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Original Article

Original Article

RTCB deficiency triggers colitis in mice by influencing the NF-κB and Wnt/β-catenin signaling pathways

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Abstract

RNA terminal phosphorylase B (RTCB) has been shown to play a significant role in multiple physiological processes. However, the specific role of RTCB in the mouse colon remains unclear. In this study, we employ a conditional knockout mouse model to investigate the effects of RTCB depletion on the colon and the potential molecular mechanisms. We assess the efficiency and phenotype of *Rtcb* knockout using PCR, western blot analysis, histological staining, and immunohistochemistry. Compared with the control mice, the *Rtcb*-knockout mice exhibit compromised colonic barrier integrity and prominent inflammatory cell infiltration. In the colonic tissues of *Rtcb*-knockout mice, the protein levels of TNF-α, IL-8, and p-p65 are increased, whereas the levels of IKKβ and IκBα are decreased. Moreover, the level of GSK3β is increased, whereas the levels of Wnt3a, β-catenin, and LGR5 are decreased. Collectively, our findings unveil a close association between RTCB and colonic tissue homeostasis and demonstrate that RTCB deficiency can lead to dysregulation of both the NF-κB and Wnt/β-catenin signaling pathways in colonic cells.

Key words RTCB, colitis, NF-κB, Wnt/β-catenin, LGR5

Introduction

RNA terminal phosphate cyclase B (RTCB), also known as FAAP, HSPC117, C22orf28, and D10Wsu52e, is a ligase that specifically connects the 2′,3′-cyclic phosphate and 5′-hydroxyl terminus of RNA [1,2]. RTCB is a highly conserved protein with a unique structure. It is present in archaea, bacteria, and animals but absent in plants and fungi [3]. In archaea and mammals, RTCB appears to serve as a catalyst in tRNA splicing [2,4]. In eukaryotes, RTCB is also involved in the unfolded protein response (UPR) by influencing the splicing of *Xbp1* mRNA [5].

RTCB has been shown to play a significant role in multiple physiological processes. In embryonic development, inhibition of RTCB in mouse embryos results in embryonic death and placental dysplasia [6]. In reproductive function, while worms can grow to adulthood but fail to generate oocytes after knockdown of *Rtcb* [7], mice have premature ovarian failure after conditional knockout of *Rtcb* [8], and RTCB is specifically highly expressed in the initial segment of the mouse epididymis [9]. In immune function, specific knockout of *Rtcb* in mouse B cells leads to abnormal cell proliferation and differentiation and disrupts the structure of the endoplasmic reticulum (ER), ultimately affecting the secretion of some antibodies [5]. In neuroprotection, RTCB could ameliorate damage to dopaminergic neurons in a worm model of Parkinson's disease [10]. A previous study showed that RTCB is widely expressed in intestinal tissues [11]; however, its function is unknown.

Colitis is an inflammatory disease of colonic tissues induced by various factors. Inflammation extends from the rectum to the proximal colon from onset, causing persistent superficial mucosal inflammation of varying degrees [12]. Inflammatory bowel disease (IBD) includes ulcerative colitis (UC) and Crohn's disease (CD). UC is most common in industrialized countries, and its incidence has been increasing in Asia [13,14]. The total incidence and prevalence of UC are reported to be 0.0012%–0.0203% and 0.0076%–0.245% per year, respectively [15]. The differences in incidence among races are more related to environment, diet, and lifestyle. There is no significant sex difference in UC patients [16]. The onset time of UC has a bimodal distribution, with the first peak at 20–30 years old

and the second peak at 50–80 years old [17]. The MAPK, NF-κB and Wnt/β-catenin signaling pathways play pivotal roles in the pathogenesis of colitis and its associated disorders [18,19]. Alterations in these signaling pathways are commonly concurrent with the onset of colitis [20,21].

Ubiquitin is a small protein that is very conserved in eukaryotes [22]. It has been shown that the promoter of ubiquitin C (*UBC*) is highly active in conferring expression of exogenous genes in various cell lines after transient transfection of appropriate expression vectors [23]. Most importantly, the *UBC* promoter retains high activities in tissues where some other promoter or enhancer sequences are inactive [24]. Cre-ERT2 is a fusion protein composed of Cre recombinase and a mutant form of the estrogen receptor that is selectively activated only in the presence of tamoxifen [25] Therefore, in *UBC-CreERT2* transgenic mice, interest genes controlled by the Cre cleavage site can be knocked out in adulthood under tamoxifen induction.

The aim of this study was to explore the function of RTCB and its molecular basis in the colon. Therefore, we constructed a conditional *Rtcb*-knockout mouse model using the *UBC-CreERT2* tool mouse and observed lesions in the colon tissues after *Rtcb* knockout. Interestingly, the colitis caused by RTCB deficiency may not depend on the XBP1-UPR pathway but on the NF-κB and Wnt/β-catenin signaling pathways.

Materials and Methods

Generation of mice model

Loxp sites were inserted on both sides of the fourth exon of the *Rtcb* gene to generate $Rtcb^{f/f}$ homozygous mice. In addition, Loxp sites were inserted on both sides of the first to fifth exons of the *Xbp1* gene to generate $Xbp1^{f/f}$ homozygous mice. $Rtcb^{f/f}$ and $Xbp1^{f/f}$ mice were purchased from GemPharmatech (Nanjing, China). The *UBC-CreERT2* mice were separately bred with both types of mice to generate $Rtcb^{f/f}$;*UBC-CreERT2* and $Xbp1^{f/f}$;*UBC-CreERT2* homozygous knockout mice. To activate CreERT2 for Rtcb knockout, these mice were intraperitoneally injected with tamoxifen (TAM, 20 mg/mL; Sigma-Aldrich, St Louis, USA) at 75 mg/kg for five consecutive days. The physiological conditions of the mice were monitored. The experiments were conducted immediately after the mice were deceased.

All mice (C57BL/6J background) used were adult males at 6 weeks after birth. The mice were raised in the animal house of Shandong Normal University with free access to food and water. The environment was a 12-h light-dark cycle system (light from 7:00 to 19:00) with a temperature of 21–24°C and humidity of approximately 55%. The mouse feed was provided by Jinan Xingkang Biotechnology (Jinan, China). This study was approved by the Animal Ethics Committee of Shandong Normal University (Approval No. AEECSDNU 2021026).

Genotyping

We generated $Rtcb^{f/f}$;*UBC-CreERT2* transgenic mice allowing for conditional deletion of *Rtcb* in adult mice. $Rtcb^{f/f}$ mice were crossed with *UBC-CreERT2* mice to obtain $Rtcb^{f/f}$;*UBC-CreERT2* mice in the offspring (Supplementary Figure S1A). To determine the genotype of the mice, the primers loxP1, loxP2, loxP3, Frt3, Frt4, Frt5, and Frt6 were designed based on the loxP sites (Supplementary Figure S1B). To detect the presence of UBC-CreERT2, the primers Universal-Cre-F and Universal-Cre-R were designed. To verify the efficiency of knockout, the primers loxP5 and Frt2 were designed. Genotyping of the mice was performed using PCR analysis, and the genotype of $Rtcb^{f/f}$;*UBC-CreERT2* transgenic mice was determined based on the size of the PCR product bands (Supplementary Figure S1C). The PCR procedure was as follows: predenaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 45 s, denaturation at 60°C for 45 s, and extension at 72°C for 30 s, followed by incubation at 72°C for 8 min and retention at 10°C. The sequences of the primers and the sizes of their products are shown in Table 1.

Western blot analysis

Colon tissues from TAM-treated mice were isolated and lysed in RIPA lysis buffer (150 mM NaCl, 1% NP-40, 0.5% deoxycholic acid, 0.1% SDS, 50 mM Tris, pH 7.4, and 1% PMSF). The lysates were then sonicated and centrifuged. Protein concentrations were quantified using a BCA Protein Concentration Determination Kit (AR0146; Boster, Wuhan, China). Equal amounts of protein samples were separated by 10%, 12.5% or 15% SDS-PAGE and transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 8% nonfat milk and incubated with primary antibodies at 4°C overnight and then with the appropriate

Table 1. The sequences of the primers used for genotyping

Primer pair	Sequence (5′→3′)	Fragment length	
		WT	Targeted
loxP1/loxP2	loxP1: TTTGCTGTGCTTCATGTCTC	0 bp	370 bp
	loxP2: ATGGCGAGCTCAGACCATAAC		
loxP2/loxP3	loxP2: ATGGCGAGCTCAGACCATAAC	0 bp	468 bp
	loxP3: ATCAGTGGGTCACTCAGAAGCTG		
Frt3/Frt4	Frt3: GTCTCTGTTGATCAGACATAGC	304 bp	493 bp
	Frt4: ACAAGCTCTCCTTGAGTTCTATG		
Frt5/Frt6	Frt5: TAGCAGCCCTCTGTCGTGATAC	493 bp	683 bp
	Frt6: GGGTGGCCTCTATGCTTAGTACCT		
Universal-Cre-F/R	F: ATTTGCCTGCATTACCGGTCG	0 bp	309 bp
	R: CAGCATTGCTGTCACTTGGTC		
loxP5/Frt2	loxP5: TCAATCCAGTCTCACCAACA	1237 bp	1406/624 bp
	Frt2: CTAGCCAAGCATGTCCTGTAG		

HRP-labelled secondary antibodies at room temperature for 1 h. The signals were developed using an Omni-ECL™ Femto Light Chemiluminescence Kit (SQ201; EpiZyme, Cambridge, USA). The images were captured with a Tanon-5200 imaging system (Tanon, Shanghai, China), and the grayscales of the blots were quantified using ImageJ software. β-Actin was used as the loading control.

Histology and immunohistochemistry

For histological analysis, the colon tissues were fixed with PFA and Bouin's solution, embedded in paraffin, cut into 5-μm sections, and finally stained with hematoxylin and eosin (H&E; Solarbio; Beijing, China).

For immunohistochemical analysis, the paraffin sections were deparaffinized and rehydrated using standard methods. The slides were treated as follows: placed in Tris-EDTA (pH 9.0) solution, heated at 95°C for 10 min, cooled at room temperature for 20 min, soaked in 3% H_2O_2 for 15 min, blocked with 1% BSA for 1 h, incubated with the primary antibody rabbit anti-E-cadherin (24E10; Cell Signaling, Danvers, USA) at 4°C overnight, incubated with the secondary antibody (AB0101; Abways, Shanghai, China) for 1 h, stained with the DAB Chromogen solution (ZLI-9018; ZSGB-BIO, Beijing, China), the Alcian Blue solution (G1027-100ML; Servicebio, Wuhan, China) for 15 min, and the Nuclear Fast Red solution (G1035-100ML; Servicebio) for 5 min, and finally observed under an E100 light microscope (Nikon, Tokyo, Japan).

Statistical analysis

Data are presented as the mean ± SEM of at least three independent experiments. The data obtained were analyzed by Student's *t* test using the GraphPad Prism 8.0 statistical software. $P < 0.05$ was considered statistically significant.

Results

TAM treatment results in weight loss and animal death of *Rtcb*$^{f/f}$;*UBC-CreERT2* mice

To induce *Rtcb* knockout, adult *Rtcb*$^{f/f}$ and *Rtcb*$^{f/f}$;*UBC-CreERT2* mice were intraperitoneally injected with TAM for 5 consecutive days. After TAM treatment, the mice were kept under normal conditions for 7 days. Their body weights were recorded daily (the weight on day 1 of injection was set as 100%). The *Rtcb*$^{f/f}$;*UBC-CreERT2* mice maintained a stable weight during the 5 days of TAM injection but suffered significant weight loss thereafter, whereas the *Rtcb*$^{f/f}$ mice maintained a stable weight throughout (Figure 1A). Furthermore, the *Rtcb*$^{f/f}$;*UBC-CreERT2* mice died after TAM treatment, whereas the *Rtcb*$^{f/f}$ mice survived (Figure 1B).

TAMs successfully induce *Rtcb* knockout in *Rtcb*$^{f/f}$;*UBC-CreERT2* mice

To check whether the *Rtcb* gene was knocked out in the transgenic mice treated with TAM, we extracted genomic DNA from various tissues, including the heart, liver, lung, kidney, and colon, of the *Rtcb*$^{f/f}$ and *Rtcb*$^{f/f}$;*UBC-CreERT2* mice and performed PCR analysis with specific primers (Table 1). In the samples obtained from the TAM-treated *Rtcb*$^{f/f}$ mice, only one DNA fragment of 1237 bp was detected. However, in the samples obtained from the TAM-treated *Rtcb*$^{f/f}$;*UBC-CreERT2* mice, two DNA fragments of 1406 bp and 624 bp were detected (Figure 2B). Furthermore, *Rtcb* knockout was confirmed at the protein level (Figure 2C,D). These results demonstrated that TAM treatment successfully induced *Rtcb* knockout in multiple tissues of *Rtcb*$^{f/f}$;*UBC-CreERT2* mice.

RTCB deficiency leads to colonic barrier damage in mice

Histological analysis was performed to examine the impact of RTCB deficiency on the colon. We dissected the TAM-treated mice and observed a shortened colon length accompanied by minor bleeding in the *Rtcb*$^{f/f}$;*UBC-CreERT2* mice but not in the *Rtcb*$^{f/f}$ mice (Figure 3A). H&E staining revealed that after *Rtcb* knockout, colonic tissues exhibited a significant loss of goblet cells and an occasional loss of epithelial cells in the mucosa, slight dilation and deformation of intestinal glands in the lamina propria, and scattered infiltration of inflammatory cells (Figure 3B).

Intestinal barrier homeostasis is closely associated with adherens junctions and tight junctions between intestinal epithelial cells (IECs). The formation of adherens junctions depends on the interaction between cadherins and catenins, with E-cadherin being a crucial molecule [26]. Then, we examined the adherent junctions of the colonic barrier using E-cadherin as a marker molecule. The results showed that RTCB deficiency resulted in a destruction of epithelial cell adhesion and a reduction in goblet cells and acidic mucus substances, indicating that the colonic barrier was damaged (Figure 3C,D). These data indicated that RTCB is involved in structure and intestinal epithelial maintain of colon.

RTCB deficiency results in colonic inflammation in mice

To investigate whether the damage to the colonic barrier is caused by increased apoptosis, we assessed the expression of cleaved

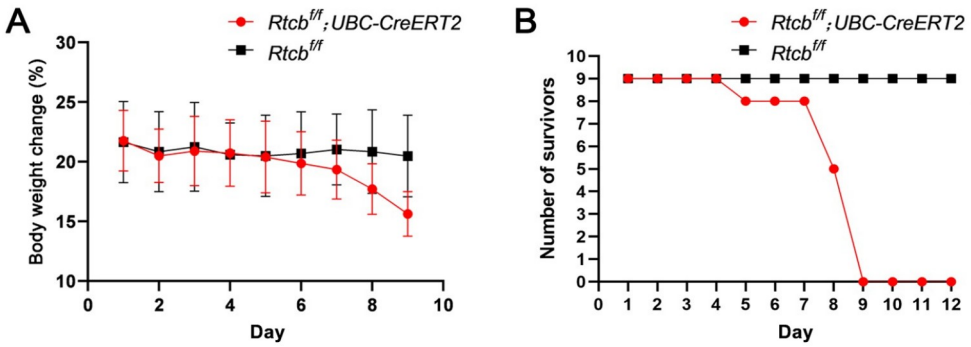

Figure 1. Growth changes in mice after TAM treatment (A) Body weight changes of the *Rtcb*$^{f/f}$ and *Rtcb*$^{f/f}$;*UBC-CreERT2* mice after TAM treatment ($n = 3$). (B) Survival rates of the *Rtcb*$^{f/f}$ and *Rtcb*$^{f/f}$;*UBC-CreERT2* mice after TAM treatment ($n = 9$).

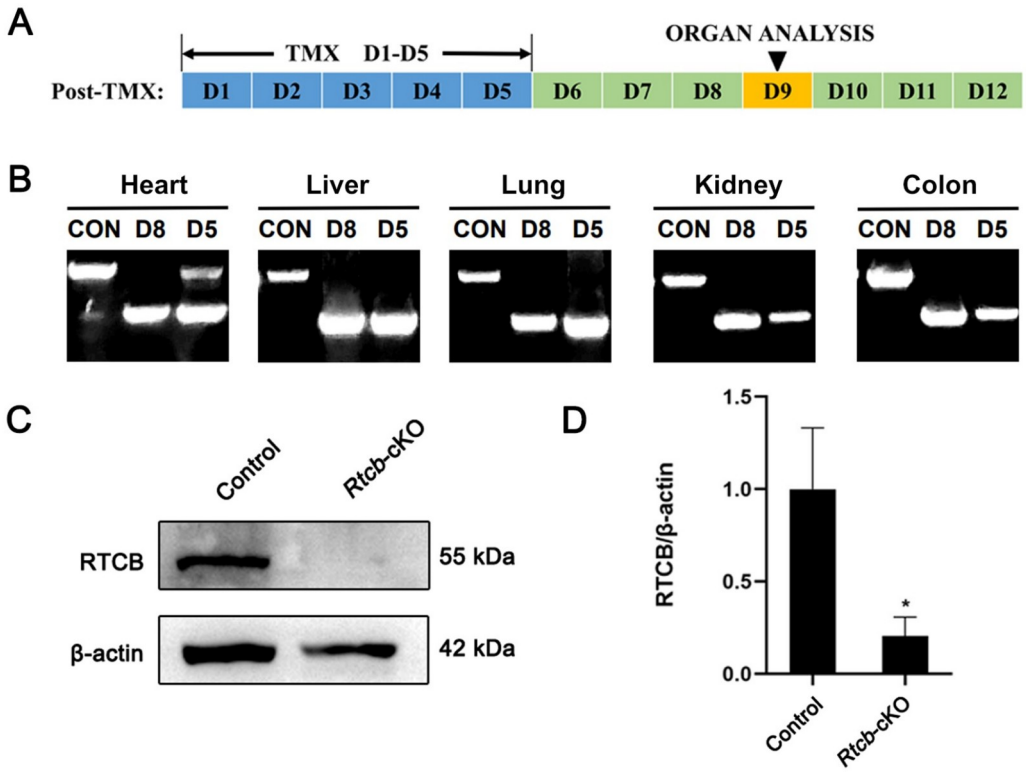

Figure 2. Experimental design and efficiency evaluation of TAM treatment (A) Experimental design. Mice were injected intraperitoneally with TAM for 5 consecutive days (D1 to D5). Body weight was measured daily until D9. Tissue collection was performed on D9. (B) PCR analysis of genomic DNA from different tissues of the TAM-treated *Rtcb*$^{f/f}$ and *Rtcb*$^{f/f}$*;UBC-CreERT2* mice using loxP5 and Frt2 primers ($n=3$). (C,D) Western blot analysis of RTCB in the colon tissues of TAM-treated *Rtcb*$^{f/f}$ (control) and *Rtcb*$^{f/f}$*;UBC-CreERT2* mice ($n=3$). Data are expressed as the mean ± SEM. *$P<0.05$.

caspase-3 (CC-3) in the colon tissues of *Rtcb*$^{f/f}$*;UBC-CreERT2* mice (Figure 4A,B). The decrease of CC3 indicated that RTCB deficiency caused a decrease rather than an increase in apoptosis. Furthermore, to examine whether RTCB deficiency increases inflammation in colon tissues, we measured the protein expressions of IL-8 and TNF-α, two markers of colonic inflammation, in the colon tissues of *Rtcb*$^{f/f}$*;UBC-CreERT2* mice. The significant increase of these two cytokines (Figure 4C–F) showed that RTCB deficiency could induce colonic inflammation in mice. These data indicated that RTCB deficiency resulted in colonic inflammation in mice rather than apoptosis.

XBP1 deficiency does not induce colonic inflammation

Xbp1 mRNA is the only known substrate of RTCB. However, we found that *Rtcb* knockout did not significantly affect the expression of the XBP1-S protein (Figure 5A,B). Additionally, to explore the effects of XBP1 deficiency on the colon, we generated *Xbp1*$^{f/f}$*;UBC-CreERT2* mice. The survival rate and body weights of the mice were monitored during TAM treatment. All mice were alive, and their body weights did not show the same dramatic loss as the *Rtcb*$^{f/f}$*;UBC-CreERT2* mice (Figure 5C,D). Then, we validated the *Xbp1* knockout at the protein level (Figure 5E,F). Histological analysis results showed that neither colon shortening nor hemorrhage occurred in the *Xbp1*$^{f/f}$*;UBC-CreERT2* mice. Moreover, there were no discernible pathological changes or damage to the colonic barrier (Figure 5G–J). These data indicated that RTCB deficiency could not lead to severe colitis through the XBP1-dependent signaling pathway.

NF-κB signaling pathway is activated in the colonic tissues of *Rtcb*-knockout mice

RTCB deficiency resulted in increased expressions of IL-8 and TNF-α (Figure 4C,E), which are downstream signaling molecules of the NF-κB pathway that is typically activated during inflammatory responses. Thus, we examined the expressions of IKKβ, IκBα and p-p65, the key molecules in the NF-κB pathway. The increase in p-p65 and the decrease in IKKβ and IκBα (Figure 6A–E) indicated that the NF-κB pathway was activated in the colonic tissues of *Rtcb*$^{f/f}$*;UBC-CreERT2* mice.

Wnt/β-Catenin signaling pathway is inhibited in the colonic tissues of *Rtcb*-knockout mice

RTCB deficiency led to impaired epithelial adhesion in colonic tissues (Figure 3), and β-catenin is one of the key molecules involved in the adherens junctions and a downstream component of the canonical Wnt signaling pathway. Thus, we examined the expressions of Wnt3a, GSK3β and β-catenin, the key molecules in the Wnt/β-catenin pathway. The increase in GSK3β and the decrease in Wnt3a and β-catenin (Figure 7A–F) indicated that the Wnt/β-catenin pathway was inhibited in the colonic tissues of *Rtcb*$^{f/f}$*;UBC-CreERT2* mice.

Intestinal stem cells (ISCs) play a crucial role in intestinal damage repair and barrier maintenance. Leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5) is a marker of colonic stem cells expressed at the crypt base and regulated by β-catenin. Thus, we also examined the expression of LGR5. The decrease in LGR5

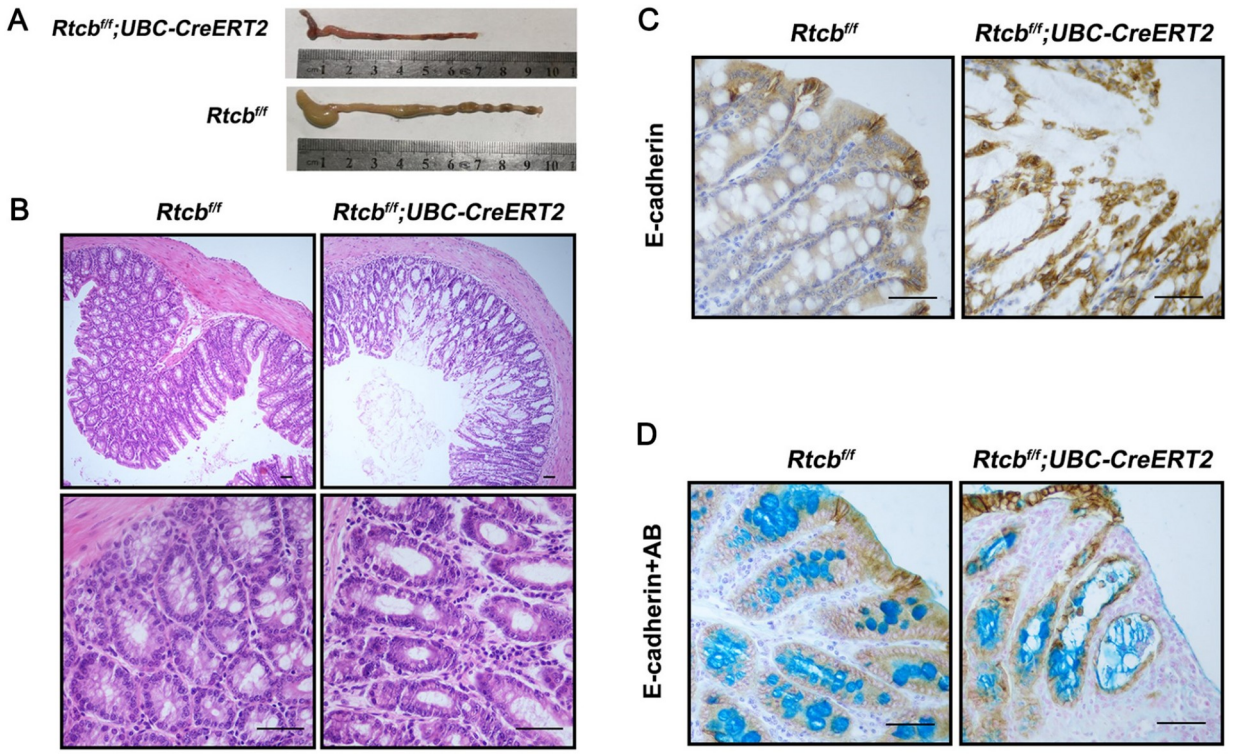

Figure 3. Histological analysis of the mouse colons after TAM treatment on D9 (A) Comparison of colon length between the TAM-treated *Rtcb*$^{f/f}$ and *Rtcb*$^{f/f}$*;UBC-CreERT2* mice (n = 3). (B) Representative H&E staining of colon sections from the TAM-treated *Rtcb*$^{f/f}$ and *Rtcb*$^{f/f}$*;UBC-CreERT2* mice ($n = 3$). (C) Representative immunohistochemical staining of E-cadherin in colon sections from TAM-treated *Rtcb*$^{f/f}$ and *Rtcb*$^{f/f}$*;UBC-CreERT2* mice ($n = 3$). (D) Representative immunohistochemical staining of E-cadherin and Alcian blue in colon sections from TAM-treated *Rtcb*$^{f/f}$ and *Rtcb*$^{f/f}$*; UBC-CreERT2* mice ($n = 3$). Scale bar: 50 μm.

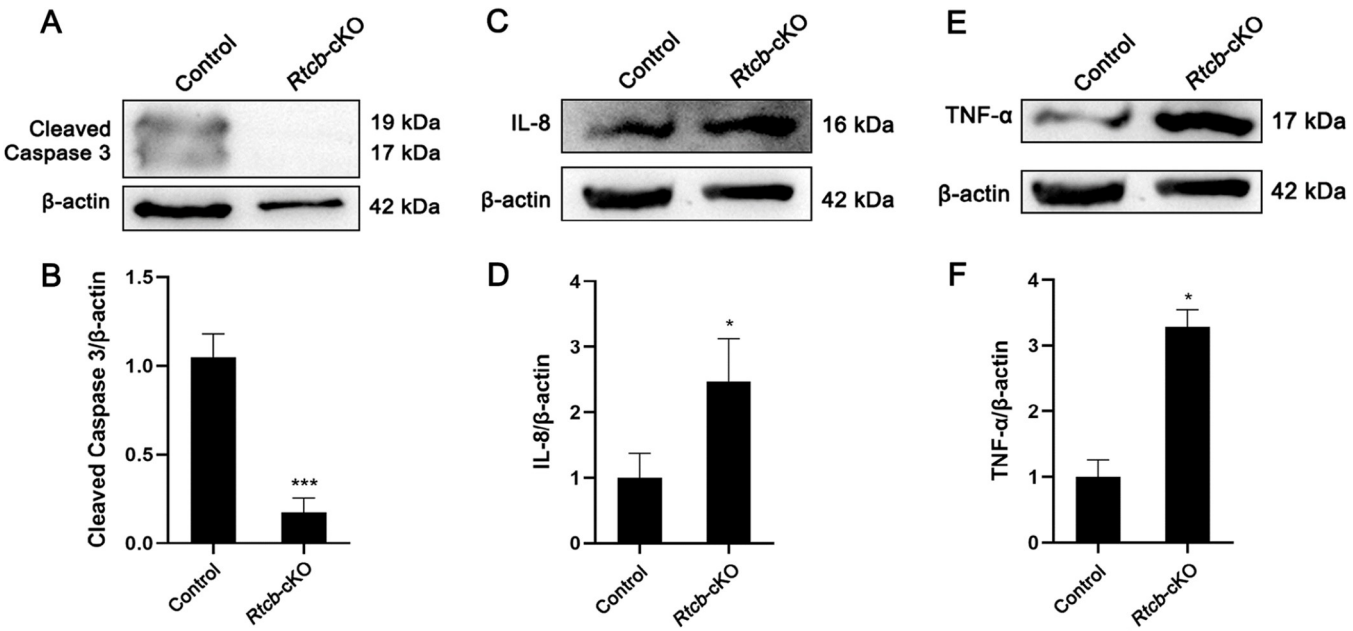

Figure 4. Analysis of colonic inflammation in mice after TAM treatment on D9 Western blot analysis of cleaved caspase 3 (A,B), IL-8 (C,D), and TNF-α (E,F) in the colon tissues of TAM-treated *Rtcb*$^{f/f}$ (control) and *Rtcb*$^{f/f}$*;UBC-CreERT2* mice ($n = 3$). Data are expressed as the mean ± SEM. $^{*}P < 0.05$, $^{***}P < 0.001$.

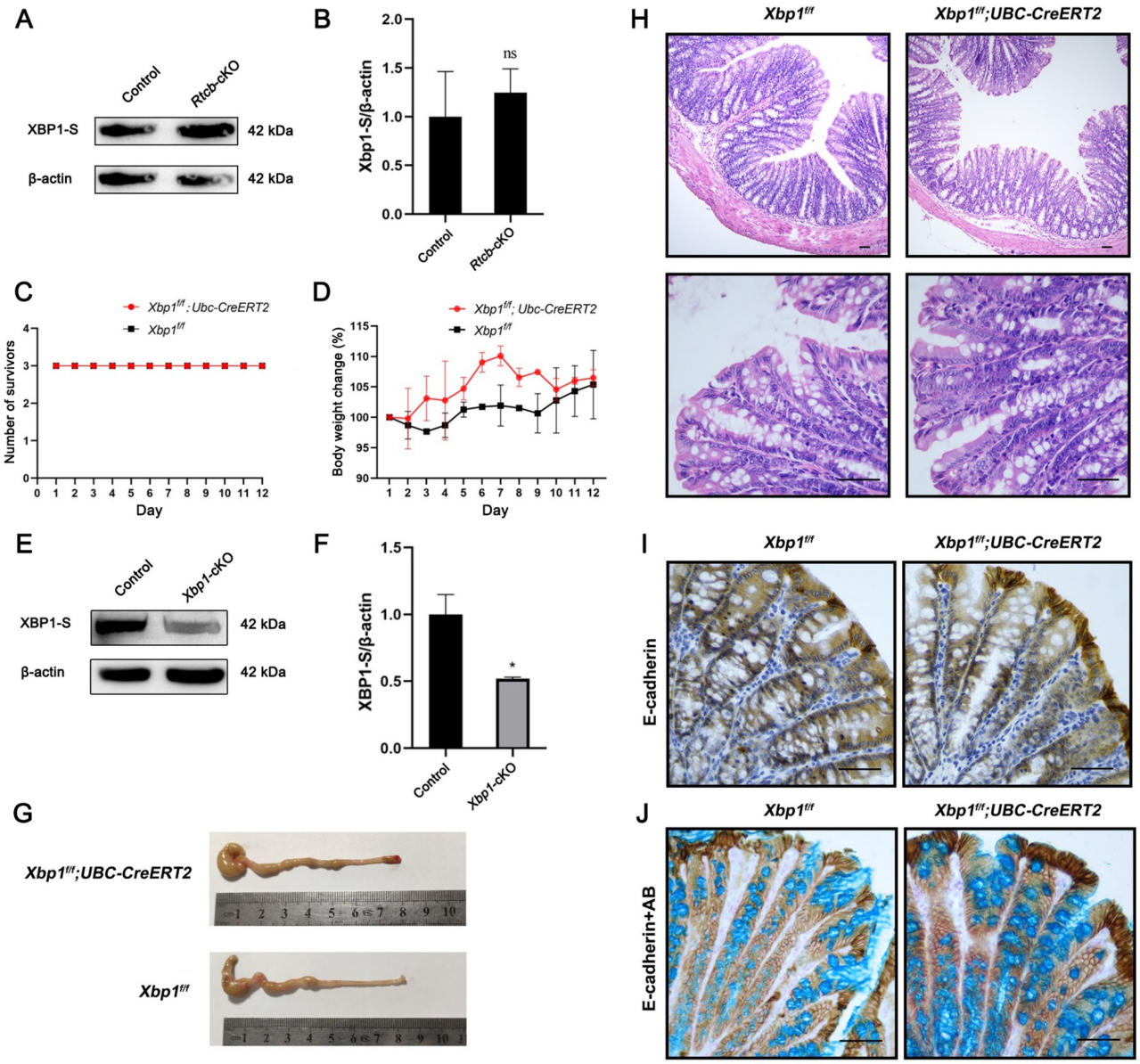

Figure 5. Analysis of the IRE1α-XBP1 signaling pathway in colon tissues after TAM treatment on D9 (A, B) Western blot analysis of XBP1-S in the colon tissues of TAM-treated *Rtcb*$^{f/f}$ (control) and *Rtcb*$^{f/f}$;*UBC-CreERT2* mice ($n=3$). (C) Survival rate of the *Xbp1*$^{f/f}$ and *Xbp1*$^{f/f}$;*UBC-CreERT2* mice after TAM treatment ($n=3$). (D) Body weight changes of the *Xbp1*$^{f/f}$ and *Xbp1*$^{f/f}$;*UBC-CreERT2* mice after TAM treatment (n = 3). (E, F) Western blot analysis of XBP1-S expression in the colon tissues of TAM-treated *Xbp1*$^{f/f}$ (control) and *Xbp1*$^{f/f}$;*UBC-CreERT2* mice ($n=3$). (G) Comparison of colon length between TAM-treated *Xbp1*$^{f/f}$ and *Xbp1*$^{f/f}$;*UBC-CreERT2* mice ($n=3$). (H) Representative H&E staining of colon sections from TAM-treated *Xbp1*$^{f/f}$ and *Xbp1*$^{f/f}$;*UBC-CreERT2* mice ($n=3$). Scale bar: 50 μm. (I) Representative immunohistochemical staining of E-cadherin in colon sections from TAM-treated *Xbp1*$^{f/f}$ and *Xbp1*$^{f/f}$;*UBC-CreERT2* mice ($n=3$). Scale bar: 50 μm. (J) Representative immunohistochemical staining of E-cadherin and Alcian blue in colon sections from TAM-treated *Xbp1*$^{f/f}$ and *Xbp1*$^{f/f}$;*UBC-CreERT2* mice ($n=3$). Scale bar: 50 μm. Data are expressed as the mean ± SEM. *$P<0.05$. ns, no significance.

(Figure 7G,H) indicated a loss of colonic stem cells in the *Rtcb*$^{f/f}$;*UBC-CreERT2* mice.

Discussion

In this study, we successfully generated *Rtcb*$^{f/f}$;*UBC-CreERT2* mice for conditional knockout of *Rtcb*, which can avoid embryonic lethality. We observed body weight loss, short-term mortality, and severe pathological changes in colon tissues upon TAM-induced *Rtcb* knockout in adult mice.

In HeLa cells, knockdown of *Rtcb* using RNA interference (RNAi) did not affect the splicing of *Xbp1* mRNA [27]. This is due to the presence of Archease, which appears to be an important auxiliary factor of RTCB in both tRNA and *Xbp1* mRNA splicing. Even a small amount of RTCB after knockdown can catalyze *Xbp1* mRNA splicing with the help of Archease [28]. Significant inhibition of *Xbp1* occurred upon simultaneous knockdown of *RTCB* and *Archease* [29].

It has been reported that although XBP1 deficiency in IECs causes severe inflammation in the small intestine, no pathological change was found in the colon [30]. To investigate whether RTCB

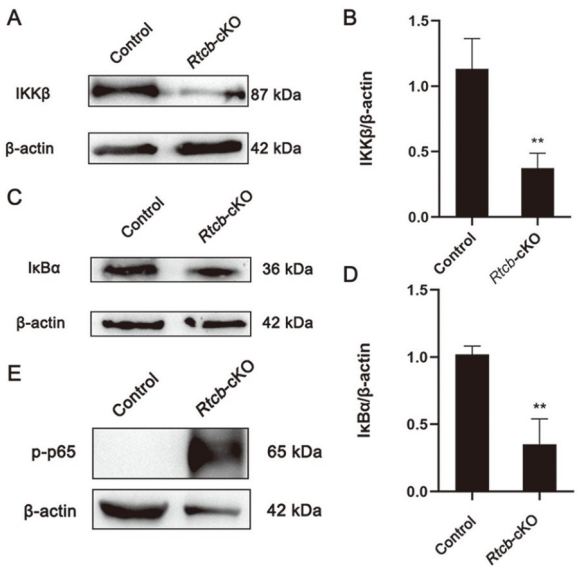

Figure 6. Analysis of the NF-κB signaling pathway in colon tissues after TAM treatment on D9 Western blot analysis of IKKβ (A,B), IκBα (C,D), and p-p65 (E) in the colon tissues of TAM-treated *Rtcb*$^{f/f}$ (control) and *Rtcb*$^{f/f}$;*UBC-CreERT2* mice ($n=3$). Data are expressed as the mean ± SEM. $^{**}P<0.01$.

deficiency causes colonic pathology through the mediation of XBP1, we also examined colonic changes in *Xbp1*-knockout mice. No colitis was observed in the adult mice with XBP1 deficiency. The IRE1α-XBP1 signaling pathway is a major component of the UPR against ER stress that is associated with various diseases. The pathway is targeted by many drugs, including activators such as tunicamycin and inhibitors such as STF-083010 [31]. We also examined the effects of activating or inhibiting the IRE1α-XBP1 pathway on the mouse male reproductive system by injecting tunicamycin or STF-083010, respectively. During these experiments, no mortality or significant body weight loss was observed (data not shown). Therefore, it appeared that the IRE1α-XBP1 signaling pathway may not be involved in the colonic inflammation or mortality caused by *Rtcb* knockout.

During IBD, cytokine production and signal transduction become dysregulated in intestinal epithelial cells, macrophages, and lymphocytes, with NF-κB being one of the key regulatory factors. In IBD patients, NF-κB is not only highly expressed but also maintained in an active state in mucosal macrophages and IECs, and the degree of NF-κB activation is significantly positively correlated with the severity of intestinal inflammation [32]. In mucosal macrophages, along with the increased expression of NF-κB, there is an increased secretion of TNF-α, IL-1, and IL-6, suggesting that NF-κB can induce proinflammatory cytokines that may account for mucosal damage in IBD [33]. Notably, there is a positive feedback mechanism where NF-κB-induced TNF-α can enhance NF-κB activation [34]. Moreover, NF-κB controls not only genes with classical proinflammatory functions but also those involved in cell protection. In IECs, NF-κB plays a protective role in acute intestinal inflammation [35]. Thus, NF-κB signaling may have different effects depending on the set of genes mobilized according to the context [36]. In addition, it has been shown that dextran sulfate sodium (DSS) induces mouse colitis mainly by causing apoptosis and damage to IECs. In the DSS mouse model of colitis, the NF-κB target genes that possess antiapoptotic and cell-protective functions are downregulated, which may aggravate the inflammatory response [35,37].

In this study, we examined the protein levels of two downstream proinflammatory cytokines (TNF-α and IL-8) and two key components (IKKβ and IκBα) of the NF-κB signaling pathway in the colonic tissues of *Rtcb*-knockout mice. The significant upregulation of TNF-α and IL-8 expression and downregulation of IKKβ and IκBα expression indicated the activation of the NF-κB pathway in the colitis induced by RTCB deficiency. Therefore, it is hypothesized that the disruption of colonic epithelial adhesion due to RTCB deficiency prevents the intestinal barrier from exerting its physiological function of resisting pathogens, leading to an increase in proinflammatory cytokines in colonic tissues and the onset of colonic inflammation. Simultaneously, NF-κB signaling is enhanced through positive feedback from TNF-α.

The Wnt signaling pathway plays a crucial regulatory role in ISCs

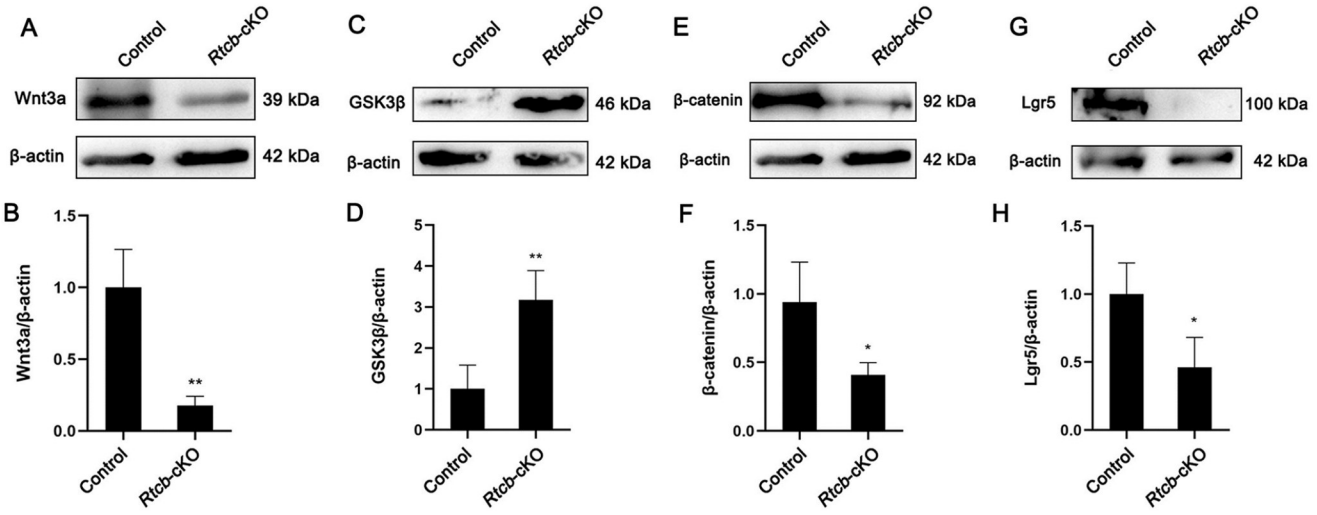

Figure 7. Analysis of the Wnt/β-catenin signaling pathway in colon tissues after TAM treatment on D9 Western blot analysis of Wnt3a (A,B), GSK3β (C,D), β-catenin (E,F), and LGR5 (G,H) in the colon tissues of TAM-treated *Rtcb*$^{f/f}$ (control) and *Rtcb*$^{f/f}$;*UBC-CreERT2* mice ($n=3$). Data are expressed as the mean ± SEM. $^{*}P<0.05$, $^{**}P<0.01$.

[38]. Inhibition of Wnt signaling in the intestines has a severe impact on epithelial cell proliferation, resulting in damage to the intestinal barrier and eventually a period of inflammation in the small intestine and colon [39]. Reducing the secretion of Wnt from colonic epithelial cells leads to impaired cell regenerative capacity and exacerbated mucosal damage [40]. In addition, removing β-catenin and TCF4 from the intestinal epithelia severely disrupts crypt formation, leading to mouse death [41]. In ISCs, the Wnt/β-catenin signaling pathway is closely related to various signaling pathways and is modulated by proinflammatory cytokines such as TNF-α and IFN-γ. During colitis, TNF-α causes intestinal epithelial barrier dysfunction and cell apoptosis. It can activate β-catenin through the AKT signaling pathway to induce the expressions of Wnt target genes in crypt base columnar cells (CBCs), the main population of ISCs [42]. After DSS treatment, loss of TNF receptors in colonic epithelial cells induces colitis in mice, leading to weakened Wnt signaling and impaired tissue damage repair. IFN-γ can induce the expression of the Wnt antagonist Dickkopf-related protein 1 (DDK1) through STAT3, thereby weakening Wnt signaling [43]. Furthermore, IFN-γ can decrease the number of LGR5-positive cells, active stem cells, and Paneth cells, exacerbating colitis damage [44].

In this study, we examined the protein levels of three key molecules (Wnt3a, GSK3β, and β-catenin) of the Wnt/β-catenin signaling pathway in the colonic tissues of *Rtcb*-knockout mice. The significant decrease in the levels of Wnt3a and β-catenin and increase in the level of GSK3β indicated the inhibition of the Wnt/β-catenin pathway in the colitis induced by RTCB deficiency. CBCs express LGR5, the R-spondin receptor that drives the continuous renewal of the intestinal epithelial barrier and gives rise to all intestinal epithelial cell lineages [45]. It has been reported that high level of β-catenin in colorectal cancer upregulates LGR5 expression, suggesting that LGR5 may be a downstream factor of β-catenin contributing to the occurrence of colorectal cancer [46]. Activation of the Wnt signaling pathway is associated with increased expression of LGR5, which may lead to malignant transformation of IECs [47]. Moreover, during the activation of the Wnt/β-catenin signaling pathway, inactivation of LGR5 in the small intestine does not affect the proliferation rate of epithelial cells, indicating the essential role of LGR5 in rapid epithelial cell proliferation [48]. Additionally, the loss of ISC function and reduced LGR5 positivity leads to rapid crypt death and impaired tissue regeneration. In this study, we detected a significant decrease in LGR5 expression in colonic tissues after *Rtcb* knockout, indicating loss of colonic stem cells.

In conclusion, our study shows that RTCB deficiency can lead to colonic barrier damage and severe colitis in mice. The NF-κB and Wnt/β-catenin signaling pathways might play a key role in this pathological process. Our findings may have important implications for understanding the pathogenesis of colitis.

Supplementary Data

Supplementary data is available at *Acta Biochimica et Biophysica Sinica* online.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Tanaka N, Shuman S. RtcB is the RNA ligase component of an escherichia coli RNA repair operon. *J Biol Chem* 2011, 286: 7727–7731
2. Englert M, Sheppard K, Aslanian A, Yates Iii JR, Söll D. Archaeal 3′-phosphate RNA splicing ligase characterization identifies the missing component in tRNA maturation. *Proc Natl Acad Sci USA* 2011, 108: 1290–1295
3. Popow J, Schleiffer A, Martinez J. Diversity and roles of (t)RNA ligases. *Cell Mol Life Sci* 2012, 69: 2657–2670
4. Popow J, Englert M, Weitzer S, Schleiffer A, Mierzwa B, Mechtler K, Trowitzsch S, *et al.* HSPC117 is the essential subunit of a human tRNA splicing ligase complex. *Science* 2011, 331: 760–764
5. Jurkin J, Henkel T, Nielsen AF, Minnich M, Popow J, Kaufmann T, Heindl K, *et al.* The mammalian tRNA ligase complex mediates splicing of *XBP1* mRNA and controls antibody secretion in plasma cells. *EMBO J* 2014, 33: 2922–2936
6. Wang Y, Hai T, Liu Z, Zhou S, Lv Z, Ding C, Liu L, *et al.* HSPC117 deficiency in cloned embryos causes placental abnormality and fetal death. *Biochem Biophys Res Commun* 2010, 397: 407–412
7. Kosmaczewski SG, Edwards TJ, Han SM, Eckwahl MJ, Meyer BI, Peach S, Hesselberth JR, *et al.* TheR tcB RNA ligase is an essential component of the metazoan unfolded protein response. *EMBO Rep* 2014, 15: 1278–1285
8. Zhang H, Jiang JC, Wu YW, Yu YS, Wang HN, Ding NZ, Fan HY. The RNA ligase RNA terminal phosphate cyclase B regulates mRNA alternative splicing and is required for mouse oocyte development and maintenance. *Development* 2022, 149: dev200497
9. Ding NZ, He M, He CQ, Hu JS, Teng J, Chen J. Expression and regulation of FAAP in the mouse epididymis. *Endocrine* 2010, 38: 188–193
10. Ray A, Zhang S, Rentas C, Caldwell KA, Caldwell GA. RTCB-1 mediates neuroprotection via XBP-1 mRNA splicing in the unfolded protein response pathway. *J Neurosci* 2014, 34: 16076–16085
11. Zhao JD, Ju XF, Zhang LQ, Zhang Y, Liang Y, An TZ, Teng CB. Spatiotemporal expression of D10Wsu52e gene in the developing mouse stomach and duodenum. *Acta Biophysica Sinica* 2015, 27: 757–764
12. Silverberg MS, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, Caprilli R, *et al.* Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a working party of the 2005 montreal world congress of gastroenterology. *Canadian J Gastroenterol* 2005, 19: 5A–36A
13. Morita N, Toki S, Hirohashi T, Minoda T, Ogawa K, Kono S, Tamakoshi A, *et al.* Incidence and prevalence of inflammatory bowel disease in Japan: nationwide epidemiological survey during the year 1991. *J Gastroenterol* 1995, 30: 1–4
14. Subasinghe D, Nawarathna NMM, Samarasekera DN. Disease characteristics of inflammatory bowel disease (IBD). *J Gastrointestinal Surg* 2011, 15: 1562–1567
15. Danese S, Fiocchi C. Ulcerative colitis. *N Engl J Med* 2011, 365: 1713–1725
16. Bernstein CN, Blanchard JF, Rawsthorne P, Wajda A. Epidemiology of Crohn's disease and ulcerative colitis in a central canadian province: a population-based study. *Am J Epidemiol* 1999, 149: 916–924
17. Langholz E, Munkholm P, Nielsen OH, Kreiner S, Binder V. Incidence and prevalence of ulcerative colitis in Copenhagen county from 1962 to 1987. *Scand J Gastroenterol* 1987, 26: 1247–1256
18. He Y, Chen L, Chen K, Sun Y. Immunohistochemical analysis of HNF4A

and β-catenin expression to predict low-grade dysplasia in the colitis-neoplastic sequence. *Acta Biochim Biophys Sin* 2021, 53: 94–101
19. E L, Li W, Hu Y, Deng L, Yao J, Zhou X. Methyl cinnamate protects against dextran sulfate sodium-induced colitis in mice by inhibiting the MAPK signaling pathway. *Acta Biochim Biophys Sin* 2023, 55: 1806–1818
20. Wei J, Feng J. Signaling pathways associated with inflammatory bowel disease. *Recent Pat Inflamm Allergy Drug Discov* 2010, 4: 105–117
21. Moparthi L, Koch S. Wnt signaling in intestinal inflammation. *Differentiation* 2019, 108: 24–32
22. Ciechanover A, Finley D, Varshavsky A. The ubiquitin-mediated proteolytic pathway and mechanisms of energy-dependent intracellular protein degradation. *J Cell Biochem* 1984, 24: 27–53
23. Wulff BS, O'Hare MMT, Boel E, Theill LE, Schwartz TW. Partial processing of the neuropeptide Y precursor in transfected CHO cells. *FEBS Lett* 1990, 261: 101–105
24. Schorpp M, Jager R, Schellander K, Schenkel J, Wagner EF, Weiher H, Angel P. The human ubiquitin C promoter directs high ubiquitous expression of transgenes in mice. *Nucleic Acids Res* 1996, 24: 1787–1788
25. Feil R, Wagner J, Metzger D, Chambon P. Regulation of cre recombinase activity by mutated estrogen receptor ligand-binding domains. *Biochem Biophys Res Commun* 1997, 237: 752–757
26. Hartsock A, Nelson WJ. Adherens and tight junctions: structure, function and connections to the actin cytoskeleton. *Biochim Biophys Acta* 2008, 1778: 660–669
27. Iwawaki T, Tokuda M. Function of yeast and amphioxus tRNA ligase in IRE1alpha-dependent XBP1 mRNA splicing. *Biochem Biophys Res Commun* 2011, 413: 527–531
28. Kosmaczewski SG, Han SM, Han B, Irving Meyer B, Baig HS, Athar W, Lin-Moore AT, *et al.* RNA ligation in neurons by RtcB inhibits axon regeneration. *Proc Natl Acad Sci USA* 2015, 112: 8451–8456
29. Desai KK, Cheng CL, Bingman CA, Phillips Jr GN, Raines RT. A tRNA splicing operon: Archease endows RtcB with dual GTP/ATP cofactor specificity and accelerates RNA ligation. *Nucleic Acids Res* 2014, 42: 3931–3942
30. Kaser A, Lee AH, Franke A, Glickman JN, Zeissig S, Tilg H, Nieuwenhuis EES, *et al.* XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. *Cell* 2008, 134: 743–756
31. Yang D, Wei Y, Lu Q, Qin D, Zhang M, Du X, Xu W, *et al.* Melatonin alleviates LPS-induced endoplasmic reticulum stress and inflammation in spermatogonial stem cells. *J Cell Physiol* 2021, 236: 3536–3551
32. Rogler G, Brand K, Vogl D, Page S, Hofmeister R, Andus T, Knuechel R, *et al.* Nuclear factor κB is activated in macrophages and epithelial cells of inflamed intestinal mucosa. *Gastroenterology* 1998, 115: 357–369
33. Neurath MF, Pettersson S, Meyer Zum Büschenfelde KH, Strober W. Local administration of antisense phosphorothiate oligonucleotides to the p65 subunit of NF–κB abrogates established experimental colitis in mice. *Nat Med* 1996, 2: 998–1004
34. Holtmann M, Neurath M. Differential TNF-signaling in chronic inflammatory disorders. *Curr Med Med* 2004, 4: 439–444
35. Steinbrecher KA, Harmel-Laws E, Sitcheran R, Baldwin AS. Loss of epithelial RelA results in deregulated intestinal proliferative/apoptotic homeostasis and susceptibility to inflammation. *J Immunol* 2008, 180: 2588–2599
36. Spehlmann ME, Eckmann L. Nuclear factor-kappa B in intestinal protection and destruction. *Curr Opin Gastroenterol* 2009, 25: 92–99
37. Eckmann L, Nebelsiek T, Fingerle AA, Dann SM, Mages J, Lang R, Robine S, *et al.* Opposing functions of IKKβ during acute and chronic intestinal inflammation. *Proc Natl Acad Sci USA* 2008, 105: 15058–15063
38. Barker N. Adult intestinal stem cells: critical drivers of epithelial homeostasis and regeneration. *Nat Rev Mol Cell Biol* 2014, 15: 19–33
39. Kuhnert F, Davis CR, Wang HT, Chu P, Lee M, Yuan J, Nusse R, *et al.* Essential requirement for Wnt signaling in proliferation of adult small intestine and colon revealed by adenoviral expression of Dickkopf-1. *Proc Natl Acad Sci USA* 2004, 101: 266–271
40. Das S, Feng Q, Balasubramanian I, Lin X, Liu H, Pellón-Cardenas O, Yu S, *et al.* Colonic healing requires Wnt produced by epithelium as well as Tagln + and Acta2 + stromal cells. *Development* 2022, 149: dev199587
41. van Es JH, Haegebarth A, Kujala P, Itzkovitz S, Koo BK, Boj SF, Korving J, *et al.* A critical role for the wnt effector Tcf4 in adult intestinal homeostatic self-renewal. *Mol Cell Biol* 2012, 32: 1918–1927
42. Bradford EM, Ryu SH, Singh AP, Lee G, Goretsky T, Sinh P, Williams DB, *et al.* Epithelial TNF receptor signaling promotes mucosal repair in inflammatory bowel disease. *J Immunol* 2017, 199: 1886–1897
43. Li W, Henderson LJ, Major EO, Al-Harthi L. IFN-γ Mediates Enhancement of HIV Replication in Astrocytes by Inducing an Antagonist of the β-Catenin Pathway (DKK1) in a STAT 3-Dependent Manner. *J Immunol* 2011, 186: 6771–6778
44. Richmond CA, Rickner H, Shah MS, Ediger T, Deary L, Zhou F, Tovaglieri A, *et al.* JAK/STAT-1 signaling is required for reserve intestinal stem cell activation during intestinal regeneration following acute inflammation. *Stem Cell Rep* 2018, 10: 17–26
45. Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegebarth A, *et al.* Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* 2007, 449: 1003–1007
46. Fan XS, Wu HY, Yu HP, Zhou Q, Zhang YF, Huang Q. Expression of Lgr5 in human colorectal carcinogenesis and its potential correlation with β-catenin. *Int J Colorectal Dis* 2010, 25: 583–590
47. Van der Flier LG, Sabates–Bellver J, Oving I, Haegebarth A, De Palo M, Anti M, Van Gijn ME, *et al.* The intestinal Wnt/TCF signature. *Gastroenterology* 2007, 132: 628–632
48. Garcia MI, Ghiani M, Lefort A, Libert F, Strollo S, Vassart G. LGR5 deficiency deregulates Wnt signaling and leads to precocious Paneth cell differentiation in the fetal intestine. *Dev Biol* 2009, 331: 58–67