

SUPPLEMENTARY MATERIAL

Figure S1: Standard curves for IgG, IgG1, IgG2, IgG3 and IgG4 and cross-reactivities. Human IgG and IgG subclass antibodies were coated in serial dilutions and incubated with monoclonal anti-IgG detection antibodies. The standard curves are fitted to a 4-parameter logistic regression model. Monoclonal antibodies directed against one subclass did not cross-react to the other IgG subclasses.

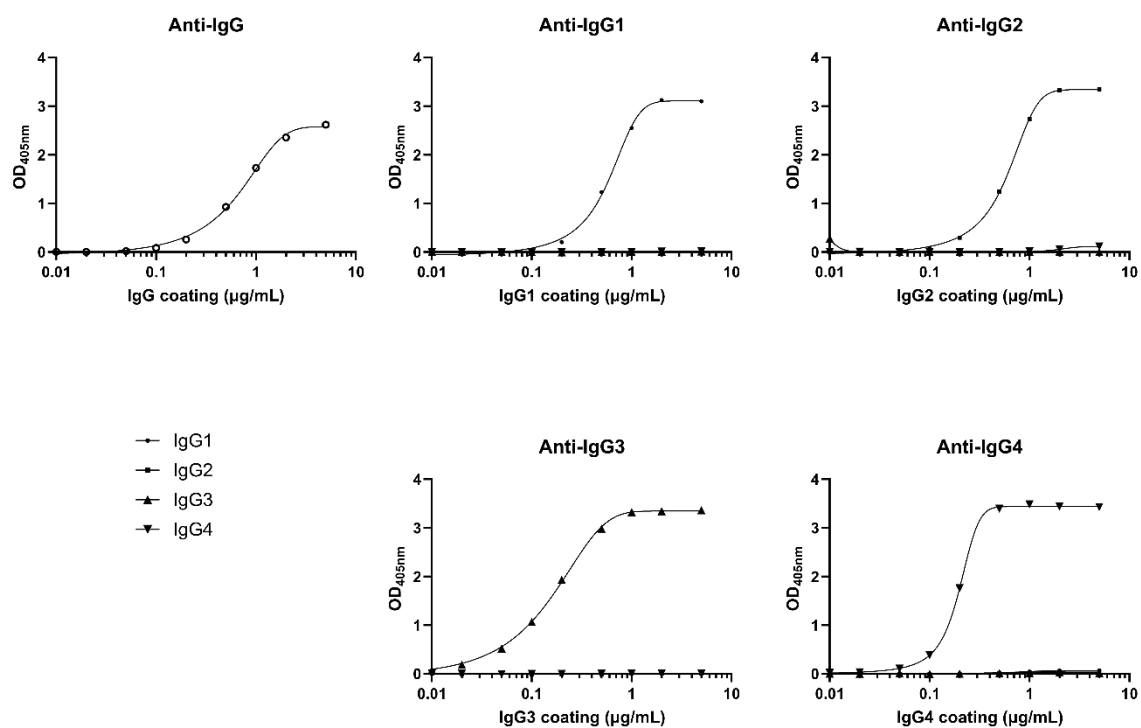


Figure S2: List of gating names. Full names and short names of the gates defined by the gating strategy.

Gating name list

Gating full name	Gating short name
Ungated	Ungated
Ungated/NoBds	NoBds
Ungated/NoBds/Sgl	Singlets
Ungated/NoBds/Sgl/Time	Time
Ungated/NoBds/Sgl/Time/45+	CD45+
Ungated/NoBds/Sgl/Time/45+/L1	Live1-Rh
Ungated/NoBds/Sgl/Time/45+/L1/L2	Live2-Pl
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+	CD66b-CD45+
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-	CD3-CD19-
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-	CD3-CD19-CD56-
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/14-16-	CD3-CD19-CD56-CD14-CD16-
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/14-16-/127+11c-	ILCs CD3-CD19-CD56-CD14-CD16-CD127+CD11c-
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/14-16-/127+11c-/117-294-	ILC1
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/14-16-/127+11c-/117-294+	ILC2
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/14-16-/127+11c-/117+294-	ILC3
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/DR-	CD3-CD19-DR-
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/DR-/56-16-	CD3-CD19-DR-CD56-CD16-
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/DR-/56-16-/123+38+	Basophils
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/DR-/56-16-/123+38+/203+294+	Basophils CD203+CD294+
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/DR-/56h16mid	NK CD56h16mid
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/DR-/56mid16hi	NK CD56midCD16hi
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/DR-/56mid16mid	NK CD56midCD16mid
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/DR+	CD3-CD19-DR+
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/DR+/14-16-	Dendritic
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/DR+/14-16-/123-11c+	DC myelo
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/DR+/14-16-/123-11c+/38-DRmid	DC myelo CD38-DRmid
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/DR+/14-16-/123-11c+/38+DR+	DC myelo act-vested
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/DR+/14-16-/123+11c-	DC plasma
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/DR+/14-16+16+	Mono noncl + intermed
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/DR+/14+16-	Mono class
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19+	Bcell CD19+
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19+/20-DR+	Bcell CD19+CD20-DR+
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19+/20-DR+/27+38+	Bcell plasmablast
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19+/20+DR+	Bcell CD19+CD20+DR+
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19+/20+DR+/27-IgD-	Bcell CD27- IgD-
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19+/20+DR+/27-IgD+	Bcell naive
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19+/20+DR+/27-IgD-	Bcell switched memory
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19+/20+DR+/27+IgD-/IgD-CCR6+	Bcell switched memory CCR6+
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19+/20+DR+/27+IgD+	Bcell IgM memory
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-	CD3+CD19-
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-	T-cell
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/TCRgd-	Tcell-TCRgd-
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/TCRgd-/4-8+	CD8+Tcell
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/TCRgd-/4-8+/45RA-CCR7-	CD8+EM
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/TCRgd-/4-8+/45RA-CCR7+	CD8+TEM
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/TCRgd-/4-8+/45RA+CCR7-	CD8+Tc
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/TCRgd-/4-8+/45RA+CCR7+	CD8+naive
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/TCRgd-/4+8-	CD4+Tcell
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/TCRgd-/4+8-/25-	CD4+CD25-
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/TCRgd-/4+8-/25-/45RA-CCR7-	CD4+EM
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/TCRgd-/4+8-/25-/45RA-CCR7+	CD4+CM
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/TCRgd-/4+8-/25-/45RA-CCR7-	CD4 Tcell Boolean: CD45RA-
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/TCRgd-/4+8-/25-/45RA-CCR7+ or 45RA-CCR7-/CCR4-CXCR5-	CD4 Tcell CCR4-CXCR5-
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/TCRgd-/4+8-/25-/45RA-CCR7+ or 45RA-CCR7-/CCR4-CXCR5-/CXCR3+CCR6-	CD4+ Th1
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/TCRgd-/4+8-/25-/45RA-CCR7+ or 45RA-CCR7-/CCR4-CXCR5-	CD4 Tcell CCR4+CXCR5-
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/TCRgd-/4+8-/25-/45RA-CCR7+ or 45RA-CCR7-/CCR4-CXCR5-/CXCR3-CCR6-	CD4+ Th2
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/TCRgd-/4+8-/25-/45RA-CCR7+ or 45RA-CCR7-/CCR4-CXCR5-/CXCR3-CCR6+	CD4+ Th17
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/TCRgd-/4+8-/25-/45RA-CCR7+ or 45RA-CCR7-/CXCR5+	CD4 Tcell CXCR5+
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/TCRgd-/4+8-/25-/45RA+CCR7-	CD4+TE
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/TCRgd-/4+8-/25-/45RA+CCR7+	CD4+ naive
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/TCRgd-/4+8-/25+127-	CD4+CD25+CD127-
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/TCRgd-/4+8-/25+127-/CCR4+45RA-	CD4+Treg
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/TCRgd+	Tcell-TCRgd+
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56-/TCRgd+/gdCD16+	Tcell-TCRgd+CD16+
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56+	CD3+CD19-CD56+
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56+/TCRgd+56+	Tcell-TCRgd+CD56+
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56+/TCRgd+/gdCD16+	Tcell-TCRgd+CD56+CD16+
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56+/56+TCRgd-	NKT
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56+/56+TCRgd-/4-8-	NKT CD4-CD8-
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56+/56+TCRgd-/4-8+	NKT CD8+
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b-45+/3-19-/56+/56+TCRgd-/4+8-	NKT CD4+
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b+45mid	CD66b+CD45mid
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b+45mid/16-294+	Eosinophils
Ungated/NoBds/Sgl/Time/45+/L1/L2/66b+45mid/16+294-	Neutrophils

Figure S3: Graphical overview of the gating strategy (illustrated on one representative sample).

Normalized CyTOF data were cleaned with several steps like excluding normalization beads, gating on single and live cells. The gate L2 (in red) was used as reference to calculate cell frequencies of single live CD45+ (indicated as number in percent below the gate name), leading to the identification of 66 cell populations within the main immune cell families, such as B cells and plasmacells, innate lymphoid cells, natural killer cells (NK), basophils, monocytes and dendritic cells (DC), NKT-like cells, CD8 and CD4 T cells, granulocytes.

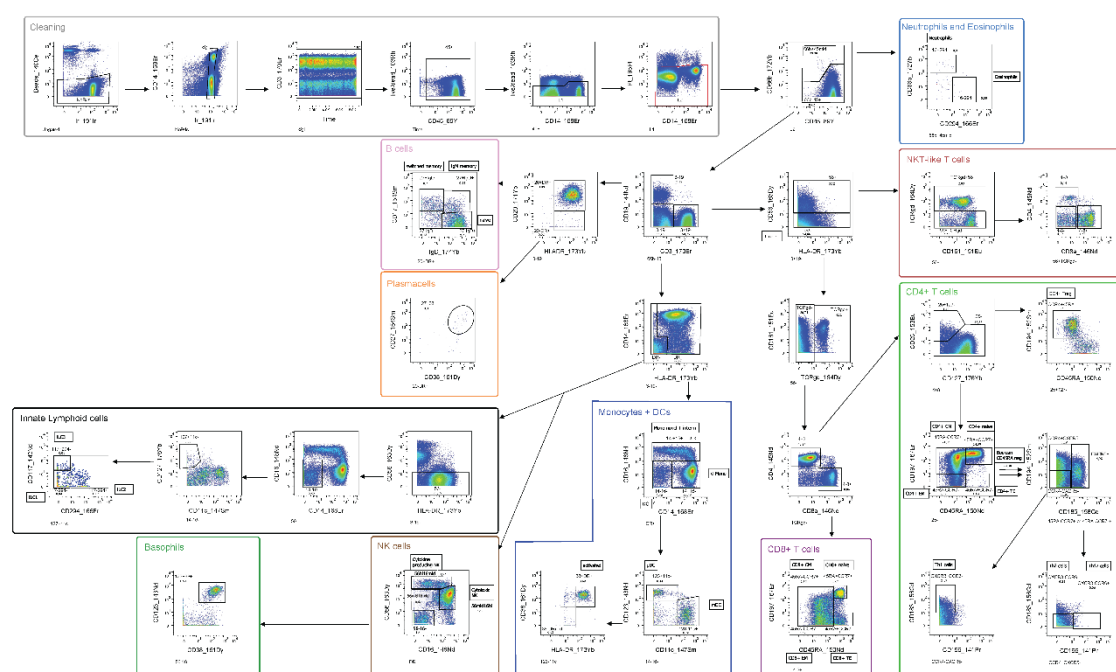


Figure S4: List of samples available and analyzed per subject and time-point.

Open symbols could not be included for flow cytometry analysis, either because the acquired sample did not fulfill the inclusion criteria (< 50% mortality rate and/or CD45+ cells >50000 events, open circle), or because the PBMC sample was not available (open diamond). Serum was available for open symbols to measure immunoglobulin levels and cytokine concentrations.

Subject	Timepoint				
	V0	V1	V2	V3	V4
Ctrl1	●				
Ctrl2	●				
Ctrl3	●				
Ctrl4	●				
Ctrl5	●				
Ctrl6		●	●	●	●
Ctrl7	○	●	●	●	
Ctrl8		◇	●	●	●
Ctrl9	●	●	◇	●	●
Pat1	●	●	●	●	●
Pat2	●	●	●	●	●
Pat3	●	●	○	●	●
Pat4	●	●	●	●	●
Pat5		○	●	●	●
Pat6		●	●	●	●
Pat7		●	●	●	●
Pat8		●	●	●	●
Pat9		●	●	●	●
Pat10		●	●	●	●
Pat11	●				
Pat12	●				
Pat13	●				
Pat14	●				
Pat15	●				
Pat16	●				

Figure S5: Longitudinal anti- α -Gal immunoglobulin profiles showing individual kinetic curves. IgE (right Y-axis), IgG and IgG subclasses (left Y-axis) were plotted for each individual subject. Sera were diluted 1/50 for IgG and 1/20 for all IgG subclasses.

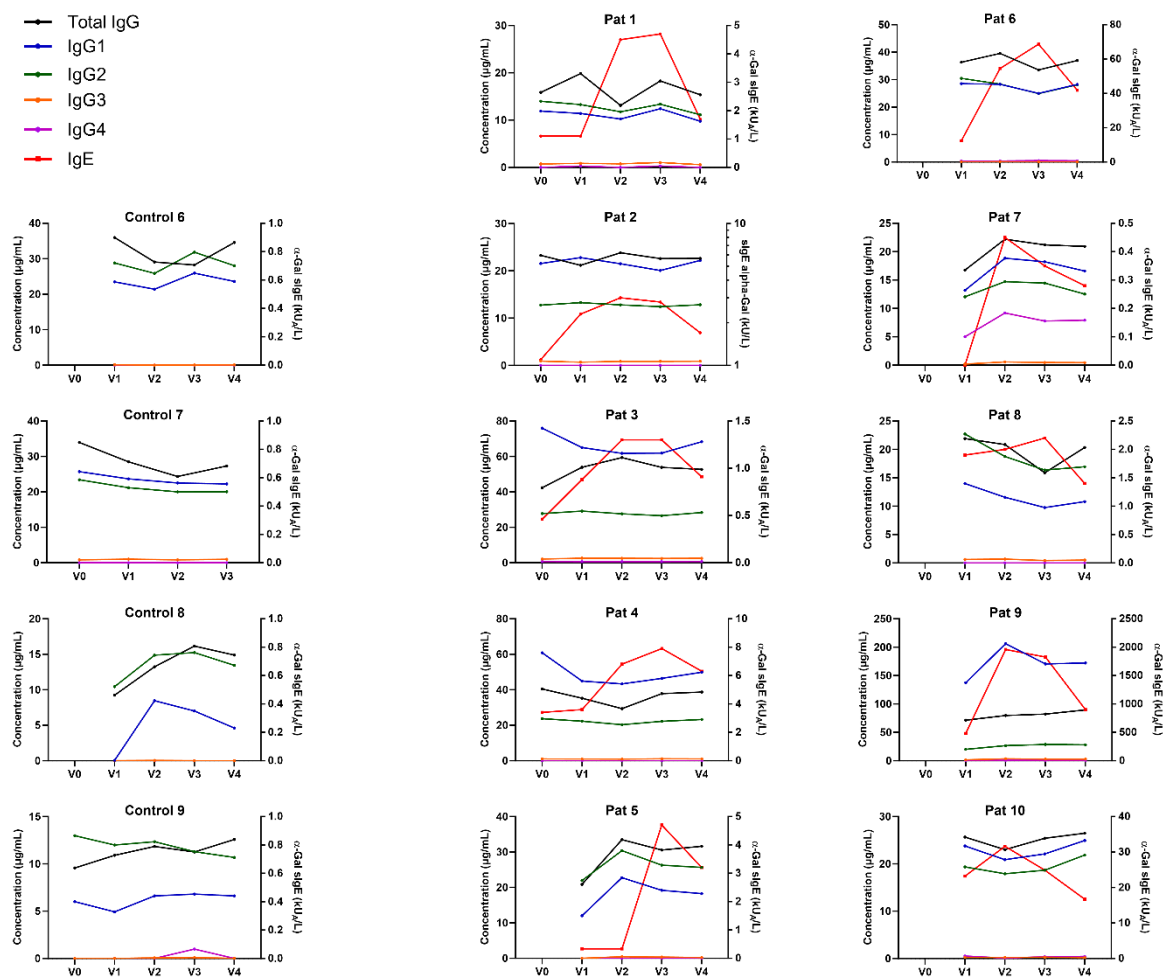


Figure S6: Proportions of sIgG1 to sIgG2 is higher in patients compared to controls. (A): The weight of sIgG1, resp. sIgG2 is expressed as percent (%) of all 4 IgG subclasses (median with interquartile range) for patients and controls. Multiple Mann-Whitney tests between patients and controls at each time-point showed only 1 significant difference (** p-value < 0.01). (B): The ratio of sIgG1 to sIgG2 is higher for patients than controls, all samples combined. The line represents the median for each group, Mann-Whitney test shows a significant difference between groups (**** p-value < 0.0001).

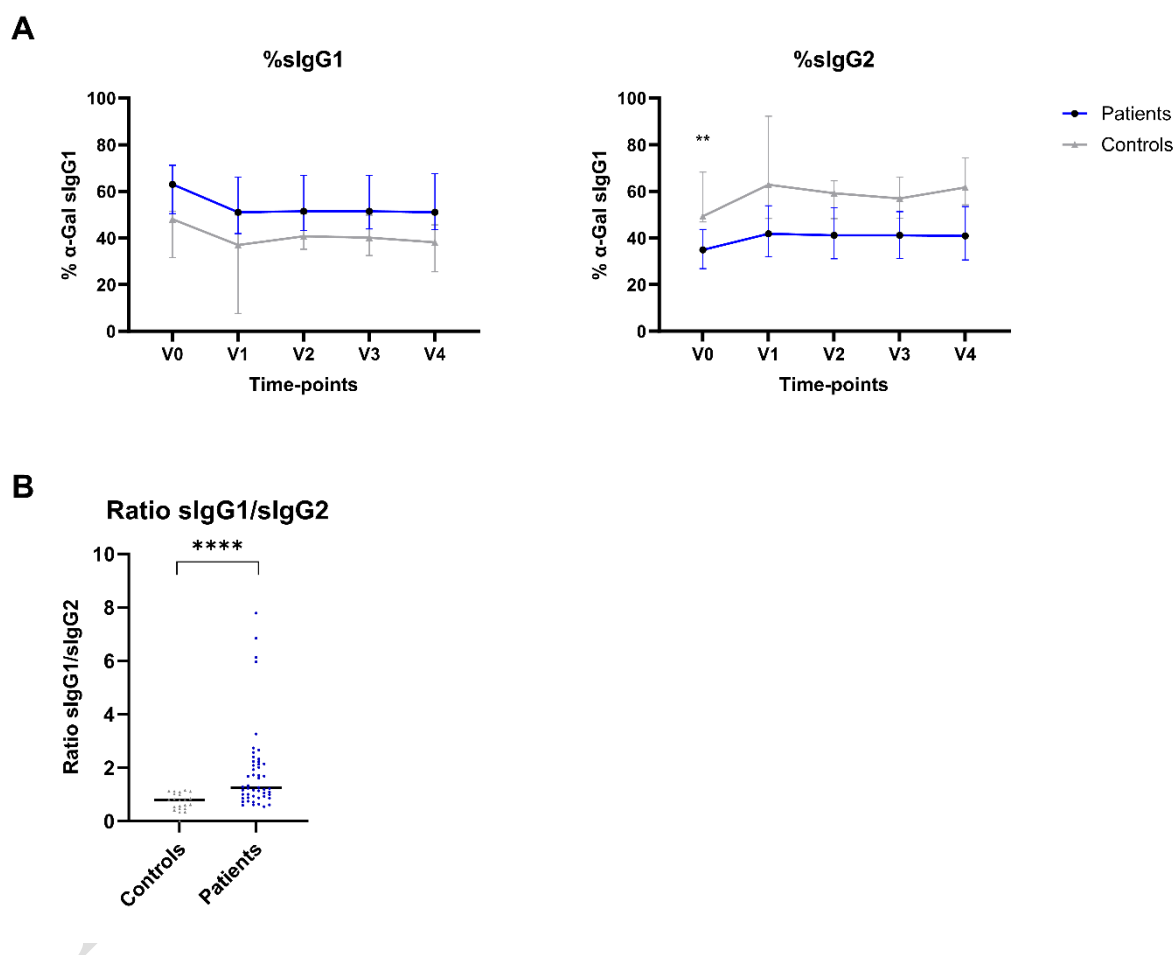


Figure S7: Longitudinal analysis of blood cell frequencies shows no significant variations.

Main cell types from total blood count, expressed as frequencies of total leucocyte counts for patient (blue line) and control group (grey line), are plotted as median with interquartile range. Friedman tests were not significant.

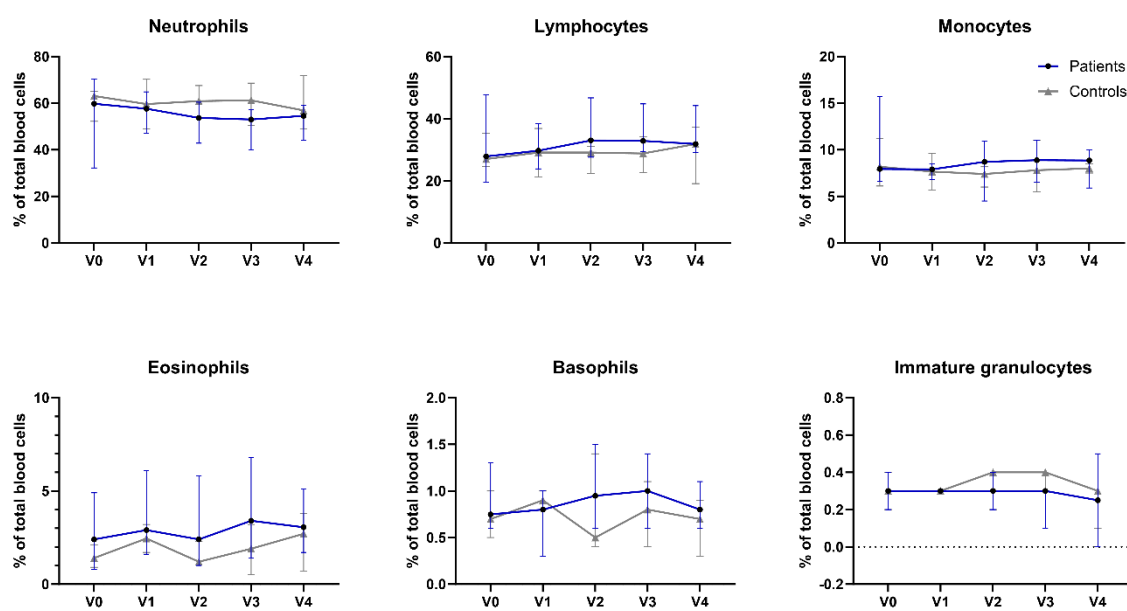


Figure S8: Plasmacytoid dendritic cell (pDC) frequencies are higher in controls regardless of the time of blood withdrawal. Frequencies of pDC cell are expressed as percent of single live CD45+ cells and divided into 2 categories of blood withdrawal before (am) or after noon (pm) for control (grey) and patient (blue) group. Boxes are delimited by interquartile range with median line and whiskers from minimal to maximal value. Pair-wise comparisons with Mann-Whitney tests show significant differences (* p-value < 0.05; ** p-value < 0.01; *** p-value < 0.001).

