

nucleocapsid proteins from SARS-CoV and SARS-CoV2 and a mutant variant from SARS-CoV2 (pos 256-261 KKPRQK→GGPRQG) (SEQ ID NOs 118 and 119) to improve protein stability were generated (**Table 1**). The complete extracellular regions of the spike protein, as well as S1 and S1B domains were generated. The full-length spike protein contained mutations to prevent cleavage between S1 and S2 (pos 682-685 RRAR→SGAG) (SEQ ID NOs 120 and 121) (Walls *et al.* (2020) Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein, Cell 181, 281-292) and to improve stability of the S2 domain (986-987 KV→PP) (SEQ ID NOs 120 and 121) (**Table 1**).

The detection of recombinant nucleocapsid and S1B proteins from SARS-CoV2 is shown in **Figure 13**. As shown in **Figure 13A**, western blots using an anti-His detection antibody of nucleocapsid (left panel) and S1B (right panel). Loaded are culture supernatants of sf21 cells after enrichment for 6His-tag containing proteins on a cobalt-loaded retention column. Calculated molecular weights of reduced proteins are ~48kDa (N) and ~30kDa (S1B). Abbreviations: NR, non-reduced; R, reduced. **Figure 13B** shows serum IgG specific to antigens as determined by ELISA using the antigen to capture. Thirty-six historic samples (sampled in 2019 and Q1 2020) of previously influenza-vaccinated healthy adults were run, as well as serum samples from 4-20 individuals after with COVID-19 or after recovery (convalescent). Statistics, Mann-Whitney U test.

The detection of B-cells with surface Ig specific for SARS-CoV2 nucleocapsid protein in a patient after recovery from COVID-19 is shown in **Figure 14**. Specifically, Figure 14A shows 2D plots showing B cells from an uninfected control stained with BUV395-conjugated and BUV737-conjugated nucleocapsid tetramers (left plot) and BV480-conjugated and BV650-conjugated S1B tetramers. Figure 14B shows 2D plots showing B cells from COVID-19 convalescent patients stained with the same NCP and S1B tetramers, showing well-defined populations for both (left and middle plot), which were mutually exclusive (right plot).

Figure 16 shows the immunophenotyping of COVID19 specific B cells. Stepwise gating of CD3⁺ T cells and CD19⁺ B cells (left panel), followed by double discrimination

of COVID-19 nucleocapsid (NCP) specific B cells, and of COVID-19 S1B specific B cells within the same flow cytometry staining is shown in **Figure 16 B and 16C**. Subsetting of total B cells in the same staining to discriminate unswitched B cells (IgD+) from Ig-class