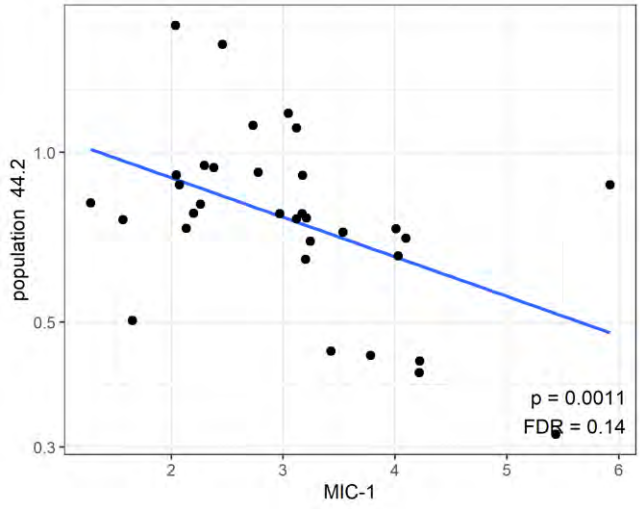
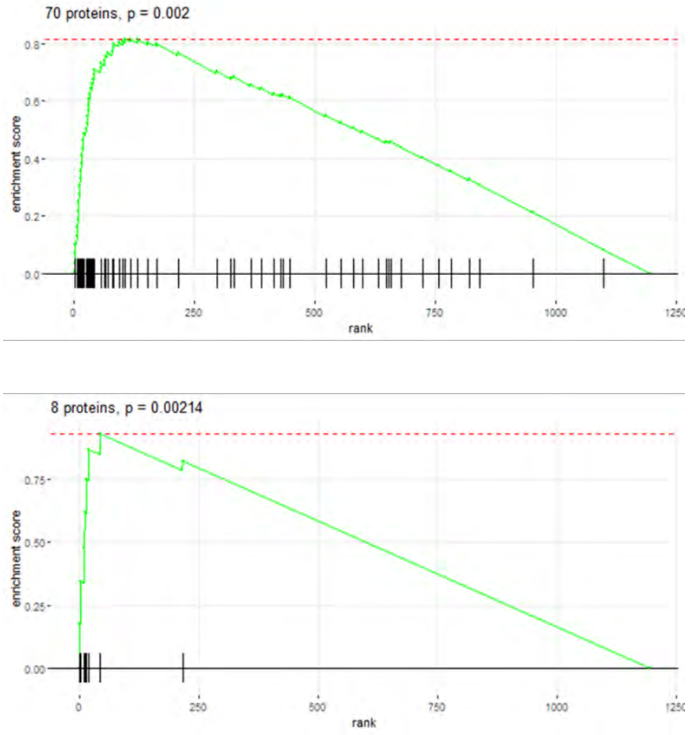
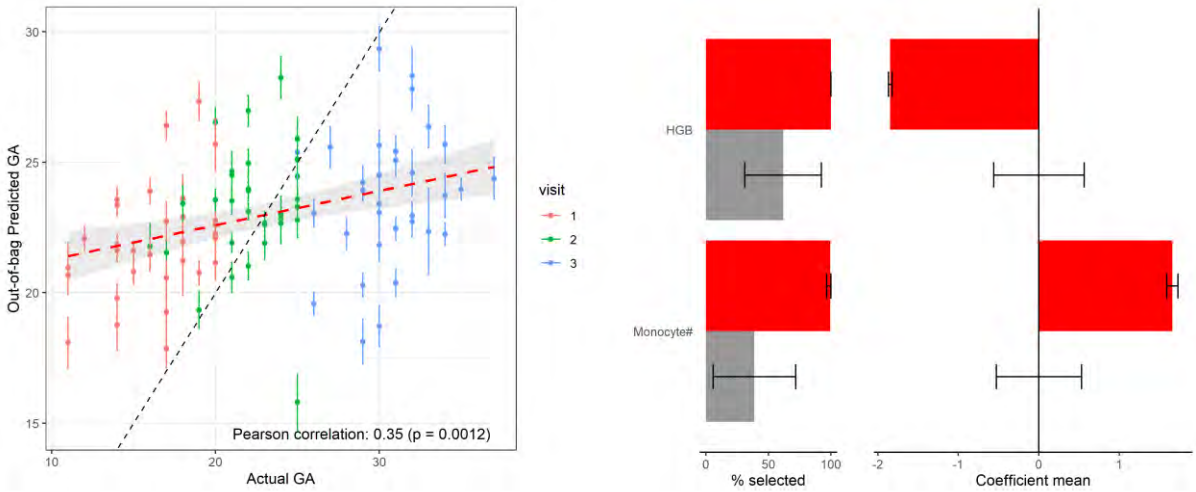
Supplementary data



Suppl Fig. 1: None of the correlations between immune populations and serum proteins that change during gestation is significant after correcting for multiple testing. 26 cell populations and 434 somamers differed between V1 and V3 significantly (FDR-adjusted $p<0.05$). A subset of 18 cell populations and 129 somamers differed with fold changes higher than 1.2 or lower than 0.8. For every pairwise combination of these selected features, a Spearman correlation was computed for the change in cell population frequency, against the change in serum protein level, using all 33 subjects. For none of the correlations was FDR adjusted $p<0.05$. The strongest correlation is shown in the plot.

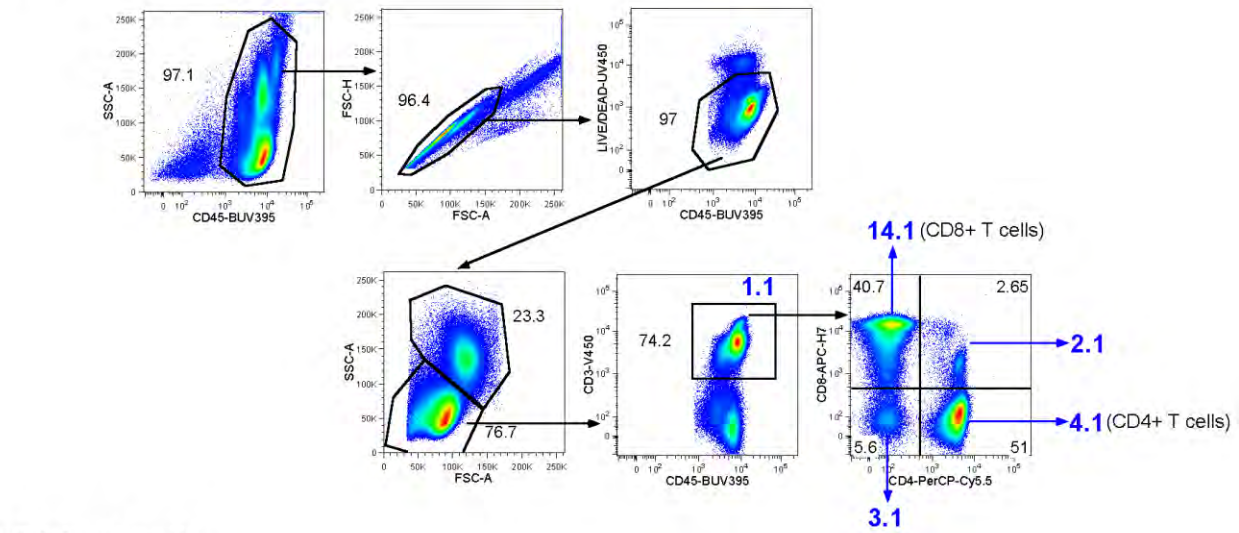


Suppl Fig. 2: Enrichment of the protein sets previously reported to predict GA in proteins ranked by their prediction power in our dataset. Ridge regression models to predict GA were generated using samples collected at 3 timepoints during pregnancy for the 33 women in our study. All somamers that passed QC (1194) were used as the features. Ridge regression was performed 100 times, each time using random 80% of samples as training set and remaining 20% as test set. The somamers were ranked by average coefficient from all models. The enrichment analysis was performed for previously reported 70 (top panel) and 8 (bottom panel) protein sets against this ranked list using *fgsea* Bioconductor package.

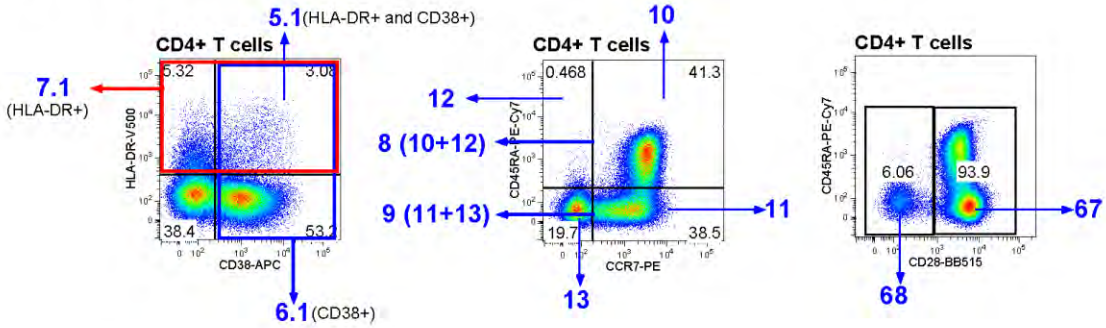


Suppl Fig. 3: Standard clinical phenotyping parameters from complete blood count (CBC) test can be used to predict gestational age. 9 parameters from CBC tests alone, without clinical flow cytometry, were used to generate an EN model of GA using data from 3 timepoints during pregnancy for the 33 women in our study. Predicted and observed GA correlated significantly (p=0.0012), where red dashed line is a linear regression with 95% confidential interval, and black dashed identity line marks equal GA (left). Frequency of selection and average weights are shown for the significant model features of hemoglobin level and monocyte count (right).

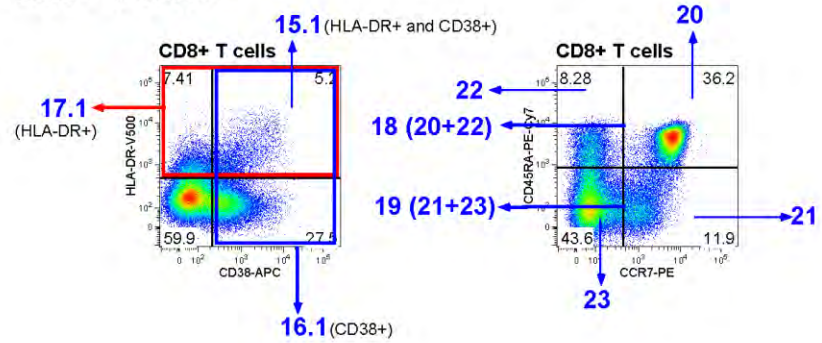
T cell Gating :



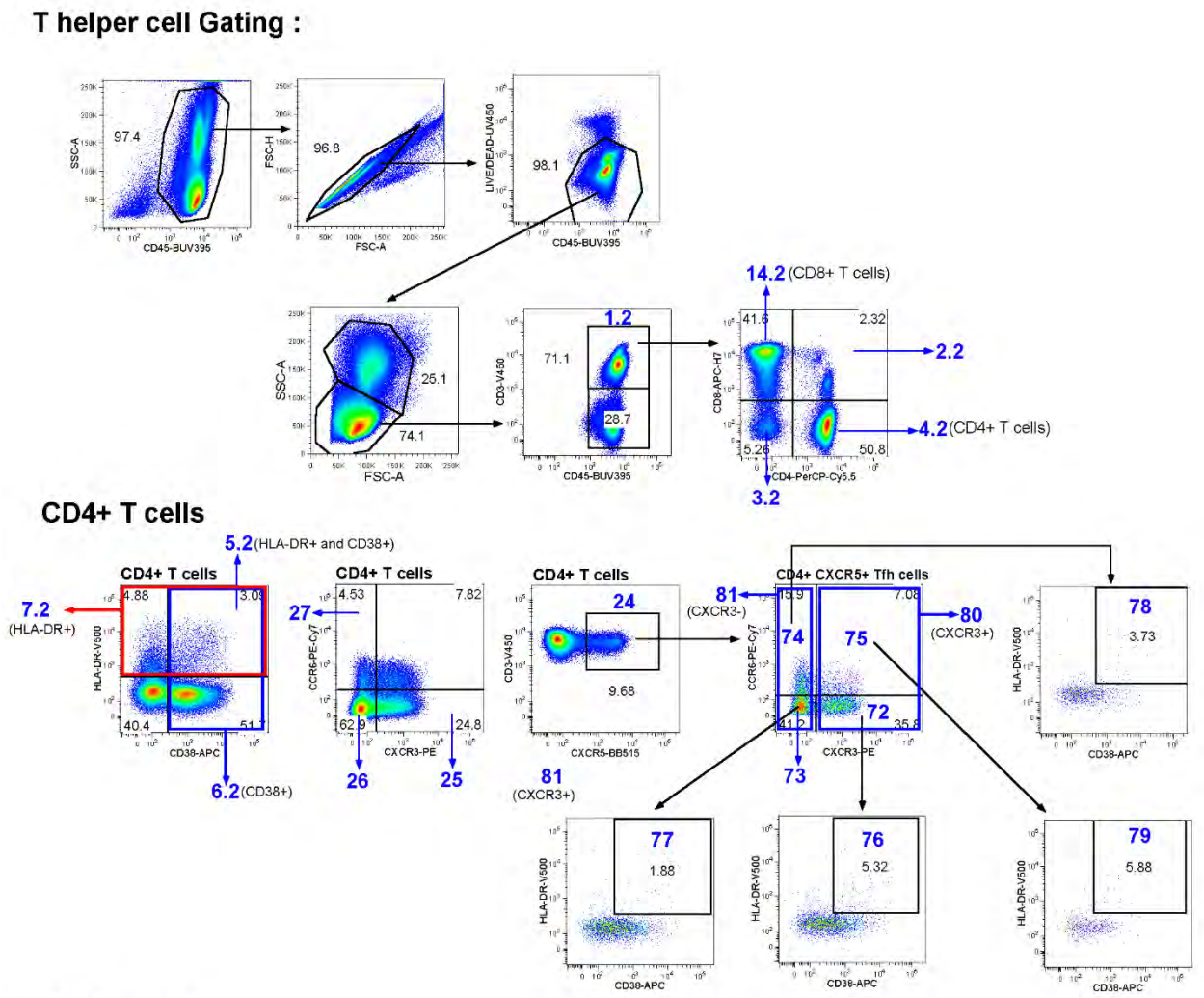
CD4+ T cells



CD8+ T cells

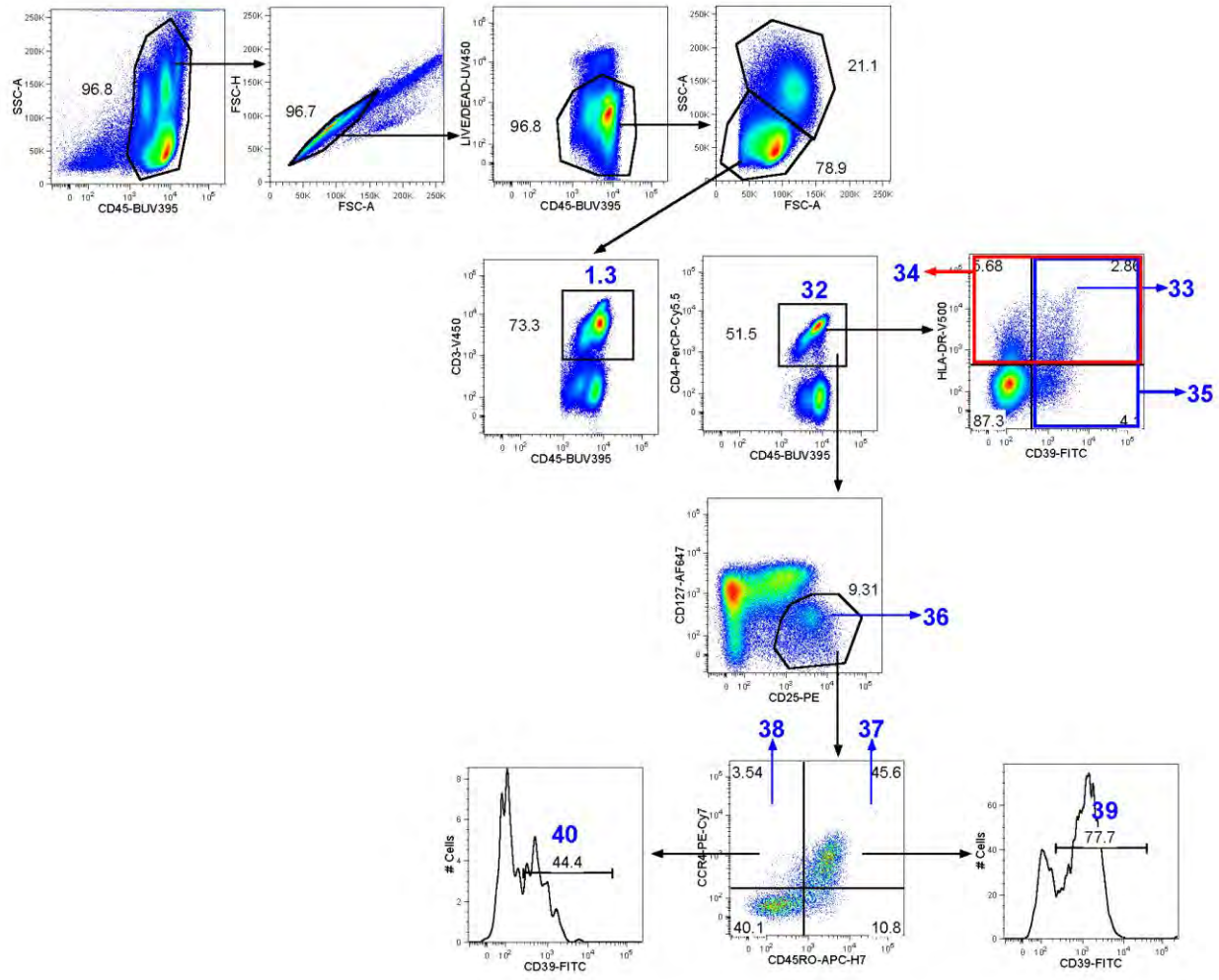


Suppl Fig. 4: Representative gating for populations identified by the T cell panel of the high parameter flow cytometry analysis (refer to Suppl Tab. 1,2).

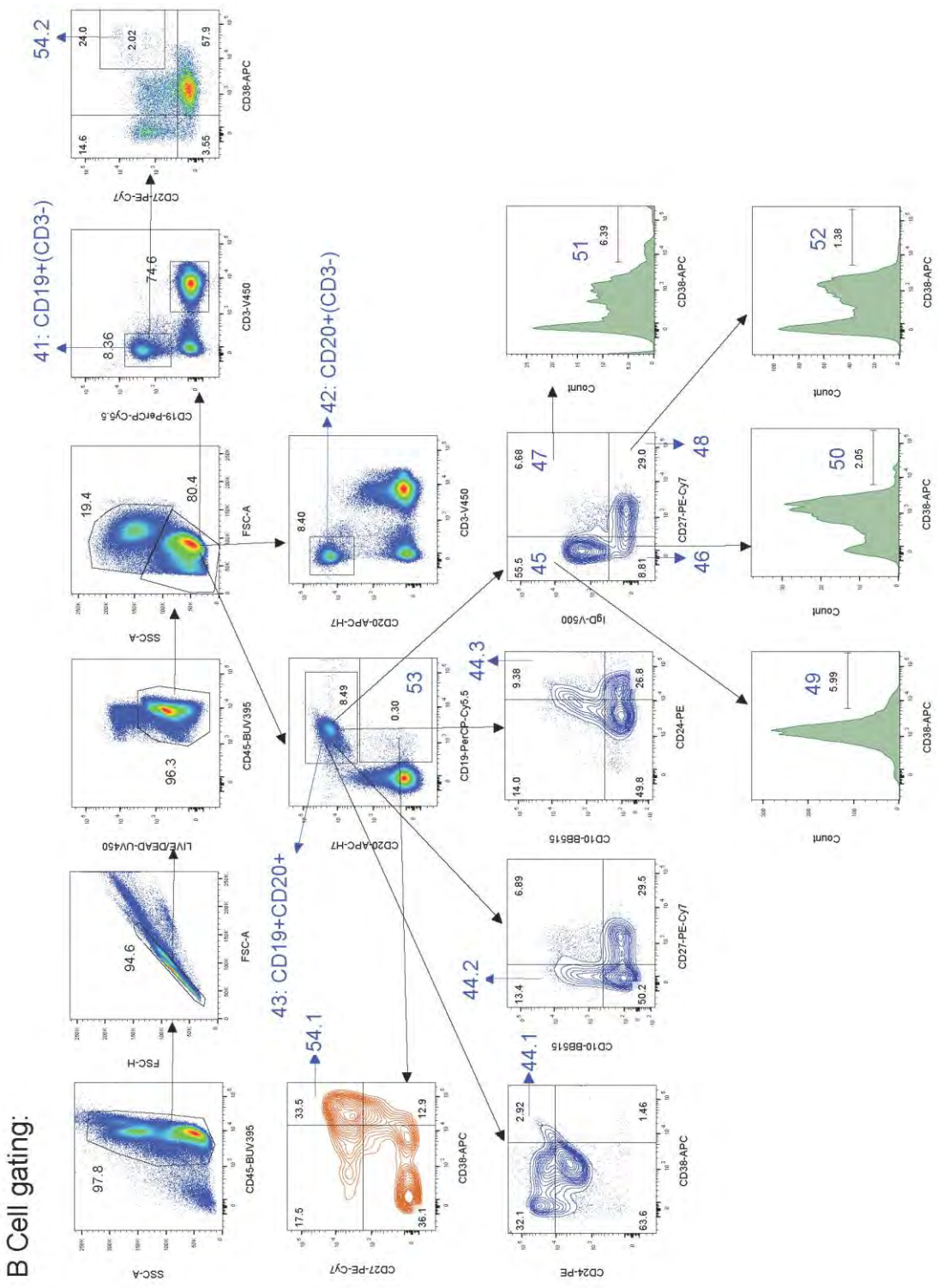


Suppl Fig. 5: Representative gating for populations identified by the T helper panel of the high parameter flow cytometry analysis (refer to Suppl Tab. 1,2).

T-reg Gating :

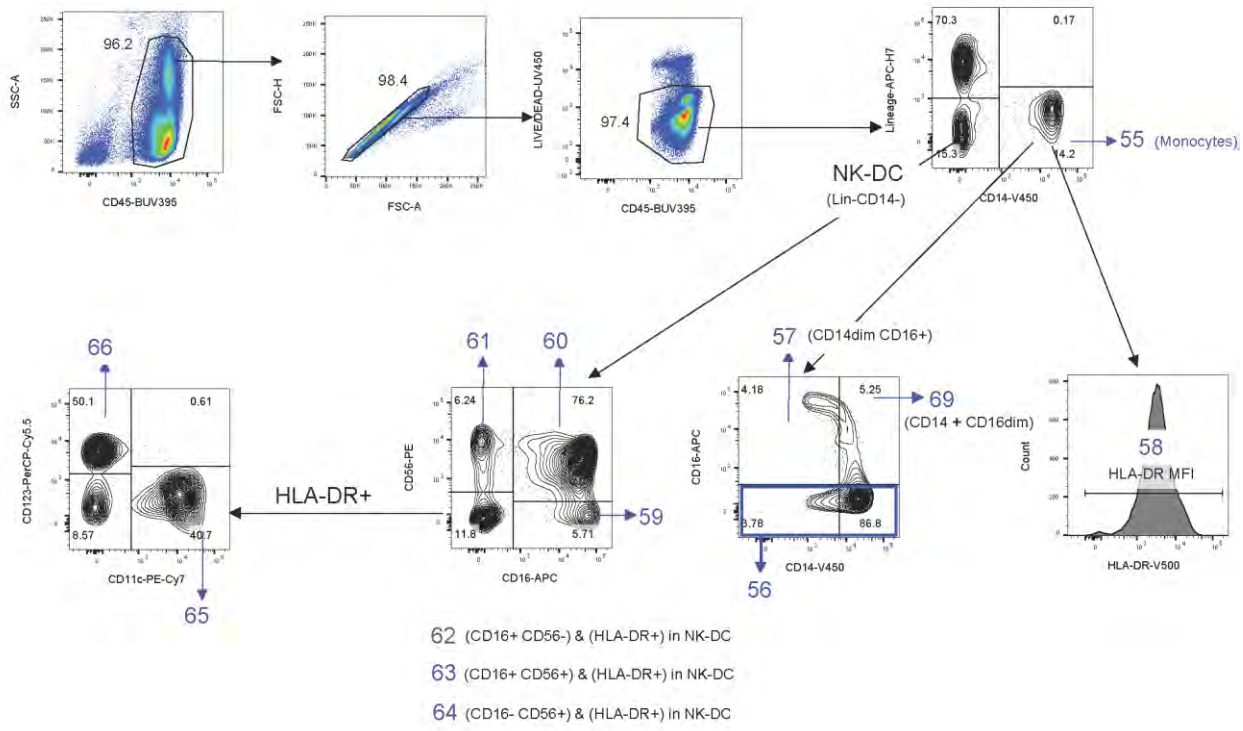


Suppl Fig. 6: Representative gating for populations identified by the T Reg panel of the high parameter flow cytometry analysis (refer to Suppl Tab. 1,2).



Suppl Fig. 7: Representative gating for populations identified by the B cell panel of the high parameter flow cytometry analysis (refer to Suppl Tab. 1,2).

DC Mon NK Cell gating:



Suppl Fig. 8: Representative gating for populations identified by the DC/Mono/NK panel of the high parameter flow cytometry analysis (refer to Suppl Tab. 1,2).

			HIPc-10c				
			T lineage			B lineage	Myeolid lineage
Excitation wavelenght	Fluorochrom	PMT name	T cell	Treg	Thelper	B cell	DC/Mono/NK
355 nm	BUV395	V450	**CD45**	**CD45**	**CD45**	**CD45**	**CD45**
			HI30	*HI30*	*HI30*	*HI30*	*HI30*
	UV450	V545	**Viability**	**Viability**	**Viability**	**Viability**	**Viability**
407 nm	V450	V605	**CD3**	**CD3**	**CD3**	**CD3**	**CD14**
			UCHT1	*UCHT1*	*UCHT1*	*UCHT1*	*MOP9*
	V500	V655	**HLA-DR**	**HLA-DR**	**HLA-DR**	**IgD**	**HLA-DR**
			G46-6	*G46-6*	*G46-6*	*IA6-2*	*G46-6*
488 nm	FITC	B515	**CD28**	**CD39**	**CXCR5**	**CD10**	**CD163**
			CD28.2	*Tu66*	*RF8B2*	*HI10a*	*GHI/61*
	PcP-Cy5.5	B710	**CD4**	**CD4**	**CD4**	**CD19**	**CD123**
			SK3	*SK3*	*SK3*	*SJ25C1*	*7G3*
532 nm	PE	G560	**CD197**	**CD25**	**CXCR3**	**CD24**	**CD56**
			150503	*2A3*	*1C6*	*ML5*	*B159*
	PE-Cy7	G780	**CD45-RA**	**CCR4**	**CCR6**	**CD27**	**CD11c**
			L48	*1G1*	*11A9*	*M-T271*	*B-Ly6*
633 nm	APC	R660	**CD38**	**CD127**	**CD38**	**CD38**	**CD16**
			HIT2	*hIL-7R-M21*	*HIT2*	*HIT2*	*B73.1*
	APC-Cy7	R780	**CD8**	**CD45-RO**	**CD8**	**CD20**	**CD3+CD19+CD20**
			SK1	*UCHL1*	*SK1*	*2H7*	*SK7+SJ25C1+2H7*

Suppl Table 1: Five parallel 10 color staining panels used for high parameter flow cytometry analysis.

Tube	Pop Code	Gate 1	Gate 2	Gate 3	Gate 4	Gate 5	Parent population	Subset name	Subset name 2
T-cell	1.1	CD3					viable mononuclear cells	T cells	CD3+ viable mononuclear cells
	2.1	CD3	CD4+/CD8+				CD3	DP T cells	CD4+CD8+ T cells
	3.1	CD3	CD4-/CD8-				CD3	DN T cells	CD4-CD8- T cells
	4.1	CD3	CD4+/CD8-	HLA-DR+/CD38+			CD3/CD4+/CD8-	CD38+HLA-DR+ activated T helper	CD4+CD8- T cells
	5.1	CD3	CD4+/CD8-	CD38+			CD3/CD4+/CD8-	CD38+ activated T helper	
	6.1	CD3	CD4+/CD8-	HLA-DR+			CD3/CD4+/CD8-	HLA-DR+ activated T helper	
	7.1	CD3	CD4+/CD8-	CD45RA+			CD3/CD4+/CD8-	CD45RA+ T helper	
	8	CD3	CD4+/CD8-	CD45RA-			CD3/CD4+/CD8-	CD45RA- T helper	
	9	CD3	CD4+/CD8-	CCR7+/CD45RA+			CD3/CD4+/CD8-	naive T helper	CD45RA+CCR7+ T helper
	10	CD3	CD4+/CD8-	CCR7+/CD45RA-			CD3/CD4+/CD8-	central memory T helper	CD45RA-CCR7+ T helper
	11	CD3	CD4+/CD8-	CCR7-/CD45RA+			CD3/CD4+/CD8-	effector T helper	CD45RA+CCR7- T helper
	12	CD3	CD4+/CD8-	CCR7-/CD45RA-			CD3/CD4+/CD8-	effector memory T helper	CD45RA-CCR7- T helper
	13	CD3	CD4+/CD8-	CD28+			CD3/CD4+/CD8-	CD28+ T helper	CD4-CD8+ T cells
	14.1	CD3	CD8+/CD4-	HLA-DR+/CD38+			CD3/CD8+/CD4-	CD38+HLA-DR+ activated T c/s	
	15.1	CD3	CD8+/CD4-	CD38+			CD3/CD8+/CD4-	CD38+ activated T c/s	
	16.1	CD3	CD8+/CD4-	HLA-DR+			CD3/CD8+/CD4-	HLA-DR+ activated T c/s	
	17.1	CD3	CD8+/CD4-	CD45RA+			CD3/CD8+/CD4-	CD45RA+ T c/s	
	18	CD3	CD8+/CD4-	CD45RA-			CD3/CD8+/CD4-	CD45RA- T c/s	
	19	CD3	CD8+/CD4-	CCR7+/CD45RA+			CD3/CD8+/CD4-	naive T c/s	CD45RA+CCR7+ T c/s
	20	CD3	CD8+/CD4-	CCR7+/CD45RA-			CD3/CD8+/CD4-	central memory T c/s	CD45RA-CCR7+ T c/s
	21	CD3	CD8+/CD4-	CCR7-/CD45RA+			CD3/CD8+/CD4-	terminal effector T c/s	CD45RA+CCR7- T c/s
	22	CD3	CD8+/CD4-	CCR7-/CD45RA-			CD3/CD8+/CD4-	effector memory T c/s	CD45RA-CCR7- T c/s
	23	CD3	CD8+/CD4-	CD28+			CD3/CD8+/CD4-	CD28+ T helper	
	67	CD3	CD4+/CD8+	CD28+			CD3/CD4+/CD8+	CD28+ T helper	
T helper	1.2	CD3					viable mononuclear cells	T cells	CD3+ viable mononuclear cells
	2.2	CD3	CD4+/CD8+				CD3	DP T cells	CD4+CD8+ T cells
	3.2	CD3	CD4+/CD8-				CD3	DN T cells	CD4-CD8- T cells
	4.2	CD3	CD4+/CD8-				CD3	T helper	CD4+CD8- T cells
	5.2	CD3	CD4+/CD8-	HLA-DR+/CD38+			CD3/CD4+/CD8-	CD38+HLA-DR+ activated T helper	
	6.2	CD3	CD4+/CD8-	CD38+			CD3/CD4+/CD8-	CD38+ activated T helper	
	7.2	CD3	CD4+/CD8-	HLA-DR+			CD3/CD4+/CD8-	HLA-DR+ activated T helper	
	24	CD3	CD4+/CD8-	CXCR5+			CD3/CD4+/CD8-	Tfh	CXCR5+ T helper
	25	CD3	CD4+/CD8-	CXCR3+/CCR6-			CD3/CD4+/CD8-	Th1	CXCR3+CCR6- T helper
	26	CD3	CD4+/CD8-	CXCR3-/CCR6-			CD3/CD4+/CD8-	Th2	CXCR3-CCR6- T helper
	27	CD3	CD4+/CD8-	CXCR3+/CCR6+			CD3/CD4+/CD8-	Th17	CXCR3+CCR6+ T helper
	14.2	CD3	CD8+/CD4-	CXCR3-/CCR6+			CD3/CD4-/CD8+	Tc17	CXCR3-CCR6+ T c/s
	15.2	CD3	CD8+/CD4-	HLA-DR+/CD38+			CD3/CD4-/CD8+	CD38+HLA-DR+ activated T c/s	CD4-CD8+ T cells
	16.2	CD3	CD8+/CD4-	CD38+			CD3/CD4-/CD8+	CD38+activated T c/s	CXCR3+CCR6- Tfh
	17.2	CD3	CD8+/CD4-	HLA-DR+			CD3/CD4-/CD8+	HLA-DR+ activated T c/s	CXCR3-CCR6+ Tfh
	28	CD3	CD8+/CD4-	CXCR5+			CD3/CD4-/CD8+	Tfh17	CXCR5+ Tc/s
	29	CD3	CD8+/CD4-	CXCR3+/CCR6-	CD38+/HLA-DR+		CD3/CD4+/CD8-/CXCR5+/CXCR3+/CCR6-	Tc1	CXCR3+CCR6- Tc/s
	30	CD3	CD8+/CD4-	CXCR3-/CCR6-			CD3/CD4-/CD8+/CXCR5+/CXCR3-/CCR6-	Tc2	CXCR3-CCR6- Tc/s
	31	CD3	CD8+/CD4-	CXCR3-/CCR6+	CD38+/HLA-DR+		CD3/CD4-/CD8+/CXCR5+/CXCR3+/CCR6+	Tc17	CXCR3-CCR6+ Tc/s
	72	CD3	CD4+/CD8-	CXCR5+	CXCR3+/CCR6-		CD3/CD4+/CD8-/CXCR5+	Tfh1	CXCR3+CCR6- Tfh
	73	CD3	CD4+/CD8-	CXCR5+	CXCR3-/CCR6-		CD3/CD4+/CD8-/CXCR5+	Tfh2	CXCR3-CCR6- Tfh
	74	CD3	CD4+/CD8-	CXCR5+	CXCR3+/CCR6+		CD3/CD4+/CD8-/CXCR5+	Tfh17	CXCR3+CCR6+Tfh
	75	CD3	CD4+/CD8-	CXCR5+	CXCR3+/CCR6-	CD38+/HLA-DR+	CD3/CD4+/CD8-/CXCR5+/CXCR3+/CCR6-	Tfh1/17	CXCR3+CCR6+ Tfh
	76	CD3	CD4+/CD8-	CXCR5+	CXCR3+/CCR6+	CD38+/HLA-DR+	CD3/CD4+/CD8-/CXCR5+/CXCR3+/CCR6+	CD38+HLA-DR+ activated Tfh1	
	77	CD3	CD4+/CD8-	CXCR5+	CXCR3-/CCR6-	CD38+/HLA-DR+	CD3/CD4+/CD8-/CXCR5+/CXCR3-/CCR6-	CD38+HLA-DR+ activated Tfh2	
	78	CD3	CD4+/CD8-	CXCR5+	CXCR3+/CCR6+	CD38+/HLA-DR+	CD3/CD4+/CD8-/CXCR5+/CXCR3+/CCR6+	CD38+HLA-DR+ activated Tfh17	
	79	CD3	CD4+/CD8-	CXCR5+	CXCR3+/CCR6+	CD38+/HLA-DR+	CD3/CD4+/CD8-/CXCR5+/CXCR3+/CCR6+	CD38+HLA-DR+ activated Tfh1/17	
	80	CD3	CD4+/CD8-	CXCR5+	CXCR3+		CD3/CD4+/CD8-/CXCR5+	CXCR3+ Tfh	
	81	CD3	CD4+/CD8-	CXCR5+	CXCR3-		CD3/CD4+/CD8-/CXCR5+	CXCR3- Tfh	
T-reg	1.3	CD3					viable mononuclear cells	T cells	CD3+ viable mononuclear cells
	32	CD3	CD4+				CD3	T helper	CD4+ T cells
	33	CD3	CD4+	HLA-DR+/CD39+			CD3/CD4+	CD39+ HLA-DR+ activated T helper	
	34	CD3	CD4+	HLA-DR+			CD3/CD4+	HLA-DR+ activated T helper	
	35	CD3	CD4+	CD39+			CD3/CD4+	CD39+ activated T helper	
	36	CD3	CD4+	CD127lo/CD25+			CD3/CD4+	Treg	CD127lowCD25+ T helper
	37	CD3	CD4+	CD127lo/CD25+	CCR4+/45RO+		CD3/CD4+/CD127lo/CD25+	memory Treg	CD45RO+CCR4+ Treg
	38	CD3	CD4+	CD127lo/CD25+	CCR4+/45RO-		CD3/CD4/CD127lo/CD25+	naive Treg	CD45RO-CCR4+ Treg
	39	CD3	CD4+	CD127lo/CD25+	CCR4+/CD45RO+	CD39+	CD3/CD4/CD127lo/CD25+/CCR4+/CD45RO+	CD39+ memory Treg	
	40	CD3	CD4+	CD127lo/CD25+	CCR4+/CD45RO-	CD39+	CD3/CD4/CD127lo/CD25+/CCR4+/CD45RO-	CD39+ naive Treg	
B-cell	41	CD3-/CD19+					viable mononuclear cells	CD19+ B cells	CD19+CD3- viable mononuclear cells
	42	CD3-/CD20+					viable mononuclear cells	CD20+ mature B cells	CD20+CD3- viable mononuclear cells
	43	CD19+/CD20+					viable mononuclear cells	CD19+CD20+ B cells	CD19+CD20+ viable mononuclear cells
	44.1	CD19+/CD20+	CD24hi/CD38hi				CD19+/CD20+	transitional B cells	CD24hiCD38hi CD19+CD20+ B cells
	44.2	CD19+/CD20+	CD10+/CD27lo				CD19+/CD20+	transitional B cells	CD10+CD27lo CD19+CD20+ B cells
	44.3	CD19+/CD20+	CD24hi/CD10+				CD19+/CD20+	transitional B cells	CD10+CD24hi CD19+CD20+ B cells
	45	CD19+/CD20+	CD27-/IgD+				CD19+/CD20+	naive B cells	CD27-IgD+ CD19+CD20+ B cells
	46	CD19+/CD20+	CD27-/IgD-				CD19+/CD20+	CD27-IgD- naive B cells	CD27-IgD- CD19+CD20+ B cells
	47	CD19+/CD20+	CD27+/IgD-				CD19+/CD20+	CD27+IgD- memory B cells	CD27+IgD- CD19+CD20+ B cells
	48	CD19+/CD20+	CD27+/IgD+				CD19+/CD20+	CD27+IgD+ memory B cells	CD27+IgD+ CD19+CD20+ B cells
	49	CD19+/CD20+	CD27-/IgD+	CD38hi			CD19+/CD20+/CD27-/IgD+	CD38high activated CD27-IgD+ naive B cells	
	50	CD19+/CD20+	CD27-/IgD-	CD38hi			CD19+/CD20+/CD27-/IgD-	CD38high activated CD27-IgD- naive B cells	
	51	CD19+/CD20+	CD27+/IgD+	CD38hi			CD19+/CD20+/CD27+/IgD+	CD38high activated CD27+IgD+ memory B cells	
	52	CD19+/CD20+	CD27+/IgD-	CD38hi			CD19+/CD20+/CD27+/IgD-	CD38high activated CD27+IgD- memory B cells	
	53	CD19+/CD20-					viable mononuclear cells	CD20- B cells	CD19+CD20- viable mononuclear cells
	54.1	CD19+/CD20-	CD27hi/CD38hi				CD19+/CD20-	plasmablast B cells	CD27hiCD38hi CD20- B cells
	54.2	CD19+/CD3-	CD27hi/CD38hi				CD19+/CD3-	plasmablast B cells	CD27hiCD38hi CD19+ B cells
DC Mono NK	55	Lin+/CD14+					viable mononuclear cells	Monocytes	CD14+ viable mononuclear cells
	56	Lin+/CD14+	CD16-	HLA-DR+			Lin+/CD14+	classical monocytes	CD14+CD16- monocytes
	57	Lin+/CD14+	CD14dim/CD16+	HLA-DR+			Lin+/CD14+	non-classical monocytes	CD14dimCD16+ monocytes
	58	Lin+/CD14+	HLA-DR+ (MFI)	HLA-DR+			Lin+/CD14+	monocytes, HLA-DR antigen density	monocyte HLA-DR MFI
	59	Lin-/CD14-	CD16+/CD56-				Lin-/CD14-	CD16+ CD56- NK cells	CD16+ CD56- Lin-CD14- cells
	60	Lin-/CD14-	CD16+/CD56+				Lin-/CD14-	CD16+CD56+ NK cells	CD16+ CD56+ Lin-CD14- cells
	61	Lin-/CD14-	CD16-/CD56+				Lin-/CD14-	CD16-CD56+ NK cells	CD16- CD56+ Lin-CD14- cells
	62	Lin-/CD14-	CD16+/CD56-	HLA-DR+			Lin-/CD14-/CD16+/CD56-	HLA-DR+ activated CD16+CD56- NK cells	
	63	Lin-/CD14-	CD16+/CD56+	HLA-DR+			Lin-/CD14-/CD16+/CD56+	HLA-DR+ activated CD16+CD56+ NK cells	
	64	Lin-/CD14-	CD16-/CD56+	HLA-DR+			Lin-/CD14-/CD16-/CD56+	HLA-DR+ activated CD16-CD56+ NK cells	
	65	Lin-/CD14-	CD16+/CD56-	HLA-DR+	CD11c+/CD123-		Lin-/CD14-/CD16+/CD56-/HLA-DR+		CD11c+CD123- HLA-DR+ CD16-CD56- Lin-CD14- cells
	66	Lin-/CD14-	CD16-/CD56-	HLA-DR+	CD123+/CD11c-		Lin-/CD14-/CD16-/CD56-/HLA-DR+	plasmacoid dendritic cells	CD123+CD11c- HLA-DR+ CD16-CD56- Lin-CD14- cells
	69	Lin-/CD14+	CD14+/CD16dim				Lin-/CD14+	CD16dim monocytes	CD14+CD16dim viable mononuclear cells

Suppl Tab 2: 79 subsets of PBMC detected by high parameter flow cytometry analysis.

Parameter	Group definition (n)	Cell populations			Serum proteins		
		p	q	ID	p	q	ID
Parity	1 (14) vs <1 (19)	0.055	0.710	X79	0.037	0.993	Transferrin
		0.090	0.710	X50	0.060	0.993	C6
		0.160	0.710	X75	0.067	0.993	FABP
Maternal age	<33 (10) vs >33 (10)	0.100	0.884	X50	0.003	0.764	OMD
		0.112	0.884	X79	0.004	0.764	FSTL3
		0.148	0.884	X1.3	0.012	0.998	Cystatin.C
Prev miscarriage	no (26) vs yes (7)	0.004	0.108	X50	0.010	0.840	HGFA
		0.021	0.213	X33	0.018	0.840	TSP4
		0.025	0.213	X49	0.022	0.840	CHL1
Gestation duration	<39 (10) vs >40 (10)	0.121	0.941	X50	0.013	1.000	VEGF.sR3
		0.235	0.941	X80	0.016	1.000	MMP.12
		0.248	0.941	X81	0.023	1.000	IL.13.Ra1
BMI at V1	<23 (10) vs >28 (10)	0.030	0.525	X1.3	0.022	1.000	granzyme.A
		0.040	0.525	X1.2	0.035	1.000	FABPL
		0.238	0.922	X44.2	0.054	1.000	HCC.4
Systolic BP at V1	<108 (10) vs >118 (10)	0.020	0.515	X44.2	0.009	0.851	DKK3
		0.107	0.746	X1.3	0.013	0.851	SLIK5
		0.128	0.746	X1.2	0.016	0.851	HCC.4

Suppl Table 3: Immune populations and serum proteins that change during gestation do not differ between individuals based on metadata characteristics. For each of the 26 flow populations and 434 somamers that changed between V1 and V3, the magnitude of change was compared between individuals segregated by metadata characteristics. The top 3 associations for each are shown for 6 characteristics, showing no FDR adjusted $p<0.05$.

Parameter	Range	Complete Blood Count	Clinical Flow Cytometry
Hemoglobin	11.2-15.7 g/dL	X	
Platelets	173-369 K/uL	X	
WBC	3.98-10.04 K/uL	X	
Neutrophil %	34-71.1%	X	
Neutrophil absolute	1.56-6.13 K/uL	X	
Lymphocytes %	19.3-51.7%	X	
Lymphocytes absolute	1.18-3.74 K/uL	X	
Monocyte%	4.7-12.5 %	X	
Monocyte absolute	0.24-0.86 K/uL	X	
CD3%	57-79%		X
CD3 absolute	615-2348 uL		X
CD4/CD3%	27-59%		X
CD4/CD3 absolute	334-1556 uL		X
CD8/CD3%	13-33%		X
CD8/CD3 absolute	149-787 uL		X
NK cells%	7-31%		X
NK Cells absolute	109-607 uL		X
CD19%	6-19 %		X
CD19 absolute	81-493 uL		X

Suppl Table 4: Parameters determined by clinical phenotyping analysis.