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	all statistical an	alyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.						
n/a	Confirmed							
	The exact	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.							
\boxtimes	A descript	A description of all covariates tested						
	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons							
\boxtimes	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)							
\boxtimes		ypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted es as exact values whenever suitable.						
\boxtimes	For Bayes	ian analysis, information on the choice of priors and Markov chain Monte Carlo settings						
\boxtimes	For hierar	chical and complex designs, identification of the appropriate level for tests and full reporting of outcomes						
\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.						
So	ftware an	d code						
Poli	cy information	about <u>availability of computer code</u>						
D	ata collection	No custom code was used for collection of data.						
D	ata analysis	Data analyses from multiplex cytokine kit was performed using provided LEGENDplex [™] data analysis software (Biolegend, USA), RNA sequencing analysis was done using NASQAR toolbox (publicly accessible at http://nasqar.abudhabi.nyu.edu/; accessed on 20 March 2022), JMP Genomics (JMP*, Version <9>. SAS Institute Inc., Cary, NC, 1989–2021) software, and Idep96 (http://bioinformatics.sdstate.edu/idep96/; accessed on 17th June 2022). Analysis of T cell infiltration into collagen gels was done using a custom-built MATLAB script (MATLAB R2019b; MathWorks Inc., USA) that can be provided upon request.						

Data

Policy information about availability of data

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

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.

- 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 datasets generated and/or analyzed from RNA seq during the current study are available and can be accessed

Field-spe	ecific	reporting				
Please select the o	ne below	that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.				
X Life sciences		Behavioural & social sciences Ecological, evolutionary & environmental sciences				
For a reference copy of	the docume	ent with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>				
Life sciences study design						
All studies must dis	sclose on	these points even when the disclosure is negative.				
Sample size	controls Surface Cytokine OVA upt Gene ex Cell mig	xperiments n=3 was chosen as the minimal replicate number. We determined this to be sufficient owing to appropriate experimental for each particular set of experiments. marker analyses, n=10 e quantification, n>=5 cake analysis via flow cytometry, n>=9 pression analyses (RNAsequencing), n=3 ration analyses, n=3 and at least 4 different positions were volumetrically imaged for quantification. are experiments, n=6				
Data exclusions	Data we	data were not excluded from analyses.				
Replication	No repli	replication studies were performed.				
Randomization		he study did not involve any human or animal subjects and the treatment samples were being compared under tightly controlled ditions, randomization was deemed unnecessary.				
Blinding	-	linding was not implemented during the experiments, and the data collected was measurable such that blinding would not have an impact n any bias in the collected data.				
We require informati	ion from a	r specific materials, systems and methods uthors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, vant 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 & ex	perime	ntal systems Methods				
n/a Involved in the study n/a Involved in the study						
☐ ☐ Antibodies ☐ ChIP-seq						
Eukaryotic cell lines						
Palaeonto	Palaeontology and archaeology MRI-based neuroimaging					
Animals ar	Animals and other organisms					
Human res						
Clinical da						
Dual use research of concern						
Antibodies						
Antibodies used		1. mouse anti-human CD11c monoclonal antibody; clone: Bu15; conjugated with PerCP; Biolegend 337234; lot: B305074				
Antibodies daed		2.mouse anti-human CCR7 monoclonal antibody; clone: G043H7; conjugated with Alexa Fluor Hospital 353206; lot: B277021				

- 3. mouse anti-human HLADR monoclonal antibody; clone L243; conjugated with Brilliant Violet 421; Biolegend 307636; lot: B311370
- 4. mouse anti-human CD80 monoclonal antibody; clone 2D10; conjugated with Brilliant Violet 711; Biolegend 305236; lot: B315414
- 5. mouse anti-human CD86 monoclonal antibody; clone BU63; conjugated with Brilliant Violet 605; Biolegend 374214; lot: B354057
- 6. mouse anti-human CD206 monoclonal antibody; clone 15-2; conjugated with Brilliant Violet 510; Biolegend 321138; lot: B322915
- 7. mouse anti-human CD209 monoclonal antibody; clone 9E9A8; conjugated with PE; Biolegend 330106; lot: B266379
- 8. mouse anti-human CD69 monoclonal antibody; clone FN50; conjugated with APC/Cyanine7; Biolegend 310914; lot:B360870

Validation

As manufacturer's website, antibody clones are tested in a variety of assays to see which applications they are suited for: https:// www.biolegend.com/en-us/quality/product-development

- 1. https://www.biolegend.com/en-us/products/percp-anti-human-cd11c-antibody-12734?GroupID=BLG6213
- 2. https://www.biolegend.com/en-us/products/alexa-fluor-488-anti-human-cd197-ccr7-antibody-7496?GroupID=BLG9611
- 3. https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-human-hla-dr-antibody-7226
- 4. https://www.biolegend.com/nl-nl/products/brilliant-violet-711-anti-human-cd80-antibody-15864
- 5. https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-human-cd86-antibody-15822?GroupID=GROUP28

6. https://www.biolegend.com/en-us/products/brilliant-violet-510-anti-human-cd206-mmr-antibody-15366
$7.\ https://www.biolegend.com/en-us/products/pe-anti-human-cd209-dc-sign-antibody-4885? Group ID=BLG58492000000000000000000000000000000000000$

8. https://www.biolegend.com/en-us/products/apc-cyanine7-anti-human-cd69-antibody-1917?GroupID=BLG10036

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

1. THP1: AddexBio; cell line C0003024, Lot:1589326 2. Jurkat: AddexBio; cell line C0003039; clone E6-1

Authentication

Cell were authenticated prior to purchase

Mycoplasma contamination

- 1. Negative from source; Cells were tested for mycoplasma using MycoAlert mycoplasma detection kit.
- 2. Negative from source; Cells were not tested for mycoplasma contamination

Commonly misidentified lines (See ICLAC register)

None of the cell types used are listed in the ICLAC database.

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

Cells were removed from within collagen matrices by digesting with collagenase (6mg/mL) for 10 mins at 37oC. Cells were then stained with appropriate antibodies for 30 mins on ice at 1:250 concentration.

Instrument

Cytek Aurora flow cytometer (4L 16V-14B-10YG-8R)

Software

FlowJo software ((Becton, Dickinson and Company, NJ, USA)

Cell population abundance

An initial gate was used to ensure a cell count of at least 20,000 cells (events) per sample.

Gating strategy

Cell populations were first gated for a live population using FSC and SSC plot to remove cell debris and dead cells (small FSC v SSC) and large clumps or aggregates of cells (large FSC or SSC) and used across all samples. This live population was then used in fluorescent histograms for the different cell surface markers.

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