Supplementary Materials and Methods

Ex vivo culture

Trop2^{+ve} cells from proximal and distal E14.5 stomachs (pool of 12, 18 and 19 embryos/litter per experiment, n=3 independent experiments) were sorted separately. At day 10 the proportion of type 1, 2 or 3 elements was quantified based on morphology and Trop2 and HGM expression on stained sections in each region with a mean of 110 elements/experiment (Fig. 1A).

For plating efficiency experiments, distal stomachs from E14.5 (pool of 10 and 16 embryos/litter per experiment, n=2 independent experiments), E15.5 (pool of 13 and 17 embryos, n=2), E17.5 (pool of 7 and 8 embryos, n=2) and P2 (pool of 12 mice, n=1) developmental stages were isolated by FACS and subsequently cultured in matrigel under ENR conditions (Fig. 1B).

Quantification of ex vivo adult antral glands survival was performed over 30-100 elements/mouse observed at day2 (Fig. 1D).

The percentage of Trop2^{+ve} elements present at day6, upon Lgr5-stem cells ablation, was calculated over a mean of 55 elements/sample (Fig.6D, bottom left). Quantification of the mean diameter size of elements grown at day12 was performed over a mean of 39 elements/sample (Fig. 6D, center and bottom).

Morphometric analysis on tissue sections

Using the AxioVision Rel 4.8 software, quantification of Trop2 and Cnx43 expression at different developmental stages was performed. The mean number of apical and basal cells analyzed per embryo was 100. For each time point, n=3 embryos (Fig. 3A).

Quantification of lineage tracing experiments was presented as the proportion of the clone area relative to the whole glandular epithelial area calculated by AxioVision Rel. 4.8 software (Fig. 3C). For clone size calculation, the area of a mean of 35 and 123 clones per animal was measured in pixels (Px^2) in glandular and squamous regions, respectively by AxioVision Rel. 4.8 software (Fig. S3C). In regeneration experiments, quantification of Trop2^{+ve} clusters/field was performed on a minimum of 20

fields/animal (Fig. 4C,G). The number of Trop2^{+ve} cells/cluster was calculated on a mean of 52 and 15 clusters/animal in the antral and corpus glands, respectively (Fig. 4D). Quantifications of the number of Ki67+ cells/gland and the gland depth were performed on a mean of 14 glands/animal (Fig. 4D; Fig. S4D). The proliferation index of Trop2^{+ve} cells was calculated on a mean of 200 and 730 Trop2^{+ve} cells at day2 and day5 of DT treatment, respectively; and a mean of 977 Trop2^{+ve} cells in indomethacin experiments (Fig. 4E right panel; Fig. 4H).

RNA seq and transcriptome analysis

The multiplexed libraries (9.5 pM) were loaded and sequences were produced using a TruSeq PE cluster and SBS-kit (200 cycles) on a HiSeq 1500 (Illumina). Approximately 25 million of paired-end reads/sample were mapped against the mouse reference genome (GRCm38.p4/mm10) using STAR software to generate read alignments for each sample. Annotations Mus_musculus.GRCm38.79.gtf were obtained from ftp.Ensembl.org. After transcripts assembling, gene level counts were obtained using HTSeq. EdgeR was then used to calculate the level of differential gene expression with biological replication (false discovery rate of 0.05, minimum counts of 50). For Venn diagram analysis genes 4 fold up-regulated were used for comparisons and GOTerm Finder was used to analyze the common genes obtained in terms of their associated biological processes. For PCA analyses, all genes above 1 count per million were considered (19468) and plots were made with ClustVis online software.

Antibodies

Goat anti-Trop2 1/50 (AF1122, R&D Systems), rabbit anti-Cnx43 1/100 (3512, Cell Signaling), mouse anti-Cdx2 1/100 (AM392, Biogenex), chicken anti-Krt14 1/1000 (SIG-3476, Covance), rabbit anti-Chromogranin A 1/200 (20085, Immunostar), rabbit anti-Sox2 1/2000 (Ab92494, Abcam), rabbit anti-KI67 1/200 (Ab15580, Abcam), chicken anti-YFP/GFP 1/2000 (Ab13970, Abcam), mouse anti-HGM 1/100 (NCL-HGM-45M1, Novocastra), mouse anti-E Cadherin 1/200 (610181, BD), rabbit anti-Epcam 1/100 (Ab71916, Abcam) and mouse anti-proton pump/K-ATPase α 1/3 (D031-3H, MBL).

Fluorochrome-conjugated anti-Trop2-APC antibody (FAB1122A, R&D systems), or the relevant isotype control-APC (IC108A, R&D systems) and anti-Epcam /CD326-PE (563477, BD Biosciences) were used for cell sorting according to manufacturer's instructions.

qPCR primer sequences

rlp13 Fw: 5'-CCCGTGGCGATTGTGAA, rlp13 Rev: 5'-TCATTGTCCTTCTGTGCAGGTT;

gapdh Fw: 5'-TTAGCCCCCCTGGCCAAGG, gapdh Rev: 5'-CTTACTCCTTGGAGGCCATG;

atp4a Fw: 5'-TGGCTCGGATGCTGCTAAAA, atp4a Rev: 5'-TCAAAGATCAGTCGGCCCTG;

cdx1 Fw: 5'-GCGGTGGCAGCGGTAAGACC, cdx1 Rev: 5'-AGCTCGGACTTGCGCCGGAT

cdx2 Fw: 5'-CTGCTCTGGGTCCCTCGCCA, cdx2 Rev: 5'- CTGCGGAGCCAGGTTCAGGC;

chga Fw: 5'-TCCCCACTGCAGCATCCAGTTC, chga Rev: 5'-CCTTCAGACGGCAGAGCTTCGG.

cnx43 Fw: 5'-TGGGGGAAAGGCGTGAGGGA, cnx43 Rev: 5'- ACCCATGTCTGGGCACCTCTCTT.

krt14 Fw: 5'-TGAAGAGCAAGATCCTGGCAGC, krt14 Rev: 5'-GCTCTGCTCCGTCTCAAACTT

lgr5 Fw: 5'-CTACTCGAAGACTTACCCAGT, lgr5 Rev: 5'-GCATTGGGGTGAATGATAG

lzp Fw: 5'-GAGACCGAAGCACCGACTATG, lzp Rev: 5'-CGGTTTTGACATTGTGTTCGC

muc2 Fw: 5'-ATGCCCACCTCCTCAAAGAC, muc2 Rev: 5'-GTAGTTTCCGTTGGAACAGTGAA

muc5ac Fw: 5'-CTCACCCTCAAGGGCATGTT, muc5ac Rev: 5'-GGTTGAAGGCTCGTACCACA

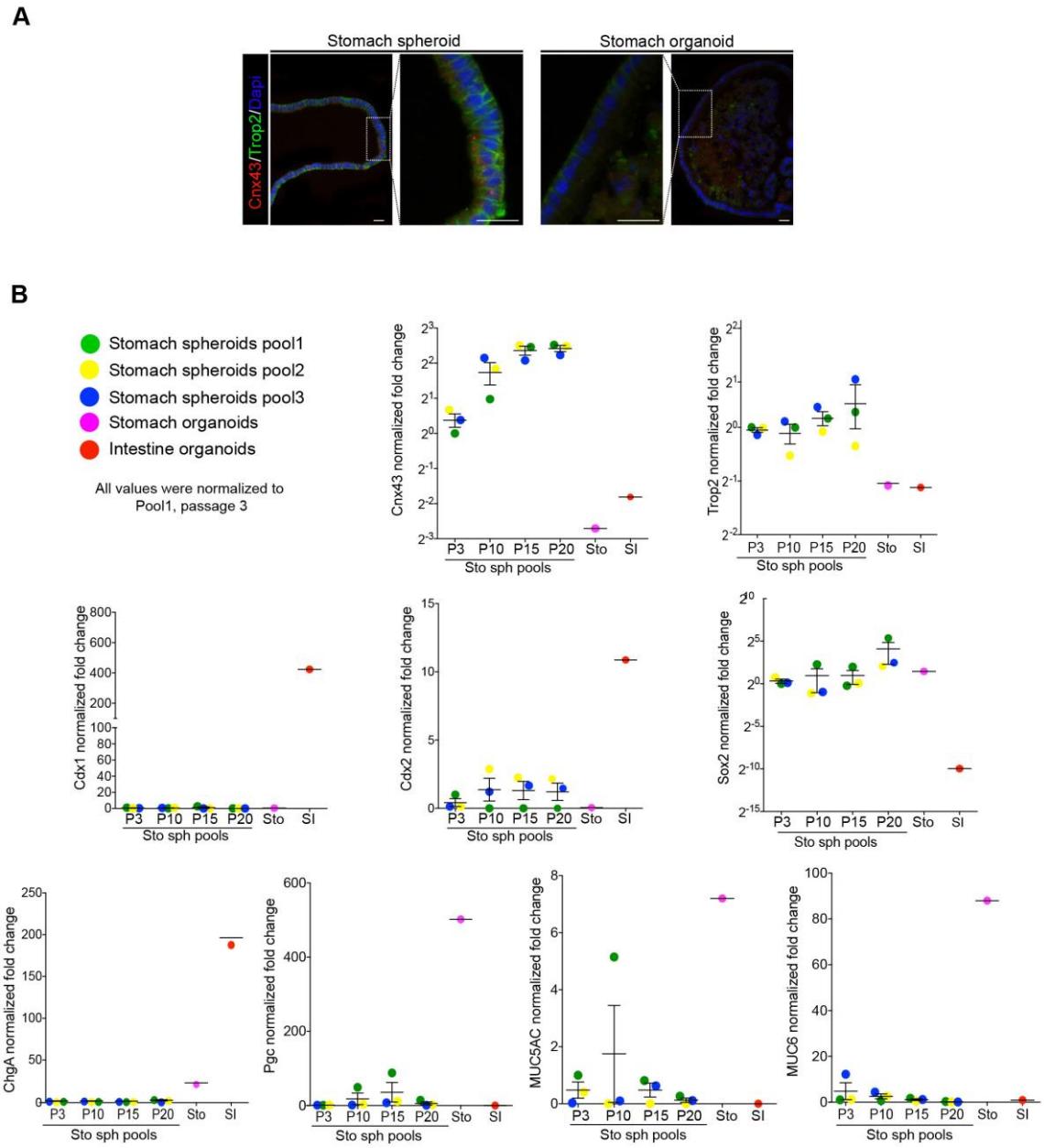
muc6 Fw: 5'-TGCATGCTCAATGGTATGGT, muc6 Rev: 5'-TGTGGGCTCTGGAGAAGAGT

pgc Fw: 5'-CCAACCTGTGGGTGTCTTCT, pgc Rev: 5'-TTAGGGACCTGGATGCTTTG

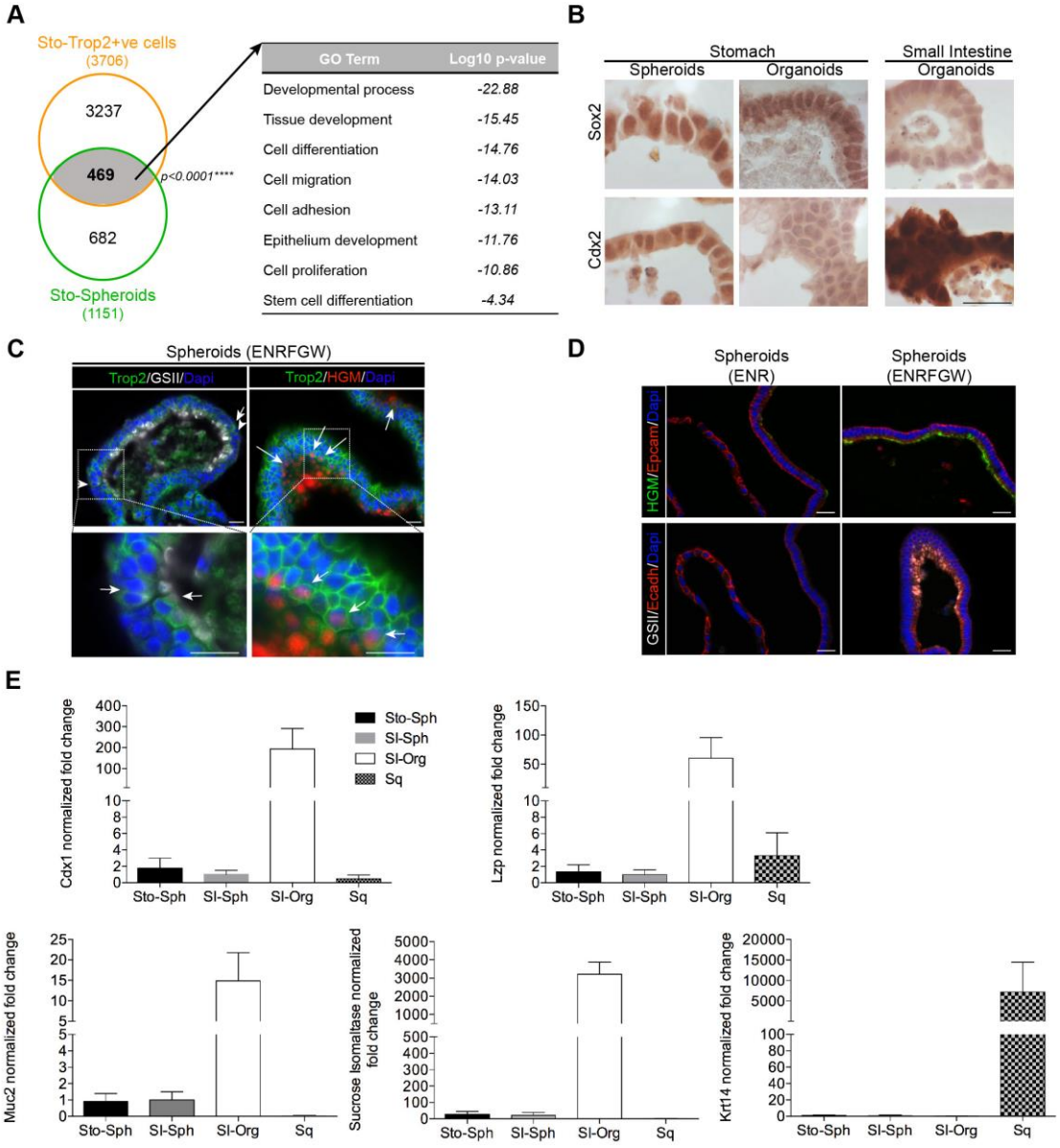
si Fw: 5'-TTCAAGAAATCACAACATTCAATTTACTAG, si Rev: 5'-CTAAAACTTTCTTTGACATTTGAGCAA

sox2 Fw: 5'-TGCCTCTTTAAGACTAGGGCT, sox2 Rev: 5'-CGCCGCGATTGTTGTGATTA

trop2 Fw: 5’-GAACGCGTCGCAGAAGGGC, trop2 Rev: 5’-CGGCGGCCCATGAACAGTGA

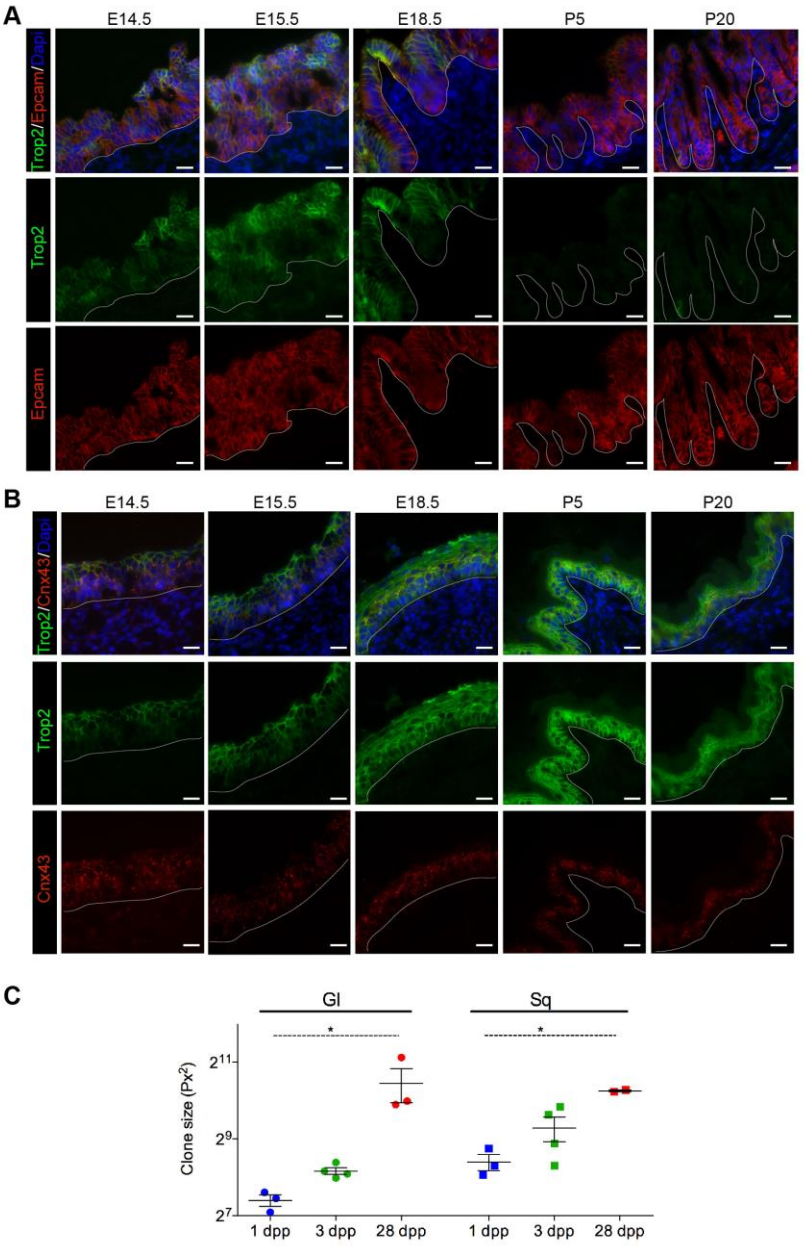


Supplementary figure S1. ***Stomach spheroid characterization*****. (A)** Immunofluorescence showing Trop2 and Cnx43 expression in stomach spheroid and organoid structures. Scale bars: 20 μm. **(B)** Stomach spheroids stability over passages 3, 10, 15 and 20 (P3, P10, P15, P20). mRNA levels of embryonic markers, transcription factors and adult differentiation markers were studied in 3 different stomach spheroid pools (Sto sph pools) by qRT-PCR. Adult-type stomach (Sto) and small intestine (SI) organoids were used as controls.

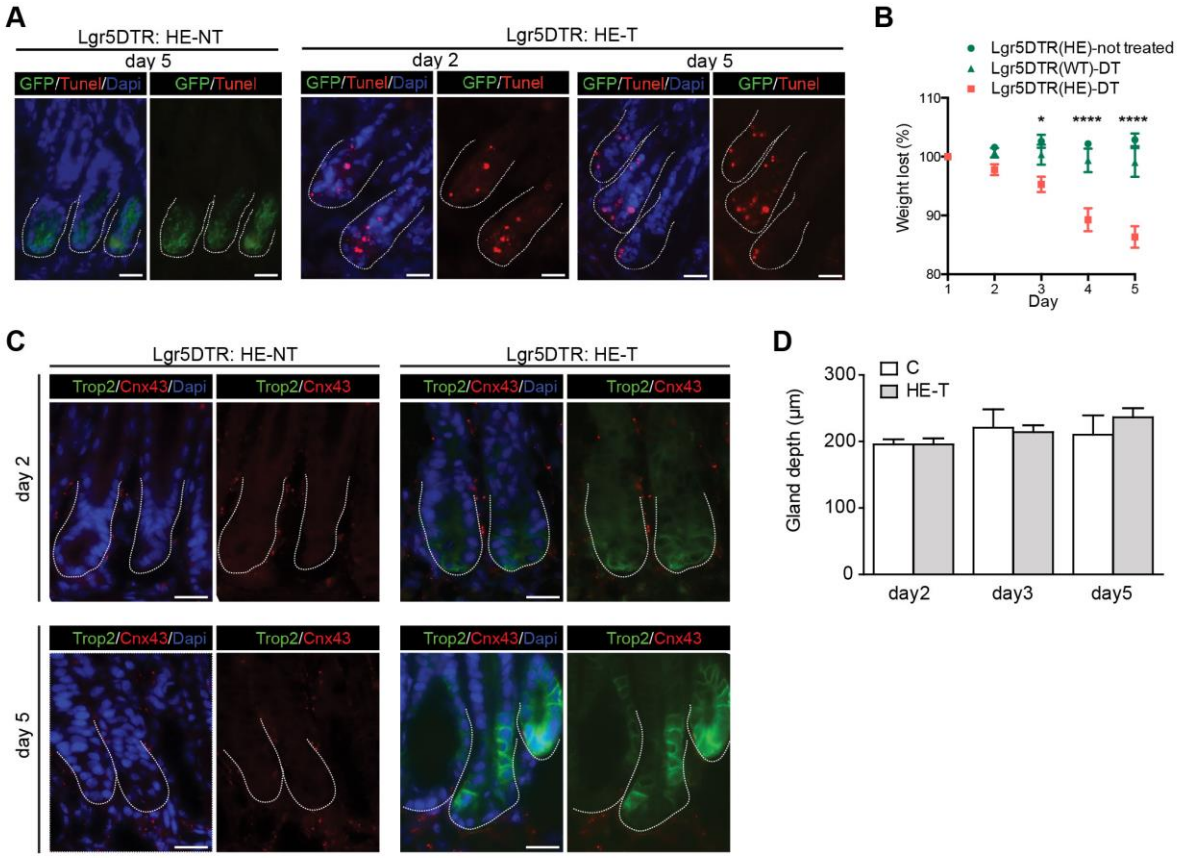


Supplementary figure S2. ***Stomach spheroids are committed to a glandular fate.*** **(A)** Venn diagram showing common genes between 4 fold up-regulated genes obtained from fetal stomach (Sto) Trop2^{+ve} cells versus gastric adult Lgr5 stem cells and stomach spheroids versus small intestine organoids. Main associated biological processes (GO Term) to this common list are shown in the table (right). Chi-square with Yates' correction. **(B)** Immunohistochemistry showing expression of Sox2 and Cdx2 in stomach spheroids and organoids and small intestine organoids. Scale bars for all panels: 20 μm.

(C) Immunofluorescence showing Trop2 and neck (GS-II) or pit (HGM) labeled-mucins co-staining over stomach spheroids cultured under ENRFGW (passage 7). Insets show magnification areas and arrows point to co-stained cells. Scale bars: 20 μm. **(D)** Immunofluorescence showing pit and neck labeled-mucins (GS-II lectin and HGM, respectively) in co-staining with Epcam or Ecadherin over stomach spheroids from passage 12 cultured under ENR or ENRFGW. Scale bars: 20 μm. **(E)** mRNA levels (mean±SEM) of differentiation markers from small intestine (SI) and squamous tissue (Sq) studied over stomach spheroids (Sto-Sph, n=7) using as controls SI spheroids (SI-Sph, n=3), SI organoids (SI-Org, n=4) and stomach squamous tissue (Sq, n=2) by qRT-PCR.



Supplementary figure S3. ***Embryonic markers during development*****. (A)** Immunofluorescence showing expression of Trop2 and Epcam at E14.5, E15.5, E18.5, P5 and P20 developmental stages in the glandular stomach. Scale bars: 20 µm. **(B)** Immunofluorescence showing expression of Trop2 and Cnx43 at E14.5, E15.5, E18.5, P5 and P20 developmental stages in squamous forestomach. Dotted lines evidence epithelial/mesenchymal boundaries in both panels. Scale bars: 20 µm. **(C)** Quantification of the clone size (pixel^2) obtained by lineage tracing at E14.5 plus 1, 3 or 28 days post pulse (dpp) in glandular (Gl) and squamous (Sq) regions. Non-parametric one-way ANOVA (* $p<0.05$). Colour dots and squares represent individual embryos/mice.



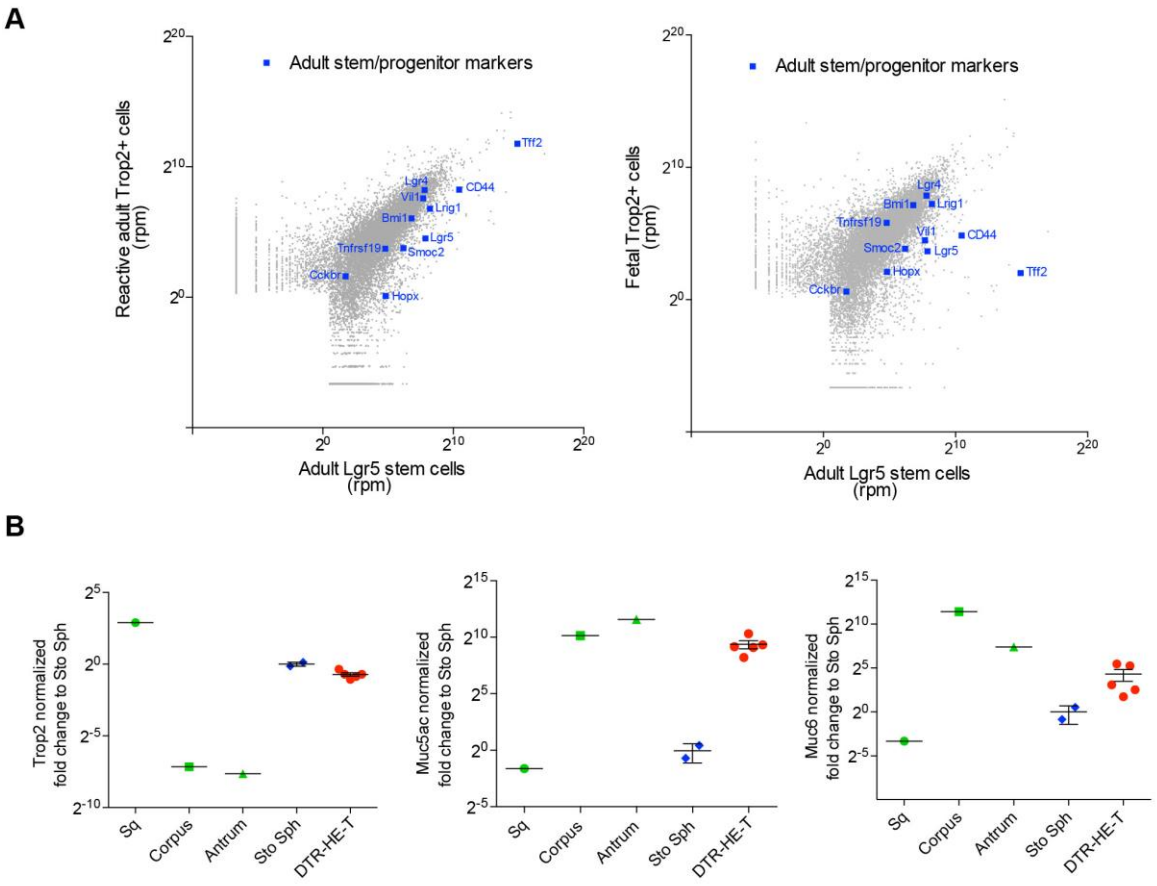
E

Mouse	Mucosal score		Sub-mucosal score		Total score	Trop2 clusters/ 10 fields antrum	Trop2 clusters/ 10 fields corpus
	# lesions	score	# lesions	score			
#2	5	5	2	4	***9***	120.8	49.5
#1	1	1	1	2	***3***	9.1	7.5
#3	1	1	1	2	***3***	7.8	0.8
#5	2	2	0	0	***2***	7.5	0.4

Mucosal score= 1 x (number mucosal lesions/whole stomach section)
Sub-mucosal score= 2 x (number mucosal lesions/whole stomach section)
Total score= mucosal score + sub-mucosal score

Supplementary figure S4. ***Epithelial injury induces Trop2 expression in adult glandular stomach.*** **(A)** Immunofluorescence showing death of Lgr5^{+ve} cells (GFP) by apoptosis (Tunel) in antral glands after diphtheria toxin (DT) treatment in Lgr5-DTR heterozygotes (HE-T) but not in vehicle-injected Lgr5-DTR heterozygotes (HE-NT). Dotted lines evidence gland limits. Scale bars 20 µm. **(B)** Modification of body weight over time after DT treatment. Two different types of controls: Lgr5DTR: HE-NT (vehicle-treated heterozygous, n=3) and Lgr5DTR: WT-T (DT-treated wild-types, n=5) were grouped for statistical analysis versus Lgr5DTR: HE-T (DT-treated heterozygous, n=7). Two-way

ANOVA, Bonferroni post-test was performed ($p<0.05$ at day 3, $p<0.0001$ at days 4 and 5). The weight loss may be caused in part by liver toxicity. **(C)** Immunofluorescence showing expression of Trop2 and Cnx43 in antral glands of Lgr5-DTR heterozygous treated (HE-T) or not (HE-NT) with DT. Dotted lines evidence the bottom of the glands. Scale bars: 20 µm. **(D)** Histogram showing the gland depth measurement (µm) at the different endpoints (day 2, 3 or 5). Lgr5-DTR HE-T mice compared with controls (C: HE-NT plus WT-T). Number of mice: n=3 at any time point for C; and n=3, 4, 7 for HE-T mice at day 2, 3 and 5. **(E)** Table showing correlation between the intensity of the tissue damage and Trop2 expression in the stomach of indomethacin-treated animals. Arbitrary scores had been used for describing the severity of the injury. Sub-mucosal lesion was scored higher than the mucosal one, since it is considered more severe.



Supplementary figure S5. ***Characterization of the reactive adult Trop2-expressing cells upon stem cell ablation.*** **(A)** Scatter plot of reactive adult Trop2^{+ve} cells (n=4 mice pooled in 2 groups) or fetal Trop2^{+ve} cells (n=2 pools of 12 and 18 embryos each) and adult Lgr5 stem cells (1 pool of 4 mice) transcriptomes, represented as the $\log_2$ mean. Rpm: reads per million. Genes representing adult stem/progenitor markers are highlighted in blue. **(B)** mRNA levels of the embryonic marker Trop2 and differentiation markers Muc6 and Muc5ac for 5 different samples of replated elements (passage 1) obtained from ex vivo culture of Lgr5DTR heterozygous mice treated with diphtheria toxin (DTR-HE-T) by qRT-PCR. Stomach spheres (Sto Sph) as well as tissue from squamous (Sq), corpus and antrum adult stomach were used as controls.

Table S1.

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Table S2.

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