Supplemental Material for

Activation and Pro-inflammatory Cytokine Production by Unswitched Memory B cells during SARS-CoV-2 infection

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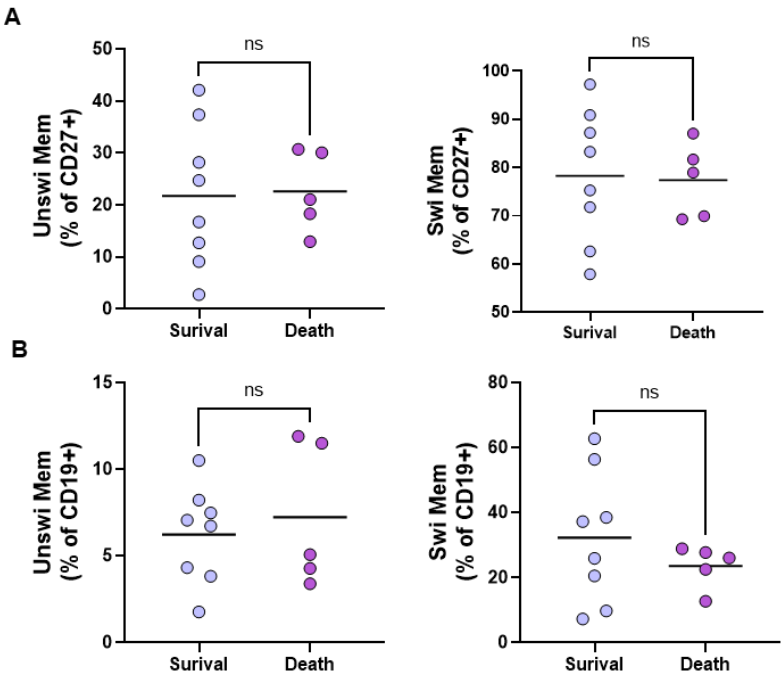
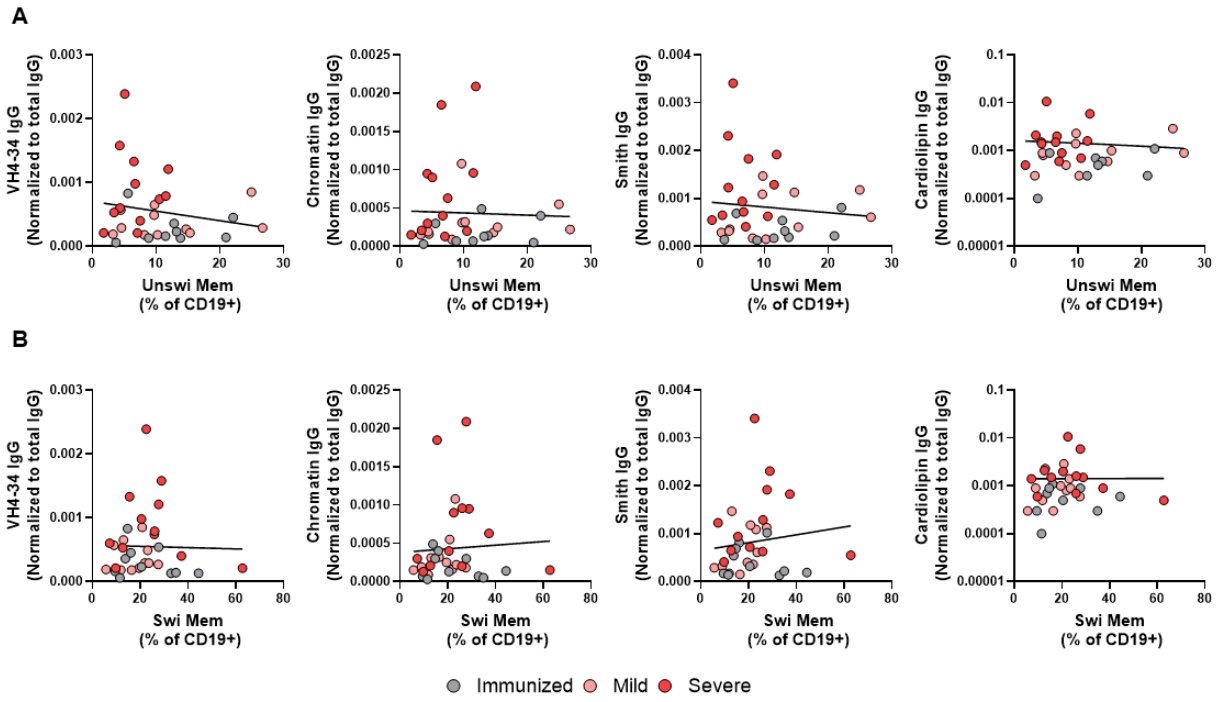
Cohort characteristics					
Parameter	**Measurement**	**Healthy**	**Immunized**	**Convalescent (Mild)**	**Severe**
Age (years)	Average (range)	42.6 (24-70)	54.1 (23-82)	40.2 (23-75)	64 (40-91)
Sex	% F (Sample size)	50 (10)	53.3 (15)	50 (12)	53.3 (15)
Time (days) of blood collection post event					
Primary vaccination	Average (range)		128 (10-535)		
Booster	Average (range)		114 (7-507)		
2nd booster	Average (range)		155 (126-193)		
Positive PCR test	Average (range)			83 (28-305)	
Admittance to hospital	Average (range)				11.2 (2-35)

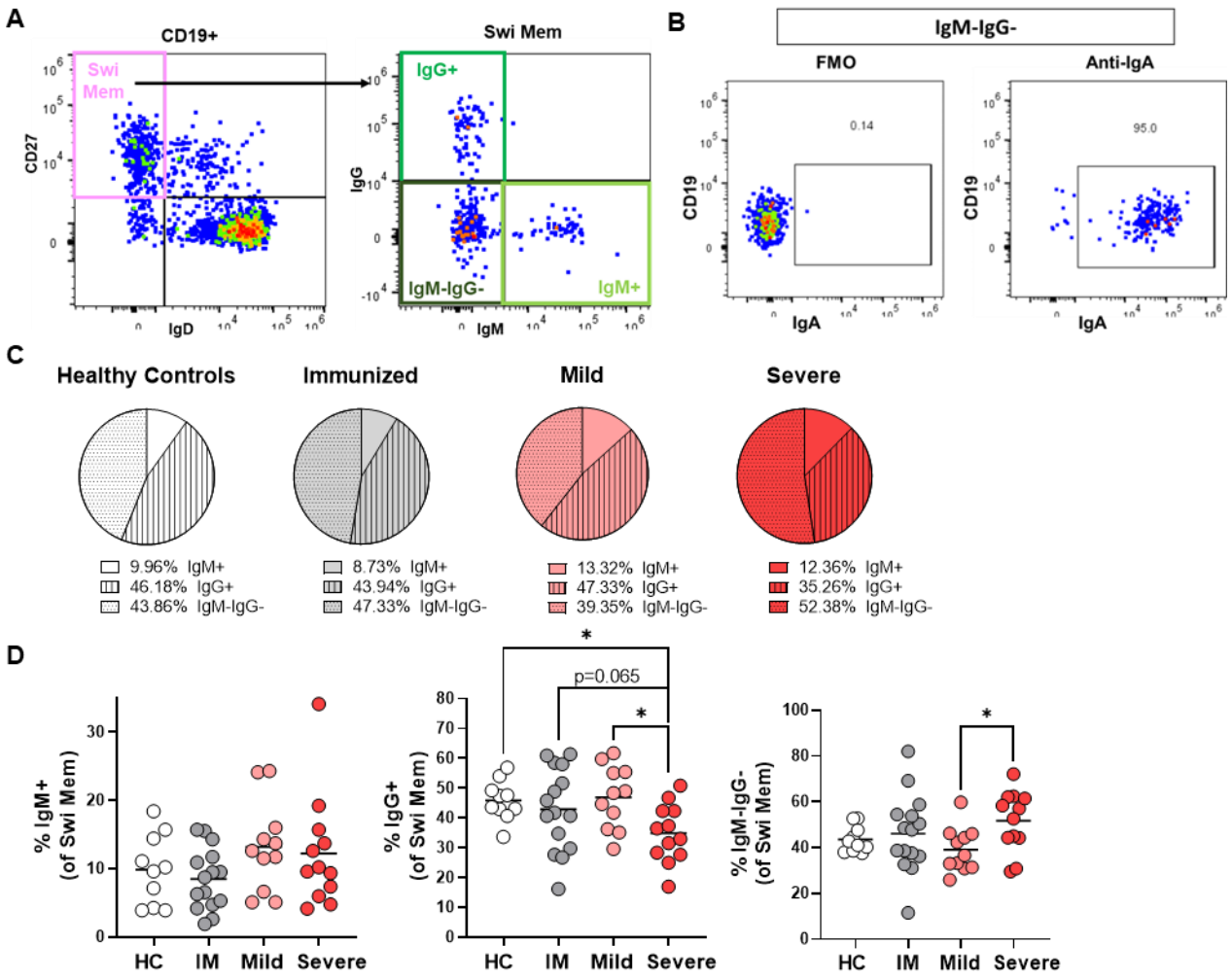
Supplemental Table 1. Demographics of individuals examined in this study. Four cohorts of individuals were examined in this study including; healthy controls, those immunized against SARS-CoV-2 with mRNA platform vaccine, those with mild SARS-CoV-2 infection not requiring hospitalization in the convalescent stage, and those with severe SARS-CoV-2 infection requiring hospitalization.



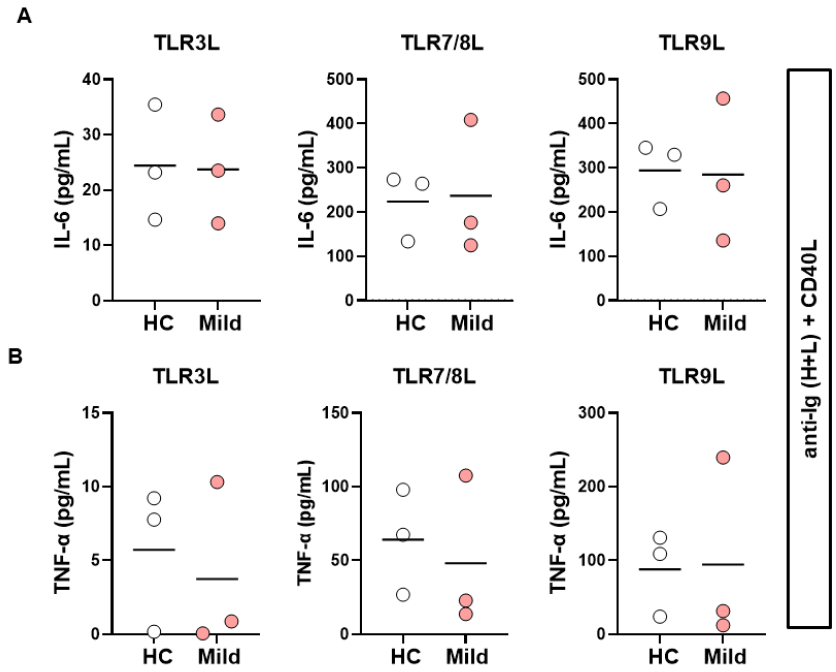
Supplemental Figure 1. Frequency of memory populations do not predict clinical outcome. (A) Frequency of unswitched or switched memory B cells as a percentage of memory (CD27+) or (B) as a percentage of total B cells (CD19+) graphed based on survival or death during hospitalization with severe SARS-CoV-2 infection (N=13). Statistics: unpaired t test, ns not significant



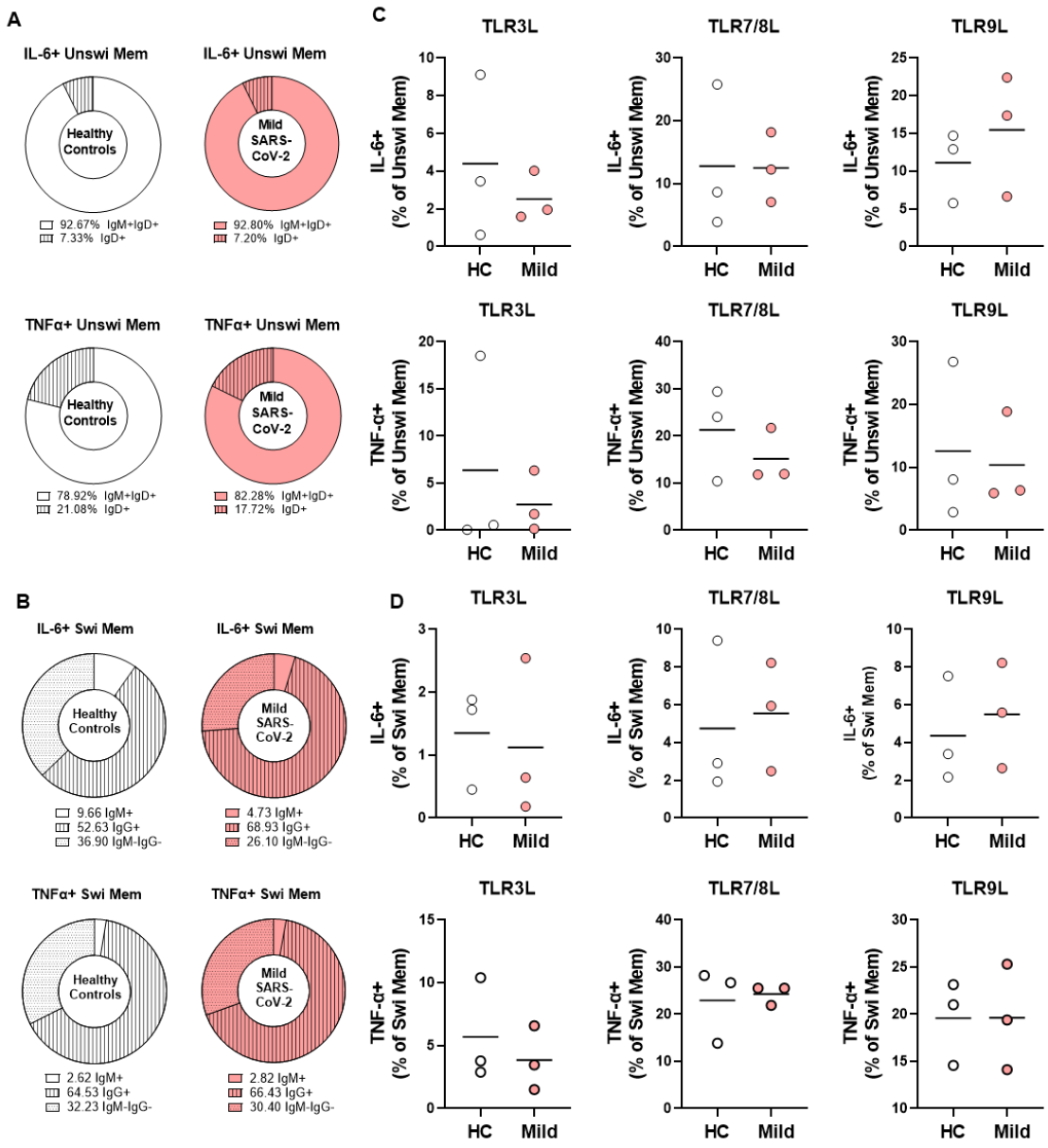
Supplemental Figure 2. Frequency of unswitched or switched memory B cells do not correlate with levels of autoreactive IgG antibodies. (A) Correlation of the frequency of unswitched memory or (B) switched memory B cells with plasma levels of autoreactive IgG antibodies (anti-VH4-34, anti-Chromatin, anti-Smith or anti-Cardiolipin) normalized to total IgG. Statistics: Pearson correlation.



Supplemental Figure 3. Alteration in isotype class of Switched Memory B cells with Severe SARS-CoV-2 infection. (A) Representative flow plot from severe SARS-CoV-2 infection depicting IgM vs IgG gating in switched memory B cells. (B) Representative flow plot from healthy control gated on IgM-IgG- in switched memory B cells showing the majority of this population is IgA+ compared to FMO. (C) Comparison of the average frequency of IgM+, IgG+ or IgM-IgG- cells out of the switched memory compartment (CD27+IgD-) within each cohort. Sample size: Healthy controls (HC; N=10), Immunized controls (IM; N=15), subjects with Mild (N=11) or Severe SARS-CoV-2 infection (N=14). (D) Frequency of IgM+, IgG+ or IgM-IgG- cells within the switched memory population (CD27+ IgD-). Statistics: One-way ANOVA, *$p<0.05$.



Supplemental Figure 4. No difference in levels of IL-6 and TNFα secretion between B cells from healthy controls or mild SARS-CoV-2 infection. (A) B cells were purified from healthy controls (HC, N=3) or subjects with mild SARS-CoV-2 infection (N=3) then stimulated in vitro overnight with 10ug/mL anti-Ig (H+L) F(ab)'2 + 100ng/mL CD40L with either 1ug/mL TL3RL (Poly I:C), 1ug/mL TLR7/8L (R848), or 2.5ug/mL TLR9L (CpG) and secretion of IL-6 or (B) TNFα was measured in cell culture supernatant. Statistics: unpaired t test.



Supplemental Figure 5. Frequency of memory isotype classes and no difference in the frequency of IL-6+ and TNFα+ between memory B cells from healthy controls or mild SARS-CoV-2 infection in response to viral TLR ligands. (A) Average frequency of IgM+IgD+ or IgD-only populations within the IL-6-producing or TNFα-producing unswitched memory B cells or (B) average frequency of IgM+, IgG+, or IgM-IgG- populations within the IL-6-producing or TNFα-producing switched memory B cells after PBMCs from healthy controls (N=3) or subjects with mild SARS-CoV-2 infection (N=3) were stimulated with 50ng/mL PMA+ 1ug/mL Ionomycin or unstimulated control (-) for 4 hours in vitro. (C) PBMCs were stimulated 4hr in vitro with 10ug/mL anti-Ig (H+L) F(ab')2 + 100ng/mL CD40L with either 1ug/mL TL3RL (Poly I:C), 1ug/mL TLR7/8L (R848), or 2.5ug/mL TLR9L (CpG) and frequency of IL-6+ or TNFα+ unswitched or (D) switched memory B cells from healthy controls (HC, N=3) or subjects with mild SARS-CoV-2 infection (N=3) were enumerated. Statistics: unpaired t test.