

Figure S1. Flowchart of image processing of immunohistochemistry stained sections. In total, ten spots (n=5 each for the center of the tumor and invasive margins) were selected, and the images were captured with a digital scanner at higher magnification (x40). The larger left image shows the selected spots for image capture. The four images in the middle indicate color deconvolution from the captured image into three colors (blue, red and green). The image in the lower right corner is a binary image acquired using ImageJ. Each red dot was counted using ImageJ.

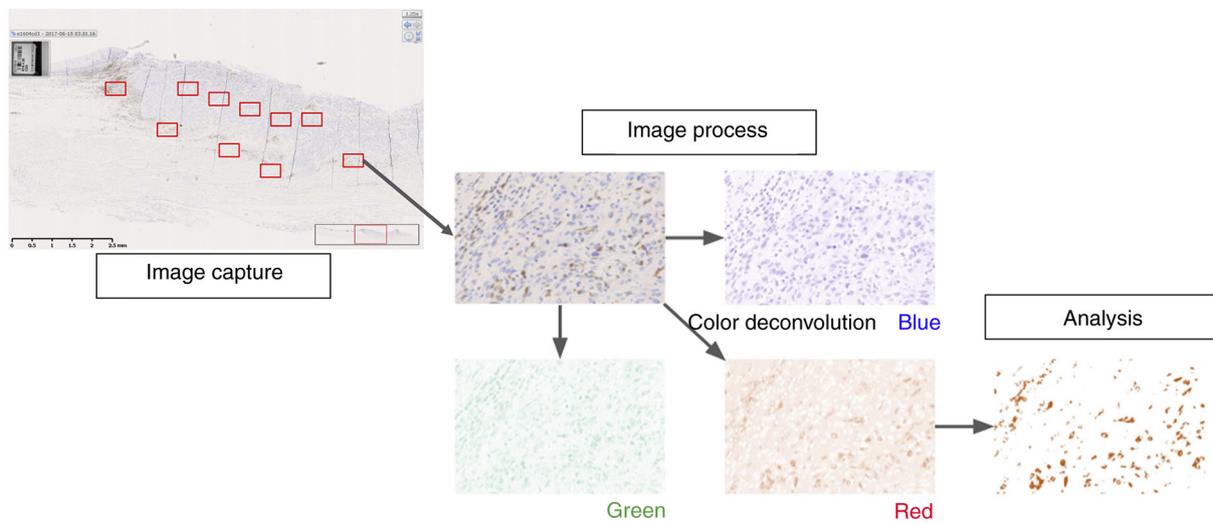


Figure S2. Image processing for AE1/AE3, CD274 and HLA IHC stained sections. The images in the upper row are IHC images for each marker. The images in the lower row are binarized images for image capture. Upper row scale bar, 2.5 mm, lower row scale bar, 100  $\mu$ m. IHC, immunohistochemistry; HLA, human leukocyte antigen.

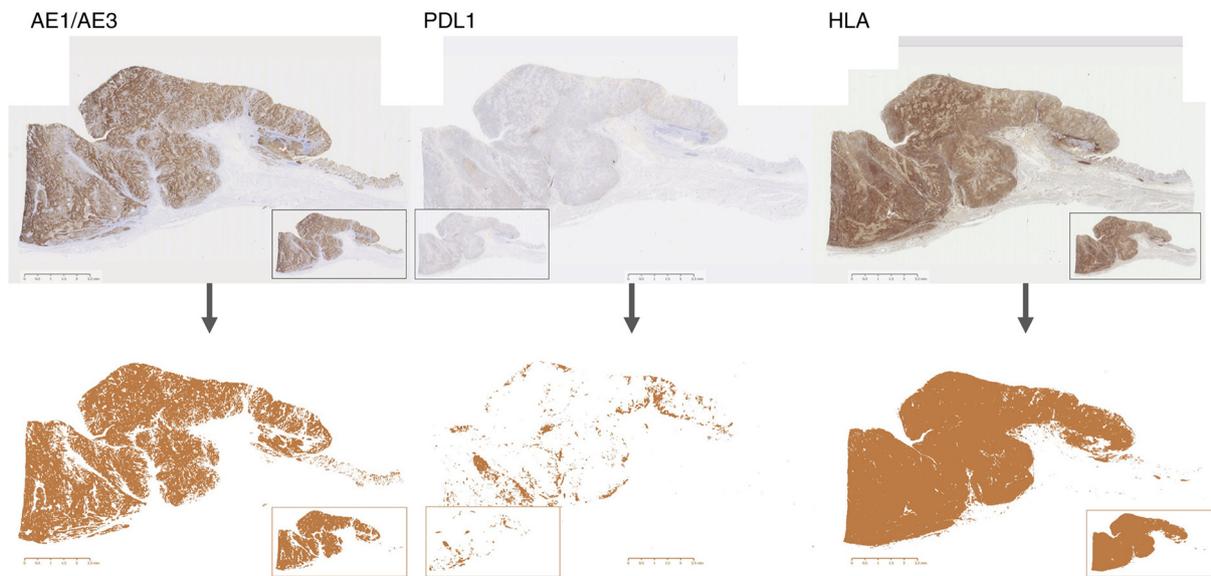


Figure S3. Example of the calculation for PD-L1 or HLA expression. The upper left image is a binary image of PD-L1 immunohistochemistry and the lower right image is a binary image of AE1/AE3. The standardized value was calculated by dividing by the area of AE1/AE3. Scale bar, 2.5 mm. PD-L1, programmed cell death 1 ligand 1; HLA, human leukocyte antigen.

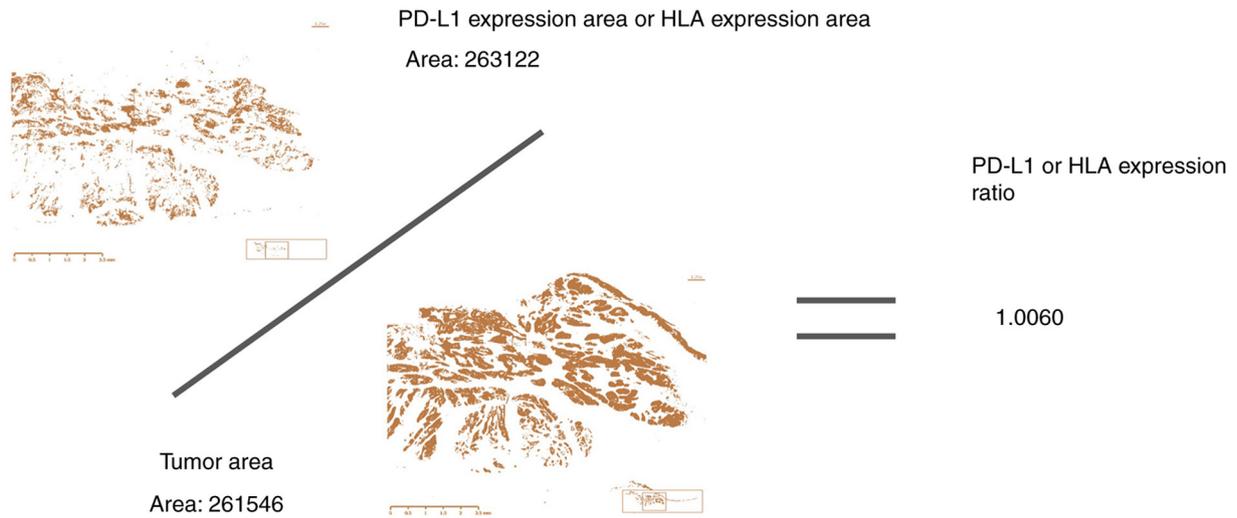


Figure S4. Comparisons of T-cell markers' expression between the center and invasive margin of the tumor. Left panels are representative macroscopic (scale bar, 2.5 mm) and microscopic views (scale bar, 100  $\mu$ m) of T-cell markers using immunohistochemistry. Right scatter plots indicate the comparison of the expression levels of the four markers between CT and IM calculated by unpaired Student's t-tests.  $P < 0.05$  was considered to indicate a statistically significant difference. CT, center of the tumor; IM, invasive margin.

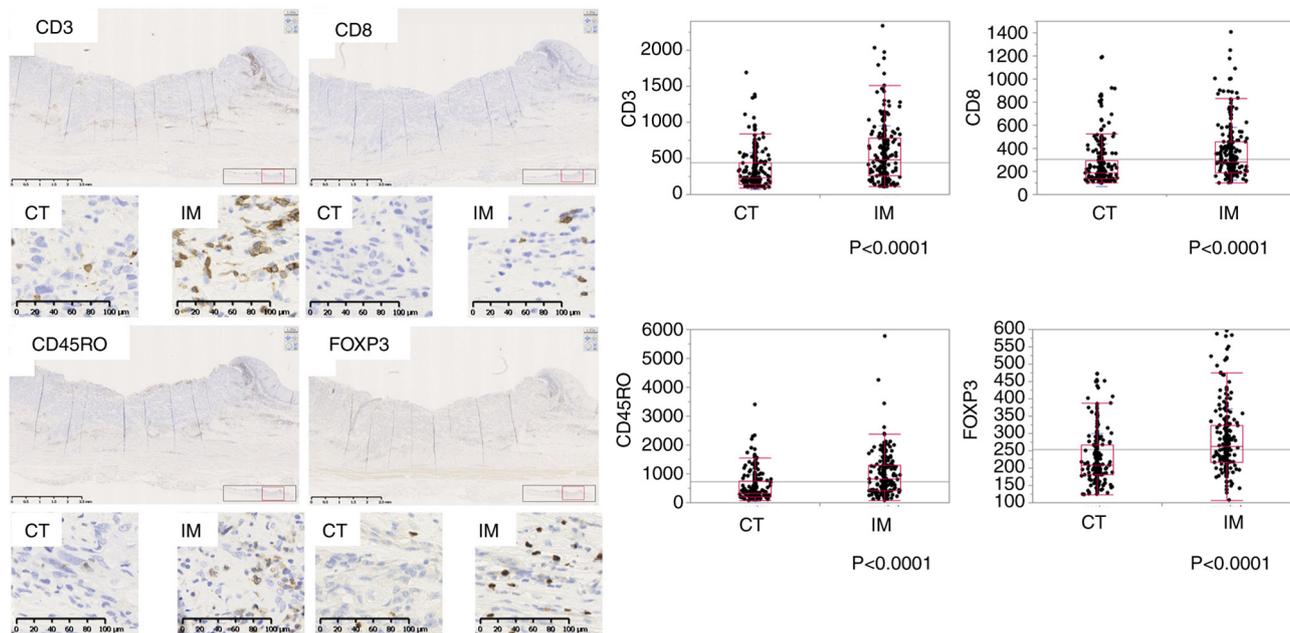


Figure S5. Scatter plot of correlation analysis for T-cell subset markers between CT and IM. The correlation was tested using linear regression analysis. CT, center of the tumor; IM, invasive margin.

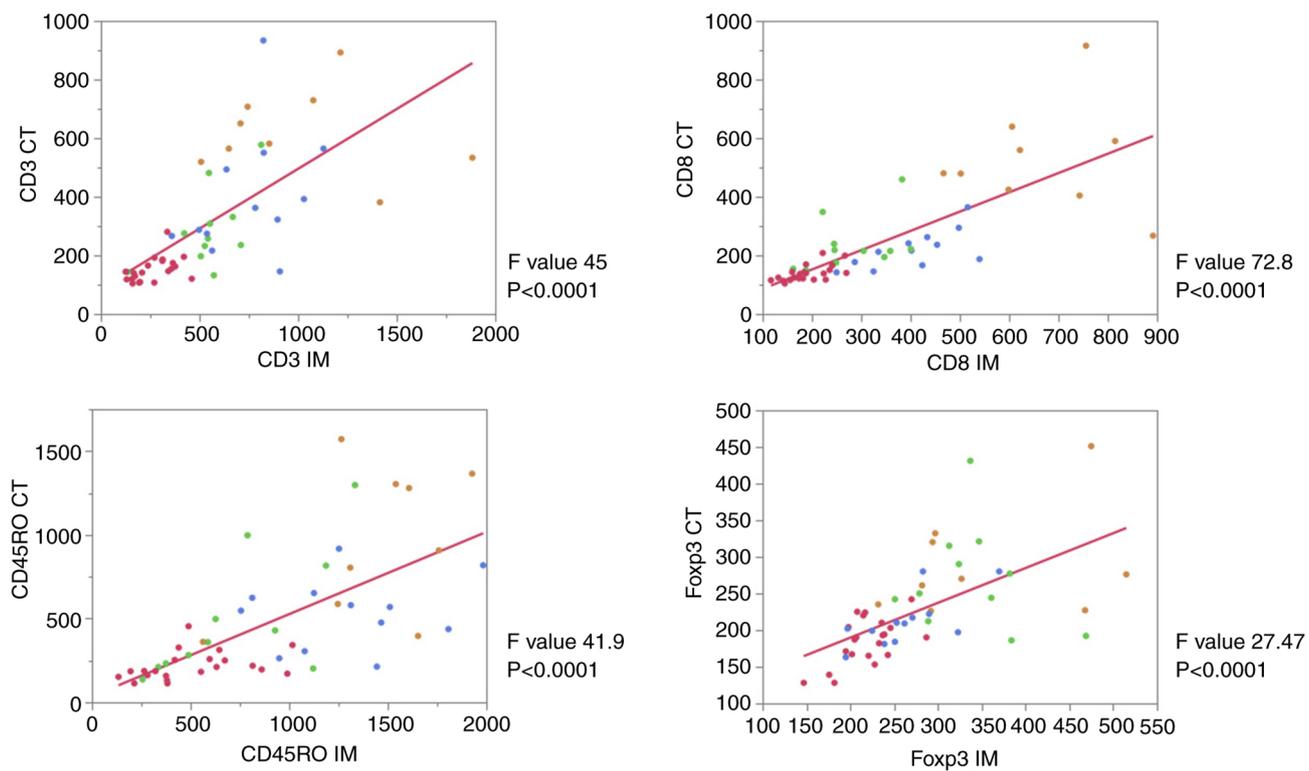


Figure S6. Microscopic tissue images for all 10 cases used in TCR analysis. Each large image is at x10 magnification (scale bar, 250  $\mu\text{m}$ ) and each right lower corner image is at x40 magnification (scale bar, 20  $\mu\text{m}$ ). IR, immuno relation; Hi, high; Lo, low; HE, hematoxylin and eosin; IHC, immunohistochemistry.

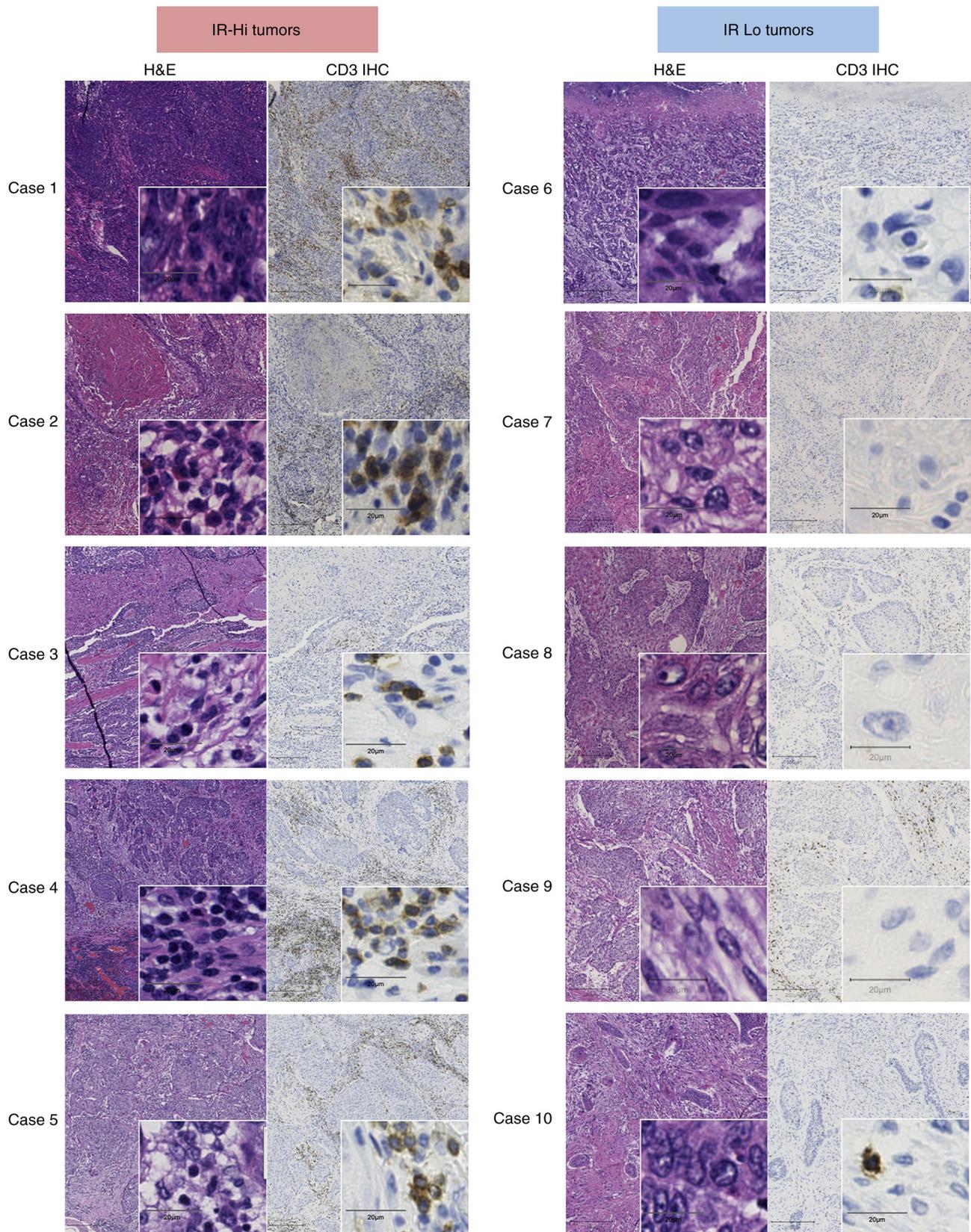




Figure S8. Comparison of cancer-specific and recurrence-free survival among Cluster\_1, Cluster\_2 and Cluster\_3 identified through hierarchical clustering analysis.  $P < 0.05$  was considered to indicate a statistically significant difference.

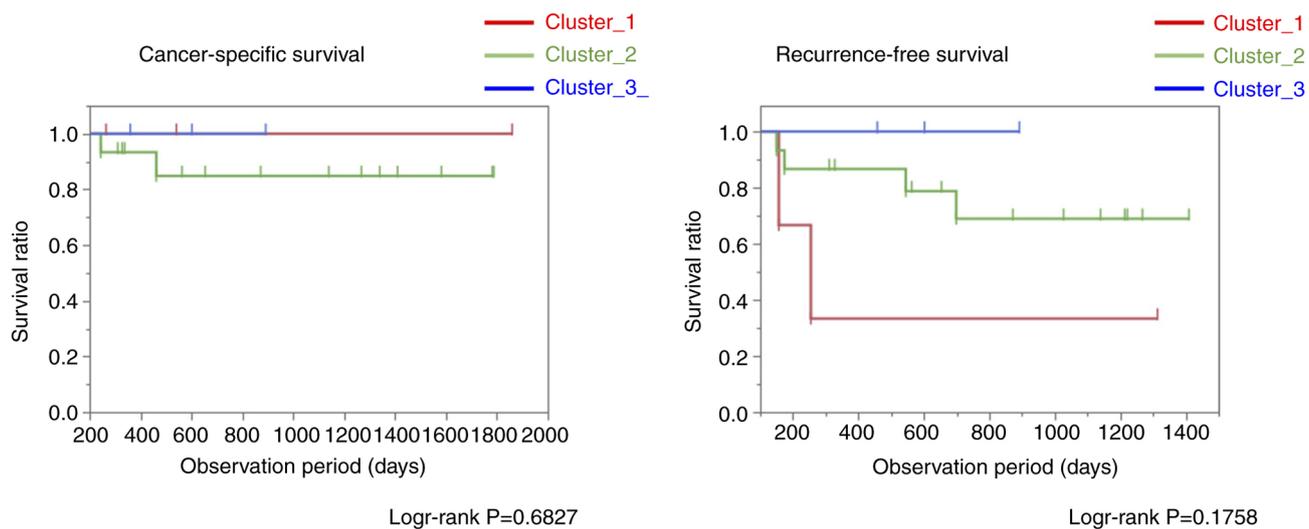


Table SI. Overview of the enrolled patients with esophageal squamous cell carcinoma (n=53).

Characteristics	Value
Mean age $\pm$ SD, years	69.4 $\pm$ 6.85
Sex, male/female	44/9
Primary tumor, T1a/T1b/T2/T3/T4a/T4b	2/4/9/33/4/1
Regional lymph nodes, N0/N1/N2/N3	20/14/13/6
Distant metastasis, M0/M1	52/1
Pathological TNM stage, IA/IB/IIA/IIB/IIIA/IIIB/IVA/IV	2/3/5/10/1/22/9/1
Histological grading, G1/G2/G3	16/29/8
Prognostic group, IA/IB/IIA/IIB/IIIA/IIIB/IVA/IVB	2/6/6/7/22/9/1
Lymph vessel invasion, ly0/ly1/ly2/ly3	19/19/12/3
Vascular invasion, v0/v1/v2/v3	9/34/7/3
INF, a/b/c	9/41/3

TNM classification and the prognostic group were determined according to the TNM Classification of Malignant Tumors 8th Edition. Tumor depth: T1a, tumor invades lamina propria or muscularis mucosae; T1b, tumor invades submucosa; T2, tumor invades muscularis propria; T3, tumor invades adventitia; T4a, tumor invades pleura, pericardium, azygos vein, diaphragm or peritoneum; T4b, tumor invades other adjacent structure such as aorta, vertebral body or trachea. Regional lymph nodes: N0, no regional lymph node metastasis; N1, metastasis in 1-2 regional lymph nodes; N2, metastasis in 3-6 regional lymph nodes; N3, metastasis in  $\geq$ 7 regional lymph nodes. Grading: G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated. INF, pattern of tumor infiltration; a, expanding growth and a distinct border with the surrounding tissue; b, in-between a and c; c, infiltrating growth and an indistinct border with surrounding tissue.

Table SII. Primer sequences used for PCR.

Primers	Sequences (5'-3')
BSL-18E	AAAGCGGCCGCATGCTTTTTTTTTTTTTTTTTTTVN
P20EA	TAATACGACTCCGAATTCCC
P10EA	GGGAATTCGG
CA1	TGTTGAAGGCGTTTGCACATGCA
CA2	GTCCATAGACCTCATGTCTAGCA
CB1	GAAGTGGACTTGACAGCGGAACT
CB2	AGGCAGTATCTGGAGTCATTGAG
P22EA-ST1-R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTAATACGACTCCGAATTCCC
CA-ST1-R	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGAGGGTCAGGGTTCTGGA
CB-ST1-R	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCTCAAACACAGCGACCTC

BSL-18E, adaptor primer containing poly T18; P20EA/P10EA, adaptors for the ligation to the 5' end of ds-cDNA; CA1/2, primers for TCR $\alpha$ -chain constant region-specific1/2; CB1/2, primers for TCR $\beta$ -chain constant region-specific1/2; P22EA-ST1-R, CA-ST1-R, and CB-ST1-R, primers for the second set of PCR product.

Table SIII. Overview of the read TCR counts analyzed by next generation sequencing.

		TCR $\alpha$					TCR $\beta$				
Case no.	Total reads, n	Assigned reads, n (%)	In frame reads, n (%)	Unique reads, n (%)	In frame/Unique, n	Total reads, n	Assigned reads, n (%)	In frame reads, n (%)	Unique reads, n (%)	In frame/Unique, n	
1	175509	155599 (88.7)	134875 (86.7)	10316 (7.6)	13.07	261933	199671 (76.2)	195172 (97.7)	13316 (6.8)	14.66	
2	101809	73316 (72.0)	64044 (87.4)	3311 (5.2)	19.34	149278	103195 (69.1)	99936 (96.8)	5939 (5.9)	16.83	
3	159393	120329 (75.5)	106293 (88.3)	2806 (2.6)	37.88	50036	25733 (51.4)	25362 (98.8)	1087 (4.3)	23.33	
4	229962	170263 (74.0)	153533 (90.2)	3604 (2.3)	42.60	41915	18928 (45.2)	18029 (96.6)	725 (4.0)	24.87	
5	312142	195764 (62.7)	175418 (89.6)	6116 (3.5)	28.68	179856	81319 (45.2)	77364 (96.0)	3541 (4.6)	21.85	

		TCR $\alpha$					TCR $\beta$				
Case no.	Total reads, n	Assigned reads, n (%)	In frame reads, n (%)	Unique reads, n (%)	In frame/Unique, n	Total reads, n	Assigned reads, n (%)	In frame reads, n (%)	Unique reads, n (%)	In frame/Unique, n	
6	98885	61415 (62.1)	55411 (90.2)	2082 (3.8)	26.61	23941	8575 (35.8)	8358 (97.5)	533 (6.4)	15.68	
7	101634	53974 (53.1)	44406 (82.3)	2231 (5.0)	19.90	140418	88417 (63.0)	81919 (92.7)	3385 (4.1)	24.20	
8	76720	63809 (83.2)	58097 (91.0)	2529 (4.4)	22.97	153237	103550 (67.6)	100537 (97.1)	4156 (4.1)	24.19	
9	310513	269749 (86.9)	227668 (84.4)	2067 (0.9)	110.14	215726	153569 (71.2)	142663 (92.9)	2148 (1.5)	66.42	
10	105175	4996 (4.8)	4342 (86.9)	202 (4.7)	21.50	75170	338 (0.4)	335 (99.4)	48 (14.3)	6.98	

IR, immuno relation; TCR, T-cell receptor.