monocyte-derived CX<sub>3</sub>CR1<sup>intermediate</sup> CD11b<sup>+</sup> cells are increased during inflammation. Thus, SO may provide these cells with M2Mϕ-like phenotypes.

SO treatment led enhanced expression of Il10 in colonic CD11b<sup>+</sup> cells including Mϕ of Atg7<sup>−/−</sup> mice, but not Atg7<sup>LysM-cre</sup> mice. In the intestinal mucosa, expression of a subset of pro-inflammatory cytokines in Mϕ is inhibited through IL-10-dependent activation of transcription factor Stat3, linking to prevention of intestinal inflammation. In addition, a previous study has shown that intestinal Mϕ-derived IL-10 is needed for maintenance of Foxp3<sup>+</sup> Treg cells. However, Mix<sub>2</sub> did not promote Il10 expression in colonic CD11b<sup>+</sup> cells, although effectively ameliorating DSS-induced colitis. These findings suggest that upregulation of Il10 expression in Mϕ may be not required for SO-mediated prevention of intestinal inflammation.

In the current study, single administration of 15 μg/mL of CA, GA, EA, or ZY equivalent to amounts contained in 1 mg/mL of SO did not show a therapeutic effect during DSS administration, while a mixture of the four compounds suppressed intestinal inflammation through activation of Atg7-dependent Mϕ autophagy. Among these compounds, CA, EA, and GA, which are categorized as tannin, induced Atg7-dependent autophagy in BMMϕ, while ZY that is categorized as saponin did not. These findings raise the possibility that the combined total quantity of tannins within SO may be essential for suppression of intestinal inflammation. Thus, it would be important to analyze whether single-administration of higher-dose CA, GA, or EA is effective to prevent colitis in the future.

To identify insights for the development of novel therapeutic intervention with either SO or its components for IBD, the following methods were performed.

**Methods**

**Mice.** C57BL/6 mice were obtained from Clea Japan, Inc. (Tokyo, Japan). Mice carrying a loxP-flanked Atg7 allele (Atg7<sup>LysM-cre</sup>) were kindly provided by Prof. M. Komatsu (Juntendo University) and Dr. K. Tanaka (Tokyo Metropolitan Institute of Medical Science). Seven-week-old female mice were used for the experiments. Mouse experiments were approved by the Animal Research Committee of the Institute of Experimental Animal Sciences Faculty of Medicine (Osaka University, Osaka, approval no. 27-099-000). All methods were carried out in accordance with guidelines and regulations of Animal Research Committee of the Institute of Experimental Animal Sciences Faculty of Medicine in Osaka University. All mice were fed a standard diet and maintained in routine light-dark cycles, and housed in specific pathogen-free conditions.

**Reagents.** Immunohistochemistry was performed following antibodies anti-rabbit LC3B (dilution 1:200, Abcam Plc., Cambridge, UK), anti-rabbit Alexa Flour 647 (Thermo Fisher Scientific K.K., Massachusetts, USA). Apoptosis of intestinal epithelial cell was detected using the ApopTag Fluorescein In situ Apoptosis Detection Kit (Merck KGaA, Darmstadt, Germany). Autophagic structures and nucleus were determined using the CYTO-ID In situ Apoptosis of intestinal epithelial cell was detected using the ApopTag Fluorescein In situ Apoptosis Detection Kit (Merck KGaA, Darmstadt, Germany). Autophagic structures and nucleus were determined using the CYTO-ID detection kit contains Hoechst33342 (Enzo Biochem Inc., New York, USA). Images of staining samples were taken by using BZ-X 700 (Keyence Corporation, Osaka, Japan) or FV1200 (BX) (Olympus Corporation, Tokyo, Japan).

**Flow cytometry.** The following antibodies were purchased from Becton, Dickinson and Company (BD) (Franklin Lakes, New Jersey, USA): 7-AAD, anti-mouse CD16/32 (clone 2.4.G2), PE-Cy7-conjugated anti-mouse-Ly-6C (clone AL-21), PE- or FITC-conjugated anti-mouse CD11b (clone M1/70), PE-conjugated anti-mouse-SiglecF (clone E50-2440) and anti-mouse Ly6G (clone 1A8). Pacific blue-conjugated anti-mouse-Ly-6C (clone AL-21), PE- or FITC-conjugated anti-mouse CD11b (clone M1/70), PE-conjugated anti-mouse-SiglecF (clone E50-2440), anti-mouse CD16/32 (clone 2.4.G2), PE-Cy7-conjugated anti-mouse-Ly-6C (clone AL-21), PE- or FITC-conjugated anti-mouse CD11b (clone M1/70), PE-conjugated anti-mouse-SiglecF (clone E50-2440), and anti-mouse Ly6G (clone 1A8). Pacific blue-conjugated anti-mouse CD11b (clone M1/70), PE-conjugated anti-mouse-SiglecF (clone E50-2440), and anti-mouse CD16/32 (clone 2.4.G2). Pacific blue-conjugated anti-mouse CD11b (clone M1/70), PE-conjugated anti-mouse-SiglecF (clone E50-2440), and anti-mouse CD16/32 (clone 2.4.G2). Pacific blue-conjugated anti-mouse CD11b (clone M1/70), PE-conjugated anti-mouse-SiglecF (clone E50-2440), and anti-mouse CD16/32 (clone 2.4.G2).

**Identification of autophagy inducers with high-throughput assay system.** 1 × 10<sup>4</sup> GFP-LC3-expressing mouse embryonic fibroblasts were cultured in each well of 96-well plate for 16 hours and subsequently treated with each substance included in a natural compound library. After 5 hours, the area of GFP-LC3 puncta was measured with Incell Analyzer 1000 (GE Healthcare UK Ltd., England).

**DSS-induced colitis.** Mice were administrated 2% DSS (160110, molecular weight 36,000–50,000; MP Biomedicals Inc., Ohio, USA; lot number Q3526) in their drinking water for 7 days. Their weight was adjusted to 16–19 g on the day of the experiment (day 0). Weight and water consumption were measured every day.
Colon length was measured 7 days after starting DSS administration. The colons were fixed in 10% formalin over 24 hours and embedded in paraffin. 3.5-µm-thick sections were stained with hematoxylin and eosin (H&E). Images of hematoxylin and eosin staining were taken using BZ-X 700 (Keyence). The histological scores were analyzed according to the method previously reported. Intestinal permeability was assessed using FITC-dextran (molecular weight: 4000, Chondrex Inc., DC, USA) according to the manufacturer's instructions. The concentration of FITC-dextran in plasma was quantified with a fluorescent plate reader (SH-9000, Corona Electric Co., Ltd, Ibaragi, Japan).

Isolation of large intestinal lamina propria cells. Large intestinal lamina propria cells were isolated using previously described protocol. Large intestinal lamina propria CD11b+ cells were collected using MACS CD11b magnetic beads (Miltenyi Biotec K.K., Bergisch Gladbach, Germany) according to the manufacturer's protocol.

Preparation of the cells. To prepare BMMϕ, bone marrow cells were isolated from mouse femurs and tibias, passed through a nylon mesh, and cultured in RPMI1640 (Nacarai Tesque Inc., Kyoto Japan) medium containing 10% fetal bovine serum (FBS; GE Healthcare Life Sciences, Illinois, USA), penicillin (10,000 unit/ml), and streptomycin (10,000 µg/ml), and 30% L929 cell culture supernatant for 7 days.

Preparation of SO and components including SO. SO roots were purchased from Tochimoto Tenkaido Co., Ltd. (Osaka, Japan). The ingredients were extracted by boiling in 10 times water for 1 hour. After filtration, the extracted components were lyophilized to powder. 0.01, 0.1, and 1 mg/mL of SO extract was co-administered with DSS orally to mice. CA, GA, EA and ZY were purchased from Namiki Shoji Co., Ltd. (Tokyo, Japan). The concentrations of these components in SO were calculated, as reported previously and 15.

Western blotting. Cell lysate and tissue sample were dissolved in RIPA buffer (Thermo) with protease inhibitor (Thermo) and phosphatase inhibitor (Thermo). Immunoblotting was performed using anti-mouse LC3B antibody (dilution 1:1000), anti-mouse beta-actin (dilution 1:1000, Sigma-Aldrich Co. I,l,c, SLE, U.S.A.) and anti-mouse GAPDH (dilution 1:2000, Abcam).

Quantitative real-time polymerase chain reactions (qRT-PCR) analysis. Total RNA was extracted from large intestinal lamina propria cells and epithelial cells using RNeasy Mini Kit (Qiagen K.K., California, USA). The RNA was reverse-transcribed into first-strand complementary DNA using the High-Capacity RNA-to-cDNA Kit (Thermo) according to the manufacturer's protocol. qRT-PCR was performed on an ABI Prism 7900HT sequence detection system (Applied Biosystems (Thermo)) using Thunderbird Probe qPCR Mix (Toyobo Co., Ltd, Osaka, Japan). The amplification conditions were 95°C (10 min), 40 cycles of 95°C (15 s) and 60°C (60 s). All values were normalized to the expression of Gapdh, encoding glyceraldehyde-3-phosphate dehydrogenase, and the fold difference in expression relative to that of Gapdh is shown. All real-time qRT-PCR reactions were performed in triplicate.

Statistical analysis. All experiments were performed using randomly assigned mice. All statistical analyses were performed using JMP Pro 13. (SAS Institute Inc., North Carolina, USA). Dunnett’s test for multiple comparisons, and unpaired, two-tailed Student’s t tests for comparisons between two groups were used to assess statistical significance. P values < 0.05 was considered to indicate a significant difference.

Received: 5 December 2019; Accepted: 4 May 2020;
Published online: 19 June 2020

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Acknowledgements
The authors are grateful to Drs. Yoshifumi Watanabe, Naotsugu Haraguchi, Miki Sakaue, Haruka Tani (Osaka University) and Hayato Urushima (Osaka-City university) for their technical support and helpful advice. We are grateful to Drs. M. Komatsu (Juntendo University) and K. Tanaka (Tokyo Metropolitan Institute of Medical Science) for kindly providing Atg7flox mice. This study was supported in part by a Grant-in-Aid for Scientific Research (A) (17H01533) and Grant-in-Aid for Scientific Research on Innovative Areas (17H06414) from the MEXT of Japan. This study was also supported by the JSPS KAKENHI (grant no. JP17859038, JP17895328), Joint Usage/Research Program of Medical Research Institute, Tokyo Medical and Dental University, and by a grant from the Takeda Science Foundation.

Author contributions
All authors contributed to the conception and study design. The roles of each author are described below. Conceptualization: [T.M., S.S., H.K.], Methodology: [S.S., J.N., H.K.], Technical Support and instruction: [H.K., M.M., T.O., N.M., H.T., M.U., C.M.], Formal analysis and investigation: [A.Y.], Material preparation [K.W., K.K.], Writing - review and editing: [H.K., T.M., T.I.], Funding acquisition: [T.M., S.S., Y.D., H.E., T.K., T.K.]. The first draft of the manuscript was written by A.Y. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41598-020-65306-4.

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