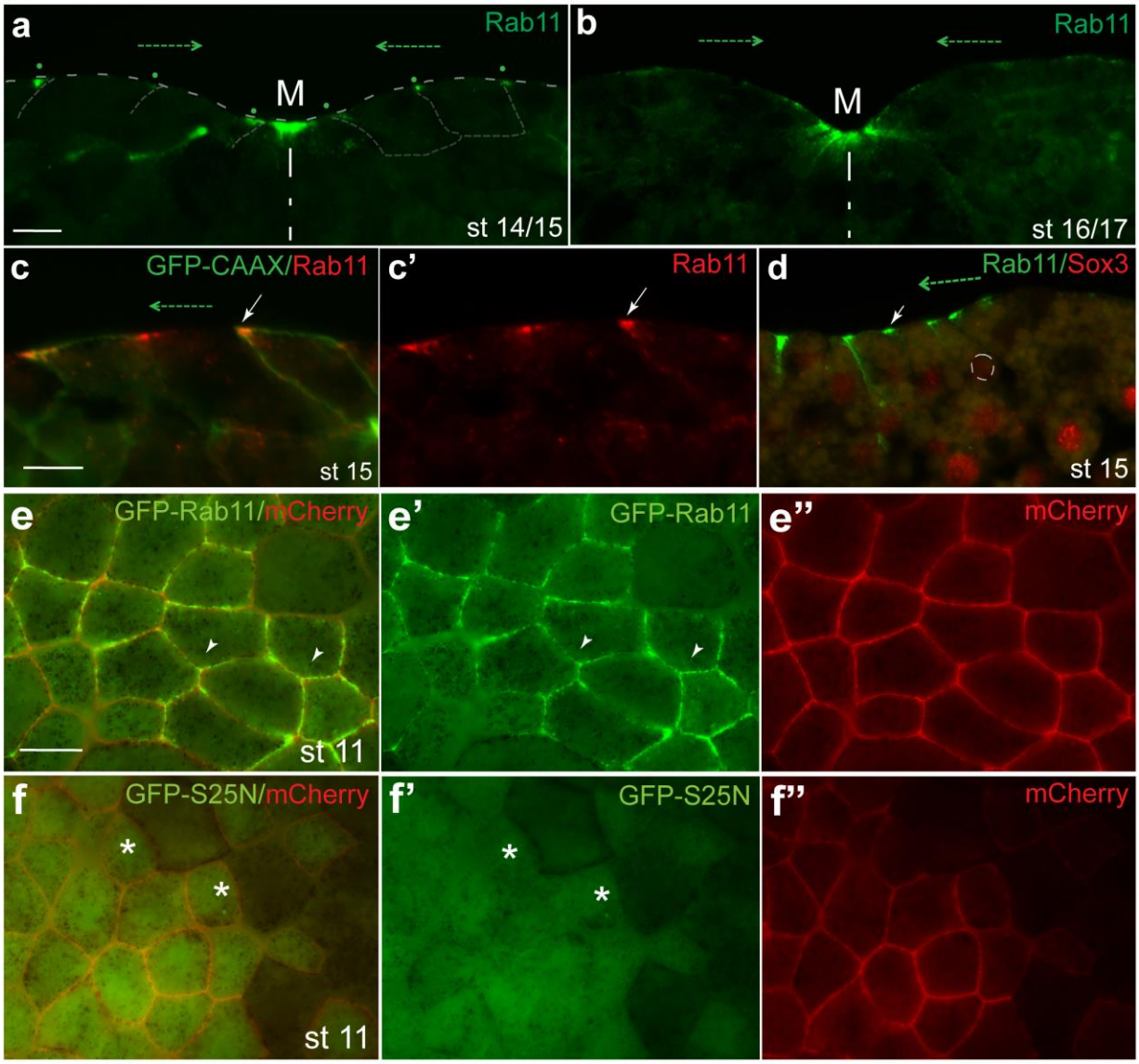
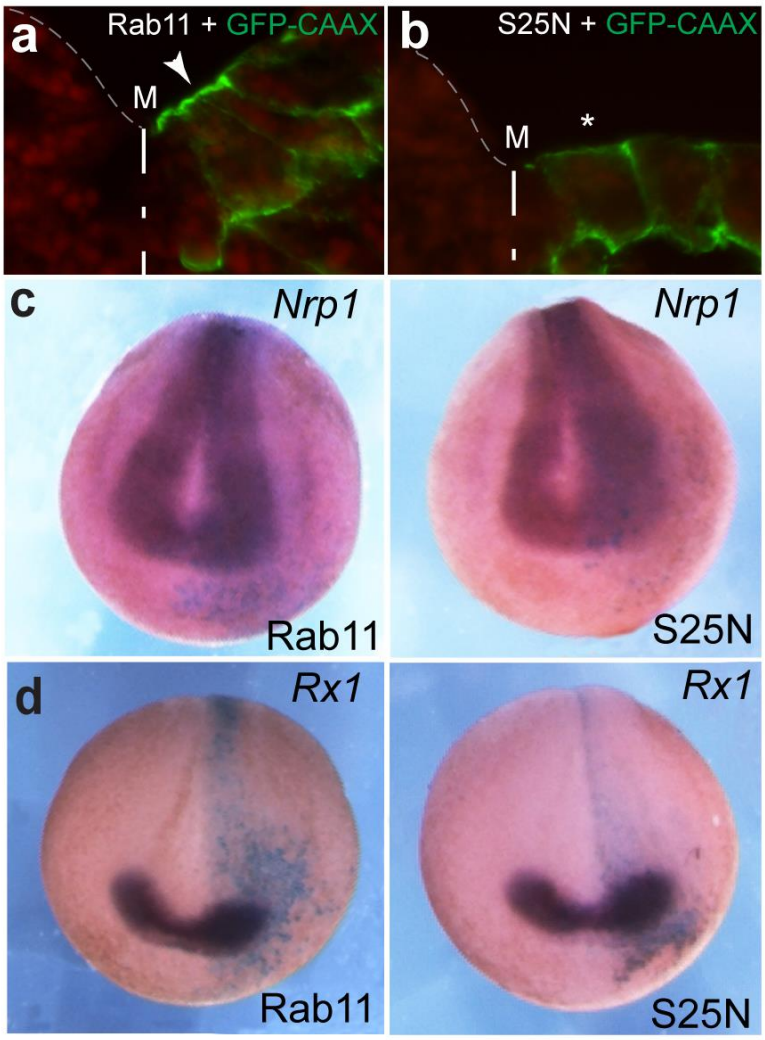
**Supplementary information:**

**Supplementary Figures 1-8**

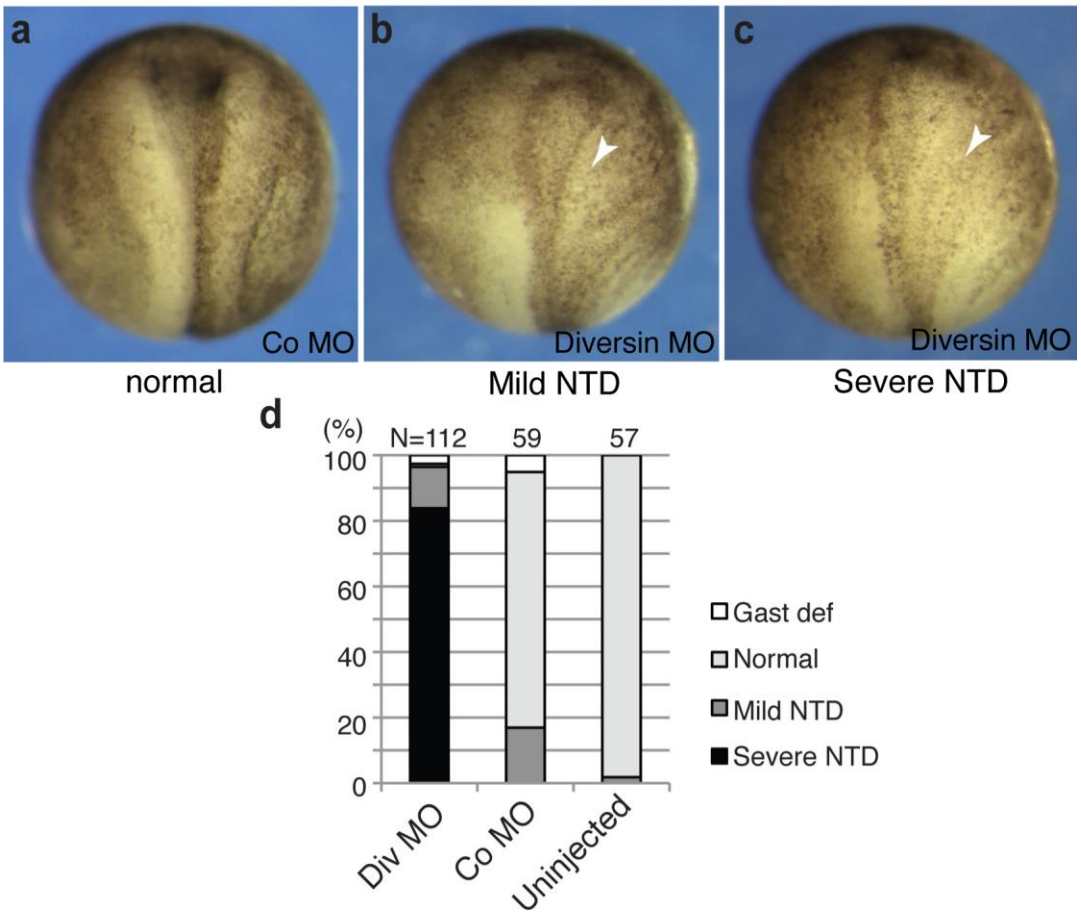


**Supplementary Fig. 1.** Localization of Rab11 along the mediolateral axis of the neural plate and in epidermis.

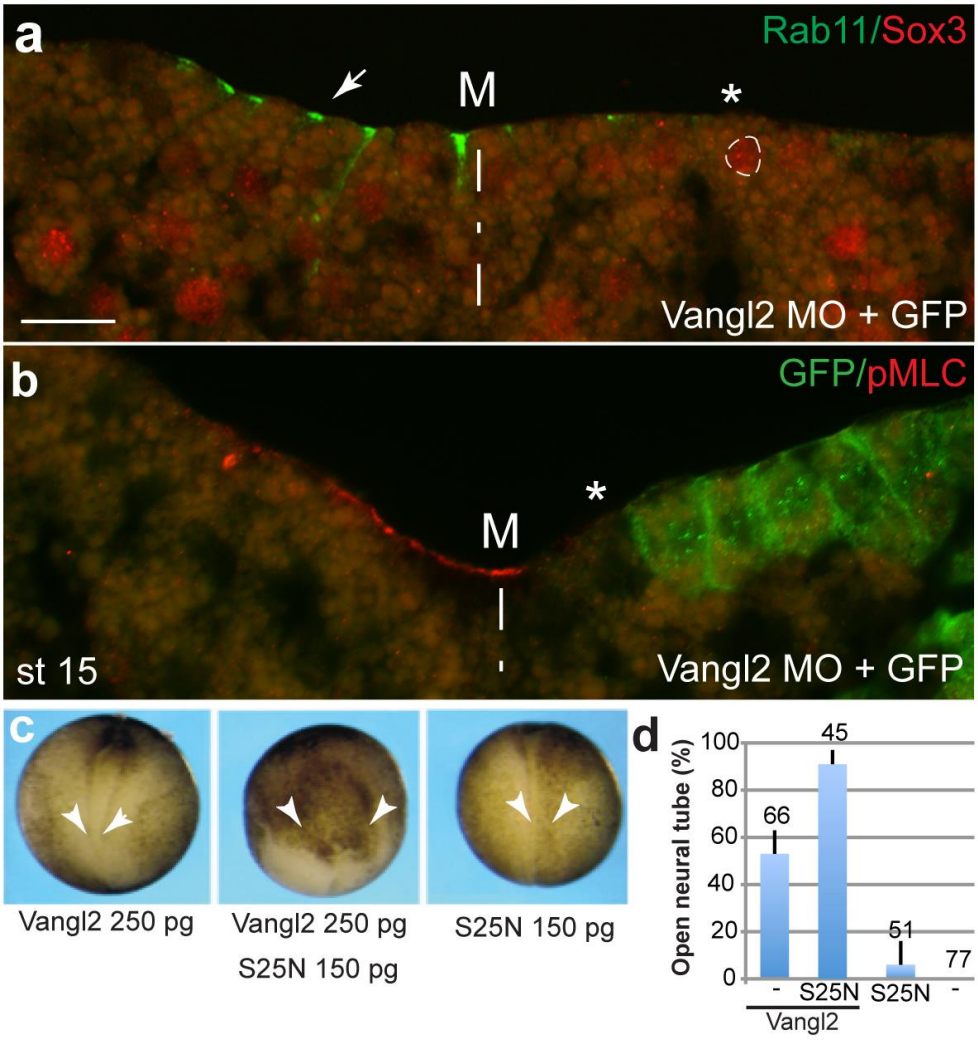
**a-d,** Transverse cryosections stained with anti-Rab11 antibodies at early neurula (a, c, d) and midneurula (b) stages. Green dots indicate Rab11 polarity. Neural plate and individual cell boundaries are shown by broken lines. M, midline position. Antibody specificity is indicated at the upper right corner of each panel. c, Mosaic GFP-expressing cells were created by injection of GFP-CAAX mRNA (50 pg). Costaining for Rab11 and GFP (c), or Rab11 and Sox3 (d) is shown. Arrows point to polarized Rab11 distribution. Sox3-positive nucleus is indicated by a broken line (d). Dashed green arrows indicate cell polarization towards the midline (a-d). Scale bar is 10 µm. **e, f**, Top view of ectoderm (st. 11) prepared from embryos injected with GFP-Rab11 or GFP-Rab11S25N RNAs (0.1 ng each). mCherry RNA (0.4 ng) was coinjected to label cell boundaries. e-e'' GFP-Rab11 is visible at cell junctions (arrows), f-f''GFP-Rab11S25N is localized throughout the cytoplasm (asterisks).


a
Rab11 + GFP-CAAX
M
b
S25N + GFP-CAAX
M
*
c
Nrp1
Rab11
Nrp1
S25N
d
Rx1
Rab11
Rx1
S25N

**Supplementary Fig. 2.** The effect of Rab11 constructs on cell shape and neural tissue markers. Wild type (**a**) and Rab11 S25N (**b**) RNAs (1 ng each) were unilaterally injected (asterisk) together with GFP-CAAX RNA to mark cell boundaries. Images demonstrate representative changes in cell shape, which were used for quantification in Fig. 2f. **c, d**, Neural tissue markers in embryos with modulated Rab11 function. Four-cell embryos unilaterally injected with indicated Rab11 RNAs (1 ng each) and LacZ RNA as a lineage tracer (100 pg) were cultured until stages 16/17, fixed and processed for *in situ* hybridization with Nrp1 (c) and Rx1 (d) antisense probes. Representative embryos from each group (7-20 embryos) do not reveal changes in Nrp1 and Rx1 expression. Note the expansion of Nrp1 on the Rab11S25N-expressing side due to unilateral neural tube closure delay.

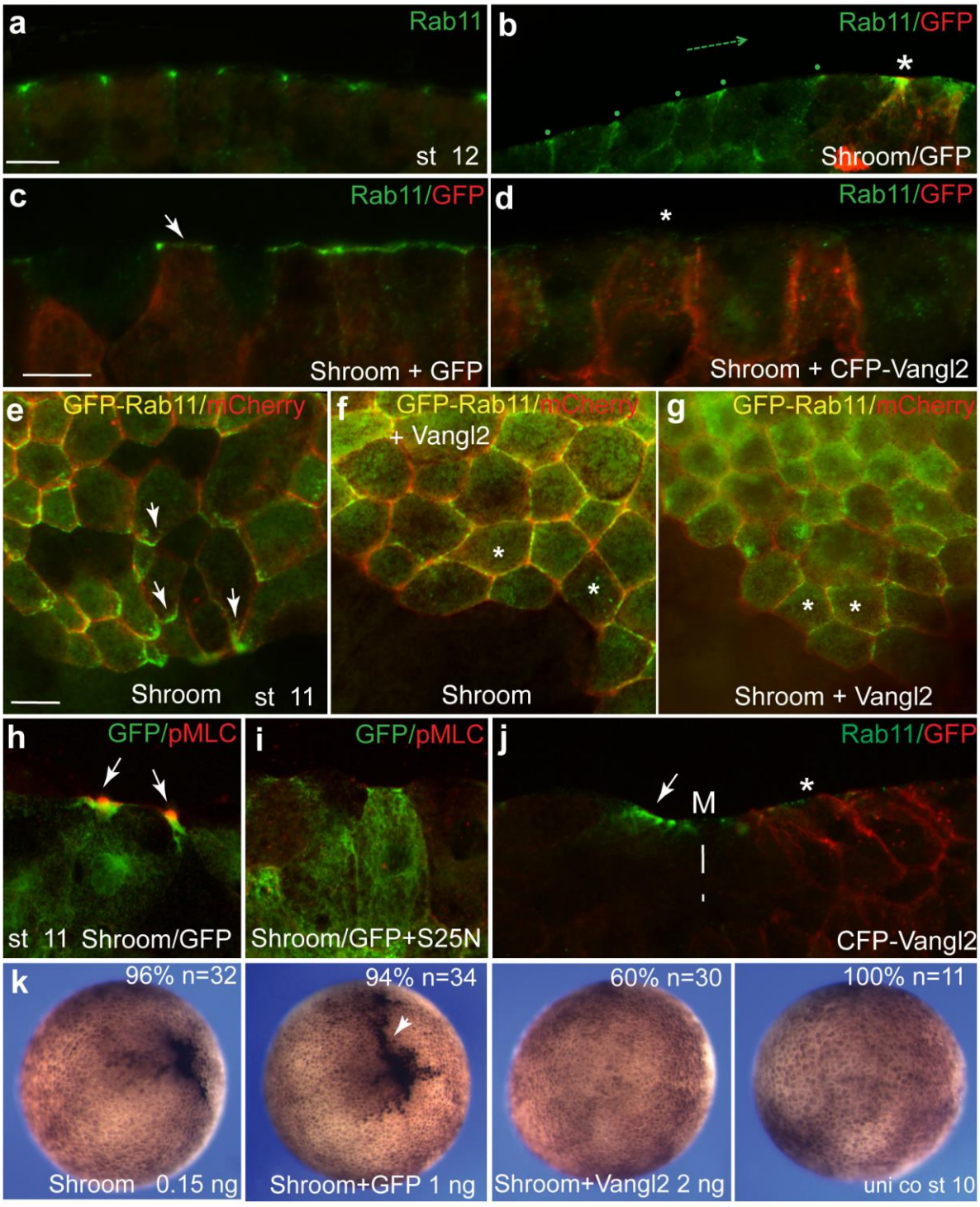



**Supplementary Fig. 3.** Diversin is required for neural tube closure. a-c, Top views of stage 16/17 embryos, unilaterally injected at the 4 cell stage with 30 ng of Div MO or CoMO . Arrowsheads mark the injected side. For quantification of defects, lack of the pigment line was scored as severe (c), and weak or discontinuous pigment line was scored as mild (b), as compared to normal or control MO-injected embryos (a). Representative phenotypes are shown. d, Frequencies of embryos in each group are shown, total number of injected embryos is shown on the top of each bar. These results are representative from 4-7 independent experiments. Gast def, gastrulation defects.

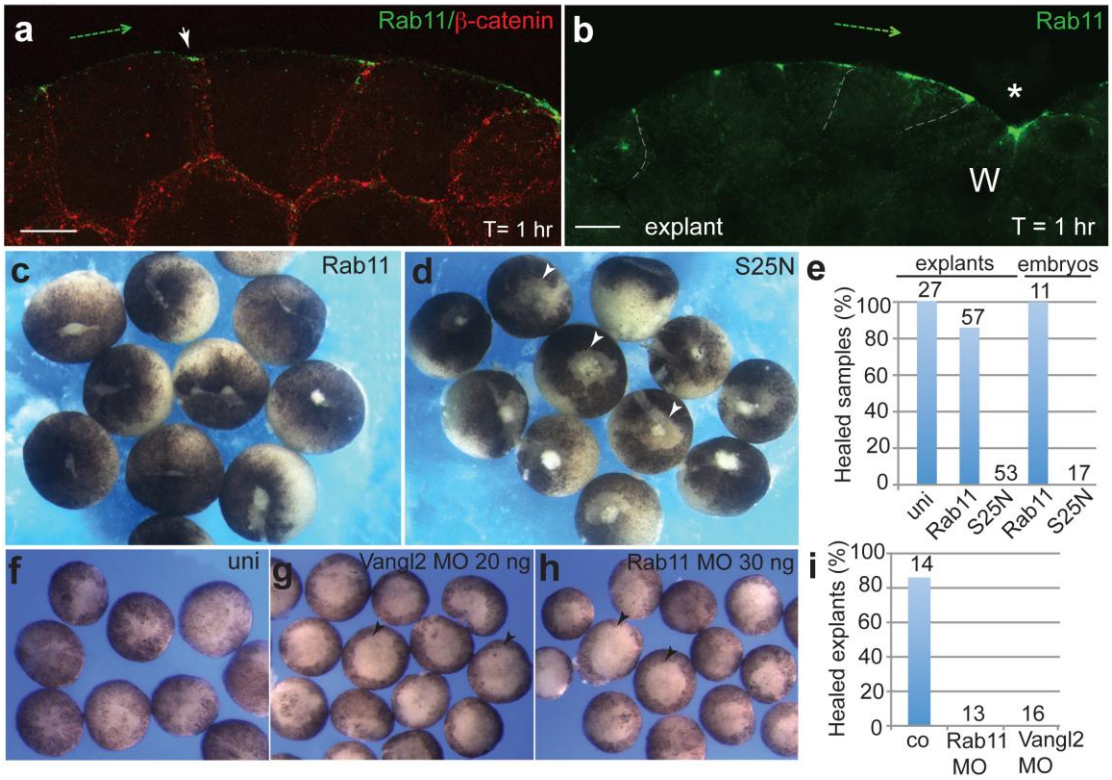

a
Rab11/Sox3
M
*
Vangl2 MO + GFP
b
GFP/pMLC
*
M
st 15
Vangl2 MO + GFP
c
Vangl2 250 pg
Vangl2 250 pg
S25N 150 pg
S25N 150 pg
d
Open neural tube (%)
100
80
60
40
20
0
66
45
51
77
-
S25N
S25N
-
Vangl2

**Supplementary Fig. 4.** Interactions of Vangl2 and Rab11 during neural tube closure.

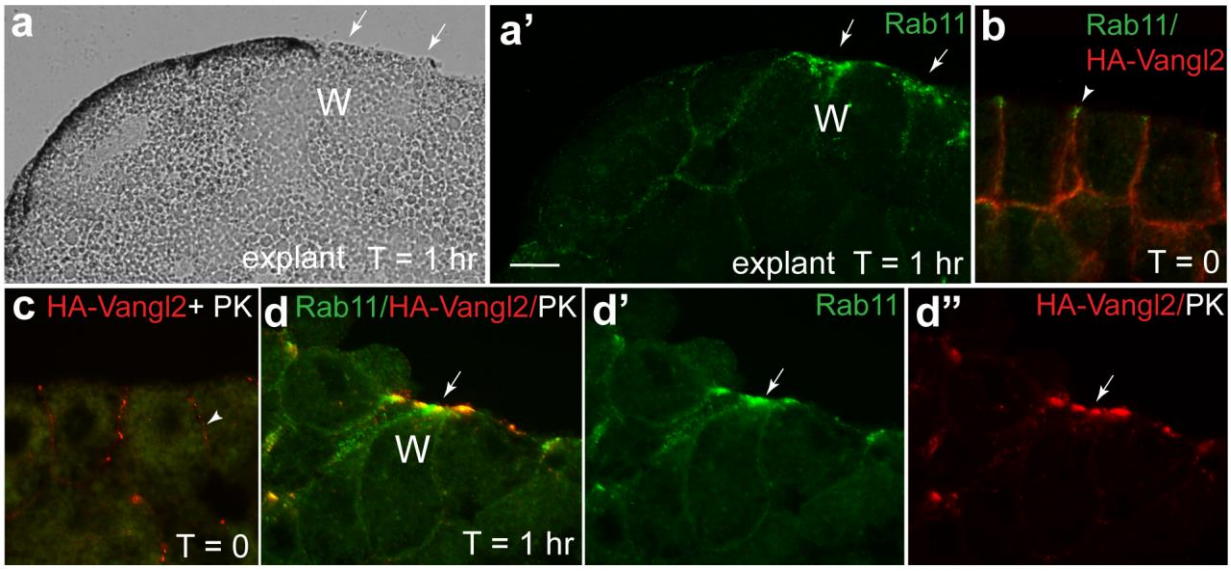
**a, b,** Immunostaining for endogenous Rab11 and pMLC in stage 15/16 embryos unilaterally injected at the 4-cell stage with Vangl2 MO (20 ng, asterisk). Vangl2 MO prevents polarization of Rab11 (a, arrow) and apical activation of pMLC (b). (a) Vangl2-depleted cells continue to express the neural progenitor marker *Sox3*. Dashed circle indicates a Sox3-positive nucleus. GFP RNA (100 pg) is a lineage tracer (a, b). Dashed line indicates the midline (M). Scale bar in (a), also refers to (b), is 20 µm. **c, d,** Functional interactions of Vangl2 and Rab11 during neural tube closure. c, Morphology of representative embryos that have been injected with the indicated doses of Vangl2 and Rab11S25N RNAs. White arrowheads demarcate neural plate boundary. d, Quantitation of the effect. Number of embryos per group is indicated above each column.


a
Rab11
st 12
b
Rab11/GFP
Shroom/GFP
c
Rab11/GFP
Shroom + GFP
d
Rab11/GFP
Shroom + CFP-Vangl2
e
GFP-Rab11/mCherry
Shroom st 11
f
GFP-Rab11/mCherry
+ Vangl2
Shroom
g
GFP-Rab11/mCherry
Shroom + Vangl2
h
GFP/pMLC
st 11 Shroom/GFP
i
GFP/pMLC
Shroom/GFP+S25N
j
Rab11/GFP
M
CFP-Vangl2
k
96% n=32
Shroom 0.15 ng
94% n=34
Shroom+GFP 1 ng
60% n=30
Shroom+Vangl2 2 ng
100% n=11
uni co st 10

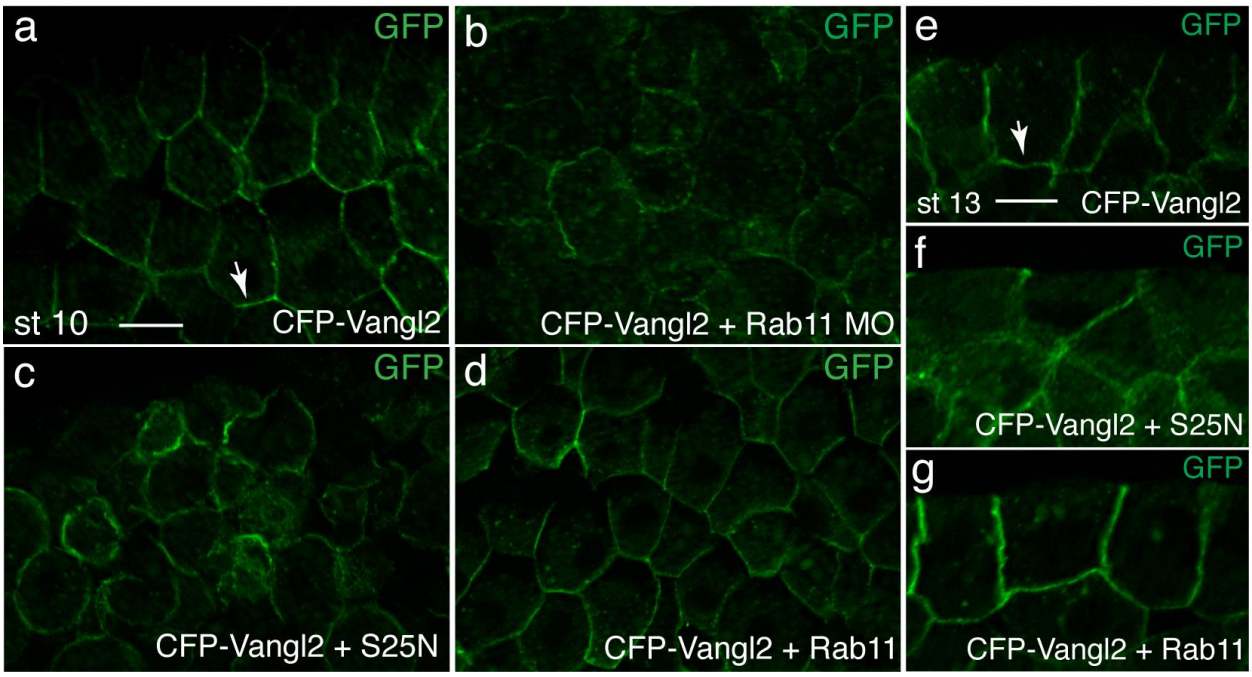
**Supplementary Fig. 5.** Rab11 and Vangl2 roles in Shroom-induced apical constriction. **a, b,** Localization of endogenous Rab11 in stage 12 control uninjected (a) and Shroom-expressing (asterisk) ectoderm cells (b). Green dots demarcate polarized Rab11, dashed arrows indicate tissue polarization towards Shroom-expressing cells. **c**, **d**, Rab11 is recruited to the apical surface of Shroom-expressing cells (c, arrow), but this recruitment is blocked by overexpressed Vangl2 RNA (2 ng) (d, asterisk). Shroom RNA was coinjected with GFP RNA (GFP, red) (a-c) or with CFP-Vangl2 RNA (GFP, red, d). **e-g,** Top view of ectoderm with polarized GFP-Rab11 near Shroom-expressing cells. GFP-Rab11 (100 pg) and Shroom (300 pg) RNA were injected into distinct blastomeres at the four-cell stage. Membrane-targeted Cherry RNA (400 pg) was co-injected with GFP-Rab11 to demarcate cell boundaries. Arrows point to polarized Rab11 in the direction of Shroom-expressing cells (strongly pigmented area at the bottom in (e). (f, g) After Vangl2 is coexpressed with GFP-Rab11 and Cherry RNAs (f) or with Shroom RNA (g), GFP-Rab11 is no longer polarized (asterisks). Scale bar in (e), also relates to (f, g), is 20 µm. **h, i,** Requirement of Rab11 for Shroom-induced phosphorylation of Myosin II light chain. Four-cell embryos were animally injected with Shroom (300 pg**)** and GFP (100 pg) as a lineage tracer, and Rab11S25N (1 ng) RNAs as indicated. Transverse cryosections of stage 11 embryos reveal ectopic pMLC staining in response to Shroom (arrows, h), which is inhibited by Rab11S25N (i). **j**, Overexpressed CFP-Vangl2 RNA inhibits Rab11 polarization (arrow) in the neural plate. Vangl2 is detected with anti-GFP antibodies. **k,** Vangl2 interferes with apical constriction induced by Shroom. Shroom RNA (150 pg) was injected with control GFP (1 ng) or Vangl2 RNA (1-2 ng) animally, into four-cell embryos, as indicated. Representative images (stages 10-10.5) are shown, with uninjected control embryos on the right. Apical constriction area is heavily pigmented (arrowhead). Frequency of embryos with indicated phenotype is shown.




**Supplementary Fig. 6.** Rab11 and Vangl2 involvement in wound healing. **a,** Confocal image of an ectodermal explant with Rab11 polarized towards the wound. Costaining with β-catenin antibodies indicates cell boundaries. Dashed green arrow indicates the direction of the wound. **b**, Embryonic explant revealing accumulation and planar polarization of Rab11 towards the wound (asterisk). **c, d**, Wound healing in late blastula embryos injected with wild-type Rab11 or Rab11S25N RNAs (1 ng each). White arrowheads point to surface healing defects. **e,** Quantitation of wound healing in ectoderm explants. Rab11-expressing explants were indistinguishable from uninjected explants. **f-i**, Requirement of Vangl2 and Rab11 for wound healing of ectodermal explants. Two-cell embryos were injected with Vangl2 MO (20 ng) or Rab11 MO (30 ng). Ectodermal explants were prepared at the midblastula stage and allowed to heal for 40 min. (f) Uninjected explants (g) Vangl2 MO-injected and (h) Rab11 MO-injected explants, i. Quantification of data from f-h.




**Supplementary Fig. 7.** Polarization of Rab11 and Vangl2 during embryonic wound healing. **a,** Brightfield image of a healing ectoderm explant discriminates inner (unpigmented) and superficial (pigmented) cells. **a**', Rab11 accumulates at the apical surface of inner cells (arrows) during wound healing of the explant in (a). **b-d**, Embryos were injected with HA-Vangl2 RNA (100 pg) (b), or HA-Vangl2 RNA and Pk (Prickle) RNA (100 pg) (c, d). (b-d) In control explants (t=0), Rab11 localizes at the cell junctions, whereas HA-Vangl2 is present at the basolateral cell cortex. (c) Vangl2 forms randomly distributed complexes with Pk. (d-d") Rab11 colocalizes with HA-Vangl2 in inner cells, one hour after healing (T=1 hr). Scale bar is 20 µm (a-d). W, wound. These results are representative from 4-6 independent experiments.




**Supplementary Fig. 8 .** Vangl2 trafficking is regulated by Rab11. Cross-sections of stage 10 or stage 13 ectoderm immunostained for GFP are shown for animal cap explants (a-d) and embryos (e-g), injected at the four-cell stage with CFP-Vangl2 RNA (100 pg) and Rab11 RNAs (1-2 ng) or Rab11 MO (20 ng) as indicated. CFP-Vangl2 is more diffuse at the cell membrane and accumulates in the cytoplasm of cells expressing Rab11S25N or Rab11 MO (arrows), but not wild-type Rab11. Apical is up. Scale bar, 15 µm. Each group included 10-15 embryos or explants. Representative images from four independent experiments are shown.