nature portfolio

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Reporting Summary

Nature Portfolio 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 Portfolio 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	ImageJ (Version 1.52a), MATLAB 2019a for projection of 3D images, preparation of videos, 3D slice view
Data analysis	GraphPad Prism 9, Version 9.5.1, GraphPad Software, Inc., Seurat 4.3.0, TFMLab Gitlab repository: https://gitlab.kuleuven.be/MAtrix/Jorge/tfmlab_public, MATLAB 2019a, Abaqus Simulia 2017. The custom codes used for image analysis and cell-cell force quantifications have been published to Zenodo and cited in the Code Availability Statement. They can be accessed at https://doi.org/10.5281/zenodo.13773083.

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 Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Single-cell RNA sequencing row datasets generated in this study have been deposited in the Gene Expression Omnibus repository under the accession series number GSE246174 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc= GSE246174]. Supplementary Information is available for this paper as a separate document. All data generated in this study are provided in the Supplementary files and the Source Data file. Raw microscopy data files used for a subset of non-

mosaic and mosaic 3D TFM trials have been published on Zenodo and can be processed using the open-source TFMLAB code to generate the results presented here. hey can be accessed at https://doi.org/10.5281/zenodo.13773083.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.* *Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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

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	Experiments were conduced with a minimum of two independent experiments and multiple biological replicates. For scRNAseq, we pooled cells from independent trials and three technical replicates. For all other experiments in PEG hydrogels after angiogenic invasion, one dish with an endothelial monolayer of 40,000 HUVECs invading the gel were considered one independent experiment when performed in independently prepared gels and independently prepared cells. Within each hydrogel, we obtained atleast 10 replicates. These replicates represented the biological variability within a hydrogel of the invading endothelial cells. We relied on our previous experience to determine these samples sizes which we deemed sufficient as they accounted for the variability inherent to pooled HUVECs and led to reproducible results. Our sample size was enough to emphasize differences between conditions of siRNA transfected cells as well as wild-type cells.
Data exclusions	3D traction force microscopy experiments are sensitive to any stage shifts and noise that are an outcome of microscopy temperature shifts or movements. We always ensured that the regions away from cells did not show spurious displacements making the given hydrogel sample unreliable as we wanted accurate assessments of only cell-induced displacements of the matrix. If such spurious noisy results were obtained, we excluded such data. For the scRNAseq analysis : cells were excluded from the analysis when the level of genes rising from the mitochondrial genome exceeded 15%. Additionally cells with fewer than 200 (low quality) and more than 7,500 (potential doublets) detected genes were also filtered out. This criteria is based on standard established protocol in the field of transcriptomic analysis and is was only applied to the scRNAseq experiment.
Replication	Data was replicated using a minimum of two and a maximum of 4 independent experiments to ensure reproducibility. The phenotypical differences between the mutant and control cell types were distinctly visible and served as a clear validation of the reproducibility of the experimental protocols on invasive behavior. All replicates were successful except for the third replicate of the siCCM2+siROCK2 treatment for staining with anti-MMP14 due to technical difficulties that spoiled the sample. For samples analyzed for filopodia and actin intensity quantifications, 2 independent trials were performed to minimize variability for comparing with previously run experiments a significant time apart. However, these results are highly reproducible which makes us confident in the quantifications.

Randomization	The culture and siRNA transfection conditions, PEG invasion assay preparation times, 3D TFM imaging times and overnight imaging times were kept constant to eliminate sample-specific characteristics that can impose variations in our data - this allowed us to reliably interpret the results and attribute them to the specific cell treatment or treatment. For selecting replicated within a given endothelial monolayer invading a given hydrogel, we imaged 10 randomly chosen locations from left to right of the monolayer ensuring a sample from each curvature of the monolayer to limit any location dependent effects.
Blinding	Investigators were not blinded to group allocation.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used	Anti-activated β1 integrin clone 9EG7 (BD Biosciences #553715, 1:100) Recombinant anti-MMP14 antibody EP1264Y (abcam #ab51074, 1:100) Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488 (Invitrogen # A-11008, 1:500) Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 546 (Invitrogen # A-11081, 1:500) Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647 (Invitrogen # 21247, 1:500) Phalloidin–Atto 647N (Sigma Aldrich #65906, 1:1000)
Validation	For antibody specificity validation, we refer to the suppliers's datasheets provided on the websites for statements on specificity and validation. β1 integrin antibodies have also been used in our previously published work mentioned below, while anti-MMP14 antibody has been used in several studies related to invasion. β1 integrin close 9EG7: Faurobert, et al. Journal of Cell Biology 202.3 (2013): 545-56 Macek Jilkova et al. Biology open 3.12 (2014): 1228-1235. Vannier, et al. Angiogenesis 24.4 (2021): 843-860. Recombinant anti-MMP14 antibody EP1264Y: Petropoulos, et al. Journal of Cell Biology 213.5 (2016): 585-599. Mori, et al. Development 140.2 (2013): 343-352.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Commercially available pooled human unbilical vein endothelial cells (HUVECs (cAP-0001)) were purchased from Angioproteomie and expanded based on protocols described in methods.
Authentication	Cell lines weren't used and did not require authentication as they were commercial HUVECs and we relied on supplier's data sheets and validation.
Mycoplasma contamination	All cells tested negative for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines listed by ICLAC were used in this work

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.