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Supplementary Materials for

Inactivation of the tumor suppressor gene *Apc* synergizes with *H. pylori* to induce DNA damage in murine gastric stem and progenitor cells

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This PDF file includes:

Figs. S1 to S5

Figure S1

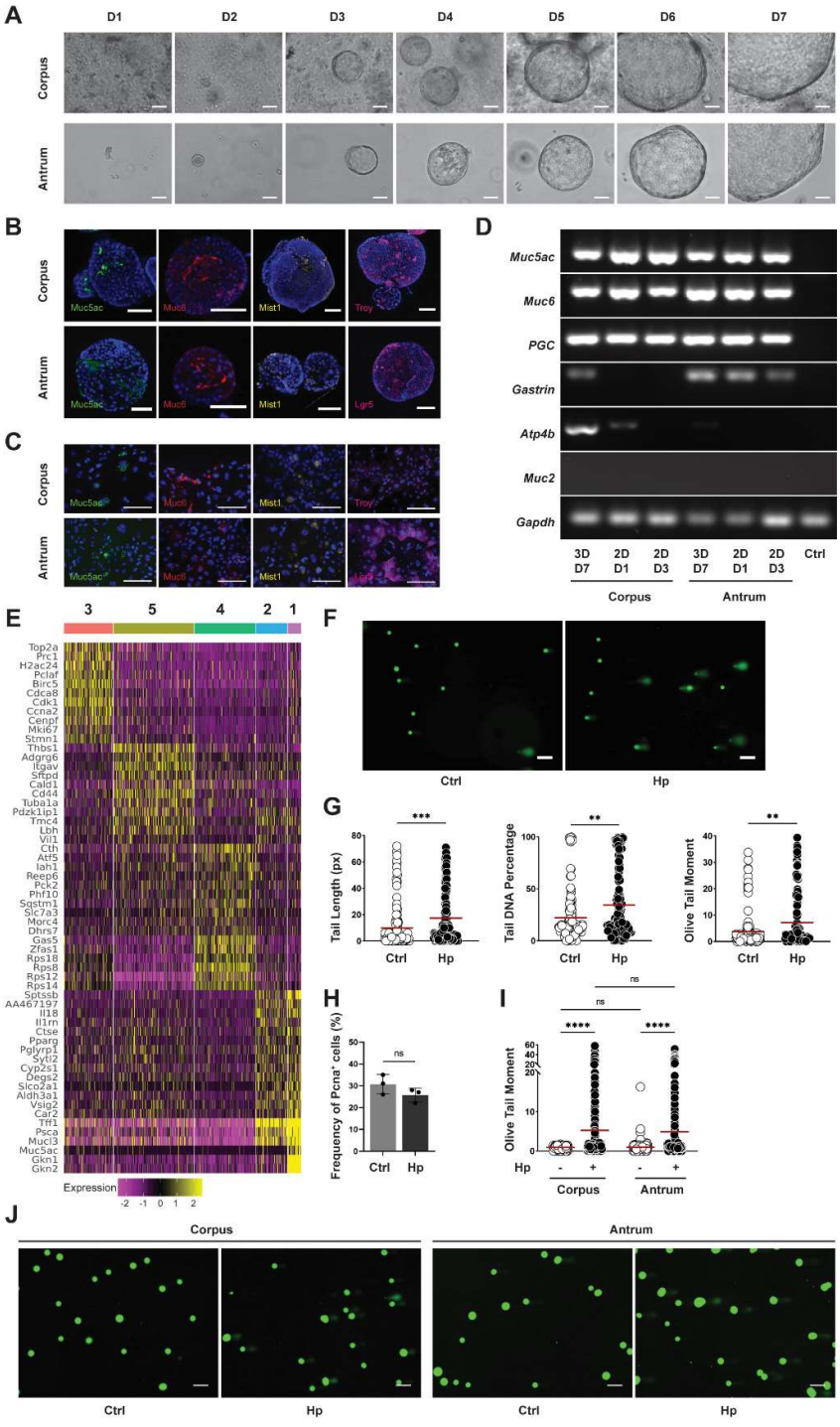


Fig. S1. Murine gastric organoids grown in 3D and 2D comprise terminally differentiated lineages as well as stem and progenitor cells. A, Time course showing the growth of antrum and corpus organoids from individual isolated glands. After seven days in culture, organoids are either injected with *H. pylori*, or harvested and seeded in 2D for further experimentation. Scale bar, 100 μm. **B,C,** Immunofluorescence staining of 3D organoids (B) or 2D cultures (C) for the indicated lineage markers. Muc5ac is a marker of mucous pit cells, Muc6 of neck and Mist1 of chief cells; Lgr5 is a stem and progenitor cell marker in antral organoid cells and Troy is a marker of corpus stem and progenitor cells. Scale bar, 100 μm. The 3D organoids were rendered in Fiji using a Maximum

Intensity Z-Projection. **D,** Reverse transcription-PCR followed by agarose gel electrophoresis, with primers specific for the indicated signature transcripts, performed using RNA extracted from antrum and corpus organoids cultured for seven days in 3D, or seeded in 2D and cultured for one or three days. Note the absence of *Muc2* transcript encoding the intestinal mucin Muc2 from all gastric organoid cultures, and the selective presence of the parietal cell marker transcript *Atp4b* only in 3D corpus, but not in antrum or 2D corpus cultures. **E,** scRNA-seq normalized expression of the top 10 marker genes of the five indicated clusters as determined by Seurat's `FindAllMarkers` function with the 'Wilcox' method, with additional selected marker genes. Cluster 1 represents mature pit cells, cluster 2 represents immature pit cells and cluster 3 represents isthmus stem and progenitor cells; the expression patterns of clusters 4 and 5 are most consistent with a basal stem and progenitor (4) and a neck and chief cell origin (5). Note that only three transcripts were selectively expressed by cluster 1. **F,G,** Representative images (F) and quantification of comet formation (G) using the alkaline comet assay, of 3D organoids injected after seven days in culture with WT *H. pylori* strain PMSS1 at a multiplicity of infection (MOI) of ~50; cells were harvested six hours later. Note that in G, the olive tail moment is the product of the % of DNA in the tail and the distance between the centers of mass of the comet head and tail regions. **H,** Quantification of $Pcna^{+}$ cells among all cells of three different antrum 2D cultures infected or not with strain PMSS1 at an MOI of ~50 for six hours. **I,J** Quantification of comet formation (I) and representative images (J) of antrum and corpus organoid cells grown in 2D and infected three days after seeding for six hours with *H. pylori* strain G27, MOI 50. Data in panels F-J are representative of at least three independently conducted experiments; p-values were calculated using student's t-test (G) and one-way ANOVA (I). p-values are indicated in ranges; *, <0.05; **, p<0.01; ***, p<0.005; **** p<0.001; ns, not significant.

Figure S2

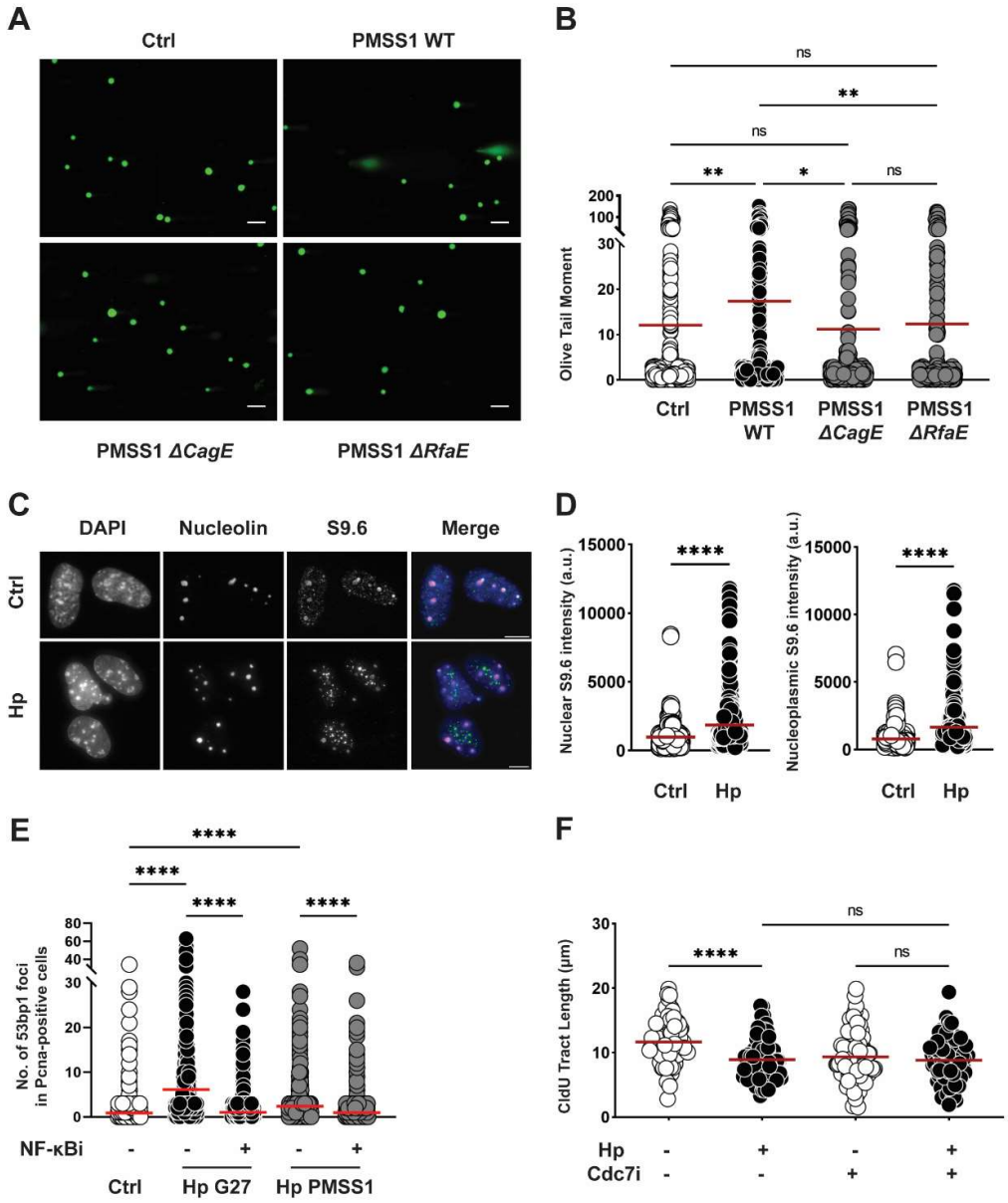
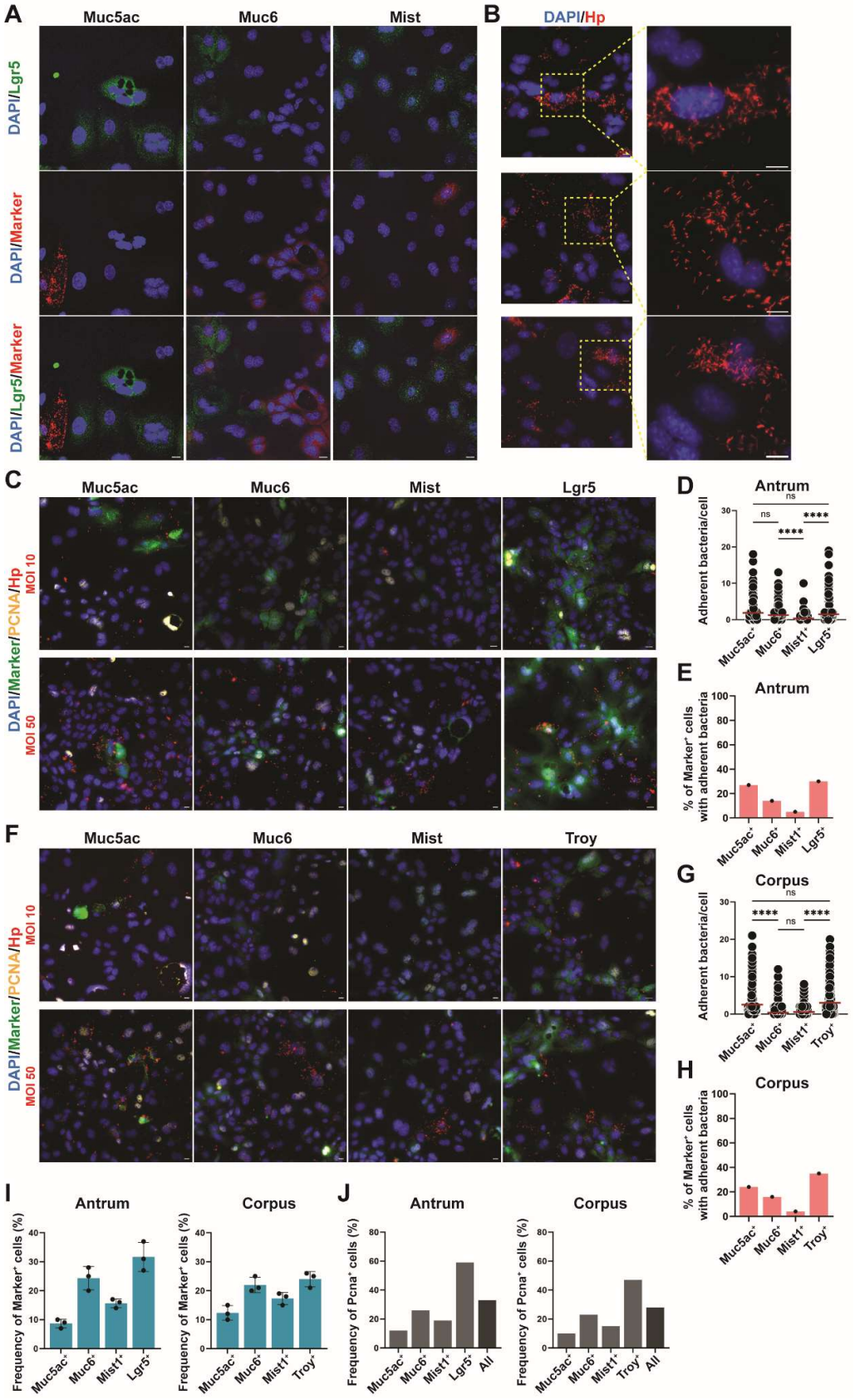


Fig. S2. *H. pylori*-induced DNA damage in gastric organoid cells is T4SS- and RfaE-, and transcription- as well as replication-dependent. A,B, Antrum organoid cells were grown as monolayers for three days and infected for six hours with the indicated WT and mutant strains of *H. pylori* (Hp) PMSS1 at MOI 50. Representative images and the quantification of comet formation, as assessed by alkaline comet assay, are shown in A and B. **C,D,** Antrum organoid cells were seeded in 2D for three days and infected for six hours with *H. pylori* G27 at MOI 50; cells were stained for RNA:DNA hybrids using the S9.6 antibody. Nuclear and nucleoplasmic S9.6 intensity are shown in D alongside representative images in C. Scale bar, 10 μm. **E,** Antrum organoid cells were seeded in 2D for three days and infected for six hours with the indicated *H. pylori* strains at MOI 50, in the presence or absence of the NF-κB inhibitor BAY 11-7082 at 1 μM final concentration. Cells were subjected to 53bp1 and Pcna staining; the number of 53bp1 foci in Pcna-positive cells are shown. **F,** Antrum organoid cells were seeded in 2D for three days and infected for six hours with *H. pylori* strain G27 at MOI 50, in the presence or absence of the Cdc7 inhibitor XL413 at 0.2 μM final concentration. Cells were subjected to DNA fiber labeling and the quantification of fiber lengths (shown in μm). Horizontal red lines indicate the means throughout. P-values were calculated by one-way ANOVA. p-values are indicated in ranges; *, <0.05; **, p<0.01; ***, p<0.005; **** p<0.001; ns, not significant.

Figure S3


A
Muc5ac
Muc6
Mist
DAPI/Lgr5
DAPI/Marker
DAPI/Lgr5/Marker
B
DAPI/Hp
C
Muc5ac
Muc6
Mist
Lgr5
DAPI/Marker/PCNA/Hp
MOI 10
MOI 50
D
Antrum
Adherent bacteria/cell
ns
****
Muc5ac+
Muc6+
Mist1+
Lgr5+
E
Antrum
% of Marker+ cells with adherent bacteria
F
Muc5ac
Muc6
Mist
Troy
G
Corpus
H
Corpus
I
Antrum
Corpus
Frequency of Marker+ cells (%)
J
Antrum
Corpus
Frequency of Pcna+ cells (%)
All

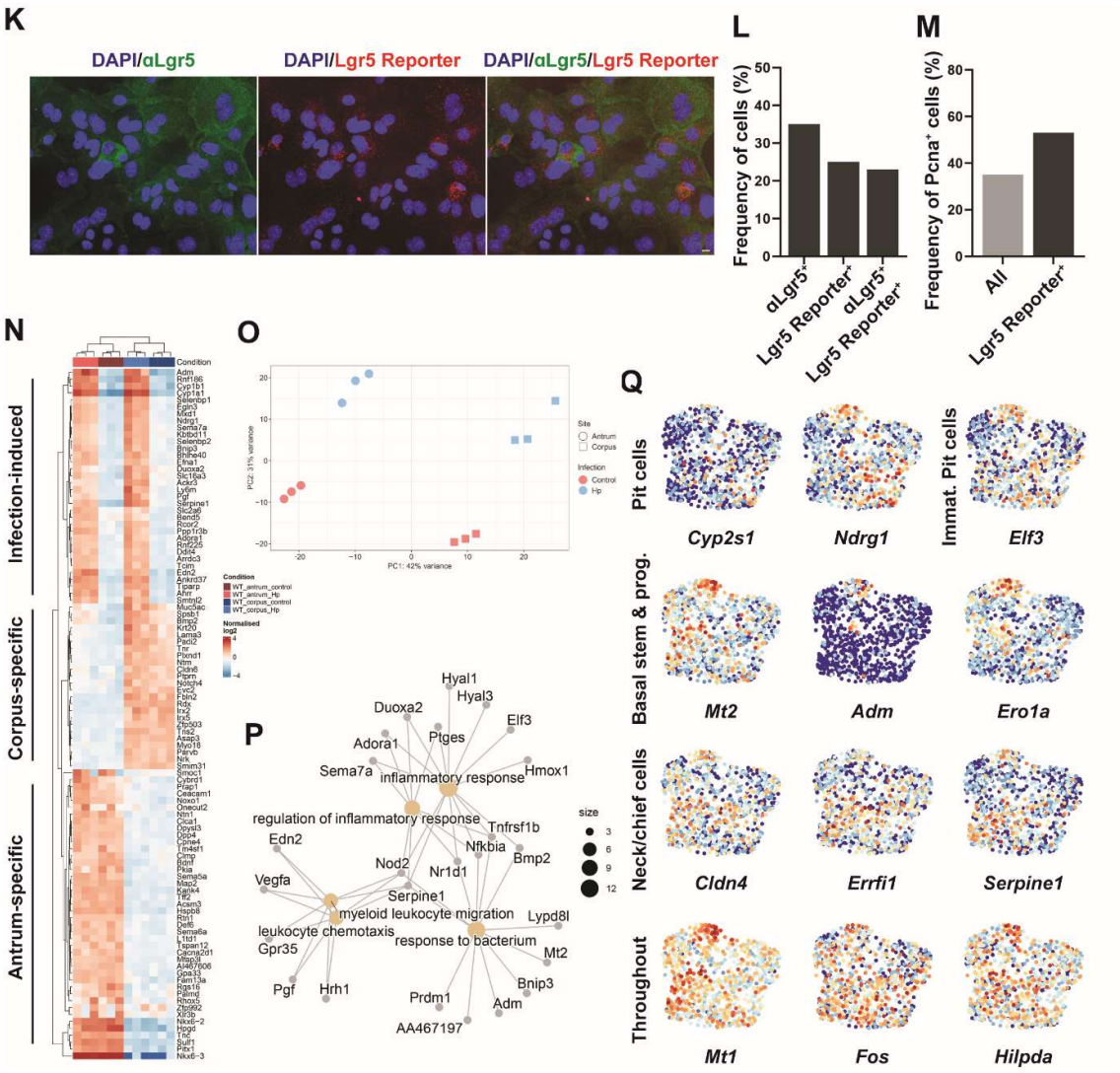


Fig. S3. *H. pylori* adheres to mucous pit and stem/progenitor cells and induces a transcriptional signature in various gastric lineages present in organoid-derived monolayers. A, The expression of Lgr5, Muc5ac, Muc6 and Mist is mutually exclusive in antral organoid cells. Antrum-derived organoids were seeded as monolayers and co-stained with antibodies for Lgr5 (in green) and the indicated lineage markers (in red). Individual channels and overlays are shown. Scale bar, 10 μm. **B,** Three fields of view of the images shown in Fig. 3C and F are shown in their original magnification, alongside a higher magnification for easier discrimination of individual bacteria. Scale bar, 10 μm. **C-H,** Antrum-derived (C-E) or corpus-derived (F-H) organoid cells seeded in 2D were infected with RFP-expressing *H. pylori* PMSS1 (MOI of 10 in upper panels in C and F; MOI of 50 in lower panels in C and F) for six hours. Cells were stained for the indicated lineage markers and subjected to quantitative automated microscopy. The number of adherent RFP^+ bacteria per cell for the infection with an MOI of 10 is shown for each lineage in D and G; the fraction of cells per lineage with any number of adherent bacteria is shown in E and H. The quantification of bacteria at MOI of 50 is shown in main Fig. 3B and E, pooled with two identical independent experiments. Representative images are shown in C and F, with lineage marker-positive cells in green. Scale bar, 10 μm. Horizontal red lines indicate the means in D and G. P-values were calculated by one-way ANOVA. **** $p<0.001$; ns, not significant. **I,** Antrum and corpus-derived organoids were seeded as monolayers and stained with antibodies for the indicated lineage markers. Frequencies of lineage marker-positive cells among all cells are shown for three independent experiments. Means +/- standard deviation are shown alongside the individual data points. **J,** Antrum and corpus-derived organoids were seeded as monolayers and stained with antibodies for the indicated lineage markers and for Pcna. Frequencies of $Pcna^+$ cells are shown for each lineage. Results are from one experiment. **K,L,** Lgr5 expression as determined by anti-Lgr5 antibody staining and *Lgr5*-mOrange

reporter expression. Antrum-derived organoid cells generated from an *Lgr5*-mOrange reporter mouse were seeded in 2D and stained for endogenous Lgr5 expression using a specific antibody (in green) and for mOrange transgene expression driven by the *Lgr5* promoter using an RFP-specific antibody (in red). Single channels as well as the overlay are shown in K, and the quantification of single and double-positive cells is shown in L. Scale bar, 10 μm. **M,** Antrum-derived organoid cells generated from an *Lgr5*-mOrange reporter mouse were seeded in 2D and stained for Pcna as well as mOrange transgene expression using an RFP-specific antibody. The frequencies of $Pcna^+$ cells among all organoid cells, and among *Lgr5*-mOrange $reporter^+$ cells are shown. **N-Q,** Corpus- and antrum-derived organoid cells were grown for three days in 2D prior to infection for six hours with *H. pylori* G27, MOI of 50. Bulk RNA isolated from three biological replicates per condition (inf and uninfected, each of antrum and corpus) was subjected to RNA sequencing; the heatmap in N shows the 100 genes with a standard deviation across samples of >2. Antrum- and corpus-specific genes, as well as infection-induced genes are annotated. Principal component analysis (in O) was conducted on the same samples as shown in N, and is based on the top 2000 genes ranked by SD. **P,** Network plot showing the genes and pathways associated with inflammatory response, response to bacterium, leukocyte chemotaxis and others. Enriched pathways were determined by Gene Ontology (GO) term pathway analysis using the hypergeometric over-representation test via the 'enrichGO' function of the clusterProfiler Bioconductor R package for the 117 intersecting genes that were upregulated (log_2 fold change >1, adjusted p-value <0.05) upon *H. pylori* infection relative to uninfected control cells in both antrum and corpus cultures. **Q,** Select genes found to be upregulated by *H. pylori* were mapped onto the Seurat cluster UMAPs of Figure 1 derived by scRNA sequencing.

Figure S4

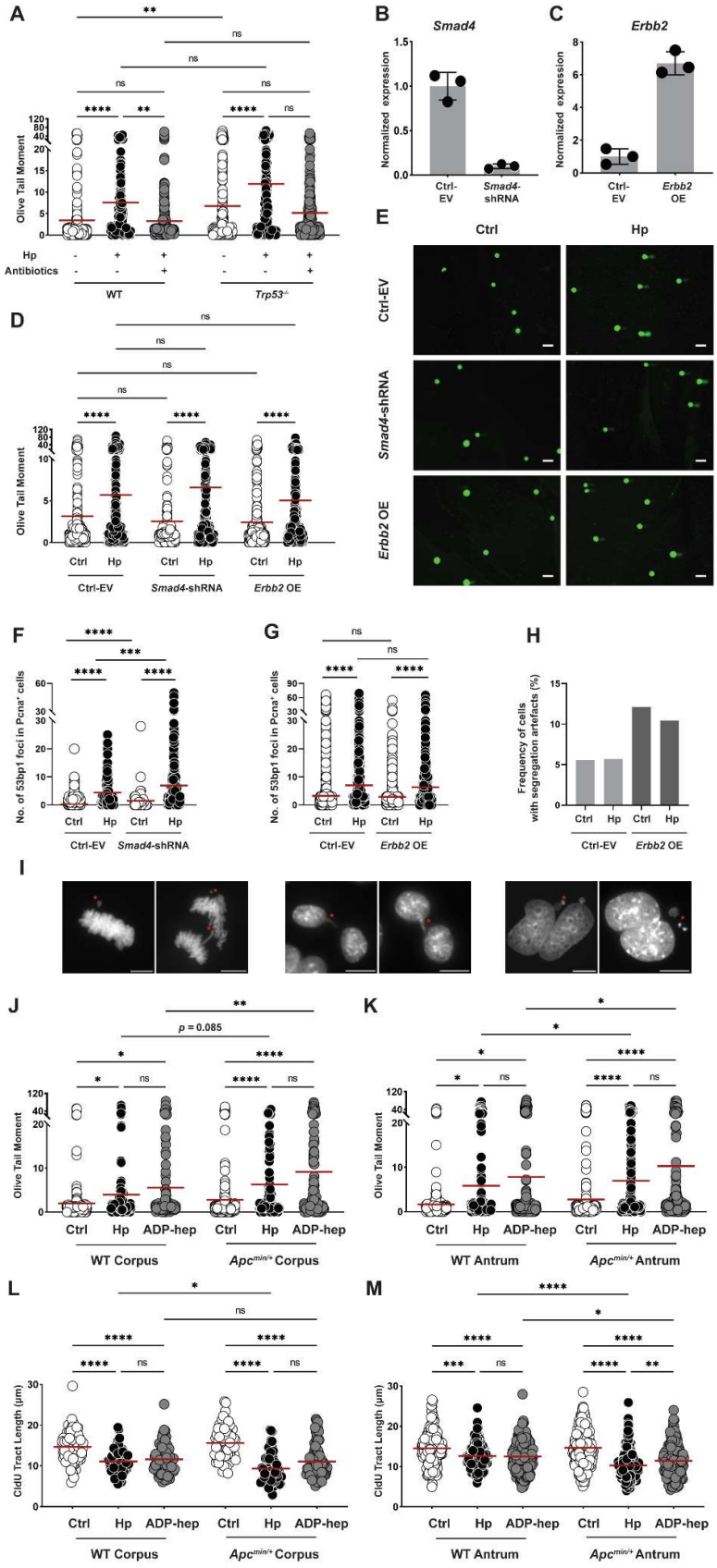

A
B
Smad4
C
Erbb2
D
E
Ctrl
Hp
Ctrl-EV
Smad4-shRNA
Erbb2 OE
F
G
H
I
J
K
L
M
Olive Tail Moment
Normalized expression
No. of 53bp1 foci in Pcna+ cells
Frequency of cells with segregation artefacts (%)
CldU Tract Length (μm)
Hp
Antibiotics
WT
Trp53-/-
ADP-hep
p = 0.085
WT Corpus
Apcmin/+ Corpus
WT Antrum
Apcmin/+ Antrum

Fig. S4. *Apc* loss, but not *Smad4* knockdown or *Erbb2* overexpression exacerbates DNA damage induced by *H. pylori* infection. A, Organoid cells derived from WT and *Trp53*$^{-/-}$ mice were infected with *H. pylori* (Hp) G27 for six hours; cells were then washed and treated (or not) with 1 μg/ml tetracycline for 24 hours to kill the bacteria and allow the cells time to repair their DNA damage. Cells were subjected to alkaline comet assay; the quantification of comet formation is shown. **B,C,** Knockdown efficiency of *Smad4* by a specific shRNA, and overexpression (OE) of *Erbb2*, as determined by qRT-PCR for three independent experiments. Treatment with empty vector (containing an irrelevant shRNA in the case of *Smad4* kd) was averaged across the three experiments and set as 1. **D-G,** Organoid cells were transduced with the indicated plasmids and infected with *H. pylori* G27 MOI 50 for six hours. Cells were subjected to alkaline comet assay and to 53bp1 and Pcna staining, followed by the quantification of comets (D) and of 53bp1 foci in Pcna-positive cells (F,G). Representative images of comets are shown in E (scale bar, 10 μm). Data in D, F and G are pooled from two independent experiments. **H,I,** Segregation artefacts caused by *Erbb2* overexpression in *H. pylori*-infected as well as uninfected control cells, as determined by DAPI staining of mitotic and post-mitotic cells. Representative images illustrating misaligned chromosomes, bulky chromosome bridges and micronuclei (all indicated by asterisks) are shown in the left, middle and right panels (I), and quantified across >100 cells from two experiments in H. Scale bar, 10 μm. **J-M,** Corpus (J,L) and antrum-derived (K,M) organoid cells derived from WT and *Apc*$^{min/+}$ mice were grown as monolayers and either infected with *H. pylori* strain G27 (MOI 50), or treated with 0.5 μM ADP-heptose for six hours. Cells in J and K were subjected to alkaline comet assay; the quantification of comet formation is shown for one representative experiment of three; ~150 cells were analyzed per condition. Cells in L and M were subjected to DNA fiber labeling and the quantification of fiber lengths (shown in μm). Horizontal red lines indicate the means throughout. P-values were calculated by one-way ANOVA. p-values are indicated in ranges; *, <0.05; **, p<0.01; ***, p<0.005; **** p<0.001; ns, not significant.

Figure S5

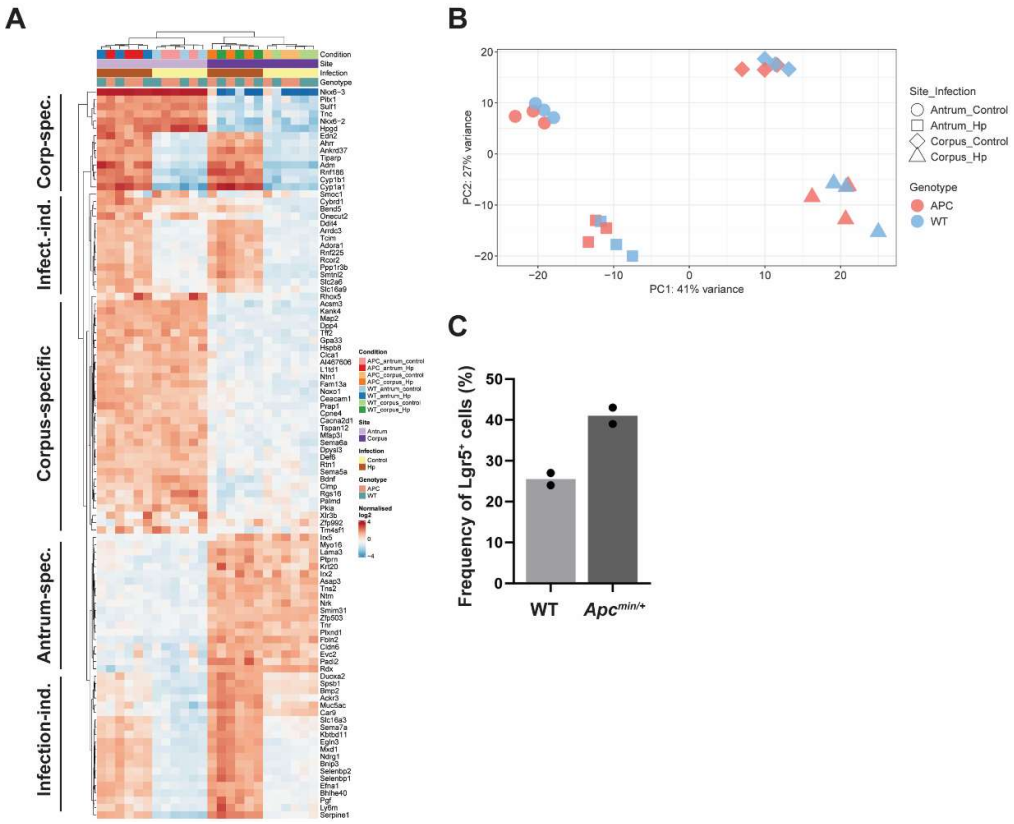


Fig. S5. Transcriptional signature of murine gastric WT and *Apc*$^{min/+}$ organoid cells infected with *H. pylori.* A,B, Corpus- and antrum-derived organoid cells derived from WT and *Apc*$^{min/+}$ mice were grown for three days in 2D prior to infection for six hours with *H. pylori* G27, MOI of 50. Bulk RNA isolated from three biological replicates per condition (WT and *Apc*$^{min/+}$, infected and uninfected, each of antrum and corpus) was subjected to RNA sequencing; the heatmap in A shows the 100 genes with a standard deviation across samples of >2. Antrum- and corpus-specific genes, as well as infection-induced genes are annotated. Principal component analysis (in B) was conducted on the same samples as shown in A, and is based on the top 2000 genes ranked by SD. Note that the WT samples are the same ones as shown in Figure S3. **C,** Antrum-derived organoid cells of the indicated genotypes were seeded in 2D and stained for Lgr5. Means are shown alongside the two replicate data points per condition.