

Figure S1 Identification of growth cone proteins forming complexes with EB1 or EB3. Proteins from growth cone cytosol (GC) that form complexes with EB1 or EB3 in GST pull-down assays were separated by one-dimensional SDS

PAGE and stained with Coomassie blue. Molecular weight marker proteins and their weights (kDa) are shown at left. GST is a control with glutathione S-transferase alone. Arrowheads point to GST-fusion proteins or to GST.

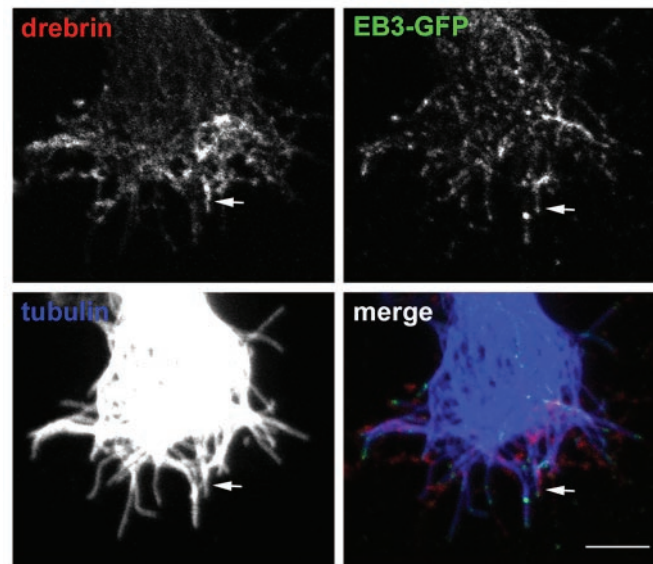


Figure S2 Co-localisation of drebrin, EB3 and microtubule plus-ends in growth cone filopodia. Following simultaneous fixation and detergent extraction, growth cones from neurons transfected with EB3-GFP were immunolabelled with anti-drebrin and anti-tubulin. EB3 is present at the

distal end of the majority of the microtubules and has a characteristic rocket-like appearance. Drebrin, presumably bound to F-actin bundles in filopodia, is occasionally seen co-localising with EB3 and dynamic microtubules (arrow). Scale, 5 μ m.

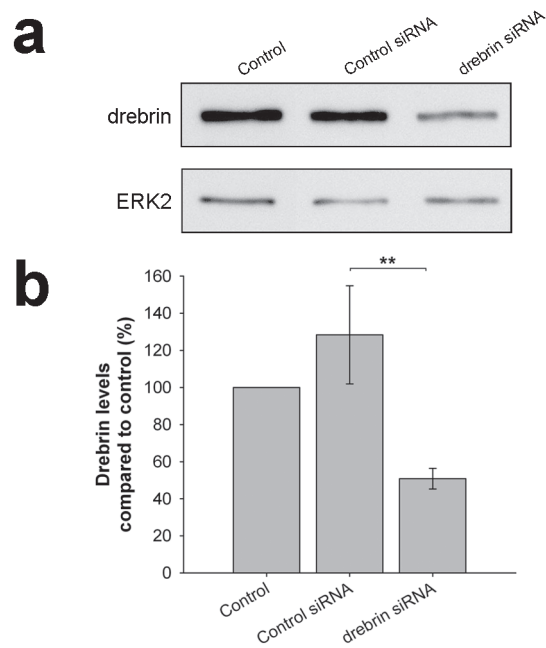


Figure S3 siRNA knockdown of drebrin in embryonic cortical cultures. **a**, Representative immunoblots from embryonic cortical cultures transfected with control siRNA or siRNA against drebrin 24 hours previously and probed with anti-drebrin and an ERK2 antibody as a loading control. Transfection with siRNA against drebrin, but not transfection with control siRNA, reduces

the levels of drebrin in cortical cultures compared to non-transfected cultures (Control). **b**, Densitometric quantification of immunoblots from cortical cultures transfected with siRNA. Blots were quantified using Phoretix software. Values are means $\pm$ s.e.m., $n = 8$. Asterisks score significant differences (unpaired Student's t-test, $**P < 0.01$).

Supplementary Movie Legends

Movie 1 Phase contrast, drebrin-YFP fluorescence and merge movies of a cerebral cortical neuron, 20 hours after transfection. Time: 60 seconds. Frame rate: 0.7 seconds.

Movie 2 Actin-GFP fluorescence, drebrin-YFP fluorescence and merge movies of a cerebral cortical neuron, 20 hours after transfection. Time: 75 seconds. Frame rate: 1.5 seconds.

Movie 3 Phase contrast, drebrin-RFP, EB3-GFP and merge movies of a growth cone from a cerebral cortical neuron, 20 hours after transfection. White arrowheads point at instances where drebrin-RFP and EB3-GFP co-localise at the base of extending filopodia. Time: 75 seconds. Frame rate: 1.5 seconds.

Supplementary Information Table 1 EB1/EB3 interacting proteins from growth cones identified by mass spectrometry

Protein	GenBank Accession No	EB1	EB3	No of independent peptides identified	Coverage
Bullous pemphigoid antigen 1b (BPAG1b)	Q91ZU6	✓	✓	71	16%
CLIP-associating protein 1 (CLASP 1)	AJ288061	✓	X	1	2%
CLIP-associating protein 2 (CLASP 2)	Q99JD4	✓	✓	43	50%
Cytoplasmic linker protein 115 (CLIP-115)	O55156	✓	✓	55	68%
Dynactin-1 (p150 ^{glued})	P28023	✓	X	33	38%
Dynein heavy chain, cytosolic (DYHC)	D13896	✓	X	2	<1%
Microtubule-actin cross-linking factor 1 (MACF)*	Q9QXZ0	✓	✓	84	21%
Microtubule-actin cross-linking factor 4 (MACF)*	XP_342907	✓	X	82	19%
Restin	NP_113933	✓	✓	19	19%
Myosin heavy chain, non-muscle type IIB	Q9JLT0	✓	✓	98	51%
Myosin heavy chain, non-muscle type 5A	Q9QYF3	✓	✓	62	40%
Myosin 18A	Q9JMH9	✓	n.d.	4	3%
Spectrin, non-erythroid α -chain (α -fodrin)	P16086	✓	✓	86	49%
Spectrin, non-erythroid β -chain (β -fodrin)	Q62261	✓	✓	67	37%
Ankyrin	Q01484	X	✓	4	3%
Drebrin (<u>D</u> evelopmentally <u>r</u> egulated <u>b</u> rain protein)	Q07266	X	✓	17	35%
Myosin heavy chain, non-muscle type IB	X68199	X	✓	20	25%

The 17 proteins identified by tandem mass spectrometry that complex with EB1 and/or EB3 in pull-down assays from growth cone cytosol are listed. Protein names are colour coded according to whether they are: known +TIPs (green), novel (blue and red) or present only in EB3 pull-downs (red). Where possible, the Accession Number is given for rat. The column labelled “Coverage” indicates the proportion of the protein that was sequenced by tandem mass spectrometry. X indicates not found. *Also known as Actin Cross-Linking Family 7. n.d.: n