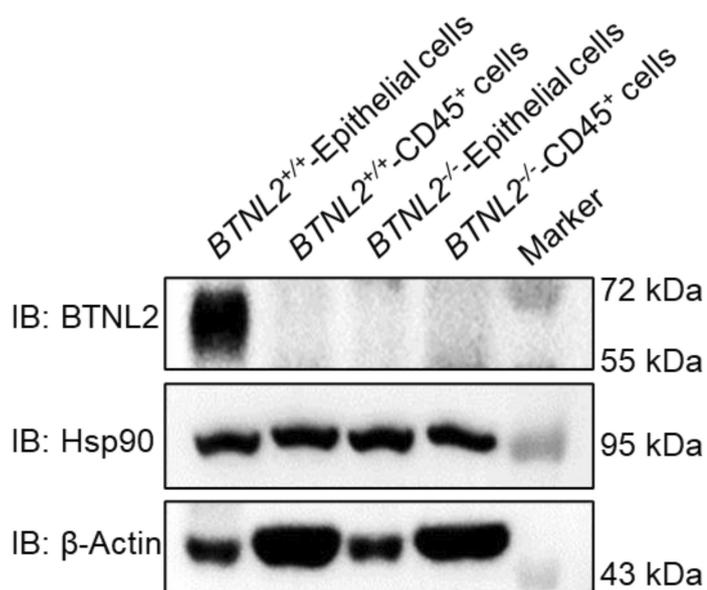


Expanded View Figures

Figure EV1. Analysis of BTNL2 expression in mouse colonic epithelial cells and immune cells.

Colonic epithelium cells were scraped with a razor blade, and CD45⁺ cells from LPLs were sorted by FACS. Cells were lysed, and BTNL2 protein level was analyzed by immunoblot.

Source data are available online for this figure.



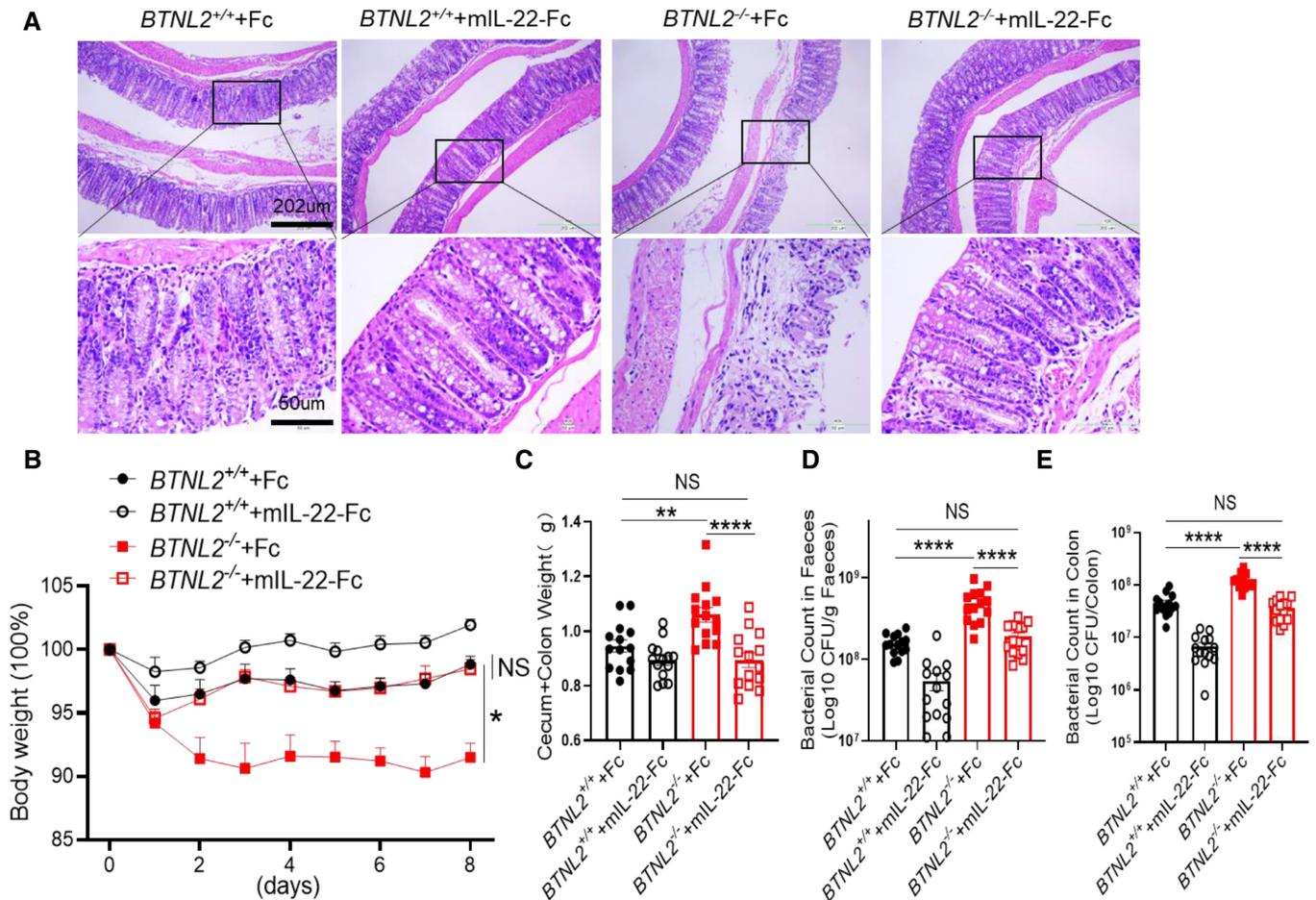


Figure EV2. mIL-22-Fc recombinant protein reversed the defective phenotype of BTNL2 KO mice against *C. rodentium* infection.

A, B Representative H&E images (A) and body weight changes (B) in wild-type control mice or BTNL2-KO mice treated with Fc or mIL-22-Fc (ip. 5 µg/mouse) at day 0, 2, 4, and 6 during *C. rodentium* infection were shown ($n = 14$).

C–E Weight of cecum and colon (C) and bacterial titers in homogenates of fecal (D) or colon (E) from mice in (A) at day 9 after infection were shown ($n = 14$).

Data information: All data are mean \pm s.e.m. NS, not significant. * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$ based on two-way ANOVA for (B) and one-way ANOVA for (C–E). Each dot represents one repetition, $n = 14$. Data are representative of three independent biological replicates.

Source data are available online for this figure.

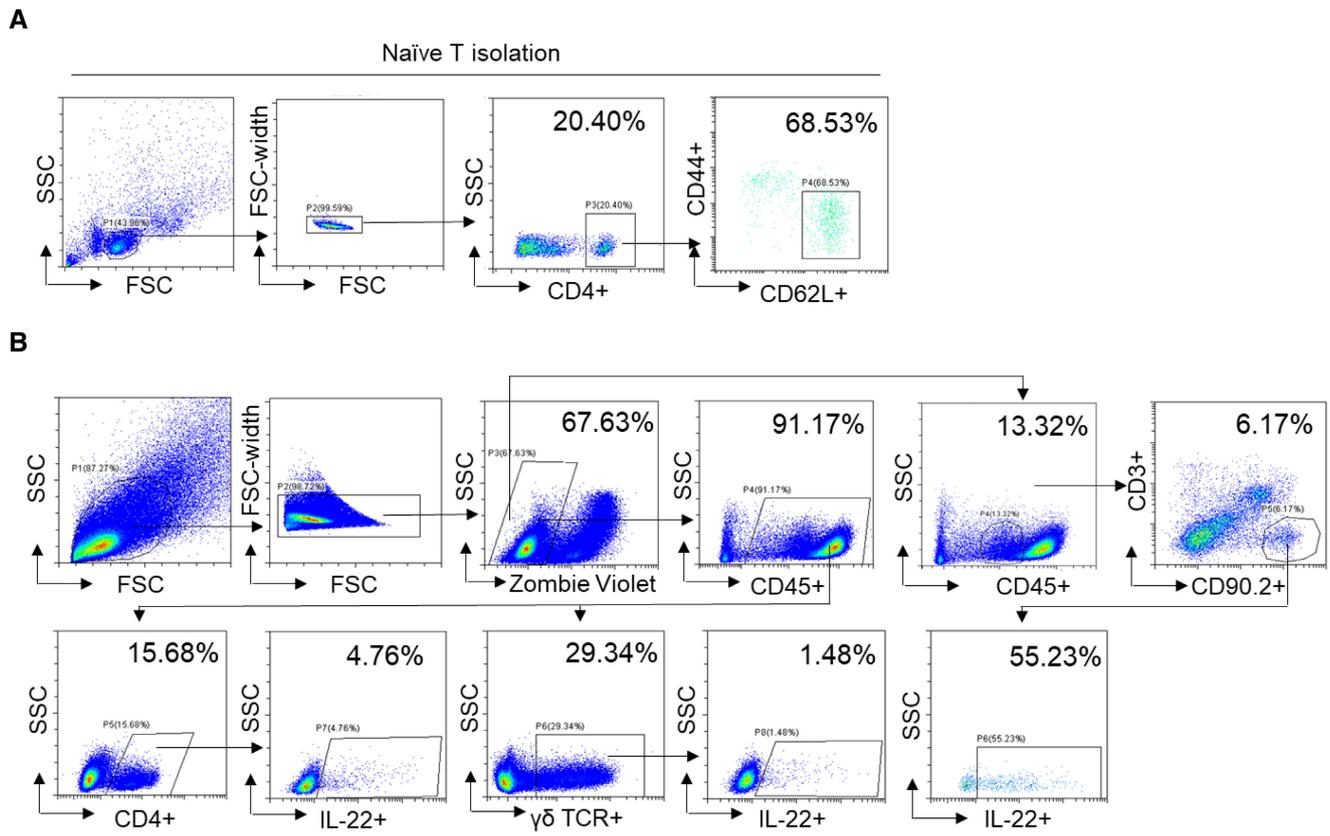


Figure EV3. Schematic diagram of flow cytometry.

A Schematic diagram of FACS sorted $CD4^+CD44^-CD62L^+$ Naïve $CD4^+$ T cells from splenocytes.

B Schematic diagram of $CD45^{Med}CD3^-CD90.2^+IL-22^+$ ILC3s, $CD45^+CD4^+IL-22^+$ $CD4^+$ T cells, and $CD45^+ \gamma\delta^+IL-22^+$ $\gamma\delta^+$ T cells in mouse colonic LPLs analyzed by flow cytometry.

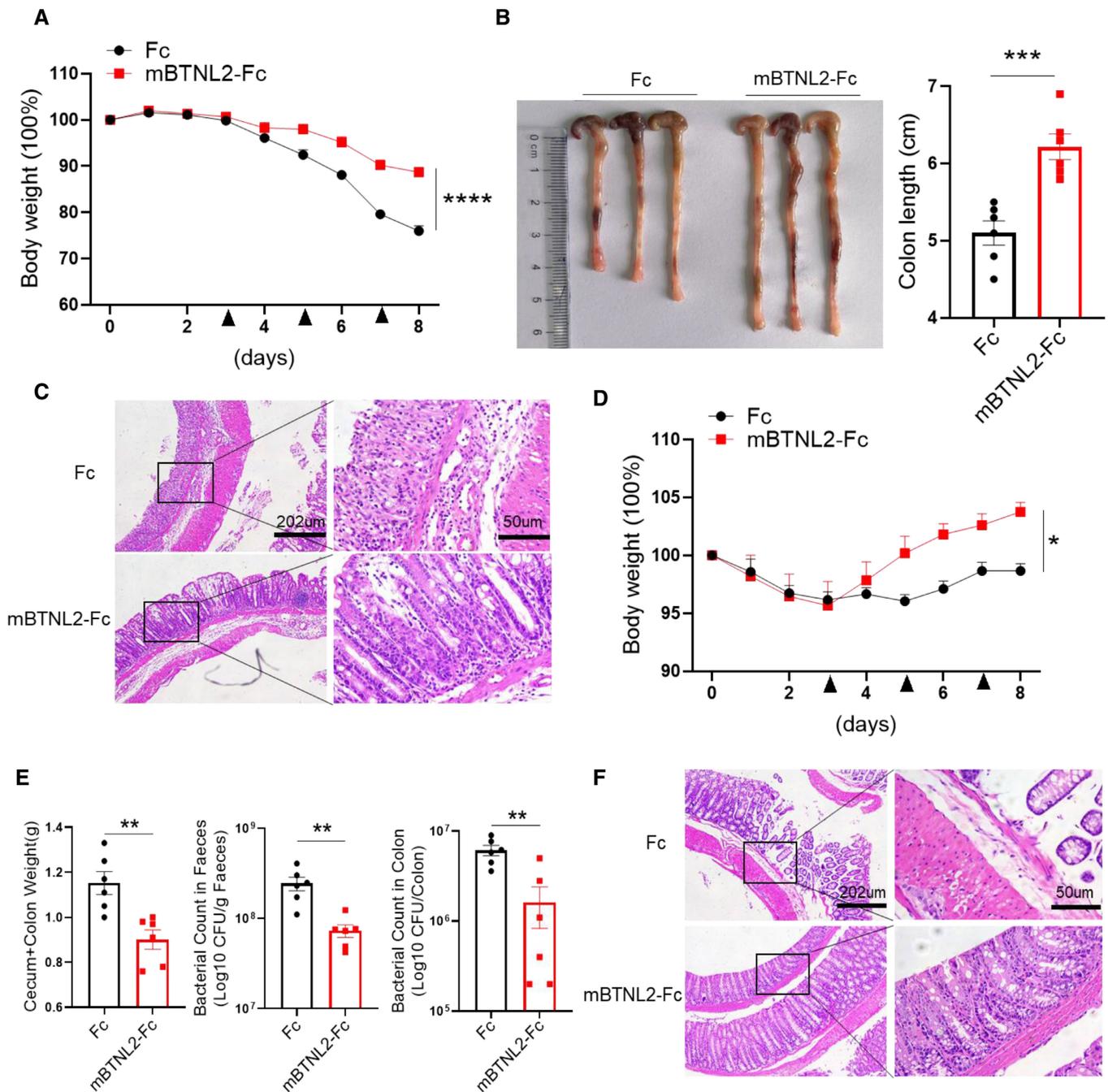


Figure EV4. BTNL2 has a protective role in mice colitis and *C. rodentium* infection after disease's onset.

A–C Body weight changes (A), representative colon image (left) and colon length (right) (B), and representative histological images (C) of wild-type mice treated with Fc or mBTNL2-Fc (ip. 50 µg/mouse) at day 3, 5, and 7 during DSS treatment were shown ($n = 6$).

D Body weight changes in wild-type mice treated with Fc or mBTNL2-Fc (ip. 50 µg/mouse) at day 3, 5, and 7 during *C. rodentium* infection were shown ($n = 6$).

E, F Weight of cecum and colon, bacterial titers in homogenates of fecal or colon (E) and representative histological images (F) from mice in (D) at day 9 after infection were shown ($n = 6$).

Data information: All data are mean \pm s.e.m. NS, not significant. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ based on two-way ANOVA for (A, D) and two-tailed Student's *t*-test for (B, E). Each dot represents one repetition, $n = 6$. Source data are available online for this figure.

Figure EV5. Anti-BTNL2 mAb aggravates DSS-induced colitis in mice.

- A–C Body weight changes (A), representative colon image (left) and colon length (right) (B), and representative histological images (C) of wild-type mice treated with isotype rat IgG1 control Ab or anti-BTNL2 mAb (ip. 200 μ g/mouse) at day 0, 2, 4, and 6 during DSS treatment were shown ($n = 6$). Data information: All data are mean \pm s.e.m. $**P < 0.01$, $***P < 0.001$ based on two-way ANOVA for (A) and two-tailed Student's t -test for (B). Each dot represents one repetition, $n = 6$.
- D Summary schematic. BTNL2 acts on Group 3 innate lymphoid cells (ILC3s), CD4⁺ T cells, and $\gamma\delta$ T cells in the gut to produce IL-22 through JAK-STAT3-HIF-1 α /RORC pathway, and a monoclonal antibody blocking BTNL2 attenuates colorectal tumorigenesis in mice by attenuating IL-22 production in the gut.

Source data are available online for this figure.

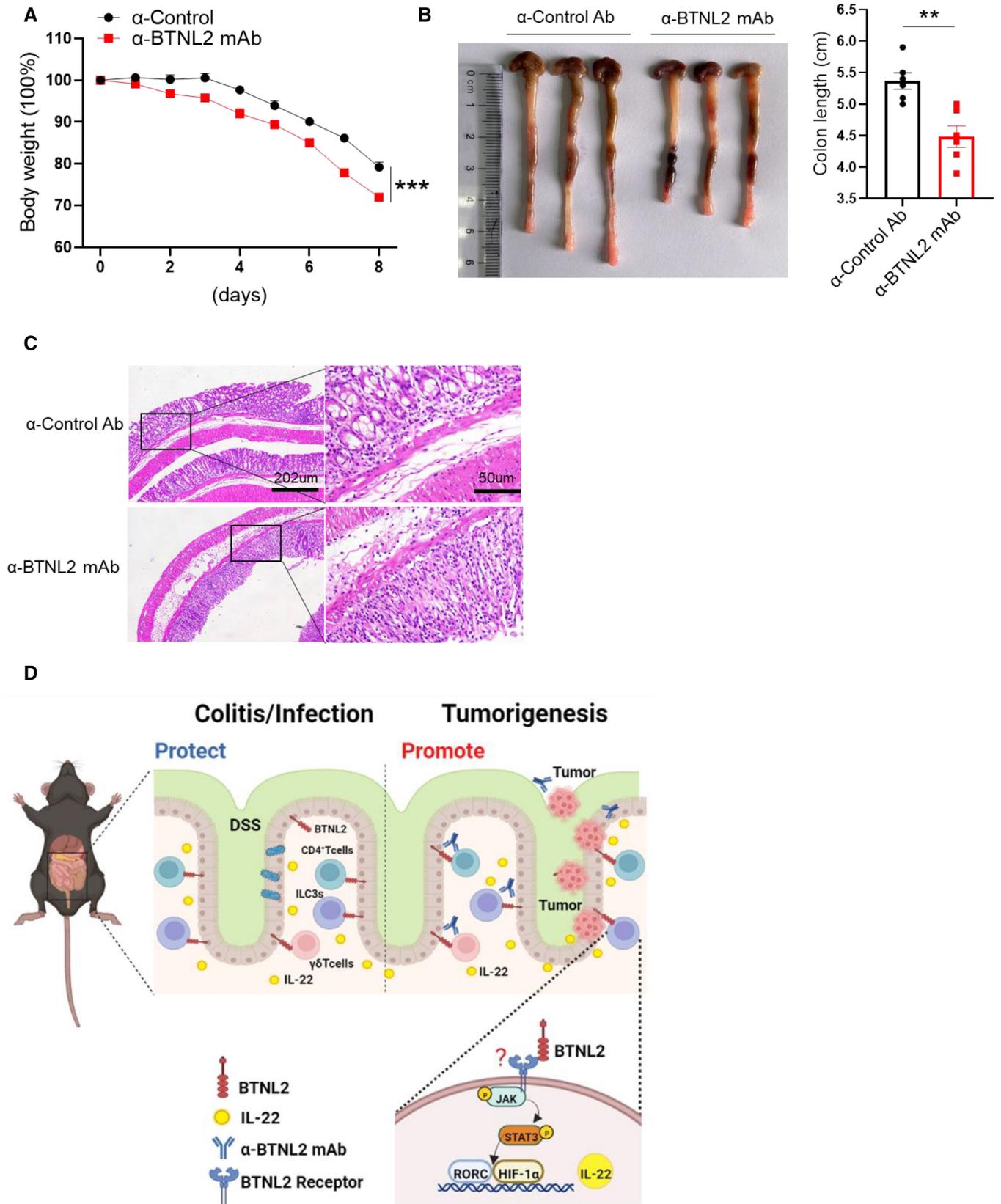


Figure EV5.