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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	Flow cytometry samples were acquired with the BD LSRFortessa™ (using FACSDiva™ v9 software) or the Cytek® Aurora spectral cytometer (using SpectroFlo® software). Plate reader data (luminescence, absorbance, fluorescence) was acquired using Agilent Gen5 software. Microscopy and histology scanning data was acquired using Zeiss ZEN blue v2.6 software. High-content imaging data was acquired using HCS Studio v2. ELISPot data was acquired using ImmunoSpot® Software version 5.3. Radiance and total flux were calculated using the Living Image v4.7 software (PerkinElmer). qPCR data was collected using the 7500 Fast Real-Time PCR System (Applied Biosystems) and the 7500 Software v2.3. Immunofluorescence Images were taken using the AxioCam HRm camera (Carl Zeiss Ltd, Toronto ON) mounted on the Zeiss Axioscope Imager M1 or the Zeiss Axio Imager M2 microscope. Immunohistochemistry tissue sections were imaged using an AxioScan.Z1 and the ZEN v2.6 (blue edition) software.
Data analysis	Flow cytometry data were analyzed using FlowJo v10.8 or OMIQ software. Dimensionality reduction plots were generated using the opt-SNE algorithm (https://doi.org/10.1038/s41467-019-13055-y). Imaging data analyses were done using Zeiss ZEN blue v2.6 software, QuPath v0.5.1, ImageJ, Fiji v2, or HCS Studio v2. Data analysis, including statistical analysis, were done using MS Excel or Graphpad Prism v10.2.1.

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

Source data are provided with this paper.

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	Patient data was collected after specimen collection, processing, and analysis. While sex and gender data were collected, they were not factored into analyses because it was no applicable and sample size was too small.
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable.
Population characteristics	Not applicable
Recruitment	Consenting of patients for specimen collection was done by the treating surgeon at the time of consent for surgery, as part of the standard procedure at The Ottawa Hospital. These specimens were collected as part of the Platform of Global Consenting for Tissue (GTC) from Surgical Specimens, under the protocol OHSN REB # 20180079-01 and 20220124-01H.
Ethics oversight	The Ottawa Hospital and The Ottawa Hospital Research Institute

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	For all in vivo animal experiments n=5-10 mice per group was selected. This group size is standard protocol based on a power calculation of 0.8., using the NIH Sample Size Calculator. Briefly, n=5 mice per group is a standard sample size given housing limitations at our animal facility. Additionally, we increase sample size if experimental outcomes are more variable. In these series of experiments we found our results consistent, and instead of increasing the n-value in a single tumour model, we repeated the experiment in 6 different tumour models. These experiments were not conducted in parallel, but sequentially. Each experiment was thus conducted independently, at a different time, using syngeneic mice sourced from different batches and colonies (Jackson Laboratory or Charles River). For patient specimen collection, all samples made available were collected, processed, and analyzed. Patient specimens are rare and often small; while experiments were designed for a power of 0.8, tumour samples were processed exhaustively to obtain the largest possible number of tumour cores per patient. Sample size was determined based on previous experiments performed in our hands that gave statistically significant data. Typically, cell culture experiments were performed using three or more independent biological replicates. Low variability within the same experimental condition indicated by tight SEM or SD confirms that the selected sample size is appropriate to detect statistical differences (if present) among treatment conditions. Where high variability among replicates was observed, sample size was increased. N-values are indicated in figure legends and defined in the Methods.
Data exclusions	Patient specimens that led to bacterial or fungal contaminations, or that was not usable due to major necrotic regions, were excluded from further processing and analysis. All attempts to repeat experiments were successful, including in multiple disease models in vivo.
Replication	A minimum of three biological replicates are used in all experiments. All animal experiments were replicated in multiple models, by 2 different operators. All in vitro experiments are the result of at least 2 independent experiments.
Randomization	Following tumour implantation in mice, all animals were randomized to treatment groups. Importantly, we ensured comparable tumour burden per treatment group prior to treatment initiation; i.e. mean tumor volume per group for experiments with subcutaneous tumours, or mean tumour burden by IVIS luminescence signal for intraperitoneal models. Ex vivo tumour cores, both murine and patient-derived, were randomized to different treatment groups. Briefly, cores were generated by biopsy punch of uniformly sliced tumour sections, and these cores were randomly allocated across the treatment groups, to ensure that tissue variability applies to all groups.

Blinding

For all in vivo studies, veterinary technicians were blinded to the treatment groups. These technicians measured tumour volumes and assessed animal wellness for objective determination of humane endpoint. Histological assessment of lungs was done by a pathologist who was blinded to the sample identity. Throughout all independently replicated experiments, investigators were blinded to treatment conditions during data acquisition and data analyses. For experiments using patient tumour specimens, the operators handling the samples, acquiring the flow cytometry data, and analyzing the readouts were blinded to treatment conditions.

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
[ ]	[x] Antibodies
[ ]	[x] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[ ]	[x] Animals and other organisms
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[ ]	[x] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used

Antibodies used for flow cytometry: We used the following antibodies: CD45-BV786 (BD, 564225; 1:1000), CD3 -Alexa Fluor 700 (BD, 561805, 1:300), CD49b-BUV395 (BD, 740250, 1:100), CD335-BUV805 (BD, 742066, 1:100), CD314-BUV496 (BD, BDB750014, 1:100), CD4-V450 (BD, 560468, 1:1000), CD8 -PerCP-Cy5.5 (BD, 551162, 1:200), TCR γ/δ-PE-Cy7 (Invitrogen, 25-5711-80, 1:200), CD25-BUV737 (Invitrogen, 367-0251-82, 1:100), CD69-BUV605 (BD, 563290, 1:100), PD-1-APC-Cy7 (Biolegend, 135223, 1:100), LAG3-BUV661 (BD, 741594, 1:100), CTLA-4-PE (Invitrogen, 12-1522-82, 1:200), CD127-PerCP-eFluor 710 (Invitrogen, 46-1273-82, 1:200), CD62L-NovaFluor Blue 555 (Invitrogen, M006T02B03, 1:300), CD44-APC (BD, 563058, 1:100), CXCR3-BV711 (BD, 740825, 1:100), CD103-BV421 (Biolegend, 121421, 1:100), CD39-PE-Fire 640 (Biolegend, 143817, 1:100), CD27-BV650 (BD, 740491, 1:200), CD3 -APC (Biolegend, 108909, 1:200), CD49b-APC (Biolegend, 100235, 1:200), CD19-PE-Cy5 (Biolegend, 115509, 1:200), CD11c-PE (BD, 553802, 1:100), CD11b-APC-Cy7 (Biolegend, 101225, 1:100), F4/80-BUV395 (BD, 565614, 1:200), MHCII (IA-IE)-BUV737 (Invitrogen, 367-5321-80, 1:500), CD86-APC-R700 (BD, 565479, 1:200), PD-L1-PE-Cy7 (Biolegend, 124313, 1:100), CD205-SB600 (Invitrogen, 63-2051-82, 1:200), CD8a-PerCP-Cy5.5 (BD, 561109, 1:200), CD1d-BV711 (BD, 740711, 1:100), IFNγ-APC (Invitrogen, 17-7311-81, 1:100), TNFa-PE (Biolegend, 506305, 1:100) , goat anti-mouse IgG-AlexaFluor™ Plus 488 (1:300, Invitrogen, Cat. # A32723), Anti-His IgG-AlexaFluor 647 (1:300, ThermoFisher Scientific, Cat. # MA1-21315-A647) or anti-human IgG-PE (1:500, Invitrogen, Cat. # PA1-86978)

Antibodies used for Western immunoblotting: Mouse anti-His antibody (Abcam, 1:1000, Cat.# ab18184, ON, Toronto, Canada). Vaccinia virus presence was confirmed with a rabbit polyclonal antibody that detects vaccinia virus proteins (LSBio, Cat. # LS-C103289, 1:1000, Seattle, WA, USA). HER2 levels were detected using a mouse monoclonal antibody against human HER2/ErbB2 (1:1000, Invitrogen, Cat. # MA5-13105). β-Actin (1:1000; 13E5; Cell Signaling Technology) was used as a loading control for immunoblots. After overnight incubation with primary antibodies, the immunoblots were probed with HRP-coupled anti-rabbit or anti-mouse antibodies (1:5000) (Jackson ImmunoResearch Laboratory, West Grove, PA, USA, Cat. # 711-035-152 (anti-rabbit) 715-035-150 (anti-mouse).

Antibodies used for microscopy or IHC: goat anti-human IgG-AlexaFluor-594 (1:200, Invitrogen, Cat. # A-11014), anti-rabbit IgG-Alexa Fluor-594 (1:300, Invitrogen, Cat. # A-11012) or anti-human IgG-Alexa Fluor-594 (1:300, Invitrogen, Cat. # A-11014), rabbit anti-vaccinia virus polyclonal antibody (1:2000, Invitrogen, Cat. # PA1-7258), rabbit anti-VSV polyclonal antibody (1:2000), rabbit anti-cleaved caspase 1 monoclonal antibody (1:500, Invitrogen, Cat. # PA5-99390), rabbit anti-cleaved caspase 3 monoclonal antibody (1:2000, Cell Signaling Technology, Cat. # 9664), trastuzumab (1:1000-1:5000)

Validation

All antibodies were previously validated in-house as part of the optimization process for flow panels. All western blotting, immunofluorescence, immunocytochemistry, and immunohistochemistry antibodies were titrated and validated prior according to manufacturer's instruction. All flow cytometry antibodies were titrated in single-stained positive and negative control samples.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

We used the following cell lines: Vero (CCL-81, ATCC; African green money, F, Kidney, DMEM), 786-O (CRL-1932, ATCC; Human, M, Kidney, DMEM), GM38 (GM00038, Coriell Institute for Medical Research; Human, F, Skin, DMEM), HT29 (HTB-38, ATCC; Human, F, Colon, RPMI), JIMT-1 (ACC589, DSMZ; Human, F, Breast, DMEM), U-2 OS (HTB-96, ATCC; Human, F, Bone, RPMI), HEK293T (CRL-3216, ATCC; Human, F, Kidney, DMEM), AF2068 and AF2028 and AF2780 (patient derived ovarian cancer ascites fluid cell lines, F, RPMI), SKOV3 (HTB-77, ATCC; Human, F, Ovary, RPMI), OVCAR-4 (SCC258, Milipore Sigma; Human, F, Ovarian cancer ascites, RPMI), J69 (Jurkat CD69-TdTomato) (Human, M, RPMI), CT26.WT (CT26) (CRL-2638, ATCC;

	Mouse, F, Colon, DMEM), CT26LacZ (CT26.CL25) (CRL-2639, ATCC; Mouse, F, Colon, DMEM), CT26-HER2T (Mouse, F, Colon, DMEM), MC38 (Mouse, F, Colon, DMEM), MC38-HER2T (Mouse, F, Colon, DMEM), MC38 (Mouse, F, Colon, DMEM), ID8 (Tp53-/- Pten-/-) (Mouse, F, Ovary, DMEM supplemented with 4% FBS and 1% ITSS (5 µg/mL insulin, 5 µg/mL transferrin, and 5 ng/mL sodium selenite; R&D Systems, MN, USA, Cat. # AR013).), ID8-PP-HER2T ((Mouse, F, Ovary, DMEM supplemented with 4% FBS and 1% ITSS (5 µg/mL insulin, 5 µg/mL transferrin, and 5 ng/mL sodium selenite; R&D Systems, MN, USA, Cat. # AR013).), ID8-HER2T ((Mouse, F, Ovary, DMEM supplemented with 4% FBS and 1% ITSS (5 µg/mL insulin, 5 µg/mL transferrin, and 5 ng/mL sodium selenite; R&D Systems, MN, USA, Cat. # AR013).), 4T1.2 (CRL-3406, ATCC; Mouse, F, Breast, RPMI), 4T1.2-HER2 (Mouse, F, Breast, RPMI), 4T1.2-HER2T (Mouse, F, Breast, RPMI), B16-F10 (CRL-6475, ATCC; Mouse, unknown sex, skin/melanoma, RPMI)
Authentication	None of the cell lines listed were present in the commonly misidentified cell lines database maintained by ICLAC. All cell lines were commercially purchased with certificates of authentication, unless specifically indicated that they were obtained from collaborating labs and documented in a Material Transfer Agreement.
Mycoplasma contamination	All cells were maintained at 370C in a 5% CO2 humidified incubator, routinely tested for mycoplasma contamination by Hoechst staining and PCR (Diamed, Mississauga, Ontario, Catalogue # ABMG238) and used within 3-10 passages since thaw
Commonly misidentified lines (See ICLAC register)	None of the cell lines listed were present in the commonly misidentified cell lines database maintained by ICLAC.

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	6-8 week-old female wildtype BALB/c or C57BL/6, and CD1 nude mice (Crl:CD1-Foxn1nu) were purchased from The Jackson Laboratory and Charles River.
Wild animals	None
Reporting on sex	All animals were used were female mice to account for sex differences, especially in murine models of ovarian and breast cancer.
Field-collected samples	None
Ethics oversight	In vivo experiments were performed via protocols OHRI-2265 and OHRI-2264 which are in good standing with the Animal Care Committee, and care and treatment of animals was in accordance with the ethical standards of the Canadian Council on Animal Care and with the Animals for Research Act.

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

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	For tumour profiling: Tumours were dissociated using the Miltenyi mouse tumour dissociation kit (Miltenyi Biotec, Cat. #

130-096-730, CA, USA) and the gentleMACS Octo Dissociator (Miltenyi Biotec, Cat. # 130-096-427). Spleens were collected and dissociated by crushing the organs through a 70 µm strainer using the plunger of a 3 mL syringe. All dissociated spleens underwent erythrocyte lysis using ACK buffer (Gibco, Cat. # A1049201). All cell suspensions were strained and counted. 2 × 106 cells were resuspended in 200 µl of FACS buffer (0.5 % BSA-PBS) and transferred to round-bottom 96-well plates for staining for flow cytometry. Alternatively, dissociated tumours or splenocytes were resuspended in 100 µl RPMI supplemented with 10% heat-inactivated FBS for in vitro culture. Next, , cells were stained with fixable viability dye FVS510 (1:1000, BD Biosciences, NJ, USA, Cat. #564406) in PBS for 15 min at room temperature. Cells were washed and then incubated with anti-CD16/32 (1:100, BD Biosciences, Cat. # 553141) in 0.5% BSA-PBS for 30 min at 40C to block non-specific antibody interaction with Fc receptors. Cells were next incubated with CellBlox™ blocking buffer (1:100, Invitrogen, Cat. # B001T06F01) to block non-specific interactions with monocytes and macrophages. Cells were subsequently stained with an antibody cocktail diluted in 0.5% BSA-PBS and Super Bright complete staining buffer (1:50, Invitrogen, Cat. # SB-4401-75). Cells were then washed and resuspended in 1% paraformaldehyde (PFA) in PBS. Samples were acquired with the BD LSRFortessa™ or the Cytek® Aurora spectral cytometer at the University of Ottawa Flow Cytometry and Virometry core facility (Director: Dr. Vera Tang), and the Ottawa Hospital Research Institute Flow Cytometry and Cell Sorting core facility (Director: Fernando Ortiz). Data were analyzed using FlowJo v10.8 or OMIQ software. Dimensionality reduction plots were generated using the opt-SNE. Unstained controls, and fluorescence-minus-one (FMO) controls were prepared in parallel. Ultracomp eBeads (Thermo Scientific, Cat. # 01-2222-42) single-stained beads were used for compensation. For the detection of virally-encoded fluorophores, samples were not fixed and were assessed by flow cytometry immediately after dissociation and staining to prevent fluorescence quenching. For the detection of cells infected by VSVΔ51-HER2T, cells were stained with trastuzumab (1:1000) followed by a goat anti-human IgG-PE (1:300, Invitrogen, PA1-86078).

Instrument

Samples were acquired with the BD LSRFortessa™ (using FACSDiva™ software) or the Cytek® Aurora spectral cytometer (using SpectroFlo® software).

Software

Analysis was done using FlowJo v10 or OMIQ softwares.

Cell population abundance

Major populations of interest fell within the CD45+ live gated leukocytes. This overarching gate represented between 20-50% of the dissociated tumour specimen. Subsequent gating depended on the subpopulation of interest. No sorting was done.

Gating strategy

For T cell populations, lymphocytes were gated using FSC/SSC, followed by doublet exclusion, then gating on CD45+ live cells. CD3+ cells were gated, followed by CD4/CD8. For NK cells, following CD45+ live gating, CD3- (or CD3+ for NKT) populations were interrogated for CD49b+ lymphocytes. For B-cells, following CD45+ live gating, CD3-/CD49b- populations were gated on CD19+ B cells. For myeloid cells, following CD45+ live gating, CD11b+ populations were selected (or CD11c+ for dendritic cells), followed by subsequent lineage-specific gating. For IgG assays, proportion of single cells positive for the secondary antibody are quantified. Gating strategy is shown in the supplemental data. Specific activation and subpopulation markers are used as indicated.
All gates were set using fluorescence-minus-one (FMO) controls.

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