SUPPLEMENTARY MATERIAL

Atractylenolide I enhances responsiveness to immune checkpoint blockade therapy by activating tumor antigen presentation

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- Supplementary Methods and References
- Supplementary Figure 1-11
- Supplementary Table 1-5

SUPPLEMENTARY METHODS

Reagents

The following antibodies were used: Immunoprecipitation antibody for pulling down PSMD4 is proteasome 19S S5A/ASF antibody (ab20239, Abcam). Western blotting antibodies including S5a/PSMD4 antibody (3336S, Cell Signaling), PSMD7 antibody (3336S, Cell Signaling), GFP(FL) Rabbit polyclonal IgG (sc-8334, Santa Cruz) and anti-β-actin (sc-8432, Santa Cruz); immunofluorescence staining antibodies including anti-human HLA-A,B,C (ab70328, Abcam) and anti-EGFR (#4267, Cell Signaling); immunohistochemical staining antibodies including anti-CD8a (98941S, Cell Signaling), anti-F4/80 (70076S, Cell Signaling) and anti-CD4 (25229S, Cell Signaling); flow cytometry antibodies including H-2Kb-FITC (Clone AF6-88.5; Biolegend), H-2Kb-PerCPCy5.5 (Clone AF6-88.5; Biolegend), H-2Kb bound to SIINFEKL-APC (clone 25-D1.16, Biolegend), CD45-BV605 (Clone 30-F11; Biolegend), TCRβ-PE/Cy7 (Clone H57-597; Biolegend), CD11b-eFluor450 (Clone M1/70; eBioscience), CD4-AF700 (Clone GK1.5; Biolegend), CD8-APC/Cy7 (Clone 53-6.7; Biolegend), 7-AAD (Biolegend), H-2k^{d}-APC (Clone SF1-1.1; Biolegend), Isotype Mouse IgG2a-APC (Clone MOPC-173, Biolegend) and HLA-A,B,C-APC/Cy7 (Clone W6/32, Biolegend); BioXCell antibodies including anti-mouse PD-1 (clone RMP1-14; BE0146), anti-mouse CD4 (clone GK1.5; BE0003-1), rat IgG2a isotype control (Clone 2A3, BE0089), anti-CD20 (Clone AISB12, BE0302), anti-CD8 (Clone 53-6.72; BE0004-1), SYTOX Blue Dead Cell Stain (S34857, ThermoFisher).The dilution of the antibodies was done using the pH 7.0 dilution buffer (BioXcell). Rat IgG2a Isotype control (InVivoMab, Clone 2A3, BE0089), anti-mouse MHC Class I (H2) (InVivoMab,Clone M1/42.3.9.8, BE0077), ATT-I (Yuanye Biotechnology Co., China, B20054; CAS#73069-13-3).

Three-dimensional protein structure analysis

The PyMol graphics program was used to depict the three-dimensional structures shown in Fig. 4. The Schrodinger drug discovery package was used to predict the structure of the covalent

complex between ATT-I and PSMD4. The Maestro module within Schrodinger was used to process the protein structures. Hydrogen atoms were added and the protonation state of ionizable residues assigned by pro-pKa. The structure was energy minimized. The CovDock module was used for the covalent docking of ATT-I to PSMD4.

PSMD4 protein expression and purification

pGEX-6P-1 vector was used to clone the recombinant protein GST-PSMD4. Mouser PSMD4 cDNA fragment (NM_001330692.2.) was amplified and ligated into the pGEX-6P-1 vector (Addgene). In order to get the recombinant protein, pGEX-6P-1/PSMD4 was transformed into E. coli BL21 (DE) (BioLab, UK). The positive clone was cultured in LB medium until OD_{600} reached approximately 0.6 at 37 °C. Isopropyl β- d-1-thiogalactopyranoside (IPTG, Sigma) was used to induce protein expression with the final concentration of 1 mM for 5 h at 37 °C. The cells were harvested and suspended in the binding buffer (140 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 1.8 mM KH2PO4, pH 7.3). The cells were lysed by sonication for 30 min, and then the cell debris was removed by centrifugation at 12,000 rpm for 20 min at 4 °C. The supernatant was collected and applied to a Glutathione Sepharose 4 FF column (Sigma, USA) to purify recombinant protein according to the manufacturer's instructions. GST-tag was digested by Prescission protease (Sigma, USA) to purify PSMD4 proteins without tag.

shRNAs and MC38 cells with stable PSMD4 knockdown

PSMD4 shRNA #1:

5’-CCGGTTATAGAACAGGGTCACATTGCTCGAGCAATGTGACCCTGTTCTATAATTTTTG-3’

PSMD4 shRNA #2:

5’-CCGGGTGAATGTTGACATCATTAATCTCGAGATTAATGATGTCAACATTCACTTTTTG-3’

PSMD7 shRNA:

5’-CCGGCCCAACTCAGTATTGGTCATTCTCGAGAATGACCAATACTGAGTTGGGTTTTTG-3’

Lentiviral PSMD4 and PSMD7 shRNA was transduced into luciferase expressing MC38-OVA cells. 48 hours after infection, the MC38 cells were cultured with the addition of 1 μg/ml puromycin and the survived cells were grown up to single colonies, from which the colonies with stable knockdown of PSMD4 or PSMD7 (expression determined by qPCR, western blotting) were selected for the *in vivo* experiment.

Proliferation assay

Luciferase expressing MC38 cell line was transfected with pCDH-CMV-OVA lentivirus plasmid ($Puro^+$) to generate single colon stably expressing OVA peptide (SIINEFKL). The OVA (pcDNA3-OVA, addgene) was constructed inhouse into the pCDH-CMV-MCS-EF1-puro plasmid (System Biosciences). Luciferase expressing MC38-OVA, MC38-OVA-$PSMD4^{loss}$ and MC38-OVA-$PSMD4^{loss}$ tumor cells were seed in 96-well microplate at 2,000 cells per well. The cells were imaged every 6 hours using the incucyte system (Essen BioScience Inc.) for 5 days and the cell confluence was analyzed by the incucyte software.

Co-immunoprecipitation

Co-immunoprecipitation was performed using the immunoprecipitation kit (ab206996, Abcam). Briefly, cells scraped from 15 cm dishes were lysed in non-denaturing lysis buffer provided by the assay kit on a rotary mixer at 4°C for 30 min. After centrifugation by 10,000 g, cell lysates were incubated with the indicated antibody overnight at 4°C. The immunoprecipitates were incubated with the protein A/G-agarose beads for 1 hour at 4°C. The beads were collected and eluted in SDS buffer elution and then immunoblotted with the indicated antibodies. Antibodies used for immunoprecipitation was proteasome 19S S5A/ASF antibody (ab20239, Abcam). Antibodies used for immunoblotting were S5a/PSMD4 antibody (Dilution: 1:2,000, 3336S, Cell Signaling), PSMD7 antibody (Dilution: 1:500, 3336S, Cell Signaling), GFP(FL) Rabbit polyclonal IgG (Dilution: 1:3,000, sc-8334, Santa Cruz) and anti-β-actin (Dilution: 1:5,000, sc-8432, Santa Cruz).

Immunofluorescence

In Brief, cells (5 x10^3 per well) were seeded and cultured overnight to Millicell EZ 8-well glass slides, rinsed with PBS and fixed with 4% paraformaldehyde (PFA) for 10 min at room temperature. Fixed cells were incubated with 0.5% Triton X-100/PBS and blocked with 0.2% BSA/PBS. Cells were then incubated with primary antibody (anti-HLA-A,B,C) overnight at 4 °C followed by Alexa Fluor 488-conjugated goat anti-mouse IgG antibody (dilution 1:300) for 1 hour at room temperature. DAPI (Sigma-Aldrich) staining was performed after antibody staining. Samples were mounted with mounting medium (Sigma-Aldrich) and fluorescent images were taken using Leica TCS SP8 (upright high-speed multiphoton) confocal imaging system. Image quantification was performed using Imaris image analysis software (Bitplane). Total intensity in green channel (HLA-A,B,C staining) per image was obtained based on the "Surfaces" image segmentation and number of nuclei (corresponding to number of cells per image) was calculated based on DAPI staining using the Imaris "Spots" segmentation module. The value of average intensity per cell was then calculated by dividing the total intensity in green channel by number of nuclei to give HLA (Intensity/cell).

Immunohistochemistry

The tissue samples were fixed in formalin for 24 hours and sequentially stored in 70% ethanol until the tissues were embedded in paraffin. Three micron sections were cut and processed for immunohistochemistry staining.

Single-cell RNA sequencing (scRNA-seq) analysis

Single cell suspension of tumors is prepared as for the CyTOF samples. Hereafter, the single cells were stained for: CD45-BV605, TCRβ-PE/Cy7, CD11b-eF450, CD4-AF700, CD8-APC/Cy7 and 7-AAD. Viable (7-AAD negative) single cells were sorted as $CD45^+$, $CD11b^-$, $TCR\beta^+$. The sorted samples were immediately submitted to the core facility for sample preparation for the

scRNA sequencing. The library for downstream scRNA sequencing is constructed using 3' v2 library preparation kit following manufacturer's protocol (10X genomics). After amplification and normalization, cDNA with index barcode were loaded on and sequenced by NovaSeq 6000 (Illumina).

Sequencing data were demultiplexed and mapped to mm10 genome for the retrieval of gene expression counts matrix by utilizing Cell Ranger (10X Genomics). Downstream analysis of these matrices were conducted by Seurat (V3.1), unless indicated otherwise. Cell filtering follows two criteria for all samples: 1) detected number of RNA in a single cell should be over 500, 2) the percentage of mitochondria genes detected in a single cell should not exceed 15%. The remaining cells from each sample were merged and log-normalized. PhenoGraph in Seurat was applied to cluster cells with resolution set as 0.5. Low dimensional t-SNE visualization was computed by Rtsne package using normalized transcriptional expression profile with perplexity setting as 30. Each cell cluster is annotated by marker genes found by FindAllMarkers function in Seurat. Heatmap is then drawn with log-normalized expression level. We further looked into T-cell activation and cytotoxicity level on representative genes with dot visualization. The color code of each codes stands for the relative mean expression level from different sample, and the size of each dot represents the percentage of expressed cells within samples. To evaluate the cytotoxic level of each cell, we used the averaged expression level of six cytotoxic and $CD8^+$ T cell marker genes, namely: *Prf1, Ifng, Tnf, Pdcd1, Sla2* and *Cd8a*. Significant expression of each cytotoxic marker gene in each cell were computed by using left truncated mixture Gaussian model. $CD8^+$ effector T cells with significant cytotoxic genes expressed were defined by the $CD8^+$ effector T cells with at least three out of the six genes significantly expressed, the proportion of which was further computed.

Mass spectrometry (MS) based cellular thermal shift assay (CETSA) to identify potential targets of ATT-I in MC38 cells using melt temperature (Tm) shifts

To screen out potential binding targets for ATT-I in a high throughput fashion, two groups of cells were compared for their protein level temperature dependent denaturation profiles using two biologically distinct replicate cell cultures for each group. Briefly, the two groups were—1) DMSO treated control MC38 cells; and 2) ATT-I (5.0 μM) treated MC38 cells. Sample preparation, mass spectrometry analysis, bioinformatics and data evaluation were performed in collaboration with the Proteomics Core Facility at the Indiana University School of Medicine Methods described below in brief were adaptations from previously published reports (1-5) and vendor provided protocols.

Cell lysis and protein assay

For cell lysis, a fresh solvent system (10 mL) was first prepared to include 50 mM HEPES, 200 mM NaCl, 10 mM $MgCl_2$,5 mM β–glycerophosphate, 0.1 mM activated sodium orthovanadate, 2 mM TCEP (tris(2-carboxyethyl)phosphine hydrochloride) and 1x EDTA-free protease inhibitor (cOmplete Mini, Roche). Pelleted cells were then mobilized in this solvent buffer system (800 μL) contained in 1.5 mL Micro Tubes (TPX Plastic for Sonication from Diagende Inc.). Mobilized cells were next subjected to sonication using a Bioruptor® sonication system (Diagende) 30 seconds/30 seconds respective on/off cycles for 15 mins in a 4 ^{o}C cold water bath. Total protein concentration of each sample was t determined using a Bradford protein assay. All lysates were then diluted to a protein concentration of 2 μg/μl for the subsequent temperature treatment.

Temperature treatment, supernatant "decanting", and proteolytic digestion

Six aliquots (50 μl) of each sample treated with either DMSO or compound were placed among six tubes designed for PCR workflows, and equilibrated at six temperature points—35.0; 45.3; 50.1; 55.2; 60.7; and 74.9 ^{o}C for 3 mins in a thermocycler (Mastercycler Pro, Eppendorf) system as described elsewhere (1, 3). Following heat treatment, lysates were centrifuged for 20 mins at 4 ^{o}C to pellet out insoluble protein and then to decant the soluble fraction. A 45 μL aliquot from each heat-treated sample was subject to protein precipitation and then reconstituted in 30 μL of 8 M Urea in Tris.HCl (pH 8.0). Samples were next subjected to reduction of Cys-Cys bonds with

5 mM TCEP, and alkylation with 10 mM chloroacetaminde (CAM) to protect the reduced Cys residues. Samples were diluted to have 2 M Urea and were digested in-solution overnight using Trypsin (Promega) to derive peptides.

Enrichment of peptides

Resulting peptides were “de-salted” using Sep-Pak® Vac 1cc C18 Cartridges, 50 mg Sorbent per Cartridge, 55-105 µm Particle Size (Waters Co.) employing a vacuum manifold. Briefly, columns adapted onto the extraction manifold were first washed sequentially with (1) ACN (500 µL)-two times, (2) ACN/H_2O 70/30 (v/v; 0.1% FA; 200 µL)-one time, and (3) MS-grade water (500 µL)-two times. Peptides from each “digestion” solution were then subjected to immobilization on C18 material by a gentle application of vacuum into the extraction manifold vacuum chamber to move each solution three times by collecting the “flow-through” fractions and running them again on to the same column. Next, the peptide-bound C18 columns were washed with 500 µL of MS-Grade H_2O and then eluted by passing 150 µL of ACN/H_2O 70/30 (v/v; 0.1% FA) three times. All elution fractions were collected into 1.5 mL Eppendorf tubes and subjected to complete dryness using a speed vacuum system.

Tandem Mass Tags (TMT) based peptide labelling and fractionation

The respective dried samples were then subjected to TMT-based labelling using sixplex kits (TMT6plex™ Isobaric Label Reagent Set, 2 x 0.8 mg). The TMT channels—TMT126, TMT127, TMT128, TMT129, TMT130, and TMT131, were employed to label peptide solutions derived from the 35.0; 45.3; 50.1; 55.2; 60.7; and 74.9 ^oC temperature treatments. Briefly, each dried sample (equivalent of 10 µg of protein digest) was reconstituted in 100 µL of 50 mM Triethylammonium bicarbonate (TEAB) and dry labeling reagents were dissolved in 40 µL of acetonitrile (ACN). Reconstituted peptide solutions were then mixed with 40 µL labelling reagent solution and kept at room temperature for overnight to label the peptides. Labelling reaction was next quenched by adding 8 µL of 5% hydroxylamine and keeping the reaction mixture at room temperature for more than 15 mins. Labelled peptide solutions were next mixed together and subjected to complete

dryness in a speed vacuum system. Dried labelled peptide mixtures were fractionated using reversed phase fractionation columns (8 fractions) employing vendor provided protocols (Pierce Biotechnology). The resulting 8 fractions were dried using a speed vacuum system and re-constituted in 0.1% formic acid (30 µL) prior to nano-LC-MS/MS analysis.

Nano-LC-MS/MS analysis

Nano-LC-MS/MS analyses were performed on a Q-Exactive Plus™ mass spectrometer (Thermo Scientific) coupled to an EASY-nLC™ HPLC system (Thermo Scientific). Ten µL equivalent volume of the re-constituted fractions from above were loaded using 300 bar as applied maximum pressure onto an in-house prepared reversed phase column. Each reversed phase column was prepared by pulling a 100 µm fused-silica column to carry 5 µm tip for the nanospray using a P-2000 laser puller, and then packing the capillary with C18 reverse phase resin (particle size: 3 µm diameter; Dr. Maisch HPLC GmbH). The peptides were eluted using a varying mobile phase (MP) gradient from 95% phase A (FA/H2O 0.1/99.9, v/v) to 24% phase B (FA/ACN 0.4/99.6, v/v) for 150 mins, from 24% phase B to 35% phase B for 25 mins and then keeping the same MP-composition for 5 more mins at 400 nL/min to ensure elution of all peptides. Nano-LC mobile phase was introduced into the mass spectrometer using a Nanospray Flex Source (Proxeon Biosystems A/S). The heated capillary temperature was kept at 275 ^{o}C and ion spray voltage was kept at 2.5 kV. During peptide elution, the mass spectrometer method was operated in positive ion mode for 180 mins, programmed to select the most intense ions from the full MS scan using a top 20 method. Additional parameters: Microscans 1; Resolution 70k; AGC target 3E6; Maximum IT 50 ms; Number of scan ranges 1; Scan range 400 to 1600 m/z; and Spectrum data type “profile”, and then to perform data dependent MS/MS scans with parameters: Microscans 1; Resolution 35k; AGC target 1E5; Maximum IT 64 ms; Loop count 20; MSX count 1; Isolation window 0.7 m/z; Fixed first mass 100 m/z; NCE 38.0; and Spectrum data type “Centroid”. The respective data dependent settings were set with parameters: Minimum AGC target of 1.00e3; Intensity threshold of 1.6e4; Apex trigger as “-”; Charge exclusion as “1,7,8, >8“; Multiple Charge

States as “all”; Peptide match as “preferred”; Exclude isotopes as “on”; Dynamic exclusion of 30.0 s; If idle “pick others”. The data were recorded using Thermo Xcalibur (4.1.31.9) software (Thermo Fisher)

Data Analysis

Resulting RAW files were analyzed using the MaxQuant software suite 1.6.0.16 (www.maxquant.org). The MS/MS spectra were searched against in silico tryptic digest of Mus musculus proteins database (FASTA format) from the UniProt sequence database (v. May 2017). Default MaxQuant parameter settings were utilized as downloaded from www.maxquant.org, except for the following: 1) in group specific parameters tab to have “type” as “Reporter ion MS2”; “Isobaric labels” selected as “6plex TMT”; “Reporter mass tol.” as “ 0.003 Da”; 2) in group specific parameters—”digestion” mode as specific for trypsin with maximum 3 missed cleavages; 3) group specific parameters—”modifications” to have variable modifications acetyl (protein N-terminus); phosphor (STY) and methionine oxidation, with peptides to carry maximum number of 5 modifications per peptide; and 4) global parameters tab— to carry a minimum peptide length of 7 residues, maximum peptide mass of 4600 Da. Please note, 4 such searches were carried out separately for each of four samples. The resulting Maxquant “peptides.txt” files were then imported to Microsoft Excel™ (Version 14.07182.5000, 32-bit) and then to JMP® 12.1.0 (64bit, Microsoft Windows 7 Enterprise 64-bit, Service Pack 1, Copyright©2015 SAS Institute Inc.) for processing. Only those peptides identified with a minimum of PEP value of 0.05 were selected for further evaluation. Table S5 summarizes the number of peptides parsed through the procedure. Protein quantification values were derived by summation of quantification values of only the unique peptides (at least one for one protein). Following methodology was utilized for procedural error correction/normalization of the quantification values obtained for each protein in each experiment.

Procedural error correction and normalization of the protein quantification values and "melt" curves

Since each TMT multiplexed experiment was carried out in separate sample preparations, and analyzed as separate mixtures, we assumed each "proteome" wide denaturation should be accounted for procedural errors separately. For the purpose, total "proteome quantifications" for each CETSA experiment were first derived by mathematical summation of protein quantification for each TMT experiment (Supplementary Figure 9, upper panel). Denaturation curves or the sigmoidal "melt curves" for these were then derived using the logistic 4P model available in JMP® 12.1.0 (64bit, Microsoft Windows 7 Enterprise 64-bit, Service Pack 1, Copyright©2015 SAS Institute Inc.). Supplementary Figure 9 (bottom panel) illustrates these curves and the associated R^2 values and "melt temperatures, T_m-values"/inflection points resulted during the derivation. Equation 1 illustrates the logistic 4P model utilized. It is apparent minimal deviation is observed from actual from the predicted, however subtle deviations are apparent (Supplementary Figure 10). Thus, using %deviation and the equation illustrated in Equation 2, TMT-quantitative values of each protein in each sample were subjected to normalization. Using these normalized values, % protein abundance values for each protein and, for each temperature treatment was derived for each experiment. Then, sigmoidal plots were derived for each protein using the same logistic 4P model available in JMP® 12.1.0 (64bit, Microsoft Windows 7 Enterprise 64-bit, Service Pack 1,

Copyright©2015 SAS Institute Inc.). This allows correction to random errors associated with assumed protein level and total proteome sigmoidal protein denaturation.

Prediction Model: Logistic 4P

$$y = c + \frac{(d-c)}{(1+Exp(-a(T-b)))}$$

$$a = Growth\ Rate$$
$$b = Inflection\ Point$$
$$c = Lower\ Asymptote$$
$$d = Upper\ Asymptote$$
$$T = Temperature$$

Equation 1. Logistic 4P model available in JMP® 12.1.0.

$$\text{Norm (TMT Response of Protein)}_e = \text{(TMT Response of Protein)}_e \, X \frac{100 - (\%\ Difference\ from\ Actual)_e}{100}$$

$$e = Experiment, e.g. C1; C2; T1\ or\ T2$$

"% Difference from Actual" *is Calculated from Total Protein Abundance Derived from Total TMT Responses for Each Channel*

Equation 2. Normalization of TMT-Quant values of each protein in each sample.

Supplementary Figure 10 illustrates the distribution plots of R^2 values derived from all sigmoidal plots constructed and parsed through the criteria $R^2 \geq 0.95$ and 35 °C ≤ Tm ≤ 90 °C. These distributions of R^2 values signified the quality of generated sigmoidal plots for the proteins isolated with applied criteria inferring the data could be used for further evaluation/processing. Nevertheless, using these parsed protein melt temperature determinations, proteins that are common in all four "CETSA samples" were next isolated, and average T_m values; $\Delta(T_m)\{$ T-C $\}$ values and the associated p-values (t-test with parameters, two-tailed distribution and two-sample unequal variance) for each common protein were calculated.

Subsequent construction of distribution plot of $\Delta(T_m)\{$ T-C $\}$ values; and "Volcano Plot" of $\Delta(T_m)\{$ T-C $\}$ values vs -log $_{10}$ p(t-test) values (Supplementary Figure 11), were then used to screen out

proteins with statistically significant positive T_m shifts due to compound treatment. It was assumed, stringent criteria, p-value (t-test) ≤ 0.05; and Δ(Tm){T-C} ≥ $(\overline{x} + 2\sigma)$ °C = +6.174 °C, would screen out/narrow down more biologically relevant group of proteins that were stabilized due to protein association.

SUPPLEMENTARY REFERENCES

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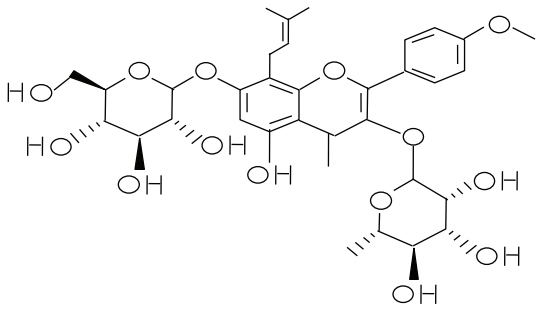
Figure S1

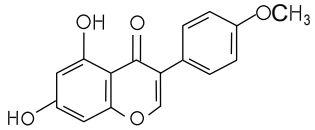
A

Icariine



B

Biochanin A



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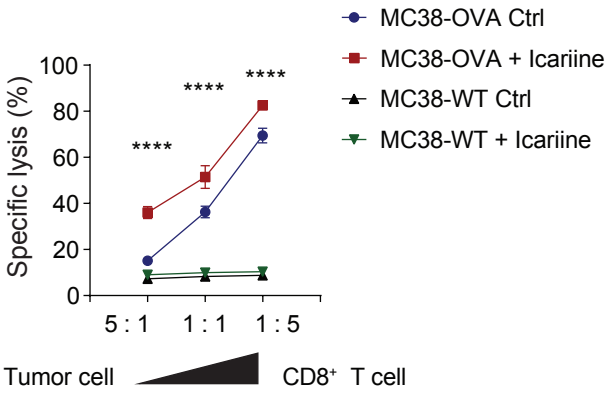

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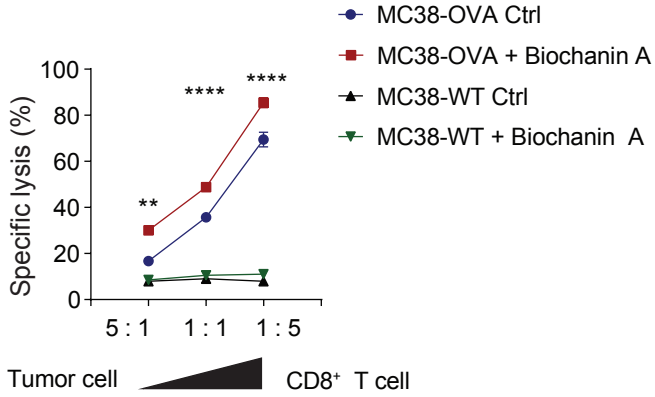


E

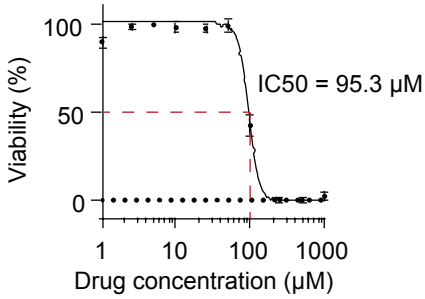
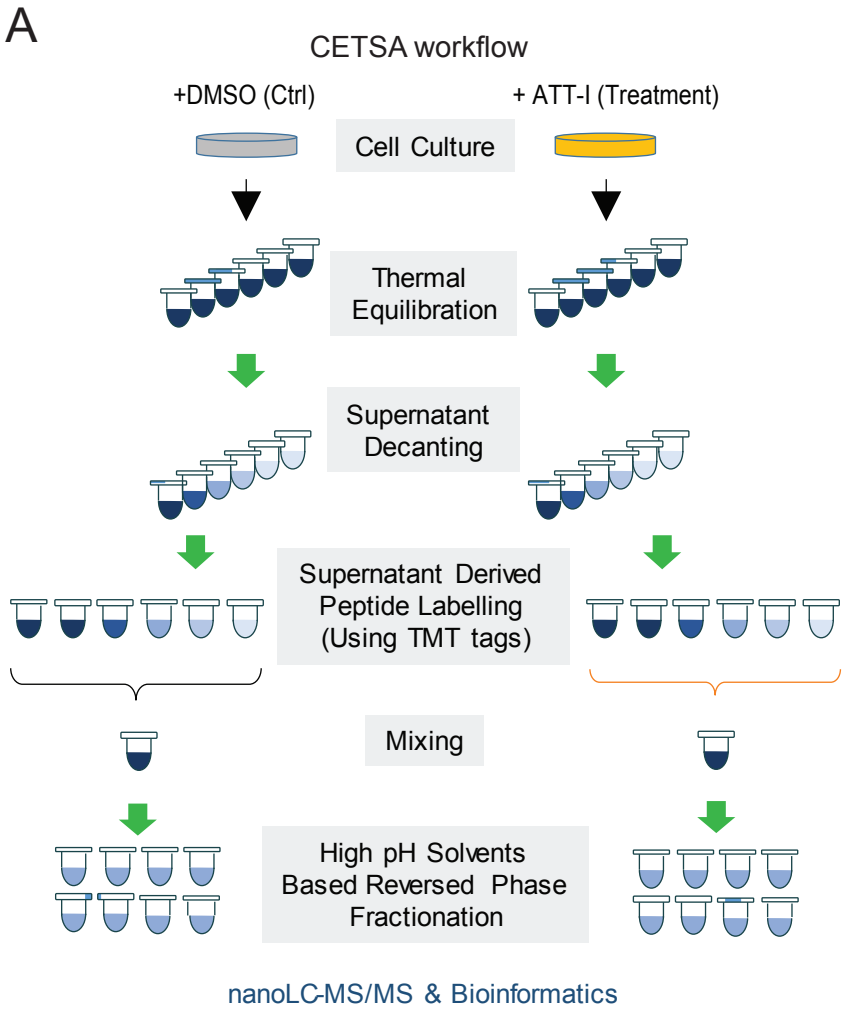
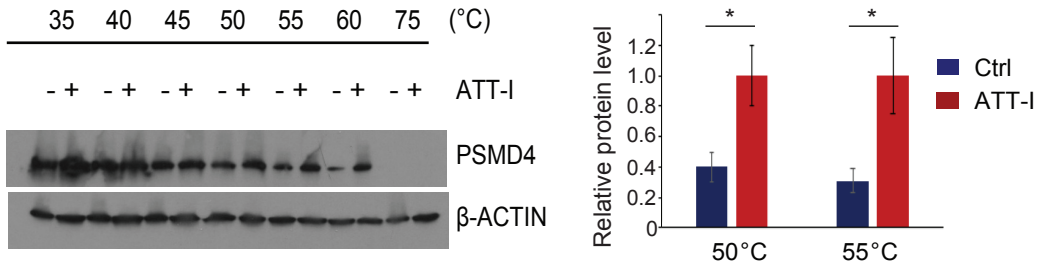
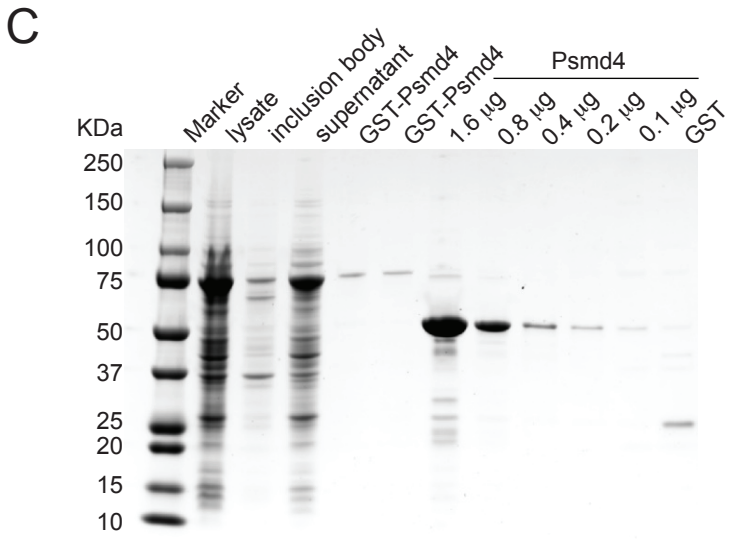


Figure S1. Small-molecule natural compounds enhance the tumor-cell killing efficiency of CD8$^+$ T cells. (**A, B**) Chemical structure of Icariine (A) and Biochanin A (B). (**C**,**D**) The effect of Icariine (C) or Biochanin A (D) treatment on the CD8$^+$ T-cell killing of MC38-OVA cells was measured under different ratios of tumor cells vs T cells as indicated. Data are presented as mean ± SD of three independent experiments. Statistical analyses were conducted using two-way ANOVA test. Statistical analyses were conducted using two-way ANOVA test. **, $p < 0.01$; ****$p < 0.0001$. (**E**) Determination for the IC50 of ATT-I on MC38 cells. MC38 cells were seeded with 2000 cells per well in 96-well plate. ATT-I with different drug doses (1, 2.5, 5, 10, 25, 50, 50, 100, 250, 500 and 1000 μM) was used to treat the cells for 3 days (n=3). WST-1 cell proliferation assay kit (Takara) was used to detect the cytotoxicity of ATT-I on cells.

Figure S2


A
CETSA workflow
+DMSO (Ctrl)
+ ATT-I (Treatment)
Cell Culture
Thermal Equilibration
Supernatant Decanting
Supernatant Derived Peptide Labelling (Using TMT tags)
Mixing
High pH Solvents Based Reversed Phase Fractionation
nanoLC-MS/MS & Bioinformatics


B
35 40 45 50 55 60 75 (°C)
- + - + - + - + - + - + - + ATT-I
PSMD4
β-ACTIN
Relative protein level
1.2
1.0
0.8
0.6
0.4
0.2
0
*
*
50°C
55°C
Ctrl
ATT-I


C
KDa
Marker
lysate
inclusion body
supernatant
GST-Psmd4
GST-Psmd4
Psmd4
1.6 μg
0.8 μg
0.4 μg
0.2 μg
0.1 μg
GST
250
150
100
75
50
37
25
20
15
10

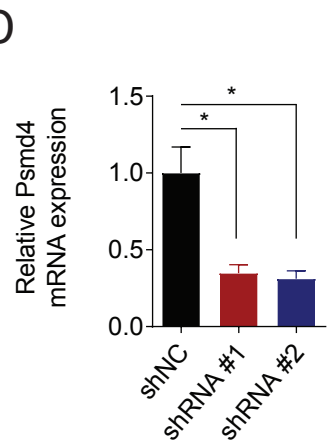
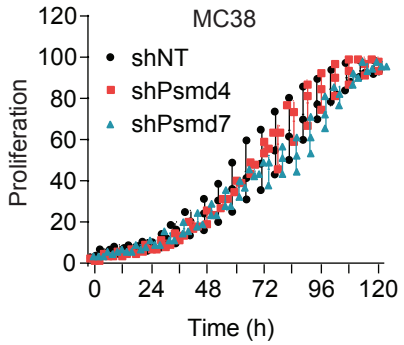

D
Relative Psmd4 mRNA expression
1.5
1.0
0.5
0.0
*
*
shNC
shRNA #1
shRNA #2

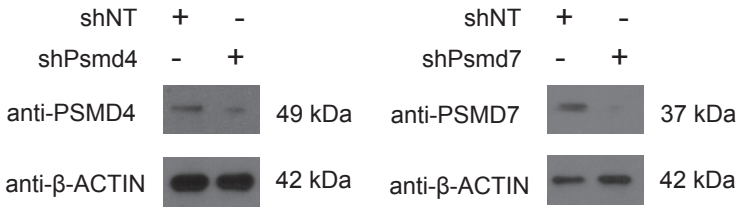
Figure S2. PSMD4 was identified as a molecular target of ATT-I by cellular thermal shift assay (CETSA). (**A**) Experimental design of CETSA. Four biologically distinct cell cultures were prepared and two were treated with DMSO (Control) and two were treated with ATT-I (Treatment). Following lysis of each cell pellet, resulting protein solutions were divided into six identical aliquots (50 µL) and equilibrated at six temperature points—35.0; 45.3; 50.1; 55.2; 60.7; and 74.9 ^{o}C for 3 minutes as described in the Methods. Resulting denatured proteins of each tube were next pelleted out and the supernatant fractions were sequentially subjected to the following procedures: proteolytic digestion (trypsin/Lys-C based), peptide labelling with different isobaric tags (TMT: Tandem Mass Tags), mixing, and high-pH solvents based reversed phase fractionation. All the fractions were analyzed using nanoLC-MS/MS as described in the Methods. All resulted files were analyzed using the MaxQuant software suite 1.6.0.16. to identify and quantify protein abundance values and JMP® Pro 14.0.0 (64 bit) to generate sigmoidal protein melt curves and subsequent data analyses to screen out potential targets of ATT-I as described in the Methods. (**B**) Representative immunoblots of PSMD4 in the HCT116 cell lysates with or without ATT-I treatment were shown on the left panel. Quantitative data are presented as mean ± SD of 3 parallel experiments. Unpaired 2-tailed t test was used for statistical analysis. (**C**) GST-PSMD4 fusion proteins were bacterially expressed, purified and processed for producing PSMD4 proteins. (**D**) Knockdown efficiency of two PSMD4 shRNAs was measured by quantitative PCR of reverse transcribed products of PSMD4 mRNA in MC38 cells. Non-target shRNA (shNC) was used as control. Data are presented as mean ± SD of three independent experiments. Statistical analyses were conducted using one-way ANOVA test. *, $p < 0.05$.

Figure S3

A



B



C

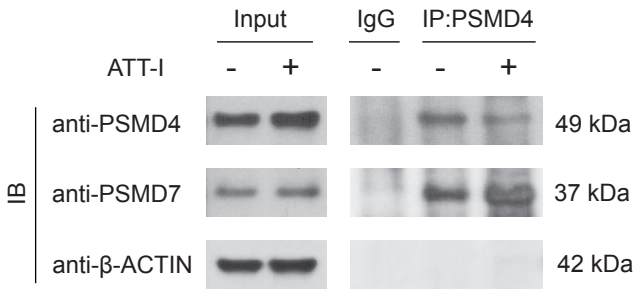


Figure S3. Validation of shPsmd4 and shPsmd7 cell lines as well as immunoblots. (**A**) Incucyte cell proliferation analysis for mouse MC38-OVA cells with shNT control, shPsmd4 and shPsmd7 knockdown (KD). No significant differences in cell proliferation were observed among the three cell lines (n=3). Statistical analyses were conducted using two-way ANOVA test. (**B**) Western blot showing shPsmd4 and shPsmd7 knockdown in MC38-OVA cells. (**C**) PSMD4 is binding with PSMD7 in 19S regulatory complexes, forming 26S immunoproteasomes. PSMD4 and PSMD7 were co-immunoprecipitated by immunoprecipitation (IP) and following immunoblotting analysis (IB). HCT116 cell lysate of 5% volume used for IP was loaded as input. GFP IgG was used as a negative control. The results showed that ATT-I enhanced or stabilized the binding between PSMD4 and PSMD7.

Figure S4

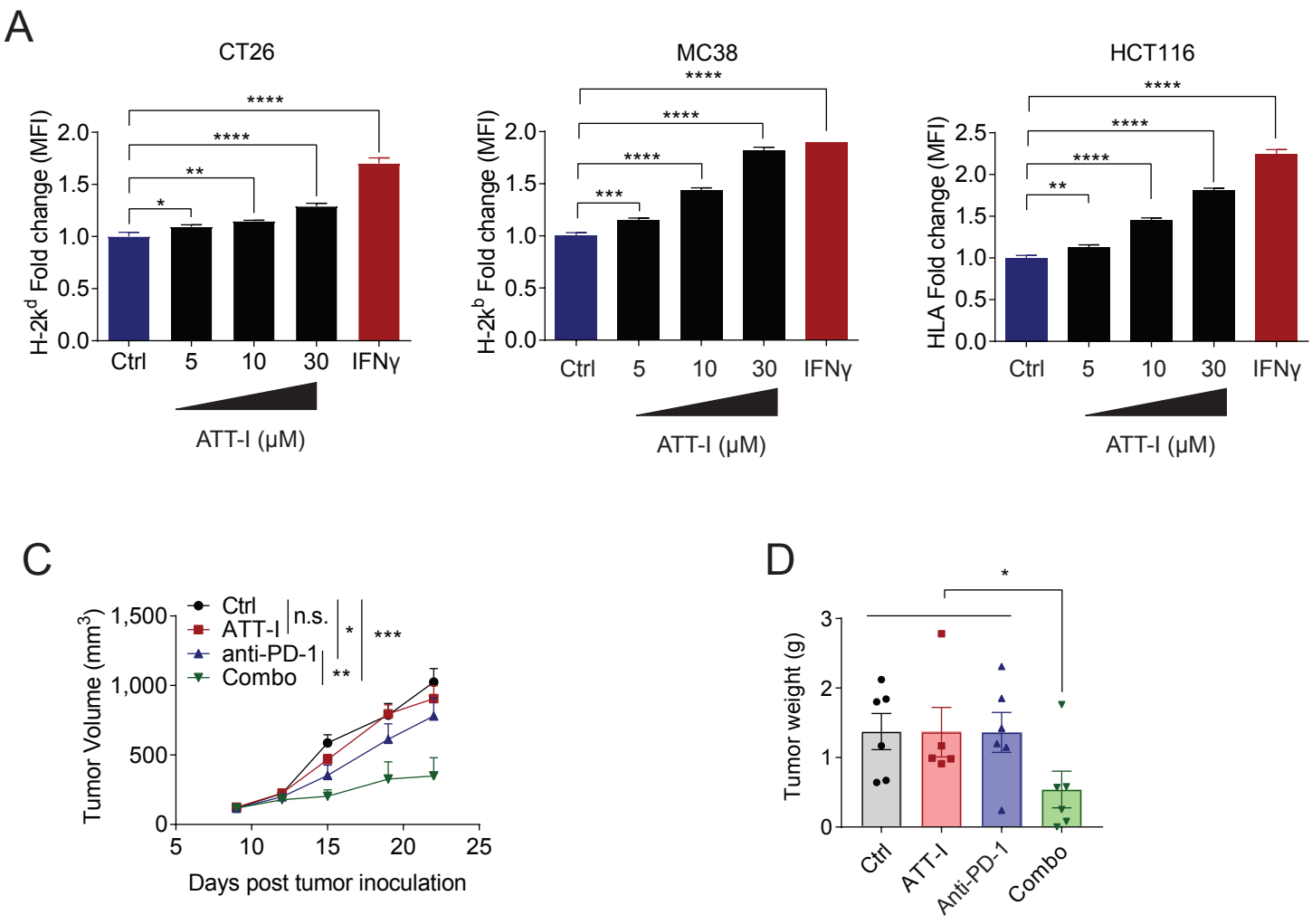
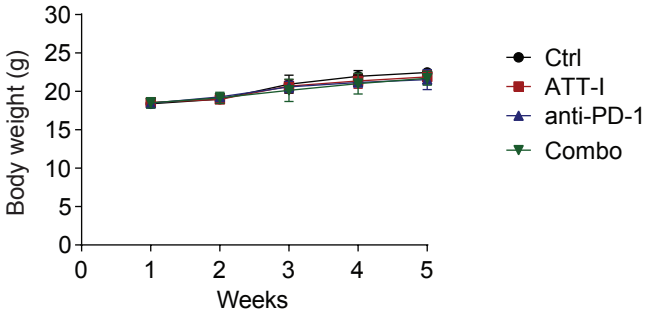

A
CT26
MC38
HCT116
H-2k^d Fold change (MFI)
H-2k^b Fold change (MFI)
HLA Fold change (MFI)
Ctrl
5
10
30
IFNγ
ATT-I (μM)
C
Tumor Volume (mm^3)
Ctrl
ATT-I
anti-PD-1
Combo
n.s.
Days post tumor inoculation
D
Tumor weight (g)
Ctrl
ATT-I
Anti-PD-1
Combo

Figure S4. ATT-I treatment enhances tumor antigen presentation and the efficiency of immune checkpoint blockade therapy. (**A**) The level of H-2k^{d} on mouse CT26 and MC38 cells and the level of HLA on HCT116 cells after ATT-I treatment were measured by flow cytometry. Quantitative data are presented as mean ± SD of 3 parallel experiments (n=3). Statistical analyses were conducted using one-way ANOVA test. (**C, D**), Effects of the ATT-I (50 mg/kg, daily) and PD-1 (200 μg/mouse, 3 times/ week, 5 injections in total) treatments on tumor growth (C) and tumor weight (D) of CT26-derived tumors in the subcutaneous BALB/c mouse model. Error bars represent SEM (n=6) and statistical analyses were conducted using two-way ANOVA test. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.005$; ****, $p < 0.001$, *n.s.*, not significant.

Figure S5

A


Body weight (g)
30
25
20
15
10
5
0
0
1
2
3
4
5
Weeks
Ctrl
ATT-I
anti-PD-1
Combo

B

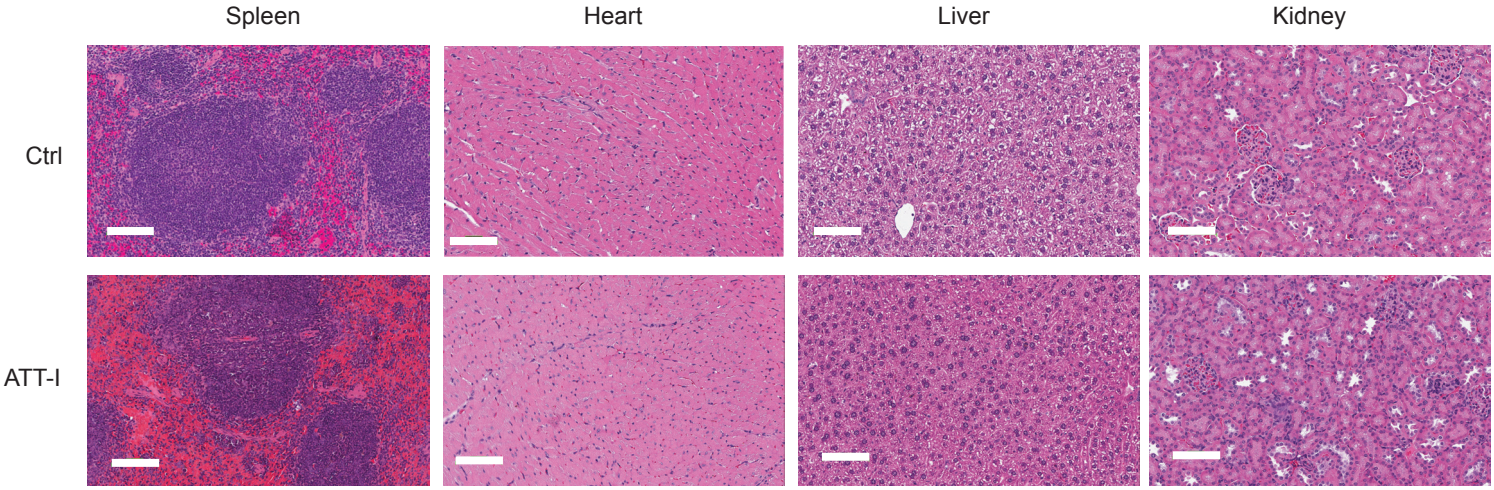

Spleen
Heart
Liver
Kidney
Ctrl
ATT-I

Figure S5. No evident systemic toxicity of the ATT-I treatment was observed on the treated C57BL/6 mice. (**A**) Body weight of mice with various treatments (n=4 in each group) showing no significant difference between the different treatments. The treatment scheme is identical to that in Fig. 3D. (**B**) Representative H&E-stained slices of major organs in mice treated with vehicle control or ATT-I (50 mg/kg, daily) on day 35. The experiments were repeated three times independently. Scale bar: 100 μm.

Figure S6

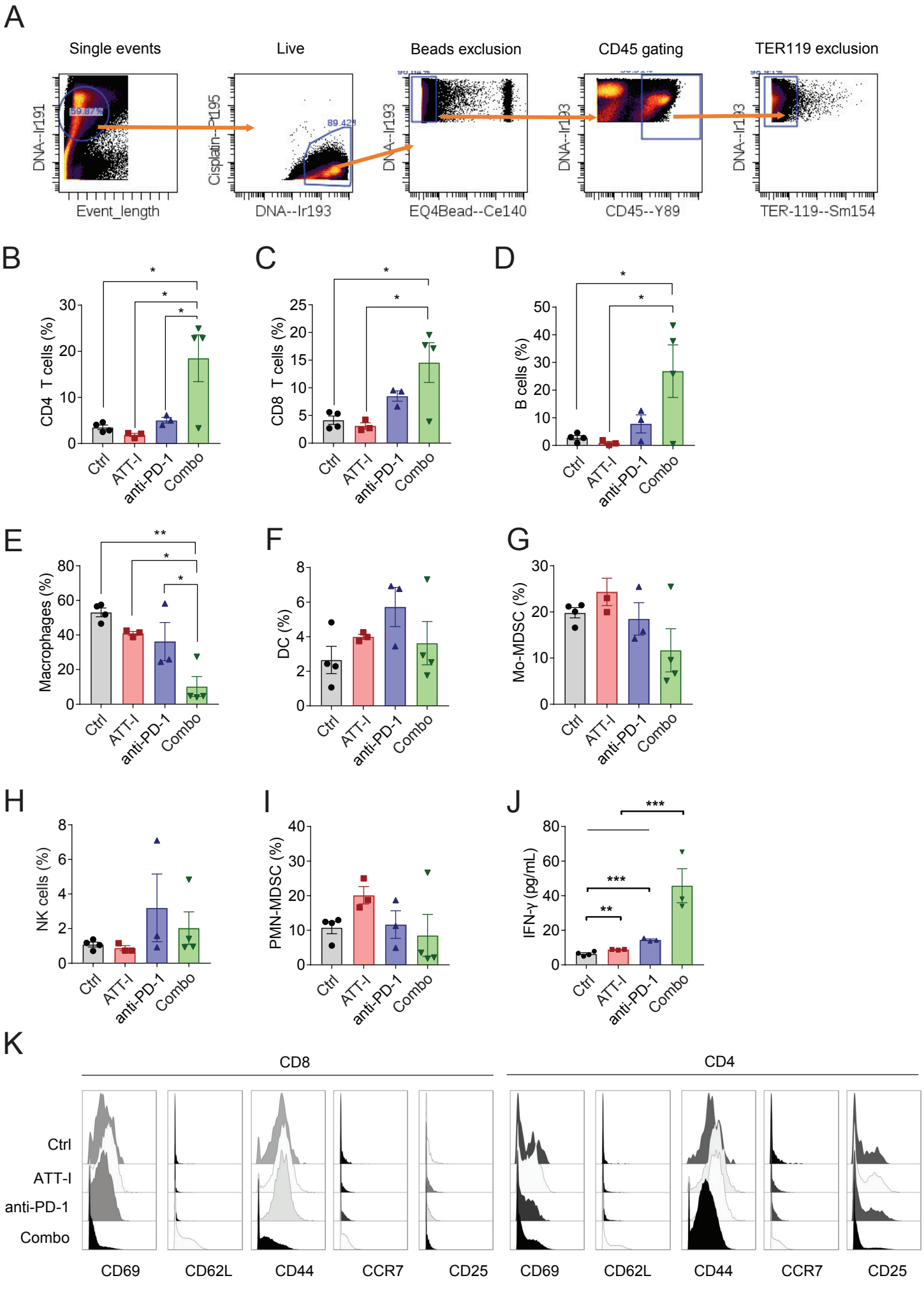

A
Single events
Live
Beads exclusion
CD45 gating
TER119 exclusion
DNA--Ir191
Event_length
Cisplatin--Pt195
DNA--Ir193
EQ4Bead--Ce140
CD45--Y89
TER-119--Sm154
B
CD4 T cells (%)
C
CD8 T cells (%)
D
B cells (%)
E
Macrophages (%)
F
DC (%)
G
Mo-MDSC (%)
H
NK cells (%)
I
PMN-MDSC (%)
J
IFN-γ (pg/mL)
Ctrl
ATT-I
anti-PD-1
Combo
K
CD8
CD4
Ctrl
ATT-I
anti-PD-1
Combo
CD69
CD62L
CD44
CCR7
CD25

Figure S6. Immune profile analysis of the MC38 tumors upon different treatments. (**A**), Gating strategy before running CyTOF viSNE analysis. Before running CyTOF viSNE analysis, events were selected as followed: single events (Ir191, Event Length), Live cells (Pt195, Ir193), Beads exclusion (Ir193, Ce140), CD45 gating (Ir193, CD45), TER119 exclusion (Ir193, TER119). (**B-I**), Statistics on the immune cell distribution of orthotopically injected MC38 cells. The figures display average frequencies of the $CD45^+$ immune cell compartment shown in Fig. 4D with the statistical significance between the different treatment groups: $CD4^+$ T cells (**B**), $CD8^+$ T cells (**C**), B cells (**D**), Macrophages (**E**), DC (**F**), Mo-MDSC (**G**), NK cells (**H**) and PMN-MDSC (**I**). Statistical analyses were conducted using one-way ANOVA. Bar plots with the scatter dots show the mean with ± SEM. (**J**) IFN-γ levels of $CD8^+$ T cells from orthotopically cecal wall implanted MC38 tumors (n=3). (**K**), Overlay histogram plots generated via cytobank on the $CD8^+$ T cells and $CD4^+$ T cells are shown for the CD69, CD62L, CD44, CCR7 and CD25 markers. *, $p < 0.05$; **, $p < 0.01$. ***, $p < 0.001$.

Figure S7

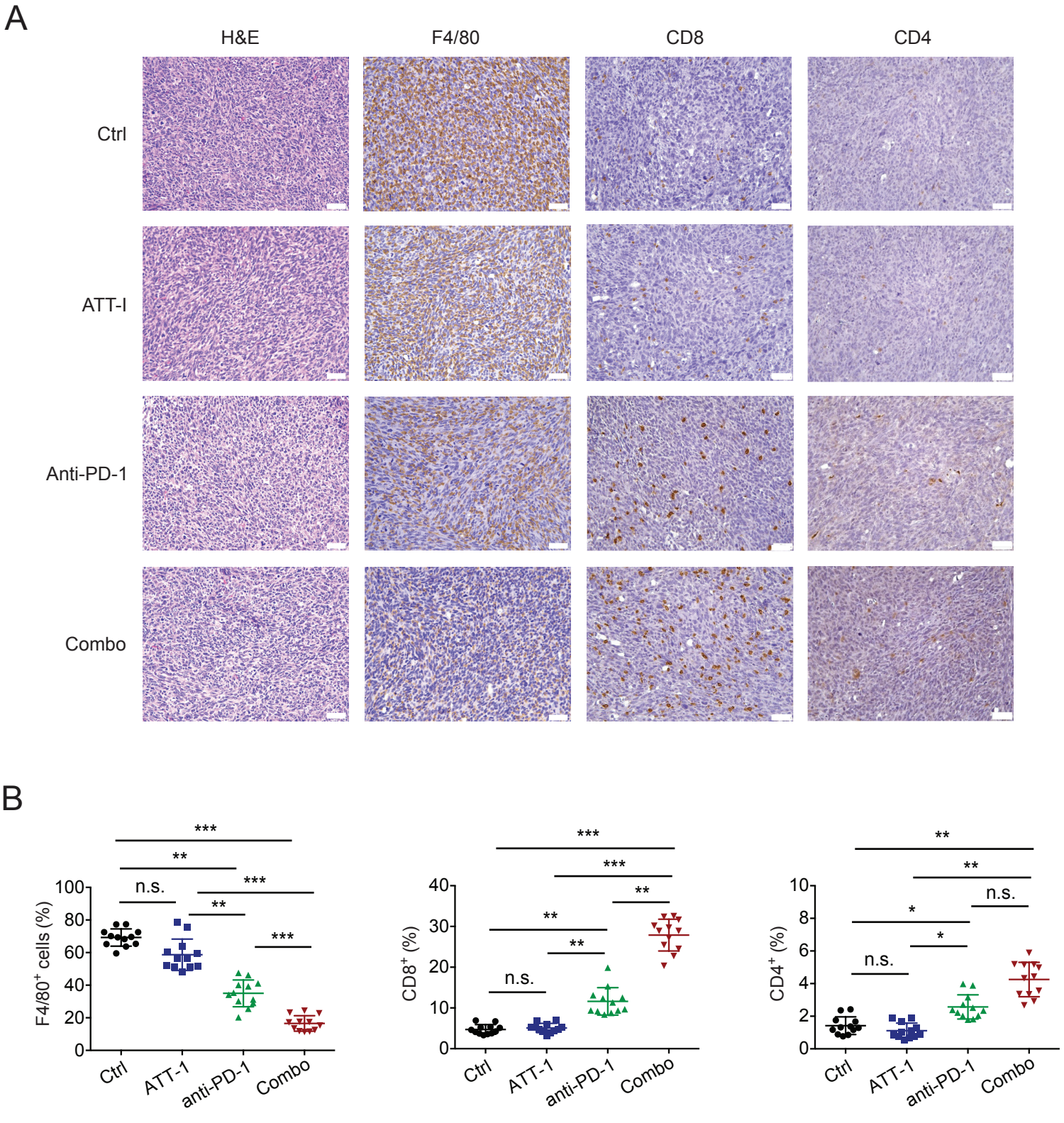

A
H&E
F4/80
CD8
CD4
Ctrl
ATT-I
Anti-PD-1
Combo
B
F4/80+ cells (%)
CD8+ (%)
CD4+ (%)
n.s.
Ctrl
ATT-1
anti-PD-1
Combo

Figure S7. ATT-I treatment in addition to PD-1 mAb treatment enhances the infiltration of $CD4^+$ and $CD8^+$ T cells while reducing the numbers of macrophages. (**A**) Representative histological images of MC38 tumors. Scale bar: 50 μm. (**B**) Quantification of the images in (A) three images quantified per mouse with a total of 4 mice per group (n=4). Each data point shown in y-axis refers to the percentage of the indicated positive cells in total cells of an image. We quantified 12 images in each group using the ImageJ software. Statistical analyses were conducted using one-way ANOVA test. .*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant.

Figure S8

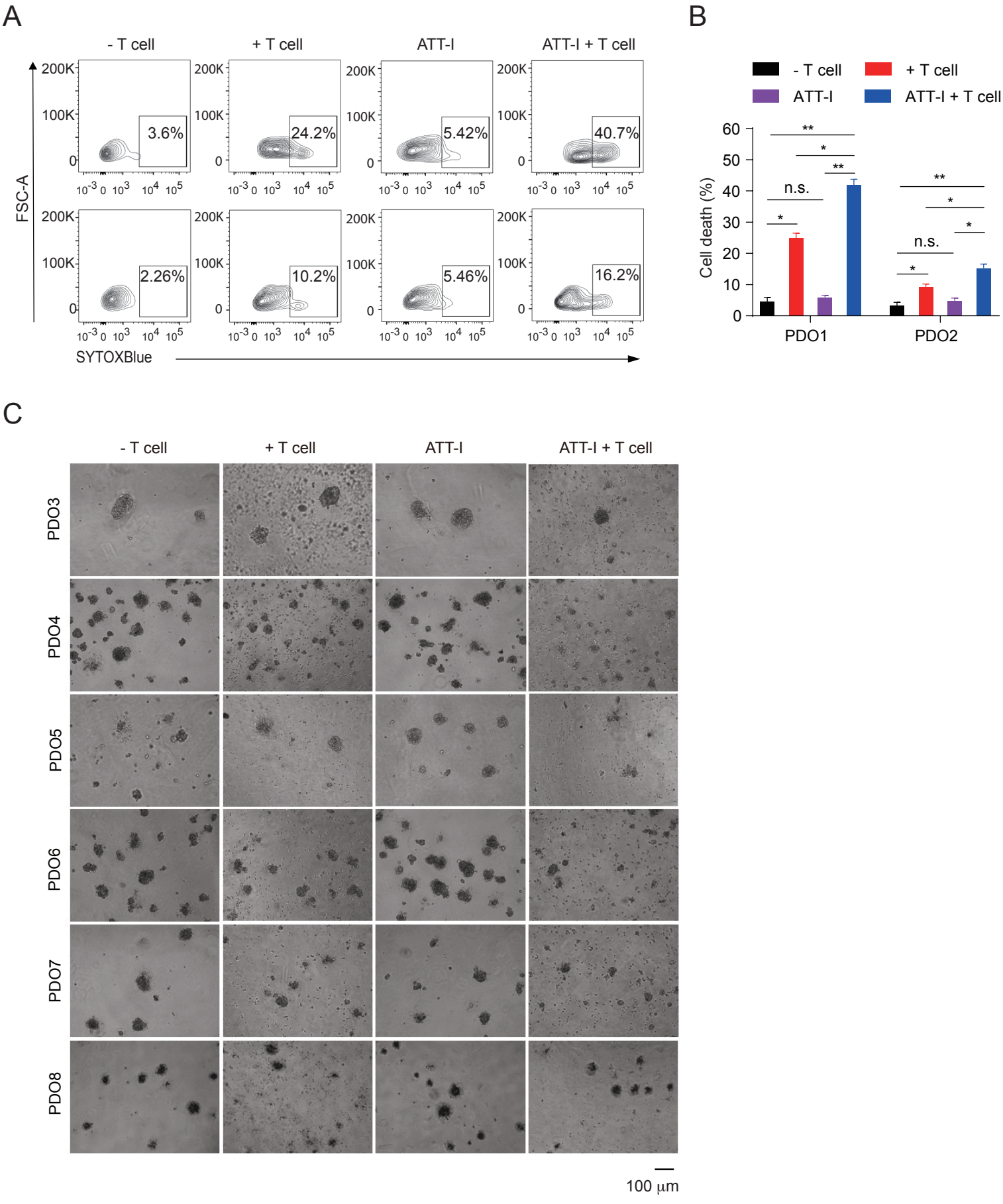

A
- T cell
+ T cell
ATT-I
ATT-I + T cell
FSC-A
SYTOXBlue
3.6%
24.2%
5.42%
40.7%
2.26%
10.2%
5.46%
16.2%
B
- T cell
+ T cell
ATT-I
ATT-I + T cell
Cell death (%)
n.s.
PDO1
PDO2
C
PDO3
PDO4
PDO5
PDO6
PDO7
PDO8
100 μm

Figure S8. The ATT-I treatment enhances the tumor killing activity of $CD8^+$ T cells against patient-derived tumor organoids. (**A**) Representative flow cytometric images of tumor cell death in the patient-derived tumor organoids (PDO1 and PDO2) using SYTOXBlue staining. (**B**) Quantification of the SYTOXBlue cell death from (A). Statistical analyses were conducted using one-way ANOVA test. (**C**) Microscopic images of PDO co-cultured with autologous $CD8^+$ T cells in the presence or absence of ATT-I (5 μM) from PDO3-8. Scale bar: 100 μm.*, $p < 0.05$; **, $p < 0.01$; ns, not significant.

Figure S9

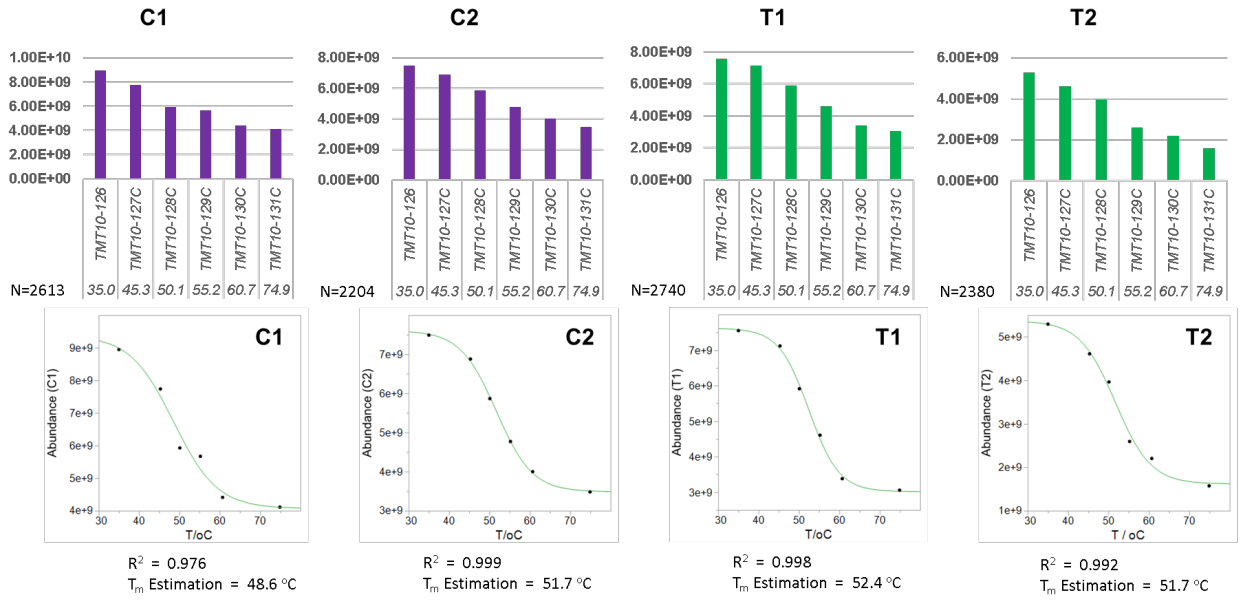
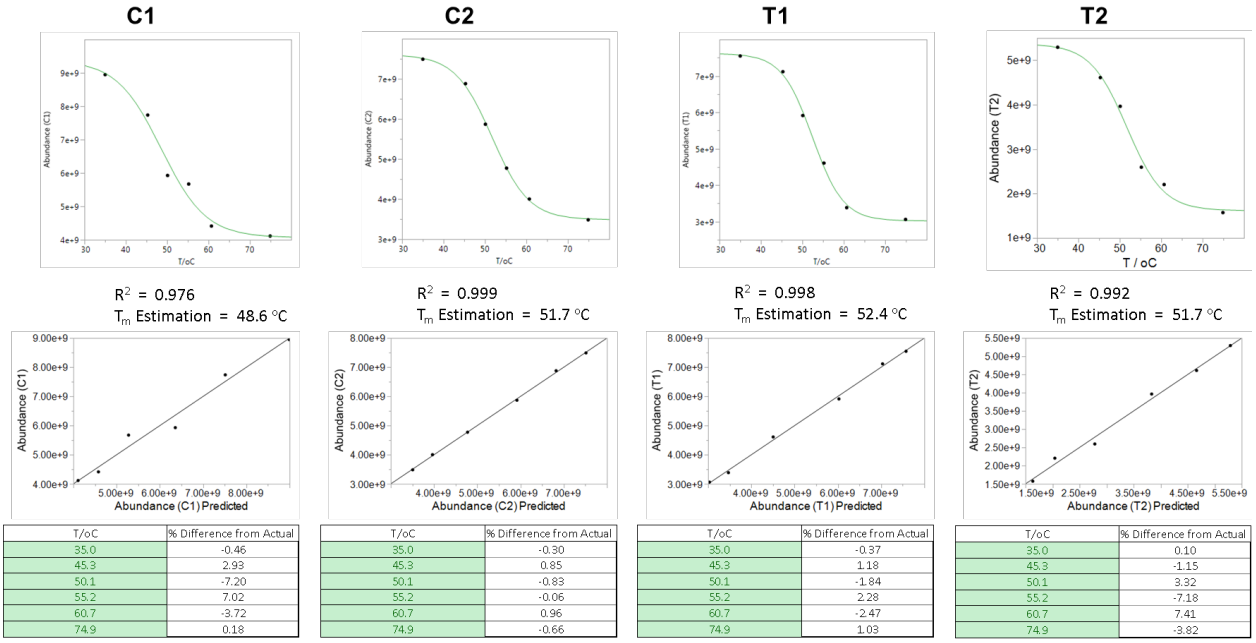


Figure S9. Total summation of TMT quantitative channels of proteins in each experiment and respective JMP GUI based simulated total proteome melt curves. C1, C2, T1, and T2 denotes, Control 1; Control 2 representing two DMSO treatments and Treatment 1 and Treatment 2 representing two compound treatments respectively. Total summation of TMT channels for each experiment was calculated using proteins quantified with at least one peptide quantified with PEP ≤ 0.05 and unique to the protein.

Figure S10



T/oC	% Difference from Actual
35.0	-0.46
45.3	2.93
50.1	-7.20
55.2	7.02
60.7	-3.72
74.9	0.18

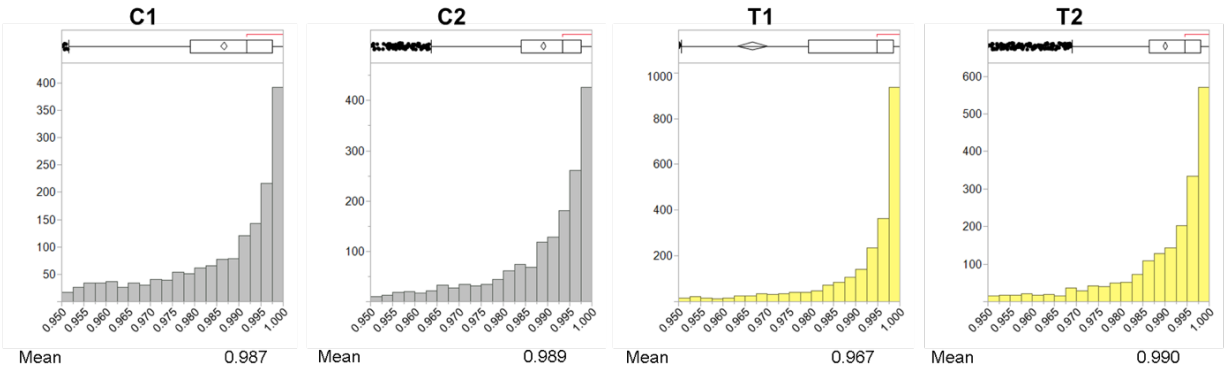
T/oC	% Difference from Actual
35.0	-0.30
45.3	0.85
50.1	-0.83
55.2	-0.06
60.7	0.96
74.9	-0.66

T/oC	% Difference from Actual
35.0	-0.37
45.3	1.18
50.1	-1.84
55.2	2.28
60.7	-2.47
74.9	1.03

T/oC	% Difference from Actual
35.0	0.10
45.3	-1.15
50.1	3.32
55.2	-7.18
60.7	7.41
74.9	-3.82

Figure S10. Correlation plots of actual and predicted protein denaturation curves and % differences from actual.

Figure S11



C1		C2		T1		T2	
Mean	0.987	Mean	0.989	Mean	0.967	Mean	0.990
Std Dev	0.013	Std Dev	0.011	Std Dev	0.188	Std Dev	0.011
Std Err Mean	0.000	Std Err Mean	0.000	Std Err Mean	0.002	Std Err Mean	0.000
Upper 95% Mean	0.987	Upper 95% Mean	0.990	Upper 95% Mean	0.970	Upper 95% Mean	0.990
Lower 95% Mean	0.986	Lower 95% Mean	0.989	Lower 95% Mean	0.963	Lower 95% Mean	0.989
N	1554	N	1596	N	2624	N	1925

Figure S11. R^2 distribution evaluation (Conditional)—$R^2 \geq 0.95$ and 35 ºC ≤ Tm ≤ 90 ºC in each sample.

Table S1. Cytotoxicity of compounds on MC38 tumor cells and mouse T cells, respectively.

The values are the percentage of viability of MC38 and $CD8^+$ T cells and their standard deviation.

NO.	Name	CAS	MC38 Mean	MC38 STDV	T Cell Mean	T Cell STDV
1	5-HTP	56-69-9	0.827	0.008	0.867	0.021
2	2-Methoxycinnamic acid	6099-03-2	0.823	0.017	0.857	0.004
3	8-Hydroxy-2-Methyl-Chromen-4-One	22105-14-2	0.823	0.013	0.751	0.011
4	1,8-Dihydroxyanthraquinone	117-10-2	0.767	0.021	0.860	0.016
5	5-Methyl-7-methoxyisoflavone	82517-12-2	0.361	0.016	0.339	0.013
6	Chelerythrine	476-32-4	0.919	0.011	0.976	0.013
7	10-Deacetylbaccatin Ⅲ	32981-86-5	0.929	0.016	0.969	0.008
8	4-Methylumbelliferone	90-33-5	0.860	0.032	0.956	0.049
9	Wolfogine		0.917	0.018	0.848	0.012
10	Punicalagin	65995-63-3	0.944	0.033	0.872	0.013
11	Arecoline	300-08-3	0.847	0.028	0.796	0.023
12	20(R)-Protopanoxadiol	7755-01-3	0.921	0.013	0.821	0.016
13	5-Hydroxymethylfurfural	67-47-0	0.744	0.017	0.774	0.011
14	Borneol	507-70-0	0.831	0.023	0.767	0.030
15	3-O-Methylgalangin	6665-74-3	0.659	0.061	0.601	0.006
16	4'-O-beta-Glucopyranosyl-5-O-Methylvisamminol	84272-85-5	0.818	0.024	0.726	0.006
17	1,5-dihydroxy-2,3,7-trimethoxyxanthen-9-one	89240-40-4	0.642	0.043	0.642	0.018
18	6-Gingerol	23513-14-6	0.859	0.047	0.876	0.020
19	Methyl 2-naphthyl ether	93-04-9	0.879	0.001	0.802	0.015
20	4-Demethylepipodophyllotoxin	6559-91-7	0.823	0.020	0.818	0.009
21	Cantharidin	56-25-7	0.813	0.028	0.852	0.028
22	Ligucyeronol		0.833	0.035	0.817	0.016
23	7-Ethyl-10-hydroxycamptothecin	86639-52-3	1.002	0.042	0.882	0.013
24	1-hydroxy,2,3,5-trimethoxyxanthone	22804-49-5	0.766	0.008	0.681	0.012
25	Pseudolaric acid-B	82508-31-4	0.987	0.006	0.980	0.013
26	Methyl 3,4-dihydroxybenzoate	2150-43-8	0.820	0.023	0.907	0.008
27	L-Epicatechin	490-46-0	0.782	0.008	0.756	0.047
28	(-)-Epigallocatechin	970-74-1	0.939	0.025	0.885	0.016
29	(-)-Epigallocatechin gallate	989-51-5	0.892	0.044	0.749	0.024
30	Epigallocatechin gallate	1257-08-5	0.498	0.045	0.557	0.057
31	Ecdysterone	5289-74-7	0.952	0.055	0.936	0.016
32	1-Acetoxy-5-deacetyl-baccatin I	119120-27-3	0.471	0.012	0.368	0.006
33	4-Methylesculetin	529-84-0	0.916	0.021	0.847	0.021
34	Bulleyaconitine A	107668-79-1	0.697	0.011	0.736	0.035
35	Oridonin	28957-04-2	0.861	0.025	0.788	0.008
36	Fraxinellone	28808-62-0	0.984	0.091	0.906	0.013
37	Docetaxel	114977-28-5	0.821	0.014	0.742	0.004
38	Ethyl 4-hydroxybenzoate	120-47-8	0.954	0.018	0.878	0.009
39	Vincristine	57-22-7	0.655	0.086	0.737	0.048
40	Indigo	482-89-3	0.865	0.037	0.850	0.026
41	Allicin	539-86-6	0.823	0.020	0.838	0.030
42	Asperosaponin Ⅵ	39524-08-8	0.251	0.057	0.248	0.033
43	Tanshinone I	568-73-0	0.955	0.059	0.854	0.025
44	Vincamine	1617-90-9	0.909	0.025	0.877	0.006
45	Cordycepin	73-03-0	0.936	0.005	0.906	0.010
46	Juniper camphor	53840-55-4	0.883	0.003	0.829	0.023
47	Ilexoside A		0.673	0.008	0.765	0.012
48	Indirubin	479-41-4	0.925	0.010	0.864	0.011
49	Catharanthine	2468-21-5	0.834	0.045	0.777	0.006
50	Scopoletin	92-61-5	0.818	0.028	0.812	0.015
51	Diosmin	520-27-4	0.862	0.042	0.849	0.021
52	Irisflorentin	41743-73-1	0.881	0.001	0.729	0.022
53	Mollugin	55481-88-4	0.657	0.002	0.546	0.015
54	Soy isoflavone		0.866	0.037	0.777	0.006
55	Farrerol	24211-30-1	0.863	0.006	0.931	0.006
56	Halofuginone	55837-20-2	0.892	0.025	0.879	0.024
57	Acetate gossypol	12542-36-8	0.851	0.029	0.721	0.018
58	Salvianolic acid B	115939-25-8	0.906	0.007	0.851	0.013
59	Hesperetin	520-33-2	0.453	0.026	0.343	0.031
60	Bavachalcone	28448-85-3	0.822	0.022	0.785	0.009
61	Febrifugine	24159-07-7	0.896	0.012	0.915	0.008
62	Digoxin	20830-75-5	0.761	0.042	0.662	0.013
63	Atractylon	6989-21-5	0.882	0.013	0.697	0.008
64	Stigmasterol	68555-08-8	0.824	0.014	0.854	0.006
65	Homoharringtonine	26833-87-4	0.870	0.007	0.884	0.011
66	Geniposide	24512-63-8	0.821	0.016	0.807	0.008
67	Koumine	1358-76-5	0.918	0.013	0.884	0.011
68	Pachymic acid	29070-92-6	0.933	0.001	0.879	0.019
69	Catechin	7295-85-4	0.882	0.015	0.829	0.011
70	Pogostone	23800-56-8	0.913	0.011	0.888	0.014
71	Cyclovirobuxine	860-79-7	0.675	0.011	0.483	0.013
72	Geniposidic acid	27741-01-1	0.858	0.021	0.855	0.018
73	Thermopsine	486-90-8	0.816	0.010	0.854	0.025
74	Picroside I	27409-30-9	0.351	0.029	0.346	0.023
75	Homovanillic acid	306-08-1	0.864	0.021	0.808	0.004
76	Echinacoside	82854-37-3	0.823	0.008	0.810	0.011
77	Buxtauine	4236-73-1	0.909	0.006	0.827	0.021
78	Phellodendrine	6873-13-18	0.863	0.037	0.825	0.018
79	Methyl hesperidin	11013-97-1	0.886	0.028	0.828	0.009
80	Galanthamine	357-70-0	0.931	0.040	0.898	0.004
81	Glabridin	59870-68-7	0.841	0.009	0.819	0.025
82	Palmatine	3486-67-7	0.954	0.023	0.819	0.017
83	Trigonelline	535-83-1	0.378	0.016	0.325	0.033
84	P-Hydroxyphenyl butanone	5471-51-2	0.849	0.025	0.846	0.034
85	Xanthoxylin	90-24-4	0.667	0.018	0.681	0.013

86	Lappaconitine	32854-75-4	0.857	0.055	0.880	0.011
87	Phloretin	60-82-2	0.931	0.040	0.855	0.008
88	Dihydroguaiaretic acid	66322-34-7	0.919	0.009	0.924	0.010
89	Fisetin	528-48-3	0.681	0.016	0.564	0.021
90	Xanthotol	2009-24-7	0.846	0.028	0.821	0.011
91	Quercitrin	522-12-3	0.652	0.021	0.748	0.018
92	Pimpinellin	131-12-4	0.820	0.016	0.694	0.018
93	Fumaric acid	110-17-8	0.857	0.028	0.766	0.047
94	Pinocembrin	480-39-7	0.885	0.025	0.838	0.028
95	Sodium tauroursodeoxycholate	14605-22-2	0.863	0.010	0.821	0.001
96	Rebaudioside A	58543-16-1	0.839	0.011	0.821	0.013
97	Icariine		0.858	0.021	0.817	0.021
98	Rosavin	84954-92-7	0.818	0.021	0.784	0.011
99	Blinin	125675-09-4	0.956	0.033	0.848	0.043
100	Theobromine	83-67-0	0.946	0.027	0.862	0.020
101	Nepetalactone	490-10-8	0.872	0.016	0.818	0.013
102	Pinostrobin	480-37-5	0.870	0.019	0.830	0.013
103	Aloperine	56293-29-9	0.825	0.017	0.796	0.009
104	Oroxin B	114482-86-9	0.868	0.035	0.850	0.004
105	Calycosin-7-glucoside	20633-67-4	0.951	0.016	0.854	0.008
106	4-Quinolone	611-36-9	0.500	0.015	0.404	0.018
107	Pomolic Acid	13849-91-7	0.928	0.028	0.854	0.010
108	Ergosterol	57-87-4	0.860	0.009	0.819	0.012
109	Veratric acid	93-07-2	0.519	0.012	0.448	0.016
110	Demethoxycurcumin	33171-16-3	0.779	0.121	0.596	0.011
111	Rosmarinic acid	20283-92-5	0.870	0.021	0.785	0.016
112	Malic acid	6915-15-7	0.819	0.012	0.762	0.013
113	5-Hydroxy-4-methoxycanthin-6-one	18110-86-6	0.822	0.018	0.810	0.003
114	Scopolamine hydrobromide	6533-68-2	0.789	0.022	0.784	0.011
115	Acteoside	61276-17-3	0.856	0.008	0.819	0.011
116	Atractylenolide I	73069-13-3	0.957	0.016	0.873	0.008
117	Reserpine	50-55-5	0.469	0.011	0.450	0.016
118	Mogroside V	88901-36-4	0.803	0.048	0.859	0.031
119	Gallic acid	149-91-7	0.918	0.021	0.883	0.015
120	Loganic acid	22255-40-9	0.587	0.025	0.526	0.013
121	Erianin	95041-90-0	0.821	0.023	0.807	0.006
122	Friedelin	559-74-0	0.928	0.034	0.876	0.006
123	Quinine	130-95-0	0.824	0.009	0.783	0.011
124	Lathyrol	34420-19-4	0.809	0.042	0.807	0.006
125	Taurochenodeoxycholic acid	516-35-8	0.887	0.008	0.827	0.006
126	Oroxin A	57396-78-8	0.829	0.005	0.773	0.011
127	Guanosine	118-00-3	0.816	0.007	0.862	0.025
128	Arecoline hydrobromide	300-08-3	0.833	0.007	0.767	0.011
129	1,8-Diacetoxy-3-carboxyanthraquinone	13739-02-1	0.821	0.027	0.808	0.004
130	Anhydroicaritin	38226-86-7	0.913	0.028	0.824	0.010
131	Camptothecin	7689-03-4	0.743	0.047	0.627	0.009
132	Aconitine	302-27-2	0.816	0.020	0.869	0.028
133	Bisdemethoxycurcumin	33171-05-0	0.840	0.023	0.817	0.006
134	Carabrone	1748-81-8	0.864	0.010	0.838	0.011
135	Gambogic acid	2752-65-0	0.917	0.035	0.831	0.005
136	Spinosin	72063-39-9	0.835	0.004	0.846	0.015
137	Orcinol glucoside	21082-33-7	0.829	0.008	0.862	0.018
138	Cimifugin	37921-38-3	0.954	0.033	0.903	0.013
139	Notoginsenoside Fe	88105-29-7	0.806	0.004	0.763	0.025
140	Notoginsenoside Fc	88122-52-5	0.788	0.035	0.720	0.001
141	Physalin L	113146-74-0	0.914	0.030	0.875	0.009
142	Stevioside	57817-89-7	0.869	0.018	0.829	0.008
143	Notoginsenoside Ft1	80418-24-2	0.877	0.021	0.853	0.009
144	Seabuckthorn flavone		1.023	0.035	0.887	0.008
145	Cephalomannine	71610-00-9	0.907	0.007	0.868	0.006
146	Phytolaccagenin	1802-12-6	0.871	0.033	0.883	0.022
147	Thymol	89-83-8	0.871	0.023	0.825	0.020
148	10,11-Dimethoxystrychnine	357-57-3	0.896	0.009	0.849	0.016
149	Harmine	442-51-3	0.882	0.013	0.814	0.005
150	Dicoumarol	66-76-2	0.920	0.022	0.870	0.004
151	Ginsenoside F3	62025-50-7	0.809	0.005	0.766	0.017
152	Esculentoside A	65497-07-6	0.949	0.031	0.922	0.015
153	Asatone	38451-63-7	0.886	0.018	0.847	0.008
154	Topotecan	123948-87-8	0.562	0.008	0.467	0.007
155	Darutoside	59219-65-7	0.828	0.009	0.881	0.014
156	Hematoxylin	517-28-2	0.880	0.016	0.872	0.014
157	Nordihydroguaiaretic acid	500-38-9	0.853	0.014	0.770	0.016
158	Rutecarpine	84-26-4	0.720	0.025	0.671	0.011
159	Asarinin	133-05-1	0.653	0.017	0.521	0.016
160	Schisantherin A	58546-56-8	0.836	0.021	0.855	0.005
161	Lycodoline	6900-92-1	0.821	0.023	0.806	0.008
162	Specnuezhenide	39011-92-2	0.970	0.032	1.006	0.034
163	Amentoflavone	1617-53-4	0.847	0.013	0.822	0.015
164	Tenuifolin	20183-47-5	0.992	0.035	0.931	0.006
165	Aucubin	479-98-1	0.836	0.017	0.837	0.006
166	Genistin	529-59-9	0.925	0.020	0.868	0.005
167	Lycorine	476-28-8	0.877	0.020	0.868	0.017
168	Ellagic acid	476-66-4	0.857	0.008	0.820	0.011
169	Dehydrocostus lactone	477-43-0	0.938	0.021	0.875	0.022
170	Huperzine A	102518-79-6	0.747	0.021	0.664	0.017
171	Chrysophanol	481-74-3	0.519	0.019	0.463	0.010
172	Daidzein	486-66-8	0.903	0.015	0.850	0.015
173	Genistein	446-72-0	0.833	0.040	0.790	0.011
174	Luteolin	491-70-3	0.863	0.022	0.830	0.013

175	Ginsenoside Re	51542-56-4	0.911	0.008	0.889	0.007
176	Apigenin	520-36-5	0.972	0.035	0.884	0.015
177	Colchicine	64-86-8	0.618	0.021	0.531	0.012
178	paclitaxtide		0.686	0.033	0.771	0.014
179	Curcumin	458-37-7	0.963	0.043	0.930	0.009
180	Sinomenine	115-53-7	0.822	0.007	0.808	0.006
181	Shikimic acid	138-59-0	0.314	0.009	0.362	0.042
182	Chlorogenic acid	327-97-9	0.897	0.008	0.868	0.006
183	Perillartine	30950-27-7	0.670	0.003	0.675	0.008
184	Oleanic acid	508-02-1	0.844	0.006	0.857	0.016
185	Chrysin	480-40-0	0.867	0.015	0.807	0.005
186	Hederagenin	465-99-6	0.824	0.014	0.702	0.015
187	Rutin	153-18-4	0.841	0.006	0.814	0.009
188	Polydatin	65914-17-2	0.619	0.017	0.553	0.021
189	Magnolol	528-43-8	0.815	0.017	0.778	0.017
190	Physcion	521-61-9	0.819	0.010	0.814	0.004
191	Emodin	518-82-1	0.925	0.034	0.825	0.011
192	Curdione	13657-68-6	1.000	0.033	0.906	0.009
193	Quercetin	6151-25-3	0.840	0.008	0.882	0.012
194	Ginsenoside Rg3	14197-60-5	0.874	0.010	0.857	0.006
195	Ginsenoside Rg1	22427-39-0	0.930	0.010	0.968	0.006
196	Resveratrol	501-36-0	0.737	0.049	0.673	0.011
197	Astragaloside A	83207-58-3	0.901	0.012	0.872	0.008
198	Honokiol	35354-74-6	0.939	0.025	0.919	0.009
199	Astragaloside II	84676-89-1	0.922	0.014	0.963	0.011
200	Sophoridine	6882-68-4	0.565	0.021	0.489	0.010
201	Imperatorin	482-44-0	0.838	0.001	0.816	0.005
202	Nodakenin	495-31-8	0.864	0.020	0.850	0.018
203	Pterostilbene	537-42-8	0.908	0.010	0.882	0.011
204	Glycyrrhetnic acid	471-53-4	0.825	0.018	0.813	0.006
205	Aloin	1415-73-2	0.689	0.029	0.721	0.027
206	Piperine	94-62-2	0.834	0.020	0.861	0.017
207	Ginsenoside Rb1	41753-43-9	0.818	0.008	0.781	0.012
208	Hyperoside	482-36-0	0.874	0.014	0.888	0.015
209	Kaempferol	520-18-3	0.827	0.014	0.816	0.008
210	Andrographolide	5508-58-7	0.890	0.001	0.809	0.008
211	Paeonol	552-41-0	0.930	0.010	0.842	0.010
212	Artemisinin	63968-64-9	0.584	0.008	0.556	0.006
213	Brucine	57-24-9	0.996	0.009	0.928	0.016
214	Tanshinone IIA	568-72-9	0.927	0.008	0.876	0.008
215	Bilobalide	33570-04-6	0.830	0.010	0.859	0.013
216	Puerarin	3681-99-0	0.887	0.006	0.814	0.009
217	Glycyrrhizic acid	1405-86-3	0.935	0.012	0.893	0.012
218	Guaiacin	36531-08-5	0.390	0.027	0.436	0.023
219	Sclareolide	564-20-5	0.926	0.008	0.882	0.013
220	Oxypeucedanin	737-52-0	0.905	0.003	0.907	0.006
221	Raceanisodamine	134355-54-7	0.887	0.006	0.876	0.008
222	Neosperidin dihydrochalcone	20702-77-6	0.825	0.011	0.810	0.003
223	Catalpol	2415-24-9	0.884	0.010	0.855	0.035
224	Lupenone	1617-70-5	0.702	0.042	0.761	0.001
225	Yohimbine	146-48-5	0.941	0.014	0.925	0.008
226	Securinine	5610-40-2	0.837	0.008	0.867	0.008
227	Isoferulic acid	537-73-5	0.867	0.018	0.866	0.030
228	Epmedin C	110642-44-9	0.981	0.016	0.823	0.023
229	Myricitrin	17912-87-7	0.761	0.015	0.656	0.008
230	Bengenin	477-90-7	0.868	0.033	0.822	0.008
231	Tectorigenin	548-77-6	0.862	0.035	0.795	0.009
232	L-abrine	21339-55-9	0.863	0.009	0.858	0.033
233	Emetine dihydrochloride	316-42-7	0.839	0.009	0.876	0.019
234	Sesamol	533-31-3	0.896	0.013	0.867	0.006
235	Isoorientin	4261-42-1	0.703	0.015	0.753	0.010
236	Leonurine hydrochloride	24697-74-3	0.784	0.011	0.710	0.003
237	Harmine hydrochloride	343-27-1	0.817	0.022	0.844	0.011
238	D-Ephedrine hydrochloride	24221-86-1	0.923	0.005	0.872	0.013
239	6-Methoxyluteolin	520-11-6	0.848	0.013	0.815	0.004
240	Quinic acid	77-95-2	0.923	0.018	0.839	0.011
241	Skimmin	93-39-0	0.870	0.019	0.820	0.004
242	Isorhamnetin-3-O-neohespeidoside	55033-90-4	0.781	0.011	0.814	0.003
243	Astragalin	480-10-4	0.877	0.018	0.843	0.011
244	Acetyl-trans-resveratrol	42206-94-0	0.919	0.009	0.914	0.016
245	Isocurcumenol	24063-71-6	0.656	0.008	0.546	0.008
246	Isorhynchophylline	6859-01-4	0.886	0.007	0.829	0.011
247	Sclareol	515-03-7	0.946	0.034	0.957	0.023
248	Typhaneoside	104472-68-6	0.839	0.011	0.825	0.006
249	Diosmetin	520-34-3	0.913	0.019	0.857	0.006
250	Ginkgolide C	15291-76-6	0.846	0.014	0.872	0.008
251	Osthole	484-12-8	0.898	0.010	0.901	0.013
252	D(-)-Salicin	138-52-3	0.864	0.024	0.817	0.008
253	Isoimperatorin	482-45-1	0.791	0.017	0.817	0.020
254	Ginkgolide B	15291-77-7	0.857	0.009	0.858	0.004
255	Ginkgolide A	15291-75-5	1.009	0.028	0.822	0.013
256	Evodiamine	518-17-2	0.678	0.016	0.708	0.006
257	Silibinin	22888-70-6	0.899	0.016	0.878	0.022
258	Naringin	10236-47-2	0.650	0.009	0.673	0.008
259	Gastrodin	62499-27-8	0.901	0.003	0.872	0.013
260	Arbutin	497-76-7	0.880	0.006	0.816	0.011
261	Dehydrocostus lactone	477-43-0	0.812	0.013	0.823	0.008
262	Betulin	473-98-3	0.886	0.001	0.832	0.006
263	Cardamonin	18956-16-6	0.850	0.004	0.816	0.008

264	2,3,5,4'-Tetrahydroxyl-diphenylethylene-2-O-beta-D-glucoside	82373-94-2	0.918	0.008	0.880	0.013
265	Hesperidin	520-26-3	0.667	0.016	0.428	0.012
266	Oroxylin A	480-11-5	0.908	0.008	0.879	0.010
267	Columbianadin	5058-13-9	0.854	0.024	0.818	0.010
268	Albiflorin	39011-90-0	0.914	0.018	0.926	0.009
269	Avicularin	572-30-5	0.890	0.001	0.823	0.005
270	Buxtauine	4236-73-1	0.919	0.025	0.856	0.013
271	Menisdaurin	67765-58-6	0.983	0.004	0.870	0.002
272	Harmine	442-51-3	0.839	0.010	0.830	0.005
273	Lupeol	545-47-1	0.906	0.021	0.935	0.011
274	Shionone	10376-48-4	0.868	0.035	0.814	0.004
275	Asarylaldehyde	14374-62-0	0.397	0.012	0.278	0.011
276	Isorhamnetin	480-19-3	0.915	0.019	0.912	0.013
277	Tetrahydropalmatine	2934-97-6	0.871	0.017	0.807	0.006
278	Isopimpinellin	482-27-9	0.929	0.011	0.874	0.009
279	Roburic acid	6812-81-3	0.827	0.021	0.756	0.015
280	Praeruptorin D	73069-28-0	0.910	0.011	0.915	0.008
281	Praeruptorin A	73069-27-9	0.738	0.041	0.821	0.003
282	Epigoitrin	1072-93-1	0.815	0.010	0.878	0.015
283	Medicagol	1983-72-8	0.835	0.011	0.865	0.008
284	β-sitosterol	64997-52-0	0.853	0.036	0.849	0.009
285	Germacrone	6902-91-6	0.967	0.013	0.925	0.011
286	Mangiferin	4773-96-0	0.820	0.011	0.815	0.004
287	Arteannuin B	50906-56-4	0.904	0.018	0.876	0.018
288	Fukinanclide		0.783	0.011	0.717	0.006
289	Atractylenolide III	73030-71-4	0.870	0.011	0.853	0.030
290	7-Ethoxy-4-Methylcoumarin	87-05-8	0.576	0.004	0.541	0.014
291	Alpinetin	36052-37-6	0.940	0.017	0.846	0.019
292	Alantolactone	546-43-0	0.757	0.021	0.530	0.013
293	3',4'-Anhydrovinblastine	38390-45-3	0.814	0.011	0.854	0.016
294	1-(3-ethyl-5,5,8,8-tetramethyl-6,7-dihydronaphthalen-2-yl)ethanone	88-29-9	0.912	0.018	0.925	0.011
295	Vindoline	2182-14-1	0.825	0.008	0.808	0.006
296	Toosendanin	58812-37-6	0.997	0.006	0.855	0.017
297	Lycorenine	477-19-0	0.826	0.013	0.872	0.014
298	Esculentoside A	65497-07-6	0.891	0.016	0.816	0.007
299	Galangin	548-83-4	4.313	5.064	0.532	0.015
300	Kirenol	52659-56-0	0.897	0.009	0.842	0.013
301	Formononetin	485-72-3	0.924	0.017	0.925	0.008
302	6-Hydroxyindole	2380-86-1	0.873	0.008	0.883	0.013
303	Bullatine B	466-26-2	0.851	0.026	0.854	0.012
304	Sanguinarine citrate	132210-34-5	0.934	0.007	0.832	0.002
305	Praeruptorin E	78478-28-1	0.752	0.040	0.637	0.002
306	Paeoniflorin	23180-57-6	0.878	0.016	0.850	0.011
307	Scoparone	120-08-1	0.880	0.018	0.815	0.002
308	taraxerol acetate	2189-80-2	0.857	0.021	0.877	0.005
309	L-Stepholidine	16562-13-3	0.817	0.029	0.716	0.010
310	Perivine	2673-40-7	0.858	0.042	0.924	0.012
311	Ilexoside B	108544-40-7	0.938	0.038	0.858	0.005
312	Bornyl acetate	76-49-3	0.835	0.004	0.816	0.006
313	Neohesperidin	13241-33-3	0.908	0.024	0.838	0.001
314	Dihydrolycorine	6271-21-2	0.614	0.016	0.719	0.013
315	Tuberostemonine HClO4		0.980	0.023	0.873	0.013
316	Lidamidine Hydrochloride	65009-35-0	0.835	0.004	0.771	0.013
317	Linderane	13476-25-0	0.823	0.006	0.696	0.011
318	Luteoloside	5373-11-5	0.880	0.008	0.922	0.015
319	Thermopsine	486-90-8	0.638	0.035	0.534	0.013
320	Rubusoside	64849-39-4	0.849	0.010	0.879	0.016
321	Poncirin	14941-08-3	0.865	0.024	0.823	0.008
322	Adicardin	103529-94-8	0.810	0.010	0.835	0.004
323	Gaultherin	490-67-5	0.890	0.001	0.820	0.011
324	1,3-diacetoxy-4,6,12-tetradecatriene-8,10-diyne	29576-66-7	0.963	0.022	0.928	0.002
325	Praeruptorin C	83382-71-2	0.929	0.011	0.880	0.004
326	Picroside II	39012-20-9	0.848	0.013	0.838	0.008
327	Kaempferol-O-glucuronide	22688-78-4	0.958	0.013	0.949	0.005
328	2-Hydroxyeupatolide	72229-33-5	0.862	0.022	0.818	0.014
329	Isobergapten	482-48-4	0.901	0.016	0.909	0.011
330	Vinblastine	865-21-4	0.828	0.010	0.863	0.015
331	Phytolaccagenin	1802-12-6	0.919	0.016	0.869	0.018
332	Atractylodinol	61642-89-5	0.824	0.017	0.810	0.012
333	Atractylodin	55290-63-6	0.894	0.010	0.921	0.004
334	Corynoxine	6877-32-3	0.911	0.014	0.825	0.010
335	Nerol	106-25-2	0.906	0.004	0.939	0.008
336	Biochanin A	491-80-5	0.865	0.039	0.871	0.014
337	Geraniol	106-24-1	0.890	0.016	0.881	0.014
338	p-Hydroxy-cinnamic acid	7400-08-0	0.859	0.021	0.830	0.012
339	Anwuligan	107534-93-0	0.949	0.013	0.907	0.005
340	Arctigenin	7770-78-7	0.888	0.013	0.829	0.009
341	3,29-Dibenzoyl karounitriol	873001-54-8	0.901	0.011	0.818	0.008
342	Cornin	548-37-8	0.864	0.019	0.723	0.016
343	Isoverbascoside	61303-13-7	0.863	0.004	0.831	0.012
344	Costunolide	553-21-9	0.850	0.015	0.817	0.006
345	Peiminine	18059-10-4	0.794	0.005	0.756	0.013
346	Neohesperidin	13241-33-3	0.825	0.021	0.815	0.004
347	Shihulimonin A	99026-99-0	0.807	0.004	0.842	0.007
348	Linarin	480-36-4	0.681	0.015	0.563	0.013
349	Suberosin	581-31-7	0.854	0.025	0.820	0.011
350	Peimine	23496-41-5	0.821	0.008	0.862	0.015
351	3,29-Dibenzoyl rarounitriol	873001-54-8	0.942	0.020	0.926	0.016
352	Tangeretin	481-53-8	0.911	0.044	0.865	0.011

353	Tenuifolin	20183-47-5	0.847	0.016	0.871	0.014
354	Darutigenol	5940-00-1	0.815	0.016	0.822	0.015
355	Norisoboldine	23599-69-1	0.980	0.012	0.914	0.003
356	Isopimpinellin	482-27-9	0.821	0.007	0.829	0.011
357	Momordin Ic	96990-18-0	0.933	0.009	0.864	0.011
358	Madecassoside	34540-22-2	0.850	0.009	0.724	0.004
359	Scoparone	120-08-1	0.960	0.011	0.973	0.067
360	Coptisine chloride	6020-18-4	0.924	0.016	0.884	0.005
361	Scoulerine	6451-72-5	0.669	0.005	0.720	0.005
362	Curcumol	4871-97-0	0.852	0.013	0.908	0.006
363	Dendrobine	2115-91-5	0.885	0.008	0.882	0.021
364	Crocin I	42553-65-1	0.940	0.011	0.878	0.006
365	Echinacoside	82854-37-3	0.820	0.016	0.813	0.006
366	4-Hydroxybenzeneacetamide	17194-82-0	0.874	0.006	0.868	0.004
367	Isocolumbin	471-545	0.885	0.008	0.809	0.001
368	Isoline	30000-36-3	0.959	0.006	0.926	0.006
369	Nuezhenide	39011-92-2	0.847	0.035	0.779	0.014
370	Ligucyperonol	105108-20-1	0.724	0.017	0.716	0.010
371	Mesaconitine	2752-64-9	0.841	0.004	0.818	0.008
372	Tiliroside	20316-62-5	0.892	0.013	0.868	0.021
373	Hispidulin	1447-88-7	0.692	0.021	0.683	0.013
374	Taraxerol		0.833	0.008	0.811	0.001
375	Liensinine HClO 4		0.923	0.006	0.866	0.010
376	Sophoricoside	152-95-4	0.920	0.006	0.908	0.006
377	Pimpinellin	131-12-4	0.843	0.006	0.874	0.010
378	(-)-Perillaldehyde	18031-40-8	0.906	0.023	0.878	0.008
379	Perillen	539-52-6	0.906	0.004	0.896	0.005
380	Neoeriocitrin	13241-32-2	0.837	0.020	0.880	0.004
381	Astragaloside II	84676-89-1	0.881	0.015	0.891	0.001
382	Crotaline	315-22-0	0.841	0.011	0.819	0.003
383	Aristolochic Acid A	313-67-7	0.836	0.028	0.822	0.012
384	Naringin	10236-47-2	0.910	0.024	0.864	0.011
385	Syringin	118-34-3	0.949	0.013	0.874	0.005
386	Salvianic acid A sodium	23028-17-3	0.903	0.001	0.850	0.009
387	Neohesperidin	13241-33-3	0.836	0.001	0.871	0.013
388	Vinorelbine	71486-22-1	0.840	0.036	0.819	0.011
389	Nitidine chloride	13063-04-2	0.924	0.017	0.853	0.020
390	Calycosin	20575-57-9	0.911	0.020	0.917	0.022
391	Protodioscin	55056-80-9	0.891	0.015	0.830	0.018
392	Potenline	53956-04-0	0.919	0.014	0.922	0.015
393	Vinblastine	865-21-4	0.860	0.032	0.853	0.012
394	Daphnetin	486-35-1	0.932	0.008	0.843	0.031
395	Curculigoside	85643-19-2	0.833	0.008	0.817	0.005
396	Palmitic acid	57-10-3	0.931	0.005	1.032	0.054
397	1-Deoxynojirimycin	19130-96-2	0.822	0.007	0.813	0.011
398	Periplocoside	13137-64-9	0.951	0.007	0.924	0.012
399	Podophyllotoxin	518-28-5	0.880	0.018	0.810	0.004
400	7-Ethylcamptothecin	78287-27-1	0.907	0.008	0.884	0.004
401	Liensinine diperchlorate	2385-63-9	0.949	0.011	0.860	0.009
402	Glycitin	40246-10-4	0.984	0.011	0.870	0.004
403	Rutin	153-18-4	0.906	0.025	0.836	0.006
404	Madecassoside	34540-22-2	0.857	0.042	0.861	0.013
405	Echinacoside	82854-37-3	0.945	0.018	0.954	0.013
406	Shikonin	517-89-5	0.873	0.026	0.869	0.005
407	Daidzin	552-66-9	0.920	0.011	0.872	0.016
408	Formononetin	485-72-3	0.828	0.012	0.868	0.035
409	Psoralen	66-97-7	0.807	0.008	0.763	0.016
410	Cucurbitacin B	6199-67-3	0.858	0.006	0.863	0.013
411	Narirutin	14259-46-2	0.894	0.011	0.848	0.019
412	Epimedin A	110623-72-8	0.880	0.010	0.832	0.025
413	Epmedin B	110623-73-9	0.827	0.021	0.875	0.003
414	Epmedin C	110642-44-9	0.988	0.008	1.000	0.029
415	6-Shogaol	555-66-8	0.889	0.004	0.869	0.005
416	Sarsasapogenin	82597-74-8	0.848	0.008	0.823	0.015
417	Magnolflorine	2141-09-5	0.826	0.009	0.811	0.002
418	Aconitine 3-acetate	77181-26-1	0.905	0.020	0.882	0.015
419	Atractylenolide I	73069-13-3	0.785	0.012	0.808	0.004
420	Tectoridin	611-40-5	0.516	0.023	0.567	0.019
421	Buddleoside	480-36-4	0.887	0.008	0.717	0.006
422	Licochalcone A	58749-22-7	0.841	0.016	0.852	0.012
423	10-Hydroxy camptothecin	19685-09-7	0.974	0.011	0.911	0.001
424	Costunolide	19685-09-7	0.835	0.012	0.857	0.014
425	Prim-O-glucosylcimifugin	80681-45-4	0.969	0.013	0.873	0.018
426	Cycloastragenol	78574-94-4	0.816	0.005	0.846	0.019
427	Betulinic acid	472-15-1	0.941	0.010	0.917	0.009
428	Harpagoside	19210-12-9	0.916	0.013	0.922	0.014
429	Vinorelbine	71486-22-1	0.849	0.025	0.831	0.004
430	Forsythoside A	79916-77-1	0.909	0.018	0.920	0.018
431	Synephrine	94-07-5	0.966	0.037	0.869	0.003
432	Salvianolic acid B	115939-25-8	0.849	0.004	0.865	0.008
433	Astragaloside III	84687-42-3	0.884	0.008	0.883	0.001
434	Astragaloside I	84680-75-1	0.905	0.004	0.824	0.019
435	Astragaloside II	84676-89-1	0.934	0.018	0.921	0.016
436	Diosgenin glucoside	14144-06-0	0.923	0.019	0.910	0.011
437	Isochlorogenic acid B	14534-61-3	0.801	0.032	0.808	0.006
438	Isochlorogenic acid A	2450-53-5	0.976	0.019	0.941	0.008
439	Sedanolide	6415-59-4	0.871	0.009	0.864	0.010
440	Atractylenolide II	73069-14-4	0.836	0.021	0.811	0.002
441	Atractylenolide III	73030-71-4	0.821	0.016	0.807	0.006

442	Isochlorogenic acid C	32451-88-0	0.956	0.006	0.871	0.016
443	Aconitine	302-27-2	0.830	0.011	0.829	0.019
444	Nomilin	1063-77-0	0.942	0.006	0.922	0.015
445	Nobiletin	478-01-3	0.863	0.011	0.848	0.006
446	Synephrine	94-07-5	0.832	0.006	0.877	0.014
447	Eriocitrin	13463-28-0	0.918	0.013	0.832	0.006
448	Morin hydrate	480-16-0	0.825	0.016	0.770	0.018
449	Biochanin A	491-80-5	0.898	0.010	0.822	0.001
450	Nuciferine	475-83-2	0.888	0.006	0.875	0.011
451	Prim-O-glucosylcimifugin	80681-45-4	0.943	0.025	0.841	0.004
452	Alantolactone	546-43-0	0.927	0.022	0.889	0.008
453	Isoalantolactone	470-17-7	0.831	0.005	0.827	0.008
454	Nerol	106-25-2	0.893	0.005	0.816	0.010
455	Dehydroandrographolide	134418-28-3	0.915	0.020	0.921	0.025
456	Matrine	519-02-8	0.920	0.026	0.902	0.000
457	Naringenin	480-41-1	0.820	0.005	0.810	0.003
458	Geniposide	24512-63-8	0.902	0.014	0.907	0.016
459	Wogonoside	51059-44-0	0.917	0.009	0.843	0.028
460	Berbamine hydrochloride	6078-17-7	0.747	0.035	0.816	0.006
461	jatrorrhizine	3621-38-3	0.828	0.015	0.832	0.000
462	Wogonin	632-85-9	0.816	0.019	0.810	0.004
463	Oxymatrine	16837-52-8	0.801	0.016	0.816	0.006
464	Berberine	2086-83-1	0.943	0.021	0.902	0.012
465	Rhein	478-43-3	0.830	0.010	0.808	0.007
466	Diosgenin	512-04-9	0.910	0.025	0.835	0.010
467	Stachydrine hydrochloride	4136-37-2	0.862	0.016	0.822	0.008
468	8-Methoxypsoralen	298-81-7	0.822	0.008	0.818	0.007
469	Evodiamine	518-17-2	0.993	0.028	0.919	0.013
470	Ursolic Acid	77-52-1	0.835	0.004	0.822	0.014
471	Lupeol	545-47-1	0.912	0.018	0.877	0.016
472	Berberine hydrochloride	633-65-8	0.840	0.009	0.821	0.016
473	glycyrrhetnic acid	471-53-4	0.719	0.025	0.787	0.008
474	Notoginseng Leaf Triterpenes	88105-29-7	0.882	0.013	0.916	0.013
475	Ginseng Leaves Extract		0.974	0.025	0.905	0.006
476	Resveratrol	501-36-0	0.832	0.006	0.821	0.016
477	Genistein	446-72-0	0.916	0.013	0.870	0.011
478	Ginsenoside Rg3	14197-60-5	0.888	0.006	0.872	0.013
479	Emodin	518-82-1	0.892	0.014	0.837	0.007
480	Ginsenoside Rh1	63223-86-9	0.843	0.035	0.901	0.011
481	Saikosaponin D	20874-52-6	0.911	0.008	0.822	0.009
482	Schisandrol A	7432-28-2	0.895	0.018	0.844	0.006
483	Ethyl ferulate	4046-02-0	0.864	0.037	0.851	0.002
484	Ginsenoside Rg2	52286-74-5	0.853	0.009	0.821	0.013
485	p-Hydroxy-cinnamic acid	7400-08-0	0.980	0.009	0.953	0.011
486	Salvianolic acid B	115939-25-8	0.822	0.008	0.805	0.007
487	Bakuchiol	10309-37-2	0.915	0.008	0.923	0.013
488	Morin hydrate	480-16-0	0.842	0.013	0.813	0.006
489	Vanillin	121-33-5	0.931	0.016	0.871	0.009
490	Ginkgolide B	15291-77-7	0.768	0.021	0.707	0.011
491	Sophocarpine	6483-15-4	0.908	0.024	0.828	0.024
492	Rhamnose	6155-35-7	0.859	0.004	0.833	0.004
493	Sesamin	607-80-7	0.929	0.011	0.828	0.006
494	Theophylline	58-55-9	0.815	0.024	0.672	0.010
495	Schizandrin B	61281-37-6	0.671	0.011	0.707	0.008
496	Notoginsenoside R1	80418-24-2	0.920	0.009	0.842	0.014
497	Astilbin	29838-67-3	0.830	0.024	0.826	0.031
498	Diammonium glycyrrhizinate	79165-06-3	0.821	0.020	0.751	0.008
499	Hyodeoxycholic acid	83-49-8	0.921	0.026	0.825	0.013
500	Cyclovirobuxine D	860-79-7	1.005	0.023	0.931	0.008
501	Hanfangichin B	436-77-1	0.908	0.022	0.829	0.004
502	Muscone	541-91-3	0.873	0.003	0.856	0.009
503	Dihydroartemisinin	71939-50-9	0.830	0.010	0.814	0.018
504	Ursodeoxycholic Acid	128-13-2	0.582	0.029	0.441	0.021
505	Sennoside A	81-27-6	0.865	0.031	0.818	0.008
506	Compound K	39262-14-1	0.943	0.005	0.882	0.013
507	Nicotinic acid	59-67-6	0.978	0.016	0.913	0.006
508	Ginsenoside Rc	11021-14-0	1.017	0.009	0.941	0.002
509	Breviscapine	116122-36-2	0.915	0.020	0.882	0.013
510	Nicotinamide	98-92-0	0.859	0.040	0.813	0.015
511	Peiminine	18059-10-4	0.760	0.011	0.690	0.013
512	Aloin	1415-73-2	0.669	0.029	0.542	0.015
513	Cinnamic alcohol	104-54-1	0.909	0.009	0.837	0.006
514	Cinnamic aldehyde	104-55-2	0.877	0.008	0.818	0.013
515	Sodium ferulic	24276-84-4	0.782	0.016	0.676	0.019
516	Isofraxidin	486-21-5	0.912	0.013	0.852	0.006
517	Psoralidin	18642-23-4	0.726	0.016	0.552	0.004
518	Ligustrazine	1124-11-4	0.981	0.024	0.826	0.016
519	p-Coumaric acid	501-98-4	0.975	0.010	0.882	0.008
520	Hordenine	539-15-1	0.929	0.015	0.831	0.008
521	Swertiamarine	17388-39-5	0.889	0.003	0.823	0.006
522	Peimine	23496-41-5	0.848	0.020	0.862	0.008
523	Ophiopogonin D	945619-74-9	0.461	0.015	0.330	0.001
524	Esculin hydrate	531-75-9	0.850	0.004	0.836	0.005
525	Artemether	71963-77-4	0.898	0.013	0.867	0.006
526	Chondroitin Sulfate	24967-93-9	0.728	0.010	0.507	0.064
527	Arctiin	20362-31-6	0.932	0.006	0.872	0.013
528	Ginsenoside Rb3	68406-26-8	0.956	0.014	0.914	0.006
529	Tectorigenin	611-40-5	0.877	0.008	0.841	0.006
530	Triptolide	38748-32-2	0.898	0.013	0.892	0.004

531	Tetrandrine	518-34-3	0.477	0.006	0.430	0.011
532	Protopanaxatriol	34080-08-5	0.868	0.022	0.832	0.008
533	Dihydromyricetin	27200-12-0	0.836	0.019	0.813	0.006
534	Tetrahydropalmatine	6024-85-7	0.746	0.010	0.669	0.005
535	Pseudoginsenoside RT5	98474-78-3	0.966	0.037	0.923	0.009
536	Ginsenoside F1	53963-43-2	0.850	0.004	0.827	0.008
537	Salicylic acid	69-72-7	0.839	0.011	0.820	0.010
538	Caffeine	58-08-2	0.880	0.016	0.833	0.005
539	Ginsenoside F2	62025-49-4	0.755	0.024	0.672	0.023
540	Lipoic acid	62-46-4	0.691	0.017	0.673	0.023
541	Lovastatin	75330-75-5	0.829	0.012	0.806	0.021
542	Ginsenoside-Rb2	11021-13-9	0.792	0.014	0.668	0.015
543	Betaine	107-43-7	0.496	0.023	0.356	0.023
544	Lycopene	502-65-8	0.985	0.010	0.923	0.006
545	Norcantharidin	5442-12-6	0.994	0.018	0.943	0.009
546	Schisandrin C	61301-33-5	0.746	0.019	0.693	0.007
547	Rhyncholphylline	76-66-4	0.888	0.008	0.816	0.006
548	Amygdalin	29883-15-6	0.960	0.016	0.929	0.005
549	Saikosaponin A	20736-09-8	0.945	0.018	0.841	0.006
550	Tangeretin	481-53-8	0.563	0.028	0.438	0.009
551	Raddeanin A	89412-79-3	0.921	0.012	0.889	0.003
552	Cichoric acid	6537-80-0	0.928	0.013	0.859	0.018
553	Salvianolic acid A	96574-01-5	0.887	0.006	0.847	0.013
554	Chenodeoxycholic acid	474-25-9	0.825	0.011	0.826	0.007
555	Celastrol	34157-83-0	0.753	0.025	0.674	0.006
556	Ginsenoside Rd	52705-93-8	0.840	0.004	0.819	0.013
557	Schizandrin A	61281-38-7	0.968	0.012	0.918	0.007
558	Asiatic acid	464-92-6	0.863	0.037	0.818	0.004
559	Protopanaxadio	30636-90-9	0.985	0.012	0.887	0.007
560	Taxifolin	480-18-2	0.807	0.008	0.719	0.008
561	Ferulic acid	1135-24-6	0.780	0.013	0.709	0.011
562	Asiaticoside	16830-15-2	0.959	0.006	0.869	0.018
563	Liquiritin	551-15-5	0.946	0.022	0.929	0.011
564	Polygalacic acid	1260-04-4	0.373	0.022	0.240	0.021
565	Phillyrin	487-41-2	0.781	0.013	0.676	0.010
566	Senegenin	2469-34-3	0.496	0.010	0.434	0.013
567	10-Gingerol	23513-15-7	0.783	0.013	0.689	0.008
568	Loganin	18524-94-2	0.923	0.005	0.897	0.008
569	Lobetyolin	136085-37-5	0.982	0.013	0.945	0.015
570	Glycitein	40957-83-3	0.821	0.011	0.813	0.017
571	8-Gingerol	23513-08-8	0.696	0.009	0.630	0.013
572	Gentiopicroside	20831-76-9	0.919	0.011	0.860	0.017
573	Dihydrocapsaicin	19408-84-5	0.777	0.008	0.717	0.021
574	Patchouli alcohol	5986-55-0	0.554	0.006	0.478	0.030
575	schisandrol B	58546-54-6	0.974	0.025	0.907	0.021
576	Ginkgolic Acid	20261-38-5	0.876	0.018	0.860	0.005
577	Salidroside	10338-51-9	0.919	0.009	0.897	0.007
578	Gypenoside		0.947	0.013	0.865	0.025
579	Sodium Aescinate	20977-05-3	0.873	0.019	0.819	0.009
580	Oleuropein	32619-42-4	0.943	0.008	0.903	0.015
581	Isopsoralen	523-50-2	0.767	0.019	0.684	0.006
582	4-Isopropyltoluene	99-87-6	0.929	0.011	0.883	0.013
583	Cytisine	485-35-8	1.013	0.001	0.983	0.022
584	Scutellarin	27740-01-8	0.815	0.018	0.828	0.013
585	Phlorizin	60-81-1	0.979	0.004	0.906	0.010
586	Isoliquiritigenin	961-29-5	0.879	0.008	0.821	0.006
587	Liquiritigenin	578-86-9	0.744	0.018	0.632	0.007
588	Jujuboside A	55466-04-1	0.599	0.015	0.482	0.023
589	Jujuboside B	55466-05-2	0.876	0.025	0.823	0.006
590	Ginsenoside Ro	34367-04-9	0.910	0.011	0.886	0.018
591	Polyphyllin Ⅰ	50773-41-6	0.703	0.015	0.631	0.006
592	Polyphyllin II	50773-42-7	0.940	0.054	0.916	0.021
593	Polyphyllin VI	55916-51-3	0.653	0.023	0.543	0.005
594	Polyphyllin VII	68124-04-9	0.943	0.021	0.895	0.009

Table S2. Effect of Compounds on the CD8+ T-cell mediated cytotoxicity against MC38 tumor cells in the co-culture.
The values shown are the Relative viability (tumor cell viability of treated group)/(tumor cell viability of control group) from the volcano plot of Figure 1B (log2[relative viability] >1; $p < 0.05$).

NO.	Name	CAS	Mean	STDV
1	5-HTP	56-69-9	1.890	0.030
2	2-Methoxycinnamic acid	6099-03-2	1.122	0.014
6	Chelerythrine	476-32-4	1.275	0.089
7	10-Deacetylbaccatin Ⅲ	32981-86-5	0.928	0.052
8	4-Methylumbelliferone	90-33-5	1.345	0.007
9	wolfogine		1.247	0.137
10	Punicalagin	65995-63-3	1.835	0.077
12	20(R)-Protopanoxadiol	7755-01-3	1.217	0.137
18	6-Gingerol	23513-14-6	0.857	0.091
19	Methyl 2-naphthyl ether	93-04-9	1.138	0.022
20	4-Demethylepipodophyllotoxin	6559-91-7	0.981	0.006
21	Cantharidin	56-25-7	1.102	0.016
22	ligucyeronol		1.382	0.107
23	7-Ethyl-10-hydroxycamptothecin	86639-52-3	0.978	0.034
25	Pseudolaric acid-B	82508-31-4	1.077	0.063
26	Methyl 3,4-dihydroxybenzoate	2150-43-8	1.701	0.016
28	(-)-Epigallocatechin	970-74-1	0.863	0.040
31	Ecdysterone	5289-74-7	1.006	0.021
33	4-Methylesculetin	529-84-0	1.671	0.074
36	Fraxinellone	28808-62-0	1.711	0.085
38	Ethyl 4-hydroxybenzoate	120-47-8	1.836	0.092
40	Indigo	482-89-3	1.218	0.008
41	Allicin	539-86-6	1.340	0.016
43	Tanshinone I	568-73-0	1.274	0.010
44	Vincamine	1617-90-9	0.978	0.062
45	Cordycepin	73-03-0	1.281	0.100
46	Juniper camphor	53840-55-4	1.292	0.030
48	Indirubin	479-41-4	1.262	0.061
50	Scopoletin	92-61-5	1.497	0.037
51	Diosmin	520-27-4	1.246	0.035
53	Mollugin	55481-88-4	1.196	0.098
55	Farrerol	24211-30-1	1.162	0.042
56	Halofuginone	55837-20-2	1.224	0.016
58	Salvianolic acid B	115939-25-8	0.989	0.060
61	Febrifugine	24159-07-7	1.286	0.100
64	Stigmasterol	68555-08-8	1.346	0.304
65	Homoharringtonine	26833-87-4	1.157	0.074
66	Geniposide	24512-63-8	1.032	0.001
67	Koumine	1358-76-5	1.156	0.022
68	Pachymic acid	29070-92-6	0.900	0.053
69	Catechin	7295-85-4	1.150	0.011
70	Pogostone	23800-56-8	1.695	0.059
72	Geniposidic acid	27741-01-1	1.026	0.019
73	Thermopsine	486-90-8	1.118	0.013
75	Homovanillic acid	306-08-1	1.115	0.031
76	Echinacoside	82854-37-3	1.270	0.168
77	Buxtauine	4236-73-1	1.852	0.196
78	Phellodendrine	6873-13-18	0.958	0.057
79	Methyl hesperidin	11013-97-1	0.707	0.041
80	Galanthamine	357-70-0	1.326	0.063
81	Glabridin	59870-68-7	1.601	0.197
82	Palmatine	3486-67-7	1.866	0.049
84	P-Hydroxyphenyl butanone	5471-51-2	1.781	0.069
86	Lappaconitine	32854-75-4	1.596	0.049
87	Phloretin	60-82-2	1.521	0.141
88	Dihydroguaiaretic acid	66322-34-7	1.465	0.078
90	Xanthotol	2009-24-7	1.705	0.078
94	Pinocembrin	480-39-7	1.846	0.091
95	Sodium tauroursodeoxycholate	14605-22-2	0.881	0.013
96	Rebaudioside A	58543-16-1	1.691	0.028
97	Icariine		2.502	0.095
99	Blinin	125675-09-4	1.762	0.153
100	Theobromine	83-67-0	1.403	0.015
101	Nepetalactone	490-10-8	0.938	0.062
102	Pinostrobin	480-37-5	1.425	0.160

104	Oroxin B	114482-86-9	1.371	0.071
105	Calycosin-7-glucoside	20633-67-4	0.803	0.013
107	Pomolic Acid	13849-91-7	0.806	0.132
108	Ergosterol	57-87-4	1.161	0.045
113	5-Hydroxy-4-methoxycanthin-6-one	18110-86-6	0.616	0.078
115	Acteoside	61276-17-3	0.379	0.074
116	Atractylenolide I	73069-13-3	2.520	0.169
118	Mogroside V	88901-36-4	0.839	0.081
119	Gallic acid	149-91-7	1.102	0.043
121	Erianin	95041-90-0	1.069	0.054
122	Friedelin	559-74-0	1.622	0.175
124	Lathyrol	34420-19-4	1.002	0.043
125	Taurochenodeoxycholic acid	516-35-8	1.888	0.135
127	Guanosine	118-00-3	1.127	0.091
129	1,8-Diacetoxy-3-carboxyanthraquinone	13739-02-1	0.880	0.014
130	Anhydroicaritin	38226-86-7	0.794	0.930
132	Aconitine	302-27-2	0.811	0.030
133	Bisdemethoxycurcumin	33171-05-0	0.725	0.076
134	Carabrone	1748-81-8	1.175	0.078
135	Gambogic acid	2752-65-0	0.771	0.014
136	Spinosin	72063-39-9	0.551	0.170
137	Orcinol glucoside	21082-33-7	1.243	0.161
138	Cimifugin	37921-38-3	1.035	0.092
141	Physalin L	113146-74-0	1.150	0.240
142	Stevioside	57817-89-7	1.816	0.204
143	Notoginsenoside Ft1	80418-24-2	0.785	0.150
144	Seabuckthorn flavone		0.907	0.036
145	Cephalomannine	71610-00-9	1.385	0.205
146	Phytolaccagenin	1802-12-6	1.061	0.057
147	Thymol	89-83-8	1.161	0.198
148	10,11-Dimethoxystrychnine	357-57-3	0.993	0.136
149	Harmine	442-51-3	1.069	0.064
150	Dicoumarol	66-76-2	0.603	0.252
152	Esculentoside A	65497-07-6	1.025	0.011
153	Asatone	38451-63-7	1.933	0.057
155	Darutoside	59219-65-7	1.053	0.006
156	Hematoxylin	517-28-2	0.946	0.095
160	Schisantherin A	58546-56-8	1.444	0.076
161	Lycodoline	6900-92-1	1.015	0.024
162	Specnuezhenide	39011-92-2	1.070	0.042
163	Amentoflavone	1617-53-4	0.779	0.158
164	Tenuifolin	20183-47-5	0.632	0.368
165	Aucubin	479-98-1	1.285	0.064
166	Genistin	529-59-9	1.671	0.155
167	Lycorine	476-28-8	0.701	0.125
168	Ellagic acid	476-66-4	1.310	0.242
169	Dehydrocostus lactone	477-43-0	1.326	0.092
172	Daidzein	486-66-8	1.096	0.062
173	Genistein	446-72-0	0.557	0.600
174	Luteolin	491-70-3	1.191	0.156
175	Ginsenoside Re	51542-56-4	0.964	0.102
176	Apigenin	520-36-5	0.498	0.559
179	Curcumin	458-37-7	1.153	0.005
180	Sinomenine	115-53-7	0.902	0.171
182	Chlorogenic acid	327-97-9	0.860	0.149
184	Oleanic acid	508-02-1	1.026	0.022
185	Chrysin	480-40-0	0.902	0.185
187	Rutin	153-18-4	1.036	0.078
190	Physcion	521-61-9	0.956	0.092
191	Emodin	518-82-1	1.077	0.135
192	Curdione	13657-68-6	0.980	0.025
193	Quercetin	6151-25-3	1.037	0.001
194	Ginsenoside Rg3	14197-60-5	0.832	0.086
195	Ginsenoside Rg1	22427-39-0	1.045	0.091
197	Astragaloside A	83207-58-3	1.286	0.133
198	Honokiol	35354-74-6	1.215	0.193
199	Astragaloside II	84676-89-1	1.417	0.050
201	Imperatorin	482-44-0	0.894	0.138
202	Nodakenin	495-31-8	0.945	0.076
203	Pterostilbene	537-42-8	1.194	0.059
204	Glycyrrhetnic acid	471-53-4	1.251	0.170

206	Piperine	94-62-2	0.486	0.414
208	Hyperoside	482-36-0	0.890	0.153
209	Kaempferol	520-18-3	1.162	0.014
210	Andrographolide	5508-58-7	0.780	0.002
211	Paeonol	552-41-0	0.692	0.030
213	Brucine	57-24-9	1.275	0.136
214	Tanshinone IIA	568-72-9	1.089	0.082
215	Bilobalide	33570-04-6	0.831	0.057
216	Puerarin	3681-99-0	1.111	0.011
217	Glycyrrhizic acid	1405-86-3	0.835	0.080
219	Sclareolide	564-20-5	0.725	0.076
220	Oxypeucedanin	737-52-0	0.908	0.090
221	Raceanisodamine	134355-54-7	1.146	0.014
222	Neosperidin dihydrochalcone	20702-77-6	1.782	0.155
223	Catalpol	2415-24-9	0.904	0.018
225	Yohimbine	146-48-5	1.040	0.011
226	Securinine	5610-40-2	1.825	0.017
227	Isoferulic acid	537-73-5	1.039	0.010
228	Epmedin C	110642-44-9	1.780	0.180
230	Bengenin	477-90-7	0.695	0.057
232	L-abrine	21339-55-9	1.166	0.202
233	Emetine dihydrochloride	316-42-7	1.492	0.313
234	Sesamol	533-31-3	0.768	0.126
237	Harmine hydrochloride	343-27-1	1.053	0.043
238	D-Ephedrine hydrochloride	24221-86-1	1.039	0.022
239	6-Methoxyluteolin	520-11-6	1.053	0.029
240	Quinic acid	77-95-2	1.056	0.047
241	Skimmin	93-39-0	1.028	0.006
243	Astragalin	480-10-4	1.689	0.131
244	Acetyl-trans-resveratrol	42206-94-0	1.252	0.028
246	Isorhynchophylline	6859-01-4	0.672	0.126
247	Sclareol	515-03-7	0.559	0.159
248	Typhaneoside	104472-68-6	1.106	0.104
249	Diosmetin	520-34-3	0.907	0.036
250	Ginkgolide C	15291-76-6	1.435	0.065
251	Osthole	484-12-8	1.596	0.107
252	D(-)-Salicin	138-52-3	1.213	0.110
254	Ginkgolide B	15291-77-7	1.346	0.064
255	Ginkgolide A	15291-75-5	1.554	0.089
257	Silibinin	22888-70-6	1.146	0.104
259	Gastrodin	62499-27-8	1.195	0.150
260	Arbutin	497-76-7	1.052	0.022
261	Dehydrocostus lactone	477-43-0	1.325	0.091
262	Betulin	473-98-3	1.509	0.223
263	Cardamonin	18956-16-6	1.381	0.499
264	2,3,5,4'-Tetrahydroxyl-diphenylethylene-2-O-beta-D-glucoside	82373-94-2	1.058	0.084
266	Oroxylin A	480-11-5	0.827	0.220
267	Columbianadin	5058-13-9	0.735	0.052
268	Albiflorin	39011-90-0	0.899	0.039
269	Avicularin	572-30-5	0.785	0.151
270	Buxtauine	4236-73-1	0.538	0.019
271	MENISDAURIN	67765-58-6	0.608	0.245
272	Harmine	442-51-3	1.136	0.001
273	Lupeol	545-47-1	1.159	0.187
274	Shionone	10376-48-4	1.219	0.264
276	Isorhamnetin	480-19-3	1.612	0.170
277	Tetrahydropalmatine	2934-97-6	0.979	0.124
278	Isopimpinellin	482-27-9	1.677	0.063
280	Praeruptorin D	73069-28-0	1.104	0.096
282	Epigoitrin	1072-93-1	0.665	0.010
283	Medicagol	1983-72-8	1.547	0.175
284	β-sitosterol	64997-52-0	1.102	0.098
285	Germacrone	6902-91-6	1.090	0.011
286	Mangiferin	4773-96-0	1.331	0.085
287	Arteannuin B	50906-56-4	1.187	0.161
289	Atractylenolide III	73030-71-4	1.527	0.195
291	Alpinetin	36052-37-6	0.902	0.115
293	3',4'-Anhydrovinblastine	38390-45-3	1.555	0.332
294	1-(3-ethyl-5,5,8,8-tetramethyl-6,7-dihydronaphthalen-2-yl)ethanone	88-29-9	0.738	0.063
295	Vindoline	2182-14-1	1.455	0.207
296	Toosendanin	58812-37-6	1.918	0.192

297	Lycorenine	477-19-0	1.034	0.018
298	Esculentoside A	65497-07-6	0.476	0.573
300	Kirenol	52659-56-0	0.921	0.144
301	Formononetin	485-72-3	0.948	0.346
302	6-Hydroxyindole	2380-86-1	1.169	0.187
303	Bullatine B	466-26-2	1.046	0.361
304	Sanguinarine citrate	132210-34-5	1.547	0.234
306	Paeoniflorin	23180-57-6	0.636	0.205
307	Scoparone	120-08-1	1.182	0.143
308	taraxerol acetate	2189-80-2	1.022	0.021
310	Perivine	2673-40-7	1.023	0.003
311	Ilexoside B	108544-40-7	1.032	0.006
312	Bornyl acetate	76-49-3	1.510	0.171
313	Neohesperidin	13241-33-3	1.027	0.149
315	Tuberostemonine HClO4		1.263	0.107
318	Luteoloside	5373-11-5	1.320	0.195
320	Rubusoside	64849-39-4	1.213	0.163
321	Poncirin	14941-08-3	1.168	0.161
322	Adicardin	103529-94-8	1.656	0.175
323	Gaultherin	490-67-5	1.015	0.166
324	1,3-diacetoxy-4,6,12-tetradecatriene-8,10-diyne	29576-66-7	1.247	0.175
325	Praeruptorin C	83382-71-2	1.037	0.106
326	Picroside II	39012-20-9	1.459	0.146
327	Kaempferol-O-glucuronide	22688-78-4	1.682	0.142
328	2-Hydroxyeupatolide	72229-33-5	1.842	0.112
329	Isobergapten	482-48-4	1.138	0.050
330	Vinblastine	865-21-4	1.040	0.059
331	Phytolaccagenin	1802-12-6	1.477	0.120
332	Atractylodinol	61642-89-5	1.338	0.132
333	Atractylodin	55290-63-6	1.297	0.081
334	Corynoxine	6877-32-3	0.775	0.002
335	Nerol	106-25-2	1.418	0.093
336	Biochanin A	491-80-5	2.154	0.089
337	Geraniol	106-24-1	1.135	0.016
338	p-Hydroxy-cinnamic acid	7400-08-0	0.922	0.099
339	Anwuligan	107534-93-0	1.121	0.006
340	Arctigenin	7770-78-7	1.053	0.030
341	3,29-Dibenzoyl karounitriol	873001-54-8	1.003	0.017
343	Isoverbascoside	61303-13-7	0.892	0.015
344	Costunolide	553-21-9	1.403	0.170
346	Neohesperidin	13241-33-3	1.090	0.124
347	Shihulimonin A	99026-99-0	1.064	0.078
349	Suberosin	581-31-7	1.614	0.082
350	Peimine	23496-41-5	1.152	0.021
351	3,29-Dibenzoyl rarounitriol	873001-54-8	1.105	0.018
352	Tangeretin	481-53-8	1.088	0.075
353	Tenuifolin	20183-47-5	1.160	0.061
354	Darutigenol	5940-00-1	1.050	0.031
355	Norisoboldine	23599-69-1	1.035	0.015
356	Isopimpinellin	482-27-9	0.945	0.103
357	Momordin Ic	96990-18-0	1.019	0.024
359	Scoparone	120-08-1	1.029	0.031
360	Coptisine chloride	6020-18-4	1.131	0.008
362	Curcumol	4871-97-0	1.017	0.013
363	Dendrobine	2115-91-5	1.032	0.022
364	Crocin I	42553-65-1	1.047	0.022
365	Echinacoside	82854-37-3	1.069	0.068
366	4-Hydroxybenzeneacetamide	17194-82-0	1.037	0.035
367	Isocolumbin	471-545	0.904	0.045
368	Isoline	30000-36-3	1.187	0.078
371	Mesaconitine	2752-64-9	0.915	0.024
372	Tiliroside	20316-62-5	1.010	0.016
374	Taraxerol		1.060	0.033
375	Liensinine HClO 4		1.163	0.013
376	Sophoricoside	152-95-4	1.752	0.170
377	Pimpinellin	131-12-4	0.942	0.021
378	(-)-Perillaldehyde	18031-40-8	1.188	0.045
379	Perillen	539-52-6	1.187	0.021
380	Neoeriocitrin	13241-32-2	1.014	0.045
381	Astragaloside II	84676-89-1	1.286	0.146
382	Crotaline	315-22-0	1.062	0.023

383	Aristolochic Acid A	313-67-7	1.102	0.016
384	Naringin	10236-47-2	1.035	0.008
385	Syringin	118-34-3	1.150	0.047
386	Salvianic acid A sodium	23028-17-3	1.247	0.105
387	Neohesperidin	13241-33-3	1.227	0.063
388	Vinorelbine	71486-22-1	1.059	0.043
389	Nitidine chloride	13063-04-2	1.051	0.008
390	Calycosin	20575-57-9	0.974	0.061
391	Protodioscin	55056-80-9	1.098	0.038
392	Potenline	53956-04-0	1.013	0.014
393	Vinblastine	865-21-4	0.965	0.047
394	Daphnetin	486-35-1	1.145	0.105
395	Curculigoside	85643-19-2	1.622	0.226
396	Palmitic acid	57-10-3	1.157	0.105
397	1-Deoxynojirimycin	19130-96-2	1.086	0.053
398	Periplocoside	13137-64-9	1.037	0.049
399	Podophyllotoxin	518-28-5	1.045	0.006
400	7-Ethylcamptothecin	78287-27-1	1.414	0.202
401	Liensinine diperchlorate	2385-63-9	1.210	0.100
402	Glycitin	40246-10-4	0.890	0.008
403	Rutin	153-18-4	1.039	0.022
404	Madecassoside	34540-22-2	1.046	0.037
405	Echinacoside	82854-37-3	1.381	0.129
406	Shikonin	517-89-5	0.929	0.015
407	Daidzin	552-66-9	1.581	0.356
408	Formononetin	485-72-3	0.838	0.078
410	Cucurbitacin B	6199-67-3	1.038	0.010
411	Narirutin	14259-46-2	1.011	0.018
412	Epimedin A	110623-72-8	1.384	0.084
413	Epmedin B	110623-73-9	1.078	0.008
414	Epmedin C	110642-44-9	1.101	0.025
415	6-Shogaol	555-66-8	1.262	0.083
416	Sarsasapogenin	82597-74-8	1.112	0.128
417	Magnolflorine	2141-09-5	1.165	0.054
418	Aconitine 3-acetate	77181-26-1	1.105	0.081
422	Licochalcone A	58749-22-7	1.103	0.051
423	10-Hydroxy camptothecin	19685-09-7	1.032	0.006
424	Costunolide	19685-09-7	1.070	0.124
425	Prim-O-glucosylcimifugin	80681-45-4	1.033	0.008
426	Cycloastragenol	78574-94-4	0.930	0.068
427	Betulinic acid	472-15-1	1.028	0.078
428	Harpagoside	19210-12-9	1.185	0.123
429	Vinorelbine	71486-22-1	1.014	0.013
430	Forsythoside A	79916-77-1	1.076	0.040
431	Synephrine	94-07-5	1.252	0.112
432	Salvianolic acid B	115939-25-8	1.207	0.071
433	Astragaloside III	84687-42-3	1.044	0.008
434	Astragaloside I	84680-75-1	1.057	0.045
435	Astragaloside II	84676-89-1	1.198	0.114
436	Diosgenin glucoside	14144-06-0	1.491	0.087
437	Isochlorogenic acid B	14534-61-3	1.006	0.045
438	Isochlorogenic acid A	2450-53-5	1.424	0.143
439	Sedanolide	6415-59-4	1.067	0.028
440	Atractylenolide II	73069-14-4	0.968	0.042
441	Atractylenolide III	73030-71-4	1.082	0.077
442	Isochlorogenic acid C	32451-88-0	1.043	0.065
443	Aconitine	302-27-2	1.023	0.021
444	Nomilin	1063-77-0	1.076	0.055
445	Nobiletin	478-01-3	1.505	0.095
446	Synephrine	94-07-5	1.238	0.091
447	Eriocitrin	13463-28-0	1.156	0.072
449	Biochanin A	491-80-5	1.026	0.028
450	Nuciferine	475-83-2	1.066	0.052
451	Prim-O-glucosylcimifugin	80681-45-4	1.056	0.024
452	Alantolactone	546-43-0	1.305	0.074
453	Isoalantolactone	470-17-7	1.043	0.006
454	Nerol	106-25-2	0.990	0.016
455	Dehydroandrographolide	134418-28-3	1.407	0.049
456	Matrine	519-02-8	1.068	0.047
457	Naringenin	480-41-1	1.025	0.011
458	Geniposide	24512-63-8	1.146	0.013

459	Wogonoside	51059-44-0	1.117	0.011
461	jatrorrhizine	3621-38-3	0.991	0.062
462	Wogonin	632-85-9	1.085	0.039
463	Oxymatrine	16837-52-8	1.037	0.077
464	Berberine	2086-83-1	1.040	0.024
465	Rhein	478-43-3	1.044	0.052
466	Diosgenin	512-04-9	0.952	0.133
467	Stachydrine hydrochloride	4136-37-2	1.152	0.028
468	8-Methoxypsoralen	298-81-7	1.053	0.008
469	Evodiamine	518-17-2	0.932	0.057
470	Ursolic Acid	77-52-1	1.119	0.019
471	Lupeol	545-47-1	1.097	0.014
472	Berberine hydrochloride	633-65-8	1.392	0.047
474	Notoginseng Leaf Triterpenes	88105-29-7	1.065	0.038
475	Ginseng Leaves Extract		1.038	0.016
476	Resveratrol	501-36-0	1.402	0.028
477	Genistein	446-72-0	1.037	0.007
478	Ginsenoside Rg3	14197-60-5	1.324	0.206
479	Emodin	518-82-1	1.442	0.071
480	Ginsenoside Rh1	63223-86-9	1.188	0.086
481	Saikosaponin D	20874-52-6	0.815	0.117
482	Schisandrol A	7432-28-2	1.280	0.074
483	Ethyl ferulate	4046-02-0	1.491	0.064
484	Ginsenoside Rg2	52286-74-5	1.168	0.072
485	p-Hydroxy-cinnamic acid	7400-08-0	1.017	0.078
486	Salvianolic acid B	115939-25-8	1.068	0.083
487	Bakuchiol	10309-37-2	1.193	0.078
488	Morin hydrate	480-16-0	0.970	0.029
489	Vanillin	121-33-5	1.763	0.141
491	Sophocarpine	6483-15-4	1.365	0.125
492	Rhamnose	6155-35-7	0.945	0.037
493	sesamin	607-80-7	1.234	0.204
496	Notoginsenoside R1	80418-24-2	1.038	0.001
497	Astilbin	29838-67-3	1.395	0.090
499	Hyodeoxycholic acid	83-49-8	1.053	0.051
500	Cyclovirobuxine D	860-79-7	1.023	0.028
501	Hanfangichin B	436-77-1	1.077	0.054
502	Muscone	541-91-3	1.019	0.019
503	Dihydroartemisinin	71939-50-9	1.164	0.013
505	Sennoside A	81-27-6	1.169	0.042
506	Compound K	39262-14-1	1.141	0.045
507	Nicotinic acid	59-67-6	1.056	0.033
508	Ginsenoside Rc	11021-14-0	1.108	0.041
509	Breviscapine	116122-36-2	1.349	0.174
510	Nicotinamide	98-92-0	1.129	0.023
513	Cinnamic alcohol	104-54-1	0.981	0.034
514	Cinnamic aldehyde	104-55-2	1.191	0.076
516	Isofraxidin	486-21-5	1.133	0.026
518	Ligustrazine	1124-11-4	1.185	0.122
519	p-Coumaric acid	501-98-4	1.114	0.059
520	Hordenine	539-15-1	1.481	0.074
521	Swertiamarine	17388-39-5	0.987	0.023
522	Peimine	23496-41-5	1.162	0.015
524	Esculin hydrate	531-75-9	1.091	0.069
525	Artemether	71963-77-4	1.110	0.025
527	Arctiin	20362-31-6	1.494	0.030
528	Ginsenoside Rb3	68406-26-8	1.048	0.013
529	Tectorigenin	611-40-5	1.152	0.041
530	Triptolide	38748-32-2	1.365	0.008
532	Protopanaxatriol	34080-08-5	1.052	0.028
533	Dihydromyricetin	27200-12-0	1.100	0.025
535	Pseudoginsenoside RT5	98474-78-3	1.014	0.022
536	Ginsenoside F1	53963-43-2	1.034	0.031
537	Salicylic acid	69-72-7	1.220	0.100
538	Caffeine	58-08-2	1.218	0.054
541	Lovastatin	75330-75-5	0.960	0.032
544	Lycopene	502-65-8	1.022	0.079
545	Norcantharidin	5442-12-6	1.510	0.068
547	Rhyncholphylline	76-66-4	1.144	0.007
548	Amygdalin	29883-15-6	1.029	0.070
549	Saikosaponin A	20736-09-8	1.167	0.057

551	Raddeanin A	89412-79-3	1.451	0.133
552	Cichoric acid	6537-80-0	1.055	0.034
553	Salvianolic acid A	96574-01-5	1.139	0.011
554	Chenodeoxycholic acid	474-25-9	1.101	0.087
556	Ginsenoside Rd	52705-93-8	1.235	0.043
557	Schizandrin A	61281-38-7	1.538	0.122
558	Asiatic acid	464-92-6	1.135	0.089
559	Protopanaxadio	30636-90-9	1.077	0.072
562	Asiaticoside	16830-15-2	1.096	0.033
563	Liquiritin	551-15-5	0.904	0.035
568	Loganin	18524-94-2	1.237	0.076
569	Lobetyolin	136085-37-5	1.113	0.006
570	Glycitein	40957-83-3	1.305	0.081
572	Gentiopicroside	20831-76-9	0.993	0.019
575	schisandrol B	58546-54-6	1.229	0.004
576	Ginkgolic Acid	20261-38-5	1.080	0.011
577	Salidroside	10338-51-9	1.081	0.030
578	Gypenoside		1.187	0.020
579	Sodium Aescinate	20977-05-3	1.246	0.066
580	Oleuropein	32619-42-4	1.025	0.010
582	4-Isopropyltoluene	99-87-6	1.166	0.039
583	Cytisine	485-35-8	1.122	0.049
584	Scutellarin	27740-01-8	1.029	0.013
585	Phlorizin	60-81-1	1.255	0.023
586	Isoliquiritigenin	961-29-5	1.169	0.033
589	Jujuboside B	55466-05-2	1.080	0.017
590	Ginsenoside Ro	34367-04-9	1.186	0.021
592	Polyphyllin II	50773-42-7	0.994	0.038
594	Polyphyllin VII	68124-04-9	1.022	0.011

Table S3. The panel of antibodies for CyTOF assay.

Marker	Clone	Tag	Ab Dilution
CD45	30- F11	89Y	200
I-A/I-E	M5/114.15.2	209Bi	100
CD274/PD-L1	10F.9G2	153Eu	200
CD3e	145-2C11	152Sm	200
CD8a	53-6.7	146Nd	100
CD25	3C7	150Nd	200
CD127/IL7Ra	A7R34	175Lu	100
CD62L	MEL-14	160Gd	300
CD69	H1.2F3	145Nd	100
TCRb	H57-597	143Nd	100
CD44	IM7	171Yb	250
CD11b	M1/70	148Nd	300
CD11c	N418	142Nd	100
F4/80	BM8	159Tb	200
Ly-6C	HK1.4	162Dy	250
Ly-6G	1A8	141Pr	100
NK1.1	PK136	170Er	100
CD45R/ B220	RA3-6B2	176Yb	100
CD19	6D5	149Sm	100
TER-119	TER-119	154Sm	200
EpCAM	G8.8	166Er	200
Vimentin	RV202	156Gd	200
CCR7	4B12	164Dy	200
Sca-1	D7	169Tm	200
CD4	RM4-5	172Yb	100
Foxp3	FJK-16s	165Ho	200

Table S4. Clinical information of human colorectal cancer samples.

Case	Sex	age	Surgery	Diagnosis
1	F	63	Lower anterior resection	Sigmoid colon adenocarcinoma
2	M	52	Colectomy	Adenocarcinoma
3	F	42	Colectomy	Adenocarcinoma
4	F	58	Hepatectomy	Metastatic colon adenocarcinoma
5	M	61	Sectionectomy	Metastatic rectal cancer
6	M	61	Ileocolic and sigmoid resection	Adenocarcinoma
7	M	63	Colectomy	Adenocarcinoma
8	F	67	Small bowel resection	Adenocarcinoma

Table S5. Parsing of peptides for quantification.

	C (Control) Prep1	*C (Control) Prep2*	*T (Treated) Prep1*	*T (Treated) Prep2*
# of peptides identified with an assigned protein name and a PEP value ≤ 0.05	33,358	26, 959	37,018	28,754
Notes: 1) PEP = Posterior Error Probability, thus 95% probability carrying PSMs were isolated 2) PSM = Peptide Spectral Matches				
# of peptides identified with a unique sequence for a protein	16,015	13, 053	17,943	14,118
Notes: 1) Some identified peptide sequences are common in multiple proteins, thus for quantification purposes, such PSMs are excluded				
# of proteins identified without contaminant peptide identifications	2613	2204	2740	2380
Notes: 1) One or more peptide spectral match can represent a protein 2) Quantification channels of peptide IDs were subjected to mathematical summation to calculate and derive protein abundance.				