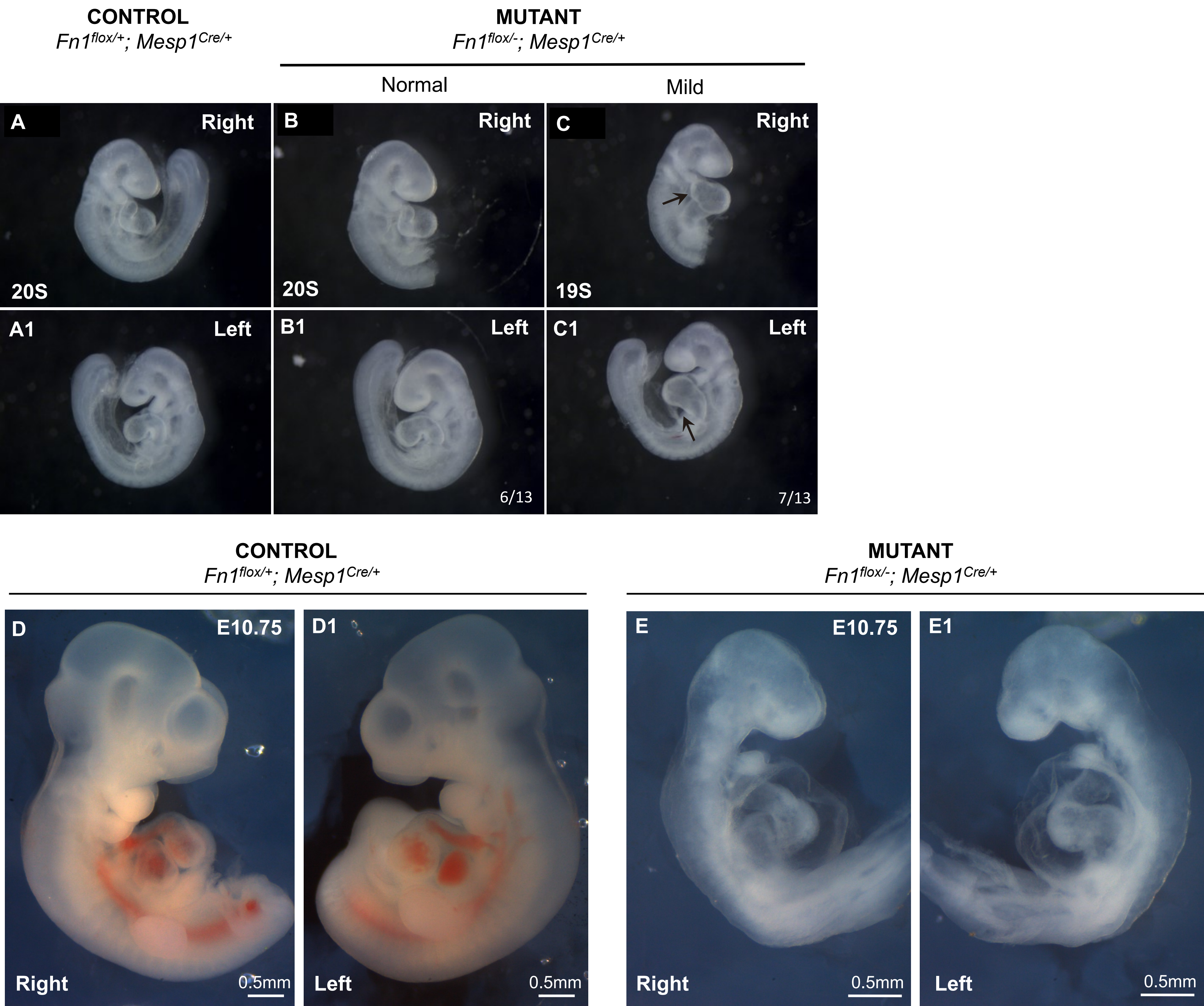


Figure S1



F

Genotype Stage	FN1 ^{flox/+} ;Mesp1 ^{Cre/-}	FN1 ^{flox/-} ;Mesp1 ^{Cre/-}	FN1 ^{flox/+} ;Mesp1 ^{Cre/+}	FN1 ^{flox/-} ;Mesp1 ^{Cre/+}	Total number of embryos genotyped
E9.5	12	10	13	13	47
E10.5	24	20	14	7*	65
E13.5-E17.5	45	36	51	0	132

* all recovered mutant embryos at E10.5 are defective
p value (χ^2 test) for E9.5 embryos is 0.94, i.e., mendelian distribution
p value (χ^2 test) for E10.5 embryos is 0.017, i.e. ~50% of embryos survive to E10.5
p value (χ^2 test) for E13-17.5 embryos is 10⁻⁴, non-mendelian distribution of genotypes

Figure S1. *Fn1^{flox/-}; Mesp1^{Cre/+}* mutant embryos do not survive past E10.5 of gestation.
A-A1 *Fn1^{flox/+}; Mesp1^{Cre/+}* (Control) and **B-C1** *Fn1^{flox/-}; Mesp1^{Cre/+}* (Mutant) embryos dissected at E9.5. **D-D1.** *Fn1^{flox/+}; Mesp1^{Cre/+}* (Control) and **E-E1** *Fn1^{flox/-}; Mesp1^{Cre/+}* (Mutant) embryos dissected at E10.75. **F.** Genotype distributions of embryos isolated at different stages of gestation.

Figure S2

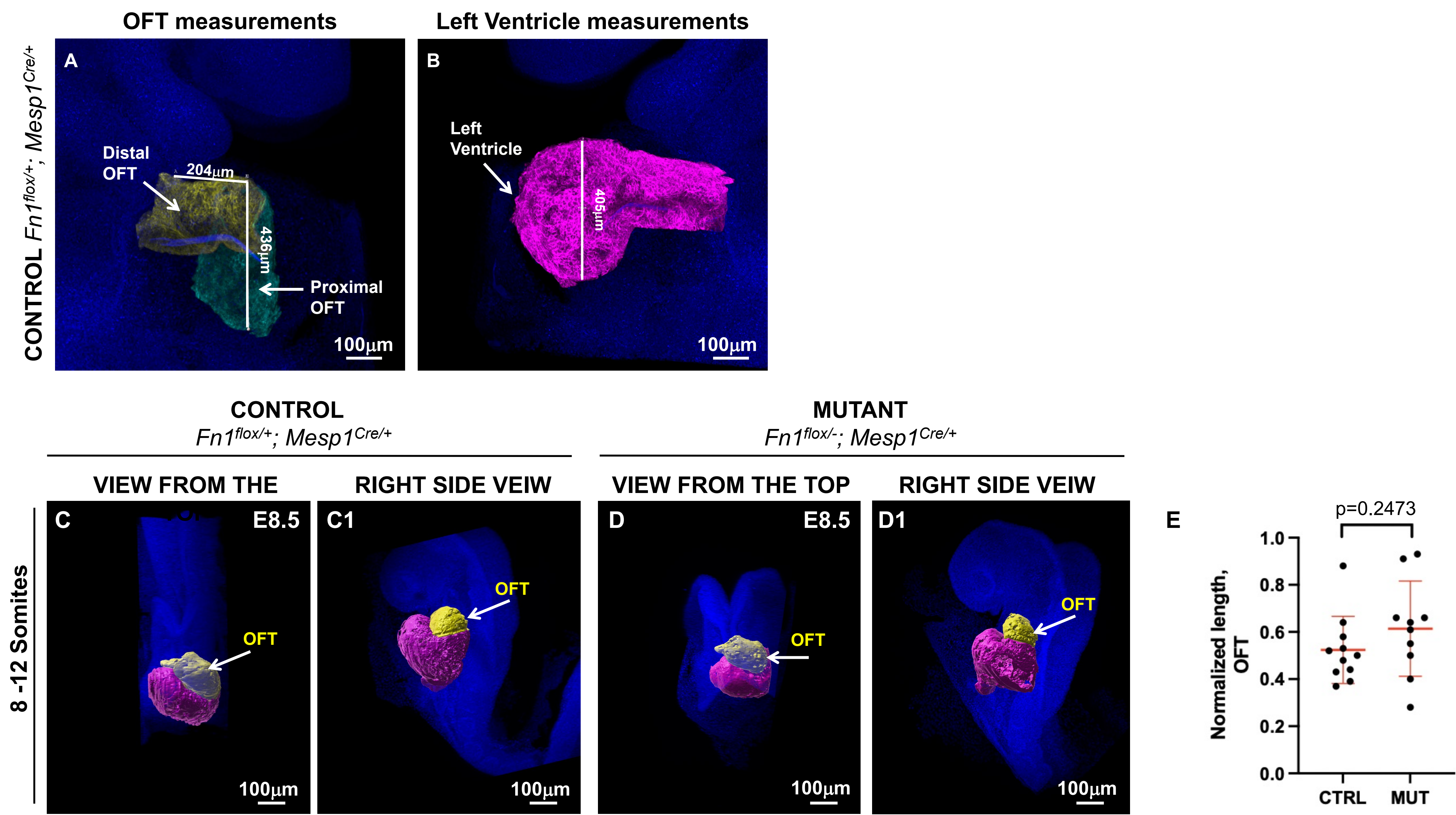


Figure S2. Initial formation of the cardiac outflow tract is not affected by the deletion of Fn1 in the mesoderm.

A-B. *Fn1^{flx/+}; Mesp1^{Cre/+}* (Control) embryo at E9.5. Imaris was used to surface the Distal OFT (yellow), Proximal OFT (turquoise) and the left ventricle and atria (pink). White lines show how the length of OFT segments and LV were measured. **C-C1** *Fn1^{flx/+}; Mesp1^{Cre/+}* (Control) and **D-D1** *Fn1^{flx/-}; Mesp1^{Cre/+}* (Mutant) embryos were dissected at E8.5. Imaris was used to surface the OFT (yellow) and the left ventricle and atria (pink). **E.** The length of the OFT was normalized to the height of the left ventricle to control for embryo size. 11 control and 11 mutant embryos were analyzed. Each dot corresponds to one embryo. Means (horizontal bars) and standard deviations (error bars) are displayed p was calculated using 2-tailed, unpaired Student's t-test. OFT- outflow tract

Figure S3

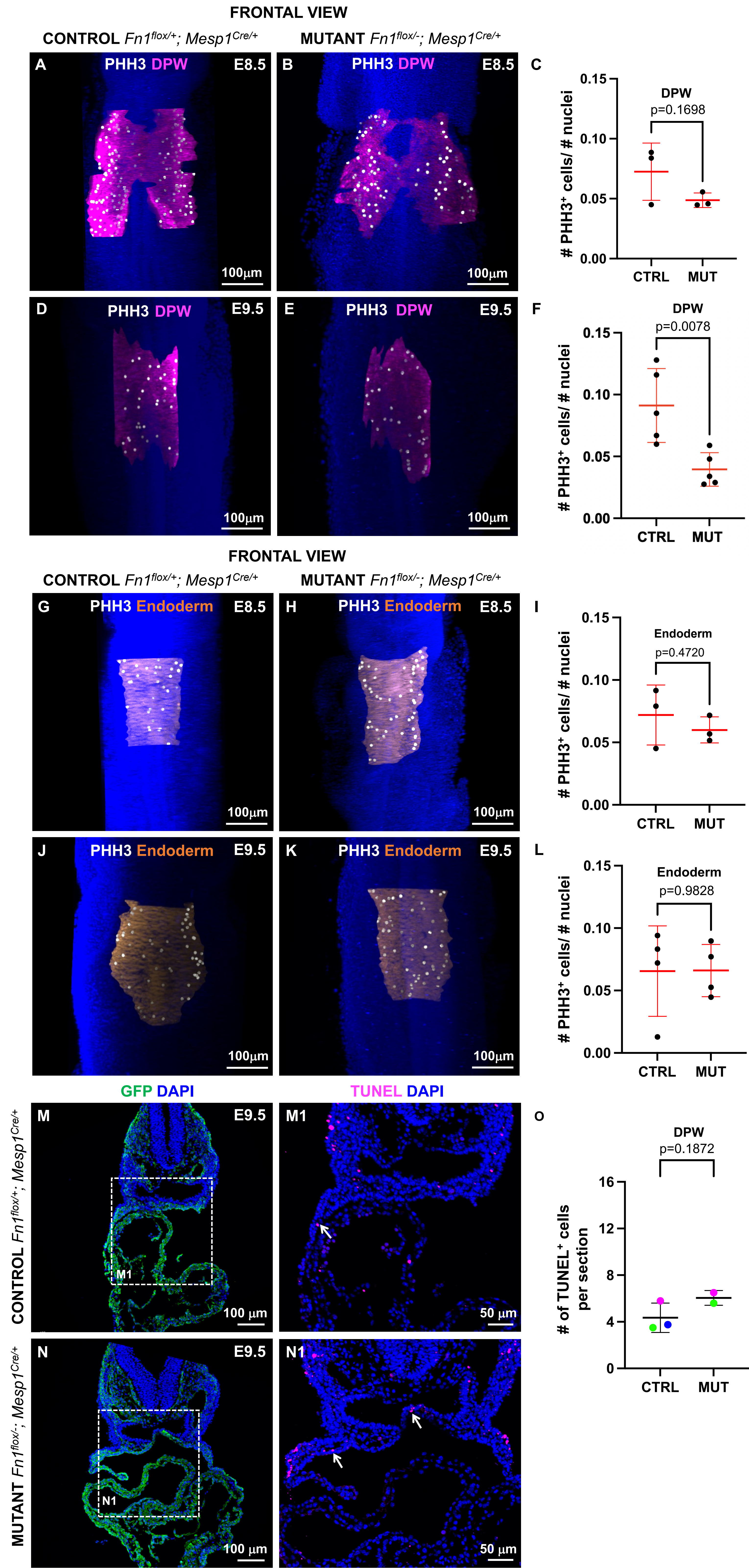


Figure S4

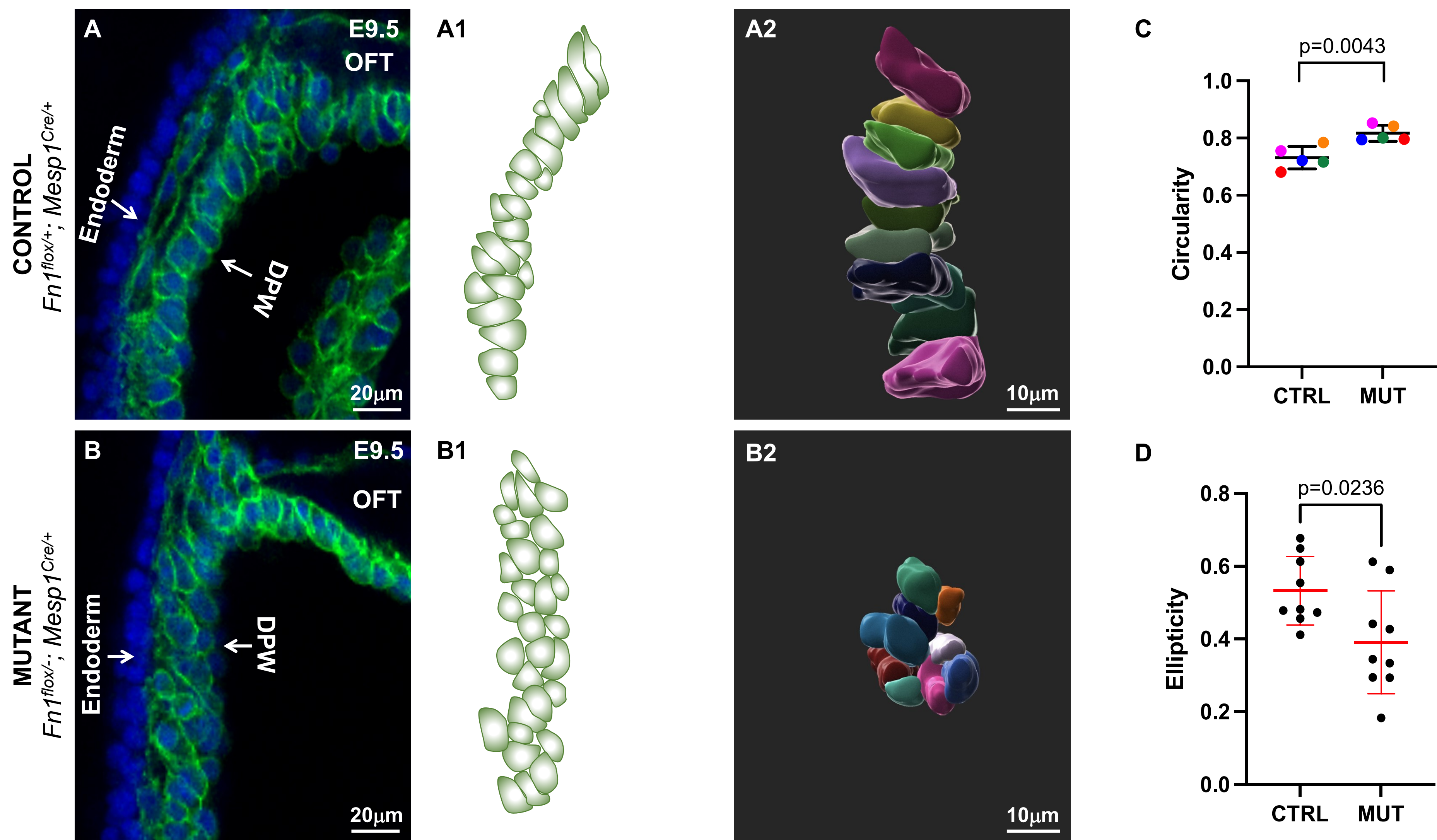


Figure S4. Mesodermal Fn1 regulates DPW cell shape at E9.5. **A.** *Fn1^{flox/+}; Mesp1^{Cre/+}* (Control) and **B.** *Fn1^{flox/-}; Mesp1^{Cre/+}* (Mutant) embryos were dissected at E9.5 (21-26s) and stained to detect GFP (green) and nuclei (DAPI, blue). **A1-B1.** Outlines of DPW cells. **A2-B2.** 3D reconstructions of anterior DPW cells. **C.** Quantification of circularity of the DPW cells. 75 cells from 5 different embryos were analyzed for each genotype. **D.** Quantification of Ellipticity of the DPW cells. 10 cells from 1 control and 1 mutant embryo were analyzed. Data points mark individual cells. Means (horizontal bars) and standard deviations (error bars) are displayed; p was calculated using 2-tailed, unpaired Student's t test. $p<0.05$ is considered significant. OFT- outflow tract, DPW- dorsal pericardial wall.

Figure S5

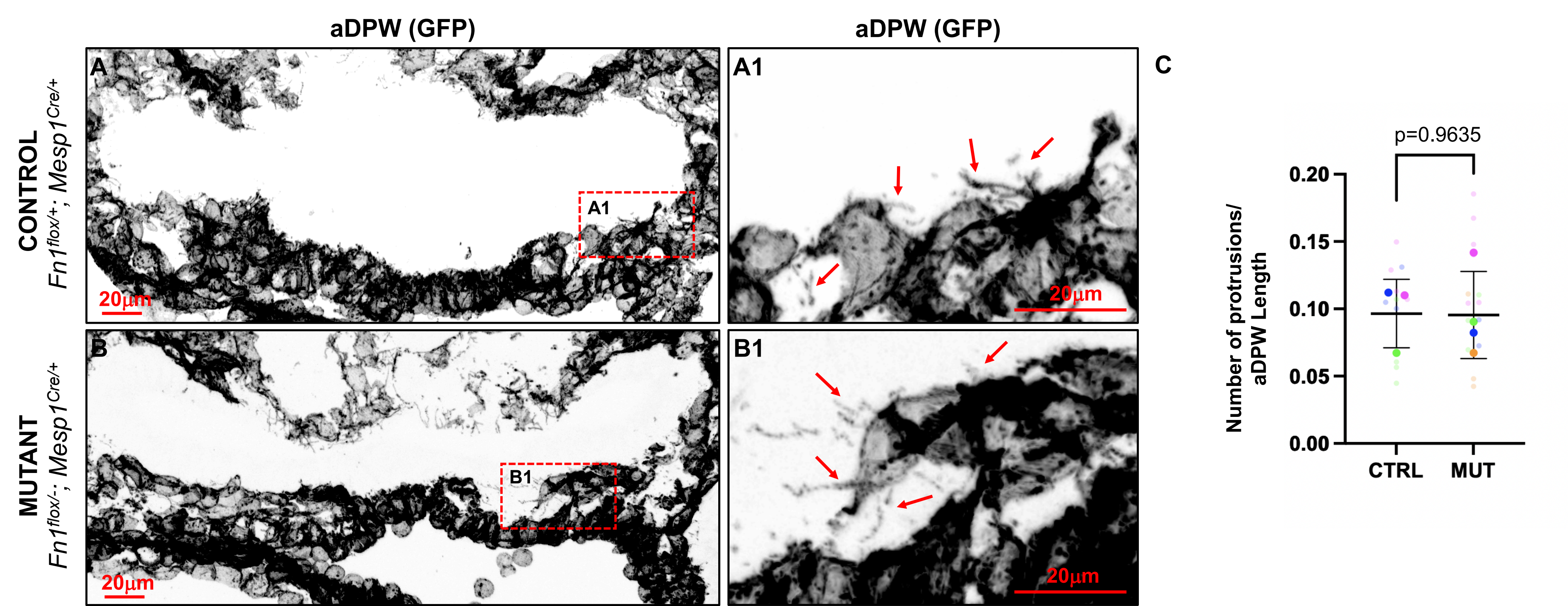


Figure S5. Mesodermal depletion of Fn1 does not alter the number of basal protrusions in the aDPW. **A.** *Fn1^{flox/+}; Mesp1^{Cre/+}* (Control) and **B.** *Fn1^{flox/-}; Mesp1^{Cre/+}* (Mutant) embryos were dissected at E9.5 (21-26s), and transverse cryosections from the anterior DPW were stained to detect GFP (black). Red rectangles in **A**, **B** are expanded in **A1** and **B1**. Arrows point to protrusion extending from the basal membranes of aDPW cells. **C.** Quantification of the number of protrusions normalized for the length of the DPW. n= 3 control and 4 mutant embryos. 2 and 7 sections per embryo were analyzed. Small dots mark the number of protrusions in each section. Large dots mark the mean in each embryo. Data from the same embryo is marked with the same color. p was calculated using 2-tailed, unpaired Student's t test. aDPW- anterior dorsal pericardial wall.

Figure S6

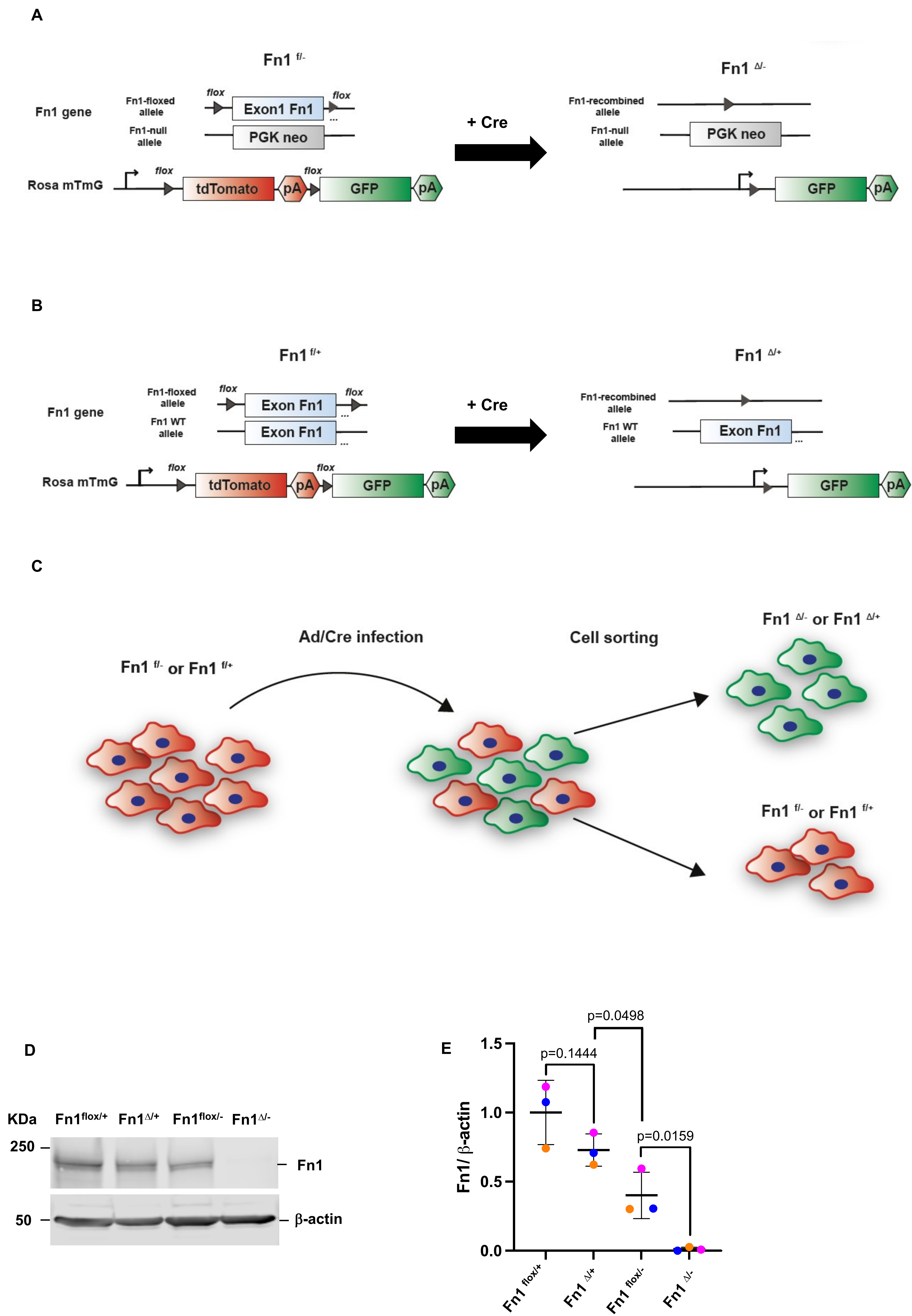


Figure S6. Generation of MEF lines . A-B. Schematic representation of resultant alleles upon Cre recombination in Fn1^{fl/+}; ROSA^{mTmG/+} and Fn1^{fl/-}; ROSA^{mTmG/+} MEFs. **A.** To delete Fn1, we infected Fn1^{fl/-}; ROSA^{mTmG/+} MEFs with adenoviruses encoding Cre recombinase to generate Fn1^{Δ/-} (Fn1-null) and Fn1^{fl/-} (control cells). **B.** Fn1^{fl/+}; ROSA^{mTmG/+} MEFs were treated with adenoviruses encoding Cre recombinase to generate control Fn1^{Δ/+} MEFs. **C.** After the infection cells were expanded and sorted. Membrane-tagged GFP-expressing cells are in green and membrane-tagged td-Tomato-expressing cells are in red. **D.** Western blot using anti-Fn1 antibody shows that we successfully ablated Fn1 in Fn1^{Δ/-} (Fn1-null) MEFs. β-actin was used as a loading control. **E.** Densitometry quantification of Fn1 levels relative to β-actin.

Figure S7

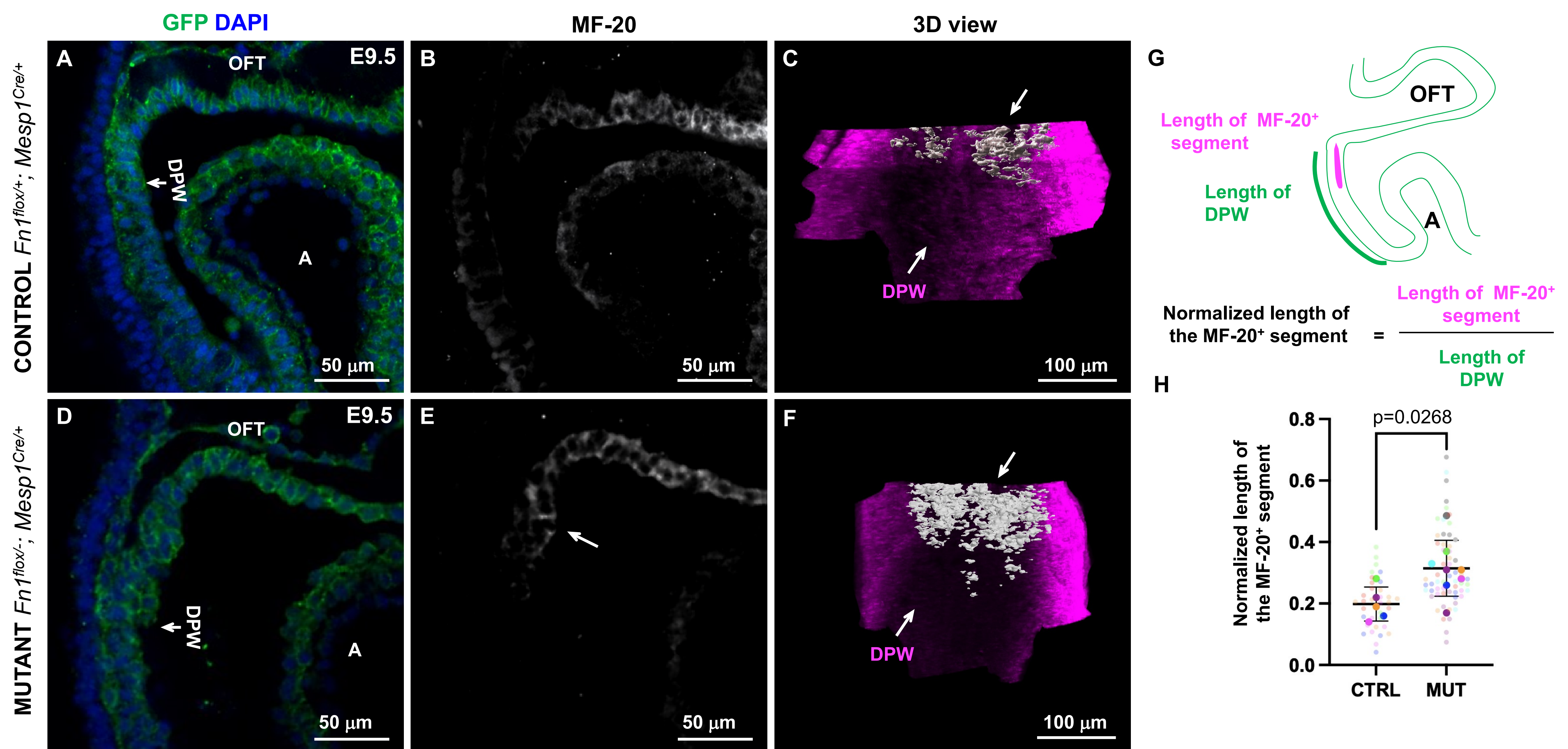


Figure S8

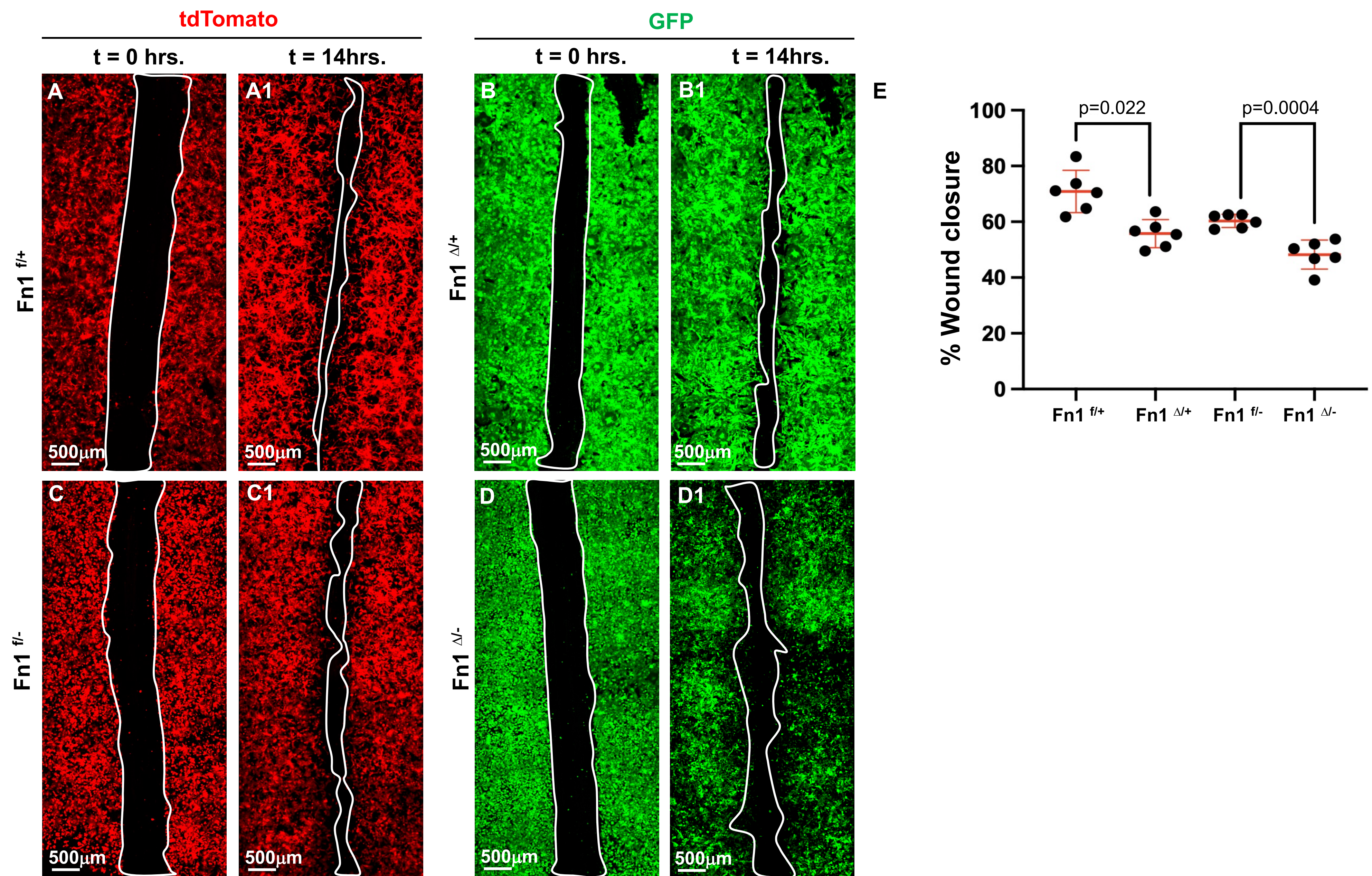


Figure S8. Wound healing assay. A-D. Cells imaged immediately after the scratch wound (time=0 hrs). **A1-D1** Cells imaged 14 hours after the scratch (time=14 hrs). White lines mark the area remaining cell-free at t=14 hours. **E.** To quantify % wound closure, cell-free area at t=14 ($A_{t=14}$) was subtracted from cell-free area at t=0 ($A_{t=0}$), and the % wound closure was calculated as $100 \times (A_{t=0} - A_{t=14}) / A_{t=0}$; n=6 independent experiments. Means (horizontal bars) and standard deviations (error bars) are displayed; p was calculated using 2-tailed, unpaired Student's t test.

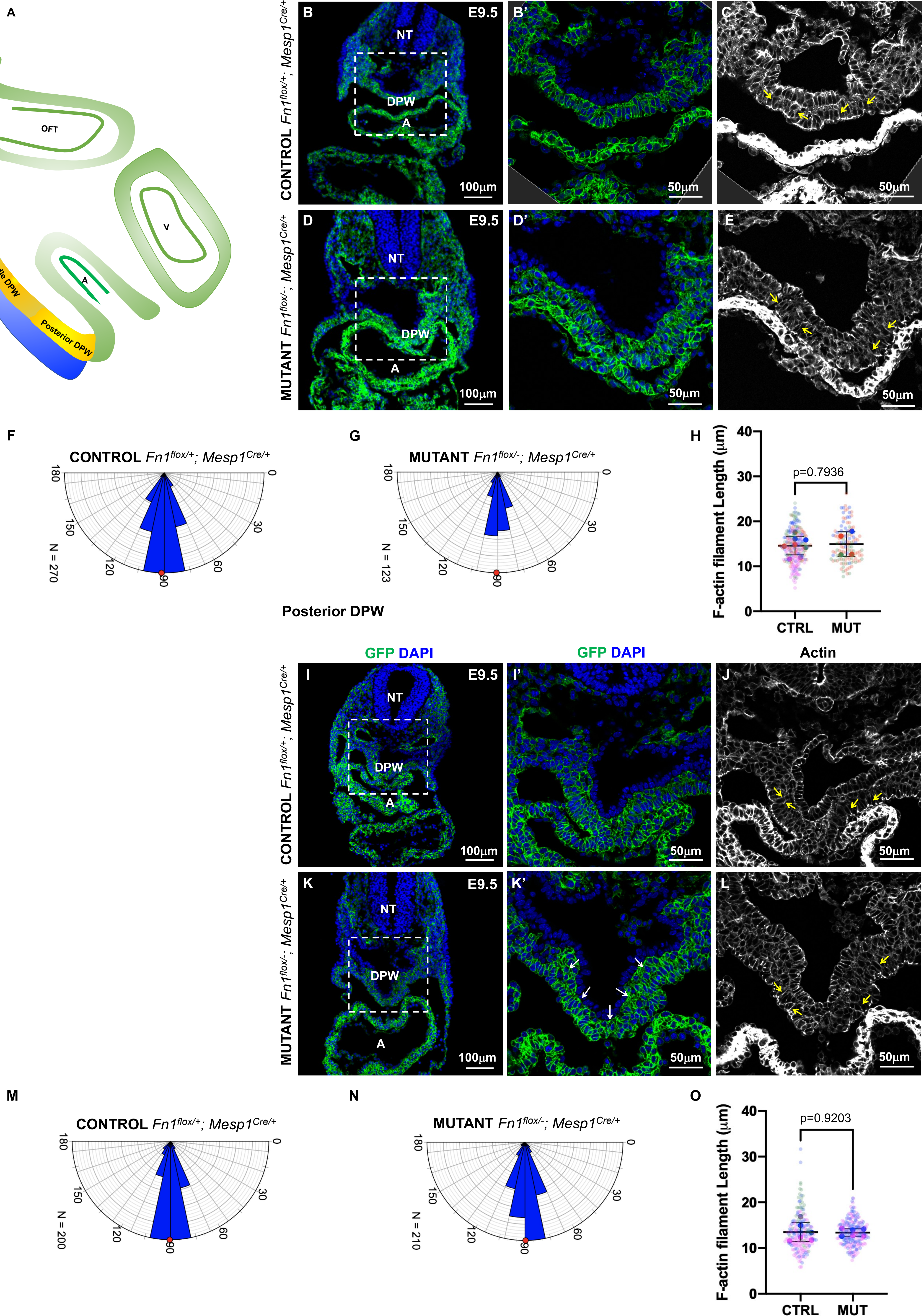
Figure S9

Figure S9. Deletion of mesodermal *Fn1* does not alter actin cytoskeleton in the middle or posterior DPW. **A.** *Fn1^{flox/+}; Mesp1^{Cre/+}* (Control) and *Fn1^{flox/-}; Mesp1^{Cre/+}* (Mutant) embryos were dissected at E9.5 (21-26s), and transverse cryosections from the middle of the DPW (**B-E**) or posterior DPW (**I-L**) were stained to detect GFP (green), F-actin (white), and nuclei (DAPI, blue). Yellow arrows point to F-actin cytoskeleton. **F-G, M-N.** Quantification of F-actin angles in the DPW cells was measured relative to the apical cell side. **F-G.** 270 cells from 4 controls and 123 cells from 3 mutants were analyzed. **M-N.** 200 cells from 3 controls and 210 cells from 2 mutants were analyzed. **H and O.** Quantification of F-actin length in DPW cells. **H.** 260 cells from 4 controls and 121 cells from 3 mutants were analyzed. In **O.** 200 cells from 3 controls and 210 cells from 2 mutants were analyzed. Small dots mark data from each cell. Large dots are means from each optical slice. Data from the same embryo is displayed with the same color. Means (horizontal bars) and standard deviations (error bars) are displayed; p was calculated using 2-tailed, unpaired Student's t test. p<0.05 is considered significant. DPW- dorsal pericardial wall, A- atrium, NT- Neural tube, OFT- outflow tract, V- ventricle

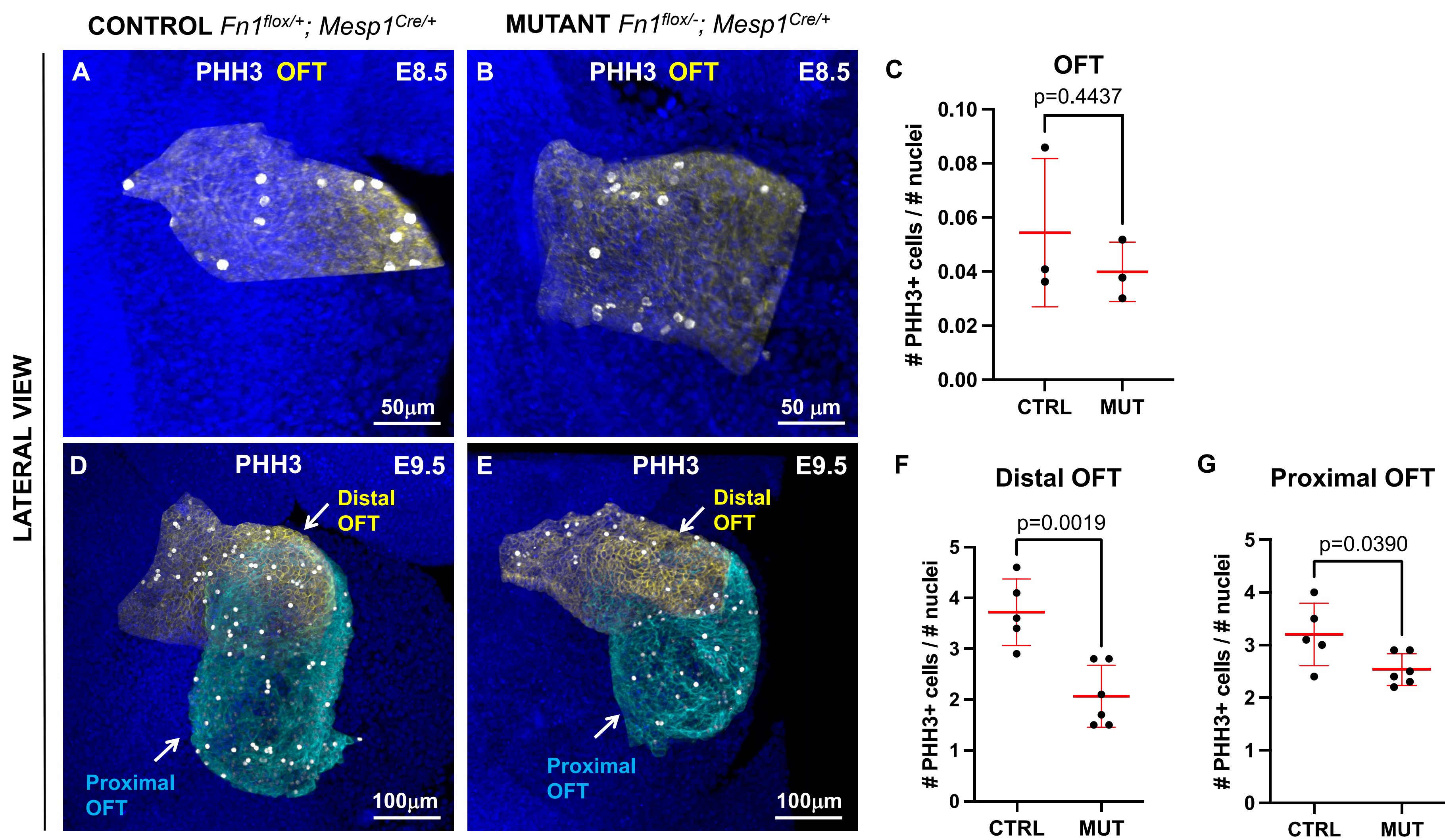
Figure S10

Figure S10. Mesodermal *Fn1* regulates OFT cell proliferation at E9.5. **A-D.** *Fn1^{flox/+}; Mesp1^{Cre/+}* (Control) and **B-E.** *Fn1^{flox/-}; Mesp1^{Cre/+}* (Mutant) embryos were dissected and stained to detect PHH3 (white), and nuclei (DAPI, blue) at E8.5 and E9.5. **A-B.** E8.5 embryos (11-12s). The OFT was segmented in yellow and the PHH3+ cells in the OFT were segmented in white using Imaris. **D-E.** E9.5 embryos (20-25s). The distal OFT was segmented in yellow, and proximal OFT – in turquoise, and the PHH3+ cells in each segment were marked in white using Imaris. **C, F, G.** The number of PHH3+ cells was normalized for the total number of nuclei in the OFT. **C.** Quantification of PHH3+ cells in the OFT of E8.5 embryos. n=3 for each genotype. **F-G.** Quantification of PHH3+ cells in the distal and proximal OFT of E9.5 embryos. n=5 control and 7 mutant embryos. Means (horizontal bars) and standard deviations (error bars) are displayed; p was calculated using 2-tailed, unpaired Student's t test. OFT- outflow tract, PHH3 – phospho Histone H3.

Figure S11

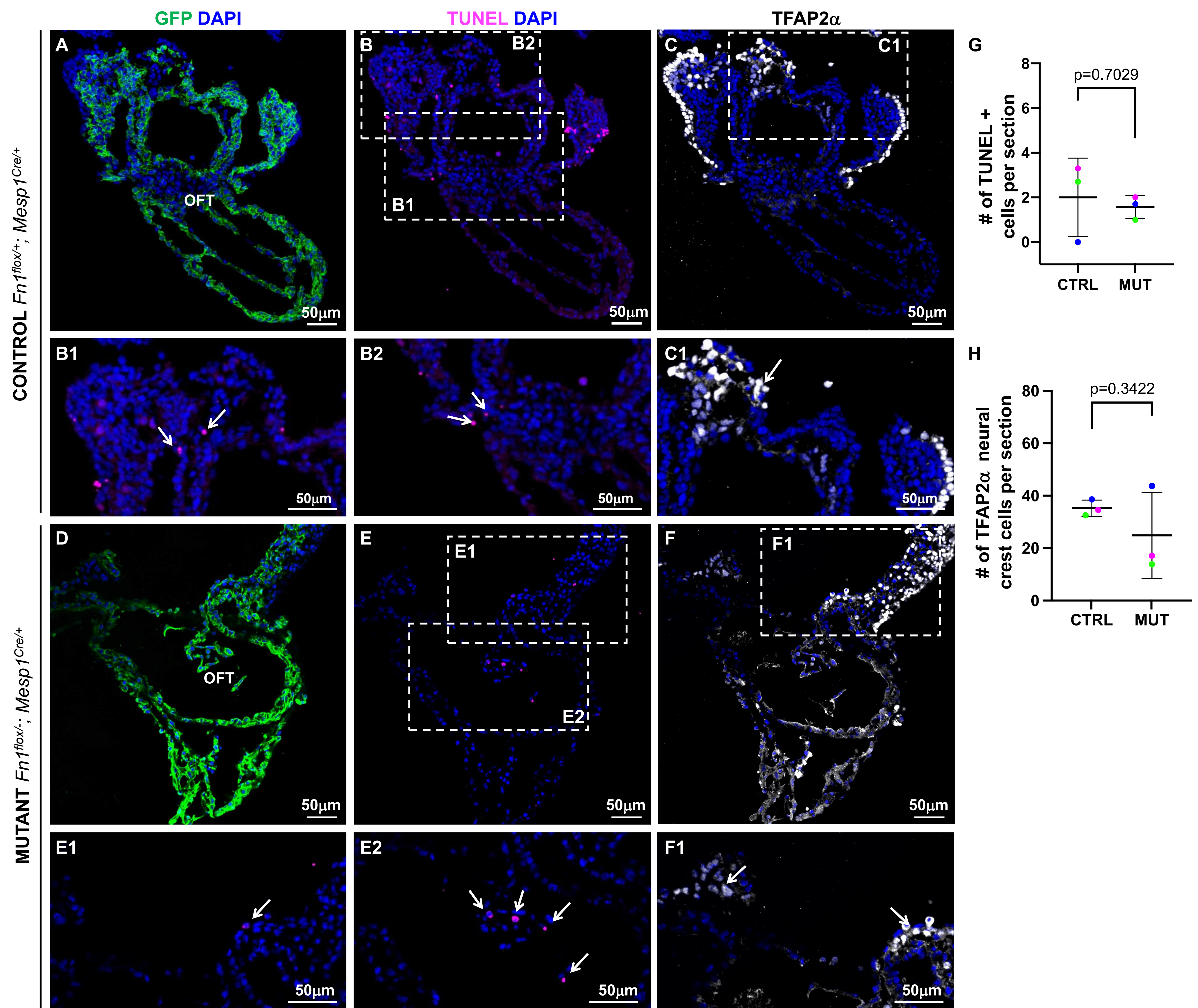


Figure S12

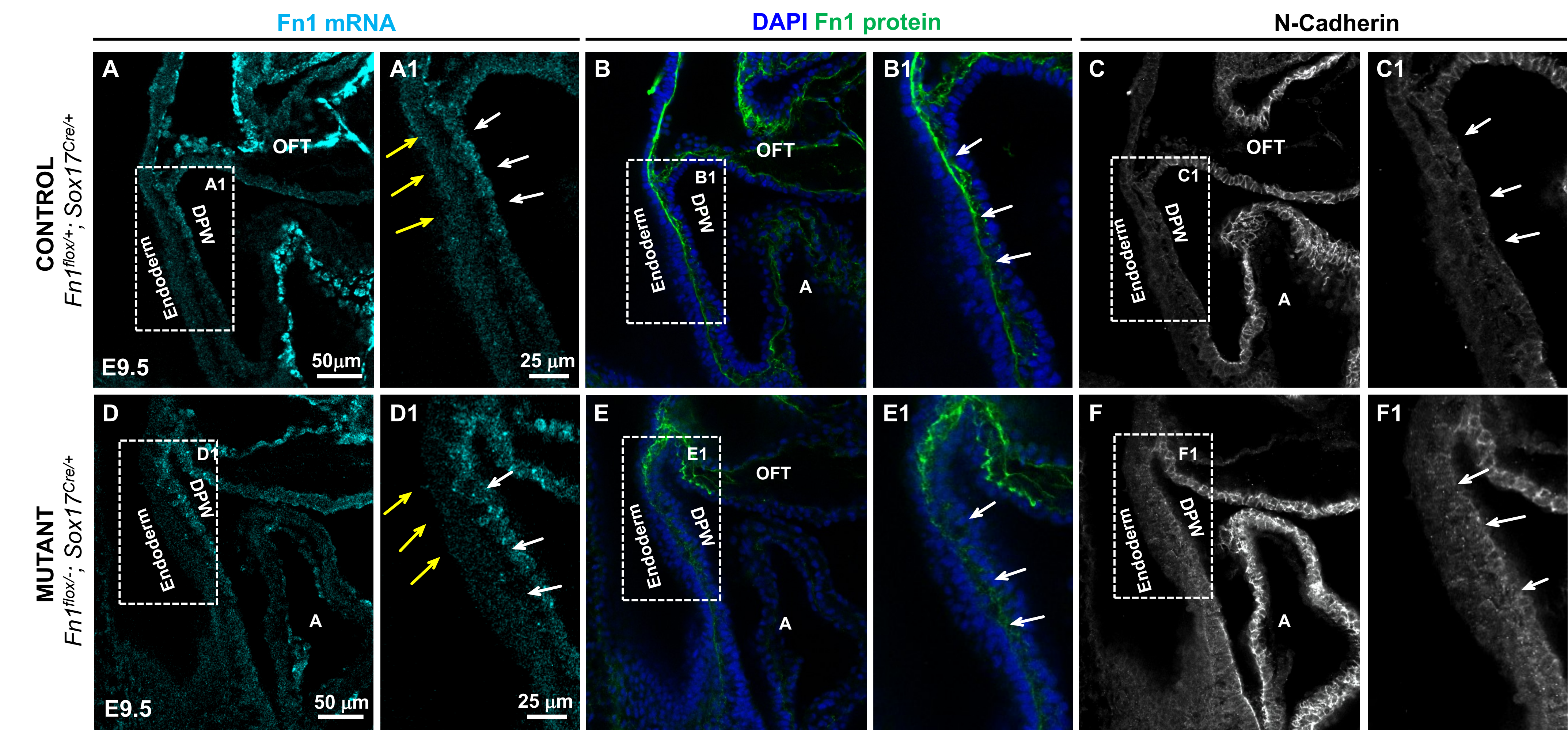


Figure S12. *Fn1* synthesized by the endoderm is not required for epithelial organization or N-Cadherin expression in the DPW. **A-C.** *Fn1^{flax/+} Sox17^{Cre/+}* (Control) and **D-F.** *Fn1^{flax/-} Sox17^{Cre/+}* (Mutant) embryos were dissected at E9.5 (20-26s). In **A** and **D** embryos were labeled to detect *Fn1* mRNA (turquoise). **A1** and **D1** are magnifications of the DWP. White and yellow arrows indicates mesodermal and endodermal expression of *Fn1* RNA respectively. In **B** and **E** embryos were stained to detect *Fn1* protein (green), and nuclei (DAPI blue). **B1** and **E1** are expanded from **B** and **E**. Arrows point to *Fn1* protein staining in the DPW. In **C** and **F** embryos were stained to detect N-cadherin (white). **C1** and **F1** are expanded from **C** and **F**. Arrows point to N-cadherin expression at SHF cell-cell junctions in the DPW. OFT- outflow tract, DPW- dorsal pericardial wall, A- atrium.