nature portfolio

Corresponding author(s): Yumeng Mao

Last updated by author(s): Jan 26, 2024

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	Live cell imaging is performed using an Incucyte instrument. Flow cytometry analysis is performed using a Cytoflex or a Fortessa instrument.
Data analysis	Genome-wide CRISPR screens were analyzed using the MaGeCK software. Differential protein expression in proteomics was calculated with R version 4.0.5. Proteins were queried for over representation analysis against the Reactome (17367534) and Gene Ontology Biological Process (10802651; 33290552) collections from the Molecular Signature Database using clusterProfiler (34557778). Flow cytometry data was analyzed using the FlowJo V10 software. Figures were generated using Graphpad Prism version 9.

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

Raw gRNA counts from the CRISPR screen and raw data from proteomics and nanostring analysis are included as supplementary materials.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data where this information has been collected, and consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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	Sample size is determined based on pilot studies for each model and previous experimental evidence. No statistical method is used to determine sample size.
Data exclusions	For in vivo studies, mice developing ulcerations in subcutaneous tumors or other abnormality are excluded due to predefined humane endpoints. For functional assays using primary immune cells, donor cells show no response to stimulation or high background activation without stimulation are excluded.
Replication	For in vitro studies, at least 3 biological replicates/donors are included. For in vivo studies, at least two biological replicates are performed with 6-10 animals per experimental group. The exact percentages of mice with small or no tumor after anti-PD1 treatment differ among experiments but the strong response in NEDD8 KO tumors to anti-PD1 therapy remains consistent.
Randomization	For in vivo studies, mice are randomly allocated to receive wild type or NEDD8 KO cells and randomly allocated to control or treatment groups.
Blinding	For most in vivo studies, animal facility staff are asked to measure tumor volumes. They are blinded from the studies. Investigators are not blinded during data collection and data 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
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used: Information regarding antibodies, e.g. clone number, catalog number, application, etc, is shown in Supplemental Table 1.

Validation: The primary antibody detecting human and murine NEDD8 was validated by KO cells as shown in the figures. Fluororescence-conjugated antibodies are selected from manufacturers that provides representative data and citations. Antibodies that are validated in KO or KD cells by vendors are prioritized.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s): Human breast cancer cell line, MDA-MB-231, was purchased from American Type Culture Collection (ATCC). HCC1937 and BT549 cell lines were a gift from Dr. Óscar Fernández-Capetillo (Karolinska Institutet, Sweden). Mouse breast cancer cell line EO771 was kindly provided by Dr. Maria Ulvmar (Uppsala University, Sweden).

Authentication: STR DNA fingerprinting was performed for human cell lines by Eurofins.

Mycoplasma contamination: Mycoplasoma contamination is routinely tested by the MycoAlert kit.

Commonly misidentified lines (See ICLAC register): Not identified.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals: Six to ten weeks old female C57BL/6NTac or C57BL/6J mice were purchased from Taconic.

Wild animals: *Provide details on animals observed in or captured in the field; report species and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.*

Reporting on sex: All mice were female.

Field-collected samples: *For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.*

Ethics oversight: All animals were housed at the animal facility at the Department of Immunology, Genetics and Pathology in the Rudbeck laboratory at Uppsala University, and all studies were approved by the Swedish Board of Agriculture at Jönköping, Sweden (Dnr: 5.8.18-06394/2020).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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 in vitro assays, CTV-treated lymphocytes were harvested from TICS assay and transferred to a 96-well V bottom plate. The cells were centrifuged at 700×g for 4 min, followed by washing them twice with PBS. After that, cell pellets were resuspended in 20 µl PBS containing aqua fixable live/dead marker (Thermo Fisher Scientific) and then incubated at room temperature for 15 min. The cells were then washed twice with PBS and resuspended in 20 µl master mix containing detection antibodies for surface markers. After 20 min incubation at 4°C, the cells were washed and resuspended in 150 µl PBS for analysis. To determine the expression of immune related surface markers on NEDD8 KO and control cells, a multi-color flow cytometer was used. In brief, triple-negative control or NEDD8 KO breast cancer cells (5x105) were cultured in 6 well flat bottom plate in culture medium and incubated overnight to allow cells to attach. Following treatment with +/- rhIFNγ (50 ng/ml) for 24 hrs, cells were harvested, and centrifuged at 350×g for 4 min. Then, the cells were resuspended in 900 µl PBS and distributed in a 96 well V bottom plate in tetraplicates (200 µl/well). Subsequently, the plate was centrifuged at 700×g for 4 min and resuspended in 20 µl PBS containing blue-fluorescent reactive dye (Thermo Fisher Scientific), detection antibodies or isotype control for 25 min at 4°C. After being washed with PBS, the cells were resuspended in 150 µl PBS and transferred into FACS tubes for analysis.
Instrument	CytoFlex or Fortessa
Software	FACSDiva
Cell population abundance	Cell sorting was performed to enrich for B2M KO MDA-MB-231 cells (Figure S1A). Cells are cultured and used for experiments when 90% confluence is reached.
Gating strategy	For in vitro co-culture assays, gating was done as follow: All cells--alive--single cells--CD3+, CD3negCD56+ (NK). From CD3+, gate on CD4+CD8neg and CD8+CD4neg. Proliferation of cells is determined by the dilution of the CTV dye in cells.

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