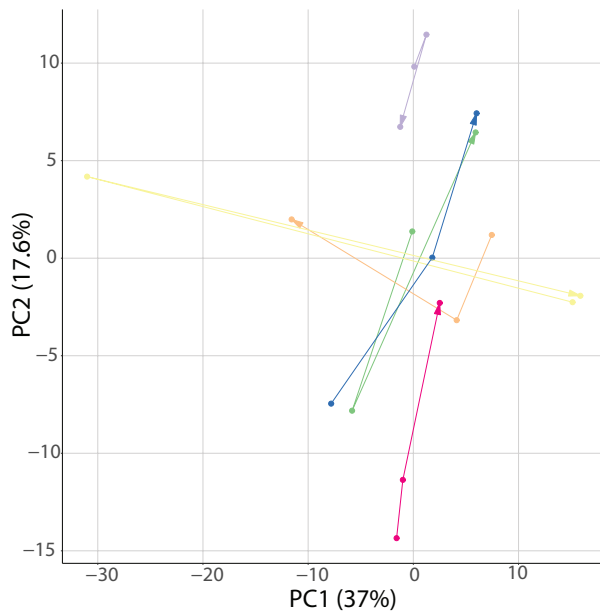
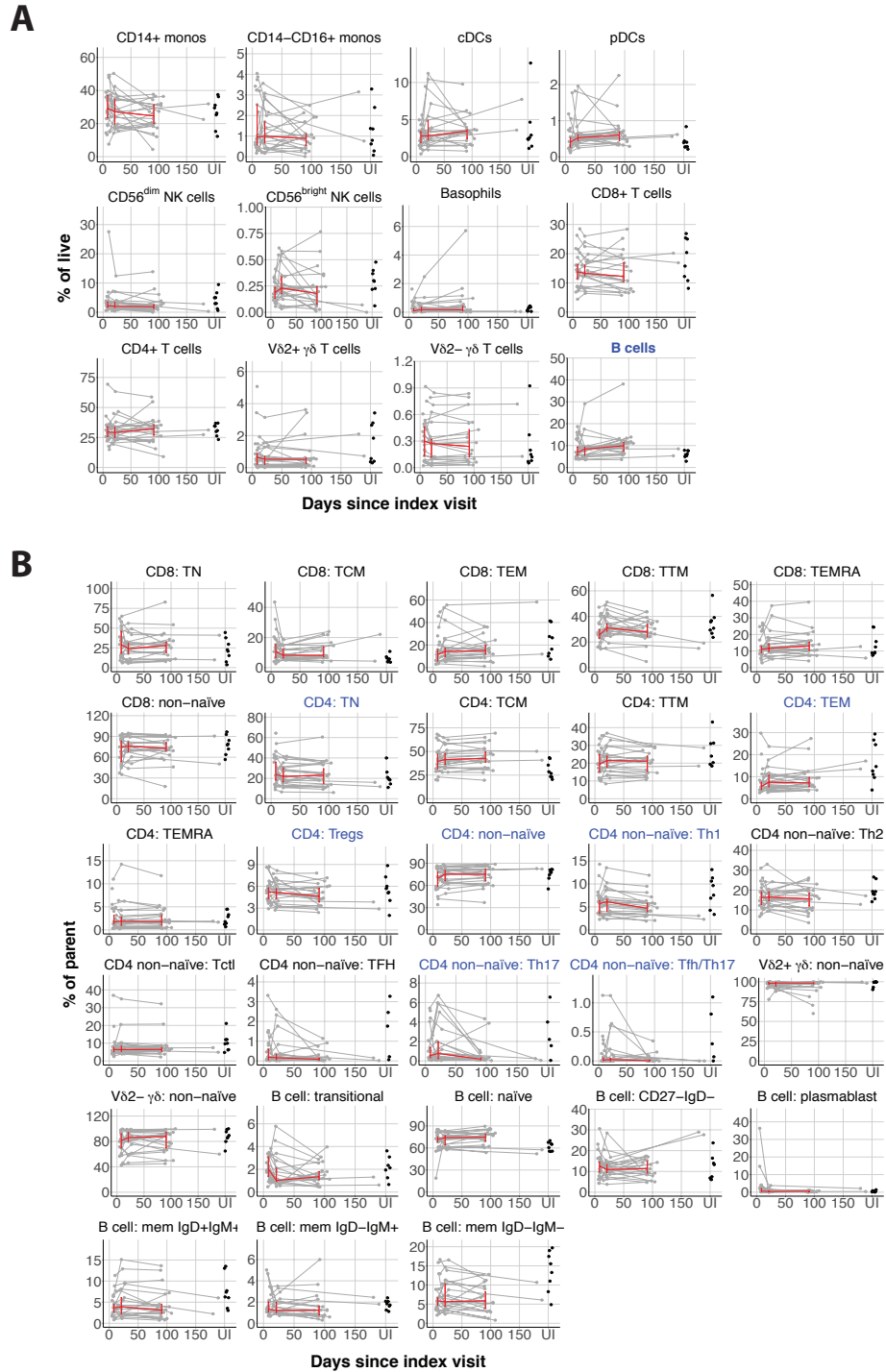


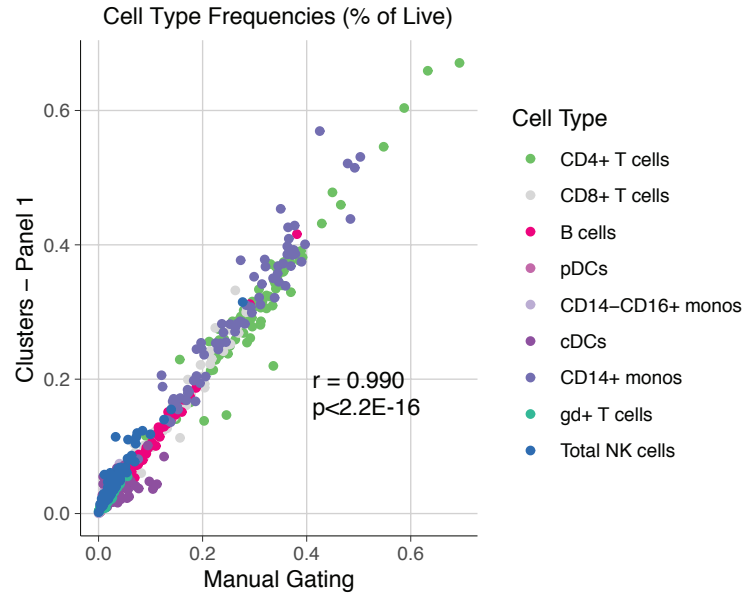
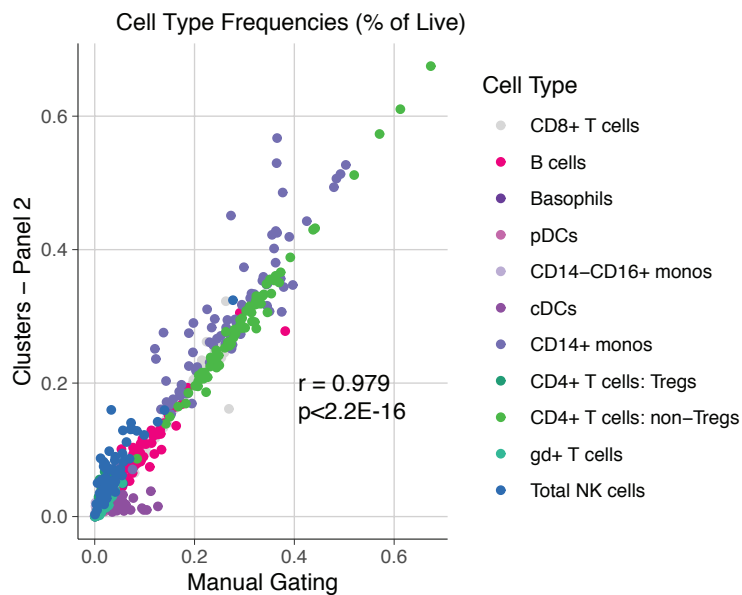
**Figure S1. CyTOF gating strategy**  
Immune cell (A) landmark population and (B) non-naïve CD4+ T cell sub-population gating strategy.



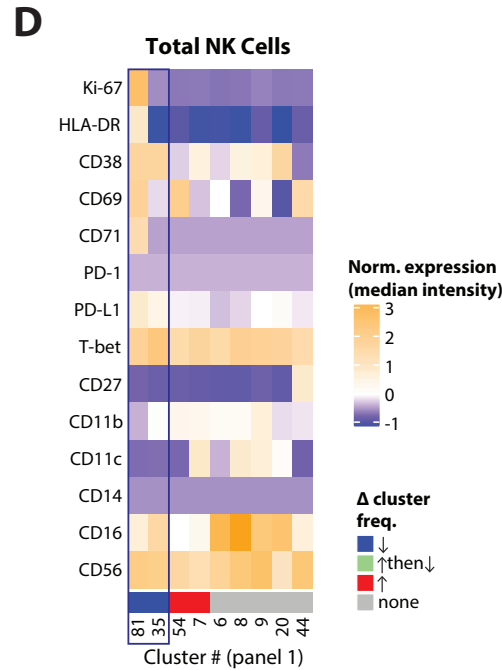
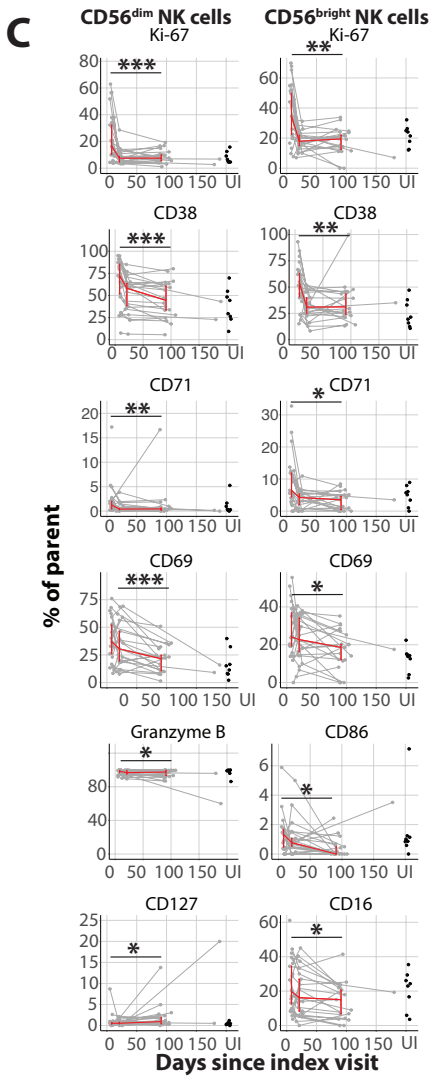
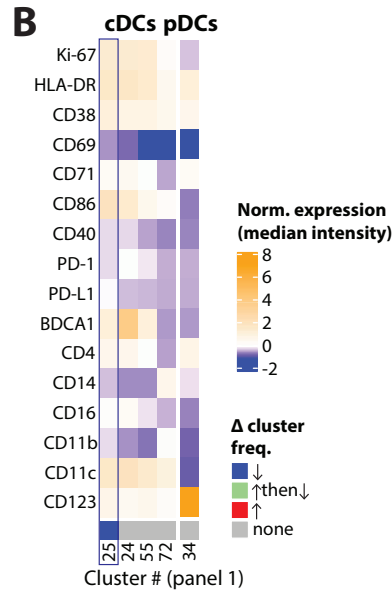
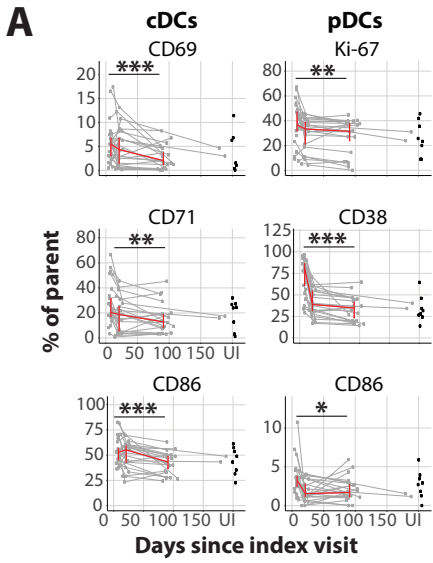
**Figure S2: Principal Component Analysis (PCA) of uninfected participants.**  
PCA representation of all manually gated parameters measured on PBMCs from ZIKV-uninfected control participants at longitudinal timepoints.



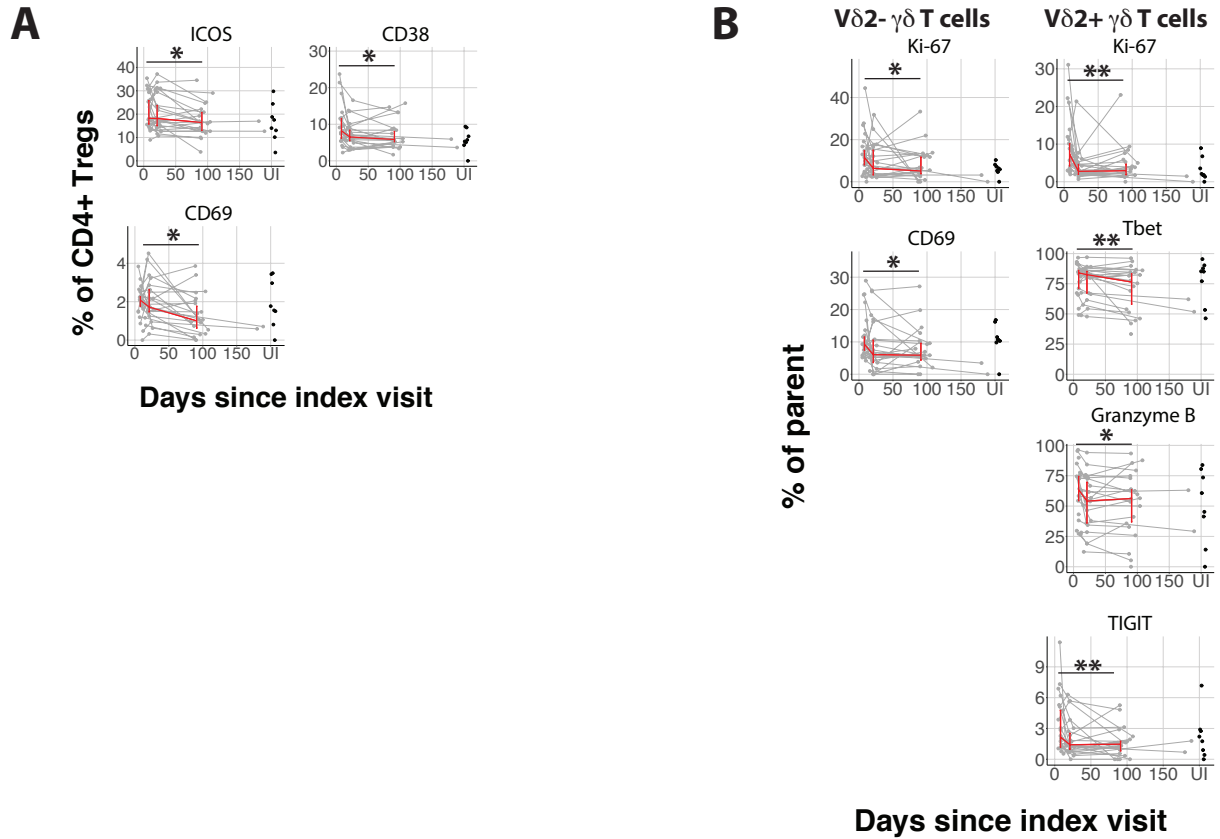
**Figure S3. Landmark and sub-landmark population abundance in acute and convalescent ZIKV infection.** Line plots of frequency of indicated (A) landmark cell type and adaptive immune subset for each participant versus time since index visit. Red line connects median values at each sampling timepoint with error bars for +/- 95% CI. Scatterplot for feature abundance from cross-sectional uninfected (UI) cohort shown on the far right. Features with  $p_{adj} < 0.05$  have blue colored titles.  $p_{adj}$  values obtained by LME model fit with Benjamini-Hochberg FDR correction.

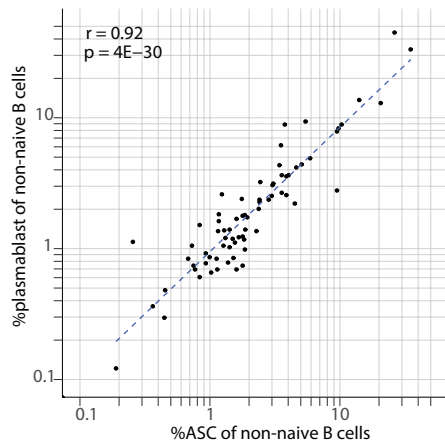
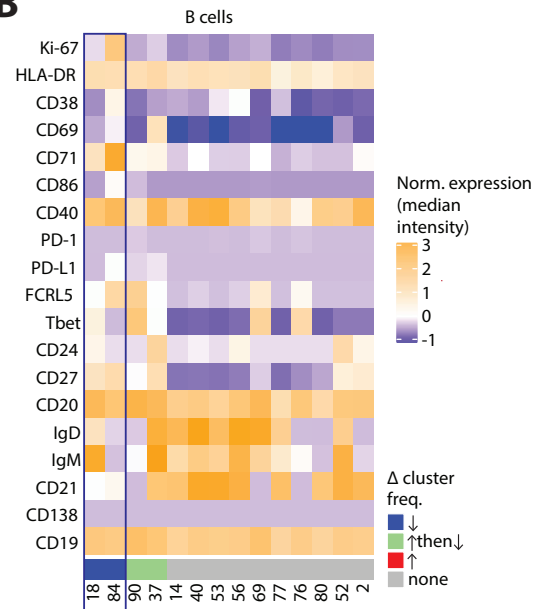
**A****B****Figure S4. Landmark cell population frequencies by manual gating vs clustering.**

High concordance in landmark cell population frequencies as measured by manual gating versus SCAFFoLD clustering analysis.

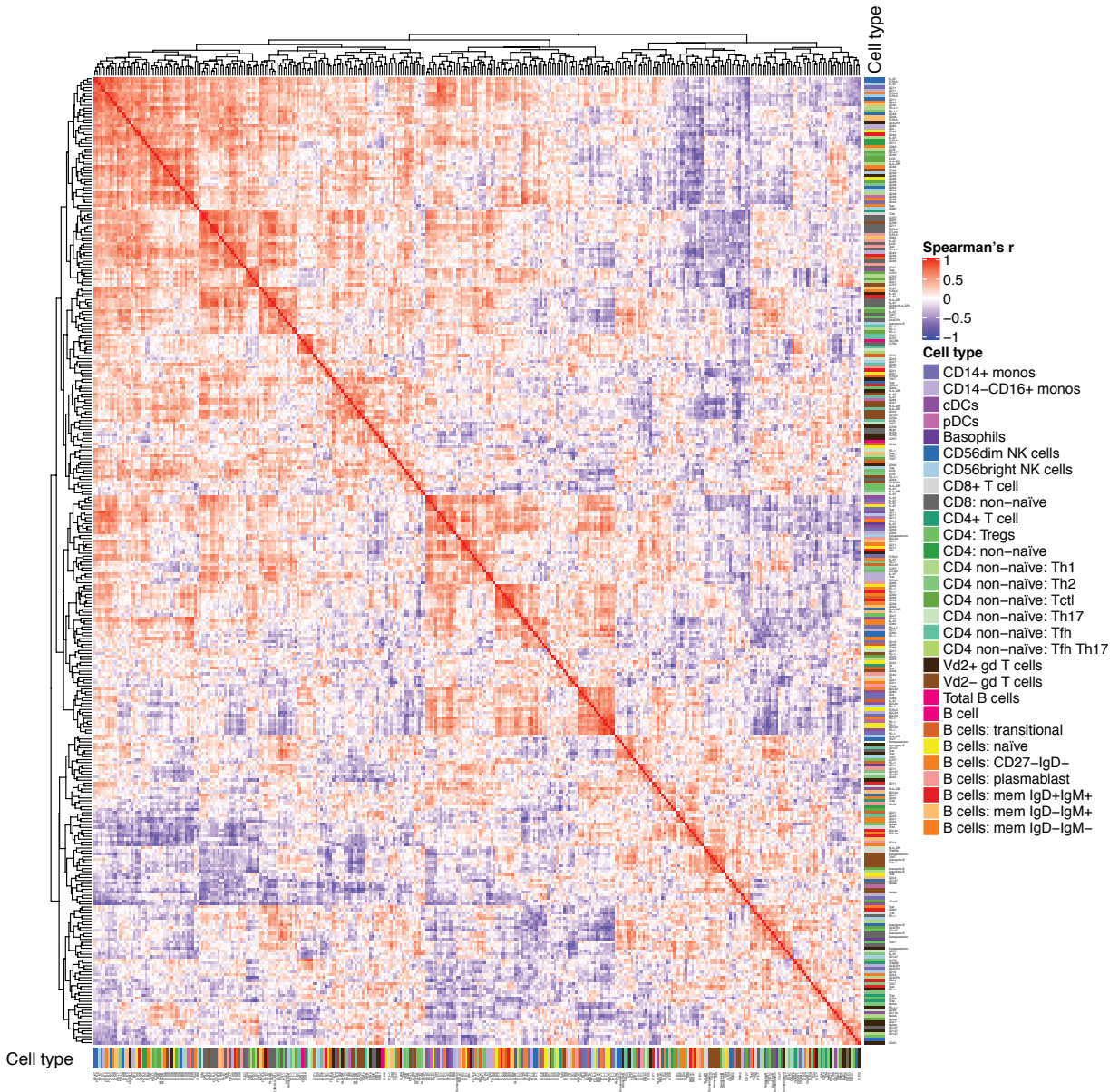


**Figure S5. cDC, pDC and NK cell features impacted by acute ZIKV infection.** (A) Line plots of frequency of phenotypic features (% of cells that express each marker) for cDCs and pDCs and (C) CD56<sup>dim</sup> and CD56<sup>bright</sup> NK cell subsets for each participant versus time since index visit. Red line connects median values at each sampling timepoint with error bars for +/- 95% CI. Scatterplot for feature abundance from cross-sectional uninfected (UI) cohort shown on the far right. \* $p_{adj} < 0.05$ , \*\* $p_{adj} < 0.01$ , \*\*\* $p_{adj} < 0.001$  ( $p_{adj}$  values obtained by LME model fit with Benjamini-Hochberg FDR correction). (B) Heatmap showing z-score normalized median expression of indicated markers (rows) for each cDC and pDC associated cell cluster (column) and (G) for each NK cell-associated column. Column annotation indicates clusters that that significantly decrease (blue), increase (red), increase and then decrease (green), or remain unchanged (grey) in abundance (as a % of the parent population;  $p_{adj} < 0.05$ ).



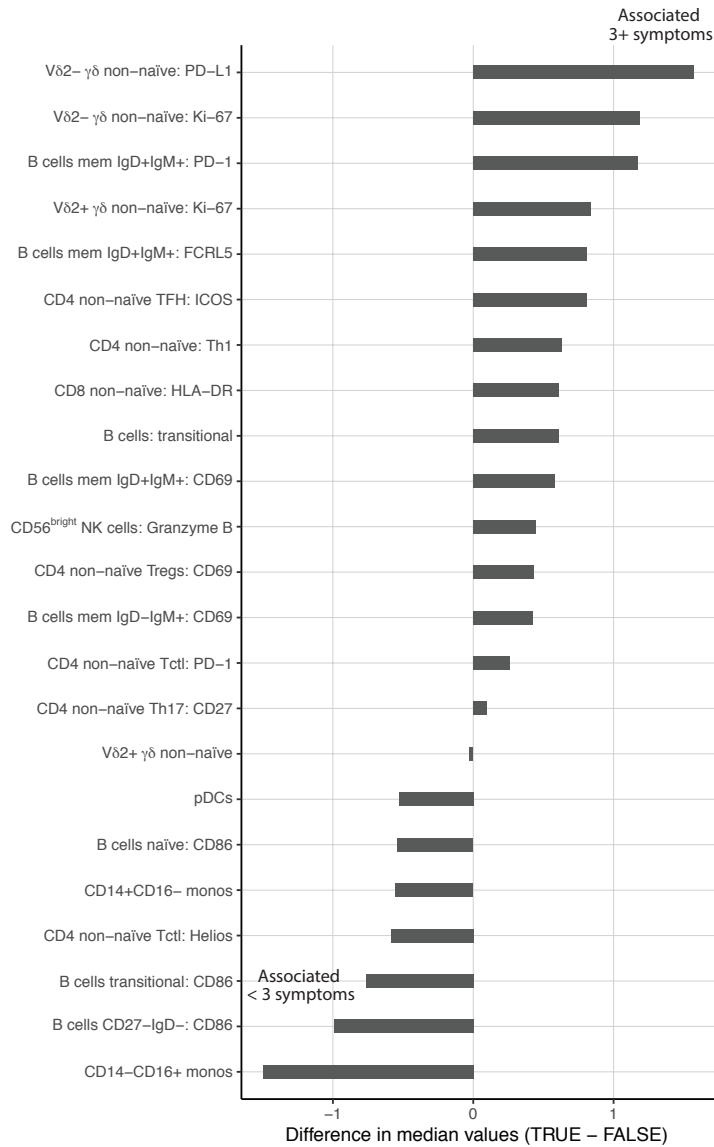
**A****B****Figure S7. B cell dynamics in ZIKV infection.**

(A) Correlation plot between the frequency of plasmablast and ASC B cell populations (as a % of non-naïve B cells; Spearman's  $r$  with regression line). (B) Phenotype (z-scored median expression of each marker) of B cell clusters that significantly decrease (blue), increase (red), increase and then decrease (green), or remain unchanged (grey) in abundance (as a % of the total B cell population;  $p_{\text{adj}} < 0.05$ ).



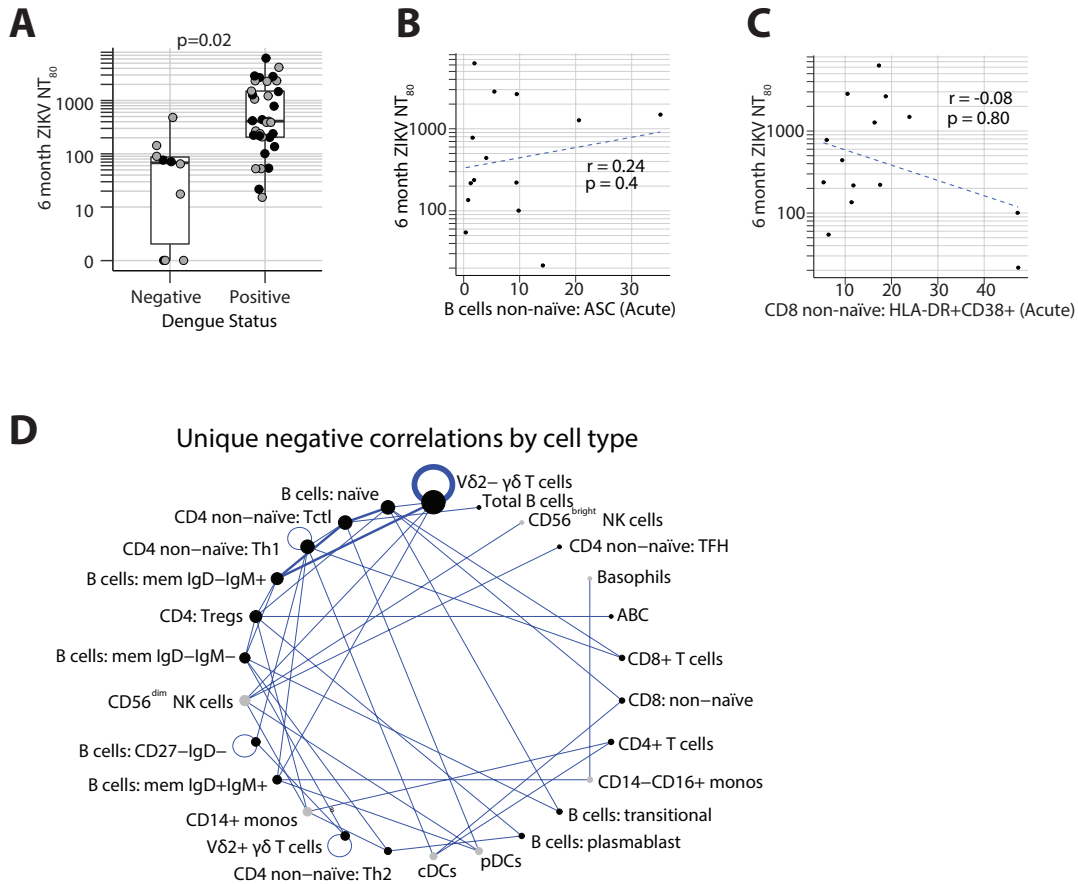
**Figure S8. Immune cell features correlated during acute ZIKV infection.**

Correlation heatmap depicting Spearman's correlation values (no significance cut-off) of all manually gated features from acute ZIKV infection (n=17 pre-IgM participants).



**Figure S9. Association of acute ZIKV infection cellular immune features with the development of symptoms.** Barplot of difference in median values at the acute visit for cellular immune features whose abundance during acute ZIKV infection is associated with the development of 3 or more symptoms reported at the acute visit (=TRUE;  $p < 0.05$  by exact permutation test).





**Figure S10. Characteristics associated with ZIKV neutralizing antibody titers 6 months after infection.** (A) Difference in 6-month ZIKV NT<sub>80</sub> between individuals with or without evidence of prior DENV infection at index visit (Wilcoxon Rank Sum test). Individuals from our sub-cohort are colored black and individuals from the larger REDSIII cohort are colored grey. Scatterplots showing 6-month ZIKV NT<sub>80</sub> titers versus the frequency of (B) ASC B cells or (C) non-naïve CD8+ T cells co-expressing HLA-DR and CD38 at the acute timepoint (Spearman's correlation). (D) Circular network graph of negative correlations unique to acute ZIKV infection. Size of node indicates number of correlations and edge thickness is proportional to the number of correlations between features classified by the indicated nodes. Nodes indicate cell type. Correlations between features of the same cell type are indicated with a circular line segment and nodes from adaptive immune cell types are colored black and from innate immune cell types are colored grey.

PANEL 1

Antigen	Metal	Clone	Vendor	Catalogue #	In-house conjugated Abs: [Ab] (mg/mL)	In-house conjugated Abs: [Staining] (ug/mL); Fluidigm: vol/100uL
CD45	89	HI30	Fluidigm	3089003B		0.5
CD14	113	M5E2	Biologend	301802	0.500	3.000
CD123	115	6H6	Biologend	306002	0.5	0.750
CD33	139	WM53	Biologend	303402	0.5	0.750
CD38	140	HIT2	Biologend	303502	0.5	3.000
CD3	141	UCHT1	Biologend	300402	0.5	1.000
CD19	142	H1B19	Biologend	302202	0.5	1.500
CXCR3	143	G025H7	Biologend	353702	0.5	1.500
CD11b	144	ICRF44	Biologend	301302	0.5	1.5
CD4	145	RPA-T4	Biologend	300502	0.5	0.250
CD8	146	RPA-T8	Biologend	301002	0.5	0.500
CD11c	147	Bu15	Biologend	337202	0.5	0.375
CD16	148	3G8	Biologend	302001	0.3	3.000
CD138	149	DL-101	Biologend	352302	0.5	1.500
CD21	151	Bu32	Biologend	313502	0.5	1.500
gdTCR	152	11F2	Fluidigm	3152008B		0.5
CD45RA	153	HI100	Biologend	304102	0.5	0.750
CD40	154	5C3	Biologend	334302	0.5	6.000
PDL1	156	29E.2A3	Biologend	329702	0.5	1.500
CD69	157	FN50	Biologend	310902	0.5	0.750
CD27	158	O323	Biologend	302802	0.5	1.500
Tbet	160	4B10	Biologend	644802	0.5	1.500
CTLA4	161	14D3	Fluidigm	3161004B		0.25
CD80	162	2D10.4	Fluidigm	3162010B		0.5
CD86	163	IT2.2	Biologend	305401	0.5	0.750
CD24	165	M15	Biologend	311102	0.5	3.000
NKG2D	166	ON72	Fluidigm	3166016B		1
FCRL5	167	509f6	Biologend	340302	0.5	3.000
Ki67	168	B56	Fluidigm	3168007B		1
CD71	169	CY1G4	Biologend	334102	0.3	0.750
IgD	170	CY1G4	Biologend	334102	0.3	0.750
CD20	171	2H7	Biologend	302302	0.3	1.500
B220	172	L161	Biologend	331502	0.3	0.375
IgM	173	MHM-88	Biologend	314502	0.5	1.500
HLA-DR	174	L243	Biologend	307602	0.5	1.500
PD-1	175	EH12.2H7	Biologend	329902	0.5	1.500
CD56	176	HCD56	Fluidigm	3176008B		0.5

PANEL 2

Antigen	Metal	Clone	Vendor	Catalogue #	In-house conjugated Abs: [Ab] (mg/mL)	In-house conjugated Abs: [Staining] (ug/mL); Fluidigm: vol/100uL
CD45	89	HI30	Fluidigm	3089003B	0.5	0.5
CD14	113	M5E2	Biologend	301802	0.500	3.000
CD123	115	6H6	Biologend	306002	0.5	0.750
CD33	139	WM53	Biologend	303402	0.5	0.750
CD38	140	HIT2	Biologend	303502	0.5	3.000
CD3	141	UCHT1	Biologend	300402	0.5	1.000
CD19	142	H1B19	Biologend	302202	0.5	1.500
CXCR3	143	G025H7	Biologend	353702	0.5	1.500
CD11b	144	ICRF44	Biologend	301302	0.5	1.5
CD4	145	RPA-T4	Biologend	300502	0.5	0.250
CD8	146	RPA-T8	Biologend	301002	0.5	0.500
CD11c	147	Bu15	Biologend	337202	0.5	0.375
CD16	148	3G8	Biologend	302001	0.3	3.000
CCR4	149	205410	R+D	MAB1567	0.5	3.000
OX40	150	A019D5	Biologend	351302	0.5	3.000
ICOS	151	C398.4A	Biologend	313539	0.5	1.500
gdTCR	152	11F2	Fluidigm	3152008B		0.5
CD45RA	153	HI100	Biologend	304102	0.5	0.750
CX3CR1	154	2A9-1	Biologend	341602	0.2	0.375
CCR6	155	G034E3	Biologend	353402	0.3	1.500
PDL1	156	29E.2A3	Biologend	329702	0.5	1.500
CD69	157	FN50	Biologend	310902	0.5	0.750
CD27	158	O323	Biologend	302802	0.5	1.500
Vd2	159	B6	Biologend	331402	0.2	0.750
Tbet	160	4B10	Biologend	644802	0.5	1.500
CTLA4	161	14D3	Fluidigm	3161004B		0.25
FOXP3	162	PCH101	Fluidigm	3162011a		0.25
EOMES	164	WD1928	ThermoFisher	14-4877-82	0.5	3.000
CD127	165	A019D5	Biologend	351302	0.5	1.500
TIGIT	166	A15153G	Biologend	372702	0.5	1.500
CCR7	167	G043H7	Biologend	353202	0.5	1.500
Ki67	168	B56	Fluidigm	3168007B		1
CD25	169	2A3	Fluidigm	3169003B		1
CXCR5	171	RF8B2	Fluidigm	3171014B		0.125
Helios	172	22F6	Biologend	137202	0.5	1.500
Granzyme B	173	GB11	BioRad	MCA2120	0.5	1.500
HLA-DR	174	L243	Biologend	307602	0.5	1.500
PD-1	175	EH12.2H7	Biologend	329902	0.5	1.500
CD56	176	HCD56	Fluidigm	3176008B		0.5

Table S1. Mass cytometry antibodies used.

**ZIKV-infected**

Participant #	Age	Sex	Pre-IgM at index visit	DENV exposed	>=3 symptoms at acute visit	Maximum ZIKV NT80 titer	6mo ZIKV NT80 titer
1	52	Male	Y	Y	TRUE	355.1	54.3
2	24	Male	N	N	FALSE	84.4	0
3	22	Male	N	Y	TRUE	9719.1	2894.4
4	62	Male	Y	Y	FALSE	1069.7	217.1
5	43	Male	Y	N	FALSE	NA	NA
6	54	Male	N	Y	FALSE	13952.6	419.9
7	51	Male	Y	Y	FALSE	2782.7	221.7
8	46	Male	Y	Y	TRUE	688	21.6
9	49	Male	N	N	FALSE	1474.1	71.8
10	37	Male	Y	Y	FALSE	37872.3	6285.7
11	46	Male	Y	Y	TRUE	3086.1	100.2
12	36	Female	N	Y	TRUE	4543	202.9
13	43	Male	Y	Y	TRUE	3405.1	1483.7
14	42	Male	Y	Y	TRUE	2238.7	134.8
15	43	Female	N	N	Unknown	1153	77.1
16	28	Female	N	N	Unknown	NA	NA
17	27	Female	Y	N	TRUE	NA	NA
18	53	Male	N	N	FALSE	1198.7	NA
19	25	Female	Y	Y	FALSE	NA	NA
20	71	Male	Y	Y	FALSE	2091.4	440.9
21	67	Male	Y	Y	FALSE	3752.8	237.6
22	24	Male	Y	Y	FALSE	2206.1	2660.2
23	56	Female	Y	Y	TRUE	9412.2	1278.7
24	44	Male	Y	Y	FALSE	23236.4	2828.5
25	21	Female	Y	Y	TRUE	1490.7	779.1

**ZIKV-uninfected**

Participant #	Age	Sex
26	49	Male
27	32	Female
28	51	Female
29	60	Male
30	53	Male
31	58	Male
32	42	Female
33	22	Male
34	32	Female
35	20	Male
36	40	Male
37	54	Male
38	44	Male
39	20	Male

**Table S2.** Study participant clinical characteristics.

Cell type	Ki-67	HLA-DR	CD38	CD69	CD71	CD86	CD16	CD40	ICOS	CTLA-4	TIGIT	PD-1	PD-L1	Granzyme B	Tbet	Eomesodermin	Helios
CD14+ monocytes	x			x	x	x	x	x				x	x		x		
CD14-CD16+ monocytes	x			x	x	x		x				x			x		
cDCs	x	x		x	x	x		x				x			x		
pDCs	x		x		x	x						x					
CD56dim NK cells	x	x	x	x	x	x					x	x		x	x	x	
CD56bright NK cells	x	x	x	x	x	x	x				x	x		x	x	x	
Basophils	x				x												
CD8: non-naïve	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x
CD4: Tregs	x	x	x	x					x	x	x	x					x
CD4: non-naïve					x												
CD4 non-naïve: Th1	x	x	x						x		x	x	x	x			x
CD4 non-naïve: Th2	x	x	x						x		x	x	x				x
CD4 non-naïve: Tfh	x	x	x						x			x					
CD4 non-naïve: Th17	x	x	x								x	x					
CD4 non-naïve: Tctl	x	x	x						x		x	x	x	x			x
Vd2+ gd T cells	x	x	x	x							x	x	x	x	x	x	x
Vd2- gd T cells	x	x	x	x							x	x	x	x	x	x	x
Total B cells																	
B cells: transitional	x		x	x	x	x		x				x			x		
B cells: naïve	x		x	x	x	x		x				x			x		
B cells: CD27-IgD-	x		x	x	x	x		x				x			x		
B cells: plasmablast	x		x	x	x	x		x				x			x		
B cells: mem IgD+IgM+	x		x	x	x	x		x				x			x		
B cells: mem IgD-IgM+	x		x	x	x	x		x				x			x		
B cells: mem IgD-IgM-	x		x	x	x	x		x				x			x		

Cell type	CD25	FCRL5	CD21	CD127	CCR4	CCR6	CXCR5	CX3CR1	BDCA1	CD4	CD11b	CD27	CCR7	CD24	total
CD14+ monocytes		x						x	x	x					13
CD14-CD16+ monocytes		x						x	x	x					11
cDCs									x		x				10
pDCs															5
CD56dim NK cells		x		x								x			14
CD56bright NK cells		x		x								x			15
Basophils															2
CD8: non-naïve	x	x		x	x	x		x				x	x		23
CD4: Tregs				x	x	x						x	x		14
CD4: non-naïve		x													2
CD4 non-naïve: Th1	x			x				x				x	x		14
CD4 non-naïve: Th2	x			x								x	x		12
CD4 non-naïve: Tfh	x			x								x	x		9
CD4 non-naïve: Th17	x			x								x	x		9
CD4 non-naïve: Tctl	x			x				x				x	x		14
Vd2+ gd T cells	x			x	x	x		x				x	x		18
Vd2- gd T cells	x			x	x	x		x				x	x		18
Total B cells							x								1
B cells: transitional		x	x						x						11
B cells: naïve		x	x						x					x	12
B cells: CD27-IgD-		x	x						x					x	12
B cells: plasmablast		x	x						x						11
B cells: mem IgD+IgM+		x	x						x					x	12
B cells: mem IgD-IgM+		x	x						x					x	12
B cells: mem IgD-IgM-		x	x						x					x	12
SUM:															286

**Table S3.** Summary of phenotypic markers assessed on each cell type for manual gating analysis.