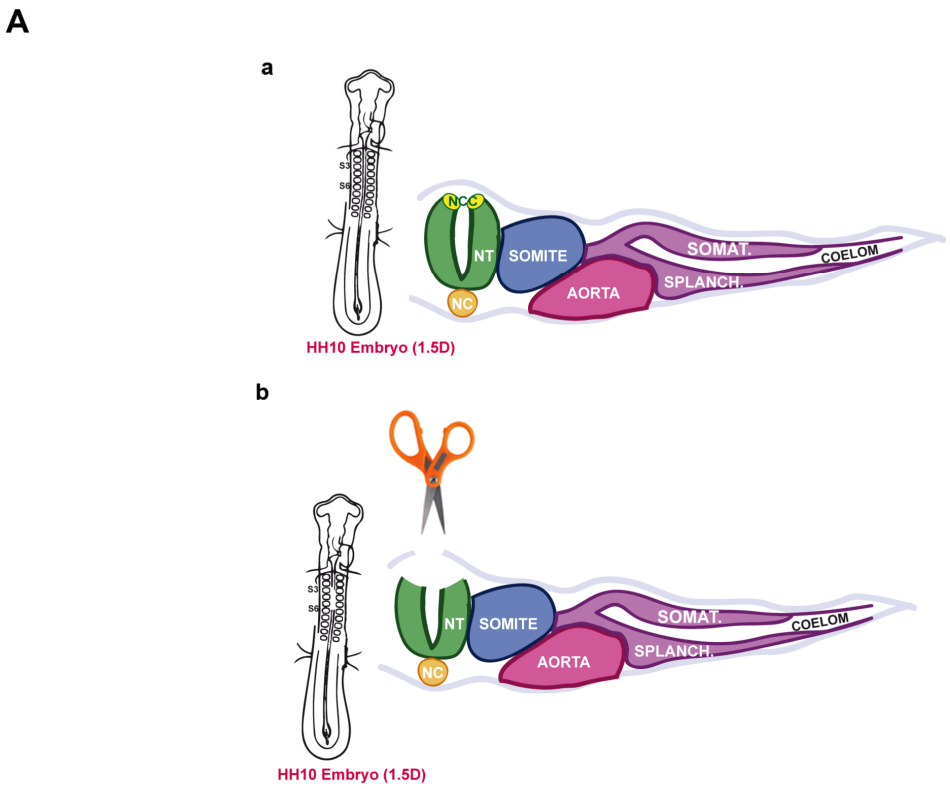

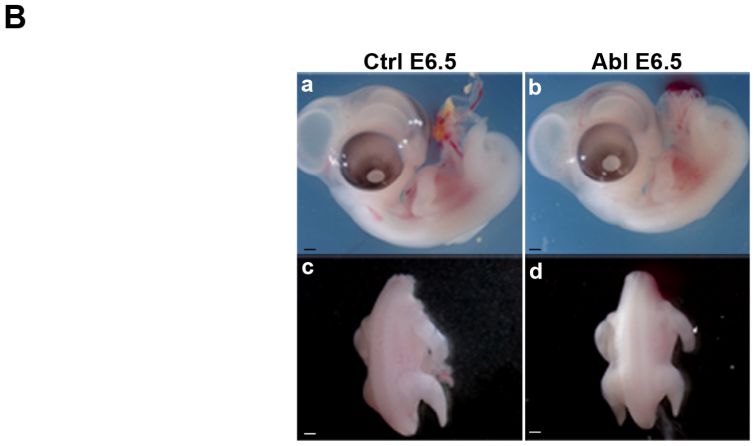

Fig. S1. Ablation of vENCCs in chick embryos. (A) Experimental design. *In ovo* ablation of the dorsal part of the neural tube between somites 3 and 6 where the neural crest cells that will colonize the GI tract are located in HH10 chicken embryos. (a) before and (b) after ablation. Abbreviations: NCC, neural crest cells; Nt, neural tube; NC, notochord; somat, somatopleura; splanch, splanchnopleura. (B) E6.5 control (Ctrl E6.5) (a,c) and vENCC-ablated embryo (Abl E6.5) (b,d). a and b: lateral views; c and d: dorsal views. Scale bars, 1mm.

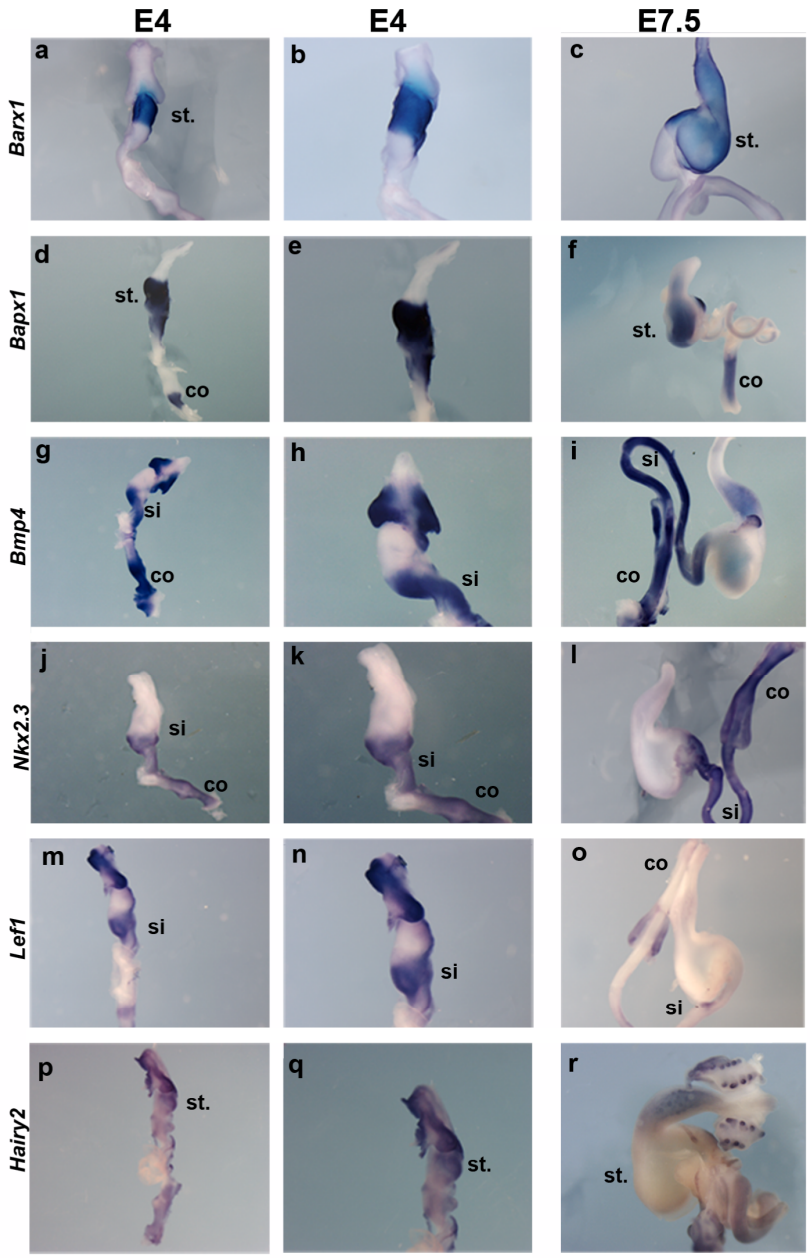

Fig. S2. Expression of *Barx1*, *Bapx1, Bmp4*,, *Nkx2.3, Lef1* and *Hairy2* in the gastrointestinal tract of chick embryos. Whole mount *in situ* hybridization analysis of E4 (a,b,d,e,g,h,j,k,m,n,p,q) and E7.5 (c,f,i,l,o,r) guts using the *Barx1* (a,b,c), *Bapx1* (d,e,f), *Bmp4* (g,h,i) *Nkx2.3* (j,k,l), *Lef1* (m,n,o) and *Hairy2* (p,q,r) probes. Abbreviations: st, stomach; si, small intestine; co, colon.

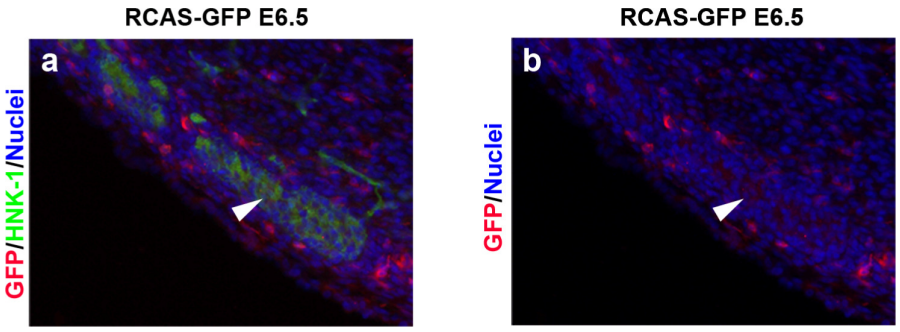

Fig. S3. Efficiency and trophism of avian RCAS retroviruses. Retroviruses expressing GFP were injected in the splanchnopleural mesoderm, which will give rise to the mesenchyme of HH10 embryos. Infected-stomachs (RCAS-GFP E6.5) were fixed at E6.5. Paraffin-embedded stomach sections were analysed by immunofluorescence using anti-GFP (red) and anti-HNK-1 (green) antibodies to specifically detect vENCCs. Nuclei were stained with Hoechst (blue). White arrowheads in a and b show that the HNK-1-positive cells do not express the GFP protein while the mesenchyme does, indicating that GFP retroviruses specifically target the mesenchyme but not the vENCCs.

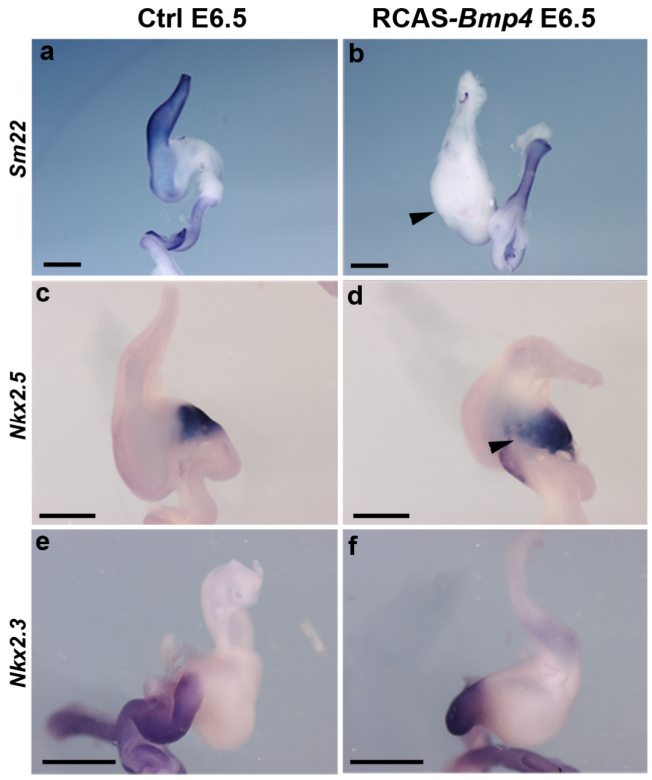

Fig. S4. Impact of *Bmp4* misexpression in the chick developing stomach. Retroviruses expressing GFP (Ctrl) or BMP4 (RCAS-*Bmp4*) were injected in the splanchnopleural mesoderm, which will give rise to the mesenchyme, of HH10 embryos. Control (CTRL E6.5) (a,c,e) and BMP4-overexpressing (RCAS-*Bmp4* E6.5) (b,d,f) guts were dissected at E6.5. Whole mount *in situ* hybridization shows that sustained BMP4 expression impairs *Sm22* expression (as shown by the black arrowhead in b) and promotes *Nkx2.5* expression (black arrowhead in d), but does not affect the expression of *Nkx2.3* (e,f). Scale bars, 1mm.

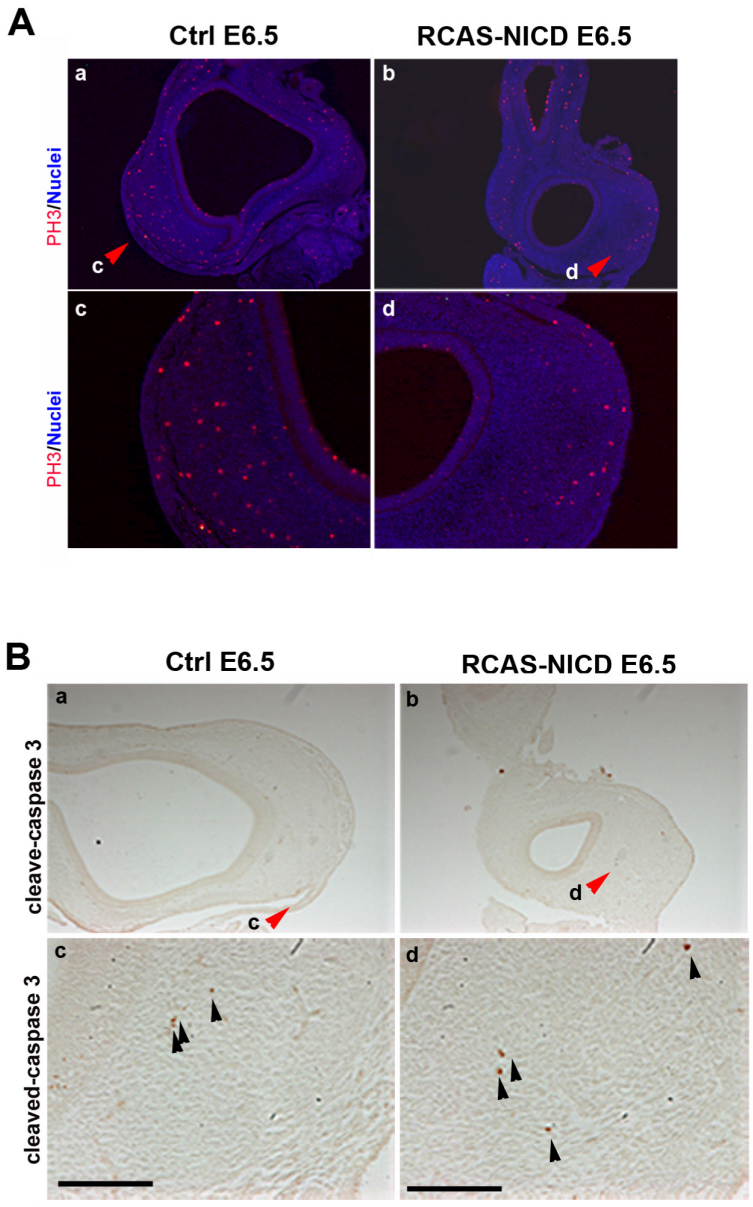

Fig. S5. Impact of NICD missexpression on mesenchymal cell proliferation and apoptosis. Retroviruses expressing control GFP (Ctrl) or NICD (RCAS-NICD E6.5) were injected in the splanchnopleural mesoderm, which will give rise to the stomach mesenchyme, of St.10 chick embryos. Control and NICD-misexpressing stomachs were dissected at E6.5. (A) Immunostaining analysis of paraffin-embedded stomach sections from (a,c) control (Ctrl E6.5) and (b,d) and NICD-misexpressing stomachs (RCAS-NICD E6.5) using anti-PH3 antibody (red; G2/M transition marker). Nuclei were labeled with Hoechst (blue); c and d are

magnified views of the regions indicated by the red arrowheads in a and b. Immunochemistry analysis of paraffin-embedded stomach sections from (a,c) control (Ctrl E6.5) and (b,d) and NICD-misexpressing stomachs (RCAS-NICD E6.5) using anti-cleaved-caspase3 antibody. c and d are magnified views of the regions indicated by red arrowheads in a and b. Black arrowheads point to cleaved-caspase 3-positive apoptotic cells. Scale bars, 100μm.

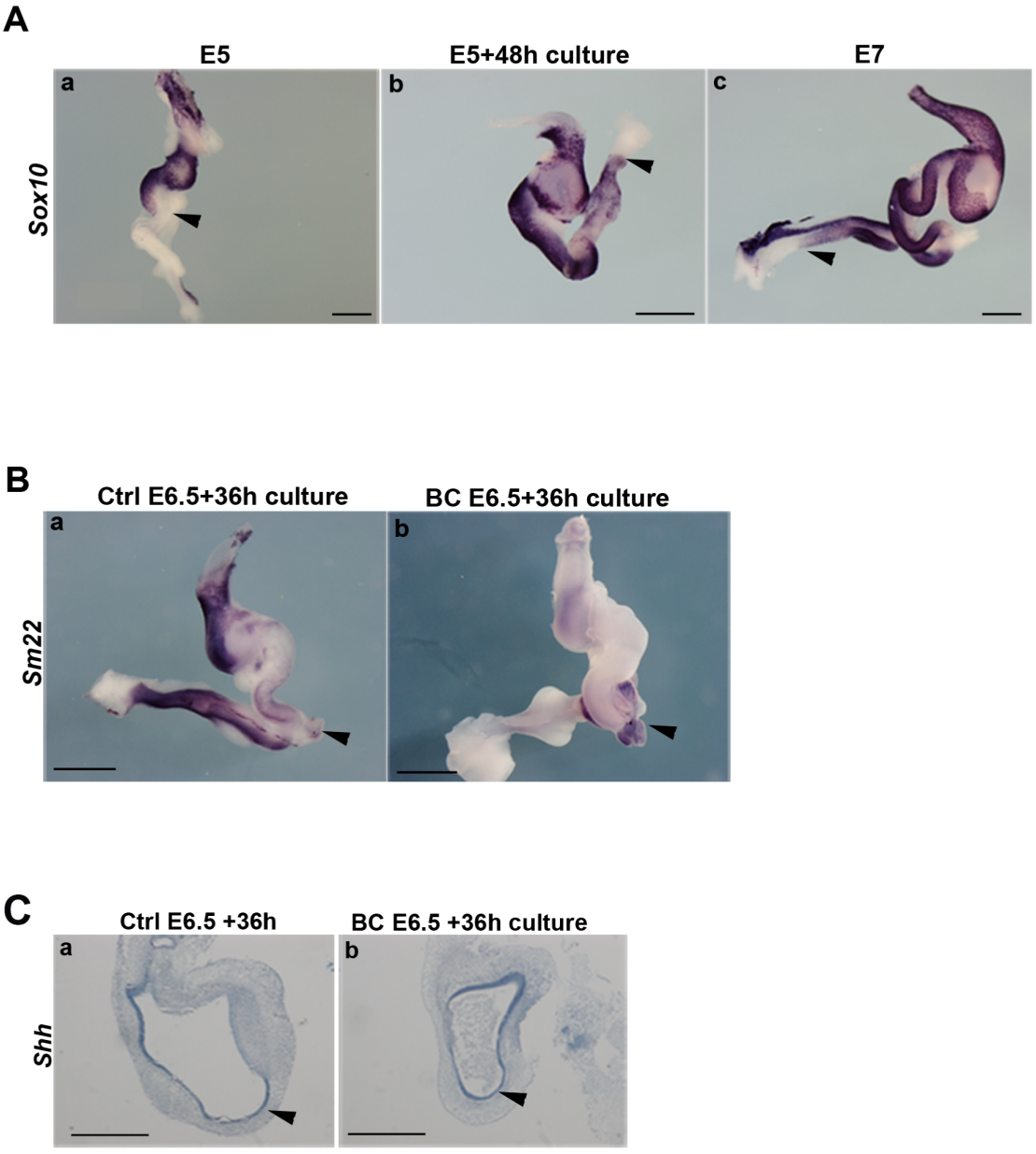

Fig. S6. Control experiments in GI organ culture. (A) Chick guts were dissected at E5 (a) and cultured in cell culture medium at 37°C for 48 hours (E5+48h culture) (b) or dissected at E7 (c). Whole mount *in situ* hybridization analysis using the *Sox10* probe. At E5, migrating vENCCs are in the duodenum (black arrowhead in a). After 48 hours of in vitro cell culture, vENCCs reached the colon (black arrowhead in b) as in E7 gut (black arrowhead in c). Scale

bars, 1mm. (B) Chick guts were dissected at E6.5, incubated (b) or not (control, a) in a 0.04% solution of Benzalkonium Chloride (BC) for 10 minutes, washed several times in cell culture medium and then cultured in cell culture dishes at 37°C for 36 hours. Whole mount *in situ* hybridization using *Sm22* probe shows that BC does not have a direct effect on SMCs as demonstrated by the comparable expression of *Sm22* in blood vessels of both embryos (black arrowheads in a and b). Scale bars, 1mm. (C) *In situ* hybridization of paraffin-embedded sections of control (a) and BC-treated stomach (b) using the *Shh* probe. Scale bars, 500 μm. BC-treated and control stomachs show comparable levels of *Shh* expression in the epithelium (black arrowheads).

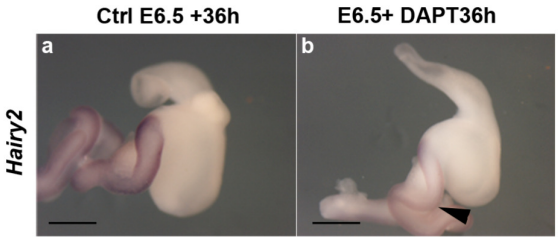

Fig. S7. Gastrointestinal organ culture in the presence of DAPT. Chick guts were dissected at E6.5, incubated or not in a 0.04% solution of Benzalkonium Chloride (BC) for 10 minutes, washed several times in cell culture medium and then incubated at 37°C with DAPT for 36 hours. Chick guts were dissected at E6.5 and cultured in cell culture medium at 37°C for 36 hours in the absence (a) or presence (b) of DAPT. *In situ* hybridization experiment using the *Hairy2* probe. In these conditions, DAPT treatment strongly reduces the expression of the Notch target gene *Hairy2* as indicated by black arrow in b. Scale bars, 1mm.

Table S1. PCR primers used to RT-qPCR analysis.

Targets	Forward primer (5'-3')	Reverse primer (5'-3')	Amplicon (bp)
aSma	CTG TAT GCT TCT GGG CG	GCA GTG GTC ACA AAG GAG	188
Sox10	CCA TCC AGC CAC CAG CA	GAC CCT CAC TCC ATG T	192
Sm22	TGA GCA GGG ATG TCC AGT	AGC CAA TGA TGT TCT TGC C	501
Myocd	CTT CTG TCA GCA ACA CCC	AAG ACT GCG ACT GGT AAC	300
Bmp4	CTT CGT CTT CAA CCT CAG CA	GAC AGC GGC TTC ATC ACT	150
Bapx1	GCA GGT GTT CGA GCT GGA	CTG TCT CTG GTC GTC GC	229
Nkx2.3	ACC TGG AGC ACC ACT TTC A	CGT AGC TGT CGGCAGAG	159
Barx1	CCG CTA CCG CAG TTT CA	GCT CCG CCT TCA GAA CG	152
CdxA	GTC TTC GGT ATT GGT AGC CC	GCT GAG ATT TAT TCT GCT TCG AG	206
Hairy1	CGT CCA ACT GCC TCC TAC	CAG AGC CAG GTG GGA AC	164
Hairy2	GTA CCG CGC CGG CTT CAG	TCA CCA GGG CCT CCA GAC	447
Lef1	AGT CCT CGC TGG TCA AC	CGA TGG TCC CTT GCT GT	174
Shh	CGT AGC TGT CGG CAG AG	TTT CGC TGC CAC TGA GTT T	180
Gapdh	CGT CCT CTC TGG CAA AG	TCA CGC TCC TGG AAG ATA G	177