

Title: IL-1-dependent enteric gliosis guides intestinal inflammation and dysmotility and modulates macrophage function

Short Title: IL-1-dependent enteric gliosis intestinal inflammation

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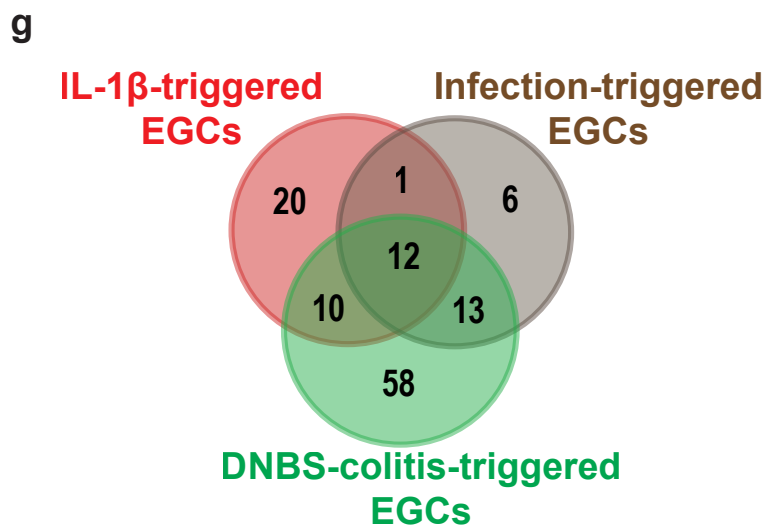
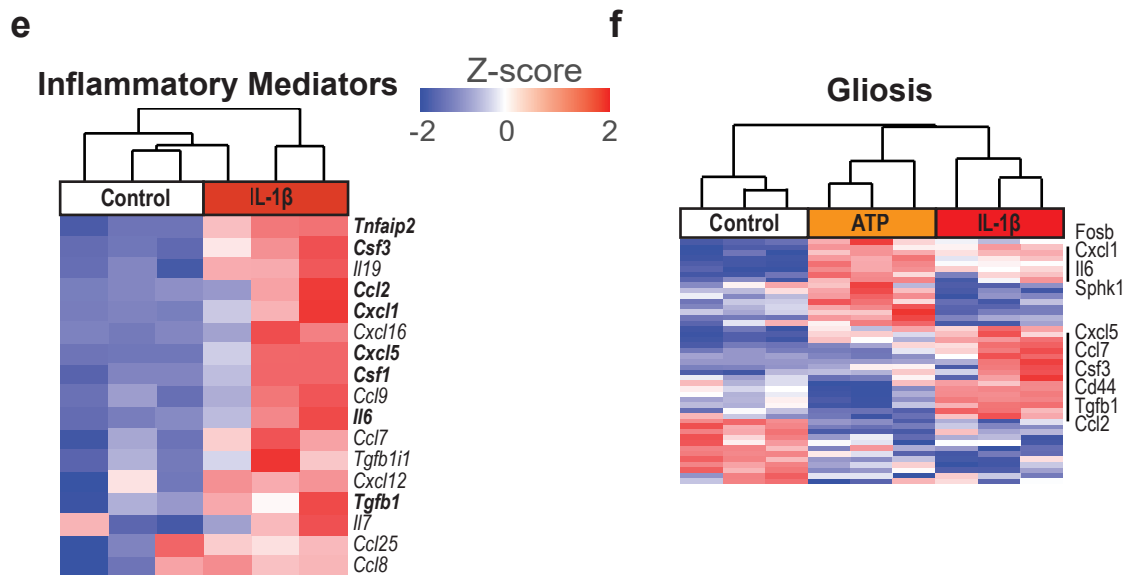
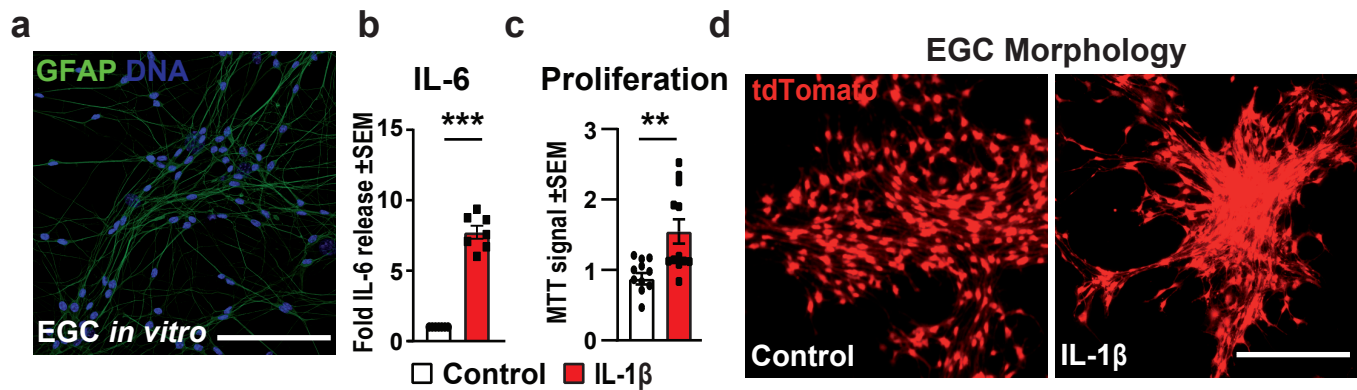


Fig. S1: IL-1 induces a specific reactive phenotype in EGCs.

(a) Immunocytochemistry for GFAP (green) and nuclei (Hoechst, blue) in primary EGCs. Scale bar 50 μ m. (b) Primary EGCs were treated with IL-1 β (10 ng/ml), or vehicle for 24h and processed for ELISA. n=8 per group. (c) Primary EGCs were treated with IL-1 β (10 ng/ml) or vehicle for 24h and processed for MTT assays to investigate proliferation. n=12 per group. (d) Live cell imaging of primary EGCs treated with IL-1 β (10 ng/ml) or vehicle for 24h. n=5. Scale bar 50 μ m. (e) Heat map of genes associated to “inflammatory mediators”. (f) Heat map of genes associated to “gliosis” showing EGCs treated with IL-1 β (10 ng/ml), ATP (100 μ M) or vehicle for 24h and processed for bulk-RNA-Seq. n=3 per group. (g) Venn diagram of induced gliosis genes showing the mutual genes between IL-1 β -triggered EGCs, infection-triggered EGCs (GSE114780) and DNBS-colitis-triggered EGCs (GSE182708). Statistics were done with Student’s t-test and Fisher’s exact test. ***<0.001, **<0.01 * were compared to untreated EGC cultures.

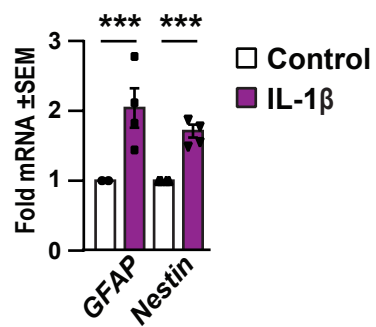
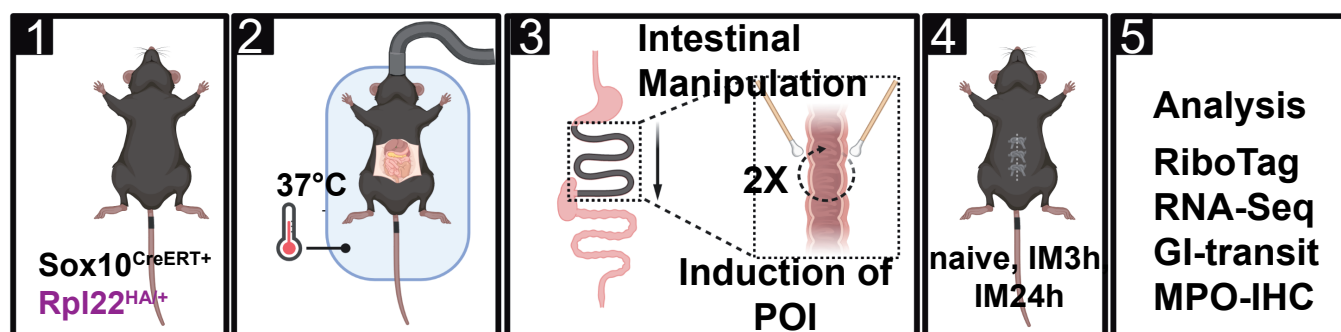
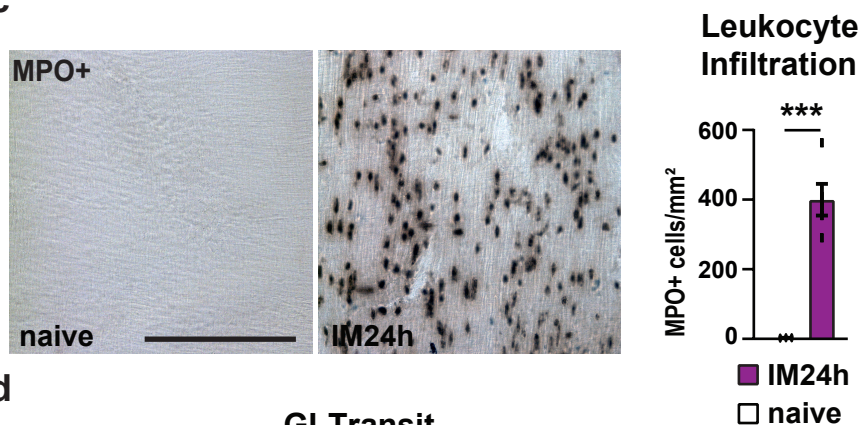
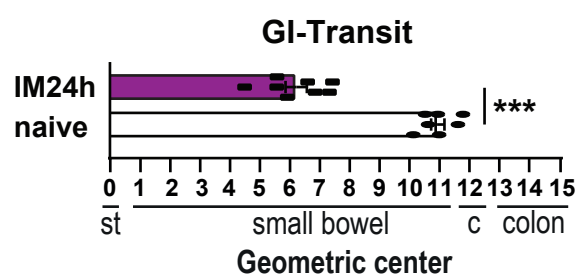
a**b****c****d**

Fig. S2: IL-1-induced enteric gliosis resembles the EGC reactivity profile after surgical trauma.

(a) qPCR analysis of EGC cultures produced from *Sox10^{CreERT2+}-Rpl22^{HA/+}* mice and treated with IL-1 β for 3h. Bars showing fold change compared to untreated EGCs. n=4. (b) Experimental scheme describing the postoperative ileus animal model, investigated disease time points, and readouts. (c) Histology of myeloperoxidase (MPO⁺) cells in the ME of naïve and IM24h *Sox10^{CreERT2+}-Rpl22^{HA/+}* animals and subsequent leukocyte infiltration quantification. n=5. Scale bar 100 μ m. (d) GI-transit analysis in naïve and IM24h animals. n=5. Statistics were done with Student's t-test. ***<0.001, All * compared to controls.

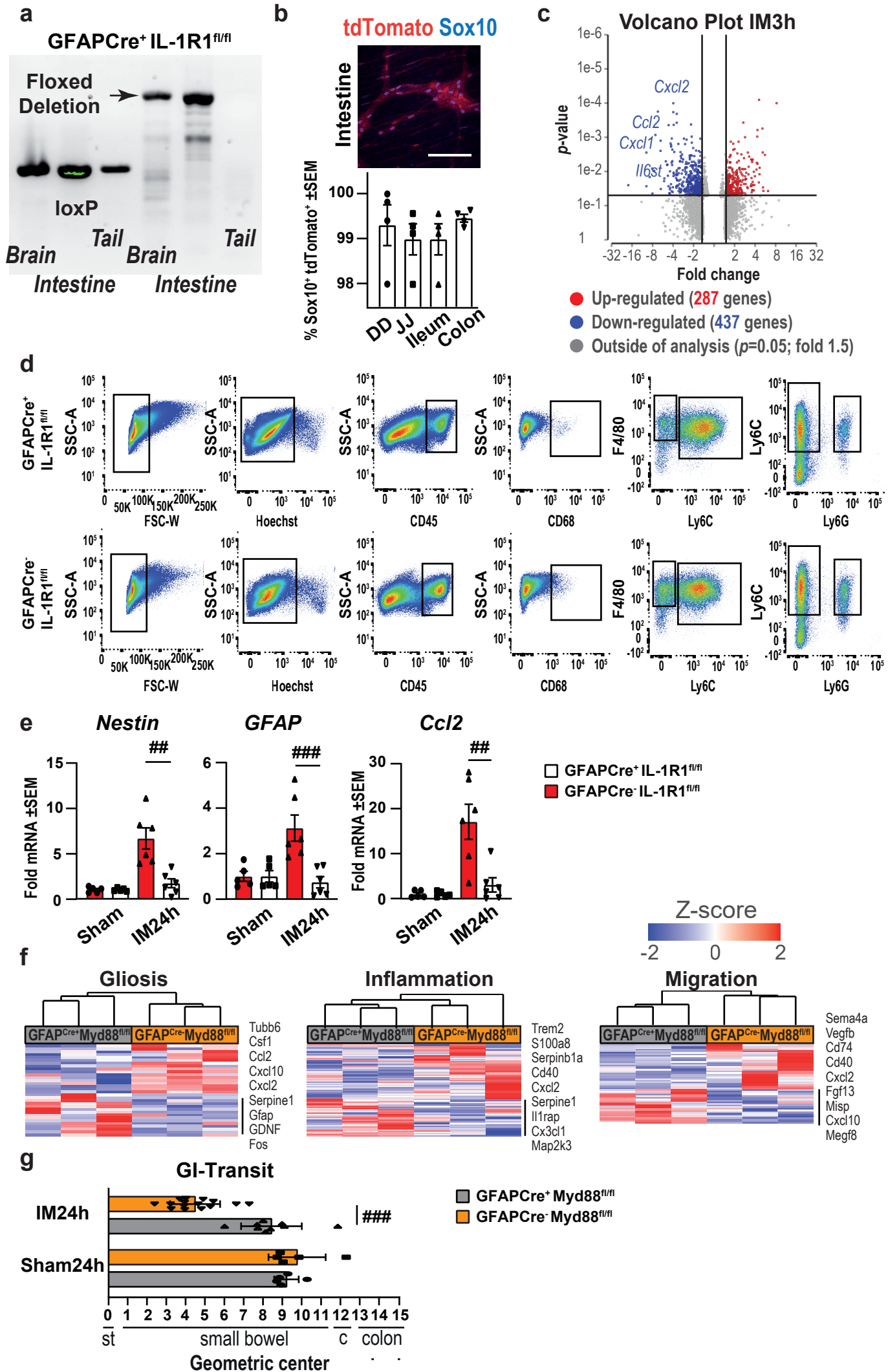


Fig. S3: EGC-restricted IL-1R1 deficiency prevents postoperative macrophage activation and protects mice from POI.

(a) PCR detecting IL1R1-loxP and IL1R1-deletion sites in the brain, intestine, and tail DNA of GFAP^{Cre+} and GFAP^{Cre-} animals. PCR proves successful Cre-loxP deletion in the IL1R1 gene locus. (b) Immunohistochemistry for EGC-marker SOX10 (blue) and tdTomato (red) in the ME of GFAP^{Cre+}Ai14^{fl/fl} mice. Quantification of double-positive cells showed a strong overlap of the SOX10⁺ tdTomato⁺ cells throughout the intestine (DD = duodenum, JJ = jejunum). n = 4. Scale bar 100µm. (c) Volcano plot showing all significantly regulated genes between Cre⁺ and Cre⁻ mice at IM3h normalized to corresponding sham groups. (d) GFAP^{Cre+}-IL1R1^{fl/fl} and GFAP^{Cre-}-IL1R1^{fl/fl} mice were analyzed at IM24h. Representative FACS analysis with gating strategy for singlets, living cells (Hoechst), CD45, CD68, Ly6C, Ly6G, F4/80 in IM24h groups of Cre⁺ and Cre⁻ mice. n=5 per group. (e) Gene expression analysis by qPCR for genes related to gliosis (*Nestin*, *GFAP*) and migration (*Csf1*) at IM24h in Cre⁺ and Cre⁻ mice normalized to corresponding sham groups. n=5 per group. (f) GFAP^{Cre+}-Myd88^{fl/fl} and GFAP^{Cre-}-Myd88^{fl/fl} mice underwent IM to induce POI. After 3h, RNA from the ME was processed for bulk-RNA-Seq analysis. Heat maps of genes involved in "gliosis", "inflammation", and "migration". n=3 per group. (g) GFAP^{Cre+}-Myd88^{fl/fl} and GFAP^{Cre-}-Myd88^{fl/fl} mice were analyzed at IM24h. GI-transit analysis in IM24h and sham groups of Cre⁺ and Cre⁻ mice. n= 5 (Sham24h); n=9-13 (IM24h). Statistics were done with Student's t-test and Fisher's exact test. ##<0.01, ###<0.001, All # compared to Cre- littermates.

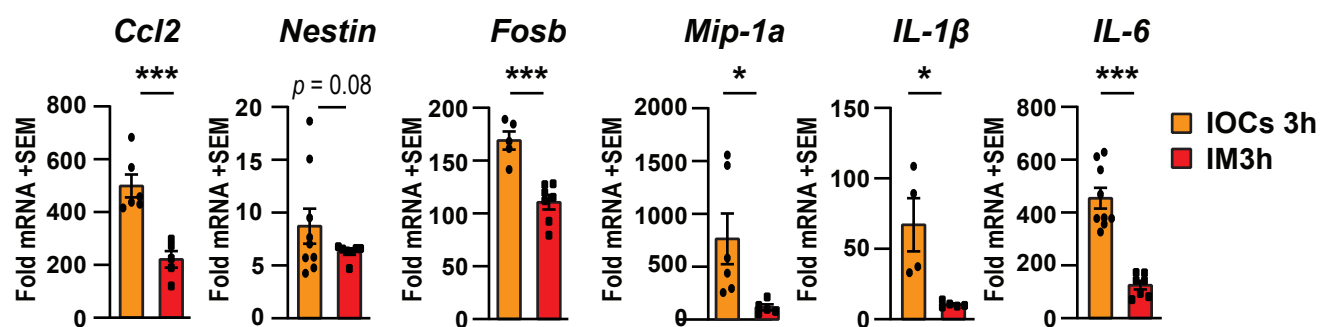
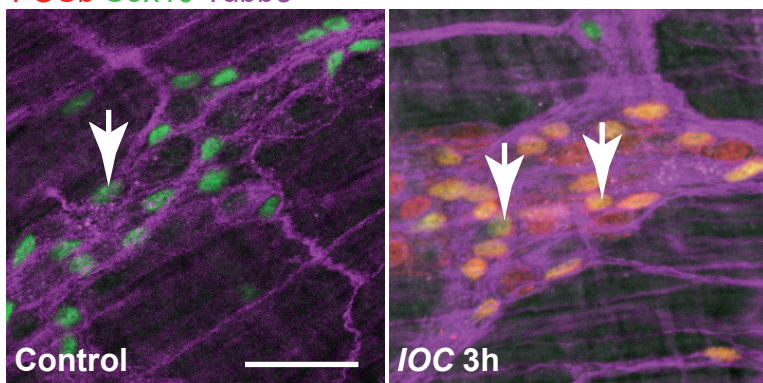
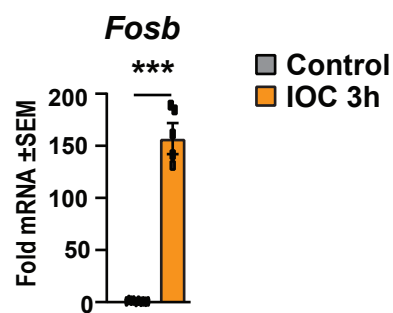
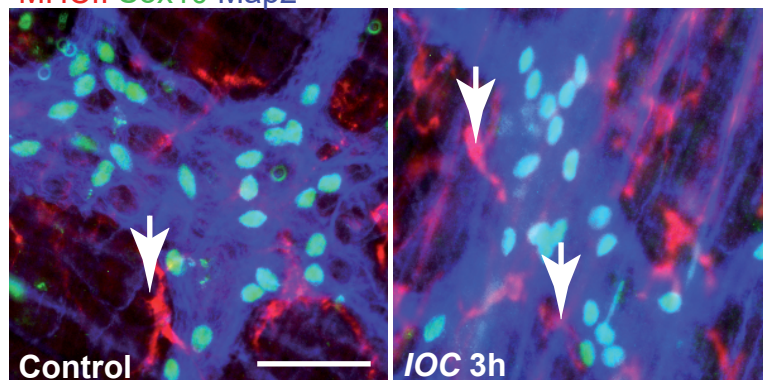
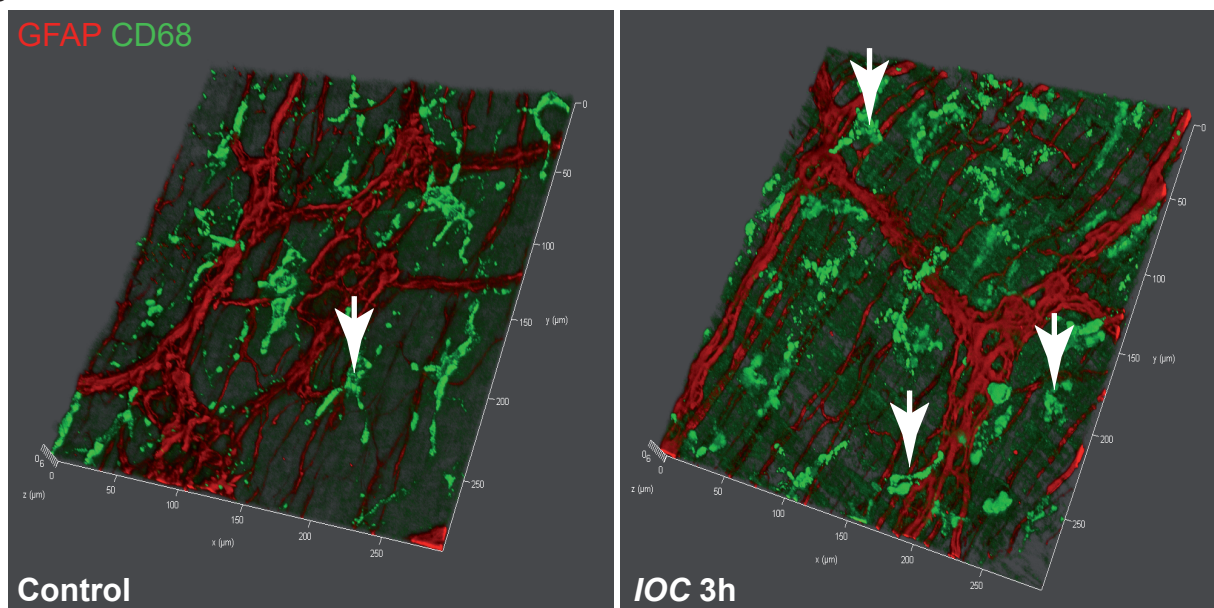
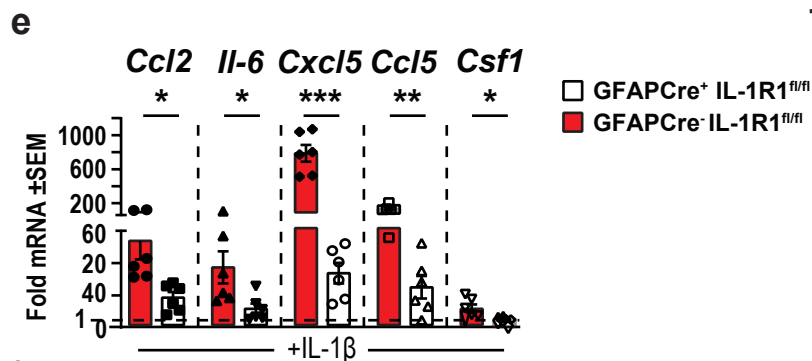
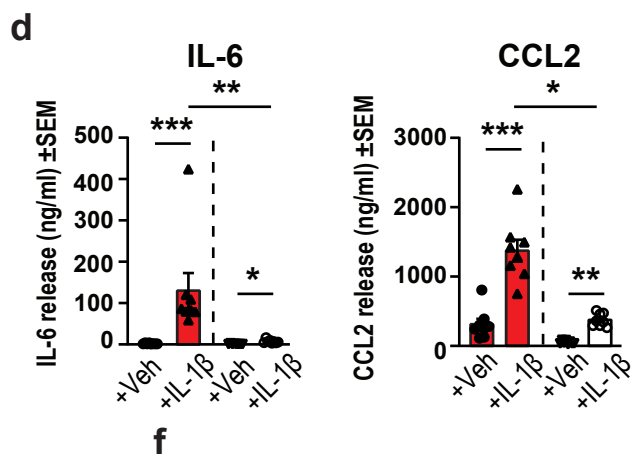
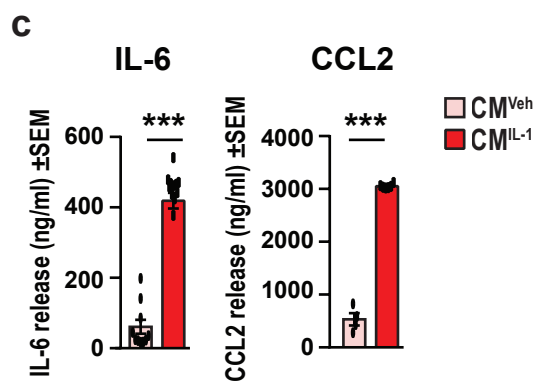
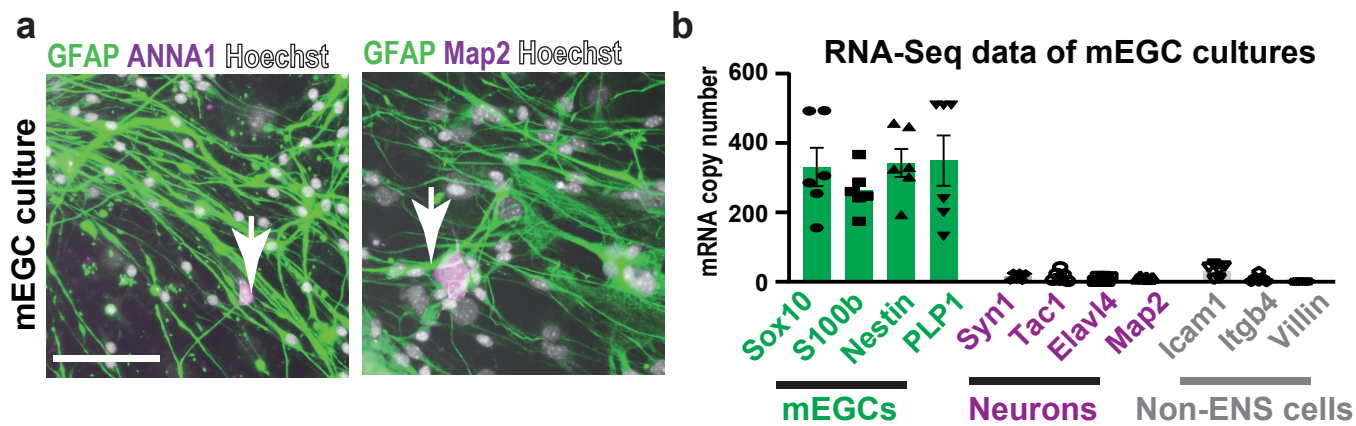
a**b****FOSb Sox10 Tubb3****c****d****MHCII Sox10 Map2****e**

Fig. S4: Intestinal organotypic cultures demonstrate IL-1-dependent involvement in ME-Mac-EGC interactions.

(a) qPCR analysis of *ME* IOCs 3h in culture and small intestine *ME* 3h after IM for the expression of inflammatory mediators and gliosis genes. IOCs show a stronger activation of enteric gliosis and a stronger expression of chemokines and cytokines. n=4-10 (IOCs); 8-10 (IM3h). Data are shown as fold induction to untreated IOCs or naïve *ME* tissue, respectively. (b-e) Intestinal organotypic cultures (IOC) from *ME* were prepared from C57BL/6 wild-type mice jejunum by surgical dissection of the *lamina propria* and mucosa tissue, a procedure that mimics the surgical trauma *in vivo*. This model allows analysis of resident cell types in the absence of any infiltrating blood-derived immune cells. IOCs were either directly processed or incubated for 3h, corresponding to the IM3h time point in the POI mouse model. n=6 per group. (b) Histology for activated (FOSb⁺, red) enteric glia (SOX10⁺, green) and neurons (TUBB3⁺, violet) in IOCs. White arrows mark activated EGCs (FOSb⁺ and SOX10⁺). Scale bar 50 μ m. (c) Gene expression analysis by qPCR for the activation marker *Fosb*. (d) Histology for macrophages (MHCII⁺, red) surrounding enteric glia (SOX10⁺, green) and neurons (MAP2⁺, violet) in IOCs. White arrows mark macrophages in close proximity to ganglia. Scale bar 50 μ m. (e) Confocal z-stacks of IOCs show activated macrophages (CD68⁺, green) surrounding EGCs (GFAP⁺, red). Statistics were done with Student's t-test. *<0.05, ***<0.001, All * compared to control IOCs.



up- and down-regulated genes in BMDMs

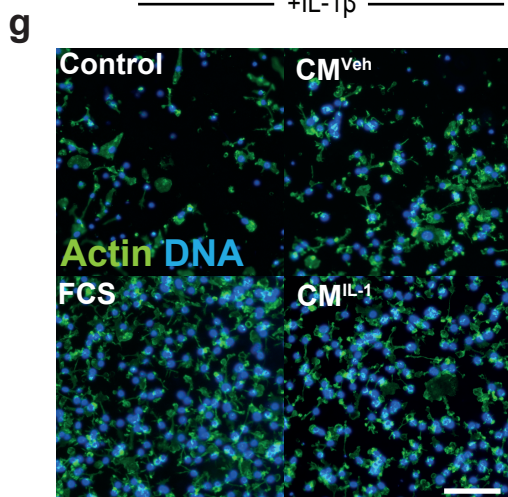
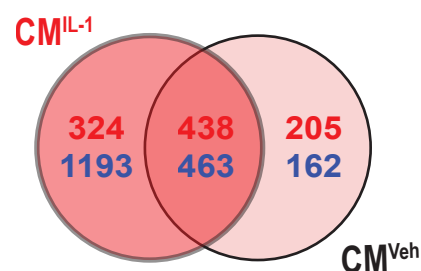


Fig. S5: The impact of EGC-derived factors on macrophage function.

(a) Immunocytochemistry of mEGC cultures for glia (GFAP, green) and neuron (MAP2 and ANNA1, violet) markers. Hoechst (white) was used for nuclei staining. Only small numbers of enteric neurons (white arrows) were detected in mEGC cultures. Scale bar = 50 μ m. (b) RNA-Seq analysis for various cell type markers in EGC cultures. Only glial markers were enriched in the EGC cultures. n=6. Data is shown as RNA copies. (c) Primary EGCs were treated with IL-1 β or vehicle for 24h. CM^{IL-1} and CM^{veh} were investigated for IL-6 and CCL2 content by ELISA. Data is shown as ng/ml $\pm$ SEM. n=10. (d+e) Primary EGCs isolated from GFAP^{Cre+}-IL1R1^{fl/fl} and GFAP^{Cre-}-IL1R1^{fl/fl} mice were treated with IL-1 β or vehicle for 24h. (d) Media from both cultures were investigated for IL-6 and CCL2 content by ELISA. Cre⁺-EGC cultures show less production of the inflammatory mediators IL-6 and CCL2. n = 3 independent EGC cultures with 2-3 technical replicates per measurements. Data is shown as ng/ml $\pm$ SEM. (e) qPCR analysis for chemokines and cytokines produced from primary EGCs after IL-1 β treatment. Cre⁺-EGC cultures show less activation and less production of inflammatory mediators. Data are shown as fold induction $\pm$ SEM. n = 6. (f) Bone-marrow-derived macrophages (BMDMs) were isolated from IL1R1-KO mice to exclude any side effects from IL1 β residues in the collected CM^{IL-1}. After BMDM maturation, cells were treated with CM^{IL-1}, CM^{veh}, or left untreated for 3 and 24h. Venn diagram of significantly up-and down-regulated genes (Fold 1.5, p-value 0.05) shared by CM^{IL-1} or CM^{veh}-treated BMDM groups. (g) Immunocytochemistry for actin (green) and nuclei (Hoechst, blue) of trans-migrating cells after CM^{IL-1}, CM^{veh}, and FCS (10%) stimulation. n=4 per group. Scale bar 50 μ m. Statistics were done with Student's t-test and Fisher's exact test. * <0.05 , ** <0.01 , *** <0.001 , All * compared to control EGCs.

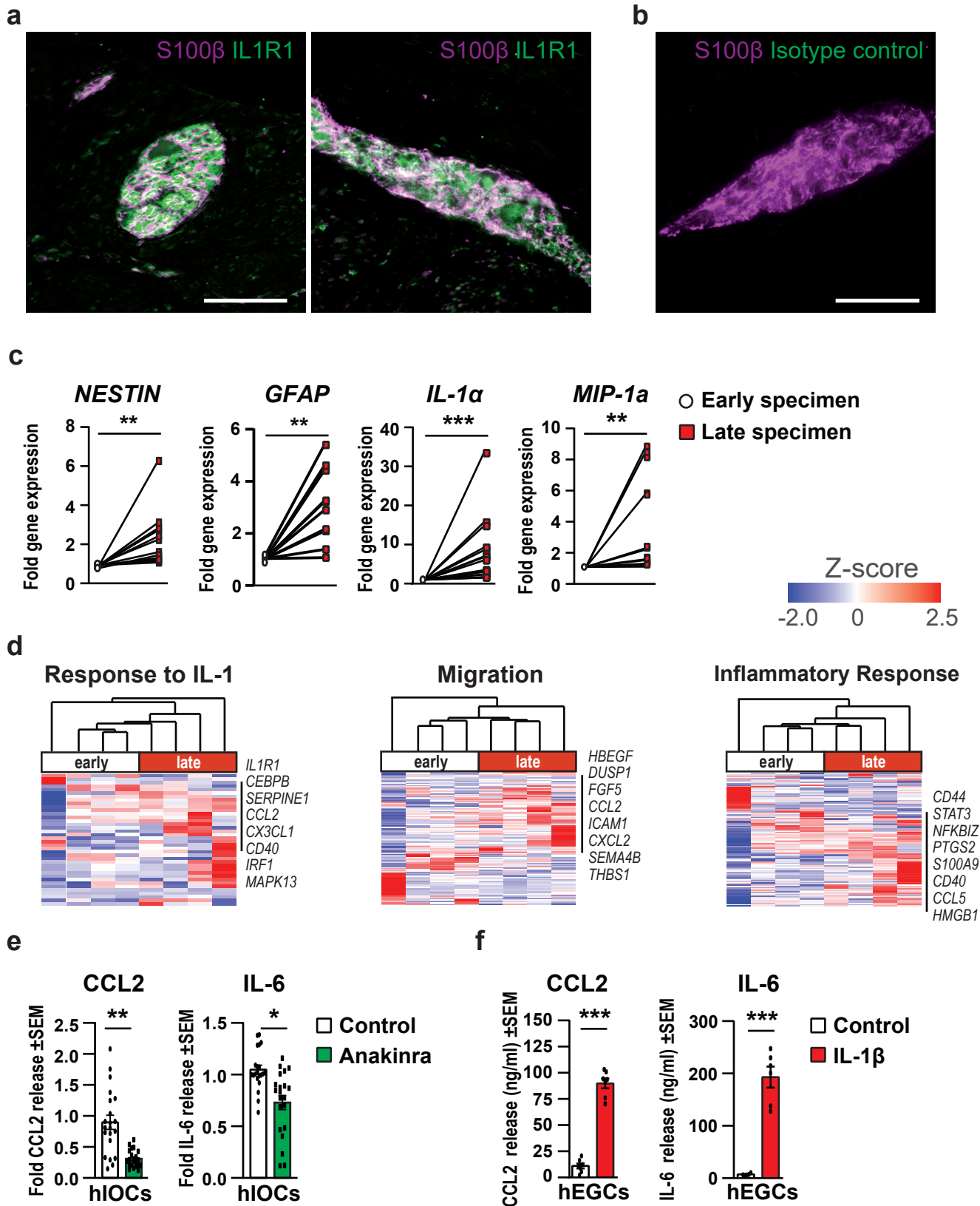


Fig. S6: Enteric gliosis and IL-1 signaling are involved in acute intestinal inflammation after abdominal surgery.

(a) IL1R1 immunoreactivity in EGCs of the myenteric plexus in the human intestine. IL1R1 (red) co-labeling occurs in S100 β ⁺ EGCs (violet). Scale bar 100 μ m. (b) Isotype control for IL1R1 antibody with S100 β ⁺ EGCs (violet). Scale bar 100 μ m. (c) Human jejunal ME specimens were collected at an early and late time point during pancreatic head resection. qPCR analysis of jejunal ME specimens for NESTIN, GFAP, IL-1 α , and MIP-1a. n=11-13; NESTIN (13), GFAP (12), IL-1 α (11), MIP-1a (13). (d) Heat maps of genes involved in “response to IL-1”, “migration”, and “inflammatory response” compared between early and late surgical specimens. (e) Human IOCs were isolated from jejunal patient samples collected at a late time point during pancreatic head resection and incubated for 24h with Anakinra (100 μ g/ml) or control. Media from both cultures were investigated for IL-6 and CCL2 content by ELISA. Anakinra treated human IOCs show less production of the inflammatory mediators IL-6 and CCL2. n = 16. (f) ELISA analyses for CCL2 and IL-6 of primary human EGCs treated with IL-1 β (0.3 ng/ml) or vehicle for 24h. n = 10. Statistics were done with Student’s t-test and Fisher’s exact test. *<0.05, **<0.01, ***<0.001, All * compared to early ME specimens or untreated hEGCs.

Supplementary tables

Supplementary Table S1: Human subjects and GI surgical specimens for IHC and hEGC cultures

#	Patient ID	Sex	Age	Surgical procedure	GI region
1	PSG154	male	64	Colectomy partial	<i>sigmoid colon</i>
2	PSG270	male	64	Colectomy partial, open	<i>sigmoid colon</i>
3	PSG271	male	50	Sigmoidectomy, laparoscopic	<i>sigmoid colon</i>
4	PSG287	female	52	Colectomy partial, laparoscopic	<i>ascending colon</i>
5	PSG288	male	68	Colectomy partial, open	<i>sigmoid colon</i>
6	PSG291	female	56	Colectomy partial, laparoscopic	<i>descending colon</i>
7	PSG294	female	66	Colectomy partial, open	<i>sigmoid colon</i>
8	ETBRPOI-002	female	43	Roux-en-Y	<i>jejunum</i>
9	ETBRPOI-003	female	34	Roux-en-Y	<i>jejunum</i>
10	ETBRPOI-004	female	42	Roux-en-Y	<i>jejunum</i>

Supplementary Table S1 summarizes information on the 14 human subjects that gave written consent to procure gut surgical tissue for *in vitro* studies. Tissue was used to isolate ganglia, prepare hEGC cultures for study, or paraffin sections for immunofluorescence staining (including jejunum IL1R1-IHC and qPCR analysis). Studies were conducted under IRB protocols.

Supplementary Table S2: Human subjects and GI surgical specimens for analysis of gliosis

#	Patient ID	Sex	Age	Start Time of procedure	Collection time points	Previous illness	Medications
1	160303_hME	male	55	09:18	11:35 and 14:00	Diabetis mellitis type II	Janumet (diabetis) Ramipril (ACE inhibitor) Cedur (blood lipids reducer) Pantozol (gastric acid inhibitor)
2	160331_hME	male	85	08:50	10:50 and 12:16	none	Ursfolk (gallstone reducer) Simvastatin (Cholesterol reducer) Pantozol Clexane (coagulation inhibitor)
3	160405_hME	male	79	09:14	11:15 and 12:40	art. hypertension polyneuropathy	Pantozol Ramipril Alna (prostate gland hyperplasia) Clexane Pregabador (neuropathy) Riopan (gastric acid inhibitor)
4	180220_hME	male	80	8:30	12:20 and 13:10	benign hyperplasia of the prostate gland art. hypertension cardiac arrhythmia duodenal ulcer	Pantozol Propafenone HCl (cardiac arrhythmia) Alna Valsartan/ Metohexal (antihypertensive drugs)
5	180221_hME	male	74	10:00	13:45 and 14:15	benign hyperplasia of the prostate gland art. hypertension hyperlipidemia	Alna Simvastatin Pantozol Beloc (antihypertensive drug) ASS Kalinor (potassium donor) Xarelto (coagulation inhibitor)
6	180424_hME	female	61	08:30	11:35 and 13:50	moderate aorta insufficiency rheumatoid arthritis reflux esophagitis art. hypertension hypercholesterolemia	Bisoprolol (β -blocker) Euthyrox (L-Thyroxin) Lantarel (arthritis) Lisinopril (blood pressure reducer) Rantudil (arthritis) Methotrexat/Golimumab (arthritis)
7	180821_hME	male	59	9:00	12:15 and 13:35	hyperplasia of the prostate gland sleep apnea syndrome kidney cyst right side	Amlodipin (antihypertensive drug) Pantozol
8	181029_hME	female	73	07:45	10:30 and 11:15	COPD Myxoma Diabetis mellitus Type II hypertension hyperlipidemia	Berotec (COPD) Inuvair (COPD) Spiriva (COPD) clexane Bisoprolol (blood pressure reducer) Ramipril HCT (diuretic drug) Marcumar Atorvastatin

9	190429_hME	female	61	9:15	13:58 and 15:13	Diabetis mellitus art. hypertension	Pantazol Amlodipin
10	190506_hME	male	76	8:55	12:12 and 13:00	Ulcera Duodeni reflux esophagitis II° sigma diverticulosis Diabetis mellitus Type II	ASS Atorvastatin (cholesterol reducer) Gabapentin (antiepileptic) Amitriptilin (antidepressant)
11	190805_hME	male	29	10:24	12:00 and 14:29	pancreas insufficiency, reflux esophagitis, opticus neuropathy, ambiguous tremor, benigne hyperplasia of prostate gland, depression	Clonazepam/Propranolol (tremor) Mitrzapin/Risperdal/Anafran il (antidepressants) Mictinorm (control of bladder function)
12	190806_hME	female	30	10:01	12:25 and 13:30	von Hippel-Lindau syndrome pheochromocytoma	Paracetamol Clexane
13	190808_hME	female	70	10:00	13:40 and 18:20	pul./art. hypertension mitral-/tricuspidal regurgitation chron. pancreatitis sec. Diabetis mellitus Type 3c kidney insufficiency (dialysis) vein thrombosis	Marcumar (antikoagulans) Atorvastatin Pantazol Bisopropol (β-blocker) Torem (urine excretion) Amitriptylin
14	190917_hME	female	51	9:21	12:07 and 12:55	none	Pantazol Sertralin (antidepressant) Paracetamol Clexane
15	191017_hME	male	75	9:11	13:38 and 15:22	silicosis gastritis	Pantazol Eliquis (coagulation inhibitor) Alina
16	191023_hME	male	57	9:11	11:20 and 14:10	cholestasis acute pancreatitis NSTEMI (myocardial infarction) art. hypertension obesity Diabetis mellitus Type II hyperlipidemia	Levemir (insulin) ASS Amlodipin Metformin (antidiabetic) Pantazol Rekawan (potassium donor) Simvastin (cholesterol reducer)
17	191119_hME	male	62	9:04	11:20 and 12:20	aortic aneurysm	Clexane
18	200109_hME	male	64	9:18	12:10 and 13:25	kidney cell carcinoma hypercholesterolemia a art. hypertension Diabetis mellitus Type II nicotine abuse	Telmisartan (blood pressure reducer) Metformin Atorvastatin Pantoprazole Actrapid (insulin) Lantus (insulin)
19	200326_hME	female	71	9:28	13:22 and 14:42	peripheral arterial disease	ASS Allopurinol (uric acid reducer)

						constriction of inner carotis art. hypertension	Bisohexal/Candesartanicilex etil (blood pressure reducer) Pantozol
20	200729_hME	female	78	9:27	10:55 and 11:45	hyperthyroidism carpal tunnel syndrome	Pantozol L-Thyroxin
21	200811_hME	female	68	8:55	10:40 and 12:25	lung carcinoma jaw angle carcinoma art. hypertension Diabetes mellitus	Pantozol L-Thyroxin Metoprolol (blood pressure reducer) Ramipril Amlodipin Mirtazapine Xipamid (urine excretion) Metform (blood sugar reducer) Kalinor Clexane
22	200818_hME	female	62	9:30	11:18 and 12:45	art. hypertension obesity chron. pain syndrome hypothyroidism	Riopan Clexane Ramipril Pantozol L-Thyroxin Levemir Metformin Actrapid

Supplementary Table S2: Information on 16 patients who underwent pylorus-preserving pancreaticoduodenectomy and gave written consent to procure jejunal tissue for qPCR studies. The tissue was used for RNA isolation, cDNA preparation, and gene expression analysis. The study was conducted under protocols permitted by the ethical committee of the University of Bonn, Germany.

Supplementary Table S3: Oligonucleotide primers

Gene	Forward	Reverse
<i>mIL-1β</i>	TTGACGGACCCCAAAAGATG	CAGGACAGCCCAGGTCAAAG
<i>mCD68</i>	TGACCTGCTCTCTCTAAGGCTACAG	AGGACCAGGCCAATGATGAG
<i>mMip-1a</i>	ACCATGACACTCTGCAACCA	CCCAGGTCTCTTTGGAGTCA
<i>mCsf-1</i>	GTGTCAGAACACTGTAGCCAC	TCAAAGGCAATCTGGCATGAAG
<i>mTnfa</i>	CTGAACTTCGGGGTGATCGG	GGCTTGCTCACTCGAATTTTGAGA
<i>mGFAP</i>	ACATCGAGATCGCCACCTAC	CCTTCTGACACGGATTTGGT
<i>mNestin</i>	AGATCGCTCAGATCCTGGAA	AGGTGTCTGCAAGCGAGAGT
<i>mIL-6</i>	AAGTCGGAGGCTTAATTACACATGT	CCATTGCACAACCTCTTTTCTCATT
<i>mFosb</i>	ATGGGCTCTCCTGTCAACAC	ACGGAGGAGACCAGAGTGG
<i>mCcl2</i>	CCCAATGAGTAGGCTGGAGA	GCTGAAGACCTTAGGGCAGA
<i>mCcl5</i>	GTGCCACGTC AAGGAGTAT	CCCACTTCTTCTCTGGGTTG
<i>mCxcl2</i>	TCCAGGTCAGTTAGCCTTGC	CGGTCAAAAAGTTTGCCTTG
<i>mCxcl5</i>	CGCTAATTTGGAGGTGATCC	AACACTGGCCGTTCTTTCC
<i>18S-RNA</i>	GTGGAGCGATTTGTCTGGTT	ACGCTGAGCCAGTCAGTGTA
<i>18S-RNA</i>	Assay ID 4319413E	
<i>hIL-1α</i>	TGGTAGTAGCAACCAACGGGA	ACTTTGATTGAGGGCGTCATTC
<i>hIL-1β</i>	AGCTACGAATCTCCGACCAC	CGTTATCCCATGTGTCGAAGAA
<i>hGFAP</i>	CTGCGGCTCGATCAACTCA	TCCAGCGACTCAATCTTCCTC
<i>hNESTIN</i>	GGGAGTTCTCAGCCTCCAG	GGAGAAACAGGGCCTACAGA
<i>hCCL2</i>	ATAACAGCAGGTGACTGGGG	CCAGCATGAAAGTCTCTGCC
<i>hMIP-1a</i>	GCTGACTACTTTGAGACGAGC	CCAGTCCATAGAAGAGGTAGC
<i>hIL-6</i>	GTCAGGGGTGGTTATTGCAT	AGTGAGGAACAAGCCAGAGC
<i>hCXCL2</i>	GGCAGAAAGCTTGTCTCAACCC	CTCCTTCAGGAACAGCCACCAA

Supplementary Table S3 summarizes information on all used qPCR primers.

Supplementary Table S4: Antibodies used in the study

Target	Dye/Dilution	Secondary antibody*	Use	Company
CD45	Pacific blue / 1:200		FACS	Biolegend Clone: 30-F11 #103126
Ly6C	APC / 1:300		FACS	eBioscience Clone: HK1.4 # 17-5932-82
CD68	Alexa647 / 1:400		FACS	Biolegend Clone: FA-11 #137003
Ly6G	FITC / 1:300		FACS	Biolegend Clone: 1A8 #127605
F4/80	PE / 1:400		FACS	Biolegend Clone: BM8 #123110
MHCII	1:400	AlexaFluor647 or Cy3	IHC	BioLegend Clone:M5/114.15.2 # 107602
GFAP	1:1000	AlexaFluor647	IHC/ICC	Synaptic System # 173002
Sox10	1:400	Cy3	IHC/ICC	Santa Cruz # sc- 17342
FOSb	1:200	Cy3	IHC	Santa Cruz # sc52
s100 β	1:500	Cy3	IHC	Sigma S2532
IL1R1	1:100	Cy2	IHC	Abcam # ab106278
HA-Tag (C29F4)	1:500	Cy5	IHC	cell signalling #3724
CD68	1:500	Cy2	IHC	Biorad # MCA1957
Phalloidin		Alexa488-coupled		Life Technologies #A12379
CD68	1:400	Cy2	IHC	Biolegend #333802
GFAP	1:1000	Cy3	IHC	DAKO #Z 0334
Map2	1:2000	Cy2	IHC	Biolegend #BLD-822501
Tubb3	1:1000	Cy5	IHC	Biolegend #801201
Hoechst	1:5000		ICC/IHC	Thermo Scientific #H3570

Supplementary Table S4 summarizes information on all used antibodies.

Supplementary Table S5: Overlap of genes between IL-1-triggered EGCs, infection-triggered EGC, and DNBS-colitis-triggered EGCs

Gene Names
<i>Cd44</i>
<i>Tubb6</i>
<i>Tnc</i>
<i>Ccl7</i>
<i>Tnfrsf12a</i>
<i>Hmox1</i>
<i>Lgals1</i>
<i>Vcan</i>
<i>Ccl2</i>
<i>Timp1</i>
<i>Serpina3n</i>
<i>Lgals3</i>

Supplementary Table S5 summarizes information on the RNA-Seq analysis of EGCs triggered with IL-1 β and infection and DNBS-colitis.

Supplementary Table S6: Overlap of genes between IL-1-triggered EGCs and IM3h-RiboTag-EGCs

Gene name
<i>Ptgs2</i>
<i>Cd44</i>
<i>Csf1</i>
<i>Gcnt2</i>
<i>Tgfb1</i>
<i>Tubb6</i>
<i>Icam1</i>
<i>Tnc</i>
<i>Tnfrsf12a</i>
<i>Rfc3</i>
<i>Cp</i>
<i>Hmox1</i>
<i>Epha4</i>
<i>Pvr</i>
<i>Ptx3</i>
<i>Vcan</i>
<i>Ccl2</i>
<i>Ctsb</i>
<i>Thbs1</i>
<i>Srxn1</i>
<i>Cxcl1</i>
<i>Sphk1</i>
<i>Timp1</i>
<i>Hmga1</i>
<i>Il6</i>
<i>Cxcl2</i>

Supplementary Table S6 summarizes information on the RNA-Seq analysis of EGCs triggered with IL-1 β and glial gene expression from *RiboTag* mice at IM3h.