

## SUPPLEMENTARY FIGURE LEGENDS

### Supplementary Figure 1. Heart defects caused by loss of LRP2 function

(A) H&E-stained transversal sections of control and *Lrp2*<sup>-/-</sup> hearts at E13.5 and E12.5. The upper panel at E13.5 demonstrates CAT formation in *Lrp2*<sup>-/-</sup> hearts (asterisk) while in control embryos aorta and pulmonary trunk are formed. In the lower panel deeper intertrabecular spaces are detected in *Lrp2*<sup>-/-</sup> hearts compared to control hearts (arrow). In addition, ventricular septal defects (VSD) are detected in *Lrp2*<sup>-/-</sup> embryos compared to control embryos (arrowhead). Also, at E12.5 the development of a CAT phenotype (asterisk) in *Lrp2*<sup>-/-</sup> embryos can be confirmed on transversal sections. Deeper intertrabecular spaces are as well visible (arrow) in *Lrp2*<sup>-/-</sup> hearts at E12.5 and formation of hemorrhages can be detected in *Lrp2*<sup>-/-</sup> epicard (arrowhead). Scale bar: 100  $\mu$ m. (B) H&E-stained coronal sections of E15.5 control and *Lrp2*<sup>-/-</sup> hearts. Semilunar valve leaflets (arrows) are even in size and shape in control embryos but are malformed and uneven in shape in *Lrp2*<sup>-/-</sup> embryos (arrows). Scale bar: 50  $\mu$ m.

### Supplementary Figure 2. Detection of SHF markers in wild-type and *Lrp2*<sup>-/-</sup> embryos

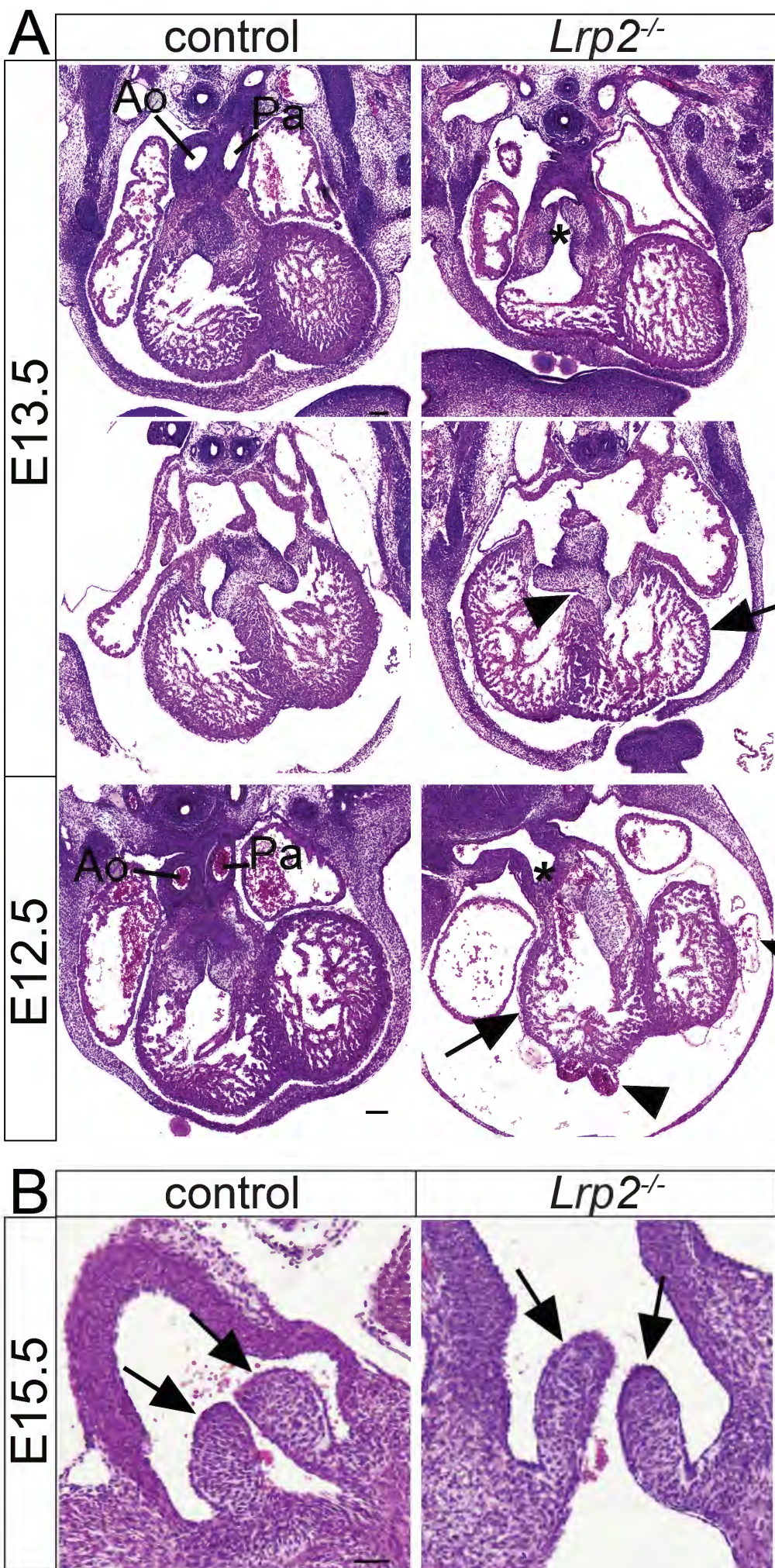
(A) *In situ* hybridization for *Semaphorin 3c* (*Sema3c*) on sagittal and coronal sections of E10.5 embryos. No difference in expression patterns in the OFT are seen comparing *Lrp2*<sup>-/-</sup> and control embryos displayed on sagittal sections. On coronal sections, *Sema3c* expression is slightly reduced in the intercalated cushions of *Lrp2*<sup>-/-</sup> embryos compared with controls (arrowheads). Scale bar: 100  $\mu$ m. (B) ISH for *T-Box transcription factor* (*Tbx1*) on sagittal sections of E10.5 embryos of the indicated genotypes. *Tbx1*, required for OFT development, displays similar expression patterns in the second heart field (asterisks) of *Lrp2*<sup>-/-</sup> and control embryos. Scale bar: 250  $\mu$ m.

**Supplementary Figure 3. Canonical / noncanonical Wnt and BMP signaling in second heart field of control and *Lrp2*<sup>-/-</sup> embryos**

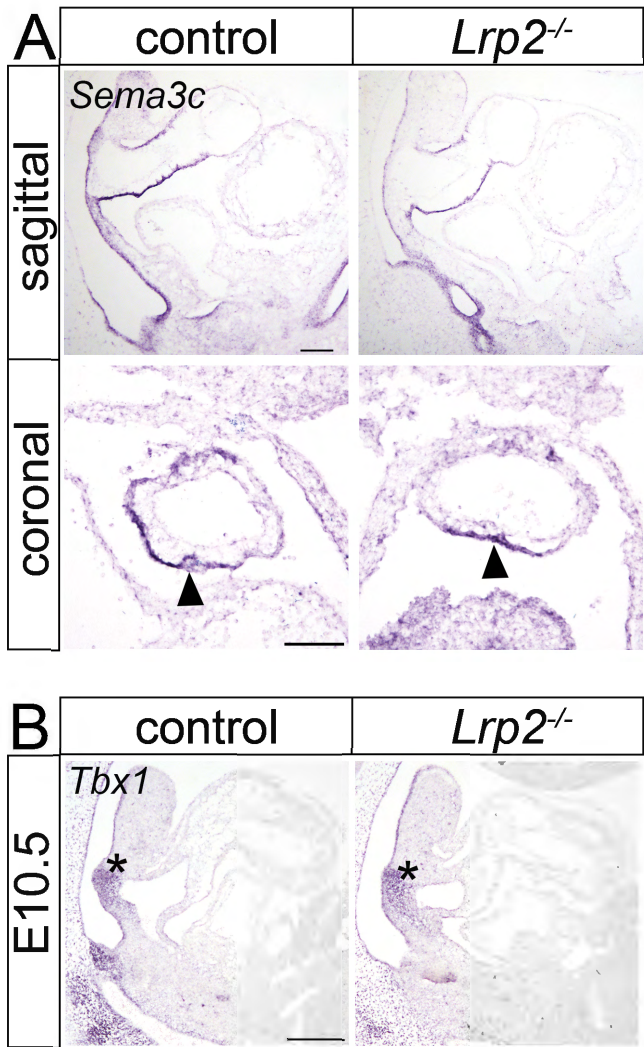
(A) Detection of lacZ activity on coronal and sagittal sections of the pharyngeal regions and OFT vessels from E10.5 *Tcf/Lef\_LacZ* reporter mice, expressing (control) or lacking LRP2 (*Lrp2*<sup>-/-</sup>). The activity of the canonical Wnt signaling pathway in the second heart field (as evidenced by lacZ activity) is comparable between *Lrp2*<sup>-/-</sup> and control embryos. Scale bar: 100 μm. (B) Upper panel *in situ* hybridization (ISH) for *Wnt11* on coronal heart sections of E10.5 embryos of the indicated genotypes. Expression of *Wnt11* in the OFT is comparable in *Lrp2*<sup>-/-</sup> and control embryos. Scale bar: 40 μm. Lower panel ISH on coronal E10.5 sections show similar patterns for *Bmp4* expression in the second heart field and distal OFT of control and *Lrp2*<sup>-/-</sup> embryos. Scale bar: 100 μm.

**Supplementary Figure 4. LRP2 deficiency does not affect development of primary cilia**

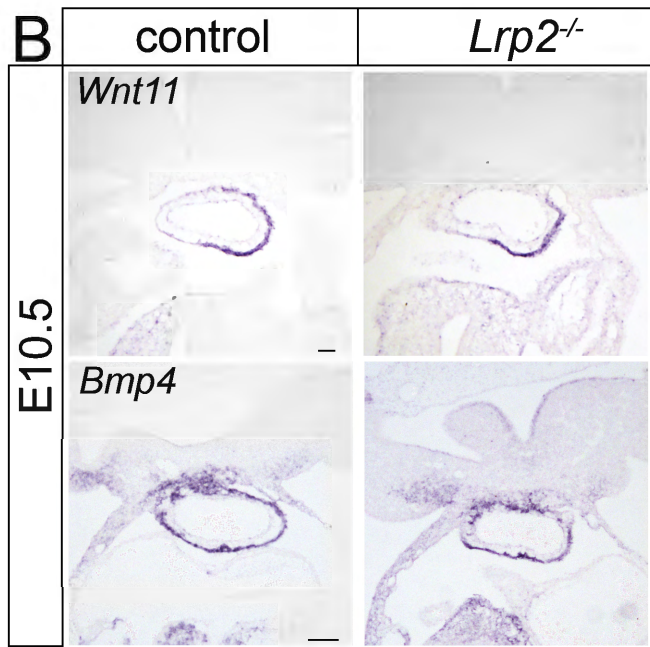
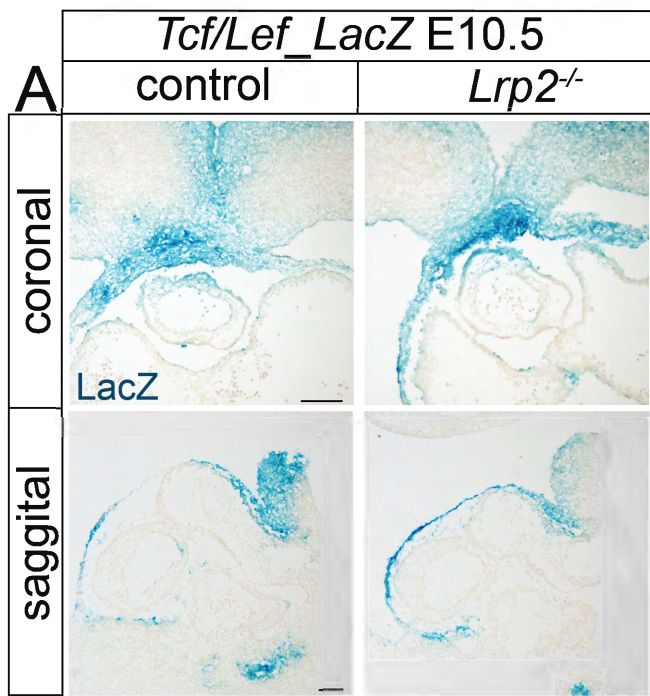
Immunohistological detection of Islet1, LRP2 and Arl13b on sagittal sections of the heart of control and *Lrp2*<sup>-/-</sup> embryos at E10.5. Similar numbers of primary cilia are detected in the DPW, Tz and in the OFT of *Lrp2*<sup>-/-</sup> and control embryos. In both genotypes Islet1 positive progenitor cells carry a primary cilium (detailed view in upper right corner of every image). Scale bar: 25 μm



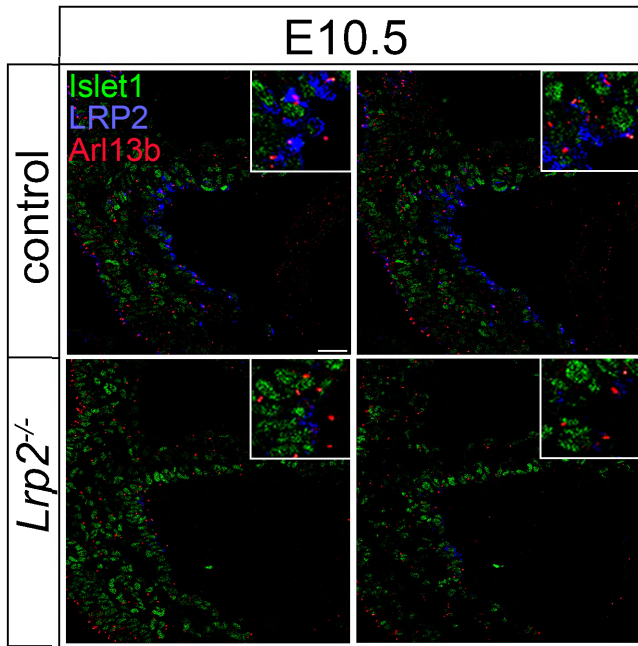
Christ *et al.*, Figure S1



Christ *et al.*, Figure S2



Christ *et al.*, Figure S3



Christ *et al.*, Figure S4