nature research

Corresponding author(s): Lydia Lynch

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	FACS Diva has been used to collect flow cytometry data. Wave Desktop version 2.6 has been used to collect Seahorse data.
Data analysis	GraphPad Prism 6 has been used to analyze the data. Expression plots were produced with GenePattern. Kegg pathway analysis were analyzed with Kegg software. FlowJo v10 has been used to analyze flow cytometry data. Wave Desktop version 2.6 has been used to analyze Seahorse experiments. ImageJ has been used for confocal images. Powerpoint has been used to edit western blot images. Adobe Photoshop has been used to edit confocal images.

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

Provide your data availability statement here.

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	Human Samples: When possible a minimum of 10 samples lean and obese were used for each experiment. Number of sample depends preliminary data which were used to calculate power calculations on the minimum sample size. As some samples were used for multiple measurements, for example all samples were used for NK cell levels, so there is increased sample size here as it combined all collections. Mouse studies: Power calculations were used to determine the minimum sample size. For HFD experiments, at least 5 mice per group per experiment were used. For the B16 tumor model, 15 mice (Figure 8B-D) or 5 (Figure 8E-J) mice per group were used. In vitro experiments including lipid and drug treatment were performed each time with at least one obese and one lean matched donor to compare. Experiments were repeated 2-5 times with different donors to verify the phenotype.
Data exclusions	Data were excluded when human cells were poor quality, for example decreased viability (>20%) due to the freezing or thawing procedure. Exclusion criteria were not pre-established. No murine data were excluded from the analysis.
Replication	All experimental findings were reliably reproduced. In many instances, the experiments have been pooled (as indicated). Figure 1: All experiments with different samples were reproducible. Figure 2: Various donors gave reproducible results. Figure 3a-c: 2 to 3 experiments, reproducible. Figure 3d: 10 replicates, 8 were reproducible, 2 didn't show changes phenotype. Thought to be due to extended length of experiment and other possibly due to donor. Figure 3e: 4 replicates, all reproducible. Figure 4a: 3 replicates, all reproducible. Figure 4b-c: 1 to 2 replicates, all reproducible. Figure 4d-e: 2 replicates, all reproducible. Figure 4f: All samples with different donors were reproducible. Figure 5a: 2 replicates, all reproducible. Figure 5b: All samples with different donor were reproducible. Figure 5c-e: 5 replicates, all reproducible. Figure 5e: 3 replicates, all reproducible. Figure 5g-i: All samples with different donors were reproducible. Figure 5j-k: 1 experiment, n=3 (HFD) n=5 (SFD) mice Figure 5l-q: 3 experiments (2 reproducible, 1 failed), 3-4 technical replicates per experiment Figure 6a: 1 experiment, reproducible. Figure 6b-d: 3 replicates, all reproducible. Figure 6e: 1 exemplary experiment of 4, all reproducible. Figure 6f: 4 experiments, all reproducible. Figure 6g: 5 experiments, all reproducible Figure 6h-n: 5 experiments, all reproducible Figure 6o: 4 experiments, all reproducible Figure 7a-b: 4 replicates, all reproducible Figure 7c: All samples with different donors were reproducible Figure 7d: 2 replicates, all reproducible Figure 7e: 1 experiment, reproducible. Figure 7f-g: 3 replicates, all reproducible. Figure 7h: 4 replicates, 3 reproducible, 1 failed. Figure 7i-j: 2 replicates, all reproducible Figure 8a: 4 experiments, reproducible Figure 8b-d: 2 experiments (pooled), reproducible Figure 8e-j: 1 experiment, 5 mice per group

Randomization	Peripheral blood was obtained from healthy donors and obese patients. Healthy lean donors have a BMI ranging from 19-25 while Obese patients have a BMI over 33. For mice, littermate controls were ear punched in order to generate experimental groups. In the B16 tumor model, mice were randomized into groups after tumor cell injection.
Blinding	For human samples, when possible, the order of acquiring the sample during imaging and flow cytometry was hidden for the investigator during the acquisition of all samples and the gating of results. The the samples were identified into groups at the stage of entering the data into the statistical package. For mouse experiments, investigators were not blinded during data collection and analysis.

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

Antibodies

Antibodies used

Human:
Anti-CD56-APC (HCD56) / BioLegend / 318310 / #B225195
Anti-IFNγ-Alexa488 (4S.B3) / BioLegend / 502515 / #B197426
Anti-IFNγ-FITC (4S.B3) / BioLegend / 502506 / #B179400
Anti-CD107a-APC (H4A3) / BioLegend / 328620 / #B168399
Anti-CD107a (H4A3) / BioLegend / 328602 / #B156594
Anti-CD3-PerCP Cy5.5 (OKT3) / BioLegend / 317336
Anti-PRF-1-PE (dg9) / BioLegend / 308106 / B160766
Anti-GZMB-FITC (GB11) / BD Bioscience / 560211 / #6168965
Anti-Perforin (Pf-80/164) / Mabtech / 3465-3-250
Anti-Pericentrin (N/A)/ Abcam / ab4448
Anti-p-S6(Ser235/236)-PE (D57.2.2E) / Cell Signaling / 5316 / #7 and #8

Mouse:
Anti-CD19 PE-CF594 (1D3) / BD Biosciences / 562291
Anti-CD45-BV605 (30-F11) / BioLegend / 103139
Anti-CD3-BV650 (17A2) / BioLegend / 100229
Anti-NKp46-BV711 (29A1.4) / BioLegend / 137621
Anti-NK1.1-BV421 (PK136) / BioLegend / 108731
Anti-IFN-gamma-PerCP/Cy5.5 (XMG1.2) / BioLegend / 505821
anti-GZMB-PE-Cy7 (NGZB) / eBioscience / 25-8898-80

Validation

Human antibodies: Validated by manufacturer using human PBMCs
Mouse antibodies: Validated by manufacturer using C57BL/6 or BALB/c splenocytes

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Cells lines K562, YT-INDY and 721.221 were obtained from Dr. Michael Brenner's Lab. Cell line B16.F10 was purchased from the ATCC.
Authentication	None of the cell lines have been authenticated.
Mycoplasma contamination	Cell lines were not tested for mycoplasma infection. However, Imaging studies with DNA staining (Hoechst) didn't show additional small nucleus that could reveal the presence of mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

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

Laboratory animals	C57Bl/6J (WT) were purchased from Jackson Laboratory and maintained in specific-pathogen-free animal facilities at Brigham and Women's Center for Comparative Medicine. In obesity experiments, male mice were used. Mice were 5-6 weeks old at the start of the experiments. In tumor experiments, C56Bl/6J (WT) female mice purchased from Envigo were used. Mice were 6-8 weeks old at the start of the experiments. All animal work was approved by and was in compliance with the Institutional Animal Care and Use Committee guidelines of Brigham and Women's Hospital and Harvard Medical School, and in Trinity Biomedical Sciences Institute.
Wild animals	N/A
Field-collected samples	N/A

Human research participants

Policy information about studies involving human research participants

Population characteristics	Peripheral blood were obtained from healthy donors and obese patients attending the weight loss clinic at Brigham and Women's Hospital, Boston medical Center or St. Vincent's University Hospital Dublin, Ireland (mean age 47, range 24–60 years; mean BMI 48). The ethics committee at Boston Medical Center, Brigham and Women's Hospital, Boston and St. Vincent's University Hospital, Dublin granted approval for this study.
Recruitment	We collected patients attending the weight management clinic, and a study coordinator collected blood for all patients that agreed to participate in the study. There was no selection bias, other than they had to have a BMI>30.

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	Human peripheral blood mononuclear cells were isolated from blood (10ml sample or leukopack collars) by Ficoll density gradient separation using ficoll-paques (GE Healthcare). Primary NK cells were purified by positive selection using the NK cell purification kit (Miltenyi Biotec). For surface staining, cells were washed twice with PBS and filtered through a 70um filter. Cells were then stained with NIR-LIVE/DEAD (Life Technologies) for 20 minutes then washed with PBS, 2% FBS, twice. Cells were then stained with antibodies diluted in PBS, FBS 2% for 0.5 to 1H at 4C. Cells were then washed twice before data collection. For intracellular staining, following surface staining, cells were fixed (20 minutes) and permeabilized (20 minutes) (Ebioscience). Fixed cells were then incubated with antibodies for 0.5 to 1H at 4C. Cells were then washed twice before data collection. For cytokine production assays, cells were stimulated with indicated stimulus and then treated with brefeldin A for 4 to 6 hours before sample processing. For cytotoxic assays, target cells were pre-stained with cell trace violet (Life technologies) or Cell Vue Maroon (Ebioscience). Target and NK cells were then co-incubated together with or without Monensin (Ebioscience) and anti-CD107a antibody for 2 to 4 hours. Two to 5 minutes before data collection, samples were stained with Propidium Iodide. For mice: Spleens and lymph nodes were strained through a 70µm cell strainer and spun. Pellets from all tissues were subjected to red blood cell lysis and subsequently resuspended in flow cytometry buffer (2% FBS and 0.02% NaN3 in phenol-free DMEM) for further staining. Tumors and draining lymph nodes were typically dissected 15-17 days after tumor induction and tumors digested with DNAse I (20 U/ml; Sigma-Aldrich) and collagenase D (1 mg/ml; Roche) in RPMI-1460 for 1H and red blood cells lysed using ammonium chloride lysis buffer. Single cell suspensions, prepared using a 100 µm nylon mesh, were stimulated with phorbol 12-myristate 13-acetate (PMA; 10 ng/ml; Sigma-Aldrich), ionomycin (500 ng/ml; Sigma-Aldrich) and brefeldin A (BFA; 5 µg/ml; Sigma-Aldrich) for 4H at 37ºC. Cells were stained with LIVE/DEAD® Fixable Aqua Dead Cell Stain (Life Technologies) and fluorochrome-conjugated antibodies for CD45, CD19, CD3, NKp46, and NK1.1, then fixed, permeabilized and incubated with antibodies for IFNγ and granzyme B.
Instrument	BD Bioscience CANTO I and BD Bioscience CANTO II and BD Bioscience Fortessa
Software	BD FACSDIVA and FLOWJO (V10) software were used for data collection and data analysis respectively.
Cell population abundance	For cytotoxicity assays, a minimum of 2,000 target cells were recorded per replicate. For others assays, a minimum of 10,000 NK cells were recorded per replicate. For human sample analysis, a minimum of 5,000 NK cells were recorded. For microarray

analysis, a minimum of 30,000 NK cells were sorted. For tumor studies, B16 cells (2 x 105 cells/mouse) were injected subcutaneously (s.c.) into the right flank. On days 3, 7 and 11 post tumor induction, mice were injected s.c. with NK cells (1.2 – 2 x 106 cells/mouse) or PBS into the tumor site.

Gating strategy

In general: Global cell population is gated on FSC-A/SSC-A. Single cells are gated on FSC-A/FSC-H. When appropriate, live cells - using Aqua or NIR LIVE/DEAD Aqua or NIR - are gated. The dead population is determined using cells pretreated with detergent (Tween20, Triton X100) to induce major cell death as a positive control. NK cells were gated as CD56+/CD3- or NK1.1+NKp46 +CD3- in mice.
The IFNg+ and p-S6+ populations were determine using the corresponding negative population in non-stimulated cells or fluorescence minus one controls.
Cell Trace Violet or Cell vue maroon population were gated using a non stained sample.

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