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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	Spectro Flo (Cytek Biosciences) was used to acquire flow cytometry data on Cytek Aurora. FluoView 1000 was used to acquire multiphoton microscopy data. Inveon PET/CT (Siemens) was used to acquire uCT data. Single cell RNA sequencing: Cellranger version version 3.0.1 for
Data analysis	Cell Ranger (v7.0.1), simpleaf (0.17.1), scanpy (1.10.1), scvi-tools (1.1.2), matplotlib (3.8.4), seaborn (0.13.2), pydeseq2 (0.4.9), gseapy (1.1.3), napari (0.4.19), aicsiamgeio (4.14.0), simpleITK (2.3.1), FlowJo (10.7.1),TRI/3D-BON64, RadiAnt DICOM Viewer, ImageJ2 (2.14.0/1.54f), GraphPad Prism (9.5.1). The data analysis code for scRNAseq is available at https://github.com/behnanNN/Behnan-NN-SM . Light sheet python script analysis is available at https://github.com/njkillian/LSM_NN

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 scRNAseq data are available at GSE235464 and GSE213309. All raw and processed files of light sheet, multiphoton, microCT imaging can be shared upon request due to their big szie. The human CT from the TCIA: TCGA-GBM, TCGA-LGG, CPTAC-GBM RTOG 0625/ACRIN 6677 can be accessed after signing agreement with TCIA because of face reconstruction possibility based on CT. Statistical Source data is provided with this paper. Analysis for Figure 1, 2,3,4,8 was based on the raw data file available in the statistic source file. The same for supplementary Figures: S3, S4,S5, S7,S8,S9,S10,S11, S15, S16

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	n/a
Reporting on race, ethnicity, or other socially relevant groupings	n/a
Population characteristics	n/a
Recruitment	n/a
Ethics oversight	n/a

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	No sample size calculation was performed. Sample size was based on pilot study. Previous publications (PMID: 27345406, PMCID: PMC5290038, DOI: 10.1038/onc.2016.230) were used to determine approximate number of cells and animals needed for experiments. A minimum of three animals were used per experiment with statistical analysis, depending on the amount of biological variability. More animals or cells were used when variability was expected to be higher. No significant inter-individual variability was observed in our results. We thus reasoned that the sample sizes were sufficient to demonstrate the findings. The precise number of animals and cells is reported in the figure legends for each figure and Statistical source of data
Data exclusions	For human CT analysis, 3 measurements (in mm) were taken at each anatomical location from 26 GBM patients and 22 age and gender matched control, but some human CT had surgical scar or poor resolution that limited the measurements at one or more locations (details of all statistical measurements can be seen in Source of statistical data). For MRI, only 21 patients out of 26 had MRI. Also, missing points in human CT for lack of clarity or covered are. Such points can be seen in statistical source data marked as NA. For scRNAseq analysis, cells expressing lower than 200 genes were excluded while genes that were expressed in less than 3 cells were also removed. In addition, cells with more than 10% of expressed genes derived from mitochondrial genome were additionally excluded. Scrublet was used to detect and remove doublets from further analysis.
Replication	All the measurements that didn't require statistical testing were taken from at least 3 animals in 2 independent experiments (otherwise 3-9 mice were used per group). All replication attempts were successful.
Randomization	No randomization was done for transgenic TRAP.Td.Tom mice, animals were assigned to the various experiments based on their genotype. C57BL/6, mice were randomized into different groups before tumor injection to receive either tumor injection or sham injection. Mice with the same age (8-12week) and gender (females), same vendor (Charles River,) housed in same clean barrier were used to reduce variability. In

figure 8, and its supplementary, mice were injected with SB28 or GL261, then randomized into various treatment group. Experimental conditions were the same for all groups

Blinding

Data collection and analysis were not performed blind to the conditions of the experiments

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

All antibodies are described in Supplementary Table S3. A list of antibodies is also provided here.
For FACS: CD8a Monoclonal Antibody (53-6.7) Brilliant Ultra Violet™ 395 eBioscience™ (Thermo Fisher Scientific Cat# 363-0081-80, RRID:AB_2920942), MHC Class II (I-A/I-E) Monoclonal Antibody (M5/114.15.2) Brilliant Ultra Violet™ 496 eBioscience™ (Thermo Fisher Scientific Cat# 364-5321-82, RRID:AB_2925347), CD44 Monoclonal Antibody (IM7) Brilliant Ultra Violet™ 563 eBioscience™ (Thermo Fisher Scientific Cat# 365-0441-82, RRID:AB_2925375), Ly-6A/E (Sca-1) Monoclonal Antibody (D7) Brilliant Ultra Violet™ 661 eBioscience™ (Thermo Fisher Scientific Cat# 376-5981-82, RRID:AB_2925469), CD274 (PD-L1, B7-H1) Monoclonal Antibody (MIH5), Brilliant Ultra Violet™ 737, eBioscience™ (Thermo Fisher Scientific Cat# 367-5982-82, RRID:AB_2896030), CD11b Monoclonal Antibody (M1/70) Brilliant Ultra Violet™ 805 eBioscience™ (Thermo Fisher Scientific Cat# 368-0112-82, RRID:AB_2896082), CD19 Monoclonal Antibody (eBio1D3 (1D3)) Super Bright™ 436 eBioscience™ (Thermo Fisher Scientific Cat# 62-0193-82, RRID:AB_2688107), IL-17A Monoclonal Antibody (eBio17B7) Brilliant Violet™ 650 eBioscience™ (Thermo Fisher Scientific Cat# 416-7177-82, RRID:AB_2925706), CD3 Monoclonal Antibody (17A2) Alexa Fluor™ 532 eBioscience™ (Thermo Fisher Scientific Cat# 58-0032-82, RRID:AB_11217479), FOXP3 Monoclonal Antibody (FJK-16s) PerCP-eFluor™ 710 eBioscience™ (Thermo Fisher Scientific Cat# 46-5773-82, RRID:AB_2811810), Rat anti mouse F4/80:StarBright Violet 570 (CI:A3-1) (Bio-Rad Cat# MCA497SBV570, RRID:AB_3101054), BD Horizon™ BV750 Rat Anti-Mouse IFN-γ (XMG1.2/R3-34) (BD Biosciences Cat# 566367, RRID:AB_2869757), BD Horizon™ BB515 Mouse Anti-Mouse CD366 (TIM-3) (5D12) (BD Biosciences Cat# 567810, RRID:AB_2916742), BD Horizon™ BB700 Rat Anti-Mouse CX3CR1 (Z8-50) (BD Biosciences Cat# 567812, RRID:AB_2916743), BD Horizon™ RB780 Mouse Anti-Ki-67 (B56) (BD Biosciences Cat# 568761, RRID:AB_3684525), BD Horizon™ R718 Rat Anti-Mouse IL-10 (JES5-16E3/R35-38) (BD Biosciences Cat# 567082, RRID:AB_2916427), BD OptiBuild™ BUV615 Rat Anti-Mouse CD56 (NCAM-1) (809220) (BD Biosciences Cat# 751046, RRID:AB_2875086), BD OptiBuild™ RY586 Rat Anti-Mouse CD14 (SA14-2) (BD Biosciences Cat# 756785, RRID:AB_3688984), BD OptiBuild™ BV711 Rat Anti-Mouse RANKL/TRANCE (IK22-5) (BD Biosciences Cat# 740820, RRID:AB_2870650), Pacific Blue™ anti-mouse Ly-6C Antibody (HK1.4) (BioLegend Cat# 128013, RRID:AB_1732090), Brilliant Violet 510™ anti-mouse CD127 (IL-7Rα) (A7R34) Antibody (BioLegend Cat# 135033, RRID:AB_2564576), Brilliant Violet 605™ anti-mouse CD117 (c-kit) Antibody (ACK2) (BioLegend Cat# 135120, RRID:AB_2650925), Brilliant Violet 785™ anti-mouse CD192 (CCR2) Antibody (SA203G11) (BioLegend Cat# 150621, RRID:AB_2721565), PerCP anti-mouse Ly-6G Antibody (1A8) (BioLegend Cat# 127654, RRID:AB_2616999), PE/Dazzle™ 594 anti-human/mouse Granzyme B Recombinant Antibody (QA16A02) (BioLegend Cat# 372216, RRID:AB_2728383), PE/Fire™ 640 anti-mouse CD279 (PD-1) Antibody (29F.1A112) (BioLegend Cat# 135257, RRID:AB_2924468), PE/Cyanine5 anti-mouse CD34 Antibody (MEC14.7) (BioLegend Cat# 119312, RRID:AB_830759), PE/Fire™ 700 anti-mouse/human CD45R/B220 Antibody (RA3-6B2) (BioLegend Cat# 103279, RRID:AB_2876408), PE/Cyanine7 anti-mouse CD45 Antibody (30-F11) (BioLegend Cat# 103114, RRID:AB_312979), PE/Fire™ 810 anti-mouse CD11c Recombinant Antibody (QA18A72) (BioLegend Cat# 161105, RRID:AB_2904307), APC anti-mouse CD265 (RANK) Antibody (R12-31) (BioLegend Cat# 119807, RRID:AB_2892276), Spark NIR™ 685 anti-mouse NK-1.1 Antibody (S17016D) (BioLegend Cat# 156529, RRID:AB_2910321), APC/Fire™ 750 anti-mouse TCR γ/δ Antibody (GL3) (BioLegend Cat# 118136, RRID:AB_2650828), APC/Fire™ 810 anti-mouse CD4 Antibody (GK1.5) (BioLegend Cat# 100479, RRID:AB_2860583), Alexa Fluor® 647 anti-mouse CD186 (CXCR6) Antibody (SA051D1) (BioLegend Cat# 151115, RRID:AB_2721358)
For Light Sheet: Polyclonal GFP antibody (N/A) (Novus Cat# NB100-62622, RRID:AB_963761), TdTomato (E3G5L) Rabbit mAb (Cell Signaling Technology Cat# 20163, RRID:AB_3662938), Donkey anti-Sheep IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488 (N/A) (Molecular Probes Cat# A-11015, RRID:AB_141362), Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 546 (N/A) (Thermo Fisher Scientific Cat# A-11035, RRID:AB_2534093)
For in vivo injection: InVivoMAb anti-mouse RANKL (CD254) (IK22/5) (Bio X Cell Cat# BE0191, RRID:AB_10949003), InVivoMAb anti-mouse PD-L1 (B7-H1) (10F.9G2) (Bio X Cell Cat# BE0101, RRID:AB_10949073), InVivoMAb anti-mouse PD-1 (CD279) (29F.1A12) (Bio X Cell Cat# BE0273, RRID:AB_2687796), InVivoMAb rat IgG2b isotype control, anti-keyhole limpet hemocyanin (LTF-2) (Bio X Cell Cat# BE0090, RRID:AB_1107780)

Validation

All antibodies were commercially purchased and validated by the manufacturer which can be accessed at manufacturer's website. For flow cytometry experimental control, single-stained and FMOs were used. The validation of all primary antibodies is described in Supplementary Table S4. A list is also provided here.

[Antigen (Rec. Dil.) Validation]
CD8 (3:2000) immunofluorescence application against C57BL/6 mouse splenocytes by the manufacturer
I-A/I-E (1:1000) immunofluorescence application against C57BL/6 mouse splenocytes by the manufacturer
CD44 (1:1000) immunofluorescence application against C57BL/6 mouse splenocytes by the manufacturer
Ly-6A (ScaI) (1:1000) immunofluorescence application against C57BL/6 mouse bone marrow cells by the manufacturer
CD274 (1:1000) immunofluorescence application against mouse splenocytes by the manufacturer
CD11b (1:1000) immunofluorescence application against mouse splenocytes by the manufacturer
CD19 (1:1000) immunofluorescence application against mouse splenocytes by the manufacturer
Ly-6C (1:1000) immunofluorescence application C57BL/6 mouse bone marrow cells by the manufacturer
CD127 (IL7R) (3:2000) immunofluorescence application C57BL/6 mouse splenocytes by the manufacturer
F4/80 (1:1000) immunofluorescence application mouse peritoneal macrophages by the manufacturer
CD117 (cKIT) (3:2000) immunofluorescence application C57BL/6 mouse bone marrow cells by the manufacturer
IL-17A/RatIgG1 (1:1000) immunofluorescence application \Th17-polarized BALB/c mouse splenocytes by the manufacturer
IFNg/RatIgG1 (1:1000) immunofluorescence application mouse splenic leucocytes by the manufacturer
CD192 (CCR2) (1:1000) immunofluorescence application C57BL/6 mouse bone marrow cells by the manufacturer
CD366 (TIM3) (3:2000) immunofluorescence application BALB/c splenic leucocytes by the manufacturer
CD3 (1:1000) immunofluorescence application mouse splenocytes by the manufacturer
Ly6G (1:1000) immunofluorescence application C57BL/6 mouse bone marrow cells by the manufacturer
CX3CR1 (1:1000) immunofluorescence application C57BL/6 Mouse splenocytes by the manufacturer
FOXP3 (1:1000) immunofluorescence application mouse splenocytes by the manufacturer
Ki-67 (1:1000) immunofluorescence application noncycling Human peripheral blood mononuclear cells or proliferating cells from the Human MOLT-4 cell line by the manufacturer
GZMB (1:1000) immunofluorescence application human peripheral blood mononuclear cells] by the manufacturer
CD279 (PD1) (1:1000) immunofluorescence application C57BL/6 mouse splenocytes by the manufacturer
CD34 (1:1000) immunofluorescence application mouse NIH/3T3 cell line by the manufacturer
B220 (1:1000) immunofluorescence application C57BL/6 mouse splenocytes by the manufacturer
CD45 (1:1000) immunofluorescence application C57BL/6 mouse splenocytes by the manufacturer
CD11c (3:2000) immunofluorescence application C57BL/6 mouse splenocytes by the manufacturer
RANK (1:1000) immunofluorescence application mouse RANK transfected cell by the manufacturer
NK1.1/CD161 (1:1000) immunofluorescence application C57BL/6 mouse splenocytes by the manufacturer
IL-10 (3:2000) immunofluorescence application mouse splenic CD4+ T cells by the manufacturer
TCR g/d (1:1000) immunofluorescence application against C57BL/6 mouse splenocytes by the manufacturer
CD4 (1:1000) immunofluorescence application against C57BL/6 mouse splenocytes by the manufacturer
CXCR6 (1:1000) immunofluorescence application against C57BL/6 mouse splenocytes by the manufacturer
CD56 (1:1000) immunofluorescence application against C57BL/6 mouse cortex cells in Li S., et. al., Nat Neurosci. 2015
CD14 (1:1000) immunofluorescence application against FVB/N mouse macrophages or DCs in Ghosh M., et.al., J Immunol. 2015
GFP (1:1000) immunohistochemistry application against GFP-expressing mouse neuron in Jennifer M., et.al., J Neurosci. 2010
TdTomato (1:800) immunohistochemistry application against tdTomato-expressing mouse liver by the manufacturer
Donkey anti-sheep (1:500) immunohistochemistry application against mouse cortex in Ross A R., et.al., Cell. 2021
Goat anti-rabbit (1:500) immunohistochemistry application against mouse heart in Cong L., et.al., Cell Prolif. 2020
mouse RANKL (CD254) (-) anti-RANKL treatment against C57BL/6 mice in Nicole Y. L., et.al., Cell. 2020
mouse PD-L1 (B7-H1) (-) anti-PD-L1 treatment against C57BL/6 mice in Stathopoulou C., et.al., Immunity. 2018
mouse PD-1 (CD279) (-) anti-PD-1 treatment against C57BL/6 mice in Wang W. et.al., Cancer Cell. 2020
rat IgG2b isotype control (-) treatment against C57BL/6 mice in Wang, W. et.al., Cancer Cell. 2020

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	The murine glioma model GL261 was provided by (NCI-Frederick). SB28 was kindly provided by Dr. Hideho Okada of UCSF. 4T1 was procured from Stem Cell Institute at the Albert Einstein College of Medicine.
Authentication	Cells were originally authenticated by the sources and further validated via scRNAseq used in our recent work (Mandal et al. Cancer Res. 2023). TRAP.Td.Tom mice were generated by our collaborator/coauthor Dr.Masaru Ishii (reference 25), Mice were genotyped by PCR, and fluorescent signal was confirmed by second method, according to their use in multiphoton or FACS. For cell lines, less than 12-passage and 80-95% viability cells were used in various in vivo experiments. Cell morphology and GFP expression were checked regularly during cell expansion for transplantation in SB28. GL261 used in current manuscript has no GFP, validated by in vitro cell morphology and histology of in vivo tumor using HE staining. In vivo tumor formation and growth pattern is reproducible in both models. mice in SB28 start showing symptoms around day 18 after tumor implant, 3-5days before GL261 tumor bearing mice start their smypoms. Also, the 2 models have distinct phenotype upon in vivo growth, SB28 has higher stiffness and mineralization shown by microCT (shown in Fig.1c), By FACS, GL261 has less than 0.5% neutrophils in total CD45+, while SB28 has around 10% Neutrophils and has half number of T cells compared to GL261.
Mycoplasma contamination	Cell cultures were tested for Mycoplasma contamination every month and no cultures were found to be contaminated.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in current work

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	8-12 weeks C57BL/6 were purchased from Charles River Laboratories (USA). Aged C57BL/6J mice (18-24month) were supplied by NIH (Division of Aging Biology). TRAP:tdTomato transgenic animals were obtained from Dr. Masaru Ishii at Osaka University, Japan upon MTA approval. TRAP:tdTomato were bred and maintained according to approved IACUC protocol (#00001516). All mice were maintained under pathogen-free conditions in individually ventilated cages, under stable conditions of humidity and temperature, and controlled vivarium on a 12:12 hours light–dark cycle with free access to food and water.
Wild animals	This study did not involve wild animals
Reporting on sex	Both male and female animals were used.
Field-collected samples	This study did not involve field-collected samples
Ethics oversight	Institutional Animal Care and Use Committee (IACUC) at the Albert Einstein College of Medicine approved the animal protocol (#00001516) according to the NIH Guidelines.

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

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	n/a
Study protocol	n/a
Data collection	n/a
Outcomes	n/a

Plants

Seed stocks	n/a
Novel plant genotypes	n/a
Authentication	n/a

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	Single cell suspensions for SM and BM were prepared as described above for scRNAseq. Cells were fixed in 4% PFA using BD Cytofix/Cytoperm Kit (Torreyana Rd, San Diego, CA, USA) for 20 minutes at 4°C and washed subsequently in FACS Buffer (PBS-4% FBS). Cells were stained with conjugated primary antibodies staining mix for 1hr at 4degC. Subsequently, cells were washed with FACS Buffer and permeabilized usign BD Cytofix/Cytoperm Kit and intracellular staining was performed

	overnight. Cells were washed and then resuspended in FACS Buffer before acquisition on 5-laser Cytek Aurora (Cytek Biosciences). Unmixed FCS files were imported in FlowJo LLC software (v10.7.1, BD, Ashland OR) for analysis. 10,000 – 635 50,000 events were recorded for all the samples.
Instrument	Cytek Aurora (5 Lasers)
Software	FlowJo v10.7.1 (BD LifeSciences)
Cell population abundance	>70% viability was confirmed post-sort for the scRNAseq samples.
Gating strategy	Described in the Manuscript under Supplementary data (Figure S4)

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