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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 Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

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

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☒	☐	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.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	SymPhoTime 64
Data analysis	SymPhoTime 64: Fitting the lifetime images. -Excel: Group of data and measurement of median and standard deviation GraphPad Prism: To plot graphs and obtain the linear regression -ImageJ: To extract the histograms of the images

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

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

The data sets generated during the current study are available from the corresponding author upon reasonable request.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The error bars and the measure of the center is mean ± SD. In each measurement was taken several GUV's and cells giving an important number of events, together with the small differences between replications were the indication of the sample size for each experiment Lifetime vs lipid composition(GUV) (fig 1c and Suppl fig a) DOPC-- R:15 DOPC:CL-- R:25 Phase separated-- R:5 SM:CL-- R:25 Lifetime vs osmotic pressure (fig 3b): GUV-- R:85 MDCK-- R:20 HeLa-- R:22 Lifetime vs osmotic shocks (fig 5d): Hela-- R:3 (Paper one exeample) lifetime vs tension (fig 3d): MDCK-- R:11 HeLa-- R:7 Lifetime vs tension (GUVs) (fig 5): GUV (POPC:SM:CL)-- R:7 GUV (SM:CL)-- R:6 GUV (DOPC:SM:CL)-- R:8 lifetime vs time during osmotic shocks: GUVs (DOPC) : R:14 (suppl fig 3a) GUVs (POPC:SM:CL) : R: 6 (suppl fig 3b) Hela (control w/o shock): R:21 (suppl fig 2a) MDCK (control w/o shock):R:19 (suppl fig 2a) Hela : R:14 (suppl fig 2b) MDCK :R:49 (suppl fig 2b)
Data exclusions	Not relevant
Replication	Lifetime vs lipid composition(GUV) (fig 1c and Suppl fig a) DOPC-- N:5 DOPC:CL-- N:5 Phase separated-- N:4 SM:CL-- N:5 Lifetime vs osmotic pressure (fig 3b): GUV-- N:4 MDCK-- N:6 HeLa-- N:20

lifetime vs tension (fig 3d):
MDCK-- N:11
HeLa-- N:7

Lifetime vs osmotic shocks (fig 5d):
Hela-- N:3 (Paper one exeample)

Lifetime vs tension (GUVs, fig 5) :
GUV (POPC:SM:CL)-- N:7
GUV (SM:CL)-- N:6
GUV (DOPC:SM:CL)-- N:8

lifetime vs time during osmotic shocks:
GUVs (DOPC) : N:2 (suppl fig 3a)
GUVs (POPC:SM:CL) : N:1 (suppl fig 3b)
Hela (control w/o shock): N:2 (suppl fig 2a)
MDCK (control w/o shock):N:2 (suppl fig 2a)
Hela : N:2 (suppl fig 2b)
MDCK :N:2 (suppl fig 2b)

Neurons--KCl and Osmotic shock (suppl fig 4): N:3

Randomization: Not relevant

Blinding: Not relevant

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☒	☐ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s): HeLa-Zerial cell line, obtained from Lukas Pelkmans published in: Pelkmans L et al. Genome-wide analysis of human kinases in clathrin- and caveolae/raft-mediated endocytosis. Nature. 2005 Jul 7;436(7047):78-86.

MDCK-II cells: bought from Sigma-Aldrich, Ref: 00062107, from Public Health England

Authentication: by the providers

Mycoplasma contamination: cells lines are tested every 6 months for mycoplasma contamination. These cells lines were tested before use for these experiments and was negative.

Commonly misidentified lines (See ICLAC register): Not misidentified lines were used