

Expanded View Figures

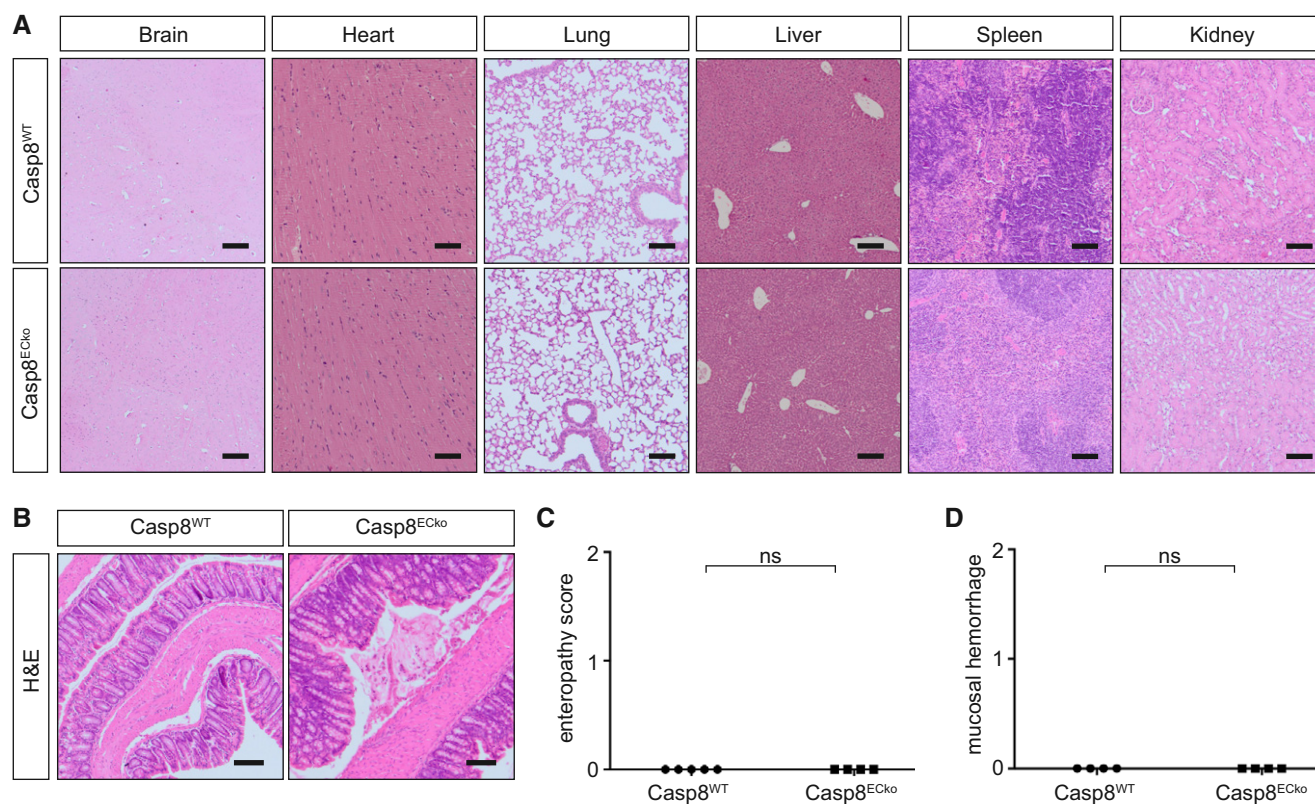


Figure EV1. Casp8^{ECKO} mice do not present defects in other organs.

A Representative images of H&E staining of the indicated organs in Casp8^{WT} and Casp8^{ECKO} mice at a late disease stage ($n = 4-5$ WT, 5 ECKO). Scale bars 100 μ m.

B-D Representative images of H&E staining of the large intestine (colon) (**B**). Quantification of intestinal pathology (**C**) and mucosal hemorrhages (**D**) in Casp8^{WT} and Casp8^{ECKO} mice at a late disease stage (enteropathy score; $n = 4$ WT, 4 ECKO; two-tailed unpaired Student's t -test). Scale bars: 100 μ m.

Data information: All data is shown as mean $\pm$ SEM. ns: not significant.

Source data are available online for this figure.

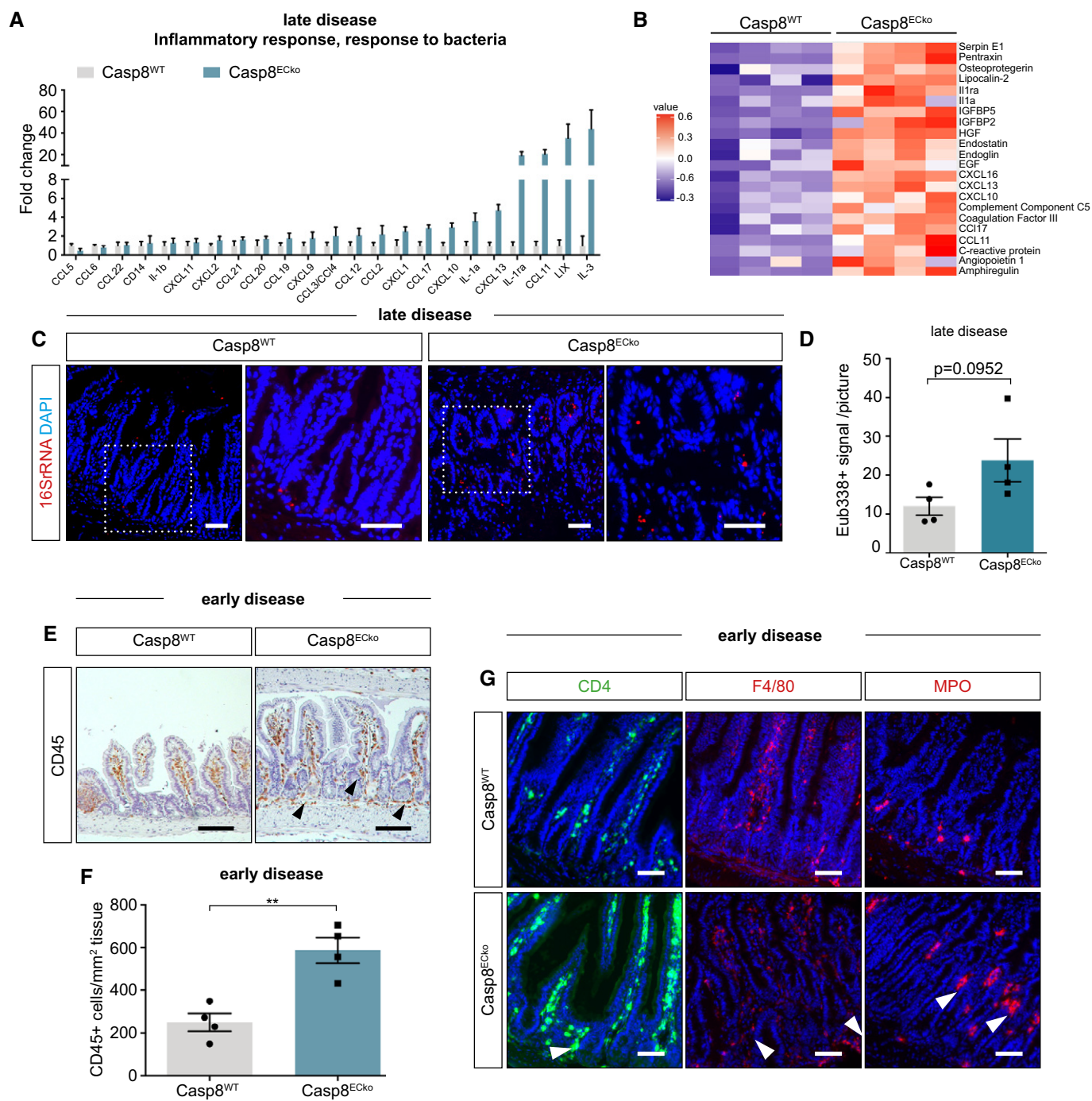


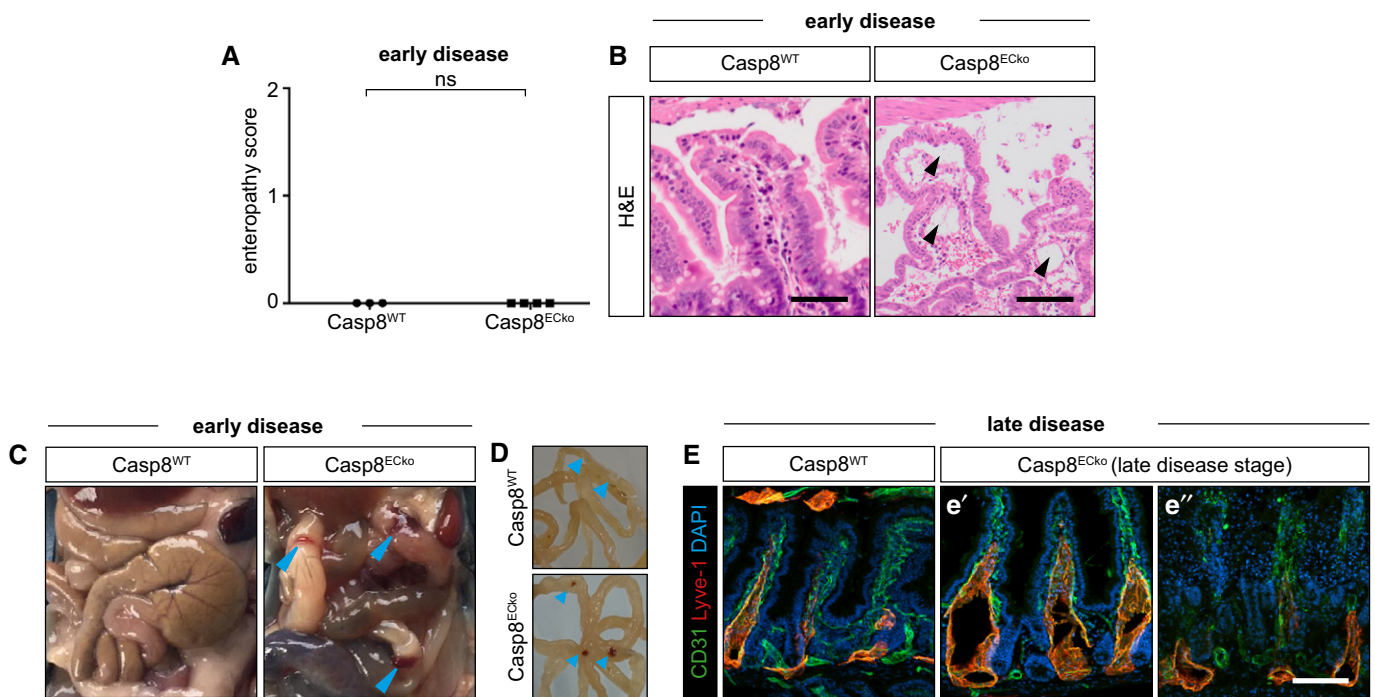
Figure EV2.

Figure EV2. Loss of Casp8 in ECs leads to small bowel inflammation.

- A Graph showing increased expression of proteins associated to the GOs inflammatory response and bacterial response in small intestinal samples of Casp8^{ECKO} mice ($n = 4$ WT, 4 ECKO), identified via a proteome profiler analysis.
- B Row-normalized heat map of significantly upregulated genes in the proteome profiler analysis of small intestinal samples of Casp8^{ECKO} compared to Casp8^{WT} mice ($n = 4$ WT, 4 ECKO, unpaired student t -test, unadjusted P -values).
- C, D Representative pictures (C) and quantification (D) of bacterial 16S rRNA in Casp8^{WT} and Casp8^{ECKO} mice by *FISH* at a late disease stage ($n = 4$ WT, 4 ECKO two-tailed unpaired student t -test). Scale bars: 50 μ m.
- E, F Representative pictures (E) and quantification (F) of CD45⁺ cells in deep layers of the lamina in the small intestine of Casp8^{WT} and Casp8^{ECKO} mice ($n = 4$ WT, 4 ECKO, two-tailed unpaired student t -test. Black arrowheads point to accumulated CD45⁺ cells). Scale bars: 50 μ m.
- G Representative pictures of T-cells (CD4⁺), macrophages (F4/80⁺), and granulocytes (MPO⁺) in the small intestine of Casp8^{WT} and Casp8^{ECKO} mice, showing accumulation of these cells in the crypt area (white arrowheads) in Casp8^{ECKO} mice already at early disease stages. Scale bars: 50 μ m.

Data information: All data is shown as mean $\pm$ SEM. ** $P < 0.01$, ns: not significant.

Source data are available online for this figure.

**Figure EV3. Casp8^{ECKO} mice present increasing vascular disintegration, including edema and loss of tissue architecture in the course of disease progression.**

- A Quantification of intestinal pathology at an early disease stage (enteropathy score; $n = 3$ WT, 4 ECKO, two-tailed unpaired student t -test).
- B Representative pictures of the small intestinal villi of Casp8^{WT} and Casp8^{ECKO} mice at an early disease stage (black arrow heads point to edema in Casp8^{ECKO} mice). Scale bars: 100 μ m.
- C, D Representative pictures of the small intestine of Casp8^{WT} and Casp8^{ECKO} mice at an early disease stage (blue arrow heads point to Peyer's Patches in Casp8^{ECKO} mice). Cleaned intestines are shown in D for better visualization of Peyer's Patches (blue arrowheads).
- E Representative images of stainings for CD31, Lyve1, and DAPI in small intestinal sections of Casp8^{WT} and Casp8^{ECKO} mice at a late disease stage. Lacteals in Casp8^{ECKO} mice are severely dilated in villi that are still present (e'). Most of the villi are blunted, showing severe tissue disintegration (e''). Scale bars: 100 μ m.

Data information: All data is shown as mean $\pm$ SEM. ns: not significant.

Source data are available online for this figure.

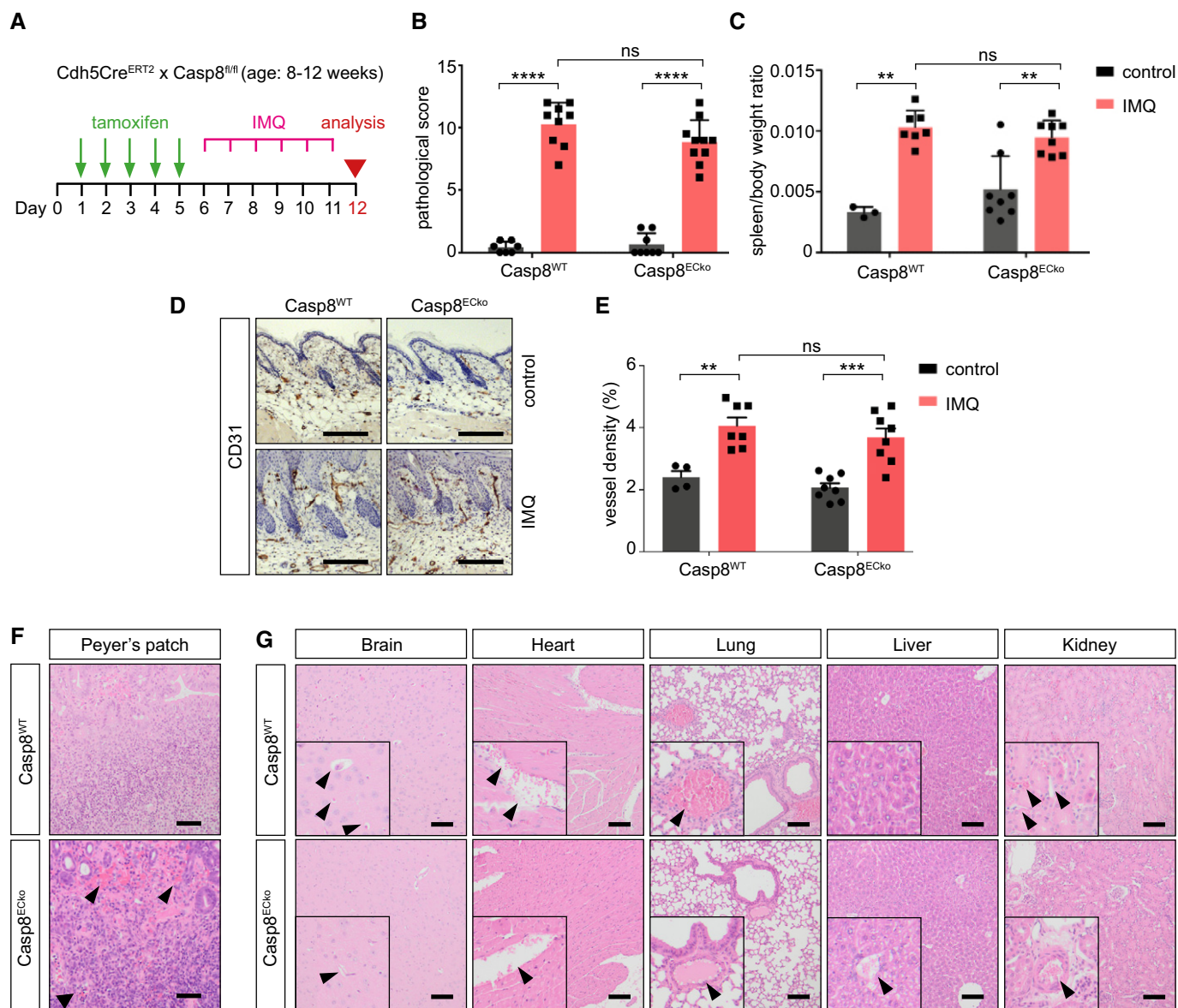


Figure EV4. Inflammation alone is not sufficient to induce vascular defects and pathology in other organs in $Casp8^{Ecko}$ mice.

- A Schematic representation of imiquimod (IMQ) treatment on the back skin of $Casp8^{WT}$ and $Casp8^{Ecko}$ mice.
- B, C Quantification of skin pathology (B) and spleen weight (C) in $Casp8^{WT}$ and $Casp8^{Ecko}$ mice with and without IMQ treatment ($n = 4-7$ WT control, 7-9 WT IMQ, 8 Ecko control, 8-10 Ecko IMQ; two-way ANOVA with Sidak's multiple comparison).
- D Representative pictures of CD31 and hematoxylin staining on skin cross sections of $Casp8^{WT}$ and $Casp8^{Ecko}$ mice with and without IMQ treatment. Scale bars: 100 μ m.
- E Graph showing quantification of CD31⁺ vessel area upon IMQ treatment in $Casp8^{WT}$ and $Casp8^{Ecko}$ mice ($n = 4$ WT control, 7 WT IMQ, 8 Ecko control, 8 Ecko IMQ; two-way ANOVA with Sidak's multiple comparison).
- F Representative images of H&E staining of Peyer's Patches from TNF- α treated $Casp8^{WT}$ and $Casp8^{Ecko}$ mice. Black arrowheads point to hemorrhages ($n = 5$ WT, 5 Ecko). Scale bars: 50 μ m.
- G Representative images of H&E staining of the indicated organs in $Casp8^{WT}$ and $Casp8^{Ecko}$ mice 24 h after TNF- α injection ($n = 5$ WT, 5 Ecko). Black arrowheads point to erythrocytes inside of (healthy) blood vessels. Scale bars: 100 μ m.

Data information: All data is shown as mean $\pm$ SEM. ** $P < 0.01$ *** $P < 0.001$, **** $P < 0.0001$; ns: not significant.

Source data are available online for this figure.

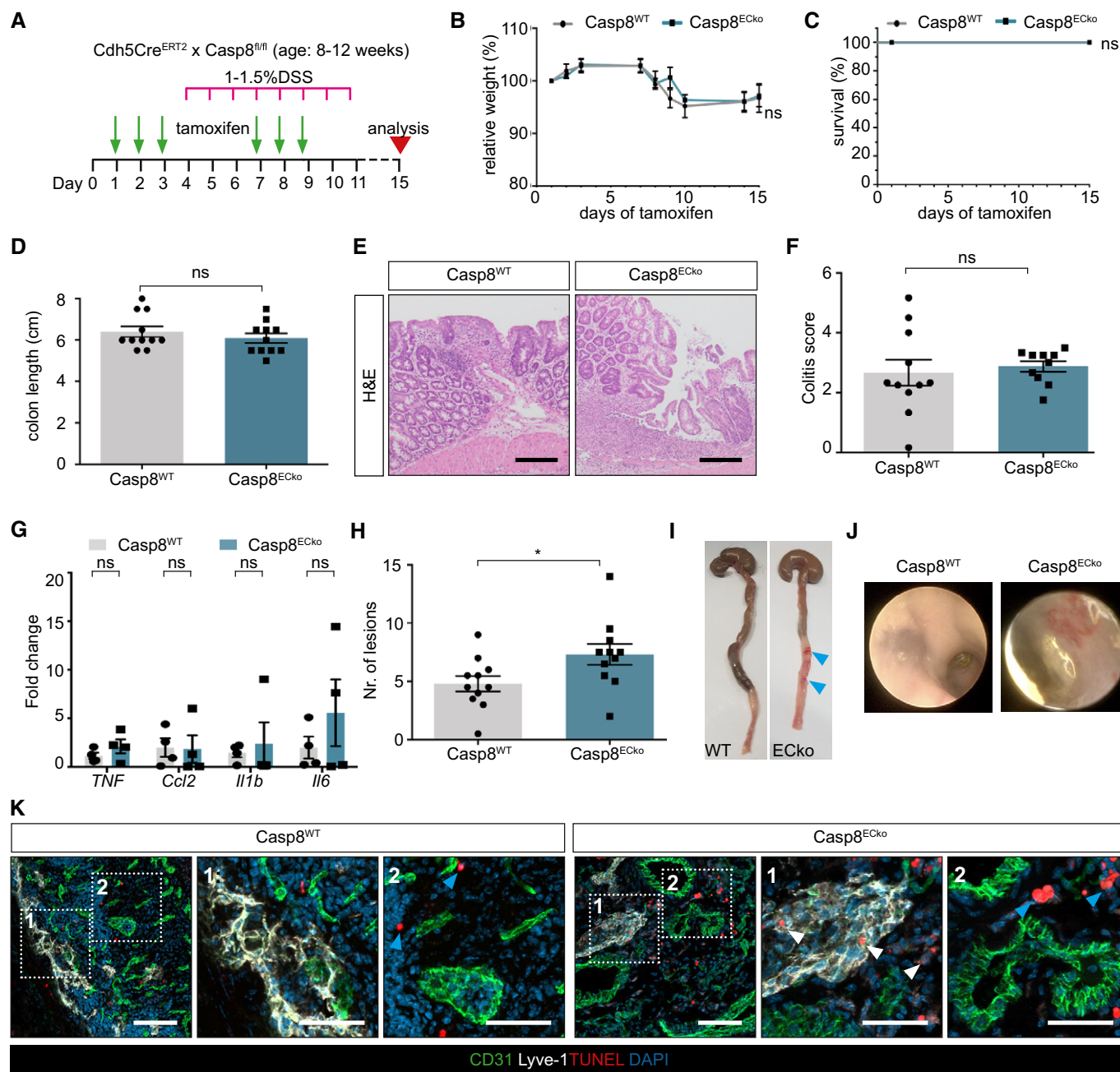


Figure EV5. *Casp8^{Ecko}* mice are more susceptible to colitis development upon DSS treatment.

A Schematic representation of low dosage DSS treatment via the drinking water in *Casp8^{WT}* and *Casp8^{Ecko}* mice.

B, C Graphs showing weight loss (**B**) and lethality (**C**) in *Casp8^{WT}* and *Casp8^{Ecko}* mice ($n = 11$ WT, 11 ECKO; **B**: curve comparison; **C**: Log-Rank test).

D Colon length of *Casp8^{WT}* and *Casp8^{Ecko}* mice after tamoxifen treatment ($n = 11$ WT, 11 ECKO; two-tailed unpaired Student's *t*-test).

E, F Representative images (**E**) and histopathological evaluation (**F**) of H&E staining of the large intestine of *Casp8^{WT}* and *Casp8^{Ecko}* mice ($n = 11$ WT, 10 ECKO; unpaired *t*-test with Welch's correction). Scale bars: 100 μ m.

G QPCR analysis of pro-inflammatory cytokines in *Casp8^{WT}* and *Casp8^{Ecko}* mice upon DSS treatment ($n = 4$ WT, 4 ECKO; multiple *t*-tests with Holm-Sidak method).

H Quantification of the total number of inflammatory lesions per section in the colon of *Casp8^{WT}* compared to *Casp8^{Ecko}* mice upon DSS treatment ($n = 11$ WT, 11 ECKO, unpaired Student's *t*-test).

I, J Representative pictures of large intestines (**I**) and colon endoscopy (**J**) of *Casp8^{WT}* mice and *Casp8^{Ecko}* mice after DSS treatment. Blue arrowheads point to hemorrhages.

K Representative pictures of TUNEL staining together with CD31 and Lyve-1 staining shows TUNEL⁺ LECs, not BECs in *Casp8^{Ecko}*, but not *Casp8^{WT}* mice upon DSS treatment. Blue arrow heads point TUNEL⁺ cells outside the vessels. White arrow heads point to TUNEL⁺ cells inside Lyve-1⁺ lymphatics. Scale bars: 50 μ m, insets 25 μ m.

Data information: Data shown as mean $\pm$ SEM. * $P < 0.05$; ns: not significant.

Source data are available online for this figure.