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Sample-size estimation

- You should state whether an appropriate sample size was computed when the study was being designed
- You should state the statistical method of sample size computation and any required assumptions
- If no explicit power analysis was used, you should describe how you decided what sample (replicate) size (number) to use

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:

This criterion is most applicable to phenotypic experiments reported in Figures 5G,H, Figure 5-figure supplement 1C,D, and Figure 6. No explicit power analysis was used to determine the number of cells scored for these experiments, and instead our sample size was guided by examples from published studies doing similar analysis of abscission defects using immunofluorescence. For Figure 5G, H and Figure 5-figure supplement 1C, D, at least 500 cells were manually scored from 5 independent replicates, making the total number of cells >2,500. This number meets and exceeds what is typically reported for this type of experiment (typically 3 replicates of ~200-500 cells each). For Figure 6, greater than 100 midbodies were inspected from each of 3 different replicates. Typical sample sizes reported from published literature for these types of experiments are between 20-50. In all cases, minimum sample sizes are reported in figure legends.

Replicates

- You should report how often each experiment was performed
- You should include a definition of biological versus technical replication
- The data obtained should be provided and sufficient information should be provided to indicate the number of independent biological and/or technical replicates
- If you encountered any outliers, you should describe how these were handled
- Criteria for exclusion/inclusion of data should be clearly stated
- High-throughput sequence data should be uploaded before submission, with a private link for reviewers provided (these are available from both GEO and ArrayExpress)

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For binding experiments reported in Figure 2 (and supplements), Figure 3, and Figure 4, each biological replicate is defined as a binding isotherm recorded from a unique preparation (and diution) of protein-peptide complexes. For experiments reported in Figure 5 (and supplements) and Figure 6, a biological replicate is defined by cells seeded and treated on separate days. The minimum number of replicates for each experiment is reported in the figure legend.

Statistical reporting

- Statistical analysis methods should be described and justified
- Raw data should be presented in figures whenever informative to do so (typically when N per group is less than 10)
- For each experiment, you should identify the statistical tests used, exact values of N, definitions of center, methods of multiple test correction, and dispersion and precision measures (e.g., mean, median, SD, SEM, confidence intervals; and, for the major substantive results, a measure of effect size (e.g., Pearson's r, Cohen's d)
- Report exact p-values wherever possible alongside the summary statistics and 95% confidence intervals. These should be reported for all key questions and not only when the p-value is less than 0.05.

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Statistical tests are described in the figure legends.

(For large datasets, or papers with a very large number of statistical tests, you may upload a single table file with tests, Ns, etc., with reference to sections in the manuscript.)

Group allocation

- Indicate how samples were allocated into experimental groups (in the case of clinical studies, please specify allocation to treatment method); if randomization was used, please also state if restricted randomization was applied
- Indicate if masking was used during group allocation, data collection and/or data analysis

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:

Microscopy data were blinded and scored as described in the Materials and methods.

Additional data files ("source data")

- We encourage you to upload relevant additional data files, such as numerical data that are represented as a graph in a figure, or as a summary table
- Where provided, these should be in the most useful format, and they can be uploaded as "Source data" files linked to a main figure or table
- Include model definition files including the full list of parameters used
- Include code used for data analysis (e.g., R, MatLab)



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Avoid stating that data files are "available upon request"

Please indicate the figures or tables for which source data files have been provided:

Source data tables have been provided for all figures with Western blots:

Figure 2 – figure supplement 6

Figure 5 – figure supplement 1

Figure 6 - figure supplement 1

Figure 6 – figure supplement 2