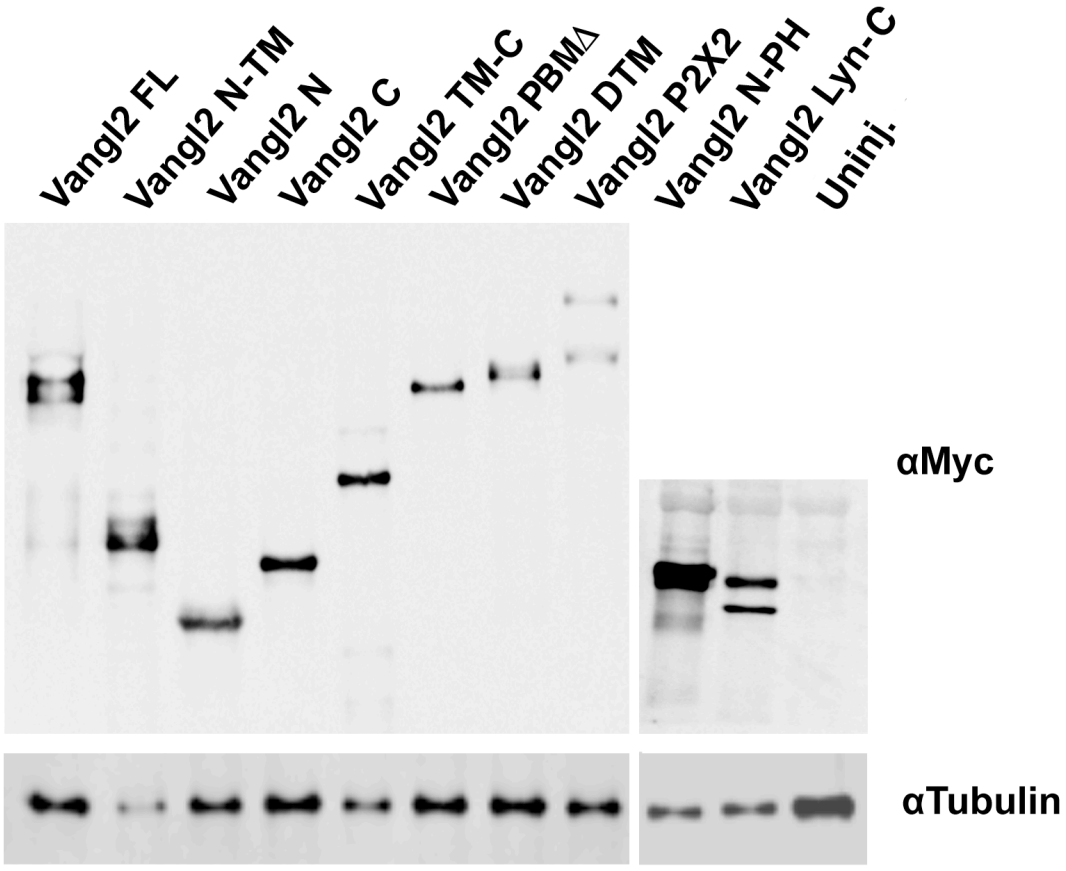
Pan et al.
Supplementary Figures S1 to S8

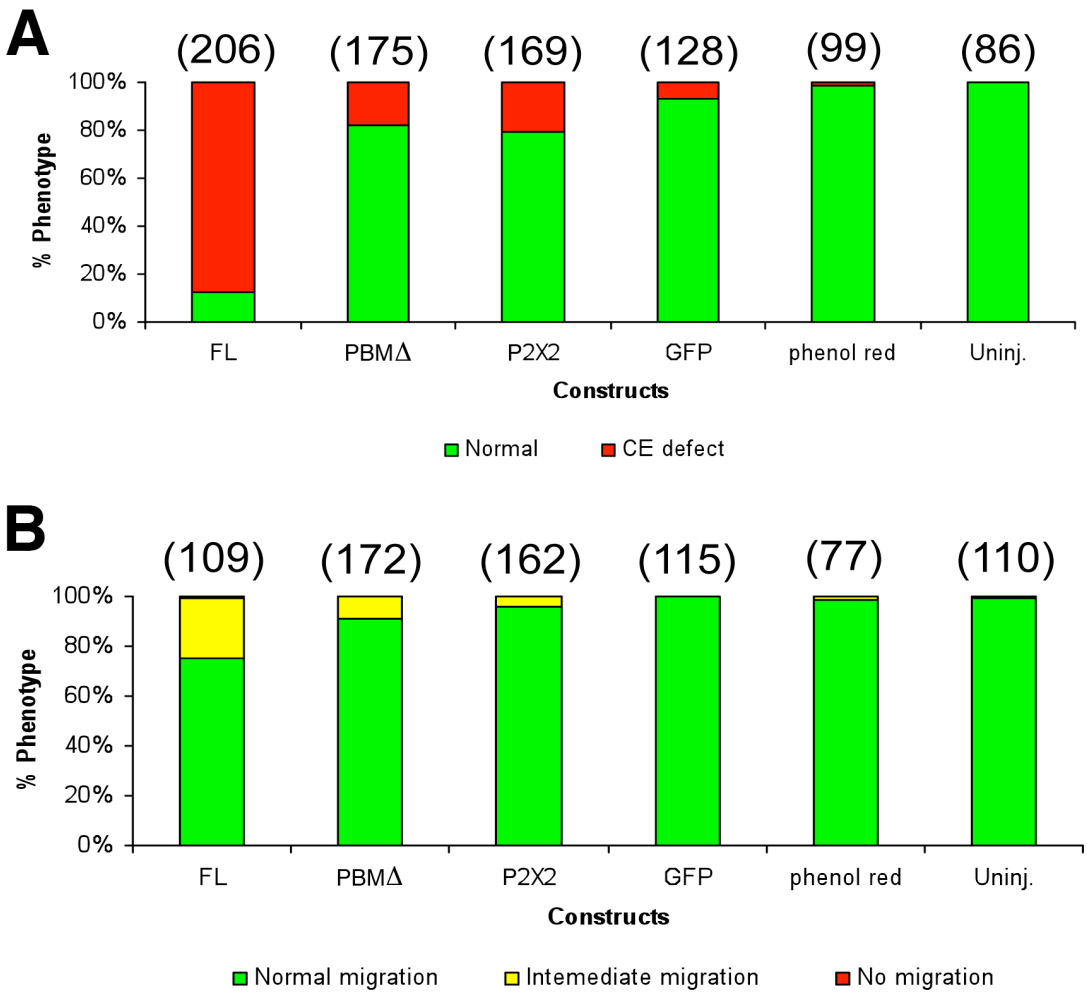
Figure S1



Validation of constructs encoding Vangl2 variants

Western blot analysis of proteins isolated from 10 hpf embryos injected with RNAs encoding various Vangl2 constructs. Myc-tagged proteins of the predicted sizes are detected. A Western for α-tubulin was performed simultaneously to detect tubulin as a loading control. For all constructs except TM-C, the dose of injected RNA was 280 pg/egg. For TM-C, a dose of 150 pg/egg was used due to the severity of CE defects at higher doses.

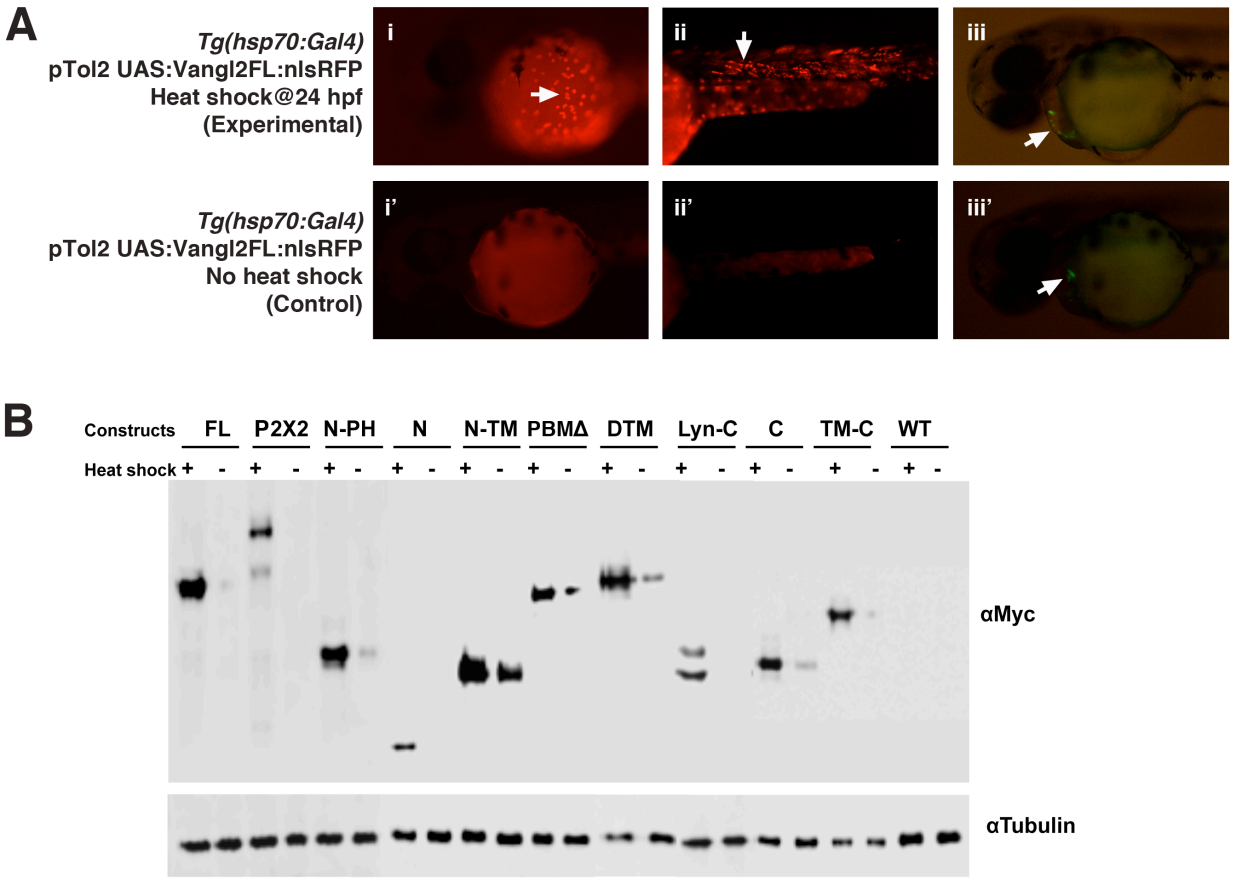
Figure S2



Evaluating the non-specific effects of RNA injections on CE movements

(A) The ability of various Vangl2 constructs to generate CE defects is variable. CE movement defects were scored by examining live 5-7 somite stage embryos. Injection dose: 280 pg RNA/embryo for all constructs. While Vangl2 FL injection generates CE defects efficiently, other constructs exhibit weaker effects (PBMΔ, P2X2) that are significantly different compared to GFP RNA or phenol red injection ($p<0.05$; Pearsons Chi square test). Number of embryos scored in parenthesis for each construct. Data pooled from 3-5 experiments. **(B)** Transient overexpression of Vangl2 variants did not cause severe defects in FBM neuron migration. See Figure 4 legend for scoring criteria.

Figure S3

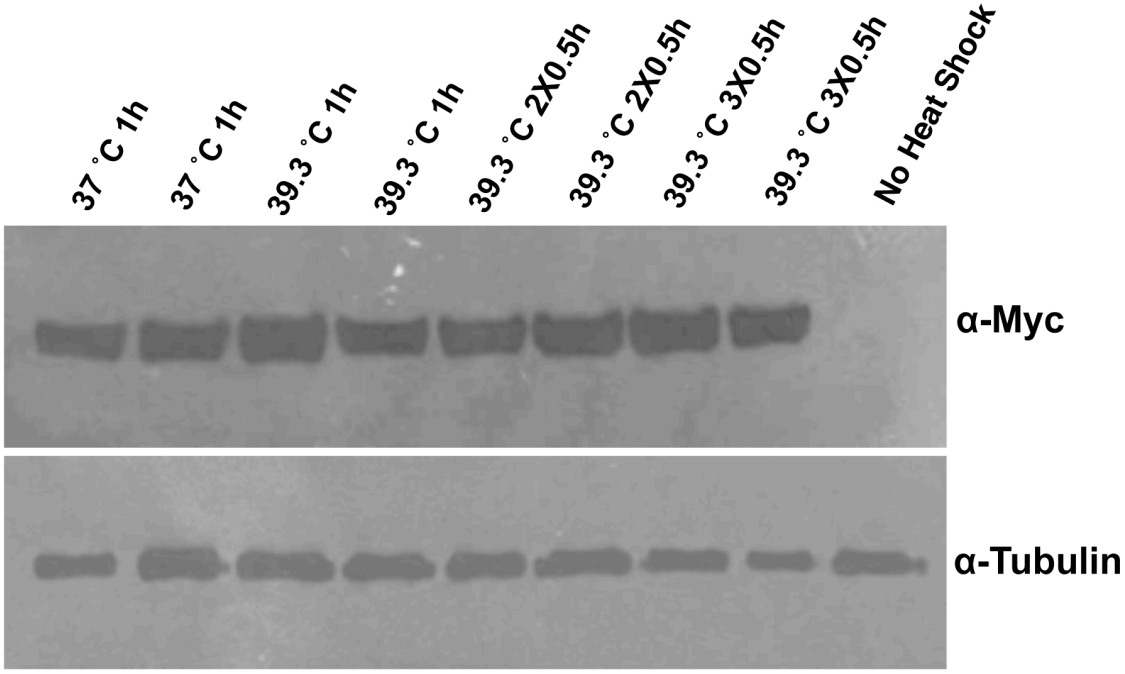


Validation of Tol2 vectors and establishment of heat shock inducible transgenic lines

(A) Transient expression of Vangl2 FL protein by injecting Tol2 plasmid into *Tg(hsp70:Gal4)* embryos (i-iii). The 24 hpf embryos were heat shocked for 1 hr at 37°C and examined at 48 hpf. Control embryos contained the Tol2 plasmid but were not subjected to heat shock (i'-iii'). Embryos carrying the injected Tol2 plasmid express GFP in the heart (arrows in iii and iii'). Heat shocked embryos express RFP in the superficial

cells of the yolk sac (i, arrow) and cells in the trunk (ii, arrow). RFP is not expressed in non-heat shocked embryos (i', ii'). **(B)** Western blotting with anti-Myc antibody shows that the various Vangl2 variants are expressed in the Tol2 plasmid-injected embryos after heat shock (+). While most constructs are not activated in the absence of heat shock (-), there is a varying amount of weak (leaky) expression for N-PH, N-TM, PBMΔ, DTM, and C. A Western using anti-tubulin antibody was performed simultaneously to detect tubulin as a loading control. **(C)** Western blotting showing different levels of Vangl2 FL protein expression among four different stable transgenic lines. Lines A and D were selected for further analysis, and most experiments were performed with line D.

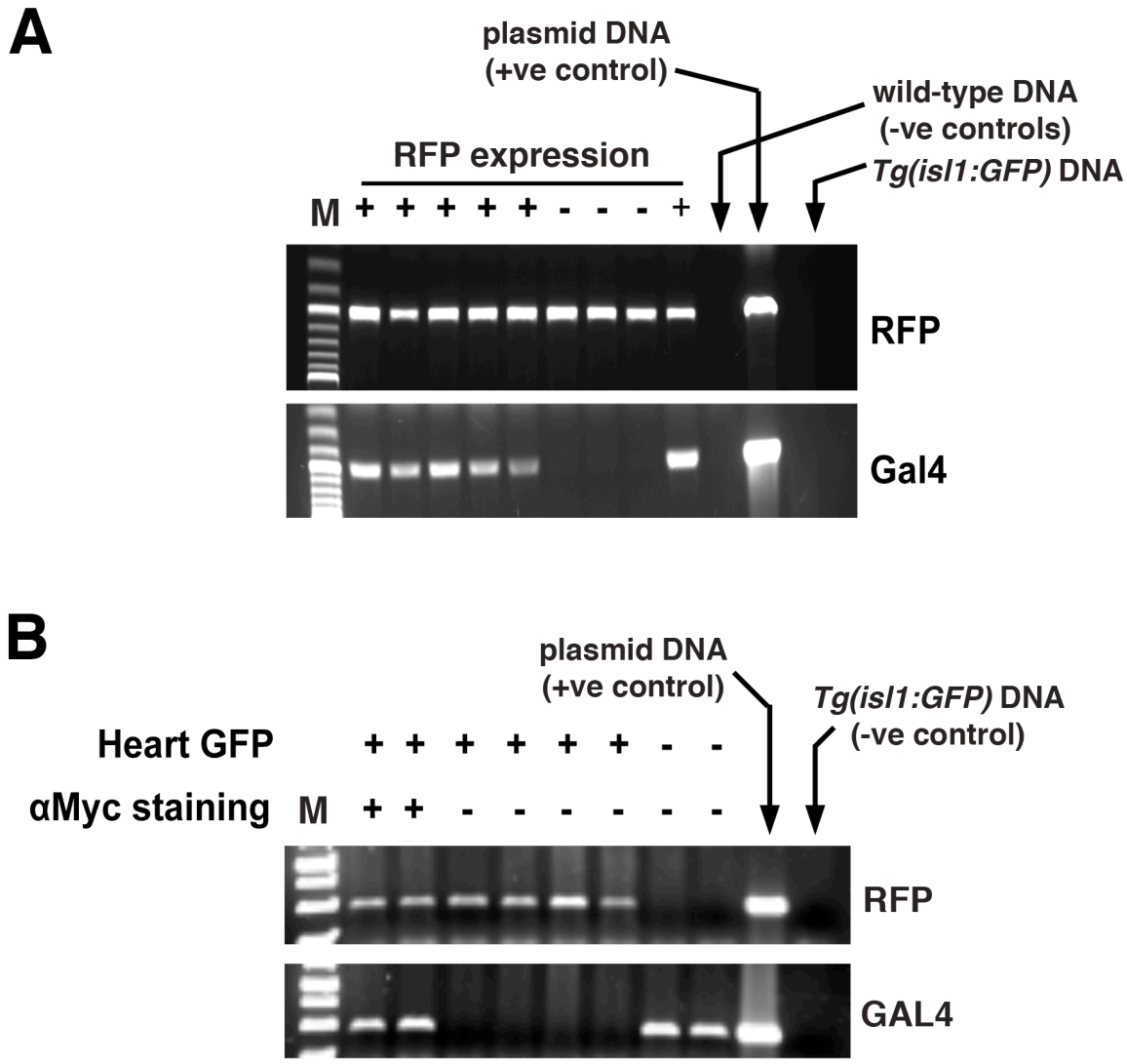
Figure S4



Defining an optimal heat shock induction protocol using the UAS:Vangl2 FL transgenic line

Western blots with anti-Myc and anti-tubulin antibodies of proteins extracted from 24 hpf embryos treated with various heat shock protocols: Two temperatures in water bath (37°C OR 39.3°C), and different heat shock durations (1 hour treatment OR two 30 min treatments, 1 hour apart OR three 30 min treatments, 1 hour apart). Vangl2 FL-expressing embryos were identified at 48 hpf by RFP expression, and protein lysates prepared for analysis. Samples were run in duplicate. Heat shock at 39.3°C generates slightly higher levels of Vangl2 FL protein, whereas the other treatment conditions did not generate significantly different amounts of protein. All subsequent analyses employed 39°C heat shock for 1 hour.

Figure S5

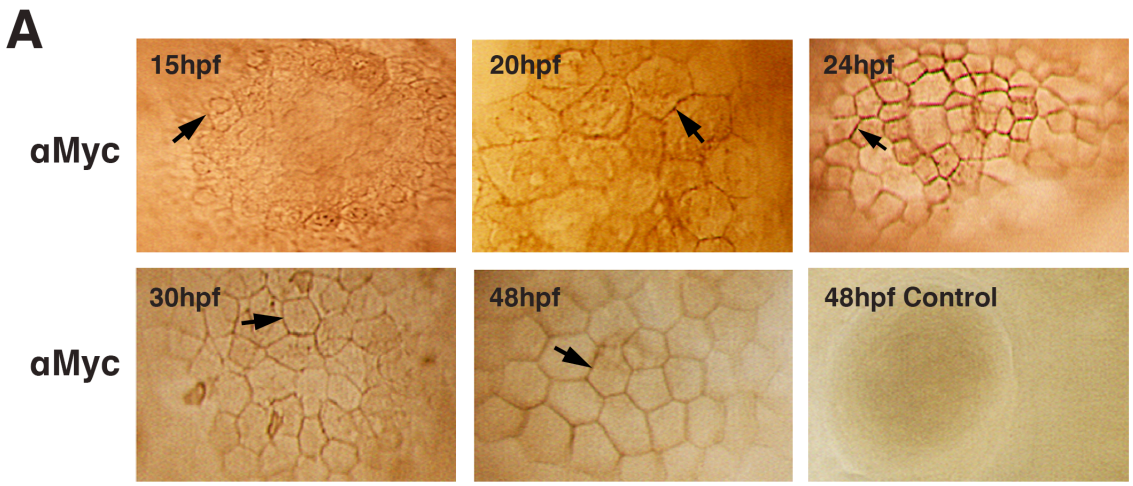


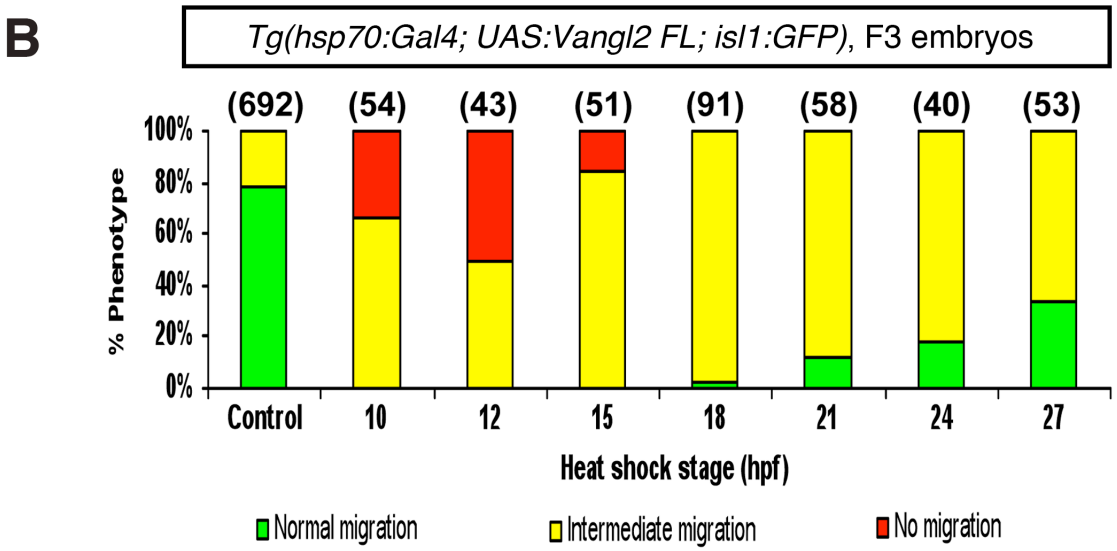
Genotypic validation of phenotypes of *Tg(hsp70:Gal4); Tg(UAS:Vangl2 FL-nlsRFP); Tg(isl1:GFP)* triple transgenic embryos

(A) *Tg(UAS:Vangl2 FL-nlsRFP); Tg(isl1:GFP)* transgenic embryos (exhibiting GFP expression in the heart and in FBM neurons) with (+) or without (-) RFP expression were selected for genotyping with primers for RFP (UAS:Vangl2 transgene) and Gal4 (hsp70:Gal4 transgene). Only RFP-expressing embryos amplified both transgenes. RFP-ve embryos lacked the *hsp70:Gal4* transgene. **(B)** *Tg(isl1:GFP)* transgenic embryos (exhibiting GFP expression in FBM neurons) were processed for anti-Myc immunostaining

(Vangl2 expression), and animals with (+) or without (-) Vangl2 expression were selected for genotyping with primers for RFP (UAS:Vangl2 transgene) and Gal4 (hsp70:Gal4 transgene). Only Myc immunopositive embryos amplified both transgenes. Vangl2-ve embryos lacked the *hsp70:Gal4* transgene. Conversely, embryos lacking heart GFP expression (no *UAS:Vangl2* transgene) never exhibited Myc immunostaining (as expected), but sometimes carried the *hsp70:Gal4* transgene.

Figure S6





(A) Appearance and perdurance of Vangl2 protein induced by heat shock treatment

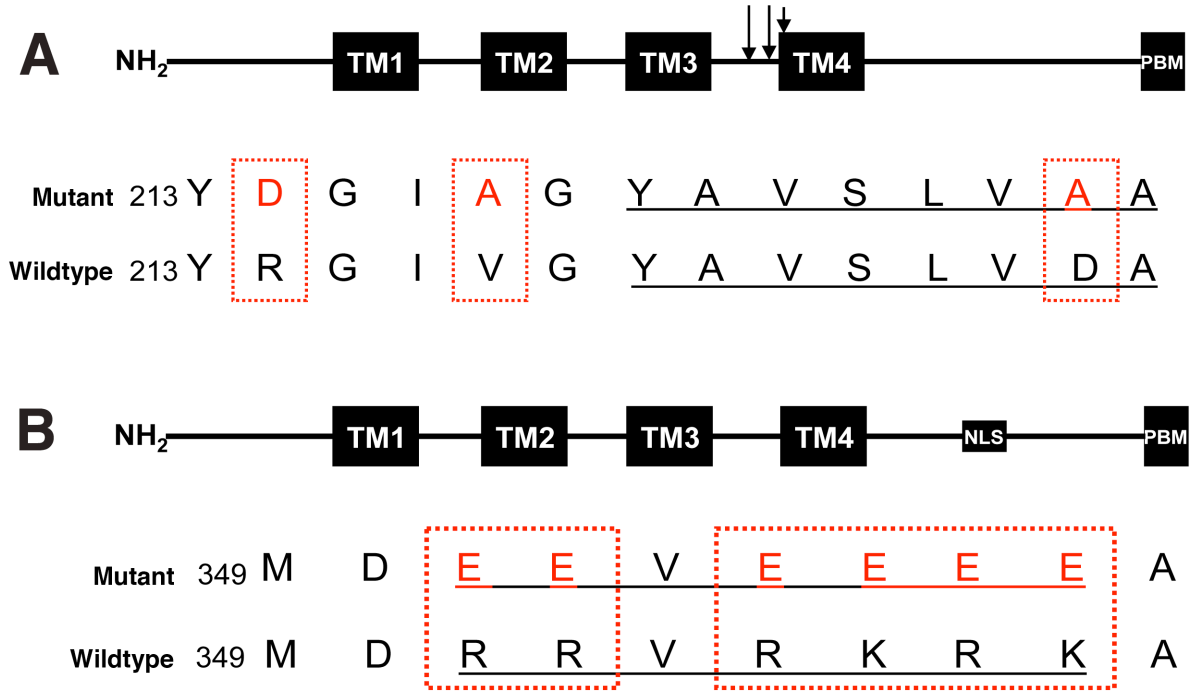
Embryos obtained by crossing *Tg(UAS:Vangl2 FL)* F1 fish to *Tg(hsp70:Gal4)* fish, were heat shocked at 10 hpf, fixed at 12-48 hpf, and processed for anti-Myc immunostaining to identify Vangl2-expressing cells. Myc-positive staining (arrows) was apparent on plasma membranes by 15 hpf (5 hours post heat shock), subsequently increased in levels, and was maintained until 48 hpf.

(B) Weaker effects on FBM neuron migration with heat shocked F3 embryos

Summary of effects on FBM neuron migration following heat shock-induced Vangl2 FL

transgene expression in F3 embryos. Embryos obtained by crossing *Tg(UAS:Vangl2 FL)* F2 fish to *Tg(hsp70:Gal4)* fish, were heat shocked at various indicated times, and migration phenotypes were scored at 48 hpf. Although induction of Vangl2 FL expression at different times interfered with FBM neuron migration, the penetrance of the phenotype was weaker than in F2 embryos (Fig.5). Nevertheless, the strongest effects (i.e., embryos with all FBM neurons stuck in r4) were observed only in embryos heat shocked between 10-15 hpf. Number of embryos scored in parenthesis. Data pooled from two or more experiments.

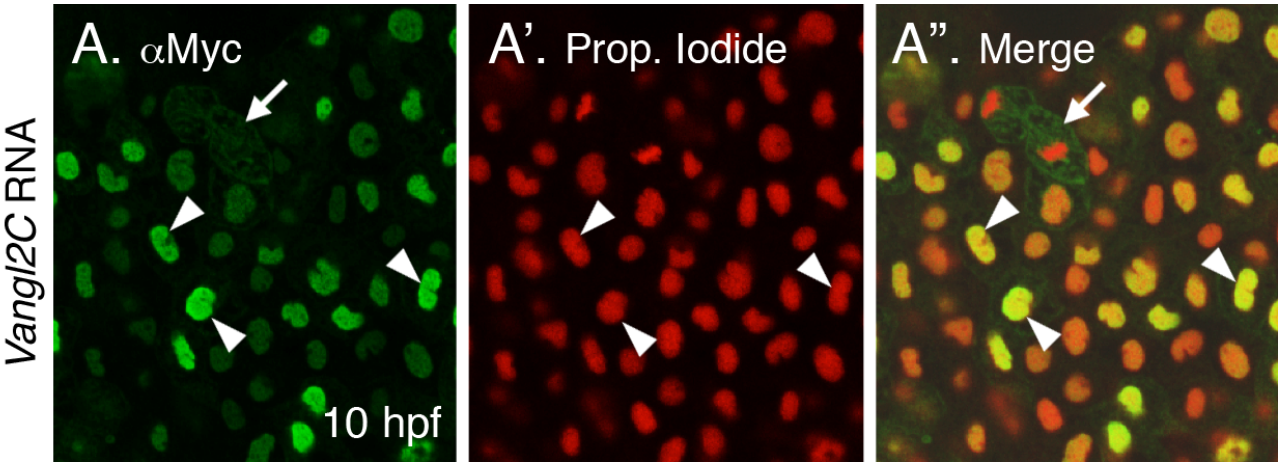
Figure S7



Site-directed mutagenesis of Vangl2 2nd extracellular loop and NLS motif

(A) Schematic representation of Vangl2 protein indicating the three point mutations introduced in the 2nd extracellular loop (arrows). The underlined sequence represents the start of the predicted fourth transmembrane domain, with the boxed regions indicating the targeted amino acids (Black, wildtype and Red, mutant). **(B)** Schematic representation of Vangl2 protein indicating the putative NLS motif where mutations were introduced. The underlined sequence indicates the NLS motif, with the boxed regions indicating the targeted amino acids (Black, wildtype and Red, mutant). Six arginines (R) or lysines (K) were substituted with six glutamic acid (E) residues.

Figure S8



Association of Vangl2 with the nucleus

(A-A") 10 hpf embryo injected with myc-tagged *Vangl2C* RNA, and processed for immunostaining with anti-Myc antibody to label Vangl2C protein (A), and counterstained with the nuclear marker propidium iodide (A'). The Vangl2C signal is essentially restricted to the nucleus (arrowheads), although some membranous and punctate staining is seen in dividing cells (arrow).