Supplemental Information

Thiel, Schmidt et al.

Supplemental Figure Legend

Supplemental figure 1:

(A) Correlation of RTX sum dose (g) with duration of B cell depletion (months). (B) Concentration of serum immunoglobulins measured in patients' serum is correlated with the duration of B cell depletion in months after RTX treatment. (C) Effect of different maintenance treatment on the duration of B cell depletion after RTX treatment. (D) Absolute B cell count/µl blood in RTX-treated AAV patients divided by disease type. (E) Absolute B cell count/µl blood and duration of B cell depletion after RTX treatment in RTX-treated AAV patients divided by CYC treatment. (F) Correlation of creatinine (mg/dl) and (G) of eGFR (ml/min/1.73qm) with duration of B cell depletion (months). Data was analyzed using Kruskal-Wallis test, corrected for multiple comparisons by Dunn's multiple comparison tests or Mann-Whitney test and depicted as $*p < 0.05$, $**p < 0.01$. Correlation analysis was performed by nonparametric spearman correlation.

Supplemental figure 2:

(A) Gating strategy to analyze peripheral B cell subpopulations. $CD19^+$ B cells were gated on after doublet and dead cell exclusion in lymphocytes. (B) Peripheral subpopulations were analyzed dividing the AAV patients according to their diagnosis (GPA, MPA or EGPA). B cell subpopulations are shown in treatment-naïve patients compared to HD. Patients are treatment-naïve for immunosuppressive treatment except for short-term glucocorticoids. Absolute count/µl blood and frequency within total B cells of Transitional B cells ($IgM^{++}CD38^{++}$), Naive B cells (IgD^+CD27^-), Marginal Zone-like B cells (MZ, IgD^+CD27^+), Switched memory B cells (IgD^-CD27^+), Double negative B cells (DN, IgD-CD27-), and Plasmablasts ($CD38^{++}CD27^{++}$). (C) Frequency of switched immunoglobulin subclasses$^+$ in IgM^-IgD^- B cells in HD and treatment-naïve patients. Data was analyzed using Kruskal-Wallis test, corrected for multiple comparisons by Dunn's multiple comparison tests or Mann-Whitney test and depicted as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Supplemental figure 3:

(A) Peripheral subpopulations were analyzed dividing the AAV patients according to their diagnosis (GPA, MPA or EGPA). B cell subpopulations are shown in patients that received RTX-induction therapy compared to HD. Patients received either no additional DMARD or either MTX or LEF or AZA or MMF maintenance therapy at time of analysis. Absolute count/µl blood and frequency within total B cells of Transitional B cells ($IgM^{++}CD38^{++}$), Naive B cells ($IgD^{+}CD27^{-}$), Marginal Zone-like B cells (MZ, $IgD^{+}CD27^{+}$), Switched memory B cells ($IgD^{-}CD27^{+}$), Double negative B cells (DN, $IgD^{-}CD27^{-}$), and Plasmablasts ($CD38^{++}CD27^{++}$).

(B) Switched immunoglobulin subclass expression was analyzed within $IgM^{-}IgD^{-}$ switched B cells. Data was analyzed using Kruskal-Wallis test, corrected for multiple comparisons by Dunn's multiple comparison tests and depicted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

Supplemental figure 4:

(A) Representative flow plot of CD86 expression (left) and bar graph of MFI of CD86 (right) in B cells. (B) Representative flow plots of CD95 expression (left) and bar graphs of frequency of $CD95^{+}$ (right) in naïve and switched memory B cells. Data was analyzed using Kruskal-Wallis test, corrected for multiple comparisons by Dunn's multiple comparison tests and depicted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

Supplemental figure 5:

(A) Gating strategy of B cell populations in CyTOF analyzed BM samples of HD and AAV. (B, C) Ex vivo BM B cells were also analyzed by flow cytometry. (B) Representative flow plots of $CD10^{+}CD38^{+}$ lymphoid precursors and CLP/pro-B, pre-B and immature B cells in HD (upper row) and AAV (lower row). (C) Frequency of $CD34^{+}$ and $CD10^{+}CD38^{+}$ cells in live and frequency of CLP/pro-B, pre-B and immature B cells in $CD10^{+}CD38^{+}$ cells. (D) Frequency of $CD33^{+}$ cells within live cells over time in *in vitro* culture of early B cell development from $CD34^{+}$ BM cells. (C) Filled circles: treatment-naïve except for short-term glucocorticoids; empty triangle: CYC+ high-dose prednisolone; empty square: RTX-induction therapy + MTX maintenance therapy. Data was analyzed using Mann-Whitney test and depicted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Supplemental figure 6:

(A) Median fluorescence intensity (MFI) of BAFF-R in peripheral B cell subpopulations in HD and treatment-naïve AAV patients. (B) Median fluorescence intensity (MFI) of BAFF-R in peripheral B cell subpopulations in HD and AAV patients that received RTX-induction treatment and either no additional DMARD or either MTX or LEF or AZA or MMF maintenance therapy at time of analysis. (C) BAFF-R expression in non-Plasmablasts in HD and AAV without and with acidic elution of BAFF before the staining. Data was analyzed using Kruskal-Wallis test, corrected for multiple comparisons by Dunn's multiple comparison tests or Mann-Whitney test and depicted as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

Supplemental Table S1

Marker	Clone	Fluorophore	Supplier
CD95	DX2	BV421	BD
CD3	OKT3	SB 436	Life Technologies
CD16	3G8	SB 436	Invitrogen
CD33	WM-53	SB 436	Invitrogen
CD20	2H7	PB	BioLegend
CD69	FN50	BV 480	BD Bioscience
BAFF-R	11C1	BV 605	BD
CD38	HB-7	BV 650	BioLegend
CD27	L128	BV 786	BD
IgG2	SAG2	FITC	Cytognos
IgG3	SAG3	FITC	Cytognos
IgG1	SAG1	PE	Cytognos
IgG2	SAG2	PE	Cytognos
TACI	1A1	PE/Dazzle 594	BioLegend
IgA1	SAA1	PerCP-Cy5.5	Cytognos
IgA2	SAA2	PerCP-Cy5.5	Cytognos
IgD	IA6-2	PerCP-eFluor 710	Invitrogen
IgE	MHE-18	PE-Cy7	BioLegend
IgG4	SAG4	APC	Cytognos
IgA1	SAA1	APC	Cytognos
IgM	polyclonal	AlexaFlour 647	Jackson Immuno Research
CD21	Bu32	AlexaFlour 700	BioLegend
zombie		zombie NIR	BioLegend
CD19	HIB19	APC-Cy7	BioLegend

Supplemental Table S2

marker	clone	tag	µl per 100 µl cell suspension	manufacturer
BARCODES				
CD45	HI30	Y89	1	Fluidigm
HLA-I	W6/32	106Cd	1	Bxcell
CD45	MEM-28	110Cd	2	Exbio
CD45	MEM-28	113In	1	Exbio
HLA-I	W6/32	116Cd	2	Bxcell
HLA-I	W6/32	175Lu	1	Bxcell
HLA-I	W6/32	198Pt	1	Bxcell
CD45	MEM-28	194Pt	2	Exbio
SURFACE MARKERS				
IgM	MHM-88	144Nd	1	BioLegend
CD24	ML5	145Nd	2	BioLegend
CD34	581	148Nd	2	Fluidigm
CD127	A019D5	149Sm	2	Fluidigm
CD38	HIT2	141Pr	2	Exbio
IgD	IA6-2	146Nd	1	Fluidigm
CD20	2H7	147Sm	1	Fluidigm
Anti- Human IgG lambda	MHL-38	151Eu	1	Fluidigm
CD135	BV10A4	156Gd	4	Exbio
CD10	HI10a	158Gd	1	Fluidigm
CD22	HIB22	159Tb	2	Fluidigm
Anti- Human IgG kappa	MHK-49	160Gd	1	Fluidigm
CD9	MEM-61	161Dy	2	Exbio
CD25	MEM-181	162Dy	4	Exbio
CD44	MEM-85	163Dy	2	Exbio
CD27	L128	167Er	1	Fluidigm
CD19	HIB19	169Tm	2	Fluidigm
CXCR5	REA103	171Yb	2	Miltenyi
CXCR4	REA649	173Yb	2	Miltenyi
HLA-DR	L243	174Yb	1	Fluidigm
CD73	AD2	176Yb	2	Exbio
LINEAGE NEGATIVE MARKERS				
CD3	UCHT1	biotin	2	Exbio
CD16	3G8	biotin	2	BioLegend
CD33	HIM3-4	biotin	2	Exbio
CD66b	G10F5	biotin	2	BioLegend
INTRACELLULAR MARKERS				
Caspase 3 (Cleaved)	D3E9	142Nd	2	Fluidigm
cPARP	F21-852	143Nd	1	Fluidigm
PAX-5	1H9	150Nd	2	BioLegend
Caspase 7 (Cleaved)	D6H1	152Sm	1	Fluidigm

BCL-2	Bcl-2/100	153Eu		2	Exbio
Tdt	E17-1519	164Dy		1	Fluidigm
Biotin	1D4-C5	165Ho		1	Fluidigm
Ki-67	B56	168Er		1	Fluidigm
CD79a	HM57	170Er		2	Exbio
IgM	MHM-88	172Yb		1	Fluidigm

Supplemental Table S3

FACS Canto II Panel				
Marker	**Clone**	**Fluorophore**	**Supplier**	**Panel version**
CD10	HI10a W8E7	FITC	BioLegend BD	v1, v2
IgD		PE	Southern Biotech	v1, v2
CD33	WM53	PerCP-Cy5.5	BioLegend	v1
CD34	581	PE-Cy7	BioLegend	v1, v2
IgM		AlexaFluor 647	Jackson Immuno Research	v1, v2
CD19	HIB19	APC-Cy7 BV510	BioLegend	v1 v2
CD38	HIT2 HB-7	Pacific Blue PerCP-Cy5.5	Exbio BioLegend	v1 v2
zombie		Zombie Aqua Zombie NIR	BioLegend	v1 v2
Cytek Aurora Panel				
Marker	**Clone**	**Fluorophore**	**Supplier**	**Panel version**
CD33	WM53	PerCP-Cy5.5 SuperBright 436	BioLegend Invitrogen	v1, v2 v3
CD10	HI10a	BV711 FITC	BioLegend	v1, v2 v3
IgD	IA6-2	PE-Dazzle 594	BioLegend	v1, v3
CD38	HB-7 HIT2 HB-7	PE-Cy7 Pacific Blue PerCP-eFluor 710	BioLegend Exbio Invitrogen	v1 v2 v3
CD34	561 581	APC PE-Cy7	BioLegend	v1 v2, v3
IgM	MHM-88	BV605	BioLegend	v1, v2, v3
zombie		zombie NIR	BioLegend	v1, v2, v3
CD19	HIB19	APC-Cy7	BioLegend	v1, v2, v3

Supplemental Table S4

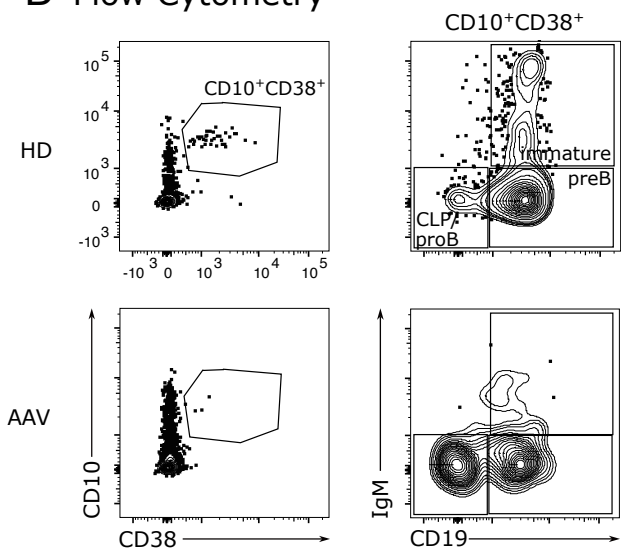| Marker	Clone	Fluorophore	Supplier
CD11c	S-HCL-3	BV 421	BioLegend
BAFF-R	11C1	BV 605	BD
IgG	G18-145	BV 650	BD
CD27	L128	BV 786	BD
IgM	MHM-88	AlexaFlour 488	BioLegend
ADAM17	111633	PE	R&D Systems
TACI	1A1	PE/Dazzle 594	BioLegend
CD86	IT2.2	PerCP-Cy5.5	BioLegend
IgD	IA6-2	PerCP-eFluor 710	Invitrogen
CD38	HB-7	PE-Cy7	BioLegend
ADAM10	SHM14	APC	BioLegend
IgA		AlexaFlour 647	Jackson Immuno Research
CD21	Bu32	AlexaFlour 700	BioLegend
zombie		zombie NIR	BioLegend
CD19	HIB19	APC-Cy7	BioLegend

suppl. Figure 1

suppl. Figure 2

A RTX-induction therapy

B

Supplemental Figure 3

suppl. Figure 4
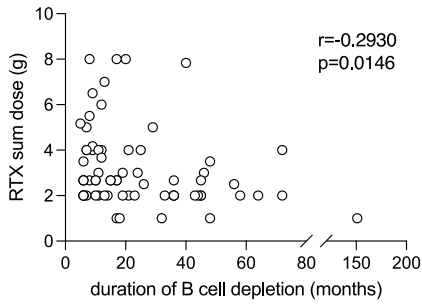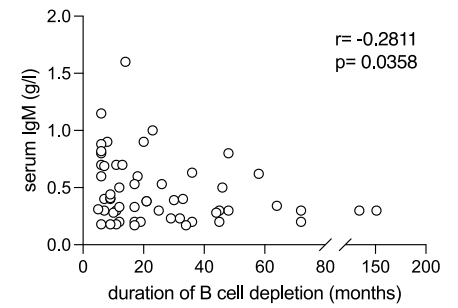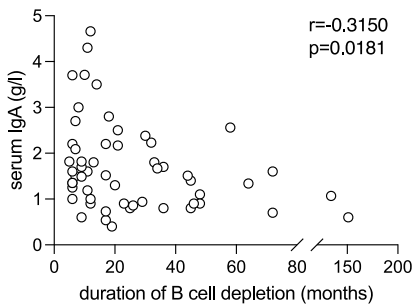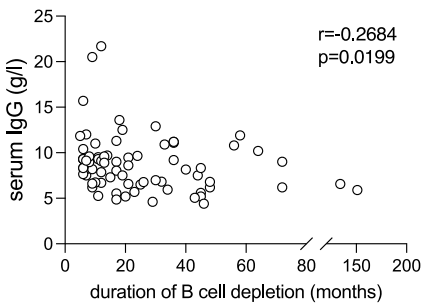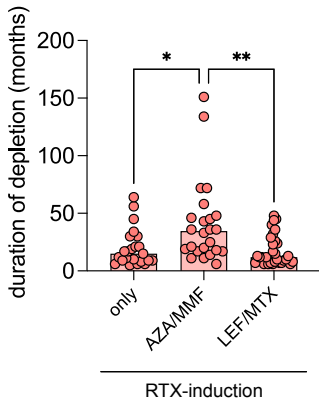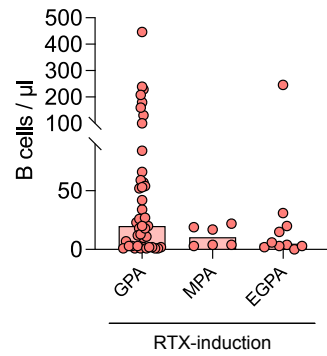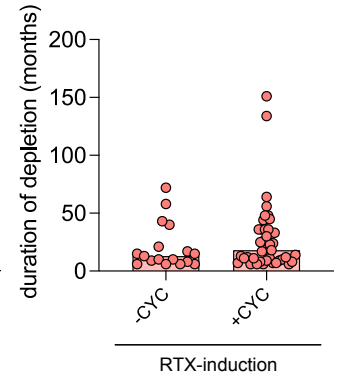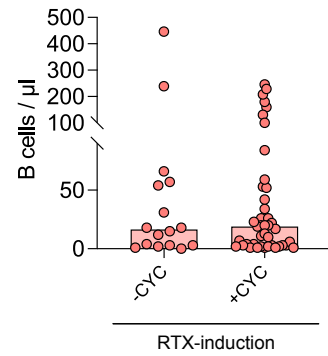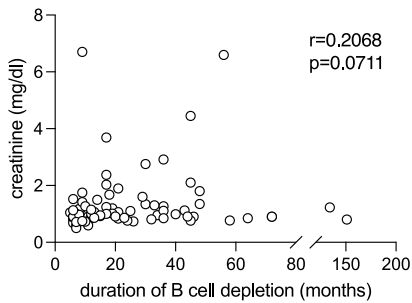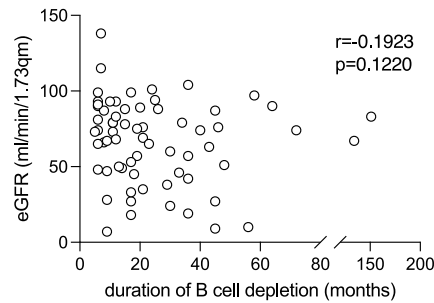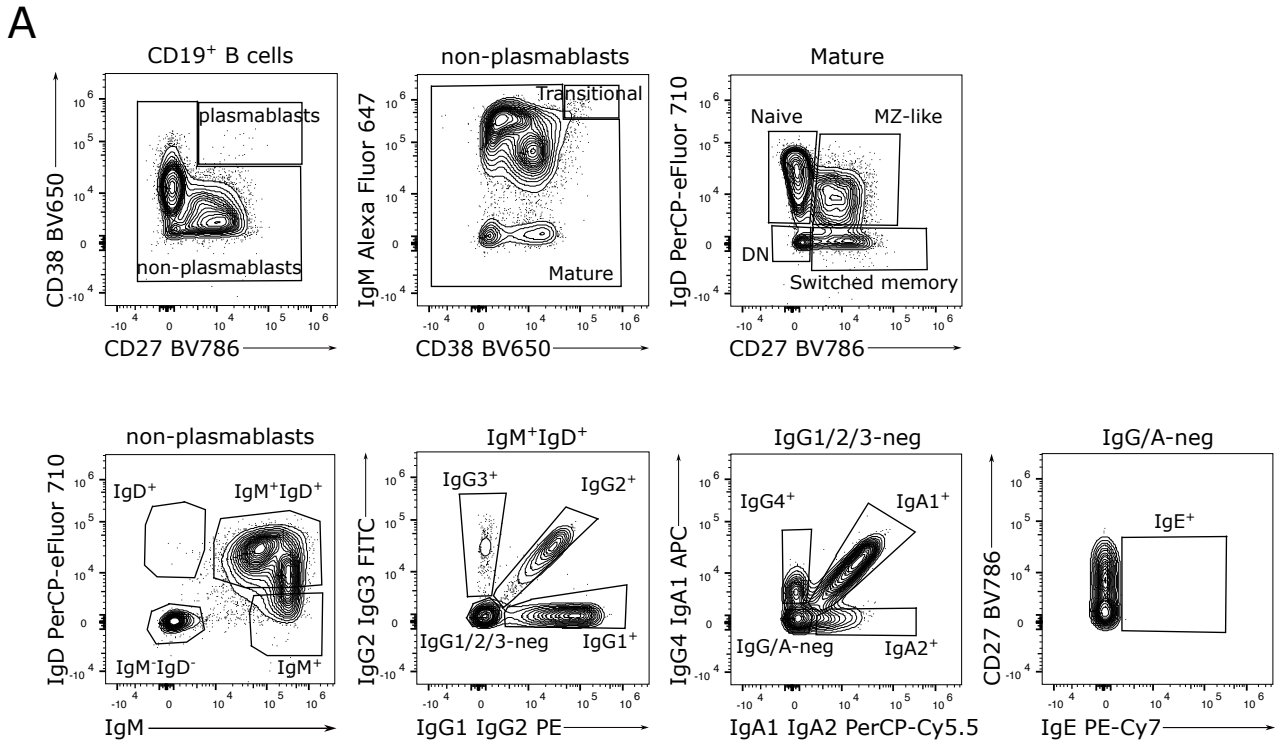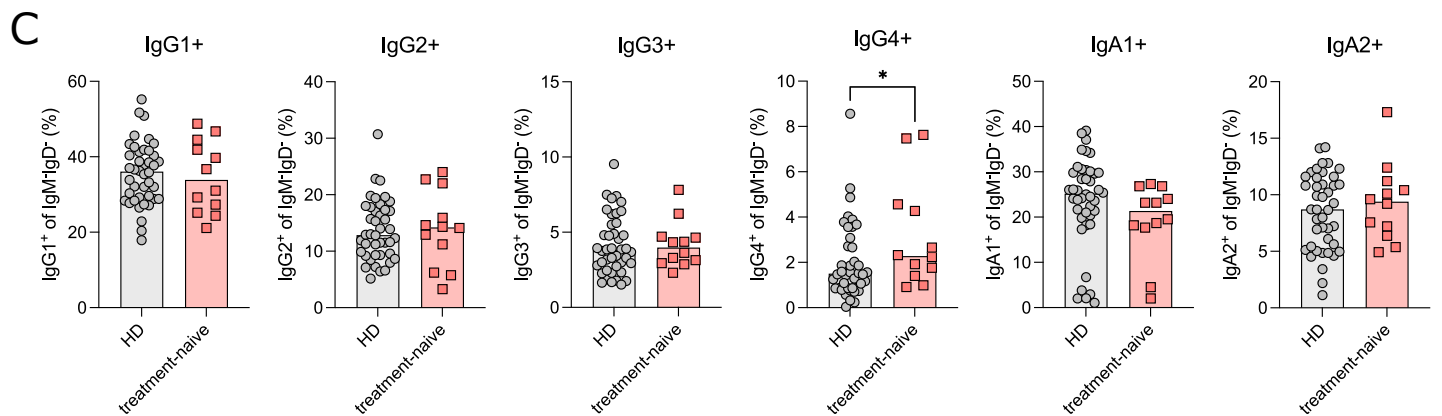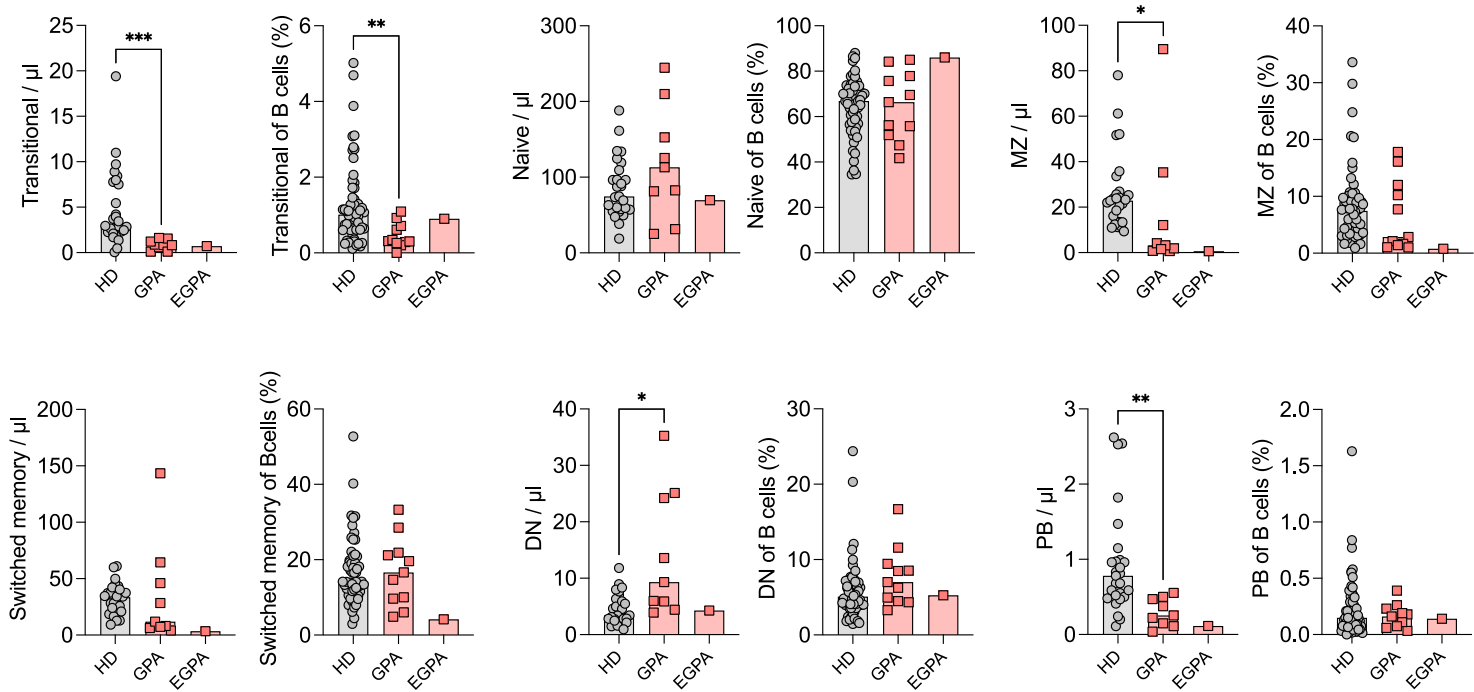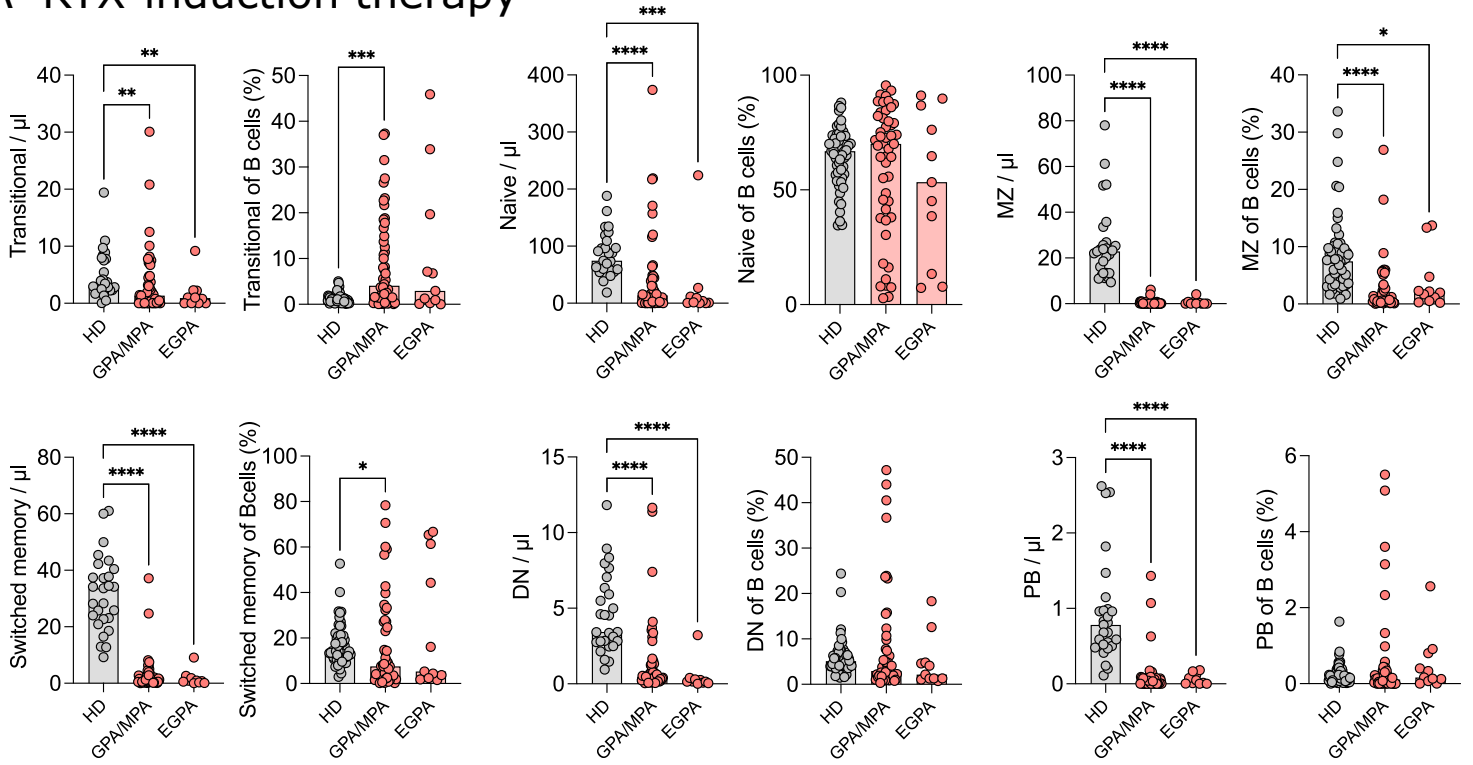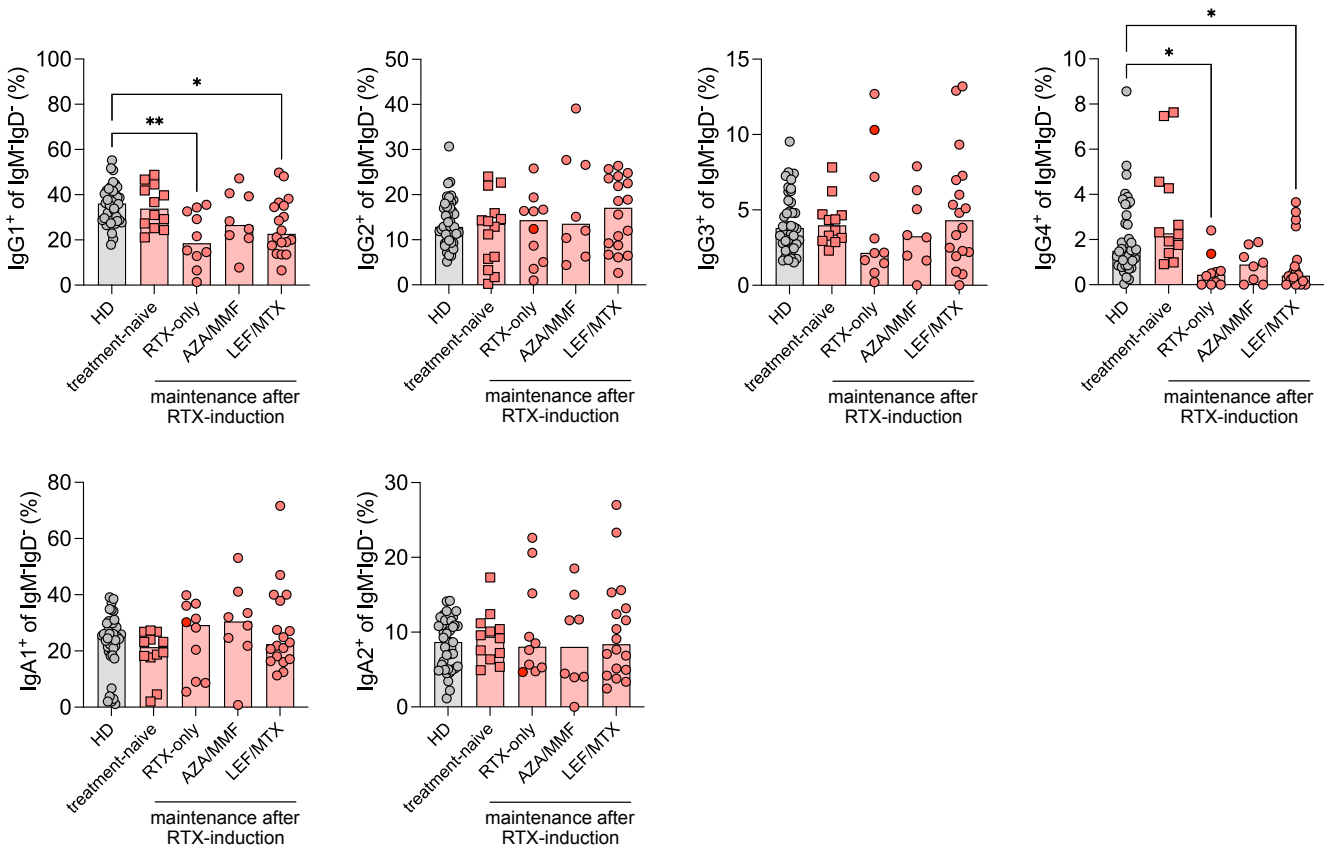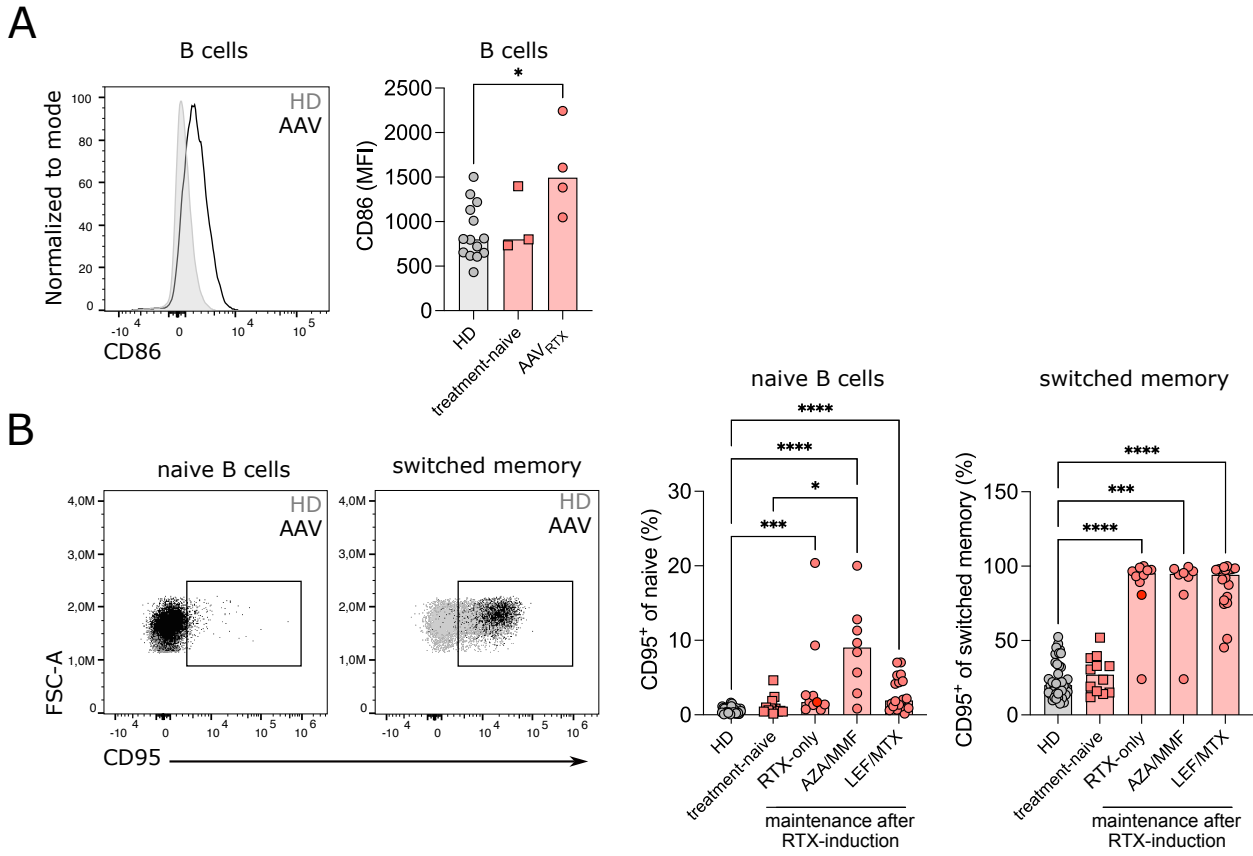
A CyTOF
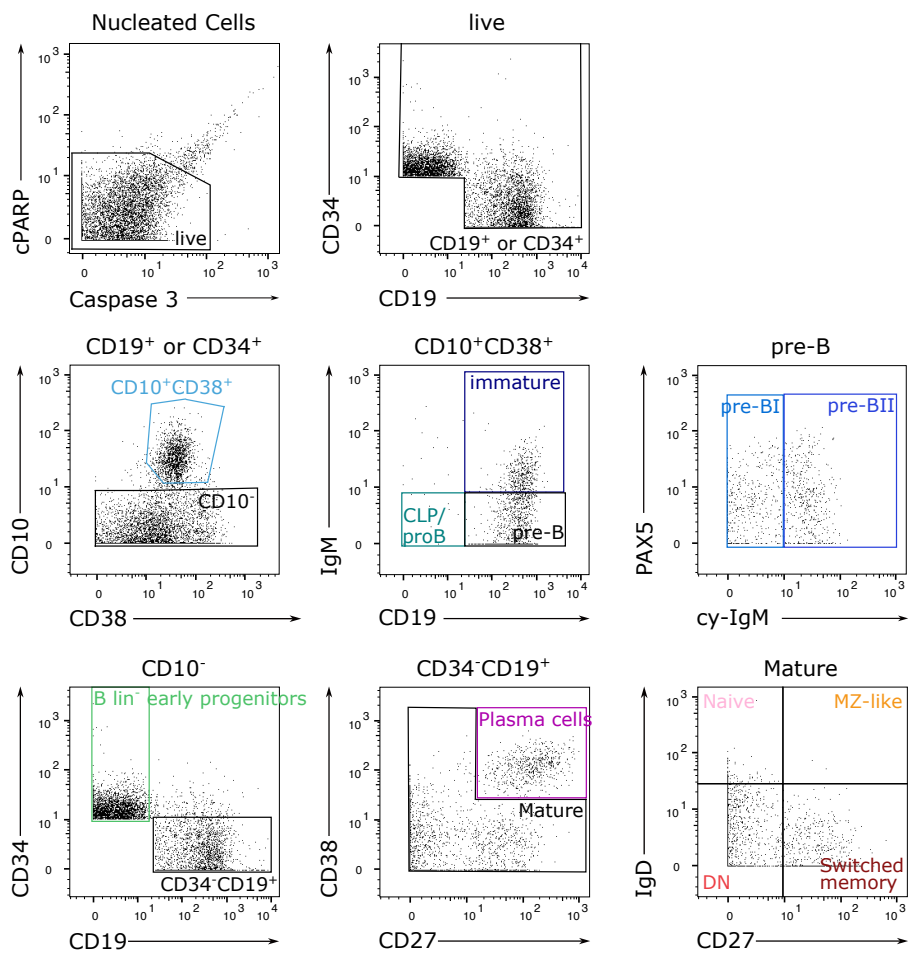
B Flow Cytometry

C

D in vitro

suppl. Figure 5

suppl. Figure 6