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Supplemental information

Autophagy controls mucus secretion from intestinal goblet cells by alleviating ER stress

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Supplementary Information

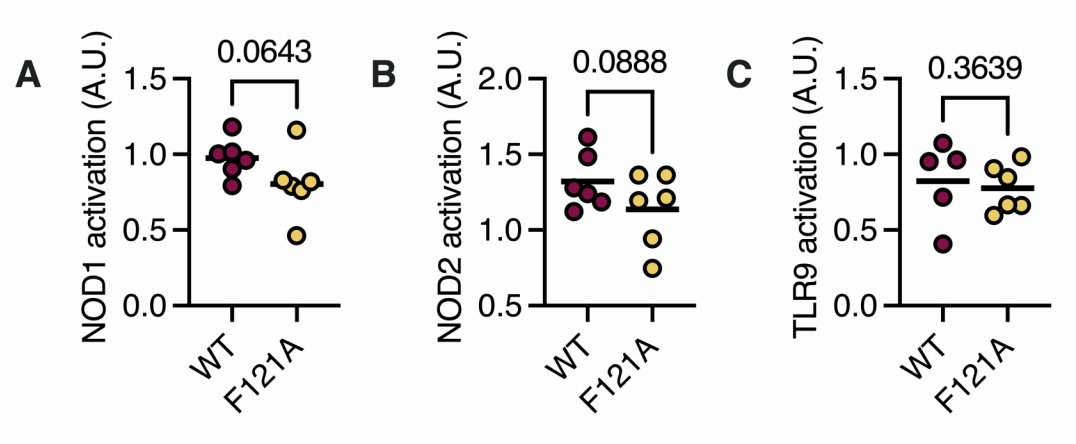

Figure S1. Reduced translocation of luminal antigens into the bloodstream of *Becn1*^{F121A} mice, Related to Figure 1. (A) Detection of NOD1, (B) NOD2 and (C) TLR9 agonist in mouse serum using reporter cell lines. Student's *t* test; *P* values are displayed. WT, wild type; F121A, *Becn1*^{F121A}; A.U., arbitrary units.

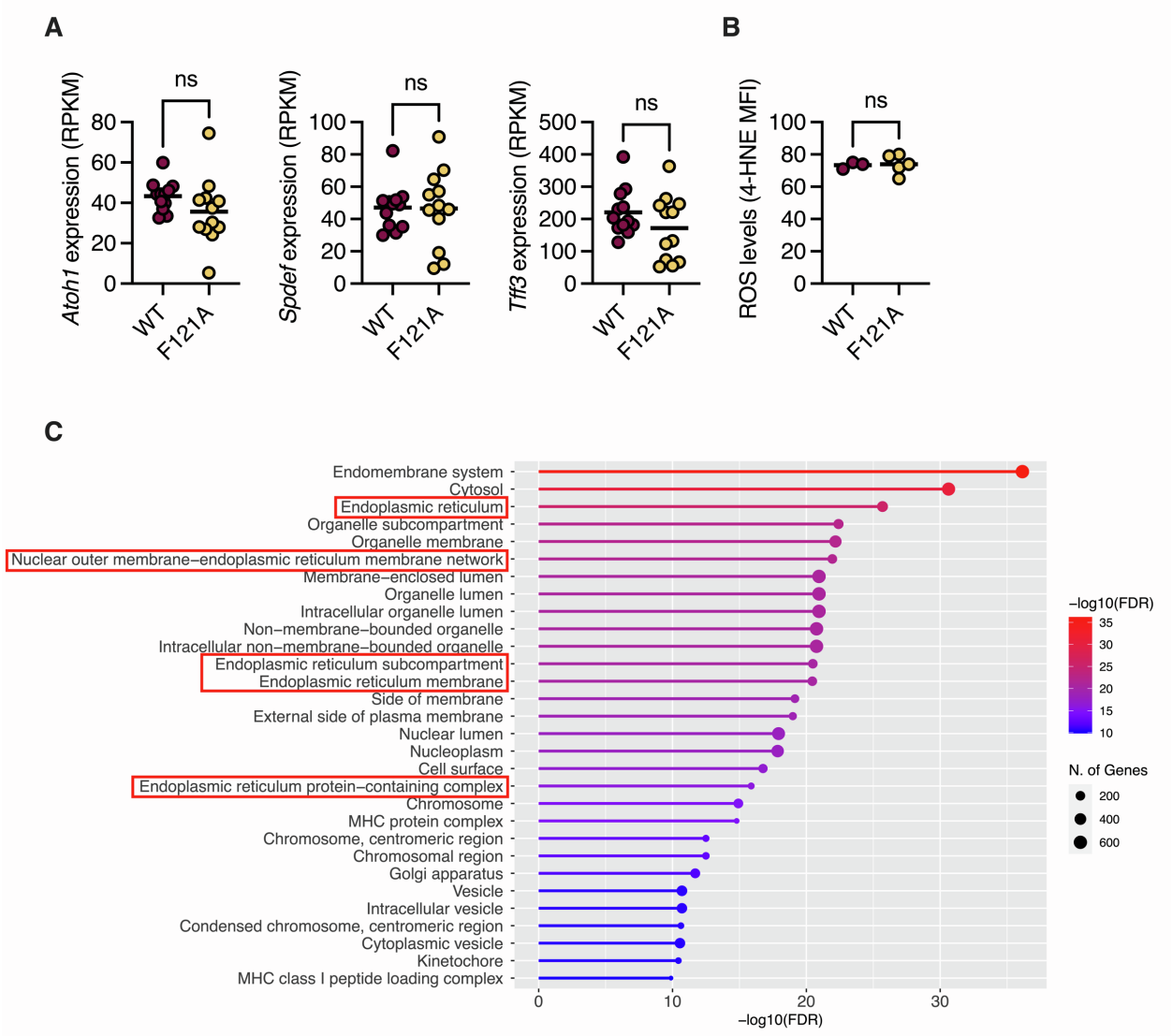

Figure S2. Transcripts of ER proteins are altered in *Becn1*^{F121A} mice, Related to Figure 2. **(A)** Expression levels of goblet cell-specific transcription factors in colons of mice using RNA sequencing. **(B)** Levels of the ROS-indicator 4 hydroxynonenal specifically in colonic goblet cell using immunohistochemistry. **(C)** Pathway analysis of differently expressed genes between wild type and *Becn1*^{F121A} mice using GO cellular components analysis. ER-related compartments are marked in red. **(A and B)** Each dot represents a mouse. Student's *t* test. ns, not significant; 4-HNE, 4 hydroxynonenal; RPKM, Reads per kilobase of transcript; WT, wild type; F121A, *Becn1*^{F121A}.

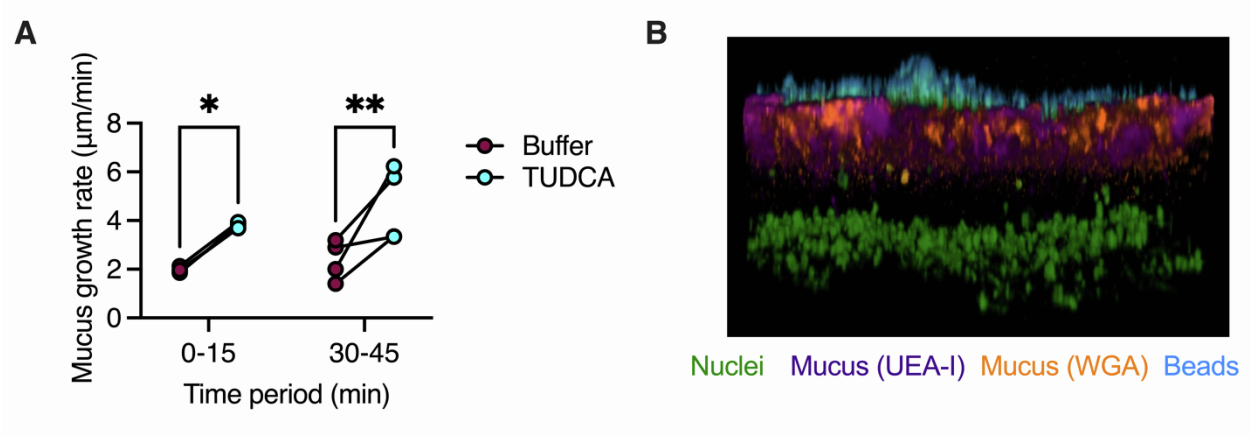

Figure S3. Reducing ER stress increases mucus secretion rate in a sustainable manner, Related to Figure 3. (A) Mucus secretion rates measured in colonic explants treated as noted. Each dot represents a mouse. *P<0.05; **P<0.01; ns, not statistically significant. Two-way ANOVA. TUDCA, tauroursodeoxycholic acid. (B) Confocal Z-stack imaging of mucus penetrability in TUDCA treated colon explants. DNA is in green, UEA-1 lectin in purple, WGA lectin in orange and beads in cyan.

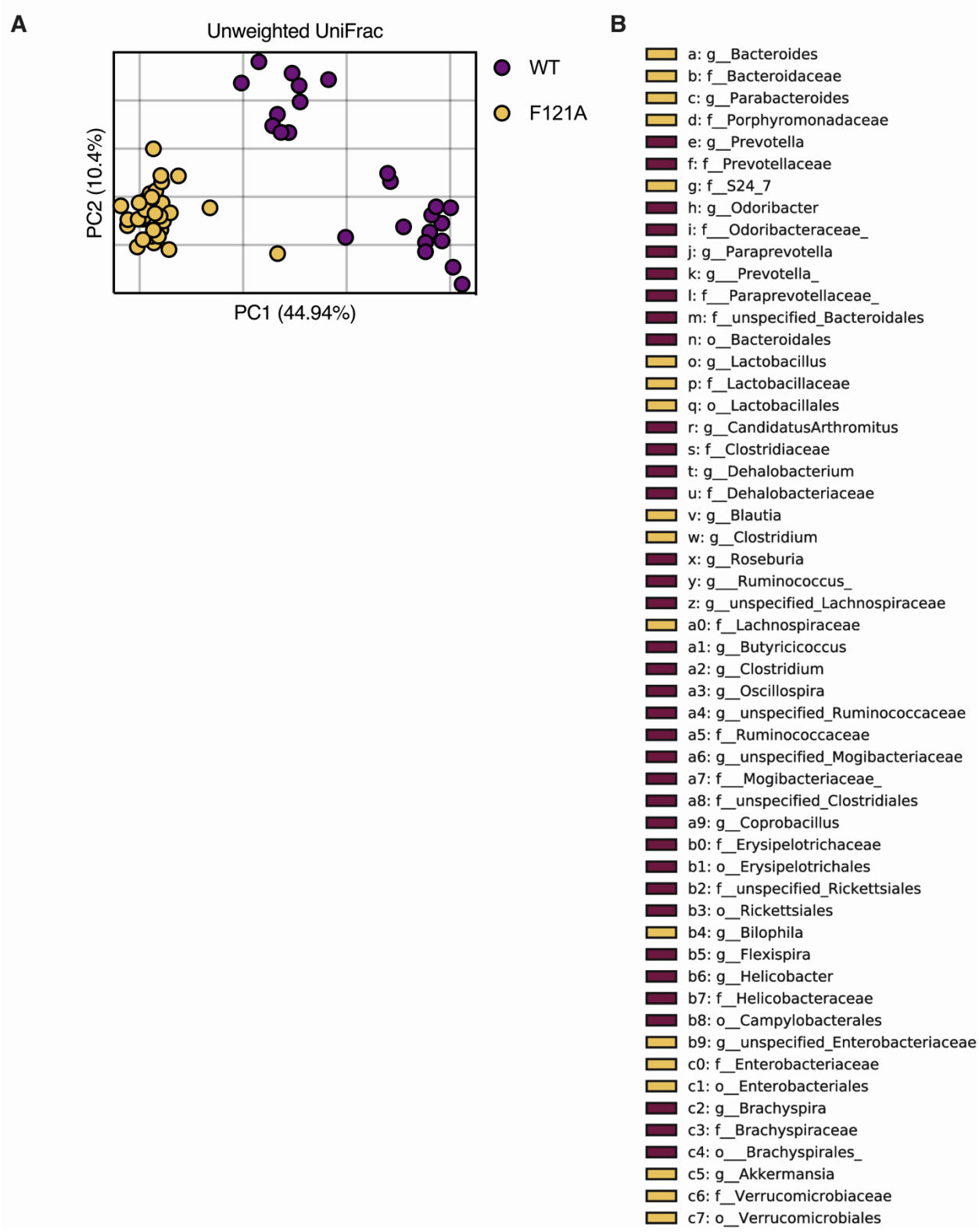

Figure S4. The gut microbiota of *Becn1*^{F121A} mice is different than that of wild type mice and enriched with mucus-utilizing bacteria, Related to Figure 5. 16 S rRNA sequencing was performed to characterize gut microbiota composition. (A) PCoA of fecal microbiota β diversity

based on unweighted UniFrac. Each dot represents a mouse. (B) Legend of cladogram depicting LEfSe analysis of differently abundant bacteria in wild type and *Becn1*^{F121A} mice in Figure 5E.

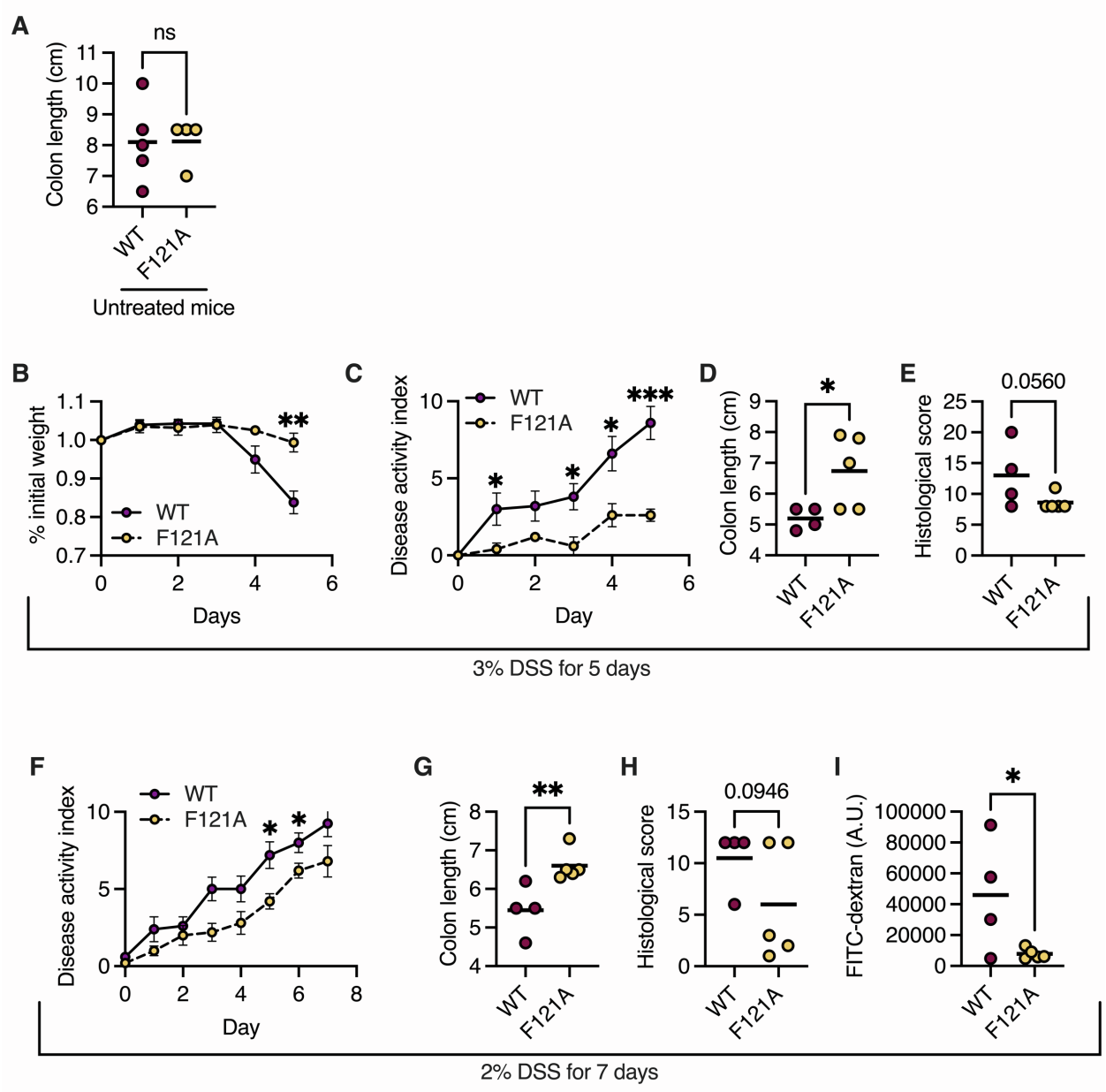

Figure S5. *Becn1*^{F121A} mice are protected from colitis, Related to Figure 6. (A) Colon length of naïve wild type and *Becn1*^{F121A} mice. (B-E) Relative weight change ±SEM (B), disease activity index ±SEM (C), colon length (D) and histological damage score (E) of mice treated with 3% DSS for 5 days. (F-I) Disease activity index ±SEM (F), colon length (G), histological damage score (H) and FITC-dextran in serum (I) of mice treated with 2% DSS for 7 days. (A, C-E, and G-I) Each symbol represents a mouse. **P*<0.05; ***P*<0.01; ****P*<0.001; (A, C-E and G-I) Student's *t* test; (B, C and F) Multiple unpaired *t* tests corrected for false discovery rate. WT, wild type; F121A, *Becn1*^{F121A}; A.U., arbitrary units.