Materials and Methods

Immunohistochemistry and Immunofluorescence

Immunohistochemistry and immunofluorescence analysis was performed on 5 μm paraffin-embedded sections. Antigen retrieval of sections was performed in citrate buffer (pH 6.0) using a pressure cooker (Biocare Medical, DC2008). Then endogenous peroxidase activity was quenched by treatment with 3% H_2O_2 for 15 min. Sections were then blocked with normal serum for 30 min. The primary antibodies in this study were as follows: Smad4 (1:200, Abcam, no. 40759); PTEN (1:100, CST, no. 9188); p-Akt (1:100, CST, no. 4060); RFP (1:1000, Rockland, no. 600-401-379); GFP (1:100, CST, no. 2956; Abcam, ab6662, 1:200); bromodeoxyuridine (BrdU, 1:300, Abcam, no. ab6326); E-cadherin (1:300, BD Biosciences, no. 610181); Ki67 (1:500, Abcam, no. 15580); cyclin D1 (1:100, CST, no. 2978); p-Smad2 (1:100, CST, no. 3108); p-EGFR (1:200, Abcam, no. 40815); p-ERK (1:100, CST, no. 4370); p-Stat3 (1:100, CST, no. 9145); Villin (1:200, Santa Cruz, no. sc-7672); Cdx1 (1:200, Sigma Aldrich, no. HPA055196); Pepsinogen (1:200, Abcam, no. 9013); TRITC-labelled UEA (1:100, Sigma Aldrich, no. L4889); and FITC-labelled DBA (1:100, Sigma Aldrich, no. L9142). The primary antibodies were incubated at 4°C overnight. For immunohistochemistry, HRP-conjugated secondary antibodies (Zhongshan Biotech, DS-0001, PV-6001, PV-9001 or PV-9003) were then applied at 37°C for 1 hour, and immunoreactive cells were visualized with DAB (Immunohistochemistry, HRP, Zhongshan Biotech, ZLI-9019). For double immunofluorescence, fluorochrome-conjugated secondary antibodies were applied at 37°C for 1 hour, stained with DAPI and observed using confocal microscopy (Zeiss, LSM 510). For triple immunofluorescence, HRP-conjugated secondary antibodies (Zhongshan Biotech, DS-0001, PV-6001, PV-9001 or PV-9003) were applied at 37°C for 1 hour, then immunoreactive cells were visualized with Fluorescein Amplification Reagent (Perkinelmer, NEL741001KT, NEL744001KT, NEL745001KT). After the first fluorescence precipitated, the primary and secondary antibodies were inactivated in citrate buffer (pH 6.0) using a pressure cooker. Then the second and third fluorescence was performed as above

separately. At last, the nucleus was staining with DAPI and observed using confocal microscopy (Zeiss, LSM 510).

In situ hybridization

Digoxigenin-11-UTP (Roche Applied Science, 11277073910) was used to label RNA probes for *Villin*, *Cdx2*, *Pepsinogen*, *Gastrin*, and *ChrgA* using the MAXIscript *in vitro* transcription kit (Ambion, T3/AM1316, T7/AM1312). In situ hybridization on 6 μm paraffin sections was performed with standard procedures. Sections were then incubated in 1:500 AP-conjugated, polyclonal sheep anti-digoxigenin antibody (Roche Applied Science, 11093274910) or HRP-conjugated, polyclonal sheep anti-digoxigenin antibody (Merck Millipore, 90420), Pat 37°C for 4 hours. Immunoreactive cells were visualized with HighDef red IHC (AP) (Enzo Life Sciences, ADI-950-140-0030) or TSA Plus Fluorescence Kits (PerkinElmer, NEL741001KT). The primers used for probes synthesis are as follows:

Villin-F	5' GGCTGGAGAGGTGGAAATGTCCTG 3'
Villin-R	5' GGTGGGTACTGCTTGGCTTTGATG 3'
Cdx2-F	5' CCTGCTGACTGCTTTCTGAG 3'
Cdx2-R	5' TGACTCGAACAGCAGCAAAC 3'
Pepsinogen-F	5' CATCATGAAGTGGATGGTGGTC 3'
Pepsinogen-R	5' GTAGTAGGTGGAGGACTTGCTGG 3'
Gastrin-F	5' CCAACTATTCCCCAGCTCTGTG 3'
Gastrin-R	5' TGTTTTGTAAGGACGGAGCTGG 3'
ChrgA-F	5' GCCAGACTACAGACCCACTCC 3'
ChrgA-F	5' CTCAAAGCTGCTGTGTTGCTG 3'

Immunoblotting

Protein lysates were separated by SDS-gel electrophoresis and transferred to PVDF membranes. Immunoblotting was performed using the following antibodies: Smad4 (1:1000, Abcam, no. 40759); PTEN (1:1,000; CST; no. 9188); p-Akt (1:1,000; CST; no. 4060); Akt

(1:1,000; CST; no. 4685); GAPDH (1:1,000; ZSGB-Bio; TA-08); SPP1 (1:1000, Abcam, no. 91655); p-ERK (1:1,000, CST, no. 4370); ERK (1:1,000, CST, no. 9102); p-p38 (1:1,000, CST, no. 9211); p38 (1:1,000, CST, no. 8690); p-JNK (1:1,000, CST, no. 4668); JNK (1:1,000, CST, no. 9258); p-Stat3 (1:1,000; CST; no. 9145); and Stat3 (1:1,000; Santa Cruz; no. 7179). Anti-rabbit or anti-mouse horseradish peroxidase conjugated secondary antibody (1:4000; Zhongshan Biotech, ZB-2301 and ZB-2305) and ECL reagents (Engreen, 29050) were used to detect the signal.

In vivo quantification

The values in Figure 5A and 5B represent the average number of GFP-positive or Ki67-positive cells per RFP-positive gland. These assays were counted from at least three sections per mouse and repeated in 4 independent mice. The percentage of $LGR5^{+}$ stem cells in normal gastric antrum or adenocarcinoma (Figure 6B) was assessed by double immunostaining of GFP and E-cadherin. Epithelial cells was counted from at least two sections of *Control* gastric antrum or adenocarcinoma in 4 independent mice per genotype. For analysis of cell proliferation in Figure S5, double immunostaining of Ki67 and E-cadherin were used in 4 independent mice per genotype.