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

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Short title: Presenilins in intestinal homeostasis

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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 homeostasis.

Design: Human inflammatory bowel disease (IBD) and control samples were analyzed 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 homeostasis.

Results: Psen1 expression was regulated in experimental gut inflammation and in IBD patients. 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 signaling and dysregulated epithelial differentiation.

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

WHAT IS ALREADY KNOWN ON THIS TOPIC

- ➢ An impaired epithelial barrier is observed in patients with IBD and disruption of the intestinal epithelial monolayer induces gut inflammation in mice.
- ➢ Many IBD patients 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 homeostasis.
- ➢ Psen1 expression is impaired in the inflamed intestine of IBD patients and mice with experimentally induced inflammation.
- ➢ Inducible deletion of both presenilins in 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 homeostasis, we suggest the presenilins as promising future targets in IBD pathogenesis for the improvement of barrier integrity and malnutrition.

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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, including the Notch receptor.[1–3] Apart from their central role in the gamma-secretase complex, presenilins have also been implicated in autophagy, apoptosis, protein trafficking, and calcium homeostasis, which appear to be independent of gamma-secretase activity.[4] Presenilins are best known as critical players in the complex pathogenesis of Alzheimer's disease (AD). Mutations in presenilin genes, particularly *PSEN1* and *PSEN2*, are known to be responsible for a subset of early-onset familial AD cases.[5] Although PSEN1 and PSEN2 share structural similarities and exhibit some functional overlap, they also have distinct expression patterns and different substrate specificities.[6–8] Genetic deletion of Psen1 causes severe morphological defects in the developing brain and hemorrhages, leading to the death of *Psen1$^{-/-}$* mice shortly after birth.[9] 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, e.g. in the gut. Interestingly, recent epidemiologic studies and experimental mouse models have uncovered a link between AD and intestinal inflammation.[10–13] 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 a highly effective barrier against potentially harmful microbes, toxins, and antigens, present in the intestinal lumen. A tight control of IEC differentiation, proliferation, and cell death are key to ensuring gut tissue homeostasis. Perturbations of epithelial homeostasis can lead to GI pathologies, including the inflammatory bowel diseases (IBD) Crohn's disease (CD) and ulcerative colitis (UC). Several pathways including Wnt- and Notch-signaling closely work together to maintain tissue homeostasis and thus prevent IBD.[14] In addition to abdominal pain and diarrhea, 70-80% of hospitalized IBD patients experience weight loss [15, 16] and 20-85% suffer from malnutrition.[17, 18] To date, the underlying mechanisms leading to IBD

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103   and especially malnutrition are still incompletely understood. However, multiple factors have

104   been identified that contribute to disease pathology, including genetic and environmental

105   factors, such as alterations in the microbiome that induce the recruitment of mucosal immune

106   cells to the inflamed areas, ultimately leading to barrier defects.[19]

107   Given the pivotal role of presenilins in both mediating the processing of the Notch receptor [2,

108   20–22] and their interaction with the Wnt signaling pathway [23–26], we hypothesized that

109   presenilins might be crucially involved in intestinal homeostasis and inflammation.

110   In this study, we identified the presenilins Psen1 and Psen2 as important molecules in

111   maintaining intestinal homeostasis. While single deletions of *Psen1* or *Psen2* were fully

112   compensated by each other, the generation of inducible Psen1/2 double knockout mice

113   resulted in spontaneous development of intestinal inflammation with barrier breakdown and

114   subsequent bacterial translocation. Lack of Psen1 and Psen2 severely impaired IEC

115   differentiation with upregulation of secretory cells including goblet, Paneth, and

116   enteroendocrine cells, at the expense of absorptive enterocytes. Deletion of *Psen1* and *Psen2*

117   caused severe impairment of nutrient uptake ultimately leading to malnutrition and death of

118   these mice. While antibiotic treatment ameliorated gut inflammation, the weight loss of mice

119   was not reversed. Our study demonstrates that presenilin expression is pivotal for intestinal

120   homeostasis as it controls IEC differentiation and nutrient absorption, both of which contribute

121   to barrier maintenance.

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 unrecognized pathways that might be involved in intestinal inflammation. To this end, we initially performed bulk RNA sequencing comparing colonic tissue samples from mice subjected to DSS-induced experimental colitis and healthy control animals. While several IBD-related pathways, such as metabolism- and cytokine-related pathways were found by KEGG pathway analysis, our colitis data surprisingly also revealed an enrichment of pathways attributed to neurodegeneration such as those involved in Alzheimer's disease (**Fig. 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 (**Suppl. Fig. 1**).[27] Thus, we next investigated *Psen1* expression levels during the course of DSS-induced colitis and recovery.[28] Strikingly, *Psen1* gene expression was significantly downregulated during the development of colitis, whereas expression levels returned to baseline levels during the recovery phase (**Fig. 1B**). Downregulation of *Psen1* expression during intestinal inflammation was also supported by assessing Psen1 protein levels in inflamed colonic tissue from DSS-treated mice (**Fig. 1C**). To compare the Psen1 expression pattern between steady state conditions and intestinal inflammation, immunofluorescence staining of Psen1 was performed in healthy and inflamed murine gut tissue. We found that Psen1 expression at the steady-state is mainly restricted to IECs, with low but detectable expression in the lamina propria. In contrast, Psen1 levels were reduced in the epithelium of DSS-treated mice during intestinal inflammation (**Fig. 1D**). To elucidate whether the observed deregulation of Psen1 can be translated to human intestinal inflammation, *PSEN1* expression in gut samples of healthy individuals was compared to those of UC and CD patients using a publicly available transcriptomic dataset.[29] Consistent with the data from our mouse model, *PSEN1* expression was significantly decreased in both IBD entities (**Fig. 1E**). Moreover, among our in-house

cohort of IBD patients, *PSEN1* was also significantly downregulated in the colon of both UC and CD patients (**Fig. 1F**). Of note, *PSEN1* expression levels also correlated with disease severity as shown in two different IBD cohorts (**Fig. 1G and H**). In contrast, the expression levels of *PSEN2* mRNA remained unchanged in colonic samples of IBD patients examined in our study (data not shown). To further confirm the observed downregulation of PSEN1 in inflamed human tissues at the protein level, immunofluorescence staining for PSEN1 in human IBD tissue samples was conducted. In line with the above data, this analysis showed a downregulation of PSEN1 in UC and CD patients, when compared to healthy individuals (**Fig. 1I**). 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 homeostasis

To study potential functions of Psen1 in the mouse intestine, *Psen1*$^{fl/fl}$ mice [30] were crossed with villin-cre mice to generate mice with IEC-specific homozygous deletion of *Psen1* (*Psen1*$^{\Delta IEC}$) (**Suppl. Fig. 2A**). Successful IEC-specific deletion of Psen1 was proven at the RNA and protein level in mouse gut tissue and in organoids generated from gut samples of these mice (**Suppl. Fig. 2B – G**). Notably, homozygous *Psen1*$^{\Delta IEC}$ mice did not develop an overt phenotype and no histological changes were observed in the intestinal architecture when compared to their littermate controls (*Psen1*$^{fl/fl}$ mice) (**Suppl. Fig. 3A**). In addition, mRNA and protein analysis of IEC subtypes and differentiation markers revealed that IEC differentiation from ISC into absorptive enterocytes or secretory cell types such as goblet, Paneth, enteroendocrine or tuft cells, did not differ between the two groups (**Suppl. Fig. 3B-D**). Moreover, no differences were observed in the levels of several inflammation-associated factors (**Suppl. Fig. 3E**). This was further supported by bulk RNA sequencing of ileum and colon tissue from *Psen1*$^{\Delta IEC}$ compared to control littermates, which confirmed that the lack of Psen1 expression had no major impact on the gut transcriptome (**Suppl. Fig. 3F**). Interestingly, however, Psen2 protein levels were significantly upregulated in the ileum and colon of

178   *Psen1*$^{\Delta IEC}$ mice (**Suppl. Fig. 3G**), suggesting a potential compensatory mechanism between

179   Psen1 and Psen2 in IECs.

Inducible Psen1/2 double knockout mice spontaneously develop intestinal

inflammation

182   Since we hypothesized that intestinal epithelial Psen2 can compensate for the loss of Psen1

183   in IECs, we crossed *Psen1*$^{\Delta IEC}$ with *Psen2*$^{-/-}$ mice to generate Psen1/2 double knockout mice

184   (**Suppl. Fig. 4A**). Noteworthy, general *Psen2*$^{-/-}$ mice were utilized in this approach, because,

185   to the best of our knowledge, conditional *Psen2* knockout mice have not yet been established.

186   Remarkably, we did not obtain any Psen1/2 double knockout mice, while the other expected

187   genotypes were born at increased ratios (**Suppl. Fig. 4B**). Of note, one allele of Psen2 was

188   sufficient to maintain intestinal homeostasis, as Psen1 knockout mice on a heterozygous

189   Psen2 background (*Psen2*$^{+/-}$ *Psen1*$^{\Delta IEC}$) were born alive and showed no detectable changes in

190   gut architecture or intestinal epithelial differentiation (**Suppl. Fig. 4B – G**). Moreover, a detailed

191   analysis of the small and large bowel of *Psen2*$^{-/-}$ mice [31] (**Suppl. Fig. 5A**) revealed no

192   detectable changes under steady-state conditions (**Suppl. Fig. 5B – G**). Together our data

193   demonstrated that neither the absence of Psen1 nor that of Psen2 alone affected gut tissue

194   homeostasis, while deletion of both presenilins caused early embryonic lethality.

195   To overcome the embryonic lethality of Psen1/2 double knockout mice and to study the

196   functions of both genes in the gut, we established a new mouse line with an inducible *Psen1*

197   knockout allele on a background of a *Psen2* germline deletion (*Psen2*$^{-/-}$ *Psen1*$^{i\Delta IEC}$; iDKO)

198   (**Suppl. Fig. 6A**). Successful deletion of *Psen1* on the *Psen2*$^{-/-}$ background was achieved by

199   injection of tamoxifen (**Fig. 2A**) and greatly reduced expression levels of Psen1 in IECs and

200   complete absence of Psen2 in whole mouse ileal and colonic tissue samples as well as in

201   isolated organoids was confirmed at the mRNA and protein level (**Suppl. Fig. 6B – F**). In sharp

202   contrast to *Psen1* and *Psen2* single knockout mice, iDKO mice showed rapid and significant

203   weight loss starting on day five to six when compared to control *Psen2*$^{-/-}$ *Psen1*$^{fl/fl}$ littermate

204   mice, with termination criteria reached on day eight after the first tamoxifen injection (**Fig. 2B**).

In addition, endoscopy of the mice demonstrated the development of spontaneous colitis in iDKO mice as assessed by the murine endoscopic index of colitis severity (MEICS) score (**Fig. 2C**).[32] Moreover, colon length measurements revealed a significant shortening of the colon in iDKO mice compared to their littermate controls (**Fig. 2D**). Furthermore, histological examination of the small and large intestine of the mice revealed pronounced histological changes, including signs of inflammation and cell death (yellow arrows). In the ileum and proximal colon, we observed structural changes in the epithelium accompanied by abundant mucus production (black arrows) (**Fig. 2E**). In addition, Psen1/2 double deficient epithelial organoids (**Suppl. Fig. 7A**) completely lacked the usual budded morphology and had a significantly reduced size (**Suppl. Fig. 7B**), further supporting aberrant IEC-intrinsic mechanisms in the absence of Psen1/2. Furthermore, assessment of the expression levels of various pro-inflammatory factors, including *S100a9*, *Il1b*, and *Tnf*, revealed a striking upregulation of these markers in the ileum and colon of iDKO mice compared to littermate controls (**Fig. 2F**). Moreover, assessment of immune cell infiltration revealed significantly increased numbers of neutrophils as determined by myeloperoxidase (MPO) staining in the ileum and colon of iDKO mice, as well as significantly increased numbers of macrophages as determined by F4/80 staining in the ileum of iDKO mice (**Fig. 2G**). In conclusion, our data demonstrate that the presenilins are key factors for intestinal homeostasis, as dual deletion of Psen1 and Psen2 leads to the development of spontaneous intestinal inflammation *in vivo*.

Presenilins are key to intestinal barrier function

Intestinal barrier breakdown is considered as the key factor underlying the immunopathogenesis of IBD.[33] To investigate whether Psen deficiency affects intestinal barrier functions, iDKO and control (*Psen2*$^{-/-}$ *Psen1*$^{fl/fl}$) 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 compared to their littermate controls, suggestive of substantial barrier defects (**Fig. 3A**). To investigate whether Psen deficiency-induced epithelial barrier disruption is independent of the cellular or microbial microenvironment, intestinal epithelial organoids were incubated with lucifer yellow to track its

233   passage into the organoid lumen.[34] An increase in dye intensity in the lumen of organoids

234   generated from iDKO but not control mice was observed, indicating that the observed barrier

235   breakdown due to presenilin loss is IEC intrinsic and independent of the intestinal environment

236   (**Fig. 3B**). Adherens junction and tight junction proteins, such as E-Cadherin and occludin

237   respectively, regulate paracellular permeability and are key guardians of the epithelial

238   barrier.[35] Several studies have already shown a downregulation of these components in both

239   UC and CD patients.[36, 37] Interestingly, immunofluorescence staining in ileal and colonic

240   tissue samples from tamoxifen-injected control and iDKO mice showed a strong reduction of

241   E-Cadherin in the membranous portion of IECs in iDKO mice (**Fig. 3C**). Moreover, Western

242   blot analysis revealed a loss of full-length occludin in the ileum of the mice, along with reduced

243   levels of cleaved occludin in the ileum and colon of iDKO mice (**Fig. 3D**). When sacrificing

244   iDKO mice at different timepoints after the first dose of tamoxifen, we determined that full-

245   length occludin was completely abolished already on day five, while the cleaved form of

246   occludin was reduced on day seven after the first tamoxifen injection (**Fig. 3E**). These

247   observations were supported by small intestinal organoid cultures (**Suppl. Fig. 7C**), suggesting

248   that epithelial-intrinsic mechanisms lead to reduced occludin expression upon presenilin

249   deletion. To functionally analyze the consequences of intestinal barrier dysfunction on

250   microbial dysbiosis in Psen iDKO mice, gut tissue sections were stained with a fluorescent

251   FISH probe detecting all eubacteria. Strikingly, while bacteria in control animals were well

252   separated from the epithelial lining, bacteria in iDKO mice infiltrated the crypt area down to the

253   crypt bottom and even into the normally tight IEC monolayer (**Fig. 3F**), suggesting that

254   presenilins are key to maintain an efficient barrier and to prevent microbial colonization of the

255   crypt regions. To examine the systemic impact of the previously observed intestinal barrier

256   breakdown in iDKO mice, tissue lysates of mesenteric lymph nodes (MLN) and livers from the

257   animals were plated on agar plates under aerobic and anaerobic conditions. While no colony-

258   forming units (CFUs) were detected in the tissues of control mice, high numbers of CFUs were

259   observed in the MLN and liver of iDKO mice (**Fig. 3G**), indicating that a Psen deficient gut

260   epithelium loses its ability to prevent a systemic spread of bacteria. In support of this,

examination of serum TNF-α levels indicated a systemic inflammatory response in iDKO mice, as evidenced by significantly elevated TNF-α levels in mice deficient in both presenilins (**Fig. 3H**). To further validate these findings, levels of pro-inflammatory markers were assessed in spleen tissue, revealing a marked increase of *Tnf* and *Nos2* (**Fig. 3I**). Finally, measurements of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels indicated liver damage (**Fig. 3J**). In conclusion, our analysis clearly demonstrated a key role of presenilins in barrier integrity, where deletion of both presenilins leads to alteration of E-Cadherin distribution, loss of the tight junction protein occludin, subsequent 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 severe wasting disease and early death of mice lacking Psen1 and Psen2 in the epithelium, mice were pre-treated with an established antibiotic cocktail to deplete the microbiota (**Fig. 4A**), which was confirmed by plating stool lysates before and after antibiotic treatment and assessing the number of CFUs (**Fig. 4B**). Unexpectedly, daily weight measurements revealed that iDKO mice treated with antibiotics displayed similar wasting disease as iDKO mice subjected to normal water (**Fig. 4C**). In contrast, endoscopic scoring of colitis severity revealed a significant reduction of inflammation in antibiotic-treated iDKO mice compared to iDKO mice receiving water only (**Fig. 4D**). Furthermore, histological examination of ileal and colonic tissue sections further confirmed the amelioration of inflammation in the colon (**Fig. 4E and F**). In addition, antibiotic treatment significantly reduced the levels of pro-inflammatory factors, including *Il6*, *Tnf*, *Il1b*, and *S100a9* in the ileum and colon of iDKO compared to water-drinking iDKO mice (**Fig. 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 that underlies the rapid and severe unrecoverable weight loss.

Psen1 and Psen2 deficiency disrupts Notch signaling, 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 homeostasis, we performed bulk RNA sequencing with ileal tissue lysates of iDKO mice and control (*Psen2*^{-/-} *Psen1*^{fl/fl}) littermates. Interestingly, this analysis revealed broad Psen-dependent transcriptional changes with 4129 differentially expressed genes between the two groups (**Fig. 5A**). Using gene ontology analysis, we observed an upregulation of pathways involved in inflammatory responses, cell migration and mucin type O-glycan biosynthesis in iDKO mice. In contrast, numerous pathways related to transport and absorption of various nutrient components, including lipids, ions, fat, sodium and minerals, were significantly reduced in the ileum of iDKO mice (**Fig. 5B, Suppl. Tab. 1 and 2**). Moreover, metabolic signatures, including lipid and fatty acid metabolic pathways, TCA cycle, gluconeogenesis, and glycolysis, were also reduced in the intestine upon induced presenilin deletion. Ingenuity® Pathway Analysis further revealed increased signatures of cell migration, epithelial cell differentiation and tumorigenic pathways, as well as decreased uptake, transport, oxidation, synthesis and metabolism of lipids and fatty acids (**Suppl. Fig. 8A and Suppl. Tab. 3**). By analyzing epithelial cell 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 (**Suppl. Fig. 8B**). To thoroughly analyze the different IEC types, several immunofluorescence stainings for unique IEC type marker proteins were performed. Remarkably, immunofluorescence staining for different stem cell markers showed a significant loss of Olfm4^{+} and Lgr5^{+} stem cell populations in the ileum and colon of iDKO mice. Furthermore, using Ki67, we detected significantly reduced numbers of proliferating cells in ileal and colonic tissue sections from iDKO mice compared to control littermates (**Suppl. Fig. 8C**). In addition, this analysis further confirmed a significant upregulation of the number of

secretory cells, including Paneth, goblet, and enteroendocrine cells (**Suppl. Fig. 8D**). Similar results were obtained *in vitro* using small intestinal organoid cultures generated from these mice, showing impaired IEC differentiation with increased numbers of secretory cells and decreased numbers of stem cells along with reduced proliferation (**Suppl. Fig. 7D and E**). In contrast, our RNA sequencing approach revealed a striking downregulation of genes associated with absorptive enterocytes (**Fig. 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 (**Fig. 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 (**Fig. 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 from control and iDKO mice were treated with Rhodamine 123, a commonly used tracer dye to investigate transport rates. Interestingly, while small intestinal organoids from control mice were able to take up Rhodamine 123 into the lumen, small intestinal organoids generated from iDKO mice almost completely failed to take up Rhodamine 123 (**Fig. 5F**). As further evidence of impaired intestinal glucose absorption *in vivo*, an oral glucose tolerance test was performed in control and iDKO mice. Remarkably, this test revealed severely impaired intestinal glucose uptake rates in iDKO mice compared to control mice (**Fig. 5G**). Notably, antibiotic-treated iDKO mice still exhibited significantly reduced serum glucose levels (**Suppl. 9A**) and impaired intestinal glucose uptake rates in an oral glucose tolerance test (**Suppl. Fig. 9B**), suggesting that the observed changes in systemic energy metabolism are not directly related to intestinal inflammation. Based on the observation by Tang *et al.* that the average adipocyte area in mesenteric white adipose tissue is almost completely reduced in fasted mice [38], we analyzed the mesenteric white adipose tissue of iDKO mice and compared it with littermate controls. The average adipose area was drastically reduced in inducible Psen1/2 double knockout mice (**Fig. 5H**). We reasoned that this was likely due to reduced lipid uptake given that the number of absorptive enterocytes was reduced. To

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343   test this hypothesis in a functional approach *in vivo*, fasted mice received corn oil as a lipid

344   source by oral gavage. Application of corn oil has been shown to result in lipid droplet formation

345   due to uptake of lipids by absorptive enterocytes, which can be detected by

346   immunofluorescence staining.[39] Strikingly, the lipid droplet accumulation in the IECs of the

347   small intestine was completely blocked in iDKO mice compared to littermate controls (**Fig. 5I**).

348   Finally, to evaluate the state of liver energy stores, we employed Periodic Acid-Schiff (PAS)

349   staining to detect glycogen levels in liver tissue of the mice. Remarkably, our analysis unveiled

350   a substantial decrease in glycogen content within the liver of iDKO mice when compared to

351   control mice (**Fig. 5J**). These analyses ultimately highlight the severity of the presenilin-

352   dependent loss of absorptive enterocytes, suggesting that the death of the inducible Psen1/2

353   double knockout mice may be due to malnutrition.

354   Since the γ-secretase multi-protein complex is critically involved in Notch signaling [20, 22, 40],

355   we hypothesized that non-functional Notch signaling might be responsible for the severely

356   impaired IEC differentiation in the inducible Psen1/2 double knockout mice, leading to reduced

357   barrier permeability on the one hand and malnutrition on the other hand. To test whether Notch

358   signaling is impaired in the intestine upon double presenilin deficiency, we performed Western

359   blot analysis using the Notch intracellular domain (NICD) as a marker of functional Notch

360   signaling. Remarkably, whereas Notch was still cleaved and NICD was released in Psen1 and

361   Psen2 single knockout mice, NICD levels were completely abolished in the colon, ileum, and

362   in small intestinal organoids generated from iDKO mice (**Fig. 5K and Suppl. Fig. 7F**). This

363   could explain the observed lack of phenotype in heterozygous Psen1/2 double knockout mice,

364   showing that one allele of Psen2 is sufficient to maintain functional Notch signaling (**Suppl.**

365   **Fig. 4H**).

366   Overall, our study identified the presenilins Psen1 and Psen2 as previously unknown players

367   in intestinal homeostasis and inflammation, where they act as key molecules in maintaining

368   balanced IEC differentiation and barrier function integrity to prevent malnutrition and intestinal

369   inflammation (**Suppl. Fig. 9C and Fig. 5L**). Thus, supporting presenilin enzymatic activity in

370   the gut epithelium or downstream signaling 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 homeostasis 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 several loss-of-function mutations in presenilin genes can cause Alzheimer's disease. Although many different studies have investigated the role of Notch signaling and general γ-secretase activity in intestinal homeostasis and inflammation, not much is known about how the presenilins are regulated during intestinal health and disease and whether they are critically involved in these processes. Interestingly, our group has already established a link between *Psen1* expression in intestinal epithelial cells and colorectal tumor development.[41] Remarkably, our present study identifies both presenilins as key molecules in intestinal homeostasis and inflammation. Strikingly, 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 signaling, ultimately resulting in malnutrition and death of the inducible Psen1/2 double knockout mice.

To date, the role of Notch signaling in intestinal inflammation is controversial. While some animal studies have shown pro-inflammatory effects of Notch signaling, as for example by using different γ-secretase inhibitors that reduced colitis severity in DSS- or TNBS-treated mice [42, 43], other studies have demonstrated anti-inflammatory roles of Notch signaling during colitis. For example, IEC-specific deletion of *Rbpj*, the major transcriptional effector of Notch signaling, in mice resulted in spontaneous development of chronic colitis with accumulation of Th17 cells [44], and deletion of Notch-1 led to more severe colitis development in DSS-treated mice.[45] Surprisingly, various studies reported a significant increase in Notch signaling in IBD patients.[46–49] However, rather than being harmful by contributing to the severity of the disease, these changes may be beneficial by helping to regenerate the damaged epithelium and thus recover from the disease.[46] Notably, several clinical trials using different γ-secretase specific inhibitors for the treatment of various diseases, including cancer and

399 Alzheimer's disease, have described the development of spontaneous gut inflammation, even

400 grade 3 colitis with ulceration in some of the patients.[50–54] These findings in humans support

401 our observation in Psen1/2 double knockout mice and our data therefore provide a potential

402 mechanistic explanation for the observed side effects in these patients. In this context, our

403 current study demonstrates the severity of double presenilin deletion in the intestine, leading

404 to the death of the mice within a few days, thus highlighting the need to avoid double presenilin

405 inhibition by γ-secretase inhibitors in the treatment of Alzheimer's disease or tumors.

406 Moreover, our study demonstrates the existence of compensatory mechanisms between the

407 two presenilins in the gut, which could be exploited to avoid Notch-related off-target effects,

408 especially in the intestine. This knowledge could be used in the future when designing new γ-

409 secretase inhibitors. Interestingly, Sannerud et al. described that PSEN2 exhibits an additional

410 structural motif in mouse embryonic fibroblasts, which is absent PSEN1. As a result, PSEN2-

411 containing γ-secretases are directed towards late endosomes/lysosomes, while PSEN1-

412 containing γ-secretases, are primarily located at the plasma membrane, thus contributing to a

413 broader subcellular localization.[6, 7] Therefore, future studies should aim to study the

414 subcellular localization of PSEN1 and PSEN2 in the IECs to better understand how

415 compartmentalization of PSEN1 and PSEN2 affects their functions in the gut epithelium.

416 Functional tight junctions are normally formed between goblet cells and enterocytes, strictly

417 limiting permeability.[55] Interestingly, the depth of tight junction formation and the number of

418 tight junction strands depend on the different IEC types between which they are formed.[56]

419 This suggests that functionally different tight junctions may be formed in the inducible Psen1/2

420 double knockout mice.

421 Interestingly, although many publications have addressed the role of Notch signaling in

422 intestinal homeostasis by IEC-specific deletion of various Notch signaling components, quite

423 different phenotypes have been observed. For example, while single *Notch1* or *Notch2*

424 knockout mice and single *Dll1* and *Dll4* knockout mice show no phenotype, dual deletion of

425 *Notch1*/*Notch2* or *Dll1*/*Dll4* resulted in increased goblet cell numbers and decreased

426 proliferative cells.[57, 58] Similarly, compensatory mechanisms were observed among *Hes*

genes, whereas triple deletion of *Hes1*/*Hes3*/*Hes5* resulted in increased secretory cell numbers and decreased proliferation.[59] Moreover, deletion of the α-secretase Adam10 also resulted in the phenotypes described above.[60] Remarkably, IEC-specific deletion of *Rbpj* not only upregulates secretory cell lineage and downregulates proliferation but also induces spontaneous colitis and bacterial translocation.[44] 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 γ-secretase independent functions beyond their involvement in Notch signaling, which has been also previously observed for the presenilins [4], further highlighting the crucial importance of the presenilins in maintaining intestinal homeostasis, 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.[61] Importantly, malnutrition has even been described to be associated with poor clinical outcome in IBD patients.[62, 63] Malabsorption in these patients is thought to be due to loss of epithelial integrity and impaired epithelial transport [61], 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 IBD patients, involving the loss of the intestinal epithelial presenilins. To date, GWAS studies have not identified presenilins or other important Notch signaling-related molecules as being associated with IBD pathogenesis.[64, 65] This might be due to the high degree of redundancy often reported for these molecules. In contrast to Psen1, our data from experimental colitis and IBD patients did not reveal a downregulation of Psen2. Additionally, not all publicly available transcriptomic datasets showed a downregulation of presenilin expression, suggesting that the expression of presenilins may vary depending on the disease, stage, sampling, and therapeutic intervention. Given that presenilin expression can be influenced by various IBD-related cytokines, including TNF-α (data not shown), it is crucial to consider the impact of

455   biologics used as treatment options in these patients. Moreover, presenilin function is not only

456   regulated on a transcriptional level but can also be activated by post-translational modification,

457   such as phosphorylation. For example, Protein Kinase A, which has been reported to mediate

458   presenilin phosphorylation [66], is significantly downregulated in UC patients (data not shown).

459   Future studies will be necessary to establish pharmacological approaches that could target

460   presenilin as a treatment option for IBD. These could involve targeting the epithelium in

461   agonistic approaches promoting presenilin enzymatic activity.

462   In conclusion, our research identifies the presenilins as key molecules of intestinal

463   homeostasis by coordinating a balanced IEC differentiation and thus a tight intestinal barrier.

464   In contrast, impaired expression or function of presenilins can lead to Notch signaling

465   disruption, with impaired IEC differentiation, resulting in malnutrition, barrier breakdown and

466   ultimately the development of spontaneous inflammation. In the future, these findings may be

467   useful in the development of IBD treatment strategies.

MATERIAL AND METHODS

Human material

Human specimens were obtained from the Department of Gastroenterology, Infectious Diseases and Rheumatology of the Charité - Universitätsmedizin Berlin, and the Universitätsklinikum Erlangen, after written informed consent of the patients and approval by the Ethics Committee of the Charité - Universitätsmedizin Berlin and the Ethics Committee of the Universitätsklinikum Erlangen. Colon specimens from healthy and IBD patients were embedded in paraffin and subsequently stained as described below. Data were anonymized. Patient's clinical information is shown in **Suppl. Tab. 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 <u>homozygous</u> conditional deletion of $Psen1$ in IECs ($Psen1^{\Delta IEC}$). 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. $Psen1^{\Delta IEC}$ were further crossed with $Psen2^{-/-}$ to generate $Psen2^{+/-} Psen1^{\Delta IEC}$ 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 ($Psen2^{-/-} Psen1^{i\Delta IEC}$; iDKO). iDKO mice were compared to $Psen2^{-/-} Psen1^{fl/fl}$ mice as littermate controls in all experiments. All mice were routinely screened for pathogens according to FELASA guidelines. Animals were sex- 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. Sample size was calculated using G*Power.[67]

<u>Histology scoring</u>

<u>Pathological scoring was performed on hematoxylin & eosin-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).</u>

Endoscopic evaluation

Endoscopic evaluation was performed using high-resolution mouse video endoscopy as previously described.[32] 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 (i.p.) with 75 mg/kg tamoxifen (Sigma) diluted 1:1 in sunflower oil on 5 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 (4,000 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 GmbH), 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 µl 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 mesenteric lymph node (MLN) and liver tissue lysates diluted in sterile PBS on MacConkey (Roth) and blood agar (Merck) plates to determine colony forming units (CFU) under aerobic and anaerobic conditions after 24 hours of incubation at 37°C. CFU were normalized to the organ weight. The anaerobic condition was established using Anaerocult™ A (Merck).

Serum glucose measurement

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

Oral glucose tolerance test

For the oral glucose tolerance test, mice were fasted overnight and baseline glucose levels were measured using a blood glucose meter (Ascensia Diabetes Care Deutschland GmbH). 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 minutes later using a blood glucose meter.

Murine organoid culture

Small intestinal organoids were established as previously described.[68] Briefly, mice were sacrificed, and the intestine was cut into small pieces, incubated in 2 mM EDTA for 30 minutes, and the released epithelial cells were filtered through a 70 µm filter. Epithelial cells were then plated in Cultrex (Bio-Techne) and cultured in culture medium in 5% CO_2 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 minutes at 37°C was used as a positive control. Images were captured using a confocal microscope and images were analyzed using ImageJ as previously described.[34]

Rhodamine 123 uptake assay

548   Organoids were incubated with 100 µM Rhodamine 123 (Sigma) for 5 minutes at 37°C, washed

549   and incubated in basal organoid culture medium for another 40 minutes at 37°C. Images were

550   captured by confocal microscopy and analyzed using ImageJ.

551   **Histology and immunofluorescence staining**

552   For evaluation of histomorphology, tissue was formalin-fixed and embedded in paraffin.

553   Sections were cut from paraffin blocks and histochemically stained with hematoxylin and eosin.

554   Immunofluorescence staining was performed on formalin-fixed paraffin-embedded sections or

555   cryosections. Cryosections and organoid cultures were fixed with 4% PFA for 30 minutes at

556   room temperature and then washed with TBS-T for 5 minutes. For paraffin sections, sections

557   were incubated for 1 hour at 65°C, paraffin was removed with ROTI®Histol, and sections were

558   rehydrated in a decreasing ethanol row. Antigen retrieval was performed using Tris/ETDA at

559   450 W for 20 minutes. After cooling, the slides were incubated with 0.1% Triton X-100 for 10

560   minutes, washed in TBS-T, and the endogenous biotin-binding sites were blocked using the

561   Avidin/Botin Blocking Kit (Vector Laboratories). The following primary antibodies were

562   incubated overnight at 4°C: Psen1 (1:300; #5643; Cell Signaling), E-Cadherin:FITC (1:200;

563   #612130; BD Biosciences), Olfm4 (1:300; #39141; Cell Signaling), Lysozyme (1:300; A0099 ;

564   Dako), Muc2 (1:500; NBP1-31231; Novus), Chromogranin A (1:300; NB120-15160; Novus),

565   Dcamkl1 (1:200; ab31704; abcam), Ki67 (1:200; 14-5698-82; eBioscience), MPO (1:200;

566   #ab9535; abcam), F4/80 (1:200; #70076; Cell Signaling), Cl. Caspase 3 (1:300; #9662; Cell

567   Signaling), E-Cadherin (1:300; #3195; Cell Signaling), Occludin (1:300; #91131; Cell

568   Signaling), and ULEX (1:50; FL-1061-2; Vector Laboratories). The next day, the slides were

569   washed three times in TBS-T and incubated with the appropriate secondary antibodies (Alexa

570   Fluor®555 anti-rabbit: 1:200, BioLegend, # 406412 for Olfm4, Lysozyme, Muc2, ChgA,

571   Dcamkl1 and Ki67; Biotin anti-rabbit IgG: 1:400, Jackson ImmunoResearch, # 111-065-144

572   combined with Streptavidin DyLight500: 1:400, Thermo Fisher, # 84542 for Psen1, MPO,

573   F4/80, Cl. Casp. 3, E-Cadherin and occludin) for 2 hours at room temperature. Slides were

574   washed in PBS, nuclear staining was performed with Hoechst (1:500; Invitrogen), and slides

575   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 ROTI®Histol, and sections were and rehydrated in a decreasing ethanol row. Antigen retrieval was performed using Tris/EDTA at 450 W for 20 minutes in a microwave. Endogenous peroxidase blocking was performed using 3% hydrogen peroxide. Endogenous biotin-binding sites were blocked using the Avidin/Biotin Blocking Kit (Vector Laboratories). The primary Psen1 antibody (1:300; #5643; Cell Signaling) was incubated overnight at 4°C. The next day, slides were washed in PBS and the secondary antibody (SignalStain®Boost IHC detection reagent (HRP, rabbit)) was incubated for 30 minutes at room temperature, followed by signal detection (SignalStain®DAB Chromogene Substrate; SignalStain®DAB Diluent) for 8 minutes at room temperature. Counterstaining was performed with hematoxylin for 5 seconds and washed with tap water. Sections were dehydrated through an increasing ethanol series and mounted with Entellan (Merck).

Intestinal alkaline phosphatase staining

Alkaline phosphatase staining was performed on cryosections fixed in ice-cold acetone for 5 minutes. Sections were air dried for 5 minutes and incubated for 30 minutes at 37°C alkaline phosphatase working solution: 0.2 M TRIS, 0.1M HCl, 0.01 g Naphtol AS-MX Phosphate (Sigma) in 500 µl N,N Dimethylformamide (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, paraffin-embedded tissue sections. Paraffin sections were incubated for 1 hour at 65°C, paraffin was removed with ROTI®Histol, and sections were rehydrated in a decreasing ethanol series. Samples were then oxidized in 0.5% periodic acid (Carl Roth) for 5 minutes, rinsed in distilled water, and incubated in Schiff's reagent for 15 minutes (Carl Roth). After washing in tap water, the slides were counterstained with hematoxylin for 1 minute. After further washing in tap water, the sections were dehydrated and mounted with Entellan (Merck).

In situ Hybridization

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 ROTI®Histol, and sections were rehydrated in a decreasing ethanol series. Slides were then incubated in hybridization buffer for 20 minutes at room temperature, followed by incubation with the Eub338 probe (3'-GCT GCC TCC CGT AGG AGT-5' Cy3; biomers) diluted 1:10 in hybridization buffer for 90 minutes at 46°C in a hybridization oven as previously described.[69] 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 ROTI®Histol, and sections were incubated in 100% ethanol. Tissues were air dried, pretreated with hydrogen peroxide (ACDBio), and then heated at 100°C for 15 minutes in Target Retrieval Buffer (ACDBio). This was followed by incubation at 40°C for 30 minutes 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 minutes, 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 Signaling), Psen2 (#9979; Cell Signaling), Na-K-ATPase (#3010; Cell Signaling), Lamin A/C (615802; Biolegend), GAPDH (#2118; Cell Signaling), β-actin HRP (ab49900; abcam), Lysozyme (A0099; Dako), Occludin (#91131; Cell Signaling), NICD (#4147; Cell Signaling). The next day, the membrane was incubated with the appropriate HRP-conjugated secondary antibody (Cell Signaling) and developed on an Amersham™ Imager 800 (GE Healthcare Life Sciences) using Western Lightning Plus ECL Substrate (Perkin Elmer).

TNF-α 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). Normalization 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 (v1.6.4) 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 (IPA; Qiagen), the Database for

658   Annotation, Visualization and Integrated Discovery (DAVID) analysis tool. Only genes with

659   adjusted p-values less than 0.05 and $\log_2$(fold change) less or greater than 1 were considered.

660   **Availability and analysis of transcriptomic datasets**

661   <u>Transcriptomic data of the IBDome cohort will be made available to the scientific community</u>

662   <u>upon acceptance of the manuscript.</u> The publicly available datasets used in this study are

663   published under the accession codes: E-MTAB-9850 [28] and GSE6731.[29] The

664   corresponding raw expression values were log-transformed. Microsoft Excel 2019 was used

665   for data sorting and comparison, while GraphPad Prism 9 was used for graphical illustrations.

666   **Statistical analysis**

667   GraphPad Prism 9 software was used for statistical analysis and graphing. As described in the

668   corresponding figure legends, significance analysis was performed using Mann-Whitney test,

669   Student's t-test or one-way ANOVA. P values below 0.05 (*), 0.01 (**), and 0.001 (***) were

670   considered significant. All data are shown as mean ± SD. Three independent replicates were

671   used in most experiments.

REFERENCES

1   Li YM, Xu M, Lai MT, et al. Photoactivated gamma-secretase inhibitors directed to the active site covalently label presenilin 1. *Nature* 2000;405(6787):689–94.

2   Strooper B de, Saftig P, Craessaerts K, et al. Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature* 1998;391(6665):387–90.

3   Güner G, Lichtenthaler SF. The substrate repertoire of γ-secretase/presenilin. *Semin Cell Dev Biol* 2020;105:27–42.

4   Duggan SP, McCarthy JV. Beyond γ-secretase activity: The multifunctional nature of presenilins in cell signalling pathways. *Cell Signal* 2016;28(1):1–11.

5   Lanoiselée H-M, Nicolas G, Wallon D, et al. APP, PSEN1, and PSEN2 mutations in early-onset Alzheimer disease: A genetic screening study of familial and sporadic cases. *PLoS Med* 2017;14(3):e1002270.

6   Sannerud R, Esselens C, Ejsmont P, et al. Restricted Location of PSEN2/γ-Secretase Determines Substrate Specificity and Generates an Intracellular Aβ Pool. *Cell* 2016;166(1):193–208.

7   Meckler X, Checler F. Presenilin 1 and Presenilin 2 Target γ-Secretase Complexes to Distinct Cellular Compartments. *J Biol Chem* 2016;291(24):12821–37.

8   Serneels L, van Biervliet J, Craessaerts K, et al. gamma-Secretase heterogeneity in the Aph1 subunit: relevance for Alzheimer's disease. *Science* 2009;324(5927):639–42.

9   Shen J, Bronson RT, Chen DF, et al. Skeletal and CNS defects in Presenilin-1-deficient mice. *Cell* 1997;89(4):629–39.

10 Zhang B, Wang HE, Bai Y-M, et al. Inflammatory bowel disease is associated with higher dementia risk: a nationwide longitudinal study. *Gut* 2021;70(1):85–91.

11 Kim GH, Lee YC, Kim TJ, et al. Risk of Neurodegenerative Diseases in Patients with Inflammatory Bowel Disease: A Nationwide Population-based Cohort Study. *J Crohns Colitis* 2022;16(3):436–43.

12 Sohrabi M, Pecoraro HL, Combs CK. Gut Inflammation Induced by Dextran Sulfate Sodium Exacerbates Amyloid-β Plaque Deposition in the AppNL-G-F Mouse Model of Alzheimer's Disease. *J Alzheimers Dis* 2021;79(3):1235–55.

13 Jang S-E, Lim S-M, Jeong J-J, et al. Gastrointestinal inflammation by gut microbiota disturbance induces memory impairment in mice. *Mucosal Immunol* 2018;11(2):369–79.

14 Baulies A, Angelis N, Li VSW. Hallmarks of intestinal stem cells. *Development* 2020;147(15).

15 Hartman C, Eliakim R, Shamir R. Nutritional status and nutritional therapy in inflammatory bowel diseases. *World J Gastroenterol* 2009;15(21):2570–78.

16 Lanfranchi GA, Brignola C, Campieri M, et al. Assessment of nutritional status in Crohn's disease in remission or low activity. *Hepatogastroenterology* 1984;31(3):129–32.

17 Goh J, O'Morain CA. Review article: nutrition and adult inflammatory bowel disease. *Aliment Pharmacol Ther* 2003;17(3):307–20.

18 Donnellan CF, Yann LH, Lal S. Nutritional management of Crohn's disease. *Therap Adv Gastroenterol* 2013;6(3):231–42.

19 Becker C, Neurath MF, Wirtz S. The Intestinal Microbiota in Inflammatory Bowel Disease. *ILAR J* 2015;56(2):192–204.

20 Levitan D, Greenwald I. Facilitation of lin-12-mediated signalling by sel-12, a Caenorhabditis elegans S182 Alzheimer's disease gene. *Nature* 1995;377(6547):351–54.

21 Annaert WG, Levesque L, Craessaerts K, et al. Presenilin 1 controls gamma-secretase processing of amyloid precursor protein in pre-golgi compartments of hippocampal neurons. *J Cell Biol* 1999;147(2):277–94.

721 22 Struhl G, Greenwald I. Presenilin is required for activity and nuclear access of Notch in
722   Drosophila. *Nature* 1999;398(6727):522–25.
723 23 Zhang Z, Hartmann H, Do VM, et al. Destabilization of beta-catenin by mutations in
724   presenilin-1 potentiates neuronal apoptosis. *Nature* 1998;395(6703):698–702.
725 24 Murayama M, Tanaka S, Palacino J, et al. Direct association of presenilin-1 with beta-
726   catenin. *FEBS Lett* 1998;433(1-2):73–77.
727 25 Yu G, Chen F, Levesque G, et al. The presenilin 1 protein is a component of a high
728   molecular weight intracellular complex that contains beta-catenin. *J Biol Chem*
729   1998;273(26):16470–75.
730 26 Kang DE, Soriano S, Frosch MP, et al. Presenilin 1 facilitates the constitutive turnover of
731   beta-catenin: differential activity of Alzheimer's disease-linked PS1 mutants in the beta-
732   catenin-signaling pathway. *J Neurosci* 1999;19(11):4229–37.
733 27 Bagaria J, Bagyinszky E, An SSA. Genetics, Functions, and Clinical Impact of Presenilin-
734   1 (PSEN1) Gene. *Int J Mol Sci* 2022;23(18).
735 28 Patankar JV, Müller TM, Kantham S, et al. E-type prostanoid receptor 4 drives resolution
736   of intestinal inflammation by blocking epithelial necroptosis. *Nat Cell Biol* 2021;23(7):796–
737   807.
738 29 Wu F, Dassopoulos T, Cope L, et al. Genome-wide gene expression differences in
739   Crohn's disease and ulcerative colitis from endoscopic pinch biopsies: insights into
740   distinctive pathogenesis. *Inflamm Bowel Dis* 2007;13(7):807–21.
741 30 Yu H, Saura CA, Choi SY, et al. APP processing and synaptic plasticity in presenilin-1
742   conditional knockout mice. *Neuron* 2001;31(5):713–26.
743 31 Herreman A, Hartmann D, Annaert W, et al. Presenilin 2 deficiency causes a mild
744   pulmonary phenotype and no changes in amyloid precursor protein processing but
745   enhances the embryonic lethal phenotype of presenilin 1 deficiency. *Proc Natl Acad Sci*
746   *U S A* 1999;96(21):11872–77.
747 32 Becker C, Fantini MC, Neurath MF. High resolution colonoscopy in live mice. *Nat Protoc*
748   2006;1(6):2900–04.
749 33 Martini E, Krug SM, Siegmund B, et al. Mend Your Fences: The Epithelial Barrier and its
750   Relationship With Mucosal Immunity in Inflammatory Bowel Disease. *Cellular and*
751   *Molecular Gastroenterology and Hepatology* 2017;4(1):33–46.
752   https://www.sciencedirect.com/science/article/pii/S2352345X1730053X.
753 34 Bardenbacher M, Ruder B, Britzen-Laurent N, et al. Investigating Intestinal Barrier
754   Breakdown in Living Organoids. *J Vis Exp* 2020(157).
755 35 Landy J, Ronde E, English N, et al. Tight junctions in inflammatory bowel diseases and
756   inflammatory bowel disease associated colorectal cancer. *World J Gastroenterol*
757   2016;22(11):3117–26.
758 36 Kucharzik T, Walsh SV, Chen J, et al. Neutrophil transmigration in inflammatory bowel
759   disease is associated with differential expression of epithelial intercellular junction
760   proteins. *Am J Pathol* 2001;159(6):2001–09.
761 37 Mehta S, Nijhuis A, Kumagai T, et al. Defects in the adherens junction complex (E-
762   cadherin/ β-catenin) in inflammatory bowel disease. *Cell and Tissue Research*
763   2015;360(3):749–60.
764 38 Tang H-N, Tang C-Y, Man X-F, et al. Plasticity of adipose tissue in response to fasting
765   and refeeding in male mice. *Nutr Metab (Lond)* 2017;14:3.
766 39 Soayfane Z, Tercé F, Cantiello M, et al. Exposure to dietary lipid leads to rapid production
767   of cytosolic lipid droplets near the brush border membrane. *Nutr Metab (Lond)*
768   2016;13(1):48.
769 40 Strooper B de, Annaert W, Cupers P, et al. A presenilin-1-dependent gamma-secretase-
770   like protease mediates release of Notch intracellular domain. *Nature*
771   1999;398(6727):518–22.

772 41 Reyes Gamez-Belmonte, Mousumi Mahapatro, Lena Erkert, et al. Epithelial presenilin-1
773   drives colorectal tumour growth by controlling EGFR-COX2 signalling. *Gut*
774   2023;72(6):1155.
775 42 Aziz M, Ishihara S, Ansary MU, et al. Crosstalk between TLR5 and Notch1 signaling in
776   epithelial cells during intestinal inflammation. *Int J Mol Med* 2013;32(5):1051–62.
777 43 Shinoda M, Shin-Ya M, Naito Y, et al. Early-stage blocking of Notch signaling inhibits the
778   depletion of goblet cells in dextran sodium sulfate-induced colitis in mice. *J Gastroenterol*
779   2010;45(6):608–17.
780 44 Obata Y, Takahashi D, Ebisawa M, et al. Epithelial cell-intrinsic Notch signaling plays an
781   essential role in the maintenance of gut immune homeostasis. *J Immunol*
782   2012;188(5):2427–36.
783 45 Mathern DR, Laitman LE, Hovhannisyan Z, et al. Mouse and human Notch-1 regulate
784   mucosal immune responses. *Mucosal Immunol* 2014;7(4):995–1005.
785 46 Okamoto R, Tsuchiya K, Nemoto Y, et al. Requirement of Notch activation during
786   regeneration of the intestinal epithelia. *Am J Physiol Gastrointest Liver Physiol*
787   2009;296(1):G23-35.
788 47 Zheng X, Tsuchiya K, Okamoto R, et al. Suppression of hath1 gene expression directly
789   regulated by hes1 via notch signaling is associated with goblet cell depletion in ulcerative
790   colitis. *Inflamm Bowel Dis* 2011;17(11):2251–60.
791 48 Dahan S, Rabinowitz KM, Martin AP, et al. Notch-1 signaling regulates intestinal epithelial
792   barrier function, through interaction with CD4+ T cells, in mice and humans.
793   *Gastroenterology* 2011;140(2):550–59.
794 49 Ghorbaninejad M, Heydari R, Mohammadi P, et al. Contribution of NOTCH signaling
795   pathway along with TNF-α in the intestinal inflammation of ulcerative colitis. *Gastroenterol
796   Hepatol Bed Bench* 2019;12(Suppl1):S80-S86.
797 50 Collins M, Michot J-M, Bellanger C, et al. Notch inhibitors induce diarrhea, hypercrinia and
798   secretory cell metaplasia in the human colon. *EXCLI J* 2021;20:819–27.
799 51 Pant S, Jones SF, Kurkjian CD, et al. A first-in-human phase I study of the oral Notch
800   inhibitor, LY900009, in patients with advanced cancer. *Eur J Cancer* 2016;56:1–9.
801 52 Massard C, Azaro A, Soria J-C, et al. First-in-human study of LY3039478, an oral Notch
802   signaling inhibitor in advanced or metastatic cancer. *Ann Oncol* 2018;29(9):1911–17.
803 53 Messersmith WA, Shapiro GI, Cleary JM, et al. A Phase I, dose-finding study in patients
804   with advanced solid malignancies of the oral γ-secretase inhibitor PF-03084014. *Clin
805   Cancer Res* 2015;21(1):60–67.
806 54 Coric V, van Dyck CH, Salloway S, et al. Safety and tolerability of the γ-secretase inhibitor
807   avagacestat in a phase 2 study of mild to moderate Alzheimer disease. *Arch Neurol*
808   2012;69(11):1430–40.
809 55 Sancho R, Cremona CA, Behrens A. Stem cell and progenitor fate in the mammalian
810   intestine: Notch and lateral inhibition in homeostasis and disease. *EMBO Rep*
811   2015;16(5):571–81.
812 56 Madara JL, Trier JS. Structure and permeability of goblet cell tight junctions in rat small
813   intestine. *J Membr Biol* 1982;66(2):145–57.
814 57 Riccio O, van Gijn ME, Bezdek AC, et al. Loss of intestinal crypt progenitor cells owing to
815   inactivation of both Notch1 and Notch2 is accompanied by derepression of CDK inhibitors
816   p27Kip1 and p57Kip2. *EMBO Rep* 2008;9(4):377–83.
817 58 Pellegrinet L, Rodilla V, Liu Z, et al. Dll1- and dll4-mediated notch signaling are required
818   for homeostasis of intestinal stem cells. *Gastroenterology* 2011;140(4):1230-1240.e1-7.
819 59 Ueo T, Imayoshi I, Kobayashi T, et al. The role of Hes genes in intestinal development,
820   homeostasis and tumor formation. *Development* 2012;139(6):1071–82.
821 60 Tsai Y-H, VanDussen KL, Sawey ET, et al. ADAM10 regulates Notch function in intestinal
822   stem cells of mice. *Gastroenterology* 2014;147(4):822-834.e13.

823   61   Balestrieri P, Ribolsi M, Guarino MPL, et al. Nutritional Aspects in Inflammatory Bowel
824   Diseases. *Nutrients* 2020;12(2).
825   62   Jansen I, Prager M, Valentini L, et al. Inflammation-driven malnutrition: a new screening
826   tool predicts outcome in Crohn's disease. *Br J Nutr* 2016;116(6):1061–67.
827   63   Takaoka A, Sasaki M, Nakanishi N, et al. Nutritional Screening and Clinical Outcome in
828   Hospitalized Patients with Crohn's Disease. *Ann Nutr Metab* 2017;71(3-4):266–72.
829   64   McGovern DPB, Kugathasan S, Cho JH. Genetics of Inflammatory Bowel Diseases.
830   *Gastroenterology* 2015;149(5):1163-1176.e2.
831   65   Jostins L, Ripke S, Weersma RK, et al. Host-microbe interactions have shaped the genetic
832   architecture of inflammatory bowel disease. *Nature* 2012;491(7422):119–24.
833   66   Maesako M, Horlacher J, Zoltowska KM, et al. Pathogenic PS1 phosphorylation at
834   Ser367. *Elife* 2017;6.
835   67   Faul F, Erdfelder E, Lang A-G, et al. G*Power 3: a flexible statistical power analysis
836   program for the social, behavioral, and biomedical sciences. *Behav Res Methods*
837   2007;39(2):175–91.
838   68   Sato T, Vries RG, Snippert HJ, et al. Single Lgr5 stem cells build crypt-villus structures in
839   vitro without a mesenchymal niche. *Nature* 2009;459(7244):262–65.
840   69   Johansson MEV, Hansson GC. Preservation of mucus in histological sections,
841   immunostaining of mucins in fixed tissue, and localization of bacteria with FISH. *Methods*
842   *Mol Biol* 2012;842:229–35.
843

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AUTHOR CONTRIBUTIONS

Study concept, design, literature search, experiments, analysis, interpretation of data, critical revision of the manuscript, manuscript drafting: LE, RGB, CB. Experimentation and analysis: LE, RGB, MK, LSc, JVP, MGA, LB, CP, ST. Material support: MM, JS, LSe, BDS, AAK. Intellectual contributions during manuscript editing, acquisition of funds and supervision: MFN, SW, CB.

COMPETING INTERESTS STATEMENT

The authors declare no conflicting financial interests.

FIGURE LEGENDS

Fig. 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 to 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, analyzed from a publicly available dataset (n = 2-3/group).[28] **(C)** Western blotting for PSEN1 in colonic tissue lysates from healthy and DSS-treated mice, with measurement of PSEN1 expression, normalized 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)** Normalized Log_2 expression levels of *PSEN1* in healthy (n= 4), Ulcerative colitis (UC) (n= 13) and Crohn's disease (CD) (n= 19) patients analyzed from a publicly available dataset.[29] **(F)** *PSEN1* normalized counts in the colon of healthy (n = 22), Ulcerative colitis (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 versus UC patients in correlation with the histopathological score of colitis (n = 60). **(I)** Representative immunofluorescence images of PSEN1 in healthy (n = 4), UC (n = 6) and CD patients (n = 4) (scale bar 50 μm) with analysis of the Psen1$^+$ area (normalized to Hoechst). Overall, data are expressed as mean ± SD. *, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$ respectively, by one-way ANOVA (B, E, G, H) Mann-Whitney test (C) or Wilcoxon rank-sum test (F).

Fig. 2: Inducible presenilin double-knockout mice spontaneously develop colitis.

(A) Schematic of intraperitoneal (i.p.) injection of tamoxifen in control (Psen2$^{-/-}$ Psen1$^{fl/fl}$) and inducible Psen1/2 double knockout (iDKO; Psen2$^{-/-}$ Psen1^{iΔIEC}) 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 = 11; iDKO: 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 3 independent experiments). **(F)** qPCR analysis of several inflammatory cytokines (normalized 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 (normalized to Hoechst). Overall, data are expressed as mean ± SD. *, **, and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively, by one-way ANOVA (B) and Mann-Whitney test (C, D, F, G).

Fig. 3: Presenilins are key to intestinal barrier function.

(A) Measurement of FITC-dextran in the serum of tamoxifen-injected control (Psen2$^{-/-}$ Psen1$^{fl/fl}$) and inducible Psen1/2 double knockout (iDKO; Psen2$^{-/-}$ Psen1^{iΔIEC}) 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 3 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 3 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 3 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 (1 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 3 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 (CFU)/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. *, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, by Mann-Whitney test.

Fig. 4: Microbiota depletion improves spontaneous inflammation but not body weight loss in inducible presenilin double-knockout mice.

(A) Schematic of antibiotic (Ab) treatment in drinking water and i.p. tamoxifen injection in control (Psen2$^{-/-}$ Psen1$^{fl/fl}$) and inducible Psen1/2 double knockout (iDKO; Psen2$^{-/-}$ Psen1$^{i\Delta IEC}$) 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 (normalized 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. *, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, by Mann-Whitney test (B) or one-way ANOVA (C, D, F).

Fig. 5: Dual presenilin deletion leads to enterocyte loss, malnutrition and death in presenilin double knockout mice due to Notch signaling disruption.

(A) Volcano plot of RNA sequencing analysis of inducible Psen1/2 double knockout (iDKO; Psen2$^{-/-}$ Psen1$^{i\Delta IEC}$) ileal tissue compared to control littermates (Psen2$^{-/-}$ Psen1$^{fl/fl}$) (p-adjusted < 0.05; log$_2$foldchange ± 1). Green dots represent downregulation, red dots represent

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upregulation, and gray indicates no gene deregulation (n = 3/group). DEG = differentially expressed genes. **(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 normalized 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 3 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 normalized 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$^{\Delta IEC}$, Psen2$^{-/-}$ and iDKO mice and their respective controls. β-actin was used as a loading control (representative of 3 independent experiments). **(L)** Schematic of the influence of a dual Psen1 and Psen2 deletion on intestinal homeostasis. Overall, data are expressed as mean ± SD. ** and *** indicate $p < 0.01$, and $p < 0.001$, respectively, by Mann-Whitney test (D, E, F, H, I, J) or two-way ANOVA (G).

Suppl. Fig. 1: Involvement of genes associated with neurodegeneration and Alzheimer's disease in intestinal inflammation.

Heatmaps of RNA sequencing data from DSS-treated mouse whole colon tissue compared to healthy mouse colons showing differentially expressed genes included in the pathways 'Pathways of neurodegeneration – multiple diseases' and 'Alzheimer's disease'. *Psen1* is highlighted with a red arrow and *Psen2* with a blue arrow (n = 3/group).

Suppl. Fig. 2: Generation of intestinal epithelial specific Psen1 deficient mice.

(A) Schematic of Psen1$^{\Delta IEC}$ mouse line generation. **(B)** *Psen1* mRNA expression levels, normalized to *Gapdh*, in the ileum and colon of Psen1$^{\Delta IEC}$ mice compared to Psen1$^{fl/fl}$ mice (Psen1$^{fl/fl}$: n = 10; Psen1$^{\Delta IEC}$: n = 10). **(C)** Western blotting for Psen1 in whole ileal and colonic tissue extracts and in isolated IEC from Psen1$^{fl/fl}$ (fl/fl) and Psen1$^{\Delta IEC}$ (ΔIEC) mice. β-actin was used as a loading control (representative of 3 independent experiments). **(D)** Representative IF images of Psen1 in the ileum and colon of the indicated mice (scale bar 100 μm) (representative of 3 independent experiments). **(E)** Psen1 protein expression levels in small intestinal organoids generated from Psen1$^{fl/fl}$ (fl/fl) and Psen1$^{\Delta IEC}$ (ΔIEC) mice. β-actin was used as a loading control (representative of 3 independent experiments). **(F)** Psen1 mRNA expression, normalized to *Gapdh*, in Si organoids established from the two mouse genotypes (3 independent experiments). **(G)** Histochemical staining images of Psen1 in paraffin-embedded Si organoids (scale bar 100 μm) (representative of 2 independent experiments). Overall, data are expressed as mean ± SD. *** indicates p < 0.001 by one-way ANOVA (B) or Mann-Whitney test (F).

Suppl. Fig. 3: Absence of Psen1 alone in IECs does not disrupt intestinal tissue or immune homeostasis.

(A) Hematoxylin & eosin (H&E) staining images of ileal and colonic sections from Psen1$^{fl/fl}$ and Psen1$^{\Delta IEC}$ mice (scale bar 250 μm) (representative of 4 independent experiments). **(B)** qPCR analysis of various IEC differentiation markers (all normalized to *Gapdh*) in the ileum and colon of Psen1$^{\Delta IEC}$ mice compared to their control littermates (Psen1$^{fl/fl}$: n = 7; Psen1$^{\Delta IEC}$: n = 6). **(C)** Representative IF images of different IEC differentiation markers: Muc2 (goblet cells), Chromogranin A (Chga; enteroendocrine cells), Dcamkl1 (tuft cells), Ki67 (proliferation), Olfm4

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995 (stem cells), intestinal alkaline phosphatase (IAP; absorptive enterocytes) and lysozyme (Lyz;

996 Paneth cells) in the ileum and colon of the different mouse genotypes (white scale bar 100 µm;

997 yellow scale bar 250 µm) (representative of 3 independent experiments). **(D)** Analysis of Chga-

998 and Dcamkl1-positive cells per high power field (HPF) in the ileum and colon of Psen1$^{\Delta IEC}$ mice

999 compared to their littermate controls (3 independent experiments). **(E)** qPCR analysis of

1000 several inflammatory markers in the ileum and colon of Psen1$^{fl/fl}$ and Psen1$^{\Delta IEC}$ mice (Psen1$^{fl/fl}$:

1001 n = 7; Psen1$^{\Delta IEC}$: n = 6). **(F)** Volcano plots of RNAseq data obtained from ileal and colonic

1002 tissue of Psen1$^{\Delta IEC}$ mice compared to their control mice. Red dots indicate upregulated genes,

1003 green dots indicate downregulation, while gray dots represent genes that are not differentially

1004 expressed (n = 3/group). **(G)** Western blot analysis of PSEN2 expression in the ileum and

1005 colon of Psen1$^{fl/fl}$ (fl/fl) and Psen1$^{\Delta IEC}$ (ΔIEC) mice (representative of 3 independent

1006 experiments) with analysis of PSEN2 protein abundance normalized to β-actin. Overall, data

1007 are expressed as mean ± SD. * and ** indicate $p < 0.05$ and $p < 0.01$, respectively, by one-

1008 way ANOVA (G).

1009 **Suppl. Fig. 4: One allele of Psen2 is sufficient to maintain intestinal homeostasis in**

1010 **heterozygous presenilin double-knockout mice.**

1011 **(A)** Schematic of the generation of conditional presenilin double-knockout mice. **(B)** Listing of

1012 the Mendelian expected genotypes of the crossing shown in (A) compared to the actual

1013 genotypes obtained. **(C)** Weekly body weight measurements of control mice (Psen2$^{-/-}$ Psen1$^{fl/fl}$)

1014 compared to heterozygous conditional presenilin double-knockout mice (Psen2$^{+/-}$ Psen1$^{\Delta IEC}$)

1015 (n = 4/group). **(D)** Representative endoscopic images of control and Psen2$^{+/-}$ Psen1$^{\Delta IEC}$ mice

1016 with corresponding MEICS scores (control: n = 10; Psen2$^{+/-}$ Psen1$^{\Delta IEC}$: n = 9). **(E)**

1017 Representative H&E images of the ileum, proximal and distal colon from control and Psen2$^{+/-}$

1018 Psen1$^{\Delta IEC}$ mice (scale bar 250 µm) (representative of 5 independent experiments). **(F)**

1019 Representative IF images for Olfm4 (stem cells) and Muc2 (goblet cells) in the ileum of control

1020 and Psen2$^{+/-}$ Psen1$^{\Delta IEC}$ mice (scale bar 100 µm) (representative of 3 independent

1021 experiments). **(G)** qPCR analysis of various IEC differentiation markers (all normalized to

1022 *Gapdh*) of ileal and colonic tissues from the indicated mouse strains (control: n = 13; Psen2$^{+/-}$

1023 Psen1$^{\Delta IEC}$: n = 7). **(H)** Western blot analysis for Notch intracellular domain (NICD) in the ileum

1024 and colon of the different genotypes. β-actin was used as a loading control (representative of

1025 3 independent experiments). Overall, data are expressed as mean ± SD.

Suppl. Fig. 5: Absence of Psen2 alone does not disrupt intestinal tissue or immune

1027 **homeostasis.**

1028 **(A)** Western blot for Psen2 expression analyzed in protein extracts of ileum and colon from

1029 Psen2$^{+/+}$ and Psen2$^{-/-}$ mice (representative of 3 independent experiments). **(B)** Hematoxylin &

1030 eosin (H&E) staining images of ileal and colonic sections from Psen2$^{+/+}$ and Psen2$^{-/-}$ mice

1031 (scale bar 250 µm) (representative of 4 independent experiments). **(C)** qPCR analysis of

1032 various IEC differentiation markers (all normalized to *Gapdh*) in the ileum and colon of Psen2^{-}

1033 $^{/-}$ mice compared to their control littermates (Psen2$^{+/+}$: n = 7; Psen2$^{-/-}$: n = 9). **(D)** Representative

1034 IF images of different IEC differentiation markers: Muc2 (goblet cells), Chromogranin A (Chga;

1035 enteroendocrine cells), Dcamkl1 (tuft cells), Ki67 (proliferation), Olfm4 (stem cells), intestinal

1036 alkaline phosphatase (IAP; absorptive enterocytes) and lysozyme (Lyz; Paneth cells) in the

1037 ileum and colon of the different mouse genotypes (white scale bar 100 µm; yellow scale bar

1038 250 µm) (representative of 3 independent experiments). **(E)** Analysis of Chga- and Dcamkl1-

1039 positive cells per high power field (HPF) in the ileum and colon of Psen2$^{-/-}$ mice compared to

1040 their littermate controls (3 independent experiments). **(F)** qPCR analysis of several

1041 inflammatory markers in the ileum and colon of Psen2$^{+/+}$ and Psen2$^{-/-}$ mice (Psen2$^{+/+}$: n = 7;

1042 Psen2$^{-/-}$: n = 9). **(G)** Volcano plots of RNAseq data obtained from ileal tissue of Psen2$^{-/-}$ mice

1043 compared to their control mice. Red dots indicate upregulated genes, green dots indicate

1044 downregulation, while gray dots represent not differentially expressed genes (n = 3/group).

1045 Overall, data are expressed as mean ± SD.

Suppl. Fig. 6: Inducible presenilin double-knockout mice lack Psen1 and Psen2 in the

1047 **IECs.**

1048 **(A)** Schematic of the generation of inducible Psen1/2 double knockout mice. **(B)** *Psen1* mRNA

1049 expression levels in ileal and colonic tissue samples from control (Psen2$^{-/-}$ Psen1$^{fl/fl}$) and

1050 inducible Psen1/2 double knockout (iDKO; Psen2$^{-/-}$ Psen1$^{i\Delta IEC}$) mice. *Gapdh* was used for

1051 normalization (control: n = 5; iDKO: n = 7). **(C)** Psen1 and Psen2 protein levels analyzed by

1052 Western blot in the ileum and colon of the indicated mouse strains. β-actin was used as a

1053 loading control (representative of 3 independent experiments). **(D)** Representative IF images

1054 for Psen1 in ileum and colon of control and iDKO mice (scale bar 100 μm) (representative of

1055 3 independent experiments). **(E)** qPCR analysis for *Psen1* in Si organoids generated from the

1056 indicated genotypes (3 independent experiments). **(F)** Western blot analysis of Si organoid

1057 protein lysates from the different mouse strains (representative of 3 independent experiments).

1058 Overall, data are expressed as mean ± SD. *** indicates $p < 0.001$ by one-way ANOVA (B) or

1059 Mann-Whitney test (E).

1060 **Suppl. Fig. 7: IECs lacking Psen1 and Psen2 have a pro-inflammatory signature and an**

1061 **impaired differentiation.**

1062 **(A)** Schematic of treatment with 50 ng/ml tamoxifen in control (Psen2$^{-/-}$ Psen1$^{fl/fl}$) and inducible

1063 Psen1/2 double knockout (iDKO; Psen2$^{-/-}$ Psen1$^{i\Delta IEC}$) small intestinal organoids for 5 days. **(B)**

1064 Representative bright field images of Si organoids generated from control and iDKO mice

1065 stimulated as depicted in (A) (scale bar 100 μm) (representative of 3 independent experiments)

1066 with organoid size measurement. **(C)** Representative IF staining images of occludin in Si

1067 organoids generated from control and iDKO mice (scale bar 100 μm) (representative of 3

1068 independent experiments). **(D)** mRNA expression levels of various IEC differentiation markers

1069 in small intestinal organoids from the two different mouse strains. *Gapdh* was used for

1070 normalization (3 independent experiments). **(E)** Representative IF staining images for different

1071 IEC differentiation markers: Olfm4 (stem cells), ULEX (goblet cells), Chromogranin A (Chga;

1072 enteroendocrine cells), lysozyme (Lyz; Paneth cells), Dcamkl1 (tuft cells), and Ki67

1073 (proliferation) (scale bar 100 μm) (representative of 3 independent experiments). **(F)** Analysis

1074 of Notch intracellular domain (NICD) protein expression by Western blotting of small intestinal

1075 (Si) organoids established from iDKO and control mice. β-actin was used as a loading control

1076 (representative of 3 independent experiments). Overall, data are expressed as mean ± SD. **

1077 and *** indicate $p < 0.01$, and $p < 0.001$, respectively, by Mann-Whitney test.

Suppl. Fig. 8: Psen1 and Psen2 deficiency leads to impaired IEC differentiation.

(A) Selected Ingenuity Pathway Analysis (IPA; Qiagen) for affected diseases and functions in the RNAseq dataset from ileal tissue of inducible Psen1/2 double knockout (iDKO; $Psen2^{-/-}$ $Psen1^{i\Delta IEC}$) and control ($Psen2^{-/-}$ $Psen1^{fl/fl}$) mice (n = 3/group). Red bars indicate a positive Z-score (upregulation), green bars indicate a negative Z-score (downregulation). **(B)** Volcano plots of RNA sequencing analysis for goblet-, Paneth-, and enteroendocrine-specific genes (n = 3/group). **(C, D)** Representative staining images for different IEC differentiation markers in the ileum and colon of the mice: **(C)** Olfm4 (stem cells), Lgr5 (stem cells), Ki67 (proliferation), **(D)** lysozyme (Lyz; Paneth cells), Muc2 (goblet cells) and Chromogranin A (Chga; enteroendocrine) (scale bar 100 µm) with analysis of cell type abundance per high power field (HPF) (n = 5-10/group). Overall, data are expressed as mean ± SD. ** and *** indicate p < 0.01, and p < 0.001, respectively, by Student's t-test (C (Olfm4), D (Lyz)) or Mann-Whitney test (C (Lgr5, Ki67), D (Muc2, Chga)).

Suppl. Fig. 9: Presenilins play a crucial role in maintaining tissue homeostasis.

(A) Measurement of serum glucose levels of antibiotic-treated control and iDKO (control: n = 12; iDKO: n = 12). **(B)** Oral glucose tolerance test in control and iDKO mice using 2.5 g glucose/kg body weight (control: n = 5; iDKO: n = 6). **(C)** Schematic of the role of presenilins in intestinal homeostasis. Overall, data are expressed as mean ± SD. ** and *** indicate p < 0.01, and p < 0.001, respectively, by Student's t-test (A) or two-way ANOVA (B).

1098 **Suppl. Tab. 1**: KEGG pathway analysis of ileal samples from iDKO mice compared to control

1099 mice on day 8 after the first tamoxifen injection (p-adjusted < 0.05; $\log_2$foldchange ± 1).

Pathways	PValue	FDR
Metabolic pathways	1,20E-31	2,99E-29
Carbon metabolism	8,76E-12	1,09E-09
Peroxisome	1,35E-10	1,12E-08
Fat digestion and absorption	9,50E-08	5,91E-06
Biosynthesis of amino acids	2,24E-07	1,12E-05
PPAR signaling pathway	4,15E-06	1,54E-04
Glycerophospholipid metabolism	4,34E-06	1,54E-04
Biosynthesis of cofactors	7,62E-06	2,37E-04
Glycolysis / Gluconeogenesis	1,17E-05	3,12E-04
Citrate cycle (TCA cycle)	1,25E-05	3,12E-04
Bladder cancer	2,49E-05	5,63E-04
Maturity onset diabetes of the young	3,36E-05	6,79E-04
Mineral absorption	3,55E-05	6,79E-04
Ascorbate and aldarate metabolism	4,13E-05	7,34E-04
Bile secretion	5,38E-05	8,93E-04
Mucin type O-glycan biosynthesis	6,37E-05	9,32E-04
Glyoxylate and dicarboxylate metabolism	6,37E-05	9,32E-04
Fatty acid degradation	6,87E-05	9,50E-04
Pyruvate metabolism	7,31E-05	9,50E-04
Retinol metabolism	7,63E-05	9,50E-04
Proximal tubule bicarbonate reclamation	8,89E-05	0,00105362
Vitamin digestion and absorption	2,38E-04	0,00268898

Sulfur metabolism	2,61E-04	0,00283071
Pentose phosphate pathway	4,10E-04	0,00425848
Parathyroid hormone synthesis, secretion and action	5,81E-04	0,00578856
Pancreatic secretion	6,79E-04	0,00650625
Cellular senescence	8,64E-04	0,00796786
Arginine biosynthesis	0,00104523	0,00889548
Pentose and glucuronate interconversions	0,00108395	0,00889548
Fructose and mannose metabolism	0,00108395	0,00889548
Glycerolipid metabolism	0,00112913	0,00889548
beta-Alanine metabolism	0,00114319	0,00889548
Small cell lung cancer	0,00132942	0,01003105
Nitrogen metabolism	0,00139984	0,01025175
Human cytomegalovirus infection	0,00153482	0,01074698
Prostate cancer	0,00155378	0,01074698
Phosphatidylinositol signaling system	0,00215034	0,01376125
Tryptophan metabolism	0,00215538	0,01376125
ABC transporters	0,00215538	0,01376125
Folate biosynthesis	0,00235303	0,01464759
Glutathione metabolism	0,00255794	0,01527716
Gastric cancer	0,00257687	0,01527716
Steroid hormone biosynthesis	0,00297108	0,01720463
Arginine and proline metabolism	0,00330297	0,01869179
Chemical carcinogenesis - receptor activation	0,00343158	0,01898805
Central carbon metabolism in cancer	0,00360391	0,01950814
Cholesterol metabolism	0,00384428	0,02036652
Arachidonic acid metabolism	0,00418117	0,02168982

2-Oxocarboxylic acid metabolism	0,0048408	0,02459915
Calcium signaling pathway	0,00531401	0,02609483
PI3K-Akt signaling pathway	0,00534472	0,02609483
Drug metabolism - other enzymes	0,00548116	0,02624635
Valine, leucine and isoleucine degradation	0,00592837	0,02718675
Inositol phosphate metabolism	0,00597724	0,02718675
Fatty acid metabolism	0,0060051	0,02718675
alpha-Linolenic acid metabolism	0,00643233	0,02860089
p53 signaling pathway	0,00700645	0,03040931
NOD-like receptor signaling pathway	0,00708329	0,03040931
Alanine, aspartate and glutamate metabolism	0,00746731	0,03151456
EGFR tyrosine kinase inhibitor resistance	0,00797674	0,03310346
Kaposi sarcoma-associated herpesvirus infection	0,00854082	0,03450642
Histidine metabolism	0,00859196	0,03450642
Glycine, serine and threonine metabolism	0,00928812	0,0367102
Linoleic acid metabolism	0,0099596	0,03874907
Insulin secretion	0,01022729	0,03917838
Human T-cell leukemia virus 1 infection	0,01051735	0,03967909
Butanoate metabolism	0,01125943	0,04184476
Nicotinate and nicotinamide metabolism	0,01143596	0,0418758
Hepatitis C	0,01236444	0,04461951
Ras signaling pathway	0,01283799	0,04500141
Glucagon signaling pathway	0,01295917	0,04500141
TNF signaling pathway	0,01301246	0,04500141
Hematopoietic cell lineage	0,01437238	0,04902359

1100

1101 **Suppl. Tab. 2**: Gene ontology biological process pathway analysis of ileal samples from iDKO

1102 mice compared to control mice on day 8 after the first tamoxifen injection (p-adjusted < 0.05;

1103 $\log_2$foldchange ± 1).

Pathways	PValue	FDR
lipid metabolic process	5,70E-27	4,00E-23
transmembrane transport	1,12E-14	3,92E-11
fatty acid metabolic process	2,38E-10	4,55E-07
lipid transport	2,59E-10	4,55E-07
cholesterol homeostasis	1,64E-09	2,31E-06
gluconeogenesis	3,84E-08	4,49E-05
ion transport	6,98E-08	7,00E-05
immune system process	2,06E-07	1,80E-04
inflammatory response	2,31E-07	1,80E-04
positive regulation of peptidyl-tyrosine phosphorylation	8,31E-07	5,83E-04
sodium ion transport	1,30E-06	8,28E-04
receptor-mediated endocytosis	1,80E-06	0,00105003
response to nutrient	2,06E-06	0,00111205
very long-chain fatty acid metabolic process	3,90E-06	0,00183662
response to xenobiotic stimulus	3,93E-06	0,00183662
steroid metabolic process	5,03E-06	0,00220575
cell migration	6,14E-06	0,00253413
epidermal growth factor receptor signaling pathway	7,31E-06	0,00274161
fatty acid beta-oxidation	7,59E-06	0,00274161
phosphorylation	7,81E-06	0,00274161
aging	1,13E-05	0,00376145
response to sodium phosphate	2,35E-05	0,0074971
response to oxidative stress	2,99E-05	0,0091168

defense response to virus	3,96E-05	0,01159348
carbohydrate metabolic process	4,49E-05	0,0123596
cell surface receptor signaling pathway	4,62E-05	0,0123596
positive regulation of apoptotic process	4,76E-05	0,0123596
positive regulation of cellular respiration	6,86E-05	0,01719422
positive regulation of MAP kinase activity	7,28E-05	0,01761366
wound healing	7,83E-05	0,01832357
cholesterol metabolic process	8,42E-05	0,01905063
positive regulation of cell migration	8,96E-05	0,0196403
ERBB2-EGFR signaling pathway	1,03E-04	0,02185839
glucose metabolic process	1,11E-04	0,0228521
triglyceride biosynthetic process	1,16E-04	0,02322057
triglyceride homeostasis	1,25E-04	0,02410963
amino acid transport	1,27E-04	0,02410963
negative regulation of viral genome replication	1,41E-04	0,0259911
regulation of cell shape	1,56E-04	0,02808965
intestinal absorption	1,60E-04	0,02815363
negative regulation of inflammatory response	1,90E-04	0,03216618
phylloquinone catabolic process	1,93E-04	0,03216618
pyruvate metabolic process	2,13E-04	0,0347233
actin filament organization	2,44E-04	0,03888719
tricarboxylic acid cycle	2,51E-04	0,0392158
negative regulation of cysteine-type endopeptidase activity involved in apoptotic process	2,62E-04	0,03996263
ureteric bud development	2,87E-04	0,04283933
metal ion transport	2,93E-04	0,04283933
axon guidance	3,43E-04	0,04875527

	3,47E-04	0,04875527
one-carbon metabolic process		

1104

1105 **Suppl. Tab. 3**: Ingenuity Pathway Analysis for diseases and functions of ileal samples from

1106 iDKO mice compared to control mice on day 8 after the first tamoxifen injection (p-adjusted <

1107 0.05; $\log_2$foldchange ± 1).

Diseases or Functions Annotation	p-value	Activation z-score
Non-hematological solid tumor	2,54E-62	3,144
Epithelial neoplasm	6,50E-62	2,712
Nonhematologic malignant neoplasm	1,39E-61	2,466
Tumorigenesis of tissue	4,39E-61	2,565
Non-melanoma solid tumor	8,44E-61	3,739
Carcinoma	2,94E-60	2,775
Abdominal carcinoma	4,91E-54	2,787
Abdominal lesion	3,58E-53	3,647
Digestive organ tumor	1,43E-52	2,302
Adenocarcinoma	6,72E-52	2,534
Formation of solid tumor	9,68E-52	2,930
Abdominal neoplasm	2,74E-51	2,243
Abdominal adenocarcinoma	1,48E-50	2,648
Extrapancreatic malignant tumor	2,69E-50	2,299
Extracranial solid tumor	1,26E-49	2,919
Abdominal cancer	5,94E-49	2,079
Intraabdominal organ tumor	1,10E-48	2,380
Malignant solid tumor	1,23E-48	3,211
Solid tumor	1,25E-48	3,811
Digestive system cancer	2,30E-48	2,083
Cancer	8,37E-48	3,955

Generation of tumor	9,67E-48	3,267
Gastrointestinal tumor	2,79E-45	2,025
Large intestine neoplasm	1,62E-42	2,180
Malignant solid organ tumor	4,83E-41	2,279
Transport of molecule	1,12E-40	-4,012
Malignant neoplasm of large intestine	5,24E-39	2,070
Incidence of tumor	2,75E-34	2,035
Tumorigenesis of epithelial neoplasm	7,89E-34	2,650
Cell movement	1,57E-32	2,068
Migration of cells	4,51E-30	2,133
Synthesis of lipid	7,05E-30	-2,930
Fatty acid metabolism	1,72E-29	-3,385
Development of carcinoma	1,90E-29	2,575
Development of malignant tumor	3,50E-29	2,594
Breast or colorectal cancer	1,92E-23	2,795
Metabolism of terpenoid	9,70E-23	-2,772
Colorectal tumor	2,04E-22	2,180
Liver lesion	8,19E-22	3,555
Colon tumor	2,81E-20	2,382
Hepatobiliary carcinoma	1,99E-19	2,025
Metabolism of membrane lipid derivative	5,67E-19	-3,017
Oxidation of lipid	1,14E-18	-4,637
Steroid metabolism	1,47E-18	-2,343
Genitourinary carcinoma	1,50E-18	2,598
Liver carcinoma	3,60E-18	2,204
Colorectal cancer	4,65E-18	2,070

Liver tumor	2,76E-17	2,112
Proliferation of epithelial cells	9,10E-17	3,543
Organismal death	1,05E-16	3,759
Growth of lesion	1,08E-16	2,300
Growth of tumor	2,29E-16	2,242
Pelvic carcinoma	2,74E-15	2,121
Development of vasculature	3,54E-15	2,538
Growth of epithelial tissue	5,14E-15	2,726
Conversion of lipid	8,70E-15	-2,628
Oxidation of fatty acid	9,34E-15	-4,431
Angiogenesis	3,28E-14	2,523
Vasculogenesis	1,74E-13	2,575
Cell death of liver cells	2,04E-13	2,231
Transport of lipid	2,13E-13	-4,123
Lipolysis	3,14E-13	-2,070
Uptake of lipid	4,29E-13	-2,730
Necrosis of liver	5,30E-13	2,272
Accumulation of lipid	5,47E-13	2,345
Growth of solid tumor	5,78E-13	2,685
Cell death of liver	6,78E-13	2,353
Cell transformation	1,11E-12	2,306
Damage of genitourinary system	1,50E-12	2,158
Cell death of parenchymal cells	2,89E-12	2,310
Neoplasia of cells	3,04E-12	2,375
Infection by RNA virus	3,91E-12	-2,248
Necrosis of liver parenchyma	7,29E-12	2,433
Cell death of hepatocytes	1,47E-11	2,338

Beta-oxidation of fatty acid	1,60E-11	-3,040
Cell movement of tumor cell lines	2,22E-11	3,158
Quantity of metal	2,78E-11	2,781
Cancer of cells	8,13E-11	2,264
Damage of urinary system	9,11E-11	2,448
Invasive cancer	1,23E-10	2,731
Viral Infection	1,36E-10	-2,752
Advanced malignant tumor	1,58E-10	2,719
Metabolism of sterol	1,64E-10	-2,206
Apoptosis of liver cells	1,87E-10	3,268
Damage of kidney	2,02E-10	2,348
Differentiation of epithelial cells	2,70E-10	2,116
Apoptosis of liver	3,21E-10	3,359
Lymphoid cancer	3,89E-10	2,145
Export of molecule	5,18E-10	-2,267
Differentiation of epithelial tissue	6,34E-10	2,006
Familial neurological disorder	7,12E-10	2,058
Quantity of metal ion	7,14E-10	2,841
Conversion of fatty acid	8,50E-10	-2,750
Flux of lipid	9,78E-10	-2,029
Apoptosis of hepatocytes	1,27E-09	2,939
Urination disorder	1,39E-09	2,210
Quantity of Ca2+	1,42E-09	2,436
Metabolism of xenobiotic	1,77E-09	-3,266
Accumulation of acylglycerol	1,85E-09	2,083

1108

1109 **Suppl. Tab. 4**: Pathoclinical characteristics of IBD patients included in the

1110 immunofluorescence staining analysis (n=14) (NA: not available).

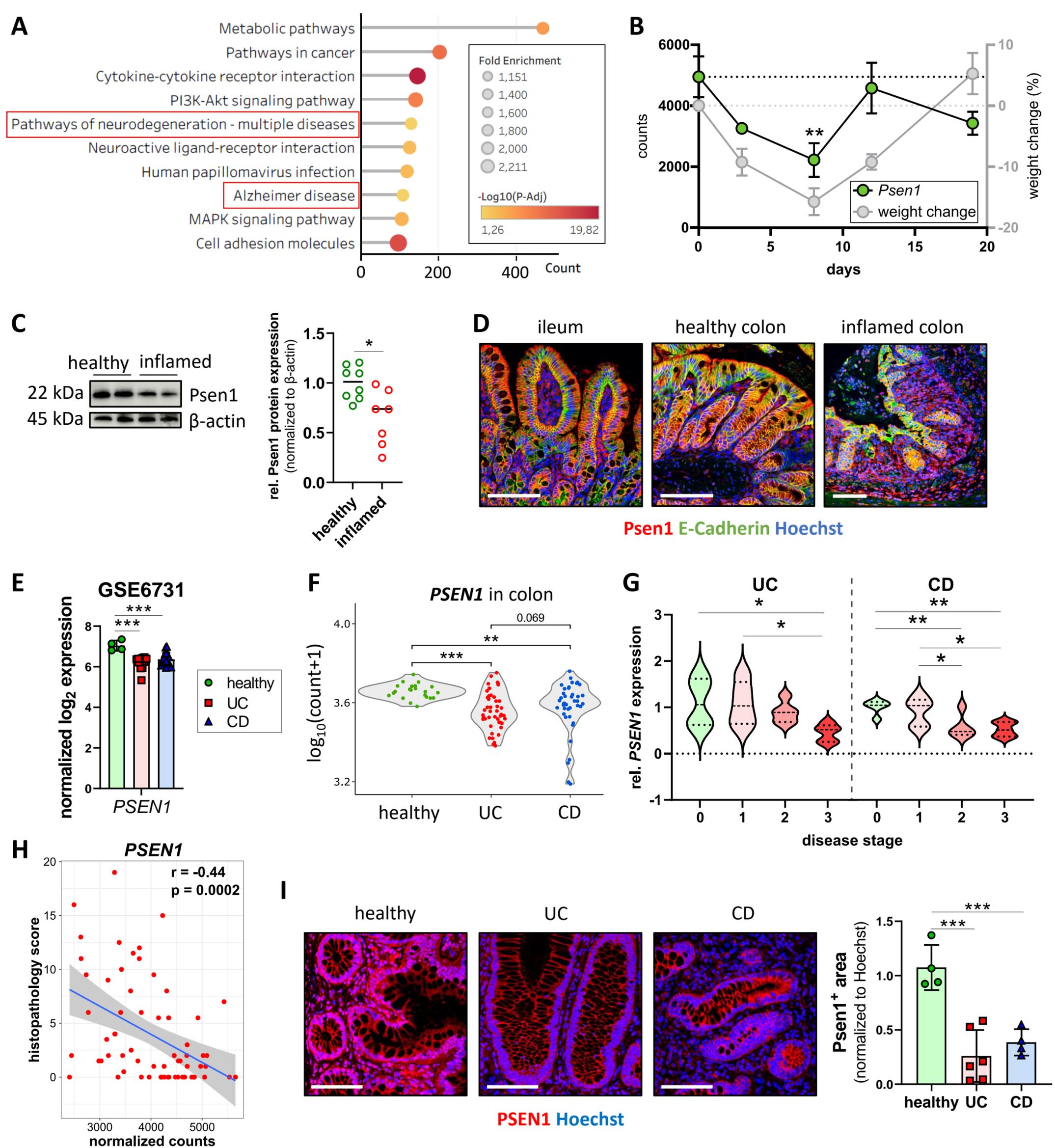| Group	No.	Localization	Histopathology score
healthy	1	Colon	0
healthy	2	Colon	0
healthy	3	Sigmoid colon	0
healthy	4	Ascending colon	0
UC	1	Colon	13
UC	2	Colon	17
UC	3	Ascending colon	17
UC	4	Transverse colon	18
UC	5	Sigmoid colon	7
UC	6	Sigmoid colon	NA
CD	1	Colon	7
CD	2	Ileum	8
CD	3	Ileum	9
CD	4	Ileum	10.5

1111

Fig. 1: Psen1 is expressed in the intestine and is differentially regulated during intestinal inflammation

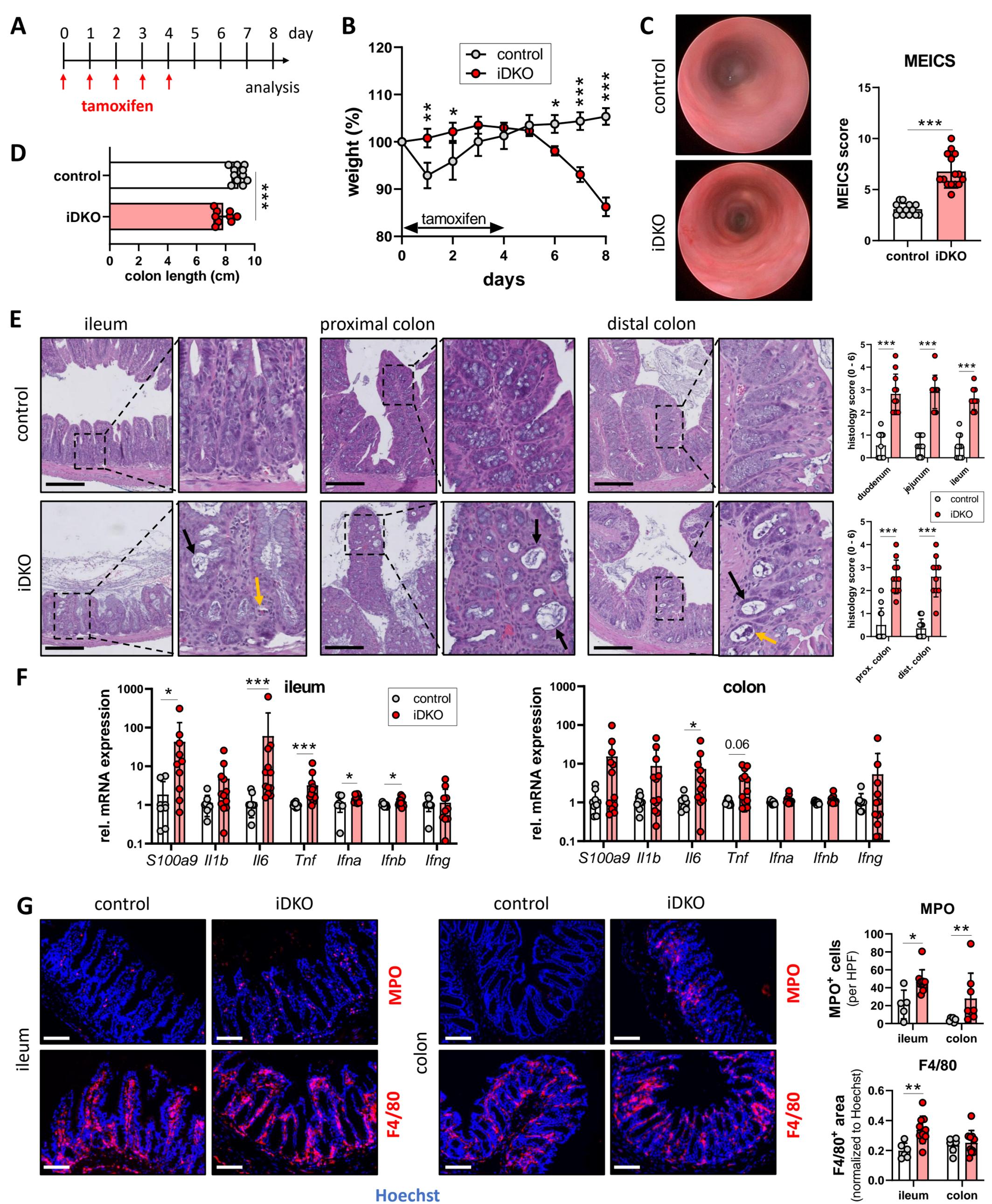Fig. 2: Inducible Psen1/2 double knockout mice spontaneously develop colitis
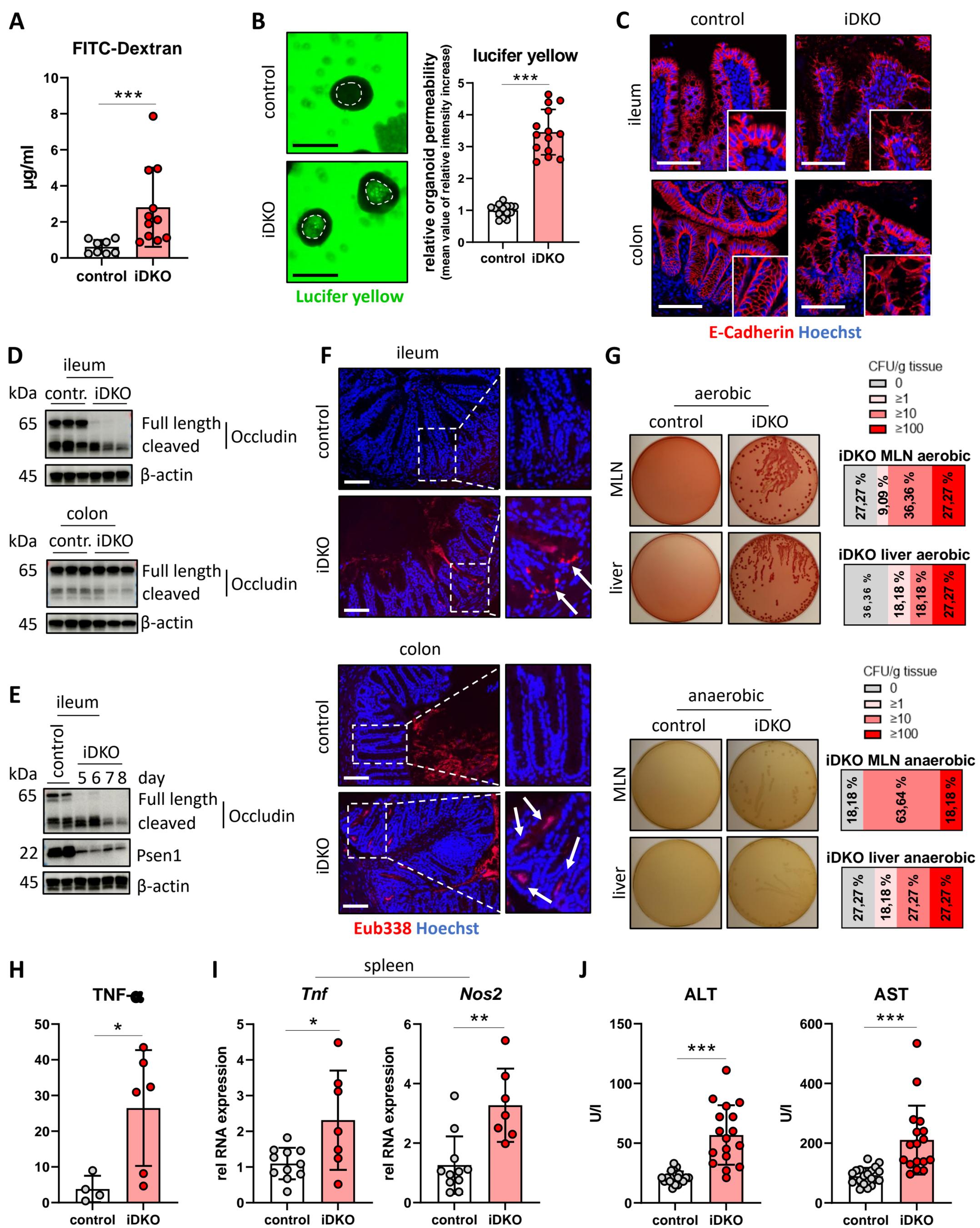
Fig. 3: Presenilins are key to intestinal barrier function

Fig. 4: Microbiota depletion improves spontaneous inflammation but not body weight loss in inducible Psen1/2 double knockout mice

Fig. 5: Dual presenilin deletion leads to enterocyte loss, malnutrition and death in presenilin double knockout mice due to Notch signaling disruption

Suppl. Fig. 1: Involvement of genes associated with neurodegeneration and Alzheimer's disease in intestinal inflammation

Suppl. Fig. 2: Generation of intestinal epithelial specific Psen1 deficient mice

A

Psen1^fl/fl   Psen1^ΔIEC   Psen1^fl/fl   Psen1^ΔIEC

ileum / colon

B

ileum — rel. mRNA expression
Psen1^fl/fl, Psen1^ΔIEC
Gpr49, Olfm4, Hes1, Atoh1, Tff3, Lyz, Dclk1, Ki67

colon — rel. mRNA expression
Gpr49, Hes1, Atoh1, Tff3, Dclk1

C

Muc2   Chga   Dcamkl1   Ki67   Olfm4   IAP   Lyz

Psen1^fl/fl / Psen1^ΔIEC (ileum, Hoechst)

Psen1^fl/fl / Psen1^ΔIEC (colon, Hoechst)

D

ileum — pos. cells/HPF
Psen1^fl/fl, Psen1^ΔIEC
Chga, Dcamkl1

colon — pos. cells/HPF
Chga, Dcamkl1

E

ileum — rel. mRNA expression
Psen1^fl/fl, Psen1^ΔIEC
S100a9, Tnf, Nos2, Il1b, Il6

colon — rel. mRNA expression
S100a9, Tnf, Nos2, Il1b, Il6

F

ileum — $-\log_{10}$(pvalue) vs $\log_2$(FoldChange)
Psen1, Mt1

colon — $-\log_{10}$(pvalue) vs $\log_2$(FoldChange)
Psen1, Mt1

G

colon | ileum
fl/fl | ΔIEC | fl/fl | ΔIEC

23 kDa — PSEN2
45 kDa — β-actin

PSEN2 — rel. protein expression (PSEN2/β-actin)
fl/fl, ΔIEC
ileum **, colon *

Suppl. Fig. 3: Absence of Psen1 alone in IECs does not disrupt intestinal tissue or immune homeostasis

A

Psen2⁻/⁻ Psen1^wt/wt ✕ Psen2⁺/⁺ Psen1^ΔIEC

Psen2⁻/⁻ Psen1^fl/fl **control**

Psen2⁺/⁻ Psen1^ΔIEC

Psen2⁻/⁻ Psen1^ΔIEC

B

Psen2⁻/⁻ Psen1^fl/fl ✕ Psen2⁺/⁻ Psen1^ΔIEC	postnatal		
	expected	obtained	
	%	%	number
Psen2⁺/⁻ Psen1^fl/fl	25	38.8	19
Psen2⁻/⁻ Psen1^fl/fl (control)	25	36.7	18
Psen2⁺/⁻ Psen1^ΔIEC	25	24.5	12
Psen2⁻/⁻ Psen1^ΔIEC	25	0	0

C weight (%) vs week; control (open), Psen2⁺/⁻ Psen1^ΔIEC (red)

D control; Psen2⁺/⁻ Psen1^ΔIEC

MEICS — MEICS score; control, Psen2⁺/⁻ Psen1^ΔIEC

E ileum / proximal colon / distal colon; control, Psen2⁺/⁻ Psen1^ΔIEC

F control; Psen2⁺/⁻ Psen1^ΔIEC — Olfm4, Muc2, Hoechst

G rel. mRNA expression: *Olfm4*, *Lyz*, *Muc2*, *Tnf*; control (open), Psen2⁺/⁻ Psen1^ΔIEC (red); ileum, colon

H ileum; control, Psen2⁺/⁻ Psen1^ΔIEC — 110 kDa NICD, 45 kDa β-actin

colon; control, Psen2⁺/⁻ Psen1^ΔIEC — 110 kDa NICD, 45 kDa β-actin

Suppl. Fig. 4: One allele of Psen2 is sufficient to maintain intestinal homeostasis in heterozygous presenilin double knockout mice

A

ileum
+/+ -/-
23 kDa — Psen2
45 kDa — β-actin

colon
+/+ -/-
23 kDa — Psen2
45 kDa — β-actin

B

ileum
Psen2$^{+/+}$ Psen2$^{-/-}$

colon
Psen2$^{+/+}$ Psen2$^{-/-}$

C

ileum

rel. mRNA expression

● Psen2$^{+/+}$
● Psen2$^{-/-}$

Gpr49 Olfm4 Hes1 Atoh1 Muc2 Tff3 Lyz Dclk1 Ki67

colon

rel. mRNA expression

Gpr49 Hes1 Atoh1 Muc2 Tff3 Dclk1 Ki67

D

Muc2   Chga   Dcamkl1   Ki67   Olfm4   IAP   Lyz

Psen2$^{+/+}$
Psen2$^{-/-}$

Ileum / Hoechst

Psen2$^{+/+}$
Psen2$^{-/-}$

colon / Hoechst

E

ileum

pos. cells/HPF

● Psen2$^{+/+}$
● Psen2$^{-/-}$

Chga   Dcamkl1

colon

pos. cells/HPF

Chga   Dcamkl1

F

ileum

rel. mRNA expression

● Psen2$^{+/+}$
● Psen2$^{-/-}$

Tnf Nos2 Il1b Il6

colon

rel. mRNA expression

Tnf Nos2 Il1b Il6

G

ileum

-log$_{10}$(pvalue)

NA

Rhox6

log$_2$(FoldChange)

Suppl. Fig. 5: Absence of Psen2 alone does not disrupt intestinal tissue or immune homeostasis

Suppl. Fig. 6: Inducible Psen1/2 double knockout mice lack Psen1 and Psen2 in the IECs

A

0 1 2 3 4 5 days

tamoxifen

analysis

split

B

control   iDKO

organoid size

area/organoid

control   iDKO

***

C

Occludin Hoechst

D

cell differentiation

rel. mRNA expression

○ control
● iDKO

Gpr49 Olfm4 Hes1 Atoh1 Muc2 Tff3 Lyz Dclk1 Ki67

*** *** *** *** *** *** ** **

E

Olfm4 ULEX Chga Lyz Dcamkl1 Ki67

control

iDKO

Hoechst

F

control iDKO

110 kDa   NICD

45 kDa   β-actin

Suppl. Fig. 7: IECs lacking Psen1 and Psen2 have a pro-inflammatory signature and an impaired IEC differentiation

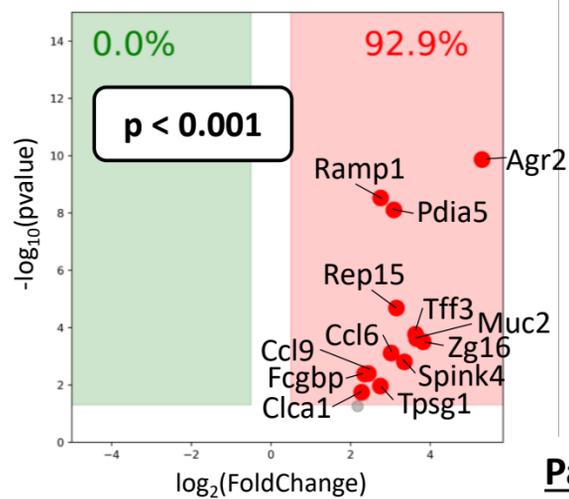**A. IPA Diseases and Functions**
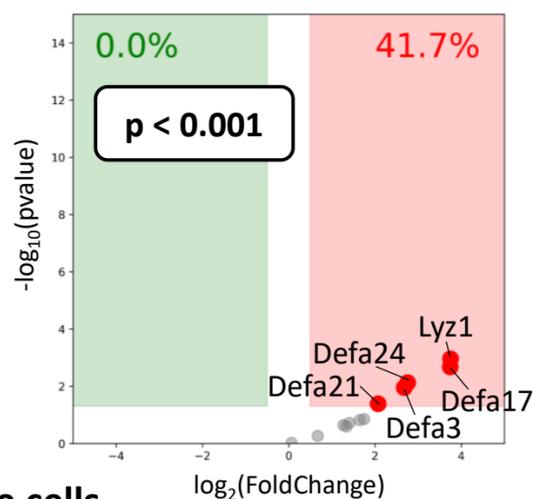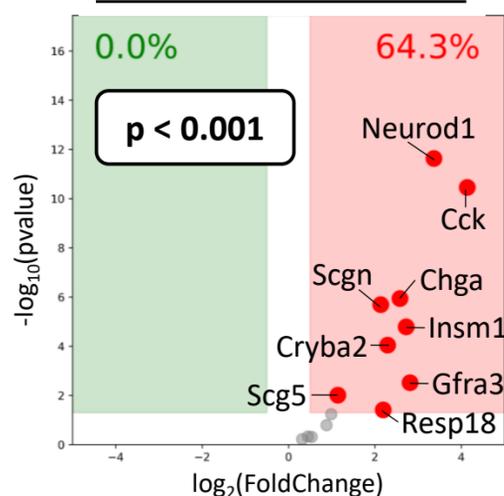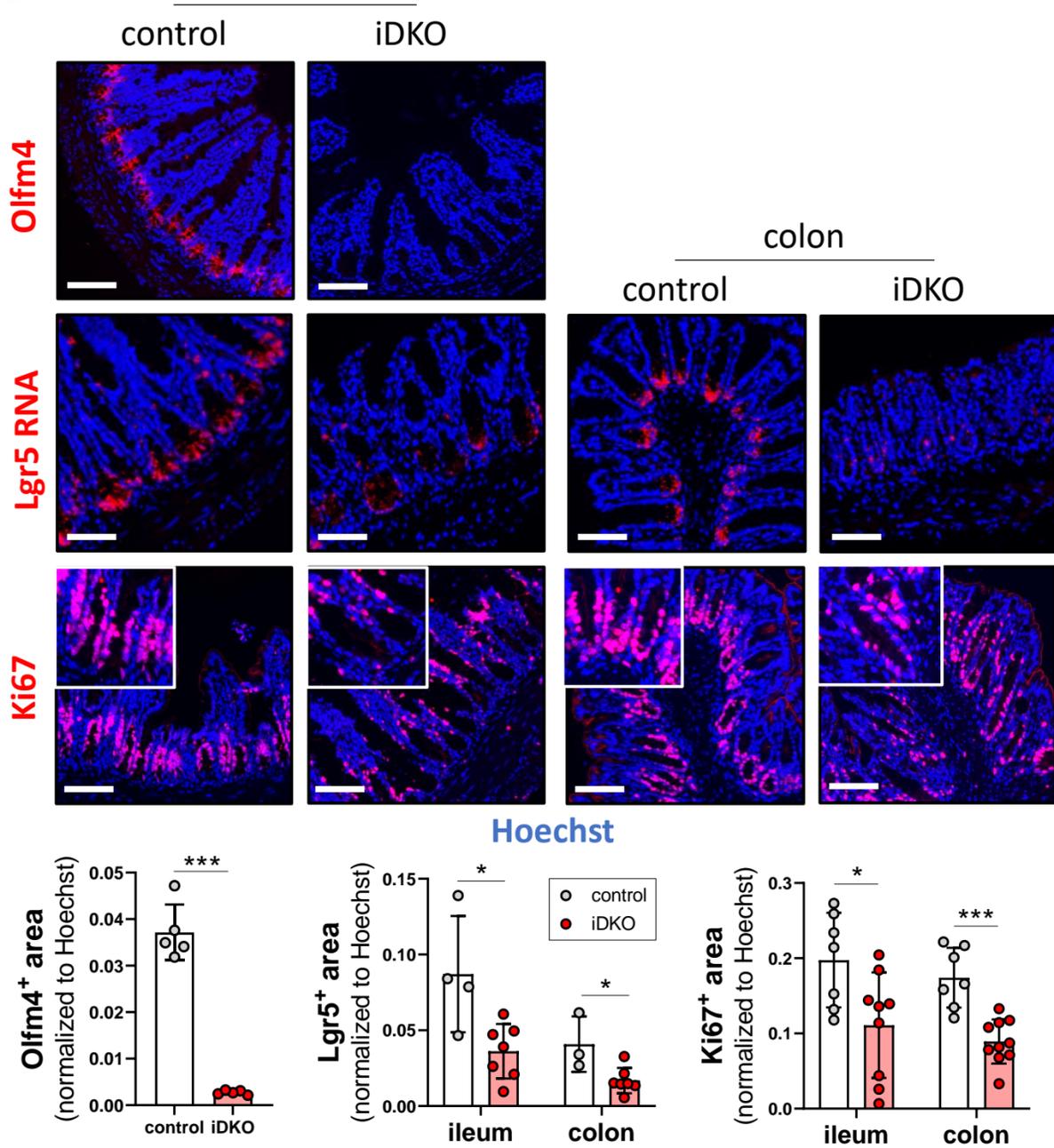
Top panel (red bars, Z-score and p-value):
- Organismal death
- Proliferation of epithelial cells
- Epithelial neoplasm
- Angiogenesis
- Large intestine neoplasm
- Migration of cells
- Differentiation of epithelial cells
- Malignant neoplasm of large intestine
- Cell movement
- Gastrointestinal tumor
- Differentiation of epithelial tissue

Bottom panel (green bars, Z-score):
- Flux of lipid
- Lipolysis
- Steroid metabolism
- Conversion of lipid
- Uptake of lipid
- Conversion of fatty acid
- Synthesis of lipid
- Metabolism of membrane lipid derivative
- Fatty acid metabolism
- Transport of molecule
- Transport of lipid
- Oxidation of fatty acid
- Oxidation of lipid

B.

Goblet cells — 0.0% / 92.9% — p < 0.001 — genes: Agr2, Ramp1, Pdia5, Rep15, Ccl6, Tff3, Muc2, Ccl9, Zg16, Fcgbp, Spink4, Clca1, Tpsg1

Paneth cells — 0.0% / 41.7% — p < 0.001 — genes: Lyz1, Defa24, Defa21, Defa17, Defa3

Enteroendocrine cells — 0.0% / 64.3% — p < 0.001 — genes: Neurod1, Cck, Scgn, Chga, Insm1, Cryba2, Gfra3, Scg5, Resp18

C. ileum / colon — Olfm4, Lgr5 RNA, Ki67, Hoechst — control vs iDKO

Olfm4$^+$ area (normalized to Hoechst): control vs iDKO ***
Lgr5$^+$ area (normalized to Hoechst): ileum *, colon *
Ki67$^+$ area (normalized to Hoechst): ileum *, colon ***

D. ileum / colon — Lyz, Muc2, Chga, Hoechst — control vs iDKO

Lyz$^+$ area (normalized to Hoechst): control vs iDKO ***
Muc2$^+$ area (normalized to Hoechst): ileum ***, colon
Chga$^+$ cells (per HPF): ileum ***, colon *

Suppl. Fig. 8: Psen1 and Psen2 deficiency leads to impaired IEC differentiation

A glucose in serum

B oral glucose tolerance test

C Presenilins functional

NOTCH signaling functional

functional barrier
functional nutrient absorption

Suppl. Fig. 9: Presenilins play a crucial role in maintaining tissue homeostasis