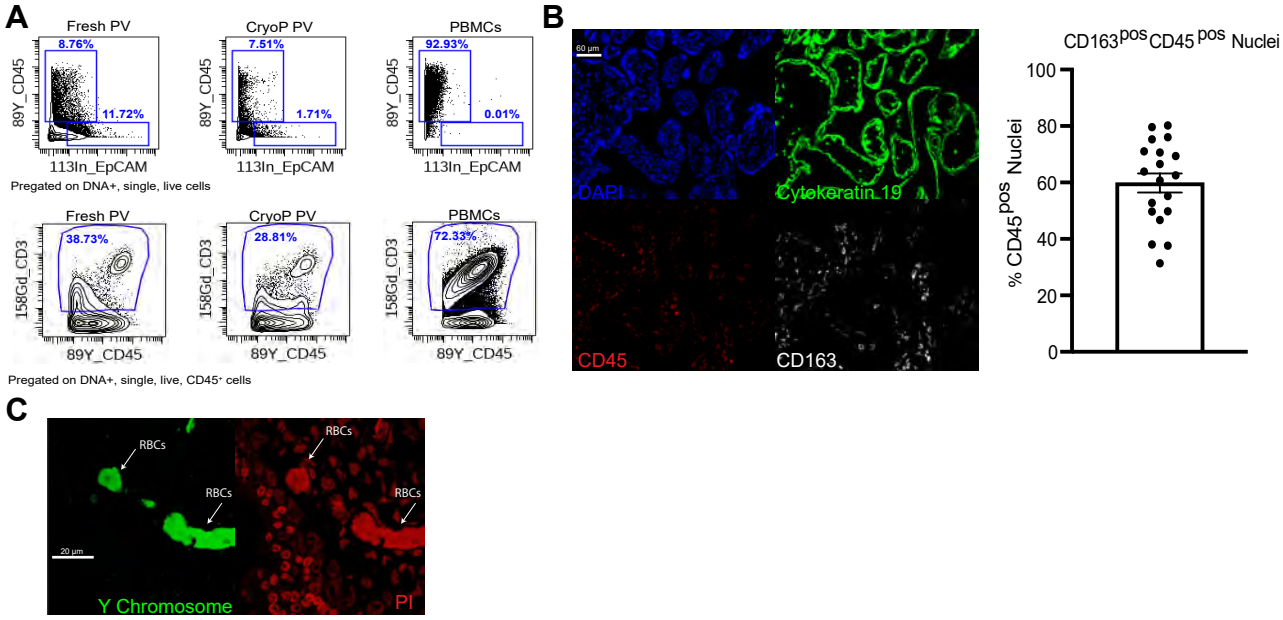

Fig. S1. (A) Comparison of CyTOF analysis of $CD45^{pos}$ and T cell abundances between fresh PV, cryopreserved PV and frozen Peripheral Blood Mononuclear Cells (PBMCs). CyTOF analysis (n=12), see Table S5 for omitted cases. (B) Splits of immunofluorescence for intravillous immune cell detection and quantification of $CD163^{hi}$ immune cells (n=19). (C) Splits for Y-chromosome *in situ* hybridization. All graphs are represented as Mean +/-SE (standard error).

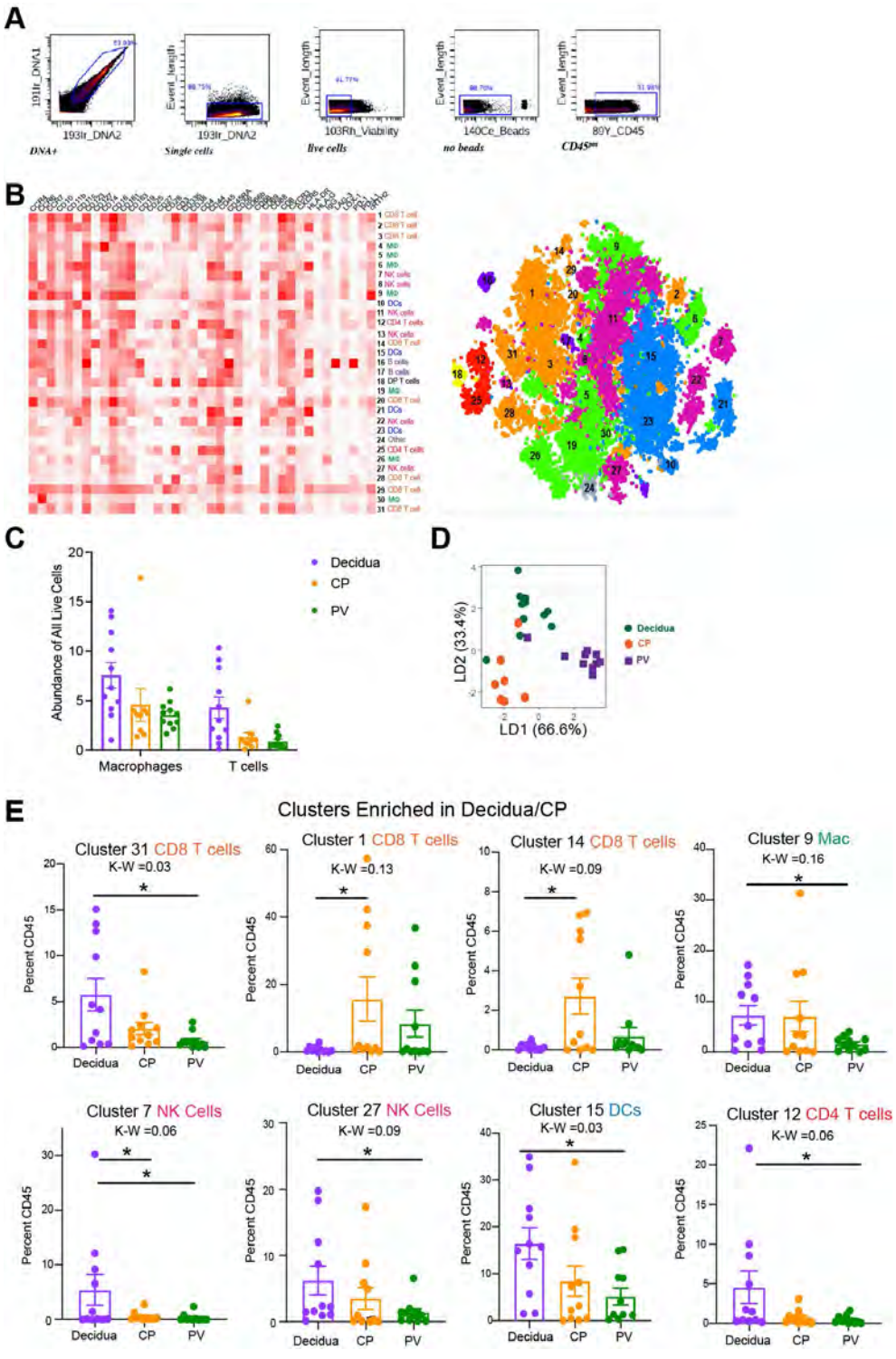

Fig. S2. (A) Pre-gating strategy for CD45^{pos} population used in automated clustering. (B) Clustergrammar heatmap used for cluster identification (left) of tsne clusters mapped (right). (C) Abundance of major immune subsets as a proportion of all live cells. (D) Linkage Disequilibrium (LD) plot confirming segregation of placental layer immune profile. (E) Abundance of decidua and CP enriched clusters. CyTOF analysis starting (n=12), see Table S5 for omitted cases. * = p value < 0.05 after posthoc analysis from Kruskal-Wallis (K-W) test. All graphs are represented as Mean +/-SE (standard error).

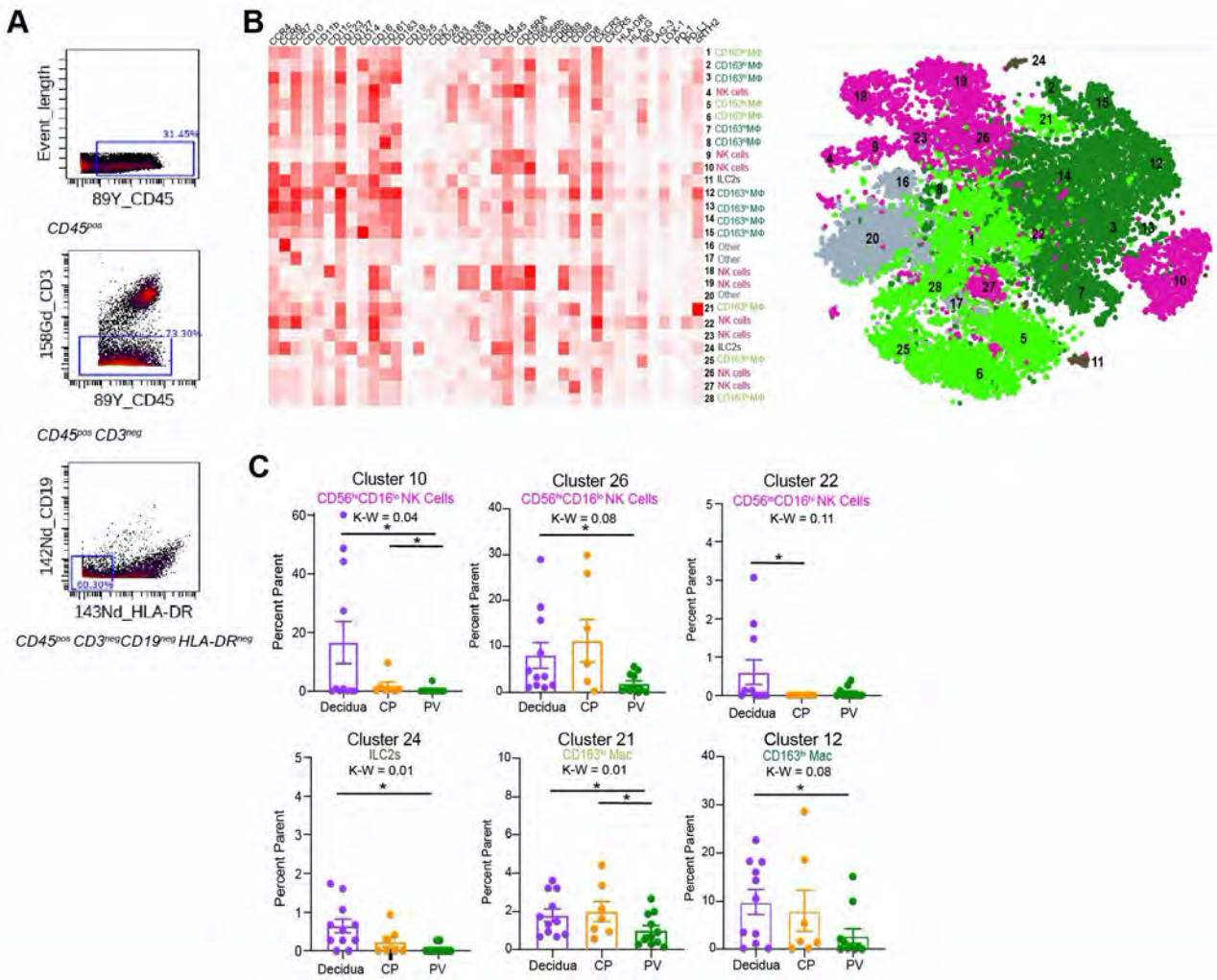

Fig. S3. (A) Pregating strategy for innate non-APC population. (B) Clustergrammar heatmap used for cluster identification (left) of tsne clusters mapped (right). (C) Cumulative data on abundance of decidua and CP enriched clusters. CyTOF analysis starting (n=12), see Table S5 for omitted cases. * = p value < 0.05 after posthoc analysis from Kruskal-Wallis (K-W) test. All graphs are represented as Mean +/-SE (standard error).

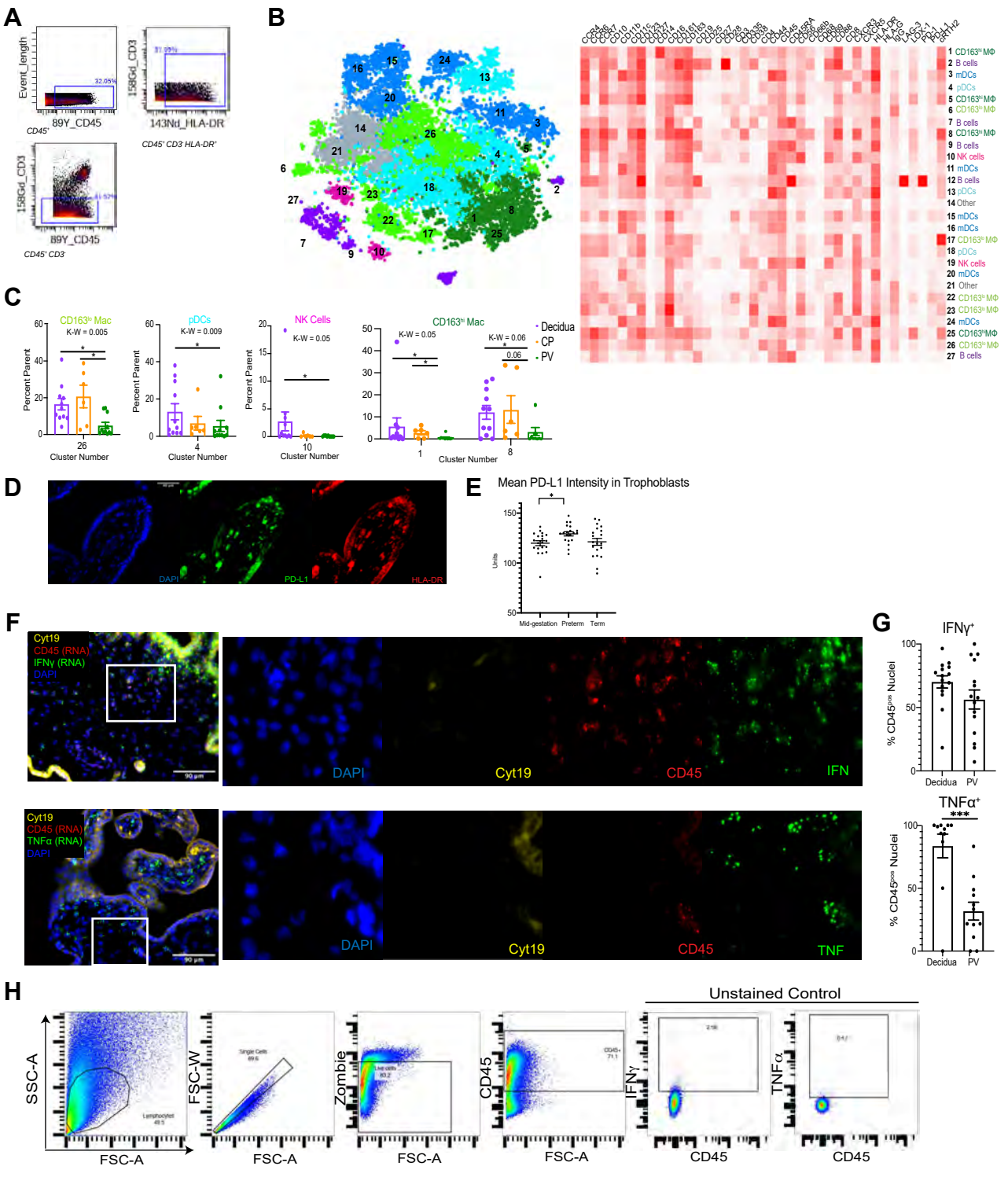

Fig. S4. (A) Pregating strategy for APC population. (B) Clustergrammar heatmap used for cluster identification (left) of tsne clusters mapped (right). (C) Cumulative data on abundance of decidua and CP enriched clusters. CyTOF analysis starting (n=12), see Table S5 for omitted cases. * = p value < 0.05 after posthoc analysis from Kruskal-Wallis (K-W) test. (D) Splits for PD-L1^{pos} PV APCs representative image. (E) Quantification of trophoblast expression of PD-L1

via IHC. (n=2) (F) Splits for dual RNA *in situ* hybridization and IF. Representative images in main figure taken from region identified in white rectangle. (G) Quantification of staining for cytokine positive immune cells in PV and decidua. (n=4) (H) Pregating for $CD45^{pos}$ population with flow cytometry. *** = p-value < 0.001 in Mann-Whitney two tailed test. All graphs are represented as Mean +/-SE (standard error).

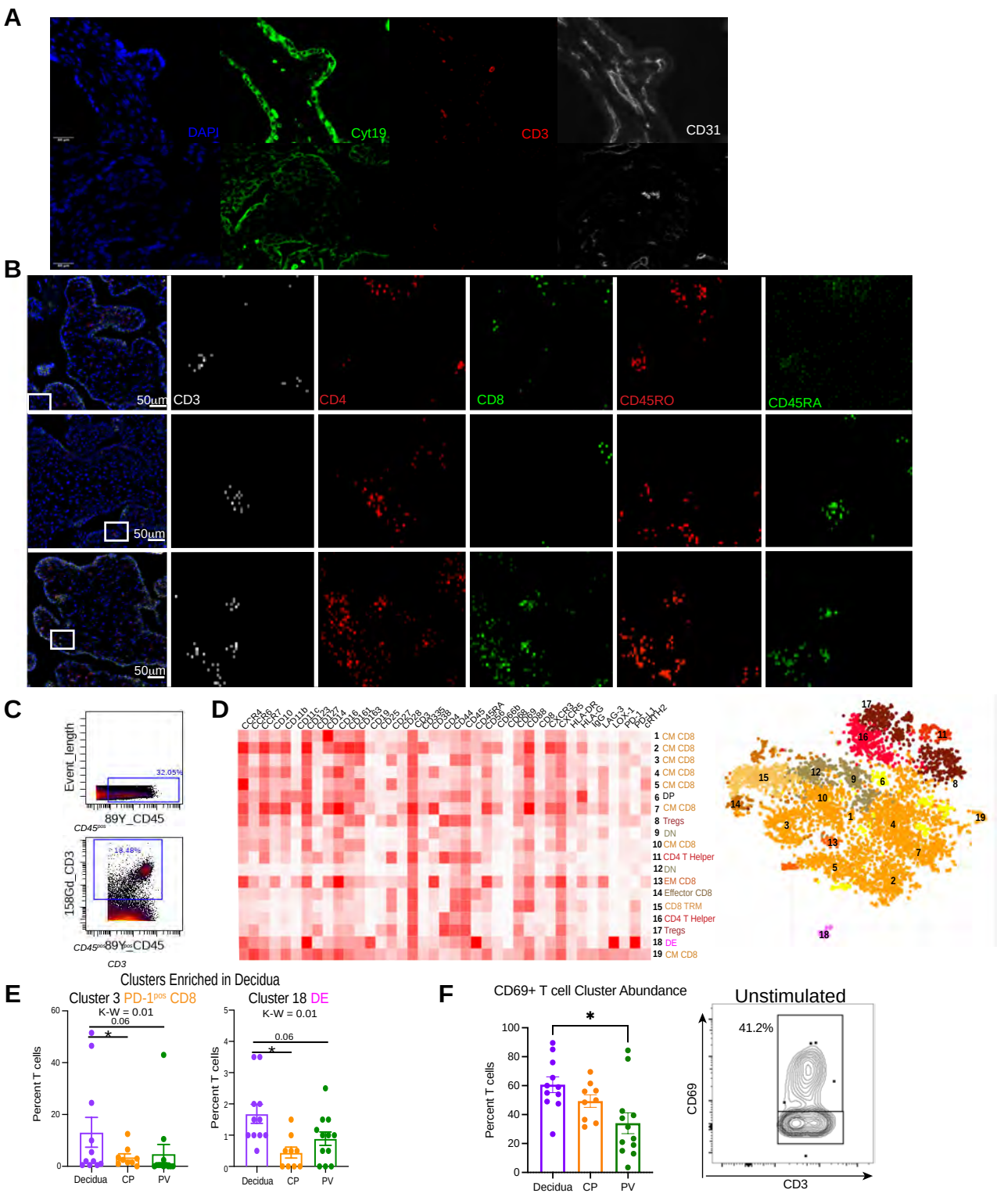

Fig. S5. (A) Splits from IF detection of T cells and PV endothelium. (B) Splits from representative IMC images of T cell subsets. T cells show in main figures identified in regions highlighted with white rectangles. (C) Pregating strategy for T cell population. (D) Clustergrammer heatmap used for cluster identification (left) of tsne clusters mapped (right). (E)

Cumulative data on abundance of decidua and CP enriched clusters. * = p value < 0.05 after posthoc analysis from Kruskal-Wallis (K-W) test. (F) Abundance of CD69+ T cells calculated by summation of all CD69+ T cell tsne clusters (left) see Table S5 for omitted cases. Representative FACS plot of $CD69^{+}$ T cells isolated from mid-gestation placenta (right). All graphs are represented as Mean +/-SE (standard error).

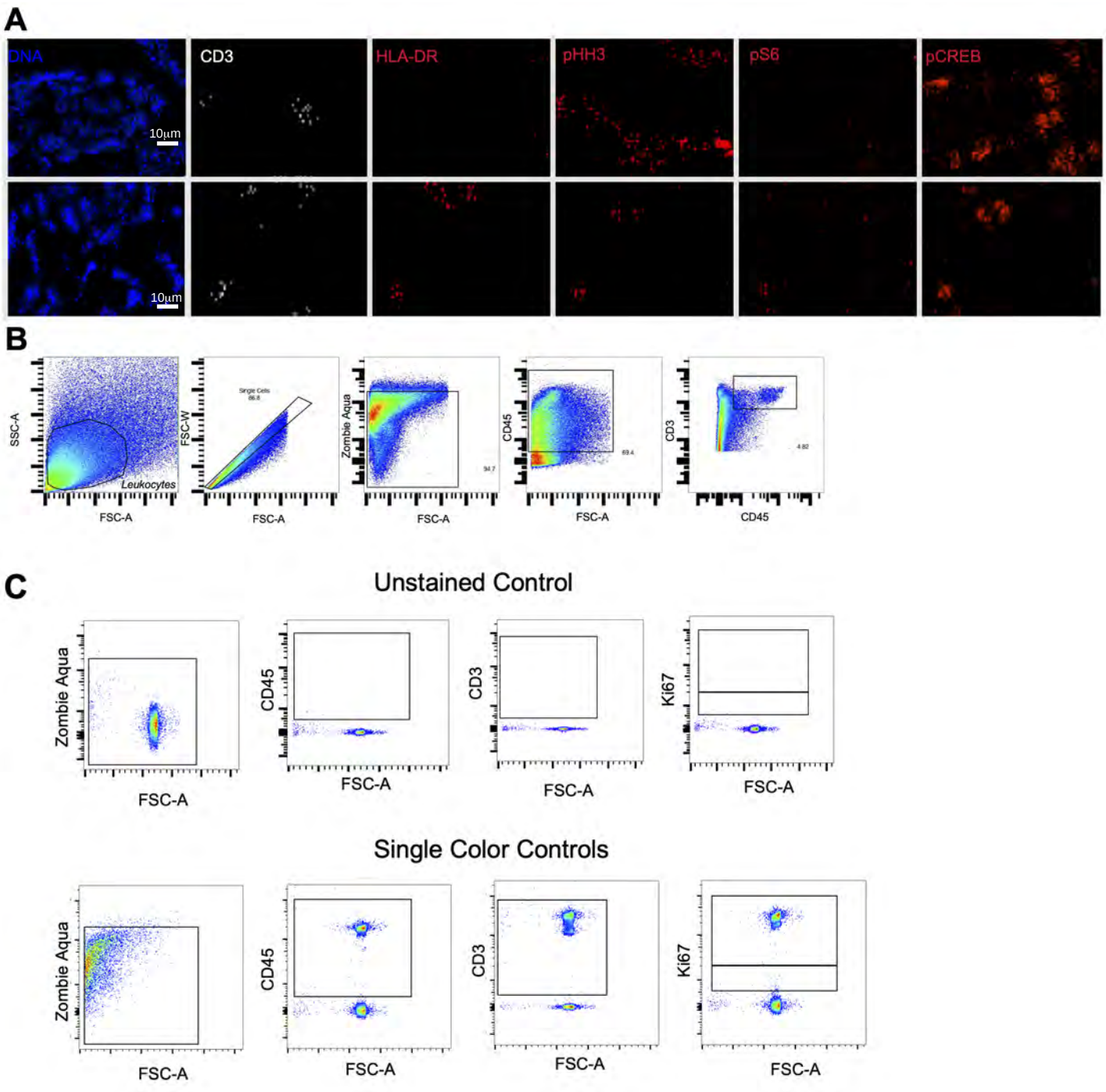

Fig. S6. (A) Splits from IMC images showing inactive and active T cells in PV. (B) Pregating strategy for $CD69^{pos}$ and $CFSE^{pos}$ T cells. (C) Gating for proliferative populations quantified in main text. All experiments are (n=3).

Table S1. Patient Cohort

Table S1: Patient Cohort

Demographic Information						
Sample Number	Gestational Age	Sex	Maternal Age Range	Diagnosis	Method of Delivery	Labor
1130	23 weeks	F	25-30	ET	N/A	N/A
1143	23 weeks	M	20-25	ET	N/A	N/A
1146	23 weeks	F	25-30	ET	N/A	N/A
1081	22 weeks	M	25-30	ET	N/A	N/A
1131	18 weeks	M	20-25	ET	N/A	N/A
1132	22 weeks	M	30-35	ET	N/A	N/A
1186	18-19 weeks	M	30-35	ET	N/A	N/A
1100A	21 weeks	N/A	34-40	ET	N/A	N/A
1102	21 weeks	F	40-45	ET	N/A	N/A
1055	23 weeks	F	35-40	ET	N/A	N/A
1108	22 weeks	F	25-30	ET	N/A	N/A
1038	21 weeks	M	N/A	ET	N/A	N/A
1042	22 weeks	M	N/A	ET	N/A	N/A
1094	17 weeks	F	25-30	ET	N/A	N/A
1106	21 weeks	F	30-35	ET	N/A	N/A
1165	18 weeks	M	20-25	ET	N/A	N/A
PO38	23 weeks	F	N/A	ET	N/A	N/A
1304	39 weeks	M	N/A	HT	C-section	N
1315	37 weeks	F	N/A	HT	C-section	N
1316	39 weeks	M	N/A	HT	C-section	N
1302	39 weeks	M	N/A	HT	C-section	N
1303	39 weeks	F	N/A	HT	C-section	N
1230	29 weeks	M	N/A	Hemorrage	Vaginal	Y
1236	35 weeks	M	N/A	D, HyT	C-section	N/A

Experimental Use								
RNAseq	qPCR	FISH	CyTOF	IMC	RISH	IHC	TCR Stim CFSE	TCR Stim Ki67
✓			✓		✓	✓		
✓		✓						
✓			✓					
	✓							
	✓		✓					
	✓		✓					
	✓			✓			✓	
	✓					✓		
	✓		✓					
	✓							
		✓	✓		✓			
			✓					
			✓				✓	
			✓		✓			
			✓	✓	✓		✓	
			✓					
								✓
								✓
								✓
						✓		
						✓		
						✓		

* genetic case
ET, Elective Termination
HTD, Healthy Term
Y, Yes
N, No
D, Diabetes
Hyt, Hypertension

Table S2. Differentially Expressed Genes Between Decidua, CP and PV

Click here to download Table S2

Table S3. Selected Immune Genes

Table S3: Selected Immune Genes

Gene Name	Expression Values								
	Decidua 1	Decidua 2	Decidua 3	CP1	CP2	CP3	PV1	PV2	PV3
PTPRC	18.25	9.53	13.57	6.64	7.22	0.21	2.84	2.84	4.34
CD3D	5.02	3.87	5.38	0	0.64	0	0	0.65	0.47
CD3G	0.63	0.3	0.67	0	0.39	0	0.12	0.12	0.21
CD3E	9.3	4.15	7.35	0.04	0.53	0	0.13	0.17	1.85
CD4	48.51	60.61	62.16	20.23	24.72	2.04	17.55	39.68	46.63
CD8A	0.38	2.85	1.73	0	0.25	0	0	0.11	0.76
IL7R	1.08	0.75	2.66	0.14	1.56	0.1	0.19	0.29	0.71
CD19	0.06	0.05	0.49	0.79	0.44	0.03	0	0.42	0.27
NCAM1	6.53	2.02	3.29	0.18	0.02	0.06	0	0.01	0.33
CD14	444.26	329.06	470.57	228.26	219.48	3.83	162.49	324.13	430.24
CD163	54.09	37.6	44.92	43.76	29.41	0.9	20.53	31.63	36.2
CD68	385.84	213.79	459.9	78.93	110.38	9.69	115.92	62.35	165.92
IL3RA	4.65	11.15	5.31	2.78	13.33	0.21	11.2	8.06	9.96
ITGAX	11.27	4.97	18.49	1.46	3.36	0.06	0.98	0.75	1.18
HLA-DRA	1,690.96	589.86	962.34	386.91	495.82	4.53	55.26	86.08	67.75
HLA-DRB5	258.75	101.54	238.74	50.52	87.15	0.99	6.51	19.9	22.32
HLA-DRB6	48.63	23.05	70.91	13.91	22.82	0.4	2.66	3.21	4.85
CD69	8.06	4.16	17.1	1.93	2.04	1.32	1.72	0.48	3.53
CD44	50.77	30.92	50.01	22.61	31.86	2	24.06	19.52	48.42
NFKB1	16.11	34.05	22.71	7.99	30.13	28.96	11.12	25.79	28.47
NFKB2	13.03	17.85	18.36	6.42	14.28	47.22	4.15	10.58	15.45
CXCL8	93.43	53.42	30.57	40.08	665	15.41	87.19	44.44	21.43
IL1B	85.82	55.8	70	4.92	60.03	0.98	4.86	3.45	15.24
IL6	2.34	5.18	3.48	5.69	14.34	22.4	16.03	6.67	12.39
IFNGR1	124.86	152.35	166.72	49.29	157.79	68.13	61.25	114.17	121.57
IFNGR2	141.04	168.79	200.74	98.05	113.48	205.37	77.54	54.45	190.82
IFNAR2	14.09	22.98	20.82	7.76	26.34	8.35	7.52	11.98	16.93
IFNAR1	26.69	33.77	34.23	14.09	29.39	29.99	12.63	17.96	32.96
TNFRSF4	4.72	4.05	10.42	2.14	7.98	0	5.1	5.42	5.79
TNFRSF14	13.04	11.26	17.46	4.76	10.05	2.69	10.73	7.98	16.69
TNFRSF1B	54.03	90.41	69.25	21.9	64.87	0.52	31.26	69.05	61.91
TNFRSF11A	2.65	3.09	1.15	3.98	2.9	0.1	2.89	2.88	1.01
TNFRSF6B	5.54	5.05	4.01	1.69	3.81	4.28	3.22	1.14	4.59

Table S4. CyTOF Panel

Table S4: CyTOF Panel

Metal	Target	Clone	Vendor
89Y	CD45	HI30	Fluidigm
113In	CD88	P12/1	BioRad
115In	CD44	IM7	BioLegend
141Pr	CD66b	G10F5	BioLegend
142Nd	CD19	HIB19	BioLegend
143Nd	HLA-DR	L243	Fluidigm
144Nd	CD69	FN50	Fluidigm
145Nd	cRTH2	BM16	BioLegend
146Nd	CD8a	RPA.T8	BioLegend
147Sm	CD10	HI10a	BioLegend
148Nd	CD28	CD28.2	BioLegend
149Sm	CD25	2A3	Fluidigm
150Nd	CD38	HIT2	BioLegend
151Eu	CD123	TUGH4	BioLegend
152Sm	CD14	M5E2	BioLegend
153Eu	CD45RA	HI100	BioLegend
154Sm	CD163	GHI/61	Fludigim
155Gd	CD27	L128	Fluidigm
156Gd	CCR4	SIDI8BEE	eBiosciences
158Gd	CD3	UCHT1	BioLegend
159Tb	CD11c	Bu15	BioLegend
160Gd	IgG	MHK49	Fluidigm
161Dy	CD16	3G8	BioLegend
162Dy	CD56	NCAM16.2	BioLegend
163Dy	CXCR3	G025H7	Fluidigm
164Dy	CD161	HP-3G10	BioLegend
165Ho	LAG3	11C3C65	Fluidigm
166Er	HLA-G	87G	BioLegend
167Er	Lox1	331212	R&D Systems
168Er	CD127	A019D5	Fludiigm
169Tm	CD11b	M1/70	BioLegend
170Er	CCR7	G043H7	BiolLegend
171Yb	CD68	Y1/82A	Fluidigm
172Yb	CD274(PD-L1)	29E.2A3	BioLegend
173Yb	CD335	9E2	BioLegend
174Yb	CD4	SK3	Fluidigm
175Lu	PD-1	EH12.2H7	Fluidigm
176Yb	IL-10	JES3-19F1	BioLegend
209Bi	CXCR5	MU5UBEE	eBiosciences

Table S5. Files Omitted from CyTOF Analyses

Table S5: Files Omitted from CyTOF Analyses

Sample Name	Figures Excluded
1102 Decidua	1,2,4,5
1165 CP	1,2,4,5
PO38 PV	1,2,4
1094 CP	2,4
1130 CP	2,4
1131 CP	2,4

Table S6. Cell Type Identification

Table S6: Cell Type Identification

Cell Type	Markers
Leukocytes/Immune Cells	CD45+
T Cells	CD45+CD3+
B Cells	CD45+CD3-CD19+
Innate Cells	CD45+CD3-CD19-
CD4 T cells	CD45+CD3+ CD4+
CD8 T cells	CD45+CD3+ CD8+
Double Positive T Cells	CD45+CD3+ CD4+ CD8+
Double Negative T Cells	CD45+CD3+ CD4- CD8-
Regulatory T cells	CD45+CD3+ CD4+ CD127lo CD25+
Macrophages	CD45+CD3-CD14+
NK Cells	CD45+CD3-CD19-CD14-CD56+
Dendritic Cells	CD45+CD3-CD19-CD14-CD56- HLA-DR+
Plasmacytoid Dendritic Cells	CD45+CD3-CD19-CD14-CD56- HLA-DR+ CD11b+
Myeloid Dendritic Cells	CD45+CD3-CD19-CD14-CD56- HLA-DR+ CD123+
Innate Lymphoid Cells	CD45+CD3-CD19- CD127+
Innate Lymphoid Cells Type 2	CD45+CD3-CD19- CD127+ cRTH2+
Dual Expressing Lymphocytes	CD45+CD3+ CD19+

Table S7. Differentially Expressed Chemokines

Table S7: Differentially Expressed Chemokines

Gene Name	Expression Values								
	Decidua 1	Decidua 2	Decidua 3	CP1	CP2	CP3	PV1	PV2	PV3
CCL20	1.8	1.82	1.54	1.86	19.35	17.19	1.92	0.6	0.82
CXCL1	13.87	30.47	10.96	10.19	63.07	200.51	10.3	54.14	6.71
CXCL2	15.56	2.21	29.92	11.11	19.91	291.33	11.76	2.57	5.55
CXCL9	8.14	6.96	6.01	2.43	0.11	0	0	0.05	0.4
CXCL10	61.44	37	50.4	17.1	4.66	0.45	4.13	1.44	18.45
CXCL11	3.71	2.37	2.43	0.37	0.31	0	0.25	0.32	2.03
CXCL13	57.5	5.58	7.54	0.08	0	0	0	0	2.3
CCR6	0.08	0.53	0.26	0.03	0	0.04	0	0	0.02
CCL19	0.67	0.65	1.41	0	0	0	0	0	0.19
CCL21	147.98	121.42	54.91	3.61	58.4	0.1	0.62	0.07	0.4
CXCL12	17.24	6.77	14.59	4.01	1.36	0.01	3.03	1.18	4
CCL17	2.51	3.02	0.67	0	0	0	0	0	0.12
CCL8	176.57	38.1	73.7	40.76	13.42	1.98	3.02	2.41	22.94
CCL5	28.58	34.01	143.58	11.25	16.63	0.12	4.67	5.99	27.3
CCL14	14.33	1.65	6.49	0.15	0.18	0	0	0.04	0.4
CCL14-15	0.17	0.41	5.36	0.91	1.52	0	0	0	0
CCL23	13.59	4.29	19.25	0	0.4	0	0.26	1.1	2.08
CCL4	173.82	72.43	292.49	51.6	61.12	3.65	28.47	17.57	77.83
CXCR3	4.35	8.74	10.61	0.06	0.11	0	0	0.14	1.68

Table S8. IMC Panel

Table S8: IMC Panel

Metal	Target	Clone	Vendor
115In	CD44	IM7	BioLegend
142Nd	CD19	6OMP31	Fluidigm
143Nd	Vimentin	D21H3	Fluidigm
144Nd	CD14	EPR3653	Fluidigm
147Sm	CD163	EDHu-1	Fluidigm
148Nd	PanKeratin	C11	Fluidigm
151Eu	CD31	EPR3094	Fluidigm
152Sm	CD45	D8M81	Fluidigm
156Gd	CD4	EPR6855	Fluidigm
160Gd	IgG	MHK49	Fluidigm
161Dy	Ki67	8D5	BioLegend
162Dy	CD8a	C8/144B	Fluidigm
164Dy	pZAP70		Cell Signaling
165Ho	pCREB	87G3	Cell Signaling
166Er	CD45RA	HI100	Fluidigm
167Er	p44/42	D13.14.4E	Cell Signaling
169Tm	pSTAT3	A1600213	Cell Signaling
170Er	CD3	polyclonal	Fluidigm
171Yb	CD66a	CD66a-B1.1	Fluidigm
173Yb	CD45RO	UCHL1	Fluidigm
174Yb	HLA-DR	LN3	Fluidigm
175Lu	pS6	D57.2.2E	Cell Signaling
176Yb	pHistoneH3	HTA28	Fluidigm

Table S9. Flow Cytometry Antibodies

Table S9: Flow Cytometry Antibodies

Fluorophore	Target	Clone	Vendor
PE-Cy7	CD3	SK7	Biolegend
PE	CD69	FN50	Biolegend
PacBlue	TNFa	Mab11	Biolegend
AF647	IFNg	4S.B3	Biolegend
Ki67	AF488		Biolegend
AF700	CD45	HI30	Biolegend