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 Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the 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
☐	☒ 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.*
☒	☐ 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. *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

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Flow-cytometry data were collected using BD FACS DIVA software. NanoSight data were collected using Malvern NTA software version 3.4. HTS data were collected on an Illumina MiSeq using MiSeq Control System v4.
Data analysis	Flow-cytometry data were analysed using FlowJo software v10. NanoSight data were analysed using Malvern NTA software version 3.4. Where indicated, microscopy images were analysed using ImageJ 1.53f51. Custom code for HTS analysis will be available on GitHub along with the final published version of the manuscript. Graphs were generated and statistical analyses were performed using Microsoft Excel 365 and GraphPad Prism 9.3.0. Figures were compiled using Adobe Illustrator 2022. The code for analysing the high-throghput screening data is available at https://github.com/leonardlab/GEMINI-HTS under an open-source license.

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 data availability statement. 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

All reported experimental data and plasmid maps for all plasmids generated in this study are freely available at Zenodo (https://doi.org:10.5281/zenodo.10022991). Key plasmids used in this study are distributed by Addgene, with complete and annotated GenBank files available at https://www.addgene.org/Joshua_Leonard. The raw and analysed datasets generated during the study are available for research purposes from the corresponding author on reasonable request.

Field-specific reporting

Please select the one 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 ☐ Ecological, evolutionary & environmental sciences

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

Life sciences study design

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

Sample size	All experiments were conducted in biological triplicates, which is standard for this type of study.
Data exclusions	In one experiment, some sample replicates were excluded owing to the unnaturally high cellular autofluorescence observed during flow cytometry. This is noted in the appropriate source-data file.
Replication	For many of the experiments performed, similar samples or conditions were included across multiple experiments to validate repeatability (for example, T-cell binding by CD2-targeted vesicles was validated across several independent experiments investigating aspects of that binding). In some cases, such as in certain high-throughput-screening experiments, the conditions were repeated using independent vesicle preparations and donor cells, to confirm trends and repeatability.
Randomization	Randomization was not relevant to the study.
Blinding	Blinding was not relevant to the study, as no conclusions were based on subjective analysis.

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

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	anti-FLAG, Sigma F1804, diluted 1:1000 anti-HA, Cell Signaling Technology 377245 C29F4, diluted 1:1000 anti-CD9, Santa Cruz Biotechnology sc-13118, diluted 1:500 anti-CD81, Santa Cruz Biotechnology sc-23962, diluted 1:500 anti-Alix, Abcam Ab117600, diluted 1:500 anti-calnexin, Abcam Ab22595, diluted 1:1000 HRP-conjugated anti-mouse, Cell Signaling Technology 7076, diluted 1:3000

HRP-conjugated anti-rabbit, Invitrogen 32460, diluted 1:3000
anti-FLAG-APC, Abcam Ab72569 at manufacturer recommended dose
anti-CD2-APC, R&D Systems FAB18561A at manufacturer recommended dose
anti-CD25-PE, Miltenyi REA945, 130-115-628 at manufacturer recommended dose
anti-SLAM-PE, Miltenyi REA151, 130-123-970 at manufacturer recommended dose
anti-mouse IgG1-APC R&D Systems IC002A at manufacturer recommended dose
anti-human IgG1-PE, Miltenyi REA293, 130-113-438 at manufacturer recommended dose
anti-CD2-APC, Beckman Coulter A60794 at manufacturer recommended dose
anti-Cas9, Cell Signaling Technology 7A9-3A3, diluted 1:1000

Validation

From Sigma: "Our standard antibody validation processes include verification for each recommended immunodetection application. Each of the thousands of antibodies in our portfolio are certified through our standard validation process to ensure quality and reproducibility."
From Cell Signaling Technology: "To ensure our antibodies will work in your experiment, we adhere to the Hallmarks of Antibody Validation™, six complementary strategies that can be used to determine the functionality, specificity, and sensitivity of an antibody in any given assay. CST adapted the work by Uhlen, et. al., ("A Proposal for Validation of Antibodies." Nature Methods (2016)) to build the Hallmarks of Antibody Validation, based on our decades of experience as an antibody manufacturer and our dedication to reproducible science."
From Abcam: "Antibody validation must be application-specific to be effective and information on which applications an antibody has been validated in can be found in the Tested Applications section on any antibody datasheet."
From Invitrogen: "Thermo Fisher Scientific is committed to adopting higher validation standards for the Invitrogen antibody portfolio. We have implemented additional specificity tests to help ensure the highest confidence levels in our products."
From R&D Systems: "R&D Systems® takes rigorous steps towards antibody validation and reproducibility. We have been since the beginning. For 30 years, we have used our industry-leading production standards and quality control specifications to develop antibodies that can be relied on for specificity and reproducibility. By developing and testing our products in-house, we can ensure a validated and specific antibody. We are confident in our antibodies and provide 100% guarantee for our products."
From Miltenyi: "All our antibodies are rigorously tested and validated before release. In the application section on the product page, you can find examples of typical performance data. In addition, we provide extended validation data highlighting details of antibody performance, specificity, and fixation compatibility."
From Beckman Coulter: "We develop and manufacture reagents according to current Good Manufacturing Practices (cGMP), the highest quality standards in the industry, ensuring optimal antibody panel performance."
Whenever possible, negative controls are included in the antibody experiments. For Western blots, this includes samples that do not contain the tag being detected. For flow cytometry, this includes cell lines lacking the antibody's target receptor or an appropriate isotype control.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

HEK293FT cells, Thermo Fisher R70007
Jurkat T cells, ATCC TIB-152
Other cell lines, which were derived from those above using lentiviral integration.

Authentication

The cell-line vendors perform routine authentication; no further authentication was performed.

Mycoplasma contamination

HEK293FT and Jurkat T cells tested negative for mycoplasma.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cells were used.

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

For EV-binding experiments, cells were incubated for 2 h at 37°C unless otherwise indicated, then washed three times in FACS buffer, centrifuging at 150 g for 5 min for Jurkat cells or 400 g for 3 min for primary T cells. Cells were resuspended in one drop of FACS buffer PBS (pH 7.4 with 2 mM EDTA and 0.05% BSA) prior to flow cytometry. For EV-uptake experiments, cells were washed twice in PBS and incubated with two drops of trypsin-EDTA for 5 min at 37°C to remove surface-bound vesicles. Cells were washed with RPMI to quench the trypsin, then washed twice more with FACS buffer prior to analysis. For surface immunoblotting, cells were washed one or three times post-staining with 1 mL of FACS buffer, centrifuging at 400 g for 3 min or 150 g for 5 min and decanting the supernatant after each wash. Cells were resuspended in two drops of FACS buffer prior to flow cytometry. Beads were diluted and analysed in PBS.

Instrument	Analytical flow cytometry was performed on a BD LSR Fortessa Special Order Research Product (Robert H. Lurie Cancer Center Flow Cytometry Core) using the 562-nm laser for dTomato (582/15 filter), the 488-nm laser for EYFP (530/30 filter), and the 488-nm and 405-nm lasers for mTFP1 (530/30 filter and 525/50 filter, respectively). FACS was performed on a BD FacsAria IIu (Robert H. Lurie Cancer Center Flow Cytometry Core) using a 488-nm laser for mTFP1 (530/30 filter) or a 562-nm laser for dTomato (582/15 filter).
Software	Data were analysed using FlowJo v10 (FlowJo, LLC).
Cell population abundance	Cells were sorted into a mTFP+ dTomato– population for Cas9 reporter experiments. Reporter construct expression was validated by analytical flow cytometry, where an average of 96.8% (stdev 0.2) of cells were classified as mTFP+ and 100% of cells were classified as dTomoto– prior to Cas9 addition.
Gating strategy	Live cells were identified by FSC-A vs. SSC-A gating, and single cells were then identified by FSC-A vs. FSC-H gating. Beads were identified by FSC-A vs. SSC-A gating. A fluorescent protein was used to identify transfected cells in relevant experiments. A sample transfected with an empty vector was used to draw a gate identifying transfected cells such that less than 1% of non-fluorescent cells were included in the analysis.

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