

Corresponding	g author(s	s): Clare	M. Waterman
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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 <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main

## Statistical parameters

text	, or I	Methods section).
n/a	Coi	nfirmed
	$\boxtimes$	The $\underline{\text{exact sample size}}(n)$ for each experimental group/condition, given as a discrete number and unit of measurement
	$\boxtimes$	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.
X		A description of all covariates tested
	$\boxtimes$	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
		A full description of the statistics including <u>central tendency</u> (e.g. means) or other basic estimates (e.g. regression coefficient) AND <u>variation</u> (e.g. standard deviation) or associated <u>estimates of uncertainty</u> (e.g. confidence intervals)
		For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
$\times$		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
$\boxtimes$		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
$\boxtimes$		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 <u>statistics for biologists</u> may be useful.

### Software and code

Policy information about availability of computer code

Data collection Data was collected using MetaMorph 7.7 (Meta Imaging), NIS Elements 5.02 (Nikon) and DeltaVision OMX AcquireSR 4.4.9800-1 (GE) softwares.

Data was analyzed using ImageJ 1.52 (NIH), MetaMorph 7.7 (Meta Imaging), MATLAB R2016a, QFSM v1.1.0 (Danuser lab), DeltaVision SoftWoRx 7.0.0 (GE), Excel v.1808 (Microsoft) and Prism 7 (GraphPad) softwares.

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

Data analysis

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

All data that support the findings of this study are available from the corresponding author upon reasonable request.

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Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.						
\(\sum_{\text{Life sciences}}\)	Behavioural & social sciences Ecological, evolutionary & environmental sciences					
For a reference copy of	For a reference copy of the document with all sections, see <u>nature.com/authors/policies/ReportingSummary-flat.pdf</u>					
Life scier	nces study design					
All studies must dis	sclose on these points even when the disclosure is negative.					
Sample size	No statistical method was used to predetermine sample size. Number of analyzed cells or measurements were chosen to equal or exceed typical standards of the field.					
Data exclusions	No data were excluded from the analyses					
Replication	All results were reproduced at least three times. All attempt for replication were successful and all experiments can be reproduced.					
Randomization	Experiments were not randomized, but independent cultures or passages were used for each independent repeat and done on different days.					
Blinding	Investigators were not blinded during data acquisition. Analysis of fluorescence intensity and quantification of particle uptake was performed					

## Reporting for specific materials, systems and methods

Ma	terials & experimental systems	Methods		
n/a	Involved in the study	n/a	Involved in the study	
$\boxtimes$	Unique biological materials	$\boxtimes$	ChIP-seq	
	Antibodies	$\boxtimes$	Flow cytometry	
	Eukaryotic cell lines	$\boxtimes$	MRI-based neuroimaging	
$\boxtimes$	Palaeontology		•	
	Animals and other organisms			
$\boxtimes$	Human research participants			

by software, independently of human factors.

### **Antibodies**

Antibodies used

- 1) Anti CD11b, clone M1/70, BD Pharmingen, cat no. 553309 (biotin), diluted at 10  $\mu$ g/mL
- 2) Anti CD11b, clone M1/70, BD Pharmingen, cat no. 553307 (No azide/low endotoxin), diluted at 10  $\mu$ g/mL
- 3) Rat IgGb2 isotype control, BD Pharmingen, cat no. 553987 (biotin), diluted at 10  $\mu$ g/mL
- 4) Rat IgGb2 isotype control, BD Pharmingen, cat no. 553985 (No azide/low endotoxin), diluted at 10 µg/mL
- 5) Anti CD18, clone GAME-46, BD Pharmingen, cat no. 553341 (No azide/low endotoxin), diluted at 10 µg/mL
- 6) Anti Syk pY348 (Y342 in mouse), BD Pharmingen, cat no. 558167, 1:200 dilution
- 7) Anti Vinculin, clone hVIN-1, Sigma-Aldrich, cat no. V9131, 1:500 dilution for IF, 1:2000 dilution for WB
- 8) Anti alpha-actinin, clone EA-53, Sigma-Aldrich, cat no. A5044, 1:500 dilution
- 9) Anti phosphotyrosine, clone 4G10, EMD Millipore, cat no. 05-321, 1:300 dilution
- 10) Anti phospho-FAK Y397, clone 141-9, Invitrogen, cat no. 44625G, 1:100 dilution
- 11) Anti phospho-Paxillin Y31, Invitrogen, cat no. 44720G, 1:100 dilution
- 12) Anti phospho-Paxillin Y118, Invitrogen, cat no. 44722G, 1:100 dilution
- 13) Anti Zyxin rabbit polyclonal antibody B71 was obtained form Mary Beckerle, 1:600 dilution
- 14) Anti iC3b, clone 013III-1.16, GeneTex, cat no. GTX40522, 1:2000 dilution
- 15) Anti sheep RBC rabbit IgM, MyBioSource, cat no. MBS524107, 1:200 dilution
- 16) Anti sheep RBC rabbit IgG, MP Bio, cat no. 55806, 1:500 dilution
- 17) Anti 1,3 beta glucan, clone 2G8, Abcam, cat no. ab233743, 1:200 dilution
- 18) Anti-bovine serum albumin, Abcam, cat no. ab186531, 1:100 dilution
- 19) Anti Syk, clone D3Z1E, Cell Signaling, cat no. 13198S, 1:1000 dilution
- 20) Anti GAPDH, clone D16H11, Cell Signaling, cat no. 5174, 1:5000 dilution

Validation

1-2) Developed and validated in: Ault KA, Springer TA. Cross-reaction of a rat-anti-mouse phagocyte-specific monoclonal antibody (anti-Mac-1) with human monocytes and natural killer cells. J Immunol. 1981; 126(1):359-364

- 3-4) Validated by BD Pharmingen. The A95-1 antibody has unknown specificity and was selected as an isotype control following screening for low background on a variety of mouse and human tissues.
- 5) Developed and validated in: Zahalka MA, Okon E, Naor D. Blocking lymphoma invasiveness with a monoclonal antibody directed against the beta-chain of the leukocyte adhesion molecule (CD18). J Immunol. 1993; 150(10):4466-4477.
- 6) Validated by BD Pharmingen for western blot, intracellular staining and immunohistochemistry-formalin.
- 7) Validated by Sigma-Adrich for western blot, indirect immunofluorescence and immunohistochemistry.
- 8) Validated by Sigma-Adrich for western blot, indirect immunofluorescence and microarray.
- 9) Validated by EMD Millipore for western blot, immunocytochemistry, immunohistochemistry and immunoprecipitation. 10-12) Validated by Invitrogen for western blot, immunofluorescence, immunocytochemistry, immunohistochemistry and immunoprecipitation.
- 13) Developed and validated in: Hoffman LM, Nix DA, Benson B, Boot-Hanford R, Gustafsson E, Jamora C, Menzies AS, Goh KL, Jensen CC, Gertler FB, Fuchs E, Fässler R, Beckerle MC. Targeted disruption of the murine zyxin gene. Mol Cell Biol. 2003 Jan;23(1):70-9.
- 14) Validated by GeneTex for western blot, immunohistochemistry, flow cytometry and ELISA.
- 15) Validated by MyBioSource for complement fixation and complement dependent haemolytic studies using sheep red blood cells as targets.
- 16) Validated by MP Bio for immunoassays, immmunoelectrophoresis and immunohistochemistry.
- 17) Validated by Abcam for western blot, immunocytochemistry, immunoprecipitation and ELISA.
- 18) Validated by Abcam for western blot, immunocytochemistry and ELISA.
- 19) Validated by Cell Signaling for western blot, immunoprecipitation, immunohistochemistry and immunofluorescence
- 20) Validated by Cell Signaling for western blot, immunohistochemistry and immunofluorescence

## Eukaryotic cell lines

Policy information about cell lines

Cell line source(s) RAW 264.7 and THP-1 cell lines were obtained directly from the American Type Culture Collection.

Authentication Cell lines were not independently authenticated.

Mycoplasma contamination Cell lines were routinely tested for mycoplasma and were certified to be negative.

Commonly misidentified lines (See ICLAC register)

No cell lines used in this study were found in the database of commonly misidentified cell lines.

### Animals and other organisms

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

Laboratory animals Female, 10 to 20 weeks old, C57BL/6 LifeAct-GFP mice were used to prepare bone marrow-derived macrophages.

Wild animals No wild animals were used in this study.

Field-collected samples No field-collected samples were used in this study.