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Supplementary Materials for

Rituximab-resistant splenic memory B cells and newly engaged naive B cells fuel relapses in patients with immune thrombocytopenia

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at stm.sciencemag.org/cgi/content/full/13/589/eabc3961/DC1)

Data file S1 (Microsoft Excel format)

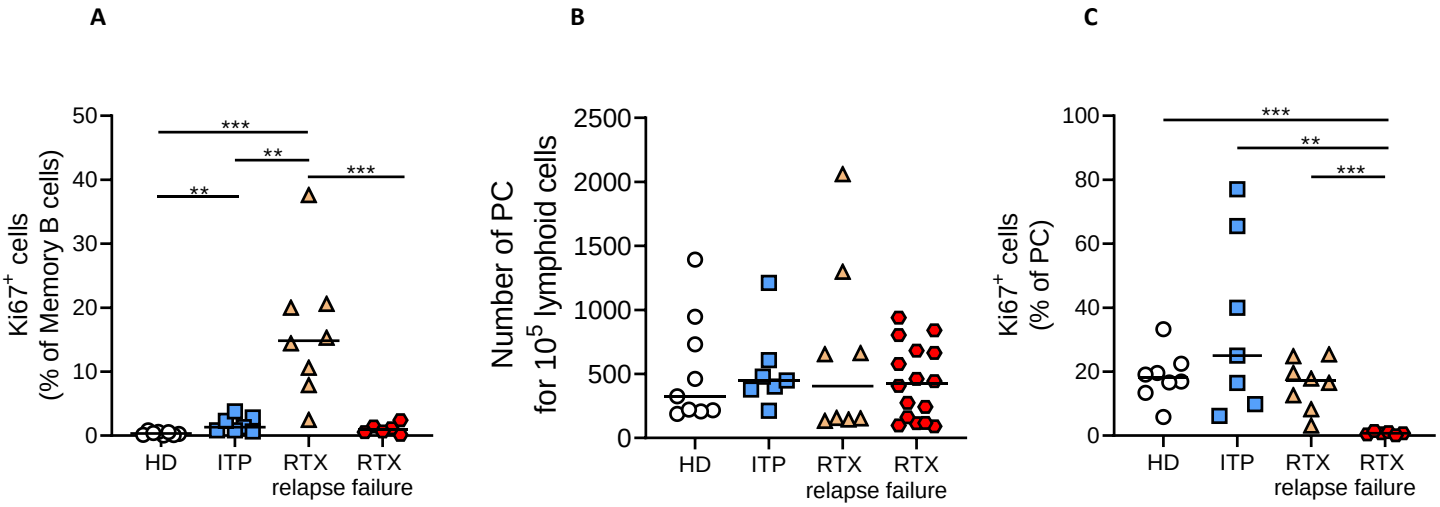

Fig. S1. Ki67 expression in splenic memory B cells and plasma cells.

Percentage of Ki67 expression on memory B cells (**A**), number of plasma cells (PC) for 10^5 lymphoid cells (**B**) and percentage of Ki67 expression and PC (**C**) in healthy donors (HD, white circles), patients with immune thrombocytopenia (ITP, blue squares), patients with relapse after RTX (RTX relapse, rose triangles) and patients with primary failure of RTX (RTX failure, red hexagons). Kruskal-Wallis and corrections for multiple comparisons were performed (***P < 0.001; **P < 0.01, *P < 0.05). Symbols indicate individual samples; horizontal bars represent median values.

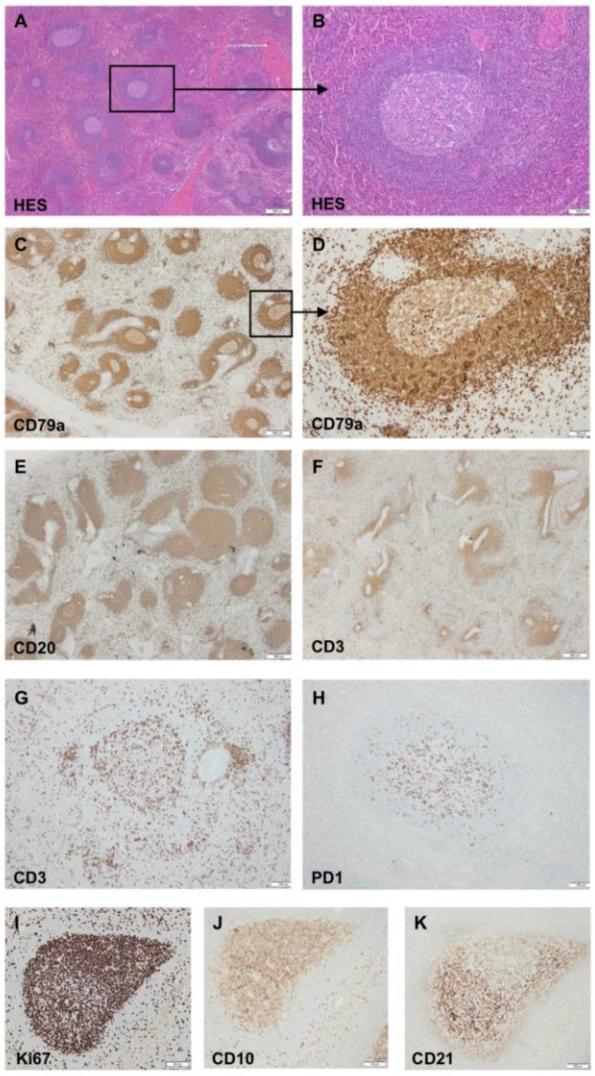

Fig. S2. Germinal centers in RTX relapse patients.

Representative spleen sections from patients with relapse after RTX (RTX relapse, n=2) stained with Hematoxylin Eosin Safran (HES) **(A-B)** and immunostained with CD79a **(C-D)**, CD20 **(E)**, CD3 **(F-G)**, PD-1 **(H)**, Ki67 **(I)**, CD10 **(J)** and CD21 **(K)** to identify germinal center (GC) B cells ($CD79a^{low}$, $CD10^{+}$, $Ki67^{+}$), T Cells ($CD3^{+}$), T follicular helper (T_{FH}) cells ($PD\text{-}1^{+}$), and follicular dendritic cells ($CD21^{+}$). (A, C, E and F) Scale bars: 500 μm. (B, D, G-K) Scale bars: 100 μm.

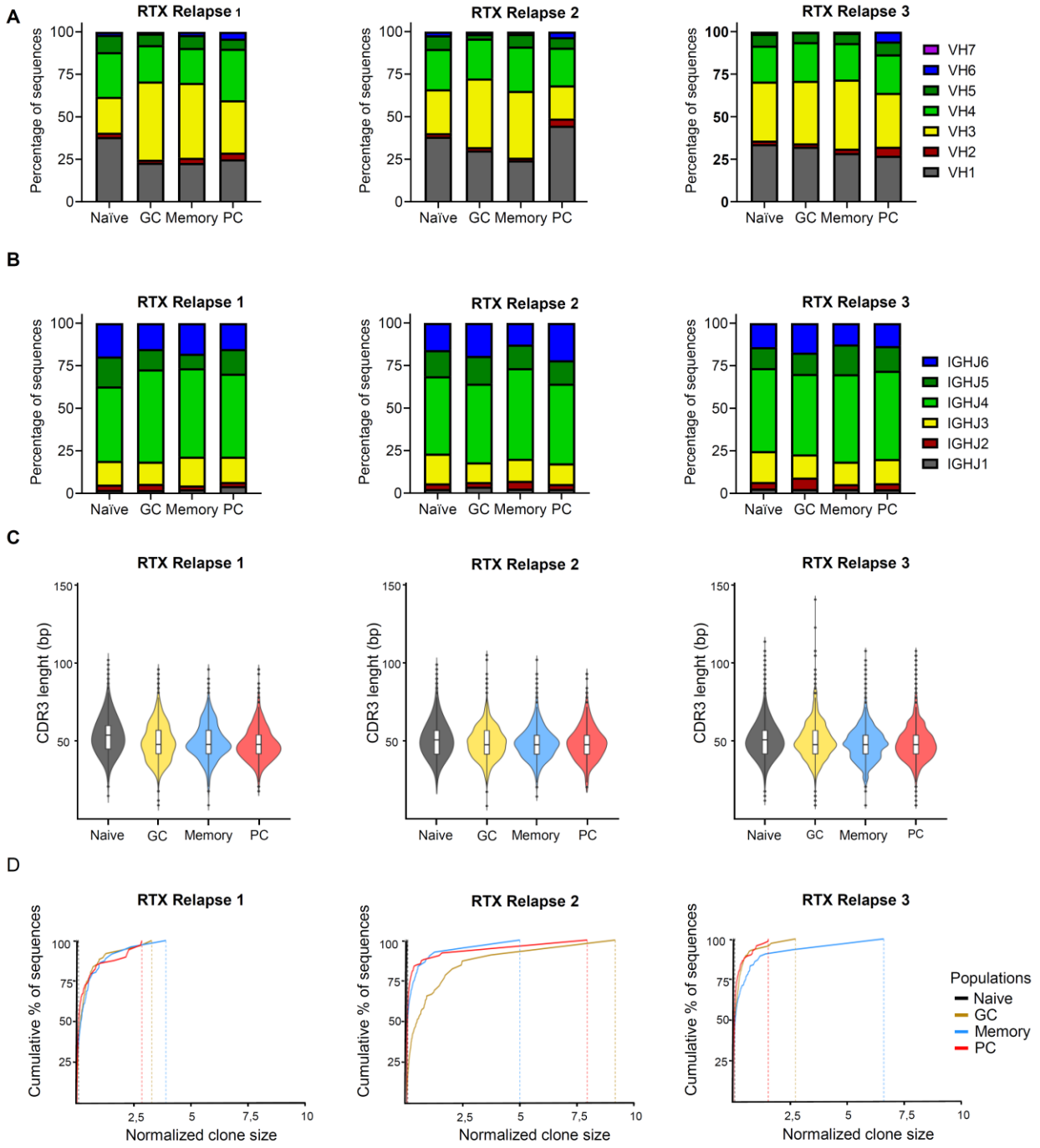

Fig. S3. Splenic B cell repertoire in RTX-relapse patients. High throughput IgH sequencing was used to analyze the splenic B cell repertoire of 3 patients with relapse after RTX (RTX relapse). VH **(A)** and JH **(B)** usage in sequences from naive, GC, memory and PC populations. **(C)** Violin plots showing CDR3 length distribution (in base pairs) in splenic B cell subsets. **(D)** Clonality of B cells subsets repertoire is shown by plotting the frequency of sequences in each individual clone among total sequences (clone size, horizontal axis), versus the contribution of individual clones to sequences (vertical axis) from smallest (bottom) to greatest (top). The naive B cell repertoire (green line), which contains only small clones, merges with the vertical axis, while antigen-experienced B cell repertoires exhibit variable clonal expansions.

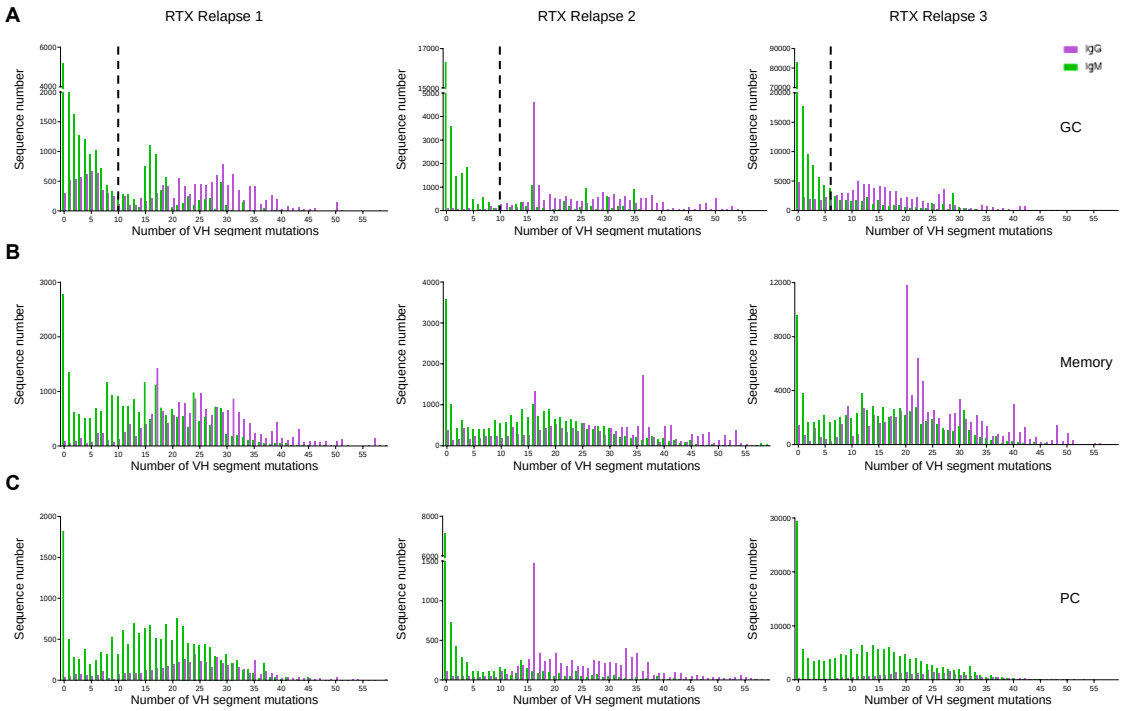

Fig. S4. IgM sequences predominate in newly generated B cells and IgG sequences in RTX-resistant cells. (A-C) VH segment mutation distribution in IgM (green) and IgG (purple) sequences from splenic GC **(A)**, memory **(B)** and PC **(C)** populations from 3 patients with relapse after RTX (RTX relapse) assessed by high-throughput IgH sequencing.

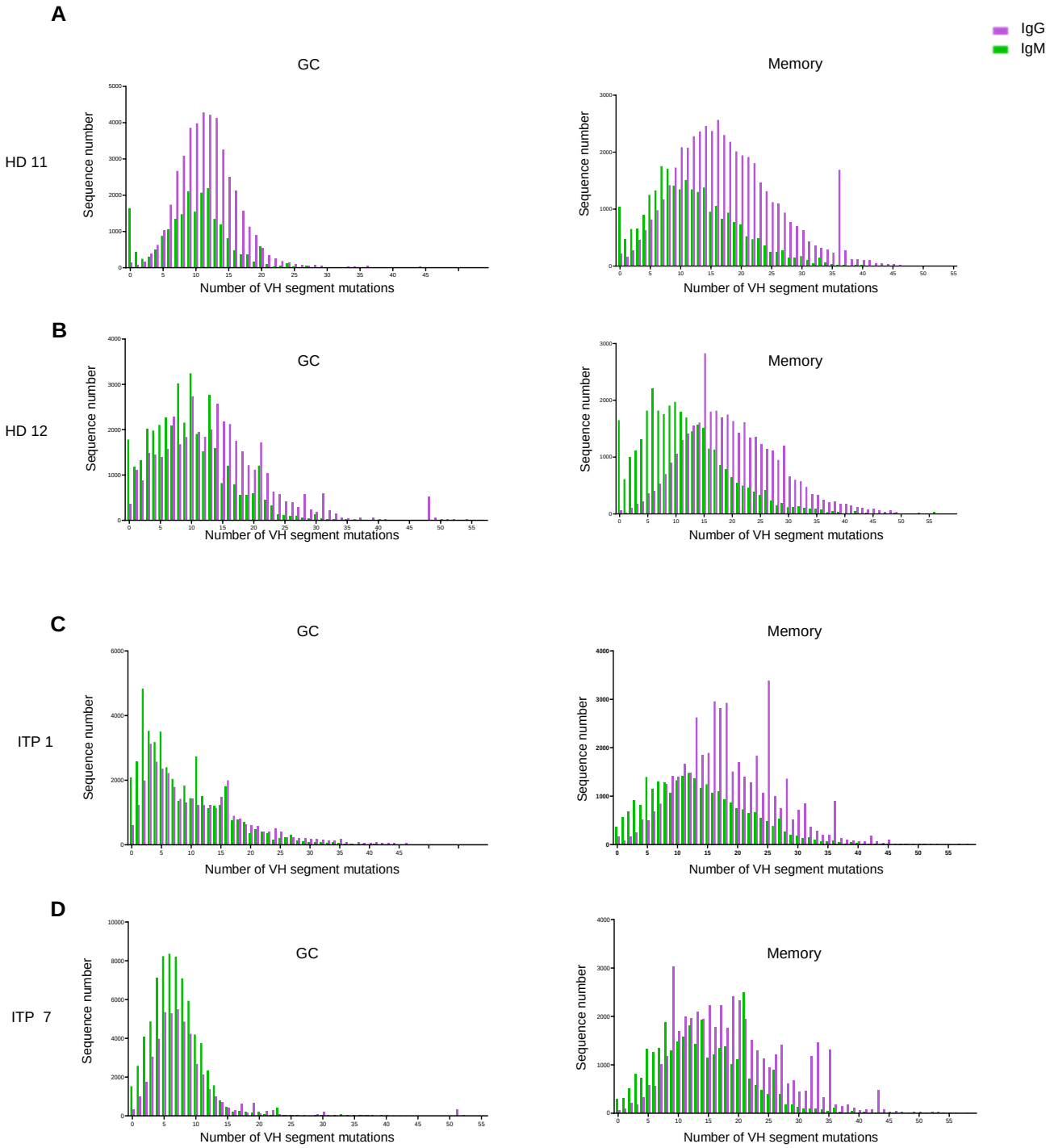

Fig. S5. VH segment mutation distribution in HD and patients with ITP. (A-D) VH segment mutation distribution in IgM (green) and IgG (purple) sequences from splenic GC and memory B cells populations from 2 HD **(A, B)** and 2 patients with ITP **(C, D)** assessed by high-throughput IgH sequencing.

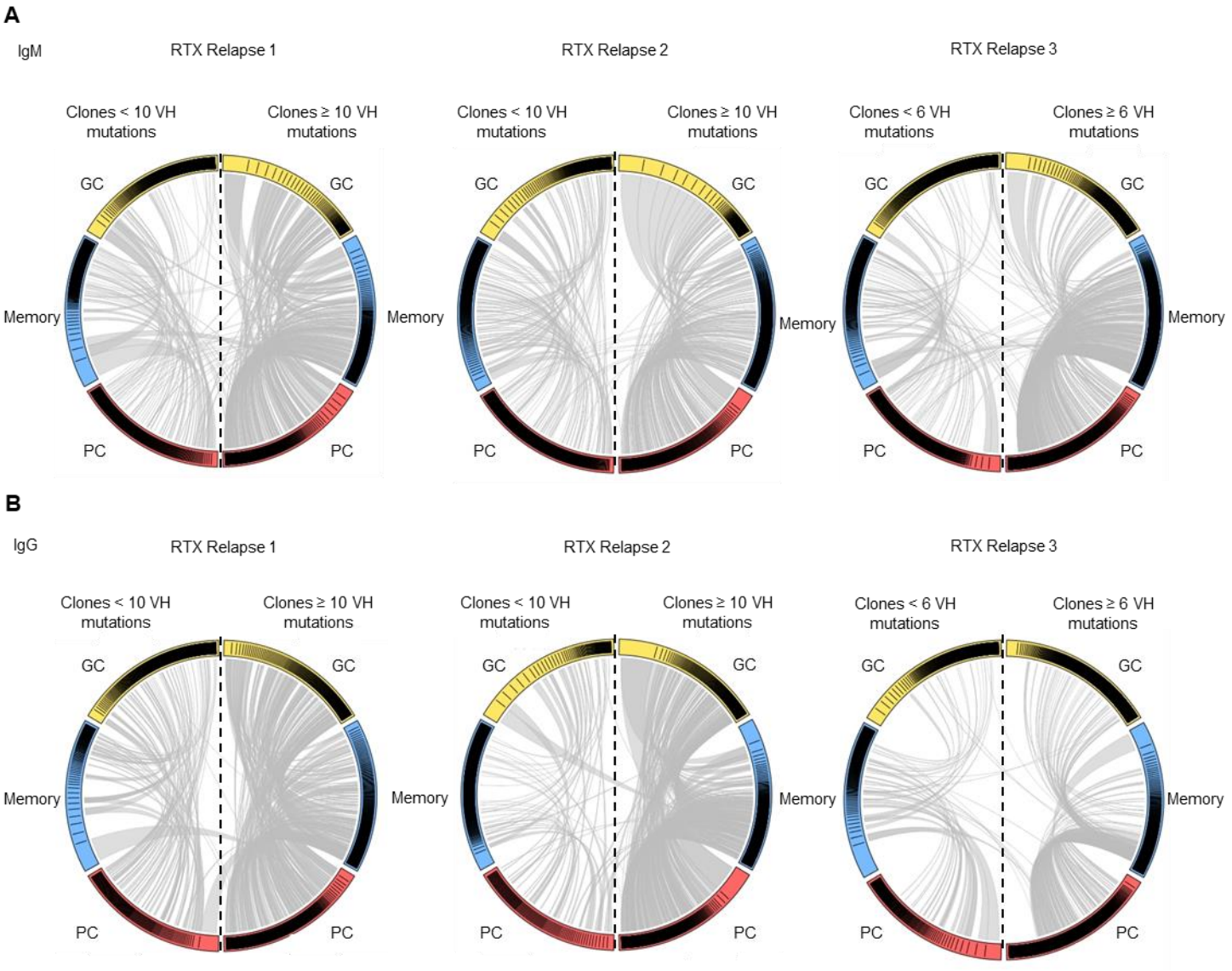

Fig. S6. Circos plots showing clonal relationships among IgM and IgG B cell populations in RTX relapse patients. (A-B) Circos plot showing clonal relationships shared between IgM **(A)** and IgG **(B)** sequences from GC, memory, and PC splenic populations. Clones from each population were classified into "lowly mutated" (left side of the plot) or "highly mutated" (right side of the plot) based on the clone median mutation number. Each colored sector represents one subpopulation and is divided into segments representing individual clones in rank-sized order. The internal connections show clones shared by multiple subpopulations.

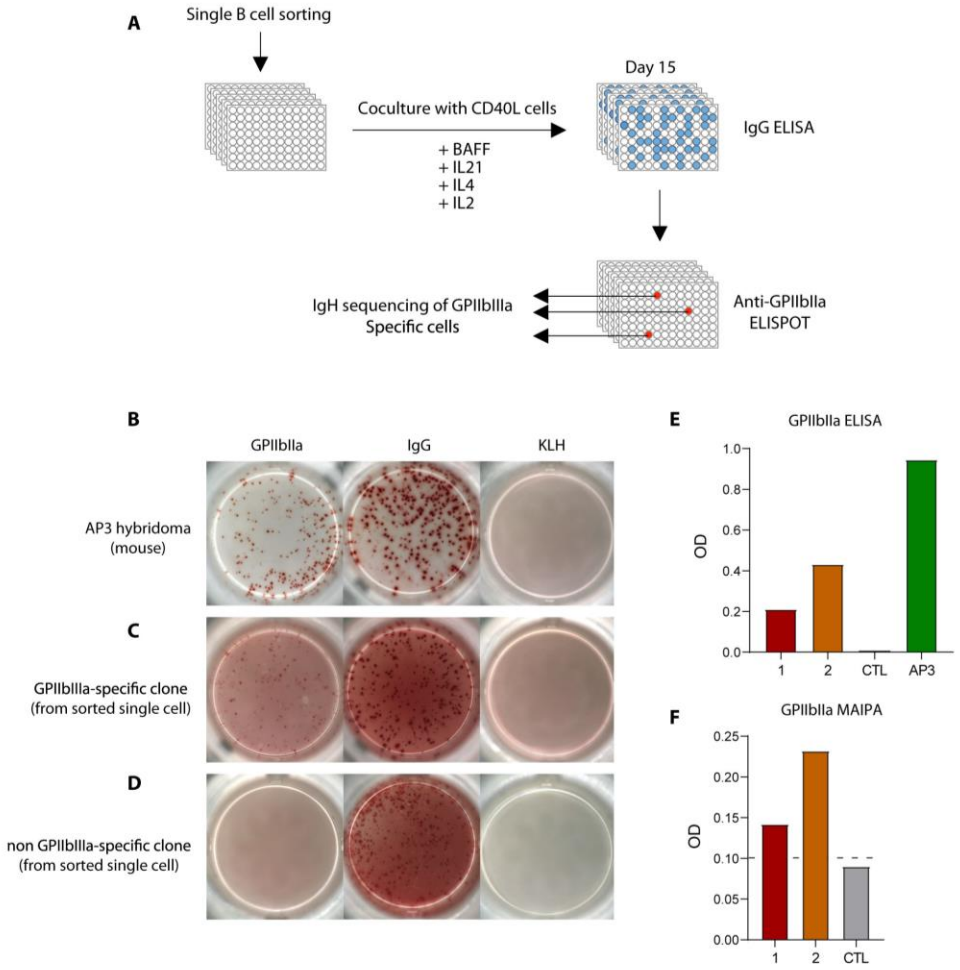

Fig. S7. Detection of autoreactive clones using IgG anti-GPIIbIIIa ELISPOT.
(A) To identify GPIIbIIIa-specific B cells, single GC and memory B cells were directly sorted into 96-well plates containing CD40L expressing cells and a cytokine cocktail and cultured for 15 days. IgG Enzyme-Linked Immunosorbent Assay (ELISA) was then performed on supernatants to select IgG secreting clones to be tested in a GPIIbIIIa ELISPOT. Cells from GPIIbIIIa-specific clones were collected for IgH sequencing. **(B-D)** IgG anti-GPIIbIIIa ELISPOT was used for the determination of clone specificities after single cell culture. The anti-GPIIbIIIa antibodies (mouse IgG1)-producing AP3 hybridoma was used for the validation of IgG anti-GPIIbIIIa ELISPOT. Representative pictures of wells containing AP3 hybridoma **(B)** or clones from GPIIbIIIa specific **(C)** or non-specific **(D)** single memory B cells cultured for 15 days on coated GPIIbIIIa (left), anti-human immunoglobulin (middle) and Keyhole Limpet Hemocyanin (KLH, right). **(E-F)** Immunoglobulin VH/VL genes from two clones (1 and 2) originating from GPIIbIIIa-specific single memory B cells were sequenced and cloned into Human Embryonic Kidney (HEK) cells. Anti-GPIIbIIIa ELISA **(E)** and Monoclonal Antibody Immobilization of Platelet Antigens (MAIPA) **(F)** performed with culture supernatants confirmed GPIIbIIIa reactivity. Irrelevant monoclonal human IgG1 was used as a negative control (CTL). Dotted line represents positive threshold for MAIPA assay.

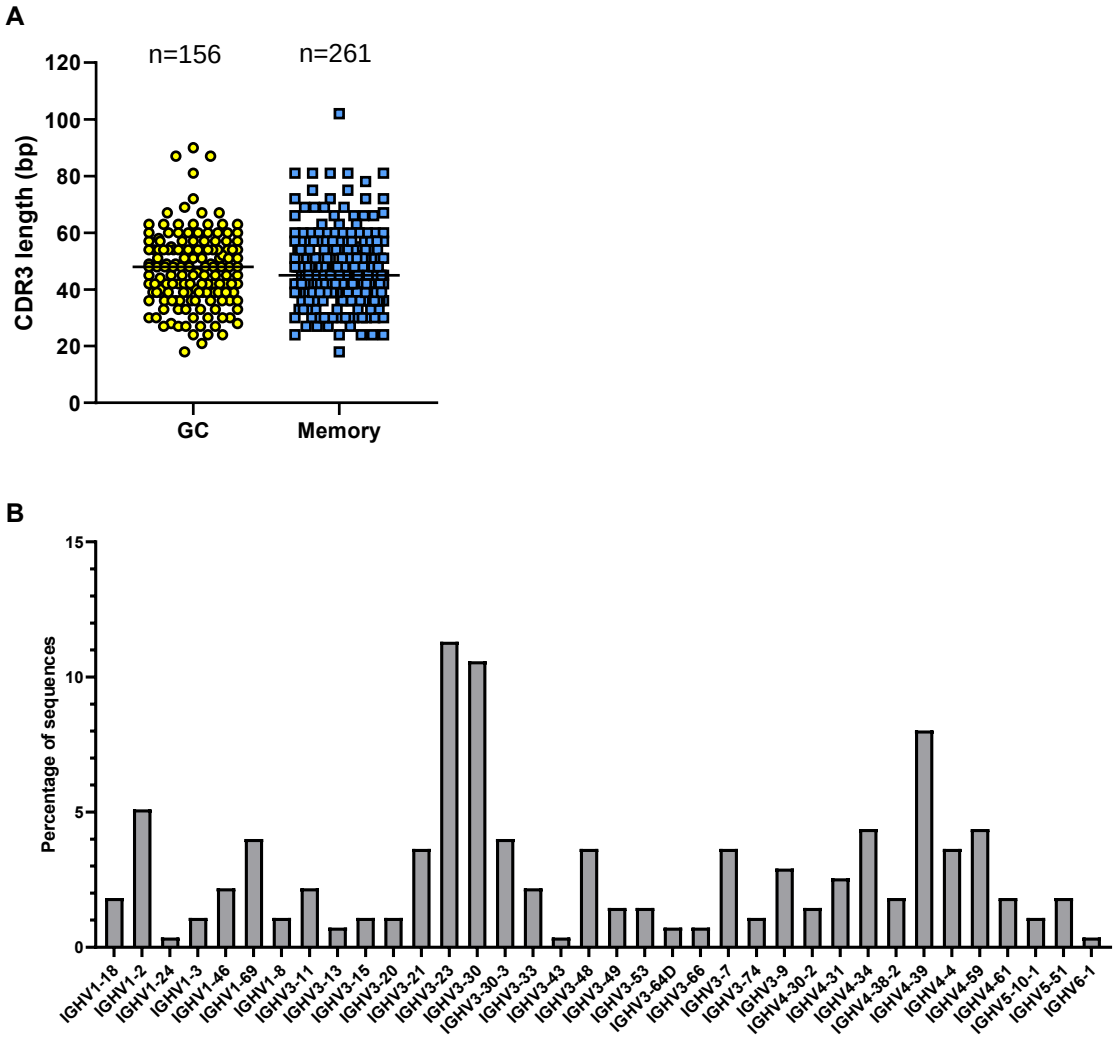

Fig. S8. GPIIbIIIa-specific B cell repertoire.

Sequences obtained from GPIIbIIIa specific GC (n= 156) and memory B cells (n=261) of patients with relapse after RTX (RTX relapse) were analyzed for CDR3 length **(A)** and VH gene distribution **(B)**.

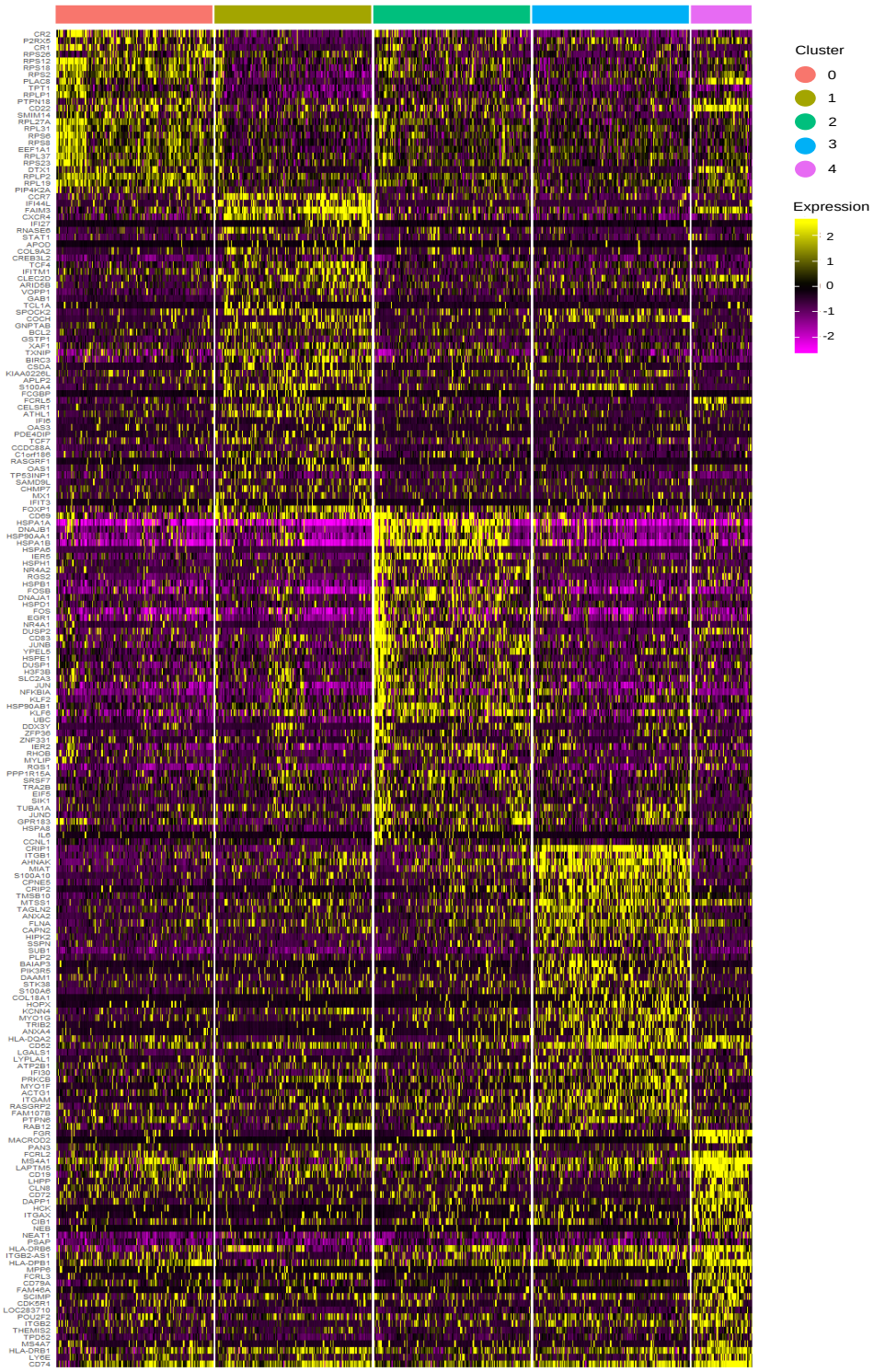

Fig. S9. Top marker genes identified for each individual memory B cell cluster.

Heatmap representing the scale expression (row-normalization) of the top markers genes identified for each of the five memory B cell clusters in the scRNA-seq dataset. Differential gene expression analysis was performed using the Model-based Analysis of Single-cell Transcriptomics (MAST) algorithm via the Seurat v3 R pipeline.

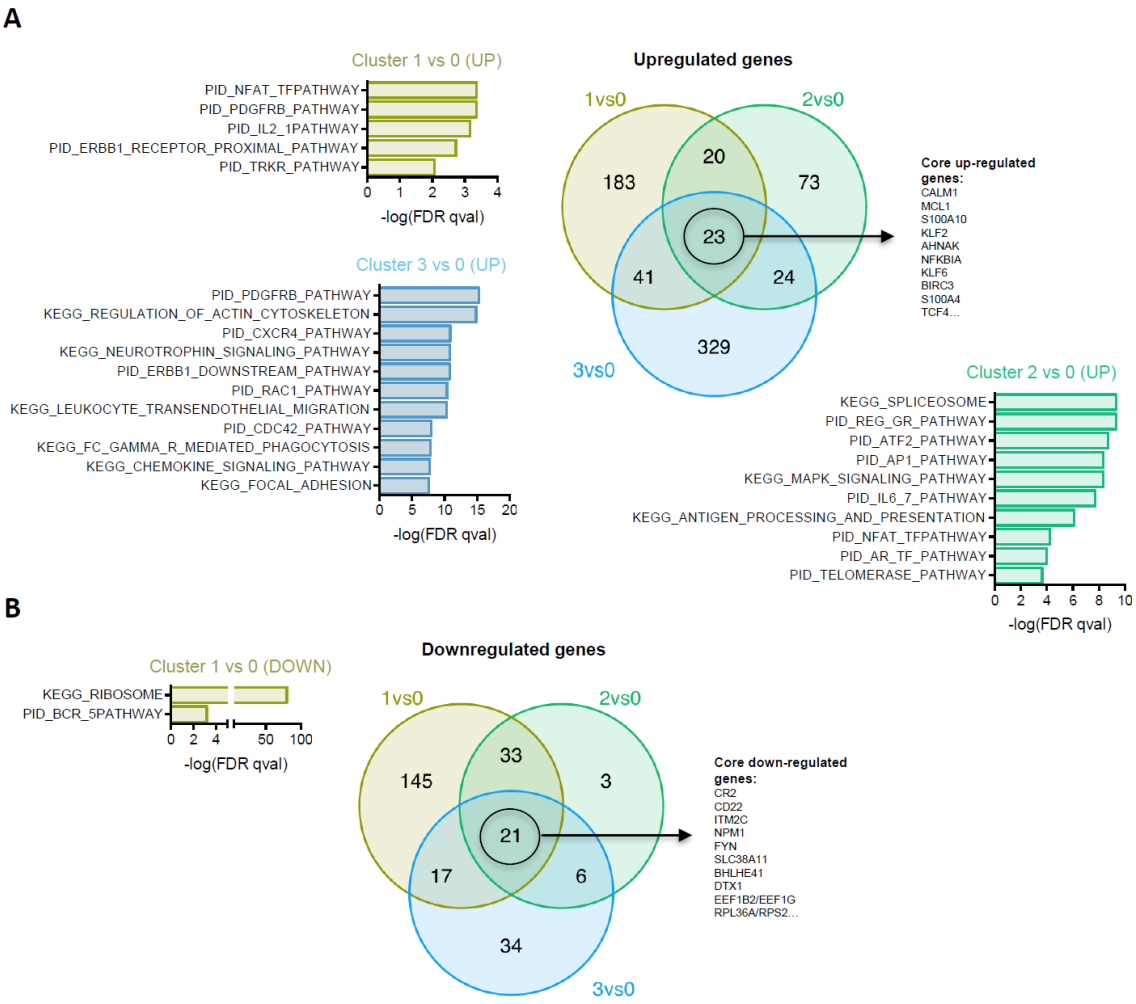
A
Cluster 1 vs 0 (UP)
PID_NFAT_TFPATHWAY
PID_PDGFRB_PATHWAY
PID_IL2_1PATHWAY
PID_ERBB1_RECEPTOR_PROXIMAL_PATHWAY
PID_TRKR_PATHWAY
0 1 2 3 4
-log(FDR qval)
Cluster 3 vs 0 (UP)
PID_PDGFRB_PATHWAY
KEGG_REGULATION_OF_ACTIN_CYTOSKELETON
PID_CXCR4_PATHWAY
KEGG_NEUROTROPHIN_SIGNALING_PATHWAY
PID_ERBB1_DOWNSTREAM_PATHWAY
PID_RAC1_PATHWAY
KEGG_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION
PID_CDC42_PATHWAY
KEGG_FC_GAMMA_R_MEDIATED_PHAGOCYTOSIS
KEGG_CHEMOKINE_SIGNALING_PATHWAY
KEGG_FOCAL_ADHESION
0 5 10 15 20
-log(FDR qval)
Upregulated genes
1vs0
2vs0
3vs0
183
20
73
23
41
24
329
Core up-regulated genes:
CALM1
MCL1
S100A10
KLF2
AHNAK
NFKBIA
KLF6
BIRC3
S100A4
TCF4...
Cluster 2 vs 0 (UP)
KEGG_SPLICEOSOME
PID_REG_GR_PATHWAY
PID_ATF2_PATHWAY
PID_AP1_PATHWAY
KEGG_MAPK_SIGNALING_PATHWAY
PID_IL6_7_PATHWAY
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION
PID_NFAT_TFPATHWAY
PID_AR_TF_PATHWAY
PID_TELOMERASE_PATHWAY
0 2 4 6 8 10
-log(FDR qval)
B
Cluster 1 vs 0 (DOWN)
KEGG_RIBOSOME
PID_BCR_5PATHWAY
0 2 4 50 100
-log(FDR qval)
Downregulated genes
1vs0
2vs0
3vs0
145
33
3
21
17
6
34
Core down-regulated genes:
CR2
CD22
ITM2C
NPM1
FYN
SLC38A11
BHLHE41
DTX1
EEF1B2/EEF1G
RPL36A/RPS2...
C
Gated on memory B cells
RTX relapse
HD
20.3 %
12.0%
0.0 %
62.4 %
RTX failure
ITP
0.0 %
79.5 %
0.8 %
75.7 %
FAS
CD29
D
FAS -
FAS +
***
*
55
40
20
0
Number of mutation in VH segment
RTX relapse
Donor 2
RTX relapse
Donor 4

Fig. S10. Characterization of memory B cells in RTX-relapse patients.

(A-B) Venn diagrams of upregulated (A) and downregulated (B) genes in memory B cells from clusters 1, 2, and 3 as compared to memory B cells from cluster 0 (MAST, logfc > 0.25 and corrected P-value>0.05). Number of uniquely or co-regulated genes are indicated inside each corresponding area of the Venn diagram. Side graphs show enriched pathways in the indicated list of uniquely upregulated (A) or downregulated (B) genes in memory B cells from clusters 1, 2, and 3 as compared to memory B cells from cluster 0. The GSEA/Molecular Signature DataBase (MSigDB) online tool was used to compute overlaps (Broad Institute). **(C)** Representative dot plots showing FAS and CD29 staining on live $CD3^-$ $CD14^-$ $CD19^+$ $CD24^+$ $CD38^-$ IgD^- $CD27^+$ memory B cells in patients with relapse after RTX (RTX relapse, top left), HD (top right), patients with failure of RTX (RTX failure, left bottom) and patients with ITP (right bottom). Gates and frequencies of FAS^+ and FAS^- populations are displayed. **(D)** Number of mutations in VH segment in sorted FAS^+ and FAS^- memory B cells from 2 patients with relapse after RTX (RTX relapse). Single cell-sorted FAS^+ and FAS^- memory B cells underwent single-cell IgH sequencing and individual IgH sequences were then analyzed using CodonCode and the IMGT/HighV-QUEST web portal. Two-tailed Mann-Whitney tests were performed (***P < 0.001; *P < 0.05).

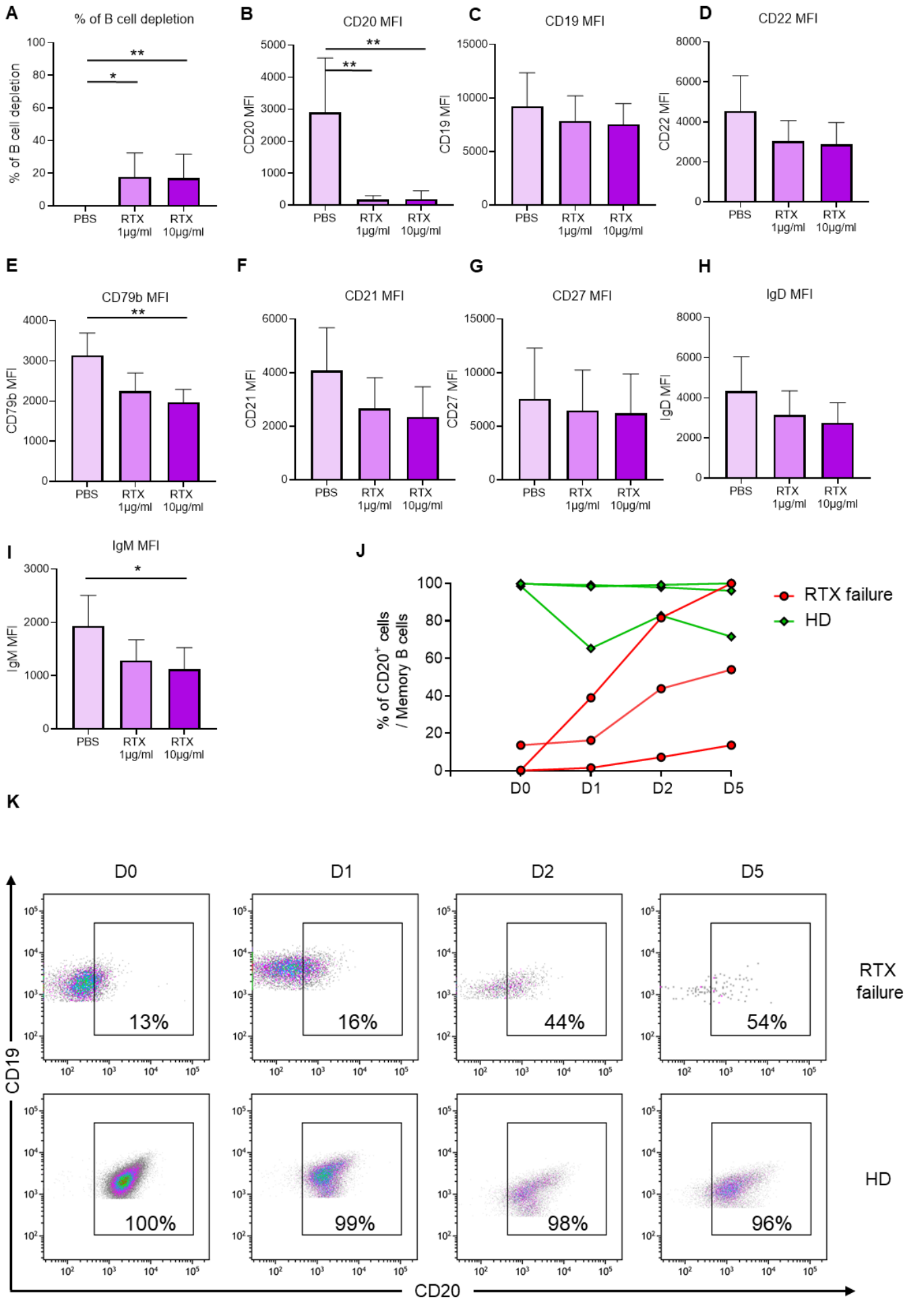
A
% of B cell depletion
% of B cell depletion
PBS
RTX 1µg/ml
RTX 10µg/ml
B
CD20 MFI
CD20 MFI
C
CD19 MFI
CD19 MFI
D
CD22 MFI
CD22 MFI
E
CD79b MFI
CD79b MFI
F
CD21 MFI
CD21 MFI
G
CD27 MFI
CD27 MFI
H
IgD MFI
IgD MFI
I
IgM MFI
IgM MFI
J
% of CD20+ cells / Memory B cells
D0
D1
D2
D5
RTX failure
HD
K
D0
D1
D2
D5
CD19
CD20
13%
16%
44%
54%
100%
99%
98%
96%
RTX failure
HD

Fig. S11. In vitro exposure to RTX induces a residual memory B cell-like phenotype in human splenic memory B cells.

Fresh total splenocytes from HD (n=5) were incubated overnight with RTX (1 or 10 µg/ml) or phosphate buffered saline (PBS). (**A**) Percentage of B cell depletion, calculated with the following formula: 100 –[(B cell:T cell ratio in sample with RTX)/(B cell:T cell ratio in sample with PBS) x 100]. (**B-G**) Histograms showing Median Fluorescence Intensity (MFI) ± standard deviation (SD) of CD20, CD19, CD22, CD79b, CD21 and CD27 on total B cells. (**H-I**) Histograms showing MFIs±SD of IgD and IgM on $CD27^-$ B cells. Kruskal-Wallis and corrections for multiple comparisons were performed (**P < 0.01, *P < 0.05). Percentage (**J**) and representative dot plots (**K**) showing CD20 expression assessed by flow cytometry analysis on memory B cells at day 0 (D0), D1, D2 and D5 of in vitro culture of splenocytes from HD (green, N=3) and patients with RTX failure (RTX failure, red, N=3). An increase in CD20 expression was observed in memory B cells from patients with RTX failure starting from D1.

Table S1. Patient characteristics. AZA: Azathioprine, CR: Complete Response CSA: Cyclosporine A, CST: Corticosteroids, IvIg: Intravenous immunoglobulins, DAPS: Dapsone, HCQ: Hydroxychloroquine, MMF: Mycophenolate mofetil, NA: Not applicable, TPO-RA: Thrombopoietin receptor agonists, RTX: Rituximab.

Patients	Age/Gender	ITP duration (months) before splenectomy	Interval between RTX and splenectomy (months)	Response to RTX (duration, months)	Treatments received before RTX and splenectomy	Treatments received during the month preceding splenectomy	Response to splenectomy
RTX_relapse1	30/F	26	12	CR (6 months)	CST, IvIg	IvIg	Failure
RTX_relapse2	61/F	28	15	CR (13 months)	CST, danazol	CST	CR
RTX_relapse3	42/F	21	12	CR (11 months)	CST	CST	CR
RTX_relapse4	51/M	60	11	CR (5 months)	CST, IvIg	IvIg	CR
RTX_relapse5	65/M	15	11	CR (6 months)	CST	CST	CR
RTX_relapse6	52/F	44	8	CR (6 months)	CST, IvIg, danazol	CST, IvIg	Failure
RTX_relapse7	45/F	21	14	CR (12 months)	CST, DAPS	CST	CR
RTX_relapse8	62/F	156	14	CR (12 months)	CST, TPO-RA, IvIg, DAPS	TPO-RA, IvIg	CR
RTX_failure1	59/F	12	4	Failure	CST, IvIg	CST, IvIg	CR
RTX_failure2	39/M	360	4	Failure	CST	CST	CR
RTX_failure3	22/F	12	3	Failure	TPO-RA, CST, Vcr	TPO-RA, CST	CR
RTX_failure4	24/M	36	1	Failure	CST, Vcr	CST, Vcr, tacrolimus	CR
RTX_failure5	48/F	24	4	Failure	CST, IvIg, AZA	CST, IvIg, AZA	Failure
RTX_failure6	51/F	3	3	Failure	CST, IvIg, Vcr, danazol	CST, IvIg, Vcr, danazol	CR
RTX_failure7	76/M	23	9	Failure	TPO-RA, CST, IvIg	CST	Failure
RTX_failure8	54/M	3	4	Failure	CST, DAPS	CST, DAPS	Failure
RTX_failure9	66/F	6	4	Failure	CST, IvIg, DAPS	CST, IvIg	CR
RTX_failure10	27/M	8	6	Failure	CST, IvIg, DAPS, HCQ	CST, IvIg, DAPS, HCQ	CR
RTX_failure11	18/M	6	2	Failure	CST, IvIg, TPO-RA, Vcr	CST, IvIg, TPO-RA	CR
RTX_failure12	36/F	6	3	Failure	CST, IvIg, TPO-RA, Vcr, AZA	CST, IvIg, TPO-RA, Vcr	CR
RTX_failure13	28/F	36	6	Failure	CST, IvIg, DAPS, Vcr	CST, IvIg, DAPS, Vcr	CR
RTX_failure14	74/F	48	6	Failure	CST, IvIg, DAPS	CST, IvIg, DAPS	Failure
RTX_failure15	67/M	6	6	Failure	CST, IvIg, TPO-RA, danazol, MMF	CST, IvIg	CR
RTX_failure16	37/M	60	1	Failure	CST, IvIg, TPO-RA, CSA, MMF	CST, IvIg, TPO-RA, CSA, MMF	CR
RTX_failure17	59/F	120	4	Failure	CST IvIg	CST IVIg	CR
RTX_failure18	37/M	12	2	Failure	CST, IVIg, TPO-RA, AZA	CST, IVIg, TPO-RA, AZA	CR
RTX_failure19	26/M	6	4	Failure	CST, IVIg, TOP-RA, MMF	CST, IVIg	Failure
ITP1	25/F	84	NA	NA	CST, IvIg, DAPS	CST	CR
ITP2	40/F	12	NA	NA	IgIV, AZA, CST, DAPS	IgIV, AZA	CR
ITP3	60/H	36	NA	NA	CST, IvIg	IgIV	CR
ITP4	50/F	28	NA	NA	CST, TPO-RA	TPO-RA	CR
ITP5	58/M	144	NA	NA	CST, IvIg, TPO-RA	IvIg, TPO-RA	CR
ITP6	22/M	24	NA	NA	CST	CST	Failure
ITP7	18/F	12	NA	NA	CST	CST	CR
HD1	52/F	NA	NA	NA	NA	NA	NA
HD2	22/M	NA	NA	NA	NA	NA	NA
HD3	32/F	NA	NA	NA	NA	NA	NA
HD4	52/F	NA	NA	NA	NA	NA	NA
HD5	28/M	NA	NA	NA	NA	NA	NA
HD6	62/M	NA	NA	NA	NA	NA	NA
HD7	64/M	NA	NA	NA	NA	NA	NA
HD8	90/F	NA	NA	NA	NA	NA	NA
HD9	68/M	NA	NA	NA	NA	NA	NA
HD10	60/F	NA	NA	NA	NA	NA	NA
HD 11	21/F	NA	NA	NA	NA	NA	NA
HD 12	34/F	NA	NA	NA	NA	NA	NA
YD1	5/F	NA	NA	NA	NA	NA	NA
YD2	4/M	NA	NA	NA	NA	NA	NA
YD3	4/F	NA	NA	NA	NA	NA	NA
YD4	4.5/M	NA	NA	NA	NA	NA	NA

Table S2. Immunoglobulin heavy chain sequencing. *d50 : number of top size-ranked clones accounting for 50% of unique sequences

Patient	Population	Number of sorted cells	Isotype	Raw reads	Submitted to IMGT	Number of productive sequences	Number of unique sequences	Number of clones	d50*
RTX relapse 1	Naive	100000	MU	113836	7912	17975	7690	7579	2775
	Memory	130000	GAMMA	104892	2773	19307	2535	1167	69
			MU	105582	5833	24859	5509	3042	35
	GC	70000	GAMMA	101734	3058	15481	2861	1294	82
			MU	105943	4082	23315	3394	1336	50
	PC	70000	GAMMA	172413	1847	5820	1817	1221	142
			MU	110724	3119	16658	3072	1546	101
RTX relapse 2	Naive	100000	MU	107764	7892	16734	7630	7518	2763
	Memory	200000	GAMMA	119216	5991	20012	5581	3625	61
			MU	103684	5814	24496	5216	2951	203
	GC	10000	GAMMA	116882	2676	23672	2484	575	24
			MU	102299	3327	34079	3123	1042	21
	PC	50000	GAMMA	147534	2223	8985	2155	1286	60
			MU	96436	2784	11445	2758	2166	391
RTX relapse 3	Naive	100000	MU	1410223	50521	119460	45552	38568	9259
	Memory	30000	GAMMA	1302391	17385	92280	15358	5157	33
			MU	1481840	16294	80997	13508	5448	349
	GC	20000	GAMMA	1384055	22692	88691	19497	5806	217
			MU	1600195	30613	160998	26058	6523	104
	PC	80000	GAMMA	1427490	12732	31536	11805	7002	451
			MU	1766698	35347	163650	33758	10485	288

Table S3. Mean standard deviation of V_H mutation numbers in the first 100 clones.

	GC	Memory	PC
RTX relapse 1	1.5295	1.9508	2.8172
RTX relapse 2	1.5959	2.2332	2.0971
RTX relapse 3	3.6396	2.2199	3.7895

Table S4. Identification of anti-GPIIbIIIa-specific single B cells. *lack of in vitro activation.

Patients	Subset	Number of sorted cells	Number of IgG secreting clones	Number of GPIIbIIIa specific clones	Percentage of GPIIbIIIa specific clones among IgG secreting clones
RTX_relapse1	GC	1152	214	47	21.96
RTX_relapse1	IgG+ Memory	1152	438	51	11.64
RTX_relapse2	GC	1440	89	31	34.83
RTX_relapse2	IgG+ Memory	576	236	61	25.85
RTX_relapse3	GC	1632	190	27	14.21
RTX_relapse3	IgG+ Memory	1248	430	68	15.81
RTX_relapse4	GC	1056	261	41	15.71
RTX_relapse4	IgG+ Memory	672	307	42	13.68
RTX_relapse5	GC	480	84	0	0.00
RTX_relapse5	IgG+ Memory	480	113	0	0.00
RTX_relapse6	GC	576	36	7	19.44
RTX_relapse6	IgG+ Memory	576	241	38	15.77
RTX_relapse7	GC	576	90	3	3.33
RTX_relapse7	IgG+ Memory	576	127	1	0.79
RTX_relapse8	GC	576	0*	0	ND
RTX_relapse8	IgG+ Memory	576	0*	0	ND
RTX_failure1	Residual Memory	1152	179	27	15.00
RTX_failure2	Residual Memory	1152	43	2	4.70
RTX_failure3	Residual Memory	768	124	13	11.00
RTX_failure4	Residual Memory	1152	134	16	12.00
HD5	GC	576	89	3	3.37
HD5	IgG+ Memory	288	157	1	0.64
HD6	GC	576	80	0	0.00
HD6	IgG+ Memory	576	230	0	0.00
HD8	GC	576	227	9	3.96
HD8	IgG+ Memory	288	183	3	1.64

Table S5. scRNA-seq statistics. YD: Young Donor.

						Number of cells per cluster				
Patient	**Sort_ID**	**High quality cells**	**Mean Reads per cell**	**Mean Transcripts (UMI) per cell**	**Mean Genes per cells**	**Cluster 0**	**Cluster 1**	**Cluster 2**	**Cluster 3**	**Cluster 4**
HD1	2019_2	217	62597	4301	1441	211	5	0	1	0
HD2	2019_3	233	90801	6586	2004	215	5	0	7	6
HD4	2019_0	53	177809	10237	2274	51	2	0	0	0
ITP1	2019_2	274	79634	5534	1690	262	2	3	4	3
ITP2	2019_3	238	73642	5138	1655	116	21	71	5	25
ITP3	2019_4/2019_1	362	91035	5276	1678	335	7	1	12	7
RTX-failure 1	2019_2	254	83192	5692	1873	29	206	12	6	1
RTX-failure 2	2019_3	259	85034	6221	1957	61	127	70	1	0
RTX-failure 3	2019_4	267	91030	4485	1620	24	241	2	0	0
RTX-relapse 2	2019_3	237	80324	5524	1791	63	7	69	90	8
RTX-relapse 3	2019_2	240	80041	5515	1717	40	8	149	42	1
RTX-relapse 4	2019_4	230	112451	5540	1726	39	8	106	76	1
YD1	2019_0	57	159489	7789	1952	1	2	54	0	0
YD2	2019_0	63	85367	5211	1518	24	3	32	3	1
YD3	2019_0	42	109403	6122	1758	26	6	8	2	0
YD4	2019_0	90	115433	7193	2056	72	6	6	6	0

					nb total cell per cluster				
Total cells	**Mean Reads per cell**	**Mean Transcripts per cell**	**Mean Genes per cell**	**Cluster 0**	**Cluster 1**	**Cluster 2**	**Cluster 3**	**Cluster 4**
3116	98580	6023	1794	1569	656	583	255	53

Table S6. Antibodies and clones.

Antigen	Fluorochrome	Origin	Clone
CD38	peridinin chlorophyll protein (PerCP)-Cyanin (Cy) 5.5	Sony	HIT2
CD38	Brilliant Blue 700	BD Biosciences	HIT2
CD27	Allophycocyanin (APC)	Sony	MT271
CD27	Phycoerythrin (PE)-Cy7	BD Biosciences	LG.3A10
CD19	PE-CF594	BD Biosciences	HIB19
CD19	Alexa Fluor 700	BD Biosciences	HIB20
CD24	Fluorescein isothiocyanate (FITC)	BD Biosciences	ML5
CD24	PE-Cy7	Sony	ML5
CD24	FITC	BD Biosciences	ML5
CD21	PE-Cy7	BD Biosciences	BLY4
CD20	APC-H7	BD Biosciences	2H7
CD3	Alexa Fluor 700	BD Biosciences	UCHT1
CD3	Brilliant Ultraviolet 395	BD Biosciences	UCHT1
CD3	PE	BD Biosciences	UCHT1
CD14	Alexa Fluor 700	BD Biosciences	MϕP9
CD14	Brilliant Ultraviolet 395	BD Biosciences	MϕP9
CD14	PE	BD Biosciences	M5E2
CD16	Brilliant Ultraviolet 395	BD Biosciences	3G8
CD16	PE	BD Biosciences	3G8
CD16	Alexa Fluor 700	BD Biosciences	3G8
CD10	PE	BD Biosciences	Hi10a
CD95	APC	BioLegend	DX2
CD29	APC	BioLegend	TS2/16
CD29	PE	BioLegend	TS2/16
IgD	Brilliant Violet 421	BD Biosciences	IA6-2
IgD	Brilliant Violet 711	BD Biosciences	IA6-2
IgD	PE-CF594	BD Biosciences	IA6-2
IgM	Brilliant Violet 605	BioLegend	MHM88
IgM	Brilliant Violet 510	BD Biosciences	G20-127
IgG	Brilliant Violet 605	BD Biosciences	G18-145
IgG	APC-H7	BD Biosciences	G18-145
IgA	APC	Miltenyi	IS11-8E10
IgA	PE	Miltenyi	IS11-8E10
Ki67	Alexa Fluor 488	BD Biosciences	B56
CD22	APC	BD Biosciences	HIB22
CD79b	PE	BD Biosciences	CB3-1
TOSO (Fcμ-R)	Brilliant Violet 421	BD Biosciences	HM14-1
pY759 -PLCγ2	Alexa 488	BD Biosciences	K86-689.37
pY84-BLNK	PE	BD Biosciences	J117-1278
Live Dead Aqua		Thermo Fisher Scientific	
Zombie Violet		BioLegend	
Sytox Blue		Thermo Fisher Scientific	

Table S7. Primers.

NGS Primers	
dsDNA Primers	
L_VH1*-r	CTCGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNNNNNCAACTACAGGTGCCCACTCC
L_VH1-46-r	CTCGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNNNNNTAGCTCCAGGTGCTCACTCC
L_VH1-69-r	CTCGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNNNNNCAGCYACAGGTGTCCASTCC
L_VH1-2-r	CTCGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNNNNNCMACAGGWGCCCACTCC
L_VH1-45-r	CTCGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNNNNNAGCCACAGATGCCTACTCC
L_VH1-24-r	CTCGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNNNNNCTACAGGCACCCACGCC
L_VH2-r	CTCGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNNNNNCCKTCCTGGGTCTTRTCC
L_VH2-70*09-r	CTCGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNNNNNCCTTCATGGGTCTTGTCT
L_VH3*-r	CTCGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNNNNNTTWAAAGGTGTCCAGTGTGARG
L_VH3-30/33/11-r	CTCGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNNNNNWTAARAGGTGTCCAGTGTCAGG
L_VH4-r	CTCGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNNNNNCCAGATGGGTCCTGYCC
L_VH5-51-r	CTCGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNNNNNTTCTCCAAGGAGTCTGTKCC
L_VH6-1-r	CTCGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNNNNNCCATGGGGTGTCCTGTCA
PCR1 primers	
Forward primer	
IL-R2	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG
Reverse primers	
CHM-2DW	CAGGAGACGAGGGGGAAAAGG
CHA-2DW	GGAAGAAGCCCTGGACCAGGC
CHG-D1	TTCGGGGAAGTAGTCCTTG
CHD-n1	TATCAAGCATGCCAGGACCAC
PCR2 primers	
Reverse primers	
HCA-n2p_ILr	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCGATGACCACGTTCCCATCT
HCGn2p_ILr	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGAAGTAGTCCTTGACCAGGCA
HCM-n2m_ILr	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAAAGGGTTGGGGCGGATGC
HCD-n2m_ILr	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGGGAACACATCCGGAGCCT
Single cell primers	
Cγ CH1	GGAAGGTGTGCACGCCGCTGGTC
L-VH mix:	
L-VH1	ACAGGTGCCCACTCCCAGGTGCAG
L-VH 3	AAGGTGTCCAGTGTGARGTGCAG
L-VH 4/6	CCCAGATGGGTCCTGTCCCAGGTGCAG
L-VH 5	CAAGGAGTCTGTTCCGAGGTGCAG
5'AgeI VH mix :	
5'AgeIVH 1/5	CTGCAACCGGTGTACATTCCGAGGTGCAGCTGGTGCAG
5'AgeIVH 3	CTGCAACCGGTGTACATTCTGAGGTGCAGCTGGTGGAG
5'AgeIVH 4	CTGCAACCGGTGTACATTCCCAGGTGCAGCTGCAGGAG
5'AgeIVH 6-1	CTGCAACCGGTGTACATTCCCAGGTACAGCTGCAGCAG
3'SalI JH mix :	
3'SalI JH 1/2/4/5	TGCGAAGTCGACGCTGAGGAGACGGTGACCAG
3' SalI JH 3	TGCGAAGTCGACGCTGAAGAGACGGTGACCATTG
3' SalI JH 6	TGCGAAGTCGACGCTGAGGAGACGGTGACCGTG