

Original Article

Wogonoside alleviates ulcerative colitis-induced senescence by activating AMPK in *in vivo* and *in vitro* models

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Abstract: Ulcerative colitis (UC), a chronic inflammatory bowel disease, causes extensive damage, primarily affecting the colonic mucosa, where persistent inflammation leads to conditions such as mucosal congestion. Recent studies suggest a reciprocal interaction between intestinal cell senescence and inflammation; however, the connection between senescence and UC remains underexplored. Wogonoside, a key active component of Huangqin Decoction, has unclear effects on intestinal cell and tissue senescence in the context of UC. Methods: Dextran Sodium Sulfate (DSS)-induced UC cell and animal models were employed to investigate the potential therapeutic effects of Wogonoside on intestinal epithelial cell senescence and its underlying mechanisms. Results: DSS treatment induced senescence in intestinal cells. Wogonoside alleviated senescence, as indicated by reduced levels of senescence markers (SA- β -gal, p16, p21, p53) and decreased epithelial damage, evidenced by increased occludin and ZO-1 expression. Mechanistic studies revealed that Wogonoside activates the AMPK signaling pathway, attenuating ROS production and thereby preventing intestinal cell senescence and injury. *In vivo*, Wogonoside similarly reduced DSS-induced senescence, as demonstrated by senescence marker analysis. Conclusion: This study demonstrates that Wogonoside mitigates UC-induced intestinal cell senescence and epithelial barrier disruption by activating the AMPK pathway and reducing ROS production, offering protective effects in both cellular and animal models. These findings suggest that Wogonoside may be a promising therapeutic strategy for the treatment of UC.

Keywords: Wogonoside, ulcerative colitis, senescence, AMPK, p16

Introduction

In recent years, advancements in science and technology have significantly improved the treatment of many refractory diseases. Current therapies for inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease, mainly rely on antibiotics and immunosuppressants, which are often associated with considerable side effects with prolonged use [1]. Therefore, identifying effective treatments or key therapeutic targets for IBD remains a major focus of research and clinical practice.

IBD is characterized by chronic inflammation of the gastrointestinal tract, with its onset and progression closely linked to genetic predisposition, gut microbiota dysbiosis, and dysregu-

lated immune responses [2]. Recent studies suggest a strong, bidirectional relationship between UC and cellular senescence, creating a vicious cycle [3]. In UC, the persistent inflammatory microenvironment plays a central role in driving cellular senescence. Inflammatory mediators, such as reactive oxygen species (ROS), cause DNA damage, triggering premature senescence in intestinal epithelial cells. Senescent cells release the Senescence-Associated Secretory Phenotype (SASP), a collection of pro-inflammatory factors that further amplify local inflammation and induce senescence in nearby healthy cells, perpetuating the cycle [4]. UC not only induces inflammation but also acts as a "time accelerator", accelerating aging processes in intestinal tissues. Senescence contributes to the initiation and progression of UC

[5]. As aging progresses, immunosenescence reduces immune surveillance and regulation, causing immune cells like T cells to adopt a pro-inflammatory phenotype, which exacerbates intestinal inflammation. Simultaneously, aging impairs intestinal barrier function and promotes gut microbiota dysbiosis, creating a conducive environment for colitis development [6]. Thus, UC-related chronic inflammation drives intestinal cell senescence, while age-related immune dysfunction, barrier compromise, and telomere attrition increase susceptibility to and severity of UC, creating a mutually exacerbating relationship [7].

Wogonoside, a flavonoid compound derived from *Scutellaria baicalensis* Georgi, is a glycosylated derivative of baicalin [8]. It exhibits a wide range of biological activities, including anti-inflammatory, antioxidant, anti-tumor, and neuroprotective effects. By modulating signaling pathways such as NF- κ B and MAPK, Wogonoside inhibits the release of inflammatory factors and mitigates oxidative stress via free radical scavenging, demonstrating notable protective effects in disease models such as UC and hepatitis [9]. Additionally, Wogonoside has been shown to inhibit the progression of various cancers [10]. However, its impact on UC-induced intestinal cell senescence remains unclear and warrants further investigation.

This study used intestinal cell models to evaluate the effects of Wogonoside on DSS-induced senescence. The results demonstrate that Wogonoside mitigates intestinal cell aging in both *in vivo* and *in vitro* models. Our findings suggest that Wogonoside alleviates UC-induced intestinal cell senescence and epithelial barrier damage by activating the AMPK signaling pathway and reducing ROS production, offering a novel therapeutic approach for UC treatment.

Materials and methods

Antibodies and chemicals

The ECL chemiluminescence solution was purchased from Millipore, USA. ZO-1 and Claudin-1 antibodies were obtained from CST, USA. RIPA lysis buffer, Goat Anti-Rabbit secondary antibody, Goat Anti-Mouse secondary antibody, and fluorescence-labeled secondary antibodies were sourced from CST, USA. TBS powder, DAPI, skimmed milk powder, BCA protein concentration assay kit, bovine serum albumin,

and protein loading buffer were obtained from Wuhan Boshide Biotechnology Co., Ltd. Wogonoside, Dextran Sodium Sulfate (DSS), PBS buffer, Hematoxylin & Eosin staining solution, and neutral balsam were purchased from MCE. Thermo Fisher Scientific provided the Cell Counting Kit-8 (CCK-8), cell detection kit, Deferoxamine (DFO), Ferrostatin-1 (Fer-1), DMSO, ECL reagent, and FBS (Fetal Bovine Serum). Anti-GPX4 (Glutathione Peroxidase 4) and anti-HO-1 (Heme Oxygenase-1) antibodies were acquired. The MDA assay kit, ZVAD-FMK, DCFH-DA, and BrdU assay kit were sourced from Beijing Solarbio Science & Technology Co., Ltd. The total protein extraction kit, isopropanol, PAGE gel rapid preparation kit, skimmed milk powder, SuperKine ultrasensitive ECL luminescent solution, β -galactosidase staining kit, and PVDF membrane were obtained from Millipore, USA. The GSH assay kit, ECL chemiluminescence kit, BCA kit, RIPA lysis buffer, DMSO, and SDS were purchased from Sigma-Aldrich. Anti-p16, anti-p21, and anti-p53 antibodies were acquired from CST, USA.

Cell culture

Caco-2 and NCM460 cells were utilized as intestinal epithelial cell models. Caco-2 cells were cultured in high-glucose DMEM with 10% FBS, while NCM460 cells, representing normal colon epithelium, were maintained in RPMI-1640 medium with 10% FBS. Both cell lines were incubated at 37°C with 5% CO₂ and controlled humidity.

Cell viability assay (CCK-8)

For cell proliferation assays, Caco-2 and NCM460 cells were digested with trypsin, resuspended into uniform cell suspensions, and seeded at 5,000 cells per well in 96-well plates. After reaching 50%-60% confluence, the medium was refreshed, and varying concentrations of the drug were added. The plate was incubated for 48 hours at 37°C. Following incubation, 20 μ L of CCK-8 solution was added to each well under light-protected conditions. After another 4 hours of incubation, absorbance was measured.

Cellular GSH, MDA, and iron assays

For further analysis, Caco-2 and NCM460 cells at 60%-70% confluence were exposed to specific concentrations of Wogonoside. After 48

hours, cells were washed three times with PBS. The final wash was removed, and a small amount of PBS was added to cover the cells. Cells were scraped, collected into centrifuge tubes, vortexed and disrupted, then centrifuged to collect the supernatant. The supernatant was stored at -80°C for subsequent BCA, GSH, and MDA analysis following the kit instructions.

Detection of intracellular ROS

Caco-2 and NCM460 cells were seeded into 6-well plates at a density of 1.2×10^6 cells per well. Upon reaching 60%-70% confluence, the cells were treated with the respective drugs and cultured for an additional 24 hours. After incubation, the medium was discarded, and cells were washed three times with PBS. Cells were then digested with trypsin, resuspended in 2 mL of serum-free medium, and pipetted to ensure complete detachment. The resulting cell suspensions were transferred to 2 mL centrifuge tubes and thoroughly mixed. All groups, except the blank control, were incubated with 10 μ M DCFH-DA dye at 37°C in the dark for 30 minutes, with gentle shaking every 5 minutes to prevent cell sedimentation. After incubation, cells were washed three times with PBS to remove any residual dye, resuspended, and intracellular ROS levels were assessed using a CLSM.

Cell immunofluorescence staining

Caco-2 and NCM460 cells were plated into confocal dishes. After drug treatment and culture, cells were fixed with 4% paraformaldehyde for 15 minutes and washed three times with PBS. Cells were then incubated with immunofluorescence blocking buffer at 37°C for 30 minutes. After removing the blocking solution and washing the cells with PBS, corresponding primary antibodies were added to cover the cells and incubated overnight at 4°C. The following day, the primary antibodies were removed, and the cells were washed three times with PBS. Fluorescent secondary antibodies were added and incubated at 37°C in the dark for 1 hour. After three additional PBS washes, DAPI staining solution was applied, and cells were incubated in the dark for 15 minutes to label the nuclei. Cells were then washed with PBS, immersed in PBS, and imaged using a laser

scanning confocal microscope at the appropriate excitation wavelengths.

Western blot analysis

Following Wogonoside treatment, total protein was extracted from cells or tissues, subjected to gel electrophoresis, and transferred to PVDF membranes. The membranes were then placed in blocking buffer and incubated with shaking at room temperature for 2 hours, followed by three washes with TBST. After blotting the membranes dry with filter paper, they were placed in incubation bags with corresponding primary antibodies and incubated overnight at 4°C. The following day, the primary antibodies were removed, and membranes were washed with TBST. Secondary antibodies were added and incubated at room temperature for 1 hour, followed by three additional washes with TBST. ECL reagent was freshly prepared according to the manufacturer's instructions (Solution A and B in a 1:1 ratio) and uniformly applied to the membranes. Images were captured and stored using a chemiluminescence imaging system.

Enzyme-linked immunosorbent assay (ELISA)

The kit was allowed to equilibrate to room temperature for 20 minutes after removal from the refrigerator. Standard and sample wells were prepared according to the manual, and the corresponding working solutions were made based on the number of wells to be tested. Then, 100 μ L of serially diluted standards or test samples were added to the respective wells within 10 minutes. The plate was sealed and incubated at 37°C for 90 minutes. After incubation, the liquid was discarded, and the plate was dried on absorbent paper without washing. Next, 100 μ L of the biotinylated antibody solution was added to each well, covered, and incubated at 37°C for 1 hour. After discarding the liquid and blotting the plate dry, 350 μ L of wash buffer was added to each well and left for 1 minute before being discarded. This washing step was repeated three times. Following the wash, 100 μ L of HRP enzyme conjugate working solution was added to each well. Subsequently, 90 μ L of substrate solution was added to each well for approximately 15 minutes. The optical density (OD) at 450 nm was measured for each well using a pre-warmed microplate reader.

BrdU cell proliferation assay

The effect of PFDA on cell proliferation was evaluated, and the BrdU assay was performed according to the manufacturer's instructions.

SA- β -gal staining

Caco-2 and NCM460 cells were seeded into appropriate culture plates. Once the cells reached optimal confluence, the medium was replaced with treatment medium containing varying concentrations of DSS (1-5%), while the control group remained untreated. The cultures were maintained at 37°C with 5% CO₂ for 24-48 hours. After treatment, the medium was discarded, and cells were gently washed twice with chilled PBS. Following the protocol of the senescence-associated β -galactosidase staining kit, cells were fixed with an appropriate amount of fixative, such as 4% paraformaldehyde, at room temperature for 10-15 minutes. After another PBS wash, cells were incubated overnight (12-16 hours) at 37°C in the dark with pre-prepared SA- β -gal staining solution containing X-gal. The following day, cells were examined under an optical microscope, and images were captured. Blue-positive cells, which displayed distinct blue staining, were considered SA- β -gal positive. The effect of DSS treatment on senescence levels in both cell types was statistically analyzed by comparing the percentage of positive cells across the groups.

Pharmacodynamic study of Wogonoside on UC mice

A UC model was established in C57BL/6 mice using oral administration of DSS. Mice were randomly assigned to five groups: normal control, 3% DSS model, Wogonoside low-dose (12.5 mg/kg), medium-dose (25 mg/kg), high-dose (50 mg/kg), and sulfasalazine positive control (500 mg/kg). Except for the normal group, all other groups received 3% DSS solution ad libitum for 6 consecutive days, along with daily oral gavage of the corresponding drugs or sterile distilled water. Body weight, water intake, food intake, and fecal characteristics were recorded daily. Animals received a deep anesthesia with an overdose of pentobarbital sodium, followed by cervical dislocation to ensure death.

Tissue immunofluorescence

Three mice from each group were randomly selected to analyze the expression and localization of ZO-1 and Occludin proteins in colon tissue. A segment of the distal colon was collected, embedded in O.C.T. compound, and frozen sections were prepared (stored at -80°C for later use). Immunofluorescence was performed by fixing tissue sections with cold acetone for 20 minutes, followed by three PBS washes (3 minutes each). The sections were blocked with 10% normal goat serum for 1.5 hours. After removing the serum, primary antibodies against ZO-1 (1:100) and Occludin (1:250) in 10% goat serum were applied and incubated overnight at 4°C. The following day, sections were washed three times with PBS and then incubated with fluorescent secondary antibodies at room temperature for 1 hour, protected from light. After three additional PBS washes, sections were mounted with an anti-fade medium containing DAPI and examined under a laser scanning confocal microscope. Images were captured, and the average fluorescence intensity was analyzed using ZEN 2 software.

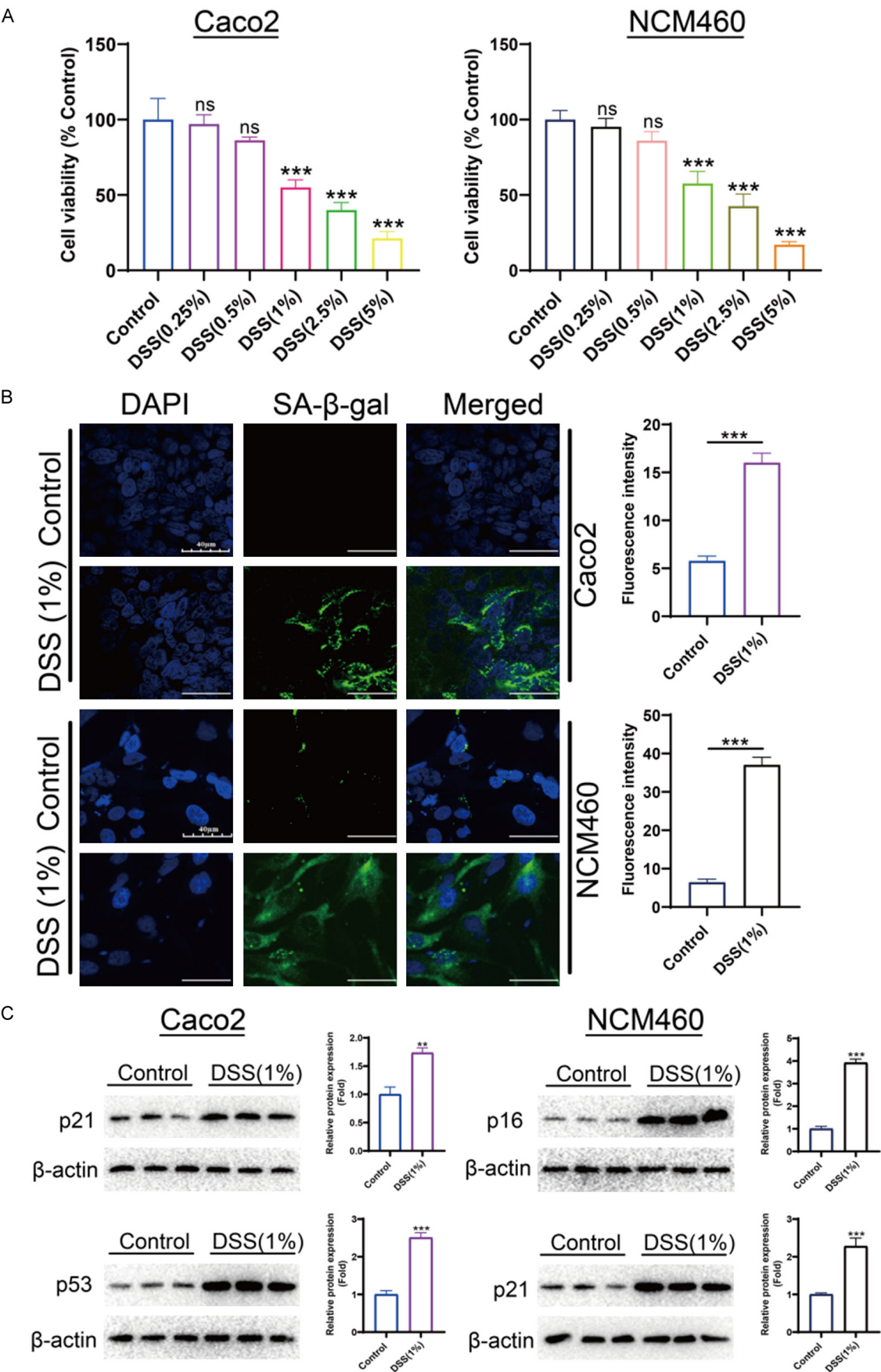
Statistical analysis

Statistical analysis of experimental data was performed using SPSS 16.0 and GraphPad Prism 5 software. Results are expressed as mean $\pm$ standard error ($\bar{x} \pm \text{SEM}$). A t-test was used for comparisons between two groups, and one-way ANOVA was employed for comparisons among multiple groups. Statistical significance was defined as a *p*-value of less than 0.05.

Results

Determination of the optimal DSS concentration

Cells were treated with DSS at varying concentrations (0.25%, 0.5%, 1%, 2.5%, 5%) for 24 hours to assess cell viability. The results demonstrated a dose-dependent reduction in cell viability with increasing DSS concentrations. Viability remained largely unaffected at 0.25-0.5% DSS compared to the control group. Treatment with 1% DSS reduced cell viability to approximately 55%, while 2.5% DSS resulted in less than 70% viability, and 5% DSS led to approximately 7% viability (**Figure 1A**). Based on these findings, 1% DSS was selected as the



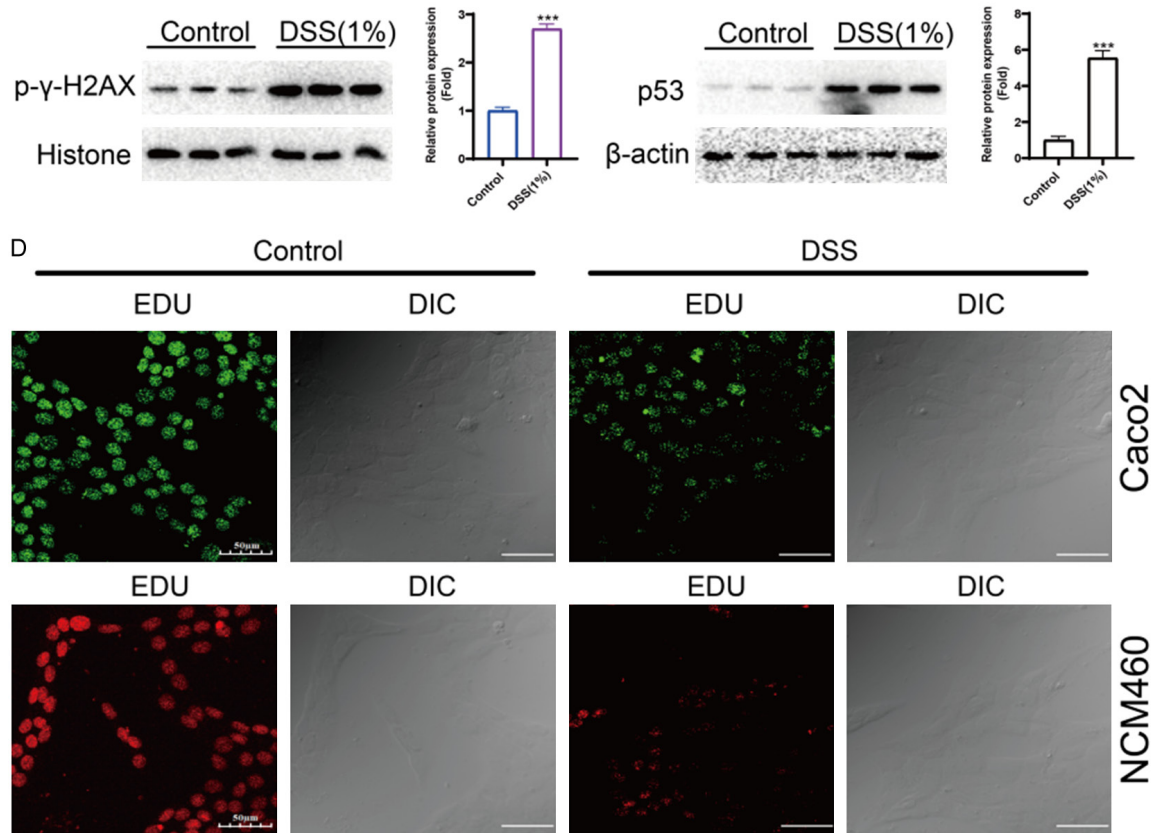


Figure 1. Determination of the optimal DSS concentration. A. Effect of different DSS concentrations on cell viability. B. DSS induced senescence in intestinal cells, as assessed by SA-β-gal staining. C. Effect of DSS on the expression of p16, p21, and p53. D. EDU staining analysis of the effect of 1% DSS on cell proliferation capacity. ns = not significant; ***P<0.001.

working concentration for further investigation into Wogonoside's potential to alleviate senescence. Subsequent analysis of cellular senescence via SA-β-gal staining revealed a gradual increase in fluorescence in intestinal cells, indicating higher levels of cellular senescence (Figure 1B). Additionally, the expression of senescence markers, including p16, p21, γ-H2AX, and p53, was significantly elevated (Figure 1C). Figure 1D shows a marked reduction in cell proliferation, as assessed by EDU staining, following treatment with 1% DSS. These results suggest that 1% DSS is an appropriate concentration for the current study.

Determination of the optimal Wogonoside concentration

The optimal concentration of Wogonoside was determined by evaluating its cytotoxicity on Caco-2 and NCM460 cells across a range of

concentrations (12.5 to 200 μM). Except for the highest concentration (200 μM), the proliferation rates in all other concentration groups (0-24 hours) were consistent with the control group. These results indicate that Wogonoside did not exhibit cytotoxic effects on intestinal cells within the 0-200 μM concentration range (Figure 2). Based on these results, 25, 50, and 75 μM concentrations of Wogonoside were selected for further experimentation.

Effect of Wogonoside on SA-β-gal activity in intestinal cells

SA-β-galactosidase staining revealed minimal senescence in the control group, with weak fluorescence intensity. In contrast, significant cell senescence was observed in the DSS group. With increasing doses of Wogonoside, a gradual reduction in senescence levels was noted in intestinal cells (Figure 3).

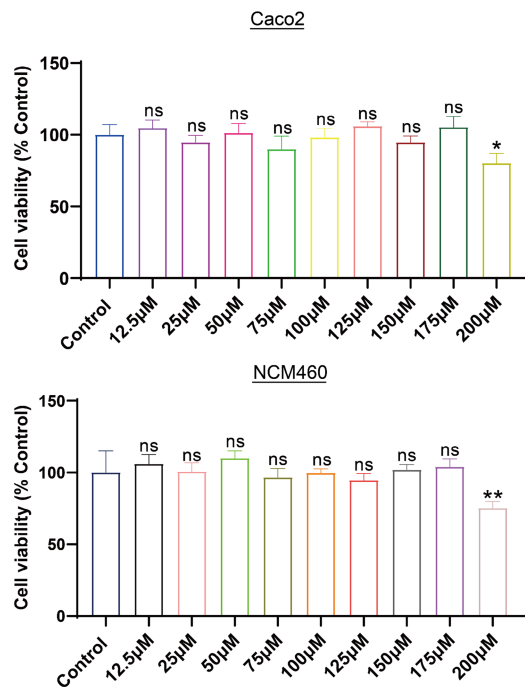


Figure 2. Determination of the optimal Wogonoside concentration. Cells were stimulated with different concentrations of Wogonoside, and then MTT assay was performed to analyze the cytotoxic effects of Wogonoside on intestinal cells. ns = not significant; * $P < 0.05$, ** $P < 0.01$.

Effect of Wogonoside on the expression of senescence-associated proteins

Western blot analysis of senescence-related proteins showed that Wogonoside treatment significantly reduced the expression of p16, p21, γ -H2AX, and p53 compared to the blank control group (**Figure 4A**). Moreover, EDU staining demonstrated a significant improvement in cell proliferation capacity in the Wogonoside-treated groups (**Figure 4B**).

Effect of Wogonoside on the expression of SASP factors in intestinal cells

ELISA results revealed a significant increase in IL-1 β and IL-6 levels in Caco-2 cells treated with DSS compared to the control group. IL-1 β and IL-6 levels were elevated in the medium- and high-dose Wogonoside groups relative to the control group (**Figure 5A**). Additionally, phosphorylated NF- κ B expression was significantly reduced in the Wogonoside-treated groups (**Figure 5B**).

Effect of Wogonoside on oxidative stress levels

Oxidative stress levels were assessed following Wogonoside treatment. The data indicated higher oxidative stress levels in the DSS model group compared to the control group. In contrast, the Wogonoside-treated groups exhibited reduced ROS and MDA levels, along with increased GSH content and enhanced total SOD activity in both Caco-2 and NCM460 cell models (**Figure 6**).

Effect of Wogonoside on intestinal cell injury

Indirect immunofluorescence analysis revealed a dose-dependent increase in the expression of tight junction proteins ZO-1, Occludin, and Claudin-1 following Wogonoside treatment (**Figure 7A**). Western blot analysis confirmed a significant restoration of ZO-1, Occludin, and Claudin-1 expression in the cell monolayer injury model (**Figure 7B**). LDH assay results showed that Wogonoside alleviated cell death induced by DSS at various concentrations (**Figure 7C**).

Molecular mechanism by which Wogonoside alleviates senescence

Several studies have demonstrated that DSS significantly increase ROS release from intestinal cells, which is a primary mechanism by which it induces colitis. ROS also plays a critical role in driving cellular senescence [11]. In the present study, mitochondrial membrane potential (MMP) was significantly reduced after DSS treatment; however, Wogonoside treatment markedly increased MMP levels (**Figure 8A**). Furthermore, MitoSOX staining indicated a significant reduction in mitochondrial ROS (**Figure 8B**).

Further mitochondrial staining revealed increased mitochondrial fission, which was notably improved by Wogonoside treatment (**Figure 9A**), suggesting that Wogonoside inhibits excessive mitochondrial fission. Western blot analysis showed a decrease in Drp1 expression, while the expression of OPA1 and Mfn1, key proteins involved in mitochondrial fusion, was significantly increased after Wogonoside treatment (**Figure 9B**). Overexpression of Drp1 (**Supplementary Figure 1**) significantly impaired the anti-senescence effects of Wogonoside (**Figure 9C**). To understand the mechanism by

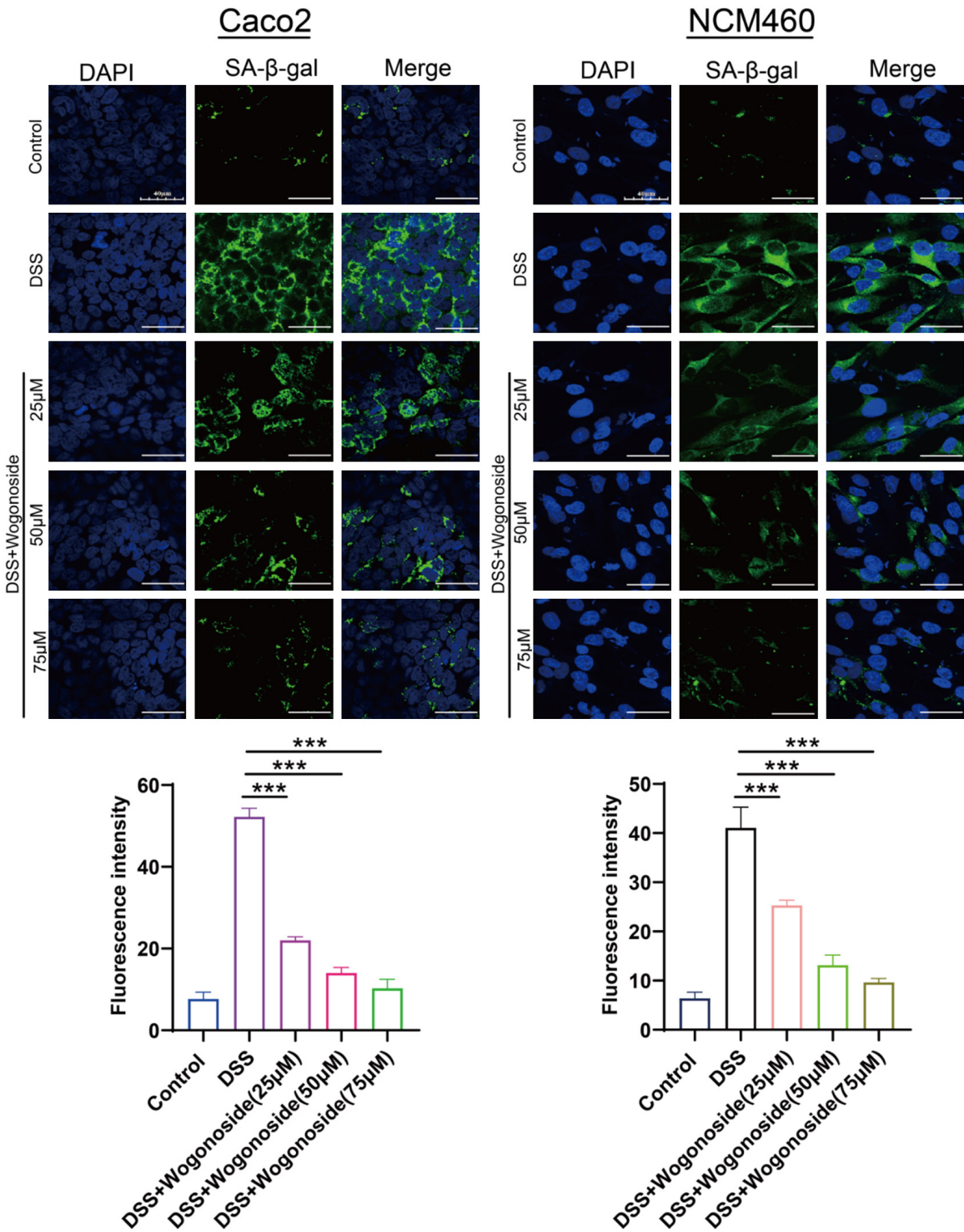


Figure 3. Effect of Wogonoside on senescence in intestinal cells. SA-β-gal staining analysis of the effect of Wogonoside on senescence. ***P<0.001.

which Wogonoside regulates Drp1 expression, this study examined AMPK, which is known to regulate mitochondrial fission and fusion.

Additionally, *Scutellaria baicalensis* and its analogs have been reported to activate AMPK. Our experimental results showed that Wogonoside

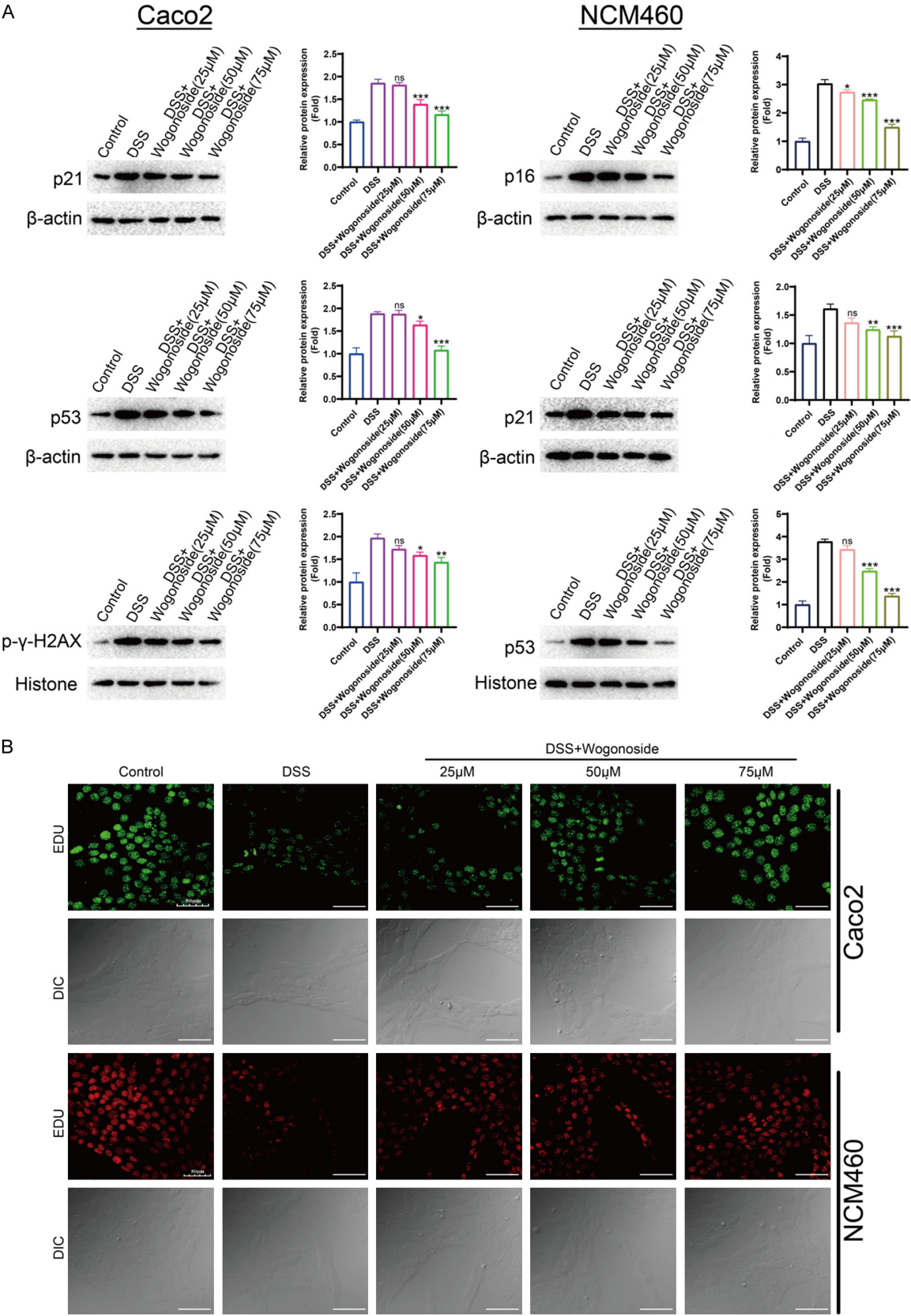


Figure 4. Effect of Wogonoside on the expression of senescence-associated proteins. A. Wogonoside inhibited the expression of p16, p21, and p53. B. Cell proliferation capacity was significantly improved in the Wogonoside groups. ns = not significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

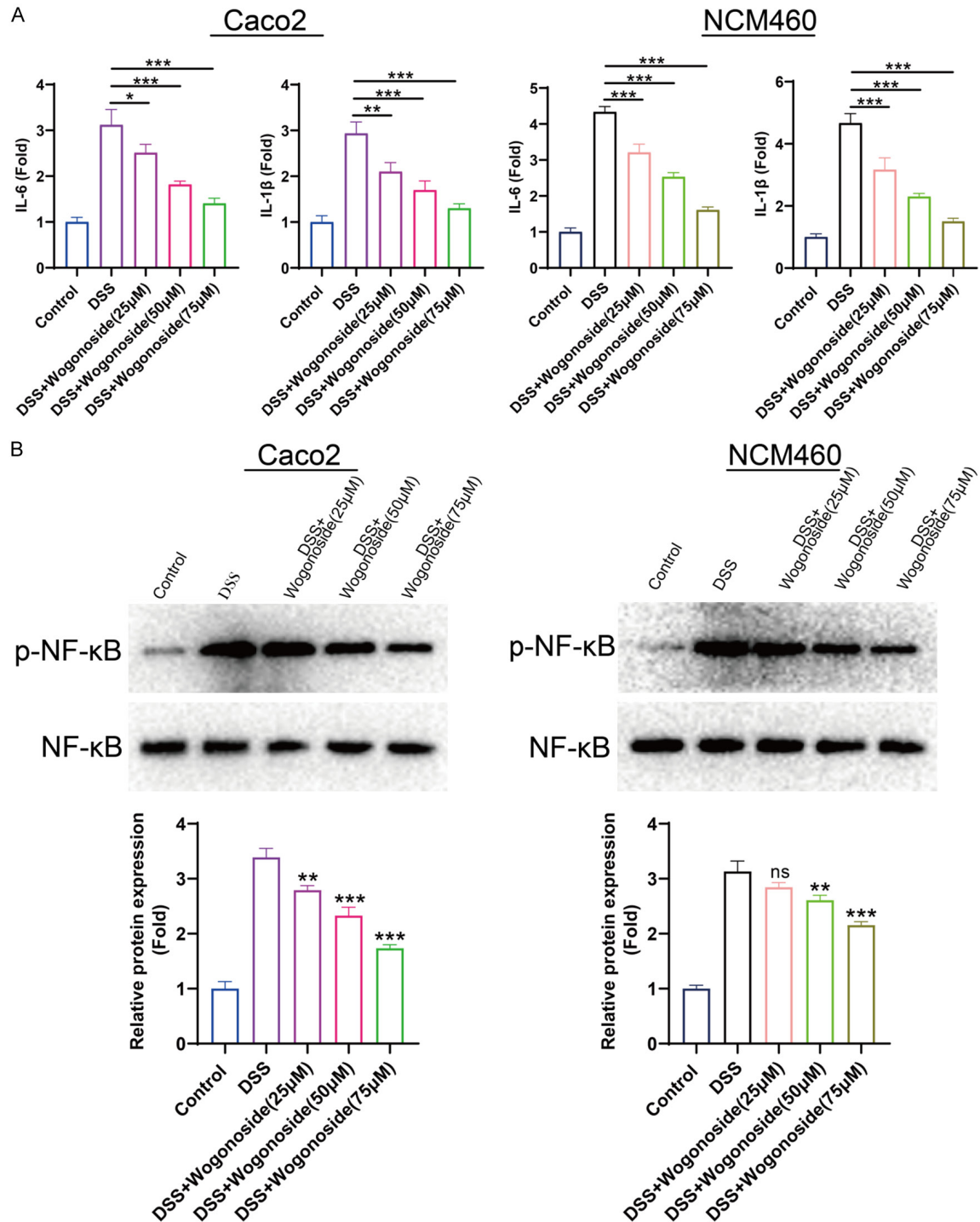


Figure 5. Effect of Wogonoside on the expression of SASP factors in intestinal cells. A. ELISA kit analysis of the effect of Wogonoside on inflammatory factors. B. Wogonoside inhibited the activation of NF-κB. ns = not significant; *P<0.05, **P<0.01, ***P<0.001.

significantly activated AMPK (Figure 9D). The use of an AMPK inhibitor (Compound C) significantly attenuated the anti-senescence effects

of Wogonoside (Figure 9E), indicating that the effects of Wogonoside are at least partially mediated by AMPK activation.

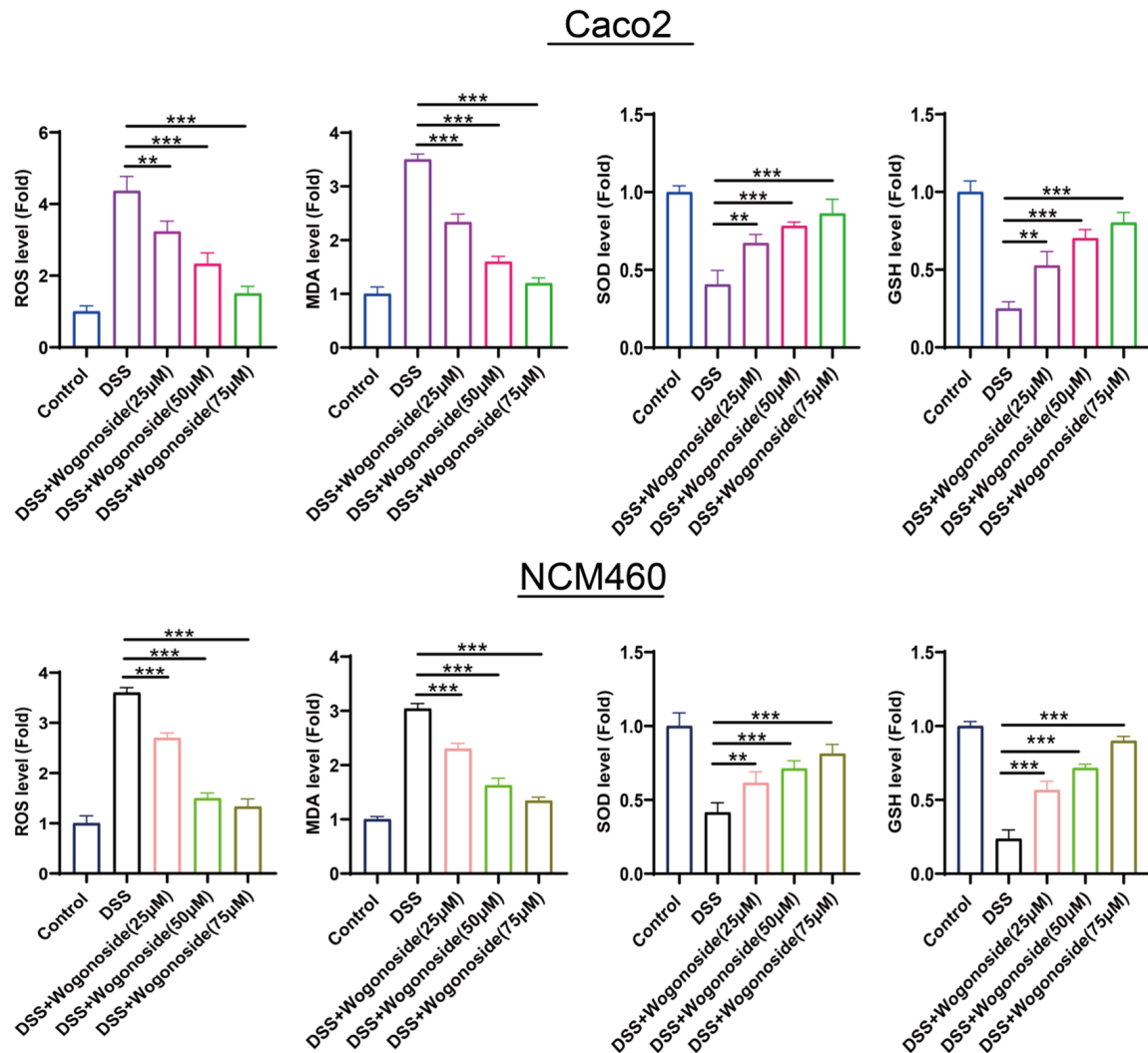


Figure 6. Effect of Wogonoside on oxidative stress in intestinal cells. Effect of Wogonoside on the expression levels of ROS, MDA, GSH, and SOD. ** $P < 0.01$, *** $P < 0.001$.

Effect of wogonoside on colonic tissue senescence in UC mice

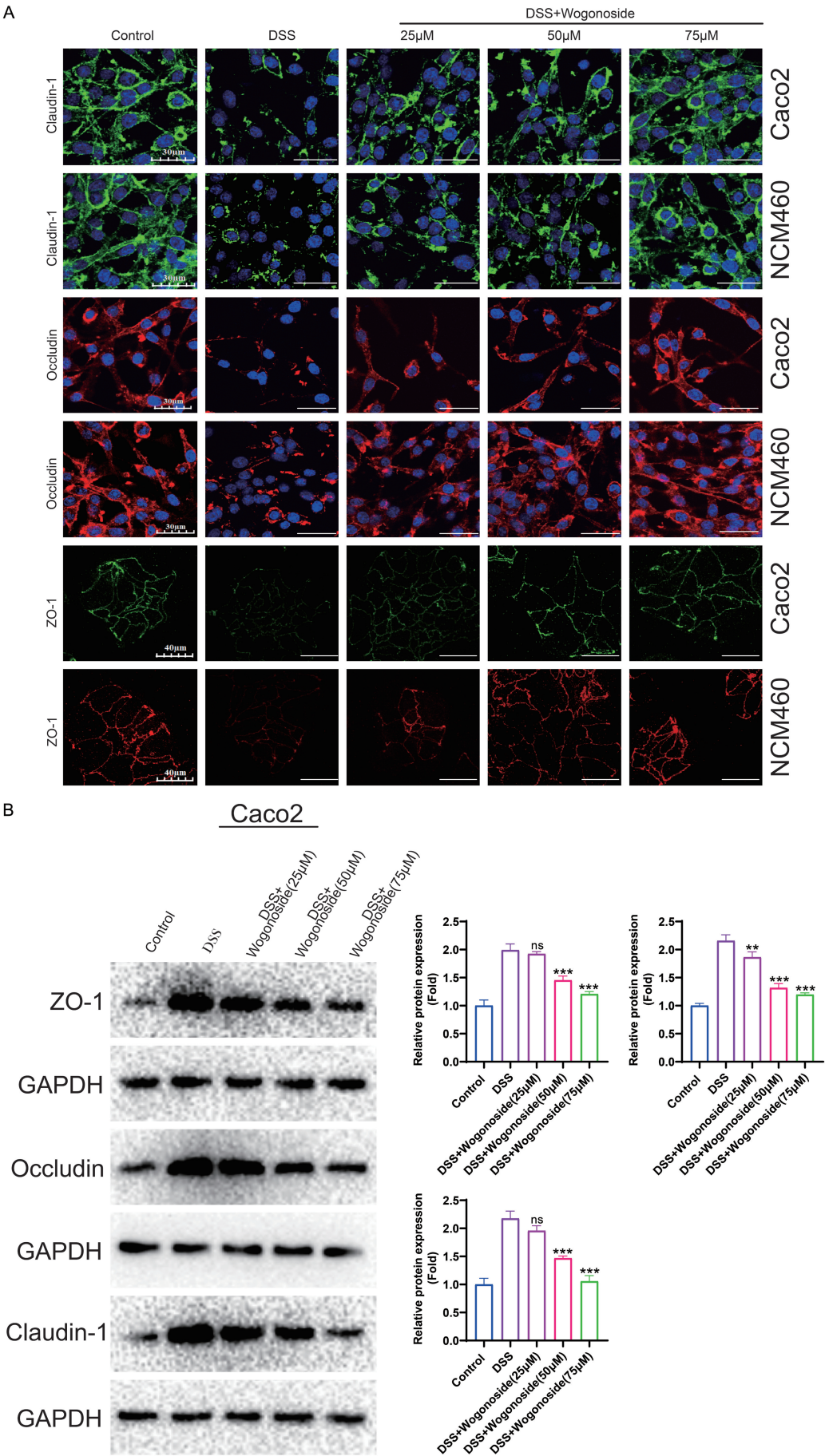
SA- β -gal staining analysis demonstrated that Wogonoside alleviated DSS-induced senescence in intestinal tissue (**Figure 10A**). Analysis of senescence markers p16, p21, and p53 confirmed that Wogonoside mitigates DSS-induced senescence (**Figure 10B**). Additionally, Western blot analysis of tight junction proteins showed that Wogonoside upregulated the expression of ZO-1, Occludin, and Claudin-1 in colon tissue (**Figure 10C**). ELISA analysis of SASP factors revealed that Wogonoside inhibited the release of SASP factors (**Figure 10D**). Furthermore, Wogonoside reduced ROS and MDA levels while increasing GSH and SOD levels (**Figure 10E**). In

the *in vivo* study, Wogonoside also activated the AMPK signaling pathway (**Figure 10F**), promoted the expression of OPA1 and Mfn1, and inhibited Drp1 expression (**Figure 10G**).

Discussion

Senescence is an inevitable process associated with the functional decline of various organs in the body. As age increases, the body's ability to cope with external stressors diminishes, resulting in a general rise in the incidence of age-related diseases [11]. This multifaceted process is linked to the gradual deterioration of physiological functions and an increased occurrence of various diseases [12]. Recent studies have highlighted a strong correlation between

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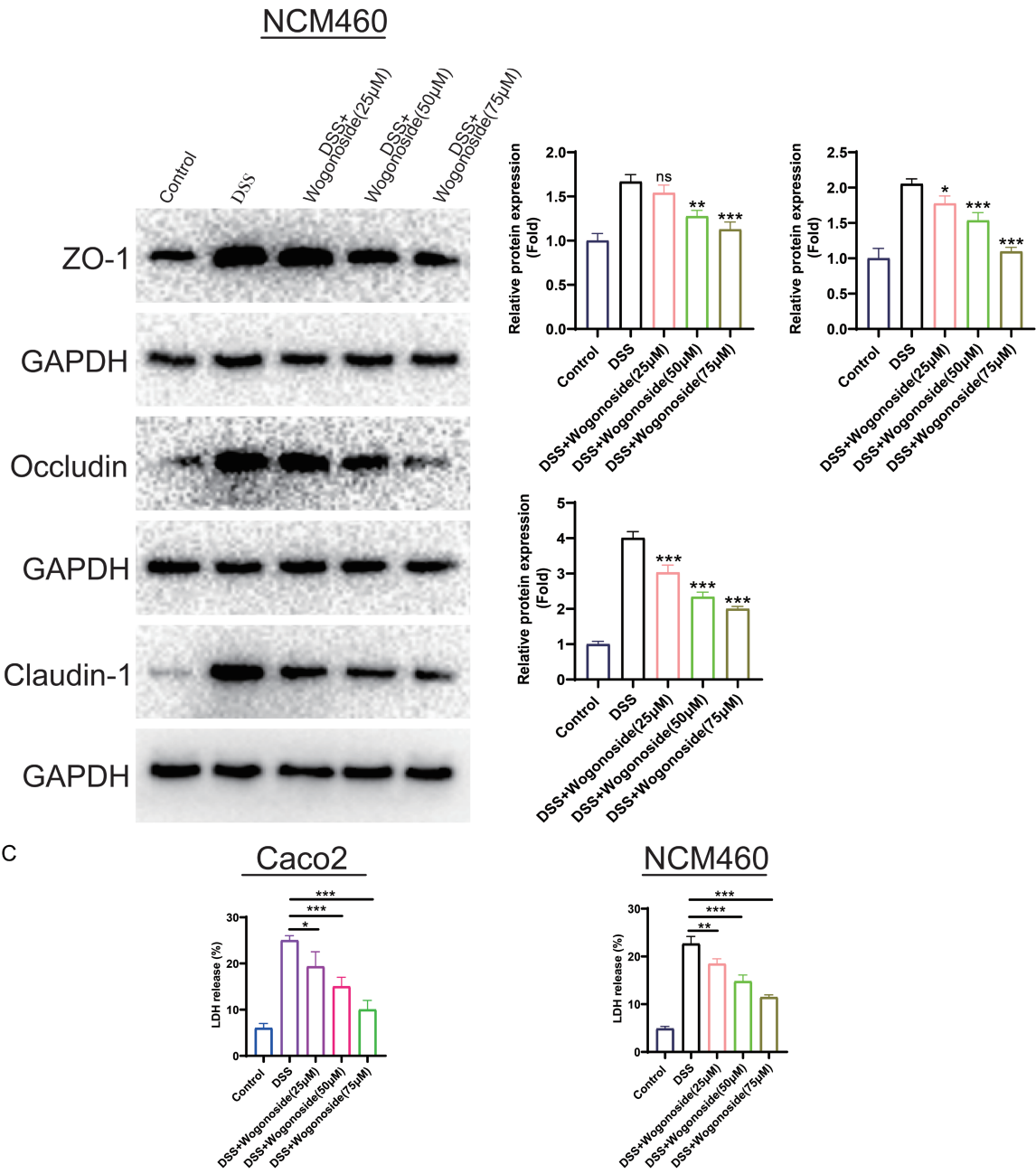


Figure 7. Effect of Wogonoside on intestinal cell injury. A. Indirect immunofluorescence analysis demonstrates Wogonoside's impact on ZO-1, Occludin, and Claudin-1 expression. B. Western Blot analysis examining Wogonoside's impact on ZO-1, Occludin, and Claudin-1 expressions. C. Wogonoside alleviated cell death. ns = not significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

UC and senescence, with a complex, bidirectional interaction between the two. UC, as a chronic systemic inflammatory condition, accelerates biological aging through various mechanisms. Chronic inflammation and oxidative stress trigger cellular senescence, leading to the SASP, which releases high levels of inflam-

matory cytokines, chemokines, and proteases. This disrupts tissue homeostasis and exacerbates intestinal mucosal barrier dysfunction [13]. Additionally, chronic inflammation accelerates telomere shortening, induces mitochondrial dysfunction, and causes DNA damage, further driving the aging process at the molecu-

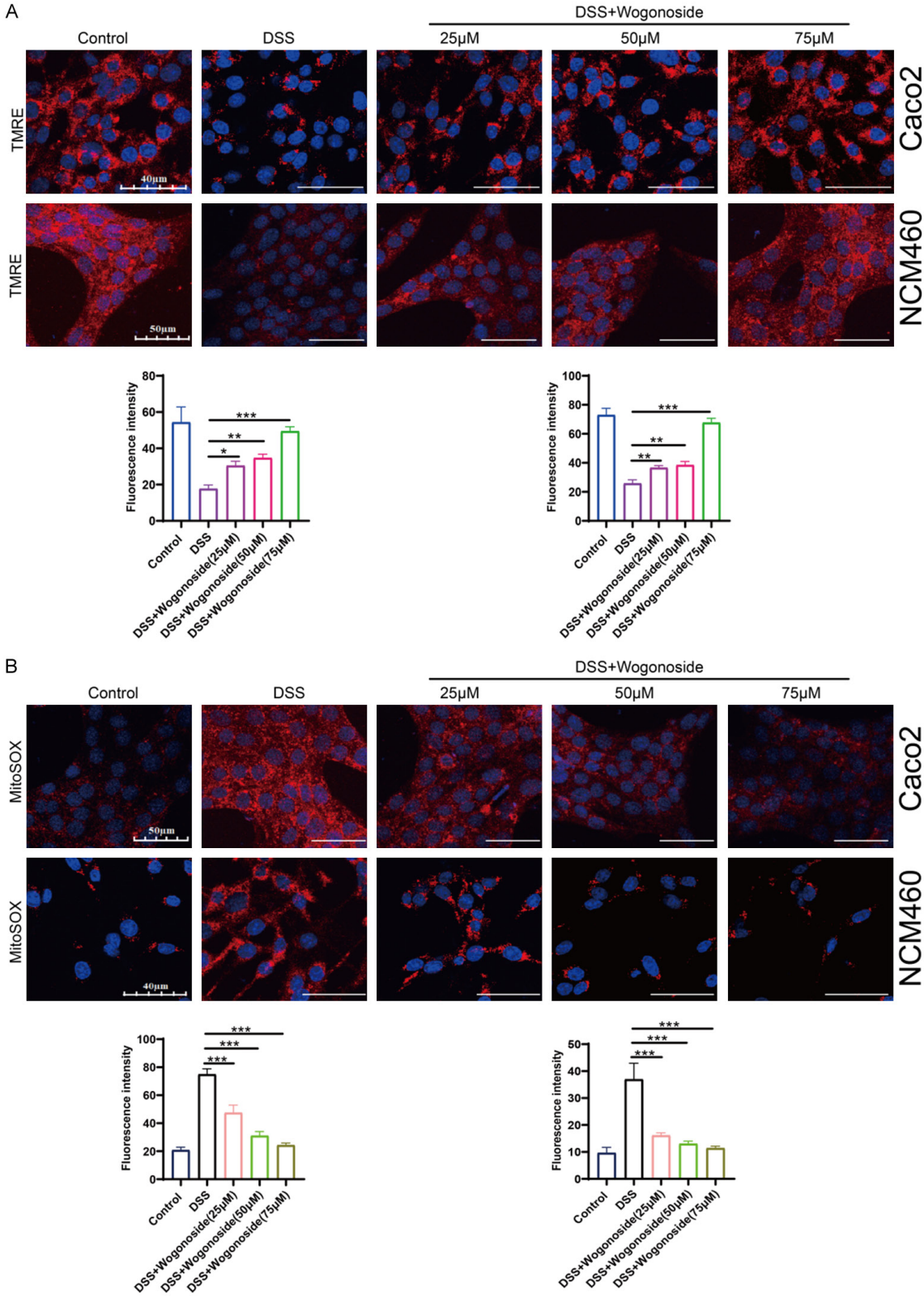
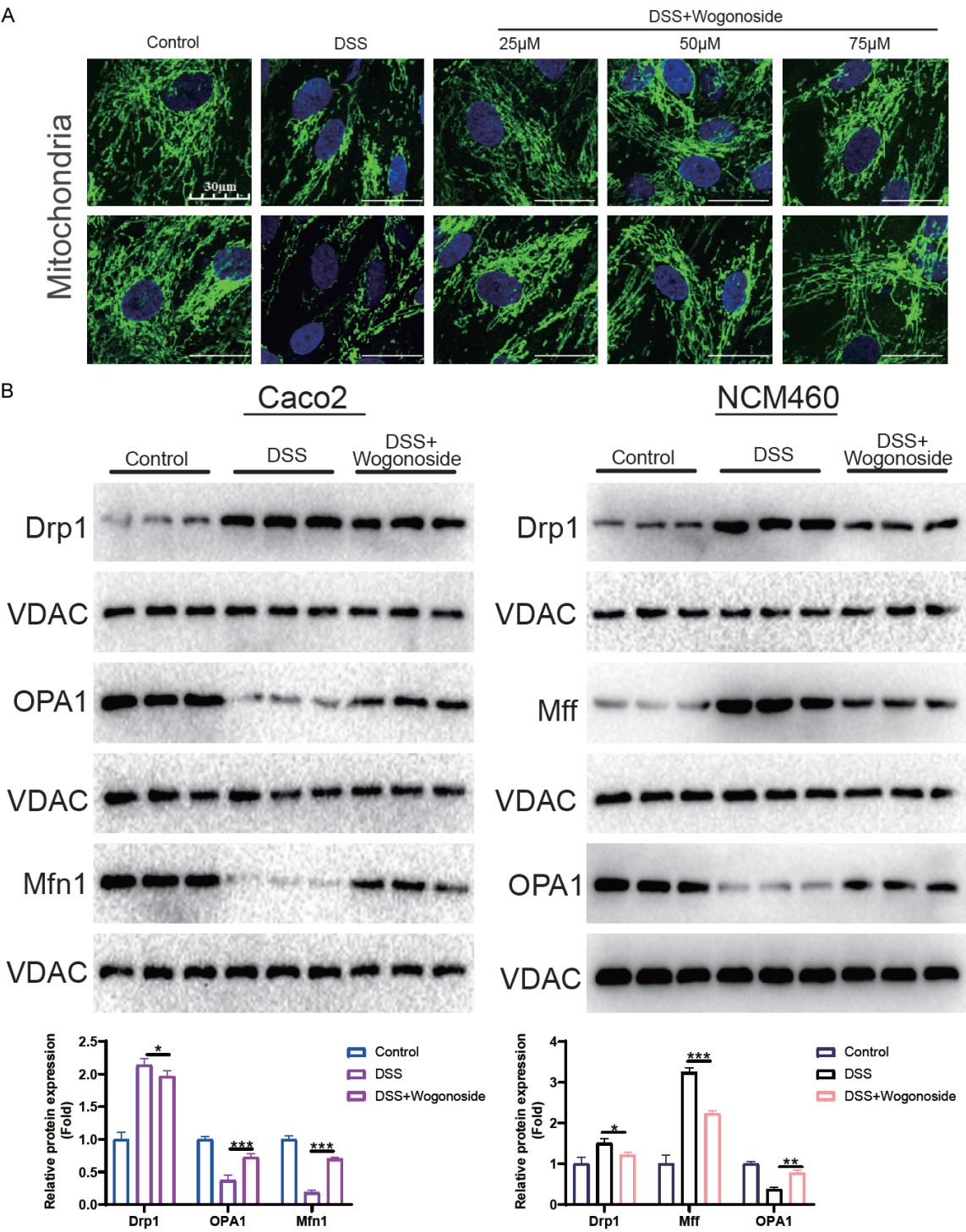


Figure 8. Elucidation of the molecular mechanism of Wogonoside action. A. MMP level was increased upon Wogonoside treatment. B. Wogonoside reduced mitochondrial ROS levels. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.



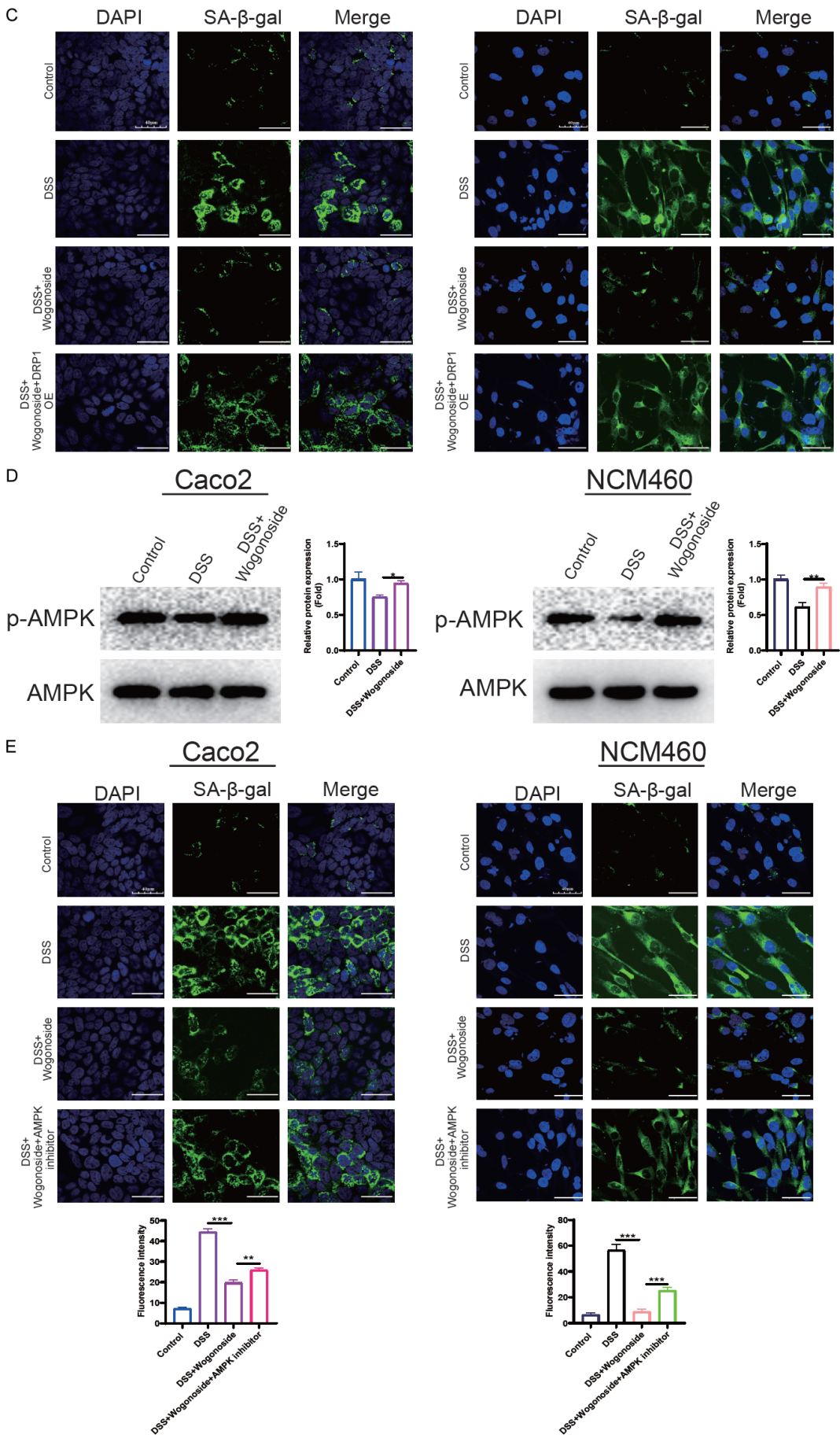


Figure 9. Wogonoside promotes mitochondrial fusion by activating AMPK. A. Effect of Wogonoside on mitochondrial fission. B. Effect of Wogonoside on the expression of mitochondrial fusion and fission proteins. C. Overexpression of Drp1 significantly inhibited the anti-senescence effect of Wogonoside. D. Wogonoside activates AMPK. E. AMPK inhibition blocked the effect of Wogonoside. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

lar level. Conversely, aging itself is a risk factor for the development and progression of UC. Immunosenescence - the age-related decline in immune function - impairs both innate and adaptive immune responses, resulting in poor inflammation control and reduced tissue repair, which may promote the onset or worsening of UC [14]. Age-related decline in intestinal stem cell function and compromised barrier integrity also makes the intestine more susceptible to inflammation. Thus, UC and senescence create a self-perpetuating cycle, where inflammation triggers senescence, which, in turn, amplifies inflammation. In this study, the effect of Wogonoside on senescence was systematically evaluated.

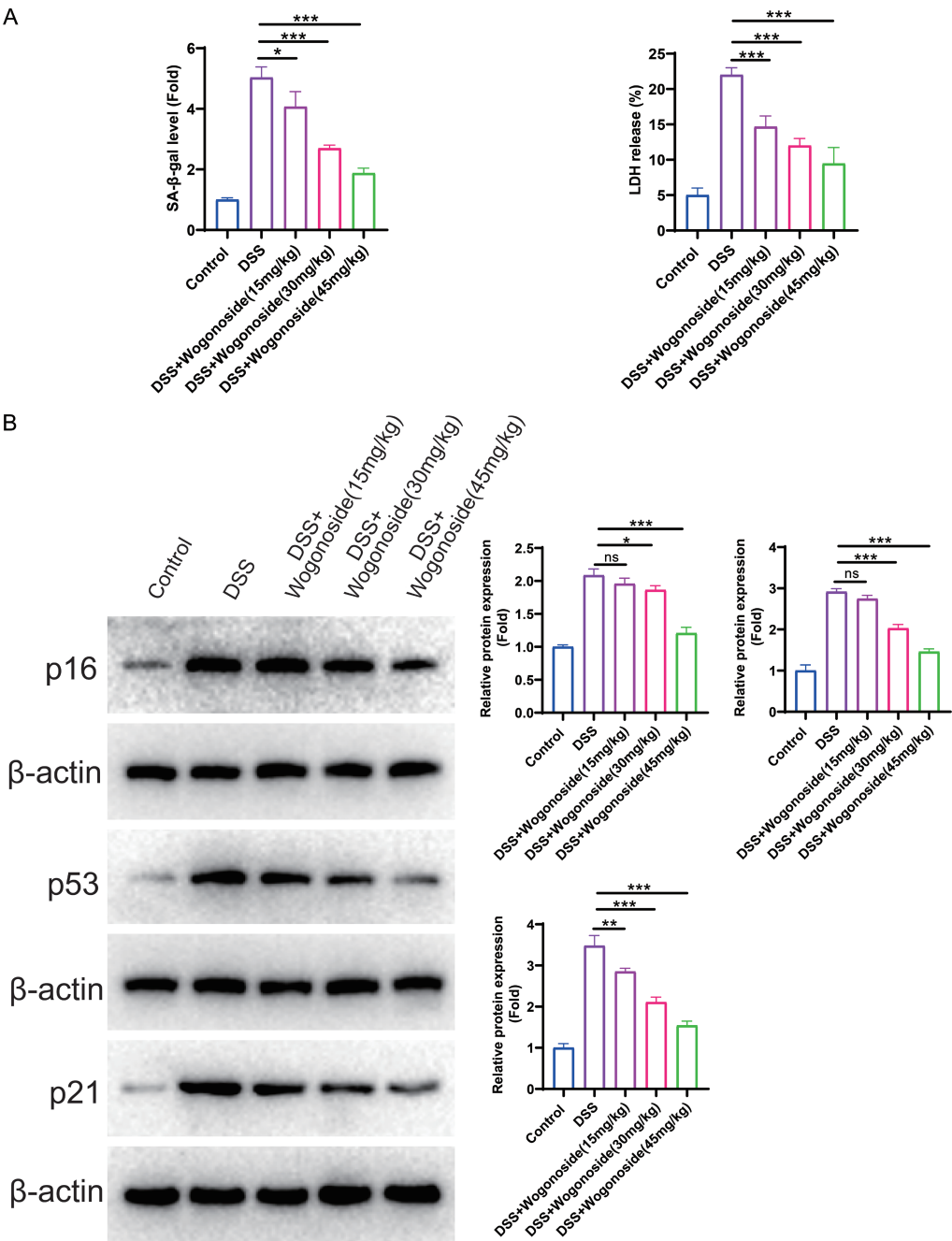
Significant progress has been made in understanding the mechanisms of aging. Current theories on aging encompass the free radical, DNA damage repair, telomerase, mitochondrial DNA damage, cross-linking, biomembrane damage, genetic programming, gene regulation, chromosome mutation, immunological, neuroendocrine, and apoptosis theories [15]. According to the free radical theory, the accumulation of ROS plays a central role in aging and age-related diseases. As organisms age, the activity of endogenous antioxidant enzymes such as SOD, CAT, and GSH-Px declines, resulting in reduced free radical scavenging capacity and increased levels of lipid peroxidation products like MDA. This oxidative damage to biological macromolecules accelerates the aging process [16]. Various herbal extracts can alleviate oxidative stress and exert anti-aging effects by enhancing antioxidant enzyme activity, increasing free radical scavenging capacity, and reducing ROS and MDA levels [17].

Wogonoside is one of the main flavonoid components of *Scutellaria baicalensis*. Current pharmacological research on it mostly focuses on anti-tumor, antioxidant, and anti-inflammatory areas. Studies indicate that it can inhibit lipid peroxidation, enhance antioxidant enzyme activity, and downregulate inflammatory responses [18]. Despite Wogonoside's promising activ-

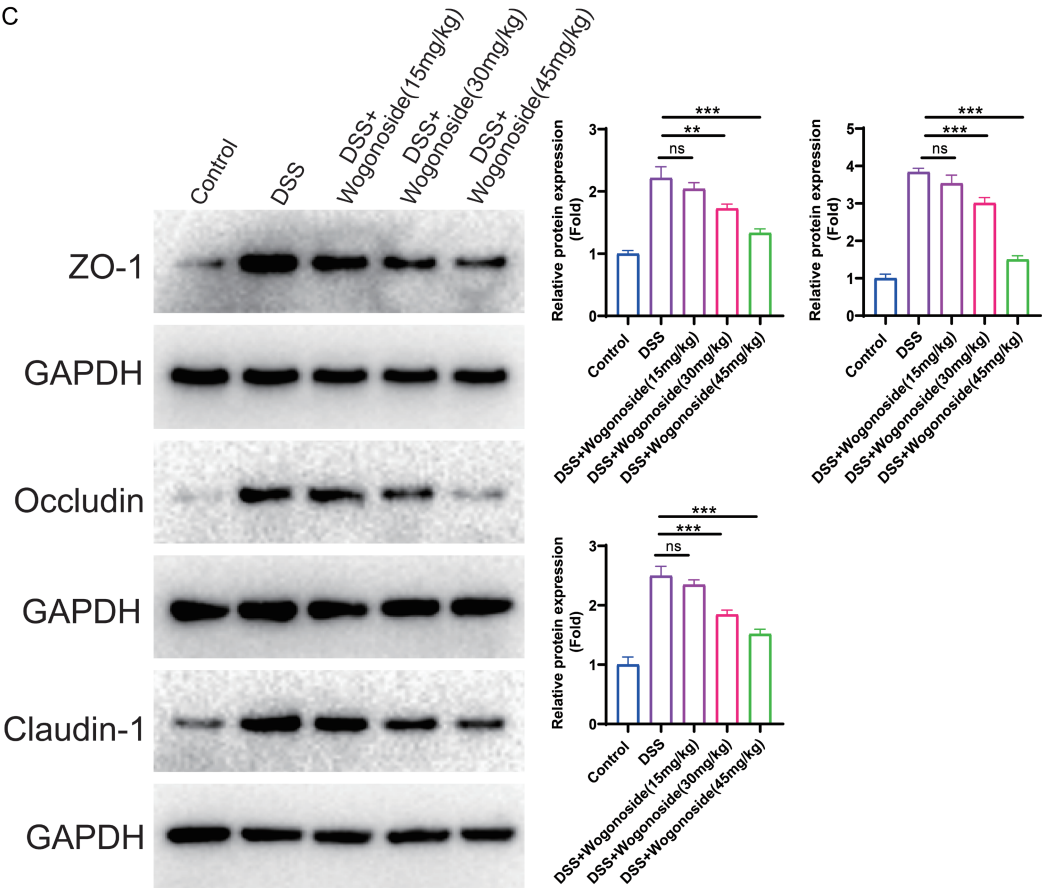
ity in various disease models, its anti-aging role remains unexplored. In the current work, we found that Wogonoside alleviates DSS-induced senescence in intestinal cells. Furthermore, we also investigated the effect of Wogonoside on the barrier function in UC mice. Our study demonstrated that Wogonoside effectively restores intestinal epithelial integrity in ulcerative colitis (UC) mice, as evidenced by both *in vivo* and *in vitro* models. Similarly, related studies have reported that *Scutellaria baicalensis*-related substances have the ability to restore intestinal integrity [19].

We explored the molecular mechanism by which Wogonoside alleviates senescence. Mitochondrial fission and ROS generation form a tight vicious cycle with cellular senescence [20]. When mitochondria are damaged, excessive fission occurs, leading to fragmentation and dysfunction, resulting in increased electron transport chain leakage and substantial ROS production. High ROS levels further exacerbate mitochondrial DNA mutations and loss of membrane potential, inducing more mitochondrial fission. This self-amplifying cycle not only promotes the cellular senescence phenotype (such as SASP factor release) but also drives overall cellular aging and tissue functional decline through oxidative damage and energy shortage [21]. Therefore, inhibiting abnormal mitochondrial fission and reducing ROS accumulation are considered potential strategies for delaying aging. We found that Wogonoside can inhibit ROS levels, suggesting that Wogonoside may alleviate senescence by reducing ROS levels.

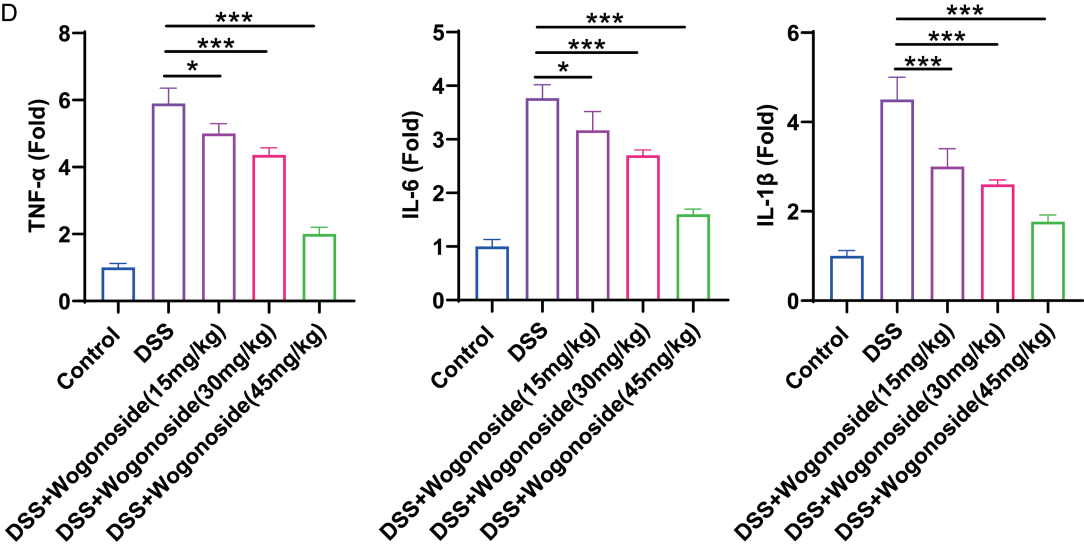
AMPK, as a central regulator of cellular energy homeostasis, effectively inhibits excessive mitochondrial fission and maintains mitochondrial network stability by coordinating various molecular mechanisms [22]. Its core role is mainly reflected in the precise regulation of key fission and fusion proteins. AMPK phosphorylates the Ser637 site (Ser600 in humans) of Dynamin-related protein 1 (Drp1) to inhibit fission. Phosphorylation at this site markedly suppresses Drp1's GTPase activity and its translocation to the mitochondrial outer membrane,



C



D



Wogonoside alleviates ulcerative colitis-induced senescence

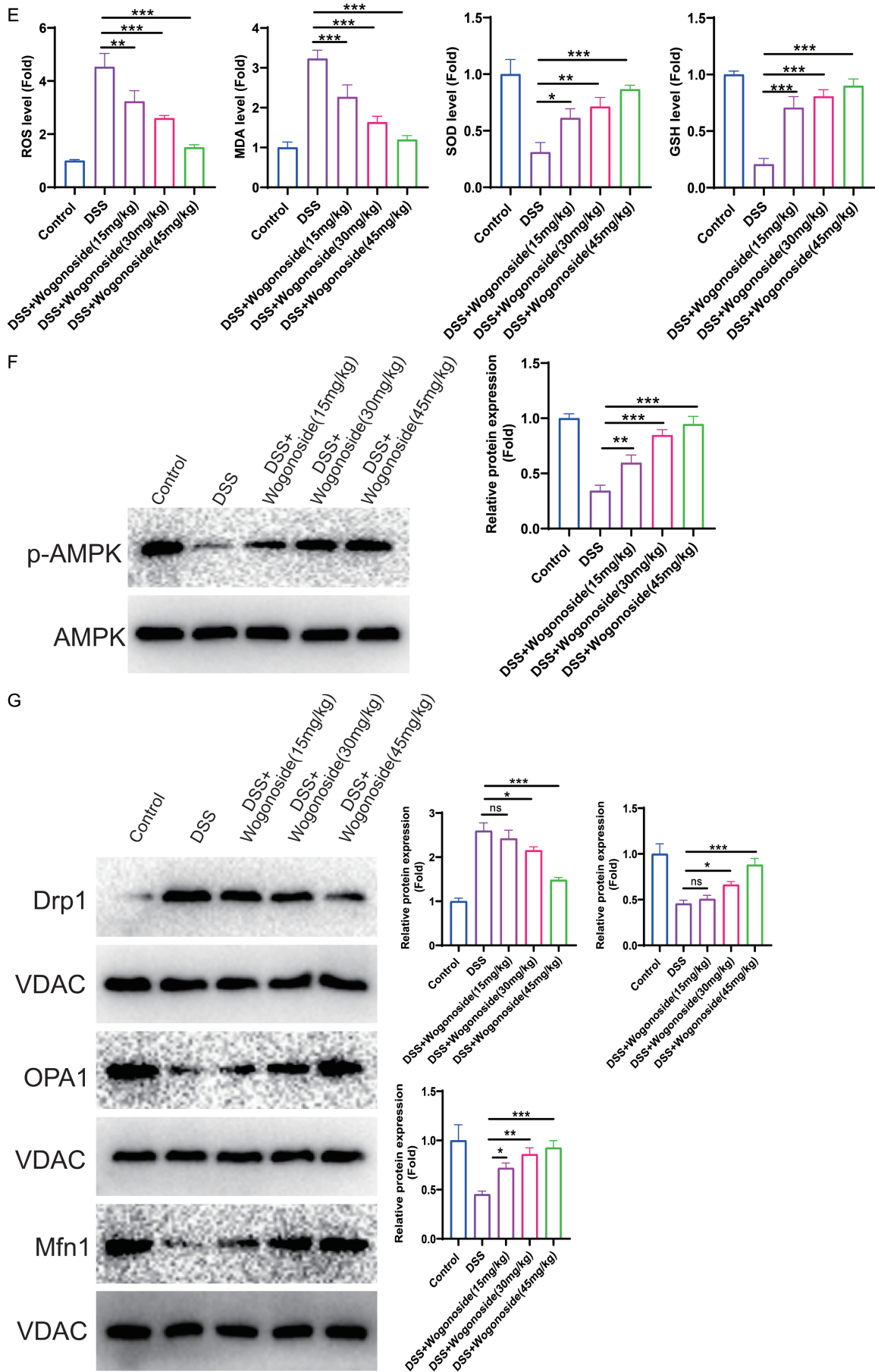


Figure 10. Effect of Wogonoside on colonic tissue senescence in UC mice in vivo. A. Effect of Wogonoside on SA- β -gal levels. B. Wogonoside alleviated DSS-induced senescence, as assessed by p16/p21/p53 expression. C. Immunofluorescence analysis examining Wogonoside's impact on ZO-1, Ocudin, and Claudin-1 expression. D. Wogonoside inhibited SASP levels. E. Effect of Wogonoside on the levels of ROS, MDA, GSH, and SOD. F. Wogonoside activated the AMPK signaling pathway. G. Effect of Wogonoside on the expression of OPA1, Mfn1, and Drp1. ns = not significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

hindering Drp1 oligomerization and constriction of ring formation, which ultimately inhibits mitochondrial fission. This direct modification is a key mechanism for AMPK's rapid inhibition of fission. In terms of promoting fusion, AMPK upregulates the expression of mitochondrial fusion proteins, such as OPA1, by activating the PGC-1 α signaling pathway. OPA1 is responsible for the fusion of the mitochondrial inner membrane and participates in maintaining cristae structure integrity [23]. AMPK activation not only increases the total amount of OPA1 but may also stabilize the long isoforms of OPA1 (L-OPA1) by improving mitochondrial membrane potential and reducing proteolytic cleavage, thereby promoting mitochondrial fusion and counteracting fission effects. Additionally, AMPK indirectly weakens the ROS-Drp1-mediated fission signaling pathway by enhancing antioxidant defense (e.g., activating the Nrf2 pathway) and clearing ROS, synergistically inhibiting abnormal fission at the microenvironment level. Our current work demonstrates that Wogonoside can inhibit the mitochondrial localization of Drp1, thereby inhibiting mitochondrial fission and promoting mitochondrial biogenesis, reducing ROS leakage, and consequently alleviating the senescence response.

In summary, the finding that Wogonoside can inhibit Ulcerative Colitis-associated cellular senescence has multifaceted potential significance. Wogonoside effectively mitigates UC-induced intestinal cell senescence and epithelial barrier damage, demonstrating protective effects in cellular and animal models, and offering a novel potential strategy for Ulcerative Colitis treatment.

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Disclosure of conflict of interest

None.

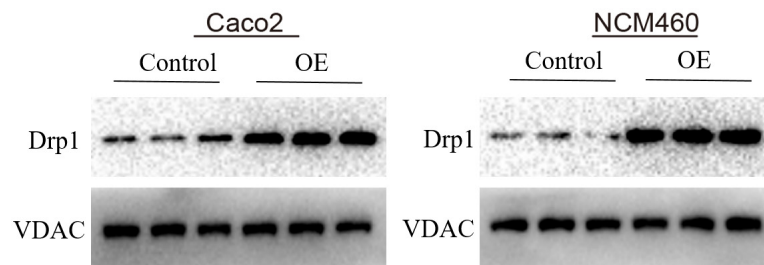
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Wogonoside alleviates ulcerative colitis-induced senescence



Supplementary Figure 1. Over-expression of Drp1 was evaluated by Western-blot.