Antigen	Antibody clone	Fluorochrome
CD3	UCHT1	Pacific Blue
CD4	SK3	APC-Cy7
CD11b	ICRF44	PE-Cy7
CD14	ΜΦΡ9	APC-Cy7
CD15	HI98	FITC
CD16	3G8	PerCP-Cy5.5
CD19	HIB19	FITC
CD25	M-A251	APC-Cy7 and APC
CD33	WM-53	APC
CD45RA	HI100 or 5H9	APC-H7 and APC
CD56	B-159 or HCD56	PE-Cy7 and Brilliant Violet 421
CD62L	SK11	PE
CD69	L78	APC
CD103	Ber-ACT8	FITC
CD124	hIL-4R M57	PE
CD127	hIL-7R-M21	PerCP-Cy5.5
CD195	3A9	APC
CD197	3D12	PE-Cy7
FoxP3	259D7C7	PE
HLA-DR	G46-6	PerCP-Cy5.5
Vδ2	B6	FITC
Vy9	B3	PE

Supplementary Table 1: Tested antigens and the respective antibody clone.

	Fluorochrome-labeled antibodies (α-)							
	FITC	PE	PerCP- Cy5.5	PE-Cy7	APC	APC- Cy7 or APC-H7	Pacific Blue or Brilliant Violet 421	Aqua
Panel-1 (B-, T-, NK cells)	Vδ2 CD19	Vy9	CD16	CD56	CD69	CD25	CD3	Live/Dead
Panel-2 (Treg)	CD103	FoxP3	CD127	CD45RA	CD25	CD4	CD3	Live/Dead
Panel-3 (MDSC)	CD15	CD124	HLA- DR	CD11b	CD33	CD14	CD19 CD56 CD3	Live/Dead
Panel-4 (γδ T- cells)	Vδ2 CD19	CD62L	CD16	CD197	CD195	CD45RA	CD3	Live/Dead

Supplementary Table 2: Antigen staining panels for immunophenotyping.

			Intra-assay variability CV [range]	Inter-assay variability CV [range]	
Panel-1 (T, B, NK cells)	1	CD19 ⁺ B cells	12.19 [5.74-9.58]	24.99 [6.69-13.36]	
	2	CD3 ⁺ T cells	9.77 [48.06-78.98]	2.37 [61.94-77.71]	
	3	CD16 ⁺ CD56 ⁺ NK cells	7.44 [4.13-9.56]	3.15 [4.51-9.75]	
	4	Vδ2 T cells	9.85 [1.33-3.76]	9.15 [1.37-3.84]	
	5	CD25 ⁺ T cells	12.58 [4.15-8.93]	14.38 [3.10-7.75]	
	6	CD69 ⁺ T cells	14.31 [0.22-0.65]	24.47 [0.12-0.72]	
	7	CD69 ⁺ NK cells	15.41 [0.84-2.33]	32.21 [0.85-2.50]	
	8	$CD25^+V\delta2^+$ T cells	29.59 [0.28-2.00]	45.42 [0.10-1.42]	
	9	$CD69^+V\delta2^+$ T cells	40.97 [0.24-1.11]	44.36 [0.18-1.34]	
	10	$CD16^+V\delta2^+$ T cells	1.80 [42.95-57.87]	7.89 [42.03-58.85]	
	11	CD4 ⁺ T cells	0.31 [52.90-78.46]	3.71 [51.28-75.84]	
gs)	12	CD4 ⁺ Treg	1.34 [6.08-9.53]	9.47 [5.69-9.44]	
(Tre	13	CD45RA ⁺ Treg	3.39 [11.60-43.43]	15.55 [11.68-52.38]	
Panel-2 (14	FoxP3 ^{hi} CD45RA ⁻ Treg	8.57 [3.54-11.71]	32.92 [3.93-11.12]	
	15	FoxP3 ^{lo} CD45RA ⁺ Treg	1.86 [49.77-74.30]	9.75 [36.88-74.11]	
	16	CD103 ⁺ Treg	11.23 [0.41-4.39]	33.06 [0.30-4.18]	
	17	MDSC1	5.26 [0.07-0.10]	10.16 [0.08-0.12]	
	18	MDSC2	6.36 [0.16-1.00]	7.17 [0.17-0.98]	
el-3 (SC)	19	MDSC3	15.55 [0.00-1.37]	58.95 [0.00-1.26]	
Pan (MD	120	MDSC4	2.16 [13.89-23.91]	6.07 [12.30-23.72]	
	21	MDSC5	3.68 [1.71-4.42]	17.43 [1.47-4.75]	
	22	MDSC6	5.72 [0.27-0.37]	10.16 [0.27-0.40]	
Panel-4 (γδ T cells)	23	CD45RA ⁺ CD62L ⁺ of Vδ2 ⁺ T cells	2.31 [15.74-44.68]	18.79 [16.36-43.62]	
	24	CD45RA ⁻ CD62L ⁺ of Vδ2 ⁺ T cells	1.80 [14.60-41.93]	11.93 [14.80-50.62]	
	25	CD45RA ⁻ CD62L ⁻ of Vδ2 ⁺ T cells	2.85 [9.21-41.23]	17.09 [9.76-40.90]	
	26	CD45RA ⁺ CD62L ⁻ of Vδ2 ⁺ T cells	3.61 [8.98-32.45]	15.05 [6.41-31.96]	

Supplementary Table 3: Overview of intra- and inter-assay variations for immune cell subpopulations.



Supplementary Figure 1: Gating strategy for Panel-1 – the major effector cell panel. Cells for analysis of Panel-1 were gated as follows: (a) setting the time gate, exclusion of doublets, gating on live cells, and furthermore on lymphocytes in FSC/SSC. (b) B cells were identified as CD19⁺ cells. T cells were identified as CD3⁺ cells. (c) NK cells were identified as CD56⁺CD16⁺ cells gated in CD3⁻CD19⁻V δ 2⁻ cells. CD56⁺CD16⁺ NK cells were further gated on activation marker CD69. (d) CD3⁺ T cells were further gated on activation markers CD25 and CD69. V δ 2-antibody was used for identification of V δ 2⁺ T cells in combination with CD3. V δ 2⁺CD3⁺ T cells were further gated on activation markers CD25 and CD69 (by transferring a gate previously set on CD3⁺ cells) as well as on FcγRIII/CD16.



Supplementary Figure 2: Gating strategy for Panel-2 – the regulatory T cell panel. (a) Cells for analysis of Panel-2 were gated as follows: setting the time gate, exclusion of doublets, gating on live cells, and furthermore on lymphocytes in FSC/SSC. (b) T cells were identified as CD3⁺ cells. CD4⁺ T cells were identified as CD3⁺CD4⁺ cells. (c) These cells were further gated on CD127^{lo} cells. (d) Among CD127^{lo}CD4⁺ T cells Tregs were identified as CD25⁺Foxp3⁺ cells. (e) Furthermore, these cells were gated on activated Treg (FoxP3^{hi}CD45RA⁻), resting Treg (FoxP3^{lo}CD45RA⁺) and non-suppressive T cells (FoxP3^{lo}CD45RA⁻). (f) Additionally the expression of CD103 and CD45RA was analyzed in Tregs.



Supplementary Figure 3: Gating strategy for Panel-3 – the myeloid derived suppressor cell (MDSC) panel. Cells for analysis of Panel-3 were gated as follows: (a) setting the time gate, exclusion of doublets, and gating on live cells (used for MDSC1 and MDSC2). (b) The MDSC1 population was identified as CD14⁺CD124⁺ cells. (c) The MDSC2 population was identified as CD15⁺CD124⁺ cells. (d) For identification of MDSC3, singlets were gated on HLA-DR⁻ lineage⁻ (i.e. CD3⁻CD19⁻CD56⁻) cells. These cells were further gated on CD33⁺SSC^{int} cells to identify MDSC3. (e) For identification of the MDSC4 population, lineage⁻ singlet cells were gated on CD14⁺ cells. These cells were further gated on HLA-DR^{lo} to identify MDSC5, lineage⁻ cells were gated on CD15⁺CD14⁻ cells. These cells were further gated on CD11b⁺ cells to identify MDSC5. (g) The MDSC6 population was identified by gating lineage⁻ cells on CD15⁺ cells which were further gated on FSC^{lo}SSC^{hi} cells.



Supplementary Figure 4: Gating strategy for Panel-4 – the V δ 2 T cell panel. Cells for analysis of Panel-4 were gated as follows: (a) setting the time gate, exclusion of doublets, gating on live cells and furthermore on lymphocytes in FSC/SSC. (b) T cells were identified as CD3⁺ cells. (c) V δ 2-antibody was used for identification of V δ 2 T cells in combination with CD3. (d) T cells were gated on CD45RA and CD62L to identify the position of the gate (left plot). The CD45RA versus CD62L gate was then transferred on V δ 2⁺CD3⁺ cells for further phenotyping (right plot).