

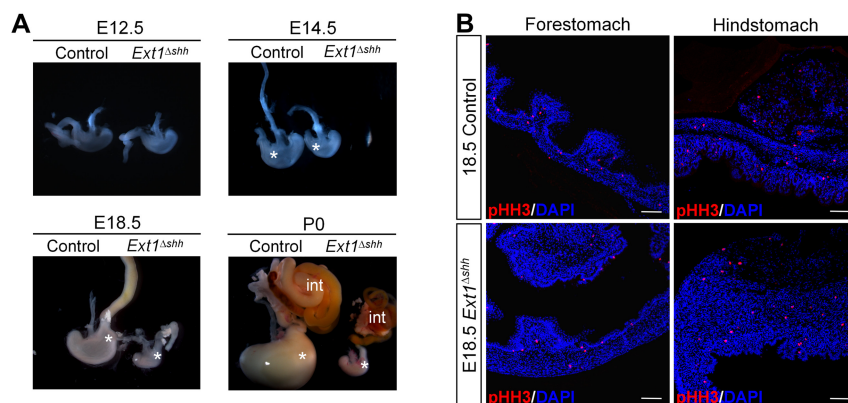
## Supplementary information

**Table S1. Primers for riboprobe synthesis**

Name	Forward sequence	Reverse sequence
<i>Shh</i>	AATTAACCCTCACTAAAGGGAG	TAATACGACTCACTATAGGGAG
	ACCAGCGGCAGATATGAAG	ACCAGGAAGGTGAGGAAGT
<i>Atp4b</i>	AATTAACCCTCACTAAAGGGAG	TAATACGACTCACTATAGGGAG
	ACCCGAATTCCGGCACTACTGT	ATGCTAAGAAGCTGTGCAGGG
<i>Bapx1</i>	AATTAACCCTCACTAAAGGGAG	TAATACGACTCACTATAGGGAG
	ACGATAATCGGCCGGGCTGTA	ACCCGCGCTCCTCTTTCTTGTT
<i>Fgf10</i>	AATTAACCCTCACTAAAGGGAG	TAATACGACTCACTATAGGGAG
	ACTTCCTCCTCGTCCTTCT	AGTACTGCATCCACCAACA
<i>Ffgr2b</i>	AATTAACCCTCACTAAAGGGAG	TAATACGACTCACTATAGGGAG
	ACCGGCCCTCCTTCAGTTTAG	ACGAGCCAGCACTTCTGCATTG
<i>Sox2</i>	AATTAACCCTCACTAAAGGGAG	TAATACGACTCACTATAGGGAG
	ACCCCAACTATTCTCCGCCAG	AGCTTCTCGGTCTCGGACAAA
<i>Etv5</i>	AATTAACCCTCACTAAAGGGAG	TAATACGACTCACTATAGGGAG
	ACGAGTGGCCGCTCAGGAGTA	AGTGGCTACAGGACGACAAC
<i>Ptch1</i>	AATTAACCCTCACTAAAGGGAG	TAATACGACTCACTATAGGGAG
	ACTTGGTGTGGTGTGGAT	AACTGTGAGGCTCTGTGTA
<i>Bmp4</i>	AATTAACCCTCACTAAAGGGAG	TAATACGACTCACTATAGGGAG
	ACTCCGTCCCTGATGGGATTCT	ACTGCTCTCCTCCTCCTCCT

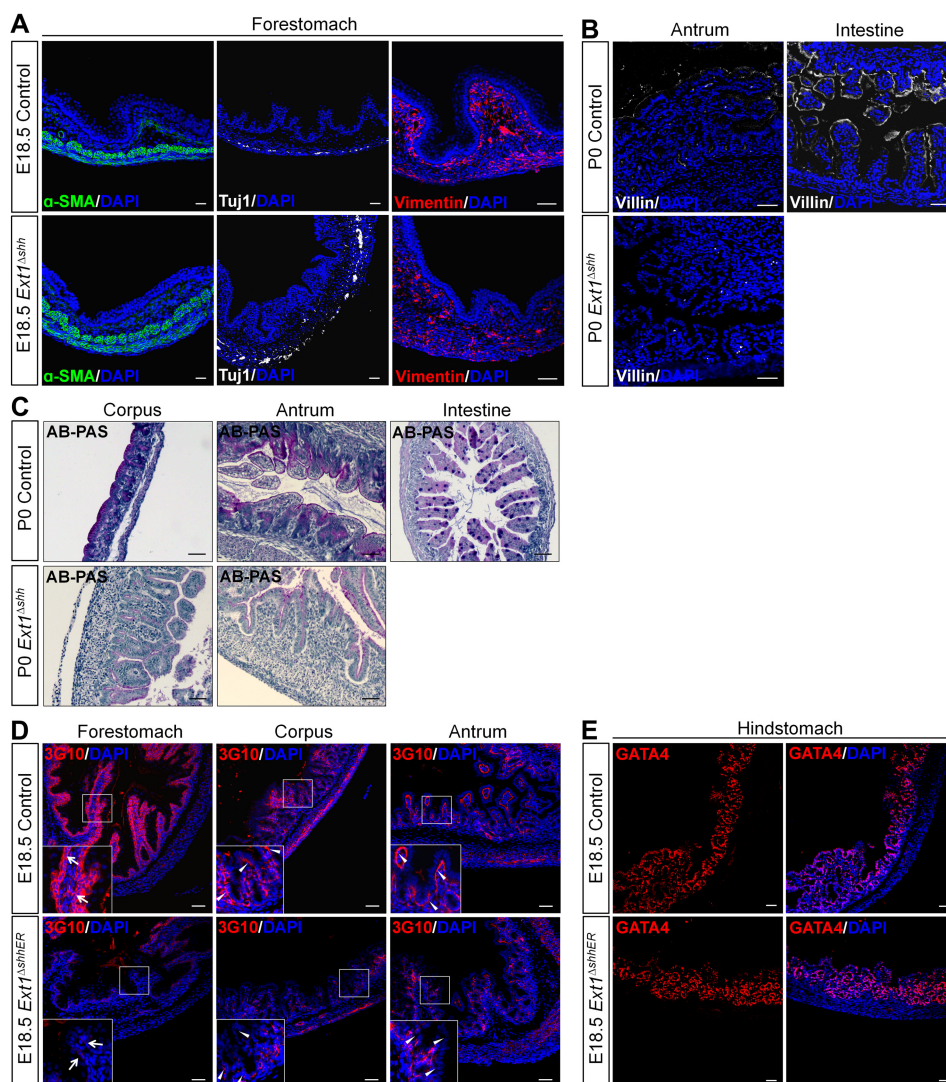
**Table S2. Primers for qPCR**

Name	Forward sequence	Reverse sequence
<i>Ext1</i>	CTCAGCTGGCTCTTGTCTCG	AGGAAAGAAAGGGCGCAGAG
<i>Atp4b</i>	CAGGAGAAGAAGTCATGCAGC	GAAACCTGCGTAGTACAGGCT
<i>Pgc</i>	ATGAAGAGTATCCGGGAGACC	TGGGCTCATAGAGTACACTGTAG
<i>Gif</i>	CCCTCTACCTCCTAAGTGTTCTC	CTGAGTCAGTCACCGAGTTCT
<i>Muc5ac</i>	CTGTGACATTATCCCATAAGCCC	AAGGGGTATAGCTGGCCTGA
<i>Fgf10</i>	TTTGGTGTCTTCGTTCCCTGT	TAGCTCCGCACATGCCTTC
<i>Etv4</i>	CGGAGGATGAAAGGCGGATAC	TCTTGGAAGTGACTGAGGTCC
<i>Etv5</i>	TCAGTCTGATAACTTGGTGCTTC	GGCTTCCTATCGTAGGCACAA
<i>Spry1</i>	ATGGATTCCCCAAGTCAGCAT	CCTGTCATAGTCTAACCTCTGCC
<i>Spry2</i>	TCCAAGAGATGCCCTTACCCA	GCAGACCGTGGAGTCTTTCA
<i>Ptch1</i>	AAAGAAGTGCAGCAAGTTTTTG	CTTCTCCTATCTTCTGACGGGT
<i>Hhip</i>	TGAAGATGCTCTCGTTTAAGCTG	CCACCACACAGGATCTCTCC
<i>Bmp4</i>	TTCCTGGTAACCGAATGCTGA	CCTGAATCTCGGCGACTTTTT
<i>Id1</i>	CCTAGCTGTTCGCTGAAGGC	CTCCGACAGACCAAGTACCAC
<i>Axin2</i>	TGACTCTCCTTCCAGATCCCA	TGCCCACACTAGGCTGACA
<i>Gapdh</i>	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA



**Fig. S1. Epithelial HS is necessary for stomach and intestine growth during embryonic development.**

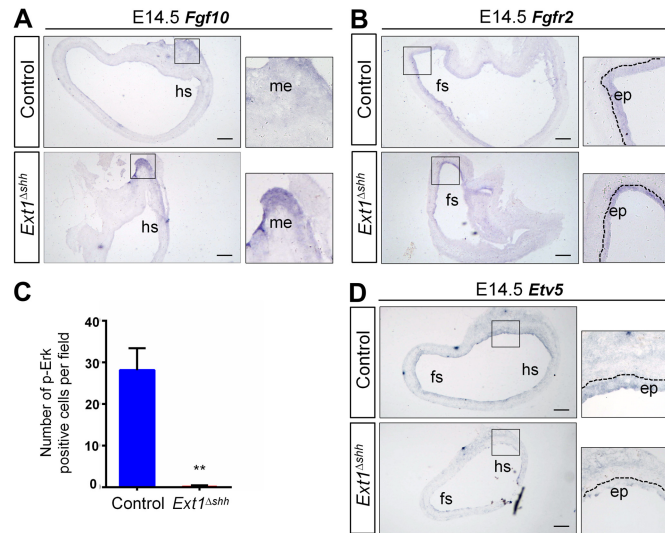
(A) Representative gross images of stomachs as indicated embryonic and postnatal stages of control and *Ext1<sup>Δshh</sup>* mice. Mutant stomach exhibits a reduction in its size. Asterisks denote the inflated glandular stomach in control and smaller glandular stomach in mutant. int, intestine. (B) Decreased epithelium proliferation at E18.5 indicated by pHH3 staining. Scale bars: 100  $\mu$ m.



**Fig. S2. Gastric identity is maintained after deletion of epithelial HS.**

(A) Immunostaining for antibodies against  $\alpha$ -SMA, Tuj1, and Vimentin indicates normal mesenchyme development in the forestomach. (B) Absence of Villin is demonstrated in control and mutant antrum, in contrast to its presence in the intestinal epithelium. (C) AB-PAS staining indicates that only pH neutral mucin (purple) is present in the stomach, while acid intestinal mucin (blue) is expressed neither in control nor in mutant stomach. (D) Examination of HS expression. After tamoxifen administration, epithelial HS is reduced both in forestomach and hindstomach. Arrows denote the epithelial HS on the cell surface in the forestomach. Arrowheads indicate

the basement membrane localization of HS in the glandular stomach. (E) Gata4 expression is not altered after inducible ablation of *Ext1*. Scale bar: 50  $\mu$ m



**Fig. S3. HS depletion leads to decreased Fgf signaling.**

(A and B) ISH of *Fgf10* (A) and *Fgfr2b* (B) on E14.5 control and mutant sections. Similar to control, distribution of *Fgf10* is observed in the mesenchyme of the posterior stomach in mutant, and strong expression of *Fgfr2b* is found in the epithelium of the anterior stomach. (C) Quantification of the number of p-Erk-positive epithelial cells. \*\*P<0.01. Error bars indicate s.e.m. n=3. (D) ISH on E14.5 paraffin sections demonstrates the reduced *Etv5* transcripts in mutant mucosa, compared to control. Right images show the high magnification micrographs of the boxed areas (A, B, and D). Dashed lines outline the epithelium and mesenchyme. fs, forestomach; hs, hindstomach; me, mesenchyme; ep, epithelium. Scale bar: 100 μm.