

SNX10-dependent LPS sensing by caspase-5 and resulting Lyn signaling causes gut barrier dysfunction

Wang *et al.*

Short title: Role of SNX10 in gut barrier function

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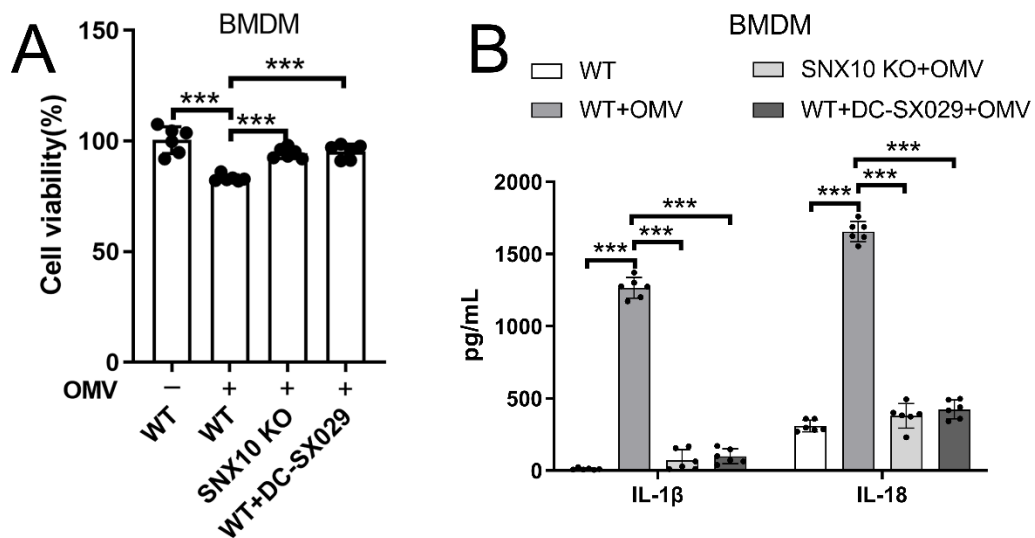
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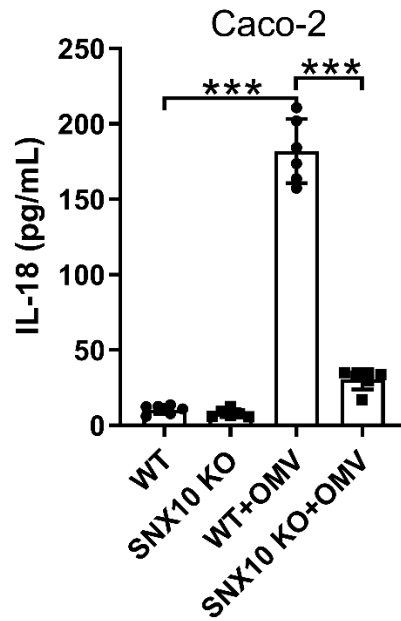
Appendix Figures and Legends



Appendix figure S1. SNX10 KO or DC-SX029 increased the cell viability and inhibited the secretion of IL-1 β and IL-18 in OMV-treated BMDMs

A and B SNX10 deficient BMDMs or WT BMDMs treated with DC-SX029 were stimulated with OMVs (100 μ g/mL) for 24 h, the cell viability (A) was detected by Cell Counting Kit-8 (CCK-8), and IL-1 β and IL-18 secretion (B) was detected by ELISA. n = 6 independent experiments.

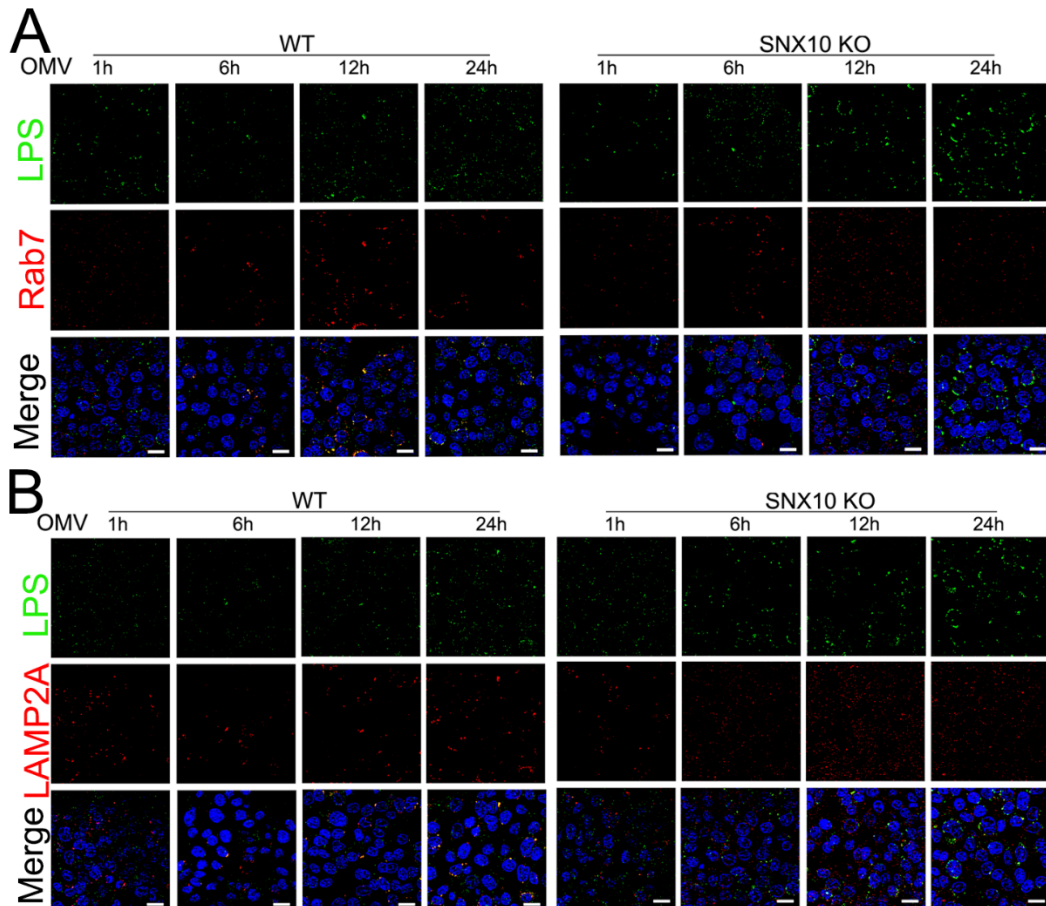
Data information: Data are means \pm SD. One-way ANOVA followed by Bonferroni post hoc test for multiple comparisons were utilized for statistical analyses. ***p < 0.001.



Appendix figure S2. SNX10 KO inhibited the secretion of IL-18 in OMV-treated Caco-2 cells

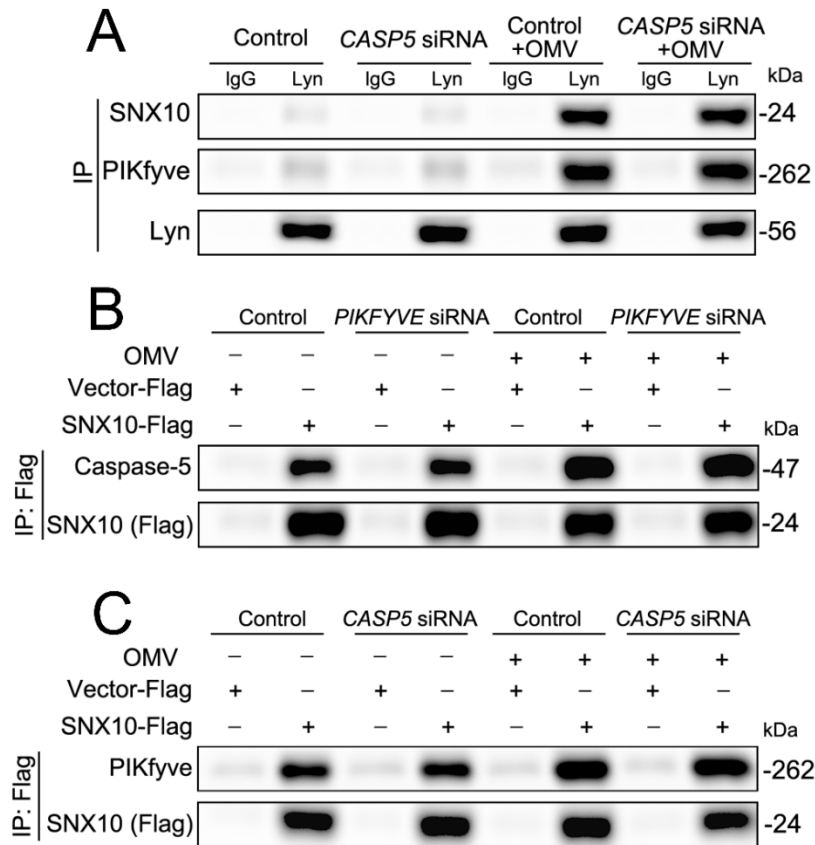
IL-18 secretion by Caco-2 cells stimulated with OMVs (100 µg/mL) for 24 h under SNX10 deficiency was detected by ELISA (n = 6 independent experiments).

Data information: Data are means ± SD. One-way ANOVA followed by Bonferroni post hoc test for multiple comparisons were utilized for statistical analyses. ***p < 0.001.



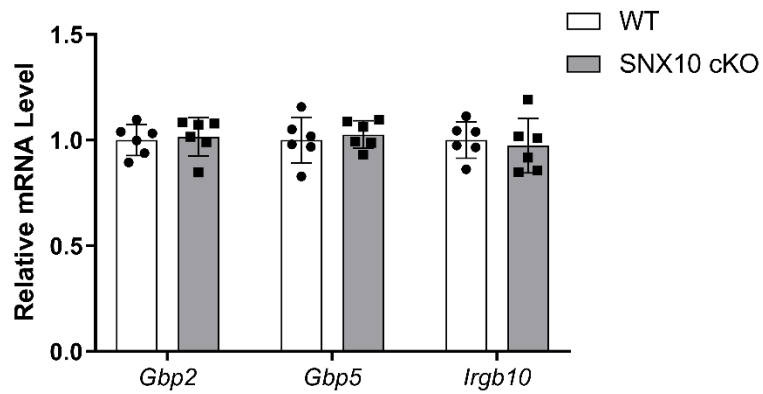
Appendix figure S3. The distribution of the cytoplasmic LPS versus later endosome marker Rab7 and lysosome marker LAMP2A

A and B WT and SNX10 KO Caco-2 cells were treated with OMVs (100 $\mu\text{g}/\text{mL}$) for the indicated time and stained with antibodies against LPS and Rab7 (A) or LAMP2A (B). Scale bar: 20 μm .



Appendix figure S4. The interaction of SNX10 with Lyn, caspase-5 or PIKfyve

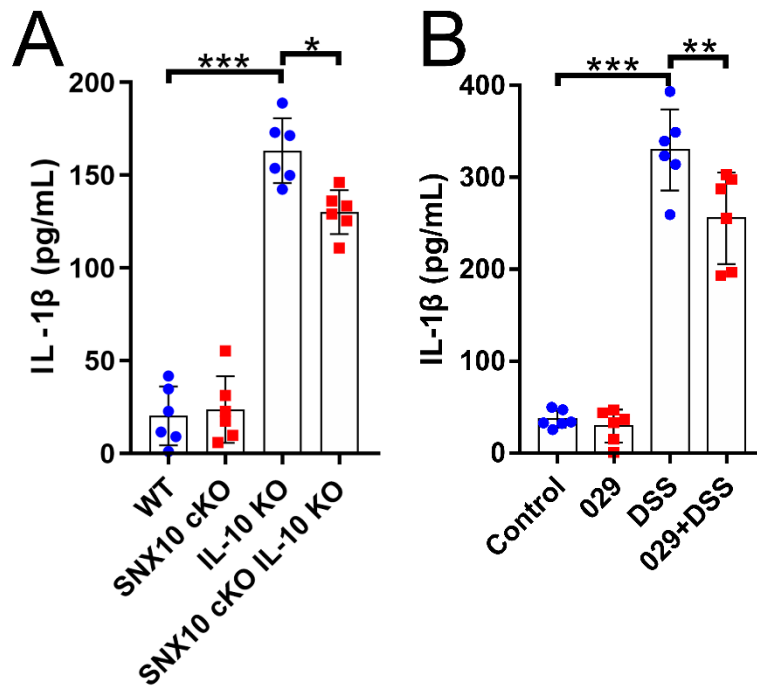
- A Caco-2 cells transfected with control siRNA or *CASP5* siRNA were treated with or without OMVs (100 µg/mL) for 24h and then subjected to IP with anti-Lyn antibody.
- B After transfected with control siRNA or *PIKFYVE* siRNA, SNX10-Flag transfected Caco-2 cells were treated with or without OMVs (100 µg/mL) for 24 h, and the cell lysates were subjected to IP.
- C After transfected with control siRNA or *CASP5* siRNA, SNX10-Flag transfected Caco-2 cells were treated with or without OMVs (100 µg/mL) for 24 h, and the cell lysates were subjected to IP.



Appendix figure S6. Effects of SNX10 deficiency on the expression of GBPs and IRGB10

The relative mRNA levels of *Gbp2*, *Gbp5* and *Irgb10* in colon epithelial tissues of WT and SNX10 cKO mice were measured (n = 6 animals, each group).

Data information: Data are means ± SD. One-way ANOVA followed by Bonferroni post hoc test for multiple comparisons were utilized for statistical analyses.



Appendix figure S7. Targeting SNX10 inhibited the secretion of IL-1 β in the serum of mice with IL-10 deficiency-induced or DSS-induced colitis

A The content of IL-1 β in the serum of WT, SNX10 cKO, IL-10 KO and SNX10 cKO IL-10 KO mice at the 16th week were measured by ELISA (n = 6 animals, each group).

B The content of IL-1 β in the serum of mice induced by DSS with or without DC-SX029 (2 mg/kg/day) were measured by ELISA at day 7 (n = 6 animals, each group).

Data information: Data are means \pm SD. One-way ANOVA followed by Bonferroni post hoc test for multiple comparisons were utilized for statistical analyses. *p < 0.05; **p < 0.01; ***p < 0.001.

Supplementary Methods

The production of the *Snx10* floxed mice

Snx10 floxed mice and *Vil1-cre* mice were purchased from Shanghai Research Center for Model Organisms (Shanghai, China). Mice containing the *Snx10*-flox (flanked by loxP) gene were established by inserting a homozygous loxP fragment into exon 4 and exon 5 of the mouse *Snx10* gene (Appendix Fig S5A), and then crossed them with *Vil1-cre* mice to obtain *Snx10*-flox homozygous (*Snx10^{f/f}*) *Vil1-cre* positive mice, in which the intestinal epithelium-specific Villin1 (*Vil1*) promoter can control Cre enzyme expression restricted to the intestinal epithelium, resulting in the specific knockout of the *Snx10* gene in the intestinal epithelium.

The *Snx10* gene was modified by flox using homologous recombination in fertilized eggs, and the specific strategy is shown in Appendix Fig S5A. The process was as follows: Cas9 mRNA and gRNA were obtained by in vitro transcription; the homologous recombination vector (donor vector) was constructed by In-Fusion cloning. Cas9 mRNA, gRNA and homologous recombinant vector (Appendix Fig S5B) were microinjected into the fertilized eggs of C57BL/6J mice, and F0 generation mice were obtained by microinjection of fertilized eggs. The genotypes were identified by long fragment PCR and the PCR products were sequenced. F1 generation mice (*Snx10^{f/+}*) were obtained by crossing F0 generation mice with wild-type C57BL/6J mice.

The target gene *Snx10* sequence was obtained from Mouse Genome Informatics (MGI) library, number 1919232; the transcript targeted by the protocol is *Snx10*-001, Ensembl number ENSMUST00000049152; loxP is inserted at both ends of exons 4 and 5.

Intron3 target sequence:

ggagggccatgccacccagggttaacactgctcattcctgtcgtcatggcttctt

The guide RNA (gRNA) target sequences:

Guide #1 GAATGAGCAGTGTTAGCCCT GGG

Guide #2 GAGCAGTGTTAGCCCTGGGT GGG

Intron5 target sequence: gtctcttaagagcacagtagatacagcgccagcatacacatctgaggcaggag

The guide RNA (gRNA) target sequences:

Guide #3 CCTGCCTCAGATGTGTATGC TGG

Guide #4 AGCGCCAGCATAACATCTG AGG

Vil1-cre mice can specifically knockout/recombine the target gene contained in the flox fragment by Vill promoter-driven expression of Cre recombinase in epithelial cells of the small and large intestine. Crosses between the two mice yielded the intestinal epithelial cell *Snx10* gene conditional knockout mice *Vil1-cre⁺Snx10^{fl/fl}* (SNX10 cKO), and negative control mice *Vil1-cre⁻Snx10^{fl/fl}* (WT).