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Reporting Summary

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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	Flow cytometry data was collected with a BD Biosciences FACSCalibur using CellQuestPro software (version 5.2.1). Western Blot data was collected using a LICOR Odyssey infrared imaging system run by software version 3.0.16. In-gel fluorescent scans were captured with a Typhoon FLA-7000 gel scanner (GE Healthcare) run by software version 1.2.1.93. Peptide mixtures were analyzed by nanoflow reversed-phase liquid chromatography tandem mass spectrometry (LC-MS/MS) on a Easy-nLC 1000 system (Thermo Fisher Scientific, Bremen, Germany) or M-Class UPLC (Waters, Milford, USA), coupled to a Q-Exactive Classic (QE) or Q-Exactive HF (QE-HF) mass spectrometer equipped with a nanoelectrospray ion source and in-source column heater (Sonation, Germany) at 40oC for automated MS/MS (Thermo Fisher Scientific). NMR spectra were recorded on a Bruker Avance DRX 300.
Data analysis	Flow cytometry data was processed using FlowJo software (version 10.5.0). Mass spectrometry data was processed with MaxQuant software (version 1.5.8.30) Data was collated and plotted for presentation in figures with Prism 7 for Mac OS X (version 7.0d).

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

Data relating to the X-ray crystal structure for mouse BAK (Figures 4e&f) was deposited into the Protein Data Bank (accession code 6MCY).
Mass spectrometry data relating to Figure 5b has been uploaded to the PRIDE database (accession PXD011150 and PXD013129).
The authors declare that all other data supporting the findings of this study are available within the paper and its supplementary information files.

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	Experiments were performed at least three independent times (exceptions: data in Supplementary Table 2 which were performed in duplicates across a number of different cell lines and different cell culture conditions with consistent results in all cases; data in Supplementary Figure 3, performed in two separate experiments, individual plots are shown for each experiment). Where knockout cell lines were engineered using CRISPR, three independent sgRNA for each gene were used. When knockout cell lines were clonally derived, at least 3 independent clones were examined. The ability of our inhibitors to block BAK-driven apoptosis differed dramatically in cells of different genotypes and these sample sizes were more than sufficient to clearly distinguish the differences.
Data exclusions	No data has been excluded.
Replication	Our findings have been replicated with several active analogues from our small molecule series and in all cases the findings have been consistent with those reported in the manuscript.
Randomization	Not relevant to our study. All experiments were performed in homogeneous cultured cell lines or primary cells derived from animals for which their was sufficient material to split the sample and expose it to various conditions (vehicle, control compounds, active analogues, inactive analogues) for comparison.
Blinding	Not relevant to our study. All data was collected in an automated fashion and computationally processed in a uniform manner to eliminate as much as possible the introduction of bias.

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	All unique materials described in the study will be made available upon reasonable request.

Antibodies

Antibodies used	BAK: rabbit polyclonal generated to aa23-32; SIGMA; catalog no. B5897; lot# 063M4765V HSP70: clone N6; gift of Robin Anderson FLAG: clone 9H1; gift of Lorraine O'Riley Cytochrome c: clone 7H8.2C12; BD Biosciences; catalog no. 556433 ; lot# 6060713
Validation	These antibodies have all been widely used and validated. Their specificity is clear from internal controls in the data presented in the manuscript.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Most data in the study was collected from SV40 LgT-transformed mouse embryo fibroblasts derived from inbred C57BL/6 mouse colonies. BAX/BAK DKO HCT-116 human cell lines (relating to Figs 2c&d) were kindly provided by Richard Youle. Human cells: RS4;11 and SW480 were sourced from ATCC; Granta-519 and DoHH2 were sourced from DSMZ, CCRF-CEM was sourced from the DTP/DCTD/NCI Tumor repository. LIM2405 was derived at the Ludwig Institute Melbourne Branch.
Authentication	The genotypes of all cell lines used in the study were confirmed by PCR, sequencing, and/or western blotting. For human cells, proof of authentication was provided by the vendors or provider (DSMZ, ATCC, NCI). LIM-2405 and SW480 were authenticated by STR typing.
Mycoplasma contamination	Mycoplasma contamination was routinely monitored using the Lonza MycoAlert Mycoplasma Detection Kit. No Mycoplasma positive cultures were used in the study.
Commonly misidentified lines (See ICLAC register)	None

Animals and other organisms

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

Laboratory animals	Primary cells (thymocytes and platelets) were collected from 8-week old wt, Bak-/- and Bax-/- C57BL/6 mice.
Wild animals	NA
Field-collected samples	NA

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	For cell viability assays, Dead (non-adherent) cells were collected by transferring cell culture media to a FACS tube. Viable (adherent) cells were collected by trypsinization them and transferring to the same corresponding FACS tube. The cells were pelleted and washed once with FACS buffer then resuspended in FACS buffer containing propidium iodide.
Instrument	BD Biosciences FACSCalibur
Software	Collection: CellQuestPro (version 5.2.1) Analysis: FlowJo (version 10.5.0)
Cell population abundance	Not relevant. All analysis was of bulk cell populations from cultured cell lines
Gating strategy	Cells were gated on the basis of forward and side scatter properties. Live/dead cell gates were established on the basis of untreated (viable) and treated (dead) control samples.

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