

FIGURE S1. *Expression of Cherry-VANGL1 WT protein in MDCK cells stably expressing GFP-VANGL1 WT, D255E, R259L and S464N proteins.* Total cell extracts were prepared from doubly-transfected MDCK cell clones expressing Cherry-VANGL1 WT in presence of either GFP-VANGL1 WT or mutant variants (D255E, R259L and S464N). Equal amounts of protein (50 µg) were separated by electrophoresis using 7.5% SDS-polyacrylamide gels and analyzed by immunoblotting with the mouse monoclonal antibody 9E10 directed against the c-Myc tag inserted in-frame near the amino terminus of Cherry and GFP recombinant VANGL1 proteins. Both GFP- and Cherry-VANGL1 proteins can be distinguished on gel: the GFP-VANGL1 proteins (90.6kDa) containing an internal HA epitope migrate slower than the Cherry-VANGL1 protein (89.1kDa). Our results show that in doubly transfected MDCK cells, the level of expression of Cherry-VANGL1 is comparable to GFP-VANGL1 variants. Actin was used as an internal loading control. Lane 1 – cells expressing GFP-VANGL1 WT; Lane 2 – cells expressing Cherry-VANGL1 WT alone; Lanes 3-6 – cells co-expressing Cherry-VANGL1 WT with GFP-VANGL1 WT, D255E, R259L, and S464N.

FIGURE S2. *Sub-cellular localization of the WT, D255E, R259L and S464N VANGL1 variants in stable doubly transfected MDCK cells co-expressing Cherry-VANGL1 WT; Z-line image analysis.* Transfected MDCK cells stably expressing GFP-VANGL1 WT, D255E, R259L or S464N mutant variants (green, Panel A) and co-expressing Cherry-VANGL1 WT (red, Panel B) were grown to confluence, fixed, permeabilized and stained for Na,K-ATPase followed by Alexafluor 647-conjugated secondary antibody (purple, Panel C). Cells were analyzed by confocal microscopy to visualize the expression of

GFP-VANGL1 variants (green), Cherry-VANGL1 WT (red) and Na,K-ATPase (purple). Representative x-z- sections are shown. The merged images show that Cherry-VANGL1 WT co-localizes with Na,K-ATPase at the basolateral membrane (pink) in all doubly transfected cells including both GFP-VANGL1 WT or GFP-VANGL1 mutants variants.

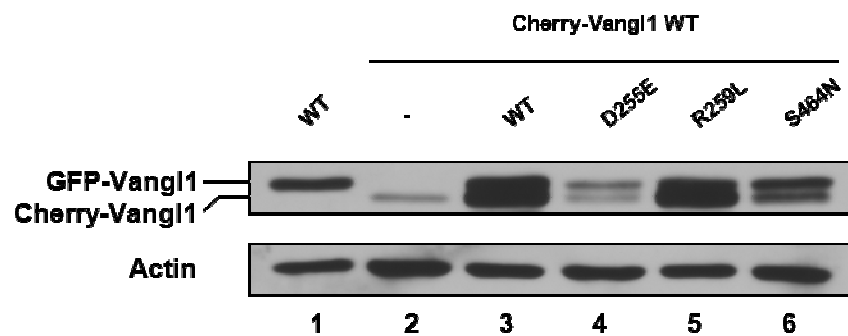


Figure S1

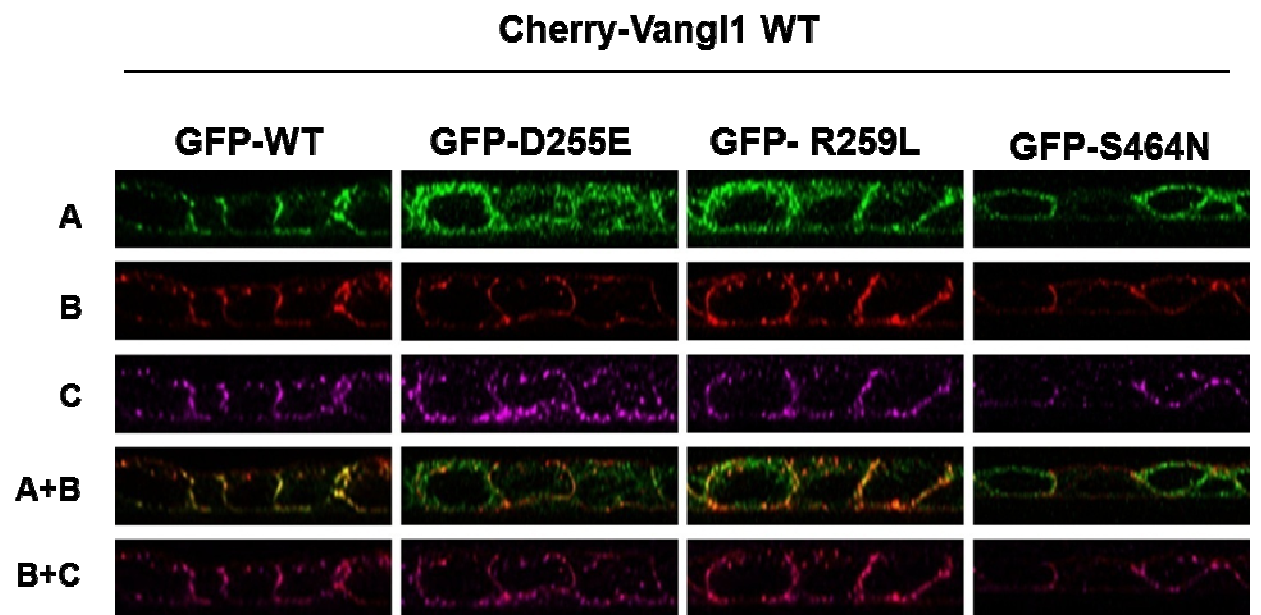


Figure S2