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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	NIS-Elements AR 4.60, 5.11 SlideBook 6.0.18 COMSOL Multiphysics 5.3 GCC 4.8 MATLAB R2014b All custom code and scripts are available online (oddelab.umn.edu and GitHub, https://github.com/cbcbcbcb123/Growth-Cone-Dynamics) or on request from the corresponding authors.
Data analysis	ImageJ/FIJI 1.52p MATLAB R2014b, R2018b CellProfiler 2.2.0 GraphPad Prism 6.05 RStudio 1.3.1073 running R 3.5.1 All custom code and scripts are available online (oddelab.umn.edu and GitHub, https://github.com/cbcbcbcb123/Growth-Cone-Dynamics) or on request from the corresponding authors.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data supporting the findings of this study are available within the article. Numerical and visual Source Data and Supplementary Data are provided with the paper. Other raw data generated during this study are available from the corresponding authors on request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to pre-determine sample sizes, which were chosen based on our previous experience on stochastic computational modeling (e.g. Nat Commun. 2017 May 22;8:15313, Sci Adv. 2020 Mar 4;6(10):eaax1909) and in vitro work on cell adhesion and mechanobiology (e.g. Nat Commun. 2017 May 22;8:15313, J Cell Biol. 2017 Apr 3;216(4):1107-1121). For all statistical analyses, three biological replicates was chosen as a self-imposed minimum. The exact replication numbers, sample sizes and statistical methods are described in detail in the text.
Data exclusions	No data were excluded from the analyses.
Replication	All experiments were repeated successfully 2+ times to ensure reproducibility. Key results were reproduced 3+ times, using different batches of reagents and/or consumables (e.g. hydrogel substrates).
Randomization	For experiments conducted using cell lines (same clonal origin), there is no need to account for additional covariates and thus no randomization was used when allocating cells to treatment groups. For immunofluorescence analyses, several fields of view (or cells) were chosen at random and imaged at different locations within each sample.
Blinding	Researchers were unblinded for data analysis. The study did not employ visual stratification of samples to support its conclusions (e.g. evaluation of immunohistochemical stains), and quantitative image and data analyses were automated to minimize the risk of human bias.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Primary antibodies: anti-paxillin (BD Biosciences, 612405, mouse monoclonal [349], 1:200 for immunofluorescence (IF)), anti-paxillin (Santa Cruz Biotechnology, sc-5574, rabbit polyclonal, 1:200 for IF), anti-vinculin (Sigma, V9131, mouse monoclonal [hVIN-1], 1:200 for IF, 1:1000 for western blotting (WB)), anti-talin-1 (Novus Biologicals, NBP2-50320, mouse monoclonal [97H6], 1:1000 for WB), anti-talin-2 (Novus Biologicals, NBP2-50322, mouse monoclonal [68E7], 1:1000 for WB), anti-FAK (BD Biosciences, 610088, mouse monoclonal [77/FAK], 1:1000 for WB), anti-p-FAK (Y397) (Cell Signaling Technology, 8556, rabbit monoclonal [D20B1], 1:100 for IF,

1:1000 for WB), anti-MLC2 (Cell Signaling Technology, 3672, rabbit polyclonal, 1:1000 for WB), anti-p-MLC2 (T18/S19) (Cell Signaling Technology, 3674, rabbit polyclonal, 1:1000 for WB), anti-ERK1/2 (Cell Signaling Technology, 9102, rabbit polyclonal, 1:1000 for WB), anti-p-ERK1/2 (T202/Y204) (Cell Signaling Technology, 4370, rabbit monoclonal [D13.14.4E], 1:1000 for WB), anti-YAP (Santa Cruz Biotechnology, sc-101199, mouse monoclonal [63.7], 1:200 for IF), anti-vimentin (Cell Signaling Technology, 5741, rabbit monoclonal [D21H3], 1:1000 for WB), anti-GAPDH (HyTest, MAb 6C5, mouse monoclonal [6C5], 1:5000 for WB), anti-fibronectin (Sigma, F3648, rabbit polyclonal, 1:500 for IF), anti-active β1-integrin (mouse monoclonal [12G10], in-house production, 5 µg/ml for IF), anti-inactive β1-integrin (rat monoclonal [Mab13], in-house production, 10 µg/ml for cell culture), and normal IgG2a kappa isotype control (eBioscience, 14-4321-85, rat monoclonal [eBR2a], 10 µg/ml for cell culture).

Secondary antibodies: Alexa Fluor 488/568-conjugated secondary antibodies raised against mouse (Invitrogen, A21202 and A10037, 1:400 for IF) and rabbit (Invitrogen, A21206 and A10042, 1:400 for IF), IRDye 800CW donkey anti-mouse IgG (LI-COR Biosciences, 926-32212, 1:5000 for WB), IRDye 800CW donkey anti-rabbit IgG (LI-COR Biosciences, 926-32213, 1:5000 for WB), and IRDye 680LT donkey anti-mouse IgG (LI-COR Biosciences, 926-68022, 1:5000 for WB).

Validation

All antibodies were used on cells of human origin. The specificity of the β1-integrin-targeting hybridoma antibodies was validated previously using integrin activity-modulating cations: Georgiadou et al. 2017, doi: 10.1083/jcb.201609066. The following phosphorylation-specific antibodies have been validated in previous studies using specific kinase inhibitors: p-FAK (Y397), Alanko et al. 2015, doi: 10.1038/ncb3250; p-ERK1/2 (T202/Y204), Al-Akhrass et al. 2021, doi: 10.1038/s41388-020-01604-5.

Additional validation statements regarding primary antibody reactivity and applications are available on the manufacturers' websites: anti-paxillin, for IF, https://www.bdbiosciences.com/en-us; anti-paxillin, for IF, https://www.scbt.com/home; anti-vinculin, for IF and WB, https://www.sigmaaldrich.com/; anti-talin-1/2, for WB, https://www.novusbio.com/; anti-FAK, for WB, https://www.bdbiosciences.com/en-us; anti-MLC2, for WB, https://www.cellsignal.com/; anti-p-MLC2, for WB, https://www.cellsignal.com/; anti-ERK1/2, for WB, https://www.cellsignal.com/; anti-YAP, for IF, https://www.scbt.com/home; anti-vimentin, for WB, https://www.cellsignal.com/; anti-GAPDH, for WB, https://www.hytest.fi/home/; anti-fibronectin, for IF, https://www.sigmaaldrich.com/.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	U-251MG human glioblastoma cells were obtained from Dr. G. Yancey Gillespie (U. Alabama-Birmingham) (originally from American Type Culture Collection, ATCC). MDA-MB-231 human breast adenocarcinoma cells were purchased from ATCC and U-2 OS human osteosarcoma cells were acquired from German Collection of Microorganisms and Cell Cultures (DSMZ).
Authentication	U-251MG and MDA-MB-231 were authenticated using short tandem repeat assays (at the University of Arizona Genetics Core and DSMZ, respectively). The U-2 OS cells were not authenticated for this study.
Mycoplasma contamination	All cell lines were confirmed negative for mycoplasma on a regular basis.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study.