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 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	ZEN microscopy software for confocal LSM700 and LSM710 microscopes (ZEISS) StepOne software V2.2 supplied by Applied Biosystems Gen5 Software for Epoch Microplate Spectrophotometer (BioTek) CellSens (Olympus)
Data analysis	Image analysis: ImageJ Fiji x64 v1.5 Statistical analysis: Graphpad prism v9.1, Excel (Microsoft 365) CRISPR screen analysis: Bowtie2 v2.5.1, BAGEL v2.0 MAGeCK v0.5.9.2, CCA v1.0 Gene ontology analysis: ShinyGo v0.77 (http://bioinformatics.sdstate.edu/go/) Mass spec analysis: MaxQuant V2.2 RNA-seq analysis: Salmon v1.10.2, DESeq2 v1.42.0

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

Raw next-generation sequencing data for RBM10 SL CRISPR-Cas9 screen have been deposited in ArrayExpress and are available through accession number E-MTAB-13626. RNA-seq sequencing data generated in this study have been deposited in ArrayExpress and are available through accession number E-MTAB-13617. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE repository with the dataset identifier PXD040109. All data supporting the findings of this study are available from the corresponding author upon request.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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

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	In-vitro cell biology, CRISPR screen, and interactome experiments were performed with 3 replicates or more if indicated. Sample sizes for in-vitro experiments were not determined by statistical power analysis but chosen according to standard practices in the field. Immunoblots were repeated at least 2 times with one representative experiment presented. For in-vivo xenograft experiments, the number of mice was calculated according to statistical power analysis and consideration of standard attrition rate, yielding N=6 mice per treatment group.
Data exclusions	No data was excluded from in vitro experiments. One mouse inoculated with NCI-H1944 cells in figure 6e died 2-weeks prior to the experiment end-point and therefore was excluded from the analysis.
Replication	The number of replicates in each experiment is indicated in the figure legends and/or methods. All replication attempts of the results in this study were successful. Beside the replicates within each experiment, we demonstrate that RBM10-WEE1 synthetic lethality is observed in 3 HCC827 RBM10-knockout clones, as well as in other cell lines such as NCI-H1299 and the RBM10-null NCI-H1975 and NCI-H1944.
Randomization	All identical untreated samples were allocated randomly into treatment conditions. All mice were randomly allocated into treatment groups. For microscopy-based analysis, a fixed number of images were obtained randomly from each sample and analyzed together with control samples using the same analysis pipeline.
Blinding	Researchers were not blinded during data collection and analysis as the study design included appropriate controls and did not require blinding. Data collection and analysis of different conditions were performed at the same time and using identical methods. Microscopy and high-throughput acquisition and analysis was performed automatically without bias between different samples.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used

RBM10 (Sigma-Aldrich cat.no. HPA034972)
FLAG-tag (Sigma-Aldrich cat.no. F7425)
β-actin (Sigma-Aldrich cat.no. A5441)
α-Tubulin (Santa Cruz Bio cat.no. sc-5286)
pRPA32-S4/S8 (Bethyl cat.no. A300-245A)
pRPA32-S33 (Bethyl cat.no. A300-246A)
RPA32 (Abcam cat.no. ab2175)
γH2AX (Cell Signaling Technology cat.no. 2577)
pChk1-S345 (Cell Signaling Technology cat.no. 2348)
pH3(Ser10) (Cell Signaling Technology cat.no. 9706)
Histone H3 (Abcam cat.no. ab1791)
BrdU FITC (BD Biosciences, cat.no. 51-23614)
Anti-biotin (Jackson ImmunoResearch cat.no. 200-002-211)
Anti-BrdU (Abcam cat. no. ab6326)
Anti-BrdU (BD Biosciences #347580)
Anti-ssDNA antibody (DSHB #AB10805144)
Rad51 (Santa Cruz Bio cat.no. sc-8349)
PARP1 (Enzo Life Sciences cat. no. ALX-210-895)
PCNA (Santa Cruz Bio cat. no. sc-56)
GAPDH (Abcam cat.no. ab8245)
GFP (Santa Cruz Bio cat.no. sc-9996)
HDAC1 (GeneTex cat.no GTX100513)
H4K16ac (Abcam cat. no. ab109463)
53BP1 (Novus biological cat. no. NB100-305)

Secondary antibodies:
anti-rabbit IgG-HRP (Jackson ImmunoResearch cat.no. 111–035-003)
anti-mouse IgG-HRP (Amersham cat.no. NXA931)
anti-rabbit IgG Alexa Fluor®488 (Invitrogen cat.no. A-21206)
anti-mouse IgG Alexa Fluor®488 (Invitrogen cat.no. A-21202)
anti-rabbit Alexa Fluor®568 (Invitrogen cat.no. A10042)
anti-mouse Alexa Fluor®568 (Invitrogen cat.no. A10037)
anti-rabbit Alexa Fluor®647 (Invitrogen cat.no. A-31573)
anti-mouse IgG Alexa Fluor®647 (Invitrogen cat.no. A32787)
anti-mouse Alexa Fluor®647 (Jackson ImmunoResearch cat. no.#115-605-166)
anti-rat Alexa Fluor®594 (ThermoFisher Scientific cat. no. A11007)
anti-mouse IgG2a Alexa Fluor®488 (ThermoFisher Scientific cat.no. A21131)

Validation

All antibodies used in this study are commercial and validated by the manufacturers. In addition, all antibodies produced the expected band size and/or subcellular localization and were used in previous publications. RBM10 antibody was further validated by the expected loss of signal upon RBM10 knockout or depletion, an in RBM10-mutated cell lines. Antibodies against damage-specific phosphorylations (e.g. phosphorylation of RPA, H2A.X, CHK1) were validated by the induction of expected bands/foci following specific types of stress. pH3(Ser10) was validated by the observed staining of early mitotic cells (determined by DAPI signal in immunofluorescence).

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HCC827 (CRL-2868), NCI-H1299 (CRL-5803), NCI-H1944 (CRL-5907), and NCI-H1975 (CRL-5908) cell lines were obtained from ATCC.
Authentication	Parental HCC827 cells were not authenticated after reception from ATCC. NCI-H1944 and NCI-H1975 were validated for RBM10-deficiency by western blot using validated RBM10 antibody. NCI-H1299 was validated as p53-deficient by DNA damage induction followed by western blot. The generated RBM10 knockout cell lines were validated by sequencing and western blot with validated RBM10 antibody.
Mycoplasma contamination	Cell lines used were free of mycoplasma.
Commonly misidentified lines (See ICLAC register)	No misidentified cell lines were used in this study.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	The study used 6-weeks old immunocompromised NOD.CB17-Prkdcscid/NcrCrl (NOD SCID) mice obtained from Envigo.
Wild animals	The study did not involve wild animals.
Reporting on sex	The findings of this study do not apply to only one sex. Sex was not considered in the study design and we did not collect sex-specific observations.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	Animal studies and protocols were approved by the Committee on the Ethics of Animal Experiments of the Technion, Israel Institute of Technology (IL-069-07-22).

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