nature portfolio

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Last updated by author(s): Oct 10, 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 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	*Provide a description of all commercial, open source and custom code used to collect the data in this study, specifying the version used OR state that no software was used.*
Data analysis	All screen analyses were performed with MAGeCK. Custom python 3.10 and matplotlib code was used to generate some figures.

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

Data Availability
All guide library information from this manuscript is provided in Supplementary Tables 1 and 2 (for NGG screen) as well as 4 and 5 (for NG screen). MAGeCK test output for all screens is provided in Supplementary Table 3. Raw sequencing data for the screens have been deposited on GEO.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

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 if 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.*
Reporting on race, ethnicity, or other socially relevant groupings	Leukopaks were purchased independently of race, ethnicity, or other socially relevant groupings
Population characteristics	T cells were sourced from Leukopaks which were bought from Stemcell. The donors were chosen without regard to sex, gender, ethnicity or race. Donors for the screen were <60 years old (with a preference for younger donors) and non-smokers.
Recruitment	Stemcell Technology recruits donors for Leukopak manufacturing. T cells were sourced from these Leukopaks using Stemcell Tecnologies magnetic isolation kits.
Ethics oversight	UCSF

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	T cells were isolated from peripheral blood of human donors. Number of donors for respective experiments represents the sample size. For all screens, a coverage of > 500 cells/sgRNA was targeted.
Data exclusions	As described in methods, screen data for samples that had very poor recovery of genomic DNA after sorting (<100x coverage relative to number of sgRNAs in library) were excluded from analyses. sgRNAs falling into the bottom 2% of read counts were not analyzed.
Replication	Each pooled screen was performed in cells from at least 2 donors. Arrayed experiments were performed at least twice, unless otherwise noted.
Randomization	T cells were sourced from Leukopaks which were purchased from Stemcell as described above.
Blinding	*Describe whether the investigators were blinded to group allocation during data collection and/or analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.*

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
☒	☐ Plants

Methods

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

Antibodies

Antibodies used	A table with all antibodies is provided as Supplementary Table 6
Validation	A table with all antibodies including links to validations is provided as Supplementary Table 6

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Cell lines were purchased from Takara Bio (HEK293T) cells or provided by the Ashworth lab, UCSF (nRFP+ A375 cells).
Authentication	Cell lines were not reauthenticated after purchase
Mycoplasma contamination	Cell lines were tested for mycoplasma. The result was negative.
Commonly misidentified lines (See ICLAC register)	*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

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	Sample preparation was performed as described in the methods section.
Instrument	BD Aria Fusion FACS machines were used for sorting. An attune Nxt (Thermo Fisher) was used for flow cytometry.
Software	FlowJo was used for analysis of flow cytometry data.
Cell population abundance	Depending on the screen, the final sorting population (eg Lymphocytes-Singlets-Live Cells-CD4-TNFapos) was 5-20% of all events.
Gating strategy	NGG screen gating: Lymphocytes (FSC-A/SSC-A) --> singlets (FSC-A/FSC-H) --> Live Cells (FSC-A/LiveDead Negative) --> screen marker (TNFa/IFNg/PD1 or CD25) final high/low gates. NGscreen gating: Lymphocytes (FSC-A/SSC-A) --> singlets (FSC-A/FSC-H) --> Live Cells (FSC-A/LiveDead Negative) --> Subtype (CD4/CD8) --> screen marker (TNFa) final high/low gates.

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