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TRIP6 deficiency inhibits inflammatory damage in a murine model of DSS-induced colitis

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Abstract

Background: TRIP6 is a zyxin family member that serves as an adaptor protein to regulate diverse biological processes. In prior reports, TRIP6 was shown to play a role in regulating inflammation by inducing the activation of the nuclear factor-κB (NF-κB) signaling cascade, though its specific role *in vivo* in inflammatory contexts remains to be clarified. Herein, we therefore employed TRIP6-deficient ($TRIP6^{-/-}$) mice in order to explore the mechanistic importance of TRIP6 in a dextran sodium sulfate (DSS)-induced model of murine colitis.

Findings: Wild-type ($TRIP6^{+/+}$) mice developed more severe colitis following DSS-mediated disease induction relative to $TRIP6^{-/-}$ mice, as evidenced by more severe colonic inflammation and associated crypt damage. From a mechanistic perspective, TRIP6 expression in wild-type mice was significantly elevated in the period of DSS treatment; TRIP6 deficiency was associated with the inhibition of IκBα degradation, thus suppressing NF-κB signaling activity and impairing the secretion of proinflammatory cytokines such as TNFα and IL-6.

Conclusions: Together, these *in vivo* data demonstrate that TRIP6 serves as a positive regulator of DSS-induced colitis by regulating inflammatory NF-κB signaling, highlighting its therapeutic promise as a protein that theoretically can be targeted to prevent or treat colitis.

Keywords

Inflammation, colitis, TRIP6, NF-κB signaling

Background

Inflammatory bowel disease (IBD) is a term used to refer to chronic inflammatory diseases affecting the colon and intestines, including both Crohn's disease (CD) and ulcerative colitis (UC) [1]. The etiological basis for IBD is complex and influenced by a range of host genetic factors, immunological dysfunction, impaired function of the intestinal epithelial barrier dysfunction, and environmental perturbations [2, 3]. While a number of anti-inflammatory drugs have been developed to facilitate IBD treatment [4], the majority of affected patients fail to achieve long-lasting relief. Genome-wide association studies related analyses have led to the identification of many genes associated with IBD susceptibility loci, including NOD2, IL23R, and ATG16L1 [5-7]. We have also recently demonstrated that the orphan nuclear receptor superfamily protein Nur77 is downregulated in colon tissue samples from UC and CD patients, with Nur77-knockout mice being more susceptible to colitis [8]. These recent research advances highlight promising novel candidate biomarkers with the potential to guide future IBD patient diagnosis and treatment.

Thyroid receptor interactor protein 6 (TRIP6, also known as ZRP-1 or OIP1), is a member of the zyxin LIM protein family that contains an N-terminal proline-rich region as well as three C-terminal cysteine-rich zinc finger domains [9]. TRIP6 functions as an adaptor protein and thereby controls processes including transcription, motility, and cytoskeletal remodeling by interacting with a variety of proteins [10-13]. For example, by interacting with the cytoplasmic receptor protein LPA2, TRIP6 can enhance lysophosphatidic acid-induced cellular migratory activity [14]. TRIP6 interactions with supervillin (SV) in the cytoplasm can also facilitate focal adhesion formation [15]. In the nucleus, TRIP6 can interact with glucocorticoid receptor (GR) to control gene transcription [13] Recent evidence also highlights the importance of

TRIP6 as a regulator of inflammatory signaling, in part owing to its ability to interact via its LIM domains with RIP2 and to thereby drive the activation of NF-κB in a manner that can be influenced by TNF and IL-1 [16]. TRIP6 can also inhibit the ability of A20 to bind to TRAF6, in turn enhancing TRAF6-mediated NF-κB activation [17]. In the glomeruli of streptozotocin-challenged mice, the expression of TRIP6 is significantly increased, and its overexpression can promote fibrosis and inflammation in podocytes exposed to high-glucose conditions [18]. Together, these prior results thus suggest that TRIP6 is an important regulator of inflammation and associated diseases processes. The functional importance of TRIP6 in the context of colitis, however, remains unknown.

As such, we herein analyzed the phenotypic characteristics of TRIP6$^{-/-}$ and wild-type (WT; TRIP6$^{+/+}$) mice in the context of a DSS-induced colitis model system. Through a series of experiments, we found that TRIP6$^{-/-}$ mice exhibited a less serious disease phenotype relative to WT controls, consistent with a role for TRIP6 as a regulator of colitis.

Materials and methods

Antibodies and reagents

Anti-IκBα was from Cell Signaling Technology (MA, USA). Anti-β-actin were purchased from Sigma-Aldrich (Sigma-Aldrich, MO, USA). Anti-TRIP6 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-CD45, anti-CD11b, anti-CD4, anti-F4/80, and anti-B220 were from BD Bioscience (CA, USA). DSS was purchased from MP Biomedicals (USA). LIVE/DEAD™ Fixable Violet Dead Cell Stain Kit was from Invitrogen (USA).

Mice

TRIP6$^{+/+}$ and TRIP6$^{-/-}$ mice were obtained from Cyagen (Taicang, Jiangsu, China) and were housed under specific pathogen-free conditions with a 12 h light/dark cycle and free access to standard food and water at the Laboratory Animal Center in Soochow University (China). The Animal Care and Use Committee of Soochow University approved all animal studies.

Tissue Collection and Assessment

Tissue samples collected from TRIP6$^{+/+}$ and TRIP6$^{-/-}$ mice for analysis included the colon, mesenteric lymph nodes, spleen, and peripheral lymph nodes. Spleen weight, colon length, and the numbers of cells in the mesenteric and peripheral lymph nodes were quantified.

DSS-induced Colitis Modelling

Mice (8-10 weeks old) were administered 2.5% DSS in their drinking water for 7 days, followed by a 6-day recovery period during which they were administered normal

water. All mice were weighed daily, and were euthanized at experimentally appropriate time points. Colon tissue samples were then collected, and colitis severity was assessed by staining paraffin-embedded colon sections with hematoxylin and eosin (H&E), with inflammation and crypt damage being quantified and given a score ranging from 0 to 4.

Histological Staining

H&E staining was performed as in previous reports [19].

qPCR

Trizol LS (Invitrogen) was used to extract total RNA from appropriate samples, after which a RevertAid™ First Strand cDNA Synthesis Kit (Fermentas) was used to synthesize cDNA. qPCR was then performed with a SYBR Green Power Master Mix based upon provided instructions (Applied Biosystems), using the primers listed in Table 1.

Western blotting

Samples of full-thickness colon tissue were homogenized in a 1 mL volume of cell lysis buffer supplemented with a protease inhibitor cocktail (Roche) for 30 min on ice, after which homogenates were centrifuged for 20 min at 13,000 xg at 4°C. Equal amounts of protein were then separated via 10% SDS-PAGE and transferred to PVDF membranes, after which protein expression was detected using appropriate antibodies, with signaling being visualized with an ECL system.

ELISAs

Serum TNFα and IL-6 levels were assessed using mouse-specific ELISA kits (R&D

Systems) based on provided directions.

Flow Cytometry

Colon tissue samples were isolated, rinsed three times using cold PBS, and digested for 45 min at 37°C in DMEM containing 2% FBS, collagenase IV (1mg/mL; Sigma-Aldrich), and DNase I (10 U/mL; Roche). The resultant digest was then passed through a 70-μm mesh filter to generate a single-cell suspension, which was stained with LIVE/DEAD, anti-CD45, anti-CD11b, anti-F4/80, anti-CD4, anti-CD8, and anti-B220 prior to analysis using a BD LSR Fortessa™ Flow Cytometer. All data were analyzed using NovoExpress or FlowJo X (TreeStar, CA, USA).

Statistical Analysis

Data are given as means±SD. Experiments were repeated in triplicate and analyzed using Prism 5.03 software (GraphPad Software), with $p < 0.05$ as the significance threshold(*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

Results and Discussion

Recent research evidence suggests that TRIP6 can control inflammatory signaling by modulating NF-κB activation [16-18], yet its *in vivo* function in inflammatory contexts has yet to be evaluated. Herein, we thus examined the functional role of TRIP6 *in vivo* by evaluating inflammatory and immune cells within the immunological organs of WT and TRIP6$^{-/-}$ mice at 3 months of age. Relative to WT (TRIP6$^{+/+}$) mice, TRIP6$^{-/-}$ animals did not exhibit any changes in cell numbers in their mesenteric lymph nodes (Fig. 1a), peripheral lymph nodes (Fig. 1b), or spleens (Fig. 1c). There were also no observed differences in colon length when comparing TRIP6$^{+/+}$ and TRIP6$^{-/-}$ mice (Fig. 1d). This suggests that TRIP6 deficiency on its own has no adverse impact on normal physiological function in these mice. To establish whether the loss of TRIP6 would alter inflammatory or immune cell activity in further detail, we performed a flow cytometry-mediated analysis of these cell populations within colon tissue samples from WT and TRIP6$^{-/-}$ mice. His analysis revealed no significant differences in the frequencies of colon-infiltrating macrophages (F4/80^{+} and CD11b^{+}), T cells (CD4^{+} and CD45^{+}), or B cells (CD45^{+} and B220^{+}) when comparing these groups (Fig. 1e-g). TRIP6 deficiency also had no impact on colon macrophage-mediated TNFα secretion (Fig. 1h). Together, these results suggested that TRIP6 deficiencies had no adverse impact on inflammation or immune function under normal physiological conditions.

Next, we assessed the role of TRIP6 in the context of inflammatory pathology by establishing a DSS-induced model of murine colitis. To that end, WT and TRIP6$^{-/-}$

mice were administered DSS-containing drinking water (2.5% w/v) for 7 days, after which a recovery period during which they were administered regular drinking water for 6 days was conducted, with body weight being recorded daily. By day 3 following DSS treatment, WT animals began to lose more weight than did TRIP6-deficient mice, with this trend remaining evident through day 13 (Fig. 2a). Consistent with this result, WT mice exhibited a significant reduction in colon length as compared to TRIP6$^{-/-}$ mice (Fig. 2b). Histological analyses further indicated that WT animals exhibited significantly higher pathological scores consistent with more severe inflammation and crypt damage as compared to TRIP6$^{-/-}$ mice on days 7 and 13 of the study period (Fig. 2c-d). These findings suggest that TRIP6 can serve as a positive regulator capable of promoting colitis in this murine model system. Chronic inflammation is also closely associated with the risk of colorectal oncogenesis [20], and our findings align well with recent research evidence stressing the oncogenic role of TRIP6 in colon cancer [21].

Unexpectedly, we found that TRIP6 expression was regulated by DSS exposure. Indeed, TRIP6 protein levels rose markedly in the colon tissues of WT mice over the course of DSS administration (Fig. 3a), further supporting a role for this adaptor protein in the context of colitic disease pathogenesis. These results are in line with prior evidence suggesting that ectopic TRIP6 expression can reverse the impact of PTPN14 silencing-mediated inhibition of fibrosis and inflammatory responses [18].

Prior evidence indicates that TRIP6 can activate inflammatory NF-κB signaling, thereby promoting resistance to apoptotic death and cellular invasion *in vitr*o [16].

Whether TRIP6 can similarly regulate the NF-κB pathway *in vivo,* however, remains to be conclusively demonstrated. We found that the loss of TRIP6 expression in mice was associated with the pronounced inhibition of IκBα degradation over the course of DSS treatment and recovery (Fig. 3b), consistent with a role for TRIP6 as an enhanced of *in vivo* NF-κB signaling in these model of experimental colitis. Inflammatory cytokine and chemokine production is a hallmark of colitis [22]. We thus further assessed TNFα and IL-6 expression in these experimental animals, revealing that TRIP6$^{-/-}$ mice exhibited decreased colon tissue TNFα and IL-6 mRNA levels relative to WT controls (Fig. 3c). We similarly observed significant reductions in serum TNFα and IL-6 levels in TRIP6$^{-/-}$ mice relative to WT animals during the DSS treatment and recovery periods (Fig. 3d). These data thus demonstrate that TRIP6$^{+/+}$ mice are highly susceptible to DSS-induced colitis, whereas the loss of TRIP6 expression compromises inflammatory signaling activity in this pathological context.

Together, our data offer robust experimental evidence for the role of TRIP6 as a positive regulator of tissue pathology in the DSS-induced colitis model system wherein it functions by promoting NF-κB signaling pathway activation. In summary, our results highlight a previously unknown role for TRIP6 as a regulator of intestinal homeostasis and underscore its promise as a therapeutic target in colitis patients.

Declarations

Ethical Approval and Consent to participate

The study was approved by The Animal Care and Use Committee of Soochow University.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

HW designed the experiments, analyzed data and prepared the manuscript. YY, XML, YL, WLY, XMY, YW and YXL performed the experiments. JRW and WJG contributed to the data analysis. All authors read and approved the final manuscript.

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Figure legends

Fig. 1 TRIP6-knockout mice do not exhibit altered inflammatory activity or immune functionality under normal physiological conditions. (a-c) Gross appearance (left) and cell numbers (right) for mesenteric lymph nodes (a), peripheral lymph nodes (b), and spleens (c) from 3-month-old TRIP6$^{+/+}$ and TRIP6$^{-/-}$ mice (n = 4/group). **(d)** Representative colon images (left) and colon length measurements (right) for 3-month-old TRIP6$^{+/+}$ and TRIP6$^{-/-}$ mice (n = 4/group). **(e-g)** Flow cytometry was

used to analyze colon-infiltrating macrophages (e), T cells (f), and B cells (g) in 3-month-old TRIP6$^{+/+}$ and TRIP6$^{-/-}$ mice (n = 6/group), with representative plots being shown. **(h)** TNFα expression in colon-infiltrating macrophages from 3-month-old TRIP6$^{+/+}$ and TRIP6$^{-/-}$ mice (n = 6/group) was assessed via flow cytometry, with representative plots being shown.

Fig. 2 Knocking out TRIP6 enhances murine resistance to DSS-induced colitis. (a) Weight loss was monitored in TRIP6$^{+/+}$ and TRIP6$^{-/-}$ mice relative to baseline following treatment with 2.5% DSS and a subsequent 6-day recovery period during which they were allowed to drink regular water (n = 12/group). Data are means ± SD. *p <0.05; **p<0.01. **(b)** Representative images of colon tissues (left) and reduced colon length (right) were assessed on days 5 and 13 following DSS treatment. Data are means ± SD. **p<0.01; ***p<0.001. **(c)** H&E staining was used to assess WT and TRIP6$^{-/-}$ murine colon tissue sections on days 5 and 13 following DSS treatment, with representative images being shown. **(d)** Inflammation, depth of inflammation, and crypt damage were scored for samples of colon tissue from TRIP6$^{+/+}$ and TRIP6$^{-/-}$ mice following treatment with DSS.

Fig. 3 TRIP6 deficiency impairs inflammatory NF-κB signaling activity in a DSS-induced model of experimental murine colitis. (a) Western blotting was used to assess the expression of TRIP6 in colon tissues from wild-type mice following DSS treatment, with representative images being shown. **(b)** Western blotting was used to assess the expression of IκBα in colon tissues from TRIP6$^{+/+}$ and TRIP6$^{-/-}$ mice following DSS treatment, with representative images and being shown (left). Protein levels of IκBα were quantified by using densitometry and normalized to those of

β-actin for comparison. Data are means ± SD. n = 3, *$p < 0.05$ and ***p<0.001. **(c-d)** qPCR (c) and ELISAs (d) were respectively used to evaluate TNFα and IL-6 mRNA levels in colon tissues and serum TNFα and IL-6 levels in TRIP6$^{+/+}$ and TRIP6$^{-/-}$ mice over the course of DSS induction and recovery. Data are means ± SD. **p <0.01 and ***p<0.001. *ns*, not significant.

Figures

Figure 1

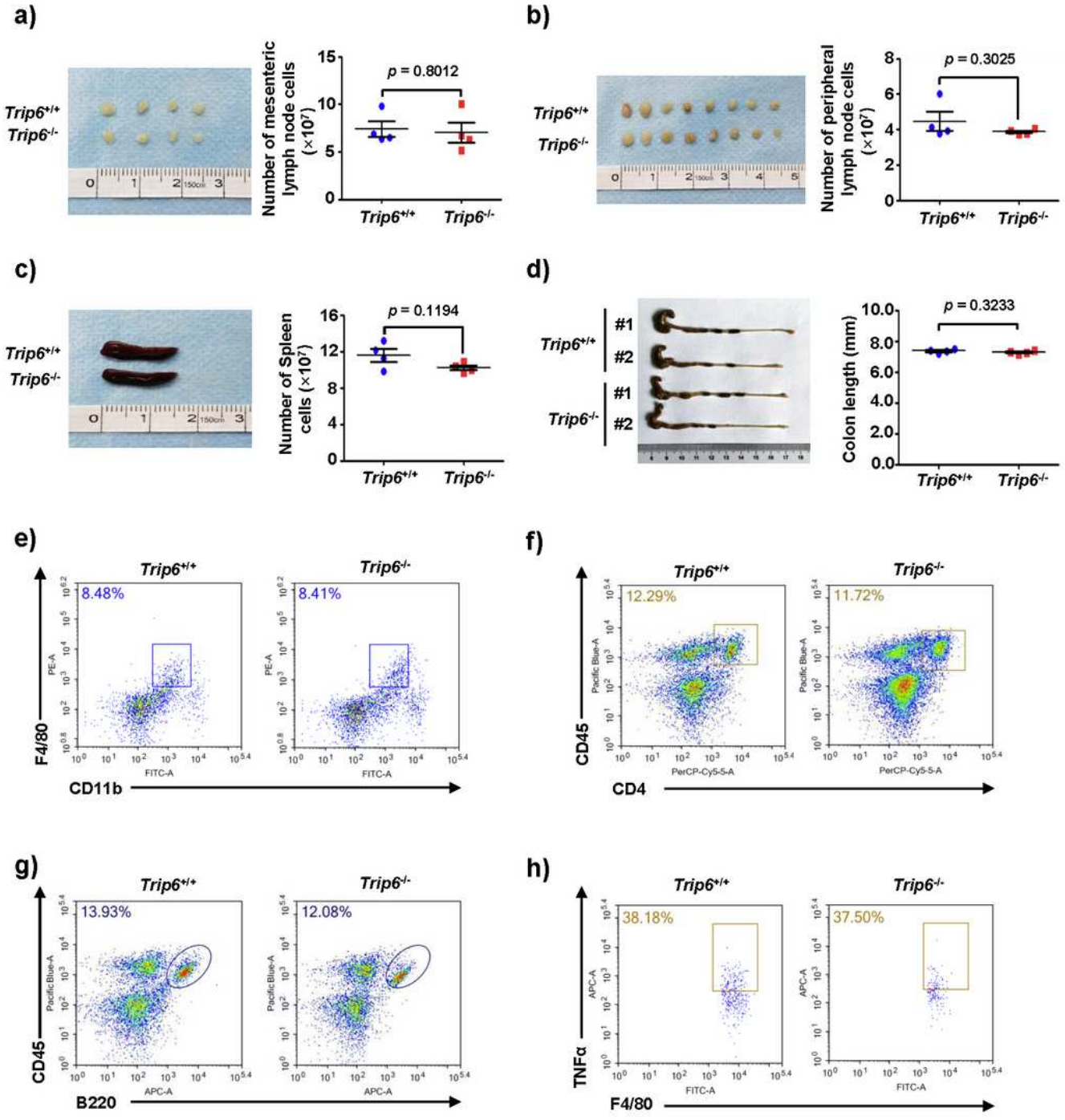

Figure 1

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Figure 2

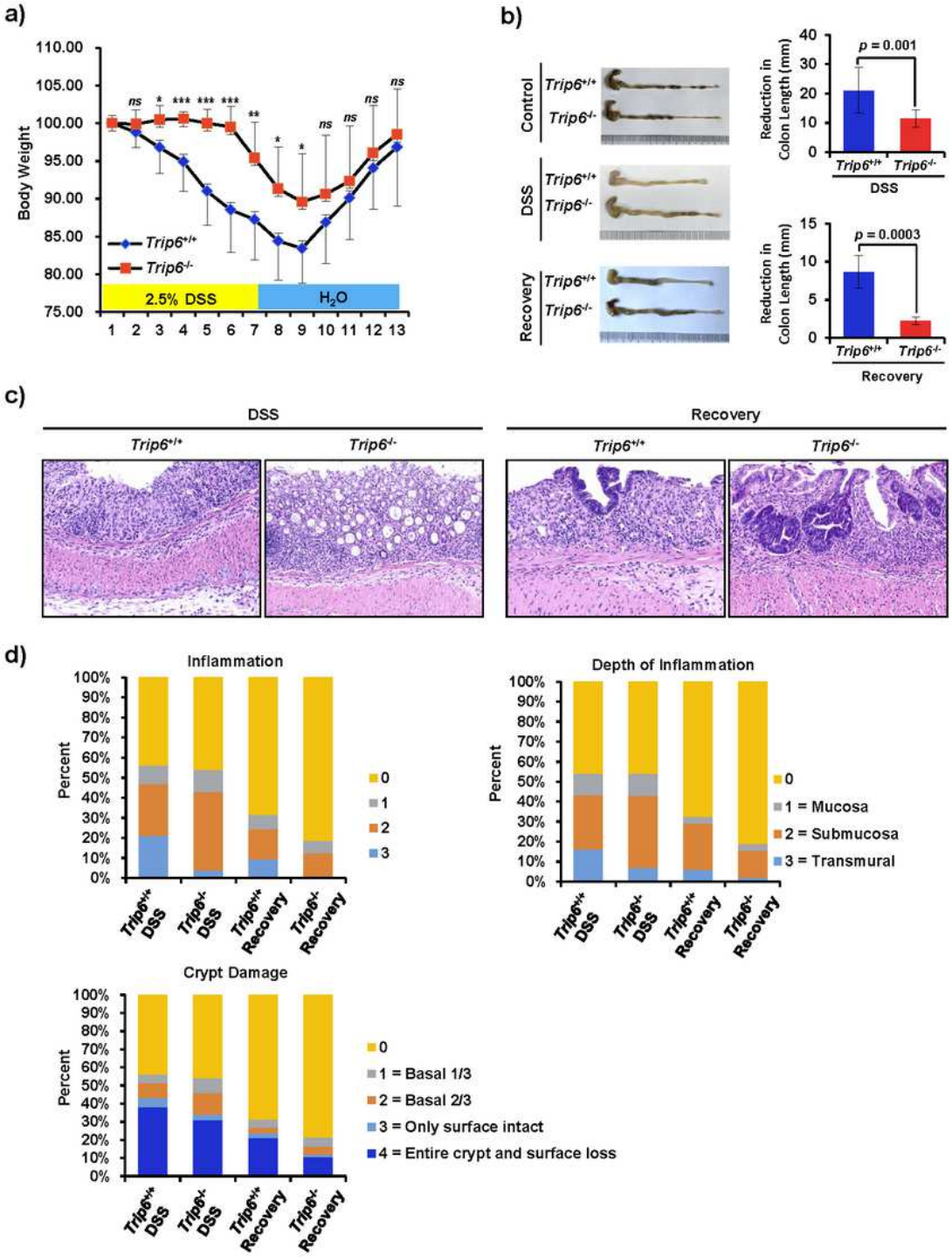

Figure 2

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Figure 3

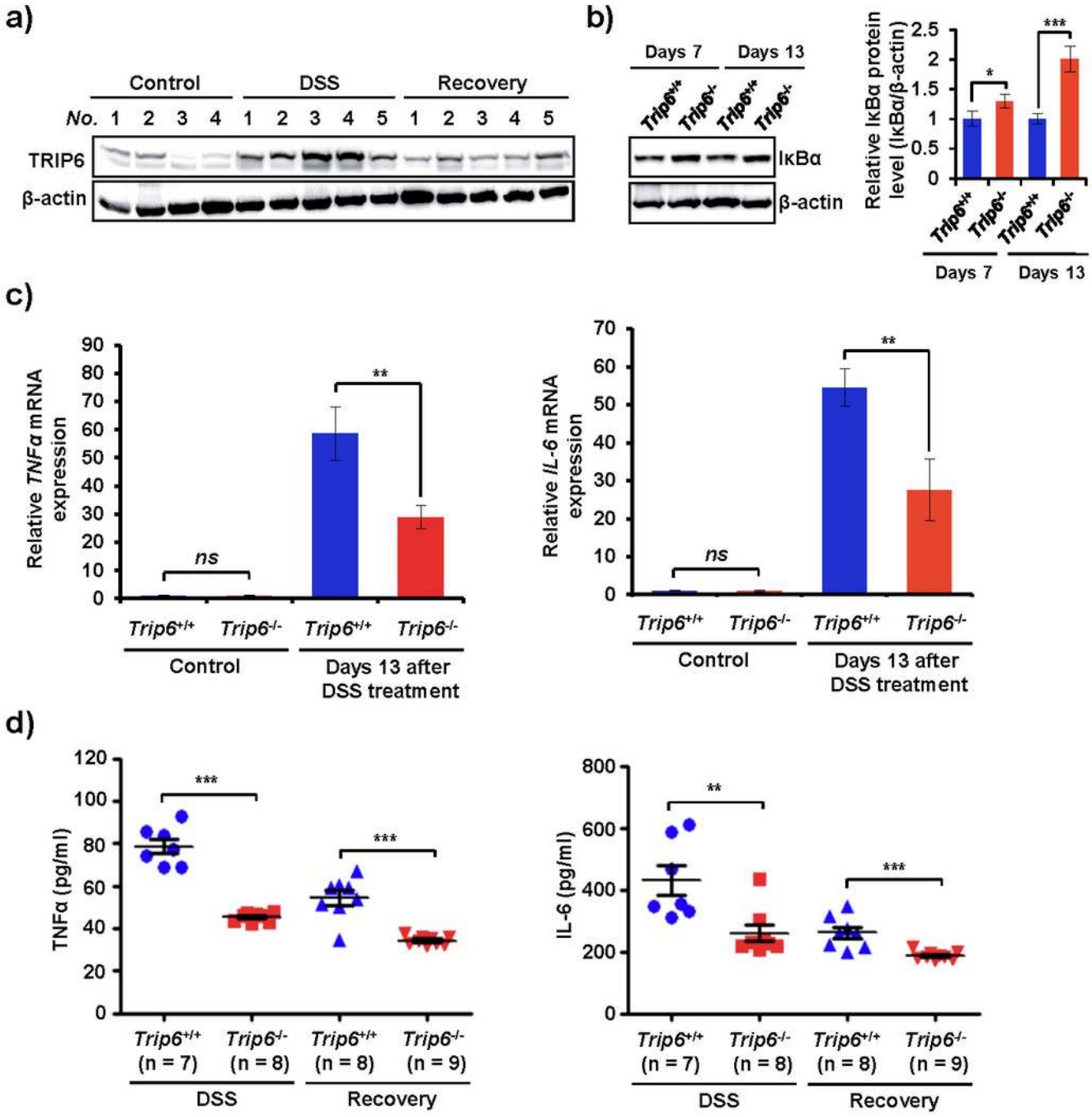

Figure 3

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