

Supplementary Materials

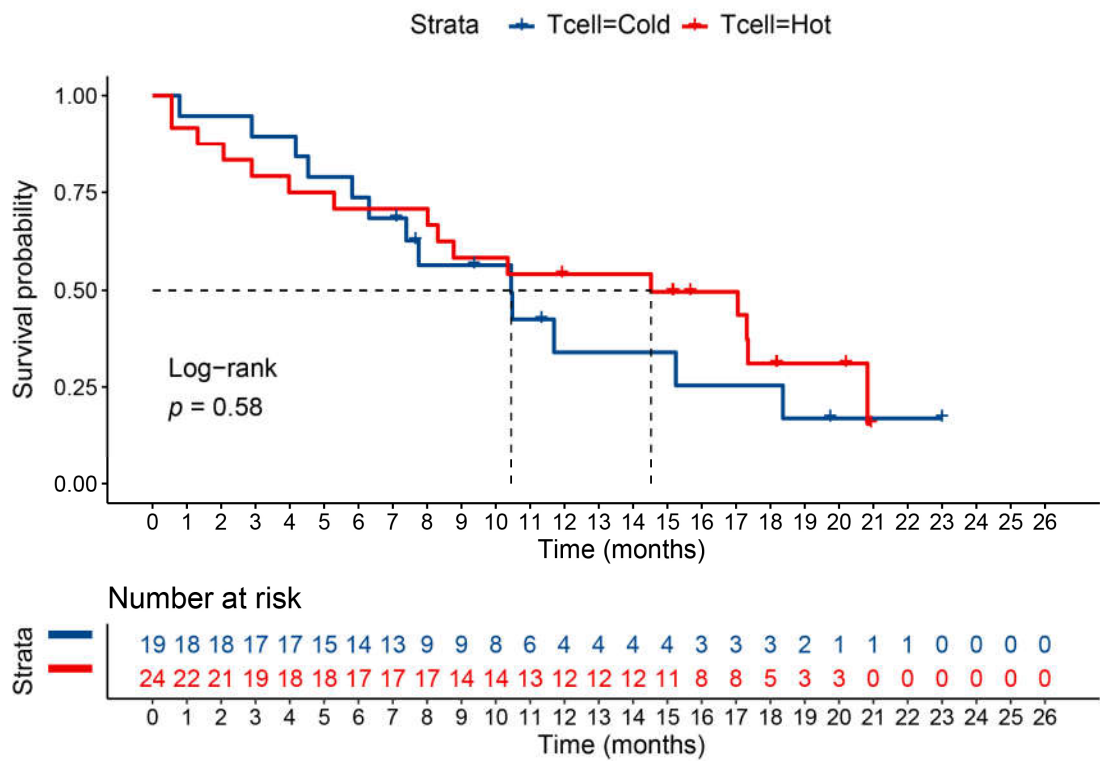
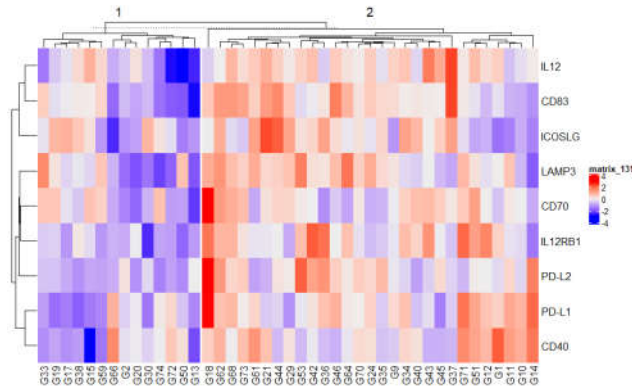
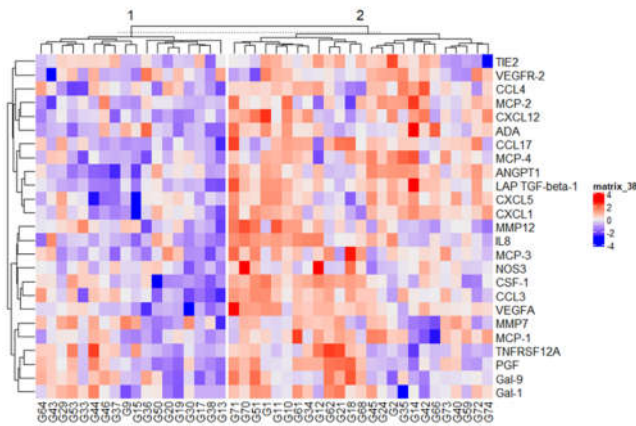


Figure S1. Kaplan–Meier overall survival curves based on hot and cold T cell activation signature. A log rank test was performed to compare the two groups.

Supervised clustering based on APC-related markers



Supervised clustering based on Myeloid/angiogenesis-related markers



Supervised clustering based on tumor-related markers

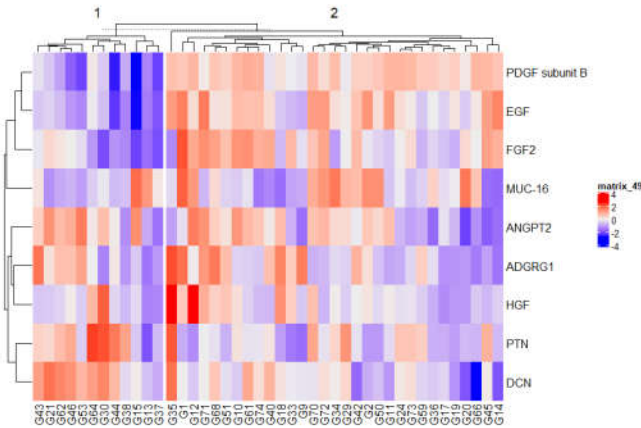


Figure S2. Hierarchical clustering analysis of protein expression. K-means clustering analysis of selected APC-related markers (9 proteins), myeloid/angiogenesis (25 proteins) and tumor-related markers (9 proteins) in the plasma.

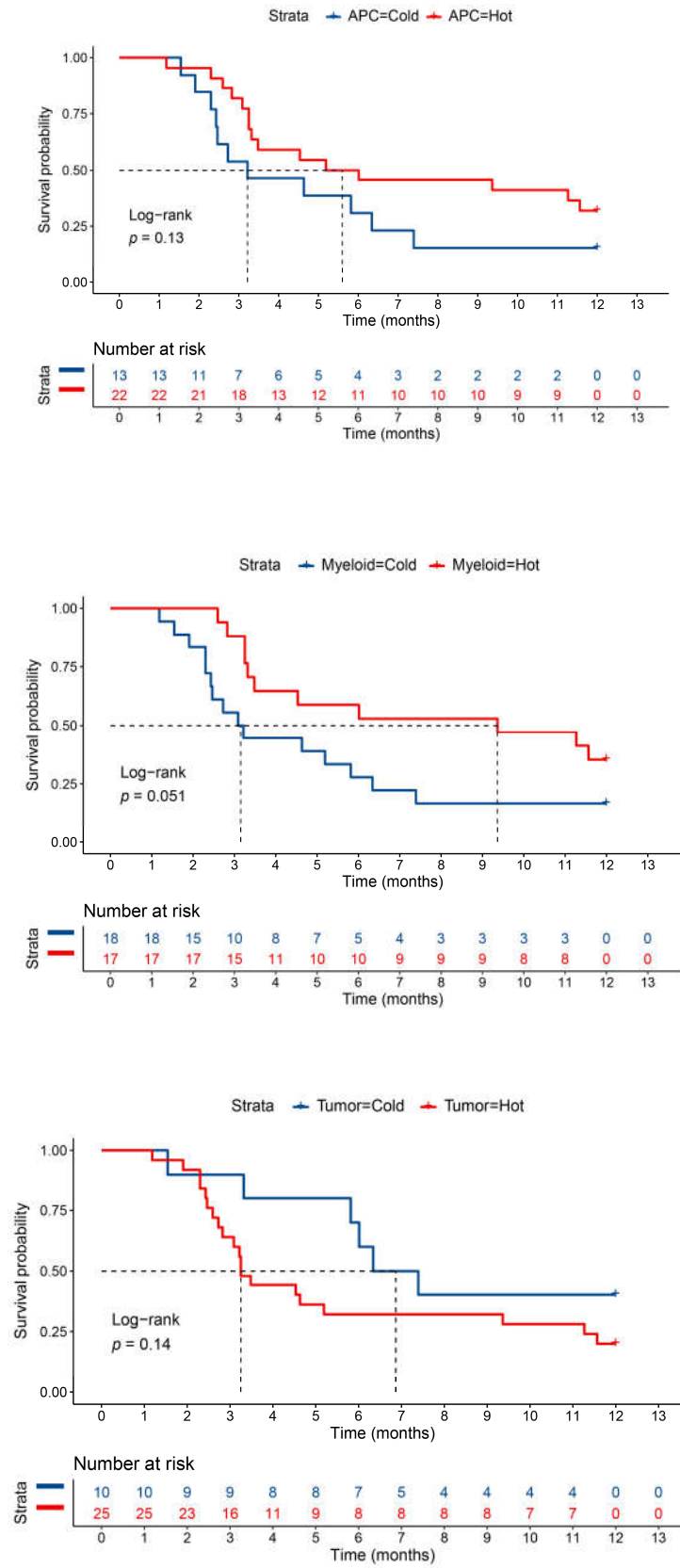


Figure S3. Progression-free survival (PFS) analysis of APC-related markers (9 proteins), myeloid/angiogenesis (25 proteins) and tumor-related markers (9 proteins) in the plasma signature (hot vs. cold). The log rank test was used to test for differences in survival.

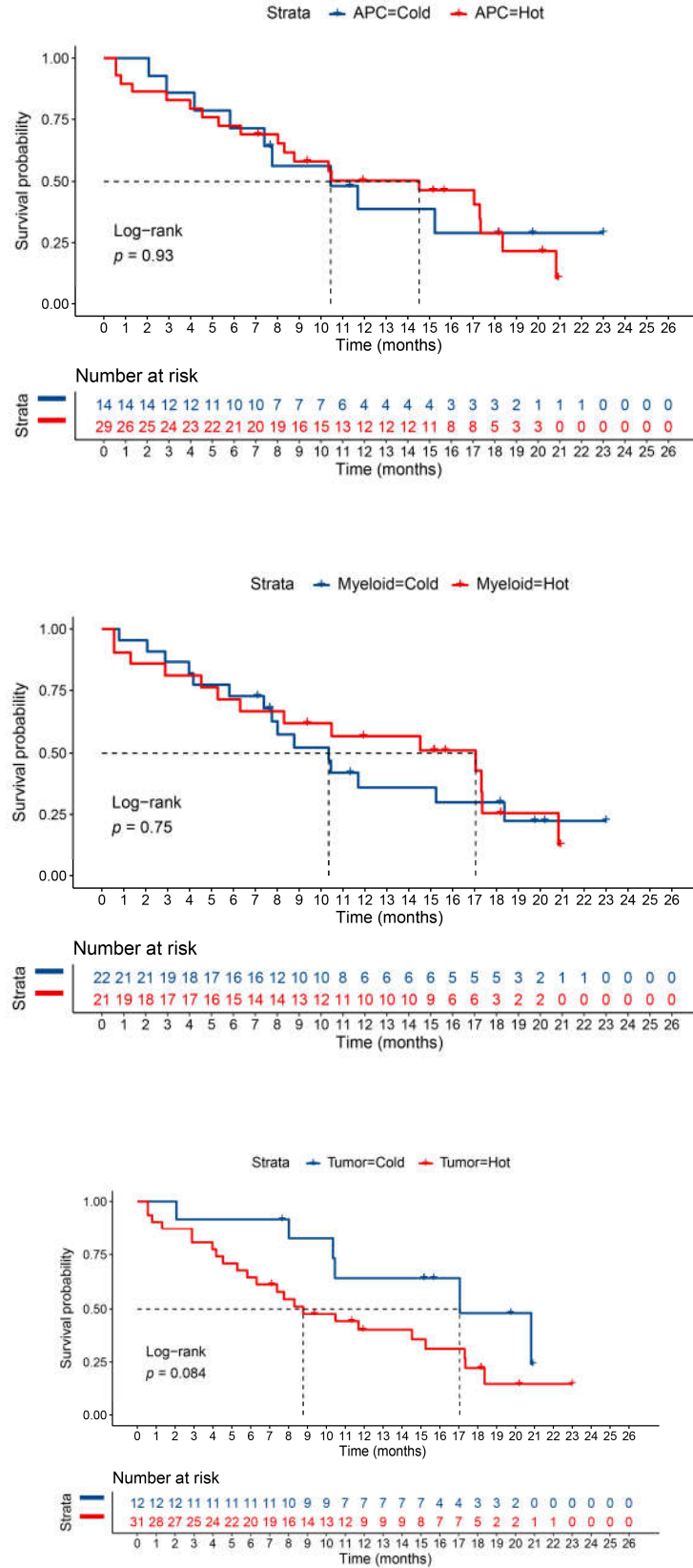


Figure S4. Overall survival (OS) analysis of APC-related markers (9 proteins), myeloid/angiogenesis (25 proteins) and tumor-related markers (9 proteins) in the plasma signature (hot vs. cold). The log rank test was used to test for differences in survival.

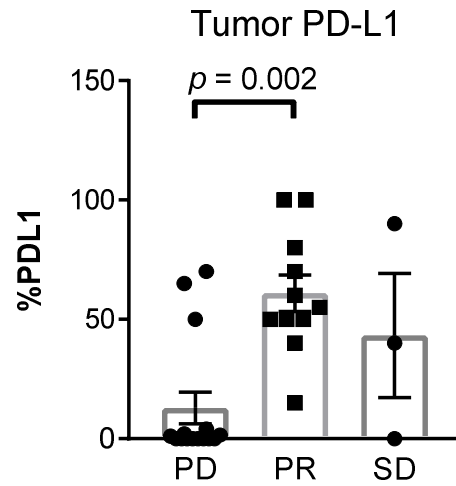


Figure S5. Comparison between patients with progressive disease (PD), spatial response (PD) and stable disease (SD) in terms of tumor expression of PD-L1, assessed by immunohistochemistry, before the initiation of anti-PD(L)1 therapy.

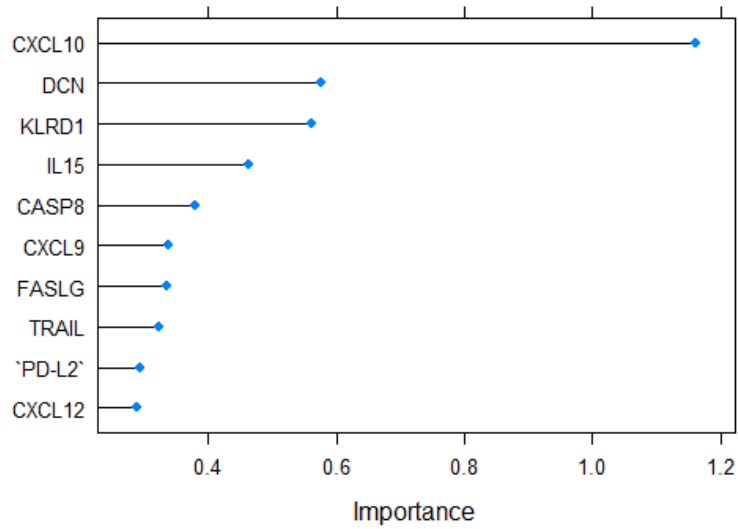


Figure S6. The top 10 proteins with the highest discrimination power in the random forest model.

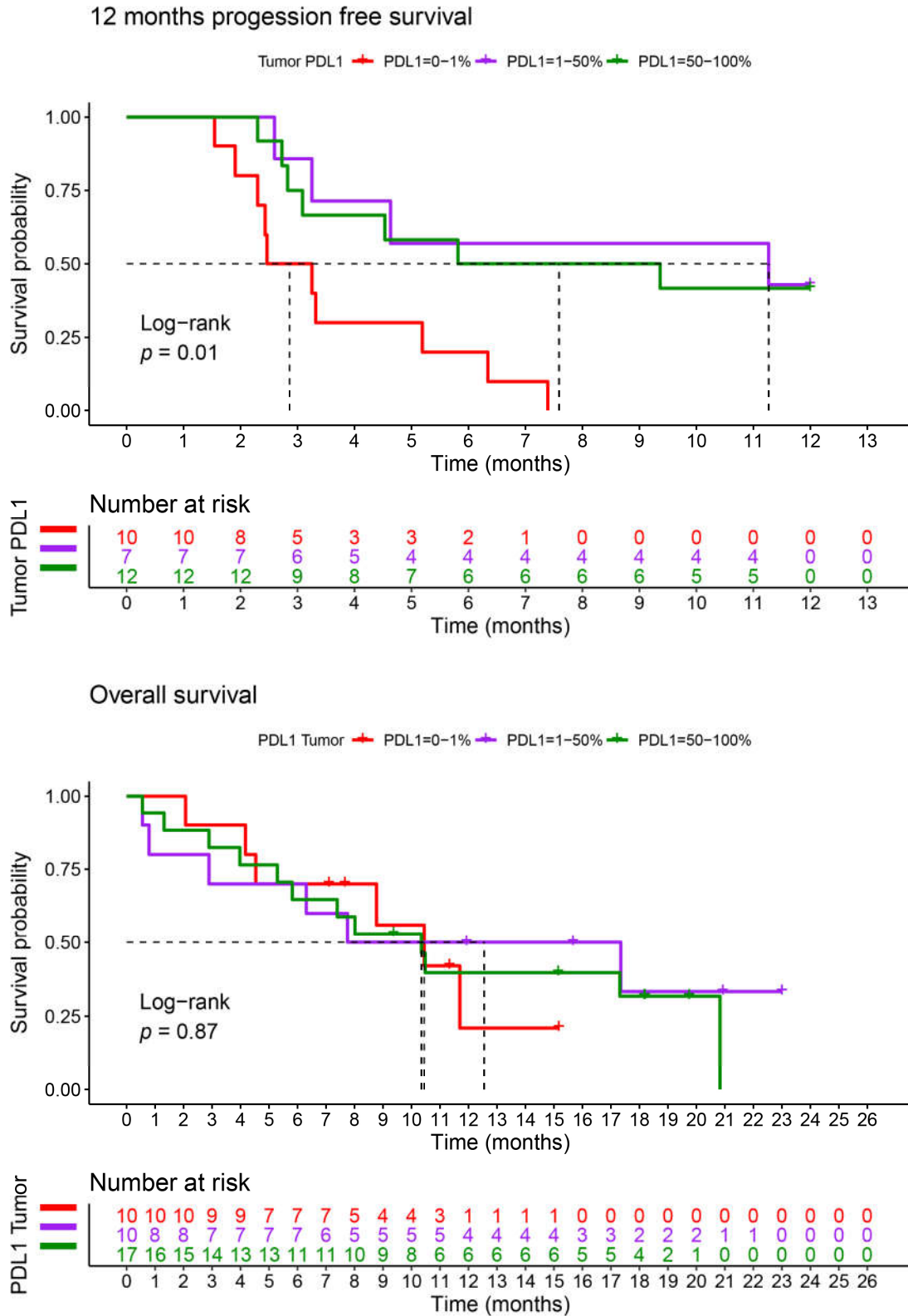


Figure S7. The association between progression-free survival (PFS) and overall survival (OS) and with the tumor PD-L1 expression. Kaplan-Meier curves of PFS and OS according to tumor PD-L1 expression groups (0-1%, 150% and 50-100%) are presented. Differences in survival between the groups were calculated by log rank test.

Table S1. Plasma proteins assessed with the multiplex proteomic analysis. The mean, median and standard deviation of the NPX value for each protein are displayed.

#	Biomarker	Mean	Median	Standard deviation
1	ADA	5.207	5.106	0.4876
2	ADGRG1	2.623	2.317	1.224
3	ANGPT1	9.029	9.198	1.003
4	ANGPT2	5.788	5.881	0.4291
5	CAIX	5.005	5.081	0.7626
6	CASP-8	5.168	5.059	0.7809
7	CCL17	9.834	9.803	1.067
8	CCL19	9.164	9.208	1.049
9	CCL20	7.606	7.498	1.018
10	CCL23	10.23	10.32	0.6841
11	CCL3	5.933	5.982	0.5311
12	CCL4	6.531	6.665	0.4534
13	CD244	6.28	6.203	0.3903
14	CD27	7.935	7.908	0.5552
15	CD4	3.509	3.482	0.3435
16	CD40	10.16	10.15	0.4434
17	CD40-L	7.08	7.281	1.463
18	CD5	5.016	5.027	0.4071
19	CD70	3.753	3.827	0.7026
20	CD83	2.738	2.774	0.469
21	CD8A	9.224	9.245	0.6812
22	CRTAM	5.611	5.432	0.7388
23	CSF-1	9.605	9.646	0.226
24	CX3CL1	6.118	6.059	0.4415
25	CXCL1	8.924	9.07	0.9046
26	CXCL10	10.31	10.24	0.9831
27	CXCL11	9.258	9.154	1.337
28	CXCL12	0.7573	0.7392	0.2913
29	CXCL13	9.058	8.972	0.8978
30	CXCL5	11.11	11.23	1.11
31	CXCL9	8.443	8.416	0.9723
32	DCN	3.953	3.959	0.3928
33	EGF	9.385	9.537	1.284
34	FASLG	5.278	5.425	0.668
35	FGF2	1.515	1.506	0.8661
36	Gal-1	6.717	6.735	0.2355
37	Gal-9	7.509	7.475	0.3624
38	GZMA	6.959	6.935	0.6409
39	GZMB	5.324	5.346	0.8977
40	GZMH	5.07	5	0.9599
41	HGF	9.177	8.951	0.8073
42	HO-1	11.19	11.24	0.4382
43	ICOSLG	4.788	4.794	0.2633
44	IFN-gamma	5.668	5.453	1.26
45	IL10	3.779	3.389	1.297
46	IL12	6.755	6.983	1.096
47	IL12RB1	1.483	1.426	0.4478
48	IL15	4.161	4.102	0.348
49	IL18	8.945	8.891	0.7163
50	IL6	4.437	4.64	1.452
51	IL7	4.557	4.632	0.9291
52	IL8	6.457	6.198	0.8853
53	KIR3DL1	1.218	1.214	1.316
54	KLRD1	6.566	6.559	0.7708
55	LAG3	2.213	2.007	0.6784

56	LAMP3	4.842	4.976	0.7136
57	LAP TGF-beta-1	9.114	9.113	0.702
58	MCP-1	10.7	10.76	0.4852
59	MCP-2	7.673	7.637	0.6686
60	MCP-3	2.341	2.298	0.7292
61	MCP-4	9.362	9.38	0.765
62	MIC-A/B	4.013	4.594	1.759
63	MMP12	8.066	7.982	1.391
64	MMP7	9.503	9.532	0.3827
65	MUC-16	5.045	4.672	1.77
66	NCR1	3.507	3.503	0.473
67	NOS3	1.647	1.582	0.6614
68	PDCD1	4.12	4.09	0.5776
69	PDGF subunit B	9.753	9.954	0.4797
70	PD-L1	5.633	5.669	0.5513
71	PD-L2	1.99	1.957	0.3543
72	PGF	8.266	8.202	0.4961
73	PTN	4.295	4.115	1.282
74	TIE2	7.042	7.099	0.289
75	TNF	2.995	2.902	0.9964
76	TNFRSF12A	4.942	4.808	0.5389
77	TNFRSF21	7.269	7.218	0.264
78	TNFRSF4	5.68	5.636	0.5821
79	TNFRSF9	6.122	6.138	0.8479
80	TNFSF14	4.79	4.587	0.883
81	TRAIL	8.254	8.241	0.3355
82	TWEAK	8.616	8.492	0.5121
83	VEGFA	8.248	8.344	0.677
84	VEGFR-2	8.016	8.027	0.2548

NPX= Normalized protein expression.

Table S2. Functional subgroups of the OLINK immuno-oncology proteins to T cell activation-, APC-, myeloid/angiogenesis- and tumor-related protein markers.

T cell activation	APC	Myeloid/angiogenesis	Tumor-related
TNFRSF9	CD40	IL8	EGF
CD27	CD70	ADA	ADGRG1
CD4	CD83	ANGPT1	ANGPT2
CD40-L	ICOSLG	ARG1	DCN
CD8A	IL12	CCL17	FGF2
CX3CL1	IL12RB1	CCL3	HGF
CXCL10	LAMP3	CCL4	MUC-16
CXCL11	PD-L1	CSF-1	PDGF subunit B
CXCL9	PD-L2	CXCL1	PTN
FASLG		CXCL12	
GZMA		CXCL5	
GZMB		Gal-1	
GZMH		Gal-9	
IFN-gamma		LAP TGF-beta-1	
IL15		MCP-1	
IL7		MCP-2	
KLRD1		MCP-3	
TNF		MCP-4	
TNFRSF21		MMP12	
TNFRSF4		MMP7	
TNFSF14		NOS3	
TRAIL		PGF	
		TIE2	

TNFRSF12A

VEGFA

VEGFR-2

APC=antigen-presenting cell.

Table S3. Plasma proteins assessed with the multiplex proteomic analysis. The median NPX values for each protein in the immune "hot" and immune "cold" clusters based on T cell activation-related markers are displayed. p-value determined by t-test with correction for multiple testing (FDR).

Rank	Protein	Hot signature median	Cold signature median	p value	q value
1	CD4	3.716	3.247	3.87×10^{-7}	1.83×10^{-6}
2	CXCL10	10.9	9.548	2.62×10^{-7}	1.83×10^{-6}
3	CXCL9	8.997	7.744	2.71×10^{-6}	8.53×10^{-6}
4	TNFRSF4	6.006	5.268	4.32×10^{-6}	1.02×10^{-5}
5	CD27	8.239	7.552	8.62×10^{-6}	1.63×10^{-5}
6	GZMA	7.271	6.542	6.66×10^{-5}	0.000105
7	CD8A	9.532	8.835	0.000415	0.00049
8	GZMH	5.505	4.521	0.000404	0.00049
9	TNFRSF9	6.481	5.667	0.001049	0.001078
10	GZMB	5.703	4.846	0.001141	0.001078
11	KLRD1	6.884	6.164	0.001508	0.001295
12	TNF	3.396	2.488	0.002008	0.001581
13	IFNg	6.154	5.053	0.003255	0.002366
14	CD40-L	6.68	7.585	0.042516	0.028698
15	TNFRSF21	7.342	7.178	0.041493	0.028698
16	CX3CL1	6.216	5.993	0.100784	0.063494
17	FASLG	5.409	5.112	0.150109	0.088658
18	TRAIL	8.31	8.184	0.229153	0.127382
19	IL7	4.437	4.71	0.343917	0.180556
20	TNFSF14	4.707	4.895	0.49519	0.246292
21	CXCL11	9.3	9.205	0.819258	0.387099
22	IL15	4.159	4.164	0.962634	0.433185

Note that NPS is a Log2 value.

Table S4. Plasma proteins assessed with the multiplex proteomic analysis. The mean and NPX values for each protein in the clinical benefit (partial response and stable disease) and progressive disease patients are displayed. p-value determined by t-test with correction for multiple testing (FDR). Note that NPS is a Log2 value.

Rank	Protein	Mean "clinical benefit"	Mean "progressive disease"	p value	q value
1	PDL1 Tumor	57.73	13.17	0.000262	0.023022
2	IL15	4.309	3.999	0.006163	0.182929
3	CXCL10	10.84	9.873	0.006236	0.182929
4	CASP-8	4.758	5.4	0.016016	0.303636
5	ADA	4.958	5.365	0.018657	0.303636
6	CXCL9	8.828	8.127	0.049878	0.521184
7	PGF	8.431	8.095	0.051795	0.521184
8	MCP-3	2.493	2.044	0.058539	0.521184
9	Gal-9	7.617	7.391	0.065608	0.521184
10	IFN-gamma	6.166	5.469	0.068418	0.521184
11	DCN	4.149	3.845	0.06970	0.303636
12	TNFRSF4	5.87	5.503	0.07107	0.521184
13	MUC-16	4.308	5.356	0.087766	0.541064
14	TNFRSF12A	5.099	4.77	0.091075	0.541064
15	CD70	3.984	3.573	0.105971	0.541064
16	HO-1	11.11	11.35	0.10808	0.541064
17	HGF	9.371	8.941	0.11093	0.541064
18	CXCL12	0.6879	0.8437	0.116672	0.541064
19	CX3CL1	6.278	6.033	0.116821	0.541064
20	PTN	4.761	4.037	0.123117	0.541715
21	PD-L2	2.098	1.913	0.150863	0.573343
22	ADGRG1	2.887	2.276	0.15439	0.573343
23	TNF	3.25	2.711	0.155873	0.573343

24	TNFRSF21	7.307	7.189	0.166476	0.573343
25	MCP-1	10.86	10.62	0.170468	0.573343
26	CXCL13	9.291	8.838	0.172898	0.573343
27	CD4	3.581	3.433	0.176223	0.573343
28	CCL3	6.032	5.78	0.182427	0.573343
29	CD244	6.144	6.311	0.20072	0.602436
30	VEGFR-2	7.967	8.088	0.205376	0.602436
31	IL12RB1	1.515	1.348	0.239934	0.665457
32	LAG3	2.326	2.038	0.241984	0.665457
33	ANGPT2	5.868	5.714	0.271646	0.689068
34	CD27	8.005	7.79	0.278714	0.689068
35	TRAIL	8.356	8.241	0.279761	0.689068
36	CD40-L	6.74	7.3	0.281891	0.689068
37	NCR1	3.591	3.424	0.310299	0.719422
38	NOS3	1.654	1.439	0.318955	0.719422
39	LAMP3	4.963	4.71	0.324723	0.719422
40	MCP-2	7.513	7.736	0.32701	0.719422
41	IL18	8.726	8.956	0.344386	0.724964
42	TNFSF14	4.589	4.865	0.346006	0.724964
43	CD8A	9.294	9.076	0.367658	0.737002
44	IL10	3.853	3.475	0.368501	0.737002
45	MMP7	9.61	9.496	0.393688	0.76988
46	CSF-1	9.644	9.583	0.408107	0.780727
47	IL12	6.817	6.547	0.437593	0.819323
48	CCL20	7.691	7.42	0.471473	0.839447
49	LAP TGF-beta-1	9.013	9.184	0.473007	0.839447
50	CD83	2.751	2.649	0.483856	0.839447
51	TNFRSF9	6.242	6.027	0.494951	0.839447
52	CD40	10.08	10.19	0.502586	0.839447
53	ICOSLG	4.839	4.775	0.505576	0.839447
54	GZMB	5.475	5.301	0.559537	0.890777
55	FGF2	1.364	1.521	0.607358	0.890777
56	PD-L1	5.522	5.626	0.607603	0.890777
57	CCL17	9.999	9.822	0.624336	0.890777
58	CD5	4.962	5.031	0.62522	0.890777
59	IL6	4.092	4.315	0.632362	0.890777
60	GZMA	6.869	6.974	0.637715	0.890777
61	MCP-4	9.481	9.362	0.655205	0.899878
62	CAIX	5.062	4.951	0.664682	0.899878
63	IL7	4.422	4.554	0.679067	0.905423
64	MIC-A/B	3.912	3.657	0.702912	0.909989
65	KIR3DL1	1.461	1.275	0.710565	0.909989
66	FASLG	5.316	5.388	0.713514	0.909989
67	PDGF subunit B	9.733	9.781	0.775351	0.968643
68	EGF	9.329	9.457	0.781519	0.968643
69	CCL23	10.21	10.16	0.807238	0.97982
70	IL8	6.432	6.356	0.812806	0.97982
71	TIE2	7.018	7.039	0.839692	0.982517
72	VEGFA	8.173	8.214	0.855355	0.982517
73	CCL4	6.488	6.515	0.858651	0.982517
74	MMP12	8.089	8.003	0.867502	0.982517
75	CXCL11	9.279	9.204	0.879076	0.982517
76	ANGPT1	9.042	9.086	0.89431	0.982517
77	CXCL5	11.17	11.22	0.903486	0.982517
78	CXCL1	8.936	8.899	0.907709	0.982517
79	PDCD1	4.106	4.084	0.918678	0.982517
80	CCL19	9.224	9.191	0.927029	0.982517
81	Gal-1	6.741	6.735	0.94756	0.982517

82	KLRD1	6.493	6.476	0.952431	0.982517
83	TWEAK	8.678	8.684	0.969791	0.982517
84	GZMH	5.003	4.991	0.971352	0.982517
85	CRTAM	5.54	5.545	0.987367	0.987367
