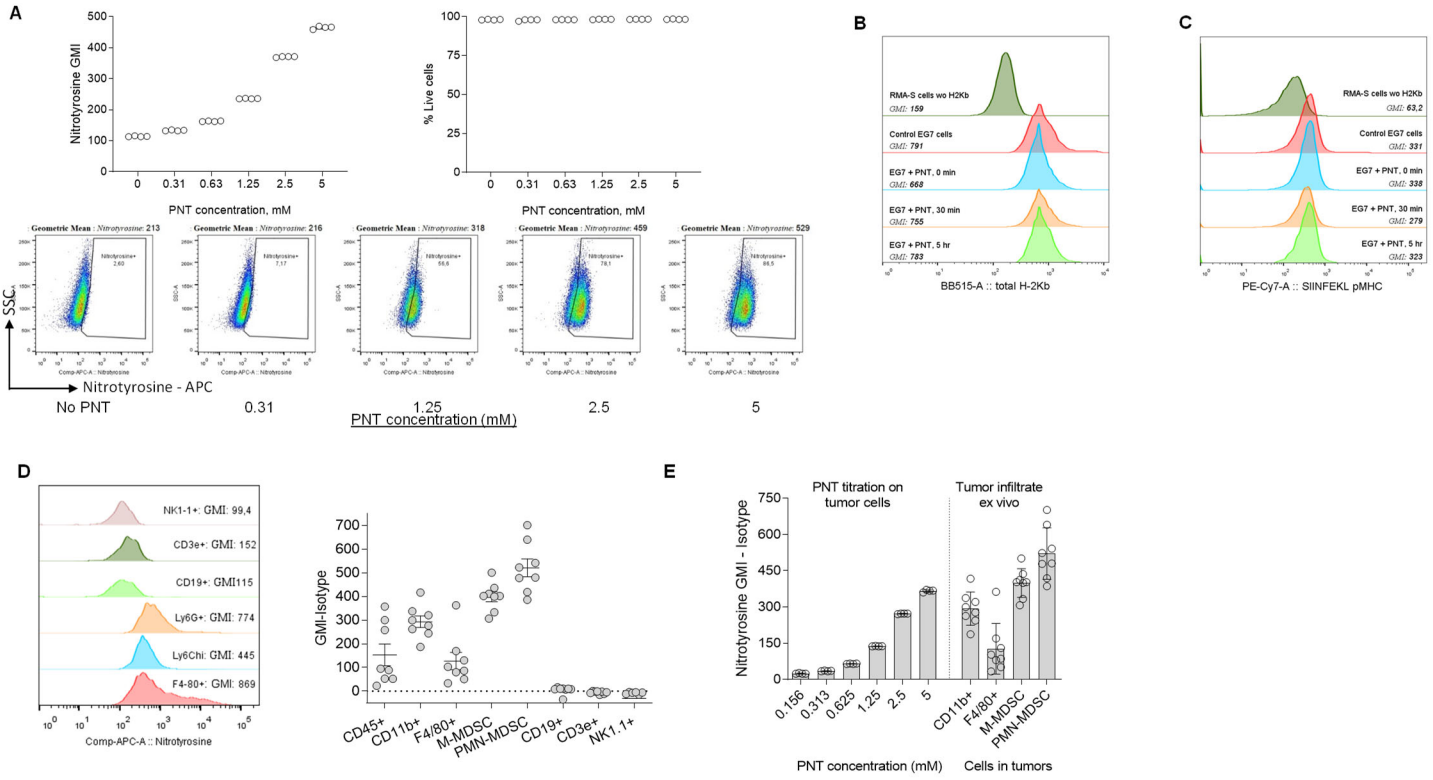
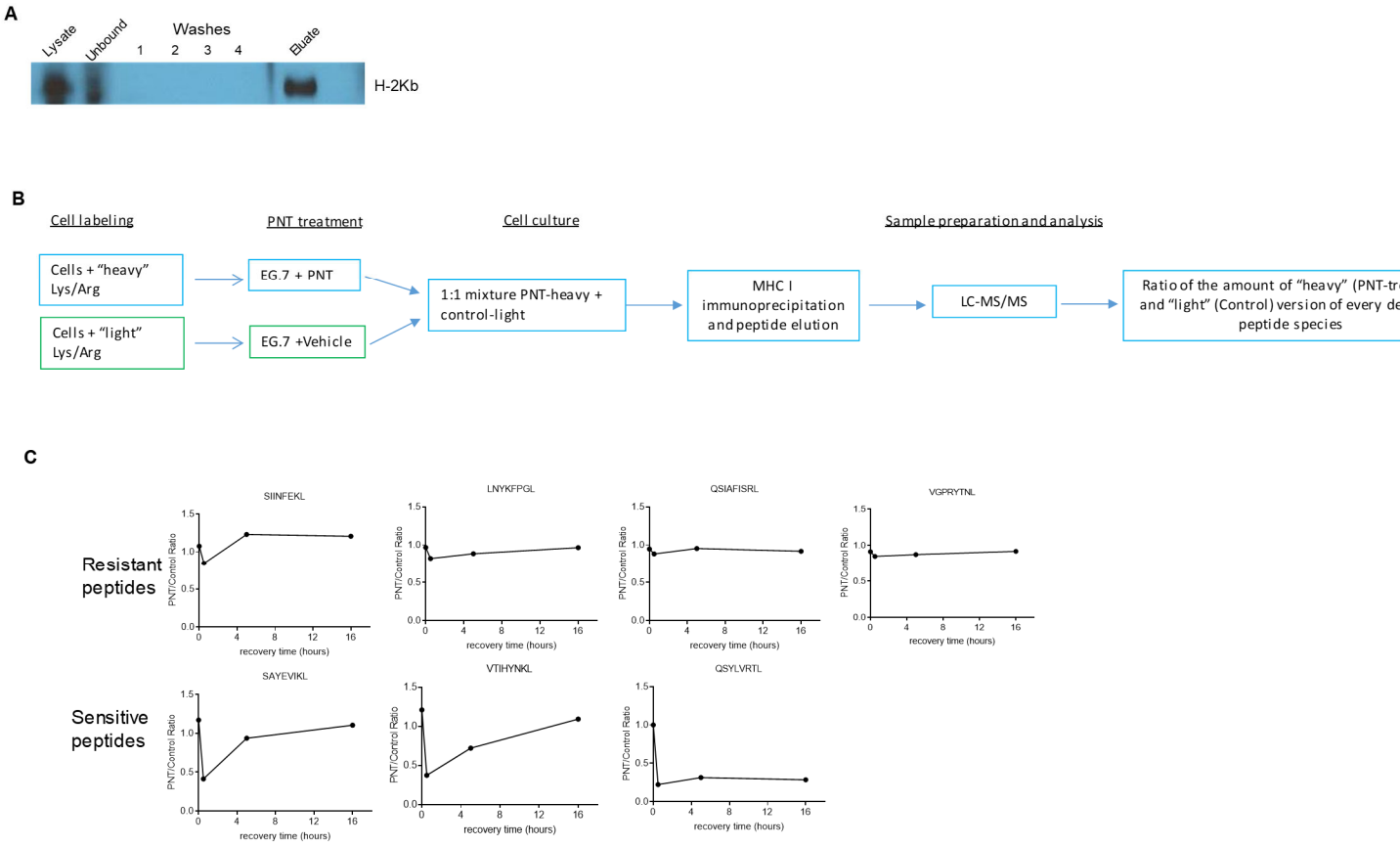
Supplementary Materials

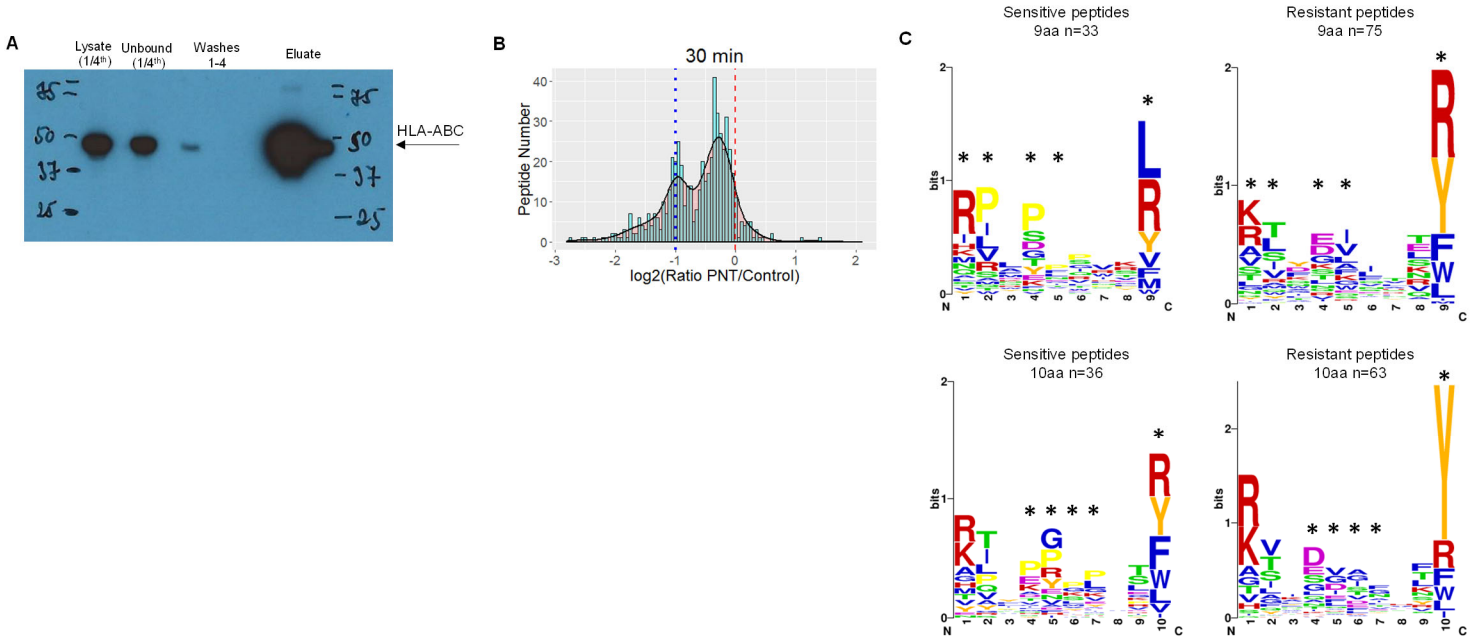


Supplementary Figure 1 related to Figure 1. PNT effects on tumor cells.
A. EG.7 cells were pre-treated with indicated concentrations of PNT and tested for viability by DAPI incorporation (right graph) and NT on cell surface (left graph). Representative flow charts for NT staining are shown on the bottom. Concentration of 2.5 mM PNT (resulting in high cell viability and high NT level) was chosen for further experiments. Typical example of 4 different experiments is shown. **B,C.** EG.7 cells were treated with PNT and expression of H-2K^b (**B**) or SIINFEKL-MHCI complex (**C**) was measured by flow cytometry at indicated time-points. RMA-S cells were used as a negative control. **D.** Mice were injected with LLC tumor cells subcutaneously. On day 19 post-injection, tumors were collected and different populations of CD45$^+$ leukocytes were analyzed for NT expression by flow cytometry. For gating the following phenotypes were used: CD11b$^+$ myeloid cells, CD11b$^+$F4/80$^+$ macrophages, CD11b$^+$ Ly6G$^-$ Ly6C^{high} M-MDSC, CD11b$^+$Ly6G$^+$ Ly6C^{low} PMN-MDSC, CD11b$^-$CD19$^+$ B cells, CD11$^+$CD3e$^+$ T cells, and CD11b$^-$NK1.1$^+$ NK-cells. The representative histogram is shown on the left and the combined data after subtraction of values of isotype Ig staining is shown on the right. N=7 for every cell population analyzed. GMI- geometric mean fluorescence intensity, **E.** EG7 cells were treated with different doses of PNT for 5 min on ice, washed 3 times and stained with anti-NT antibody followed by flow cytometry. Data shown after subtraction of background (GMI after staining with isotype Ig) and compared to the values of NT expression observed on different subsets of myeloid cells from tumor cell infiltrates. n=4 for PNT titration experiment, n=7 for the staining of NT in tumor cell infiltrate populations. GMI- geometric mean fluorescence intensity.



Supplementary Figure 2 related to Figure 1. Schema of MHC I peptide isolation and analysis.

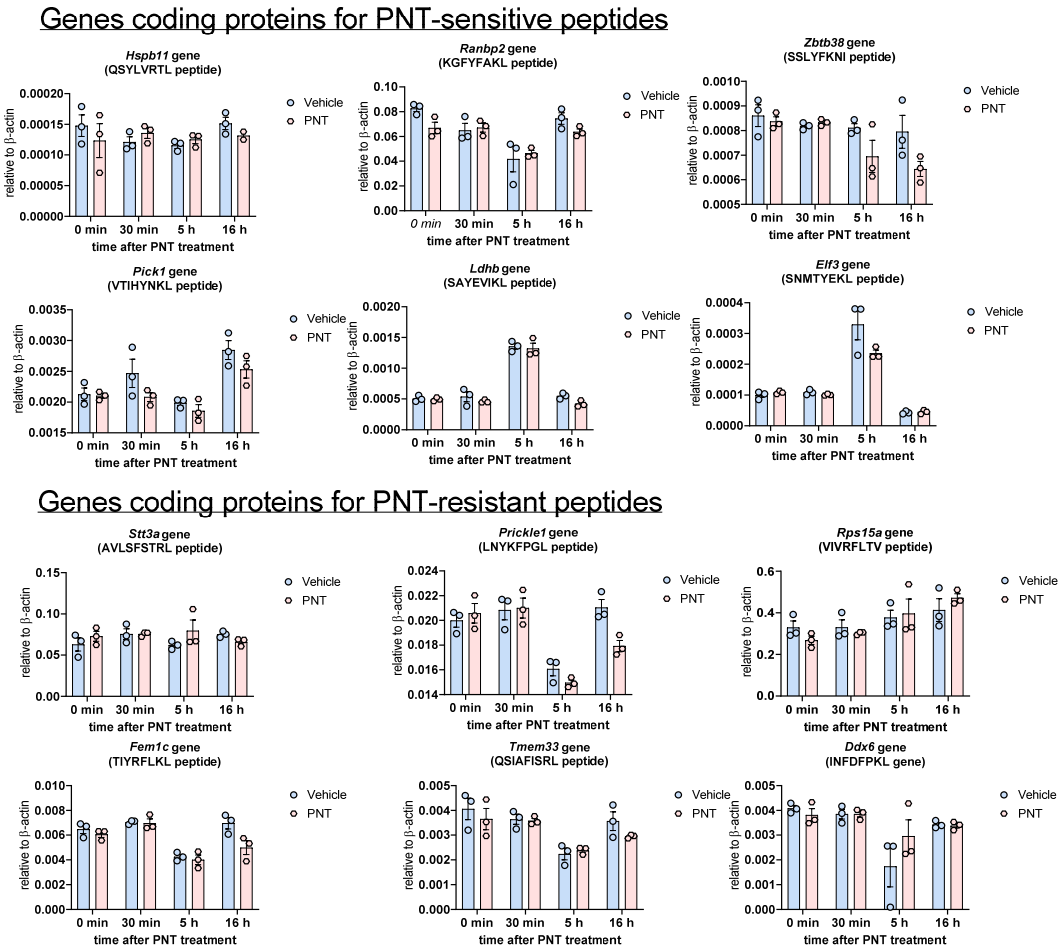
A. Representative western blot for H-2K^{b} immunoprecipitation by Y-3 antibody. **B.** The protocol for MHC I peptide analysis and study of PNT effect on MHC I peptide profile. **C.** Kinetics of ratios of representative PNT-resistant and PNT-sensitive peptides detected by LC-MS/MS in experiments in **Fig.1A**.



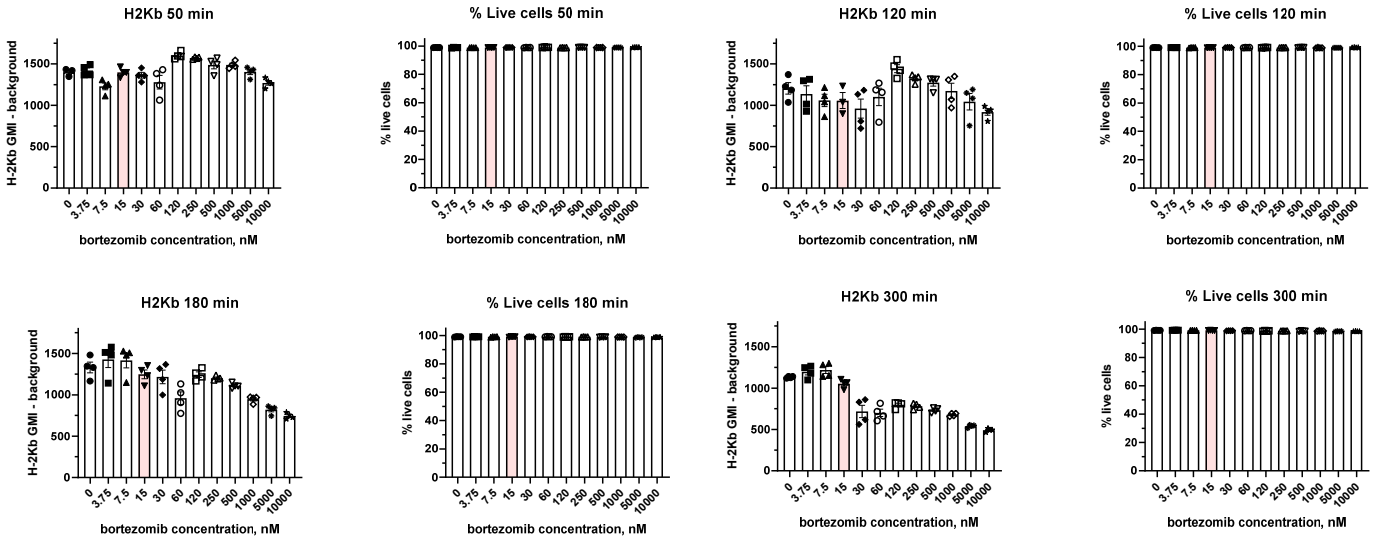
Supplementary Figure 3 related to Figure 1. The effect of PNT on MHC I peptide profile of human melanoma UACC903 cells. The experiments were performed with human melanoma UACC903 cells as described in Fig. 1A and Fig. S2B. **A.** The representative western blot for MHC I immunoprecipitation from UACC903 cells with the use of anti-HLA-A, -B, -C (clone TP25.99.8.4) antibody. **B.** the histogram of log2 (PNT/Control) ratios for the abundance of the detected peptide species. Red dashed line designates 1:1 PNT/Control ratio, blue dotted line shows the decrease of PNT-treated peptide counterpart more than 2-fold. **c.** MHC I peptides are grouped according to their lengths and PNT/control ratio (less than 0.5 are designated as "PNT-sensitive" and more than 0.8 as "PNT-resistant"), the numbers of peptides in each group are indicated. The height of each bar is proportional to the degree of amino acid conservation, and the height of each letter composing the column is proportional to its frequency at the given position. The analysis was conducted using https://weblogo.berkeley.edu/logo.cgi website. Amino acids are colored as follows: hydrophobic (blue), polar uncharged side chains (green), with electrically charged side chains (red), proline and tyrosine are marked as yellow and orange. For each position the amino acid content was compared between sensitive and resistant peptides and the significance of difference was tested with Fisher's exact test with FDR adjustment, * $p<0.05$.

Supplemental Table 3 related to Figure 1. List of peptides selected for further study.

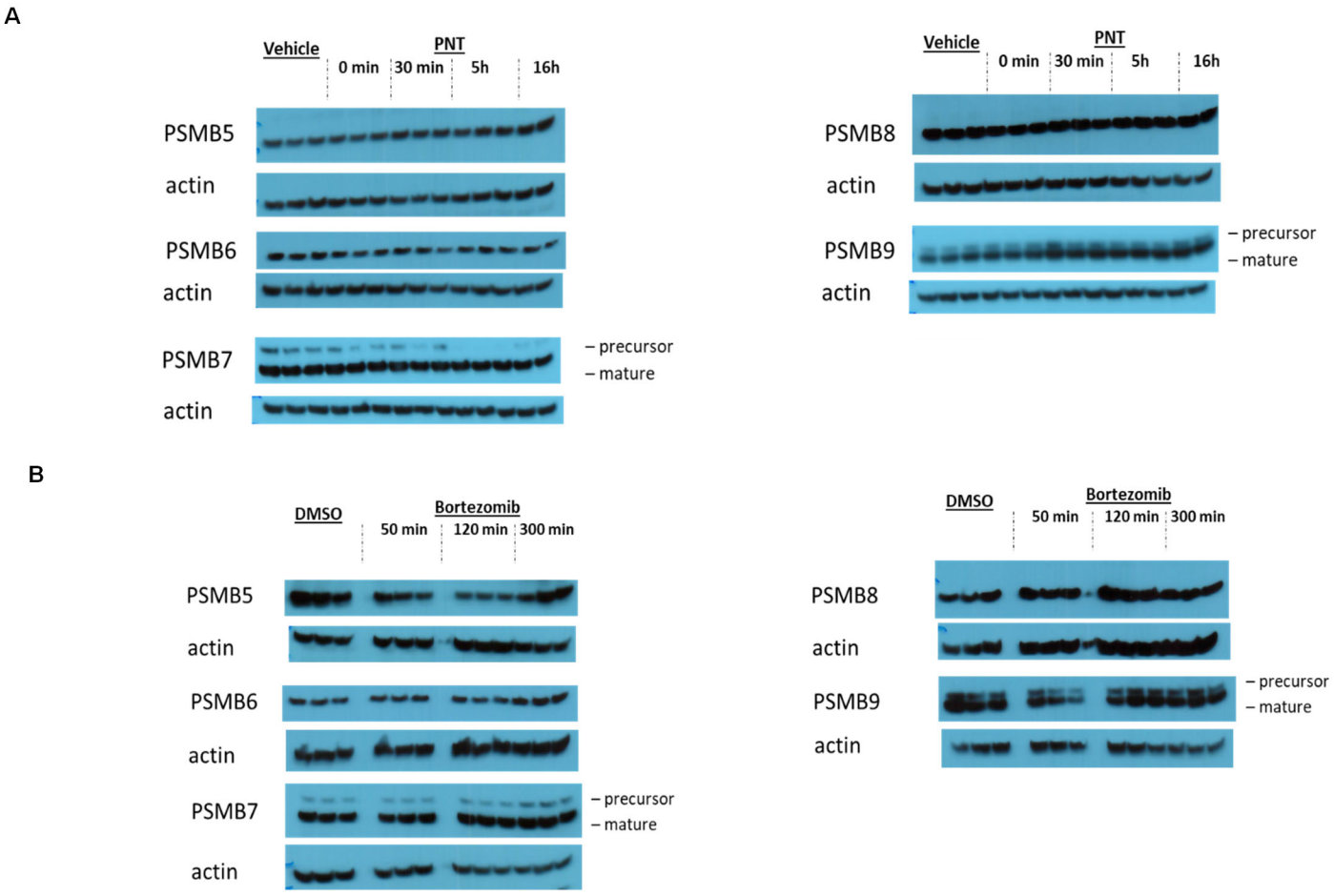
Sequence	Length	Group	IC50 NetMHC	IC50 SMM	Protein names
QSYLVRTL	8	Sensitive	30.51	80.3	Intraflagellar transport protein 25 homolog
SSLYFKNI	8	Sensitive	10.22	66.64	Zinc finger and BTB domain-containing protein 38
KGFYFAKL	8	Sensitive	3.77	11.01	E3 SUMO-protein ligase RanBP2
VTIHYNKL	8	Sensitive	14.57	31.17	Galectin;PRKCA-binding protein
SAYEVIKL	8	Sensitive	56.79	132.97	L-lactate dehydrogenase;L-lactate dehydrogenase A chain;L-lactate dehydrogenase B chain
SSYNYRGF	8	Sensitive	9.7	34.18	Choline/ethanolamine kinase;Choline kinase alpha
INLIFRYL	8	Sensitive	3.37	6.66	Small nuclear ribonucleoprotein E
VSFTYRYL	8	Sensitive	1.9	0.93	Vacuolar protein sorting-associated protein 16 homolog
SNMTYEKL	8	Sensitive	39.52	146.13	ETS-related transcription factor Elf-3
AVLSFSTRL	9	Resistant	20.1	116.17	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit STT3A
QILSDFPKL	9	Resistant	1193.63	665.44	Nucleolar GTP-binding protein 1
LNYKFPGL	8	Resistant	3.48	11.09	Prickle-like protein 1
VIVRFLTV	8	Resistant	39.99	68.67	40S ribosomal protein S15a
VGPRYTNL	8	Resistant	4.62	11.74	Mitogen-activated protein kinase;Mitogen-activated protein kinase 1
TIYRFLKL	8	Resistant	3.73	6.22	Protein fem-1 homolog C
TSLAFESRL	9	Resistant	13.29	101.88	Cytospin-B
SVIKFENL	8	Resistant	7.11	36.88	Palmitoyltransferase ZDHHC6
QSIAFISRL	9	Resistant	13.5	132.47	Transmembrane protein 33
SALRFLNL	8	Resistant	3.29	9.05	Serine/threonine-protein kinase 11-interacting protein
SSYTFPKM	8	Resistant	3.4	12.38	Enoyl-CoA delta isomerase 2, mitochondrial
INFDFPKL	8	Resistant	6.22	7.98	Probable ATP-dependent RNA helicase DDX6
TSYRFLAL	8	Resistant	2.13	1.66	HAUS augmin-like complex subunit 3
VIVEFRDL	8	Resistant	18.43	54.17	Protein arginine N-methyltransferase 7



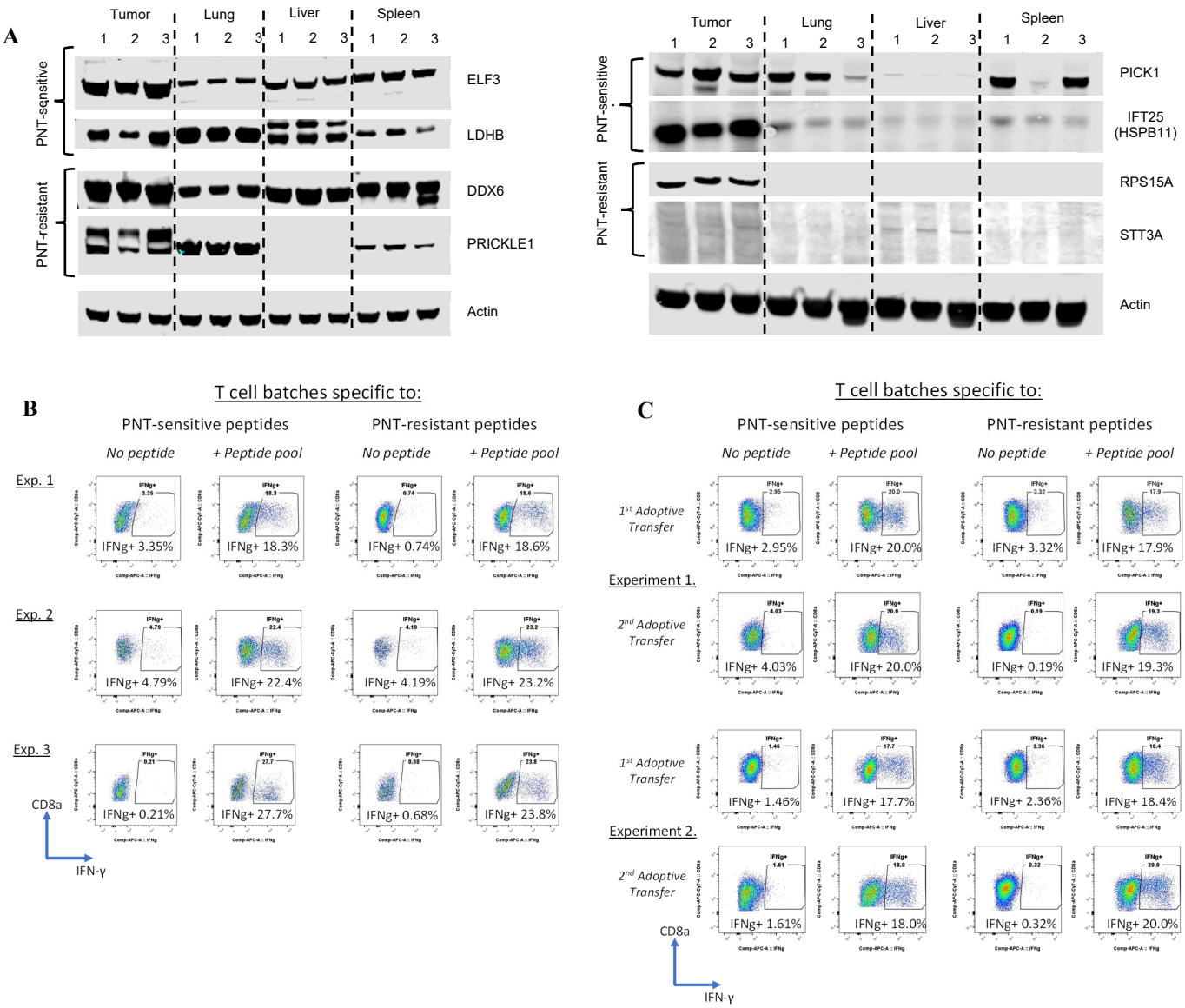
Supplementary Figure 4 related to Figure 2. Expression of genes encoding proteins containing PNT-resistant and PNT-sensitive peptides. EG.7 cells were treated with PNT or vehicle, washed, cultured for the indicated time, and lysed. The expression of the genes encoding the proteins of the representative PNT-sensitive and resistant peptide was analyzed by qRT-PCR. n=3. Mean ± SEM are shown.



Supplemental Figure 5 related to Figure 3. Effect of bortezomib on tumor cells. EG.7 cells were incubated with various concentrations of bortezomib for the indicated time, cells were washed and analyzed for H-2Kb expression and viability by flow cytometry. Based on the results, 15 nM bortezomib concentration was selected for further experiments as a concentration that doesn't affect H-2Kb expression and cell viability for the time of experiment (300 min). N=3. Mean ± SEM are shown.



Supplementary Figure 6 related to Figure 3. The effect of PNT and bortezomib on catalytic subunits of proteasomes. A. EG.7 cells were treated with PNT or vehicle, washed, cultured for the specified time and lysed for WB analysis of the indicated proteasome/immunoproteasome subunits. **B**. EG.7 cells were treated 15 nM bortezomib or vehicle for the specified time, washed, and lysed for WB analysis of the indicated proteasome/immunoproteasome subunits. Marks on the left edges of some images reflect the placement of molecular weight markers to align blots and films.



Supplementary Figure 7 related to Figure 4. Expression of proteins – source of PNT-resistant and PNT-sensitive peptides and the specificity of CD8$^+$ T cells to PNT-sensitive and resistant peptides. A. Western Blotting of different proteins that generates PNT-sensitive peptide (ELF3, LDHB, PICK1 and HSPB11) or PNT-resistant peptides (DDX6, PRICKLE1, RPS15A and STT3A) in different mouse tissue. Tissues lysates were generated from EL4-bearing mice. Results of individual mice are shown. **B,C.** Naïve C57BL/6 mice were immunized with the pool of 3 PNT-sensitive or 3 PNT-resistant peptides (50 µg/injection each) in combination with CpG (20 µg/injection s.c.) or poly-ICLC (50 µg/injection i.v.) and on day 12 after immunization mice were boosted with the same peptide pool, adjuvant and 50 µg anti-CD40 antibody. On day 23-28 after immunization, splenic and lymph node cells were collected and re-stimulated with 2 µg/ml of specific peptides pool in the presence of 5 ng/ml murine IL2. On day 6 cells were tested for specificity by overnight re-stimulation with the same pool of specific peptides in the presence of monensin and followed by intracellular staining for IFN-γ in live CD3$^+$ CD8$^+$ NK1.1$^-$ T cells. **B**. Examples of cells used in experiments *in vitro*. **C.** Examples of cells used in experiments *in*

vivo.

Supplemental Table 5 related to Figure 5. Characteristics of Peptide-DimerX^{+} CD8^{+} T cells generated by vaccinations and restimulation

Peptide loaded to DimerX multimer	Peptide group	% Peptide Dimer+ out of all CD8+	%CD62L-CD44+ (Effectors)	%CD62L+ CD44- (Naive)	%CD62L+ CD44+ (Central memory)	%CTLA4+	%PD1+	GMI CD28
QSYLVRTL	PNT-sensitive	2.59	74.4	3.4	20	9.63	27.8	897
SAYEVIKL	PNT-sensitive	3.83	80.7	3.04	14.8	8	23.1	907
SSLYFKNI	PNT-sensitive	1.98	69	7.03	22.4	15.2	34.8	1067
LNYKFPGL	PNT-resistant	2.31	66.9	1.66	29.6	10.1	26.3	1107
TIYRFLKL	PNT-resistant	1.83	82.6	1.72	13	7.78	35.9	1326
QSIAFISRL	PNT-resistant	5.4	66.7	6.81	23.2	3.11	20.5	977

CTLs were generated by vaccination of mice with pool of PNT-sensitive and resistant peptides. Peptide specific CD8^{+} T cells were evaluated by flow cytometry by using staining with indicated peptide-DimerX multimer. Two experiments for each peptide were performed.

Supplemental Table 7 Related to Figure 8. Characteristics of patients enrolled to study at University of Lausanne

Patient	Gender	Age	Melanoma type‡	TNM	Stage	Breslow
1	M	55	SSM	pT3bN3M0	IIIC	3
2	F	52	SSM	pT3aN0M0	IIA	2.7
3	M	24	SSM	pT2aN1bM0	IIIB	1.4
4	M	34	SSM	pT1aN0M0	IA	0.7
5	F	20	OM	pT2aN0M0	IB	6.8
6	M	49	SSM	pT3bN1aM0	IIIC	2.23
7	M	51	NM	pT4aN1bM0	IIIC	1.2
8	M	29	SSM	pT3aN1bM0	IIIB	2.38
9	F	64	UK	pTxN0M0	uk	n.a.
10	M	68	NM	pT3aN2bM0	IIIB	3.1
11	M	43	ARM	pT2bN3M0RO	IIIC	between 1 and 2
12	F	61	NM	pT4aN0M0	IIB	4.05
13	M	57	NM	pT3aN0M0	IIA	2.15
14	M	47	SSM	pT2aN2bM0	IIIB	1.35
15	M	61	SSM	pT4bN0M0	IIC	6.5
16	M	58	UK	pT3aN0M0	IIA	2.44
17	M	52	SSM	pT3N0M0	II	2.5

‡ARM: anorectal melanoma, NM: nodular melanoma, OM: ocular melanoma, SSM: superficial spreading melanoma;

Supplemental Table 8 related to Figure 8. Characteristics of patients enrolled to study at University of Pennsylvania

Age	Gender	Race	Ethnicity	Tissue Type/Site	Treatment Agent
69	F	White	Non-His or Latino	Small bowel	Pembrolizumab
54	M	White	Non-His or Latino	Primary skin (head), Lymph node (neck)	Pembrolizumab
45	F	White	Non-His or Latino	Primary skin/head & neck	Pembrolizumab
57	M	White	Non-His or Latino	Lymph node/head & neck	Pembrolizumab
40	F	White	Non-His or Latino	Lung	Pembrolizumab
29	F	White	Non-His or Latino	Lymph node/head & neck	Pembrolizumab
52	M	White	Non-His or Latino	Brain	Pembrolizumab
36	M	White	Non-His or Latino	Lymph node/neck	Pembrolizumab
53	M	Unknown	Non-His or Latino	Small bowel & colon	Pembrolizumab
30	F	White	Non-His or Latino	Brain	Pembrolizumab
44	F	White	Non-His or Latino	Spine	Pembrolizumab
68	M	White	Non-His or Latino	Lymph node/head & neck	Pembrolizumab
70	M	White	Non-His or Latino	Bladder	Pembrolizumab
61	M	White	Non-His or Latino	Bladder	Pembrolizumab

Supplemental Table 9 related to STAR methods. Sequence of primers used in experiments.

Protein	Primer	5' --> 3'
Hspb11	m_QSYLVRTL Fw	5' GAGGCAATGCAATGTAAAGGCTA 3
	m_QSYLVRTL Rev	5' CCTGTGGTGGTCCAAAAGGT 3'
Zbtb38	m_SSLYFKNI Fw	5' CGTCTGTCCGTTGGGACAAT 3'
	m_SSLYFKNI Rev	5' ACTGTCATCTCAGAACACGCC 3'
Ranbp2	m_KGFYFAKL Fw	5' TAACTCCCACCAAGGGTCCA 3'
	m_KGFYFAKL Rev	5' TGGTGGTGTGTGCATCTGTT 3'
Pick1	m_VTIHYNKL Fw	5' CGAGTGAGCACAGGCAACTA 3'
	m_VTIHYNKL Rev	5' CGATGTCCTGGACGTGCTT 3'
Ldhb	m_SAYEVIKL Fw	5' GTACACGGAGACCTCGGTATTAT 3
	m_SAYEVIKL Rev	5' TCCGCCAAGTCCTTCATTAAGAT 3'
Elf3	m_SNMTYEKL Fw	5' CTCCTCCGACTACCTTTGGC 3'
	m_SNMTYEKL Rev	5' GGATCTTGTCTGAGGTCCTGG 3'
Stt3a	m_AVLSFSTRL Fw	5' TGCTGGTATTGGCACCTGTT 3'
	m_AVLSFSTRL Rev	5' CCACTCGCCACCTCATTCTT 3'
Prickle1	m_LNYKFPGL Fw	5' TGTGGGGAGCATATTGGTGTG 3'
	m_LNYKFPGL Rev	5' CAGCAACGAGGCCTTACACT 3'
Rps15a	m_VIVRFLTV Fw	5' AAGGTTGAACAAGTGTGGCG 3'
	m_VIVRFLTV Rev	5' AAACTGACGTGAAGGGAGCA 3'
Fem1c	m_TIYRFLKL Fw	5' AGTGTTAAAGGCAACACTGCAT 3'
	m_TIYRFLKL Rev	5' TGTCTTGCTTGTCTGGGCAT 3'
Tmem33	m_QSIAFISRL Fw	5' CATAGGGGGACTTCTGCGG 3'
	m_QSIAFISRL Rev	5' GTCATCATGAATTGCACAGCG 3'
Ddx6	m_INFDFPKL Fw	5' AATAAAGGACCGAGGGTGCG 3'
	m_INFDFPKL Rev	5' ACTCACTTCAATCCCACGCC 3'