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# Downregulation of OCTN2 by Cytokines Plays an Important Role in the Progression of Inflammatory Bowel Disease

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**ABSTRACT**

Inflammatory bowel diseases (IBD) are characterized by chronic relapsing disorders of the gastrointestinal tract. OCTN2 (SLC22A5) and its substrate L-carnitine (L-Car) play crucial roles in maintaining normal intestinal function. An aim of this study was to delineate the expression alteration of OCTN2 in IBD and its underlying mechanism. We also investigated the impact of OCTN2 on IBD progression and the possibility of improving IBD through OCTN2 regulation. Our results showed decreased OCTN2 expression levels and L-Car content in inflamed colon tissues of IBD patients and mice, which negatively correlated with the degree of colonic inflammation in IBD mice. Mixed proinflammatory cytokines TNF-α, IL-1β and IFNγ downregulated the expression of OCTN2 and subsequently reduced the L-Car content through PPARγ/RXRα pathways in FHC cells. OCTN2 silencing reduced the proliferation rate of the colon cells, whereas OCTN2 overexpression increased the proliferation rate. Furthermore, the ability of PPARγ agonist, luteolin, to increase OCTN2 expression resulted in the alleviation of colonic inflammatory responses. In conclusion, OCTN2 was downregulated in IBD by proinflammatory cytokines via the PPARγ/RXRα pathways, which reduced L-Car concentration and subsequently induced IBD deterioration. Upregulation of OCTN2 by the PPARγ agonist alleviated colonic inflammation. Our findings suggest that, OCTN2 may serve as a therapeutic target for IBD therapy.

*Keywords*: Inflammatory Bowel Diseases; OCTN2; L-carnitine; Proinflammatory

Cytokines; Luteolin

**Abbreviations**

| | |
|---|---|
| IBD | Inflammatory bowel diseases |
| UC | Ulcerative colitis |
| CD | Crohn's disease |
| DSS | Dextran sodium sulfate |
| TNBS | Trinitrobenzenesulfonic acid |
| L-Car | L-carnitine |
| d3-L-Car | d3-L-carnitine |
| OCTN2 | The carnitine/organic cation transporter 2 |
| $ATB^{0,+}$ | Amino acid transporter $B^{0,+}$ |
| CT1 | Carnitine transporter 1 |
| TNF-α | Tumor necrosis factor α |
| IL-1β | Interleukin 1β |
| ICAM-1 | Intercellular adhesion molecule 1 |
| IFN-γ | Interferon γ |
| PPARα | Peroxisome proliferator-activated receptor α |
| PPARγ | Peroxisome proliferator-activated receptor γ |
| RXRα | Retinoid X receptor α |

| | |
|---|---|
| qRT-PCR | Quantitative real-time polymerase chain reaction |

## 1. Introduction

Inflammatory bowel disease (IBD), which includes two immune-mediated conditions, Crohn's disease (CD) and ulcerative colitis (UC), are chronic relapsing disorders of the gastrointestinal tract with unknown etiology [1]. In recent years, the incidence and prevalence of IBD continue to increase worldwide in adults [2] and children [3], especially in emerging industrialized countries, such as Asia, Africa, and Latin America [4]. IBD has been classified as a prototypical complex disease, in which biological complexity arises from intricate interactions between multiple factors, such as susceptibility genes, environmental impact, inappropriate immune responses, and the microbiome [5], but its specific pathogenesis remains poorly understood. The ambiguity of the pathogenesis and the individual differences between the different patients create enormous challenges; therefore, effective therapeutic approaches are of great importance.

Carnitine/organic cation transporter 2 (OCTN2, SLC22A5), a member of the solute carrier (SLC) transporter superfamily, is a sodium-dependent high-affinity transporter for L-carnitine (L-Car) [6]. OCTN2 is physiologically the most important transporter for absorbing L-Car from the diet, which provides about 75% of the daily L-Car requirements for adults. The main function of L-Car is to transfer long-chain fatty acids across the inner mitochondrial membrane for subsequent β-oxidation [7]. L-Car also contributes to the oxidation of butyric acid, which provides colonocytes with 70% energy, and is the primary metabolic fuel required for the maintenance and functional

integrity of normal human colonic epithelial cells [8]. Additionally, L-Car also displays immunosuppressive properties [9]; supplementation of L-Car is beneficial for the treatment of gut inflammation [10]. Deficiency of L-Car would result in lipid metabolism disorders, skeletal weakness and even death [11].

OCTN2 is closely associated with different diseases, such as primary carnitine deficiency, IBD, diabetes and asthma [12]. Studies have shown that mutations in the promoter region of the *OCTN2* gene on chromosome 5q31 increase the susceptibility of Crohn's disease [13, 14]. However, changes in OCTN2 expression in IBD still remain controversial. Fujiya and colleagues demonstrated that colonic epithelial OCTN2 expression was increased in actively inflamed areas of both CD and UC [15]. Studies have suggested a decreased tendency of OCTN2 mRNA expression in UC patients [16] whereas others did not observed significant differences in OCTN2 mRNA expression [17].

Taken together, OCTN2 may be essential for the renewal of epithelial cells, but its role and regulatory mechanisms in IBD have not yet been clearly elucidated. In addition, the relationship between inflammation and OCTN2 remains to be determined. Thus, the aim of this study was to first demonstrate the comprehensive expressions and regulatory mechanisms of OCTN2 in IBD. Additionally, to clarify the impact of OCTN2 on the cell proliferation rate by *OCTN2* knockdown and overexpressed cells. Finally, we sought to determine whether IBD could be alleviated by upregulation of OCTN2.

## 2. Materials and methods

### *2.1. Materials*

Fetal bovine serum (FBS), trypsin, RPM 1640 medium and Dulbecco's modified Eagle medium (DMEM) were purchased from Gibco Invitrogen Corporation (Carlsbad, CA, USA). $d_3$-L-carnitine ($d_3$-L-Car) was obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). L-carnitine (L-Car) was provided by Meilun biological Co., Ltd. (Dalian, China). Luteolin and Trinitrobenzenesulfonic acid (TNBS) were purchased from Aladdin (Shanghai, China). Dextran sodium sulfate (DSS) was purchased from MP Biomedicals (Solon, OH). Tumor necrosis factor-α (TNF-α), Interferon-γ (IFN-γ), and Interleukin-1β (IL-1β) were purchased from R&D Systems (Abingdon, UK). OCTN2 antibody (species reactivity: human, mouse, rat) was obtained from Sigma-Aldrich (St. Louis, MO, USA. (SAB4300885)). PPARγ antibody was obtained from Diagvio (Hangzhou, China). GAPDH antibody, the secondary anti-mouse and anti-rabbit antibodies were obtained from Multi Sciences Biotech Corporation (Hangzhou, China). 1-Methyl-4-phenylpyridiniumiodide ($MPP^+$) and formic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was obtained from Sangon Biotech Co., Ltd. (Shanghai, China). Acetonitrile was obtained from Tedia (Fairfield, OH, USA). All other chemicals or solvents were of the highest grade commercially available.

Blank vector (pEnter), hOCTN2 (SLC22A5) expression plasmid were obtained

from ViGene Biosciences Inc. (Shandong, China.) SiRNAs were synthesized by Gene Pharma (Shanghai, China), the target sequences are "GGAUGUUAAAAGAUGGUCAdTdT" for sense and "UGACCAUCUUUUAACAUCCdTdT" for anti-sense.

*2.2. Clinical tissue collection*

Human IBD tissues were obtained from the First Affiliated Hospital of Zhejiang University at Hangzhou, China. The tissues were approved for use by the Institutional Review Board of the Hospital Ethics Committee 2020-70. A total of 47 patients, with UC (n = 21) or with CD (n = 26) were included. Tissues were obtained by experienced clinicians. For patients undergoing surgery, intestinal tissues were taken both from the inflamed and the non-inflamed regions (paired biopsies). Non-inflamed areas were defined as mucosa regions without any macroscopic/endoscopic signs of inflammation (ulceration, edema, hemorrhagic appearance, or mucinous/fibrinous coating); the other tissues were obtained by endoscopic biopsies. The tissue samples were frozen in liquid nitrogen immediately after collection and stored at -80˚C for qRT-PCR and western blot analysis. Total RNA and proteins were isolated from biopsies of non-inflamed and inflamed colon or ileal tissues.

*2.3. Animals and cell culture*

All animal procedures were performed in accordance with the 'Principles of laboratory animal care' (http://grants1.nih.gov/grants/olaw/references/phspol.htm) and were approved by the Institutional Animal Care and Use Committee of Zhejiang University (2015-0026). All experimental procedures were conducted as humanely as possible. C57BL/6 mice (8 weeks; 22± 2 g) and Balb/c mice (8 weeks; 22± 2 g) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. [SCXK(Hu)2017-0005] and housed in a specific pathogen-free facility at Zhejiang University. The animals were

housed in cages at controlled temperature (22.0 ± 1˚C) and humidity (50 ± 10) % with a 12-h light-dark cycle and free access to food and water throughout the study.

HT29 cells (human colon cancer cell line) and FHC cells (human normal colon cell line ) were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA) and cultured in RPMI 1640 supplemented with 10% FBS (v/v) and 1% penicillin/streptomycin (v/v) at 37˚C with 5% $CO_2$. All cells used in this study were between passages 3 and 8.

*2.4. DSS-induced colitis*

Colitis induced by dextran sodium sulfate (DSS; molecular weight 36,000-50,000) is a well-established experimental model that shares many symptoms with UC in humans [18]. For acute colitis, mice were given DSS (3.0% w/v) dissolved in drinking water for 7 days. For chronic colitis, mice were given three 7-day cycles of DSS (2.0% w/v) in drinking water, with intervals of 14 days of only water administration between the DSS cycles. The control mice were given normal drinking water only. The physical characteristics: body weight, stool consistency and stool blood content were recorded daily [19]. Severity of colitis was assessed by DAI based on the scoring system reported by Benoit Chassaing [20]. At the end of the DSS administration, mice were sacrificed by cervical dislocation. Tissues were collected and stored at –80°C until analysis.

To explore the relationship between OCTN2 and inflammation, adult male mice were given 2.5% DSS in drinking water for 5 days followed by regular drinking water. Control mice were given normal drinking water only. DSS-treated animals were sacrificed at Day 5, Day 9 and Day 20. The intestinal samples were collected and stored

163 at –80 ◦C prior to analysis.

164 To study the effect of luteolin on DSS-induced colitis, adult male mice were
165 randomly allocated into three groups, with eight mice in each group receiving daily
166 doses of vehicle (0.5% CMC-Na), vehicle (0.5% CMC-Na) or 50 mg/kg luteolin
167 (dissolved in CMC-Na) via oral gavage for 14 days successively. Between Days 7 to
168 14, mice in group II and III were given DSS (3.0% w/v) in their drinking water; mice
169 in group I were given water only. Body weight for all mice was recorded daily. On Day
170 15, all mice were sacrificed by cervical dislocation, the colon was excised and measured.
171 Tissues samples were collected and stored at –80 ◦C until further analysis. All efforts
172 were made to minimize suffering of mice.

173 *2.5. TNBS-induced colitis*

174 TNBS-induced colitis is a well-established experimental model that shares many
175 symptoms with CD in human [21]. Adult male mice were anesthetized with 8%
176 ketamine after fasting for 24 h, then rapidly injected with 100 μl of 2.75 mg TNBS
177 solution (100 μl of 2.75% (m/v) TNBS in anhydrous ethanol) through the rectum. The
178 control mice administered ethanol only. Physical characteristics of all mice were
179 recorded daily. Four days after TNBS administration, mice were sacrificed by cervical
180 dislocation, tissues were collected and stored at –80◦C until further analysis.

181 *2.6. Small interfering RNA and plasmid transfection*

182 HT29/FHC cells were cultured to 60% confluence in 24-well plates and transfected
183 for 48 h with OCTN2 siRNA or control siRNA using Jetprime according to the
184 manufacturer's instructions. At the end of the incubation, cell proliferation rate was
185 analyzed by MTT assay.

HT29/FHC cells were seeded at 60% confluence in 96-well plates and transiently transfected with pEnter or hOCTN2 plasmid for 24 h using Jetprime according to the manufacturer's instructions. After 24 h, cells were cultured in medium containing 0.5% FBS with or without designated concentrations of L-Car.

### 2.7. *Cellular accumulation experiment*

The functional activity of OCTN2 in control and OCTN2-overexpressed cells was analyzed by conducting d3-L-Car accumulation experiments. HT29/FHC cells were seeded at 60% confluence in 24-well plates and transfected as described earlier. 24 h after transfection, cells were washed twice and pre-incubated with Krebs-Ringer-Henseleit buffer (KRH) for 15 min at 37°C. Subsequently, 500 µL KRH containing d3-L-Car with or without L-Car was added to cells and left to accumulate for 3 min. Accumulation was terminated by removing the incubation buffer and immediately adding ice-cold buffer, after which the cells were washed 3 times with ice-cold buffer and finally lysed using 200 µL 0.1% sodium dodecyl sulfate (SDS). The concentration of d3-L-Car was determined by LC-MS/MS assay developed in our laboratory [22]. The uptake rates were normalized to the protein content of each sample lysate.

### *2.8. Treatment of FHC cells with proinflammatory cytokines*

Breakdown of immunological tolerance to exogenous antigens or luminal flora in IBD leads to excessive secretion of proinflammatory cytokines, including IL-1β, IFN-γ, and TNF-α [23]. To establish the inflammatory environment, FHC cells were seeded at a density of $2 \times 10^5$ cells/ml in 12-well plates and left to culture overnight. Cells were then incubated with or without the following proinflammatory cytokines TNF-α: INF-γ: IL -1β at a 2:2:1 concentration ratio for 24 h. These stimuli concentrations can be considered as physio-pathologically relevant, reflecting increased cytokine production in the gut mucosa during active IBD [24]. The mRNA levels of OCTN2 were measured

211 by qRT-PCR, and the concentration of L-Car was measured by LC-MS/MS as
212 described previously [22].

213 *2.9. MTT assay*

214 Cell proliferation rate was measured by MTT assay [25]. After treatments, cell
215 supernatants were removed and 200 μl of MTT reagent was added to each well at a
216 final concentration of 0.5 mg/ml and left to incubate for 4 h at 37 ◦C. Subsequently, the
217 incubation mixture was removed and 200 μl of DMSO was added to each well to
218 dissolve the formazan crystals. Finally, wells were analyzed at 570 nm optical density
219 using a microplate reader (SpectraMaxM2, Molecular Devices, CA, USA). Cell
220 proliferation rate was expressed as a percentage of the vehicle group (% control).

221 *2.10. Quantitative real-time PCR analysis*

222 Total RNA was extracted from tissues and cells using RNA simple Total RNA Kit
223 (Tiangen, Beijing, China). Subsequently, cDNAs were synthesized using PrimeScript
224 RT Master Mix (Takara, Tokyo, Japan) according to the manufacturer's instructions.
225 The quantitative real-time PCR (qRT-PCR) conditions were as follows: denaturation at
226 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s. The resulting
227 cDNA was amplified using qRT-PCR with SYBR® Premix EX Taq (Takara, Tokyo,
228 Japan). Relative mRNA levels of target genes were normalized to the housekeeping
229 gene GAPDH or β-ACTIN. The specific primers are listed in **Table 1.**

230 *2.11. Western blot analysis.*

231 Tissues were homogenized in RIPA lysis buffer (Beyotime, Shanghai, China), and
232 protein concentrations were determined using a BCA Protein Assay Kit (Beyotime,

233 Shanghai, China). Equal amounts of denatured protein (50 μg) were separated by SDS-
234 PAGE and subsequently transferred onto PVDF membranes (Millipore Corporation,
235 Billerica, MA, USA). The membranes were blocked with 5% non-fat milk in TBST
236 buffer (100-mM Tris–HCl, pH 7.4, 150-mM NaCl, and 0.1% Tween 20) and incubated
237 with primary anti-OCTN2 antibody (1:1000), anti-PPARγ (1:1000), and anti-GAPDH
238 antibody (1:5000) for 24 h at 4°C. Membranes were then washed 5 times with TBST,
239 each wash taking 5 min, and then incubated with secondary antibodies for 2 h at room
240 temperature. After incubation, secondary antibodies were washed 5 times with TBST,
241 each wash taking 5 min, and proteins were detected on X-ray film using the enhanced
242 chemiluminescence (ECL) by Western blotting detection system (LI-COR Biosciences,
243 Lincoln, NE) [26]. The Western blot experiments were performed at least 3 times.

244 *2.12. Data analysis*

245 All *in vitro* experiments were conducted at least twice, in triplicate. Data were
246 analyzed by unpaired Student's *t* test was performed between two groups, and one-way
247 analysis of variance followed with Dunnett's or Tukey's post hoc test was applied for
248 more than two groups, using GraphPad Prism version 5.0 (GraphPad Software Inc., San
249 Diego, CA, USA; RRID:SCR_002798). For all analysis, data are expressed as mean ±
250 standard error of the mean (SEM). *P* values < 0.05 were considered statistically
251 significant.

252

253 **3. Results**

254 *3.1. OCTN2 mRNA and protein expression were downregulated in the colon of IBD*

*patients and mice*

Firstly, we measured the expression of OCTN2 in the colon of non-inflamed or inflamed regions of IBD patients, including CD and UC patients. Compared to the non-inflamed areas, OCTN2 mRNA expression downregulated in the inflamed regions (**Fig. 1A**). Western blot analysis also revealed a significant downregulation of OCTN2 at the protein level in inflamed regions of IBD patients (**Fig. 1B-D**). Mouse Octn2 has 85.5% identity with human OCTN2 [27]. Moreover, the characterization of OCTN2/Octn2 as a high-affinity, sodium ion-dependent transporter of carnitine was confirmed [6, 28]. We further detected mRNA and protein levels of OCTN2 in the colon of DSS-induced acute and chronic UC male mice. The DSS-induced mice showed significant weight loss and short colon length, as well as an increased expression of proinflammatory cytokines in the colon. Histological analysis also showed obvious damage and epithelial immune cell infiltration (data not shown). The presentation of these symptoms proved that our mouse models were successful. Corroborating with the results in IBD patients, a significant downregulation of Octn2 mRNA and protein levels were observed in the colon specimens of DSS-treated male mice with acute or chronic colitis, when compared to untreated male mice ($p < 0.01$, **Fig. 2A, 2B and 2D**). In addition, Octn2 expression was also reduced in the colon of acute UC female mice, indicating that gender differences did not impact Octn2 alteration ($P < 0.001$, **Fig. 2C**). To investigate the expression of Octn2 in mice of TNBS-induced acute colitis, we developed a successful CD mouse model based on the weight loss, short colon length as well as higher proinflammatory cytokines expression in the colon (**Fig. 3A-C**). In line with the results of DSS-treated mice, Octn2 mRNA expression level was significantly downregulated in the colon of TNBS-treated male mice ($P < 0.001$, **Fig. 3D**).

*3.2. Reduction of L-Car concentrations in the colon of IBD patients and mice*

Furthermore, L-Car, a typical substrate of OCTN2, was detected in the colon tissues. **Figures 4A-C** demonstrate the drastic reduction in L-Car concentrations in the colon of IBD patients, DSS-induced acute UC mice and TNBS-induced acute CD mice, similar to the reduction of OCTN2 in colon. Additionally, we detected mRNA levels of carnitine transporters, including CT1/Ct1, CT2/Ct2, OCTN2/Octn2 and $ATB^{0,+}$/$Atb^{0,+}$ in the colon of mice and human (**Fig. 4D**). Our results revealed that OCTN2/Octn2 and $ATB^{0,+}$/$Atb^{0,+}$ was highly expressed in the colon, whereas the mRNA level of CT1/Ct1 were extremely low, and CT2/Ct2 was undetectable. The results also showed that the mRNA expression of the $ATB^{0,+}$ in the inflamed areas tended to be higher than in the non-inflamed areas of IBD patients (**Fig. 4E**). Considering the affinity to L-Car was much lower than OCTN2, we deduced that the decreased L-Car concentration in the colon of IBD patients is attributed to the reduction of OCTN2.

*3.3. OCTN2 expression was negatively correlated with severity of inflammation*

To clarify the association between OCTN2 and inflammation, we determined the expression levels of OCTN2 and pivotal proinflammatory cytokines in mice of DSS-induced UC on Day 5, Day 9, Day 20. **Figures 5A** revealed that in mice treated with 3.0% DSS for 5 days successively, colon inflammation was observed on Day 5, developed to severest on Day 9 and almost recovered by Day 20, based on the expression levels of proinflammatory cytokines ICAM-1, IL-1β and IL-6 (**Fig. 5A**). Meanwhile, the mRNA levels of OCTN2 initially decreased on Day 5, were lowest on Day 9, and increased to a degree on Day 20 (**Fig. 5A, B**). These findings suggest the

negative correlation of OCTN2 expression with the levels of ICAM-1, IL-1β and IL-6 (**Fig. 5C-E**).

*3.4. Influence of OCTN2 on the proliferation of FHC and HT29 cells*

To characterize the role of OCTN2 in colon cells, the proliferation rate of FHC and HT29 cells with or without knockdown of OCTN2 by siRNA was evaluated. As shown in **Fig. 6A-D**, 11, 33, and 55 pM of siRNA significantly reduced the expression and function of OCTN2. Following this, 33 pM of siRNA was selected in subsequent experiments for its efficient knockdown effect. The results showed that knockdown of OCTN2 significantly inhibited the proliferation rate of FHC cells, as well as in HT29 cells ($P$<0.001, **Fig. 6E, F**). We further investigated the effect of OCTN2 overexpression on cell proliferation. Transfection with hOCTN2 significantly increased the function of OCTN2 ($P$< 0.001, **Fig. 6G, H**). In addition, the proliferation rate of hOCTN2-overexpressed cells was much higher than that of control cells, whereas L-Car was found to slightly increase the proliferation rate of hOCTN2-overexpressed cells ( **Fig. 6I, J**).

*3.5. OCTN2 was downregulated by proinflammatory cytokines via PPARγ*

To elucidate the mechanism of OCTN2 downregulation in IBD, we explored the effect of a mixture of IBD-related proinflammatory cytokines TNF-α, IFN-γ, and IL-1β on OCTN2 expression in FHC (human normal colon cell line) cells. The expression of inflammatory mediator IL-18, was substantially increased in cells treated with these mixed proinflammatory cytokines (**Fig. 7A**). The results revealed that 10-30 ng/ml of mixed proinflammatory cytokines, in the following ratio TNF-α: INF-γ: IL -1β = 2:2:1,

323 reduced the levels of OCTN2 and cellular accumulation of L-Car content in a
324 concentration dependent manner (**Fig. 7B, C, E**).

325 PPARα and PPARγ are reported to contribute to OCTN2 regulation [29, 30]. Here,
326 we detected the expression of those genes in FHC cells with or without
327 proinflammatory cytokines treatment. Our results showed that the mRNA levels of
328 PPARα and PPARγ were concentration-dependent downregulated, indicating that
329 inflammation inhibited the expression of OCTN2 through PPARα/γ mediated pathways
330 (**Fig. 7D**).

331 It has been reported that ligand-activated PPARα/γ act as transcription factors and
332 assemble into a heterodimer complex with retinoid X receptor α (RXRα) binding to the
333 peroxisome proliferator responsive element (PPRE) within the promoter of OCTN2 [29,
334 30]. In this study, we determined whether the regulation of OCTN2 in IBD was also
335 mediated by PPARα/γ. The results demonstrated that the mRNA levels of PPARα,
336 PPARγ, RXRα were all significantly downregulated in the colon of DSS-treated mice.
337 Since the expression of PPARα was much lower than PPARγ in mice colon, we
338 deduced that the inflammation-induced downregulation of OCTN2 might be mediated
339 by PPARγ/RXRα pathways (**Fig. 7F**, **G**).

340 *3.6. Luteolin, an PPARγ activator, attenuated DSS-induced colitis in mice*

341 Based on our findings that the downregulation of OCTN2 in the colon might
342 contribute to the progression of IBD, and the probable involvement of PPARγ in the
343 downregulation of OCTN2, we subsequently studied the effects of luteolin, an agonist
344 of PPARγ, on DSS-induced colitis. As shown in **Fig. 8A-C**, luteolin co-treated mice

had less loss in colon length, body weight and expressed lower levels of inflammatory mediators, such as IL-1β and IL-6. Increases in Pck1, a downstream target gene of PPARγ, indicated that luteolin successfully stimulated PPARγ. Our data revealed that luteolin increased the Octn2 mRNA, protein expression and L-Car concentration in the colon (**Fig. 8D-G**). Moreover, these results also imply that luteolin could potentially protect against acute DSS-colitis as an agent to attenuate IBD progression.

## 4. Discussion

The present study demonstrated that the expression of OCTN2 in the colon was significantly decreased in IBD mice and IBD patients, which was likely mediated through PPARγ/RXRα pathways. Additionally, we showed that downregulation of OCTN2 led to L-Car deficiency and caused a reduction in the colon cell proliferation rate, whereas the reversal of OCTN2 downregulation by luteolin resulted in attenuating inflammatory response. Therefore, this finding suggests a potential strategy for improving IBD.

We observed that the OCTN2 expression and tissue L-Car concentration were significantly reduced in the colon of IBD mice and patients. This finding is inconsistent with reports from Fujiya et al, whose data based on a small sample analysis ($n \leq 11$), showed that colonic epithelial OCTN2 mRNA and protein expression were increased in actively inflamed areas of both CD and UC [15]. However, our findings corroborate previous reports in which they demonstrated a downregulation of OCTN2 mRNA level in IBD patients [31, 32]. Although CT1, CT2, OCTN2 and $ATB^{0,+}$ can mediate L-Car

uptake, our results revealed that only Octn2/OCTN2 and $Atb^{0,+}$/$ATB^{0,+}$ mRNA were highly expressed in mouse and human colon (Fig. 4D). $ATB^{0,+}$ is a low- affinity transporter for L-Car with an apparent $K_m$ of 800 μM [33] while the $K_m$ of OCTN2 is 4.3 μM [34]. Additionally, the mRNA expression of $ATB^{0,+}$ in IBD patients tended to be higher in inflamed areas compared to non-inflamed areas. Although our observation was non-significant, similar findings have been previously reported, in which an increase in $ATB^{0,+}$ mRNA expression in IBD patients was observed [35, 36]. Thus, we considered that OCTN2 mediated most of L-Car transport and was responsible for the L-Car deficiency in colons of IBD patients.

Even though the absorption of L-Car by the colon may be not relevant for the maintenance of systemic L-Car homeostasis in physiological conditions, it is crucial for colonocytes. Increasing evidence has demonstrated that OCTN2 and L-Car play a vital role in energy production and fatty acid metabolism [37, 38]. It is reasonable to speculate that the downregulation of OCTN2, and hence the reduced L-Car concentration during intestinal inflammation, may lead to failure of fatty acid β-oxidation in the epithelium of the colonic mucosa as well as cell injury.

In the present study, we explored the influence of OCTN2 on cell proliferation. We demonstrated that the knockdown of OCTN2 caused a deficiency of L-Car and significantly reduced the proliferation rate of FHC cells. Meanwhile, the proliferation rate of OCTN2-overexpressed cells was obviously higher than that of control cells, indicating an immeasurable role of OCTN2 in cell growth (Fig. 6). It is known that butyrate is the primary energy source for colonocytes, and that L-Car is involved in

389 colonic butyrate oxidation. Since feces from active CD patients present similar levels
390 of fecal short-chain fatty acids [39], impaired butyrate metabolism in IBD patients may
391 be due to impaired oxidation in IBD patients [40]. Our results support the view of
392 Roediger, that it is not decreased butyrate uptake but rather abnormal butyrate
393 utilization that amplifies colonocyte damage in IBD patients [41]. Taken together, we
394 suggest that the decrease of L-Car levels may be responsible for disorders of colonic
395 energy metabolism and disturb internal milieu.

396 To date, little is known about the regulation mechanisms of intestinal epithelial
397 OCTN2 in disease. Here, we report for the first time that the expression of OCTN2
398 negatively correlated with the expression of proinflammatory cytokines, suggesting that
399 the downregulation of OCTN2 in IBD may be attributed to active mucosal
400 inflammation (Fig. 5). We analyzed how the endogenous mediators IL-1β, TNF-α, IFN-
401 γ, whose mucosal levels are increased during IBD, affect OCTN2 expression. The
402 concentration of stimuli used here is considered to represent the acute inflammatory
403 reaction and was verified as non-cytotoxic [24, 42]. As expected, the mixed
404 proinflammatory cytokines significantly downregulated the expression of OCTN2 and
405 reduced L-Car content in FHC cells (Fig. 7A-E). While this finding contradicts a
406 previous report stating that proinflammatory cytokines TNFα and IFNγ stimulate
407 OCTN2 expression in Caco-2 BBE cells [15], our results are in line with data from Li
408 et al. showing that LPS downregulated OCTN2 in alveolar epithelial cells [43].

409 Furthermore, we found that inflammation downregulated the expression of PPARα/γ
410 in FHC cells in a concentration-dependent manner, which was consistent with the report

411 that cytokines decrease the expression of RXRα, PPARα, PPARγ in Hep3B human
412 hepatoma cells and 3T3-L1 adipocytes [44, 45]. In addition, the mRNA levels of
413 PPARα/γ and RXRα were obviously lower in the colon of DSS-treated mice. The
414 expression of PPARα in the colon was much lower than that of PPARγ; thus, our data
415 suggests that PPARγ plays a major role in the regulation of OCTN2 in the colon (Fig.
416 7F-7G). Further studies are needed to elucidate the mechanism by which inflammation
417 regulates PPARγ. Studies have shown that PDZK1/2 also participates separately in the
418 regulation of OCTN2 by regulating the function of OCTN2 and stimulating transport
419 activity of OCTN2. We have observed a decrease in PDZK1 and PDZK2 mRNA levels
420 in DSS-induced mice; however, the mixed proinflammatory cytokine stimulation had
421 no effect on their expression in FHC cells (data not shown). Based on this, the role of
422 PDZK1 and PDZK2 in the regulation of OCTN2 in IBD requires further investigation.
423 Together these results indicate that the reduction of L-Car reabsorption may be the
424 result of inflammation-induced OCTN2 downregulation through PPARγ/RXRα
425 pathways.

426 Based on our finding that PPARγ was downregulated in the colon of IBD mice and
427 it mediated the downregulation of OCTN2, we explored the effects of luteolin, an
428 agonist of PPARγ, on the progression of IBD. Our results demonstrated that luteolin
429 could protect mice from DSS-induced colitis, while OCTN2 expression levels and L-
430 Car content had increased (Fig. 8). The study by Li et al suggested a protective role of
431 luteolin in IBD through activation of the Nrf2 signaling pathway [46]. We suggest that
432 the upregulation of OCTN2 levels may be another mechanism to protect IBD

deterioration by luteolin.

In summary, our findings suggest that OCTN2 may participate in the restoration of intestinal homeostasis under conditions of inflammation. A decrease in colonic OCTN2 expression in the IBD state resulted in a decrease in L-Car uptake by intestinal epithelial cells, causing disorders of renewal and repair, leading to the deterioration of inflammation and poor prognosis. The promising results on the PPARγ agonists, such as luteolin, in relieving inflammation provide strong support for further research on OCTN2, with the aim of improving IBD.

## Ethics statement

The human intestinal tissues were approved for use by the Ethics Committee (2020-70) of the First Affiliated Hospital of Zhejiang University. And all patients signed their informed consent.

## Author contributions

P. L., Y. Q. W., and H. D. J. designed the research. P. L., J. L., M. J. W. performed the research. P. L., M. R. B. analyzed data. P. L., Q. Q. Z and H. D. J. wrote the paper. All authors read and approved the final manuscript.

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**Conflict of interest**

The authors declare that they have no conflict of interest.

**References**

[1] M. F. Neurath, Current and emerging therapeutic targets for IBD, Nat. Rev. Gastro. Hepat. 14(5) (2017) 269-278.

[2] N. A. Molodecky, I. S. Soon, D. M. Rabi, W. A. Ghali, M. Ferris, G. Chernoff, E. I. Benchimol, R. Panaccione, S. Ghosh, H. W. Barkema, G. G. Kaplan, Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review, Gastroenterology. 142(1) (2012) 46-54.

[3] J. Sýkora, R. Pomahačová, M. Kreslová, D. Cvalínová, P. Štych, J. Schwarz, Current global trends in the incidence of pediatric-onset inflammatory bowel disease, World J. Gastroentero. 24(25) (2018) 2741-2763.

[4] S. C. Ng, H. Y. Shi, N. Hamidi, F. E. Underwood, W. Tang, E. I. Benchimol, R. Panaccione, S. Ghosh, J. C. Y. Wu, F. K. L. Chan, J. J. Y. Sung, G. G. Kaplan, Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: A systematic review of population-based studies, The Lancet. 390(10114) (2017) 2769-2778.

[5] P. Olivera, S. Danese, N. Jay, G. Natoli, L. Peyrin-Biroulet, Big data in IBD: A look into the future, Nat. Rev. Gastro. Hepat. 16(5) (2019) 312-321.

[6] I. Tamai, R. Ohashi, J. Nezu, Y. Sai, D. Kobayashi, A. Oku, M. Shimane, A. Tsuji,

Molecular and functional characterization of organic Cation/Carnitine transporter family in mice, J. Biol. Chem. 275(51) (2000) 40064-40072.

[7]S. P. Chapela, N. Kriguer, E. H. Fernandez, C. A. Stella, Involvement of L-carnitine in cellular metabolism: Beyond Acyl-CoA transport, Mini Rev Med Chem. 9(13) (2009) 1518-1526.

[8]G. D. Argenio, M. Calvani, A. Casamassimi, E. Al, Experimental colitis: Decreased Octn2 and Atb0+ expression in rat colonocytes induces carnitine depletion that is reversible by carnitine-loaded liposomes, The Faseb Journal. 20(14) (2006) 2544-2546.

[9]G. Famularo, C. Somone, V. TrinchieriRI, L. Mosca, Carnitines and its congeners: A metabolic pathway to the regulation of immune response and inflammation, Ann. Ny. Acad. Sci. 1033(1) (2004) 132-138.

[10]G. Fortin, K. Yurchenko, C. Collette, M. Rubio, A. C. Villani, A. Bitton, M. Sarfati, D. Franchimont, L-carnitine, a diet component and organic cation transporter OCTN ligand, displays immunosuppressive properties and abrogates intestinal inflammation, Clinical & Experimental Immunology. 156(1) (2009) 161-171.

[11]P. L. Magoulas, A. W. El-Hattab, Systemic primary carnitine deficiency: An overview of clinical manifestations, diagnosis, and management, Orphanet J. Rare Dis. 7(1) (2012) 68.

[12]L. Pochini, M. Scalise, M. Galluccio, C. Indiveri, OCTN cation transporters in health and disease: Role as drug targets and assay development, J. Biomol. Screen. 18(8) (2013) 851-867.

[13]L. N. Colin, R. N. Elaine, H. Drummond, G. T. Ho, A. Tenesa, L. Smith, N. Anderson, I. D. R. Arnott, J. Satsangi, The contribution of OCTN1/2 variants within the IBD5 locus to disease susceptibility and severity in crohn ’ s disease,

Gastroenterology. 129(6) (2005) 1854-2186.

[14]S. Waller, Evidence for association of OCTN genes and IBD5 with ulcerative colitis, Gut. 55(6) (2006) 809-814.

[15]M. Fujiya, Y. Inaba, M. W. Musch, S. Hu, Y. Kohgo, E. B. Chang, Cytokine regulation of OCTN2 expression and activity in small and large intestine, Inflamm. Bowel Dis. 17(4) (2011) 907-916.

[16]J. K. Yamamoto-Furusho, E. J. Mendivil-Rangel, M. A. Villeda-Ramírez, G. Fonseca-Camarillo, R. Barreto-Zuñiga, Gene expression of carnitine organic cation transporters 1 and 2 (OCTN) is downregulated in patients with ulcerative colitis, Inflamm. Bowel Dis. 17(10) (2011) 2205-2206.

[17]M. Girardin, S. Dionne, P. Goyette, J. Rioux, A. Bitton, I. Elimrani, P. Charlebois, I. Qureshi, E. Levy, E. G. Seidman, Expression and functional analysis of intestinal organic cation/l-carnitine transporter (OCTN) in Crohn's Disease, Journal of Crohn's and Colitis. 6(2) (2012) 189-197.

[18]R. M. Gadaleta, O. Garcia-Irigoyen, A. Moschetta, Exploration of inflammatory bowel disease in mice: Chemically induced murine models of inflammatory bowel disease (IBD), Current Protocols in Mouse Biology. 7(1) (2017) 13-28.

[19]Y. Wang, Y. Hu, P. Li, Y. Weng, N. Kamada, H. Jiang, D. E. Smith, Expression and regulation of proton-coupled oligopeptide transporters in colonic tissue and immune cells of mice, Biochem. Pharmacol. 148(2018) 163-173.

[20]B. Chassaing, J. D. Aitken, M. Malleshappa, E. Al, Dextran sulfate sodium (DSS)-induced colitis in mice, Current Protocols in Immunology. 104(15/25) (2014) 15-25.

[21]R. M. Gadaleta, O. Garcia-Irigoyen, A. Moschetta, Exploration of inflammatory bowel disease in mice: Chemically induced murine models of inflammatory bowel disease (IBD), Current protocols in mouse biology. 7(1) (2017) 13-28.

[22] W. Wang, M. Bai, T. Jiang, C. Li, P. Li, H. Zhou, Z. Wang, L. Li, H. Jiang, Clozapine-induced reduction of l-carnitine reabsorption via inhibition/down-regulation of renal carnitine/organic cation transporter 2 contributes to liver lipid metabolic disorder in mice, Toxicol. Appl. Pharm. 363(2019) 47-56.

[23] M. Nakamura, H. Saito, J. Kasanuki, Y. Tamura, S. Yoshida, Cytokine production in patients with inflammatory bowel disease, Gut. 33(7) (1992) 933-937.

[24] J. Van, A. Hendrickx, B. Romier, Infammatory parameters in Caco-2 cells: Effect of stimuli nature, concentration, combination and cell diferentiation, Toxicol. In Vitro. 24(5) (2010) 1441-1449.

[25] Z. Ma, S. Lu, D. Sun, M. Bai, T. Jiang, N. Lin, H. Zhou, S. Zeng, H. Jiang, Roles of organic anion transporter 2 and equilibrative nucleoside transporter 1 in hepatic disposition and antiviral activity of entecavir during non‐pregnancy and pregnancy, Brit. J. Pharmacol. 176(17) (2019) 3236-3249.

[26] T. Jiang, Y. Zhang, M. Bai, P. Li, W. Wang, M. Chen, Z. Ma, S. Zeng, H. Zhou, H. Jiang, Up-regulation of hepatic fatty acid transporters and inhibition/down-regulation of hepatic OCTN2 contribute to olanzapine-induced liver steatosis, Toxicol. Lett. 316(2019) 183-193.

[27] Primary systemic carnitine deficiency is caused by mutations in a gene encoding sodium ion-dependent carnitine transporter, Na Genet. 1(21) (1999)91-94.

[28] I. Tamai, R. Ohashi, J. Nezu, H. Yabuuchi, A. Oku, M. Shimane, Y. Sai, A. Tsuji, Molecular and functional identification of sodium ion-dependent, high affinity human carnitine transporter OCTN2, J. Biol. Chem. 273(32) (1998) 20378-20382.

[29] G. D'Argenio, O. Petillo, S. Margarucci, E. Al, Colon OCTN2, gene expression is up-regulated by peroxisome proliferator-activated receptor γ in humans and mice and contributes to local and systemic carnitine homeostasis, Journal of Biological

Chemistry. 285(35) (2010) 27078.

[30]G. Wen, R. Ringseis, K. Eder, Mouse OCTN2 is directly regulated by peroxisome proliferator-activated receptor α (PPAR α) via a PPRE located in the first intron, Biochem. Pharmacol. 79(5) (2010) 768-776.

[31]K. Repnik, U. Potocnik, Haplotype in the IBD5 region is associated with refractory Crohn's disease in Slovenian patients and modulates expression of the SLC22A5 gene, J. Gastroenterol. 46(9) (2011) 1081-1091.

[32]K. A. Wojtal, J. J. Eloranta, P. Hruz, H. Gutmann, J. Drewe, A. Staumann, C. Beglinger, M. Fried, G. A. Kullak-Ublick, S. R. Vavricka, Changes in mRNA expression levels of solute carrier transporters in inflammatory bowel disease patients, Drug Metab. Dispos. 37(9) (2009) 1871-1877.

[33]T. Nakanishi, T. Hatanaka, T. Huang, P. D. Prasad, F. H. Leibach, M. E. Ganapathy, V. Ganapathy, Na+- and Cl- -coupled active transport of carnitine by the amino acid transporter ATB(0,+) from mouse colon expressed in HRPE cells and Xenopus oocytes, J. Physiol.-London. 532(2) (2001) 297-304.

[34]I. Tamai, Pharmacological and pathophysiological roles of carnitine/organic cation transporters (OCTNs: SLC22A4, SLC22A5 and Slc22a21), Biopharm. Drug Dispos. 34(1) (2013) 29-44.

[35]J. T. Bjerrum, C. Nyberg, J. Olsen, O. H. Nielsen, Assessment of the validity of a multigene analysis in the diagnostics of inflammatory bowel disease, J. Intern. Med. 275(5) (2014) 484-493.

[36]C. Flach, A. Eriksson, E. Jennische, S. Lange, C. Gunnerek, I. Lönnroth, Detection of elafin as a candidate biomarker for ulcerative colitis by whole-genome microarray screening, Inflamm. Bowel Dis. 12(9) (2006) 837-842.

[37]P. Rinaldo, D. Matern, M. J. Bennett, Fatty acid oxidation disorders, Annu. Rev.

Physiol. 64(1) (2002) 477-502.

[38]S. Yildirim, A. Yildirim, S. Dane, E. Aliyev, R. Yigitoglu, Dose-Dependent protective effect of L-Carnitine on oxidative stress in the livers of hyperthyroid rats, The Eurasian Journal of Medicine. 45(1) (2013) 1-6.

[39]E. Ferrer-Picon, I. Dotti, A. M. Corraliza, A. Mayorgas, M. Esteller, J. C. Perales, E. Ricart, M. C. Masamunt, A. Carrasco, E. Tristan, M. Esteve, A. Salas, Intestinal inflammation modulates the epithelial response to butyrate in patients with inflammatory bowel disease, Inflamm. Bowel Dis. 26(1) (2020) 43-55.

[40]V. De Preter, I. Arijs, K. Windey, W. Vanhove, S. Vermeire, F. Schuit, P. Rutgeerts, K. Verbeke, Impaired butyrate oxidation in ulcerative colitis is due to decreased butyrate uptake and a defect in the oxidation pathway, Inflamm. Bowel Dis. 18(6) (2012) 1127-1136.

[41]W. E. W. Roediger, The colonic epithelium in ulcerative colitis: An energy-deficiency disease? The Lancet. 316(8197) (1980) 712-715.

[42]P. Rosenstiel, M. Fantini, K. Bräutigam, TNF-alpha and IFN-gamma regulate the expression of the NOD2 (CARD15) gene in human intestinal epithelial cells, Gastroenterology. 124(4) (2003) 1001-1009.

[43]C. Q. J. Z. Dalang Li, X. J. Song, LPS-induced inflammation delays the transportation of ASP + due to down-regulation of OCTN1/2 in alveolar epithelial cells, Journal of Drug Targeting. 3 (2019) 1-31.

[44]M. S. Kim, T. R. Sweeney, J. K. Shigenaga, Tumor necrosis factor and interleukin 1 decrease RXR α, PPAR α, PPAR γ, LXR α, and the coactivators SRC-1, PGC-1 α, and PGC-1 β, in liver cells, Metabolism-clinical & Experimental. 56(2) (2007) 267-279.

[45]B. Lu, A. H. Moser, J. K. Shigenaga, K. R. Feingold, C. Grunfeld, Type II nuclear

hormone receptors, coactivator, and target gene repression in adipose tissue in the acute-phase response, J. Lipid Res. 47(10) (2006) 2179-2190.

[46] Y. Li, L. Shen, H. Luo, Luteolin ameliorates dextran sulfate sodium-induced colitis in mice possibly through activation of the Nrf2 signaling pathway, Int. Immunopharmacol. 40(2016) 24-31.

**Figure Legends**

**Fig. 1.** Colonic OCTN2 expression is reduced in human CD and human UC. (**A**) mRNA level in CD (n = 26) and UC (n = 21) patients; (**B**) Protein level in IBD patients (n = 8, paired tissues) (**C**) Qualitative assessment of OCTN2 protein expression in human CD, (paired tissues). (**D**) Qualitative assessment of OCTN2 protein expression in human UC, (paired tissues). NI, non-inflamed region; I, inflamed region; UC, ulcerative colitis; CD, Crohn's diseases; Data are expressed as mean ± SEM, compared with the non-inflamed region of the same patient, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

**Fig. 2.** Expression of Octn2 in the colon tissues from normal mice (Ctr) and DSS-induced colitis mice. (**A**) mRNA level in male mice with acute UC (n = 10). (**B**) mRNA level in male mice with chronic UC (n = 6). (**C**) mRNA level in female mice with acute UC (n = 8). (**D**) Qualitative assessment of OCTN2 protein expression in male mice with acute UC. Colitis was induced with 3.0% DSS for acute UC and 2.0% DSS for chronic UC. Data are expressed as mean ± SEM, compared to the control group (Ctr), $**P < 0.01$, $***P < 0.001$.

**Fig. 3.** mRNA expression of Octn2 in mice of TNBS-induced colitis. (**A**) Weight loss

627 was evaluated daily. Colon length (**B**) and mRNA levels of proinflammatory cytokines
628 (**C**) were evaluated at Day 4 after TNBS administration. (**D**) mRNA expression of
629 Octn2. Data are expressed as mean ± SEM, n = 5, male mice, compared to the control
630 group (Ctr), $^{**}P < 0.01$, $^{***}P < 0.001$.

631

632 **Fig. 4.** L-Car concentrations and mRNA levels of carnitine transporters in the colon of
633 IBD patients and mice. (**A**) L-Car concentration in colonic tissues of IBD patients with
634 CD (n = 10) and UC (n = 5), compared to the non-inflamed region of the same patient.
635 (**B**) L-Car concentrations in colons of normal mice (Ctr) and DSS-induced acute colitis
636 male mice (n = 10). (**C**) L-Car concentrations in colons of normal mice (Ctr) and TNBS-
637 induced acute colitis male mice (n = 5). Data are expressed as mean ± SEM, compared
638 to the control group (Ctr), $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. (**D**) Carnitine
639 transporter mRNA levels in mice and human colons (n = 5). (**E**) Colonic $ATB^{0,+}$ mRNA
640 expression in IBD patients with CD (n = 15) and with UC (n = 5), compared to the non-
641 inflamed region of the same patient.

642

643 **Fig. 5.** The association between inflammation and Octn2. (**A**) mRNA expression of
644 Octn2 and inflammatory cytokines in mice at different stages of DSS-induced colitis.
645 (**B**) Mice were given 3.0% DSS in their drinking water for 5 days followed by regular
646 drinking water. The correlation between Octn2 and ICAM-1(**C**), IL -1β (**D**), IL-6 (**E**),
647 respectively. Data are expressed as mean ± SEM (n = 5) in male mice, compared to the
648 control group (Ctr), $^{**}P < 0.01$, $^{***}P < 0.001$.

649

650 **Fig. 6.** Influence of OCTN2 on proliferation rate of FHC and HT29 cells. (**A-F**) Cells
651 were transfected with either scrambled control siRNA or OCTN2 specific siRNA for
652 48 h. The mRNA levels of OCTN2 (**A, B**) and the concentrations of L-Car (**C, D**).
653 Proliferation rate of FHC cells (**E**) and HT29 cells (**F**). Cell proliferation rate was
654 normalized to cells transfected with scrambled control siRNA (100%). Data are
655 expressed as mean ± SEM (n = 6) compared to the control group (NC), $^{*}P < 0.05$, $^{**}P$
656 $< 0.01$, $^{***}P < 0.001$. (**G, H**) d3-L-Car accumulation in hOCTN2-overexpressed cells.
657 Cells were transfected with either pEnter or hOCTN2 plasmid for 24 h. L-Car was used
658 as inhibitor of OCTN2. Data are expressed as mean ± SEM (n = 3). Compared with
659 cells transfected with pEnter, $^{###}P < 0.001$, compared with the accumulation without
660 inhibitor, $^{***}P < 0.001$. (**I, J**) Cell proliferation rate of hOCTN2-overexpressed cells.
661 Cells were transfected with either pEnter or hOCTN2 plasmid for 24 h and incubated
662 with designed concentrations of L-Car for another 24 h. Cell proliferation rate was
663 normalized to Ctr-pEnter cells. Data are expressed as mean ± SEM (n = 5). Compared
664 to cells transfected with pEnter plasmid, $^{#}P < 0.05$, $^{##}P < 0.01$, $^{###}P < 0.001$.

665

666 **Fig. 7.** Mechanisms of OCTN2 downregulation by inflammation. The effect of
667 proinflammatory cytokines on mRNA expression of IL-18 (**A**), OCTN2 (**B**) and mRNA
668 expression of PPARα and PPARγ (**D**) in FHC cells. (**C**) The concentration of L-Car.
669 (**E**) Protein levels of OCTN2 in FHC cells. Cells were incubated with or without
670 concentrations of mixed proinflammatory cytokines (TNF-α: INF-γ: IL -1β = 2:2:1) for

671 24 h. Data are expressed as mean ± SEM (n = 6) compared to the control group (Ctr),
672 $^{**}P < 0.01$, $^{***}P < 0.001$. (**F**) The mRNA levels of PPARα, PPARγ, RXRα in DSS-
673 induced mice. (**G**) Protein levels of PPARγ in DSS-induced mice. Data are expressed
674 as mean ± SEM (n = 10) compared to with the control group (Ctr), $^{*}P < 0.05$, $^{***}P <$
675 0.001.
676
677 **Fig. 8.** The influence of luteolin on DSS-induced colitis. Effect of Octn2 agonist
678 luteolin on colon length (**A**), body weight (**B**), the mRNA levels of proinflammatory
679 cytokines (**C**) and Octn2, Pck1 and PPARγ (**D**) in colons. (**E**) The concentrations of L-
680 Car in colons. (**F**) The protein expression of OCTN2. (**G**) Qualitative assessment of
681 OCTN2 protein expression in colons. Data are expressed as mean ± SEM (n = 8), male
682 mice. compared to the control group (Ctr), $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$,
683 $^{\#}P<0.05$, $^{\#\#}P<0.01$.
684
685 **Table 1. Primer list for real-time PCR**

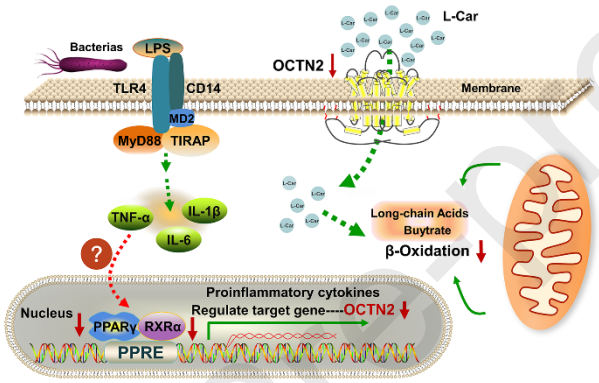
| Gene | Forward primer | Reverse prime |
|---|---|---|
| Mus PPARα | TTTTAGACCCCCAGGGAAAC | CCCTGTCCCTGGAACCTC |
| Mus PPARβ | GGGAAAAGTTTTGGCAGGA | TGCCCAAAACACTGTACAACA |
| Mus PPARγ | TGACAGGAAAGACAACAGACAAA | GAGGACTCAGGGTGGTTCAG |
| Mus RXRα | GCGTACTGCAAACACAAGTACC | CCCGATGAGCTTGAAGAAGA |
| Mus PDZK1 | ACTCTGTTGGTGCTGGACAA | GCAGTTCTTGACTTTGGCAGTA |
| Mus PDZK2 | TTTGGCTTCAGCGTCACC | CCCCATTCACTTCCAGCA |

| | | |
|---|---|---|
| Human PPARα | AAGCTGTCACCACAGTAGCTTG | AACGAATCGCGTTGTGTGAC |
| Human PPARβ | ACCAACGAGGGTCTGGAAT | TCTGAACGCAGATGGACCTC |
| Human PPARγ | GGAAGGTGGGTGTGTAGTCG | AGAGGTTAAGGCCCCTTCCT |
| Human RXRα | CCTTCACTTCCTGGCCATCCA | TCCCTACAGACCACAGGCAC |

686

687 **Graphical Abstract:**

688




689

690 Schematic diagram of OCTN2 regulation mechanism and the role of OCTN2 in mitochondrial fatty
691 acid oxidation in intestinal epithelial cells.

692

693 **CRediT authorship contribution statement**

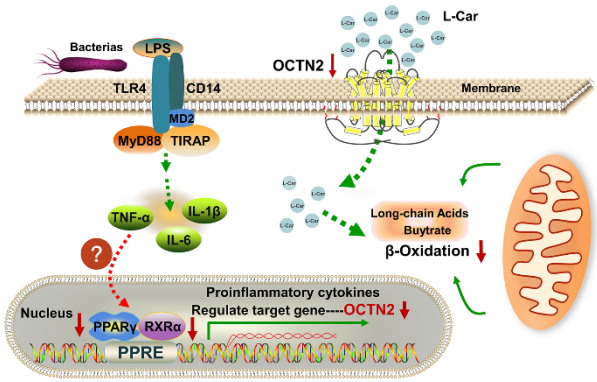
694 **Ping Li:** Conceptualization, Methodology, Validation, Investigation, Formal analysis,
695 Writing - Original Draft, Writing - Review & Editing. **Yuqing Wang:**
696 Conceptualization, Methodology, Writing - Review & Editing. **Jun Luo:** Methodology,
697 Investigation. **Qingquan Zeng:** Methodology, Investigation. **Miaojuan Wang:**
698 Investigation. **Mengru Bai:** Conceptualization. **Hui Zhou:** Project administration,
699 Funding acquisition. **Jinhai Wang:** Conceptualization, Project administration,
700 Resources. **Huidi Jiang:** Conceptualization, Funding acquisition, Project

701 administration, Supervision, Writing - Original Draft, Writing - Review & Editing.

702

703

704

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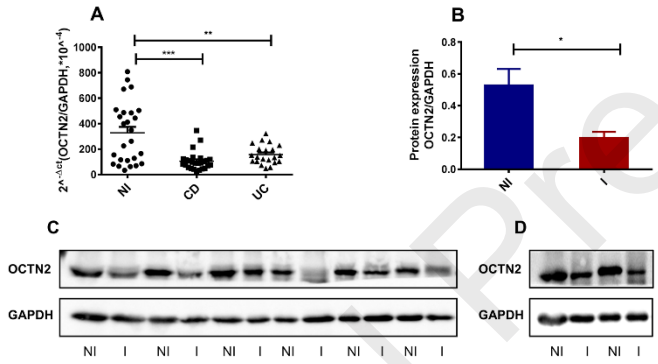


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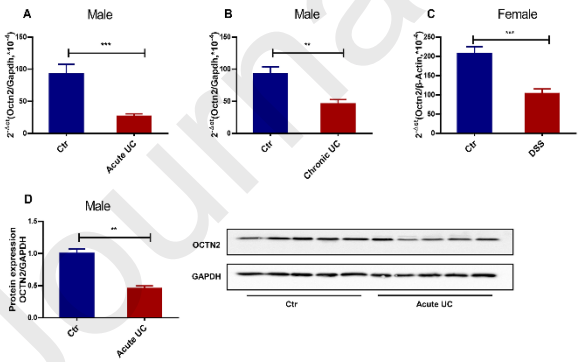

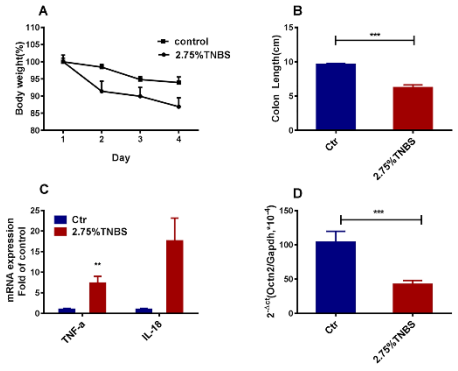


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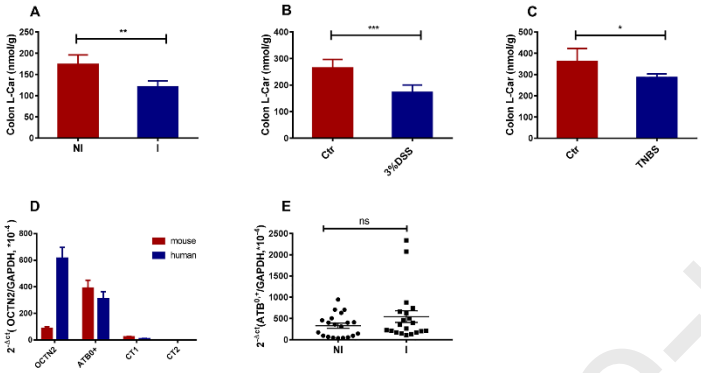


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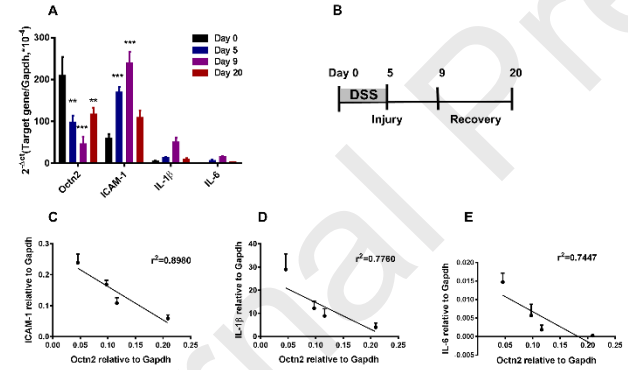


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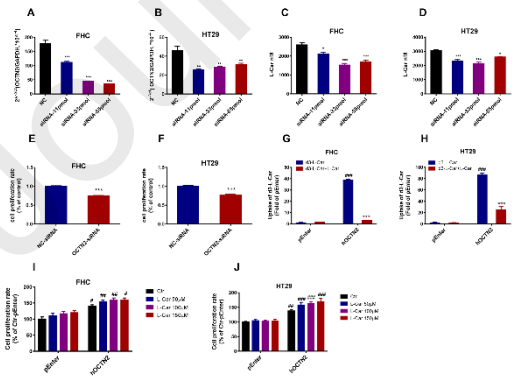


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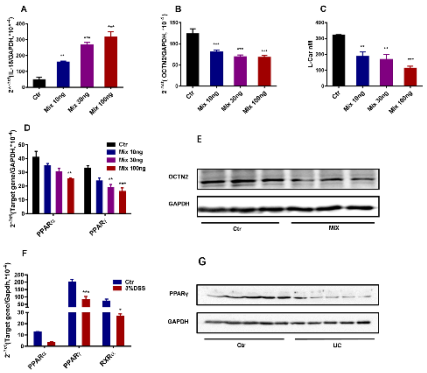

712