nature portfolio

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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	SpectroFlo Software (3.3.0) to collect the flow cytometry data; Excel (16.63.1) for tumour size measurements, CRi Panoramic SCAN 40x Whole Slide Scanner to collect H&E and IHC data; NovaSeq6000 (Illumina) to collect scRNA-seq raw data; NovaSeqX (Illumina) to collect RNA-seq raw data; Incucyte Base software (v2019A) to collect live cell imaging data
Data analysis	FlowJo (10.8.1) for flow cytometry analysis with FlowJo plugins Phenograph (2.4), ClusterExplorer (1.7.4), and FlowSOM (3.0.18). GraphPad Prism (10.3.1) for statistical data analysis of in vivo tumor, flow and metastasis data; Qupath (0.3.0) and ImageJ (1.54g) for H&E and IF data, Incucyte Base software (v2019A) for live cell imaging data. For scRNA-seq: Cellranger (7.1.0), Python Pegasus (1.8.1), scanpy (1.8.1), Seurat (4.4.0), Slingshot (3.18), Velocyto (0.17.17), scVelo (0.3.2), R (4.3). For RNA-seq: STAR (2.7.10b, Subread (2.0.5) DESeq2 in Bioconductor 1.46.0, GSEA (4.3.2). A custom code has been developed for AI-assisted monocyte detection in IF stainings using the human-in-the-loop pipeline of Cellpose (2.0) and has been deposited on Github (https://github.com/apiffko/IF.mono.quantif) and Zenodo (https://doi.org/10.5281/zenodo.14982759) - please see Code reporting summary for more details.

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

All data generated or analyzed during this study are included in this published article (and its supplementary information files). Source data are provided with this paper. The single-cell RNA sequencing data have been deposited at the Gene Expression Omnibus (GEO) repository with the accession number GSE250375. The bulk-RNA sequencing data have been deposited at the GEO repository with the accession number GSE291151. The microarray data of the patients with advanced solid tumors enrolled in the clinical trial (NCT02608385) are available from the corresponding author on reasonable request. The human LUSC genomic and clinical data were derived from the TCGA Research Network (http://cancergenome.nih.gov) using the Xena platform (https://xena.ucsc.edu/). Mouse reference genome (mm10-2020A): https://www.ncbi.nlm.nih.gov/grc/mouse. Molecular Signature Database: https://www.gsea-msigdb.org/gsea/msigdb/index.jsp. Reactome datasets: https://reactome.org/.

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	Does not apply.
Reporting on race, ethnicity, or other socially relevant groupings	Does not apply.
Population characteristics	NCT02608385 is a phase I/II trial designed to evaluate the safety and efficacy of pembrolizumab with multisite stereotactic body radiation therapy in patients with advanced solid tumors. The COSINR study (NCT03223155) is a randomized phase I/II trial designed to evaluate the safety and efficacy of combination immune checkpoint blockade (ICB) and stereotactic body radiotherapy (SBRT) as first-line treatment for patients with stage IV NSCLC.
Recruitment	Eligibility criteria for NCT02608385 are described in: Luke, J.J. et al. Safety and Clinical Activity of Pembrolizumab and Multisite Stereotactic Body Radiotherapy in Patients with advanced solid tumors. JCO 36, 1611-1618 (2018). Eligibility criteria for NCT03223155 are described in: Bestvina, C.M. et al. A Phase 1 Trial of Concurrent or Sequential Ipilimumab, Nivolumab, and Stereotactic Body Radiotherapy in Patients With Stage IV NSCLC Study. JTO 17, 130-140 (2022).
Ethics oversight	NCT02608385 was approved University of Chicago Biological Sciences Division IRB Committee (IRB15-1130) NCT03223155 was approved by the University of Chicago Biological Sciences Division IRB Committee (IRB17-0547-CR003). All patients treated on the trials provided written informed consent.

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

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample sizes were chosen based on previous studies; Yang at al. 2022 (https://doi.org/10.1038/s41565-022-01225-x), and Wang et al. 2023 (https://doi.org/10.1016/j.ccell.2023.04.019). This estimate informed our power analysis and allowed us to choose a sample size balancing statistical power with practical considerations such as time, cost and resource availability. Ethical considerations (3R) played a role in determining sample size for in vivo experiments, by aiming to use the smallest number of subjects necessary to achieve reliable results.
Data exclusions	For in vivo experiments, individual mice were removed from the study either prior to treatment, if found to be an engraftment outlier or from the final analysis if, at end point, the mouse was found to be a significant outlier with regards to tumor growth. These exclusion criteria were established prior to tumor engraftment. All outlier calculations for in vivo experiments were performed using the GraphPad Outlier Calculator (https://www.graphpad.com/quickcalcs/Grubbs1.cfm).
Replication	The murine experiments were performed independently for two or three times as indicated in respective figure legends. The human PBMC

Replication	and biopsy gene array analysis, as well as the scRNA-seq were performed as single experiments. Number of biological / technical replicates is indicated in the figure legends.
Randomization	Mice were allocated to treatment or control groups based on the average tumor size to achieve similar tumor burden between groups at the time of treatment initiation. Age- and sex matched mice were used within an experiment, and age- and sex-matched littermate controls were used for studies involving conditional KO mice. For all other experiments, samples were randomly allocated.
Blinding	Drug administration and tumor measurements were performed by two different groups of investigators. Data analysis (flow cytometry / H&E) was independently assessed in a blinded manner by a second investigator. Blinding was not fully implemented for in vitro assays because treatment conditions required adjustments during handling, such as media changes and incubation times.

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

Antibodies used for IF stainings:
pEGFR, Tyr 992, #44786G, Invitrogen, 1:100
Ly6C-PE/Dazzle 594, HK1.4, #128044, Biolegend, 1:100
CD47, BV421, miap301 #127527, Biolegend, 1:100
SIRPα, AF647, P84, #144028, Biolegend, 1:100
Phospho-Myosin Light Chain 2 (Ser19), #3671, Cell Signaling, 1:100
Myosin IIa Antibody, #3403, Cell Signaling, 1:100
Anti-Rabbit IgG (H+L), AF488, #A32731, Invitrogen, 1:500

Hashtag antibodies for scRNA-seq:
B0301 Hashtag 1, M1/42, 155831, Biolegend, 1:200
B0302 Hashtag 2, M1/42 155833, Biolegend, 1:200
B0303 Hashtag 3, M1/42 155835, Biolegend 1:200
B0304 Hashtag 4, M1/42 155837, Biolegend 1:200

Antibodies used in vivo
CD47, BE0270, BioXcell, IAP, 200 ug x 4
AREG, AF989, R&D Systems 1.5ug/ml blood volume, x 4
Rat IgG2a BE0089 BioXcell 2A3 200 ug x 4
Goat IgG BE0130 BioXcell200 ug x 4

Murine antibodies used for flow cytometry:
CD16/32 BE0307 BioXcell 2.4G2 unconjugated 1:100
CD11b 101241 BioLegend M1/70 BV711 1:200
CD11c 117329 BioLegend N418 BV421 1:200
CD3 100249 BioLegend 17A2 BV750 1:200
CD4 100410 BioLegend GK1.5 PE-Cy5 1:200
CD45 58-0451-82 invitrogen 30-F11 AF532 1:200
CD8a 100759 BioLegend 53-6.7 BV711 1:200
CD8a 53-6.7, 563786, BD, BUV395, 1:200
CD8a 100714 BioLegend 53-6.7 APC-Cy7 1:200
CD8a 563786 BD Biosciences 53-6.7 BUV395 1:200
F4/80 123110 BioLegend BM8 PE 1:200
F4/80 123141 BioLegend BM8 BV785 1:200
F4/80 749282 BD Biosciences T45-2342 BUV805 1:200
LIVE/DEAD™ Fixable Yellow Dead Cell Stain L34959 invitrogen 1:2000
Zombie NIR Fixable Viability Kit Biolegend 423105 1:2000
Ly-6C 128012 BioLegend HK1.4 PerCP-Cy5.5 1:200
Ly-6G 560601 BD Biosciences 1A8 PE-Cy7 1:200
MHCII (I-A/I-E) 107622 BioLegend M5/114.15.2 AF700 1:200

NK-1.1 741233 BD Biosciences PK136 BUV563 1:200
NK-1.1 108748 BioLegend PK136 PE-CF594 1:200
PD-1 135225 BioLegend 29F.1A12 BV785 1:200
phospho-EGFR (Tyr992) polyclonal antibody (44-786G), 1:200 - conjugated with LYNX rapid antibody conjugation kit (LNK082C, BioRad)
AREG, AF989, R&D Systems, 1:200 - conjugated with LYNX rapid antibdoy conjugation kit (LNK142PERCPCY5.5, BioRad)
FoxP3 126419 BioLegend MF14 BV421 1:200
PD-L1 124336, BioLegend 10F9G2 BV650 1:200
CD206 141734, BioLegend C068C2 AF700 1:200
CCR7 120123, BioLegend 4B12 PE/Cy7 1:200
CD19 566167, BD, 6D5, BV480, 1:200
Tyr705 STAT3 Phospho, BioLegend 651004, 13A3-1, PE, 1:200
CD47, BV421, miap301 #127527, Biolegend, 1:200
CD47, APC, miap301 #127514, Biolegend, 1:200

Human antibodies used for flow cytometry:
FoxP3 (236A/E7), 14-4777-82, Invitrogen, PE/Cy7 1:200
CD20 (2H7), 14-0209-82, Invitrogen, SB436 1:200
CD16 (eBioCB16), 16-0168-85, Invitrogen, eFluor450 1:200
CD4 (SK3) 344602, Biolegend, BV750 1:200,
CCR7 (G043H7), 353207, Biolegend, BV421 1:200
PD-1 (EH12.2H7), 329950, Biolegend, BV650 1:200
PD-L1 (B7-H1), 329706, Biolegend, PE, 1:200
HLA-DR (L243), 980412, Biolegend, APC/Fire 750, 1:200
CD11c (3.9), 301636, Biolegend, BV605, 1:200
CD303 (BDCA-2), 354234, Biolegend, BV711, 1:200
CD304 (12C2) 354540, Biolegend, APC/Fire810 1:200
CD56 (NCAM16.2), 612928, BD, BUV563, 1:200
CD141 (1A4) 746604, BD, BV480, 1:200
CD45 (HI30), 612891, BD, BUV805, 1:200
CD3 (UCHT1), 58-0038-42, ThermoFisher, AF532, 1:200
CD33 (WM53), BDB740293, Fisher Scientific, BUV395, 1:200
CD8a (SK1), 46-0087-42, ThermoFisher, PerCP-eFluor 710, 1:200
CD1c (L161), 331544, Biolegend, BV785,1:200
CD71 (CY1G4), 334120, Biolegend, PE-Dazzle 594, 1:200
CD45RA (HI100), 304120, Biolegend, AlexaFluor 700, 1:200

Validation

All antibodies used are commercially available and validated by the manufacturers. Additional information can be found on the manufacturers websites.
Antibodies used for IF stainings:
pEGFR, Tyr 992, #44786G, Invitrogen https://www.thermofisher.com/antibody/product/Phospho-EGFR-Tyr992-Antibody-Polyclonal/44-786G
Ly6C-PE/Dazzle 594, HK1.4, #128044, Biolegend https://www.biolegend.com/nl-be/products/pe-dazzle-594-anti-mouse-ly-6c-antibody-12513
CD47, BV421, miap301 #127527, Biolegend https://www.biolegend.com/fr-ch/products/brilliant-violet-421-anti-mouse-cd47-antibody-15866
SIRPα, AF647, P84, #144028, Biolegend https://www.biolegend.com/fr-lu/products/alexa-fluor-647-anti-mouse-cd172a-sirpalpha-antibody-15378
Phospho-Myosin Light Chain 2 (Ser19), #3671, Cell Signaling https://www.cellsignal.com/products/primary-antibodies/myosin-iia-antibody/3403?srsltid=AfmBOorTUaCD5y787N6Iy33hn4zaqENIQGUOmHCmOYt4xHePp4M96Khp
Myosin IIa Antibody, #3403, Cell Signaling https://www.cellsignal.com/products/primary-antibodies/myosin-iia-antibody/3403?srsltid=AfmBOopTnkWSRJKug-k23v7eW9cfJ8_HNgCQD11Ufo9Hr7n_vXSpXJFF
Anti-Rabbit IgG (H+L), AF488, #A32731, Invitrogen, https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32731

Hashtag antibodies for scRNA-seq:
B0301 Hashtag 1, M1/42, 155831, Biolegend https://www.biolegend.com/fr-ch/products/totalseq-b0301-anti-mouse-hashtag-1-antibody-17771
B0302 Hashtag 2, M1/42, 155833, Biolegend https://www.biolegend.com/fr-ch/products/totalseq-b0302-anti-mouse-hashtag-2-antibody-17772
B0303 Hashtag 3, M1/42, 155835, Biolegend https://www.biolegend.com/fr-ch/products/totalseq-b0303-anti-mouse-hashtag-3-antibody-17773
B0304 Hashtag 4, M1/42, 155837, Biolegend https://www.biolegend.com/fr-ch/products/totalseq-b0304-anti-mouse-hashtag-4-antibody-17774

Antibodies used in vivo (Target molecule, catalog number, supplier, webpage)
CD47, BE0270, BioXcell https://bioxcell.com/invivomab-anti-mouse-cd47-iap-be0270
AREG, AF989, R&D Systems https://www.rndsystems.com/products/mouse-amphiregulin-antibody_af989
Rat IgG2a BE0089 BioXcell https://bxcell.com/product/rat-igg2a-isotype-control/
goat IgG BE0130 BioXcell https://bioxcell.com/invivomab-polyclonal-goat-igg-be0130

Antibodies used iin murine flow cytometry (Target molecule, catalog number, supplier, webpage)
CD16/32 BE0307 BioXcell 2.4G2 https://bioxcell.com/invivomab-anti-mouse-cd16-cd32-be0307
CD11b 101241 BioLegend https://www.biolegend.com/en-us/products/brilliant-violet-711-anti-mouse-human-cd11b-antibody-7927
CD11c 117329 BioLegend https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-mouse-cd11c-antibody-7149
CD3 100249 BioLegend https://www.biolegend.com/en-us/products/brilliant-violet-750-anti-mouse-cd3-antibody-15755

CD4 100410 BioLegend https://www.biolegend.com/en-us/products/pe-cyanine5-anti-mouse-cd4-antibody-251
CD45 58-0451-82 invitrogen https://www.thermofisher.com/antibody/product/CD45-Antibody-clone-30-F11-Monoclonal/58-0451-82
CD8a 100759 BioLegend https://www.biolegend.com/en-us/products/brilliant-violet-711-anti-mouse-cd8a-antibody-7926
CD8a 563786 BD Biosciences https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv395-rat-anti-mouse-cd8a.563786
CD8a 100714 BioLegend https://www.biolegend.com/en-us/products/apc-cyanine7-anti-mouse-cd8a-antibody-2269
F4/80 123110 BioLegend https://www.biolegend.com/en-us/products/pe-anti-mouse-f4-80-antibody-4068
F4/80 123141 BioLegend https://www.biolegend.com/en-us/products/brilliant-violet-785-anti-mouse-f4-80-antibody-9919
F4/80 749282 BD Biosciences https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv805-rat-anti-mouse-f4-80.749282
LIVE/DEAD™ Fixable Yellow Dead Cell Stain L34959 invitrogen https://www.thermofisher.com/order/catalog/product/L34959?SID=srch-srp-L34959
Zombie NIR™ Fixable Viability Kit 423105 Biolegend https://www.biolegend.com/fr-lu/products/zombie-nir-fixable-viability-kit-8657
Ly-6C 128012 BioLegend https://www.biolegend.com/fr-lu/products/percp-cyanine5-5-anti-mouse-ly-6c-antibody-5967
Ly-6G 560601 BD Biosciences https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-cy-7-rat-anti-mouse-ly-6g.560601
MHCII(I-A/I-E) 107622 BioLegend https://www.biolegend.com/fr-lu/products/alexa-fluor-700-anti-mouse-i-a-i-e-antibody-3413
NK-1.1 741233 BD Biosciences https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv563-mouse-anti-mouse-nk-1-1.741233
NK1.1 108748 Biolegend PK136 https://www.biolegend.com/en-us/products/pe-dazzle-594-anti-mouse-nk-1-1-antibody-10320
PD-1 135225 BioLegend https://www.biolegend.com/fr-lu/products/brilliant-violet-785-anti-mouse-cd279-pd-1-antibody-9874
FoxP3 126419 BioLegend BV421 1:200 https://www.biolegend.com/nl-be/products/brilliant-violet-421-anti-mouse-foxp3-antibody-12143?GroupID=BLG5706
PD-L1 124336, BioLegend BV650 1:500 https://www.biolegend.com/ja-jp/products/brilliant-violet-650-anti-mouse-cd274-b7-h1-pd-l1-antibody-16055
CD206 141734, BioLegend AF700 1:500 https://www.biolegend.com/en-gb/products/alexa-fluor-700-anti-mouse-cd206-mmr-antibody-13456?GroupID=BLG9506
CCR7 120123, BioLegend PE/Cy7 1:500 https://www.biolegend.com/en-gb/products/pe-cyanine7-anti-mouse-cd197-ccr7-antibody-13133?GroupID=BLG4001
CD19 566167, BD, BV480, 1:500 https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv480-rat-anti-mouse-cd19.566167?tab=product_details
AREG AF989, R&D Systems https://www.rndsystems.com/products/mouse-amphiregulin-antibody_af989
Tyr705 STAT3 Phospho, 651004, Biolegend, 13A3-1 https://www.biolegend.com/en-us/products/pe-anti-stat3-phospho-tyr705-antibody-12914
CD47, BV421, miap301 #127527, Biolegend, 1:200 https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-mouse-cd47-antibody-15866
CD47, APC, miap301 #127514, Biolegend, 1:200 https://www.biolegend.com/en-us/products/apc-anti-mouse-cd47-antibody-9119

Antibodies used in human flow cytometry:
FoxP3 (236A/E7), 14-4777-82, Invitrogen, PE/Cy7 1:500 https://www.thermofisher.com/antibody/product/FOXP3-Antibody-clone-236A-E7-Monoclonal/25-4777-42
CD20 (2H7), 14-0209-82, Invitrogen, SB436 1:500 https://www.thermofisher.com/antibody/product/CD20-Antibody-clone-2H7-Monoclonal/14-0209-82
CD16 (eBioCB16), 16-0168-85, Invitrogen, eFluor450 1:500 https://www.thermofisher.com/antibody/product/CD16-Antibody-clone-eBioCB16-CB16-Monoclonal/16-0168-\85
CD4 (SK3) 344602, Biolegend, BV750 1:500, https://www.biolegend.com/de-at/products/purified-anti-human-cd4-antibody-6205
CCR7 (G043H7), 353207, Biolegend, BV421 1:500 https://www.biolegend.com/en-ie/products/brilliant-violet-421-anti-human-cd197-ccr7-antibody-7497?GroupID=BLG9610
PD-1 (EH12.2H7), 329950, Biolegend, BV650 1:500 https://www.biolegend.com/fr-ch/products/brilliant-violet-650-anti-human-cd279-pd-1-antibody-12364
PD-L1 (B7-H1), 329706, Biolegend, PE, 1:500 https://www.biolegend.com/en-gb/products/pe-anti-human-cd274-b7-h1-pd-l1-antibody-4375?GroupID=BLG5402
HLA-DR (L243), 980412, Biolegend, APC/Fire 750, 1:500 https://www.biolegend.com/de-de/products/apc-fire-750-anti-human-hla-dr-antibody-13051
CD11c (3.9), 301636, Biolegend, BV605, 1:500 https://www.biolegend.com/fr-ch/products/brilliant-violet-605-anti-human-cd11c-antibody-8603
CD303 (BDCA-2), 354234, Biolegend, BV711, 1:500 https://www.biolegend.com/ja-jp/products/brilliant-violet-711-anti-human-cd303-bdca-2-antibody-16057
CD304 (12C2) 354540, Biolegend, APC/Fire810 1:500 https://www.biolegend.com/fr-ch/products/apc-fire-810-anti-human-cd304-neuropilin-1-antibody-22109
CD56 (NCAM16.2), 612928, BD, BUV563, 1:500 https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv563-mouse-anti-human-cd56.612928?tab=product_details
CD141 (1A4) 746604, BD, BV480, 1:500 https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv480-mouse-anti-human-cd141.746604?tab=product_details
CD45 (HI30), 612891, BD, BUV805, 1:500 https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv805-mouse-anti-human-cd45.612891?tab=product_details
CD3 (UCHT1), 58-0038-42, ThermoFisher, AF532, 1:500 https://www.thermofisher.com/antibody/product/CD3-Antibody-clone-UCHT1-Monoclonal/58-0038-42
CD33 (WM53), BDB740293, Fisher Scientific, BUV395, 1:500 https://www.fishersci.com/shop/products/mouse-anti-human-cd33-buv395-clone-wm53-also-known-as-wm-53-bd-optibuild/BDB740293
CD8a (SK1), 46-0087-42, ThermoFisher, PerCP-eFluor 710, 1:500 https://www.thermofisher.com/antibody/product/CD8a-Antibody-clone-SK1-Monoclonal/46-0087-42
CD1c, 331544, Biolegend, BV785,1:500, https://www.biolegend.com/nl-nl/products/brilliant-violet-785-anti-human-cd1c-antibody-17375
CD71 334120, Biolegend, PE-Dazzle 594, 1: 500 https://www.biolegend.com/fr-lu/products/pe-dazzle-594-anti-human-cd71-

antibody-15988
CD45RA, 304120, Biolegend, AlexaFluor 700, 1:500 https://www.biolegend.com/de-at/products/alexa-fluor-700-anti-human-cd45ra-antibody-3421

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Cell lines: LLC (CRL-1642) and E0771-LMB (CRL-3405) cells were purchased from ATCC (Manassas, VA) and were maintained according to the method of characterization used by ATCC. To enhance metastatic homing to the lungs, both LLC and EO771-LMB cell lines underwent two consecutive rounds of in vivo passaging via spontaneous lung metastases. For each passage, 1 × 10^6 cells were injected subcutaneously into the flanks of WT mice. After 28 days, spontaneous lung metastases were dissected, and metastatic tumour cells were isolated and re-cultured. CRISPR-Cas9 mediated Areg-depletion was conducted using amphiregulin CRISPR KO plasmid from Santa Cruz (sc-419185). sc-419185: amphiregulin CRISPR/Cas9 KO Plasmid (m) is a pool of 3 different gRNA plasmids:A:GCCAGAAGGCATTTCGCTTA. B: AGGGGACTACGACTACTCAG, C: TGCCGATGCCAATAGCTGCG. The cell lines were authenticated by flow cytometry, RT-qPCR and murine AREG-ELISA of conditioned cell culture media and tumour cell lysates.
Authentication	Cell lines were not independently authenticated beyond the identity provided from the ATCC. AR+ and AR- cell lines were authenticated by flow cytometry, RT-qPCR and murine AREG-ELISA of conditioned cell culture media and tumour cell lysates.
Mycoplasma contamination	All cell lines were mycoplasma negative. All cell lines were checked for pathogen contamination via Charles River Research Animal Diagnostic Services before use.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	NCT02608385 & NCT03223155
Study protocol	The full trial protocol of NCT02608385 is described in: Luke, J.J. et al. Safety and Clinical Activity of Pembrolizumab and Multisite Stereotactic Body Radiotherapy in Patients with advanced solid tumors. JCO 36, 1611-1618 (2018). The full COSINR trial protocol (NCT03223155) is described in: Bestvina, C.M. et al. A Phase 1 Trial of Concurrent or Sequential Ipilimumab, Nivolumab, and Stereotactic Body Radiotherapy in Patients With Stage IV NSCLC Study. JTO 17, 130-140 (2022).
Data collection	11 patients were analyzed from NCT02608385 and 15 and 21 patients from NCT03223155 for flow cytometry and serum ELISA, respectively. All clinical data were obtained and stored at the University of Chicago Medical Center.
Outcomes	Clinical evaluation of patients was performed during radiotherapy and with every immunotherapy treatment. Radiographic evaluation was performed using CT scans every 12 weeks, or earlier if clinically warranted. a modified version of RECIST version 1.1 was used to assess overall response, allowing for the incusion of both irradiated and nonirradiated metastases as target and nontarget metastases. The rest of the RECIST principles were applied. Tumor control was defined as any response other than progressive disease. The Kaplan-Meier method was used to estimate progression-free survival (PFS) and overall survival (OS), defined as the time from SBRT planning to either progression per RECIST criteria or death from any cause.

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	Lungs were minced and digested with 1 mg/mL collagenase IV (#C4-22-1G, Sigma-Aldrich) and 200 µg/mL DNase I (#04536282001, Millipore Sigma) at 37°C for 45-60 min on a shaker at 150 rpm/min. For some experiments, cells were then pelleted by centrifugation at 400 × g for 10 min and further purified with 40 and 70% Percoll (GE) solutions by centrifuging at 800 × g (no brake) for 20 min at room temperature. Non-tumor cells were collected from the interface. Red blood cells were lysed using ACK lysis buffer (#A1049201, Gibco). The resulting cell suspensions were filtered through 70 µm cell strainers. Samples were then washed with PBS and incubated with Zombie NIR (BioLegend, 423105) or LIVE/DEADTM yellow (L34959, Invitrogen) fixable viability dye (1:2000) for 10 min at 4°C. Fc blocking was performed using anti-FcR (2.4G2, #CP025, BioXcell,

	1:100). For extracellular staining, surface marker antibodies were added in staining buffer (PBS with 2% FBS and 0.5 mM EDTA (#324506, Millipore-Sigma)) at a 1:200 dilution. For intracellular staining, cells were fixed and permeabilized using the BD Cytofix/Cytoperm solution (#554722, Fisher Scientific) for 60 minutes at 4°C and maintained in BD Perm/Wash buffer (#BDB554723, Fisher Scientific) per the manufacturer's instructions, followed by incubation with intracellular antibodies (1:100 dilution, 30 min, 4°C).
Instrument	Cytek Aurora (5-laser)
Software	SpectroFlo Software (3.3.) and FlowJo (10.8.1).
Cell population abundance	After antibody staining of single cells, FSC - SSC gating was used to determine pure live cell population to be analyzed.
Gating strategy	Gating was performed using fluorescence intensity which is distinct from negative control cells (no-staining).

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