# nature portfolio

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## **Reporting Summary**

Nature Portfolio 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 Portfolio policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

		ics

For all statistical ar	nalyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.							
n/a Confirmed								
☐ ☐ The exact	exact sample size $(n)$ for each experimental group/condition, given as a discrete number and unit of measurement							
A stateme	🔀 A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly							
The statis	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 descrip	A description of all covariates tested							
A descrip	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons							
A full des	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 h	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>							
For Bayes	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings							
For hiera	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes							
Estimates	Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i> ), indicating how they were calculated							
,	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.							
Software an	d code							
Policy information	about availability of computer code							
Data collection	Leica Application Suite 3 (TCS SP8 STED 3X FALCON FLIM)							
Data analysis	Graphpad prism 6. Fiji for Mac OS X							
For manuscripts utilizin	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 and							

#### Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our  $\underline{\text{policy}}$

All authors confirm that all relevant data are included in the paper and its supplementary information file. Additional files can be provided upon request.

Human rese	arch part	ticipants				
Policy information	about <u>studies</u>	involving human research participants and Sex and Gender in Research.				
Reporting on sex and gender		Not applicable				
Population characteristics		Not applicable				
Recruitment		Not applicable				
Ethics oversight		Not applicable				
Note that full informa	ation on the app	proval of the study protocol must also be provided in the manuscript.				
Field-spe	ecific re	eporting				
Please select the o	ne below that	is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.				
X Life sciences		Behavioural & social sciences				
For a reference copy of	the document wit	h all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>				
Life scier	nces st	udy design				
All studies must dis	sclose on these	e points even when the disclosure is negative.				
Sample size	No statistical i	methods were used to predetermine sample size. Standard sample size from the field were used.				
Data exclusions	No data was e	was excluded.				
Replication	All experiments were repeated 3 times. FLIM analysis was done on random cells with foci. Microscopy images and western blots are representative of at least three individual experiments. All image quantifications are shown as the mean of replicates acquired in randomly chosen microscopy areas.					
Randomization	mization Not relevant in our study as it only involves experiments with cell lines.					
Blinding	_	vas done but bias was impossible because only the FLIM analysis revealed the aggregate species composition in the cells, which is fore starting the FLIM analysis. No data was excluded.				
We require informatisystem or method lis  Materials & ex  n/a Involved in the Antibodies  Eukaryotic	oon from authors ted is relevant to perimental ne study	n/a Involved in the study  ChIP-seq  Flow cytometry				
Antibodios						

#### Antibodies

Antibodies used

anti-HSP40/Hdj1 (2E1, Enzo), anti-DNAJA2 (ab147216, Abcam), Anti-GAPDH antibody (clone GAPDH-71.1, Sigma-Aldrich

Validation

These antibodies are commercial antibodies and have been previously used and validated. See individual information provided online on the company website.

### Eukaryotic cell lines

Policy information about <u>cell lines and Sex and Gender in Research</u>

Cell line source(s)  $\mbox{EK293T} \ \alpha\mbox{-SynA53T-YFP was kindly provided by Marc Diamond (MTA)}$ 

HEK293T

Authentication The original HEK293T cells were received from ATCC with authentication certificate from supplier. The HEK293T α-SynA53T-YFP was kindly provided by Marc Diamond (MTA). α-SynA53T-YFP expression was validated in microscopy and western blot.

Mycoplasma contamination Cell lines tested negative for mycoplasma contamination.

Commonly misidentified lines (See <u>ICLAC</u> register)

Not applicable.