

Impact of BECLIN1 haploinsufficiency on goblet cell function and susceptibility to colitis

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Abstract

BECLIN1 is a central regulator of autophagy and endocytic trafficking essential for epithelial homeostasis. While complete intestinal epithelial loss of BECLIN1 causes fatal enteritis, the consequence of its partial loss in the gut remains unclear. Given that BECLIN1 expression can vary in human disease, we investigated whether reduced BECLIN1 is sufficient to impair gut barrier function. Heterozygous *Becn1* deletion (*Becn1*^{+/-}) in the mouse intestinal epithelium caused subtle but functionally important defects, including shortened small intestines, reduced colonic crypt length, altered epithelial architecture, and loss of goblet cells with reduced mucin production, particularly in mature goblet cells. These changes occurred despite preservation of basal autophagy, implicating trafficking-related functions. Supporting this conclusion, *Becn1*^{+/-} intestinal epithelial cells showed modest increases in RAB5⁺ vesicles, redistribution of E-CADHERIN with F-actin along lateral membranes, increased apico-basal cell length and reduced basal width. Following dextran sulfate sodium (DSS) treatment, *Becn1*^{+/-} mice exhibited greater weight loss, higher disease activity, more severe histological colitis score, and disproportionate loss of neutral mucins, with inflammation confined to the mucosa. Goblet cell dysfunction likely underpinned these barrier defects. These findings establish that BECLIN1 insufficiency destabilises epithelial organisation and barrier defence, thereby sensitising the gut to inflammatory challenge and further positioning BECLIN1 as a key determinant of intestinal homeostasis.

INTRODUCTION

Genome-wide association studies have identified polymorphisms in autophagy regulators, such as *ATG16L1* and *IRGM*, as strongly associated with inflammatory bowel disease (IBD) (1–7). These findings highlight the importance of autophagy in intestinal homeostasis and implicate its disruption in IBD pathogenesis. Functional studies in mouse and cell-based models have revealed both epithelial-intrinsic roles, including maintenance of barrier integrity and cell survival (2, 8–16), and immune-mediated extrinsic roles, such as regulating inflammatory responses and microbial balance (17–21).

Recent studies have shown that the prototypical autophagy regulator BECLIN1 is critical for intestinal homeostasis. Constitutive activation of BECLIN1-dependent autophagy alleviates goblet cell ER stress, thickening the mucus barrier and protecting against chemical- and infection-driven inflammation (11). Beyond autophagy, BECLIN1 regulates endocytic trafficking, maintaining barrier integrity via correct junctional protein localisation, cytoskeletal organisation, and epithelial remodelling (22–24). Complete intestinal epithelial deletion of BECLIN1 is rapidly fatal due to severe enterocolitis with extensive epithelial cell loss, impaired specialised IEC functions, inflammation, and barrier breakdown (23).

BECLIN1 undergoes extensive post-translational modifications that fine-tune its activity and interactions (25–28). In diseases, however, it is reduced BECLIN1 expression rather than mutation that contributes to pathogenesis (29–31), with changes arising from altered protein interactions, cleavage, epigenetic regulation, or monoallelic deletion (32–35). Despite this, the consequences of partial BECLIN1 loss in the intestinal epithelium remain poorly understood. Here, we demonstrate that partial loss of BECLIN1

impairs goblet cell function and increases susceptibility to inflammation. By dissecting its roles in endocytic trafficking, cytoskeletal organisation and mucin secretion, we provide new insights into how BECLIN1 insufficiency destabilises epithelial integrity and compromises barrier defence.

MATERIALS AND METHODS

Mice

All mouse strains used in this study were bred on the C57BL/6 J background. *Becn1*^{tm1b(KOMP)Wtsi} mice were purchased from the European Conditional Mouse Mutagenesis Program (EUCOMM). *Becn1*^{fl/fl} mice were generated by breeding *Becn1*^{tm1b(KOMP)Wtsi} mice onto CAG-FLPe mice. *Becn1*^{+/+}, *Becn1*^{+/fl}, and *Becn1*^{fl/fl}; *Vil1-CreERT2*^{Cre/+} mice were then subsequently generated by breeding *Becn1*^{+/+}, *Becn1*^{fl/+} and *Becn1*^{fl/fl} mice to the *Vil1-CreERT2* mice (36).

Mice were housed at the La Trobe Animal Research and Teaching Facility (LARTF, La Trobe University, VIC, Australia) under Specific Pathogen Free (SPF) conditions. All experiments performed were approved by the La Trobe University animal ethics committees (approvals AEC18024, AEC18036) in accordance with the Australian code for the care and use of animals for scientific purposes. All research with these mice has complied with all relevant ethical regulations for animal use. To induce gene deletion, male and female mice aged six weeks or older, were selected indiscriminately and intraperitoneally injected with 4 mg tamoxifen (T5648, Sigma-Aldrich, St. Louis, Missouri, USA) in sunflower seed oil (25007, Sigma-Aldrich, St. Louis, Missouri, USA), delivered as one 200 µl injection per day of a 10 mg/ml stock, over two consecutive days. Mice were humanely euthanised by CO₂ asphyxiation.

Dextran Sulphate Sodium (DSS) treatment

Following Tamoxifen treatment to induce gene deletion, mice were administered DSS (2% (w/v), MP Biomedicals, Irvine, California, USA) in autoclaved drinking water provided *ad libitum* for 5 days. Control groups received autoclaved drinking water without DSS for the same duration. During the experimental period, mice were monitored daily and weighed. The average daily intake of 2% (w/v) DSS water was calculated by measuring the weight difference of drinking bottles at the start and end of the experiment and dividing by the number of treatment days. The disease activity index (DAI) was assessed using a cumulative scoring system based on parameters outlined in Table 1, including weight loss, stool consistency and the presence of hematochezia (presence of blood in stool). At the experimental endpoint, mice were humanely euthanised using CO₂ asphyxiation. The small and large intestine were harvested, and their lengths were measured prior to rolling and fixation in 10% (v/v) neutral buffered formalin for 48 hours.

Table 1

Scoring system for the evaluation of colitis severity in mice based on weight loss, stool consistency and haematochezia parameters.

Score	Weight loss (%)	Stool consistency	Haematochezia
0	Normal	Normal	No blood
1	< 5		Visible blood in rectum
2	6–10	Pasty, semiformed	Visible blood on fur
3	11–20		
4	> 20	Liquid, sticky or unable to defecate after 5 min	

Intestinal organoid culture

Organoids were established by culturing crypt-enriched fractions from the duodenum of untreated mice as described previously (23). To induce *Becn1* deletion, organoids were seeded into media containing 200 nM 4-hydroxytamoxifen (4-HT) (H7904, Sigma-Aldrich, St. Louis, Missouri, USA) for three days and then maintained as per normal. For organoid re-passaging experiments, 4-HT-treated organoids were passaged at day 7 and maintained as above.

Intestinal epithelial cell isolation

The mouse small intestine was opened longitudinally, rinsed with Dulbecco's Phosphate Buffered Saline (dPBS), and incubated in dPBS + 15 mM EDTA for 15 minutes at 37°C with agitation. Dissociation of IECs was achieved by vortexing. The cell suspension was then washed once with ice-cold dPBS, and the cell pellet snap-frozen on dry ice and stored at – 80°C until use.

Histology, immunohistochemistry and organoid whole-mount immunofluorescence

Preparation of intestinal “Swiss-rolls”, haematoxylin and eosin (H&E), Periodic acid-Schiff, Alcian blue (PAS-AB) and Ki67 staining were all performed as described previously (23). All slides were scanned using the Aperio AT2 (Leica, Wetzlar, Germany) and images captured and analysed using Aperio ImageScope v12.4.2.5010 and Fiji ImageJ. Whole-mount staining of intestinal organoids was performed as previously described (22).

Immunoblotting of mouse tissues

Preparation of mouse cell lysates and immunoblotting was performed as described previously (23). Primary antibodies were: Beclin1, 1:500 (3495, Cell Signaling Technology, Danvers, Massachusetts, USA);

p62/SQSTM1, 1:500 (5114, Cell Signaling Technology, Danvers, Massachusetts, USA); LC3B, 1:500 (NB100-2220, Novus Biologicals, Centennial Colorado, USA); β -Actin, 1:5 000 (A2228, Sigma-Aldrich, St. Louis, Missouri, USA); GAPDH, 1:5 000 (MA5-15738, Invitrogen, Waltham, Massachusetts, USA). Secondary antibodies used were: Donkey anti-Rabbit IgG, 1:10 000 (NA943V, GE Healthcare, Chicago, Illinois, USA); Goat anti-Mouse IgG, 1:10 000 (A0168, Sigma-Aldrich, St. Louis, Missouri, USA).

RESULTS

Generation of mice with inducible monoallelic deletion of *Becn1* in the intestinal epithelium

We previously reported that homozygous *Becn1* deletion in the intestinal epithelium results in rapid, fatal enterocolitis, with severe epithelial disruption (23). To model partial BECLIN1 loss, we generated mice in which monoallelic *Becn1* deletion could be induced in the intestinal epithelium using mice heterozygous for the *Becn1* floxed allele (*Becn1^{fl/+}*) and carrying the *Vil1-Cre^{ERT2}* transgene (*Becn1^{fl/+};Vil1-CreERT2^{Cre/+}*). Littermates lacking the floxed allele (*Becn1^{+/+}*) but positive for *Vil1-CreERT2^{Cre/+}* (*Becn1^{+/+};Vil1-CreERT2^{Cre/+}*) served as controls. Intraperitoneal Tamoxifen injections induced deletion in the small intestine (duodenum, jejunum, ileum) and colon of *Becn1^{fl/+};Vil1-CreERT2^{Cre/+}* mice, confirmed genomically and by protein analysis showing approximately 50% reduced BECLIN1 levels (Fig. 1A, Supplementary Fig. 1A). Tamoxifen-treated *Becn1^{+/+};Vil1-CreERT2^{Cre/+}* and *Becn1^{fl/+};Vil1-CreERT2^{Cre/+}* mice are referred to as *Becn1^{+/+}* and *Becn1^{+/-}* mice respectively throughout.

Mice with reduced BECLIN1 have shortened small intestines and reduced crypt lengths without other overt phenotypes

At 7 days post-BECLIN1 deletion, there were no significant body weight differences between *Becn1^{+/+}* and *Becn1^{+/-}* mice (Supplementary Fig. 1B), unlike the marked weight loss previously seen with homozygous *Becn1* deletion in the intestinal epithelium (23). Despite appearing grossly normal, *Becn1^{+/-}* mice displayed significantly shorter small intestines than *Becn1^{+/+}* controls, while colon lengths were unchanged (Fig. 1B). Histological examination by H&E staining revealed largely normal small and large intestinal morphology in *Becn1^{+/-}* mice (Fig. 1C). At this time point, distal colonic crypt length (Fig. 1D) and width (Supplementary Fig. 1C) did not differ between genotypes. However, by 14- and 35-days post deletion, *Becn1^{+/-}* mice exhibited significantly shorter colonic crypts and reduced crypt volume fraction (37) compared to controls (Fig. 1D). Observation over one month post deletion also revealed a persistently shorter small intestine (Supplementary Fig. 1D) without weight loss (Supplementary Fig. 1B), and otherwise normal intestinal morphology (Supplementary Fig. 1E). These phenotypes contrasted sharply with *Becn1^{-/-}* mice, where small intestines were shortened, swollen, and lytic, with villus stunting along the entire length within 7 days of gene deletion (Figs. 1B, C) (23). The need to euthanise *Becn1^{-/-}* mice within 7 days precluded investigation of equivalent colonic changes. In summary, apart from reduced small intestinal and colonic crypt lengths, heterozygous *Becn1* deletion does not cause overt

abnormalities under basal conditions up to one month post deletion, in contrast to its homozygous loss (23).

Intestinal epithelial cells with reduced BECLIN1 levels display no disruption to basal autophagy, differentiation, or proliferation

Monoallelic *Becn1* deletion caused minimal changes in basal autophagy, with total P62 and LC3B levels comparable to wild-type IECs (Fig. 1E). In contrast, *Becn1*^{-/-} IECs showed pronounced P62 and LC3B accumulation (Fig. 1E) (23). We previously identified an essential role for BECLIN1 in endocytic trafficking in intestinal-derived organoids (22, 23). To test whether monoallelic *Becn1* loss induced similar defects, we generated organoids from *Becn1*^{+/+}; *Becn1*^{fl/+}; and *Becn1*^{fl/fl}; *Vil1-CreERT2*^{Cre/+} mice. Treatment with 4-hydroxytamoxifen (4-HT) led to reduced BECLIN1 expression in *Becn1*^{fl/+} organoids which did not impact basal autophagy (as with intestinal epithelial cells from mice), unlike homozygous deletion (Supplementary Figs. 2A, B).

At 7 days post 4-HT treatment, *Becn1*^{-/-} organoids showed significantly reduced budding crypt formation (Fig. 1F) (23), whereas *Becn1*^{+/+} organoids formed similar crypt-like projections as *Becn1*^{fl/+} organoids, indicating normal IEC proliferation and differentiation (Fig. 1F). Re-passaging of *Becn1*^{+/+} and *Becn1*^{fl/+} yielded mature organoids by Day 5, with similar numbers of budding crypts, further indicating minimal defects in proliferation or differentiation (Fig. 1G). This is consistent with Ki67 staining of intestinal tissues from *Becn1*^{+/+} mice showing no proliferation differences, including at the crypt bases (Supplementary Fig. 2C). In contrast, surviving *Becn1*^{-/-} organoids failed to generate viable organoid structures upon re-passaging, producing significantly fewer crypt-like projections by Day 5 (Fig. 1G). Thus, reduced BECLIN1 expression does not impair basal autophagy, survival, differentiation, or proliferation of IECs.

Intestinal epithelial cells with reduced BECLIN1 levels display changes in early endocytic trafficking and E-CADHERIN distribution

BECLIN1 loss has been reported to affect endocytic trafficking in other tissues, similar to the effects of homozygous loss in the gut (22, 23, 38–42). To determine whether this also occurs following heterozygous loss in intestinal epithelial cells, we examined *Becn1*^{+/+} organoids and found a modest but significant increase in cytoplasmic RAB5⁺ early endosomes (Figs. 2A, B, Supplementary Fig. 3) though without the aberrant enlargement seen in *Becn1*^{-/-} organoids (Figs. 2A, C). As the junctional protein E-CADHERIN can become trapped within enlarged RAB5⁺ early endosomes in *Becn1*^{-/-} IECs (Figs. 2A, D, E) (23), we examined its localisation in *Becn1*^{+/+} IECs. Here, E-CADHERIN levels were increased at apical and lateral membranes (Figs. 2F, G) and in the cytoplasm (Fig. 2D), but notably, co-localisation with RAB5 was unchanged at all subcellular sites (Figs. 2E, H, I). Thus, reduced BECLIN1 alters early endosomal dynamics, but E-CADHERIN localisation changes appear independent of RAB5-mediated trafficking.

Monoallelic *Becn1* deletion alters F-actin organisation and intestinal epithelial cell architecture

As homozygous BECLIN1 loss disrupts the cytoskeleton and compromises the intestinal epithelial barrier (22), we examined whether altered F-actin organisation, critical for adhesion and endocytosis (43, 44) contributes to the E-CADHERIN changes in *Becn1*^{+/-} organoids. Here, *Becn1*^{+/-} IECs displayed significantly increased F-actin and co-localised E-CADHERIN along the lateral membrane but not apical membrane (Figs. 3A-E), consistent with an F-actin-driven “cadherin flow” mechanism, whereby cadherins are transported along remodelling actin networks (45), potentially contributing to altered E-CADHERIN localisation (Figs. 2A, E). Notably, E-CADHERIN was redistributed within the lateral membrane toward apicolateral junctions, possibly to maintain cell-cell contacts and preserve epithelial integrity. Given this increased lateral membrane localisation of both E-CADHERIN and F-actin in *Becn1*^{+/-} IECs (Fig. 3E), we assessed cell morphology and found *Becn1*^{+/-} organoids had a longer apico-basal axis and shorter basal IEC width (Figs. 3A, F, G), likely contributing to the shorter small intestinal length (Fig. 1B). Reduced BECLIN1 therefore alters adhesion dynamics, cytoskeletal organisation, and epithelial architecture.

Reduced BECLIN1 levels in intestinal epithelial cells increases susceptibility to DSS-induced colitis

To test whether the changes in endocytic trafficking, intercellular adhesion, and cytoskeleton architecture observed in *Becn1*^{+/-} mice increase susceptibility to inflammation, 7- to 14-week-old mice were treated with dextran sulphate sodium (DSS) to induce colitis, a widely used model of IBD pathogenesis (46). Following Tamoxifen-induced gene deletion, mice received either 2% DSS in drinking water for 5 days or normal water (Fig. 4A). There were no genotype-dependent differences in starting body weight or DSS-water consumption (Supplementary Figs. 4A, B).

At endpoint, both DSS-treated genotypes displayed expected colitic changes, including darker, reddish areas in the colon consistent with hyperemia/inflammation (Figs. 4B, C) and colon shortening with swelling and constriction (Figs. 4C, D), but gross colonic changes were similar (Figs. 4B-D). The small intestines of *Becn1*^{+/-} mice were significantly shorter than *Becn1*^{+/+} controls regardless of DSS treatment (Supplementary Fig. 4C). Both genotypes lost weight after DSS exposure, but this was greater in *Becn1*^{+/-} mice (Fig. 4E). They also developed more severe diarrhea, with a higher proportion producing pasty to liquid stools (Fig. 4F), though hematochezia did not differ (Fig. 4G). Importantly, the composite disease activity index (DAI), incorporating weight loss, stool consistency, and rectal bleeding (Table 1) (47, 48), was significantly higher in *Becn1*^{+/-} mice, indicating greater colitis severity (Fig. 4H).

Histological assessment of DSS-treated colons was performed using a blinded scoring system evaluating crypt architecture, inflammatory cell infiltration, and epithelial integrity, with the composite score reported as the histological colitis score (HCS) (49). Untreated mice of both genotypes had minimal mucosal damage and low histological colitis scores (HCS) (Figs. 4I, J, K). DSS treatment increased the HCS scores in both genotypes, but *Becn1*^{+/-} mice scored approximately 2.2-fold higher (Fig. 4K), with more severe epithelial injury and crypt attenuation, epithelial loss, and immune infiltration (Figs. 4I, J). These mice also had more regions of epithelial erosion (Fig. 4L), characterised by complete

crypt loss and dense localised immune cell infiltration. Lymphoid follicle counts and sizes did not differ between groups (Supplementary Figs. 4D, E), indicating that inflammation was largely confined to the epithelial layer. Hence, monoallelic *Becn1* loss worsens DSS-induced colitis, indicating increased sensitivity to intestinal inflammation (Figs. 4E-L).

Monoallelic *Becn1* deletion causes goblet cell defects and compromises mucosal immunity under basal conditions

Goblet cells are secretory IECs specialised for mucus production, forming a critical barrier against pathogens and mechanical damage (50). Disruption of this barrier is a hallmark of chronic inflammatory conditions, including IBD, often arising from goblet cell loss or defects in mucin formation, storage, or secretion (50–53). Periodic-Acid-Schiff (PAS) and Alcian Blue (AB) staining revealed a significant reduction in PAS-AB staining in the distal colon (Figs. 5A, B), but not the proximal colon (Fig. 5B), of untreated *Becn1*^{+/-} mice compared to untreated *Becn1*^{+/+} mice (Figs. 5A, B), suggesting either fewer goblet cells and/or reduced mucin production (13). As goblet cell maturation involves migration to the crypt surface, we also quantified goblet cells in the lower (immature) and upper (mature) colonic crypt compartments (Fig. 5C, Supplementary Fig. 5) (13, 55). PAS-AB^{+ve} mucin staining was reduced in both lower and upper crypt compartments, but more so in the latter (50% versus 15%) (Fig. 5C). This maturation defect was further supported by the enlarged cytoplasmic mucin (theca) areas in remaining mature goblet cells of untreated *Becn1*^{+/-} mice compared with *Becn1*^{+/+} mice (Fig. 5D), consistent with impaired mucin secretion. These results show that reduced BECLIN1 results in goblet cell loss and defective mucin production, potentially compromising mucosal barrier function even under basal conditions.

Reduction of BECLIN1 levels exacerbates DSS-induced barrier dysfunction

We next investigated goblet cell responses in DSS-treated *Becn1*^{+/+} and *Becn1*^{+/-} mice. The epithelial damage in both DSS-treated *Becn1*^{+/+} and *Becn1*^{+/-} mice (Figs. 4I-L) was accompanied by a significant reduction in PAS-AB^{+ve} mucin area in the distal colon compared with their respective untreated controls (Figs. 5A, B), consistent with the distal colon being the primary site of DSS-induced injury (54). The epithelial damage in the distal colon was further exacerbated in DSS-treated *Becn1*^{+/-} mice, where the PAS-AB^{+ve} mucin area was significantly reduced when compared with DSS-treated *Becn1*^{+/+} mice (Figs. 5A, B). We also assessed PAS-AB staining across the entire colon, which revealed a loss of PAS^{+ve} neutral mucins in *Becn1*^{+/-} mice compared to *Becn1*^{+/+} in the untreated groups, with no significant change in AB^{+ve} acidic mucins (Fig. 5E). Notably, following DSS treatment, *Becn1*^{+/-} mice exhibited a loss of both neutral and acidic mucins, consistent with the exacerbated epithelial damage observed (Figs. 5E, 4I-L). This reveals a vulnerability of the mucosal barrier when BECLIN1 is reduced, reinforcing its role in epithelial homeostasis. The selective baseline loss of neutral mucins, and subsequent loss of

both mucin types after DSS treatment, highlights the critical importance of neutral mucins in protecting against DSS-induced colitis.

DISCUSSION

We previously identified an essential role for BECLIN1 in maintaining intestinal homeostasis and barrier integrity mediated through its autophagy and endocytic trafficking functions (22, 23). Given that homozygous *Becn1* deletion causes fatal enteritis in adult mice (23), we investigated whether partial reduction, mimicking clinically relevant haploinsufficiency, also impairs intestinal function.

Unlike homozygous BECLIN1 deletion, monoallelic loss did not trigger overt spontaneous disease. However, *Becn1*^{+/-} mice did display subclinical abnormalities, including shortened small intestines, reduced distal colonic crypt length, and goblet cell loss – hallmarks of compromised epithelial integrity. These changes occurred despite minimal changes in basal autophagy, suggesting BECLIN1's trafficking and epithelial organisation roles may be more critical in this context (22, 23). Altered tissue architecture was marked by increased apico-basal length, reduced basal width, and redistribution of E-CADHERIN and F-actin along the lateral membranes, likely reflecting cytoskeletal remodelling. These features mirror BECLIN1-null IECs, where E-CADHERIN becomes trapped in early endosomes (22, 23).

While cytoplasmic RAB5⁺ vesicles were modestly increased, early endosome enlargement was absent in *Becn1*^{+/-} cells, suggesting a threshold effect in trafficking disruption. Increased co-localisation of E-CADHERIN with lateral F-actin in *Becn1*^{+/-} IECs may stabilise junctional integrity under impaired trafficking (43, 44), though altered F-actin organisation can also impair IEC contractility, crypt architecture (56), and mucin granule exocytosis (57, 58).

Goblet cells were disproportionately affected, with reduced numbers, altered maturation (larger thecae), and diminished mucin staining, especially in the upper crypt. This aligns with the known role of autophagy in promoting goblet cell function via ER stress alleviation and mucin handling (11). Following DSS challenge, *Becn1*^{+/-} mice failed to mount the same level of compensatory mucin response as wild-type controls, showing depletion of both neutral and acidic mucins. The baseline vulnerability due to selective mucin loss, compounded by the broad depletion after DSS treatment, highlights the critical role of neutral mucins in maintaining the buffering capacity of the mucus barrier. Loss of the major secreted mucin, MUC2, similarly causes spontaneous colitis in mice (53) and comparable mucin shifts occur in human IBD (59–64).

Together, our findings suggest that beyond its canonical autophagy role, BECLIN1 integrates trafficking, cytoskeletal dynamics, and mucosal defence. The heightened DSS susceptibility of *Becn1*^{+/-} mice, despite inflammation remaining confined to the mucosa, supports a barrier-specific defect consistent with goblet cell dysfunction and epithelial disorganisation.

Although IBD GWAS have not identified BECLIN1 single nucleotide polymorphisms (SNPs), emerging evidence implicates both IEC-intrinsic and -extrinsic mechanisms that lower BECLIN1 levels, thereby exacerbating colitis. For instance, cyclic GMP-AMP synthase (cGAS) promotes autophagy in IECs through interaction with BECLIN1, and its loss worsens colitis (65). Similarly, the m⁶A nuclear reader, YTHDC1, stabilises *Becn1* mRNA and is downregulated in DSS colitis and in IBD patient microbiota (35, 66). Moreover, while no IBD-associated SNPs have been identified within *Becn1* itself, variants in upstream regulators such as STAT3, a transcriptional regulator of *Becn1*, have been implicated in IBD pathogenesis (67–69). Caspase-mediated cleavage of BECLIN1 during apoptosis may also contribute to reduced protein levels in inflamed tissue, amplifying epithelial injury (70, 71).

Looking forward, resources such as atlases of IBD patient samples (72, 73) provide opportunities to examine BECLIN1 expression at endoscopically defined disease margins. Expanding clinical analyses will be critical to determine whether there are changes in BECLIN1 levels across broader clinical cohorts and to assess its potential as a biomarker of IBD pathogenesis and severity.

In summary, partial BECLIN1 reduction compromises epithelial homeostasis and exacerbates inflammation in the absence of complete autophagy loss. These findings establish BECLIN1 as a critical epithelial defence regulator. Future studies should explore whether restoring BECLIN1 levels or mimicking its trafficking-related functions could preserve barrier integrity and enable patient stratification for precision therapeutic interventions in IBD.

Declarations

CONFLICT OF INTEREST

J.M.Murphy, A.L.S., K.M.P., and S.N.Y. contribute to, or have contributed to, the development of necroptosis pathway inhibitors in collaboration with Anaxis Pharma Pty. Ltd. J.M.Murphy has also received research funding from Anaxis Pharma Pty. Ltd. All other authors declare no competing interests.

AUTHOR CONTRIBUTIONS

J.J., S.T., designed, performed, and analysed experiments and wrote the paper; T.J.H., Y.W.N.R., S.L.E., A.H.A-A, K.P., S.N.Y., M.E., D.B., C.M.R., R.N., L.J.J., designed, performed experiments, analysed and interpreted data; P.D.C., K.D., B.T.K., A.S.Y., J.M.M., B.C., A.L.S., J.M.Murphy analysed and interpreted data; W.D.F., E.F.L. designed the project, analysed, interpreted data, and wrote the paper. All authors commented on the manuscript.

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AVAILABILITY OF DATA AND MATERIALS

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Figures

Figure 1

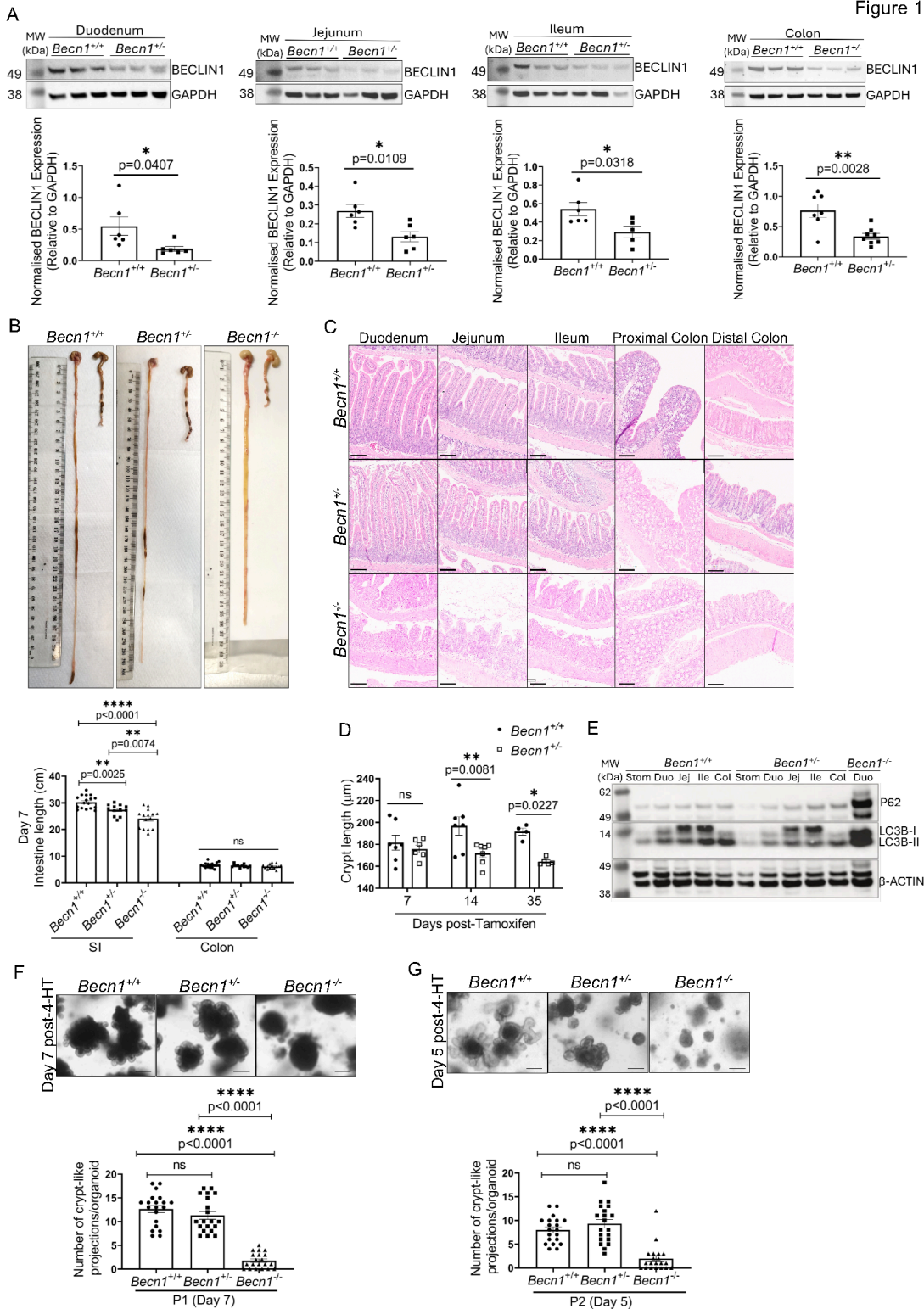


Figure 1

Monoallelic *Becn1* deletion induces mild intestinal epithelial alterations under basal conditions, distinct from the severe defects observed with homozygous *Becn1* deletion. (A) Representative Western blots (top) showing BECLIN1 levels in the indicated tissues, with densitometry quantification (bottom). Data represent a minimum of $n = 5$ animals per genotype from three independent experiments. GAPDH was used as a loading control. **(B)** Representative images of intestinal tracts of *Becn1*^{+/+}, *Becn1*^{+/-} and

Becn1^{-/-} mice (top) with the measurements of intestinal length at Day 7 post Tamoxifen administration (bottom). Data represent $n > 12$ animals per genotype from $n > 3$ independent experiments **(C)** H&E-stained FFPE sections of the intestinal tracts from *Becn1*^{+/+}, *Becn1*^{+/-} and *Becn1*^{-/-} mice. Scale bars = 100 μ m. Data represent $n > 6$ biologically independent mice of each genotype from $n = 3$ independent experiments. **(D)** Quantification of distal colon crypt length in *Becn1*^{+/+} and *Becn1*^{+/-} mice at days 7, 14 and 35 post-tamoxifen administration. Only visible full-length crypts were included in the analysis, with a minimum of $n > 5$ crypts measured per mouse and averaged. Data represent $n > 4$ animals per genotype from $n = 3$ independent experiments. **(E)** Western blot showing markers of basal autophagy across different sections of the gastrointestinal tract in *Becn1*^{+/+} and *Becn1*^{+/-} mice. The duodenum of *Becn1*^{-/-} mice was included as a positive control for defective autophagy. β -ACTIN was used as a loading control. **(F)** Representative phase contrast microscopy images of *Becn1*^{+/+}, *Becn1*^{+/-} and *Becn1*^{-/-} organoids at Day 7 post-4-HT treatment and **(G)** re-passaged surviving *Becn1*^{+/+}, *Becn1*^{+/-} and *Becn1*^{-/-} organoids at Day 5, along with respective quantification of the number of budding crypt-like projections per organoid. Scale bar = 100 μ m. Data in (F) and (G) were obtained from $n = 20$ organoids per genotype across $n = 3$ independent experiments. Graphs show the mean $\pm$ S.E.M. Statistical significance was determined by unpaired (Student's) t-test, except in (F) and (G) where ordinary one-way ANOVA was used. SI: small intestine. Stom: stomach. Duo: duodenum. Jej: jejunum. Ile: ileum. Col: Colon. MW: molecular weight. 4-HT: 4-hydroxytamoxifen. ns: not significant ($p > 0.05$).

Figure 2

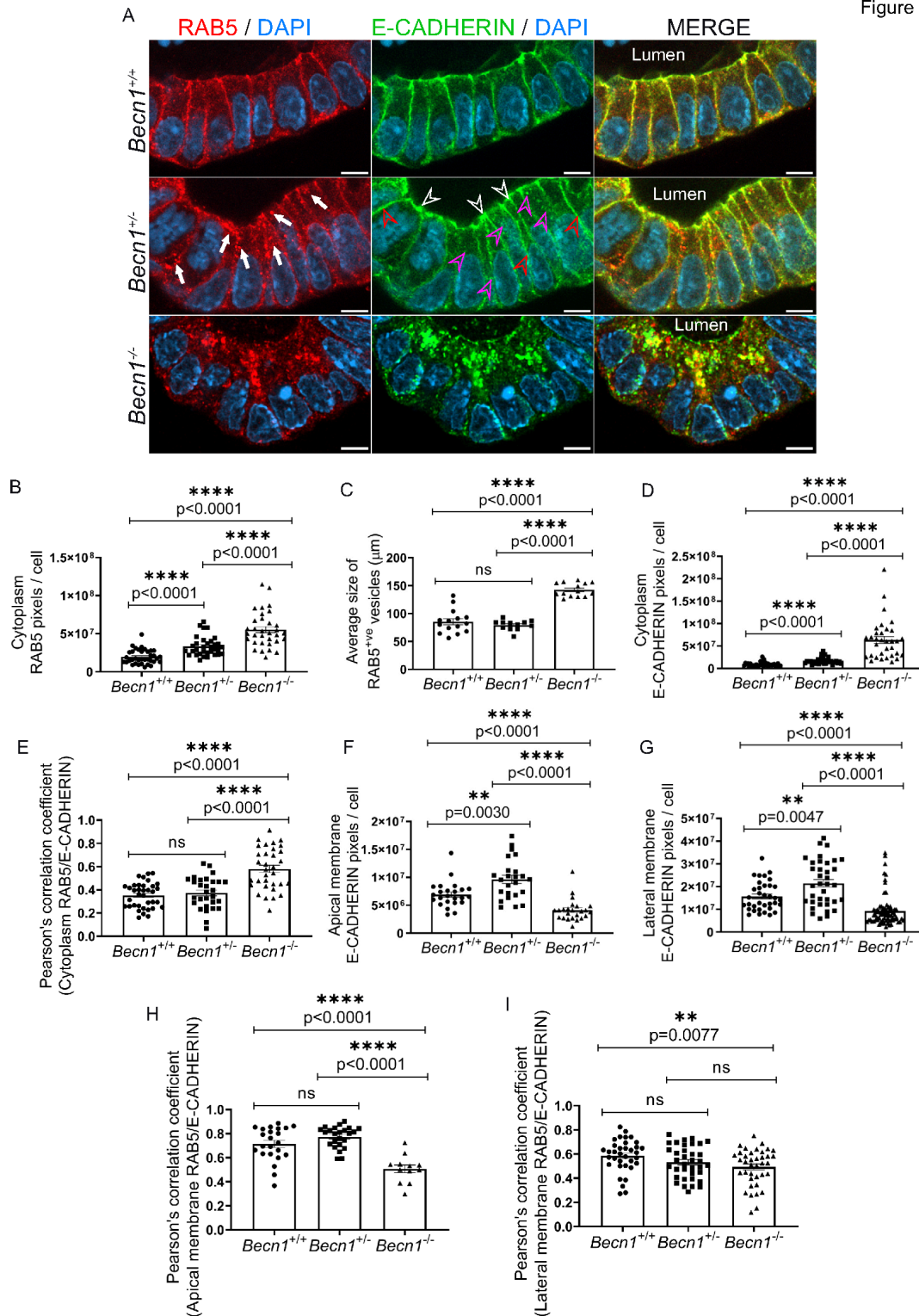


Figure 2

BECLIN1 reduction alters E-CADHERIN trafficking in a RAB5-independent manner. (A) Representative whole-mount immunofluorescence staining for RAB5 (red), E-CADHERIN (green) and DAPI (blue) in *Becln1*^{+/+}, *Becln1*^{+/-} and *Becln1*^{-/-} organoids. Increased cytoplasmic RAB5 is indicated by white arrows. Aberrant E-CADHERIN localisation is denoted by white (apical), red (lateral), and purple (cytoplasmic) open arrowheads. Scale bar = 5 μ m. **(B)** Quantification of cytoplasmic RAB5 pixels per cell. **(C)**

Measurement of the average size of RAB5^{+ve} vesicles. **(D)** Quantification of cytoplasmic E-CADHERIN pixels per cell, along with its **(E)** co-localisation with cytoplasmic RAB5, measured using Pearson's correlation coefficient. Quantification of **(F)** apical and **(G)** lateral E-CADHERIN pixels in intestinal epithelial cells. Measurement of the degree of colocalisation between RAB5 and E-CADHERIN on the **(H)** apical and **(I)** lateral membrane of intestinal epithelial cells. Data are representative of at least $n = 3$ different slices per organoid and of at least $n = 3$ biologically independent organoids. For each z-section, at least $n = 3$ individual IECs with clear apical to basal delineation were used. Graphs indicate the mean $\pm$ S.E.M. Statistical significance was determined using unpaired (Student's) t-test. ns = not significant ($p > 0.05$).

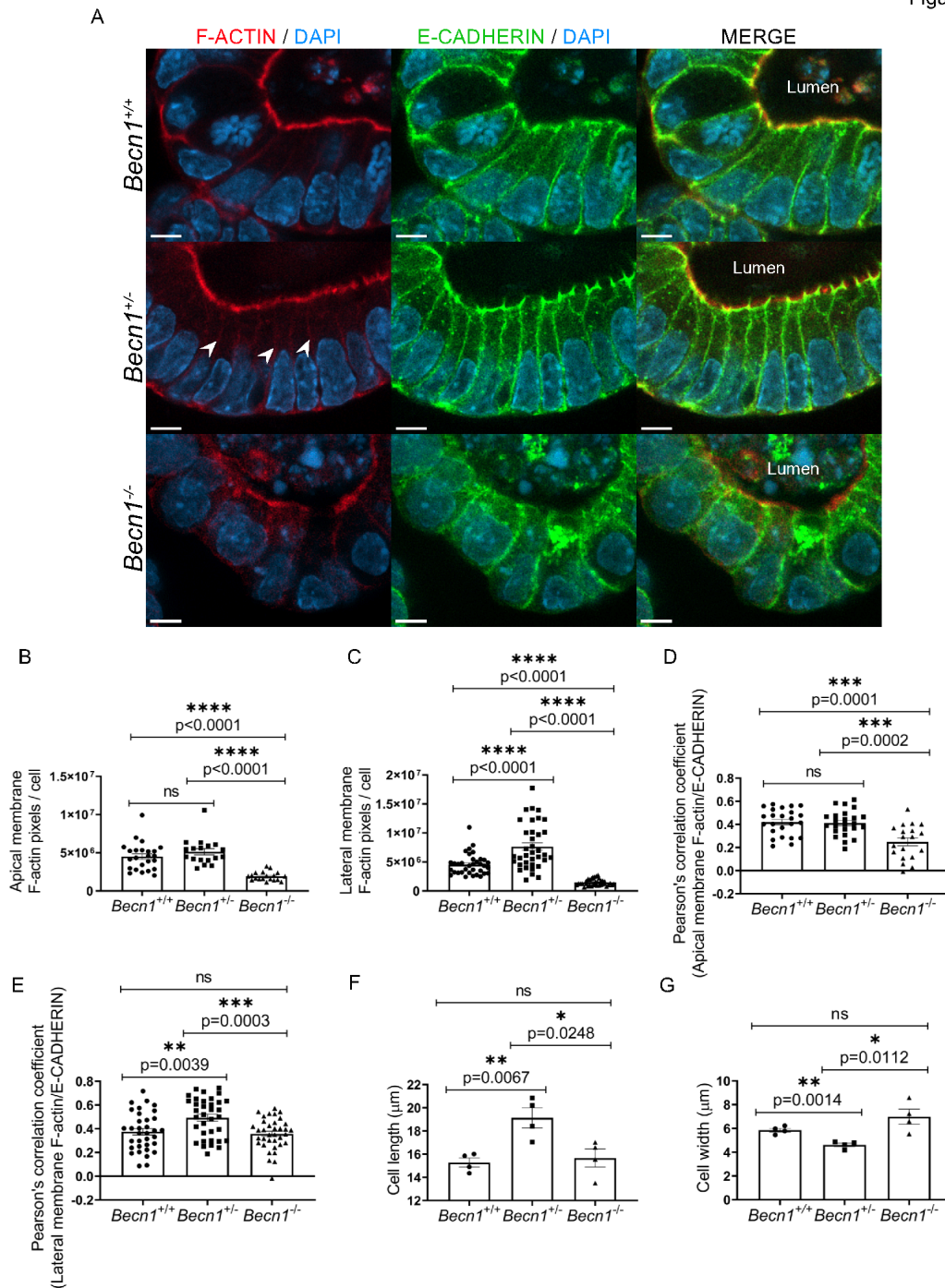


Figure 3

Monoallelic *Becn1* deletion alters F-actin organisation in intestinal epithelial cells. (A) Representative whole-mount immunofluorescence staining for F-actin (red), E-CADHERIN (green) and DAPI (blue) in *Becn1*^{+/+}, *Becn1*^{+/-} and *Becn1*^{-/-} organoids. Increased lateral F-actin in *Becn1*^{+/-} organoids is indicated by white arrowheads. Scale bar = 5 μm. Quantification of (B) apical and (C) lateral F-actin signals per cell. Colocalisation between F-actin and E-CADHERIN on the (D) apical and (E) lateral membranes of

intestinal epithelial cells, assessed using Pearson's correlation coefficient. **(F)** Measurement of cell length (apical to basal membrane) and **(G)** cell width (lateral to lateral membrane). Data are representative of at least $n = 3$ different z-slices per organoid and of at least $n = 3$ biologically independent organoids. For each z-slice, at least $n = 3$ individual cells with clear apical to basal delineation were used for quantification. Graphs indicate the mean $\pm$ S.E.M. Statistical significance was determined using unpaired (Student's) t-test for all graphs. ns = not significant ($p > 0.05$).

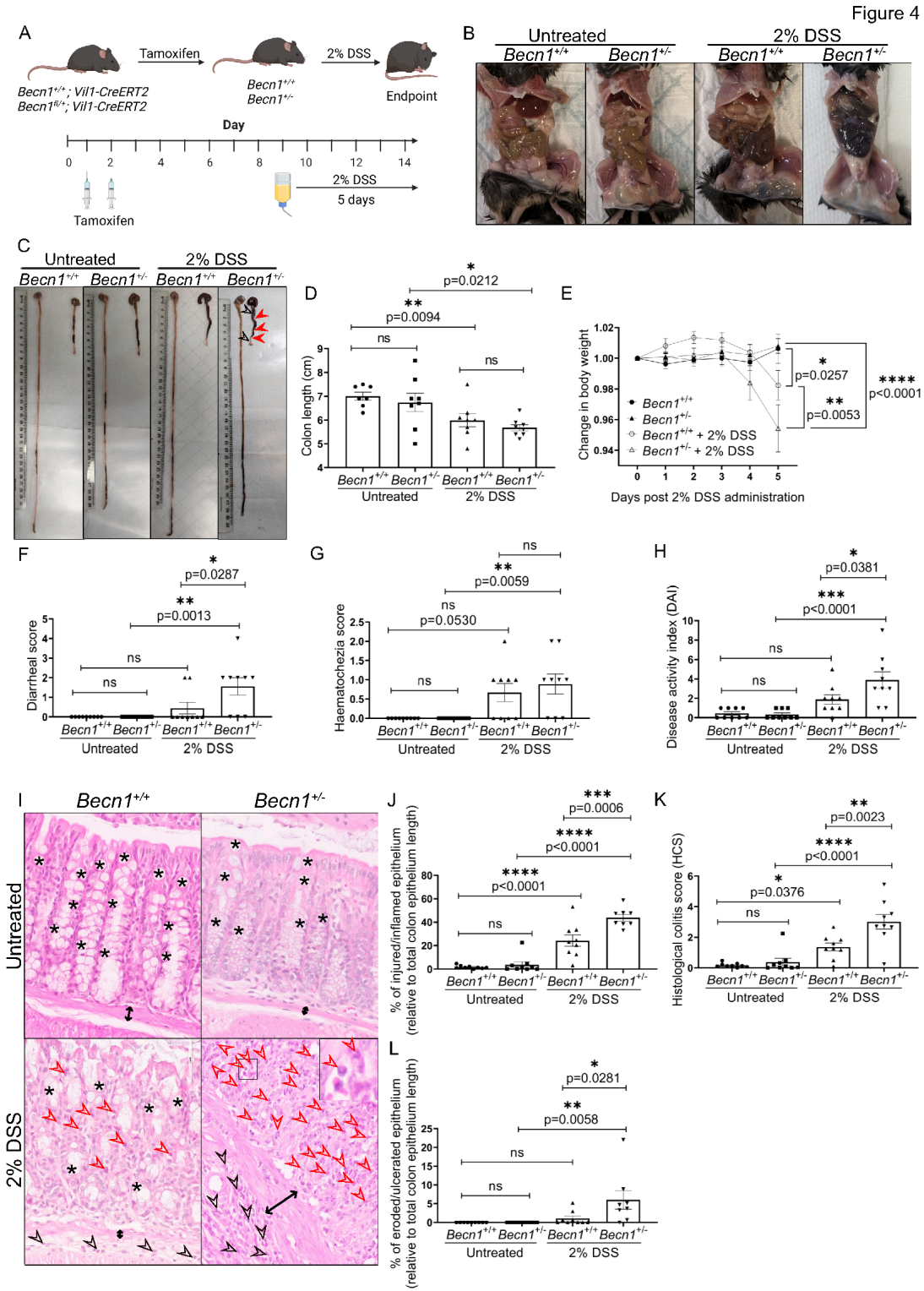


Figure 4

Monoallelic *Becn1* deletion exacerbates colitis severity following Dextran Sulphate Sodium administration.

(A) Schematic diagram depicting the timeline of Tamoxifen-induced *Becn1* gene deletion followed by 5 days of 2% Dextran Sulphate Sodium (DSS) treatment. Image created in BioRender.com.

(B) Representative images of abdominal necropsy and **(C)** intestinal tracts of *Becn1*^{+/+} and *Becn1*^{+/-} mice, who received normal drinking water (untreated) or 2% DSS drinking water, at endpoint. Red arrowheads and black open arrowheads highlight points of colon constriction and swelling, respectively.

(D) Colon lengths of mice from each treatment group at endpoint. **(E)** Changes in body weight of *Becn1*^{+/+} and *Becn1*^{+/-} mice during the 2% DSS treatment period, normalised to body weight at the start of DSS treatment (day 9). **(F)** Diarrheal and **(G)** haematochezia scores of *Becn1*^{+/+} and *Becn1*^{+/-} mice who received either normal drinking water or 2% DSS drinking water. Scores were determined based on parameters outlined in Table 1. **(H)** Disease Activity Index (DAI) score of *Becn1*^{+/+} and *Becn1*^{+/-} mice receiving either normal drinking water or 2% DSS drinking water, calculated by summing the scores of the parameters outlined in Table 1. **(I)** H&E-stained FFPE distal colon sections from *Becn1*^{+/+} and *Becn1*^{+/-} mice who received normal drinking water (untreated) or 2% DSS drinking water (2% DSS). The black double-headed arrow denotes the muscularis mucosae, and goblet cells are indicated by *. Immune cell infiltration in the submucosa and mucosa is marked by black and red open arrowheads, respectively. A magnified area (indicated by the box) provides a closer view of the mucosa, illustrating the increased immune infiltration in DSS-treated *Becn1*^{+/-} mice. **(J)** Percentage of injured or inflamed epithelium, normalised to the length of the muscularis mucosae, in the colon of *Becn1*^{+/+} and *Becn1*^{+/-} mice receiving either normal drinking water or 2% DSS drinking water. **(K)** The histological colitis scores (HCS) of *Becn1*^{+/+} and *Becn1*^{+/-} mice with and without DSS treatment was calculated based on described methods (49). **(L)** Percentage of eroded epithelium, normalised to the length of the muscularis mucosae, in the colon of *Becn1*^{+/+} and *Becn1*^{+/-} mice receiving either normal drinking water or 2% DSS drinking water. Data are representative of at least $n = 9$ biologically independent mice from $n = 3$ independent experiments. Graphs indicate the $\pm$ S.E.M. Statistical significance was determined using ordinary one-way ANOVA except in (E) where changes in body weight was determined using two-way ANOVA with Tukey's post-hoc test.

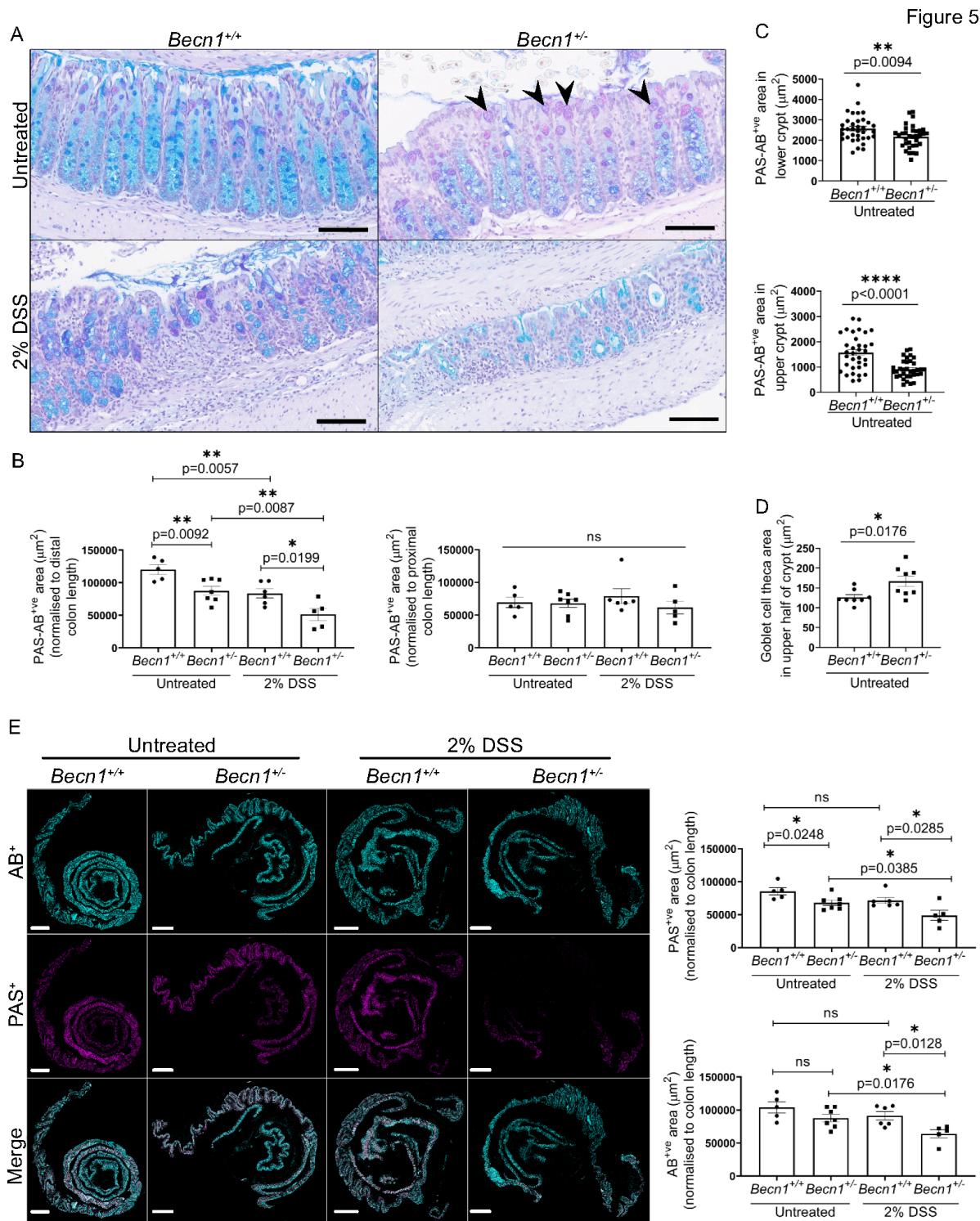


Figure 5

Monoallelic *Becn1* deletion disrupts goblet cell function and predisposes to DSS-induced colitis. (A) Representative PAS-AB-stained distal colon sections from untreated and DSS-treated *Becn1*^{+/+} and *Becn1*^{+/-} mice. Scale bar = 100 μm. Black arrowheads depicts enlarged goblet cells. **(B)** Quantification of PAS-AB⁺ve area (measured per μm of muscularis mucosae) normalised to gross colon length in the

distal and proximal colon of untreated and DSS-treated *Becn1*^{+/+} and *Becn1*^{+/-} mice. **(C)** Quantification of PAS-AB^{+ve} area in the lower half and upper half of distal colon crypts, as delineated in Supplementary Figure 5, in *Becn1*^{+/+} and *Becn1*^{+/-} mice. **(D)** Quantification of goblet cell theca size in the upper half of distal colonic crypts of *Becn1*^{+/+} and *Becn1*^{+/-} mice. **(E)** Graphical representation of acidic (AB^{+ve}), neutral (PAS^{+ve}), and total (Merge) mucins in untreated and DSS-treated *Becn1*^{+/+} and *Becn1*^{+/-} mice. Scale bars = 1 mm. The areas of acidic (AB^{+ve}) and neutral (PAS^{+ve}) mucus were quantified per μm of muscularis mucosae and normalised to gross total colon length. Data represent $n > 5$ biologically independent mice from $n = 2$ independent experiments. Only animals that received DSS treatment from the same lot number were included for analysis. Graphs show the $\pm$ S.E.M. Statistical significance was determined by unpaired (Student's) t-test. For crypt-specific quantifications in (C), full-length intact crypts with at least three contiguous intact crypts were analysed, with $n > 3$ crypts analysed per animal. The average goblet cell theca area in (D) was obtained by measuring >100 goblet cells per animal. DSS: dextran sulphate sodium. PAS: periodic acid Schiff. AB: alcian blue. ns: not significant ($p > 0.05$).

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