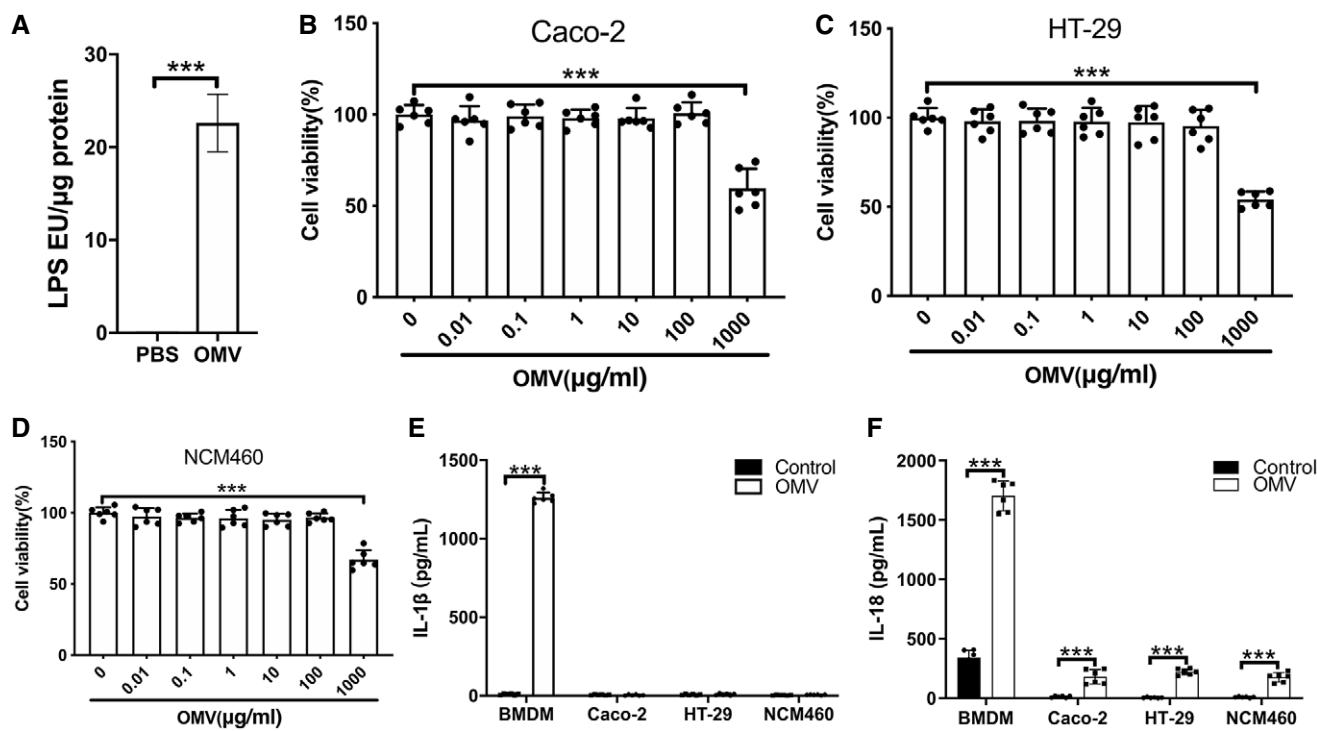


## Expanded View Figures



**Figure EV1. Effects of OMV treatment on the viability of intestinal epithelial cells.**

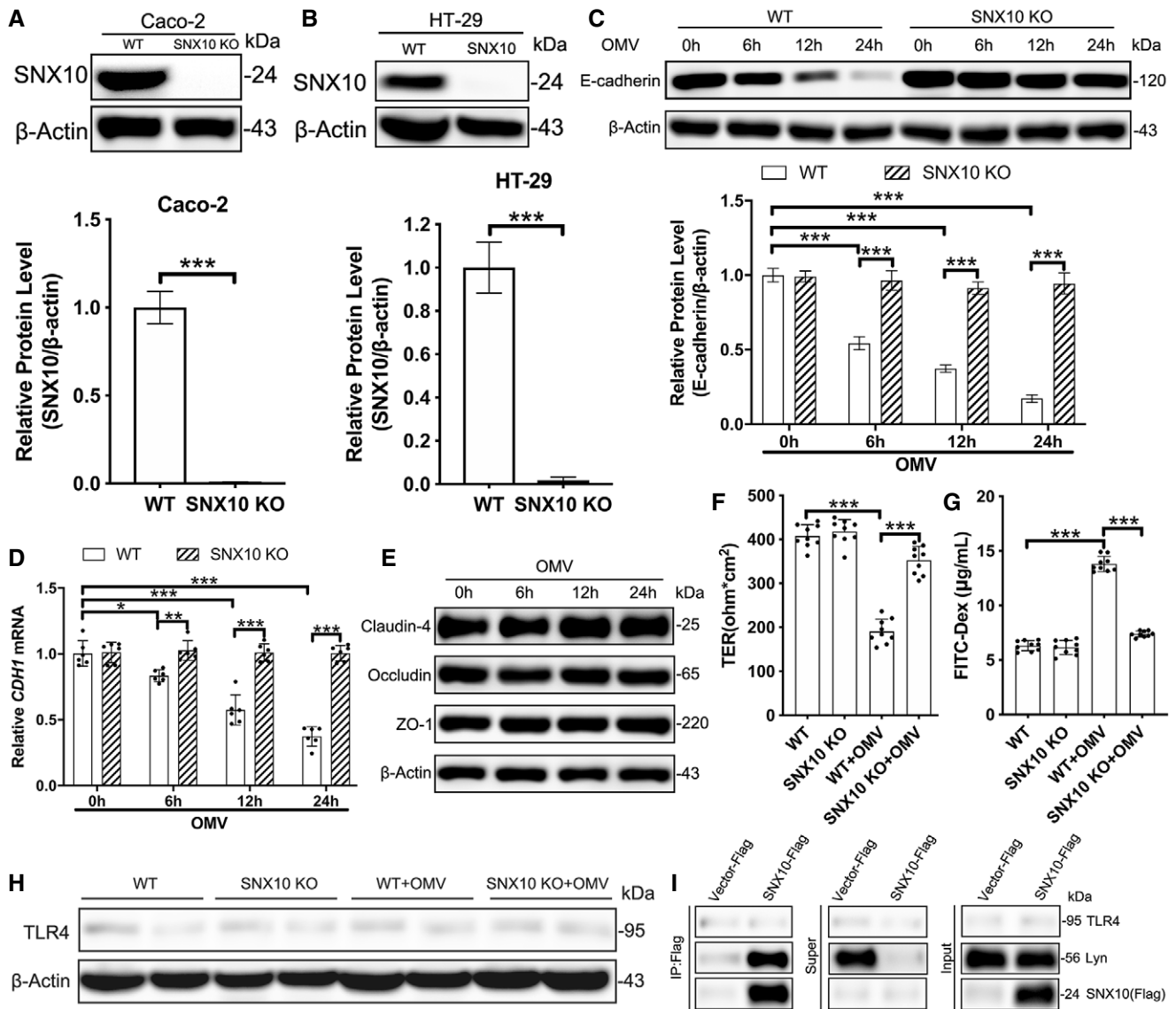
A LPS levels in purified OMVs were determined by LAL assay ( $n = 6$  independent experiments).

B–D The cell viability of Caco-2 (B), HT-29 (C), and NCM460 (D) cells treated with indicated doses of OMVs for 24 h was detected by Cell Counting Kit-8 (CCK-8;  $n = 6$  independent experiments).

E IL-1 $\beta$  secretion by indicated cell types stimulated with OMVs (100  $\mu\text{g}/\text{ml}$ ) for 24 h was detected by ELISA ( $n = 6$  independent experiments).

F IL-18 secretion by indicated cell types stimulated with OMVs (100  $\mu\text{g}/\text{ml}$ ) for 24 h was detected by ELISA ( $n = 6$  independent experiments).

Data information: Data are means  $\pm$  SD. Two-tailed unpaired t-test between two groups and one-way ANOVA followed by Bonferroni *post hoc* test for multiple comparisons were utilized for statistical analyses. \*\*\* $p < 0.001$ .

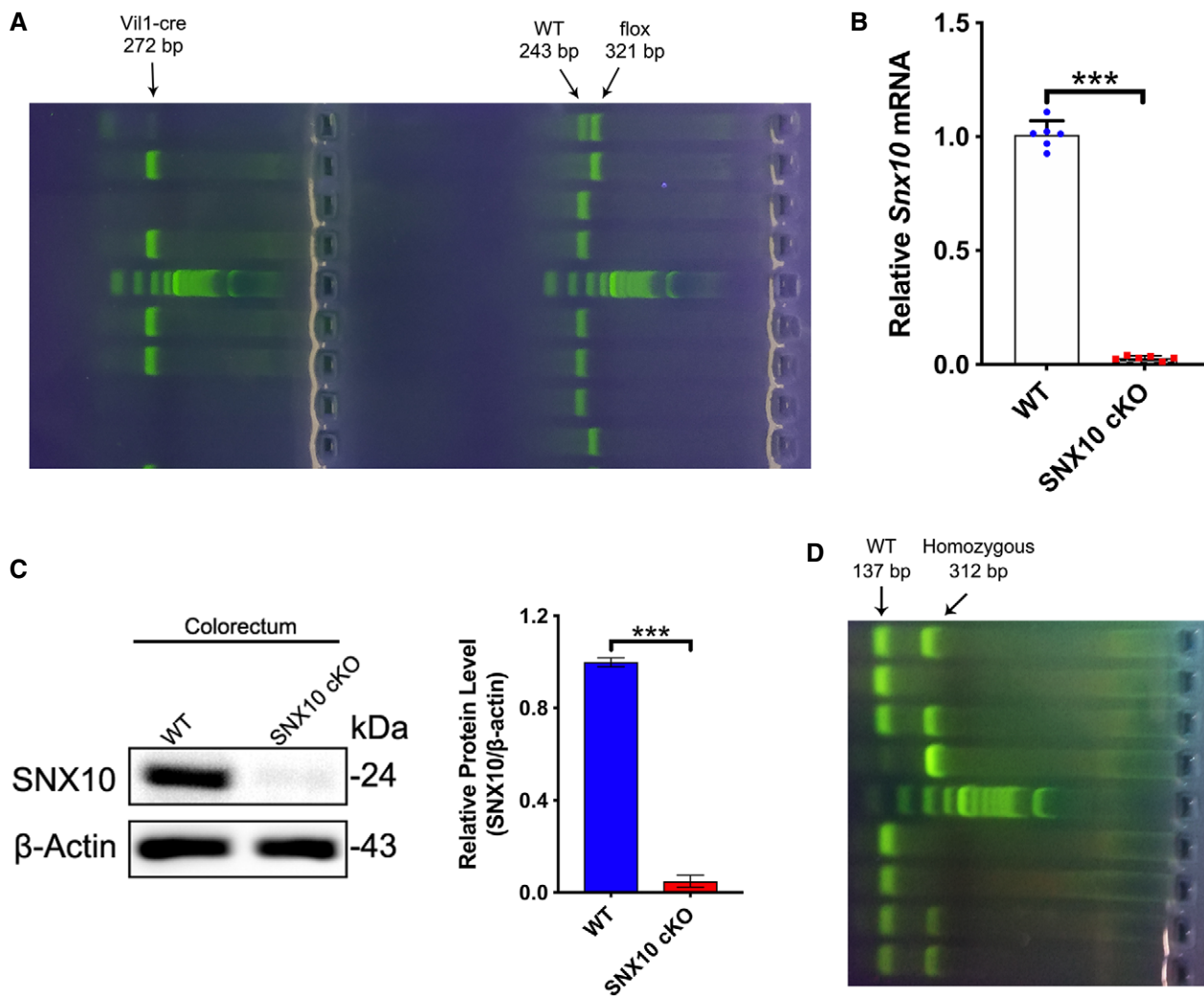


**Figure EV2. SNX10 deficiency maintains the E-cadherin expression and intestinal epithelial barrier function in HT-29 cells treated with OMVs.**

- A SNX10 was deleted in Caco-2 cells by CRISPR/Cas9 technology, and the efficiency was confirmed by immunoblots and quantified by ImageJ software ( $n = 6$  independent experiments).
- B SNX10 was deleted in HT-29 cells by CRISPR/Cas9 technology, and the efficiency was confirmed by immunoblots and quantified by ImageJ software ( $n = 6$  independent experiments).
- C Protein levels of E-cadherin in HT-29 cells were measured by western blots and quantified by ImageJ software ( $n = 3$  independent experiments).
- D The relative mRNA levels of *CDH1* (encoding E-cadherin) in HT-29 cells were determined by RT-qPCR method ( $n = 6$  independent experiments).
- E The expression of tight junction proteins in Caco-2 cells treated with OMVs (100  $\mu\text{g}/\text{ml}$ ) was determined by immunoblots.
- F TEER value of HT-29 cell monolayers incubated with or without OMVs (100  $\mu\text{g}/\text{ml}$ ) for 24 h was measured ( $n = 9$  independent experiments).
- G HT-29 cell monolayers on transwell membranes were treated with or without OMVs (100  $\mu\text{g}/\text{ml}$ ) for 24 h. FITC-dextran was added to these cells (top of the membrane). After 2 h, FITC-dextran levels in the bottom chamber wells were detected ( $n = 9$  independent experiments).
- H Lysates from WT and SNX10 KO Caco-2 cells treated with or without 100  $\mu\text{g}/\text{ml}$  OMVs for 24 h were analyzed by immunoblots.
- I Lysates from Caco-2 cells transfected with SNX10-Flag were subjected to pull-down assay with anti-Flag antibody-conjugated agarose, followed by immunoblots with indicated antibodies.

Data information: Data are means  $\pm$  SD. Two-tailed unpaired *t*-test between two groups (A and B), and one-way ANOVA followed by Bonferroni *post hoc* test for multiple comparisons were utilized for statistical analyses (C, D, F and G). \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

Source data are available online for this figure.



**Figure EV3. The confirmation of SNX10 deficiency and IL-10 deficiency in mice.**

A Identification of SNX10 intestinal epithelium conditional knockout (SNX10 cKO) mice (*Vil1-cre<sup>+</sup>Snx10<sup>fl/fl</sup>*) and WT mice (*Vil1-cre<sup>-</sup>Snx10<sup>fl/fl</sup>*).

B The mRNA levels of *Snx10* in intestinal epithelium of WT and SNX10 cKO mice were detected by RT-qPCR ( $n = 6$  animals, each group).

C Protein expression of SNX10 in intestinal epithelium of WT and SNX10 cKO mice was determined by immunoblots and quantified by ImageJ software ( $n = 3$  animals, each group).

D Identification of IL-10 knockout (IL-10 KO) mice (*Il10<sup>-/-</sup>*) and WT mice (*Il10<sup>+/+</sup>*).

Data information: Data are means  $\pm$  SD. Two-tailed unpaired  $t$ -test between two groups was utilized for statistical analyses. \*\*\* $P < 0.001$ .

Source data are available online for this figure.

**Figure EV4. Small molecule compound DC-SX029 targets SNX10 to block its protein–protein interaction and improves DSS-induced mouse colitis.**

- A The structure of DC-SX029.
- B Cellular Thermal Shift Assay (CETSA) was conducted with intact Caco-2 cells transfected SNX10-Flag in the presence of 50  $\mu$ M DC-SX029. Representative immunoblots for the stabilization of SNX10 protein under 45.5–64.5°C were shown. The protein levels were quantified by Image J ( $n = 3$  independent experiments).
- C Caco-2 cells transfected with SNX10-Flag were treated with vehicle or 50  $\mu$ M DC-SX029. Cell lysates were subjected to pull-down by anti-FLAG M2 agarose beads, followed by immunoblots with the indicated antibodies.
- D LPS levels in the cytosolic and residual fractions of Caco-2 cells stimulated by OMVs (100  $\mu$ g/ml) with or without DC-SX029 (50  $\mu$ M) for the indicated time were detected by LAL assay ( $n = 6$  independent experiments).
- E Cytoplasmic and nuclear proteins were extracted from Caco-2 cells and the indicated proteins were detected by immunoblots.
- F TEER value of Caco-2 cell monolayers was analyzed ( $n = 9$  independent experiments).
- G Caco-2 cell monolayers on transwell membranes were stimulated by OMVs (100  $\mu$ g/ml) with or without DC-SX029 (50  $\mu$ M) for 24 h. FITC-dextran was added to these cells (top of the membrane). After 2 h, FITC-dextran levels in the bottom chamber wells were measured ( $n = 9$  independent experiments).
- H Body weight of mice was measured daily ( $n = 6$  animals, each group). One-way ANOVA followed by Bonferroni *post hoc* test was used for statistical analyses.  $**P < 0.01$  and  $***P < 0.001$  versus Control group;  $###P < 0.001$  versus DSS stimulation group.
- I, J Length of colons from mice induced by DSS with or without DC-SX029 (2 mg/kg/day) was measured (I) and analyzed (J) on day 7 ( $n = 6$  animals, each group).
- K Clinical score was evaluated every day for 1 week ( $n = 6$  animals, each group). One-way ANOVA followed by Bonferroni *post hoc* test was used for statistical analyses.  $***P < 0.001$  versus Control group;  $###P < 0.001$  versus DSS stimulation group.
- L, M Representative H&E images of colon tissues of mice induced by DSS with or without DC-SX029 (2 mg/kg/day) on day 7 were shown (L) and histological changes were analyzed (M) ( $n = 6$  animals, each group). Scale bar: 100  $\mu$ m.
- N The content of TNF- $\alpha$ , IL-6, IL-12/IL-23 p40, and IL-17A in the serum of mice was measured by ELISA ( $n = 6$  animals, each group).
- O The relative mRNA levels of *Tnf*, *Il6*, *Il23a*, and *Il17a* in colon epithelial tissues of mice were measured ( $n = 6$  animals, each group).

Data information: Data are means  $\pm$  SD. One-way ANOVA followed by Bonferroni *post hoc* test was used for statistical analyses.  $*P < 0.05$ ;  $**P < 0.01$ ;  $***P < 0.001$ .

Source data are available online for this figure.

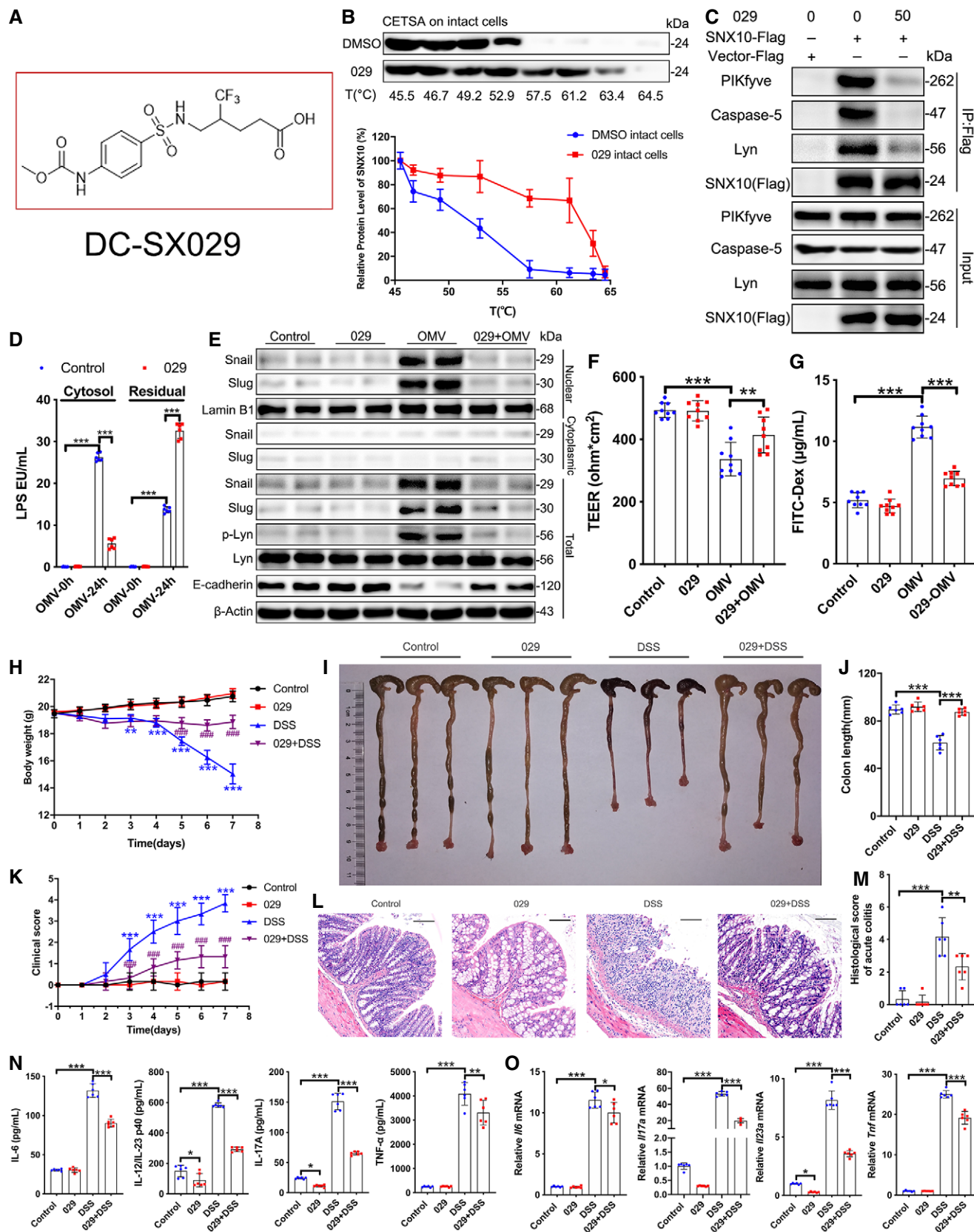
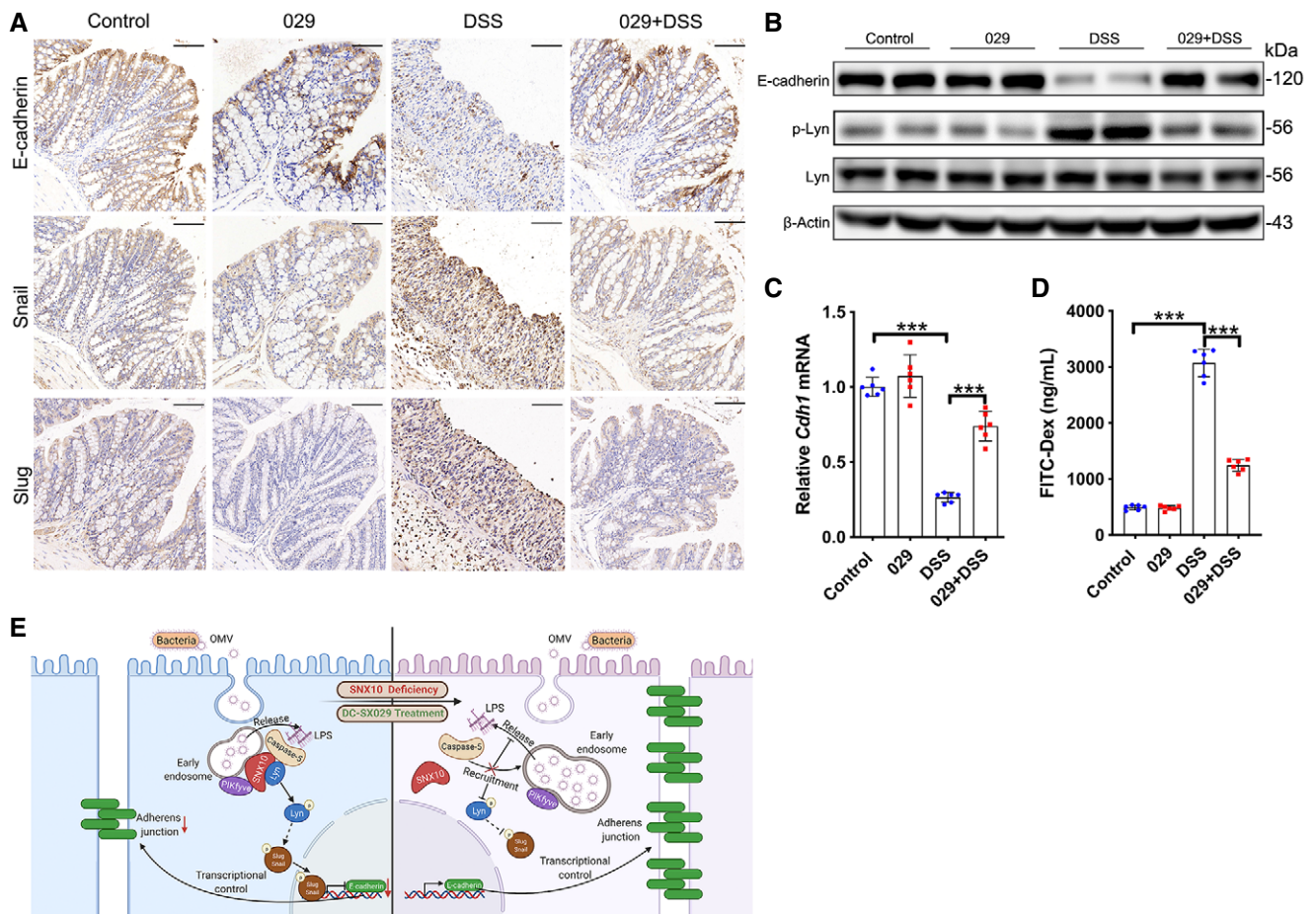


Figure EV4.





**Figure EV5. The SNX10 PPI inhibitor DC-SX029 maintains the intestinal epithelial barrier function in DSS-induced mouse colitis.**

- A Representative immunohistochemistry images of E-cadherin, Snail and Slug in colon tissues on day 7 from mice induced by DSS with or without DC-SX029 (2 mg/kg/day) were shown. Scale bar: 100  $\mu$ m.
- B Protein expression of E-cadherin, p-Lyn, and Lyn in colon epithelial tissues was determined by immunoblots.
- C The relative mRNA levels of *Cdh1* (encoding E-cadherin) in colon epithelial tissues were measured ( $n = 6$  animals, each group).
- D The intestinal permeability *in vivo* was evaluated using FITC-Dextran ( $n = 6$  animals, each group).
- E Proposed mechanisms for SNX10 in integrating liberation and sensing of cytoplasmic LPS to control PIKfyve-caspase-5-Lyn-Snail/Slug-E-cadherin signaling activation cascade.

Data information: Data are means  $\pm$  SD. One-way ANOVA followed by Bonferroni *post hoc* test was used for statistical analyses. \*\*\* $P < 0.001$ .

Source data are available online for this figure.