



**Figure S1. Impact of SB431542 treatment on *pitx* expression.** In-situ results showing typical *pitx* expression in the heads of tailbud stage embryos (St. 22) dechorionated shortly before fixation and treated as indicated. (**a'**) Wildtype expression pattern. (**a''**) 5 $\mu$ M treatment leads to loss of lateral *pitx* expression in the epidermis and internal germ layers, note that Nodal-independent expression in the anterior neural boundary (Yoshida and Saiga, 2008) is not impacted. (**a'''**) 0.05 $\mu$ M treatment appears to reduce *pitx* expression particularly in the left epidermis, but does not eliminate nodal-dependent lateral expression. The *Ci-Pitx* probe was generated using T7 polymerase on the *Ci-Pitx* cDNA in Gene Collection library clone GC30b01 (Satou et al., 2002) according to standard protocols. To ensure that dechoronation did not interfere with left-right patterning, embryos were dechorionated just prior to fixation using the same protocol as employed for zygotic dechoronation. Standard in-situ hybridization protocols were also employed (Cooley et al., 2011).

**To view supplemental videos, please use the following link:**

<https://drive.google.com/drive/folders/0B39IHb5TppUKZ2duVjc5ZklXQzA?usp=sharing>

**Video S1. Ion flux is required for neurula rotation.** Time-lapse of DMSO-treated (left panel) and 40 $\mu$ M omeprazole-treated (right panel) neurula stage embryos. Rotation is apparent on the left, while rotation is absent on the right. Movement at the end of the video in the right panel is associated with tail morphogenesis.

**Videos S2-4. Live-imaging of *Ciona* heart progenitor cells.** Ventral projections from a confocal time-lapse of 3 different embryos expressing *Mesp>GPI::GFP* to label heart progenitors. No consistent protrusive activity is observed. Protrusive activity after heart progenitors have shifted is associated with ASMPs.