nature research

Corresponding author(s): Ton N. Schumacher

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.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	BD FacsDiva, ImageStream, BD BioRad ChemiDoc
Data analysis	FlowJo (v10.1), IDEAS (v6.2), (ImageLab v4.0.1), Typhoon FLA 9500 (Image Quant TL1Dv8.1), Prism (v7.0), FACS Diva software (v8.0.1.)

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 upon request. 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 sequencing datasets have been deposited in the NCBI Sequence Read Archive under accession numbers SRP144590 and PRJNA505977 and will be released upon publication. In addition, all processed screen results are accessible in an interactive database (https://phenosaurus.nki.nl/).

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	No expected effect size was pre-specified. Generally accepted samples sizes were used, with a significant difference between conditions indicating that the sample size is sufficient.
Data exclusions	In one in vivo experiment with WT and QPCTL KO cells, one mouse has been excluded due to a puncture in the muscle layer of the stomach, as no peritoneal lavage could be performed after this (so no analysis could be performed on this mouse). In analysis of chromium release assays, large outliers within triplicate measurements have been excluded. In analysis of ADCP experiment with Raji cells, one large outlier (4 times measured) has been excluded. Exclusion criteria were pre-established by historical data.
Replication	Every figure states how many times each experiment had been repeated. To ensure experiments could be reliably reproduced, fully independent experiments were performed as defined by commonly accepted standards.
Randomization	Allocation was random
Blinding	Experiments were not performed blindly, as preparation of the cell mixes for in vivo experiments, and the actual in vivo experiments were prepared by the same person due to logistical reasons.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants

Methods

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	All the materials are commercially available.

Antibodies

Antibodies used	Antibodies used (name, clone, company, conjugate and supplier-ID); - anti-human CD47 antibody CC2C6, BioLegend FITC (cat#323106), for flow cytometry dilution 1:50 or 1:80 - anti-human CD47 antibody B6H12, eBioscience APC (cat#17-00479-41), for flow cytometry dilution 1:10, 1:25, 1:50 or 1:80 as indicated - anti-human CD47 antibody 2D3, eBioscience FITC (cat#14-0478-82), for flow cytometry dilution 1:50 - anti-mouse CD47 antibody MIAP301, BioLegend PE (cat#127508), for flow cytometry dilution 1:50 - Recombinant human SIRP alpha/CD172a Fc Chimera Protein, R&D Systems (cat#4546-SA-050), for flow cytometry 4.0 ug/mL - Recombinant human SIRP alpha/CD172a His-tag Protein, R&D Systems (cat#9378-SA-050), for flow cytometry 4.0 ug/mL, 12 ug/mL or 32 ug/mL, as indicated - Recombinant human SIRP gamma/CD172g Fc Chimera Protein, R&D Systems (cat#4486-SBB-100), for flow cytometry 4.0 ug/mL, 12 ug/mL or 32 ug/mL, as indicated - Recombinant mouse SIRP alpha/CD172a Fc Chimera Protein, R&D Systems (cat#7154-SA-050), for flow cytometry 2.0 ug/mL

- anti-mouse IgG (minimal x-reactivity) antibody Poly4053, BioLegend AF488 (cat#405319), for flow cytometry dilution 1:100 or 1:200
- anti-human IgG Fc antibody HP6017, BioLegend APC (cat#409306), for flow cytometry dilution 1:100
- anti-human IgG Fc antibody HP6017 BioLegend BV421 (cat#409318), for flow cytometry dilution 1:200
- anti-human IgG Polyclonal, Jackson Immuno Research AlexaFluor 633 (cat#109-136-098), for flow cytometry dilution 1:1000
- anti-FLAG M2, Sigma Aldrich (cat#F3165), for western blotting 1:1000
- purified mouse IgG1 kappa isotype control MOPC-21 BioLegend (cat#400102), for pre-clearing 3ug per 50uL beads
- anti-human CD47 antibody B6H12.2, Novus Biologicals (cat#NBP2-31106), for IP 3ug per 50uL beads
- purified anti-human CD47 antibody CC2C6, BioLegend (cat#323102), for IP 3ug per 50uL beads
- anti-human CD11b antibody M1/70 eBioscience, for ImageStream dilution 1:100
- anti-mouse B220 RA3-6B2, BioLegend FITC (cat#103205), for flow cytometry dilution 1:100
- anti-mouse cd3e 145-2C11, BioLegend PE (cat#100308), for flow cytometry dilution 1:200
- anti-mouse MHC class II M5/114.15.2, BioLegend Pe/Cy7 (cat#107630), for flow cytometry dilution 1:2000
- anti-human CD89 A59, BioLegend PerCp/Cy5,5 (cat#354109), for flow cytometry dilution 1:80
- anti-mouse CD8a 53-6.7, BioLegend Pe/Cy7 (cat#100721), for flow cytometry dilution 1:400
- anti-mouse Ly-6G 1A8, BioLegend pacific blue (cat#127612), for flow cytometry dilution 1:400
- anti-mouse CD45 30-F11, BioLegend BV510 (cat#103137), for flow cytometry dilution 1:100
- anti-mouse CD4 RM4-5, BioLegend BV421 (cat#100563), for flow cytometry dilution 1:800
- anti-mouse F4/80 BM8, BioLegend APC/Cy7 (cat#123118), for flow cytometry dilution 1:200
- anti-mouse CD11b m1/70, BD Biosciences Al488 (cat#557672), for flow cytometry dilution 1:750
- anti-mouse Siglec-F E50-2440, BD Biosciences PE (cat#552126), for flow cytometry dilution 1:200
- anti-mouse F4/80 BM8, BioLegend BV650 (#cat123149), for flow cytometry dilution 1:200
- anti-mouse I-A/I-E (MHC II) M5/114.15.2, BioLegend BV650 (#cat107641), for flow cytometry dilution 1:100
- anti-mouse FOXP3 150D, BioLegend AF647 (#cat320013), for flow cytometry dilution 1:50
- anti-mouse CD8a 53-6.7, eBioscience AF700 (#cat557945), for flow cytometry dilution 1:200
- anti-mouse CD4 GK5.1, eBioscience PE (#cat557308), for flow cytometry dilution 1:200
- anti-human CD8 SK1, BioLegend AF700 (#cat344723), for flow cytometry dilution 1:50
- anti-human CD4 SK3, eBioscience PerCP eF710 (#cat46-0047-42), for flow cytometry dilution 1:100
- anti-human IFNg B27, BD Biosciences APC 544702 (#cat5162798), for flow cytometry dilution 1:50
- anti-human TNF MAb11, BD Biosciences FITC (#cat554512), for flow cytometry dilution 1:50
- anti-human IL-2 MQ1-17H12, BD Biosciences PE (#cat559334), for flow cytometry dilution 1:100
- anti-human CD4 SK3, BD Biosciences APC-H7 (#cat566003), for flow cytometry dilution 1:200
- anti-human CD27 L128, BD Biosciences BUV737 (#cat564301), for flow cytometry dilution 1:50
- anti-human CD28 CD28.2, BD Biosciences BUV395 (#cat740308), for flow cytometry dilution 1:25
- anti-human CD45RA MEM-56, Invitrogen PE-Cy5.5 (#catMHCD45RA18), for flow cytometry dilution 1:200
- anti-human CD45RO UCHL1, Invitrogen APC (#catMA1-19782), for flow cytometry dilution 1:100
- anti-human CCR7 150503, BD Biosciences PE-CF594 (#cat562381), for flow cytometry dilution 1:100
- anti-human PD-1 J105, eBioscience PE (#cat12-2799-41), for flow cytometry dilution 1:100
- anti-human CD62L SK11, BD Biosciences FITC (#cat347443), for flow cytometry dilution 1:100
- anti-human IFNg B27, BD Biosciences PE (#cat554701), for flow cytometry dilution 1:100
- anti-human TNF Mab11, BD Biosciences Pe-Cy7 (#cat557647), for flow cytometry dilution 1:200
- anti-human IL-2 MQ1-17H12, BD Biosciences APC (#cat554567), for flow cytometry dilution 1:200
- anti-human CD3 SK7, BD Biosciences APC-H7 (cat#560176), for flow cytometry dilution 1:30
- anti-human CD4 SK3, BD Biosciences BUV661 (cat#566003), for flow cytometry dilution 1:500
- anti-human CD4 SK3, BD Biosciences FITC (#cat347413), for flow cytometry dilution 1:30
- anti-human IL-4 MP4-25D2, Biolegend BV421 (#cat500825), for flow cytometry dilution 1:100
- anti-human IL17A N49-653, BD Pharmingen AF700 (#cat560613), for flow cytometry dilution 1:100
- anti-human CD20 2H7, BioLegend PE (#cat302306), for flow cytometry dilution 1:100
- anti-human CD340 (erbB2/HER-2) 24D2, BioLegend AF488 (#cat324410), for flow cytometry dilution 1:25
- anti-human Her2 24D2, BioLegend AF657 (#cat324412), for flow cytometry dilution 1:100
- anti-mouse Ly6G 1A8, BioXCell (#catBP0075-1), for in vivo use 200ug/injection

Validation

All antibodies are commercially available and were only used for applications validated by the manufacturer.
Additional validations include;
- Antibodies against human and mouse CD47 and human and mouse SIRPα were validated by comparison in WT and KO cells.
- Antibodies used for in vitro effector assays were validated by comparison in negative and positive cells.
- Antibodies used for immune infiltrate were validated by comparison in negative and positive cells.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Sources of all the cell lines is indicated in the methods section. HAP1 cells have been described previously (Carette et al. 2011). A375, A431, A549, DLD1, Raji, RKO, SKBR3 and JY cells were purchased from American Type Culture Collection (ATCC). B16F10 cells were kindly provided by D. Peeper. Ba/F3 cells are bone-marrow-derived, immortalized cells which are IL-3-dependent, as described in (Bracke et al. 2001). NKIRTIL006 cell line was generated from a patient treated at the Netherlands Cancer Institute.

Authentication

Human tumor cell lines and Ba/F3-Her2 have been validated by STR analysis. B16F10 cells were directly obtained from ATCC and have not been further validated. JY cells and NKIRTIL006 cells have validated by their capacity to express loaded peptide or to be a target for NKIRTIL006 TCR-specific T cells.

Mycoplasma contamination	Cell lines have been tested negative for Mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used in this study

Animals and other organisms

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

Laboratory animals	Species; Mice Strains; hCD89 Tg on a BALB/cByJ background (the peritoneal Ba/F3 tumor model in human FcaRI transgenic mice) and littermate controls have been described previously (Boross et al., 2013) Sex; male and female for both strains Age; 10-35 weeks old for both strains
Wild animals	This study did not involve wild animals
Field-collected samples	This study did not involve samples collected from the field

Human research participants

Policy information about studies involving human research participants

Population characteristics	Peripheral blood was obtained from healthy individuals collected by Sanquin Blood Supply (Amsterdam, The Netherlands)
Recruitment	Healthy individuals were recruited by Sanquin Blood Supply and no biases were present.

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	Cell lines were cultured, and when attaching cells, harvested by trypsin EDTA treatment. All cells were stained in PBS containing 0.5% w/v bovine serum albumin and 0.2% w/v sodium azide.
Instrument	BD LSRII, Fortessa or FACSCantoII
Software	Data were collected using BD FacsDiva and analyzed using FlowJo
Cell population abundance	B16F10 CD47 KO and QPCTL KO bulk population were sorted after transient transfection with sgRNA's targeting CD47 or QPCTL locus. Sorting strategy was based on αmCD47-MIAP301 negative/mSIRPα-Fc negative (for CD47 KO) or αmCD47-MIAP301 positive/mSIRPα-Fc low.
Gating strategy	Experiments were gated first by morphology to exclude cell debris, then in case of non-permeabilized cells by DAPI or PI negative to exclude dead cells.

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