

Reporting Summary

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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 MESO QuickPlex SQ 120 (MSD), FACSDiva v8.02 (BD Biosciences), BioTek Gen5 v3.09, REDCap version 9.5.22; Vanderbilt University, Nashville, TN, USA

Data analysis DISCOVERY WORKBENCH 4.0 (MSD), GraphPad Prism V8.4.3, IBM SPSS Statistics, BioTek Gen5 v3.09, SpectroFlo v2.2 (Cytek Biosciences) FlowJo v10.6.2 (BD BioSciences)

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 Research [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 list of figures that have associated raw data
- A description of any restrictions on data availability

Individual participant data will be made available when the trial is complete, upon requests directed to the corresponding author; after approval of a proposal, data can be shared through a secure online platform.

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](https://www.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	Power and sample size calculations were done for the primary efficacy only, which is not presented here. No specific power calculations were carried out for these immunogenicity subgroups which are secondary endpoints in the study and mostly descriptive in nature. The results presented in this manuscript are from an interim analysis of secondary or exploratory outcomes for the described groups of this phase I/II randomised control trial (NCT04324606). The primary efficacy analysis power calculation is described in the attached study protocol.
Data exclusions	All available data from eligible volunteers was included in the manuscript, with the exception of any individual data points which failed QC criteria described in the 'Replication' section below.
Replication	Aurora cytometry for tSNE analysis – Single technical replicates for each biological sample were ran. Experiments were ran on frozen PBMC samples so repeats were not possible. Instead, quality control criteria were applied to all samples, and samples that failed were excluded from analysis. QC criteria included: exclusion of any samples with a viable lymphocyte event count of <100,000 cells. All FACS plots were manually inspected to confirm quality of staining and cytometric acquisition. To maintain reproducibility between sample batches, the same cytometer was used for every run, the same lot of antibodies were used for all samples and gating and analysis was performed by a single operator. Aurora Intracellular cytokine staining - Single technical replicates for each biological sample were ran. Experiments were ran on frozen PBMC samples so repeats were not possible. Instead, quality control criteria were applied to all samples, and samples that failed were excluded from analysis. QC criteria included: exclusion of any samples with a viable CD4+ event count of <20,000 cells and any sample with <1% of cells being positive in the SEB sample for either IFNγ, TNFα or IL2. All FACS plots were manually inspected to confirm quality of staining and cytometric acquisition. To maintain reproducibility between sample batches, the same cytometer was used for every run, the same lot of antibodies were used for all samples and gating and analysis was performed by a single operator. MSD - test samples were plated out in duplicate. Data was analysed using MSD discovery workbench 4.0. Samples were repeated if any sample a replicate with a coefficient of variations (CV) greater than 20%. Replicates were read off the standard curve, multiplied by dilution factor, and concentration was reported as an average of the replicates in pg/ml. Concentration from stimulated sample was subtracted from concentration from unstimulated (background subtract). Negative values of background subtract have been replaced by zeros. An arbitrary value of 0.0001 has been added to the background subtracts across all the samples to overcome the presence of null values raised from samples too low to be read off the standard curve. For the isotype and subclass standardised ELISAs (IgA, IgM, IgG1, and IgG3), test samples were plated in technical triplicates and a standard curve was included on every microplate, as well as 3 independent dilutions (internal controls) of the same standard curve serum diluted to correspond to the fourth dilution of the standard curve and plated in duplicate. Using Gen5 ELISA software v3.04 (BioTek), the standard curve OD values at 405 nm were fitted to a four-parameter model. Quality control was then performed on this curve as follows: the R2 value for the standard curve must be >0.994, the average interpolated AU of the internal control must be within 20% of the target value (equal in value to the dilution factor the internal control was plated at), the average OD at 405 nm of the blank control wells must be <0.15 OD405. Failure to meet these parameters results in the rejection of the experiment data, and the plate will be repeated. This ensures that the curve, interpolations and background of the assay are the same for every plate, and ensures that test samples are interpolated similarly between experiments. In addition, test samples must have a coefficient of variance in their OD405 values of <20%. Failure to meet this parameter results in the rejection of the data for that sample and the sample will be repeated. For the OD ELISAs (IgG2, IgG4 and IgE) samples were plated in technical duplicate. For the avidity ELISAs, samples were plated in technical duplicate. In addition, a standard serum pool was included on every plate and the IC50 calculated, data from any plates where the IC50 of the standard deviated >20% from the target value were discarded. Data for any samples for which the OD at 0M NaSCN across two replicates deviated by more than 20% was also discarded. ELISpot - plates were counted using an AID automated ELISpot counter (AID Diagnostika GmbH, algorithm C), using identical settings for all plates and spot counts were adjusted only to remove artefacts. Responses were averaged across triplicate wells and the mean response of the unstimulated (negative control) wells were subtracted. Results are expressed as spot forming cells (SFC)/106 PBMCs. Assays were ran on fresh cells only, therefore samples could not be repeated. Instead, QC criteria were applied to samples before inclusion in analysis. Responses to a peptide were considered positive if background subtracted responses were >40 SFC/106 PBMCs. If responses were >80 SFC/106 PBMC in the negative control (PBMC without antigen) or <800 SFC/106 PBMC in the positive control wells (pooled Staphylococcal enterotoxin B at 0.02 μg/mL and phytohaemagglutinin-L at 10 μg/mL), results were excluded from further analysis. Intracellular cytokine staining (Standard) – Single technical replicates for each biological sample were ran. Experiments were ran on freshly isolated samples so repeats were not possible. Instead, quality control criteria were applied to all samples, and samples that failed were excluded from analysis. QC criteria included: exclusion of any samples with a viable CD3+ event count of <100,000 cells and any sample with <1% of cells being positive in the SEB sample for either IFNγ, TNFα or IL2. All FACS plots were manually inspected to confirm quality of staining and cytometric acquisition. To maintain reproducibility between sample batches, the same cytometer was used for every run, the same lot of antibodies were used for all samples and gating and analysis was performed by a single operator.
Randomization	Volunteers were randomized 1:1 to receive ChAdOx1 nCoV-19, except for a subset of 10 individuals who were not initially randomized, as detailed in the main study protocol which has been previously published.

The study is single blinded (participant blinded). Investigators working on sample processing in the lab were blinded to group allocation. Investigators performing data collection and analysis were blinded to volunteer vaccination status.

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

ELISPOT
Anti-human IFN-γ Monoclonal antibody, MABTECH, 3420-25A, 1-DIK, Lot 102, 1:100
Anti-IFN-γ Biotinylated monoclonal antibody, MABTECH, 3420-25A, 7-B6-1, Lot 102, 1:1000
Streptavidin-Alkaline Phosphatase, MABTECH, 3420-25A, Lot 102, 1:1000

MSD multiplex cytokine panel
Anti human IFN-γ antibody (50x) SulfoTag, MSD, K15010B-4, Clone: D21Q0-3, lot: D0081379, 1:50 dilution
Anti human IL-1β antibody (50x) SulfoTag, MSD, K15010B-4, Clone: D21AG-3, lot: D0081380, 1:50 dilution
Anti human IL-2 antibody (50x) SulfoTag, MSD, K15010B-4, Clone: D21QQ-3, lot: D0081320, 1:50 dilution
Anti human IL-4 antibody (50x) SulfoTag, MSD, K15010B-4, Clone: D21QR-3, lot: D0081302, 1:50 dilution
Anti human IL-8 antibody (50x) SulfoTag, MSD, K15010B-4, Clone: D21AN-3, lot: D0081255, 1:50 dilution
Anti human IL-10 antibody (50x) SulfoTag,MSD, K15010B-4, Clone: D21QU-3, lot: D0081343, 1:50 dilution
Anti human IL-12p70 antibody (50x) SulfoTag, MSD, K15010B-4, Clone: D21QV-3, lot: D0081307, 1:50 dilution
Anti human IL-13 antibody (50x) SulfoTag, MSD, K15010B-4, Clone: D21OD-3, lot: D0081381, 1:50 dilution
Anti human TNF-α antibody (50x) SulfoTag, MSD, K15010B-4, Clone: D21BH-3, lot: D0081382, 1:50 dilution

Flow Cytometry (Aurora and standard ICS):

Live/Dead Zombie UV, BioLegend, 423107, 1:250 dilution, lot 2176884
CD4 AF700, OKT4, BioLegend, 317425, 1:400 dilution lot B247388
CD19 Spark NIR 685, V CD19.11 BioLegend, 302269, 1:200 dilution, lot B311830
CD56 APC, 5.1H11, BioLegend, 362503, 1:100 dilution, lot B280822
CCR7 PER-CP/Cy5.5, G043H7 BioLegend, 353219, 1:50 dilution, lot B286436
PD1 PE/Dazzle 594, EH12.2H7, BioLegend, 329940, 1:100 dilution, lot B285543
CD57- PE/Cy7, HNK-1 BioLegend, 359624, 1:200 dilution, lot B277980
Interferon gamma BV650, 4S.B3, BioLegend, 502537, 1:100 dilution, lot B291938
IL2 BV605, MQ1-17H12, BioLegend, 500331, 1:100 dilution, lot B284324
CD107a BV785 H4A3, BioLegend, 328644 1:100 dilution, lot B284309
CD8 AF405, 3B5, ThermoFisher Scientific, MHCD0826, 1:100 dilution, lot 2201611
CD45RA SuperBright 702, HI100, ThermoFisher Scientific, 67-0458-41, 1:100 dilution, lot 2124807
CD27 PerCP eF710, O323, ThermoFisher Scientific, 46-0279-41, 1:100 dilution, lot 2129920
CD20 AF532, 2H7, ThermoFisher Scientific, 58-0209-42, 1:50 dilution, lot 2247567
Ki-67 APC eF780, SolA15, ThermoFisher Scientific, 47-5698-80, 1:100 dilution, lot 2162717
CD16 BUV496 BD BioSciences, 3G8, 564654, 1:200 dilution, lot 0051270
CD3 BUV661, OKT3, BD BioSciences 48-0037-41, 1:800 dilution, lot 0157849
CD138 BUV805, MI15, BD BioSciences, 748350, 1:100 dilution, lot 0156566
NKG2A BV480, 131411, BD BioSciences, 747923, 1:50 dilution, lot 0154607
IgM BB515, G20-127, BD BioSciences, 564622, 1:100 dilution, lot 9318220
IgG BV421, G18-145, BD BioSciences, 562581, 1:100 dilution, lot 9259706
TNF alpha BUV395, mAb11, BD BioSciences, 563996, 1:100 dilution, lot 0010280
CD69 BV750, FN50, BD BioSciences, 747522, 1:100 dilution, lot 0156563
CD71 BUV563, L01.1, BD BioSciences, 748314, 1:100 dilution, lot 0156565
CD25 BV737, M-A251, BD BioSciences, 356137, 1:800 dilution, lot 8218884
NKG2C PE, REA205, Miltenyi, 130-119-776, 1:200 dilution, lot 5200601467
KLRG1 VioBlue, 2F1/KLRG1, Miltenyi, 138421, 1:100 dilution, lot 5200704650
Anti-human CD28 Functional grade, eBioscience, 16-0289-85, 1:1000 dilution, lot 2197855
Anti-human CD49d Functional grade, eBioscience, 16-0499-85, 1:1000 dilution, lot 2062915
CD107a PE-Cy5, eBioH4A3, eBioscience, 15-1079-42, 1:1000 dilution, lot 2087212

CD8a APC-eF780, RPA-T8, eBioscience, 47-0088-42, 1:20 dilution, lot 2121889
 Interferon gamma FITC, 4S.B3, eBioscience, 11-7319-82, 1:1000 dilution, lot 2204969
 IL-2 BV650, MQ1-17H12, Biolegend, 500334, 1:100 dilution, lot B293289
 IL-5 PE, JES1-39D10, Biolegend, 500904, 1:80 dilution, lot B60242
 IL-13 APC, JES10-5A2, Biolegend, 501907, 1:40 dilution, lot B301070
 TNF alpha PE-Cy7, MAb11, eBioscience, 25-7349-82, 1:4000 dilution, lot 2007278
 CD3 AF700, UCHT1, eBioscience, 56-0038-82, 1:200 dilution, lot 2102327
 CD4 PerCP Cy5.5, RPA-T4, eBioscience, 17-0049-42, 1:40 dilution, lot 1995457
 CD14 eF450, 61D3, eBioscience, 48-0149-42, 1:400 dilution, lot 2146887
 CD19 eF450, H1B19, eBioscience, 48-0199-42, 1:400 dilution, lot 2018427
 CD45RA BV605, HI100, Biolegend, 304134, 1:4000 dilution, lot B254373
 CCR7 BV711, G043H7, Bioscience, 353228, 1:100 dilution, B284686
 LIVE/DEAD Fixable Aqua (405nm), Invitrogen, L34966, 1:1600 dilution, lot 2157201

ELISA:

Anti-Human IgG (γ-chain specific)–Alkaline Phosphatase antibody produced in goat, Sigma-Aldrich, A3187, Polyclonal, SLCG3196, 1:1000
 Mouse Anti-Human IgG1 Hinge-AP, Southern Biotech, 9052-04, 4E3, G4015-MK18Z, 1:1000
 Mouse Anti-Human IgG2 Fc-AP, Southern Biotech, 9060-04, 31-7-4, E2013-T035F, 1:500
 Mouse Anti-Human IgG3 Hinge-AP, Southern Biotech, 9210-04, HP6050, F1818-TD48E, 1:500
 Mouse Anti-Human IgG4 Fc-AP, Southern Biotech, 9200-04, HP6025, B3317-M788G, 1:1000
 Goat Anti-Human IgA-AP, Southern Biotech, 2050-04, Polyclonal, C5213-R1660, 1:1000
 Goat Anti-Human IgM-AP, Southern Biotech, 2020-04, Polyclonal, L4206-Q408D, 1:1000
 Mouse Anti-Human IgE Fc-AP, Southern Biotech, 9160-04, B3102E8, J1617-X949D, 1:500
 Recombinant Human IgG2 Lambda, Bio-Rad Laboratories Ltd, HCA108, AbD00264_hlgG2, 1607, 1:6400
 Recombinant Human IgG4 Lambda, Bio-Rad Laboratories Ltd, HCA050A, AbD00264_hlgG4, 151154, 1:8000
 Recombinant Human IgE Lambda, Bio-Rad Laboratories Ltd, HCA171, AbD00264_hlgE, 1807, 1:16000

Validation

Flow Cytometry (Aurora and standard ICS):

Each primary antibody was titrated in-house as a single stain on frozen naive human PBMC to determine the optimal concentration for separation between positive and negative cell populations. The full panel of antibodies were then tested together at working concentrations to confirm the quality of staining is similar when compensation is applied.

The Aurora antibodies: Live/Dead Zombie UV, BioLegend, 423107, lot 2176884. Zombie UV™ is an amine reactive fluorescent dye that is non-permeant to live cells, but permeant to the cells with compromised membranes. For flow cytometry, the suggested dilution is 1:100-1:1000 for 1-10 million cells.

Source (<https://www.biolegend.com/en-us/products/zombie-uv-fixable-viability-kit-9336>)

CD4 AF700, OKT4, BioLegend, 317425, 1:400 dilution lot B247388. CD4, also known as T4, is a 55 kD single-chain type I transmembrane glycoprotein expressed on most thymocytes, a subset of T cells, and monocytes/macrophages. The OKT4 antibody binds to the D3 domain of CD4. This antibody is routinely tested by flow cytometric analysis. Source (<https://www.biolegend.com/en-us/search-results/alexa-fluor-700-anti-human-cd4-antibody-3661>)

CD19 Spark NIR 685, V CD19.11 BioLegend, 302269, 1:200 dilution, lot B311830. CD19 is a 95 kD type I transmembrane glycoprotein also known as B4. It is a member of the immunoglobulin superfamily expressed on B-cells. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 µL per million cells in 100 µL staining volume

Cells. This antibody is routinely tested by flow cytometric analysis. Source (<https://www.biolegend.com/en-us/products/spark-nir-685-anti-human-cd19-antibody-18623>)

CD56 APC, 5.1H11, BioLegend, 362503, 1:100 dilution, lot B280822. CD56 is a single transmembrane glycoprotein also known as NCAM (neural cell adhesion molecule), Leu-19, or NKH1. It is a member of the Ig superfamily. The 140 kD isoform is expressed on NK and NKT cells. This antibody is routinely tested by flow cytometric analysis. Source (<https://www.biolegend.com/en-gb/products/apc-anti-human-cd56-ncam-antibody-14587>).

CCR7 PER-CP/Cy5.5, G043H7 BioLegend, 353219, 1:50 dilution, lot B286436. CCR7, also known as CD197, is a chemokine receptor that binds CCL19 and CCL21. CCR7 and its ligands link innate and adaptive immunity by affecting interactions between T cells and dendritic cells and their downstream effect. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 µL per million cells in 100 µL staining volume. This antibody is routinely tested by flow cytometric analysis. Source (<https://www.biolegend.com/en-us/products/percp-cyanine5-5-anti-human-cd197-ccr7-antibody-7539>)

PD1 PE/Dazzle 594, EH12.2H7, BioLegend, 329940, 1:100 dilution, lot B285543. Programmed cell death 1 (PD-1), also known as CD279, is a 55 kD member of the immunoglobulin superfamily. CD279 contains the immunoreceptor tyrosine-based inhibitory motif (ITIM) in the cytoplasmic region and plays a key role in peripheral tolerance and autoimmune disease. CD279 is expressed predominantly on activated T cells, B cells, and myeloid cells.

Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 µL per million cells in 100 µL staining volume. This antibody is routinely tested by flow

cytometric analysis. Source (<https://www.biolegend.com/en-us/search-results/pe-dazzle-594-anti-human-cd279-pd-1-antibody-9834>).

CD57- PE/Cy7, HNK-1 BioLegend, 359624, 1:200 dilution, lot B277980. CD57, also known as HNK-1, NK-1, and Leu-7 is a 100-115 kD oligosaccharide antigenic determinant expressed on a variety of proteins, lipids, and chondroitin sulfate proteoglycans. CD57 is expressed on a subset of peripheral blood lymphocytes, including NK cells and CD8+ T cells. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 µl per million cells in 100 µl staining volume. This antibody is routinely tested by flow cytometric analysis. Source (<https://www.biolegend.com/en-us/search-results/pe-cyanine7-anti-human-cd57-antibody-13715>).

Interferon gamma BV650, 4S.B3, BioLegend, 502537, 1:100 dilution, lot B291938. Interferon-γ is a potent multifunctional cytokine which is secreted primarily by activated NK cells and T cells. Each lot of this antibody is quality control tested by intracellular immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 µl per million cells in 100 µl staining volume. This antibody is routinely tested by flow cytometric analysis. Source (<https://www.biolegend.com/en-us/search-results/brilliant-violet-650-anti-human-ifn-gamma-antibody-7678>)

IL2 BV605, MQ1-17H12, BioLegend, 500331, 1:100 dilution, lot B284324. IL-2 is a potent lymphoid cell growth factor which exerts its biological activity primarily on T cells, promoting proliferation and maturation. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 µl per million cells in 100 µl staining volume. This antibody is routinely tested by flow cytometric analysis. Source (<https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-human-il-2-antibody-7676>).

CD107a BV785 H4A3, BioLegend, 328644 1:100 dilution, lot B284309. CD107a, also known as Lysosome-Associated Membrane Protein 1 (LAMP-1) or LGP-120, is a 110-140 kD type I membrane glycoprotein. Mature CD107a is heavily glycosylated from a 40 kD core protein. This molecule is located on the luminal side of lysosomes. Upon activation, CD107a is transferred to the cell membrane surface of activated platelets, activated lymphocytes, macrophages, epithelial cells, endothelial cells, and some tumor cells. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 µl per million cells in 100 µl staining volume. This antibody is routinely tested by flow cytometric analysis. Source (<https://www.biolegend.com/en-us/products/brilliant-violet-785-anti-human-cd107a-lamp-1-antibody-12095>)

CD8 AF405, 3B5, ThermoFisher Scientific, MHCD0826, 1:100 dilution, lot 2201611. CD8 molecule is composed of two chains termed alpha and beta. CD8 is found on a T cell subset of normal cytotoxic / suppressor cells which make up approximately 20 to 35% of human peripheral blood lymphocytes. The CD8 antigen is also detected on natural killer cells, 80% of thymocytes, on a subpopulation of 30% of peripheral blood null cells and 15 to 30% of bone marrow cells. This antibody is routinely tested by flow cytometric analysis. Source (<https://www.thermofisher.com/antibody/product/CD8-Antibody-clone-3B5-Monoclonal/MHCD0826>).

CD45RA SuperBright 702, HI100, ThermoFisher Scientific, 67-0458-41, 1:100 dilution, lot 2124807. Description: The HI100 monoclonal antibody reacts with human CD45RA, a 220 kDa molecule expressed by subpopulations of CD4+ peripheral T lymphocytes, CD8+ peripheral T lymphocytes, and B cells. The CD45RA+ T cell populations are mainly naive/virgin allowing the use of HI100 mAb as a phenotypic marker to discriminate T cell subsets. This HI100 antibody has been pre-diluted and tested by flow cytometric analysis of normal human peripheral blood cells. This may be used at 5 µL (0.06 µg) per test This antibody is routinely tested by flow cytometric analysis. Source (<https://www.thermofisher.com/antibody/product/CD45RA-Antibody-clone-HI100-Monoclonal/67-0458-42>)

CD27 PerCP eF710, O323, ThermoFisher Scientific, 46-0279-41, 1:100 dilution, lot 2129920. The O323 monoclonal antibody reacts with human CD27, a lymphocyte-specific member of the TNFR superfamily. CD27 is expressed by a subset of thymocytes and virtually all mature T cells and is upregulated upon T-cell stimulation. CD27 binds to CD70, and through this interaction, plays an important role in T cell-B cell interaction. This O323 antibody has been pre-titrated and tested by flow cytometric analysis of normal human peripheral blood cells. This can be used at 5 µL (0.06 µg) per test. This antibody is routinely tested by flow cytometric analysis. Source (<https://www.thermofisher.com/antibody/product/CD27-Antibody-clone-O323-Monoclonal/46-0279-42>).

CD20 AF532, 2H7, ThermoFisher Scientific, 58-0209-42, 1:50 dilution, lot 2247567. The 2H7 monoclonal antibody reacts with human CD20, a 33-36 kDa transmembrane protein. CD20 is expressed by developing B cells as well as mature B cells but not plasma cells. CD20 has been detected at low levels on a small subset of mature T cells. It is suggested that CD20 plays a role in B-cell activation. This 2H7 antibody has been pre-diluted and tested by flow cytometric analysis of normal human peripheral blood cells. This may be used at 5 µL (0.06 µg) per test. This antibody is routinely tested by flow cytometric analysis. Source (<https://www.thermofisher.com/antibody/product/CD20-Antibody-clone-2H7-Monoclonal/58-0209-42>).

Ki-67 APC eF780, SolA15, ThermoFisher Scientific, 47-5698-80, 1:100 dilution, lot 2162717. The monoclonal antibody SolA15 recognizes mouse and rat Ki-67, a 300 kDa nuclear protein. Ki-67 is present during all active phases of the cell cycle (G1, S, G2, and mitosis), but is absent from resting cells (G0). Ki-67 is detected within the nucleus during interphase but redistributes to the chromosomes during mitosis. Ki-67 is used as a marker for determining the growth fraction of a given population of cells. This antibody can be used at less than or equal to 0.06 µg per test. Source (<https://www.thermofisher.com/antibody/product/Ki-67->

Antibody-clone-SolA15-Monoclonal/47-5698-82).

CD16 BUV496 BD BioSciences, 3G8, 564654, 1:200 dilution, lot 0051270. The 3G8 monoclonal antibody specifically recognizes CD16a and CD16b, low affinity receptors for the Fc region of IgG. The recommended volume is 5µL/test. This antibody is routinely tested by flow cytometric analysis. Source (<https://www.bdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/buv496-mouse-anti-human-cd16-3g8/p/612944>)

CD3 BUV661, OKT3, BD BioSciences 48-0037-41, 1:800 dilution, lot 0157849. The OKT3 monoclonal antibody specifically recognizes the CD3 epsilon subunit (CD3ε/CD3e) of the CD3 complex which consists of four transmembrane proteins (γ, δ, ε, ζ) that are associated with the T cell antigen receptor (TCR) to form the CD3/TCR complex. The CD3 complex associates with either TCR αβ or TCR γδ heterodimers that are alternatively expressed by some thymocytes, T cells or NKT cells. The CD3 complex is required for the cell surface expression and signal-transducing functions of the TCR. This antibody is routinely tested by flow cytometric analysis. Source (<https://www.bdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/buv661-mouse-anti-human-cd3-okt3/p/750972>).

CD138 BUV805, MI15, BD BioSciences, 748350, 1:100 dilution, lot 0156566. The MI15 monoclonal antibody specifically binds to CD138 (Syndecan-1), an 85-92 kDa single chain transmembrane protein. It is also expressed on pre-B cells, immature B cells, and plasma cells, but not on mature circulating B-lymphocytes. This antibody was developed for use in flow cytometry. Source (<https://www.bdbiosciences.com/eu/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/buv805-mouse-anti-human-cd138-mi15/p/748350>)

NKG2A BV480, 131411, BD BioSciences, 747923, 1:50 dilution, lot 0154607. The 131411 monoclonal antibody specifically recognizes NKG2A which is also known as NK cell receptor A, Natural killer cell lectin, Natural killer group protein 2, C-lectin type II protein, or CD159 antigen-like family member A (CD159a). NKG2A (CD159a) is encoded by KLRC1(killer cell lectin like receptor C1) which belongs to the killer cell lectin-like receptor (KLR) family. This antibody was developed for use in flow cytometry. Source (<https://www.bdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/bv480-mouse-anti-human-nkg2a-cd159a-131411/p/747923>)

IgM BB515, G20-127, BD BioSciences, 564622, 1:100 dilution, lot 9318220. The G20-127 monoclonal antibody binds to the heavy chain of human IgM. The G20-127 antibody is not thought to react with other immunoglobulin heavy chain isotypes. This reagent has been pre-diluted for use at the recommended Volume per Test (5µL/test). This antibody is routinely tested by flow cytometric analysis. Source (<https://www.bdbiosciences.com/us/applications/research/b-cell-research/immunoglobulins/human/bb515-mouse-anti-human-igm-g20-127/p/564622>)

IgG BV421, G18-145, BD BioSciences, 562581, 1:100 dilution, lot 9259706. The G18-145 monoclonal antibody specifically binds to the heavy chain of human immunoglobulin G subclasses: IgG1, IgG2, IgG3 and IgG4. The G18-145 antibody has been reported not to react with the heavy chains of other human immunoglobulin isotypes. This reagent has been pre-diluted for use at the recommended Volume per Test (5µL/test). This antibody is routinely tested by flow cytometric analysis. Source (<https://www.bdbiosciences.com/us/applications/research/b-cell-research/immunoglobulins/human/bv421-mouse-anti-human-igg-g18-145/p/562581>)

TNF alpha BUV395, mAb11, BD BioSciences, 563996, 1:100 dilution, lot 0010280. The MAb11 monoclonal antibody specifically binds to human tumor necrosis factor (TNF, also known as TNF-α) protein. TNF is an efficient juxtacrine, paracrine and endocrine mediator of inflammatory and immune functions. This reagent has been pre-diluted for use at the recommended Volume per Test (5µL/test). This antibody is routinely tested by flow cytometric analysis. Source (<https://www.bdbiosciences.com/us/applications/research/t-cell-immunology/th-1-cells/intracellular-markers/cytokines-and-chemokines/human/buv395-mouse-anti-human-tnf-mab11/p/563996>).

CD69 BV750, FN50, BD BioSciences, 747522, 1:100 dilution, lot 0156563. The FN50 monoclonal antibody specifically binds to human CD69. CD69 is also known as activation-induced molecule (AIM), early activation antigen (EA-1), very early activation antigen (VEA), C-type lectin domain family 2 member C (CLEC2C), MLR-3, GP32/28 and Leu-23. CD69 is a transmembrane type II homodimer receptor. CD69 is comprised of disulfide-linked, differentially glycosylated core protein subunits that are approximately 28 and 34 kDa in size. Each subunit contains a C-type lectin domain. CD69 is expressed on activated T, B, and natural killer (NK) lymphocytes, thymocytes, neutrophils, eosinophils and platelets. This antibody was developed for use in flow cytometry. This antibody is routinely tested by flow cytometric analysis. Source (<https://www.bdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/bv750-mouse-anti-human-cd69-fn50-also-known-as-fn-50/p/747522>).

CD71 BUV563, L01.1, BD BioSciences, 748314, 1:100 dilution, lot 0156565. The L01.1 monoclonal antibody specifically binds to CD71 which is also known as the transferrin receptor (TFR). This type II transmembrane glycoprotein is expressed on cells as a disulfide-linked homodimer comprised of 95 kDa monomers. CD71 is expressed on activated lymphocytes, monocytes, macrophages, brain endothelium, and most proliferating cells. This antibody was developed for use in flow cytometry. This antibody is routinely tested by flow cytometric analysis. Source (<https://www.bdbiosciences.com/eu/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/buv563-mouse-anti-human-cd71-l011/p/748314>).

CD25 BV737, M-A251, BD BioSciences, 356137, 1:800 dilution, lot 8218884 The M-A251 monoclonal antibody specifically binds to the 55 kDa type I transmembrane glycoprotein known as low-affinity interleukin-2 receptor alpha chain subunit (IL-2Rα). CD25 is expressed on regulatory T cells, activated lymphocytes (T and B), and monocytes. This antibody was developed for use in flow cytometry. This antibody is routinely tested by flow cytometric analysis. Source (<https://www.bdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/buv737-mouse-anti-human-cd25-m-a251/p/741832>).

NKG2C PE, REA205, Miltenyi, 130-119-776, 1:200 dilution, lot 5200601467. Clone REA205 recognizes the CD159c antigen, a calcium-dependent (C-type) lectin which is also known as NKG2C type II integral membrane protein or KLRC2. This antibody is recommended at a dilution of 1:50. Source (<https://www.miltenyibiotec.com/GB-en/products/cd159c-nkg2c-antibody-anti-human-reafinity-rea205.html#gref>)

KLRG1 VioBlue, 2F1/KLRG1, Miltenyi, 138421, 1:100 dilution, lot 5200704650. Clone REA261 recognizes the killer cell lectin-like receptor subfamily G member 1 (KLRG1) antigen, a transmembrane protein, which is also known as C-type lectin domain family 15 member A (CLEC15A) or ITIM-containing receptor MAFA-L. KLRG1 is expressed by basophils, CD4 and CD8 T cells that exhibit a memory cell phenotype, and by a large proportion of peripheral blood NK cells. This antibody is recommended at a dilution of 1:50. Source (<https://www.miltenyibiotec.com/GB-en/products/klrg1-antibody-anti-human-reafinity-rea261.html#gref>).

Anti-human CD28 Functional grade, eBioscience, 16-0289-85, 1:1000 dilution, lot 2197855. The CD28.6 monoclonal antibody reacts with the human CD28 molecule, a 44 kDa homodimer expressed by thymocytes, mature T cells and plasma cells. This antibody is used frequently for functional T cell stimulation. Source (<https://www.thermofisher.com/antibody/product/CD28-Antibody-clone-CD28-6-Monoclonal/16-0288-81>)

Anti-human CD49d Functional grade, eBioscience, 16-0499-85, 1:1000 dilution, lot 2062915. The 9F10 monoclonal antibody reacts with human CD49d, the 150 kDa integrin alpha4 subunit. The complex of CD49d non-covalently associated with integrin beta1 (CD29), also known as VLA-4, is a receptor for fibronectin and VCAM-1 (CD106). This complex is expressed by thymocytes, peripheral lymphocytes, monocytes and eosinophils. This antibody is used frequently for functional T cell stimulation. Source (<https://www.thermofisher.com/antibody/product/CD49d-Integrin-alpha-4-Antibody-clone-9F10-Monoclonal/16-0499-81>).

CD107a PE-Cy5, eBioH4A3, eBioscience, 15-1079-42, 1:1000 dilution, lot 2087212

Description: The eBioH4A3 monoclonal antibody reacts with human CD107a, also known as lysosomal-associated membrane protein-1 (LAMP-1). CD107a is a highly glycosylated protein of approximately 110kDa. It is predominantly expressed intracellularly in the lysosomal/endosomal membrane in nearly all cells. CD107a is transiently expressed on the cell surface of degranulating cytolytic T cells, and is also upregulated on the surface of activated platelets and some cancer cells.

his eBioH4A3 antibody has been reported for use in intracellular staining followed by flow cytometric analysis. It has also been reported for use in surface staining in a flow cytometric based degranulation assay.

Source: <https://www.thermofisher.com/antibody/product/CD107a-LAMP-1-Antibody-clone-eBioH4A3-Monoclonal/15-1079-42>

CD8a APC-eF780, RPA-T8, eBioscience, 47-0088-42, 1:20 dilution, lot 2121889

The RPA-T8 monoclonal antibody reacts with the human CD8a molecule, an approximately 32-34 kDa cell surface receptor expressed either as a heterodimer with the CD8 beta chain (CD8 alpha/beta) or as a homodimer (CD8 alpha/alpha). A majority of thymocytes and a subpopulation of mature T cells and NK cells express CD8a. CD8 binds to MHC class I and through its association with protein tyrosine kinase p56lck plays a role in T-cell development and activation of mature T cells.

This RPA-T8 antibody has been reported for use in flow cytometric analysis.

Source: <https://www.thermofisher.com/antibody/product/CD8a-Antibody-clone-RPA-T8-Monoclonal/47-0088-42>

Interferon gamma FITC, 4S.B3, eBioscience, 11-7319-82, 1:1000 dilution, lot 2204969

The 4S.B3 monoclonal antibody reacts with interferon-gamma (IFN gamma). Human IFN gamma is a 17 kDa factor produced by activated T and NK cells and is an anti-viral and anti-parasitic cytokine. IFN gamma in synergy with other cytokines, such as TNF alpha, inhibits proliferation of normal and transformed cells. Immunomodulatory effects of IFN gamma are exerted on a wide range of cell types expressing the high affinity receptors for IFN gamma. Glycosylation of IFN gamma does not affect its biological activity.

The 4S.B3 antibody has been reported for use in intracellular staining for flow cytometric analysis.

Source: <https://www.thermofisher.com/antibody/product/IFN-gamma-Antibody-clone-4S-B3-Monoclonal/11-7319-82>

IL-2 BV650, MQ1-17H12, Biolegend, 500334, 1:100 dilution, lot B293289

IL-2 is a potent lymphoid cell growth factor which exerts its biological activity primarily on T cells, promoting proliferation and maturation. Additionally, IL-2 has been found to stimulate growth and differentiation of B cells, NK cells, LAK cells, monocytes, and oligodendrocytes.

Each lot of this antibody is quality control tested by intracellular immunofluorescent staining with flow cytometric analysis.

Source (<https://www.biolegend.com/en-us/products/brilliant-violet-650-anti-human-il-2-antibody-7768>)

IL-5 PE, JES1-39D10, Biolegend, 500904, 1:80 dilution, lot B60242

L-5 is a homodimeric, disulphide-linked protein produced by T-cells. Monomeric human IL-5 is a 126 amino acid protein with a reported molecular weight of 26 kD for the homodimeric protein. Mouse and human IL-5 are approximately 70% identical. IL-5 has been shown to promote the growth of immature hematopoietic BFU-E progenitors, stimulate the activation and differentiation of eosinophils, and promote the generation of cytotoxic lymphocytes. The JES1-39D10 antibody can neutralize the bioactivity of natural or recombinant IL-5.

Each lot of this antibody is quality control tested by intracellular immunofluorescent staining with flow cytometric analysis.

Source (<https://www.biolegend.com/en-us/products/pe-anti-human-il-5-antibody-934>)

IL-13 APC, JES10-5A2, Biolegend, 501907, 1:40 dilution, lot B301070

IL-13 is an immunoregulatory cytokine produced primarily by activated Th2 lymphocytes. IL-13 shares 30% amino acid sequence homology with IL-4 and demonstrates similar biological activities. The biological activities of IL-13 include: suppression of macrophage cytotoxic activity, upregulation of IL-1RA expression, and suppression of proinflammatory cytokine secretion. The JES10-5A2 antibody reacts with human interleukin-13 (IL-13). The JES10-5A2 antibody can neutralize the bioactivity of natural or recombinant IL-13.

Each lot of this antibody is quality control tested by intracellular immunofluorescent staining with flow cytometric analysis.

Source <https://www.biolegend.com/en-us/products/apc-anti-human-il-13-antibody-938>

TNF alpha PE-Cy7, MAb11, eBioscience, 25-7349-82, 1:4000 dilution, lot 2007278

The MAb11 antibody reacts with human tumor necrosis factor-alpha (TNF alpha), a 17 kDa cytokine produced by monocytes, macrophages, neutrophils, NK cells and CD4+ T cells. TNF alpha has cytolytic activity against a range of tumor cells and is important in immune regulation. TNF alpha forms dimers and trimers and also exists as a 26 kDa membrane bound form. The MAb11 antibody is a neutralizing antibody.

This MAb11 antibody has been reported for use in intracellular staining followed by flow cytometric analysis.

Source <https://www.biolegend.com/en-us/products-2/alexa-fluor-488-anti-human-tnf-alpha-antibody-2750>

CD3 AF700, UCHT1, eBioscience, 56-0038-82, 1:200 dilution, lot 2102327

CD3ε is a 20 kD chain of the CD3/T-cell receptor (TCR) complex which is composed of two CD3ε, one CD3γ, one CD3δ, one CD3ζ (CD247), and a T-cell receptor (α/β or γ/δ) heterodimer. It is found on all mature T lymphocytes, NK-T cells, and some thymocytes. CD3, also known as T3, is a member of the immunoglobulin superfamily that plays a role in antigen recognition, signal transduction, and T cell activation.

Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.

Source: <https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-human-cd3-antibody-3417>

CD4 PerCP Cy5.5, RPA-T4, eBioscience, 17-0049-42, 1:40 dilution, lot 1995457

CD4, also known as T4, is a 55 kD single-chain type I transmembrane glycoprotein expressed on most thymocytes, a subset of T cells, and monocytes/macrophages. CD4, a member of the Ig superfamily, recognizes antigens associated with MHC class II molecules, and participates in cell-cell interactions, thymic differentiation, and signal transduction. CD4 acts as a primary receptor for HIV, binding to HIV gp120. CD4 has also been shown to interact with IL-16.

Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.

Source: <https://www.biolegend.com/en-us/products/alexa-fluor-488-anti-human-cd4-antibody-2727>

CD14 eF450, 61D3, eBioscience, 48-0149-42, 1:400 dilution, lot 2146887

The 61D3 monoclonal antibody reacts with human CD14, a 53-55 kDa GPI-linked glycoprotein. CD14 is expressed on monocytes, interfollicular macrophages and some dendritic cells. Complexes of LPS and LBP (LPS-Binding Protein) bind with high affinity to monocytes through the surface CD14.

This antibody is routinely tested by flow cytometric analysis.

Source: <https://www.thermofisher.com/antibody/product/CD14-Antibody-clone-61D3-Monoclonal/48-0149-42>

CD19 eF450, HIB19, eBioscience, 48-0199-42, 1:400 dilution, lot 2018427

The HIB19 monoclonal antibody reacts with human CD19, a 95 kDa transmembrane glycoprotein. CD19 is expressed by B cells during all stages of development excluding the terminally differentiated plasma cells. Follicular dendritic cells also express CD19. Together CD21, CD81, Leu13, MHC class II, and CD19 form a multimolecular complex that associates with BCR. Signaling through CD19 induces tyrosine phosphorylation, calcium flux and proliferation of B cells. The SJ25C1 antibody and the HIB19 monoclonal antibody recognize overlapping epitopes.

This HIB19 antibody has been reported for use in flow cytometric analysis.

Source <https://www.thermofisher.com/antibody/product/CD19-Antibody-clone-HIB19-Monoclonal/25-0199-42>

CD45RA BV605, HI100, Biolegend, 304134, 1:4000 dilution, lot B254373

The HI100 monoclonal antibody reacts with human CD45RA, a 220 kDa molecule expressed by subpopulations of CD4+ peripheral T lymphocytes, CD8+ peripheral T lymphocytes, and B cells. The CD45RA+ T cell populations are mainly naive/virgin allowing the use of HI100 mAb as a phenotypic marker to discriminate T cell subsets.

This HI100 antibody has been reported for use in flow cytometric analysis.

Source: <https://www.thermofisher.com/antibody/product/CD45RA-Antibody-clone-HI100-Monoclonal/25-0458-42>

CCR7 BV711, G043H7, Bioscience, 353228, 1:100 dilution, B284686

CCR7, also known as CD197, is a chemokine receptor that binds CCL19 and CCL21. CCR7 and its ligands link innate and adaptive immunity by affecting interactions between T cells and dendritic cells and their downstream effect. Naïve T cells enter the lymph node through high endothelial venules, which express CCL21. Dendritic cells and macrophages enter the lymph node through afferent lymphatics. The encounter of T cells and dendritic cells in the T cell zone is CCR7-dependent. In addition, during immunological surveillance, B cells recirculate between B-cell-rich compartments (follicles or B cell zones) in secondary lymphoid organs, surveying for antigen. After antigen binding, B cells move to the boundary of B and T zones to interact with T-helper cells; this B cell migration is directed by CCR7 and its ligands. CCR7-positive cancer cell expression has been associated with lymph node metastasis.

Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.

Source <https://www.biolegend.com/en-us/products/alexa-fluor-488-anti-human-cd197-ccr7-antibody-7496>

LIVE/DEAD Fixable Aqua (405nm), Invitrogen, L34966, 1:1600 dilution, lot 2157201

The Live/Dead Cell Double Staining Kit is utilized for simultaneous fluorescence staining of viable and dead cells. This kit contains calcein-AM and propidium iodide (PI) solutions, which stain viable and dead cells, respectively. Calcein-AM, acetoxymethyl ester of calcein, is highly lipophilic and cell membrane permeable. Though calcein-AM itself is not a fluorescent molecule, the calcein generated from Calcein-AM by esterase in a viable cell emits a strong green fluorescence (λ_{ex} 490 nm, λ_{em} 515 nm). Therefore, calcein-AM only stains viable cells.

Source: <https://www.sigmaaldrich.com/catalog/product/sigma/04511?lang=en®ion=GB>

ELISA:

Anti-Human IgG (γ -chain specific)–Alkaline Phosphatase antibody produced in goat, Sigma-Aldrich, A3187, Polyclonal, SLCG3196. The A3187 antibody specifically binds to human IgG γ -chain. This antibody is routinely tested in ELISA (Source: <https://www.sigmaaldrich.com/catalog/product/sigma/a3187?lang=en®ion=GB>)

Mouse Anti-Human IgG1 Hinge-AP, Southern Biotech, 9052-04, 4E3, G4015-MK18Z. The 9052-04 specifically binds to human IgG1 hinge region. This antibody is routinely tested in ELISA (Source: <https://www.southernbiotech.com/?catno=9052-04&type=Monoclonal#&panel2-1>)

Mouse Anti-Human IgG2 Fd-AP, Southern Biotech, 9080-04, 31-7-4, E2013-T035F. The 9080-04 specifically binds to human IgG2 Fd region. This antibody is routinely tested in ELISA (Source: <https://www.southernbiotech.com/?catno=9080-04&type=Monoclonal#&panel2-1>)

Mouse Anti-Human IgG3 Hinge-AP, Southern Biotech, 9210-04, HP6050, F1818-TD48E. The 9210-04 specifically binds to human IgG3 hinge region. This antibody is routinely tested in ELISA (Source: <https://www.southernbiotech.com/?catno=9210-04&type=Monoclonal#&panel2-1>)

Mouse Anti-Human IgG4 Fc-AP, Southern Biotech, 9200-04, HP6025, B3317-M788G. The 9200-04 specifically binds to human IgG4 Fc region. This antibody is routinely tested in ELISA (Source: <https://www.southernbiotech.com/?catno=9200-04&type=Monoclonal#&panel2-1>)

Goat Anti-Human IgA-AP, Southern Biotech, 2050-04, Polyclonal, C5213-R1660. The 2050-04 specifically binds to human IgA heavy chain. This antibody is routinely tested in ELISA (Source: <https://www.southernbiotech.com/?catno=2050-04&type=Polyclonal#&panel2-1>)

Goat Anti-Human IgM-AP, Southern Biotech, 2020-04, Polyclonal, L4206-Q408D. The 2020-04 specifically binds to human IgM heavy chain. This antibody is routinely tested in ELISA (Source: <https://www.southernbiotech.com/?catno=2020-04&type=Polyclonal#&panel2-1>)

Mouse Anti-Human IgE Fc-AP, Southern Biotech, 9160-04, B3102E8, J1617-X949D. The 9160-04 specifically binds to human IgE Fc region. This antibody is routinely tested in ELISA (Source: <https://www.southernbiotech.com/?catno=9160-04&type=Monoclonal#&panel2-1>)

Recombinant Human IgG2 Lambda, Bio-Rad Laboratories Ltd, HCA108, AbD00264_hlgG2, 1607. The HCA108 is a recombinant antibody that has no known reactivity with mammalian proteins or other antigens. This antibody is routinely tested in ELISA (Source: <https://www.bio-rad-antibodies.com/protein/human-igg2-recombinant-protein-abd00264-higg2-hca108.html?f=purified>)

Recombinant Human IgG4 Lambda, Bio-Rad Laboratories Ltd, HCA050A, AbD00264_hlgG4, 151154. The HCA050A is a recombinant antibody that has no known reactivity with mammalian proteins or other antigens. This antibody is routinely tested in ELISA (Source: <https://www.bio-rad-antibodies.com/protein/human-igg4-recombinant-protein-abd00264-higg4-hca050.html?f=purified>)

Recombinant Human IgE Lambda, Bio-Rad Laboratories Ltd, HCA171, AbD00264_hlgE, 1807. The HCA171 is a recombinant antibody that has no known reactivity with mammalian proteins or other antigens. This antibody is routinely tested in ELISA (Source: <https://www.bio-rad-antibodies.com/protein/human-ige-recombinant-protein-abd00264-hige-hca171.html?f=purified>)

ELISPOT

All antibody used in this assay were titrated in house.

Anti-human IFN- γ acts as a capture antibody that binds specifically to human IFN- γ
Anti-IFN- γ (biotinylated) acts as a detection antibody which specifically binds to IFN γ

Both antibodies are routinely used and tested across ELISpot IFN γ . Further information can be found https://www.mabtech.com/products/human-ifn-gamma-elispot-basic-kit-kit-alp_3420-2a#tabs-min-1

MSD multiplex cytokine panel

MSD offers V-PLEX assays for customers who require unsurpassed performance and quality. V-PLEX products are developed under rigorous design control and are fully validated according to fit-for-purpose39 principles in accordance with MSD's Quality Management System. They offer exceptional sensitivity, simple protocols, reproducible results, and lot-to-lot consistency. In addition

to the analytical validation, robustness of the assay protocol is assessed during development along with the stability and robustness of the assay components and kits. Specific details of their antibody validation is confidential. Further information can be found at <https://www.mesoscale.com/en/products/human-th1-th2-10-plex-tissue-culture-kit-k15010b/>

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	Expi293™ (Thermo Fisher Scientific)
Authentication	None
Mycoplasma contamination	Cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	None

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics	Male and Female healthy adult volunteers aged 18-55 years old were recruited from 5 sites across the UK
Recruitment	The results described are for a phase I/II trial in a healthy volunteer population with minimal co-morbidity. Recruitment was carried out through general advertisement including the study website and online social media adverts. Participants were pre-screened through an online questionnaire covering the main eligibility criteria. A formal in-person screening visit then took place and participants were enrolled if meeting the eligibility criteria. Participants without access to the internet were able to answer the pre-screening questions via phone. Individuals without access to the internet, phone communication and who were solely reliant on public transport were unable to take part. As a phase I/II healthy volunteer trial this is likely to have had minimal impact on the validity of the results. Further assessment of the vaccine in the target population will take place in phase III trials (not presented here) which will increase the generalisability of results.
Ethics oversight	This study was approved in the UK by the Medicines and Healthcare products Regulatory Agency (reference 21584/0424/001-0001) and the South Central Berkshire Research Ethics Committee (reference 20/SC/0145).

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

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	ISRCTN [15281137] and ClinicalTrials.gov [NCT04324606]
Study protocol	The trial protocol can be accessed as supplementary data in Folegatti PM and Ewer KJ et al., Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. Lancet. 2020 Aug 15;396(10249):467-478. doi: 10.1016/S0140-6736(20)31604-4. PMID: 32702298; PMCID: PMC7445431.
Data collection	Participants were recruited between April 23 and May 21, 2020 from 5 different sites across the UK (Centre for Clinical Vaccinology and Tropical Medicine, University of Oxford; NIHR Southampton Clinical Research Facility, University Hospital Southampton NHS Foundation Trust, Southampton; Clinical Research Facility, Imperial College London; St Georges University of London and University Hospital NHS Foundation Trust; and University Hospitals Bristol and Weston NHS Foundation Trust
Outcomes	This manuscript reports on secondary immunogenicity outcomes. Immunogenicity outcomes were assessed through tSNE analysis, multiplex cytokine analysis, ELISA, IFN-gamma ELISPOT, flow cytometry and intracellular cytokine staining.

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	Aurora samples:
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Sample preparation

The samples used for flow cytometry on the Cytex Aurora spectral analyser were frozen aliquots of peripheral blood mononuclear cells (PBMCs) of 30 donors from days 0, 7, 14 and 28 after vaccination with ChAdOx1 nCoV19 (n=26). Cells were defrosted in media containing >5U/mL benzonase and re-suspended in complete RPMI media supplemented with 10% FCS, L-glutamine and Penicillin/streptomycin at a concentration of 2×10^7 cells/mL. 2×10^6 PBMCs per well were plated in a 96-well plate and stimulated with synthetic peptides spanning the SARS-CoV-2 Spike protein split into two separate pools for the S1 and S2 subunits (Supplementary table S2) at a final concentration of $2 \mu\text{g/mL}$, or media as a control. One well per donor was stimulated with Phorbol 12-myristate 13-acetate (PMA, BioLegend) as a positive control. PBMCs were co-stimulated in the presence of anti-human CD28, CD49d ($1 \mu\text{g/mL}$, Life Technologies Ltd), and CD107a-BV786 (BioLegend) for two hours at 37°C with 5% CO_2 , and then incubated for a further 16 hours after the addition of $1 \mu\text{g/mL}$ Brefeldin A and Monensin to each well (BioLegend).

Standard ICS samples:

Intracellular cytokine staining (ICS) was performed on freshly isolated PBMCs stimulated with pooled S1 and S2 peptides. 3×10^6 PBMCs were resuspended in 5 ml polypropylene FACS tubes to a volume of 1 ml in R10 media supplemented with $1 \mu\text{g/mL}$ anti-human CD28 and CD49d and $1 \mu\text{L}$ CD107a PE-Cy5 (eBioscience). S1 and S2 peptide pools (Supplementary table S2) were added at a concentration of $2 \mu\text{g/mL}$. Each sample also included a positive control (Staphylococcal enterotoxin B at $1 \mu\text{g/mL}$, Sigma Aldrich) and an unstimulated (media only) control. Cells were incubated at 37°C with 5% CO_2 for 16-20 hours with Brefeldin A ($3 \mu\text{g/mL}$) and monensin (2mM) (eBioscience) added after 2 hours.

At the end of the incubation, cells were washed in FACS buffer (PBS containing 0.1% bovine serum albumin and 0.01% NaN_3) and transferred to a 96 well U-bottom tissue culture plate for staining. A surface staining cocktail was first added containing $2.5 \mu\text{L}$ of a 1:40 dilution of Aqua Live/Dead stain (ThermoFisher Scientific) and $1 \mu\text{L}$ of BV711 CCR7 (Biolegend) in $46.5 \mu\text{L}$ FACS buffer. Cells were incubated in the dark for 20 minutes and washed with FACS buffer. $100 \mu\text{L}$ CytoFix/CytoPerm solution (BD Biosciences) was added to each well and left to incubate for a further 20 minutes. Cells were then washed with Perm/Wash buffer before intracellular cytokine staining. The ICS cocktail contained $0.025 \mu\text{L}$ CD45RA BV605, $0.025 \mu\text{L}$ TNF α PE-Cy7, $0.1 \mu\text{L}$ IFN γ FITC, $0.025 \mu\text{L}$ CD14 e450, $0.025 \mu\text{L}$ CD19 e450, $0.5 \mu\text{L}$ CD3 AF700, $1 \mu\text{L}$ IL-2 BV650, $1.25 \mu\text{L}$ IL-5 PE, $2.5 \mu\text{L}$ IL-13 APC, $3.5 \mu\text{L}$ CD4 PerCP Cy5.5 and $5 \mu\text{L}$ CD8 APC-eF780 to a total volume of $50 \mu\text{L}$ diluted in FACS buffer. Samples were stained in the dark for 30 minutes. Cells were washed twice with perm/wash buffer and twice with FACS buffer before being resuspended in $100 \mu\text{L}$ of 1% paraformaldehyde.

Compensation controls were prepared fresh for each batch using OneComp eBeads (eBioscience). Cells were kept on ice and strained through a $35 \mu\text{m}$ filter before acquisition. Cells were acquired on a 5-laser BD LSRFortessa flow cytometer (BD Biosciences) and data analysed in FlowJo v10.7. A hierarchical gating strategy was applied for sample analysis (Supplementary figure 7). A QC process was applied to remove samples with fewer than 100,000 events in the live CD3+ gate, samples with <1% cytokine response to SEB (CD4+ and CD8+ IFN γ , CD8+ TNF α). A lower limit of detection was applied and only samples with an ELISPOT response greater than 200 SFC/106 PBMC were included in the analysis).

Instrument

Cytex Aurora, Custom build with 4 lasers (Cytex Biosciences) and LSRFortessa-X20, 5 laser (Becton Dickinson)

Software

SpectroFlo v2.2 (Cytex Biosciences)
FACSDiva v 8.02 (BD Biosciences)
FlowJo v10.6.2 (BD Biosciences)
Prism v8 (GraphPad)

Cell population abundance

For Aurora tSNE analysis, live single cell lymphocytes were the cell type of interest. The median event count across all samples analysed was 435000 [Range: 107274-741000, IQR: 271137-588000].

For Aurora Intracellular cytokine staining live single cell lymphocytes were the cell type of interest. The median event count across all samples analysed was 465500 [Range: 42886-923000, IQR: 254193-694250].

For standard intracellular cytokine staining experiments, the cell population of interest was single cell viable CD3+ lymphocytes. The median event count across all samples analysed was 603000 [range: 163693-918000, IQR: 462250-703250].

Gating strategy

Cytex Aurora analysis:

1. Y=Live/Dead Zombie UV X=FSC-A. Live population gated
2. Y=FSC-H, X= FSC-A, Cells along the diagonal axis gated
3. Y= SSC-A, X= FSC-A, Lymphocytes gated and defined as the population of cells within the X=0.5M-2.0M and Y=0.3M-1.0M range.
4. Y=CD19 Spark NIR685 X= CD3 BUV661.

- a) B cells defined as Y> -7×10^3 , X= $-102 - 3 \times 10^3$
- b) CD3+ cells defined as Y= $-9 \times 10^3 - 1$, X> 6×10^3
- c) CD19-CD3- cells defined as Y= $-8 \times 10^3 - 0$, X= $-102 - 3 \times 10^3$.

5. CD3+ T cells

- a) CD8+ T cells defined as Y= $2 \times 10^4 - 2 \times 10^5$, X= $-7 \times 10^3 - 3 \times 10^3$
- b) CD4+ T cells defined as Y= $-104 - 104$, X= $4 \times 10^3 - 3 \times 10^4$.

Within each population of CD4 or CD8 T cells:

- c) Y=CD45RA = 7×10^3 X=CCR7 = 3×10^3
- d) Y=Ki67 Y> 7×10^3 X=FSC-A
- e) Y=CD57 = 2×10^4 , X= CD57 = 2×10^3
- f) Y=CD69 = 104, X= CD25 = 5×10^3

g) $Y = \text{IFN-}\gamma = \text{diagonal from } 3 \times 10^4 - 10^4$, $X = \text{CD107a} = 10^5$

h) $Y = \text{TNF-}\alpha = 2 \times 10^3$, $X = \text{IL2} = 9 \times 10^3$

6. CD19+ B cells

a) $Y = \text{IgG} = 9 \times 10^3$, $X = \text{CD27} = 3 \times 10^3$

b) $Y = \text{CD71} = 10^4$, $X = \text{Ki67} = 6 \times 10^4$

7. CD19-CD3- cells

NK cells defined as $Y = \text{CD56} = \text{diagonal from } 2 \times 10^4 \text{ to } 5 \times 10^3$ when $X = 6 \times 10^4$. Then $Y = 2 \times 10^3$. $X = \text{CD16} = > 0$.

Within NK cells

$Y = \text{CD57} = 2 \times 10^3$, $X = \text{NKG2C} = 3 \times 10^3$

$Y = \text{Ki67} \Rightarrow 5 \times 10^4$, $X = \text{CD56} \Rightarrow 3 \times 10^3$.

Standard ICS analysis:

(Parent lymphocyte gating)

1: $X = \text{FSC-A}$, $Y = \text{SSC-A}$, lymphocyte population gated

2: $X = \text{FSC-A}$, $Y = \text{FSC-H}$, single cell population gated

3: $X = \text{Live/Dead Aqua} = 2 \times 10^3$ (upper limit), $Y = \text{CD3 AF700} = 2 \times 10^2$

4: $X = \text{Dump}(\text{CD14-}/\text{CD19-}) \text{ e450} = 1 \times 10^3$ (upper limit), $Y = \text{SSC-H}$ (all events)

(CD4+ T cell gating)

5: $X = \text{CD4 PerCP Cy5.5} = 7 \times 10^2$, $Y = \text{IFN}\gamma \text{ FITC}$ (all events)

6: $X = \text{CD8 APC-eF780} = 3 \times 10^2$ (upper limit), $Y = \text{IFN}\gamma \text{ FITC}$ (all events)

6a: $X = \text{IFN}\gamma \text{ FITC} = 5 \times 10^2$, $Y = \text{IL-2 BV650} = 3 \times 10^3$

6b: $X = \text{IFN}\gamma \text{ FITC}$ (all events), $Y = \text{TNF}\alpha \text{ PE-Cy7} = 5 \times 10^2$

6c: $X = \text{IFN}\gamma \text{ FITC}$ (all events), $Y = \text{IL-5 PE} = 1 \times 10^3$

6d: $X = \text{IFN}\gamma \text{ FITC}$ (all events), $Y = \text{IL-13 APC} = 1 \times 10^3$

(CD8+ T cell gating)

7: $X = \text{CD8 APC-eF780} = 2 \times 10^2$, $Y = \text{IFN}\gamma \text{ FITC}$ (all events)

8: $X = \text{CD4 PerCP Cy5.5} = 1 \times 10^3$ (upper limit), $Y = \text{IFN}\gamma \text{ FITC}$ (all events)

6a: $X = \text{IFN}\gamma \text{ FITC} = 5 \times 10^2$, $Y = \text{IL-2 BV650} = 3 \times 10^3$

6b: $X = \text{IFN}\gamma \text{ FITC}$ (all events), $Y = \text{TNF}\alpha \text{ PE-Cy7} = 9 \times 10^2$

6c: $X = \text{IFN}\gamma \text{ FITC}$ (all events), $Y = \text{IL-5 PE} = 1 \times 10^3$

6d: $X = \text{IFN}\gamma \text{ FITC}$ (all events), $Y = \text{IL-13 APC} = 1 \times 10^3$

6e: $X = \text{IFN}\gamma \text{ FITC}$ (all events), $Y = \text{CD107a PE-Cy7} = 1 \times 10^4$

n.b Gate 4 represents the parent gate for both gates 5 and 7.

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