**Supplementary information**

**Supplementary figure legends to Figures S1-S5.**

Fig. S1

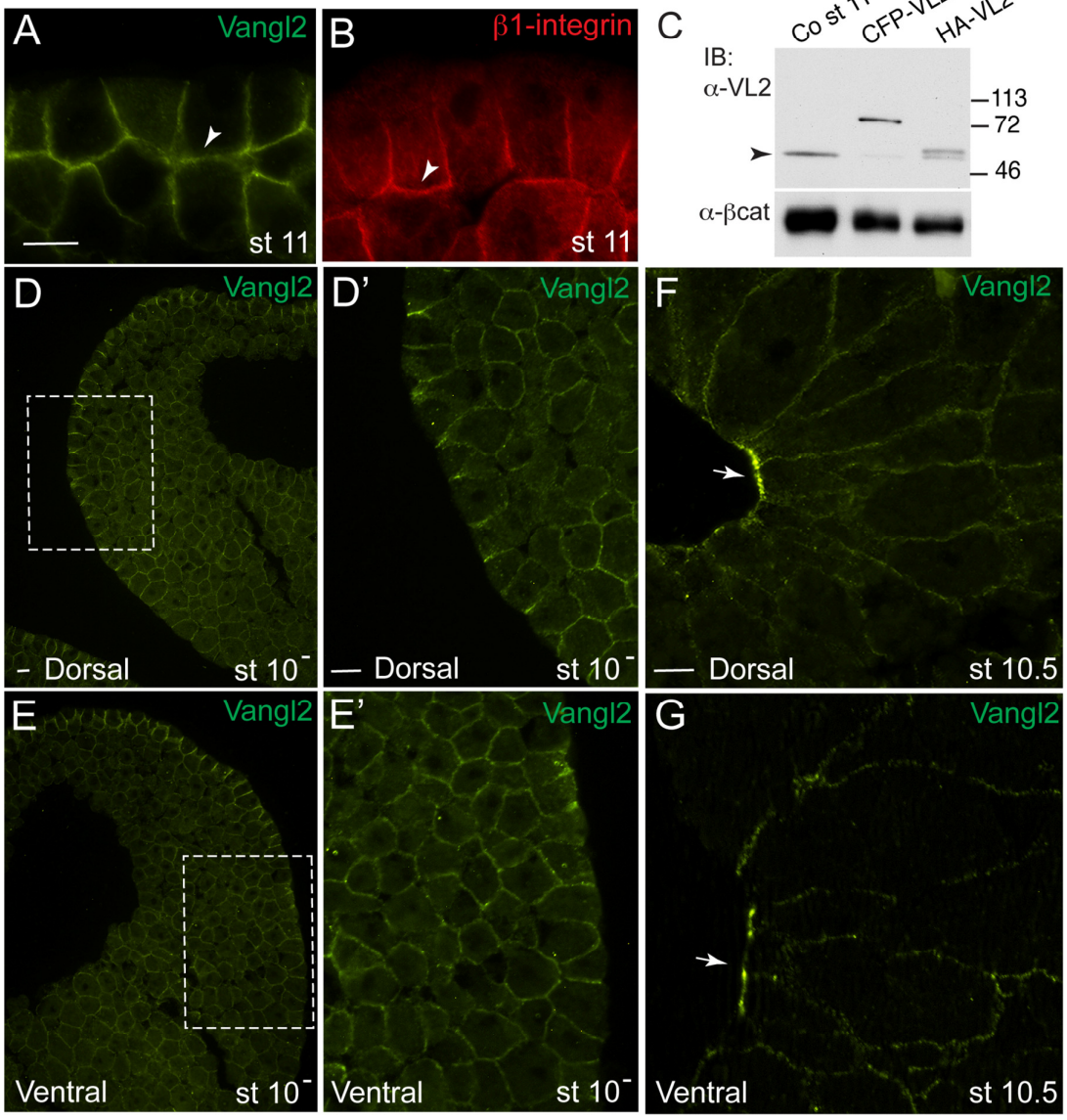


**Fig. S1. Immunodetection of endogenous Vangl2 protein.**

(A, B) Basolateral localization (arrowhead) of Vangl2 (A) and β1-integrin (B) in embryonic ectoderm at stage 11. (C) Immunoblot of ectodermal cell lysates with anti-Vangl2 antibody reveals a 60 kDa band (arrowhead) corresponding to endogenous Vangl2, misexpressed *Xenopus* CFP-Vangl2 and mouse HA-VL2, as indicated.

(D-G) Vangl2 immunostaining on cross-sections of st. 10$^{-}$ and st. 10.5 embryos. Dashed boxes in D and E correspond to the magnified images shown in D' and E'. Vangl2 is detected at the basolateral membrane and in cytoplasmic vesicles near the apical cortex, and appears more abundant in the superficial cell layer. Scale bar in A is 20 μm. Both apical (arrow) and basolateral staining is detected in the constricting blastopore cells of st. 10.5 embryo. D-G, Scale bar is 20 μm.

Fig. S2

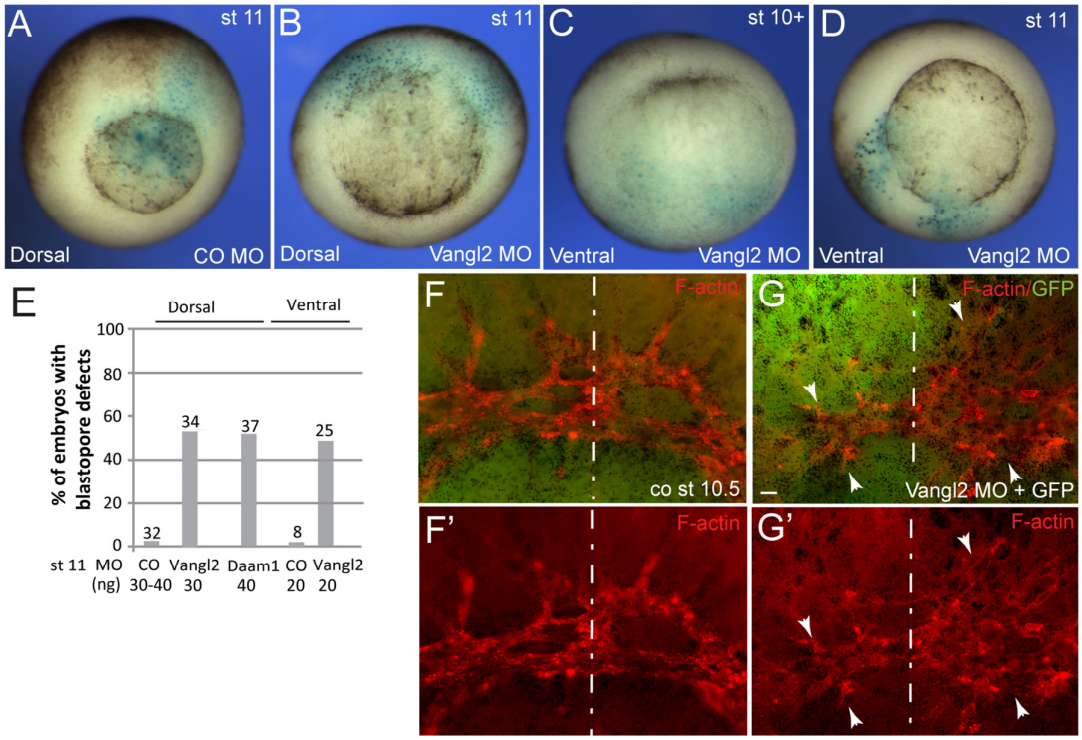


**Fig. S2.** Inhibition of blastopore lip formation in Vangl2-depleted embryos. (A-E) Early embryos were injected with Vangl2 MO or control MO either dorsally or ventrally as indicated. LacZ RNA (150 pg) has been coinjected a lineage tracer. Vegetal views of stage 11 embryos are shown. E, Quantitation of the experiments shown in A-D. (F, G) *En face* view of the dorsal lip in stage 10+ embryos. Vangl2 was depleted by a unilateral injection of control or Vangl2 MO (30 ng each). F-actin staining in control (F) and Vangl2-depleted (G) blastopore cells. Arrow points to reduced apical F-actin in Vangl2-depleted cells (marked by GFP, green). Animal pole is at the top. Scale bar in F (also applies to G) is 10 μm. Dashed line indicates the midline. Arrowheads point to F-actin-enriched areas, which are reduced by Vangl2 depletion.

Fig. S3

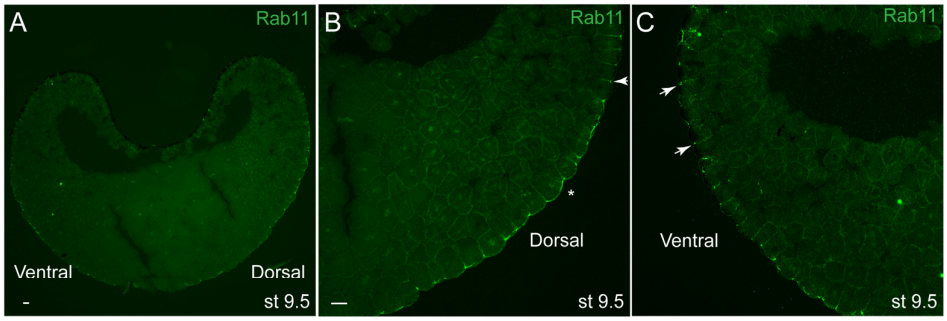


**Fig. S3. Rab11 subcellular localization before gastrulation.** (A-C) Cross-sections of late blastula embryos (n=10) stained for endogenous Rab11. A, Representative embryo at stage 9.5 is shown, B, Dorsal marginal zone area reveals enriched apical (asterisk) and apical junctional (arrowhead) staining. C, Rab11 is mostly at the junctions (arrowheads) in the ventral marginal zone. Scale bar is 20 μm in all panels.

Fig. S4

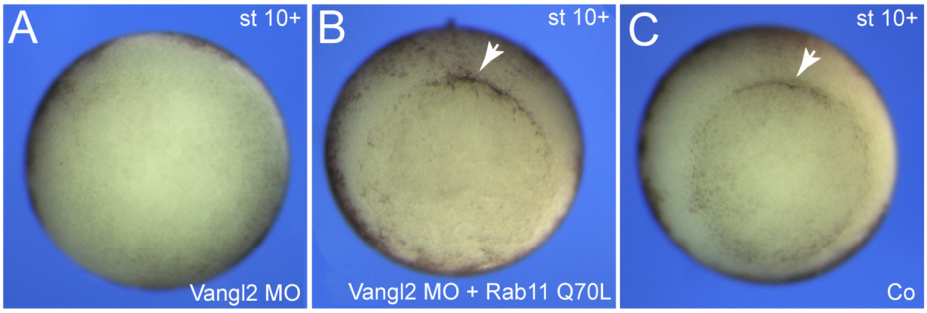


**Fig. S4. Rescue of dorsal blastopore in Vangl2-depleted embryos by Rab11 RNA.** (A, B) Embryos were injected with 30 ng of Vangl2 MO and 1 ng of Rab11Q70L RNA as indicated. (C) Uninjected control embryo. Vegetal views reveal the comparative morphology of the dorsal lip (arrow) for representative embryos from data in Fig. 6I.

Fig. S5

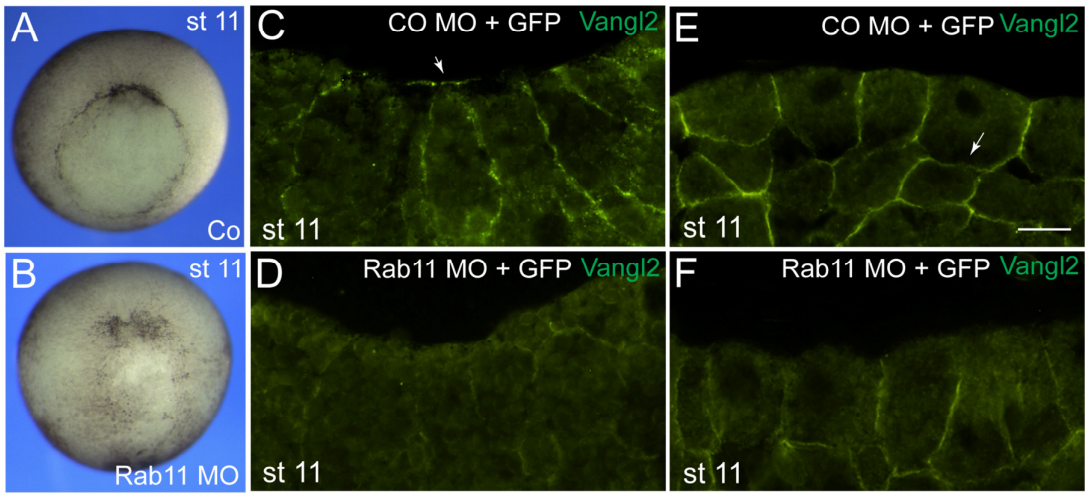


**Fig. S5. Vangl2 is dissociated from the cell membrane in Rab11-depleted embryos.**

Embryos were coinjected with CO MO or Vangl2 MO (30 ng each) and 100 pg of GFP RNA as lineage tracer (not shown). A, B, Morphology of control (A) and Rab11-depleted (B) embryos at st. 11, vegetal views are shown. C, D, Rab11 depletion interferes with the membrane localization of Vangl2, including the apical surface of the constricting blastopore cells (arrow). E, F, Basolateral membrane localization of Vangl2 is inhibited in ectoderm cells depleted of Rab11. Scale bar in E, also refers to C, D and F, is 20 μm.