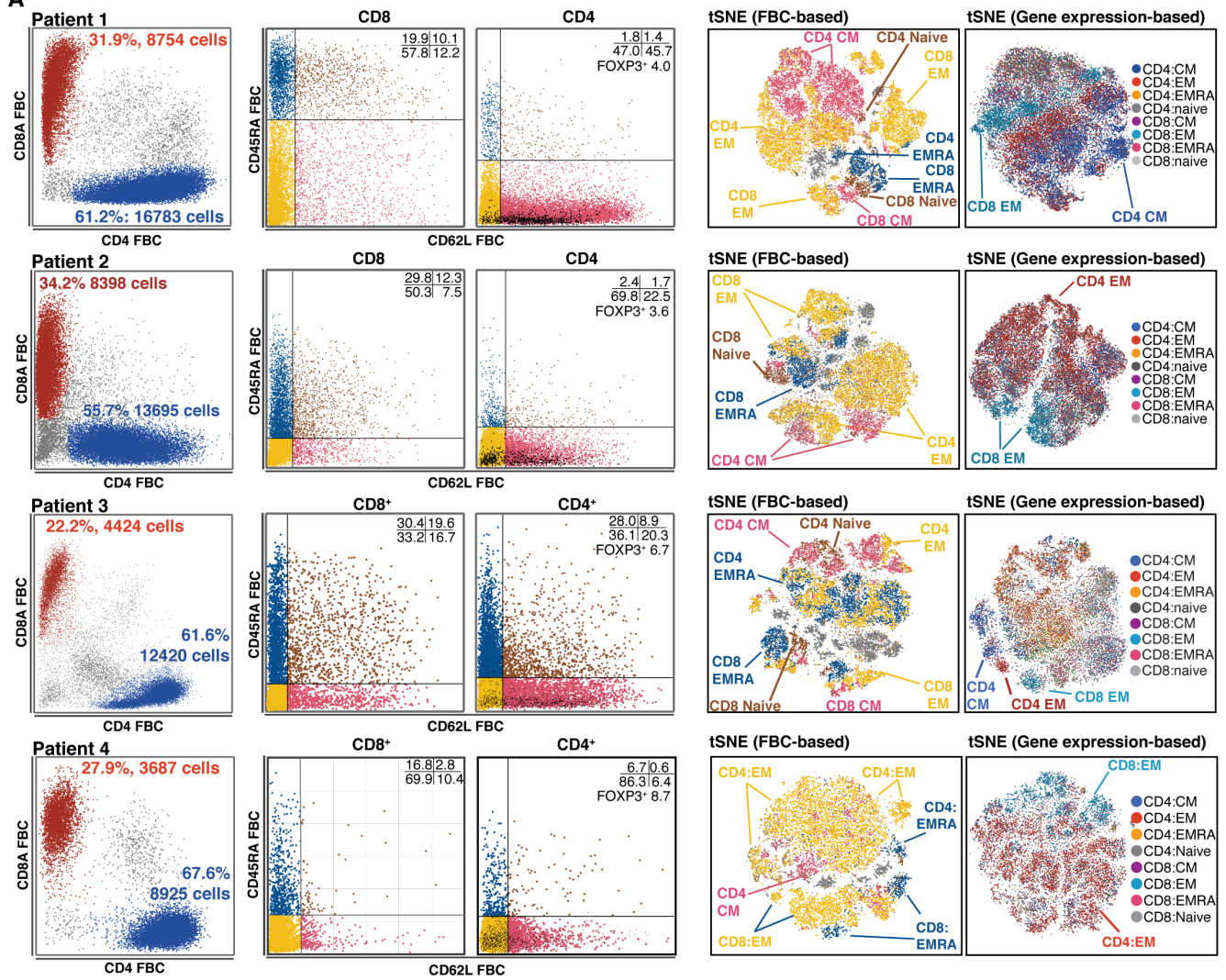
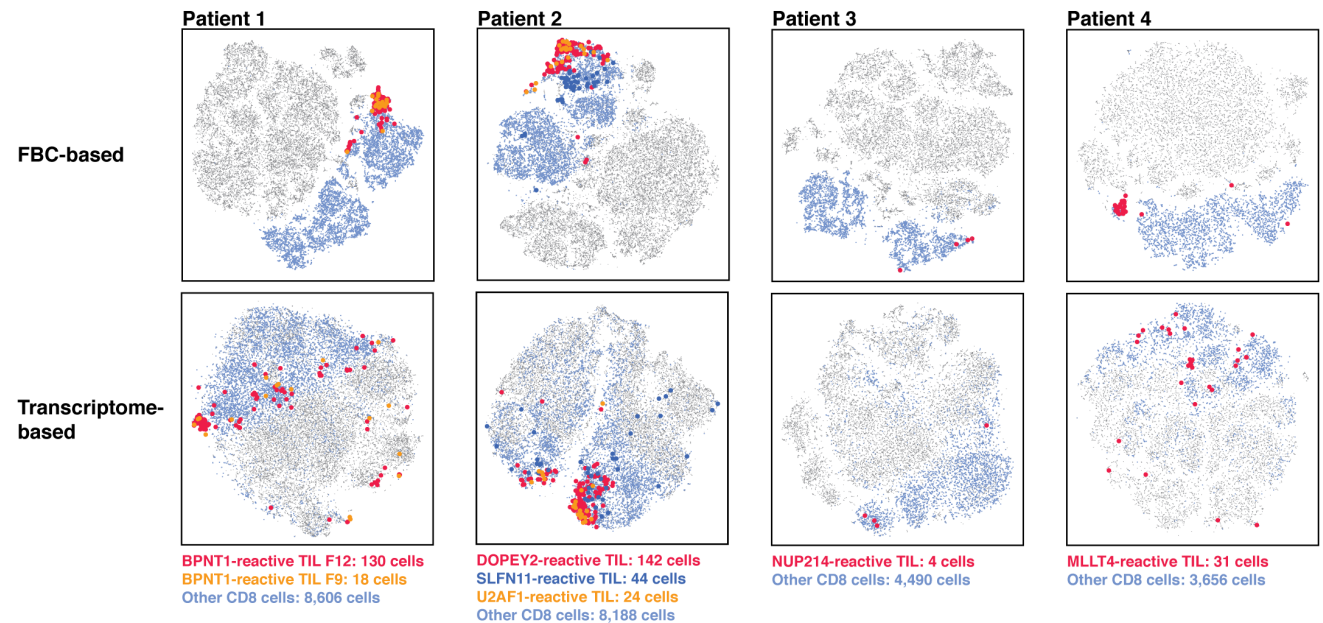


**Figure S1**

**A**



**B**



## Figure S1 (Related to Figure 2)

### CITE-seq analysis of uncultured T-cells from NSCLC tumor

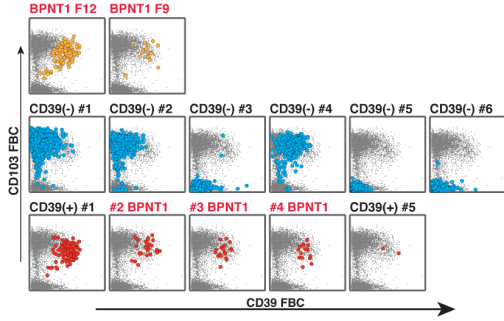
(A) First column: CD4:CD8 ratio. Second column: differentiation status of CD8 T cells as assessed by CD62L by CD45RA. Brown dots: naïve, Pink dots: CM, Yellow dots: EM, and blue dots: EMRA. Third column: differentiation status of CD4 T cells as assessed by 6D62L by CD45RA plot. Brown dots: naïve, Pink dots: CM, Yellow dots: EM, blue dots: EMRA, and Black dots: *FOXP3*<sup>+</sup> Tregs. Fourth column: tSNE plot of T cells based on the cell surface protein expression. Fifth column: tSNE plot of T cells based on the transcriptome. In the fourth and the fifth columns, differentiation status are color-coded, showing the better segregation of differentiation status by the cell-surface protein-based tSNE plots.

(B) Comparison of the clustering of neoantigen-reactive CD8<sup>+</sup> T cells. The top tSNE plots are FBC (cell surface protein)-based, and the bottom tSNE plots are transcriptome-based. Antigen specificities are color-coded as shown on the bottom.

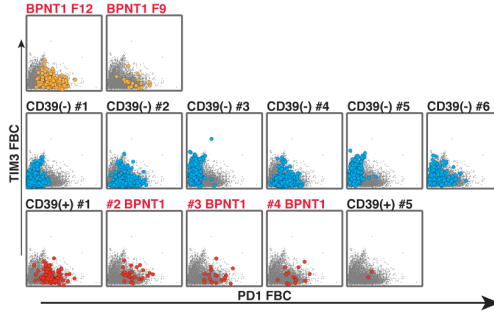
Figure S2

(A)

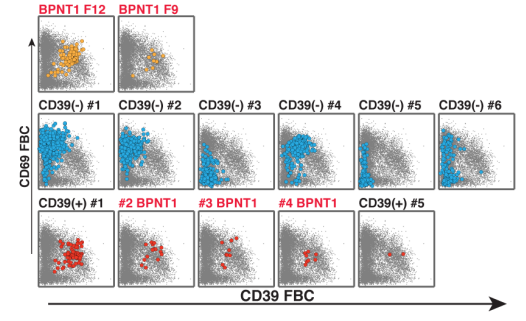
Patient 1



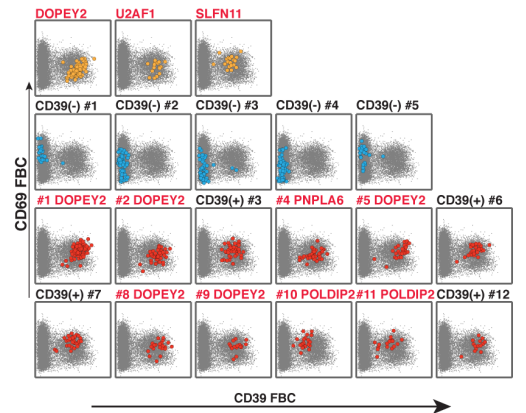
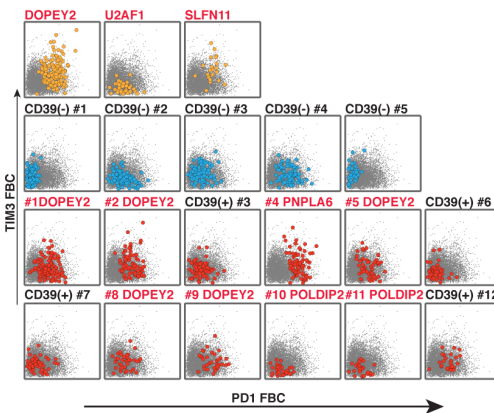
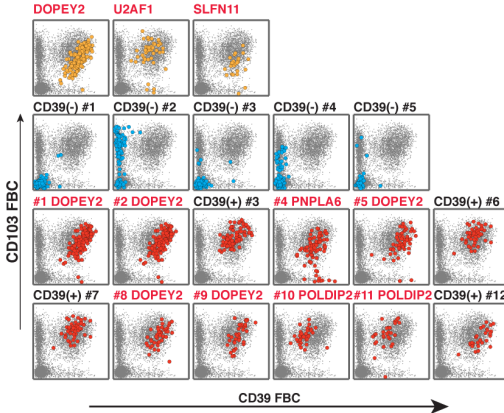
(B)



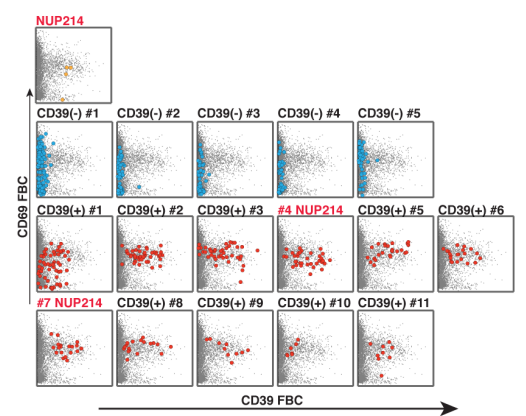
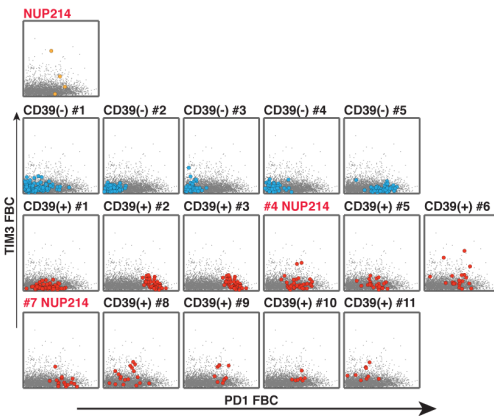
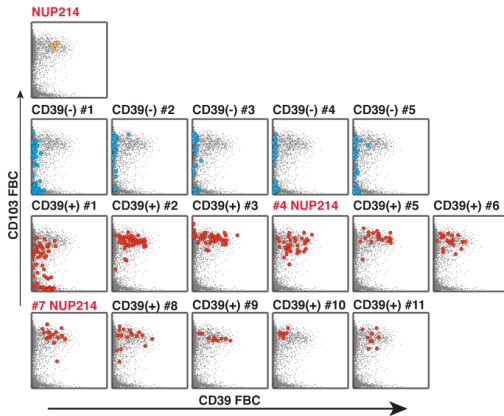
(C)



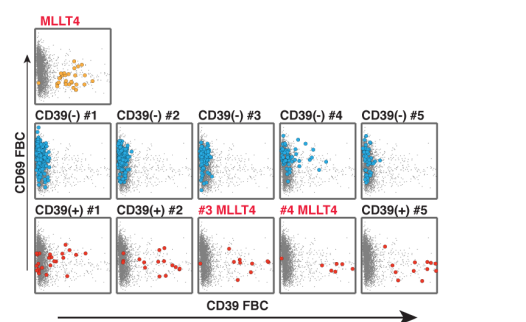
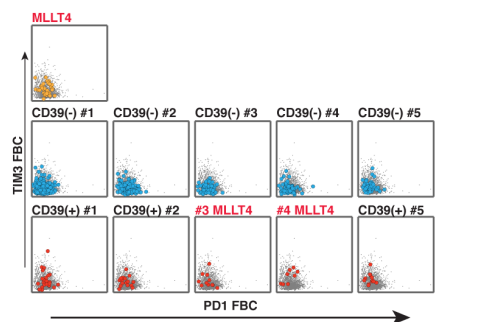
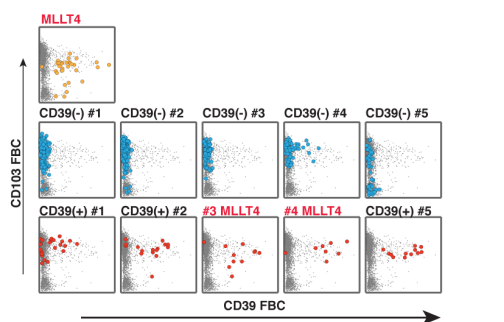
Patient 2



Patient 3



Patient 4



**Figure S2 (Related to Figure 4)**

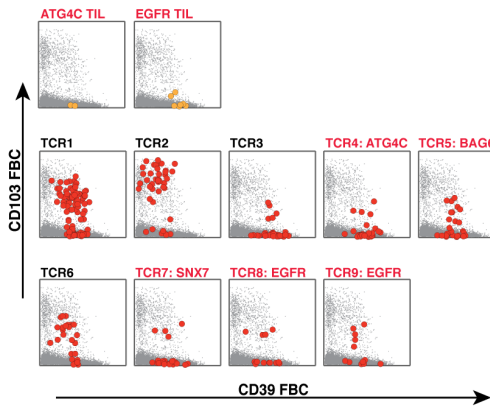
**Cell surface protein expression on CD8<sup>+</sup> T-cells as analyzed by CITE-seq.**

In each patient, orange dots are neoantigen-reactive T-cells identified by the conventional TIL culture; blue dots are CD39(-) clonotypes; red dots are CD39(+), *CXCL13*<sup>+</sup> clonotypes. For clonotypes whose antigens were identified, antigen names are shown on boxes. (A) CD39 FBC by CD103 FBC, (B) PD1 FBC by TIM3 FBC, and (C) CD39 FBC by CD69 FBC

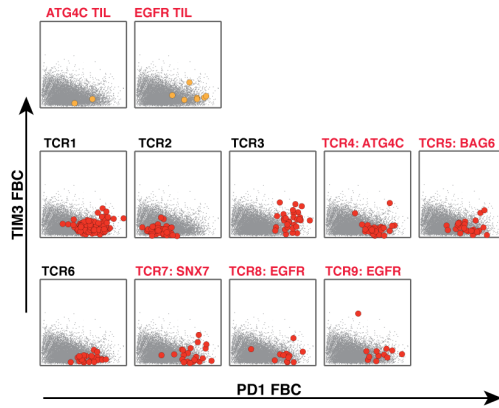
Figure S3

(A)

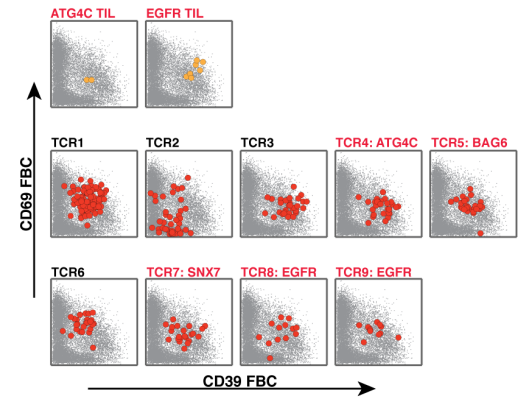
Patient 1



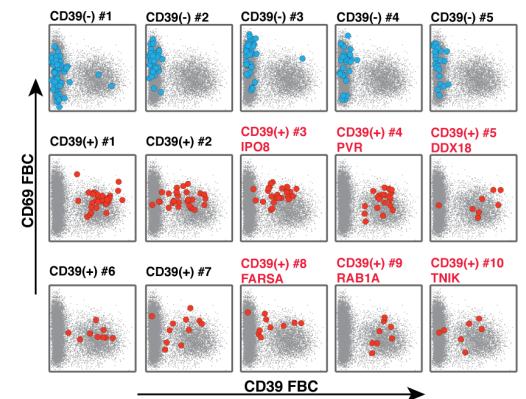
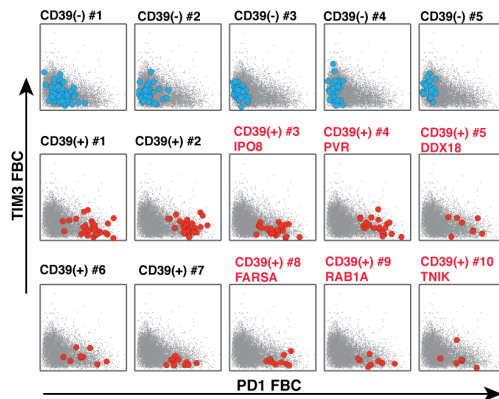
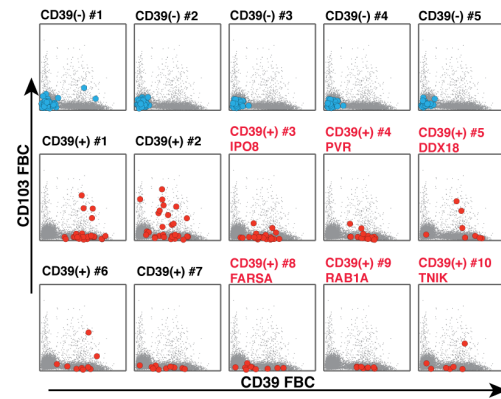
(B)



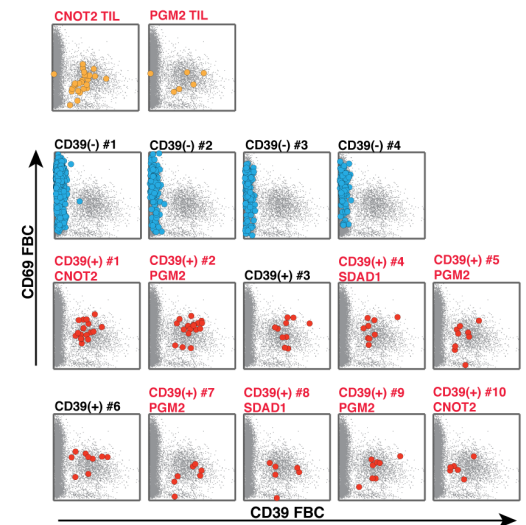
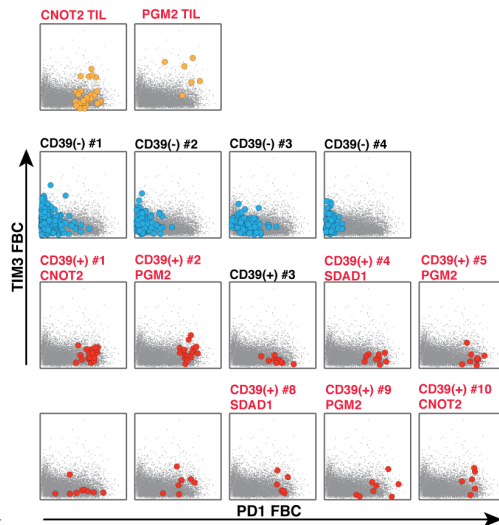
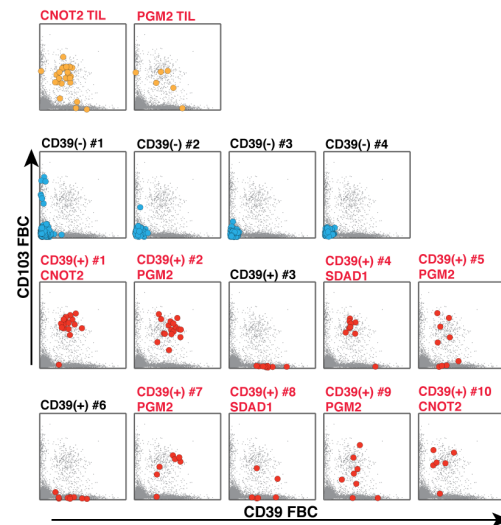
(C)



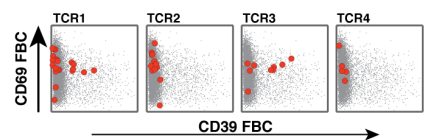
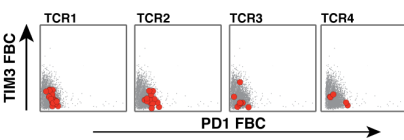
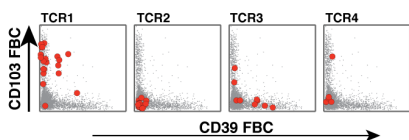
Patient 2



Patient 3



Patient 4

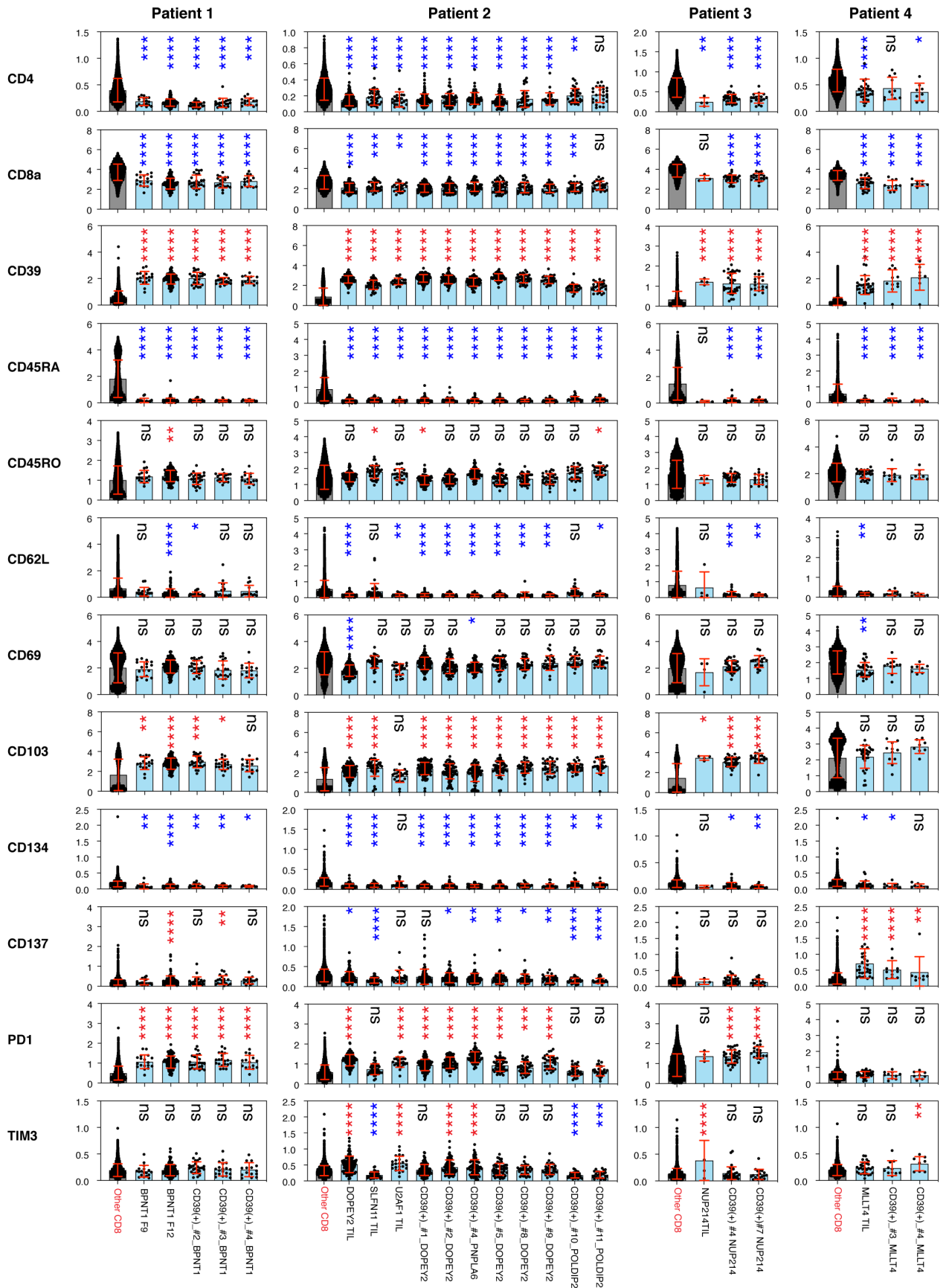


**Figure S3 (Related to Figure 5)**

**Cell surface protein expression on CD4<sup>+</sup> T-cells analyzed by CITE-seq.**

In each patient, orange dots are neoantigen-reactive T-cells identified by the conventional TIL culture; blue dots are CD39(-) clonotypes; red dots are CD39(+), *CXCL13*<sup>+</sup> clonotypes. For clonotypes whose antigens were identified, antigen names are shown on boxes. (A) CD39 FBC by CD103 FBC, (B) PD1 FBC by TIM3 FBC, and (C) CD39 FBC by CD69 FBC

Figure S4



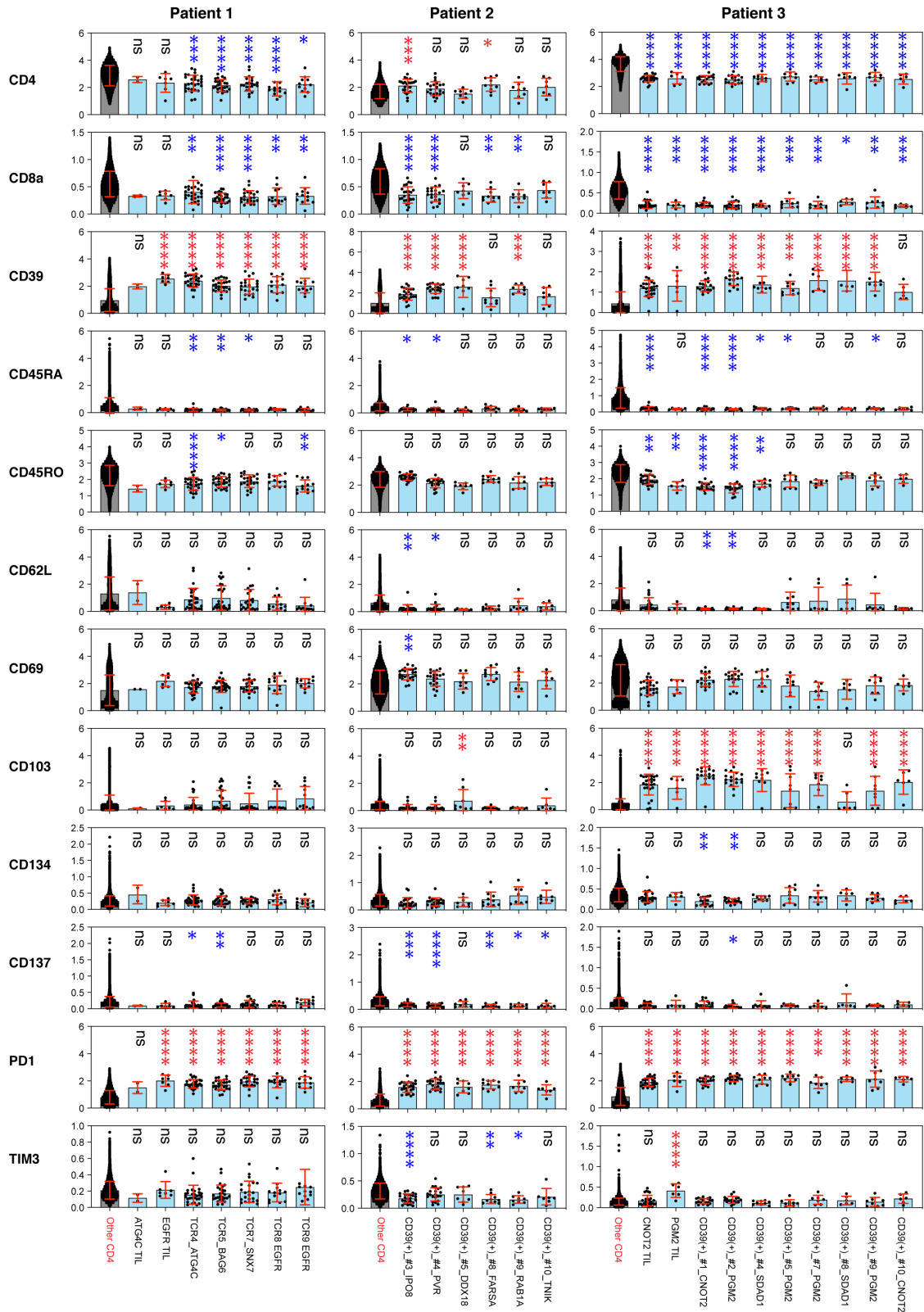
**Figure S4 (Related to Figure 4)**

**Cell surface protein expression on neoantigen-reactive CD8<sup>+</sup> T cell clonotypes compared with other CD8<sup>+</sup> T cells from the same tumor.**

Expression of cell surface molecules as analyzed by CITE-seq is shown. Molecule names are shown on the left, and Clonotype IDs and antigens recognized are shown on the bottom. Red stars represent up-regulation and blue stars down-regulation compared with “Other CD8” T cells from the same patient. Statistical analysis was done by ANOVA using Graphpad Prism (P-value formats: \*\*\*\* P≤0.0001, \*\*\* P≤0.001, \*\* P≤0.01, \* P≤0.05, ns P>0.05)



Figure S5



**Figure S5 (Related to Figure 5)**

**Cell surface protein expression on neoantigen-reactive CD4<sup>+</sup> T cell clonotypes compared with other CD4<sup>+</sup> T cells from the same tumor.**

Expression of cell surface molecules as analyzed by CITE-seq is shown. Molecule names are shown on the left, and Clonotype IDs and antigens recognized are shown on the bottom. Red stars represent up-regulation and blue stars down-regulation compared with “Other CD4” T cells from the same patient. Statistical analysis was done by ANOVA using Graphpad Prism (P-value formats: \*\*\*\* P≤0.0001, \*\*\* P≤0.001, \*\* P≤0.01, \* P≤0.05, ns P>0.05)

Table S1, Patient demographics, related to Figure 1.

ID	Age/ sex	Pathology	smoking history	# of non- synonymous variants	common mutations found	Notes
Patient 1 Tumor ID: 4237	51/F	adenocarcinoma	(-)	183	EGFR (p.E746_A750 del, p.T790M)	Erlotinib(2 years)
						Osimertinib
Patient 2 Tumor ID: 4234	49/F	adenocarcinoma	0.5pack/day 30 yrs	463	KRAS (p.G12C)	
					TP53 Stopgain (p.E287*)	
					U2AF1 (p.S34F)	
Patient 3 Tumor ID: 4129	52/F	adenocarcinoma	1 pack/day 20 yrs	250	KRAS (G12C), TP53 (p.M160I)	
Patient 4 Tumor ID: 4369	52/F	adenocarcinoma	10 pack-year	106	KRAS (G12V)	pembrolizumab (stopped 62 days before tissue procurement)

**Table S2** Feature Barcoding (FBC) antibodies used for the study, related to Figure 2

	pattern	barcode sequence
CD4	5PNNNNNNNNNN (BC) NNNNNNNNN	GAGGTTAGTGATGGA
CD8a	5PNNNNNNNNNN (BC) NNNNNNNNN	GCTGCGCTTTCCATT
CD45RA	5PNNNNNNNNNN (BC) NNNNNNNNN	TCAATCCTTCCGCTT
CD45RO	5PNNNNNNNNNN (BC) NNNNNNNNN	CTCCGAATCATGTTG
CD62L	5PNNNNNNNNNN (BC) NNNNNNNNN	GTCCCTGCAACTGA
CD69	5PNNNNNNNNNN (BC) NNNNNNNNN	GTCTCTTGGCTTAAA
CD103	5PNNNNNNNNNN (BC) NNNNNNNNN	GACCTCATTGTGAAT
CD134	5PNNNNNNNNNN (BC) NNNNNNNNN	AACCCACCGTTGTTA
CD137	5PNNNNNNNNNN (BC) NNNNNNNNN	CAGTAAGTTCGGGAC
CD39	5PNNNNNNNNNN (BC) NNNNNNNNN	TTACCTGGTATCCGT
PD1	5PNNNNNNNNNN (BC) NNNNNNNNN	ACAGCGCCGTATTTA
TIM3	5PNNNNNNNNNN (BC) NNNNNNNNN	TGTCCTACCCAACTT