Original research

Alzheimer's disease-related presenilins are key to intestinal epithelial cell function and gut immune homoeostasis

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ABSTRACT

Objective Mutations in presenilin genes are the major cause of Alzheimer's disease. However, little is known about their expression and function in the gut. In this study, we identify the presenilins Psen1 and Psen2 as key molecules that maintain intestinal homoeostasis.

Design Human inflammatory bowel disease (IBD) and control samples were analysed for Psen1 expression. Newly generated intestinal epithelium-specific Psen1-deficient, Psen2-deficient and inducible Psen1/Psen2 double-deficient mice were used to dissect the functional role of presenilins in intestinal homoeostasis.

Results Psen1 expression was regulated in experimental gut inflammation and in patients with IBD. Induced deletion of Psen1 and Psen2 in mice caused rapid weight loss and spontaneous development of intestinal inflammation. Mice exhibited epithelial barrier disruption with bacterial translocation and deregulation of key pathways for nutrient uptake. Wasting disease was independent of gut inflammation and dysbiosis, as depletion of microbiota rescued Psen-deficient animals from spontaneous colitis development but not from weight loss. On a molecular level, intestinal epithelial cells lacking Psen showed impaired Notch signalling and dysregulated epithelial differentiation.

Conclusion Overall, our study provides evidence that Psen1 and Psen2 are important guardians of intestinal homoeostasis and future targets for barrier-promoting therapeutic strategies in IBD.

INTRODUCTION

Presenilins are transmembrane proteins that act as the catalytic subunit of the gamma-secretase complex, which plays a central role in the processing of amyloid precursor protein (APP) and several other substrates.^{1–3} Presenilins have also been implicated in autophagy, apoptosis, protein trafficking and calcium homoeostasis, which appear to be independent of gamma-secretase activity.⁴ Presenilins are best known as critical players in the pathogenesis of Alzheimer's disease (AD). Mutations in presenilin genes, particularly *PSEN1* and *PSEN2*, are known to be responsible for early-onset familial AD.⁵ Although PSEN1 and PSEN2 share

WHAT IS ALREADY KNOWN ON THIS TOPIC

- ⇒ An impaired epithelial barrier is observed in patients with inflammatory bowel disease (IBD) and disruption of the intestinal epithelial monolayer induces gut inflammation in mice.
- ⇒ Many patients with IBD experience weight loss and malnutrition.
- ⇒ Psen1 and Psen2 are key molecules in the pathogenesis of Alzheimer's disease while their role in the gut remains largely unknown.

WHAT THIS STUDY ADDS

- ⇒ This is the first study describing the functional role of Psen1 and Psen2 in intestinal homoeostasis.
- ⇒ Psen1 expression is impaired in the inflamed intestine of patients with IBD and mice with experimentally induced inflammation.
- ⇒ Inducible deletion of both presenilins in intestinal epithelial cell (IECs) results in spontaneous intestinal inflammation with barrier breakdown and bacterial translocation.
- ⇒ Epithelial Psen1 and Psen2 are crucial molecules for Notch-mediated IEC differentiation.
- ⇒ Mice with an IEC-specific deletion of Psen1/ Psen2 develop lethal severe wasting.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Since our study demonstrates that presenilin expression is pivotal for intestinal homoeostasis, we suggest the presenilins as promising future targets in IBD pathogenesis for the improvement of barrier integrity and malnutrition.

structural similarities and exhibit some functional overlap, they also have distinct expression patterns and different substrate specificities.^{6–8} While studies on PSEN1 and PSEN2 have largely focused on their role in neurologic diseases, far less is known about the functional role of the presenilins in other organs, for example, in the gut. Interestingly, recent

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epidemiological studies and experimental mouse models have uncovered a link between AD and intestinal inflammation.^{9–12} In this context, our own research data show dysregulation of the presenilins in experimental colitis in mice, providing a rational for studying these molecules in the gut.

For efficient protection of the gastrointestinal (GI) tract, intestinal epithelial cells (IECs) form an effective barrier against potentially harmful microbes, toxins and antigens, present in the intestinal lumen. Perturbations of epithelial homoeostasis can lead to GI pathologies, including the inflammatory bowel diseases (IBD) Crohn's disease (CD) and ulcerative colitis (UC). Several pathways including Wnt-signalling and Notch-signalling closely work together to maintain tissue homoeostasis and thus prevent IBD.¹³ In addition to abdominal pain and diarrhoea, 70%–80% of hospitalised patients with IBD experience weight loss^{14 15} and 20%–85% suffer from malnutrition.^{16 17} To date, the underlying mechanisms leading to IBD and especially malnutrition are still incompletely understood.

Given the pivotal role of presenilins in both mediating the processing of the Notch receptor^{2 18–20} and their interaction with the Wnt-signalling pathway,^{21–24} we hypothesised that presenilins might be crucially involved in intestinal homoeostasis and inflammation.

In this study, we identified Psen1 and Psen2 as important molecules in maintaining intestinal homoeostasis. The generation of inducible Psen1/2 double knockout mice resulted in spontaneous development of intestinal inflammation with barrier breakdown and subsequent bacterial translocation. Lack of Psen1 and Psen2 severely impaired IEC differentiation with upregulation of secretory cells including goblet, Paneth and enteroendocrine cells, at the expense of absorptive enterocytes. Deletion of both presenilins caused severe impairment of nutrient uptake ultimately leading to malnutrition and death of these mice. While antibiotic treatment ameliorated gut inflammation, the body weight loss was not reversed. Our study demonstrates that presenilin expression is pivotal for intestinal homoeostasis as it controls IEC differentiation and nutrient absorption.

METHODS

Methods are available as online supplemental file.

RESULTS

Presenilins are expressed in the intestinal epithelium and are differentially regulated during intestinal inflammation

To identify underlying mechanisms of IBD pathogenesis, we sought to identify novel and previously unrecognised pathways that might be involved in intestinal inflammation. To this end, we performed bulk RNA sequencing comparing colonic tissue samples from mice subjected to dextran sulfate sodium (DSS)induced experimental colitis and healthy control animals. While several IBD-related pathways, such as metabolism-related and cytokine-related pathways were found by Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, our colitis data surprisingly also revealed an enrichment of pathways attributed to neurodegeneration such as those involved in AD (figure 1A) Among the differentially expressed genes in both pathways, we found the presenilins Psen1 and Psen2, which are key molecules in the genetics of familial AD (online supplemental figure 1).²⁵ Strikingly, during the course of DSS-induces colitis,²⁶ Psen1 gene expression was significantly downregulated during the development of colitis, whereas expression levels returned to baseline levels during the recovery phase (figure 1B). Downregulation of Psen1 expression during intestinal inflammation was further

supported by assessing Psen1 protein levels (figure 1C). Comparison of Psen1 expression pattern between steady state conditions and intestinal inflammation using immunofluorescence staining revealed that Psen1 expression is mainly restricted to IECs. In contrast, Psen1 levels were reduced in the epithelium of mice during intestinal inflammation (figure 1D). Consistent with the data from our mouse model, PSEN1 expression was significantly decreased in both UC and CD patients in a publicly available transcriptomic dataset²⁷ (figure 1E). Moreover, among our in-house cohort of patients with IBD, PSEN1 was also significantly downregulated in the colon of both UC and CD patients (figure 1F). Of note, PSEN1 expression levels also correlated with disease severity as shown in two different IBD cohorts (figure 1G,H). The observed downregulation of PSEN1 in inflamed human tissues was further confirmed by immunofluorescence staining for PSEN1 at the protein level in UC and CD patients compared with healthy individuals (figure 1I). Interestingly, an integrated analysis approach of four different single-cell datasets from patients with IBD²⁸⁻³¹ revealed a unique downregulation of *PSEN1* expression in inflamed enterocytes (figure 1]), which was not observed in immune or mesenchymal cell subsets (online supplemental figure 2A,B). Noteworthy, similar to PSEN1, the expression levels of PSEN2 were greatly reduced in enterocytes of UC and CD patients (online supplemental figure 2C). Taken together, our analysis demonstrates intestinal epithelial expression of Psen1, together with marked deregulation during intestinal inflammation in mice and humans, suggesting Psen1 as an important epithelial molecule involved in intestinal inflammation.

Lack of epithelial Psen1 alone does not disrupt intestinal homoeostasis

To study potential functions of Psen1 in the mouse intestine, $Psen1^{fl/fl}$ mice³² were crossed with villin-cre mice to generate mice with IEC-specific homozygous deletion of Psen1 (*Psen1* $^{\Delta IEC}$) (online supplemental figure 3A–G). Notably, homozvgous $Psen1^{\Delta IEC}$ mice did not develop an overt phenotype and no histological changes were observed in the intestinal architecture when compared with their littermate controls (online supplemental figure 4A). In addition, mRNA and protein analvsis of IEC subtypes and differentiation markers revealed that IEC differentiation from intestinal stem cells into absorptive enterocytes or secretory cell types such as goblet, Paneth, enteroendocrine or tuft cells, did not differ between the two groups (online supplemental figure 4B-D). Moreover, no differences were observed in the levels of several inflammation-associated factors (online supplemental figure 4E), further supported by the lack of changes in bulk RNA sequencing of ileum and colon tissue from $Psen1^{\Delta IEC}$ compared with control littermates (online supplemental figure 4F). Interestingly, however, PSEN2 protein levels were significantly upregulated in the ileum and colon of $Psen1^{\Delta IEC}$ mice (online supplemental figure 4G), suggesting a potential compensatory mechanism between both presenilins in IECs.

Inducible Psen1/2 double knockout mice spontaneously develop intestinal inflammation

Since we hypothesised that intestinal epithelial Psen2 can compensate for the loss of Psen1, we crossed $Psen1^{\Delta \text{IEC}}$ with $Psen2^{-/-}$ mice to generate Psen1/2 double knockout mice (online supplemental figure 5A). Remarkably, we did not obtain any Psen1/2 double knockout mice while the other expected genotypes were born at increased ratios (online supplemental figure



Figure 1 Psen1 is expressed in the intestine and is differentially regulated during intestinal inflammation. (A) KEGG analysis of RNA sequencing data from DSS-treated mouse whole colon tissue compared with healthy mouse colons (n=3/group). (B) *Psen1* counts in the colon of DSS-treated mice at different stages of inflammation with body weight change (%) of the mice, analysed from a publicly available dataset (n=2–3/group).²⁶ (C) Western blotting for PSEN1 in colonic tissue lysates from healthy and DSS-treated mice, with measurement of PSEN1 expression, normalised to β -actin (n=7–8/group). (D) Representative immunofluorescence images for Psen1 (red) and E-Cadherin (green) in the ileum and colon of healthy and severely inflamed DSS-treated mice (scale bar 100 µm) (representative of 3 independent experiments). (E) Normalised Log₂ expression levels of *PSEN1* in healthy (n=4), ulcerative colitis (UC) (n=13) and Crohn's disease (CD) (n=19) patients analysed from a publicly available dataset.²⁷ (F) *PSEN1* normalised counts in the colon of healthy (n=22), UC (n=44) and Crohn's disease (n=41) patients. (G) Relative *PSEN1* expression levels in different disease stages of UC and CD patients (n=5/group). (H) Linear regression analysis of *PSEN1* in colonic samples of healthy (n=4), UC (n=6) and CD patients (n=4) (scale bar 50 µm) with analysis of the Psen1⁺ area (normalised to Hoechst). (J) *PSEN1* relative expression across epithelial cell subsets without downsampling the number of cells per subset, sorted by disease status in the cecum, appendix, colon and rectum tissues, obtained from http://scibd.cn. Overall, data are expressed as mean±SD. *p<0.05, **p<0.01, ***p<0.001, respectively, by one-way ANOVA (B, E, G, H) Mann-Whitney U test (C) or Wilcoxon rank-sum test (F). ANOVA, analysis of variance.

5B). Of note, one allele of Psen2 was sufficient to maintain intestinal homoeostasis, as Psen1 knockout mice on a heterozygous Psen2 background ($Psen2^{+/-} Psen1^{\Delta IEC}$) were born alive and showed no detectable changes in gut architecture or IEC differentiation (online supplemental figure 5C–G). Moreover, a detailed analysis of the intestine of $Psen2^{-/-}$ mice³³ (online supplemental figure 6A) revealed no detectable changes under steady-state conditions (online supplemental figure 6B–G). Together our data demonstrated that neither the absence of Psen1 nor that of Psen2 alone affected gut tissue homoeostasis while deletion of both presenilins caused early embryonic lethality.

To overcome the embryonic lethality of Psen1/2 double knockout mice and to study the functions of both genes in the gut, we established a new mouse line with an inducible *Psen1* knockout allele on a background of a *Psen2* germline deletion (*Psen2^{-/-} Psen1*^{i Δ IEC}; iDKO) (online supplemental figure 7A).



Figure 2 Inducible Psen1/2 double knockout mice spontaneously develop colitis. (A) Schematic of intraperitoneal (i.p.) injection of tamoxifen in control (Psen2^{-/-} Psen1^{il/II}) and inducible Psen1/2 double knockout (iDKO; Psen2^{-/-} Psen1^{il/IIC}) mice. (B) Daily weight measurements of tamoxifen-injected control and iDKO mice. Weight is expressed as percentage of initial weight (control: n=5; iDKO: n=7). (C) Representative endoscopic images and murine endoscopic index of colitis severity (MEICS) scoring in control and iDKO mice (control: n=13; iDKO: n=15). (D) Measurement of colon length in the different mouse strains (control: n=9). (E) Representative H&E images of ileum and proximal and distal colon from control and iDKO mice (scale bar 250 µm) with histology scoring of duodenum, jejunum, ileum, proximal and distal colon. Yellow arrows indicate cell death, and black arrows indicate mucus accumulation (representative of three independent experiments). (F) qPCR analysis of several inflammatory cytokines (normalised to *Gapdh*) from ileum and colon of the shown mice (control: n=8; iDKO: n=11). (G) Representative IF images for MPO (neutrophils) and F4/80 (macrophages) in the ileum and colon of the different genotypes (scale bar 100 µm) (control: n=5–6; iDKO: n=7–10) with analysis of the MPO⁺ cells (per high power field (HPF)) and F4/80⁺ area (normalised to Hoechst). Overall, data are expressed as mean±SD *p<0.05, **p<0.01, ***p<0.001, respectively, by one-way ANOVA (B) and Mann-Whitney U test (C, D, F, G). ANOVA, analysis of variance.

Successful deletion of *Psen1* on the *Psen2^{-/-}* background was achieved by injection of tamoxifen (figure 2A), resulting in greatly reduced expression levels of Psen1 in IECs and complete absence of Psen2 in intestinal tissue samples as well as in isolated organoids (online supplemental figure 7B–F). In contrast to *Psen1* and *Psen2* single knockout mice, iDKO mice showed rapid and significant weight loss when compared with control $Psen2^{-/-} Psen1^{fl/fl}$ littermate mice (figure 2B). In addition, endoscopy of the mice demonstrated the development of spontaneous colitis in iDKO mice (figure 2C).³⁴ Moreover, we observed a significant shortening of the colon in iDKO mice compared with

their littermate controls (figure 2D). Furthermore, histological examination of the small and large intestine showed histological changes, including signs of inflammation and cell death. In the ileum and proximal colon, we observed structural changes in the epithelium accompanied by abundant mucus production (figure 2E). In addition, Psen1/2 double-deficient epithelial organoids (online supplemental figure 8A) completely lacked the usual budded morphology and had a significantly reduced size (online supplemental figure 8B), further supporting aberrant IEC-intrinsic mechanisms in the absence of Psen1/2. Furthermore, proinflammatory markers, including *S100a9*, *Il1b* and *Tnf*, showed a striking upregulation in the ileum and colon of iDKO mice compared with littermate controls (figure 2F). Moreover, assessment of immune cell infiltration revealed significantly increased numbers of neutrophils and macrophages in the intestine of iDKO mice (figure 2G). In conclusion, our data demonstrate that the presenilins are key factors for intestinal homoeostasis, as dual deletion of Psen1 and Psen2 leads to the development of spontaneous intestinal inflammation in vivo.

Presenilins are key to intestinal barrier function

To investigate whether Psen deficiency affects intestinal barrier functions, iDKO and control littermate animals were orally gavaged with FITC-Dextran to determine its passage across the epithelium. Interestingly, significantly higher levels of FITC-Dextran were measured in the serum of iDKO mice, suggestive of substantial barrier defects (figure 3A). Increased permeability was also demonstrated on the level of epithelial organoids using lucifer yellow.³⁵ An increase in dye intensity in the lumen of organoids generated from iDKO but not control mice was observed, indicating that the observed barrier breakdown due to presenilin loss is IEC intrinsic and independent of the intestinal environment (figure 3B). Adherens junction and tight junction proteins, such as E-Cadherin and occludin, respectively, regulate paracellular permeability and are key guardians of the epithelial barrier.³⁶ Several studies have already shown a downregulation of these components in patients with IBD.^{37 38} Interestingly, immunofluorescence staining in ileal and colonic tissue samples from tamoxifen-injected control and iDKO mice showed a strong reduction of E-Cadherin in the membranous portion of IECs in iDKO mice (figure 3C). Moreover, Western blot analysis revealed a loss of full-length occludin in the ileum of the mice, along with reduced levels of cleaved occludin in the ileum and colon of iDKO mice (figure 3D). When sacrificing iDKO mice at different timepoints after the first dose of tamoxifen, we determined that full-length occludin was completely abolished already on day 5, while the cleaved form of occludin was reduced on day seven after the first tamoxifen injection (figure 3E). These observations were supported by small intestinal organoid cultures (online supplemental figure 8C), suggesting that epithelialintrinsic mechanisms lead to reduced occludin expression on presenilin deletion. To analyse the consequences of intestinal barrier dysfunction on microbial dysbiosis in iDKO mice, gut tissue sections were stained with a fluorescence in situ hybridisation (FISH) probe detecting all eubacteria. Strikingly, while bacteria in control animals were well separated from the epithelial lining, bacteria in iDKO mice infiltrated the crypt area down to the crypt bottom and even into the normally tight IEC monolayer (figure 3F), suggesting that presenilins are key to maintain an efficient barrier and to prevent microbial colonisation of the crypt regions. To examine the systemic impact of the previously observed intestinal barrier breakdown in iDKO mice, tissue lysates of mesenteric lymph nodes (MLN) and livers from the animals were plated on agar plates. While no colony-forming units (CFUs) were detected in the tissues of control mice, high numbers of CFUs were observed in the MLN and liver of iDKO mice (figure 3G), indicating that a presenilin deficient gut epithelium loses its ability to prevent a systemic spread of bacteria. In support of this, elevated levels of serum TNF- α (figure 3H) and proinflammatory markers in spleen tissue (figure 3I) of iDKO mice indicated a systemic inflammatory response. Finally, measurements of serum aspartate aminotransferase and alanine aminotransferase (ALT) levels indicated liver damage (figure 3]). In conclusion, our analysis clearly demonstrated the key role of presenilins in barrier integrity, where deletion of both presenilins leads to breakdown of the intestinal barrier and bacterial translocation to distant organs, eliciting a systemic inflammatory response.

Spontaneous inflammation but not rapid body weight loss in presenilin-deficient mice is driven by microbiota

To investigate whether barrier breakdown with subsequent bacterial translocation could be chiefly responsible for the severe wasting disease and early death of mice lacking Psen1/2 in the epithelium, mice were pretreated with an antibiotic cocktail to deplete the microbiota (figure 4A), which was confirmed by plating stool lysates before and after antibiotic treatment and assessing the number of CFUs (figure 4B). Unexpectedly, daily weight measurements revealed that iDKO mice treated with antibiotics displayed similar wasting disease as iDKO mice subjected to normal water (figure 4C). In contrast, endoscopic scoring of colitis severity revealed a significant reduction of inflammation in antibiotic-treated iDKO mice compared with iDKO mice receiving water only (figure 4D). Furthermore, histological examination of ileal and colonic tissue sections confirmed the amelioration of inflammation in the colon (figure 4E,F). In addition, antibiotic treatment significantly reduced the levels of proinflammatory factors, including Il6, Tnf, Il1b and S100a9 in the ileum and colon of iDKO compared with water-drinking iDKO mice (figure 4G). Taken together, antibiotic treatment of iDKO mice could ameliorate signs of inflammation and barrier breakdown, but could not rescue the weight loss in these mice, suggesting that microbial translocation in iDKO is a driver of colitis development, but there might be another potential mechanism underlying the rapid and severe unrecoverable weight loss.

Psen1/2 deficiency disrupts Notch signalling, alters IEC differentiation and leads to malnutrition in inducible Psen1/2 double knockout mice

To further improve our mechanistic understanding of the functions of presenilins in gut homoeostasis, we performed bulk RNA sequencing with ileal tissue lysates of iDKO mice and control littermates. Interestingly, this analysis revealed broad presenilindependent transcriptional changes with 4129 differentially expressed genes between the two groups (figure 5A). Using gene ontology analysis, we observed upregulation of pathways involved in inflammatory responses, cell migration and mucin type O-glycan biosynthesis in iDKO mice, whereas numerous pathways related to transport and absorption of nutrient components, including lipids, ions, fat, sodium and minerals, were significantly reduced. Moreover, metabolic signatures, including lipid and fatty acid metabolic pathways, TCA cycle, gluconeogenesis and glycolysis, were also reduced on induced presenilin deletion (figure 5B and online supplemental figure 9A, online supplemental tables 1-3). By analysing IEC differentiation in more detail, we observed an influence of dual presenilin deletion on secretory IEC differentiation, with increased numbers of goblet, Paneth and enteroendocrine cells (online supplemental figure 9B). To thoroughly analyse the different IEC types, several immunofluorescence stainings for unique IEC type marker proteins were performed, showing a significant loss of Olfm4⁺ and Lgr5⁺ stem cell populations in the intestine of iDKO mice, as well as significantly reduced numbers of Ki67⁺ proliferating cells (online supplemental figure 9C). In addition, this analysis further confirmed an upregulation of the number of secretory cells, including Paneth, goblet and enteroendocrine cells (online supplemental figure 9D). Similar results were



Figure 3 Presenilins are key to intestinal barrier function. (A) Measurement of FITC-dextran in the serum of tamoxifen-injected control (Psen2^{-/-} Psen1^{I/ΔIEC}) mice (control: n=8; iDKO: n=11). (B) Lucifer yellow uptake in Si organoids with analysis of the mean value of relative intensity increase (scale bar 100 µm) (representative of three independent experiments). Dashed lines indicate the lumen of the organoids. (C) Representative IF images for E-Cadherin in the ileum and colon of control and iDKO mice (scale bar 100 µm) (representative of three independent experiments). (D) Western blot analysis for Occludin in ileal and colonic tissue extracts from the indicated mice. β-actin was used as a loading control (representative of three independent experiments). (E) Occludin and Psen1 protein expression levels in the ileum of control and iDKO mice; control mice were sacrificed on day 8 after the first dose of tamoxifen, iDKO mice were sacrificed on day 5, day 6, day 7 and day 8 after the first dose. β-actin was used as a loading control (one experiment). (F) Representative FISH staining images for Eub338 (white arrows) in the ileum and colon of the indicated mouse strains (scale bar 100 µm) (representative of three independent experiments). (G) Tissue plating of mesenteric lymph node (MLN) and liver tissue extracts from the different genotypes cultured under aerobic and anaerobic conditions, including assessment of colony-forming units (CFUs)/g stool (n=≥7/group). (H) Analysis of TNF-α levels in serum of control and iDKO mice (control: n=4; iDKO: n=6). (I) qPCR analysis of *Tnf* and *Nos2* in spleen tissue of the different genotypes (control: n=11; iDKO: n=7). (J) Assessment of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels (control: n=18; iDKO: n=17). Overall, data are expressed as mean±SD *p<0.05, **p<0.01, ***p<0.01, respectively, by Mann-Whitney U test.

obtained in vitro using small intestinal organoids generated from these mice (online supplemental figure 8D,E). In contrast, our RNA sequencing approach revealed a striking downregulation of genes associated with absorptive enterocytes (figure 5C). In line with this, staining for the enterocyte-specific marker intestinal alkaline phosphatase (IAP) revealed a largely diminished number of absorptive enterocytes in the small intestine of iDKO mice (figure 5D). To functionally investigate the loss of enterocytes in the intestine of inducible Psen1/2 double knockout mice, serum glucose measurements revealed a highly significant downregulation of glucose levels in iDKO mice (figure 5E), suggesting that the lack of Psen1/2 diminishes the ability of the intestine to properly absorb vitally important nutrients. To test whether the impaired uptake rate is IEC-specific, small intestinal organoids



Figure 4 Microbiota depletion improves spontaneous inflammation but not body weight loss in inducible Psen1/2 double knockout mice. (A) Schematic of antibiotic (Ab) treatment in drinking water and i.p. tamoxifen injection in control (Psen2^{-/-} Psen1^{i/JIE}) and inducible Psen1/2 double knockout (iDKO; Psen2^{-/-} Psen1^{i/JIEC}) mice. (B) Stool lysates plated on agar plates with corresponding evaluation of CFU/g stool (control: n=9; iDKO: n=10). (C) Daily weight measurements presented as percentage of initial weight in control and iDKO animals treated with or without antibiotics (n≥3/group). (D) Representative endoscopic images and MEICS score (n≥3/group). (E) Representative H&E staining images of ileum and colon sections of the indicated mice and treatments (scale bar 250 µm) (n≥3/group). (F) Histological scoring of ileal and colonic tissue sections of the different treatment groups indicated (n≥3/group). (G) qPCR analysis for various inflammatory markers (normalised to *Hprt*) in the ileum and colon of control and iDKO mice treated with and without antibiotics (n≥3/group). Overall, data are expressed as mean±SD *p<0.05, **p<0.01, ***p<0.001, respectively, by Mann-Whitney U test (B) or one-way ANOVA (C, D, F). ANOVA, analysis of variance; CFU, colony-forming unit; i.p., intraperitoneally; MEICS, Murine Endoscopic Index of Colitis Severity Score.

from control and iDKO mice were treated with Rhodamine 123. Interestingly, in contrast to control organoids, small intestinal organoids generated from iDKO mice almost completely failed to take up Rhodamine 123 (figure 5F). As further evidence of impaired intestinal glucose absorption in vivo, an oral glucose tolerance test revealed severely impaired intestinal glucose uptake rates in iDKO mice compared with control mice (figure 5G). Notably, antibiotic-treated iDKO mice still exhibited significantly reduced serum glucose levels (online supplemental

figure 10A) and impaired intestinal glucose uptake rates (online supplemental figure 10B), suggesting that the observed changes in systemic energy metabolism are not directly related to intestinal inflammation. In agreement with the observation by Tang *et al* that the average adipocyte area in mesenteric white adipose tissue is almost completely reduced in fasted mice,³⁹ we observed that the average adipose area was drastically reduced in inducible Psen1/2 double knockout mice (figure 5H). We reasoned that this was likely due to reduced lipid uptake given that the number

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Figure 5 Dual presenilin deletion leads to enterocyte loss, malnutrition and death in Psen1/2 double knockout mice due to Notch signalling disruption. (A) Volcano plot of RNA sequencing analysis of inducible Psen1/2 double knockout (iDKO; Psen2^{-/-} Psen1^{iΔIEC}) ileal tissue compared with control littermates (Psen2^{-/-} Psen1^{fl/fl}) (p-adjusted<0.05; log,foldchange±1). Green dots represent downregulation, red dots represent upregulation and grey indicates no gene deregulation (n=3/group). (B) Selected gene ontology biological process (GO_BP) and KEGG pathways of the described RNAseq dataset (n=3/group). Red bars indicate upregulation, green bars indicate downregulation. (C) Heatmap of RNA sequencing analysis of ileal tissue from control and iDKO mice (n=3/group) for absorptive enterocyte-specific genes (n=3/group). (D) Images of intestinal alkaline phosphatase (IAP: bordeaux: enterocyte marker) staining in the ileum of control and iDKO mice (scale bar 100 µm) with analysis of IAP+area normalised to hematoxylin (n=5–7/group). (E) Measurement of serum glucose levels of indicated mice (control: n=31; iDKO: n=28). (F) Rhodamine 123 uptake and relative measured Rhodamine 123 intensity in the lumen of Si organoids generated from control and iDKO mice treated with 50 ng/mL tamoxifen for 5 days (scale bar 100 µm) (representative of three independent experiments). (G) Oral glucose tolerance test in control and iDKO mice using 2.5 g glucose/kg body weight (control: n=10; iDKO: n=6). (H) Representative H&E staining images of mesenteric fat and analysis of the area of fat cells from the two mouse strains (scale bar 250 µm) (control: n=15; iDKO: n=8). (I) Representative immunofluorescence staining images of HCS LipidTOX in the duodenum of control and iDKO mice with analysis of HCS LipidTOX positive area normalised to Hoechst (scale bar 100 µm) (control: n=9: iDKO: n=6). (J) Representative images of periodic acid-schiff (PAS) staining on liver tissue samples from control and iDKO mice with analysis of PAS⁺ staining (fold change) (scale bar 100 µm) (control: n=17; iDKO: n=9). (K) Analysis of Notch intracellular domain (NICD) protein expression by Western blotting of ileal and colonic tissue extracts from Psen1^{ΔIEC}, Psen2^{-/-} and iDKO mice and their respective controls. β -actin was used as a loading control (representative of three independent experiments). (L) Schematic of the influence of a dual Psen1 and Psen2 deletion on intestinal homoeostasis. Overall, data are expressed as mean±SD **p<0.01, ***p<0.001, respectively, by Mann-Whitney U test (D, E, F, H, I, J) or two-way ANOVA (G). ANOVA, analysis of variance; DEG, differentially expressed genes.

of absorptive enterocytes was reduced. To test this hypothesis in a functional approach in vivo, fasted mice received corn oil as a lipid source by oral gavage. Application of corn oil has been shown to result in lipid droplet formation due to uptake of lipids by absorptive enterocytes, which can be detected by immunofluorescence staining.⁴⁰ Strikingly, lipid droplet accumulation in IECs of the small intestine was completely blocked in iDKO mice compared with littermate controls (figure 51). Finally, to assess the state of hepatic energy stores, periodic acid–Schiff (PAS) staining to detect glycogen levels in liver tissue of the mice unveiled a substantial decrease in glycogen content in the liver of iDKO mice when compared with control mice (figure 5 J). These analyses ultimately highlight the severity of the presenilindependent loss of absorptive enterocytes, suggesting that the death of the inducible Psen1/2 double knockout mice may be due to malnutrition.

Since the γ -secretase is critically involved in Notch signalling,^{18–20} we hypothesised that non-functional Notch signalling might be responsible for the severely impaired IEC differentiation in the inducible Psen1/2 double knockout mice, leading to reduced barrier permeability on the one hand and malnutrition on the other hand. To test whether Notch signalling is impaired in the intestine on Psen1/2 deficiency, we performed Western blot analysis using the Notch intracellular domain (NICD) as a marker of functional Notch signalling. Remarkably, whereas NICD was still released in Psen1 and Psen2 single knockout mice, NICD levels were completely abolished in the colon, ileum and in organoids generated from iDKO mice (figure 5K and online supplemental figure 8F). This could explain the observed lack of phenotype in heterozygous Psen1/2 double knockout mice, showing that one allele of Psen2 is sufficient to maintain functional Notch signalling (online supplemental figure 5H).

Overall, our study identified the presenilins Psen1 and Psen2 as previously unknown players in intestinal homoeostasis and inflammation, where they act as key molecules in maintaining balanced IEC differentiation and barrier function integrity to prevent malnutrition and intestinal inflammation (online supplemental figure 10C and figure 5L). Thus, supporting presenilin enzymatic activity in the gut epithelium or downstream signal-ling may provide new targets for novel IBD therapies.

DISCUSSION

Our study reveals for the first time the key role of the presenilins Psen1 and Psen2 for intestinal homoeostasis by controlling the balance of IEC differentiation to sustain barrier integrity and to avoid malnutrition and the development of intestinal inflammation. To date, presenilins have been extensively studied in the brain, where mutations in presenilin genes cause AD. We have previously established a link between Psen1 and colorectal tumour development.⁴¹ However, not much is known about how the presenilins are regulated during intestinal health and inflammation and whether they are critically involved in these processes. Remarkably, our present study identified that double deletion of Psen1 and Psen2 in mice leads to spontaneous development of intestinal inflammation, with barrier breakdown and bacterial translocation. These outcomes are associated with impaired IEC differentiation, non-functional Notch signalling, ultimately resulting in malnutrition and death of the inducible Psen1/2 double knockout mice.

To date, the role of Notch signalling in intestinal inflammation is controversial. While some animal studies have shown proinflammatory effects of Notch signalling, for example, by using different γ -secretase inhibitors that reduced colitis severity in DSS-treated or TNBS-treated mice,^{42 43} other studies have demonstrated anti-inflammatory roles of Notch signalling during colitis.^{44 45} Surprisingly, various studies reported a significant increase in Notch signalling in patients with IBD.⁴⁶⁻⁴⁹ These changes may be beneficial by helping to regenerate the damaged epithelium and thus recover from the disease.⁴⁶ Notably, several clinical trials using γ -secretase specific inhibitors in patients with cancer and AD, have described the development of gut inflammation.⁵⁰⁻⁵⁴ These findings in humans support our observation in Psen1/2 double knockout mice and our data, therefore, provide a potential mechanistic explanation for the observed side effects in these patients. Our study demonstrates the existence of compensatory mechanisms between the two presenilins in the gut, which could be exploited to avoid Notch-related offtarget effects, especially in the intestine. This knowledge could be used in the future when designing new γ -secretase inhibitors.

Interestingly, although many publications have addressed the role of Notch signalling in intestinal homoeostasis by IEC-specific deletion of various Notch signalling components, quite different phenotypes have been observed. For example, while single Notch1 or Notch2 knockout mice and single Dll1 and Dll4 knockout mice show no phenotype, dual deletion of Notch1/Notch2 or Dll1/Dll4 resulted in increased goblet cell numbers and decreased proliferative cells.55 56 Similarly, compensatory mechanisms were observed among Hes genes, whereas triple deletion of Hes1/Hes3/Hes5 resulted in increased secretory cell numbers and decreased proliferation.⁵⁷ Moreover, deletion of the a-secretase Adam10 also resulted in the phenotypes described above.⁵⁸ Remarkably, IEC-specific deletion of Rbpj not only upregulates secretory cell lineage and downregulates proliferation but also induces spontaneous colitis and bacterial translocation.⁴⁵ Notably, none of these studies investigated the loss of absorptive enterocytes and associated malnutrition as seen in the mouse model presented in the current study. On the one hand, these studies demonstrate the importance of compensatory mechanisms within protein families to ensure the proper functioning of vitally important pathways. More importantly, they suggest y-secretase independent functions beyond their involvement in Notch signalling, which has been also previously observed for the presenilins,⁴ further highlighting the crucial importance of the presenilins in maintaining intestinal homoeostasis, particularly nutrient uptake and barrier function. At present, it is well-established that IBD pathogenesis and malnutrition are closely linked and are thought to result from reduced oral food intake, enteric nutrient loss, increased energy requirements and malabsorption.⁵⁹ Importantly, malnutrition has even been described to be associated with poor clinical outcome in patients with IBD.^{60 61} Malabsorption in these patients is thought to be due to loss of epithelial integrity and impaired epithelial transport,⁵⁹ but the underlying molecular mechanisms are poorly understood. Thus, our study suggests a molecular origin for the commonly observed reduced nutrient absorption and thus malnutrition in patients with IBD. To date, GWAS studies have not identified presenilins or other important Notch signalling-related molecules as being associated with IBD pathogenesis.^{62 63} This might be due to the high degree of redundancy often reported for these molecules.

Future studies will be necessary to establish potential pharmacological interventions that could target presenilins as a treatment option for IBD. These could involve targeting the enzymatic activity of Psen1 and Psen2 in the intestinal epithelium using an agonistic approach. However, such a pharmacological substance has not yet been reported, and the feasibility of such an approach would have to be established.

In conclusion, our research identifies the presenilins as key molecules of intestinal homoeostasis by coordinating a balanced IEC differentiation and thus a tight intestinal barrier. In contrast, impaired expression or function of presenilins can lead to Notch signalling disruption, with impaired IEC differentiation, resulting in malnutrition, barrier breakdown and ultimately the development of spontaneous inflammation. In the future, these findings may be useful in the development of IBD treatment strategies.

MATERIAL AND METHODS Human material

Colon specimens from healthy and patients with IBD were embedded in paraffin and subsequently stained as described below. Data were anonymised. Patient's clinical information is shown in online supplemental table 4.

Mice

Mice carrying loxP-flanked *Psen1* alleles (*Psen1*^{fl/fl}) (Jackson strain #004825) were crossed with villin-cre mice (Jackson strain #004586) to generate mice with a homozygous conditional deletion of *Psen1* in IECs (*Psen1*^{ΔIEC}). The generation and maintenance of *Psen2*^{-/-} has been described previously (Jackson strain #005617). *Psen1*^{fl/fl} and *Psen2*^{+/+} mice were used as littermate controls, respectively. *Psen1*^{ΔIEC} were further crossed with *Psen2*^{-/-} to generate *Psen2*^{+/-} *Psen1*^{ΔIEC} mice. In addition, *Psen1*^{fl/fl} mice were crossed with *Psen2*^{-/-} and villin-creERT2 (Jackson strain #020282) to generate inducible Psen1/2 double knockout mice (*Psen2*^{-/-} *Psen1*^{fl/fl} mice as littermate controls in all experiments. All mice were routinely screened for pathogens according to FELASA guidelines. Animals were sex-matched and age-matched and littermates were used for each experiment in accordance with German law and with the approval of the local animal care committee. The sample size was calculated using G*Power.⁶⁴

Histology scoring

Pathological scoring was performed on H&E-stained tissue sections. The scoring included evaluating the integrity of the intestinal epithelium (0: intact; 1–3: mild, moderate or severe destruction, respectively) and mucosal inflammation (0: no inflammatory infiltration; 1–3: rare, moderate or massive inflammatory infiltration, respectively).

Endoscopic evaluation

Endoscopic evaluation was performed using high-resolution mouse video endoscopy as previously described.³⁴ The murine endoscopic index of colitis severity score (MEICS) evaluates colonic wall thickening, changes in normal vascular pattern, presence of fibrin, mucosal granularity, and stool consistency. Scores ranged from 0 to 3 for each parameter, and the sum of all the parameters is displayed as MEICS. The colon was scored for visible damage by a blinded observer.

Tamoxifen administration

Inducible Psen1/2 double knockout mice were injected intraperitoneally with 75 mg/kg tamoxifen (Sigma) diluted 1:1 in sunflower oil on five consecutive days.

FITC-dextran administration

FITC-dextran administration was performed after the mice were fasted for 4 hours. 0.4 mg/kg body weight of FITC-dextran (4000 g/mol average molecular weight; Sigma) was administered by oral gavage. After 4 hours, the mice were sacrificed, and serum FITC-dextran levels were measured using a fluorimeter.

Antibiotic treatment

For microbiome depletion, mice were treated with 1 g/L metronidazole (Braun), 1 g/L ampicillin (ratiopharm), 0.5 g/L vancomycin (Dr. Friedrich Eberth Arzneimittel) and 1 g/L neomycin (Caelo) in drinking water. 8 g/L sweetener (Splenda) was added to mask the bitter taste of the antibiotics.

In vivo HCS LipidTOX assay

Mice were fasted for 4 hours and then given $200 \ \mu L$ of corn oil (Sigma) by oral gavage. After 4 hours, the mice were sacrificed. Cryosections were fixed in 4% PFA for 30 min and lipid droplets were detected with HCS LipidTOX Green (1:200; Thermo Fisher). Nuclear staining was performed with Hoechst

(1:500; Invitrogen) and slides were mounted with fluorescence mounting medium (Dako).

Bacterial translocation studies

Bacterial translocation was assessed by plating MLN and liver tissue lysates diluted in sterile PBS on MacConkey (Roth) and blood agar (Merck) plates to determine CFU under aerobic and anaerobic conditions after 24 hours of incubation at 37°C. CFUs were normalised to the organ weight. The anaerobic condition was established using Anaerocult A (Merck).

Serum glucose measurement

Serum glucose was measured using a blood glucose metre (Ascensia Diabetes Care Deutschland).

Oral glucose tolerance test

For the oral glucose tolerance test, mice were fasted overnight and baseline glucose levels were measured using a blood glucose metre (Ascensia Diabetes Care Deutschland). Mice then received 2.5 g glucose/kg body weight by oral gavage and blood glucose levels were measured 10, 15, 20, 30, 45, 60 and 90 min later using a blood glucose metre.

Murine organoid culture

Small intestinal organoids were established as previously described.⁶⁵ Briefly, mice were sacrificed, and the intestine was cut into small pieces, incubated in 2 mM EDTA for 30 min, and the released epithelial cells were filtered through a 70 μ m filter. Epithelial cells were then plated in Cultrex (Bio-Techne) and cultured in culture medium in 5% CO₂ at 37°C and passaged twice a week. Psen1 was deleted in small intestinal organoids by incubation with 50 ng/mL tamoxifen for 5 days, organoids were split on day 3 and fresh tamoxifen was added.

Organoid permeability assay

Organoids were incubated with 1 mM Lucifer yellow (Sigma) for 1 hour at 37° C. 2 mM EGTA incubated for 10–15 min at 37° C was used as a positive control. Images were captured using a confocal microscope and images were analysed using ImageJ as previously described.³⁵

Rhodamine 123 uptake assay

Organoids were incubated with 100μ M Rhodamine 123 (Sigma) for 5 min at 37°C, washed and incubated in basal organoid culture medium for another 40 min at 37°C. Images were captured by confocal microscopy and analysed using ImageJ.

Histology and immunofluorescence staining

For evaluation of histomorphology, tissue was formalin-fixed and embedded in paraffin. Sections were cut from paraffin blocks and histochemically stained with H&E. Immunofluorescence staining was performed on formalin-fixed paraffin-embedded sections or cryosections. Cryosections and organoid cultures were fixed with 4% PFA for 30 min at room temperature and then washed with TBS-T for 5 min. For paraffin sections, sections were incubated for 1 hour at 65°C, paraffin was removed with ROTIHistol, and sections were rehydrated in a decreasing ethanol row. Antigen retrieval was performed using Tris/ETDA at 450 W for 20 min. After cooling, the slides were incubated with 0.1% Triton X-100 for 10 min, washed in TBS-T, and the endogenous biotin-binding sites were blocked using the Avidin/Botin Blocking Kit (Vector Laboratories). The following primary antibodies were incubated overnight at 4°C: Psen1 (1:300; #5643; Cell Signalling), E-Cadherin:FITC (1:200; #612130; BD Biosciences), Olfm4 (1:300; #39141; Cell Signalling), Lysozyme (1:300; A0099 ; Dako), Muc2 (1:500; NBP1-31231; Novus). Chromogranin A (1:300; NB120-15160; Novus), Dcamkl1 (1:200; ab31704; abcam), Ki67 (1:200; 14-5698-82; eBioscience), MPO (1:200; #ab9535; abcam), F4/80 (1:200; #70076; Cell Signalling), Cl. Caspase 3 (1:300; #9662; Cell Signalling), E-Cadherin (1:300; #3195; Cell Signalling), Occludin (1:300; #91131; Cell Signalling), and ULEX (1:50; FL-1061-2; Vector Laboratories). The next day, the slides were washed three times in TBS-T and incubated with the appropriate secondary antibodies (Alexa Fluor555 anti-rabbit: 1:200, BioLegend, # 406 412 for Olfm4, Lysozyme, Muc2, ChgA, Dcamkl1 and Ki67; Biotin anti-rabbit IgG: 1:400, Jackson ImmunoResearch, # 111-065-144 combined with Streptavidin DyLight500: 1:400, Thermo Fisher, # 84 542 for Psen1, MPO, F4/80, Cl. Casp. 3, E-Cadherin and occludin) for 2 hours at room temperature. Slides were washed in PBS, nuclear staining was performed with Hoechst (1:500; Invitrogen), and slides were mounted with fluorescence mounting medium (Dako).

Immunohistochemistry

For immunohistochemical staining, small intestinal organoids were pelleted, embedded in Histogel (Thermo Fisher), and then embedded in paraffin. Paraffin sections were incubated for 1 hour at 65°C, paraffin was removed with ROTIHistol, and sections were and rehydrated in a decreasing ethanol row. Antigen retrieval was performed using Tris/EDTA at 450 W for 20 min in a microwave. Endogenous peroxidase blocking was performed using 3% hydrogen peroxide. Endogenous biotinbinding sites were blocked using the Avidin/Biotin Blocking Kit (Vector Laboratories). The primary Psen1 antibody (1:300; #5643; Cell Signalling) was incubated overnight at 4°C. The next day, slides were washed in PBS and the secondary antibody (SignalStainBoost IHC detection reagent (HRP, rabbit)) was incubated for 30 min at room temperature, followed by signal detection (SignalStainDAB Chromogene Substrate; SignalStainDAB Diluent) for 8 min at room temperature. Counterstaining was performed with haematoxylin for 5 s and washed with tap water. Sections were dehydrated through an increasing ethanol series and mounted with Entellan (Merck).

IAP staining

Alkaline phosphatase staining was performed on cryosections fixed in ice-cold acetone for 5 min. Sections were air dried for 5 min and incubated for 30 min at 37°C alkaline phosphatase working solution: 0.2 M TRIS, 0.1M HCl, 0.01 g Naphtol AS-MX Phosphate (Sigma) in 500 μ L N,N Dimethylforma-mide (Merck), 0.036 g Fast Red Violet LB Salt (Sigma), total pH=8.74.

Periodic acid–Schiff staining

PAS staining was performed on formalin-fixed, paraffinembedded tissue sections. Paraffin sections were incubated for 1 hour at 65°C, paraffin was removed with ROTIHistol, and sections were rehydrated in a decreasing ethanol series. Samples were then oxidised in 0.5% periodic acid (Carl Roth) for 5 min, rinsed in distilled water, and incubated in Schiff's reagent for 15 min (Carl Roth). After washing in tap water, the slides were counterstained with haematoxylin for 1 min. After further washing in tap water, the sections were dehydrated and mounted with Entellan (Merck).

In situ hybridisation

For bacterial detection, the Eub338 probe was stained on formalin-fixed, paraffin-embedded tissue sections. Paraffin sections were incubated for 1 hour at 65°C, paraffin was removed with ROTIHistol, and sections were rehydrated in a decreasing ethanol series. Slides were then incubated in hybridisation buffer for 20 min at room temperature, followed by incubation with the Eub338 probe (3'-GCT GCC TCC CGT AGG AGT-5' Cy3; biomers) diluted 1:10 in hybridisation buffer for 90 min at 46°C in a hybridisation oven as previously described.⁶⁶ Counterstaining was performed with Hoechst (1:500; Invitrogen), and slides were mounted with fluorescence mounting medium (Dako).

RNAScope

RNAScope (ACDBio) staining was performed on formalin-fixed paraffin-embedded sections according to the manufacturer's instructions. Briefly, sections were incubated for 1 hour at 65°C, paraffin was removed with ROTIHistol, and sections were incubated in 100% ethanol. Tissues were air dried, pretreated with hydrogen peroxide (ACDBio), and then heated at 100°C for 15 min in Target Retrieval Buffer (ACDBio). This was followed by incubation at 40°C for 30 min in a HybEZ Oven (ACDBio) with Protease Plus Reagent (ACDBio), after which the slides were washed and a murine Lgr5 probe (ACDBio) was applied and incubated at 40°C for 2 hours. Signal amplification and detection were performed using RNAScope 2.5 HD Red Detection Reagent (ACDBio) according to the manufacturer's instructions. Nuclei were detected with Hoechst (1:500; Invitrogen) and slides were mounted with fluorescence mounting medium (Dako).

Microscopy

Images of staining were obtained using a NanoZoomer 2.0 (Hamamatsu), a DMI 4000 B (Leica) or a DMI 6000 CS (Leica) microscope.

Western blotting

Tissue and organoid protein extracts were isolated using Tissue or Mammalian Protein Extraction Reagent (Thermo Fisher). Protein concentration was determined by the Bradford assay. Protein samples were diluted in NuPage LDS Sample Buffer (Thermo Fisher), denatured at 95°C for 5 min, and then separated on Mini-PROTEAN Precast Gels (Bio-Rad) at 200 V for approximately 30 min. Proteins were blotted onto PVDF membranes, blocked in 5% milk and incubated with the following antibodies at 4°C overnight: Psen1 (#5643; Cell Signalling), Psen2 (#9979; Cell Signalling), Na-K-ATPase (#3010; Cell Signalling), Lamin A/C (615802; Biolegend), GAPDH (#2118; Cell Signalling), β -actin HRP (ab49900; abcam), Lysozyme (A0099; Dako), Occludin (#91131; Cell Signalling), NICD (#4147; Cell Signalling). The next day, the membrane was incubated with the appropriate HRP-conjugated secondary antibody (Cell Signalling) and developed on an Amersham Imager 800 (GE Healthcare Life Sciences) using Western Lightning Plus ECL Substrate (Perkin Elmer).

TNF- ELISA

For quantification of TNF- α in serum samples, the Mouse TNF ELISA Kit (BD Biosciences) was used according to the manufacturer's instructions.

RNA isolation, cDNA transcription and qPCR

The NucleoSpin RNA Kit (Macherey Nagel) was used to isolate total RNA from tissues or organoids. Subsequently, cDNA synthesis was performed using the Script cDNA Synthesis Kit (Jena Bioscience). For gene expression measurements, SensiFAST SYBR No-ROX (BioCat) and QuantiTect primers (QIAGEN) were used in a real-time PCR cycler (Bio-Rad). Normalisation was performed with *Gapdh* or *Hprt* as described in the corresponding figure legend.

Bulk RNA sequencing and analysis

For bulk RNA sequencing, QC quality control was performed and samples were sequenced on an Illumina NovaSeq platform (Novogene, Cambridge, UK). STAR (2.7.0d) and feature counts (V.1.6.4) were used for mapping to the reference genome (mm10) and quantification, respectively. Differential expression of the groups of samples was performed using DESeq2 (1.24.0). Enrichment, clustering and other analyses were performed using in-house bioinformatic tools and Ingenuity Pathway Analysis (Qiagen), the Database for Annotation, Visualisation and Integrated Discovery analysis tool. Only genes with adjusted p values less than 0.05 and $\log_2(fold change)$ less or greater than 1 were considered. Bulk RNA sequencing data of Psen1 and Psen2 deficient mice have been submitted to ArrayExpress.

Availability and analysis of transcriptomic datasets

Transcriptomic data of the IBDome cohort will be made available to the scientific community on acceptance of the manuscript (https://doi.org/10.5281/zenodo.10868289). The publicly available datasets used in this study are published under the accession codes: E-MTAB-9850²⁶ and GSE6731.²⁷ The corresponding raw expression values were log-transformed. Microsoft Excel 2019 was used for data sorting and comparison. Single-cell datasets were obtained from (http://scibd.cn), which included four different datasets: Huang *et al*²⁹; Parikh *et al*³⁰; Kong *et al*²⁸ and Friedrich *et al*.³¹ The relative expression of *PSEN1* and *PSEN2* in epithelial, immune and mesenchymal cell subsets without downsampling the number of cells per subset was evaluated, sorted by disease status in the cecum, appendix, colon and rectum tissues. GraphPad Prism V.9 was used for graphical illustrations.

Statistical analysis

GraphPad Prism V.9 software was used for statistical analysis and graphing. As described in the corresponding figure legends, significance analysis was performed using Mann-Whitney test, Student's t-test or one-way analysis of variance. P values below 0.05 (*), 0.01 (**) and 0.001 (***) were considered significant. All data are shown as mean±SD. Three independent replicates were used in most experiments.

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Data availability statement Data are available in a public, open access repository. Data are available on reasonable request. Transcriptomic data of the IBDome cohort will be made available to the scientific community on acceptance of the manuscript. Bulk RNA sequencing data of Psen1 and Psen2 deficient mice have been submitted to ArrayExpress. The publicly available datasets used in this study are published under the accession codes: E-MTAB-9850 and GSE6731.

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