

Supplemental Information: Early Immunologic Correlates of HIV Protection can be Identified from Computational Analysis of Complex Multivariate T-cells Flow Cytometry Assays

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Introduction

This document contains the supplemental information which are referred to in the main text.

Per-channel Cell Population Identification

The following movie was used to visually confirm that the single dimensional gates are partitioning the cells to correct positive and negative populations:

- AVI: <http://www.terryfoxylab.ca/flowsite/flowType/AutomaticGates.avi>
- YouTube: <http://www.youtube.com/watch?v=SDwub9PPN0Y>

Marker Impacts

To provide a more detailed understanding of the marker impacts, here we have provided an example: Consider the KI-67⁺CD4⁻CD8⁺ phenotype ($p = 1.89 \times 10^{-6}$, $R^2 = 0.05$). Changing KI-67's status to neutral will result in CD4⁻CD8⁺ ($p = 2.6 \times 10^{-2}$, $R^2 = 0.02$). Therefore, the impact of KI-67 is $0.05 - 0.02 = 0.03$. Repeating this process and averaging over the impacts for all phenotypes in a given group results in the impact values discussed in the main manuscript.

Supplemental Tables

The Tables included in this section (S1, S2, S3) are discussed in the main text.

Table S1. Statistically significant phenotypes.

Phenotype	p-value	p-value CI	adjusted p-value	CPhR Coefficient	R ²	Cell Frequency
1 CD28-CD45RO+CD57-CCR5+	5.3e-07	(4.3e-14, 1.3e-02)	2e-02	20.5	0.056	0.03048
2 CD28-CD8+CD57-CD127-	2.5e-07	(2.3e-14, 3.8e-04)	1e-02	12.3	0.060	0.05975
3 CD28-CD45RO+CD57-CCR7-	5.1e-07	(2.3e-14, 6.1e-04)	2e-02	15.7	0.057	0.03829
4 CD28-CD45RO+CD4-CD57-	3.5e-07	(2.3e-14, 1.1e-03)	1e-02	13.2	0.058	0.04357
5 CD45RO+CD4-CD57-CD127-	2.7e-07	(1.2e-13, 7.1e-03)	1e-02	12.8	0.059	0.05062
6 CD28-CD45RO+CD57-CD127-	4.7e-08	(1.7e-14, 6.8e-04)	2e-03	16.0	0.067	0.03732
7 CD45RO+CD4-CD27-CD127-	4.4e-07	(5.8e-14, 1.1e-03)	2e-02	14.3	0.057	0.04830
8 CD28-CD45RO+CD57-	5.6e-07	(4.4e-14, 4.1e-04)	2e-02	12.4	0.056	0.05015
9 CD45RO+CD4-CD127-	6.5e-07	(4.7e-15, 2.9e-03)	2e-02	9.6	0.056	0.07176
10 CD28-CD45RO+CD4-CD127-	3.1e-07	(0.0e+00, 5.7e-03)	1e-02	11.7	0.059	0.05300
11 CD28-CD45RO+CD57-CCR5+CD27-CCR7+CD127-	4.7e-07	(5.7e-14, 7.7e-03)	2e-02	171.4	0.057	0.00315
12 CD28-CD45RO+CD4-CD57-CCR5+CD27-CCR7+CD127-	4.5e-07	(1.8e-13, 3.9e-04)	2e-02	176.2	0.057	0.00294
13 CD28-CD57-CD127-	3.3e-07	(3.4e-15, 8.0e-03)	1e-02	8.0	0.058	0.12341
14 CD28-CD4-CD57-	8.8e-07	(2.2e-15, 2.9e-03)	3e-02	7.2	0.054	0.15525
15 CD57-CD27-CD127-	6.2e-08	(2.4e-14, 4.7e-03)	2e-03	9.5	0.065	0.12173
16 CD4-CD57-CD27-CD127-	4.7e-08	(4.2e-14, 3.3e-03)	2e-03	9.7	0.067	0.09721
17 CD28-CD57-CCR7-CD127-	2.8e-07	(9.7e-15, 1.0e-02)	1e-02	9.8	0.059	0.08417
18 CD28-CD4-CD57-CD127-	3.3e-08	(2.0e-12, 5.7e-04)	1e-03	9.1	0.068	0.10852
19 CD4-CD57-CCR7-CD127-	6.5e-07	(3.8e-15, 2.3e-03)	2e-02	8.8	0.056	0.09501
20 CD45RO-CD4-CD57-CCR5-CD27+CCR7-CD127-	6.1e-07	(1.2e-12, 2.6e-03)	2e-02	498.4	0.056	0.00097
21 CD28-CD45RO-CD4-CD57+CCR5-CD27+CCR7-CD127-	2.5e-07	(0.0e+00, 7.7e-03)	1e-02	561.2	0.060	0.00074
22 CD45RO-CD8+CD57+CCR5-CD27+CCR7-CD127-	1.2e-07	(4.6e-14, 3.3e-04)	5e-03	638.6	0.063	0.00068
23 CD45RO-CD8+CD4-CD57+CCR5-CD27+CCR7-CD127-	1.2e-07	(5.1e-14, 2.0e-03)	5e-03	638.6	0.063	0.00068
24 CD28-CD45RO-CD4-CD57+CCR5-CD27+CD127-	5.7e-07	(1.1e-13, 2.3e-03)	2e-02	298.3	0.056	0.00099
25 KI-67+CD28-CCR5+	1.0e-11	(2.9e-13, 2.8e-03)	4e-07	96.1	0.101	0.00547
26 KI-67+CD28-CCR5+CD27-	8.7e-12	(1.5e-14, 8.9e-04)	3e-07	115.3	0.102	0.00453
27 KI-67+CCR5+	1.3e-11	(2.4e-14, 7.0e-03)	5e-07	53.4	0.100	0.01192
28 KI-67+CD28+CD45RO+CD57-CCR7-CD127-	4.2e-09	(5.6e-16, 3.0e-03)	2e-04	241.3	0.077	0.00209
29 KI-67+CD45RO-CD4-CD27-CCR7-CD127-	1.2e-09	(2.0e-14, 4.4e-03)	4e-05	161.9	0.082	0.00297
30 KI-67+CD28-CD45RO-CD8-CD4-	5.0e-09	(2.9e-12, 1.7e-03)	2e-04	176.0	0.076	0.00225
31 KI-67+CD8-CD4-	8.1e-09	(6.1e-13, 4.5e-02)	3e-04	58.1	0.074	0.00738
32 KI-67+CCRS+CD27-CCR7-	2.0e-11	(3.8e-14, 6.0e-04)	8e-07	109.8	0.099	0.00532
33 KI-67+CD8-CCRS+CCR7-	1.3e-10	(3.1e-13, 2.0e-03)	5e-06	147.3	0.091	0.00392
34 KI-67+CD28-CD8-CCR5+CCR7+CD127-	2.6e-09	(1.6e-14, 1.1e-02)	1e-04	625.8	0.079	0.00061
35 KI-67+CD28+CD45RO+CD8+CD57-CD27+CCR7+	6.7e-07	(3.8e-13, 1.5e-03)	3e-02	585.4	0.055	0.00051
36 KI-67+CD28+CD45RO+CD8+CD4-CD57-CD27+CCR7+	6.7e-07	(1.1e-16, 4.7e-03)	3e-02	585.4	0.055	0.00051
37 KI-67+CD8+CD27-CCR7-CD127-	4.7e-11	(1.3e-13, 1.4e-03)	2e-06	141.3	0.095	0.00292
38 KI-67+CD8+CD4-CD27-CCR7-CD127-	4.7e-11	(1.3e-13, 1.3e-03)	2e-06	141.3	0.095	0.00292
39 KI-67+CD28-CD8+CD27-CCR7-CD127-	2.7e-11	(1.0e-13, 7.6e-04)	1e-06	164.5	0.097	0.00241
40 KI-67+CD28-CD8+CD4-CD27-CCR7-CD127-	2.7e-11	(2.7e-13, 1.4e-03)	1e-06	164.5	0.097	0.00241
41 KI-67+CD28-CD8+CCR7-CD127-	6.6e-11	(5.6e-14, 1.5e-02)	3e-06	132.9	0.094	0.00293
42 KI-67+CD28-CD8+CD4-CCR7-CD127-	6.6e-11	(1.2e-14, 8.4e-04)	3e-06	132.9	0.094	0.00293
43 KI-67+CD45RO+CD8+CD27-CCR7-	1.2e-09	(4.0e-12, 2.8e-03)	5e-05	143.6	0.082	0.00216
44 KI-67+CD45RO+CD8+CD4-CD27-CCR7-	1.2e-09	(1.0e-12, 1.2e-02)	5e-05	143.6	0.082	0.00216
45 KI-67+CD28-CD45RO+CD8+CD27-CCR7-	1.0e-09	(1.9e-15, 7.3e-04)	4e-05	188.5	0.082	0.00155
46 KI-67+CD28-CD45RO+CD8+CD4-CD27-CCR7-	1.0e-09	(1.7e-13, 2.0e-03)	4e-05	188.5	0.082	0.00155
47 KI-67+CD45RO+CD8+CD27-CD127-	7.1e-10	(1.2e-14, 6.8e-03)	3e-05	152.4	0.084	0.00221
48 KI-67+CD45RO+CD8+CD4-CD27-CD127-	7.1e-10	(3.4e-14, 1.5e-03)	3e-05	152.4	0.084	0.00221
49 KI-67+CD28-CD45RO+CD8+CD27-CD127-	5.0e-10	(6.0e-13, 3.1e-03)	2e-05	201.3	0.085	0.00163
50 KI-67+CD28-CD45RO+CD8+CD4-CD27-CD127-	5.0e-10	(4.6e-14, 2.7e-03)	2e-05	201.3	0.085	0.00163
51 KI-67+CD28-CD45RO+CD8+CD4-CD127-	1.0e-09	(1.2e-15, 3.2e-03)	4e-05	150.5	0.083	0.00222
52 KI-67+CD28-CD45RO+CD8+CD4-CD127-	1.0e-09	(1.5e-11, 3.6e-03)	4e-05	150.5	0.083	0.00222
53 KI-67+CD45RO+CD8+CD4-CD127-	2.2e-09	(2.8e-13, 2.1e-03)	9e-05	99.8	0.079	0.00362
54 KI-67+CD28-CD45RO+CD8+CD4-CCR7-	8.0e-09	(2.7e-12, 7.2e-04)	3e-04	133.6	0.074	0.00209
55 KI-67+CD28-CD45RO+CD57-CCR7+CD127-	5.9e-08	(4.0e-15, 4.5e-03)	2e-03	376.6	0.066	0.00075
56 KI-67+CD28-CD45RO+CD4-CD57-CCR7+CD127-	5.0e-08	(4.8e-13, 3.9e-03)	2e-03	409.6	0.066	0.00070
57 KI-67+CD57-CD27-CD127-	5.9e-10	(3.2e-14, 2.7e-03)	2e-05	44.9	0.085	0.00806
58 KI-67+CD28-CD27-CD127-	4.8e-10	(7.3e-15, 2.5e-03)	2e-05	50.6	0.086	0.00711
59 KI-67+CD4-CD127-	1.3e-10	(4.4e-16, 9.7e-03)	5e-06	37.1	0.091	0.01159
60 KI-67+CD28-CD127-	4.9e-10	(1.1e-12, 1.4e-03)	2e-05	41.4	0.086	0.00823
61 KI-67+CD4-CD27-	5.6e-09	(2.1e-14, 2.6e-03)	2e-04	28.6	0.075	0.01122
62 KI-67+CD28-CD4-CD27-	1.8e-09	(3.6e-13, 5.3e-03)	7e-05	40.2	0.080	0.00785
63 KI-67+CD27-CD127-	1.3e-09	(9.8e-15, 1.1e-03)	5e-05	33.0	0.082	0.01052
64 KI-67+CD4-CD27-CCR7-	6.5e-11	(1.4e-15, 9.6e-04)	2e-06	47.3	0.094	0.00947
65 KI-67+CD4-CD27-CCR7-	9.6e-11	(1.1e-16, 1.5e-03)	4e-06	52.1	0.092	0.00764
66 KI-67+CD4-CCR7-	1.7e-10	(3.0e-14, 1.0e-02)	7e-06	41.4	0.090	0.00987
67 KI-67+CD45RO+CD57-CCR7-	1.4e-09	(6.6e-13, 1.2e-03)	5e-05	49.6	0.081	0.00695
68 KI-67+CD45RO+CD57-CD27-CCR7-	9.1e-10	(8.6e-12, 2.5e-03)	3e-05	66.4	0.083	0.00505
69 KI-67+CD45RO+CD4-	2.0e-09	(8.0e-13, 2.5e-03)	8e-05	45.3	0.080	0.00851
70 KI-67+CD28-CD45RO+	1.3e-08	(1.2e-12, 2.4e-03)	5e-04	54.9	0.072	0.00525
71 KI-67+CD45RO+CD127-	1.1e-09	(4.4e-16, 1.5e-02)	4e-05	42.5	0.082	0.00834
72 KI-67+CD45RO+CD57-CD127-	2.9e-10	(1.5e-14, 6.4e-04)	1e-05	55.0	0.088	0.00719
73 KI-67+CD28-CD45RO+CD8+CD27-	9.2e-09	(2.6e-15, 2.3e-03)	4e-04	138.0	0.073	0.00201
74 KI-67+CD28-CD45RO+CD8+CD4-CD27-	9.2e-09	(1.0e-15, 4.6e-03)	4e-04	138.0	0.073	0.00201
75 KI-67+CD8+CD4-CD57-CD27-CD127-	1.9e-09	(5.9e-14, 7.0e-03)	7e-05	113.8	0.080	0.00274
76 KI-67+CD28-CD45RO+CD8+	9.3e-09	(5.9e-13, 1.4e-03)	4e-04	102.7	0.073	0.00279
77 KI-67+CD28-CD45RO+CD8+CD4-	9.3e-09	(0.0e+00, 1.6e-03)	4e-04	102.7	0.073	0.00279
78 KI-67+CD45RO+CD8+	2.1e-08	(6.9e-15, 6.8e-04)	8e-04	59.1	0.070	0.00512
79 KI-67+CD8+CCR7-	3.0e-08	(7.7e-13, 2.8e-03)	1e-03	49.5	0.068	0.00530
80 KI-67+CD8+CD27-CCR7-	8.3e-09	(1.0e-13, 3.6e-03)	3e-04	70.7	0.074	0.00377
81 KI-67+CD4-	2.8e-08	(1.0e-13, 2.3e-03)	1e-03	17.1	0.069	0.01627
82 KI-67+CD28-CD4-	1.1e-08	(5.9e-14, 4.0e-03)	4e-04	26.7	0.073	0.00950
83 KI-67+CD127-	2.7e-08	(1.2e-12, 2.1e-03)	1e-03	19.1	0.069	0.01460
84 KI-67+CCR7-	8.4e-08	(3.4e-15, 2.3e-03)	3e-03	18.3	0.064	0.01311
85 KI-67+CD27-CCR7-	3.5e-08	(1.7e-13, 1.2e-03)	1e-03	25.2	0.068	0.00998
86 KI-67+CD45RO+CD27-	7.5e-07	(5.4e-13, 1.8e-03)	3e-02	24.0	0.055	0.00862

Table S1. Statistically significant phenotypes.

Phenotype	p-value	p-value CI	adjusted p-value	CPhR Coefficient	R ²	Cell Frequency
87 KI-67+CD45RO+CD57-	1.2e-07	(2.1e-13, 3.1e-03)	5e-03	22.9	0.062	0.01123
88 KI-67+CD4-CD57-	1.3e-08	(3.8e-15, 2.1e-03)	5e-04	25.3	0.072	0.01209
89 KI-67+CD28-CD4-CD57-	9.7e-09	(5.5e-12, 1.2e-03)	4e-04	37.7	0.073	0.00698
90 KI-67+CD57-CD127-	3.3e-09	(1.3e-13, 3.3e-03)	1e-04	28.1	0.078	0.01128
91 KI-67+CD45RO+CCR7-	4.2e-09	(7.8e-15, 2.5e-03)	2e-04	37.5	0.077	0.00819
92 KI-67+CD57-CCR7-	2.7e-08	(2.8e-13, 2.8e-03)	1e-03	26.6	0.069	0.01008
93 KI-67+CD57-CD27-CCR7-	1.2e-08	(4.9e-13, 2.6e-03)	5e-04	36.8	0.072	0.00762
94 KI-67+CD28-CCR7-	3.3e-09	(4.6e-14, 5.7e-03)	1e-04	37.7	0.078	0.00739
95 KI-67+CD28-CD27-CCR7-	3.3e-09	(2.6e-14, 6.5e-04)	1e-04	43.0	0.078	0.00647
96 KI-67+CD28-	1.9e-07	(4.0e-15, 2.7e-03)	7e-03	18.3	0.061	0.01053
97 KI-67+CD28-CD27-	7.1e-08	(1.5e-12, 8.6e-04)	3e-03	26.3	0.065	0.00874
98 KI-67+CD28-CD8-	8.3e-08	(5.5e-14, 2.5e-03)	3e-03	44.2	0.064	0.00523
99 KI-67+CD45RO+	8.9e-07	(1.9e-13, 2.5e-03)	3e-02	15.4	0.054	0.01343
100 KI-67+CD8+CD57-	1.1e-06	(4.4e-14, 3.1e-03)	4e-02	28.3	0.053	0.00648
101 KI-67+CD8+CD27-	6.4e-07	(2.3e-14, 1.1e-02)	2e-02	35.2	0.056	0.00560

Table S2. The identified phenotypes, projected into the Cytotoxic and Helper T-cell compartments.

Phenotype	p-value	p-value CI	adjusted	CPhR Coefficient	R ²	Cell Frequency
Original Phenotypes:						
1 KI-67+CD127-	2.7e-08	(0.0e+00, 7.3e-06)	1e-03	19.1	0.06886	1e-02
2 CD45RO-CD8+CD57+CCR5-CD27+CCR7-CD127-	3.1e-07	(1.7e-13, 3.4e-03)	1e-02	633.0	0.05869	6e-04
3 CD28-CD45RO+CD57-	5.6e-07	(1.2e-14, 2.6e-04)	2e-02	12.4	0.05620	5e-02
Projected to the Cytotoxic Compartment (CD8+CD4-):						
4 KI-67+CD8+CD4-CD127-	6.4e-08	(4.2e-14, 2.7e-05)	2e-03	43.6	0.06528	6e-03
5 CD45RO-CD8+CD4-CD57+CCR5-CD27+CCR7-CD127-	3.1e-07	(5.6e-14, 2.7e-03)	1e-02	633.0	0.05869	6e-04
6 CD28-CD45RO+CD8+CD4-CD57-	2.6e-06	(2.2e-10, 2.9e-03)	1e-01	15.3	0.04982	3e-02
Projected to the Helper Compartment (CD8-CD4+):						
7 KI-67+CD8-CD4+CD127-	2.7e-04	(2.4e-12, 1.2e-03)	1e+01	31.9	0.03023	3e-03
8 CD45RO-CD8-CD4+CD57+CCR5-CD27+CCR7-CD127-	4.3e-01	(5.7e-03, 9.3e-01)	2e+04	-163.1	0.00144	5e-05
9 CD28-CD45RO+CD8-CD4+CD57-	6.3e-01	(1.4e-02, 9.4e-01)	2e+04	4.7	0.00054	7e-03

Table S3. The representative immunophenotypes. The markers within Figure 1D with a positive impact on the predictive power were combined to form these immunophenotypes.

Immunophenotype	p-value	p-value CI	Adjusted p-value	CPhR Coefficient	R ²	Cell Frequency
1 Ki-67 ⁺ CD4 ⁻ CCR5 ⁺ CD127 ⁻	1.7×10^{-10}	(0, 1.0×10^{-5})	6.5×10^{-6}	78	0.090	0.00704
2 CD45RO ⁻ CD8 ⁺ CD4 ⁻ CD57 ⁺ CCR5 ⁻ CD27 ⁺ CCR7 ⁻ CD127 ⁻	1.2×10^{-7}	(0, 7.7×10^{-5})	4.6×10^{-3}	639	0.063	0.00068
3 CD28 ⁻ CD45RO ⁺ CD4 ⁻ CD57 ⁻ CD27 ⁻ CD127 ⁻	6.5×10^{-8}	(2.2×10^{-16} , 1.9×10^{-5})	2.4×10^{-3}	22	0.065	0.02456

Supplemental Figures

The Figures included in this section (S1, S2, S3, S4, S5, S6) are discussed in the main text.

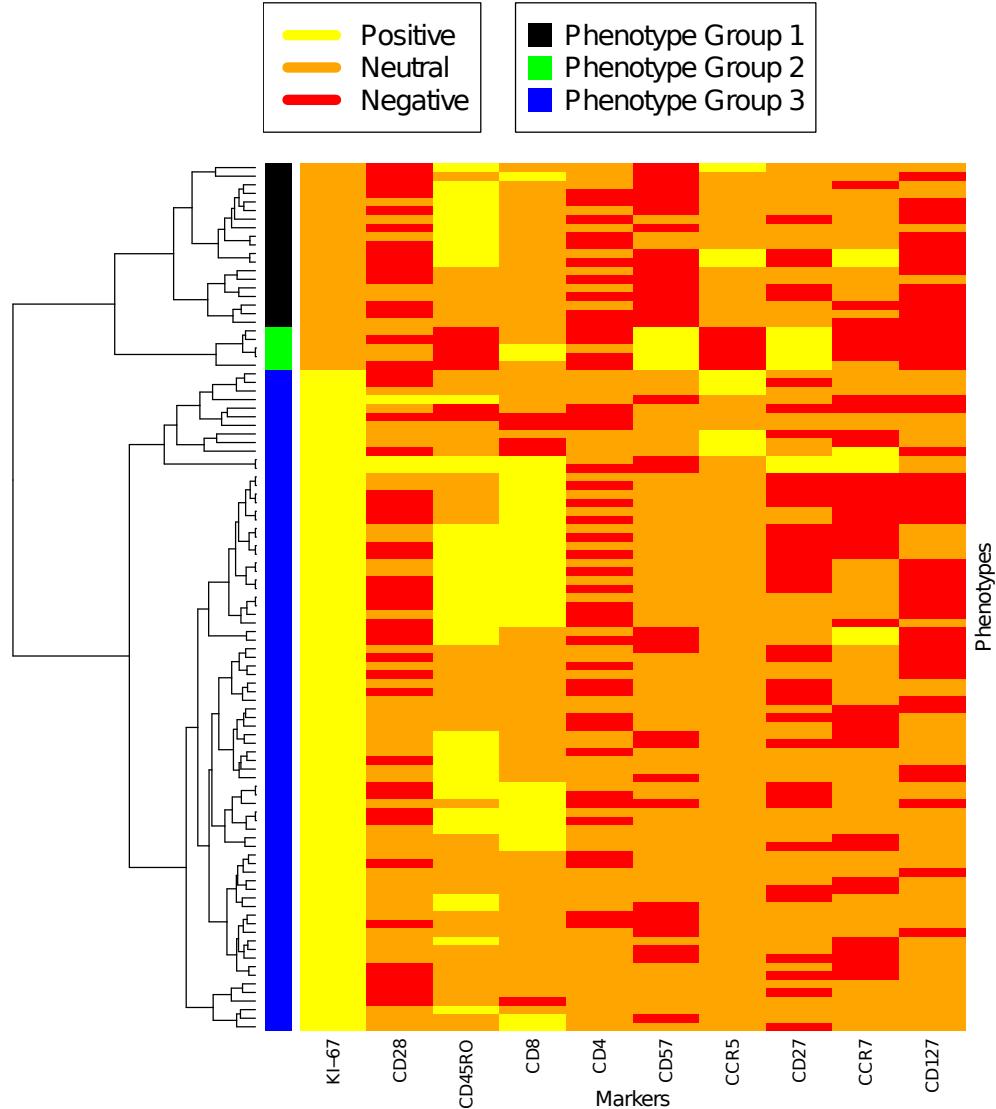


Figure S1. Hierarchical clustering of the statistically significant phenotypes based on the correlation between them. The phenotype names are replaced with a heatmap to make it easier to observe the patterns. The colours denote the “state” of each marker (column) for each phenotype (row).

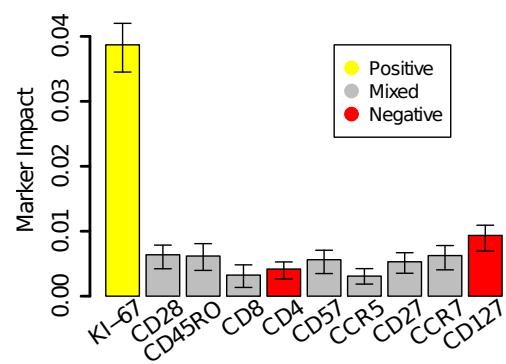


Figure S2. Bulk (over all phenotypes) measurement of the impact of each marker.

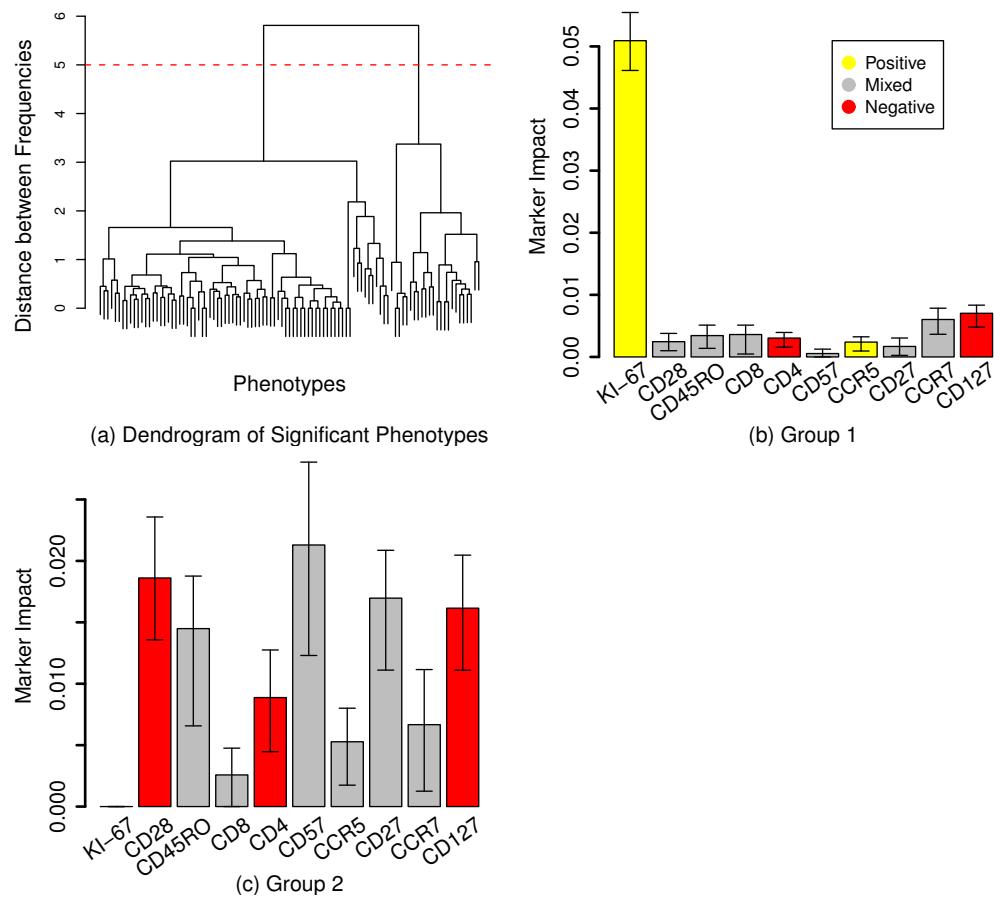


Figure S3. (a) Hierarchical clustering of phenotypes. The red dashed line shows the threshold which results in five groups of phenotypes. (b) and (c) The impact of each of the markers inside the groups of phenotypes.

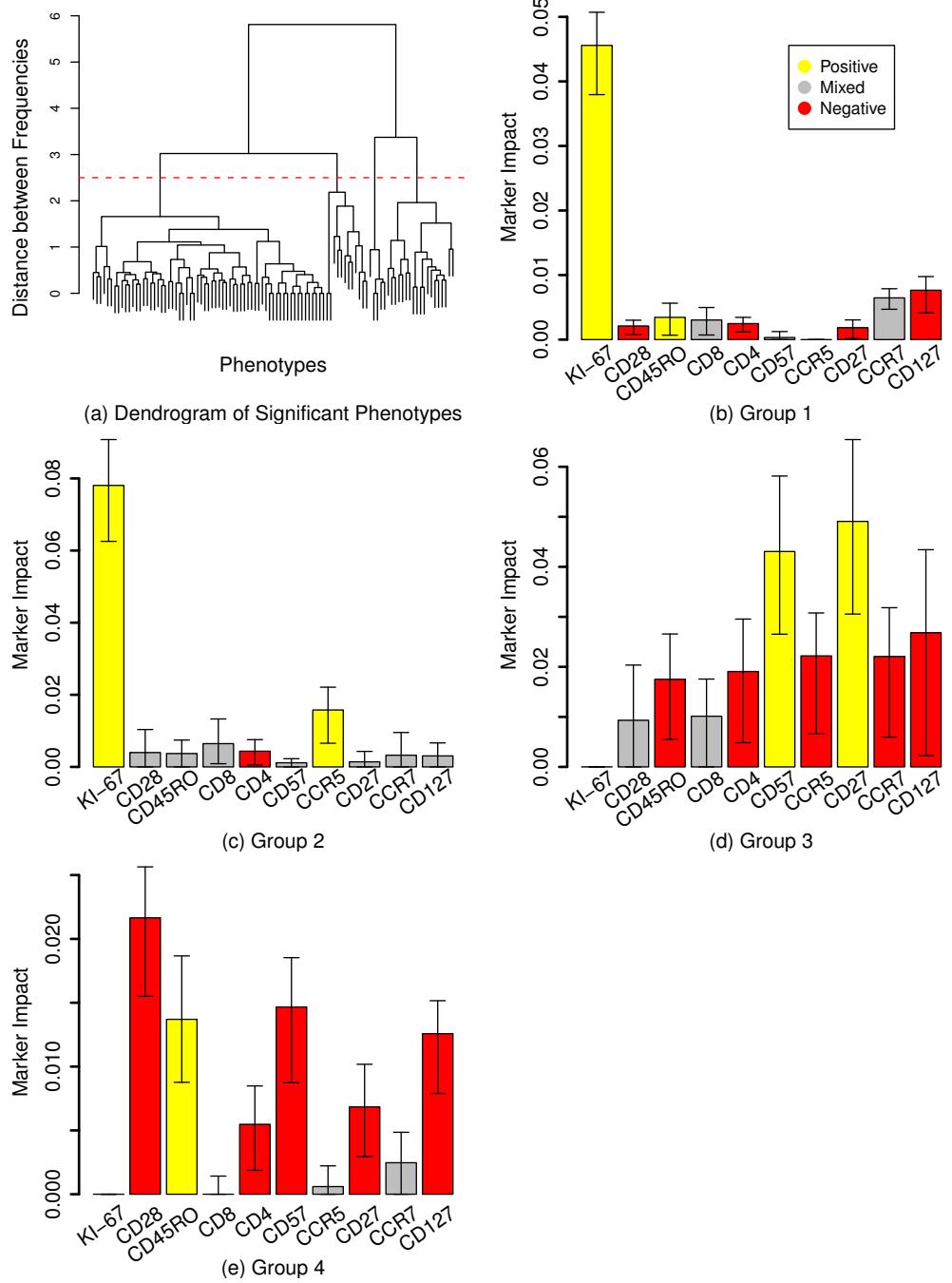


Figure S4. (a) Hierarchical clustering of phenotypes. The red dashed line shows the threshold which results in five groups of phenotypes. (b), (c), (d), and (e) The impact of each of the markers inside the groups of phenotypes.

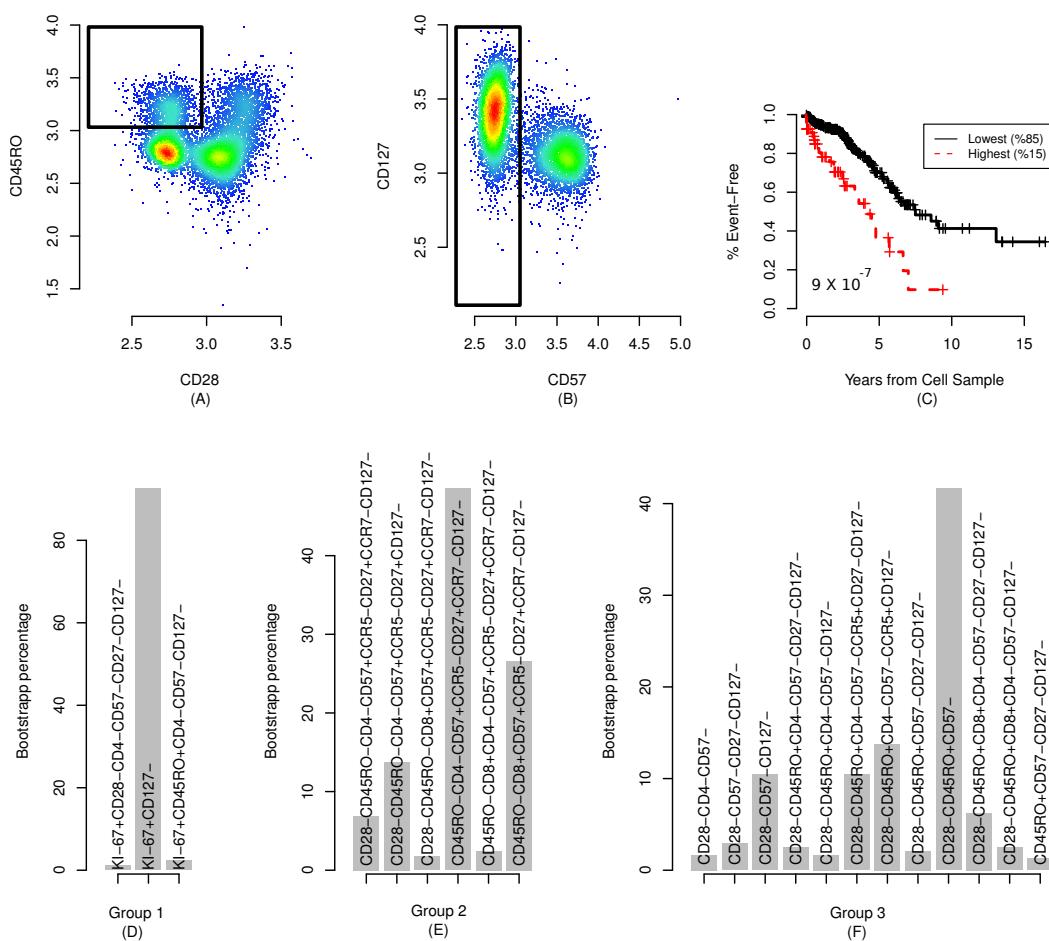


Figure S5. Confirmatory analysis. (A,B) The CD28⁻CD45RO⁺CD57⁻ immunophenotype was identified by manual analysis of all samples. (C) Kaplan-Meier curves confirm the predictive power of the manually measured immunophenotype. (D,E, and F) The immunophenotypes originally selected by the pipeline were dominant in bootstrapping-based sensitivity analysis of the entire pipeline.

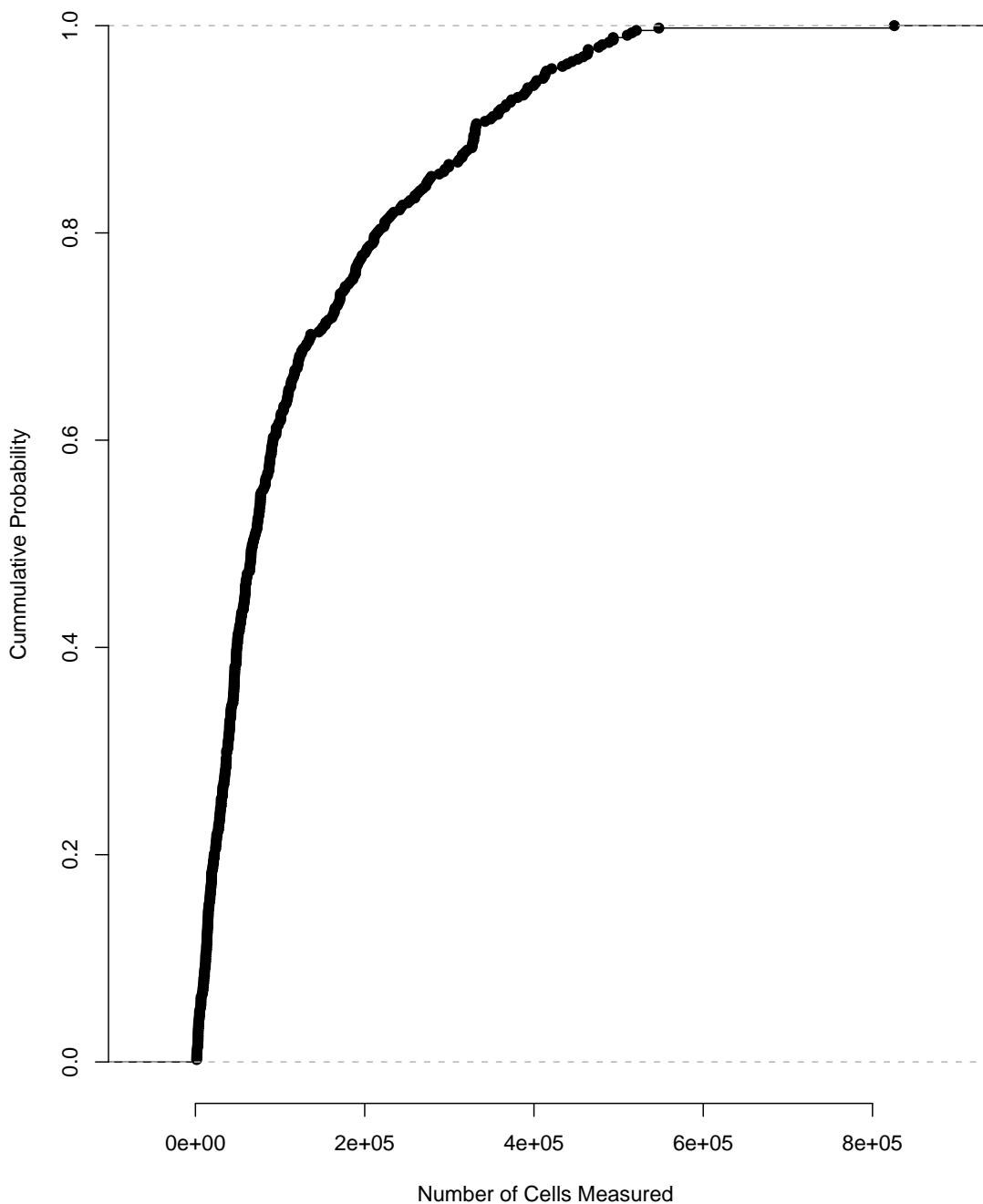


Figure S6. Empirical CDF of the number of T-cells measured for each sample. Minimum, maximum, mean and median of the distribution are 144, 825739, 123682, and 68095, respectively.

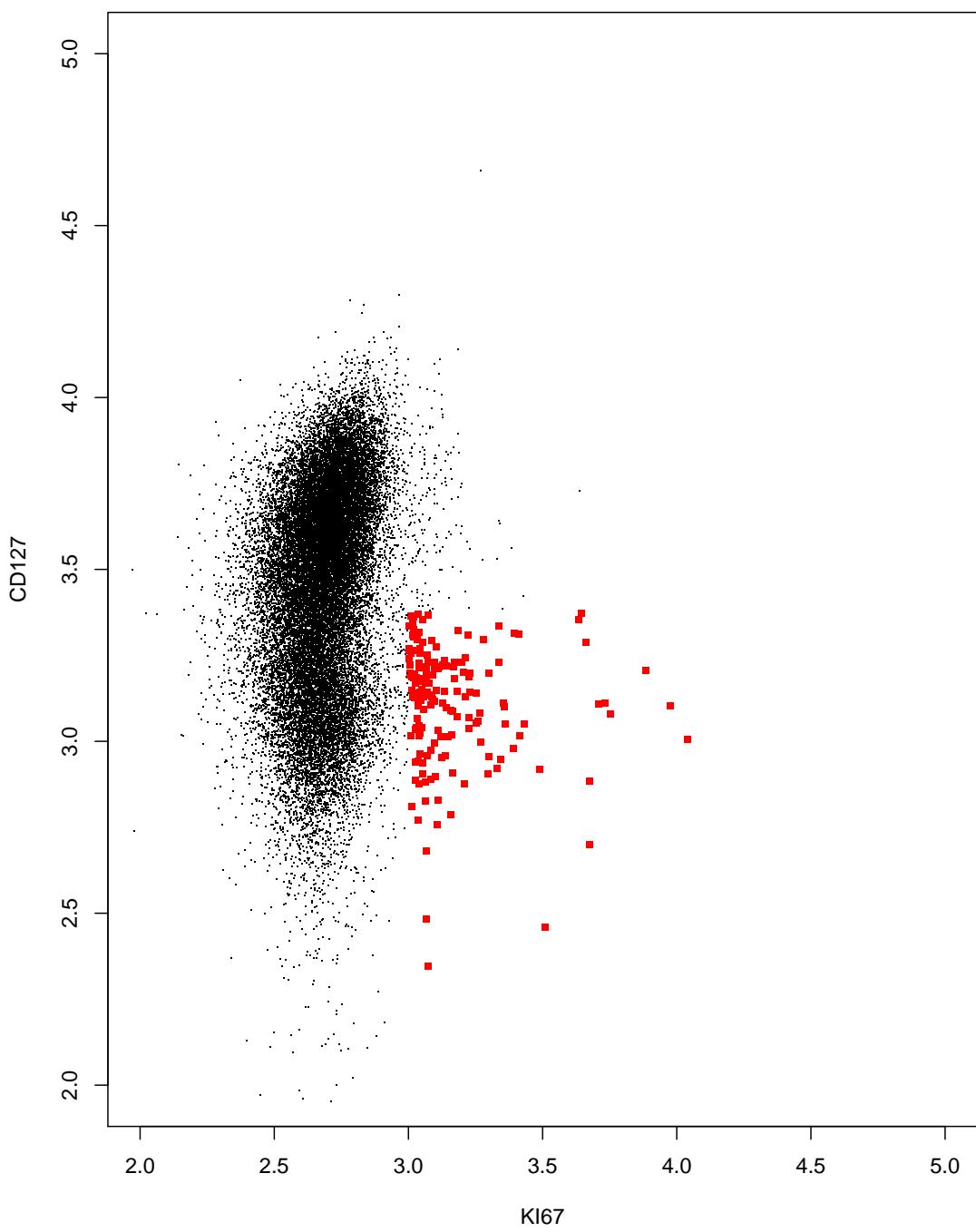


Figure S7. Scatter plot of the Ki-67^+ CD127^- immunophenotype (similar to Figure S8) for sample 299987. Cell frequency: 0.0327 ; Survival time: 11 days

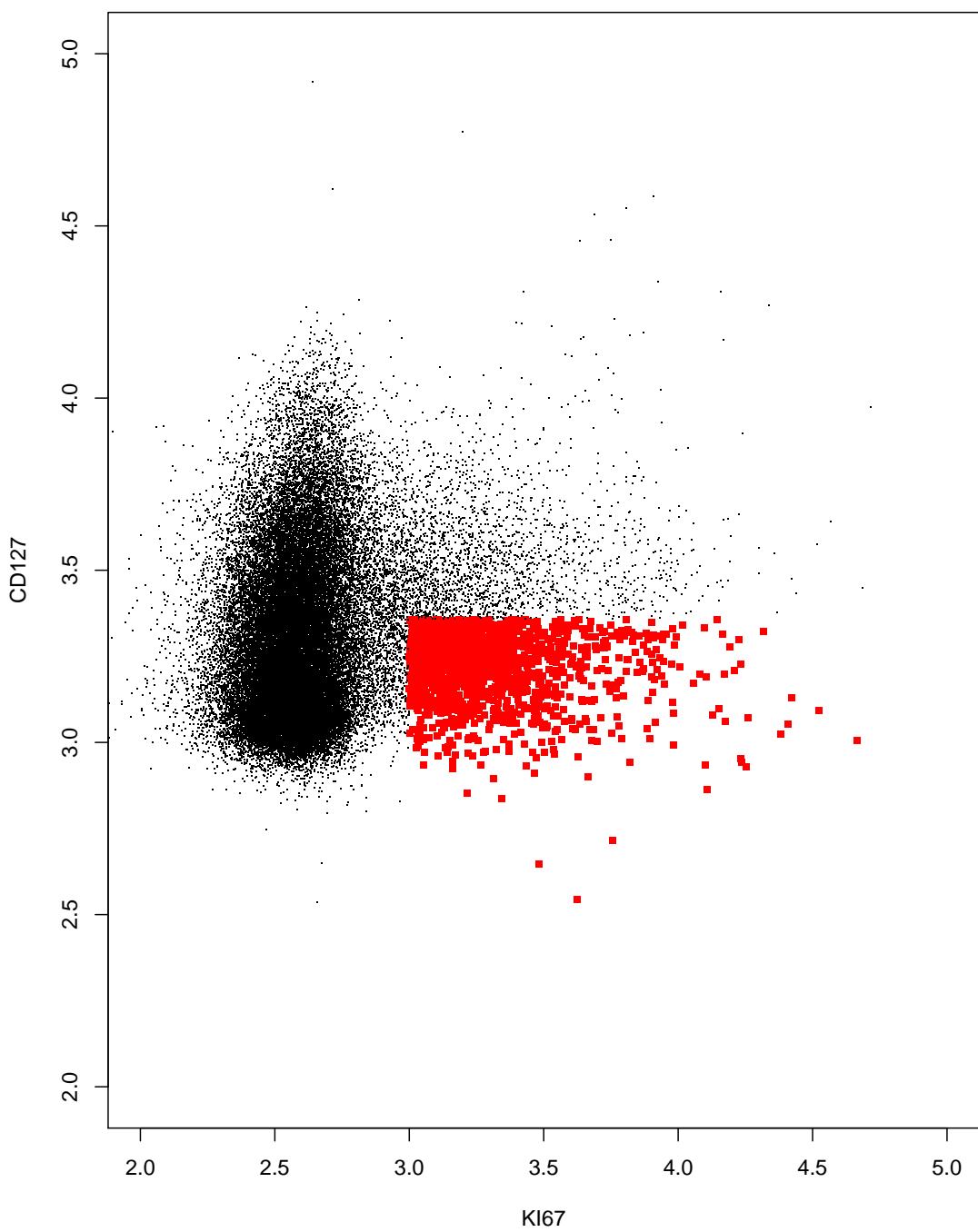


Figure S8. Scatter plot of the Ki-67^+ CD127^- immunophenotype (similar to Figure S7) for sample 548988. Cell frequency: 0.0059 ; Survival time: 6004 days

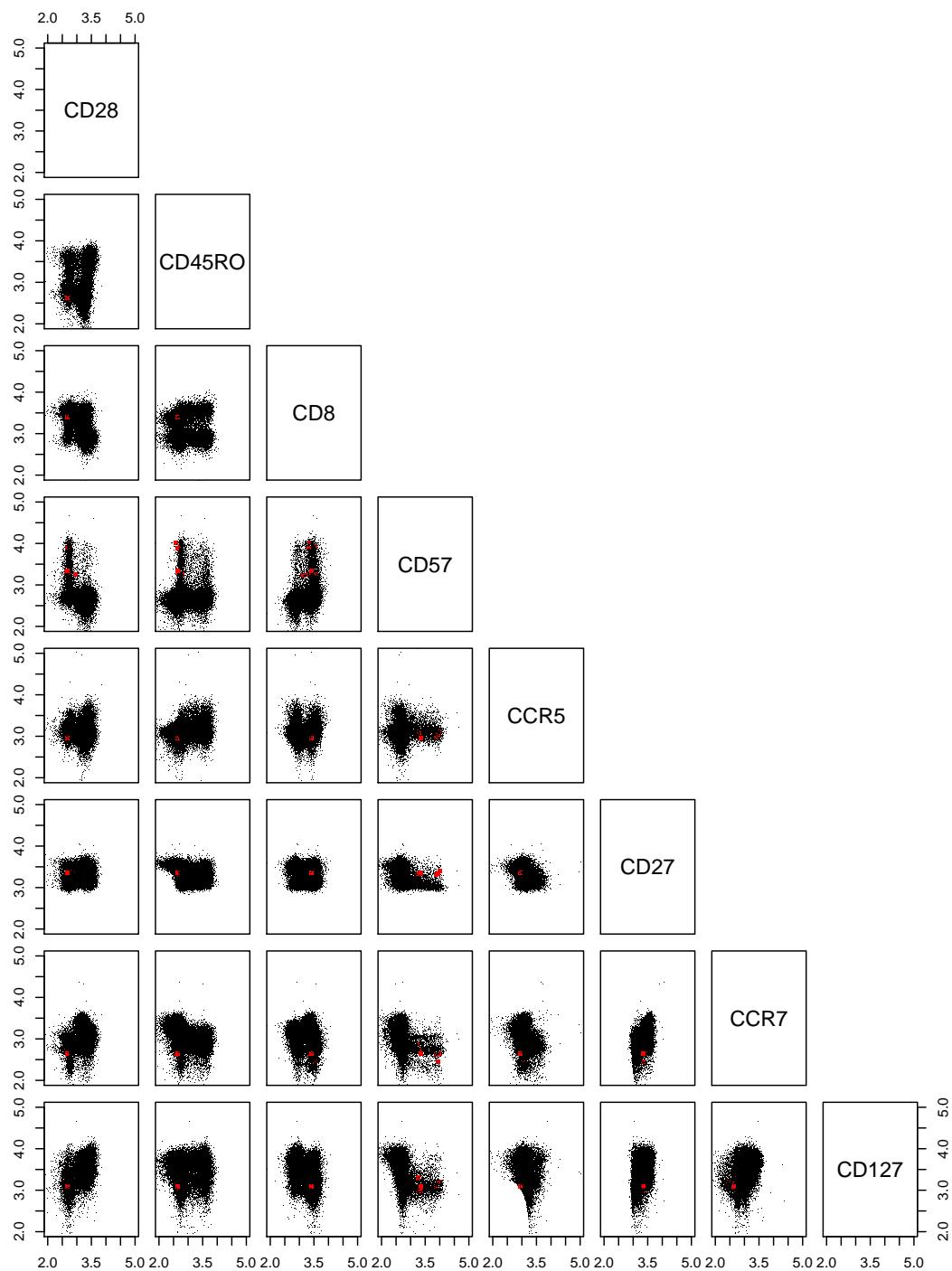


Figure S9. Scatter plot of the $\text{CD45RO}^- \text{CD8}^+ \text{CD57}^+ \text{CCR5}^- \text{CD27}^+ \text{CCR7}^- \text{CD127}^-$ immunophenotype (similar to Figure S10) for sample 299987. Cell frequency: 0.0067 ; Survival time: 11 days

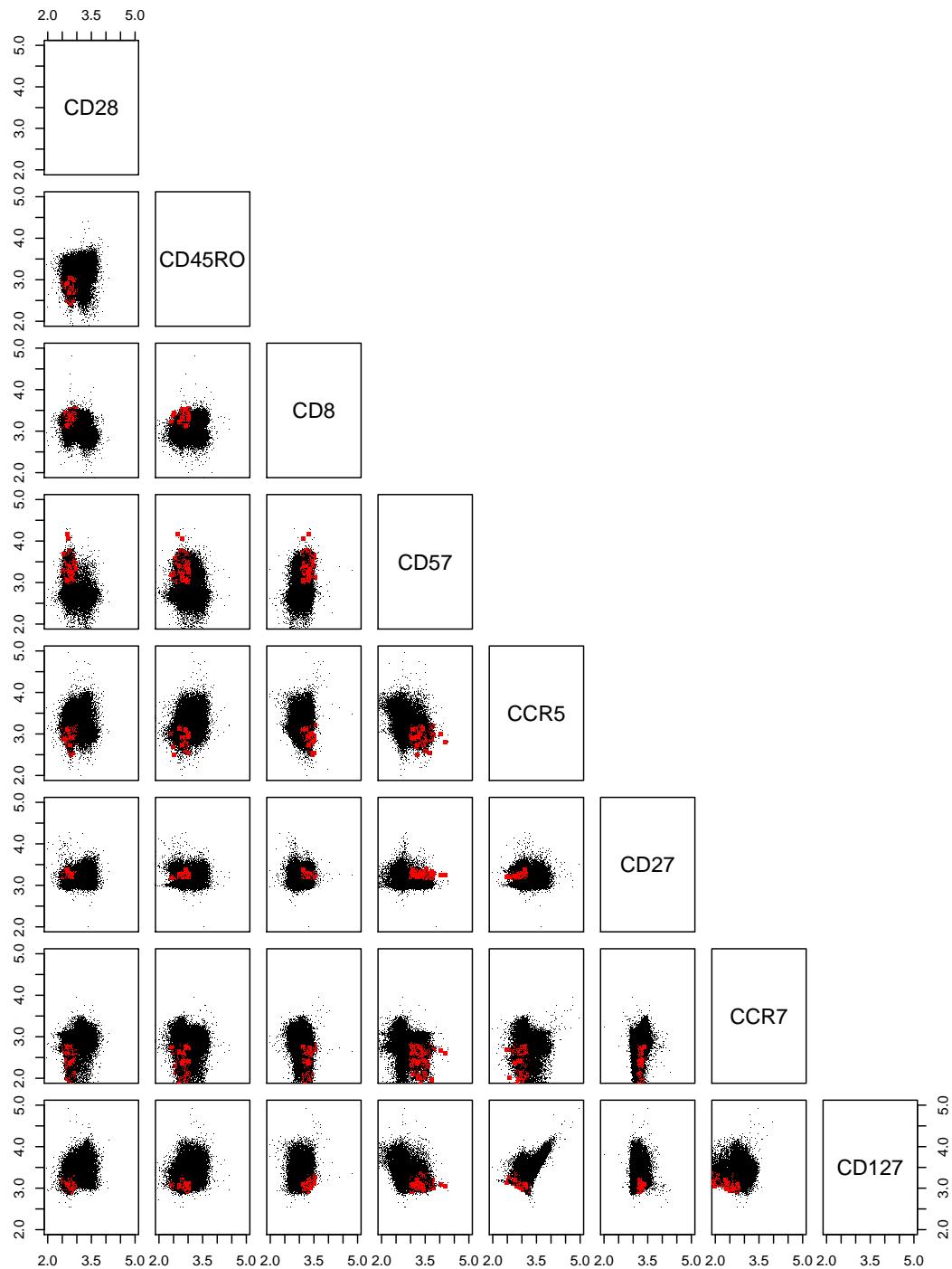


Figure S10. Scatter plot of the CD45RO⁻CD8⁺CD57⁺CCR5⁻ CD27⁺CCR7⁻CD127⁻ immunophenotype (similar to Figure S9) for sample 548988. Cell frequency: 0.0003 ; Survival time: 6004 days

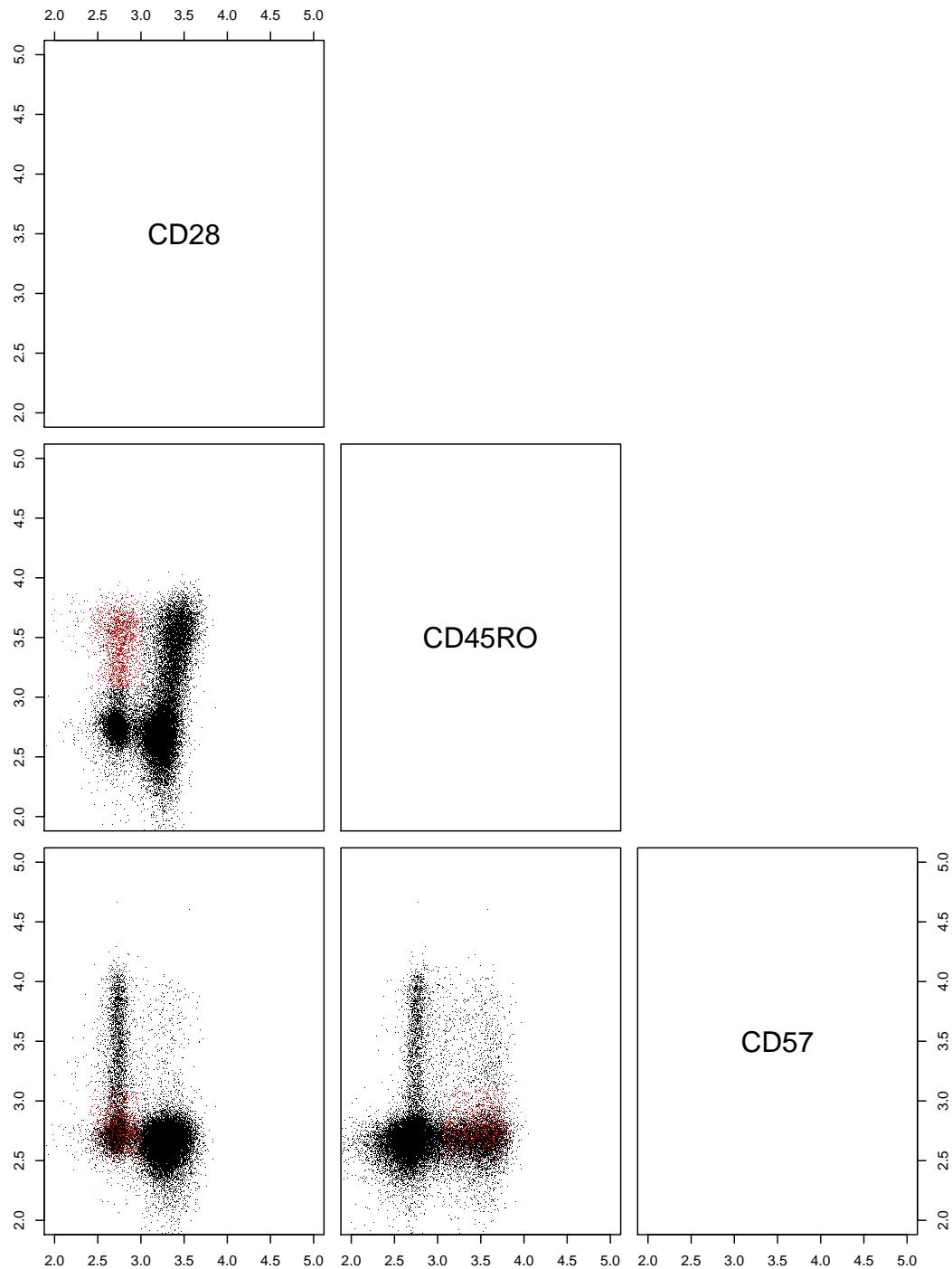


Figure S11. Scatter plot of the $\text{CD28}^- \text{CD45RO}^+ \text{CD57}^-$ immunophenotype (similar to Figure S12) for sample 299987. Cell frequency: 0.1928 ; Survival time: 11 days

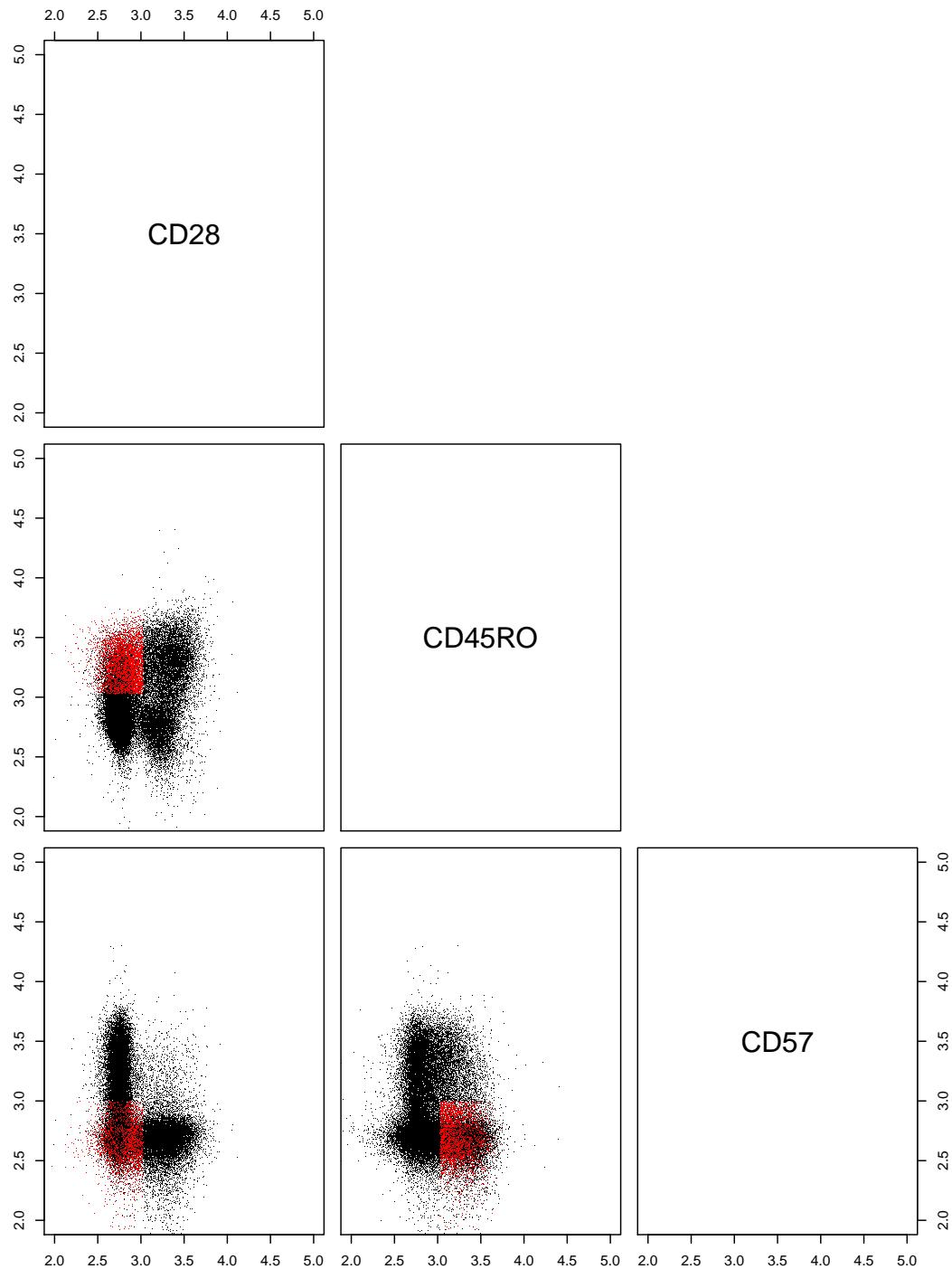


Figure S12. Scatter plot of the $\text{CD28}^- \text{CD45RO}^+ \text{CD57}^-$ immunophenotype (similar to Figure S11) for sample 548988. Cell frequency: 0.0517 ; Survival time: 6004 days