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Corresponding author(s):	Witold K. Surewicz
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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>.

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St	at.	ıct.	ics

all st	atistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
Cor	nfirmed
\boxtimes	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
\boxtimes	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
\boxtimes	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
1	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

Tecan SPARKControl (ver. 2.3), BZ-X Viewer (Keyence, ver. 1.3.1.1), Malvern Zetasizer Software (ver. 7.13), Leica Application Suite X (3.3.0.16799), www.chemspider.com; https://pubchem.ncbi.nlm.nih.gov; ChemDraw Professional (ver. 17.0)

Data analysis

BZ-X Analyzer (Keyence, ver. 1.3.1.1), Excel (ver. 2013, 2017), Origin (ver. 2017, 2019), ImageJ (ver. 1.53c)

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 $% \left(1\right) =\left(1\right) \left(1\right) \left($
- A description of any restrictions on data availability

The data generated in this study are available from the corresponding authors upon reasonable request. The source data underlying Fig. 1-7 and Supplementary Fig. 1,2,4-8 are provided as a Source Data file.

Field-specific reporting					
Please select the or	ne below tha	t 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			
For a reference copy of t	he document wi	th all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>			
Life scier	nces st	tudy design			
All studies must dis	close on the	se points even when the disclosure is negative.			
Sample size	performing s	analysis was used to predetermine sample size because no group comparisons were made. Consistent with prior studies imilar assays (PMID 28819146, PMID 32499559, PMID 27545621, PMID 30814253), each experiment was performed with a three technical replicates.			
Data exclusions	Data were no	ot excluded from the analyses.			
Replication	Reproducibility was validated by repeating experiments using independent sample preparations (as noted in figure legends, where rele addition to technical replicates for each individual preparation. For recombinant protein studies, independent preparations are defined separate purification batches and, for cellular studies, independent preparations (biological replicates) are defined by separate passage cells.				
Randomization	Randomization Randomization was not relevant to experiments involving recombinant protein as samples were not assigned to specific groups. For cellular studies, images were quantified in the absence of treatment group identifiers.				
Blinding	Blinding was	not applicable to this study. The authors who performed each experiment also analyzed the data.			
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 Methods n/a Involved in the study Antibodies ChIP-seq Flow cytometry Palaeontology Animals and other organisms Human research participants					
Clinical data					
Antibodies					
Antibodies used		Mouse anti-G3BP (Sigma; WH0010146M1), anti-mouse CF488 (Sigma; SAB4600237)			
Validation		For mouse anti-G3BP (Sigma; WH0010146M1), validation was performed by manufacturer and deemed "suitable" for immunofluorescence (https://www.sigmaaldrich.com/catalog/product/sigma/wh0010146m1). For anti-mouse CF488 secondary antibody (Sigma; SAB4600237), validation for immunofluorescence was performed by manufacturer (https://www.sigmaaldrich.com/catalog/product/sigma/sab4600237).			
Eukaryotic c	ell lines				
Policy information about <u>cell lines</u>					
Cell line source(s)		ATCC (CCL-247)			

Cell line source(s)

ATCC (CCL-247)

Authentication

Authentication performed by STR profiling by distributor

Mycoplasma contamination

Commonly misidentified lines (See ICLAC register)

ATCC (CCL-247)

Authentication performed by STR profiling by distributor

PCR testing indicated cell lines were free of mycoplasma contamination

No commonly misidentified cell lines were used in this study.