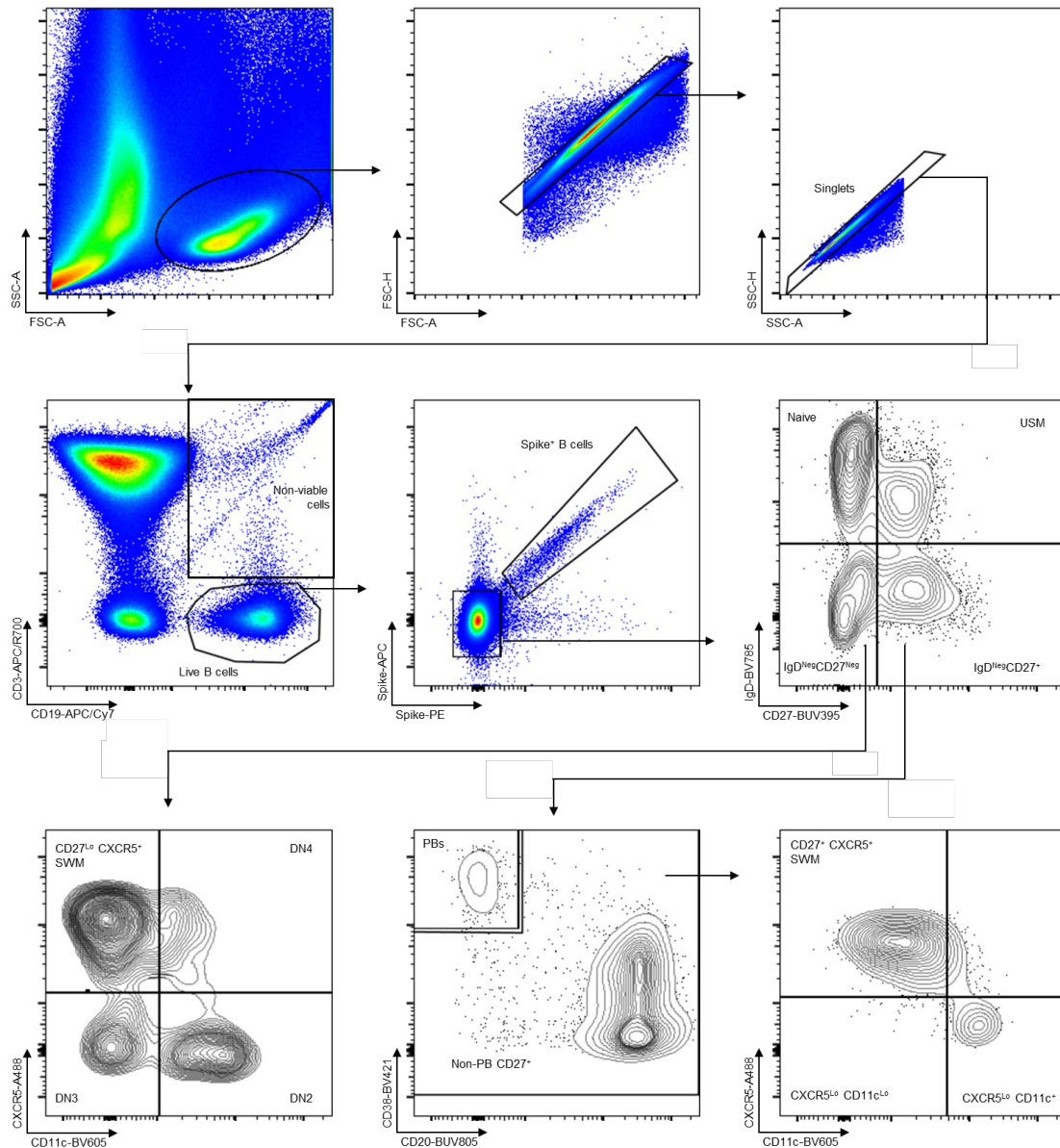
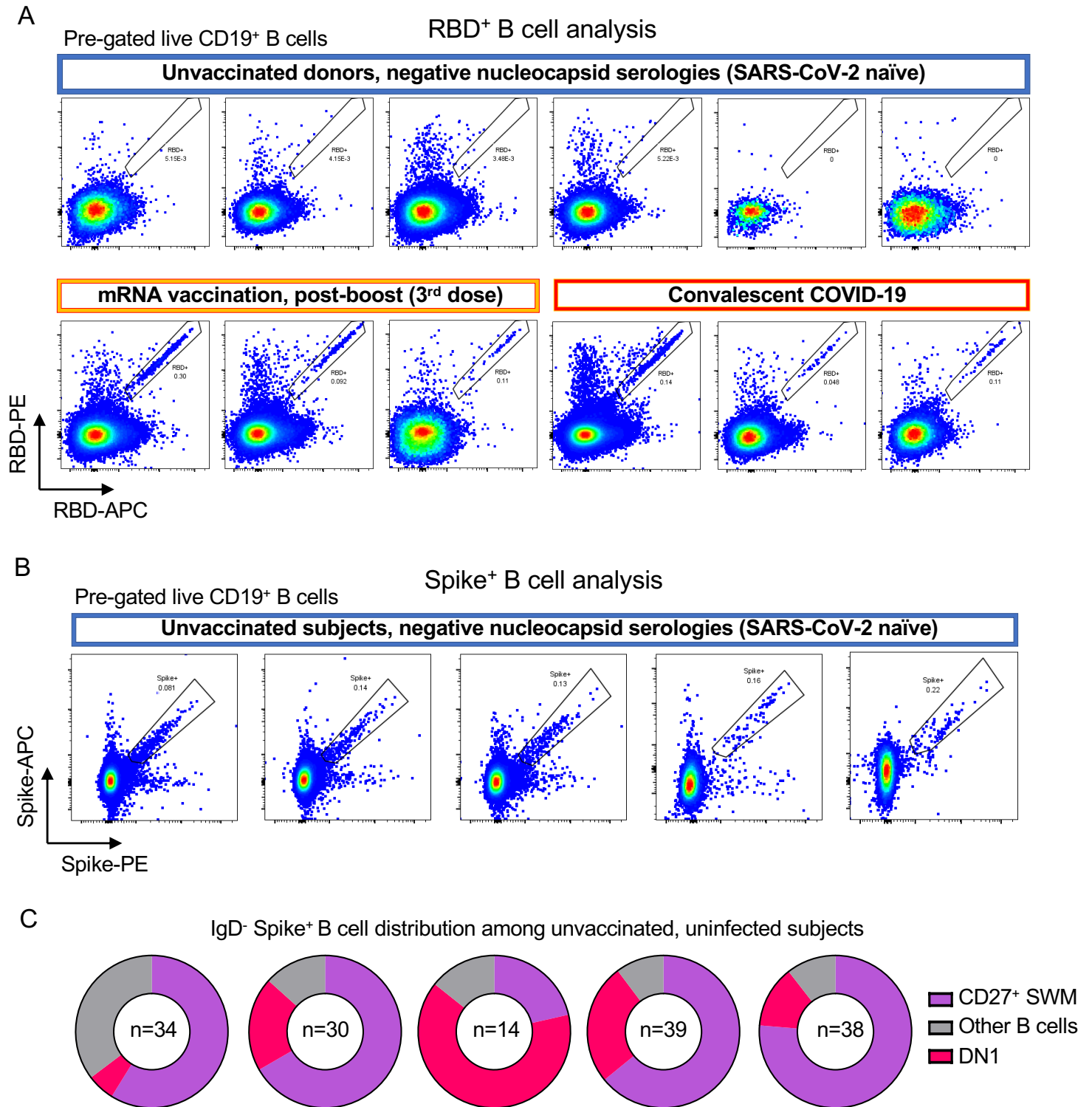


Supplemental Figure 1



Flow cytometry hierarchical gating strategy. Pseudocolor and contour plots displaying hierarchical gating strategy used to quantify B cell subsets. For each sample, gating was defined using total B cells and then applied to antigen-specific B cells.

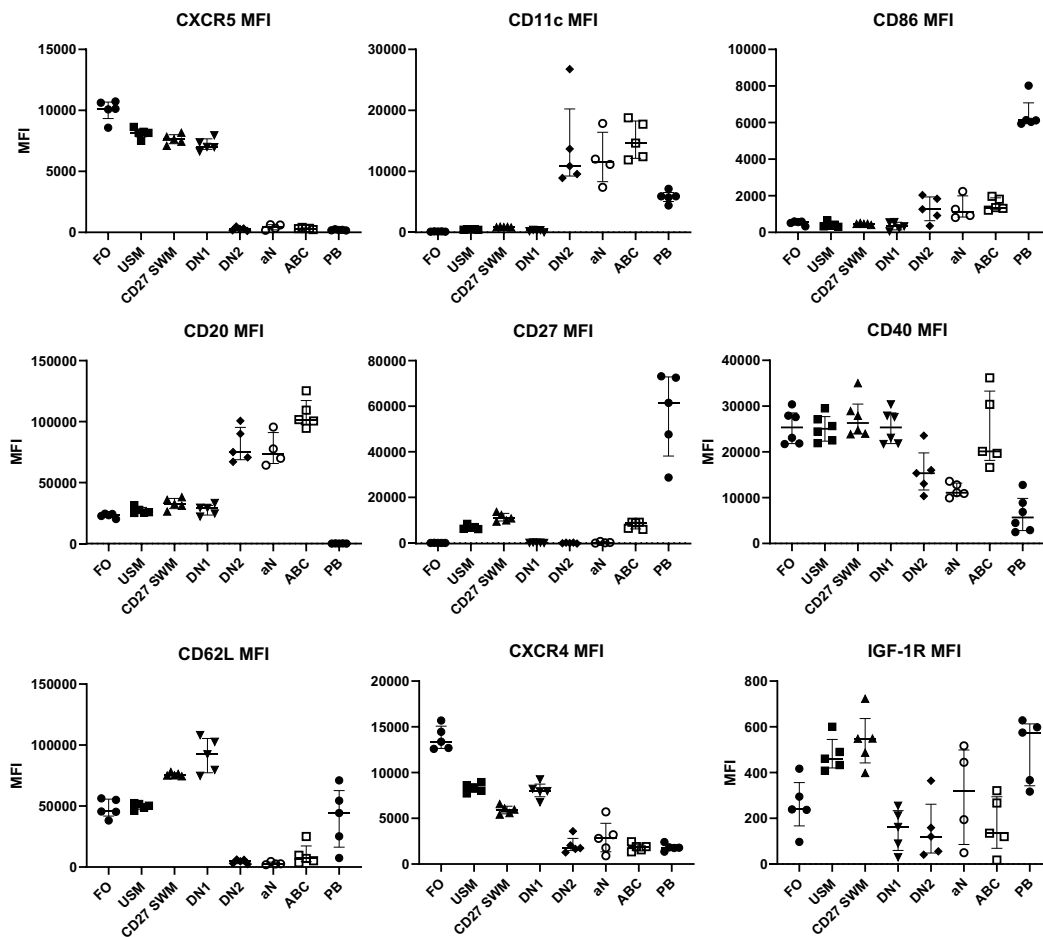
Supplemental Fig 2



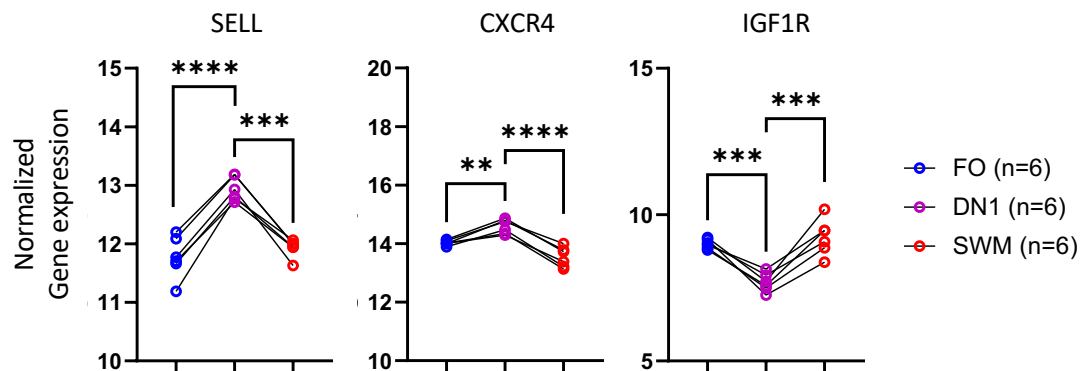
SARS-CoV-2 RBD-specific B cells are absent while CD27⁺ SWM and DN1 B cells dominate spike-specific B cells among SARS-CoV-2 naïve hosts. A) Representative pseudocolor plots of RBD-specific B cells among unvaccinated, SARS-CoV-2 naïve hosts (N=6), mRNA vaccine recipients post-boost (N=3), and convalescent COVID-19 patients (N=3) demonstrating the absence of RBD-specific B cells among SARS-CoV-2 naïve hosts. B) Representative pseudocolor plots of spike-specific B cells among unvaccinated, SARS-CoV-2 naïve hosts (N=6). C) Donut charts displaying the proportion of CD27⁺ SWM and DN1 B cells among total spike-specific B cells from unvaccinated, SARS-CoV-2 naïve hosts (N=6). Each donut chart represents the B cell profile from one individual donor. Numbers provided within each donut chart represent the total number of spike-specific B cells profiled for each donor.

Supplemental Figure 3

A

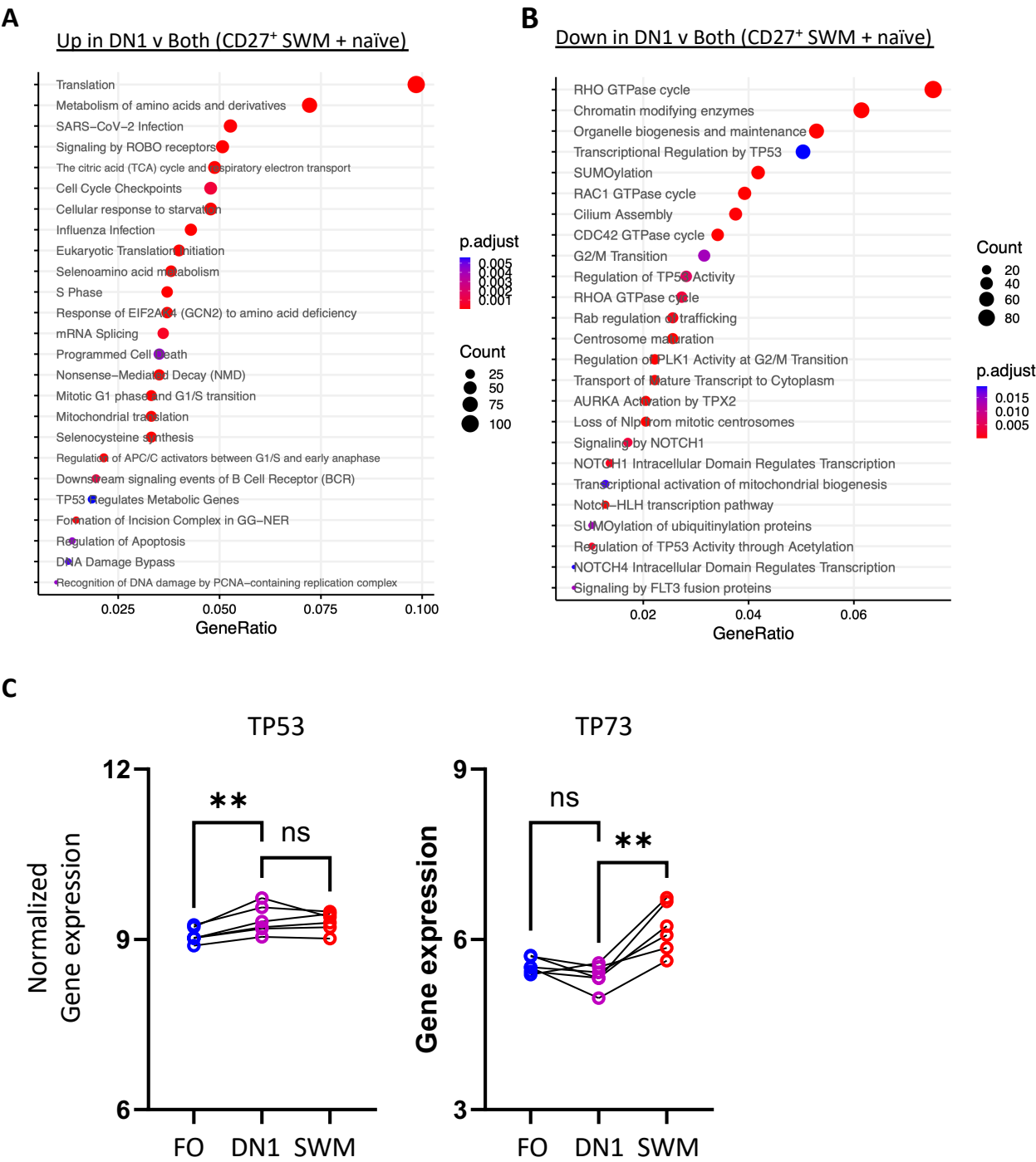


B



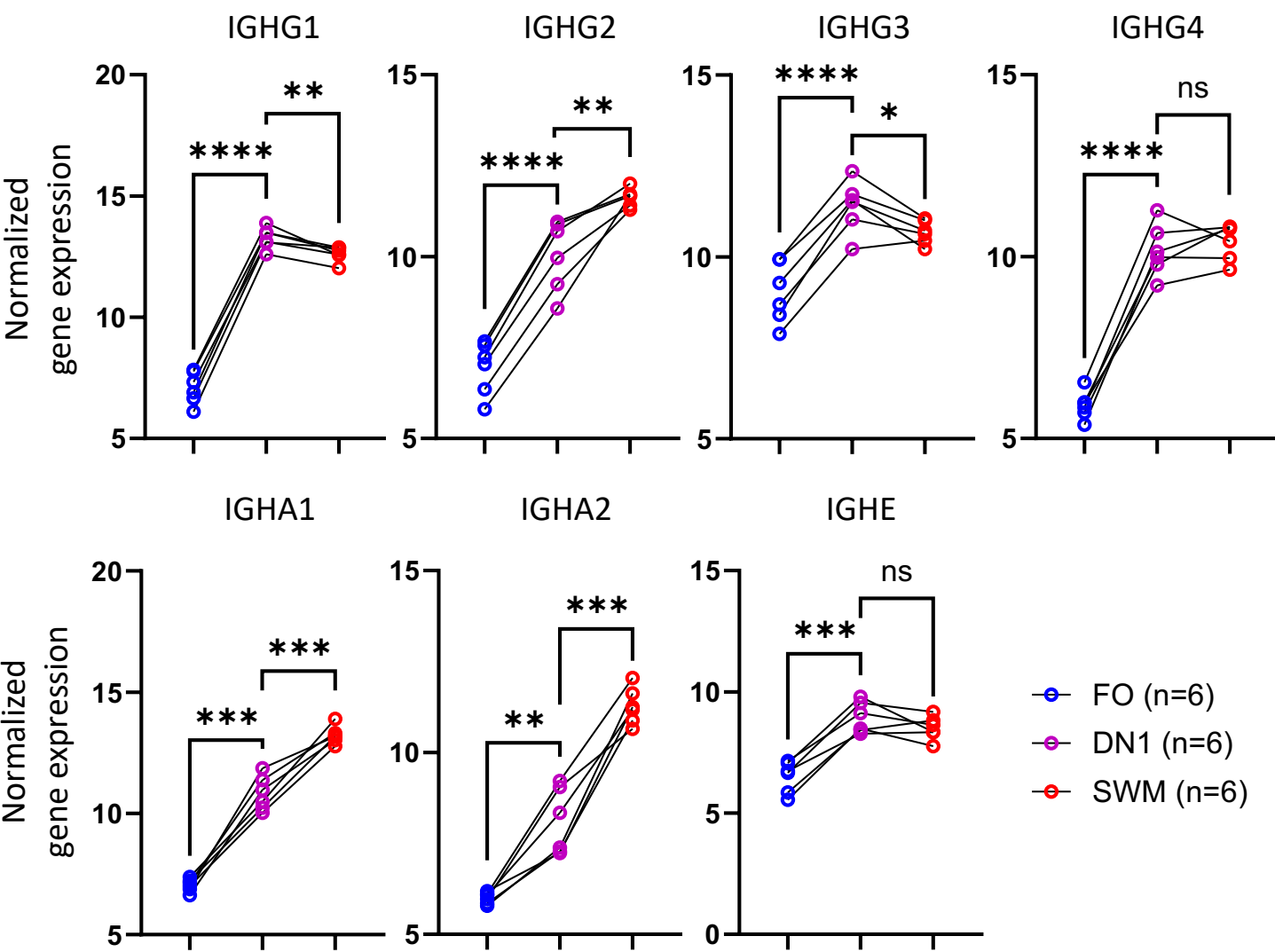
DN1 B cells are distinguished from canonical CD27+ SWM by higher expression of CD62L and CXCR4 with lower expression of IGF-1R. A) Mean fluorescent intensity (MFI) data for CXCR5, CD11c, CD86, CD20, CD27, CD40, CD62L, CXCR4, and IGF-1R across B cell subsets. B) Normalized gene expression data for SELL, CXCR4, and IGF1R from bulk RNA sequencing of B cell subsets.

Supplemental Figure 4



Preferential usage of different metabolic pathways may distinguish memory B cell subsets. Dot plot displaying gene set enrichment analysis comparing DN1 B cells to CD27⁺ SWM and naïve B cells. Size of dot indicates number of genes enriched within respective gene set. A) Dot plot displaying gene sets over-expressed by DN1 B cells. B) Dot plot displaying gene sets under-expressed by DN1 B cells. C) Normalized gene expression data for *TP53* and *TP73* from bulk RNA sequencing of B cell subsets.

Supplemental Figure 5



Memory B cell subsets may differ in their predilection for class switching to IgA and IgG2. Normalized gene expression data for *IGHG1*, *IGHG2*, *IGHG3*, *IGHG4*, *IGHA1*, *IGHA2*, and *IGHE* from bulk RNA sequencing of B cell subsets.

Supplemental Table 1

**Vaccinee Cohort
Demographic Data**

Vaccinee Cohort Demographic Data	
Pre-vaccination baseline (n=11)	
Male, n (%)	3 (27)
Female, n (%)	8 (73)
Caucasian, n (%)	6 (55)
African American, n (%)	2 (18)
Hispanic, n (%)	3 (27)
Age, median (IQR)	51 (31-63)
Prior SARS-CoV-2 infection, n (%)	0 (0%)
Week 1-2 post-dose 1 (n=9)	
Male, n (%)	5 (56)
Female, n (%)	4 (44)
Caucasian, n (%)	8 (89)
African American, n (%)	1 (11)
Hispanic, n (%)	0 (0)
Age, median (IQR)	56 (43-64)
Prior SARS-CoV-2 infection, n (%)	0 (0)
Days from last immunization, median (IQR)	9 (7.5-10)
Paired samples from baseline, n (%)	1 (11)
Week 3-4 post-dose 1 (n=19)	
Male, n (%)	9 (47)
Female, n (%)	10 (53)
Caucasian, n (%)	17 (89)
African American, n (%)	2 (11)
Hispanic, n (%)	0 (0)
Age, median (IQR)	60 (45-71)
Prior SARS-CoV-2 infection, n (%)	0 (0)
Days from last immunization, median (IQR)	21 (20-27)
Paired samples from baseline, n (%)	5 (26)
Paired samples from Week 1-2 post-dose 1, n (%)	9 (47)
Week 1-2 post-dose 2 (n=19)	
Male, n (%)	8 (42)
Female, n (%)	11 (58)
Caucasian, n (%)	17 (89)
African American, n (%)	2 (11)
Hispanic, n (%)	0 (0)
Age, median (IQR)	61 (52-74)
Prior SARS-CoV-2 infection, n (%)	0 (0)
Days from last immunization, median (IQR)	29 (28-35)
Paired samples from baseline, n (%)	5 (26)
Paired samples from Week 1-2 post-dose 1, n (%)	9 (47)
Paired samples from Week 3-4 post-dose 1, n (%)	17 (89)
Week 4-8 post-dose 2 (n=12)	
Male, n (%)	7 (58)
Female, n (%)	5 (42)
Caucasian, n (%)	12 (100)
African American, n (%)	0 (0)
Hispanic, n (%)	0 (0)
Age, median (IQR)	66 (62-71)
Prior SARS-CoV-2 infection, n (%)	0 (0)
Days from last immunization, median (IQR)	62 (58-87)
Paired samples from baseline, n (%)	1 (8)
Paired samples from Week 1-2 post-dose 1, n (%)	7 (58)
Paired samples from Week 3-4 post-dose 1, n (%)	11 (92)
Paired samples from Week 1-2 post-dose 2, n (%)	11 (92)
Week 24 post-dose 2 (n=10)	
Male, n (%)	3 (30)
Female, n (%)	7 (70)
Caucasian, n (%)	8 (80)
African American, n (%)	0 (0)
Hispanic, n (%)	2 (20)
Age, median (IQR)	44 (38-65)
Prior SARS-CoV-2 infection, n (%)	0 (0)
Days from last immunization, median (IQR)	223 (180-287)
Paired samples from baseline, n (%)	0 (0)
Paired samples from Week 1-2 post-dose 1, n (%)	0 (0)
Paired samples from Week 3-4 post-dose 1, n (%)	0 (0)
Paired samples from Week 1-2 post-dose 2, n (%)	0 (0)
Paired samples from Week 4-8 post-dose 2, n (%)	0 (0)

Supplemental Table 2**COVID-19 Cohort
Demographic Data**

COVID-19 Cohort Demographic Data		
Acute COVID-19 (n=13)		
	Male, n (%)	7 (54)
	Female, n (%)	6 (46)
	Caucasian, n (%)	7 (54)
	African American, n (%)	1 (8)
	Hispanic, n (%)	7 (54)
	Age, median (IQR)	62 (45-73)
	Outpatient symptomatic infection, n (%)	0 (0)
	Hospital admission, regular floor, n (%)	0 (0)
	Hospital admission, intensive care, n (%)	13 (100)
	Vaccinated, n (%)	0 (0)
	Days from symptom onset, median (IQR)	23 (15-29)
Convalescent COVID-19 (n=66)		
	Male, n (%)	24 (36)
	Female, n (%)	42 (64)
	Caucasian, n (%)	48 (72)
	African American, n (%)	1 (2)
	Hispanic, n (%)	16 (24)
	Age, median (IQR)	39 (32-56)
	Asymptomatic infection, n (%)	8 (12)
	Outpatient symptomatic infection, n (%)	46 (70)
	Hospital admission, regular floor, n (%)	7 (11)
	Hospital admission, intensive care, n (%)	5 (8)
	Vaccinated, n (%)	0 (0)
	Days from symptom onset, median (IQR)	217 (174-248)