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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	FACS DIVA v6.1.3, FlowJo v10.1 for flow cytometry, living Image 4.5.2. for bioluminescence analysis, Visual Molecular Dynamics (VMD) software (version 1.9.1), GROMACS simulation package (version 2016.4), NAMD version 2.10, Acpype, AmberTools, LigPlot+ and PLIP version 1.3.0 for in silico modeling, MaxQuant, MaxLFQ and Perseus for proteomic analysis, QuantStudio 6 and 7 Flex Software for qRT-PCR
Data analysis	ImageJ and Definiens developer XD 2.5. for image analysis, Morpheous for heatmap generation, brainmetgpa for estimating survival . All of them are available online. FACS DIVA v6.1.3, FlowJo v10.1 for flow cytometry, living Image 4.5.2. for bioluminescence analysis, Visual Molecular Dynamics (VMD) software (version 1.9.1), GROMACS simulation package (version 2016.4), NAMD version 2.10, Acpype, AmberTools, LigPlot+ and PLIP version 1.3.0 for in silico modeling, MaxQuant, MaxLFQ and Perseus for proteomic analysis, QuantStudio 6 and 7 Flex Software for qRT-PCR

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All published non-commercial reagents would be made available upon request. Proteomics data is available at PRIDE (PXD008956).

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences

Study design

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

Sample size

Sample size was determined according to our previous experience with both brain organotypic cultures (Martini, Valiente et al. Development. 2009; Valiente et al. Journal of Neuroscience. 2011; Valiente et al. Cell. 2014; Chen et al. Nature. 2016) and in vivo models of brain metastasis (Valiente et al. Cell. 2014; Chen et al. Nature. 2016). Organotypic culture includes, at least, 4 individual replicas measured with bioluminescence and histological validation. In vivo brain metastasis models requires, at least, 8 individual animals measured with bioluminescence ex vivo and histological validation. This sample size requirements were achieved in each individual experiment presented in the manuscript. In vitro experiments (astrosphere assays, T cell co-culture, culture of cancer cells) include at least 3 replicas.

Data exclusions

No data has been excluded from any of the experiments presented in the manuscript

Replication

All data incorporated in the paper has been replicated with reproducible results.

Randomization

Brain cultures or animals receiving treatments were randomized into control or experimental arms according to:
-Brain organotypic cultures: Bioluminiscence imaging at day 0 allowed to generate two groups with homogeneous values so no biased in any of them could interfere with the hypothesis being tested through the use of the inhibitor or genetic manipulations.
-Mice were homogenized regarding age (4-8 weeks) and sex to be present in both control and experimental groups at similar percentages so no biased can influence the effect of the inhibitor or genetic manipulation.

Blinding

Neuropathologists were blinded to interpret the staining of human samples. Investigators were not blinded during data collection, however data collection occurs simultaneously to both control and experimental group in the case of organotypic brain cultures, given that both are located within the same plate that was imaged, so exactly the same settings were applied to both groups. The same simultaneous data collection applies to in vitro cancer cell culture with bioluminescence or astrosphere imaging for number/ size analysis. Data collection was blinded in the case of in vivo brain metastasis experiments.

Materials & experimental systems

Policy information about availability of materials

n/a	Involved in the study
☐	☒ Unique materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☐	☒ Research animals
☐	☒ Human research participants

Unique materials

Obtaining unique materials

Al BrM models are available at the MSKCC cell repository and the one generated in this study (B16/F10-BrM) will be made available upon request. The mouse model generated (cKO-Stat3) could be made available upon authorization from its original sources (Jax lab, and Valeria Poli). Legasil and silibinin use in the formulation of Legasil was provided by MEDA.

Antibodies

Antibodies used

All antibodies used are commercially available and we indicate their specific dilution and lot number if available. For human samples pSTAT3 (Tyr705) was initially validated using a positive control as suggested in the antibody data sheet. The same was

applied to the ICAM1 antibody applied to human samples. Both validations were performed in an automated manner and by personnel belonging to our core facilities. GFP, GFAP, pSTAT3 (Tyr705), BrdU, cleaved Caspase 3, Nestin are very well established antibodies in the field with a very specific staining pattern that we initially confirmed. Nestin was validated using embrionic brain tissue. pSTAT3 staining was validated by the use of various inhibitors targeting the activation of this pathway. BrdU staining was only observed upon previous administration of the thymidine analog. GFAP was validated staining normal brains, where no staining can be detected. Primary antibodies for immunofluorescence: GFP (Aves Labs, ref. GFP-1020, 1:1,000), STAT3 (Tyr705) (Cell Signaling, ref. 9145, 1:100), GFAP (Millipore, ref. MAB360, 1:1000), Ibal (Wako, ref. 019-19741, 1:500), Nestin (BD Bioscience, ref. 556309, 1:100), NeuN (Millipore, ref. MAB377, 1:500), cleaved caspase-3 (1:500, ref. 9661; Cell Signaling), BrdU (Abcam, ref.ab6326, 1:500), CD8 (Novus Biologicals, ref. NB200-578, 1:100), CTGF (L-20) (Santa Cruz, ref. sc-14939, 1:200), ICAM1 (BD Pharmigen, ref. 553249, 1:100), PD-L1 (B7-H1, clone 10F.9G2)(BioXcell, ref. BE0101, 1:100), CD74 (BD Biosciences, ref. 555318, 1:100), CD31 (BD Pharmingen, ref. 550274, 1:100), Collagen IV (Millipore, ref. AB756P, 1:500), CD68 (KP1) (Abcam, ref. ab955 1:200), STAT3α (D1A5) (Cell Signaling, ref. 8768, 1:100), Beta III Tubulin (2G10) (Tuj1) (Abcam, ref. ab78078, 1:100), Olig 2 (Millipore, ref. AB9610, 1:500). Secondary antibodies: Alexa-Fluor anti-chicken488, anti-rabbit555, anti-mouse555, anti-mouse633, anti-mouse647 (Invitrogen, dilution 1:300). Primary antibodies for immunohistochemistry: GFAP (Dako, ref. IR524, ready to use), p-STAT3 (Tyr705) (D3A7, Cell Signaling, ref. 9145, 1:100), ICAM1 (Sigma-Aldrich, ref. HPA004877, 1:500), STAT3 (F-2, Santa Cruz, ref. sc-8019, 1:100), Nestin (EPR1301(2), Abcam, ref. ab176571, 1:350). Antibodies for flow citometry: anti-mouse CD8a-FITC (Tonbo Biosciences, ref.35-008-1, 1:200), anti-mouse NK1.1-PE (e-Bioscience, ref. 12-5941-82, 1:200) and anti-mouse CD11C-APC (BD Biosciences, ref. 550261, 1:200), CD44-PE (BD Biosciences, ref. 553134, 1:200) and CD25-PerCP-Cyanine5.5 (e-Bioscience, ref. 45-0251-80, 1:200), CD74-AF647 (1:200; Biolegend ref. 151003) and CD11b-PerCP-Cyanine5.5 (1:200; e-Bioscience ref. 45-0112-82).

Validation

All antibodies used have been used based on its previous validation as provided by the vendor and/or previous publications. When genetic models were available (cKO-Stat3) antibodies were validated on them as well.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

MDA231-BrM2, ErbB2-BrM2, 373N1, 393N1, 482N1, 2691N1, H2030-BrM3, PC9-BrM3 and HCC1954-BrM1 were obtained from MSKCC (Joan Massagué lab). B16/F10-BrM3 was generated in Valiente lab.

Authentication

Cancer cell lines used have been obtained from the same batches that were previously published (Valiente et al. Cell 2014). Cell lines were validated by morphological analysis and their behavior in vivo. To discard any cell line specific mechanism we have incorporated to our study a large variety of brain metastatic models.

Mycoplasma contamination

All cell lines were tested to be free from Mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were use in this study

Research animals

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

Animals/animal-derived materials

We have used nude animals (Hsd:Athymic Nude-Foxn1nu) bough to Envigo and C57BL/6 animals obtained from the inbred colony at CNIO (This colony was established from C57BL/6JOlaHsd from Envigo and it is renewed every two years with new animals from the original colony). The cKO-Stat3 was established from founder animals obtained from Jackson Labs (ref. 012849) and founder animals from Valeria Poli and then maintained with C57BL/6 animals. The Cx3cr1-EGFP mice was established from founder animals obtained from Jackson Labs (ref. 005582). Mice used in experiments have 4-10 weeks of age. Nude mice are females. Singeneic strains are males and females, with an equal distribution between control and experimental cohorts.

Human research participants

Policy information about studies involving human research participants

Population characteristics

Human patients included in the analysis of pSTAT3 by immunohistochemistry included 91 samples from lung adenocarcinoma (44 samples), breast adenocarcinoma (40 samples), melanoma (2 samples) and other primary tumors (5 samples) obtained through surgical resection at three different institutions (Vall d´Hebron Hospital, University and City of Health and Science University Hospital of Turin, Hospital Universitario 12 de Octubre. Clinical information regarding diagnosis of brain metastasis and exact date of death were included, when available, also in the analysis to determine survival. Both control and Legasil® groups were treated at the same institution (Dr. Josep Trueta University Hospital). Detailed information regarding sex (P=0.457), age (P= 0.986), Karnosfky PS (P= 0.008), extracranial metastases (P= 0.693), histology (P= 0.725), BM status (P= 0.394), GPA prognostic class (P= 0.066), gene status (P= 0.047), number of BM (P= 0.484), size of biggest BM (P= 0.806) as well as specific chemotherapy receiving previous to diagnosis of brain metastases, 1st, 2nd, 3rd and 4th lines of therapy is provided. Note that no differences in clinical variables were notice with the exception of Karnofsky PS, in which the control group had a better index, and the gene status, in which the Legasil® cohort had more patients with mutations in the primary tumor susceptible to be treated with targeted therapies. This last consideration was discarded to be responsible for the benefit derived of using Legasil® because removal of theses subpopulation of patients did not change the main conclusion.

Method-specific reporting

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ Magnetic resonance imaging

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	T cells/GFP+ cells were obtained from the spleen/ brain of 10-15 week-old C57BL/6 mice/Cx3cr1-EGFP mice. Organs were dissected and pressed through a 70 µm cell strainer and red blood cells were lysed with ACK Lysing Buffer (Lonza, ref. 10-548E). Cells were concentrated by centrifugation for 5 min at 2,000 rpm and resuspended in BSA 0.1% in PBS in single cell condition after passage through a mesh (50 µm). Then, samples were incubated with Fc-Block (BD, ref. 553141) and stainings were performed. Cultured CD8+ T cells/primary mouse astrocytes infected with the EF.STAT3C.Ubc.GFP plasmid were collected/trypisinized and washed with PBS 1X. Cells were resuspended in BSA 0.1% in PBS and incubated with Fc-Block (BD, ref. 553141) for later staining as described above. For cell sorting the following buffer was used: FBS 2%, HEPES 25 mM, 10 U/ml DNase in PBS.
Instrument	BD FACSAria IIu cell sorter (BD, San Jose CA) and BD FACSCanto II (BD, San Jose CA) equipped with 488 nm, 633 nm and 407 nm lasers.
Software	All data was collected and performed with BD FACSdiva v6.1.3 (BD, San Jose CA) and analyzed using FlowJo v10.1 (Treestar, OR).
Cell population abundance	Between 2,500 and 20,000 single live events from the populations of interest were acquired to analyze and between 5,000 and 70,000 events were sorted to culture.
Gating strategy	Pulse processing and DAPI were used to exclude cell aggregates and dead cells and Fluorescence minus one controls were used to perform the gating of the different subpopulations analysed. For the experiments shown in figure 4b and supplementary 4b, the live single population, excluding debris, was gated to select the doble negative CD11c-APC NK1.1-PE subpopulation that was used to sort CD8-FITC positive cells (>10x3). Sorted CD8+ cells were then used for the experiments to analyze CD44-PE and CD25-PerCP-Cyaninne5.5 expression. Analysis include percentage of cells above 10x3 for both cell surface markers. Last steps of the gating strategy are shown in figure supplementary 4b, the complete strategy is provided in the attached file.

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