nature portfolio

Corresponding author(s): Sarah M. Fortune
Galit Alter

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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	FACSDiva version 9.0; NextSeq500 (Illumina)
Data analysis	GraphPad Prism (version 8.4.0); R (version 4.1.1); Microsoft Excel (version 16.43); FlowJo (version 10.3); Columbus Image Data Storage and Analysis System; CellRanger (version 6.1.2); Python (version 3.8.8); GlycanAssure Data Acquisition Software (version 1.0) R packages used: corrplot (version 0.92); ggplot2 (version 3.3.5); gplots (version 3.1.1); factoextra (version 1.0.7); ggpubr (version 0.4.0); Seurat (version 4.0.0) Custom code to reproduce computational analyses available at: https://github.com/eirvine94/tb_fc_engineering_manuscript

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

RNAseq data have been deposited in the Gene Expression Omnibus under accession number GSE271079. All other data and metadata associated with this study are available in the main text, Supplementary Information and/or at: https://fairdomhub.org/studies/1089.

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	Investigators did not have access to information on sex and gender. The specimens used in this study were provided either coded or anonymized to protect the identity of the donors. For any coded specimens, the investigators did not have access to the key to the code and did not seek out the key. An agreement is in place between the investigators and the sample provider, ensuring that these identifiers will never be made known. As such, this study does not involve human subjects research.
Reporting on race, ethnicity, or other socially relevant groupings	Investigators did not have access to information on race, ethnicity, or other socially relevant groupings. The specimens were provided either coded or anonymized to protect the identity of the donors. For any coded specimens, the investigators did not have access to the key to the code and did not seek out the key. An agreement is in place between the investigators and the sample provider, ensuring that these identifiers will never be made known. As such, this study does not involve human subjects research.
Population characteristics	The samples used in this study were from the blood of healthy, human immunodeficiency virus-negative subjects. The specimens were provided anonymized or coded.
Recruitment	The samples used in this study are primary immune cells derived from human whole-blood and buffy coats. These specimens were obtained from healthy, human immunodeficiency virus (HIV)-negative subjects, who were recruited through a voluntary donation program conducted at Massachusetts General Hospital. Participants were informed about the study through informational sessions and written materials, and those interested underwent screening for eligibility. Eligibility criteria included the absence of clinical signs of illness and negative test results for active HIV, hepatitis C virus (HCV), and hepatitis B virus (HBV) infections. No demographic characteristics, such as age, gender, or ethnicity, were used as selection criteria to ensure a diverse and representative sample. The specimens were provided either coded or anonymized to protect the identity of the donors. For any coded specimens, the investigator did not have access to the key to the code and did not seek out the key. An agreement is in place between the investigator and the sample provider, ensuring that these identifiers will never be made known. Additionally, the samples were provided by individuals who did not have any role in the research study.
Ethics oversight	All donors provided written, informed consent. The study was approved by the institutional review board at Massachusetts General Hospital.

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 sizes were determined was based on guidelines and findings from previously published studies using similar systems serology and Fc engineering approaches. Specifically, we reviewed other studies such as Gunn...Alter. Immunity 2021 (PMC8111768) and Irvine...Alter. Nature Immunology 2021 (PMC8642241), which employed similar experimental frameworks, taking into consideration the effect sizes, variability and statistical power reported. These studies provided a benchmark for selecting sample sizes that would ensure robust and reproducible results while maintaining ethical considerations regarding the use of biological samples.
Data exclusions	No data were excluded from the analyses.
Replication	Reported experiments were reproducible. Systems serology measurements were captured over 2 independent runs in technical duplicate;

Replication

replicates were minimally different. Mtb infection assays were performed in triplicate using cells from at least 2 healthy human donors. Single cell RNA-seq experiment was performed using cells from 3 healthy human donors.

Randomization

Randomization was not applied to this study because it did not involve research subjects or participants. The study was conducted using in vitro assays and controlled laboratory experiments with predefined conditions and parameters. The focus was on the functional consequences of antibody Fc engineering in the context of Mtb infection, where randomization is not typically a requirement. Instead, we ensured experimental rigor through replication, the use of appropriate controls, and standardized procedures to minimize bias and variability.

Blinding

Investigators were not blinded during the experiments herein because the nature of the experimental design required the investigators to be aware of the specific treatments and conditions being applied. This awareness was necessary for the accurate preparation, handling, and application of the antibodies, as well as for the precise monitoring of experimental variables. However, to mitigate potential bias, we employed standardized protocols, included appropriate controls, and conducted multiple replicates.

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

BioLegend:
anti-human CD66b-Pacific Blue | clone G10F5 | lot B256448| cat 305112
anti-IFNγ | clone B27 | cat 506502

BD Biosciences:
PE-Cy7 anti-human CD56 | clone B159 | lot 0274120 | cat 557747
APC-Cy7 anti-human CD16 | clone 3G8 | lot 9289979| cat 557758
Alexa Fluor 700 anti-human CD3 | clone UCHT1 | lot 7145618| cat 557943
PE anti-human MIP-1β | clone D21-1351 | lot 0065243 | cat 550078
FITC anti-human IFNγ | clone 25723.11 | lot 0342682| cat 340449
PE-Cy5 anti-human CD107a | clone H4A3 (RUO) | lot 0149826 | cat 555802

BioXcell:
Human IgG1 isotype control | clone N/A | lot 659518A1 | cat BE0297

MP Biomedicals:
FITC anti-guinea pig complement C3 | lot 07927 | cat 55385

ThermoFisher:
HRP anti-human kappa light chain | cat A18853 | lot 67-50-041519

Immune Technology:
Human IgG1 isotype control | clone 37G12 | lot 090327-AB005-01-18 | cat IT-001-37G12

Invivogen:
Anti-IL-1β | clone 4H5 | cat mabg-hil1b-3

Validation

All antibodies were used according to manufacturer's instructions and previously published methods. They were validated and titrated for specificity prior to use.

Antibody-dependent neutrophil phagocytosis, validation of CD66b antibody described in: PMID 27667685, PMID 29605231, PMID 30096313, PMID 30092199 PMID 30029854, and PMID 30629918.

Antibody-dependent NK cell activation, validation of antibodies described in: PMID 23468501, PMID 24648341, PMID 26745376, PMID 27667685, PMID 30096313, PMID 30092199, and PMID 30029854.

Antibody-dependent complement deposition, validation of antibodies described in: PMID 33852832 and PMID 31301278.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	THP1 from ATCC cat TIB202; 293F from ThermoFisher cat R79007
Authentication	Cell lines commercially purchased. THP1 authentication was performed via STR Profiling service ATCC 135-XV. 293F cells were not authenticated.
Mycoplasma contamination	THP1 cell line was tested and negative for mycoplasma via ATCC Univ Mycoplasma kit 30-1012K. 293F cells were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*

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	Fresh peripheral blood was collected from healthy donors in acid citrate dextrose (ACD) anti-coagulant tubes.
Instrument	BD LSRII
Software	FACSDiva (version 9.0) and FlowJo (version 10.3)
Cell population abundance	Cell sorting not performed.
Gating strategy	The flow cytometry gating strategy employed in this study began with initial gating on forward scatter (FSC) and side scatter (SSC) to identify single cells and exclude doublets. Specifically, an FSC-A versus FSC-H gate was used to select single cells, followed by an FSC-A versus SSC-A gate to exclude debris and dead cells, thereby focusing on the main population of viable cells. Negative controls were used to establish baseline autofluorescence and non-specific binding, setting thresholds for positive staining. Experimental samples were then gated using these thresholds to accurately define positive and negative populations. Marker-specific gating was applied to identify relevant cell populations. For instance, neutrophils were identified by gating on CD66b (PacBlue-A) following the selection of single, viable cells. Similarly, NK cells were gated using CD56 (PE-Cy7-A) and CD16 (APC-Cy7-A). Fluorescence Minus One (FMO) controls were utilized to set precise gates for positive and negative populations, accounting for background fluorescence. Sequential gating was then performed to further refine specific populations, such as CD107a, IFNγ, and MIP1b for functional assays. All gates were consistently applied across samples using FlowJo software to ensure reproducibility and accuracy. This comprehensive gating strategy enabled precise identification and quantification of cell populations, minimizing background noise and non-specific signals. See Extended Data Figure 7 for exact gating strategy.

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