

Supplemental Information

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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/ μ l blood in RTX-treated AAV patients divided by disease type. (E) Absolute B cell count/ μ l 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/ μ l 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/ μ l 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

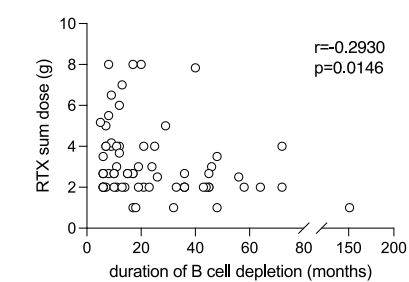
Supplemental Table S3

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
Cytex 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

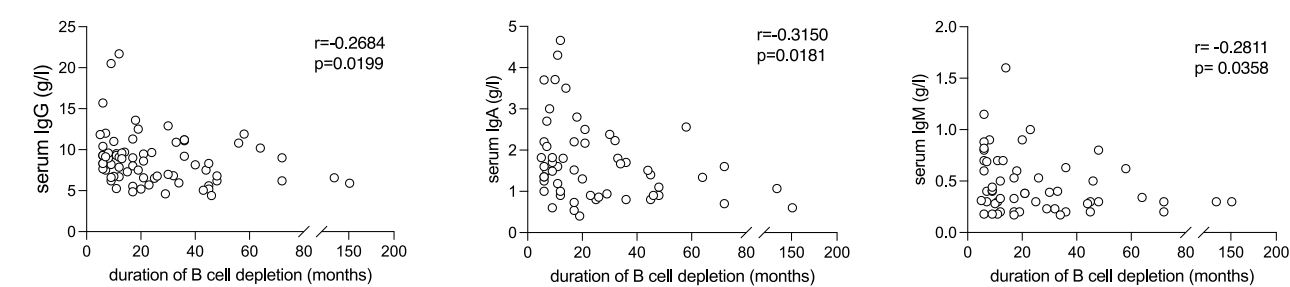
Supplemental Table S4

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

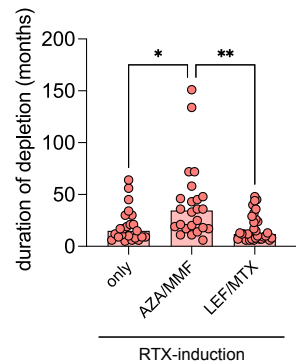
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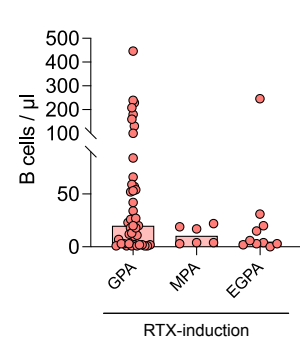
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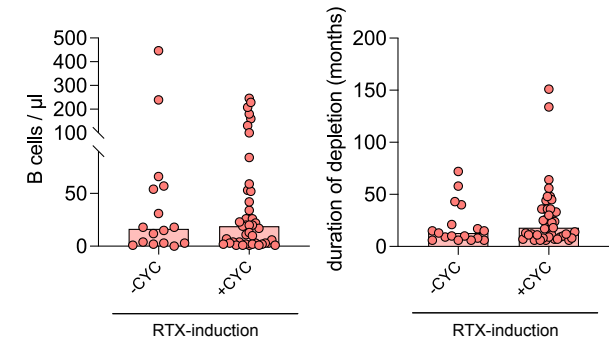
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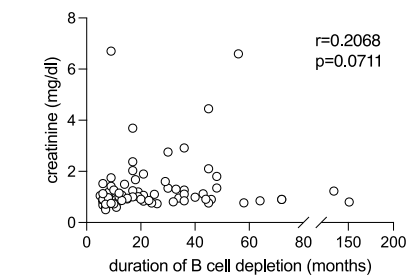
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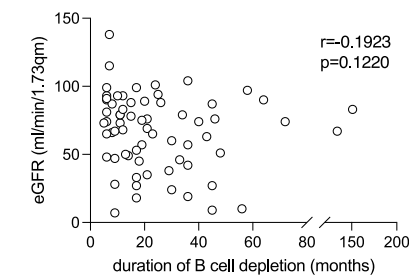
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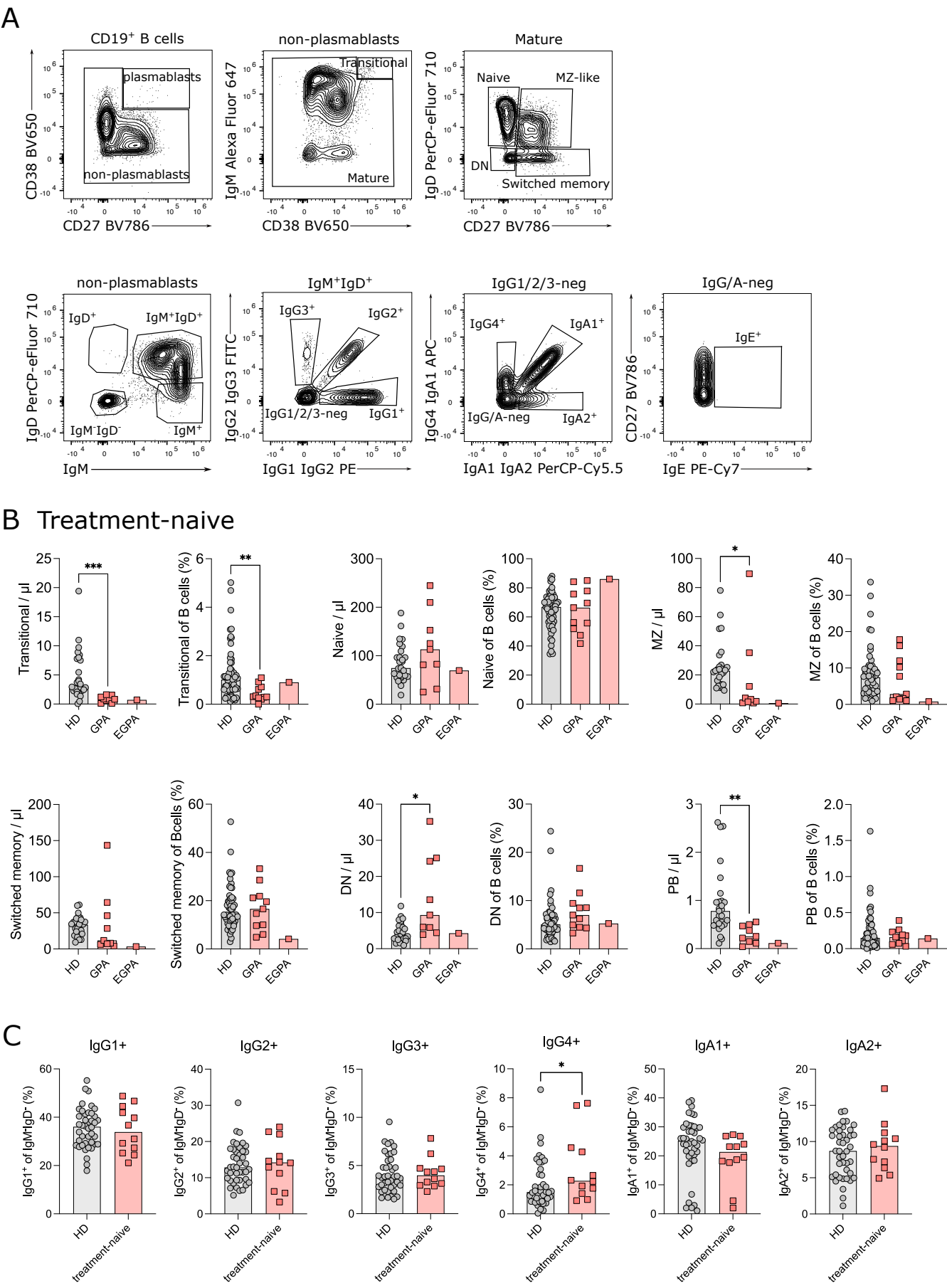
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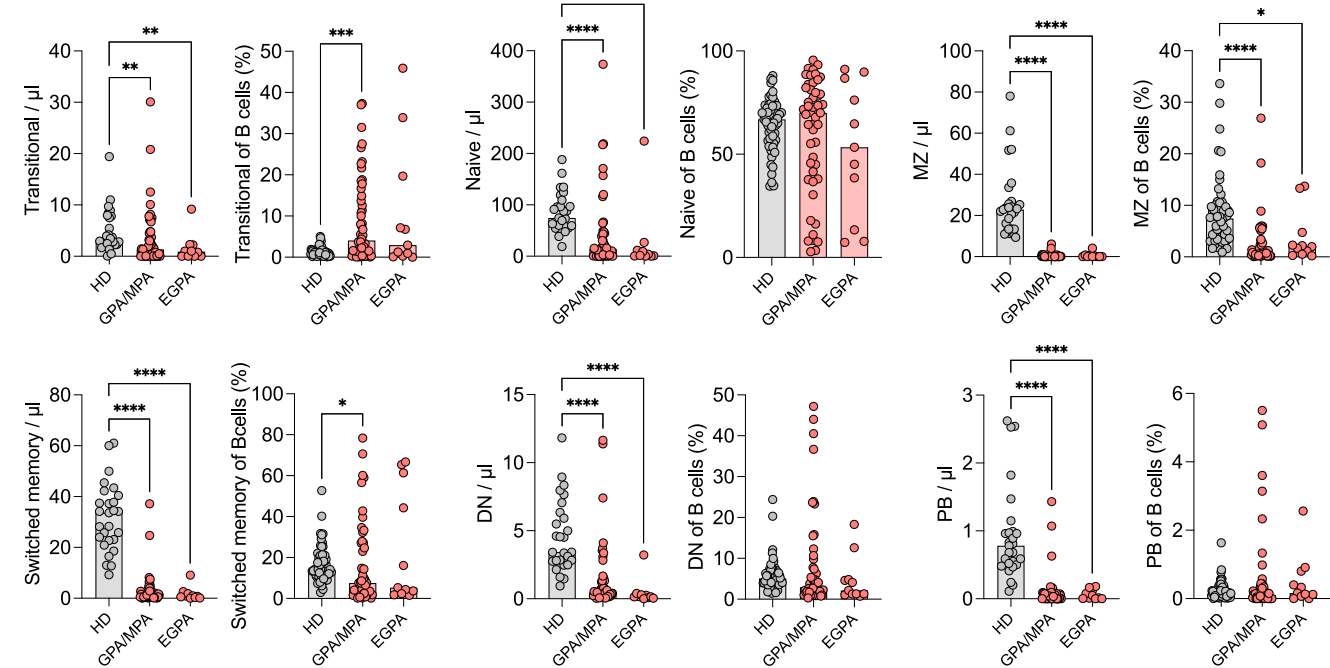


suppl. Figure 1

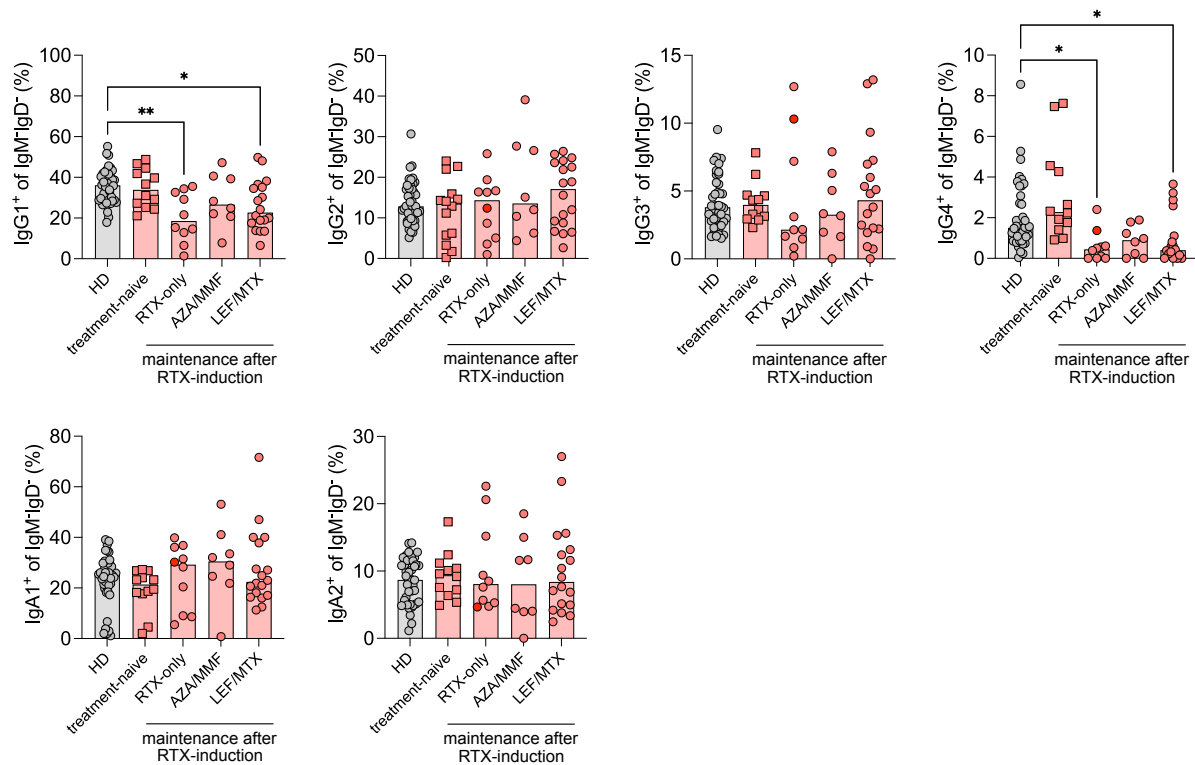


suppl. Figure 2

A RTX-induction therapy



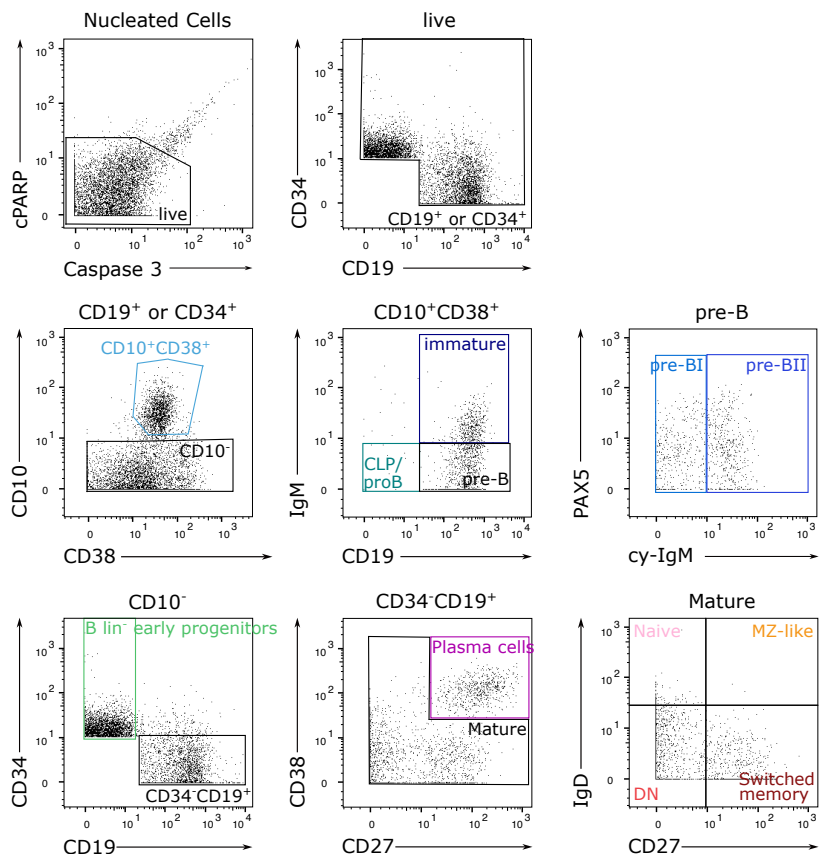
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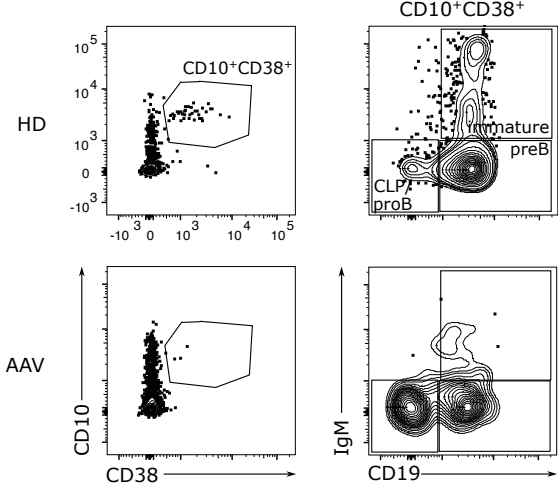
Supplemental Figure 3



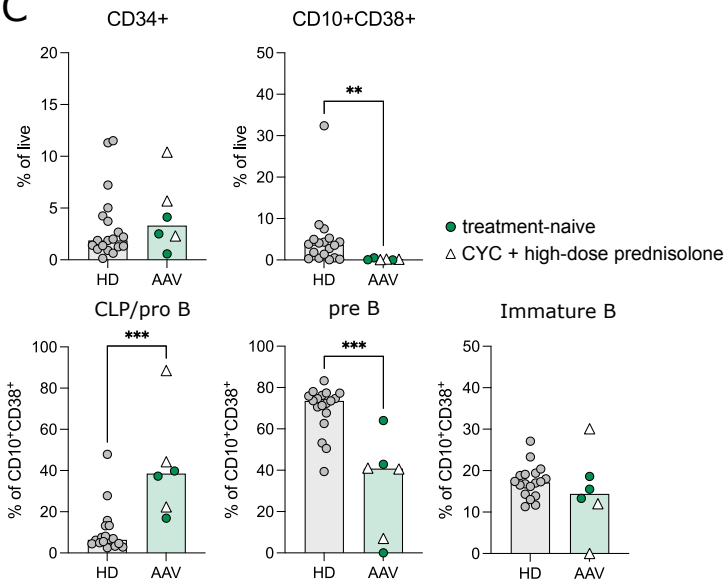
A CyTOF



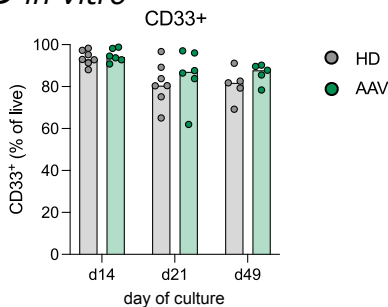
B Flow Cytometry



C



D in vitro



suppl. Figure 5

