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<br>*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<br>*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 | No software was used for data collection. |
| Data analysis | The analysis pipeline is available at Github:<br>https://cole-trapnell-lab.github.io/monocle3/docs/citations<br>https://sekalylab.github.io/unsupflowhelper/guides/trajectory_vignette<br><br>The code used for analysis is available at Github and Zenodo:<br>https://github.com/sekalylab/dual_blockade_IL-10_PD-1_SIV<br>https://zenodo.org/doi/10.5281/zenodo.10211327 |

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

The data generated and analyzed in the current study are available online long with the code availability, and upon request from the corresponding author.

## 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 | Not applicable. |
| Reporting on race, ethnicity, or other socially relevant groupings | Not applicable. |
| Population characteristics | Not applicable. |
| Recruitment | Not applicable. |
| Ethics oversight | Not applicable. |

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 was determined using power analyses based on previous in vivo Rhesus Macaque studies to ensure statistically significant results |
| Data exclusions | Data are excluded for one animal in the aIL-10 alone group due to early removal from the study. |
| Replication | No attempts were made to replicate this data given the scale and resource investment required for these studies. |
| Randomization | Animals were stratified into experimental groups to have similar average animal age, weight, peak viral load, viral load at ART initiation and time to viral suppression on ART across all groups. |
| Blinding | Blinding of primary investigators was not possible given the need to properly handle and identify samples. Secondary collaborators were blinded to treatment groups prior to analysis of shipped materials. |

# 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

Supplementary table 3 contains all the following information along with each antibody titration specific for our A5 Symphony machine. BCL-2 Clone BCL-2/100 Company BD Cat# 624296, BCL6 Clone K112-91 Company BD Cat# 563582, CCR7 Clone 3D12 Company BD Cat# 751103, CD101 Clone BB27 Company Biolegend Cat# 331014, CD20 Clone 2h7 Company BD Cat# 560631, CD200 Clone MRC OX-104 Company BD Cat# CUSTOM, CD25 Clone BC96 Company Biolegend Cat# 302634, CD27 Clone M-T271 Company BD Cat# 741609, CD28 Clone CD28.2 Company BD Cat# 612815, CD3 Clone SP34-2 Company BD Cat# 564117, CD38 Clone AT-1 Company Stemcell Technologies Cat# 60131FI, CD39 Clone A1 Company Biolegend Cat# 328228, CD4+ Clone OKT4 Company Biolegend Cat# 317412, CD49b Clone AK-7 Company BD Cat# 748595, CD69 Clone FN50 Company BD Cat# 748763, CD8+ Clone RPA-T8 Company BD Cat# 612942, CD95 Clone DX2 Company Biolegend Cat# 305628, CMAF Clone T54-853 Company BD Cat# 565795, CX3CR1 Clone 2A9-1 Company BD Cat# 749357, EOMES Clone WD1928 Company Invitrogen Cat# 47-4877-42, CXCR5 Clone RF8B2 Company BD Cat# 747111, FoxP3 Clone 150d Company Biolegend Cat# 320014, GARP Clone 7B11 Company BD Cat# 751507, GzmB Clone GB11 Company Thermo Cat# GRB17, HLA-DR Clone L243 Company Biolegend Cat# 307636, ID2 Clone Company THEERMOFISHER Cat# 48-9475-82, IFN-g Clone B27 Company BD Cat# 566394, IGD Clone IA6-2 Company BD Cat# 742039, Il2 Clone MQ1-17H12 Company BD Cat# 566361, IRF4 Clone IRF4.3E4 Company Biolegend Cat# 646408, Ki-67 Clone B56 Company BD Cat# 566109, LAG3 Clone C9B7W Company BD Cat# Custom, Live/Dead Company BD Cat# 564406, Live/Dead Company BD Cat# 564997, PD-1 Clone EH12.2H7 Company Biolegend Cat# 329930, SATB1 Clone 14/SATB1 Company BD Cat# Custom, SLAMF6 Clone hSF6.4.20 Company BD Cat# 624294, Tbet Clone O4-46 Company BD Cat# 561316, Tcf-1 Clone C63D9 Company Cell Signaling Technologies Cat# 6444S, TNF Clone Mab11 Company BD Cat# 563418, Tox Clone TXRX10 Company Thermo Cat# 12-6502-82

Validation

Every antibody was titrated independently in ex vivo or stimulated cells when appropriate. Fluorescence minus one was performed for non-dichotomic stains and positive and negative controls were also added.

# 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 | All animals in this study were Indian rhesus macaques (Macaca mulatta) between 3-4 years of age at the time of infection. |
| Wild animals | This study did not include wild animals. |
| Reporting on sex | Sex of animals was considered in the design of the study. All efforts were made to balance animal inclusion by sex, but were limited due to availability of animals at time of study initiation. The study included 3 female and 25 male macaques stratified equally across the treatment groups. |
| Field-collected samples | This study did not include samples collected from the field. |
| Ethics oversight | This study was approved by the Emory University Institutional Animal Care and Use Committee (IACUC) via permits 201800047. Experiments were conducted following guidelines set forth by the NIH and Animal Welfare Act in regard to the housing and welfare of laboratory Rhesus macaques (RMs). All possible efforts were taken to minimize pain experienced by the RMs. |

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 | Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by density gradient centrifugation. For lymph node biopsies, the skin over the axillary or inguinal region was clipped and surgically prepped. An incision was then made in the skin over the LN, which was exposed by blunt dissection and excised over clamps. Half of the LN was placed in freshly prepared neutral buffered 4% paraformaldehyde for 24h at room temperature (RT) for fixation, followed by transfer to 80% ethanol and subsequent embedding of tissue in paraffin. The remaining LNs were then homogenized and passed through a 70-µm cell strainer to isolate lymphocytes and cryopreserved for downstream analysis. |
| Instrument | A5 Symphony (BD Biosciences) |
| Software | BD FACSDiva software |
| Cell population abundance | This study was performed using whole PBMCs or whole LN cell suspension. No sorting was performed. |
| Gating strategy | In the manuscript figures (Fig 4, 5, and Extended data Fig 1, 4 and 6), subsets were generated by manual gating or by UMAP. Mononuclear cells were defined via the diagonal of FSC-H vs FSC-A and lymphocytes were gated from SSC-A vs FSC-A. A live gate based on viability die was then performed. CD4 and CD8 T cells were gated as live, CD3+ CD8+ or CD3+ CD4+ lymphocytes and memory T cells were gated as CD95+ CD28 +/-.Subsequent gates for the different markers were performed and MFI (median intensity of fluorescence) was added. For the UMAPS, single cells/lymphocytes/live pure CD4 or CD8 T cells were exported as csv files. The manual gated populations are labeled as follows: Tissue_Subset_Phenotype_Stim. Ex. LN_CD3p_CD4p_MFI_BCL-2_UNS; if more than one timepoint is shown in the same plot, the population name is preceded by the timepoint. Ex. Wk24_LN_CD3p_CD4p_MFI_BCL-2_UNS. The letter "p" after the subsetting means "+" for that marker; the letter "n" after the subsetting means "-" for that marker.<br>Trajectory analysis was performed using Unsupflowhelper, using Monocle3 in unstimulated CD4+ and CD8+ T cells from LNs (https://cole-trapnell-lab.github.io/monocle3/docs/citations). Cells were ordered based on assessment of cluster phenotypes, with a starting node set within Naïve T cell clusters 49,50 (CD95- CD28+). The progression of the trajectory analysis was defined by the positioning of cells based on the modulation of the relative expression (MFI) of the markers included in the panel. Example: Naiveness/Stemness (i.e. CD95, CD28, TCF-1); Activation/Differentiation (i.e ID2, Tbet, GrzB); Exhaustion (i.e TOX, IRF4, SLAMF6). Same statistics analysis as above was applied to identify the CD4+ and CD8+ T cells clusters modulated by the combo therapy in the different time points (week 0,9 and 24 post-ATI) and associated with LN CA-vRNA in LNs 24 weeks post-ATI. |

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