

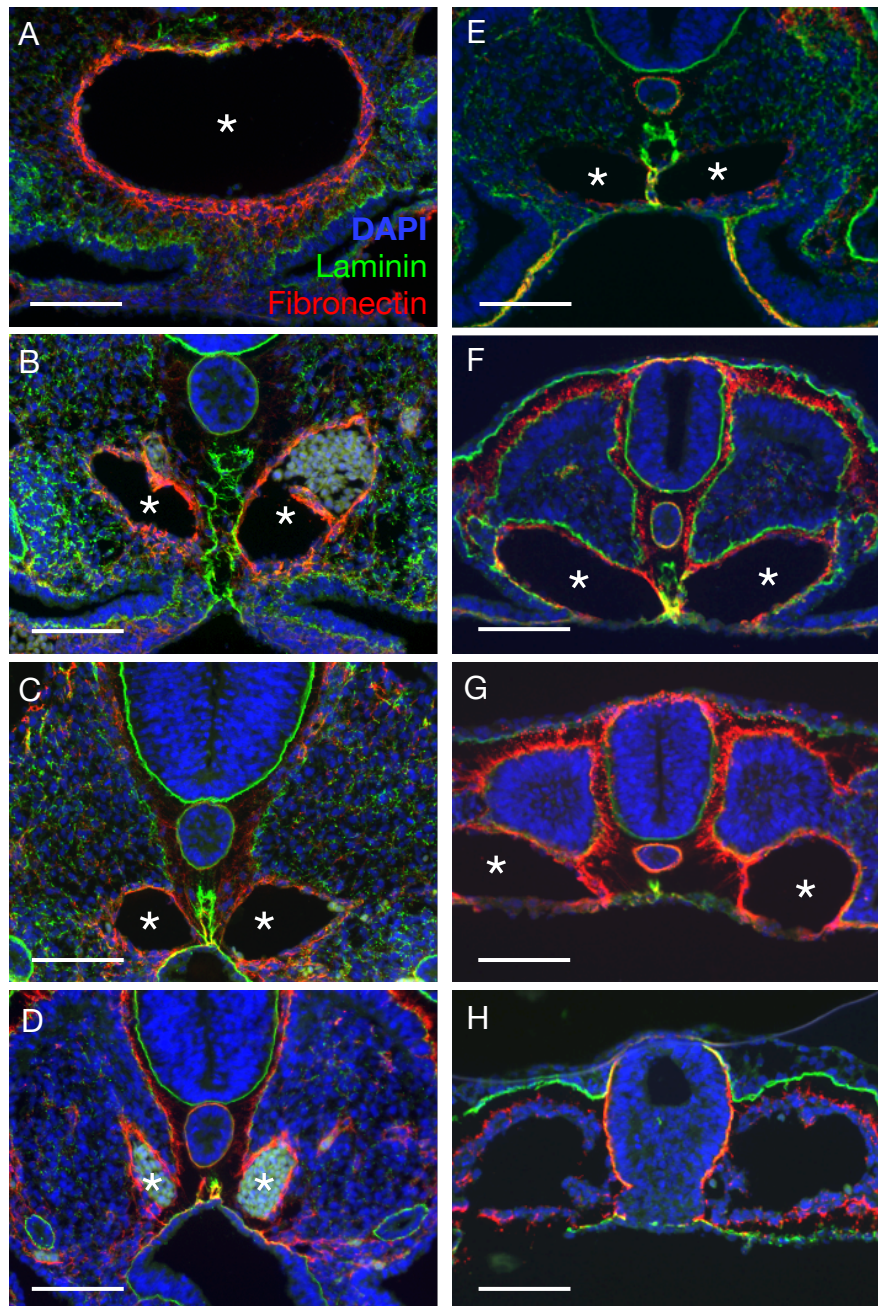
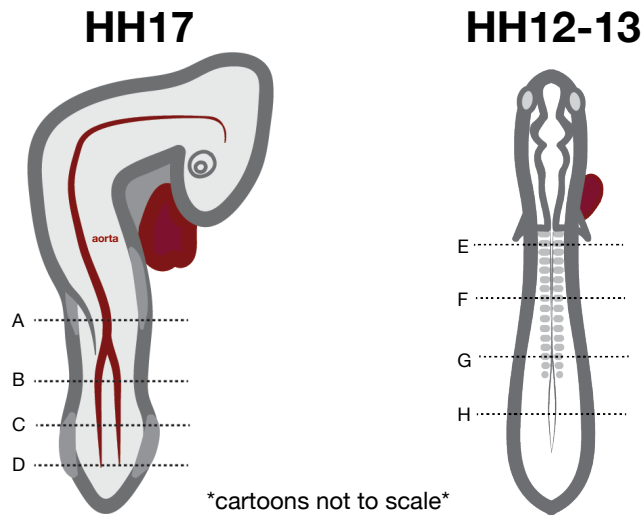
**Supplemental Figure 1. SEM of cross-sections of chicken embryos at different stages of midline development.** Yellow boxes indicate position of higher magnification images on right.

## Supplemental Figure 2.

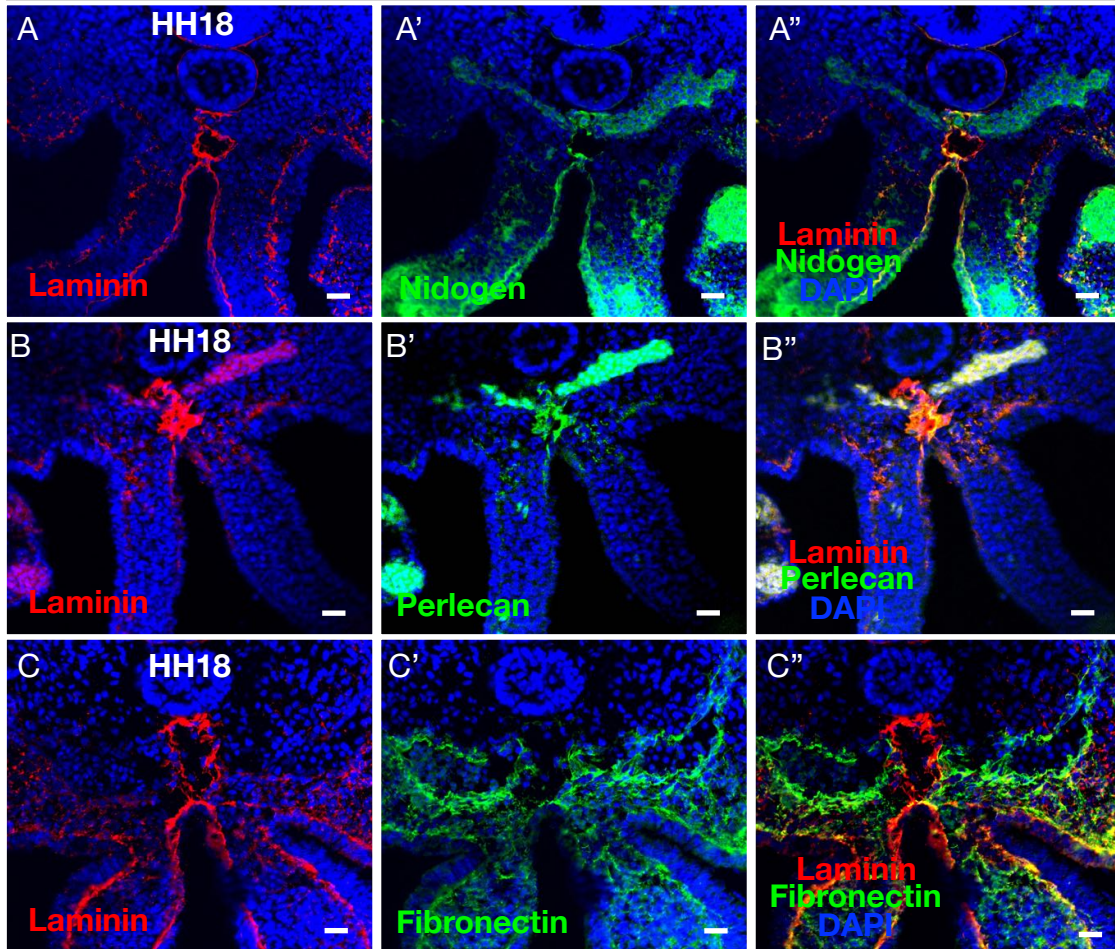
### Pseudotime kinetics of the midline along the anterior-posterior axis.

(A-D) Laminin (green) and fibronectin (red) highlight the midline and aorta/e, respectively, in this HH17 embryo. The maturation of the midline occurs in an anterior-to-posterior wave (from **A** to **D**), as does the fusing of the two branches of the aorta into one (A-D, asterisks).

Immunohistochemistry images were taken at approximately the axial level shown in the above cartoon. Notice that the midline at a more anterior position in a younger HH12-13 (E-H) embryo appears similar to the midline at a more posterior position in an older embryo (i.e., compare C to F and D to G). Scale bars = 100  $\mu$ m.



Localization of other basement membrane components relative to the midline



**Supplemental Figure 3. The required basement membrane components nidogen and perlecan co-localize with laminin at the midline. (A)** Nidogen co-localizes with laminin at the midline. **(B)** Perlecan co-localizes with laminin at the midline. **(C)** Fibronectin surrounds the dorsal aortae and is only found in the midline nearest the endoderm. Scale bars = 25  $\mu\text{m}$ .