

E15 Control stomach

E15 Evans Blue injection stomach after 1 hour

**Fig. S1. Intra-oral administration of Evans Blue solution.** After opening a small window in the extraembryonic membranes close to the head, the solution was deposited directly in the E15 chick embryo beak with a capillary pipette and the egg returned to the 38°C incubator. One hour later, the whole GI tract was dissected and the dye was observed in the stomach lumen (black arrows).

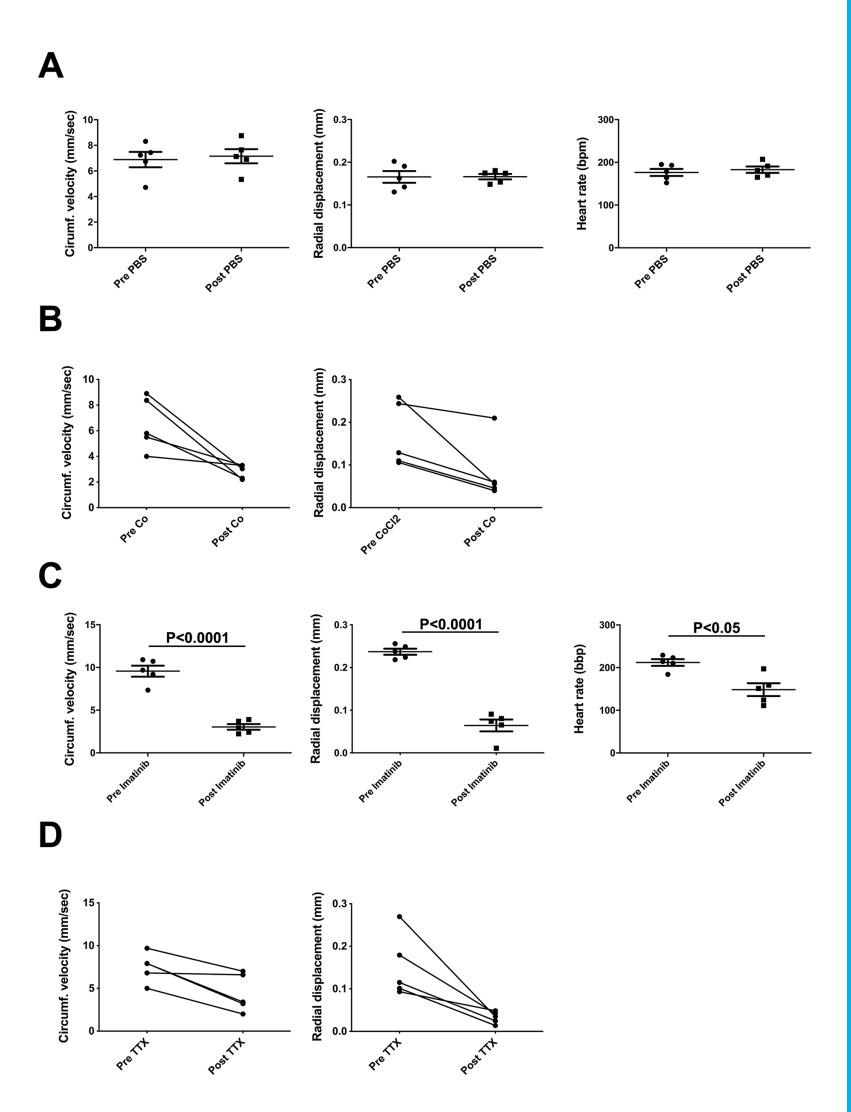
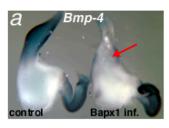
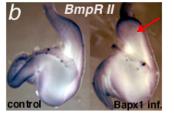


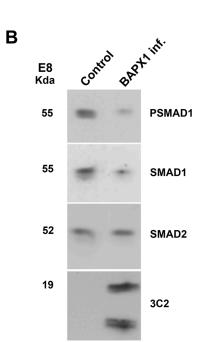
Fig. S2. Impact of intra-oral administration of PBS and drugs on stomach motility. (A) Evaluation of the impact of intra-oral administration of PBS (negative control), compared to untreated, on the stomach circumferential strain velocity, stomach radial strain changes, and heart rate in E15 chick embryos (n=5). PBS administration did not alter the stomach circumferential velocity (from 6.88  $\pm$  0.92 mm/sec before to 7.15  $\pm$  0.83 mm/sec after PBS administration, not significant) and radial displacement (from 0.165  $\pm$  0.02 mm before to 0.166  $\pm$  0.012 mm after treatment, not significant) and the heart rate (176.4  $\pm$  14.7 bpm before and 182.8  $\pm$  12.9 bpm after treatment, not significant). (B) Changes of circumferential strain velocity and radial strain in individual stomachs before and after Cobalt Chloride (CoCl2) administration in E15 chick embryos (n=5). (C) Impact of imatinib (20  $\mu$ M) on stomach circumferential strain velocity, stomach radial strain changes, and heart rate in E15 chick embryos (n=5). (D) Changes of circumferential strain velocity and radial strain in individual stomachs before and after tetrodotoxin (TTX) administration in E15 chick embryos (n=5).

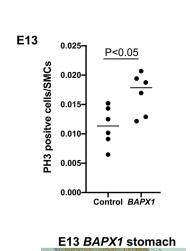


E8



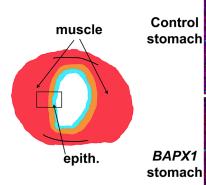


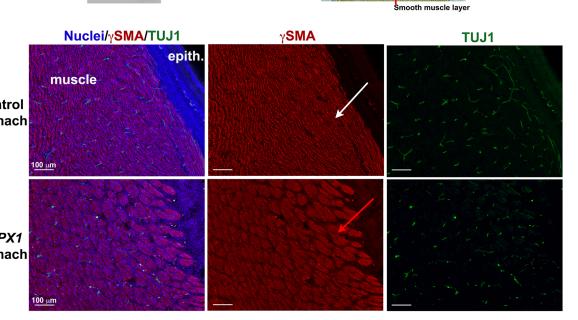




ENCDCs







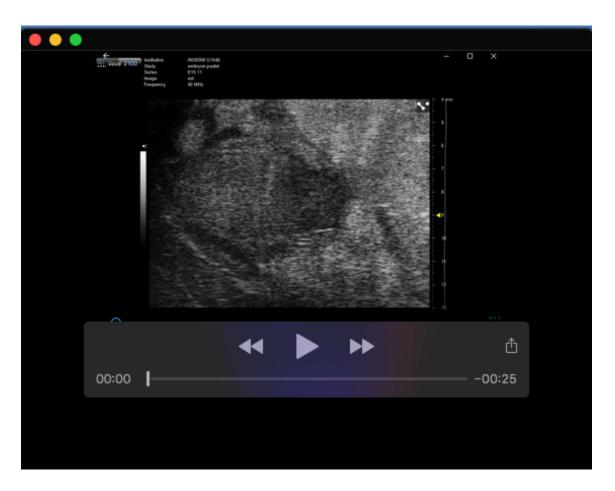
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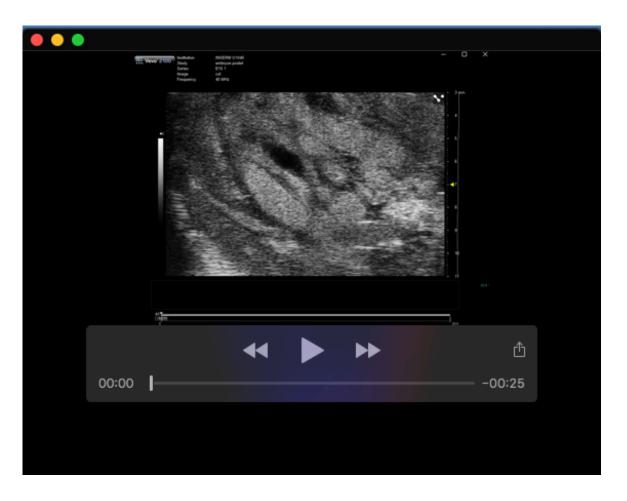
**Fig. S3. Inhibition of BMP signaling activation in** *BAPX1*-overexpressing stomach. (A) *In situ* hybridization analysis of whole-mount E8 stomach overexpressing BAPX1 demonstrated that BAPX1 leads to *BMP4* and *BMPRII* mRNA inhibition (red arrows in panels a, b). (B) Western blot analysis of protein extracts from E8 stomach overexpressing BAPX1 showed inhibition of PSMAD1/5/8 (namely PSMAD1) and SMAD1 expression. (C) Quantification of PH3-positive cells in Control and the *BAPX1*-overexpressing stomach smooth muscle layer at E13. Proliferation rates were assessed by counting the number of PH3-positive cells relative to the total number of aSMA-positive nuclei in the section. Six control stomachs and six *BAPX1*-overexpressing stomachs were analyzed. One slide for each stomach was analyzed. \*P<0.05 (Student's *t*-test). Mean ratio of PH3-positive cells/SMCs was 0.01130 (for Control) and 0.01681 (for BAPX1). Error bars indicate s.e.m. (D) Transversal paraffin sections of E13 *BAPX1*-expressing stomach analyzed by immunohistochemistry with an anti-gag (3C2) antibody. Red arrow indicates the presence of retroviral infection in the smooth muscle layer and black arrows its absence in the stomach epithelial layer and vENCDCs. Scale bars: 100µm. (E) Transversal paraffin sections of E13 *BAPX1*-expressing and Control stomachs close to the epithelium (box drawn in the left panel). Nuclei were visualized with Hoechst. Antibodies against smooth muscle cells (γSMA), and neuronal cells (TUJ1) were used. Scale bars, 100 µm.



Movie 1. Movie obtained using the Vevo2100 ultrasound system to visualize stomach motility at E15.

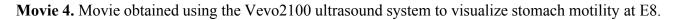


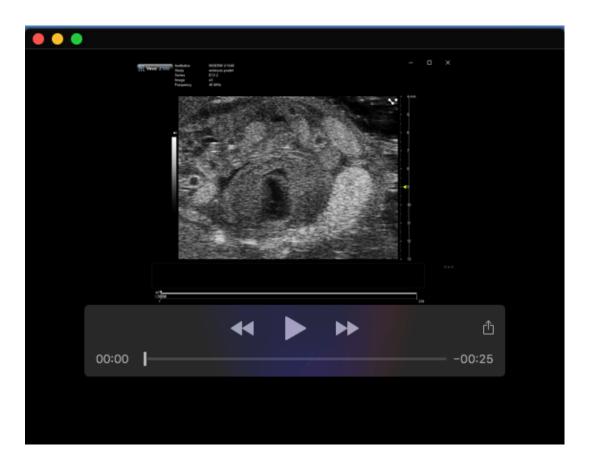
Movie 2. Movie obtained using the Vevo2100 ultrasound system to visualize small intestine motility at E15.



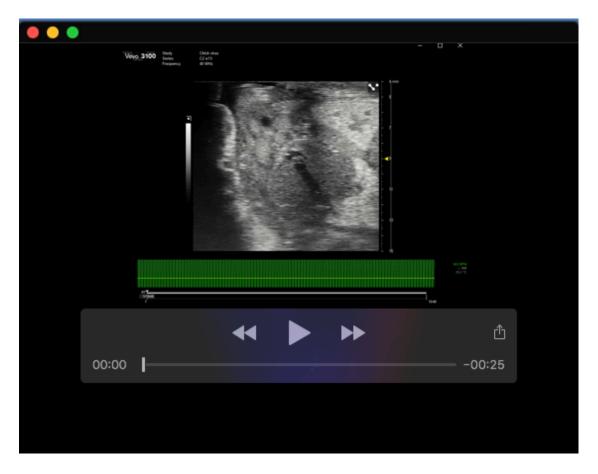
Movie 3. Movie obtained using the Vevo2100 ultrasound system to visualize colon motility at E15.







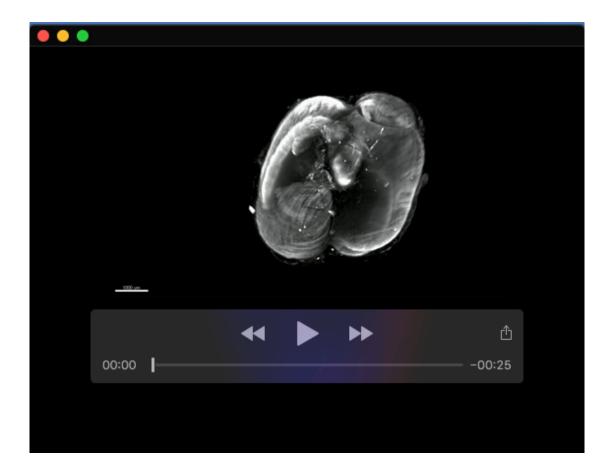
Movie 5. Movie obtained using the Vevo2100 ultrasound system to visualize stomach motility at E13.

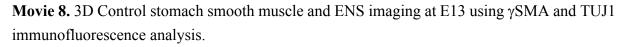


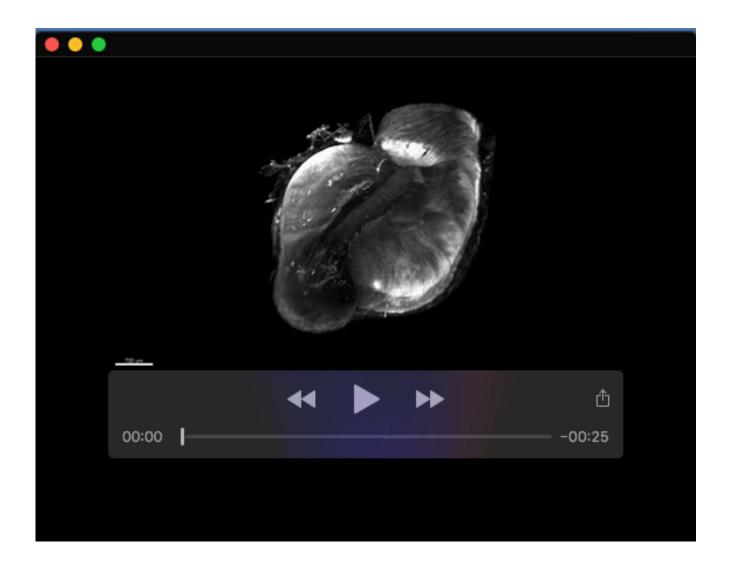
**Movie 6.** Movie obtained using the Vevo3100 ultrasound system to visualize stomach motility of E13 Control-stomach.



**Movie 7.** Movie obtained using the Vevo3100 ultrasound system to visualize stomach motility of E13 *BAPX1*-overexpressing stomach.







**Movie 9.** 3D *BAPX1*-overexpressing stomach smooth muscle and ENS imaging at E13 using  $\gamma$ SMA and TUJ1 immunofluorescence analysis.