Supplementary Materials

Materials & Methods

Animals

Mongolian gerbils were purchased from SLC Japan. Five-week-old male Mongolian gerbils were housed in plastic cages with bedding in an air-conditioned biohazard room with a 12 hr light/12 hr dark cycle. They were given food and autoclaved distilled water freely.

Bacterial Culture and *H. pylori* Infection

Helicobacter pylori strain ATCC 43504 was purchased from American Tissue Culture and cultures were grown as previously described.[15] *H. pylori* cultures were diluted with culture medium to 1×10^8 colony-forming units/ml and used immediately in animal experiments. Gerbils were separated into *H. pylori* infected group (n=24) and no infected group (n=6). Gerbils of *H. pylori* infected group were infected with *H. pylori* by oral gavage two times per day for 2 days (total four times) and were then maintained for a year of infection. At sacrifice after 4 weeks treatment, *H. pylori* infection was confirmed with a CLO test.

Study Design

After one year of *H. pylori* infection, the gerbils were randomly assigned to receive subcutaneous implantation of either placebo pellets (n=13) or Selumetinib-containing slow release pellets (n=11) for a total of 4 weeks of dosing. In addition to these infected gerbils, uninfected control gerbils were similarly divided into either placebo administration (n=3) or Selumetinib administration (n=3). All gerbils were sacrificed after 4 weeks treatment and their stomachs were removed immediately. The excised stomachs were opened along the greater curvature, separated into halves, and one half of was fixed in 4% paraformaldehyde solution for 24 h, embedded in paraffin, and cut into 5 μm sections.

Selumetinib Treatment

Selumetinib was provided by Astra-Zeneca Corporation or purchased from Selleckchem. Selumetinib-containing pellets were formulated with 8.5 mg of Selumetinib per pellet for a 14 day release (Innovative Research of America). The pellet was implanted subcutaneously in the flank and after 2 weeks, a second pellet was implanted in the other side subcutaneously, so the gerbils were treated with Selumetinib for four weeks total. Placebo pellet containing no Selumetinib was similarly implanted subcutaneously twice in four weeks for the placebo treated group of gerbils. The protocol was approved by the animal experiment committee of the University of Tokyo, Graduate School of Medicine (P14-140).

Immunohistochemistry

For all immunohistochemistry, paraffin sections (5μm) were used. Sections were deparaffined, rehydrated and then antigen-retrieved in the Target Retrieval solution (DAKO North America, Inc, Carpinteria, CA) using a pressure cooker. After blocking the sections with Protein Block Serum-Free (DAKO North America, Inc) at room temperature for 90 min, the primary antibody incubation was performed using Antibody Diluent with Background Reducing Components (DAKO North America, Inc) at 4°C overnight. The primary antibodies used were as follows: rabbit anti-phospho-p44/42 MAPK (Erk1/2) (Cell Signaling, Danvers, MA), rabbit anti-Ki67 (Cell Signaling), rabbit anti-MUC2 (Santa Cruz Biotechnology, Dallas, TX), mouse anti-MUC4 (Santa Cruz Biotechnology), mouse anti-H/K-ATPase (Fitzgerald, Acton, MA), goat anti-intrinsic factor (gift from David Alpers, Washington University, St. Louis), and mouse anti-TFF2 (a gift from Dr. Nicholas Wright, Barts Cancer Center, London, UK). For immunohistochemistry, primary antibodies were detected with the Dako Envision+ System-

Horseradish-Peroxidase 3,3'-diaminobenzidine tetra hydrochloride (DAB). Sections were counterstained with haematoxylin. For immunofluorescence, sections were incubated with secondary antibodies conjugated with Cy2, Cy3, and Cy5 (Jackson ImmunoResearch Laboratories, West Grove, PA), Alexa-488 or 647-conjugated *Griffonia Simplicifolia* lectin II (GSII lectin) (Molecular Probes, Eugene, OR), and UEA1, FITC conjugated (Sigma, St. Louis, MO) at room temperature for 1 hour. After that, 4',6-diamidino-2-phenylindole (DAPI) was used for nuclear DNA staining. Then, sections were mounted using ProLong Gold Antifade Reagent (Invitrogen, Carlsbad, CA).

Quantitation

All the sections were scanned by with an Aperio Versa or Leica SCN400 digital imager (Vanderbilt Digital Histology Shared Resource). The immunofluorescence images were captured using an Axio Imager 2 microscope (Carl Zeiss AG, Oberkochen, Germany). The phosphor-ERK1/2-positive cell counting was performed as described before[3]. Briefly, all stained sections were scanned using a Leica SCN400 Slide Scanner (Leica, Buffalo Grove, IL). Whole slides were imaged at 20× magnification to a resolution of 0.5 µm/pixel. The numbers of phosphor-ERK1/2-positive cells (brown) and negative nuclei (blue) were determined by analysis of the high-resolution images in the Versa software only in the corpus mucosa. For immunofluorescence, all the stained tissue slides were imaged on an Aperio Versa 200 automated slide scanner (Leica). Tissue cores were imaged at 20× magnification to a resolution of 0.323 µm/pixel. The number of parietal cells (H/K-ATPase positive) and all the cells (recognized by DAPI) in the corpus mucosa were determined by analysis of the high resolution images using CellProfiler software and the percentage of H/K-ATPase positive cells were calculated. For MUC4 and UEA1-positive glands, 30-60 glands were counted. All the fluorescence images were

analyzed using CellProfiler.

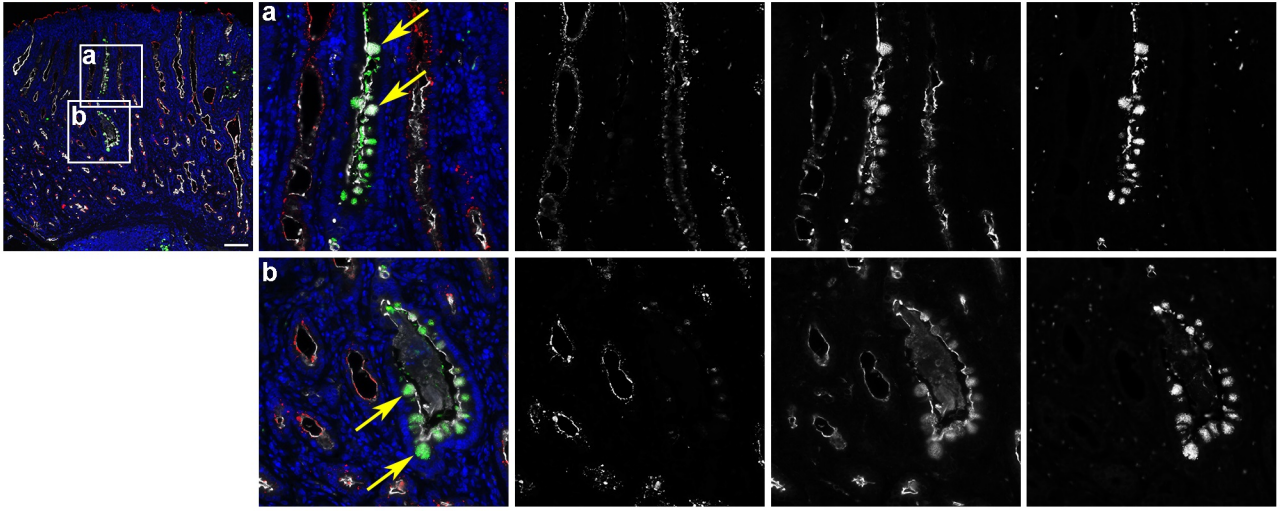
For all comparisons, ANOVA was utilized with post hoc analysis using Fisher's LSD test for comparison of significant groups.

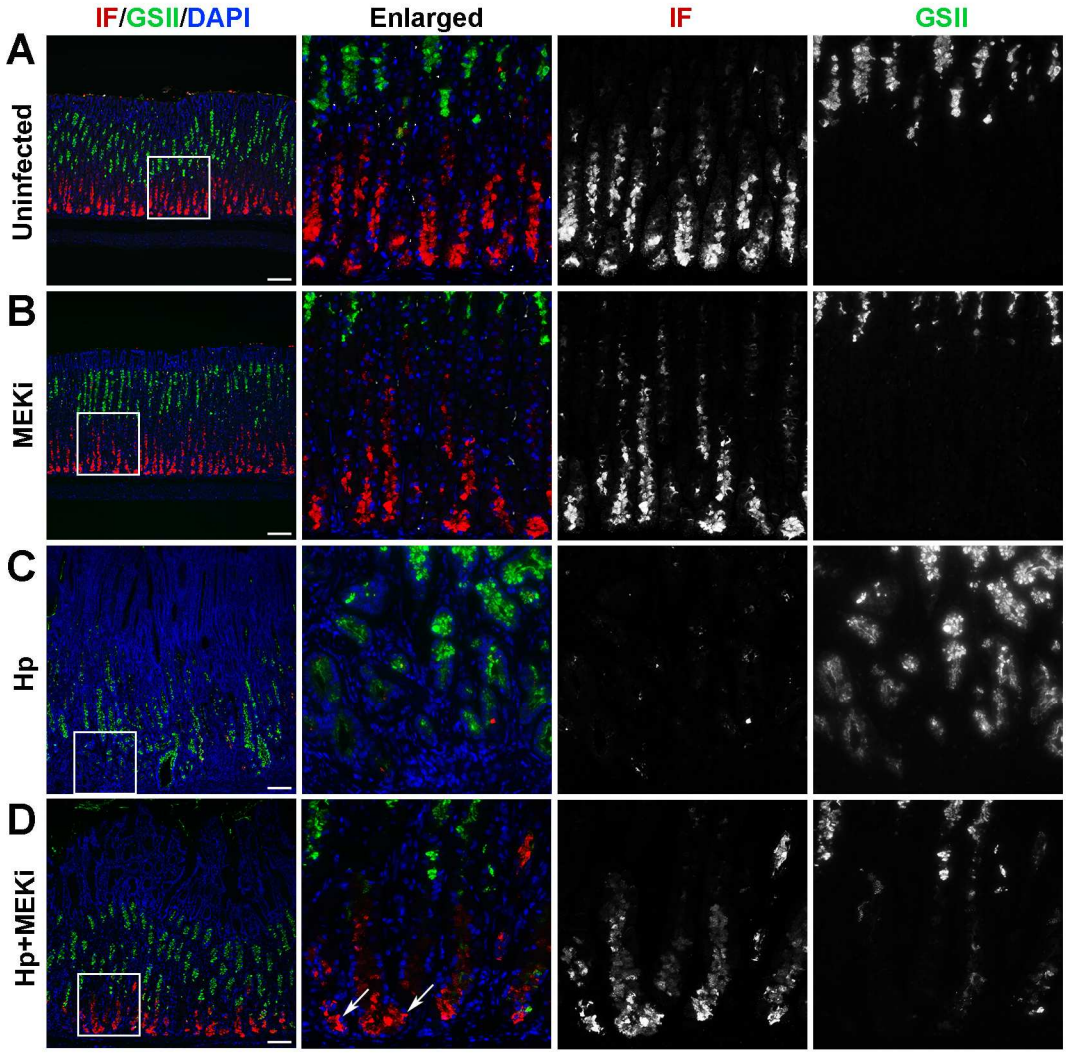
Supplementary Figure Legends

Supplementary Figure 1. Expression pattern of UEA1 in *H pylori*-infected gerbil gastric corpus mucosa. Immunofluorescence staining of gerbil corpus mucosa with various SPEM and IM markers. Red: UEA1, white: MUC4, green: MUC2 and blue: DAPI. IM (UEA1-/ MUC4+/ MUC2+) is indicated by yellow arrows. Scale bars: 100 μm.

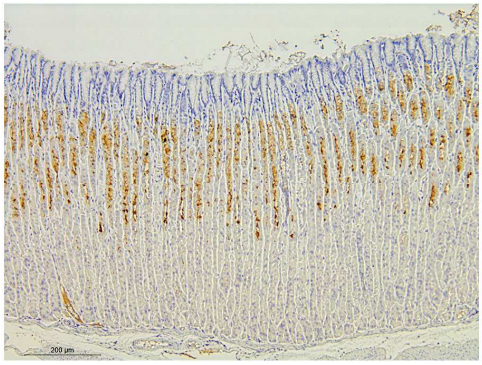
Supplementary Figure 2. Re-establishment of normal gastric chief cells after Selumetinib treatment. Immunofluorescence staining of gastric corpus mucosa of gerbils from Uninfected (*A*), MEKi (*B*), Hp (*C*), and Hp+MEKi (*D*) groups. Mature chief cells (IF+/GSII-, white arrow) were observed in the Selumetinib-treated gerbils. Scale bars: 100 μm.

Supplemental Figure 3. TFF2 staining in uninfected and *H pylori*-infected gerbils without or with Selumetinib treatment. Sections of stomach from uninfected gerbils or gerbils infected with *H pylori* and treated with either vehicle pellets or Selumetinib pellets were immunostained for TFF2. Higher magnification insets are shown in the lower panels. Note that the Selumetinib treatment led to reappearance of small triangular TFF2-staining cells similar in morphology to normal mucous neck cells (see inset in lower right panel).

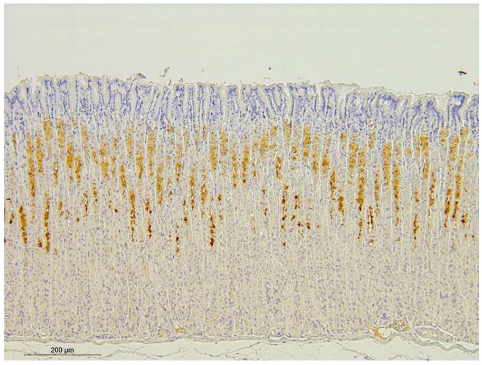

UEA1/MUC4/MUC2/DAPI
Enlarge
UEA1
MUC4
MUC2
a
b
a
b


IF/GSII/DAPI
Enlarged
IF
GSII
A
Uninfected
B
MEKi
C
Hp
D
Hp+MEKi

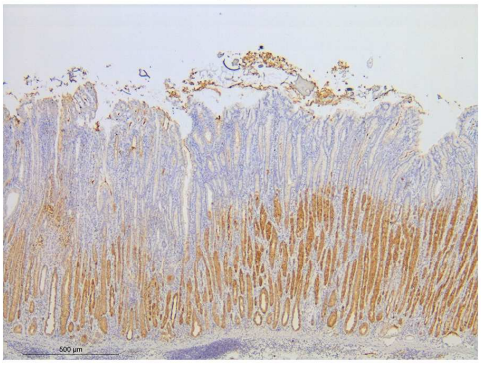
Uninfected



Uninfected + Selumetinib



H. pylori



H. pylori + Selumetinib