Supplementary Methods

Generation of CCK2R-CreERT BAC transgenic mice

The Cre-ERTM-FrtNeoFrt cassette was ligated into murine CCK2R BAC genomic DNA (BACPAC, RP23-477L18). The BAC genomic DNA was purified and electroporated into SW105 competent cells. The correct sequence was confirmed by using restriction enzyme digestion and PCR in the region of interest. The PCR amplified CreTM-FrtNeoFrt DNA with 50bp BAC homologue on both ends was electroporated into SW105 CCK2R-BAC containing cells. The BAC DNA was isolated, linearized with PI-SceI, and was then microinjected into the pronucleus of fertilized CBA x C57BL/6J oocytes in the Columbia University Transgenic Animal Core. Six positive founders were mated with C57BL/6J mice, and F4 or F5 generations were crossed to Rosa-LacZ (B6.129S4-Gt(ROSA)26Sor^{tm1Sor}/J), -Tomato/GFP (B6.129S4-Gt(ROSA)26Sortm4 (ACTB-tdTomato,-EGFP) Luo/J), or -tdTomato (B6;129S6-Gt(ROSA)26Sortm9 (CAG-tdTomato) Hze/J) reporter strains from Jackson Laboratories.

Mouse gastric cancer model

Age- and sex-matched 8 to 12-week old mice in a C57BL/6 background were infected by oral gavage with *H. felis* in 0.2ml trypticase broth three times per week on every other day for a total dose of 100 million colony-forming units (CFU) per mouse. MNU (Sigma Chemical Co, St Louis, MO) was dissolved in distilled water at a concentration of 240 ppm and freshly

prepared thrice per week for administration in drinking water in light-shielded bottles ad libitum. Mice were given drinking water containing 240 ppm MNU on alternate weeks for a total of 10 weeks (total exposure of 5 weeks). In the combination of *H. felis* and MNU, we administered MNU 2 weeks after *H felis* inoculation as described previously (1).

Tissue collection and histological analysis

Following isoflurane inhalation, blood was immediately collected into serum collection vials (BD Biosciences) by incision of the brachial artery or vein, and mice were then euthanized by cervical dislocation. The stomach and proximal duodenum were removed and the stomach incised along the greater curvature. Linear gastric strips from the lesser curvature were fixed overnight in 10% phosphate-buffered formalin or 4% paraformaldehyde, embedded into paraffin block or OCT compounds. Histological scoring was performed as published criteria by board certified veterinary pathologists (S.M.) blinded to sample identity (2). A dysplasia score of 3.0 was considered carcinoma *in situ* or low-grade gastrointestinal intraepithelial neoplasia (GIN), and a score of 4.0 represented invasive gastric cancer. The remainder of the gastric tissue was snap-frozen in dry ice and stored at –80°C for mRNA analysis.

Quantitative analysis of mRNA expression

Longitudinal strips of gastric tissue from the anterior wall as well as the posterior wall were harvested and snap-frozen in dry ice and kept in -80 °C freezer until processed for analysis. Total RNA was extracted with Nucleospin RNA II kit (Clontech) and cDNA was synthesized

by Superscript III First-strand Synthesis System for RT-PCR (Invitrogen, CA). Expression levels of indicated genes were quantified by realtime PCR assays using SYBR Green and 7300 Real Time PCR System. Primer sequences used in this experiment are available upon request.

In vitro culture system

Antrum was removed from mouse stomachs and the tissue chopped into approximately 5 mm pieces. Afterwards, tissue was washed with cold PBS, and incubated in 2.5 mM EDTA in PBS for 60 minutes on ice. The tissue fragments were suspended vigorously with a 10-ml pipette in cold PBS containing 10% FBS, yielding supernatants enriched in crypts. Crypt fractions were centrifuged at 900 rpm for 5 minutes at 4 °C. Crypt fraction samples were passed through 100 μm filters (BD Biosciences), and centrifuged at 720 rpm for 5 minutes. Crypts were embedded in extracellular matrix (provided from NCI) and seeded on pre-warmed 24-well or 48-well plates. After the matrix solidified, advanced DMEM/F12 medium containing penicillin/streptomycin, 50 μg/ml gentamicin, 10 mM HEPES, GlutaMAX, N2, B27 (all from Invitrogen), and 1 μM N-acetylcysteine (Sigma-Aldrich), 50 ng/mL EGF (Invitrogen), 100 ng/mL Noggin (Peprotech) and R-spondin1 was overlaid as indicated. In culture medium, 100 ng/ml Wnt3A (Peprotech) or Wnt3A-conditioned medium (1:1 concentration) was added. Wnt3A-conditioned medium was prepared as previously (3). Growth factors were added every other day and the entire medium was changed twice a

week. Passaging of organoids was performed at day 7 as described previously (4). When passaging organoids, we dissociate organoids mechanically by 1mL tip and pipet, and replate them. The number of organoids per well was counted on microscopic images. Gastric single cells from antrum were isolated and cultured as described previously (5). Crypts were dissociated with TrypLE express (Invitrogen) including 1 mg/ml DNase I (Roche) for 10 min at 37 °C. Dissociated cells were passed through a 20-μm cell strainer and washed with 2% FBS/PBS. Viable epithelial single cells were gated by forward scatter, side scatter and a pulse-width parameter, and negative staining for propidium iodide. Sorted cells were collected, pelleted and embedded in extracellular matrix, followed by seeding on a 48-well plate (100-3000 singlets per well). Y-27632 (10 μM, Sigma) was included for the first two days. The images of organoids were acquired using fluorescent microscopy (Nikon, TE2000-U) or 2-photon microscopy (Nikon, A1P).

Immunohistochemistry and Immunofluorescence

Tissues were fixed in 10% formalin or 4% paraformaldehyde overnight, embedded in paraffin, and processed by standard histological methods. Immunohistochemical staining was performed with avidin-biotin-peroxidase complex kits (Vector Laboratories, CA) according to the manufacturer's instructions. The following primary antibodies were used: anti-Ki67 (1:100, rabbit polyclonal, Abcam), anti-TFF1 antibody (1:200, Santa Cruz), anti-mouse CD44 antibody (1:200, rat monoclonal clone IM-7, BD Biosciences), anti-BrdU antibody

(1:200, rat monoclonal, Abcam), anti-H/K-ATPase antibody (1:1000, mouse monoclonal, Abcam), anti-Chromogranin A antibody (1:200, rabbit polyclonal, Abcam), anti-Sox2 antibody (1:200, rabbit polyclonal, Abcam), anti-gastrin antibody (1:200, rabbit polyclonal, Santa Cruz), anti-somatostatin antibody (1:200, Abcam), anti-Dclk1 antibody (1:200, Abcam), anti-cleaved caspase-3 antibody (1:200, Cell Signaling Technology) and anti-GFP antibody (1:200, rabbit polyclonal, Invitrogen). Primary antibodies were incubated at room temperature for one hour or at 4 ^{o}C overnight, in a humidified chamber. Subsequently, the sections were incubated with biotinylated secondary antibodies (Vectastain ABC kit; Vector Laboratories, Burlingame, CA) for 30 minutes, followed by incubation with avidin-coupled peroxidase (Vector Laboratories) for 30 minutes. Diaminobenzidine (DAB; Dako) as the chromogen and slides were counterstained with Mayer's hematoxylin. For immunofluorescence, Alexa fluor 488 or 594 secondary antibodies (Invitrogen) were used and then counterstained with 4', 6-diamidino-2-phenylindole (Vector Laboratories). The number of Ki67-, BrdU-, or Lgr5-GFP-positive cells in the stomach glands was measured at 20 different locations in the each of three group mice under the Nikon TE2000 microscope (Nikon Inc., Melville, NY). All values were expressed as mean ± SE (standard error). *: $p< 0.05$. For X-gal staining, intracardiac perfusion of a solution containing 4% paraformaldehyde and 2% glutaraldehyde in 0.1 M Sorensen's phosphate buffer (pH 7.4) (containing 2 mM $MgCl_2$ and 5 mM EGTA) was performed, then tissues were fixed in 4%

PFA at 4°C for two to three hours and cryopreserved in 30% sucrose before embedding in OCT compound. Tissues were then sectioned on a cryostat for enzyme histochemical analysis. Frozen sections (5 μm) were washed (0.01% sodium deoxycholate and 0.02% Nonidet P-40) and incubated overnight at room temperature in a 0.1% X-gal solution (4% 4-chloro-5-bromo-3-indolyl-D-galactopyranoside (X-gal) dissolved in dimethylformamide, 5 mM K3Fe(CN)6, 5 mM K4Fe(CN)6.6H_2O). Sections were then counterstained with nuclear fast red and visualized using standard light microscopy.

Statistical analysis

The differences between means were compared using Student's *t*-test or Mann-Whitney *u*-test. P values < 0.05 were considered to indicate statistical significance.

References

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