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Supplemental information

Tfh cells and the germinal center are required for memory B cell formation & humoral immunity after ChAdOx1 nCoV-19 vaccination

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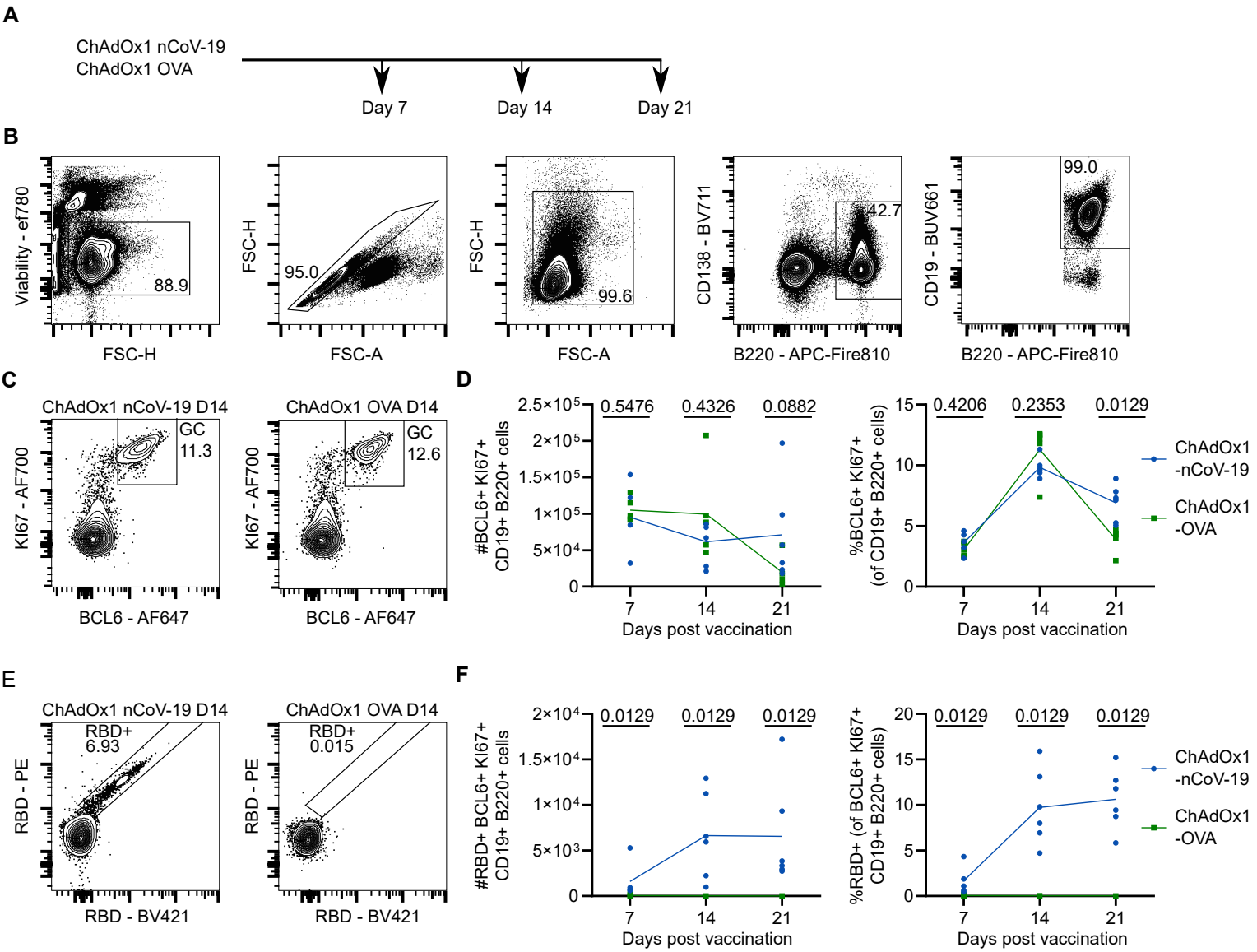
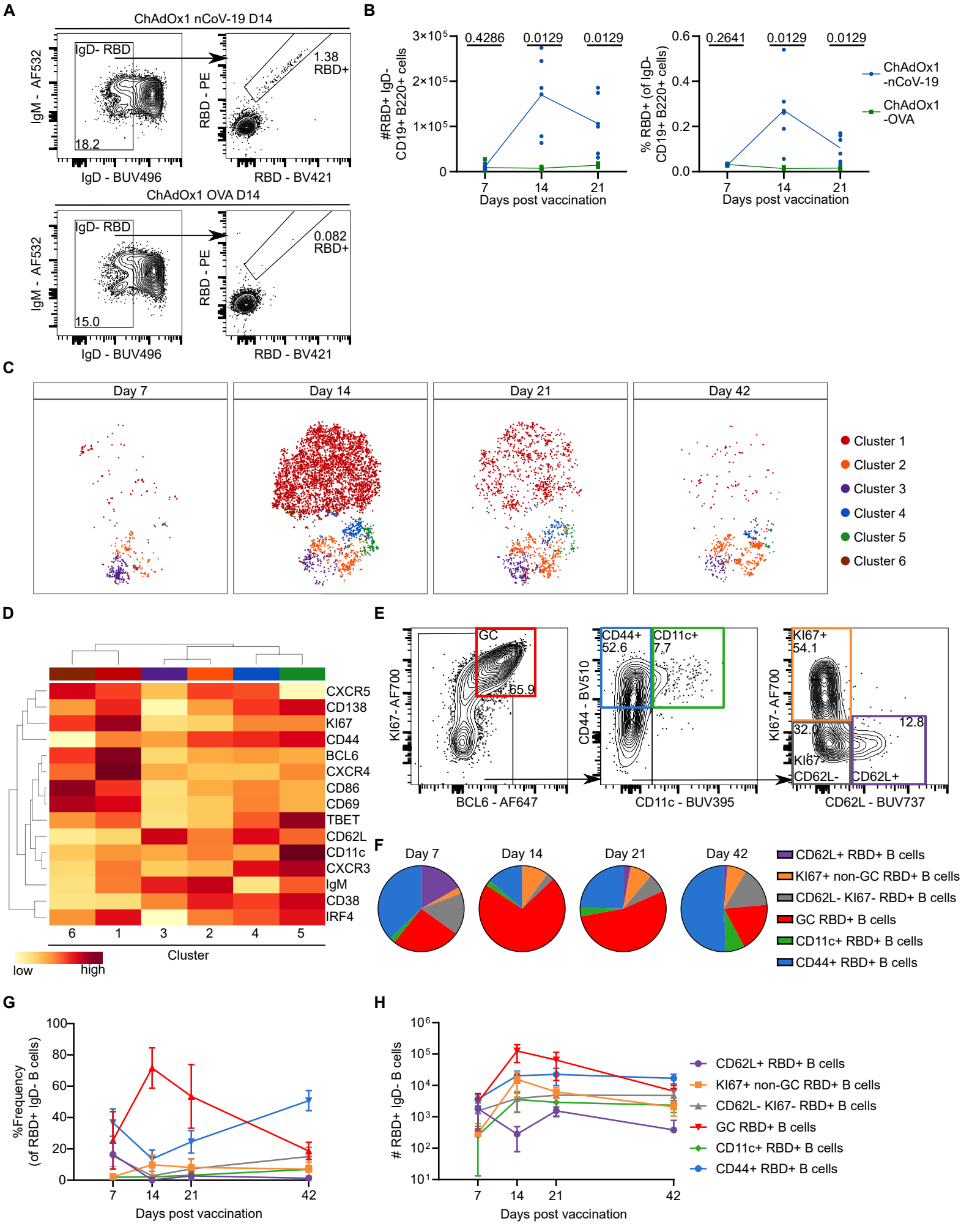
Supplementary Figure 1



Supplementary Figure 1 - ChAdOx1 nCoV-19 elicits an RBD-specific GC response. Related to Figure 1.

(A) Mice were immunised with 50µL of either ChAdOx1 nCoV-19 or ChAdOx1 OVA intramuscularly, with mILN being analysed at indicated timepoints. **(B)** Median flow cytometry gating to define live, single, B cells. **(C)** Median flow cytometry plots for GC B cell staining, pre-gated on live, single, CD19+ B220+ cells. **(D)** Total number and relative frequency of GC B cells after immunisation. **(E)** Median flow cytometry plots for RBD+ GC B cells, pre-gated on live single, CD19+ B220+, BCL6+ KI67+ cells. **(F)** Total number and relative frequency of RBD+ GC B cells after immunisation. For each timepoint and condition, n=5 or 6 per group, with each symbol representing a single biological replicate. For (**D and F)** Multiple Mann-Whitney tests per row were used, with P-values corrected for multiple comparison analysis with the Holm-Šídák method. Data representative of two individual experiments.

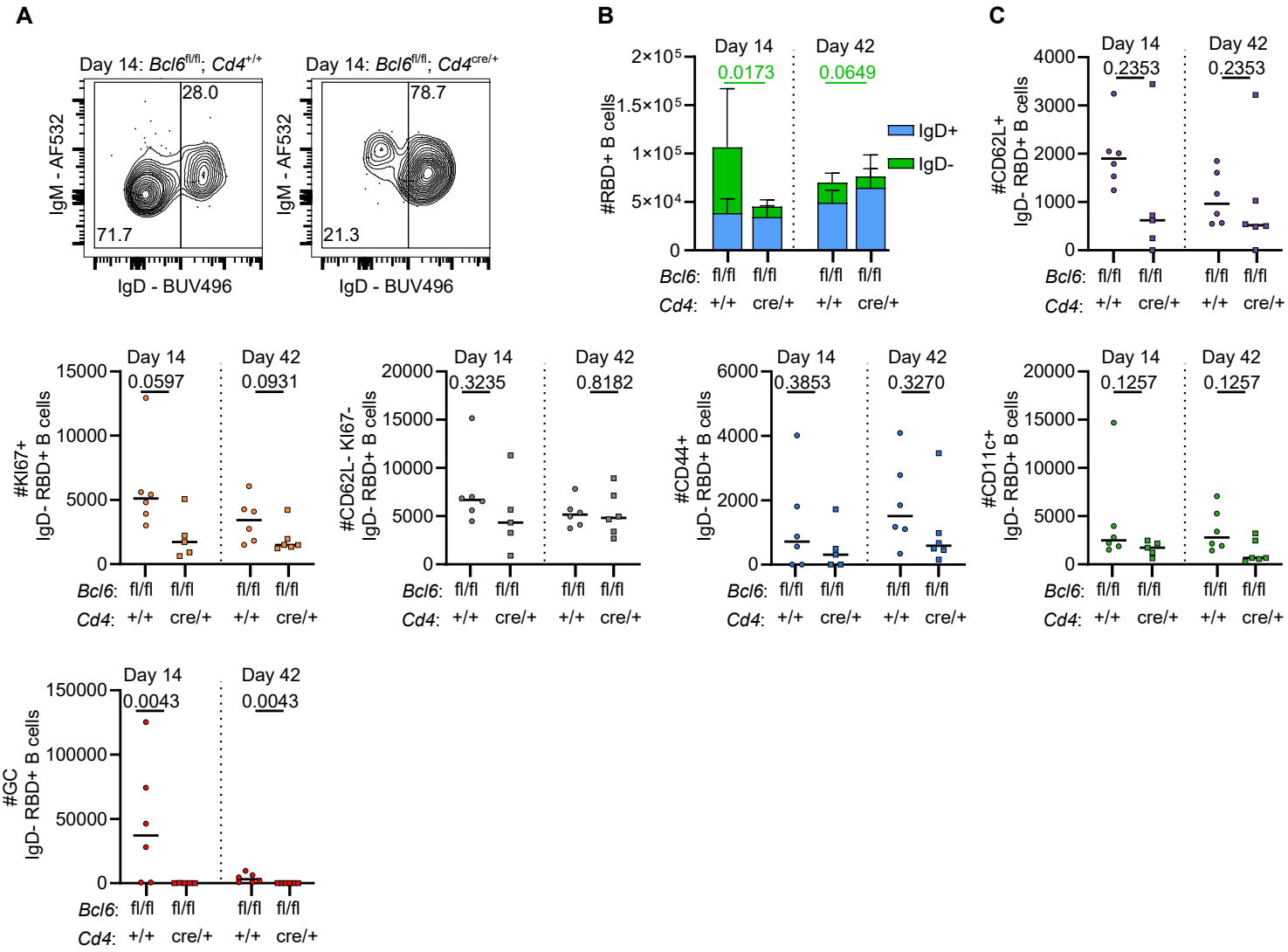
Supplementary Figure 2



Supplementary Figure 2 - ChAdOx1 nCoV-19 immunisation generates a mix of RBD-specific GC and memory B cells in the spleen. Related to Figure 1.

(A) Median flow cytometry plots for IgD- RBD+ Spleen B cell staining, pre-gated on live, single, CD19+ B220+ cells. **(B)** Total number and relative frequency of IgD- RBD+ B cells. **(C)** tSNE analysis of IgD- RBD+ B cells separated by timepoint. FlowSOM analysis was used to identify 6 clusters of cells. **(D)** Heatmap showing mean MFI of each marker used in **C** for clustering analysis. **(E)** Flow cytometry gating of 6 IgD- RBD+ sub-populations based on a concatenated sample of all IgD- RBD+ B cells shown in **C**. **(F)** Pie charts showing relative frequency of sub-populations identified in **E.** for each of the 4 timepoints. **(G-H)** Line graphs showing relative frequency **(G)** and quantification **(H)** of sub-populations identified in **E.** Error bars show mean and standard deviation. For each timepoint and condition, n=5 or 6 per group. For **(B)** Multiple Mann-Whitney tests per row were used, with P-values corrected for multiple comparison analysis with the Holm-Šídák method. Data representative of two individual experiments.

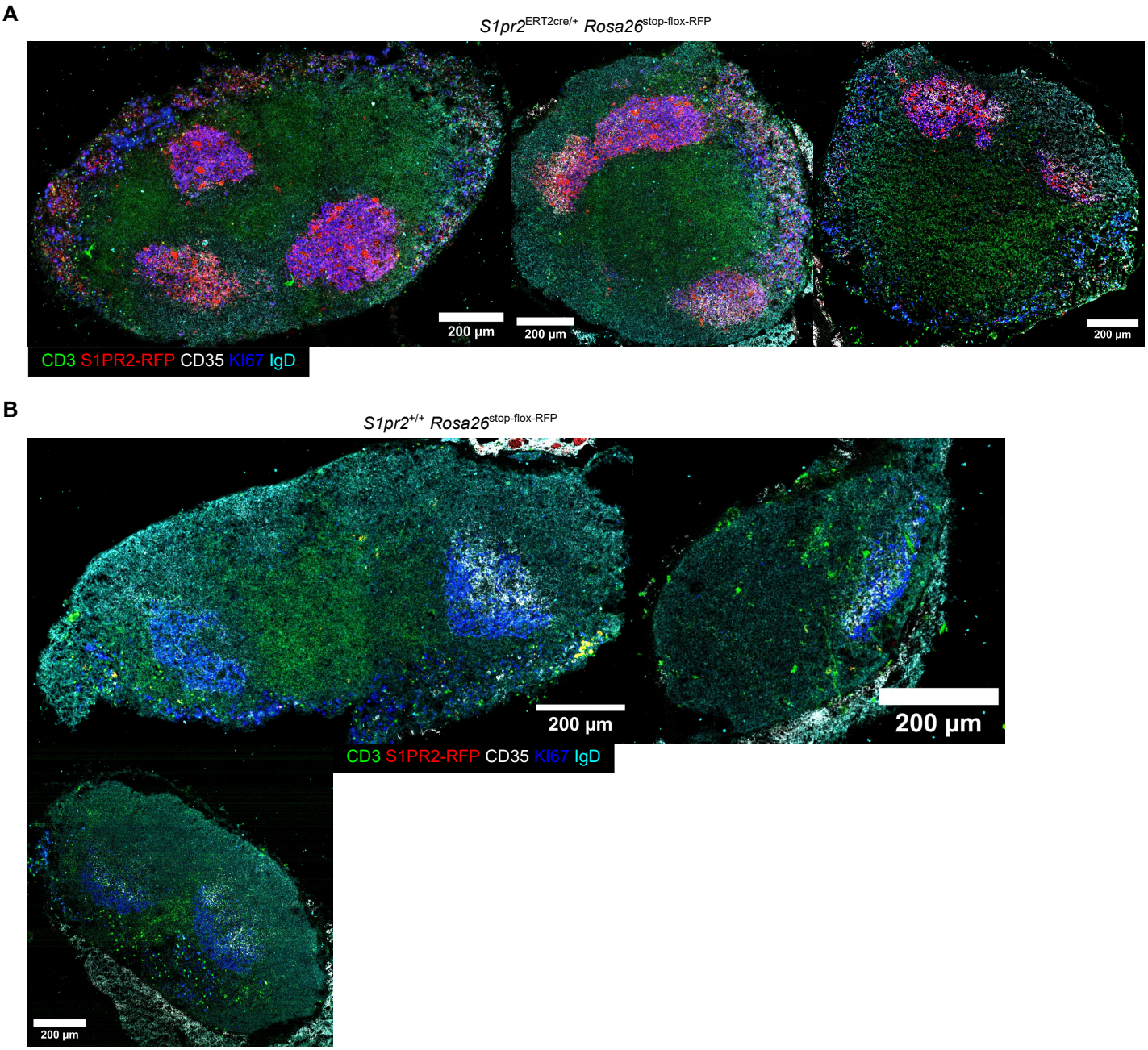
Supplementary Figure 3



Supplementary Figure 3 – The spleen contains Tfh cell independent IgD- RBD+ memory B cells after ChAdOx1 nCoV-19 immunisation. Related to Figure 3.

(A) Day 14 median flow cytometry plots for IgD staining of spleen RBD+ B cells, pre-gated on live, single, CD19+ B220+, RBD+ cells. **(B)** Total number of RBD+ B cells at indicated timepoint. p-value shown is from comparison of the number of IgD-RBD+ cells, bar height shows the mean, and the error bars the standard deviation. **(C)** IgD- RBD+ B cell subsets were enumerated using the gating strategy as shown in Figure 2E. For each timepoint and condition, n=4-6 respectively per group. For **(B and C)** multiple Mann-Whitney tests per row were used, with P-values corrected for multiple comparison analysis with the Holm-Šídák method. In dot plots, each symbol represents a biological replicate and the bar height the mean. Data representative of two individual experiments.

Supplementary Figure 4

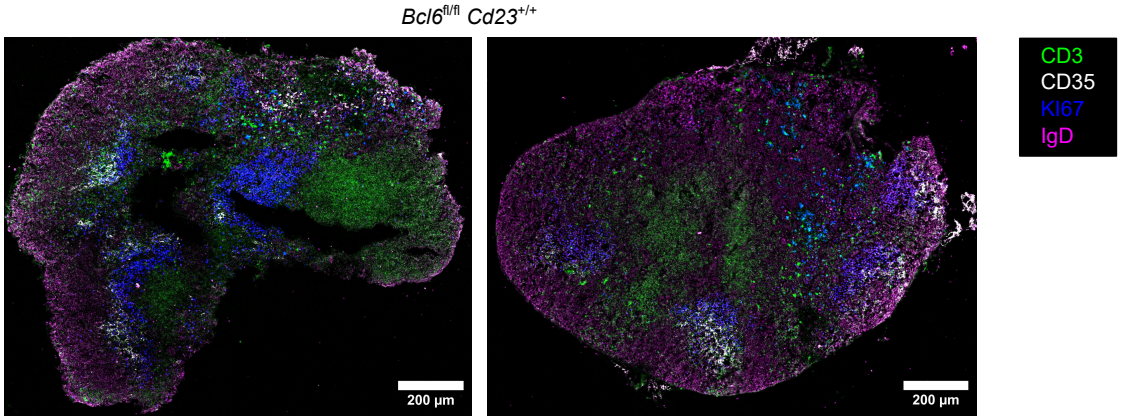


Supplementary Figure 4 – S1PR2-RFP labelled lymphocytes localise to secondary lymphoid follicles. Related to Figure 3.

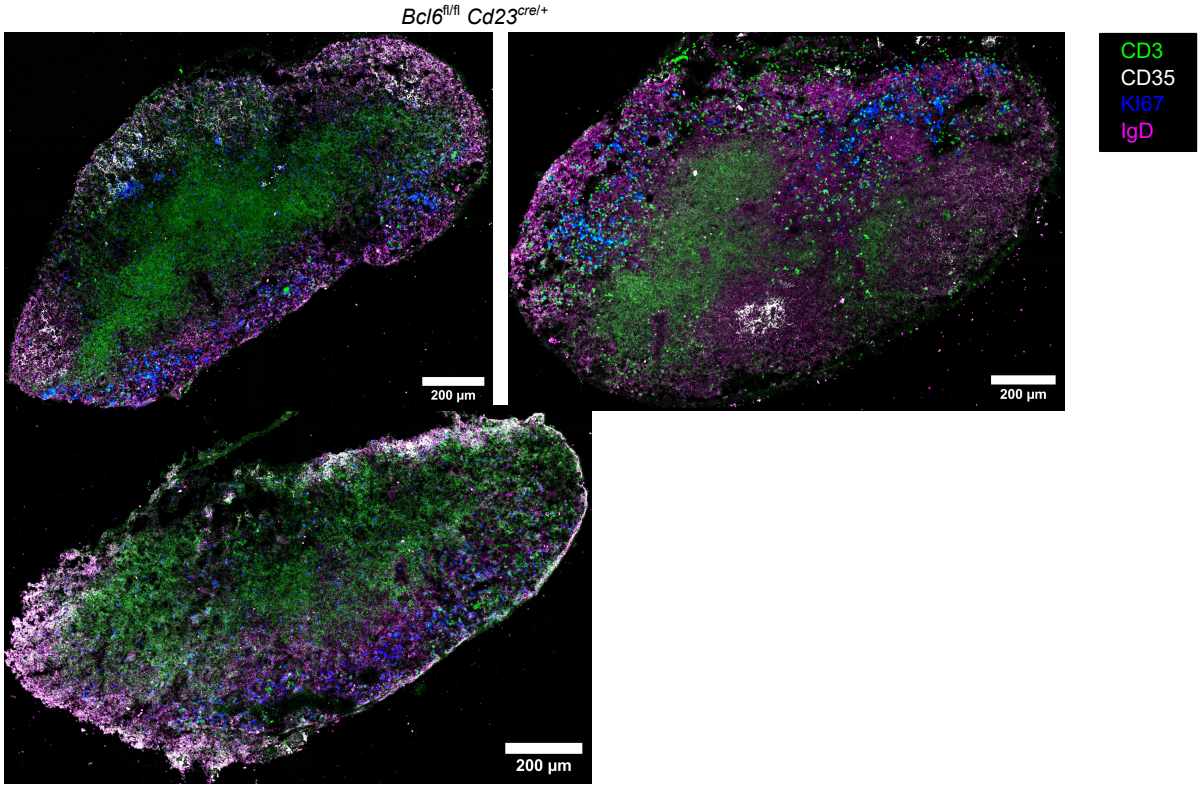
(A) Day 14 confocal microscopy of whole mILNs from *S1pr2*$^{ERT2\text{-}cre}$ *Rosa26*$^{stop\text{-}flox\text{-}RFP}$ mice. **(B)** Day 14 confocal microscopy of whole mILNs from *S1pr2*$^{+/+}$ *Rosa26*$^{stop\text{-}flox\text{-}RFP}$ mice.

Supplementary Figure 5

A



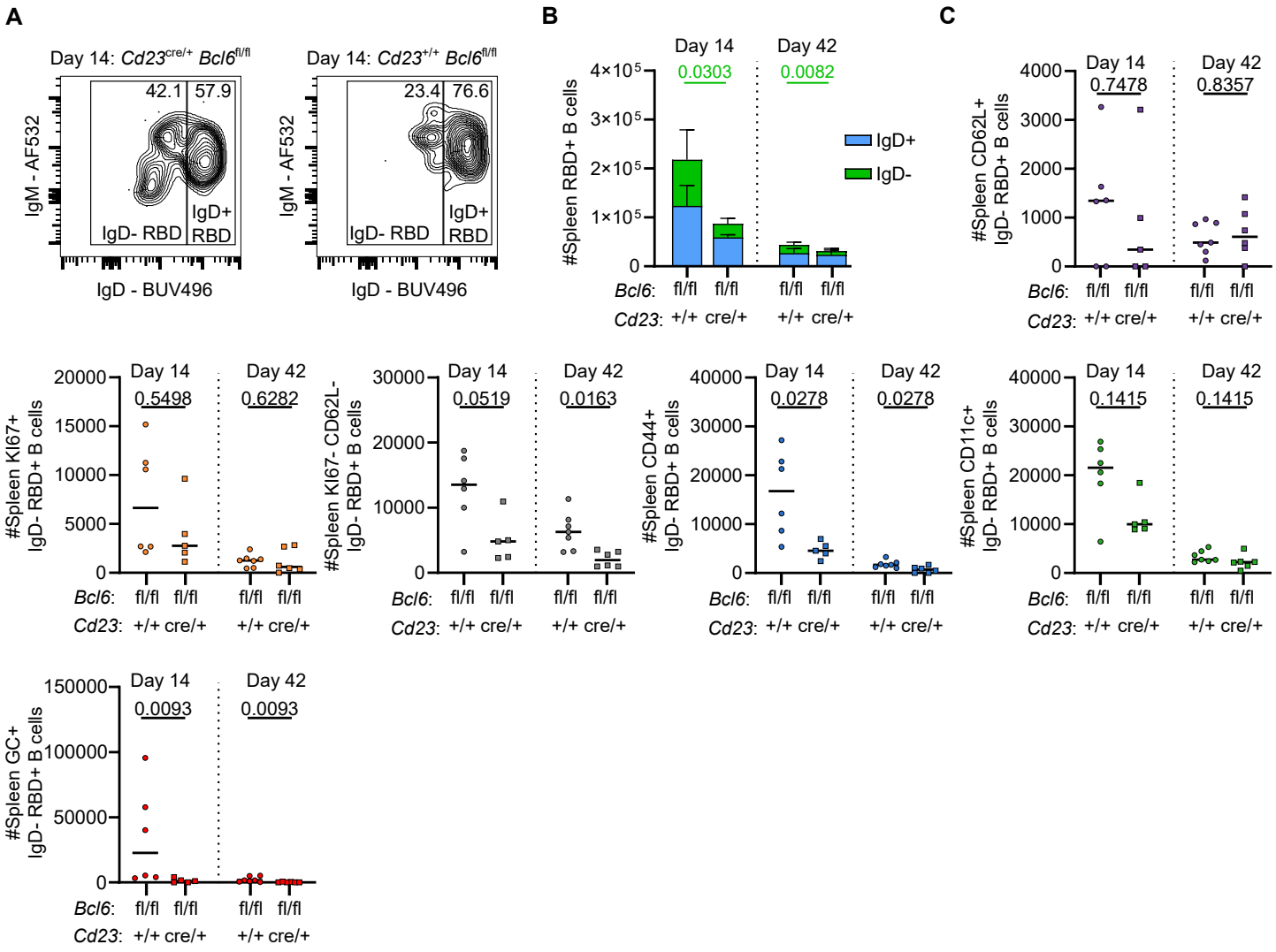
B



Supplementary Figure 5 - *Cd23*$^{cre/+}$; *Bcl6*$^{fl/fl}$ mice have disorganised lymph node secondary follicles. Related to Figure 5.

(A) Day 14 confocal microscopy of whole mILNs from *Cd23*$^{+/+}$; *Bcl6*$^{fl/fl}$ mice. **(B)** Day 14 confocal microscopy of whole mILNs from *Cd23*$^{cre/+}$; *Bcl6*$^{fl/fl}$ mice.

Supplementary Figure 6



Supplementary Figure 6 – GC B cells are required for splenic CD44+ RBD-specific memory B cell formation. Related to Figure 6.

(A) Day 14 median flow cytometry plots for IgD staining of spleen RBD+ B cells, pre-gated on live, single, CD19+ B220+, RBD+ cells. **(B)** Total number of RBD+ B cells at indicated timepoint, p-value shown is from comparison of the number of IgD- RBD+ cells, bar height shows the mean, and the error bars the standard deviation. **(C)** IgD- RBD+ B cell subsets were enumerated using the gating strategy as shown in Supplementary Figure 2E. For **(B and C)** multiple Mann-Whitney tests per row were used, with P-values corrected for multiple comparison analysis with the Holm-Šídák method. In dot plots, each symbol represents a biological replicate and the bar height the mean. Data representative of two individual experiments.