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Supplemental information

A booster dose enhances immunogenicity of the COVID-19 vaccine candidate ChAdOx1 nCoV-19 in aged mice

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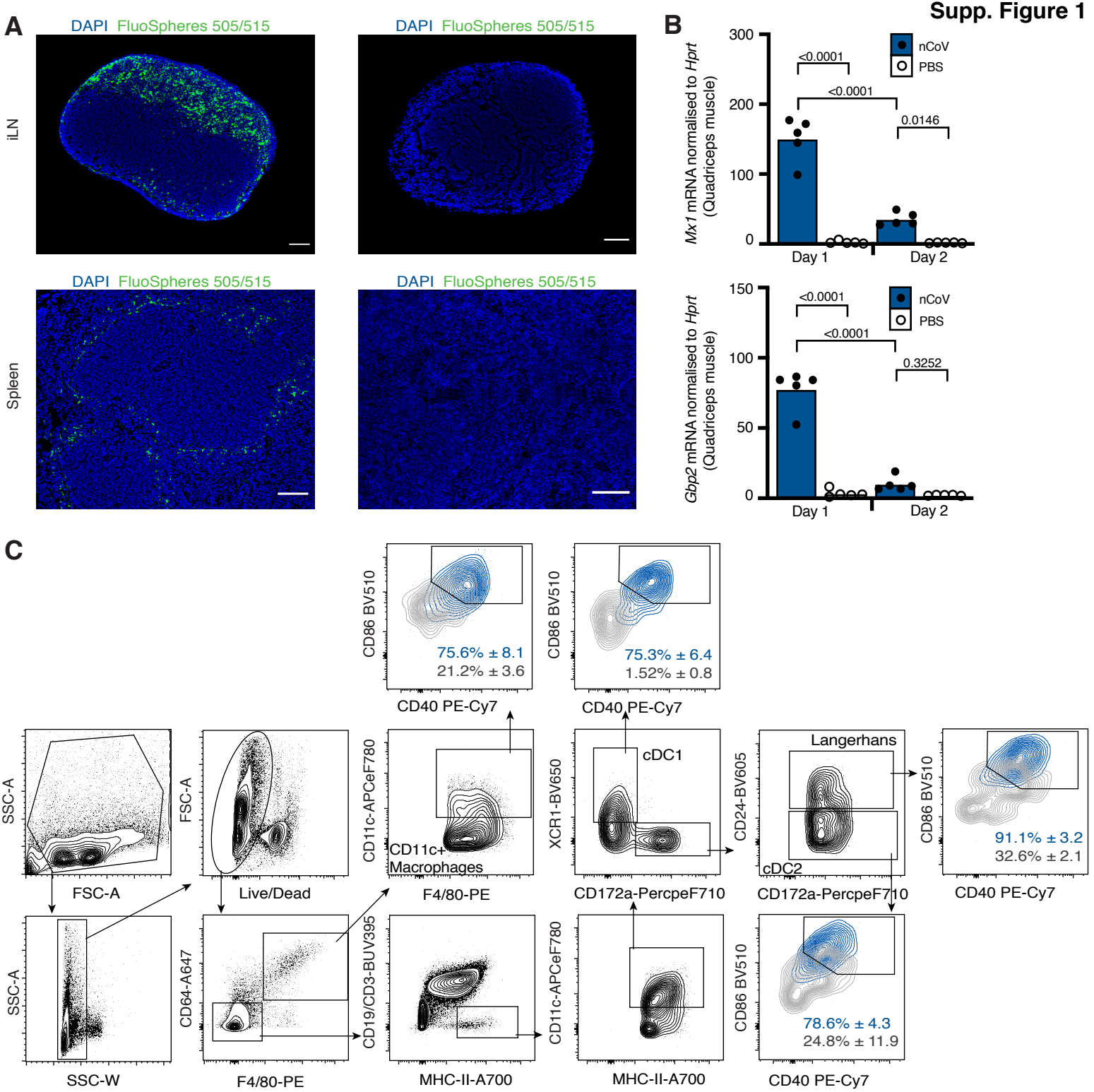
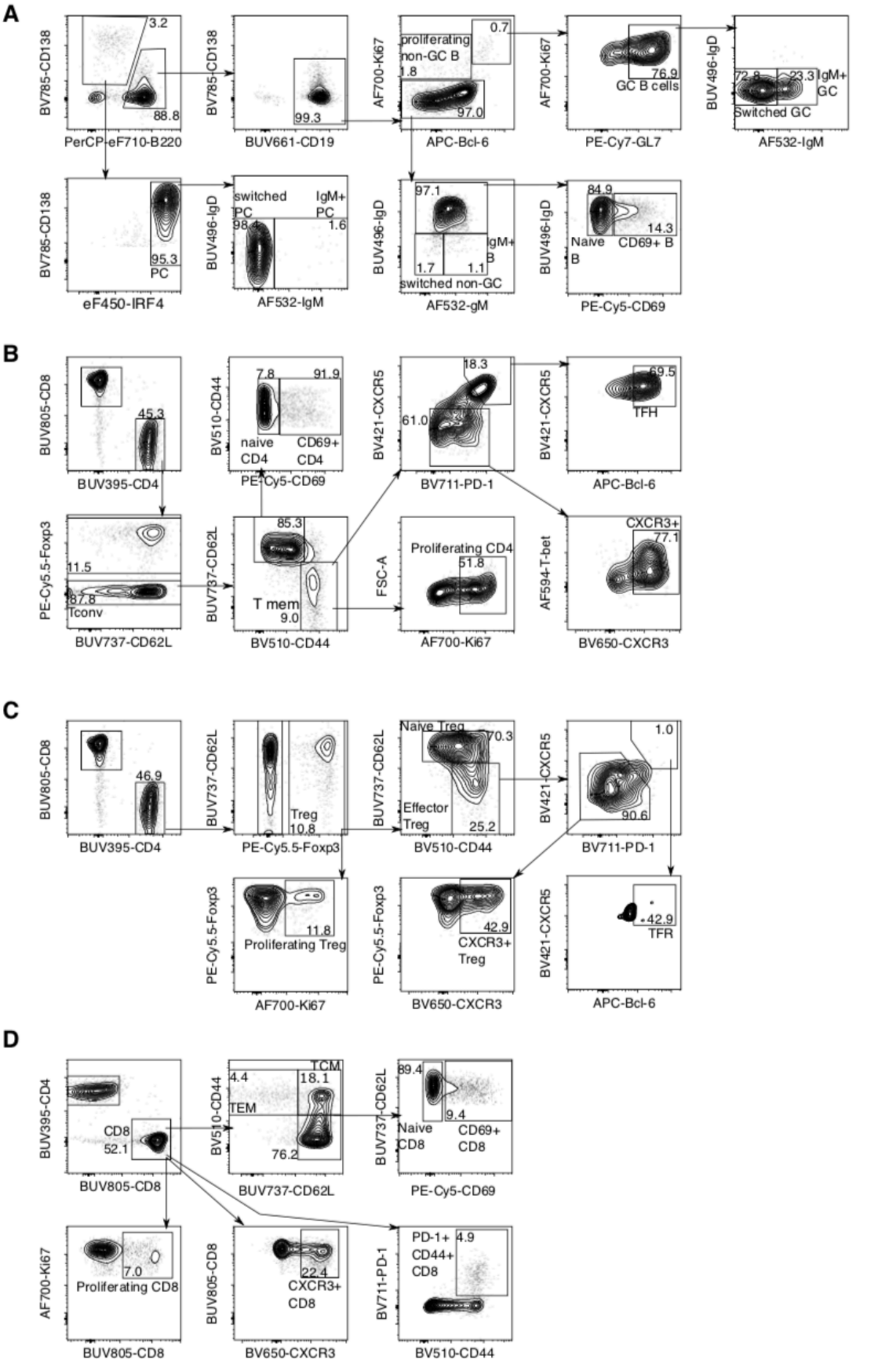
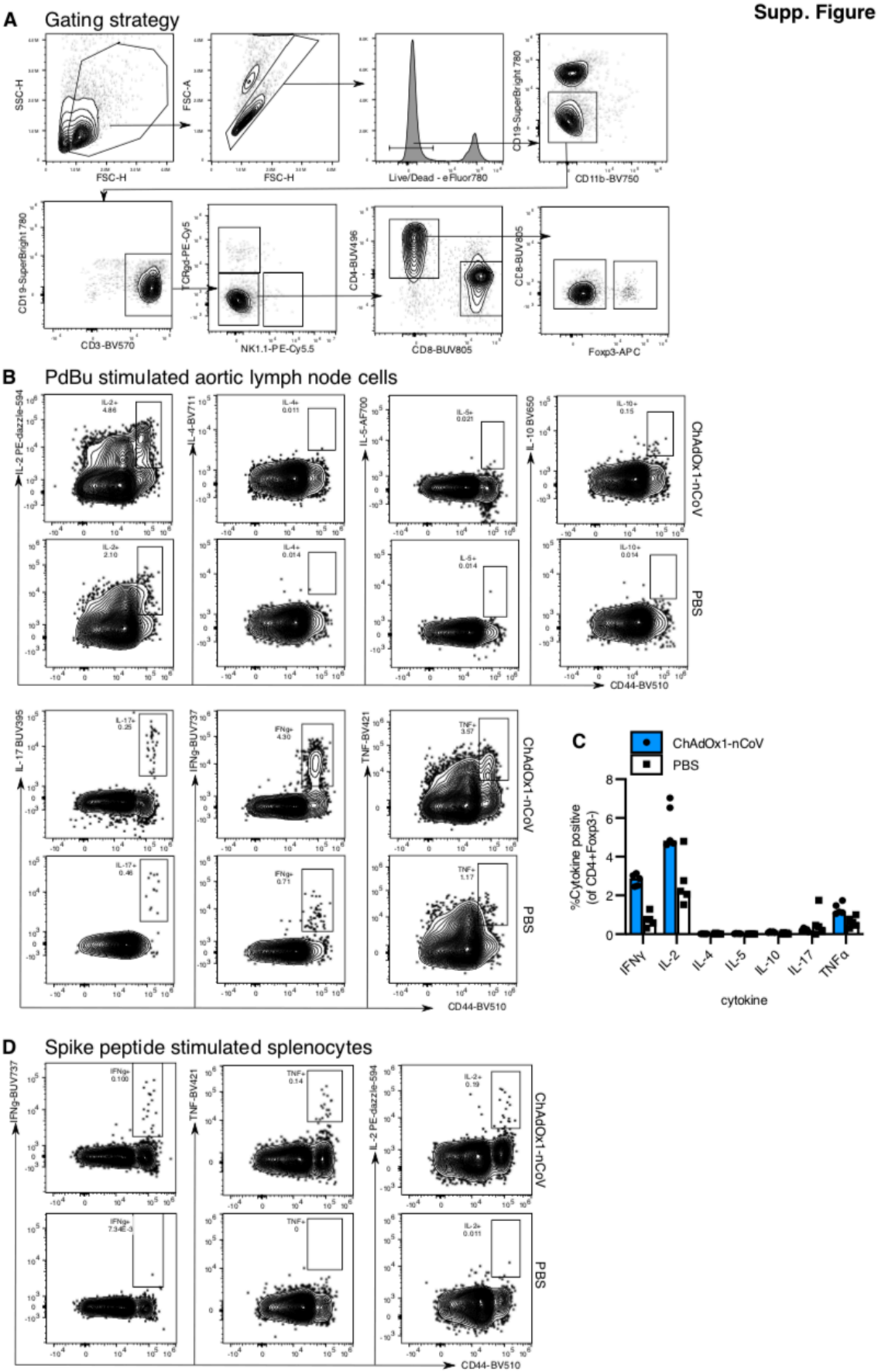

A
DAPI FluoSpheres 505/515
iLN
Spleen
B
Mx1 mRNA normalised to Hprt (Quadriceps muscle)
Gbp2 mRNA normalised to Hprt (Quadriceps muscle)
nCoV
PBS
<0.0001
0.0146
0.3252
Day 1
Day 2
C
CD86 BV510
CD40 PE-Cy7
75.6% ± 8.1
21.2% ± 3.6
75.3% ± 6.4
1.52% ± 0.8
SSC-A
FSC-A
SSC-W
Live/Dead
CD11c-APCeF780
CD11c+ Macrophages
F4/80-PE
XCR1-BV650
cDC1
CD172a-PercpeF710
CD24-BV605
Langerhans
cDC2
91.1% ± 3.2
32.6% ± 2.1
CD64-A647
CD19/CD3-BUV395
MHC-II-A700
78.6% ± 4.3
24.8% ± 11.9
D
iLN Macrophages
iLN cDC2
iLN Langerhans
iLN cDC1
#CD86+ CD40+ (CD11c+F4/80+CD64+)
#CD86+ CD40+ (CD24- CD11c+)
#CD86+ CD40+ (CD24+ CD11c+)
#CD86+ CD40+ (XCR1+ CD172a-)
0.7943
0.0035
0.0010
0.8766
0.7530
0.0384
0.9960
0.9880
0.1792
0.0189
0.1681
0.0981
E
Spleen Macrophages
Spleen cDC2
Spleen Langerhans
Spleen cDC1
#CD86+ CD40+ (CD11c+F4/80+CD64+)
#CD86+ CD40+ (CD24- CD11c+)
#CD86+ CD40+ (CD24+ CD11c+)
#CD86+ CD40+ (XCR1+ CD172a-)
0.0279
0.9503
0.0223
0.2363
0.0270
0.0007
0.0546
>0.9999
0.0113
0.0279
0.9503
0.0223

Supplementary Figure 1. Intramuscular immunisation results in the activation of antigen presenting cells in the iLN and spleen upon immunisation with ChAdOx1 nCoV-19. Related to figures 1-3. A. Representative immunofluorescence confocal images of DAPI expression and FluoSpheres^{TM} (505/515) localisation in the iLN (top) and spleen (bottom) of mice immunised with yellow-green 20nm fluorescent FluoSpheres^{TM} (left) or PBS (right) at 24hr post intramuscular immunisation. Scale bars, 100μM for both iLN and spleen. Data are representative of two independent experiments (n=2-5 per group/experiment). **B.** Expression of *Mx1* (top) and *Gbp2* (bottom) in the quadriceps muscle of mice immunised with ChAdOx1 nCoV or PBS at one- and two-days post immunisation, as determined by RT-qPCR. **c.** Gating strategy used for the identification of antigen presenting cell (APC) populations in the iLN and spleen of mice immunised with ChAdOx1 nCoV-19 or PBS at one- and two-days post immunisation. Macrophages were defined as $CD11c^{+}F4/80^{+}CD64^{+}$ cells. cDC1s were defined as $XCR1^{+}CD172a^{-}CD11c^{+}MHC\text{-}II^{+}CD3/CD19^{-}CD64^{-}F4/80^{-}$ cells. cDC2s $CD24^{-}CD172a^{+}XCR1^{-}CD11c^{+}MHC\text{-}II^{+}CD3/CD19^{-}CD64^{-}F4/80^{-}$ cells. Langerhans cells were defined as $CD24^{+}CD11c^{+}CD172a^{+}XCR1^{-}CD11c^{+}MHC\text{-}II^{+}CD3/CD19^{-}CD64^{-}F4/80^{-}$ cells. Activated cells within each of these populations were identified as $CD86^{+}CD40^{+}$. These flow cytometry plots are representative of cells in the iLN of mice immunised with ChAdOx1 nCoV-19 (blue) or PBS (grey) at two-days post immunization, and indicate the median as a percentage as well as the standard deviation. **D-E.** Quantification of the absolute number of $CD86^{+}CD40^{+}$ cells for each APC population in the iLN (**D**) and in the spleen (**E**). Bar height in **D-E** corresponds to the mean and each circle represents one biological replicate. P-values were determined using a Shapiro-Wilk normality test followed by either an ordinary one-way ANOVA test for data with a normal distribution or a Kruskal Wallis test for non-normally distributed data alongside a multiple comparisons test. Data are representative of two independent experiments (n=4-5 per group/experiment).

Supp. figure 2

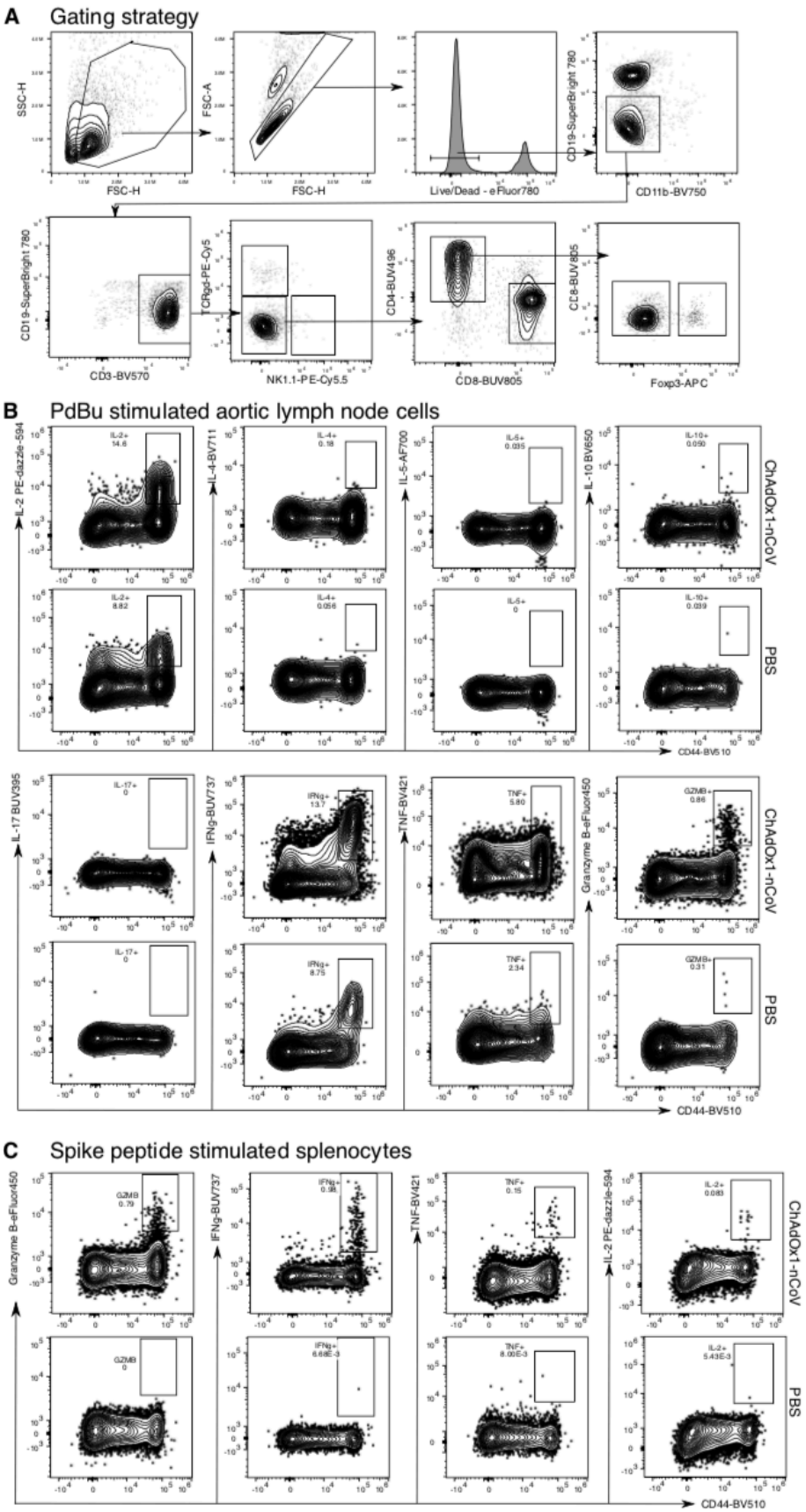


Supplementary Figure 2. Gating strategy for ex vivo lymphocyte analyses. Related to figures 1-3. Representative flow cytometric contour plots showing the gating strategy for B cell subsets (**A**), $CD4^{+}Foxp3^{-}$ T cell subsets (**B**), Treg cell subsets (**C**) and CD8 cell subsets (**D**). Samples are taken from an iliac lymph node seven days after ChAdOx1 nCoV-19 immunisation.

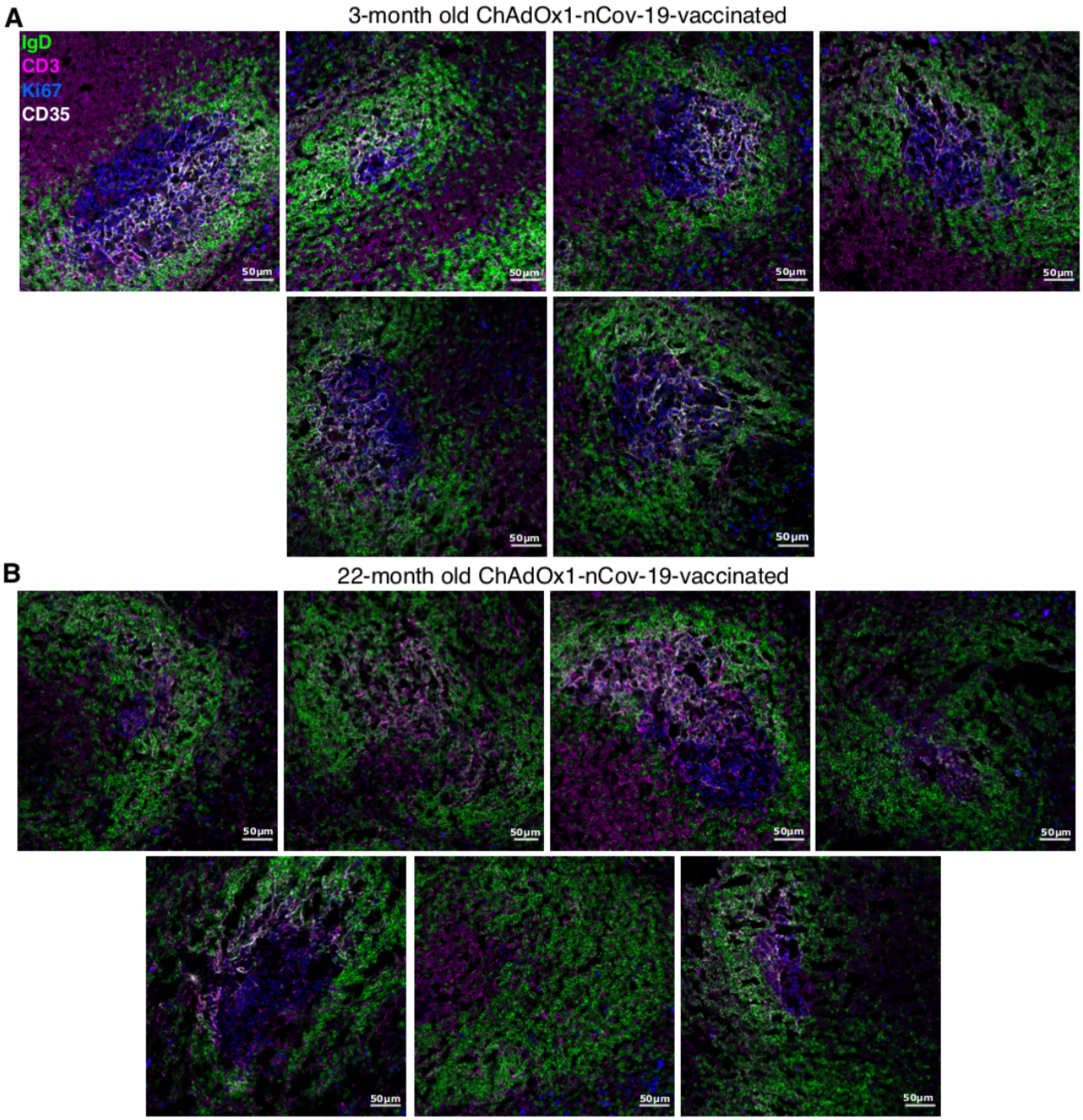


Supplementary Figure 3. Gating strategy CD4 T cell cytokine staining. Related to figures 2 and 5. Flow cytometric contour plots showing the gating strategy to identify T cell subsets (**A**), cytokine production in $CD4^{+}Foxp3^{-}$ iliac lymph node T cells six hours after PdBu/Ionomycin restimulation (**B, C**), and in SARS-CoV-2 peptide restimulated $CD4^{+}Foxp3^{-}$ splenocytes (**D**).

Supp. Figure 4



Supplementary Figure 4. Gating strategy CD8 T cell cytokine and granzyme B staining. Related to figures 3 and 4. Flow cytometric contour plots showing the gating strategy to identify T cell subsets (**A**), cytokine and granzyme B production in CD8$^+$ iliac lymph node T cells six hours after PdBu/Ionomycin restimulation (**B**), and in SARS-CoV-2 peptide restimulated CD8$^+$ splenocytes (**C**).



Supplementary Figure 5. Germinal centre images nine days after ChAdOx1 nCoV-19 immunisation in adult and aged mice. Related to figure 6.
Confocal images of the spleen of ChAdOx1 nCoV-19 immunised 3-month-old (**A**) and 22-month-old (**B**) mice. 10 µm spleen sections were stained with anti-IgD (green), anti-CD3 (pink), anti-Ki67 (blue) and anti-CD35 (white) antibodies. Each image is from a different mouse. The scale bars indicate 50µm.

Supplementary methods

Related to star methods and supplementary figure 1.

Flow cytometry of dendritic cells

For dendritic cell analysis, secondary lymphoid tissues were harvested and incubated with 10 mg/ml Collagenase D (Roche #11088866001) in plain Roswell Park Memorial Institute (RPMI) (Gibco #11875093) for 30-45 min at 37 °C, followed by gentle pipetting to disrupt the tissue. Cells were washed with PBS containing 2% FBS, before cell numbers were determined using a CASY® TT Cell Counter (Roche). 4×106 cells were transferred to 96-well plates for antibody staining. After a wash with PBS, cells were stained with Live/Dead Fixable Blue Dead Cell Stain (Invitrogen #L23105; diluted 1:1000 in PBS) on ice for 10min after which they were washed in 2% FBS in PBS and treated with unlabelled anti-CD16/32 (eBioscience #14-0161-82; diluted 1:100 in 2% FBS in PBS) for 20min at 4ºC. Surface antibody staining was then performed for 1hr at 4ºC in Brilliant Stain Buffer (BD Biosciences #563794). Samples were acquired on an LSRFortessa 5 with stained UltraComp eBeads Compensation Beads (Invitrogen #01-2222-41) as compensation controls.

RNA isolation and quantitative Real-Time PCR

The right quadriceps muscle was dissected and weighed. The muscle tissue was snap frozen in liquid nitrogen and homogenized in 2mL TRIzol reagent (Thermo Fisher Scientific #15596026), then 1 mL of the homogenized muscle in TRIzol was processed following the manufacturer's instructions. RNA concentrations obtained from the RNA isolation were measured using the NanoDrop system (Thermo Fisher Scientific). Real time quantitative polymerase chain reaction (RT-qPCR) was performed using TaqMan Gene Expression Assays for *Mx1* (Mm 00487796_m1), *Gbp2* (Mm 00494576_g1) and *Hprt* (Mm 03024075_m1) directly on RNA using Thermo Fisher Scientific's TaqMan RNA-to-CT 1-Step Kit (#4392656) following the manufacturer's protocol. All RT-qPCR reactions were assembled in 384-well plates (Bio-Rad #HSP3805), adding 10ng of template RNA per reaction to 8µl of a master mix containing the appropriate TaqMan Gene Expression Assay. All samples were run in triplicates on a BioRad CFX384 Real-Time System. The $2^{-\Delta\Delta Ct}$-method was applied for relative quantification of mRNA levels. Samples from PBS immunised mice were used as calibrators. Cq values were exported from the CFX Manager software (Bio-rad).