Lrp6 is required for convergent extension during *Xenopus* gastrulation

DEV010272 Supplementary Material

Supplemental Material

Figure S1. β-catenin fails to rescue inhibition of convergent-extension mediated by LRP6MO. LPR6MO (30 ng)-induced block of activin-mediated animal cap elongation (A) and Keller sandwich elongation (B) is not rescued by co-injection of β-catenin mRNA at a concentration (80 pg) that normally induces complete secondary axis formation. In contrast, the LRP6MO-induced block of activin-mediated animal cap and Keller sandwich elongation can be rescued by co-injection of LRP6 mRNA (500 pg). The Student's t test was used for statistical analysis. Error bars indicate standard deviation. Asterisks mark differences that are statistically significant from control ($p<0.01$). Numbers of explants scored are indicated in parentheses. The same control explants were used in Figures S3 because the experiments were performed simultaneously.

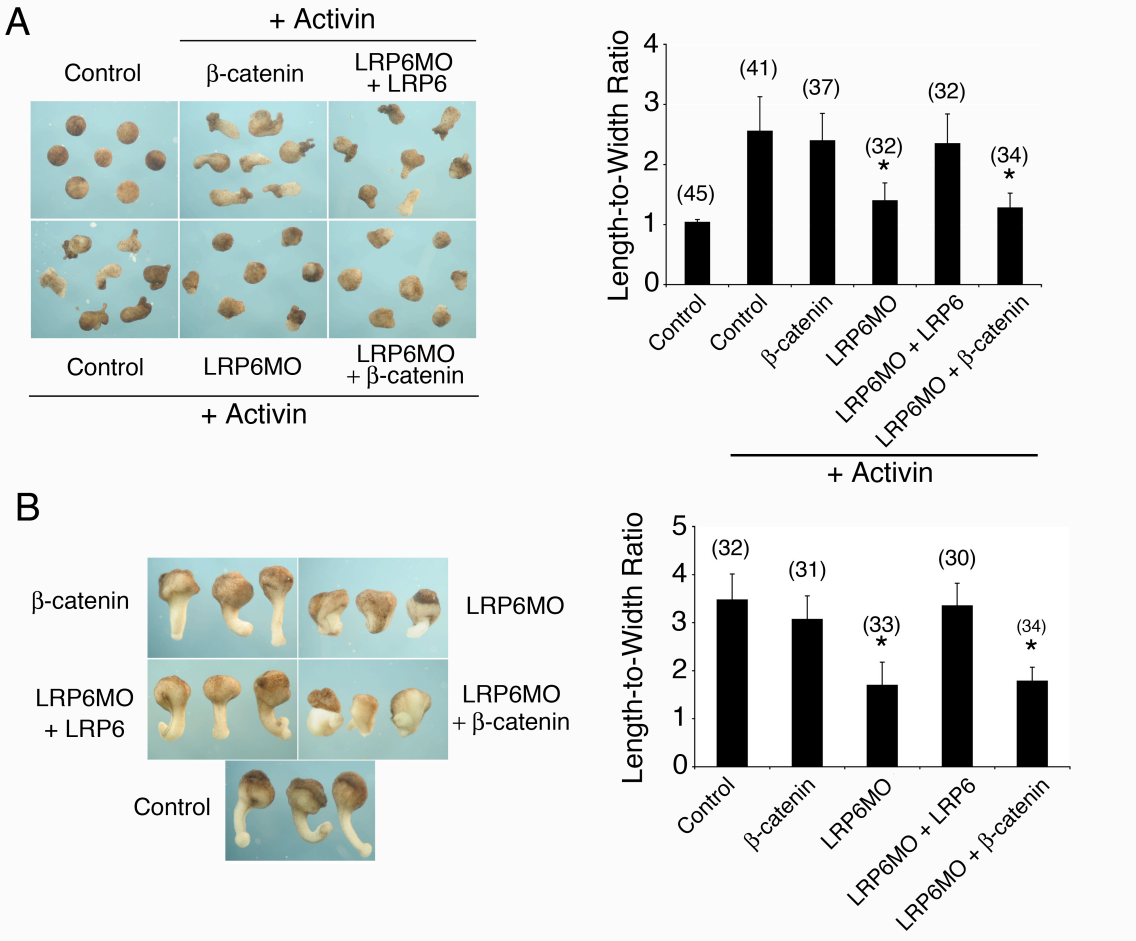
Figure S2. Localization of VSVG-LRP6 in whole embryo sections parallels its localization in Xenopus explants. Cryosections of stage 10.5 Xenopus embryos injected with VSVG-LRP6 (2 ng) show polarized distribution of LRP6 (red) in DMZ cells and uniform distribution in cells of the animal pole and VMZ. Cells were also stained for DNA with DAPI (blue). The positions of the cell areas of the VMZ and DMZ shown relative to the whole section areas are indicated in the schematic. The section plane was

chosen so that it transverses cells of the marginal zone that are immediately underneath the superficial endodermal epithelium. The relative position of the blastopore lip near the anterior-most edge of the dorsal section is indicated with an arrow. Error bars indicate standard deviation. Asterisks show differences that are statistically significant ($p<0.01$). Numbers of cells analyzed are shown in parentheses. Scale bars: 30 μm in micrographs and 500 μm in schematic.

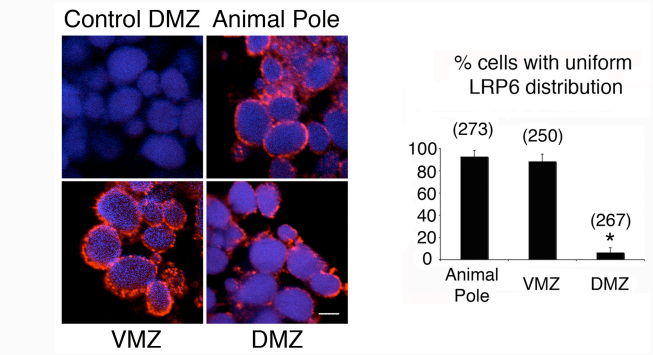
Figure S3. LRP6-B rescues the effect of LRP6MO on explant elongation and mimics the effects of LRP6 in controlling cell shape and behavior. LRP6MO (30 ng)-induced block in activin-mediated animal cap (A) and Keller sandwich (B) elongation is rescued by co-injection of LRP6-B mRNA (800 pg). The Student's t test was used for statistical analysis. Error bars indicate standard deviation. Asterisks mark differences that are statistically significant from control ($p<0.01$). Numbers of explants scored are indicated in parentheses. The same control explants were used in Figure S1 because the experiments were performed simultaneously. (C) Phalloidin staining reveals that DMZ cells from stage 10.5 embryos expressing LRP6-B (1.5 ng) have smaller length-to-width ratios and form greater numbers of cytoplasmic protrusions along their long and short axes compared to control cells. (D) Shaved Keller explants of the DMZ from embryos expressing LRP6-B show impaired motility during early gastrulation (stage 10.5). VMZ cells expressing LRP6-B have similar motility to control VMZ cells. (E) Similar to LRP6, LRP6-B does not cause XDsh-GFP cortical translocation or enhanced nuclear JNK staining in animal caps. Experiments and data analysis for LRP6-B were conducted together with LRP6 and LRP6MO studies (Figs. 3,5). Statistical analyses were carried

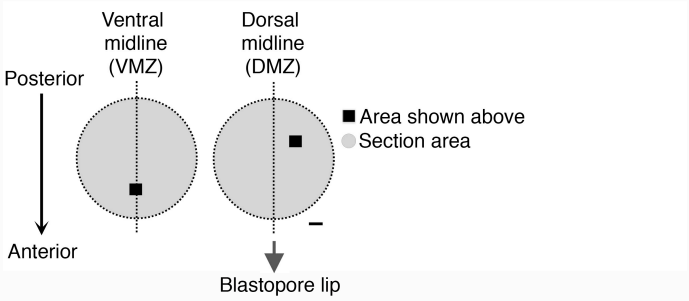
out using the Student's t test, and error bars indicate standard deviation. Asterisks show differences that are statistically significant ($p<0.01$). Numbers of cells analyzed are shown in parentheses. DMZ and VMZ cells used to determine length-to-width ratios were also used to determine the number of protrusions per cell. Scale bars: 30 μm in (A), 15 μm in (B), 25 μm in (D top panels), and 100 μm in (D lower panels).

Tahinci et al_Fig.S1



Tahinci et al_Fig.S2





Tahinci et al_Fig.S3