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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

For in vivo experiments, samples sizes were determined based on previous experience with the models utilized, including experience in variability of tumor growth.

2. Data exclusions

Describe any data exclusions.

No data were excluded from analysis.

3. Replication

Describe whether the experimental findings were reliably reproduced.

Experimental findings were reliably reproduced.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Prior to treatment initiation, mice were randomized into groups of equal average tumor volume.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

In vivo experiments were performed in a blinded fashion when possible. Downstream analyses of mouse tissue (immunohistochemistry, image analysis, flow cytometry) all experiments were performed in a blinded fashion such that the people conducting and/or analyzing the assay were not aware of treatment groups until data gathering was complete.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	No custom code was used.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	There are no restrictions on availability of materials.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Immunostaining antibodies: Ki-67 (Vector), HER2 (AB16901; Abcam), STAT1 (AB47425; Abcam), FoxP3 (clone FJK-16s; eBioscience 14-5773-80), CD8 (clone 4SM15; eBioscience 14-0808-82), and Ki-67 (clone SP6; Thermo Scientific MA5-14520). Immunostaining secondary antibodies: AF488 AffiniPure donkey anti-mouse IgG (Jackson ImmunoResearch Laboratories 715-545-150), Cy3 AffiniPure donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories 711-165-152), AF488 donkey anti-rabbit IgG (Life Technologies R37118), and AF647 goat anti-rat IgG (Life Technologies A21247). Western blotting antibodies: cleaved PARP (CST 9541), cleaved caspase-3 (CST 9661), phospho-STAT1 Y701 (CST 9167), STAT1 (CST 9172), FLAG (CST 2368), vinculin (V9131, Sigma) and DNMT1 (92314, Abcam). Murine flow cytometry antibodies: Antibodies were purchased from Biolegend unless otherwise indicated: CD45 (clone 30-F11; 103126 and 103131), CD3 (clone 145-2C11; 100320), CD8 (clone 53-6.7; 100714, 100743), CD4 (clone RM4-5, 100529), PD-1 (clone 29F.1A12; 135223), Tim-3 (clone RMT3-23; 119705), CTLA-4 (clone UC10-4B9; 106313), LAG-3 (clone C9B7W; 125207), B220 (clone RA3-6B2; 103245), NK1.1 (clone PK136; 108739), CD11b (clone M1/70; 101206), Ly6G (clone 1A8; 127639), Ly6C (clone AL-21; BD Pharmigen 560595), and FoxP3 (clone FJK-16s; eBioscience 17-5773-82). Human flow cytometry antibodies: 2-microglobulin (clone 2M2; 316304) and HLA-A,B,C (clone W6/32; 311425). dsRNA was detected by the K1 antibody (English and Scientific Consulting (Hungary). All antibodies were previously validated by their manufacturers.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	BT474, SKBR3, MDA-MB-361, MDA-MB-453, and MCF7 human breast cancer cell lines were obtained from ATCC. MMTV-PyMT-S2WTP3 cells were a gift from Dr. Andreas Möller, QIMR Berghofer Medical Research Institute, and CT-26 cells were a gift from Dr. Steve Elledge, Harvard University.
b. Describe the method of cell line authentication used.	All cell lines were authenticated by short tandem repeat analysis.
c. Report whether the cell lines were tested for mycoplasma contamination.	All cell lines tested negative for mycoplasma.
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

MMTV-rtTA/tetO-HER2 mice1 were maintained in house. Female FVB MMTV-PyMT, Balb/c (6-7 weeks old), and Foxn1nu (8 weeks old) mice were purchased from Jackson Labs. Female FVB mice (7 weeks old) were purchased from Taconic Biosciences. P14-TCR transgenic mice (gift from Dr. Kai Wucherpfennig, Harvard Medical School), and OT-I mice (C57BL/6 Tg(TcraTcrb)1100Mjb/J; Jackson Labs) were used as a source of CD8+ T cells for in vitro co-culture assays. FVB CD45.2+ mice (gift from Dr. Daniel Tenen, Harvard Medical School) were used as a source of T cells for in vitro studies.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

This study did not involve human research participants.

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Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. 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).

☒ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	Tumor cell lines –1x106 cells per condition were stained with the appropriate antibodies diluted in PBS (Hyclone) plus 2% FBS (Life Technologies) for 30 minutes on ice. Matched fluorescence minus one (FMO) staining for each condition was performed as a control. Blood – Obtained by retro-orbital sampling at intermediate time points and by cardiac puncture at experimental endpoints. Blood cells and plasma were separated by centrifugation at 1,500 x g for 8 minutes at 4C. Spleen, thymus, and lymph nodes – Single cell suspensions were obtained by mechanical digestion. Tumor - Tumors were first mechanically disrupted by chopping, then chemically digested in dissociation buffer (2 mg/mL collagenase type IV (Worthington Biochemical), 0.02 mg/mL DNase (Sigma Aldrich) in DMEM (Life Technologies) containing 5% FBS (Life Technologies), PenStrep (Hyclone)) with agitation at 37C for 45 minutes. Following RBC lysis if necessary (blood, spleen, thymus, tumor; PharmLyse, BD Biosciences), single cell suspensions were blocked with anti-CD16/32 (Biolegend) for 20 minutes on ice and then incubated with appropriate antibodies for 30 min on ice.
6. Identify the instrument used for data collection.	Flow cytometry was performed on a LSR II SORP 5 Laser Analyzer (BD Biosciences) or BD FACSCanto II Flow Cytometer (BD Biosciences, Ref. 338960).
7. Describe the software used to collect and analyze the flow cytometry data.	FACSDiva software (BD Biosciences) was used to collect the data, and the data was analyzed using FlowJo (TreeStar).
8. Describe the abundance of the relevant cell populations within post-sort fractions.	No FACS sorting was performed for this work.
9. Describe the gating strategy used.	For all experiments, debris was first excluded by a morphology gate based on FSC-A and SSC-A. Then, non-singlets were eliminated from analysis by a single cell gate based on FSC-H and FSC-A. Next, dead cells were eliminated by an appropriate viability gate: 7AAD was used to distinguish live/dead cells, except in cases requiring intracellular staining in which case eFluor 450 (eBioscience) or Zombie Yellow (Biolegend) fixable viability dyes were used. When appropriate, all lymphocytes were identified using a CD45+ gate. For identification of regulatory T cells, CD45+ cells were gated for CD3+ CD4+ FoxP3+ cells. For analysis of checkpoint inhibitor

expression, CD3+ CD4+ and CD3+ CD8+ populations were then examined for PD-1, CTLA-4, Tim-3, and LAG-3 expression.

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