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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	Flow cytometry data was collected using FACSDiva 8.0.1 (BD). Bioluminescence data was collected using Living Image 4.2.
Data analysis	Flow cytometry data was analyzed using FACSDiva 8.0.1 (BD) and FlowJo 10 (Tree Star). Bioluminescence data was analyzed using Living Image 4.2. All graphs were generated and analyzed using GraphPad Prism 8. Indel analysis for gene knockouts was performed using TIDE 2.0.1. Single-cell RNA sequencing analysis was performed with STAR 2.5.3a. Contour plots were generated using Plotly 2018.

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. 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 primary data for all figures and supplementary figures are available from the corresponding authors upon request.

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 study design

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

Sample size	Sample sizes were modeled after those from existing publications regarding in vitro immune killing assays and in vivo tumor growth assays, and an independent statistical method was not used to determine sample size. In our experience with in vitro measurements of phagocytosis, we have found that assaying human macrophages from 3 donors is sufficient for studies of antibody efficacy based off of observed variability among donors.
Data exclusions	As listed in the Methods, phagocytosis assays were performed in a minimum of technical triplicate for a minimum of 3 human donors per treatment group. In some cases, donors or specific technical replicates were excluded on the pre-established criterion that they were found to be a significant outlier by the GraphPad Outlier Calculator (https://www.graphpad.com/quickcalcs/Grubbs1.cfm). In some cases, additional replicates of specific phagocytosis assay conditions were repeated as part of pilot experiments, or as confirmatory replicates, but only a discrete set of data performed under identical conditions was specifically reported. For in vivo experiments, individual mice were removed from the study either prior to treatment, if found to be an engraftment outlier by bioluminescence imaging, 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). In some cases across additional experiments, including pilot experiments, additional mice were engrafted subcutaneously with relevant cell lines and followed for non-standard periods of time, or assessed for tumor growth at non-standard intervals, but only a discrete set of mice assessed under identical conditions was reported.
Replication	In vitro phagocytosis assays were performed in technical triplicate for a minimum of 3 human donors per treatment group with similar results and responses observed across donors and replicates. In vitro phagocytosis assays were performed across multiple experimental replicates, when possible, with the exceptions of the phagocytosis assays shown in Figure 2d (4 biological replicates, one experimental replicate), Figure 2g (4 biological replicates, one experimental replicate), Figure 2b (U-87 only; 3 biological replicates, one experimental replicate), Extended Data Figure 2e (3 biological replicates, one experimental replicate), Extended Data Figure 3c (4 biological replicates, one experimental replicate), Extended Data Figure 5c (3 biological replicates, one experimental replicate), Extended Data Figure 5d,f (4 biological replicates, one experimental replicate), Extended Data Figure 9a (4 biological replicates, one experimental replicate). Staining and recombinant Siglec binding experiments were performed in at least 2 experimental replicates. Automated live cell microscopy experiments were performed across at least technical and biological duplicates. Whenever practical for in vivo experiments, multiple cohorts across experimental replicates were performed. The number of cohorts performed is listed in the figure legends pertinent for each in vivo experiment. We observed similar results across cohorts and across individual mice within each cohort, as represented in the figures.
Randomization	For macrophage depletion experiments, mice pre-treated with either vehicle or anti-CSF1R mAb were randomized amongst treatment cohorts prior to engraftment with WT or CD24 KO MCF-7 tumors. Similarly, mice engrafted with MCF-7 tumors were randomized prior to treatment with anti-human CD24 mAb.
Blinding	All experiments, including in vivo experiments, were performed by unblinded investigators as all experiments in this work contained internal controls to allow for quantification and data analysis.

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
[ ]	[x] Animals and other organisms
[ ]	[x] Human research participants
[x]	[ ] Clinical data

Methods

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

Antibodies

Antibodies used	All antibodies used in this work, clone, application, and supplier are listed in Supplementary Table 1.
Validation	The anti-human CD24 antibody (Clone SN3, Novus Bio (NB100-64861) and Creative Biolabs (CSC-S170)) used for staining and treatment studies in this work was validated by Novus Bio in human peripheral blood granulocytes. This antibody was also validated by staining unmodified MCF-7 cells versus CD24 knockout MCF-7 cells (dilution assessed in this work 1:50). The SN3

antibody was confirmed to not bind to mouse CD24a-expressing ID8 cells by flow cytometry. The CD24a antibody (Clone M1/69, BioLegend (101814)) was validated by staining unmodified ID8 cells versus CD24a knockout ID8 cells (dilution assessed in this work 1:100). The anti-human CD47 antibody used for treatments (Clone 5F9-G4, in house) is a clinical trial-grade humanized antibody which was validated as described in Liu et al. nature research | reporting summary October 2018 PLoS One (2015). The anti-human CD47 antibody used for staining (Clone B6H12, eBioscience (17-0479-42)) was validated by Barkal et al. Nature Immunology (2018) by comparing staining (dilution assessed in this work 1:100) of unmodified versus CD47 knockout cells. The Siglec-10 antibody (Clone 5G6, Thermo Scientific (MA5-28236)) has been validated by Thermo Fisher Scientific by staining CHO cells modified to express human Siglec-10 (dilution assessed in this work 1:50). The anti-human CD45 antibody (Clone HI30, BioLegend (304008)), the anti-human CD56 antibody (Clone HCD56, BioLegend (318316)), the anti-human CD3 antibody (Clone UCHT1, BioLegend (300415)), and the anti-human CD19 antibody (Clone SJ25C1, BioLegend (363011)) were all validated by the manufacturer by staining human peripheral lymphocytes (dilution assessed in this work 1:100). The anti-human/mouse CD11b antibody (Clone M1/70, BioLegend (101220)) was validated by the manufacturer by staining C57BL/6 mouse bone marrow cells (dilution assessed in this work 1:100). The anti-human CD14 antibody (Clone M5E2, BioLegend (301819)) was validated by the manufacturer by staining human peripheral blood monocytes (dilution assessed in this work 1:100). The anti-human EpCAM antibody (Clone 9C4, BioLegend (324204)) and the anti-human EpCAM antibody (Clone VU-1D9, ThermoFisher Scientific (BMS171)) were validated by the manufacturer by staining the HT29 human colon carcinoma cell line (dilution assessed in this work 1:100). The anti-human Siglec-5 antibody (Clone 1A5, BioLegend (352003)) was validated by the manufacturer by staining human peripheral blood granulocytes. The anti-human Siglec-9 antibody (Clone K8, BioLegend (351503)) was validated by the manufacturer by staining human peripheral blood monocytes. The anti-mouse CD45 antibody (Clone 30-F11, BioLegend (103106)) was validated by the manufacturer by staining C57BL/6 mouse splenocytes (dilution assessed in this work 1:100). The anti-mouse CD80 antibody (Clone 16-10A1, BioLegend (104725)) and the anti-mouse F4/80 antibody (Clone BM8, BioLegend (123114)) were validated by the manufacturer by staining thioglycolate-induced Balb/c mouse peritoneal macrophages (dilution assessed in this work 1:100). The anti-mouse CSF1R antibody (Clone AFS98, BioXCell (BE0213)) was validated by the investigators through FACS measurements of the frequency of tissue resident macrophages after 18 days of IP treatment with CSF1R antibody as compared to vehicle-treated mice.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	All cell lines used in this work were obtained from ATCC, with the exception of the APL1 human pancreatic neuroendocrine tumor line which was derived from a primary patient tumor as described in Krampitz et al. PNAS (2016) and the ID8 murine ovarian carcinoma cell line which was a gift from the laboratory of O. Dorigo.
Authentication	Cell lines were not independently authenticated beyond the identity provided from ATCC. The APL1 cell line was not independently authenticated beyond that performed in Krampitz et al. PNAS (2016). The ID8 murine ovarian carcinoma cell line was not independently authenticated.
Mycoplasma contamination	Stocks of all cell lines were tested for mycoplasma contamination prior to submission. All were negative.
Commonly misidentified lines (See ICLAC register)	None of the cell lines used in this study are listed in the database of commonly misidentified cell lines.

Animals and other organisms

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

Laboratory animals	Animals used in xenograft experiments were 6-10 week old females of the NOD-scid IL2rγ-null (NSG) background obtained from in house breeding stocks. Animals used for syngeneic experiments were 6-8 week old females of the C57BL/6 background obtained from the Jackson Laboratory.
Wild animals	This study did not involve wild animals.
Field-collected samples	This study did not involve samples collected in the field.
Ethics oversight	All experiments were carried out in accordance with ethical care guidelines set by the Stanford University Administrative Panel on Laboratory Animal Care. Specific protocol numbers available on request.

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	The primary human samples used in this work were all collected from female patients who had been diagnosed with ovarian cancer or breast cancer and who were operated on at Stanford University Medical Center. All patients were above 30 years of age and female. Information not protected by HIPAA (i.e. age, genotypic/molecular information) available on request.
Recruitment	Female patients with ovarian cancer and breast cancer identified by the surgeons (I. Wapnir, breast cancer; O. Dorigo, ovarian cancer; Human Immune Monitoring Center Biobank and Stanford Tissue Bank; breast cancer) were recruited for the IRB approved studies reported here.

Ethics oversight	The Human Immune Monitoring Center Biobank, the Stanford Tissue Bank, and Dr. Oliver Dorigo all received IRB approval from the Stanford University Administrative Panels on Human Subjects Research and complied with all ethical guidelines for human subjects research to obtain patient samples of ovarian cancer and breast cancer, and received informed consent from all patients. Specific IRB protocol numbers are available on request.

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

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	Please also see description of sample preparation included in the Methods. Briefly: FACS of primary human tumors/mouse tumors: Solid tumors were excised and mechanically dissociated using a straight razor prior to incubation with 10 mL RPMI supplemented with 10 mL of RPMI + 10 µg/mL DNaseI (Sigma Aldrich) + 25 µg/mL Liberase (Roche) for 30-60 min at 37°C. Single cell suspensions were blocked using species-matched anti-CD16/32 antibodies (TruStain fcX, BioLegend) for 10 minutes on ice prior to staining. Gates were set by fluorescence minus one controls for markers other than CD24 and Siglec-10 which were set based off of isotype controls. All samples were analyzed while in FACS buffer containing 1 µg/mL DAPI in order to exclude dead cells. Channel compensations were performed using single-stained UltraComp eBeads (Affymetrix) or cells. In vitro phagocytosis assay: All phagocytosis assay wells were stained with anti-human/mouse CD11b antibody (Clone M1/70, BioLegend) for 30 minutes on ice, prior to analysis. All samples were analyzed while in FACS buffer containing 1 µg/mL DAPI in order to exclude dead cells. Gates were set based off of fluorescence minus one controls. Data was analyzed using FlowJo (Treestar) and outliers among technical replicates in each treatment group were removed using GraphPad Outlier Calculator (http://graphpad.com/quickcalcs/Grubbs1.cfm).
Instrument	All samples were analyzed on an LSR Fortessa (BD) or Aria II SORP (BD).
Software	FACS data was collected using FACS Diva (BD) and analyzed using FACS Diva (BD) or FlowJo (Tree Star). Statistical analyses and plots were generated using GraphPad Prism 8.
Cell population abundance	The CD24-null MCF7-7 and ID8 CD24a-null cell lines were sorted based off of positive controls (WT versions of each cell line). After the initial knockout was performed, the CD24-null cell lines approximately 10% of the population, while in successive purification sorts, the CD24-null population was >70% of the population. No other populations were sorted for this manuscript.
Gating strategy	All gating strategies used in this work are included in the Extended Data Figures. Briefly: FACS of primary human tumors: The frequency of CD24+ cancer cells was measured as the number of DAPI–CD14–EpCAM+CD24+ cells out of total DAPI–CD14–EpCAM+ cells as determined by isotype controls. The frequency of Siglec-10+ TAMs was measured as the number of DAPI–EpCAM–CD14+CD11b+Siglec-10+ cells out of total DAPI–EpCAM–CD14+CD11b+ cells, as determined by isotype controls (Gating Strategy Extended Data Figure 2a). In vitro phagocytosis assays: Phagocytosis was defined as the frequency of the DAPI-CD11b+GFP+ population among all DAPI-CD11b+ cells (Gating Strategy Extended Data Figure 3). In vivo TAM phagocytosis assays: In vivo phagocytosis was defined as the DAPI-CD45+ CD11b+F480+GFP+ population out of total TAMs defined as DAPI-CD45+ CD11b+F480+ cells (Gating Strategy Extended Data Figure 5a). M1-like mouse TAMs: The frequency of M1-like mouse TAMs was measured as DAPI–, CD45+, CD11b+, F480+, CD80+ TAMs out of total TAMs defined as DAPI-CD45+ CD11b+F480+, as defined by fluorescence minus one controls (Gating Strategy Extended Data Figure 5a).

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