nature portfolio

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Last updated by author(s): Feb 16, 2022

# 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 Editorial Policies and the Editorial Policy Checklist.

## Statistics

For all statistical analyses, confirm that the following items are present in the 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 |
| ☐ | ☒ | 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<br>*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 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<br>*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 |

*Our web collection on statistics for biologists contains articles on many of the points above.*

## Software and code

Policy information about availability of computer code

| | |
|---|---|
| Data collection | AxioVision, Rel. 4.9.1 (Carl Zeiss®), IncuCyteTM Live-Cell Imaging System (IncuCyte HD), Leica TCS-SP5 and Las-AF software, Fortessa flow cytometer (BD Biosciences). |
| Data analysis | Graphad 9, Flow Jo, Axiovision 4.9, ImageJ (find maxima, quantification western blot and analyze particules process and a macro adapted from Daniel J. Shiwarski as described in the manuscript), IncuCyteTM Live-Cell software, Microsoft Excel 2019, iDraw |

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 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.

## 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The authors declare that the data supporting the findings of this study are available within the paper and its supplementary information files. If needed, additional information is available from the corresponding author upon reasonable request.

# Field-specific reporting

Please select the one below that 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 ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

# Life sciences study design

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

| | |
|---|---|
| Sample size | The chosen sample size is based on numbers used for previous publiations, which is most optimal to generate statisticaly significant results. |
| Data exclusions | No data were excluded. |
| Replication | All attempts at replication were successful. |
| Randomization | Sample/Cells were randomized to be examined. |
| Blinding | Blinding was not relevant because all samples/cells were analyzed in the same way |

# 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

| n/a | Involved in the study |
|---|---|
| ☐ | ☒ Antibodies |
| ☐ | ☒ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology and archaeology |
| ☒ | ☐ Animals and other organisms |
| ☒ | ☐ Human research participants |
| ☒ | ☐ Clinical data |
| ☒ | ☐ Dual use research of concern |

## Methods

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

## Antibodies

| | |
|---|---|
| Antibodies used | The following antibodies were used for the study: Beta-Actin (AC14) (abcam, AB6276); Atg16L1 (D6D5) (Cell Signaling, 8089T); anti-phospho-Histone H2A.X (Ser139) (Sigma-Aldrich, JBW301); LC3 A/B (D3U4C) (Cell Signaling, 12741S); phospho-mTOR (Ser2448) (D9C2) (Cell Signaling, 5536T); SQSTM1/p62 (Cell Signaling, 5114T) and (abcam, ab56416); Rad51 (114B4) (abcam, ab213); and BRCA1 (Sigma Millipore, 07-434); Atg5 (D5F5U) (Cell Signaling, 12994S); Atg12 (D88H11) (Cell Signaling, 4180S); Beclin-1 (D40C5) (Cell Signaling, 3495S); Atg7 (D12B11) (Cell Signaling, 8558S); β-Tubulin (D2N5G) (Cell Signaling, 15115S); Filamin A (Cell Signaling, 4762S), SP1 (Sigma, PLA0307), PARP1 (proteintech, 66250), PAR/pADR (R&D systems, 4335-MC-100), Goat anti-mouse (Millipore, AP124P), Goat anti-rabbit (Millipore, AP156P), Rabbit anti-goat (Milliporte, AP106P), anti-mouse Cy5 (Life Technologies Inc., A10524) and anti-rabbit 488 (ThermoFisher Scientific, A-11008). |
| Validation | Antibody validation was deferred to the manufacturers and was supported by multiple publications. |

## Eukaryotic cell lines

Policy information about cell lines

| | |
|---|---|
| Cell line source(s) | Human PC cell lines, LNCaP and PC-3, were purchased from the American Type Culture Collection (ATCC CRL-174, ATCC CRL-250, respectively). C4-2B cells were gifted by Dr. Gleave. PC CRISPR KO Atg16L1 cell lines were developed from the parental ones. |
| Authentication | PC cell lines were authenticated by Short Tandem Repeat (STR) profiling |
| Mycoplasma contamination | All cell lines were tested negative for mycoplasma. |

| | |
|---|---|
| Commonly misidentified lines (See ICLAC register) | No commonly misidentified cell lines were used in this study. |

# Flow Cytometry

## Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

## Methodology

| | |
|---|---|
| Sample preparation | Cell lines |
| Instrument | BD LSRFortessa |
| Software | Flow-Jo |
| Cell population abundance | A maximum of 30,000 events was counted for cell cycle, 15,000 for HR/NHEJ analyzes and 10,000 for siRNA experiement |
| Gating strategy | We removed element under 50 FSC and under 50 SSC which are considered as debris. Under FSC-H/FSC-W we gate the low population to eliminated doublet. |

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.