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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 (*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	NMR spectra were acquired using Bruker 600 Mhz NMR Spectrometer. HPLC spectra were acquired by Waters E2695 Seperation Module and UV/Vis detector E2489. Real-time PCR data were acquired by 7900HT Fast Realtime PCR System. DLS size and zeta potential data were acquired by Zetasizer. Cryo-EM images were acquired by ThermoFisher TF20 electron microscope with TVIPS XF416 CMOS camera. Confocal florescence images were acquired by Olympus FluoView 1000 confocal laser scanning microscope. Florescence images were acquired by Keyence BZ-X710 Fluorescence Microscope. In vitro luciferase data were acquired by Wallac Victor2 1420 Multilabel Counter (Perkin Elmer). In vivo bioluminescence and fluorescence images were acquired by IVIS Lumina XR (Perkin Elmer). Platinum (Pt) concentration were measured using a PerkinElmer NeXION 300X Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). Flow cytometry samples were analyzed using SpectroFlo® Software on Cytek® Aurora or High-Performance BD FACSDiva Software on BD LSR II.
Data analysis	NMR spectra were analyzed using TopSpin. HPLC data and spectra were analyzed by Empower 3 Chromatography Data System. RNA sequencing files were analyzed with STAR 2.6.1a and Cufflinks 2.2.1. Cryo-EM images were acquired with TVIPS Emplified software. In vitro luciferase data were analyzed by Wallac 1420 Workstation. In vivo bioluminescence and fluorescence images were analysed by Live image software (Perkin Elmer). Flow cytometry data were analyzed using FlowJo software package (version 10.6.2; BD, USA). All statistical analyses were performed with Graphpad Prism 9.4.0.

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 bulk messenger RNA-seq data mapped to the mouse genome (GRCm38: https://www.ncbi.nlm.nih.gov/assembly/GCF_000001635.20/) are available in the NCBI Gene Expression Omnibus (GEO) under accession no. GSE214881 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE214881). Source data are provided with this paper. All data generated or analyzed during this study are included in this published article and its Supplementary information files.

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 in vitro or ex vivo studies, sample sizes were based on our previous experiments or previous publication (Nature Communications 7, 1-12 2016; Acta Biomaterialia 43, 282-291 2016). We used n=3 as a minimum to obtain statistically meaningful and significant results. Similarly, for in vivo studies, when monitoring tumor growth or survival in a setting of treatment, as long as the volume in each group at the beginning of the treatment is comparable to each other, n=5-8 is sufficient to detect significant biological differences with good reproducibility. An independent statistical method was not used to determine sample size. Details regarding sample size of all experiments are provided in the Methods section and Figure legends.
Data exclusions	No data were excluded.
Replication	Experiments were repeated and experimental findings were reproducible. In Figure 1, data are representative of 2 independent experiments in b, j, k, l, m and n, and 3 independent experiments in c-i, o and p. In Figure 2, data are representative of 2 independent experiments in h and 3 independent experiments in b-g, i and j. In Figure 3, data are representative of 2 independent experiments in a-h and j, and 3 tumor tissues in k-n. In Figure 4, data are representative of 2 independent experiments in all panels. In Figure 5, data are representative of 2 independent experiments in all panels. In Figure 6, data are representative of 2 independent experiments in panels a-l.
Randomization	The experimental groups were allocated randomly.
Blinding	No formal blinding was used due to the predetermined nature and protocol of measurements and the work does not involve participant groups. Most of the data of this study are qualitative. Imaging data were acquired using a computer controlled instrument with the same setting through the entire experiment. The image processing parameters were kept the same for all samples.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used

Detailed information on antibodies is listed below.

Western blotting Experiments:
Human Xkr8 Polyclonal Antibody (ThermoFisher, Cat# PA5-46668, Lot# XI3723982, dilution: 1/2000)
Human Xkr8 Polyclonal Antibody (ThermoFisher, Cat# PA5-98929, Lot# WL3455335, dilution: 1/1500)
β-Tubulin Antibody (Cell Signaling Technology, Cat# 2146S, Lot# 7, dilution: 1/2000)
Anti-rabbit IgG, HRP-linked Antibody (Cell Signaling Technology, Cat# 7074S, Lot# 31, dilution: 1/5000)

Immunofluorescence Experiments:
Alexa Fluor® 488 Phalloidin (Cell Signaling Technology, Cat# 8878S, Lot# 9, dilution: 1/20)
Human/Mouse/Rat EEA1 Alexa Fluor® 488-conjugated Antibody (R&D Systems, Cat# IC8047G, Lot# ADWI0217091, Sheep IgG, dilution: 1/10)

Flow cytometry Experiments:
BV605 Mouse Anti-Human CD44 (BD Biosciences, Cat# 747751, Clone# G44-26, Lot# 2066901, Mouse IgG2b, κ, dilution: 1/200)
APC Rat Anti-Mouse CD44 (BD Biosciences, Cat# 561862, Clone# IM7, Lot# 1334565, Rat IgG2b, κ, dilution: 1/200)
Brilliant Violet 421 Annexin V Antibody (Biolegend, Cat# 640923, Lot# B373982, dilution: 1/200)
Zombie NIR (Biolegend, Cat# 423105, Lot# B328963, dilution: 1/1000)
PerCP anti-mouse CD45 Antibody (Biolegend, Cat# 103130, Clone# 30-F11, Lot# B280746, Rat IgG2b, κ, dilution: 1/200)
Brilliant Violet 785 anti-mouse CD4 Antibody (Biolegend, Cat# 100453, Clone# GK1.5, Lot# B354442, Rat IgG2b, κ, dilution: 1/200)
FITC anti-mouse CD8 Antibody (eBioscience, Cat# 11-0081-82, Clone# 30-F11, Lot# B280746, Rat IgG2a, κ, dilution: 1/200)
PE-CF594 anti-mouse Foxp3 Antibody (BD Biosciences, Cat# 562466, Clone# MF23, Lot# 1138849, Rat IgG2b, dilution: 1/200)
PE-Cy7 anti-mouse IFN-γ Antibody (BD Biosciences, Cat# 557649, Clone# XMG1.2, Lot# 1243760, Rat IgG1, κ, dilution: 1/200)
Alexa Fluor 647 anti-human/mouse Granzyme B Antibody (Biolegend, Cat# 515405, Clone# GB11, Lot# B301363, Mouse IgG1, κ, dilution: 1/200)
PE anti-mouse CD279 (PD-1) Antibody (BD Biosciences, Cat# 561788, Clone# J43, Lot# 1337063, Armenian Hamster IgG2, κ, dilution: 1/200)
APC anti-mouse CD11b Antibody (eBioscience, Cat# 17-0112-81, Clone# M1/70, Lot# 2376137, Rat IgG2b, κ, dilution: 1/200)
Brilliant Violet 711 anti-mouse Ly-6G/Ly-6C (Gr-1) Antibody (Biolegend, Cat# 108443, Clone# RB6-8C5, Lot# B293445, Rat IgG2b, κ, dilution: 1/200)
BV510 anti-mouse CD24 Antibody (BD Biosciences, Cat# 747717, Rat DA, Clone# M1/69, Lot# 1139643, also known as DA/HA IgG2b, κ, dilution: 1/200)
APC/Cyanine7 anti-mouse F4/80 Antibody (Biolegend, Cat# 123118, Clone# BM8, Lot# B352753, Rat IgG2a, κ, dilution: 1/200)
Pacific Blue anti-mouse I-A/I-E Antibody (Biolegend, Cat# 107620, Clone# M5/114.15.2, Lot# B347113, Rat IgG2b, κ, dilution: 1/200)
FITC anti-mouse CD206 (MMR) Antibody (Biolegend, Cat# 141703, Clone# MR5D3, Lot# XF3624224, Rat IgG2a, κ, dilution: 1/200)

Validation

All antibodies were verified by the supplier and each lot has been quality tested. Validation statements are shown on the manufacturer's website.

Western blotting Experiments:
Human Xkr8 Polyclonal Antibody (ThermoFisher, Cat# PA5-46668, https://www.thermofisher.com/antibody/product/XKR8-Antibody-Polyclonal/PA5-46668)
Human Xkr8 Polyclonal Antibody (ThermoFisher, Cat# PA5-98929, https://www.thermofisher.com/antibody/product/XKR8-Antibody-Polyclonal/PA5-98929)
β-Tubulin Antibody (Cell Signaling Technology, Cat# 2146S, https://www.cellsignal.com/products/primary-antibodies/b-tubulin-antibody/2146)
Anti-rabbit IgG, HRP-linked Antibody (Cell Signaling Technology, Cat# 7074S, https://www.cellsignal.com/products/secondary-antibodies/anti-rabbit-igg-hrp-linked-antibody/7074)

Immunofluorescence Experiments:
Alexa Fluor® 488 Phalloidin (Cell Signaling Technology, Cat# 8878S, https://www.cellsignal.com/products/buffers-dyes/alexa-fluor-488-phalloidin/8878)
Human/Mouse/Rat EEA1 Alexa Fluor® 488-conjugated Antibody (R&D Systems, Cat# IC8047G, https://www.rndsystems.com/products/human-mouse-rat-eea1-alexa-fluor-488-conjugated-antibody_ic8047g)

Flow cytometry Experiments:
BV605 Mouse Anti-Human CD44 (BD Biosciences, Cat# 747751, https://www.bdbiosciences.com/en-ca/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv605-mouse-anti-human-cd44.747751)
APC Rat Anti-Mouse CD44 (BD Biosciences, Cat# 561862, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-rat-anti-mouse-cd44.561862)
Brilliant Violet 421 Annexin V Antibody (Biolegend, Cat# 640923, https://www.biolegend.com/fr-lu/products/brilliant-violet-421-annexin-v-9286)
Zombie NIR (Biolegend, Cat# 423105, https://www.biolegend.com/fr-lu/products/zombie-nir-fixable-viability-kit-8657)
PerCP anti-mouse CD45 Antibody (Biolegend, Cat# 103130, https://www.biolegend.com/fr-fr/products/percp-anti-mouse-cd45-antibody-4265)
Brilliant Violet 785 anti-mouse CD4 Antibody (Biolegend, Cat# 100453, https://www.biolegend.com/de-at/products/brilliant-violet-785-anti-mouse-cd4-antibody-11948)
FITC anti-mouse CD8 Antibody (eBioscience, Cat# 11-0081-82, https://www.thermofisher.com/antibody/product/CD8a-Antibody-clone-53-6-7-Monoclonal/11-0081-82)
PE-CF594 anti-mouse Foxp3 Antibody (BD Biosciences, Cat# 562466, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-cf594-mouse-anti-human-cd4.566914?gclid=Cj0KCQjwhY-aBhCUARIsALNIC06tfrqWRsir_izFg9s-XiDntW8fXLcezaYbybHmcu6lwUxmqSv5n0waAoF4EALw_wcB)

PE-Cy7 anti-mouse IFN-γ Antibody (BD Biosciences, Cat# 557649, https://www.biolegend.com/en-us/search-results/pe-cyanine7-anti-mouse-ifn-gamma-antibody-5865?gclid=Cj0KCQjwhY-aBhCUARIsALNIC04DMAxf_6ZlCJUITo3U-celAxIHgpGoPDkkk3ep-p_ZQYycNR3MS9IaAjSLEALw_wcB)
Alexa Fluor 647 anti-human/mouse Granzyme B Antibody (Biolegend, Cat# 515405, https://www.biolegend.com/nl-nl/products/alexa-fluor-647-anti-human-mouse-granzyme-b-antibody-6067)
PE anti-mouse CD279 (PD-1) Antibody (BD Biosciences, Cat# 561788, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-hamster-anti-mouse-cd279-pd-1.551892)
APC anti-mouse CD11b Antibody (eBioscience, Cat# 17-0112-81, https://www.biolegend.com/en-us/search-results/apc-anti-mouse-human-cd11b-antibody-345?GroupID=BLG10530&gclid=Cj0KCQjwhY-aBhCUARIsALNIC07gHgaYhMiOlZe-VzqDSd6ZqA2x6ER1gYyRdZb4QrRMG1mGn-pyoDgaAorcEALw_wcB)
Brilliant Violet 711 anti-mouse Ly-6G/Ly-6C (Gr-1) Antibody (Biolegend, Cat# 108443, https://www.biolegend.com/en-us/products/brilliant-violet-711-anti-mouse-ly-6g-ly-6c-gr-1-antibody-8896)
BV510 anti-mouse CD24 Antibody (BD Biosciences, Cat# 747717, https://www.bdbiosciences.com/en-tw/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv510-rat-anti-mouse-cd24.747717)
APC/Cyanine7 anti-mouse F4/80 Antibody (Biolegend, Cat# 123118, https://www.biolegend.com/fr-fr/products/apc-cyanine7-anti-mouse-f4-80-antibody-4072)
Pacific Blue anti-mouse I-A/I-E Antibody (Biolegend, Cat# 107620, https://www.biolegend.com/en-us/search-results/pacific-blue-anti-mouse-i-a-i-e-antibody-3136)
FITC anti-mouse CD206 (MMR) Antibody (Biolegend, Cat# 141703, https://www.biolegend.com/fr-lu/products/fitc-anti-mouse-cd206-mmr-antibody-7318)

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Cell line sources were provided under "Method: Tumor cell lines" section. CT26 murine CRC cell lines, HT29 and WiDr human CRC cell lines, Panc02 murine PCa cell line, PANC-1 human PCa cell line, 4T1.2 murine BCa cell line, and BT-474 human BCa cell line were obtained from ATCC (Manassas, VA). MC38 and MC38-Luc cell lines were kindly given by Dr. Zongsheng Guo (University of Pittsburgh Cancer Institute, University of Pittsburgh, Pittsburgh, PA 15261, USA). MC38 cell lines were originally obtained from Kerafast (MA, USA).
Authentication	Cell lines were used without any modification once received from respective suppliers and therefore were not authenticated. Engineered cell line was verified using negative control and maintained according to manufacturer's suggestion.
Mycoplasma contamination	Cell lines were all tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study.

Animals and other organisms

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

Laboratory animals	As reported under "Method: Mice" section. Female C57BL/6, BALB/c, NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) and B6.129(Cg)-Cd44tm1Hbg/J (CD44-/-) mice aged between 4–6 weeks were purchased from The Jackson Laboratories (CT, USA). Mice were housed under pathogen-free conditions according to AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care) guidelines. The mouse-related experiments were performed in full compliance with institutional guidelines and approved by the Animal Use and Care Administrative Advisory Committee at the University of Pittsburgh under Protocol #: 21099779. Mice were housed at an ambient temperature of 22 °C (22–24 °C) and humidity of 45%, with a 14/10 day/night cycle (on at 6:00, off at 20:00), and allowed access to food ad libitum.
Wild animals	The study did not involve wild animals.
Field-collected samples	No Field-collected samples were used in this study
Ethics oversight	The mouse-related experiments were performed in full compliance with institutional guidelines and approved by the Animal Use and Care Administrative Advisory Committee at the University of Pittsburgh.

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	Tumors were cut mechanically with scissors and digested with Liberase TL and DNase I. Tissues were further grinded and filtered through a 70 µm cell strainer with red blood cells lysed by ACK lysis buffer. Detailed methods for sample preparation were provided under "Methods: Analysis of tumor-infiltrating lymphocytes and monocytes" section.
Instrument	Cytek® Aurora and BD LSR II
Software	SpectroFlo® Software or High-Performance BD FACSDiva Software were used for data collection and FlowJo software package (version 10.6.2; TreeStar, USA) was used for data analysis.
Cell population abundance	Sorting was not done. Cell populations of interest were collected as much as possible in the intratumoural immunology assay.
Gating strategy	Tumor cells were gated under Zombie NIR- & CD45- cell population and further stained by Annexin V+ for PS expression. T cells were gated under Zombie NIR- & CD45+ cells. CD4 and CD8 cell were gated under T cells. Tregs were gated as CD4+ FoxP3+ cells. IFNγ+, GzmB+ and PD-1+ cells were gated under CD8+ cells. Macrophage population were characterized by using Gr-1-, CD11b+, MHCII+ and F4/80 gating. M1-like cells (CD206-) and M2-like (CD206+) were gated under macrophage populations. Detailed gating strategy were provided under "Methods: Analysis of tumor-infiltrating lymphocytes and monocytes" section and Fig. S17.

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