Supplementary figures

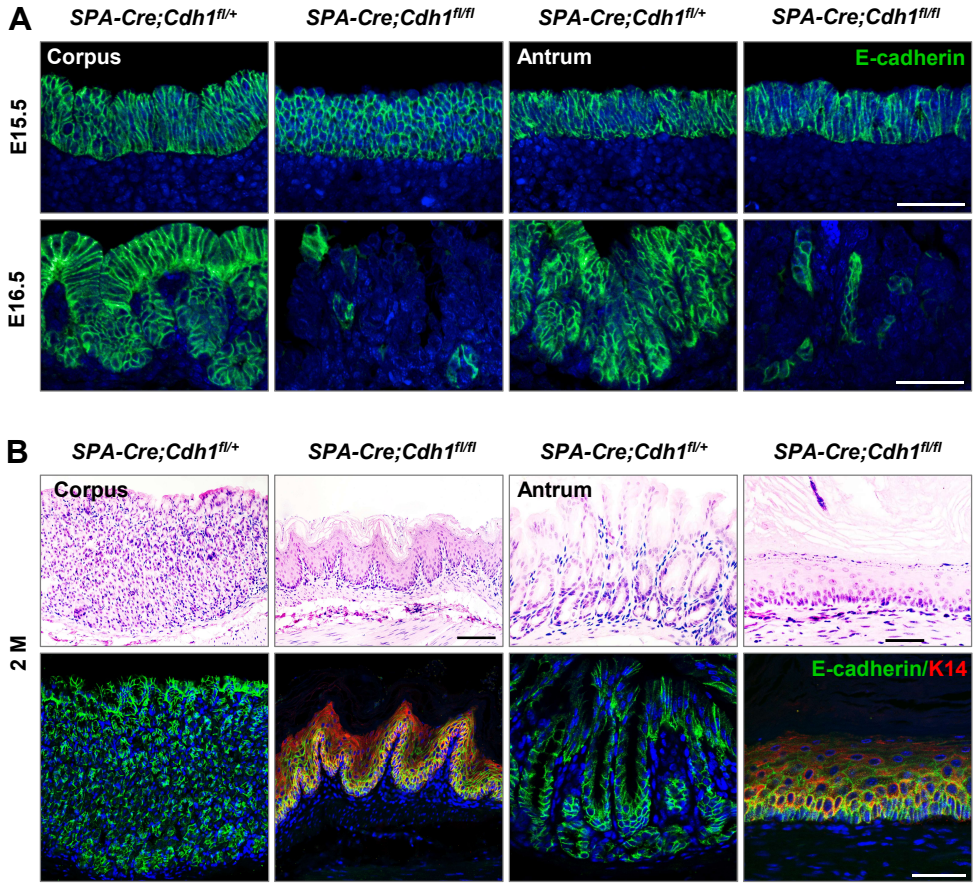

Figure S1. SPA-Cre-mediated deletion of E-cadherin in gastric disrupted the glandular epithelia.

(A) Immunofluorescence staining (IF) for E-cadherin in the gastric epithelium of *SPA-Cre;Cdh1*$^{fl/+}$ and *SPA-Cre;Cdh1*$^{fl/fl}$ mice at E15.5 and E16.5. (B) H&E staining and co-immunofluorescence staining (Co-IF) for E-cadherin and Keratin 14 (K14) in gastric corpus and antral region of *SPA-Cre;Cdh1*$^{fl/+}$ and *SPA-Cre;Cdh1*$^{fl/fl}$ mice at the age of 2 months. Nuclei are dyed using DAPI (blue). Scale bars: 50 μm.

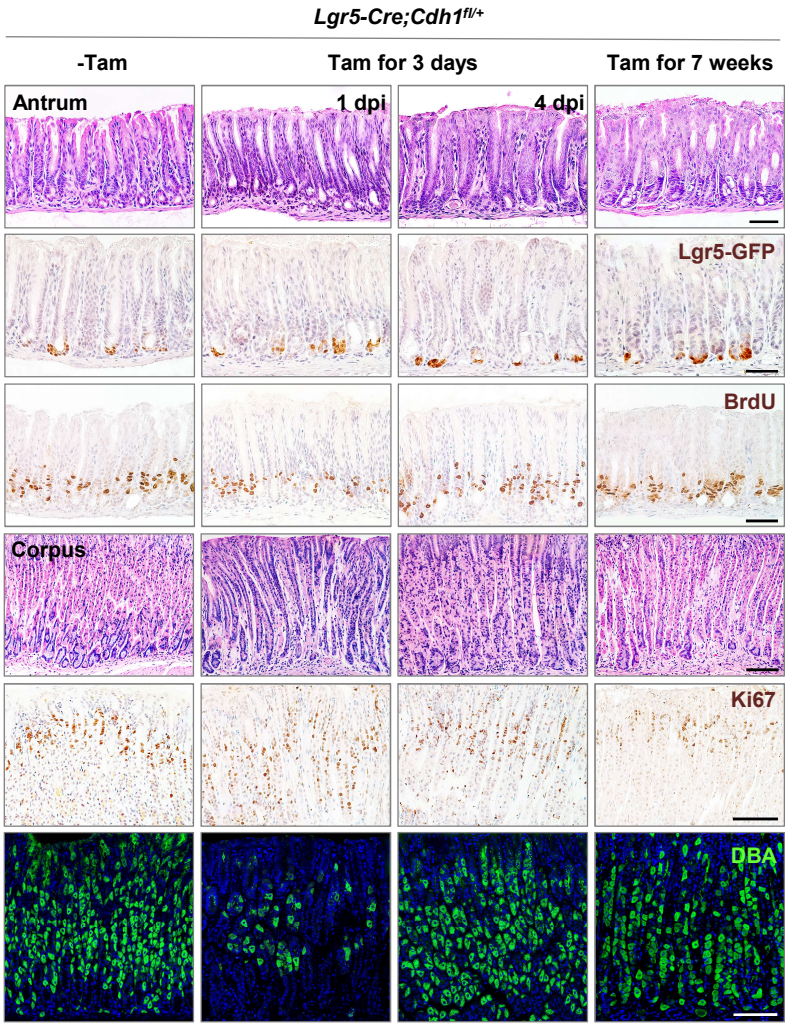

Figure S2. Tamoxifen has no detrimental effect on the gastric antrum. Following Tam treatment, the antral epithelium was unaffected, as shown by H&E staining, and no changes in the number of $Lgr5^{+}$ stem cells (Lgr5-GFP) and proliferative cells (BrdU), suggesting that the Tam dose has no detrimental effects on the gastric antrum. Consistent with previous observations [1, 2], treatment with Tam led to abnormalities in the epithelium of the gastric corpus, as shown by the impaired epithelial structure (H&E), increase in the number of proliferative cells (Ki67), and a reduction in that of parietal cells (DBA). Nuclei are dyed using DAPI (blue). Scale bars: 50 μm.

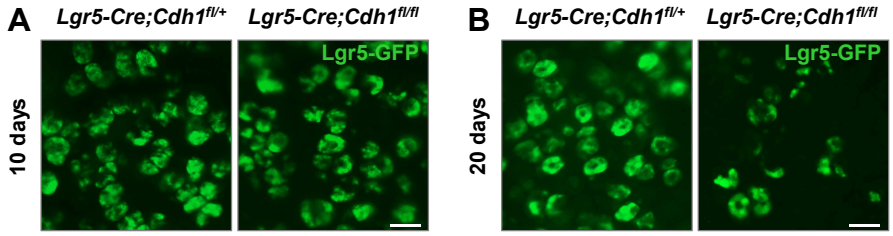

Figure S3. The number of antral Lgr5^{+} stem cells after continued Tam treatment.

Direct imaging for endogenous Lgr5-GFP fluorescence on whole mount tissues in *Lgr5-Cre;Cdh1$^{fl/+}$* and *Lgr5-Cre;Cdh1$^{fl/fl}$* mice (A) after 10-days Tam treatment or (B) after 20-day Tam treatment. Nuclei are dyed using DAPI (blue). Scale bars: 50 μm.

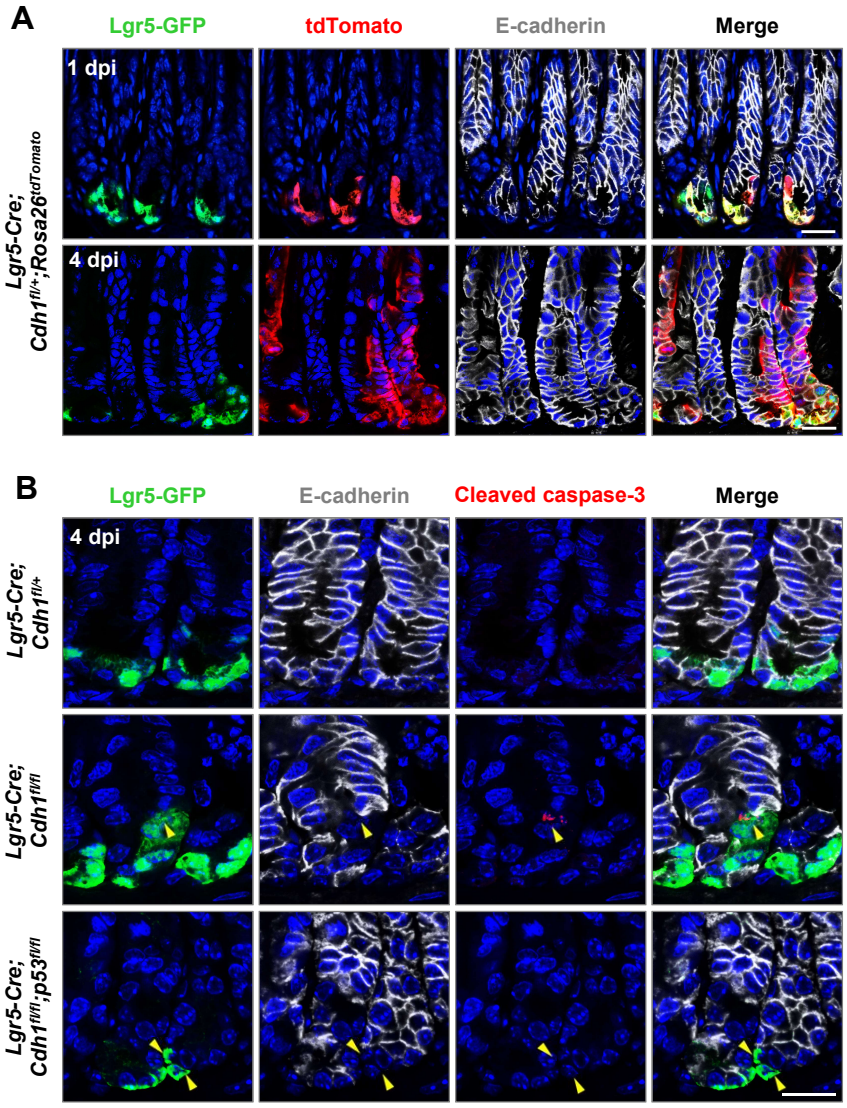

Figure S4. Additional deletion of p53 in E-cadherin-deficient Lgr5^{+} stem cells abolished their apoptosis.

(A) Co-IF for Lgr5-GFP (green), tdTomato (red), and E-cadherin (white) in the gastric antrum of *Lgr5-Cre;Cdh1$^{fl/+}$;Rosa26^{tdTomato}* mice at 1 and 4 dpi after 3-day Tam treatment. (B) Co-IF for Lgr5-GFP (green), E-cadherin (white), and cleaved caspase-3 (red) in the gastric antrum of *Lgr5-Cre;Cdh1$^{fl/+}$*, *Lgr5-Cre;Cdh1$^{fl/fl}$*, and *Lgr5-Cre;Cdh1$^{fl/fl}$; p53$^{fl/fl}$* mice at 4 dpi after 3-day Tam treatment. Yellow arrowheads indicate Lgr5-GFP^{+}E-cadherin^{-} cells. Nuclei are dyed using DAPI (blue). Scale bars: 20 μm.

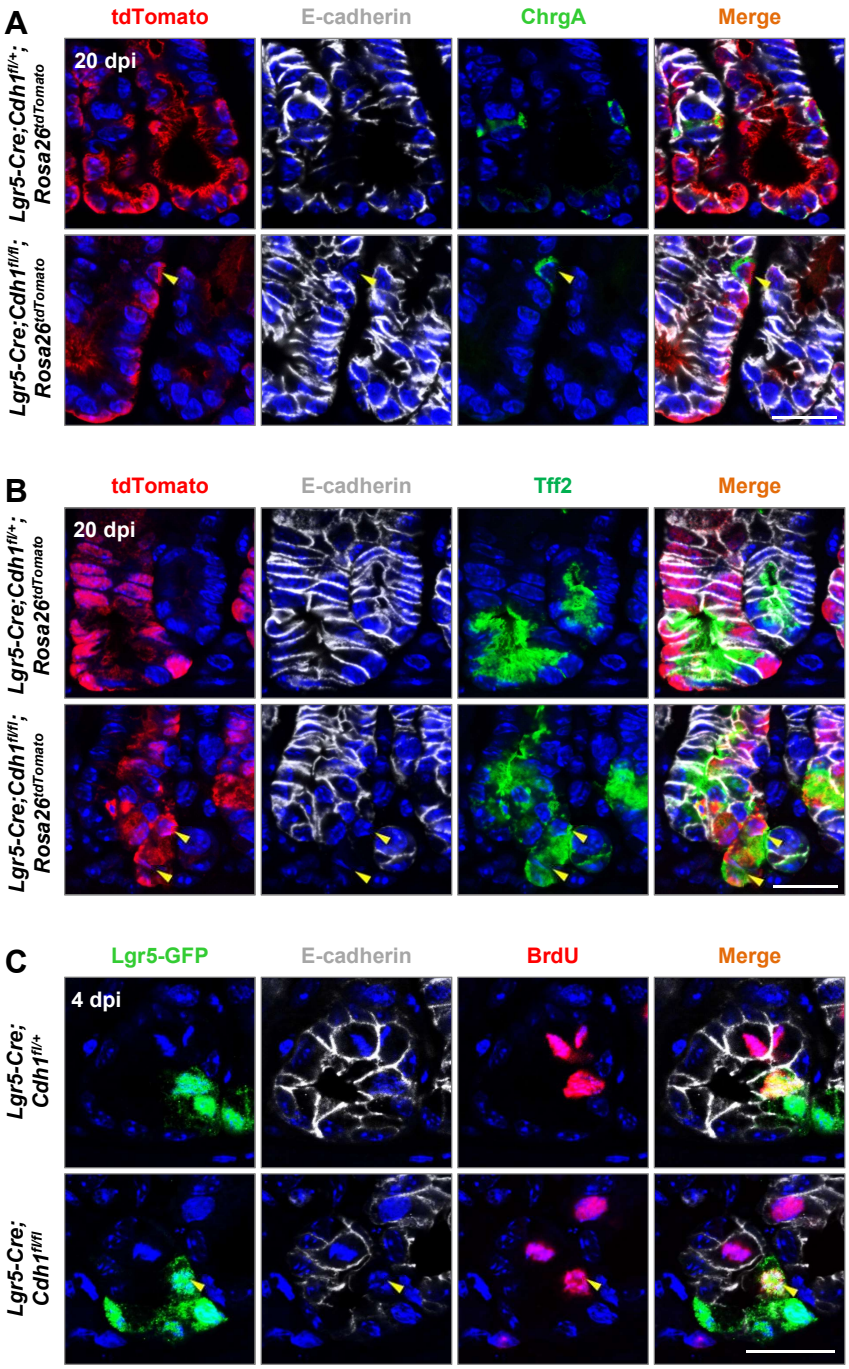

Figure S5. The differentiation and proliferation of E-cadherin-deficient Lgr5^{+} stem cell. (A) and (B) Co-IF for tdTomato (red), and E-cadherin (white), and ChrgA (green) or Tff2 (green) in the gastric antrum of *Lgr5-Cre;Cdh1$^{fl/+}$;Rosa26^{tdTomato}* and *Lgr5-Cre;Cdh1$^{fl/fl}$;Rosa26^{tdTomato}* mice at 20 dpi after 3-day Tam treatment. (C) Co-IF for Lgr5-GFP (green), E-cadherin (white), and BrdU (red) in the gastric antrum of *Lgr5-Cre;Cdh1$^{fl/+}$* and *Lgr5-Cre;Cdh1$^{fl/fl}$* mice at 4 dpi after 3-day Tam treatment. Yellow arrowheads indicate E-cadherin-deficient cells. Nuclei are dyed using DAPI (blue). Scale bars: 20 μm.

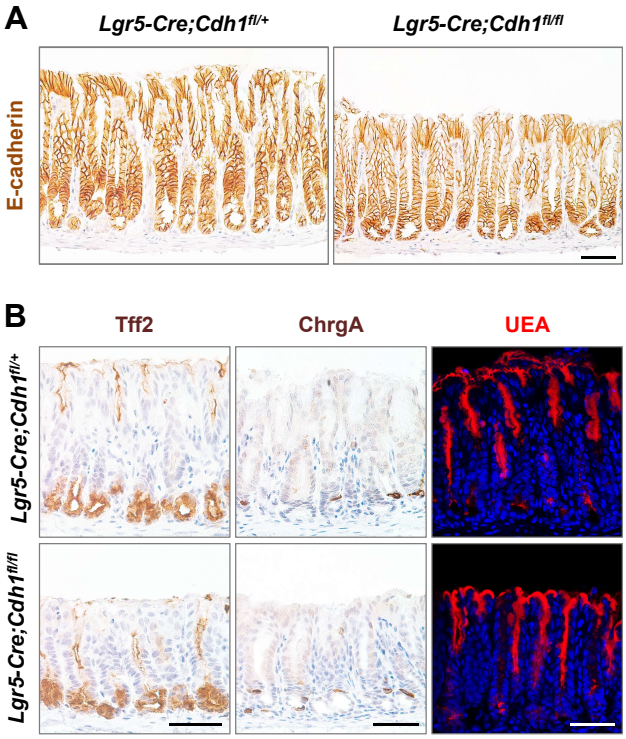

Figure S6. The loss of Lgr5^{+} stem cells does not affect the cellular differentiation of the gastric antral epithelium.

(A) IHC for E-cadherin in the gastric antrum of *Lgr5-Cre;Cdh1$^{fl/fl}$;Rosa26^{tdTomato}* mice after 7 weeks of Tam treatment. (B) IHC for Tff2 (a marker of mucous neck cells) and ChrgA (a marker of endocrine cells), as well as IF for UEA (a marker of surface mucous cells) in the gastric antrum of *Lgr5-Cre;Cdh1$^{fl/+}$* and *Lgr5-Cre;Cdh1$^{fl/fl}$* mice after 7 weeks of Tam treatment. Nuclei are dyed using DAPI (blue). Scale bars: 50 μm.

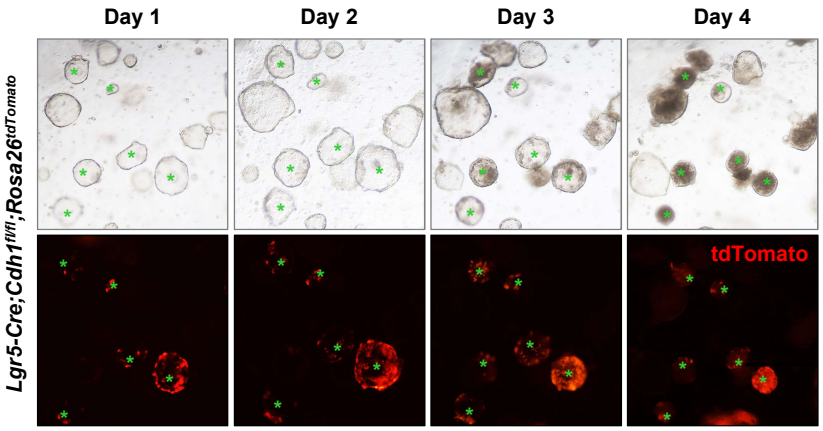

Figure S7. Organoid growth in the *Lgr5-Cre;Cdh1$^{fl/fl}$;Rosa26^{tdTomato}* antrum. Morphology and direct tdTomato imaging of antral organoids in *Lgr5-Cre; Cdh1$^{fl/fl}$;Rosa26^{tdTomato}* antrum after treatment with 4H-Tam for 1, 2, 3, and 4 days. Green asterisks indicate the tdTomato^{+} shrunken organoids. Scale bars: 100 μm.

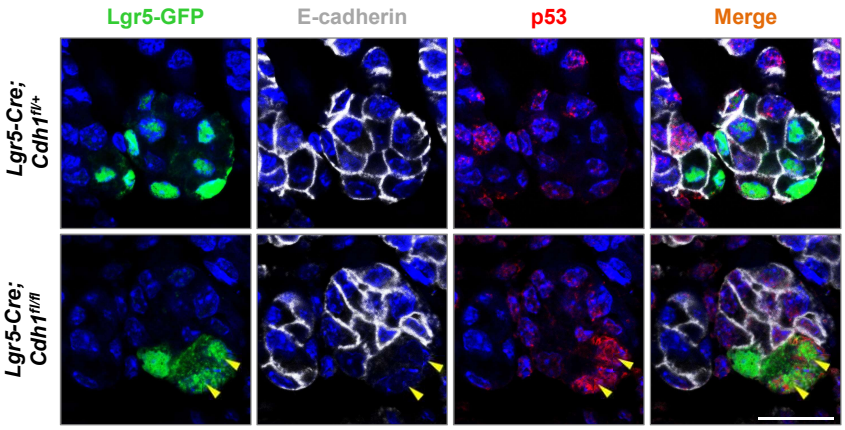

Figure S8. p53 expression in E-cadherin-deficient Lgr5^{+} stem cells.

Co-IF for Lgr5-GFP (green), E-cadherin (White), and p53 (Red) in the *Lgr5-Cre;Cdh1$^{fl/+}$* and *Lgr5-Cre;Cdh1$^{fl/fl}$* mice. Yellow arrowhead indicating the E-cadherin-deficient Lgr5^{+} stem cells. Nuclei are dyed using DAPI (blue). Scale bars: 20 μm.

References

1. Huh WJ, Khurana SS, Geahlen JH, Kohli K, Waller RA, Mills JC. Tamoxifen induces rapid, reversible atrophy, and metaplasia in mouse stomach. Gastroenterology. 2012; 142: 21-4 e7.
2. Leushacke M, Tan SH, Wong A, Swathi Y, Hajamohideen A, Tan LT, et al. Lgr5-expressing chief cells drive epithelial regeneration and cancer in the oxyntic stomach. Nat Cell Biol. 2017; 19: 774-86.