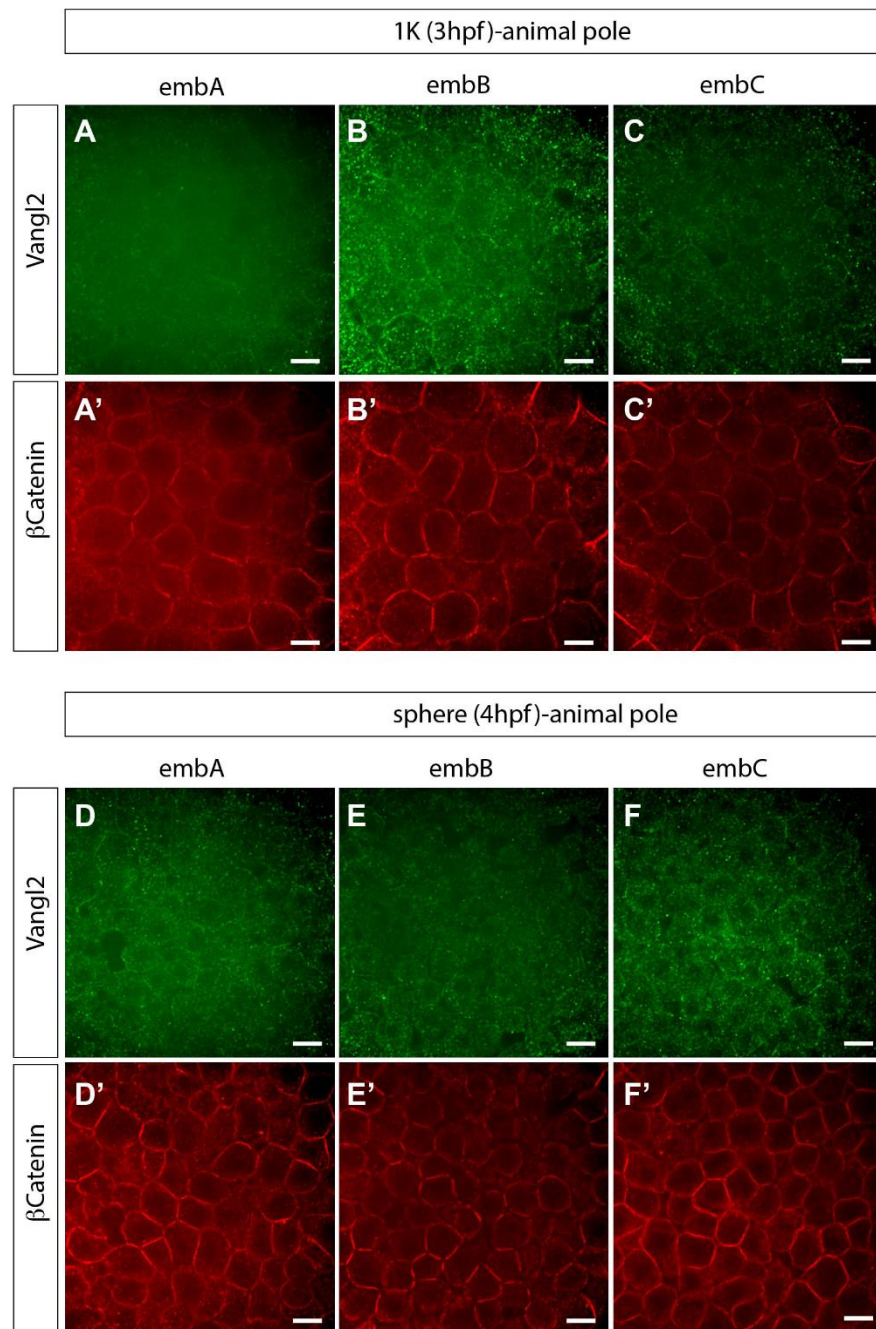
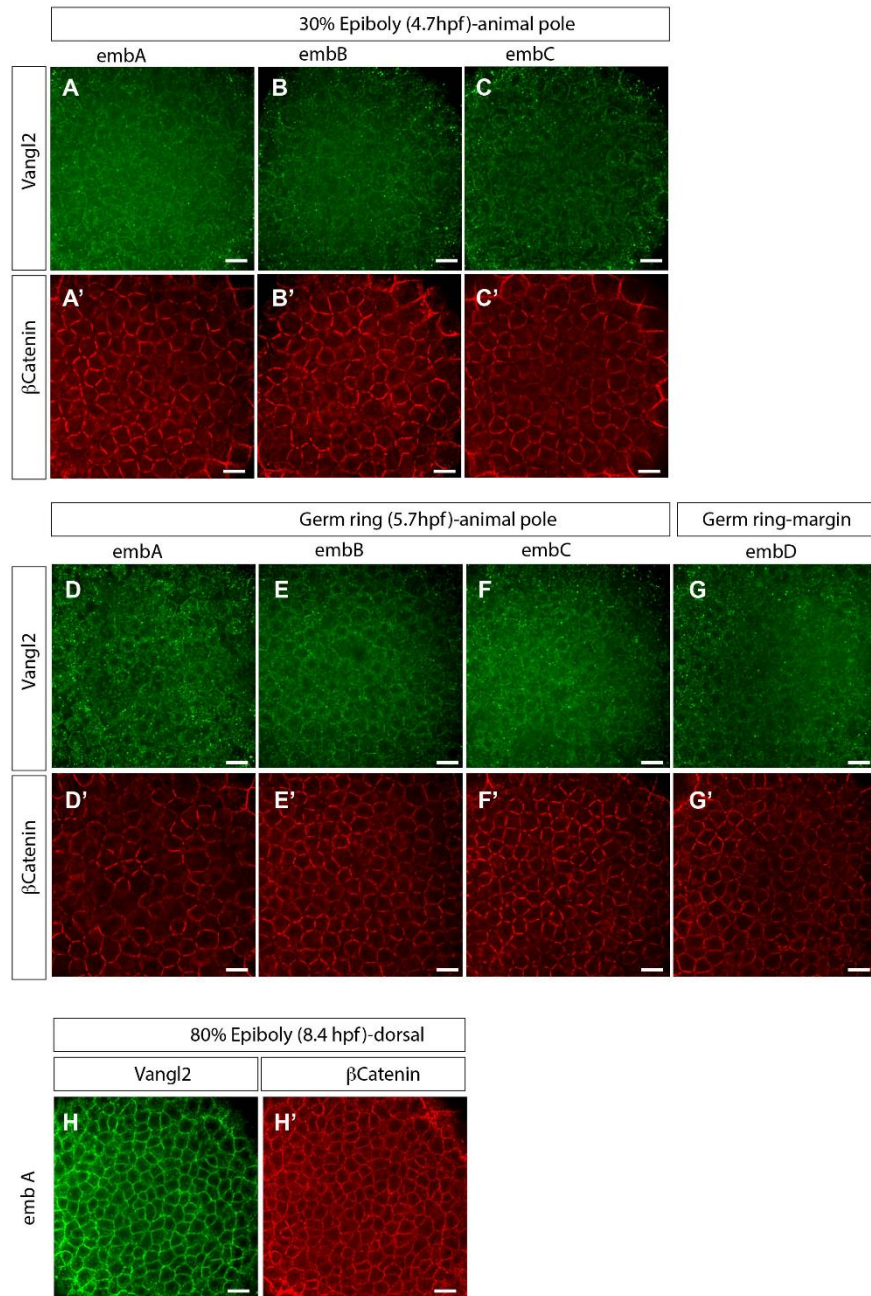


**Fig. S1. Rescue of the *MZvangl2*<sup>vu67/vu67</sup> phenotype with *GFP-VANGL2* synthetic mRNA.**  
(A) 30hpf WT embryo. (B) 30hpf *MZvangl2*<sup>vu67/vu67</sup> embryo. (C,D,E) 30hpf *MZvangl2*<sup>vu67/vu67</sup> embryos injected at 1-cell stage with 25 pg, 50 pg or 75 pg synthetic *GFP-VANGL* mRNA.



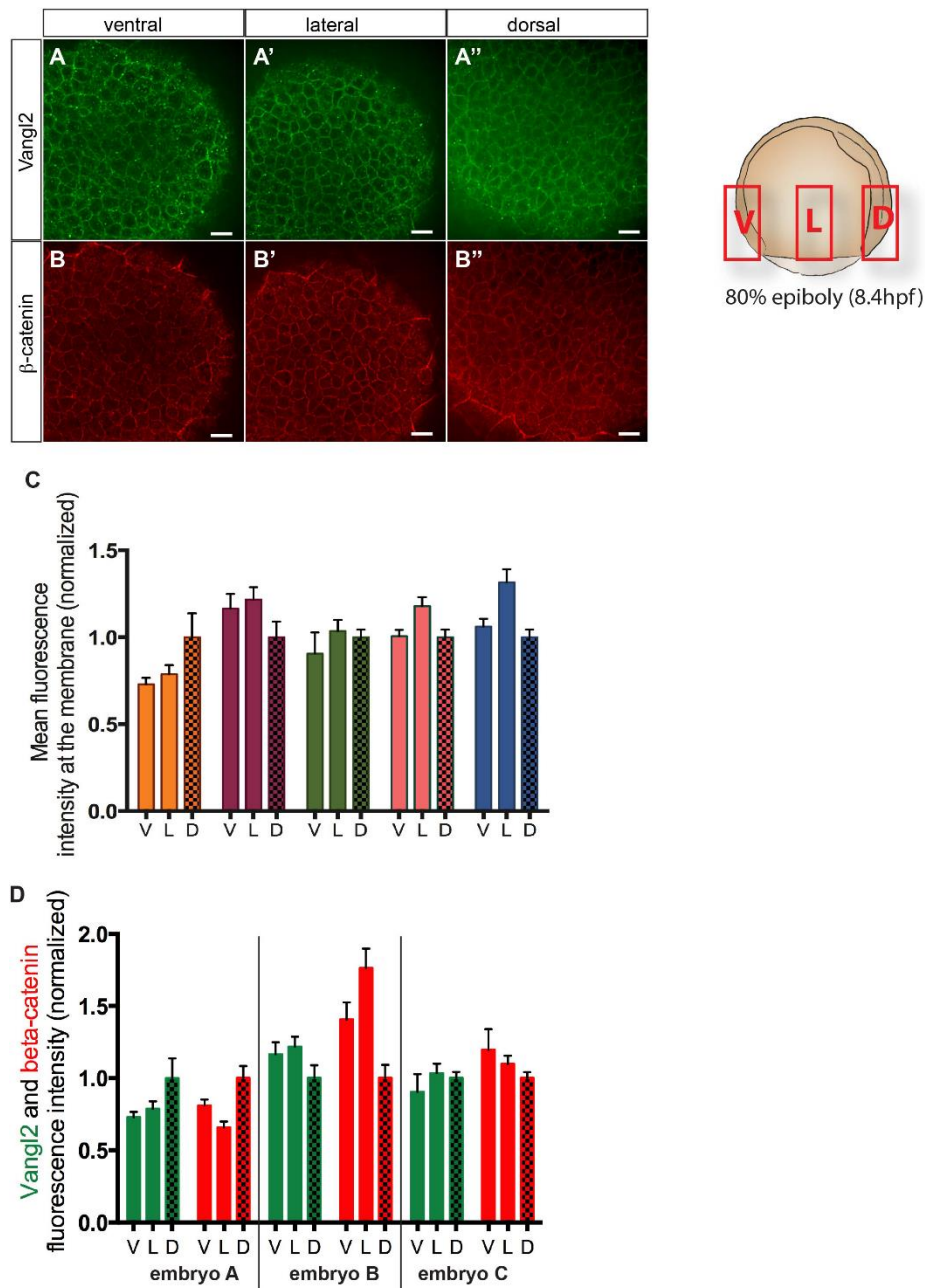
**Fig. S2. Vangl2 protein expression at blastula stages.**

Confocal images of embryos after immunostaining using the anti-C-terminal Vangl2 antibody (green) and anti- $\beta$ -catenin (red).  $\beta$ -catenin staining was used as control in order to visualize the cell membranes. Several embryos are shown in order to represent the variability of the anti-Vangl2 staining at the membrane versus cytoplasm between embryos at the same developmental stage. All images were acquired at the animal pole location. (A-C') 3 different embryos at sphere stage (4hpf). (D-F') 3 different embryos at 1K stage (3hpf). Note the weak Vangl2 staining (green) at the membrane and the abundant puncta in the cytoplasm. Scale bars, 20  $\mu$ m.



**Fig S3. Vangl2 protein expression from 30% epiboly to 80% epiboly stages.**

Confocal images of embryos after immunostaining using the anti-C-terminal zVangl2 antibody (green) and anti- $\beta$ catenin (red).  $\beta$ -catenin staining was used as control in order to visualize the cell membranes. Several embryos are shown in order to represent the slight variability of the anti-Vangl2 staining at the membrane versus cytoplasm between embryos at the same developmental stage. (A-C') 3 different embryos at 30% epiboly stage (4.7hpf). Images were acquired at the animal pole location. (D-F') 3 different embryos at germ ring stage (5.7hpf) (images acquired at the animal pole location). (G-G') embryos at germ ring stage (images acquired at the embryonic margin location). (H-H') embryos at 80% epiboly stage (8.4hpf) (images acquired at the dorsal location). Scale bars, 20  $\mu$ m.



**Fig S4. Vangl2 membrane localization in ventral, lateral or dorsal domain of the embryo does not show domain specific preference.**

(A-B'') Confocal images acquired in the ventral (A,B), lateral (A'B') and dorsal (A'',B'') domains in the embryo. Images were taken in all 3 domains of the same embryo and analyzed separately. Embryos at 80% epiboly stage (8.4hpf) were analyzed. (C) Quantitative data obtained from the analysis of the Vangl2 staining fluorescence intensity at the cell membrane in 5 different embryos (each embryo's D, V, L data set is in different color on the histogram). (D) Quantitative data comparing βcatenin (control) and Vangl2 staining for 3 different embryos in all D, L V domains, showing no domain specific preference for neither protein. All data were normalized such as dorsal=1. Scale bars, 20 μm.