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Corresponding author(s): Wenyi Wei

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	Zen (Zeiss) software was used to collect image. FACSDiva (BD Bioscience) software was used to collect flow cytometry data.
Data analysis	We used the Image J software to quantify the protein bands intensity and used the GraphPad (PRISM 8) to generate the graph figures and statistic analyses. We used Mascot 2.5.1 and vScaffold 4.4.8 to analyze the mass spectrometry results. We used BD FACSDiva 6.1.3 software for Flow analysis.

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

Source data for Fig. 1a and Supplementary Fig. 1a have been provided as Supplementary Table 1. The mass spectrometry-based screening data generated in this

study have been deposited in ProteomeXchange under the accession code PXD011657 and are available to the public. All other data supporting the findings of this study are available from the corresponding author on reasonable request.

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	No sample size calculations were performed. Sample size was determined according to our experience as well as literature reporting in terms of specific experiment.
Data exclusions	No samples or animals were excluded from the analyses
Replication	Multiple independent repeats were included for related experiments. Each experiment was performed for at least twice to make sure similar results are reproducible. Animal-related experiments and mass spectrometry experiments have been done once.
Randomization	6-8 week female nude mice were chosen as xenograft hosts. 10 days female and male WT and AKT1-K140/142R-Knockin mice were chosen to monitor the body weight. 4 weeks female and male WT and AKT1-K140/142R-Knockin mice were chosen for carcinogen treatment. All these mice were randomly allocated into experimental groups.
Blinding	For cell-based experiments Western blotting, immunostaining and FACS, cell types were known when prepare the samples or start to treat cells at the beginning of experiments. Data measurement for cell number and colony formation and soft agar assays were blinded to different person who processed assay at the time. IHC staining analysis were performed and analyzed blindedly.

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

Antibodies

Antibodies used

All antibodies were used at a 1:1000 dilution in TBST buffer with 5% non-fat milk for western blot.

Anti-phospho-Ser473-Akt antibody (Cell Signaling Technology, 4060), anti-phospho-Thr308-Akt antibody (Cell Signaling Technology, 2965), anti-Akt1 antibody (Cell Signaling Technology, 2938), anti-Akt total antibody (Cell Signaling Technology, 4691), anti-phospho-Ser9-GSK3b antibody (Cell Signaling Technology, 5558), anti-GSK3b antibody (Cell Signaling Technology, 12456), anti-phospho-FOXO1 (Thr24)/FOXO3A (Thr32) antibody (Cell Signaling Technology, 9464), anti-FOXO3A antibody (Cell Signaling Technology, 2497), anti-GST antibody (Cell Signaling Technology, 2625), anti-pS6K1 (Thr389) antibody (Cell Signaling Technology, 9205), anti-S6K1 antibody (Cell Signaling Technology, 2708) and anti-pS240/244-S6 antibody (Cell Signaling Technology, 5364), Anti-SETDB1 antibody (Proteintech, 11231), Anti-KDM4A antibody (Bethyl, A300-860A), anti-KDM4B antibody (Bethyl, A301-478A), polyclonal anti-HA antibody (Santa Cruz, 805), Polyclonal anti-Flag antibody (Sigma, F-2425), monoclonal anti-Flag antibody (Sigma, F-3165, clone M2), anti-Tubulin antibody (Sigma, T-5168), anti-Flag agarose beads (Sigma, A-2220), anti-HA agarose beads (Sigma, A-2095), peroxidase-conjugated anti-mouse secondary antibody (Sigma, A-4416) and peroxidase-conjugated anti-rabbit secondary antibody (Sigma, A-4914), Monoclonal anti-HA antibody (Covance, MMS-101P) . The polyclonal Akt1-K140-me3 antibodies generated by Cell Signaling Technology (CTS) were derived from rabbit, with each hydroxylation residue produced four clones. The antigen sequence used for immunization was Akt1 aa130-152 (GAEEMEVSLAKPKHRVTMNEFEY). K stands for tri-methylation residue in this synthetic peptide. The antibodies were affinity

purified using the antigen peptide column, but they were not counter selected on unmodified antigen.

Validation

All antibodies used in our study have been validated and detailed information could be found on the website from manufactures as listed below. Some of them have also been validated by our experiments as shown in this manuscript using either over-express, knockout or knockdown strategies.
phospho-Ser473-Akt, https://www.cellsignal.com/products/primary-antibodies/phospho-akt-ser473-d9e-xp-rabbit-mab/4060

phospho-Thr308-Akt, https://www.cellsignal.com/products/primary-antibodies/phospho-akt-thr308-c31e5e-rabbit-mab/2965?site-search-type=Products&N=4294956287&Ntt=%282965%29%2C&fromPage=plp&_requestid=740425

Akt1 antibody, https://www.cellsignal.com/products/primary-antibodies/akt-pan-c67e7-rabbit-mab/4691?site-search-type=Products&N=4294956287&Ntt=%284691%29%2C&fromPage=plp&_requestid=740463

Akt total antibody, https://www.cellsignal.com/products/primary-antibodies/akt1-c73h10-rabbit-mab/2938?site-search-type=Products&N=4294956287&Ntt=%282938%29%2C&fromPage=plp&_requestid=740451

phospho-Ser9-GSK3b antibody, https://www.cellsignal.com/products/primary-antibodies/phospho-gsk-3b-ser9-d85e12-xp-rabbit-mab/5558?site-search-type=Products&N=4294956287&Ntt=%285558%29%2C&fromPage=plp&_requestid=740494

GSK3bantibody, https://www.cellsignal.com/products/primary-antibodies/gsk-3b-d5c5z-xp-rabbit-mab/12456?site-search-type=Products&N=4294956287&Ntt=%2812456%29&fromPage=plp&_requestid=740502

phospho-FOXO1 (Thr24)/FOXO3A (Thr32) antibody, https://www.cellsignal.com/products/primary-antibodies/phospho-foxo1-thr24-foxo3a-thr32-antibody/9464?site-search-type=Products&N=4294956287&Ntt=+%289464%29&fromPage=plp&_requestid=740513

FOXO3A antibody, https://www.cellsignal.com/products/primary-antibodies/foxo3a-75d8-rabbit-mab/2497?site-search-type=Products&N=4294956287&Ntt=%282497%29&fromPage=plp&_requestid=740526

GST antibody, https://www.cellsignal.com/products/primary-antibodies/gst-91g1-rabbit-mab/2625?site-search-type=Products&N=4294956287&Ntt=%282625&fromPage=plp&_requestid=740541

pS6K1 (Thr389) antibody, https://www.cellsignal.com/products/primary-antibodies/phospho-p70-s6-kinase-thr389-antibody/9205?site-search-type=Products&N=4294956287&Ntt=%289205%29&fromPage=plp&_requestid=740549

S6K1 antibody, https://www.cellsignal.com/products/primary-antibodies/p70-s6-kinase-49d7-rabbit-mab/2708?site-search-type=Products&N=4294956287&Ntt=%282708%29&fromPage=plp

pS240/244-S6 antibody, https://www.cellsignal.com/products/primary-antibodies/phospho-s6-ribosomal-protein-ser240-244-d68f8-xp-rabbit-mab/5364?site-search-type=Products&N=4294956287&Ntt=5364%29&fromPage=plp&_requestid=740580

SMYD3 antibody, https://www.cellsignal.com/products/primary-antibodies/smyd3-d2q4v-rabbit-mab/12859?site-search-type=Products&N=4294956287&Ntt=smyd3&fromPage=plp

H3K9me3 antibody, https://www.cellsignal.com/products/primary-antibodies/tri-methyl-histone-h3-lys9-d4w1u-rabbit-mab/13969?site-search-type=Products&N=4294956287&Ntt=h3k9me3+&fromPage=plp

Pan-Kme3 antibody (14680), https://www.cellsignal.com/products/primary-antibodies/tri-methyl-lysine-motif-tme-k-d1l1x-rabbit-mab/14680?_=1541688963603&Ntt=Tri-Methyl-lys&tahead=true

AIF antibody, https://www.cellsignal.com/products/primary-antibodies/aif-d39d2-xp-rabbit-mab/5318?site-search-type=Products&N=4294956287&Ntt=aif&fromPage=plp

Histone H3 antibody, https://www.cellsignal.com/products/primary-antibodies/histone-h3-d1h2-xp-rabbit-mab/4499?site-search-type=Products&N=4294956287&Ntt=histone+h3&fromPage=plp

PDK1 antibody, https://www.cellsignal.com/products/primary-antibodies/pdk1-d4q4d-rabbit-mab/13037?site-search-type=Products&N=4294956287&Ntt=pdk1&fromPage=plp

anti-SETDB1, https://www.cellsignal.com/products/primary-antibodies/eset-d4m8r-xp-rabbit-mab/93212

H3K4me2, https://www.cellsignal.com/products/primary-antibodies/di-methyl-histone-h3-lys4-c64g9-rabbit-mab/9725?_=1541689459579&Ntt=H3K4me2,&tahead=true

SETDB1 antibody (11231), https://www.ptglab.com/products/SETDB1-Antibody-11231-1-AP.htm

KDM4A, https://www.bethyl.com/product/A300-860A/JMJD2A+Antibody

KDM4B, https://www.bethyl.com/product/A301-478A?referrer=search

Akt1 agarose beads, https://www.scbt.com/scbt/product/akt1-antibody-b-1

polyclonal anti-HA antibody (sc-805), https://www.scbt.com/scbt/product/ha-probe-antibody-y-11?requestFrom=search

Polyclonal anti-Flag antibody (F-2425), https://www.sigmaaldrich.com/catalog/product/sigma/f7425?lang=en&region=US

monoclonal anti-Flag antibody (F-3165, clone M2), https://www.sigmaaldrich.com/catalog/product/sigma/f3165?lang=en&region=US

Tubulin antibody (T-5168), https://www.sigmaaldrich.com/catalog/product/sigma/t5168?lang=en&region=US

Flag agarose beads (A-2220), https://www.sigmaaldrich.com/catalog/product/sigma/a2220?lang=en&region=US

HA agarose beads (A-2095), https://www.sigmaaldrich.com/catalog/product/sigma/a2095?lang=en&region=US

peroxidase-conjugated anti-mouse secondary antibody (A-4416), https://www.sigmaaldrich.com/catalog/product/sigma/a4416?lang=en&region=US

peroxidase-conjugated anti-rabbit secondary antibody (A-4914), https://www.sigmaaldrich.com/catalog/product/sigma/a4914?lang=en&region=US

Monoclonal anti-HA antibody (MMS-101P), https://www.biolegend.com/en-us/products/purified-anti-ha-11-epitope-tag-antibody-11374

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK293, HEK293T, DLD1, OVCAR5, HIM and A375 cells were obtained from American Type Culture Collection (ATCC). HCT116 PTEN+/+ and PTEN-/- cells were obtained from Dr. Todd Waldman in School of Medicine, Georgetown University. Setdb1f/f-ER-Cre mouse embryonic fibroblasts (MEFs) were obtained as gifts from Drs. Yoich Shinakai and Matthew C. Lorincz. DLD1-AKT1-/-AKT2-/- (termed AKT1/2-/-) and counterpart cells were kindly provided by Dr. Bert Vogelstein (Johns Hopkins University School of Medicine). Jmjd2bflox/flox MEFs were generated by Dr. Hitoshi Okada (Kindai University of Medicine). Kras;p53 and Kras;p53;sMYD3-/- cells derived from mouse lung and pancreas were obtained from Pawel K. Mazur at Stanford University School of Medicine.
Authentication	Cell lines were not authenticated.
Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	HEK-293T cells were used to for lentiviral and retroviral production.

Animals and other organisms

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

Laboratory animals	NU/J nude female mice at 4-6 week old were purchased from Taconic mouse facility. For Tumor xenograft models, tumor cells were injected subcutaneously into both flanks of 6-8 week old female nude mice. All animal experiments were approved by All experimental procedures were approved by the Institutional Animal Care & Use Committee (IACUC, RN150D) at Beth Israel Deaconess Medical Center with protocol #043-2015. The research projects that are approved by the IACUC are operated according the applicable Institutional regulations. AKT1-K140/142R knockin mice were generated by BIDMC transgenic facility with C57BL6 mice (Jackson Lab). 10 days female and male WT and AKT1-K140/142R-Knockin mice were genotyped and chosen to monitor the body weight. 4 weeks female and male WT and AKT1-K140/142R-Knockin mice were chosen for carcinogen treatment. All these mice were randomly allocated into experimental groups.
Wild animals	No wild animals involved in this study.
Field-collected samples	This study didn't involve samples collected from field.

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	Cells were cultured in 60 cm plates, and were starved for 24 hrs with serum-free and glucose-free DMEM, then subjected to 20 uM 2-NBDG (Sigma 72987) containing glucose-free DMEM for different time points. Last, the cellular glucose uptake was

	quantified by FACS analysis.
Instrument	Using BD FACSCanto II Flow instrument
Software	Using BD FACSDiva 6.1.3 software to collect data and analyze data.
Cell population abundance	At least 10000 cells were analyzed for each sample.
Gating strategy	Cell population gating (FSC-Area VS FSC-Height) was adopted to make sure doublet exclusion and only single cell was used for analysis. A figure exemplifying the gating strategy is provided in the Supplementary Table 2.

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