

Reporting Summary

Nature Portfolio 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 Portfolio 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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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 v9.0,Delta Vision OMX v3.70, AMT Imaging System v.7
Data analysis	FlowJo v10.10, Graphpad Prism 10, Gene Set Enrichment Analysis (GSEA) v4.2.3, ImageJ v1.54g, Burrows-Wheeler Aligner(BWA) v0.7.17-r1188, Circle-Map v.1.1.2, CNVkit v0.9.10, circlize v 0.4.15, Bowtie2 v 2.3.5.1, FastQC v0.11.9 , SAMBLASTER v0.1.26, SAMTOOLS v1.13, SAMBAMBA v0.6.6, MACS2 v2.2.8, CHIPseeker v1.30.3, clusterProfiler v4.2.2, Manorm v1.1.4, deepTools v3.5.1, ChromHMM v1.24, STAR v2.7.3a, Trimmomatic v0.36, HTseq v2.0.2, EdgeR v3.36.0, maftools v2.10.05, ggplot2 v3.5.0 , R v4.3.2 , Peak Scanner Software v1.0, Wound healing size tool, Aperio eSlide manager v12.4.2.5010, Colour Deconvolution2, Bio-Formats v7.3.1, OpenComet v1.3.1 , Seahorse Wave Software v2.6, Biorender, Proteome Discoverer v2.4

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 Portfolio [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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

The ChIP-seq, RNA-seq and WGS data supporting this study have been deposited in Sequence Read Archive (SRA) under accession code PRJNA1173195 (<https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA1173195>). The mouse reference genome (mm10) coordinates are available in GENCODE (https://www.gencodegenes.org/mouse/release_M10.html). The human reference genome (hg19) coordinates are available in GENCODE (https://www.gencodegenes.org/human/release_19.html). The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD065234 (<https://www.ebi.ac.uk/pride/archive/projects/PXD065234>). The clinical and genomic data for patients with resectable and advanced bladder cancer receiving cisplatin-based chemotherapy is available in cBioPortal as blca_msk_tcga_2020 (https://www.cbioportal.org/study/summary?id=blca_msk_tcga_2020) and paired_bladder_2022 (https://www.cbioportal.org/study/summary?id=paired_bladder_2022). The amplicon intervals for The Cancer Genome Atlas (TCGA) cohort are available as Supplementary Table 1 of previously reported study (<https://www.nature.com/articles/s41588-020-0678-2#Sec20>). The overall survival, tumor mutation burden and mRNA expression data for IMvigor210 study is available as IMvigor210CoreBiologies R package (<http://research-pub.gene.com/IMvigor210CoreBiologies/>). The clinical and genomic data for MSK_2018 cohort is available in cBioPortal as tmb_mskcc_2018 (http://www.cbioportal.org/study?id=tmb_mskcc_2018). The tumor mutations burden and mRNA expression data for TCGA-BLCA cohort is available at cBioPortal (https://www.cbioportal.org/study/summary?id=blca_tcga_pub_2017). Somatic variants summary for bladder cancer patients is available at TCGA (<https://portal.gdc.cancer.gov/projects/TCGA-BLCA>). The genomic and mRNA expression data for HCRN cohort is available in cBioPortal with identifier blca_bcan_hcrn_2022 (https://www.cbioportal.org/study/clinicalData?id=blca_bcan_hcrn_2022). The remaining data are available within the Article, Supplementary Information or Source Data file.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	The present study does not involve any human research participants.
Reporting on race, ethnicity, or other socially relevant groupings	The present study does not involve any human research participants.
Population characteristics	The present study does not involve any human research participants.
Recruitment	The present study does not involve any human research participants.
Ethics oversight	The present study does not involve any human research participants.

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

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](https://www.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 murine and human cell line experiments, no statistical method was used to predetermine sample size but our sample sizes are similar to those reported in previous publications (PMID: 37081259, PMID: 36646856, PMID: 36585453).
Data exclusions	No data was excluded.
Replication	All the experiments were replicated 2-3 times with similar results.
Randomization	For murine in-vivo tumor experiments, mice were randomly assigned to different treatment groups. For all other in-vitro experiments random allocation of samples was done to minimize biased assessments.
Blinding	The Investigators conducting the sample preparation and data collection were not blinded to experimental conditions. Conversely, the bioinformatician who performed the analysis of the ChIP-seq, bulk RNA-seq and WGS-seq data was blinded to the experimental conditions, as she was not involved in the upstream sample preparation or processing. The lack of blinding in the sample preparation and data collection phases was considered inconsequential to the study's conclusions, as the observed experimental results would remain consistent irrespective of the blinding status.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

Antibody; Clone; Company; Catalogue Number;RRID,Dilution

KDM6A / UTX Primary Antibody, Clone D3Q1I,Cell Signaling Technology,Cat# 33510, RRID: AB_2721244,1:2000
 Beta Actin Rabbit Secondary Antibody,Cell Signaling Technology,Cat# 4967, RRID: AB_330288,1:2000
 Anti-Mouse CD45 Antibody,Clone 30-F11,Standard BioTools,Cat# 3089005B, RRID: AB_2651152,1:200
 KLRG1,clone2F1,Millipore Sigma,Cat#MABF467, 1:200
 Anti-Mouse CD11c Antibody,Clone N418,Standard BioTools, Cat# 3142003B, RRID: AB_2814737, 1:50
 Anti-mouse CD4 Antibody,Clone RM4-5, BioLegend, Cat# 100561, RRID: AB_2562762, 1:200
 Anti-mouse CD62L Antibody,Clone MEL-14,BioLegend, Cat# 104402, RRID: AB_313089, 1:100
 Anti-mouse CD73 Antibody,Clone TY/23,BioLegend,Cat# 117202, RRID: AB_2936539, 1:400
 Anti-Mouse CD8a Antibody, Clone 53-6.7, Standard BioTools Cat# 3146003B, RRID: AB_2687833, 1:100
 Anti-mouse Ly-6G Antibody, Clone 1A8, BioLegend Cat# 127637, RRID: AB_2563784, 1:200
 Anti-Mouse CD11b Antibody, Clone M1/70, Standard BioTools Cat# 3148003B, RRID: AB_2814738, 1:1600
 Anti-Mouse CD19 Antibody ,Clone 6D5, Standard BioTools Cat# 3149002B, RRID: AB_2814679, 1:200
 Anti-Mouse Ly-6C Antibody, Clone HK1.4, Standard BioTools Cat# 3150010B, RRID: AB_2895118, 1:400
 Anti- CD25 Antibody ,Clone 3C7, Standard BioTools Cat# 3151007B, RRID: AB_2827880, 1:50
 Anti-Mouse CD3e Antibody ,Clone 145-2C11, Standard BioTools Cat# 3152004, RRID: AB_2687836, 1:100
 TIGIT, clone 1G9, Millipore Sigma Cat#MABF2657 , 1:50
 CTLA-4, clone UC10-4B9, Standard BioTools Cat#3154008B , 1:100
 Anti-T-bet Antibody ,Clone 4B10, BioLegend Cat# 644825, RRID: AB_2563788,1:150
 Anti-human/mouse Granzyme B Recombinant Antibody ,Clone QA16A02, BioLegend Cat# 372202, RRID: AB_2686929, 1:50
 Anti-Mouse Foxp3 Antibody ,Clone FJK-16s, Standard BioTools Cat# 3158003A, RRID: AB_2814740, 1:100
 PD-1, clone J43, Standard BioTools Cat#3159023B, 1:100
 Anti-mouse CD38 Antibody ,clone 90, BioLegend Cat# 102702, RRID: AB_312923 , 1:400
 Anti-Mouse iNOS Antibody ,Clone CXNFT, Standard BioTools Cat# 3161011B, RRID: AB_2922920, 1:100
 Anti-mouse CD204 ,Clone 7G5C33, BioLegend Cat# 676202, RRID: AB_2565623, 1:100
 Anti-mouse CD273/PD-L2 ,Clone TY25,BioLegend Cat# 107202, RRID: AB_345250, 1:100
 Anti-Mouse LAP/TGFB Antibody ,Clone TW7-16B4, Standard BioTools Cat# 3164014, RRID: AB_2687842 , 1:200
 Anti-mouse IFN-γ ,Clone XMG1.2,BioLegend Cat# 505843, RRID: AB_2562847,1 :75
 EOMES Monoclonal Antibody ,Clone Dan11mag,Thermo Fisher Scientific, Cat# 14-4875-82, RRID: AB_11042577, 1:150
 CD80 Monoclonal Antibody ,Clone 16-10A1, Thermo Fisher Scientific, Cat# 14-0801-82, RRID: AB_467346, 1:800
 Arginase I Antibody ,Clone 8C9, Santa Cruz Biotechnology, Cat# sc-47715, RRID: AB_626697, 1:300
 Anti-Mouse CD206/MMR ,Clone C068C2, Standard BioTools ,Cat# 3169021B, RRID: AB_2832249, 1:75
 Anti-Mouse NK1.1 Antibody ,Clone PK136, Standard BioTools ,Cat# 3170002B, RRID: AB_2885023, 1:100
 Anti-Human/Mouse CD44 ,Clone IM7, Standard BioTools, Cat# 3171003B, RRID:AB_2895121, 1:400
 Gata-3 Monoclonal Antibody, Clone TWAJ, Thermo Fisher Scientific, Cat# 14-9966-82, RRID: AB_1210519, 1:100
 Anti Mouse CD278/ICOS BioLegend, Cat# 117408, RRID: AB_2122603, 1:100
 LAG3, clone C9B7W, Standard BioTools Cat#3174019B ,1:100
 F4/80 Monoclonal Antibody ,Clone BM8, Thermo Fisher Scientific ,Cat# 14-4801-82, RRID: AB_467558, 1:100
 Anti-mouse CD86 Antibody ,Clone GL-1, BioLegend Cat# 105001, RRID: AB_313144, 1:100
 Rabbit Anti-mouse EXO1, Bioss ,Cat#BS-13119R, 1:200
 Mouse Anti-Phospho-Histone H2A.X (Ser 139) Abcam ,Cat#ab242296 , 1:100
 Goat anti-mouse FITC-conjugated secondary Abcam ,Cat#ab242296 , 1:100
 H3K4me3 Histone antibody Active Motif Cat#39159, RRID:AB_2615077, 10μg per pull down reaction
 H3K27ac Histone antibody Active Motif Cat#39133, RRID:AB_2561016, 10μg per pull down reaction
 H3K27me3 Histone antibody Active Motif Cat#39155, RRID:AB_2561020, 10μg per pull down reaction

H3K9la Histone Antibody, Clone 13H2L1, PTM-BIO Cat# PTM-1419RM, RRID:AB_3076695, 10µg per pull down reaction
H3K18la Histone Antibody ,Clone 22h2L3, PTM-BIO Cat# PTM-1406RM, RRID:AB_2909438, 10µg per pull down reaction
H3K9ac Histone antibody Active Motif Cat#39137, RRID:AB_2561017, 10µg per pull down reaction
Anti-mouse MSH2 Antibody ,Clone 2L9I4, conjugated in-house with AF647 Bio-Techne Cat #NBP3-16692, 1:50
Anti-mouse MSH6 Antibody, Clone 035, conjugated in-house with AF647 Bio-Techne Cat # NBP2-89265 , 1:50
Purified Anti-Mouse CD16/CD32 (Mouse BD Fc Block) ,Clone 2.4G2, BD Biosciences Cat # 553142, RRID:AB_394657,1:200
Annexin V Pacific Blue ,ThermoFisher Scientific Cat # A35122, 1:20
Alexa Fluor 532 anti-mouse CD45 Antibody ,Clone 30-F11, eBioscience Cat # 58-0451-82, RRID:AB_11218871, 1:50
Alexa Fluor 488 anti-mouse CD3 Antibody ,Clone 17A2, BioLegend Cat# 100210, RRID:AB_493530, 1:50
Alexa Fluor 700 anti-mouse CD4 Antibody ,Clone RM4-5, BioLegend Cat# 100536,RRID:AB_493701, 1:50
Brilliant Violet 650 anti-mouse CD25 Antibody ,Clone PC61, BioLegend Cat# 102038, RRID: AB_2563060, 1:50
PerCP-Cyanine5.5 anti-mouse FOXP3 Antibody ,Clone FJK-16s, eBioscience Cat# 45-5773-82, RRID: AB_914351, 1:25
Brilliant Violet 605 anti-Mouse PD-1 ,Clone J43, BD Biosciences Cat # 563059, RRID: AB_2737980, 1:50
Brilliant Violet 421 anti-mouse TGF-β1 Antibody, Clone TW7-16B4, BioLegend Cat# 141407, RRID:AB_2561580, 1:25
H3K9la Histone Antibody, Clone 13H2L1, conjugated in-house with AF647, PTM-BIO Cat# PTM-1419RM, RRID:AB_3076695, 1:100
H3K18la Histone Antibody ,Clone 22h2L3, conjugated in-house with AF647 PTM-BIO Cat# PTM-1406RM, RRID:AB_2909438, 1:100
Pacific Blue anti-mouse CD45 Clone 30-F11, BioLegend Cat#103126, RRID: AB_493535, 1:50
Phycoerythrin anti-mouse E-Cadherin ,Clone 4A2, Cell Signaling Technology Cat#82718, RRID: AB_2799998, 1:50
Anti-PD-1 ,Clone RMP1-14, Bio X Cell Cat# BE0146, RRID: AB_10949053, 200 µg, 100 µg, and 100 µg
Anti-mouse CD3e, Clone 145-2C11, BD Biosciences Cat # 553057, RRID: AB_394590, 3 µg/ml
Anti-mouse CD28, Clone: 37.51 BD Biosciences Cat # 553294, RRID: AB_394763, 2 µg/ml
Pan Anti-Kla Antibody (Anti-L-Lactyllysine Rabbit pAb) PTM BIO Cat #PTM-1401, RRID:AB_2868521,10µg per pull down reaction

Validation

These were all previously validated commercially available antibodies. . Manufacturer validation and references for each can be found : Antibody, Supplier name , Catalog number, Clone name,Manufacturer information.
KDM6A / UTX Primary Antibody (Clone D3Q1I) Cell Signaling Technology Cat# 33510, RRID: AB_2721244,<https://www.cellsignal.com/products/primary-antibodies/utx-d3q1i-rabbit-mab/33510>
Beta Actin Rabbit Secondary Antibody Cell Signaling Technology Cat# 4967, RRID: AB_330288,<https://www.cellsignal.com/products/primary-antibodies/b-actin-antibody/4967>
Anti-Mouse CD45 Antibody (Clone 30-F11) Standard BioTools Cat# 3089005B, RRID: AB_2651152,https://store.standardbio.com/product_detail/guest-catalog/3089005B
KLRG1 Millipore Sigma Cat#MABF467, clone2F1,<https://www.sigmaaldrich.com/US/en/product/mm/mabf467>
Anti-Mouse CD11c Antibody (Clone N418) Standard BioTools Cat# 3142003B, RRID: AB_2814737
Anti-mouse CD4 Antibody (Clone RM4-5) BioLegend Cat# 100561, RRID: AB_2562762,<https://www.biolegend.com/en-gb/products/purified-anti-mouse-cd4-maxpar-ready-antibody-9153>
Anti-mouse CD62L Antibody (Clone MEL-14) BioLegend Cat# 104402, RRID: AB_313089,<https://www.biolegend.com/en-ie/products/purified-anti-mouse-cd62l-antibody-388>
Anti-mouse CD73 Antibody (Clone TY/23) BioLegend Cat# 117202, RRID: AB_2936539,<https://www.biolegend.com/de-at/products/purified-anti-mouse-cd73-antibody-22685>
Anti-Mouse CD8a Antibody (Clone 53-6.7) Standard BioTools Cat# 3146003B, RRID: AB_2687833
Anti-mouse Ly-6G Antibody (Clone 1A8) BioLegend Cat# 127637, RRID: AB_2563784,<https://www.biolegend.com/nl-nl/products/purified-anti-mouse-ly-6g-antibody-4767?GroupID=BLG7232>
Anti-Mouse CD11b Antibody (Clone M1/70) Standard BioTools Cat# 3148003B, RRID: AB_2814738,https://store.standardbio.com/product_detail/guest-catalog/3143015B
Anti-Mouse CD19 Antibody (Clone 6D5) Standard BioTools Cat# 3149002B, RRID: AB_2814679,https://store.standardbio.com/product_detail/guest-catalog/3149002B
Anti-Mouse Ly-6C Antibody (clone HK1.4) Standard BioTools Cat# 3150010B, RRID: AB_2895118
Anti- CD25 Antibody (Clone 3C7) Standard BioTools Cat# 3151007B, RRID: AB_2827880
Anti-Mouse CD3e Antibody (Clone 145-2C11) Standard BioTools Cat# 3152004, RRID: AB_2687836
TIGIT, clone 1G9 Millipore Sigma Cat#MABF2657,<https://www.sigmaaldrich.com/US/en/product/mm/mabf2657>
CTLA-4, clone UC10-4B9 Standard BioTools Cat#3154008B
Anti-T-bet Antibody (Clone 4B10) BioLegend Cat# 644825, RRID: AB_2563788,<https://www.biolegend.com/ja-jp/products/purified-anti-t-bet-maxpar-ready-antibody-10169>
Anti-human/mouse Granzyme B Recombinant Antibody (Clone QA16A02) BioLegend Cat# 372202, RRID: AB_2686929,<https://www.biolegend.com/nl-nl/products/apc-anti-human-mouse-granzyme-b-recombinant-antibody-14429>
Anti-Mouse Foxp3 Antibody (Clone FJK-16s) Standard BioTools Cat# 3158003A, RRID: AB_2814740,https://store.standardbio.com/product_detail/guest-catalog/3158003a/
PD-1, clone J43 Standard BioTools Cat#3159023B,
Anti-mouse CD38 Antibody (clone 90) BioLegend Cat# 102702, RRID: AB_312923,<https://www.biolegend.com/nl-be/products/purified-anti-mouse-cd38-antibody-185>
Anti-Mouse iNOS Antibody (Clone CXNFT) Standard BioTools Cat# 3161011B, RRID: AB_2922920
Anti-mouse CD204 (Clone 7G5C33) BioLegend Cat# 676202, RRID: AB_2565623,<https://www.biolegend.com/ja-jp/products/purified-anti-mouse-cd204-antibody-11787>
Anti-mouse CD273/PD-L2 (Clone TY25) BioLegend Cat# 107202, RRID: AB_345250,<https://www.biolegend.com/nl-be/products/purified-anti-mouse-cd273-b7-dc-pd-l2-antibody-2545>
Anti-Mouse LAP/TGFB Antibody (Clone TW7-16B4) Standard BioTools Cat# 3164014, RRID: AB_2687842
Anti-mouse IFN-γ (Clone XMG1.2) BioLegend Cat# 505843, RRID: AB_2562847,<https://www.biolegend.com/fr-lu/products/purified-anti-mouse-ifn-gamma-maxpar-ready-antibody-9213>
EOMES Monoclonal Antibody (Clone Dan11mag) Thermo Fisher Scientific Cat# 14-4875-82, RRID: AB_11042577,<https://>

www.thermofisher.com/antibody/product/EOMES-Antibody-clone-Dan11mag-Monoclonal/14-4875-82
 CD80 Monoclonal Antibody (Clone 16-10A1) Thermo Fisher Scientific Cat# 14-0801-82, RRID: AB_467346, <https://www.thermofisher.com/antibody/product/CD80-B7-1-Antibody-clone-16-10A1-Monoclonal/14-0801-82>
 Arginase I Antibody (Clone 8C9) Santa Cruz Biotechnology Cat# sc-47715, RRID: AB_626697, <https://www.scbt.com/p/arginase-1-antibody-8c9?srltid=AfmBOop9XNpijwIA6gnoaBuO44WcteoKEP7QSQ-uZSriMeZshPa9BRL2>
 Anti-Mouse CD206/MMR (Clone C068C2) Standard BioTools Cat# 3169021B, RRID: AB_2832249, <https://www.citeab.com/antibodies/12122124-3169021b-anti-mouse-cd206-mmrc068c2-169tm-100-te>
 Anti-Mouse NK1.1 Antibody (Clone PK136) Standard BioTools Cat# 3170002B, RRID: AB_2885023, <https://www.citeab.com/antibodies/12121492-3170002b-anti-mouse-nk1-1-pk136-170er-100-tests>
 Anti-Human/Mouse CD44 (Clone IM7) Standard BioTools Cat# 3171003B, RRID: AB_2895121
 Gata-3 Monoclonal Antibody (Clone TWAJ) Thermo Fisher Scientific Cat# 14-9966-82, RRID: AB_1210519, <https://www.thermofisher.com/antibody/product/Gata-3-Antibody-clone-TWAJ-Monoclonal/14-9966-82>
 Anti Mouse CD278/ICOS BioLegend Cat# 117408, RRID: AB_2122603, <https://www.biolegend.com/en-ie/products/pe-anti-mouse-cd278-icos-antibody-37>
 LAG3, clone C9B7W Standard BioTools Cat#3174019B
 F4/80 Monoclonal Antibody (Clone BM8) Thermo Fisher Scientific Cat# 14-4801-82, RRID: AB_467558
 Anti-mouse CD86 Antibody (Clone GL-1) BioLegend Cat# 105001, RRID: AB_313144, <https://www.biolegend.com/en-gb/products/purified-anti-mouse-cd86-antibody-257>
 Rabbit Anti-mouse EXO1 Bioss Cat#BS-13119R, https://www.biossusa.com/products/bs-13119r?srltid=AfmBOopifri-vBNYjB7rUnYDCdWqKy5il7qOn251rMgRKPVB2gJD_RYS
 Mouse Anti-Phospho-Histone H2A.X (Ser 139) Abcam Cat#ab242296, <https://www.abcam.com/en-us/products/assay-kits/gamma-h2ax-staining-kit-ab242296>
 Goat anti-mouse FITC-conjugated secondary Abcam Cat#ab242296, <https://www.abcam.com/en-us/products/assay-kits/gamma-h2ax-staining-kit-ab242296>
 H3K4me3 Histone antibody Active Motif Cat#39159, RRID: AB_2615077, <https://www.activemotif.com/catalog/details/39159>
 H3K27ac Histone antibody Active Motif Cat#39133, RRID: AB_2561016, <https://www.activemotif.com/catalog/details/39133/histone-h3-acetyl-lys27-antibody-pab>
 H3K27me3 Histone antibody Active Motif Cat#39155, RRID: AB_2561020, <https://www.activemotif.com/catalog/details/39155>
 H3K9la Histone Antibody (Clone 13H2L1) PTM-BIO Cat# PTM-1419RM, RRID: AB_3076695, <https://ptmbio.com/products/anti-l-lactyl-histone-h3-lys9-rabbit-mab/PTM-1419RM.htm>
 H3K18la Histone Antibody (Clone 22h2L3) PTM-BIO Cat# PTM-1406RM, RRID: AB_2909438, <https://ptmbio.com/products/anti-l-lactyl-histone-h3-lys18-rabbit-mab/PTM-1406RM.htm>
 H3K9ac Histone antibody Active Motif Cat#39137, RRID: AB_2561017, <https://www.activemotif.com/catalog/details/39137/histone-h3-acetyl-lys9-antibody-pab>
 Anti-mouse MSH2 Antibody (Clone 2L9I4) Bio-Techne Cat #NBP3-16692, <https://www.fishersci.nl/shop/products/msh2-rabbit-anti-human-mouse-rat-clone-2l9i4-novus-biologicals/p-7248183>
 Anti-mouse MSH6 Antibody (Clone 035) Bio-Techne Cat # NBP2-89265, https://www.novusbio.com/products/msh6-antibody-035_nbp2-89265?srltid=AfmBOoobU5gA2HvWhpmGgtjyr1aloeweykovBSOTJNNGv_2vtXnmviX1
 Purified Anti-Mouse CD16/CD32 (Mouse BD Fc Block) Clone 2.4G2 BD Biosciences Cat # 553142, RRID: AB_394657, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/purified-rat-anti-mouse-cd16-cd32-mouse-bd-fc-block.553141?tab=product_details
 Annexin V Pacific Blue ThermoFisher Scientific Cat # A35122, <https://www.thermofisher.com/order/catalog/product/A35122>
 Alexa Fluor 532 anti-mouse CD45 Antibody (Clone 30-F11) eBioscience Cat # 58-0451-82, RRID: AB_11218871, <https://www.thermofisher.com/antibody/product/CD45-Antibody-clone-30-F11-Monoclonal/58-0451-82>
 Alexa Fluor 488 anti-mouse CD3 Antibody (Clone 17A2) BioLegend Cat# 100210, RRID: AB_493530, <https://www.biolegend.com/de-de/products/alexa-fluor-488-anti-mouse-cd3-antibody-2835>
 Alexa Fluor 700 anti-mouse CD4 Antibody (Clone RM4-5) BioLegend Cat# 100536, RRID: AB_493701, <https://www.biolegend.com/fr-lu/products/alexa-fluor-700-anti-mouse-cd4-antibody-3386>
 Brilliant Violet 650 anti-mouse CD25 Antibody (Clone PC61) BioLegend Cat# 102038, RRID: AB_2563060, <https://www.biolegend.com/nl-be/products/brilliant-violet-650-anti-mouse-cd25-antibody-7640>
 PerCP-Cyanine5.5 anti-mouse FOXP3 Antibody (Clone FJK-16s) eBioscience Cat# 45-5773-82, RRID: AB_914351, <https://www.thermofisher.com/antibody/product/FOXP3-Antibody-clone-FJK-16s-Monoclonal/45-5773-82>
 Brilliant Violet 605 anti-Mouse PD-1 (Clone J43) BD Biosciences Cat # 563059, RRID: AB_2737980,
 Brilliant Violet 421 anti-mouse TGF- β 1 Antibody (Clone TW7-16B4) BioLegend Cat# 141407, RRID: AB_2561580, <https://www.biolegend.com/fr-ch/products/brilliant-violet-421-anti-mouse-lap-tgf-beta1-antibody-8180>
 Pacific Blue anti-mouse CD45 (Clone 30-F11) BioLegend Cat#103126, RRID: AB_493535, <https://www.biolegend.com/de-at/products/pacific-blue-anti-mouse-cd45-antibody-3102>
 Phycoerythrin anti-mouse E-Cadherin (Clone 4A2) Cell Signaling Technology Cat#82718, RRID: AB_2799998, <https://www.cellsignal.com/products/antibody-conjugates/e-cadherin-4a2-mouse-mab-pe-conjugate/82718?srltid=AfmBOopslq78muw7AXwSimR2slggjwja8COUnqBDIwjgsJpJ4Hy3Vo1>
 Anti-PD-1 Clone:RMP1-14, Bio X Cell Cat# BE0146, RRID: AB_10949053, <https://bioxcell.com/invivomab-anti-mouse-pd-1-cd279-be0146>
 Anti-mouse CD3e Clone: 145-2C11 BD Biosciences Cat # 553057, RRID: AB_394590, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/purified-na-le-hamster-anti-mouse-cd3e.553057?tab=product_details
 Anti-mouse CD28 Clone: 37.51 BD Biosciences Cat # 553294, RRID: AB_394763, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/purified-na-le-hamster-anti-mouse-cd28.553294?tab=product_details
 Pan Anti-Kla Antibody (Anti-L-Lactyllysine Rabbit pAb) PTM BIO Cat #PTM-1401, RRID: AB_2868521, <https://www.ptmbio.com/products/anti-l-lactyllysine-rabbit-pab/PTM-1401.htm>

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	The murine bladder cancer cell line MB49 was obtained from Millipore Sigma (Catalog number - SCC148). RT4 (Catalog-HTB-2), and SCaBER (Catalog- HTB-3) cell lines were purchased from ATCC. KDM6A KO clones were generated as described in the methods section
Authentication	We performed human and mouse cell lines authentication using STR (short tandem repeat) profiling
Mycoplasma contamination	All the cell lines were negative for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	5–7-weeks old C57BL/6 mice were purchased from the National Cancer Institute (NCI)
Wild animals	No wild animals were used in this study.
Reporting on sex	The findings do not apply to only one sex. Age and sex matched mice were used for the experiments in manuscript. Sex was not considered in the study design. The study involves understanding the role of KDM6A in the regulation of response to therapeutic perturbations in bladder cancer. Therefore sex and gender based analyses were not performed.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal experiments were conducted according to protocols approved by the Animal Resource Center at The University of Texas MD Anderson Cancer Center. The maximum tumor size for subcutaneous tumors permitted by the Institutional Animal Care and Use Committee (IACUC protocol no. 00002153-RN01) at The University of Texas MD Anderson Cancer Center (MDACC) is 15mm. The maximal tumor size was not exceeded for any of experiments.

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

Plants

Seed stocks	Not Applicable
Novel plant genotypes	Not Applicable
Authentication	Not Applicable

ChIP-seq

Data deposition

- ☒ Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- ☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links <i>May remain private before publication.</i>	https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA1173195
Files in database submission	1. CHIP-seq with anti-KDM6A antibody pulldown experiment in sgScramble cells (sgScramble_KDM6A.fastq.gz) 2. CHIP-seq with anti-H3K4me3 antibody pulldown experiment in sgScramble cells (sgScramble_H3K4me3.fastq.gz) 3. CHIP-seq input control for sgScramble cells (sgScramble_INPUT_CTRL.fastq.gz) 4. CHIP-seq with anti-H3K9la antibody pulldown experiment in in-vitro generated (murine) Tregs (Tregs_H3K9la.fastq.gz) 5. CHIP-seq with anti-H3K18la antibody pulldown experiment in in-vitro generated (murine) Tregs (Tregs_H3K18la.fastq.gz) 6. CHIP-seq with anti-H3K4me3 antibody pulldown experiment in in-vitro generated (murine) Tregs (Tregs_H3K4me3.fastq.gz) 7. CHIP-seq with anti-H3K27ac antibody pulldown experiment in in-vitro generated (murine) Tregs (Tregs_H3K27ac.fastq.gz) 8. CHIP-seq with anti-H3K9ac antibody pulldown experiment in in-vitro generated (murine) Tregs (Tregs_H3K9ac.fastq.gz)

9. CHIP-seq input control for in-vitro generated (murine) Tregs (Tregs_INPUT_CTRL.fastq.gz)
 10. CHIP-seq with anti-KDM6A antibody pulldown experiment in sgKdm6a cells (sgKdm6a_KDM6A.fastq.gz)
 11. CHIP-seq with anti-KDM6A antibody pulldown experiment in sgKdm6a (clone2) cells (sgKdm6a_C2_KDM6A.fastq.gz)
 12. CHIP-seq with anti-H3K4me3 antibody pulldown experiment in sgKdm6a cells (sgKdm6a_H3K4me3.fastq.gz)
 13. CHIP-seq with anti-H3K4me3 antibody pulldown experiment in sgKdm6a (clone2) cells (sgKdm6a_C2_H3K4me3.fastq.gz)
 14. CHIP-seq input control for sgKdm6a cells (sgKdm6a_INPUT_CTRL.fastq.gz)
 15. CHIP-seq with anti-H3K27me3 antibody pulldown experiment in sgKdm6a cells (sgKdm6a_H3K27me3.fastq.gz)
 16. CHIP-seq with anti-H3K27me3 antibody pulldown experiment in sgScramble cells (sgScramble_H3K27me3.fastq.gz)
 17. CHIP-seq with anti-H3K9la antibody pulldown experiment in in-vitro generated (murine) Tregs treated with exogenous sodium lactate (Na-La) for 48 hours (Tregs_exogenous_lactate_H3K9la.fastq.gz)
 18. CHIP-seq with anti-H3K18la antibody pulldown experiment in in-vitro generated (murine) Tregs treated with exogenous sodium lactate (Na-La) for 48 hours (Tregs_exogenous_lactate_H3K18la.fastq.gz)
 19. CHIP-seq with anti-H3K4me3 antibody pulldown experiment in in-vitro generated (murine) Tregs treated with exogenous sodium lactate (Na-La) for 48 hours (Tregs_exogenous_lactate_H3K4me3.fastq.gz)
 20. CHIP-seq with anti-H3K27ac antibody pulldown experiment in in-vitro generated (murine) Tregs treated with exogenous sodium lactate (Na-La) for 48 hours (Tregs_exogenous_lactate_H3K27ac.fastq.gz)
 21. CHIP-seq input control for in-vitro generated (murine) Tregs treated with exogenous sodium lactate (Na-La) for 48 hours (Tregs_exogenous_lactate_INPUT_CTRL.fastq.gz)

Genome browser session
 (e.g. [UCSC](#))

Not Applicable

Methodology

Replicates	02
Sequencing depth	Single-End
Antibodies	Antibodies used have been mentioned in Supplementary Table 1.
Peak calling parameters	"macs2 callpeak" was used to call narrow peaks and broad peaks from "--nomodel" and "-p 0.05" options. Differential peaks were called with MANorm with bed and respective bam files.
Data quality	Library and purity was confirmed on Bioanalyzer prior to sequencing. Peaks with significant p-values and fold enrichment between 5 to 30 were taken into account for differential analysis.
Software	FastQC v0.11.9, bowtie2 v2.3.5.1, SAMBLASTER v0.1.26, SAMTOOLS v1.13, SAMBAMBA v0.6.6, ChromHMM v1.24, Integrative Genomics Viewer (IGV) v2.16.0, MACS2 v2.2.8, CHIPseeker v1.30.3, clusterProfiler v4.2.2, ChIPpeakAnno v3.28.1, deepTools v3.5.1, EdgeR v3.36.0, Homer v4.11.1, MANorm v1.1.4

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	2X10 ⁶ cells were washed once in PBS containing 5% FBS (FACS buffer) by centrifugation at 200Xg for 5 minutes at 4 °C. Following the wash cells were incubated in blocking buffer containing bovine, murine, rat, hamster, rabbit serum and 25 µg/mL 2.4G2 antibody (Fc block) in PBS for 15 minutes on ice. Cells were then incubated for 30 minutes on ice in an antibody cocktail for surface staining (Supplementary Table 1). Following surface staining, cells were washed twice in FACS buffer before being subjected to fixation and permeabilization for 45 minutes on ice. After a wash with 1X permeabilization buffer, cells intracellular staining was performed by incubating the cells for 20 minutes in a cocktail of antibodies (Supplementary Table 1) at room temperature. Then the cells were washed twice in FACS buffer, fixed in 1% paraformaldehyde and acquired on a BD LSRFortessa flow cytometer.
Instrument	BD LSR II
Software	FlowJo v10.10
Cell population abundance	No FACS sorting of cell populations was done in this study. The individual values have been plotted as graphs in the manuscript main and extended data figures.

Gating strategy

Cells were gated based on FSC-A and SSC-A followed by singlet/doublet discrimination. Positive populations were gated using unstained controls.

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