SUPPLEMENTARY MATERIAL

Supplementary Figure 1. Requirement of Scrib in zebrafish posterior hindbrain organization

(A) Posterior hindbrain structure in *mzscrib* mutants as analyzed by aPKCζ staining. Labeled apical cell membranes are aligned at the midline of *wt* embryos and misaligned in *mzscrib* mutants. **(B)** Alignment of the subapically localized tight junctional component Mpp5a at the midline in *wt* and aberrant alignment in *mzscrib* mutants. These are the same embryos as shown in Fig. 1D, imaged in a different channel. **(C)** Abnormal organization of the neural tube lumen in *mzscrib* compared to *wt* in transverse sections at posterior hindbrain level as analyzed by aPKCζ (green), F-actin (red) and DAPI (blue) staining. Dorsal is to the top. **(D)** *mzscrib* mutants display aberrant vagus motor neuron positioning visualized in tg(*isl1*:GFP) transgenic zebrafish. Dorsal view. Anterior is to the top in A,B,D.

Supplementary Figure 2. Scrib is not essential for proper velocity of hindbrain neural keel cell convergence and Scrib shows different effects on zebrafish hindbrain organization than Vangl2

(A) Single optical slices of *wt* and *scrib* morphant hindbrain neural plate architecture in dorsal horizontal view (upper row) and transverse view (lower row) with membrane-bound GFP at 1-2 somites (10.5 hpf). **(B)** Hindbrain neural keel convergence analyzed by multi-photon imaging of membrane-bound GFP in dorsal horizontal view starting at 4 somites (11.3 hpf). An image was acquired every 3 minutes for 105 minutes, ending at the 8 somite stage. Pictures were taken at all *z*-levels of the neural keel and the broadest keel positions were used for the analysis. Note abnormal convergence in the *vangl2* morphant and only slightly slower convergence in *scrib* morphant compared to *wt*. Arrows indicate the same cells at different time points. Anterior is to the left in all panels. Average neural keel convergence velocity in the posterior hindbrain noted on top of each panel (n=2 for each genotype). **(C)** Medial positioning of mitotically dividing cells

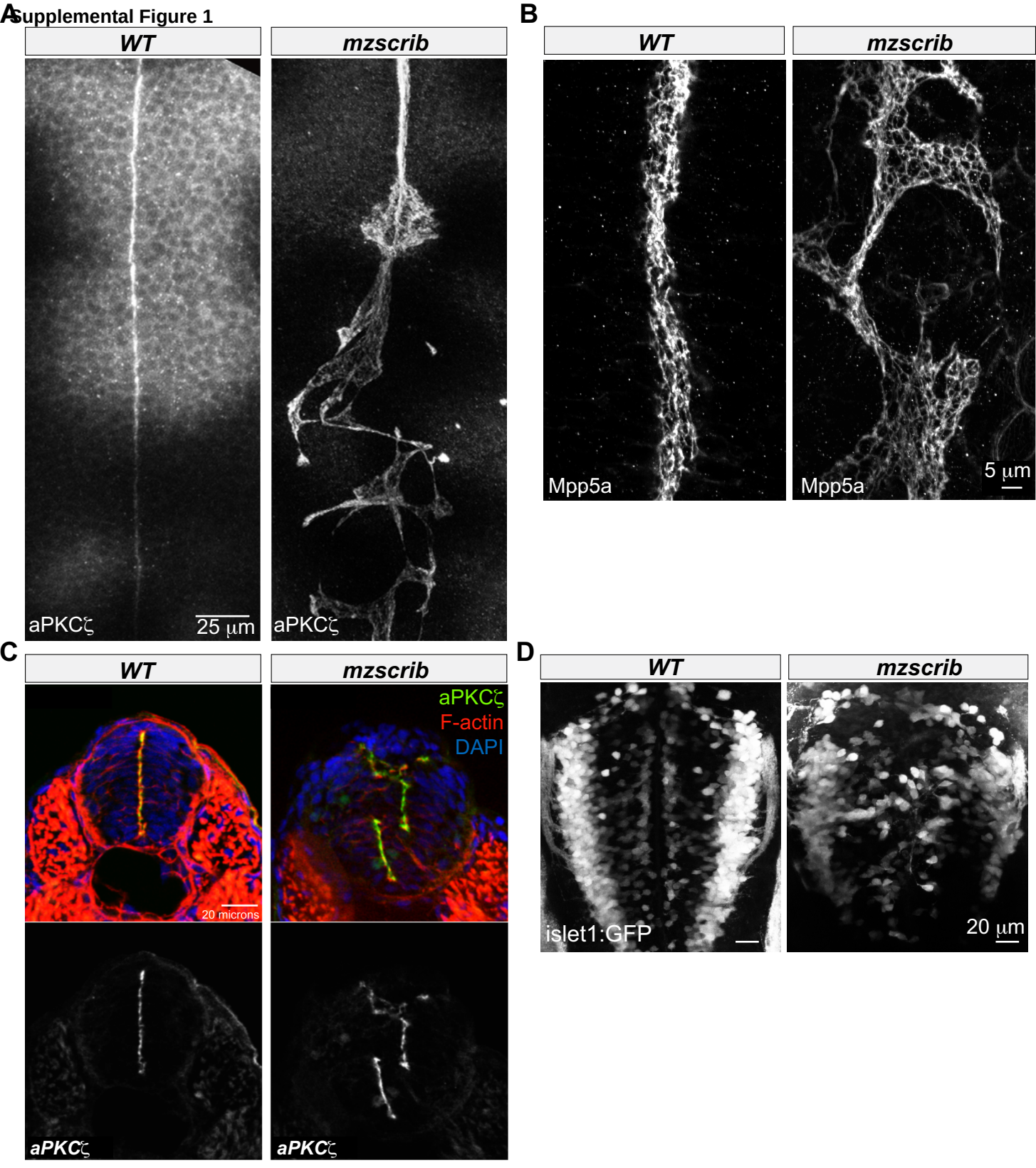
marked with anti phospho-Histone H3 (Ser10) medially along the midline in the posterior hindbrain neural keel of *wt* as well as in *mzscrib* mutants at 14 hpf. Images present maximal intensity projections of 4.8 µm confocal optical sections in dorsal horizontal view, anterior is to the top. **(D)** Quantification of the positioning of phospho-Histone H3 positive mitotic cells relative to the midline in confocal sections of the dorsal 30 µm of the neural keel plotted in microns on the horizontal axis. The *y*-axis displays the distribution frequency. Mitotic cells were positioned at an average distance of 11.36 ± 7.16 µm (*n*=196 cells, 9 embryos) in the *wt* (grey) and 12.28 ± 8.64 µm (*n*=238 cells, 9 embryos) in the *mzscrib* mutants (green). The differences between the two populations were not significant (*P*>0.2, *t*-test). **(E)** Organization of hindbrain neural progenitors in horizontal dorsal view showing the abnormally branched midline in *mzscrib* compared to the double midlines of *vangl2*$^{-/-}$ mutants. aPKCζ (red), γ-tubulin (green) and DAPI (blue). Anterior to the top in all pannels.

Supplementary Figure 3. GFP-Prickle localizes to cytoplasm, GFP-Scrib shows cortical localization in *wt* mitotic neural keel progenitors and Scrib is not required for maintenance of subapical α-Catenin localization in the mature neural tube epithelium. (A) Optical sections from timelapse imaging (min'sec") revealing anterior polarization of GFP-Pk foci (arrows) in interphase and subsequent to cytokinesis (cells followed marked by stars), but absence of GFP-Pk foci in mitotic cells. Time of optical sections given in min'sec". **(B)** Persistent cortical GFP-Scrib localization in *wt* neural keel progenitors with arrowhead noting a mitotic cell and arrows pointing to a cell undergoing cytokinesis. Dorsal horizontal view with anterior to the top in all panels. Double arrowheads indicate apico-basal axis. **(C)** Ctnna-citrine fusion protein of *Gt(ctnna-citrine)* transgenic line colocalizes with the endogenous Ctnna protein in immunofluorescent stainings. Single confocal images taken in the region of nasal epithelium (n.e.) and neuroepithelium of the retina. **(D)** Ctnna-citrine localization (arrows) in the mature neural tube reveals residual Ctnna-citrine foci in the

disorganized hindbrain epithelium of *scrib* morphants. Double arrowheads indicate apico-basal axis. Anterior-posterior axis is horizontal.

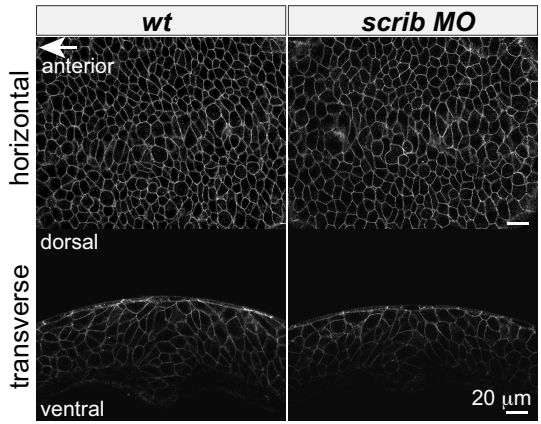
Supplementary Figure 4. Rescue of apico-basal cell division orientation of a single *cdh2*$^{-/-}$ mutant cell in a wildtype environment. Selected time points from an *in vivo* imaging of a genetic mosaic in which a *cdh2*$^{-/-}$ donor derived cell (expressing H2B-GFP (green) and membrane bound mRFP1 (purple)) was transplanted into the presumptive posterior hindbrain of a *wt* host embryo. Double arrowhead indicates the apico-basal axis of the neuroepithelium, yellow arrows indicate the final orientation of cell division. Anterior is to the top. The times of the optical sections are given in min'sec".

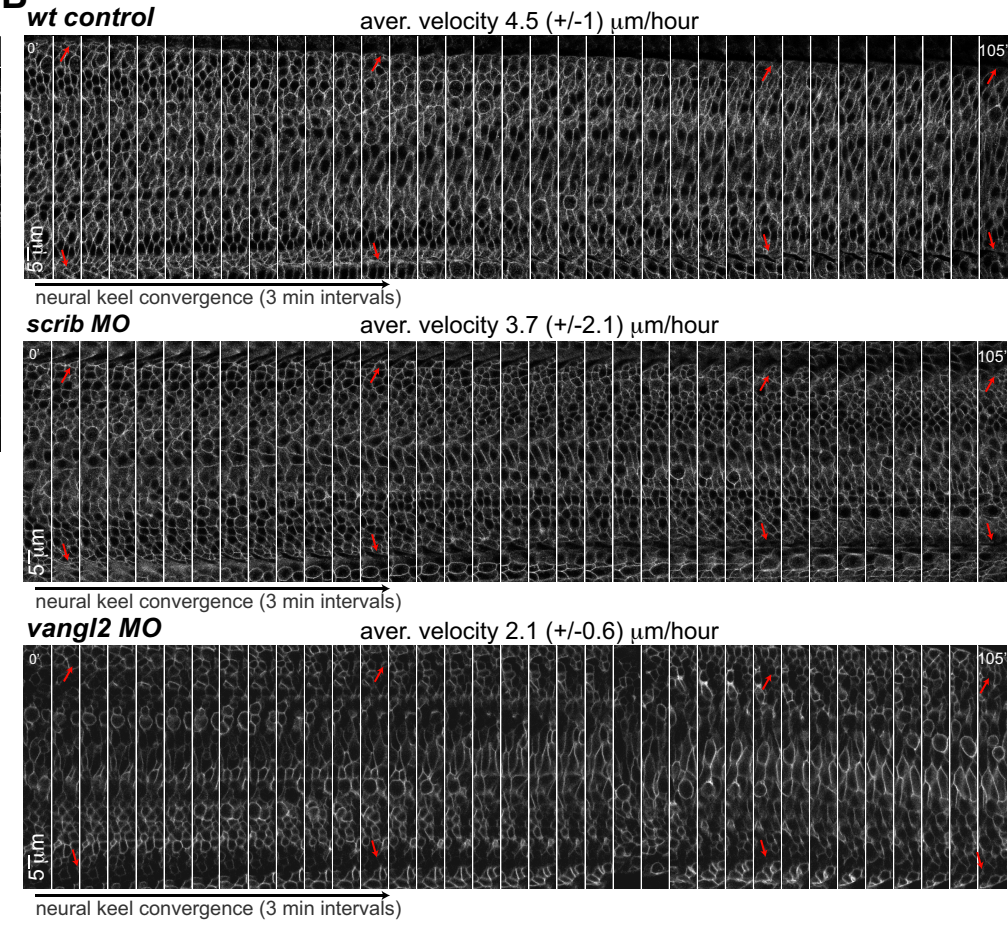
Supplemental Figure 1

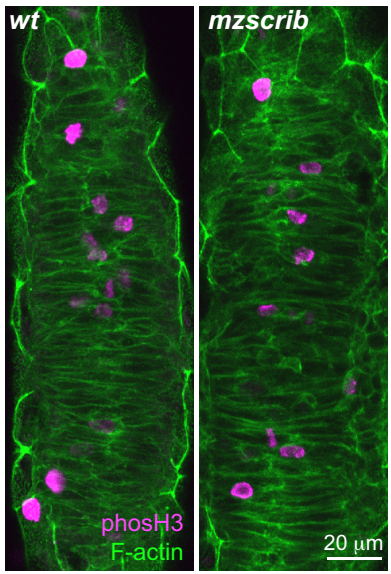


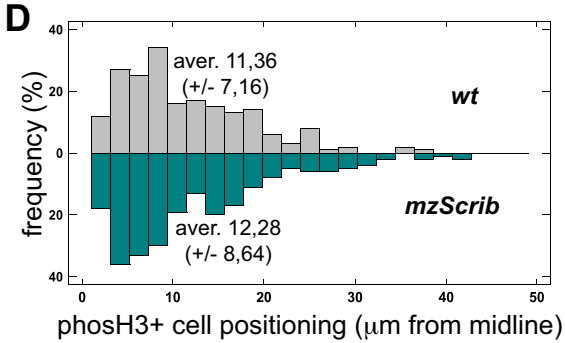
Supplementary Fig.1, Zigman et al.

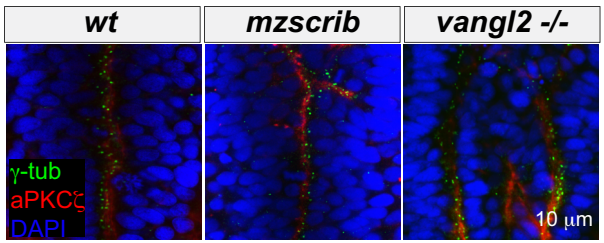
Supplemental Figure 2



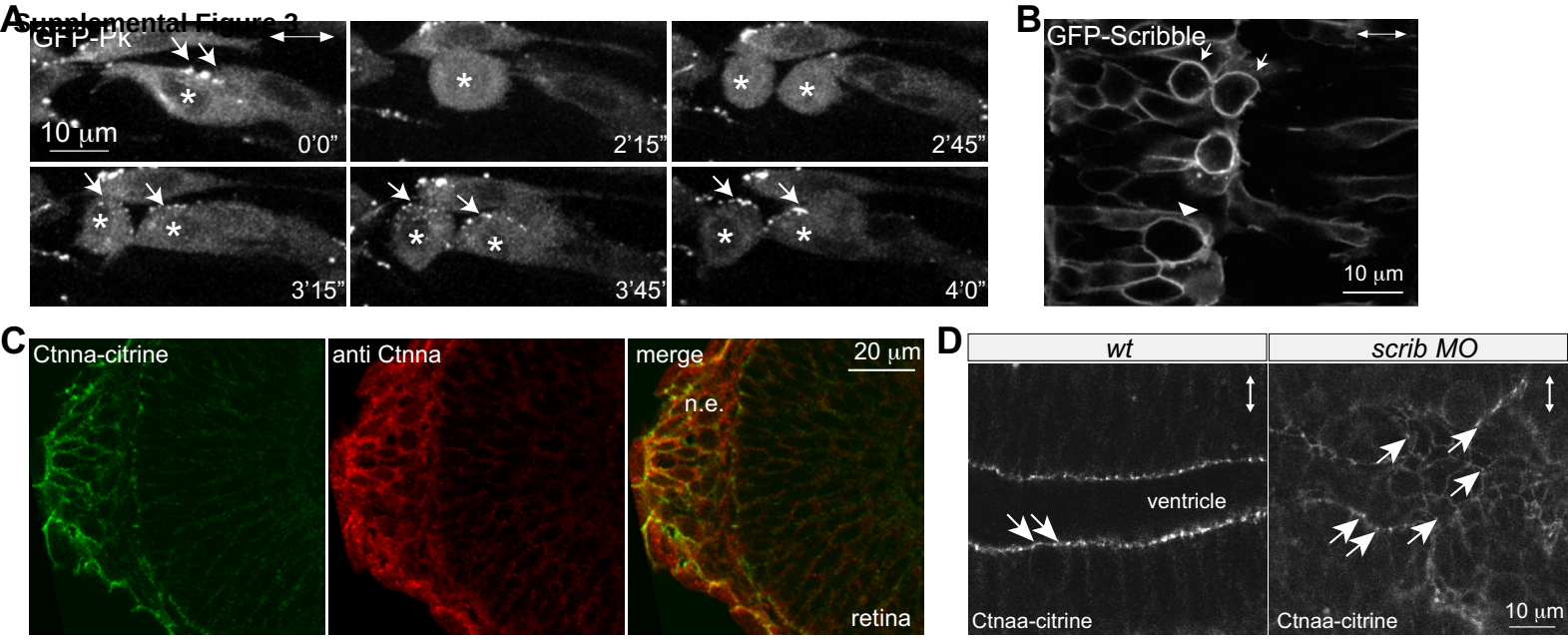




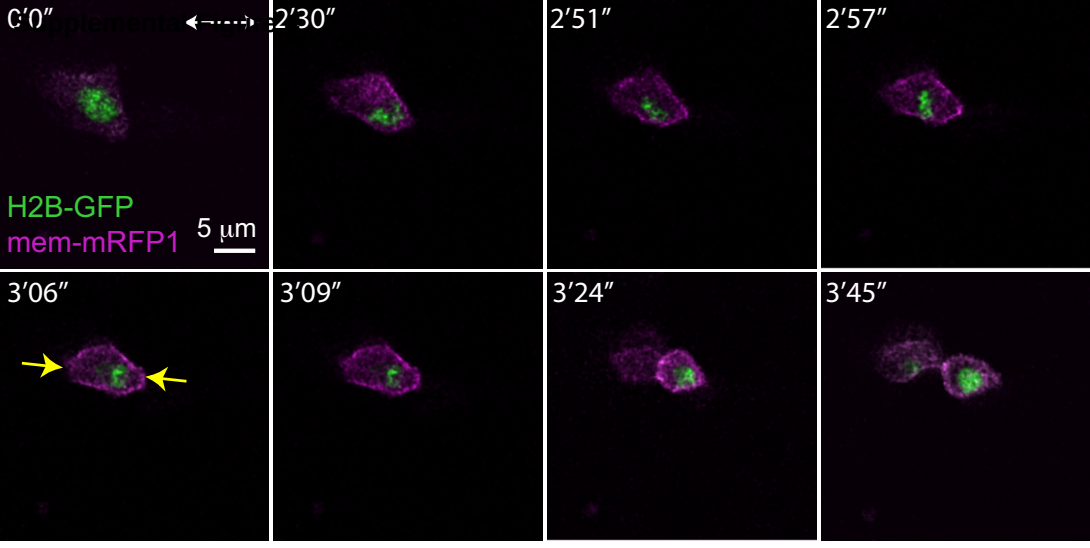




Supplementary Fig.2, Zigman et al.



Supplementary Fig.3, Zigman et al.



Supplementary Fig.4, Zigman et al.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Zebrafish lines

The *scrib*$^{-/-}$ and maternal-zygotic *mzscrib* allele used was originally described as *llk*^{rw16} [13]. Since *mzscrib* mutants were phenotypically indistinguishable from *scrib* morphants, the *scrib* morpholino [13] was used in some experiments. The *cdh2*$^{-/-}$ allele was originally described as *glo*^{m117} [41] and the *vangl2*$^{-/-}$ mutant was described as *tri*^{m209} allele [42]. The *pard6gb*^{fh266} mutant was generated by TILLING as described [43]. To visualize chromosomes we used the previously decribed *Tg(h2a.f/z-GFP)* line [44].

The *GT(ctnna-citrine)*^{ct3a} line was isolated in a gene trap screen in which an artificial internal exon encoding citrine (variant of yellow fluorescent protein, YFP) flanked by a splice acceptor and donor was integrated randomly throughout the genome via Tol2 transposition. In the *ctnna-citrine* line, the citrine exon is inserted between exons 7-8 of the α-catenin (*ctnna*) gene.

RNA expression constructs

Capped sense RNA was synthesized and 1 nl was microinjected into 1, 2 or 16-cell stage embryos. To label chromatin we used Histone2B-GFP mRNA at 25 ng/µl, for plasma membranes we injected mRNA encoding membrane-bound mRFP1 at 100 ng/µl [45]. GFP-Pk mRNA was used as described [18]. For visualization of F-actin the calponin homology domain of utrophin fused to mCherry (Utr-CH-RFP) mRNA was injected at 100 ng/µl [24]. For localization of Scribble, a GFP-Scrib mRNA was injected at 50-100 ng/µl [13].

Transplants

Gastrula stage transplantation [46, 47] was used both to mark cells unilaterally in *wt* and mutant embryos (Fig. 3) and to assess cell autonomy in genetic mosaics (Fig. 6). In the former case *wt* cells were transplanted into *wt* hosts and *mzscrib* cells into *mzscrib* hosts. In the latter case, cells were transplanted from *wt*, *mzscrib* or *cdh2*$^{-/-}$ mutant embryos into *wt* hosts. To analyze the behavior of single transplanted cells in genetic mosaics, 2-3 donor cells were transplanted, and to analyze the behavior of larger transplanted cell communities approx. 50 cells were transplanted. To track transplanted cells, donor embryos were injected with a rhodamine dextran conjugate or membrane-mRFP1 mRNA.

Knockdown by antisense morpholino oligonucleotides

The following previously described morpholinos from Gene Tools were used: *scrib* ATG MO [13], *vangl2* [48], *cdh2* MO: [21], *dvl2* MO [17], *Pk1a MO* [49], *Pk1b I3E4* MO and *Pk1b E6I6* MO [50]. We confirmed that we could recapitulate the phenotypes described in those studies before we went on to assess cell division in the neural keel.

Cell proliferation inhibitors

Embryos were incubated in embryo medium with 150 mM aphidicolin (Sigma) and 20 mM hydroxyurea (Sigma) in 4% dimethylsulphoxide as previously described [17, 18].

Immunofluorescent labeling

The following primary antibodies were used: rabbit anti-aPKC (C-20) (Santa Cruz, 1:1000), mouse anti-ZO-1 (Zymed, 1:200), mouse and rabbit anti γ-tubulin (Sigma), rabbit phospho-Histone H3 (Ser10) (Milipore, 1:1000).

Live imaging

To image live embryos with a time series of *z*-stacks, embryos were mounted in 1.2% low-melting point agarose and analyzed on Zeiss LSM510 Meta, Zeiss

LSM5 Pascal or Nikon A1R multiphoton confocal microscope. For multiphoton measurements we used an 880 nm laser wavelength of a Chameleon laser (Coherent). Zeiss LSM, NIS-Elements Nikon software, Volocity and ImageJ were used to analyze images. To quantify the distribution of angles of mitoses first the axis of separating chromosomes at anaphase onset was determined by plotting a line in the orientation of chromosome separation and then the angle between this axis and the midline axis was measured. For quantification of mitosis rotation a line along the condensed chromosomes was manually plotted for every time point. Spindle orientation was inferred from this measurement (final spindle angle = absolute value of the angle between the chromosome plate axis and the midline axis - 90˚). Angle measurements were performed using Zeiss LSM software and plotted in Oriana software.

For fluorescence intensity measurements (in Fig.5B'') 0.5μm^2 squares were laid over different cortical positions covering the cell cortex, and the ratio between the equatorial and other cortical positions was measured. The equatorial positions of the cortex (as in Fig.5B' and Fig.5B'' measurements) were defined as the regions displaying the highest focal Ctnna-catenin intensity levels around the cell cortex, corresponding to the subsequent cell division cleavage plane (observed at later time points).