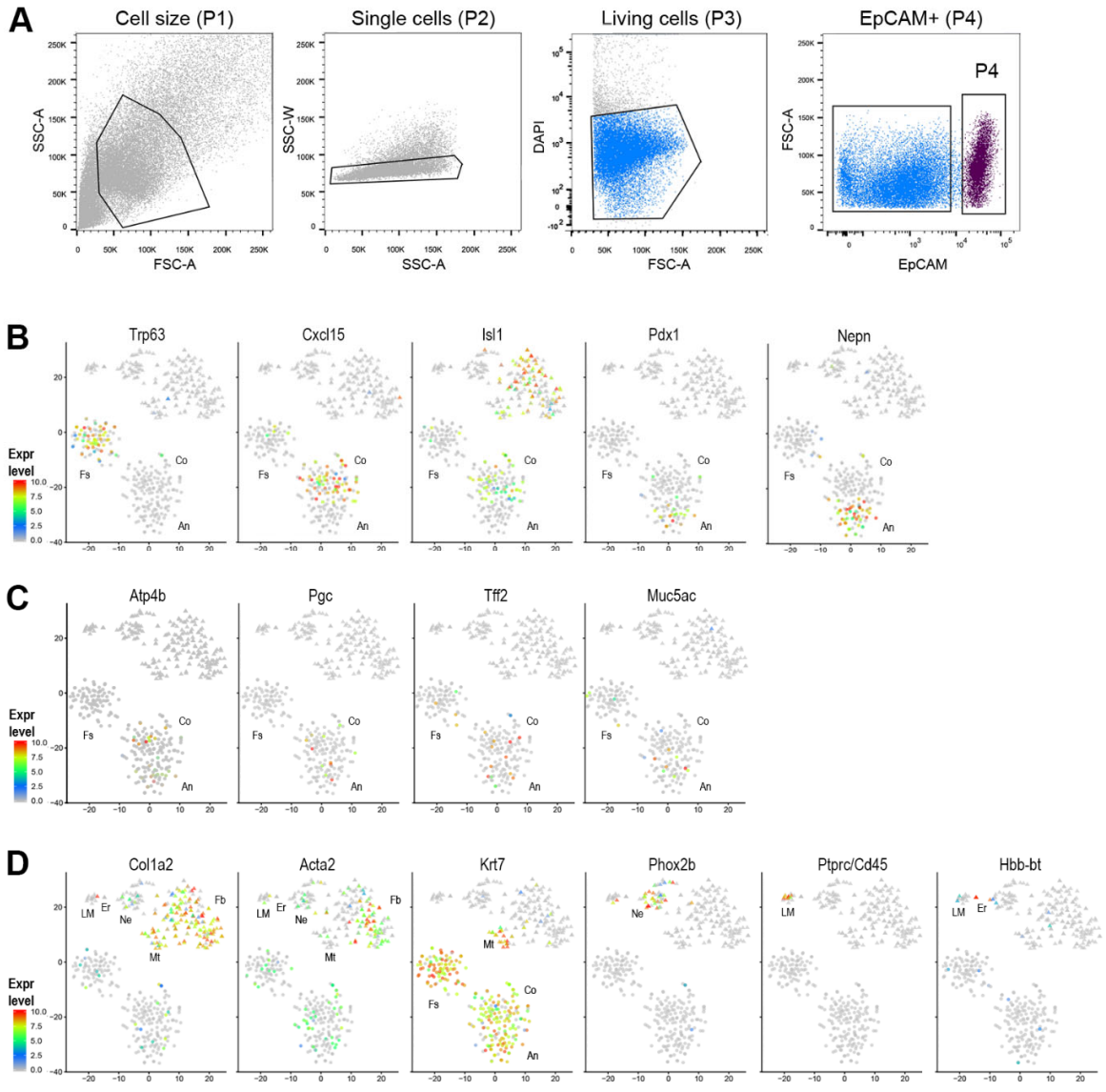

Fig. S1. Cellular composition of mouse embryonic stomach. (A) FACS strategy used to isolate EpCAM^{+} and EpCAM^{-} cells from the embryonic stomach. Cells from the dissected stomachs at E13.5 were dissociated by enzymatic digestion and stained with EpCAM antibody. These cells were selected based on size (P1 and P2), DAPI dye exclusion (P3, living cells) and EpCAM^{+} labelling (P4) (n=39). (B) T-stochastic neighbour embedding (t-SNE) plots of mouse embryonic gastric cells. Single cells coloured by expression of marker genes *Trp63* for forestomach (Fs),

Cxcl15 and *Isl1* for corpus (Co), *Pdx1* and *Nepn* for antrum (An). (C) t-SNE plots of mouse embryonic gastric cells showing expression of marker genes *Atp4b* for parietal, *Pgc* for zymogenic chief, *Tff2* for mucous neck, and *Muc5ac* for mucous pit cells. (D) t-SNE plots of mouse embryonic gastric cells showing expression of marker genes *Col1a2* for fibroblasts (Fb) and *Acta2* for myofibroblasts, both *Col1a2* and *Krt7* for mesothelial cells (Ms), *Phox2b* for enteric neural cells (Ne), *Ptprc/CD45* for lympho-myeloid cells (LM) and haemoglobin *Hbb-bt* for erythroid cells (Er). Colour bar: log2(TPM+1). See also Fig. 1.

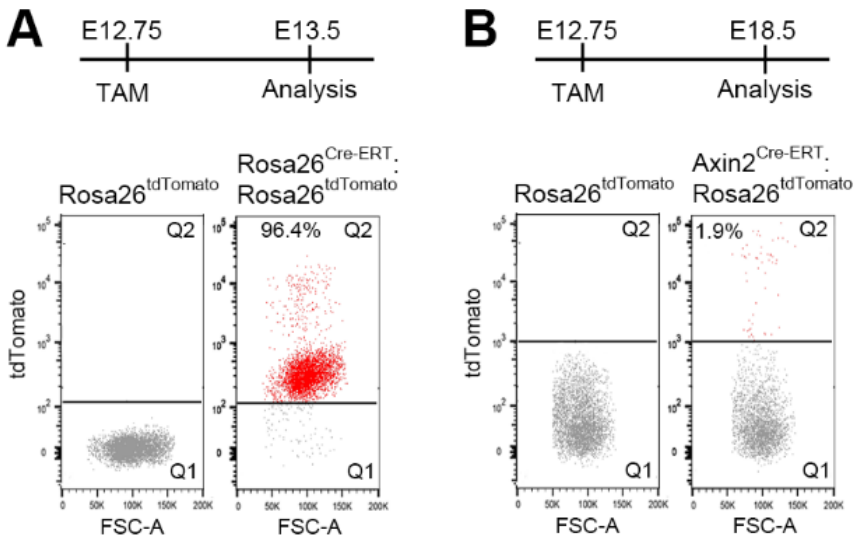

Fig. S2. Adult gastric stem cell markers are expressed in the embryonic stomach epithelium. (A) Representative FACS plots used to analyse tdTomato^{+} cells from the small intestine of *Rosa26*$^{Cre\text{-}ERT}$*/Rosa26*^{tdTomato} embryos 16 hours after tamoxifen (TAM) treatment (n=3 embryos analysed). (B) Representative FACS plots used to analyse the percentages of tdTomato^{+} cells from the small intestine of *Axin2*$^{Cre\text{-}ERT}$*/Rosa26*^{tdTomato} embryos at E18.5 after a single treatment with tamoxifen at E12.75 (n=7 embryos analysed). See also Fig. 2.

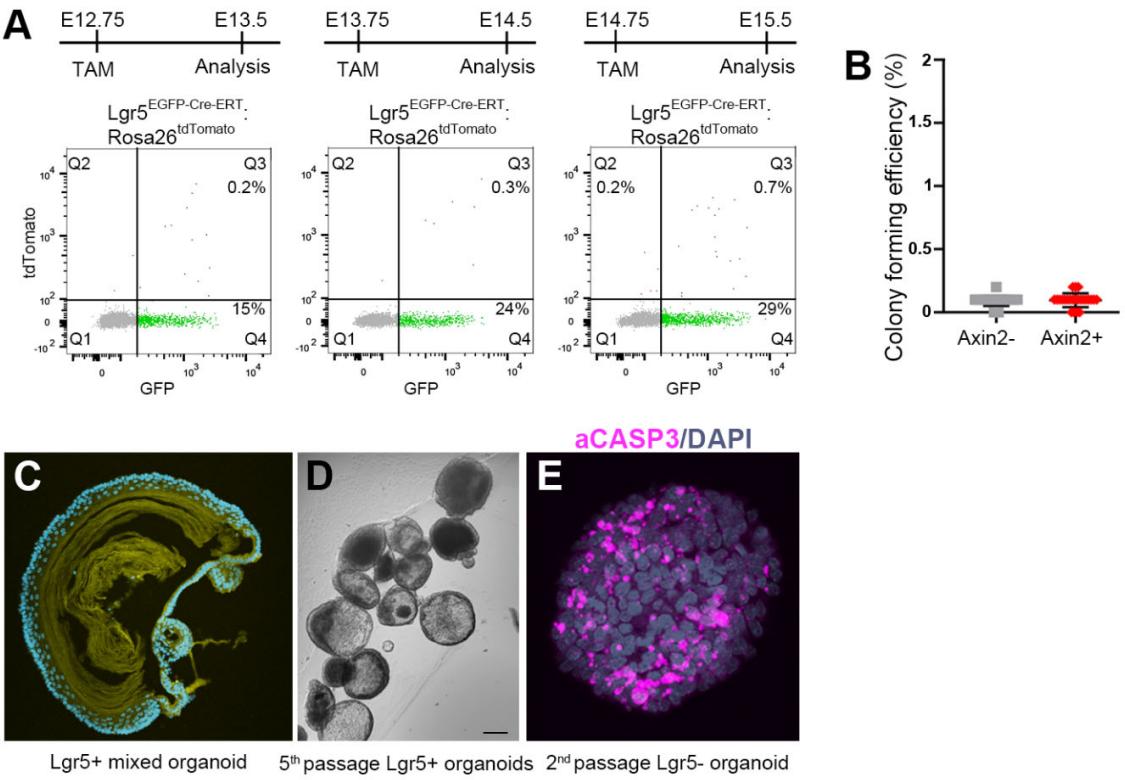

Fig. S3. Lgr5-expressing embryonic cells form organoids ex vivo. (A) Representative FACS plots showing EpCAM$^+$ (grey, Q1), Lgr5-EGFP$^+$EpCAM$^+$ (green, Q4), tdTomato$^+$EpCAM$^+$ (red, Q2) and Lgr5-EGFP$^+$:tdTomato$^+$EpCAM$^+$ (purple, Q3) cell populations isolated from the embryonic stomach at various developmental stages 16 hours after a single treatment with TAM (n=5 embryos analysed). (B) Colony-forming efficiency of Axin2$^-$EpCAM$^+$ and Axin2:tdTomato$^+$EpCAM$^+$ cells isolated by FACS at E13.5. Error bars are ± SD, n=24. (C) Section of the gastric "mixed" organoid derived from *Lgr5*-expressing epithelial progenitors labelled with plasma membrane stain (green) and DAPI for nuclei (blue). Note the presence of the nuclei-free squamous epithelium within the organoid lumen. (D) Representative image of gastric organoids derived from Lgr5-positive embryonic progenitors after 5 passages (35 days of culture). (E) A confocal image of Lgr5$^-$ derived embryonic organoid grown in WERNF medium for 2 passages labelled for activated Caspase3 showing apoptotic cells (magenta). Nuclei stained with DAPI (blue). Scale bar: 100 µm (B and D) and 0.5 mm (C). See also Fig. 3.

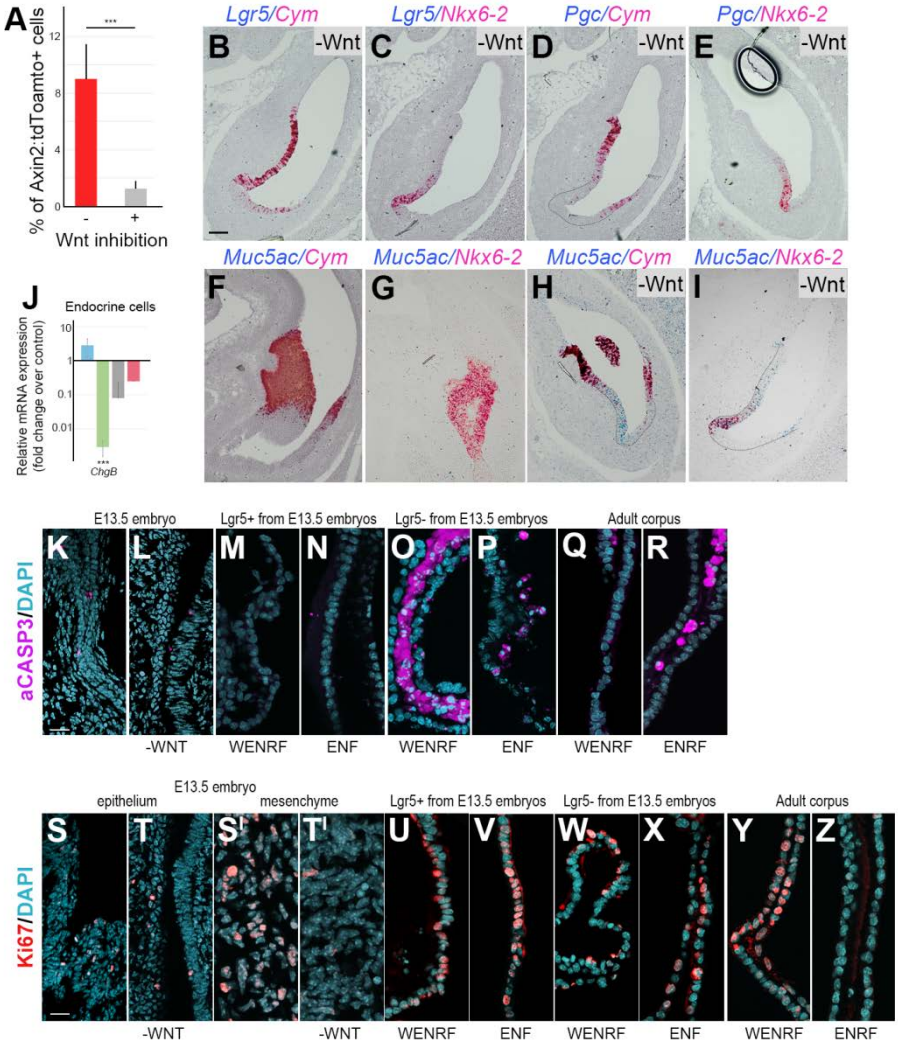

Fig. S4. Functions of WNT signalling in the embryonic gastric epithelium. (A) Quantification of Axin2:tdTomato^{+}EpCam gastric epithelial cells from Wnt-59C treated and control embryos at E13.5. Error bars are ± SD, n=19. ***P<0.001 by Student´s t-test. (B-I) Single-molecule RNA ISH for *Lgr5* (cyan)/*Cym* (magenta, B), *Lgr5* (cyan)/*Nkx6-2* (magenta, C), *Pgc* (cyan)/*Cym* (magenta, D), *Pgc* (cyan)/*Nkx6-2* (magenta, E) on E13.5 stomach from Wnt-59C treated embryos (n=3), and *Muc5ac* (cyan)/*Cym* (magenta, F), *Muc5ac* (cyan)/*Nkx6-2* (magenta, G), *Muc5ac* (cyan)/*Cym* (magenta, H), *Muc5ac* (cyan)/*Nkx6-2* (magenta, I) on E13.5 stomach from wild type (F-G) and Wnt-59C treated (H-I) embryos (n=3). (J) qRT-PCR analysis showing expression of

entero-endocrine cell marker in stomach epithelium of the embryos treated for 16 hours with WNT inhibitor Wnt-59C compared to vehicle-treated controls (blue), in $Lgr5^+$ derived organoids grown for 7 days in ENF medium (green), $Lgr5^-$ derived organoids grown for 7 days in ENF medium (grey) and in adult corpus organoids grown for 48 hours without or with Gsk3β inhibitor (pink) (n=3). *Tbp* expression was used as a normalizing control. Error bars are ± SD. ***P<0.001, **P<0.01 and *P<0.05 by Student´s t-test. (K-R) Confocal images of E13.5 mouse stomach from embryos either treated with control vehicle (K) or Wnt-59C inhibitor (L) for 16 hours, $Lgr5^+$ derived organoids (M-N), $Lgr5^-$ derived organoids (O-P) or adult corpus organoids (Q-R) cultured either with Gsk3β inhibitor and R-spondin (WENRF) or without (ENF) labelled for apoptotic cells (aCASP3+, magenta) (n=3). (S-Z) Confocal images of E13.5 mouse stomach from embryos either treated with control vehicle (S-S´) or Wnt-59C inhibitor (T-T´) for 16 hours, $Lgr5^+$ derived organoids (U-V), $Lgr5^-$ derived organoids (W-X) or adult corpus organoids (Y-Z) cultured either with Gsk3β inhibitor and R-spondin1 (WENRF) or without (ENF) and labelled for proliferating cells (Ki67+, red) (n=3). Nuclei stained with DAPI (blue). Scale bar: 100 µm (B-I), 25 µm (M-R, U-Z), 40 µm (K-L, S-T) and 15 µm (S´-T´). See also Fig. 4.

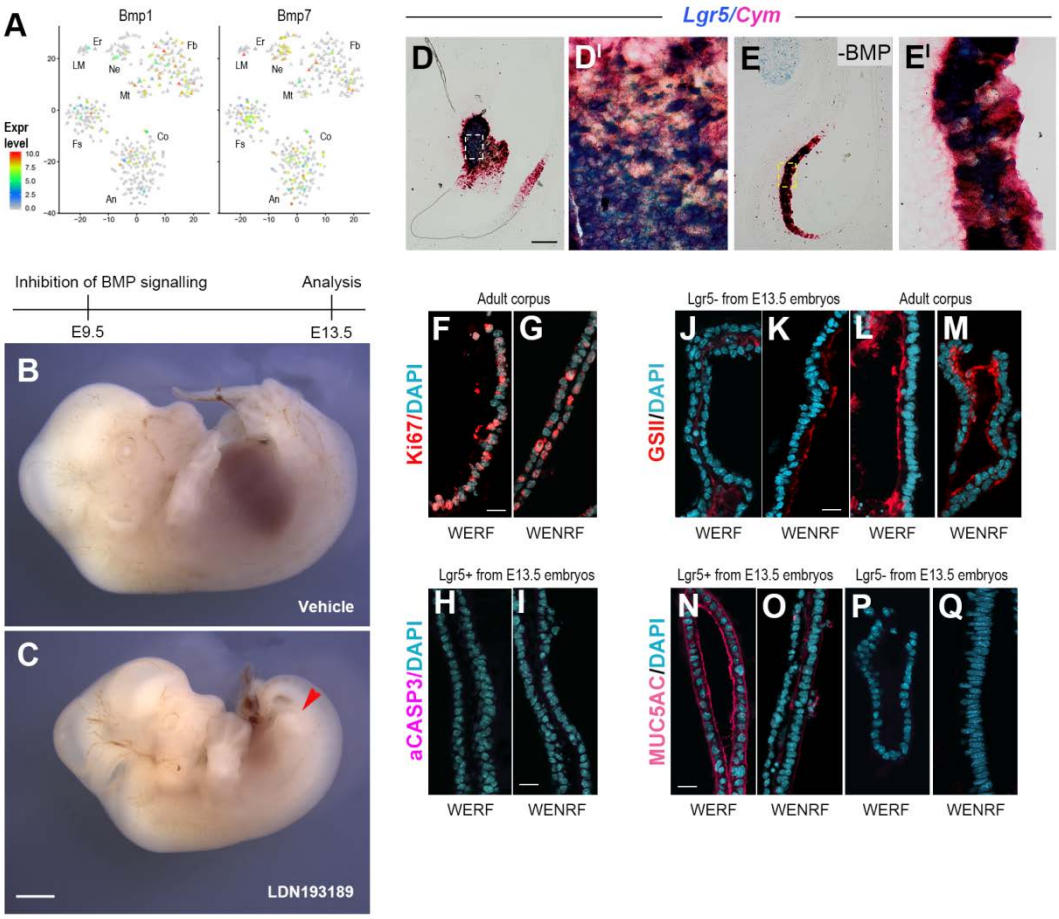

Fig. S5. Effects of BMP signalling inhibition on gastric epithelial cells. (A) t-SNE plots of mouse embryonic gastric cells. Single cells coloured by expression of *Bmp1* and *Bmp7* in forestomach (Fs), corpus (Co) and antral (An) epithelium as well as in fibroblast (Fb), mesothelial (Ms), enteric neural (Ne), lympho-myeloid (LM) and erythroid (Er) cell lineages. Colour bar: log2(TPM+1). (B-C) Whole-mount view of mouse embryos at E13.5 treated with either vehicle (B) or BMP receptor 1A inhibitor LDN193189 (C) at E9.5. Arrowhead points to the absent hindlimb caused by inhibition of BMP signalling (n>10 embryos analysed). (D-E) smRNA ISH for *Lgr5* (cyan)/*Cym* (magenta) on E13.5 stomach from control (D-D') and LDN193189 (E-E') treated embryos (n=3). D'-E' show magnified views of the boxed areas. (F-G) Confocal images of adult corpus derived organoids cultured either with Noggin (WENRF) or without (WERF) labelled for proliferating cells Ki67 (red) (n=3). (H-I) Confocal images of $Lgr5^{+}$ derived organoids cultured either with Noggin (WENRF) or without (WERF) labelled for apoptotic cells ($aCASP3^{+}$, magenta) (n=3). (J-M) Confocal images of $Lgr5^{-}$ (J-K) and adult corpus derived organoids (L-M)

cultured either with Noggin (WENRF) or without (WERF) labelled for GSII showing mucous neck cells (red) (n=3). (N-Q) Confocal images of $Lgr5^{+}$ (N-O) and $Lgr5^{-}$ derived organoids (P-Q) cultured either with Noggin (WENRF) or without (WERF) labelled for MUC5Ac showing mucous pit cells (n=3). Nuclei stained with DAPI (blue). Scale bar: 0.75 mm (B-C), 100 µm (D-E), 15 µm (D′-E′), 25 µm (H-I, L-M, N-U). See also Fig. 5.

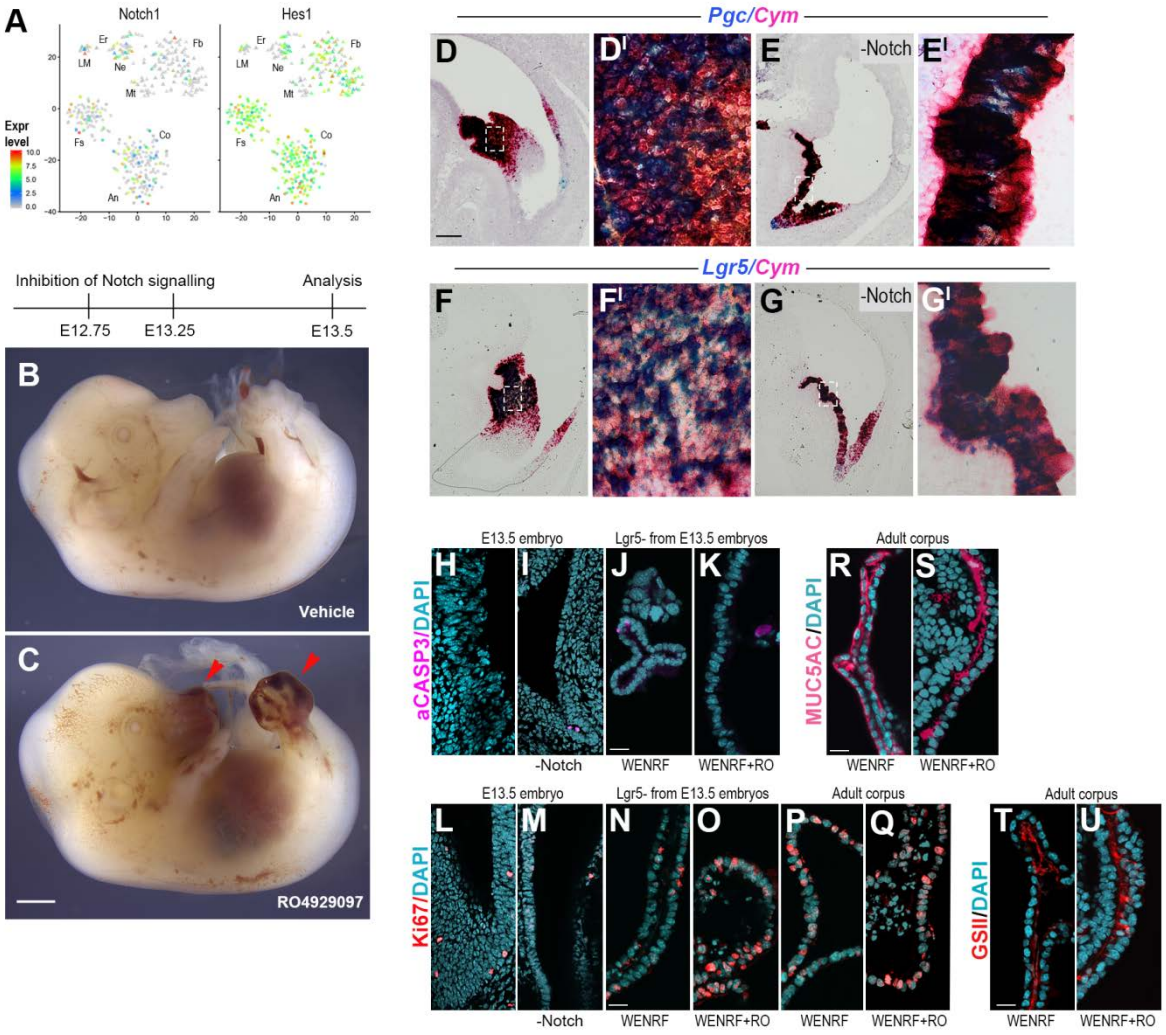

Fig. S6. Effects of Notch signalling inhibition on gastric epithelial cells. (A) tSNE plots of mouse embryonic gastric cells. Single cells coloured by expression of *Notch1* and *Hes1* in forestomach (Fs), corpus (Co) and antral (An) epithelium as well as in fibroblast (Fb), mesothelial (Ms), enteric neural (Ne), lympho-myeloid (LM) and erythroid (Er) cell lineages. Colour bar: log2(TPM+1). (B-C) Whole-mount view of mouse embryos at E13.5 treated with either vehicle (B) or Notch inhibitor RO4929097 (C) 16 and 4 hours before the analysis. Arrowheads point to haemorrhages caused by inhibition of Notch signalling (n>10 embryos analysed). (D-G) Single-molecule RNA ISH for *Pgc* (cyan)/*Cym* (magenta) (D-E) and *Lgr5* (cyan)/*Cym* (magenta) (F-G) on E13.5 stomach from control (D, F) and RO4929097 (E, F) treated embryos (n=3). D'-G' show magnified views of the boxed areas. (H-K) Confocal images of E13.5 mouse stomach from embryos either treated with control vehicle (H) or RO4929097 inhibitor (I) for 16 hours and Lgr5^{-}

derived organoids (J-K) grown either with DMSO (J) or with Notch inhibitor RO4929097 (K) labelled for activated Caspase3 showing apoptotic cells (magenta). (L-Q) Confocal images of E13.5 mouse stomach from embryos either treated with control vehicle (L) or RO4929097 inhibitor (M) for 16 hours, $Lgr5^{-}$ derived organoids (N-O) and adult corpus derived organoids (P-Q) grown either with DMSO (N, P) or with Notch inhibitor RO4929097 (O, Q) labelled for Ki67 showing proliferating cells (red) (n=3). (R-S) Confocal images of adult corpus derived organoids grown either with DMSO (R) or with Notch inhibitor RO4929097 (S) labelled for MUC5Ac showing mucous pit cells (pink). (T-U) Confocal images of adult corpus derived organoids grown either with DMSO (T) or with Notch inhibitor RO4929097 (U) labelled GS-II showing mucous neck cells (red). Scale bar: 0.75 mm (B-C), 100 µm (D-G), 15 µm (D'-G'), 25 µm (J-K, N-U), 40 µm (H-I, L-M). See also Fig. 6.