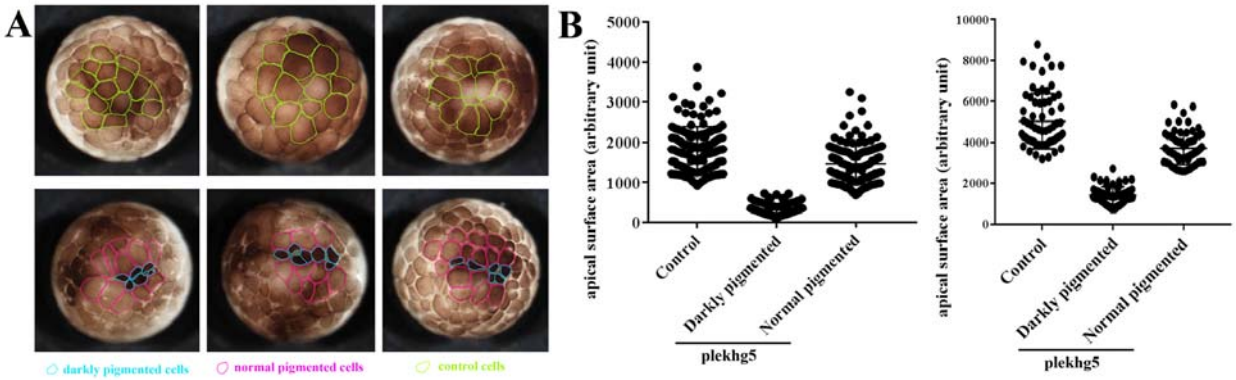


Fig. S1. Quantitative analysis of apical cell surface area in control and *plekhg5*-injected blastula embryos. A) Individual animal cells at the blastula stages were marked and their surface areas were measured using the NIH ImageJ software. B) Scatter plots of two individual experiments with mean and standard deviation are shown. GraphPad Prism7 software was used for the plot. Student t-test was also performed and showed that the differences between samples were significant.

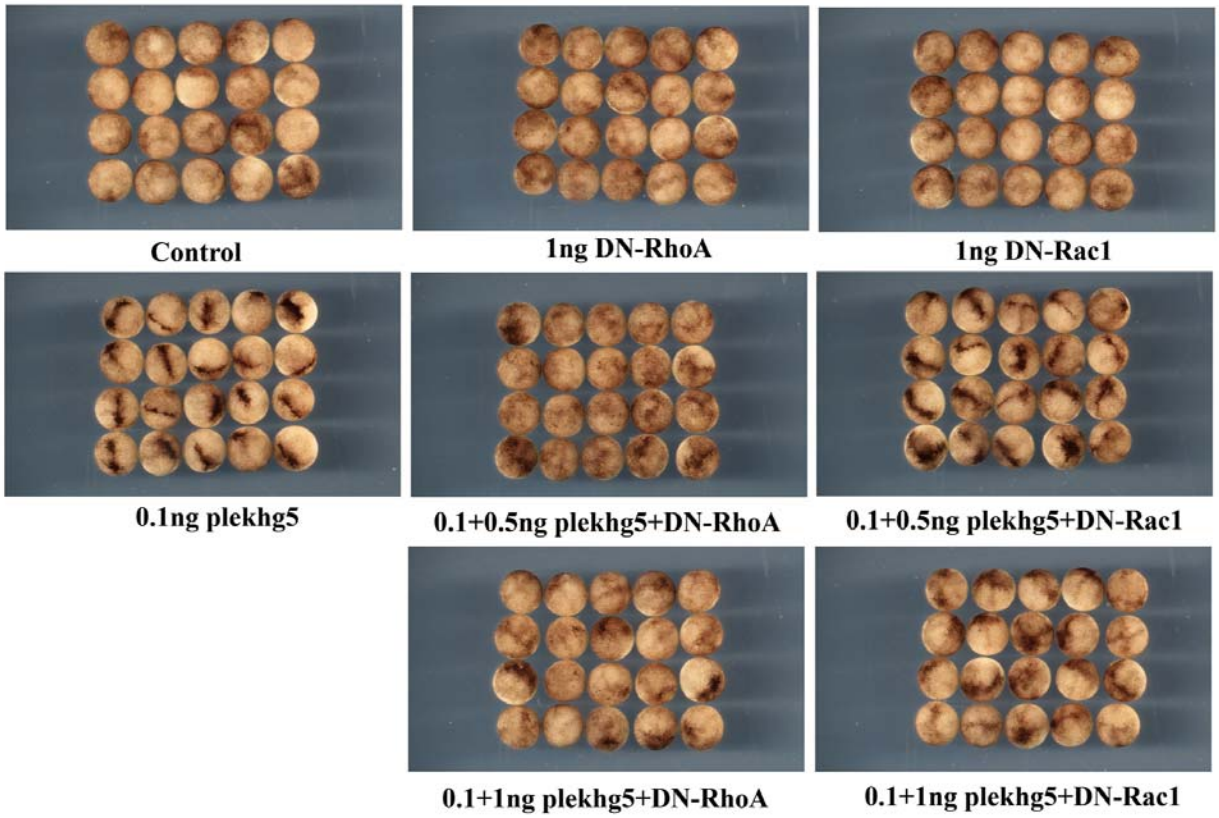


Fig. S2. Dominant negative (DN) RhoA, but not DN-Rac1, efficiently blocks ectopic blastopore lip induction by *plekhg5*. While expression of 1ng DN-RhoA or DN-Rac1 does not change animal cell morphology, 0.5ng to 1ng of DN-RhoA prevents ectopic blastopore lip-like morphology induced by *plekhg5*, whereas DN-Rac1 is not effective in inhibiting *plekhg5*.

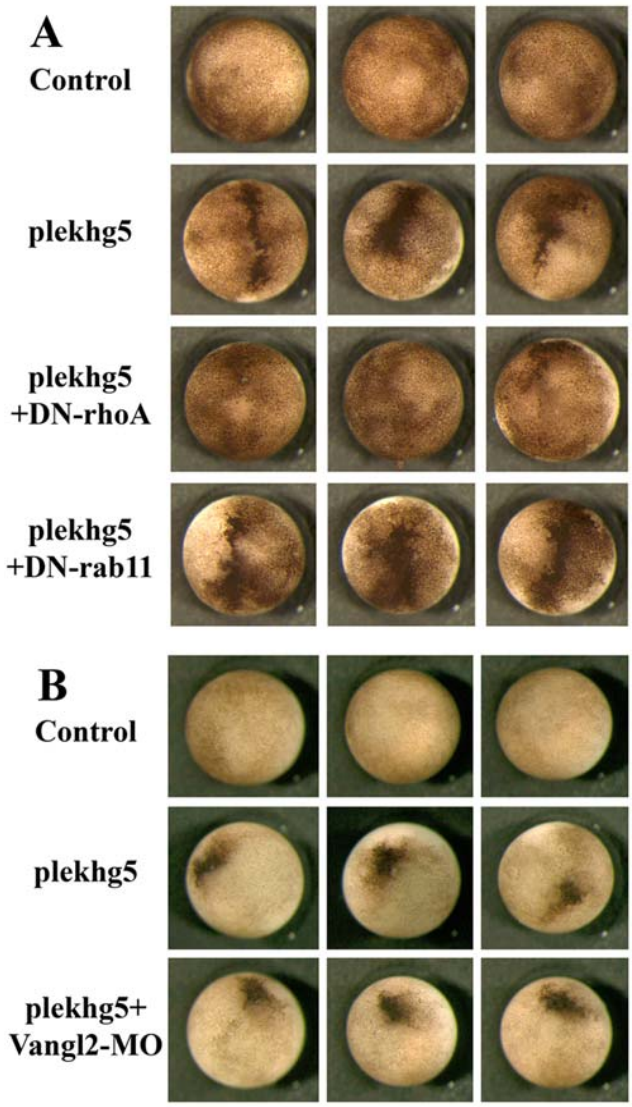


Fig. S3. Unlike DN-rhoA, neither DN-Rab11 (panel A), nor Vangl2-MO (panel B), blocks ectopic blastopore lip-like morphology by plekhg5. *plekhg5* RNA, 0.1ng, 22/22 embryos with ectopic blastopore lip; with DN-rhoA, 1-2ng, 2/23; with DN-Rab11, 1-2ng, 34/35; with Vangl2-MO, 25ng, 18/18 embryos.

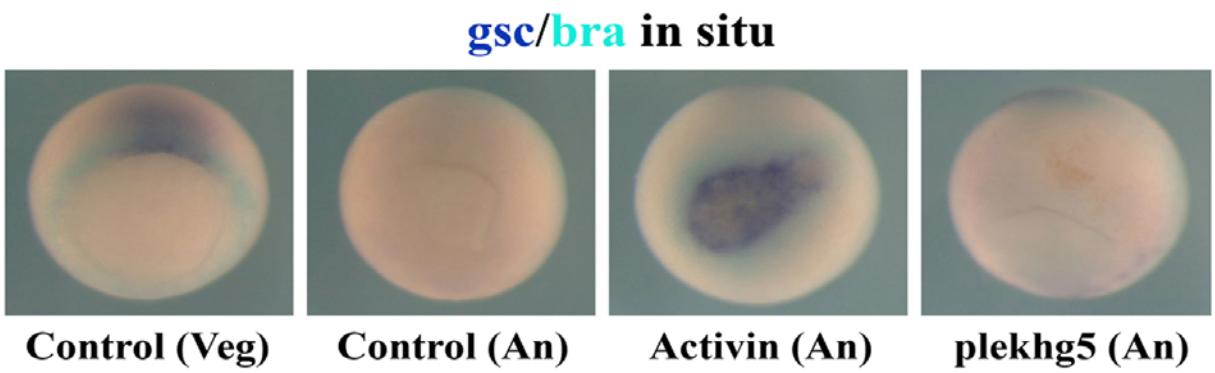


Fig. S4. Unlike activin, *plekhg5* does not induce the mesodermal markers gsc (*goosecoid*) and *bra* (*brachyury*) in the animal region. The vegetal view (Veg) of the left panel *shows* the endogenous expression of the mesodermal markers, and the animal view (An) of the other panels shows the ectopic expression of the markers.

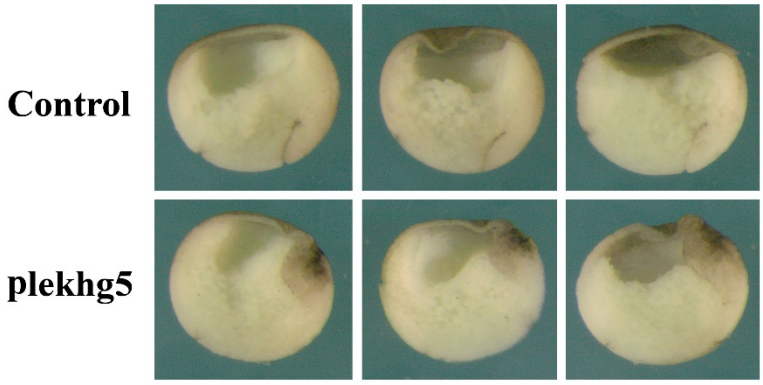


Fig. S5. *Plekhg5* interferes with radial cell intercalation in the ectoderm. Side view of bisected embryos reveals that ectopic expression of *plekhg5* blocks radial cell intercalation, resulting in thick mass of multi-layered cells underneath the darkly pigmented, apically constricting, superficial epithelial cells.

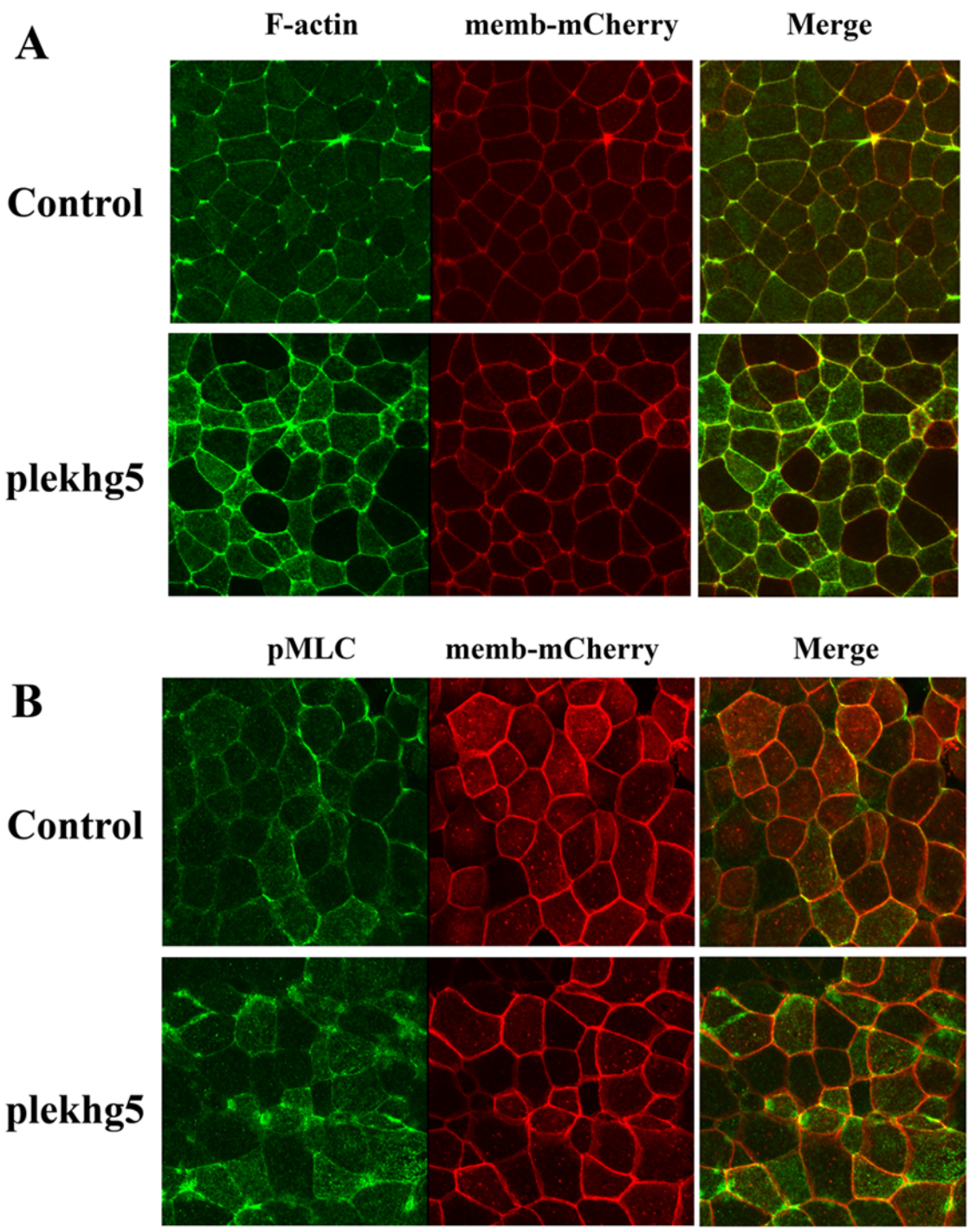


Fig. S6. *plekhg5* induces apical accumulation of F-actin and pMLC. En face view of the animal cells from the control or the *plekhg5*-injected embryos shows that both F-actin and pMLC preferentially localize to the cell junctions in control embryos, but their signals are enhanced at the apical cortex in *plekhg5*-injected embryos.

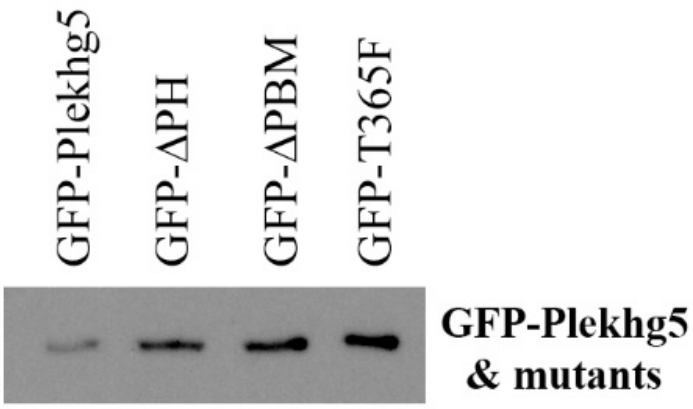


Fig. S7. Western blot analysis shows that GFP-Plekhg5 mutants are expressed at similar levels as GFP-Plekhg5.

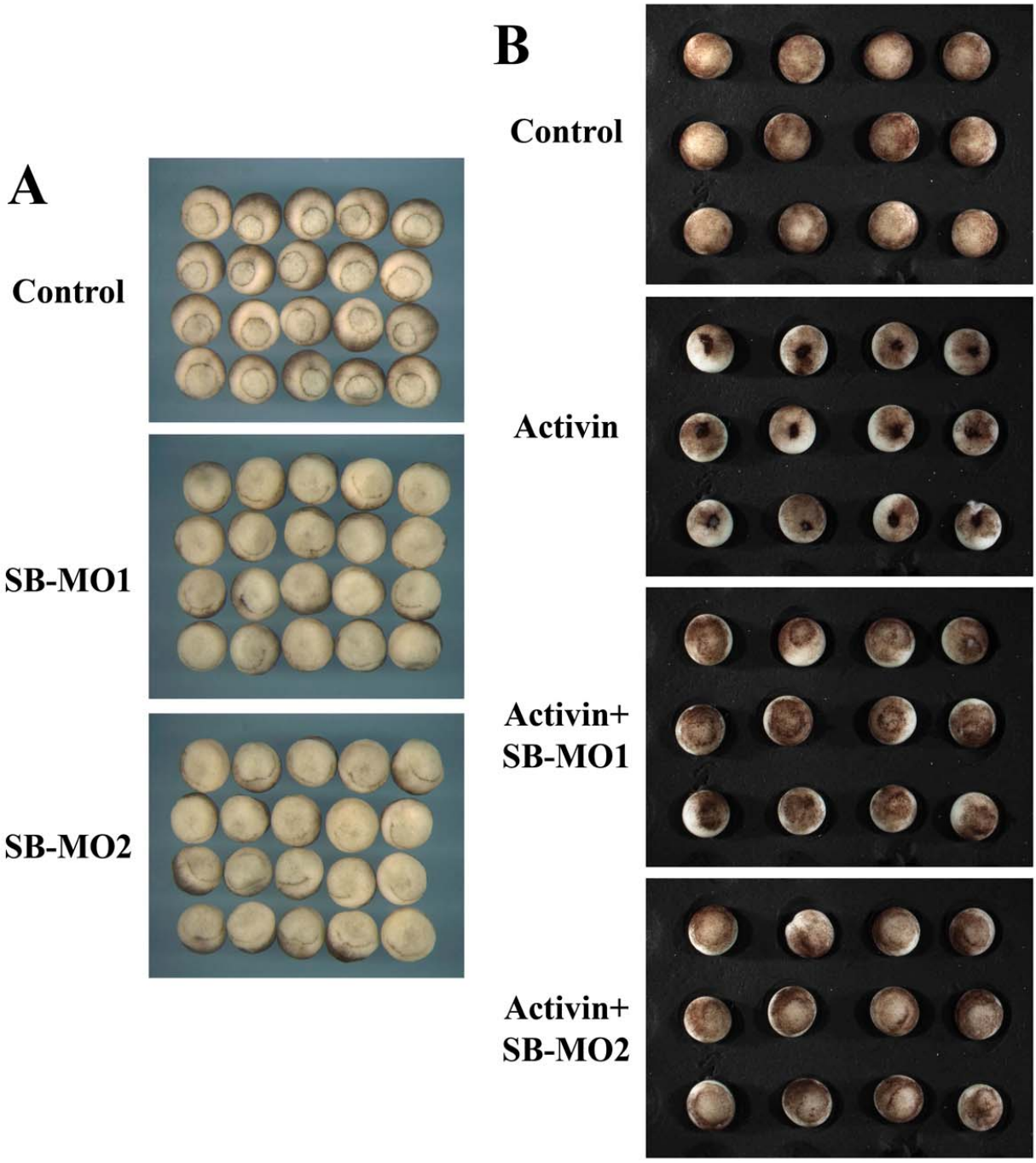


Fig. S8. Both *plekhg5* SB-MOs induce similar phenotypes in early Xenopus embryos. A) Injection of either SB-MO1 or SB-MO2 into the marginal zone region of early Xenopus embryos leads to defects in blastopore lip formation. B) Both *plekhg5* SB-MO1 and SB-MO2 block the ectopic blastopore lip induction by activin. Doses of reagents used: SB-MO1 and SB-MO2, 50ng; activin, 5pg.

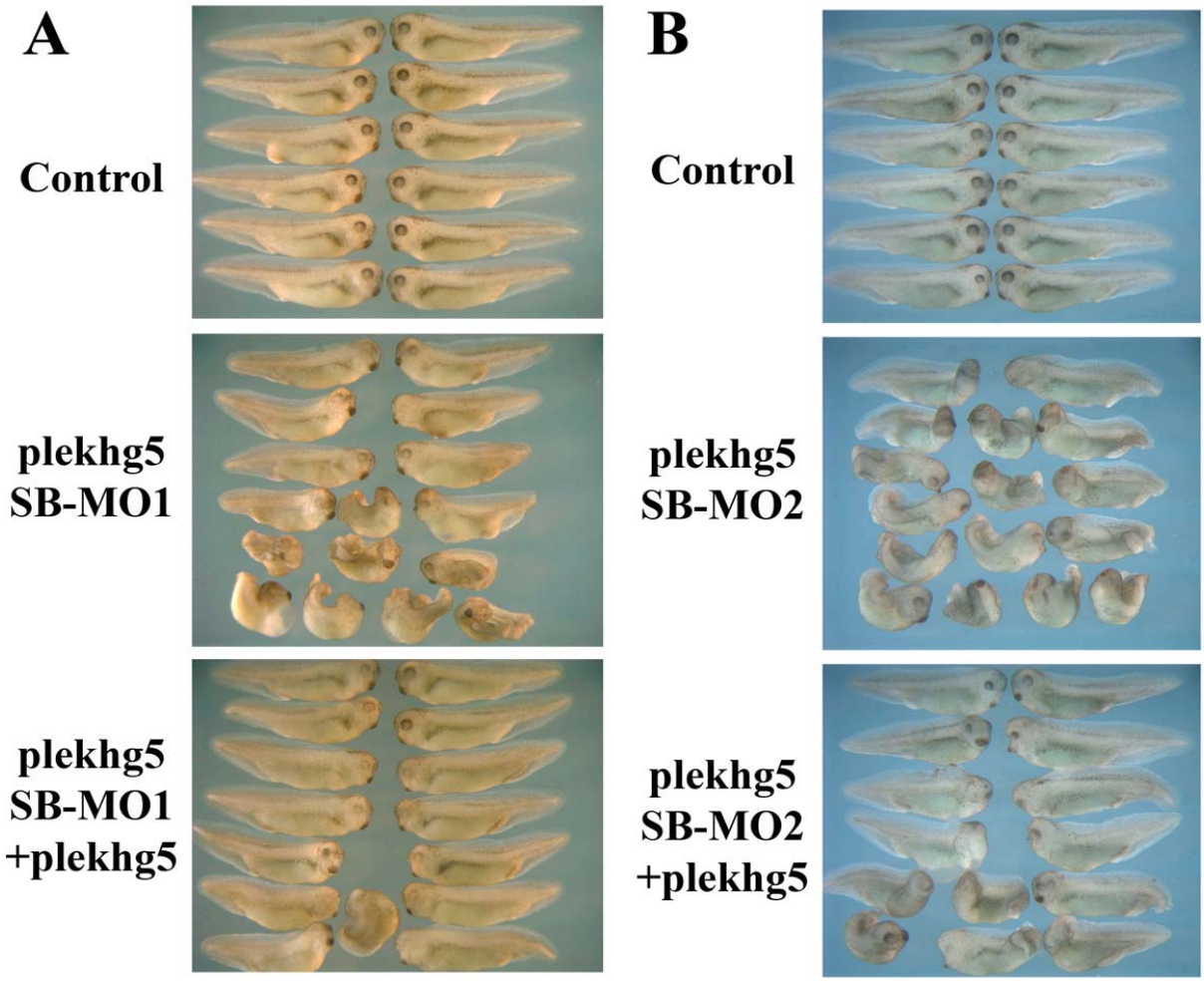


Fig. S9. *plekhg5* SB MOs induce axial defects at the tadpole stages that are largely rescued by co-expressed wild type *plekhg5* RNA. *plekhg5* SB-MOs (50ng) induce axial defects, including small head, shortened axis, and some with failure in blastopore closure. The defects are largely rescued when SB-MOs are co-expressed with wild type *plekhkg5* RNA (25-100pg).

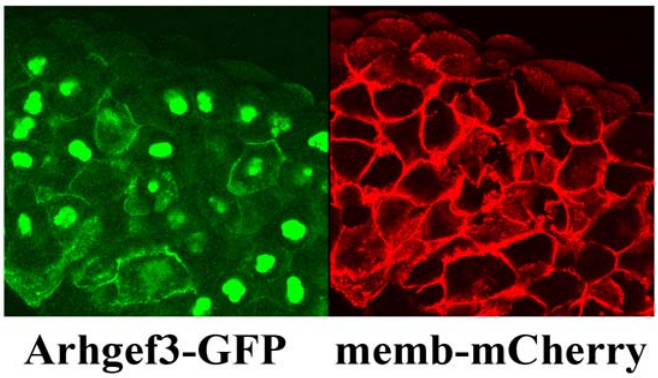


Fig. S10. Arhgef3, another organizer-enriched, PH-domain-containing RhoGEF, is localized strongly in the cell nucleus in addition to some membrane signals.

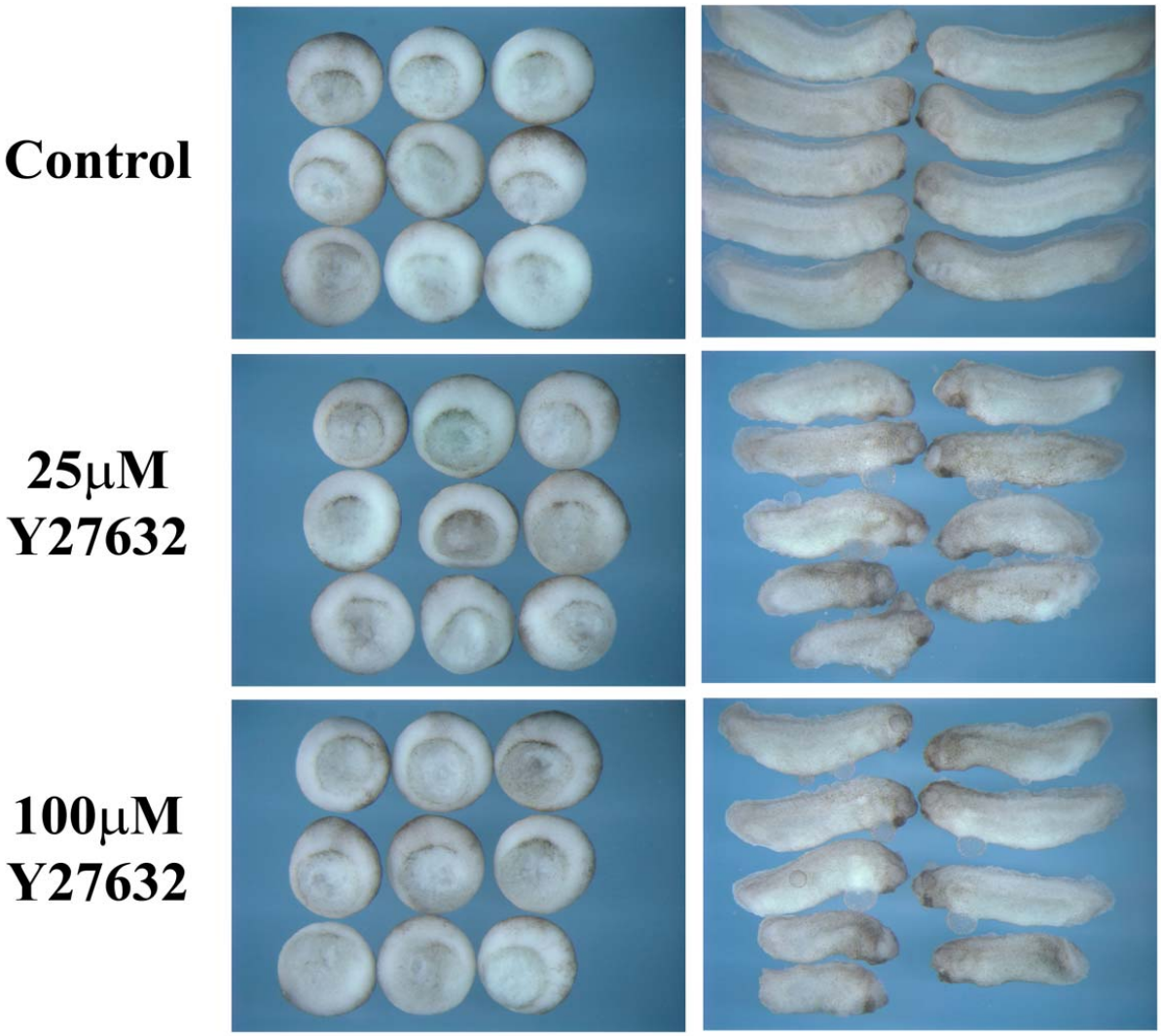
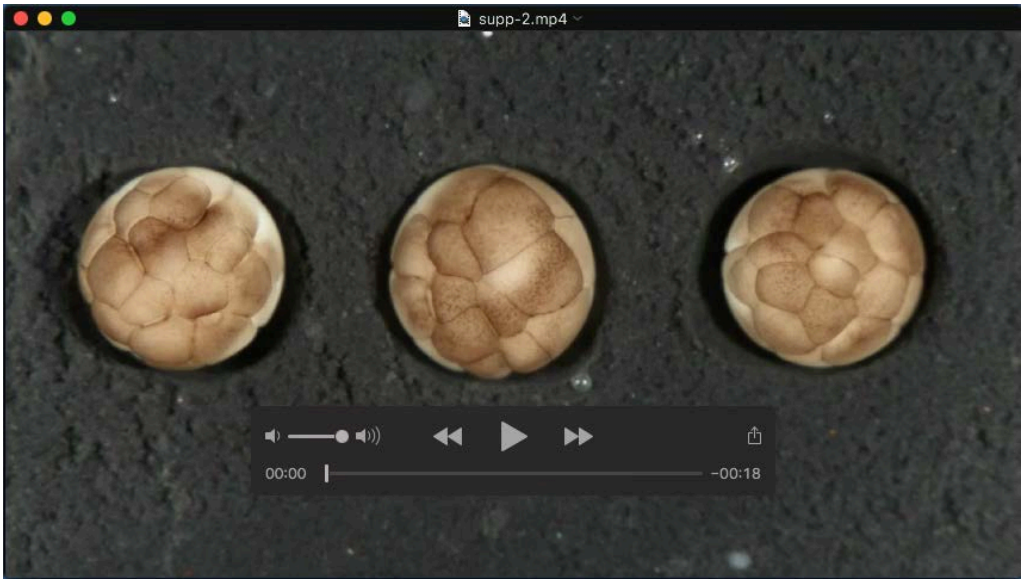
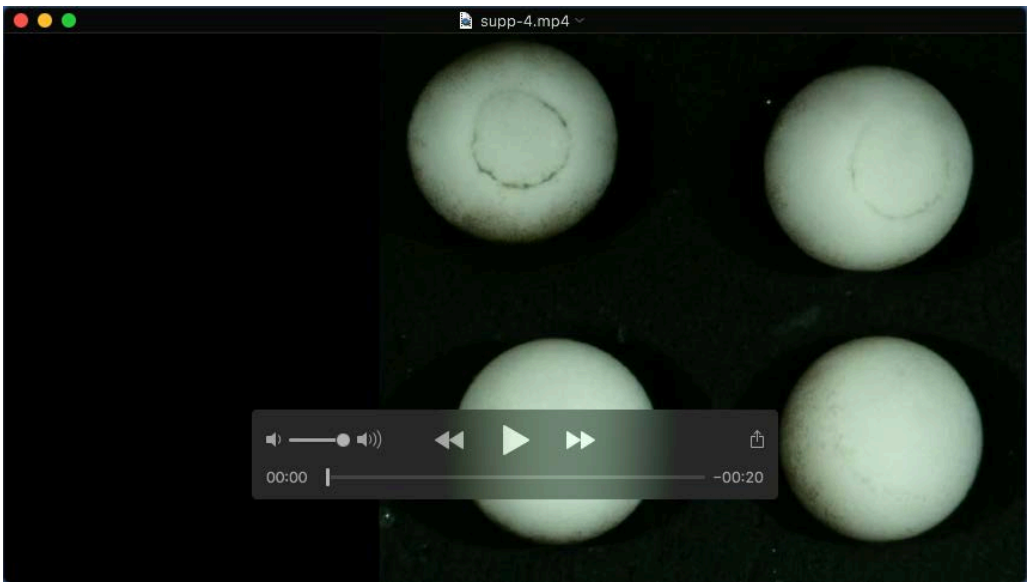


Fig. S11. Treatment of the blastula embryos with the ROCK inhibitor Y27632 does not prevent formation of the blastopore lip, though the embryos display multiple defects at later stages, including smaller head and skin blistering.



Movie 1. *plekhg5* induces apical constriction at blastula stages (embryo on the right), whereas activin induces ectopic blastopore lip only during gastrulation (embryo in the center). The control embryo is shown on the left.

Movie 2. Gastrulation movements in embryos with altered levels of *plekhg5*. The control embryo is shown on the left, the morphant embryo with *plekhg5* SB-MO injected into the dorsal marginal zone is shown in the center, and the embryo injected with the *plekhg5* RNA is shown on the right.



Movie 3. Injection of *plekhg5* SB-MO into the marginal zone of all 4 blastomeres at the 4-cell stages results in minimal blastopore lip formation during gastrulation. The embryos nonetheless accomplish blastopore closure when control siblings reach the neurula stages. Convergent extension tissue movements seem to drive the blastopore closure in the morphant embryos. Top two embryos are the controls and the bottom two embryos are the morphants.

Movie 4. Gastrulation movements of *plekhg5* morphant embryos with the MO injected either into the dorsal (left embryo) or the ventral (right embryo) side. The dorsally injected embryo shows defects in blastopore closure. This happens in a minority of the morphant embryos, suggesting a lack of precision in cell movements during gastrulation.