

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](#).

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
- ☐ ☒ An indication of 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 statistics 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
- ☐ ☒ Clearly defined error bars
State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on [statistics for biologists](#) may be useful.

Software and code

Policy information about [availability of computer code](#)

Data collection

NIS elements software (Nikon), LCS software (Leica) and LSM (Zeiss).

Data analysis

Patch Morphology Analysis Dynamic software (Digital Cell Imaging Laboratories, Belgium) was used for analysis of static (fixed) and dynamic (live) cell imaging data in most cases. FIJI was used for analysis of mitotic axis alignment. KNIME (v3.6.0) was used for analysis for siRNA- as well as PIP and Arp2/3 drug-responses. NIS elements was used for 3D / 4D image data rendering. R (v3.5.1) was used for statistical data analyses and visualisation via RStudio (v1.1.453), although some analyses and graphical outputs were generated directly within Excel. STORM data were analysed using Insight3 software. Mass spectrometry quantification was performed using Progenesis LC-MS software (Progenesis Q1, Nonlinear Dynamics, Newcastle, UK).

R code as well as Knime image analysis and data integration workflows are available via the GitHub repository: <https://github.com/locus/lock-et-al-NCB-2018-Reticular-Adhesions-Data-and-Analysis-Repository>

This repository also includes all quantitative source data (as presented in Supplementary Table 1) and sample image data from Arp2/3 drug inhibition studies (for use in Knime image analysis workflows). Instructions as to the content and use of this repository are included in an associated .pdf file "Instructional Workflow for Data Exploration and Reproduction.pdf".

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 upon request. 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 mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifiers PXD008645 and PXD008680.

Quantitative data underlying graphical figure panels are available via the GitHub repository: <https://github.com/locusJ/Lock-et-al-NCB-2018-Reticular-Adhesions-Data-and-Analysis-Repository>

in the file "Supplementary Table 1 Statistics Source Data.xlsx"

Figures with associated raw quantitative data include: 1A; 1H; 2A; 2B; 2C; 2D; 2N; 2S; 2T; 2V; 2W; 3C; 3D; 3E; 3H; 3I; 5B; 5C; 5D; 6A; 6B; 6M; 6N; 7E; 7F; 8A; 8B; 8C; Supp F5C; Supp F6A.

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Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf](https://www.nature.com/authors/policies/ReportingSummary-flat.pdf)

Life sciences study design

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

Sample size	Reticular adhesions are previously uncharacterised, in terms of their morphology, dynamics, content and functional influence. Therefore, no prior knowledge existed to guide prediction of sample sizes that might optimally mediate comparisons between reticular and classical adhesion characteristics. For this reason, sample sizes were based on standards in the field (such as for FRAP analysis), or based on the number of cells / adhesions accessible through automated imaging and analysis (where sample number typically exceeds current gold standards). At no time were the results of statistical comparisons (e.g. Mann-Whitney U test) used to motivate additional experiments aiming to achieve statistical significance.
Data exclusions	As described in text, 4 proteins detected in raw mass spectrometry data were excluded due to over-representation in a database of common contaminant proteins (CRAPome database, Mellacheruvu et al., 2013).
Replication	All experiments were successfully replicated. The number of replicates is specified for each experiment.
Randomization	Sample randomization was not relevant to this study because analysed populations were assigned to classes based on objective measures of identity (e.g. reticular versus classical adhesions defined by consistent quantitative criteria).
Blinding	Analysis of mitotic division axis alignment was manual and therefore computationally blinded. All other analyses were automated and therefore non-blinded.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

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

Antibodies

Antibodies used	Primary antibodies used are described in Supplementary Table 4. Anti-mouse and anti-rabbit secondary antibodies conjugated with Alexa 488, 568 or 647 were used as appropriate (Thermo Fisher Scientific). For fixed F-actin labelling, phalloidin pre-conjugated with Alexa 488, 568 or 647 was used as appropriate (Thermo Fisher Scientific). DAPI (4', 6-Diamidino-2-Phenylindole, Dihydrochloride; Thermo Fisher Scientific) nucleic acid stain was used as a nuclear marker as appropriate.
Validation	Details per Ab provided in Supplementary Table 4.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	All cell lines were acquired directly from either ATCC or ECACC, with the exception of H1299, CS-1 and talin 1 null mouse embryonic stem cells.
Authentication	Cell lines acquired directly from either ATCC or ECACC have been authenticated by the provider. H1299, CS-1 and talin 1 null mouse embryonic stem cells were not authenticated.
Mycoplasma contamination	All cell lines were tested and found to be negative for mycoplasma
Commonly misidentified lines (See ICLAC register)	No cell lines used in this study were found in the database of commonly misidentified cell lines (v8.0) that is maintained by ICLAC and NCBI Biosample