Methods

Genetic mapping and positional cloning

To put *tri* on the genetic map, a genome scan was performed using the γ-irradiation-induced deletion allele *tri*^{v7} and a panel of 125 simple-sequence length polymorphism markers representing 25 linkage groups. The linkage group 7 marker Z9067 was absent in *tri*^{v7} embryos. Further genetic analysis on a panel of 50 *tri*^{m209} embryos linked *tri* to a cluster of four linkage group 7 markers, also deleted in *tri*^{v7} embryos (Supplementary Fig. S1). High-resolution mapping using Z17411 and a panel of 2,400 *tri*^{m209} and *tri*^{m747} embryos placed this marker less than 0.02 cM from the *tri* locus. The ends of BAC clones were sequenced directly and YAC ends recovered by self-circularization. BAC and YAC end sequencing uncovered polymorphic markers used to determine a critical interval for the *tri* locus. Sequencing of BAC137K24 identified a *stbm* homologue[1] located ~9 kb from Z17411.

Morpholino and RNA injections

For *tri* gain- and loss-of-function experiments, 50-300 pg *tri* RNA and 1-32 ng *tri* MO were used. The *dishevelled-ΔN*, *rho kinase 2* (*rok2*), and *dominant negative-rok2* constructs were previously described[2,3]. For epistatic analyses, doses of 30-100 pg *dishevelled-ΔN*, 60-180 *rok2*, 1-50 pg *dominant negative-rok2* , and 0.5-50 pg *tri* RNA were used. Doses of 1 pg-1 ng *tri* MO were used to "knock-down" *tri* function in *slb*^{zf216} embryos.

Whole-mount in situ hybridization and immunostaining

Antisense RNA probes *opl*[4], *krox-20* (ref. 5), *myoD*[6], *emx1* (ref. 7), *pax2b*[8], *hgg1* (ref. 9), *dlx3*[10], *shh*[11], and *deltaC*[12] were used for in situ hybridization as described[13]. To monitor rat Rok2 protein distribution, 50 pg of rat *rok2* RNA was injected alone, or with 200 pg *tri*, 60 pg *wnt11*, or *tri* plus *wnt11* RNA. Embryos were fixed and stained as described[14] using the following antibodies: primary C20 anti-Rok2 polyclonal antibody (1:100) (Research Antibodies) and anti-goat IgG secondary antibody conjugated to Alexa green 488 (1:250) (Molecular Probes). Images were acquired using a Zeiss LSM 510 laser scanning inverted confocal microscope.

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Movies S1 and S2

These movies are continuous Nomarski time-lapse recordings (approximately 12 minutes) of mesodermal cells converging dorsally. (Movie S1) By the end of gastrulation (tailbud-1 somite), WT mesodermal cells in dorsolateral regions are elongated, closely packed, and move dorsally (to the right, in the movie) along straighter paths achieving greater net speeds compared to earlier gastrulation stages. (Movie S2) In contrast, by the end of gastrulation dorsolateral mesodermal cells in *tri* mutant embryos are not elongated and move more slowly towards dorsal while taking indirect paths.

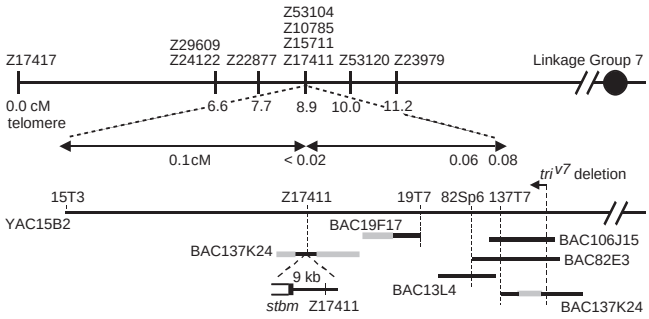

Figure S1 **Positional cloning of *tri*.** Integrated genetic and physical maps of the *tri* locus. The γ-irradiation-induced deletion allele *tri*^{v7} (breakpoint indicated) was used to map *tri* to linkage group 7. Linkage analysis placed marker Z17411 less than 0.02 cM from *tri*. Chimeric BACs 137K24 and 19F17 contained disparate genetic elements (indicated in grey) that did not map to this genetic region. Sequencing of BAC137K24 identified a *stbm* homologue located ~9 kb from Z17411. cM, centimorgan.

Movies S3 and S4

These movies are continuous time-lapse recordings of GFP-expressing facial (nVII) motor neurons acquired every 5 minutes for periods ranging from 4-8 hours. Images show dorsal views of the hindbrain with anterior to the left. In WT embryos (Movie S3), facial motor neurons are born in rhombomere 4 (center) and several cells migrate posteriorly toward rhombomeres 6 and 7. In *tri* mutant embryos (Movie S4), facial motor neurons are born in rhombomere 4 (center left). Mutant cells change their shapes frequently but do not migrate effectively toward rhombomeres 6 and 7.

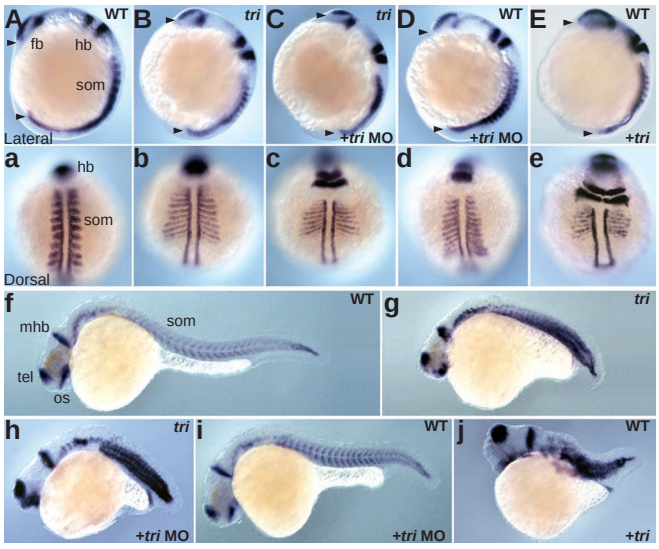

Figure S2 **Tri/Stbm does not regulate anteroposterior neural patterning. A,a-E,e,** Eight-ten somite embryos labeled with the forebrain marker *opl*, the hindbrain marker *krox-20*, and the somitic marker *myoD*. Arrowheads indicate anteroposterior length. WT (**A,a**) and *tri*^{m209} (**B,b**) embryos. *tri* (**C,c**) and WT (**D,d**) embryos injected with *tri* MO, and WT embryos (**E,e**) injected with *tri* RNA show inhibited convergence and extension, as evidenced by mediolaterally broadened *opl*, *krox-20*, and *myoD* domains but normal anteroposterior neural patterning. **f-j,** Lateral views of 1 day post-fertilization embryos labeled with *myoD* and the telencephalon marker *emx1* and the midbrain/hindbrain marker *pax2b*. WT (**f**) and *tri*^{m209} (**g**) embryos. *tri* (**h**) and WT (**i**) embryos injected with *tri* MO, and WT (**j**) embryos injected with *tri* RNA show essentially normal expression of neural markers. fb, forebrain; hb, hindbrain; som, somites; tel, telencephalon; mhb, midbrain/hindbrain boundary; os, optic stalks.

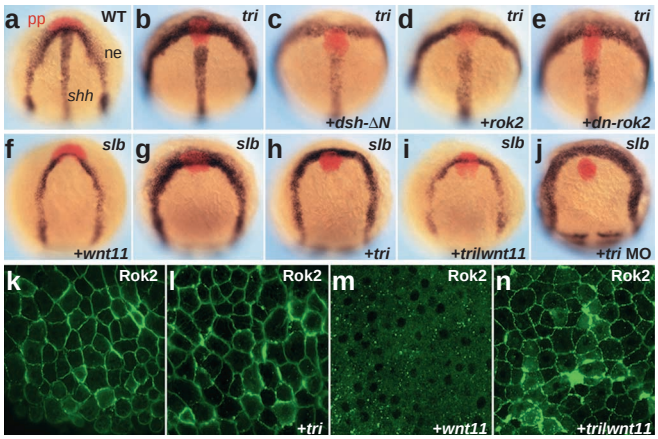

Figure S3 **Epistatic analysis of *tri* and PCP pathway components. a-e,** Two-three somite embryos labeled with the prechordal plate (pp) marker *hgg1* (red), *dlx3* marker delimiting the neuroectoderm (ne), and midline marker *shh*. WT (**a**) and *tri*^{m209} (**b**) embryos. Injection of *dishevelled-ΔN* (**c**), *rho kinase 2* (*rok2*) (**d**), or *dominant-negative rok2* (**e**) RNA did not suppress the *tri* convergence and extension phenotype. **f-j,** One-two somite *slb*^{tz216} embryos (verified by *deltaC* somitic staining, out of focus) labeled with *hgg1* (red) and *dlx3*. **f,** *slb*^{tz216} (*wnt11*) embryos rescued with *wnt11* RNA show *hgg1* staining anterior to a narrow *dlx3* domain. **g**, *slb*^{tz216} embryo. Injection of *tri* RNA (**h**), *tri* plus *wnt11* RNA (**i**), or low doses of *tri* MO (**j**) did not suppress the *slb* convergence and extension phenotype. **k-n,** Distribution of rat Rok2 upon overexpression in zebrafish blastulae was juxtamembranous when injected alone (**k**) or with *tri* RNA (**l**). Co-injection of *wnt11* with rat *rok2* removed Rok2 from the membrane (**m**) whereas co-injection of *rok2/wnt11* plus *tri* did not (**n**).