

Supplementary Materials: Inhibition of Autophagic Degradation Process Contributes to Claudin-2 Expression Increase and Epithelial Tight Junction Dysfunction in TNF- α Treated Cell Monolayers

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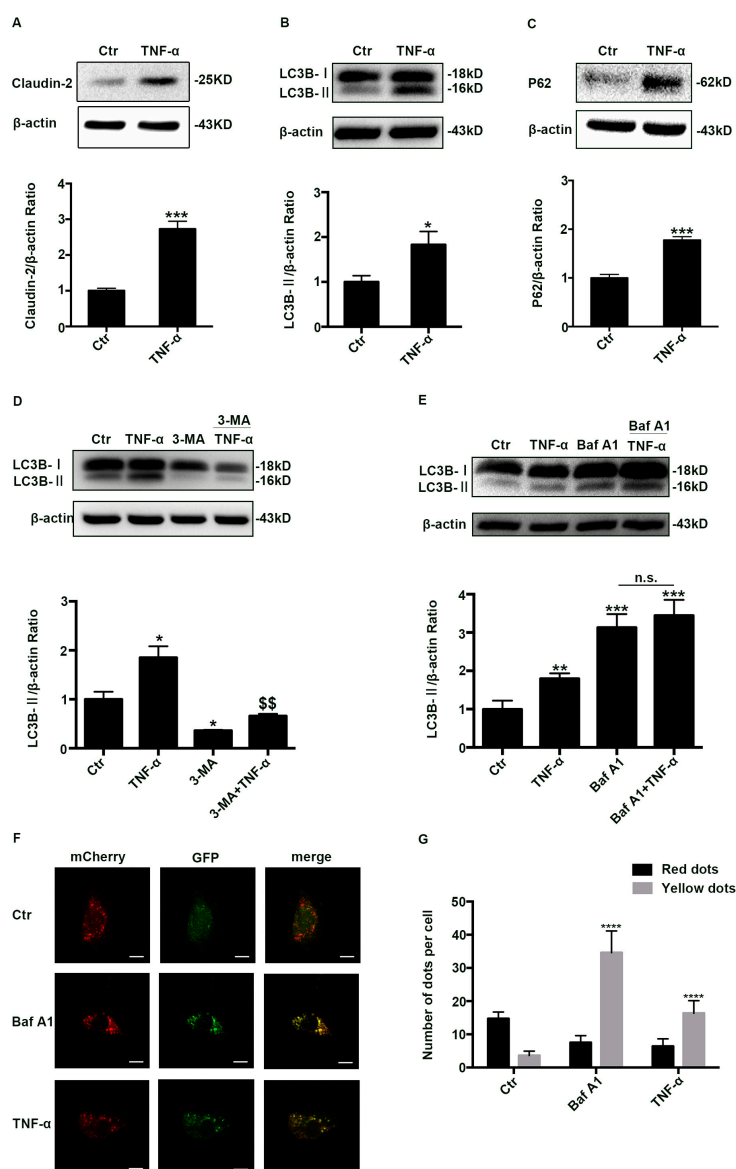


Figure S1. Claudin-2 increase and impaired autophagic flux in TNF- α treated IEC-6 cell monolayers. (A–C) Western blotting analysis of claudin-2, LC3B-II and P62 protein in IEC-6 cells after TNF- α (20 ng/mL, 48 h) treatment. (D,E) Western blot analysis of LC3B-II in cells treated with TNF- α (20 ng/mL) in the presence or absence of 3-MA (5 mM) or Baf A1 (100 nM) pretreatment. (F) Representative images of IEC-6 cells transfected with ad-mCherry-GFP-LC3B adenovirus after TNF- α (20 ng/mL) or Baf A1 treatment (100 nM). Scale bar: 10 μ m. (G) The number of red and yellow LC3 dots per cell were counted under confocal microscope (>30 cells/group). Data were shown as mean \pm SD and replicated in 3 times. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ versus control group. \$\$ $p < 0.01$ versus 3-MA group. n.s., no significant versus TNF- α group. Ctr, control group; 3-MA, 3-methyladenine; Baf A1, Bafilomycin A1.

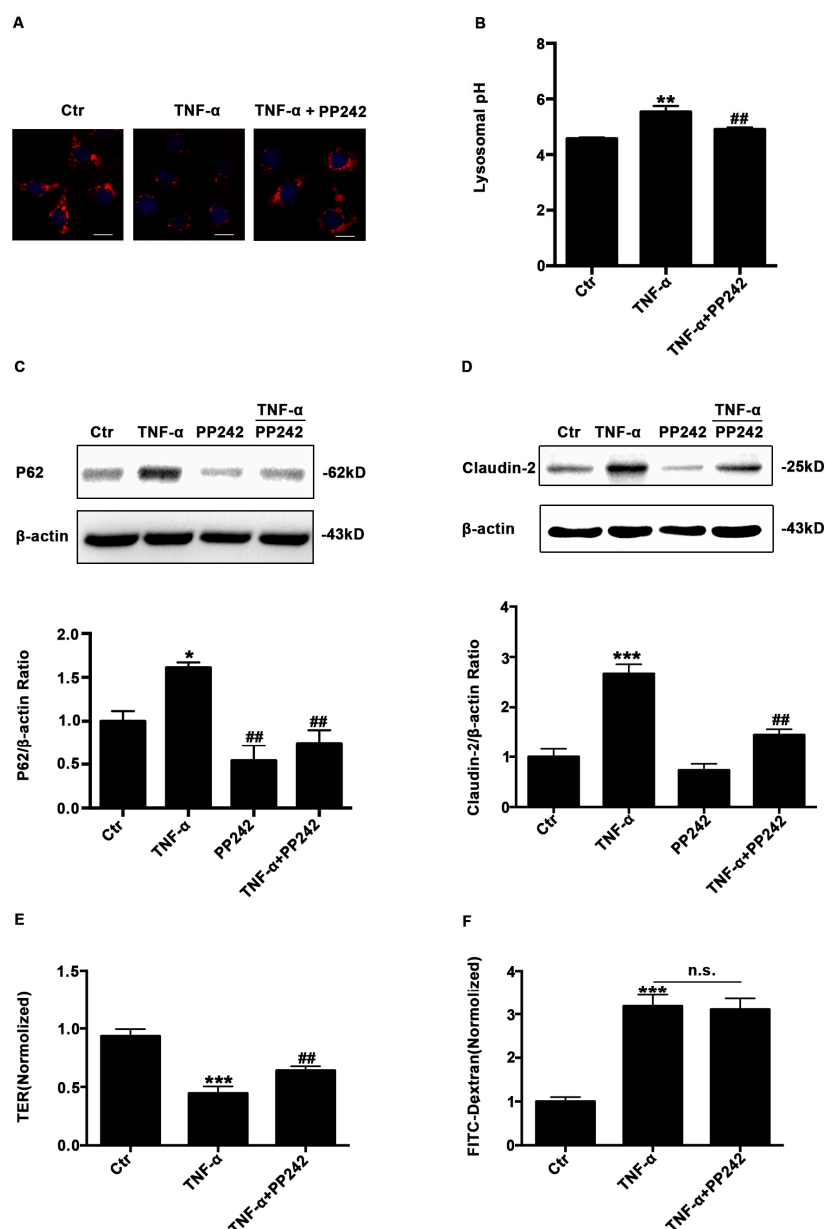


Figure S2. The effect of PP242 on the change of lysosomal pH, autophagic flux, claudin-2 and intestinal barrier function induced by TNF- α in IEC-6 cell monolayers. **(A)** Images of IEC-6 cells labeled with Lysotracker Red DND-99 dye after TNF- α (20 ng/mL, 48 h) and/or PP242 (1 μ M, 24 h) treatment. Scale bar: 15 μ m. **(B)** Lysosomal pH changes of IEC-6 cells labeled with Lysosensor Yellow/Blue DND-160 after TNF- α and/or PP242 treatment. **(C,D)** Western blot analysis of P62 and claudin-2 protein during TNF- α and/or PP242 treatment. **(E,F)** The effect of PP242 in TER and the permeability of the FITC-dextran in TNF- α treated IEC-6 cell monolayers. Data were shown as mean \pm SD and replicated in 3 times. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus control group. ## $p < 0.01$ versus TNF- α group. n.s., no significant versus TNF- α group. Ctrl, control group.