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Supplemental Information

Intestinal Epithelial Autophagy Is Essential for Host Defense against Invasive Bacteria

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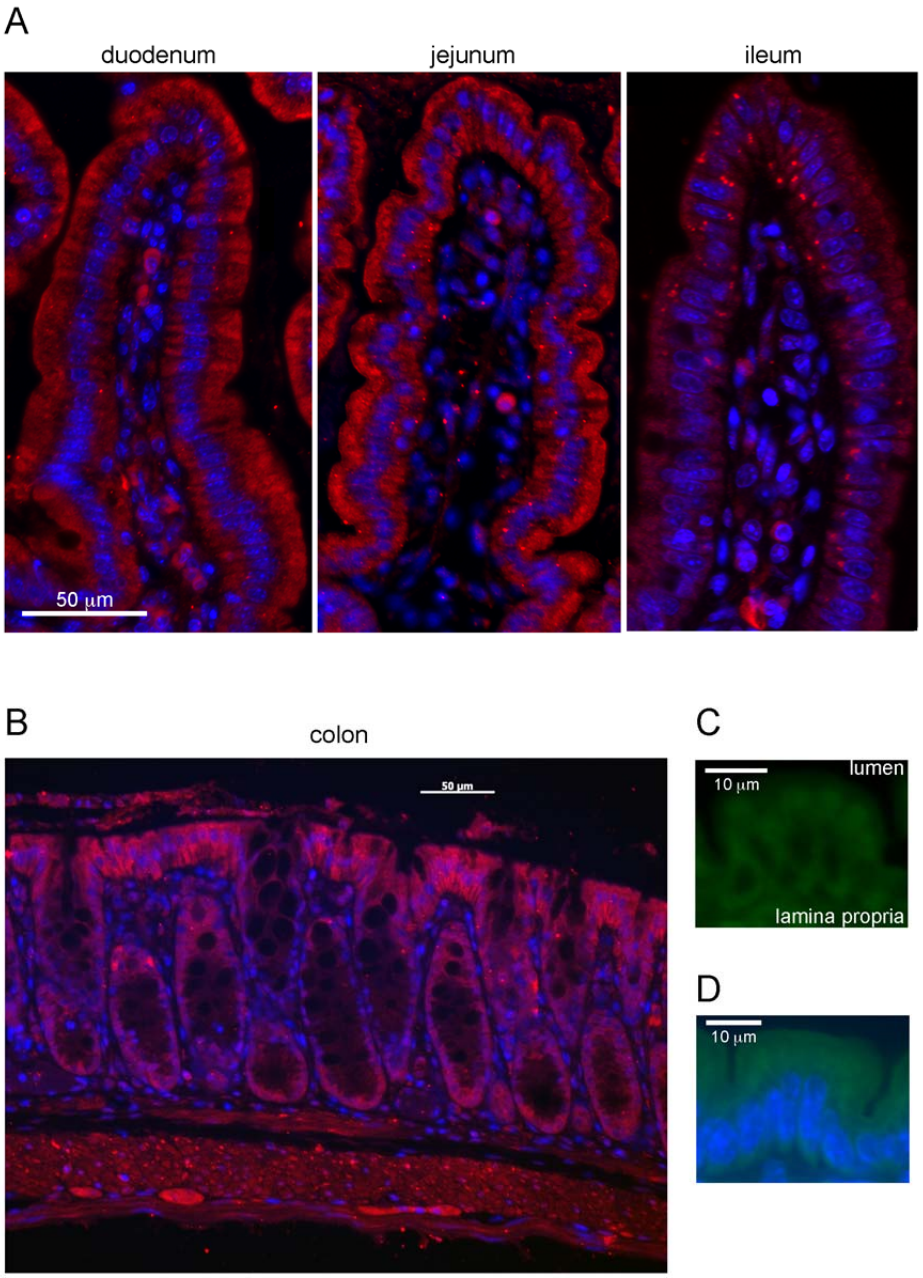

Figure S1. Cephalocaudal Distribution of LC3^{+} Autophagosomes in the Intestinal Epithelium Following *S.* Typhimurium Oral Challenge, Related to Figure 1

Germfree mice were orally challenged with 10^{9} CFU of *S.* Typhimurium. Small intestinal tissues (duodenum, jejunum, ileum) **(A)**, and colon **(B)** were fixed and probed with an anti-LC3 antibody. Scale bars= 50 μm. **(C)** To verify that the GFP signal in Figure 1F was not due to nonspecific autofluorescence, we analyzed a serially-cut section with a no primary antibody (anti-GFP) control. **(D)** Sections of germfree distal small intestine (ileum) were stained with anti-GFP to show that the GFP signal in Figure 1F is specific to *S.* Typhimurium.

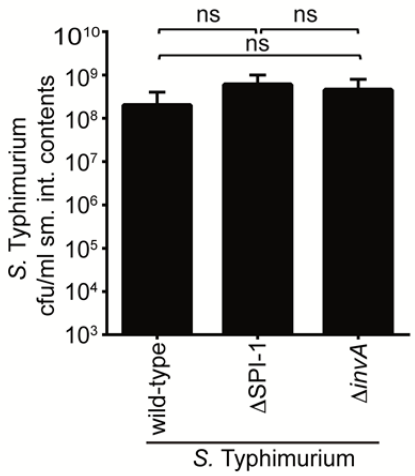

Figure S2. Intestinal Colonization Levels of Wild-Type and Mutant *S.* Typhimurium Strains, Related to Figure 4

Wild-type or isogenic mutant *S.* Typhimurium (ΔSPI-1 or Δ*invA*) was introduced into germ-free mice and small intestinal colonization levels were quantified 24 hours later by dilution plating. ns, not significant. Data are represented as mean±SEM.

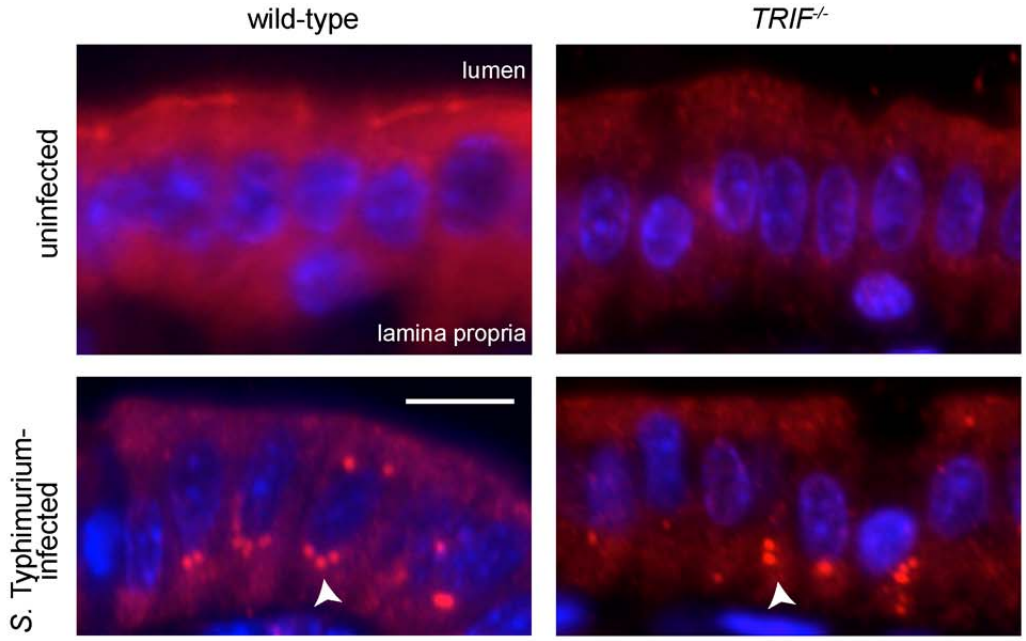

Figure S3. Autophagy Induction in the Intestinal Epithelium Is Not TRIF-Dependent, Related to Figure 5

Conventionally-raised wild-type and *TRIF*$^{-/-}$ mice were orally gavaged with 10^9 CFU of *S.* Typhimurium. Ileal tissues were taken after 24 hours and LC3^{+} puncta were visualized by immunofluorescence.

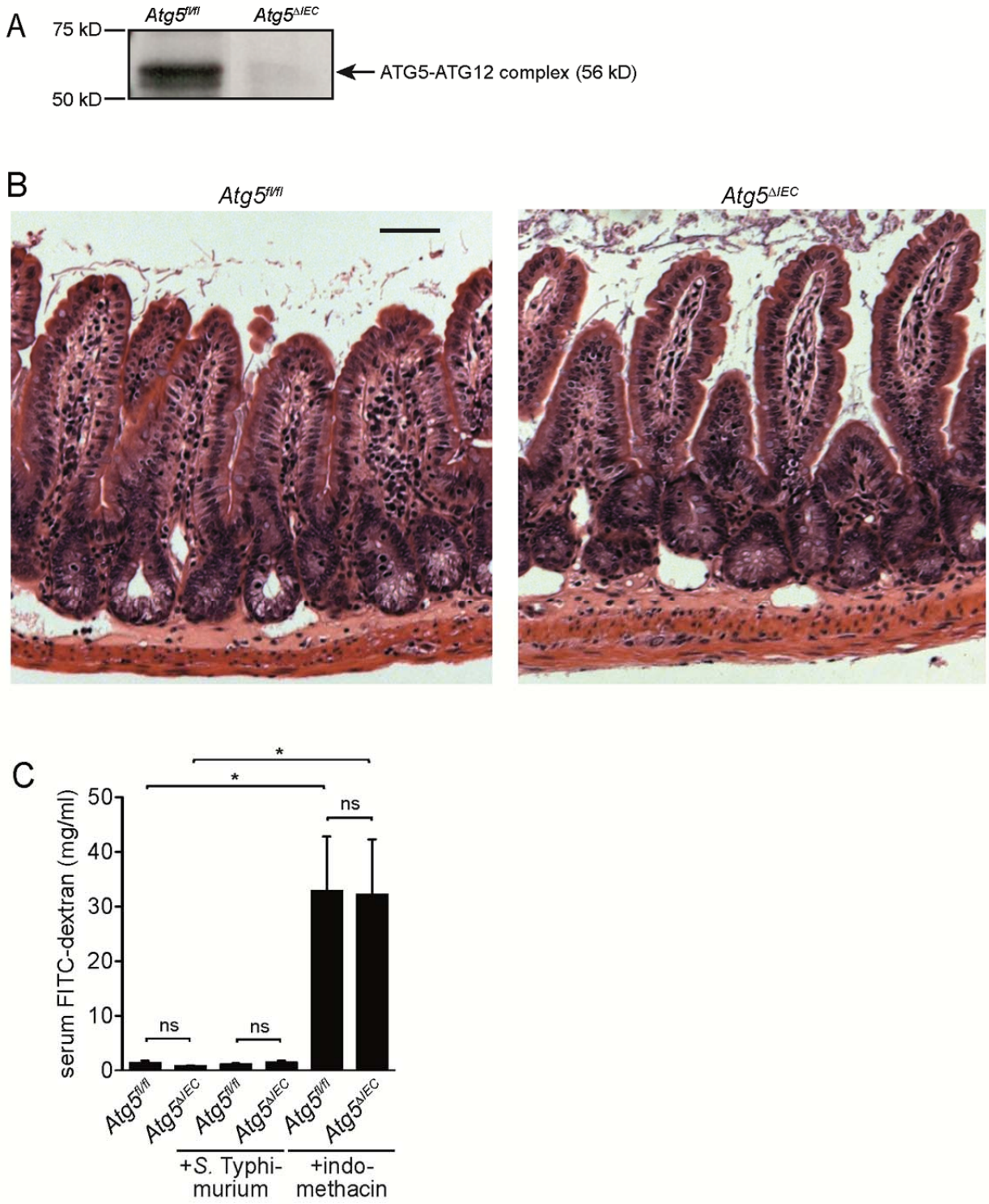

Figure S4. Characterization of *Atg5*$^{\Delta IEC}$ Mice, Related to Figure 6

(A) Western blot of isolated intestinal epithelial cell proteins, probed with anti-ATG5 antibody (Novus Biologicals). ATG5 exists in the ATG12-conjugated form within cells (Mizushima et al., 2001) and is predicted to migrate at 56 kDa.

(B) Small intestines from *Atg5*$^{fl/fl}$ and *Atg5*$^{\Delta IEC}$ mice, stained with hematoxylin and eosin. The images show that there is no overt pathology or inflammation in the unchallenged *Atg5*$^{\Delta IEC}$ mice.

(C) Intestinal permeability measurements in *Atg5*$^{fl/fl}$ and *Atg5*$^{\Delta IEC}$ mice. Serum levels of FITC-dextran 4 hours after oral gavage are shown. One set of mice was orally inoculated with 10^9 CFU of *S.* Typhimurium. As a positive control, intestinal epithelial damage was induced by pretreatment with indomethacin (or 10% DMSO as control) for 1 hour prior to FITC-dextran administration (600 mg/kg body weight; 4 kDa; Sigma). Data are represented as mean±SEM; *, $p<0.05$; ns, not significant; n=3-4 mice/group.

Supplemental References

Mizushima, N., Yamamoto, A., Hatano, M., Kobayashi, Y., Kabeya, Y., Suzuki, K., Tokuhisa, T., Ohsumi, Y., and Yoshimori, T. (2001). Dissection of autophagosome formation using Apg5-deficient mouse embryonic stem cells. J. Cell Biol. *152*, 657–668.