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☐ Initial submission   ☐ Revised version   ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

▶ Experimental design

1. **Sample size**

   Describe how sample size was determined.

   > All sample sizes are listed in detail in the figure legends and main text. The number of cells imaged are consistent with previous live cell imaging studies (and from N≥3 independent experiments).

2. **Data exclusions**

   Describe any data exclusions.

   > No data were excluded from the analyses.

3. **Replication**

   Describe whether the experimental findings were reliably reproduced.

   > All data presented were from biological replicates. All attempts at replication were successful.

4. **Randomization**

   Describe how samples/organisms/participants were allocated into experimental groups.

   > Aside from the strong premise for the proposed research, additional steps were taken to ensure rigor and reproducibility, as follows: 1) scientific questions were addressed using complementary technical approaches to ensure that the findings were robust; 2) for studies involving multiple different experimental conditions in the same cell line, studies were performed on cells originating from the same cell line batch.

5. **Blinding**

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   > Whenever possible, experimenters were blinded to condition and examiner to exclude bias. For example, for our preliminary analyses, we asked other researchers who were either involved or not involved in the study to examine blinded samples for biological effects.

   Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6.   Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	A statement indicating how many times each experiment was replicated
☐	☒	The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted
☐	☒	A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒	Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

▶ Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

> Statistics and graphing were performed using Prism 7 (GraphPad) software. All videos and images were assembled using ImageJ 1.51j8 (NIH).

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

▶ Materials and reagents

Policy information about availability of materials

8.   Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

> All unique materials used are readily available from the authors or from standard commercial sources detailed in the Methods section.

9.   Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

> Lamp1 rabbit antibody (Sigma, L1418), Tom20 mouse antibody (BD biosciences, 612278), Flag rabbit antibody (Sigma, F7425), HA rabbit antibody (Cell Signaling, 3724S), HA mouse antibody (Cell Signaling, 2367S) and Alexa fluorophore-conjugated secondary antibodies from Molecular Probes (Invitrogen) were used. All antibodies have been previously validated for use in the system under study (immunofluorescence in mammalian cell lines).

10. Eukaryotic cell lines

a.   State the source of each eukaryotic cell line used.

> HeLa (ATCC), HEK293 (Life Technologies), HCT116 (from Richard Youle) and H4 cells (from Pamela McLean) were used. Further details are provided in the Methods section (Cell Culture and Transfection subsection).

b.   Describe the method of cell line authentication used.

> Cell lines were previously authenticated by cytochrome c oxidase subunit I (COI) and short tandem repeat (STR) testing.

c.   Report whether the cell lines were tested for mycoplasma contamination.

> Cells lines were tested negative for mycoplasma contamination.

d.   If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

> No commonly misidentified cell lines were used.

▶ Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

> No animals were used in this study.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

> This study did not involve human research participants.