nature portfolio

Corresponding author(s):	Maartje van den Biggelaar, PhD		

Last updated by author(s): Apr 19, 2023

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>.

\sim						
✓.	t	2	1	ıc:	ŀι	CS
J	L.	а	ı.	I.O.	L I	LO

FOL	all statistical analyses, confirm that the following items are present in the figure regend, table regend, main text, of interhoos section.
n/a	Confirmed
	\square 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 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
\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	Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated
	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

MS data collection was performed on a nanoscale C18 reverse chromatography coupled on-line to either an Orbitrap Fusion Lumos Tribrid or Orbitrap Fusion Tribrid mass spectrometer (Thermo Fisher). RNA sequencing data collection was performed by GeneWiz (Azenta life sciences)

Data analysis

MS data was processed with Maxquant (1.6.2.10) using the Andromeda engine while RNA sequencing was processed with STAR (2.7.8a). Data was analysed in R (3.5.2) / Rstudio (1.1.456), for specific packages used see methods. Data was visualized in Cytoscape (3.8.0)

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

Policy information about availability of data

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

- 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 MS raw and output files have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD036582. mRNA sequencing data has been deposited in NCBI's Gene Expression Omnibus and is accessible through GE Series accession number (GSE213111).

Human rese	arch p	articipants				
Policy information about studies involving human research participants and Sex and Gender in Research.						
Reporting on sex	and gend	er No human research participants were involved in this study				
Population characteristics		-				
Recruitment		-				
Ethics oversight -		-				
	ation on the	e approval of the study protocol must also be provided in the manuscript.				
Field-spe	cific	reporting				
Please select the or	ne 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				
For a reference copy of t	the documer	nt with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>				
Life scier	nces	study design				
		these points even when the disclosure is negative.				
Sample size		n 19 different donors were used, pooled per three for experiments. Stimulations were performed in 3 biological replicates				
Data exclusions	No data v	o data was excluded				
Replication	Proteomi	roteomic signatures of unstimulated, TNFa and IFNy stimulated cells were reproducible throughout experiments and endothelial cell pools.				
Randomization	Endotheli	elial cell pools were made randomly				
Blinding	No blindi	lo blinding was applied				
Poportin	a for	conscisis materials systems and methods				
		specific materials, systems and methods thors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material.				
'		ant 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	perimen	ital systems Methods				
		n/a Involved in the study				
Antibodies ChIP-seq						
Eukaryotic cell lines Flow cytometry						
Palaeontology and archaeology MRI-based neuroimaging Animals and other organisms						
Animals an	,	;allistilis				
Dual use re		concern				
ı						
Antibodies						
Antibodies used anti HLA-DR, L243 (InVivoMAb, BE0306, 688119S1), anti pan-HLA-A,-B,-C, W conjugated antibody (Invitrogen, A21200, 1696214)		anti HLA-DR, L243 (InVivoMAb, BE0306, 688119S1), anti pan-HLA-A,-B,-C, W6/32 (ATCC, HB-95), secondary Alexa Fluor 488 conjugated antibody (Invitrogen, A21200, 1696214)				
Validation HLA-DR (L243) RRID: AB_2736986, the antibody registry pan HLA-A,-B,-C (W6/32)		RRID: AB_2736986, the antibody registry				

-Brodsky FM, Parham P. Monomorphic anti-HLA-A,B,C monoclonal antibodies detecting molecular subunits and combinatorial

determinants. J. Immunol. 128: 129-135, 1982. PubMed: 6172474

Eukaryotic cell lines

Cell line source(s)

Policy information about <u>cell lines and Sex and Gender in Research</u>

oney information about <u>centimes and sex and Gender in Nescard</u>

Blood derived endothelial cells were isolated from healthy donors (Martin-Ramirez, J. et al., Establishment of outgrowth endothelial cells from peripheral blood. Nat. Protoc. 7, 1709–15 (2012)). For overview of cell source donor see supplemental

table 2

Authentication Endothelial cell marker VWF expression was checked by immunostaining for all isolated cells

Commonly misidentified lines (See ICLAC register)

This study does not use commonly misidentified cell lines