Supplemental material

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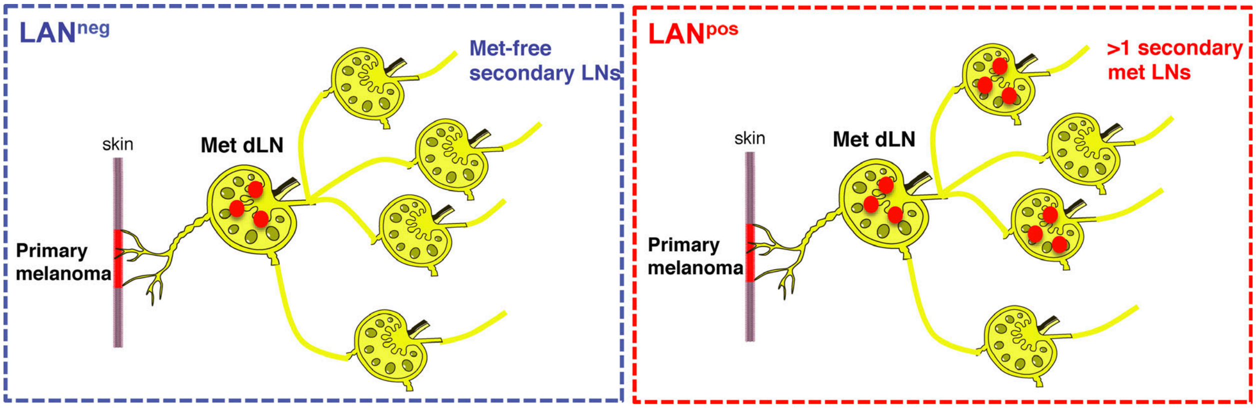


Figure S1. **Schematic explaining the collection of lymphatic exudate samples in LAN^{neg} and LAN^{pos} patients.** Met, metastasis.

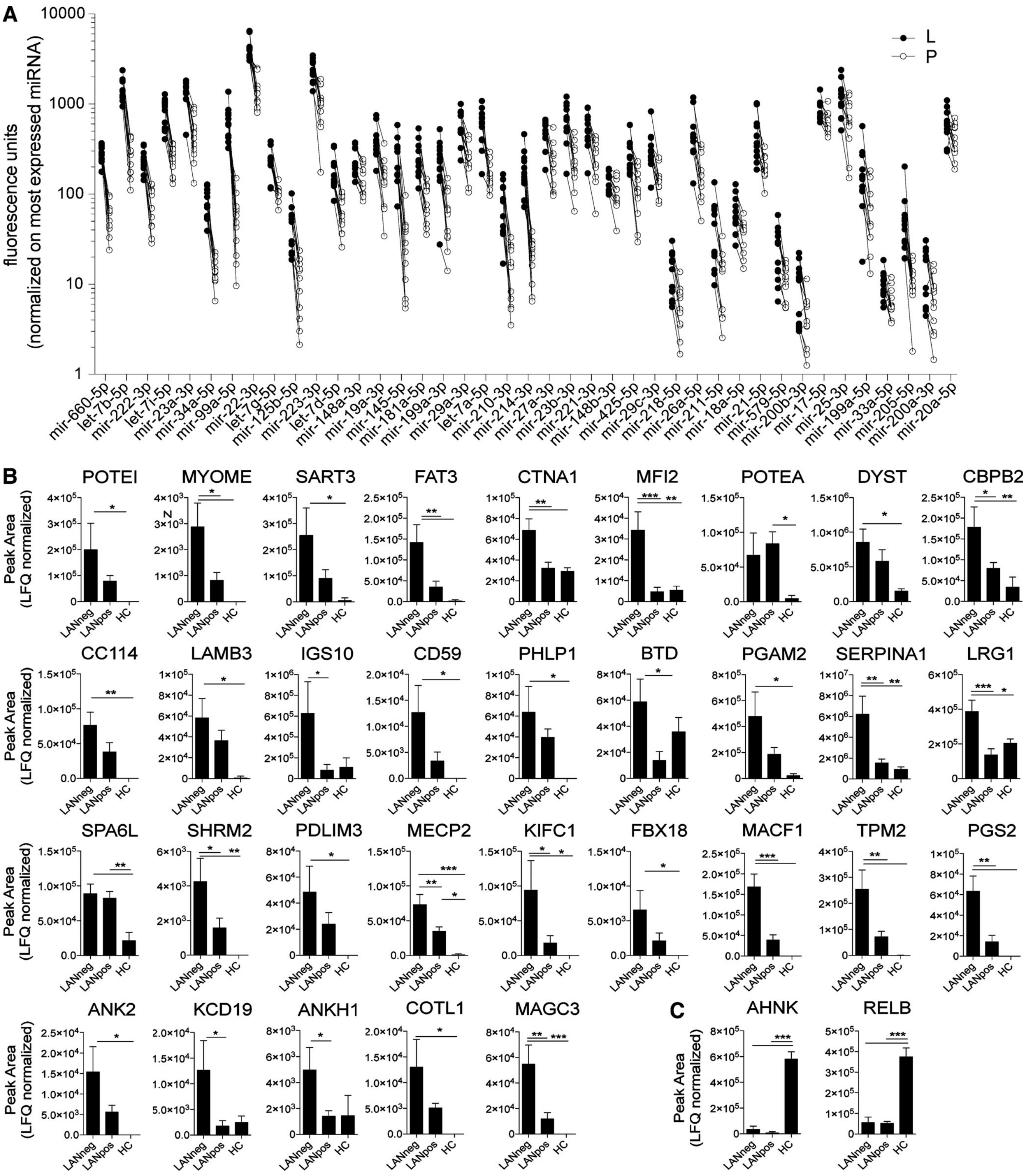
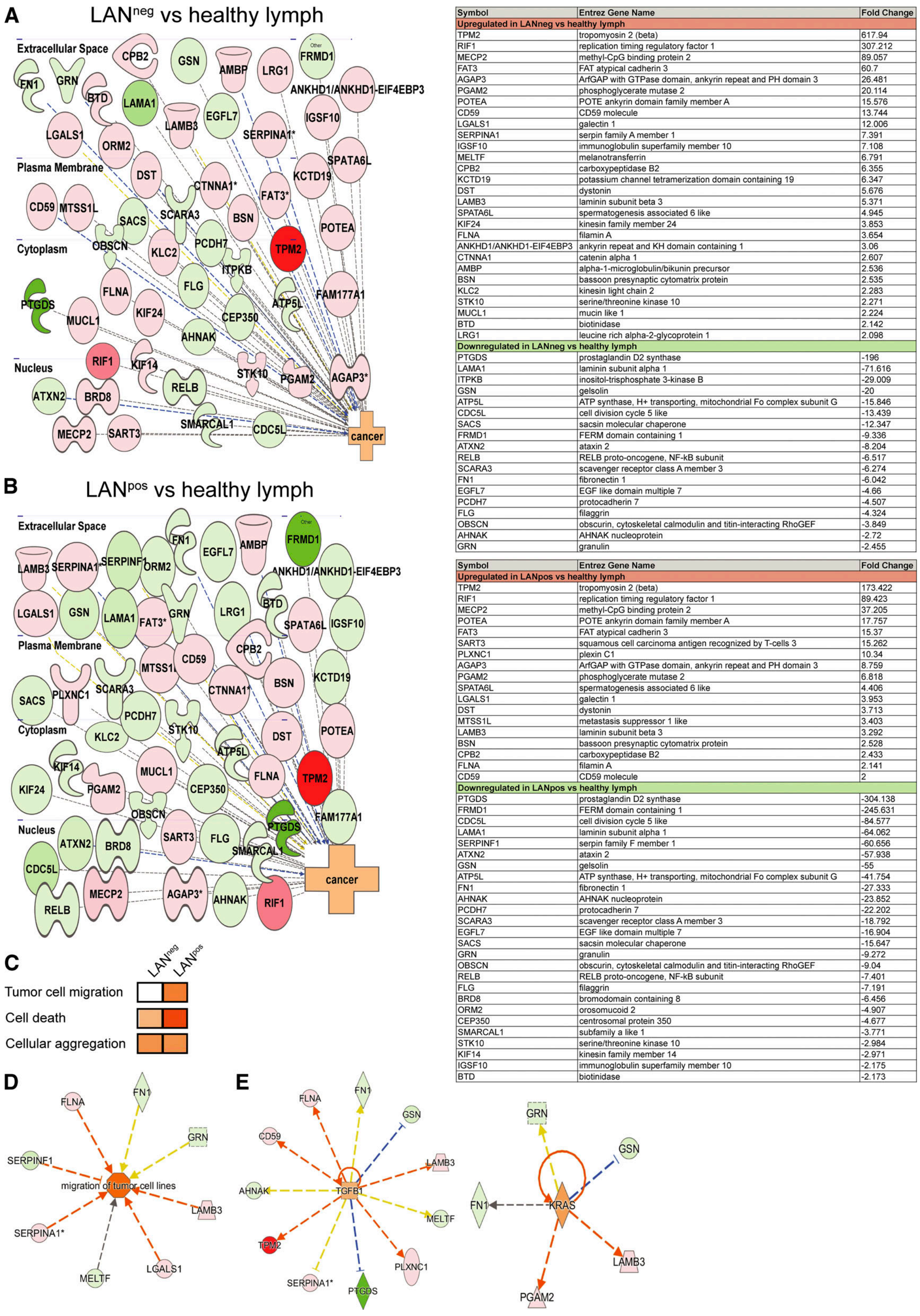


Figure S2. **Lymphatic exudate is enriched in miRNA and proteins associated with metastatic melanoma. (A)** Paired tumor-associated miRNA expression of lymphatic exudate (L) and plasma (P) of pooled LAN^{neg} (*n* = 5) and LAN^{pos} (*n* = 7) melanoma patients (P values are listed in Table S1). **(B and C)** Expression of melanoma-associated proteins reported in publicly available Human Protein Atlas to be (B) up- or (C) down-regulated in melanoma tumors and identified by LFQ analysis to be significantly expressed in the lymphatic exudate of LAN^{neg} and LAN^{pos} patients as compared with HCs (*n* = 6 for LAN^{neg}, *n* = 12 for LAN^{pos}, and *n* = 5 for HC. Data shown as mean ± SEM. *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ using one-way ANOVA with Tukey's multiple comparison test.



Symbol	Entrez Gene Name	Fold Change
Upregulated in LANneg vs healthy lymph		
TPM2	tropomyosin 2 (beta)	617.94
RIF1	replication timing regulatory factor 1	307.212
MECP2	methyl-CpG binding protein 2	89.057
FAT3	FAT atypical cadherin 3	60.7
AGAP3	ArfGAP with GTPase domain, ankyrin repeat and PH domain 3	26.481
PGAM2	phosphoglycerate mutase 2	20.114
POTEA	POTE ankyrin domain family member A	15.576
CD59	CD59 molecule	13.744
LGALS1	galectin 1	12.006
SERPINA1	serpin family A member 1	7.391
IGSF10	immunoglobulin superfamily member 10	7.108
MELTF	melanotransferrin	6.791
CPB2	carboxypeptidase B2	6.355
KCTD19	potassium channel tetramerization domain containing 19	6.347
DST	dystonin	5.676
LAMB3	laminin subunit beta 3	5.371
SPATA6L	spermatogenesis associated 6 like	4.945
KIF24	kinesin family member 24	3.853
FLNA	filamin A	3.654
ANKHD1/ANKHD1-EIF4EBP3	ankyrin repeat and KH domain containing 1	3.06
CTNNA1	catenin alpha 1	2.607
AMBP	alpha-1-microglobulin/bikunin precursor	2.536
BSN	bassoon presynaptic cytomatrix protein	2.535
KLC2	kinesin light chain 2	2.283
STK10	serine/threonine kinase 10	2.271
MUCL1	mucin like 1	2.224
BTD	biotinidase	2.142
LRG1	leucine rich alpha-2-glycoprotein 1	2.098
Downregulated in LANneg vs healthy lymph		
PTGDS	prostaglandin D2 synthase	-196
LAMA1	laminin subunit alpha 1	-71.616
ITPKB	inositol-trisphosphate 3-kinase B	-29.009
GSN	gelsolin	-20
ATP5L	ATP synthase, H+ transporting, mitochondrial Fo complex subunit G	-15.846
CDC5L	cell division cycle 5 like	-13.439
SACS	sacsin molecular chaperone	-12.347
FRMD1	FERM domain containing 1	-9.336
ATXN2	ataxin 2	-8.204
RELB	RELB proto-oncogene, NF-kB subunit	-6.517
SCARA3	scavenger receptor class A member 3	-6.274
FN1	fibronectin 1	-6.042
EGFL7	EGF like domain multiple 7	-4.66
PCDH7	protocadherin 7	-4.507
FLG	filaggrin	-4.324
OBSCN	obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF	-3.849
AHNAK	AHNAK nucleoprotein	-2.72
GRN	granulin	-2.455

Symbol	Entrez Gene Name	Fold Change
Upregulated in LANpos vs healthy lymph		
TPM2	tropomyosin 2 (beta)	173.422
RIF1	replication timing regulatory factor 1	89.423
MECP2	methyl-CpG binding protein 2	37.205
POTEA	POTE ankyrin domain family member A	17.757
FAT3	FAT atypical cadherin 3	15.37
SART3	squamous cell carcinoma antigen recognized by T-cells 3	15.262
PLXNC1	plexin C1	10.34
AGAP3	ArfGAP with GTPase domain, ankyrin repeat and PH domain 3	8.759
PGAM2	phosphoglycerate mutase 2	6.818
SPATA6L	spermatogenesis associated 6 like	4.406
LGALS1	galectin 1	3.953
DST	dystonin	3.713
MTSS1L	metastasis suppressor 1 like	3.403
LAMB3	laminin subunit beta 3	3.292
BSN	bassoon presynaptic cytomatrix protein	2.528
CPB2	carboxypeptidase B2	2.433
FLNA	filamin A	2.141
CD59	CD59 molecule	2
Downregulated in LANpos vs healthy lymph		
PTGDS	prostaglandin D2 synthase	-304.138
FRMD1	FERM domain containing 1	-245.631
CDC5L	cell division cycle 5 like	-84.577
LAMA1	laminin subunit alpha 1	-64.062
SERPINF1	serpin family F member 1	-60.656
ATXN2	ataxin 2	-57.938
GSN	gelsolin	-55
ATP5L	ATP synthase, H+ transporting, mitochondrial Fo complex subunit G	-41.754
FN1	fibronectin 1	-27.333
AHNAK	AHNAK nucleoprotein	-23.852
PCDH7	protocadherin 7	-22.202
SCARA3	scavenger receptor class A member 3	-18.792
EGFL7	EGF like domain multiple 7	-16.904
SACS	sacsin molecular chaperone	-15.647
GRN	granulin	-9.272
OBSCN	obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF	-9.04
RELB	RELB proto-oncogene, NF-kB subunit	-7.401
FLG	filaggrin	-7.191
BRD8	bromodomain containing 8	-6.456
ORM2	orosomucoid 2	-4.907
CEP350	centrosomal protein 350	-4.677
SMARCAL1	subfamily a like 1	-3.771
STK10	serine/threonine kinase 10	-2.984
KIF14	kinesin family member 14	-2.971
IGSF10	immunoglobulin superfamily member 10	-2.175
BTD	biotinidase	-2.173

Figure S3. **Melanoma-associated proteins define LAN^{neg} and LAN^{pos} patients. (A and B)** IPA predicted of cellular and molecular networks and listed melanoma associated proteins that are mostly up- or down-regulated in lymphatic exudate of LAN^{neg} (A) or LAN^{pos} versus HCs (B). **(C)** IPA predicted activation z-score comparison of cellular functions associated with melanoma in the proteome of lymphatic exudate from LAN^{neg} versus LAN^{pos} patients. **(D and E)** Main proteins predicting tumor cell migration (D) and main predicted upstream master gene regulators (E) for the top expressed proteins in the lymphatic exudate of LAN^{pos} patients (orange line: leads to activation; blue line: leads to inhibition; yellow line: finding inconsistent; gray line: effect not predicted; red proteins: up-regulated; green proteins: down-regulated; different shades indicate the extent of the up- or down-regulation; *n* = 6 LAN^{neg}, *n* = 12 LAN^{pos}, HC *n* = 5 for both lymphatic exudate and plasma). Raw data in Table S1.

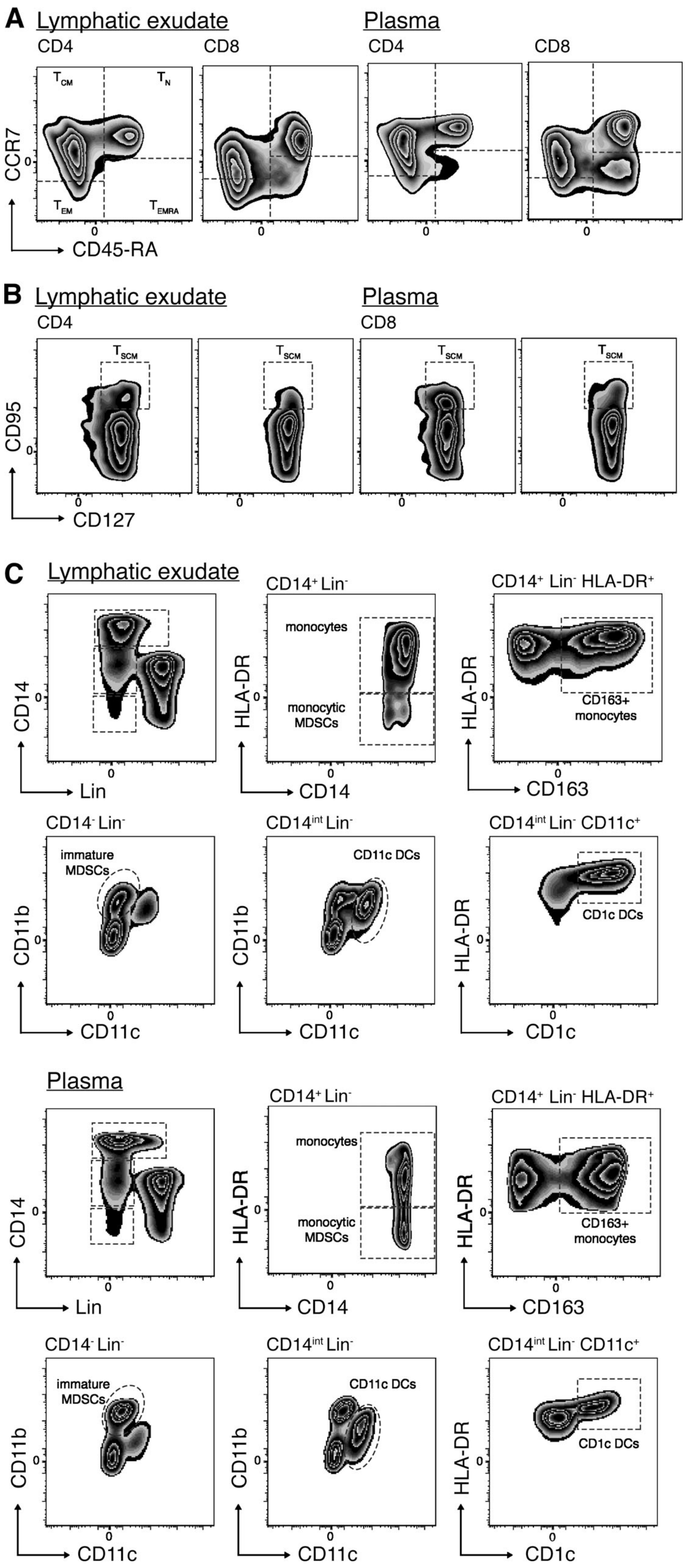


Figure S4. **Gating strategy for flow cytometric analysis of immune cells in lymphatic exudate and plasma. (A)** $CD4^+$ and $CD8^+$ T cell subsets T_{CM}, T_N, T_{EM}, and T_{EMRA}, after gating for $CD3^+$ cells. **(B)** From the naive subsets shown in A, T_{SCM} were defined as $CD95^{hi}$ and $CD127^{hi}$. **(C)** Monocyte subsets after live/dead and doublets exclusion. Lin, lineage.

Table S1 is provided online as a separate Excel file, and lists clinical data of analyzed patients and the raw proteomic, miRNA, and IPA data.