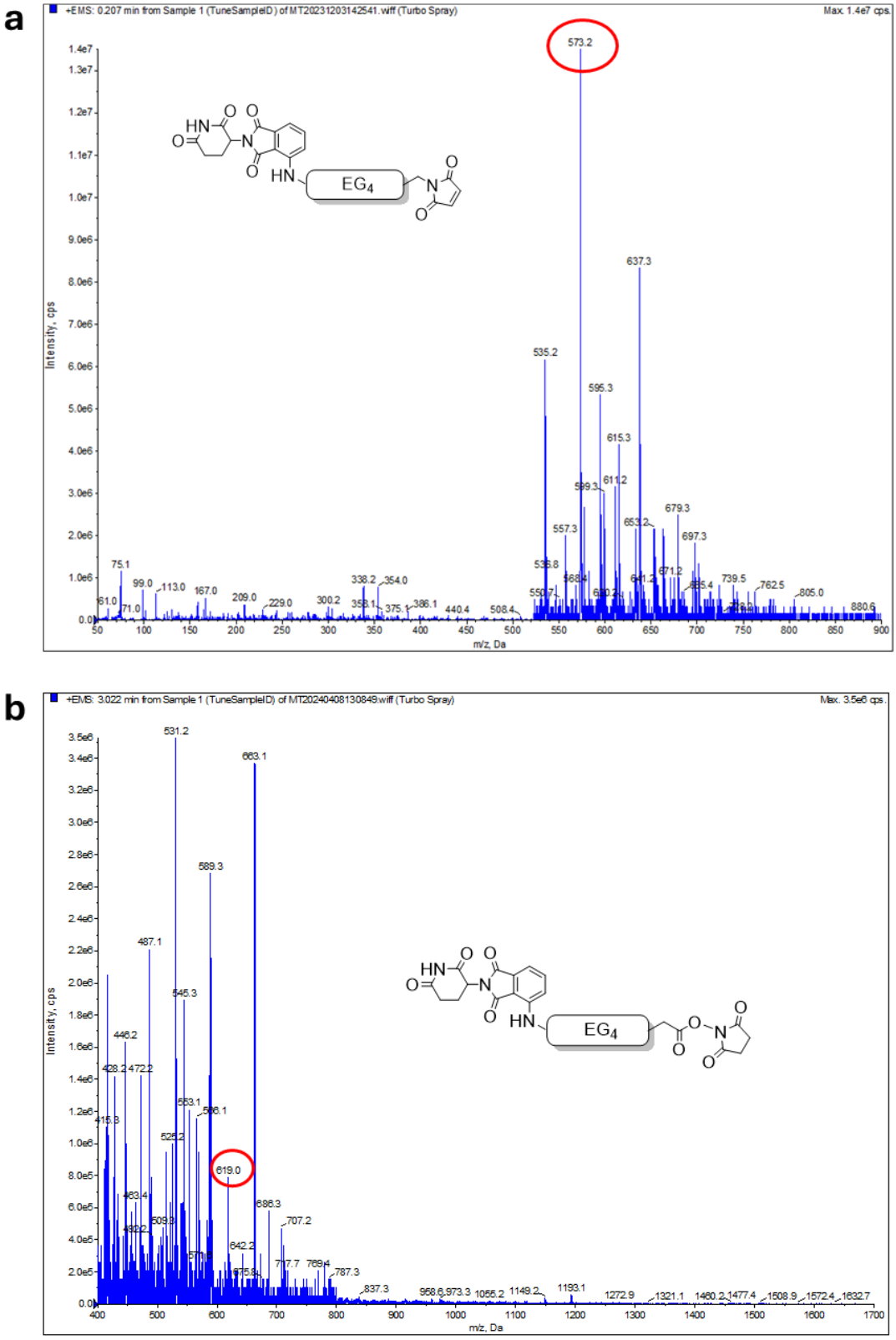
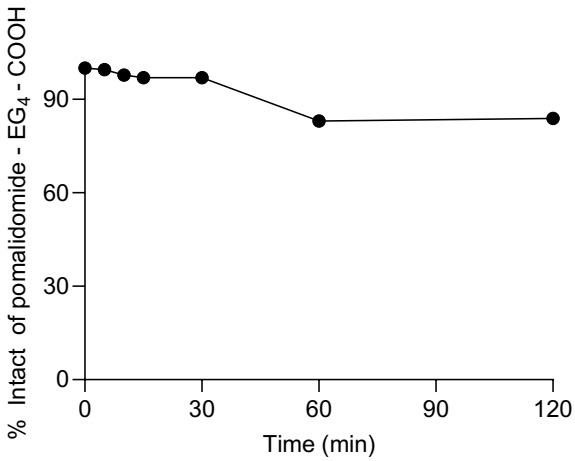
Supplementary Information

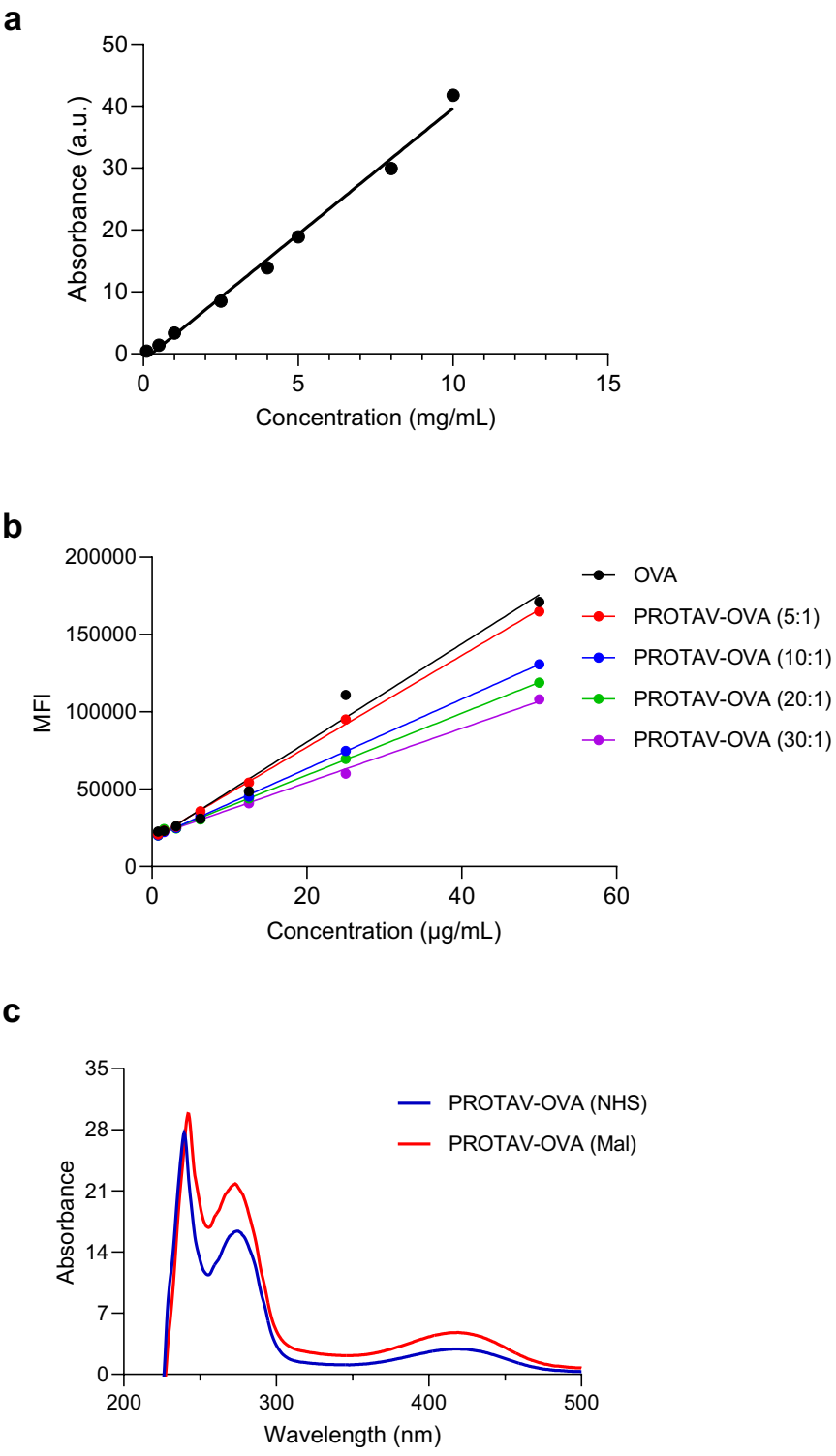
Table of Content



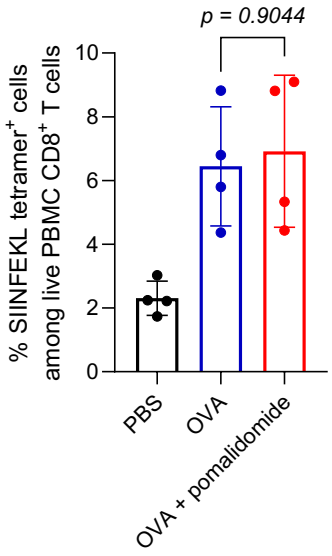
Supplementary Fig. 1 | Chemical structures and ESI MS spectra of pomalidomide-EG$_4$-Mal (a) and pomalidomide-EG$_4$-NHS (b).



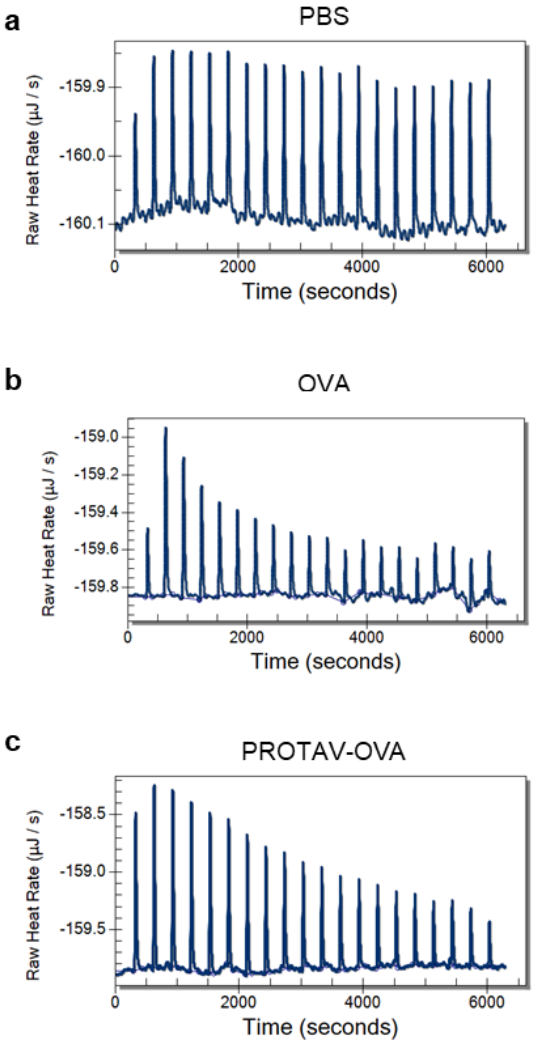
Supplementary Fig. 2 | Stability of pomalidomide-EG_4-COOH in serum measured by LC-MS. Pomalidomide-EG_4-COOH was incubated in serum for a series of durations up to 2 h, followed by LC-MS measurement to quantify the remaining intact pomalidomide-EG_4-COOH.



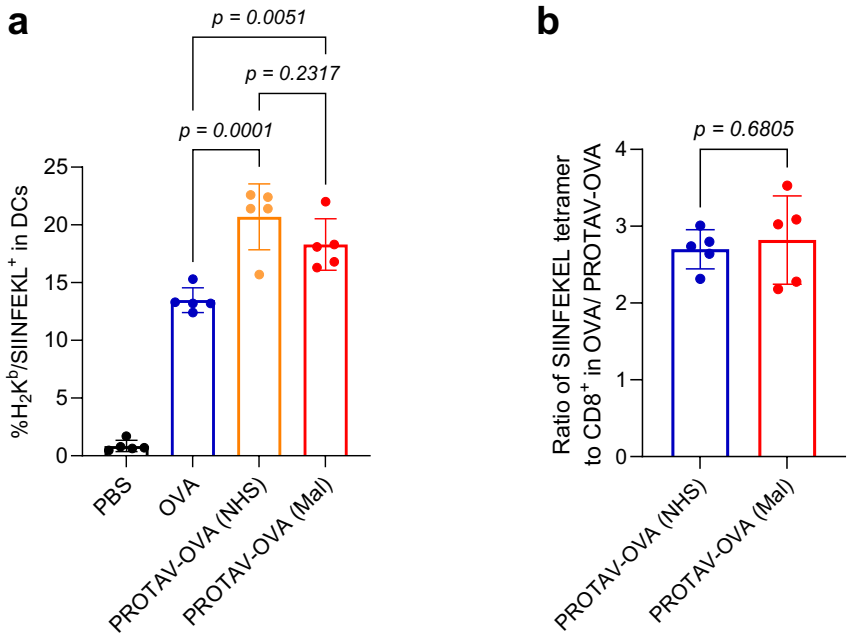
Supplementary Fig. 3 | Characterization of PTOTAV-OVA conjugation ratio. a, Standard curve of pomalidomide-EG_4 absorbance (420 nm) *vs.* concentration. **b**, MFI of amine-reactive beads used in an amine-reactive fluorescent assay. The results were used to determine the copy numbers of pomalidomide-EG_4-NHS conjugated per OVA with a series of pomalidomide-EG_4-NHS: OVA feeding molar ratios. **c**, UV-vis spectra of PROVA-OVA with two synthesis methods *via* NHS-EDC and maleimide-thiol conjugations, respectively.



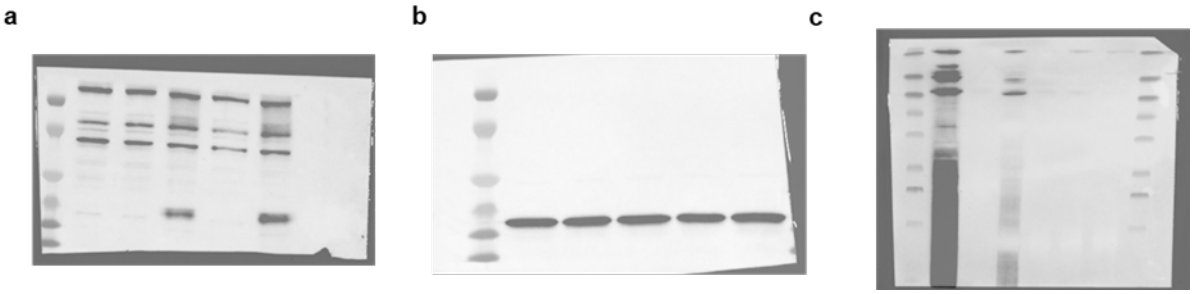
Supplementary Fig. 4 | Tetramer staining of mouse PBMC CD8+ T cells from mice immunized with OVA or physical mixture of OVA and pomalidomide (day 21). C57BL/6 mice (6-8 weeks; *n* = 4) were immunized by *s.c.* administration at tail base (day 0, day 14). CpG (2 nmole) adjuvant was mixed with OVA or pomalidomide + OVA (10 μg).



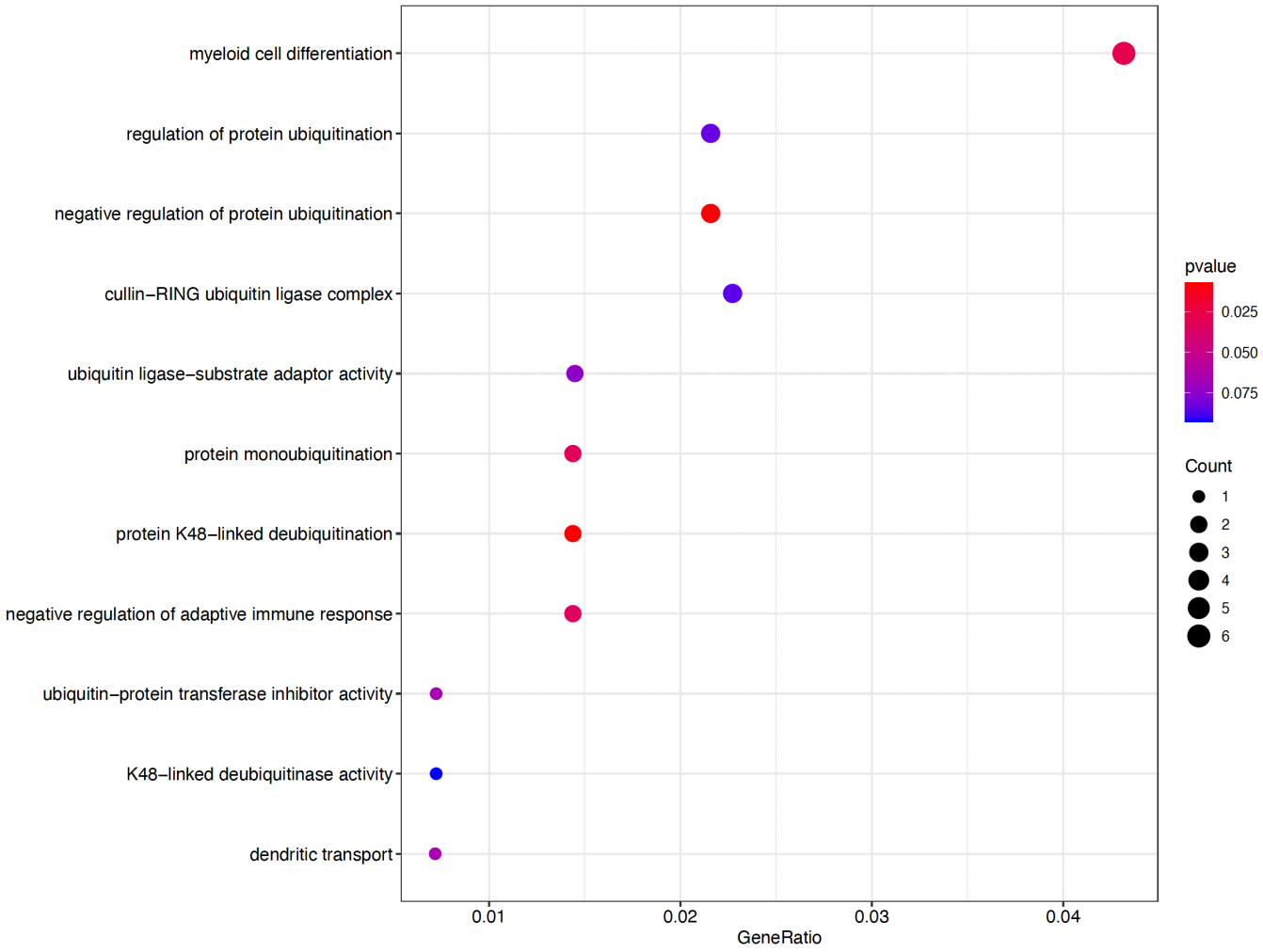
Supplementary Fig. 5 l Nano ITC results showing the CRBN binding between PROTAV-OVA and CRBN protein. Shown are nano ITC graphs of binding kinetics between CRBN and PBS (**a**), OVA (**b**), and PROTAV-OVA (**c**).



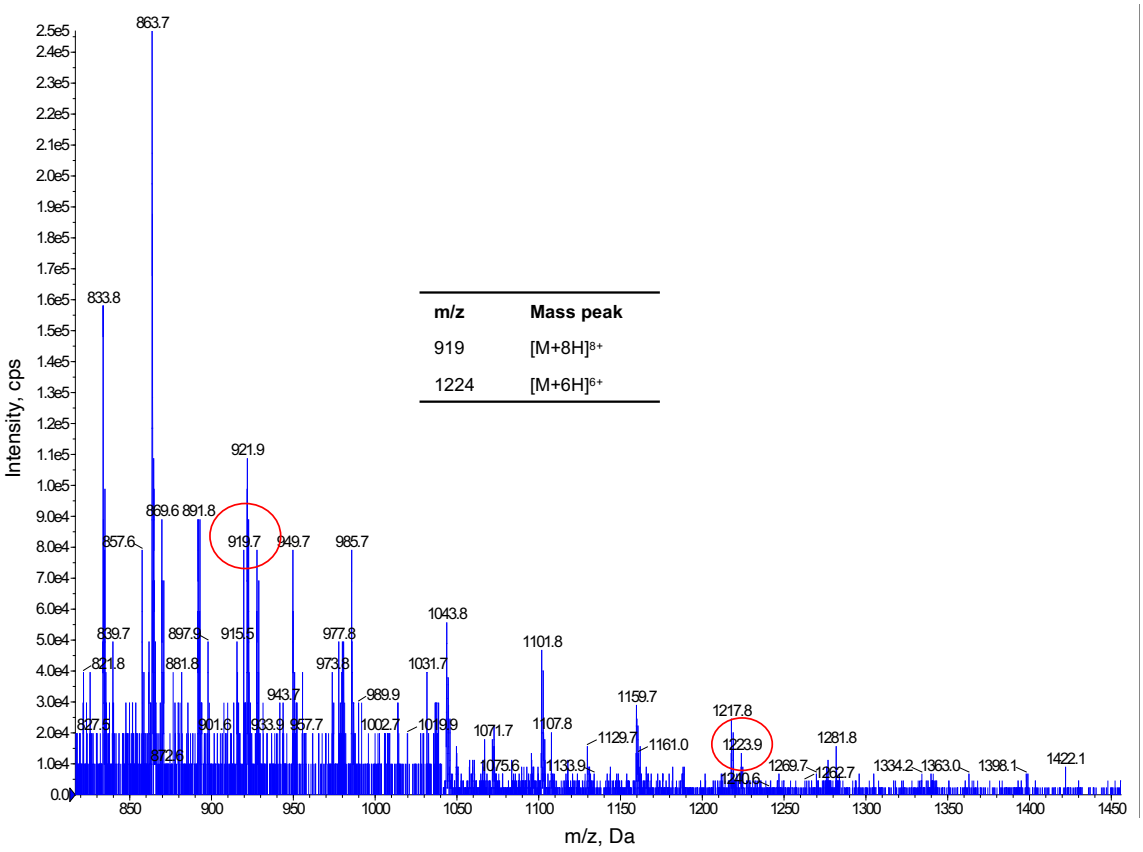
Supplementary Fig. 6 l Tetramer staining of mouse PBMC CD8⁺ T cells from mice immunized with PROVA-OVA synthesized using two conjugation methods. a, MFI of H-2Kᵇ/SIINFEKL on DCs quantified from flow cytometry data showing that PROTAV-OVA promoted the presentation of SIINFEKL antigen epitope on DC2.4 cells following a 24-h treatment. **b**, The PROTAV-OVA: OVA ratio of H-2Kᵇ/SIINFEKL tetramer-positive PBMC CD8⁺ cell fractions elicited by two PROTAV-OVA synthesized by two different conjugation methods (NHS-EDC and maleimide-thiol). These results suggest that these two conjugation chemistries enabled PROTAV-OVA for comparable enhancement of T cell responses over OVA. Mice were immunized by *s.c.* administration at tail base (day 0, day 14). PBMCs were collected for T cell analysis on day 21. CpG (2 nmole) adjuvant was mixed with OVA or PROTAV-OVA (10 μg).



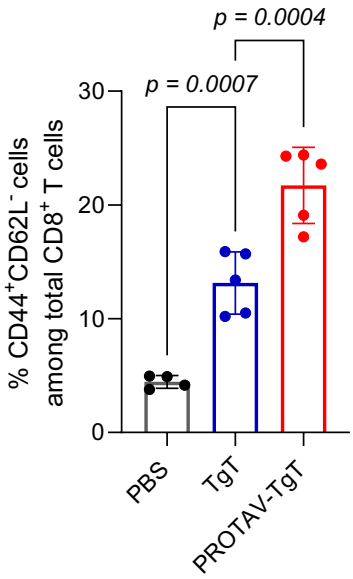
Supplementary Fig. 7 l Complete images of Western blot results shown in Fig. 3. (**a**, **b**) correspond to **Fig. 3e**, and (**c**) corresponds to **Fig. 3f**.



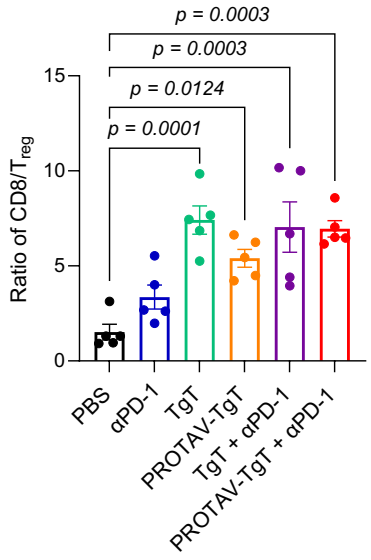
Supplementary Fig. 8 | RNA-seq GO analysis of the top 140 differentially expressed genes from BMDCs treated with PROTAV-OVA *vs.* OVA. The results indicate significant enrichment in pathways associated with ubiquitination and immunomodulation in BMDCs treated with PROTAV-OVA relative to OVA. 2 nmole/well CpG was used as an adjuvant, and all RNAseq GO analysis was conducted by using data from the treatment of CpG alone as background. Treatment: 24 h.



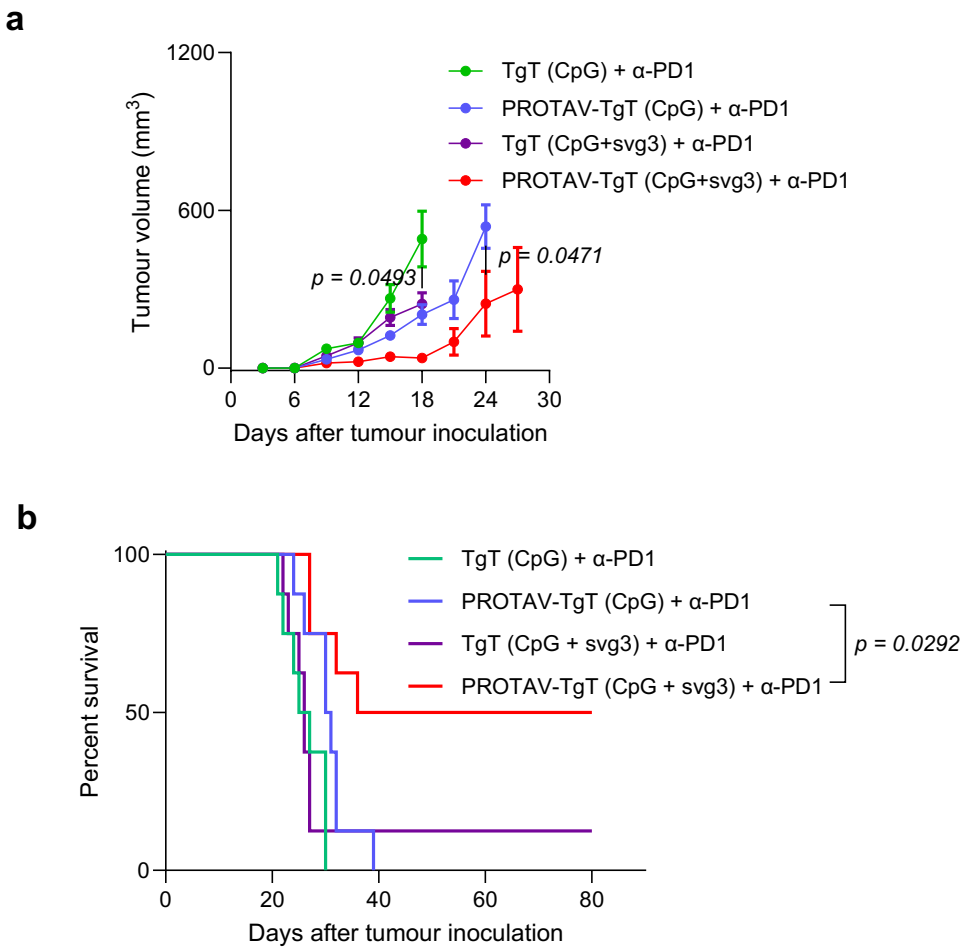
Supplementary Fig. 9 | ESI-MS spectra of PROTAV-TgT.



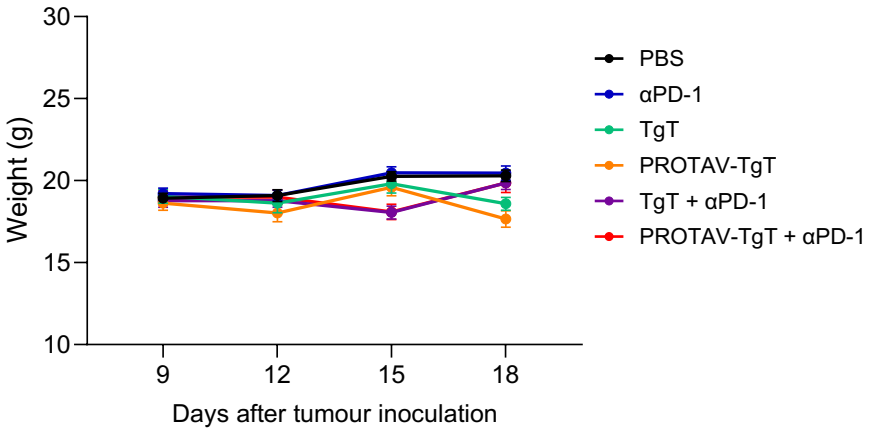
Supplementary Fig. 10 | Flow cytometry analysis of CD44 and CD62L levels on PBMC CD8⁺ T cells demonstrates that PROTAV-TgT elicited CD8⁺ T cell memory. Vaccines were delivered by SM-102 LNPs (dose: 20 µg antigen, 2 nmole CpG, 1 nmole Svg3).



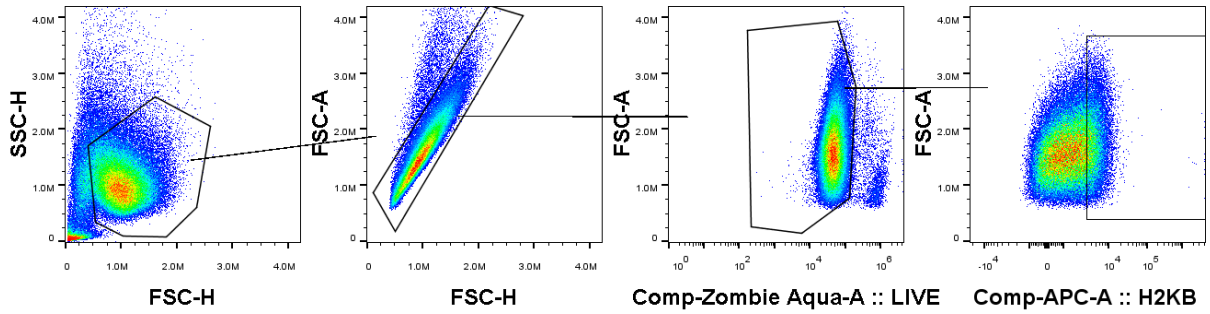
Supplementary Fig. 11 | CD8/Treg ratio in B16F10 TME following treatment with PROTAV-TgT and ICB. Vaccines were loaded in SM-102 LNPs (dose: 50 µg antigen, 2 nmole CpG, 1 nmole Svg3) and were *s.c.* injected at mouse tail base. αPD-1: 150 µg, *i.p.* administration.



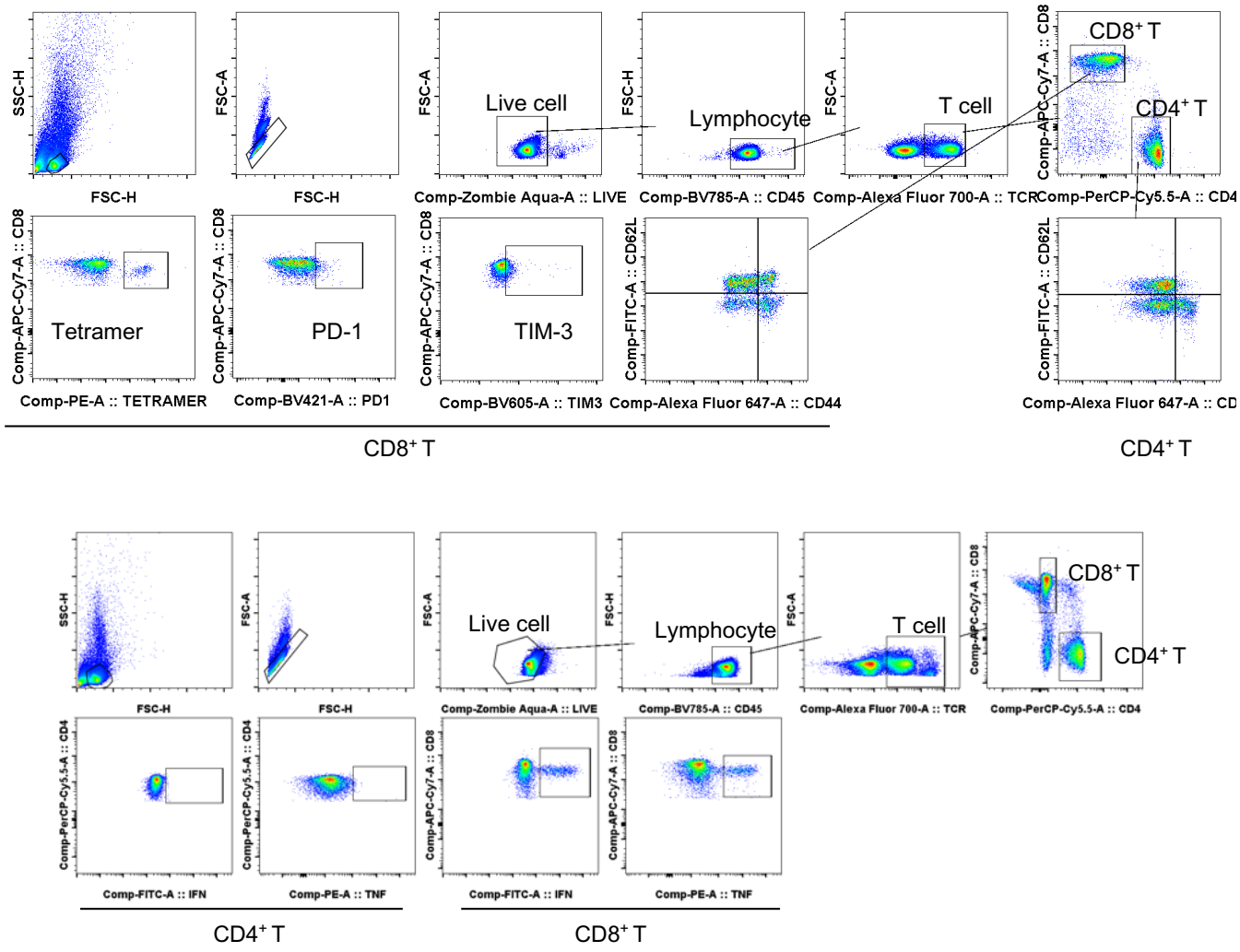
Supplementary Fig. 12 | Tumor growth curves (a) and Kaplan-Meier mouse survival curves (b) of B16F10 melanoma-bearing C57BL/6 mice after the indicated αPD-1 combination therapies with PROTAV-TgT or TgT with CpG single adjuvant or Svg3/CpG biadjuvant. PROTAV-TgT with biadjuvant Svg3/CpG outperformed PROTAV-TgT with single adjuvant CpG to inhibit tumor growth. Vaccines were loaded in SM-102 LNPs (dose: 50 μg antigen, 2 nmole CpG, 1 nmole Svg3) and were *s.c.* injected at mouse tail base. αPD-1: 150 μg, *i.p.* administration.



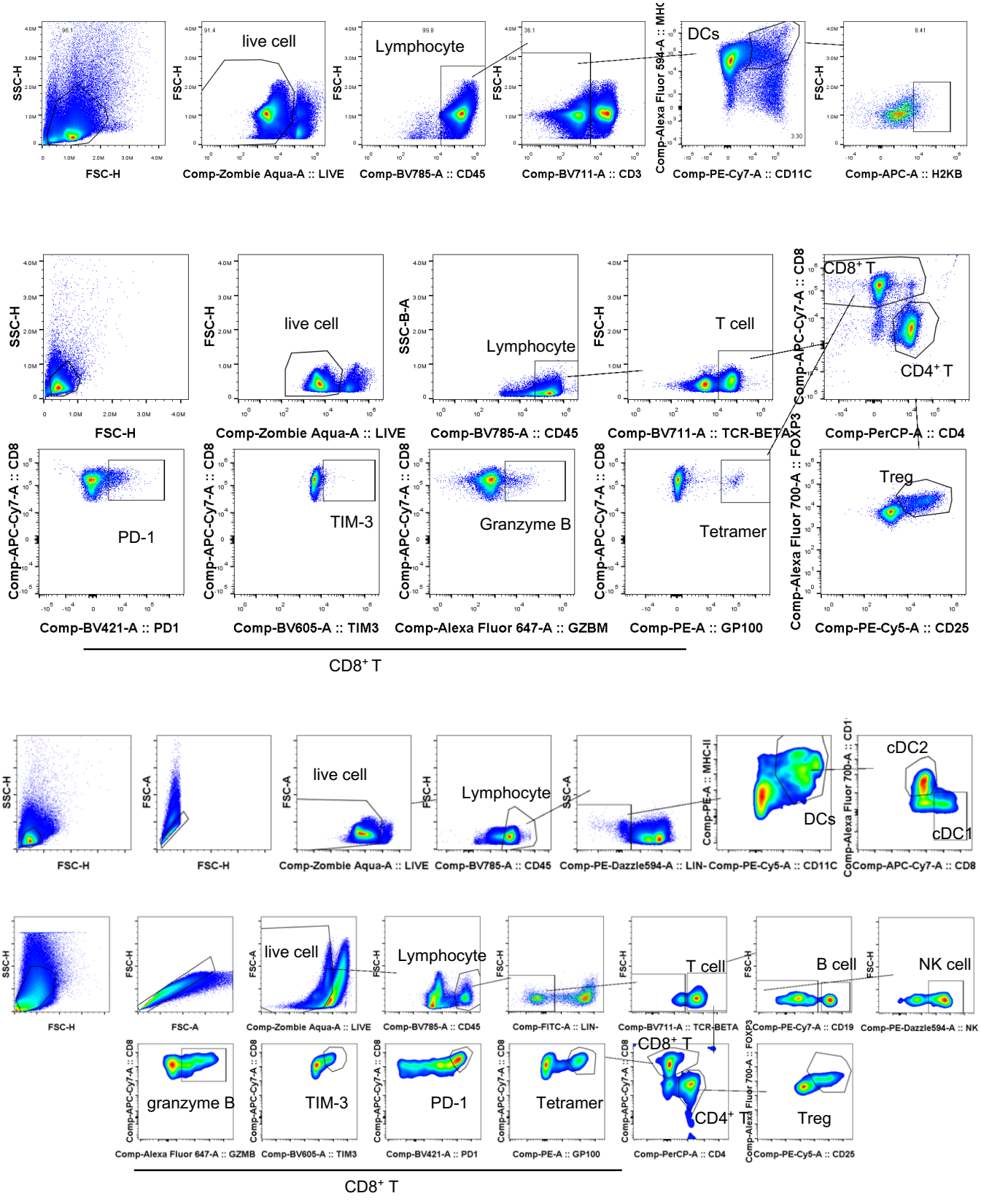
Supplementary Fig. 13 l Body weight of B16F10 tumor-bearing mice after treatment with PROTAV + ICB or controls. Vaccines were loaded in SM-102 LNPs (dose: 50 µg antigen, 2 nmole CpG, 1 nmole Svg3) and were *s.c.* injected at mouse tail base. αPD-1: 150 µg, *i.p.* administration.



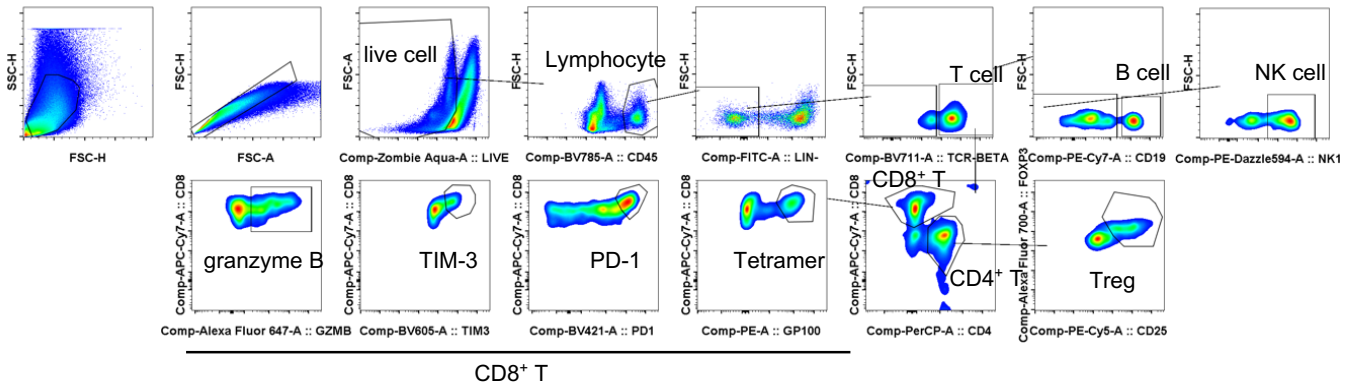
Supplementary Fig. 14 l Gating strategy for H-2K^b/SIINFEKL staining for cultured DCs.



Supplementary Fig. 15 | Gating strategy for PBMC T cell staining.



Supplementary Fig. 16 | Gating strategy for staining DCs and T cells from lymph nodes.



Supplementary Fig. 17 | Gating strategy for TME T cell staining.

Supplementary Tables

Supplementary Table 1 | Oligonucleotide sequences.

Name	Sequence (5' → 3')
CpG	TCCATGACGTTCCTGACGTT
Svg3	CAGGGGGGACCACTCTTAAGCCTCAAGGGAAGCTGGGTTGAGGCTTAAGAGTGGTCCCGGGT

Supplementary Table 2 | A list of peptides used in this study.

Peptides	Sequences
SIINFEKL	SIINFEKL
$Trp2_{180\text{-}188}$	SVYDFFVWL
$gp100_{25\text{-}33}$	KVPRNQDWL
$Trp1_{456\text{-}462}$	TAPDNLGY

Supplementary Table 3 | A list of antibodies used in this study.

Targets	Fluorochromes	Clones	Vendors	Catalogue #
CD45	Brilliant Violet 421	30-F11	BioLegend	103133
CD11c	Alexa Fluor 594	N418	BioLegend	117346
CD11b	FITC	M1/70	BioLegend	101205
CD8α	APC-Cy7	53-6.7	BioLegend	100713
CD4	PerCP-Cy5.5	GK1.5	BioLegend	100433
CD103	Brilliant Violet 605	2E7	BioLegend	121433
CD205	PE-Cy7	NLDC-145	BioLegend	138209
F4/80	APC-Cy7	BM8	BioLegend	123117
CD11b	PE-Cy5	M1/70	BioLegend	101209
NK1.1	APC	S17016D	BioLegend	156505
CD3	PerCP-Cy5.5	17A2	BioLegend	100217
CD279 (PD-1)	Brilliant Violet 421	29F.1A12	BioLegend	135217
IFN-γ	PE	XMG1.2	BioLegend	505807
TNF-α	FITC	MP6-XT22	BioLegend	506303
CD44	Alexa Fluor 647	IM7	BioLegend	103018
CD62L	FITC	MEL-14	BioLegend	104405
CD25	FITC	3C7	BioLegend	101907
FoxP3	Alexa Fluor 647	MF-14	BioLegend	126407
IL-10	Brilliant Violet 711	JES5-16E3	BioLegend	505041
MerTK	APC	2B10C42	BioLegend	151508
XCR1	APC-Cy7	ZET	BioLegend	148224
CD19	PE-Dazzle 594	6D5	BioLegend	115554
Granzyme B	APC	QA16A02	BioLegend	372203
Ubiquitin	N/A	P4D1	BioLegend	646302
CD279 (PD-1)	N/A	RMP1-14-CP162	Bio X Cell	CP162
CD152 (CTLA-4)	N/A	9H10-CP146	Bio X Cell	CP146
β-actin	N/A	15G5A11/E2	Thermo Fisher	MA1-140
OVA	N/A	N/A	Thermo Fisher	PA1-196
GAPDH	N/A	5-E10	Thermo Fisher	MA5-45076