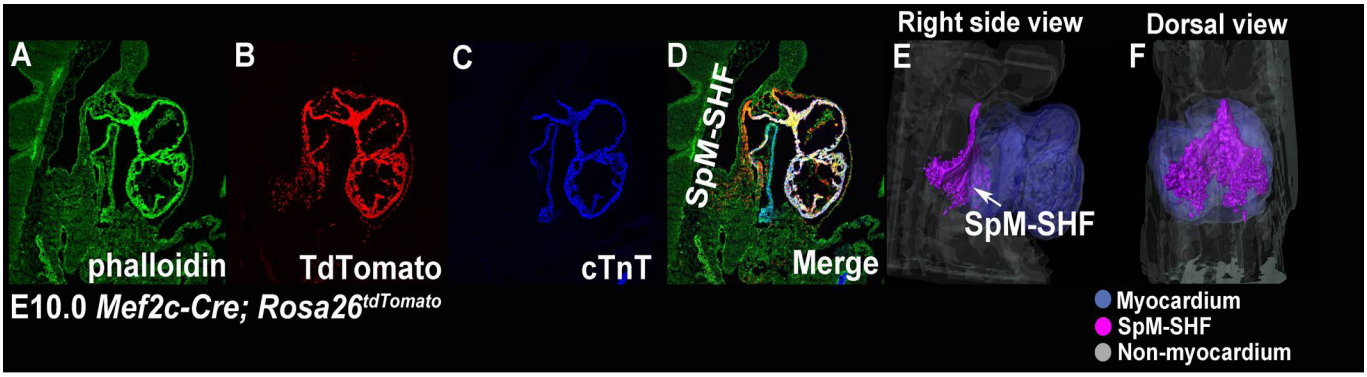


Figure S1. 3D reconstruction to analyze morphogenesis of the SpM-SHF
To genetically label and identify SpM-SHF progenitor cells, *Mef2c-Cre*; *Rosa26*$^{td\text{-}Tomato}$ embryos were serially sectioned sagittally and stained with phalloidin (A) and myocardial marker cTnT (C). Confocal images of tdTomato labeled cells (B), as well as phalloidin and cTnT staining, were merged (D) and imported into Amira for 3D reconstruction. In the right side (E) and dorsal (F) view of the reconstructed 3D models, the differentiated myocardium in the heart is colored in blue, while the td-Tomato positive, cTnT negative SpM-SHF progenitor population is colored in purple.

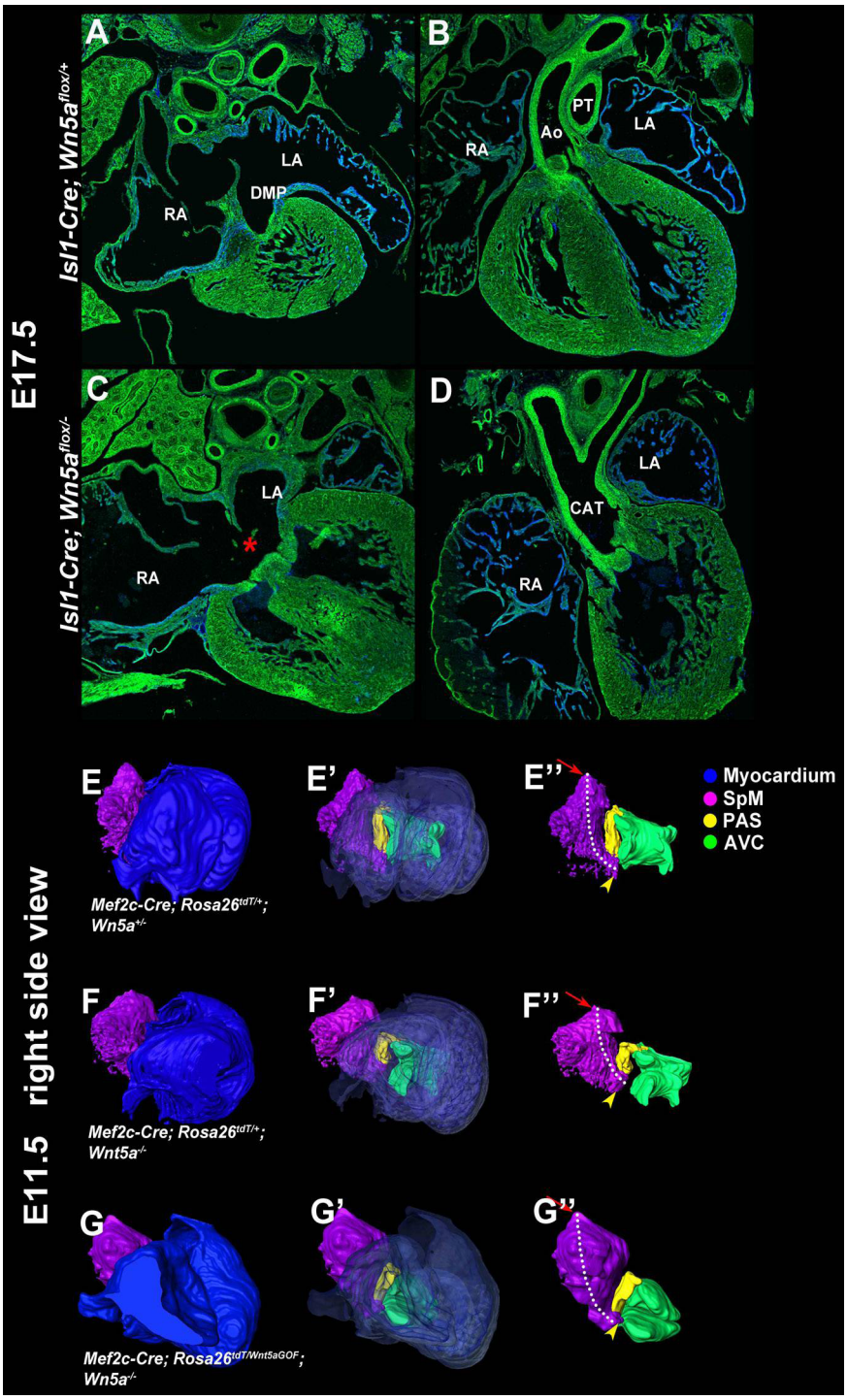


Figure S2. Expression of Wnt5a within the SHF is critical for DMP and atrial septum formation.

(A-D) E17.5 heart from control and *Isl1-Cre; Wn5a*$^{flox/-}$ mutant embryos. Deletion of *Wnt5a* specifically in the SHF in *Isl1-Cre; Wn5a*$^{flox/-}$ mutants is sufficient to recapitulate the primum atrial septal defects (asterisk in C) and OFT defects (D, common arterial trunk (CAT)) observed in *Wnt5a* null mutants. (E-G") 3D reconstructions show that the SpM-SHF is shortened and the DMP fails to extend into the atria in *Mef2c-Cre; Rosa26*$^{tdT/+}$*; Wnt5a*$^{-/-}$ mutants at E11.5 (compare dotted lines and yellow arrowheads in E" and F"). Both defects can be rescued by specifically expressing Wnt5a in the SHF in *Mef2c-Cre; Rosa26*$^{tdT/Wnt5a\text{-}GOF}$*; Wnt5a*$^{-/-}$embryos (compare dotted lines and yellow arrowheads in F" and G").

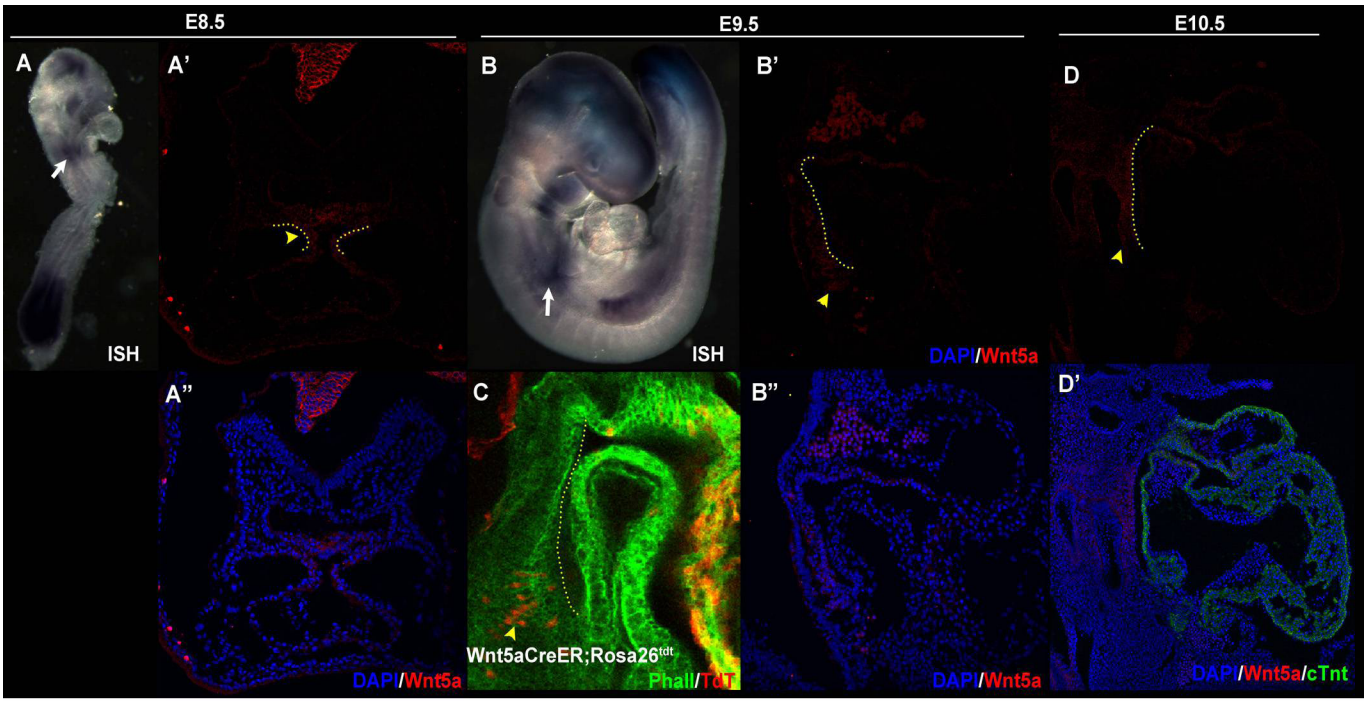


Figure S3. The spatial-temoral expression pattern of Wnt5a in the SpM-SHF.
In situ hybridization (ISH) of E8.5 and E9.5 embryos shows restricted expression of high level *Wnt5a* mRNA in caudal SpM-SHF (white arrows in A, B). Immunostaining of sagittal (B', B", D, D') or transvers (A', A") sections show that Wnt5a protein is also restricted in the caudal SpM at E8.5 and E9.5. The caudally restricted Wnt5a expression at E8.5 and 9.5 resembles the pattern of *Wnt5a* lineage labeled genetically using *Wnt5a-CreER* (C). At E10.5, however, Wnt5a expression becomes more widespread and extends to the anterior SpM (D, D'). The yellow dotted lines in B' and D outline and entire length of the SpM-SHF. The yellow arrowheads in B' and D indicate the caudal boundary of Wnt5a protein expression.

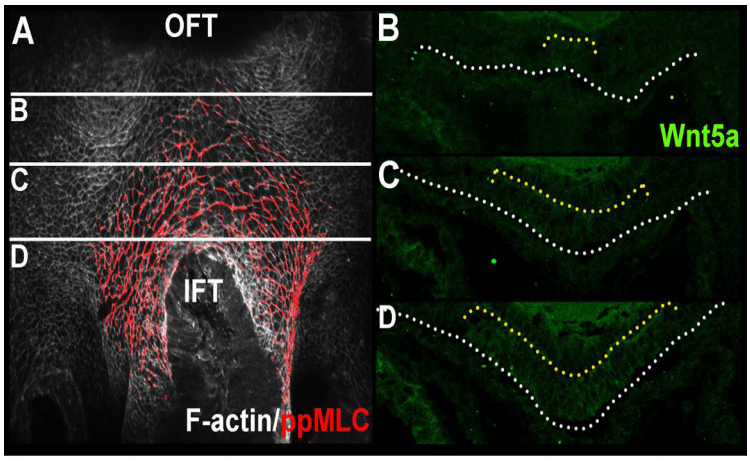


Figure S4. Restricted expression of Wnt5a in the medial and posterior SpM-SHF.
E9.5 SpM-SHF (A) is serially sectioned transversely from anterior (B) to posterior (C and D), and stained with anti-Wnt5a antibody. Wnt5a protein is expressed specifically in the medial and posterior region of the SpM-SHF, coinciding with where the M-L oriented actomyosin cables are observed.

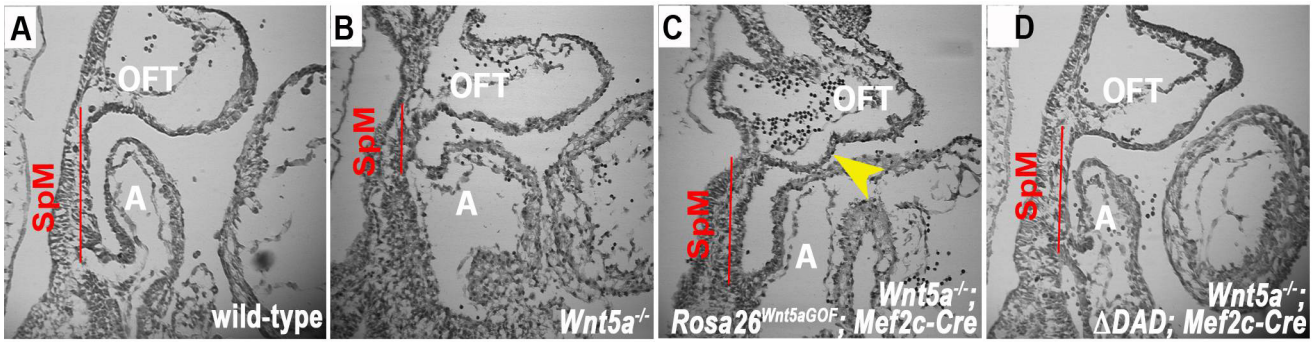


Figure S5. Different effect of over-expression of *ΔDAD* vs. *Wnt5a*.
In *Wnt5a* null mice, SHF morphogenetic defect causes the SpM between the OFT and atrium (A) to be shortened (A,B). This defect is rescued by activating ΔDAD expression in the SHF using *Rosa26-ΔDAD*; *Mef2c-Cre* (H), or Wnt5a expression with *Rosa26^{Wnt5aGOF}; Mef2c-Cre* (C). Wnt5a expression causes SHF cells to accumulate into aberrant bulges in the rostral SpM (yellow arrow in C), but ΔDAD does not (D).

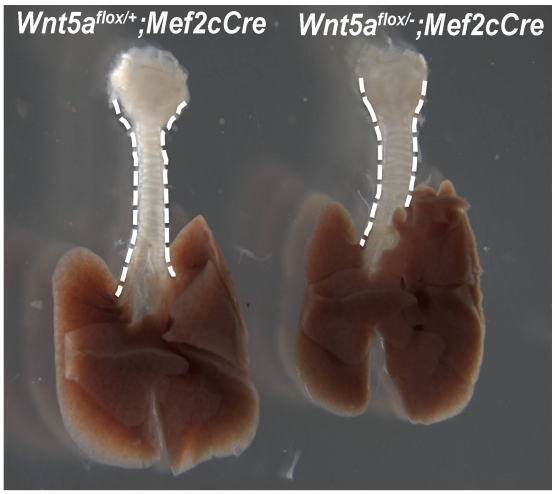
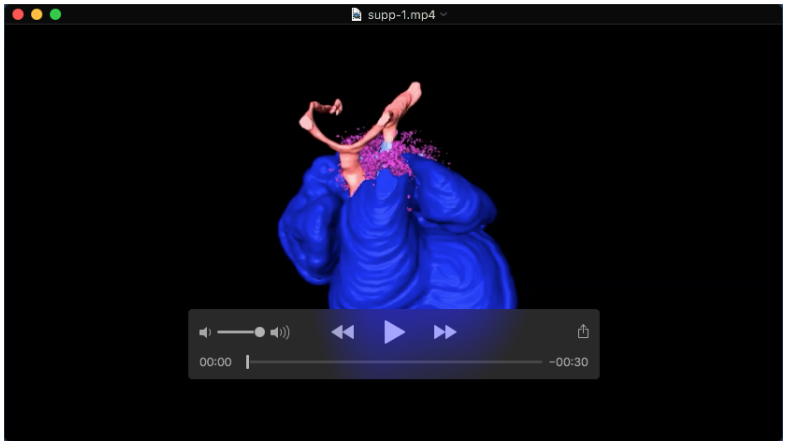
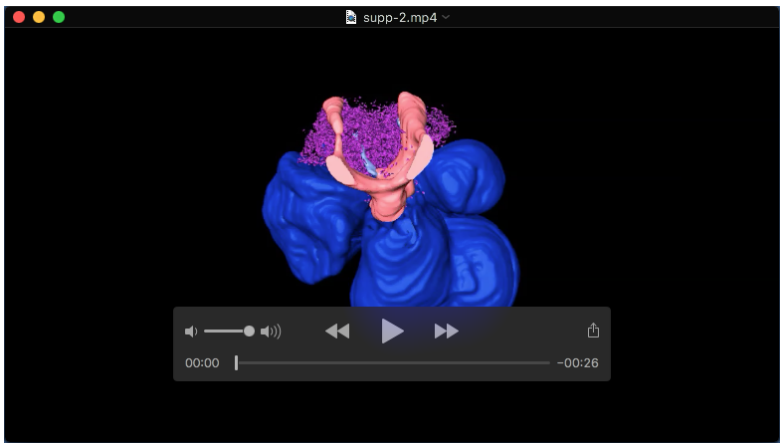


Figure S6. Conditional deletion of *Wnt5a* with *Mef2cCre* leads to pulmonary defects.
Deleting *Wnt5a* specifically in the SHF using *Mef2cCre* (*Wnt5a$^{flox/-}$; Mef2cCre*) leads to shortening of the trachea and bronchi.



Movie 1. 3D movie of Wnt5a lineage in wild-type E13.5 embryos

Wild-type embryos in *Wnt5a-CreER; Rosa26*$^{td\text{-}Tomato/+}$ background were treated with a single dose of tamoxifen at E7.5 to label specifically *Wnt5a* expressing cells at this stage. Embryos were then collected to generate 3D reconstructed movie at E13.5. Color labels for different structures are the same as in Fig. 4I-I".



Movie 2. 3D movie of Wnt5a lineage in *Wnt5a*$^{-/-}$ E13.5 embryos

Wnt5a$^{-/-}$ embryos in *Wnt5a-CreER; Rosa26*$^{td\text{-}Tomato/+}$ background were treated with a single dose of tamoxifen at E7.5 to label specifically *Wnt5a* expressing cells at this stage. Embryos were then collected to generate 3D reconstructed movie at E13.5. Color labels for different structures are the same as in Fig. 4J-J".