Heat shock transcription factor 2 promotes mitophagy of intestinal epithelial cells through PARL/PINK1/Parkin pathway in ulcerative colitis

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Research Article

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Abstract

Objective and background

The overactivation of NLRP3 inflammasome is a critical factor for ulcerative colitis (UC). We found that heat shock transcription factor 2 (HSF2), which is highly expressed in UC, could inhibit the activation of NLRP3 inflammasome and reduce IL-1β in intestinal epithelial cells(IECs), but the mechanisms were still unclear. We aimed to elucidate the mechanisms of HSF2 inhibiting the activation of NLRP3 inflammasome of IECs.

Methods:

TEM was used to detect the number of damaged mitochondria and the level of mitophagy in intestinal mucosa of UC patients, healthy controls, mice and Caco-2 cells. ROS levels were detected by immunofluorescence or flow cytometry. RT-PCR, WB and immunohistochemistry were used to detect the level of PARL, PINK1 and Parkin.

Results:

The levels of mitophagy and ROS in intestinal mucosa of UC patients were positively correlated with disease activity. In IECs, after downregulation of HSF2, mitophagy reduced and number of damaged mitochondria and ROS increased, while overexpression of HSF2 showed opposite results. In addition, HSF2 might regulate mitophagy through PARL/PINK1/Parkin signaling pathway.

Conclusions:

HSF2 may promote mitophagy and decrease the level of mtROS through the PARL/PINK1/Parkin signaling pathway, which might be a potential mechanism of HSF2 inhibiting inflammation in UC.

1. Introduction

Ulcerative colitis (UC) is a chronic, recurrent inflammatory disease. In recent years, the incidence of UC is growing rapidly around the world, especially in Asia, including China[1, 2]. Although previous studies have shown that the occurrence of UC is related to genetic susceptibility, immune overreaction, intestinal flora disorder, and an urban lifestyle, its exact etiology and pathogenesis are still unclear[3, 4]. Due to the unclear pathogenesis of UC and the lack of effective therapeutic drugs, most UC patients still have repeated disease activity, although various biological agents have shown some improved efficacy. Therefore, there is an urgent need to explore the pathogenesis of UC and find new therapeutic targets.

In recent years, studies have shown that a moderate inflammatory response is an important part of the intestinal mucosa to resist the invasion of pathogenic microorganisms and maintain the homeostasis of intestinal epithelial cells, but excessive and continuous inflammation is a critical factor in the occurrence and development of UC[5, 6]. Innate immune disorder of the intestinal mucosa has become one of the

important characteristic markers of UC[7]. It has been reported that the NLRP3 inflammasome, an indispensable component of innate immunity, plays a vital role in innate immune re-sponse and disease occurrence[8]. Proper activation of NLRP3 inflammasome is fundamental for the body to fight against microbial infection and regulate the mucosal immune response[9]; however, excessive activation can cause a severe inflammatory response, damage the intestinal epithelial barrier, and participate in the process of UC by cleaving caspase1 and promoting the massive secretion of proinflammatory factors such as IL-1β[10, 11]. In short, proper activation of the NLRP3 inflammasome is an important factor in maintaining intestinal mucosa and body homeostasis. Therefore, further exploration of the regulatory mechanism of the NLRP3 inflammasome in UC is significant for understanding the pathogenesis of UC.

Our team carried out a series of studies on intestinal mucositis in UC and found that HSF2, which can maintain cell homeostasis, is specifically expressed at a high level in UC and can inhibit LPS-induced IL-1β in Caco-2 cells, but the specific mechanism is unclear[12]. We further found that HSF2 reduced secretion of IL-1β by inhibiting NLRP3 activation, but the specific mechanism of HSF2 inhibiting the activation of NLRP3 inflammasome is still unclear[13].

The NLRP3 inflammasome can be activated by a variety of endogenous or exogenous stimuli in the body, including mitochondrial DNA, mtROS, and potassium ion efflux[14–16]. As an important activator, the ROS level determines the activation of the NLRP3 inflammasome and the inflammation level of intestinal mucosa. Studies have reported that excessive accumulation of ROS in the intestinal mucosa is one of the characteristics of UC[17]. In addition, it has been reported that both patients with UC and mice with DSS-induced colitis showed an increase in damaged mitochondria in IECs[18, 19], and damaged mitochondria are an important source of ROS[20]. Therefore, timely removal of damaged mitochondria is essential to reduce intracellular ROS, in-hibit inflammation, and maintain intestinal mucosal homeostasis.

Mitophagy is a crucial way to remove intracellularly damaged mitochondria, but its function and mechanism in UC are not well studied. As reported[21], mitophagy can regulate the activation of NLRP3 inflammasome in the intestinal mucosa of mice with DSS-induced colitis and play a protective role, which is similar to the role of HSF2 in UC. The PARL/PINK1/Parkin signaling pathway plays an important role in the regulation of mitophagy[22]. Studies have suggested that HSF2 regulation of HSP72 can affect the expression of Parkin protein and promote mitophagy[23–26]. Therefore, we speculate that HSF2 inhibits the activation of NLRP3 inflammasome of IECs in UC by regulating the level of mitophagy. In order to confirm this conjecture, we carried out this study.

In this study, we found that HSF2 in UC can enhance mitophagy, reduce intracellular ROS, inhibit the activation of NLRP3 inflammasome and relieve mucosal inflammation through the PARL/PINK1/Parkin signaling pathway, which provides a new direction for exploring the pathogenesis of UC and developing therapeutic targets.

2. Materials And Methods

2.1. Patient selection and sample processing

A total of 90 UC patients were selected for the study according to the opinions of the third European Crohn's and Colitis Organization (ECCO) consensus on the diag-nosis and management of UC. The patients were treated at the First Affiliated Hospital of Kunming Medical University from January 2018 to December 2019. Disease severity was classified by the Mayo Score as mild, moderate, or severe. The lesions were all of left semicolon type and only mesalazine was used for treatment within 3 months before hospitalization (all patients who needed other drugs, such as glucocorticoids and immunosuppressants, to control the disease underwent colonoscopy and biopsy as soon as possible before medication, so as to reduce the interference of those drugs on the results of this study). At the same time, 60 healthy volunteers for colon cancer screening were chosen as controls. The required age range was between 18 and 60 years. Table 1 shows the information and characteristics of enrolled participants. All colonoscopy examinations were performed by specialists in the Department of Gastroenterology, and the same number of tissue samples were collected from the same fixed site (sigmoid colon). All enrollees signed the informed consent form.

2.2. Mice

The *hsf2*$^{-/-}$ C57BL/6 mice that had been successfully established were reared in the SPF laboratory animal house. The genotypes of the mice were identified by PCR. Homozygous mice were used in this study. A total of 40 male mice (7–8 weeks) were divided into wild-type (WT, 20) and knockout (KO, 20) groups. Then each group was randomly divided into 2 subgroups, with 10 mice in each (WT + H_2O, WT + DSS, KO + H_2O, KO + DSS). The WT + H_2O and KO + H_2O groups were given distilled water for 7 days, and the WT + DSS and KO + DSS groups were given 3% dextran sulfate sodium(DSS) for 7 days. The disease activity index(DAI) of all mice was measured every day. On the 8th day, all mice were killed, and colon specimens were taken to measure their length. Each mouse colon was cut into 5 segments: one was immediately put into 2% glutaraldehyde for electron microscope observation, 2 were stained with hematoxylin eosin(HE) and detected by immunohistochemistry, and 2 were stored at -80 ℃ for RT-PCR and Western blotting.

2.3. Cell culture

Human colon adenocarcinoma(Caco-2) cells were used in this study. The Caco-2 cells were obtained from the cell culture library of the Kunming Institute of Zoology, Chinese Academy of Sciences. After the cells were resuscitated in a water bath at 37 ℃ constant temperature, they were cultured in 1640 medium containing 10% fetal bovine serum and then incubated under 5% CO2 at 37 ℃.

2.4. Regulating HSF2 expression by transfecting lentiviral

HSF2 overexpression and knockdown and negative control lentiviruses were purchased from Shanghai GeneChem Co., Ltd. Viruses were transfected into Caco-2 cells according to the lentivirus product instructions. The MOI of Caco-2 cells was set at 20. In addition, a certain amount of HittransG P was added to the transfection group to make a final concentration of 1 ug/mL, so as to improve transfection

efficiency. Ac-cording to the experimental method of Wang et al.[27], an appropriate amount of LPS was added to the cell culture medium to stimulate cell inflammation.

2.5. Transmission electron microscope (TEM) cell culture

TEM (JEM-1400 Flash, Nippon Electronics Corporation) was provided by Kunming Medical University. The collected colonic mucosal tissues were immediately placed in 2.5% glutaraldehyde and stored at 4 ℃ in a refrigerator. PBS was first used for repeated washing 3 times, then osmium acid(1%) was used for fixation for 2 hours, and then washing again 3 times for 10 min each time. Dehydration was then carried out by eth-anol and acetone(paying attention to the temperature). Finally, after handling the embedding solution, staining by uranyl acetate and lead citrate, and drying, the speci-mens were observed under TEM.

2.6. Immunohistochemistry

The intestinal mucosal tissue specimens were embedded with paraffin, fixed with 4% paraformaldehyde, and sliced continuously with a slicer. They were then defatted and hydrated in a solution of xylene, then treated with a citrate buffer(0.01M) under 800 W microwave, and incubated for 10 minutes in 3% hydrogen peroxide at room temperature. After that, 50 uL of primary antibody (PARL or PINK1 or Parkin, diluted 1:200) was added to the slices, followed by incubation at 4 ℃ overnight. Another 50 uL DAB was added for color development, and the dyeing time and degree were controlled under microscope observation. Double steam water was used to wash twice, one minute each time, and hematoxylin was used for re-staining(1 minute), followed by rinsing with 1% ammonia after removal. The slides were successively immersed in 95% and 100% eth-anol, and were dehydrated twice in total. After blow-drying, they were sealed with neutral resin and the results were observed under the microscope.

2.7. Immunofluorescence

The ROS levels in tissues were observed by laser confocal microscopy. First, appropriate OCT encapsulating agent was poured into the premarked specimen box. Then, the mucosal colon tissues were quickly placed in the specimen box and stored at -80 ℃. The second part was staining. After the frozen section was taken out, ROS dye was added, and the sample was incubated in darkness for 30 minutes at 37 ℃. Second, nuclear staining was carried out. Finally, the tablets were sealed with anti-fluorescence sealing tablets. The slices were observed under a laser confocal microscope and images were collected (blue light: DAPI ultraviolet excitation wavelength 330–380 nm, emission wavelength 420 nm; red light: CY3 excitation wavelength 510–560 nm, emission wave-length 590 nm).

2.8. Reverse transcription polymerase chain reaction (RT-PCR)

This part of the experiment was conducted according to the Platinum® SYBR® Green qPCR kit (TaKaRa, Japan) instructions. Each part of the experiment was repeated at least 3 times. The primer sequence in this study was designed and synthesized by TaKaRa Bio Inc. as follows:

PARL (Forward): 5’-CCTATAAGAACACTCGTGAAGCC-3’

PARL (Reverse): 5’-CCAGTCAGCTTTTATGCCATC-3’

PINK (Forward): 5’-GGTGTCAGGCTGGGGCAA-3’

PINK (Reverse): 5’-TGGCTTCATACACAGCGGC-3’

Parkin (Forward): 5’-TCTTCGGCATCTTGTCTG-3’

Parkin (Reverse): 5’- CTGGGAGTCGTAGTTCTAACG – 3’

GAPDH (Forward): 5’-CAAGTTCAACGGCACAGTCA-3’

GAPDH (Reverse): 5’-CACCCCATTTGATGTTAGCG-3’

2.9. Western blotting

Mouse tissue samples or cells were homogenized in lysis buffer containing 1% protease inhibitors. Protein assay kit was used to determine the protein concentration. The antibodies used in this study were anti-PARL (1:1000, Santa Cruz), anti-PINK1 (1:1000, Santa Cruz), anti-Parkin (1:1000, Santa Cruz), anti-β-actin (1:5000, Abcam), and anti-GAPDH (1:5000, Abcam). The results were analyzed by ImageJ.

2.10. Statistical analysis

SPSS 25.0 software was used for statistical analysis of all data. Measurement data were expressed as mean ± standard deviation. One-way ANOVA was used for measurement of data between groups, and LSD was used for pairwise comparison between groups. In the figures, * $P < 0.05$, ** $P < 0.01$and **** $P < 0.0001$.

3. Results

3.1. The clinical part

Details of UC patients and healthy controls were summarized in the Table 1.

Firstly, we detected the damaged mitochondria in the intestinal mucosa of normal control group and UC patients with different disease severity by TEM, and found that the number of damaged mitochondria in the IECs of UC in each group was significantly increased compared with the normal controls, and the number of damaged mitochon-dria was positively correlated with the severity of UC (Fig. 1-A,B). Secondly, we used immunofluorescence to detect the level of ROS in the intestinal mucosa of normal con-trol group and UC patients with different disease severity. It was found that the level of ROS in the intestinal mucosa of UC was significantly increased in each group, and was positively correlated with the disease severity (Fig. 1-C,D). Thirdly, we used TEM to detect the intestinal mucosa of UC patients with different disease severity and found that the level of mitophagy in IECs of UC in each group was significantly higher than

that in the normal control group, and was positively correlated with the severity of UC (Fig. 1-A,B). In addition, since the PARL/PINK1/Parkin signaling pathway is the most studied mitophagy pathway at present, in order to further clarify whether the level of mitophagy in UC patients is related to this pathway, RT-PCR and IHC were used to detect the mRNA and protein expression of PARL,PINK1 and Parkin in the intestinal mucosa of UC patients and healthy controls. Compared with the healthy controls, the levels of PINK1 and Parkin in the intestinal mucosa of UC patients were increased, while the level of PARL was decreased (Fig. 2).

These results once again demonstrated that there was significant mitochondrial damage in the IECs of UC patients, and a large amount of ROS accumulated in the intestinal mucosa of UC patients. In addition, significant mitophagy exists in the IECs, which may be closely related to the PARL/PINK1/Parkin signaling pathway. Moreover, the above changes in the intestinal mucosa and IECs of UC are closely related to the severity of disease.

Mitophagy, as an important way to remove damaged mitochondria and mtROS, can inhibit the activation of NLRP3 inflammasome by reducing the level of ROS, which is an important activator of NLRP3 inflammasome. The increased level of mitophagy in UC patients was also confirmed in this study. Combined with our previous studies, HSF2 expressed highly in UC IECs, and can reduce IL-1β by inhibiting the activation of NLRP3 inflammasome. Therefore, we speculate that the protective effect of HSF2 on inhibiting NLRP3 inflammasome activation and intestinal mucositis may be achieved by promoting intracellular mitophagy and reducing mtROS. In order to test this hypothesis, we conducted further in vitro and in vivo experiments.

3.2. The animal experiments

DSS was used to induce colitis in *hsf2*$^{-/-}$mice(Fig. 3). Transmission electron microscopy showed that the number of damaged mitochondria in intestinal mucosa of *hsf2*$^{-/-}$ DSS colitis mice was significantly higher than that of wild-type mice, while the level of mitophagy was lower than that of wild-type mice (Fig. 4-A,B). This result is consistent with our expectation and similar to the results of clinical part: the level of *hsf2* in intestinal mucosa is positively correlated with the level of mitophagy in IECs. Compared with wild-type mice, the level of ROS in intestinal mucosa of *hsf2*$^{-/-}$ DSS colitis mice was significantly increased (Fig. 4-C,D). By RT-PCR, immunohistochemistry and Western blotting, we found that compared with wild-type mice, the mRNA and protein expression levels of PARL, PINK1 and Parkin in intestinal mucosa of *hsf2*$^{-/-}$ DSS colitis mice were significantly changed. The level of PARL was increased, while the levels of PINK1 and Parkin were decreased (Fig. 5,6).

These results suggest that downregulating *hsf2* can reduce the level of mitophagy and increase the number of damaged mitochondria in IECs of DSS colitis mice, which may be closely related to the PARL/PINK1/Parkin signaling pathway. To further explore how *hsf2* regulates intestinal inflammation by promoting mitophagy in inflammatory state, we conducted in vitro experiments.

3.3. The cell experiments

First, we successfully regulated the expression of HSF2 in Caco-2 cells by lentivirus transfection (Fig. 7), and stimulated Caco-2 cells with LPS to generate inflammation. Second, we observed Caco-2 cells with TEM, and found that increased damaged mitochondria and mitophagy showed in the LPS stimulated Caco-2 cells; Overexpression of HSF2 could increase the level of LPS induced mitophagy and decrease the number of damaged mitochondria in Caco-2 cells. On the contrary, downregulating HSF2 could decrease the level of mitophagy and increase the number of damaged mitochondria in Caco-2 cells (Fig. 8). Third, flow cytometry showed that ROS content in Caco-2 cells in-creased significantly when LPS was used to stimulate the cells, and decreased after overexpression of HSF2. On the contrary, knockdown of HSF2 could increase the level of ROS (Fig. 9). Finally, RT-PCR and Western blotting were used to detect the gene and protein levels of PARL,PINK1 and Parkin, which is one of the important regulatory signaling pathways of mitophagy. It was found that the level of PARL,PINK1 and Parkin was closely related to the level of LPS induced inflammation and HSF2. After LPS stimulated Caco-2 cells, the gene and protein levels of PARL,PINK1 and Parkin were significantly changed: the level of PARL was decreased, However, the level of PINK1 and Parkin increased (mitophagy increased). Overexpression of HSF2 could further enhance the effect of LPS on the gene and protein levels of PARL,PINK1 and Parkin: the level of PARL was further decreased, and the level of PINK1 and Parkin was further increased (mitophagy was decreased); On the contrary, downregulation of HSF2 attenuated the effect of LPS on the gene and protein levels of PARL/PINK1/Parkin signaling pathway (Fig. 10). In addition, as an important downstream molecule of NLRP3 inflammasome, we detected the level of IL-1β and IL-18 in each group by ELISA. The level of IL-1β and IL-18 increased significantly after LPS and ATP was used to stimulate the cells. Moreover, after overexpression of HSF2, the intracellular IL-1β and IL-18 decreased; On the contrary, after downregulation of HSF2, the level of IL-1β and IL-18 increased.(Fig. 11)

4. Discussion

Intestinal mucosal homeostasis is the basis of maintaining normal intestinal func-tion. Moderate inflammation is important for maintaining homeostasis, while excessive inflammation can damage IECs, which is also a critical factor in the pathogenesis of UC. How to maintain the balance between inflammation and anti-inflammation of IECs may be question to investigate the pathogenesis of UC. In this study, we found that HSF2 can regulate mitophagy of IECs, inhibit intestinal mucosal inflammation, and maintain in-testinal homeostasis in UC. This study reveals a new regulatory mechanism for the intestinal mucosal homeostasis of HSF2 in UC.

NLRP3 inflammasome can be activated by a variety of endogenous or exogenous stimuli, including mtROS[14–16]. Previous studies have reported that the level of ROS in intestinal mucosa of UC patients is significantly increased due to long-term inflam-matory stimulation, and there are many damaged mitochondria[19, 28]. Our study found that the number of damaged mitochondria in IECs of UC patients increased with disease severity, and the level of mtROS in intestinal mucosa increased continuously, which was consistent with previous studies, further proving the correlation between the level of damaged mitochondria and mtROS and the severity of UC. These results suggest that the damage to mitochondria and the increased mtROS level may be important factors in the overactivation of NLRP3 inflammasome

and the promotion of UC development. Therefore, timely removal of damaged mitochondria may be an important way to effectively inhibit the overactivation of NLRP3 inflammasome.

Mitophagy plays an important role in limiting the accumulation of mtROS and maintaining cell homeostasis by clearing damaged mitochondria[29]. In our study, we found that with increasing severity of UC, the level of mitophagy in IECs of UC patients rose accordingly. Similar results were found in mice with DSS-induced colitis and LPS-stimulated Caco-2 cells. This suggests that IECs may reduce mitochondrial injury under severe stress of UC by mitophagy, but it is not enough to reverse the disease process. These results are consistent with the physiological function of mitophagy and suggest that the level of mitophagy in IECs may be closely related to the activation of the NLRP3 inflammasome in UC, but the specific regulatory mechanism still needs to be further explored.

The signaling pathways of selective autophagy in damaged mitochondria have not been fully elucidated, including the PARL/PINK1/Parkin signaling pathway[30], the Nix protein mediated pathway[31], and other pathways mediated by dynamic related protein 1 (Drp1)[32], among which the most recognized is the PARL/PINK1/Parkin pathway. Some studies have found that HSP72 regulated by HSF2 can regulate the level of mitophagy through Parkin protein[23–26]. Our study also found that the PARL/PINK1/Parkin pathway has significant changes in UC patients, and changes further with the severity of the disease. These results suggest that the PARL/PINK1/Parkin pathway plays an important role in the regulation of mitophagy in UC intestinal mucosa. In addition, in mice with DSS colitis, compared with wild-type mice, the level of PARL in intestinal mucosa of *hsf2*$^{-/-}$ mice was increased, while the level of PINK1 and Parkin was decreased. In Caco-2 cells, with overexpression of HSF2, the level of PARL decreased, but the levels of PINK1 and Parkin increased. However, when HSF2 was knocked down, the opposite occurred. Therefore, we speculate that HSF2 may promote mitophagy in IECs through the PARL/PINK1/Parkin signaling pathway and timely remove damaged mitochondria, thus reducing the accumulation of mtROS in cells and inhibiting the activation of NLRP3 inflammasome, alleviating the inflamma-tion of intestinal mucosa, and playing a protective role in UC.

Although we further elucidated the mechanism of HSF2 regulating the activation of NLRP3 inflammasome in UC, there are still some unanswered questions. As an important transcription factor of HSPs, HSF2 may play a role by regulating the expression level of HSP. However, in our study, we did not continue to explore the relationship and regulatory mechanism between HSP and the PARL/PINK1/Parkin signaling pathway, mitophagy, and mtROS. In the future, we will further explore the specific relationship and potential regulatory mechanism of HSP regulated by HSF2 with PARL/PINK1/Parkin signaling pathway, mitophagy, mtROS and NLRP3 inflammasome in UC IECs, so as to lay a new theoretical foundation for further elucidating the mechanism of HSF2 in UC.

5. Conclusions

In UC, HSF2 may promote mitophagy and decrease the level of mtROS through the PARL/PINK1/Parkin signaling pathway. These results further elucidate the mechanism of high-level specific expression of

HSF2 in UC inhibiting the activation of NLRP3 inflammasome and alleviating the inflammation of intestinal mucosa.

Abbreviations

UC, Ulcerative colitis; NLRP3, Nod-like recepter 3; HSPs, Heat shock proteins; HSF2, Heat shock transcription factor 2; ROS, Reactive oxygen species; DSS, Dextran sulfate sodium.

Declarations

Author Contributions: Conceptualization, Fengrui Zhang and Junkun Niu; methodology, Hao Liang; validation, Wen Wang, Wei Zhao and Yuran Feng; investigation, Jing Wu, Maojuan Li and Xinyu Bai; data curation, Jiao Zhou; writing-original draft preparation, Hao Liang.; writing-review and editing, Fengrui Zhang and Junkun Niu; supervision, Yinglei Miao and Zhong Zeng; project administration, Yinglei Miao. All authors have read and agreed to the published version of the manuscript.

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References

1. Kaplan GG, Ng SC. Understanding and Preventing the Global Increase of Inflammatory Bowel Disease [published correction appears in Gastroenterology. 2017 Jun;152(8):2084]. Gastroenterology. 2017;152(2):313–321.e2. doi: 10.1053/ j. gas-tro.2016.10.020
2. Ng SC, Shi HY, Hamidi N, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies [published correction appears in Lancet. 2020 Oct 3;396(10256): e56]. Lancet. 2017;390(10114):2769–2778. doi:10.1016/S0140-6736(17)32448-0

3. Ordás I, Eckmann L, Talamini M, et al. Ulcerative colitis. Lancet. 2012;380(9853):1606–1619. doi:10.1016/S0140-6736(12)60150-0
4. Zuo T, Kamm MA, Ng SC, et al. Urbanization and the gut microbiota in health and inflammatory bowel disease. Nat Rev Gastroenterol Hepatol. 2018;15(7):440–452. doi:10.1038/s41575-018-0003-z
5. Sánchez de Medina F, Romero-Calvo I, Mascaraque C, et al. Intestinal inflammation and mucosal barrier function. Inflamm Bowel Dis. 2014;20(12):2394–2404. doi:10.1097/MIB.0000000000000204
6. Yao D, Dong M, Dai C, et al. Inflammation and Inflammatory Cytokine Contribute to the Initiation and Development of Ulcerative Colitis and Its Associated Cancer. Inflamm Bowel Dis. 2019;25(10):1595–1602. doi:10.1093/ibd/izz149
7. Geremia A, Biancheri P, Allan P, Corazza GR, Di Sabatino A. Innate and adaptive immunity in inflammatory bowel disease. Autoimmun Rev. 2014;13(1):3–10. doi:10.1016/j.autrev.2013.06.004
8. Próchnicki T, Latz E. Inflammasomes on the Crossroads of Innate Immune Recognition and Metabolic Control. Cell Metab. 2017;26(1):71–93. doi:10.1016/j.cmet.2017.06.018
9. Strowig T, Henao-Mejia J, Elinav E, et al. Inflammasomes in health and disease. Nature. 2012;481(7381):278–286. doi:10.1038/nature10759
10. Ranson N, Veldhuis M, Mitchell B, et al. NLRP3-Dependent and -Independent Processing of Interleukin (IL)-1β in Active Ulcerative Colitis. Int J Mol Sci. 2018;20(1):57. doi:10.3390/ijms20010057
11. Zhen Y, Zhang H. NLRP3 Inflammasome and Inflammatory Bowel Disease. Front Immunol. 2019;10:276. doi:10.3389/fimmu.2019.00276
12. Miao J, Niu J, Wang K, et al. Heat shock factor 2 levels are associated with the severity of ulcerative colitis. PLoS One. 2014;9(2):e88822. doi:10.1371/journal.pone.0088822
13. Zhang F, Zhao W, Zhou J, et al. Heat shock transcription factor 2 reduces the secretion of IL-1β by inhibiting NLRP3 in-flammasome activation in ulcerative colitis. Gene. 2021; 768:145299. doi: 10.1016/j.gene.2020.145299
14. Swanson KV, Deng M, Ting JP. The NLRP3 inflammasome: molecular activation and regulation to therapeutics. Nat Rev Immunol. 2019;19(8):477–489. doi:10.1038/s41577-019-0165-0
15. Kelley N, Jeltema D, Duan Y, et al. The NLRP3 Inflammasome: An Overview of Mechanisms of Activation and Regulation. Int J Mol Sci. 2019;20(13):3328. doi:10.3390/ijms20133328
16. Yang Y, Wang H, Kouadir M,et al. Recent advances in the mechanisms of NLRP3 inflammasome activation and its inhibitors. Cell Death Dis. 2019;10(2):128. doi:10.1038/s41419-019-1413-8
17. Wang Z, Li S, Cao Y, et al. Oxidative Stress and Carbonyl Lesions in Ulcerative Colitis and Associated Colorectal Cancer. Oxid Med Cell Longev. 2016;2016:9875298. doi:10.1155/2016/9875298
18. Spoorthi BC, More SS, Gautham SA, et al. Role of free radical scavenging activity of vasoactive intestinal peptide in the at-tenuation of mitochondrial dysfunction to ameliorate dextran sulphate sodium-induced colitis in mice: Implications in ul-cerative colitis. J Dig Dis. 2020;21(12):711–723. doi:10.1111/1751-2980.12932

19. Chandraiah SB, Ghosh S, Saha I, et al. Substance P failed to reverse dextran sulfate sodium-induced murine colitis mediated by mitochondrial dysfunction: implications in ulcerative colitis. 3 Biotech. 2021;11(4):199. doi:10.1007/s13205-021-02755-2
20. Kudryavtseva AV, Krasnov GS, Dmitriev AA, et al. Mitochondrial dysfunction and oxidative stress in aging and cancer. Oncotarget. 2016;7(29):44879–44905. doi:10.18632/oncotarget.9821
21. Guo W, Sun Y, Liu W, et al. Small molecule-driven mitophagy-mediated NLRP3 inflammasome inhibition is responsible for the prevention of colitis-associated cancer. Autophagy. 2014;10(6):972–985. doi:10.4161/auto.28374
22. Ashrafi G, Schwarz TL. The pathways of mitophagy for quality control and clearance of mitochondria. Cell Death Differ. 2013;20(1):31–42. doi:10.1038/cdd.2012.81
23. Kwong JM, Lalezary M, Nguyen JK, et al. Co-expression of heat shock transcription factors 1 and 2 in rat retinal ganglion cells. Neurosci Lett. 2006;405(3):191–195. doi:10.1016/j.neulet.2006.06.070
24. Ahn J, Piri N, Caprioli J, Munemasa Y, et al. Expression of heat shock transcription factors and heat shock protein 72 in rat retina after intravitreal injection of low dose N-methyl-D-aspartate. Neurosci Lett. 2008;433(1):11–16. doi:10.1016/j.neulet.2007.12.045
25. Drew BG, Ribas V, Le JA, et al. HSP72 is a mitochondrial stress sensor critical for Parkin action, oxidative metabolism, and insulin sensitivity in skeletal muscle. Diabetes. 2014;63(5):1488–1505. doi:10.2337/db13-0665
26. Ostling P, Björk JK, Roos-Mattjus P, et al. Heat shock factor 2 (HSF2) contributes to inducible expression of hsp genes through interplay with HSF1. J Biol Chem. 2007;282(10):7077–7086. doi:10.1074/jbc.M607556200
27. Wang W, Zhang F, Li X, et al. Heat shock transcription factor 2 inhibits intestinal epithelial cell apoptosis through the mi-tochondrial pathway in ulcerative colitis. Biochem Biophys Res Commun. 2020;527(1):173–179. doi: 10.1016/j.bbrc.2020. 04.10
28. Liu Z, Ren Z, Zhang J, et al. Role of ROS and Nutritional Antioxidants in Human Diseases. Front Physiol. 2018;9: 477. Pub-lished 2018 May 17. doi: 10.3389/fphys.2018.00477
29. Larabi A, Barnich N, Nguyen HTT. New insights into the interplay between autophagy, gut microbiota and inflammatory responses in IBD. Autophagy. 2020;16(1):38–51. doi:10.1080/15548627.2019.1635384
30. Dombi E, Mortiboys H, Poulton J. Modulating Mitophagy in Mitochondrial Disease. Curr Med Chem. 2018;25(40):5597–5612. doi:10.2174/0929867324666170616101741
31. Zhang M, Shi R, Zhang Y, et al. Nix/BNIP3L-dependent mitophagy accounts for airway epithelial cell injury induced by cigarette smoke. J Cell Physiol. 2019;234(8):14210–14220. doi:10.1002/jcp.28117
32. Feng ST, Wang ZZ, Yuan YH, et al. Dynamin-related protein 1: A protein critical for mitochondrial fission, mitophagy, and neuronal death in Parkinson's disease. Pharmacol Res. 2020;151:104553. doi:10.1016/j.phrs.2019.104553

Tables

Table 1. Details of UC patients and healthy controls.

Patient details	Number of patients (%)	Healthy controls details	Number of healthy controls □%□
Gender			
Male	48 (53.3%)	Male	15 (50%)
Female	42 (46.7%)	Female	15 (50%)
Mean age (years)	40.5 (19-58)	Mean age (year)	42.2 (18-57)
Diagnosis		Diagnosis	
UC (Mayo score)		Healthy	
Mild	33 (36.7%)	-	-
Moderate	30 (33.3%)	-	-
Severe	27 (30%)	-	-
Actual disease extent			
E1	0 (0%)	-	-
E2	90 (100%)	-	-
E3	0 (0%)	-	-
Mayo Score			
0	0 (0)	-	-
1	33 (36.7%)	-	-
2	29 (32.2%)	-	-
3	28 (31.1%)	-	-
Medication			
5-ASA (mesalazine)	90 (100%)	-	-

Figures

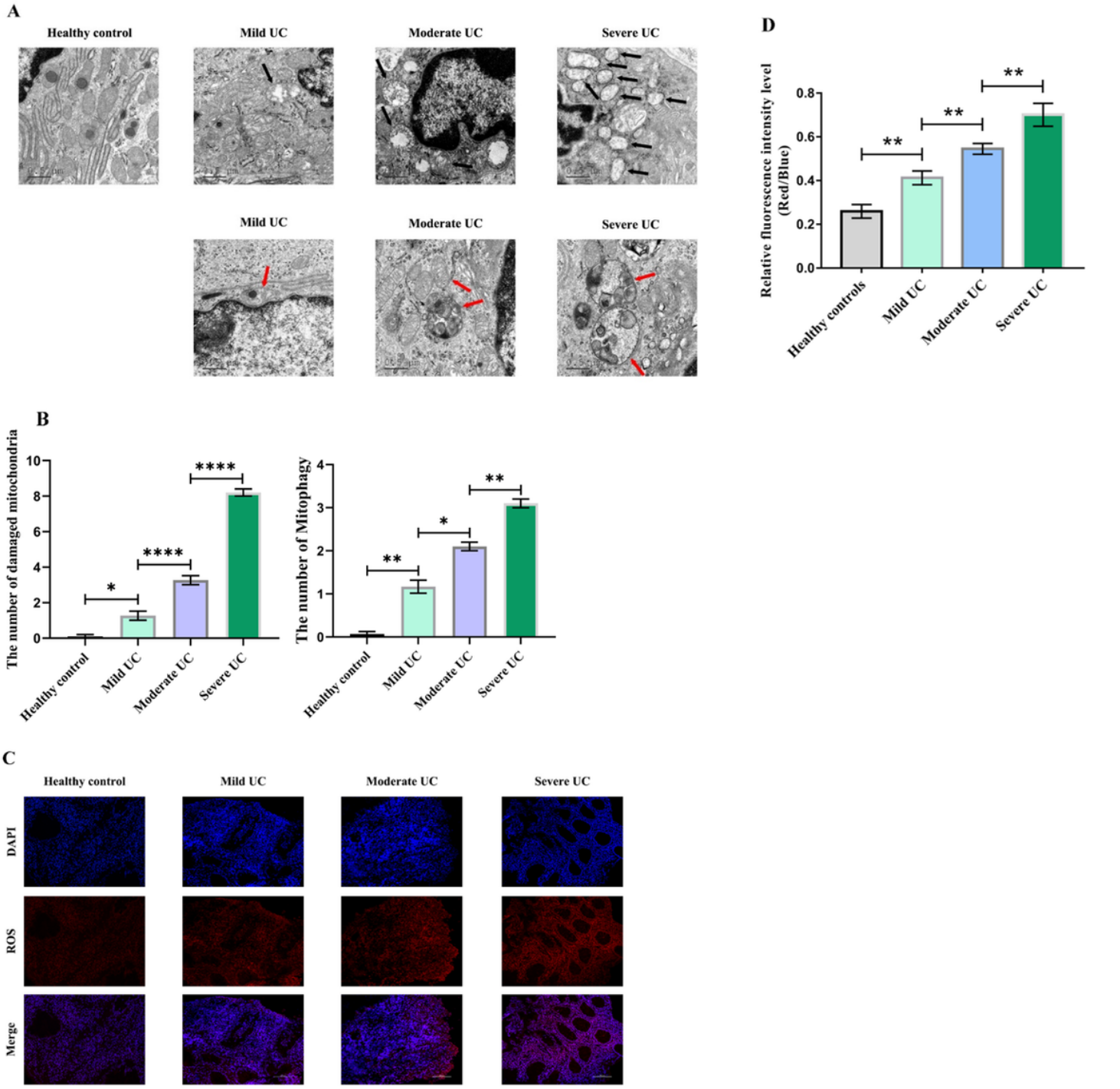

Figure 1

Compared with healthy controls, obvious morphological abnormalities were present in mitochondria of IECs of UC patients, and mitophagy level of IECs rose with increased disease activity, showing a positive correlation. ROS level was higher in intestinal mucosal tissues of UC patients than healthy controls. With increased of disease severity, ROS levels in intestinal mucosal tissues increased continuously. (A) Mitophagy level in mouse IECs were observed by TEM. (Black arrows show damaged mitochondria, red arrows show mitophagy). (B)The number of damaged mitochondria and the level of mitophagy in

healthy controls and patients were shown in the graph. Statistical significance at * P < 0.05, ** P < 0.01 and **** P < 0.0001. (C) Immunofluorescence was used to detect ROS levels in intestinal mucosal tissues of patients in each group. (D) The ROS levels are expressed as means ± standard deviation, ROS level was positively correlated with disease activity. Statistical significance at ** P < 0.01.

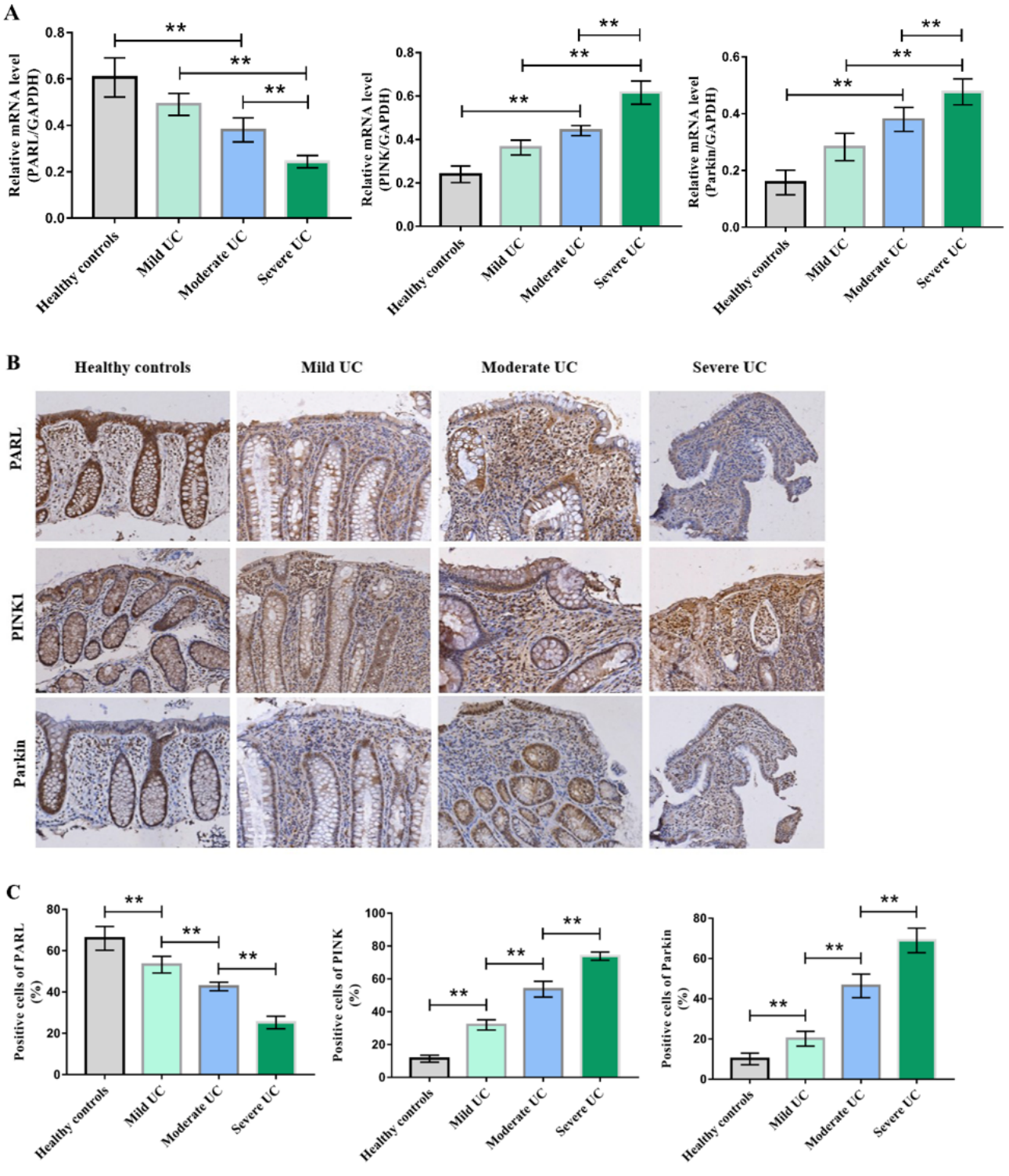

Figure 2

Higher severity of UC means lower PARL; PARL level in intestinal mucosa of UC patients decreased and PINK1 and Parkin levels rose. (A) Expression levels of PARL, PINK1, and Parkin genes in intestinal mucosa of each group were measured by RT-PCR. Statistical significance at ** P < 0.01. (B) Immunohistochemistry was used to detect levels of PARL, PINK1, and Parkin proteins in intestinal mucosa of mice. (**C**) These data of immunohistochemistry was expressed as means ± standard deviation, and statistical results were obtained by one-way ANOVA. Statistical significance at ** P < 0.01.

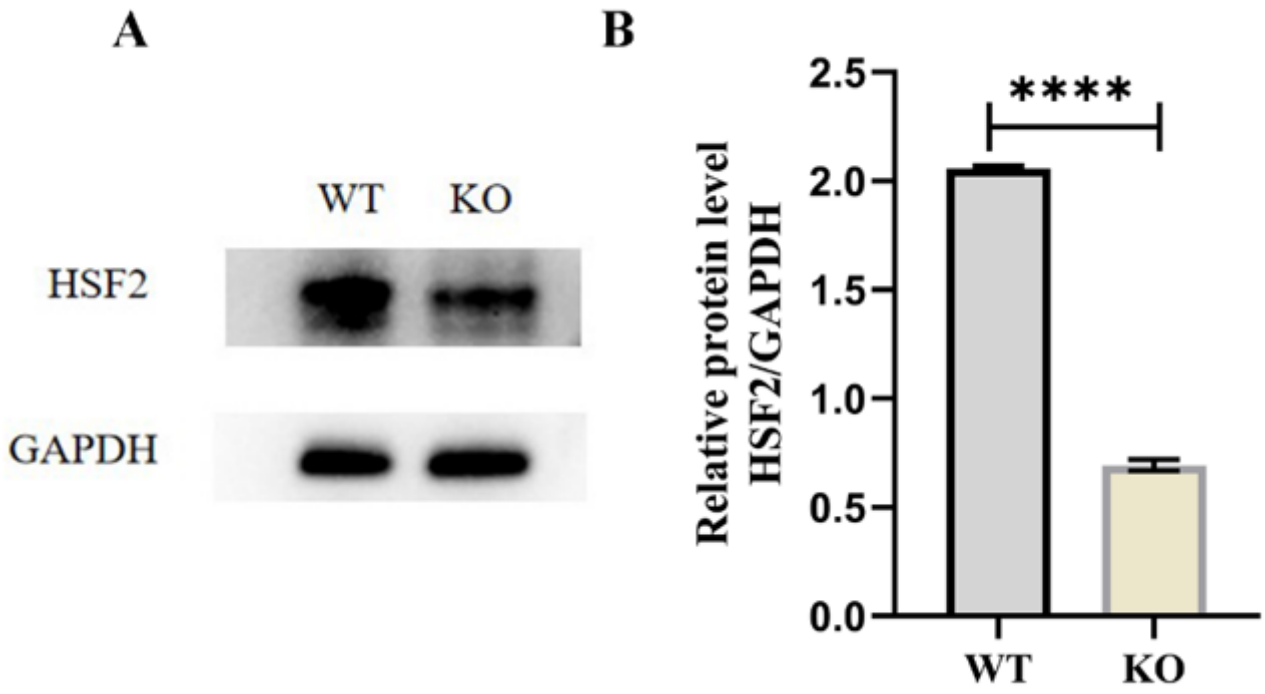

Figure 3

There are significant differences about the *hsf2* protein level between the wild type mice and the *hsf2*$^{-/-}$ mice. (A) Level of *hsf2* protein in mice was detected by Western blot. (WT, wild type; KO, HSF2 knockout mice) (B) These part data expressed as means ± standard deviation. Statistical significance at **** P < 0.0001.

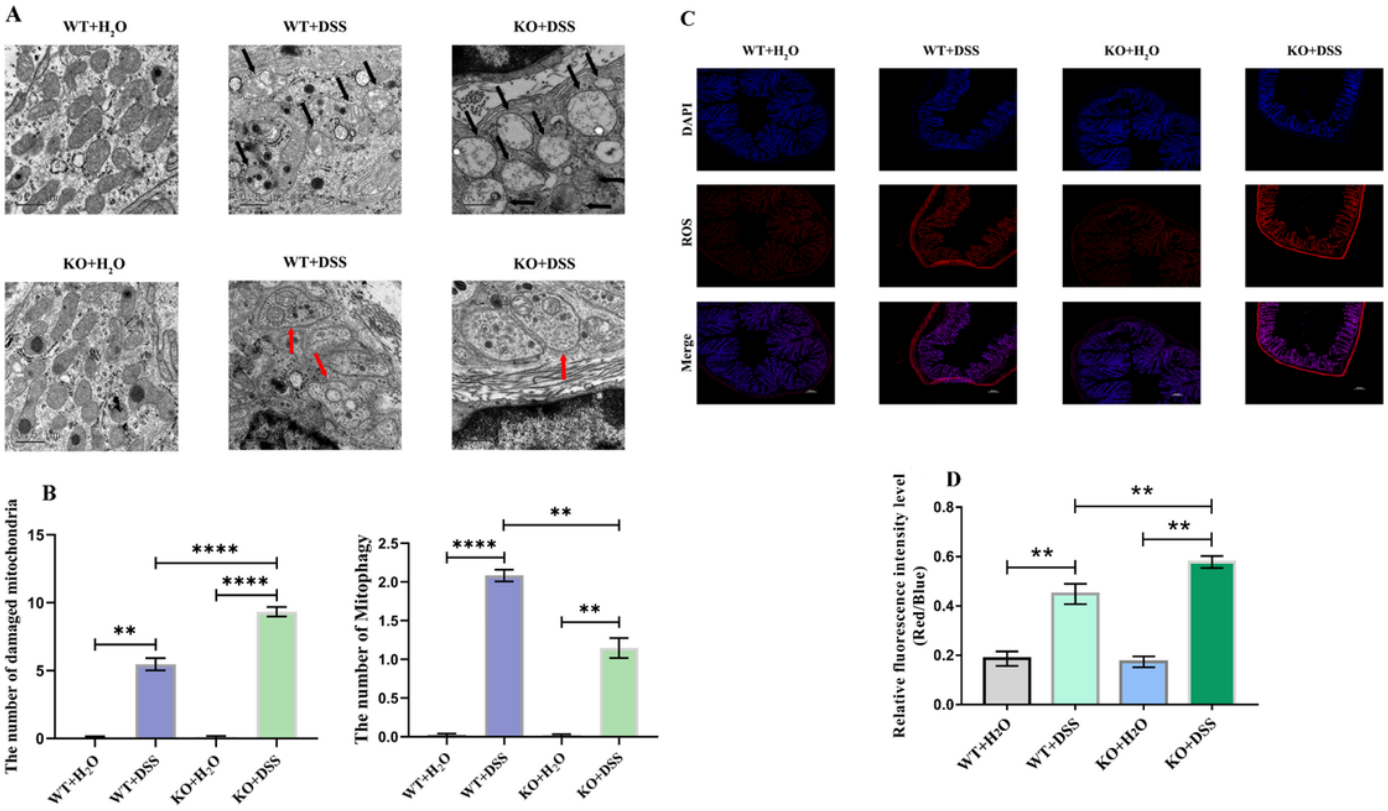

Figure 4

Compared with WT+DSS group, mitophagy of intestinal mucosa of KO+DSS group was less. No obvious mitophagy occurred in WT+H_2O and KO+H_2O groups, which were used as controls. Compared with WT + DSS group, ROS level of KO + DSS group was higher. (A) Transmission electron microscope was used to observe level of mitophagy in intestinal mucosa of mice. In mice given DSS, compared with wild-type mice, *hsf2*$^{-/-}$ mice showed decreased mitophagy levels. (Black arrows show damaged mitochondria, red arrows show mitophagy). (B)The number of damaged mitochondria and the level of mitophagy in wild type mice and the *hsf2*$^{-/-}$ mice were shown in the graph. Statis-tical significance at ** $P < 0.01$ and **** $P < 0.0001$. (C) Immunofluorescence was used to detect ROS levels in intestinal mucosa of mice. (D) The ROS levels expressed as means ± standard deviation, and statistical results were obtained by one-way ANOVA. Statistical significance at ** $P < 0.01$.

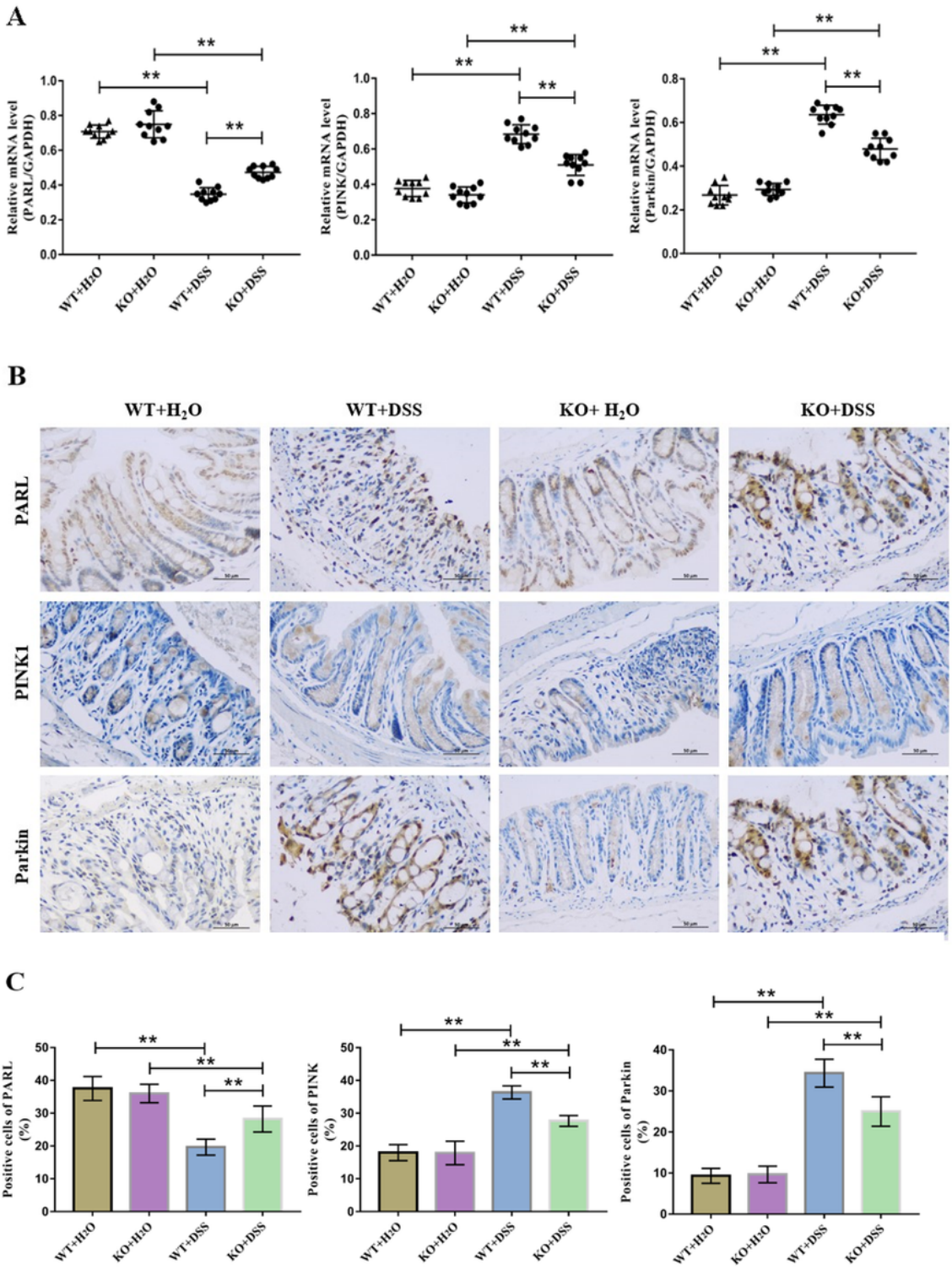

Figure 5

With hsf2 gene knocked out, level of mitophagy of intestinal mucosa decreased. (A) RT-PCR was used to detect expression of PARL, PINK1, and Parkin genes in intestinal mucosa of mice in each group. Compared with WT + DSS group, higher levels of PARL gene presented in KO+DSS group, but levels of PINK1 and Parkin genes were lower. Statistical significance at ** $P < 0.01$. (B) Immunohistochemistry was used to detect levels of PARL, PINK1, and Parkin proteins in intestinal mucosa of mice in each group.

Compared with WT+DSS group, level of PARL protein in KO+DSS group was higher, but levels of PINK1 and Parkin protein were decreased. (C) The data of immunohistochemistry was expressed as means ± standard deviation, and statistical results were obtained by one-way ANOVA. Statistical significance at ** P < 0.01.

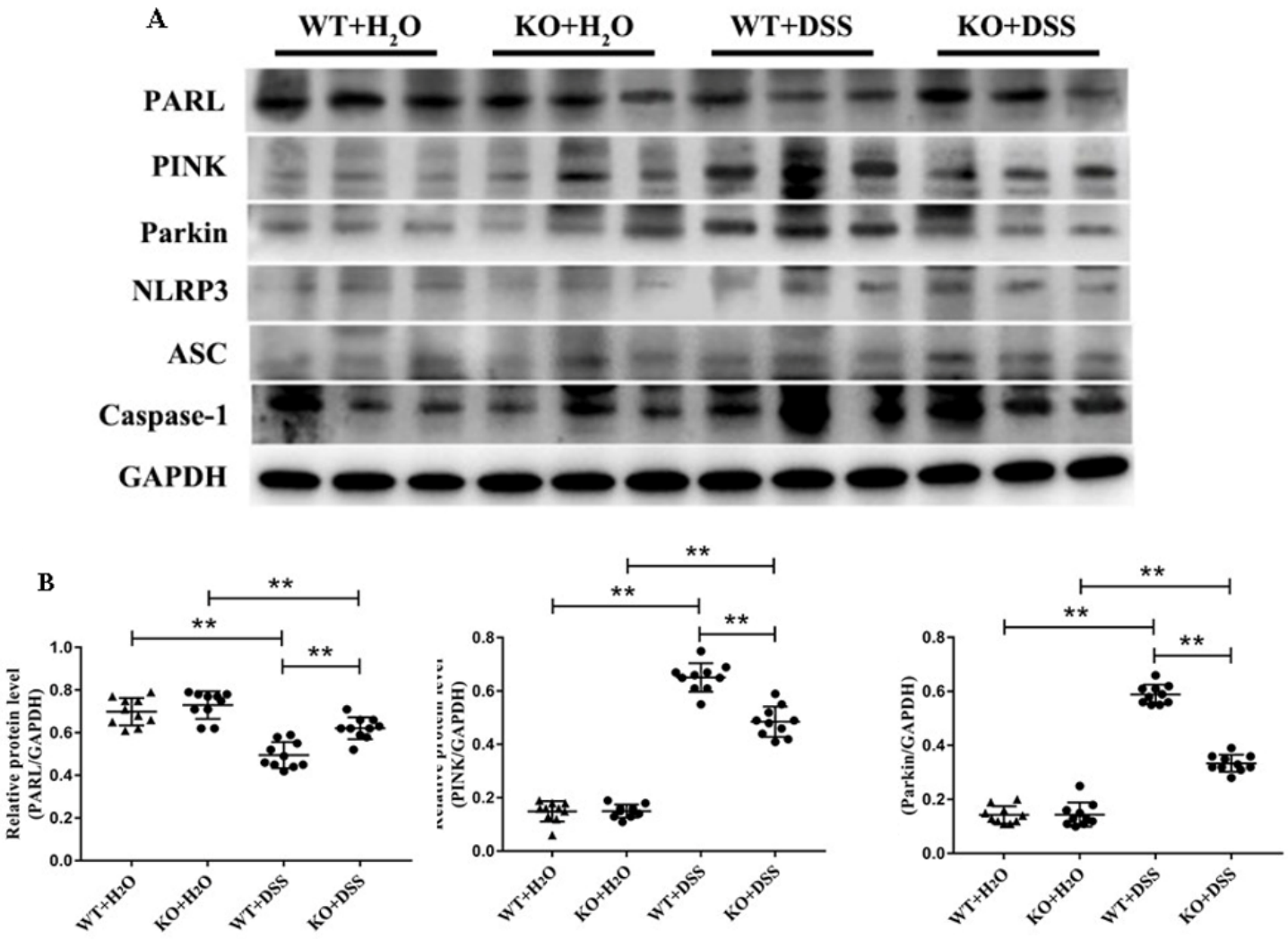

Figure 6

hsf2$^{-/-}$ mice presented lower mitophagy levels in intestinal mucosa compared with WT group. (A) Western blotting was used to detect levels of PARL, PINK1, and Parkin proteins of intestinal mucosa of mice in each group. (B) These data of western blotting was expressed as means ± standard deviation, and statistical results were obtained by one-way ANOVA. Statistical significance at ** P < 0.01.

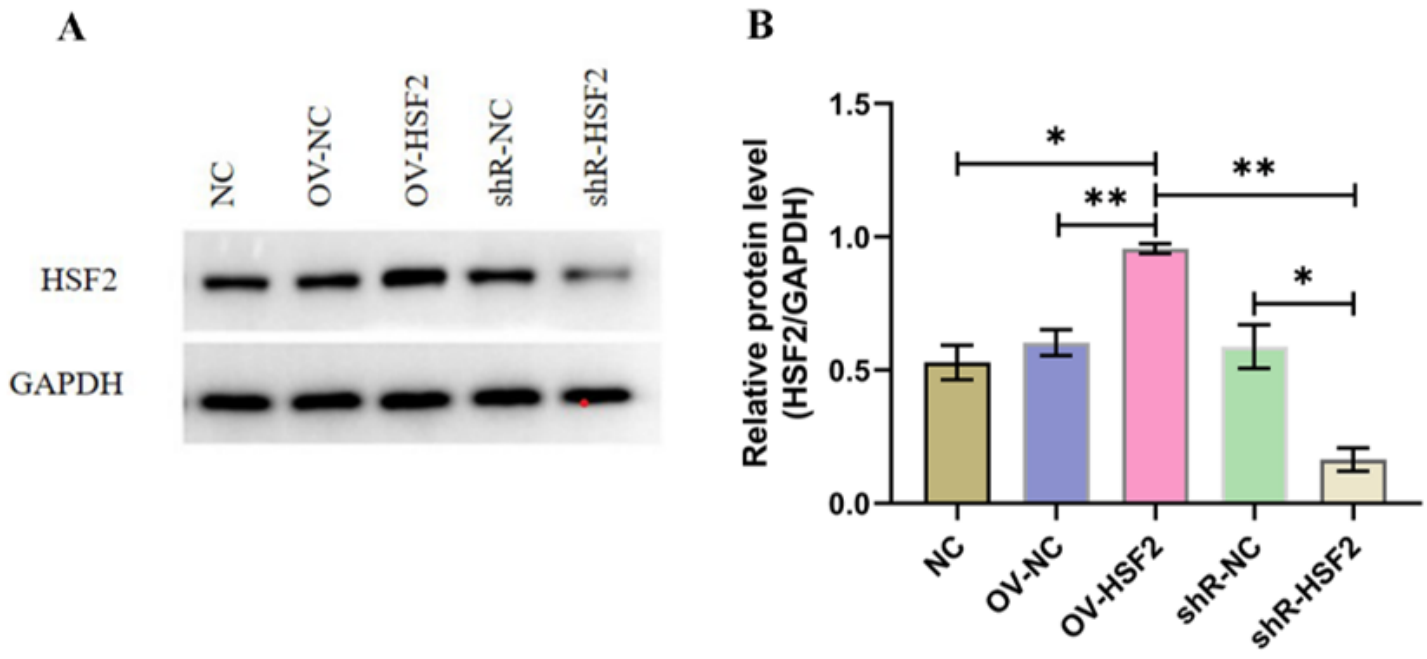

Figure 7

HSF2 gene level in Caco-2 cells was regulated by lentivirus. (A) Level of HSF2 protein in Caco-2 cells was detected by Western blotting.(NC, normal controls; OV-HSF2, HSF2 overexpression group; OV-NC, negative overexpression controls; shR-HSF2, HSF2 knockdown group; shR-NC, negative knockdown controls). (B) These data expressed as means ± standard deviation, and statistical results were obtained by one-way ANOVA. Statistical significance at * P <

0.05 and ** P < 0.01.

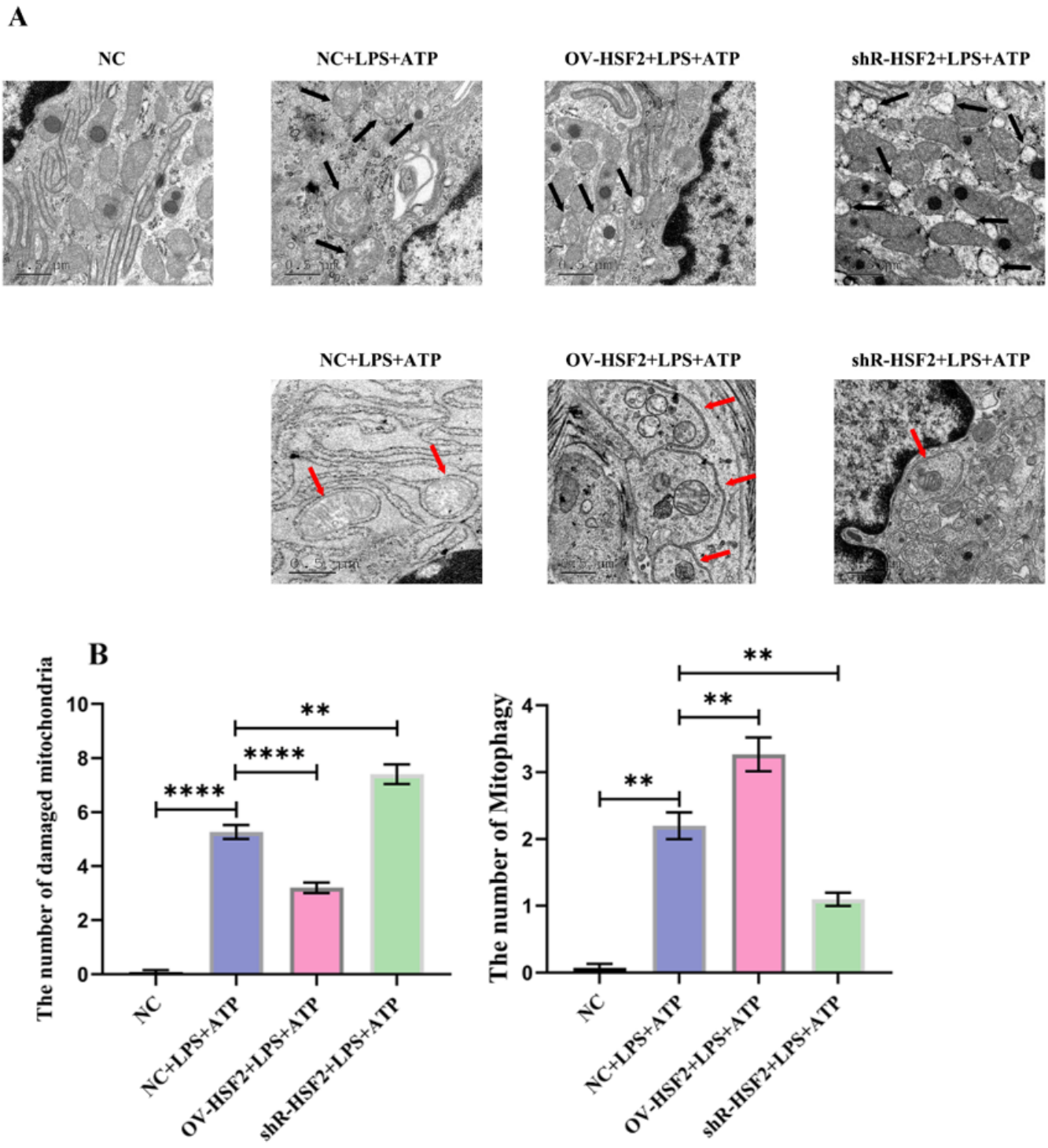

Figure 8

Number of damaged mitochondria and mitophagy in Caco-2 cells was significantly increased after LPS stimulation. Overexpression of HSF2 increased mitophagy level in Caco-2 cells and reduce number of intracellularly damaged mitochondria. Conversely, downregulation of HSF2 reduced mitophagy level in Caco-2 cells and increased number of intracellularly damaged mitochondria.(A) Mitochondrial morphology and mitophagy of Caco-2 cells in each group were detected by TEM. (Black arrows show

abnormal morphology of damaged mitochondria, red arrows show mitophagy). (B)The number of damaged mitochondria and the level of mitophagy in each group Caco-2 cells were shown in the graph.

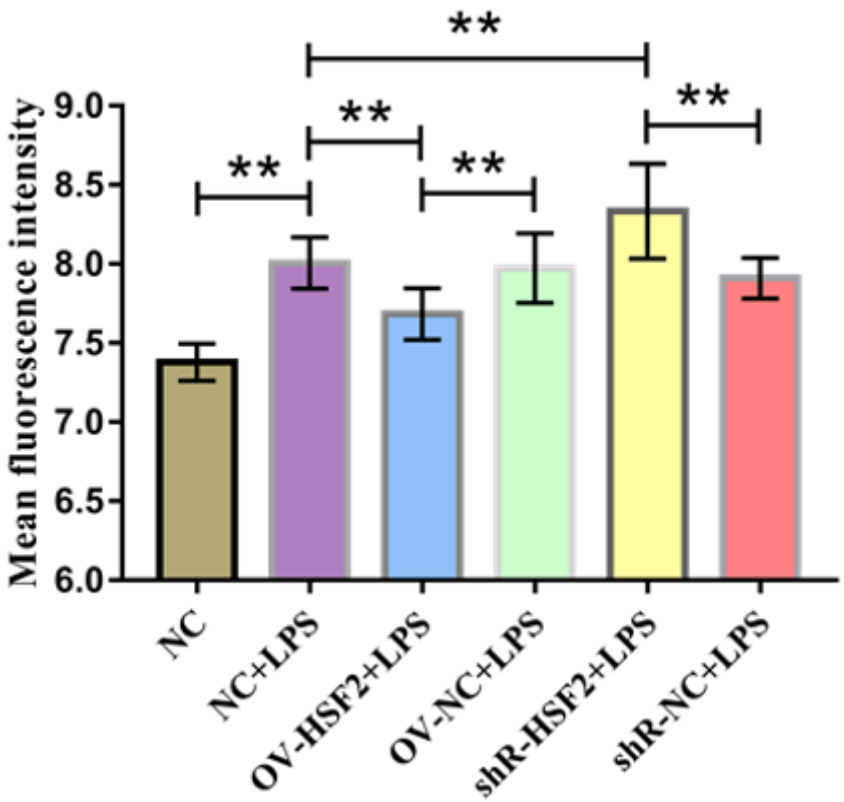

Figure 9

Intracellular mtROS levels of Caco-2 cells treated differently in each group were detected by DHE-ROS detection kit and flow cytometry. After stimulation with LPS, intracellular mtROS level was significantly increased. In addition, overexpression of HSF2 reduced intracellular mtROS levels. On the contrary, after downregulation of HSF2, intracellular mtROS level increased. These data are expressed as means ± standard deviations, and statistical results were obtained by one-way ANOVA. Statistical significance at ** $P < 0.01$.

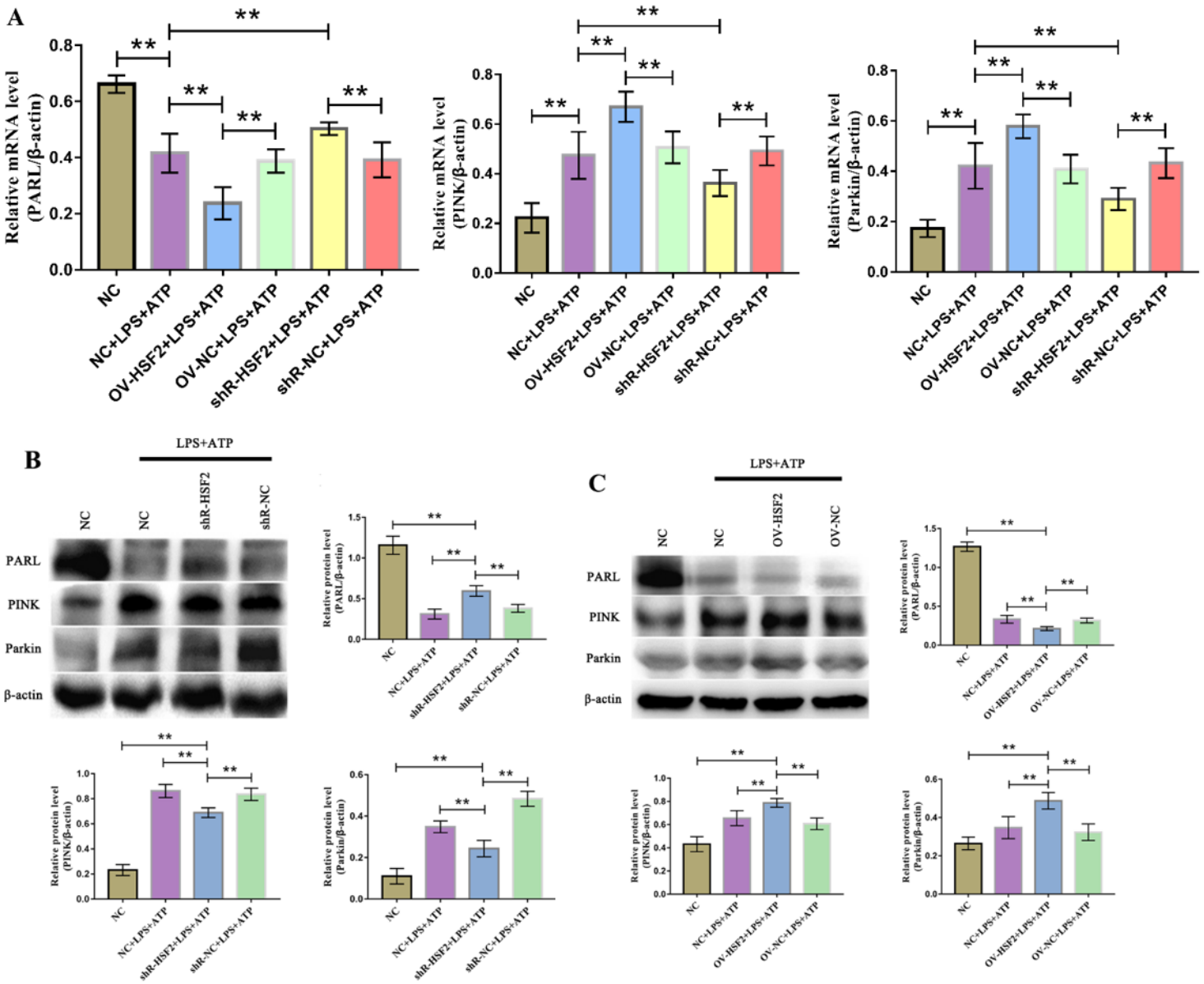

Figure 10

HSF2 may regulate mitophagy in Caco-2 cells through PARL/PINK1/Parkin signaling pathway. When HSF2 overexpressed, level of PARL gene in cells decreased, and levels of PINK1 and Parkin genes increased; by comparison, with overexpressed cells, level of PARL gene was higher after HSF2 knockdown, and levels of PINK1 and Parkin genes were lower. (A) RT-PCR was used to detect levels of PARL, PINK1, and Parkin genes in Caco-2 cells of each group. These expressed as means ± standard deviation, and statistical results were obtained by one-way ANOVA. Statistical significance at * $P < 0.05$ and ** $P < 0.01$. (B,C) Western blotting was used to detect levels of PARL, PINK1, and Parkin proteins in Caco-2 cells of each group. Results were consistent with RT-PCR.

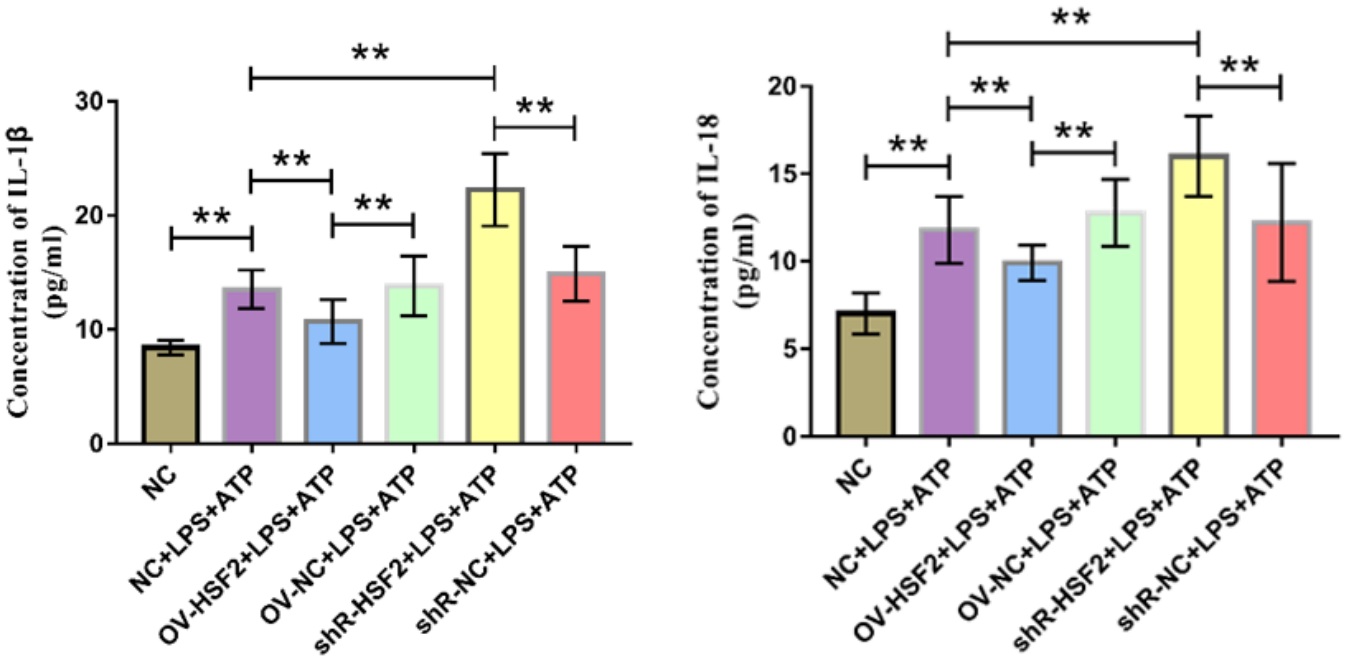

Figure 11

Level of IL-1β and IL-18 in each group Caco-2 cells was detected by ELISA, and increased significantly after LPS and ATP were used to stimulate cells. In addition, after HSF2 overexpression, intracellular IL-1β and IL-18 decreased; on the contrary, after HSF2 downregulation, intracellular IL-1β and IL-18 increased, and difference was statistically significant. Statistical significance at ** $P < 0.01$.

Supplementary Files

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- Additionalfigure.docx