Figures and figure supplements

Inter-dependent apical microtubule and actin dynamics orchestrate centrosome retention and neuronal delamination

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Figure 1. Characterisation of the sub-apical microtubule architecture. (A) Representative image of a 3-day-old chick embryo neural tube stained with acetylated α-tubulin, phalloidin and N-Cadherin. (A’–A’’’’) Magnification of the boxed region in (A). (B) En face imaging of neuroepithelial end-feet with acetylated α-tubulin and IFT88. (B’–B’’’) Magnification of boxed region in (B). (C–C’) Another example as in (B’). (D–D’) End-foot stained with α-tubulin and γ-tubulin. (E) En face imaging of E12.5 mouse embryo spinal cord and cortex stained with acetylated α-tubulin and IFT88. (F) Stills of a neuroepithelial cell (dotted lines show cell outline) en face imaging from apical to more basal (left to right). Tissue explan stained for α-tubulin, N-Cadherin and phalloidin. (G) Neural progenitor cell expressing EMTB-GFP (and nuclear localised GFP from pCIG-Neurog2) imaged with SIM. The boxed region was magnified in (G’–G’’’). Three different angles off the boxed region in G generated by 3D reconstruction. (H) Diagram of microtubule

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organization at the apical end-feet and relationship with the acto-myosin ring and the AJs. For all figures, images were captured by wide-field microscopy, unless otherwise stated. Scale bars, (A) (B) (E) (G) (A’–A””) 10 μm, (B’–B’’) (C–C’) (D–D’) (F) (G’–G””) 2 μm.
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Figure 1—figure supplement 1. Actin-tubulin co-alignment at the adhesion belt level. (A) Representative en face image of apical end-feet stained with α-tubulin, phalloidin and N-Cadherin. The yellow line is a representative region to measure fluorescence intensity at a single point on the adhesion belt. (B) Line graphs of normalised fluorescence intensity across the adhesion belt at a single position as in (A). Dashed lines indicate the AJs as defined by N-Cadherin. Scale bar, 5 μm.

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Figure 2. Microtubule dynamics at the apical end-foot and alignment with the actin belt. (A) Microtubule nucleation from the centrosome. The apical end-foot outline and tracking of EB3-GFP comets over time are shown below. (B) Movement of polymerising microtubules along the actin cable. Lines Figure 2 continued on next page
Figure 2 continued

track movement of two EB3-GFP comets. (C) Microtubules nucleated from the centrosome bend and travel along the actin cable. Lines follow the movement of two EB3-GFP comets. (D) Trail tracking of EB3-GFP comets over time along the F-tractin-mKate2 belt. Three timepoints are shown. The arrowhead represents the starting point of EB3-GFP comet movement. The yellow line shows its position at different timepoints and the method for the measurement of fluorescence intensity at that particular point for both channels. (E) Example of fitted Guassian curves for the calculation of inter-peak distance between the two channels. For the purpose of this example, both fitted fluorescence intensity calculations were normalised from 0 to 1. (F) Box-plots of the microtubule (EB3-GFP)- actin (F-tractin-mKate2) inter-peak distance over time (paired t-test: tp 1 vs tp 2, p=0.84; tp 2 vs tp 3, p=0.72; tp 1 vs tp 3, p=0.96). Scale bars, (A) (B) (C) 2 μm.

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Figure 3. Effects of small molecule treatments on the adhesion belt and microtubules. (A – A’’, B – B’’, F – F’’, G – G’’). En face imaging of apical end-feet following treatment of chick embryo neural tube explants with Nocodazole (Noc) or Latrunculin-A (Lat-A). Boxed areas indicate how a line is drawn across the adhesion belt for measurement of fluorescence intensity. Letters next to the boxes refer to the corresponding line graphs. (C, D, H, I) Line graphs of normalised fluorescence intensity across the adhesion belt. For Nocodazole a distance of 4 μm and for Latrunculin-A 2 μm was measured. Boxed area represents the adhesion belt and the letter refers to the box plot quantifications from that area. Error bars = SEM. (C’, D’, H’, I’) Box plots Figure 3 continued on next page
of the area under the curve (adhesion belt) from the line graphs. The median value, as well as the upper and lower quartiles are represented. T-test, (C) \( p < 0.0001 \) (DMSO [Nocodazole control]: 210 measurements, 8 explants in 3 experiments), (D) \( p = 0.51 \) (DMSO [Nocodazole control]: 180 measurements, 6 explants in 3 experiments; Nocodazole: 244 measurements, 8 explants in 3 experiments), (H) \( p < 0.0001 \) (DMSO [Latrunculin-A control]: 140 measurements, 5 explants in 2 experiments; Latrunculin-A: 213 measurements, 7 explants in 3 experiments) and (I) \( p < 0.0001 \) (DMSO [Latrunculin-A control]: 140 measurements, 5 explants in 2 experiments; Latrunculin-A: 213 measurements, 7 explants in 3 experiments). When the entire curve is considered in (D), the area of the Nocodazole treatment is statistically larger than that of the DMSO treatment, \( p < 0.0001 \). (E, K) End-foot area measurements for DMSO and small molecule treatments, as outlined by the N-Cadherin staining (A‴, B‴, F‴, G‴). T-test, (E) \( p = 0.73 \) (DMSO [Nocodazole control]: 276 measurements in 3 experiments; Nocodazole: 304 measurements in 3 experiments) and (K) \( p < 0.0001 \) (DMSO [Latrunculin-A control]: 222 measurements in two experiments; Latrunculin-A: 334 measurements in 3 experiments). Error bars = SEM. (J) Normalised tubulin fluorescence following DMSO or Latrunculin-A treatment. T-test, \( p < 0.0001 \) (DMSO: 110 measurements in 2 experiments; Latrunculin-A: 205 measurements in 3 experiments). Error bars = SEM, scale bars, 10 μm.

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Figure 4. Apical cytoskeletal changes in delaminating cells. (A) In cells with small apical end-feet, EB3-GFP comets still radiate towards and become closely associated with the actin cable (white arrowheads). (B) STED image of a differentiating neuron end-foot mis-expressing EMTG-GFP (green) and F-tractin-mKate2 (red). (C) Time-lapse sequence of microtubule dynamics during apical abscission. Embryo neural tubes were electroporated with EMTB-GFP (green), pCIG-Neurog2 (nuclear, green) and mKate2-GPI (red). Abscission site (white arrowheads), withdrawing apical process (white arrows), abscessed particle (yellow arrows) and apical side (white dashed line). Scale bars, (A) 2 μm, (B) 1 μm, (C) 10 μm, enlarged regions, 2 μm.

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Figure 5. Apical abscission depends on dynamic microtubules. (A) Time-lapse sequence of cell imaged in medium containing DMSO vehicle control undergoing apical abscission. (B) Time-lapse sequence of cell imaged in medium containing nocodazole. (C) Time-lapse sequence of cell imaged in medium containing taxol. Embryo neural tubes were electroporated with GFP-GPI (cell membrane, green) and pCIG-Neurog2 (nucleus, green). Here and Figure 5—figure supplement 5: Apical end process (purple arrowhead), abscission site (white arrowheads), withdrawing apical process (white arrows), abscised particle (yellow arrows) and apical side (white dashed line). Scale bars: 10 μm; enlarged region, 2 μm.

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Figure 5—figure supplement 1. Assessment of nocodazole treatment efficacy in neural tube slices. (A–B) Two examples of mitotic arrest following nocodazole treatment. Arrested cells indicated with yellow star. (C) An example of mitotic progression following DMSO control vehicle treatment. Red and yellow dashed lines indicate two daughter cells. Nocodazole or DMSO added at the beginning of imaging (0 hr 0 min). Scale bars, 10 μm.
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**Figure 5—figure supplement 2.** Assessment of taxol treatment efficacy in neural tube slices. (A–B) Two examples of mitotic arrest following taxol treatment. Arrested cells indicated with yellow star. (C) An example of mitotic progression following DMSO vehicle control treatment. Red and yellow dashed lines indicate two daughter cells. Taxol or DMSO added at the beginning of imaging (0 hr 0 min). Scale bars, 10 μm.

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Figure 5—figure supplement 3. Stathmin-GFP mis-expression results in reduced delamination. (A) Time lapse sequence of a cell mis-expressing Stathmin-GFP (green) and pCIG-Neurog2 (nuclear, green). (B) Time lapse of a cell mis-expressing GFP and pCIG-Neurog2 (nuclear, green).

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Figure 6. Coordination of sub-apical actin and microtubule dynamics. (A) Live imaging of sub-apical actin (F-tractin td-Tomato) and microtubule (EMTB-GFP) dynamics during apical abscission. (B–B’, C–C’, D–D’). Time-lapse sequences of neural tube in embryo slices electroporated with EMTB-GFP/pCIG-Neurog2/mKate2 GPI or F-tractin-mKate2/pCIG-Neurog2/GFP GPI and treated with taxol (B, C) or ML-7 (D) or control vehicle (B’, C’, D’). Abscission site (white arrowheads), withdrawing apical process (white arrows), abscised particle (yellow arrows) and apical side (white dashed line) (B’’, C’’, D’’). Line graphs of normalised fluorescence intensities of EMTB-GFP or F-tractin-mKate2 dynamics in taxol or ML-7 (grey dashed line) and their control vehicles (black dashed line), quantified for 3 hr 30 min at 30 min intervals. EMTB-GFP dynamics are significantly affected by the taxol and ML-7.

Figure 6 continued on next page
Figure 6 continued

Treatment (2-way ANOVA, p<0.001 for each of the treatments, error bars = SEM). F-actin-mKate2 dynamics are significantly affected by ML-7 treatment (2-way ANOVA, p=0.002, error bars = SEM). Black arrowhead is abscission point for controls. Scale bars, (A) 2 μm, (B–B') (C–C') (D–D') 10 μm; enlarged regions, 2 μm. Figure

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Figure 7. Drebrin localisation in the neural tube. (A) Representative image of HH17-18 chick embryo neural tube labelled with antibodies to detect drebrin and acetylated α-tubulin and stained with phalloidin. Magnified boxed region shown in A’-A’′′. End-foot of a neuroepithelial cell mis-expressing (B) Drebrin-mCherry and (B’) EMTB-GFP and stained with (B’’) phalloidin. (C) Representative line graphs of normalised fluorescence intensity at the level of the actin cable (B–B’’). (D) En face imaging of neuroepithelial end-feet electroporated with Drebrin-YFP and stained for phalloidin. Boxed areas are magnified. White arrowheads indicate Drebrin-YFP and phalloidin co-localisation. Scale bars, (A) 20 μm and boxed region 5 μm, (B) 2 μm, (C) 10 μm.

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Figure 7—figure supplement 1. Drebrin knockdown reduces the incidence of delamination. (A) Time lapse sequence of a cell mis-expressing Drebrin-shRNA (green) and pCIG-Neurog2 (nuclear, green). (B) Time lapse sequence of a cell mis-expressing Drebrin-shScrambled (green) and pCIG-Neurog2 (nuclear, green). Apical end process (purple arrowhead), abscission site (white arrowheads), withdrawing apical process (white arrows), abscised particle (yellow arrows) and apical side (white dashed line). Scale bars, 10 μm; enlarged regions, 2 μm.

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Figure 8. Centrosome translocation during apical abscission depends on actin and microtubule dynamics. (A) The centrosome (labelled with PACT-TagRFP, red) undergoes a basal translocation through a thinned region of membrane (labelled with GFP-GPI, green). (B) The centrosome (labelled with PACT-TagRFP, red) translocates through a condensed microtubule tunnel-like configuration (labelled with EMTB-GFP, green). (C–E) Time-lapse sequences of centrosome dynamics in cells imaged in medium containing ML-7 (C), taxol (D) or DMSO control (E). Embryo neural tubes electroporated with GFP-GPI/pCIG-Neurog2/PACT TagRFP. Apical end process (purple arrowhead), abscission site (white arrowheads), withdrawing apical process (white arrows), abscised particle (yellow arrows) and apical side (white dashed line). Scale bars, (A) (B) 2 μm, (C) (D) (E) 10 μm; enlarged regions, 2 μm. DOI: https://doi.org/10.7554/eLife.26215.047
**Figure 8—figure supplement 1.** Measurement of apical microtubule rim and centrosome diameter. The Z-stack section in which IFT88 appears largest was selected for measurement of acetylated tubulin ring (yellow dashed line around cell) and IFT88 labelled structure. Capped lines represent the diameter of the microtubule ring and IFT88. Scale bar: 1 μm.

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Figure 9. Compromised microtubule nucleating potential of the centrosome blocks delamination from the apical surface. (A) Green light irradiation-mediated photobleaching of PACT-KillerRed, which localises to the centrosome, is accompanied by a corresponding depletion of PACT-YFP fluorescence. Arrows point to the centrosome. (B) Photobleaching of PACT-TagRFP following green light irradiation does not result in a corresponding reduction of PACT-YFP fluorescence. Arrows point to the centrosome. (C) Time-lapse sequence of neural progenitors following CALI. Cells poised to differentiate remain attached to the apical surface. Cells were electroporated with pCIG-Neurog2, GFP-GPI and PACT-KillerRed. White arrows point to the apical tips of cells that have been subjected to CALI. (D) Time-lapse sequence of neural progenitors following the imaging regime used for CALI. Two out of three cells underwent apical abscission during the 8 hr post-irradiation imaging period. Cells were electroporated with pCIG-Neurog2, GFP-GPI and PACT-TagRFP in place of the PACT-KillerRed construct. Abscission site (white arrowheads), withdrawing apical process (white arrows), abscised particle (yellow arrows) and apical side (white dashed line). (E) Reduction in the microtubule nucleation potential (48% reduction) of the centrosome, 3 hr post-CALI. Stills of EB3-GFP comets pre- and post-irradiation of a single end-foot (en face). Scale bars, (A–D) 10 μm; enlarged regions, 2 μm, (E) 2 μm.

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Centrosome generated apical microtubules have a wheel-like configuration in neuroepithelial cells and are required to maintain adherens junctions, while an intact actin cable is required to maintain apical microtubules, adherens junctions and end-foot shape.

N-Cadherin/AJ downregulation triggers delamination.

Interdependent acto-myosin constriction and microtubule dynamics mediate condensation of the apical cytoskeleton, which acquires a tunnel-like configuration, with the centrosome at its centre.

Centrosome and ciliary membrane dissociate and the centrosome begins to transit basally, this movement relies on acto-myosin constriction and dynamic microtubules, which are nucleated by the centrosome.

Centrosome moves basally and the actin/microtubule condensation persists distal to the now retained centrosome.

Final abscission, including membrane scission, takes place, abscised particle contains ciliary and apical membrane. The actin/microtubule condensation disappears from the withdrawing cell-process tip.

Figure 10. Summary of cytoskeletal configuration and dynamic changes in the apical end-foot during neuronal delamination.
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