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Reporting Summary

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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	BD FACSDiva Software version 6.0 for flow cytometry data collection Image Lab 5.2.1 for viewing and processing of Western Blot images Zeiss Observer D1 was used for immnuofluorescence analysis, the accompanying Zeiss AxioVision Release 4.8.2 software was used to capture the resultant images.
Data analysis	Graphpad Prism (Graphpad Software) v 7.0 was used for statistical analyses. FlowJo Version 10.1 was used to analyze flow cytometry data XCMS: version 1.30.3 (Scripps Institute) and TargetLynx module in MassLynx software (version 4.1 SCN810, Waters Corp) was used to analyze Mass Spectrometry Data

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

Correspondence and requests for materials should be addressed to B.L (bing.lim@merck.com) or W.L.T (tamwl@gis.a-star.edu.sg). The metabolomics datasets generated or analyzed during this study are included in this published article in Supplementary Tables 2 and 3. Additional datasets are also 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	For all Experiments at least 3 or more replicates was used in order to obtain statistical significance. For all animal studies, at least 3 animals were used for each experimental group; this is specified in the figure legends.
Data exclusions	No data was excluded from the analysis
Replication	All experiments were replicated at least 3 times using the same experimental conditions. In the case of knockdown experiments, two or more shRNAs were used per gene target. All replication attempts were successful.
Randomization	4-6 week old mice were randomly selected in animal experiments. There was no sex bias in our selection of mice.
Blinding	IHCs performed on tumor microarrays was done in a blinded fashion, where the researcher was unaware of the tumor stage of the individual tumor section.

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

All antibodies are used at 1:1000 dilution unless otherwise stated.

Supplier: Santa Cruz
anti-β-Actin mouse monoclonal clone C4 (sc-47778). Lot no: G0213
anti-GAPDH mouse monoclonal clone 6C5 (sc-32233). Lot no: F2712
Donkey anti-goat secondary antibody, conjugated with HRP-(sc-2304) Lot no: F1803. Dilution 1:5000

Supplier: Abcam
anti-GLDC rabbit polyclonal (ab97625). Lot no: GR277520-6
anti-H3 rabbit polyclonal (ab1791). Lot no: GR150473-1
anti-H3(dimethylK36) rabbit polyclonal (ab9049). Lot No: GR75522-3
anti MAT2A rabbit polyclonal (ab189208). Lot no: GR179402-12
anti MTR goat polyclonal (ab9209). Lot no: GR65779-8
anti-H3(trimethylK79) rabbit polyclonal (ab2621). Lot no: GR188147-1
anti MTHFR rabbit polyclonal (ab125707). Lot no: GR289909-1
Anti-GFP chicken polyclonal (ab13970). Lot no: GR236651-10
Anti-Histone H4 rabbit polyclonal (ab 7311). Lot no: GR325224-1
Anti-MTAP mouse monoclonal (ab 55517). Lot no: GR305460-1

Supplier: Active Motif
anti H3(trimethyl K4) rabbit polyclonal (39915). Lot no: 12613005

anti H3(trimethyl K27) rabbit polyclonal (39155). Lot no: 12912013
anti-H3(trimethylK36) rabbit polyclonal (61101). Lot no: 12912002
anti-H3(trimethylK9) rabbit polyclonal (39765). Lot no: 31610002

Supplier: BD Biosciences
anti-β-catenin mouse monoclonal Clone 14/Beta-Catenin (BD610154). Lot no: 6176992

Supplier: Genetex
anti-MAT2A mouse monoclonal Clone AT3A2 (GTX50027). Lot no: 821803570

Supplier: Sigma
Anti-MAT2A rabbit polyclonal (HPA043028). Lot no:R40523
Anti-SHMT2 rabbit polyclonal (HPA020549). Lot no: P09754
Anti-AHCY mouse monoclonal antibody clone M1, (WH0000191M8). Lot no:08183-M1
Anti-CD166/ALCAM rabbit polyclonal (HPA010926). Lot no:B75231

Supplier: R&D Biosystems
Anti ALCAM-PE conjugate clone 105902 (FAB6561P). Lot no: LAR0413011

Supplier: CST
Anti-Symmetric Di-Methyl Arginine Motif rabbit monoclonal (13222). Lot no: 1.
Goat anti-rabbit IgG, HRP-linked Antibody (7074P2). Lot no: 25. Dilution 1:5000
Goat anti-mouse IgG, HRP-linked Antibody (7076S). Lot no: 33. Dilution 1:5000

Supplier: Thermofisher
Goat anti-Chicken IgY (H+L) Secondary Antibody, Alexa Fluor 488. Polyclonal (A-11039). Lot no: 1759025
Donkey anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor 594. Polyclonal (A21203). Lot no: 1820027
Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 647. Polyclonal (A21245). Lot no: 1558736

Validation

Previously published antibodies are cited in the Methods section and below. Human and species cross-reactivity was determined by Western Blots. Manufacturer validation statements of specific antibodies are stated below.
MTHFR antibody was validated by us in IHC and shRNA knockdown experiments using TS cell lysates in Extended Figures 4b and 3f.
Sigma AHCY (SAHH,WH0000191M8) was validated by us in WB experiments using TS cell lysates in Figure 1i.
Sigma MAT2A antibody (HPA043028) was validated by us in IHC experiments in Figures 4b and 4c.
Genetex MAT2A antibody (GTX50027) was validated by us in immunofluorescence (IF) experiments in Figures 4e.
MAT2A antibody (ab 189208) was validated by us in shRNA knockdown experiments in TS cell lysates in Extended Figure 4a.

Supplier: Santa Cruz
anti-β-Actin(1) mouse monoclonal clone C4 (sc-47778). Detects human and mouse β-Actin by WB, IP, IF, IHC(P) and ELISA .
anti-GAPDH(2) mouse monoclonal clone 6C5 (sc-32233). Detects human and mouse GAPDH by WB, IP, and IF.
Donkey anti-goat secondary antibody, conjugated with HRP-(sc-2304) Manufacturer validates detection of goat IgG by WB.

Supplier: Abcam
anti-GLDC(3) rabbit polyclonal (ab97625). Manufacturer validates detection of human GLDC by WB, ICC/IF.
anti-H3(4) rabbit polyclonal (ab1791). Manufacturer validates detection of human and mouse H3 by WB.
anti-H3(dimethylK36)(5) rabbit polyclonal (ab9049). Manufacturer validates detection of human H3 and mouse (dimethylK36) by WB.
anti MAT2A rabbit polyclonal (ab189208). Manufacturer validates detection of human and mouse MAT2A by WB. Validated by us in shRNA knockdown experiments in TS cell lysates in Extended Figure 4a
anti MTR(6) goat polyclonal (ab9209). Manufacturer validates detection of human MTR by WB and IHC-P.
anti-H3(trimethylK79)(7) rabbit polyclonal (ab2621). Manufacturer validates detection of human and mouse H3(trimethylK79) by ICC/IF, ChIP, WB, ChIP/Chip.
anti MTHFR rabbit polyclonal (ab125707) Manufacturer validates detection of human and mouse MTHFR by WB (manufacturer).
MTHFR antibody was validated by us in IHC and shRNA knockdown experiments in TS cell lysates in Extended Figures 4b and 3f.
Anti-GFP(8) chicken polyclonal (ab13970). Manufacturer validates detection of GFP by IF.
Anti-Histone H4(9) rabbit polyclonal (ab 7311) Manufacturer validates detection of human and mouse H4 by WB.
Anti-MTAP(10) mouse monoclonal (ab 55517) Manufacturer validates detection of human MTAP by WB.

Supplier: Active Motif
anti H3(trimethyl K4)(11) rabbit polyclonal (39915). Manufacturer validates detection of human and mouse H3(trimethyl K4) by WB.
anti H3(trimethyl K27)(12) rabbit polyclonal (39155). Manufacturer validates detection of human and mouse H3(trimethyl K27) by WB.
anti-H3(trimethylK36)(13) rabbit polyclonal (61101). Manufacturer validates detection of human and mouse H3(trimethylK36) by WB.
anti-H3(trimethylK9)(14) rabbit polyclonal (39765). Manufacturer validates detection of human and mouse H3(trimethylK9) by WB.

Supplier: BD Biosciences
anti-β-catenin(15) mouse monoclonal Clone 14/Beta-Catenin (BD610154). Manufacturer validates detection of human and mouse β-catenin by WB.

Supplier: Genetex

anti-MAT2A(16) mouse monoclonal Clone AT3A2 (GTX50027). Manufacturer validates detection of human MAT2A by WB and IHC-P. Validated by us in immunofluorescence (IF) experiments in Figure 4e.

Supplier: Sigma
Anti-MAT2A rabbit polyclonal (HPA043028). Manufacturer validates detection of human MAT2A by IHC in the Human Protein Atlas. Validated by us in IHC experiments in Figures 4b and 4c
Anti-SHMT2(17) rabbit polyclonal (HPA020549). Manufacturer validates detection of human SHMT2 by WB.
Anti-SAHH mouse monoclonal antibody clone M1, (WH0000191M8). Manufacturer validates detection of human SAHH by WB. Validated by us in WB experiments using TS cell lysates in Figure 1i
Anti-CD166/ALCAM(18) rabbit polyclonal (HPA010926). Manufacturer validates detection of human CD166/ALCAM by IHC in the Human Protein Atlas.

Supplier: R&D Biosystems
Anti ALCAM-PE(3) conjugate clone 105902 (FAB6561P). Manufacturer validates detection of human CD166/ALCAM for flow-cytometry.

Supplier: CST
Anti-Symmetric Di-Methyl Arginine Motif(19) rabbit monoclonal (13222). Manufacturer validates detection of human SDMA for WB.
Goat anti-rabbit IgG, HRP-linked Antibody (7074P2). Manufacturer validates detection of rabbit IgG in WB.
Goat anti-mouse IgG, HRP-linked Antibody (7076S). Manufacturer validates detection of rabbit IgG in WB.

Supplier: Thermofisher
Goat anti-Chicken IgY (H+L) Secondary Antibody, Alexa Fluor 488. Polyclonal (A-11039). Manufacturer validates use in IF.
Donkey anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor 594. Polyclonal (A21203). Manufacturer validates use in IF.
Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 647. Polyclonal (A21245). Manufacturer validates use in IF.

References:
1. Davis, M.A., et al. Calpain drives pyroptotic vimentin cleavage, intermediate filament loss, and cell rupture that mediates immunostimulation. Proceedings of the National Academy of Sciences of the United States of America (2019).
2. Zizza, P., et al. TRF2 positively regulates SULF2 expression increasing VEGF-A release and activity in tumor microenvironment. Nucleic Acids Res (2019).
3. Zhang, W.C., et al. Glycine decarboxylase activity drives non-small cell lung cancer tumor-initiating cells and tumorigenesis. Cell 148, 259-272 (2012).
4. Garcia-Lopez, S., et al. Deregulation of the imprinted DLK1-DIO3 locus ncRNAs is associated with replicative senescence of human adipose-derived stem cells. PloS one 13, e0206534 (2018).
5. Wang, L., et al. H3K36 trimethylation mediated by SETD2 regulates the fate of bone marrow mesenchymal stem cells. PLoS Biol 16, e2006522 (2018).
6. Fofou-Caillierez, M.B., et al. Interaction between methionine synthase isoforms and MMACHC: characterization in cblG-variant, cblG and cblC inherited causes of megaloblastic anaemia. Hum Mol Genet 22, 4591-4601 (2013).
7. Zhu, B., et al. The protective role of DOT1L in UV-induced melanomagenesis. Nat Commun 9, 259 (2018).
8. Papal, S., Monti, C.E., Tennison, M.E. & Swaroop, A. Molecular dissection of cone photoreceptor-enriched genes encoding transmembrane and secretory proteins. J Neurosci Res 97, 16-28 (2019).
9. Cooper, S., et al. Jarid2 binds mono-ubiquitylated H2A lysine 119 to mediate crosstalk between Polycomb complexes PRC1 and PRC2. Nat Commun 7, 13661 (2016).
10. Limm, K., Dettmer, K., Reinders, J., Oefner, P.J. & Bosserhoff, A.K. Characterization of the Methylthioadenosine Phosphorylase Polymorphism rs7023954 - Incidence and Effects on Enzymatic Function in Malignant Melanoma. PloS one 11, e0160348 (2016).
11. Chen, X., Zhi, X., Wang, J. & Su, J. RANKL signaling in bone marrow mesenchymal stem cells negatively regulates osteoblastic bone formation. Bone Res 6, 34 (2018).
12. Li, Y., et al. Genome-wide analyses reveal a role of Polycomb in promoting hypomethylation of DNA methylation valleys. Genome Biol 19, 18 (2018).
13. Fang, D., Gan, H., Wang, H., Zhou, H. & Zhang, Z. Probe the function of histone lysine 36 methylation using histone H3 lysine 36 to methionine mutant transgene in mammalian cells. Cell Cycle 16, 1781-1789 (2017).
14. Zhang, X., et al. H3 ubiquitination by NEDD4 regulates H3 acetylation and tumorigenesis. Nat Commun 8, 14799 (2017).
15. Freeman, T.J., et al. Inhibition of Pannexin 1 Reduces the Tumorigenic Properties of Human Melanoma Cells. Cancers (Basel) 11(2019).
16. Zhang, W., et al. Fluorinated N,N-dialkylaminostilbenes repress colon cancer by targeting methionine S-adenosyltransferase 2A. ACS chemical biology 8, 796-803 (2013).
17. Kim, D., et al. SHMT2 drives glioma cell survival in ischaemia but imposes a dependence on glycine clearance. Nature 520, 363-367 (2015).
18. Kahlert, C., et al. Increased expression of ALCAM/CD166 in pancreatic cancer is an independent prognostic marker for poor survival and early tumour relapse. Br J Cancer 101, 457-464 (2009).
19. Gerhart, S.V., et al. Activation of the p53-MDM4 regulatory axis defines the anti-tumour response to PRMT5 inhibition through its role in regulating cellular splicing. Scientific reports 8, 9711 (2018).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Tumor sphere lines were derived by us from freshly resected human non-small cell lung cancer patient samples. Adh and GLDC KD lines were further derived from Tumor sphere lines. All other human cancer and murine cell lines (NIH 3T3, NIH

	3T3-L1, HLF, MRC-5,PC-9, OVCAR-5, HT-29, SW 480, CACO-2, HCT-116, MCF-7, MDA-MB-436 and SUM-159) were obtained from ATCC.
Authentication	Tumor sphere lines were derived from us from human primary tissue samples. We confirmed their origin and cell type by human protein expression analysis and genomic exon-sequencing analysis (See Supplementary Table 1). All other human cancer and murine cell lines were obtained from ATCC, and not directly authenticated by us.
Mycoplasma contamination	All cell lines were tested regularly (e.g, every 6 months for mycoplasma contamination) and results were negative.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

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

Laboratory animals	4-6 weeks male and female NOD.Cg-Prkdc(scid) Il2rg(tm1wjl) SzJ (Jackson Laboratories). Mice were randomly allocated and not selected by sex or age into experimental groups.
Wild animals	Study did not involve wild animals.
Field-collected samples	Study did not involve field-collected samples.
Ethics oversight	All animal experiments were approved by the Agency for Science Technology and Research Singapore – Biological Resource Centre IACUC (Protocol number 171286). The use of patient derived xenografts (PDX) has been approved by the SingHealth Centralised Institutional Review Board (CIRB Ref: 2007/444/B).

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	Relevant clinical information is provided in Supplementary Table 1.
Recruitment	Written informed consent was obtained from all participating patients diagnosed with Non-Small Cell Adenocarcinoma prior to surgical resection or biopsy. Clinical material was then used to derive patient derived xenografts in immunocompromised mice. Self selection bias is unlikely to be present and is unlikely to affect downstream results.
Ethics oversight	The use of patient derived xenografts (PDX) has been approved by the SingHealth Centralised Institutional Review Board (CIRB Ref: 2007/444/B).

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	Human tumor sphere cells previously derived from patient xenografts were used. These cells were first dissociated with accutase prior to analysis. For human patient sample derived PDX cells, , tumors were harvested in cold PBS with antibiotics, chopped with a sterile blade and incubated in 1mg/ml collagenase/dispase (Sigma Aldrich) in DMEM/F12 media (Thermo Fisher Scientific) at 37 °C with agitation. Suspensions were then washed in PBS and passed through 70 and 40 µm cell strainers, centrifuged and evaluated for cell viability by tryphan blue exclusion before downstream assays. Dissociated cells were stained with antibodies conjugated with PE against human CD166 (R&D Systems FAB6561P) in DPBS containing 5 % FBS for 1 hour at 4°C. Cells were then washed and resuspended in DPBS. The fluorescence intensity was measured on a flow cytometer (BD LSR II). Cell sorting was performed on a FACSAria II (BD).
Instrument	SRII (BD) for cytometry. FACSARiaII (BD) for FACS sorting
Software	Collection: BD FACSDiva Software version 6.0 Analysis: Flowjo 10.1

Cell population abundance	%CD166+ cells within tumors were reported in Supplementary Figures and FIgures. The purity was verified by flow cytometry. and sorted cells were immediately used for downstream analysis.
Gating strategy	Refer to Supplementary Figure 1 for gating strategy. An 'Unstained' sample is used as a negative control.

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