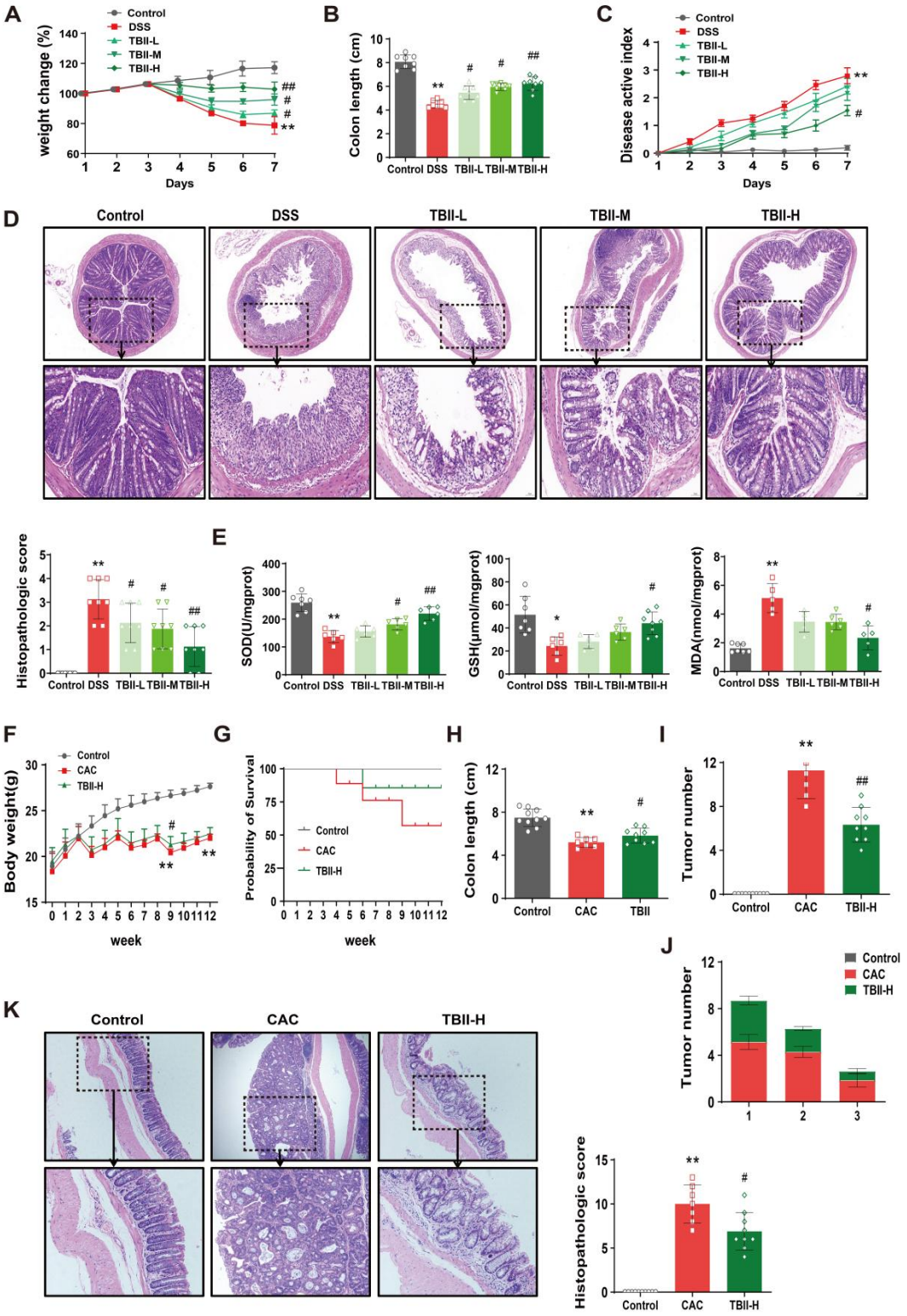


Figure 1. TBⅡ alleviated DSS-induced colitis and prevent AOM/DSS-induced colon carcinogenesis in mice. (A) Body weight change. (B) Colon length. (C) Score of disease activity index. (D) Representative images of hematoxylin and eosin (H&E) staining in the colon tissues and

histopathologic score (100x and 400x). (E) Colonic SOD, GSH AND MDA. (F) Body weight. (G) survival rate. (H) Colon length. (I) Tumor number. (J) Tumor size. (K) Representative images of H&E staining in the colon tissues and histopathologic score (100x and 400x). Values represent mean ± standard error of the mean $^{*}P<0.05$, $^{**}P<0.01$ vs. control group; $^{\#}P< 0.05$, $^{\#\#}P<0.01$ vs. DSS or CAC group.

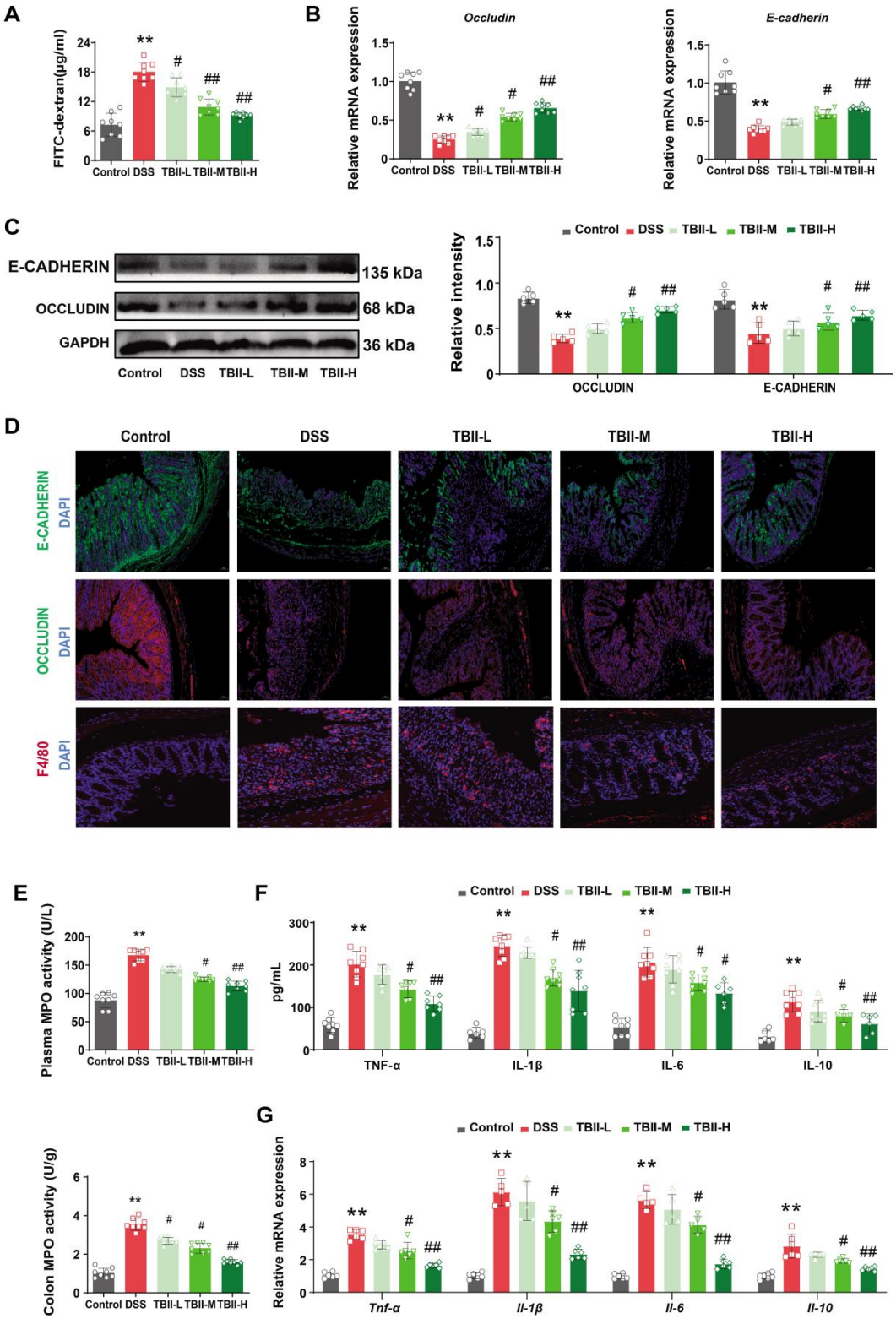


Figure 2. TBⅡ prevented DSS-induced the disruption of colonic epithelial integrity and attenuated DSS-induced the inflammation of colon tissue. (A) FITC-dextran levels in each group. (B) The mRNA expression levels of E-cadherin and Occludin in the colon tissue. (C) The

protein expressions of E-CADHERIN and OCCLUDIN were detected by Western blot in colonic tissue. (D) Colonic tissues were immunofluorescently stained for E-CADHERIN, OCCLUDIN and F4/80, and the nuclei were stained with DAPI (200x). (E) Plasma and Colonic MPO. (F) Serum cytokine secretion. (G) The mRNA expression of cytokine in colon. Values represent mean ± standard error of the mean $^{*}P<0.05$, $^{**}P<0.01$ vs. control group; $^{\#}P< 0.05$, $^{\#\#}P<0.01$ vs. DSS group.

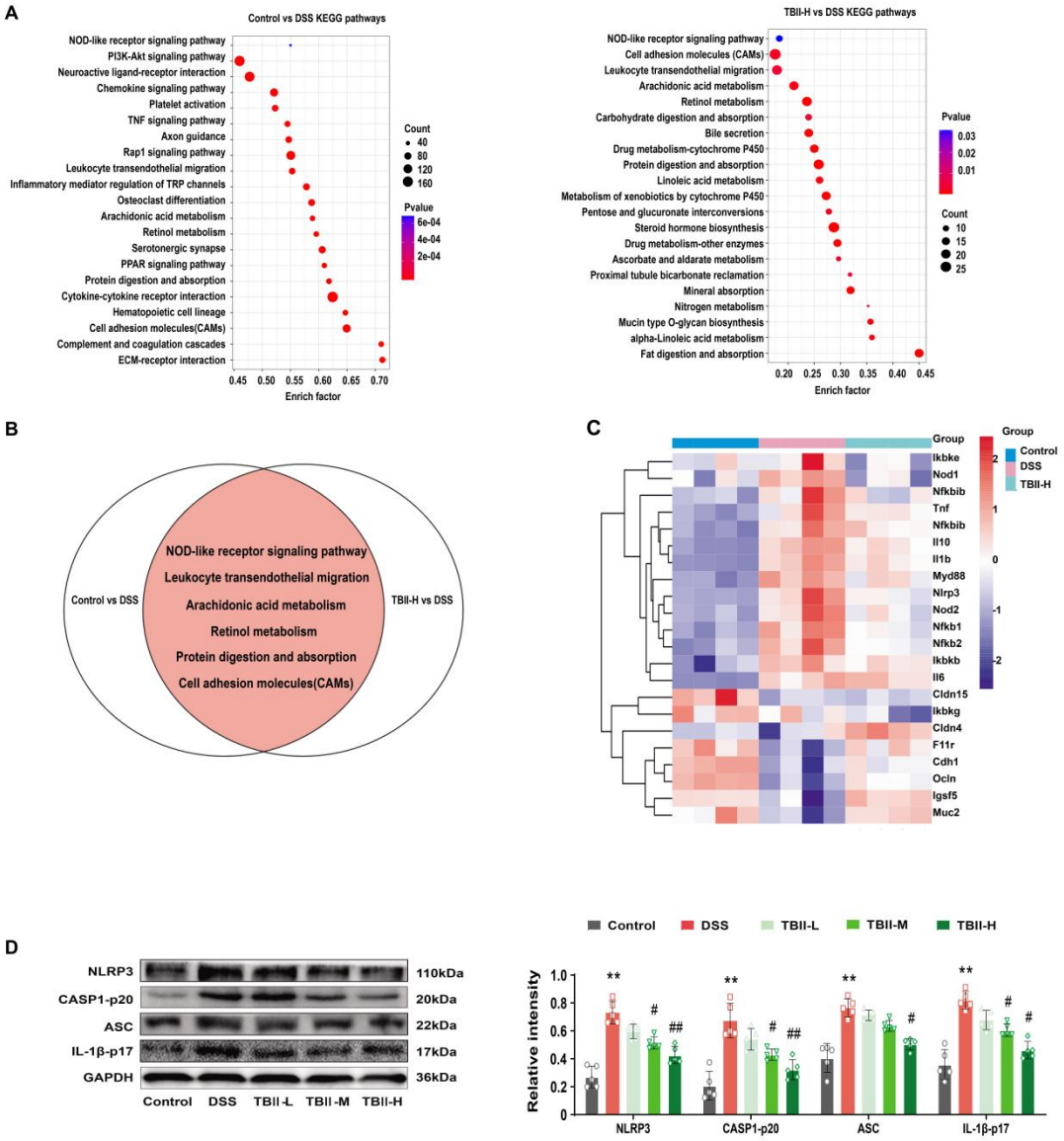


Figure 3. TBⅡ mitigated DSS-induced colitis via inhibiting NLRP3 signaling. (A) Analysis of KEGG enrichment signaling pathway in mice. (B) KEGG enrichment signaling pathway intersected by Control vs DSS and TBⅡ-H vs DSS. (C) Heat map demonstrating pathway specific transcript

expression in mice. (D) The expression of NLRP3 inflammasome proteins detected by western blot and GAPDH was used as a loading control. Values represent mean ± standard error of the mean $^{*}P$<0.05, ** P<0.01 vs. control group; $^{\#}P$< 0.05, $^{\#\#}P$<0.01 vs. DSS group.

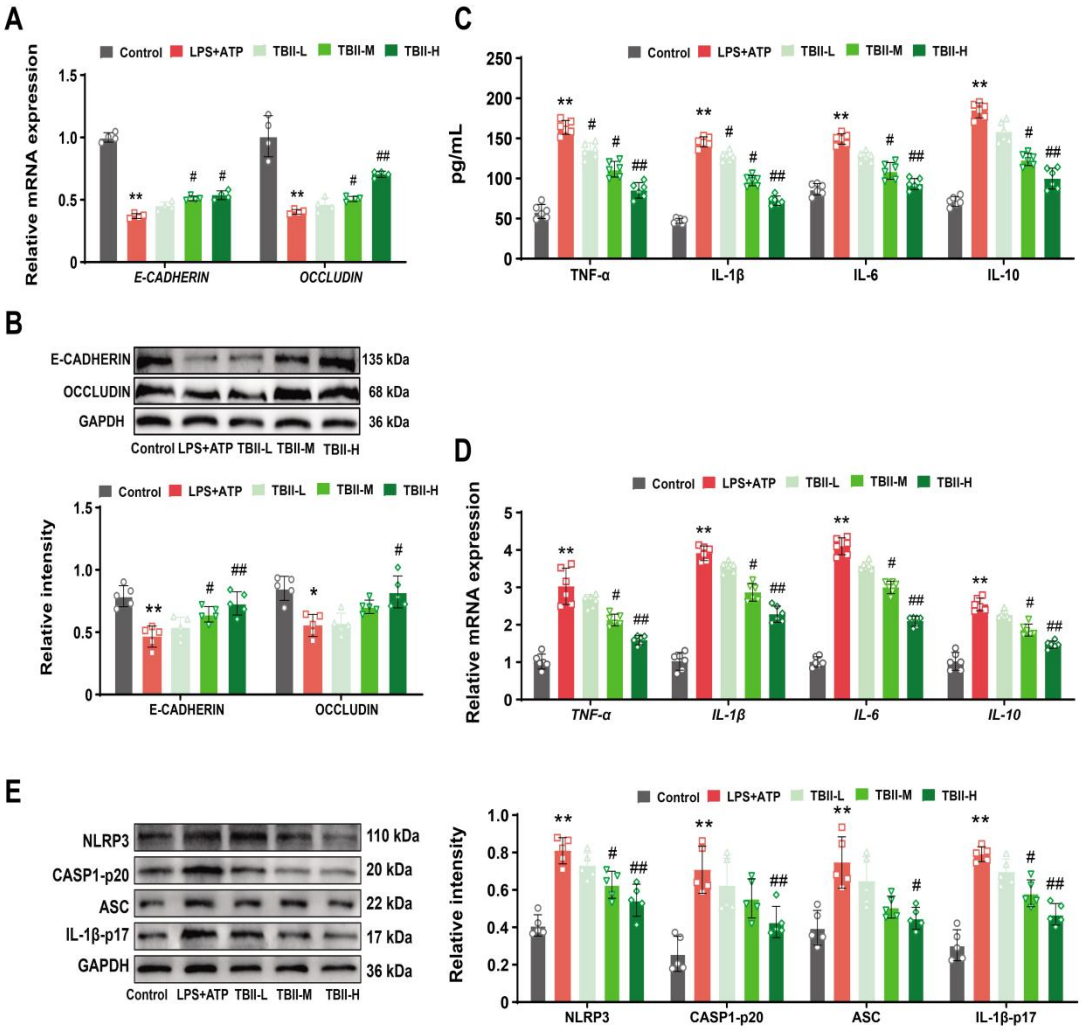


Figure 4. TBⅡ alleviates LPS+ATP-induced intestinal epithelial cell damage. (A) The mRNA expression of E-cadherin and Occludin. (B) The expression of E-cadherin and Occludin proteins detected by western blot and GAPDH was used as a loading control. (C) The production of cytokine in cells by ELISA. (D) The mRNA expression of cytokine. (E) The expression of NLRP3 inflammasome proteins detected by western blot. Values represent mean ± standard error of the mean Values represent mean ± standard error of the mean $^{*}P$<0.05, ** P<0.01 vs. control group; $^{\#}P$< 0.05, $^{\#\#}P$<0.01 vs. LPS+ATP group.

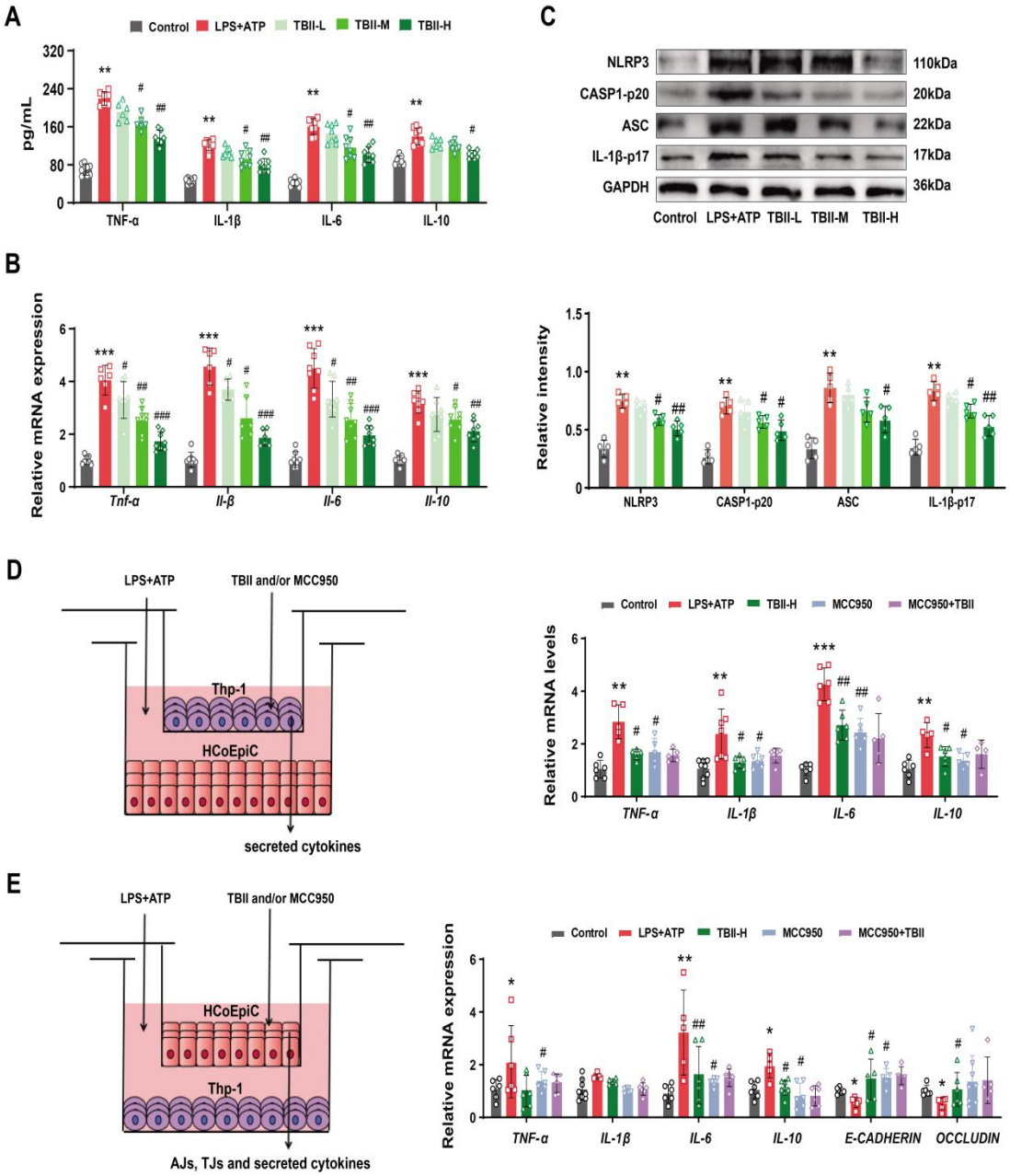


Figure 5. TBⅡ alleviates inflammatory response via blocking the communitation between epithelial cells and macrophages. (A) The production of cytokine in BMDMs by ELISA. (B) The mRNA expression of cytokine. (C) The expression of NLRP3 inflammasome proteins detected by by western blot and GAPDH was used as a loading control. (D)The mRNA expression of cytokines in THP-1 cells. (E) The mRNA expression of cytokines, E-cadherin and Occludin in HCoEpiC cells. Values represent mean±standard error of the mean $^{*}P<0.05$, $^{**}P<0.01$ vs. Control group; $^{\#}P< 0.05$, $^{\#\#}P<0.01$ vs. LPS+ATP group.

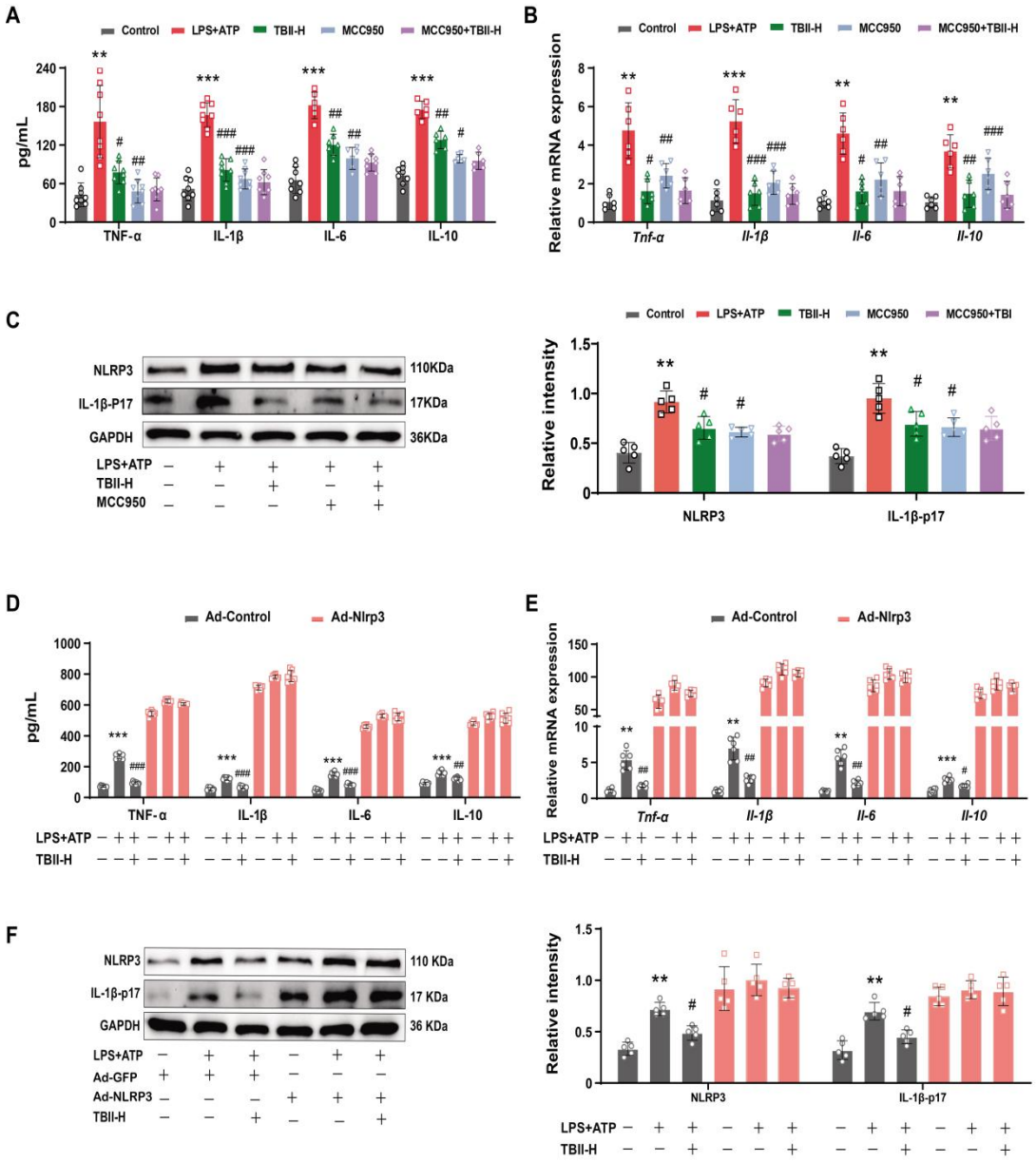


Figure 6. TBⅡ mitigated LPS+ATP-induced inflammation via suppressing NLRP3. (A) The production of cytokine in BMDMs by ELISA. (B) The mRNA expression of cytokine. (C) The expression of NLRP3 inflammasome proteins detected by by western blot and GAPDH was used as a loading control. (D) The production of cytokine in control (empty vector) or Nlrp3-overexpressed cells. (E)The mRNA expression of cytokine. (F) The expression of NLRP3 and IL-1β-p17. Values represent mean ± standard error of the mean $^{*}P<0.05$, $^{**}P<0.01$ vs. Control group; $^{\#}P< 0.05$, $^{\#\#}P<0.01$ vs. LPS+ATP group.

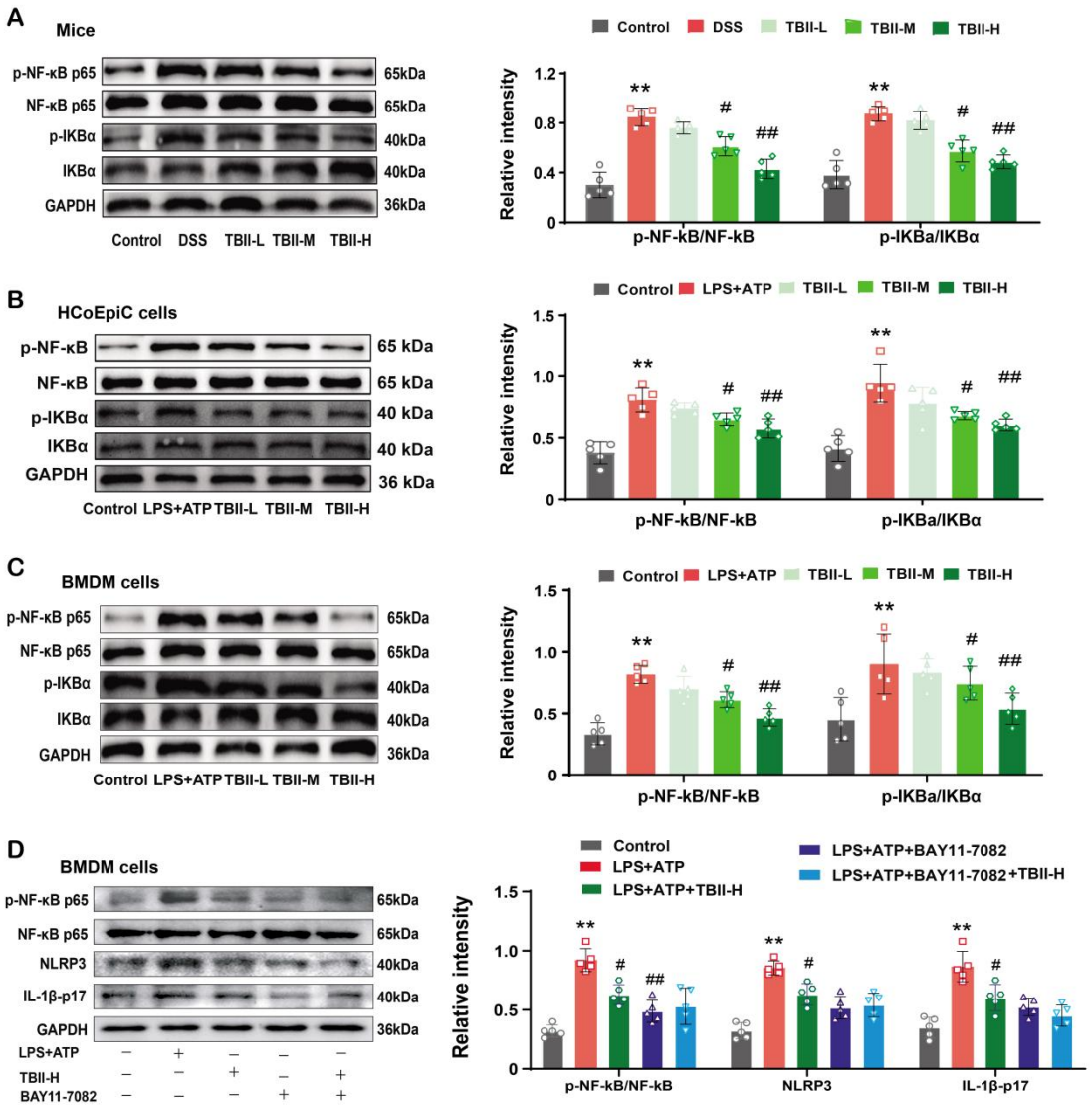


Figure 7. TBⅡ reduces NLRP3 expression by suppressing NF-κB signaling. (A) The expression of NF-κB signaling proteins detected by western blot and GAPDH was used as a loading control in mice. (B) The expression of NF-κB signaling proteins detected by western blot and GAPDH was used as a loading control in HCoEpiC cells (C) The expression of NF-κB signaling proteins detected by western blot and GAPDH was used as a loading control in BMDMs. (D) The expression of NF-κB signaling pathway and NLRP3 inflammasome proteins detected by western blot and GAPDH was used as a loading control with or without BAY11-7082. Values represent mean ± standard error of the mean $^{*}P<0.05$, $^{**}P<0.01$ vs. Control group; $^{\#}P< 0.05$, $^{\#\#}P<0.01$ vs. DSS or LPS+ATP group; $^{\$}P< 0.05$, $^{\$\$}P<0.01$ vs. LPS+ATP+BAY11-7082 group.

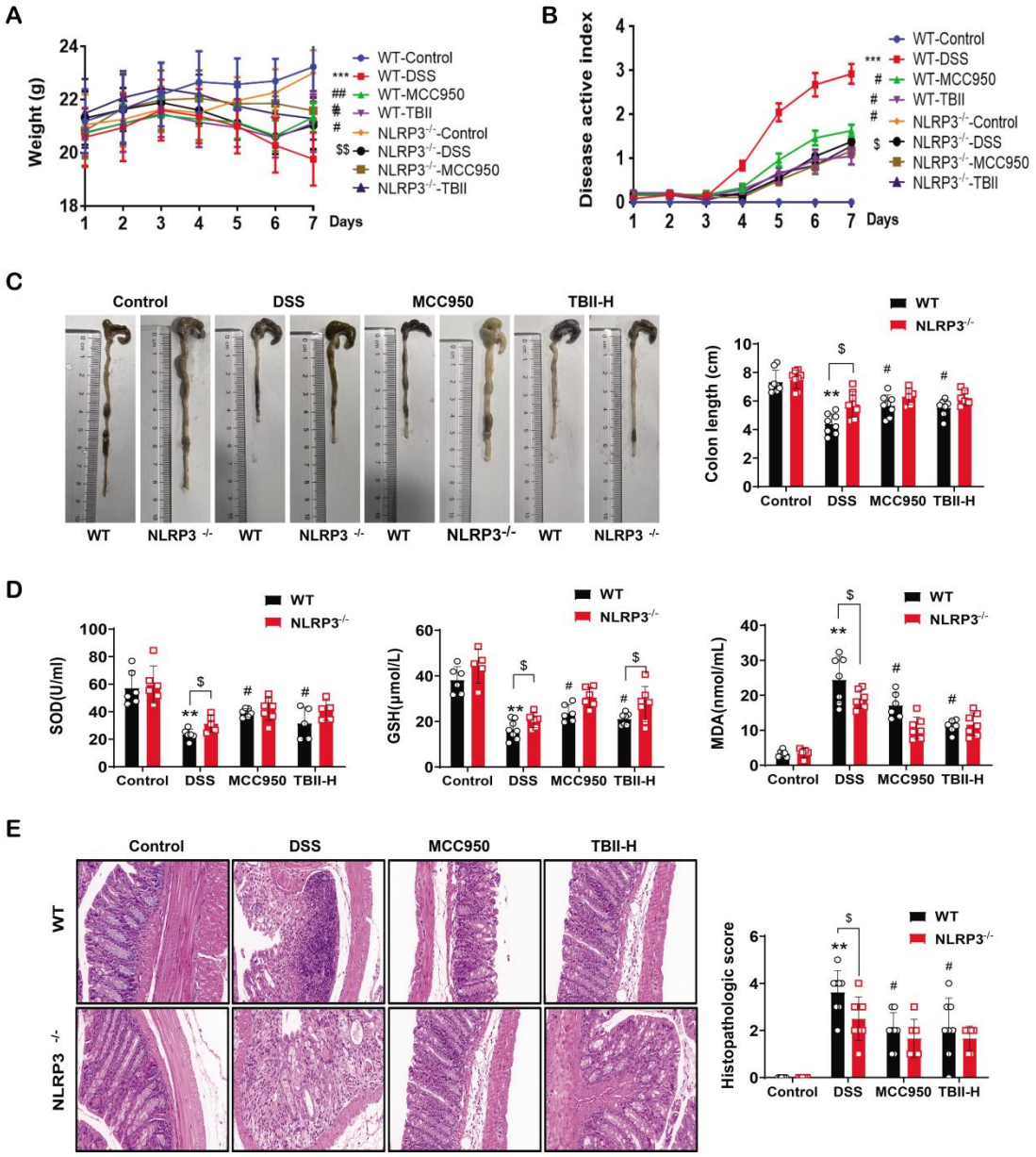


Figure 8. Inhibition of NLRP3 is indispensable of TBⅡ-mediated protective effects against DSS-induced colitis (A) Body weight. (B) Score of disease activity index. (C) Colon length. (D) Serum SOD, MDA AND GSH. (E) Representative images of hematoxylin and eosin (H&E) staining in the colon tissues and histopathologic score (400x). Values represent mean±standard error of the mean $^{*}P<0.05$, $^{**}P<0.01$ vs. WT-Control group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs. WT-DSS group; $^{\$}P<0.05$, $^{\$\$}P<0.01$ vs. WT mice group.

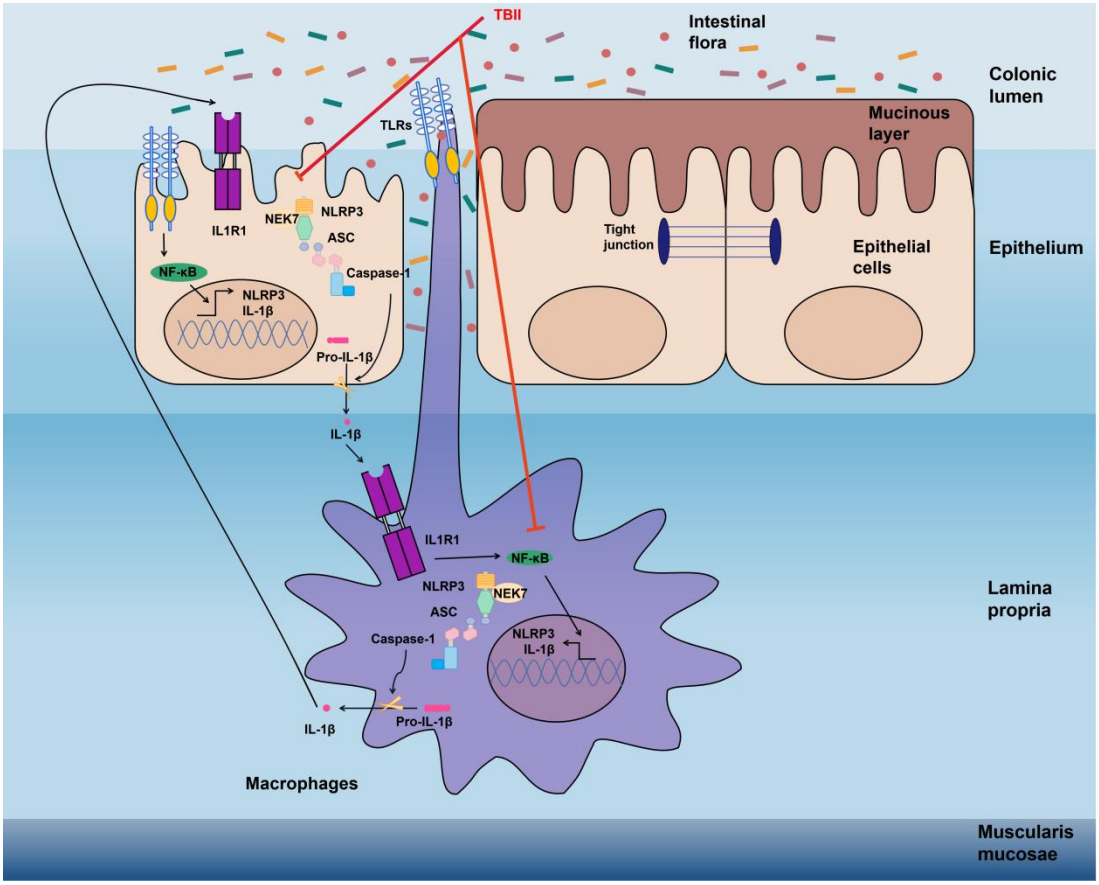
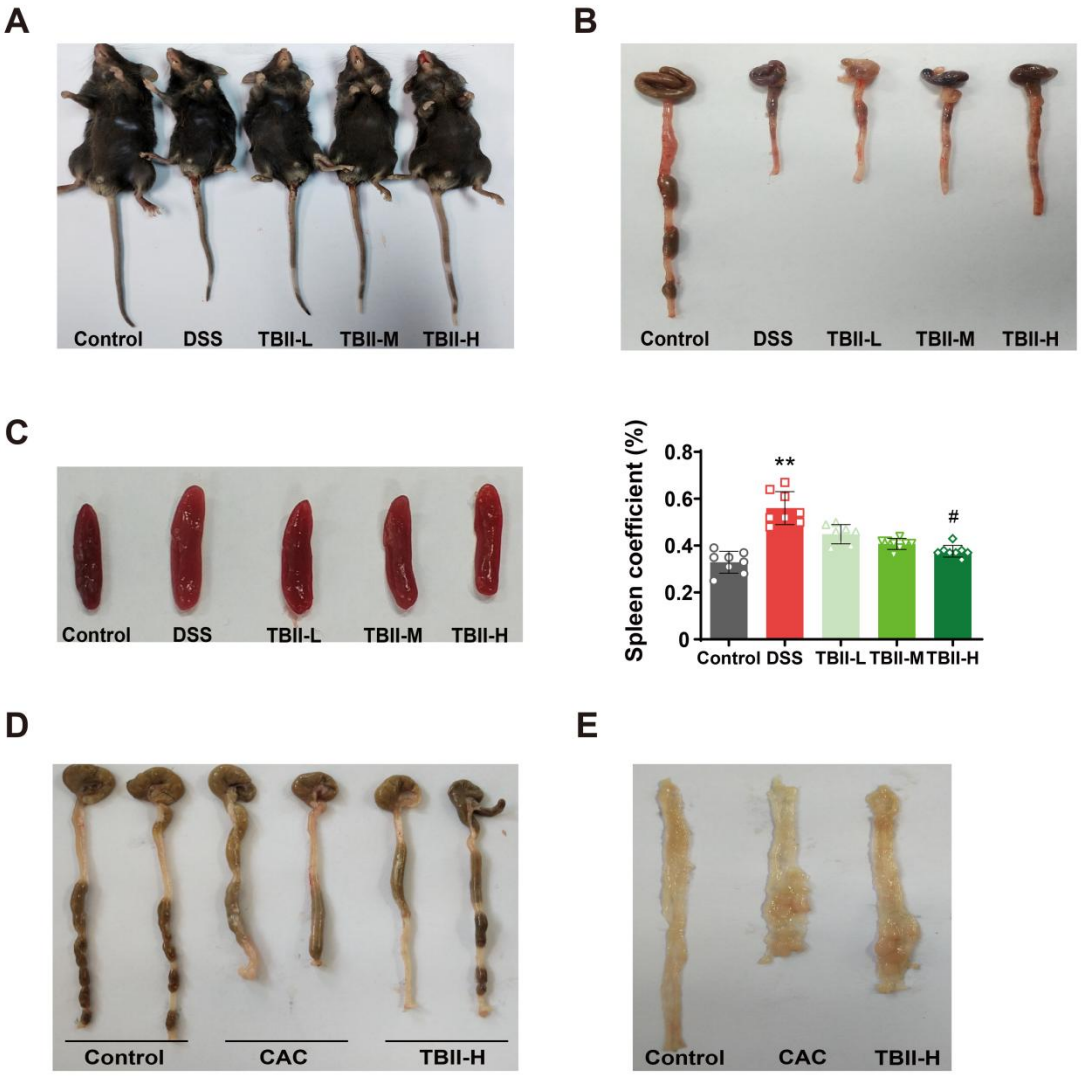
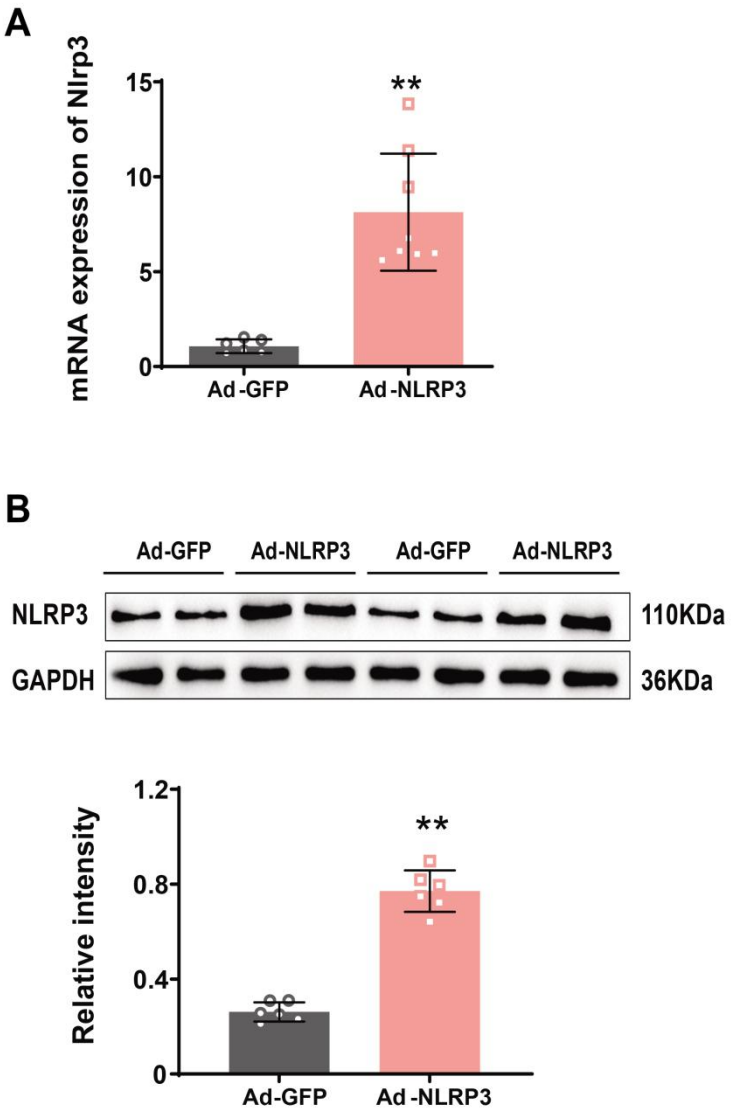


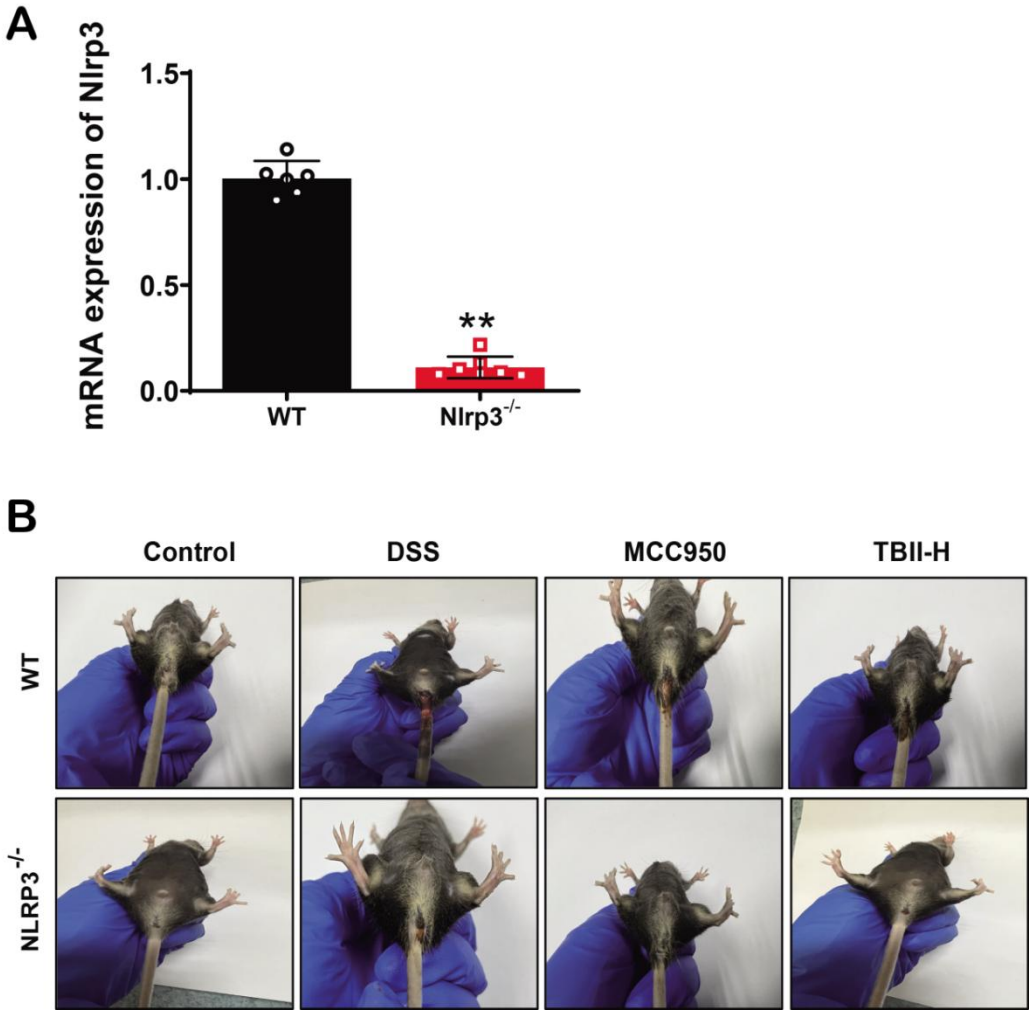
Figure 9. Diagram of potential mechanisms of TBⅡ in attenuating intestinal inflammation. In mice with DSS induced UC, mucin synthesis and secretion are impaired, pathogenic bacteria proliferate and invade, and tight junction regulation is disrupted, causing increased permeability of intestinal epithelium. Intestinal epithelial TLRs recognize antigens, activate NF-κB and transcribe NLRP3 and IL-1β. NLRP3 assembles with ASC and Caspase-1 into NLRP3 inflammasome in the cytoplasm, which cleaves pro-IL-1β into bio-active IL-1β and releases it into the extracellular. This IL-1β is recognized by IL1R1 in lamina propria macrophages, activates NF-κB/NLRP3 signal, releases massive pro-inflammatory cytokines (such as IL-1β), thereafter intensifying the colonic inflammation and accelerating the damage to intestinal epithelial cells. Besides, TBⅡ could reduce NLRP3 expression by inhibiting NF-κB signaling, an upstream event of NLRP3's key transcriptional regulator, and consequently blocking NlRP3-mediated the cross-talk between epithelial cells and macrophage, thereby decreasing the production of pro-inflammatory cytokines and alleviating DSS-induced barrier damage and colitis.



Supplementary Figure 1. Further characterization the effect of TBⅡ on colitis and colon cancer. (A) Representative body type pictures of colitis mice. (B) Representative colon pictures of mice in each group. (C) Representative spleen pictures of mice and spleen coefficient. (D) Representative pictures of colon cancer mice. (E) Representative diagram of colon tumors in colon cancer mice. Values represent mean±standard error of the mean $^{**}P<0.01$ vs. Control group,$^{\#}P<0.05$ vs. DSS group.



Supplementary Figure 2. Further characterization the effect of TBⅡ on colitis and colon cancer. (A) The mRNA expression of Nlrp3. (B) The expression of NLRP3 inflammasome proteins detected by western blot and GAPDH was used as a loading control in BMDMs. Values represent mean ± standard error of the mean $^{**}P<0.01$ vs. Ad-GFP group.



Supplementary Figure 3. Characterization of Nlrp3$^{-/-}$ mice. (A) The mRNA expression of Nlrp3. (B) Representative body type pictures of colitis mice. Values represent mean ± standard error of the mean ***P*<0.01 vs. WT group.

Supplementary Table 1 The sequences of primersfor qRT-PCR analysis

Gene	Forward	Reverse
mTnf-α(ID:21926)	CAGGCGGTGCCTATGTCTC	CGATCACCCCGAAGTTCAGTAG
mIl-1β(ID:16716)	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG
mIl-6(ID:16193)	CTGCAAGAGACTTCCATCCAG	AGTGGTATAGACAGGTCTGTTGG
mIl10(ID:16153)	CTTACTGACTGGCATGAGGATCA	GCAGCTCTAGGAGCATGTGG
mE-cadherin(ID:12	CAGTTCCGAGGTCTACA	TGAATCGGGAGTCTTCC

550)	CCTT	GAAAA
mOccludin(ID:18260)	TGAAAGTCCACCTCCTTACAGA	CCGGATAAAAAGAGTACGCTGG
mNlrp3(ID:216799)	ATTACCCGCCCGAGAAAGG	CATGAGTGTGGCTAGATCCAAG
hTnf-α(ID:7124)	CCTCTCTCTAATCAGCCCTCTG	GAGGACCTGGGAGTAGATGAG
hIl-1β(ID:3553)	ATGATGGCTTATTACAGTGGCAA	GTCGGAGATTCGTAGCTGGA
hIl-6(ID:3569)	ACTCACCTCTTCAGAACGAATTG	CCATCTTTGGAAGGTTCAGGTTG
hIl10(ID:3586)	GACTTTAAGGGTTACCTGGGTTG	TCACATGCGCCTTGATGTCTG
hE-cadherin(ID:999)	CGAGAGCTACACGTTCACGG	GGGTGTCGAGGGAAAAATAGG
hOccludin(ID:100506658)	ACAAGCGGTTTTATCCAGAGTC	GTCATCCACAGGCGAAGTTAAT