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Corresponding author(s): Rohan Beckwith

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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 Authors & Referees 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

- Flow cytometry: LSR Fortessa cell analyzer (BD Biosciences)
- Cmpd mass spec: Sciex 6500+ triple quad mass spectrometer
- Chemoprot mass spec: LTQ Orbitrap Elite (Thermo)
- RT-qPCR: ViiA-7 qPCR instrument (Applied Biosystems)
- Cell viability screening assay: Perkin Elmer Viewlux or Envision plate readers
- Gel or film analysis: ChemiDoc (BioRad)
- Imaging smFISH and IHC: Axioimager Z1 microscope (Zeiss)
- RNA sequencing: HiSeq2500 platform (Illumina)
- X-ray data: Pilatus 6M detector at the Advanced Photon Source beamline

Data analysis

- Flow cytometry: FlowJo v10 (Flowjo LLC)
- siRNA screening: RSA, GeneMANIA (genemania.org), Spotfire 6.5 (Tibco)
- X-ray data analysis: autoPROC, PHASER, coot, BUSTER
- Image analysis: Zen (Zeiss), Matlab (Mathworks), ImageJ
- Chemoprot mass spec: Trans-proteomic pipeline (TPP) modules (Institute for Systems Biology), Mascot v 2.5.1 (Matrix Science), ProteinProphet (proteinprophet.sourceforge.net)
- RNA-seq data processing
  - o STAR v2.5.0a, read alignments (to hg19)
  - o SAMtools v1.3.1, indexing of aligned/sorted reads
- Differential gene expression and read-through analysis
  - o QuasR v1.14.0, read counts
  - o edgeR v3.22.3, differential expression analysis
  - o R v3.5.0 (statistical language platform for running QuasR and edgeR)

o Metascape (metascape.org)

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. 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 datasets generated during and/or analyzed during the current study are included in this published article (and its supplementary information files). The raw RNA-sequencing reads are available in the NCBI Sequence Read Archive under accession number SRP158650. X-ray structure data has been deposited to wwPDB with pdb code 6M8Q. All other relevant data are available from the corresponding author on 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	Experiments were performed at least two, (typically three), independent times (exceptions: Xenograft study used a group size of 8 mice per treatment, as is standard. The siRNA library was tested as one replicate with each gene represented by n=8 siRNAs on average per condition.)
Data exclusions	No data was excluded
Replication	Each experiment has been conducted two or more times in this study and was reproduced. In each case, the experiment was conducted with at least three biological replicates. Exceptions is CETSA experiment which is N=1 as workflow becoming patent-protected (March 3 2015), unfortunately we cannot repeat these experiments because of license restrictions
Randomization	Randomization was not relevant in this study as items involved in the study were identical
Blinding	There was no group allocation performed in this study

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
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	PE-conjugated CD11b Manufacturer: BioLegend Catalog number: 301306 Lot number:B183220 PE-conjugated CD44 Manufacturer: BioLegend Catalog number: 338808 Lot number:B189534 PE-conjugated CD54 Manufacturer: BioLegend Catalog number: 353106 Lot number:B169127 PE-conjugated CD73 Manufacturer: BioLegend Catalog number: 344004 Lot number:B189876 Alexa 568 goat anti mouse Manufacturer: ThermoFisher Scientific Catalog number: A11031 Lot number:2026148 Alexa 647 goat anti rabbit Manufacturer: ThermoFisher Scientific Catalog number: A21245 Lot number:2051068

Nucleolin Manufacturer: Abcam Catalog number: ab22758 Lot number:GR303746-3
DNA-RNA Hybrid clone S9.6 Manufacturer: Millipore Catalog number: MABE1095 Lot number:06131_4
CPSF3 Manufacturer: Abgent Catalog number: AT1610a Lot number:11175
FLI1 Manufacturer: Abcam Catalog number: ab124791 Lot number:YH112404C
MEK1/2 Manufacturer: Cell Signaling Technologies Catalog number: 8727 Lot number:5
MYB Manufacturer: Cell Signaling Technologies Catalog number: 12319 Lot number:1
MYC Manufacturer: Cell Signaling Technologies Catalog number: 13987 Lot number:5
NKX2.2 Manufacturer: Abcam Catalog number: ab187375 Lot number:GR299445-4
VIMENTIN Manufacturer: Cell Signaling Technologies Catalog number: 5741 Lot number:1
BETA-ACTIN Manufacturer: Sigma Catalog number: A5441 Lot number:127M4866V

Validation

Link to manufacture's validation data:
PE-conjugated CD11b https://www.biolegend.com/en-us/products/pe-anti-human-cd11b-antibody-768
PE-conjugated CD44 https://www.biolegend.com/en-us/products/pe-anti-human-cd44-antibody-5745
PE-conjugated CD54 https://www.biolegend.com/en-us/products/pe-anti-human-cd54-antibody-7447
PE-conjugated CD73 https://www.biolegend.com/en-us/products/pe-anti-human-cd73-ecto-5-nucleotidase-antibody-6092
Alexa 568 goat anti mouse https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11031
Alexa 647 goat anti rabbit https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21245
Nucleolin https://www.abcam.com/nucleolin-antibody-ab22758.html
DNA-RNA Hybrid clone S9.6 http://www.merckmillipore.com/CH/de/product/Anti-DNA-RNA-Hybrid-Antibody-clone-S9.6,MM_NF-MABE1095
CPSF3 http://www.abgent.com/products/AT1610a-CPSF3-Antibody-monoclonal-M01
FLI1 https://www.abcam.com/fli1-antibody-epr4645-ab124791.html
MEK1/2 https://www.cellsignal.com/products/primary-antibodies/mek1-2-d1a5-rabbit-mab/8727
MYB https://www.cellsignal.com/products/primary-antibodies/c-myb-d2r4y-rabbit-mab/12319
MYC https://www.cellsignal.com/products/primary-antibodies/c-myc-d3n8f-rabbit-mab/13987
NKX2.2 https://www.abcam.com/nkx22-antibody-nx2294-ab187375.html
VIMENTIN https://www.cellsignal.com/products/primary-antibodies/vimentin-d21h3-xp-rabbit-mab/5741
BETA-ACTIN https://www.sigmaaldrich.com/catalog/product/sigma/a5441

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

All cell lines were sources from the CCLE (https://portals.broadinstitute.org/ccle) as previously described (PMID: 22460905)
MONO-MAC-1
EOL-1
U-937
OCI-AML3
MOLM-13
NOMO-1
SKM-1
MHH-ES-1
A-673
MONO-MAC-6
MV-4-11
ML-2
OCI-AML2
NB-4
SK-ES-1
THP-1
WSU-AML
KBM-7
PL-21
AML-193
HL-60
KASUMI-1
SNU-878
BEN
OCI-LY3
BC-3C
P31/FUJ
CMK-11-5
MOLM-16
MOLT-16
KO52
Hep G2
GSS
BDCM
huH-1

OCI-AML5
CADO-ES1
HuH-6
JHH-5
GDM-1
SYO-1
CFPAC-1
OCI-M1
CMK-86
CMK
NCI-H2172
QGP-1
M-07e
HCC-1588
JHOM-1
NCI-H520
RMUG-S
SK-N-MC
COLO-320
SEM
HEL 92.1.7
RS4;11
TC-71
COLO 205
MM603
HCT 116
VM-CUB1
SW 1353
TF-1
KMS-11
SW982
MM473
MM138
Li-7
NCI-H1573
HuH-7
NCI-H661
BFTC-909
JIMT-1
KYSE-510
Hs 695T
Hs 936.T
KYSE-150
BICR 31
LS1034
HCC1143
BICR 18
SNU-C4
NCI-H1373
OC 314
SH-4
CL-14
SNU-349
MM576
UKE1
Kasumi3
SK-MEL-2

Authentication	Cell lines were authenticated by single nucleotide polymorphism (SNP) testing
Mycoplasma contamination	All cell lines tested negative for Mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	The only ICLAC commonly misidentified cell line used in this study was SK-N-MC. This was included to increase Ewing's Sarcoma cell line sample size.

Animals and other organisms

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

Laboratory animals	For xenograft studies female athymic nude mice (Hsd:Athymic Nude-Foxn1nu; from Harlan Laboratories) at age 8 weeks were used. For the PK studies male B6 mice (C57BL/6NCrl; from Charles River Laboratories) at age 8 weeks were used.

Wild animals	The study did not involve wild animals
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal studies were carried out in accordance with federal, state, local and institutional guidelines governing the use of laboratory animals in research, and were approved by the Novartis Institutes for BioMedical Research Institutional Animal Care and Use Committee.

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	For cell surface antibody staining, 1x 106 either A-673 (ATCC CRL-1598) Ewing's sarcoma cells, A-673 cells with FLI1 shRNA1917, or THP-1 (ATCC TIB-202) AML cells were plated, incubated overnight (37°C, 5% CO2, 95% relative humidity), and treated with compound 1 (1µM), DMSO (0.1%) or doxycycline (100 ng mL-1) the following day for 72 h (THP-1), 96 h (A-673), or 120 h (A-673 FLI1 shRNA1917). Cells were washed into FACS staining buffer (PBS, with 1% BSA) and stained with either PE-conjugated CD73 (BioLegend 344004), CD44 (BioLegend 338808), CD54 (BioLegend 353106), or CD11b (BioLegend 301306). For cell cycle and Annexin V staining, 5x 105 NOMO-1 cells were plated, incubated overnight (37°C, 5% CO2, 95% relative humidity), and treated the following day with either compound 1 (1 or 10 µM) or DMSO (0.1%) for 1.5, 4, or 24 h. Cells were lifted, washed with PBS and each sample's volume was split, half for Annexin V staining and half for cell cycle analysis. To assess apoptosis levels, cells were stained with Annexin V Dead Cell Apoptosis Kit (Thermo Fisher Scientific) according to manufacturer's instructions. To assess cell cycle status, cells were permeabilized in 70% ethanol at -20°C for 15 min, followed by PBS washes and staining with BD Pharmingen PI/RNase Staining Buffer (BD Biosciences).
Instrument	LSR Fortessa cell analyzer (BD Biosciences)
Software	Data acquisition using BD FACS Diva V8. Data was analyzed using FLowJo V10 (FLOWJO, LLC)
Cell population abundance	Samples were not sorted, they were analyzed. Analysis was performed on 1x104 live, single cells.
Gating strategy	Samples were processed by gating cells based on SSC-A/FSC-A for live cells and FSC-W/FSC-A for single cells. For Annexin V analysis, PI-A/FITC-A scatter plots were generated with quadrant parameters based on DMSO-treated controls. For cell cycle analysis, cell count/PI-A histograms were generated and analyzed using the FlowJo cell cycle analysis module.

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