nature portfolio

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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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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For a	all st	atistical an	alyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.		
n/a	Cor	nfirmed			
	\boxtimes	The exact	sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement		
	\boxtimes	A stateme	nt on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly		
	\boxtimes	The statist	ical test(s) used AND whether they are one- or two-sided on tests should be described solely by name; describe more complex techniques in the Methods section.		
\boxtimes		A descripti	ion of all covariates tested		
\boxtimes		A descripti	on of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons		
\boxtimes			ription of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) cion (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)		
\boxtimes			pothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted as as exact values whenever suitable.		
\boxtimes		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings			
\boxtimes		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes			
\boxtimes	\boxtimes Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated				
Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.					
Sof	tw	vare and	d code		
Policy information about <u>availability of computer code</u>					
Da	Data collection RTCA xIMT ver 1.2. Perkin-Elmer In Vivo Imaging System (IVIS). BioTek Gen5 ver 3.1. EnVision Manager ver 1.14. RTCA eSight ver 1.12. BD FACSverse				

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

Data analysis

Policy information about availability of data

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

Graphpad Prism ver 9.5.0. Living Image 4.7.2. GastroPlus ver 9.6. FlowJo ver 10.8.1

- 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

The authors will comply with requests for access to the datasets necessary to interpret, verify and extend the research. Requests should be submitted to the corresponding author (MDL).

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender

Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data where this information has been collected, and consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Recruitment

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Ethics oversight

Identify the organization(s) that approved the study protocol.

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	w that is the best fit for your research.	If you a	are not sure, read the appropriate sections before making your selection.
X Life sciences	Behavioural & social sciences	E	cological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

Life sciences study design

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

Sample size

The sample size of n=8-10 mice per group was based on a preliminary dose ranging experiment with this tumor model, where this sample size was able to distinguish treatment effect of 5 mg/kg trastuzumab vs isotype control. No formal sample size calculation was conducted.

Data exclusions No data were excluded from the analyses.

Replication The in vivo tumor model was a single experiment with n=8-10 mice per group. Tumor size was monitored by 2 orthogonal methods, caliper

measurement and imaging

Randomization

Naive SCID mice were randomly allocated to treatment groups for surgical implantation of the SKOV3 cells in matrigel.

Blinding

Tumor size determinations were conducted by investigators blinded to treatment assignment.

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	Methods	
n/a Involved in the study	n/a Involved in the study	
Antibodies	ChIP-seq	
Eukaryotic cell lines	Flow cytometry	
Palaeontology and archaeology	MRI-based neuroimaging	
Animals and other organisms		
Clinical data		
Dual use research of concern		

Antibodies

Antibodies used

Human CD66b (BD, Cat. Nu. 564679; final dilution ratio 1:24); Ly-6C (BioLegend, Clone HK1.4, 1:40 dilution), Ly-6G (BD, Clone 1A8, 1:50 dilution ratio), CD45 (BioLegend, clone 30-F11, 1:40 dilution ratio). F4/80 (BioLegend, clone BM8, 1:250 dilution ratio). CD11b (BioLegend, clone M1/70, 1:250 dilution ratio). I-A/I-E (MHC-II) (BioLegend, clone M5/114.15.2, 1:250 dilution ratio).

Validation

Antibodies were not independently validated.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s) SKOV3 cells were obtained from ATCC, (SK-OV-3 [SKOV-3;SKOV3] (ATCC® HTB77™)), female

Primary human neutrophils were isolated from normal human donor blood and used on the day of blood collection.

Authentication SKOV3 cells were not independently authenticated

Cell lines were not tested for mycoplasma contamination Mycoplasma contamination

Commonly misidentified lines (See ICLAC register)

Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

female C.B.17 SCID mice, 20-22 gm Laboratory animals

Wild animals Provide details on animals observed in or captured in the field; report species and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released,

say where and when) OR state that the study did not involve wild animals.

Reporting on sex Studies were conducted in female mice to minimized potential variability due to sex differences.

For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, Field-collected samples photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.

The experiments were conducted in accordance with US legislation governing animal studies and the experimental protocols were Ethics oversight approved by the Lilly Animal Care and Use Committee and performed in accordance with approved guidelines

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

Mouse SKOV3 tumors were harvested and placed in 24 well dishes in PBS on ice. A 40 micron cell strainer was placed on top of a 50ml conical tube and prewet with ice cold PBS. Each tumor was placed in an individual cell strainer and dissociated using the tip of a plunger from a 3ml syringe to process the tumor through the cell strainer, followed by wash with 1-5mls of ice cold PBS. The process was repeated until no tissue remained on the filter. Each tube was then filled to 30mls with ice cold PBS and centrifuged at 320xg for 10 minutes. Supernatant was removed and the cell pellet resuspended in 5mls of ice cold PBS. 10ul of cell suspension was removed for cell counts and 1x106 cells were removed for staining and characterization by flow. The cells were stained for viability and markers to detect infiltrating neutrophils. Neutrophils were identified as CD45+/CD11b+/LY6C+/LY6G+ cells. 10K live CD45+ events (cells) per sample were captured on the BD FACSVerse cytometer for analysis of forward scatter

Instrument

BD FACSVerse cytometer

Software FlowJo ver 10.8.1

Cell population abundance

CD45+ cells were 1% - 19% of live cells in tumors from SCID mice. Tumor infiltrating neutrophils were 12% to 76% of CD45+ positive cells in tumors in SCID mice.

Gating strategy

Neutrophils were identified as live CD45+/CD11b+/LY6C+/LY6G+ cells.