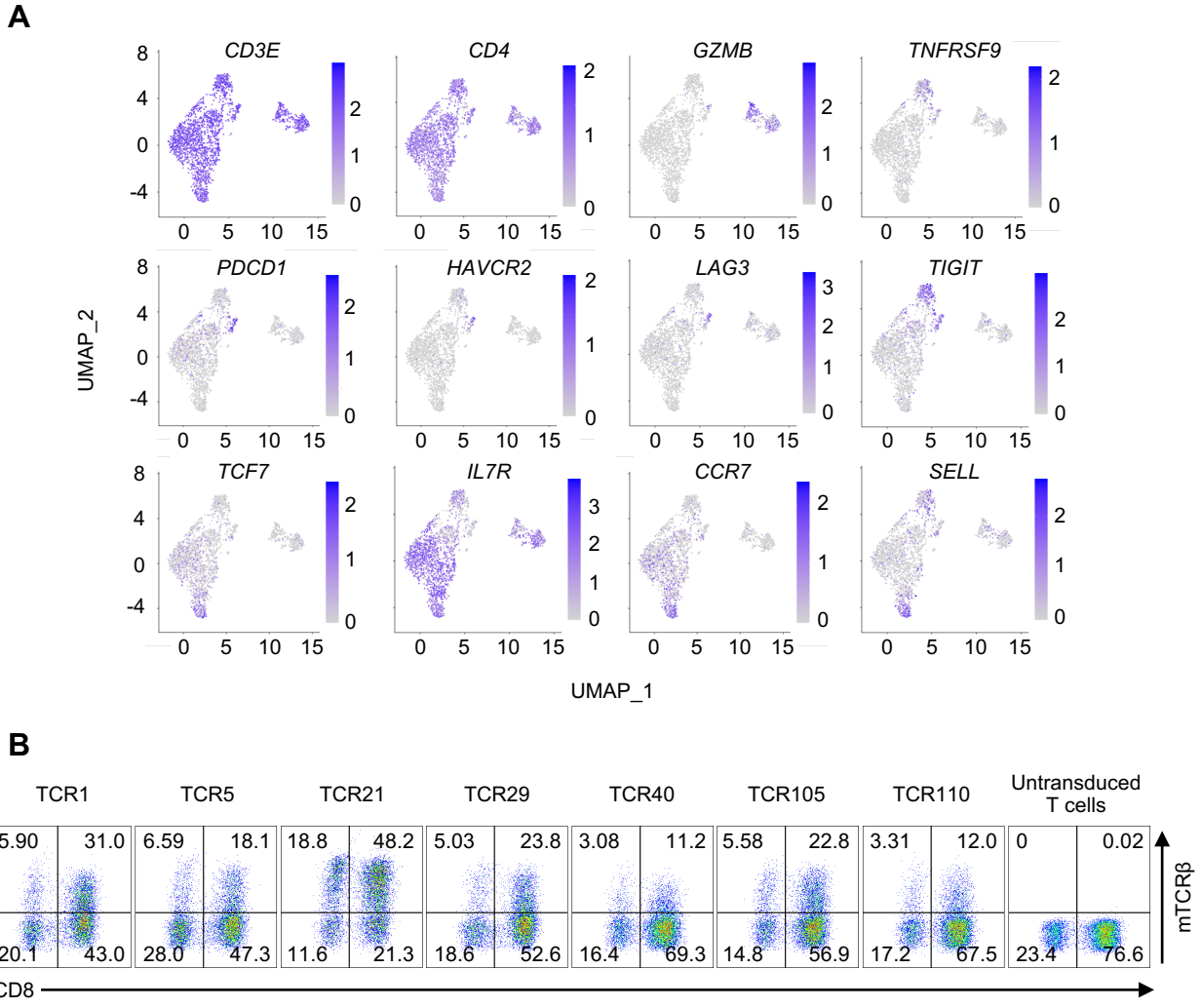
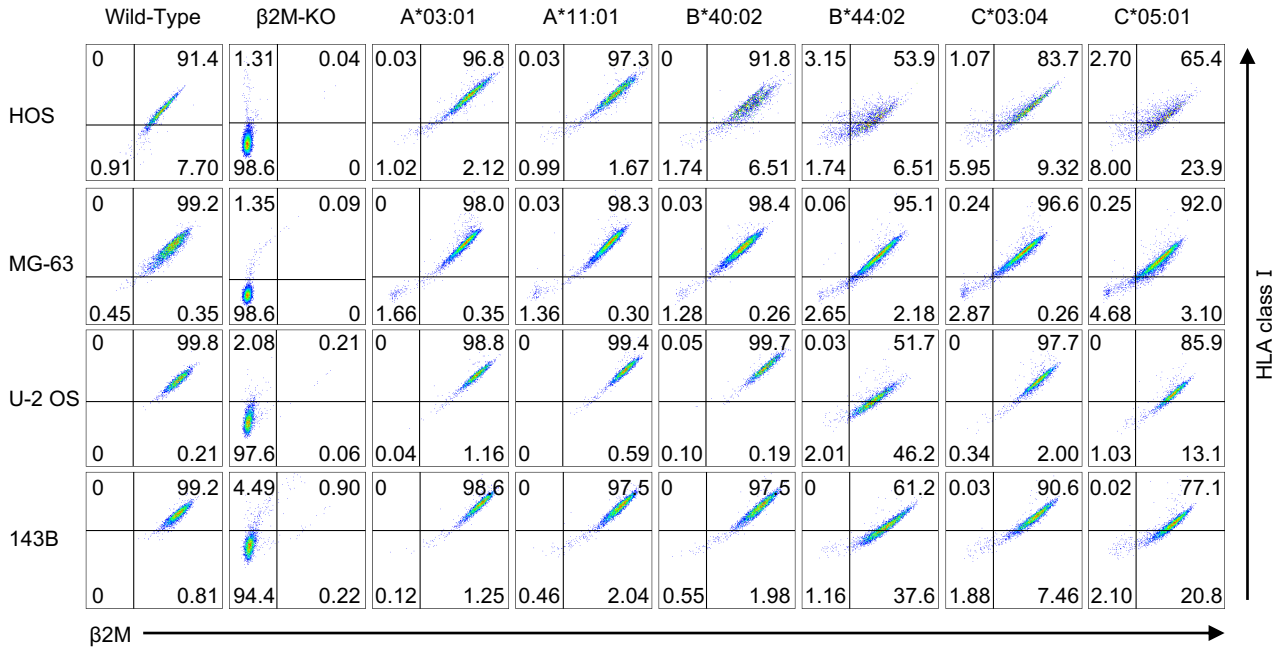


Supplementary Figure 1. Single-cell transcriptome profiling of CD4⁺ TILs in osteosarcoma.

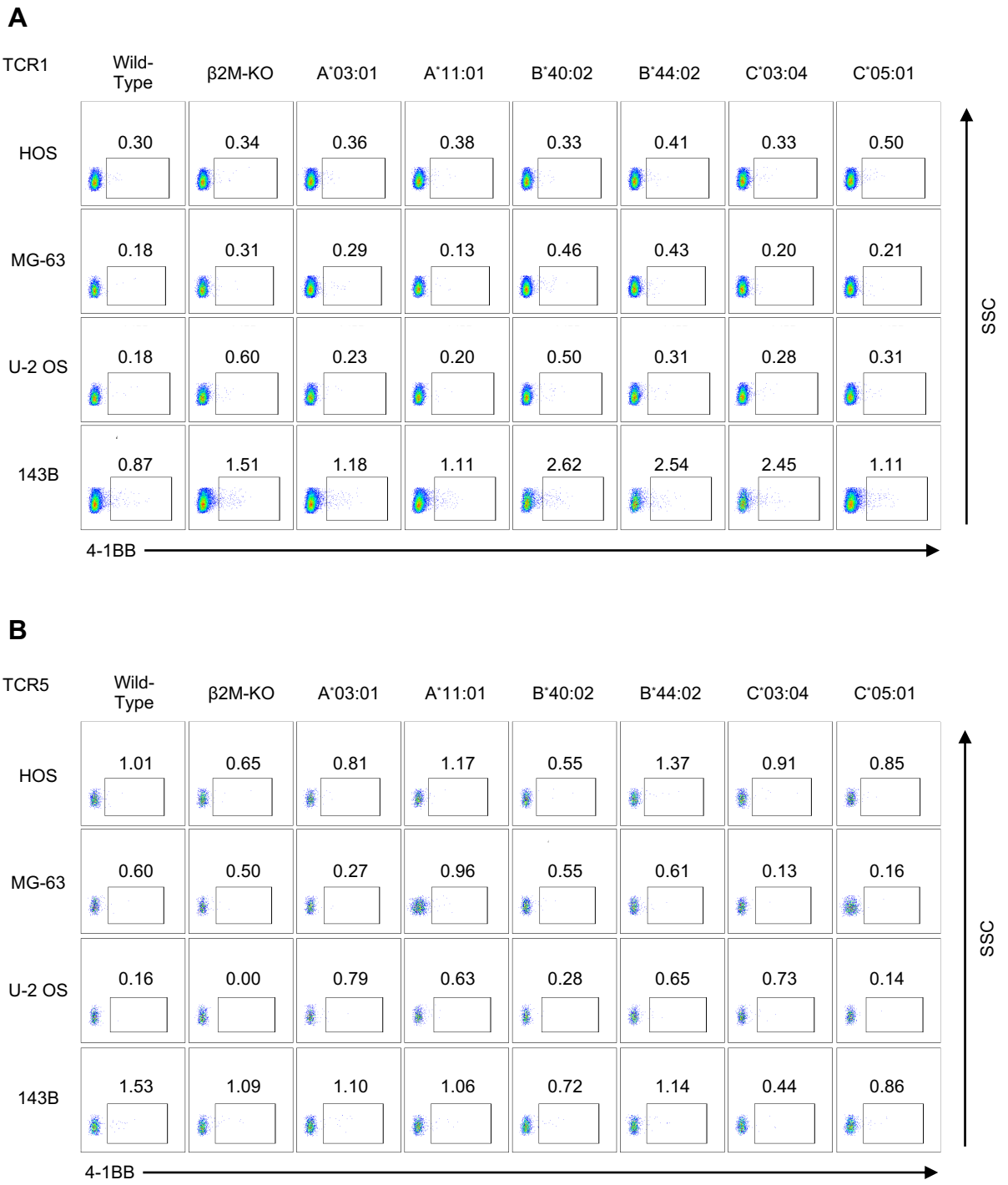
(A) Feature plots of CD4⁺ T cells showing representative marker genes associated with T cell identity, effector function, activation, exhaustion, and memory states.

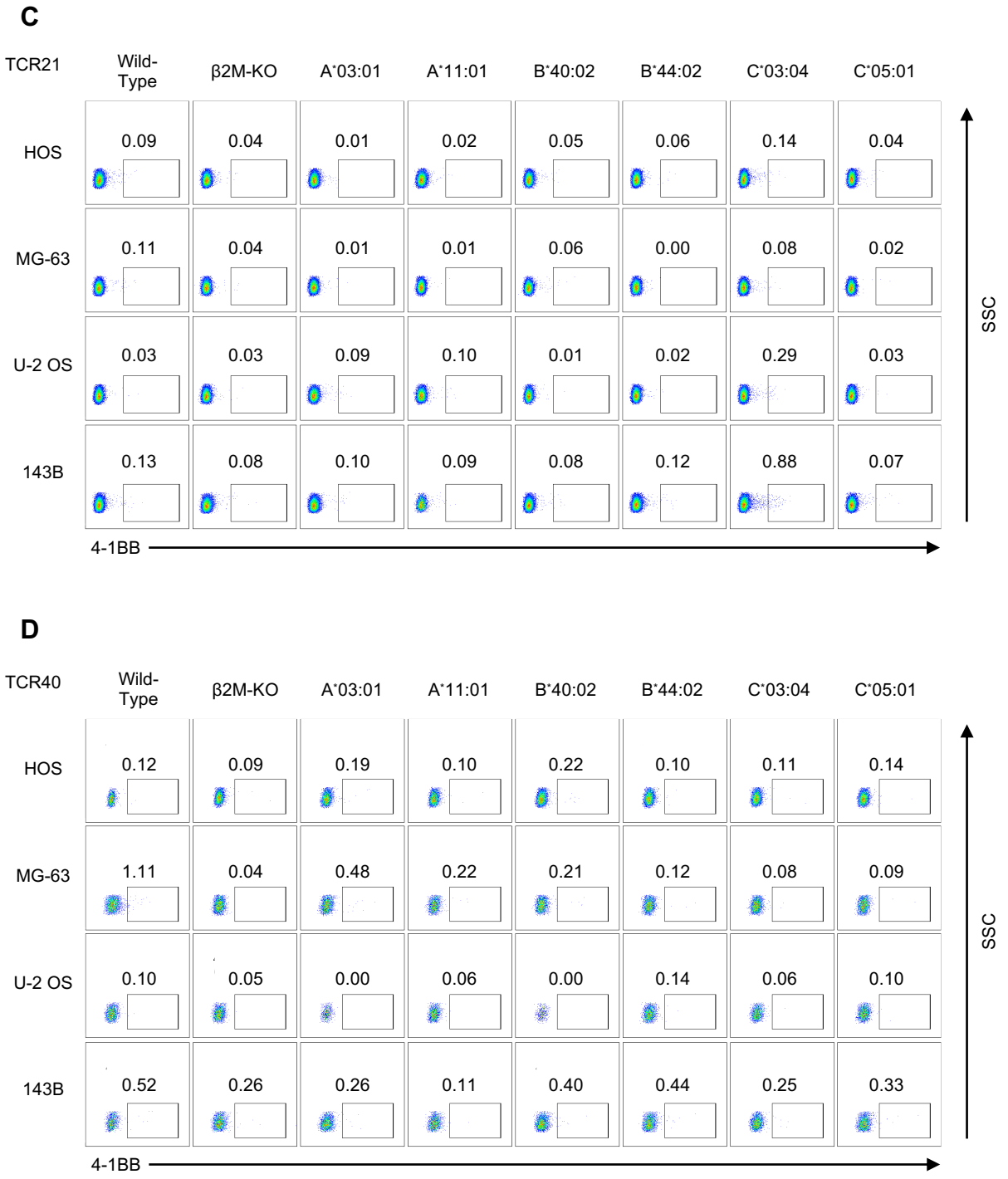
(B) Representative flow cytometry plots showing the transduction efficiency of TCRs.



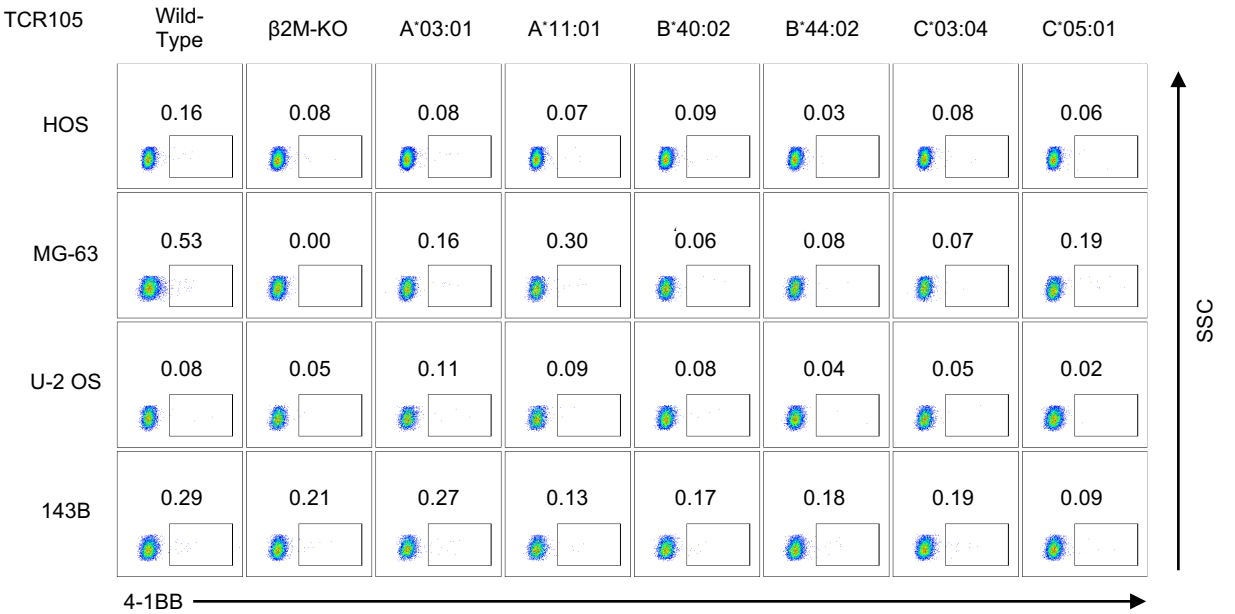
Supplementary Figure 2. Generation of osteosarcoma cell lines expressing a single HLA class I.

Expression of β2M and HLA class I in osteosarcoma cell lines transduced with a single HLA class I allele.


A
TCR1
Wild-Type
β2M-KO
A*03:01
A*11:01
B*40:02
B*44:02
C*03:04
C*05:01
HOS
0.30
0.34
0.36
0.38
0.33
0.41
0.33
0.50
MG-63
0.18
0.31
0.29
0.13
0.46
0.43
0.20
0.21
U-2 OS
0.18
0.60
0.23
0.20
0.50
0.31
0.28
0.31
143B
0.87
1.51
1.18
1.11
2.62
2.54
2.45
1.11
SSC
4-1BB
B
TCR5
Wild-Type
β2M-KO
A*03:01
A*11:01
B*40:02
B*44:02
C*03:04
C*05:01
HOS
1.01
0.65
0.81
1.17
0.55
1.37
0.91
0.85
MG-63
0.60
0.50
0.27
0.96
0.55
0.61
0.13
0.16
U-2 OS
0.16
0.00
0.79
0.63
0.28
0.65
0.73
0.14
143B
1.53
1.09
1.10
1.06
0.72
1.14
0.44
0.86
SSC
4-1BB


C
TCR21
Wild-Type
β2M-KO
A*03:01
A*11:01
B*40:02
B*44:02
C*03:04
C*05:01
HOS
0.09
0.04
0.01
0.02
0.05
0.06
0.14
0.04
MG-63
0.11
0.04
0.01
0.01
0.06
0.00
0.08
0.02
U-2 OS
0.03
0.03
0.09
0.10
0.01
0.02
0.29
0.03
143B
0.13
0.08
0.10
0.09
0.08
0.12
0.88
0.07
SSC
4-1BB
D
TCR40
Wild-Type
β2M-KO
A*03:01
A*11:01
B*40:02
B*44:02
C*03:04
C*05:01
HOS
0.12
0.09
0.19
0.10
0.22
0.10
0.11
0.14
MG-63
1.11
0.04
0.48
0.22
0.21
0.12
0.08
0.09
U-2 OS
0.10
0.05
0.00
0.06
0.00
0.14
0.06
0.10
143B
0.52
0.26
0.26
0.11
0.40
0.44
0.25
0.33
SSC
4-1BB

E

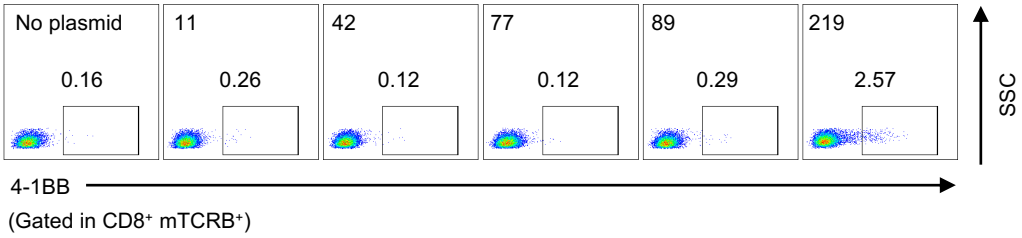

TCR105
Wild-Type
β2M-KO
A*03:01
A*11:01
B*40:02
B*44:02
C*03:04
C*05:01
HOS
MG-63
U-2 OS
143B
0.16 0.08 0.08 0.07 0.09 0.03 0.08 0.06
0.53 0.00 0.16 0.30 0.06 0.08 0.07 0.19
0.08 0.05 0.11 0.09 0.08 0.04 0.05 0.02
0.29 0.21 0.27 0.13 0.17 0.18 0.19 0.09
SSC
4-1BB

F


TCR110
Wild-Type
β2M-KO
A*03:01
A*11:01
B*40:02
B*44:02
C*03:04
C*05:01
HOS
MG-63
U-2 OS
143B
0.16 0.08 0.08 0.08 0.09 0.03 0.08 0.06
0.53 0.00 0.16 0.30 0.06 0.08 0.07 0.19
0.08 0.05 0.11 0.09 0.08 0.04 0.05 0.02
0.29 0.21 0.27 0.13 0.17 0.18 0.19 0.09
SSC
4-1BB

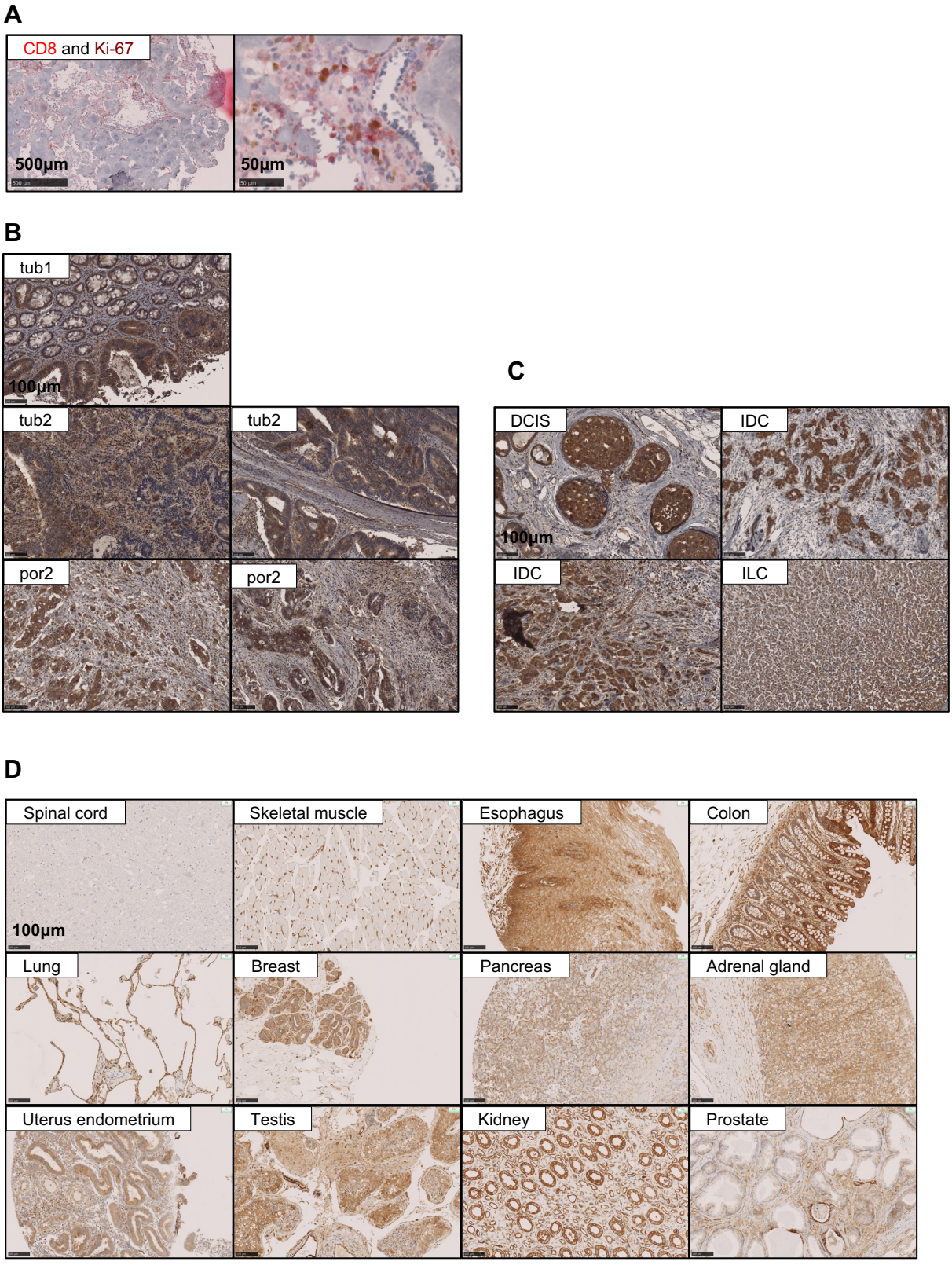
Supplementary Figure 3. Co-culture assay of TCR-T cells and osteosarcoma cell lines.

(A–F) Representative flow cytometry plots showing 4-1BB upregulation in TCR-T cells following co-culture with osteosarcoma cell lines. TCR1 (**A**), TCR5 (**B**), TCR21 (**C**), TCR40 (**D**), TCR105 (**E**), and TCR110 (**F**) were co-cultured with wild-type, β2M-KO, or the indicated monoallelic HLA–expressing osteosarcoma cell lines. The percentages indicate the proportion of 4-1BB^{+} cells among TCR-T cells. Data are representative of two independent experiments.



Supplementary Figure 4. Flow cytometric analysis of 4-1BB upregulation in cDNA library screening.

Representative flow cytometry plots showing 4-1BB upregulation in TCR29 co-cultured with 293T/β2m-KO/B*40:02 cells transfected with the indicated cDNA library pools. The heatmap shown in Fig. 2B was generated from these flow cytometry data.


A
CD8 and Ki-67
500µm
50µm
B
tub1
100µm
tub2
tub2
por2
por2
C
DCIS
IDC
100µm
IDC
ILC
D
Spinal cord
100µm
Skeletal muscle
Esophagus
Colon
Lung
Breast
Pancreas
Adrenal gland
Uterus endometrium
Testis
Kidney
Prostate

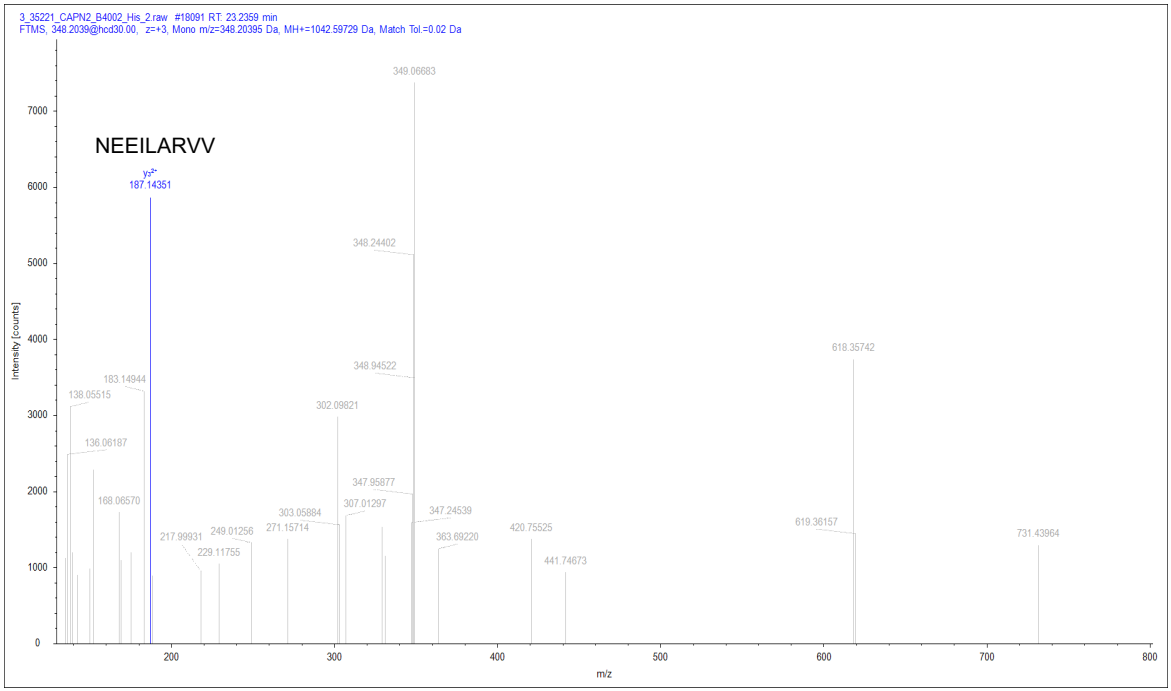
Supplementary Figure 5. Immunohistochemical analysis of calpain-2 in normal and cancer tissues.

(A) CD8 and Ki-67 double immunohistochemical staining in osteosarcoma specimens.

(B) Calpain-2 staining in colon cancer tissues of different histological subtypes: tub1 (well-differentiated tubular adenocarcinoma), tub2 (moderately differentiated tubular adenocarcinoma), and por2 (non-solid-type poorly differentiated adenocarcinoma).

(C) Calpain-2 staining in breast cancer tissues of different histological subtypes: DCIS (ductal carcinoma in situ), IDC (invasive ductal carcinoma), and ILC (invasive lobular carcinoma).

(D) Calpain-2 immunohistochemical staining in normal tissues.



Supplementary Figure 6. MS/MS spectrum of the calpain2-derived peptide.

Representative MS/MS spectrum of the calpain2-derived peptide NEEILARVV identified by LC–MS/MS using higher-energy collisional dissociation (HCD). Fragment ions corresponding to b and y-ion series are indicated.

Supplementary Table 1. CDR3 sequences of TCR genes isolated from TILs

Clonotype	TRAV	CDR3α	TRAJ	TRBV	CDR3β	TRBJ
1	35*02	CAGQAGANNLFF	36*01	4-2*01	CASSLAGKNEGGYTF	1-2*01
5	1-2*01	CAVMDSNYQLIW	33*01	28*01	CASSPPTEVSGAFF	1-1*01
21	12-1*01	CVVRNSGYALNF	41*01	6-5*01	CASSYSGGDEQFF	2-1*01
29	3*01	CGFSVNTPLVF	9*01	19*01	CASSPGQIGYTF	1-2*01
40	3*01	CAVRDTSGGKLIF	23*01	7-8*01	CASKSPGVGYEQYF	2-7*01
105	20*02	CAVQANRGSTLGRLYF	18*01	19*01	CASSRNRGDYEAFF	1-1*01
110	12-2*02	CAVKAYAGNMLTF	39*01	7-3*01	CASSLSQGYEQYF	2-7*01