ONLINE SUPPLEMENTARY MATERIAL

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Supplementary Methods

Organoid generation, culture, differentiation and drug treatment

Details of gastric epithelial cell isolation and organoid culture were described previously[1]. Gastric epithelial single cells were mixed with ice-cold Matrigel (Corning) and plated on 24-well plates (Thermo Fisher Scientific), and mouse gastric culture medium (Advanced Dulbecco's Modified Eagle Medium/F12 supplemented with 10 mM of HEPES (Thermo Fisher Scientific), GlutaMAX (Thermo Fisher Scientific), penicillin/streptomycin (Thermo Fisher Scientific), N2 (Thermo Fisher Scientific), B27 (Thermo Fisher Scientific), 10 mM Y27632, 1 mM of N-acetylcysteine (Merck) and 1% BSA) was overlaid. The cells were also cultured with growth factors such as 100 ng/ml of Noggin (Miltenyi Biotec), 50 ng/ml of EGF (Merck), 100 ng/ml of FGF10 (Peprotech), 10 nM of gastrin I (Merck) and R-spondin 1-conditioned medium and Wnt3a-conditioned medium (WENFRG). The medium supplemented with growth factors was replaced every 3-4 days. All organoids were maintained in the condition of 5% CO_2 and 37 ^{o}C in a humidified incubator. To induce tissue stem cell differentiation in organoid, Wnt3a and R-spondin 1 were removed from day 5 organoids (ENFG). The organoids in ENFG culture condition were maintained for 2 days and analysed by qPCR. Ras-Erk pathway inhibitors, U0126 (Selleckchem) and MK-8353 (Selleckchem), were added at day 0 of organoid culture. Organoids were cultured until day 4 (96 h).

The SMARTvector Inducible shRNA with tRFP (Horizon Discovery) was used to suppress *Iqgap3* expression in mouse organoid. Three different targets of shRNA were prepared. The target of shRNA1 is ATGTTGGCTTTGTCATCAA. The target of shRNA2 is TCAGAGTTCTTGTCTTGCC. The target of shRNA3 is AGATTGGTCTGCTCGTCAA. Details of organoid transfection was described previously [2]. The single cells from organoids were mixed with BTXpress electroporation buffer (BTX) and shRNA plasmids. Electroporation was performed with NEPA21 Super Electroporator (NEPA gene). The cells were cultured in the

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WENFRG-culture condition to generate organoids. Next, 4 μg/ml of puromycin (Thermo Fisher Scientific) was added to the culture medium. After 4 days of puromycin selection, organoids were cultured in WENFRG -culture condition with 2 μg/ml of puromycin (Thermo Fisher Scientific) and 4 μg/ml of doxycycline (Merck) to induce shRNA. Passage of organoids was performed every 7-10 days. Organoids were visualized by Eclipse TS100 (Nikon) and counted at days 5-8.

Cell culture and shRNA/siRNA knock-down

NTERA-2, human malignant pluripotent embryonal carcinoma cell line, obtained from ATCC (NTERA-2 cl.D1) was cultured in DMEM (Dulbecco's Modified Medium, Nacalai Tesque) with 10% FBS (fetal bovine serum) at 37 °C in a humidified 5% CO2 incubator according to the manufacturer's instructions. Cells were maintained at high density and the medium was changed every 2-3 days. HGC-27, human gastric cancer cell line, obtained from CellBank Australia, the European Collection of Cell Cultures (Cat#94042256), was cultured in RPMI 1640 (Nacalai Tesque) with 10% FBS 37 °C in a humidified 5% CO2 incubator according to the manufacturer's instructions. Cells were passaged when the confluency reached 80-90%. ON-TARGET plus SMARTpool siRNAs targeting human IQGAP3 (Dharmacon, Cat# L-009077-00-0005), and a non-targeting siRNA pool (Dharmacon, Cat#D001810-10-50) as the control siRNA were used for the knockdown experiments. At 24 h after seeding in a 6-well culture plate, cells were transfected with 35 nM of siRNAs using jetPRIME (Polypus Transfection) according to the manufacturer's protocol. At 48 h after transfection, cells were reseeded into a 6-well plate and a 6 cm dish which were then transfected in a similar manner at 6-8 h after seeding, and harvesting the cells at 72 h later for RNA and protein quantification.

HEK293T cells and NIH3T3 cells were cultured with DMEM (Nacalai Tesque) supplemented with 10% FBS and 1% Penicillin/streptomycin. Cells were maintained at 5% CO_2 and 37 ^{o}C in a humidified incubator. For attenuation of Iqgap3 mRNA expression in NIH3T3 cells, the SMARTvector Inducible shRNA with tRFP (Horizon Discovery) was used. The shRNA plasmids were transfected into NIH3T3 cells using jetPRIME (Polypus Transfection). Proteins were extracted from knockdown cells to perform immunoblot.

Human clinical samples

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Primary healthy or cancer stomach tissues and clinical information were collected with patient consent in the GASCADII (Gastric Cancer Biomarker Discovery II) study, which was conducted at National University Hospital (Singapore) and approved by the National Healthcare Group Domain Specific Review Board (ref. no. 2005/00440). Gastritis tissues were collected with patient consent from the National University Hospital tissue repositories or pathology archives and with the approval of the respective Institutional Research Ethics Review Committees in accordance with local regulations and legislations. Clinical information was collected with the approval of the Institutional Review Board.

Immunofluorescence staining

To prepare paraffin tissue sections, mice or human stomachs were washed with PBS containing 10% FBS and fixed with PBS containing 4% paraformaldehyde (PFA). Fixed tissues were embedded in paraffin after incubation with ethanol and butanol. For preparing frozen tissue sections, mice or human stomach were washed with PBS containing 10% FBS and fixed with PBS containing 1% or 4% PFA. Fixed tissues were embedded in OCT compound (Leica Biosystems) after incubation with sucrose in PBS. Organoids were embedded in HistoGel (Thermo Fisher Scientific) and fixed with PBS containing 4% PFA. Fixed organoids were embedded in paraffin after incubation with ethanol and butanol. 5-μm paraffin embedded tissue sections or 5-μm OCT frozen tissue sections were processed using a standard histological protocol.

To stain paraffin or frozen tissue slides, blocking was performed with Dako protein Block (Agilent, Santa Clara, CA), 3% BSA (Merck) in PBST, 2% Goat serum (Merck) in PBST or 5% skim milk in PBS. Primary antibodies were diluted in the blocking reagent and incubated with the tissue slides overnight at 4 ºC. Secondary antibodies were diluted in 5% skim milk in PBST and incubated with the slides for 1 h at room temperature.

The antibodies used to stain mouse Iqgap-family proteins, namely anti-IQGAP3 (1:200), anti-IQGAP1 (1:200), and anti-IQGAP2 antibodies (1:200), were as described previously [3]. The other primary antibodies for immunofluorescence staining are as follows: anti-IQGAP3 (PA5-56363, Thermo Fisher Scientific, 1:100), anti-IQGAP1 (sc-376021, Santa Cruz Biotechnology, 1:200), anti-IQGAP2 (sc-17835, Santa Cruz Biotechnology, 1:200), anti-GFP (598, MBL, 1:500), anti-RFP (PM005, MBL, 1:500), anti-RFP (MBS448122, MyBioSource.com, 1:200), anti-Ki67 (14-5698-82, Thermo Fisher Scientific, 1:2000), anti-Ki67 (ab16667, Abcam, 1:500), anti-Ki67

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(M7240, Agilent, 1:100), anti-E-cadherin Alexa488/555/647-conjugated (560061/64/62, BD Bioscience, 1:200), anti-Nras (10724-1-AP, Proteintech, 1:200), anti-Hras (sc-35, Santa Cruz Biotechnology, 1:100), anti-Kras (12063-1-AP, Proteintech, 1:200), anti-phosphorylated ERK (4370, Cell Signaling Technologies, 1:100), anti-HER2 (2165, Cell Signaling Technologies, 1:200), anti-CD44v10 (LKG-M002, Cosmo Bio, 1:500), anti-CD44v9 (LKG-M001, Cosmo Bio, 1:500), anti-PDX1 (ab47267, Abcam, 1:200), anti-Tff2 (13681-1-AP, Proteintech, 1:100), anti-CD45 (14-0451-82, Thermo Fisher Scientific, 1:500), anti-Muc5ac (sc-16903, Santa Cruz Biotechnology, 1:200), anti-H,K-ATPase (D031-3, MBL, 1:1000), anti-Pepsinogen C (ab31464, Abcam, 1:200), anti-GIF (provided by D. H. Alpers, 1:1000), Lectin GS-II Alexa647-conjugated (L-32451, Thermo Fisher Scientific, 1:1000). Following primary antibody incubation, slides were washed in PBST then incubated with Alexa Fluor 488-, 546/555- or 633/647-conjugated secondary antibody (Thermo Fisher Scientific, 1:200) for 1 h at room temperature. Tissue slides were counterstained with 4',6-diamidino-2-phenylindole (DAPI) and visualized by Eclipse Ti (Nikon) or LSM880 with Airyscan (Zeiss) confocal microscope.

Flow cytometry

Detail of gastric glands isolation was described previously [1]. Isolated mouse stomach was chopped into 2 mm^2 pieces and incubated with chelating buffer (5 mM EDTA and 0.52 mM DTT in PBS) for 2 h at 4 ^oC on a shaking platform, then gastric units were released by vigorous shaking in cold dissociation buffer (54.8 mM D-sorbitol and 44 mM Sucrose in PBS). Isolated mouse gastric units were incubated in TrypLE Express (Thermo Fisher Scientific) with Y-27632 (Merck) for 12 minutes at 37 ^oC with repetitive pipetting for digestion into single cells. The cells were passed through a 30 μm cell strainer (Miltenyi Biotech) and labeled with antibodies in HBSS with 2% FBS. Antibodies for the flow cytometry are as follows: anti-EpCAM APC/Cy7-conjugated (BioLegend) and anti-ErbB2 PE-conjugated (R&D Systems). Dead cells were excluded by DAPI staining, and labeled cells were sorted by using FACSAria II (BD Bioscience). Data were analyzed with FlowJo 10.6 (BD Bioscience).

Quantitative PCR (qPCR)

Total RNA from the cell lines was extracted using RNeasy Kit (Qiagen) with the removal of genomic DNA using the RNase-free DNase Set (Qiagen) according to the manufacturer's

instructions. Complementary DNA (cDNA) was prepared from 1 μg of total RNA extracted using iScript reverse transcription supermix for RT-qPCR (Bio-Rad). qPCR was performed using SYBR Green qPCR kit (Bio-Rad) and QuantStudio3 Real-Time PCR systems (Thermo Fisher Scientific). RNA in FACS-sorted Iqgap3^{high} and Iqgap3$^{low/neg}$ cells of *Iqgap3-2A-tdTomato* mice was extracted using NucleoSpin RNA XS (MACHEREY-NAGEL) by following the manufacturer's manual. RNA from Iqgap3^{high}/Iqgap3$^{low/neg}$ cells was used for cDNA synthesis with RT-RamDA cDNA Synthesis Kit (Toyobo). RNA in HDT-treated mice stomach was extracted using the RNeasy Mini Kit (QIAGEN) with on-column genomic DNA digestion using the RNase-free DNase Set (QIAGEN); cDNA synthesis was done using PrimeScript RT-PCR kit (Takara Bio). RNA in organoids and FACS-sorted corpus epithelial cells was extracted using NucleoSpin RNA XS (MACHEREY-NAGEL); cDNA synthesis was performed with High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). All were done according to the manufacturers' instructions. qPCR was performed with KAPA SYBR FAST (Merck) and QuantStudio3 Real-Time PCR systems (Thermo Fisher Scientific). Gene expression levels in each sample were normalized by glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) expression. All experiments were performed in triplicate, and primer sequences are listed in Table S1.

Single-molecule RNA in situ hybridization

Tissue sections (5 μm thickness) were processed for RNA in situ detection using RNAscope 2.5 HD Reagent Kit according to the manufacturer's instructions (Advanced Cell Diagnostics). All probes, including controls, were designed by the manufacturer (Advanced Cell Diagnostics). Probes for ISH are the following: *Iqgap1* (542081), *Iqgap2* (542101), *Iqgap3* (539811), *Mki67* (416771-C2), *Lgr5* (312171-C2), Duplex positive control (321651) and negative control (320751). Images were acquired by TissueFAXS (TissueGnostics).

Immunoprecipitation and immunoblot

The plasmids (pCAG-Venus-Iqgap3, pCAG-Myc-Hras and pCAG-Myc-Hras^{G12V}) were transfected into HEK293T cells by using TransIT reagent (Mirus Bio) (7). Cell lysates were extracted by using lysis buffer (20 mM Tris-HCl, 150 mM NaCl, 5 mM MgCl2, Halt phosphatase inhibitor (Thermo Fisher Scientific), 1% Triton-X-100, 2 mM Dithiothreitol, Complete protease inhibitor (Merck)). Cell lysates were incubated with GFP-Trap (ChromoTek). The cell lysates or

GFP-Trap-affinity-purified proteins were analyzed by immunoblot with 4–20% Mini-PROTEAN TGX Precast Protein Gels (Bio-Rad). After electrophoresis, the proteins were transferred to PVDF membrane (Merck) and detected by primary antibodies and HRP-conjugated secondary antibodies. Antibodies for immunoblots are as follows: anti-Myc (sc-40, Santa Cruz Biotechnology), anti-GFP (D153-3, MBL, 1:1000), anti-phosphorylated ERK (4370, Cell Signaling Technologies, 1:1000), anti-ERK (9102, Cell Signaling Technologies, 1:1000), anti-α-tubulin (T9026, Merck, 1:1000), anti-IQGAP3 (25930-1-AP, Proteintech, 1:1000), anti-Rabbit IgG HRP-conjugate (GE Healthcare Life Sciences) and anti-Mouse IgG HRP-conjugate (GE Healthcare Life Sciences). Blots were visualized by Immobilon Western Chemiluminescent HRP Substrate (Merck) and ImageQuant LAS500 (GE Healthcare Life Sciences).

The siRNA transfected NTERA-2 and HGC-27 cell pellets were first lysed by resuspending of the cell pellets with the lysis buffer (50 mM Tris pH 8, 150 mM NaCl, 0.1% SDS, 0.5% Sodium Deoxycholate and 1% NP-40) supplemented with Halt phosphatase inhibitor (Thermo Fisher Scientific), Complete protease inhibitor (Merck) and PMSF (Sigma-Aldrich), and incubating at 4 °C for 45 minutes. After centrifugation of the lysates at 13500 rpm for 15 minutes at 4 °C, the supernatant of each lysate was collected and the protein concentrations were measured using the Pierce BCA Protein Assay kit (Thermo Fisher Scientific) according to the manufacturer's protocol. 40 μg of protein was loaded into each well of 8% SDS/PAGE. The antibodies used were as follow: anti-IQGAP3 (25930-1-AP, Proteintech, 1:1000), anti-NANOG (4903, Cell Signalling Technology, 1:1000), anti-OCT4 (ab19857, Abcam, 1:1000), anti-KLF4 (ab106629, Abcam, 1:1000), anti-CD44v9 (LKG-M001, Cosmo Bio, 1:1000), anti-GFAP (16825-1-AP, Proteintech, 1:1000), anti-PGC (ab93886, Abcam, 1:1000) and anti-GAPDH (2118, Cell Signalling Technology, 1:1000). Blots were visualized by Immobilon Western Chemiluminescent HRP Substrate (Merck) and ImageQuant LAS500 (GE Healthcare Life Sciences).

RNA-sequencing and data analysis

RNA extracted from FACS-sorted Iqgap3^{high} and Iqgap3$^{low/neg}$ cells of *Iqgap3-2A-tdTomato* mice were sent to Beijing Genomics Institute for PCR amplification, transcriptome library preparation and sequencing. Raw reads were aligned to mouse reference genome version GRCm38 gencode M23 (downloaded from https://www.gencodegenes.org/) using STAR aligner version 2.7.1a with default parameters [4]. Read counts per gene were generated using featureCounts function in the

subread package version 2.0.0. [5]. Counts per million (cpm) values were calculated using cpm function of the edgeR R package [6]. Normalized gene expression data were subjected to Gene Set Enrichment Analysis (GSEA) using the broad institute GSEA tool (http://software.broadinstitute.org/gsea/index.jsp) with the Molecular Signature Database v6.0, FAC-sorted Lgr5^{high} or Lgr5^{neg} corpus epithelial cells public datasets (GSE86603) and HSC gene signature [7] to identify enriched gene sets/pathways in Iqgap3^{high} and Iqgap3$^{low/neg}$ cells.

RNA extracted from the HDT-treated stomach was sent to BGI for transcriptome library preparation and sequencing. Sequenced reads were aligned with the STAR software to mm10, and mapped counts were employed to generate the raw expression counts using the FeatureCounts with GENCODE transcriptome annotation. The raw expression counts were then further normalized using the Cross-Correlation method [8]. Normalized gene expression data were subjected to GSEA using the broad institute GSEA tool with the Molecular Signature Database v6.0 to identify enriched gene sets/pathways in HDT versus untreated.

The RNA-seq datasets (Figures 3 and S3) revealed that Iqgap3 mRNA was unexpectedly low in the tdTomato^{high} fraction – only its 3'UTR was detected. Because of the *Iqgap3-2A-CreERT2;Rosa-tdTomato* cassette design, the presence of tdTomato protein and the enrichment of Ki67 and Stmn1, Iqgap3 mRNA expression in the tdTomato^{high} fraction can be inferred. Of note, Iqgap3 mRNA in Iqgap3^{high} fraction was detected by qPCR analysis (figure 1G). As to why the Iqgap3 mRNA was poorly detected by the RNA sequencing technology, we posit that mRNA transcribed from an artificially manipulated genome might be more susceptible to nonsense-mediated mRNA decay [9] during stem cell isolation and lysis. Moreover, different RNA amplification methods, such as SMART-seqII and RT-RamDA were used for RNA-seq and qPCR respectively. This might also have affected Iqgap3 mRNA detection.

Transcriptomic analysis for human gastric cancer and normal tissue samples

Microarray gene expression data for 185 primary gastric cancer (GC) and 89 normal gastric samples were generated as described previously [10]. RNA-seq for 27 primary GC and 18 matched normal gastric samples were performed and analyzed as described previously [11]. For each cohort, *IQGAP3* expression was compared between primary GC and matched normal samples using the two-sided Wilcoxon's rank-sum test ($p < 0.05$).

Tissue-microarray for human gastric cancer

Tissue microarrays (TMAs) were constructed from the archival formalin fixed paraffin embedded (FFPE) gastric cancer tissue specimens (between the year 2000-2013) obtained from the Department of Pathology, National University Hospital, Singapore. TMAs with 2 mm cores were constructed from the tumor and the adjacent normal area. Consecutive 4 μm sections were obtained from each TMA and mounted on coated glass slides for immunohistochemical staining. Ethics approval was obtained from the Domain Specific Review Board of the hospital (2015/0209).

Database analysis

IQGAP3, *MKI67* and *PCNA* expression dataset in human gastric cancer were obtained by using cBioportal for cancer genomics (https://www.cbioportal.org/) [12]. RNA-sequencing dataset from The Cancer Genome Atlas (TCGA)[13] was used. Annotated data was visualized by GraphPad Prism7 (GraphPad software).

Quantification and statistical analysis

All statistical analyses including cell number counts, organoid sizes, qPCR and gene expression in cancer patients were shown with standard deviation (SD) or standard error of mean (SEM). Two-tailed student's t-test, One-way ANOVA or two-sided Wilcoxon's rank sum test were used for statistical analysis. P-values less than 0.05 were considered statistically significant.

Data and code availability

All data relevant to the study are included in the article or uploaded as online supplemental information. RNA-sequencing datasets generated in this study are deposited in Gene Expression Omnibus (GEO) database under accession codes GSE161443 for *Iqgap3-2A-tdTomato* reporter mice and GSE161442 for HDT treatment respectively. All supporting data are available from the corresponding authors upon reasonable request.

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Supplementary Figure Legends

Figure S1. Expression of Iqgap1, Iqgap2 and Iqgap3 in the corpus epithelium.

(A) Knock-in strategy for generation of *Iqgap3-2A-tdTomato* mouse model.

(B) Quantification of Ki67+ cells in the tdTomato+ (Iqgap3+) population of *Iqgap3-2A-tdTomato* mice (n=3). Error bar represents standard deviation (SD) from 796 cells of 3 mice. Data were analyzed by Student's t-test.

(C) IF staining for Iqgap3 and tdTomato on the corpus of *eR1-CreERT2;Rosa-tdTomato* mice at 16 h post tamoxifen induction (p.i.) (n=3). eR1-LT, eR1-derived cell lineage tracing. Arrow, eR1+ cell.

(D-E) IF staining for Iqgap1, Iqgap2, Ki67 and E-cad on the corpus of wild type (WT) mice (n=3).

(F-G) ISH for *Iqgap1*, *Iqgap2* and *Mki67* on corpus gastric units of WT mice (n=2).

Scale bar = 100 μm.

Figure S2. Expression of Iqgap3 marks stem cells in corpus of stomach.

(A) Knock-in strategy for generation of *Iqgap3-2A-CreERT2* mouse model.

(B) IF staining for tdTomato on corpus of *Iqgap3-2A-CreERT2;Rosa-tdTomato* mice at 1 day (1 d) post tamoxifen treatment (TMX 2 mg) and untreated control (TMX 0 mg) (n=3). Dashed box, Co-IF staining for tdTomato and Ki67 is shown in Fig.2. LT , Lineage tracing.

(C) IF staining for tdTomato, GS-II and Gif on the corpus of *Iqgap3-2A-CreERT2;Rosa-tdTomato* mice at 1 year post tamoxifen induction (n=3). Image were shown as X-Z plane of neck (#1: GS-II+ and Gif-), neck-base transition (#2: GS-II+ and Gif+) and base (#3: GS-II- and Gif+).

(D) Microscopic image for induction of differentiation in organoids from WT mouse. The organoids were generated with WENFRG (Wnt3a, Egf, Noggin, Fgf10, R-spondin1, Gastrin) culture condition. On day 5, the organoids were cultured with ENFG (Egf, Noggin, Fgf10, Gastrin) for 2 days to induce differentiation (n=3).

(E) qPCR analysis for *Iqgap3* and *Muc5ac* mRNA on organoids with WENFRG condition and organoids with ENFG condition (n=3). The mRNA expression was normalized by *Gapdh* expression. Error bars represent SD from each population. Data sets were analyzed by Student's t-test.

(F) IF staining for tdTomato and Muc5ac on WENFRG- and ENFG-cultured organoids from Iqgap3+ cells of *Iqgap3-2A-CreERT2;Rosa-tdTomato* mice (n=2).

(G-J) IF staining for tdTomato, PgC, Gif, GS-II, Ki67 and HK-ATPase in organoids from Iqgap3+ cells of *Iqgap3-2A-CreERT2;Rosa-tdTomato* mice (n=2). Arrow, PgC+/GS-II- or Gif+/GS-II- cell. Arrow head, PgC+/GS-II+ or Gif+/GS-II+ cell. Open arrow head, PgC-/GS-II+ cell. Scale bar, 100 μm.

Figure S3. Transcriptome analysis for Iqgap3-expressing cells in isthmus.

(A) The average gene expression level of *Iqgap3, Mki67, Stmn1, Kitl, PgC and Gpr30* from RNA sequencing of tdTomato^{high} (Iqgap3^{high}) and tdTomato$^{low/neg}$ (Iqgap3$^{low/neg}$) cell fractions from *Iqgap3-2A-tdTomato* mice. Iqgap3 mRNA reads comprised mainly 3'-UTR (See RNA-sequencing and data analysis in Supplementary Methods). CPM, counts per million.
(B) Heat map showing the top 30 genes from Iqgap3^{high} cell fractions upregulated in the Lgr5-negative (LGR5neg) gene signature.

(C) GSEA showing enrichment of the short term hematopoietic stem cell (ST-HSC) gene signature in Iqgap3^{high} and Iqgap3$^{low/neg}$ cell fractions.

(D) Heat map showing the top 30 genes from Iqgap3^{high} cell fractions upregulated in the ST-HSC gene signature.

(E-F) GSEA showing enrichment of the HSC self-renewal associated gene signature, GO asymmetric cell division gene signature, Hallmark Notch signaling gene signature, Hallmark Hedgehog gene signature and Hallmark Wnt-β-catenin signaling gene signature in Iqgap3^{high} and Iqgap3$^{low/neg}$ cell fractions.

Figure S4. Ras-Erk pathway regulators are expressed in the isthmus.

(A-B) IF staining for Kras, Nras, Ki67 and E-cad on the corpus of wild type mice (n=3).

(C) FACSort to isolate $Her2^{High}$ and $Her2^{Low}$ gastric epithelial cell fractions (n=3).

(D-E) qPCR analysis for *Her2 (Erbb2)*, *Iqgap3*, *Mki67*, *Lgr5*, *Mist1*, *Hras*, *Kras and Nras* mRNA in $Her2^{high}$ and $Her2^{low}$ fractions of gastric epithelial cells (n=3). Error bars represent SD from each population. Data sets were analyzed by Student's t-test.

Scale bar, 100 μm.

Figure S5. Iqgap3 regulates stem cell proliferation via Ras-Erk pathway.

(A) *Iqgap3* shRNA knock-down in NIH3T3 cells (n=2).

(B-D) Representative microscopic image of Iqgap3 shRNA knock-down (KD) organoids generated from WT mice (n=2). Organoids from 0 passage (no passage), 1^{st} passage and 2^{nd} passage are shown. RFP expression indicated shRNA plasmid transfected organoids.

(E) IF staining for p-Erk and E-cad on U0126 and MK-8353 treated organoids from corpus of WT mice (n=2).

(F-G) Microscopic images of U0126 and MK-8353 treated organoids from corpus of WT mice (n=2).

Figure S6. Iqgap3-expressing corpus cells proliferate in response to tissue damage.

(A) IF staining for E-cad and H,K-ATPase on untreated and 48 h post-HDT treated WT corpus.
(B) IF staining for Ki67 on untreated, 24 h, 48 h and 14 days post-HDT treated WT corpus.

(C) ISH for *Iqgap3*(green) and *Lgr5*(red) on 48 h post-HDT treated WT corpus (X-Z plane). Boxes indicate enlarged regions.

(D) IF staining for tdTomato and Ki67 on 48 h post-HDT treated corpus from *Iqgap3-2A-tdTomato* mice.

(E) IF staining for Ki67, CD45 and E-cad on the bottom part of corpus gastric units from untreated and 48 h post-HDT treated WT mice.

(F) Quantification of CD45+ cells and Ki67+/CD45+ cells per 20× fields (n=3). Data were analyzed by Student's t-test.

(G) IF staining for tdTomato, H,K-ATPase and Ki67 on 14 days post-HDT treated corpus from *Iqgap3-2A-CreERT2;Rosa-tdTomato* mice.

Scale bars = 100 μm.

Figure S7. HDT-induced tissue damage promotes stem cell activity.

(A) GSEA showing enrichment of Mitotic spindle, Myc targets V1, E2F targets and G2M checkpoint gene signatures in 48 h post-HDT corpus (n=2).

(B) Heat map showing top 20 genes upregulated in (A) based on RNA-seq data from untreated and 48 h post-HDT corpus tissue.

(C-D) GSEA showing enrichment of Inflammatory response and Wnt/β-catenin signaling gene signatures in 48 h post-HDT corpus (n=2).

(E) Heat map showing top 20 genes upregulated in (C-D) based on RNA-seq data from untreated and 48 h post-HDT corpus tissue.

P values determined by a weighted Kolmogorov-Smirnov-like statistic and adjusted for multiple hypothesis testing.

Figure S8. Robust expression of IQGAP3 in human cancer tissue.

(A) Difference of IQGAP3 Tissue Microarray (TMA) score in gastric cancer and paired-adjacent normal stomach tissue.

(B) Representative image of IQGAP3 expression in adjacent normal stomach tissue from the TMA

(C) qPCR for *IQGAP3, NANOG, OCT4, KLF4, cMYC, SOX2, CD44v9 and PGC* mRNA from IQGAP3 knockdown gastric cancer cell line HGC-27. The mRNA expression was normalized by *GAPDH* expression (n=3). Error bars represent SD. Data sets were analyzed by Student's t-test. * $P < 0.05$, ** $P < 0.01$. n.s., not significant.

(D) Immunoblot for IQGAP3, CD44v9, PGC and GAPDH from IQGAP3 knockdown HGC-27 cells (n=3).

(E) Correlation of expression between *IQGAP3* and proliferating markers, *MKI67* and *PCNA* in human gastric cancer. Gene expression dataset was obtained from the TCGA database.

Scale bar, 100 μm.

Figure S9. IQGAP3 is expressed in proliferative CD44v9+ gastric cancer cells.

(A-C) IF staining for IQGAP3, Ki67 and CD44v9 in the human gastric tumor (n=7). Scale bar, 100 μm.

Supplementary Table 1. Primer sequencing for qPCR

Gene	Forward sequence	Reverse sequence
	Primers for mouse mRNA	
Atp4b	CAGCCTGTACTACGCAGGTT	TCTCAAGGTTACCCCTGGTG
Bhlha15 (*Mist1*)	GCTGACCGCCACCATACTTAC	TGTGTAGAGTAGCGTTGCAGG
Cdh1	CCCAGAGACTGGTGCCATTT	TGGCAATGGGTGAACCATCA
Chga	TCTGCCGTCTGAAGGGAAG	TCCTGCTTATGTTCCAGCTCC
Erbb2	GAGACAGAGCTAAGGAAGCTGA	ACGGGGATTTTCACGTTCTCC
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Gif	CCTGGGGCCTTATTGTCTCTTC	TGAAGTTGGCTGTGATGTGC
Hras	CGTGAGATTCGGCAGCATAAA	GACAGCACACATTTGCAGCTC
Iqgap3	AGAGCCAACGAGGACACAAG	TCCTCAACACACGCTCTGTC
Kras	ACATTGGTGAGAGAGATCCGA	CACAGCCAGGAGTCTTTTCTTC
Lgr5	CAGCCTCAAAGTGCTTATGCT	GTGGCACGTAACTGATGTGG
Mki67	CCAAGACTCATCAGGGCATGT	TTCCCTGTAACTGCTCCCTG
Muc5ac	CGTACCATGAACACCGCTCT	TTGAAGGCTCGTACCACAGG
Muc6	TGCATGCTCAATGGTATGGT	TGTGGGCTCTGGAGAAGAGT
Nras	ACTGAGTACAAACTGGTGGTGG	TCGGTAAGAATCCTCTATGGTGG
PgC	GCTGCCAAGGCATTGTAGAC	CGCAGCTCACAAAATACTGTC
Stathmin1 (Stmn1)	TCTGTCCCCGATTTCCCCC	AGCTGCTTCAAGACTTCCGC
tdTomato	CCGGCTACTACTACGTGGAC	CCGTACAGGAACAGGTGGTG
Tnfrsf19 (Troy)	TTCTGTGGGGGACACGATG	AGAAAATTCAGCGCAGATGGAA
	Primers for human mRNA	
CD44v9	TCCCAGACGAAGACAGTCCCTGGAT	CACTGGGGTGGAATGTGTCTTGGTC
cMYC	CCTGGTGCTCCATGAGGAGAC	CAGACTCTGACCTTTTGCCAGG
GAPDH	GTCAGTGGTGGACCTGACCT	AGGGGTCTACATGGCAACTG
GFAP	ATCAACTCACCGCCAACA	CGACTCAATCTTCCTCTCCAG

IQGAP3	AGGGTGATCAGGAACAAGCC	ACAGGGTACACTGGAGGCAG
KLF4	ACCAGGCACTACCGTAAACACA	GGTCCGACCTGGAAAATGCT
NANOG	CTGGACACTGGCTGAATCC	GACTGGATGGGCATCATGG
OCT4	TGGGTGGAGGAAGCTGACAACAAT	TTCGGGCACTGCAGGAACAAATTC
PGC	AGAGCCAGGCCTGCACCAGT	GCCCCTGTGGCCTGCAGAAG
SOX2	CCTACTCGCAGCAGGGCACC	CTCGGCGCCGGGGAGATACA