

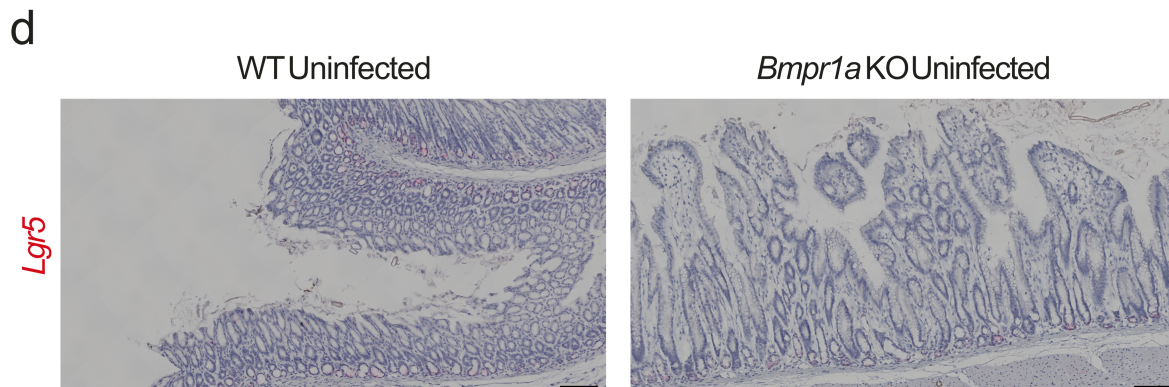
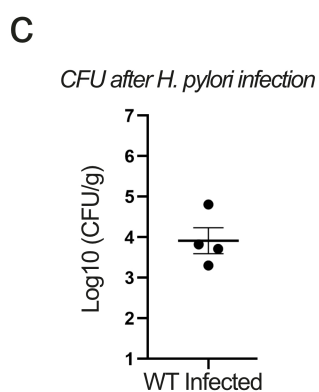
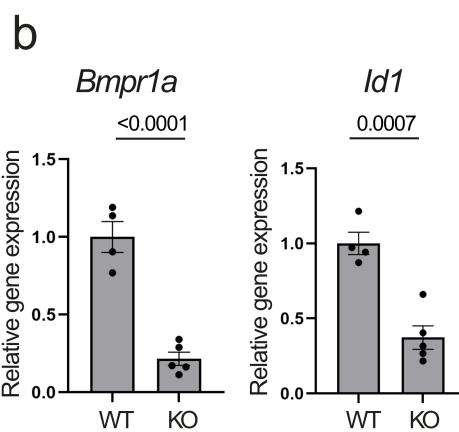
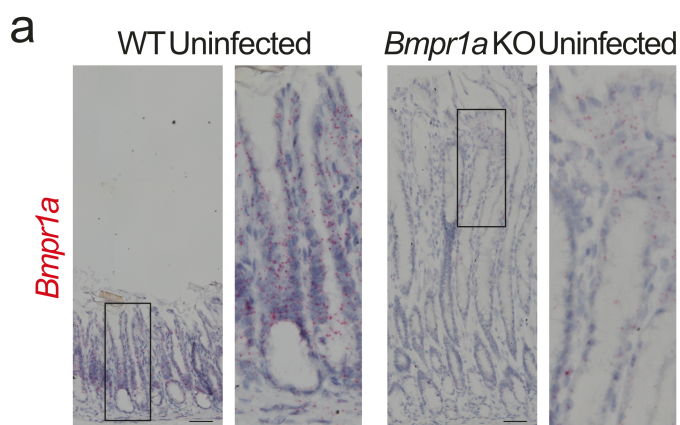
Supplementary Information

***Helicobacter pylori* triggers gastric mucosal remodeling toward a fetal-like transcriptional program via stromal IL-1 β signaling**

Giulia Beccaceci, Stefanie Müllerke, Hilmar Berger, Christian Täger, Ronja Möbius, Anne-Sophie Fischer, Kimberly Hartl, Jonas Wizenty, Hans-Joachim Mollenkopf, Michael Naumann, Manqiang Lin, Michael Sigal

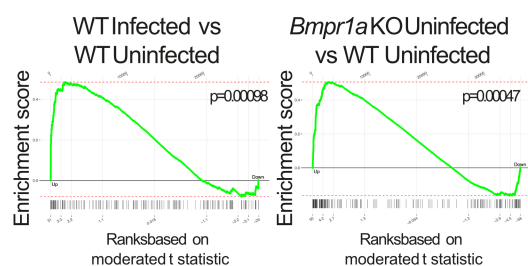
This PDF file includes:

Supplementary Figures 1-11



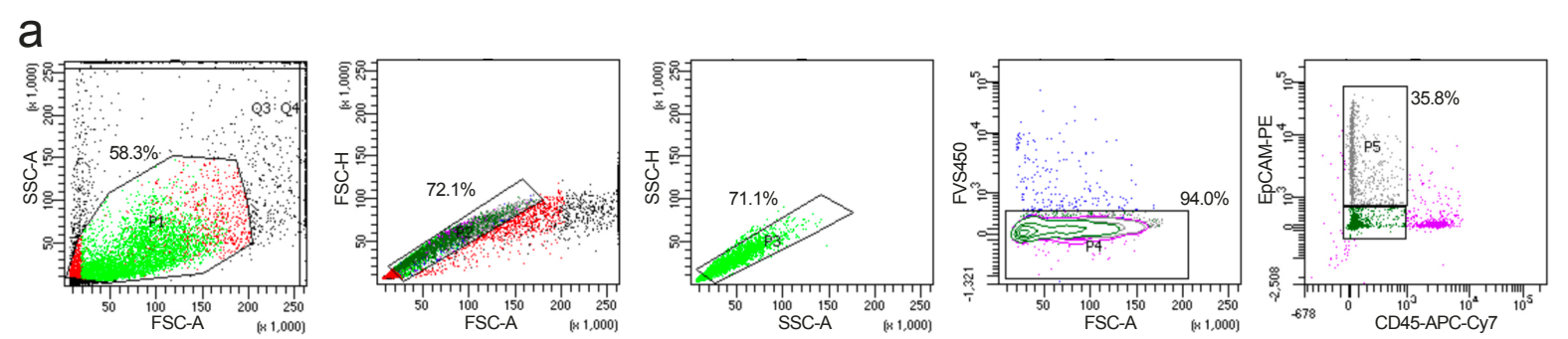
e

YAP KO down (Gregorieff et al.)

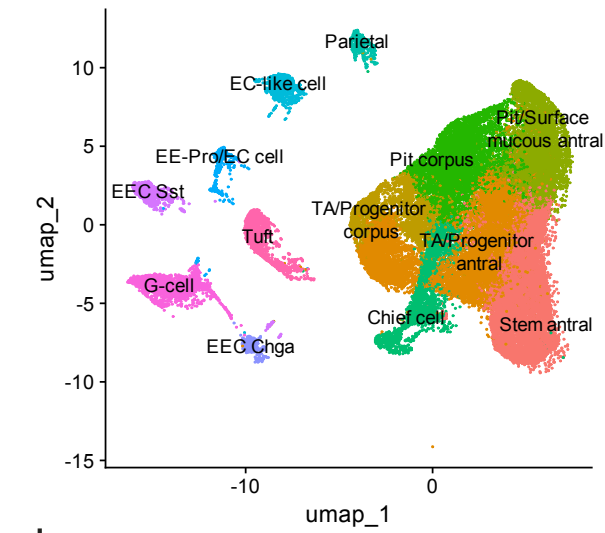


Supplementary Fig.1 Characterization of *Bmpr1a* KO mice and *H. pylori* infection

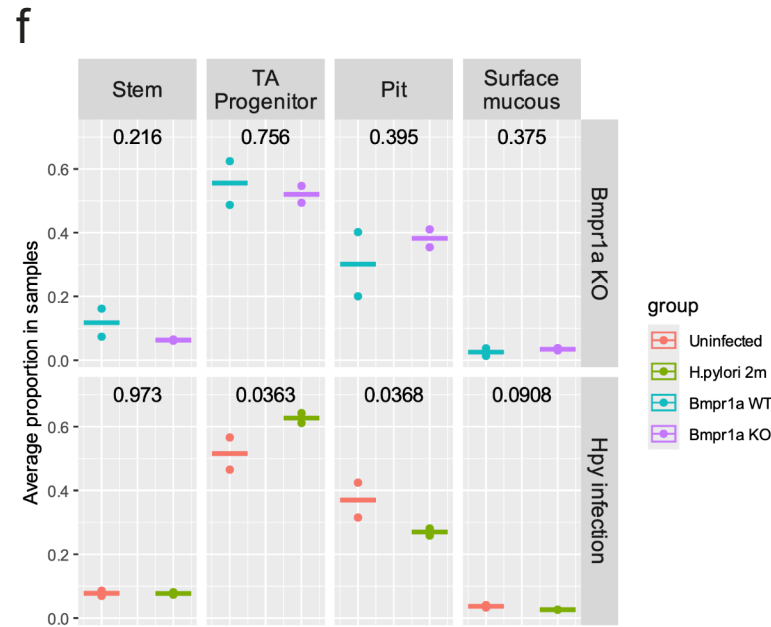
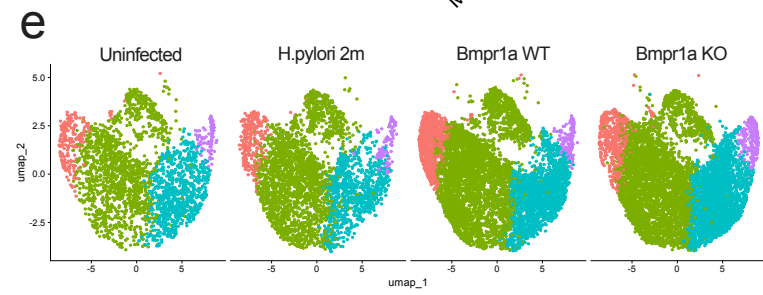
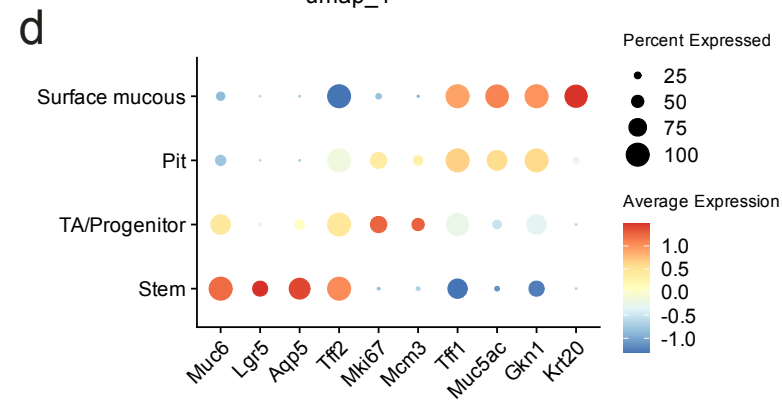
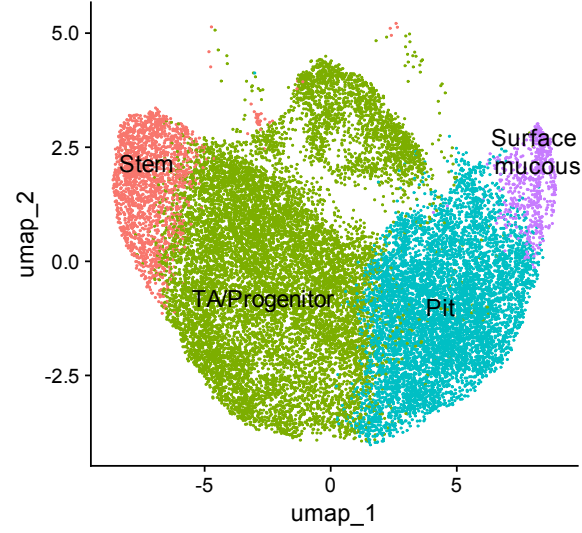
a ISH images for *Bmpr1a* in antral tissues from uninfected WT ($n = 4$) and *Bmpr1a* KO ($n = 5$) mice. Scale bars: 50 μm . **b** mRNA expression of *Bmpr1a* and *Id1* in uninfected WT ($n = 4$ mice) and *Bmpr1a* KO ($n = 5$ mice) antral tissues. **c** Colony-forming unit (CFU) quantification for gastric tissues of 2-month-infected WT mice ($n = 4$ mice). **d** ISH images for *Lgr5* in sections from uninfected WT and *Bmpr1a* KO mice. **e** GSEA of microarray data comparing antral tissue from infected WT vs. uninfected WT mice, as well as from uninfected *Bmpr1a* KO vs. WT mice ($n = 2$ mice per group), for the YAP target gene signature (YAP KO down). Scale bar: 100 μm . Data are presented as mean $\pm$ SEM. Statistical analyses were performed using Student's *t*-test (two-tailed) for **b**; using unadjusted *p*-values of permutation test provided by R package fgsea for **e**. Images are representative of at least three biological replicates. Source data are provided as a Source Data file.



b Murine Gastric Epithelium

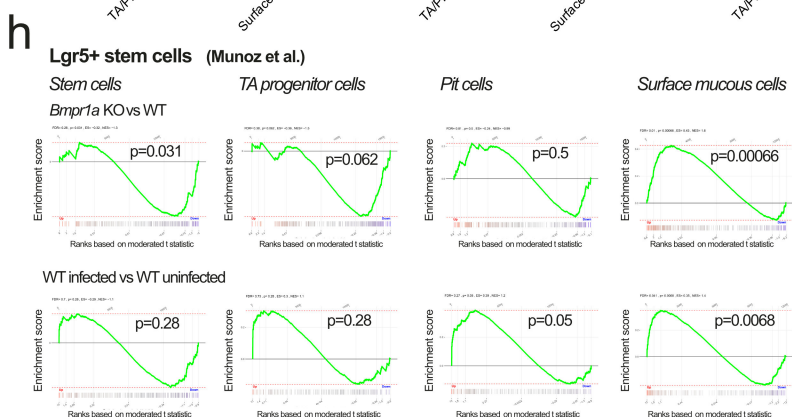
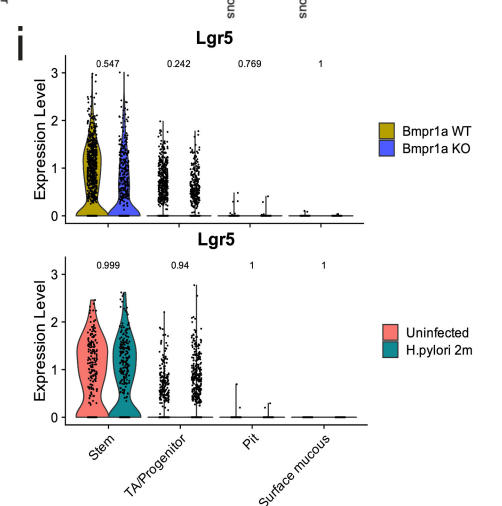
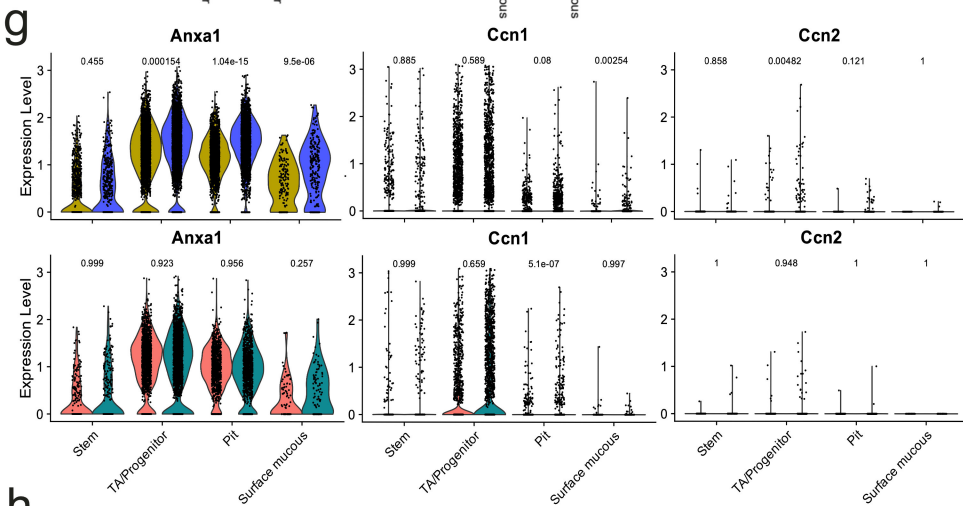
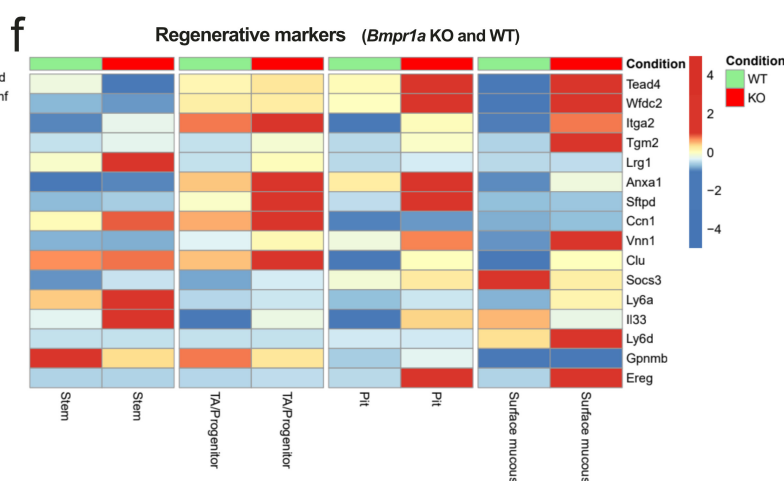
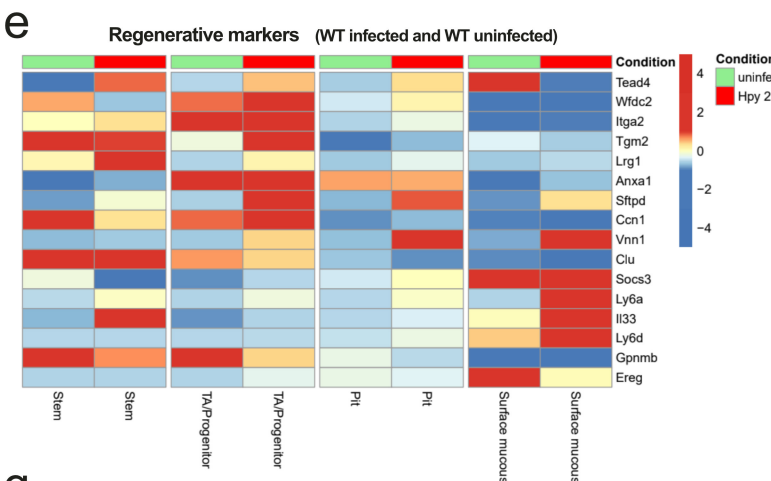
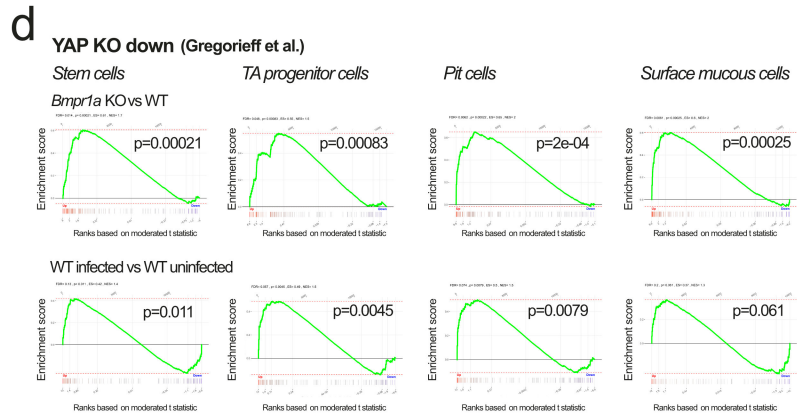
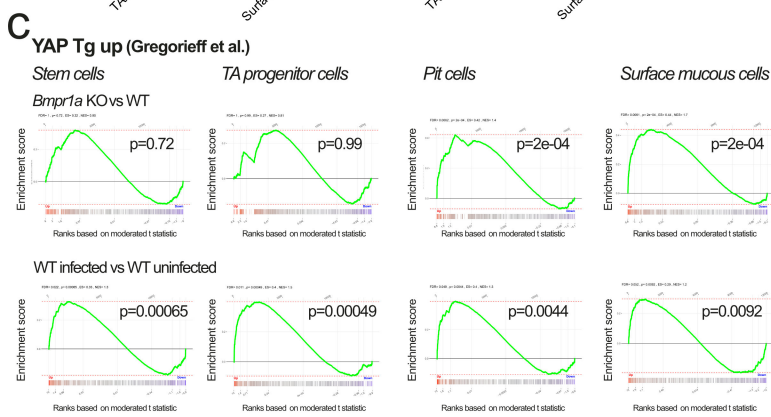
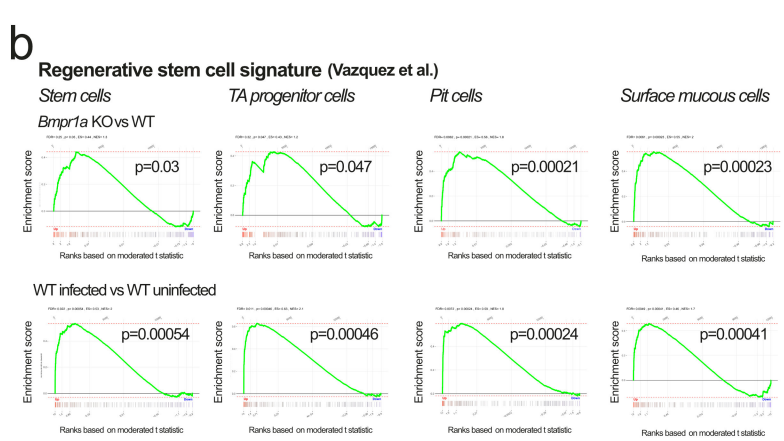
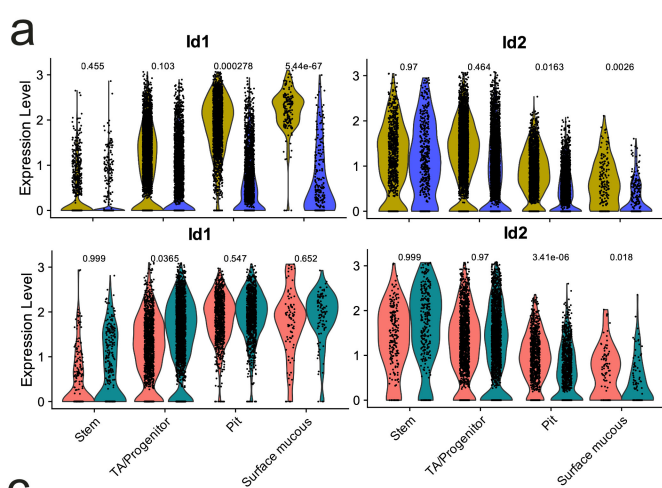


c Murine Antral Epithelium



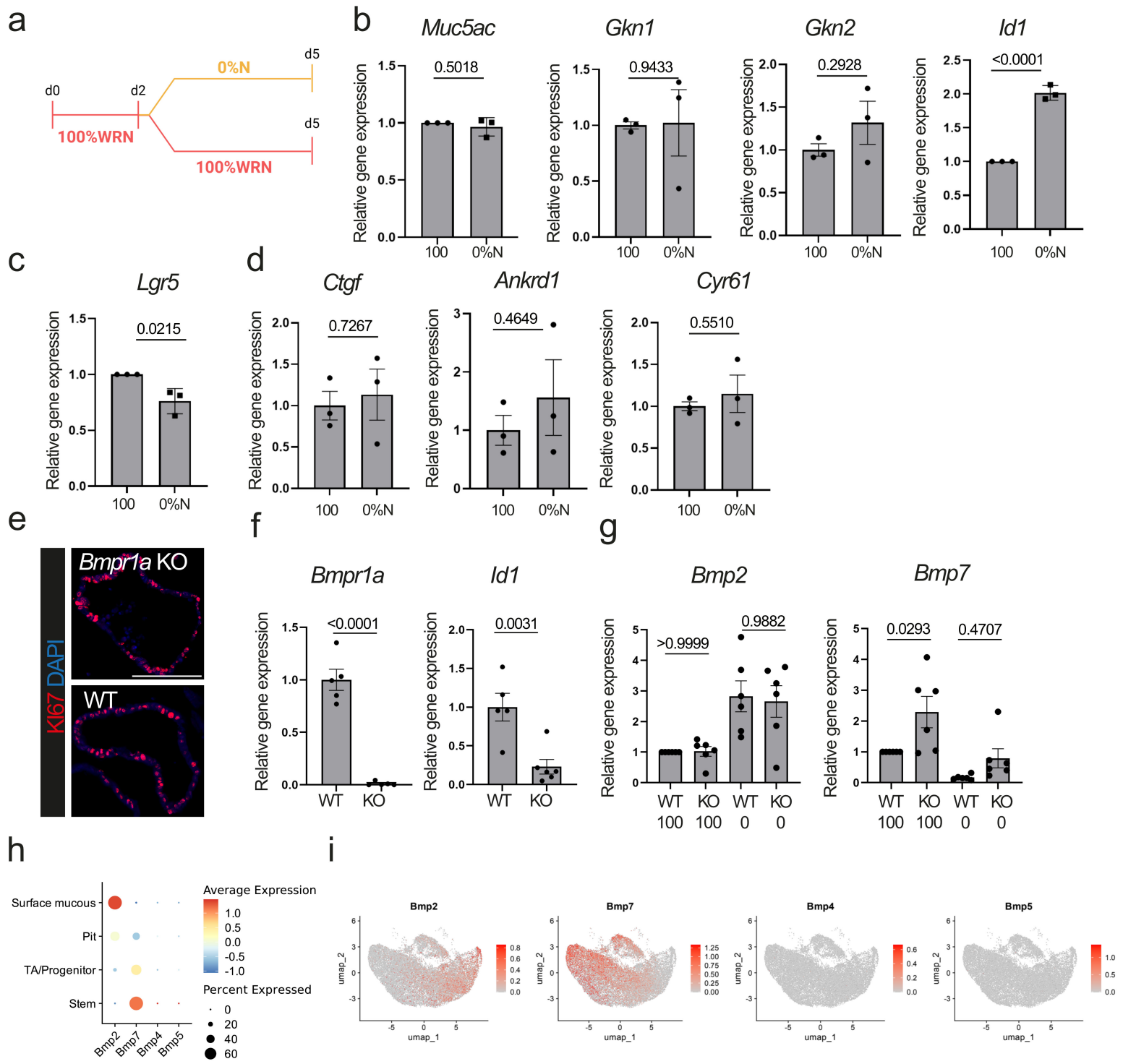
Supplementary Fig. 2 Single-cell RNA-sequencing of murine gastric epithelium

a Single cells were gated based on SSC-A, FSC-A, FSC-H, and SSC-H; live cells were identified by negative FVS450 staining, and epithelial cells were selected as EpCAM-positive and CD45-negative. Gate P5 indicates the epithelial cell population selected for downstream processing. **b-c** UMAP plots of the scRNA-seq dataset showing integrated clusters and cells from uninfected WT vs. infected WT mice, as well as from uninfected *Bmpr1a* KO vs. WT mice. Main epithelial cell clusters from the antrum were isolated for further analysis (**c**). **d** Dot plot showing the expression of cluster marker genes across identified subpopulations in the antral epithelium. Circle size indicates the within-cluster proportion of gene detection; fill color represents the scaled average expression level. **e** Individual UMAP plots showing antral epithelial cells from uninfected WT vs. infected WT mice, as well as from uninfected *Bmpr1a* KO vs. WT mice. **f** Comparison of the proportions of antral epithelial subpopulations in uninfected WT vs. infected WT mice, as well as from uninfected *Bmpr1a* KO vs. WT mice. Numbers indicate *p*-values of proportion differences between groups ($n = 2$ mice per group; moderated *t*-test after logit-transform of proportions).

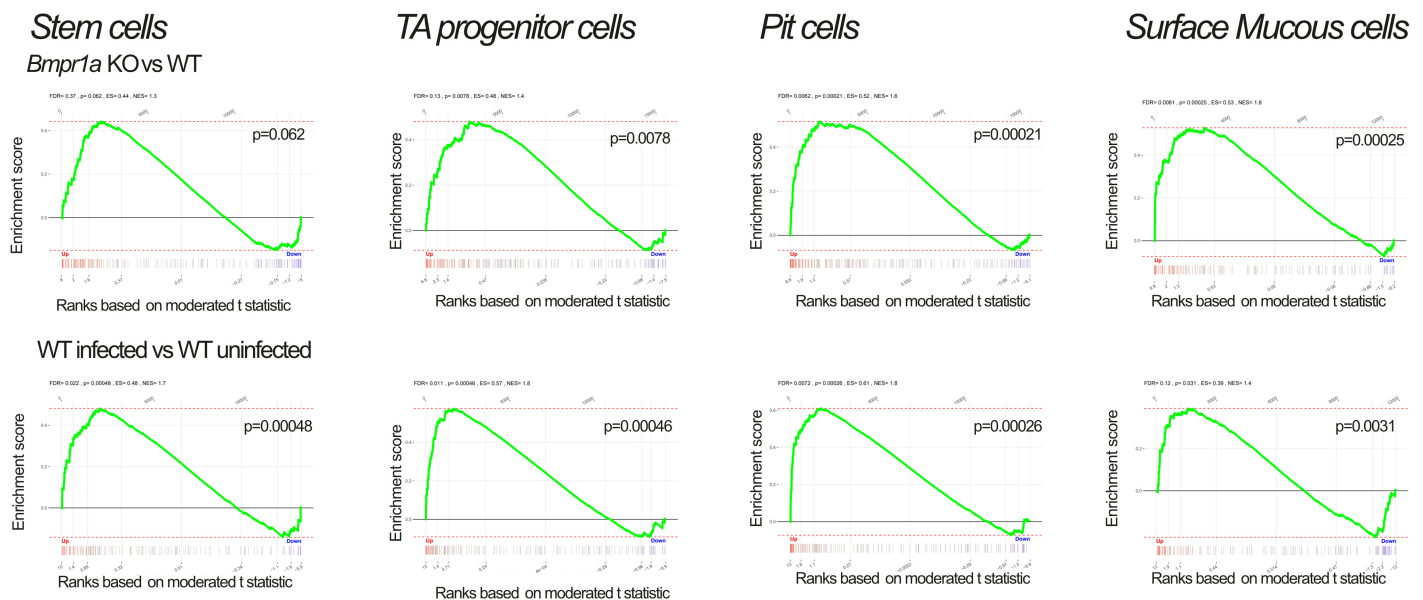


Supplementary Fig. 3 Gene expression and signature analyses of murine gastric epithelium

a Violin plot showing the expression level of *Id1* and *Id2* in antral epithelial cells from uninfected *Bmpr1a* KO vs. WT mice, as well as from infected WT vs. uninfected WT mice. **b-d** GSEA of scRNA-seq pseudo-bulk expression differences comparing antral tissue from uninfected *Bmpr1a* KO vs. WT mice, as well as from infected WT vs. uninfected WT mice, for regenerative stem cell signature (**b**), and YAP target gene signatures YAP Tg up (**c**) and YAP KO down (**d**). **e-f** Heatmap showing average expression of regenerative marker genes in antral epithelial cell populations. **g** Violin plot showing the expression level of *Anxa1*, *Ccn1* (*Cyr61*), and *Ccn2* (*Ctgf*) in antral epithelial cells from uninfected *Bmpr1a* KO vs. WT mice, as well as from infected WT vs. uninfected WT mice. **h** GSEA of scRNA-seq comparing antral tissue from uninfected *Bmpr1a* KO vs. WT mice, as well as from infected WT vs. uninfected WT mice, for *Lgr5*+ stem cell signature. **i** Violin plot showing the expression level of *Lgr5* in antral epithelial cells from uninfected *Bmpr1a* KO vs. WT mice, as well as from infected WT vs. uninfected WT mice. For **a**, **g**, **i**, numbers at the top indicate *p*-values for mean differences between conditions for each cluster (*n* = 2 mice per group), derived from genome-wide pseudo-bulk differential expression analysis of antral scRNA-seq data. Statistical analyses were performed using unadjusted *p*-values of permutation test provided by R package fgsea for **b-d** and **h**.

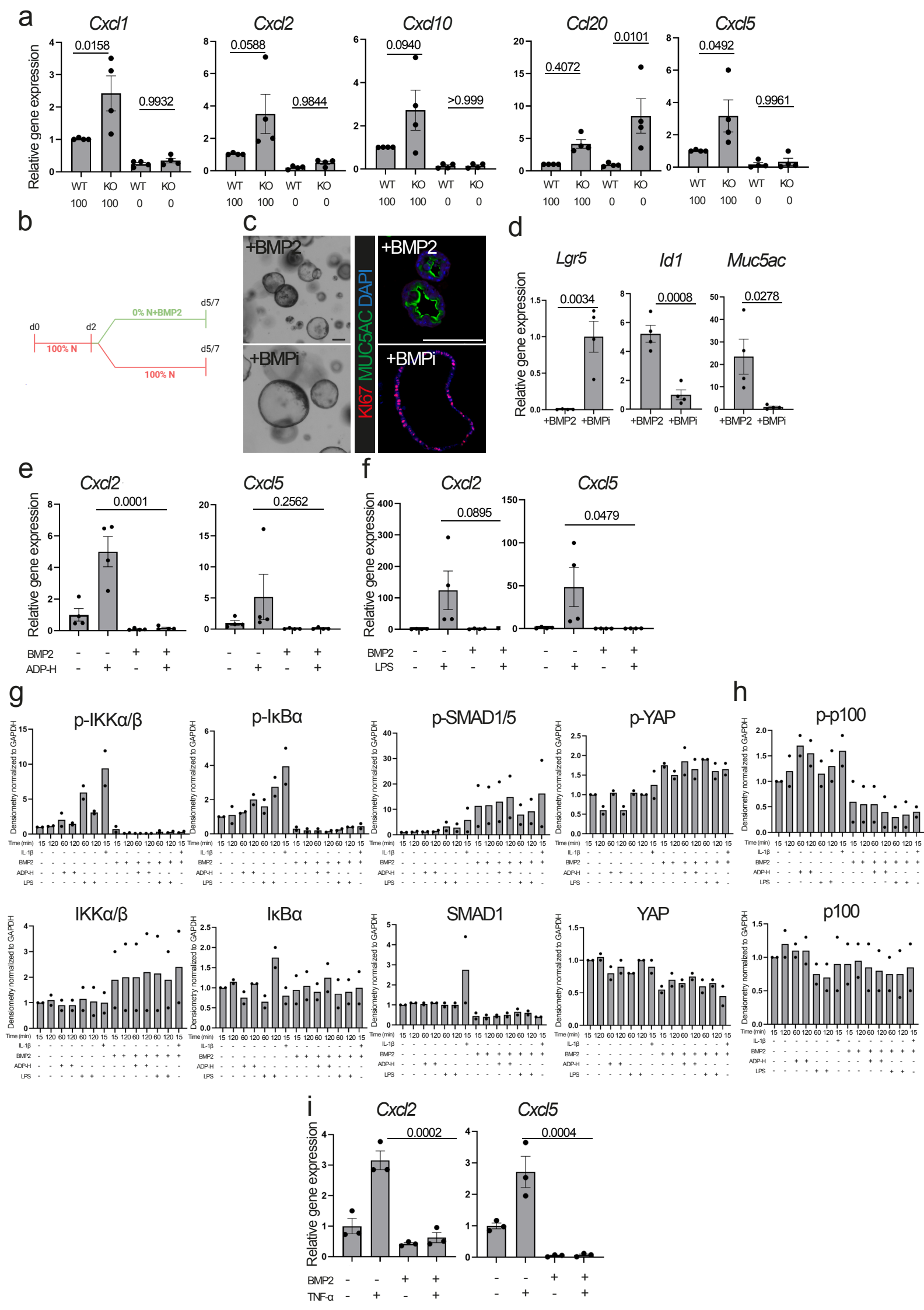


j Hallmark Inflammatory Response



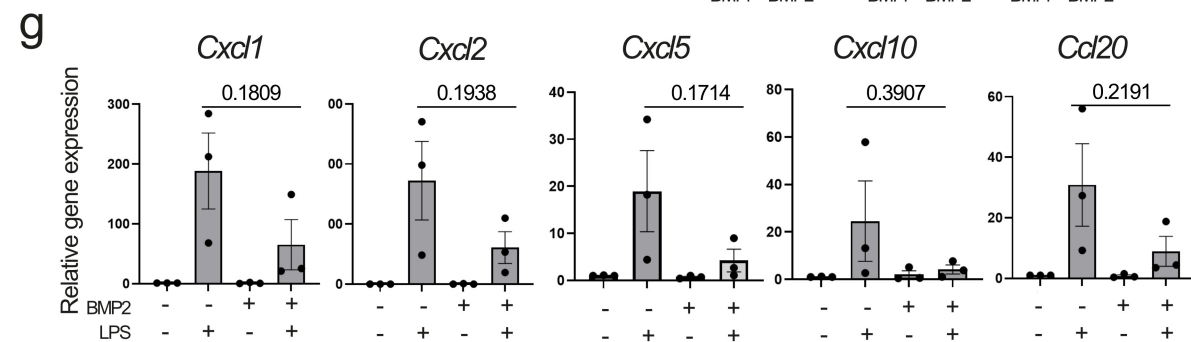
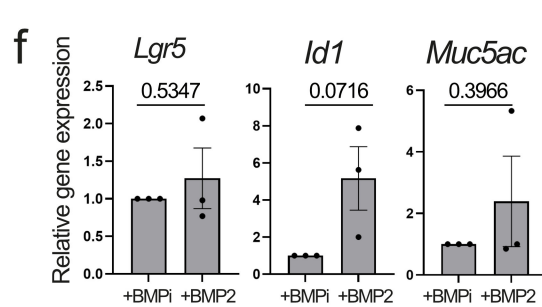
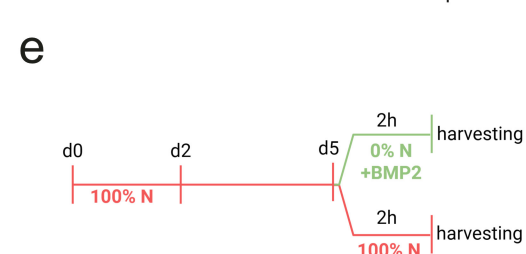
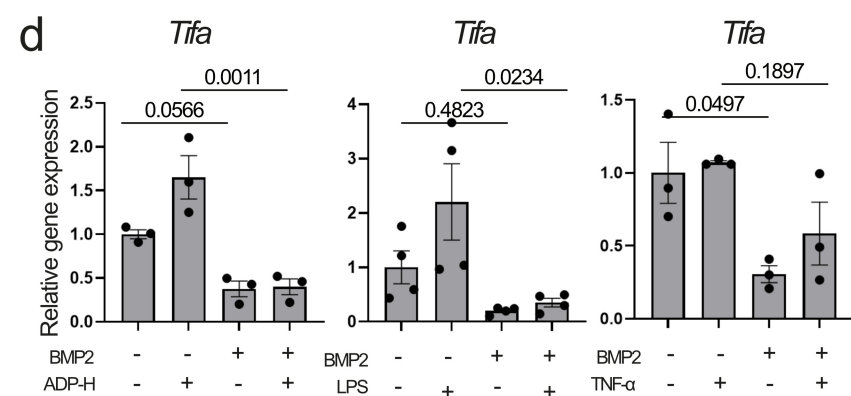
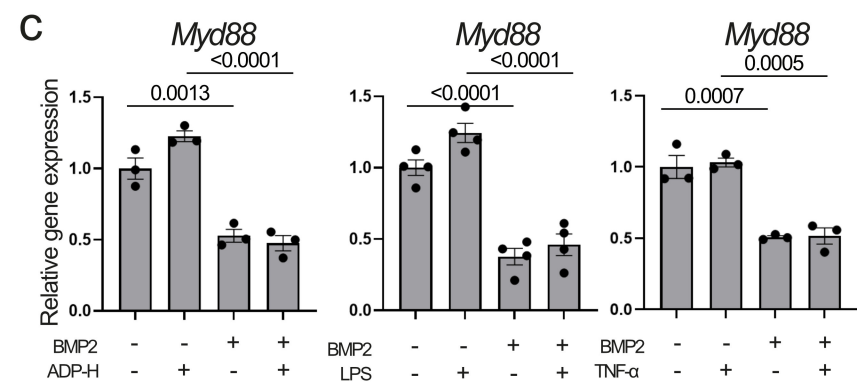
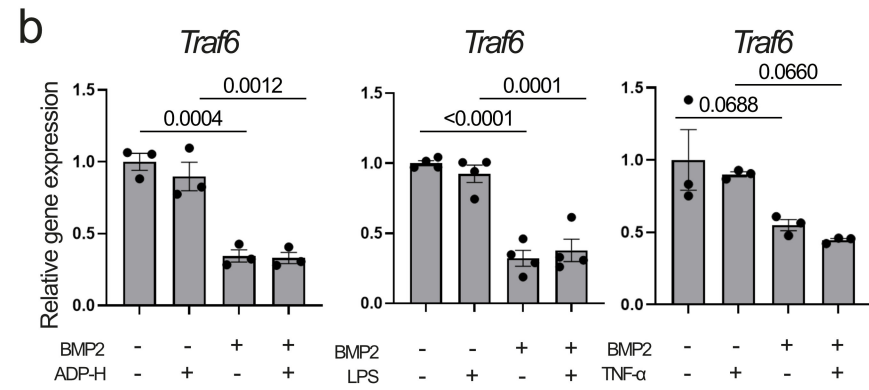
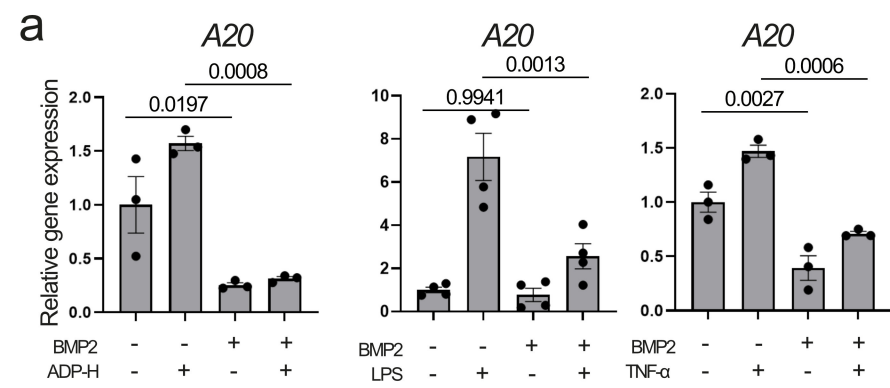
Supplementary Fig. 4 Inhibition of epithelial BMP signaling and the epithelial inflammatory response upon infection or *Bmpr1a* KO

a Organoid treatment schematic. Created in BioRender. Müllerke, S. (2026) <https://BioRender.com/l9g4426>. **b-d** mRNA expression of the differentiated cell markers *Muc5Ac*, *Gkn1* and *Gkn2* (**b**), the BMP signaling target *Id1* (**b**), the stem cell marker *Lgr5* (**c**), and YAP target genes *Ctgf*, *Ankrd1* and *Cyr61* (**d**) in WT organoids grown in 100%WRN or 0%N medium ($n = 3$ biological replicates per group). **e** Confocal microscopy images of KI67 and DAPI staining in WT and *Bmpr1a* KO organoids. Scale bar: 100 μ m. The experiment was performed three times independently with similar results. **f** mRNA expression of *Bmpr1a* and *Id1* in WT ($n = 5$ biological replicates) and *Bmpr1a* KO ($n = 6$ biological replicates) organoids. **g** mRNA expression of the BMP ligands *Bmp2* and *Bmp7* in WT and *Bmpr1a* KO organoids grown in 100%WRN or 0%WRN medium ($n = 6$ biological replicates per group). **h** Dot plot showing the expression of BMP ligands across identified cellular components in the murine antral epithelium. Circle size indicates the within-cluster proportion of gene detection; fill color represents the scaled average expression level. **i** UMAP plots of scRNA-seq showing expression levels of BMP ligands in antral epithelial cells. **j** GSEA of scRNA-seq pseudo-bulk expression differences comparing antral tissue from uninfected *Bmpr1a* KO vs. WT mice, as well as from infected WT vs. uninfected WT mice, for MsigDB HALLMARK_INFLAMMATORY_RESPONSE signature. Data are presented as mean $\pm$ SEM. Statistical analyses were performed using Student's *t*-test (two-tailed) for **b-d** and **f**; using one-way ANOVA, followed by Tukey's multiple comparisons test for **g**; using unadjusted *p*-values of permutation test provided by R package fgsea for **j**. Source data are provided as a Source Data file.



Supplementary Fig. 5 Epithelial BMP signaling regulates proinflammatory response

a mRNA expression of the proinflammatory cytokines *Cxcl1*, *Cxcl2*, *Cxcl10*, *Ccl20* and *Cxcl5* in WT and *Bmpr1a* KO organoids grown in 100%WRN or 0%WRN ($n = 4$ biological replicates per group). **b** Organoid treatment schematic. Created in BioRender. Müllerke, S. (2026) <https://BioRender.com/ijz9u7g>. **c** Brightfield and confocal images for KI67, MUC5AC, and DAPI co-staining in antral gastric organoids cultured in standard medium containing the BMP inhibitor Noggin (+BMPI) or in Noggin-deprived medium supplemented with BMP2 (+BMP2). Scale bars: 100 μ m. The experiment was performed three times independently with similar results. **d** mRNA expression of stem cell marker *Lgr5*, BMP target gene *Id1*, and differentiated pit cell marker *Muc5ac* in murine gastric organoids cultured in standard full medium (+BMPI) or in Noggin-deprived medium supplemented with BMP2 (+BMP2) ($n = 4$ biological replicates per group). **e-f** mRNA expression of *Cxcl2* and *Cxcl5* in organoids treated with BMP2 and/or ADP heptose (ADP-H) (**e**), and treated with BMP2 and/or LPS (**f**) ($n = 4$ biological replicates per group). ADP-H/LPS treatment was performed for 2 hours. **g-h** Densitometric quantification of the Western blot shown in **Figures 3g-h** ($n = 2$ biological replicates per group). **i** mRNA expression for *Cxcl2* and *Cxcl5* in organoids treated with BMP2 and/or TNF- α . TNF- α treatment was performed for 2 hours ($n = 3$ biological replicates per group). Data are presented as mean $\pm$ SEM. Statistical analyses were performed using Student's *t*-test (two-tailed) for **d**; using one-way ANOVA, followed by Tukey's multiple comparisons test for **a**, **e**, and **i**. Source data are provided as a Source Data file.

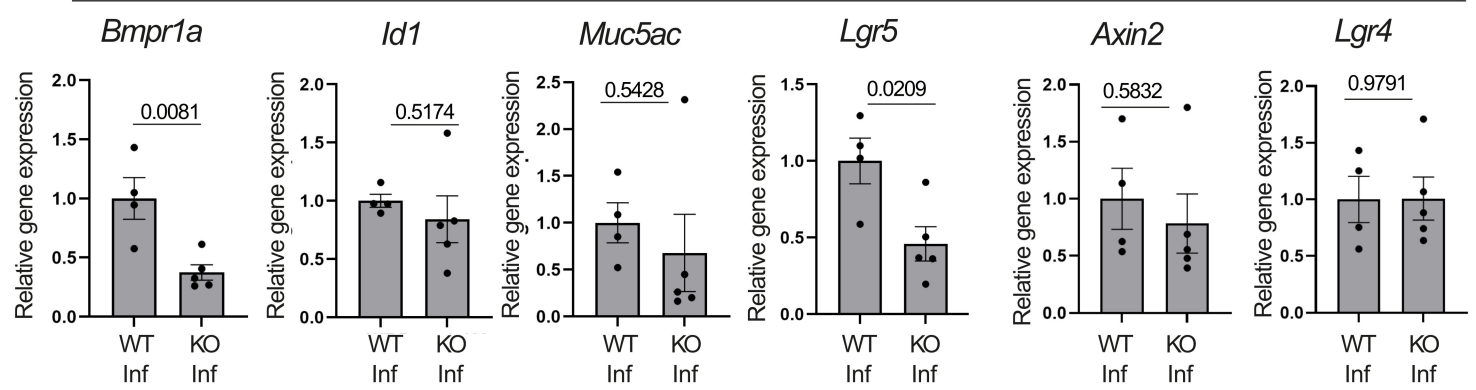


Supplementary Fig. 6 BMP signaling inhibits the expression of NF- κ B signaling mediator genes

a-d mRNA expression of *A20*, *Traf6*, *Myd88*, and *Tifa* in organoids treated with BMP2 and/or ADP-H ($n = 3$ biological replicates per group), LPS ($n = 4$ biological replicates per group) or TNF- α ($n = 3$ biological replicates per group). Treatment with ADP-H, LPS, or TNF- α was performed for 2 hours. **e** Organoid treatment schematic. Created in BioRender. Müllerke, S. (2026) <https://BioRender.com/nskn9y8>. **f** mRNA expression of stem cell marker *Lgr5*, BMP target gene *Id1*, and pit cell marker *Muc5ac* in murine gastric organoids cultured in standard full medium (+BMPi) or in Noggin-deprived medium supplemented with BMP2 (+BMP2) ($n = 3$ biological replicates per group). **g** mRNA expression of NF- κ B target genes (*Cxcl1*, *Cxcl2*, *Cxcl5*, *Cxcl10*, *Ccl20*) in organoids treated with BMP2 and/or LPS ($n = 3$ biological replicates per group). LPS and BMP2 treatments were performed for 2 h. Data are presented as mean $\pm$ SEM. Statistical analyses were performed using Student's *t*-test (two-tailed) for **f**; using one-way ANOVA, followed by Tukey's multiple comparisons test for **a-d** and **g**. Source data are provided as a Source Data file.

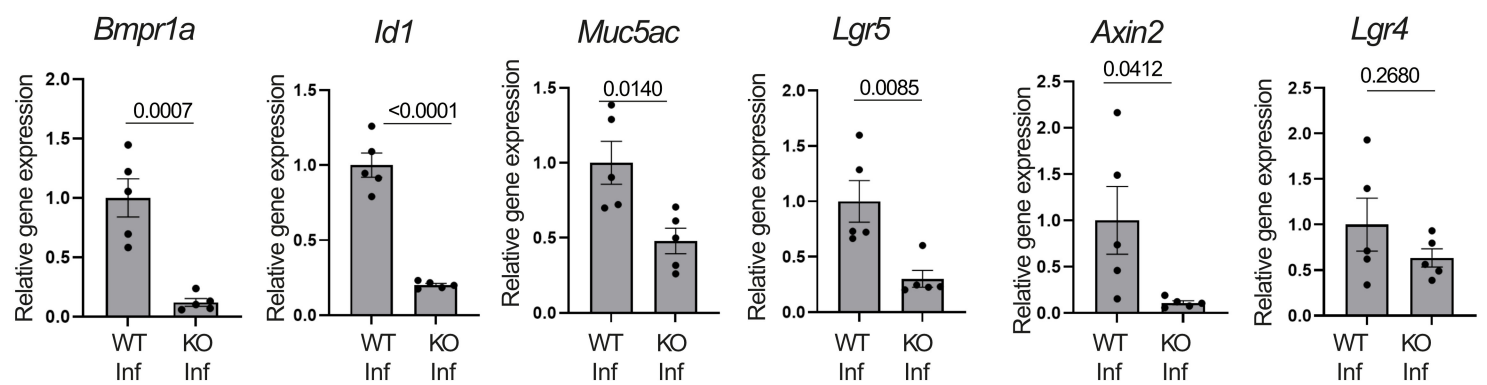
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2 weeks infection (3 weeks KO)

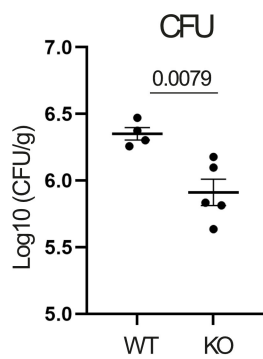


b

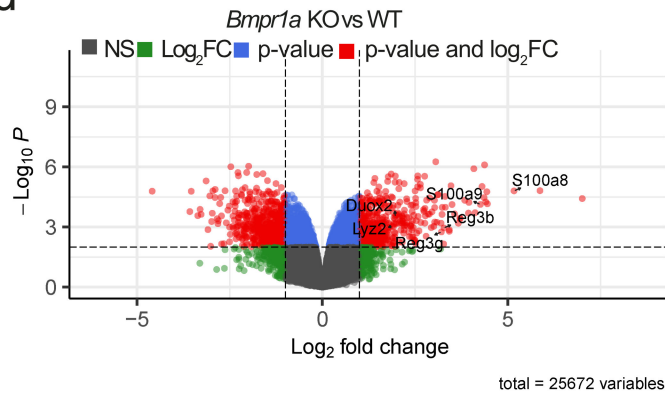
2 months KO and infection



c

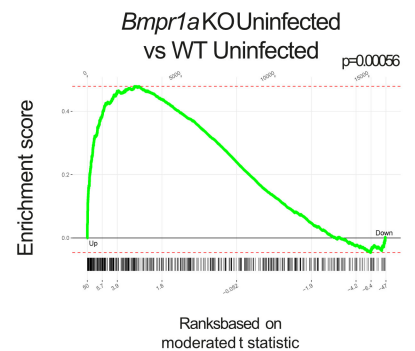


d



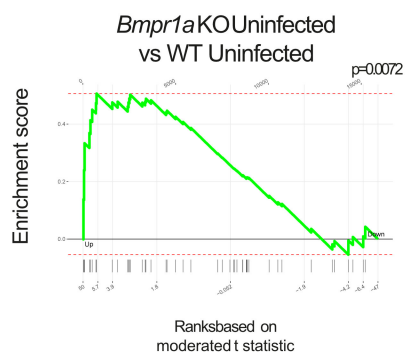
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GO Response to bacterium

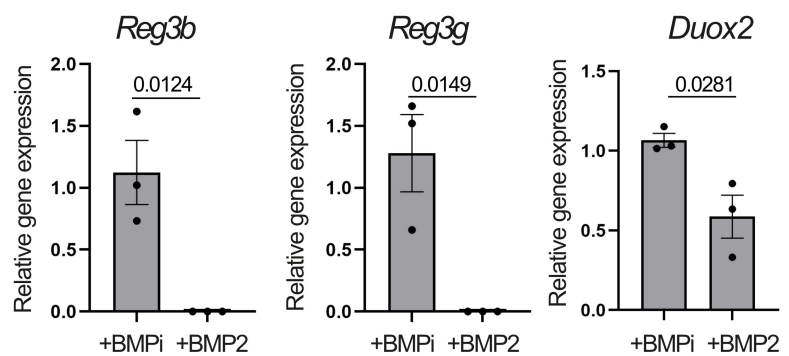


f

Reactome antimicrobial peptides

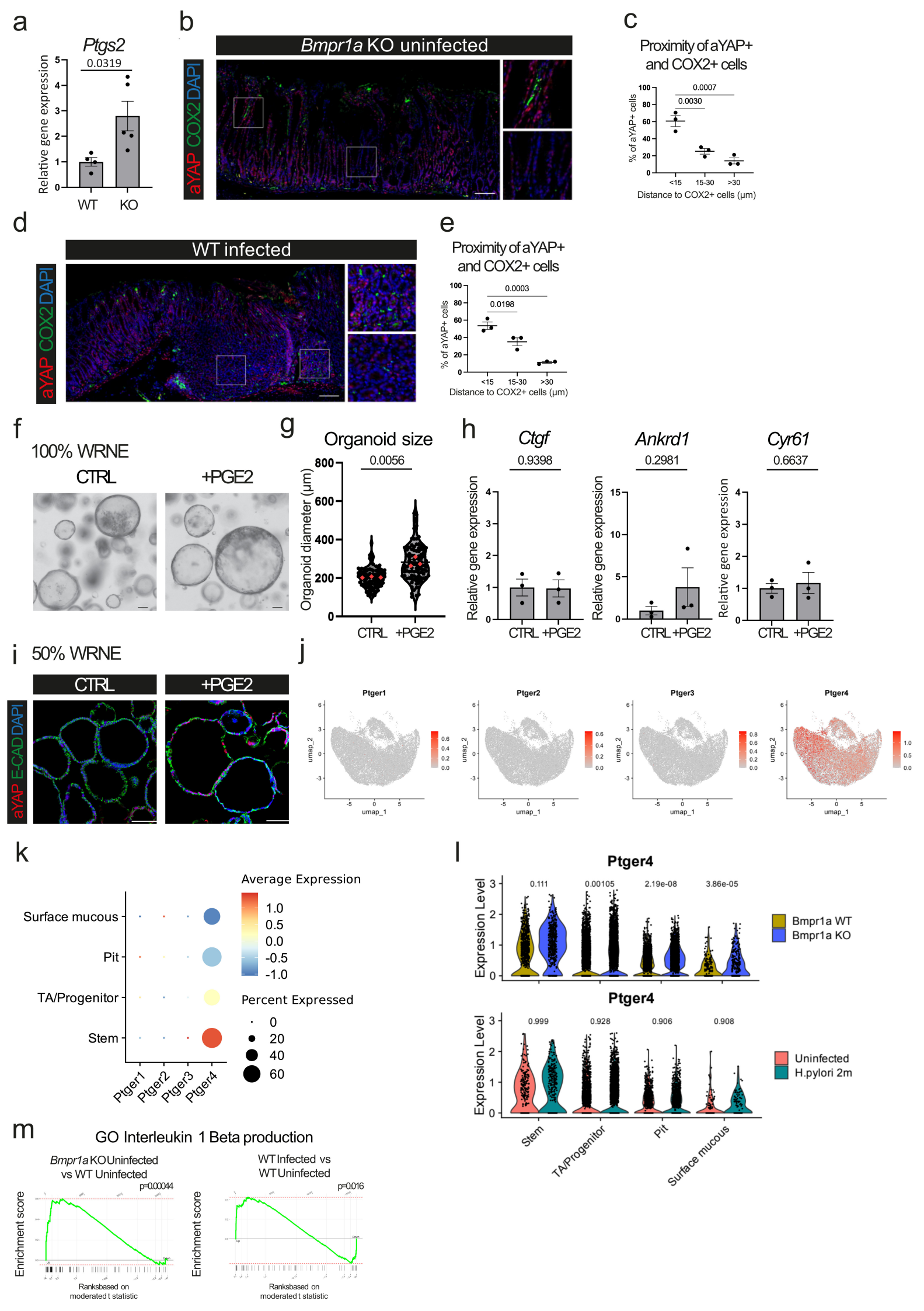


g



Supplementary Fig. 7 Inhibition of BMP signaling promotes antimicrobial response

a-b mRNA expression of *Bmpr1a*, *Id1*, *Muc5Ac*, *Lgr5*, *Axin2*, *Lgr4* in WT and *Bmpr1a* KO antral tissue after 2 weeks (WT: $n = 4$ mice; KO: $n = 5$ mice) (**a**) or 2 months (WT: $n = 5$ mice; KO: $n = 5$ mice) (**b**) of infection with *H. pylori*. **c** Colony-forming unit (CFU) quantification from 2-week-infected WT ($n = 4$) and *Bmpr1a* KO ($n = 5$) mice. **d** Volcano plot comparing microarray data of uninfected *Bmpr1a* KO vs WT antral tissue. Antimicrobial genes are highlighted. **e-f** GSEA of microarray data comparing antral tissues from uninfected *Bmpr1a* KO and WT mice, for response-to-bacterium (**e**), and antimicrobial peptide (**f**) gene signatures ($n = 2$ mice per group). **g** mRNA expression of the antimicrobial genes *Reg3b*, *Reg3g*, and *Duox2* in murine gastric organoids cultured in standard medium containing the BMP inhibitor Noggin (+BMPi) or in Noggin-deprived medium supplemented with BMP2 (+BMP2) ($n = 3$ biological replicates per group). Data are presented as mean $\pm$ SEM. Statistical analyses were performed using Student's *t*-test (two-tailed) for **a-c** and **g**; using unadjusted *p*-values of permutation test provided by R package fgsea for **e-f**. Source data are provided as a Source Data file.



Supplementary Fig. 8 Stromal PGE2 activates YAP in gastric epithelial cells

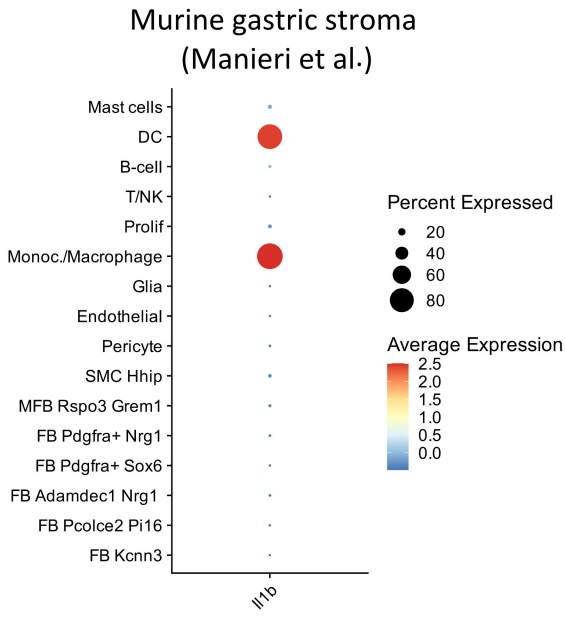
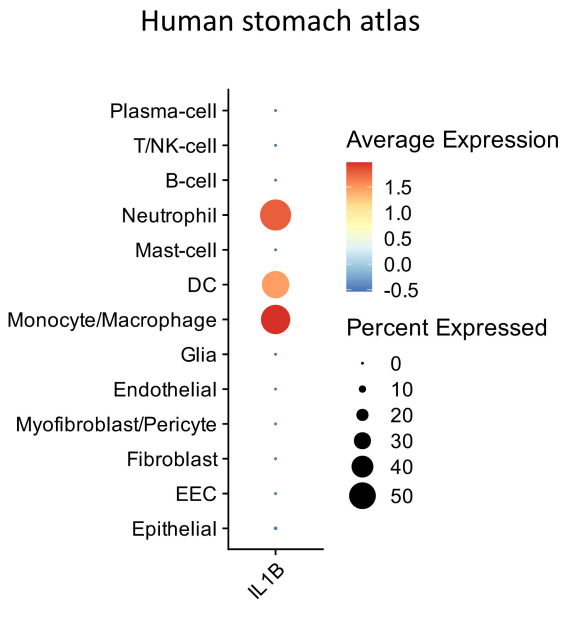
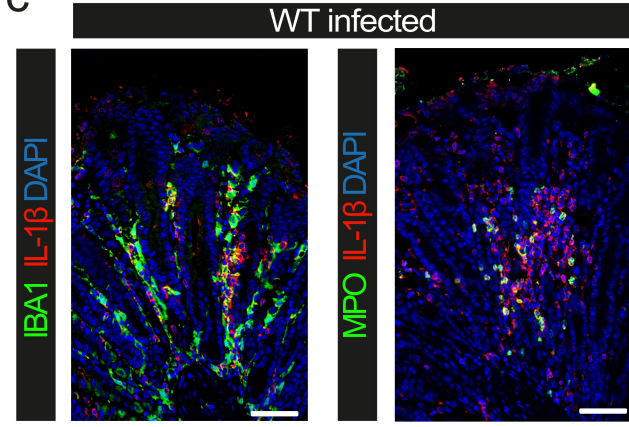
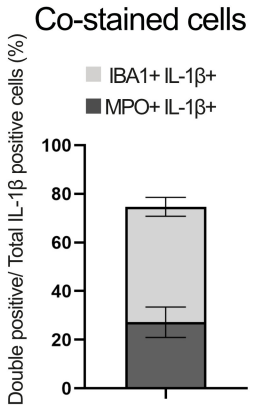
a mRNA expression of *Ptgs2* in WT ($n = 4$ mice) and *Bmpr1a* KO ($n = 5$ mice) uninfected antral gastric tissues. **b-e** Confocal images and proximity analysis of aYAP+ cells and COX2+ cells in uninfected *Bmpr1a* KO (**b-c**) and infected WT (**d-e**) gastric tissues ($n = 3$ mice per group). **f** Brightfield images of WT gastric antral organoids grown for 4 days in standard medium (100%WRNE) with or without PGE2. Scale bars: 100 μ m. The experiment was performed three times independently with similar results. **g** Quantification of organoid diameter. The mean values for each biological replicate are highlighted in red ($n = 3$ biological replicates per group). **h** mRNA expression analysis of the YAP target genes *Ctgf*, *Ankrd1* and *Cyr61* in WT organoids grown in standard medium with or without PGE2 ($n = 3$ biological replicates per group). **i** Confocal microscopy images of E-CAD+ aYAP+ cells in control and PGE2-treated gastric organoids grown in 50%WRNE medium. Scale bars: 100 μ m. **j** UMAP plots of scRNA-seq showing expression levels of PGE2 receptors (*Ptger1-4*) in antral epithelial cells. **k** Dot plot showing the expression of *Ptger1-4* across cell subpopulations in the murine antral epithelium. Circle size indicates the within-cluster probability of gene detection; fill color represents the normalized average expression level. **l** Violin plot showing the expression level of *Ptger4* in antral epithelial cells from uninfected *Bmpr1a* KO vs. WT mice, as well as from infected WT vs. uninfected WT mice. Numbers at the top indicate p-values for mean differences between conditions for each cluster ($n = 2$ mice per group), derived from genome-wide pseudo-bulk differential expression analysis of antral scRNA data. **m** GSEA of microarray data comparing antral tissues from uninfected *Bmpr1a* KO vs. WT mice, as well as from infected WT vs. uninfected WT mice, for IL-1 β production gene signatures ($n = 2$ mice per group). Data are presented as mean $\pm$ SEM. Statistical analyses were performed using Student's *t*-test (two-tailed) for **a** and **g-h**; using one-way ANOVA, followed by Tukey's multiple comparisons test for **c** and **e**; using unadjusted *p*-values of permutation test provided by R package fgsea for **m**. Images are representative of at least three biological replicates. Source data are provided as a Source Data file.

t-SNE plot showing cell clusters. The x-axis is labeled 'umap_1' and the y-axis is labeled 'umap_2'. The clusters are labeled with cell types: Prolif, Monoc, Macrophage, DC, T/NK, B-cell, Pericyte, Mast cells, Endothelial, FB Pdgfra+ Sox6, FB Kcnk3, FB Adamdec1 Nrg1, FB Pdgfra+ Nrg1, SMC Hsp, MFB Rspo3 Grem1, and Glia.

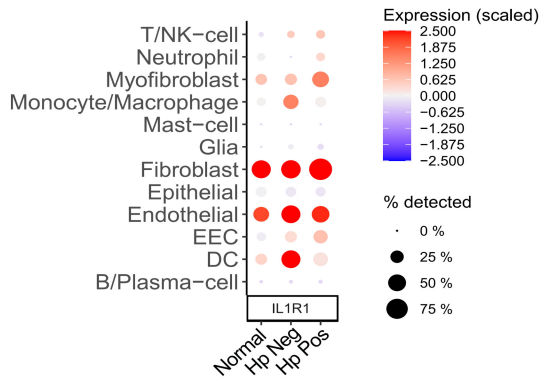
Figure 2 is a heatmap visualization showing the expression of IL1R1, PTGS2, and PTGES across various cell types. The y-axis lists the cell types: Plasma-cell, T/NK-cell, B-cell, Neutrophil, Mast-cell, DC, Monocyte/Macrophage, Glia, Endothelial, Myofibroblast/Pericyte, Fibroblast, EEC, and Epithelial. The x-axis shows the three genes: IL1R1, PTGS2, and PTGES. The size of the bubbles represents the 'Percent Expressed' (0, 20, 40, 60), and the color represents the 'Average Expression' (0.8, 0.4, 0.0, -0.4). The data shows that IL1R1 and PTGS2 are highly expressed in Neutrophils, Mast cells, DCs, Monocytes/Macrophages, and Fibroblasts, while PTGES is highly expressed in Fibroblasts. The expression of these genes is generally low or negative in other cell types like Plasma cells, T/NK cells, B cells, Glia, Endothelial cells, Myofibroblasts/Pericytes, EECs, and Epithelial cells.

Supplementary Fig. 9 scRNA-seq of murine gastric stroma and human stomach tissue

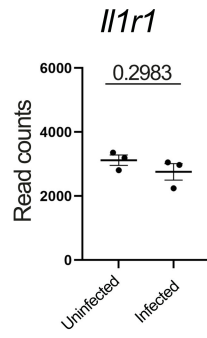
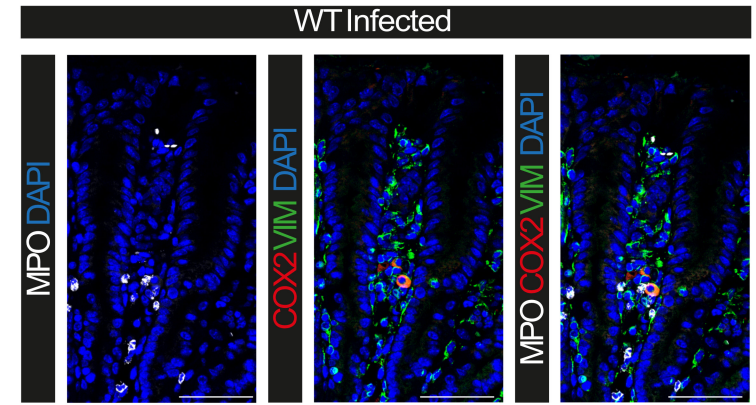
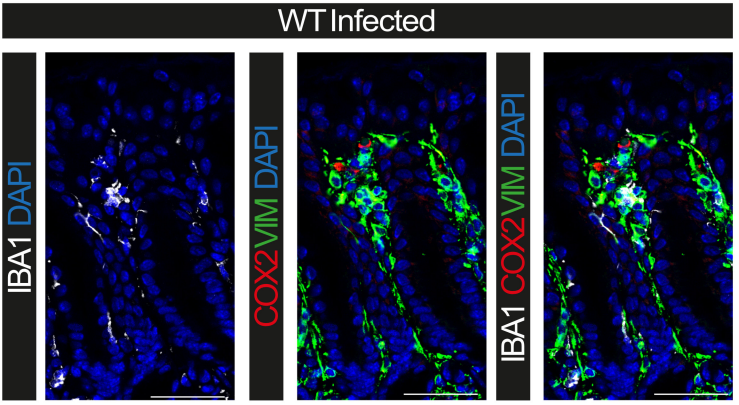
a UMAP showing cell subsets in the stromal compartment from healthy murine WT stomach tissue. **b** Dot plot showing the expression of cluster marker genes across identified subpopulations in the gastric stroma. Circle size indicates the within-cluster proportion of gene detection; fill color represents the scaled average expression level. **c** UMAP showing cell subsets from human stomach tissue. **d** Dot plot showing the expression of cluster marker genes across identified subpopulations in human stomach tissue. **e** Dot plot showing the expression of *IL1R1*, *PTGS2*, and *PTGES* in cell subpopulations from human stomach tissue.

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

Human stomach atlas

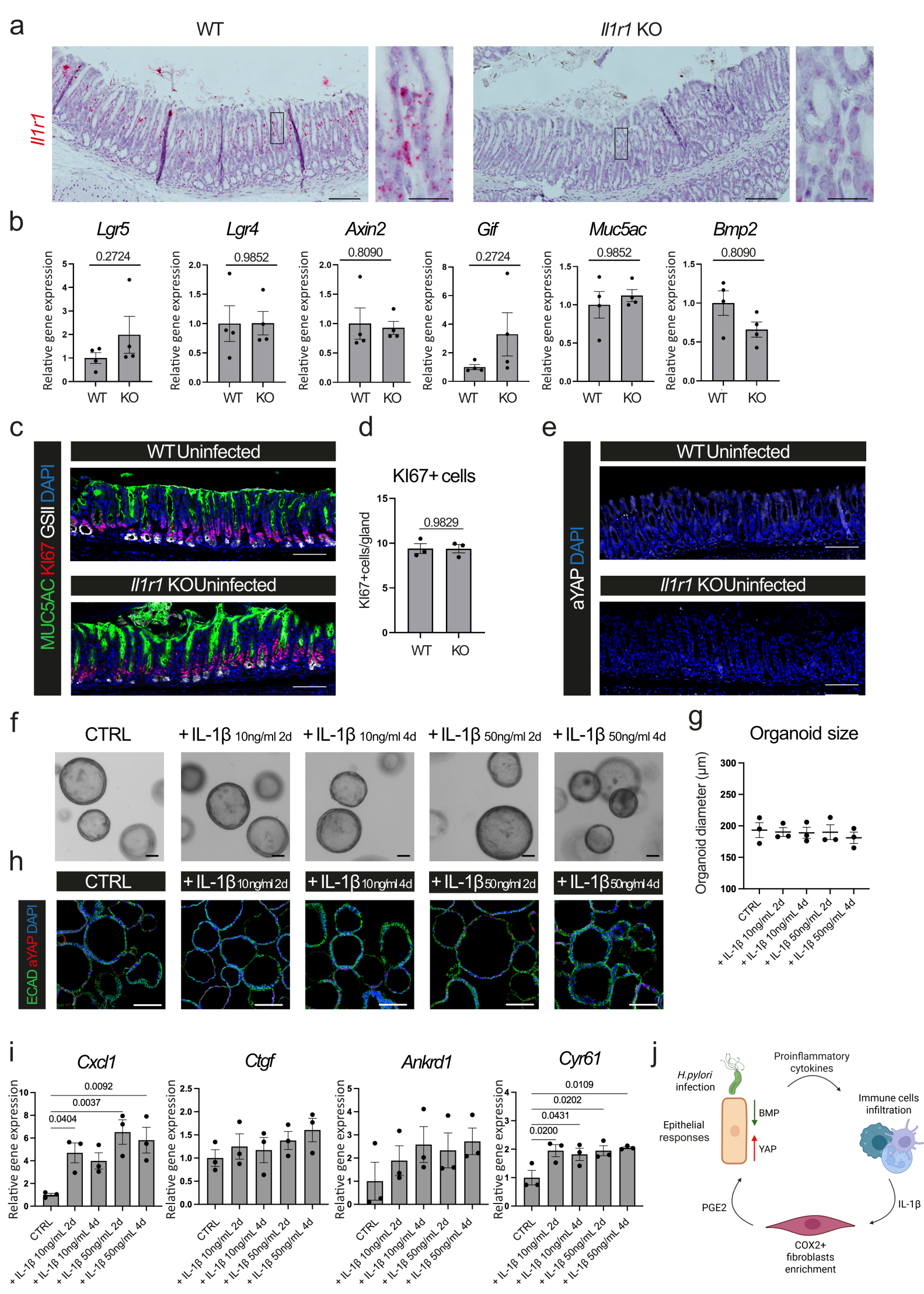
**f**

Murine gastric stroma bulk RNAseq (Kapalcynska, Lin et al.)

**g****h**

Supplementary Fig. 10 Analysis of IL-1 β -producing cells and their interaction with stromal cells

a-b Dot plot showing the expression of IL-1 β in cell subpopulations from murine (**a**) and human (**b**) stomach tissues. **c** Confocal microscopy images of co-staining of IL-1 β with IBA1 or MPO in antral gastric tissue from infected WT mice. Scale bars: 50 μ m. **d** IL-1 β +MPO+ ($n = 4$ mice) or IL-1 β +IBA1+ ($n = 3$ mice) co-stained cells normalized to the total IL-1 β + cells per section, expressed in percentage. **e** Dot plot of human stomach scRNA-seq showing the cell populations that express *IL1R1* under normal and gastritis (*H. pylori*-negative or -positive) conditions. **f** The expression level of *Il1r1* in the bulk RNA-seq dataset of murine stromal cells under uninfected and infected conditions ($n = 3$ mice per group). **g-h** Confocal microscopy images of COX2, VIM, and MPO, and images of COX2, VIM, and IBA1 in antral gastric tissue from infected WT mice. Scale bars: 50 μ m. Data are presented as mean $\pm$ SEM. Statistical analyses were performed using Student's *t*-test (two-tailed) for **f**. Images are representative of at least three biological replicates. Source data are provided as a Source Data file.



Supplementary Fig. 11 Characterization of *Il1r1* KO mouse model and the direct effect of IL-1 β on epithelial cells

a ISH images of *Il1r1* in tissue from uninfected WT and *Il1r1* KO mice. Scale bars: 100 μ m, inserts: 20 μ m. **b** mRNA expression of *Lgr5*, *Lgr4*, *Axin2*, *Gif*, *Muc5ac*, and *Bmp2* in uninfected WT and *Il1r1* KO antral gastric tissue ($n = 4$ mice per group). **c** Confocal microscopy images of MUC5AC, KI67, GSII, and DAPI staining in antral gastric tissue from uninfected WT and *Il1r1* KO mice. Scale bars: 100 μ m. **d** Quantification of KI67+ cells in antral gastric glands of uninfected WT and *Il1r1* KO mice ($n = 3$ mice per group). **e** Confocal microscopy images of aYAP and DAPI staining in antral gastric tissue from uninfected WT and *Il1r1* KO mice. Scale bars: 100 μ m. **f-g** Brightfield images and diameter quantification of gastric organoids grown for 4 days in 50% WRNE medium following IL-1 β treatment at the indicated concentrations and time points ($n = 3$ biological replicates per group). Scale bars: 100 μ m. The experiment was performed three times independently with similar results. **h** Confocal microscopy images of E-CAD+ aYAP+ cells in control and IL-1 β -treated organoids grown in 50%WRNE medium. Scale bars: 100 μ m. The experiment was performed three times independently with similar results. **i** mRNA expression of NF- κ B target genes *Cxcl1* and YAP target genes *Ctgf*, *Ankrd1*, and *Cyr61* in control and IL-1 β -treated organoids grown in 50%WRNE medium ($n = 3$ biological replicates per group). **j** Schematic representation of the epithelial-immune-stromal crosstalk during *H. pylori* infection. Created in BioRender. Müllerke, S. (2026) <https://BioRender.com/tuugyrh>. Infection downregulates BMP activity in gastric epithelial cells, driving increased production of proinflammatory cytokines that recruit IL-1 β -producing immune cells. This immune infiltration leads to the enrichment of COX2+ fibroblasts via IL-1 β signaling. The resulting stromal PGE2 is sensed by neighboring epithelial cells, triggering YAP activation and fetal-like transcriptional responses. Data are presented as mean $\pm$ SEM. Statistical analyses were performed using Student's *t*-test (two-tailed) for **b** and **d**; using one-way ANOVA, followed by Tukey's multiple comparisons test for **g** and **i**. Images are representative of at least three biological replicates. Source data are provided as a Source Data file.