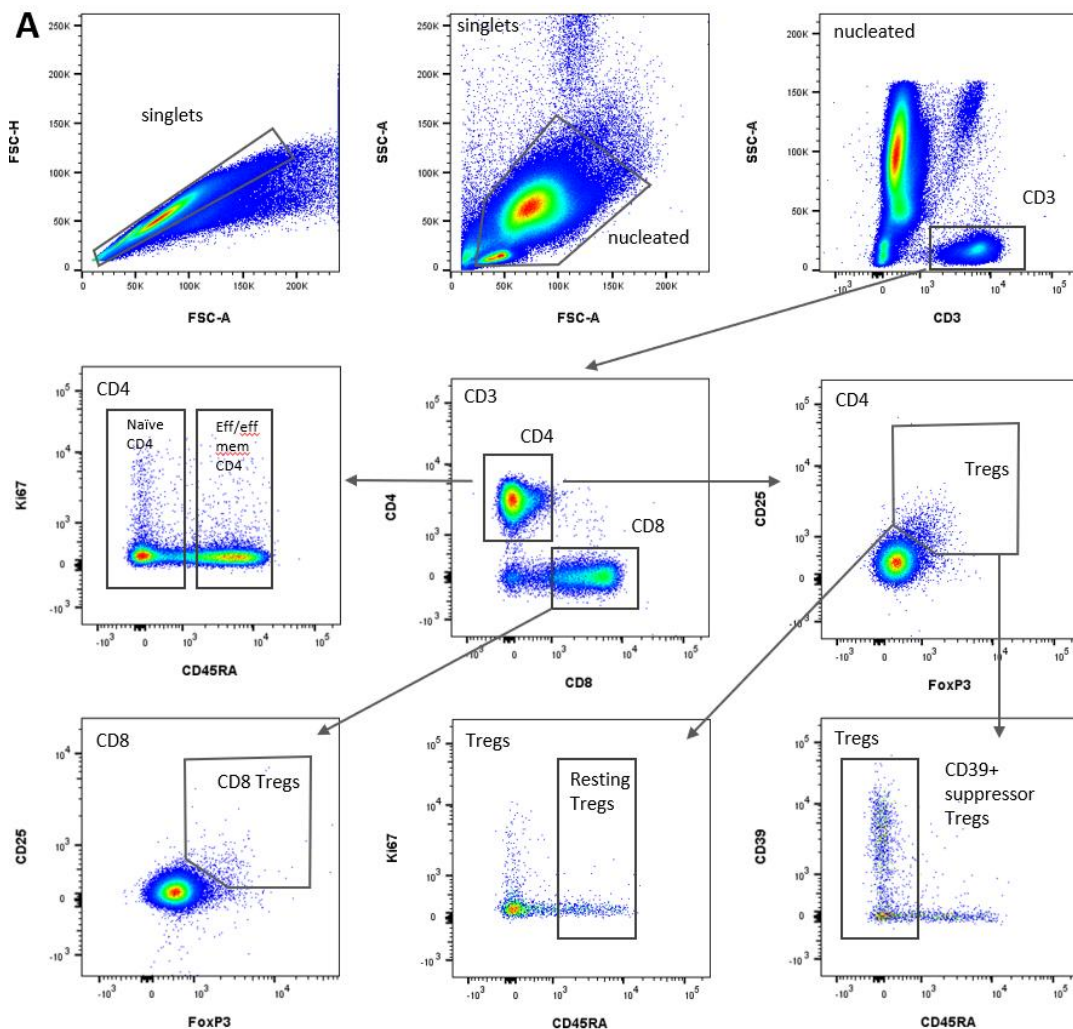
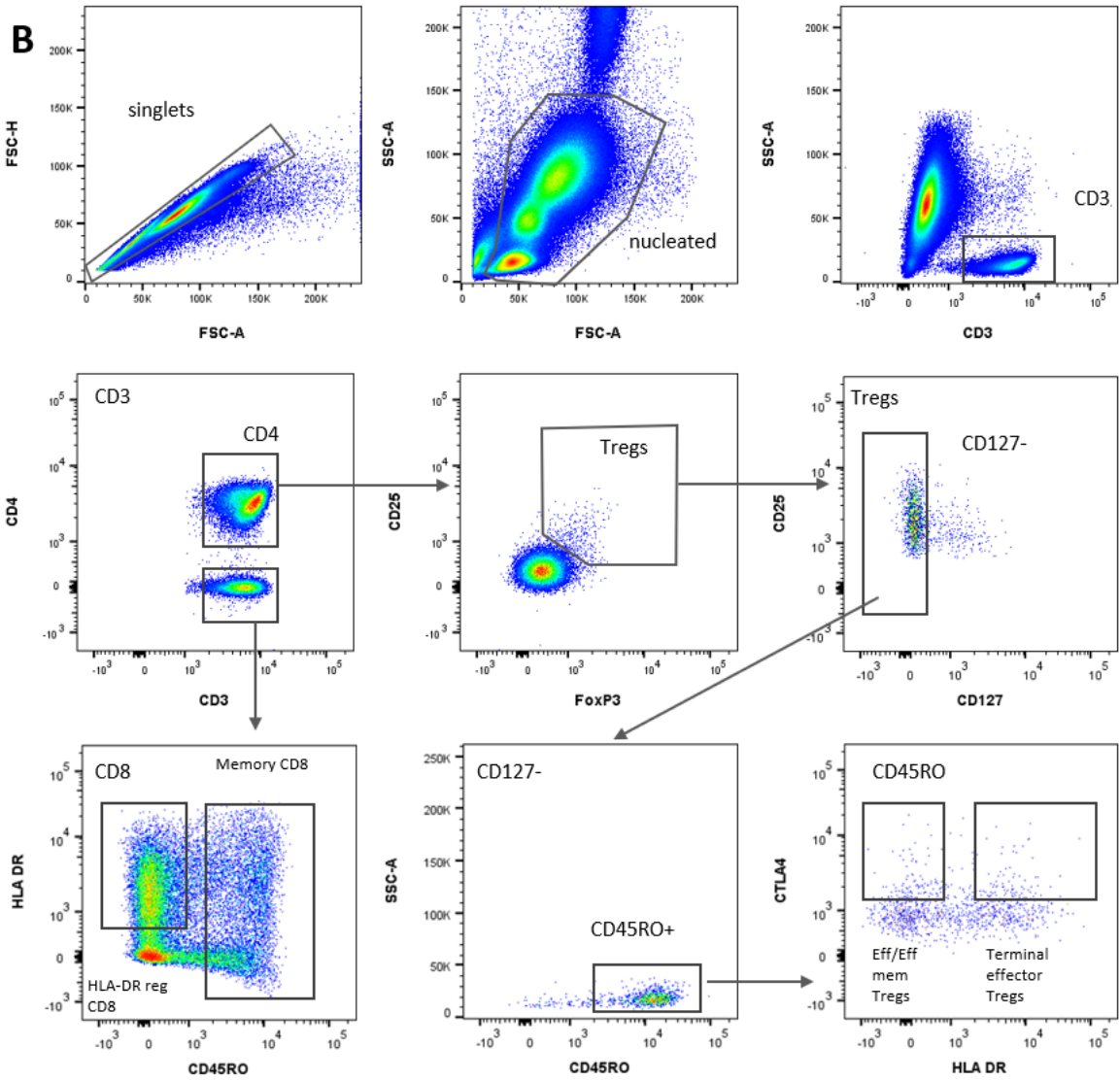


Supplementary Materials:

Deep Phenotyping Reveals Distinct Immune Signatures Correlating with Prognostication, Treatment Responses, and MRD Status in Multiple Myeloma

Konstantinos Papadimitriou, Nikolaos Tsakirakis, Panagiotis Malandrakis, Panagiotis Vitsos, Andreas Metousis, Nikolaos Orologas-Stavrou, Ioannis Ntanasis-Stathopoulos, Nikolaos Kanellias, Evangelos Eleutherakis-Papaiakovou, Panagiotis Pothos, Despina Fotiou, Maria Gavriatopoulou, Efsthathios Kastritis, Meletios-Athanasios Dimopoulos, Evangelos Terpos, Ourania E. Tsitsilonis and Ioannis V. Kostopoulos





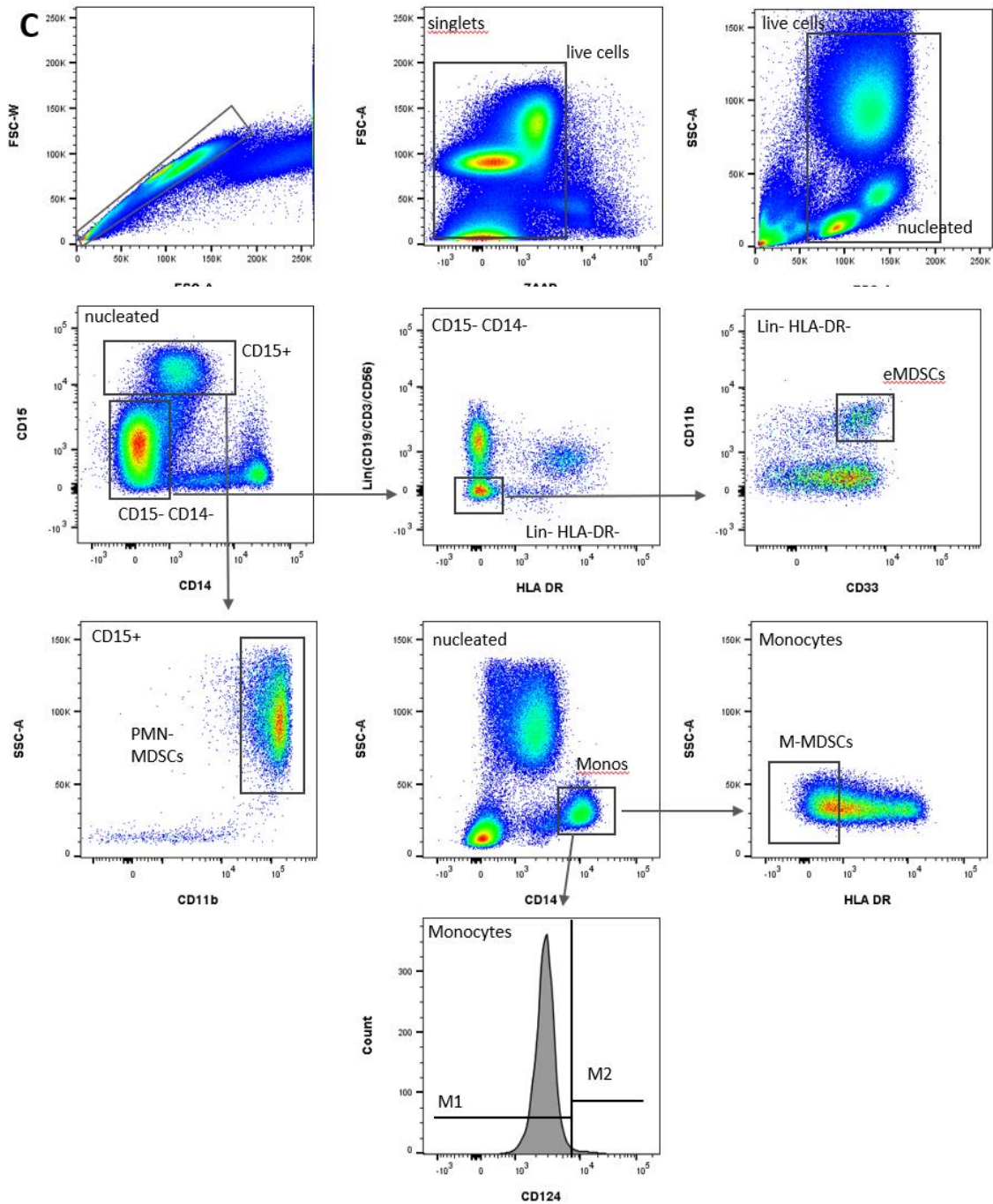


Figure S1. (A) Gating strategy of panel 1 for T cells and regulatory T cells analysis (B) Gating strategy of panel 2 for T cells and regulatory T cells analysis. (C) Gating strategy of panel 3 for MDSCs analysis. Live PBMCs were initially selected. Nucleated cells in a CD14–CD15 dot plot were gated for further discrimination of PMN-MDSCs and eMDSCs. The gating strategy followed the recommendations for MDSC characterization [1].

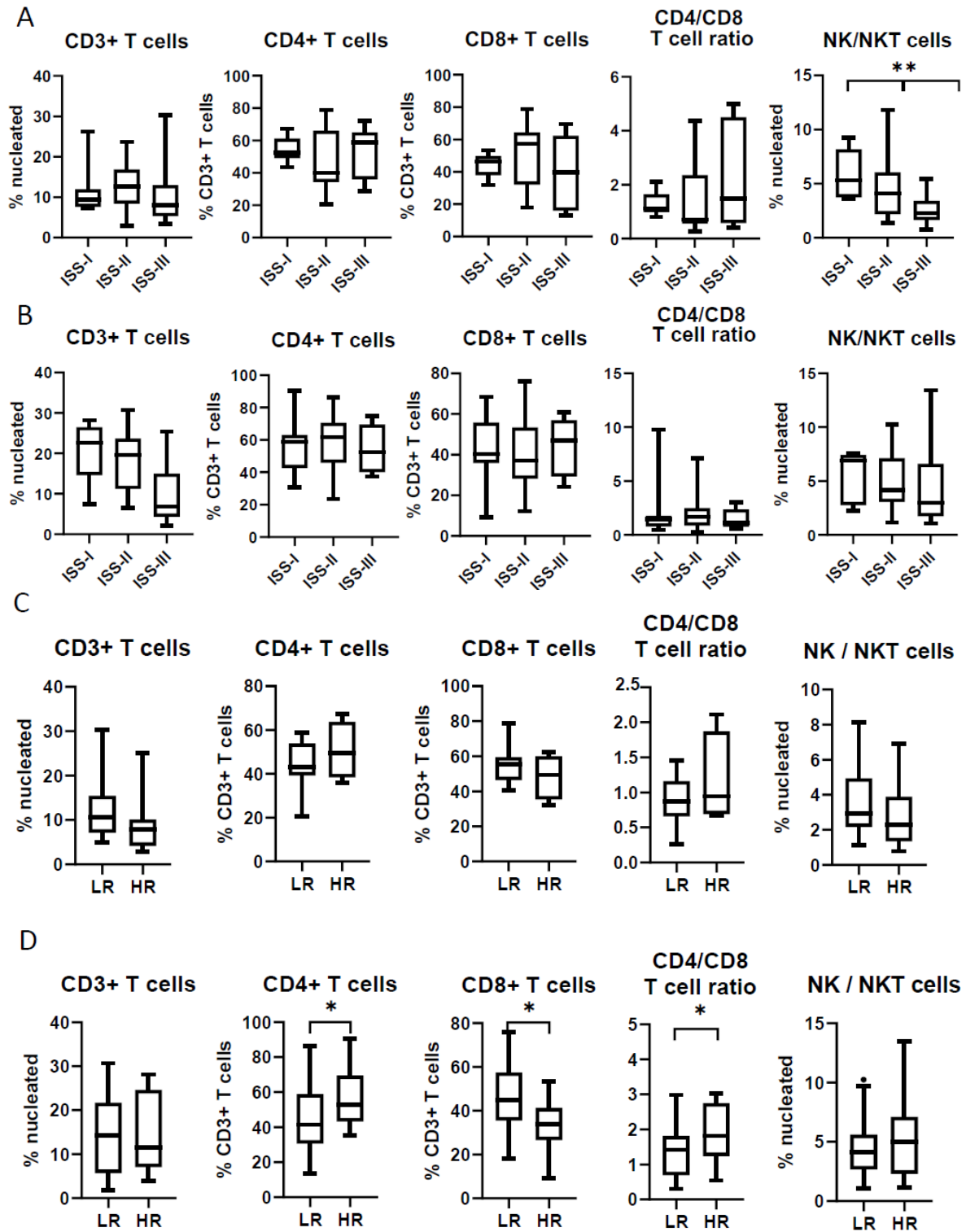


Figure S2. Distribution of total T cells, their CD4+ and CD8+ counterpart, the CD4/CD8 ratio and NK/NKT frequency in the bone marrow (BM) and peripheral blood (PB) of MM patients at the time of diagnosis. (A) Distribution in BM according to the International Staging System (ISS); (B) distribution in PB according to ISS stage; (C) distribution in BM according to cytogenetics; (D) distribution in PB according to cytogenetics. * $p < 0.05$; ** $p < 0.01$; HR: high-risk; LR: low-risk.

Table S1: Clones and relative providers of antibodies used in the study.

Antibody	Clone	Provider
CD38-FITC	Multi-epitope	Cytogonos, Salamanca, Spain

CD56-PE	C5.9	Cytognos, Salamanca, Spain
CD45-PerCPCy5.5	HI30	BD Bioscience, San Jose, CA, USA
CD138-BV421	MI15	BD Bioscience, San Jose, CA, USA
CD27-BV510	O323	BD Bioscience, San Jose, CA, USA
CD19-PC7	J3-119	Beckman Coulter, Brea, CA, USA
CD117-APC	104D2	BD Bioscience, San Jose, CA, USA
CD81-APCC750	M38	Cytognos, Salamanca, Spain
Kappa-APC	Polyclonal	Agilent, Santa Clara, CA, USA
Lambda-APCC750	Polyclonal	Cytognos, Salamanca, Spain
CD3-FITC	HIT3a	BD Bioscience, San Jose, CA, USA
CD4-APC-Cy7	RPA-T4	BD Bioscience, San Jose, CA, USA
CD8-PerCPCy5.5	SK1	BD Bioscience, San Jose, CA, USA
CD25-APC	555434	BD Bioscience, San Jose, CA, USA
FoxP3-PE	236A/E7	BD Bioscience, San Jose, CA, USA
CD127-BV510	HIL-7R-M21	BD Bioscience, San Jose, CA, USA
CD39-BV421	TU66	BD Bioscience, San Jose, CA, USA
Ki67-BV510	B56	BD Bioscience, San Jose, CA, USA
CD45RA-PC7	HI100	BD Bioscience, San Jose, CA, USA
CD45RO-PerCPCy5.5	UCHL1	BD Bioscience, San Jose, CA, USA
CTLA4-BV421	BNI3	BD Bioscience, San Jose, CA, USA
HLA-DR-PC7	G46-6	BD Bioscience, San Jose, CA, USA
CD14-FITC	M5E2	BD Bioscience, San Jose, CA, USA
CD11b-PE	Mac-1	BD Bioscience, San Jose, CA, USA
7-AAD		BD Bioscience, San Jose, CA, USA
CD124-BV421	hIL4R-M57	BD Bioscience, San Jose, CA, USA
CD33-BV510	WM53	BD Bioscience, San Jose, CA, USA
HLA-DR-PE	TU36	BD Bioscience, San Jose, CA, USA
CD15-APC	HI98	BD Bioscience, San Jose, CA, USA
CD3-APC-Cy7	SK7	BD Bioscience, San Jose, CA, USA
CD19-APC-Cy7	557791	BD Bioscience, San Jose, CA, USA
CD56-APC-Cy7	HCD56	Biolegend, San Diego, CA, USA

Table S2. Relative distribution of various immune subsets in paired bone marrow (BM) and peripheral blood (PB) samples.

Immune subset	At diagnosis		<i>p</i> value	In complete remission		<i>p</i> value
	BM	PB		BM	PB	
T cells (% of nucleated cells)	10.52 ± 6.52	13.57 ± 8.09	0.004*	8.64 ± 7.30	11.25 ± 9.85	0.85
CD27+ T cells (% of T cells)	69.80 ± 20.41	67.31 ± 20.29	0.26	50.52 ± 19.12	47.58 ± 26.41	0.74
CD4+ T cells (% of CD3+ cells)	51.10 ± 14.70	61.40 ± 15.7	<0.001	40.94 ± 16.06	46.33 ± 18.2	<0.001
Naïve CD4+ T cells (% of CD4+ cells)	28.43 ± 21.16	34.3 ± 21.5	0.002	15.48 ± 14.02	14.50 ± 12.81	0.39
Proliferating naïve CD4+ T cells (% of CD4+ cells)	0.59 ± 0.72	0.90 ± 0.71	0.014	0.34 ± 0.45	0.21 ± 0.33	0.006

Effector / effector memory CD4+ T cells (% of CD4+ cells)	68.44 ± 21.47	62.13 ± 21.70	<0.001	82.79 ± 14.84	83.89 ± 13.5	0.36
Proliferating Effector/effector memory CD4+ T cells (% of CD4+ cells)	1.55 ± 0.85	1.94 ± 0.90	0.06	2.04 ± 0.91	1.9 ± 0.88	0.34
Tregs (% of CD4+ cells)	1.19 ± 0.86	1.10 ± 0.80	0.57	1.74 ± 1.48	1.71 ± 1.52	0.88
Resting Tregs (% of Tregs)	15.33 ± 16.44	16.30 ± 19.75	0.92	8.11 ± 19.24	9.03 ± 19.21	0.51
Proliferating resting Tregs (% of Tregs)	3.11 ± 4.69	6.25 ± 10.54	0.26	1.16 ± 1.98	0.79 ± 1.79	0.54
Effector/effector memory Tregs (% of Tregs)	14.60 ± 8.23	9.33 ± 8.03	0.03	12.18 ± 7.83	12.06 ± 11.42	0.51
Proliferating effector/effector memory Tregs (% of Tregs)	0.15 ± 0.09	1.16 ± 0.62	0.43	1.48 ± 0.43	1.41 ± 1.32	0.74
Terminal effector Tregs (% of Tregs)	16.01 ± 10.9	7.43 ± 6.5	0.01	14.62 ± 12.15	14.06 ± 10.4	0.59
CD39+ suppressor Tregs (% of Tregs)	36.13 ± 31.89	47.2 ± 22.2	0.14	48.12 ± 30	50.94 ± 29.46	0.32
Proliferating CD39+ suppressor Tregs (% of Tregs)	4.54 ± 6.28	8.1 ± 6.3	0.003	6.19 ± 4.66	6.89 ± 4.66	0.41
CD8+ T cells (% of CD3+ cells)	46.50 ± 15.06	36.8 ± 15.9	<0.001	53.14 ± 15.91	43.82 ± 18.04	<0.001
Proliferating CD8+ T cells (% of CD8 cells)	2.17 ± 1.23	2.52 ± 1.73	0.27	1.74 ± 1.19	1.61 ± 1.13	0.39
HLA-DR regulatory CD8+ T cells (% of CD8 cells)	11.93 ± 7.65	7.30 ± 5.74	<0.001	16.72 ± 14.34	17.66 ± 17.65	0.93
Memory CD8+ T cells (% of CD8 cells)	27.48 ± 15.41	27.1 ± 13.8	0.87	34.56 ± 19.97	31.79 ± 20.90	0.22
CD8+ Tregs (% CD8+ cells)	0.25 ± 0.82	0.29 ± 1.02	0.28	0.34 ± 1.39	0.27 ± 1.17	0.27
B cells (% of nucleated cells)	1.49 ± 1.15	1.42 ± 0.93	0.69	3.51 ± 4.34	4.2 ± 5.24	0.54
Memory B cells (% of B cells)	34.21 ± 21.7	36.90 ± 20.73	0.03	9.21 ± 15.28	10.34 ± 12.47	0.67
Naïve B cells (% of B cells)	46.74 ± 21.79	61.61 ± 20.56	<0.001	48.73 ± 25.18	63.47 ± 25.14	0.74
B cell precursors (% of B cells)	19.09 ± 16.54	2.02 ± 2.11	<0.001	37.17 ± 27.07	1.07 ± 1.25	<0.001
PMN-MDSCs (% of mononuclear cells)	2.38 ± 3.53	9.86 ± 11.82	0.03	3.85 ± 5.02	14.18 ± 13.31	0.07
M-MDSCs (% of mononuclear cells)	6.12 ± 4.02	10.11 ± 5.38	0.04	11.92 ± 10.81	7.64 ± 10.21	0.58
eMDSCs	0.32 ± 0.49	0.55 ± 1.01	0.32	0.51 ± 0.65	0.85 ± 0.80	0.36

(% of mononuclear cells)						
Monocytes PB / TAMS BM	3.16 ± 1.67	6.63 ± 2.63	<0.001	5.24 ± 2.91	6.87 ± 4.41	0.12
(% of nucleated cells)						
M1 monocytes	97.8 ± 0.94	98.26 ± 0.64	0.10	98.11 ± 1.24	97.27 ± 1.49	0.84
(% of monocytes)						
M2 monocytes	2.20 ± 0.94	1.72 ± 0.64	0.09	1.89 ± 1.24	2.73 ± 1.49	0.06
(% of monocytes)						
NK/NKT cells	3.23 ± 1.84	4.43 ± 2.73	0.005	3.02 ± 2.76	3.94 ± 3.14	0.08
(% nucleated cells)						
CD27+ NK/NKT cells	17.01 ± 10.23	14.20 ± 9.91	0.016	13.64 ± 7.15	15.24 ± 8.32	0.12
(% of NK/NKT cells)						
Neutrophils	68.11 ± 11.99	65.19 ± 13.04	0.55	60.88 ± 23.66	63.88 ± 19.23	0.24
(% nucleated cells)						

* *p* values in bold characters denote statistically significant differences.

Reference

1. Bronte, V.; Brandau, S.; Chen, S.-H.; Colombo, M.P.; Frey, A.B.; Greten, T.F.; Mandruzzato, S.; Murray, P.J.; Ochoa, A.; Ostrand-Rosenberg, S.; et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat. Commun.* **2016**, *7*, 12150, doi:10.1038/ncomms12150.