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## The PCP Protein Vangl2 Regulates Migration of Hindbrain Motor Neurons by Acting in Floor Plate Cells, and Independently of Cilia Function

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### Abstract

Vangl2, a core component of the Planar Cell Polarity pathway, is necessary for the caudal migration of Facial Branchiomotor (FBM) neurons in the vertebrate hindbrain. Studies in zebrafish suggest that *vangl2* functions largely non-cell autonomously to regulate FBM neuron migration out of rhombomere 4 (r4), but the cell-type within which it acts is not known. Here, we demonstrate that *vangl2* functions largely in floor plate cells to regulate caudal neuronal migration. Furthermore, FBM neurons fail to migrate caudally in the mouse *Gli2* mutant that lacks the floor plate, suggesting an evolutionarily conserved role for this cell type in neuronal migration. Although hindbrain floor plate cilia are disorganized in *vangl2* mutant embryos, cilia appear to be dispensable for neuronal migration. Notably, Vangl2 is enriched in the basolateral, but not apical, membranes of floor plate cells. Taken together, our data suggest strongly that Vangl2 regulates FBM neuron migration by acting in floor plate cells, independently of cilia function.

### Keywords

Hindbrain; Facial Branchiomotor Neuron; Floor plate cells; Van gogh-like 2; Neuronal migration; Cilia

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## INTRODUCTION

During nervous system development, newborn neurons migrate away from the germinal zone to their final locations where they generate functional neural networks. Neuronal migration disorders in humans can lead to severe physiological and cognitive problems (Copp and Harding, 1999; Baraban, 2007). Cortical neurons in the forebrain migrate extensively along radial and tangential pathways (Nadarajah and Parnavelas, 2002; Marin and Rubenstein, 2003). Similarly, facial branchiomotor (FBM) neurons, a subset of cranial motor neurons, migrate along tangential and radial pathways from their birthplace in rhombomere 4 (r4) of the hindbrain to caudal rhombomeres (r6/r7) in most vertebrates (Chandrasekhar, 2004; Gilland and Baker, 2005).

Studies in zebrafish and mice have identified several molecules necessary for the migration of FBM neurons, including components of the Wnt/Planar Cell Polarity (Wnt/PCP) pathway. Wnt/PCP signaling regulates convergence and extension cell movements during gastrulation (Roszko et al., 2009) and establishes the polarity of neural progenitors (Ciruna et al., 2006). Inactivation of core Wnt/PCP components such as Van gogh-like 2 (*Vangl2*) (Bingham et al., 2002; Jessen et al., 2002; Vivancos et al., 2009; Glasco et al., 2012), Prickle1a (*Pk1a*) (Carreira-Barbosa et al., 2003), Prickle1b (*Pk1b*) (Rohrschneider et al., 2007), Scribble1 (*Scrib*) (Wada et al., 2005) and Frizzled3a (*Fzd3a*) and *Celsr* 1–3 (Wada et al., 2006; Qu et al., 2010) abolishes FBM neuron migration in zebrafish and mouse. Interestingly, inactivation of Wnt/PCP components like *Wnt11*, *Wnt5a*, *Glypican4* and *Dishevelled* have little effect on FBM neuron migration (Bingham et al., 2002; Jessen et al., 2002; Vivancos et al., 2009; Glasco et al., 2012), suggesting redundant or non-essential roles in this process. Furthermore, mosaic analyses suggest that *vangl2*, *fzd3a*, *celsr2* and *scrib* largely function non-cell autonomously during FBM neuron migration (Jessen et al., 2002; Wada et al., 2005; Wada et al., 2006). These observations suggest that some core PCP genes may regulate FBM neuron migration through novel cellular mechanisms. These mechanisms must also be compatible with the behavior of “pioneer” and “follower” FBM neurons in zebrafish (Wanner and Prince, 2013).

Wada et al (2006) proposed that zebrafish *fzd3a* and *celsr2* function in neuroepithelial cells adjacent to the FBM neurons, and regulate their caudal migration by preventing their integration into the neuroepithelium in rhombomere 4. In contrast, *pk1b* functions within the FBM neurons to regulate their polarity and midline-directed protrusions during their migration (Mapp et al., 2010; Mapp et al., 2011). Although *vangl2* largely functions non-autonomously for neuronal migration (Jessen et al., 2002), the cell-type within which it acts is not known. Walsh et al. (2011) also suggested an FBM neuron-autonomous role for *vangl2* during migration. Our analyses employing genetic mosaics, an inducible transgene, and mutants suggest strongly that *vangl2* functions primarily in floor plate cells to regulate FBM neuron migration. Our data also indicate that floor plate cilia are not required for migration.

## MATERIALS AND METHODS

### Animals

Zebrafish were maintained following standard protocols and IACUC guidelines as described previously (Westerfield, 1995; Sittaramane et al., 2009). Embryos were developed at 28.5°C and staged by hours post fertilization (hpf) (Kimmel et al., 1995). *Tg(isll:gfp)* and *Tg(zcrest:mRFP)* fish (Higashijima et al., 2000; Mapp et al., 2010), were used to analyze FBM neuron migration. SAGFF187A/*Tg(Fp:Gal4FF; UAS:GFP)* was generated by the gene trap method using the SAGFF Tol2 construct (Asakawa et al., 2008), and expressed

Gal4FF and GFP in the floor plate from 16–48 hpf. *Tg(vangl2:GFP-Vangl2)* and *Tg(UAS:Myc-vangl2)* were generated using the Tol2 Gateway cloning kit (X.P. and A.C., unpublished data; Tol2 kit provided by Kristen Kwan and Chi-Bin Chien, University of Utah). The following mutant lines were used: *trilobite* (*trilobite<sup>trilobite</sup>*; (Hammerschmidt et al., 1996)), *detour* (*detour<sup>ts269</sup>* and *detour<sup>ts370a</sup>*; (Brand et al., 1996)), *iguana* (*iguana<sup>ts294e</sup>*; (Brand et al., 1996)), and *oval* (*oval<sup>ts288b</sup>*; (TsujiKawa and Malicki, 2004; Huang and Schier, 2009)).

Mouse *Gli2*<sup>+/-</sup> mice were maintained according to IACUC guidelines at UMDNJ. Embryos were staged and processed as described previously (Matise et al., 1998; Glasco et al., 2012).

### Morpholino and mRNA Injections

The following morpholinos were obtained from Gene Tools and injected at the indicated doses: *sox32* MO ((Sakaguchi et al., 2001); 4–8 ng/embryo), *no tail* MO ((Nasevicius and Ekker, 2000); 4 ng/embryo) and *vangl2* MO ((Jessen et al., 2002); 4 ng/embryo). The following mRNAs were used: *TARAM-A<sup>d</sup>/TAR* RNA ((Thisse and Thisse, 1999); 50 pg/embryo) and mRFP RNA (100 pg/embryo).

### Mesoderm transplantations

Donor cells were targeted to the host mesoderm by injecting *TARAM-A<sup>d</sup>/TAR* RNA into donor embryos. *TARAM-A<sup>d</sup>/TAR* is a constitutively active activin type I receptor that cell autonomously induces mesendodermal fates, and *TAR*-expressing cells preferentially form mesoderm at low doses (50 pg RNA/embryo) (Thisse and Thisse, 1999) (Fig. S4). Donor embryos were injected at the one-cell stage with a mixture of 2% rhodamine dextran, 3% lysine-fixable biotin dextran (Molecular Probes), and *TAR* RNA. At the late blastula stage, cells were transplanted to the margin of 50% epiboly stage hosts. Host embryos were screened at 24 hpf for those containing donor-derived cells in the cranial mesoderm. In some experiments, the donor cells also contained *vangl2* MO, resulting in the knockdown of *vangl2* expression in donor-derived mesodermal cells, a strategy employed previously for knocking down BMP function in the endoderm (Holzschuh et al., 2005).

### Floor plate transplantations

Donor cells were targeted to the floor plate by injecting *no tail* (*ntl*) MO into donor embryos, since *ntl*-deficient cells preferentially contribute to the floor plate (Halpern et al., 1993). Donor embryos were injected with *ntl* MO (4–6 ng/embryo) and 2% rhodamine dextran, and late blastula stage cells were transplanted to the margin of *Tg(isl1:gfp)* hosts (3 hpf). Host embryos (Fig. S5) containing donor-derived cells in the hindbrain floor plate were selected for further analysis. We verified that *ntl* morphant donor cells did not differentiate into motor neurons by transplanting *Tg(isl1:gfp)*, *ntl* MO cells into wildtype non-transgenic host embryos. In three independent experiments (80 embryos), we obtained 38 host embryos with donor-derived cells in the hindbrain floor plate. Importantly, no GFP-expressing FBM neurons were found in any of these embryos, indicating that *ntl* MO donor cells are highly unlikely to differentiate into FBM neurons.

### Quantification of FBM neuron migration phenotypes

Phenotypes were scored by examining the distribution of FBM neurons in rhombomeres 4–7. “Normal migration” indicates >90% (qualitative estimate) of neurons migrated out of r4, (e.g., Fig. 2A, B). “Abrogation/abrogated” in the transplant experiments indicates >20% of neurons failed to migrate out of r4 on one or both sides of the host wildtype hindbrain (e.g., Fig. 3A, C). Control *vangl2*-deficient embryos exhibit complete migration block since FBM neurons never exited r4 (e.g., Fig. 6D and 3F). “Rescued migration” in transplant experiments indicates at least 50% of neurons migrated out of r4 in *vangl2* mutant embryos,

(e.g., Figs. 2F, 3D and 3F). Rhombomere assignments (particularly in Fig. 4) were made by dividing the anterior-posterior axis of the hindbrain from r2 to r7 into six roughly equal parts. The anterior limit of r2 was defined by the presence of trigeminal motor neurons (in most embryos), and the caudal limit of r7 was defined by the presence of vagal motor neurons (in wildtype and *vangl2*-deficient embryos). When visible, the characteristic genu and/or exit point of the facial motor axons reliably marked r4. In *detour* mutant host embryos, r2 and r4 were readily identified, and the dimensions of r4-r6 were borrowed from wildtype siblings because rhombomere patterning is not affected in these mutants (Chandrasekhar et al., 1999).

### Genotyping of rescued embryos

Rescued *vangl2* mutant host embryos (short trunks with thin somites) could usually be definitively identified by examining the distribution of FBM neurons (e.g., Fig. 3D), and were confirmed to be *vangl2*<sup>-/-</sup> by genotyping. We amplified a 449 bp genomic region spanning the point mutation in the *trilobite* (*tritic240a*) allele (Jessen et al., 2002) (Forward: 5'-GCCTGGATGGTCACAGATTT-3' and Reverse: 5'-CCGGAAGTTTATCAGTATGGGAAACAC-3'), and identified mutants by sequencing.

### Immunohistochemistry and in situ hybridization

Immunohistochemistry was performed as described previously (Bingham et al., 2002; Sittaramane et al., 2009) using the following antibodies: acetylated  $\alpha$ -tubulin (Sigma, 1:500), GFP (Invitrogen, 1:1000 for DAB reaction and 1:2000 for Alexafluor), rhodamine dextran (Invitrogen, 1:500), RFP and Myc (Cell Signaling Technology, 1:250), and Alexafluor-conjugated secondary antibodies (Invitrogen, 1:500). Mesoderm transplant embryos were processed for anti-GFP immunostaining and biotin-streptavidin chemistry to simultaneously visualize donor-derived mesodermal cells and GFP-expressing host motor neurons. Floor plate transplant embryos were processed for anti-GFP and anti-rhodamine immunostaining to visualize donor-derived floor plate cells and host FBM neurons.

Synthesis of digoxigenin labeled probes and wholemount in situ hybridization were performed as described previously (Vanderlaan et al., 2005). For cross-sections, embryos were embedded in OCT (EMS), and processed (30  $\mu$ m thickness) on a Leica CM1900 cryostat. Fluorescent imaging was performed on a Zeiss LSM 510 confocal microscope. Mouse in situs and NeuroVue labeling were performed as described previously (Qu et al., 2010; Glasco et al., 2012). Images were processed in Adobe Photoshop to adjust brightness and contrast only.

### Analysis of motor neuron protrusions

Optical sections were obtained on Zeiss LSM 510 and 710 confocal microscopes. Cell protrusions and their angles relative to the midline were measured in LSM Image browser or Zen Black software (Zeiss, Inc.). For comparing angular distributions between wild type and mutant embryos, protrusions were placed in two groups (0°–180° and 180°–360°) for each genotype. The differences in distributions were tested for significance using Watson-Williams F-test (JMP software, SAS Institute Inc.).

## RESULTS

### *Vangl2* expression in non-neural tissues is dispensable for FBM neuron migration

*Van gogh-like 2* (*vangl2*) is expressed broadly in the hindbrain and mesendoderm between 18–24 hpf, spanning the period of FBM neuron migration (Figs. 1 and S1). We showed previously that *vangl2* function largely functions non-cell autonomously for FBM neuron

migration (Jessen et al., 2002). We hypothesized that neuroepithelial tissues co-expressing *vangl2* and *prickle1a* (*pk1a*) would be good candidates for *vangl2* site of action since 1) *pk1a* is expressed along the lateral margins of the hindbrain, except in rhombomeres 4–6 (Carreira-Barbosa et al., 2003; Veeman et al., 2003), 2) *vangl2* and *pk1a* interact genetically during for FBM neuron migration (Carreira-Barbosa et al., 2003), and 3) *Drosophila* Van gogh and Prickle interact physically (Bastock et al., 2003; Jenny et al., 2003). Surprisingly, cross-sections revealed that *pk1a* is mostly expressed outside the neural tube, in endodermal and mesodermal tissues (Fig. S2). Since *vangl2* is also expressed in these tissues, in close proximity to the migrating FBM neurons, we tested whether *vangl2* functions in the mesoderm or endoderm to regulate FBM neuron migration.

To test whether endoderm played a role, we generated embryos lacking endoderm using morpholinos (MO) against *casanova* (*cas*), which encodes Sox32, which is essential for endoderm specification (Kikuchi et al., 2001; Sakaguchi et al., 2001). In *cas/sox32* MO-injected embryos, endodermal cells at the hindbrain level were absent, while mesoderm was not affected (Fig. S3). Nevertheless, caudal migration of FBM neurons was largely unaffected (Fig. 2A–C), with >90% (qualitative estimate) of motor neurons exiting r4 in 36 hpf morphant embryos, demonstrating that the endoderm (and expression of *vangl2* and *pk1a* within) is not required for initiating caudal migration. However, while the FBM neurons were organized in two distinct clusters in r6 and r7 in control embryos, the neurons were more evenly distributed between r5–r7 in morphant embryos, suggesting that the endoderm may influence subsequent phases of caudal migration.

Next, we tested whether *vangl2* expression in the mesoderm played a role in FBM neuron migration. Cells from embryos co-injected with *vangl2* MO and *TAR* RNA were transplanted into *Tg(isll:GFP)* hosts to generate mosaic embryos containing *vangl2*-deficient mesoderm (see Materials and Methods). Host wildtype FBM neurons migrated normally even when donor-derived *vangl2* morphant mesodermal cells were located adjacent to the motor neurons in r4 (Fig. S4; 219/243 embryos), suggesting that mesodermal expression of *vangl2* is not required for FBM neuron migration. Conversely, the presence of donor-derived wildtype mesodermal cells at the r4 level in *vangl2* mutant hosts was unable to induce (rescue) the migration of host mutant FBM neurons (137/167 embryos; data not shown). Intriguingly, the migration of host wildtype neurons was abrogated (partially blocked) (Fig. 2D, E; 24/243 embryos) only when the donor-derived, *vangl2* MO + *TAR* RNA-injected cells formed ventral neuroepithelial cells, including the floor plate. Similarly, the migration of host *vangl2*-morphant (or *vangl2*<sup>-/-</sup>) neurons was partially rescued (Fig. 2F–H; 30/167 embryos) only when the donor-derived, *TAR* RNA-injected wildtype cells formed ventral neuroepithelial cells, including the floor plate and possibly FBM neurons. This unanticipated location of *TAR* RNA-injected donor cells in the ventral neural tube and their ability to influence the migration of host FBM neurons suggested a role for *vangl2* in floor plate cells or other ventral neuroepithelial cells in regulating FBM neuron migration.

### ***Vangl2* can function in floor plate cells to regulate FBM neuron migration**

To directly test a role for *vangl2* in floor plate cells, we used cell transplantation to target *vangl2*-deficient cells to the floor plate in *Tg(isll:GFP)* host embryos. Donor cells were targeted to the floor plate by injecting *no tail* (*ntl*) MO into donor embryos, since *ntl*-deficient cells preferentially contribute to the floor plate (Halpern et al., 1993) (see Materials and Methods for details). In control experiments, donor-derived wildtype cells differentiating into floor plate in wildtype hosts did not affect the migration of host FBM neurons (Fig. 3C; 35 embryos). However, when *vangl2* mutant donor cells contributed to the floor plate in the r4 region of 36 hpf wildtype hosts, a large number of host neurons failed to migrate out of r4 in a majority of the embryos (Figs. 3A–C and S6; 30/44 embryos),



consistent with a requirement for *vangl2* function in floor plate cells for neuronal migration. The blocking ability of *vangl2* mutant cells was variable since host neurons migrated normally in several embryos containing donor floor plate cells in r4 (Fig. 3C; 14/44 embryos). Importantly, *vangl2* mutant donor cells were able to block the migration of wildtype FBM neurons only if they contributed to the floor plate in the r4 region of the host embryos, since donor-derived cells in other locations in the hindbrain, including the floor plate in other rhombomeres failed to block migration even partially (Fig. 3C; 201 embryos).

Next, we used the Gal4-UAS system to ask whether floor plate-specific expression of a *vangl2* transgene could rescue FBM neuron migration in *vangl2* mutant embryos. We employed a Tol2 gene trap line SAGFF187A (henceforth called *Tg(Fp:Gal4FF)*; *Tg(UAS:GFP)*), which expresses Gal4FF and GFP (Asakawa et al., 2008) in the floor plate from 16–48 hpf, to drive expression of a *UAS:Myc-vangl2* transgene (Fig. S7; X.P. and A.C., unpublished data). *Vangl2* mutant (*tri*<sup>-/-</sup>) embryos obtained from a cross of *Tg(Fp:Gal4FF)*; *Tg(UAS:GFP)*; *tri*<sup>+/-</sup> and *Tg(UAS:Myc-vangl2)*; *Tg(islet1:gfp)*; *tri*<sup>+/-</sup> fish were processed for anti-MYC (Vangl2 expression) and anti-GFP (FBM neurons and floor plate) immunostaining at 48 hpf and scored for the presence of caudally migrating FBM neurons outside r4. Consistent with a role for *vangl2* in the floor plate, >50% of *vangl2* mutant FBM neurons migrated out of r4 in mutant embryos containing at least 1–2 Myc-Vangl2 expressing floor plate cells in the r4 region (12/12 embryos; Figs. 3D–F and S8; Table S1). Migration of mutant neurons was not rescued either in embryos not expressing Myc-Vangl2 (146/146) or in embryos containing Myc-Vangl2 expressing floor plate cells outside the r4 region (148/148; Fig. 3F). Taken together, the floor plate transplant and transgene experiments suggest strongly that *vangl2* functions primarily in floor plate cells to regulate FBM neuron migration.

It is also possible that *vangl2* functions in part in other cell types such as the FBM neurons themselves to regulate migration. Indeed, the inability of transplanted wildtype FBM neurons to migrate in *vangl2*-deficient hosts (Fig. 4B) (Jessen et al., 2002) may reflect a partial requirement for *vangl2* in the FBM neurons, since non-migrating host motor neurons may inhibit the migration of donor-derived wildtype neurons through *vangl2*-mediated cell interactions. Recently, Walsh et al. (2011) performed elegant transplant experiments that indicated that *vangl2* function is required in the environment (non cell-autonomous role) and in FBM neurons (cell autonomous role) for migration. Specifically, the authors suggest that *vangl2* acts in FBM neurons, mediating interactions between motor neurons needed to migrate out of r4 (collective migration mode). To definitively resolve this issue, one must eliminate the influence of host FBM neurons on the behavior of donor-derived motor neurons. Therefore, we performed similar transplant experiments in *detour* mutant (*gli1*<sup>-/-</sup>) host embryos that generate few or no branchiomotor neurons (Chandrasekhar et al., 1999; Vanderlaan et al., 2005).

In control experiments, ~85% of wildtype donor-derived FBM neurons migrated out of r4 in wildtype host embryos (Fig. 4A). Next, as expected, all wildtype FBM neurons failed to migrate out of r4 in *vangl2* morphant embryos (Fig. 4B), and ~85% of *vangl2* mutant FBM neurons migrated out of r4 in wildtype hosts (Fig. 4D), similar to previous results (Jessen et al., 2002; Walsh et al., 2011), and consistent with a non-autonomous role for *vangl2*. About 70% of wildtype FBM neurons migrated out of r4 in *detour* (*dtr*) mutant (but *vangl2*<sup>+/+</sup>) hosts (Fig. 4C). Importantly, ~60% of *vangl2* mutant FBM neurons migrated out of r4 in *dtr* mutant hosts (Fig. 4E), which is comparable in efficiency to the ability of wildtype FBM neurons to migrate in *dtr* mutants (Fig. 4C), indicating that *vangl2*<sup>-/-</sup> neurons do not require the presence of *vangl2*<sup>+/+</sup> neurons to migrate caudally (one interpretation of Fig. 4D). Further discounting a motor neuron-autonomous role for *vangl2*, donor-derived wildtype FBM neurons completely failed to migrate out of r4 in *vangl2* morphant, *dtr* mutants (Fig.

4F), identical to the results in *vangl2* morphant hosts (Fig. 4B), and ruling out an influence of host motor neurons on the behavior of donor wildtype FBM neurons in experiment 4B. Lastly, since the migration of wildtype FBM neurons was completely blocked by inactivating *vangl2* in an environment lacking FBM neurons (compare Figs. 4C and F), we conclude that *vangl2* regulates FBM neuron migration out of r4 by acting largely outside motor neurons, likely in neuroepithelial cells such as the floor plate.

### **FBM neurons fail to migrate caudally in the mouse *Gli2* mutant, which fails to induce the floor plate**

To test whether a potential role for the floor plate in FBM neuron migration was evolutionarily conserved, we analyzed *Gli2* mutant mice, which fail to induce the floor plate but still generate motor neurons (Matise et al., 1998). *Gli2* mutant hindbrains did not express the floor plate marker *Foxa2* (Fig. 5A–D), as shown previously in the spinal cord (Matise et al., 1998). In situ hybridization with *Tbx20*, a branchiomotor neuron marker (Song et al., 2006), showed that in wildtype E12.5 siblings, FBM neurons formed a continuous longitudinal stream of caudally migrating cells spanning r4 to r6, with radial migration within r6 to form the facial motor nucleus (nVII) (Figs. 5E and S9). In *Gli2* mutants, FBM neurons were greatly reduced in number (Fig. 5F). Importantly, however, they failed to migrate caudally out of r4, but still migrated radially within r4 (Figs. 5F and S9). Consistent with this, the nVII nucleus at E14.5, detected by *Tbx20* and *Phox2b* in situ (Glasco et al., 2012), was located rostrally in the r4 region of *Gli2* mutants, rather than in r6, as in wildtype siblings (Fig. S9). The neuronal populations were fused across the midline at E12.5 due to the absence of the floor plate, as shown previously for spinal motor neurons (Matise et al., 1998). To confirm that the *Tbx20*-expressing cells in mutants were FBM neurons, we performed retrograde labeling with NeuroVue dyes (Fritzsche et al., 2005). As expected, the cell bodies of the nV and nVII nuclei were located in r2 and r4–r6, respectively, in wildtype embryos (Fig. 5G). In *Gli2* mutants, a small number of back-filled FBM neurons were located within r4 at the midline, with axons extending laterally (Fig. 5H), indicating the failure of caudal migration.

### **Potential role for Vangl2-dependent floor plate polarity, but not for floor plate cilia function, in FBM neuron migration**

We showed previously that FBM neurons in *vangl2*-deficient embryos generate protrusions and change shapes at the same rate as neurons in wildtype embryos, but fail to migrate out of r4 because they are unable to maintain caudally directed protrusions (Jessen et al., 2002; Sittaramane et al., 2009). Since our data suggested strongly that *vangl2* functions in floor plate cells in r4 to regulate caudal migration, we wondered whether FBM neurons located in r4 exhibit previously undetected protrusive behaviors. Therefore we quantified protrusions of FBM neurons located in r4 in fixed wildtype and *vangl2* mutant *Tg(zCREST:mRFP)* embryos at 19 and 24 hpf (Fig. 6). Protrusions of wildtype neurons were directed largely toward the midline at both ages, as shown previously (Mapp et al., 2010). Imaging of *Tg(Fp:Gal4FF); Tg(UAS:GFP); Tg(zCREST:mRFP)* embryos to simultaneously examine floor plate cells (GFP+ve) and motor neurons (mRFP+ve) revealed that some midline-directed protrusions contacted floor plate cells (Fig. S10). Importantly, protrusions on *vangl2* mutant neurons arose from all around the cell periphery (Fig. 6B, E, D), with a majority of protrusions directed away from the midline at 19 hpf (Fig. 6E; significant difference from wildtype at  $p < 0.001$ ). These data suggest that *vangl2* acting non-autonomously, likely in floor plate cells, affects the ability of FBM neurons to generate or preferentially maintain midline-directed protrusions.

Vangl2 may function in, and influence the polarity of, floor plate cells to regulate FBM neuron migration. Indeed, Borovina et al. (2010) showed that floor plate polarity, defined by

the position of the centrosome generating the primary cilium and by the orientation of the cilium, is disrupted in the spinal cord floor plate of *MZvangl2* mutants. We confirmed that the organization of floor plate cilia was also disrupted in the hindbrain region of *vangl2* mutants (Fig. 7A, B). To test whether floor plate cilia are necessary for FBM neuron migration, analogous to the role of ependymal cilia in olfactory neuroblast migration (Sawamoto et al., 2006), we first examined *iguana* (*igu*) mutants, which form very few floor plate cilia (Kim et al., 2010). In wildtype siblings, floor plate cilia were longitudinally oriented, and a majority of FBM neurons had migrated out of r4 (Fig. 7C). In *igu* mutants, although floor plate cilia were absent (37/44 embryos; Fig. 7D) or greatly reduced (7/44) in number, most FBM neurons migrated normally out of r4, suggesting that cilia are not required for migration. Since floor plate cilia eventually form in *iguana* mutants by 48 hpf (Kim et al., 2010), we sought to rule out any involvement of cilia by analyzing maternal zygotic mutants of the intraflagellar transport protein Ift88/Polaris/Oval (*MZovl*), which lack all cilia (Huang and Schier, 2009). We verified that floor plate cilia were absent in the hindbrain region of *MZovl* mutants (Fig. 7E, F). *Tag1* in situ revealed that in spite of the complete absence of cilia, a majority of FBM neurons migrated out of r4 in *MZovl* mutants (Fig. 7H). The numbers of trigeminal (r2, r3) and facial (r4–r7) branchiomotor neurons cells were reduced in *MZovl* mutants, likely reflecting dampened hedgehog signaling (Huang and Schier, 2009). A small number of *tag1*-expressing neurons consistently remained in the r4 region of *MZovl* embryos, reflecting either a partial dependence of caudal migration on cilia function or subtle defects in PCP signaling.

### Vangl2 is enriched in basolateral membranes of floor plate cells

Our data suggest that *vangl2* functions in floor plate cells to regulate FBM neuron migration independently of floor plate cilia function. To explore this issue further, we asked whether Vangl2 itself exhibited asymmetric localization in floor plate cells. We generated a transgenic line driving an N-terminally fused GFP-Vangl2 protein from a 5 kb *vangl2* promoter element (X.P. and A.C., unpublished data). The GFP-Vangl2 transgene was expressed in a similar pattern to the endogenous gene, but also in some ectopic tissues such as the somites (Fig. 8B). Importantly, the *vangl2* transgene was able to efficiently rescue FBM neuron migration in *vangl2* morphants (Fig. 8A, B; 16/16 embryos), indicating that the fusion protein is expressed at appropriate levels and retains the biochemical activity of the endogenous protein. To examine localization in floor plate cells, we analyzed *Tg(vangl2:GFP-Vangl2)* embryos injected with RNA encoding membrane-RFP. In >90% of floor plate cells, GFP-Vangl2 was enriched in the anterior, posterior, and ventral membranes compared to the apical membrane (Fig. 8C; 131/141 cells, 6 embryos). To determine whether GFP-Vangl2 showed preferential accumulation in the anterior or posterior membranes, we tried to generate isolated transgene-expressing floor plate cells using the *ntl* MO to generate floor plate mosaics. We found again that GFP-Vangl2 was enriched in all floor plate cell membranes except the apical (Fig. 8D; 21/31 cells, 3 embryos), but did not observe consistent differences between the anterior and posterior membranes. We also could not detect preferential enrichment of GFP-Vangl2 on anterior or posterior membranes of migrating FBM neurons (Fig. 8E; 60 cells, 4 embryos). Together, these data suggest that differential enrichment of GFP-Vangl2 on floor plate membranes reflects an aspect of floor plate polarity that may influence FBM neuron migration.

## DISCUSSION

Vangl2 is a core component of the PCP signaling pathway, and is essential for directional movement of gastrulating cells and FBM neurons (Jessen et al., 2002; Roszko et al., 2009). Consistent with its broad expression pattern, previous studies have suggested cell autonomous and non-cell autonomous roles for *vangl2* in regulating FBM neuron migration



(Jessen et al., 2002; Walsh et al., 2011). Here, we provide multiple lines of evidence that suggest strongly that *vangl2* functions primarily in floor plate cells to regulate migration of FBM neurons out of rhombomere 4. Importantly, we also find that floor plate cilia function is not required for neuronal migration.

### Site of action of PCP genes for FBM neuron migration

Several broadly expressed PCP genes (*vangl2*, *celsr2*, *fzd3a*, *scrib*) appear to function non cell-autonomously for FBM neuron migration (Jessen et al., 2002; Wada et al., 2005; Wada et al., 2006). Wada and colleagues performed mosaic analyses to propose that *fzd3a* (and possibly *celsr2* and *scrib*) functions in neuroepithelial cells surrounding the FBM neurons to inhibit their radial migration (and integration into the neuroepithelium) within r4, consequently enabling their caudal migration into r6. One caveat of these mosaic analyses is that the donor-derived FBM neurons of one genotype (e.g., *scrib* mutant) were surrounded by host FBM neurons of the second genotype (e.g., wildtype), leaving open the possibility that the migratory behaviors of the donor-derived neurons were being dictated by interactions with host motor neurons (motor neuron-autonomous) and not by interactions with neuroepithelial cells in the environment (non cell-autonomous). This issue was addressed recently (Walsh et al., 2011) by examining the behaviors of donor-derived *vangl2*- and *scrib*-deficient FBM neurons in *vangl2*+ve and *scrib*+ve host embryos, respectively, where the host FBM neurons failed to migrate due to *pk1b* knockdown. Whereas *vangl2*-deficient FBM neurons frequently migrated out of r4 in wildtype hosts, consistent with a non-autonomous role for *vangl2*, *vangl2*-deficient motor neurons failed to migrate out of r4 in *pk1b* morphant hosts, indicating that the migratory behavior of the host motor neurons was influencing the migratory ability of the donor-derived FBM neurons (motor neuron autonomous effect). Two mechanisms are consistent with this motor neuron autonomous effect (Walsh et al., 2011). Firstly, migratory FBM neurons in r4 may interact and influence each other's decision to migrate out of r4. Importantly, these interactions would not be dependent on the function of core PCP genes (collective migration, independent of PCP function). Secondly, PCP proteins may also function within FBM neurons to facilitate their migration (PCP-dependent migration), but this function would be masked by the collective mode of migration. Our mosaic analysis with *detour* mutants to resolve this issue suggests strongly that *vangl2* does not function in FBM neurons for migration.

### Vangl2 functions in floor plate cells to regulate FBM neuron migration

Since the collective mode of FBM neuron migration (Walsh et al., 2011) can confound mosaic analysis of *vangl2* function, we performed chimeric analysis in *detour* mutant (*gli1*<sup>-/-</sup>) host embryos, which fail to induce branchiomotor neurons (Chandrasekhar et al., 1999; Vanderlaan et al., 2005). This allowed us to examine the migratory behavior of donor-derived FBM neurons in the absence of host-derived branchiomotor neurons, thereby removing the contribution of collective migration to mosaic analysis phenotypes (Fig. 4). *Vangl2* mutant FBM neurons migrated out of r4 in *detour* mutant hosts in a similar fashion to wildtype neurons, indicating that *vangl2* function in FBM neurons is dispensable for migration. Importantly, wildtype FBM neurons failed to migrate out of r4 in *vangl2* morphant, *detour* mutant hosts, similar to their behavior in *vangl2* morphant hosts, demonstrating an exclusive requirement for *vangl2* function outside of motor neurons. Interestingly, FBM neuron migration out of r4 was less efficient in *detour* mutant hosts compared to wildtype hosts (Fig. 4A, C-E), suggesting that the *dtr* mutant hindbrain may be less conducive to migration. Consistent with this, expression of chemokine genes *cxcl12a* and *cxcr7b*, which play roles in FBM neuron migration (Cubedo et al., 2009), was altered in putative *detour* mutant hindbrains (Fig. S11).

While the *detour* transplant experiments argue against a motor neuron-autonomous role for *vangl2* in FBM neurons, the *no tail* transplant and *UAS:Myc-vangl2* transgene experiments strongly favor a non cell-autonomous role for *vangl2* in floor plate cells for regulating migration (Fig. 3). In the transplant studies, the moderate efficiency of abrogating wildtype neuron migration (Fig. 3A) may be attributed to variability in the position and number of donor-derived floor plate cells in host embryos. Similarly, while expression of the *UAS:Myc-vangl2* transgene in floor plate cells in the r4 region rescued neuronal migration in every *vangl2* mutant (Fig. 3F), rescue efficiency ranged from ~50 to >90%. This variable efficiency is likely due to the mosaic expression of the transgene in floor plate cells resulting from methylation-mediated transcriptional silencing of the 10XUAS element in the *UAS:Myc-vangl2* transgene (Goll et al., 2009; Akitake et al., 2011). Nevertheless, taken together, the transplantation and transgenic data suggest strongly that *vangl2* functions primarily in floor plate cells for FBM neuron migration. However, we cannot definitively rule out that *vangl2* may also function in the neuroepithelial cells surrounding the FBM neurons to regulate their migration (Walsh et al., 2011), as proposed for *fzd3a* (Wada et al., 2006).

Our observation that caudal migration of FBM neurons is lost in mouse *Gli2* mutants, which fail to induce the floor plate (Matise et al., 1998), lends further support to the idea that floor plate cells play an evolutionarily conserved role in this migration. However, since other ventral cells, in addition to the floor plate, are also missing in *Gli2* mutants, the migration phenotype cannot be attributed exclusively to floor plate-associated defects. Nevertheless, because *Vangl2* is expressed in the mouse hindbrain floor plate (Glasco et al., 2012), and *Vangl2* mutant mice exhibit defective floor plate development (Greene et al., 1998; Murdoch et al., 2001) and defective FBM neuron migration (Glasco et al., 2012), we propose that *Vangl2* functions in the floor plate to regulate FBM neuron migration in mice, as in zebrafish. Demonstration of a specific role for *Vangl2* in the mouse floor plate will require analyses of conditional knockouts using the *Vangl2 flox* allele (Song et al., 2010). However, because most FBM neurons in mice are positioned several cell diameters away from the floor plate (Qu et al., 2010), it is unlikely that floor plate cells (and *Vangl2* function within) will directly regulate caudal migration of all FBM neurons, and other cell types including ventral neuroepithelial cells adjacent to the floor plate and motor neurons may also play a role.

### Role of floor plate polarity and cilia function in FBM neuron migration

Given the importance of the floor plate for FBM neuron migration, it will be critical to identify the aspects of floor plate structure and function that are necessary for migration. One attractive possibility is that motile floor plate cilia (Yu et al., 2008; Cruz et al., 2010) produce directed fluid flow in the fourth ventricle that could influence FBM neuron migration by generating gradients of floor plate-secreted molecules within the neural tube. Indeed, Sawamoto et al. (2006) demonstrated that fluid flow caused by ependymal cilia generates a gradient of the chemorepulsive Slit proteins in the subventricular zone, and that cilia function and Slits were necessary for the directed migration of olfactory neuroblasts in the rostral migratory stream toward the olfactory bulb. Our results with *iguana* and *MZovl* mutants argue against an analogous role for floor plate cilia in the caudal migration of FBM neurons.

If cilia are not involved, how might *Vangl2* regulate the properties of floor plate cells to regulate migration? We found that a GFP-*Vangl2* fusion protein is enriched in the basolateral, but not the apical, membranes of floor plate cells. The differential distribution of GFP-*Vangl2* on floor plate membranes is likely to reflect the distribution of endogenous *Vangl2* since the fusion protein is biologically active (i.e., able to rescue FBM neuron

migration in *vangl2* mutants), and its expression is controlled by *vangl2* promoter elements. Since Vangl2 physically interacts with Wnt/PCP molecules like Prickle and Dishevelled (Bastock et al., 2003; Jenny et al., 2003; Torban et al., 2004), these molecules may also exhibit differential subcellular localization in floor plate cells. However, neither Prickle nor Dishevelled is likely to be involved in the floor plate-mediated regulation of neuronal migration since *prickle* genes are not expressed in the floor plate (Carreira-Barbosa et al., 2003; Tissir and Goffinet, 2006; Rohrschneider et al., 2007) (this report), and Dishevelled-mediated signaling is largely dispensable for migration (Jessen et al., 2002; Glasco et al., 2012).

Our observation that the protrusions of premigratory wildtype FBM neurons in r4 are preferentially directed toward the midline, while those of non-migrating *vangl2* mutant neurons are less directed suggests that the floor plate and/or adjacent neuroepithelial cells may be the source of an orientating cue, as suggested previously (Fig. 8F; Mapp et al., 2010). We propose that this putative orientating cue is closely associated with (sequestered by) asymmetrically distributed Vangl2 on floor plate cells to orientate FBM neurons in r4 and to make them competent (primed) to respond to other environmental cues (Step 1). These primed FBM neurons may respond to cell adhesive or cell repulsive cues in a Pk1b- and Fzd3a-dependent manner (Step 2), get excluded from the neuroepithelium in r4, and migrate caudally, as proposed previously (Wada et al., 2006). In *vangl2* mutants, the orientating cue is not localized, and the FBM neurons do not become competent (Step 1 fails), resulting in their integration into the neuroepithelium in r4 and consequent failure to migrate caudally. It will be of great interest to identify the putative orientating cue(s), and to elucidate its postulated association with Vangl2.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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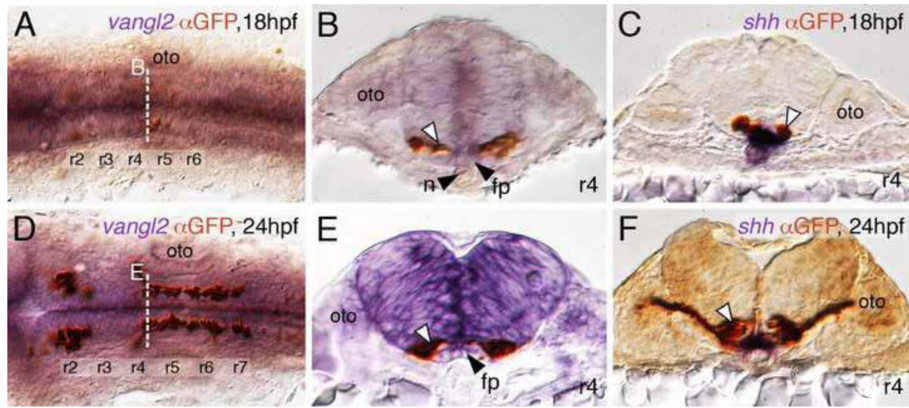
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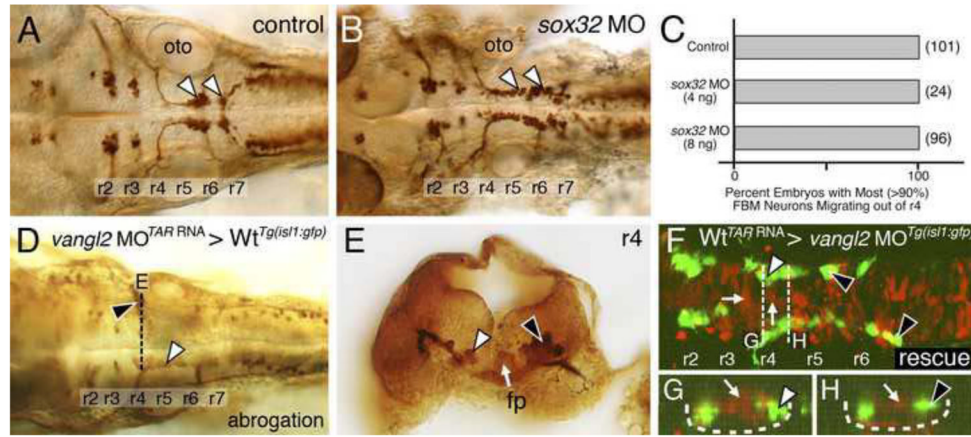
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### Highlights

- *vangl2* largely functions in the floor plate for facial motor neuron migration.
- Floor plate cilia are dispensable for neuronal migration.
- Vangl2 is enriched in the basolateral membranes of floor plate cells.
- Facial motor neurons fail to migrate in a mouse mutant lacking the floor plate.

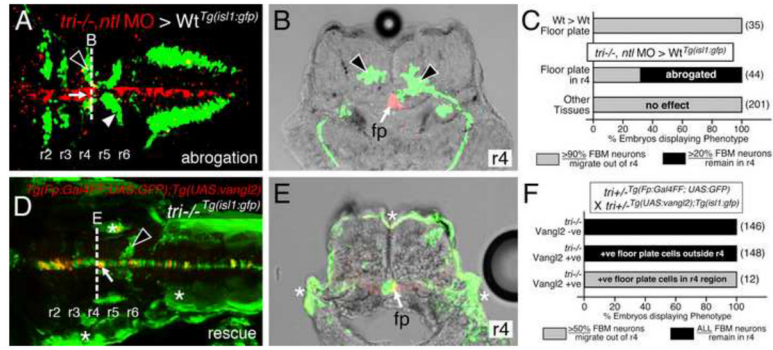


**Figure 1. FBM neurons contact *vangl2*-expressing floor plate cells in rhombomere 4**  
 (A, D) Dorsal views of *Tg(islet1:GFP)* hindbrains processed for *vangl2* in situ (purple) and anti-GFP immunostaining to label FBM neurons (brown). The level of the cross-sections is indicated by the broken lines. (B, E) *vangl2* is expressed broadly in the endoderm, mesoderm and within the neural tube, including the floor plate (fp, black arrowhead). Some FBM neurons (white arrowhead) are immediately adjacent to the floor plate cells. (C, F) FBM neuron cell bodies and processes (white arrowhead) contact floor plate cells marked by *shh* expression (purple). n, notochord; oto, otocyst.



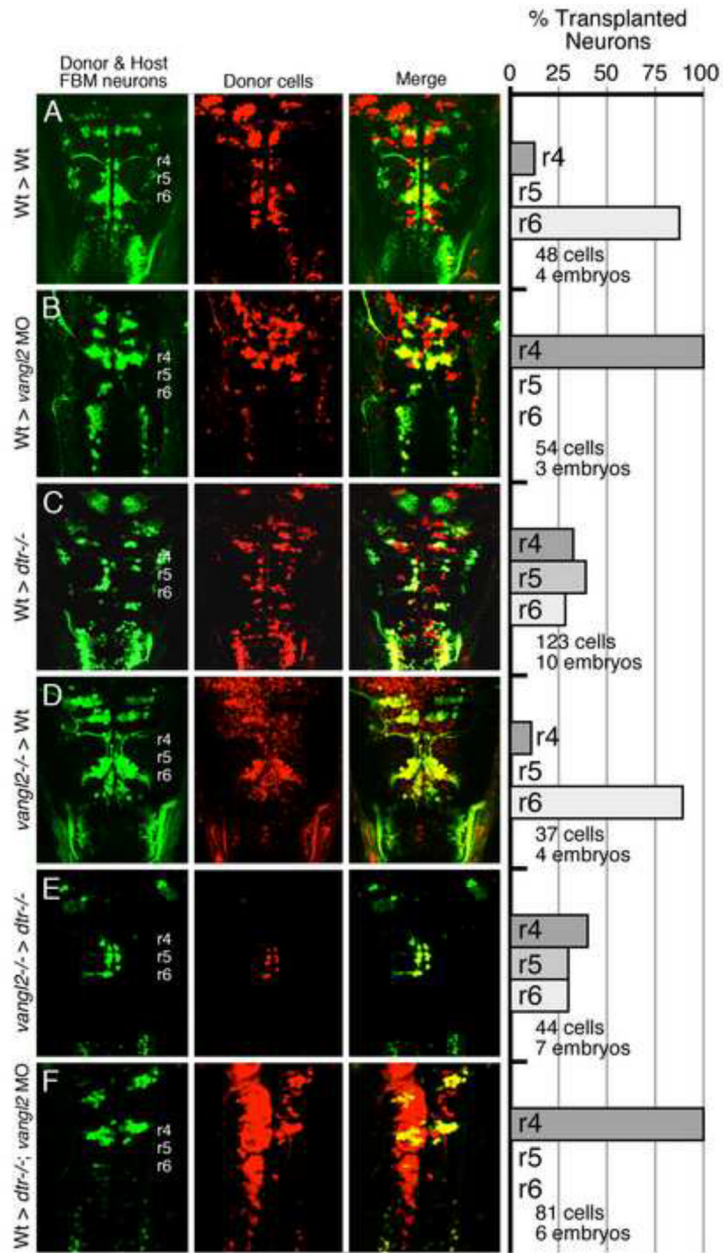
**Figure 2. Ventral neural tube expression of *vangl2*, but not the endoderm, is required for FBM neuron migration**

(A, B) Dorsal views of 36 hpf *Tg(isll:GFP)* hindbrains stained for FBM neurons (arrowheads, GFP antibody). In *sox32* morphant embryos, which lack endoderm, FBM neurons largely migrate normally out of r4, as in wildtype embryos. (C) Quantification of the FBM neuron migration phenotype in *sox32* morphant embryos. Number of embryos in parentheses. (D–H) Dorsal views of 36 hpf *Tg(isll:GFP)* host hindbrains (D, F), with the level of r4 cross-sections (E, G, H) indicated by broken lines. In D and E, motor neurons and donor-derived cells are both labeled brown due to streptavidin-conjugated alkaline phosphatase reactivity used to detect anti-GFP antibody binding (motor neurons) and biotinylated rhodamine dextran (*vangl2* MO donor cells). In F–H, the endogenous fluorescence from GFP and rhodamine were used to identify the motor neurons and donor-derived cells, respectively. Inadvertant targeting of *vangl2* morphant cells to the ventral neural tube (white arrow, E) in a wildtype host (D) results in failure of host FBM neurons to migrate (black arrowhead in D, E) on one side, with normal migration on the other (white arrowheads). Conversely, inadvertant targeting of wildtype cells (rhodamine dextran tracer, red) to the ventral neural tube (white arrows, F–H), including the floor plate (not visible due to very weak fluorescence), in a *vangl2* morphant host (F) rescues the migration defect of host FBM neurons (black arrowheads, F–H). fp, floor plate; oto, otic vesicle.



**Figure 3. *Vangl2* functions in floor plate cells in r4 to regulate FBM neuron migration**  
 Panels **A, D** show dorsal views of 36 hpf (**A**) and 48 hpf (**D**) *Tg(isl1:GFP)* host hindbrains, and panels **B, E** show cross-sections at the levels indicated by the broken lines. *Vangl2* mutants are indicated as *tri-/-* throughout. (**A, B**) Presence of *vangl2* mutant donor cells in the floor plate (fp) in r4 (arrows) of a wildtype host can abrogate (partially block) the migration of some host FBM neurons (black arrowheads) out of r4. A large number of host neurons (white arrowhead) migrate caudally. (**C**) Quantification of *vangl2* mutant > Wt transplantation data. Number of embryos in parentheses. (**D, E**) Floor plate-specific expression of *vangl2* (arrows) in a *vangl2* mutant completely rescues the migration of host FBM neurons (arrowhead). Gal4+ve floor plate cells are green, Myc-*Vangl2*+ve cells are red or yellow. Asterisks indicate Gal4FF-induced GFP expression in otic vesicle, trunk muscles, and skin. Many GFP-expressing cells are Myc-*Vangl2*-ve (and vice versa) probably due to independent and variable silencing of the *UAS:GFP* and *UAS:Myc-vangl2* transgenes integrated at different sites. (**F**) Quantification of Gal4-UAS rescue data. Number of embryos in parentheses. fp, floor plate.

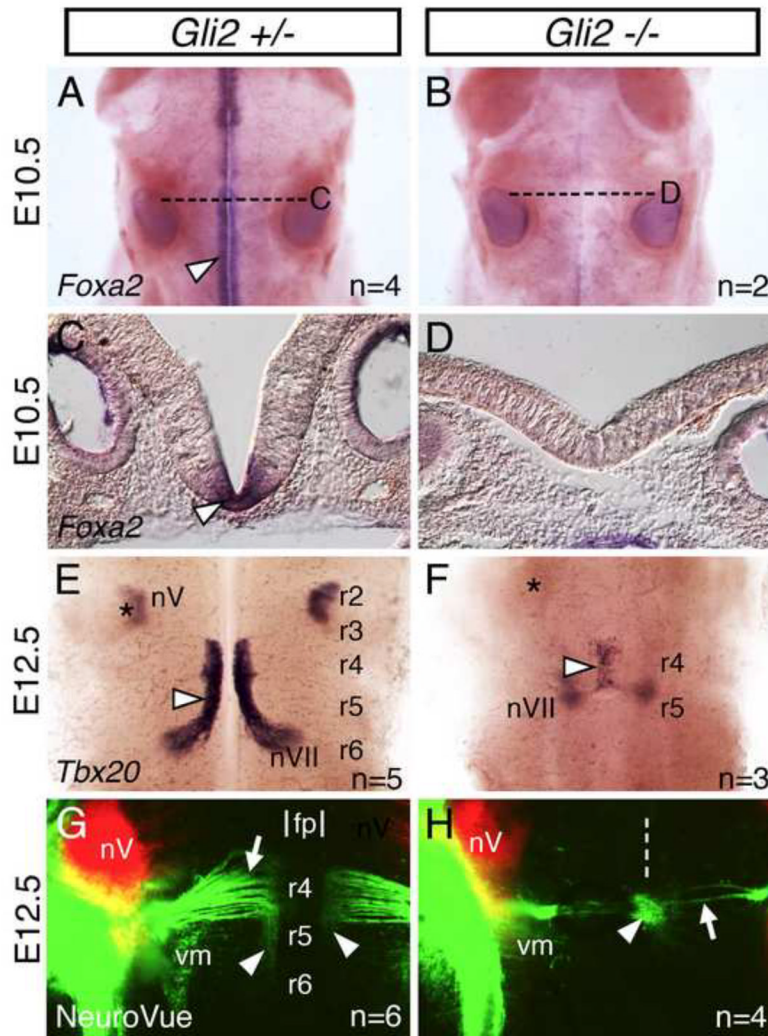




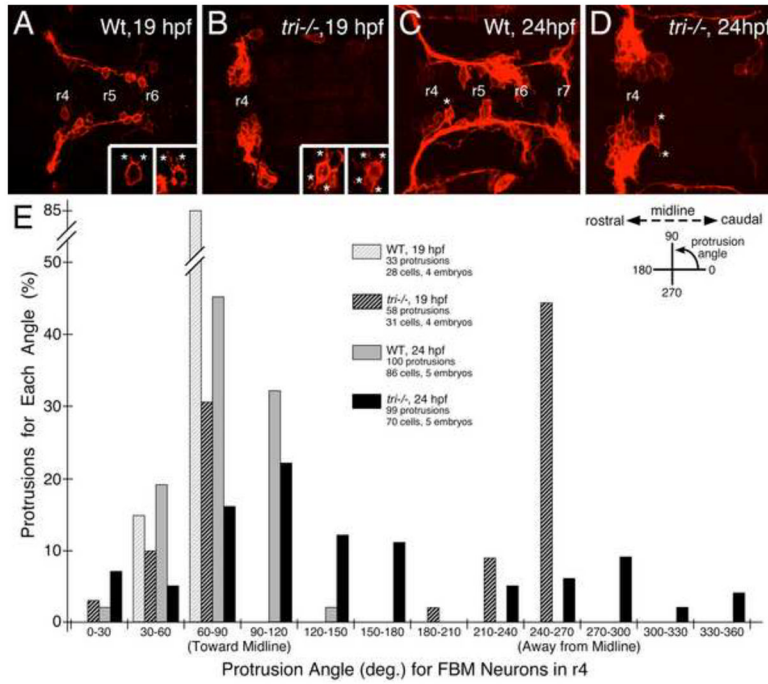
**Figure 4. *Vangl2* functions outside FBM neurons to regulate their migration**

(A–F) Dorsal views of the hindbrain in 48 hpf host embryos with anterior to the top. Both the donor and host embryos are *Tg(isll:GFP)*. Donor derived cells contain the lineage tracer rhodamine dextran (red), hence the donor-derived motor neurons are yellow, while the host motor neurons are green. Quantification of non-migrated FBM neurons in r4 and migrated FBM neurons in r5 and r6 is shown in the histograms. Different transplant conditions are indicated on the far left, and written as Donor > Host. *Vangl2*<sup>-/-</sup> refers to *trilobite* mutants. Wildtype, donor-derived motor neurons (yellow cells) in r6 of *vangl2* morphant (B) and *dtr*<sup>-/-</sup>; *vangl2* morphant (F) host embryos are not scored as FBM neurons because they do not have anteriorly directed axons exiting in r4, and are likely glossopharyngeal motor neurons. (A–D) depict control experiments. (E) Many *vangl2* mutant FBM neurons migrate out of r4 in a *vangl2*<sup>+/+</sup> environment, even in the absence of host wildtype FBM neurons. (F)

Conversely, wildtype FBM neurons never migrate out of r4 in a *vangl2*-deficient environment, even in the absence of host mutant FBM neurons (compare to B).



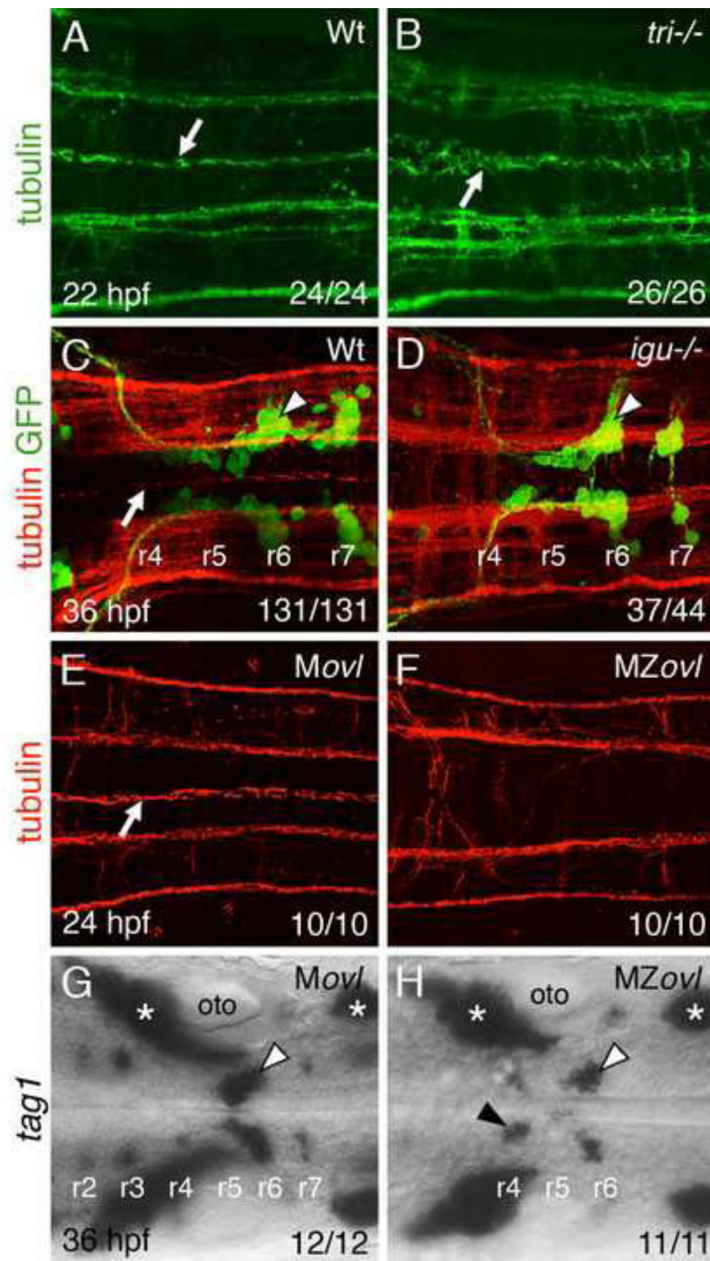
**Figure 5. FBM neurons fail to migrate caudally in mouse *Gli2* mutants, which lack the floor plate (A, B, E–H)** Dorsal views of hindbrains with anterior to the top. (C, D) Cross-sections (dorsal up) at the levels indicated by broken lines (A, B). (A–D) *Foxa2* in situ. In a *Gli2*<sup>+/-</sup> embryo (A, C), *Foxa2* is expressed along the midline, corresponding to the floor plate (arrowheads), at all axial levels. In a *Gli2*<sup>-/-</sup> embryo (B, D), *Foxa2* expression is missing, indicating the absence of a floor plate. (E–H) *Tbx20* in situ (E, F) or NeuroVue injection at the cranial nerve V (red) and VII (green) exit points (G, H). In a *Gli2*<sup>+/-</sup> embryo (E), FBM neurons migrate caudally from r4 into r6 (arrowhead). In a *Gli2*<sup>-/-</sup> embryo (F), FBM neurons fail to migrate out of r4, and are found in a single cluster (arrowhead) fused across the midline. Asterisks in E, F indicate location of the trigeminal motor nucleus (nV) in r2. In a *Gli2*<sup>+/-</sup> embryo (G), many retrogradely-labeled FBM neurons (arrowheads) are located in r5, and the motor axons (arrow) exhibit a characteristic fan shape (genu) indicative of caudal migration. The approximate location of the floor plate (fp) is indicated. In a *Gli2*<sup>-/-</sup> embryo (H), FBM neurons (arrowhead) are found in a single cluster at the midline (broken line), and their axons (arrow) lack the genu reflecting the failure to migrate. The locations of the trigeminal motor (nV) and visceromotor (vm) neurons in r2 and r5, respectively, are similar between wildtype and mutant embryos.



#### Figure 6. Loss of orientated FBM neuron protrusions in *vangl2* mutants

(A–D) Dorsal views of *Tg(zCREST:mRFP)* hindbrains processed for RFP immunostaining. In wildtype embryos (A, C and insets), protrusions (asterisks) generated by FBM neurons in r4 are oriented toward the midline. In *vangl2* mutant (*tri*<sup>-/-</sup>) embryos (B, D and insets), the protrusions of FBM neurons in r4 are oriented in various directions, with a large number directed away from the midline. (E) Quantification of the orientation of protrusions of FBM neurons in r4 of wildtype and *vangl2* mutant embryos. Whereas the orientation angles for all wildtype protrusions at 19 and 24 hpf are limited to the quadrants directed toward the midline, a large number of protrusions of mutant neurons are directed 180 degrees away from the midline, especially at 19 hpf (significant difference from wildtype at  $p < 0.001$ ; Watson-Williams F-test).

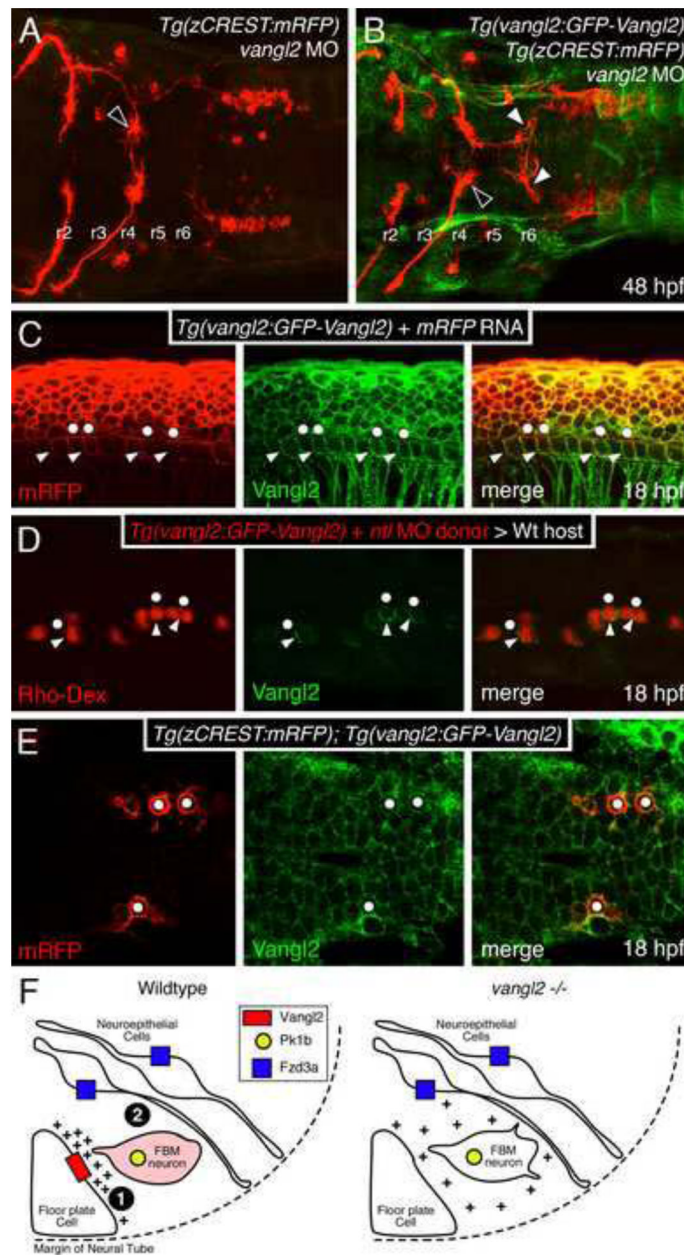




**Figure 7. Floor plate cilia are largely dispensable for FBM neuron migration**  
 (A–H) Dorsal views of hindbrains with anterior to the left. The ratio of the number of embryos exhibiting a phenotype to the total number of embryos examined is indicated in each panel. (A, B) Anti-tubulin immunostaining (green). In a wildtype hindbrain (A), floor plate cilia (arrow) are uniformly orientated along the anterior-posterior axis, whereas in a *vangl2* mutant (*tri*<sup>-/-</sup>) mutant (B), the floor plate cilia (arrow) are disorganized and misorientated. (C, D) *Tg(isll:GFP)* embryos processed for anti-tubulin (red) and anti-GFP (green) immunostaining. In a wildtype sibling (C), floor plate cilia (arrow) are uniformly orientated, and FBM neurons (arrowhead) migrate out of r4. In an *iguana*<sup>-/-</sup> embryo (D), although floor plate cilia are absent, FBM neurons (arrowhead) migrate normally out of r4. (E, F) While floor plate cilia (white arrow) are present and uniformly orientated in the *Movl* hindbrain (E), they are absent in the *MZovl* hindbrain (F). Prominent longitudinal axon tracts



appear unaffected in *MZovl* embryos. (**G, H**) *Tag1* in situ to label branchiomotor and sensory neurons. FBM neurons (white arrowheads) migrate out of r4 in both *Movl* and *MZovl* embryos, although a small number of neurons (black arrowhead) consistently remained in r4 in *MZovl* embryos. Asterisks indicate the location of sensory ganglia, which develop normally in both genotypes.



### Figure 8. Vangl2 is enriched in basolateral membranes of floor plate cells

Anterior is to the left in panels A–E. (A, B) Dorsal views of hindbrains processed for RFP and GFP immunostaining. In a control *vangl2* morphant embryo (A), FBM neurons fail to migrate out of r4 (black arrowhead). In a *Tg(vangl2:GFP-Vangl2)* *vangl2* morphant (B), many FBM neurons migrate out of r4 (white arrowheads). (C) Lateral view of an embryo processed for immunostaining to detect mRFP and GFP-Vangl2. Whereas mRFP is present on basolateral (arrowheads) and apical (dots) surfaces of floor plate cells, the apical surfaces are mostly devoid of GFP-Vangl2. (D) Lateral view of a host embryo containing donor-derived cells targeted to the floor plate, and processed for Rhodamine and GFP immunostaining. Donor-derived floor plate cells (Rho-Dex) exhibit basolateral enrichment of GFP-Vangl2 (arrowheads) with weaker signal at the apical surface (dots). (E) Dorsal view of the hindbrain showing uniform distribution of GFP-Vangl2 on the surface (mRFP)

of FBM neurons (dots). **(F)** Model for regulation of FBM neuron migration by floor plate-derived Vangl2. In the wildtype rhombomere 4 hemi cross-section, Vangl2 on basolateral membranes of floor plate cells sequester a floor plate-derived orientating cue (+). Orientated protrusions on FBM neurons and associated signaling make them competent (primed; denoted by pink shading) to respond to other environmental cues (Step 1). These primed FBM neurons may respond to cell adhesive and/or cell repulsive cues in a Pk1b- and Fzd3a-dependent manner (Step 2), get excluded from the neuroepithelium in r4, and migrate caudally, as proposed previously (Wada et al., 2006). In rhombomere 4 of *vangl2* mutants, the floor plate-derived cue does not get sequestered at the basolateral membranes of floor plate cells, and the FBM neurons do not become competent (Step 1 fails). The neurons are unable to respond to adhesive/repulsive cues on neuroepithelial cells, resulting in their integration into the neuroepithelium in r4 and consequent failure to migrate caudally. The depiction of Vangl2 and Fzd3a on floor plate and neuroepithelial cells, respectively, indicates their functional requirement for neuronal migration, and not their expression pattern, which is ubiquitous in the neural tube.