

Figure 1. The expression of *Frl* in *Meis1* lineage is required for the OFT elongation.

Frl^{fl/y}; Meis1^{fl/y} (Control) and *Frl^{fl/y}; Meis1^{fl/y}* (Mutant) embryos were dissected at E8.5 and staged according to somite number. **A-D**, Embryos between 18-19 somites, *n*=5 controls and 4 mutants. **E-H**, Embryos between 20-25 somites, *n*=5 controls and 7 mutant embryos. Images were used to surface distal OFT (yellow), proximal OFT (purple), and the left ventricle and atria (pink). **C-D**, **G-H**, The length of the distal and proximal OFT in each embryo was normalized to the height of the left ventricle, to control for embryo size. **I-J**, *Frl^{fl/y}; Meis1^{fl/y}* (Control) and *Frl^{fl/y}; Meis1^{fl/y}* (Mutant) embryos were dissected at E8.5 (20-25s) and stained with O4P1 and the MF-20 antibody. **K**, Quantification of angles in controls and mutants as shown in **I-J**, *n*=5 controls and 13 mutants. Means (horizontal bars) and standard deviations (error bars) are displayed; *p* was calculated using 2-tailed, unpaired Student's *t* test. OFT-surface tract, MF-20- antibody detecting sarcomeric myosin heavy chain.

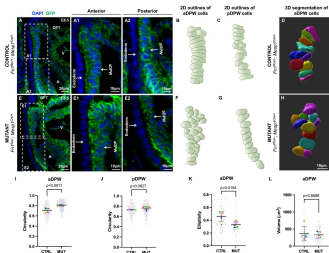


Figure 2. The expression of *Pit1* in the mesoderm is important for the regulation of cell shape in the anterior DPM at E8.5. **A**, *Pit1*^{Cre/+}; *Meis2*^{Cre/+} (Control) and **E**, *Pit1*^{Cre/+}; *Meis2*^{Cre/+} (Mutant) embryos were dissected at E8.5 (9–11 h) and stained to detect GFP (green) and nuclei (DAPI, blue). Regions marked by dashed rectangles in **A**, **E** are magnified in **A1-E1** and **A2-E2**. **B-C**, **F-G**, Outlines of aDPW (and pDPW) cells. **D-H**, 3D shapes of aDPW cells. **I-L**, Shape analyses. Small data mark a data point from each cell, large dots mark average per embryo. Data points from one embryo are marked with the same color. **I-J**, Cell circularity. **I**, 320 cells from 5 control and 317 cells from 5 mutant embryo were analyzed. **J**, 311 cells from 5 control and 308 cells from 5 mutant embryo were analyzed. **K-L**, Ellipticity and volume of aDPW cells. **K**, 311 cells from 5 control and 50 cells from mutant embryos were analyzed. Means (horizontal bars) and standard deviations (error bars) are displayed. p was calculated using 2-tailed, unpaired Student's *t* test. ODT-outflow tract, aDPW-anterior dorsal pericardial wall, pDPW-posterior dorsal pericardial wall, Atrium, V-ventricle.

Figure 3

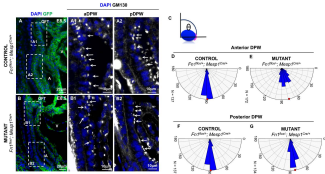


Figure 3. Expression of *Frl* in the mesoderm regulates Golgi apparatus orientation in the anterior DPM. A-B, *Frl*^{+/+}; *Mesp1*^{+/+} (Control) and C-B, *Frl*^{-/-}; *Mesp1*^{+/+} (Mutant) embryos were dissected at E9.5 (p-11d), and stained to detect GFP (green), GM130 (white), and nuclei (DAPI, blue). A1-B2, Magnification of the aDPM. A2-B2, Magnification of the pDPM. D-G, Semicircular histograms show the distribution of the Golgi orientation angles in DPM cells, measured as is indicated in C. N=number of cells analyzed. 3 different control and mutant were analyzed. GFP-outflow tract, aDPM-anterior dorsal pericardial wall, pDPM-posterior dorsal pericardial wall, A-atrium, V-ventricle

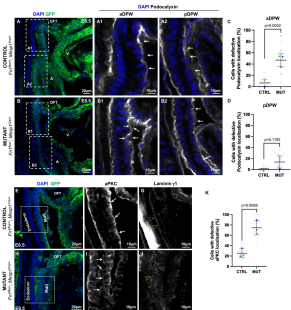


Figure 4. Expression of Pnl in the mesoderm regulates cell polarity in the anterior DPW. A, *Pnl^{+/+}; Mesp1^{+/+}* (Control) and B, *Pnl^{+/+}; Mesp1^{-/-}* (mutant) embryos were dissected at E8.5 (9–11s), and stained to detect GFP (green), pectocapsin (white), and nuclei (DAPI, blue). Boxes in A and B were expanded in A1 and B1 to show the anterior DPW and in A2 and B2 to show the posterior DPW. Arrows in A1 indicate cells with normal apical localization of pectocapsin. Arrows in B1 show a defective basolateral localization of pectocapsin in mutants. A2, B2. Pectocapsin is localized apically in the posterior DPW. C–D. Quantification of cells with defective pectocapsin localization in the anterior and posterior DPW. C, 148 cells in the aDPW from 3 controls and 224 cells in aDPW from 3 mutants were analyzed. D, 148 cells in the pDPW from 3 controls and 162 cells in the pDPW from 3 mutants were analyzed. Each small dot represents data from one slice, and each large dot is an average of all slices per embryo. Data from the same embryo is marked by the same color.

E–G. *Pnl^{+/+}; Mesp1^{+/+}* (Control) and E–G, *Pnl^{+/+}; Mesp1^{-/-}* (Mutant) embryos were dissected at E8.5 (7–8s), and stained for GFP (green), aPNC, laminin-γ1, and nuclei (DAPI, blue). Arrows in F point to apical localization of aPNC in control GFP cells. 1 Arrow points to defective basolateral localization of aPNC in mutants. Arrowheads in G point to basal localization of laminin-γ1 in controls and in J to disorganized localization of laminin-γ1 in mutants. G. Quantification of cells in which aPNC was re-distributed away from the apical surface. DPW cells from 5 different slices were evaluated in 3 control and 3 mutant embryos. Each small dot represents data from one slice, and each large dot is an average of all slices per embryo. Data from the same embryo is marked by the same color. Means (horizontal bars) and standard deviations (error bars) are displayed; p was calculated using 2-tailed, unpaired Student's t test.

Ctrl-outflow tract, aDPW-anterior dorsal pericardial wall, pDPW-posterior dorsal pericardial wall, A-aorta, V-ventricle.

Figure 5

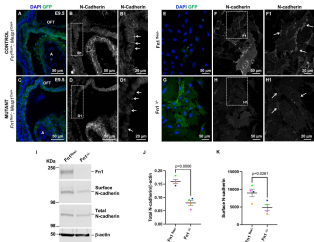
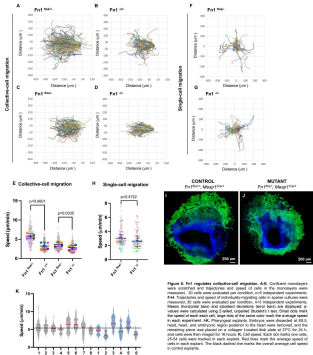


Figure 5. Mesodermal Fcrl regulates the expression of N-Cadherin at cell-cell junctions in the BMP. A-D, *Fcrl*^{+/+}; *Mesp1*^{Cre} (Control) and E-D, *Fcrl*^{-/-}; *Mesp1*^{Cre} (Mutant) embryos were dissected at E9.5 (21-28h) and stained to detect GFP (green), N-cadherin (yellow) and nuclei (DAPI, blue). Junctional N-cadherin is enriched in the anterior DPW (boxed region in B). Boxes in B-D are expanded in E1-E3. Arrows point to DPW. E-H, Immunostaining to detect GFP (green), N-cadherin (yellow) and nuclei (DAPI, blue) in *Fcrl*^{+/+} (*Fcrl*^{+/+}) and *Fcrl*^{-/-} (*Fcrl*^{-/-}) MEFs. Boxes in F-H are expanded in F1-F3. Arrows point to N-cadherin at cell junctions in control MEFs (F1) and to the absent N-cadherin in *Fcrl*^{-/-} MEFs (H1). I, Western blot analysis of total cell lysates and cell surface proteins. Positions of molecular mass markers is indicated on the left. J-K, Densitometrical quantification of immunoblot signals. n = 4 for each genotype. Each experiment is represented by a dot of different color. Means (horizontal bars) and standard deviations (error bars) are displayed. 2-tailed, unpaired Student's t test was used to determine p values. O/E-outflow tract, A-atrium.

Findings



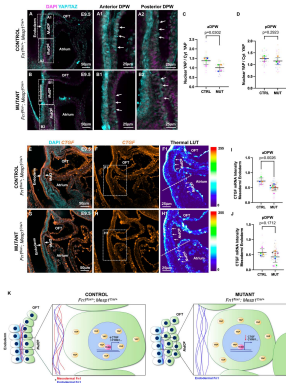


Figure 8. Bimodal Pax6 regulates nuclear translocation and transcriptional activity of YFP. A, B. Pax6^{Cre}/YFP^{loxP} (Control) and Pax6^{Cre}/YFP^{loxP} (Mutant) embryos were dissected at E9.5 (E9.5-10.5) and stained to detect nuclear (DAPI, magenta) and YFP (cyan). Regions outlined by dashed rectangles are expanded in A1, A2, B1, B2 (anterior DPW) and A3, A4 (posterior DPW). C-G. Quantification of the intensity ratio between nuclear and cytoplasmic YFP in the aDPW and pDPW. 7-8 optical slices from 4 control and 7-8 optical slices from 4 mutant embryos were analyzed. Each small dot represents one slice, and each large dot is an average of all slices per embryo. Data from the same embryo is marked by the same color. E-F. Pax6^{Cre}/YFP^{loxP} (Control) and Pax6^{Cre}/YFP^{loxP} (Mutant) embryos were dissected at E9.5 (E9.5-10.5) and labeled to detect CTGF mRNA (orange) and nuclei (cyan). Rectangles in F1-F4 are expanded in F1-F4 and color-coded according to fluorescence intensity (scale is on the right). G. Quantification of CTGF mRNA fluorescence intensity ratio between the aDPW (mesoderm) and anterior endoderm. J. Quantification of CTGF mRNA fluorescence intensity ratio between the pDPW (mesoderm) and posterior endoderm. H-I. 8 optical slices from 4 control and 8 mutant embryos were analyzed. Each small dot represents one slice, and each large dot is an average of all slices per embryo. Data from the same embryo is marked by the same color. Slices (horizontal bars) and standard deviations (error bars) are displayed. 2-tailed, unpaired Student's t-test was used to determine p-values. K. Summary in the presence of both the mesodermal Pax6, nuclear translocation and activity of YFP are decreased. aDPW, anterior dorsal paraxial wall; pDPW, posterior dorsal paraxial wall; CP, outflow tract.

Figure 9

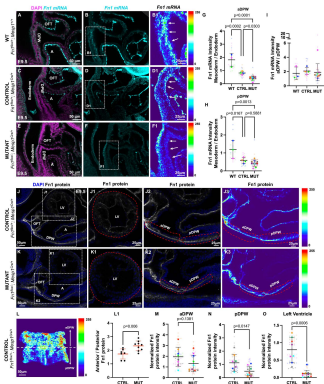


Figure 9. Expression patterns of *Per1* mRNA and protein in controls and mutants. A-F, Expression of *Per1* mRNA. A-B *Per1*^{+/+}; *Magd*^{+/+} (WT), C-D, *Per1*^{+/+}; *Magd*^{+/+} (Control), and E-F *Per1*^{+/+}; *Magd*^{+/+} (Mutant) embryos were dissected at E9.5 (E9.5-E10) and labeled with DAPI (magenta) and anti-sense *Per1* probe (cyan/blue). Regions outlined by the dotted rectangles in E, F, G are expanded in H, I, J and K. L, Quantification of the *Per1* mRNA intensity ratio between the aDPW (muscle) and anterior endoderm. G, Quantification of the *Per1* mRNA intensity ratio between the aDPW (muscle) and posterior endoderm. H, *Per1* mRNA intensity ratio between aDPW and pDPW. I, *Per1* mRNA intensity ratio between aDPW and pDPW. J, *Per1* protein expression in the left ventricle. K, *Per1* protein expression in the DPAV color-coded according to fluorescence intensity in J. L, *Per1* protein expression in the DPAV. L1, Quantification of *Per1* protein intensity ratio between aDPW and pDPW, according to L. Each dot marks one embryo. M-O, Quantification of *Per1* intensity in the aDPW, pDPW and LV regions outlined by the red dashed lines in J1-K2, normalized by DAPI intensity. L1 optical slices from 8 control and 10 mutant embryos were evaluated. Each small dot represents one slice, and each legend is an average of all slices per embryo. Data from the same embryo is marked by the same color. Means (horizontal bars) and standard deviations (error bars) are displayed. 2-tailed, unpaired Student's t test was used to determine p values. aDPW, anterior dorsal pericardial wall; pDPW, posterior dorsal pericardial wall; CPT, caudal heart; A, atrium; LV, left ventricle.

Figure 10

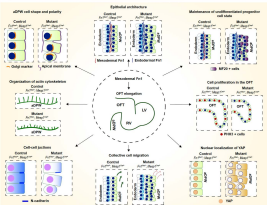


Figure 10. Summary of cellular and molecular processes regulated by mesodermal Fgf1 in the DRPF. Fgf1 synthesized by the mesoderm is a central regulator of epithelial properties in the anterior dorsal pericardial wall (aDRPF) and outflow tract elongation.