Supplementary Figure legends

Supplementary Figure 1.

(A) Efficiency of XLRP5/6 MO and mLrp5/6 overexpression in Xenopus embryos. Cell lysates were injected as indicated and analyzed by Western Blotting with phospho Lrp6 antibody, which also recognizes unphosphorylated Lrp5 and Lrp6, or XLRP5 and XLRP6, respectively. Levels of E-/N-cadherin and total protein were used as a loading control.

(B) Scheme of Xenopus injections used in the study.

(C) Effects of XLRP5/6 MOs on ventralization. Xenopus embryos were injected as indicated and the ventralization has been scored using DAI index (Kao and Elinson, 1988). Ventralization induced by XLRP5 MO is rescued by mLrp5 and β-catenin.

Supplementary Figure 2. Heart morphology in *Lrp6* and *Wnt5a* mutants at E10.5.

Heart of E10.5 wild type, Lrp6-/- and Wnt5a-/- mouse embryos was dissected and the morphology was analyzed. No obvious abnormalities were detected at this stage. Representative examples are shown. RV – right ventricle, LV – left ventricle, RA - right atrium, LA - left atrium, OFT – outflow tract.

Supplementary Materials and Methods

Mouse genotyping

Ear or embryonic tissues were boiled at 95ºC for 40 minutes in 100-200 µl of 25mM NaOH/0,2mM EDTA and an equal volume of 40mM Tris HCl pH 5 was added. 3 µl of this solution was used for PCRs. *Lrp6* wild type allele was identified with the previously described primers LRP6-U1 and LRP6-D1, while mice with the gene trap insertion (*Lrp6* knockout allele) were recognized with the following primer set: CD4mix forward (5´-

GCACGGATGTCTCAGATCAAGAGG-3') and CD4mix reverse (5'-CGGGATCATCGCTCCCATATATG-3'), with an annealing temperature of 63ºC and an amplicon of 108bp. *Wnt5a* WT and null alleles were identified with the following primers: 5a WT For (5´-GACTTCCTGGTGAGGGTGCGTG-3´), Wnt5a WT Rev (5´-GGAGAATGGGCACACAGAATCAAC-3´), Wnt5a null For (5´-GGGAGCCGGTTGGCGCTACCGGTGG-3´) and Wnt5a null Rev (5´-GGAGAATGGGCACACAGAATCAAC-3´).

Sequences of antisense MOs

XLRP5 MO (5'-ctc cca tgg cct cgt acc cct ctcc), XLRP6 MO (5'-gct caa tgc tcc ccc gta acc cgac) or standard control MO directed against Lpr5 and Lrp6 were used at 0.8 pmol (all MOs: GeneTools, Philomath, OR, USA). Sequences of XWnt5a and XWnt11 MOs were published before (Pandur et al., 2002; Schambony and Wedlich, 2007).

Antibodies

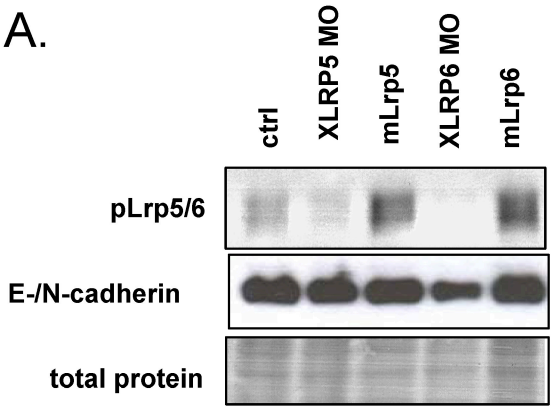
Following antibodies were used: anti P-Ser1490-Lrp6 (#2568), anti Lrp6 (#2560) from Cell Signaling Technologies), anti-Dvl2, anti-Dvl3 (sc-8027), anti-c-Myc (sc-40) (from Santa Cruz Biotechnology), anti-active β-catenin (ABC, #05-389) and anti-Rac1 (-389) from Upstate Biotechnology , anti-β-actin (Ab6276) and anti HA (Ab9110) for IP from Abcam, anti HA (HA.11) for WB from Nordic Biosite, and anti-human IgG Fc fragment (109-005-098, Jackson ImmunoResearch Laboratories). Cadherin levels in Xenopus lysates were detected using the mixture of antibodies against E- and N-cadherin (610182 and 610921, BD Biosciences).

Quantitative-PCR

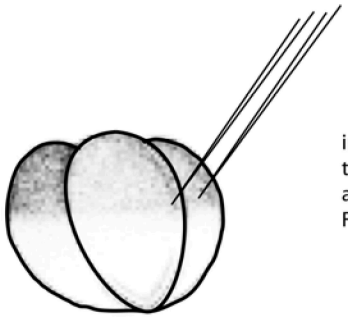
cDNA was generated as described previously (Castelo-Branco et al., 2003) RNA from. ventral midbrains of *lrp6*+/+, *lrp6*+/- and *lrp6*-/- mouse embryos at E11.5 and E13.5.5 (where both Lrp6 and Wnt5a are known to be expressed) was extracted using RNeasy Mini Kit (Qiagen). 1 μg of RNA was reverse-transcribed using SuperScript II Reverse Transcriptase (Invitrogen) and random primers (Invitrogen). Wnt5a primers used in this study have been described previously (Castelo-Branco et al., 2003), Lrp6 primers were as follows: forward 5-GCTACAAATGGCAAAGAGAATGC-3, reverse CAGTATACAAGCCATGACCAAACA.

Supplementary Figure 1

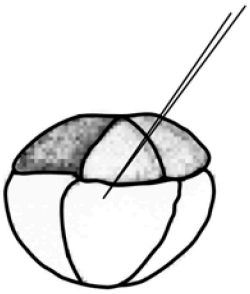
A.



B.

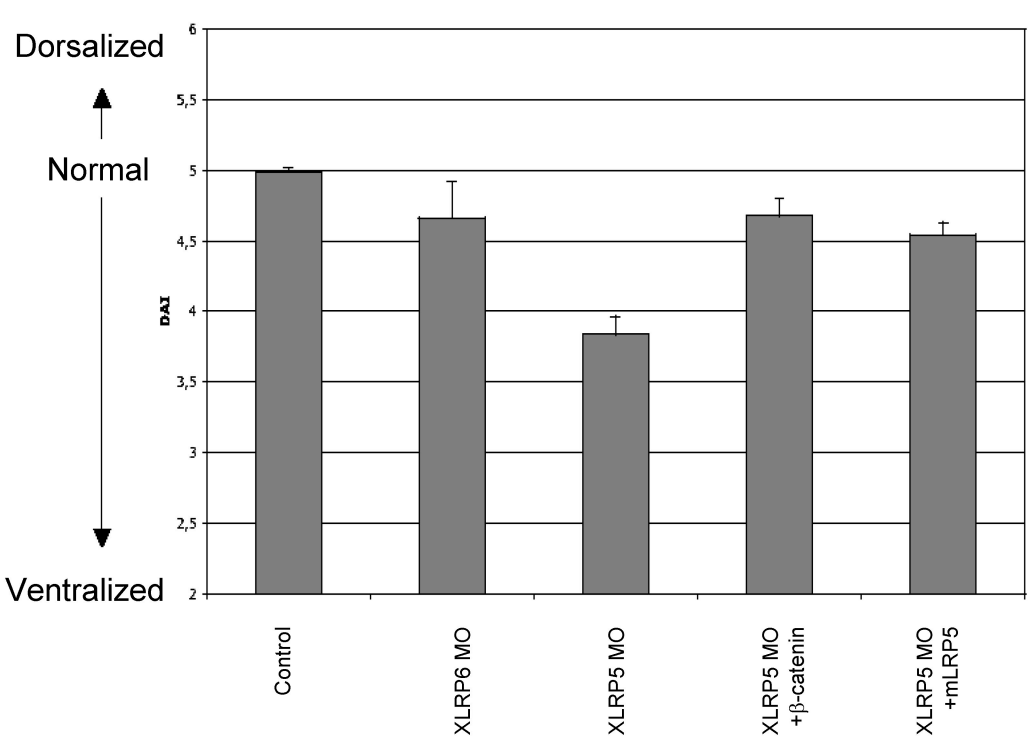


injection in 4-cell stage embryo
targets Dorsal Marginal Zone
affects primary axis formation (ventralization) and CE movements
Figures 2, 3, and Suppl Figure 1



injection in 8-cell stage embryo
targets presumptive cardiac mesoderm
affects cardiac development, but avoids earlier effects on primary axis and CE
Figure 4

C.



Supplementary
Figure 2