

Figure S1 Gating strategy for flow cytometry assay. Representative flow cytometry plots from pleural effusion showing the gating scheme for isolating (8) Tex^{stem} , (6) Tex^{term} , (9) $\text{Tex}^{\text{stem}} \text{TCF1}^+$, (7) $\text{Tex}^{\text{term}} \text{TCF1}^+$ and (5) $\text{PD1}^+ \text{CD8}$ T cells. PD1, programmed death 1; Tex^{stem} , stem-like exhausted CD8 T cells; Tex^{term} , terminally exhausted CD8 T cells; TCF1, T cell factor 1.

Table S1 Reagents and resources

Reagent or resource	Source	Identifier
Antibodies		
Flow cytometry		
7-AAD	BD Biosciences	559925
PE streptavidin	biolegend	Cat#405204
eFluor 780 Fixable viability dye	eBiosciences	Cat#65-0865-14
APC/Cyanine7 anti-human CD14 Antibody (clone: M5E2)	biolegend	301820
APC/Cyanine7 anti-human CD19 Antibody (clone: HIB19)	biolegend	302218
BUV395 CD3 Mouse anti-Human (clone: UCHT1)	BD Biosciences	563546
BV510 CD3 Mouse anti-Human (clone: UCHT1)	biolegend	300448
BUV805 CD4 Mouse anti-Human (clone: RPA-T4)	BD Biosciences	742000
BB515 CD4 Mouse anti-Human (clone: RPA-T4)	BD Biosciences	564419
BV711 CD4 Mouse anti-Human (clone: SK3)	BD Biosciences	563028
BV650 CD8 Mouse anti-Human (clone: RPA-T8)	BD Biosciences	563821
PeCy7 PD1 Mouse anti-Human (clone: EH12.1)	BD Biosciences	561272
PE CD39 Mouse anti-Human (clone: TU66)	BD Biosciences	555464
APC CD28 Mouse anti-Human (clone: CD28.2)	biolegend	302912
AF488 TCF1 Rabbit anti-Human (clone: C63D9)	Cell Signalling	6444S
CytoF		
89 - CD45 (clone: HI30)	Fluidigm	Cat# 3089003, RRID:AB_2661851
112 - CD14 (clone: TuK4)	ThermoFisher	Cat# MHCD1400, RRID:AB_10371749
115 - CD57 (clone: HNK-1)	Biolegend	359602
141 - CD56 (clone: REA196)	Miltenyi Biotec	130-108-016
142 - HLA-DR (clone: L243)	Biolegend	Cat# 307602, RRID:AB_314680
143 - CD103 (clone: B-Ly7)	ThermoFisher	Cat# 14-1038-80, RRID:AB_467411
144 - TIGIT (clone: MBSA-43)	ThermoFisher	16-9500-82
145 - CD69 (FN50)	Biolegend	310902
146 - CD8a (clone: RPA-T8)	Biolegend	Cat# 301002, RRID:AB_314120
147 - CD4 (clone: RPA-T4)	Biolegend	Cat# 300502, RRID:AB_314070
148 - CD45RO (clone: UCHL1)	Biolegend	Cat# 304202, RRID:AB_314418
149 - 4-1BB (clone: 4B4-1)	Biolegend	309802
150 - KLRG1 (clone: 13F2F12)	ThermoFisher	Cat# 16-9488-85, RRID:AB_2637116
151 - CD27 (clone: O323)	Biolegend	302802
152 - ICOS (clone: C398.4A)	ThermoFisher	14-9949-82
155 - Va7.2 (clone: 3C10)	Biolegend	351702
156 - CD3 (clone: UCHT1)	Biolegend	Cat# 300402, RRID:AB_314056
160 - PD-1 (clone: eBioJ105)	ThermoFisher	Cat# 14-2799-80, RRID:AB_763476
161 - CD38 (clone: HIT2)	Biolegend	Cat# 303502, RRID:AB_314354
162 - CD161 (clone: HP-3G10)	Biolegend	Cat# 339902, RRID:AB_1501090
166 - CD127 (clone: AO19D5)	Biolegend	351302
168 - CCR7 (clone: 150503)	R&D	Cat# MAB197, RRID:AB_2072803
169 - CD25 (clone: M-A251)	Biolegend	356102
170 - CD71 (clone: CY1G4)	Biolegend	334102
171 - anti-PE (clone: PE001)	Biolegend	408102
TCR gamma/delta Monoclonal Antibody (clone: 5A6.E9)	ThermoFisher	MHGD04
173 - CD28 (clone: CD28.2)	Biolegend	302937
176 - CD39 (clone: A1)	Biolegend	328202
209 - CD16 (clone: 3G8)	Fluidigm	Cat# 3209002B, RRID:AB_2756431
10x CITEseq		
TotalSeq-C0034 anti-human CD3 (clone: UCHT1)	Biolegend	300479
TotalSeq-C0053 anti-human CD11c (clone: S-HCL-3)	Biolegend	371521
TotalSeq-C0063 anti-human CD45RA (clone: HI100)	Biolegend	304163
TotalSeq-C0072 anti-human CD4 (clone: RPA-T4)	Biolegend	300567
TotalSeq-C0080 anti-human CD8 (clone: RPA-T8)	Biolegend	301071
TotalSeq-C0081 anti-human CD14 (clone: M5E2)	Biolegend	301859
TotalSeq-C0083 anti-human CD16 (clone: 3G8)	Biolegend	302065
TotalSeq-C0084 anti-human CD56 (clone: QA17A16)	Biolegend	392425
TotalSeq-C0085 anti-human CD25 (clone: BC96)	Biolegend	302649
TotalSeq-C0087 anti-human CD45RO (clone: UCHL1)	Biolegend	304259
TotalSeq-C0088 anti-human CD279 (PD-1) (clone: EH12.2H7)	Biolegend	329963
TotalSeq-C0089 anti-human TIGIT (VSTM3) (clone: A15153G)	Biolegend	372729
TotalSeq-C0100 anti-human CD20 (clone: 2H7)	Biolegend	302363
TotalSeq-C0139 anti-human TCR γ/δ (clone:B1)	Biolegend	331231
TotalSeq-C0140 anti-human CD183 (CXCR3) (clone: G025H7)	Biolegend	353747
TotalSeq-C0141 anti-human CD195 (CCR5) (clone: J418F1)	Biolegend	359137
TotalSeq-C0143 anti-human CD196 (CCR6) (clone: G034E3)	Biolegend	353440
TotalSeq-C0144 anti-human CD185 (CXCR5) (clone: J252D4)	Biolegend	356939
TotalSeq-C0145 anti-human CD103 (Integrin α E) (clone: Ber-ACT8)	Biolegend	350233
TotalSeq-C0146 anti-human CD69 (clone: FN50)	Biolegend	310951
TotalSeq-C0148 anti-human CD197 (CCR7) (clone: G043H7)	Biolegend	353251
TotalSeq-C0149 anti-human CD161 (clone: HP-3G10)	Biolegend	339947
TotalSeq-C0151 anti-human CD152 (CTLA-4) (clone: BNI3)	Biolegend	369621
TotalSeq-C0152 anti-human CD223 (LAG-3) (clone: 11C365)	Biolegend	369335
TotalSeq-C0154 anti-human CD27 (clone: O323)	Biolegend	302853
TotalSeq-C0156 anti-human CD95 (Fas) (clone: DX2)	Biolegend	305651
TotalSeq-C0158 anti-human CD134 (OX40) (clone: Ber-ACT35)	Biolegend	350035
TotalSeq-C0159 anti-human HLA-DR (clone: L243)	Biolegend	307663
TotalSeq-C0168 anti-human CD57 (clone: QA17A04)	Biolegend	393321
TotalSeq-C0169 anti-human CD366 (Tim-3) (clone: F38-2E2)	Biolegend	345049
TotalSeq-C0170 anti-human CD272 (BTLA) (clone: MIH26)	Biolegend	344527
TotalSeq-C0176 anti-human CD39 (clone: A1)	Biolegend	328237
TotalSeq-C0179 anti-human CX3CR1 (clone: K0124E1)	Biolegend	355705
TotalSeq-C0180 anti-human CD24 (clone: ML5)	Biolegend	311143
TotalSeq-C0189 anti-human CD244 (2B4) (clone: C1.7)	Biolegend	329529
TotalSeq-C0250 anti-mouse/human KLRG1 (MAFA) (clone: 2F1/KLRG1)	Biolegend	138433
TotalSeq-C0355 anti-human CD137 (4-1BB) (clone: 4B4-1)	Biolegend	309839
TotalSeq-C0366 anti-human CD184 (CXCR4) (clone: 12G5)	Biolegend	306533
TotalSeq-C0386 anti-human CD28 (clone: CD28.2)	Biolegend	302963
TotalSeq-C0389 anti-human CD38 (clone: HIT2)	Biolegend	303543
TotalSeq-C0390 anti-human CD127 (IL-7R α) (clone: A019D5)	Biolegend	351356
TotalSeq-C0394 anti-human CD71 (clone: CY1G4)	Biolegend	334125
TotalSeq-C0396 anti-human CD26 (clone: BA5b)	Biolegend	302722
TotalSeq-C0397 anti-human CD193 (CCR3) (clone: 5E80)	Biolegend	310733
TotalSeq-C0577 anti-human CD73 (Ecto-5'-nucleotidase) (clone: AD2)	Biolegend	344031
TotalSeq-C0581 anti-human TCR V α 7.2 (clone: 3C10)	Biolegend	351735
TotalSeq-C0815 anti-human CCR10 (clone: 6588-5)	Biolegend	341507
TotalSeq-C0830 anti-human CD319 (CRACC) (clone: 162.1)	Biolegend	331823
TotalSeq-C0844 anti-human CD45RB (clone: MEM-55)	Biolegend	310211
Critical commercial assays		
Chromium Next GEM Single Cell 5' Kit v2	10x Genomics	PN-1000263
Library Construction Kit	10x Genomics	PN-1000190
5' Feature Barcode Kit	10x Genomics	PN-1000256
Chromium Single Cell Human TCR Amplification Kit	10x Genomics	PN-1000252
Chromium Next GEM Chip K Single Cell Kit,	10x Genomics	PN-1000286
Dual Index Kit TT Set A	10x Genomics	PN-1000215
Dual Index Kit TN Set A	10x Genomics	PN-1000250
Software and algorithms		
FlowJo	BD Biosciences	V9.9.4, V10.6.0 and V10.8.0
R	R Foundation for Statistical Computing	V4.2.1
GraphPad Prism	GraphPad	V9
SPSS	IBM	V28.0

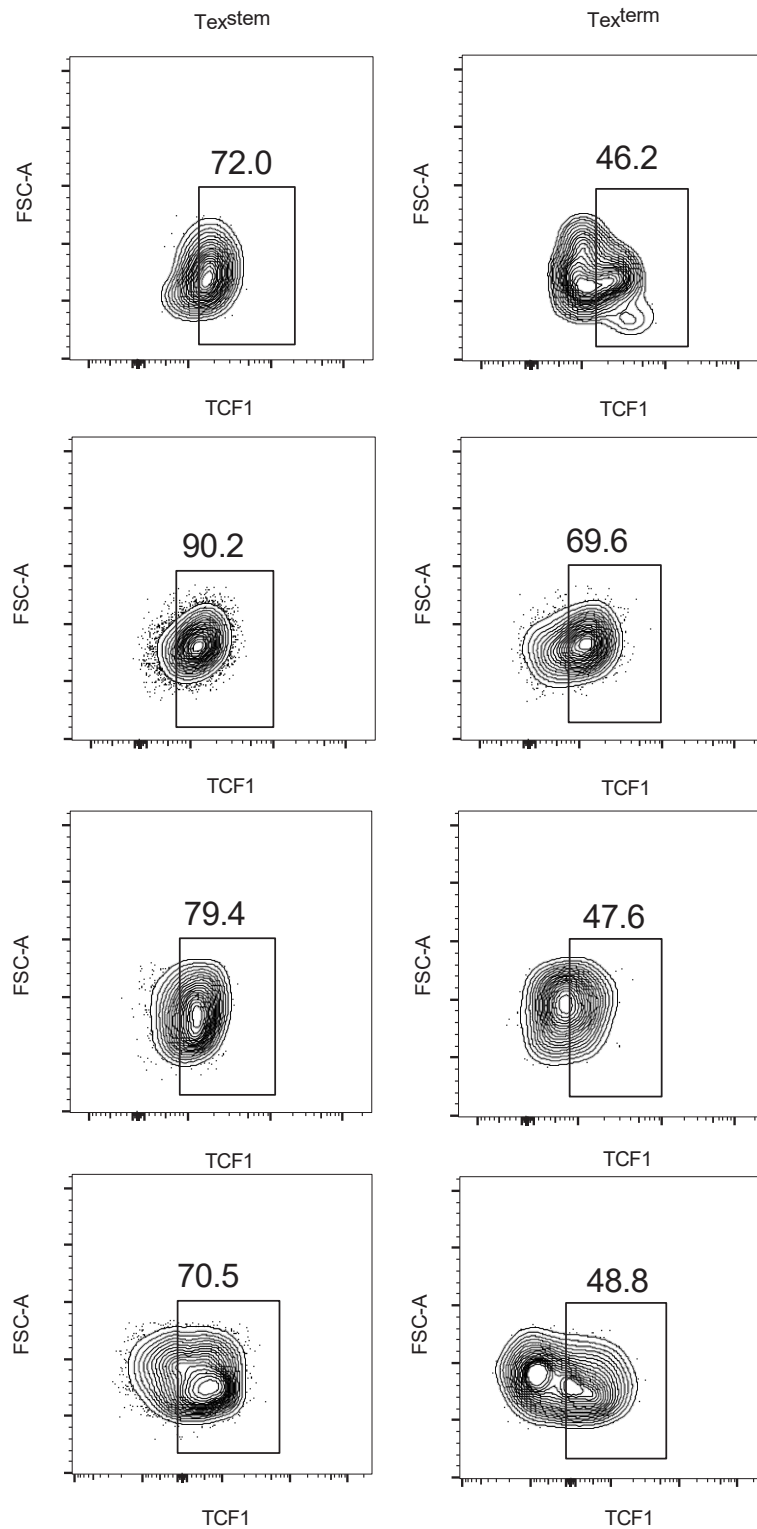


Figure S2 Flow cytometry analysis of TCF1 expression of Tex^{stem} and Tex^{term} in 3 mesothelioma and 1 NSCLC patients. TCF1, T cell factor 1; Tex^{stem}, stem-like exhausted CD8 T cells; Tex^{term}, terminally exhausted CD8 T cells; NSCLC, non-small cell lung cancer.

Table S2 Cox regression model showing hazard ratios for overall survival conferred by variables in NSCLC non-squamous carcinoma patients

Variable	Category	Univariate			Multivariate ^a		
		HR	95% CI	P	HR	95% CI	P
Age	Per unit increase	1.00	0.97–1.03	0.972	0.93	0.89–0.98	0.008
Sex	M vs. F	1.12	0.59–2.10	0.729	2.09	0.85–5.14	0.107
EGFR	Mutant vs. WT	0.49	0.20–1.20	0.120	1.01	0.19–5.42	0.990
KRAS	Mutant vs. WT	1.33	0.65–2.73	0.612	1.40	0.40–4.93	0.601
Smoking	Former/current vs. never	2.32	0.95–5.67	0.064	3.39	0.15–0.82	0.083
ECOG	2–4 vs. 0–1	2.38	1.2–4.71	0.012	3.80	1.03–13.99	0.045
Charlson Comorbidities Index	10–13 vs. 6–9	0.94	0.50–1.76	0.842	0.68	0.23–2.01	0.488
Lines of therapy	0						
	1	0.50	0.22–1.11	0.087	0.61	0.17–2.16	0.442
	≥2	0.16	0.06–0.43	<0.001	0.17	0.03–0.92	0.039
ICPB treatment	Yes vs. No	0.42	0.19–0.93	0.034	0.38	0.08–1.72	0.209
Targeted therapy	Yes vs. No	0.26	0.08–0.89	0.032	0.94	0.08–11.66	0.964
Tex ^{stem}	High vs. Low	0.39	0.19–0.81	0.011	0.35	0.15–0.82	0.016
Tex ^{term}	High vs. Low	1.86	0.96–3.63	0.068	1.18	0.49–2.80	0.714
Tex ^{stem} :Tex ^{term} ratio	Per unit increase	0.97	0.95–0.99	0.006	0.96	0.93–0.99	0.005
PD1 ⁺	High vs. Low	0.63	0.31–1.24	0.180	0.70	0.29–1.69	0.425

^a, multivariate cox regression analysis adjusted for age, sex, smoking, ECOG, Charlson Comorbidities Index, number of lines of systemic therapy, molecular status, ICPB treatment and targeted therapy. NSCLC, non-small cell lung cancer; HR, hazard ratio; CI, confidence interval; M, male; F, female; EGFR, epidermal growth factor receptor; WT, wild type; KRAS, Kirsten rat sarcoma virus; ECOG, Eastern Cooperative Oncology Group; ICPB, immune checkpoint blockade; Tex^{stem}, stem-like exhausted CD8 T cells; Tex^{term}, terminally exhausted CD8 T cells; PD1, programmed death 1.

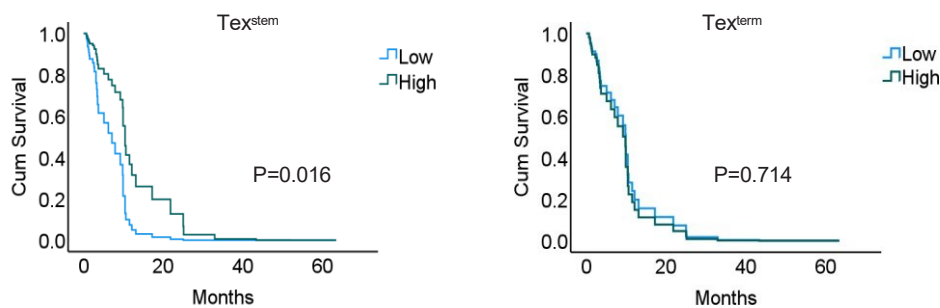


Figure S3 Tex^{stem}, but not Tex^{term}, cells correlate with improved survival in non-squamous carcinoma patients (n=41). Multivariate Cox regression analysis of overall survival plotted according to abundance of pleural effusion exhausted CD8 T-cell subsets dichotomized at the median. Tex^{stem}, stem-like exhausted CD8 T cells; Tex^{term}, terminally exhausted CD8 T cells.

Table S3 Clinical characteristics of patients included in the longitudinal, CyTOF and 10x analyses

ID	Age (years)	Sex	Histology	Smoking	OS (mths)	Analysis
Mesothelioma						
M1	65	M	Epithelioid	Ex	38	Longitudinal
M2	67	M	Epithelioid	Never	40	Longitudinal
M3	69	M	Epithelioid	Ex	43	Longitudinal, CyTOF, 10x
M4	89	M	Epithelioid	Ex	18	Longitudinal
M5	65	M	Epithelioid	Never	N/A ^a	Longitudinal
M6	70	M	Epithelioid	Ex	27	Longitudinal
M7	57	M	Epithelioid	Ex	102	Longitudinal, CyTOF
M8	75	M	Epithelioid	Ex	N/A	Longitudinal
M9	74	M	Epithelioid	Current	62	Longitudinal, CyTOF
M10	59	M	Epithelioid	Ex	25	Longitudinal, CyTOF, 10x
M11	70	F	NOS	Current	23	Longitudinal
NSCLC						
L1	62	F	Adeno	Ex	25	Longitudinal, CyTOF, 10x
L2	89	M	Adenosquamous	Ex	17	Longitudinal
L3	74	F	Adeno	Ex	33	Longitudinal, CyTOF
L4	64	M	Adeno	Never	52	Longitudinal, CyTOF, 10x

^a, not applicable as patient still alive. CyTOF, cytometry by time of flight; OS, overall survival; mths, months; M, male; F, female; NOS, not otherwise specified; NSCLC, non-small cell lung cancer; Adeno, adenocarcinoma.

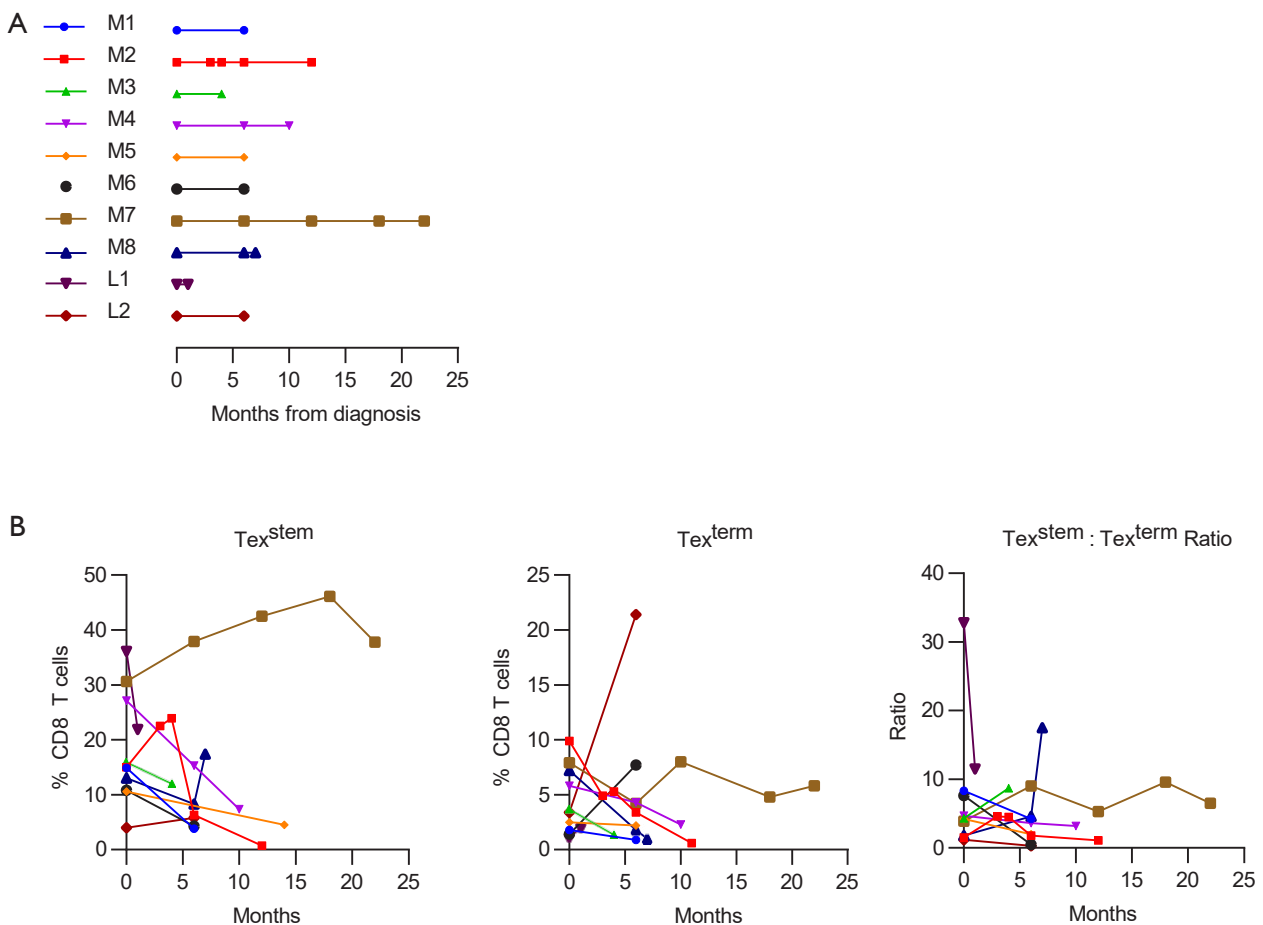


Figure S4 Longitudinal analysis of Tex^{stem} and Tex^{term} in malignant pleural effusions of mesothelioma and NSCLC patients. (A) Number and timepoints of longitudinal samples collected from 8 mesothelioma and 2 lung cancer patients. (B) Longitudinal changes in the frequency of Tex subsets. Tex^{stem}, stem-like exhausted CD8 T cells; Tex^{term}, terminally exhausted CD8 T cells; NSCLC, non-small cell lung cancer.

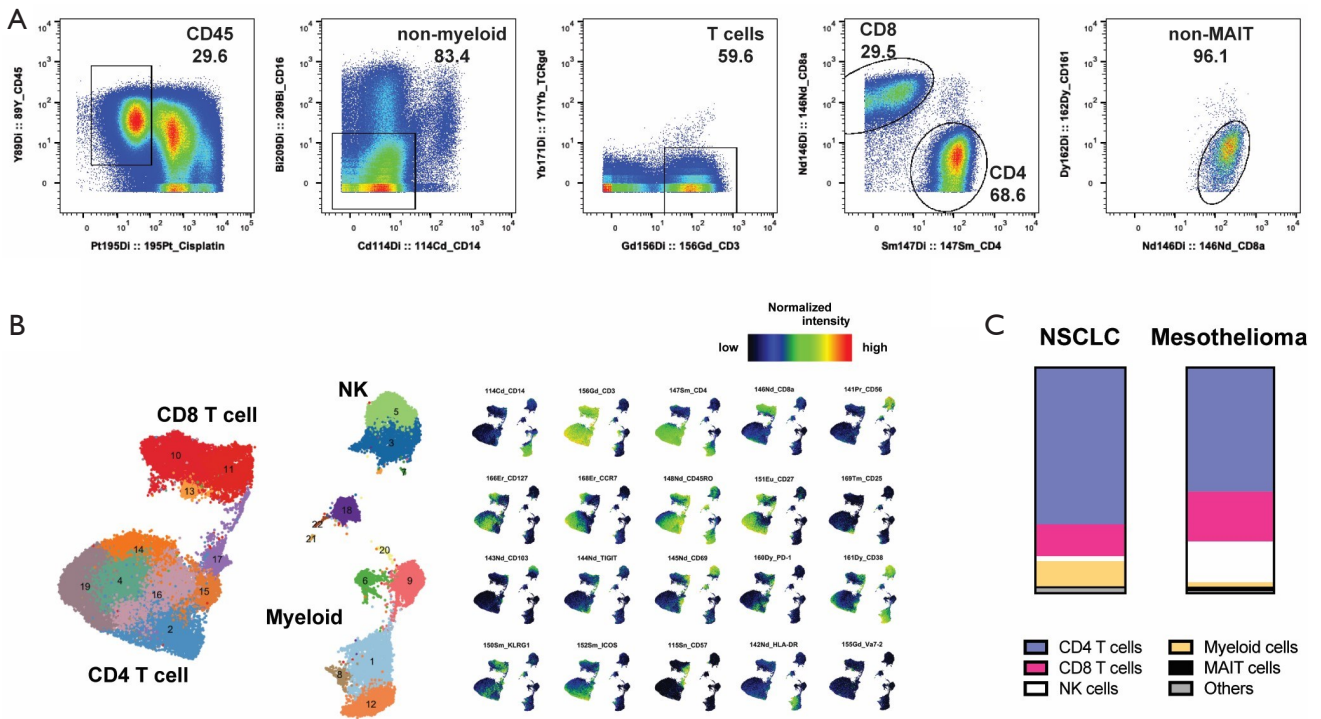


Figure S5 Mass cytometry based phenotypic profiling identifies heterogeneity of CD8 T cells in pleural effusions of mesothelioma and NSCLC patients. (A) Gating strategy for mass cytometry. Representative data from one patient. (B) UMAP projection of CD45⁺ immune cells derived from malignant pleural effusions of mesothelioma and NSCLC patients. Normalized intensity of individual markers. Gated on DNA + cisplatin-CD45⁺. (C) Proportion of each immune cell subsets within pleural effusions of mesothelioma (n=5) and NSCLC (n=3). The median value is presented. NSCLC, non-small cell lung cancer; UMAP, uniform manifold approximation and projection; MAIT, mucosal-associated invariant T; NK, natural killer.

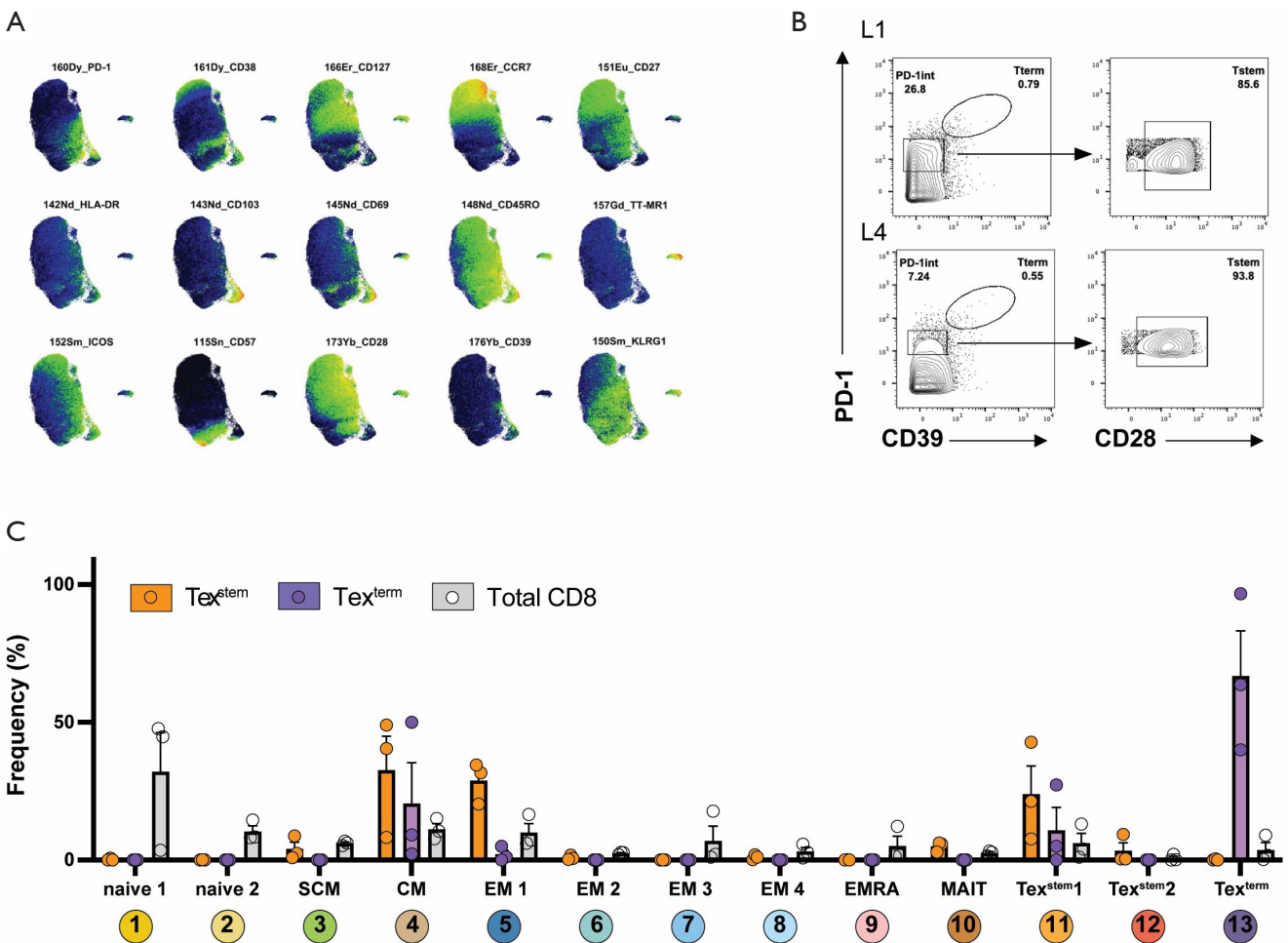


Figure S6 Mass cytometry based phenotypic profiling identifies heterogeneity of CD8 T cells in pleural effusions of NSCLC patients. (A) Marker expression of CD8 T cells derived from malignant pleural effusions of NSCLC patients (n=3). (B) Gating strategy for Tex^{stem} and Tex^{term} by mass cytometry. Representative data. (C) Cluster frequencies of Tex^{stem} (orange), Tex^{term} (violet), and total CD8 T cells (gray) in malignant pleural effusions. NSCLC, non-small cell lung cancer; PD-1, programmed death 1; Tex^{stem}, stem-like exhausted CD8 T cells; Tex^{term}, terminally exhausted CD8 T cells; SCM, memory stem cell; CM, central memory; EM, effector memory; EMRA, effector memory re-expressing CD45RA; MAIT, mucosal-associated invariant T.

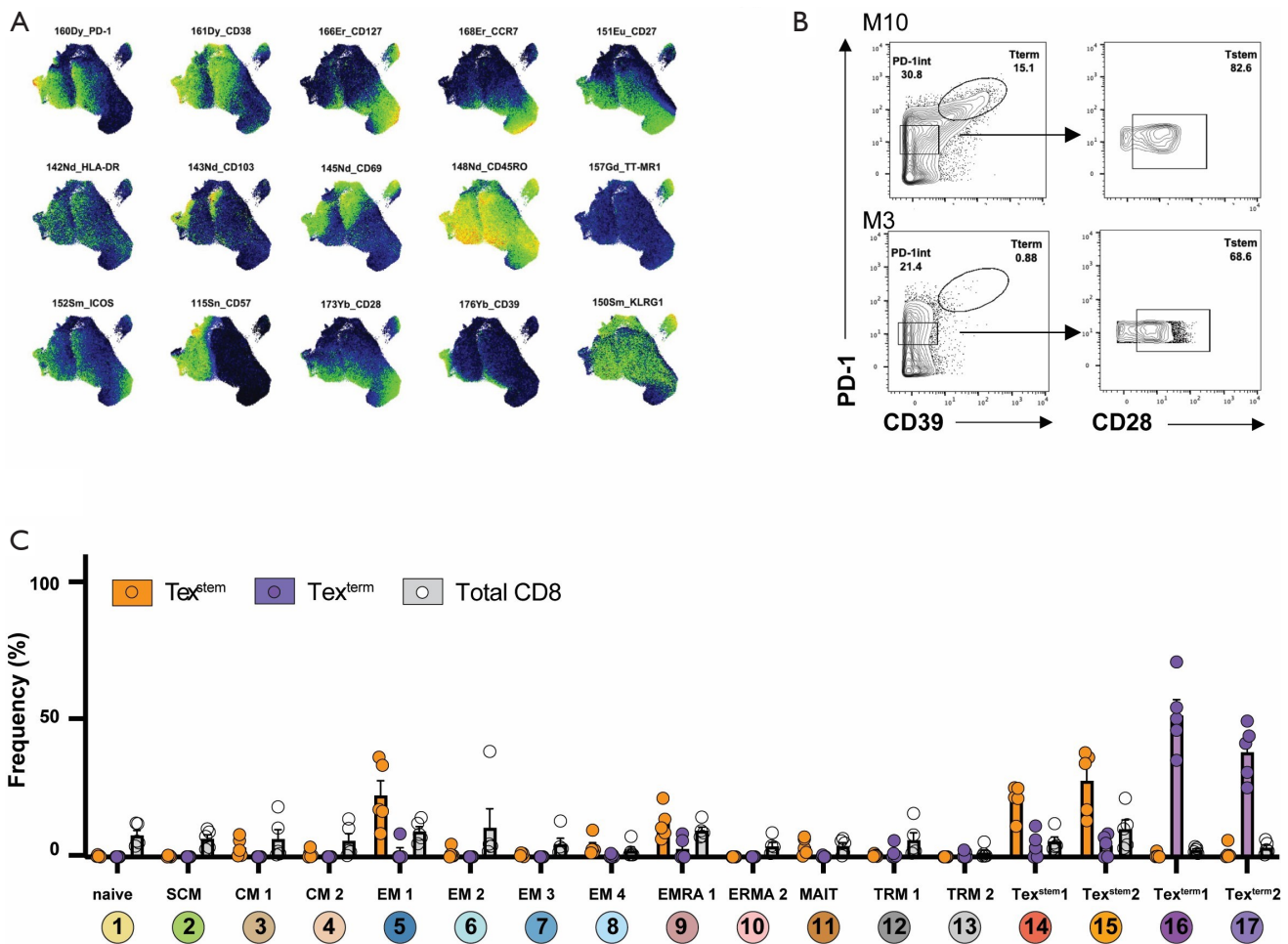


Figure S7 Mass cytometry based phenotypic profiling identifies heterogeneity of CD8 T cells in pleural effusions of mesothelioma patients. (A) Marker expression of CD8 T cells derived from malignant pleural effusions of mesothelioma patients (n=5). (B) Gating strategy for Tex^{stem} and Tex^{term} by mass cytometry. Representative data. (C) Cluster frequencies of Tex^{stem} (orange), Tex^{term} (violet), and total CD8 T cells (gray) in malignant pleural effusions. PD-1, programmed death 1; Tex^{stem}, stem-like exhausted CD8 T cells; Tex^{term}, terminally exhausted CD8 T cells; SCM, memory stem cell; CM, central memory; EM, effector memory; EMRA, effector memory re-expressing CD45RA; MAIT, mucosal-associated invariant T.

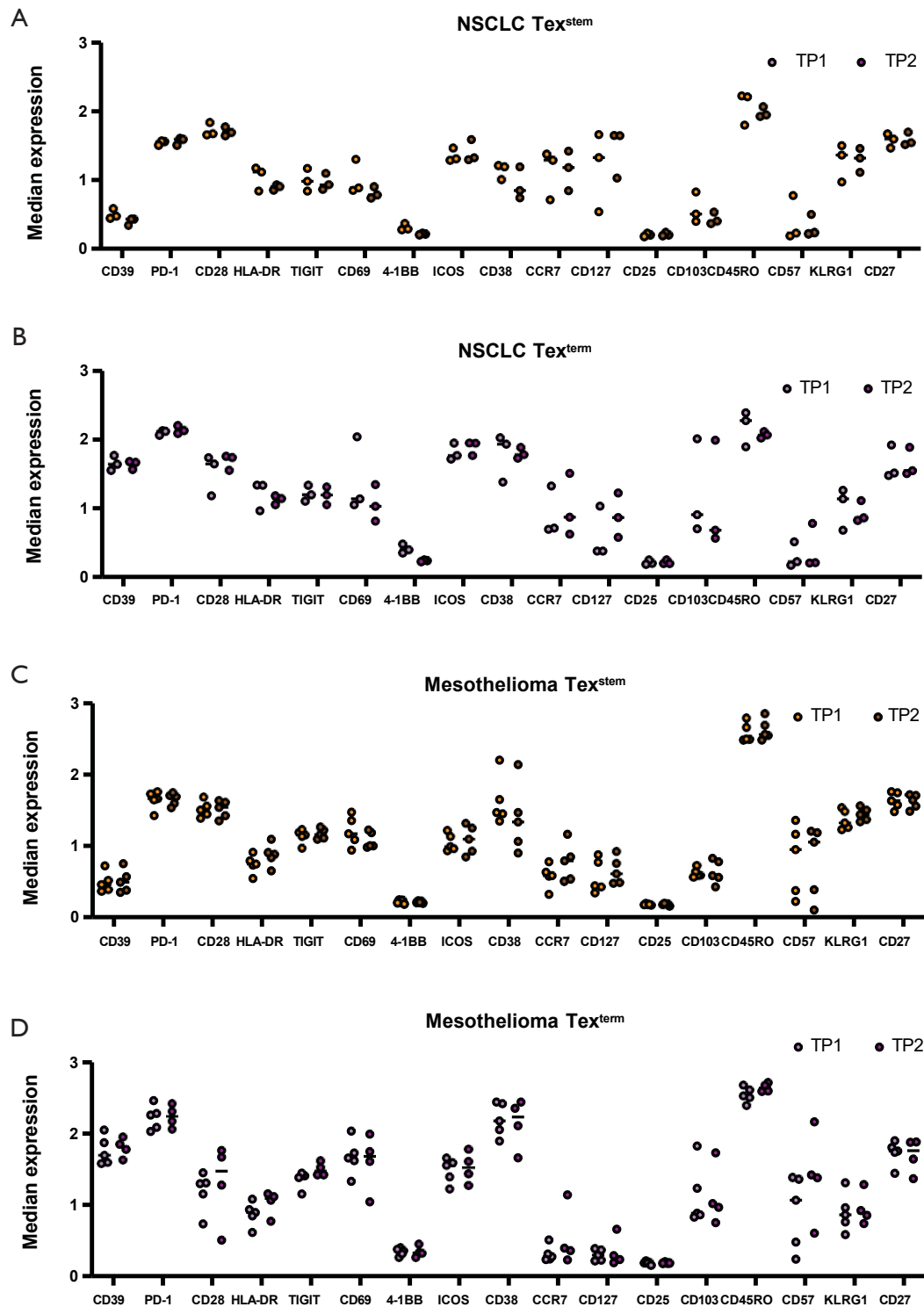


Figure S8 Longitudinal analysis of Tex^{stem} and Tex^{term} from pleural effusions. (A,B) Median marker expression of Tex^{stem} (A) and Tex^{term} (B) from malignant pleural effusions of NSCLC patients (n=3) at two respective TP. (C,D) Median marker expression of Tex^{stem} (C) and Tex^{term} (D) from malignant pleural effusions of mesothelioma patients (n=5) at two respective timepoint (TP). Tex^{stem} , stem-like exhausted CD8 T cells; Tex^{term} , terminally exhausted CD8 T cells; NSCLC, non-small cell lung cancer; TP, timepoint.

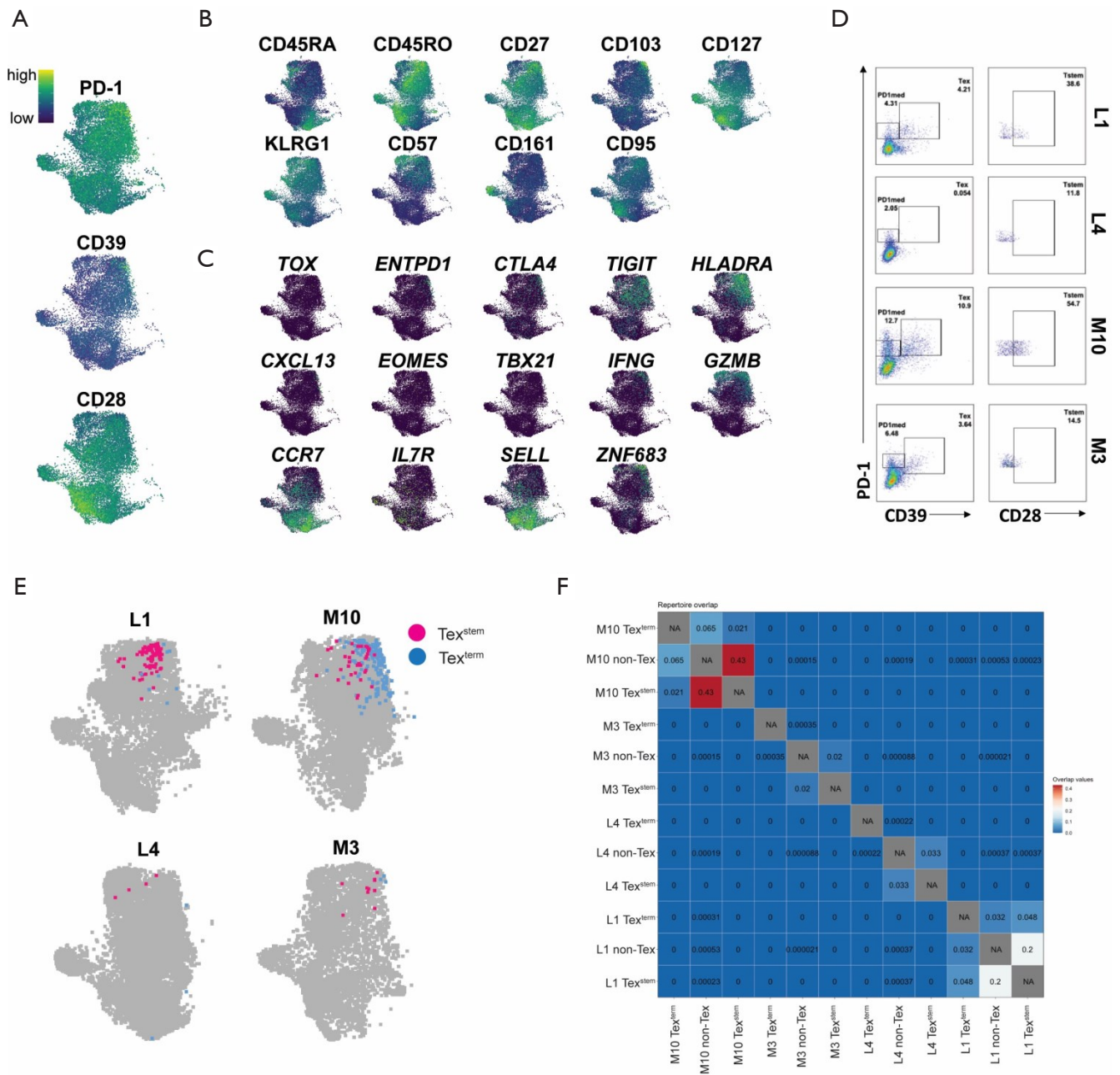


Figure S9 Phenotypic and clonotypic profiling of Tex^{stem} and Tex^{term} CD8 T cells in pleural effusions of NSCLC and mesothelioma patients using single-cell analyses. (A-C) UMAP plot showing the expression of selected markers among CD8 T cells isolated from malignant pleural effusions. Selected markers from scADT (A,B) and scRNA (C). (D) Gating strategy for Tex^{stem} and Tex^{term} by scADT. (E) UMAP projection of Tex^{stem} (red) and Tex^{term} (blue) onto total CD8 (gray). (F) Heatmap of Morisita-Horn indices from each sample. Tex^{stem} , stem-like exhausted CD8 T cells; Tex^{term} , terminally exhausted CD8 T cells; NSCLC, non-small cell lung cancer; UMAP, uniform manifold approximation and projection; scADT, single-cell antibody-derived tag; scRNA, single-cell ribonucleic acid.