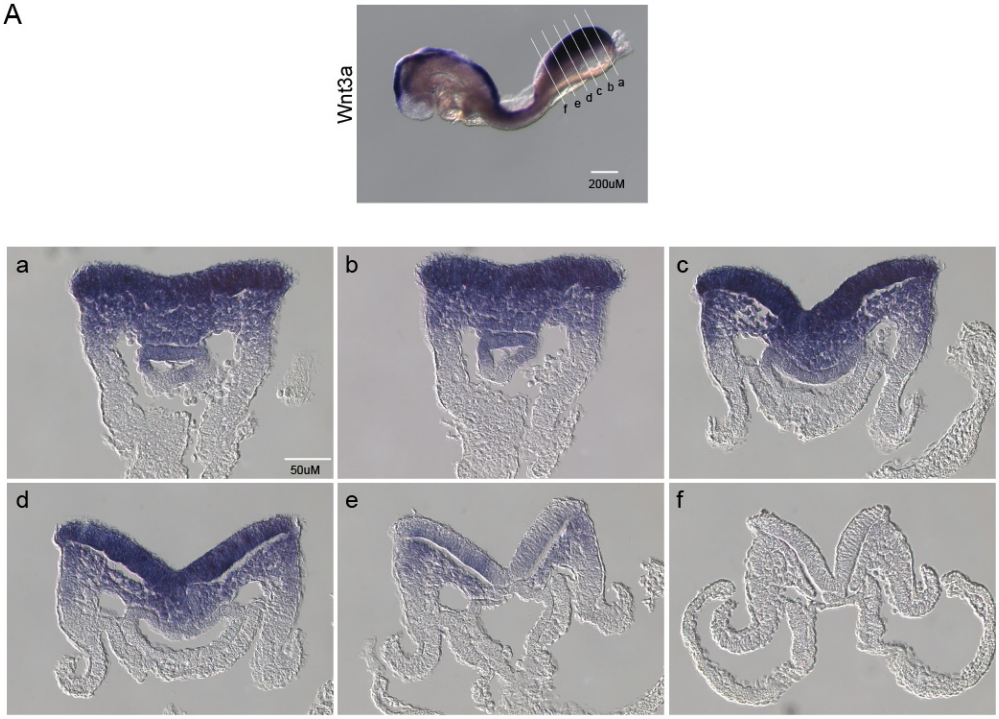
Supplemental Data

A



B

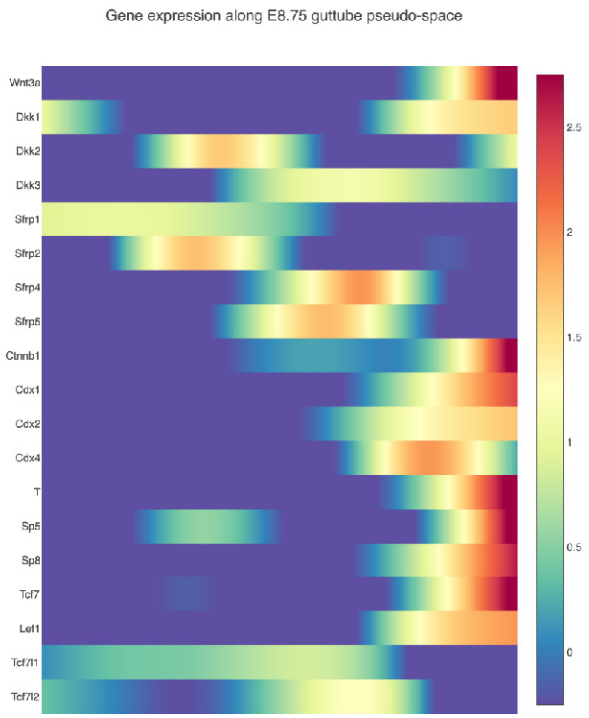


Fig.S1. The expression of Wnt3a/β-catenin signaling pathway components in the extending gut.

A. WISH of *Wnt3a* expression in e8.5 (8-10 somite) wildtype embryo. (a-f) Transverse serial sections through the *Wnt3a* expression domain in the posterior region of the embryo, from posterior to anterior, illustrate the graded expression along the DV axis.

B. Heat map of expression of select components of the Wnt3a/β-catenin signaling pathway along the anterior-posterior pseudo-space. Columns represent single cells ordered by pseudo-space, rows represent a single gene. Heat map generated from the endoderm explore website (Nowotschin et al., 2019).

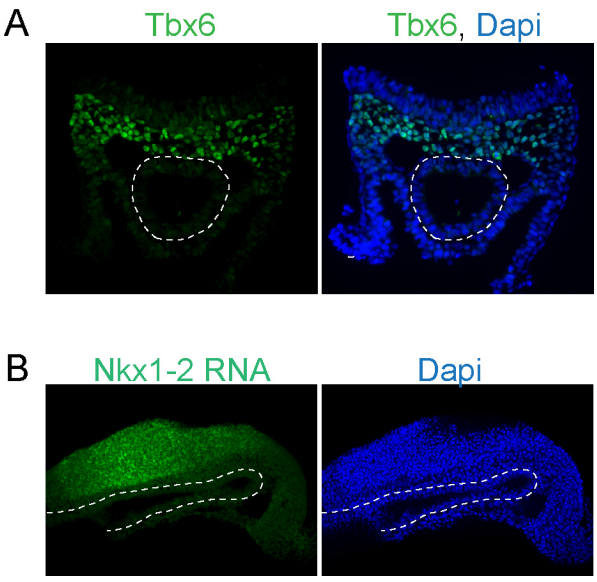


Fig. S2. The PSM and NMP markers Tbx6 and *Nkx1.2* are not expressed in the E8.5 hindgut epithelium.

A. Immunofluorescent detection of Tbx6 protein expression in a cross-section through the caudal end of an E8.5 embryo. The hindgut is outlined with dashed lines.

B. Sagital optical section of fluorescent HCR in situ of *Nkx1.2* RNA expression in E8.5 embryo.

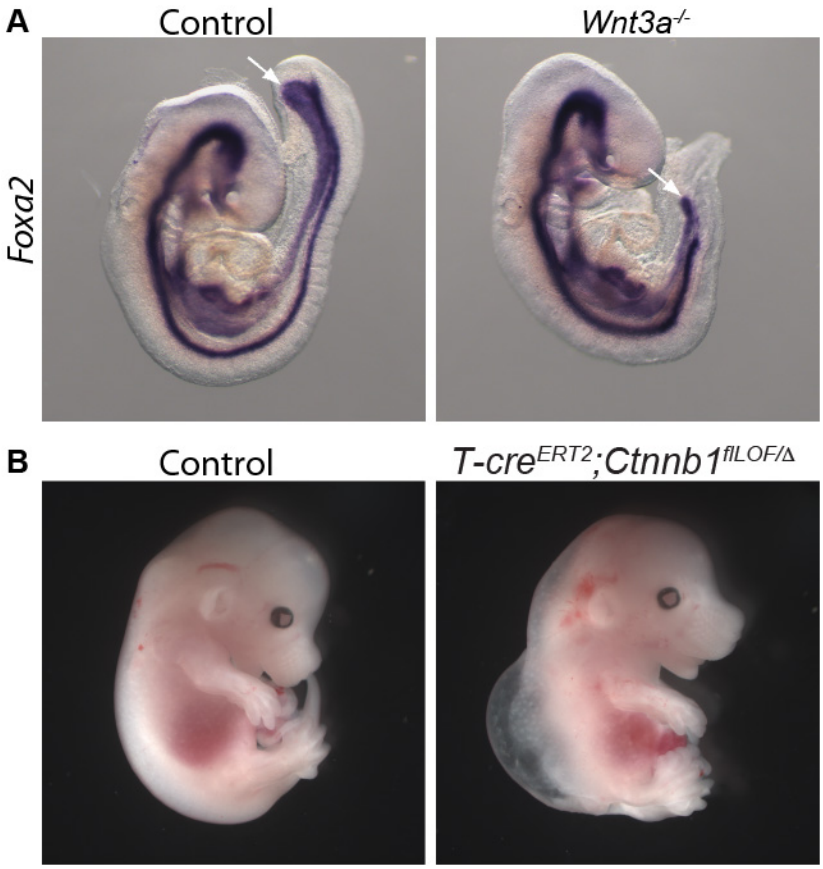
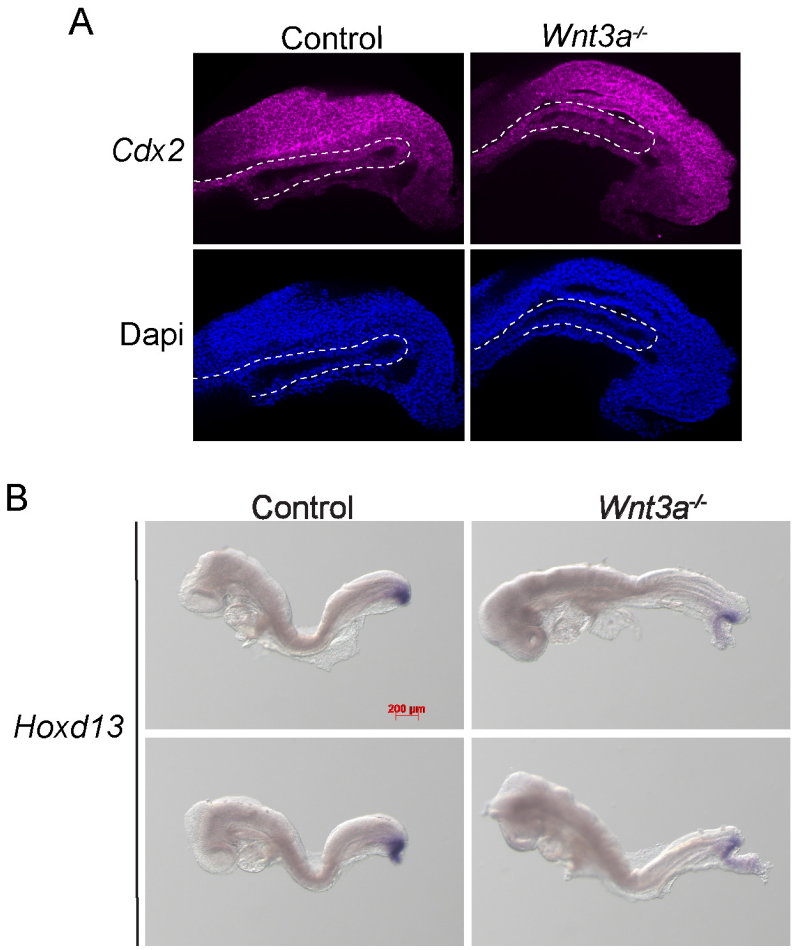


Fig.S3. *Wnt3a*$^{-/-}$ and *T-Cre*^{ERT2}*;Ctnnb1*$^{flLOF/\Delta}$ mutants.

A. WISH of *Foxa2* expression in e9.5 control littermate and *Wnt3a*$^{-/-}$ mutants illustrating defective hindgut and notochord elongation. Arrows indicate caudal terminus of the hindgut.

B. E14.5 control and *T-Cre*^{ERT2}*;Ctnnb1*$^{flLOF/\Delta}$ fetuses. A single dose of TAM was administered at E7.5.



A. **Fig. S4. *Cdx2* and *Hoxd13* remain caudally localized in *Wnt3a*$^{-/-}$ mutants.** Fluorescent in situ HCR of *Cdx2* expression in E8.5 control littermate and *Wnt3a*$^{-/-}$ mutants show persistent caudally localized *Cdx2* expression in the mutant hindgut and PS.

B. Chromogenic WISH showing *Hoxd13* expression in E8.5 control littermate and *Wnt3a*$^{-/-}$ mutants.

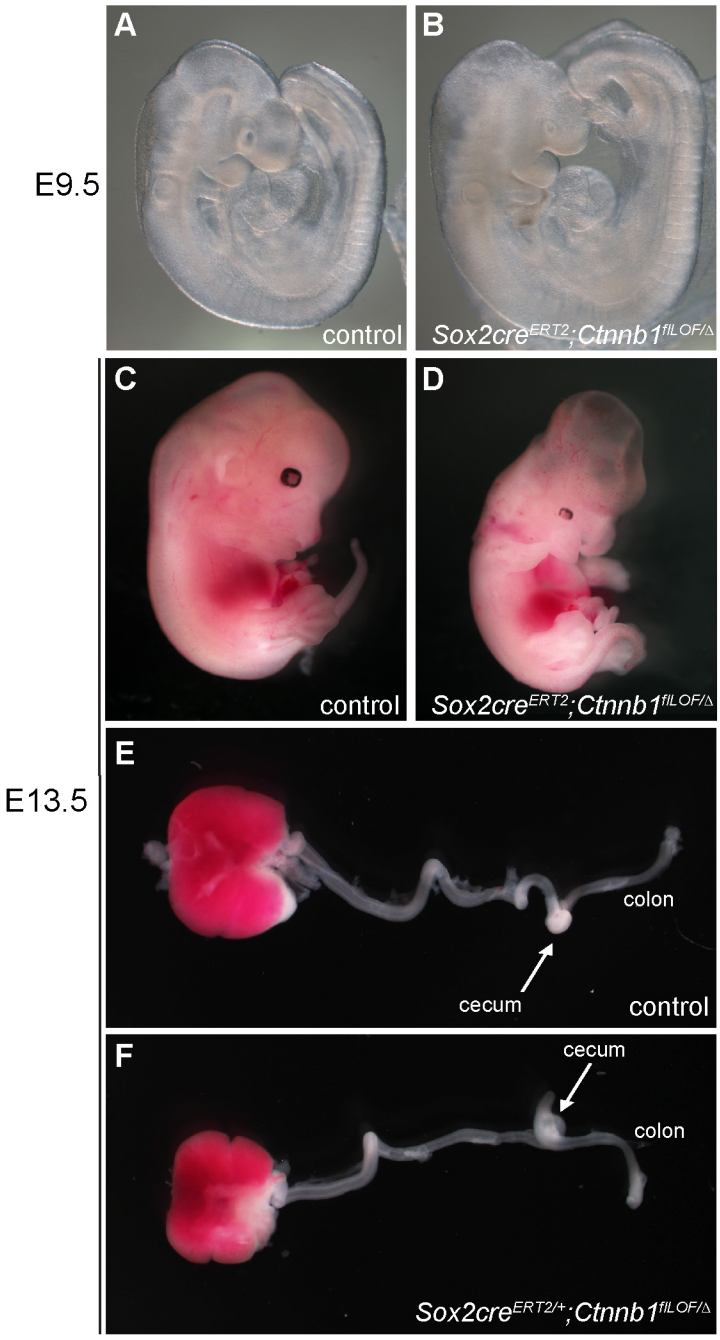


Fig. S5. Targeting β-catenin in dorsal hindgut progenitors using *Sox2-CreER.*

(A,B) E9.5 control *Sox2-CreER/+;Ctnnb1*$^{flLOF/+}$ embryo (A) and *Sox2-CreER/+;Ctnnb1*$^{flLOF/\Delta}$ (β-catenin loss-of-function) mutant (B).

(C,D) E13.5 control *Sox2-CreER/+;Ctnnb1*$^{flLOF/+}$ fetus (C) and *Sox2-CreER/+;Ctnnb1*$^{flLOF/\Delta}$ mutant (D).

(E,F) Isolated GI tracts from control *Sox2-CreER/+;Ctnnb1*$^{flLOF/+}$ fetus (E) and *Sox2-CreER/+;Ctnnb1*$^{flLOF/\Delta}$ mutant (F) showing no loss of the colon.

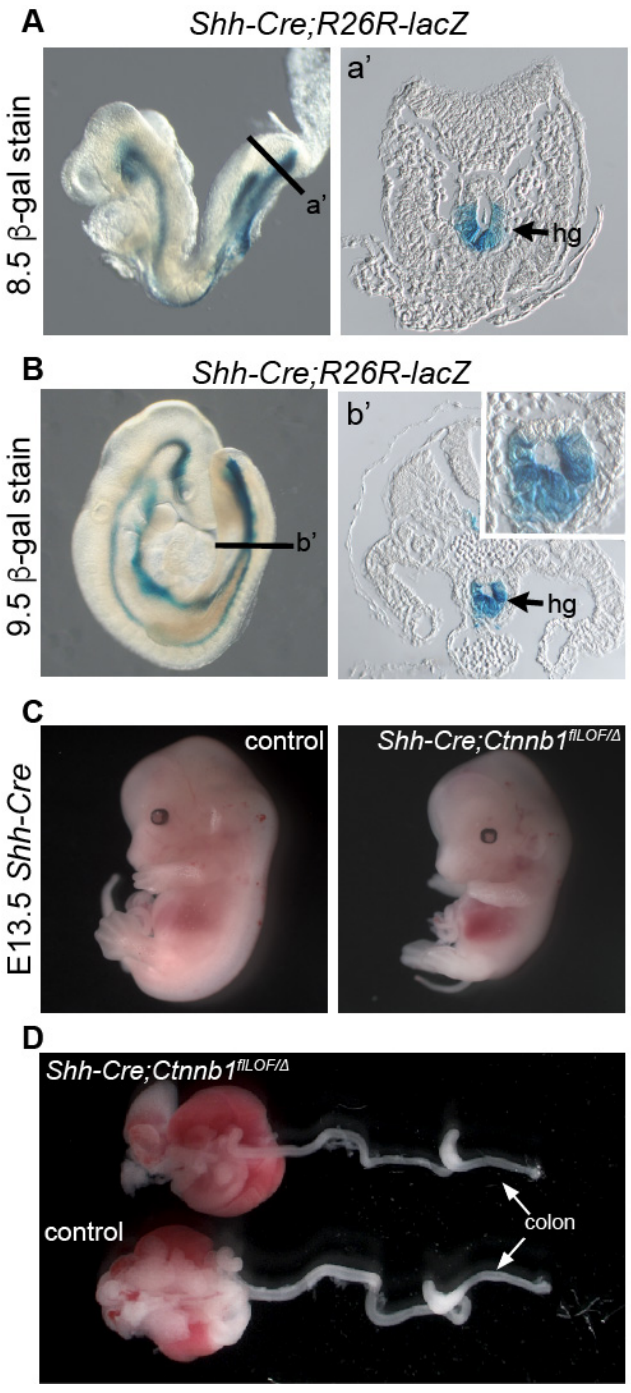


Fig. S6. Targeting β-catenin in the ventral hindgut using *Shh-Cre*.

(A) Whole-mount β-gal stain of E8.5 *Shh-Cre;R26R-lacZ* embryo and transverse section through distal hindgut (a').

(B) Whole-mount β-gal stain of E9.5 *Shh-Cre;R26R-lacZ* embryo and transverse sections through the hindgut region (b'). Inset: high power magnification of hindgut.

(C) Whole E13.5 control (left) and *Shh-Cre;Ctnnb1*$^{flLOF/\Delta}$ (β-catenin loss-of-function) mutant.

(D) Isolated GI tracts from control (bottom) and *Shh-Cre;Ctnnb1*$^{flLOF/\Delta}$ (top) showing no loss of the colon.

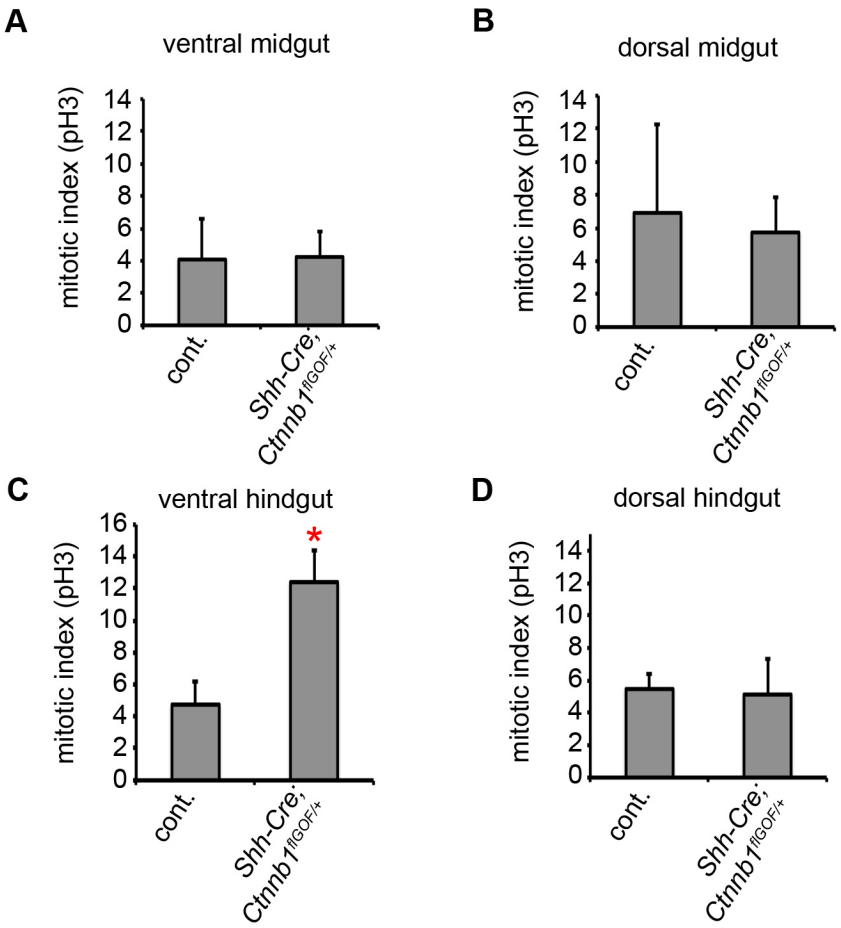


Fig. S7. Cell proliferative effects of activating of β-catenin signaling in the ventral hindgut in *Shh-Cre;Ctnnb1$^{flGOF/+}$* embryos.

Graph of the mitotic index determined by pH3 detection in the following regions of E9.5 control and *Shh-Cre;Ctnnb1$^{flGOF/+}$* embryos:

(A) ventral midgut

(B) dorsal midgut

(C) ventral hindgut, showing significantly higher mitotic index in the ventral hindgut of *Shh-Cre;Ctnnb1$^{flGOF/+}$* embryos (Data are mean±s.e.m. $P<2.05x10^{-4}$).

(D) dorsal hindgut.