Gastric stem cells promote inflammation and gland remodeling in response to *Helicobacter pylori* via Rspo3-Lgr4 axis

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### Abstract

*Helicobacter pylori* is a pathogen that colonizes the stomach and causes chronic gastritis. *Helicobacter pylori* can colonize deep inside gastric glands, triggering increased R-spondin 3 (Rspo3) signaling. This causes an expansion of the “gland base module,” which consists of self-renewing stem cells and antimicrobial secretory cells and results in gland hyperplasia. The contribution of Rspo3 receptors Lgr4 and Lgr5 is not well explored. Here, we identified that Lgr4 regulates Lgr5 expression and is required for *H. pylori*-induced hyperplasia and inflammation, while Lgr5 alone is not. Using conditional knockout mice, we reveal that R-spondin signaling via Lgr4 drives proliferation of stem cells and also induces NF-κB activity in the proliferative stem cells. Upon exposure to *H. pylori*, the Lgr4-driven NF-κB activation is responsible for the expansion of the gland base module and simultaneously enables chemokine expression in stem cells, resulting in gland hyperplasia and neutrophil recruitment. This demonstrates a connection between R-spondin-Lgr and NF-κB signaling that links epithelial stem cell behavior and inflammatory responses to gland-invading *H. pylori*.

**Keywords** *Helicobacter pylori*; Lgr4; Lgr5; NF-κB; R-spondin 3

**Subject Categories** Digestive System; Microbiology; Virology & Host Pathogen Interaction; Stem Cells & Regenerative Medicine

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### Introduction

*Helicobacter pylori* is a gram-negative bacterium that has evolved to colonize and persist in the stomach for decades. It is found in approx. 50% of the world’s population and infection is the most important risk factor for gastric cancer. It is able to establish free-swimming colonies that survive within the protective mucus layer in direct contact with the surface epithelium (Amieva & El-Omar, 2008). In addition, a subpopulation of *H. pylori* can form colonies deep inside the gastric glands, where stem and progenitor cells reside (Sigal et al., 2015; Fung et al., 2019). It has been shown that gland-colonizing bacteria, not the bacteria colonizing the surface, induce the typical pathology associated with *H. pylori* infection, that is, stem cell expansion, gastritis, hyperplasia, and metaplasia—precursor lesions for gastric cancer (Howitt et al., 2011; Sigal et al., 2015; Fischer & Sigal, 2019; Liabeuf et al., 2022).

Upon attachment, highly pathogenic strains of *H. pylori* use the bacterial type IV secretion system (T4SS) to inject virulence factors into the host cell cytoplasm, including the effector molecule CagA (Backert et al., 2000; Amieva et al., 2003), as well as the LPS biosynthesis metabolite ADP-glycero-β-D-manno-heptose (ADP heptose) (Pfänkuch et al., 2019). ADP heptose, as well as its derivative D-glycero-β-D-manno-heptose 1,7-bisphosphate (HBP), has been shown to act as a central mediator of *H. pylori*-driven inflammation by inducing activation of the transcription factor NF-κB via alpha kinase 1 (ALPK1) and TRAF-interacting protein with forkhead-associated domain (TIFA) (Zimmermann et al., 2017; Pfänkuch et al., 2019). However, it remains unclear why these inflammatory responses are triggered specifically by bacteria that have invaded the glands and not by those that remain on the surface.

We have previously shown gland responses to *H. pylori* infection to be driven by increased expression of Rspo3 in the stroma beneath the glands, which drives stem cell expansion and hyperplasia (Sigal et al., 2017). During normal gland homeostasis, a molecular signaling network established by close interplay between epithelial and stromal cells controls epithelial turnover and differentiation (Sigal et al., 2017; Fischer & Sigal, 2019; Kapalczynska et al., 2022). The antral gland base and lower isthmus contains gastric stem cells that are characterized by high Wnt signaling and expression of stem cell markers such as Axin2 and Lgr5 (Barker et al., 2010; Sigal...
et al., 2017). The epithelium can be divided into the “gland base module” consisting of stem cells as well as gland base secretory cells (Tan et al., 2020), and the “pit module” consisting of surface mucous cells. The base module relies on stromal-derived Rspo3, which binds to Lgr receptors that associate with and prevent turnover of the Wnt/ Lrp/ Frizzled receptors complex, thereby stabilizing Wnt signaling (de Lau et al., 2011). Lgr4 is expressed broadly throughout the gland, while Lgr5 is itself a target gene of Rspo3 signaling and is found exclusively in the base (Sigal et al., 2017). Loss of Rspo3 results in a loss of the gland base module, while the increased Rspo3 expression upon infection with H. pylori drives stem cell expansion and accumulation of gland base secretory cells.

We have previously shown that Rspo3 counterbalances colonization and gland infiltration of H. pylori and mechanistically drives expansion of rapidly cycling Axin2+/Lgr4+/Lgr5- cells in the isthmus, as well as slowly cycling Axin2+/Lgr4+/Lgr5+ basal cells that differentiate into secretory cells leading to hyperplasia in the antrum (Sigal et al., 2015, 2017, 2019). While this suggests that both Lgr4 and Lgr5 are likely to play a role in mediating the effects of Rspo, their distinct roles in driving antral pathobiology remain elusive.

Here, we applied conditional knockout mice that lack Lgr4 or Lgr5 to explore their involvement in antral mucosal responses to H. pylori infection. We reveal that Lgr4 expression is required to maintain expression of Lgr5 in the gland base. Depletion of Lgr4, but not Lgr5 alone, results in a diminished hyperplasia as well reduced inflammatory response, and we demonstrate that the Rspo-Lgr axis does not only promote hyperplasia but is also important for the ability of gland base cells to induce epithelial pro-inflammatory NF-κB signaling upon infection. Our data reveal an explanation for the unique ability of the stem cell compartment to respond to H. pylori infection and demonstrate a novel link between epithelial stem cells and innate immunity in the stomach.

Results

**Lgr4/5, but not Lgr5 alone, is required for Helicobacter pylori-driven gland base cell expansion and hyperplasia**

Lgr5 and Lgr4 are the Rspo receptors expressed in gastric epithelial cells. While Lgr5 itself is a Wnt target gene, Lgr4 is regulated differently (Barker et al., 2007). Expression of Lgr5 is thus limited to base cells, where Wnt/ Rspo concentrations are highest, while Lgr4 is broadly expressed throughout the antral gland. In situ hybridization and analysis of publicly available single-cell RNA-seq data confirmed the broader expression of Lgr4 in the stomach, and we noticed the highest concentration of Lgr4 in the isthmus (Fig EV1A-C). Rspo3 triggers expansion of slower-cycling Lgr5+ base cells and rapid-cycling Lgr4+ isthmus cells, leading to hyperplasia in the antrum upon infection with H. pylori. Both cell types are considered progenitor cells that co-express the classic Wnt target gene Axin2 and are in direct contact with H. pylori micro-colonies (Sigal et al., 2015, 2017, 2019). To examine the specific roles of Lgr4 and Lgr5 under homeostatic conditions and upon infection with H. pylori, we generated conditional knockout (KO) mice, achieved by floxed sites, which enable a depletion of Lgr5 or Lgr4 (Plana-Paz et al, 2016) in Axin2+ stem cells upon injection of tamoxifen (B6-Axin2CreER+/Lgr5fl/fl and B6-Axin2CreER+/Lgr4fl/fl, hereafter referred to as Lgr5−/− and Lgr4−/−), while B6-Axin2CreER+/Lgr4+ and B6-Axin2CreER+/Lgr4+/+ littermates served as controls. For experiments without infection, mice were treated with tamoxifen 14 days before sacrifice, allowing repopulation of glands by mutant cells. For infection experiments, some mice were infected 14 days after tamoxifen treatment with H. pylori and sacrificed 2 months later. All infected mice showed deep gland colonization (examples are shown in Fig EV2A).

We first examined the effects of Lgr5 depletion. Infection led to hyperplasia in both the control mice and in the Lgr5−/− mice (Fig 1A and B). We earlier described that infection also leads to upregulation of Lgr5 (Sigal et al., 2019). To visualize mucous gland base cells in the antrum, we used immunofluorescence labeling for lectin GSI (GSI) and gastric intrinsic factor (GIF). Only a subpopulation of GSI+ cells were GIF positive (Figs 1A and EV2B). Proliferating ishmsus cells were identified by anti-Ki67 staining and pit cells by anti-MUC5AC. In Lgr5−/− mice, we did not detect changes in GSI+, GIF+, or Ki67+ cells, nor change in the morphology of the gland base. Upon infection, immunofluorescence showed an expansion of the GSI+ GIF+ compartment in both the control and the Lgr5−/− mice. Hyperplastic glands in the Lgr5−/− mice as well as in the littermate controls were populated by an increased number of Ki67+ cells (Fig 1A and C). We did not observe any significant alterations in H. pylori colony-forming units (CFUs) in Lgr5−/− mice compared with control mice (Fig 1D). Gene expression analysis under homeostasis

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**Figure 1. Lgr5 is dispensable for gastric gland hyperplasia, gland base expansion, and antimicrobial defense upon infection with Helicobacter pylori.**

Lgr5+/+Axin2fl/fl (Lgr4−/−) and control mice were infected for 2 months with H. pylori.

A Representative H&E staining from antral glands (upper row). The dotted line marks the gland base cell area, which expands upon infection in lgr5−/− and control mice. Immunofluorescence labeling for GSI and Ki67 (middle row) and GIF and Muc5ac (lower row) of antral epithelium from Lgr5−/− and control mice uninfected and after 2 months of infection. Scale bars: 50 μm.

B Quantification of antral gland height in Lgr5−/− compared with control mice. Uninfected control (n = 5) versus uninfected lgr5−/− (n = 4) versus infected control (n = 4) versus infected lgr5−/− (n = 4).

C Quantifications of GSI+ area (n = 4–5 biological replicates per group, n = 10 glands per mouse), Ki67 as percentage of total DAPI-stained cells (n = 3–4 biological replicates per group, n = 20 glands per mouse), GIF+ area (n = 4–5 biological replicates per group, n = 10 glands per mouse), Muc5ac+ area (n = 4–5 biological replicates per group, n = 10 glands per mouse).

D Helicobacter pylori CFUs in Lgr5−/− (n = 5) and control mice (n = 5).

E qPCR expression levels of Lgr4, Lgr5, and Axin2, gland base cell markers (Gif, Pgc, Tff2, and Muc6), pit cell marker Muc5ac, and Itln1 in uninfected Lgr5−/− (n = 6–10) and control mice (n = 4–10).

F qPCR expression levels of Lgr4, Lgr5, gland base cell markers (Gif, Pgc, and Muc6), and Itln1 in Lgr5−/− (n = 5) and control mice (n = 5) after 2 months of infection.

Data information: For B, C, D, E, and F, data are mean + SD. *P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant (one-way ANOVA + Tukey’s multiple comparison test for B, C, t-test for D, E and F).

Source data are available online for this figure.
Figure 1.
and upon infection similarly revealed no significant changes in base cell markers (Axin2, Gif, Pgc, Tff2, and Muc6) or pit cell marker Muc5ac in control versus in Lgr5−/− mice (Fig 1E and F). Expression of Itln1, which we previously found to be expressed in gland base secretory cells and which binds H. pylori to counterbalance infection (Sigal et al, 2019), was also not significantly reduced. Although in an earlier study we showed that selective deletion of Lgr5-expressing cells leads to increased colonization with H. pylori (Sigal et al, 2019), the current data suggest that this phenomenon as well as the epithelial response to infection does not require Lgr5 expression.

We next explored the responses of Lgr4−/− mice to H. pylori and found that they failed to develop hyperplasia (Fig 2A and B). Immunofluorescence showed an expansion of both the Ki67+ and the GSII+ / GIF + compartment in control, but not in Lgr4−/− mice (Fig 2A and C). H. pylori CFUs were significantly higher in Lgr4−/− compared with control mice (Fig 2D), mimicking the effects of Rspo3 depletion that we described previously (Sigal et al, 2019). Notably, even in the absence of infection, gland bases of Lgr4−/− mice contained fewer GSII+ cells. A significant reduction in expression of Lgr5, Axin2, and Gif was detected in uninfected Lgr4−/− mice compared with uninfected control littermates (Fig 2E), which indicated that Lgr4 is required for Lgr5 expression and Lgr5+ cell differentiation. Our finding that Lgr4−/− mice harbor fewer stem and secretory cells upon infection was further corroborated by gene expression analysis where we observed a significant decrease in expression of Lgr4 alone, our data indicate that expression of Lgr4 is essential for gastric gland hyperplasia upon infection, gland base expansion, and antimicrobial defense upon H. pylori infection, while these parameters are unaffected upon loss of Lgr5 alone.

**R-spondin 3-Lgr signaling enables inflammatory responses to H. pylori**

To obtain deeper insight into the effects of Lgr4 depletion in the stomach, we performed whole-transcriptome microarray analysis on whole antral tissue from 2-month H. pylori-infected Lgr4−/− mice and littermate controls. Gene set enrichment analysis (GSEA) confirmed that the loss of Lgr4 (which, as shown above also leads to the loss of Lgr5 expression) led to a reduction in the antral stem cell signature (Barker et al, 2010) and genes associated with beta-catenin signaling (Herbst et al, 2014) (Fig 3A and B). The downregulation of the Wnt target genes Axin2 and Sox9 was validated by qPCR (Fig 3C). In infected wildtype control, but not in infected Lgr4−/− mice, we identified nuclear beta-catenin (Ctnnb1) in Ki67+ and GSII+ cells in the gland (Fig EV2C). Intriguingly, we found that various inflammation-associated genes were differentially expressed in control versus Lgr4−/− mice and GO enrichment analysis further revealed that 63 out of the 157 downregulated genes were linked to innate immune signaling via NF-κB and to TNF-α signaling via NF-κB (Table EV1, Fig 3D).

We analyzed by immunofluorescence the key NF-κB subunit mediating innate immune response p65 as well as p65 acetylated K310 (Fig 3E–G). Acetylation of p65 at lysine 310 on p65 is required for full transcriptional activation of NF-κB (Chen et al, 2002). Total p65 localized predominantly in the lower Ki67+ gland isthmus compartment in uninfected mice. In infected control mice, p65 level was increased in the expanded Ki67+ compartment and at less intensity in the GSII+ cells. In contrast, p65 level was not increased in H. pylori-infected Lgr4−/− mice (Fig 3E and F). Inhibition of H. pylori-induced NF-κB signaling by deletion of Lgr4 became obvious by staining for the activated p65 (p65 at K310), which revealed a strong expression exclusively in the glands of infected control, but not of infected Lgr4−/− mice (Fig 3G).

To corroborate this revelation, we tested for expression of NF-κB target genes in uninfected and infected Lgr4−/− mice and controls by qPCR. Expressions of Cxcl1 and Cxcl2, considered murine homologs of IL-8, as well as Cxcl10 and Ccl2, both prominently regulated in the microarray data (Table EV1), were upregulated upon infection only in control, but not in Lgr4−/− mice (Fig 3H). Immunofluorescence for Cxcl1 further confirmed activation of NF-κB signaling in the lower gland isthmus proliferative cell compartment (Fig 3I). Taken together, our in vivo experiments reveal that Lgr4 is required for NF-κB activation and expression of pro-inflammatory chemokines in response to H. pylori infection.

![Figure 2. Lgr4 regulates hyperplasia and expansion of gland base cells upon infection with Helicobacter pylori.](https://example.com/figure2.png)

Lgr4+/+/Axin2+/−/−/− (Lgr4−/−) and control mice were infected for 2 months with H. pylori.
A Representative H&E staining from antral glands (upper row). The dotted line marks the gland base cell area, which does not expand in infected Lgr4−/− but does expand in the corresponding control mice. Immunofluorescence labeling for GSII and Ki67 (middle row) and GIF and Muc5ac (bottom row) of antral epithelium from Lgr4−/− and control mice (n = 2) after 2 months of infection. Scale bars: 50 μm.
B Quantification of antral gland height in Lgr4−/− compared with control mice. Uninfected control (n = 2) versus uninfected Lgr4−/− (n = 4) versus infected Lgr4−/− (n = 4).
C Quantifications of GSII+ area (n = 2–4 biological replicates per group, n = 10 glands per mouse), Ki67 as percentage of total DAPI-stained cells (n = 2–4 biological replicates per group, n = 10 glands per mouse), GIF+ area (n = 2–4 biological replicates per group, n = 10 glands per mouse), Muc5ac + area (n = 4–5 biological replicates per group, n = 10 glands per mouse).
D Helicobacter pylori CFUs show higher colonization in Lgr4−/− (n = 5) compared with control mice (n = 4).
E qPCR expression levels of Lgr4, Lgr5, Axin2, gland base cell markers (Gif, Pgc, Tff2, and Muc6), pit cell marker Muc5ac, and Itln1 in uninfected Lgr4−/− (n = 4–10) and control mice (n = 4–6).
F qPCR expression levels of Lgr4, Lgr5, gland base cell markers (Gif, Pgc, and Muc6), and Itln1 in Lgr4−/− (n = 5) and control mice (n = 5) after 2 months of infection.

Data information: For (B, C, D, E, and F), data are mean ± SD. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; ns, not significant (one-way ANOVA + Tukey’s multiple comparison test for B, C, t-test for D, E, and F).

Source data are available online for this figure.
Figure 2.
Figure 3.
To validate that these effects are directly related to the Rspo3-Lgr regulatory axis, we analyzed NF-κB expression and target genes in tamoxifen-treated, 2-week- or 2-month-infected B6-Myh11CreERT2/Rspo3+/− mice (here referred to as: Rspo3−/−), in which Rspo3 is knocked out specifically in myofibroblasts beneath the gland base, as described previously (Sigal et al., 2019). B6-Myh11CreERT2/Rspo3+/− littermates served as controls. In 2-week-infected control mice, we observed a strong expression of p65 specifically in the gland isthmus with a pronounced nuclear translocation of p65, whereas the infection. Rspo3 KO also led to reduced expression of Cxcl1 and Cxcl2 at 2 weeks and 2 months of infection, as determined by qPCR (Fig 4C and D). We also generated mice to conditionally overexpress Rspo3 in Myh11+ mesenchymal cells (Myh11CreERT2/Rosa26SorflflGAG-Rspo3), here referred to as: Rspo3 KI) and found that infection in these mice leads to a strong upregulation of p65, GSI, and Ki67 compared with infected control mice (Fig 4E-H). Notably, Rspo3 KI upregulated p65 also in uninfected mice in the gland as well as in the colon (Figs 4F and EV3A). The massive hyperplastic glands in Rspo3 KI mice revealed throughout the epithelium intense signals for nuclear p65 acetyl K310 (Fig 4I). Collectively, our results reveal that the Rspo3-Lgr axis not only induces Wnt but also enables an activation of NF-κB signaling baseline and upon infection, facilitating the induction of epithelial pro-inflammatory chemokines in response to bacteria in vivo.

R-spondin 3-Lgr4–NF-κB signaling is responsible for neutrophil infiltration in H. pylori gastritis

In H. pylori-infected mice, we observed a manifestation of pro-inflammatory processes, including neutrophil chemotaxis and immune response in dependence of Lgr4, by separately examining genes that are experimentally validated as targets of NF-κB (see https://www.bu.edu/nf-kb/gene-resources/target-genes/) and whose expression was decreased in Lgr4−/− mice (Fig EV3B). We, therefore, asked if infiltration of immune cells upon infection is dependent on Lgr4 or Lgr5. Using immunofluorescence, we stained for MPO, IBA1, and CD3 to analyze the presence of neutrophils, macrophages, and T cells, respectively, in Lgr4−/− mice in uninfected and infected conditions as well as in control mice. As expected, infection led to high mucosal influx of all analyzed immune cells, predominantly in the proximal antrum. Interestingly in infected Lgr4−/− mice, neutrophil infiltration was significantly diminished, indicating that a lack of proper epithelial Lgr signaling has a functional consequence with regard to the recruitment of inflammatory cells (Fig 5A and B). Recruitment of macrophages and T cells was not
Figure 4.
significantly altered in these mice, which is in line with our similar observations in Rsps3 KO mice (Sigal et al., 2019) and may point toward a distinct immune cell infiltration pattern with various influence factors in gastritis that warrants further investigation (Fig EV4A and B). Equivalent analysis of infected Lgr5−/− mice failed to detect altered immune cell infiltration (Figs 5A and B, and EV4A and B).

**Stem cells are the primary epithelial sensors of ADP heptose and promote pro-inflammatory signaling**

To discriminate between effects by immune cells and direct epithelial responses, we took advantage of the epithelial gastric organoid cultures. Gastric organoids were grown in the standard full medium (FM) containing Rsps and Wnt, which resembles the gland base environment and drives stem cell turnover. To test whether these growth factors affect the epithelial ability to mediate pro-inflammatory responses, some organoids were cultured in medium without Rsps, without Wnt and Rsps, or with the addition of Bmp2, which rapidly induces pit cell differentiation. Organoids grown in FM showed robust growth, while removal of growth factors or addition of Bmp2 led to reduced growth and a more spherical appearance (Fig 6A). Immunofluorescence analysis revealed that organoids grown in FM formed small invaginations, which corresponded to GSII+ gland base cells. As expected, upon removal of

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Figure 5. Lgr4 controls mucosal neutrophil infiltration.

A Immunofluorescence labeling for MPO in uninfected and 2-month-infected Lgr4−/− and control mice (top panel) and 2-month-infected Lgr5−/− and control mice (bottom panel). Asterisks indicate nonspecific staining. Scale bars: 50 μm.

B Quantification of MPO+ cells in uninfected and 2-month-infected Lgr4−/− and control mice (n = 2 biological replicates per group, n = 5–10 FOVs per mouse) (left panel) and 2-month-infected Lgr5−/− and control mice (n = 2 biological replicates per group, n = 6–8 FOVs per mouse) (right panel).

Data information: For (B) data are mean ± SD. **P < 0.01; ***P < 0.001; ns, not significant (one-way ANOVA + Tukey’s multiple comparison test).

Source data are available online for this figure.
Figure 6.
growth factors, invaginations and GSII + cells were lost, while more MUC5AC + pit cells were detected (Fig 6B). Expression of the stem cell marker Lgr5, as well as the gland base mucous cell marker Gif, was strongly downregulated in all other conditions compared with stem cell-enriched organoids (FM), as analyzed by qPCR (Fig 6C and D).

To investigate the mechanism by which NF-κB could be induced upon *H. pylori* infection, we treated gastric organoids with ADP heptose, a bacterial sugar, which was previously shown to induce NF-κB in the context of *H. pylori* infection (Pfannkuch et al., 2019). We thus exposed organoids grown in the different medium conditions to ADP heptose for 2 h, to examine their ability to activate NF-κB. The inflammatory response was measured via qPCR of Cxcl1 and Cxcl2, which are classic murine ADP heptose readouts (Brooks et al., 2020) as well as Ccl2 and Cxcl10, which we found to be upregulated in an Rspo/Lgr4-dependent manner after *H. pylori* infection in vivo. ADP heptose triggered NF-κB target gene expression in organoids grown in FM, while organoids grown in the absence of Rspo and Wnt signaling remained unresponsive (Fig 6E). Notably, the absence of Rspo alone already prevented full activation of NF-κB signaling, demonstrating the critical role of Rspo signaling for maintaining the ability of the cells to induce epithelial pro-inflammatory signaling. To address whether these responses are limited to ADP heptose or also affect other pro-inflammatory pathways, we treated organoids with TNF-α. We found similar effects of growth factor withdrawal, indicating that NF-κB activation in gland base cells is not limited to ADP heptose-induced signaling and that activation via the classical TNF-α pathway also depends on Rspo signaling (Fig 6F).

To determine whether this phenotype is indeed due to the activation of NF-κB, we quantitated DNA binding activity of the transcription factor by electrophoretic mobility shift assay (EMSA) in organoids treated as above (Fig 7A and B). We observed baseline activity of NF-κB in dependence of Rspo and demonstrated that the presence of Rspo is necessary for the pronounced induced activation of NF-κB and of its upstream kinase, IKK (IκB kinase). Removal of Rspo or Wnt led to a partial loss of a bona fide target of NF-κB, IκBα (Nfkbia). Supershift analysis in FM organoids + ADP heptose with antibodies against the five subunits confirmed p65 as the principal mediator (Fig 7C). These findings confirm that induction of the canonical IKK-NF-κB pathway in response to ADP heptose in gastric epithelium requires functional Wnt/ Rspo signaling.

To determine which cells in ADP heptose-treated organoids harbor nuclear p65, we stained organoids treated as above with antibodies against total p65 and Ki67 or Gif (Fig EV5A). Nuclear translocation of p65 was detected in nuclei of Ki67+ and GSII+ cells in organoids grown in FM with treated with ADP heptose compared with untreated controls within the respective medium conditions (n = 4 biological replicates per condition).

Data information: For (C, D, E, and F) data are mean ± SD. *P < 0.05; **P < 0.01; ****P < 0.0001. (one-way ANOVA + Tukey’s multiple comparison test for C and D; t-test for E and F). Genes without asterisks are not significantly altered. Source data are available online for this figure.
Discussion

Here, we demonstrate that the stem cell compartment in the stomach antrum is uniquely equipped to respond to infection and initiate mucosal inflammation. Our data reveal a link between Rspo-Lgr signaling and NF-κB, which enables the gland base compartment to rapidly initiate a pro-inflammatory response and simultaneously structurally adapt to *H. pylori* infection.

Most investigations of epithelial homeostasis that explore Rspo-Wnt signaling axis put a focus on Lgr5, which was originally identified as a marker of intestinal and later of gastric stem cells (Barker *et al.*, 2007, 2010). Lgr5 is a Wnt target gene that requires high levels of Wnt signaling (Segditsas *et al.*, 2008). Such high Wnt activity depends in vivo in the stomach on the Wnt signaling enhancer Rspo3 produced by stromal myofibroblasts (Sigal *et al.*, 2017; Harnack *et al.*, 2019). Rspo molecules bind to Lgr4 and Lgr5 and prevent turnover of the Wnt receptor Frizzled (de Lau *et al.*, 2011). Thus, Rspo signaling itself drives expression of its own receptor Lgr5, implying that a positive autocrine feedback loop that stabilizes Wnt/ Rspo signaling could exist (Yan *et al.*, 2017).
contrast, unlike Lgr5, Lgr4 is not a Wnt target gene and broadly expressed in the epithelium of the gastrointestinal tract, including the stomach (Sigal et al., 2017; Harnack et al., 2019). It has been shown that Lgr5 and Lgr4 act in concert to maintain homeostasis in the intestine (de Lau et al., 2011). While loss of Lgr5+ cells does not impact epithelial homeostasis and injury repair (Tian et al., 2011; Castillo-Azofeifa et al., 2019), loss of Lgr4, similar to loss of Rspo3, renders the epithelium more susceptible to injury (Liu et al., 2013; Greicius et al., 2018; Harnack et al., 2019). Depletion of Lgr5+ cells is followed by their recovery in the intestine and stomach, and this process has been shown to require Rspo3, which is likely through interaction with Lgr4 (Sigal et al., 2017; Harnack et al., 2019). Our data here demonstrate that Lgr4 represents a critical receptor for mucosal responses to H. pylori in the antrum. Lgr4 also controls Lgr5 expression; however, Lgr5 alone does not impact H. pylori infection, indicating that while Lgr5+ cells are critical in the context of infection, their maintenance and function is not driven by Lgr5 alone, but requires Lgr4 expression. Therefore, the Rspo-Lgr4 axis is highly relevant for epithelial responses, while Lgr5 expression is a marker of Rspo signaling, but is largely dispensable for Rspo effects.

_Helicobacter pylori_ colonizes the stomach of about 50% of the world’s population and is the main risk factor for gastric cancer.

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**Figure 8.** NF-κB activity defines gland base compartment in the antrum.

A Bright-field images of antrum organoids from 129.129P2-ctnnb1tm1M19/LacZtm1M19/LacZ (ΔN) mice and control mice, passage 0, day 6. Scale bars: 500 μm.

B, C qPCR expression levels of Lgr4, Lgr5, Gif, Pgc, Muc6 (B) and Cxcl1, Cxcl2 (C) of organoids (n = 3 biological replicates).

D Representative H&E staining and immunofluorescence labeling for GSII, Ki67, MUC5AC, DAPI in uninfected and control mice. Scale bars: 50 μm.

E Quantification of GSII and Ki67 as percentage of total DAPI-stained cells in ΔN and control mice (n = 5 biological replicates per group, n = 20 glands per mouse).

Data information: For (B, C, and E) data are mean + SD. *P < 0.05; **P < 0.01; ns, not significant (t-test).

Source data are available online for this figure.
Infection causes a chronic active gastritis, characterized by mucosal infiltration of immune cells such as neutrophils (Eck et al., 2000), which can progress to atrophic or hyperplastic gastritis, metaplasia and, in about 1% of patients, gastric cancer (Correa, 1995). Neutrophils are associated with mucosal damage in *H. pylori* gastritis (Ernst et al., 1997; Shimoyama & Crabtree, 1998). It is well established that the inflammatory response to infection is linked to epithelial pathology and mouse experiments have demonstrated that the degree of immune responses against *H. pylori* correlates with the degree of epithelial premalignant pathology, while the degree of inflammation correlates negatively with *H. pylori* colonization (Wunder et al., 2006; Arnold et al., 2011). The link between infection, inflammation, and epithelial pathology is not fully resolved. We have previously observed that colonization of gastric glands (in contrast to colonization of the surface pits) triggers inflammation, gland hyperplasia as well as metaplasia (Sigal et al., 2015). These responses result in an accumulation of antimicrobial gland base secretory cells, which limit gland colonization but fail to clear the infection (Sigal et al., 2019). Here, we demonstrate and mechanistically resolve the unique ability of the epithelial gland base module to initiate a pro-inflammatory NF-kB response that promotes an accumulation of gland base cells and simultaneously induces an upregulation of epithelial chemokines leading to mucosal neutrophil infiltration that can initiate and maintain a chronic active gastritis. These data provide an explanation for selective pro-inflammatory responses to gland-associated bacteria and link epithelial and inflammatory responses observed upon infection.

It is likely that this unique ability of gland and crypt base cells is not restricted to *H. pylori* infection in the stomach. Glands and crypts show a similar organization throughout the gastrointestinal tract, with stem cells located in the base. It will be interesting to investigate mechanisms of Rspo-NF-kB interactions in response to intestinal pathogens. While crypt colonization may be the most beneficial niche for chronic bacterial colonization as it may provide nutritional and physical benefits for colonization (Keilberg et al., 2016), bacterial penetration into this protected compartment that harbors stem cells is likely highly detrimental for the host. Indeed, several pathogens have evolved to target this compartment. In the colon *Shigella flexneri* reach the bottom of crypts to target stem cells leading to long-term repercussions for tissue regeneration (Arena et al., 2015). Moreover, *Vibrio cholerae* in the small intestine and *Campylobacter jejuni* in the cecum establish crypt colonization by directed and efficient swimming. *Vibrio cholerae* infect intestinal progenitor cells with a type VI secretion system blocking growth and repair pathways in addition to extensive tissue damage upon infection (Fast et al., 2020). Sensing of bacteria and the ability to initiate inflammation upon gland base cell colonization is, therefore, likely to be a conserved property of gland and crypt base cells, enabling a rapid response to these undesired events.

Progenitor cells in the gland are, therefore, not only responsible for gland repopulation but also play a key role in mucosal immunity. Modulation of gastrointestinal stem cell behavior by immune-derived signals has been shown previously. Stem cells in the small intestine express NOD2 receptors, and microbiota-derived peptidoglycans that activate NOD2 support stem cell survival (Nigro et al., 2014). T cell-derived cytokines have also been shown to modulate crypt differentiation, enabling epithelial adaptation to infectious stimuli (Biton et al., 2018). Here, we demonstrate that antral base cells not only respond to immune signals but act as critical components of the innate immune system by initiating responses to pathogenic bacteria via NF-kB signaling.

Molecular links between NF-kB and Wnt signaling in carcinogenesis have been established using genetic cancer models (Greten & Grivennikov, 2019), for example, in Wnt-induced intestinal cancer, enhanced NF-kB activity promotes carcinogenesis, while loss of NF-kB results in prolonged survival and diminished tumor growth (Schwitalla et al., 2013). Constitutive Wnt activation following APC loss leads to hyperproliferation of stem cells and tumor formation via RAC1 (Ras-related C3 botulinum toxin substrate 1)-ROS-NF-kB pathway activation (Myant et al., 2013). In the small intestine, NF-kB is required for proper secretory Paneth cell differentiation and epithelial homeostasis by regulating Wnt signaling and Sox9 expression downstream of NF-kB (Brischetto et al., 2021). Recently, activation of NF-kB via ALPK1 and TIFA in proliferative intestinal cells has been linked to DNA damage and abrogated replication stress, which predisposes to mutagenesis (Bauer et al., 2020). Our data now reveal that an interplay between these two signaling pathways is in fact an integral part of antral gland physiology. Under healthy conditions, this signaling unit is restricted to the stem cell compartment. This restriction is the basis of selective immune responses if the gland becomes infected. In contrast, mutations in the Wnt signaling pathway may result in an expansion of cells with high Wnt signaling, and thus NF-kB competence, to the gland surface, where cells are more likely to be exposed to various signals from luminal bacteria. This would in turn lead to a loss of biogeographic selectivity and initiation of inflammation even by surface epithelial cells, which in turn would perpetuate the regenerative process and may contribute to tumor cell proliferation and progression.

**Materials and Methods**

**Mouse experiments**

All procedures that involved animals were approved by the institutional, local, and national legal authorities at the Max Planck Institute for Infection Biology (LAGeSo, Berlin, Reg. G0084/17) and were compliant with all of the relevant ethical regulations regarding animal research. Axin2<sup>CreERT2</sup> mice were described before (van Amerongen et al., 2012) and were obtained from the Jackson Laboratories. Lgr<sub>4</sub>/<sup>fl/fl</sup> mice and Lgr<sub>5</sub>/<sup>fl/fl</sup> mice were kindly provided by Dr. Jan Tchorz, Novartis. Mouse models were designed for both genes by flanking exon1 withloxP elements facilitating Cre-mediated recombination and excision of exon1 including part of the 5’UTR. For Lgr<sub>4</sub>, Cre-mediated excision results in a deletion of 1.9 kb including Lgr4 exon1 plus 900 bp of 5’UTR. For Lgr<sub>5</sub>, Cre-mediated excision results in a deletion of 1.6 kb including Lgr5 exon1 plus 600 bp of the 5’UTR (Planas-Paz et al., 2016). Rspo3<sup>fl/fl</sup> mice (Neufeld et al., 2012) were a kind gift from John Cobb. Myh11<sup>CreERT2</sup> mice (Herring et al., 2014) were a kind gift from Stefan Offermanns. Mice that conditionally overexpress Rspo3 under the Rosa26 promoter (Rosa26Sor<sup>1(CAG-Rspo3)</sup>) were described previously (Hilkens et al., 2017) and were a gift from John Hilkens and Elvira Bakker. These animals were bred with Myh11<sup>CreERT2</sup> mice to generate double-heterozygous Myh11<sup>CreERT2/Rosa26Sor</sup> mice. (Parsonnet et al., 1997).
mice (AN) (Schmidt-Ullrich et al., 2001) and B6-Tg(k-EGFP)3Pl/Rsu (k-EGFP) (Tomman et al., 2016) were a kind gift from Ruth Schmidt-Ullrich.

All animals were maintained in autoclaved microisolator cages and provided with sterile drinking water and chow ad libitum. Six-to-eight-week-old male mice were used for this study. Sample size was estimated by own experience in this field (Sigal et al., 2017, 2019). Tamoxifen (Sigma) was injected intraperitoneally into mice on two to three consecutive days (4 mg 25 g body weight; diluted in 200 μl corn oil) at the indicated time points.

At the time of collection, the forestomach was removed, and the glandular stomach was opened along the lesser curvature and laid flat. The stomach contents were removed, and the tissue was divided into two halves along the greater curvature. For RNA analysis, the tissue was divided between antrum and corpus, and pieces were snap-frozen. For microscopy analysis, a similar longitudinal section at the midline along the greater curvature was used for all animals to minimize sampling error. For all mouse experiments, mice were randomized to experimental groups.

**Helicobacter pylori infection and CFU analysis**

Animals were infected with a single oral dose of 1 × 10^6 H. pylori strain PMSS1 and euthanized at the indicated time points as previously described (Sigal et al., 2015). Longitudinal sections of stomach tissue (antrum and corpus) were weighed and then mechanically homogenized in brain-heart-infusion medium. Serial dilutions of the homogenates were plated for H. pylori CFU enumeration, and bacterial counts were expressed as CFU g^{-1} of stomach. Lgr5^{-/-} and Lgr5^{-/-} mice plus littermate controls were infected with separate H. pylori cultures leading to variability in CFUs between two different experiments. Experiments were performed in at least three biological replicates per condition. The investigator was blinded for CFU analysis.

**Tissue processing and microscopy**

Tissue samples were processed for microscopy as previously described, with minor modifications (Sigal et al., 2015). For paraffin embedding, tissues were fixed in 2% paraformaldehyde for 1 day. Paraffin embedding and sectioning, as well as H&E staining, were performed by the Charité Core Unit Immunopathology for Experimental Models. Paraffin-embedded sections were hydrated, followed by an antigen retrieval step, then incubated with primary antibodies, followed by a washing step, and incubated with secondary antibody for 1 h. Samples were imaged using a Leica Sp8 confocal microscope (LAS X acquisition software) or a Zeiss Observer 7 microscope (ZEN acquisition software). The investigator performing staining was blinded for treatment and genotype.

The following antibodies were used for this study: rabbit anti-GIF (custom-made by David H. Alpers); mouse anti-Muc5ac (Invitrogen, MA5-12178, lot RL2313146B); rabbit anti-Ki67 (Cell Signaling, 9129S, clone D3B5, lot 3); rabbit anti-NF-kB p65 (Cell Signaling, D14E12, #8242, lot 16); mouse anti-E-Cadherin (BD Biosciences, 610,181); DAPI (Roche, Cat. No. 10236276001); goat anti-GFP (Abcam, ab6673); rabbit anti-CD3 (Abcam, ab16669); goat anti-Iba1 (VWR, 100369-764); rabbit anti-MPO (Abcam, ab208670); mouse anti-beta-catenin (Becton Dickenson, 610,153); rabbit anti-H. pylori (custom-made and described previously (Sigal et al., 2015)); Alexa Fluor 647 Phalloidin (Invitrogen, A22827); rabbit anti-NF-kB p65 acetyl K310 (Cell Signaling, #3045); rabbit anti-Cxcl1 (Ptlab, 12335). GSI + cells were stained with Lectin GSI from Grifonia simplicifolia, Alexa Fluor 647 conjugate (Life Technologies, L32451, lot 1774229), nuclei were counterstained with DAPI (Roche, Cat. No. 10236276001).

**Organoid cultures**

Antrum tissue was sterilized in 0.04% (w/v) sodium hypochlorite (Roth) for 15 min at room temperature, followed by 90-min incubation in buffered saline solution containing 0.5 mM DTT and 3 mM EDTA to dissociate gastric glands. Isolated glands were washed with PBS and mixed with 50 μl Matrigel (BD) and plated in 24-well plates. After polymerization of Matrigel, murine gastric culture medium (Advanced Dulbecco's Modified Eagle Medium/F12 supplemented with B-27 (Invitrogen), N-2 (Invitrogen), N-acetyl cysteine (Sigma), 100 U/ml penicillin-streptomycin (Invitrogen) and containing 50 ng ml^{-1} epidermal growth factor (Invitrogen), 100 ng ml^{-1} Noggin (Peprotech), 100 ng ml^{-1} fibroblast growth factor 10 (Peprotech), 10 μg/gm gastrin (Sigma), 7.5 μg/ml Y-27632 (Sigma), Rspo1-conditioned medium and Wnt3A-conditioned medium) was overlaid. The medium supplemented with growth factors was replaced every 2–3 days. After 2–3 days in culture, for some experiments Wnt3a and/or Rspo1 were removed or 50 ng ml^{-1} BMP2 (R&D Systems) was added for a further 3–5 days. Subsequently, organoids were treated with 0.5 μM ADP heptose (Invigogen) or 10 ng/ml TNF-α (Peprotech) for 2 h. For qPCR analysis, RNA from the organoids was then extracted as described below. For immunofluorescence, organoids were fixed with 3.7% formaldehyde for 1–2 h at room temperature and embedded in paraffin as described before (Grinat et al., 2022).

**Microarray analysis**

For microarray analysis, RNA of infected Lgr4^{-/-} mice and the corresponding controls treated with tamoxifen 2 weeks before euthanasion were isolated from the antrum. Total RNA from bulk tissue was isolated using the RNEasy mini kit (Qiagen) following the manufacturer’s protocol.

Microarray experiments were performed as independent color dye hybridizations using two biological replicates. Quality control and quantification of total RNA were carried out using an Agilent 2100 Bioanalyzer (Agilent Technologies) and a NanoDrop 1,000 UV–Vis spectrophotometer (Kisker). RNA labeling was performed using the dual-color Quick-Amp Labeling Kit (Agilent Technologies). In brief, mRNA was reverse transcribed and amplified using an oligo-dT-T7 promoter primer, and the resulting complementary RNA (cRNA) was labeled with cyanine 3-CPT or cyanine 5-CPT. After precipitation, purification, and quantification, 1.25 μg of each labeled cRNA was fragmented and hybridized to whole mouse genome 4 × 44 K multipack microarrays (Agilent-014868, whole mouse genome 4 × 44 K microarray kit) according to the supplier’s protocol (Agilent Technologies). Scanning of microarrays was performed with 5 μm resolution using a G2565CA high-resolution laser microarray scanner (Agilent Technologies) with extended dynamic range. Microarray image data were analyzed.
using the Image Analysis/Feature Extraction software G2567AA v.4.11.5.1.1 (Agilent Technologies) using the default settings and the GE2_1105_Oct12 extraction protocol. The extracted MAGE-ML files were analyzed further using the Rosetta Resolver Biosoftware, build 7.2.2 SP1.31 (Rosetta Biosoftware). Ratio profiles comprising single hybridizations were combined in an error-weighted manner to create ratio experiments. A 1.5-fold-change expression cut-off for ratio experiments was applied together with anti-correlation of dye-swapped ratio profiles, yielding microarray analysis results that were highly significant ($P < 0.01$), robust and reproducible (Churchill, 2002).

GSEA analysis

We used antral stem cell signature genes (Barker et al., 2010), betacatenin target genes (Herbst et al., 2014), and NF-$kappaB$ signature genes from the MsigDB (Liberzon et al., 2015) on genes pre-ranked by gene-expression-based $t$-score between the antrum tissue of Lgr4$^{fl/fl}$/Axin2$^{CreERT2}$ and Lgr4$^{-/-}$/Axin2$^{CreERT2}$ animals using the gsea R package (preprint: Sersgushichev, 2016) with 5,000 permutations; $P$-values were calculated using FDR (Benjamini & Hochberg, 1995). For further information regarding GSEA and data interpretation, please refer to the original publication (Subramaniam et al., 2005).

The computational code for GSEA analysis of microarray in this manuscript data can be accessed under https://github.com/Sigal-Lab/Wizenty_et_al_Lgr4_stomach_gland.

DAVID and Revigo analysis

Genes showing significant negative changes in expression ($P < 0.05$) were analyzed by DAVID 6.7 BP direct (https://david.ncifcrf.gov). For NF-$kappaB$ target genes, Gilmore database (https://www.bu.edu/nf-kb/gene-resources/target-genes/) was used to select genes showing a significant decrease in expression in Lgr4 KO ($P < 0.05$). Separate GO terms were obtained for these from DAVID 6.7 BP direct. GO terms were refined by Revigo (Uniprot mus musculus, Resnik Large; Supek et al., 2011).

RT–PCR

For qPCR from mouse tissue, RNA was extracted from the snap-frozen antrum tissue using the RNeasy RNA Purification Kit (Qiagen). For the analysis of organoid RNA, organoids were released from Matrigel with cold Dulbecco’s phosphate-buffered saline (DPBS) and pelleted by centrifugation (7 min at 4°C, 300 g), and RNA was then isolated using the TRizol (Invitrogen) and the RNeasy RNA Purification Kit according to the manufacturer’s protocol. Quality and quantity controls were performed for each extract using the NanoDrop 1,000 Spectrophotometer (Thermo Scientific). qPCR was performed using the Power SYBR Green RNA-to-CT 1-Step Kit (Applied Biosystems). Reactions were performed in 25 µl containing 50 ng RNA, 12.5 µl SYBR Green mix, 0.2 µl RT mix, and 0.2 µM primer.

The cycles used were as follows: 30 min at 48°C; 10 min at 95°C; followed by 40 cycles of 15 s at 95°C; and 60 s at 60°C. For each oligonucleotide pair and RNA sample, the reaction was performed in duplicate. The expression levels of the target genes were normalized to the levels of Gapdh gene expression in each individual sample and expressed as fold-change compared with the expression in control tissue (WT, uninfected or nontreated), using the $2^{-\Delta\Delta C_{\text{T}}}$ equation (Pfaffl, 2001).

The following murine primers (Sigma) were used: Axin2 forward 5’-TGAACCTCTCCTTTAGCCTTCA-3’, reverse 5’-TGCCCCACTATGAGCTTCA-3’; Ccl2 forward 5’-CAGTGCCTTGATGGTCTTC-3’, reverse 5’-GTTGGGCATTTAAGCCTCT-3’; Cxcl1 forward 5’-CCCAAAACCGAAGTCTACAC-3’, reverse 5’-CTTCGGTTGGAGGAGACC-3’; Cxcl10 forward 5’-CAAGCCACTGTCCTGAAGCA-3’, reverse 5’-TGACCTAGGAGGACCAAGGA-3’; Cxcl11 forward 5’-TCACCATCTCCAGGAGCC-3’, reverse 5’-AAGCCATGTGTTGGTGAGC-3’; Cg3-3 forward 5’-AGCAACCTCCTATCAAGGA-3’, reverse 5’-CAAGCCCCCTCCTATCAAGGA-3’; Il1n1 forward 5’-GTGCTTACACAGGAGGTTTACCT-3’, reverse 5’-AGACCATCTTGGCTCCTTTGT-3’; Lgr5 forward 5’-TACAAACTGTGTCGTAACACCC-3’, reverse 5’-VGGAGGTATGGTGCTTGA-3’; Muc5ac forward 5’-ATACTGTGGGATAGCTGACC-3’, reverse 5’-Muc5ac forward 5’-CCATGGGATAGCTGACC-3’, reverse 5’-Muc5ac forward 5’-CCATGGGATAGCTGACC-3’, reverse 5’-Muc5ac forward 5’-CCATGGGATAGCTGACC-3’, reverse 5’-Muc5ac forward 5’-CCATGGGATAGCTGACC-3’, reverse 5’-Muc5ac forward 5’-CCATGGGATAGCTGACC-3’, reverse 5’-Muc5ac forward 5’-CCATGGGATAGCTGACC-3’, reverse 5’-Muc5ac forward 5’-CCATGGGATAGCTGACC-3’. For each experiment, $P$-values from all gene sets were jointly adjusted for multiple testing using FDR—the method of Benjamini–Hochberg (Benjamini & Hochberg, 1995). For further information regarding GSEA and data interpretation, please refer to the original publication (Subramaniam et al., 2005).

Electrophoretic mobility shift assay (EMSA) and sodium dodecyl sulfate-polyacrylamide gel electrophoreses (SDS–PAGE)

The gel electrophoresis mobility shift assay (EMSA) is used to detect protein complexes with nucleic acids. Here, EMSA was used to determine the affinity of NF-$kappaB$ to the specific sequence on DNA (the $kappaB$ sequence). EMSA provides a readout of the activation state of the NF-$kappaB$ pathway. The 32p radiolabeled DNA probe containing the $kappaB$ sequence was incubated with the protein lysate and then separated on a gel.

EMSA was performed with whole-cell lysates (WCL). Whole-cell lysates were prepared using Baeuerle buffer (20 mM HEPES pH 7.9; 350 mM NaCl; 20% glycerol; 1 mM MgCl$_2$; 0.5 mM EDTA; 0.1 mM EGTA; 1% NP-40), including complete protease inhibitor cocktail (Roche), 10 mM NaF, 8 mM b-glycerophosphate, 0.2 mM Na$_3$VO$_4$, and 1 mM DTT. H2K$b$ probe from MHC promoter was used and incubated with 5 µg of whole-cell lysate for 30 min. The protein and DNA was then separated on a non-denaturing TBE-polyacrylamide gel over 90 min. The gel was vacuum dried, and signal was visualized on an X-ray film.

EMSA protocol and SDS–PAGE protocol was described previously (Mikuda et al., 2018). Supershift protocol was described previously (Kolesnichenko et al., 2021).

The following antibodies were used for this study: Ix5r2 C-15 (Santa Cruz, sc-203), IKK-p (S176-180) (Cell Signaling, #2697), alpha-tubulin DM1A (Santa Cruz, sc-32,293), p65 C-20 (Santa Cruz, sc-372 X), p50 E-10 (Santa Cruz, sc-8,414 X), RelB D-4 (Santa Cruz, sc-48,366 X), p52 (Millipore, 06–413), and cRel (Cell Signaling, #4727).
Single-molecule RNA ISH

Tissue sections cut at 5 μm thickness were processed for RNA in situ detection using the RNAscope Red Detection Kit according to the manufacturer’s instructions (Advanced Cell Diagnostics). Positive and negative control probes were used for each experiment according to the manufacturer’s instructions. The following probes were used in this study: Lgr4 (Order no.: 318321, Lot: 18053A, Target region: 1973–2895); Lgr5 (Order no.: 312171, Lot: 16351A, Target region: 2165–3082).

ScRNA-seq analysis

ScRNA-seq data from epithelial cells of murine gastric antrum were used from a published data set (Busslinger et al., 2021b). GEO accession number for the source data is GSE157694 (Data ref: Busslinger et al., 2021a). Seurat R package (Butler et al., 2018) was used to perform t-distributed stochastic neighbor embedding (t-SNE) analysis. t-SNE analysis was applied to visualize cell clusters. Datasets were filtered (> 50 transcripts, < 5% mitochondrial reads per cell), integrated with SCTransform to eliminate the batch effect of samples, and log-normalized, and the first 20 principal components were used to reduce dimensionality by t-SNE with a resolution of 0.1.

Statistics

GraphPad Prism (v.8.4.0) was used for statistical analysis. Data are displayed as mean ± SD. P < 0.05 was considered to be statistically significant.

For comparison of two groups, we used an unpaired two-sided t-test, for multiple comparisons one-way ANOVA as indicated. Data were filtered (> 50 transcripts, < 5% mitochondrial reads per cell), integrated with SCTransform to eliminate the batch effect of samples, and log-normalized, and the first 20 principal components were used to reduce dimensionality by t-SNE with a resolution of 0.1.

Data availability

The microarray data have been deposited in the Gene Expression Omnibus (GEO) of the National Center for Biotechnology Information under GEO accession number GSE165028 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE165028). Quantitative data that support the findings in this study are available within the paper and in the source data. All other data supporting these findings are available from the corresponding author upon reasonable request.

References

van Amerongen R, Bowman AN, Nusse R (2012) Developmental stage and time dictate the fate of Wnt/beta-catenin-responsive stem cells in the mammary gland. Cell Stem Cell 11: 387–400

Amieva MR, El-Omar EM (2008) Host-bacterial interactions in Helicobacter pylori infection. Gastroenterology 134: 306–323

Amieva MR, Vogelmann R, Covacci A, Tompkins LS, Nelson WJ, Falkow S (2003) Disruption of the epithelial apical-junctional complex by Helicobacter pylori CagA. Science 300: 1430–1434

Arena ET, Campbell-Valois FX, Tinevez JY, Nigro G, Sachse M, Moya-Nilges M, Nothelfer K, Marteyn B, Shorte SL, Sansonetti PJ (2015) Bioimage analysis of shigella infection reveals targeting of colonic crypts. Proc Natl Acad Sci U S A 112: E3282–E3290

Arnold IC, Lee JY, Amieva MR, Roers A, Flavell RA, Sparwasser T, Müller A (2011) Tolerance rather than immunity protects from Helicobacter pylori-induced gastric preneoplasia. Gastroenterology 140: 199–209

Bakert S, Ziska E, Brinkmann V, Zimny-Arndt U, Faucconier A, Jungblut PR, Naumann M, Meyer TF (2000) Translocation of the Helicobacter pylori CagA protein in gastric epithelial cells by a type IV secretion apparatus. Cell Microbiol 2: 155–164

Barker N, Huch M, Kujala P, van de Wetering M, Snippert HJ, van Es JH, Sato T, Stange DE, Begthel H, van den Born M et al (2010) Lgr5(+ve) stem cells
drive self-renewal in the stomach and build long-lived gastric units in vivo. Cell Stem Cell 6: 25–36
Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegeman A, Korving J, Begthel H, Peters PJ et al (2007) Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 449: 1003–1007
Bauer M, Nascakova Z, Mihai A-I, Cheng PF, Levesque MP, Lampart S, Hurwitz R, Pfannkuch L, Dobrovolna J, Jacobs M et al (2020) The ALPK1/ TIFANF-kB axis links a bacterial carcinogen to R-loop-induced replication stress. Nat Commun 11: 5117
Benjamin Y, Hochberg Y (1995) Controlling the false discovery rate: A practical and powerful approach to multiple testing. J R Stat Soc B Methodol 57: 289–300
Biton M, Haber AL, Rogel N, Burgin G, Beyaz S, Schnell A, Ashenberg O, Su CW, Smilie C, Shekhar K et al (2018) T helper cell cytokines modulate intestinal stem cell renewal and differentiation. Cell 175: 1307–1320.e1322
Brischetto C, Krieger K, Klotz C, Krahn J, Kunz S, Kolesnichenko M, Mucka P, Heuberger J, Scheideret C, Schmidt-Ullrich R (2021) NF-kB determines Paneth versus goblet cell fate decision in the small intestine. Development 148: dev.199683
Brooks P, Zur Bruegge T, Boyle EC, Kalies S, Villarreal SN, Liese A, Bleich A, Buettner T (2020) CD14 and ALPK1 affect expression of tight junction components and proinflammatory mediators upon bacterial stimulation in a colonic 3D organoid model. Stem Cells Int 2020: 4069354
Busslinger GA, Weusten BLA, Bogte A, Begthel H, Brosens LAA, Clevers H (2021a) Gene Expression Omnibus GSE157694 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE157694). [DATASET]
Busslinger GA, Weusten BLA, Bogte A, Begthel H, Brosens LAA, Clevers H (2021b) Human gastrointestinal epithelia of the esophagus, stomach, and duodenum resolved at single-cell resolution. Cell Rep 34: 108819
Butler A, Hoffman P, Smibert P, Papalexi E, Satija R (2019) Atoh1(+) cells during colonic regeneration. Cell Stem Cell 25: e2020–e2021
Cameron S, W above d, Stove J, Sluijsen R, Koppens MAJ, De Laet W, Barker N, Low TY, Koo BK, Li VS, Teunissen H, Kujala P, Haegebarth A, Peters PJ, van de Wetering M et al (2011) Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. Nature 476: 293–297
ChePep controls Wnt/β-catenin signalling. Nature 495: 3000231
Greicius G, Kabiri Z, Sigmundsson K, Liang C, Bunte R, Singh MK, Virshup DM (2018) PDGFrAipha(+)- pericytial stromal cells are the critical source of Wnts and RSP03 for murine intestinal stem cells in vivo. Proc Natl Acad Sci U S A 115: E3173–E3181
Grenet FR, Grivennikov SI (2019) Inflammation and cancer: Triggers, mechanisms, and consequences. Immunity 51: 27–41
Grinat J, Kosel F, Goveas N, Kranz A, Alexopoulos D, Rajewsky K, Sigal M, Stewart AF, Heuberger J (2022) Epigenetic modifier balances Mapk and Wnt signalling in differentiation of goblet and Paneth cells. Life Sci Alliance 5: e202101187
Harnack C, Berger H, Antanaviciute A, Vidal R, Sauer S, Simmons A, Meyer TF, Sigal M (2019) R-spondin 3 promotes stem cell recovery and epithelial regeneration in the colon. Nat Commun 10: 4368
Herbst A, Jurinovic V, Krebs S, Thieme S, Blum H, Göke B, Kolligs FT (2014) Comprehensive analysis of β-catenin target genes in colorectal carcinoma cell lines with deregulated Wnt/b-catenin signaling. BMC Genomics 15: 74
Herring BP, Hoggatt AM, Burlak C, Offermanns S (2014) Previously differentiated mediatal vascular smooth muscle cells contribute to neointima formation following vascular injury. Vasc Cell 6: 21
Hilken J, Tilmer NC, Boer M, Iink GJ, Schewe M, Sacchetti A, Koppens MAJ, Song JY, Bakker ERM (2017) RSP03 expands intestinal stem cell and niche compartments and drives tumorigenesis. Gut 66: 1095–1105
Howitt MR, Lee JY, Lertsethtakarn P, Vogelmann R, Joubert LM, Ottemann KM, Amieva MR (2011) ChePeps controls Helicobacter pylori infection of the gastric glands and chemotaxis in the Epsilonproteobacteria. MBio 2: e00098-11
Kapalszcynska M, Lin M, Maertzdorf J, Heuberger J, Muellerke S, Zuo X, Vidal R, Shureiqi I, Fischer AS, Sauer S et al (2022) BMP feed-forward loop promotes terminal differentiation in gastric glands and is interrupted by H. pylori-driven inflammation. Nat Commun 13: 1577
Keilberg D, Zavros Y, Shepherd B, Salama NR, Ottemann KM (2016) Spatial and temporal shifts in bacterial biogeography and gland occupation during the development of a chronic infection. mBio 7: e01705-16
Kolesnichenko M, Mikuda N, Höpken UE, Kargel E, Uyar B, Tufan AB, Milanovic S, Wos W, Krahn I, Schleich K et al (2021) Transcriptional repression of NFKBIA targets constitutive IKK- and proteasome-independent p65/RelA activation in senescence. EMBO J 40: e104296
de Lau W, Barker N, Low TY, Koo BK, Li VS, Teunissen H, Kujala P, Haegeman A, Peters PJ, van de Wetering M et al (2011) Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. Nature 476: 293–297
Liu S, Qian Y, Li L, Wei G, Guan Y, Pan H, Guan X, Zhang L, Lu X, Zhao Y et al (2019) Lgr5 gene deficiency increases susceptibility and severity of dextran sodium sulfate-induced inflammatory bowel disease in mice. J Biol Chem 298: 8794–8803 discussion 8804
Mikuda N, Kolesnichenko M, Beaudette P, Popp O, Uyar B, Sun W, Tufan AB, Perder B, Akalin A, Chen W et al (2018) The IkB kinase complex is a regulator of mRNA stability. EMBO J 37: e98658
Myant KB, Cammareri P, McChee EJ, Ridgway RA, Huels DJ, Cordero JB, Schwitalla S, Kalina G, Ogg EL, Athineos D et al (2013) ROS production and NF-kB activation triggered by RAC1 facilitate WNT-driven intestinal stem cell transcriptional reprogramming. EMBO J 32: 4252–4264
Miyanishi K, Hellou E, Uyar B, Tufan AB, Milanovic S, Wos W, Krahn I, Schleich K et al (2018) Transcriptional repression of NFKBIA targets constitutive IKK- and proteasome-independent p65/RelA activation in senescence. EMBO J 40: e104296
cell proliferation and colorectal cancer initiation. Cell Stem Cell 12: 761–773

Neufeld S, Rosin JM, Ambasta A, Hui K, Shaneman V, Crowder R, Vickerman L, Cobb J (2012) A conditional allele of Rspon3 reveals redundant function of R-spondins during mouse limb development. Genesis 50: 741–749

Nigro G, Rossi R, Commere PH, Jay P, Sansonetti PJ (2014) The cytotoxic bacterial peptidoglycan sensor Nod2 affords stem cell protection and links microbes to gut epithelial regeneration. Cell Host Microbe 15: 792–798

Parsonnet J, Friedman GD, Orentreich N, Vogelman H (1997) Risk for gastric cancer in people with CagA positive or CagA negative Helicobacter pylori infection. Gut 40: 297–301

Pfaafi MW (2003) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29: e45

Pfannkuch L, Hurwitz R, Traulsen J, Sigulla J, Poeschke M, Matzner L, Kosma P, Schmid M, Meyer TF (2019) ADP heptose, a novel pathogen-associated molecular pattern identified in Helicobacter pylori. FASEB J 33: 9087–9099

Planas-Paz L, Orsini V, Boulter L, Calabrese D, Pikiolik M, Nigsh F, Xie Y, Roma G, Donovan A, Martí Pé et al (2016) The RSPO-LSGR4/5-ZNRF3/RNF43 module controls liver zonation and size. Nat Cell Biol 18: 467–479

Schmidt-Ullrich R, Aebischer T, Hulsenk J, Birchmeier W, Klemm U, Scheidereit C (2001) Requirement of NF-kappaB/Rel for the development of hair follicles and other epidermal appendages. Development 128: 3843–3853

Schwitalla S, Fingerle AA, Cammareri P, Nebelsiek T, Cöktuna SI, Ziegler PK, Canli O, Heijmans J, Huels DJ, Moreaux G et al (2013) Intestinal tumorigenesis initiated by dedifferentiation and acquisition of stem-cell-like properties. Cell 152: 25–38

Segditsas S, Sieber O, Deheragoda M, East P, Rowan A, Jeffery R, Nye E, Clark S, Spencer-Dene B, Stamp G et al (2008) Putative direct and indirect Wnt targets identified through consistent gene expression changes in APC-mutant intestinal adenomas from humans and mice. Hum Mol Genet 17: 3864–3875

Sergushichev AA (2016) An algorithm for fast preranked gene set enrichment analysis using cumulative statistic calculation. bioRxiv https://doi.org/10.1101/060012 [PREPRINT]

Shimoyama T, Crabtree JH (1998) Bacterial factors and immune pathogenesis in Helicobacter pylori infection. Gut 43: S2–S5

Sigal M, Logan CY, Kapalczynska M, Mollenkopf HJ, Berger H, Wiedenmann B, Nusse R, Amieva MR, Meyer TF (2017) Stromal R-spondin orchestrates gastric epithelial stem cells and gland homeostasis. Nature 548: 451–455

Sigal M, Reines MD, Mullerke S, Fischer C, Kapalczynska M, Berger H, Bakker EURM, Mollenkopf HJ, Rothenberg ME, Wiedenmann B et al (2019) R-spondin-3 induces secretory, antimicrobial Lgr5(+) cells in the stomach. Nat Cell Biol 21: 812–823

Sigal M, Rothenberg ME, Logan CY, Lee JY, Honaker RW, Cooper RL, Passarelli B, Camorlinga M, Bouley DM, Alvarez G et al (2015) Helicobacter pylori activates and expands Lgr5(+) stem cells through direct colonization of the gastric glands. Gastroenterology 148: 1392–1404.e1321

Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomero SL, Golub TR, Lander ES et al (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 102: 15545–15550

Supek F, Bošnjak M, Šmuc N, Šmuc T (2011) REVIGO summarizes and visualizes long lists of gene ontology terms. PLoS One 6: e21800

Tan SH, Swathi Y, Tan S, Koh J, Seishima R, Murakami K, Oshima M, Tsuji T, Phuah P, Tan LT et al (2020) AQPS enriches for stem cells and cancer origins in the distal stomach. Nature 578: 437–443

Tian H, Bihehs B, Warming S, Leong KG, Rangell L, Klein OD, de Sauvage FJ (2011) A reserve stem cell population in small intestine renders Lgr5-positive cells dispensable. Nature 478: 255–259

Tomann P, Paus R, Millar SE, Scheideiret C, Schmidt-Ullrich R (2016) Lhx2 is a direct NF-xB target gene that promotes primary hair follicle placode down-growth. Development 143: 1512–1522

Wunder C, Churin Y, Winau F, Warnecke D, Vieth M, Lindner B, Zähringer U, Mollenkopf HJ, Heinz E, Meyer TF (2006) Cholesterol glucosylation promotes immune evasion by Helicobacter pylori. Nat Med 12: 1030–1038

Yan KS, Janda CY, Chang J, Zheng GXY, Larkin KA, Luca VC, Chia LA, Mah AT, Han A, Terry JM et al (2017) Non-equivalence of Wnt and R-spondin ligands during Lgr5(+) intestinal stem-cell self-renewal. Nature 545: 238–242

Zimmermann S, Pfannkuch L, Al-Zeer MA, Bartfeld S, Koch M, Liu J, Rechner C, Soerensen M, Sokolova O, Zamyatina A et al (2017) ALPK1- and TIFA-dependent innate immune response triggered by the Helicobacter pylori type IV secretion system. Cell Rep 20: 2384–2395

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