Supplementary Methods

FITC-labeled dextran assay

To assess the functionality of tight junctions in 3-D cultures, spheroids were incubated for three hours with fluorescein isothothiocyanate-labeled dextran (FITC-dextran, 4 kDa, Sigma) as previously described [1]. Briefly, cultures cultures were grown in expansion medium for five days before FITC-labeled dextran (1 mg/ml) was added to the medium. Cultures were incubated for three hours, then tight junctions were disrupted by addition of EGTA (2 mM, Roth). The influx of labeled dextran into the lumen of spheroids was analysed by confocal microscopy (Olympus IX81 attached to a Crest Visitron spinning disc system) and pictures were taken every minute with VisiView (Visitron; version 2.1.0) software. Color intensities in and outside of the spheroids were determined for every picture with ImageJ [2] and used for the analysis of time-dependent influx of FITC-dextran into the lumen.

Infections with H. pylori

H. *pylori* P12 (strain collection no. P243) and its isogenic mutant P12ΔcagPAI (strain collection no. P387), cloned by replacing the pathogenicity island with a kanamycin cassette [3], were grown on GC agar plates supplemented with 10% horse serum (Biochrom) and corresponding antibiotics for 48 h under microaerobic conditions at 37°C. After growth, bacteria were passaged every day by harvesting with a sterile cotton swab followed by 2 washes in PBS and resuspension in RPMI 1640 medium (Gibco) containing 2 mM L-glutamine and 25 mM HEPES. Bacterial number was determined by measuring the optical density at 550 nm. Infection was carried out in RPMI medium. Cells were serum starved for the duration of infection.

Lentiviral manipulation

Replication deficient lentiviral particles were produced by $CaCl_2$-transfection of 293-T cells with the packaging vector psPAX2 (Addgene, #12260), the envelope vector pMD2.G (Addgene, #12259) and pLVTHM (Addgene, #12247) harboring a GFP signal. After two days, the supernatant containing the produced lentiviral particles was filtered (0.45 µm), concentrated with Lenti-X Concentrator (Clontech) and the pellet dissolved in ADF medium.

Spheroid cultures were prepared as described for passaging and collected in 250 µl infection medium/sample (expansion medium with ADF containing lentiviral particles instead of normal ADF plus 8 µg/ml polybrene (Sigma)) and transferred to a 48-well plate, spinoculated for 1 h at 32°C at 600 x g and incubated for 4 h at 37°C. Afterwards, infected cells were collected, resuspended in Matrigel and cultivated under normal culture conditions with expansion medium.

Paraffin Embedding and Microtome Sections of 3-D Cultures

Gastric spheroids/organoids were washed with cold PBS 5-10 times to remove Matrigel, fixed in 3.7% PFA overnight at 4°C, washed 3 times with PBS and stored in PBS at 4°C until use. Fixed samples were dehydrated in an ascending alcohol series up to absolute ethanol, stored once for 20 min in isopropanol and twice for 20 min in acetone before being transferred to a metal paraffin embedding chamber and, after careful removal of the acetone, placed on a hot plate at 60°C. Pre-heated, molten Paraffin (60°C) was added and samples were left at 60°C for 20 min. Thereafter, samples were moved to a cooling plate for 1h until solidified. Specimens were cut with a Microm Paraffin Rotation Microtome into 5 µm slices. Sections were collected on object slides and dried at 37°C before use.

Immunofluorescence Staining and Image Analysis

For de-waxing and antigen retrieval, paraffinized samples slides were washed twice with xylene (10 min) followed by a descending series of alcohols (20 sec each), followed by two washes with water. Afterwards, slides were incubated for 30 min in target retrieval solution (Dako) at 95°C, 20 min at room temperature (RT) and 5 min under running water.

Immunofluorescence labeling was carried out as previously described [4]. In short, samples were washed twice with PBS and incubated with blocking solution (PBS, 1% bovine serum albumin, 2% FCS) for 2 h followed by incubation with the primary antibody (in blocking solution) for 2 h at RT. After 3 washes with PBS, samples were incubated with fluorescently labeled secondary antibodies and Draq5 (1:1000; Cell Signaling) (in blocking solution) for 90 min in the dark at RT. Antibodies and dilutions are listed in Supplementary Table 1. Samples were washed three times with PBS, mounted in Mowiol (Sigma) and analyzed by confocal microscopy using a Leica TCS SP-8 microscope and Leica confocal software. Single channel images were merged with Photoshop (Adobe).

Protein Lysates and Immunoblot Analysis

Cells were directly harvested with 2x Laemmli buffer (4% SDS, 20% glycerol, 120 mM Tris-Cl (pH 6.8) and 0.02% bromphenol blue) and boiled for 10 min at 95°C. Samples were separated on a 10% SDS-polyacrylamide gel and transferred to a polyvinylidene difluoride membrane by Western blotting. Membranes were blocked with TBS buffer containing 0.1% Tween-20 and 3% BSA for 2 h and incubated with primary antibodies overnight at 4°C, followed by HRP-conjugated secondary antibodies for 2 h. Membranes were covered with Hyperfilm ECL (Amersham) and signals detected with X-ray films. Antibodies and dilutions used are listed in Supplementary Table 1.

RNA Isolation and Reverse Transcription PCR Analysis

Gastric tissue pieces were transferred to 2 ml tubes with 0.3 ml ice-cold RNAse-free water and homogenized with three 20 sec pulses of an ULTRA-TURRAX T8 (IKA) at maximum speed. RNA was extracted from gastric cell cultures or freshly homogenized tissue using the TRIzol (Invitrogen) method and subsequently purified with the GeneJET RNA Purification kit (Thermo Fisher) in accordance to manufacturer's protocol. DNA was removed with the DNAfree kit from Ambion.

For semi-quantitative RT-PCR, reverse transcription was carried out with SuperScript III First strand Synthesis System for RT-PCR (Invitrogen) and cDNA was amplified in a thermal cycler (annealing temperature 55°C, 30 cycles). Primer sequences are given in Supplementary Table 2. The resulting fragments were analyzed by agarose gel electrophoresis.

Microarray eypression profiling and data analysis

Microarray experiments were performed similar to Koch et al. [5] as independent dual-color dye-reversal color-swap hybridizations. Quality control and quantification of total RNA was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies) and a NanoDrop 1000 UV-Vis spectrophotometer (Kisker). Total RNA was isolated with TRIzol (Life Technologies) according the supplier's protocol. RNA labeling was performed with 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 resulting cRNA was labeled with Cyanine 3-CTP or Cyanine 5-CTP. After precipitation, purification, and quantification, 1.25 µg of each labeled cRNA was fragmented and hybridized to whole genome human 4 × 44k multipack microarrays 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 XDR extended range. Microarray image data were analyzed with the Image Analysis/Feature Extraction software G2567AA v. A.11.5.1.1 (Agilent Technologies) using default settings. The extracted MAGE-ML files were analyzed further with the Rosetta Resolver Biosoftware, Build 7.2.2 SP1.31 (Rosetta Biosoftware). Ratio profiles comprising single hybridizations were combined in an error-weighted fashion to create ratio experiments. A 1.5-fold change expression cut-off for ratio experiments was applied together with anti-correlation of ratio profiles, rendering the microarray analysis highly significant ($P < .01$), robust, and reproducible. Microarray data have been deposited in the Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/geo/) of the National Center for Biotechnology Information and can be assessed with the GEO accession number GSE58473.

Gene set enrichment analysis (GSEA)

H.pylori infected and uninfected corpus tissues were derived from data published previously [8]. Gene set enrichment analysis [6, 7] for those signatures and for all curated gene sets from the

MSigDB (www.broadinstitute.org/gsea/msigdb/collections.jsp, c2.all.v4.0) was performed on genes pre-ranked by gene expression based t-score between corpus and antrum organoids and between infected and uninfected 2D primary cells. Standard settings with 1000 permutations were used for all analyses.

Statistical Analysis

Statistical significance was analyzed using Student´s t test.

Supplementary References

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Supplementary Figure Legends

Supplementary Figure 1. Human gastric spheroid cultures can be stored at -80°C. Representative micrographs of spheroid cultures before and after freezing and thawing. Examples from two different donors are shown. Scale bars: 500 µm.

Supplementary Figure 2. 3-D gastric cultures are correctly polarized and harbor functional tight junctions. (A) Confocal micrographs of human gastric spheroids (cross sections), fluorescently labeled with antibodies against the tight junction marker occludin (green) and the polarization marker β-catenin (red); nuclei were counterstained with Draq5 (blue). L= lumen. Scale bars: 25 µm. (B) Live imaging of a whole spheroid after 3 h in culture medium containing FITC-labeled dextran. Following EGTA addition, labeled dextran enters the spheroid through disrupted tight junctions. Scale bar: 100 µm (C) Influx of dextran over time after addition of EGTA, as measured by relative fluorescence intensity in the lumen of the spheroid relative to the outside.

Supplementary Figure 3. Addition of Notch signaling inhibitor DBZ to gastric spheroid cultures leads to down-regulation of stem cell markers. (A) Expression changes for a selection of regulated genes as determined by microarray analysis comparing corpus spheroids incubated with DBZ for five days to non-treated spheroids. (B) Expression changes for a selection of regulated genes as determined by microarray analysis comparing antrum spheroids incubated with DBZ for five days to non-treated spheroids.

Supplementary Figure 4. Gene expression in human gastric organoids correlates to that in the corresponding stomach region. (A) GSEA analysis of genes upregulated in corpus vs antrum tissue samples [8] shows only limited enrichment among genes upregulated in corpus organoids. Genes were ranked by t-score of corpus vs. antrum gene expression in organoids. (B) GSEA reveals that genes higher expressed in antrum tissue compared to corpus are also strongly enriched among those upregulated in antrum organoids. Genes were ranked by t-score of antrum vs. corpus gene expression in organoids. ES= enrichment score; NES= normalized enrichment score; FDR= false discovery rate

Supplementary Figure 5: Infected 2-D gastric primary cells show an inflammatory response characteristic for infection with *H. pylori*. GSEA reveals significant enrichment of gene sets of up-regulated TNF targets, up-regulated NF- κB targets and inflammatory response to LPS among genes up-regulated in 2D primary cells after *H.pylori* infection. ES= enrichment score; NES= normalized enrichment score; FDR= false discovery rate

Supplementary Figure 6. Human gastric spheroid cultures can be manipulated with lentivirus. Representative micrographs of spheroids infected with a GFP-transducing lentivirus (upper panel) and non-infected spheroids (lower panel). Pictures were taken five days after lentivirus infection. Scale bars: 500 µm.

Supplementary Movie 1: Representative example of a growing spheroid culture kept in expansion medium. Growth of the spheroids culture was recorded over seven days. Original magnification: 4x objective; time is displayed as days: hours: minutes.

Supplementary Table 1: Antibodies used in the study.

Supplementary Table 2: Sequences of primers used in the study.