MEK inhibition enhances presentation of targetable MHC-I tumor antigens in mutant melanomas

Stopfer & Rettko et al.

SI Appendix

This file contains:
Supplementary Figures & legends
Supplementary Methods
Supplementary References
Supplementary Data legends
Supplementary Tables & legends

SUPPLEMENTARY FIGURES

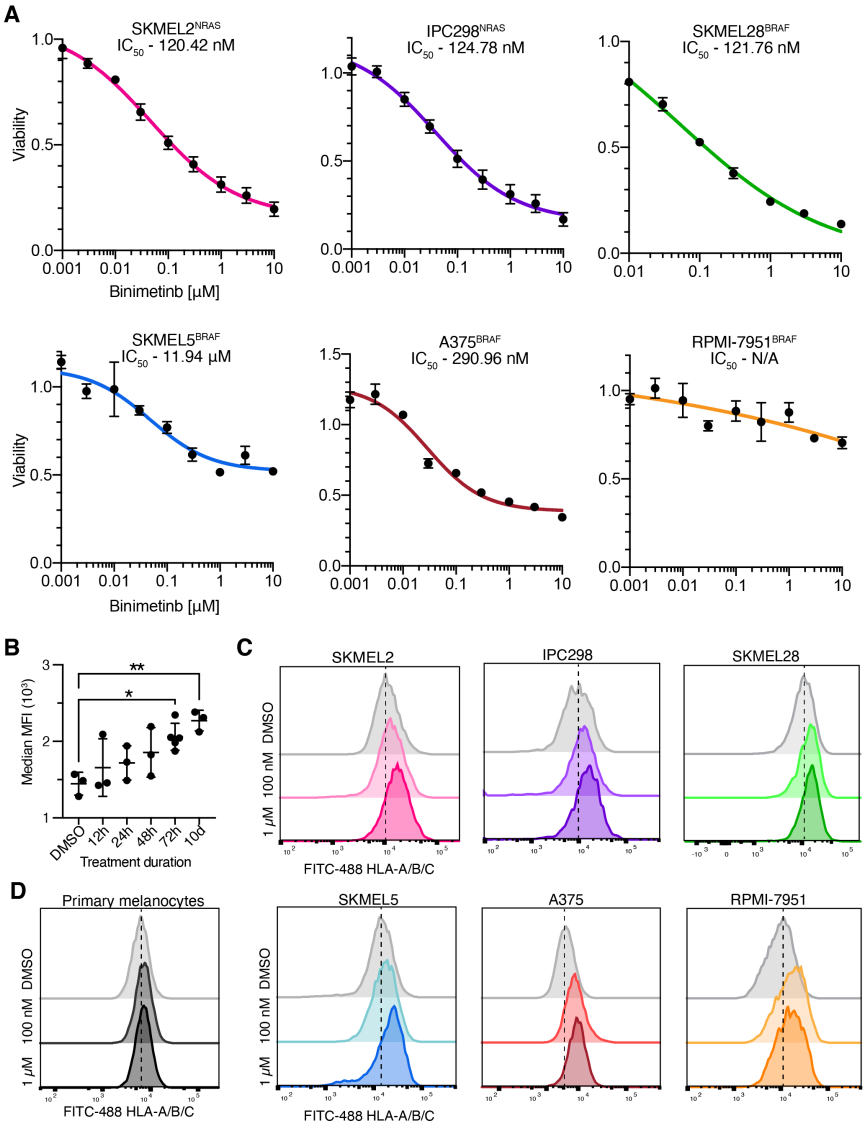

Fig. S1. Phenotypic characterization of cellular response to binimetinib.
(A) Cell viability (fraction of DMSO control) at 72 hr after binimetinib treatment. Data are represented as mean values +/- SD for n=3 replicates. Lines represents a four-parameter nonlinear regression curve fit. **(B)** Surface HLA expression of SKMEL5 cells treated with 100 nM binimetinib measured by flow cytometry. Data are represented as mean values +/- SD for n=3 replicates and significance between replicates (*p<0.05, **p<0.01) is calculated using a one-way ANOVA test comparing each value to the DMSO control. **(C)-(D)** Flow cytometry measurements of surface HLA expression in cells after 72 hours of treatment with DMSO, 100 nM MEKi, or 1 µM MEKi. Data are represented as % of maximum signal, and the distributions are representative of three independent experiments.

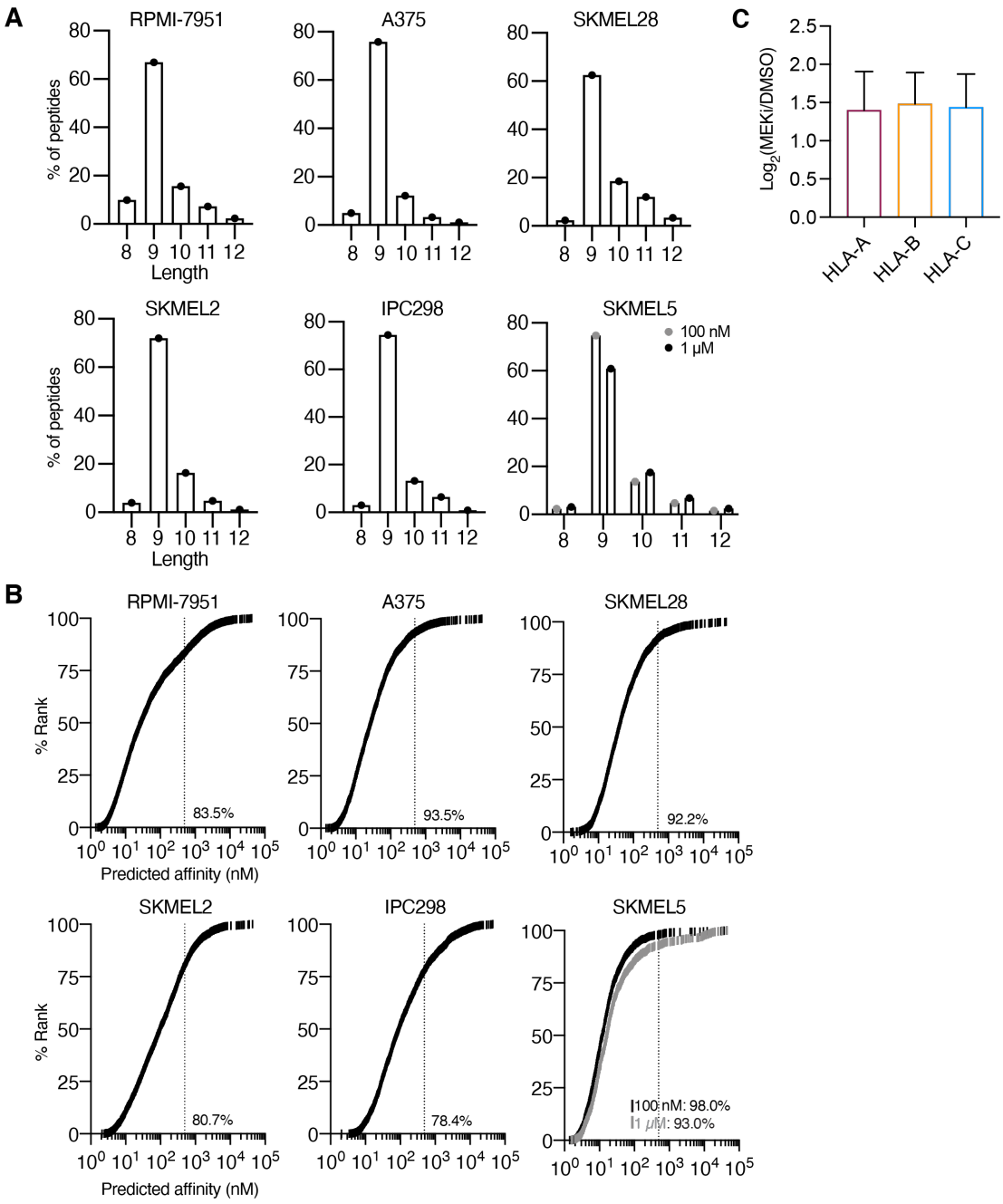

Fig. S2. MHC peptide characterization from *in vitro* analyses.
(A) Peptide length distribution for each cell line from multiplexed mass spectrometry analyses where n=3 biological replicates were treated with either DMSO or MEKi. **(B)** Predicted binding affinity of 9-mer peptides, rank ordered. Dotted line represents threshold for binding at ≤500 nM. Percentage of peptides ≤500 nM are listed on each plot. **(C)** Average fold change in presentation for SKMEL5 9-mers +/- 1 µM MEKi segregated by highest predicted affinity to HLA-A/B/C. Error bars represent +/- standard deviation. Tukey's multiple comparisons test shows no significant difference between pairwise comparisons.

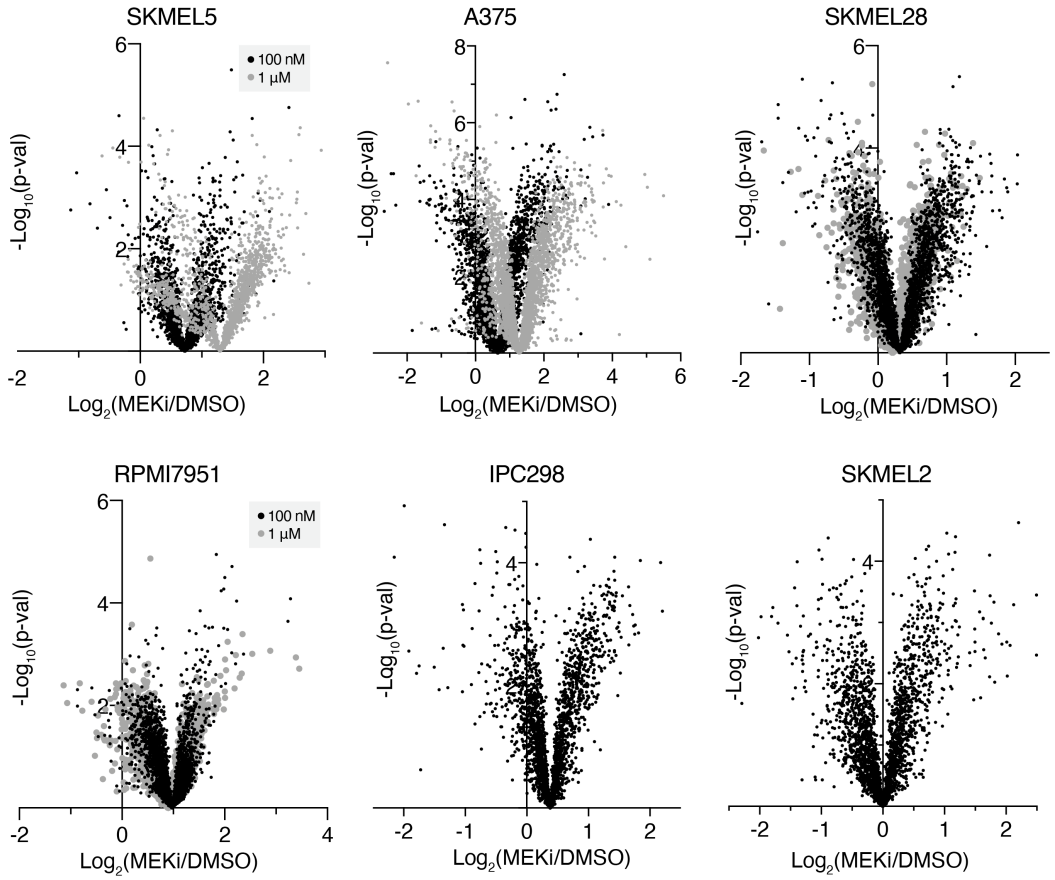

Fig. S3 Volcano plots of *in vitro* pMHC analyses.
Volcano plots of the average fold change in pMHC expression with binimetinib treatment (n=3 biological replicates for DMSO and MEKi treated cells) versus significance (mean-adjusted p value, unpaired two-sided t test).

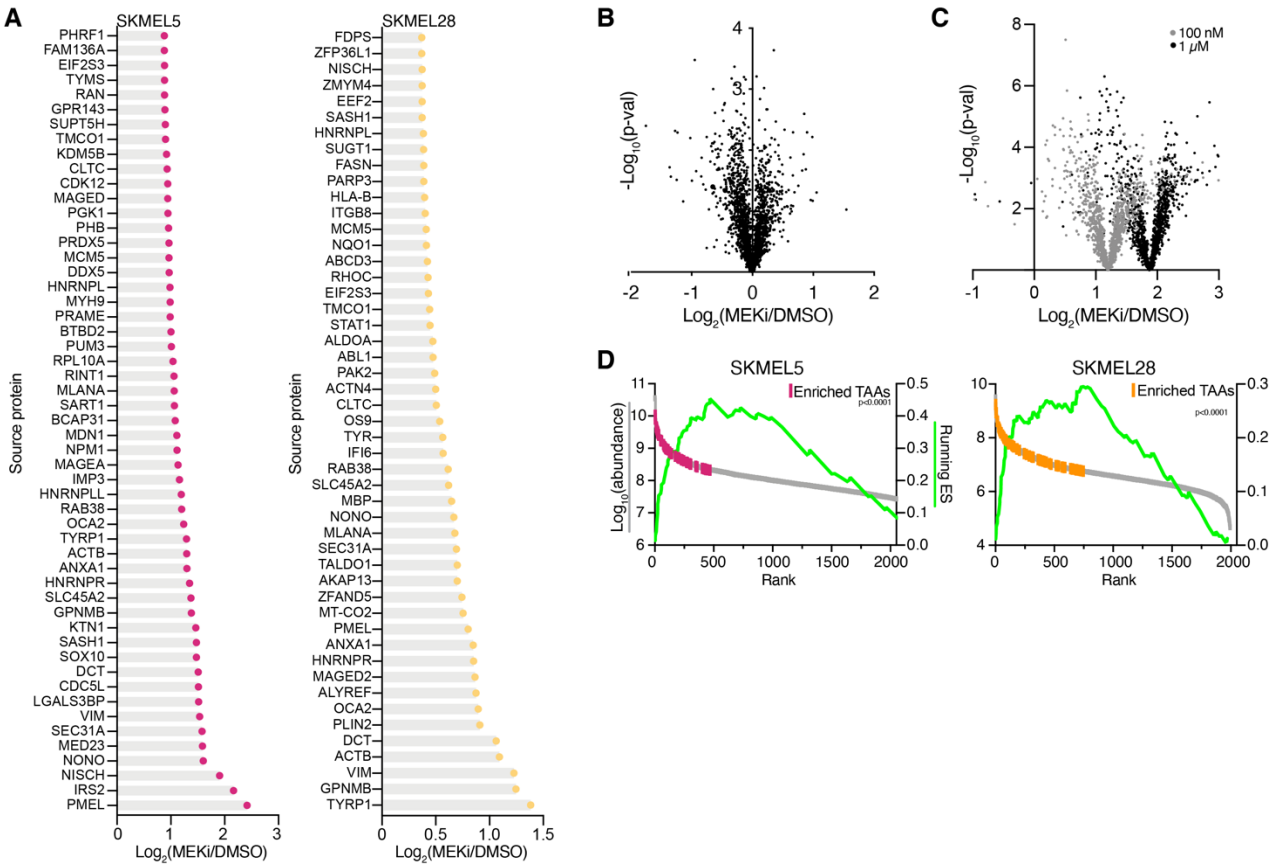

Fig. S4 TAA pMHC enrichment following binimetinib treatment.
(A) Enriched TAA pMHC expression changes with 100 nM MEKi. **(B)-(C)** Volcano plots of the average fold change in pMHC expression with 10 nM binimetinib treatment **(B)** and 100 nM and 1 μM trametinib treatment **(C)**. Data shown are the mean of n=3 biological replicates per condition versus significance (mean-adjusted p value, unpaired two-sided t test). **(D)** Enrichment plots with peptides rank ordered by precursor ion abundance. $p<0.0001$ for both SKMEL5 100 nM and SKMEL28 100 nM analyses.

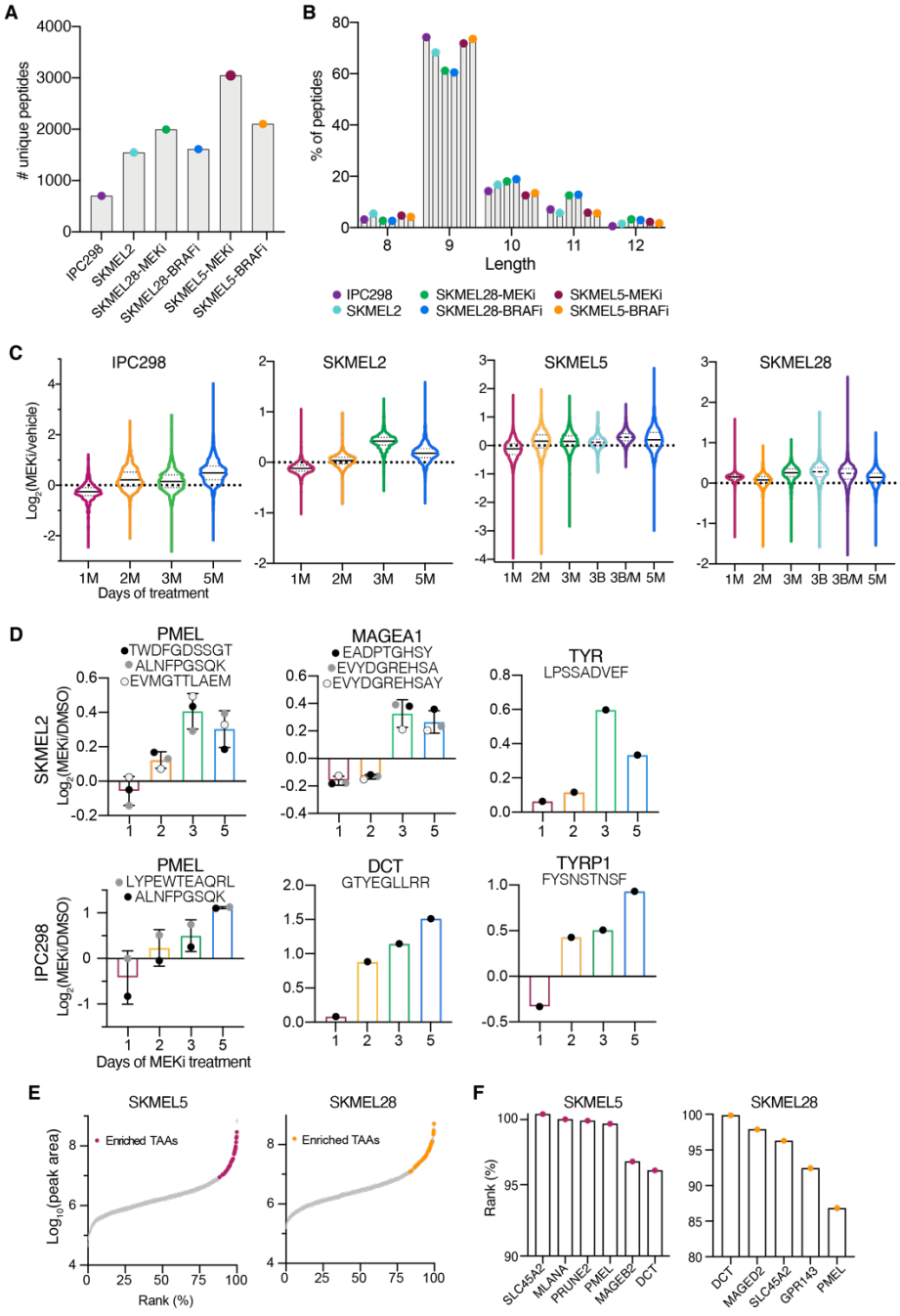

Fig. S5 TAA enrichment following MEKi treatment *in vivo.*

(A) Number of unique pMHCs identified in each analysis. **(B)** Length distribution of pMHCs represented as a percentage of the total pMHCs identified. **C)** Violin plot of distribution of fold changes in presentation of pMHCs following MEK inhibition (M, binimetinib), BRAF inhibition (B, encorafinib) or both (B/M). Solid line represents median, dotted lines define the first and third quartiles. **(D)** Changes in pMHC expression for select melanoma differentiation antigens, x-axis = number of days of binimetinib treatment. Errors bars represent standard deviation when >1 peptide from each source protein was identified. **(E)** Rank-ordered average abundance (n=3 biological replicates) of pMHCs in SKMEL5 and SKMEL28 analyses. Positively enriched TAAs are highlighted in color. **(F)** Select TAA peptide rank-ordered average abundance for CLXs.

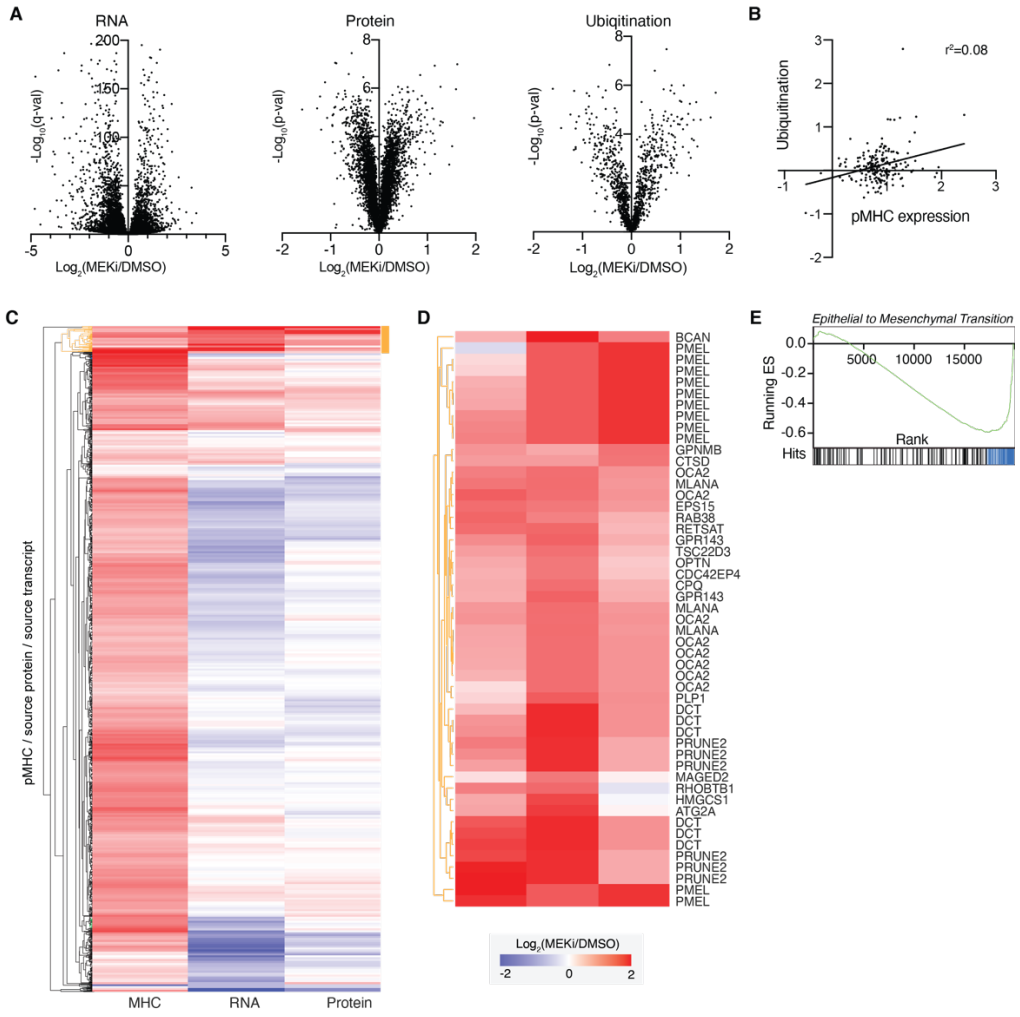

Fig. S6 Correlation between pMHC, protein, and transcript expression with MEKi treatment.

(A) Volcano plots of changes in RNA and protein expression, and abundance of ubiquitylated peptides. The y-axis represents significance values. Significance: RNA: Wald test, Benjamini Hochberg adjusted. Protein/Ubiquitination: unpaired two-sided T-test. **(B)** Correlation between pMHC expression and ubiquitination levels in SKMEL5 cells +/- 100 nM MEKi. Values represent Log_2(MEKi/DMSO). **(C)-(D)**. Hierarchical clustering of pMHC, RNA, and protein expression, represented as the change in expression following 100 nM MEKi. **(D)** displays subcluster highlighted in orange in **(C)**. **(E)** Enrichment plot of EMT genes using for RNA-seq data, SKMEL5 cells +/- 100 nM binimetinib. p & q-values < 0.0001.

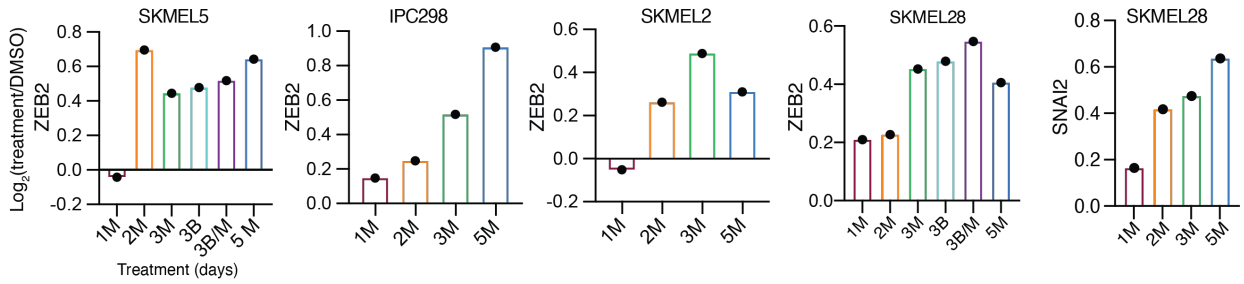

Fig. S7 pMHC presentation changes in EMT-derived epitopes.
Maximum change in expression of pMHCs derived from ZEB2 and SNAI2 source proteins from CLX analyses. X-axis describes days of therapy and drug treatment (M=MEKi, binimetinib and B=BRAFi, encorafinib.

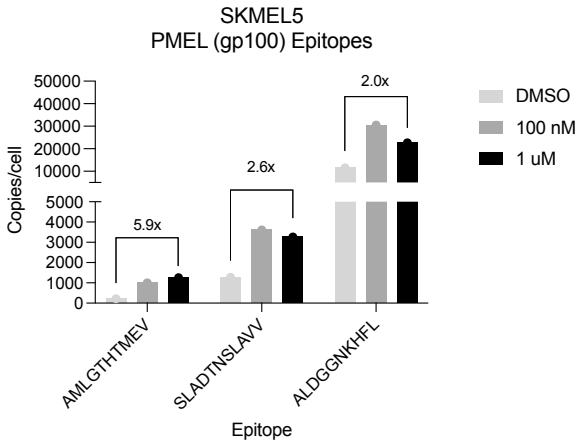

Fig. S8 PMEL-derived epitopes on SKMEL5 cells.
Copies-per-cell estimations of three HLA-A*02:01 PMEL-derived epitopes treated with DMSO or 100 nM/1uM binimetinib for 72 hours.

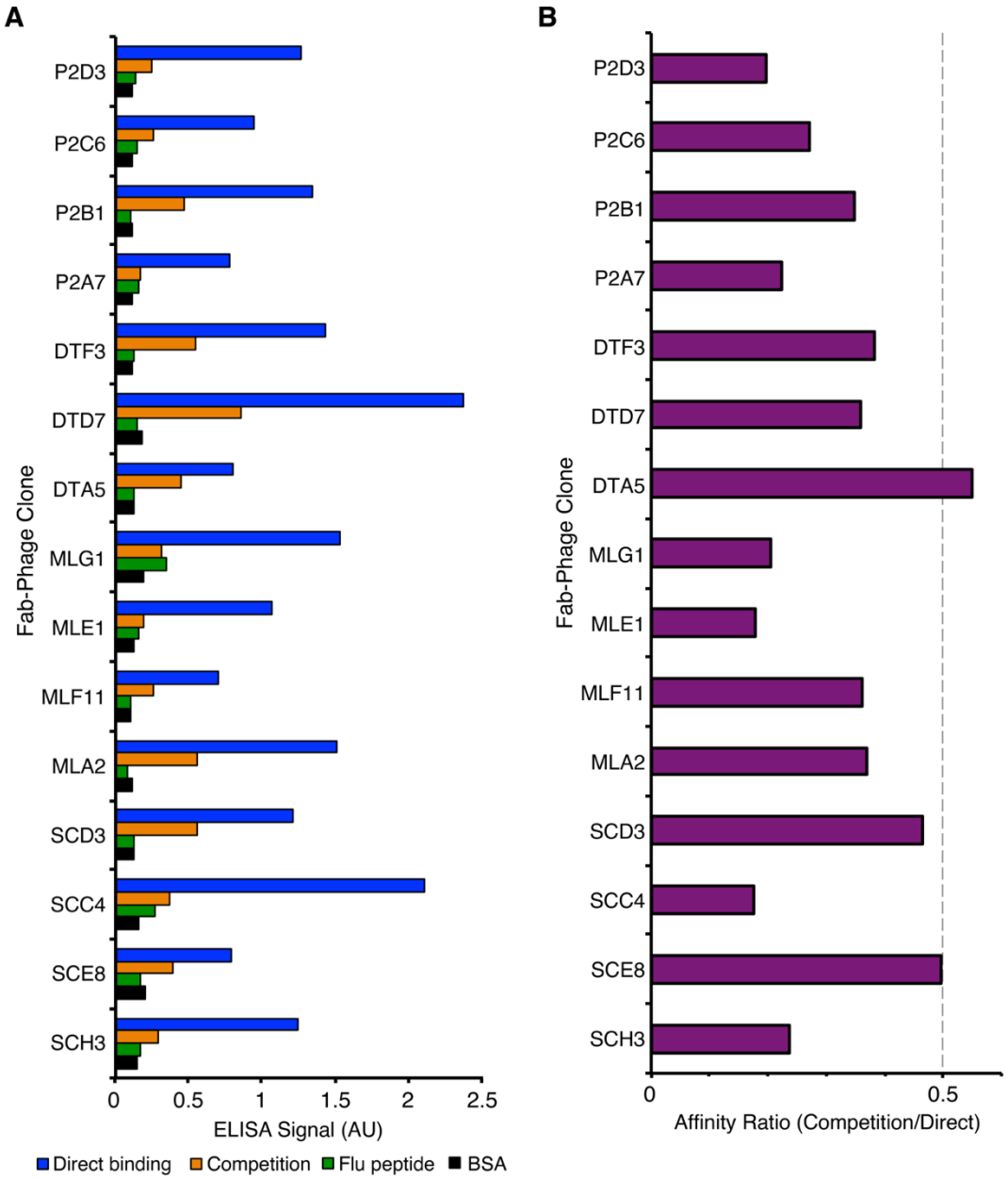

Fig. S9 Characterization of Fab-phage clones.
(A) Fab-phage ELISA screen of each MHC-peptide complex. Clones with signal above 0.5 AU and competition ratios less than 0.5 were considered high affinity, with predicted relative affinities of <20 nM. Passing clones (green) were further evaluated for binding to the FLU MHC-peptide complex and sequenced. **(B)** ELISA values for all unique Fab-phage clones across all phage-display selections for MHC-peptide complexes.

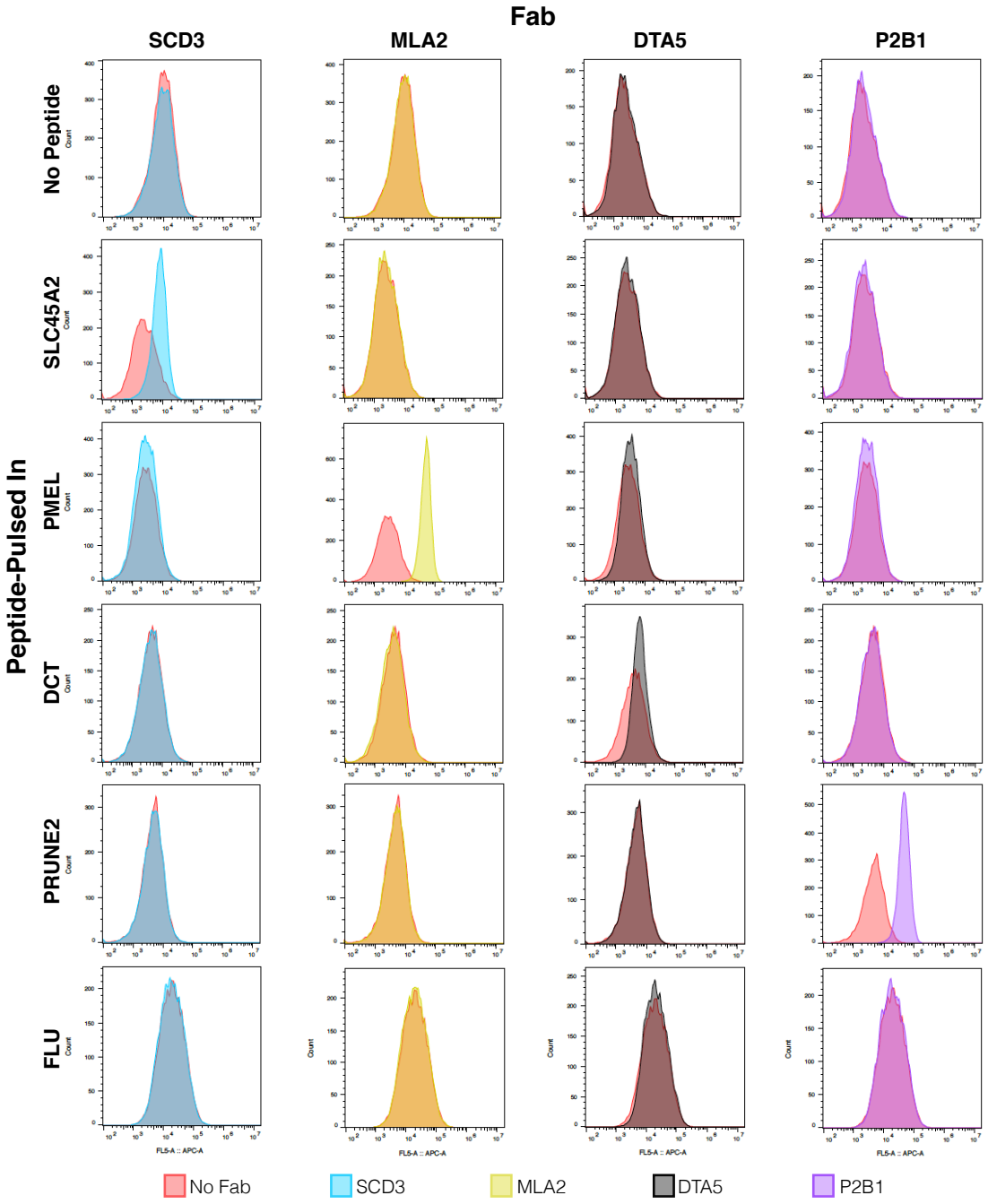

Fig. S10 Peptide specificity of pMHC-specific Fabs.
Fluorescence intensity of T2 cells loaded with no peptide (negative control), a decoy FLU peptide, or peptide of interest stained with a pMHC-specific Fab.

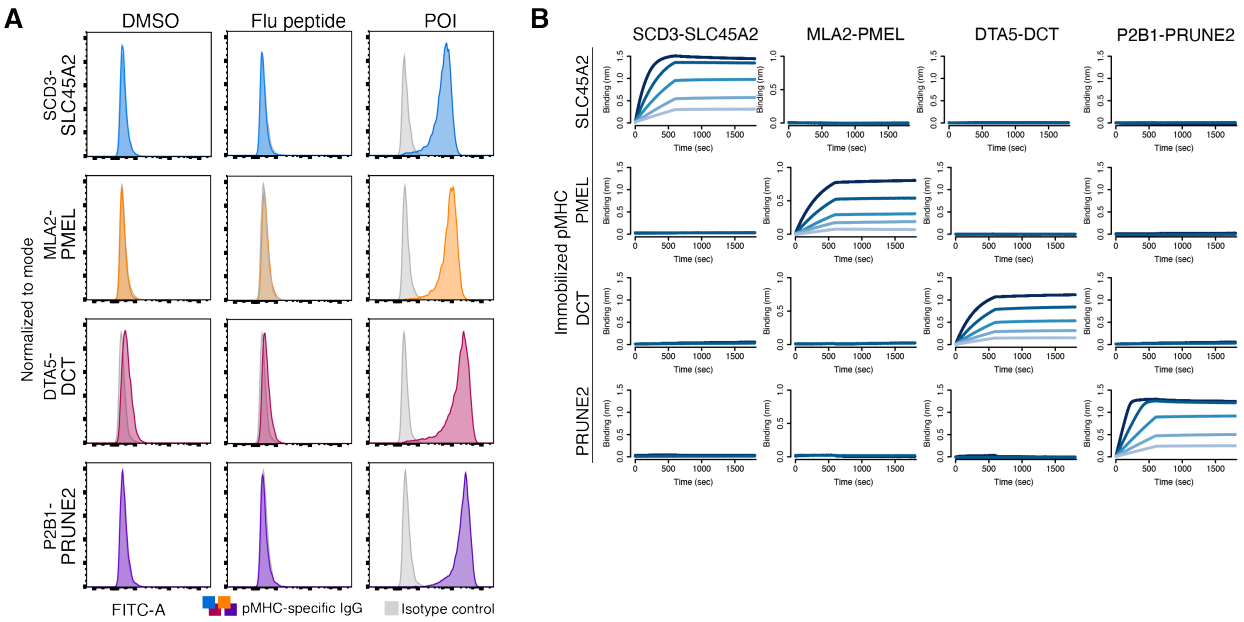

Fig. S11 Peptide specificity of pMHC-specific IgGs.

(A) Fluorescence intensity of T2 cells loaded with DMSO (negative control), a decoy FLU peptide, or peptide of interest (POI) stained with a pMHC-specific IgG (color) or an isotype control (grey). **(B)** Bio-layer interferometry (BLI) analysis of IgG's (two-fold dilutions starting at 20 nM) against selected HLA-A*02:01 MHC-peptide complexes.

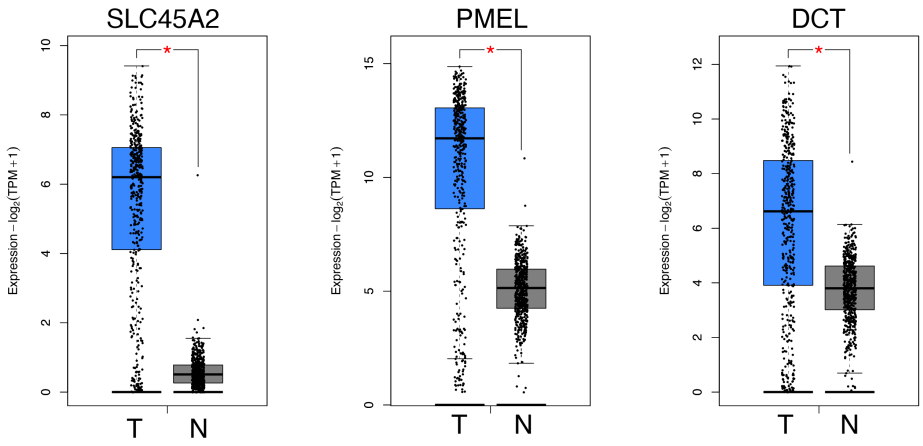

Fig. S12 Tumor versus normal gene expression profiles of select TAAs.
Gene Expression profiles (RNA-seq) in skin cutaneous melanoma for n=461tumors (T, TCGA) and n=558 normal tissues (N, GTEX). *p ≤ 0.01, one way ANOVA.

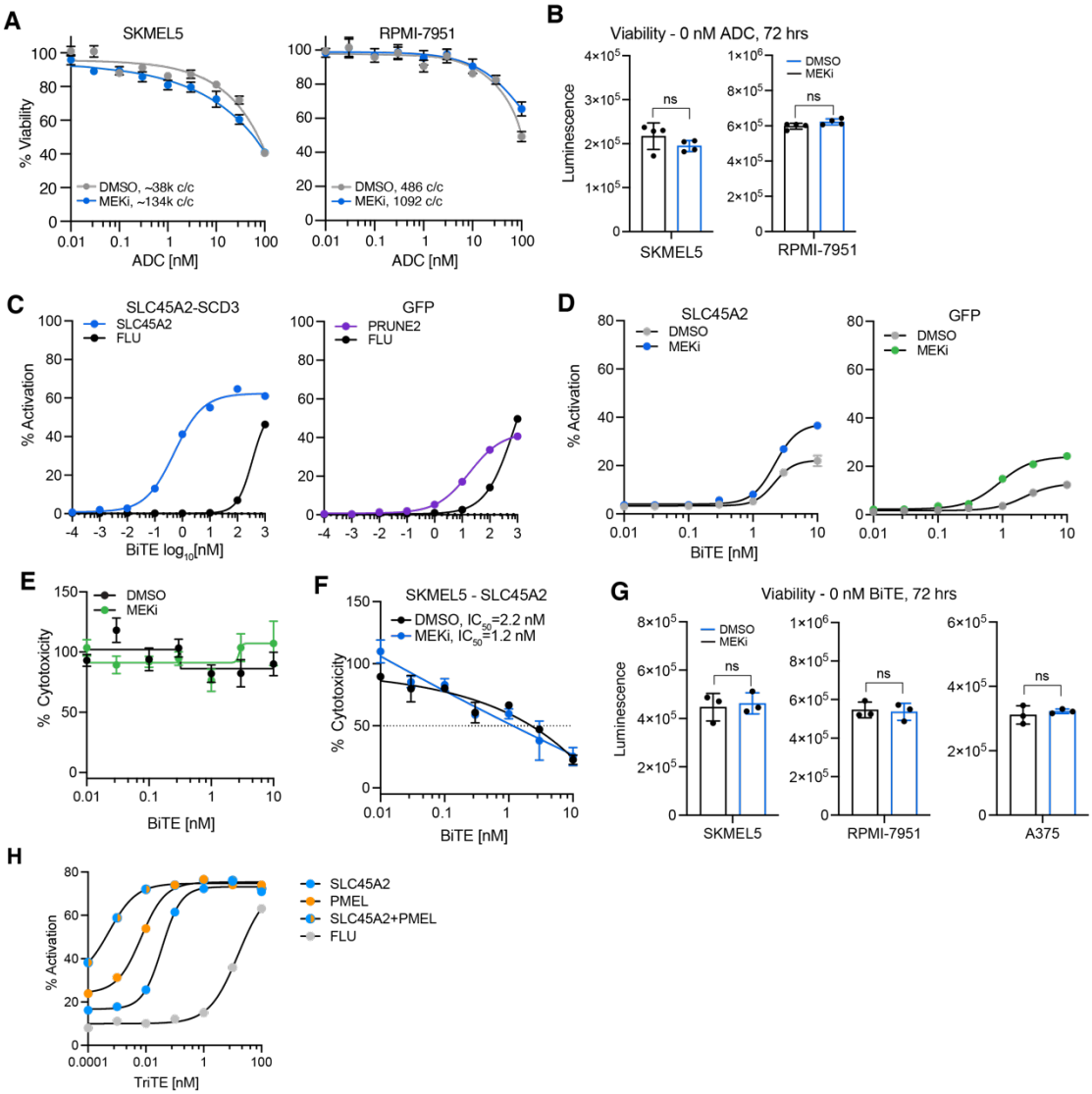

Fig. S13 Characterization of pMHC-specific ADCs and BiTEs *in vitro*.
(A) Cell viability after 72 hr incubation with SLC45A2-ADC. Error bars represent +/- SD for n=4 biological replicates. Lines represents a four parameter logistic (4PL) nonlinear regression curve. c/c denotes average copies per cell. (**B, G**) Luminescence readout from Cell Titer Glo assay following 72 hours of 0 nM ADC (**B**) or 0 nM BiTE (**G**) treatment of cells pre-treated with DMSO or MEKi. "ns" denotes no significance, paired, two-tailed t-test.
(C-D) Percent of GFP+ Jurkat cells following incubation with peptide-pulsed T2 cells **(C)** or SKMEL5 cells **(D)** and a pMHC-specific BiTE or negative control BiTE (anti-GFP) for 24 hours. **(E)** Cell viability (percentage of untreated control) of target cells incubated with normal human T cells (effector:target 2:1) & a negative control anti-GFP BiTE for 48 hours. **(F)** Cell viability (percentage of untreated control) of target cells incubated with normal human T cells (effector:target 2:1) & a pMHC-specific BiTE or 48 hours. **(H)** Percent GFP+ Jurkat cells following incubation with Peptide-pulsed T2 cells and TriTEs for 24 hours.

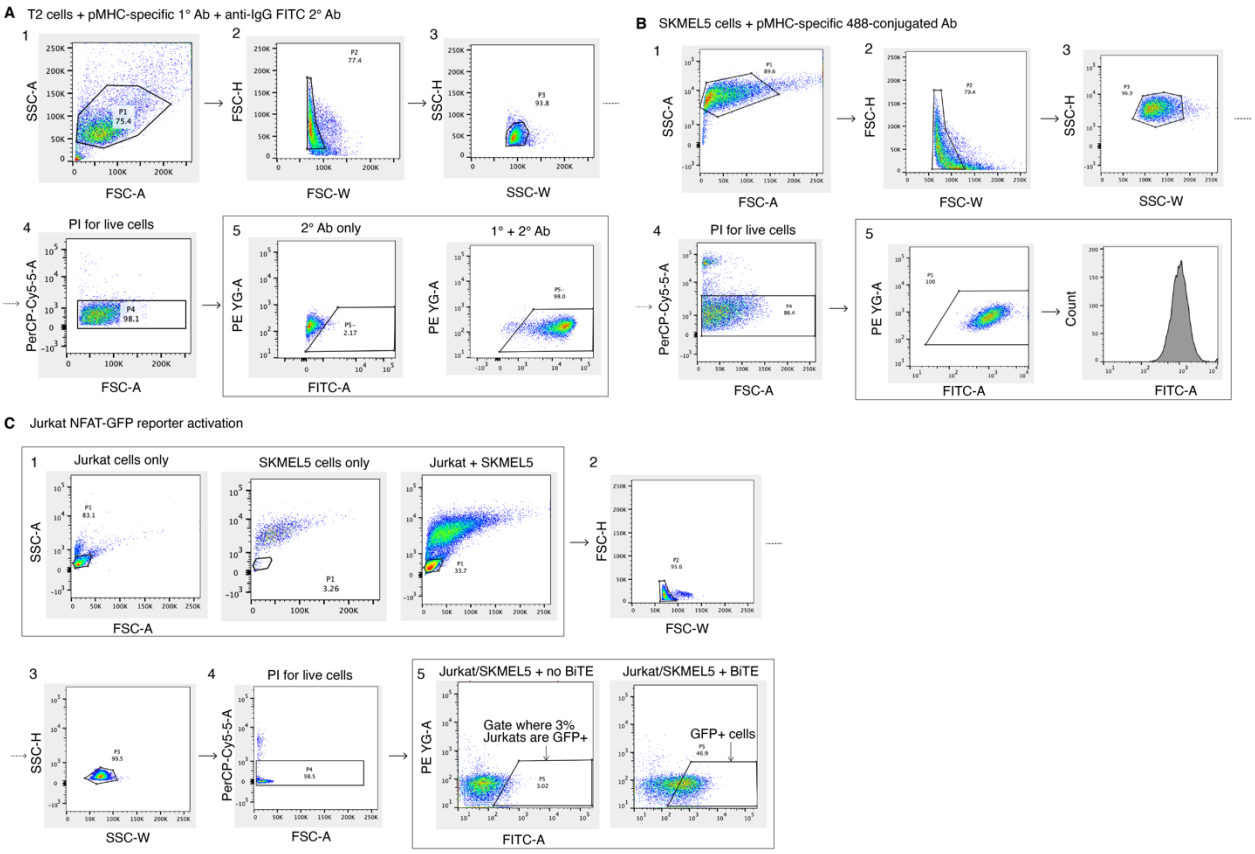

Figure S14 Flow cytometry gating strategies.
Flow cytometry gating strategies for **(A)** Peptide-loaded T2 cells + pMHC-specific 1° Ab plus anti-IgG 2° Ab, **(B)** SKMEL5 cells + pMHC-specific Alexa Fluor 488-conjugated Ab, and **(C)** Jurkat NFAT-GFP reporter activation assays.

SUPPLEMENTARY METHODS

Peptide synthesis

Heavy leucine-containing peptides for hipMHC quantification correction (ALNEQIARL^{+7}, SLPEEIGHL$^{+7,}$ and SVVESVKFL^{+7} were synthesized at the MIT-Koch Institute Swanson Biotechnology Center in Biopolymers and Proteomics Facility using standard Fmoc chemistry using an Intavis model MultiPep peptide synthesizer with HATU activation and 5 µmol chemistry cycles as previously described.(1) Standard Fmoc amino acids were procured from NovaBiochem and Fmoc-Leu (13C6, 15N) was obtained from Cambridge Isotope Laboratories. Light peptides for pMHC-antibody generation (PMEL, DCT, PRUNE2, SLC45A2) were synthesized on a Gyros-Protein Technologies Tribute with UV feedback at a 100 micromole scale using standard Fmoc chemistry and HATU/NMM activation chemistry. Both light and heavy leucine-containing peptides were purified on a Gilson GX-271 preparative HPLC system by reverse phase, and quality assured with MS on a Bruker MicroFlex MALDI-TOF and by RP-HPLC on an Agilent model 1100 HPLC.

Isotopologue peptides for SureQuant-IsoMHC analyses were synthesized using HeavyPeptide AQUA Custom Synthesis Service (Thermo Fisher Scientific) and were purified to >97% and validated with amino acid analysis as previously described (2).

UV-mediated peptide exchange for hipMHCs

UV-mediated peptide exchange was performed using recombinant, biotinylated Flex-T HLA-A*02:01 monomers (BioLegend), using a modified version of the commercial protocol. Briefly, 2-4 µL of 500 µM peptide stock, 2 µL of Flex-T monomer, and 32 µL of 1X PBS were combined in a 96-well U bottom plate. On ice, plates were illuminated with ultraviolet light (365 nm) for 30 minutes, followed by a 30-minute incubation at 37 °C protected from light. Concentration of stable complexes following peptide exchange was quantified using the Flex-T HLA class I ELISA assay (Biolegend) per manufacturer's instructions for HLA-A*02:01. ELISA results were acquired using a Tecan plate reader Infinite 200 with Tecan icontrol version 1.7.1.12.

Peptide MHC binding affinity

Binding affinity of 9-mer pMHCs was estimated using NetMHCpan-4.0 against each cell line's allelic profile (SI Appendix, **Table S1**) (3, 4). The minimum predicted affinity (nM) of each peptide was used to assign peptides to their best predicted allele. The threshold for binding was set to 500 nM.

Flow cytometry

Surface HLA expression in melanoma cells

Cells were seeded and treated with DMSO or binimetinib in 10 cm plates, then lifted with 0.05% Trypsin-EDTA and 10^6 cells/mL were spun at 300 g for 3 minutes, washed with ice cold flow buffer [1X PBS supplemented with 3% bovine serum albumin (BSA)] and incubated with fluorophore-conjugated antibody at 0.5 µg mL^{-1} in flow buffer for 30 minutes on ice. After incubation, cells were washed again, and resuspended in flow buffer plus 5 µL of propidium iodide (PI) staining solution (10 µg mL^{-1}, Invitrogen) per sample. Analyses were performed on an LSRII (BD Biosciences) and all flow cytometry data was analyzed using FlowJo (version 10.7.2). Antibody: Alexa Fluor 488 HLA-A, B, C, clone W6/32 [Biolegend, cat # 311413]. The gating strategy previously described (1).

pMHC-Fab and pMHC-antibody staining

T2 lymphoblasts: the day prior to Fab staining, T2 lymphoblasts were cultured in RPMI serum-free media containing 50 µg/mL peptide of interest at a concentration of 1e^6 cells/mL. Cells were collected by centrifugation and washed 1X in flow buffer. Each sample was resuspended in 10 µg/mL Fab for 30 minutes, and then washed 3x in flow buffer. Each sample was then stained with an anti-human Fab goat mAb Alexa Fluor 647 conjugate (Jackson ImmunoResearch) for 30 minutes, and then washed 3x in flow buffer. Samples were resuspended in 200 µL sterile PBS pH 7.4 and analyzed on a CytoFLEX (Beckman Coulter). For pMHC-antibody staining (full length IgG), T2 cells were incubated with the peptide of interest

overnight, harvested, and stained with either primary pMHC specific IgG antibodies or a human IgG isotype control (Abcam, ab20619) at 10 μg/mL for 20 minutes on ice. Cells were then washed with flow buffer 1X and incubated with protein A-488 secondary antibody conjugate (Invitrogen, P11047) for 20 minutes (1:1000 dilution). Cells were washed again with flow buffer and resuspended in PI staining solution prior to analysis on the LSRII. Gating strategy for T2 cells is shown in **Fig. S14A**.

SKMEL5 cells: SKMEL5 cells were pre-treated with DMSO or 1 μM binimetinib for 72 hours in 10 cm plates and were subsequently harvested (10^6 cells/mL), washed, and stained with fluorophore conjugated pMHC-antibodies at 2 μg/mL, and analyzed using the LSRII. **Fig. S14B** describes the gating strategy for SKMEL5 cells.

Jurkat NFAT-GFP activation

SKMEL5 cells were treated in 10 cm plates with DMSO or 1 μM binimetinib for 72 hours, after which cells were seeded in a 24 well plate at a ratio of 250,000 SKMEL5 cells to 50,000 Jurkat NFAT-GFP cells (5:1) in Jurkat culture medium with n=3 technical replicates per condition and incubated with a pMHC-specific or anti-GFP (control) BiTE for 24 hours. T2 cells were seeded at 1:1 ratio ($5e^4$ cells to $5e^4$ cells) in a 96-well round bottom plate. Cells were washed 2x with flow buffer and resuspended in PI staining solution. Cells were gated according to the stagey described in **Fig. S14C**, where the percentage of GFP positive cells were gated so ~97% of Jurkat cells with no BiTE were classified as GFP negative. SKMEL5 cells were analyzed on the LSRII, T2 on the CytoFLEX.

RNA-sequencing

RNA was isolated from 10 cm plates of SKMEL5 cells with 3 biological replicates per condition (DMSO, 100 nM binimetinib, 1 μM binimetinib) using Direct-zol RNA miniprep kit (Zymo Research), as previously described (1). RNA were confirmed for quality using the Agilent Fragment Analyzer and 300 ng of material was polyA-selected using NEBNext Poly(A) mRNA Magnetic Isolation Module (E7490) modified to include two rounds of polyA binding and 10 minute incubations. cDNA was generated using the NEB Ultra II directional kit (E7760) following manufacturer instructions using 12 cycles of PCR and and a 0.9X SPRI clean. The resulting libraries were quality assessed using the Fragment Analyzer and quantified by qPCR prior to be sequenced on the Illumina HiSeq2000. The 40nt single-end reads with an average depth of 5 million reads per sample were sequenced for all conditions.

RNAseq reads were aligned to the human transcriptome prepared with the hg38 primary assembly and the Ensembl version 95 annotation using STAR version 2.5.3a (5). Gene expression was summarized with RSEM version 1.3.0 and SAMtools version 1.3.(6, 7) Differential expression analysis was performed with DESeq2 version 1.24.0 running under R version 3.6.0 with normal log fold change shrinkage (8). Significance values (adjusted p-value, Wald test) were multiple hypothesis corrected using Benjamini-Hochberg (BH) method. The resulting data were parsed and assembled using Tibco Spotfire Analyst version 7.11.1.

Cloning

Fabs were subcloned from the Fab-phagemid into an *E. coli* expression vector pBL347. The heavy chain of the IgG was cloned from the Fab plasmid into a pFUSE (InvivoGen) vector with a human IgG1 Fc domain. The light chain of the IgG was cloned from the Fab plasmid into the same vector but lacking the Fc domain. The light chain of the BiTE was cloned from the Fab plasmid into a pFUSE (InvivoGen) vector with an anti-CD3 scFv (OKT3). The heavy chain of the BiTE was cloned into the same vector lacking the OKT3. SCD3-arm of the TriTE was converted into a scFab and cloned into a pFUSE (InvivoGen) vector with the KIH strategy “knob” human Fc domain(9) MLA2-arm of the TriTE was converted into a scFab and cloned into a pFUSE (InvivoGen) vector with the KIH strategy “hole” human Fc domain followed by OKT3. All constructs were sequence verified by Sanger sequencing.

Protein expression and purification

MHC-peptide complexes were expressed and refolded as previously described.(10) Briefly, MHC-peptide complexes were refolded at 10°C for 3 days and SEC-purified on a HiLoad 16/600 Superdex 75 pg column equilibrated in 10 mM Tris pH 8. After purification, MHC-peptide complexes were biotinylated

using a BirA reaction kit (Avidity) per manufacturer's instructions in the presence of excess peptide and β_2M at 25°C for 4 hours. After biotinylation, MHC-peptide complexes were purified again via SEC to remove excess biotin. Proper folding was assessed by SDS-PAGE. Biotinylation was assessed by pre-incubating MHC-peptide complexes with NeutrAvidin and subsequently assessed by SDS-PAGE.

Fabs were expressed in E. coli C43 (DE3) Pro+ as previously described using an optimized autoinduction medium and purified by protein A affinity chromatography (11). IgGs, BiTEs, and TriTEs were expressed in Expi293 BirA cells using transient transfection (Expifectamine, Thermo Fisher Scientific). After transfection for 3–5 d, media was harvested, IgGs and TriTEs purified by Ni-NTA affinity chromatography and BiTEs were purified using protein A affinity chromatography. All proteins were buffer exchanged into PBS pH 7.4 and stored in 10% glycerol at -80°C and assessed by SDS-PAGE.

All proteins were then buffer exchanged into phosphate-buffered saline (PBS) containing 20% glycerol, concentrated, and flash frozen for storage. All other proteins were buffer exchanged into PBS by spin concentration and stored in aliquots at –80°C. The purity and integrity of all proteins were assessed by SDS-PAGE. Fabs were subsequently buffer exchanged into PBS pH 7.4 and stored in 10% glycerol at -80°C and assessed by SDS-PAGE.

Fab-phage selection

Phage selections were run as previously described (Hornsby et al. 2015). Selections were performed on a KingFischer™ System (Thermo Fisher Scientific). Biotinylated antigens were immobilized using streptavidin-coated magnetic beads (Promega). In each round, phage was first cleared by incubation with beads loaded with MHC-peptide complexes loaded with FLU peptide. Unbound phage was next incubated with beads loaded with MHC-peptide complex of interest. Beads were washed and bound phage was eluted with 50 µg/mL of TEV protease. Four rounds of selection were performed with decreasing amounts of MHC-peptide complex of interest. Selections were performed in PBS+0.02% Tween-20+0.2% bovine serum albumin (PBSTB). Individual phage clones from the fourth round of selections were analyzed by ELISA.

Phage ELISA

For each phage clone, four different conditions were tested – Direct: MHC-peptide complex of interest, Competition: MHC-peptide complex of interest with an equal concentration of MHC-peptide complex in solution, Negative selection: FLU MHC-peptide complex, and Control: PBSTB. 384-well Nunc Maxisorp flat-bottom clear plates (Thermo Fisher Scientific) were coated with 0.5 µg/mL of NeutrAvidin in PBS overnight at 4°C and subsequently blocked with PBSTB. Plates were washed 3x with PBS containing 0.05% Tween-20 (PBST) and were washed similarly between each of the steps. 20 nM biotinylated MHC-peptide complex was diluted in PBSTB and immobilized on the NeutrAvidin-coated wells for 30 minutes at room temperature, then blocked with PBSTB + 10 µM biotin for 10 minutes. For the competition samples, phage supernatant was diluted 1:5 into PBSTB with 20 nM MHC-peptide complex of interest for 30 minutes prior to addition to the plate. For the direct samples, phage supernatant was diluted 1:5 in PBSTB. Competition and direct samples were added to the plate for 30 minutes at room temperature. Bound phage was detected by incubation with anti-M13-horseradish peroxidase conjugate (Sino Biologics, 1:5000) for 30 minutes, followed by the addition of TMB substrate (VWR International). The reaction was quenched with the addition of 1 M phosphoric acid and the absorbance at 450 nm was measured using a Tecan M200 Pro spectrophotometer. Clones with high binding to MHC-peptide complex of interest, low binding to PBSTB/FLU MHC-peptide complex, and a competition ratio (Competition AU/Direct AU) ≥0.5 were carried forward.

Bio-layer Interferometry

BLI measurements were made using an Octet RED384 (ForteBio) instrument. MHC-peptide complex was immobilized on an streptavidin biosensor and loaded for 200 seconds. After blocking with 10 µM biotin, purified binders in solution were used as the analyte. PBSTB was used for all buffers. Data were analyzed using the ForteBio Octet analysis software and kinetic parameters were determined using a 1:1 monovalent binding model.

IgG NHS-Fluorophore Conjugation

Purified IgG's were buffer exchanged into PBS pH 8.3. Concentrated IgG to ~11 mg/mL (with the exception of P2B1 which was only 2 mg/mL), and added 20 mM NHS-AF488 (Fluoroprobes) at either a 10:1 or 5:1 (Dye:IgG) ratio. Conjugation reactions were incubated at room temperature for 1 hour, and then quench by adding equivalent volume of 1 M glycine pH 8.4 as dye. Reactions were further incubated for 1 hour and then buffer exchanged into PBS pH 7.4 until all excess dye was removed. IgG and dye concentration was determined by UV.

ADC conjugation

Purified IgG was buffer exchanged into PBS pH 7.4 and concentrated to 35µM. 20x 100 mM piperidine-derived oxaziridine molecule (12) was added to PBS pH 7.4, and subsequently added to IgG for a final IgG concentration of 35µM. Labeling was conducted at room temperature for 2 hours, and buffer exchanged with PBS pH 7.4 to remove unconjugated oxaziridine. 5% v/v 5 mM DBCO-PEG4-Glu-vc-PAB-MMAF (Levena Biopharma) was added to oxaziridine-labeled IgG and incubated overnight at room temperature. IgG was buffer exchanged into PBS pH 7.4 to remove unconjugated MMAF. Conjugation efficiency was assessed by intact protein mass spectrometry using a Xevo G2-XS Mass Spectrometer (Waters).

Enrichment analyses

For pMHC pathway and TAA enrichment analyses, gene names from peptide source proteins were extracted and rank ordered according to the average $\log_2$ fold change over DMSO treated cells. In cases where more than one peptide mapped to the same source protein, the maximum/minimum was chosen, depending on the directionality of enrichment analysis. For RNAseq & protein expression data, data sets were rank ordered according to the mean $\log_2$ fold change value with only protein encoding genes considered.

We utilized gene set enrichment analysis (GSEA) 4.0.3 pre-ranked tool against the Molecular Signatures Database hallmarks gene sets with 1000 permutations, weighted enrichment statistic (p=1), and a minimum gene size of 15 (13–15). Results were filtered for FDR q-value ≤ 0.25, and nominal p-value ≤ 0.05. P-values > 0.05 in reported analyses are noted.

TCGA/gTEX/Cell line expression analysis

mRNASeq normalized gene expression data (MD5) from the TCGA skin cutaneous melanoma study (SKCM) for was obtained from Firebrowse (16). Expression for all tumors was z-score normalized, and *BRAF* mutant tumor data was extracted for subsequent analyses. Pairwise gene expression significance comparisons were calculated using an un-paired, two-tailed T test, and significance values for HLA expression between MITF-low and immune subtypes were calculated using Sidak's multiple comparisons test. Tumor versus normal expression profiles for SKCM (Fig. S11) were generated using the Gene Expression Profiling Interactive Analysis 2 (GEPIA2) (17) using data from TCGA and gTEX studies with a jitter size of 0.4 and a p-value cutoff of 0.01 for significance, calculated using a one-way ANOVA statistical test. Expression data for 57 BRAF/NRAS melanoma cell lines was obtained from TRON Cell line Portal (4).

Global protein expression profiling sample preparation

For a quantitative global proteomics analysis, 300 µg of supernatant from DMSO and 100 nM MEKi sample for SKMEL5 cells was diluted 8-fold in 8M urea, reduced with 10 mM dithiothreitol in 100 mM ammonium acetate (pH 8.9) at 56°C for 45 minutes, and subsequently alkylated with 50 mM iodoacetamide for 45 minutes rotating at RT in the dark. Lysates were diluted 4-fold with 100 mM ammonium acetate and digested with sequence-grade trypsin (Promega) overnight at RT at an enzyme:substrate ratio of 50:1 (w/w). The reaction was quenched with formic acid (5% total volume) and desalted on C18-based STAGE tips. Solvents: 0.1% formic acid, 90% acetonitrile (MeCN) in 0.1% formic acid, and 60% acetic MeCN in 0.1% formic acid. Volumes were reduced with vacuum centrifugation and lyophilized in 150 ug aliquots. Peptide aliquots were labeled with TMT10-plex reagents in 70%

ethanol/150 mM triethylammonium bicarbonate (TEAB) for 1 hour at room temperature, pooled, brought to dryness with vacuum centrifugation, and stored at -80°C.

The labeled mixture was resuspended in 0.1% formic acid, and 25% was loaded onto an Agilent Zorbax 300Extend-C18 5 µm 4.6 × 250 mm column on an Agilent 1200 operating at 1 ml/min for fractionation, as previously described (18). Briefly, peptides were eluted with the following gradient: 1% B to 5% B for 10 mins, 5–35% B for 60 mins, 35-70% B for 15 min, held at 70% B for 5 mins, and was followed by equilibration back to 1% B. Fractions were collected with a Gilson FC203B fraction collector at 1 minute intervals and fractions 10-90 were concatenated to 20 fractions. The fraction volumes were next reduced by vacuum centrifugation, lyophilized, and stored at -80°C prior to analysis.

Ubiquitination sample preparation

SKMEL5 cells were seeded in 10 cm plates and allowed to adhere overnight. Cells were then treated with DMSO or 100 nM binimetinib for 72 hours. Prior to harvest, cells were treated with 100 nM bortezomib (PS-341, SelleckChem) to halt protease activity. Cells were next washed with ice cold 1X PBS and lysed in 8M Urea. Lysates were processed to tryptic peptides as described in the global protein expression methods and desalted using SepPak plus cartridges. Five mg aliquots per sample were lyophilized and stored at 80 °C prior to analysis.

PRMScan ubiquitin remnant motif (anti–K-ε-GG) antibody beads (Cell Signaling Technology, #5562) were crosslinked as previously described.(19) Briefly, beads were washed 3x with 100 mM sodium borate pH 9, incubated in cross linking buffer (20 mM DMP in 100 mM sodium borate pH9) for 30 mins (RT, rotation). Beads were next washed 3x with blocking buffer (200 mM ethanolamine, pH 8) and incubated for 2 hours at 4°C rotating. Crosslinked beads were washed 3x with immunoprecipitation buffer (100 mmol/l Tris–HCl, 1% Nonidet P-40 at pH 7.4) and stored in 1X PBS with 0.02% sodium azide at 4°C prior to use.

Each sample was resuspended in 1 mL IP buffer and added to 40 uL bead slurry of conjugated anti–K-ε-GG beads and incubated for 2 hours rotating at 4°C.(20) Peptides were washed 2x with IP buffer and 3x with 1X PBS, and diGly peptides were eluted 2x with 0.2% TFA for 5 minutes. To improve specificity, each lysate was IP'd twice, following the same IP protocol with the first elution. Finally, peptides were dried with vacuum centrifugation and lyophilized.

Lyophilized samples were next labeled with 100 µg of TMT-6plex, as described in the MHC labeling methods section. A high pH reverse-phase peptide fraction kit was used to separate labeled peptides into six fractions, according to manufacturer's instructions (17.5%, 20%, 22.5%, 25%, 30%, and 70% MeCN, Thermo Scientific). Peptide fraction volume was reduced with vacuum centrifugation, lyophilized, and stored at -80°C prior to analysis.

HF-X LC-MS/MS data acquisition

Chromatography:

Peptides were resuspended in 0.1% acetic acid and loaded on a precolumn packed in-house (100 µm ID × 10 cm packed with 10 µm C18 beads (YMC gel, ODS-A, 12 nm, S-10 µm, AA12S11)). The precolumn was then washed with 0.1% acetic acid and connected in series to an analytical capillary column with an integrated electrospray tip (~1 µm orifice) with 5µM C18 beads, prepared in house ((50 µm ID × 12 cm with 5 µm C18 beads (YMC gel, ODS-AQ, 12 nm, S-5 µm, AQ12S05)).

Labeled pMHC analyses:

Peptides were eluted using a 130-minute gradient with 10-45% buffer B (70% Acetonitrile, 0.2M acetic acid) from 5-100 minutes and 45-55% buffer B from 100-120 minutes at a flow rate of 0.2 mL/min for a flow split of approximately 10,000:1. Peptides were analyzed using a Thermo Q Exactive HF-X Hybrid Quadrupole-Orbitrap mass spectrometer, and data was acquired using Thermo Fisher Scientific Xcalibur version 2.9.0.2923. Standard mass spectrometry parameters were as follows: spray voltage, 2.5 kV; no sheath or auxiliary gas flow; heated capillary temperature, 250 °C.

The HF-X was operated in data-dependent acquisition (DDA) mode for LF and TMT analyses. LF: Full-scan mass spectrometry spectra (mass/charge ratio (*m/z*), 350 to 2,000; resolution, 60,000) were detected in the Orbitrap analyzer after accumulation of ions at $3e^6$ target value with a maximum IT of 50

ms. For every full scan, the top 20 most intense ions were isolated (isolation width of 0.4 *m/z*) and fragmented (collision energy (CE): 28%) by higher energy collisional dissociation (HCD) with a maximum injection time of 350 ms, AGC target $1e^5$, and 30,000 resolution. Charge states < 2 and > 4 were excluded, and dynamic exclusion was set to 45 seconds. TMT: Full-scan mass spectrometry spectra (mass/charge ratio (*m/z*), 400 to 2,000; resolution, 60,000) were detected in the Orbitrap analyzer after accumulation of ions at $3e^6$ target value with a maximum IT of 50 ms. For every full scan, the 20 most intense ions were isolated (isolation width of 0.4 *m/z*) and fragmented (collision energy (CE): 31%) by higher energy collisional dissociation (HCD) with a maximum injection time of 350 ms, AGC target $1e^5$, and 30,000 resolution. Charge states < 2 and > 4 were excluded, and dynamic exclusion was set to 60 seconds.

Global protein expression profiling:

Peptides were analyzed using a Thermo Q Exactive HF-X Hybrid Quadrupole-Orbitrap mass spectrometer. Standard mass spectrometry parameters were as follows: spray voltage, 2.5 kV; no sheath or auxiliary gas flow; heated capillary temperature, 250°C. Peptides were eluted with 80% acetonitrile in 0.1% formic acid (buffer B) in following gradient: 0–10% buffer B for 5 min, 10–30% for 100 min, 30–40% for 14 min, 40–60% for 5 min, 60–100% for 2 min, held at 100% of 10 mins, and equilibrated back to 0.1% formic acid. All twenty fractions were analyzed back-to-back.

The HF-X was operated in data-dependent acquisition (DDA) mode. Full-scan mass spectrometry spectra (mass/charge ratio (*m/z*), 300 to 2,000; resolution, 60,000) were detected in the Orbitrap analyzer after accumulation of ions at $3e^6$ target value with a maximum IT of 50 ms. For every full scan, the 15 most intense ions were isolated (isolation width of 0.4 *m/z*) and fragmented (collision energy (CE): 31%) by HCD with a maximum injection time of 350 ms, AGC target $1e^5$, and 30,000 resolution. Charge states of 1 and >7 were excluded, and dynamic exclusion was set to 20 seconds.

Ubiquitination analyses:

The same gradient and standard instrument parameters from global protein expression profiling were used for ubiquitination analyses. The HF-X was operated in data-dependent acquisition (DDA) mode. Full-scan mass spectrometry spectra (mass/charge ratio (m/z), 400 to 1,250; resolution, 60,000) were detected in the Orbitrap analyzer after accumulation of ions at 5e5 target value with a maximum IT of 100 ms. For every full scan, the 20 most intense ions were isolated (isolation width of 0.4 m/z) and fragmented (collision energy (CE): 33%) by HCD with a maximum injection time of 300 ms, AGC target 1e5, and 60,000 resolution. Charge states of <3 and >7 were excluded, and dynamic exclusion was set to 30 seconds. The six fractions were analyzed back-to-back to minimize effects from instrument performance variation.

Exploris 480 LC-MS/MS data acquisition

pMHC samples were analyzed using an Orbitrap Exploris 480 mass spectrometer (Thermo Scientific) coupled with an UltiMate 3000 RSLC Nano LC system (Dionex), Nanospray Flex ion source (Thermo Scientific), and column oven heater (Sonation). Samples were resuspended in 0.1% formic acid and directly loaded onto a 10-15 cm analytical capillary chromatography column with an integrated electrospray tip (~1 µm orifice), prepared and packed in house (50 µm ID 1.9 µM C18 beads, ReproSil-Pur). Unless otherwise defined, Standard mass spectrometry parameters were as follows: spray voltage, 2.0 kV; no sheath or auxiliary gas flow; heated capillary temperature, 275 °C.

<u>*Labeled DDA pMHC analyses*</u>: pMHC elutions were injected in 15-25% fractions for improved coverage of the immunopeptidome. TMT-6/10 chromatography: Peptides were eluted using a gradient with 8-25% buffer B for 50 minutes, 25-35% for 25 minutes, 35-55% for 5 minutes, 55-100% for 2 minutes, hold for 1 minutes, and 100% to 3% for 2 minutes. TMT-Pro chromatography: Peptides were eluted using a gradient with 8-25% buffer B for 50 minutes, 25-45% for 30 minutes, 45-100% for 2 minutes, hold for 1 minutes, and 100% to 3% for 2 minutes.

The Exploris was operated in data dependent acquisition (DDA) mode. Full scan mass spectra (350-1200 m/z, 60,000 resolution) were detected in the orbitrap analyzer after accumulation of $3e^6$ ions

(normalized AGC target of 300%) or 25 ms. For every full scan, MS^2 were collected during a 3 second cycle time. Ions were isolated (0.4 m/z isolation width) for a maximum of 150 ms or 75% AGC target and fragmented by HCD with 32% CE (TMT-6/10) or 30% (TMT-pro) at a resolution of 45,000. Charge states < 2 and > 4 were excluded, and precursors were excluded from selection for 30 seconds if fragmented n=2 times within 20 second window.

Isotopologue absolute quantification analyses:

Survey analyses of 4H peptides: Peptides were eluted with 6-25% buffer B for 53 minutes, 25-45% for 12 minutes, 45-97% for 3 minutes, and 97% to 3% for 1 minute. The Exploris was operated in data dependent acquisition (DDA) mode with an inclusion list(2). Full scan mass spectra (300-1500 m/z, 120,000 resolution) were detected in the orbitrap analyzer after accumulation of $3e^6$ ions or 50 ms. For each full scan, up to 20 ions were subsequently isolated for targets on the inclusion with (+/- 5 ppm of targets m/z) with a minimum intensity threshold of $1e^6$. Ions were collected with a 10s maximum injection time, AGC target: 1000%, and fragmented by HCD with 30% nCE.

SureQuant-IsoMHC targeted analyses: Standard mass spectrometry parameters for SureQuant acquisition are as follows: spray voltage: 1.6kV, heated capillary temperature: 280°C. A custom SureQuant acquisition method was built using the Thermo Orbitrap Exploris Series 2.0 software. Full-scan mass spectra were collected with scan range: 350-1200 m/z, AGC target value: $3e^6$, maximum IT: 50 ms, resolution: 120,000. 4H peptides matching the m/z (+/- 3 ppm) and exceeding the defined intensity threshold (1% apex intensity from the survey analysis) were isolated (isolation width 1 m/z) and fragmented by HCD (nCE: 27%) with a scan range: 150-1700 m/z, maximum IT: 10 ms, AGC target: 1000%, resolution: 7,500.

A product ion trigger filter next performs pseudo-spectral matching, where an MS^2 scan of the 1H, 2H, 3H, and endogenous peptides are triggered at the defined mass offsets if the 4H trigger peptide contains $n \geq 5$ product ions from the defined list. Scan parameters are the same as the first MS2 scan but with 250 ms max IT, resolution 120,000. The inclusion list, ions for pseudo-spectral matching, and additional method paramters and details have been previously reported.(2)

LC-MS/MS data analysis:

All mass spectra were analyzed with Proteome Discoverer (PD, version 2.5) and searched using Mascot (version 2.4) against the human SwissProt database. MS/MS spectra were matched with an initial mass tolerance of 10 ppm on precursor masses and 20 mmu for fragment ions. Data analyses were performed using Matlab version R2019b, and Microsoft Excel version 16.34

pMHC analyses: No enzyme was used, static modifications included N-terminal and lysine TMT, and variable modifications included oxidized methionine for all analyses and phosphorylated serine, threonine, and tyrosine for cell treatment analyses. Treatment analyses were also searched against a previously published catalog of over 40,000 predicted antigenic mutations in cancer cell lines.(21) Heavy leucine-containing peptides were searched for separately with heavy leucine (+7), c-terminal amidation, and methionine oxidation as dynamic modifications against a custom database of the synthetic peptide standards. All analyses were filtered with the following criteria: search engine rank =1, isolation interference ≤ 30%, and length between 8 and 15 amino acids. Label-free analyses were filtered with ion score ≥ 20, and labeled samples were filtered with ion score ≥ 15 and percolator q-value ≤ 0.05. Area under the curve (AUC) quantitation was performed using the minora feature detector in PD with match between runs enabled and filtered for ion score ≥ 20.

For TMT-labeled *in vitro* samples, ratios against a reference channel (usually TMT126) were calculated and the median of all ratios for correction hipMHCs was used to determine the final correction parameters. Only PSMs of heavy leucine-coded peptides with an average reporter ion intensity within 10-fold of the interquartile range of endogenous PSM reporter ion intensities were used for correction. To evaluate differences between conditions, the $\log_2$ transformed ratio of arithmetic mean intensity for drug- and DMSO-treated samples (n=3) was calculated. To determine if peptides were significantly increasing, an unpaired, 2-sided t-test was performed, and peptides with p □ 0.05 were considered significantly increasing/decreasing. To evaluate which peptides were significantly enriched above the mean, treated

samples were mean centered by dividing the ion intensity of each peptide by the mean fold-change across all peptides, after which a student's 2-tailed t-test was performed on adjusted values. Peptides with a mean-adjusted p-value 0.05 were considered significantly enriched. Mean centering was not performed on samples where the mean $\log_2$ fold change was between -0.07 and 0.07.

Global protein expression profiling: Enzyme: trypsin, allowing for up to 2 missed cleavages. Cysteine carbamidomethylation, TMT-labeled lysine, and peptide N-termini were searched as fixed modifications, and oxidated methionine was set as a variable modification. PSMs from all fractions were filtered according to search engine rank = 1, ion score ≥ 20, precursor isolation interference ≤30%. Reporter ion abundances for peptides mapping to the same protein were summed, and quantification was corrected by normalizing with the median fold change in TMT abundances over TMT-126 to account for variations in sample input. To determine differences in protein expression, an unpaired, 2-sided t-test was performed, and peptides with $p \leq 0.05$ were considered significantly changing.

Ubiquitination analysis: Enzyme: trypsin, allowing for up to 2 missed cleavages. Cysteine carbamidomethylation was set as a static modification, and dynamic modifications were set as diGly-TMT on lysine residues (monoisotopic: 343.20), and N-terminal TMT. PSMs from 6 fractions were filtered to the following criteria: di-Gly modification, search engine rank = 1, ion score ≥ 15, precursor isolation interference ≤ 30%. Reporter ion intensities were summed for PSMs mapping to the same peptide, and the fold change in abundance was calculated by taking the average reporter ion abundance for n=3 replicates per condition. Variation in sample input was account for by normalizing each reporter channel to the median fold change over TMT-126 across all peptides. When multiple peptides mapped to a source protein, the maximal fold change value was used for pMHC comparisons. Significance values were calculated using an unpaired, 2-sided t-test was performed, and peptides with $p \leq 0.05$ were considered significantly changing.

Isotopologue absolute quantification analyses:

Peak areas of 6 preselected product ions for each peptide (endogenous and 1-3H isotopologues) were exported from Skyline ((version 20.2.1.28)(22) and summed for all ions quantifiable across the endogenous and isotopologues as previously described.(2) 1-3H peptides were used to generate a calibration curve, from which endogenous pMHC concentrations were determined. Concentrations outside of the standard curve were extrapolated.

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SUPPLEMENTARY DATA

Data S1. In vitro quantitative immunopeptidomics datasets for all cell lines and treatment conditions.

Data S2. In vivo Quantitative immunopeptidomics for all cell line xenografts and treatment conditions.

Data S3. Transcript expression of SKMEL5 cells +/- 100 nM and 1 uM binimetinib treatment for 72 hours.

Data S4. Protein expression of SKMEL5 cells +/- 100 nM binimetinib treatment for 72 hours.

Data S5. Peptide ubiquitination levels of SKMEL5 cells +/- 100 nM binimetinib treatment for 72 hours.

Data S6. Absolute quantification of 18 tumor associated antigens

SUPPLEMENTARY TABLES

Table S1. Allelic profile of melanoma cell lines.

Cell line	HLA-A	HLA-B	HLA-C
SKMEL5	02:01, 11:01	40:01, 07:02	03:04, 07:02
SKMEL28	11:01	40:01	03:04
IPC298	24:02, 03:01	07:02, 35:02	04:01, 07:02
SKMEL2	26:01, 03:01	38:01, 35:08	04:01, 12:03
A375	01:01, 02:01	44:03, 57:01	06:02, 16:01
RPMI-7951	01:01, 02:01	08:01, 13:02	07:01, 06:02

Table S2. Tumor associated antigen peptide library for enrichment analyses.

AAAAAIFVI	ALIHHNTHL	AMLDLLKSV	AVFDGAQVTSK	DLTSFLLSL	ESDPIVAQY
AAANIIRTL	ALISKNPV	AMLERQFTV	AVLTKQLLH	DLWKETVFT	ESFSGSLGHL
AAFDGRHSQTL	ALKDSVQRA	AMLGTHTMEV	AVMALENNYEV	DNGAKSVVL	ESLFRAVITK
AAGIGILTV	ALKDVEERV	AMLGTHTMEVTV	AVQEFGLARFK	DPARYEFLW	ESVMINGKY
AARAVFLAL	ALLALTSAV	AMTKDNNLL	AVTNVRTSI	DPKDAEKAI	ETAGPQGPPHY
AAVEEGIVLGGG	ALLAVGATK	AMVGAVLTA	AVVDLQGGGHSY	DPSTDYYQEL	ETFTEGQKL
ACDGERPTL	ALLEIASCL	AMYDKGPFRSK	AVVGILLVV	DPYKATSAV	ETHLSSKRY
ACDPHSGHFV	ALLESSLRQA	ANADLEVKI	AVYGQKEIHRK	DQYPYLKSV	ETILTFHAF
AEEAAGIGIL	ALLKDTVYT	ANDPIFVVL	AWISKPPGV	DRASFIKNL	ETLGFLNHY
AEEAAGIGILT	ALLMPAGVPL	APAGRPSAS	AWLVAAAEI	DSDPDSFQDY	ETVELQISL
AEEHSIATL	ALLNIKVKL	APAGRPSASR	AYACNTSTL	DSFPMEIRQY	ETVSEQSNV
AEHIESRTL	ALLPSLSHC	APAGVREVM	AYDFLYNYL	DSFPMEIRQYL	EVAPDAKSF
AEINNIIKI	ALLPTALDAL	APDGAKVASL	AYGLDFYIL	DTEFPNFKY	EVAPPASGTR
AELESKTNTL	ALMDKSLHV	APLLRWVL	AYIDFEMKI	DVNGLRRVL	EVDPASNTY
AELLNIPFLY	ALMEQQHYV	APLQRSQSL	AYTKKAPQL	DVTSAPDNK	EVDPIGHLY
AELVHFLLL	ALMVRQARGL	APNTGRANQQM	AYVPQQAWI	DVWSFGILL	EVDPIGHLYIF
AEPINIQTW	ALNFPGSQK	APRGVRMAV	CHILLGNYC	DYIGPCKYI	EVDPIGHVY
AFLPWHRLF	ALPPPLMLL	APVIKARMM	CIAEQYHTV	DYKSAHKGF	EVFEGREDSVF
AFLRHAAL	ALPSFQIPV	AQAEHSITRV	CILGKLFTK	DYLQCVLQI	EVFPLAMNY
AGDGTTTATVLA	ALQDIGKNIYTI	AQCQETIRV	CITFQVWDV	DYLQYVLQI	EVFPLAMNYL
AGFKGEQGKGEP	ALQKAKQDL	AQDPHSLWV	CLAFPAPAKA	DYLRSVLEDF	EVFQNANFRSF
AGYLMELCC	ALRCASPWL	AQPDTAPLPV	CLGHNHKEV	DYPSLSATDI	EVHNLNQLLY
AHVDKCLEL	ALREEEEGV	AQYEHDLEVA	CLLSGTYIFA	DYSARWNEI	EVIGRGHFGCVY
AIDELKECF	ALSDHHIYL	ARGPESRLL	CLLWSFQTSA	EAAGIGILTV	EVIPYTPAM
AIIDPLIYA	ALSEDLLSI	ARGQPGVMG	CLVFLAPAKA	EADPTGHSY	EVIPYTPAMQR
AIISGDSPV	ALSVMGVYV	ARHRRSLRL	CLVFPAPAKA	EAFIQPITR	EVIPYTPAMQRY
AISANIADI	ALTAVAEEV	ARSVRTRRL	CLVFPAPAKAV	EEFGRAFSF	EVIQWLAKL
AIYDHINEGV	ALTDIDLQL	ARTDLEMQI	CMHLLLEAV	EEKLIVVLF	EVISCKLIKR
AIYDHVNEGV	ALTEHSLMGM	ASERGRLLY	CMLGDPVPT	EEYLQAFTY	EVISSRGTSM
AIYKQSQHM	ALTERSLMGM	ASFDKAKLK	CMTWNQMNL	EEYNSHQSL	EVITSSRTTI
AKYLMELTM	ALTPVVVTL	ASGPGGGAPR	CQWGRLWQL	EFKRIVQRI	EVKLSDYKGKYV
ALAGLSPV	ALVDAGVPM	ASLDSDPWV	CTACRWKKACQ	EFQKMRRDL	EVLDSLLVQY
ALAPAPAEV	ALVSIIKV	ASLIYRRRLMK	CTACRWKKACQR	EGDCAPEEK	EVLLRPGLHFR
ALARGAGTVPL	ALWGPDPAAA	ASSTLYLVF	CYMEAVAL	EILGALLSI	EVMSNMETF
ALASHLIEA	ALWGPDPAAAF	ASYLDKVRA	CYTWNQMNL	ELAEYLYNI	EVRGDVFPSY
ALAVLSVTL	ALWKEPGSNV	ATAGDGLIELRK	DAKNKLEGL	ELAGIGILTV	EVTFVPGLY
ALCQNGYHGT	ALWMRLLPL	ATAGIIGVNR	DALVLKTV	ELAPIGHNRMY	EVTSSGRTSI
ALCQNGYHGTI	ALWMRLLPLL	ATAQFKINK	DCLVFLAPA	ELFQDLSQL	EVVEKYEIY
ALCRWGLLL	ALWPWLLMA	ATATPCWTWLL	DEKQQHIVY	ELHLLQDEEV	EVVHKIIEL
ALDEKLLNI	ALWPWLLMA(T)	ATFSSSHRYHK	DELEIKAY	ELHLLQDKEV	EVVRIGHLY
ALDGGNKHFL	ALWPWLLMAT	ATGFKQSSK	DEVYQVTVY	ELSDSLGPV	EVYDGREHSA
ALDVYNGLL	ALYGDIDAV	ATIIDILTK	DFMIQGGDF	ELTLGEFLK	EYILSLEEL
ALEEANADL	ALYLVCGER	ATLPLLCAR	DIKAKMQAS	ELTLGEFLKL	EYLQLVFGI
ALENNYEVL	ALYSGVHKK	ATQIPSYKK	DLDVKKMPL	ELVRRILSR	EYLSLSDKI
ALFDIESKV	ALYVDSLFFL	ATSPPASVR	DLILELLDL	ELWKNPTAF	EYRGFTQDF
ALGDLFQSI	AMAPIKVRL	ATTNILEHY	DLKGFLSYL	EPLARLEL	EYSKECLKEF
ALGGHPLLGV	AMAQDPHSL	ATVGIMIGV	DLLSHAFFA	EQYEQILAF	EYSRRHPQL
ALIDCNPCTL	AMAQDPHSLWV	AVAANIVLTV	DLPAYVRNL	ERGFFYTPK	EYTAKIALL
ALIEVGPDHFC	AMARDPHSL	AVASLLKGR	DLPPPPPLL	ERLERQERL	EYYLQNAFL
ALIGGPPV	AMARDPHSLWV	AVCPWTWLR	DLSPGLPAA	ERSPVIQTL	FALQLHDPSGY

Table S2 continued

FATPMEAEL	FLPETEPPEM	FVGEFFTDV	GLWRHSPCA	HTMEVTVYHR	IMIHDLCLA
FATPMEAELAR	FLPETEPPEML	FVSGSGIAIA	GLYDGMEHL	HTRTPPIIHR	IMIHDLCLV
FAWERVRGL	FLPHFQALHV	FVSGSGIATA	GMVTTSTTL	HTYLEPGPVTAQ	IMIHDLCLVFL
FEITPPVVL	FLPRNIGNA	FVWLHYYSV	GMWESNANV	HVDSTLLQ	IMLCLIAAV
FGLATEKSR	FLQDVMNIL	FYTPKTRRE	GPFGAVNNV	HVDSTLLQV	IMNDMPIYM
FGLFPRLCPV	FLRAENETGNM	GADGVGKSA	GPFGPPMPLHV	HVYDGKFLAR	IMPGQEAGL
FIASNGVKLV	FLRNFSLML	GADGVGKSAL	GPRESRPPA	HYTNASDGL	IMPKAGLLI
FIDKFTPPV	FLRNFSLMV	GAFEHLPSL	GQHLHLETF	IALNFPGSQK	IPSDLERRIL
FIDNTDSVV	FLRNLVPRT	GAIAAIMQK	GRAPQVLVL	IARNLTQQL	IPSNPRYGM
FIDSYICQV	FLSSANEHL	GASGVGSGL	GSHLVEALY	ICLHHLPFWI	IQATVMIIV
FIFPASKVYL	FLSTLTIDGV	GCELKADKDY	GSPATWTTR	IESRTLAIA	IRRGVMLAV
FIFSILVLA	FLTGNQLAV	GDFGLATEK	GSSDVIIHR	IEVDGKQVEL	ISGGPRISY
FIIENLKAA	FLTKRGGQV	GEISEKAKL	GTAAIQAHY	IIGGGMAFT	ISKPPGVAL
FILPVLGAV	FLTKRGRQV	GERGFFYT	GTADVHFER	IIMFDVTSR	ISSVLAGASCPA
FINDEIFVEL	FLTKRSGQV	GEVDVEQHTL	GTATLRLVK	IISAVVGIL	ISTQQQATFLL
FIQVYEVERA	FLTKRSGQVCA	GFKQSSKAL	GTMDCTHPL	IITEVITRL	ITARPVLW
FKNIVTPRT	FLTKRSRQV	GIMAIELAEL	GTMDCTHSL	ILAKFLHWL	ITDFGLAKL
FLAELAYDL	FLTPKKLQCV	GIPPAPHGV	GTSSVIVSR	ILAKFLHWLe	ITDFGLARL
FLAKLNNTV	FLTPLRNFL	GIPPAPRGV	GTWESNANV	ILAVDGVLSV	ITDQVPFSV
FLALIICNA	FLTSGTQFSDA	GIVEQCCTSI	GTYEGLLRR	ILDEKPVII	ITKKVADLVGF
FLAPAKAVV	FLWGPRALA	GLAPPQHLIRV	GVALQTMKQ	ILDFGLAKL	ITQPGPLAPL
FLAPAKAVVYV	FLWGPRALV	GLASFKSFLK	GVFIQVYEV	ILDKKVEKV	ITQPGPLVPL
FLASESLIKQI	FLWGPRAYA	GLCEREDLL	GVLVGVALI	ILDKVLVHL	IVDCLTEMY
FLDEFMEGV	FLWSVFMLI	GLEALVPLAV	GVNPVVSYAV	ILDSSEEDK	IVDSLTEMY
FLDRFLSCM	FLWSVFWLI	GLEKIEKQL	GVRGRVEEI	ILDTAGREEY	IYMDGTADFSF
FLEGNEVGKTY	FLYDDNQRV	GLFDEYLEMV	GVYDGREHTV	ILFGISLREV	KAFLTQLDEL
FLFAVGFYL	FLYGALLLA	GLFGDIYLA	GYCASLFAIL	ILGALLSIL	KAFQDVLYV
FLFDGSPTY	FLYTLLREV	GLGLPKLYL	GYDQIMPKI	ILHNGAYSL	KAKQDLARL
FLFLLFFWL	FMHNRLQYSL	GLGNRWTSRT	GYDQIMPKK	ILIDWLVQV	KALRLSASALF
FLGMESCGI	FMNKFIYEI	GLGPVAAV	HAIPHYVTM	ILKDFSILL	KASEKIFYV
FLGYLILGV	FMTRKLWDL	GLIEKNIEL	HIAGSLAVV	ILLEAPTGLA	KAYGASKTFGK
FLHHLIAEIH	FMTSSWWGA	GLKAGVIAV	HLCGSHLVEA	ILLEAPTGLV	KCDICTDEY
FLIIWQNTM	FMTSSWWRA	GLLDKAVSNV	HLFGYSWYK	ILLRDAGLV	KCQEVLAWL
FLIVLSVAL	FMTSSWWRAPL	GLLDQVAAL	HLLTSPKPSL	ILLWAARYD	KEADPTGHSY
FLLDILGAT	FMVEDETVL	GLLETTVQKV	HLSTAFARV	ILMEHIHKL	KEAGNINTSL
FLLENAAYL	FMVELVEGA	GLLGQEGLVEI	HLSYHRLLPL	ILMEHIHKLK	KECVLHDDL
FLLENAAYLD	FPALRFVEV	GLLQVHHSCPL	HLSYHWLLPL	ILMEHIHKLKA	KECVLRDDL
FLLFIFKVA	FPSDSWCYF	GLMDVQIPT	HLVEALYLV	ILMHCQTTL	KEFEDDIINW
FLLGLIFLL	FPYGTTVTY	GLPAGAAAQA	HLWVKNMFL	ILNAMIAKI	KEFEDGIINW
FLLKAEVQKL	FQRQGQTAL	GLPGQEGLVEI	HLWVKNVFL	ILPLHGPEA	KEFKRIVQR
FLLKLTPLL	FRSGLDSYV	GLPPDVQRV	HLYQGCQVV	ILSAHVATA	KEFTVSGNILTI
FLLQMMQICL	FSIDSPDSL	GLPPDVQRVh	HMYHSLYLK	ILSLELMKL	KELEGILLL
FLLQMMQVCL	FSYMGPSQRPL	GLPPPPPLL	HPLVFHTNR	ILTVILGVL	KELPSLHVL
FLLSLFSLWL	FTHNEYKFYV	GLQHWVPEL	HPRQEQIAL	ILVLASTITI	KEPSEIVEL
FLMLVGGSTL	FTWAGKAVL	GLQLGVQAV	HPRYFNQLST	ILYENNVITV	KEWMPVTKL
FLMSSWWPNL	FTWAGQAVL	GLREDLLSL	HQILKGGSGTY	ILYENNVIV	KFHRVIKDF
FLNQTDETL	FTWEGLYNV	GLREREDLL	HRWCIPWQRL	IMAIELAEL	KFLDALISL
FLPATLTMV	FVEHDDESPGL	GLRRVLDEL	HSATGFKQSSK	IMDQVPFSV	KGSGKMKTE
FLPEFGISSA	FVEHDLYCTL	GLSPNLNRFL	HSSSHWLRLP	IMFDVTSRV	KIADPICTFI
FLPETEPPEI	FVFLRNFSL	GLSTILLYH	HSWITRSEA	IMIGVLVGV	KIDEKTAELK

Table S2 continued

KIFDEILVNA	KLQELNYNL	KTWDQVPFS	LLDGTATLRL	LLMPAGVPL	LMLGEFLKL
KIFGSLAFL	KLQQKEEQL	KTWDQVPFSV	LLDKAVSNVI	LLMPAGVPLT	LMLQNALTTM
KIFSEVTLK	KLQVFLIVL	KTWDQVPFSVSV	LLDRFLATV	LLMWITQCF	LMVLMLAAL
KILDAVVAQK	KLSEGDLLA	KTWGQYWQ	LLDTNYNLF	LLNAFTVTV	LMWAKIGPV
KINKNPKYK	KLSEQESLL	KTWGQYWQV	LLDTNYNLFY	LLNATIAEV	LNIDLLWSV
KIQEILTQV	KLTQINFNM	KVAELVHFL	LLDVAPLSL	LLNLPDKMFL	LNIYEKDDKL
KIQRNLRTL	KLVERLGAA	KVAELVRFL	LLDVPTAAV	LLNLPVWVL	LNLPDKMFL
KIWEELSVLE	KLVMSQANV	KVFGSLAFV	LLEAPTGLV	LLNQPDKMFL	LPAVVGLSPGEQ
KIWEELSVLEV	KLVVVGAVGV	KVHPVIWSL	LLEEMFLTV	LLPENNVLSPV	LPGEVFAI
KIYSENLKL	KLYSENLKL	KVIDQQNGL	LLESAFPGGL	LLPPLLEHL	LPHAPGVQM
KIYSENLKLA	KLYSENLKLA	KVLEFLAKL	LLFETVMCDT	LLQAEAPRL	LPHNHTDL
KIYSENLTL	KLYSENLTL	KVLEHVVRV	LLFGLALIEV	LLQDSVDFSL	LPHSEITTL
KIYSENLTLA	KLYSENLTLA	KVLEYVIKV	LLFLLQMMQI	LLQEEEEEL	LPHSSSHWL
KLADQYPHL	KMAAFPETL	KVLHELFGMDI	LLFLLQMMQV	LLQEYNWEL	LPLLALLAL
KLAEAERVGLHK	KMAELVHFL	KVNIVPVIAK	LLFPYILPPKA	LLQGWVMYV	LPMEVEKNSTL
KLAKPLSSL	KMDAEHPEL	KVSAVTLAY	LLFSFAQAV	LLQLGYSGRL	LPPPPPLLDL
KLATAQFKI	KMFVKGAPDSV	KVVEFLAML	LLGATCMFV	LLQLYSGRL	LPQKKSNAL
KLCKVRKITV	KMFVKGAPESV	KYDCFLHPF	LLGCPVPLGV	LLQMMQICL	LPRWPPPQL
KLCPVQLWV	KMISAIPTL	KYIQESQAL	LLGDLFGV	LLQMMQVCL	LPSSADVEF
KLDETGNSL	KMLDHEYTT	KYLATASTM	LLGNCLPTV	LLQVHHSCPL	LQSRGYSSL
KLDETGNSLK	KMLKSFLKA	KYLKLSSSEL	LLGPGRPYR	LLRGYHQDAY	LRAGRSRRL
KLDETGNSLKV	KMNVFDTNL	KYVGIEREM	LLGPTVML	LLSAEVQQHL	LRRYLENGK
KLDVGNAEV	KMQASIEKA	LAALPHSCL	LLGRFELIGI	LLSAVLPSV	LSIGTGRAM
KLEGLEDAL	KMRRDLEEA	LAAQERRVPR	LLGRNSFEV	LLSDDDVVV	LSRLSNRLL
KLFGSLAFV	KMVELVHFL	LALWGPDPAA	LLHVHHSCPL	LLSDEDVAL	LTLGEFLK
KLFGVLRLK	KMYAFTLES	LAMPFATPM	LLIADNPQL	LLSDEDVALM	LTLGEFLKL
KLGDCIWTYL	KMYAFTLESV	LAPAKAVVYV	LLIDLTSFL	LLSDEDVALMV	LTLTTGEWAV
KLGDCIWTYLS	KNKRILMEH	LASEKVYTI	LLIDLTSFLL	LLSDEDVEL	LTYNDFINK
KLGDCIWTYPS	KPIVVLHGY	LATEKSRWS	LLIGATIQV	LLSDEDVELM	LTYVSFRNL
KLIETYFSK	KPQQKGLRL	LATEKSRWSG	LLIGATIQVT	LLSDEDVELMV	LVALLACLTV
KLIGDPNLEFV	KPRQSSPQL	LAVDGVLSV	LLIGATMQV	LLSETVMCDT	LVALLVCLTV
KLIKDGLIIRK	KPSGATEPI	LCGSHLVEAL	LLIGATMQVT	LLSGQPASA	LVCGERGFFY
KLKHYGPGWV	KPSPPYFGL	LDKVRALEE	LLIGGFAGL	LLSHGAVIEV	LVFGIELMEV
KLLDISELDMV	KQDFSVPQL	LEEKKGNYV	LLIKKLPRV	LLSILCIWV	LVFLAPAKAVV
KLLEYIEEI	KQDNSTYIMRV	LEEYNSHQSL	LLLDDLLVSI	LLSLFSLWL	LVHFLLLKY
KLLGPHVEGL	KQLPEEKQPLL	LEKQLIEL	LLLEAVPAV	LLSPLHCWA	LVLKRCLLH
KLLGPHVLGV	KQPAIMPGQSY	LGYGFVNYI	LLLELAGVTHV	LLSPLHCWAV	LVMAPRTVL
KLLMVLMLA	KQSSKALQR	LGYGFVNYV	LLLGIGILV	LLSSGAFSA	LVQENYLEY
KLLQIQLCA	KRIQEIIEQ	LHHAFVDSIF	LLLGPLGPL	LLTSRLRFI	LVVVGAVGV
KLLQIQLCAKV	KRTLKIPAM	LIAHNQVRQV	LLLGTIHAL	LLTTLSNRV	LWMRLLPLL
KLLQIQLRA	KSEMNVNMKY	LIFDLGGGT	LLLHCPSKTV	LLVALAIGCV	LYATVIHDI
KLLQIQLRAKV	KSLNYSGVK	LIFDLGGGTFD	LLLLDVAPL	LLVSEIDWL	LYAWEPSFL
KLLSSGAFSA	KSMNANTITK	LIYDSSLCDL	LLLLTVLTV	LLWWIAVGPV	LYLVCGERGF
KLMPPDRTAV	KTCPVQLWV	LIYRRRLMK	LLLPAEVQQHL	LLYKLADLI	LYSACFWWL
KLMSPKLYVW	KTIHLTLKV	LKLSGVVRL	LLLPALAGA	LMAGCIQEA	LYSDPADYF
KLNVPATFML	KTLGKLWRL	LLAAVAALL	LLLPGPSAA	LMALPPCHAL	LYVDSLFFL
KLPNSVLGR	KTLTSVFQK	LLAGIGTVPI	LLLRSPAGV	LMASSPTSI	LYVDSLFFLc
KLQAPVQEL	KTPFVSPLL	LLAGPPGV	LLLSAEVQQHL	LMETHLSSK	MALENNYEV
KLQATVQEL	KTVDLILEL	LLASSMSSQL	LLMEGVPKSL	LMFWSPSHSCA	MAQKRIHAL
KLQEELNKV	KTVNELQNL	LLDDSLVSI	LLMEKEDYHSL	LMGDKSENV	MAVPPCCIGV

Table S2 continued

MEGEVWGL	NLFDTAEVYA	PLTSIISAV	RAPPTTPAL	RLLYPDYQI	RTFHHGVRV
MEIFIEVFSHF	NLFDTAEVYAA	PPSACSPRF	RASHPIVQK	RLMKQDFSV	RTGEVKWSV
MEKEDYHSL	NLFETPVEA	PTLDKVLEL	RAYQQALSR	RLNAALREK	RTIAPIIGR
MEVDPIGHLY	NLFLFLFAV	PTLDKVLEV	RCHELTVSL	RLPRIFCSC	RTIPTPLQPL
MFPEVKEKG	NLIKLAQKV	QCSGNFMGF	RELEETNQKL	RLQGISPKI	RTKQLYPEW
MGNIDSINCK	NLKLKLHSF	QFITSTNTF	REPVTKAEML	RLQREWHTL	RTLAEIAKV
MIAVFLPIV	NLLDSLEQYI	QGQHFLQKV	REQFLGALDL	RLQTPMQVGL	RTLDKVLEV
MIHDLCLAFPA	NLLEREFGA	QIAKGMSYL	RESEEESVSL	RLQVPVEAV	RTNWPNTGK
MIHDLCLVFL	NLLGRFELI	QIEGLKEEL	RFEEKHAYF	RLRAPEVFL	RTTEINFKV
MIMQGGFSV	NLLGRFELIGI	QILKGGSGT	RFKMFPEVK	RLRPLCCTA	RVFQGFFTGR
MLAVISCAV	NLPDKMFLPGA	QILPLHGPEA	RIAECILGM	RLSCPSPRA	RVHAYIISY
MLGDPVPTPT	NLQGSPVYV	QIRPIFSNR	RIDITLSSV	RLSCSSPRA	RVKAPNKSL
MLGTHTMEV	NLSALGIFST	QLARQQVHV	RIGQRQETV	RLSSCVPVA	RVLRQEVAAPL
MLLAVLYCL	NLSSAEVVV	QLCAKVPLL	RIKDFLRNL	RLTSTNPTM	RVLRQEVEAPL
MLLDKNIPI	NLVRDDGSAV	QLCPICRAPV	RILGPGLNK	RLTSTNPTT	RVPGVAPTL
MLLKTSEFL	NLWDLTDASVV	QLEERTWLL	RILMEHIHKLK	RLVDDFLLV	RVQEAVESMVK
MLLSVPLLLG	NLYPFVKTV	QLFEDNYAL	RINEFSISSF	RLVELAGQSLLK	RVRFFFPSL
MLMAQEALAFL	NMQDLVEDL	QLFNHTMFI	RIVQRIKDF	RLWQELSD	RVSLPTSPR
MLPSQPTLL	NMVAKVDEV	QLFNKHTMFI	RLAEYQAYI	RLWTTTRPRV	RVTSIRLFEV
MLTNSCVKL	NPATPASKL	QLGPTCLSSL	RLARLALVL	RLYDEKQQHI	RVWDLPGVLK
MLVGGSTLCV	NPIVVFHGY	QLGPVGGVF	RLASFYDWLP	RLYDEKQQHIVY	RWPSCQKKF
MLWSCTFCRI	NPKAFFSVL	QLGRISLLL	RLASSVLRCGK	RLYEMILKR	RYAMTVWYF
MLWSCTFCRM	NSELSCQLY	QLIMPGQEA	RLASYLDKV	RLYPWGVVEV	RYCNLEGPPI
MLYYPSVSR	NSQPVWLCL	QLLALLPSL	RLDFNLIRV	RMFPNAPYL	RYGSFSVTL
MMKMMCIKDL	NTDSPLRY	QLLDGFMITL	RLDQLLRHV	RMLPHAPGV	RYMPPAHRNF
MMLPSQPTL	NTYASPRFK	QLLDQVEQI	RLFAFVRFT	RMMEYGTTMV	RYQLDPKFI
MMLPSQPTLL	NTYASPRFKf	QLLIKAVNL	RLFFYRKSV	RMMLPSQPTL	RYQQWMERF
MMLPSQPTLLT	NVIRDAVTY	QLLKLNVPA	RLFVGSIPK	RMPEAAPPV	SACDVSVRV
MMLPSRPTL	NVLHFFNAPL	QLLNSVLTL	RLGGAALPRV	RMTDQEAIQ	SACDVSVRVV
MMLPSRPTLL	NVMPVLDQSV	QLMAFNHLV	RLGLQVRKNK	RMTDQEAIQDL	SAFPTTINF
MMLPSRPTLLT	NYARTEDFF	QLQGLQHNA	RLGNSLLLK	RNGYRALMDKS	SAGPPSLRK
MMQICLHHL	NYKHCFPEI	QLSLLMWIT	RLGPTLMCL	RPHVPESAF	SASVQRADTSL
MMQVCLHHL	NYKRCFPVI	QLSSGVSEIRH	RLGPVARTRV	RPKSNIVL	SAWISKPPGV
MMSEGGPPGA	NYNNFYRFL	QLVFGIEVV	RLIDLGVGL	RPKSNIVLL	SAYGEPRKL
MMYKDILLL	NYSVRYRPGL	QLVIQCEPL	RLIGDAAKNQV	RQAGDFHQV	SEHLDTQKELL
MPFATPMEA	PAFSYSFFV	QLYALPCVL	RLLASLQDL	RQFVTQLY	SEIWRDIDF
MPFATPMEAEL	PLADLSPFA	QMFFCFKEL	RLLCALTSL	RQKKIRIQL	SEIWRDIDFd
MPGEATETV	PLALEGSLQK	QMMQICLHHL	RLLDLAQEGL	RQKRILVNL	SELFRSGLDSY
MQLIYDSSL	PLDGGVAAA	QMMQVCLHHL	RLLIKKLPRV	RQLAQEQFFL	SESIKKKVL
MSLQRQFLR	PLFDFSWLSL	QQITKTEV	RLLKEYQEL	RQVGDFHQV	SESLKMIF
MTSALPIIQK	PLFQVPEPV	QQLDSKFLEQV	RLLPLLALL	RRFFPYYV	SFSYTLLSL
MTVDSLVNK	PLHCWAVLL	QRPYGYDQIM	RLLPLLALLAL	RRKWRRWHL	SGMGSTVSK
MVIGIPVYV	PLHCWVVLL	QVFPGLLERV	RLLPLWAAL	RRQRRSRRL	SHETVIIEL
MVKISGGPR	PLLALLALWG	QVLDLRLPSGV	RLLPLWAALPL	RRRWHRWRL	SHLVEALYLV
MVWESGCTV	PLLENVISK	QYSWFVNGTF	RLLQETELV	RSCGLFQKL	SIFDGRVVAK
MVYDLYKTL	PLPEAPLSL	RAGLQVRKNK	RLLSDEDVAL	RSDSGQQARY	SIFTWAGKAVL
MYIFPVHWQF	PLPPARNGGL	RALAETSYV	RLLSDEDVALM	RSKFRQIV	SIFTWAGQAVL
NCLKLLESL	PLPPARNGGLg	RALAKLLPL	RLLSDEDVEL	RSRRVLYPR	SILEDPPSI
NLAQDLATV	PLQPEQLQV	RALEEANADLEV	RLLSDEDVELM	RSYHLQIVTK	SIQNYHPFA
NLATYMNSI	PLTEYIQPV	RALRLTAFASL	RLLVPTQFV	RSYVPLAHR	SISVLISAL

Table S2 continued

SIVKIQSWFRM	SLMSWSAIL	SQGFSHSQM	THFPDETEI	TMTRVLQGV	VLHDDLLE
SLAAGVKLL	SLNYSGVKEL	SQKTYQGSY	TIADFWQMV	TPGNRAISL	VLHDDLLEA
SLAAYIPRL	SLPGGTAS	SQLTTLSFY	TIHDSIQYV	TPNQRQNVC	VLHELFGMDI
SLADEAEVYL	SLPKHSVTI	SQQAQLAAA	TILLGIFFL	TPRLPSSADVEF	VLHWDPETV
SLADTNSLAV	SLPPPGTRV	SRASRALRL	TIMIHDLCLA	TPRTPPPQ	VLLESAFPGGL
SLADTNSLAVV	SLPRGTSTPK	SRDSRGKPGY	TINPQVSKT	TQPGPLAPL	VLLESAFPGRL
SLAMLDLLHV	SLQALKVTV	SRFGGAVVR	TIPTPLQPL	TQPGPLVPL	VLLGMEGSV
SLASLLPHV	SLQDVPLAAL	SRFTYTALK	TIRYPDPVI	TRPWSGPYIL	VLLLVLAGV
SLAVVSTQL	SLQEEIAFL	SSADVEFCL	TLADFDPRV	TRVLAMAIY	VLLQAGSLHA
SLCPWSWRAA	SLQEKVAKA	SSDNYEHWLY	TLAKYLMEL	TSALPIIQK	VLLRHSKNV
SLDDYNHLV	SLQKRGIVEQ	SSDYVIPIGTY	TLDEKVAELV	TSDQLGYSY	VLMIKALEL
SLDDYNHLVTL	SLQPLALEG	SSFGRGFFK	TLDSQVMSL	TSEHSHFSL	VLNSLASLL
SLDKDIVAL	SLQRMVQEL	SSKALQRPV	TLDWLLQTPK	TSEKRPFMCAY	VLNSVASLL
SLEEEIRFL	SLQRTVQEL	SSLSLFFRK	TLEEITGYL	TSTTSLELD	VLPDVFIRC
SLEENIVIL	SLQSMVQEL	SSPGCQPPA	TLEGFASPL	TTINYTLWR	VLPDVFIRCV
SLFEGIDIYT	SLQSTVQEL	SSSGLHPPK	TLGEFLKL	TTLITNLSSV	VLPDVFIRCV
SLFEGVDFYT	SLRILYMTL	SSVPGVRLL	TLITDGMRSV	TTNAIDELK	VLQELNVTV
SLFGKLQLQL	SLSKILDTV	STALRLTAF	TLKCDCEIL	TVASRLGPV	VLQVGLPAL
SLFLGILSV	SLSPLQAEL	STAPPAHGV	TLKKYFIPV	TVFDAKRLIGR	VLQWLPDNRL
SLFPNSPKWTSK	SLSRFSWGA	STAPPVHNV	TLLASSMSSQL	TVSGNILTIR	VLQWLSDNRL
SLFRAVITK	SLVEELKKV	STDPQHHAY	TLLIGATIQV	TYACFVSNL	VLRDDLLEA
SLFVSNHAY	SLWGGDVVL	STIKFQMKK	TLLIGATIQVT	TYLPTNASL	VLRENTSPK
SLGEQQYSV	SLWSSSPMA	STLCQVEPV	TLLIGATMQV	TYSEKTTLF	VLRKEEEKL
SLGIMAIEL	SLWSSSPMAT	STLQGLTSV	TLLIGATMQVT	VAANIVLTV	VLRQEVAAPL
SLGSPVLGL	SLWSSSPMATT	STMPHTSGMNR	TLLLEGVMAA	VAELVHFLL	VLSVNVPDV
SLGWLFLLL	SLYHVYEVNL	STPPPGATRV	TLLNLPDKMFL	VAVKAPGFGD	VLTSESMHV
SLIAAAAFCLA	SLYKFSPFPL	STSQEIHSATK	TLLNQPDKMFL	VCGERGFFYT	VLVEGSTRI
SLIKQIPRI	SLYQLENYC	STVASRLGPV	TLLPATMNI	VCLHHLPFWI	VLVPPLPSL
SLKLLESLTPI	SLYSFPEPEA	STVASWLGPV	TLLSNIQGV	VEETPGWPTTL	VLWDRTFSL
SLLDRFLATV	SMCRFSPLTL	SVAQQLLNGK	TLMSAMTNL	VEGSGELFRW	VLYGPDAPTV
SLLGLALLAV	SMLIRNNFL	SVASLLPHV	TLPGYPPHV	VEIEERGVKL	VLYPRVVRR
SLLGQLSGQV	SMPPPGTRV	SVASTITGV	TLPPAWQPFL	VFLPCDSWNL	VLYRYGSFSV
SLLKFLAKV	SMPQGTFPV	SVGSVLLTV	TLPPRPDHI	VIMPCSWWV	VLYRYGSFSVTL
SLLLELEEV	SMSKEAVAI	SVHSLHIWSL	TLRTGEVKWSV	VISNDVCAQV	VMALENNYEV
SLLMWITQA	SMSSQLGRISL	SVKPASSSF	TLSSRVCCRT	VIVMLTPLV	VMIIVSSLAV
SLLMWITQC	SNDGPTLI	SVPQLPHSSSHW	TLTNIAMRPGL	VIWEVLNAV	VMLDKQKEL
SLLMWITQCFL	SPASSRTDL	SVQGIIIYR	TLTTGEWAV	VLAGGFFLL	VMNILLQYV
SLLNLPVWV	SPAVDKAQAEL	SVSPVVHVR	TLWVDPYEV	VLAGVGFFI	VMNILLQYVV
SLLNLPVWVLM	SPGSGFWSF	SVVKIQSWFRM	TLYEAVREV	VLASIEAEL	VPGWGIALL
SLLPAIVEL	SPHPVTALL	SVYDFFVWL	TLYNPERTITV	VLASIEAELPM	VPLDCVLYRY
SLLQHLIGL	SPLFQRSSL	SYLDKVRA	TMASTSVSRSA	VLASIEPEL	VPRSAATTL
SLLQSREYSSL	SPQNLRNTL	SYLDSGIHF	TMESMNGGKLY	VLASIEPELPM	VPYGSFKHV
SLLQSRGYSSL	SPRFSPITI	SYRNEIAYL	TMGGYCGYL	VLCSIDWFM	VRIGHLYIL
SLLSGDWVL	SPRPPLGSSL	SYTRLFLIL	TMHSLTIQM	VLDFRLPSGV	VRLGSLSTK
SLLSLPVWV	SPRWWPTCL	TALRLTAFASL	TMKIYSENLTL	VLDGLDVLL	VRSRRCLRL
SLLSLPVWVLM	SPSKAFASL	TCQPTCRSL	TMKLYSENLTL	VLEGMEVV	VSDFGGRSL
SLLSPLHCWA	SPSSNRIRNT	TEAASRYNL	TMLARLASA	VLFGLGFAI	VSLLSLPVWV
SLLSPLHCWAV	SPSVDKARAEL	TETEAIHVF	TMLGRRAPI	VLFSSDFRI	VTLLIGATIQV
SLLTSSKGQLQK	SPTSSRTSSL	TFDYLRSVL	TMLGRRPPI	VLFYLGQY	VTLLIGATMQV
SLMASSPTSI	SQFGGGSQY	TFPDLESEF	TMNGSKSPV	VLFYLGQYI	VTTDIQVKV

Table S2 continued

VVEGTAYGL	YLDPAQQNL	YVDPVITSI
VVHFFKNIV	YLEPGPVTA	YVFTLLVSL
VVLGVVFGI	YLEYRQVPV	YVIPIGTYGQM
VVMSWAPPV	YLFSEEITSG	YYNAFHWAI
VVMVNQGLTK	YLGSYGFRL	YYSVRDTLL
VVPCEPPEV	YLIELIDRV	YYWPRPRRY
VVPEDYWGV	YLLKPVQRI	RVASPTSGVK
VVQNFAKEFV	YLLLRVLNI	
VVTGVLVYL	YLLPAIVHI	
VVVGAVGVG	YLLPEAEEI	
VYDFFVWLHY	YLLQGMIAAV	
VYDYNCHVDL	YLMDTSGKV	
VYFFLPDHL	YLNDHLEPWI	
VYGIRLEHF	YLNKEIEEA	
VYLDKFIRL	YLQGMIAAV	
VYSDADIFL	YLQLVFGIEV	
VYVKGLLAKI	YLQQNTHTL	
WATLPLLCAR	YLSGANLNL	
WGPDPAAA	YLSGANLNLG	
WLDEVKQAL	YLSGANLNV	
WLDPNETNEI	YLVGNVCIL	
WLEYYNLER	YLVPQQGFFC	
WLLPLWAAL	YLYDRLLRI	
WLLPLWAALPL	YLYGQTTTY	
WLPFGFILI	YMDGTMSQV	
WLPKILGEV	YMFDVTSRV	
WLQYFPNPV	YMFPNAPYL	
WLSLKTLLSL	YMIAHITGL	
WLSLLFKKL	YMIDPSGVSY	
WMRLLPLLAL	YMIMVKCWMI	
WQYFFPVIF	YMIPSIRNSI	
WYEGLDHAL	YMMPVNSEV	
WYQTKYEEL	YMNGTMSQV	
YAVDRAITH	YMNSIRLYA	
YEDIHGTLHL	YPFKPPKV	
YEGSPIKVTL	YQGSYGFRL	
YGHSGQASGLY	YQLDPKFIV	
YGYDNVKEY	YRPRPRRY	
YIDEQFERY	YRYGSFSVTL	
YIFAVLLVCV	YSDHQPSGPYY	
YIGEVLVSV	YSLEYFQFV	
YLAMPFATPME	YSLKLIKRL	
YLAPENGYL	YSWMDISCWI	
YLCDKVIPG	YTCPLCRAPV	
YLCDKVVPG	YTDFHCQYV	
YLCSGSSYF	YTDFVGEGL	
YLCSGSSYFV	YTDQPSTSQIAY	
YLDLFGDPSV	YTLDRDSLYV	
YLDLLFQIL	YTMKEVLFY	
YLDLLFQILL	YVDFREYEYY	

Table S3. Custom library of tumor associated antigen source proteins.

ABCA1	BING4	CLTC	DUSP22	GNTK	IMP3
ABCA6	BIRC5	CLYBL	EEF2	GPC3	INPP5D
ABCC3	BIRC7	CML28	EFTUD2	GPCPD1	INS
ABCD3	BIRC8	CNMD	EGFR	GPNMB	INSM2
ABL1	BIRC9	COA1	EHD2	GPR143	INTS11
ACPP	BRAF	COL2A1	EIF2S3	HAO2	INTS13
ACRBP	BST2	COL4A3	EIF3D	HAUS3	IQGAP2
ACTB	BTBD2	COL6A2	ELAC2	HBD	IRS2
ACTN4	BTG1	CORO1A	ELAVL1	HCG	ITGAL
ADAM17	BTK	COX2	ELAVL4	HDAC1	ITGAM
ADAMTSL5	C18orf21	CPSF	EML6	HDGF	ITGB2
ADRP	C2CD4A	CPSF1	ENAH	HEPACAM	ITGB8
AFP	C5	CPVL	EPCAM	HHAT	KAAG1
AIM2	CA9	CR2	EPHA2	HIFPH3	KCNAB1
AIMP1	CACNG	CRABP1	ERAP1	HIST4H4	KDM2B
AKAP13	CADH3	CRNKL1	ERBB2	HIVEP1	KDM5B
ALDH1A1	CALCA	CSAG2	ERVK3-1	HLA-A	KDM5C
ALDOA	CALR	CSF1	ETV5	HLA-B	KDM5D
ALK	CAMP	CSF3R	EVI2B	HLA-DOB	KDR
ALYREF	CASP5	CSNK1A1	EZH2	HLA-DPA1	KIAA1551
AML1	CASP8	CSPG4	EZR	HMMR	KIF20A
AMZ2	CCDC110	CT83	FAM136A	HMOX1	KLK10
ANKRD30A	CCL3	CTAG1A	FASN	HMSD	KLK3
ANO7	CCL3L1	CTDP1	FBXW11	HNF4G	KLK4
ANXA1	CCLA2	CTNNB1	FCER1A	HNRNPL	KRAS
APOBEC3H	CCNA1	CTPS1	FDPS	HNRNPLL	KRI1
ARF1	CCNB1	CTSH	FGF5	HNRNPR	KRT16
ARHGAP15	CCND1	CYP1B1	FGF6	HOXD3	KRT18
ARHGAP25	CCNI	CYP21A2	FLT3	HPN	KRT6C
ARHGAP4	CD19	CYP2A6	FLT3LG	HPSE	KTN1
ARHGAP45	CD274	CYP2A7	FMOD	HSDL1	LAGE1
ARL4D	CD33	CYP2C8	FMR1NB	HSP90AB1	LAGE3
ART4	CD48	CYP2C9	FNDC3B	HSPA1A	LAS1L
ART5	CD69	CYP2D6	FOLH1	HSPA1B	LCK
ASH1L	CD79B	CYPB	FOXO1	HSPA1L	LCP2
ATIC	CDC5L	DAPK2	G3BP1	HSPA6	LGALS1
ATP2A3	CDCA7L	DCT	G6PC2	HSPB1	LGALS3BP
ATXN10	CDH13	DDX21	GAD2	HSPD1	LGSN
B2A2	CDK12	DDX3Y	GAGE1	ICAM3	LPGAT1
B3A2	CDK4	DDX5	GAS7	ICE	LRMP
BA46	CDKN1A	DKK1	GATA2	IDNK	LRRC8A
BAD	CDKN2A	DLAT	GC	IDO1	LTB
BAGE1	CDR2	DMD	GCGR	IER3	LY6K
BCAP31	CEACAM5	DMXL1	GEMIN4	IFG2BP3	LYN
BCHE	CELF6	DNAJC2	GFAP	IFI30	MAG
BCL2	CELSR1	DNMBP	GINS1	IFI6	MAGEA1
BCL2A1	CENPM	DNMT1	GLRX3	IGF2BP2	MAGEA12
BCL2L4	CEP55	DOCK2	GLS	IGF2BP3	MAGEB2
BFAR	CLCA2	DOK2	GNAO1	IL13RA2	MAGEC1
BID	CLP	DSE	GNL3L	IL2RG	MAGEC2

Table S3 continued

MAGEC2	NELFA	PSMB1	SIRT2	UBE2C
MAGED2	NFYC	PSMB10	SLC25A5	UBE2D2
MAGEE1	NISCH	PTHLH	SLC30A8	UGT2B17
MAGEF1	NLRP5	PTPN11	SLC41A3	UQCR10
MAGEF1	NOB1	PTPN21	SLC45A2	UQCRH
MALL	NONO	PTPRC	SLC45A3	USP11
MAP4K1	NPM1	PTPRN	SLCO2A1	USP9X
MARK3	NQO1	PTTG1IP	SNRNP70	USP9Y
MATN2	NRAS	PUM3	SNRPD1	UTY
MBP	NUDCD1	PWWP3A	SNX14	VEGFA
MC1R	NUF2	PXDN	SOX10	VENTXP1
MCF2	NUF3	RAB38	SP110	VGF
MCM5	NUP210	RAN	SPA17	VIM
MCMBP	NUP37	RASGRF1	SPARC	VIPR1
MDK	OCA2	RASGRP2	SPATA5L1	VPS13B
MDM2	OGT	RASSF10	SSX1	VSIG10L
MDN1	OS9	RBAF600	SSX2	WNK2
ME1	P2RX5	RBBP4	STAT1	WT1
MED23	PAK2	RBL2	STEAP1	XAGE1B
MED24	PARP10	RFA1	SUGT1	XBP1
MET	PARP3	RGS5	SUPT5H	ZFAND5
METTL21A	PASD1	RHOC	SYNGR1	ZFHX3
MFGE8	PAX3	RINT1	TAG1	ZFP36L1
MICA	PAX5	RNF19B	TALDO1	ZFY
MLANA	PCDH11Y	RNF43	TBC1D22A	ZMYM4
MMP2	PCDH20	RPA1	TCHH	ZNF395
MMP7	PDGFRA	RPL10A	TEK	
MOK	PFKM	RPL19	TEP1	
MPL	PGK1	RPS2	TERT	
MRPL19	PHB	RPS4Y1	TG	
MS4A1	PHRF1	RPSA	TGRBR2	
MSCP	PIM1	RUBCNL	THEM6	
MSLN	PLAC1	SAGE1	TMCO1	
MT-ATP6	PLIN2	SART1	TMED4	
MT-CO2	PLP1	SART3	TMSB10	
MTRR	PMEL	SASH1	TMSB4Y	
MUC1	POP1	SCGB2A2	TOP1	
MUC16	PP2A	SCGB2A7	TOP2A	
MUC5AC	PPFIBP1	SCRN1	TP53	
MUM2	PPIB	SEC31A	TP53I11	
MUM3	PPP1R3B	SELL	TPBG	
MYH1	PRAME	SELPLG	TPO	
MYH2	PRDM1	SEPT2	TRIM22	
MYH9	PRDX2	SEPT6	TRIM68	
MYO1G	PRDX5	SERPINB5	TTK	
N4BP2	PRELID1	SF1	TTN	
N4BP2L1	PRKCB	SFMBT1	TYMS	
NACA2	PRTN3	SGT1B	TYR	
NCF4	PSD4	SH3GLB2	TYRP1	
NECTIN4	PSMA3	SIRPD	UBD	

Table S4. CLX treatment groups and dosing schedule. Groups 6 and 7 were not included in SKMEL2/IPC298 studies (*NRAS* mutant).

Study groups	Schedule	Route
1. Vehicle (1%CMC/0.5%Tween80)	BID1-3, 2hr	PO
2. 3.5 mg/kg MEK162	QD, 2hr	PO
3. 3.5 mg/kg MEK162	BID1-2, 2hr	PO
4. 3.5mg/kg MEK162	BID1-3, 2hr	PO
5. 3.5mg/kg MEK162	BID1-5, 2hr	PO
6. 20mg/kg LGX818	QD1-3, 2hr	PO
7. 3.5 mg/kg MEK162/ 20mg/kg LGX818	BID1-3, 2hr / QD1-3	PO