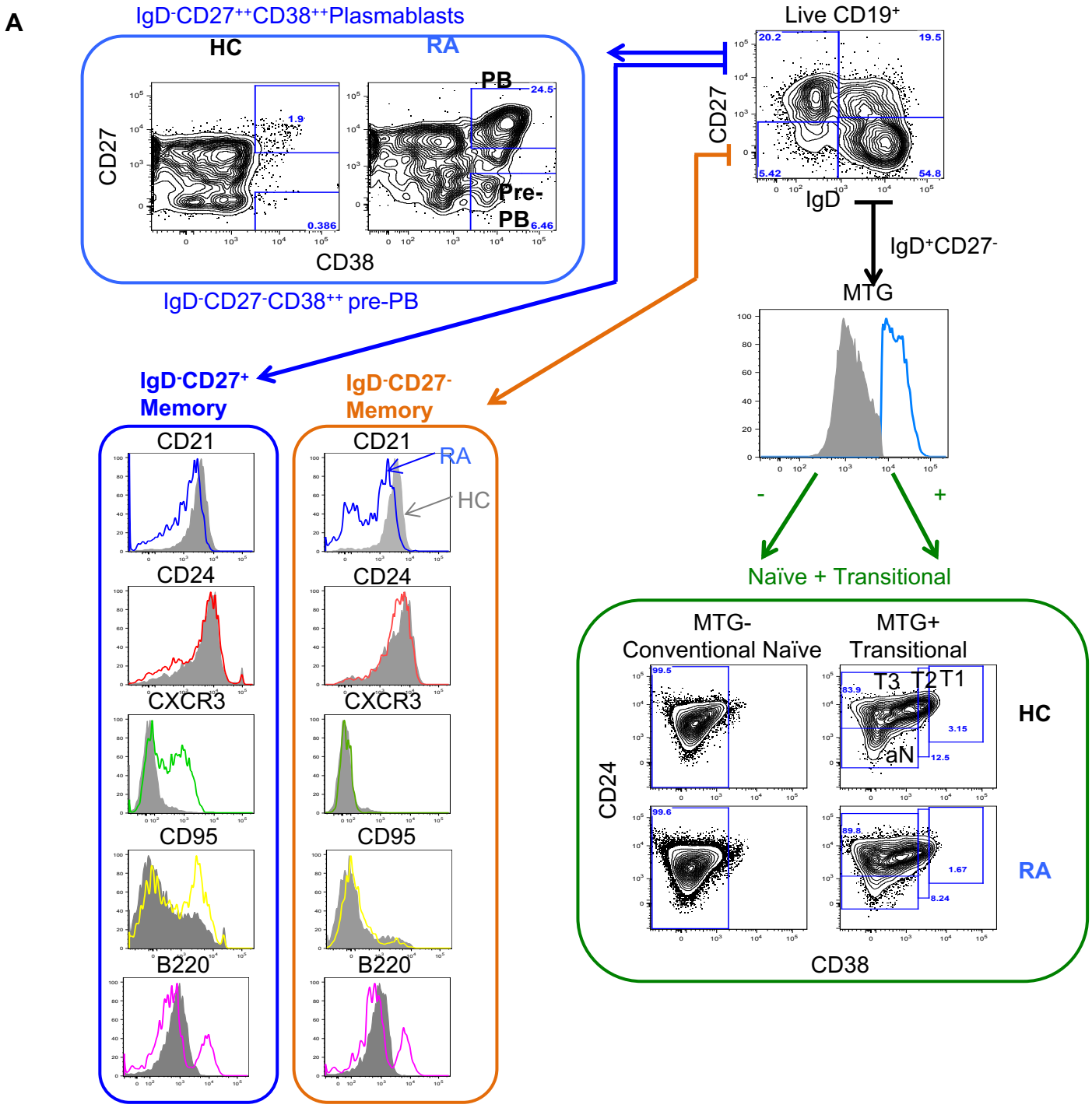
B cell relationship to anti-TNF in RA

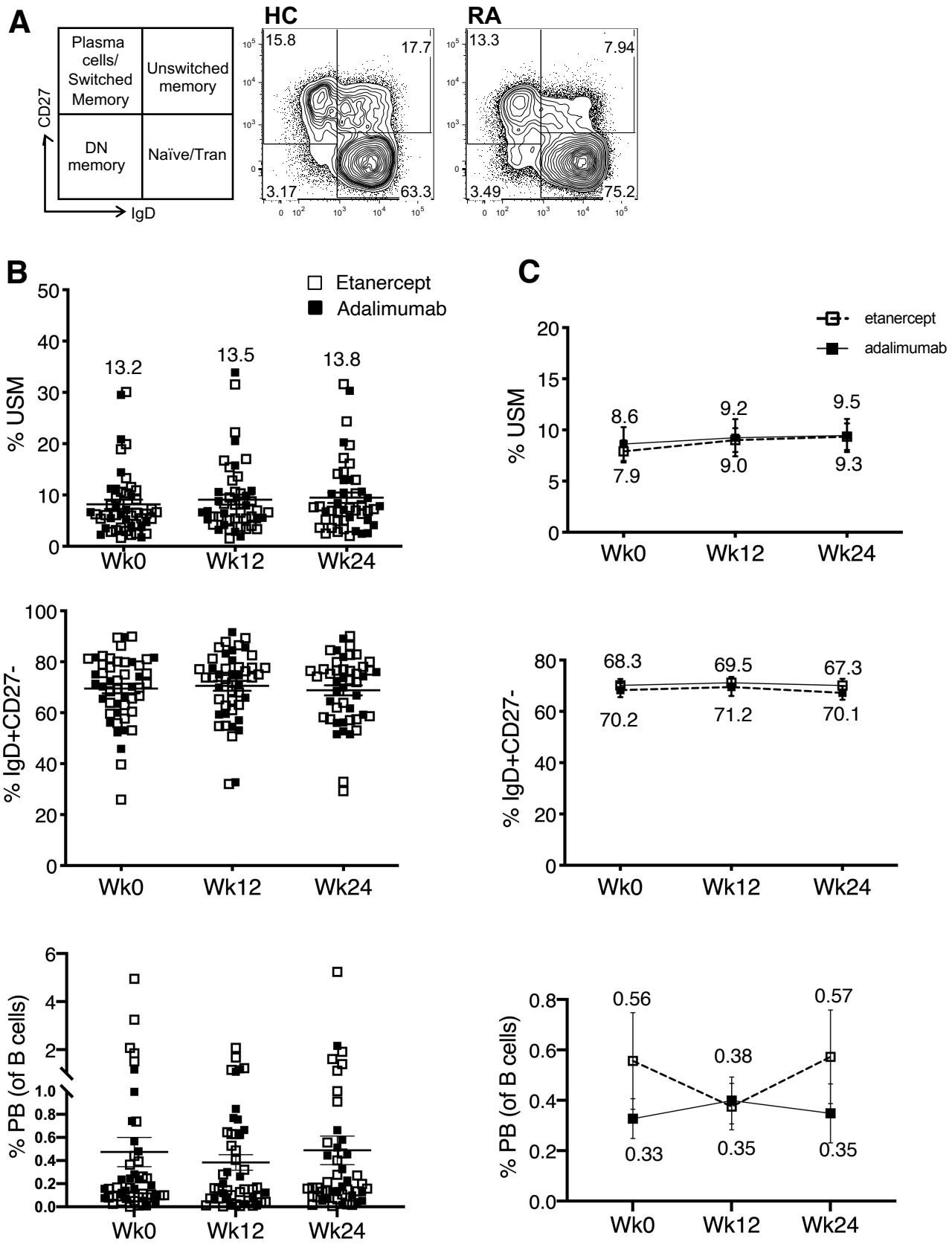
Supplementary figures and tables

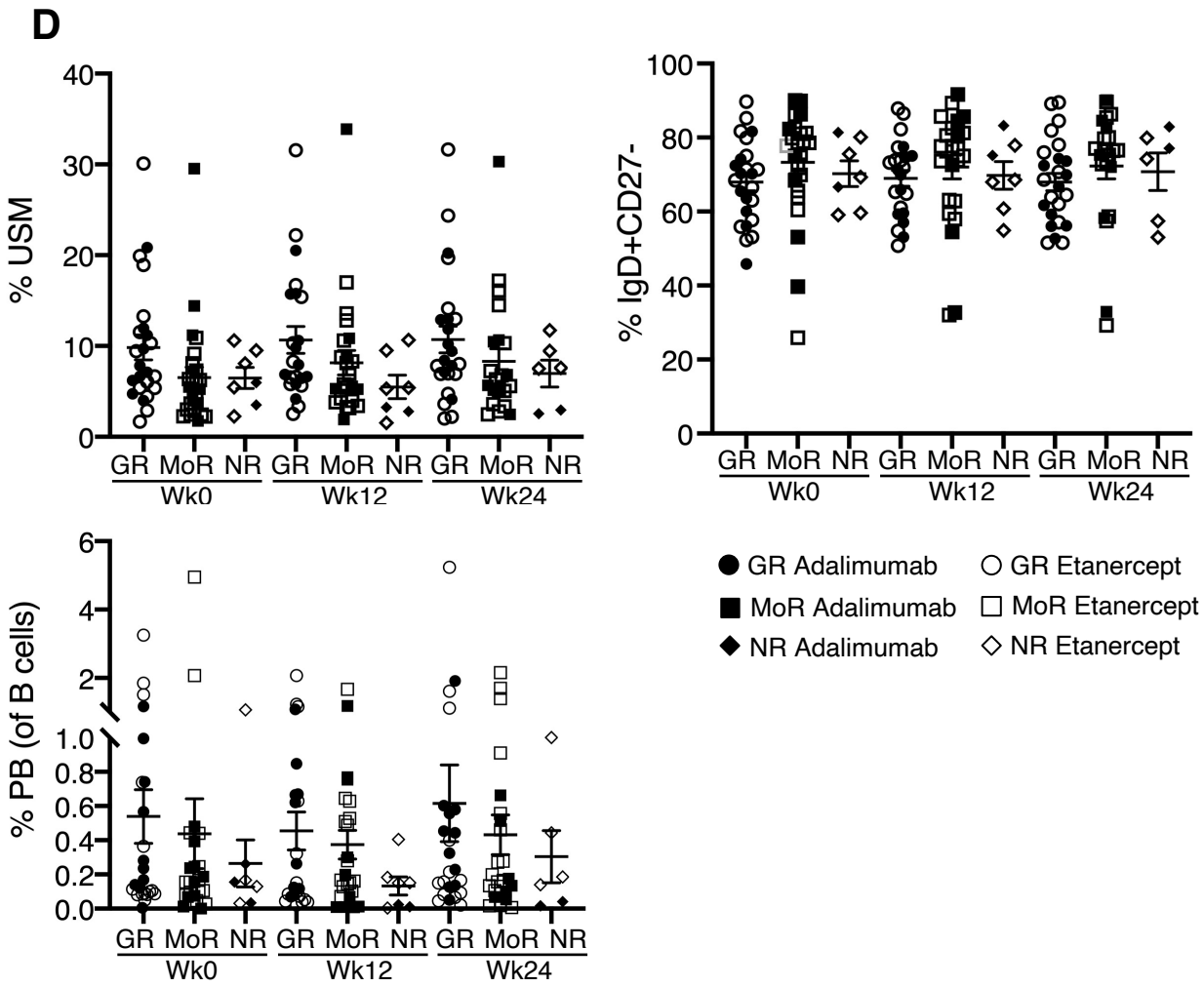


B

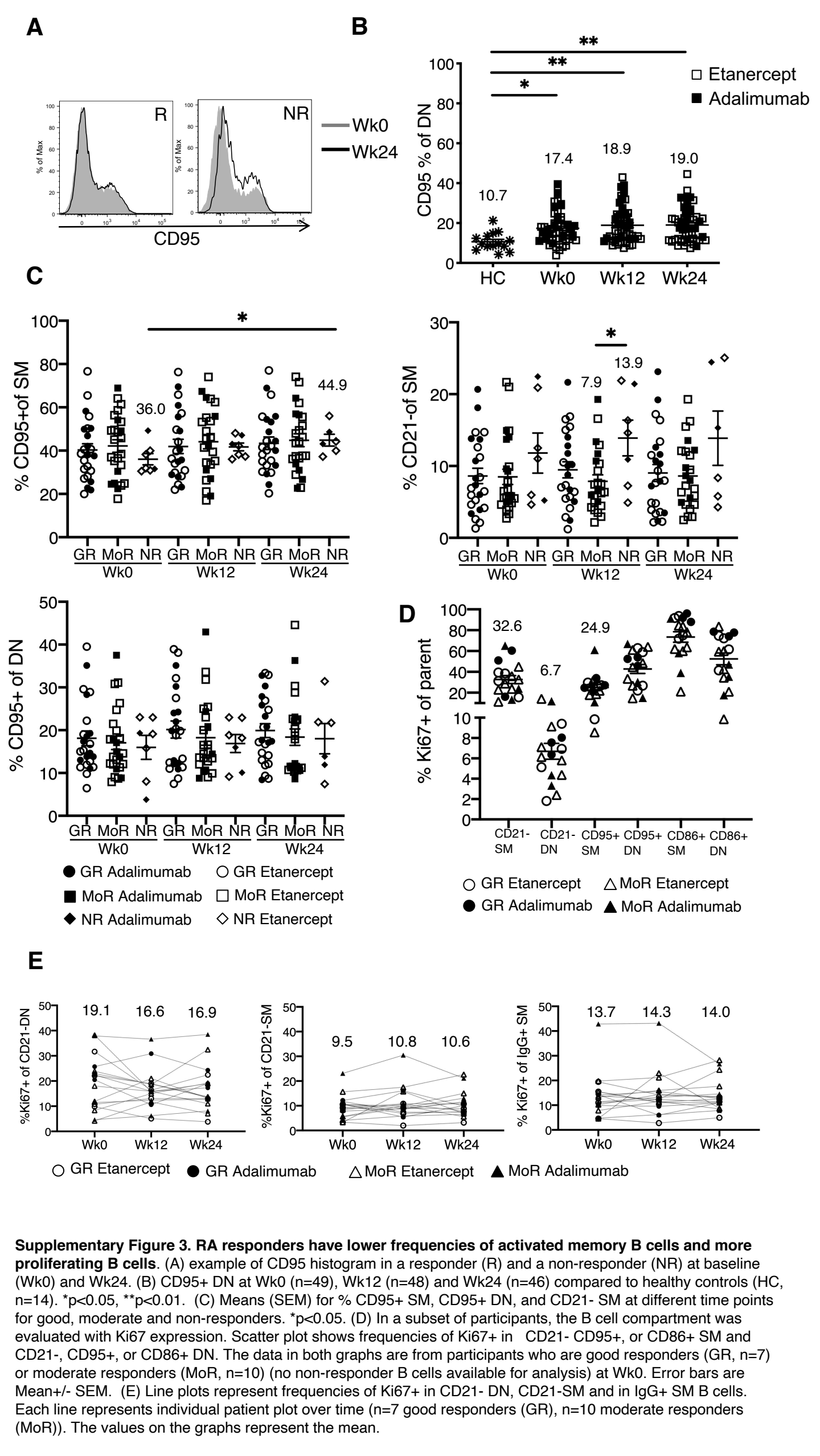
B CELL SUBSET	IDENTIFICATION MARKERS	PANEL	ADDITIONAL MARKERS
Naïve	IgD+CD27-MTG-CD24+/-CD38+/-	Memory	CXCR3, CD21, CD95, B220
Unswitched Memory	IgM+IgD+CD27+	Transitional	CD10, IgM, CD23
Switched Memory	IgD-CD27+ (exclude CD38++)	B cell-Ki67	Ki67, IgA, IgG
Double Negative	IgD-CD27-		
Transitional 1/2	IgD+CD27-MTG+CD24++CD38++		
Transitional 3	IgD+CD27-MTG+CD24+CD38+/-		
Pre-plasmablast	IgD-CD27-CD24-CD38++		
Plasmablasts	IgD-CD27++CD38++		
Activated naïve	IgD+CD27-MTG+CD24+/-CD38+/-		

Supplementary Figure 1. Gating strategy for B cell subsets. (A) Gating scheme for core B cell subsets. IgD and CD27 are used to define Naive, Unswitched memory, Switched memory and Double negative B cells. Within IgD- B cells, plasmablast and pre-plasmablast are defined as CD27++CD38++ and CD27-CD38++ respectively. Within IgD+CD27-, mitotracker (MTG) is used to separate transitional B cells from conventional naive B cells. CD38 and CD24 are used to delineate transitional B cell subsets and identify activated naïve B cells. In addition, additional markers are evaluated in core B cell subsets including CD21, CD24, CD95, CXCR3 and B220. Examples of healthy control (HC) and RA participants are shown. (B) Definitions of B cell subsets and additional makers evaluated in this study.

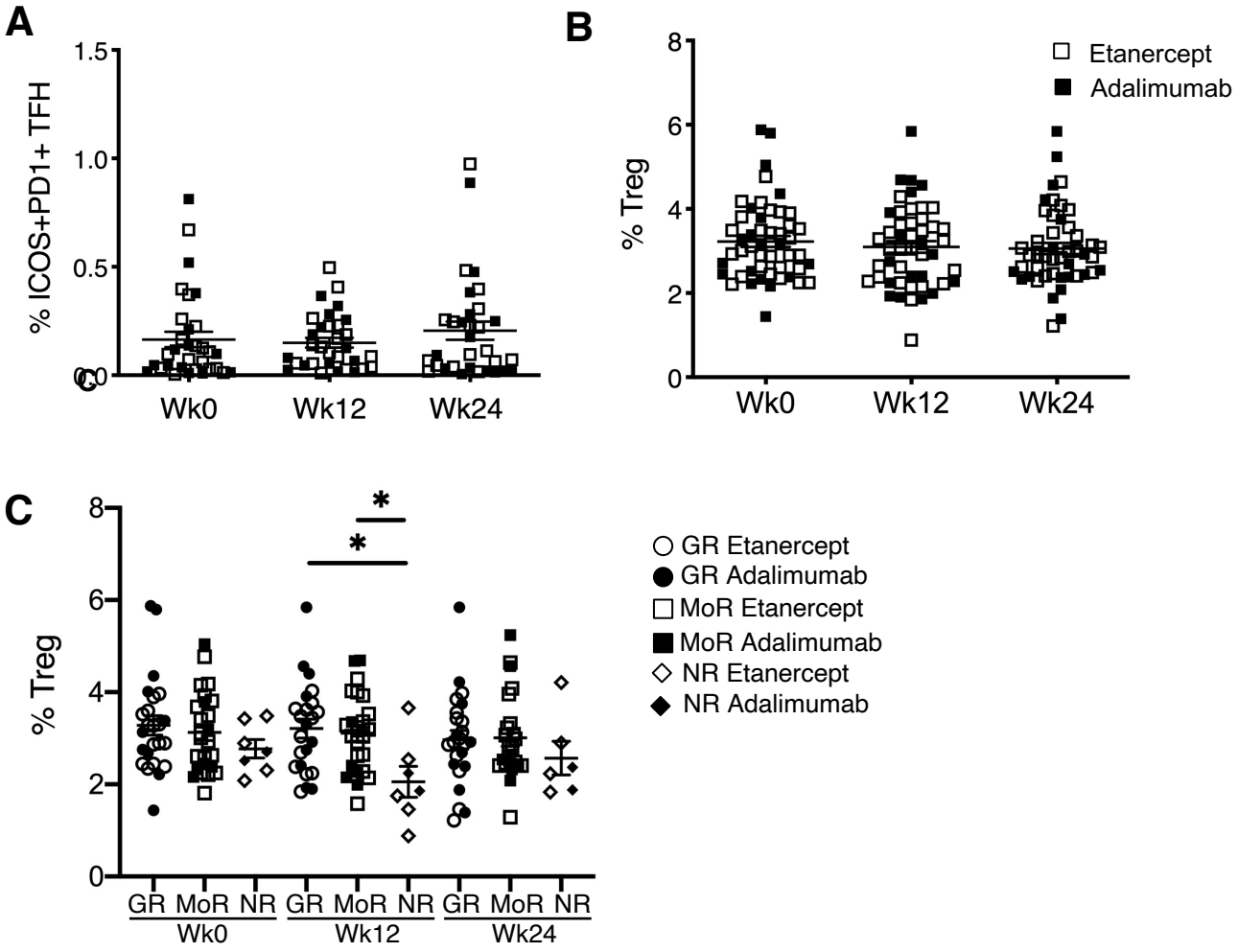

A
Plasma cells/ Switched Memory
Unswitched memory
DN memory
Naïve/Tran
CD27
IgD
HC
RA
15.8
17.7
3.17
63.3
13.3
7.94
3.49
75.2
B
Etanercept
Adalimumab
% USM
13.2
13.5
13.8
Wk0
Wk12
Wk24
% IgD+CD27-
% PB (of B cells)
C
etanercept
adalimumab
% USM
8.6
9.2
9.5
7.9
9.0
9.3
% IgD+CD27-
68.3
69.5
67.3
70.2
71.2
70.1
% PB (of B cells)
0.56
0.38
0.57
0.33
0.35
0.35



Supplementary Figure 2. Core B cell subsets over time. (A) Dot plot example of B cell subsets based on CD27 and IgD expression from healthy control (HC) and RA participants. (B) Scatter plots of frequencies of unswitched memory (USM), and IgD+CD27- (Naïve+T) of total CD19+ B cells over time, n=49. (C) Line plot of USM, and IgD+CD27- frequencies over time (mean±SEM) in each treatment group. n=18 adalimumab, n=31 etanercept. (D) Scatter plots represent frequencies of USM, IgD+CD27- and plasma cell (PC) over time for non-responders (NR: n=7 Wk0, n=7 Wk12, n=6 Wk24), good responders (GR: n=24 Wk0, n=23 Wk12, n=24 Wk24) and moderate responders (MoR:, n=25 Wk0, n=24 Wk12, n=22 Wk24). Error bars depict Mean+/- SEM. All longitudinal comparisons resulted in p-value > 0.05.



Supplementary Figure 3. RA responders have lower frequencies of activated memory B cells and more proliferating B cells. (A) example of CD95 histogram in a responder (R) and a non-responder (NR) at baseline (Wk0) and Wk24. (B) CD95+ DN at Wk0 (n=49), Wk12 (n=48) and Wk24 (n=46) compared to healthy controls (HC, n=14). *p<0.05, **p<0.01. (C) Means (SEM) for % CD95+ SM, CD95+ DN, and CD21- SM at different time points for good, moderate and non-responders. *p<0.05. (D) In a subset of participants, the B cell compartment was evaluated with Ki67 expression. Scatter plot shows frequencies of Ki67+ in CD21- CD95+, or CD86+ SM and CD21-, CD95+, or CD86+ DN. The data in both graphs are from participants who are good responders (GR, n=7) or moderate responders (MoR, n=10) (no non-responder B cells available for analysis) at Wk0. Error bars are Mean+/- SEM. (E) Line plots represent frequencies of Ki67+ in CD21- DN, CD21-SM and in IgG+ SM B cells. Each line represents individual patient plot over time (n=7 good responders (GR), n=10 moderate responders (MoR)). The values on the graphs represent the mean.



Supplementary Figure 4. Frequency of T cell subsets. (A) Circulating TFH cells were defined as CXCR5+ICOS+PD1+ cells. Scatter plots show frequencies of TFH express as % of CD3+ T cells from a subset of participants (n=32) regardless of treatment at Wk0, Wk12 and Wk24. **(B)** Regulatory T cells (Treg) were classified as CD4+CD25+CD127low/-. Scatter plots represent percent of Treg express as % of CD3+ T cells from all participants (n=56 Wk0, n=54 Wk12, n=52 Wk24) regardless of treatment at Wk0, Wk12 and Wk24. **(C)** Scatter plots represent frequencies of Treg as % of CD3+ T cells in participants separated by response status and time points, Good responder (GR: n=24 Wk0, n=23 Wk12, n=24 Wk24), moderate responder (MoR: n=25 Wk0, n=24 Wk12, n=24 Wk24) and non-responders (NR: n=7 Wk0, n=7 Wk12, n=6 Wk24). * $p<0.01$ non-responders vs. good and moderate responders.

Supplemental table 1. Changes in B cell populations over time. B cell subset by treatment group (PP)

		Etanercept	Adalimumab	P-value*
Primary Analysis:				
CD27+ SM (% parent)		N=30	N= 18	
Week 0	Mean (SE)	12.4 (1.5)	14.1 (1.9)	
Week 12	Mean (SE)	13.2 (1.3)	13.8 (1.7)	
Adjusted Change	Mean (SE)	0.7 (0.5)	-0.1 (0.6)	0.301
Secondary Analyses:				
CD27+ USM (% parent)		N=30	N=18	
Week 0	Mean (SE)	7.6 (1.1)	8.6 (1.7)	
Week 12	Mean (SE)	9.0 (1.2)	9.2 (1.8)	
Adjusted Change	Mean (SE)	1.5 (0.5)	0.6 (0.6)	0.237
DN (% parent)		N=30	N=18	
Week 0	Mean (SE)	6.1 (0.6)	6.8 (0.7)	
Week 12	Mean (SE)	5.7 (0.6)	5.4 (0.8)	
Adjusted Change	Mean (SE)	-0.5 (0.4)	-1.3 (0.5)	0.227
Naïve (% parent)		N=30	N=18	
Week 0	Mean (SE)	72.0 (2.5)	68.8 (3.2)	
Week 12	Mean (SE)	70.6 (2.2)	70.3 (3.4)	
Adjusted Change	Mean (SE)	-1.3 (0.9)	1.2 (1.1)	0.074

*P-value is testing for treatment effect for an analysis of covariance with adjustments for baseline.

Supplemental table 2. Changes in B cell populations over time. Core B cell subsets.

Response*	Time (Wk)	Naïve+T#	USM#	SM#	DN#
GR (n=24)	0	68.0±2.3	9.8±1.4	13.6±1.4	6.5±0.5
(n=23)	12	69.0±2.2	10.7±1.5	13.4±1.1	5.4±0.4
(n=24)	24	68.0±2.3	10.7±1.5	13.9±1.3	5.5±0.5
MoR (n=25)	0	73.3±3.1	6.7±1.1	12.5±1.8	6.0±0.6
(n=24)	12	72.0±3.2	8.2±1.4	13.4±1.8	5.2±0.7
(n=22)	24	72.3±3.4	8.3±1.4	13.7±2.1	4.6±0.5
NR (n=7)	0	70.2±3.5	6.0±1.3	13.5±1.9	8.7±1.5
(n=7)	12	69.8±3.7	5.5±1.3	14.3±2.6	8.6±1.8
(n=6)	24	70.8±5.1	7.0±1.5	13.1±3.1	7.1±1.9

* the frequencies of B cell subsets were compared between response groups at each time point using Tukey's comparison test. p-value for all comparisons were greater than 0.05.
# Naïve+T=IgD+CD27-, USM=unswitched memory=IgD+CD27+, SM=switched memory=IgD-CD27+, DN=double negative=IgD-CD27- (expressed as frequency of parent CD19+ population). GR=good responder, MoR=moderate responder, NR=non-responder. N's provided as a range as the number available for each visit varied by cell subset.

Supplemental table 3. Changes in B cell populations over time. Additional B cell subsets

Response*	Time (Wk)	T1/2[#]	T3[#]	Pre-PB[#]	PB[#]	acN [#]
GR (n=24)	0	1.2±0.1	19.6±2.0	1.4±0.4	2.8±1.0	6.4±0.7
(n=23)	12	1.0±0.2	20.3±2.4	1.1±0.3	2.3±0.6	6.9±0.8
(n=23-24)	24	1.5±0.3	22.3±2.6	1.3±0.3	3.1±1.0	6.5±0.8
MoR (n=24-25)	0	1.4±0.3	15.5±1.3	1.3±0.3	2.9±1.6	6.8±1.0
(n=23-24)	12	1.2±0.2	18.0±2.2	1.0±0.2	2.5±0.6	6.5±0.9
(n=21-22)	24	1.3±0.2	16.8±1.9	1.0±0.2	2.1±0.6	6.6±1.1
NR (n=7)	0	0.3±0.1	19.2±4.4	0.9±0.4	1.2±0.5	14.6±1.7
(n=7)	12	0.5±0.3	20.1±4.5	0.8±0.6	1.0±0.7	15.6±1.8
(n=6)	24	0.7±0.4	18.7±4.4	0.9±0.6	2.6±1.9	14.2±3.1

* the frequencies of B cell subsets were compared between response groups at each time point using Tukey's comparison test. p-value for all comparisons were greater than 0.05.

[#] T1/2=transitional 1 and 2, T3=transitional 3 (expressed as frequency of CD19+). Pre-PB, PB=plasmablast (expressed as frequency of parent CD19+MTG+IgD-) (Mean±SEM). GR=good responder, MoR=moderate responder, NR=non-responder. N's provided as a range as the number available for each visit varied by cell subset.