Effects of rosuvastatin on the immune system in healthy volunteers with normal serum cholesterol

Peer W. F. Karmaus, Min Shi, Shira Perl, Angélique Biancotto, Julián Candia, Foo Cheung, Yuri Kotliarov, Neal Young, Michael B. Fessler & the CHI Consortium[Ψ]

[Ψ]CHI Consortium: Jinguo Chen, Rongye Shi, Huizhi Zhou, Richard Apps, John Tsang; Trans-NIH Center for Human Immunology, Autoimmunity, and Inflammation, NIH, Bethesda, MD 20892

SUPPLEMENTAL INFORMATION

Table S2. Baseline (day 0) immunophenotypic differences between CRP-low and CRP-high subjects

Analyte	Median in CRP-low group	Median in CRP-high group	P-value [A]	Relative direction in CRP high group	FDR
CRP [B]	88.03	567.60	6.29×10^{-5}	↑	0.012
IL-1Rα	149.58	280.78	4.40×10^{-4}	↑	0.033
CRP_btris [C]	0.54	3.58	5.76×10^{-4}	↑	0.033
IFNγ	169.25	259.99	7.54×10^{-4}	↑	0.033
IL-6	7.09	14.64	1.11×10^{-3}	↑	0.033
IL-7	10.26	20.79	1.19×10^{-3}	↑	0.033
Resistin	3092.22	5618.02	1.19×10^{-3}	↑	0.033
G-CSF	26.09	40.59	1.89×10^{-3}	↑	0.037
IL-8	26.42	160.43	1.89×10^{-3}	↑	0.037
SAP	5144.70	15560.53	1.89×10^{-3}	↑	0.037
IL-4	5.10	8.71	2.40×10^{-3}	↑	0.042
TNFα	34.03	57.79	2.83×10^{-3}	↑	0.042
IL-10	5.43	20.33	3.23×10^{-3}	↑	0.042
IL-18	13.97	88.09	3.58×10^{-3}	↑	0.042
C-Peptide	645.84	1211.98	4.15×10^{-3}	↑	0.042
FGF basic	16.01	26.88	4.15×10^{-3}	↑	0.042
Glucagon	140.49	194.06	4.15×10^{-3}	↑	0.042
IL-17	15.24	34.67	4.15×10^{-3}	↑	0.042
SCGFβ	3576.09	9672.58	4.15×10^{-3}	↑	0.042
IL-2	12.31	20.11	5.72×10^{-3}	↑	0.051
GLP-1	218.14	361.50	5.91×10^{-3}	↑	0.051
IL-12p70	23.62	55.97	5.91×10^{-3}	↑	0.051
GROα	31.12	209.93	6.04×10^{-3}	↑	0.051
HGF	399.64	705.56	8.29×10^{-3}	↑	0.067
Insulin	288.03	476.72	0.011	↑	0.085
MIP-1β	90.33	196.12	0.011	↑	0.085
Leptin	2710.21	15038.80	0.015	↑	0.101
sCD40L	571.00	1022.83	0.015	↑	0.101
X16.1: $CD8^+/CD38^+$	3.41	15.90	0.015	↑	0.101
IL-16	0.00	238.54	0.016	↑	0.101
IL-13	13.80	27.23	0.018	↑	0.112
VEGF	60.18	215.19	0.018	↑	0.112
Neutrophil Differential (%)	51.90	62.10	0.023	↑	0.133
IL-12p40 [D]	0.00	0.00	0.026	↑	0.133
GM-CSF [E]	0.00	0.00	0.026	↑	0.133
IL-17A2 [F]	0.00	0.00	0.026	↑	0.133
IL-15	0.00	5.40	0.026	↑	0.133
RANTES	5775.42	6779.47	0.027	↑	0.133
X12:$CD4^+CD28/CCR7^+CD45RA^-$	7.65	19.80	0.027	↑	0.133
TRAIL	0.00	48.20	0.029	↑	0.139
MIP-1α	4.28	11.34	0.030	↑	0.140
IL-9	23.97	41.86	0.035	↑	0.155
X27: $CD4^+/CXCR3^-CCR6^+$	8.99	15.20	0.035	↑	0.155
IL-5	0.67	6.62	0.035	↑	0.155
X121: $CD8^+/CXCR3^+CCR6^+$	8.64	3.08	0.037	↓	0.160
X20:$CD8^+CD28/CCR7^+CD45RA^+$	5.44	15.30	0.044	↑	0.175
X25: $CD4^+/CXCR3^+CCR6^-$	14.40	9.06	0.044	↓	0.175
X29: $CD8^+/CXCR3^+CCR6^-$	50.30	27.80	0.044	↓	0.175
X30: $CD8^+/CXCR3^-CCR6^-$	37.50	56.10	0.044	↑	0.175
Lymphocyte Differential (%)	39.30	30.30	0.046	↓	0.176
IL-31	43.76	64.82	0.046	↑	0.176

[A] Wilcoxon rank sum test; [B] hsCRP measured by Luminex; [C] hsCRP measured by NIH Clinical Center assay; [D] Mean values: CRP-low=0, CRP-high=74.02; [E] Mean values: CRP-low=0, CRP-high=150.87; [F] Mean values: CRP-low=0, CRP-high=5.52.

CCL = Chemokine (C-C motif) ligand; G-CSF = granulocyte-colony stimulating factor; GM-CSF = Granulocyte-macrophage CSF; FGF = fibroblast growth factor; CRP = C-reactive protein; FGF = fibroblast growth factor; GLP = glucagon-like peptide; GRO = growth-related protein; IFN = interferon; IL = interleukin; MIP = macrophage inflammatory factor; SAP = Serum Amyloid P; sCD40L = soluble CD40 ligand; SCGF = stem cell growth factor; TRAIL = TNF-related apoptosis-inducing ligand; VEGF = vascular endothelial growth factor. False discovery rate (FDR)-adjusted p-values are also shown. Cell populations with 'X' identifications are percentages by FACS.

Table S3. Variables changed by rosuvastatin treatment in interaction with baseline CRP [A]

	Estimate	Std. Error	df	t value	Pr(≥|t|)	LRT.p
X135: $CD3^{-}/CD19^{+}/CD20^{-}/CD38^{-}CD24^{-}$	-11.963	4.524	45	-2.644	0.011	0.008
X17.1: $CD8^{+}/HLA\text{-}DR^{+}$	0.269	0.105	45	2.555	0.014	0.010
X16.1: $CD8^{+}/CD38^{+}$	0.260	0.113	45	2.310	0.026	0.019

[A] Variables that were significantly changed after commencing rosuvastatin (nominal p-value by likelihood ratio test [LRT.p]) ≤0.05), as assessed by linear regression including an interaction term for baseline CRP. P-value by Wald test [Pr(≥|t|)] is also shown. None of the variables shown had FDR-adjusted p-value ≤0.05. Cell populations are percentages quantified by FACS.

Table S4. Variables changed by rosuvastatin discontinuation in interaction with baseline CRP [A]

	Estimate	Std. Error	df	t value	Pr(≥|t|)	LRT.p
Eotaxin	0.462	0.128	45	3.620	7.44×10^{-4}	4.29×10^{-4}
X121: $CD8^+/CXCR3^+CCR6^+$	-0.727	0.252	45	-2.883	6.02×10^{-3}	3.90×10^{-3}
CD19 (%)	0.182	0.066	41	2.744	8.97×10^{-3}	5.97×10^{-3}
IL-25	-1.550	0.614	45	-2.525	0.015	0.011
$CD4^+$ $CD3^+$ cell count	267.252	105.341	41	2.537	0.015	0.011
IL-1β	0.370	0.150	45	2.459	0.018	0.013
X35: $CD4^+/CD39^+$	-0.236	0.100	45	-2.359	0.023	0.017
IL-17A2	-1.256	0.544	45	-2.308	0.026	0.018
X123: Transitional B cells	0.433	0.189	45	2.298	0.026	0.020
X127: $CD19^+$	0.423	0.186	45	2.270	0.028	0.021
X128: $CD20^+$	0.412	0.188	45	2.198	0.033	0.026
X25: $CD4^+/CXCR3^+CCR6^-$	-4.779	2.183	45	-2.189	0.034	0.026
X117: $CD8^+CD28^+/CCR7^+CD45RA^+$	4.394	2.183	45	2.012	0.050	0.040
X30: $CD8^+/CXCR3^-CCR6^-$	15.595	7.803	45	1.999	0.052	0.040
$CD19^+$ cell count	0.321	0.161	41	1.996	0.053	0.044
G-CSF	0.993	0.500	45	1.986	0.053	0.044
X119: $CD8^+CD28^+/CCR7^-CD45RA^-$	-3.696	1.886	45	-1.959	0.056	0.046

[A] Variables that were significantly changed after discontinuing rosuvastatin (nominal p-value by likelihood ratio test [LRT.p]) ≤0.05), as assessed by linear regression including an interaction term for baseline CRP. P-value by Wald test [Pr(≥|t|)] is also shown. None of the variables shown had FDR-adjusted p-value ≤0.05. Cell populations indicated with an 'X' are percentages and were quantified by FACS; other cell measures were quantified by clinical cytometry.

G-CSF = granulocyte-colony stimulating factor; IL = interleukin.

Table S5. Baseline (day 0) immunophenotypic differences between CRP-low and CRP-high subjects excluding three CRP-high subjects assayed in separate batch.

Analyte	P-value [A]	Relative Direction in CRP high group	FDR
CRP	0.001	↑	0.142
C-peptide	0.001	↑	0.142
Insulin	0.003	↑	0.189
CRP_btris	0.005	↑	0.241
GROα	0.010	↑	0.263
IL-1Rα	0.010	↑	0.263
IL-6	0.011	↑	0.263
Resistin	0.010	↑	0.263
IFNγ	0.018	↑	0.341
IL-2	0.026	↑	0.341
IL-4	0.026	↑	0.341
IL-7	0.026	↑	0.341
PAI-1	0.018	↑	0.341
SAA	0.022	↑	0.341
Neutrophil Differential (%)	0.022	↑	0.341
G-CSF	0.040	↑	0.366
HGF	0.041	↑	0.366
IL-8	0.040	↑	0.366
IL-10	0.037	↑	0.366
IL-18	0.041	↑	0.366
PCT	0.042	↑	0.366
SAP	0.040	↑	0.366

[A] Wilcoxon rank sum test; **hsCRP measured by Luminex; ***hsCRP measured by NIH Clinical Center assay.
CRP = C-reactive protein; G-CSF = granulocyte-colony stimulating factor; GM-CSF = Granulocyte-macrophage CSF; HGF = human growth factor; GRO = growth-related protein; IFN = interferon; IL = interleukin; PAI = plasminogen activator inhibitor; PCT = procalcitonin; SAA = Serum Amyloid A; SAP = Serum Amyloid P. False discovery rate (FDR)-adjusted p-values are shown. Cell populations with 'X' identifications are percentages by FACS.

Table S6. Medication and supplement use reported by study participants*

	CRP-low subjects (N)	CRP-high subjects (N)
Multivitamin	4	3
Fish oil	2	0
Butalbital/acetaminophen	1	1
Aspirin/paracetamol	0	1
Ibuprofen	0	2
Hormone replacement	1	0
Lisinopril	0	1
Birth control	1	1

*Medications/supplements reported by study participants at time of enrollment, as well as the number (N) of subjects taking these, is indicated.

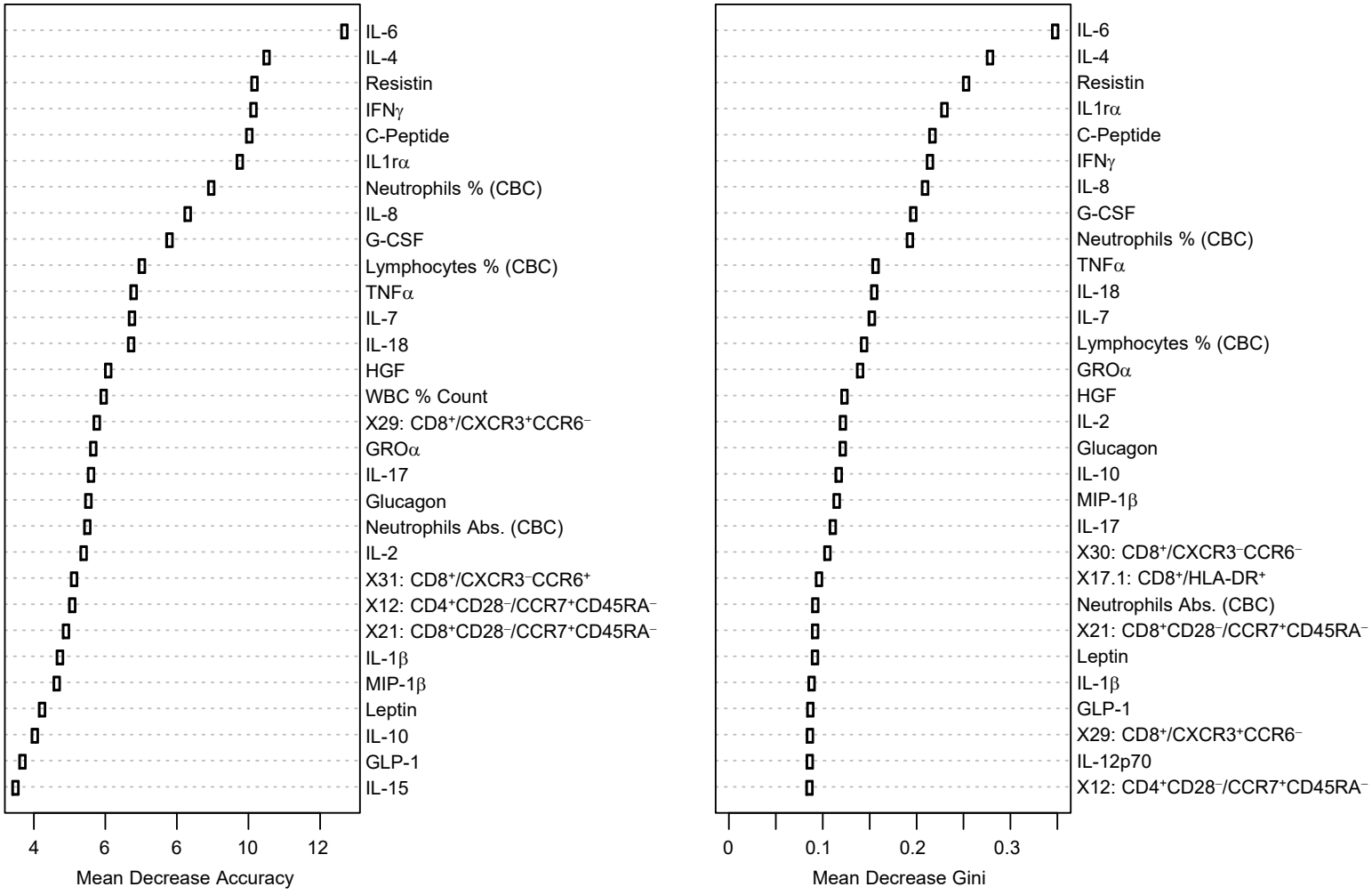


Figure S1. Random forests analysis of CRP-high vs. CRP-low participant subgroups. Random forests analysis was used to identify variables of greatest importance (top of list) in discriminating between CRP high and low study participants. As shown, IL-6 was found to be the top discriminating biomarker.

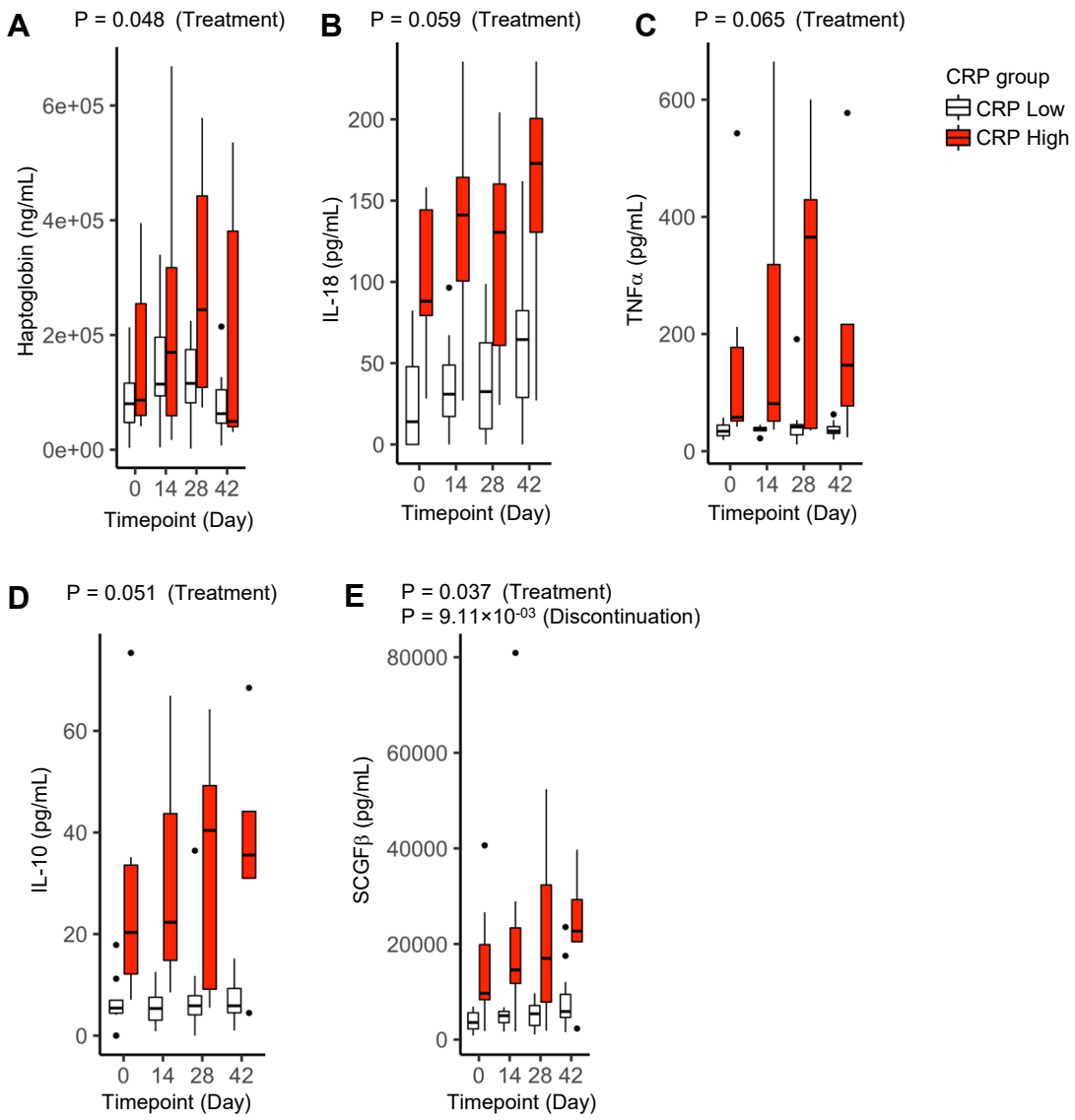


Figure S2. Effect of rosuvastatin on immune measures. Measures were plotted in study subjects at the indicated trial timepoints (baseline [day 0], rosuvastatin treatment [days 14, 28], and 14 days after rosuvastatin discontinuation [day 42]). Subjects with low vs. high CRP at baseline are plotted separately. Boxes depict the interquartile range (IQR) around the median. The upper whisker extends from the hinge to the largest value no further than 1.5 * IQR from the hinge; the lower whisker extends from the hinge to the smallest value at most 1.5 * IQR of the hinge. Outlying points are plotted individually. Nominal P values for rosuvastatin treatment and/or discontinuation, which were determined for the overall study group by linear regression, are shown in the figure panels (also listed in Tables II and III).

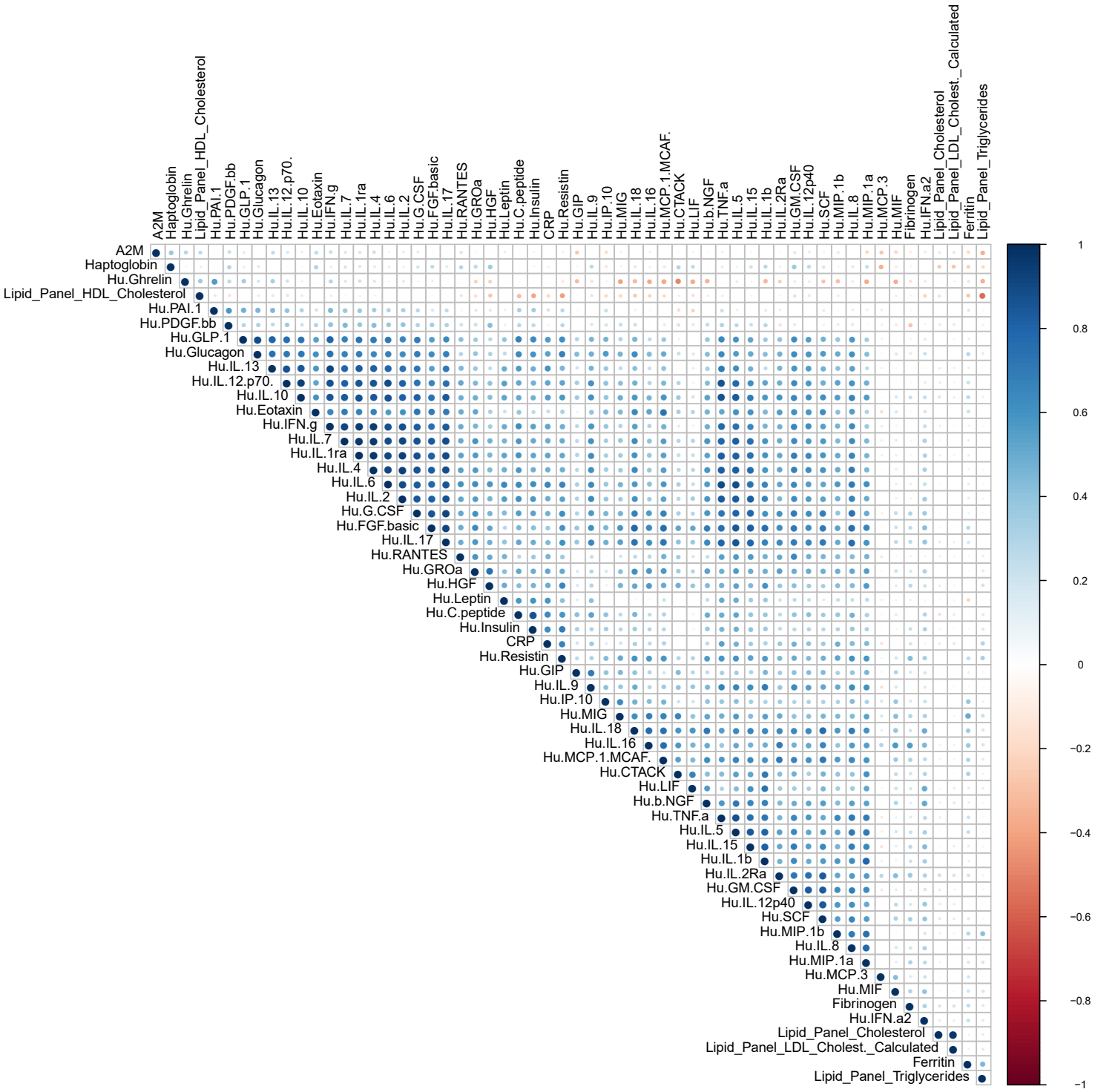


Figure S3. Spearman correlation of Luminex parameters. Spearman correlation was performed across all timepoints and individuals for each Luminex parameter using the "rcorr" function of the "Hmisc" package in R. The blue color denotes positive correlation, while the red color denotes negative correlation with a range of values from 1 to -1, denoting complete positive correlation or complete negative correlation, respectively. As expected, all parameters have an correlation value of 1 (dark blue) when compared to themselves.

Figure S4A.

B cell gating:

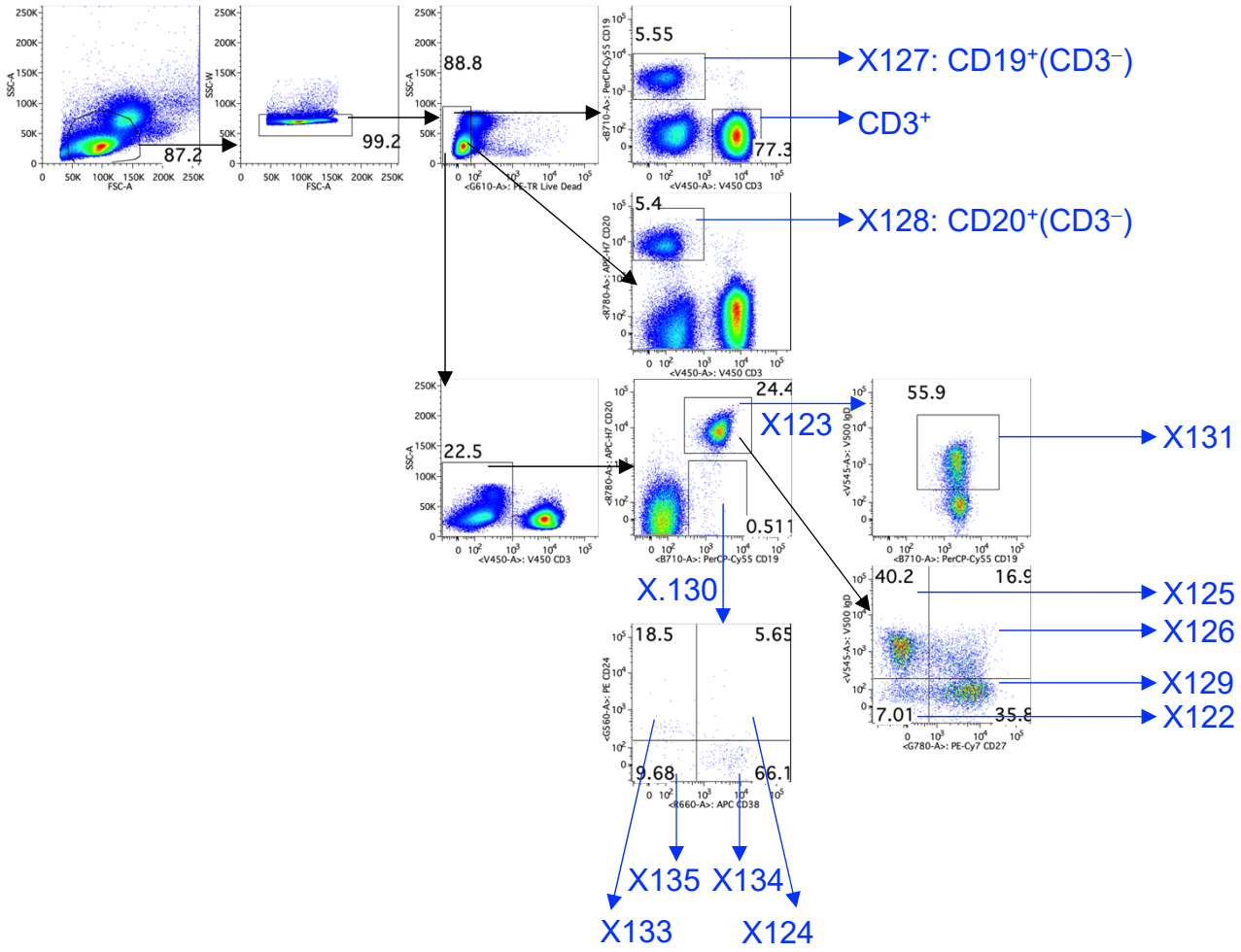
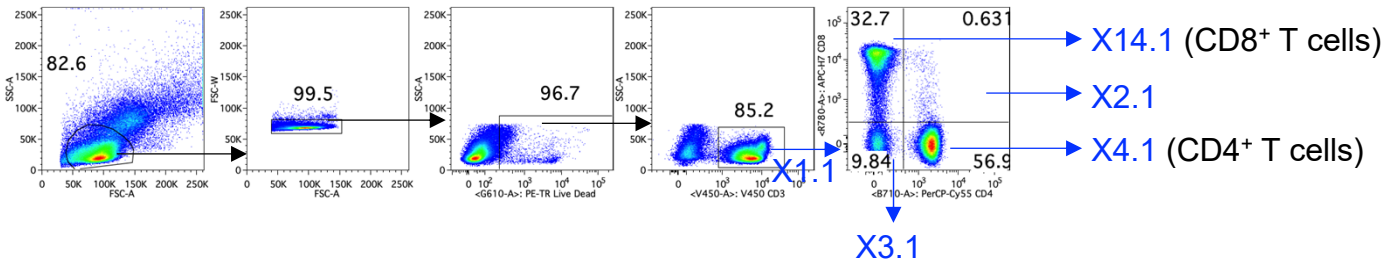
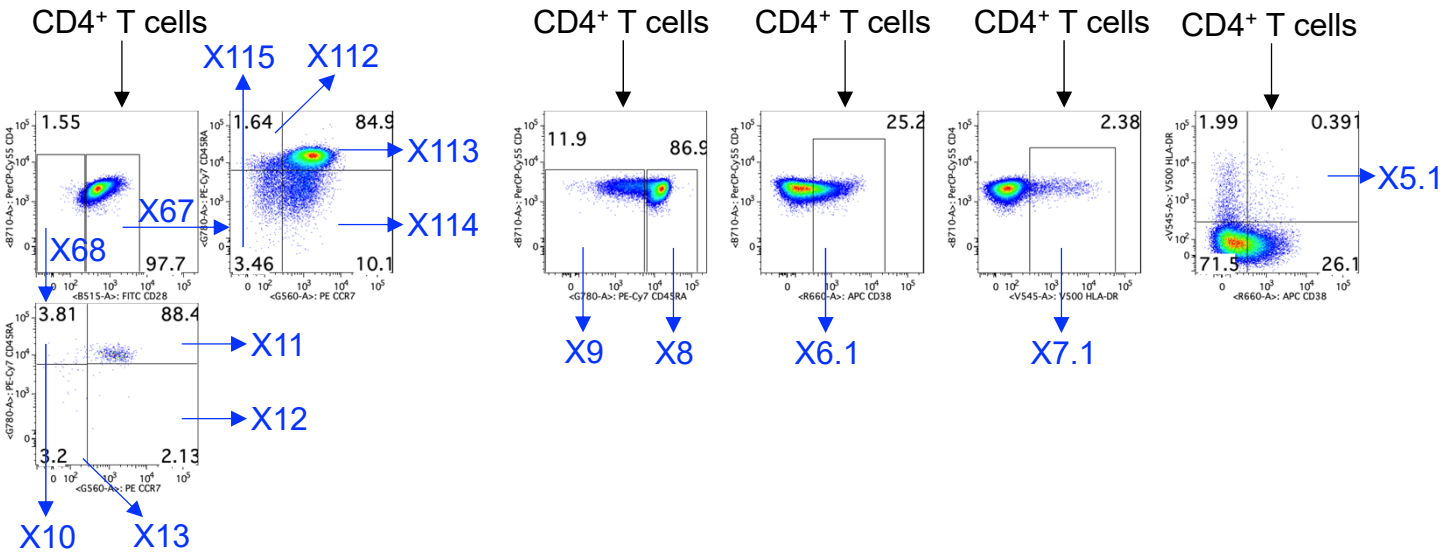


Figure S4B.

T cell Gating:



CD4⁺ T cells



CD8⁺ T cells

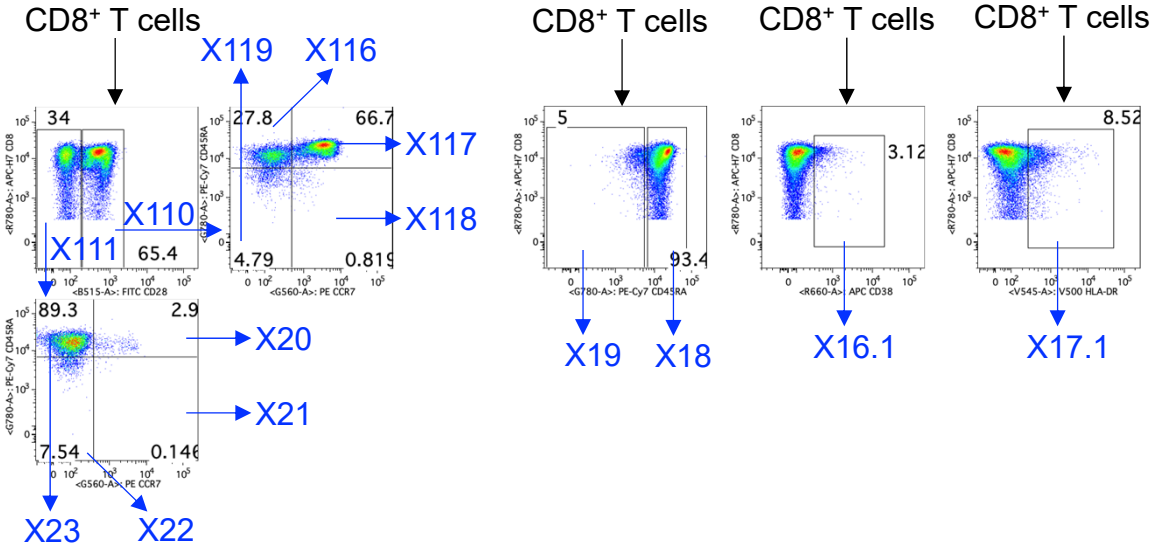


Figure S4C.

T helper cell Gating:

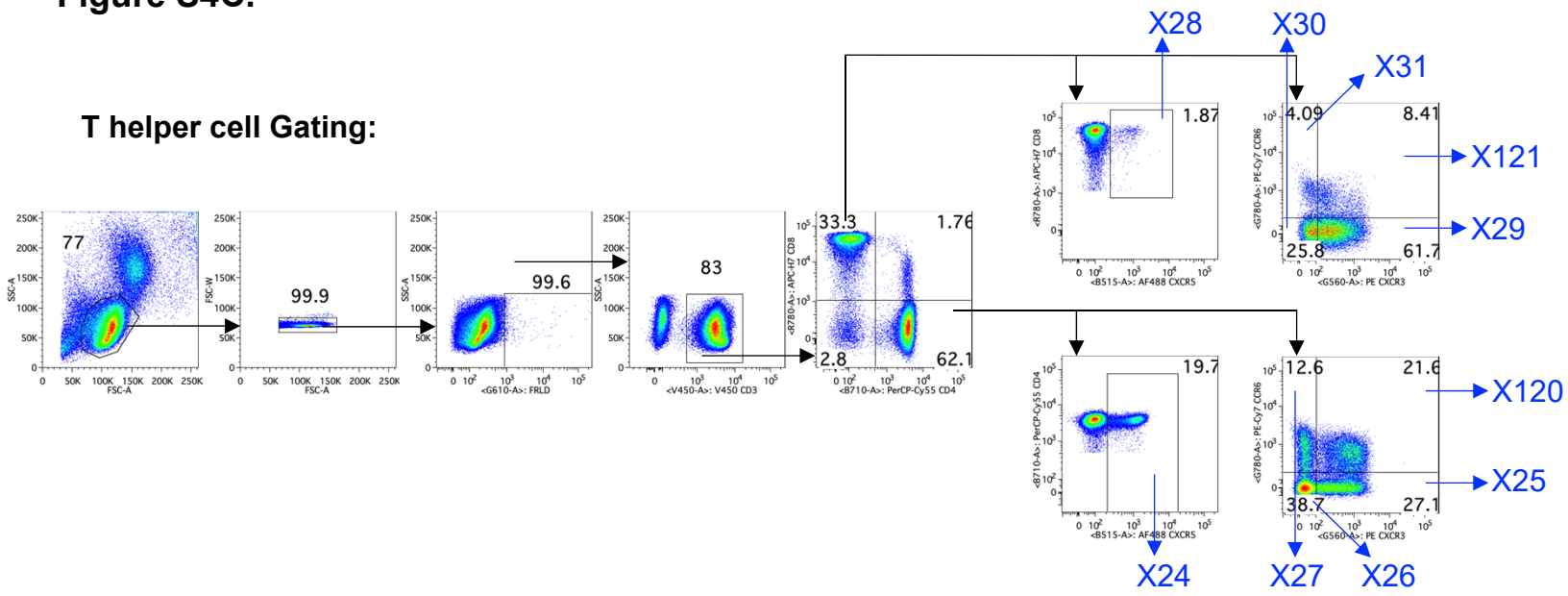


Figure S4. Flow cytometry gating strategy. Representative flow cytometric gates are shown for leukocyte subtypes assayed in this study, including B cells (A) and T cell subsets (B-C).