Unbiased screening identifies functional differences in NK cells after early life psycho-sociological stress

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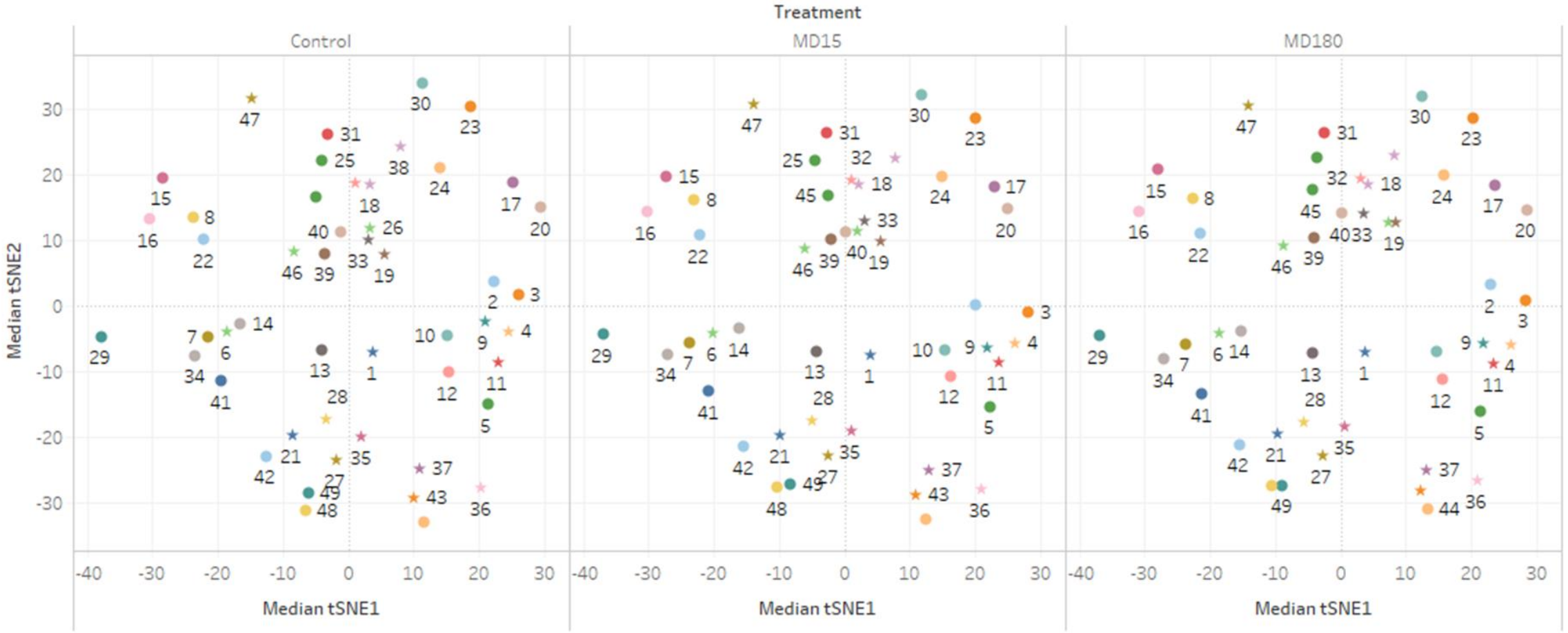
Supplementary figure 1: viSNE clusters definition based on the used markers. Clusters colored by CD3, CD4 and CD8 cell markers to identify immune populations associated. Blue to orange: low to high expression of the marker.



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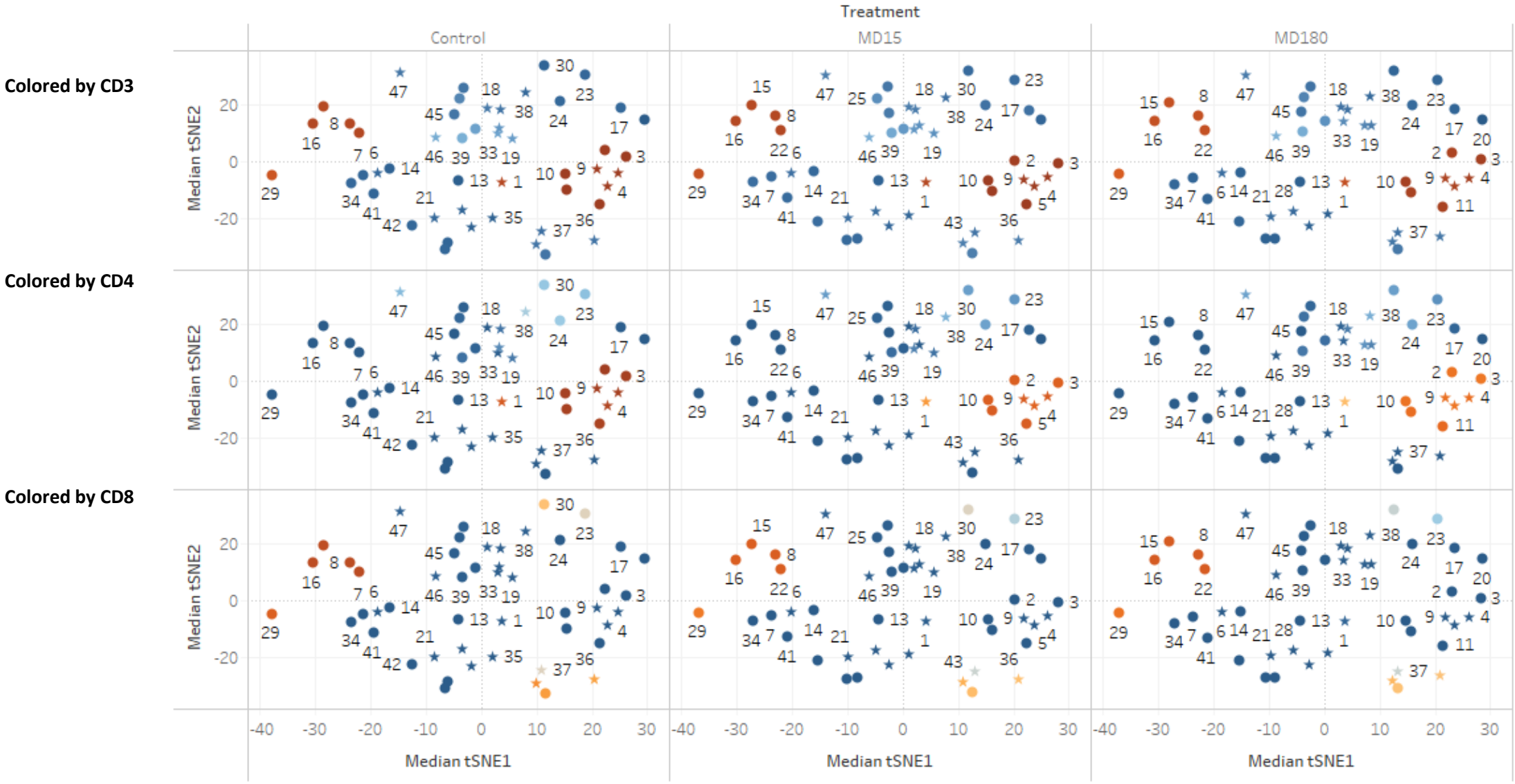
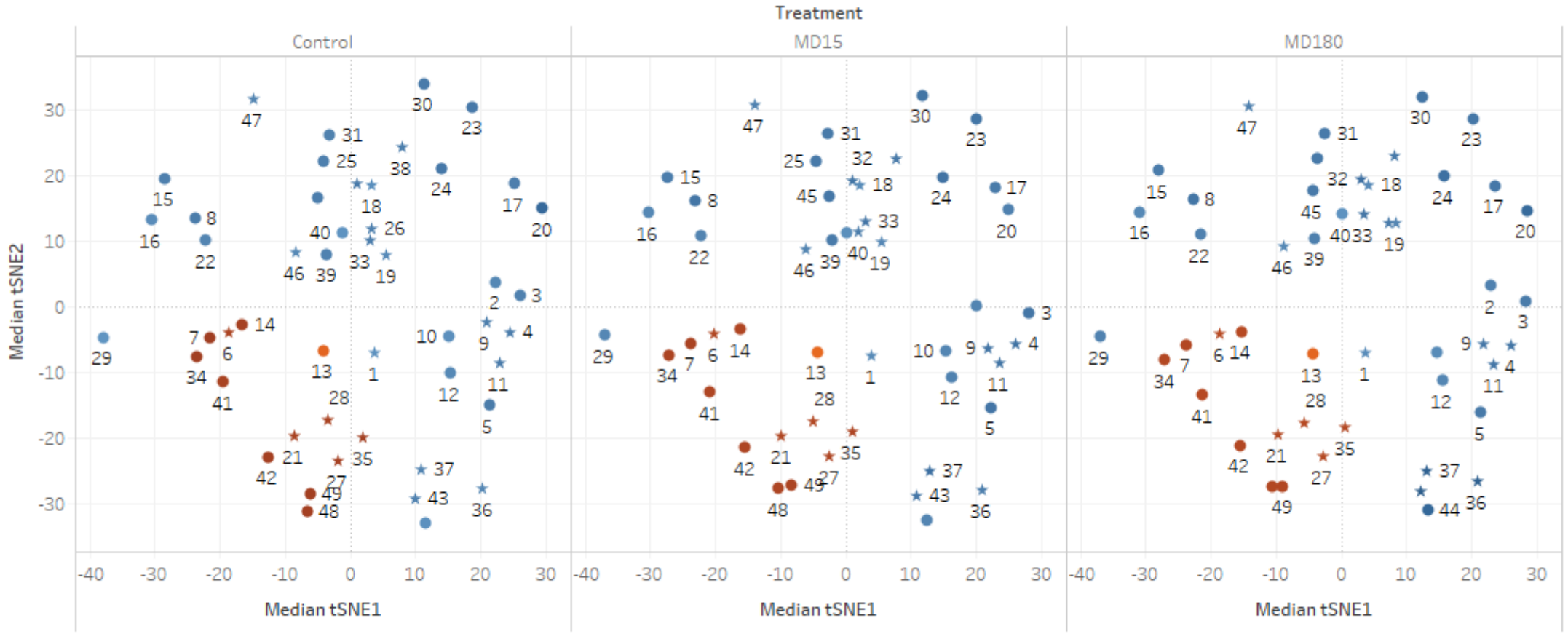


Supplementary figure 2: Clusters colored by CD45RA, CD11b/c, CD25 and FoxP3 cell markers to identify immune populations associated. Blue to orange: low to high expression of the marker.

Colored by CD45RA



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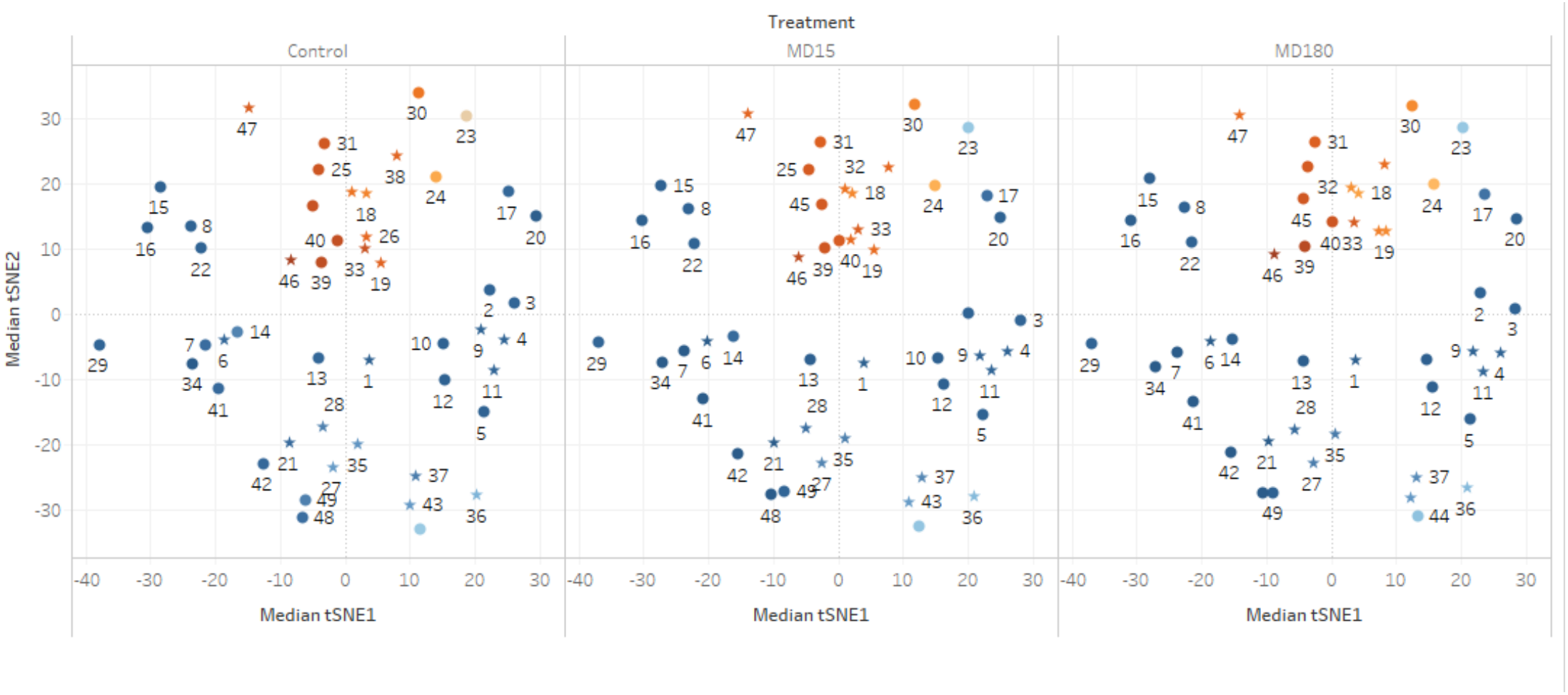
Colored by CD11b/c



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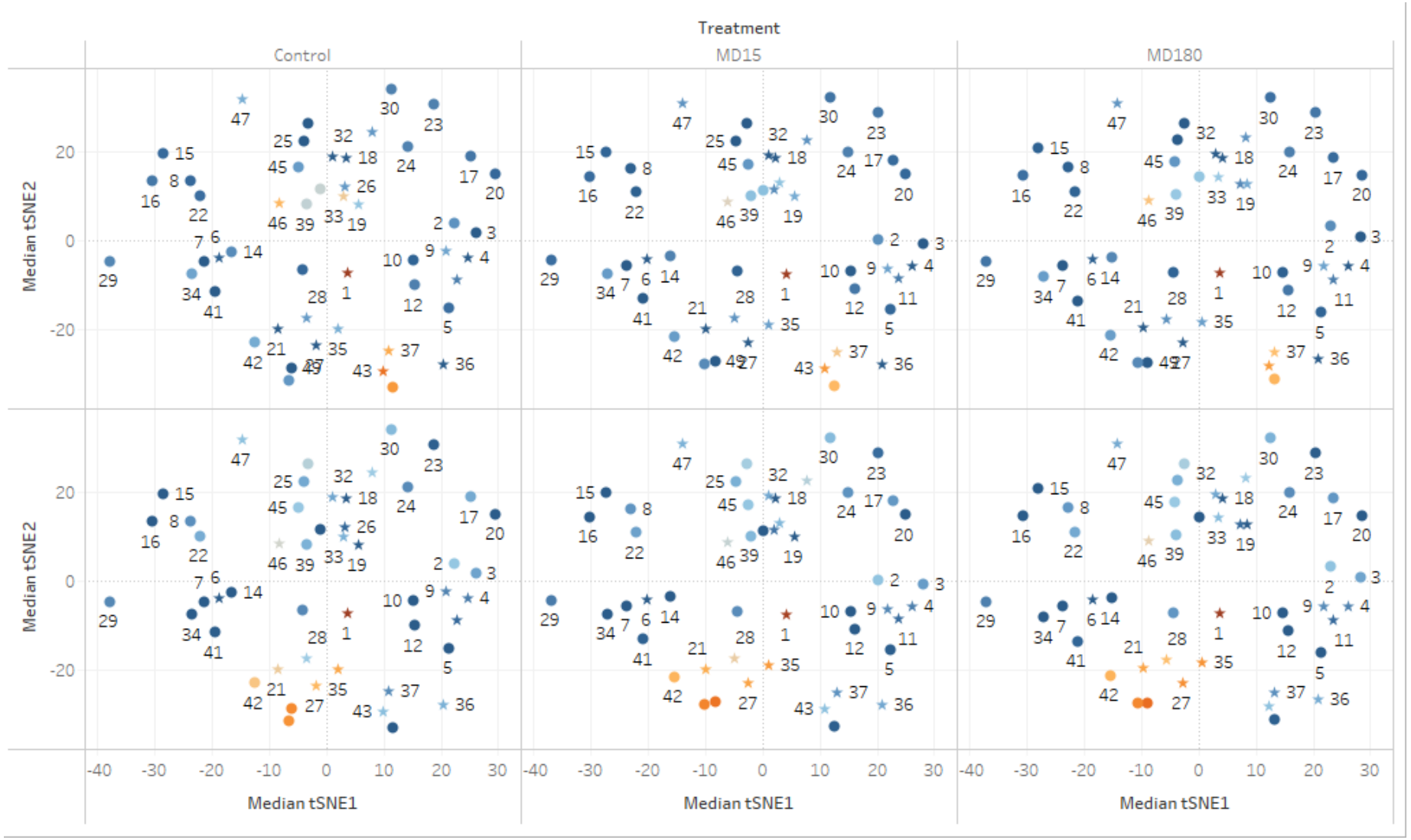
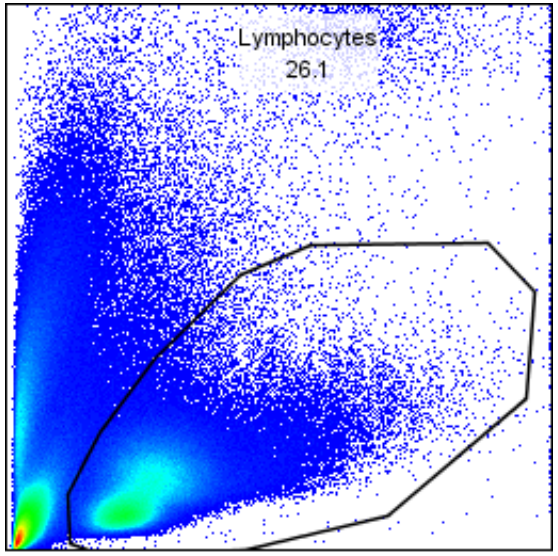
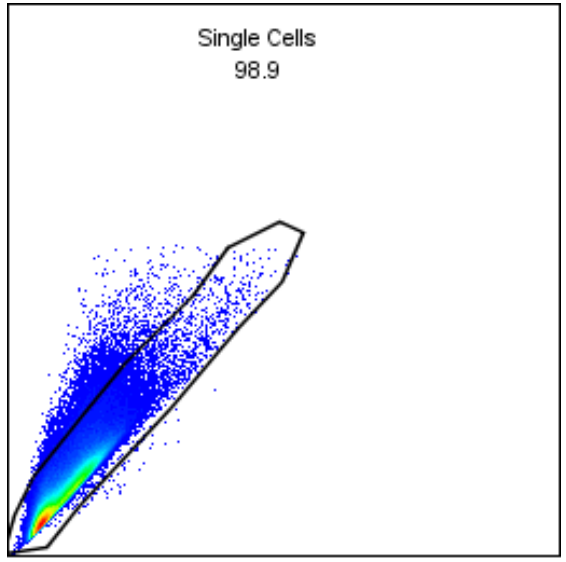
Colored by CD25

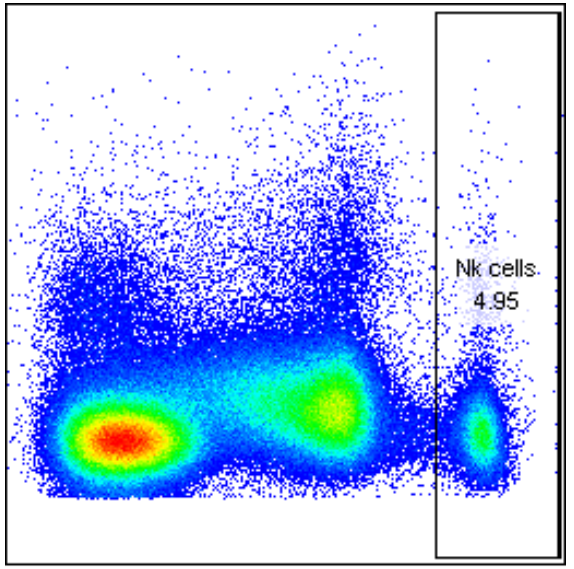
Colored by FoxP3

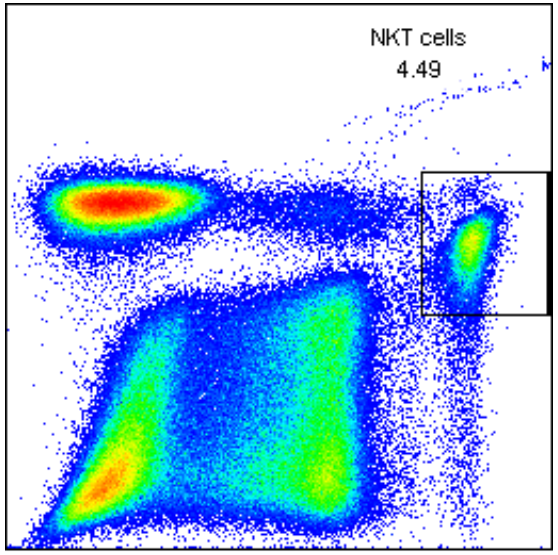


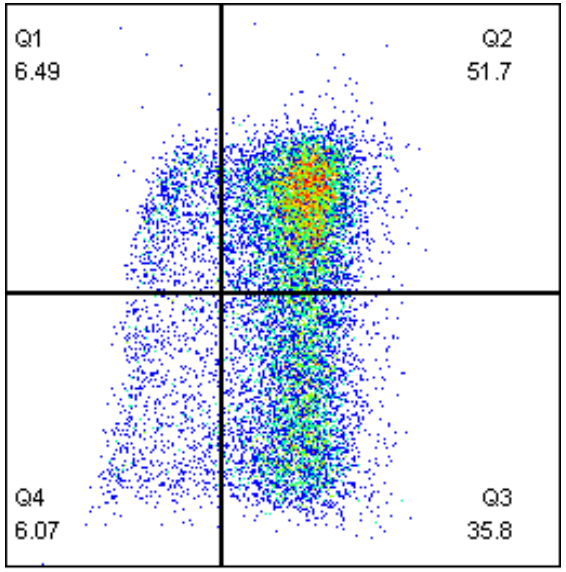
Supplementary figure 3: FlowJo gating strategy for the quantification of NK and NKT-like cells



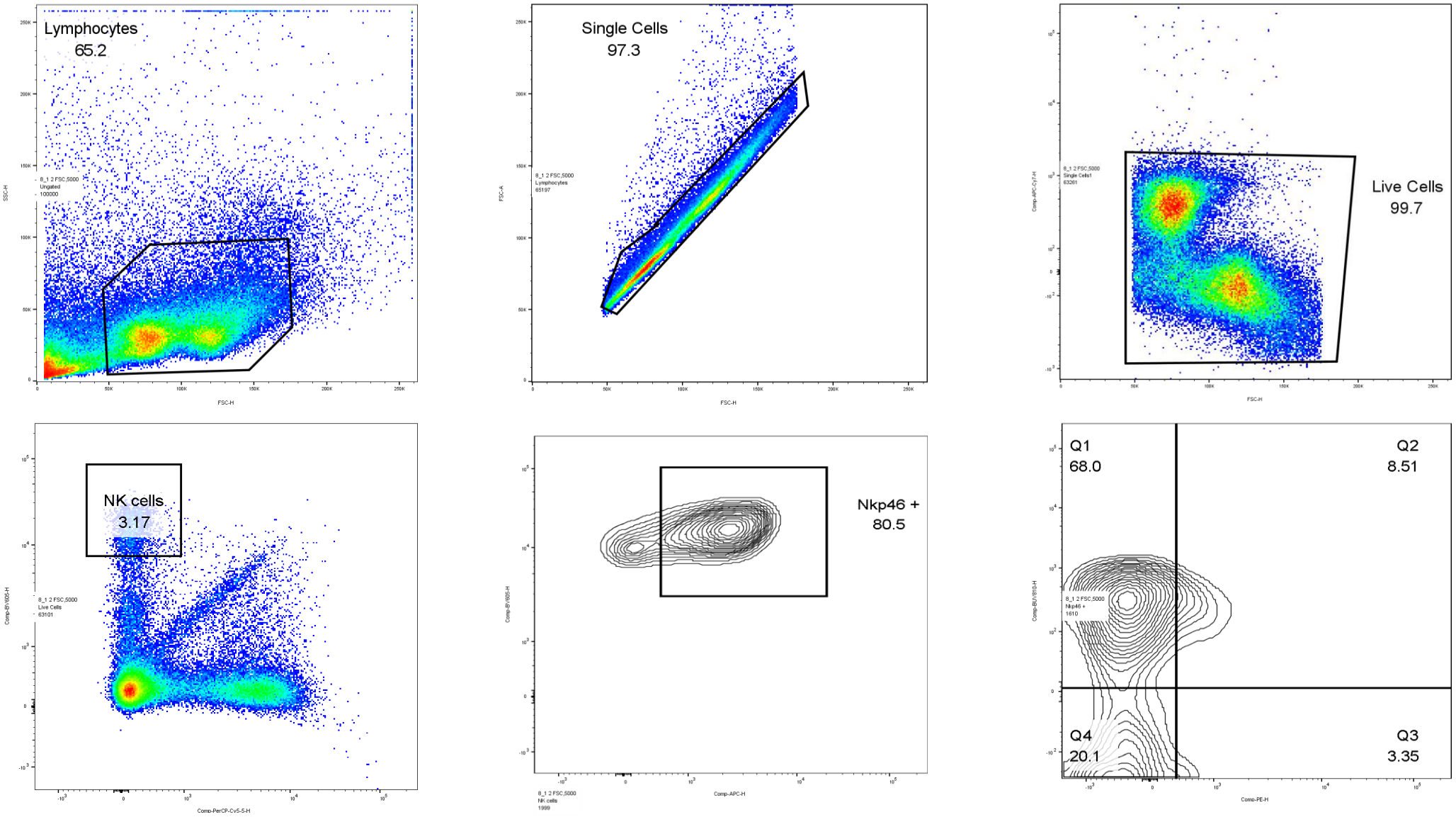




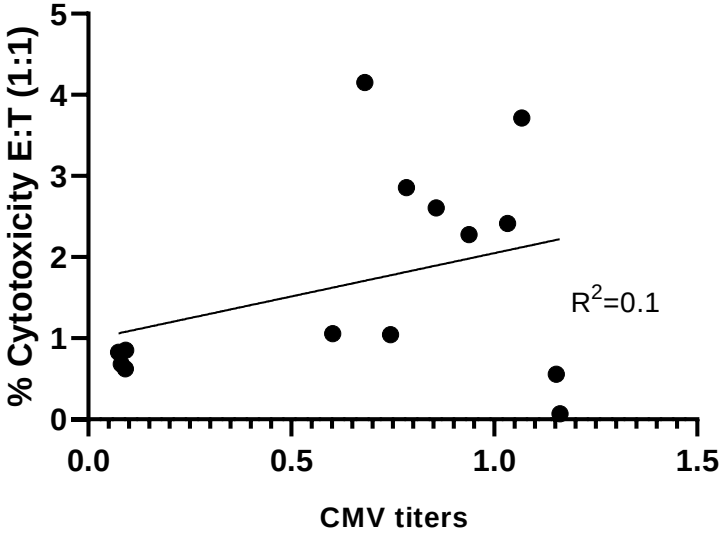


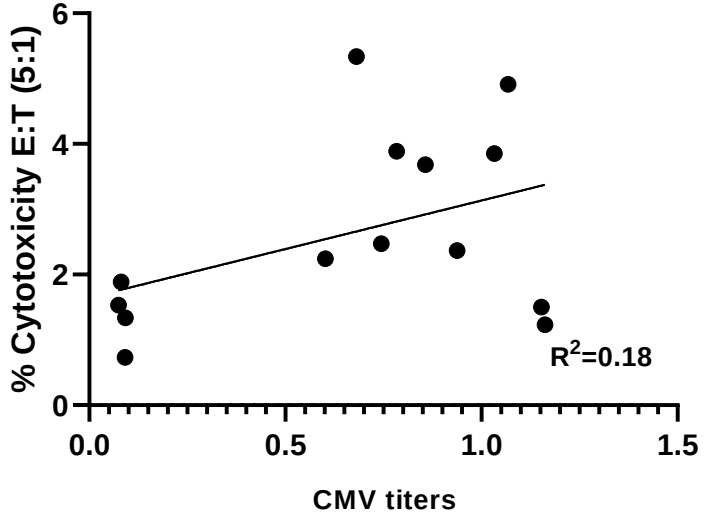


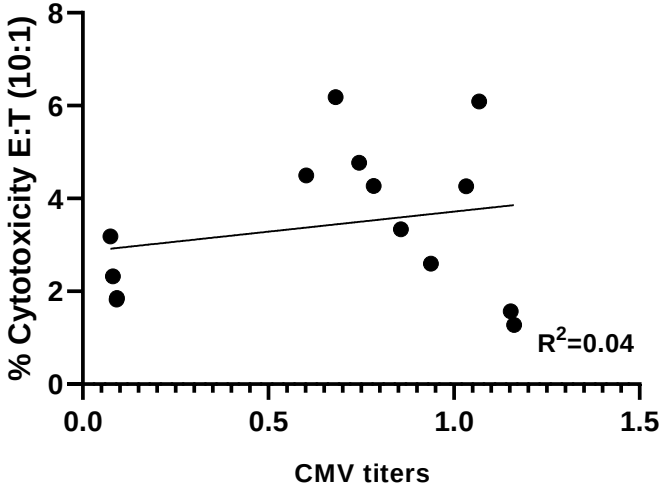
Supplementary figure 4: FlowJo gating strategy for the quantification of NK cell maturation state.

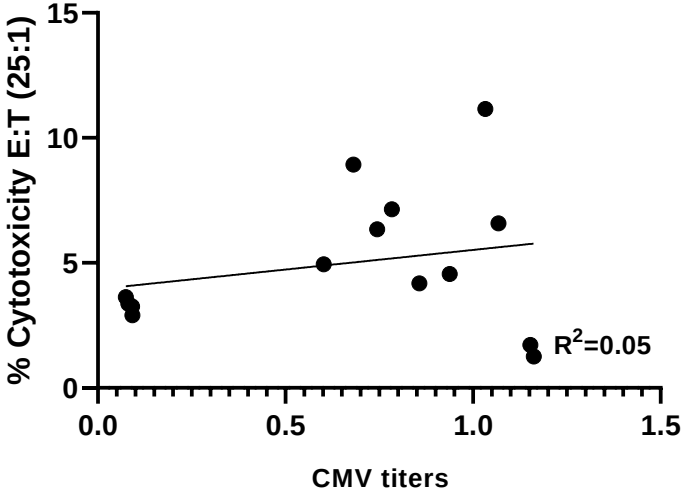


Supplementary figure 5: Correlation analysis between CMV titers and cytotoxic response of NK cells of institutionalized individuals. .

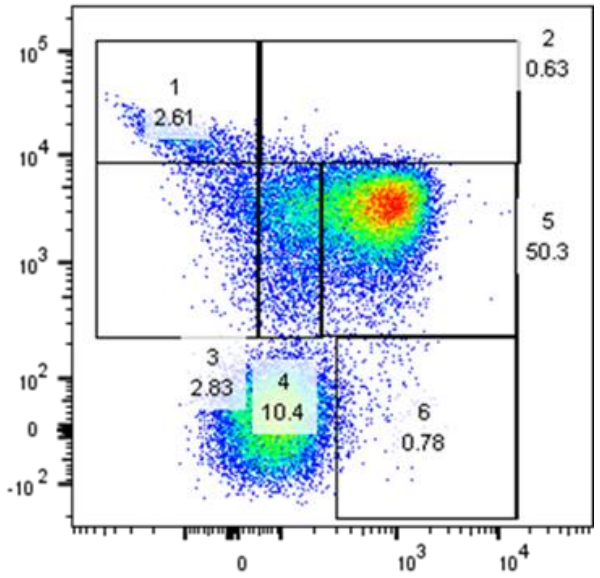


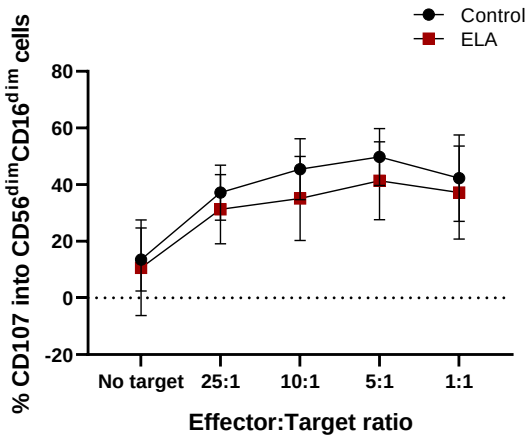


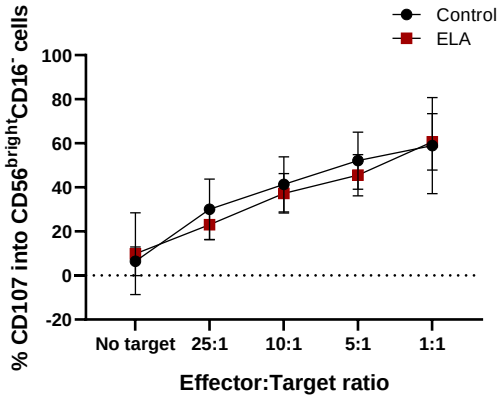


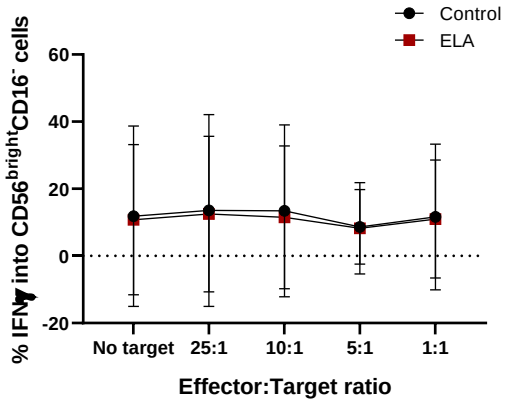


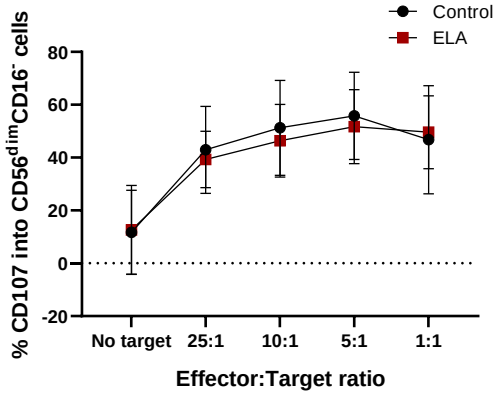
Supplementary figure 6: (A): representative image of the NK cell population gating strategy: 1– CD56brightCD16-; 2 –CD56brightCD16+; 3- CD56dimCD16+; 4– CD56dimCD16dim; 5- CD56dimCD16bright; 6 – CD56-CD16bright. Rest of the figures show the release of IFN-γ and CD107a by the different NK cell populations. No significant differences were found. Data is presented as mean ± SEM of 14 donor per group.

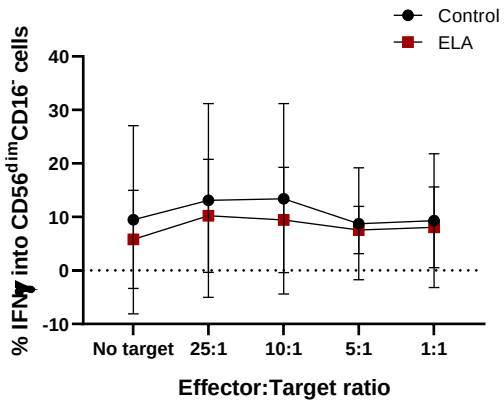


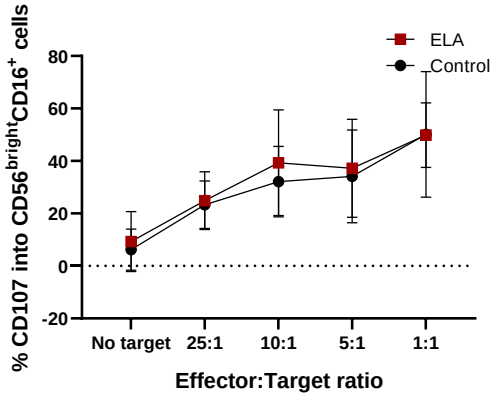












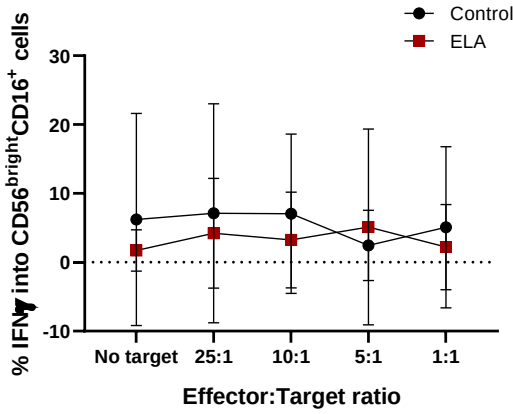


Table 1 – Description of the antibodies used for immunepehotyping.

IMMUNOPHENOTYPING			
Cell type	**Antibody**	**Laser**	**Reference**
T cells	CD3	FITC	BD 559975
T helper cell	CD4	BV510	BD 740138
T cytototxic cell	CD8	BUV395	BD 740257
B cells	CD45RA	PE Cy5	BD 561624
Activation marker	CD25	BV650	BD 742755
NK cells	CD161a	BV605	BD 744051
Macrophages, dendritic cells	CD11b/c	BB700	BD 746165
Activation marker B cells	RT1B	BV786	BD 744131
T regulatory cells	FoxP3	APC	BD 566527
Viability marker	Live/Dead	APC Cy7	Biolegend 423105
NK CELL MATURATION ASSAY			
T cells	CD3	PerCP Cy5.5	ThermoFisher 46-0030-82
NK cell	CD161a	BV605	BD BV605
NK cell phenotypic marker	NKp46 (CD335)	APC	Biolegend, 250808
NK cells maturation marker	CD27	BUV737	BD 612831
NK cells maturation marker	CD11b	PE	BD 562105
Viability marker	Live/Dead	APC-Cy7	Biolegend 423105
NK CELL CYTOTOXICITY ASSAY			
Viability marker	TO-PRO	APC	ThermoFisher Scientific T3605
Viability marker	Cell Tracer Violet	Pacific Blue	ThermoFisher Scientific C34571
NK CELL DEGRANULATION ASSAY			
T cells	CD3	BV510	BD 563109
NK cell phenotypic marker	CD19	PE-Cy5	Biolegend 302210
NK cell phenotypic marker	CD16	BUV496	BD 564653
NK cell phenotypic marker	CD56	BV711	BD 563169
NK cell functional marker	CD107a	FITC	Biolegend 328606
Cytokine	IFNγ	PE	BD 502509
Viability marker	Cell Tracer Violet	Pacific Blue	ThermoFisher Scientific C34571
Viability marker	Live/Dead	APC Cy7	Biolegend 423105

Table 2 - *Clusters obtained from viSNE unbiased phenotyping. Association of the markers used with each cluster and p values obtained after statistical analysis.*

Cluster	Markers	CTR vs MD_{15}	CTR vs MD_{180}	MD_{15} vs MD_{180}
1	$CD3^+$; $CD4^+$; $CD25^+$; $FoxP3^+$	3.73E-10	1.86E-08	3.29E-01
2	$CD3^+$; $CD4^+$	5.39E-05	2.55E-01	5.10E-03
3	$CD3^+$; $CD4^+$	5.30E-03	9.31E-01	1.34E-02
4	$CD3^+$; $CD4^+$	4.91E-03	2.82E-03	9.77E-01
5	$CD3^+$; $CD4^+$	6.70E-01	7.41E-02	9.92E-03
6	$CD45RA^+$; $RT1B^+$	3.44E-03	4.86E-02	5.40E-01
7	$CD45RA^+$; $RT1B^+$	4.13E-01	1.25E-02	3.82E-04
8	$CD3^+$; $CD8^+$	7.56E-01	1.65E-03	1.10E-02
9	$CD3^+$; $CD4^+$	2.62E-14	1.73E-13	6.59E-01
10	$CD3^+$; $CD4^+$	9.93E-01	2.18E-03	3.00E-03
11	$CD3^+$; $CD4^+$	7.33E-07	1.27E-05	5.99E-01
12	$CD3^+$; $CD4^+$	ns	ns	ns
13	$CD45RA^+$	7.18E-05	3.01E-01	5.00E-03
14	$CD45RA^+$; $RT1B^+$	3.05E-05	7.55E-01	2.47E-04
15	$CD3^+$; $CD8^+$	ns	ns	ns
16	$CD3^+$; $CD8^+$	3.31E-02	7.98E-02	9.16E-01
17	$ROR\gamma T^+$	ns	ns	ns
18	$CD11b/c^+$	1.70E-06	5.57E-09	1.25E-01
19	$CD11b/c^+$	1.73E-07	6.70E-05	1.19E-01
20	$CD11b/c^+$	9.10E-01	3.13E-02	1.15E-02
21	$CD45RA^+$; $RT1B^+$	4.42E-06	1.55E-07	4.90E-01
22	$CD3^+$; $CD8^+$	8.44E-04	1.52E-01	9.81E-02
23	CD8+;	1.89E-02	1.23E-01	6.75E-01
24	$CD11b/c^+$	ns	ns	ns
25	$CD11b/c^+$	4.30E-03	3.79E-01	1.05E-01
26	$CD11b/c^+$	2.45E-03	4.49E-04	8.18E-01
27	$CD45RA^+$; $RT1B^+$	1.33E-12	2.01E-06	1.79E-05
28	$CD45RA^+$; $RT1B^+$	1.28E-06	2.85E-06	9.60E-01
29	$CD161a^+$	6.92E-01	9.65E-03	6.71E-02
30	$CD8^+$; $CD11b/c^+$	1.40E-01	3.12E-02	7.61E-01
31	$CD11b/c^+$	ns	ns	ns
32	$CD11b/c^+$	8.62E-13	3.12E-07	6.76E-05
33	$CD11b/c^+$	4.58E-10	8.47E-04	4.29E-05
34	$CD45RA^+$; $RT1B^+$	1.87E-02	4.19E-04	3.44E-01
35	$CD45RA^+$; $RT1B^+$	4.30E-14	4.45E-09	1.06E-04
36	$CD8^+$; $CD161a^+$; $Tbet^+$	5.63E-05	1.14E-05	8.51E-01
37	$CD8^+$; $CD161a^+$	2.08E-03	4.46E-07	1.70E-02
38	$CD11b/c^+$	5.88E-07	1.68E-05	4.94E-01
39	$CD11b/c^+$	2.45E-01	6.00E-03	2.24E-01
40	$CD11b/c^+$	ns	ns	ns
41	$CD45RA^+$; $RT1B^+$	1.43E-02	9.37E-01	3.25E-02
42	$CD45RA^+$; $RT1B^+$	1.64E-01	6.57E-01	2.55E-02
43	$CD8^+$; $CD161a^+$; $Tbet^+$	5.87E-07	3.89E-03	1.21E-02
44	$CD8^+$; $CD161a^+$; $Tbet^+$	4.94E-01	1.23E-02	1.54E-01
45	$CD11b/c^+$	ns	ns	ns
46	$CD11b/c^+$	3.28E-07	2.49E-05	3.14E-01
47	$CD161a^+$	6.67E-08	3.21E-07	8.48E-01
48	$CD45RA^+$; $RT1B^+$	2.61E-04	1.82E-01	3.23E-02
49	$CD45RA^+$; $RT1B^+$	2.94E-01	4.86E-03	1.60E-01