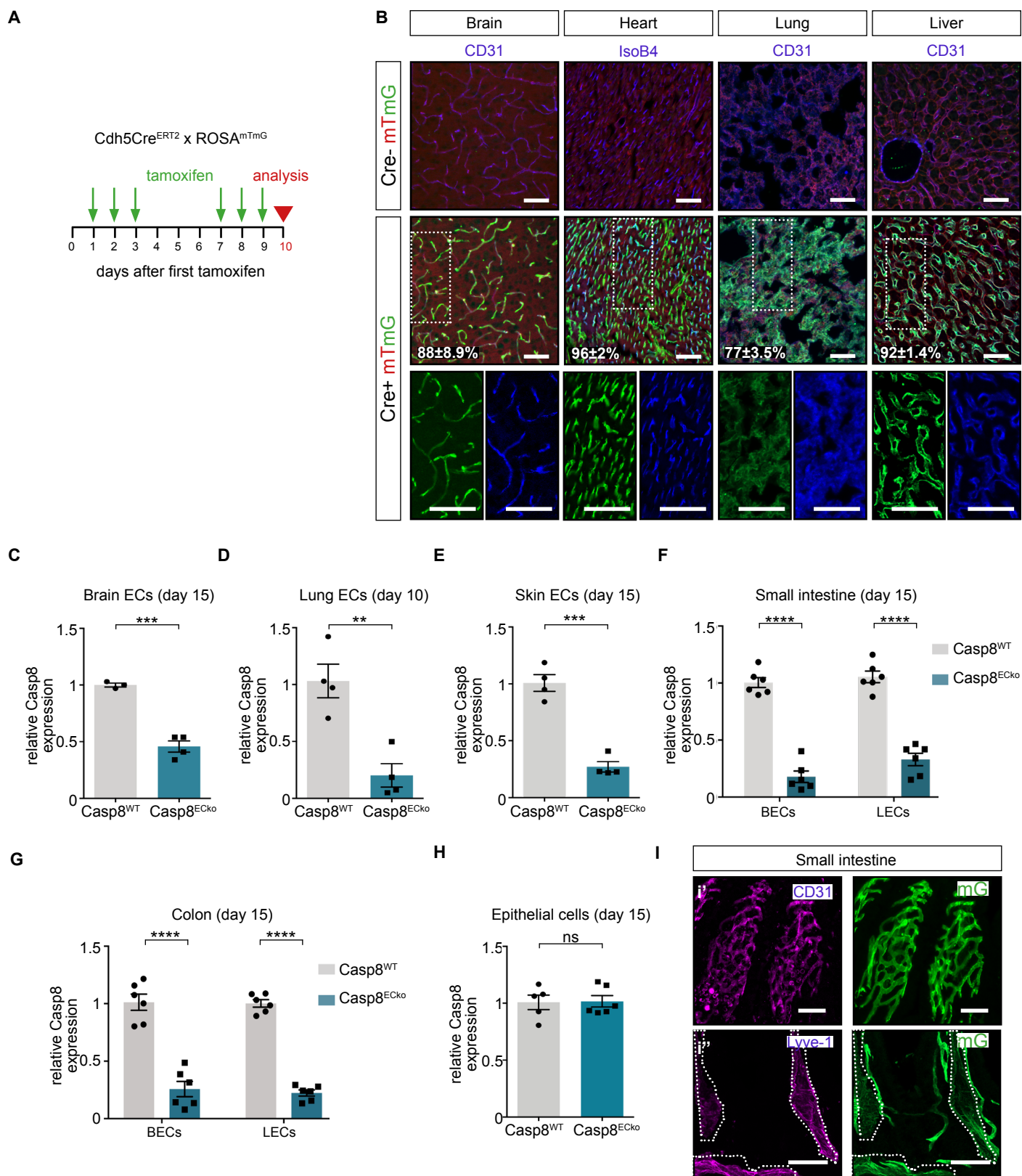


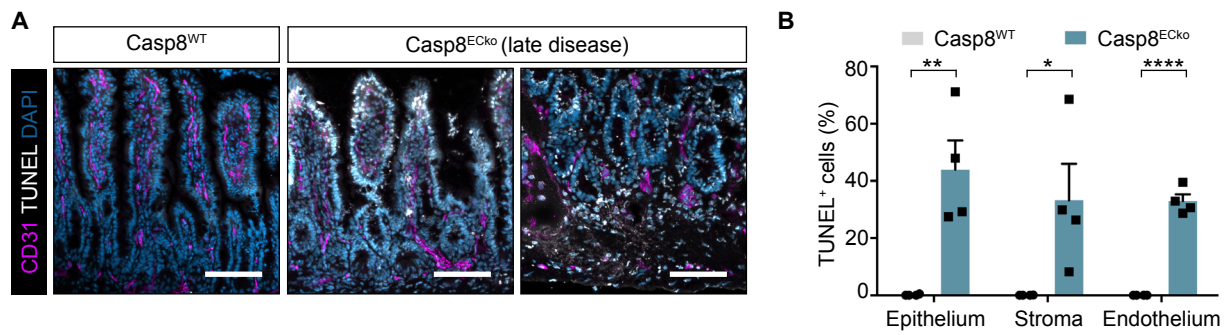
Table of Content

1. Appendix Supplementary Figures S1-S7
2. Appendix Supplementary Figure Legends
3. Appendix Table S1
4. Appendix Table S2

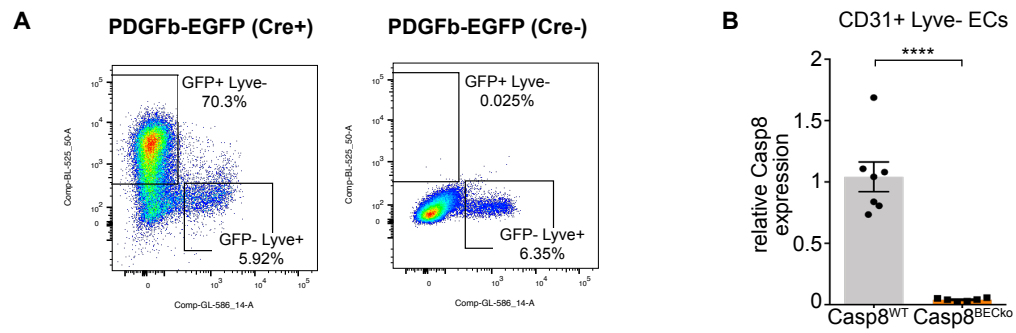
1. Appendix Supplementary Figures S1-S7



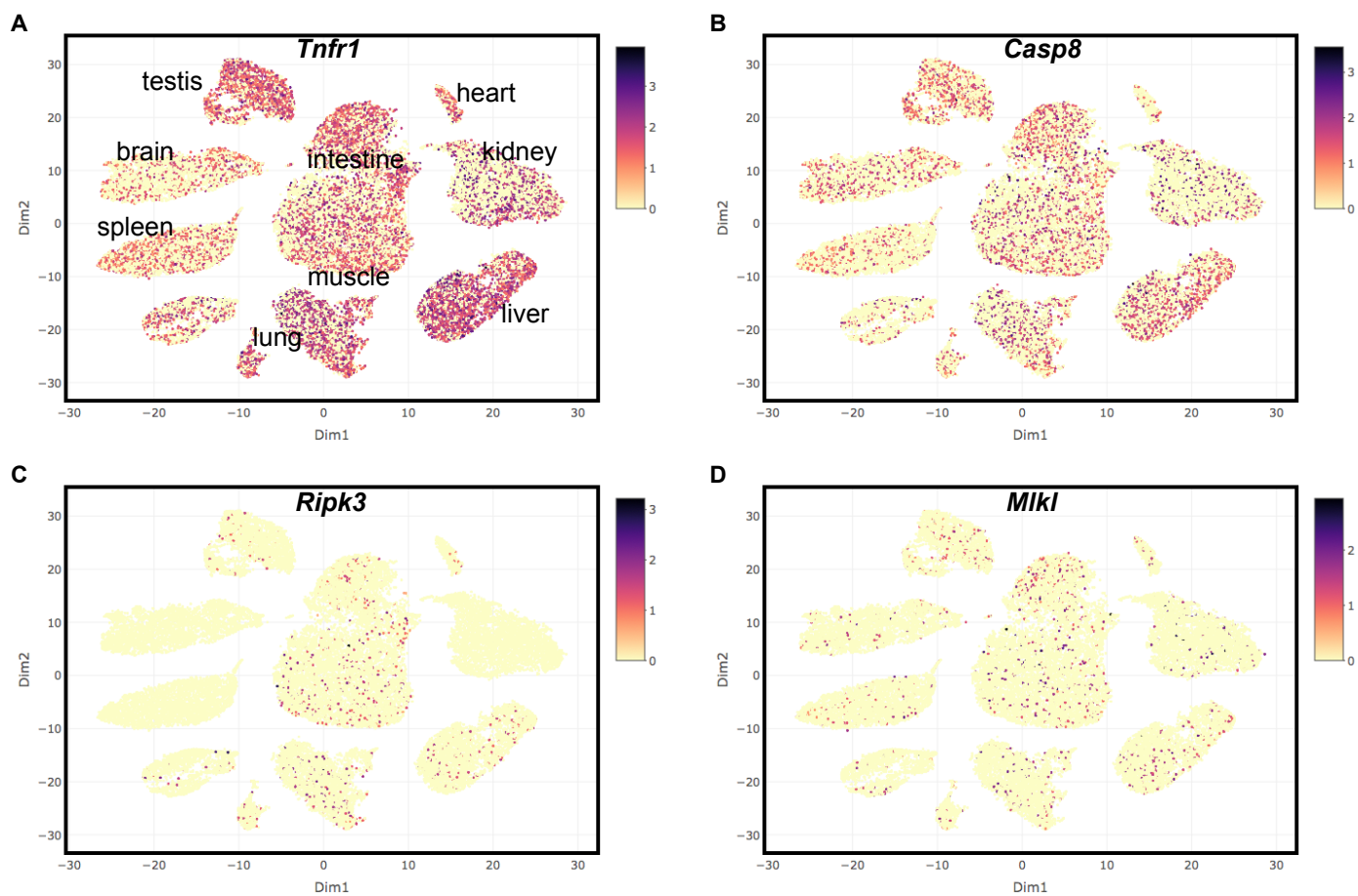
Appendix Fig. S1:



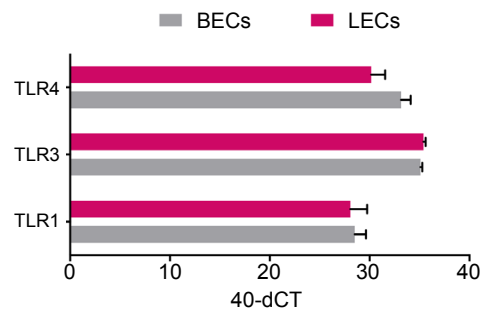
Appendix Fig. S2:



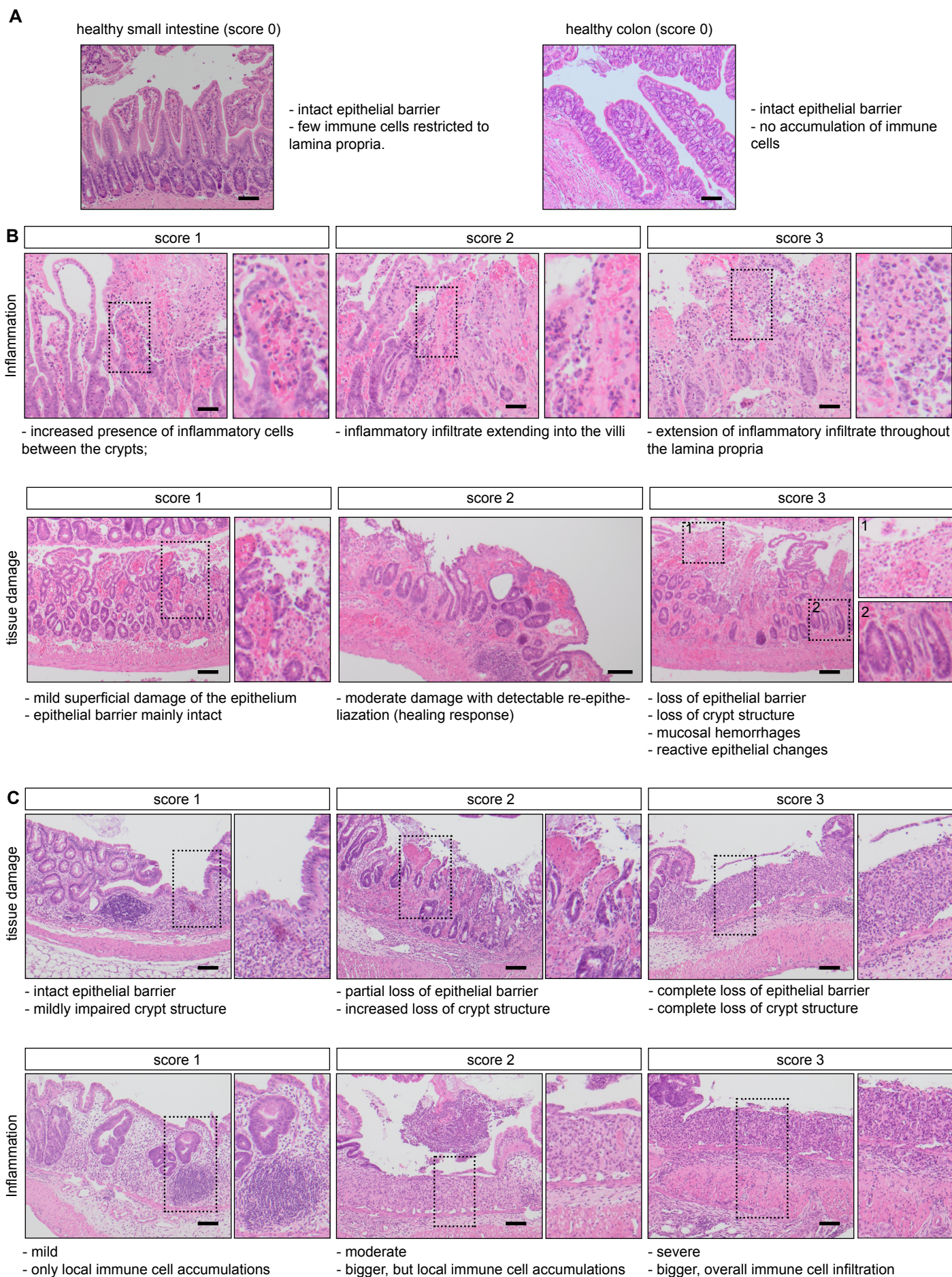
Appendix Fig. S3:



Appendix Fig. S4:



Appendix Fig. S5:



Appendix Fig. S7:

2. Appendix Supplementary Figure Legends

Appendix Fig. S1: Efficient Cre recombination and Casp8 deletion in ECs from different organs in Casp8^{ECKO} mice.

A. Scheme showing the tamoxifen treatment protocol and the day of analysis for Cre activity in Cdh5Cre^{ERT2} x ROSA^{mTmG} reporter mice.

B. Representative images of vessel staining (CD31 or IsoB4) and mT/mG signal in the brain, heart, lung and liver of Cre⁺ and Cre⁻ mice. Boxed areas are shown in higher magnifications and split channels. Numbers indicate the percentage of CD31⁺/GFP⁺ vasculature (n=3 Cre⁺). Scale bars: 100µm.

C-G. QPCR quantification of Casp8 expression in ECs from brain (C; n= 3 WT, 4 ECKO; two tailed unpaired Student's t-test), lung (D; n= 4 WT, 4 ECKO; two tailed unpaired Student's t-test), skin (E; n= 4 WT, 4 ECKO; two tailed unpaired Student's t-test), small intestine (F; n= 6 WT, 6 ECKO; two-way ANOVA with Sidak's multiple comparison) and colon (G; n= 6 WT, 6 ECKO; two-way ANOVA with Sidak's multiple comparison) of Casp8^{WT} and Casp8^{ECKO} mice.

H. QPCR quantification of Casp8 expression in isolated small intestinal epithelial cells from Casp8^{WT} and Casp8^{ECKO} mice (n= 5 WT, 6 ECKO; two tailed unpaired Student's t-test).

I. Representative pictures of CD31 (i') and Lyve-1 (i'') staining and mG signal in the small intestine of Cdh5Cre^{ERT2} x ROSA^{mTmG} reporter mice (n= 3 Cre⁺ mice) showing GFP⁺ cells in blood and lymphatic ECs. Scale bars: 50µm.

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

Appendix Fig. S2: Characterization of cell death upon Casp8 deletion in ECs.

A. Representative images of CD31, TUNEL and DAPI staining of sections of small intestine from Casp8^{WT} and Casp8^{ECKO} mice at a late disease stage. Scale bars: 100µm.

B. Quantification of TUNEL+ cells in Casp8^{WT} and Casp8^{ECKO} small intestines at a late disease stage (n= 4 WT, 4 ECKO, multiple t-tests with Holm-Sidak method).

Data information: All data is shown as mean ± SEM. * P<0.05, ** P <0.01, *** P <0.001, **** P <0.0001; ns: not significant.

Appendix Fig. S3: Expression of PDGFB-EGFP is restricted to BECs in the small intestine.

A: Assessment of EGFP expression in Pdgfb-iCreER+ and Pdgfb-iCreER- mice by flow cytometry, showing that EGFP expression was absent from the Lyve1+ population (plots are representative of n=6 mice per group).

B: Casp8 expression levels determined by qPCR in small intestinal BECs of Casp8^{WT} and Casp8^{ECKO} mice (n=6 mice per genotype; two tailed unpaired Student's t-test).

Data information: All data is shown as mean ± SEM. **** P<0.0001.

Appendix Fig. S4: Genes associated to the necroptosis signaling pathway are equally expressed in ECs of different organs.

A-D. *Tnfr1* expression (A). *Casp8* expression (B). *Ripk3* expression (C). *Mkl1* expression (D). Note that except of brain, spleen and kidney, all molecules are co-expressed in different EC clusters.

Data information: Data has been extracted from a publicly available scRNA-Seq database (Kalucka et al. 2020; https://endotheliomics.shinyapps.io/ec_atlas/).

Appendix Fig. S5: TLRs are expressed in small intestinal BECs and LECs. QPCR expression analysis of the main TLRs in sorted small intestinal BECs and LECs (n= 6 mice).

Data information: All data is shown as mean $\pm$ SEM.

Appendix Fig. S6: Microbiome sequencing of Casp8^{ECKO} early disease intestine.

A-B. Principal component analysis (PcoA) of weighted UniFrac distances (A) and heat map (B) of small intestinal microbiome analysis between Casp8^{ECKO} and Casp8^{WT} mice at an early disease stage (n= 8 WT, 7 ECKO, A: Permanova test and Mann-Whitney U test)

Appendix Fig. S7: Illustration of the different parameters of the histopathological score for small intestine and DSS-induced colitis samples.

A. Representative pictures of healthy small intestine and colon tissue. Scale bars: 100 μ m.

B. Exemplary pictures of different severities (score 1-3) in the small intestine in the two evaluated parameters: inflammation and tissue damage. Insets highlight the specific features explained below the pictures. Scale bar: 100 μ m.

C. Exemplary pictures of different severities (score 1-3) in the DSS-induced colitis model in the two evaluated parameters: inflammation and tissue damage. Insets highlight the specific features explained below the pictures. Scale bar: 100 μ m.

3. Appendix Table S1

qPCR primers

<i>Gene</i>	<i>Primer</i>
Mouse <i>Angpt2</i>	
Mouse <i>Casp8</i>	1. 5'-GGTCATGCTCTTTAAGCTCTCA-3' 2. 5'-TCATCTTCCAGCTTACATTTGG-3'
Mouse <i>Ccl2</i>	1. 5'-GATGCAGTTAACGCCCCACT-3' 2. 5'-ACCCATTCCCTTCTTGGGGTC-3'
Mouse <i>c-myc</i>	1. 5'-CAGCGACTCTGAAGAAGAGCA-3' 2. 5'-TTGTGCTGGTGAGTGGAGAC-3'
Mouse <i>Cxcl1</i>	1. 5'-ACTCAAGAATGGTCGCGAGG-3' 2. 5'-GTGCCATCAGAGCAGTCTGT-3'
Mouse <i>Cxcl5</i>	1. 5'-CCCTACGGTGGGAAGTCATAGC-3' 2. 5'-TTCAGTGGGGTCAGAGTCCT-3'
Mouse <i>Icam1</i>	1. 5'-AAGGTGGTTCTTCTGAGCGG-3' 2. 5'-TCCAGCCGAGGACCATAACAG-3'
Mouse <i>Il1b</i>	1. 5'-TGCCACCTTTTGACAGTGATG-3' 2. 5'-AAGGTCCACGGGAAAGACAC-3'
Mouse <i>Il1m</i>	1. 5'-TCACCAACACACCGGAAGAG-3' 2. 5'-GCCAGAGTGATCAGGCAGTT-3'
Mouse <i>Il6</i>	1. 5'-CCCCAATTTCGAATGCTCTCC-3' 2. 5'-CGCACTAGGTTTGCCGAGTA-3'
Mouse <i>Ki67</i>	1. 5'-CGGCCAGAGCTAACTTGCG-3' 2. 5'-TTCAATACTCCTTCCAAACAGGCA-3'
Mouse <i>Mkl</i>	1. 5'-AGATCCCATTTGAAGGCTGTGA-3' 2. 5'-TGCCAGAAAGACTCCTACCG-3'
Mouse <i>Ripk3</i>	1. 5'-GCCTTCCTCTCAGTCCACAC-3' 2. 5'-ACGCACCAGTAGGCCATAAC-3'
Mouse <i>Tlr1</i>	1. 5'-GTGTGCAGCTGATTGCTCAT-3' 2. 5'-CAAACCGATCGTAGTGCTGA-3'
Mouse <i>Tlr 3</i>	1. 5'-ATATGCGCTTCAATCCGTTTC-3' 2. 5'-CAGGAGCATACTGGTGCTGA-3'
Mouse <i>Tlr 4</i>	1. 5'-TTTGCTGGGGCTCATTCACT-3' 2. 5'-GACTCGGCACTTAGCACTGT-3'
Mouse <i>TNFA</i>	1. 5'-GACGTGGAAGTGGCAGAAGAG-3' 2. 5'-TGCCACAAGCAGGAATGAGA-3'
Mouse <i>Tnfr1</i>	1. 5'-AAGGCTGGAAGCCCCCTAAC-3' 2. 5'-GAACTAAAGCCTGGGGTGCT-3'
Mouse <i>Vcam1</i>	1. 5'-GTCACGGTCAAGTGTGTTGGC-3' 2. 5'-TCCTGGGAGAGATGTAGACTTGT-3'
Mouse <i>Vegfa</i>	1. 5'-CGTTCACTGTGAGCCTTGTT-3' 2. 5'-CTTGGCTTGTACATCTGCA-3'
Human <i>Mkl</i>	1. 5'-CCCTCAGGTAGGGATCGGG-3' 2. 5'-TTTCCATGCCTTCGCGCCT-3'
Human <i>TNFA</i>	1. 5'-ATCTTCTCGAACCCGAGTGA-3' 2. 5'-CGGTTCAAGCACTGGAGCT-3'
Human <i>Tnfr1</i>	1. 5'-CCCAGTCTCAACCCTCAAC-3' 2. 5'-ATTCCACCAACAGCTCCAG-3'
Human <i>Ripk3</i>	1. 5'-CAAGATCGTAAACTCGAAGG-3' 2. 5'-CCGTTCTCATGAATTTAGT-3'
16S rDNA Bacteroidetes 1	1. 5'-GGAGAGTACCCGAGAAAAAGC-3' 2. 5'-TTCCGCATACTTCTCGCCCA-3'
16S rDNA Bacteroidetes 2	1. 5'-CCAGCAGCCGCGGTAATA-3' 2. 5'-CGCATTCCGCATACTTCTC-3'
16S rDNA Firmicutes	1. 5'-TGAAACTYAAAGGAATTGACG-3' 2. 5'-ACCATGCACACCTGTC-3'

4. Appendix Table S2

P values for Fig. 1-6, Fig. EV 1-5, Appendix Fig. S1, S2

Figure	Comparison	<i>P</i> value
Fig. 1B	Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,0032
Fig. 1C	Casp8 ^{WT} vs. Casp8 ^{ECKO}	<0,0001
Fig. 1F	Casp8 ^{WT} vs. Casp8 ^{ECKO}	>0,9999
Fig. 1G	Casp8 ^{WT} vs. Casp8 ^{ECKO}	<0,0001
Fig. 1H	Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,0003
Fig. 2A	Ccl2: Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,00004
Fig. 2A	Il1b: Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,003908
Fig. 2A	TNA: Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,024796
Fig. 2A	Il1rn: Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,00137
Fig. 2B	VCAM: Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,001718
Fig. 2B	ICAM: Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,014235
Fig. 2B	VEGF: Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,001437
Fig. 2B	Ang2: Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,000059
Fig. 2C	Complement Factor D: Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,817708
Fig. 2C	Complement Component C5/C5a: Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,021638
Fig. 2C	C-Reactive Protein: Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,023798
Fig. 2D	EGF: Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,016627
Fig. 2D	HGF: Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,000003
Fig. 2D	Amphiregulin: Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,000984
Fig. 2D	IGFBP-2: Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,013671
Fig. 2D	IGFBP-5: Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,000239
Fig. 2F	Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,0074
Fig. 2G	c-myc: Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,0062
Fig. 2G	Ki67: Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,0085
Fig. 3B	Casp-8 ^{WT} vs. Casp8 ^{ECKO} no hem	0,0112
Fig. 3B	Casp-8 ^{WT} vs. Casp8 ^{ECKO} mild hem	0,0152
Fig. 3B	Casp-8 ^{WT} vs. Casp8 ^{ECKO} strong hem	<0,0001
Fig. 3E	Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,0018
Fig. 3F	Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,0125
Fig. 3H	Intestine: Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,0003
Fig. 3H	Brain: Casp8 ^{WT} vs. Casp8 ^{ECKO}	>0,9999
Fig. 4B:	Epithelium: Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,008097
Fig. 4B:	Stroma: Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,027191
Fig. 4B:	Endothelium: Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,001177
Fig. 4D:	Casp8 ^{WT} /MLKL ^{ko} vs. Casp8 ^{ECKO} /MLKL ^{ko}	>0,9999
Fig. 4E:	Casp8 ^{WT} /MLKL ^{ko} vs. Casp8 ^{ECKO} /MLKL ^{ko}	0,1210
Fig. 4H:	Casp8 ^{WT} /MLKL ^{ko} vs. Casp8 ^{ECKO} /MLKL ^{ko}	0,3060
Fig. 4I:	Casp8 ^{WT} /MLKL ^{ko} vs. Casp8 ^{ECKO} /MLKL ^{ko}	0,4110
Fig. 4J:	Casp8 ^{WT} /MLKL ^{ko} vs. Casp8 ^{ECKO} /MLKL ^{ko}	0,1738
Fig. 4K:	Casp8 ^{WT} /MLKL ^{ko} vs. Casp8 ^{ECKO} /MLKL ^{ko}	0,8435
Fig. 4L:	Casp8 ^{WT} /MLKL ^{ko} vs. Casp8 ^{ECKO} /MLKL ^{ko}	0,5301
Fig. 4N:	Casp8 ^{WT} /MLKL ^{ko} vs. Casp8 ^{ECKO} /MLKL ^{ko}	0,8903
Fig. 4P:	Casp8 ^{WT} /MLKL ^{ko} vs. Casp8 ^{ECKO} /MLKL ^{ko}	>0,9999
Fig. 4Q:	Casp8 ^{WT} /MLKL ^{ko} vs. Casp8 ^{ECKO} /MLKL ^{ko}	>0,9999
Fig. 5C:	Casp8 ^{WT} vs. Casp8 ^{BECKO}	>0,9999
Fig. 5F:	Casp8 ^{WT} vs. Casp8 ^{BECKO}	>0,9999
Fig. 5G:	Casp8 ^{WT} vs. Casp8 ^{BECKO}	>0,9999
Fig. 5I:	Casp8 ^{WT} vs. Casp8 ^{BECKO}	0,2097
Fig. 5J:	Casp8 ^{WT} vs. Casp8 ^{BECKO}	0,0677
Fig. 5L:	Casp8 ^{WT} vs. Casp8 ^{BECKO}	0,3351
Fig. 6B:	Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,9731
Fig. 6E:	Casp8 ^{WT} vs. Casp8 ^{ECKO}	0,0005

Fig. 6E:	Casp8 ^{Ecko} vs. Casp8 ^{Ecko} /MLKL ^{ko}	<0,0001
Fig. 6H:	Casp8 ^{WT} vs. Casp8 ^{Ecko} + IgG	0,0003
Fig. 6H:	Casp8 ^{WT} vs. Casp8 ^{Ecko} + Enbrel	0,0133
Fig. 6K:	Casp8 ^{WT} vs. Casp8 ^{Ecko} + IgG	<0,0001
Fig. 6K:	Casp8 ^{WT} vs. Casp8 ^{Ecko} + Enbrel diseased	<0,0001
Fig. 6K:	Casp8 ^{WT} vs. Casp8 ^{Ecko} + Enbrel healthy	>0,9999
Fig. 7B:	Bacteroidetes 1: Control vs. Antibiotics	<0,0001
Fig. 7B:	Bacteroidetes 2: Control vs. Antibiotics	<0,0001
Fig. 7C:	Casp8 ^{WT} vs. Casp8 ^{Ecko}	0,0001
Fig. 7C:	Casp8 ^{Ecko} vs. Casp8 ^{Ecko} antibiotics	0,0002
Fig. 7D:	Casp8 ^{WT} vs. Casp8 ^{Ecko}	0,0001
Fig. 7D:	Casp8 ^{Ecko} vs. Casp8 ^{Ecko} antibiotics	0,0025
Fig. 7G:	Casp8 ^{WT} vs. Casp8 ^{Ecko}	<0,0001
Fig. 7G:	Casp8 ^{Ecko} vs. Casp8 ^{Ecko} antibiotics	<0,0001
Fig. 7H:	Casp8 ^{WT} vs. Casp8 ^{Ecko}	<0,0001
Fig. 7H:	Casp8 ^{Ecko} vs. Casp8 ^{Ecko} antibiotics	<0,0001
Fig. EV1C:	Casp8 ^{WT} vs. Casp8 ^{Ecko}	>0,9999
Fig. EV1D:	Casp8 ^{WT} vs. Casp8 ^{Ecko}	>0,9999
Fig. EV2D:	Casp8 ^{WT} vs. Casp8 ^{Ecko}	0,0951
Fig. EV2F:	Casp8 ^{WT} vs. Casp8 ^{Ecko}	0,0037
Fig. EV3A:	Casp8 ^{WT} vs. Casp8 ^{Ecko}	>0,9999
Fig. EV4B:	Casp8 ^{WT} + Casp8 ^{WT} + IMQ	<0,0001
Fig. EV4B:	Casp8 ^{Ecko} + Casp8 ^{Ecko} + IMQ	<0,0001
Fig. EV4B:	Casp8 ^{WT} + IMQ vs. Casp8 ^{Ecko} + IMQ	0,1908
Fig. EV4C:	Casp8 ^{WT} + Casp8 ^{WT} + IMQ	0,0013
Fig. EV4C:	Casp8 ^{Ecko} + Casp8 ^{Ecko} + IMQ	0,0025
Fig. EV4C:	Casp8 ^{WT} + IMQ vs. Casp8 ^{Ecko} + IMQ	0,9680
Fig. EV4E:	Casp8 ^{WT} + Casp8 ^{WT} + IMQ	0,0023
Fig. EV4E:	Casp8 ^{Ecko} + Casp8 ^{Ecko} + IMQ	0,0002
Fig. EV4E:	Casp8 ^{WT} + IMQ vs. Casp8 ^{Ecko} + IMQ	0,8412
Fig. EV5B:	Casp8 ^{WT} vs. Casp8 ^{Ecko}	0,3686
Fig. EV5C:	Casp8 ^{WT} vs. Casp8 ^{Ecko}	>0,9999
Fig. EV5D:	Casp8 ^{WT} vs. Casp8 ^{Ecko}	0,3714
Fig. EV5F:	Casp8 ^{WT} vs. Casp8 ^{Ecko}	0,3715
Fig. EV5G:	TNFA: Casp8 ^{WT} vs. Casp8 ^{Ecko}	0,270271
Fig. EV5G:	Ccl2: Casp8 ^{WT} vs. Casp8 ^{Ecko}	0,933552
Fig. EV5G:	Il1b: Casp8 ^{WT} vs. Casp8 ^{Ecko}	0,700199
Fig. EV5G:	Il16: Casp8 ^{WT} vs. Casp8 ^{Ecko}	0,359654
Fig. EV5H:	Casp8 ^{WT} vs. Casp8 ^{Ecko}	0,0371
Appendix Fig. S1C:	Casp8 ^{WT} vs. Casp8 ^{Ecko}	0,0003
Appendix Fig. S1D:	Casp8 ^{WT} vs. Casp8 ^{Ecko}	0,0037
Appendix Fig. S1E:	Casp8 ^{WT} vs. Casp8 ^{Ecko}	0,0001
Appendix Fig. S1F:	BEC: Casp8 ^{WT} vs. Casp8 ^{Ecko}	<0,0001
Appendix Fig. S1F:	LEC: Casp8 ^{WT} vs. Casp8 ^{Ecko}	<0,0001
Appendix Fig. S1G:	BEC: Casp8 ^{WT} vs. Casp8 ^{Ecko}	<0,0001
Appendix Fig. S1G:	LEC: Casp8 ^{WT} vs. Casp8 ^{Ecko}	<0,0001
Appendix Fig. S1H:	Casp8 ^{WT} vs. Casp8 ^{Ecko}	0,915
Appendix Fig. S2B:	Epithelium: Casp8 ^{WT} vs. Casp8 ^{Ecko}	0,000008
Appendix Fig. S2B:	Stroma: Casp8 ^{WT} vs. Casp8 ^{Ecko}	0,005076
Appendix Fig. S2B:	Endothelium: Casp8 ^{WT} vs. Casp8 ^{Ecko}	0,039503
Appendix Fig. S3B:	Casp8 ^{WT} vs. Casp8 ^{BEcko}	<0,0001