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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 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	GloMax Discover Microplate Reader (Promega), CLARIOstar (BMG Labtech), GloMax Discover luminometer (Promega), ChemiDoc MP imaging system (BioRad), PHERAstar FS plate reader (BMG Labtech), Q Exactive HF Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Scientific), Epson Perfection V800 Photo scanner, ÄKTA pure system (GE Healthcare), MicroCal iTC200 (Malvern), Biacore T200 (GE Healthcare), BD FACS Canto II flow cytometer (BD Biosciences).
Data analysis	Image Studio Lite v.5.2, Graphpad PRISM v.7.0c, v.7.03, v.7.05, or v.8, MaxQuant software v.1.6.0.16, ImageJ software (NIH) v. 1.52n, Microcal ITC200 Origin analysis software (Malvern), Biacore insight evaluation software(GE Healthcare), FlowJo™ 10.7.1. Software.

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

The authors declare that all data supporting the findings of this study are available within the paper and its supplementary information files. Mass spectrometry proteomics data (Fig. 2e and Extended Data Fig. 2f) are provided in Supplementary Data Set 1. The study report of pharmacokinetics of SIM1 following Single IV and SC administrations to mice provided by Chempartner Co. Ltd, is provided in Supplementary Data Set 2. The study report of BromoScan profiling service data (Fig. 5a) is provided as Supplementary Data Set 3. Source data for Figures 1 and 3, and Extended Data Figures 1, 3 and 4 are provided with this paper.

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	Sample sizes were not predetermined using statistical analyses. For biochemical, biophysical and cellular assays, sample sizes were based on prior experience in the field and from our previous studies (Zengerle et al. ACS Chem Biol 2015; Gadd et al. Nat. Chem. Biol. 2017; Riching et al. ACS Chem Biol. 2018). To confirm results with biological repeats, experiments were performed in most cases as either in duplicates or triplicates.
Data exclusions	No data were excluded
Replication	Unless stated in figure legends or method sections, all experiments were done at least twice and the reproduction were successful. The number of technical replicates and independent biological experiments are specified in the manuscript. Most results were validated by alternative techniques as demonstrated in the manuscript.
Randomization	No animal or behavioral study was performed in this study. No large data set and/or compound libraries were used in this study. Randomization was thus not necessary in our study and was not performed.
Blinding	Similar to what is mentioned above, no animal or behavioral study was performed in this study, and no large data set and/or compound libraries were used in this study. Randomization was thus not necessary in our study and was not performed.

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	ant-BRD2 (abcam #ab139690) anti-BRD3 (abcam #ab50818) anti-BRD4 (abcam #ab128874) anti-c-myc (abcam #32072) anti-PARP (CST #9542S) anti-cleaved PARP (BD Pharmingen #51-9000017) anti-caspase-3 (CST #9662S) anti-tubulin (Bio-Rad #12004165) anti-β-actin (CST #4970S) IRDye® 800CW Goat anti-Mouse: LI-COR #925-32210 IRDye® 800CW Donkey anti-Rabbit: LI-COR #926-32213
Validation	- anti-BRD2 (abcam #ab139690): Knockdown validated, disappearance of the band in immunoblotting upon MZ1 / siRNA treatment (Zengerle et al. ACS Chem. Biol. 2015). - anti-BRD3 (abcam #ab50818): Knockdown validated, disappearance of the band in immunoblotting upon MZ1 / siRNA treatment (Zengerle et al. ACS Chem. Biol. 2015). - anti-BRD4 (abcam #ab128874): Knockdown validated, disappearance of the bands in immunoblotting upon MZ1 / siRNA treatment

(Zengerle et al. ACS Chem. Biol. 2015).
- anti-c-myc (abcam #32072): Knockout validated, from the manufacturer's website. https://www.abcam.com/c-myc-antibody-y69-ab32072.html; see also: https://www.labome.com/product/Abcam/ab32072.html
- anti-PARP (CST #9542S): Disappearance of the peak in Western blot analysis by staurosporine treatment, as per information on the manufacturer's website: https://www.cellsignal.com/products/primary-antibodies/parp-antibody/9542?_=1626576061358&Ntt=9542s&tahead=true&country=USA
- anti-cleaved PARP (BD Pharmingen #51-9000017): Identified of the band in Western blot analysis in only camptothecin treatment, as per the manufacturer's website: https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/purified-mouse-anti-cleaved-parp-asp214.552596
Additional validations and relevant citations can be found in the vendors' web-pages using the catalogue numbers specified above for all the antibodies used.

More detailed information for each primary antibody:

anti-c-myc (abcam #32072)
Describe validation of primary antibody: KO validated by manufacturer
Species: Host – Rabbit; human c-myc peptide (ab166837) used for immunisation
Validation statement for species and application: Reacts with: Mouse, Rat, Human
Application: Flow Cyt (Intra), WB, ICC/IF, IHC-P, IP. Abpromise guarantee covers the use of ab32072 in the following tested applications.
Validation statement: WB validation performed in (as can be found on manufacturer's website): Jurkat, HeLa, HEK-293T, Raji, MCF-7, K562, THP1, A20, AR42J, Rat-1, rat spleen and pancreas, L6, Neuro-2a, Raw264.7 cell lysates, L363 MM and CA46 cells. ICC/IF: HEK293 and HeLa cells. IHC-P: Human Burkitt lymphoma, diffuse large B cell lymphoma, adenocarcinoma of the colon, lung adenocarcinoma, gastric adenocarcinoma, urinary bladder transitional carcinoma, esophagus, glioblastoma and low-grade glioma tumor tissues. IP: Jurkat whole cell lysate (ab7899). Flow Cyt (intra): HeLa cells.
Relevant citations: ab32072 has been referenced in 747 publications
KO validation of c-Myc antibody: Littler S, Sloss O, Geary B, Pierce A, Whetton AD, Taylor SS. Oncogenic MYC amplifies mitotic perturbations. Open Biol. 2019 Aug 30;9(8):190136. doi: 10.1098/rsob.190136.
Source:
Product datasheet https://www.abcam.com/c-myc-antibody-y69-ab32072.html
https://www.labome.com/product/Abcam/ab32072.html

CASPASE 3, CELL SIGNALLING, #9662
Describe validation of primary antibody: validated by manufacturer in Caspase 3 KO HCT116 cells - Western blot analysis of extracts from HCT116 cells (lane 1) or CASP3 knock-out cells (lane 2) using Caspase-3 Antibody #9662 (upper), and α-Actinin (D6F6) XP® Rabbit mAb #6487 (lower). The absence of signal in the CASP3 knock-out HCT116 cells confirms specificity of the antibody for CASP3.
Species: Human, Mouse, Rat, Monkey; Species predicted to react based on 100% sequence homology:Pig
Validation statement for species and application: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding the cleavage site of human caspase-3. Antibodies are purified by protein A and peptide affinity chromatography. Species reactivity is determined by testing in at least one approved application (e.g., western blot).
Application: western blotting, immunoprecipitation, immunohistochemistry (parrafin)
Relevant citations:
CST 9662 has been referenced in 2723 publications.
Cell Signaling Technology caspase-3 antibody (Cell Signaling, 9662S) was used in western blot knockout validation on human samples McComb S, Chan PK, Guinot A, Hartmannsdottir H, Jenni S, Dobay MP, Bourquin JP, Bornhauser BC. Efficient apoptosis requires feedback amplification of upstream apoptotic signals by effector caspase-3 or -7. Sci Adv. 2019 Jul 31;5(7):eaau9433. doi: 10.1126/sciadv.aau9433.
Cell Signaling Technology caspase-3 antibody (Cell Signaling, 9662) was used in western blot knockout validation on human samples Wang Y, Gao W, Shi X, Ding J, Liu W, He H, Wang K, Shao F. Chemotherapy drugs induce pyroptosis through caspase-3 cleavage of a gasdermin. Nature. 2017 Jul 6;547(7661):99-103. doi: 10.1038/nature22393. Epub 2017 May 1. PMID: 28459430.
Antibody profile (online databases, data from the manuscript):
https://www.phosphosite.org/proteinAction.action?id=4672&showAllSites=true
Source:
https://www.cellsignal.co.uk/products/primary-antibodies/caspase-3-antibody/9662
https://www.labome.com/review/gene/human/caspase-3-antibody.html

anti-PARP (CST #9542S)
Describe validation of primary antibody: Confirmed by western blot analysis of extracts from NIH/3T3 cells, untreated or staurosporine-treated (1 µM), and Jurkat cells, untreated or etoposide-treated (25 µM). PARP cleavage detected in treated samples and full-length PARP detected in untreated samples, both at expected Mw.
Species: Human, Mouse, Rat, Monkey
Validation statement for species and application: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to the caspase cleavage site in PARP. Antibodies are purified by protein A and peptide affinity chromatography. PARP Antibody detects endogenous levels of full length PARP1 (116 kDa), as well as the large fragment (89 kDa) of PARP1 resulting from caspase cleavage. The antibody does not cross-react with related proteins or other PARP isoforms.
Application: Western blotting
Relevant citations:
Antibody profile (online databases, data from the manuscript):
https://www.phosphosite.org/proteinAction.action?id=9166&showAllSites=true

https://www.cellsignal.co.uk/products/primary-antibodies/parp-antibody/9542 -

anti-cleaved PARP (BD Pharmingen #51-9000017)

Describe validation of primary antibody: Jurkat cells were either left untreated or treated with camptothecin (4 µM, 4 hours) to induce apoptosis. Lysates were probed with anti-PARP (clone F21-852, Cat. No. 552596) at concentrations of 0.25 (lanes 1, 4), 0.125 (lanes 2, 5), and 0.06 µg/ml (lanes 3, 6). Cleaved PARP is identified as a band of ~89 kDa in only the treated cells.

Validation statement for species and application: A peptide corresponding to the N-terminus of the cleavage site (Asp 214) of human PARP was used as the immunogen. The F21-852 monoclonal antibody reacts only with the 89-kDa fragment of human PARP-1 that is downstream of the Caspase-3 cleavage site (Asp214) and contains the automodification and catalytic domains. It does not react with intact human PARP-1. Cross-reactivity with other members of the PARP superfamily is unknown. Recognition of cleaved PARP in mouse cells has been demonstrated, and it may also cross-react with a number of other species due to the conserved nature of the molecule.

Application: western blot, intracellular staining (flow cytometry), immunoprecipitation

References:

D'Amours D, Desnoyers S, D'Silva I, Poirier GG. Poly(ADP-ribosyl)ation reactions in the regulation of nucelar functions. Biochem J. 1999; 342:249-268. (Biology)

Kaufmann SH, Desnoyers S, Ottaviano Y, Davidson NE, Poirier GG. Specific proteolytic cleavage of poly(ADP-ribose) polymerase: an early marker of chemotherapy-induced apoptosis. Cancer Res. 1993; 53(17):3976-3985. (Biology) Lamarre D, Talbot B, de Murcia G, et al. Structural and functional analysis of poly(ADP ribose) polymerase: an immunological study. Biochim Biophys Acta. 1988; 950 (2):147-160. (Biology)

Lamarre D, Talbot B, Leduc Y, Muller S, Poirier G. Production and characterization of monoclonal antibodies specific for the functional domains of poly(ADP-ribose) polymerase. Biochem Cell Biol. 1986; 64(4):368-376. (Biology)

Patel T, Gores GJ, Kaufmann SH. The role of proteases during apoptosis. FASEB J. 1996; 10(5):587-597. (Biology) Tewari M, Quan LT, O'Rourke K, et al. Yama/CPP32 beta, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly(ADP-ribose) polymerase. Cell. 1995; 81(5):801-809. (Biology)

Source: https://www.bdbiosciences.com/ds/pm/tds/552596.pdf

β-Actin (13E5) Rabbit mAb #4970

Application: WB, IHC-P, IF-IC, F

Specificity: β-Actin (13E5) Rabbit mAb detects endogenous levels of total β-actin protein. Depsite the high sequence identity between the cytoplasmic actin isoforms, β-actin and cytoplasmic γ-actin, β-Actin (13E5) Rabbit mAb #4970 does not cross-react with cytoplasmic γ-actin, or any other actin isoforms.

Species Reactivity: Human, Mouse, Rat, Monkey, Bovine, Pig. Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Species predicted to react based on 100% sequence homology:

Hamster, Chicken, Dog, Horse

hFAB™ Rhodamine Anti-Tubulin Primary Antibody, 200 µl #12004165

Cross-reactivity

Human, mouse, and rat

Antigen

Recombinant human β-tubulin expressed in E.coli

Recombinant Anti-BRD2 antibody [EPR7642] - ChIP Grade (ab139690)

Describe validation of primary antibody by manufacturer: was shown to specifically react with BRD2 when BRD2 knockout samples were used. Wild-type and BRD2 knockout samples were subjected to SDS-PAGE. ab139690 and ab8245 (loading control to GAPDH) were diluted 1/1000 and 1/2000 respectively and incubated overnight at 4°C. Blots were developedwith Goat anti-Rabbit IgG H&L (IRDye 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye 680RD)n preadsorbed (ab216776) secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging;

Validation of primary antibody by us: Validated by siRNA knockout in https://pubs.acs.org/doi/10.1021/acschembio.5b00216; disappearance of the BRD2 band upon the treatment with MZ1;

Species: Reacts with human

Application: western blot, immunohistochemistry, immunocytochemistry, flow cytometry, ChiP; unsuitable for IP. Abpromise guarantee covers the use of ab139690 in the following tested applications - western blot, immunohistochemistry, immunocytochemistry, flow cytometry, ChiP.

Validation statement for species and application: Synthetic peptide within Human BRD2 aa 1-100 (N terminal). The exact sequence is proprietary. Database link: P25440

ab139690 has been referenced in 18 publications.

Anti-BRD3 antibody [2088C3a] (ab50818)

Describe validation of primary antibody by manufacturer: ab50818 Anti-BRD3 antibody [2088C3a] was shown to specificallyreact with BRD3 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266793 (knockout cell lysate ab258335) was used. Wild-type and BRD3 knockout samples were subjected to SDS-PAGE. ab50818 and Anti-GAPDH antibody[EPR16891] - Loading Control (ab181602) were incubated overnight at 4°C at 1 in 500 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye 680RD) preadsorbed (ab216777) and Goat anti-MouseIgG H&L (IRDye 800CW) preadsorbed (ab216772) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Describe validation of primary antibody by us: Validation of primary antibody by us: Validated by siRNA knockout in https://pubs.acs.org/doi/10.1021/acschembio.5b00216; disappearance of the BRD2 band upon the treatment with MZ1;

Application: Flow Cyt, WB, IP, ICC/IF
Abpromise guarantee covers the use of ab50818 in the following tested applications (Flow Cyt, WB, IP, ICC/IF). The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.
Reacts with: Mouse, Rat, Human; immunogen: recombinant fragment of BRD3 (human)

Recombinant Anti-Brd4 antibody [EPR5150(2)] (ab128874)
Describe validation of primary antibody by manufacturer: ab128874 was shown to recognize Brd4 when Brd4 knockout samples were used, along with additional cross-reactive bands. Wild-type and Brd4 knockout samples were subjected to SDSPAGE. ab128874 and ab8245 (loading control to GAPDH) were diluted at 1/1000 and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye 680RD) preadsorbed (ab216776) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.
Describe validation of primary antibody by us: Validation of primary antibody by us: Validated by siRNA knockout in https://pubs.acs.org/doi/10.1021/acschembio.5b00216; disappearance of the BRD2 band upon the treatment with MZ1;
Abpromise guarantee covers the use of ab128874 in the following tested applications. Suitable for: Flow Cyt (Intra), WB, IHC-P, ICC/IF
Reacts with: Mouse, Human Predicted to work with: Rat; Immunogen: Synthetic peptide within Human Brd4 aa 150-250. The exact sequence is proprietary. Database link: O60885
ab128874 has been referenced in 83 publications.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK293, MV4;11, HL-60, A549 and 22Rv1 cell lines were obtained from ATCC. CRISPR HiBiT-BRD2, BRD3, and BRD4 HEK293 cells lines and cMyc-HiBiT MV4;11 cells lines were made at Promega Corporation. All cell lines (and the CRISPR versions generated) are listed in the 'cell lines and culture' methods section.
Authentication	All cell lines were authenticated by STR analysis. All CRISPR edited cell lines were additionally confirmed by Sanger Sequencing.
Mycoplasma contamination	All cell lines were tested for mycoplasma and found negative.
Commonly misidentified lines (See ICLAC register)	none.

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	MV4;11 cells were counted on a Countess 3 Automated Cell Counter (Thermo Fisher, UK) with the addition of trypan blue
Instrument	BD FACS Canto II flow cytometer (BD Biosciences)
Software	Data were analysed on FlowJo™ 10.7.1. Software and Prism (GraphPad, version 7.05).
Cell population abundance	1x10^6 cells were aliquoted, spun down and resuspended in RPMI media containing test compounds at indicated concentrations
Gating strategy	Gating was performed based on Dmso control and applied unchanged to other conditions, see Supplementary Information Figure 1

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