nature research

Corresponding author(s): Jiri Bartek, Francesco Cecconi

Last updated by author(s): 02/02/2021

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 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 *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 *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	Zeiss ZEN software, for LSM microscope image acquisition scanR acquisition software, for images taken in Olympus high-content screening microscope BC Cytoflex, for flow cytometry data DiVa, version 6.1, for flow cytometry data Chemidoc, for WB acquisition iBright CL1500, for WB acquisition NDP.view 2, versione 2.7, for tissues IF ImageXpress Micro Confocal High-Content Imaging System live imaging for cell cycle lenght quantification Comet Assay IV software, for comet assay experime
Data analysis	ZEN lite 2012 software, confocal microscopy analysis scanR analysis software, analysis of high-content microscope acquired images NDP.view 2, versione 2.7, for tissues IF Comet Assay IV software FlowJo_V10, flow cytometry analysis Image Lab, gel densitometry analysis Image J, gel densitometry analysis GraphPad Prism 7, numerical and statistical analysis Tibco Spotfire 7.6.1 box plots with distribution and dot plots Adobe Illustrator CC, 2020 Ingenuity Pathway Analysis, Quiagen, 2020

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 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

All the computer scripts and source codes used to generate and analyse the results from TCGA analysis presented in the manuscript are available at E. Papaleo Group Github repository [https://github.com/ELELAB/AMBRA_low].
Differential expression analysis from RNA seq analysis is present as Extended Data table.

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	No statistical method was used to predetermine sample size. Untreated control cells, with drug- vehicle, or cells transfected with siSCRAMBLE siRNA were included as appropriate controls for each specific experiment.
Data exclusions	No exclusion was applied.
Replication	Error bars in bar plots represent means ± SEM/SD. Each experiment was performed ≥3 independent times with similar results. All representative experimental findings were verified in ≥3 independent experiments. In Extended data Fig. 4g , instead, n = 2. All the measurements were taken from distinct samples. The exact number of repeats, the number of analyzed cells per repeat as well as the exact p values and degrees of freedom are indicated in each Figure graph or/and in Source Data.
Randomization	Samples were organized into different treatments (cell type/ chemical treatment/knockdown conditions).
Blinding	No blind experiments were performed in this study. Quantification of cell foci counts and signal intensities in different experiments/ experimental conditions were performed by applying same parameters in an unbiased 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	b-tubulin, Sigma-Aldrich, T4026 (IB 1:1000) g-tubulin, Sigma-Aldrich, T3559 (IF 1:200) b-actin, Sigma-Aldrich, A2066 (IB 1:5000) Ambra1, Merck Millipore, ABC131 (IB 1:1000) Ambra1 (G-6), Santa Cruz, sc-398204 (IB 1:1000) n-Myc, Cell Signaling, 84406 (IB 1:1000) n-Myc, Santa Cruz, sc-53993 (IB 1:1000, IP 1:100)

Cyclin D1, Cell Signaling, 2978 (IB 1:1000)
Cyclin D1, Abcam, 16663 (IF 1:300, IP 1:100)
Cyclin D2, Cell Signaling, 3741 (IB 1:1000)
Cyclin A2 (Santa Cruz sc-596 IB 1:500),
Cyclin E2 (Cell Signaling 4132 IB 1:1000),
Cyclin B1 (Abcam ab7957-1 IB 1:1000),
Ki67 (Abcam 15580 1:200),
NCL-Ki67p (Leica Biosystems IHC 1:6000),
PARP (Cell Signaling 9542 IB 1:1000),
pRb 780 (Cell Signaling 8307 1:1000)
pRb 807/811 (Cell Signaling 9308 IB 1:1000),
Rb (Cell Signaling 9313 1:1000),
ULK1 (Cell Signaling Technology, 8054, IB 1:1000),
Chk1 (G4, Santa Cruz, sc-8408 IB 1:1000)
Chk1(Ser345) (Cell Signaling, 2341, IB 1:1000),
H2AX (Merck-Millipore 07-627 IB 1:1000),
H2AX p-S139 (Merck Millipore 05-636 IF 1:1000 / IB 1:1000),
H2AX p-S139 (Cell Signaling 2577 IHC 1:400),
c-Myc (9E10) (Santa Cruz sc-40 1:1000),
SOD1 (Santa Cruz sc-11407 IB 1:1000),
LDH (Santa Cruz sc-33781 IB 1:1000),
GAPDH (Merck-Millipore CB1001 IB 1:10000),
Histone H3 p-S10 (Abcam ab5176 IF 1:1000),
BRCA1 (Bethyl laboratories, IHC-00278, IF 1:500 IB 1:1000),
Rad51 (Abcam, ab213, IB 1:1000)
RPA pSer4, pSer8 (Novus Biologicals NBP1-23017 IHC 1:2000),
RPA pS4-S8 (Bethyl A300-245A IB 1:1000),
RPA (abcam ab2175 IF 1:200),
CDK2 (Santa Cruz sc-6248 1:400),
CDK2 pT160.(Santa Cruz sc-101656 1:500)
Sox2 (Santa Cruz sc-17320 IF 1:100)
Tbr2 (Abcam ab216870 IF 1:100)
cul1: (Thermo Fisher Scientific 32-2400 WB 1:1000)
cul2: (Bethyl Laboratories A302-475A WB 1:1000)
cul3: (Bethyl Laboratories A301-109A WB 1:1000)
cul4a (Bethyl Laboratories A300-739A WB 1:1000)
cul5: (Bethyl Laboratories A302-173A WB 1:1000)
NeuN (Abcam ab104224 IF 1:100)
ATG7 (D12B11 Cell Signaling 8558S)
Cyclin D3 (Thermo Fisher Scientific MA5-12717 WB 1:1000)
α-tubulin (Sigma-Aldrich T6074 WB 1:5000)
p-cyclin D1 (T286) (Cell Signaling Technology Cat. No. 3300S WB 1:1000)
pMyc S62 (1:100)

Validation

b-tubulin, validated by the company, 628 citations
g-tubulin, validated by the comapny, 127 citations,
b-actin, validated by the company, 2194 citations
Ambra1, validated by the company and by gt/gt mouse model see Fig. 1g
Ambra1 (G-6), validated by the company, 6 citations
n-Myc, CS, validated by the company, product citations 2
n-Myc, SC, validated by the company, 114 citations
Cyclin D1, CS, validated by the company, 563 citations
Cyclin D1, abcam, validated by the company, 135 citations
Cyclin D2, validated by the company, 52 citations,
Cyclin A2, validated by the company, 271 citations,
Cyclin E1, validated by the company, 124 citations,
Cyclin B1, validated by the company, 67 citations
Ki67, validated by the company, 1637 citations,
NCL-Ki67p, Leica Biosystems, validated by the company, 128 citations
PARP, validated by the company, 1818 citations,
pRb 780, validated by the company, 183 citations
pRb 807/811, validated by the company, 277 citations
Rb, validated by the company, 56 citations
ULK1, validated by the company, 197 citations
Chk1, validated by the company, 592 citations
Chk1, validated by the company, 214 citations
H2AX, validated by the company, 29 citations
H2AX p-S139, validated by the company, 494 citations
H2AX p-S139 CS, validated by the company, 438 citations
c-Myc (9E10), validated by the company, 6327 citations
SOD1, discontinued, 184 citations
LDH, discontinued, 44 citations
GAPDH, validated by the company
Histone H3 p-S10, validated by the company, 183 citations
BRCA1, validated by the company, 2 citations
Rad51, validated by the company, 65 citations

RPA pS4-S8, validated by the company, 208 citations
RPA, validated by the company, 125 citations
CDK2, validated by the company, 338 citations
CDK2 pT160, validated by the company, 7 citations
Sox2, discontinued, 119 citations
Tbr2 validated by the company, 1 reference
cul1, validated by the company, 13 citations
cul2, validated by the company, 4 citations
cul3, validated by the company, 27 citations
cul4a , validated by the company, 32 citations
cul5: (Bethyl Laboratories A302-173A WB 1:1000), 13 citations
NeuN, validated by the company, 204 citations
ATG7 (D12B11 Cell Signaling 8558S), 255 citations
Cyclin D3 (Thermo Fisher Scientific MA5-12717 WB 1:1000), 30 citations
α-tubulin (Sigma-Aldrich T6074 WB 1:5000) 962 citations
p-cyclin D1 (T286) (Cell Signaling Technology Cat. No. 3300S WB 1:1000), citations 23
pMyc S62 (1:100) validated by Prof. Sears (see citation)

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	U2-OS (ATCC HTB-96) SH-Sy5y ATCC® CRL-2266 U-87 MG (U87) (ATCC® HTB-14™) SK-N-BE(2) ATCC® CRL-2271 BJ-5ta (BJhTERT) (ATCC® CRL-4001™) HCC827 cells are from ATCC and validate by the company. https://www.lgcstandards-atcc.org/Products/All/CRL-2868.aspx?geo_country=dk Saos-2 cells are from ATCC. https://www.lgcstandards-atcc.org/products/all/HTB-85.aspx SK-UT-1B cells are also from ATCC. And described previously in our paper PMID: 8710382 https://www.lgcstandards-atcc.org/products/all/HTB-115.aspx A549 cells are from ATCC and validate by the company. https://www.lgcstandards-atcc.org/products/all/CCL-185.aspx?geo_country=dk H1299 cells are from ATCC and validate by the company. https://www.lgcstandards-atcc.org/products/all/crl-5803.aspx?geo_country=dk
Authentication	Cells lines were purchased from ATCC and authenticated by STR method.
Mycoplasma contamination	All cell lines used in this study were negative for mycoplasma. We regularly check by PCR the presence of mycoplasma in all cell lines used in our laboratory.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	To generate Ambra1flox/flox/Nestin-cre and KrasG12D/Ambra1flox/flox animals, homozygous Ambra1flox/flox females were bred onto Ambra1wt/flox /Nestin-cre or Ambra1wt/flox/KRasG12D. Nestin-cre and KrasG12D mice were purchased from Jackson Lab and maintained in the animal facility Plaisant Castel Romano (RM, Italy) and at the Danish Cancer Society Research Center (Copenhagen, DK), respectively. Athymic Nude-Foxn1nu mice, male, were purchased from Envigo. NOG mice were purchased from Taconics.
Wild animals	The study did not involve wild animals.
Field-collected samples	the study did not involve field-collected samples.
Ethics oversight	All in vivo experiments were approved by and performed in accordance with the ethical international, EU and national requirements and were approved by the Italian Health Ministry (D.lgs 26/2014; N°. 737 03/2013; N°88/2016-PR), the Danish Health Authorities (Dyreforsøgstilsynet, 2019-15-0201-01668, Dyreforsøgstilsynet, 2018-15-0201-01391, C2) and Authorization code from Madrid Region Government (PROEX 193/15)

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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	U2OS FUCCI cells were collected, resuspended in PBS and directly mesured with BC Cytoflex (Extended Data Fig.3p). For annexin V staining neural stem cells or U87 cells were washed in PBS and resuspended in Annexin V 1x binding buffer (BD Biosciences catalog number 556454). Then 5 uL of APC-Annexin V were added to each sample. Depending on experimental conditions also 0.1 mg/ml iodure propide was added to the solution and samples were analysed with FACS Canto II within 30 minutes (Ext. Data Fig. 5g, 5h, 8n, 9c) . For cell cycle analysis neur al stem cells were washed with PBS and fixed with 80% methanol / 20% acetone cold solution and stored overnight at 4°C. Before analysis cells were treated with RNase and resuspended with 0.1 mg/ml iodure propide solution in PBS and analysed with FACS Canto II (Exteded data Fig. 2e).
Instrument	BC Cytoflex FACS Canto II
Software	FlowJo_V10 DiVa 6.2
Cell population abundance	10000 events were collected in each analysis
Gating strategy	All flow cytometry experiments were gated and analyzed similarly. The same gates were used for the control and experimental conditions.

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