SUPPLEMENTARY MATERIALS

Belimumab promotes negative selection of activated autoreactive B cells in systemic lupus erythematosus patients

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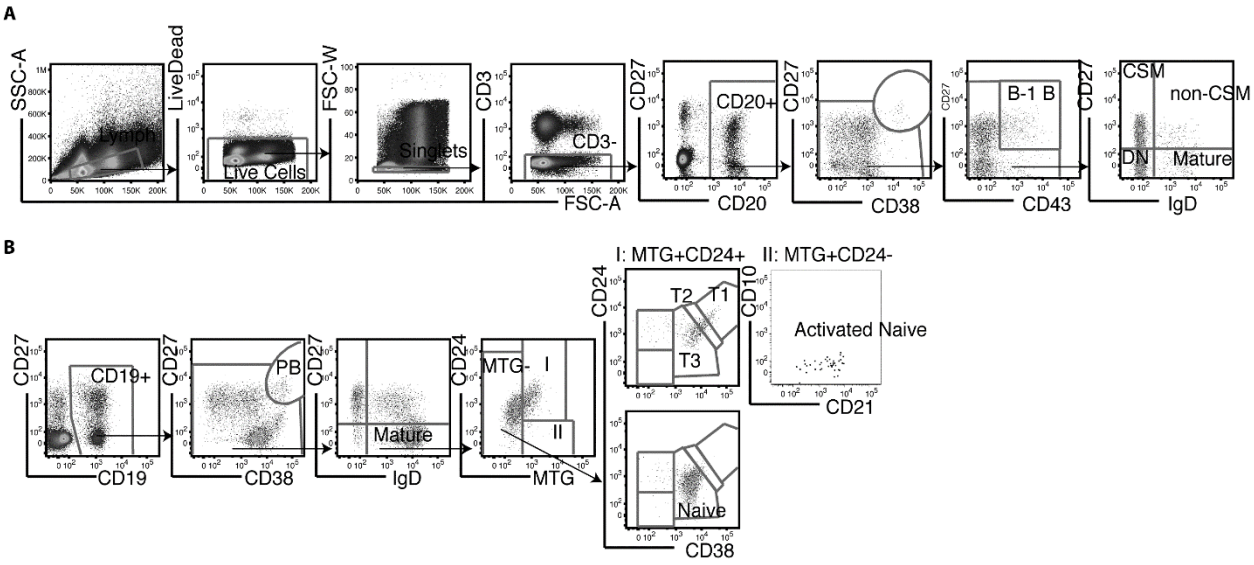
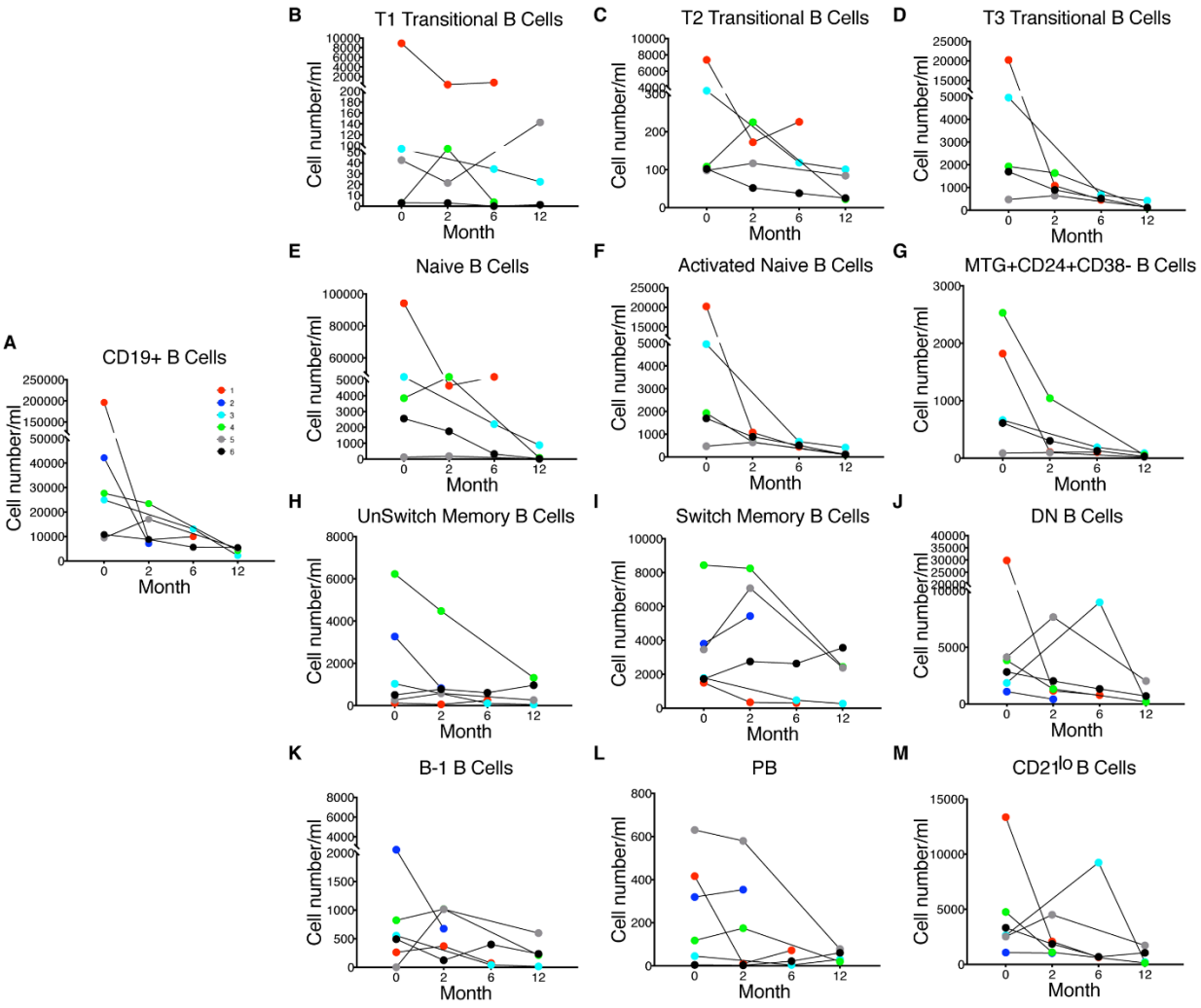
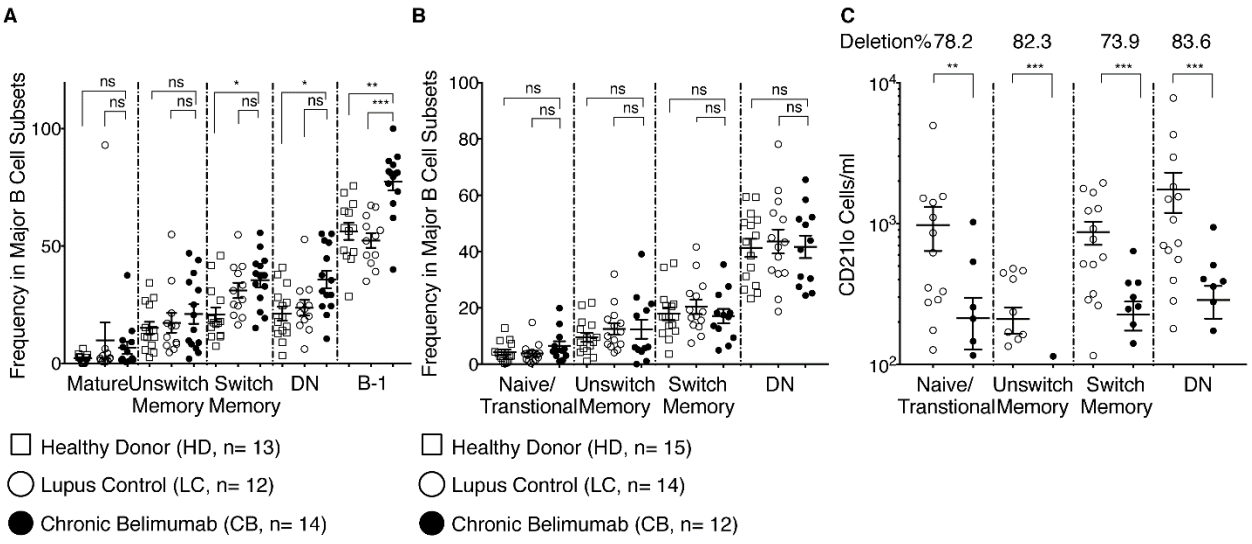


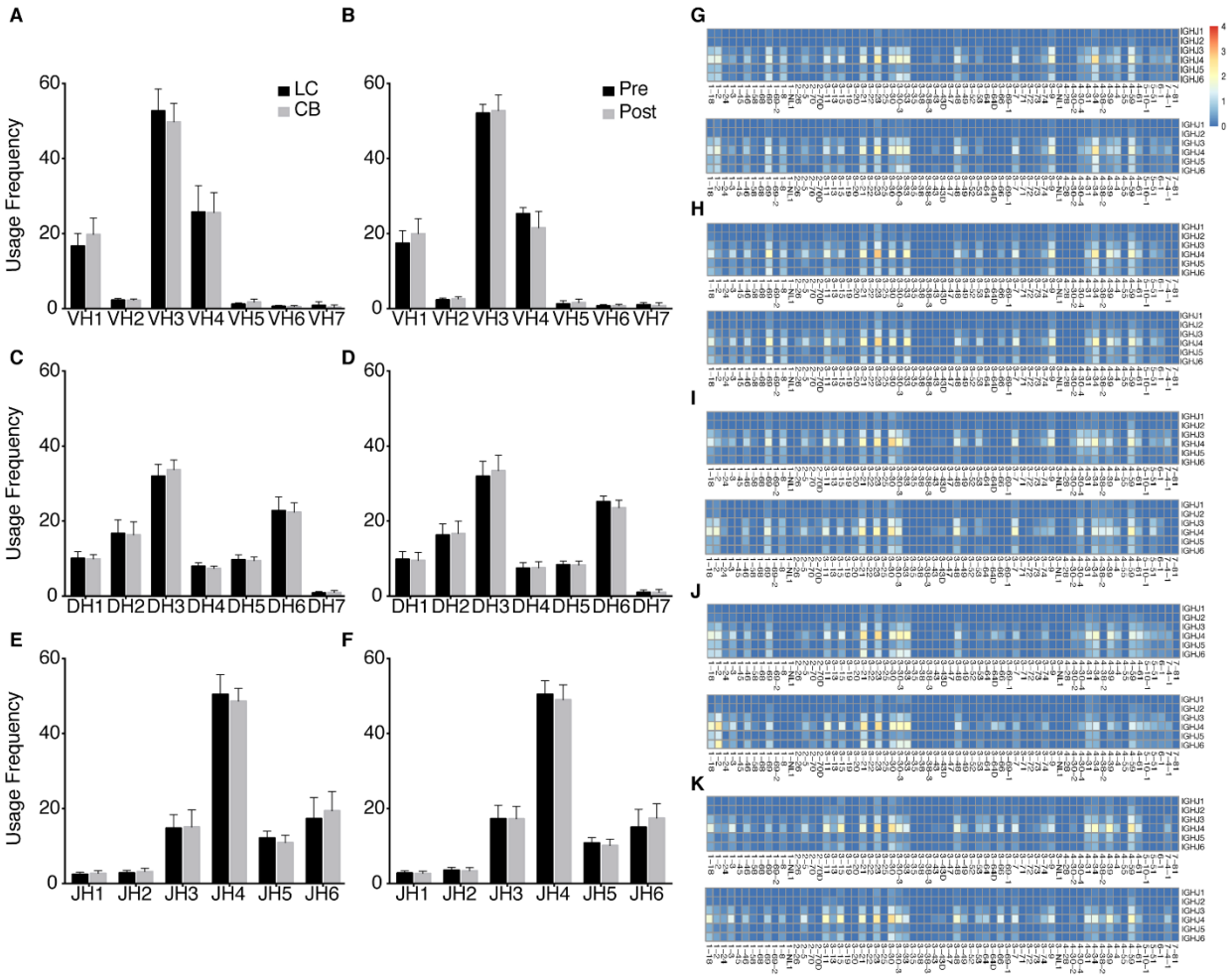
Supplementary Figure 1: Gating strategy. (A) Plots show the gating strategy for major B cell populations, including $CD27^{-}IgD^{+}$ (transitional and naïve), $CD27^{+}IgD^{+}$ (unswitched memory - non-CSM) $CD27^{+}IgD^{-}$ (switched memory - CSM) and $CD27^{-}IgD^{-}$ (double negative - DN) B cells as well as $CD20^{+}CD38^{hi}CD27^{hi}$ (pre-plasmablast and plasmablast) and $CD20^{+}CD27^{+}CD43^{+}$ (B1) B cells. (B) Plots show the gating strategy for major B cell populations, including $CD27^{-}IgD^{+}$ (transitional and naïve), $CD27^{+}IgD^{+}$ (unswitched memory) $CD27^{+}IgD^{-}$ (switched memory) and $CD27^{-}IgD^{-}$ (double negative - DN) B cells and $CD19^{+}CD27^{hi}CD38^{hi}$ $CD38^{hi}CD27^{hi}$ (pre-plasmablast and plasmablast - PB). From the mature $CD27^{-}IgD^{+}$ B cell compartment , cells were further subsetted into transitional T1 ($MTG^{+}CD38^{+++}CD24^{+++}$), T2 ($MTG^{+}CD38^{++}CD24^{++}$), T3 ($MTG^{+}CD38^{+}CD24^{+}$), activated naïve ($MTG^{+}CD24^{-}$), naïve ($MTG^{-}$) and $MTG^{+}CD24^{+}CD38^{-}$ B cells.



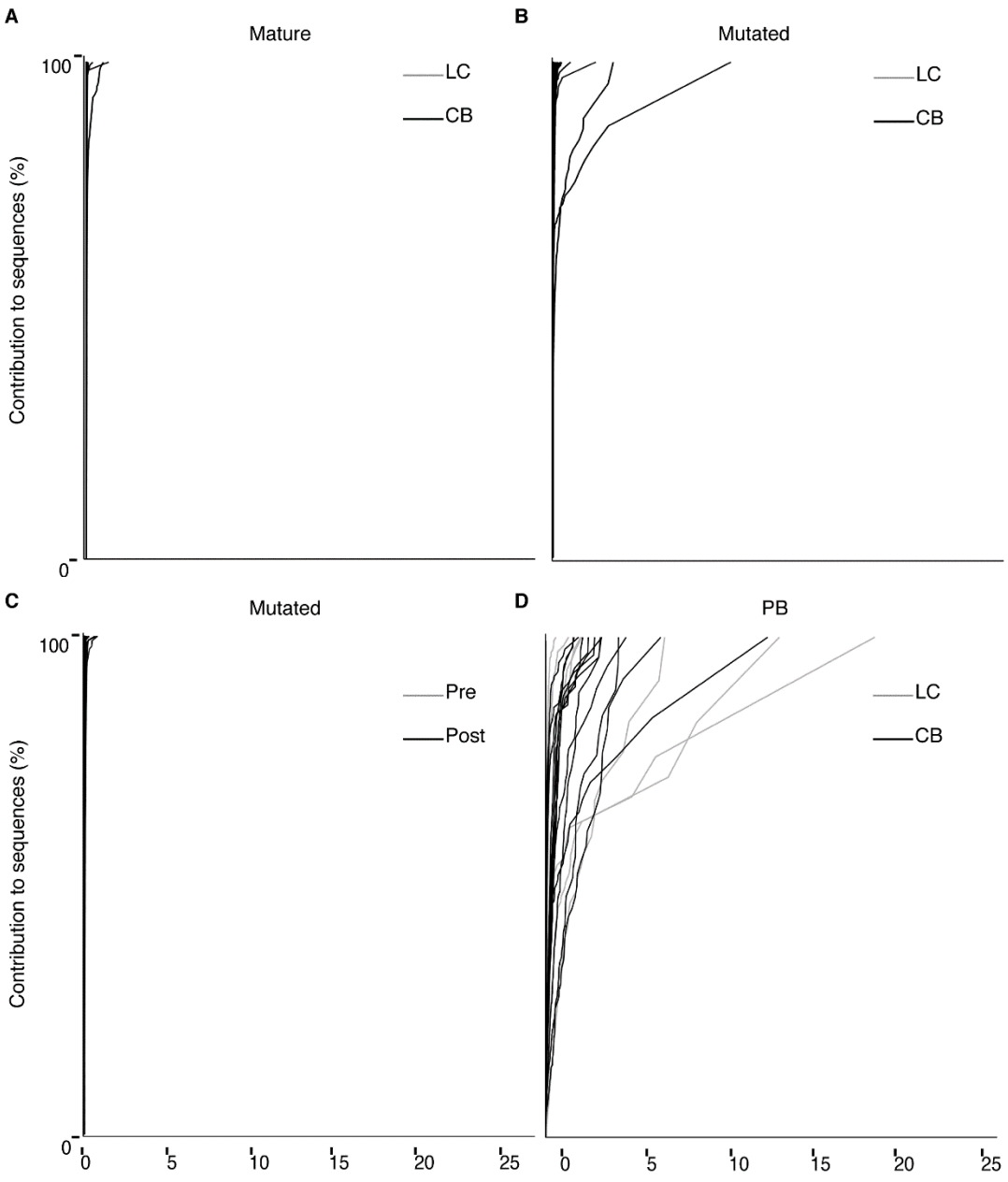
Supplementary Figure 2: Comparison of absolute B cell count/ml before and after belimumab treatment. Connected dot graphs show changes in the number of total B cells (A), and B cell subsets (B-M) at indicated times (in months) after treatment. Each color represents an individual donor.



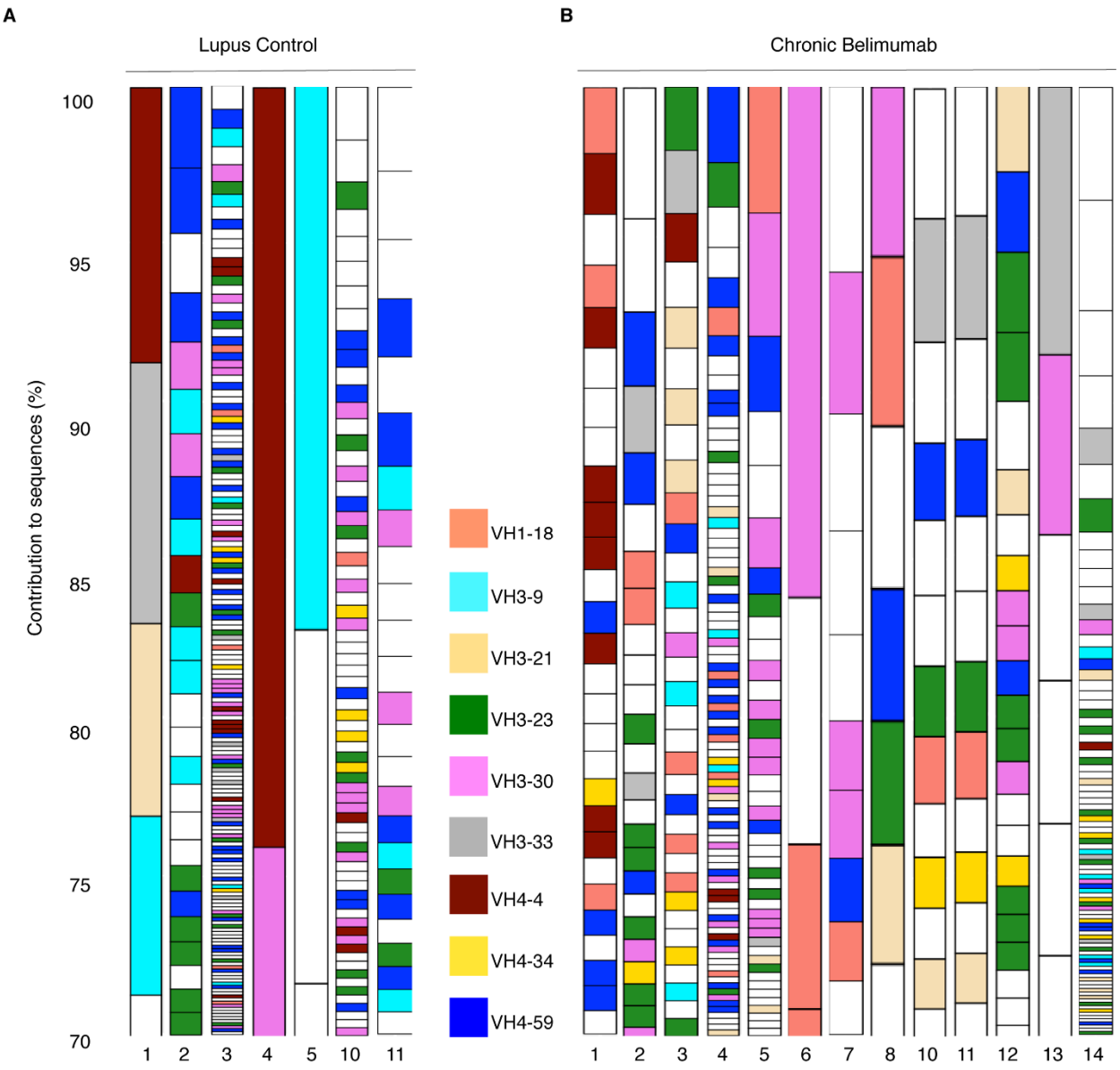
Supplementary Figure 3: The frequency and number of CD95 and CD21 expressing B cell subsets. A-C. The frequency and number of CD95 and CD21 expressing B cell subsets. Percent (A) of CD95 positive B cells within the major subsets (HD: n=13, LC: n=12, CB: n=14). Frequency (B) and total number (C) of $CD21^{lo}$ cells in major subsets of B cells (HD: n=15, LC: n=14, CB: n=12). *p <0.05; **p < 0.01; ***p <0.001; ****p<0.0001; ns: not significant. Comparisons were performed using Mann Whitney analysis (Suppl Fig 3C) and Kruskal-Wallis Test (Suppl Fig 3A and 3B).



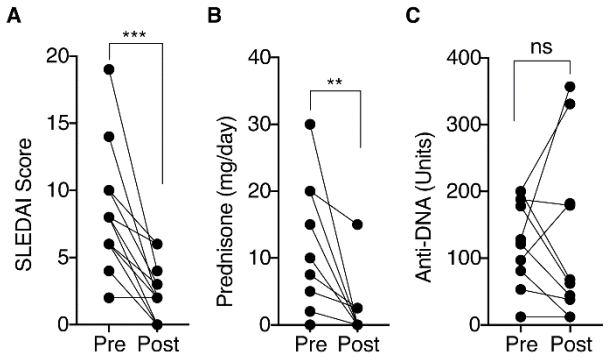
Supplementary Figure 4: VH, DH, JH and VH-JH paired usage. Bar graphs show frequencies of VH, DH and JH family usage in patients with (n=14) and without (n=10) belimumab treatment (A, C, E) and 5 individual patients before and after 6 months of treatment (B, D, F). Heatmaps (G-K) show the VH-JH paired usage from individual patients before (top) and after (bottom) treatment.



Supplementary Figure 5: Clonotype distribution of different B cell subsets with and without treatment. Clonotype size ranking is shown on the y-axis, and the frequency of sequences for each clone as a percent of total repertoire is shown on the x-axis. Each line represents an individual patient (lupus control (LC) n=10; chronic belimumab (CB) n=14; pre and post belimumab n=5).



Supplementary Figure 6. Clonal representation of PB in patients with and without belimumab treatment. Stacked bar graphs display the size distribution of the most common individual clonotypes constituting 30% of the repertoire (D30) of plasmablasts from (A) lupus controls (n=7) and (B) chronic belimumab-treated patients (n=13). Individual donors are shown at the bottom of each graph and the most frequently represented VH are color coded. Comparisons of individual genes were performed using Mann Whitney analysis.



Supplementary Figure 7. Characteristics of chronic belimumab treated patients at inception. Data from the pre-belimumab screening visit are compared with the study visit (n=15). After >7 years of belimumab treatment patients manifested a significant decrease in SLEDAI score and prednisone dose but changes in anti-dsDNA antibody titers were variable. Comparisons were performed using Wilcoxon Signed Rank test for paired samples. *p <0.05; **p < 0.01; ***p <0.001; ****p<0.0001; ns: not significant.

Supplementary Table 1: Antibodies used for flow cytometry and sorting

Panel 1	Source	Catalog	Clone
Mitotracker green	Invitrogen	M-7514	
IgD PE	BD	BD 555779	IA6-2
CD3 PE/Texas Red	Beckman Coulter	IM2705U	UCHT1
CD38 PerCP/Cy5.5	Beckman Coulter	A70205	LS198.4.3
CD21 PECy7	BD	BD 561374	B-ly4
CD27 APC	Beckman Coulter	B09983	1A4CD27
CD10 APC/AF700	Beckman Coulter	A86353	ALB1
CD24 APC/AF750	Beckman Coulter	B10738	ALB9
CD19 V450	BD	BD 560354	HIB19
Live/Dead Aqua	Invitrogen	L34957	
Panel 2			
IgM FITC	BD	BD 555782	G20-127
CD38 APC/AF700	Beckman Coulter	B23489	LS198.4.3
CD3 PE/Texas Red	Beckman Coulter	IM 27050	UCHT1
CD95 PerCP/Cy5.5	BD	BD 561655	DX2
IgD PE Cy7	BD	BD 561314	IA6-2
CD27 APC	Beckman Coulter	B09983	1A4CD27
TACI PE	BD	BD 558414	1A1-K21-M22
CD43 APC.AF750	Beckman Coulter	A89307	DFT1
CD20 Pacific Blue	BioLegend	302320	2H7
Live/Dead Aqua	Invitrogen	L34957	
Sorting Panel			
CD19 PE Cy7	BD	341103	SJ25C1
CD27 APC	Beckman Coulter	B09983	1A4CD27
IgD PE	BD	555779	IA6-2
CD38 PerCP/Cy5.5	Beckman Coulter	A70205	LS198.4.3
IgM FITC	BD	555782	G20-127
CD10 APC/AF750	Beckman Coulter	A889310	ALB1
CD43 APC/AF750	Beckman Coulter	A89307	DFT1
CD11b Pacific Blue	BD	558123	ICRF44
CD56 V450	BD	560361	B159
CD3 Pacific Blue	BD	558117	UCHT1

Supplementary Table 2: Summary of sequences at each stage of analysis per donor

CD27- Cells: LC vs CB									
ID	Cells	Reads	Productive Sequences	Mature(<4 Mutations) [A]	Mutated [B]	MatureUniq CDR3VDJ [C]	MutatedUniq CDR3VDJ [D]	Mutated Clone 0.85 [D]	Mutated D20 [E]
CB1	68241	235401	117902	89770	28132	27988	13167	12007	358
CB2	25340	213666	97555	68321	29234	13896	8782	6836	144
CB3	199459	212373	149315	135193	14122	67937	12227	12037	993
CB4	25214	117312	53449	45866	7583	11572	4279	4003	101
CB5	72850	212545	142107	128857	13250	50672	10968	10713	671
CB6	38642	228491	130188	118281	11907	32859	8767	8473	361
CB7	52159	122027	62587	46666	15921	18555	6993	6191	3
CB8	10641	291917	97002	77115	19887	12295	5885	4750	7
CB9	40824	133403	82187	64929	17258	20455	8811	7997	245
CB10	26727	111993	66878	60109	6769	21086	5443	5306	256
CB11	154436	142253	87278	80460	6818	29818	5881	5779	478
CB12	84132	631989	342526	295882	46644	80993	32183	29954	875
CB13	37832	414721	216548	197236	19312	41666	13205	12606	2327
CB14	48213	503570	259373	227004	32369	56579	20698	19302	289
LC1	749220	153828	106727	97255	9472	54277	7692	7547	432
LC2	301686	468192	314531	266314	48217	156396	37309	34709	1386
LC3	1E+06	201513	90635	81439	9196	53368	7713	7510	547
LC4	631028	198173	151369	142799	8570	98195	7970	7902	994
LC5	251900	373421	232126	203841	28285	91367	23165	22078	1201
LC6	1E+06	241943	132564	116593	15971	39382	9965	9365	255
LC7	179823	578097	377849	315964	61885	117536	39431	35703	831
LC8	1E+06	303041	228547	212554	15993	98751	14078	13849	1081
LC9	63199	227353	147264	126996	20268	39105	11234	10184	128
LC10	1E+06	338344	256174	227263	28911	111551	22090	21101	1055

CD27- Cells: Pre vs. Post Treatment									
ID	Cells	Reads	Productive Sequences	Mature(<4 Mutations) [A]	Mutated [B]	MatureUniq CDR3VDJ [C]	MutatedUniq CDR3VDJ [C]	Mutated Clone 0.85 [D]	Mutated D20 [E]
Pre1	280347	415267	304941	273387	31554	155363	28282	27335	2327
Pre2	307673	515651	382670	347876	34794	82314	32132	31125	3114
Pre3	128179	364022	138804	106908	31896	69231	20360	18908	780
Pre4	74266	314445	182630	121227	61403	34481	22831	18482	243
Pre5	376757	365427	244024	215481	28543	113482	23379	22433	1385
Post1	126845	282410	177495	151500	25995	57205	17762	16752	615
Post2	80067	490998	305209	274153	31056	63601	21622	20329	678
Post3	60896	306749	128338	83786	44552	17201	14666	11797	257
Post4	14703	314865	180288	89573	90715	13614	15622	8482	132
Post5	170745	328001	202810	136701	66109	51599	33592	29139	972

Plasmablasts: LC vs. CB							
ID	Cells	Reads	Productive Sequences	UniqCDR3 VDJ [C]	Clone 0.85 [D]	D20 [E]	D50 [E]
LC1	1896	24072	1510	161	122	2	11
LC2	2382	193157	11057	896	561	12	64
LC3	27311	1010530	58286	7394	5484	82	484
LC4	411	5278	252	93	87	1	3
LC5	12122	99575	5669	561	272	1	9
LC10	7065	449622	25270	2586	1619	34	179
LC11	1915	687147	36609	2005	522	9	46
CB1	638	879130	46321	2494	608	11	50
CB2	1037	834462	45948	2858	738	8	48
CB3	1957	322060	17137	1384	714	14	70
CB4	5293	932475	46025	3836	2046	23	176
CB5	9206	746076	38484	4098	2510	6	126
CB6	2244	840141	43912	2558	915	1	10
CB7	630	490265	26195	1362	309	4	20
CB8	4856	141356	7584	437	146	3	13
CB10	1066	796269	43433	2279	609	7	36
CB10	899	858262	47219	2632	693	5	26
CB11	914	408268	22091	1543	605	7	40
CB13	261	733574	42020	1716	367	2	9
CB14	46610	1130375	61538	10698	6722	10	332

A: Sequences with < 4 nucleotide mutations in the whole VH sequence
B: Sequences with ≥ 4 nucleotide mutations in the whole VH sequence
C: Number of sequences that are 100% identical in VH, DH, JH and CDR3
D: Number of sequences with the same VH, JH CDR3L and ≥ 85% identical in the CDR3
E: The number of clonotypes in a size-ordered list that span 20% (or 50%) of the sequences

Supplementary Table 3: Statistics for Figure 4[A]

LC: Mutated/Mature

VH	p-Value	FDR
IGHV1_18	0.0039	0.0105
IGHV1_24	0.0039	0.0105
IGHV1_3	0.0039	0.0105
IGHV1_46	0.0039	0.0105
IGHV1_58	0.0020	0.0105
IGHV1_69	0.0020	0.0105
IGHV2_26	0.0195	0.0412
IGHV2_5	0.0020	0.0105
IGHV2_70	0.0039	0.0105
IGHV3_15	0.0039	0.0105
IGHV3_20	0.0039	0.0105
IGHV3_21	0.0195	0.0412
IGHV3_23	0.0020	0.0105
IGHV3_30	0.0020	0.0105
IGHV3_49	0.0020	0.0105
IGHV3_53	0.0059	0.0144
IGHV3_64	0.0020	0.0105
IGHV3_69_1	0.0039	0.0105
IGHV3_73	0.0098	0.0230
IGHV3_74	0.0020	0.0105
IGHV3_9	0.0039	0.0105
IGHV4_28	0.0059	0.0144
IGHV4_39	0.0020	0.0105
IGHV4_4	0.0195	0.0412
IGHV4_59	0.0039	0.0105
IGHV4-34	0.0488	0.0823
IGHV5_51	0.0020	0.0105
IGHV7_4_1	0.0039	0.0105

CB: Mutated/Mature

VH	p-Value	FDR
IGHV1_46	0.0004	0.0017
IGHV1_58	0.0052	0.0141
IGHV2_26	0.0002	0.0016
IGHV2_5	0.0002	0.0016
IGHV3_15	0.0006	0.0024
IGHV3_20	0.0005	0.0021
IGHV3_21	0.0004	0.0017
IGHV3_23	0.0001	0.0012
IGHV3_48	0.0009	0.0028
IGHV3_49	0.0001	0.0012
IGHV3_53	0.0004	0.0017
IGHV3_64	0.0009	0.0028
IGHV3_69_1	0.0004	0.0017
IGHV3_74	0.0001	0.0012
IGHV3_9	0.0002	0.0016
IGHV4_30_2	0.0085	0.0219
IGHV4_30_4	0.0052	0.0141
IGHV4_34	0.0001	0.0012
IGHV4_39	0.0203	0.0478
IGHV4_4	0.0023	0.0068
IGHV4_59	0.0009	0.0028
IGHV5_10_1	0.0156	0.0384
IGHV5_51	0.0001	0.0012
IGHV7_4_1	0.0017	0.0053

CB: PC/Mature[B]

VH	p-Value	FDR
IGHV1_46	0.0017	0.0154
IGHV1_69	0.0005	0.0099
IGHV1_8	0.0007	0.0099
IGHV3_43	0.0005	0.0099
IGHV3_7	0.0046	0.0313
IGHV3_72	0.0007	0.0099
IGHV3_74	0.0024	0.0188
IGHV4_34	0.0012	0.0132

CB: PC/Mutated[B]

IGHV1_3	0.0034	0.0185
IGHV1_46	0.0002	0.0044
IGHV1_8	0.0024	0.0185
IGHV2_26	0.0105	0.0405
IGHV2_5	0.0134	0.0453
IGHV3_11	0.0134	0.0453
IGHV3_23	0.0012	0.0132
IGHV3_30	0.0081	0.0363
IGHV3_43	0.0034	0.0185
IGHV3_64	0.0046	0.0228
IGHV3_7	0.0105	0.0405
IGHV3_72	0.0007	0.0099
IGHV4_39	0.0034	0.0185
IGHV4_4	0.0002	0.0044
IGHV4_59	0.0017	0.0154
IGHV5_51	0.0002	0.0044

A: only those V genes with a statistically significant p value <0.05 and false discovery rate (FDR) <0.05 are shown

B: no significant differences were seen in lupus controls