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A mast cell specific receptor-Mrgprb2 knockout aggravates colitis by exacerbating immune system, mucosal barrier and gut microbiota disorders

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Abstract: Ulcerative colitis (UC) is a chronic immune-related disease which changes in intestinal microbiota and damage to the intestinal barrier contribute to its pathogenesis. Human mas-related G protein-coupled receptor X2 (MRGPRX2) and its mice homolog Mrgprb2 are selectively expressed on mast cells (MCs) to recruit immune cells and modulate host defense against microbial infection. To investigate the role of Mrgprb2 in UC, we compared the differences between Mrgprb2 knockout (b2 KO) mice and wild-type (WT) mice with dextran sulfate sodium (DSS)-induced colitis in the severity of clinical symptoms, inflammatory cells infiltration, degree of intestinal barrier damage, and composition of intestinal flora. The results showed that the weight loss, disease activity index (DAI) score, colon shortening, colonic pathological damage were significantly increased in b2 KO mice while MCs activation, cytokines and chemokines secretion, and inflammatory cell infiltration were decreased. In addition, the abundance and diversity of intestinal microbiota were reduced in b2 KO mice. B2 KO mice also exhibited the reduction of probiotics such as norank_F_Muribaculaceae and Lactobacillus and increase of harmful bacteria like Escherichia-Shigella. Intestinal mucosal barrier damage of b2 KO mice was more severe than WT mice due to the attenuated expression of mucin2(MUC2), occludin, junctional adhesion molecules (JAM)-A. These results demonstrated that Mrgprb2 may have protective effect on DSS-induced colitis by regulating intestinal flora disorder, participating in barrier repair, and recruiting inflammatory cells to eliminate pathogens.

Keywords: Ulcerative colitis · Mast cells · Mrgprb2 · Inflammatory cells · Intestinal barrier · Gut microbiota

Introduction

UC is a kind of chronic inflammatory bowel disease (IBD) initiating in the rectum and colon(Kobayashi et al., 2020). Multiple factors including genes, environment, immune system and gut microbiota closely interact with each other and changes to one factor can affect the intestinal homeostasis based on a delicate equilibrium and lead to the occurrence of disease such as UC(Friedrich, Pohin, & Powrie, 2019).Of all the factors that affect intestinal homeostasis, more and more emerging evidences support the assumption that MCs play an important role(Albert-Bayo et al., 2019; De Zuani, Dal Secco, & Frossi, 2018; Lyons & Pullen, 2020).

MCs are widely distributed in the gastrointestinal tract and numerous intestinal diseases including

UC are thought to be related to the role of uncontrolled or disordered MCs activation (Lyons & Pullen, 2020; Wouters, Vicario, & Santos, 2016). Upon stimulation, MCs release cytokines, chemokines, growth factors, and mitogens to adjust vascular and epithelial permeability, tissue repair, innate and adaptive immunity and bacterial clearance, which is important for maintaining intestinal homeostasis (Bischoff, 2007; Gupta & Harvima, 2018; Mukai, Tsai, Saito, & Galli, 2018). Degranulation and cytokines release of MCs are induced by activation of various cell surface receptors such as high-affinity IgE receptor (FcεRI) and G-protein-coupled receptors (GPCRs) (Bulfone-Paus, Nilsson, Draber, Blank, & Levi-Schaffer, 2017). Recently, researchers began to focus on MRGPRX2 which activated MCs through a non-IgE mechanism and was found only expressed in MCs except dorsal root ganglia (Fujisawa et al., 2014; Kajiwara et al., 2010; Meixiong & Dong, 2017; Tatemoto et al., 2006).

MRGPRX2 is specifically expressed on MCs and *Mrgprb2* is the mouse ortholog of human MRGPRX2 (McNeil et al., 2015). Studies showed that MRGPRX2/*Mrgprb2* mediates neurogenic inflammation. The substance P (SP)-mediated MCs activation via MRGPRX2 led to recruitment of innate immune cells at the injury site and the release of multiple pro-inflammatory cytokines and chemokines which induce inflammatory mechanical and thermal hyperalgesia. *Mrgprb2*-deficient mice had a striking decrease in recruitment of immune cells at the injured site (Green, Limjunyawong, Gour, Pundir, & Dong, 2019). MRGPRX2/*Mrgprb2* also has an effect on the immune response to bacterial infections. Competence-stimulating peptide (CSP)-1, one of Gram-positive quorum-sensing molecules (QSMs), activated MCs through *Mrgprb2* and MRGPRX2 and led to inhibition of bacterial growth and biofilm formation (Pundir et al., 2019). In models of nasopharynx, peritoneum, and skin infection, *Mrgprb2* deficient mice showed reduced bacterial clearance, while activation of *Mrgprb2* eliminated bacteria and improved disease scores (Pundir et al., 2019). At present, researchers have found some associations between MRGPRX2 and UC, for example, in patients of UC, expression of the proteolytic precursors of the MRGPRX2 agonist was increased in inflamed compared to uninfamed ulcerative colitis (Chen et al., 2021). A new therapeutic target may be obtained by studying the effect of *Mrgprb2* on UC and related studies are still insufficient.

The aim of this work is to investigate the effect of MRGPRX2/*Mrgprb2* on UC and explore the mechanism through which MRGPRX2/*Mrgprb2* participates in the pathogenesis of UC.

Materials and Methods

Animals and DSS induced colitis

B2 KO C57BL/6J mice were purchased from GemPharmatech Co., Ltd. (Jiangsu, China). Sexually mature female and male mice backcrossed to C57BL/6 mice for more than 5 generations. Mice were bred and raised in SPF environment in Renmin Hospital of Wuhan University Animal Experiment Center. Animal experiments were approved by the Animal Care and Ethics Committee of Wuhan University according to the legal requirements (Approval No. 20210303, 16 March 2021).

Identify the genotypes of newly born mice, and select 16 WT male mice and 16 homozygous b2 KO male mice. Thirty-two mice were assigned to two control groups received no DSS: control group, b2KO group, and two DSS treated groups: DSS group and b2KODSS group. Mice were raised under a 12 h/12 h dark/light cycle with an ambient temperature of 22°C±1°C, 50%±10% humidity, and with free access to food and water. After one week of adaptive feeding, mice of two DSS treated groups

were given 3% DSS (36 to 50 kDa, MP Biomedicals, Santa Ana, CA, USA) in the drinking water for 7 days to induce acute colitis and control groups received normal water. Body weight, the stool consistency and hematochezia were recorded daily to determine the disease activity index (DAI)(Murthy et al., 1993). At day 8 the mice were sacrificed by CO₂ narcosis and their intestinal tracts were removed to measure the length and collect the intestinal contents. The distal colon was fixed with 4% paraformaldehyde for hematoxylin-eosin (H&E) staining, and the rest was cryopreserved in liquid nitrogen for other tests.

Histological evaluation of colon sections

The colon tissues fixed with paraformaldehyde were embedded in paraffin wax and a 4µm section of each sample was placed on a glass slide and stained with HandE. Histological damage scores for HandE stained colon sections were assessed according to published guidelines:(a) crypt distortion and loss (normal to severe, 0–3), (b) inflammatory cell infiltration (normal to intensive, 0–3), (c) muscle thickening (presence of significant muscle thickening, 0–3), (d) goblet cell depletion (absence and presence, 0–1), and (e) crypt abscess (absence and presence, 0–1)(Cooper, Murthy, Shah, & Sedergran, 1993). Five visual fields were selected for each section and evaluated by an experienced pathologist in a blinded manner.

Immunofluorescence of intestinal barrier related proteins

Paraffin-embedded colon sections were deparaffinized and rehydrated through a series of xylene and ethanol washes. After washed with PBS, sections were blocked in 10% goat preimmune serum in PBS for 1 h in the dark in a humid chamber at room temperature and then incubated overnight with rabbit anti-occludin antibody (1:100)(Abcam, Cambridge, UK)and anti-mucin2 (MUC2) antibody (1:1000)(Abcam, Cambridge, UK). Tissue sections were then incubated with secondary antibody in the dark at room temperature for 50 min. Then add DAPI to the slides for incubation in the dark at room temperature for 10 min. Finally, the slides were washed three times in PBS and mounted with Antifade Mounting Medium and stored at 4 °C in the dark until imaging.

Measurement of relative mRNA expressions in colon tissues

Total RNA was isolated using TRIzol reagent (Servicebio, Wuhan, China). The supernatant was removed after centrifugation. The RNA was reverse-transcribed into cDNA by using Servicebio® RT First Strand cDNA Synthesis Kit (Servicebio, Wuhan, China) in a 20 µL reaction volume. Then cDNA samples were subjected to quantitative real time PCR using CFX Opus Real-Time PCR System (Bio-rad, California, USA) and SYBR Green qPCR Master Mix (None ROX) (Servicebio, Wuhan, China) according to the manufacturer's directions. After the PCR reaction system was configured, amplification was carried out according to the steps of 95°C for 10min, 40 cycles, 95°C for 15sec and 60°C for 30 sec. During the temperature rise from 65°C to 95°C, a fluorescence signal was collected every 0.5°C. The results were analyzed by $2^{-\Delta\Delta CT}$ method. The primers in this experiment are listed in Table 1.

Analyses of inflammatory cytokines in colon tissues

The colon tissues were accurately weighed and grinded into homogenate under the condition of ice bath. After centrifugation, the supernatant was taken for determination. The levels of tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), IL-6, IL-10, chemokines-CCL2, CCL3, Granulocyte colony-stimulating factor (GM-CSF), SP, nerve growth factor (NGF), histamine in colon tissue were measured with the ELISA kits (Multisciences (Lianke) Biotech, Co., Ltd, Hangzhou, China), and specific experimental steps were carried out according to the instructions.

Analysis of colonic contents by 16S RNA gene sequencing

Colon contents of mice were collected and rapidly frozen with liquid nitrogen and then stored at -80°C . Microbial community genomic DNA was extracted using the E.Z.N.A.®soil DNA kit (Omega Bio-tek, GA, USA) according to manufacturer's instructions. The DNA quality was checked on 1% agarose gel electrophoresis and the concentration and purity of the obtained DNA were determined by Nano Drop 2000 UV-Vis spectrophotometer (Thermo Scientific, Wilmington, USA). The V3–V4 hypervariable regions of the bacteria 16s rRNA gene were amplified by an ABI GeneAmp® 9700 PCR thermocycler (ABI,CA,USA) with primers 338F (5'-ACTCCT ACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT -3'). After PCR amplification and purification, PCR products were quantified and homogenized using a Picogreen dye fluorometer, and sequenced on Illumina MiSeq PE300 platform/NovaSeq PE250 platform (Illumina, San Diego, USA). The obtained data were analyzed on the cloud platform of Majorbio. (Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China).

Statistical Analyses

All experimental data were analyzed and visualized using GraphPad Prism version 9.20 for Windows (GraphPad Software, San Diego, CA, USA.) and presented as the mean \pm standard error of the mean. Statistical significance of differences was assessed with Student's t test for two groups or one-way ANOVA for multiple groups. A p-value of <0.05 was considered statistically significant.

Results

B2 KO mice expressed more severe clinical symptoms

Drinking water added with DSS induced colitis in mice, resembling the pathogenesis of human UC. We evaluated clinical symptoms of colitis in b2 KO mice and wild mice. After 7 days 3% DSS treatment, both groups of mice suffered severe colonic inflammation and the body weight of mice in both DSS treatment groups decreased at the end of the experiment (Fig. 1A, B). We observed that mice in b2KODSS group began to lose weight from day 3 and the loss in the next few days was greater than the DSS group ($p < 0.01$) (Fig. 1C). Colon length and DAI score were important indicators of the severity of colon inflammation. Compared with DSS group, the b2KODSS group mice had significantly shorter colons ($p < 0.001$) and higher DAI score ($p < 0.01$) (Fig. 1D, E, F). Then we performed pathological analysis of the colon tissue and found that the colonic mucosa, submucosa, muscular layer and outer membrane of the two control groups were intact. However, the colonic tissue sections of the two experimental groups treated with DSS exhibited crypt distortion, goblet cells loss,

inflammatory cell infiltration and severe mucosal damage and b2KODSS group had more severe colonic injury($p < 0.05$) (Fig. 1G, H). These results suggested that the clinical manifestations of DSS-induced colitis in mice were more severe when Mrgprb2 was deficient.

Fig.1 Lack of Mrgprb2 aggravated symptoms of DSS-induced colitis in mice. (A) The diagram of the animal experimental design. (B) Daily mouse body weight. (C) Loss of body weight. (D) DAI score. (E, F) Colon length. (G) Representative H&E images of colon sections. (a) control group, (b) DSS group, (c) b2KO group, (d) b2KODSS group. Scale bar, 100 μ m. (H) Histological scores. All data are expressed as mean \pm SD (n = 8 mice/group). Statistical significance is indicated as follows: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

B2 KO mice recruited fewer inflammatory cells and exhibited attenuated MCs degranulation and cytokines expression

As Mrgprb2 is a major driver of the recruitment of inflammatory cells (Subramanian, Gupta, & Ali, 2016), we hypothesized that b2 KO mice had a reduced ability to recruit inflammatory cells to the site of injury. We tested the colonic mRNA expression levels of myeloperoxidase (MPO) and found that MPO expression increased in both DSS treatment groups while the expression of MPO in b2KODSS group was lower than that in DSS group ($P < 0.01$) (Fig. 2A). In addition, the levels of GM-CSF, CCL2 and CCL3 in b2KODSS group were also lower than DSS group ($p < 0.05$) (Fig. 2B), which might suggest that b2 KO mice recruited fewer inflammatory cells. Histamine levels in all four groups were analyzed to measure the mast cell activation. The results showed the level of histamine in b2KODSS group was lower than DSS group ($p < 0.05$) (Fig. 2C). Then we tested the levels of inflammatory factors in intestinal samples including TNF- α , IL-6, IL-1 β , IL-10. As shown in Figure 2D, the expressions of TNF- α , IL-6, IL-1 β , IL-10 in b2KODSS group were significantly lower than that in DSS group ($p < 0.001$). Mrgprb2 receptor also plays an important role in the process of the interaction between MCs and nerves (Green et al., 2019). We verified NGF and SP expression levels. The expression of NGF in b2KODSS group was reduced ($p < 0.001$) (Fig. 2E). SP level was no significant difference between the two groups (Fig. 2F).

Fig. 2 Mrgprb2 deficiency attenuates inflammatory cell infiltration, mast cell activation and cytokine secretion. (A) The colonic mRNA expression levels of MPO. (B) Elisa analysis of GM-CSF, CCL2 and CCL3. (C) Elisa analysis of histamine. (D) Elisa analysis of TNF- α , IL-6, IL-1 β , IL-10. (E) Elisa analysis of NGF. (F) Elisa analysis of SP. All data are expressed as mean \pm SD (n = 8 mice/group). Statistical significance is indicated as follows: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Damage to the colonic mucosal barrier was more serious in b2 KO mice

In the colon of UC patients, various factors lead to decreased synthesis of colonic mucin, especially mucin 2, and altered expression of tight junction (TJs) protein, which ultimately leads to impaired intestinal barrier function (D'Alessio et al., 2021; Kobayashi et al., 2020). Immunofluorescence examination showed that the expressions of MUC2 and occludin in b2KODSS group were weaker than that in DSS group (Fig. 3A, B). The mRNA expression level of JAM-A in DSS group was higher than b2KODSS group ($p < 0.05$) while the expression of ZO-1, E-cadherin, Claudin-3

were no significant difference between DSS group and b2KODSS group. Compared with b2KO group, mice in b2KODSS group exhibited strikingly enhanced expression of zona occludens (ZO-1), E-cadherin, Claudin-3(p<0.01). DSS group also had higher expression of ZO-1, E-cadherin than control group (p<0.05) (Fig. 3C).

Fig. 3 B2 KO mice expressed fewer mucins and TJs proteins. (A) Immunofluorescence analysis on MUC2 in colon sections. (a) control group, (b) DSS group, (c) b2KO group, (d) b2KODSS group, (e) Average fluorescence intensity of MUC2. Representative images are shown. Scale bar, 50µm. (B) Immunofluorescence analysis on occludin in colon sections. (a) control group, (b) DSS group, (c) b2KO group, (d) b2KODSS group, (e) Average fluorescence intensity of occludin. Representative images are shown. Scale bar, 50 µm. (C) The colonic mRNA expression levels of JAM-A, ZO-1, E-cadherin, Claudin-3. All data are expressed as mean ± SD (n = 8 mice/group). Statistical significance is indicated as follows: * p < 0.05, ** p < 0.01, and *** p < 0.001.

Community structure of the gut microbiota was significantly changed in b2 KO mice

Mrgprb2 contributes to host defense in mice and induces protective immunity against bacterial infection(Pundir et al., 2019). QSMs including CSPs are secreted by bacteria to signal population density, we found the mRNA expression levels of CSP-1 was higher in b2KODSS group than DSS group (p<0.01) (Fig. 4A). Previous studies had suggested that intestinal microbiota changes played an important role in the pathogenesis of IBD(Imhann et al., 2018; Sartor & Wu, 2017). In order to verify whether b2 KO had an effect on the diversity of intestinal microbes in mice, we conducted a 16sRNA study on the collected intestinal contents. As expected, the number of operational taxonomic units (OTUs) in both DSS treatment groups was significantly lower than that in both control groups and b2KODSS group had the lowest OTU level. The characteristics and common taxa of the four groups were determined by using Venn diagram. Venn diagram revealed that 180 OTUs coexisted in the four groups and the numbers of unique OTUs in the control group, DSS group, b2KO group and b2KODSS group were 14, 20, 40 and 12, respectively (Fig. 4B).

Fig. 4 B2 KO decreased the gut microbiota abundance. (A) The mRNA expression levels of CSP-1. (B) Venn diagram based on OTU level. All data are expressed as mean ± SD (n = 7 mice/group). Statistical significance is indicated as follows: * p < 0.05, ** p < 0.01, and *** p < 0.001.

The rarefaction curve of observed OTUs plateaued with the current sequencing indicated that the sequencing depth was sufficient enough to reflect the diversity information of the sample, and the sequencing result was credible (Fig. 5A). Then the alpha diversity of the samples was analyzed and according to the Sobs and Chao indices we found the community abundance of the two DSS treatment groups decreased compared with the two control groups. Shannon index, which reflects community diversity, declined in b2KODSS group indicating the species diversity of b2KODSS group was low (p < 0.05) (Fig. 5B).

Fig. 5 Rarefaction curves and alpha-diversity of gut microbiota. (A) Rarefaction curves based on the OTU level. (B) Sobs, Chao and Shannon indices of OTU level. All data are expressed as mean ± SD (n = 7 mice/group). Statistical significance is indicated as follows: * p < 0.05, ** p < 0.01, and *** p <

0.001.

The species abundance of each sample was counted at the phylum level, and the community composition was studied by drawing histogram (Figure 6A). According to the analysis results, a total of 8 phyla were detected, among which Bacteroidetes, Firmicutes, Proteobacteria and Actinobacteriota accounted for the largest proportion. Compared with the two control groups, the content of Proteobacteria in the samples of the two DSS treatment groups was significantly increased. Among the four groups, b2KODSS group had the lowest proportion of Firmicutes and the highest proportion of Proteobacteria (Figure 6B). The comparison between DSS group and b2KODSS group showed that the proportion of Proteobacteria, Actinobacteriota, and Desulfobacterota in the samples of b2KODSS group were higher (Figure 6C). At the genus level, the dominant bacteria in each group were norank_F_Muribaculaceae which had been shown to improve intestinal mucositis in mice (Liu et al., 2021) (Figure 6D). Norank_F_Muribaculaceae decreased in both DSS treatment groups and the reduction was more significantly in b2KODSS group. Another beneficial bacterium, Lactobacillus, were also strikingly reduced in both DSS treatment groups (Fig. 6E). The proportion of harmful bacteria escherichia-shigella was elevated in DSS group and b2KODSS group, among which b2KODSS group was higher (Fig. 6F).

Fig. 6 Knockout of Mrgprb2 changed the composition of gut microbiota. (A) Composition of microbiota in mice at the phylum level. (B) One-way anova analysis of microbiota in four group at the phylum level. (C) Differences of microbiota between DSS group and b2KODSS group by student's t-test at the phylum level. (D) Composition of microbiota in mice at the genus level. (E) One-way anova analysis of microbiota in four group at the genus level. (F) Differences of microbiota between DSS group and b2KODSS group by student's t-test at the genus level. All data are expressed as mean \pm SD (n = 7 mice/group). Statistical significance is indicated as follows: * p < 0.05, ** p < 0.01, and *** p < 0.001.

Beta diversity analysis is an inter-group comparative analysis of species diversity in different habitats or microbial communities to explore the similarity or difference in community composition among different groups. Hierarchical cluster analysis at OTU level showed that there were significant differences in microbial community structure between the two experimental groups and the two control groups (Fig. 7A), and the bacterial composition in the groups was similar. Principal coordinates analysis (PCoA) is the projection of sample distance matrix through different distance algorithms. PCoA analysis of four groups of samples at the OTU level showed that DSS treatment significantly altered the intestinal microbial structure and knockout of Mrgprb2 gene also affected intestinal microbiota (Fig. 7B, C).

Fig. 7 Beta diversity analysis of gut microbiota. (A) Hierarchical cluster analysis. (B) PCoA plot on OTU level based on the Bray-Curtis index.

Fig. 8 B2 KO aggravates DSS-induced colitis by exacerbating gut microbiota dysbiosis, mucosal barrier disruptions and immune system disorders.

Discussion

MCs are constitutively found in the gastrointestinal tract and thought to be associated with many intestinal diseases including UC, irritable bowel syndrome, and food allergies(Wouters et al., 2016). MRGPRX2/Mrgprb2 is a receptor specifically expressed on MCs except dorsal root ganglion cells and mediates degranulation of MCs(Redegeld, Yu, Kumari, Charles, & Blank, 2018). We constructed b2 KO mice models and treated them with DSS to induce colitis and found that the b2 KO mice showed more severe symptoms. MCs activation attenuated because of the lack of Mrgprb2. Cytokines and chemokines secreted by MCs reduced resulting in the less infiltration of phagocytic immune cells. On the other hand, the abundance and diversity of intestinal flora in b2 KO mice were declined, the growth of pathogenic bacteria increased while the proportion of beneficial bacteria decreased, and the severe destruction of intestinal barrier made it easier for pathogens to invade (Fig. 8). These results suggest that the presence of Mrgprb2 may have a protective effect on mice with colitis.

MCs are one of the key effector cells in the inflammatory process(Forsythe, 2019),and MRGPRX2/Mrgprb2 plays an important role in mast cell activation. The loss of Mrgprb2 on MCs lead to a reduction in its activation, fewer cytokines secretion and inflammatory cells recruitment. In model of colitis we found the expression of chemokines such as CCL2 and CCL3 and infiltration of neutrophils was decreased in b2 KO mice. Less histamine and TNF-a released from MCs activate dendritic cells weakly result in attenuated antigen presentation to T cells(Subramanian et al., 2016). Reduced infiltration and activation of innate and adaptive immune cells attenuates host resistance to pathogens. Mrgprb2 is also involved in the interaction between MCs and nerves. In our study, b2 KO mice showed reduced NGF secretion. The neuroprotective and neurotrophic properties of NGF are impaired under pathological conditions(Xu et al., 2019).

The inner mucus layer of human separates bacteria from coming in contact with the epithelial cells while active UC patients have a penetrable inner mucus layer allows bacteria to penetrate and reach the epithelium(Johansson et al., 2014). In patients with UC, the thickness of these mucous layers is reduced due to the loss of goblet cells and the goblet cell secretory response to microbial challenge attenuate(Birchenough, Johansson, Gustafsson, Bergstrom, & Hansson, 2015). TJs composed of claudins, ZO-1, members of the occludin family, and JAMs are intercellular adhesion complexes essential for epithelial and endothelial barrier function(Kurashima & Kiyono, 2017; Zeisel, Dhawan, & Baumert, 2019). In UC patients as well as in colitis mice model, epithelial JAM-A expression decreased and JAM-A deficiency led to a strikingly increased susceptibility to DSS colitis(Vetrano et al., 2008). Mucosal biopsy of patients with UC exhibited lower occludin levels than the controls and the decrease was correlated with declined levels of caspase 3 gene(Kuo et al., 2019).In our study, the expression of MUC2, JAM-A and occludin in b2 KO mice were decreased which means the gut barrier was damaged, making it easier for bacteria to invade.

In models of infection, b2 KO mice exhibited impaired bacterial clearance and displayed higher bacterial loads than WT mice while activation of Mrgprb2 sufficiently eliminated bacteria and reduced disease scores (Pundir et al., 2019). CSP-1 inhibits bacterial growth and prevents biofilm formation by inducing degranulation, TNF-a secretion, and reactive oxygen species and prostaglandin D2 generation in mouse peritoneal MCs via Mrgprb2(Pundir et al., 2019; Roy, Chompunud Na Ayudhya, Thapaliya, Deepak, & Ali, 2021). In our study, although the expression of CSP-1 was elevated in b2 KO mice, the lack of Mrgprb2 prevented it from mediating antibacterial action.

The microbial patterns among UC patients exhibit reduced microbial diversity, increased instability of the gut microbiota composition over time, decreased relative abundance of Firmicutes,

and an increase in Proteobacteria(Lee & Chang, 2021). Reduced gut microbial diversity may result in impaired integrity of the intestinal barrier and damaged regulation of the host immune system. Mucolytic bacteria and pathogenic bacteria are also on the rise, leading to degradation of the mucosal barrier, which allows pathogens to move further into intestinal tissues(Alipour et al., 2016; Chassaing & Darfeuille-Michaud, 2011; Ng et al., 2013). Our results showed that b2 KO mice induced colitis exhibited reduced microbial abundance and diversity. Compared with other three groups, the abundance of Firmicutes in b2KODSS group was significantly declined, and the abundance of Proteobacteria was elevated. At the genus level, the dominant bacteria in each group was norank_F_Muribaculaceae and in b2KODSS group the abundance of norank_F_Muribaculaceae was strikingly reduced. Some strains of norank_f_Muribaculaceae use intestinal mucus polysaccharides as growth nutrients and can inhibit the growth and colonization of pathogenic bacteria such as *Flavobacterium difficile* in the intestine by occupying ecological space(Pereira et al., 2020). Another probiotic in UC treatment, *Lactobacillus*, which are SCFAs generation bacteria related to a lower abundance of pathogenic bacteria and decreased inflammatory markers (Nascimento, Machado, Galvez, Cazarin, & Marostica Junior, 2020)was lower in both DSS treatment groups. *Escherichia-Shigella* is the typical genus of Proteobacteria and includes LPS-producing, Gram-negative bacteria. Its relative abundance in the IBD patients was higher than healthy people (Santoru et al., 2017)and there was a dramatically increased abundance of this classic pathogen in the b2KODSS group. Increased pathogenic bacteria are more likely to invade the damaged intestinal barrier and cause disease.

Conclusions

In this study we demonstrated b2 KO mice with colitis had more severe symptoms. *Mrgprb2* deletion mice had altered intestinal flora and more serious damage to the intestinal barrier. The reduced infiltration of inflammatory cells attenuated the body's ability to eliminate invading pathogens and ultimately led to more severe disease. Future studies are needed to verify the specific molecular mechanism of *Mrgprb2*.

Author Contributions

Conceptualization, Ming Shao; Data curation, Ming Shao and Yinzhi Deng; Formal analysis, Ming Shao; Funding acquisition, Hesheng Luo; Investigation, Ming Shao, Jingwen Liu and Fangting Yuan; Methodology, Ming Shao; Writing original draft, Ming Shao.

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Data Availability Statement

The 16S rRNA gene sequences were deposited in the GenBank Sequence Read Archive (SRA) database (ID PRJNA812349).

Conflicts of Interest

The authors declare no conflict of interest.

Ethics approval

Animal experiments were approved by the Animal Care and Ethics Committee of Wuhan University according to the legal requirements (Approval No. 20210303, 16 March 2021).

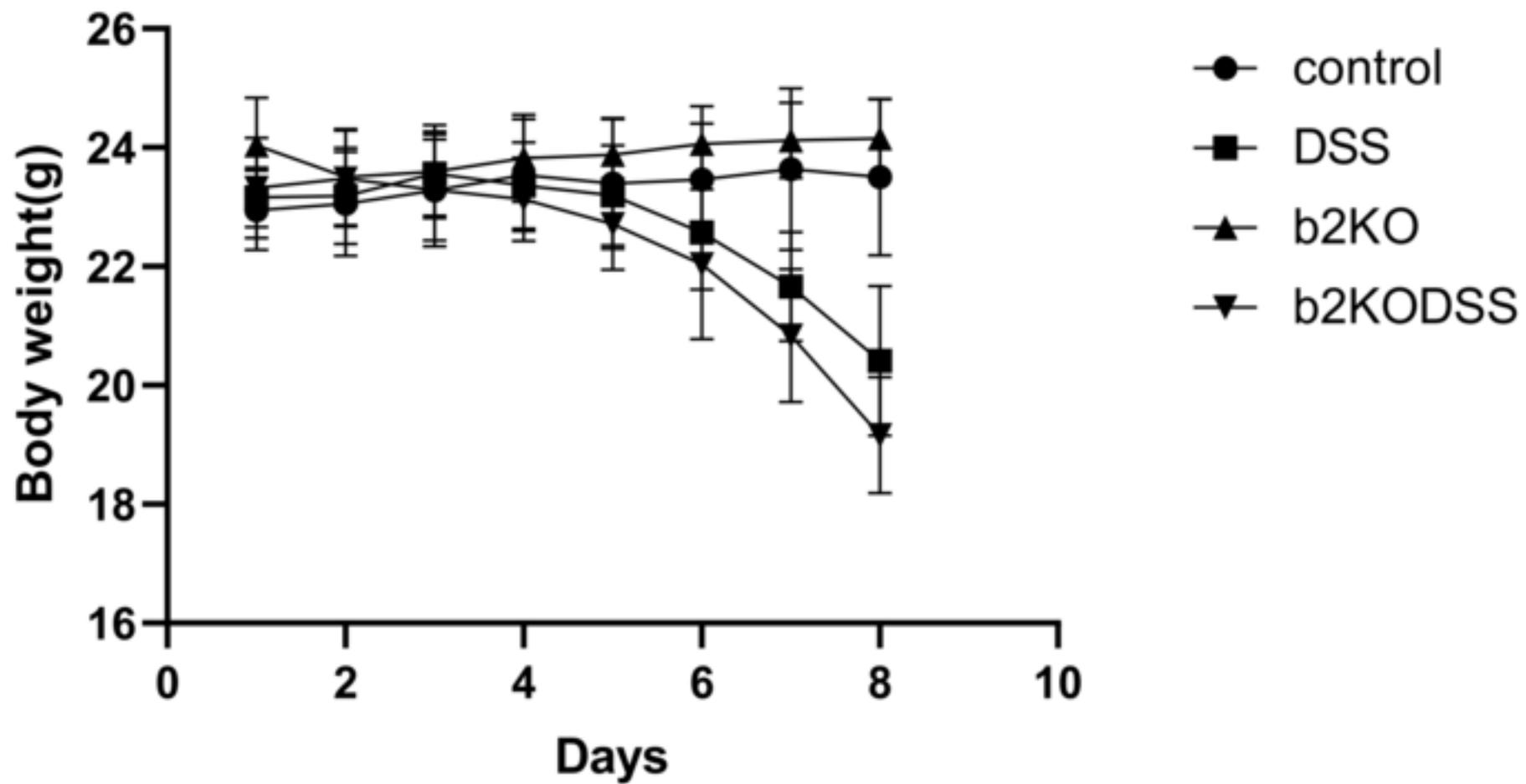
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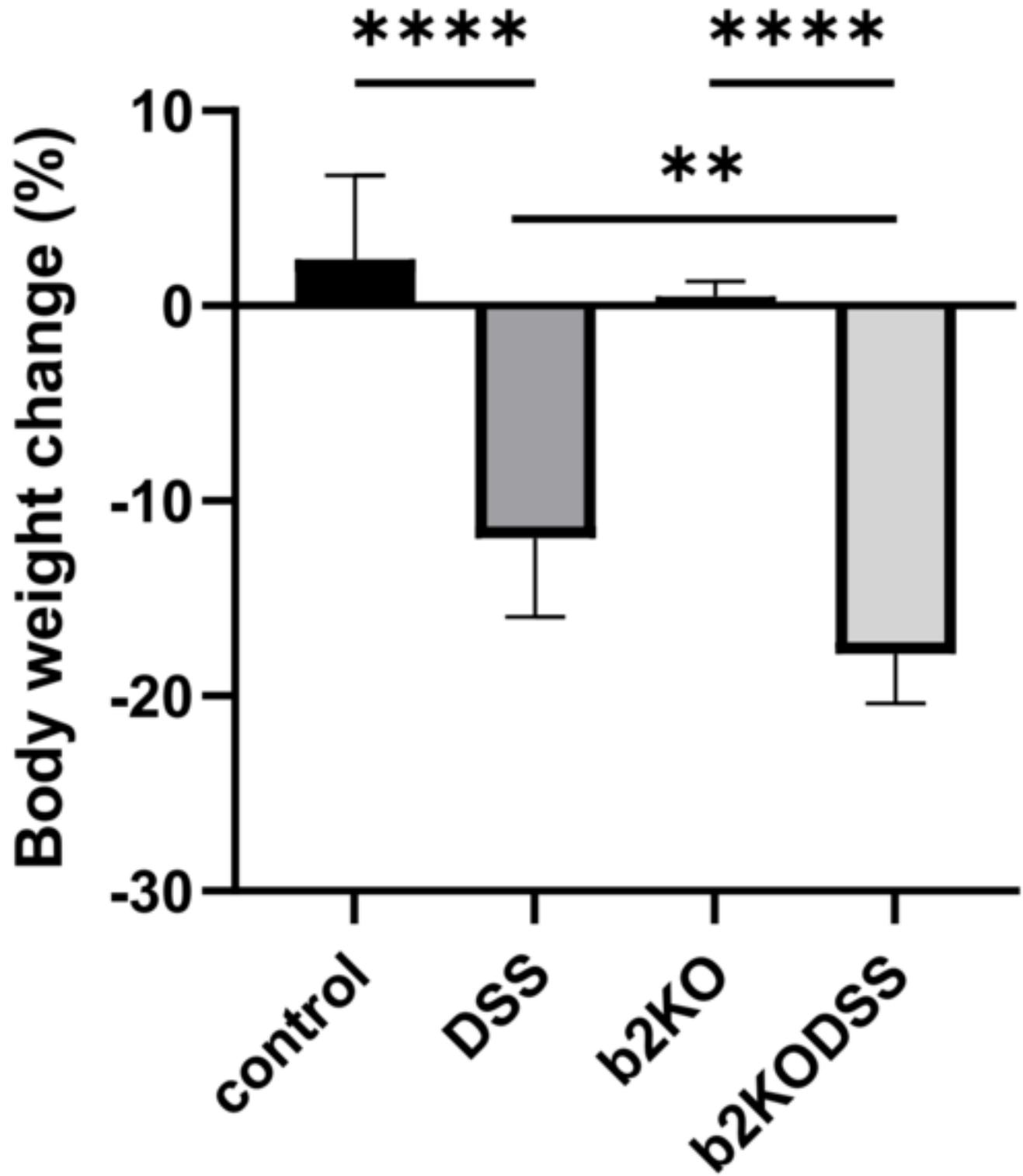
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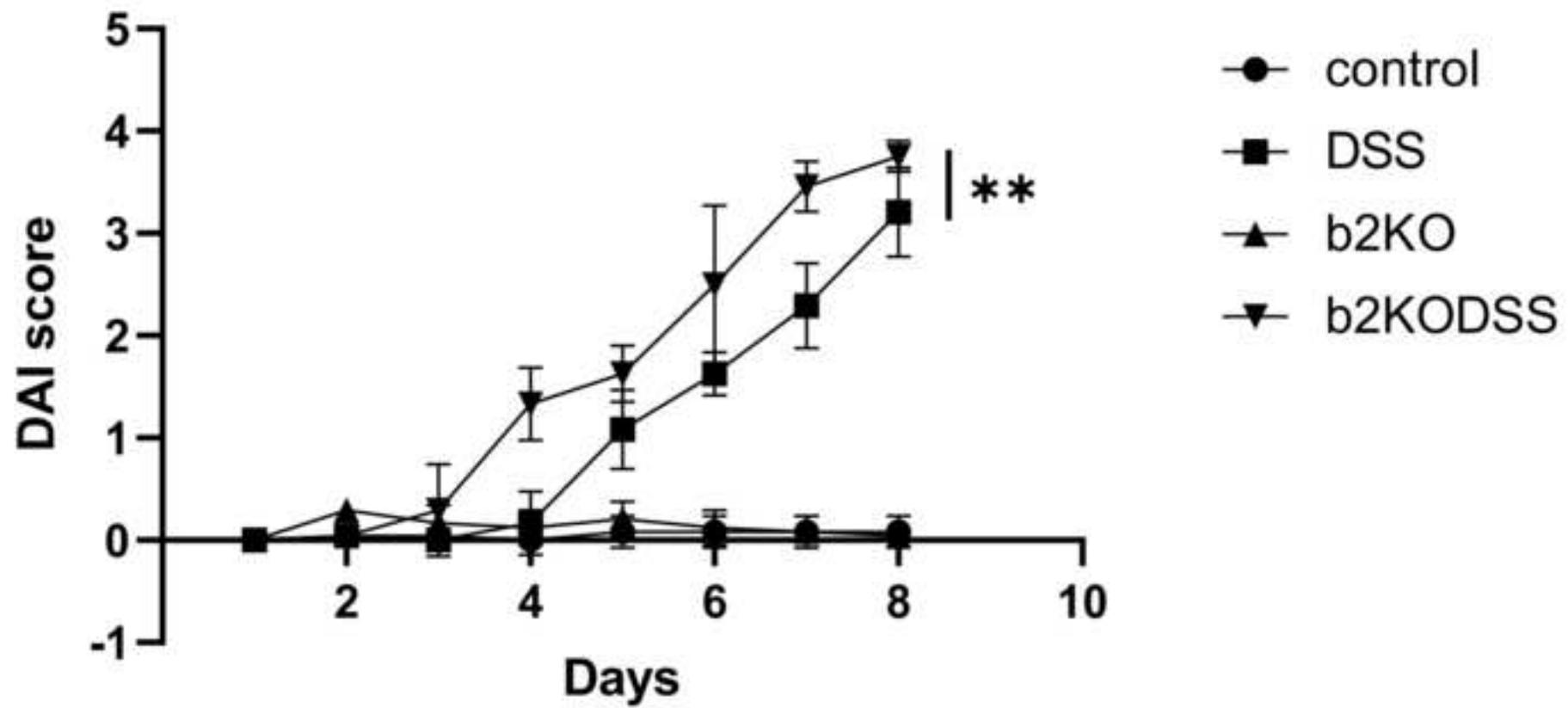
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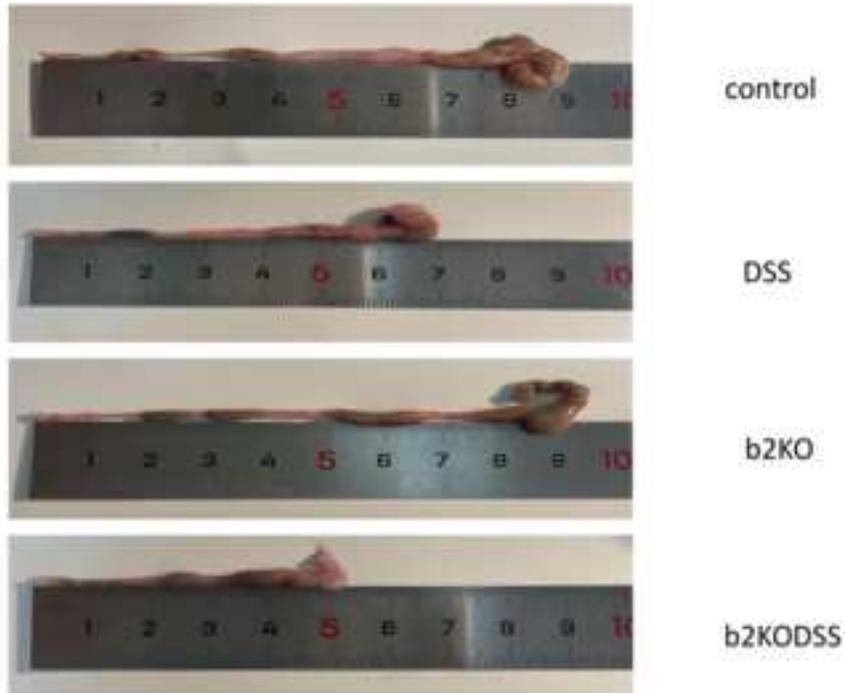
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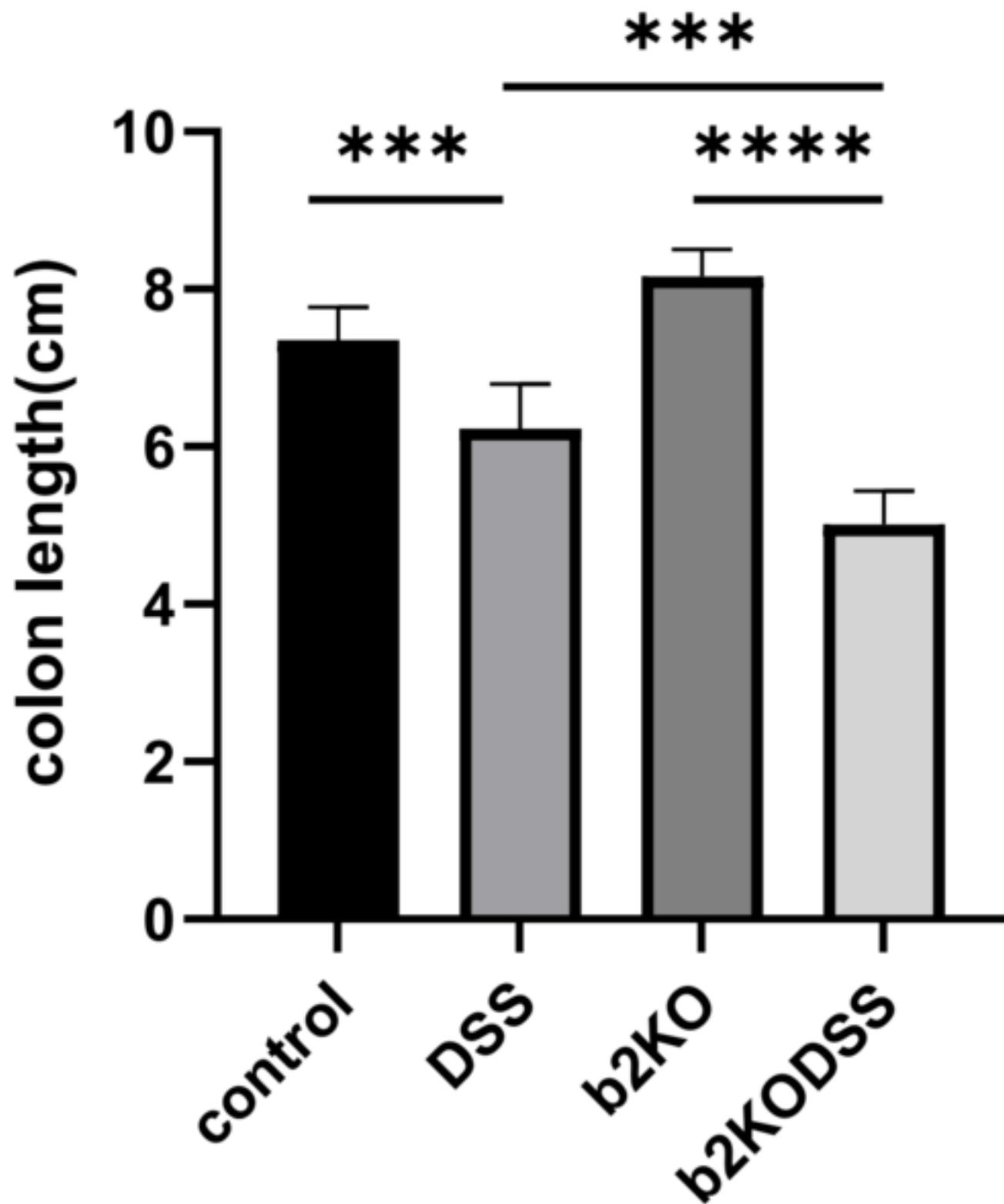
Groups	Day1-Day7	Day8
control	normal water	sacrifice
DSS	3%DSS	sacrifice
b2KO	normal water	sacrifice
b2KODSS	3%DSS	sacrifice

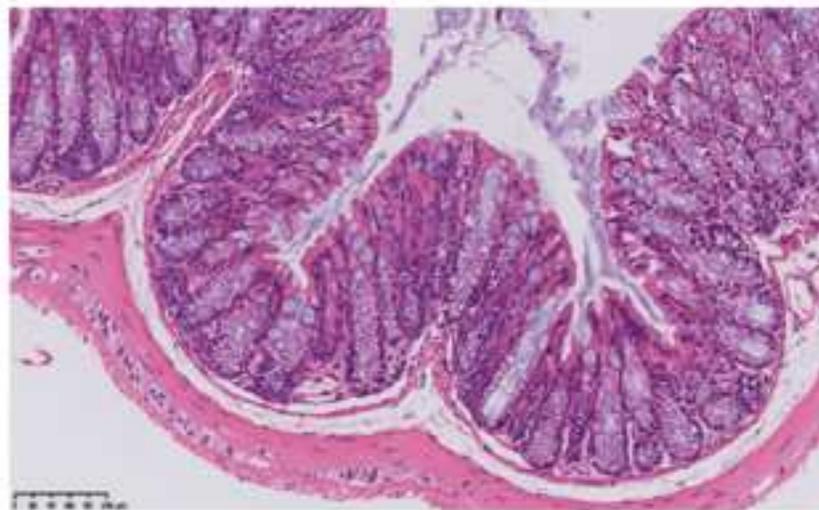


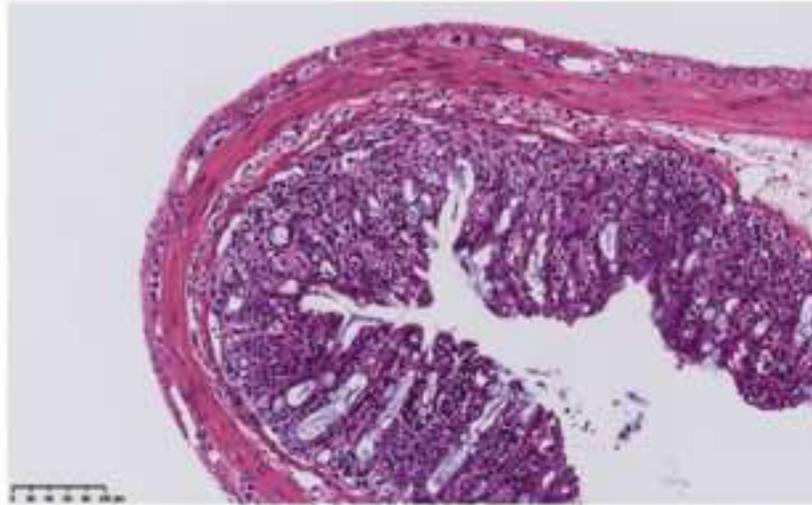




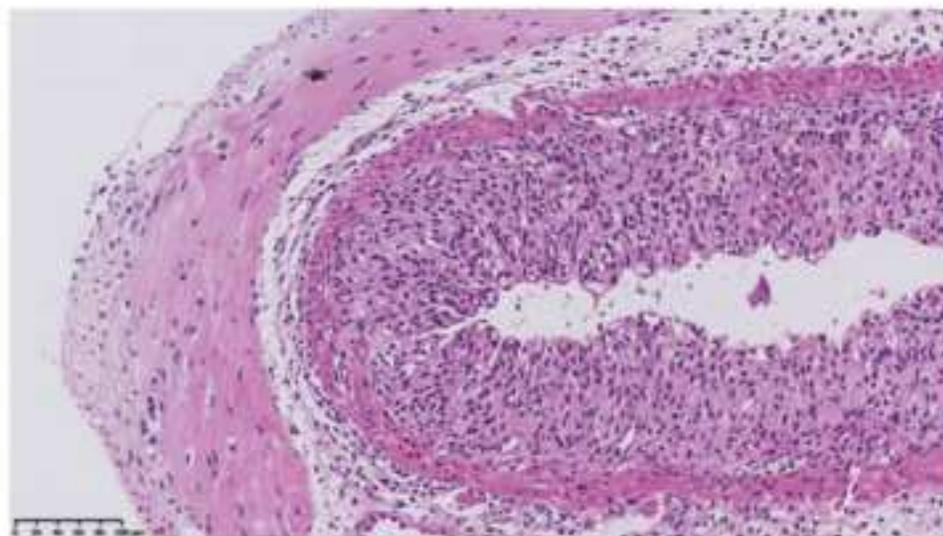


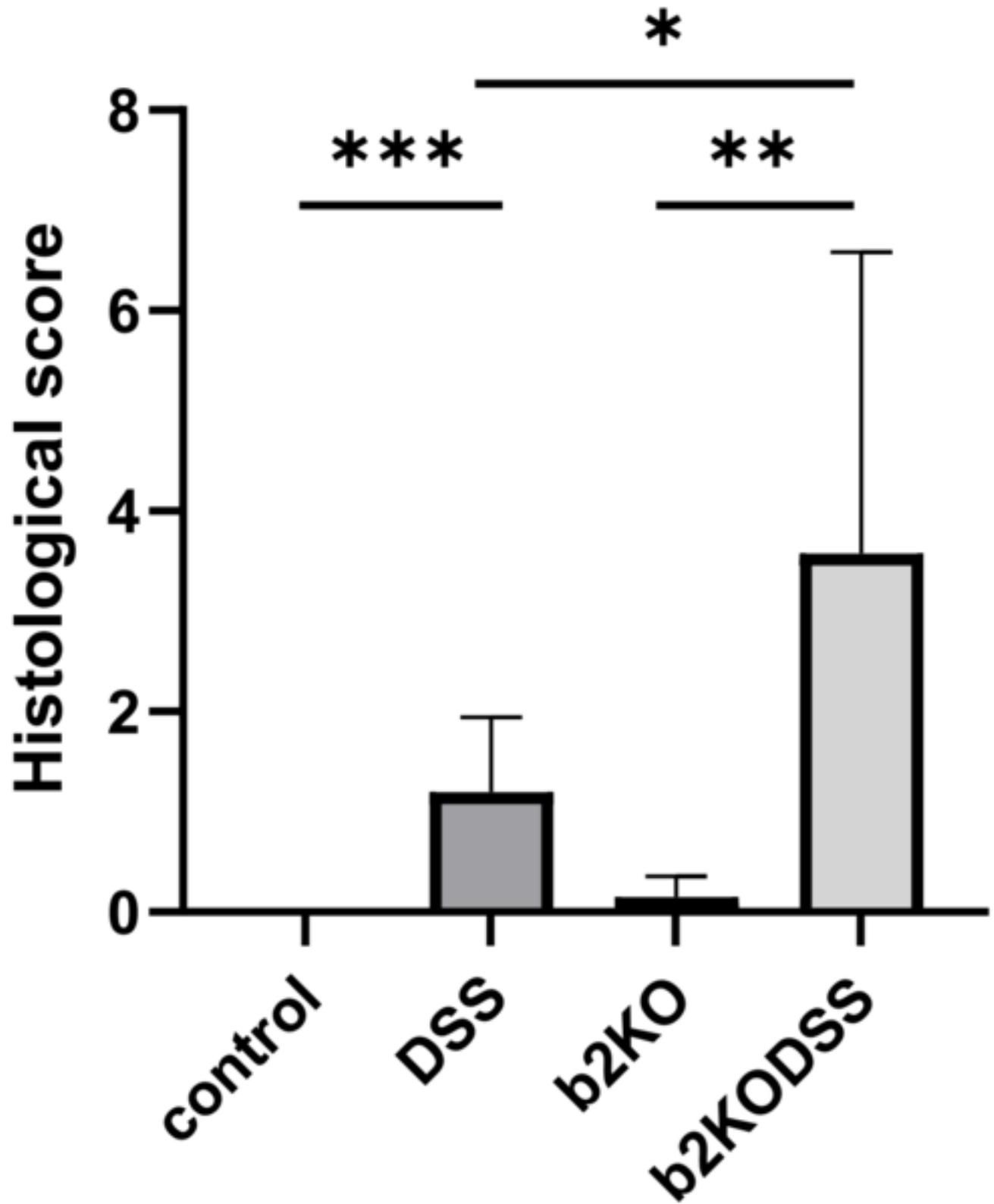


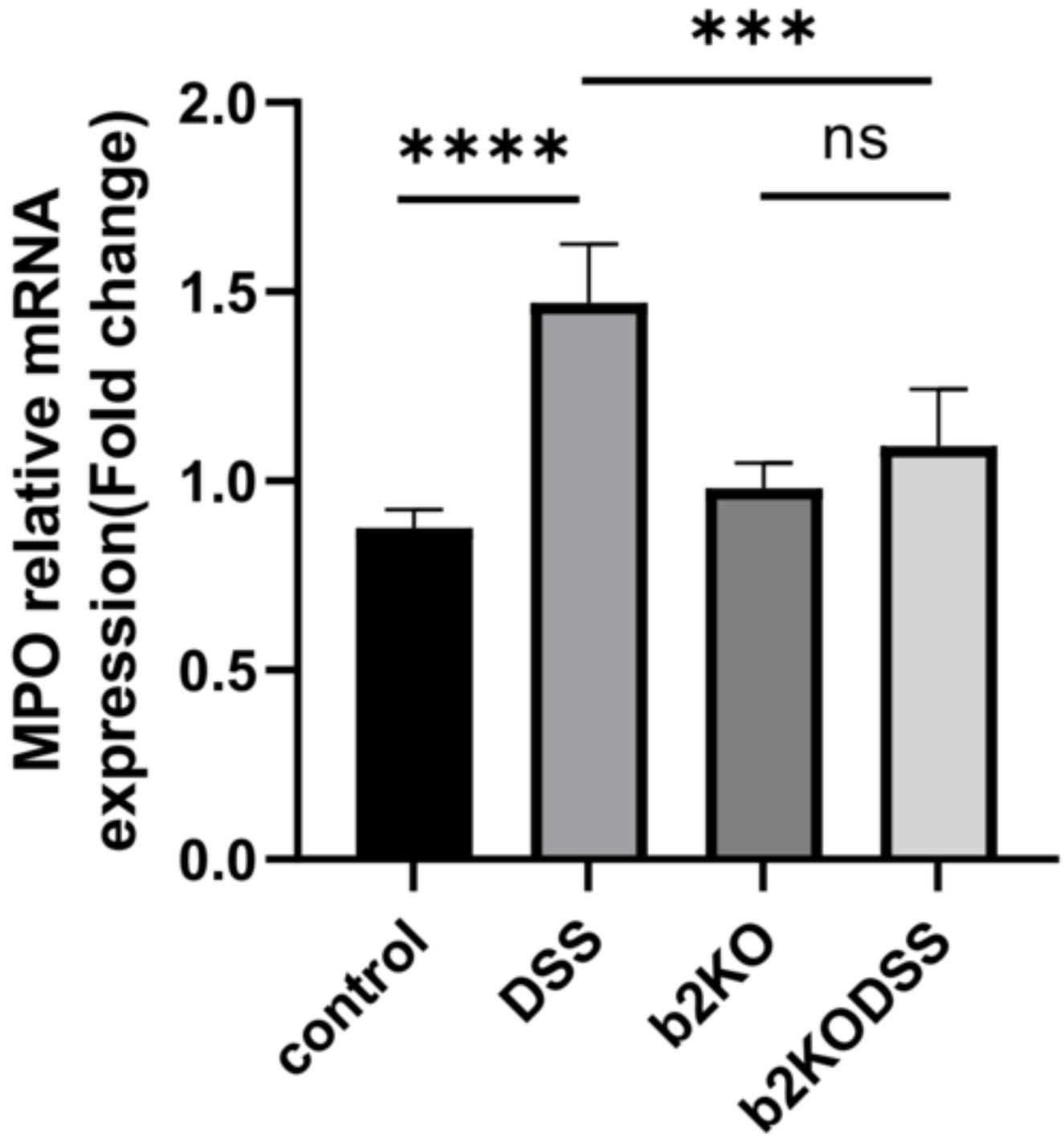


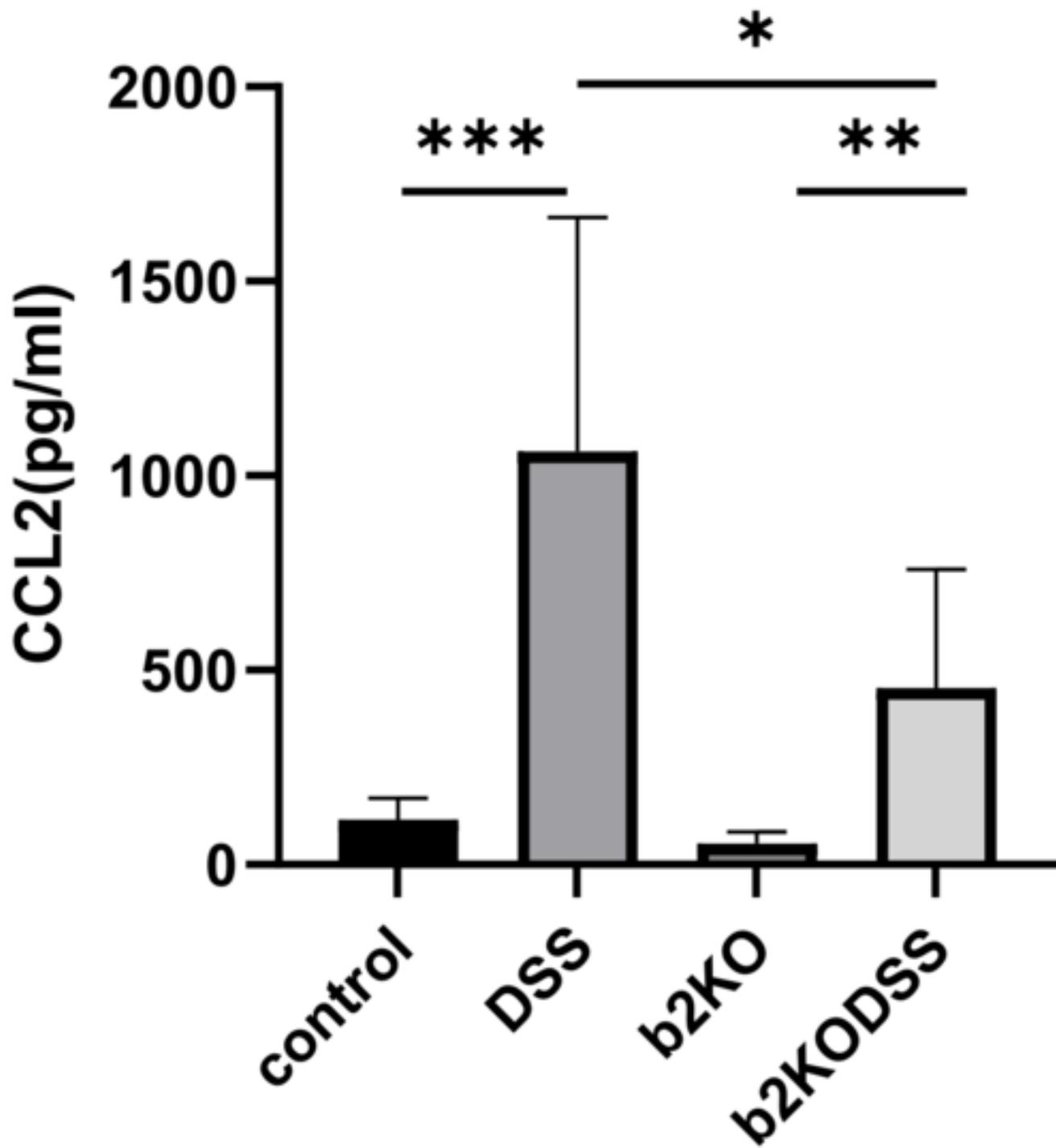


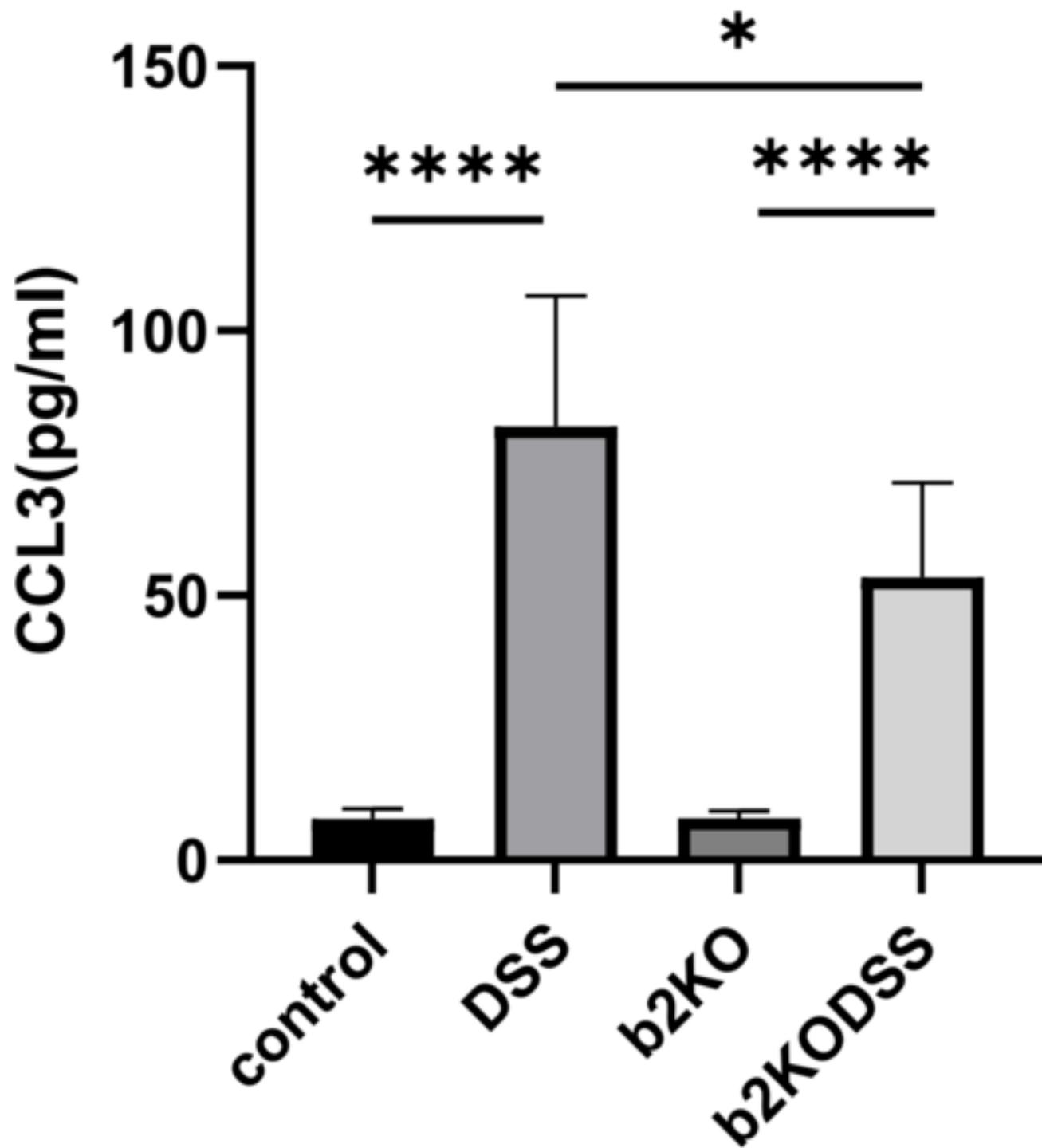


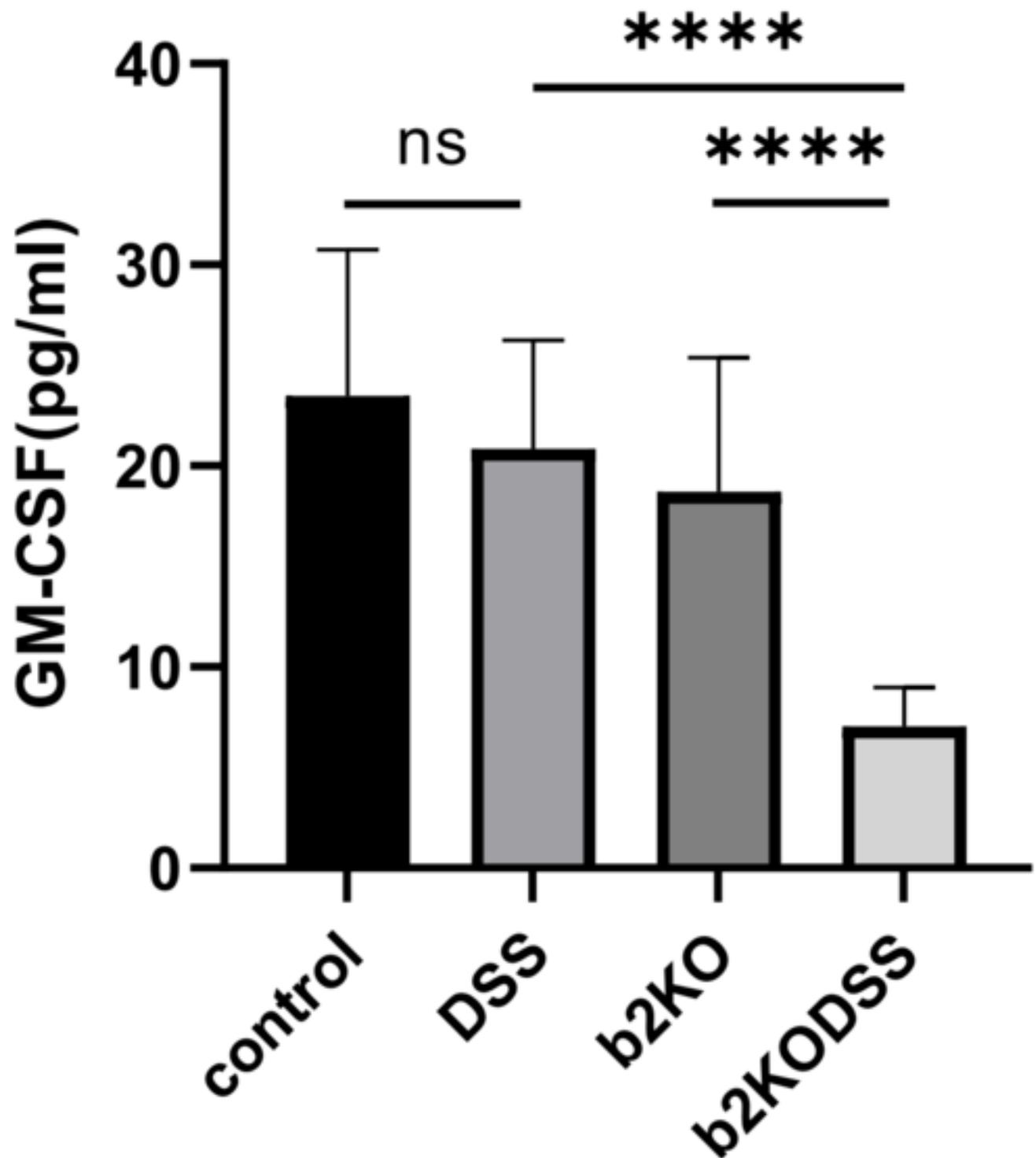


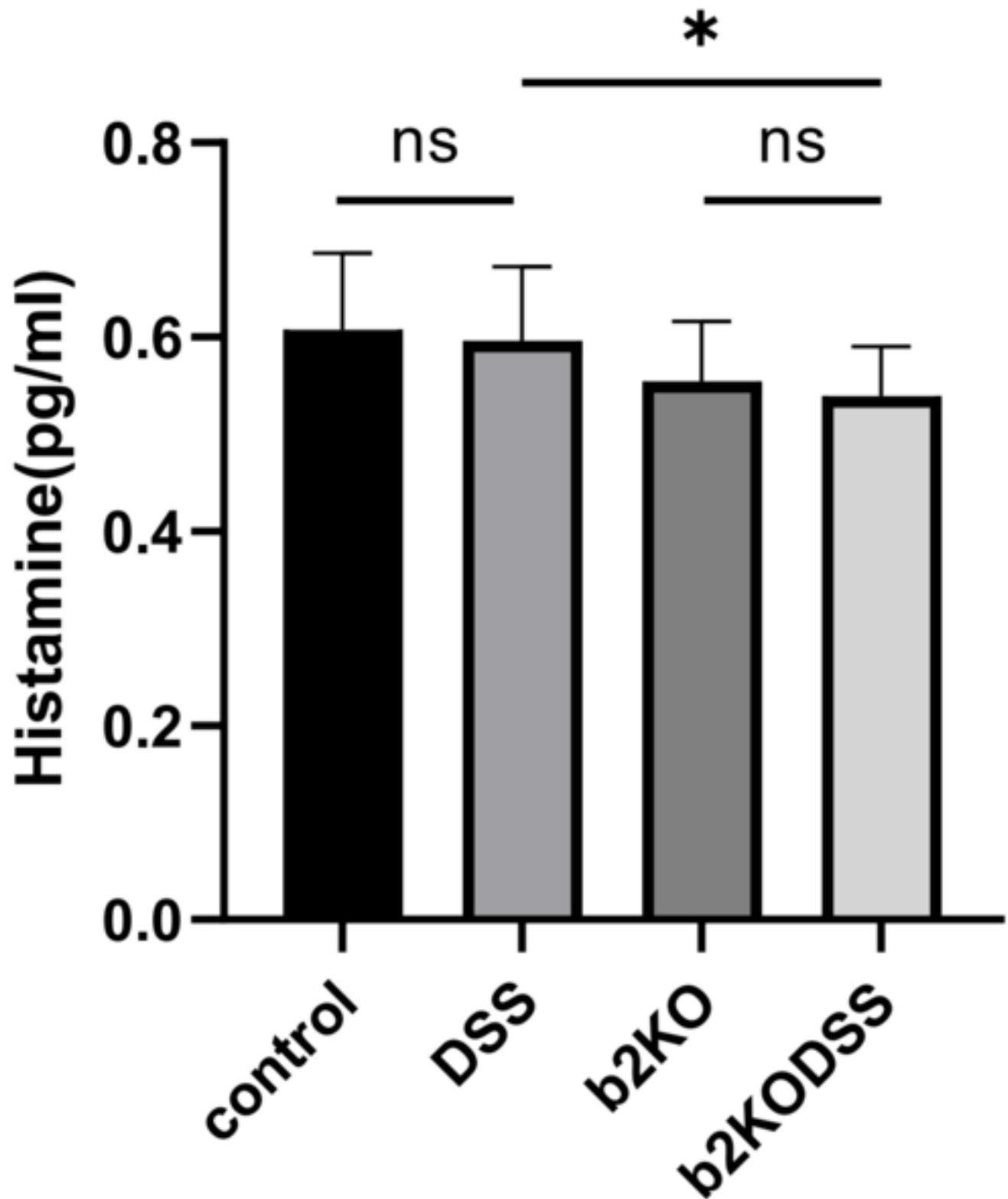


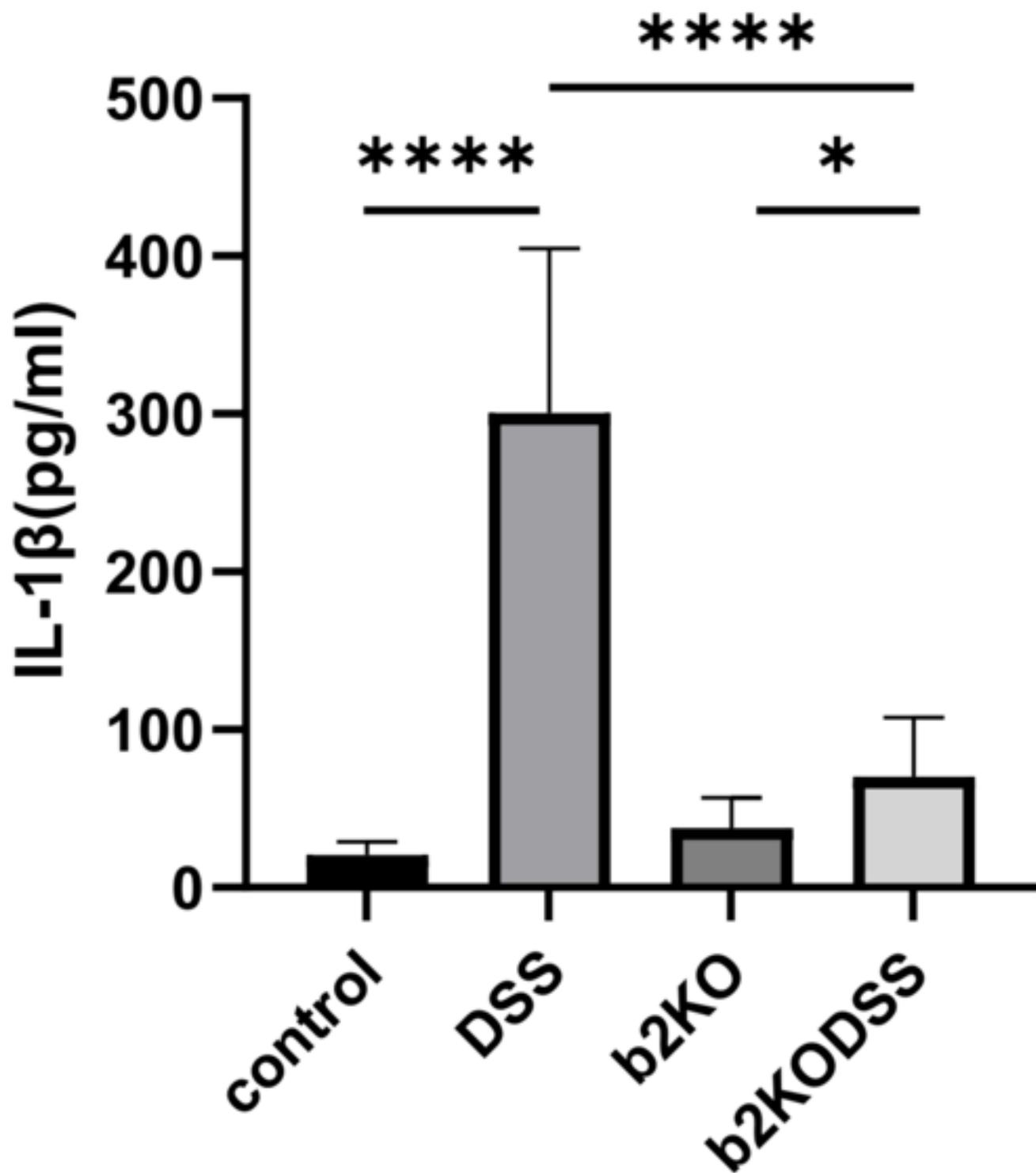


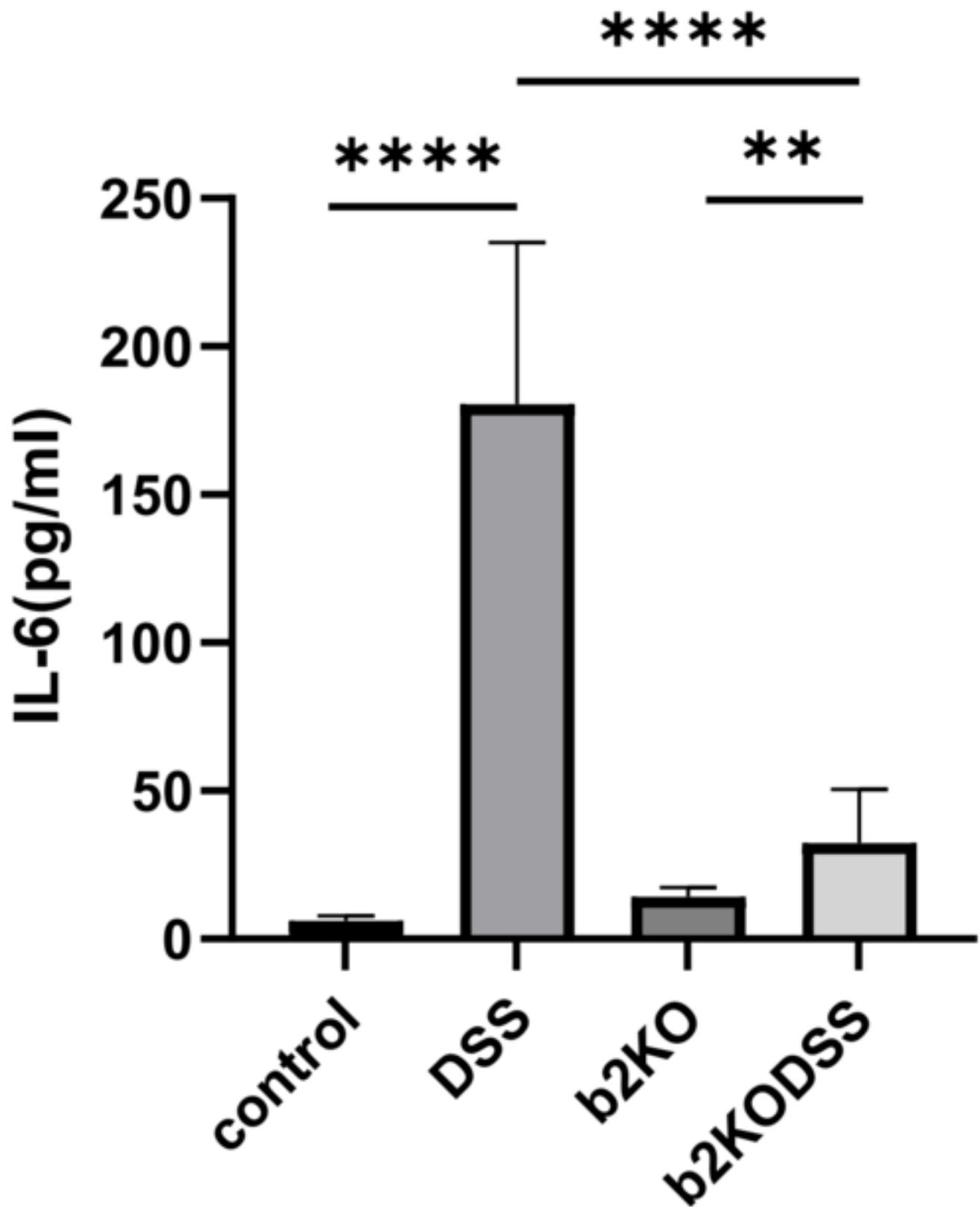


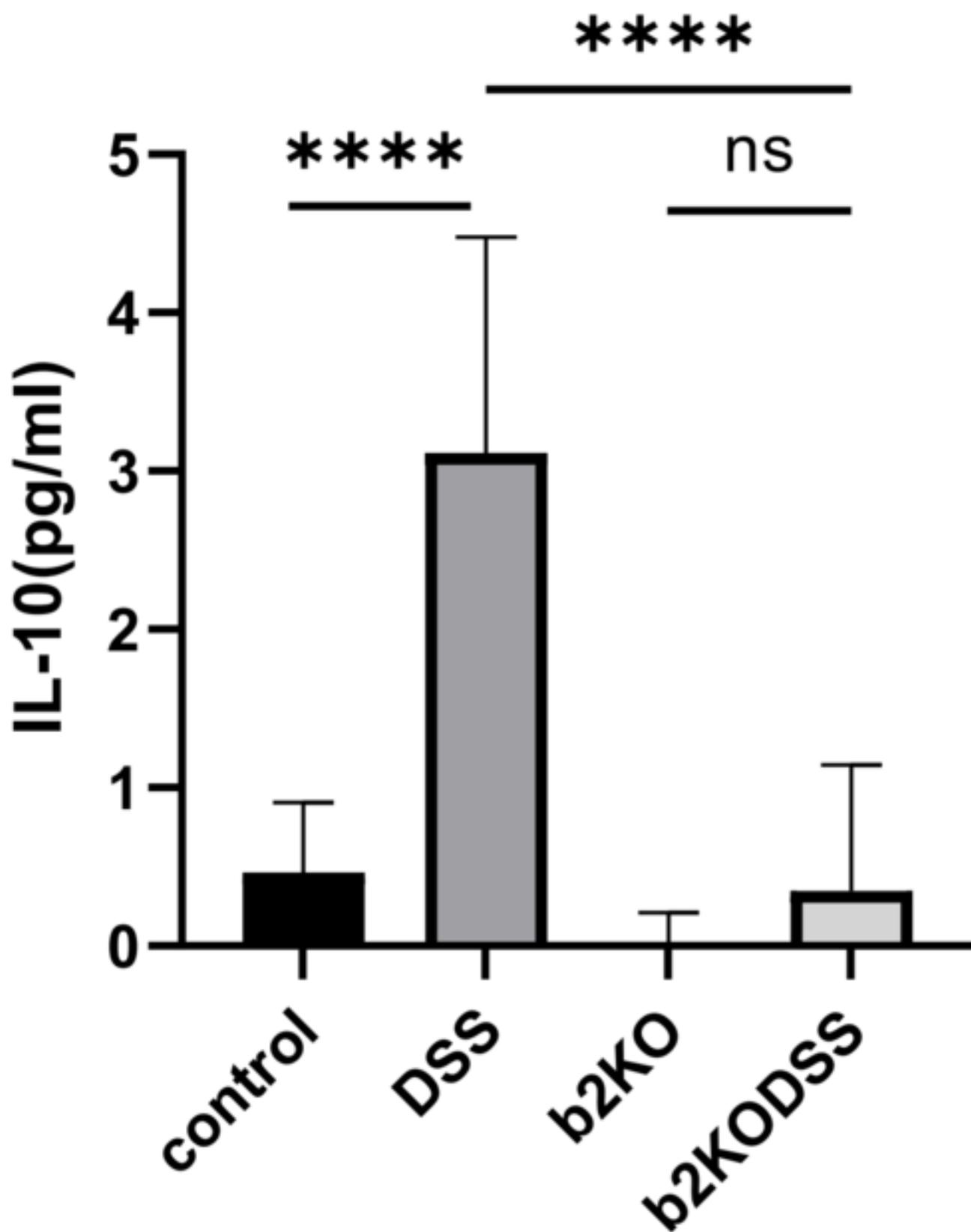


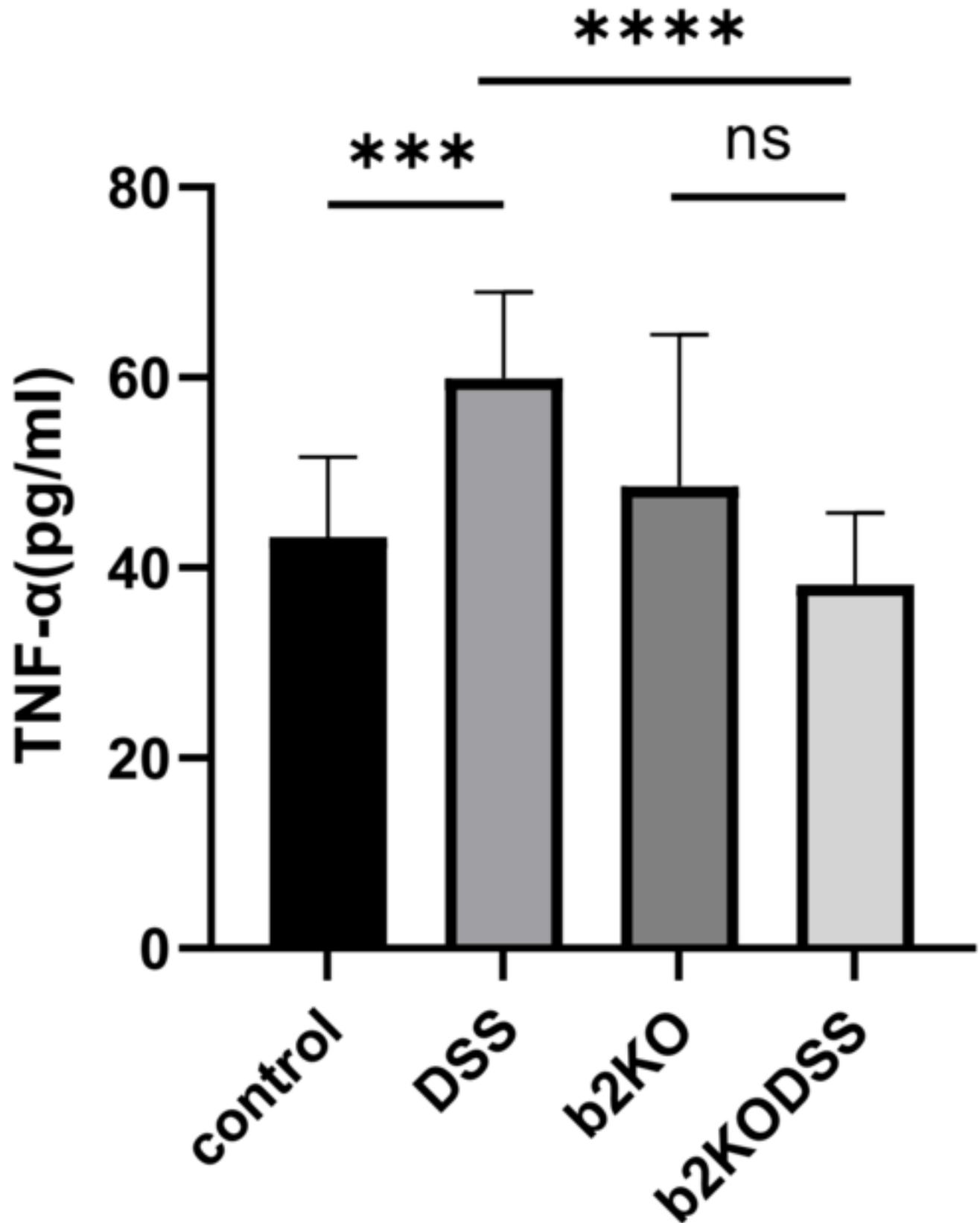


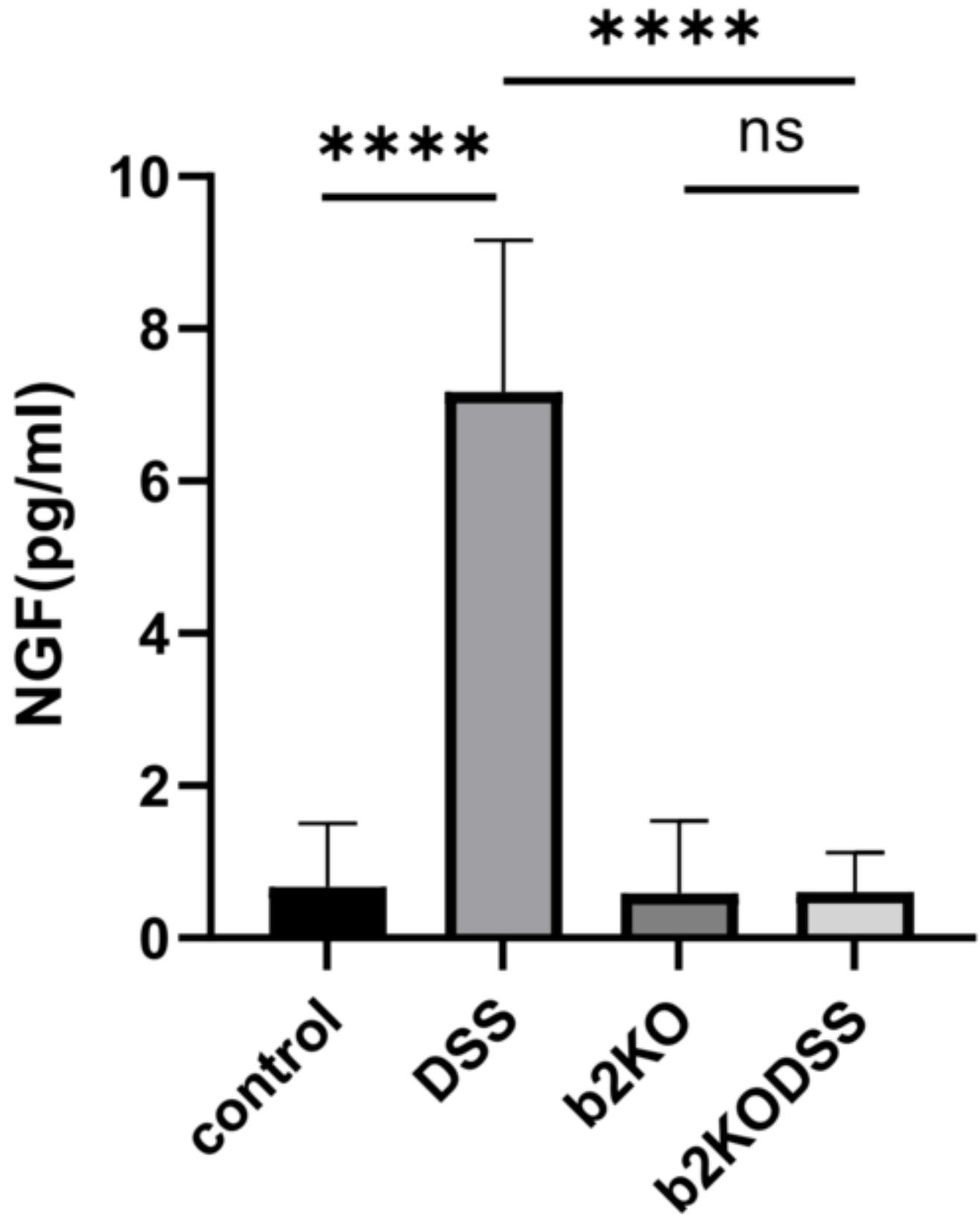


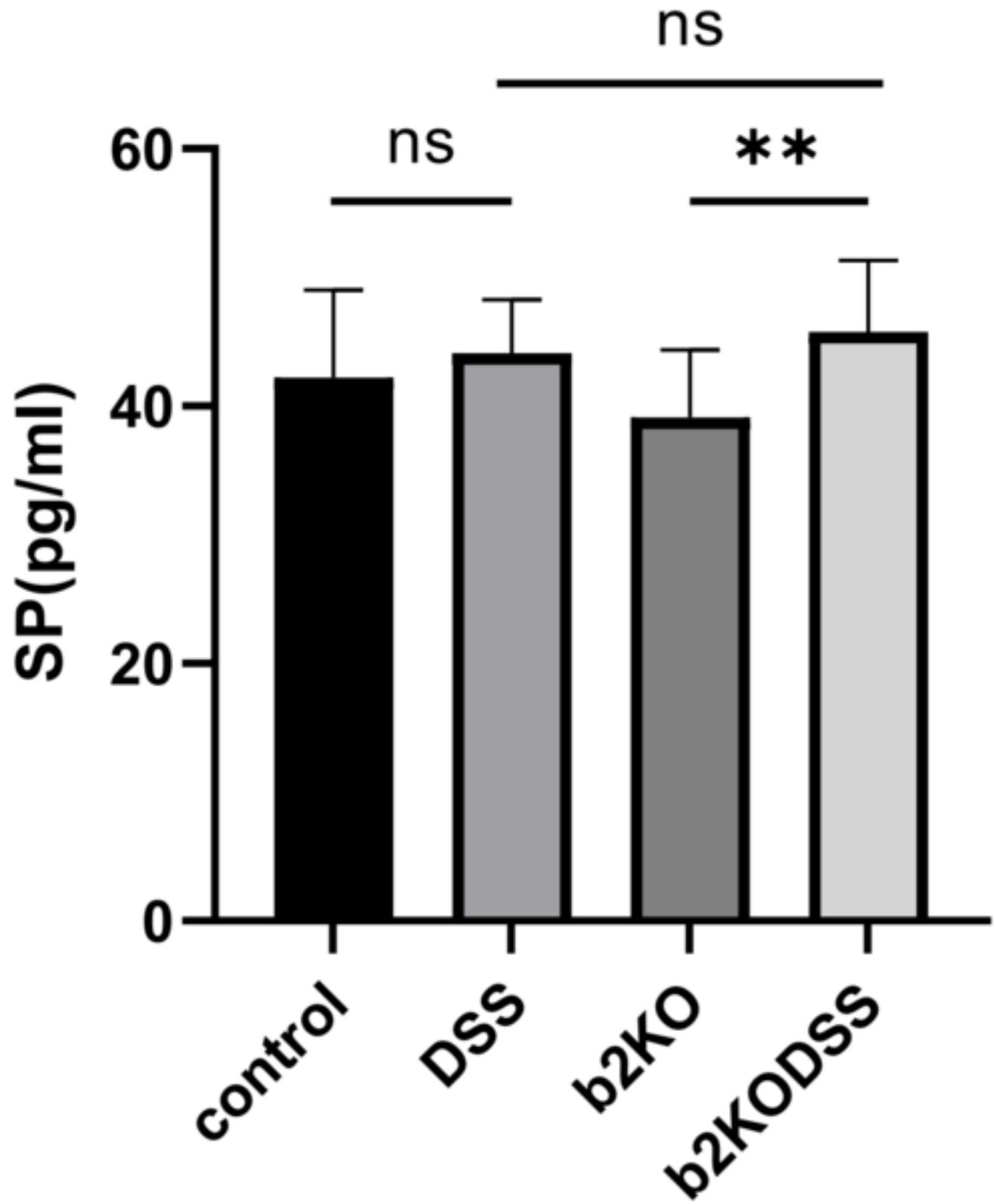


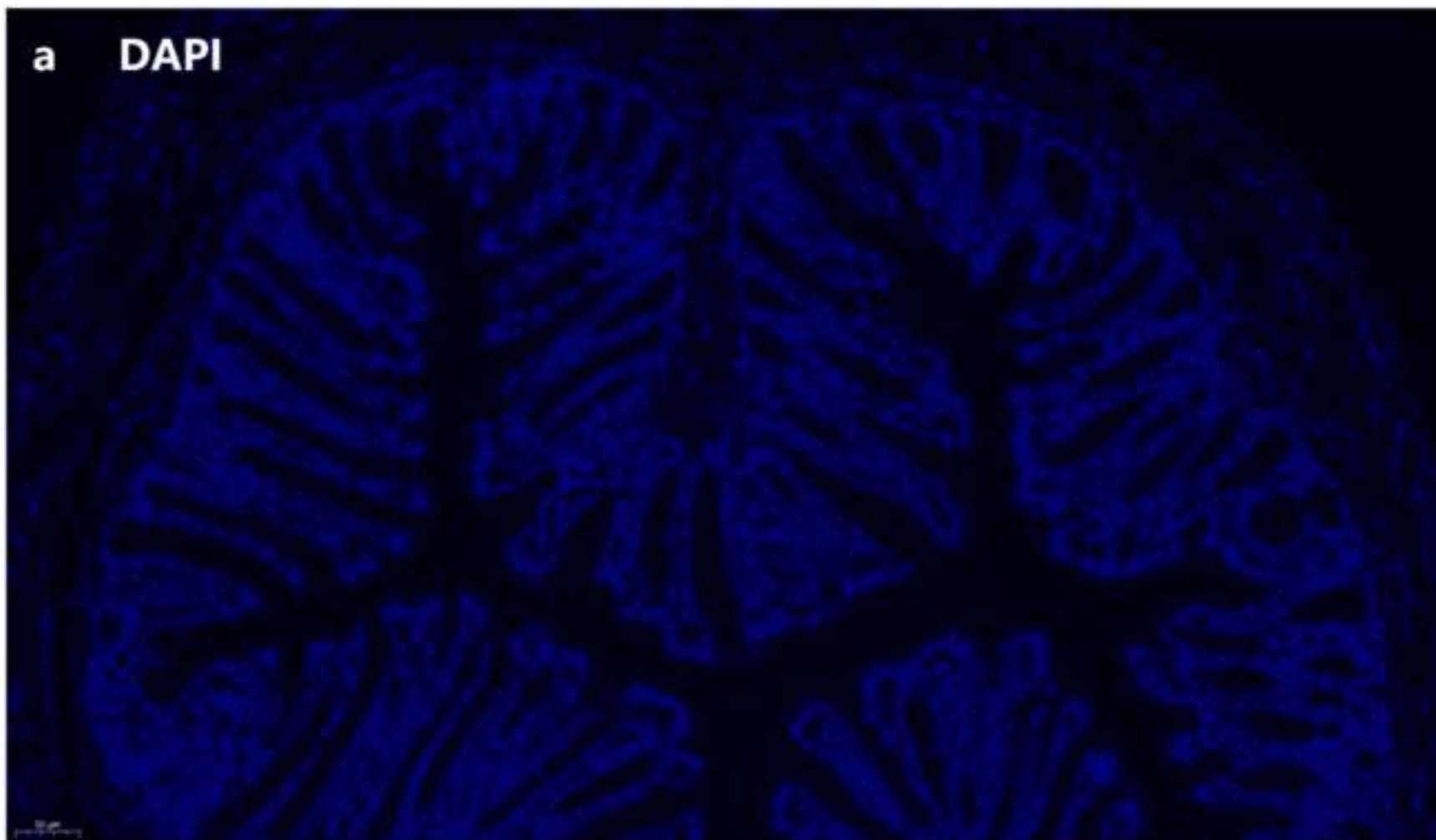


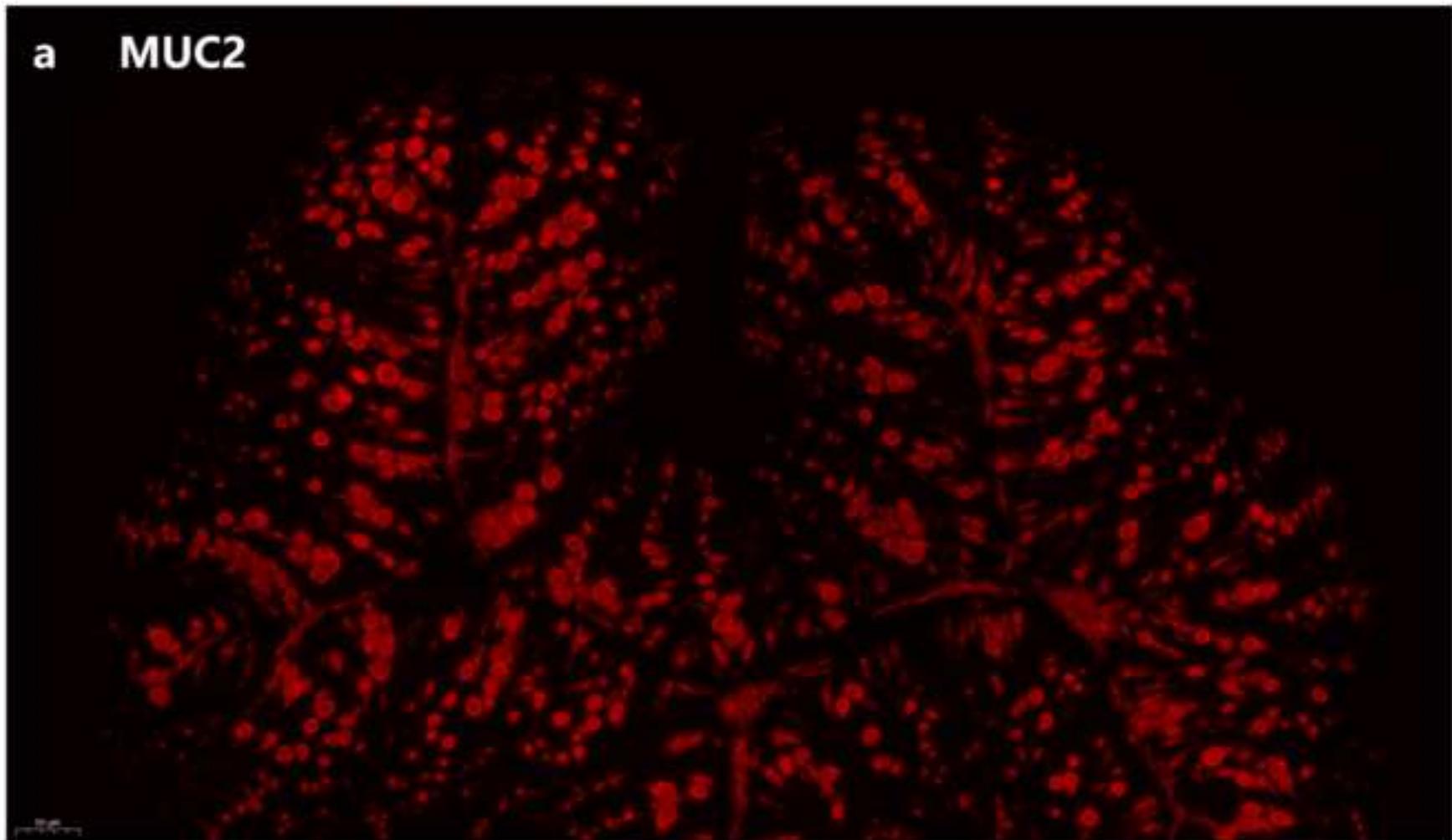


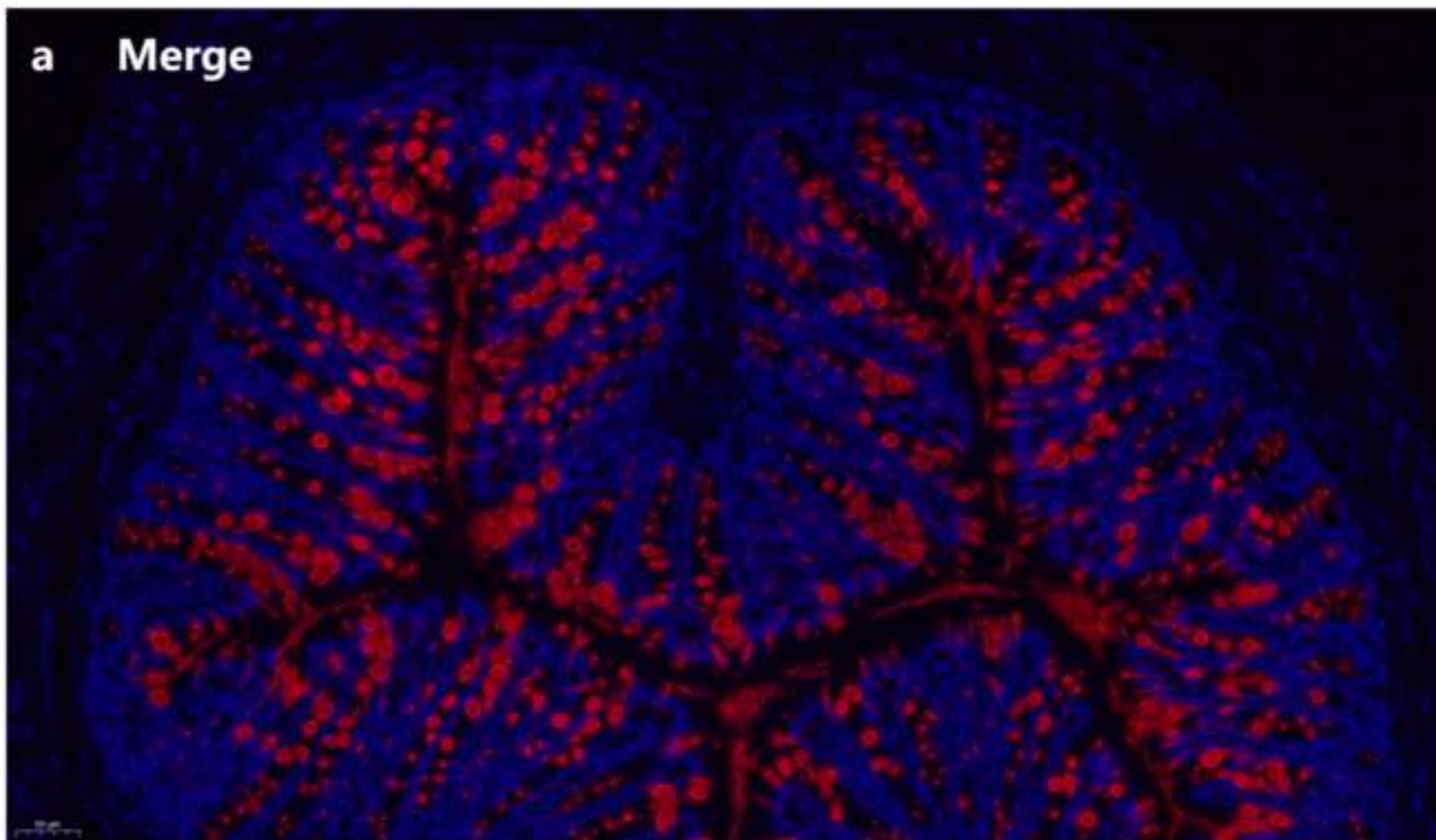


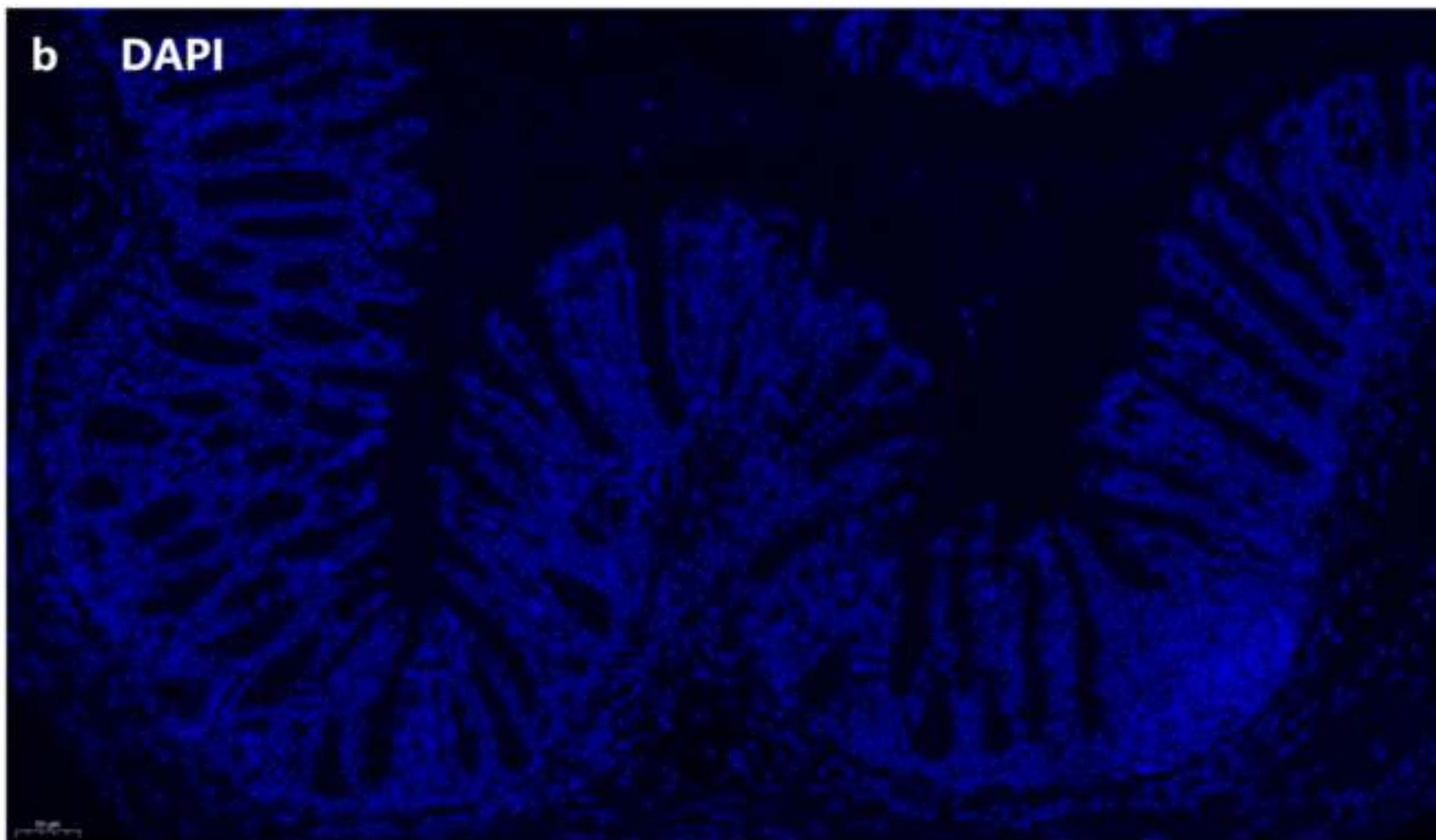


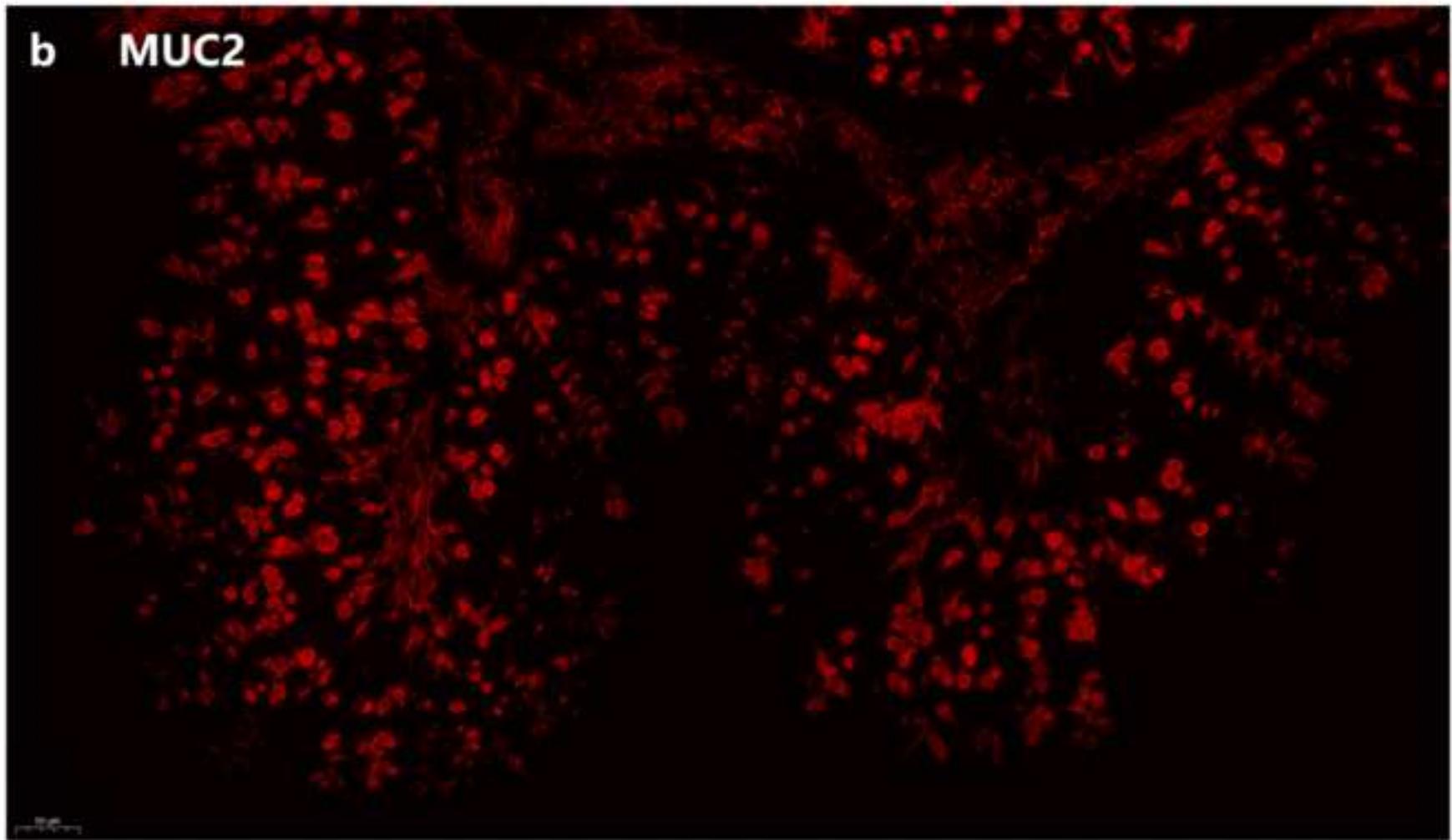


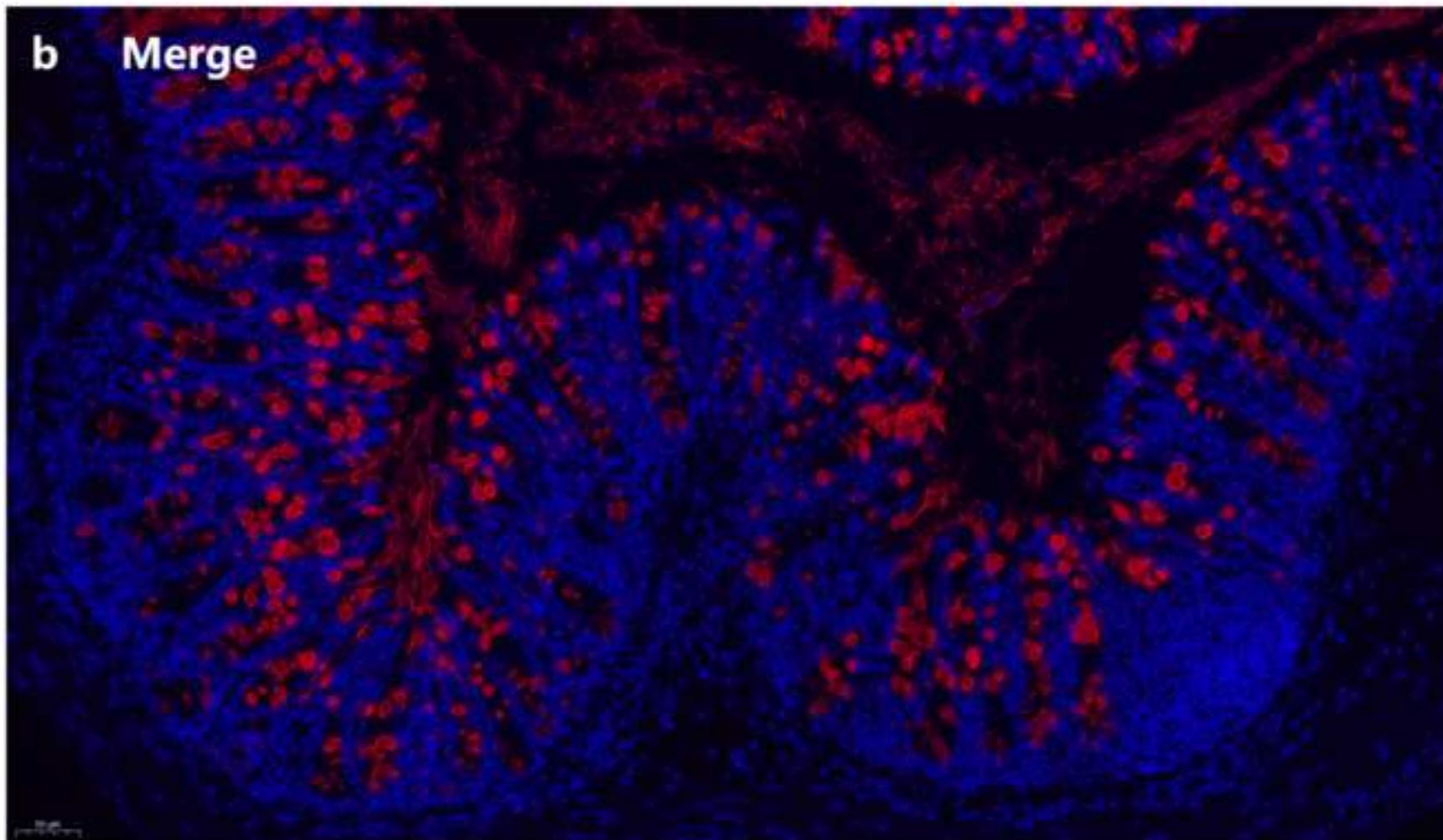


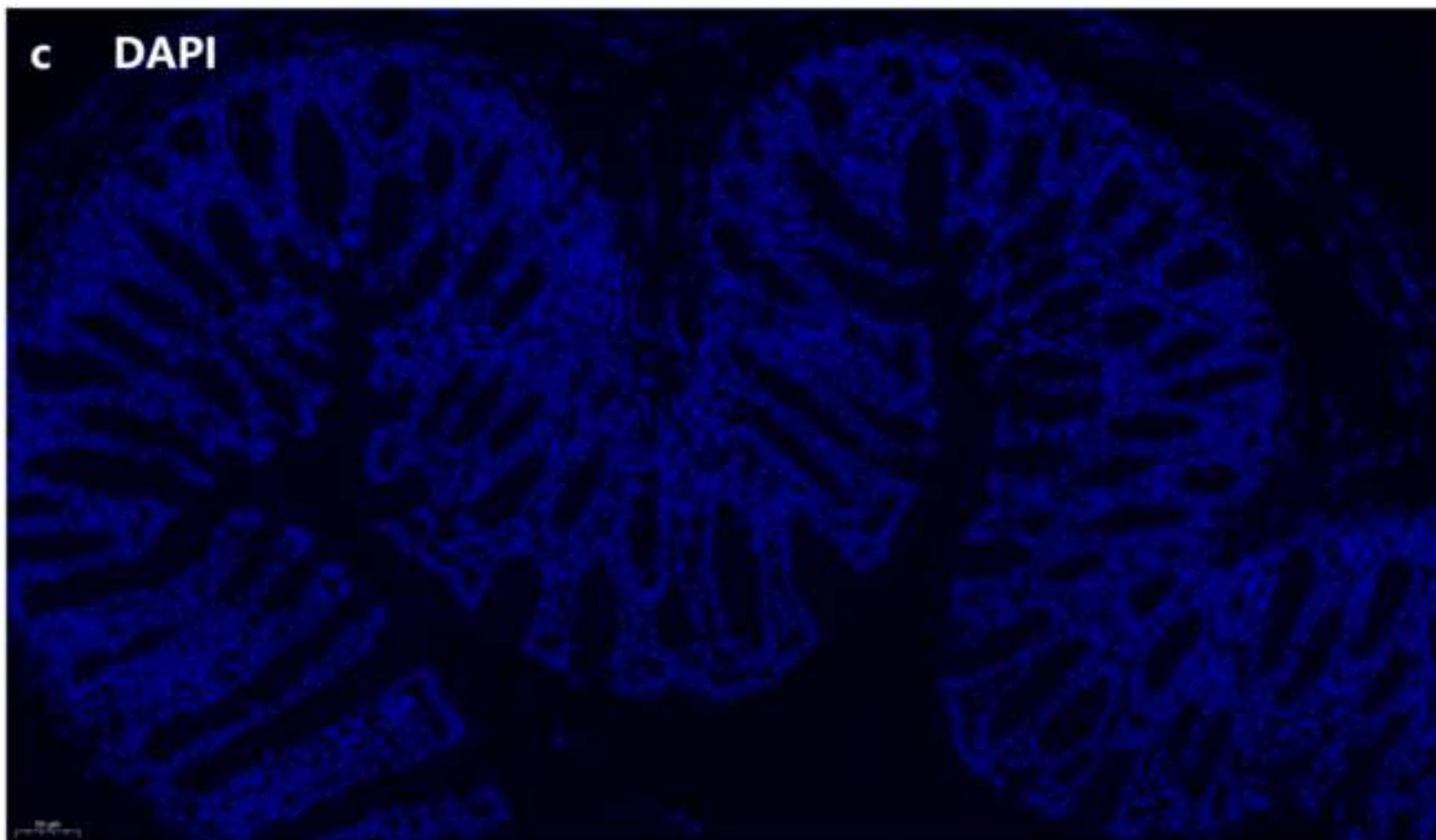


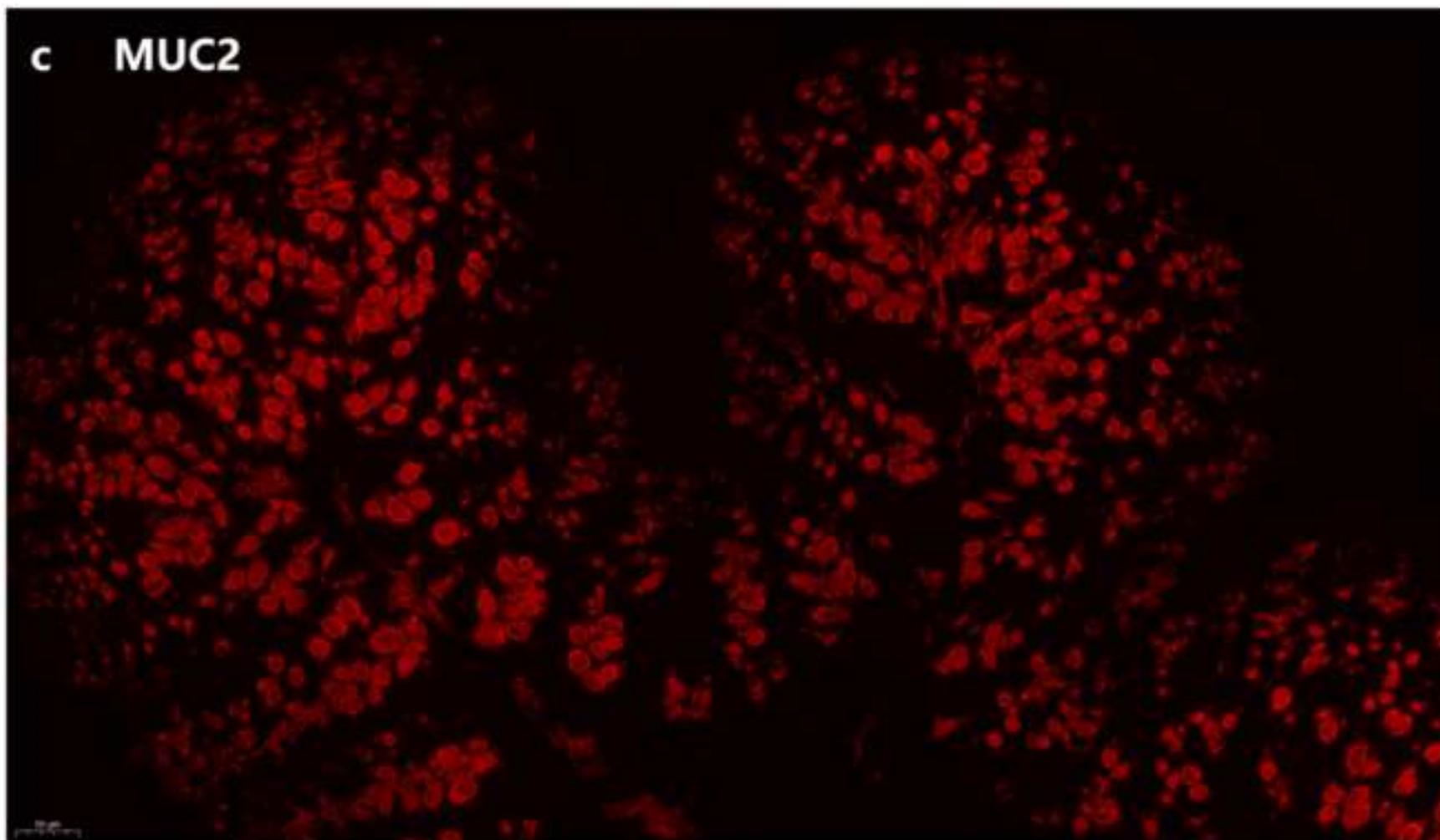


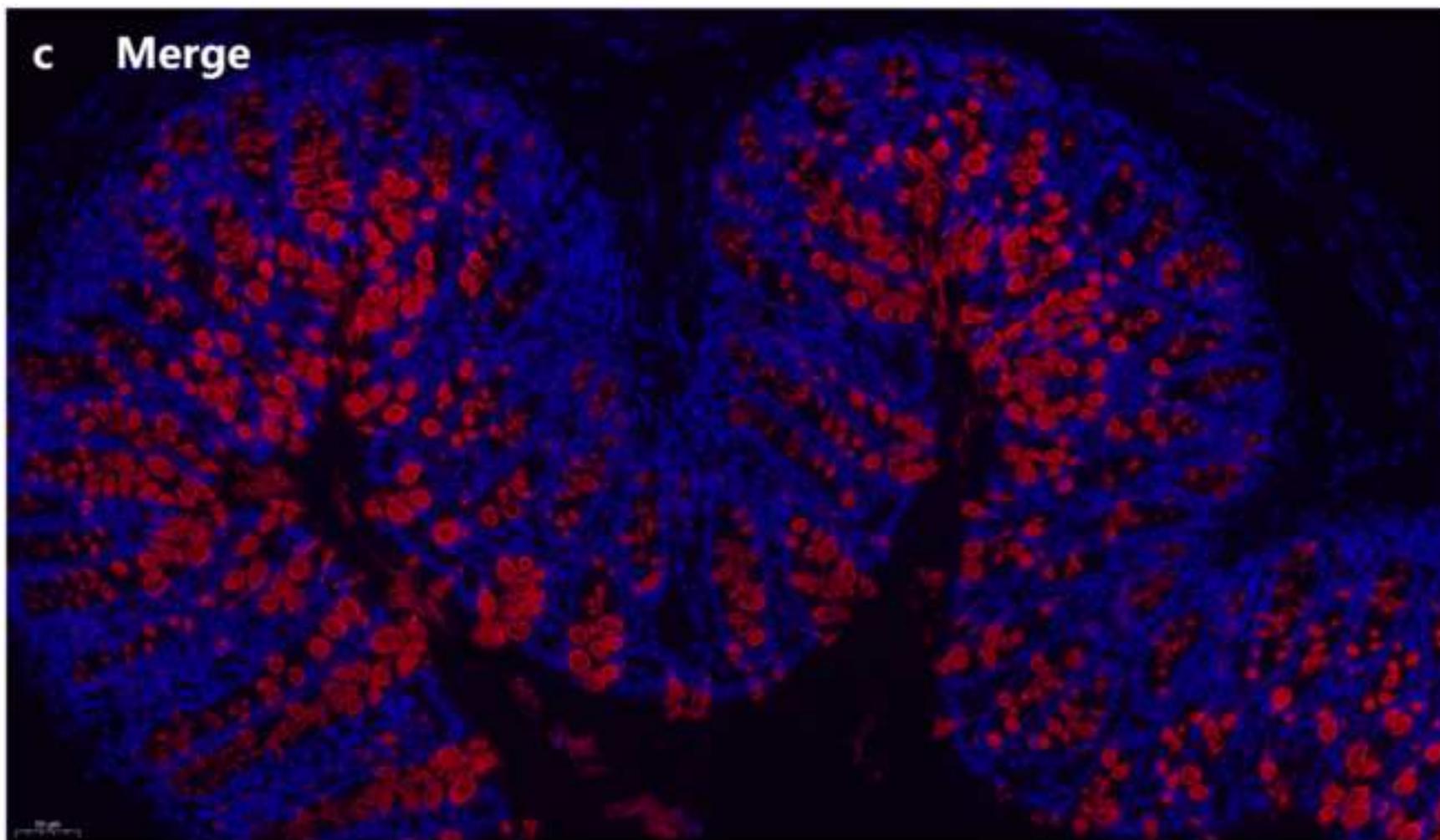


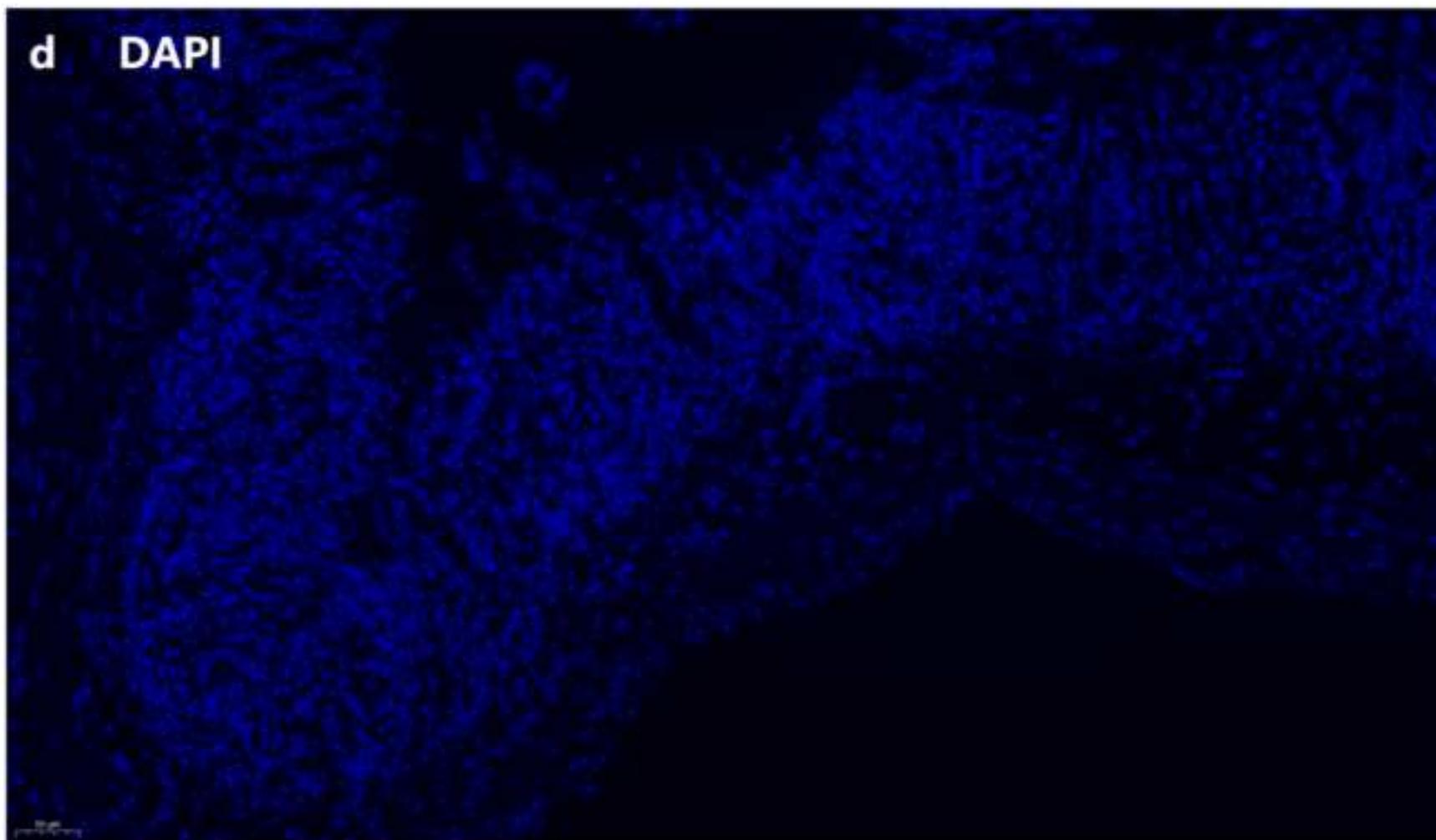


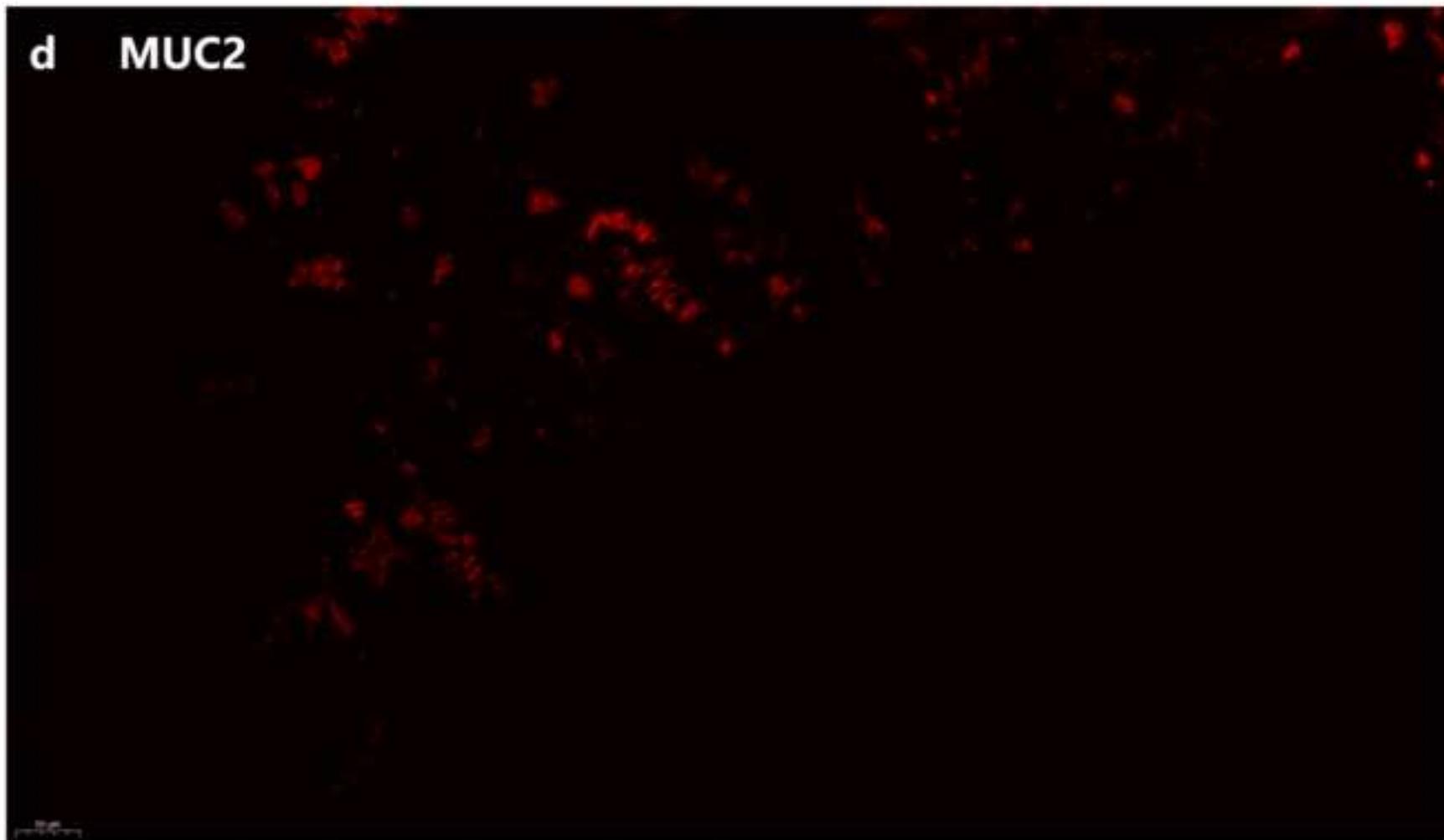


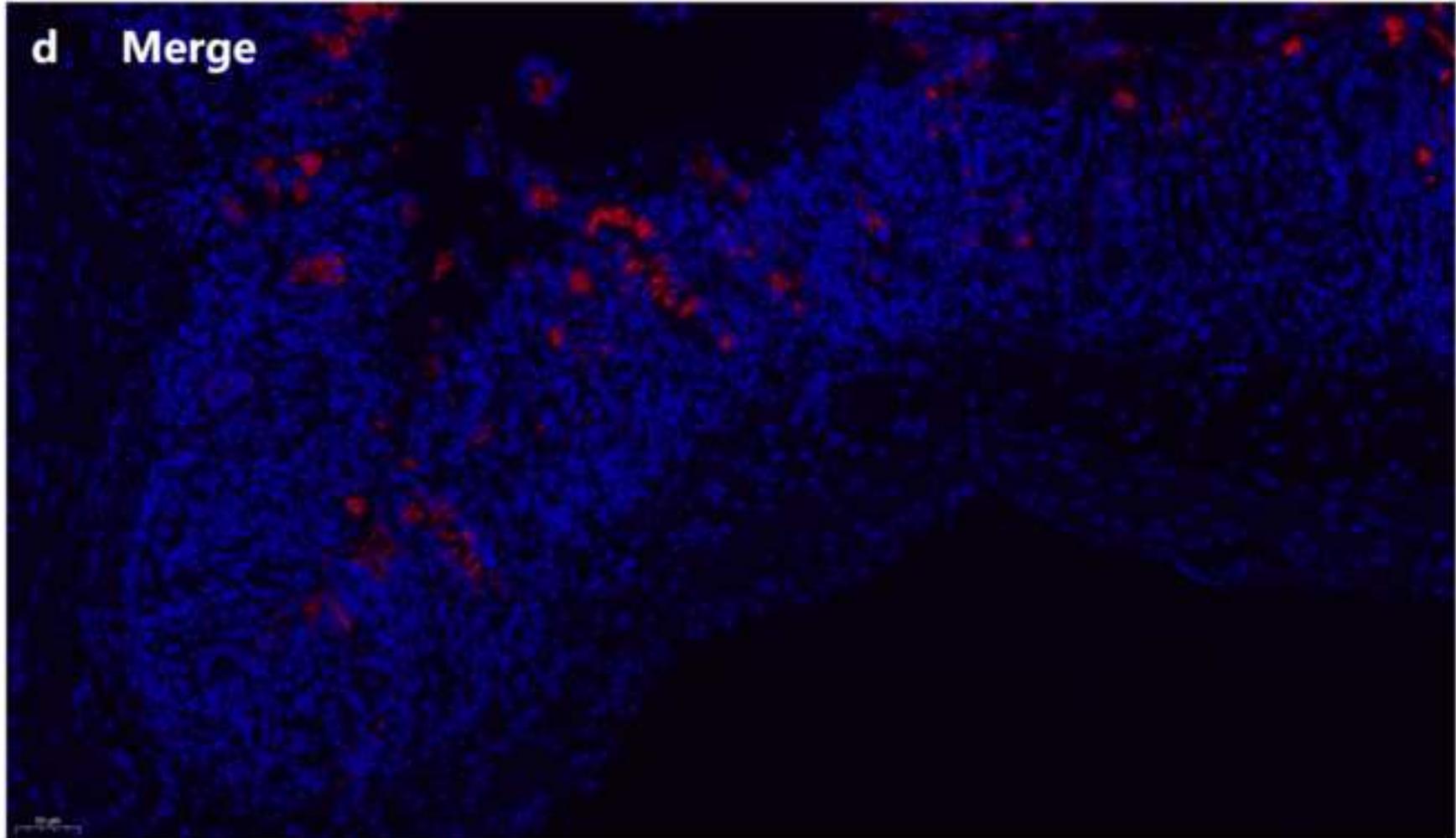


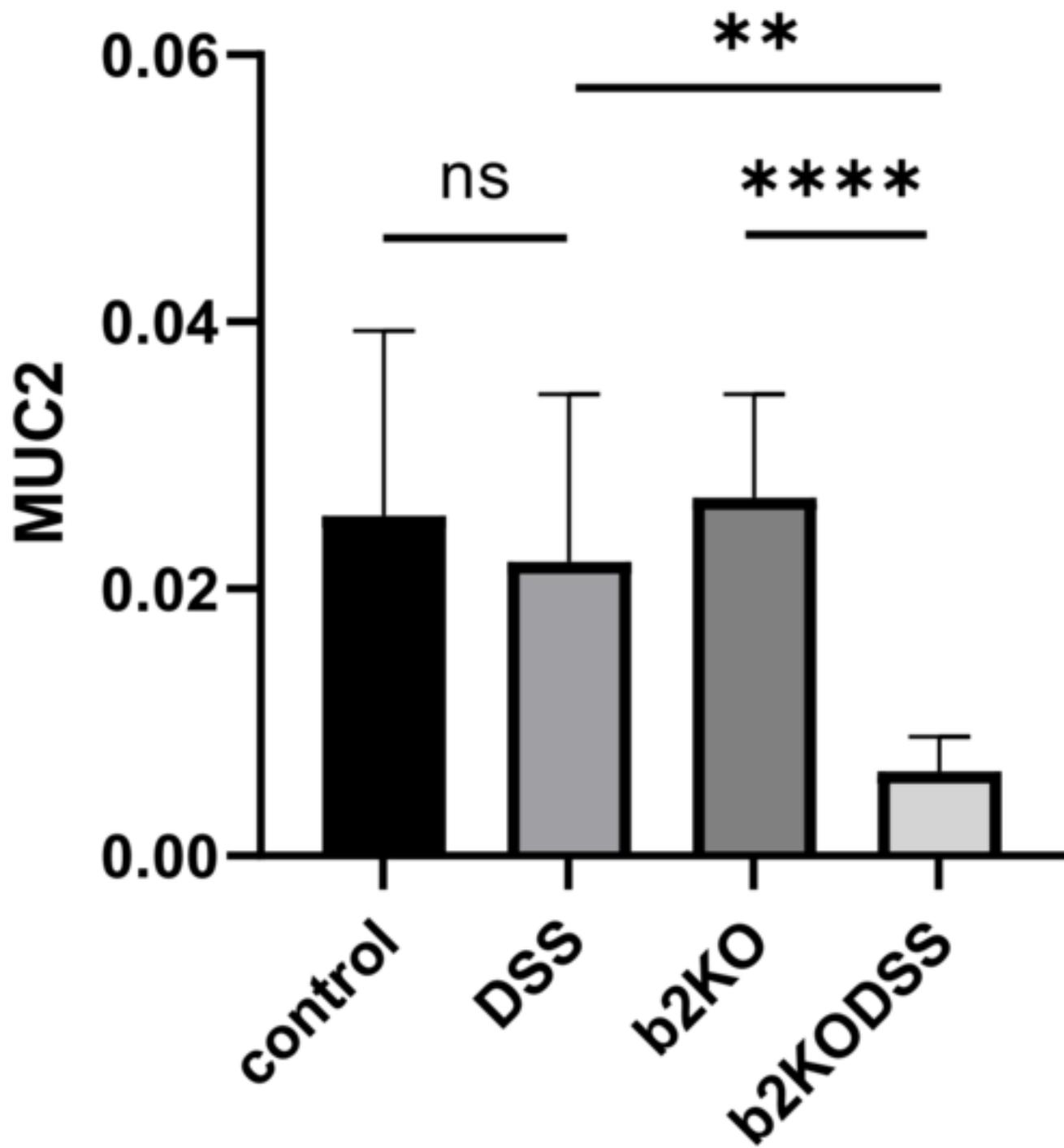


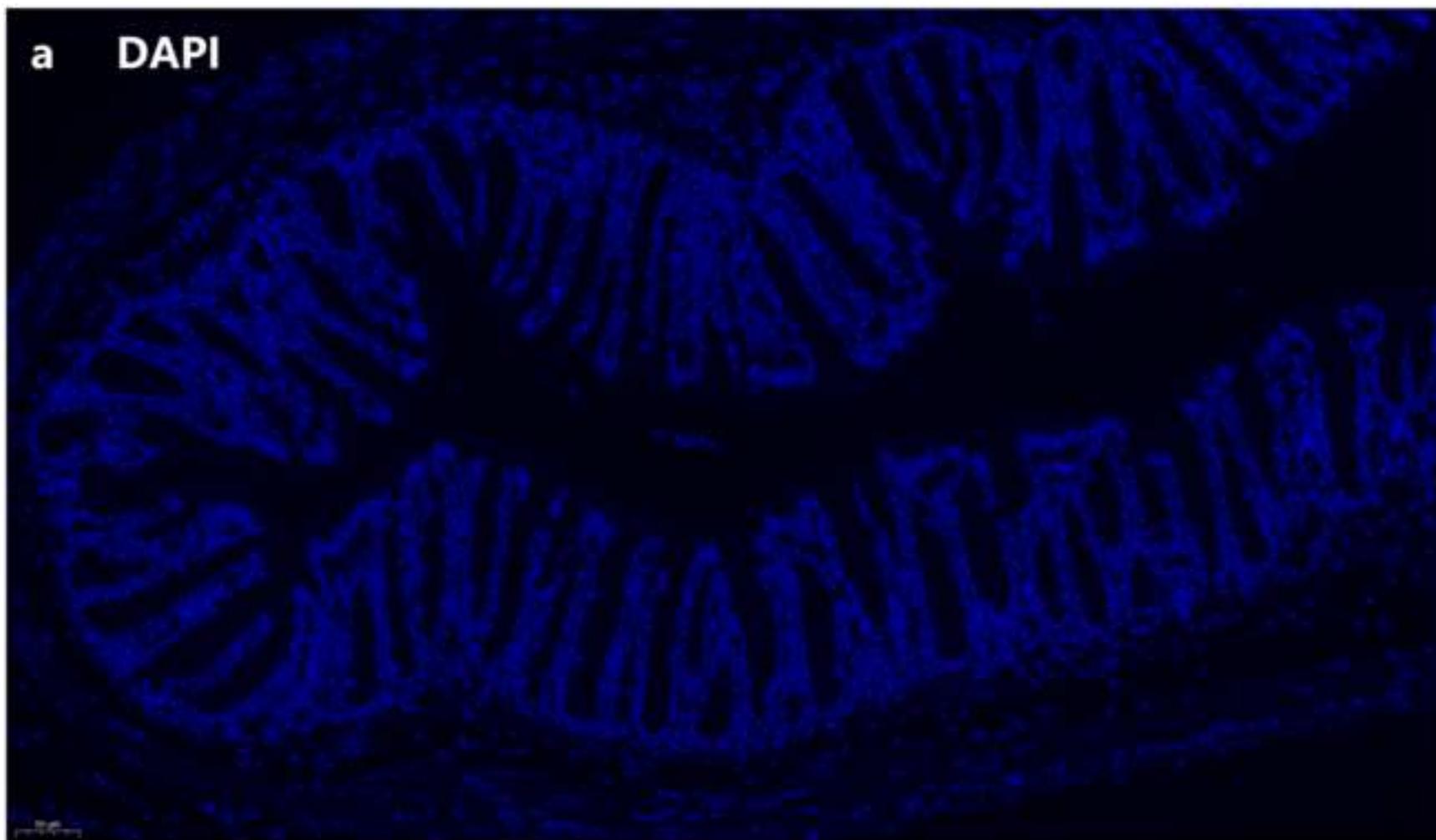


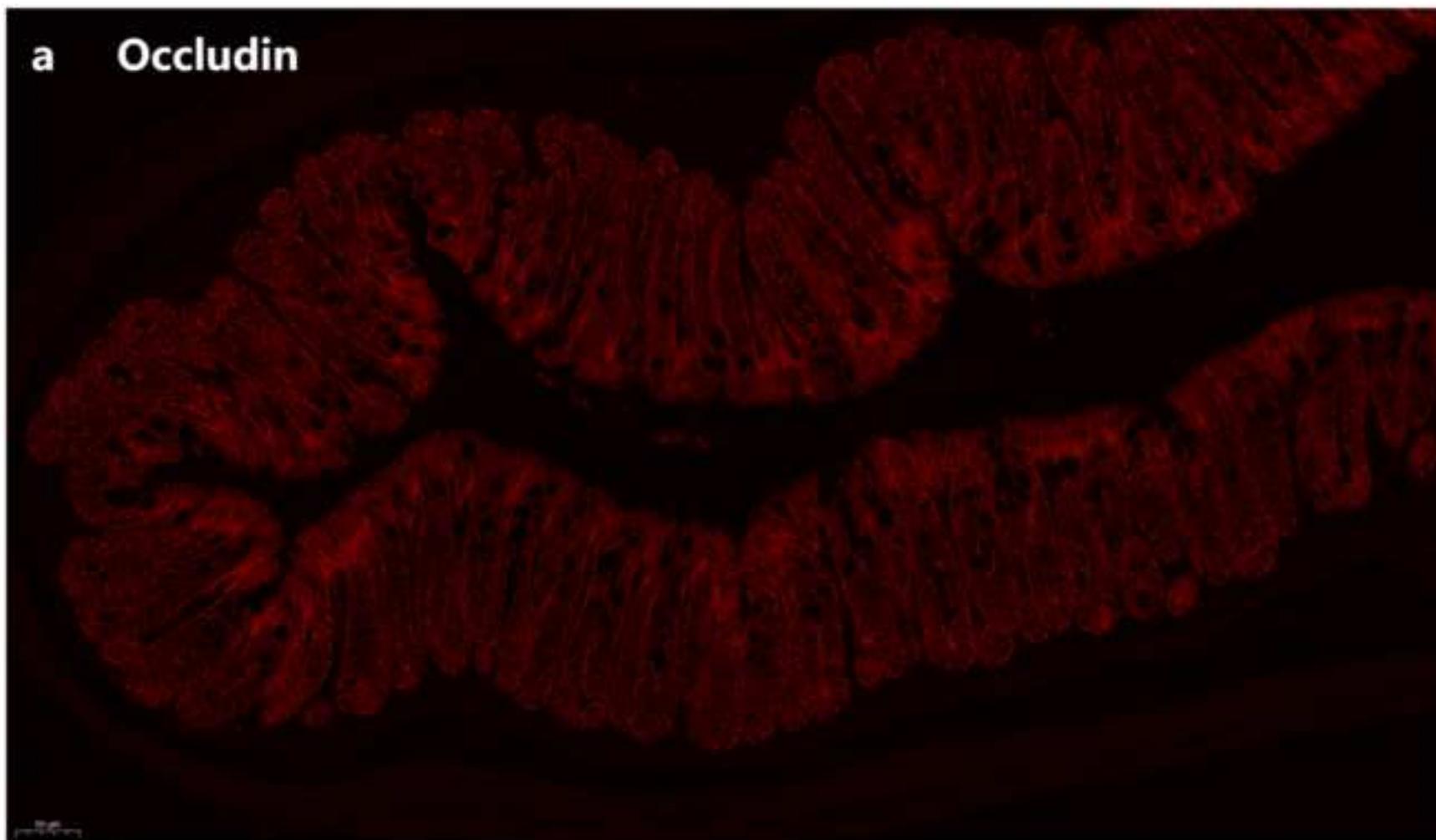


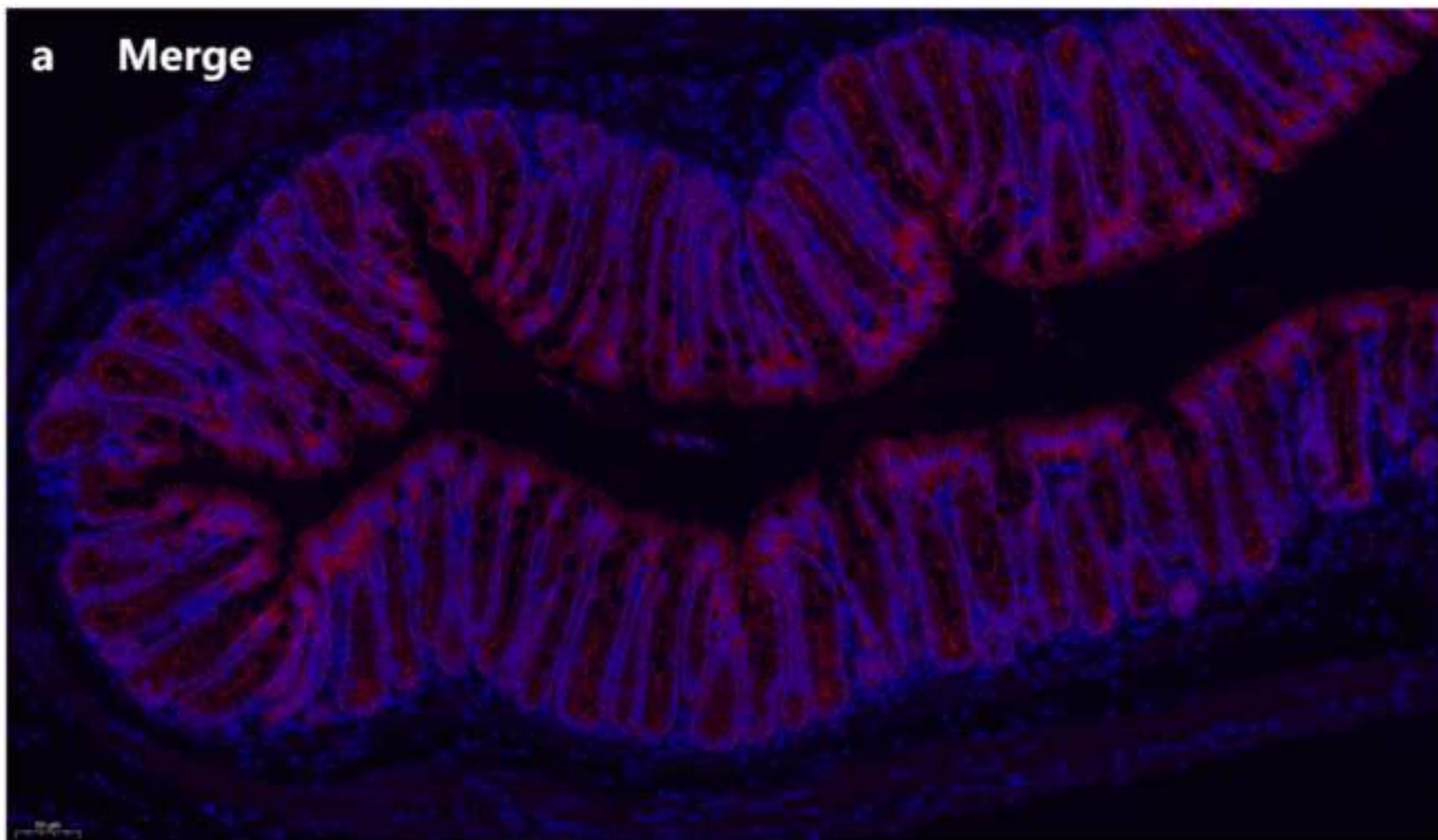


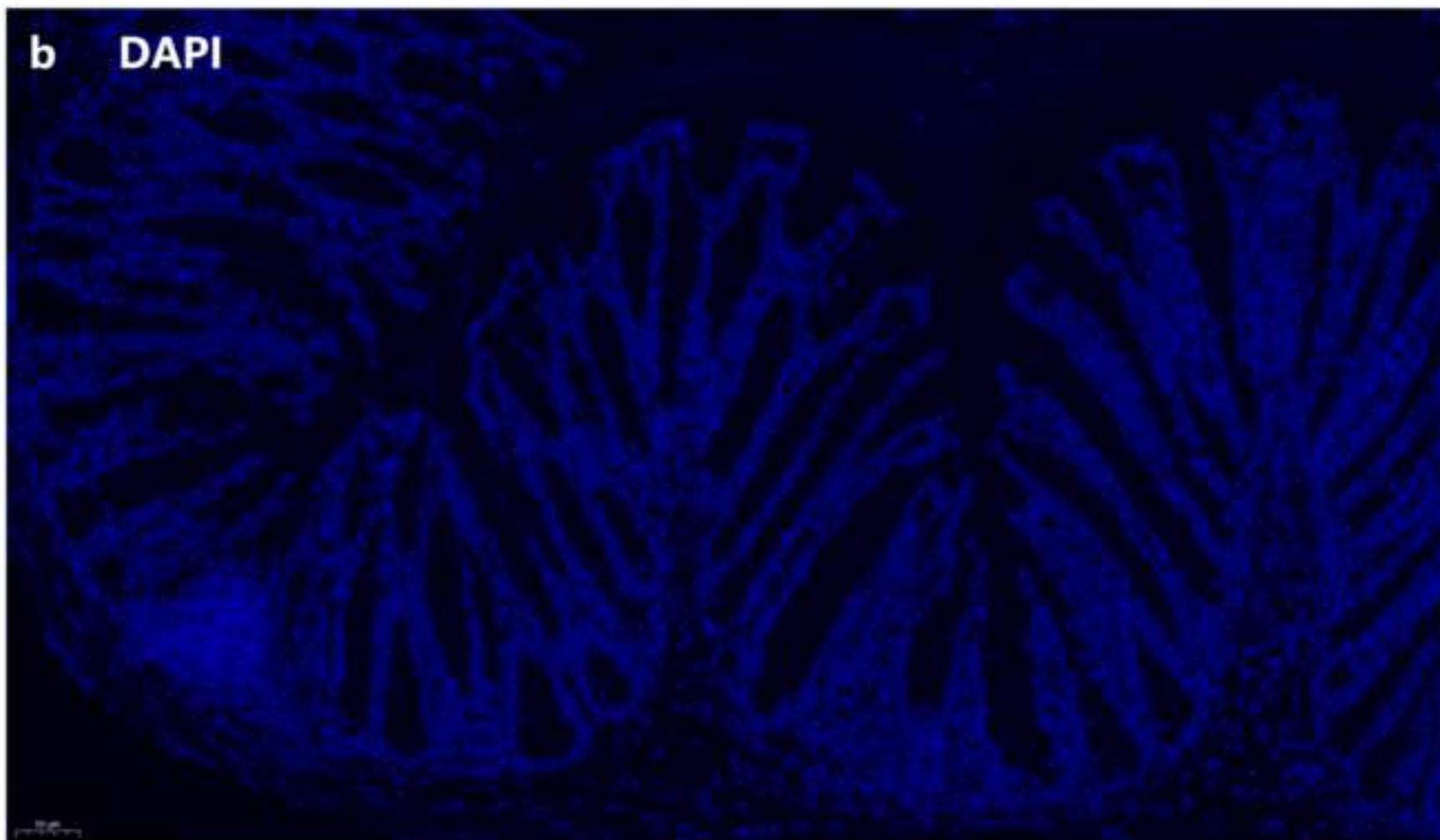


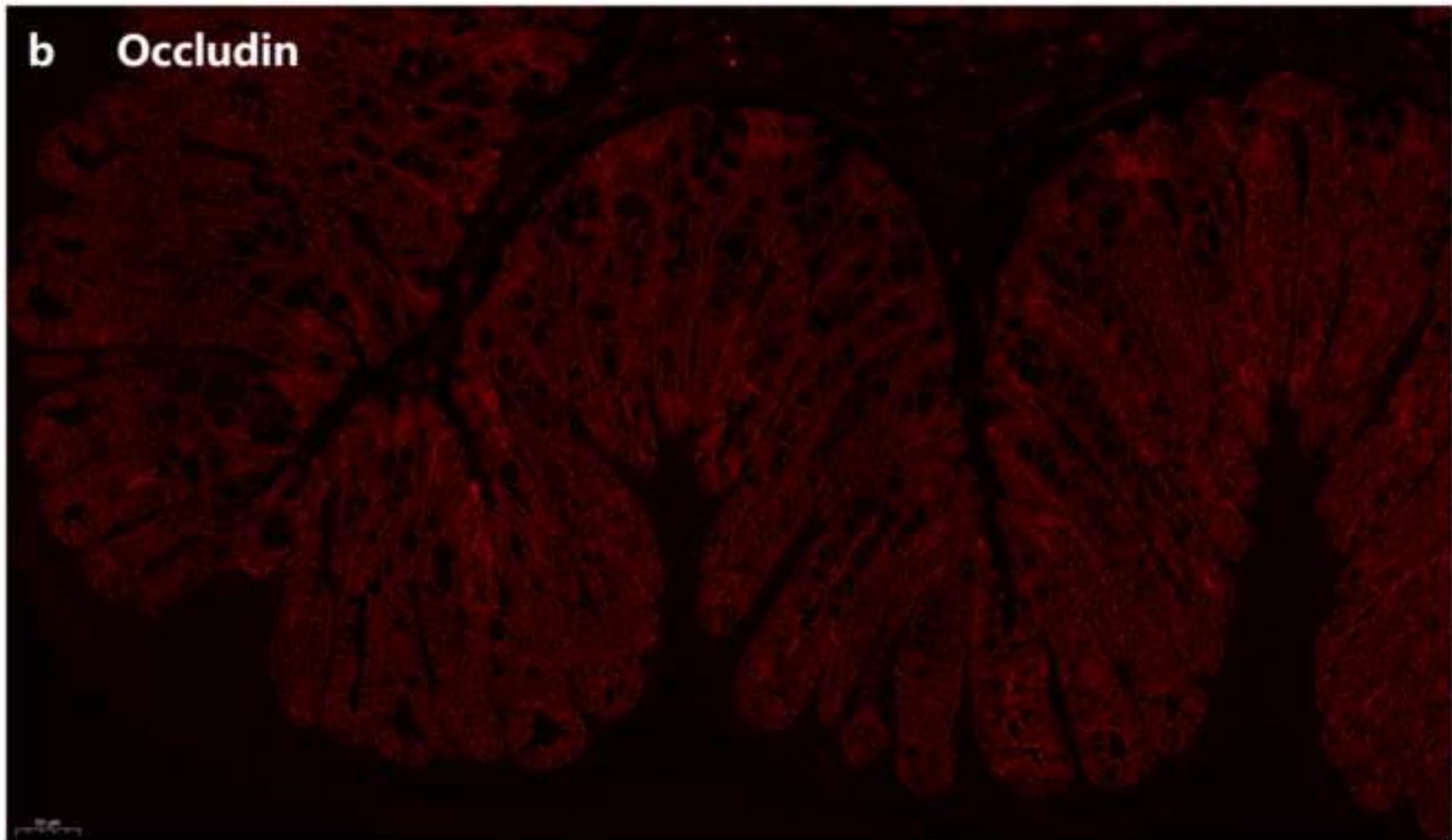


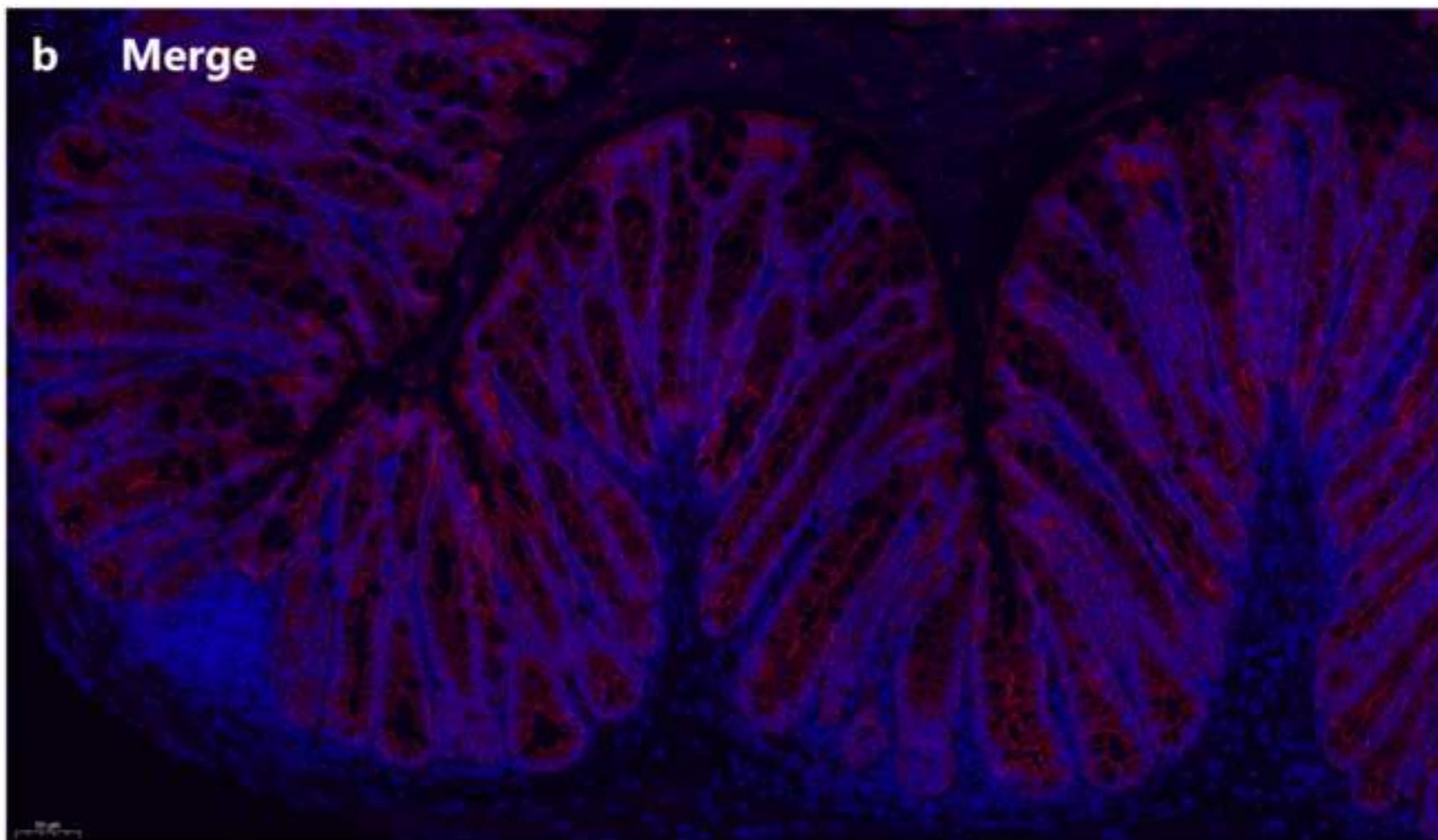


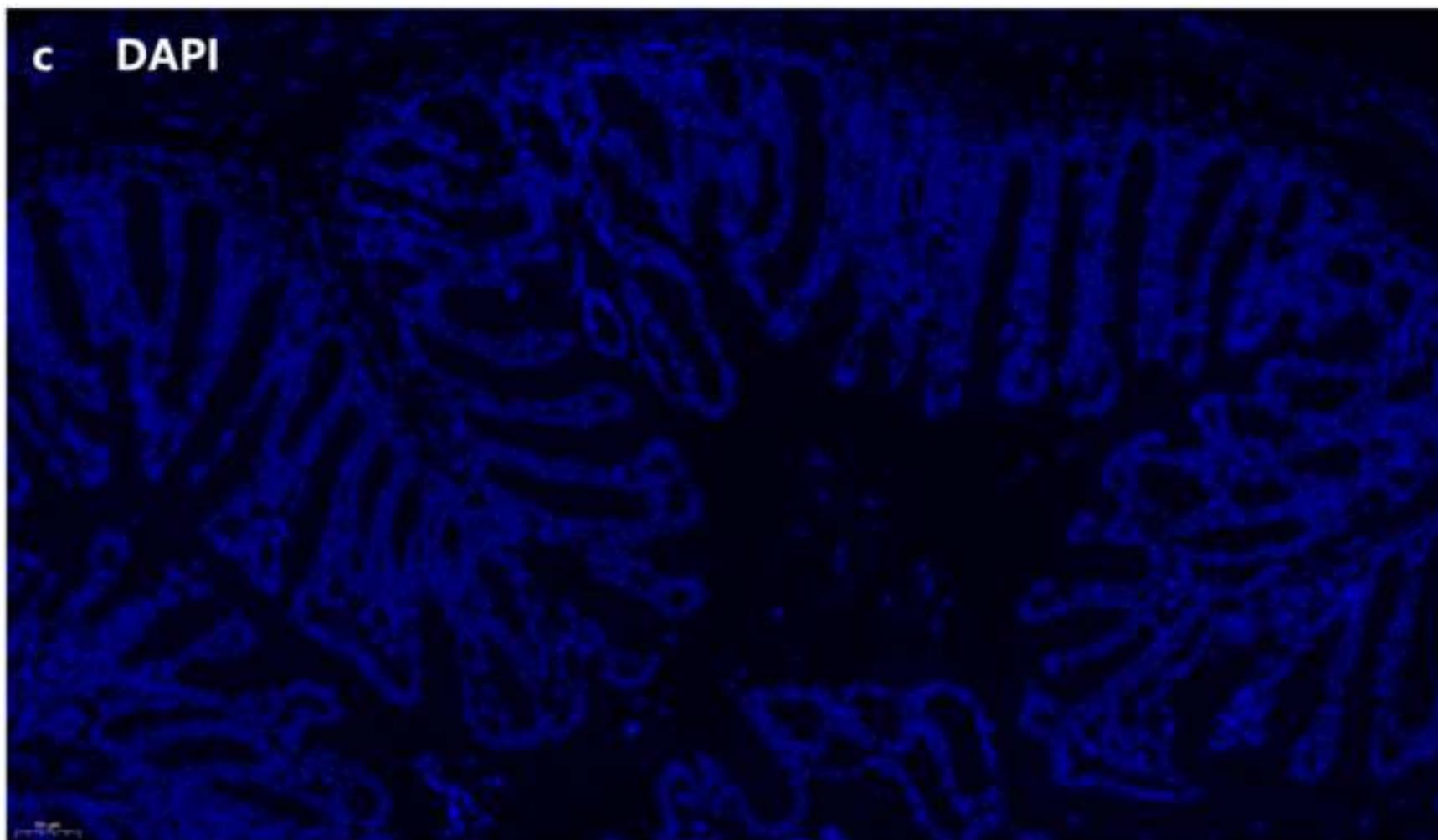


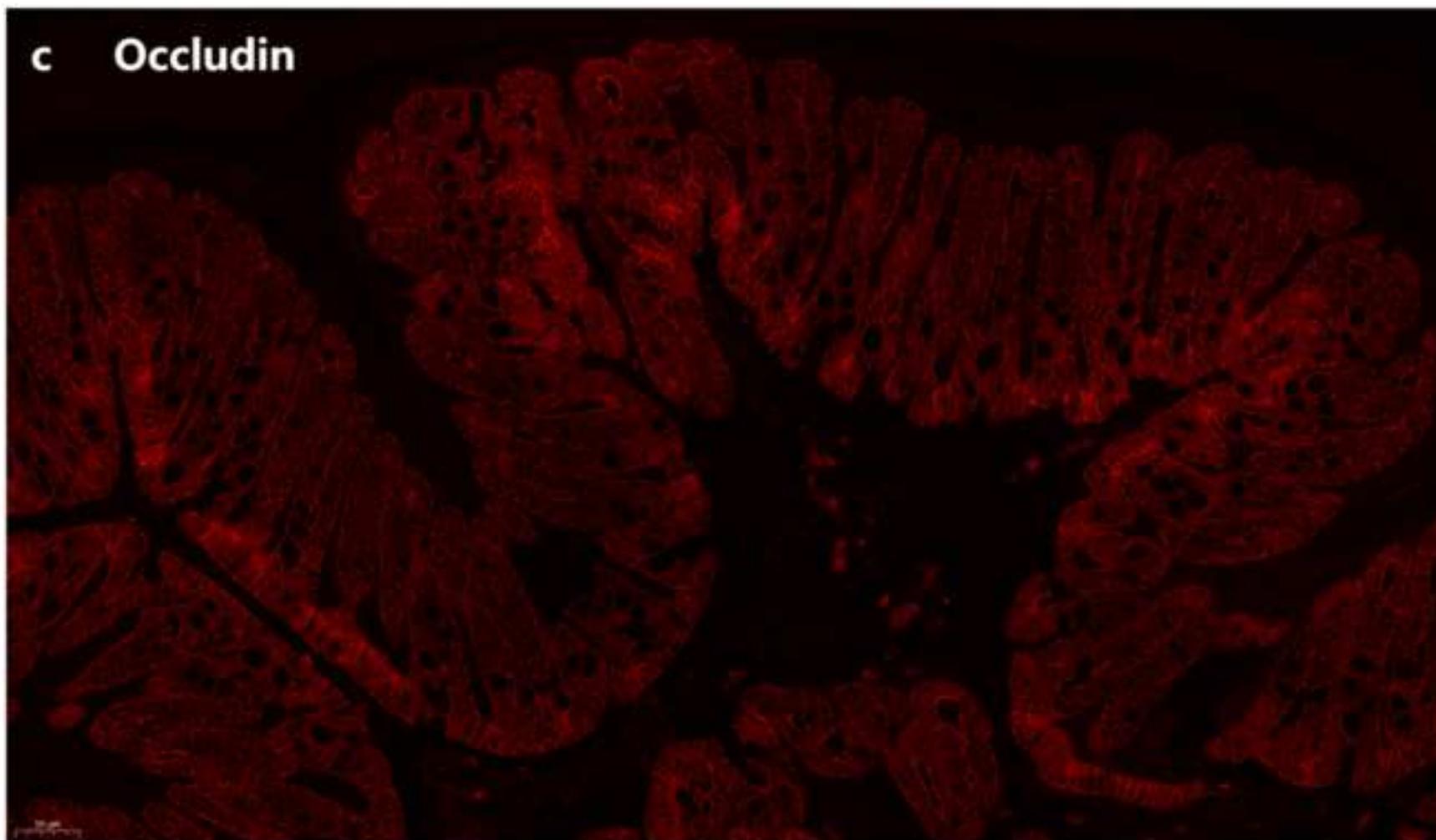


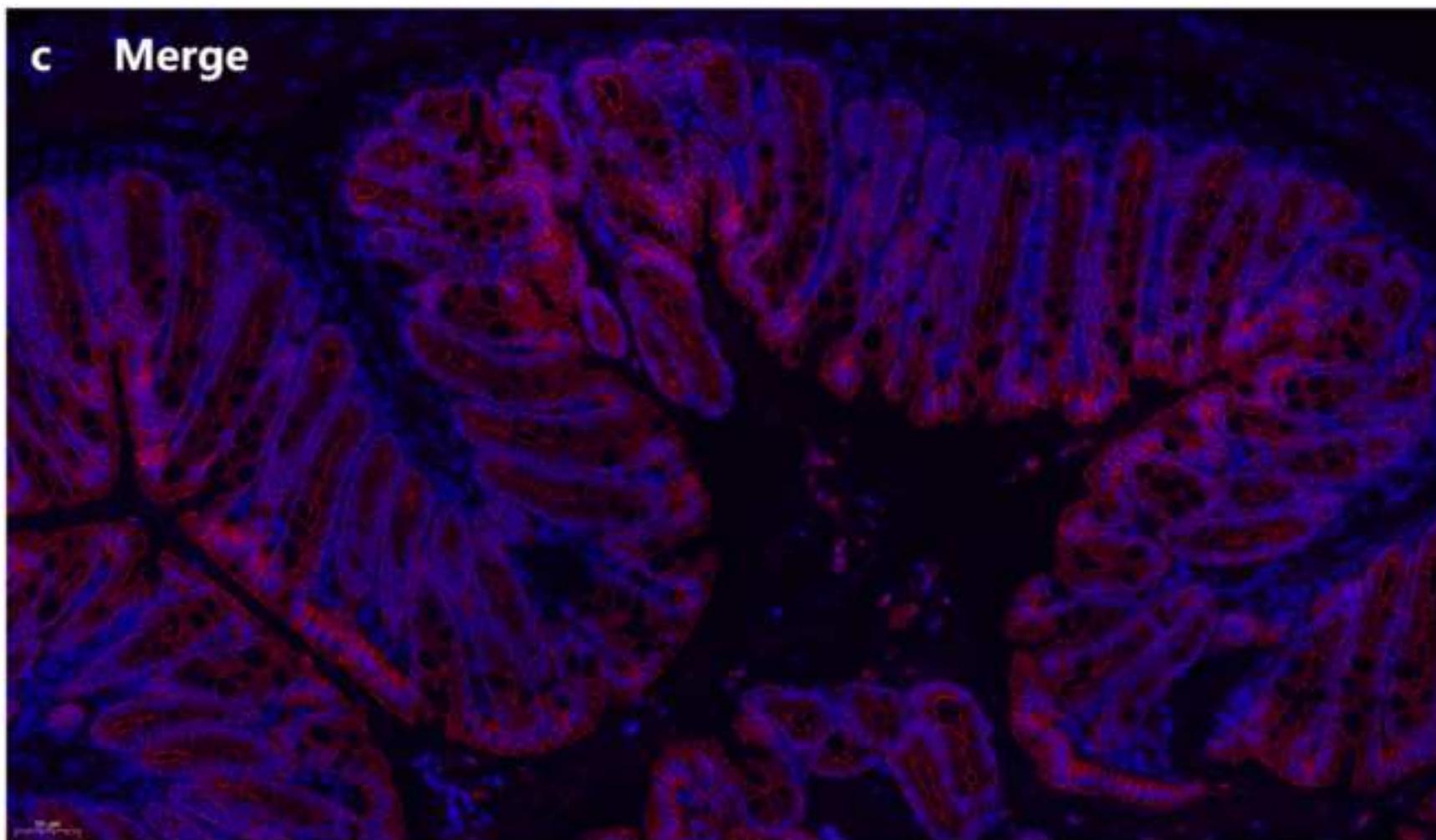


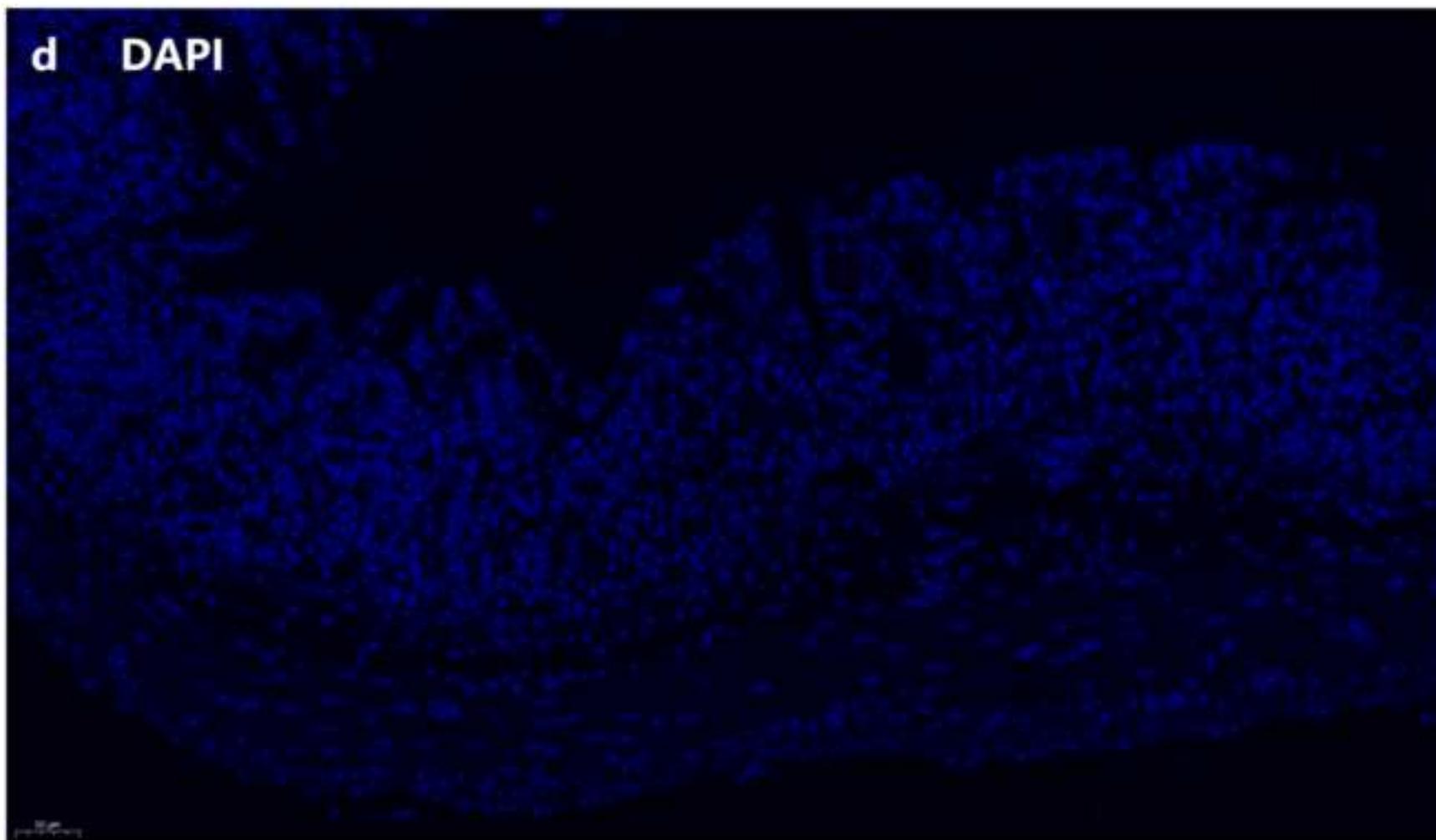


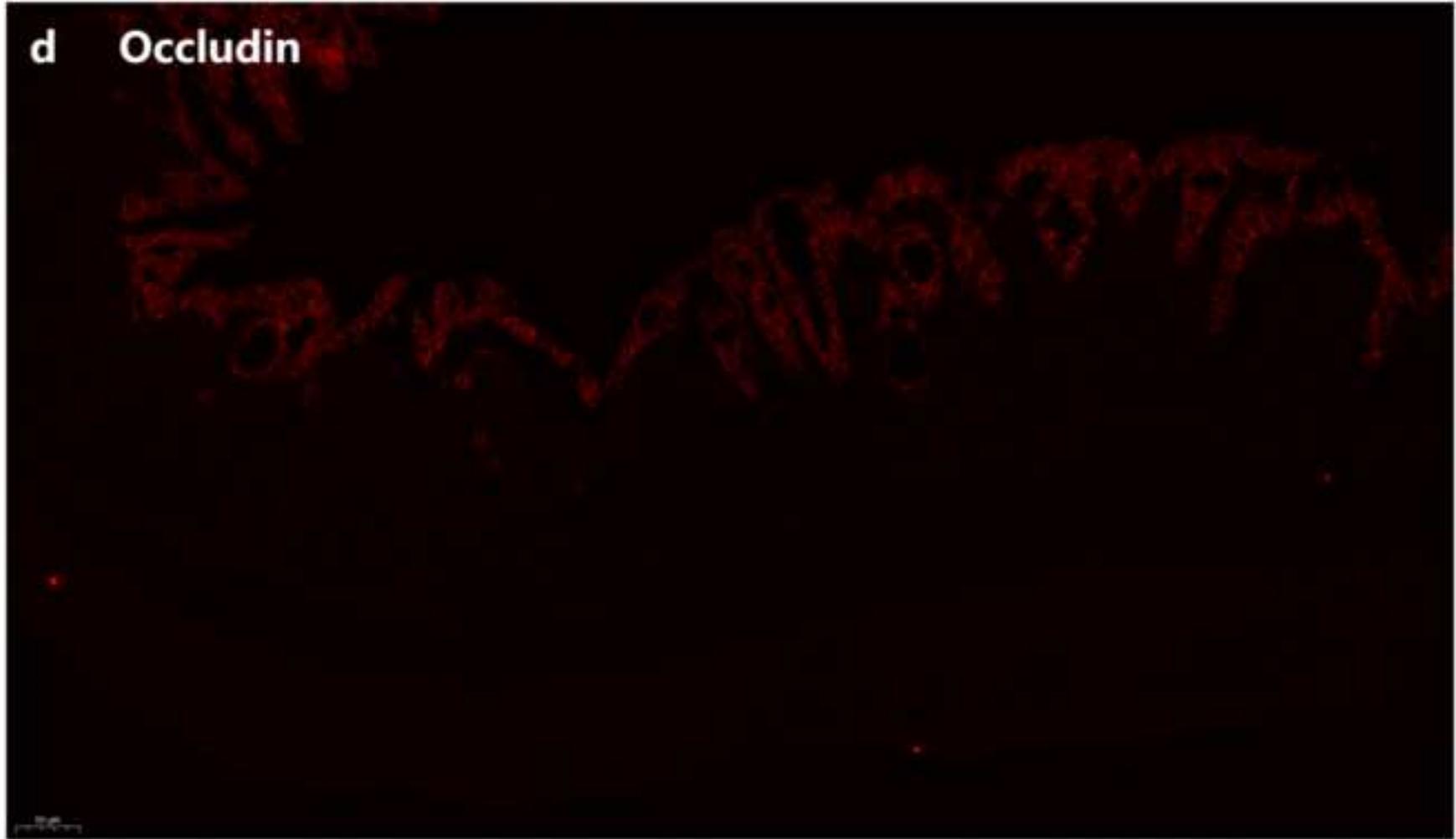


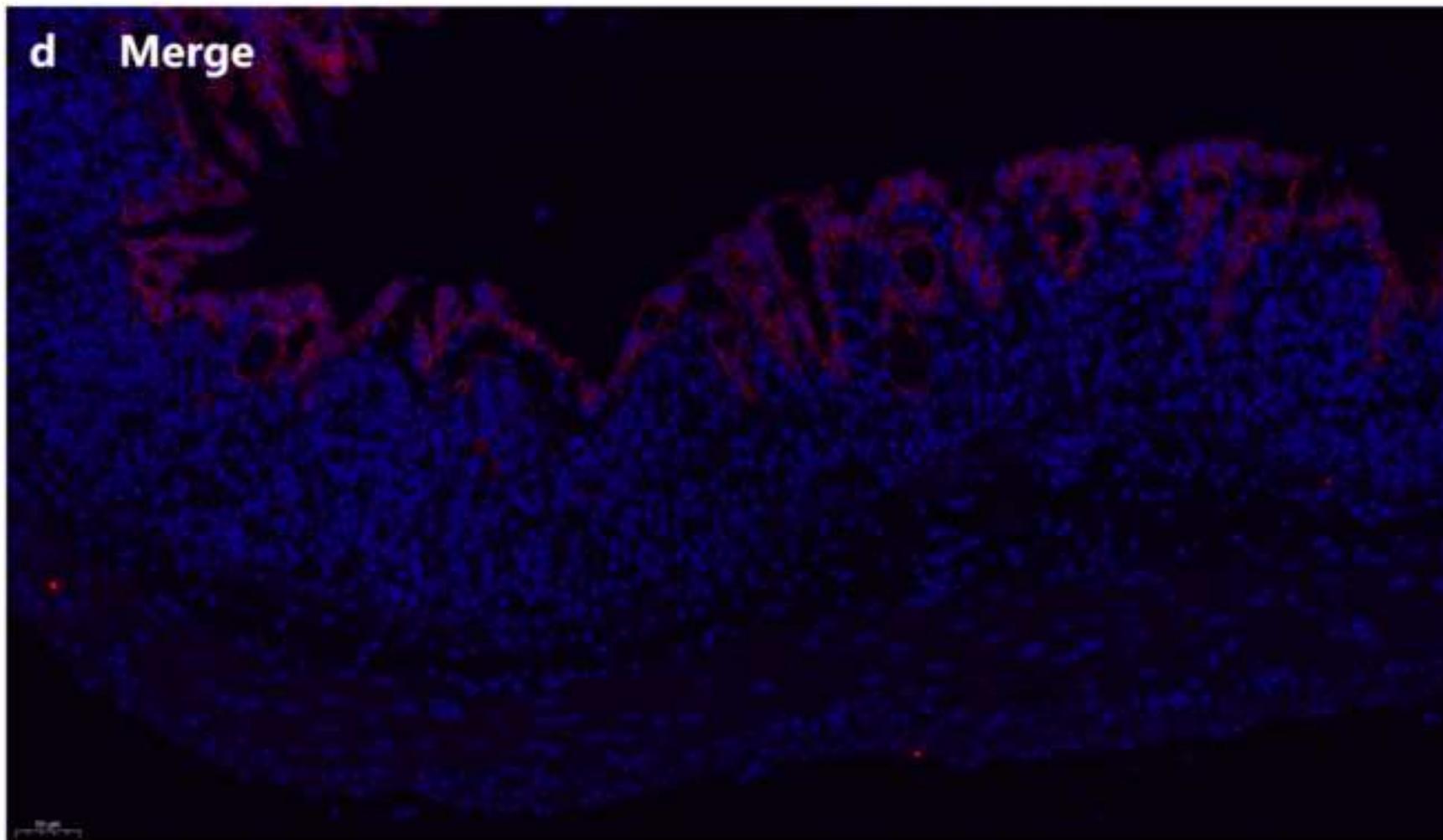


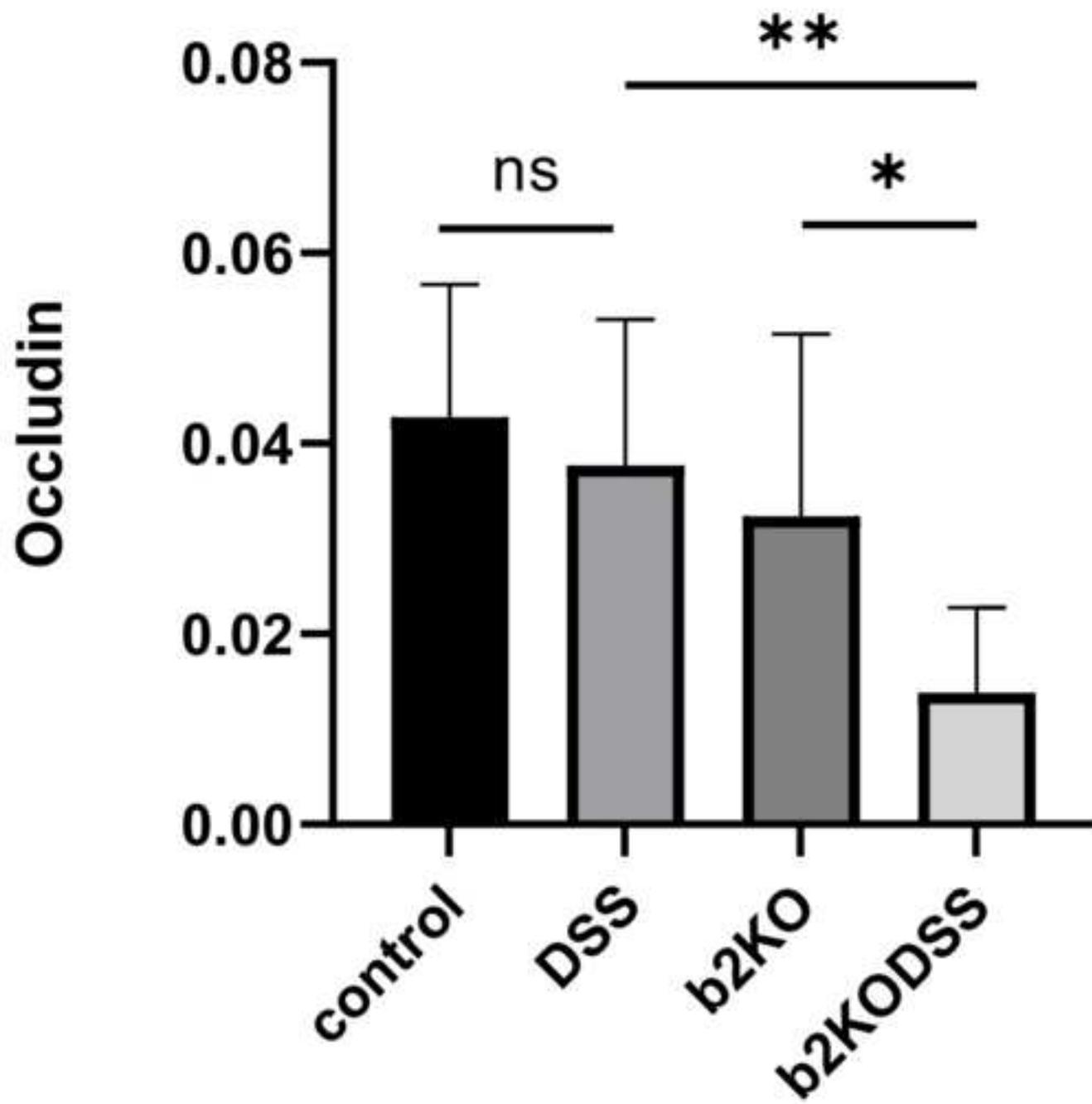


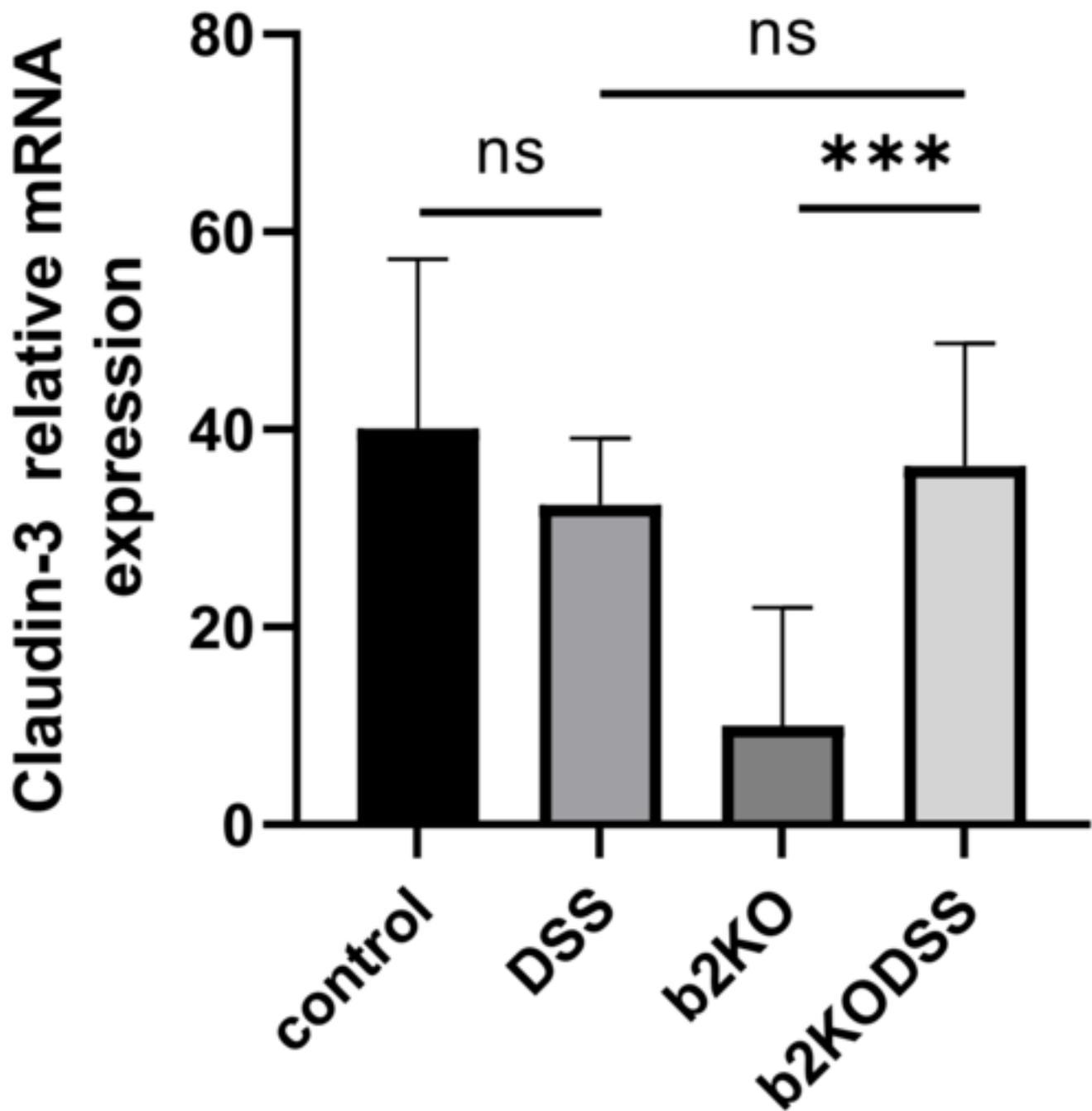


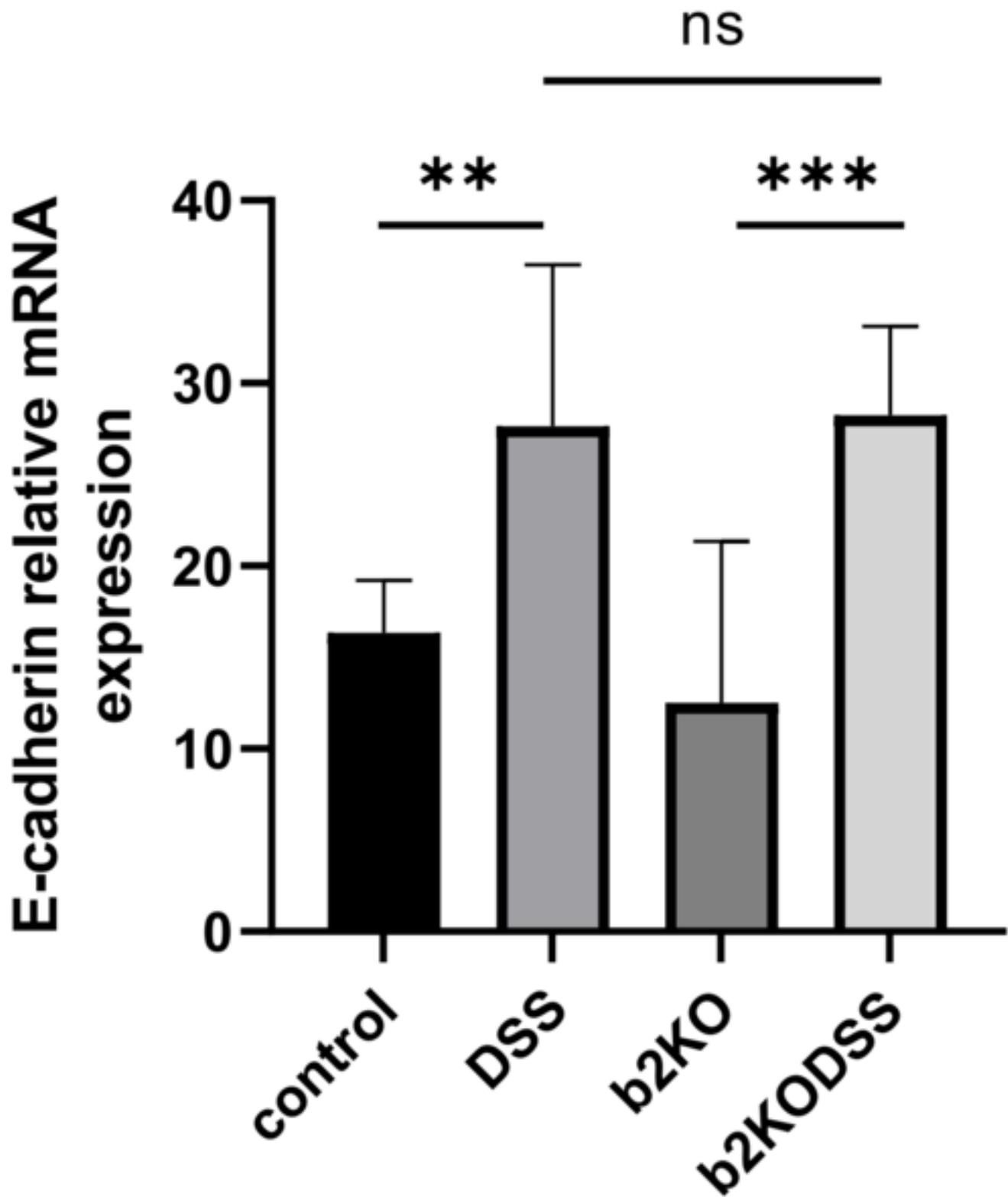


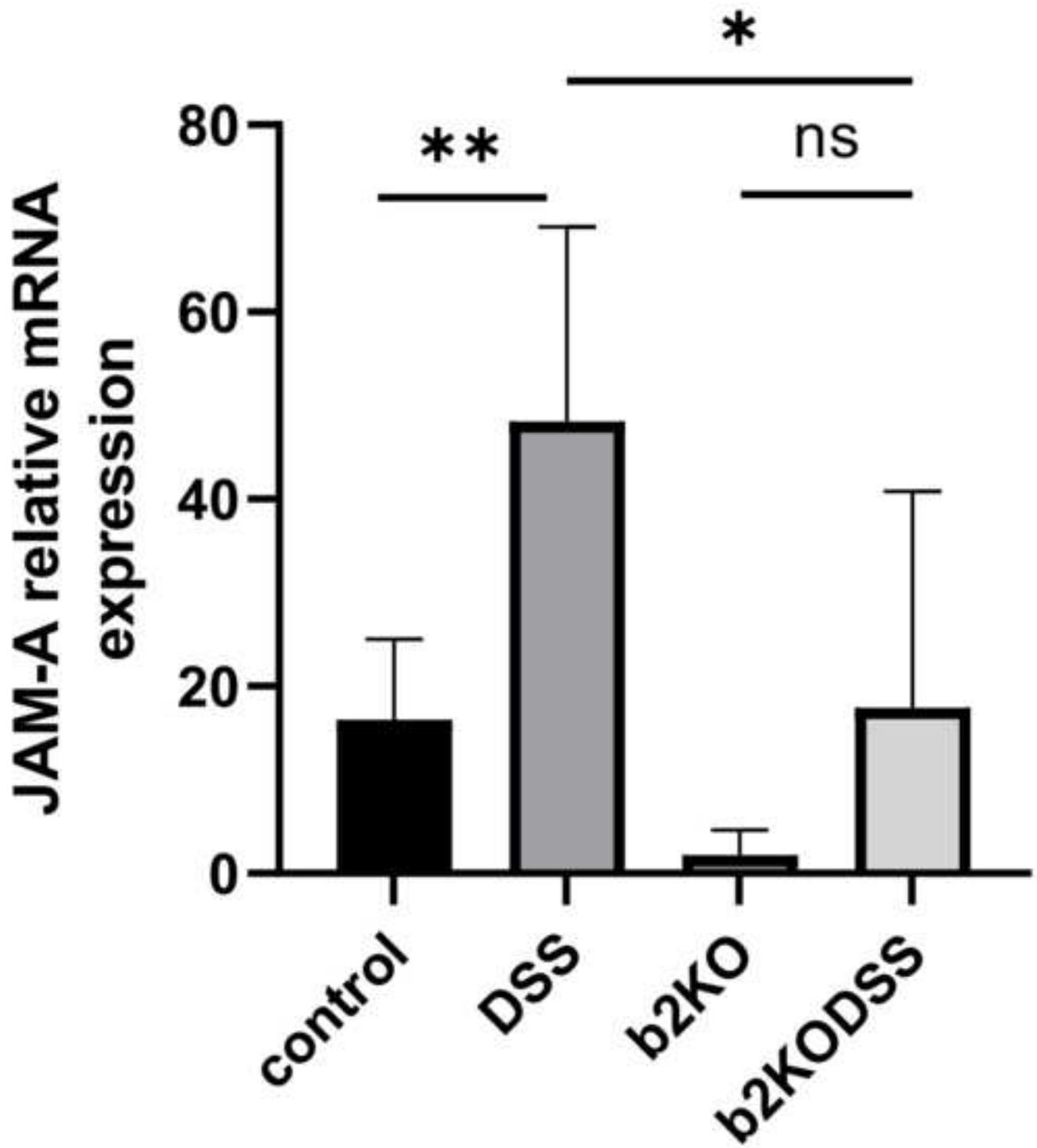


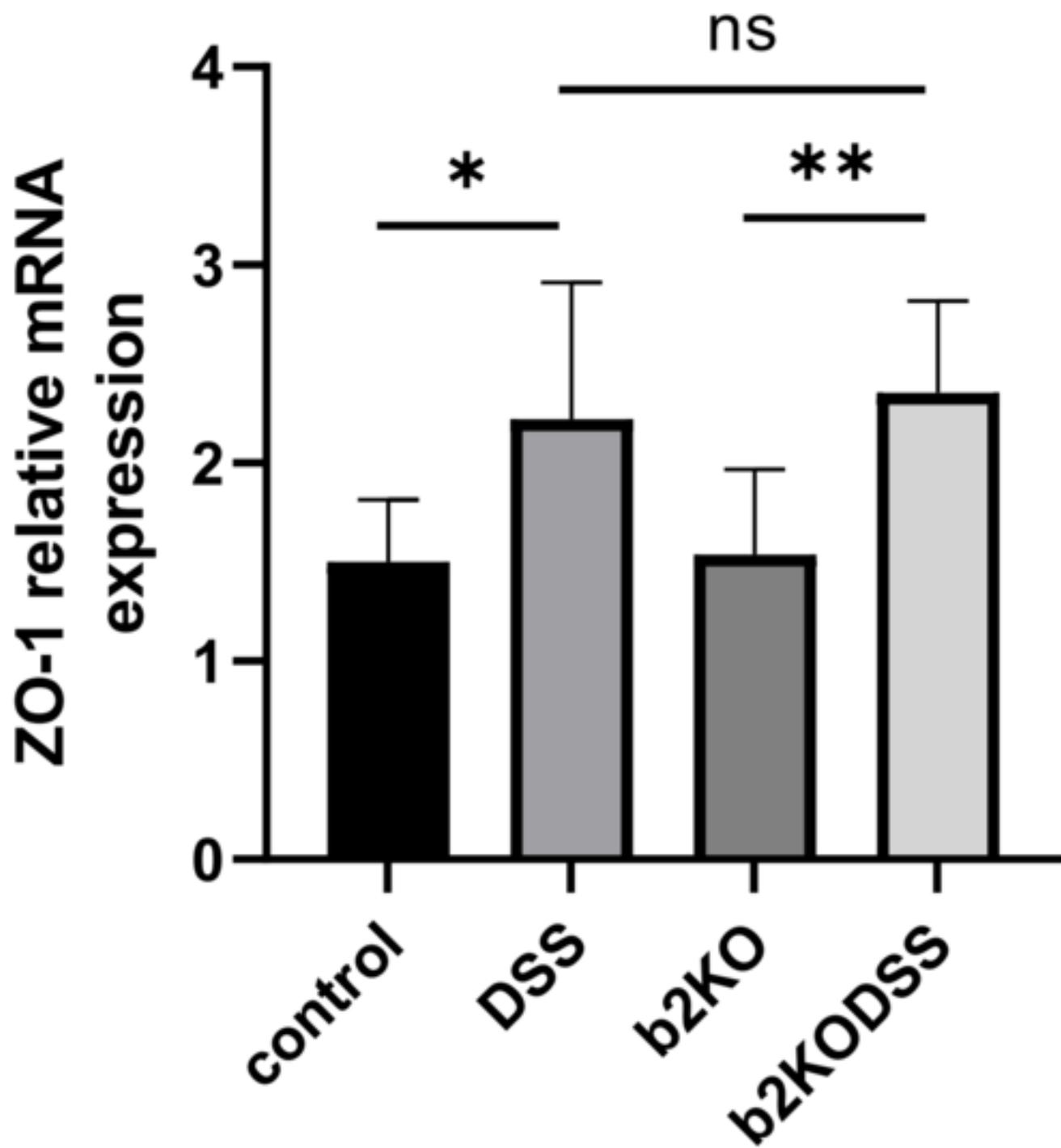












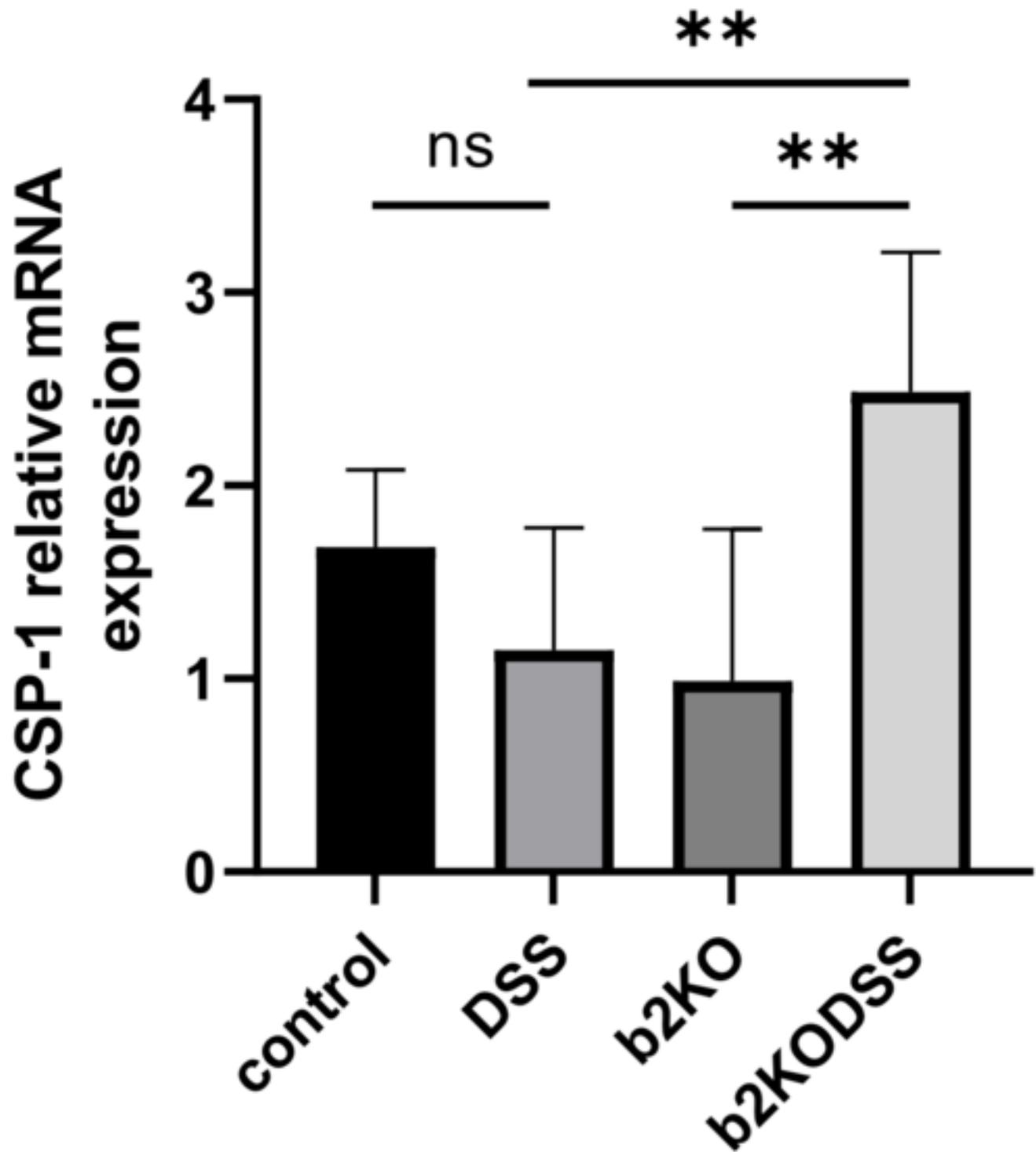
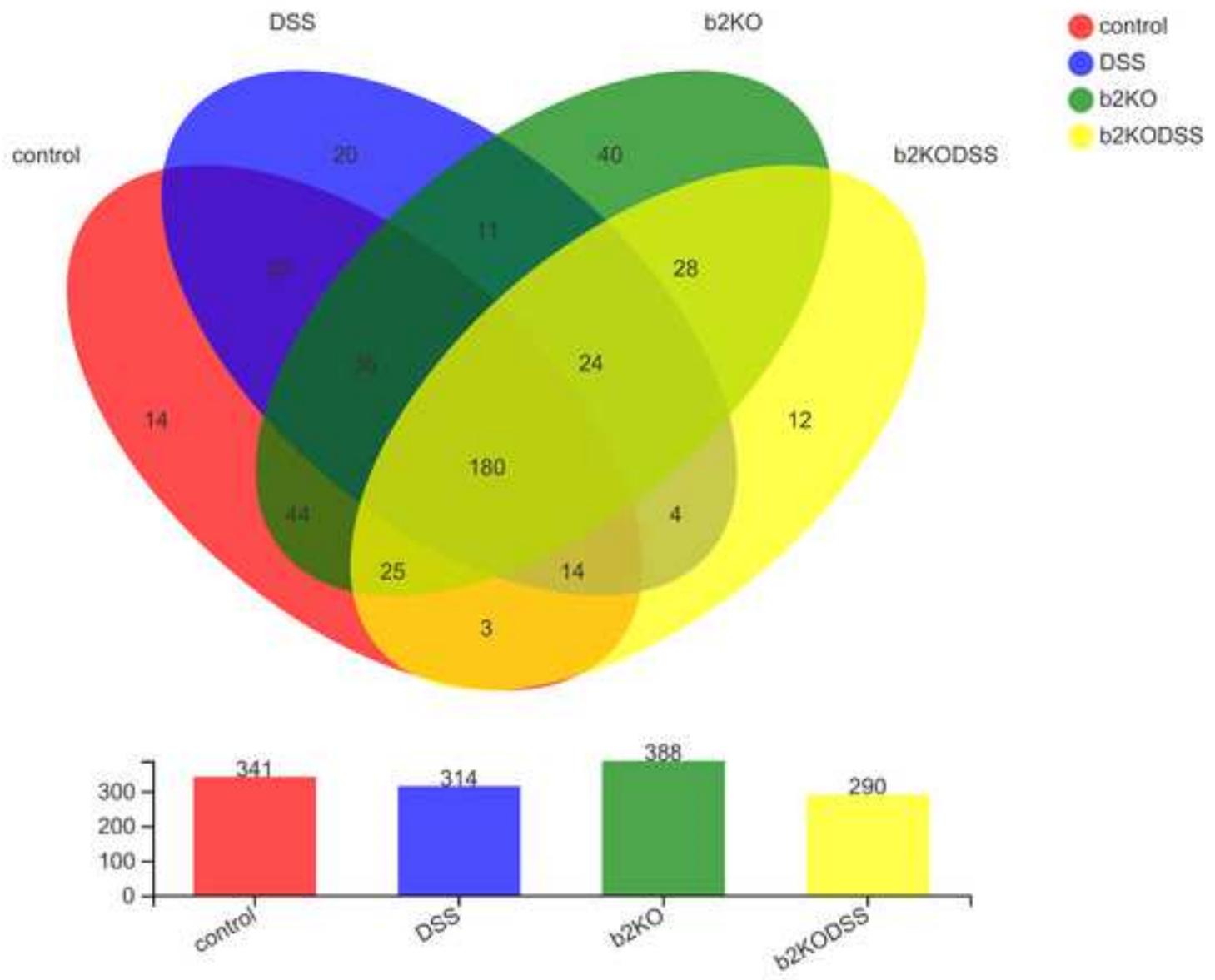
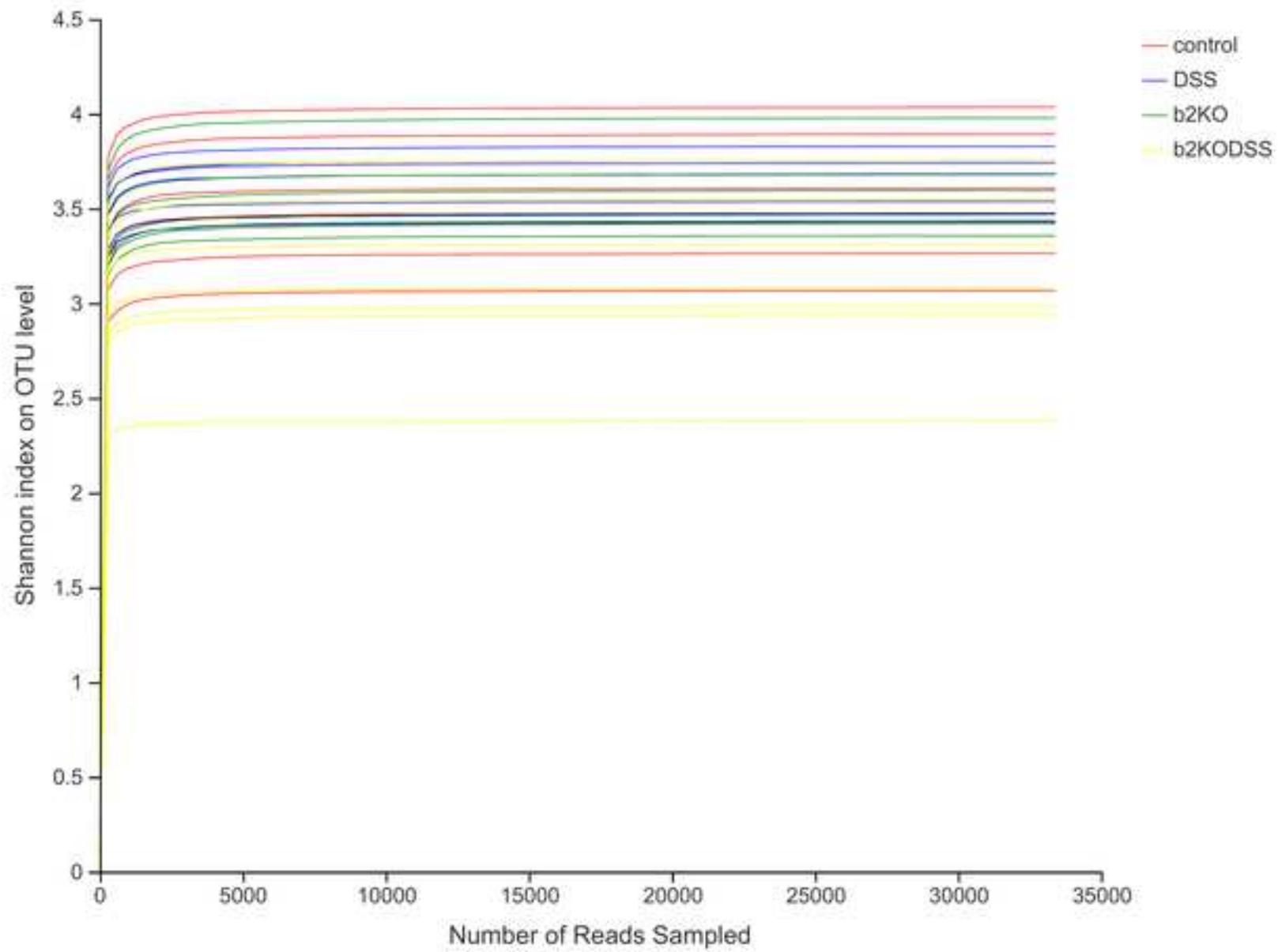
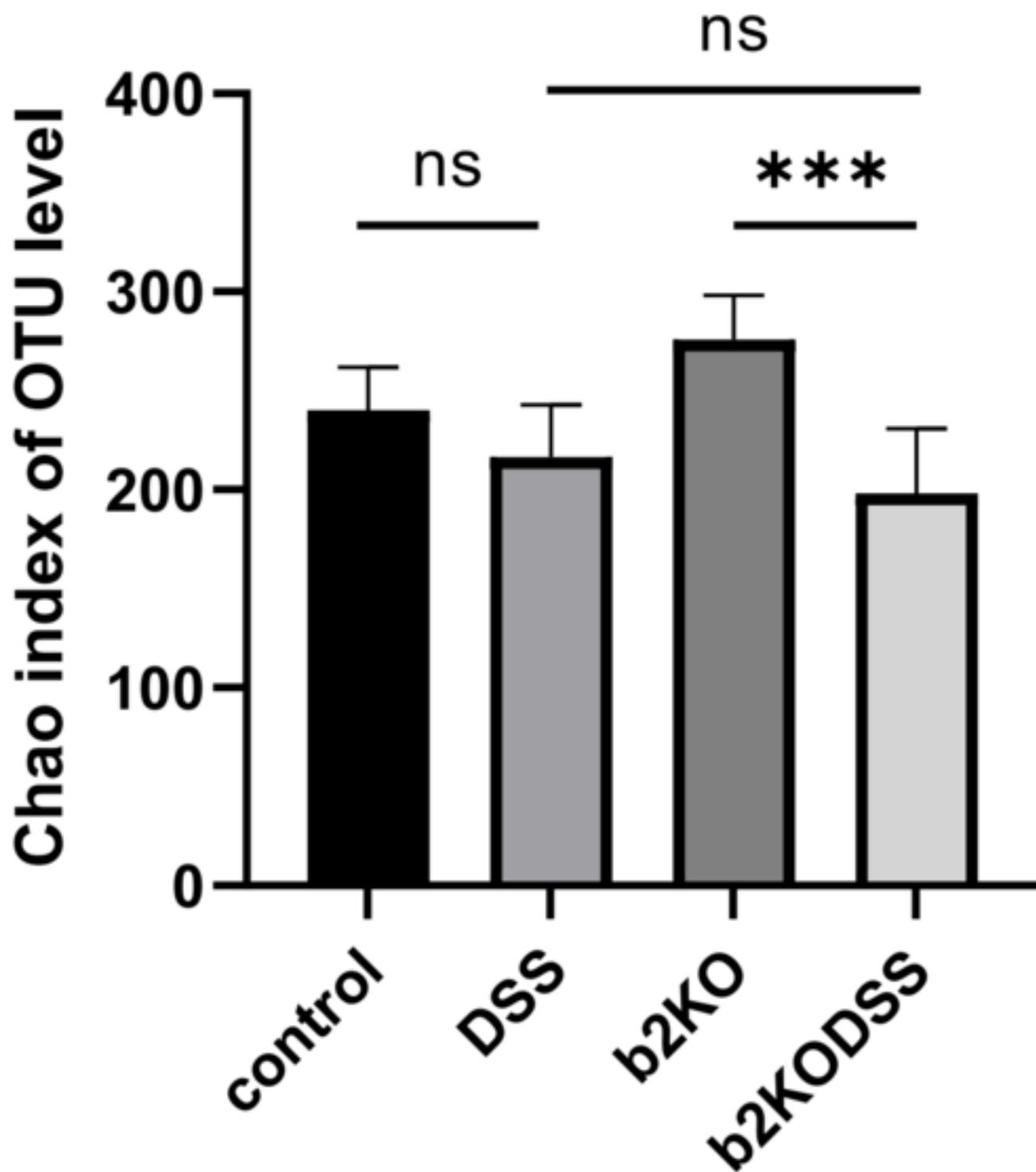
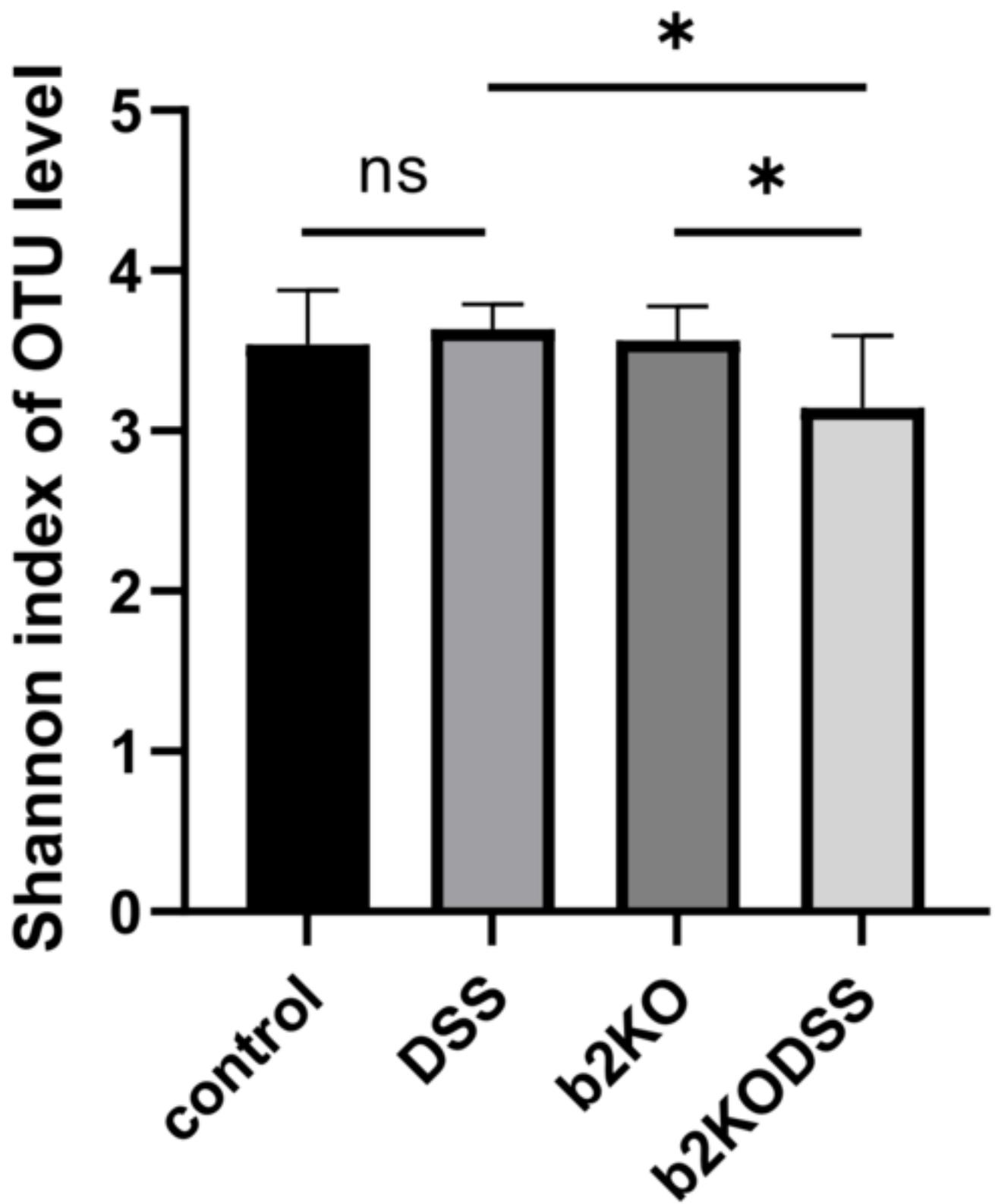


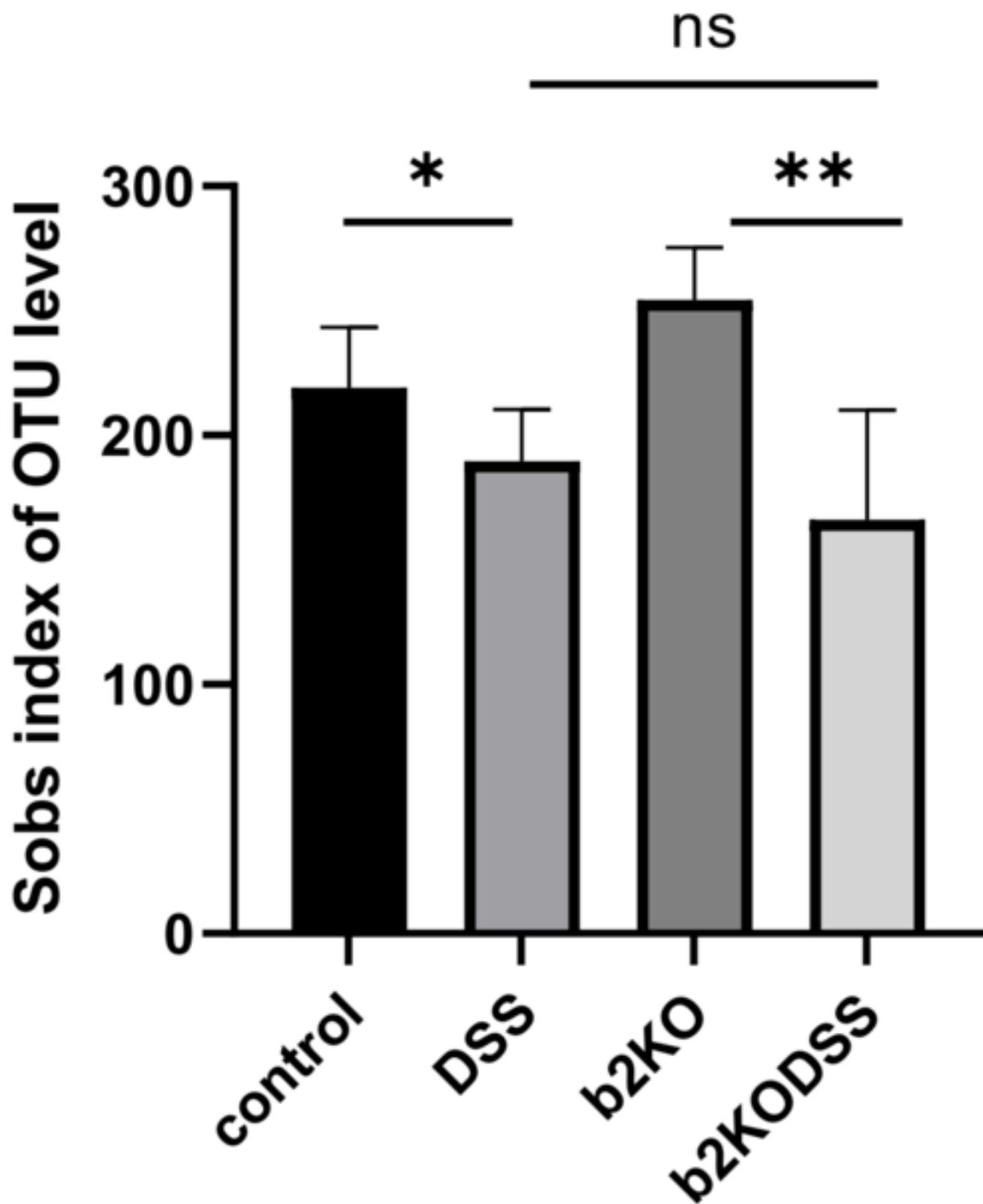
Fig. 4B

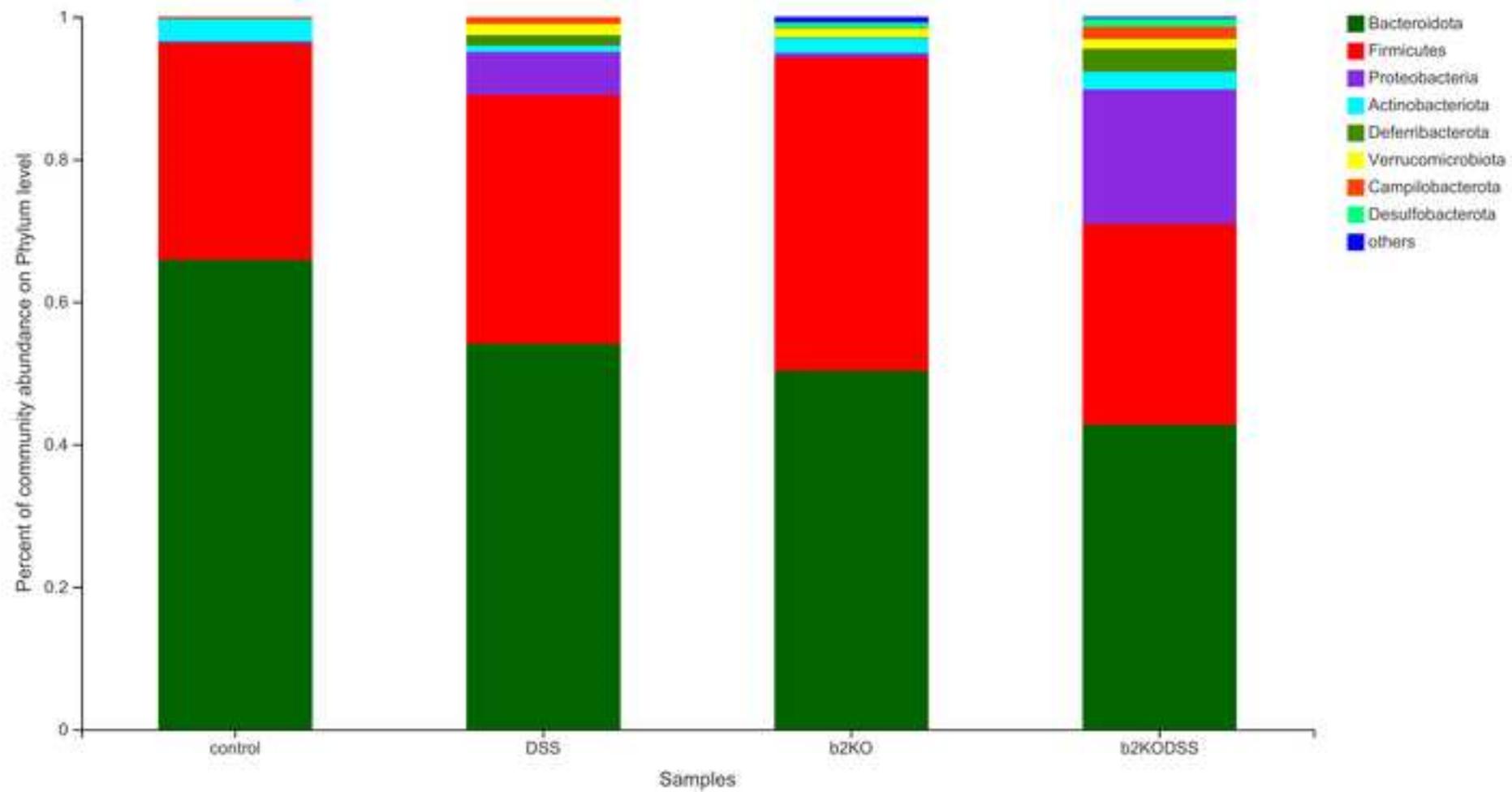


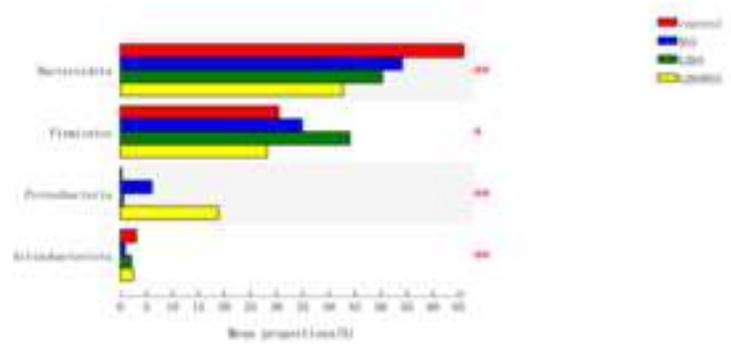


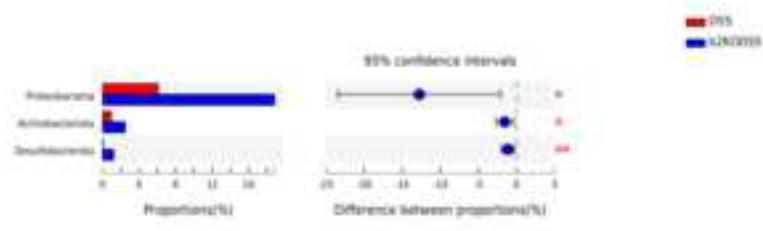


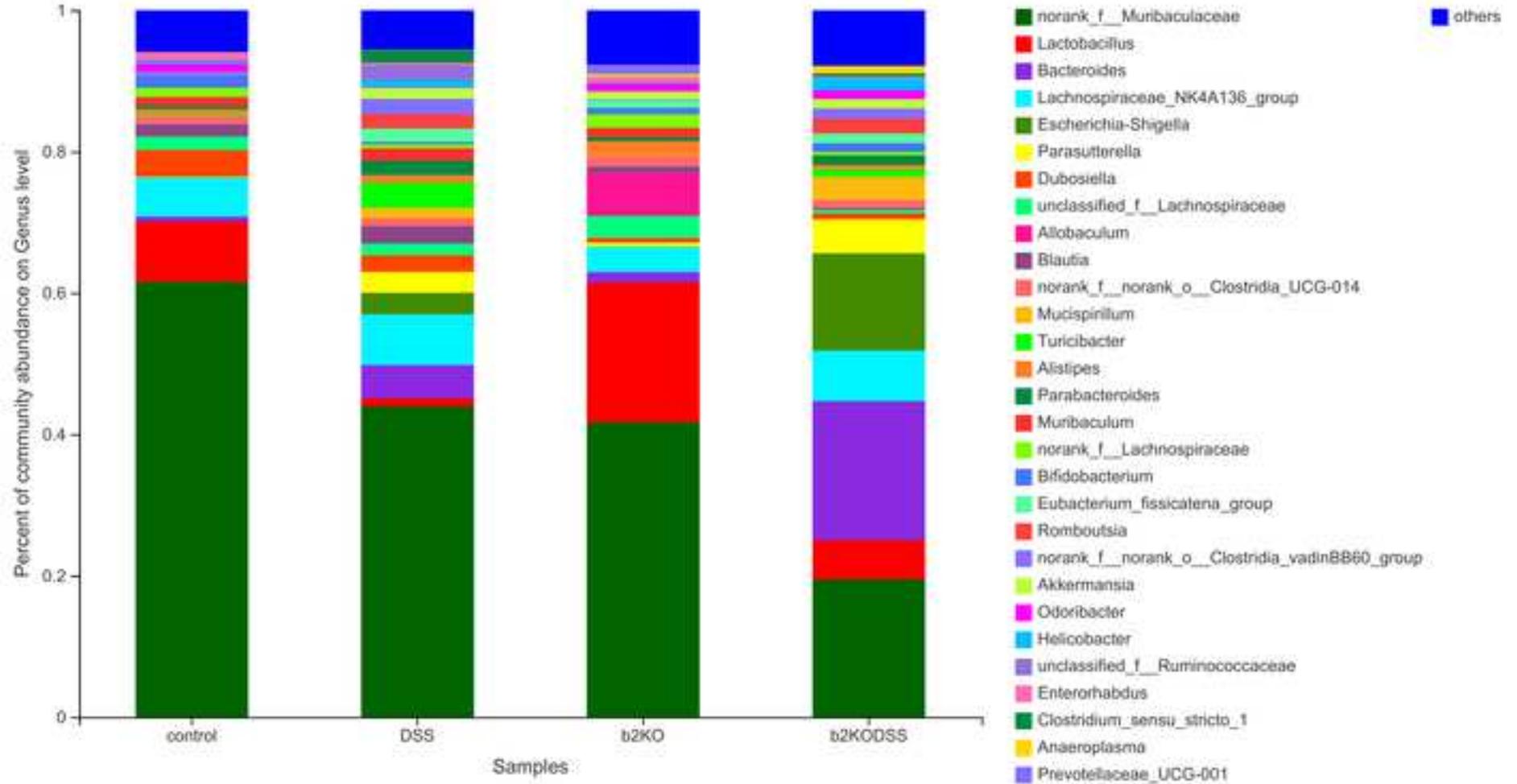


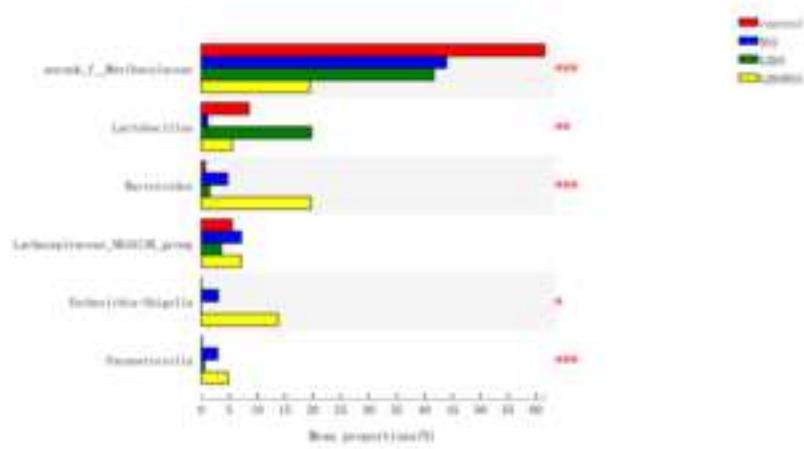


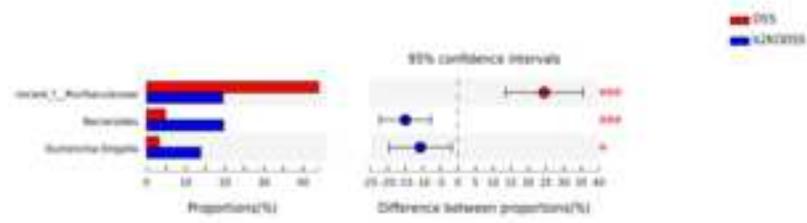


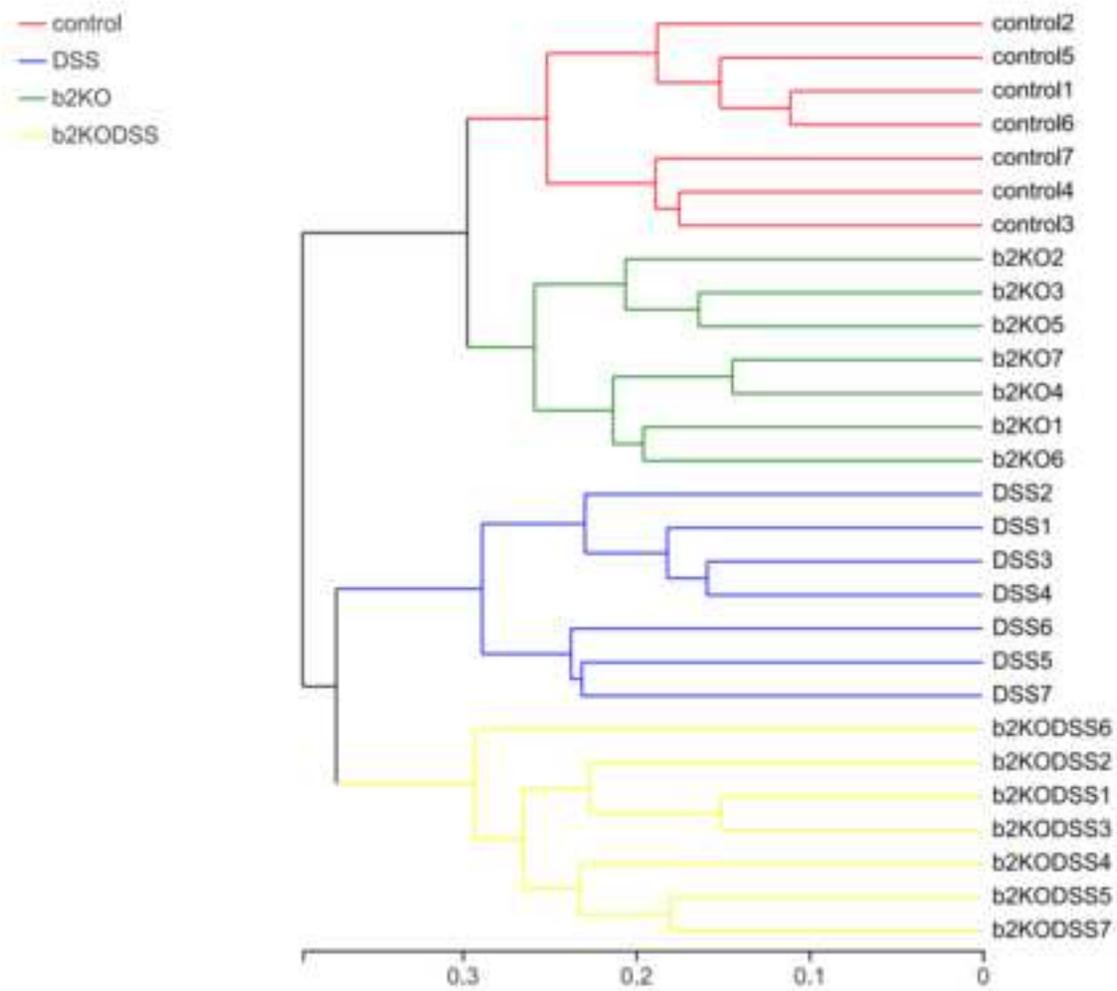


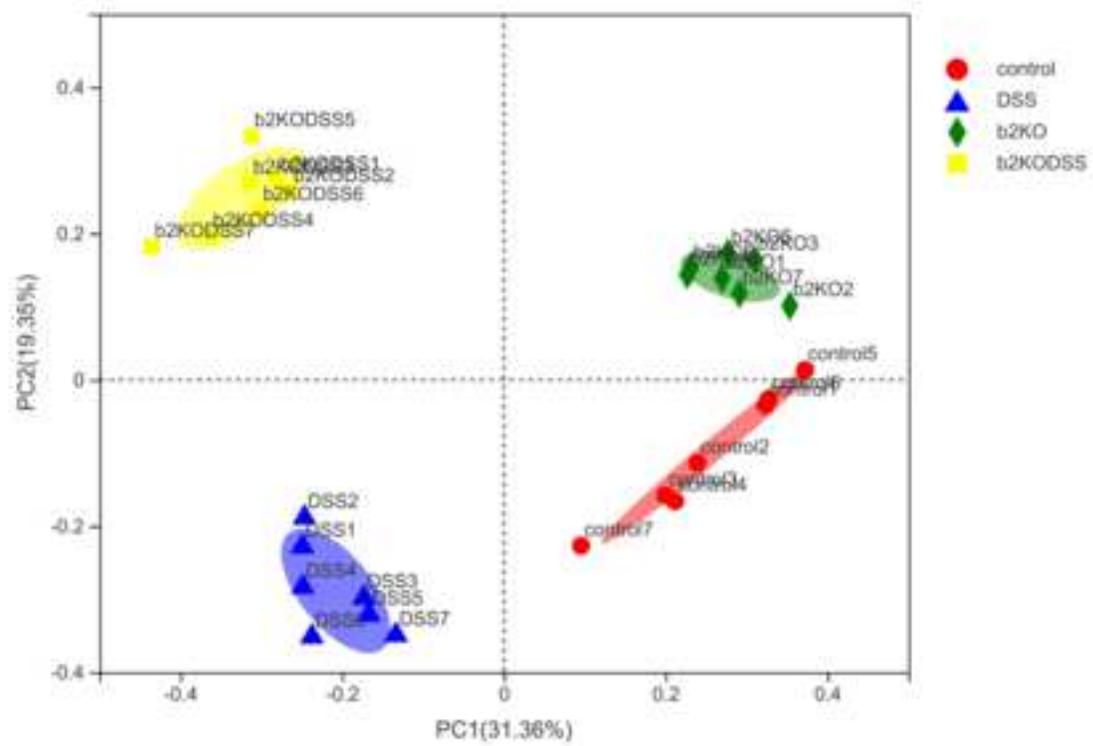


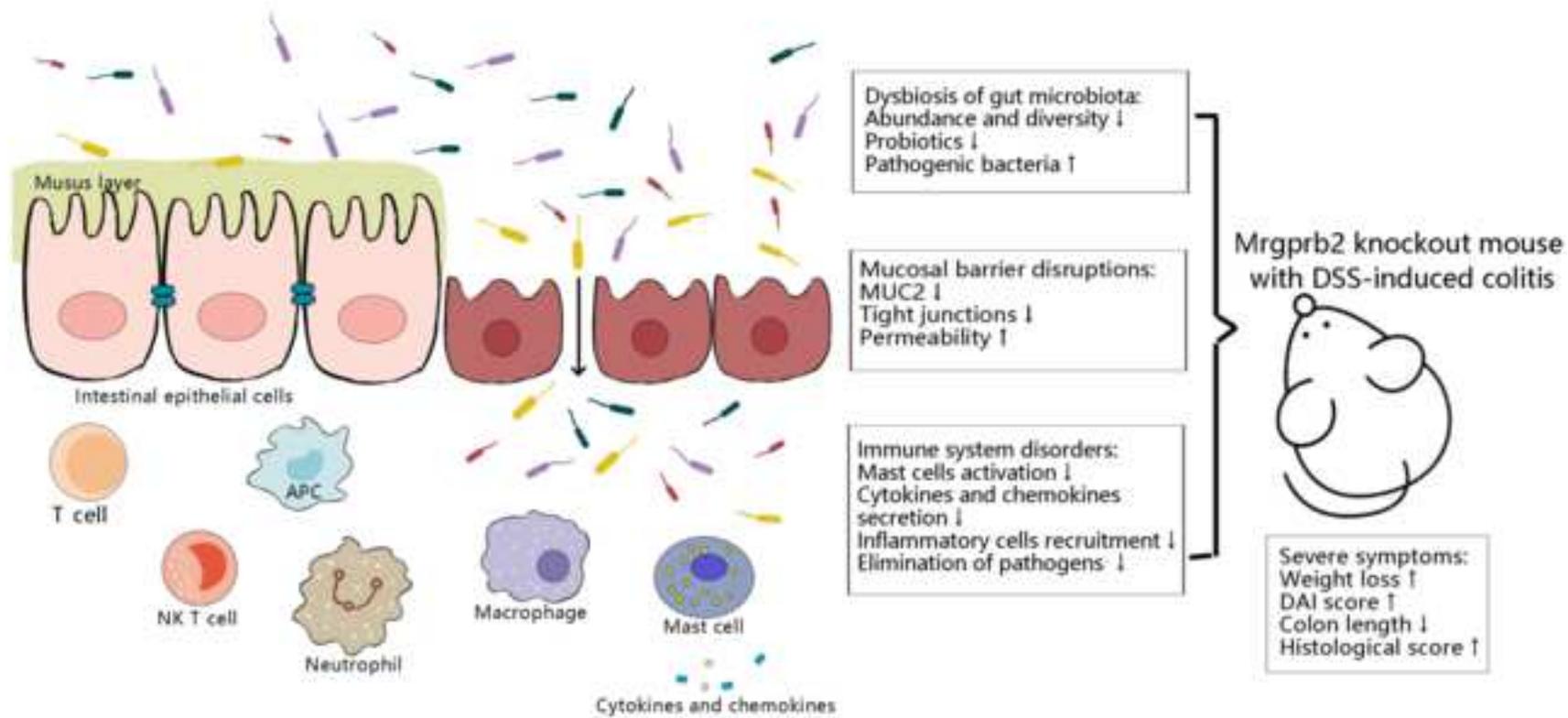












Name	Sequence (5' → 3')	Target Products
M-GAPDH-S	CCTCGTCCCGTAGACAAAATG	GAPDH
M-GAPDH-A	TGAGGTCAATGAAGGGGTCGT	
M-mpo-S	CAATGTCTTCACCAACGCTTTC	MPO
M-mpo-A	AATGCCACCTTCCAACACGA	
M-MUC2-S	CCTGTCCCGACTTCAACCCA	MUC2
M-MUC2-A	TGACGGAGACAGCAGAGCAAG	
M-occludin-S	CACCTCCTTACAGACCTGATGAAT	occludin
M-occludin-A	AGCCACCTCCGTAGCCAAA	
M-JAM-A-S	CGTGACAGCCTTTGATAGTGGTG	JAM-A
M-JAM-A-A	TGGGCTGGCTGTAATGACC	
M-ZO1-S	GGGAAAACCCGAAACTGATG	ZO-1
M-ZO1-A	GCTGTACTGTGAGGGCAACG	
M-claudin3-S	GAGTGCTTTTCCTGTTGGCG	claudin3
M-claudin3-A	GTAGTCCTTGCGGTCGTAGG	
M-Cdh1-S	ATGAAGGCGGGAATCGTG	E-cadherin
M-Cdh1-A	CCGTAGAAACAGTAGGAGCAGC	
M-Rcan1-S	TGGCTGGAAACAAGTAGAAGATG	CSP-1
M-Rcan1-A	GTCTCCGTGTCTGGATGATTTG	