Supplementary Figure 1

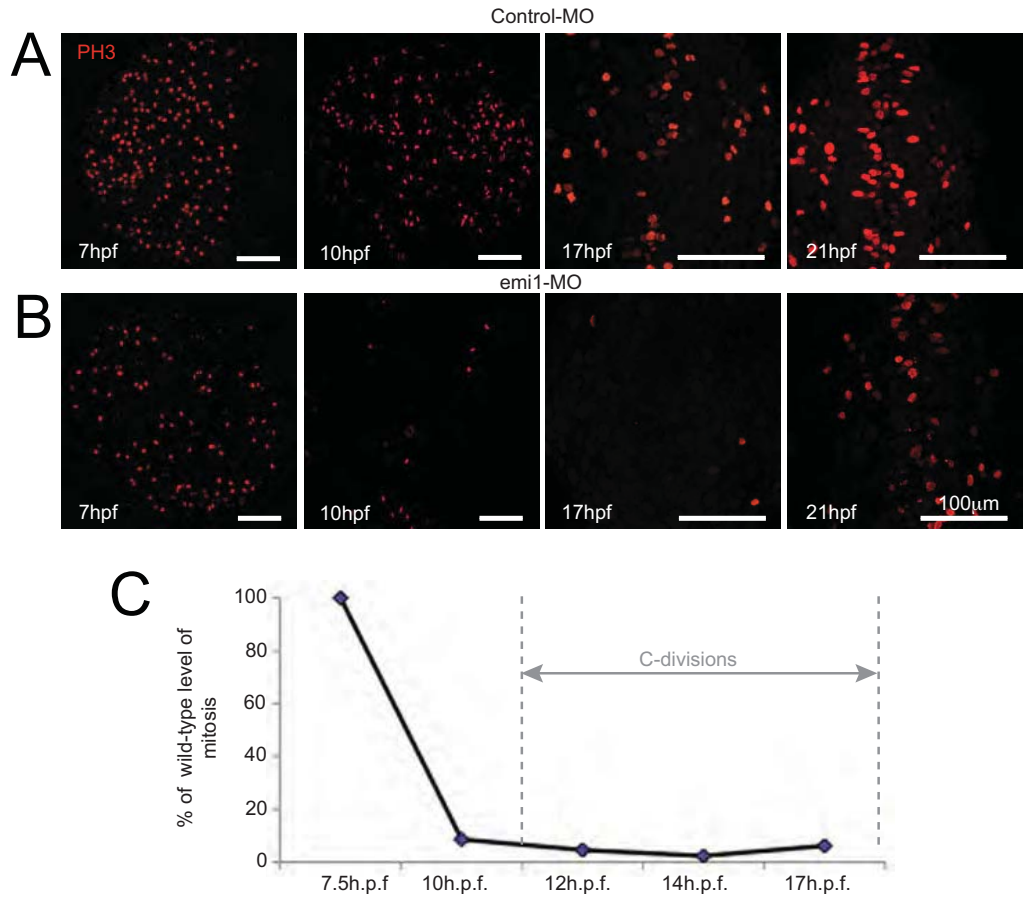

Figure S1. *Emi1* morpholino and Ap/Hu efficiently block divisions in the neural keel and rod, related to figure 2.

Horizontal midline views of control embryos (**A**) and *emi1*MO injected embryos (**B**), stained with PH3. *emi1*MO efficiently blocked divisions between 10 and 17 h.p.f., coinciding with the period of C-division (approximately 11-18 h.p.f.) without any obvious toxic effects.

C. Quantification of M-phase profiles in emi1MO embryos shown as percentage of control data. *emi1* MO reduced the overall number of mitotic cells by over 90% of control levels during the period of C-division.

Supplementary Figure 2

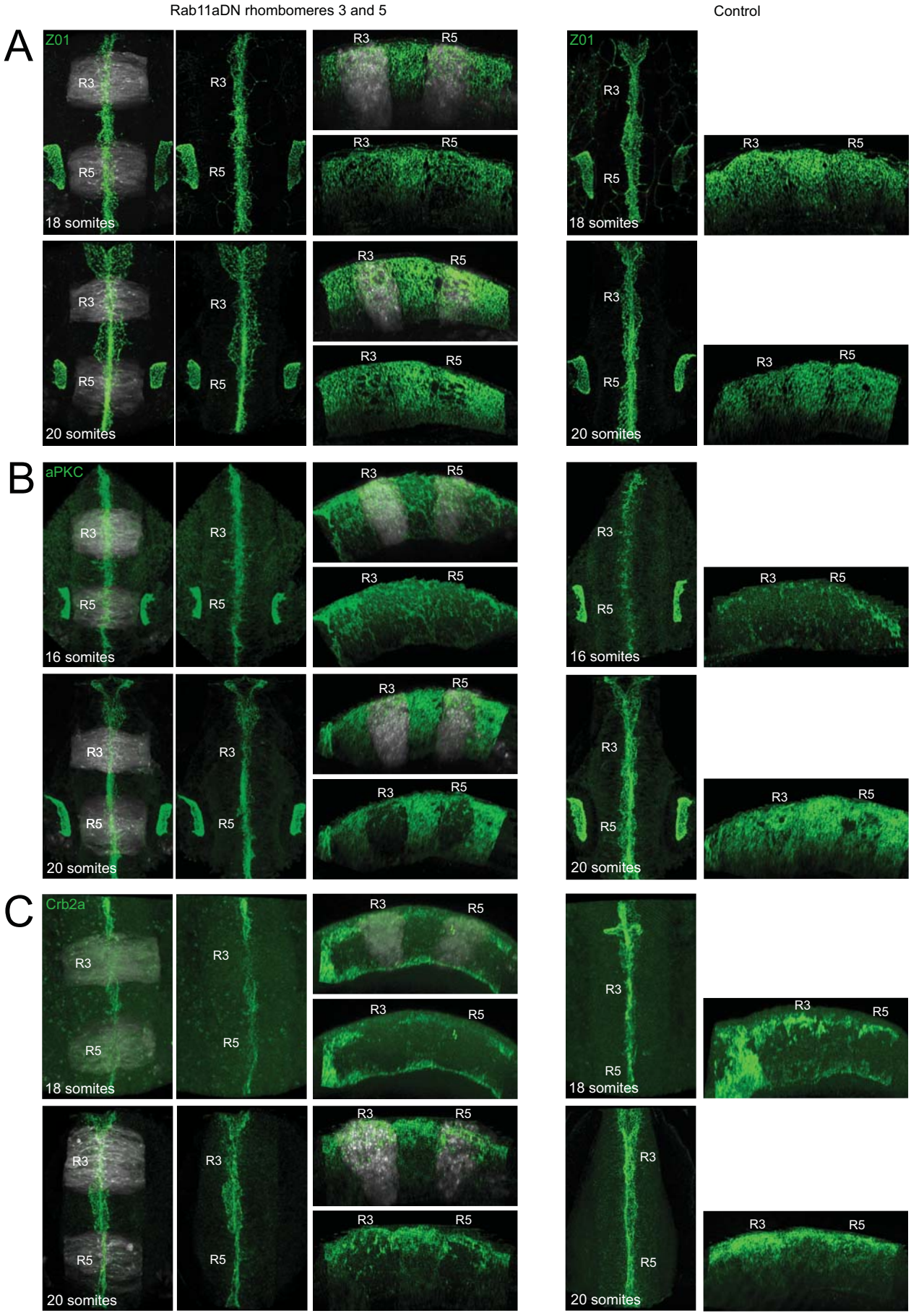

Figure S2. Rab11a is required for the maintenance of apical junctional complexes, related to figure 5.

Immuno-staining of late rod (16 or 18 somite) and early lumen opening (20 somite) stage embryos. Images are 3D reconstructions of z-stacks through the whole embryo, viewed horizontally and sagitally. *Krox20-RFP-KalTA4*x*UAS:mCherry-Rab11a-S25N* embryos, which have Rab11aDN rhombomeres 3 and 5, are shown on the left and control embryos on the right. Rhombomeres 3 and 5 are labelled 'R3, R5'. **A.** ZO1 staining is slightly more disorganised in Rab11aDN rhombomeres 3 and 5 in comparison with control embryos at the 18-somite stage. This disorganisation is more pronounced by 20 somites. **B.** aPKC staining was present at slightly lower levels in Rab11aDN rhombomeres 3 and 5 than in rhombomere 4, in comparison with control embryos, where staining between rhombomeres is more uniform across rhombomeres. However, at 20 somites there is a markedly lower level of aPKC staining in Rab11aDN rhombomeres 3 and 5 in comparison to controls and irregularly spaced clumps of staining are present dorsally. **C.** Crb2a staining was lower in dorsal DNRab11a rhombomeres at the 18-somite stage. At 20 somites Crb2a staining was present in irregularly spaced clumps, similar to aPKC staining.

Movie Legends

Movie S1. Pard3-GFP puncta localise near the tissue centre before during and after C-division, related to figure 1A. Each time-point is a projection from a small stack of confocal planes, taken from horizontal view of neural rod. Green channel is Pard3-GFP, red channel is H2B-RFP and mRFP.

Movie S2. Pard3-GFP localization to the tissue midline in division-blocked cell, related to figure 2B-D. Each time-point is a projection from a small stack of confocal planes, taken from a horizontal view of the neural rod. Green channel is Pard3-GFP, red channel is H2B-RFP and mRFP.

Movie S3. EB3-GFP polarity is mirrored around the tissue midline in two division-blocked cells, related to Figure 3C. Each time-point is a single z-plane, taken from a horizontal view of the neural rod. Green channel is EB3-GFP, red channel is H2B-RFP and mRFP.

Movie S4. EB3-GFP polarity is mirrored around tissue midline in a cell before C-division, related to Figure 3D. Each time-point is a projection of six confocal planes, taken from horizontal view of the neural rod. Green channel is EB3-GFP, red channel is H2B-RFP and mRFP.

Movie S5. RAB11A-EGFP puncta progressively distribute around the tissue midline through C-division, related to Figure 6A. Each time-point is a projection from a stack of confocal planes, taken from a horizontal view of the neural rod. A single empty frame is inserted at a gap in time-lapse collection. Green channel is RAB11A-EGFP, red channel is H2B-RFP and mRFP. Bright field is shown in grey.

Movie S6. RAB11A-EGFP puncta progressively localize to tissue midline in a division-blocked cell, related to Figure 6B. Each time-point is a projection from a stack of confocal planes, taken from a horizontal view of the neural rod. Single empty frames have been inserted at time points where we have removed frames to reduce file size. Green channel is RAB11A-EGFP, red channel is H2B-RFP and mRFP. Bright field shown in grey.

Supplementary Methods

Embryo Care

Embryos were collected, staged and cultured according to standard protocols (Kimmel et al, 1995; Westerfield, 2000). All procedures were carried out with Home Office approval and were subject to local Ethical Committee review.

Fish Lines

The Tg(UAS:mCherry-Rab11a S25N)^{mw35} line was generated according to established protocols (Clark et al, 2011; Kawakami, 2004; Kawakami, 2005). The tg(*Krox20-RFP-KalTA4*) line (Distel et al, 2009) was kindly given to us by David Wilkinson. The Gt(Ctnna-citrine)ct3a line (Zigman et al, 2011) was kindly given to us by Mihaela Zigman.

Morpholino and expression constructs.

Antisense morpholinos were made by Genetools. These were: *early mitotic inhibitor 1* (*emi1-MO*), designed against a region containing the ATG start site (5' GTA GTT TGG ACA CTT CAT ATT GAGG 3'), Laminin C1 (5′-TGTGCCTTTTGCTATTGCGACCTC-3′) (Parsons et al, 2002), p53 (5' GCGCCATTGCTTTGCAAGAATTG 3') and standard control (control-MO, 5'-CCT CTT ACC TCA GTT ACA ATT TATA 3'). pCS2+ vectors containing cDNA for the following genes were linearised and mRNA synthesised with Ambion mMessage mMachine System from the sp6 promoter (AM1340): *partitioning defective 3-GFP* and *RFP* from zebrafish (*pard3-GFP* and *pard3-RFP),* a mutant version of this gene, *pard3-Δ6-EGFP, CENTRIN-GFP* and *RFP* from human*, end binding protein 3-GFP* from human *(EB3-GFP), rab protein 11a-EGFP* from human *(RAB11A-EGFP),* the dominant-negative form of this protein (*RAB11A-S25N-EGFP), histone H2B-RFP* from human *(H2B-RFP), CHERRY-CAAX* from human *(CH-CAAX or mRFP), or GPI anchored GFP (mGFP), GFP-Zonula Occludens* from human (*GFP-ZO1*). *Doublecortin-GFP* from human *(DCX-GFP)* DNA was expressed from a Tol2 transposon element vector (pT2KXIGΔin).

Embryo Injections

Embryos were injected using standard injection protocols (Westerfield, 2000). For ubiquitous distribution of mRNA and Morpholino oligonucleotides, embryos were injected at a 1 to 4-cell stage. In order to image individual cells, embryos were mosaically labelled by injecting mRNA into 1 blastomere of a 32 to 128-cell stage embryo. DNA constructs were injected at an early 1-cell stage, within 30 minutes of egg laying. *pard3* and *CENTRIN* construct mRNA was injected at 50 to 100pg per embryo. *pard3-Δ6-EGFP* mRNA was injected at 300pg per embryo. *mGFP*, *mRFP, CH-CAAX* and *H2B-RFP* were injected at 50-100pgs per embryo. *EB3-GFP* mRNA was injected at 10-30pg per embryo. *RAB11A-EGFP* and *RAB11A-S25N-EGFP* were injected at 50pg per embryo. *GFP-ZO1* was injected at 100-150pgs per embryo. To label cells with the microtubule-associated protein DOUBLECORTIN-GFP (*DCX-GFP*) (Francis et al, 1999; Gleeson et al, 1999), 20pg plasmid DNA was injected at the 1 cell stage.

Immunohistochemistry

For immunostaining, embryos were fixed with 4% paraformaldehyde for 2 hours at room temperature. We used antibodies against the following proteins: ZO1 (mouse monoclonal, Invitrogen 33-9100, 1:500 dilution), Phospho-Histone-H3 (PH3, rabbit polyclonal, Upstate (Millipore) 06-570, 1:500 dilution), PKCζ (aPKC, rabbit polyclonal, Santa Cruz sc-216, 1:300 dilution), Crb2a (mouse monoclonal, ZIRC zs-4, 1:200 dilution). Appropriate alexa conjugated secondary antibodies (Invitrogen) were used at a 1:500 dilution. Nuclei were counter stained with sytox nuclei stains (Invitrogen) at a 1:10,000 dilution for 1 hour after secondary antibody incubation.

Time-lapse confocal imaging and processing

Embryos were mounted in low melting point agarose and confocal time-lapse movies were made at 28.5°C as previously described (Tawk et al, 2007) using a Leica SP5 confocal and water dipping x20, x25, x40 or x65 objectives. Data was collected from the hindbrain and anterior spinal cord regions. Some adjacent cells have been removed from the images to increase clarity of the cells of interest. Images were processed using Volocity and ImageJ.

Supplementary references

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