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Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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	LivingImage software v. 4.3.1 (Caliper LifeSciences), FCS Diva (BD), Illumina NextSeq platform, EVOS FL2 Auto (Life Sciences), Incucyte S3 Software (Sartorius), Attune NXT Software Version 3.1 (Thermo Fisher), MSD Discovery Workbench Version 4.0 (Meso Scale Diagnostics)
Data analysis	GraphPad Prism 6.05 (GraphPad Software, Inc.), FlowJo v.10 (FlowJo LLC), Fiji software (ImageJ), LivingImage software v. 4.3.1 (Caliper LifeSciencies), Excel (Microsoft), Trimmomatic (v0.36), STAR (v2.6.0c), featureCounts (v1.6.1), bedtools (v2.27.1), makeUCSCfile, IGV (Broad Institute), RUM, Ingenuity Pathway Analysis (Qiagen Bioinformatics), Incucyte S3 Software (Sartorius), MSD Discovery Workbench Version 4.0 (Meso Scale Diagnostics)

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 requests for raw and analyzed data and materials are promptly reviewed by the University of Pennsylvania Center for Innovation to see if the request is subject to any intellectual property or confidentiality obligations. Any data and materials that can be shared will be released via a Material Transfer Agreement. All raw sequencing data can be found at the NCBI Sequence Read Archive (accession number: not yet available, data actively being uploaded).

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	Sample size was calculated by estimating the confidence interval and margin of error, or experiments were ran in at least duplicate/triplicate.
Data exclusions	No relevant data were excluded. A priori criteria for exclusion were developed.
Replication	All experiments were conducted in duplicate or triplicate. When available and possible we used macrophages derived from multiple normal donors and different cell lines. All attempts at replication were successful.
Randomization	Randomization was not relevant to this work. For in vivo models, baseline tumor burden was verified as equivalent (i.e. there was no statistically significant difference amongst the groups via ANOVA) between treatment groups.
Blinding	Blinding analysis was implented in quantification of tumor cells in immunohistochemistry sections.

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
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Primary human macrophages were tested for CAR-HER2 expression using a two-step staining protocol: human HER2/ ERBB2 Protein-His tag (Sino Biological Inc, 10004-H08H-100) primary stain followed by Human TruStain FcX (Biolegend, 422302) and Anti-His Tag APC (R&D Systems, IC050A) secondary stain. TruStain FcX (Biolegend, 422302) was always used for FACS staining of monocytes, macrophages, or monocytic cell lines expressing Fc receptors. Macrophage purity was tested using the following panel: Anti-CD11b PE (Biolegend, 301306), Anti-CD14 BV711 (Biolegend, 301838), Anti-CD3 FITC (eBioscience, 11-0038-42), Anti-CD19 PE-CY7 (eBioscience, 25-0198-42), Anti-CD66b PerCP-CY5.5 (Biolegend, 305108), Anti-CD56 BV605 (Biolegend, 318334), and Live/Dead Fixable Aqua (L/D aqua) Dead Cell Stain Kit (ThermoFisher, L34957). The same panel was used for testing the monocyte purity post CD14 MACS selection, prior to seeding for differentiation. M1/M2 markers on primary human macrophages were detected with the following panel: Anti-CD11B PE (Biolegend, 301306), Anti-CD80 BV605 (Biolegend, 305225), Anti-CD86 BV711 (Biolegend, 305440), Anti CD206 BV421 (Biolegend, 321126), Anti CD163 APC-CY7 (Biolegend, 333622), anti HLA-DR BV785 (Biolegend, 307642), Anti-HLA ABC PE/CY7 (Biolegend, 311430) and Live/Dead Fixable Aqua Dead Cell Stain Kit. CD46 expression was detected with Anti-CD46 APC (Biolegend, 352405) and CXADR was detected with Anti-CAR PE (EMD Millipore, FCMAB418PE-I). Appropriate fluorescence matched isotype controls were acquired from Biolegend. Surface HER2 was detected using Anti-Human CD340/HER2 APC (Biolegend, 324408). In phagocytosis assays, macrophages were stained with anti-CD11b APC-CY7 (Biolegend, 301342) and tumor cells, which were all sorted for >99% GFP positivity, were detected on FACS by GFP. The following antibodies were used: human CD4 PerCP/Cy5.5 (BioLegend #344608), human CD8 BV421 (Biolegend #344748), human CD3 BV711 (BioLegend #317328), human CD11b FITC (BioLegend #101206), human CD69 PE (BioLegend #3110906), and L/D Aqua (Thermo). Flow cytometry data were acquired on a BD Fortessa with HTS (BD Biosciences, USA), and analyzed with FlowJo X10 (FlowJo, LLC).
Validation	Antibodies were validated using positive and negative cells or isotype controls. Validation reports were provided by the antibody manufacturers (BioLegend). Appropriate compensation controls were used for every experiment. The BD Fortessa and Attune Nxt were calibrated daily using CS&T beads (BD Biosciences) or manufacturer recommended methods.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Cell lines were purchased from and validated by the American Type Culture Collection (ATCC).
Authentication	Cell lines were authenticated as by routine practice by ATCC. Cell lines cultured in-house were validated by ATCC services. Relevant antigen expression was routinely validated during culture.
Mycoplasma contamination	Cell lines were tested for the presence of mycoplasma contamination (MycoAlert™ Mycoplasma Detection Kit, LT07-318, Lonza, Basel, Switzerland). All the cell line were negative for mycoplsma.
Commonly misidentified lines (See ICLAC register)	None of the cell lines used in this manuscript are listed in the ICLAC Database of Cross-contaminated or Misidentified Cell Lines (Version 8.0).

Animals and other organisms

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

Laboratory animals	NOD/SCID Il2rg-/- hIL3-hGMCSF-hSF (NSG-SM3 or NSGS) mice originally obtained from Jackson Laboratories were purchased and bred by the Stem Cell and Xenograft Core at the University of Pennsylvania. Male and female mice were used, age 6-8 weeks.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All in vivo studies were conducted under IACUC approved protocols under established policies at the University of Pennsylvania.

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	Standard flow cytometry protocol was used in this study. Cells were washed before and after staining, and Fc block was used (Human TruStain FcX) when staining Fc receptor expressing cells (macrophages, PBMC's, THP-1s). Samples were acquired on an LSRII Fortessa (BD) or an Attune Nxt using the high-throughput system add-on. Compensation was performed with every experiment and the instrument was calibrated daily using CS&T beads. Sample preparation details on individual experiments are in the Methods section.
Instrument	Flow cytometry was performed on a Fortessa-LSR II cytometer (Becton-Dickinson) or Attune NxT (Thermo Fisher).
Software	FCS files were analyzed with FlowJo X 10.0.7r2 (Tree Star) or later.
Cell population abundance	Target cell lines were sorted for >99% GFP positivity on a BD Influx Cell Sorter (BD Biosciences). THP-1's were sorted for >99% mRFP positivity and >99% CAR positivity on a BD Influx cell sorter by staff at the UPenn Flow Core or the Wistar Flow Core at The Wistar Institute. Purity was determined by flow cytometry during and after the sort. Purity was periodically checked if cells were kept in culture. Primary effector cells were not sorted.
Gating strategy	Generally, FACS gating was performed as follows: FSC/SSC -> Singlets -> Live cells (L/D Aqua negative) -> gating of interest. The appropriate negative control was used for generating gates of interest. For example, for CAR expression, the gate was drawn based on untransduced macrophages that were stained with the same reagents. In other cases, unstimulated macrophages were used to draw the gates of interest, such as in induction of phenotypic markers. In other cases, such as HER2 antigen staining, fluorophore-matched isotype controls were used. For phagocytosis assays, gating was performed as follows: FSC/SSC -> live -> CD11b+ -> CD11b+/GFP+. Gates were drawn using macrophages alone as a control.

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