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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 Authors & Referees 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	Data collection for imaging was performed with NIS-Elements 5.02.00 (Nikon Eclipse microscope), VisiView 2.1.1 (Visitron TIRF/ epifluorescence microscope), Andor iQ 2.6 (Andor spinning-disc microscope), and iMIC Live Acquisition Software (TILL Photonics, FEI, Hillsboro, OR) ; see also the methods section 'Imaging'. Cell sorting was performed using FACS Diva 6.1.3. Design of microfluidic devices was performed with Coreldraw X8 (Corel Corporation, USA). A custom script was used to control the valves (Matlab, MathWorks, USA).
Data analysis	Analysis was performed with Fiji (ImageJ) 1.0; Matlab R2018a (MathWorks, USA); ilastik v1.1.5. Custom-made scripts for analysis (Matlab, MathWorks, USA) are available upon request. All statistical analyses were performed with GraphPad Prism 7 (GraphPad Software, USA).

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/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

Data that support the findings of this study are available within the article and its Supplementary Information. Source Data for Figs. 1, 2, 4 and Extended Data Figs 1–8, are provided with the paper. Any additional information and related data are available from the corresponding authors upon reasonable 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	We indicate in supplementary table Statistics and Reproducibility an exhaustive summary of all sample size and statistics for each experiment. No predeterminations of samples sizes were done; every experimental repetition included parallel imaging of multiple cells, resulting in samples sizes of mostly in the range of tens to hundreds.
Data exclusions	Data were only excluded in the case of microfluidic experiments based on one pre-established criterium: when microfluidic-based cell pushing was unsuccessful, such as valve leakage or high cell damage, the experiment was removed from analysis
Replication	Replications of all data was successful. All data were replicates at least 3 times, see supplementary table Statistics and Reproducibility for recapitulations of experiments.
Randomization	No randomization was performed : all cells assigned to control and experimental groups were analyzed.
Blinding	Investigators were not blinded to group allocation for majority of the study as data collection and analysis was performed by the same individual assigning the groups; however, key findings (e.g. cell velocities within different zones) were reproduced in an unbiased-(blinded) manner by the use of Fiji and custom-made analysis scripts.

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
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	Mouse Talin (anti-pan-talin, clone 8d4, T3287, Sigma-Aldrich, USA; dilution 1/200). Mouse GAPDH (clone GA1R, ab125247, Abcam, UK; dilution 1/3000). Goat anti-mouse IgG HRP conjugate (170-6516; Biorad, USA; dilution 1:5000)
Validation	All antibodies represent commercial standard validated antibody markers, validation data are available on vendor websites. https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Datasheet/1/t3287dat.pdf https://www.abcam.com/gapdh-antibody-ga1r-loading-control-ab125247.pdf https://www.bio-rad.com/webroot/web/pdf/lsr/literature/LIT372.pdf

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	The LMR7.5 T cell hybridoma was a gift from A.M. Lennon-Duménil (Institut Curie, Paris, France) and created by N. Glaichenhaus (Université Côte d'Azur, Nice, France). The Lenti-X™ 293T Cell Line was obtained from TBUSA, formerly known as Clontech Laboratories, Inc. (632180). PLB-985 cells were obtained from the DSMZ (ACC 139)

Authentication	Cell line authentication was performed on the Lenti-X™ 293T by the seller. For the LMR7.5 T cell hybridoma, RNA sequencing was performed. PLB-985 (ACC-139) was validated by the DSMZ (cell surface markers, DNA fingerprint).
Mycoplasma contamination	All cell lines were tested and negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly mis-identifed cell line were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	6–12-week-old, male or female WT or Lifeact-GFP-expressing C57BL/6J mice were used for (1) primary T cell selection from lymph nodes and spleen, and (2) dendritic cell differentiation from bone marrow progenitors.
Wild animals	The study did not involved wild animals.
Field-collected samples	The study did not involve sample collection from the field.
Ethics oversight	All animal experiments at IST Austria were in accordance with Austrian law for animal experiments. Permission was granted by the Austrian Federal Ministry of Science, Research and Economy (identification code: BMWF-66.018/0005-II/3b/2012).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	All cell suspensions were filtered through a cotton mesh to remove cell aggregates and clumps, no staining was performed
Instrument	FACS Aria III was used for cell sorting (with nozzle 85µm and laser lines 488 and 561nm).
Software	Data were collected with FACS Diva 6.1.3.
Cell population abundance	Lifeact-GFP or Lifeact-mCherry positive cells were collected.
Gating strategy	Doublets and debris were excluded based on FSC and SSC characteristics.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.