

Supplemental information

**ILC3s restrict the dissemination of intestinal
bacteria to safeguard liver
regeneration after surgery**

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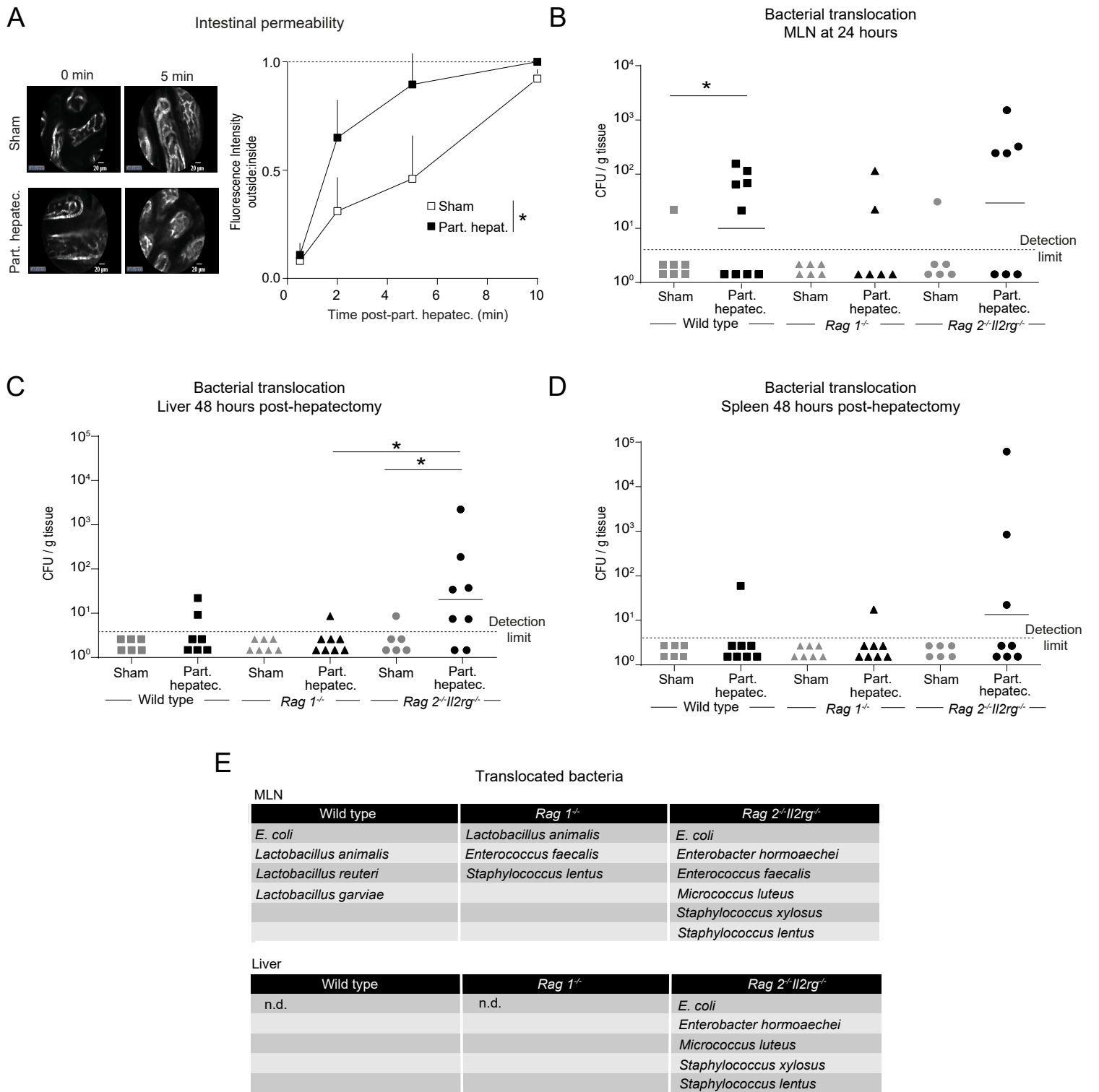


Figure S1. A surgery-induced leaky intestine is associated with systemic bacteria with an enteric profile, related to Figure 2.

A) Immediately post-partial hepatectomy, intestinal permeability was visualized using endomicroscopy for 30 min after intravenous injection of 70kDa FITC-Dextran. (B-E) SPF wild-type, *Rag1^{-/-}*, *Rag2^{-/-}Il2rg^{-/-}* mice underwent two-third partial hepatectomy. Bacterial titers in (B) mesenteric lymph nodes (MLN), (C) liver and (D) spleen were determined 24 (B) or 48 (C, D) hours post-PH. Geometric means for log scales and arithmetic means for linear scales are shown. (E) Bacteria from single isolated colonies identified by full 16S rRNA Sanger sequencing. Data in A and E are representative of n=3-4 mice per group in two independent experiments, and data in B-D are pooled from two independent experiments with n=3-4. P-values are indicated as follow: *p≤0.05.

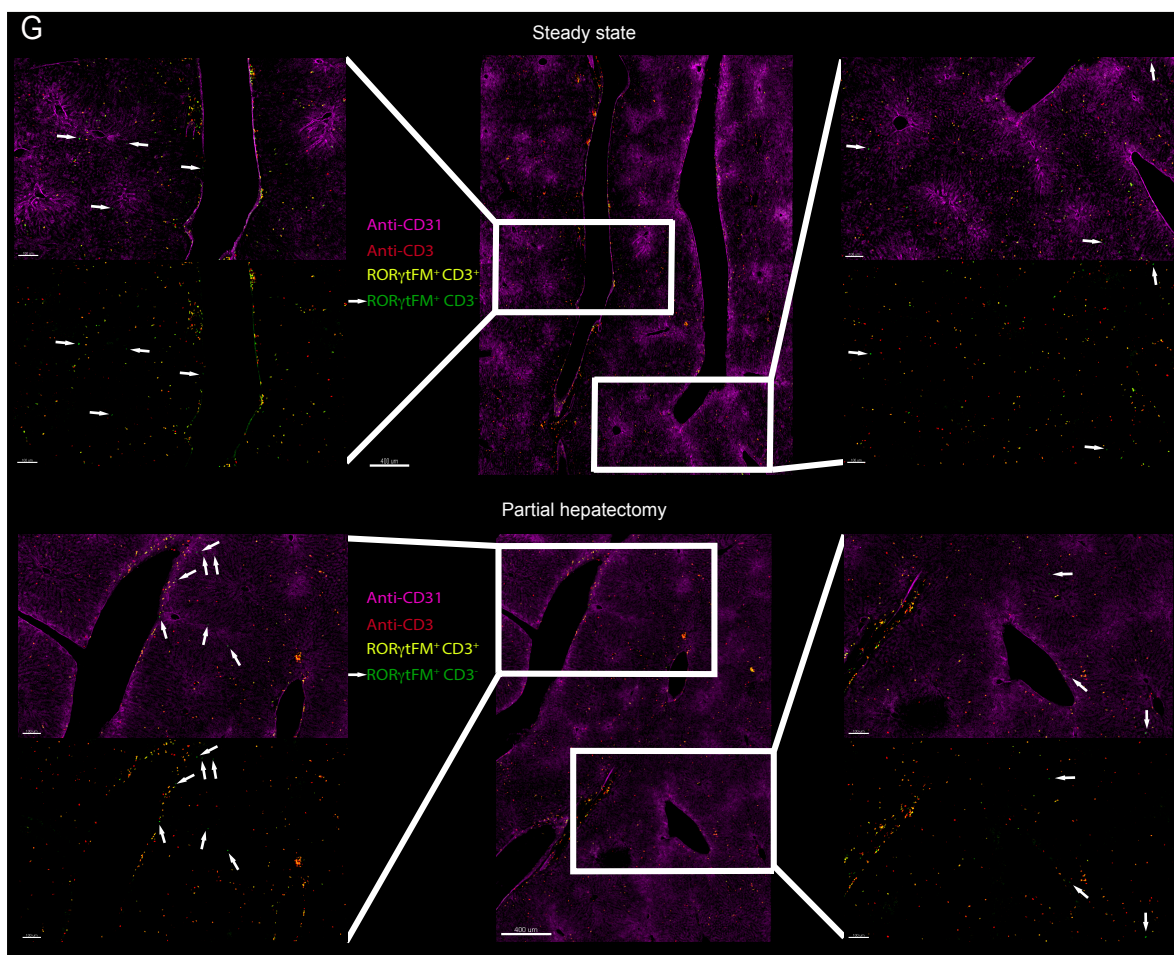
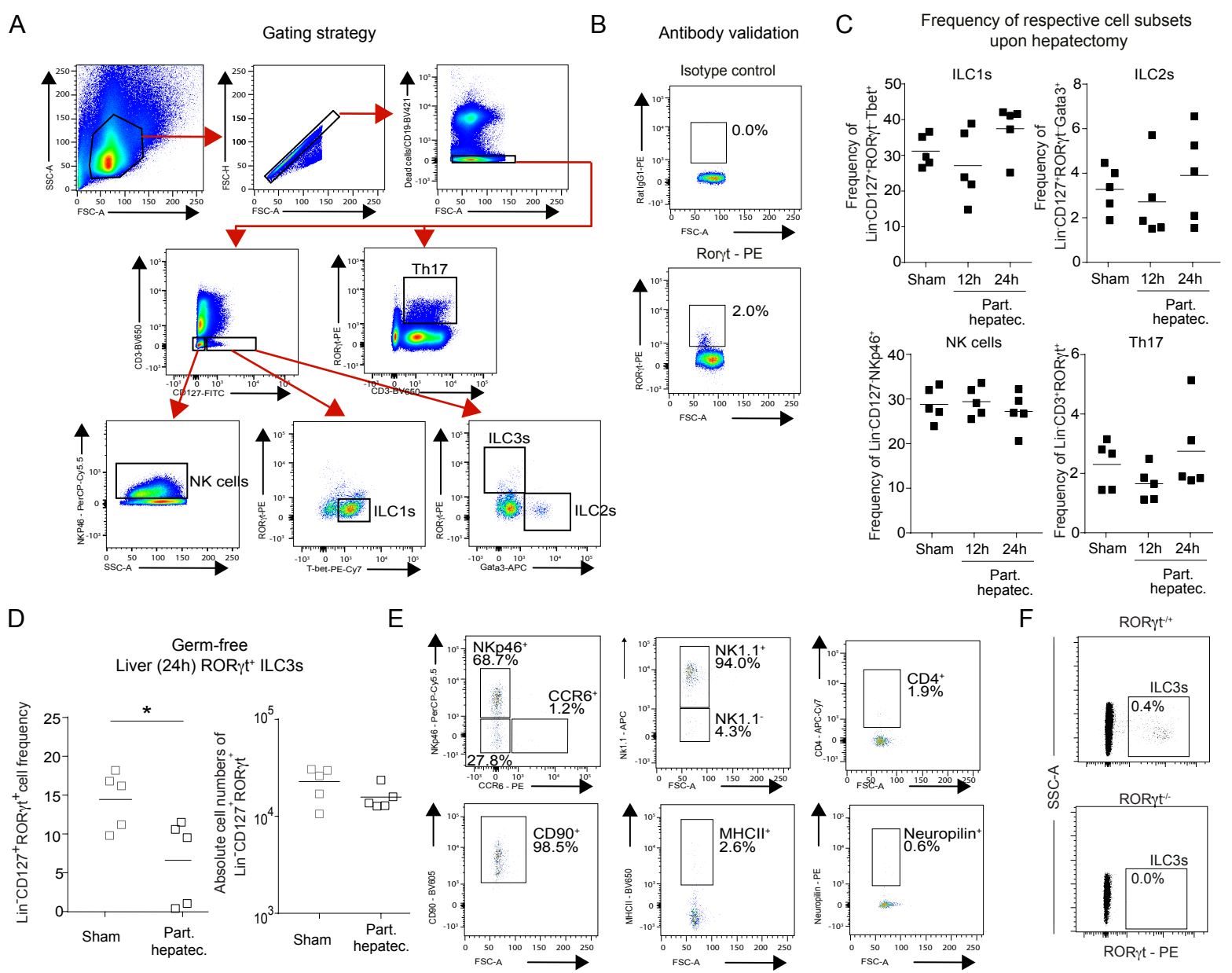


Figure S2. Hepatic ILC3s express classical ILC3 markers, depend on the transcription factor ROR γ t and increase only in the presence of microbiota upon hepatectomy, related to Figure 3.

(A) Gating strategy to identify innate lymphoid cells (ILCs) and Th17 cells in the liver by multidimensional flow cytometry. (B) Antibody validation of the ROR γ t antibody in PE with the appropriate isotype control IgG1. (C, D) SPF-colonized (C) and germ-free (D) wild-type mice underwent two-third partial hepatectomy and hepatic ILCs were analyzed at 12 and 24 hours post-PH by flow cytometry. (E) Multidimensional flow cytometry of ILC3s in the liver using *Rorc-Cre*⁺ *Rosa26R*^{EGFP/+} mice. Lin⁻ (CD19, Fc ϵ r1, Ly6G, CD3, CD5) ROR γ tFM⁺ (YFP) cells and their expression of NKp46, CCR6, NK1.1, CD90, MHCII, CD4, Neuropilin1 and NKG2D is shown. (F) ROR γ t⁺ ILC3s were analyzed in *Rorc*(γ t)^{+/+} and *Rorc*(γ t)^{-/-} mice. (G) Confocal microscopy pictures of *Rorc-Cre*⁺ *Rosa26R*^{EGFP/+} mice (ROR γ tFM⁺) following at steady state or 24h after PH. Embed livers were stained with anti-CD3, anti-GFP and anti-CD31. Arrows indicate ROR γ tFM⁺CD3⁻ ILCs. Geometric means for log scales and arithmetic means for linear scales are shown. Data in A-G are representative of n=3-4 mice per group in two independent experiments. P-values are indicated as follow: *p \leq 0.05.

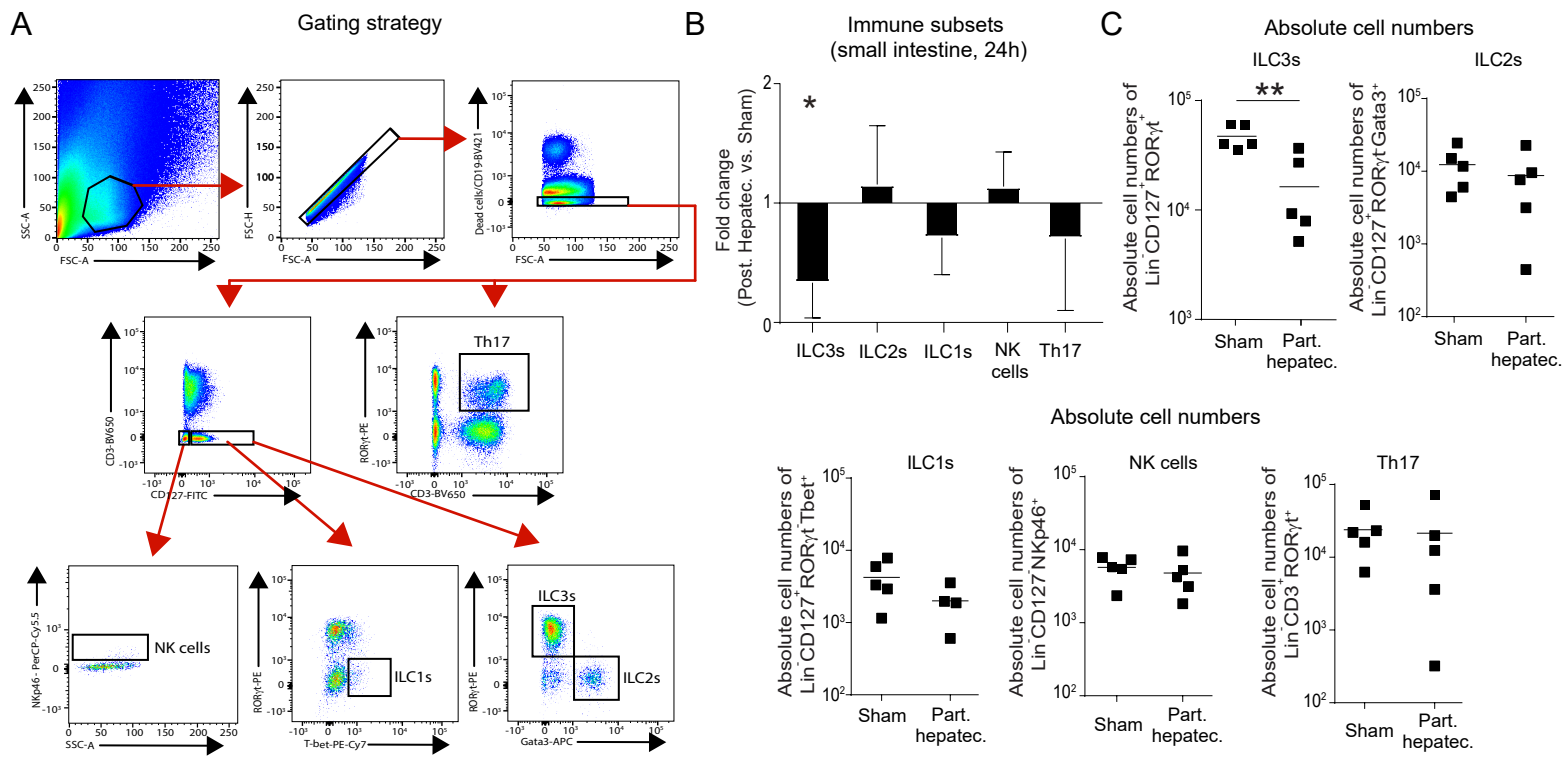


Figure S3. Partial hepatectomy induces a decrease of ILC3s in the lamina propria of the small intestine, related to Figure 3

SPF wild-type mice underwent two-third partial hepatectomy and were analysed by flow cytometry at indicated time points after surgery. (A) Gating strategy to identify innate lymphoid cells (ILCs) and Th17 cells in the lamina propria of the small intestine. (B) Relative cellular changes in comparison to sham-operated mice of different ILC subsets and Th17 cells analyzed in small intestines of SPF wild-type mice 24 hours after hepatectomy. (C) Absolute cell numbers of different ILC subsets and Th17 cells in the small intestine 24 hours after partial hepatectomy or sham surgery. Geometric means for log scales and arithmetic means for linear scales are shown. Normalized values were analysed by Student's t test where two experimental groups were compared or by ANOVA where more than two groups were analysed in parallel. Data in A-C are representative of n=5 mice per group in two independent experiments. P-values are indicated as follow: *p ≤ 0.05; ** p ≤ 0.01.

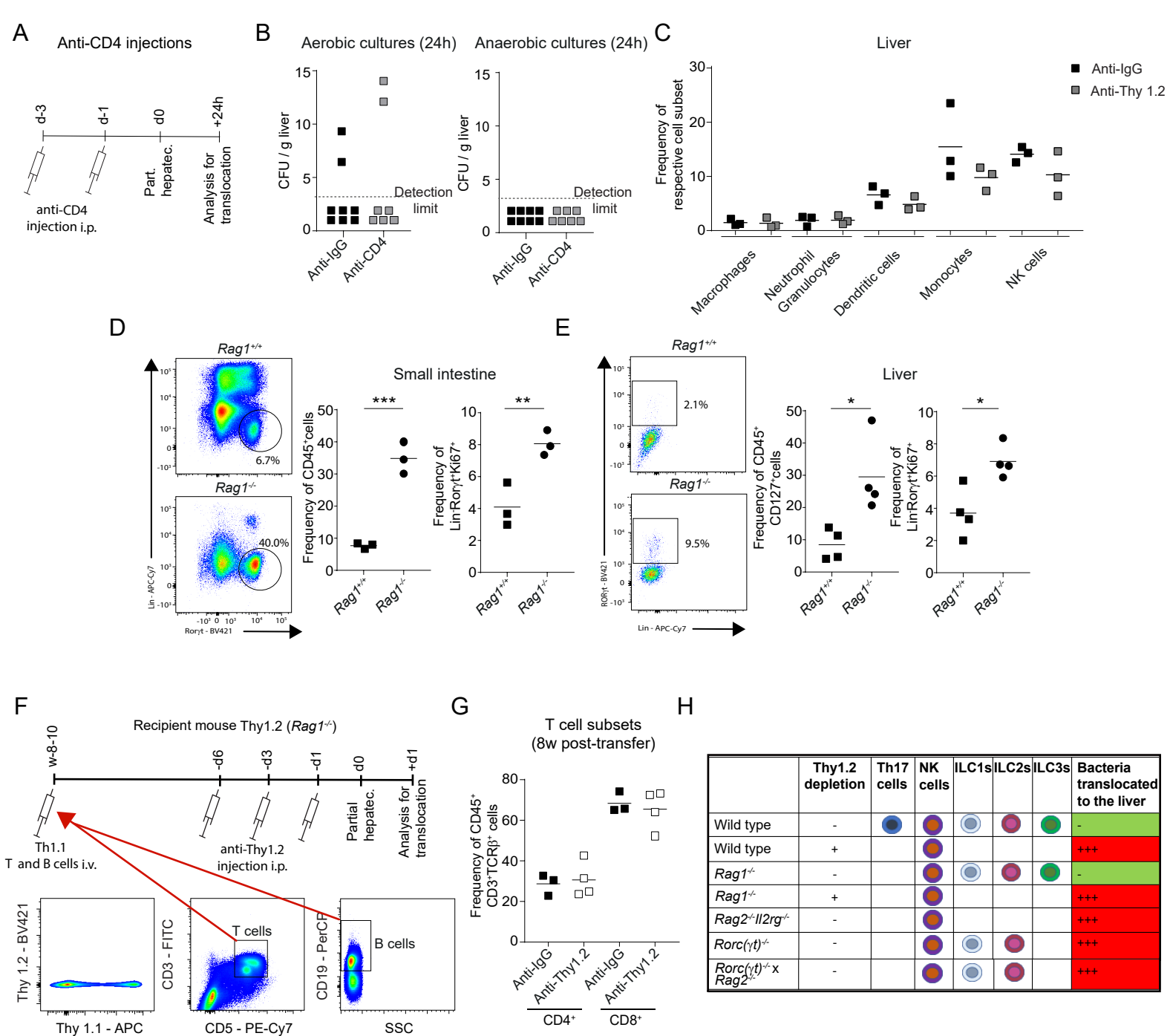


Figure S4. T cells are not required to prevent systemic bacterial spread, related to Figure 4.

(A, B) Anti-CD4 injection i.p. 2x prior to partial-hepatectomy and analysis for bacterial translocation to the liver in aerobic and anaerobic conditions 24 hours after partial hepatectomy. (C) Thy 1.2 cell depletion using anti-Thy1.2 antibody or anti-IgG administered 6 days, 3 days and 1 day prior to partial hepatectomy in *Rag1*^{-/-} mice. Depletion of different myeloid subsets was assessed by flow cytometry. ROR γ t⁺ ILC3s from (D) the small intestine and (E) the liver in *Rag1*^{+/+} and *Rag1*^{-/-} were analyzed by flow cytometry in steady state. Plots represent the quantification of total ILC3s and Ki67⁺ILC3s. (F) Sort-purification of CD19⁺ B cells and CD3⁺CD5⁺ T cells from the spleen and MLN of *Thy1.1* mice. 40-60 \times 10⁶ Thy 1.1 cells were transferred i.v. to *Rag1*^{-/-} Thy1.2 recipient mice. I.p. injection of anti-Thy1.2 was performed three times prior to partial hepatectomy. (G) Validation of successful transfer of T cells by flow cytometry. (H) Table summarizing the different experimental approaches, which suggest that the loss of control of systemic bacterial dissemination segregates with the presence of ILC3. Data in A-G are representative of n=3-4 mice per group in two independent experiments, data in B are pooled from two independent experiments. Geometric means for log scales and arithmetic means for linear scales are shown. Normalized values were analysed by Student's t test. The p-values are indicated as follows: *p \leq 0.05, **p \leq 0.01, ***p \leq 0.001.

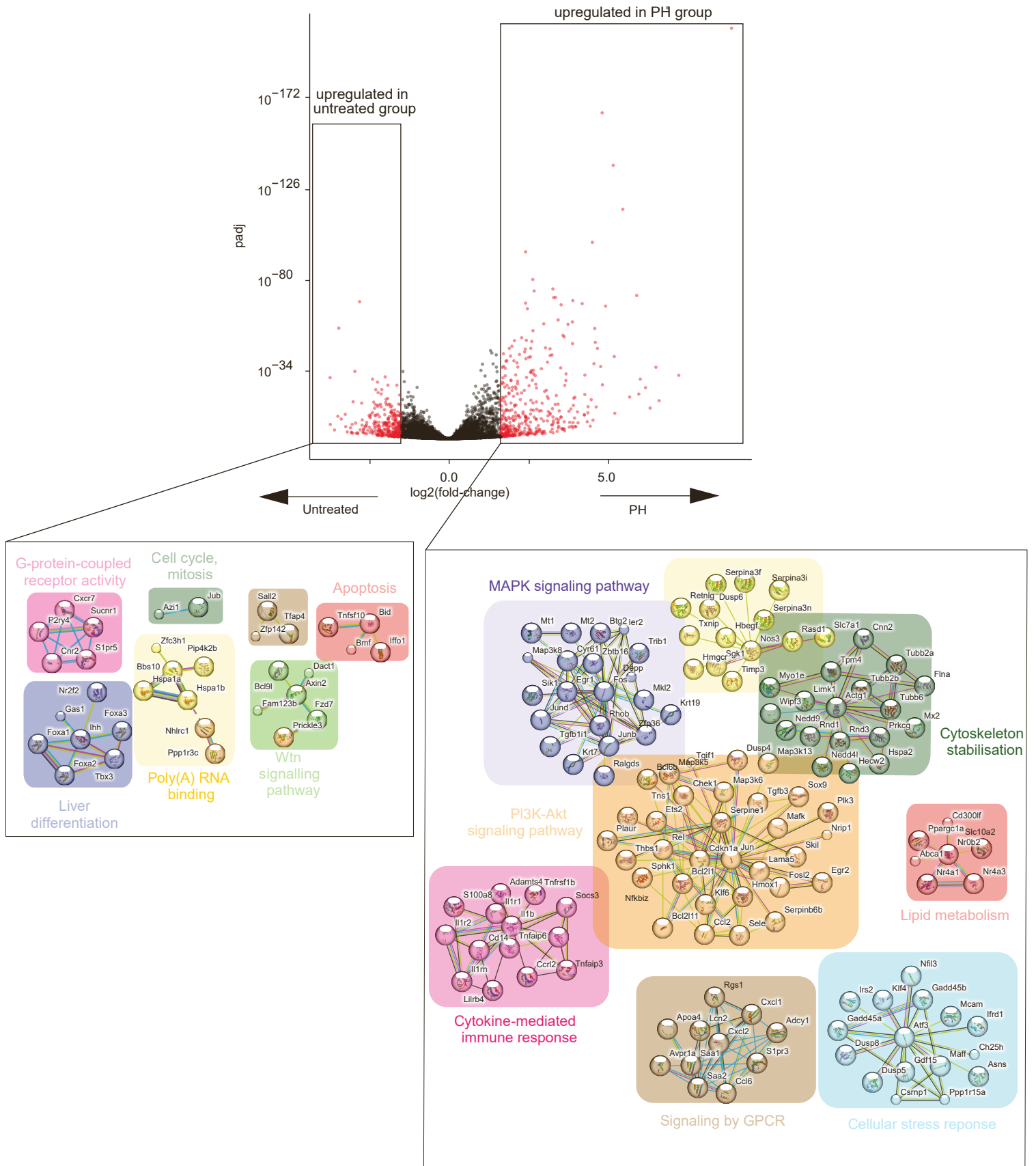


Figure S5. RNA sequencing of liver tissue reveals activation of the inflammasome early after hepatectomy, related to Figure 4
 RNA-Sequencing of whole liver tissue before and 3 hours post-partial hepatectomy in SPF-colonized wild-type mice

A

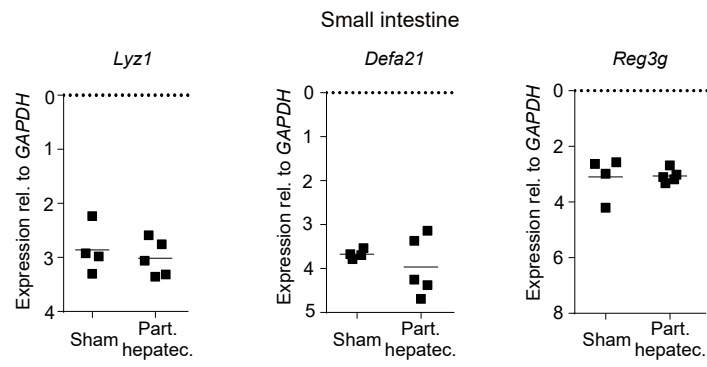


Figure S6. Expression of antimicrobial peptide encoding genes in the small intestine are not affected following partial hepatectomy, related to Figure 5 SPF wild-type mice underwent two-third partial hepatectomy. Whole tissue RNA was isolated from full tissue of the small intestine and analysed for gene expression of antimicrobial peptides at 24 hours post-PH. *Lyz1* (Lysozyme 1), *Defa21* (Alpha-defensin 21), *Reg3g* (Reg-3-gamma) by RT-qPCR. The graphs show expression relative to the housekeeping gene Gapdh. Data in A are representative of n=4-5 mice per group in two independent experiments.

		Sham		12h post-hepatec		24h post-hepatec	
		Average	SD	Average	SD	Average	SD
MLN	CD11b ⁺ Ly6G ⁺ Neutrophils (%)	0.63	0.35	0.35	0.28	0.12	0.05
	CD11c ⁺ F4/80 ⁺ Macrophages (%)	12.39	1.91	7.30	2.07	7.22	3.02
	Ly6C ⁺ Monocytes (%)	21.62	3.75	23.98	1.20	23.20	2.92
	CD11c ⁺ Dendritic cells (%)	29.76	3.99	26.94	3.36	28.78	8.51
	NK cells	8.53	3.51	9.37	5.26	10.71	3.41
	ILC1s	36.70	3.19	38.9	7.72	36.65	3.92
	ILC2s	4.92	1.69	5.12	1.47	9.25	2.70
	ILC3s	17.60	6.24	12.43	3.48	23.94	8.61
	Th cells	1.87	0.86	2.22	1.00	2.37	0.58
Spleen	CD11b ⁺ Ly6G ⁺ Neutrophils (%)	0.19	0.12	0.12	0.03	0.18	0.04
	F4/80 ⁺ CD11c ⁺ Macrophages (%)	10.63	2.45	6.07	1.12	6.33	1.31
	Ly6C ⁺ Monocytes (%)	13.45	1.94	17.48	1.27	19.72	1.87
	CD11c ⁺ Dendritic cells (%)	22.46	11.83	44.73	3.25	49.98	3.62
	NK cells	41.95	4.96	41.42	2.01	42.53	5.27
	ILC1s	14.1	2.58	15.13	7.60	13.11	2.60
	ILC2s	7.19	1.16	3.72	0.34	5.08	2.41
	ILC3s	0.15	0	0.15	0.02	0.16	0.04
	Th17 cells	0.77	0.05	0.62	0.04	0.48	0.23

Table S1: Myeloid cells and ILCs do not change following hepatectomy in mesenteric lymph nodes (MLN) and spleen
Cellular subsets were analyzed by flow cytometry at indicated time points. Data show average and standard deviation (SD).
Data are representative of two independent experiments.

Table S2. Oligonucleotides and RNA sequences		
<i>DEFA21</i> F: 5'- AGG CTG TGT CTG TCT CCT TTG; R: 5'- TGC AAG CAT CCA TCA CAC TGG	Sigma	Custom made
<i>GAPDH</i> F: 5'- CAT CAA GAA GGT GGT GAA GC; R: 5'- CCT GTT GCT GTA GCC GTA TT	Sigma	Custom made
<i>Il1B</i> F: 5'- ACCTGTCCTGTGTAATGAAAGACG; R: 5'- TGGGTATTGCTTGGGATCCA	Sigma	Custom made
<i>Il18</i> F: 5'- ACA ACT TTG GCC GAC TTC AC; R: 5'- TGG ATC CAT TTC CTC AAA GG	Sigma	Custom made
<i>IL22</i> F: 5' - ATG AGT TTT TCC CTT ATG GGG AC; R . 5'- GCT GGA AGT TGG ACA CCT CAA	Sigma	Custom made
<i>Reg3g</i> F: 5' - TTC CTG TCC TCC ATG ATC AAA; R . 5'- CAT CCA CCT CTG TTG GGT TC	Sigma	Custom made
<i>Reg3b</i> F: 5'- GCA GAA CCC AAT GGA GGT GG; R : 5'- CAC CCA GGG ATG TGA GAA GAG	Sigma	Custom made
<i>Lyz1</i> F: 5'- CTT GTC ACT CCT CAC CCC TG; R : 5'- AGC CGT TCC CCT TCC AAT G	Sigma	Custom made
<i>CCNA2</i>	Thermo Fisher Scientific	Mm00438063_m1
<i>FOXM1B</i>	Thermo Fisher Scientific	Mm00514925_m1
<i>TBP</i>	Thermo Fisher Scientific	Mm01277042_m1