

SUPPLEMENTARY MATERIALS

Analysis of bee venom

Apis mellifera crude bee venom was collected from Zonguldak province located in the Black Sea region in Turkey. 5 mg of bee venom was dissolved in 1 mL of solvent consisting of 90% Solvent A + 10% Solvent B [(Solvent A: H₂O:0.05% trifluoroacetic acid (TFA) (v/v), Solvent B: 90% ACN:10% H₂O:0.045% TFA (v/v/v)]. The mixtures were vortexed and then sonicated for about ten minutes. After centrifugation for two minutes, the mixtures were filtered through a 0.45 µm regenerated cellulose filter. The phospholipase A2 (PLA₂) enzyme content was analyzed with Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) as reported in the literature [14]. The analysis was performed by AutoFlex Speed (Bruker Daltonics, Germany), and the α-Cyano-4-hydroxycinnamic acid (CHCA) solution was used as a matrix solution. The monoisotopic molecular mass was estimated in a range of m/z 5000 - 20000 for PLA₂. The peptide content of the bee venom was analyzed with a quadrupole-time-of-flight liquid chromatography-mass spectrometer (Agilent G6530B Q-TOF LC-MS). 80 µL of the sample was injected, and the separation was performed on an Agilent Zorbax 300SB-C18 column (2.1 × 150 mm, 5 µm particle size). A 2% Solvent D gradient method with a flow rate of 0.3 mL/min for 60 min was used [Solvent C: H₂O:0.1% formic acid (FA) (v/v) and Solvent D: 90% ACN:10% H₂O:0.1% FA (v/v/v)]. The column temperature was adjusted to 25 °C. The instrument settings for the mass spectrometer were as follows; 10 L/min drying gas flow rate and 45 psi nebulizer pressure were used. The nebulizing gas temperature was set at 350 °C, capillary potential 3500 V, and the sheath gas flow rate was 10 L/min. The fragmentor voltage was set to 165 V and the skimmer voltage was 65 V of MS TOF analyzer. The mass spectra were recorded in positive ion mode and the mass/charge ratio (m/z) was set to 100-3000 Da. LC-MS chromatograms and spectra were analyzed by Agilent Qualitative Analysis B.06.00 software.

Cell culture

Cancer cells experienced in this study were 1) MDA-MB-231 (American Type Cell Collection ATCC, Cat No. HTB-26, VA, USA) human metastatic breast, 2) HEPG2 (ATCC, Cat. No. HB-8065) human liver and 3) MCF-7 (ATCC, Cat No. HTB-22) human breast cancer cells. Normal cells used as control were 1) ARPE-19 (ATCC, Catalog No. CRL-2302) human epithelial from retina, 2) MCF10A (ATCC, Catalog No. CRL-10318) human breast, and 3) NIH3T3 (ATCC, Cat. No. CRL-1658) embryonic mouse fibroblast. MCF-7/MDA-MB-231/ARPE-19, HEPG2, and NIH3T3/MCF10A cells were cultured in RPMI (Wisent Inc. Multicell, USA, Cat. No. 350000CL), EMEM (Cat. No. 320026CL) and DMEM (Cat. No. 319005CL) media, respectively. These media contained 10% (v/v) fetal bovine serum (Sigma-Aldrich Co. St. Louis, US, Cat. No: 12103C) for MCF-7/MDA-MB-231/ARPE-19/HEPG2/MCF10A cells and 10% (v/v) bovine calf serum (Sigma-Aldrich, Cat. No. 12133C) for NIH3T3 cells, and 1% (v/v) penicillin-streptomycin (Capricorn, Cat. No. PS-B) was added to each media. Cells were incubated at 37 °C incubator with 5% CO₂. The passage

numbers of these cells used throughout the study were 19 for MCF-7, 8 for MDA-MB-231, 8 for HEPG2, 18 for ARPE19, 13 for MCF-10A, and 12 for NIH3T3.

MTT cytotoxicity assay

5000 cells per well were cultured until they reached full confluency followed by treatment with bee venom at 8, 12, 25, 50, or 100 µg/mL for 24h. Bee venom was obtained and prepared as previously ^[15]. After 24h incubation with bee venom, cells were treated with 200µl media including 10 µL of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide, 5mg/mL) (Sigma-Aldrich, U.S., Cat. No. M2272) per well for 4h at 37°C ^[15,16]. After incubation with MTT, 200 µL DMSO was added to each well and incubated for 2h on a shaker (in dark) at room temperature. After incubation, color development (from purple to yellow) was observed and absorbances were measured at 570 nm using a microplate reader. IC₅₀ values for each cell line were calculated as previously according to the logarithmic slope formula ^[16]. Each treatment was performed as at least three repeats and also each experiment was repeated independently as triplicates.

Anastasis experimental design

All cells were treated separately with bee venom or cisplatin for 24h as stated above, and then washed once with 1x PBS (phosphate buffered saline) (Wisent, 311-010-CL). Cells were trypsinized and collected by centrifugation at 230 rpm. The supernatant was removed, and the cells were resuspended in a fresh medium. Cell viability (%) and total live cell numbers were determined by trypan blue staining using Countess FL II automatic cell counter (Thermofisher) ^[17]. 10µL of cell suspension was mixed (1:1 ratio) with 10µL of 0.4% trypan blue (Biological Industries, B 103-102-1B) and incubated for approximately 10 mins at room temperature. 10 µL of cell-dye mixture was loaded on the coverslip of the device. Trypan blue is a negatively charged dye that is used to determine cell viability. Since the membrane structure is intact in living cells, the dye cannot enter the cell, while dead cells absorb the dye dead cells appear blue/black under the light microscope, and live/dead cells stained by trypan blue can be also detectable using automated counters. The rest of the cells were washed three times with 1x PBS to remove bee venom/cisplatin completely, and the washed cells were cultured again in fresh media without bee venom/cisplatin for 24 hours. The same method was repeated for each of the next 24 and 48 hours. The viability rates of the treated cells with bee venom/cisplatin in the first 24 hours were determined at the end of the next 24, 48 and 72 hours in the media that did not contain bee venom/cisplatin (**Figure 1**). It should be noted that all untreated cells proliferated too much therefore half of the cell suspensions were discarded after counting and seeded in fresh media.

Population doubling times

Population doubling times were calculated using the formula below where N_t is the number of cells at time t , N_0 is the number of cells initially at time 0, t is time (hours), and GR is the growth rate ^[18].

$$N_t = N_0 \times e^{grt}$$

$$GR = \frac{\ln \left(\frac{N_t}{N_0} \right)}{t}$$

$$\text{Doubling time} = \frac{\ln(2)}{\text{GR}}$$

Statistical analyses

All experiments were performed in at least three independent repeats. Each repeat also included at least three intra-experimental repeats for a treatment. The reliability of the automated counter was tested by measuring each sample at least three times within an experimental repeat. Standard errors of viabilities (S.E. +/- standard error of the mean) were calculated using SPSS software, and viabilities (%) were compared using UNIANOVA, and *p* values less than 0.05 were considered significant.

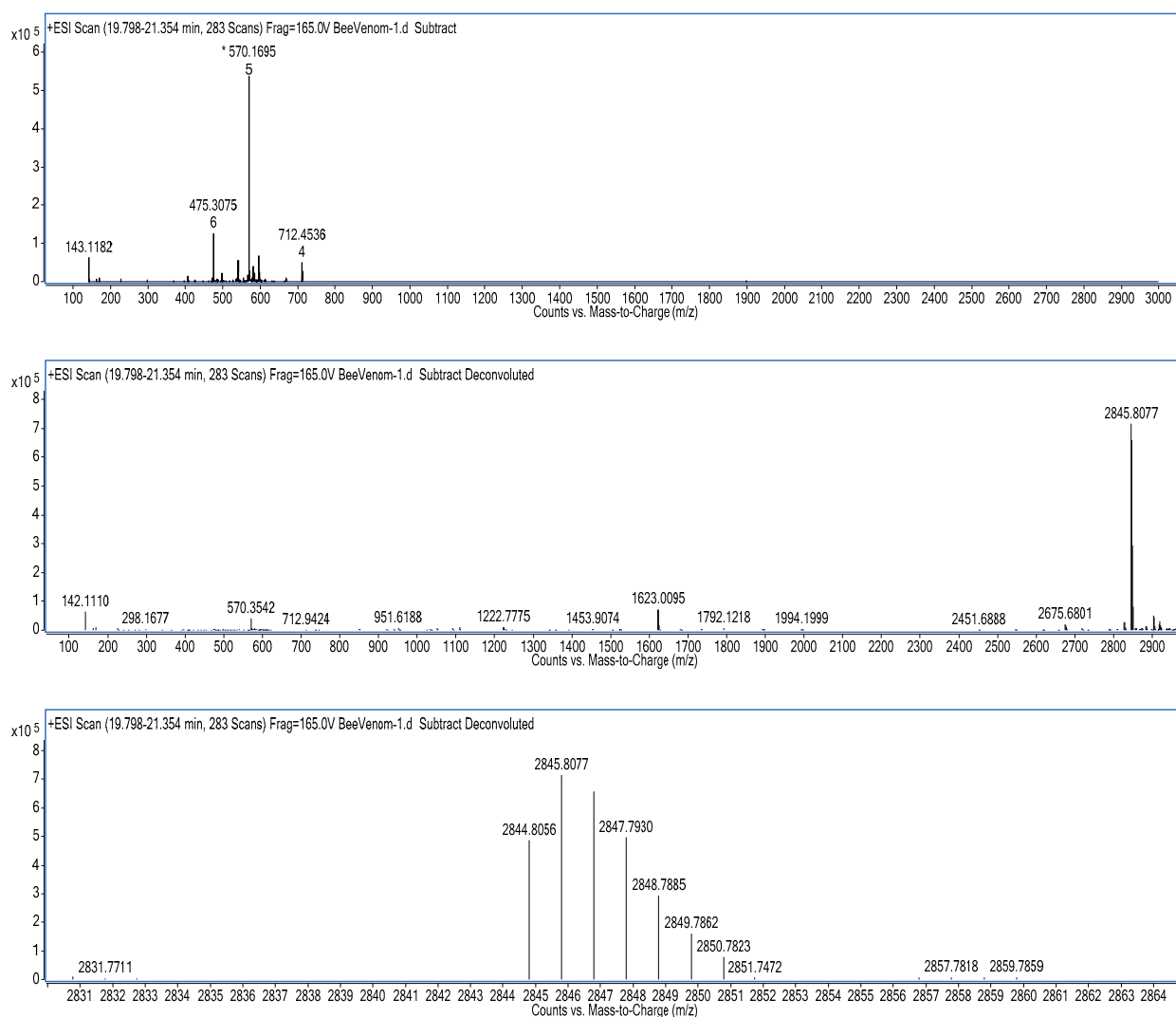


Fig. S1 LC-MS spectrum and deconvoluted spectrum of Melittin.

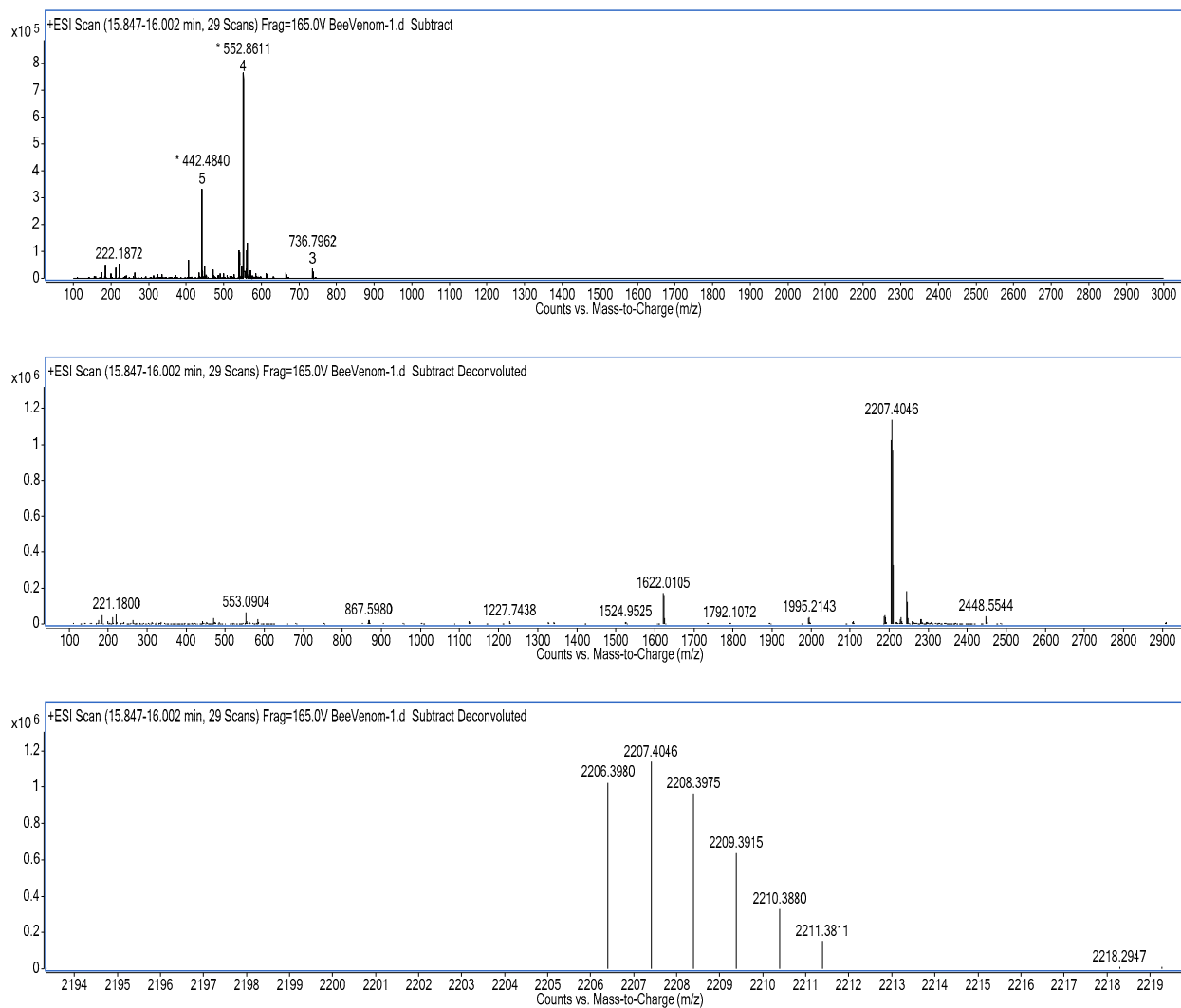


Fig. S2 LC-MS spectrum and deconvoluted spectrum of Melittin-F.

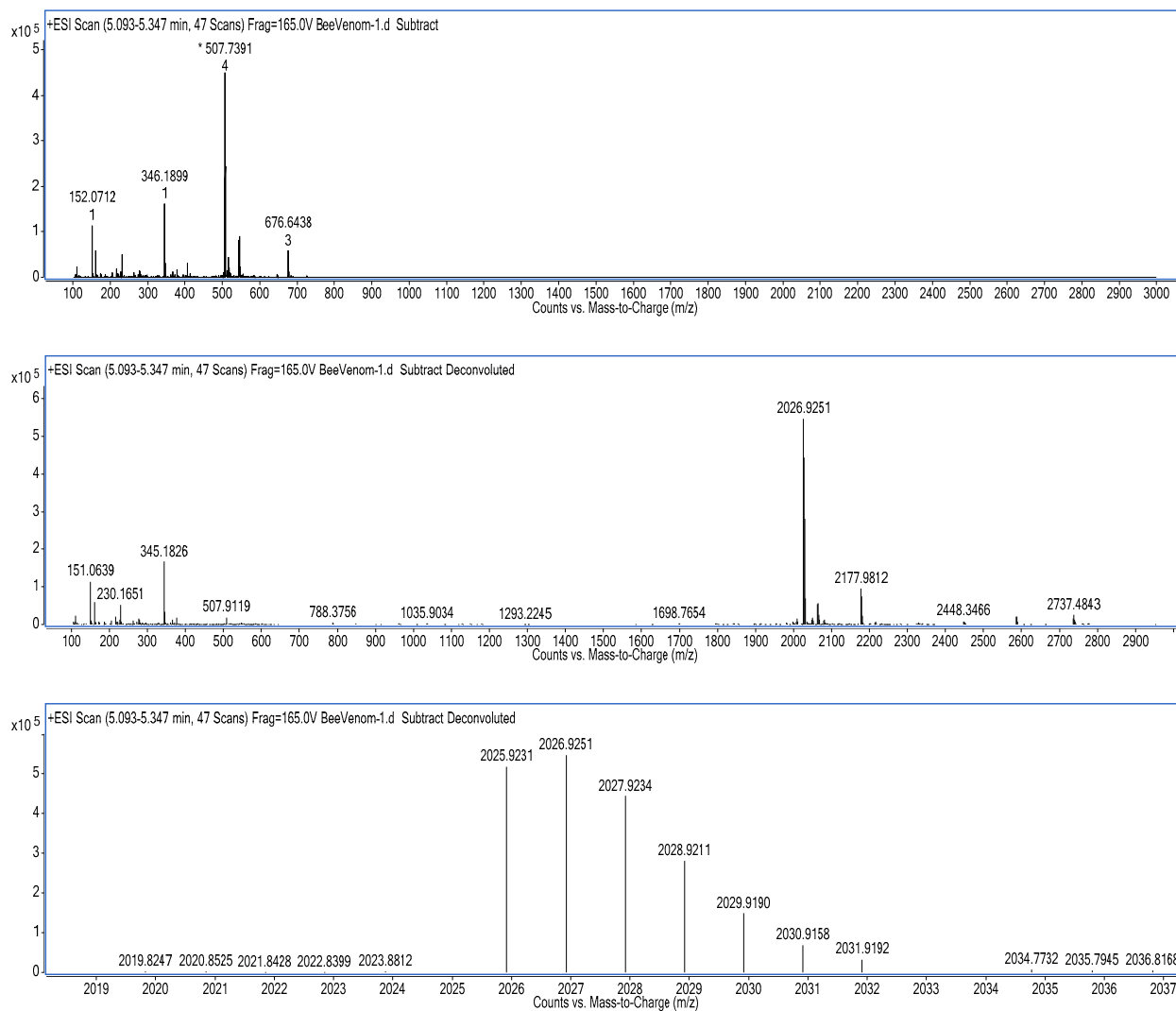


Fig. S3 LC-MS spectrum and deconvoluted spectrum of Apamin.

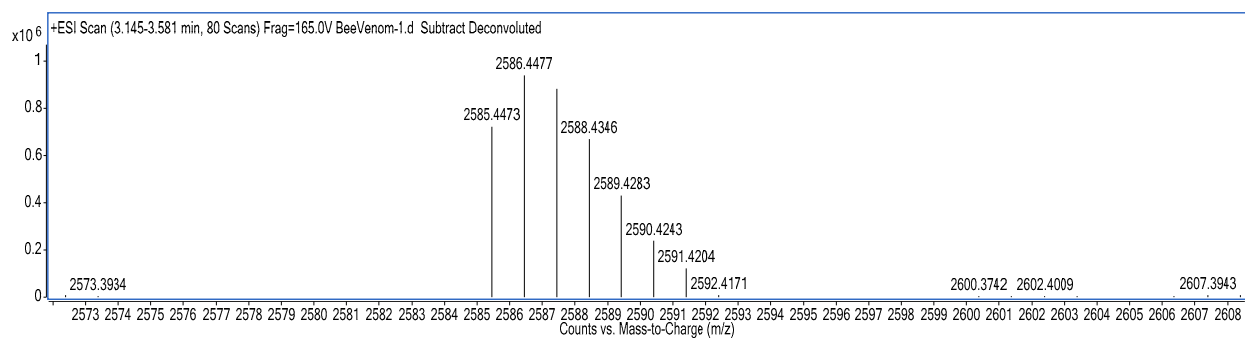
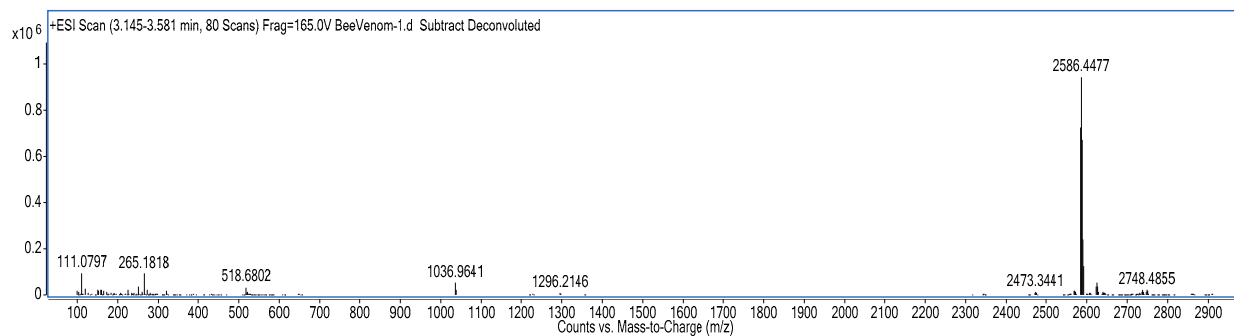
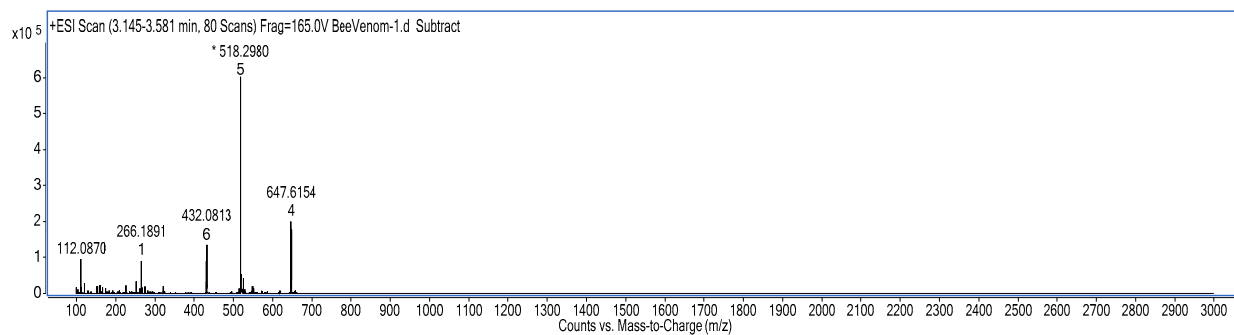


Fig. S4 LC-MS spectrum and deconvoluted spectrum of Mast Cell-Degranulating Peptide.

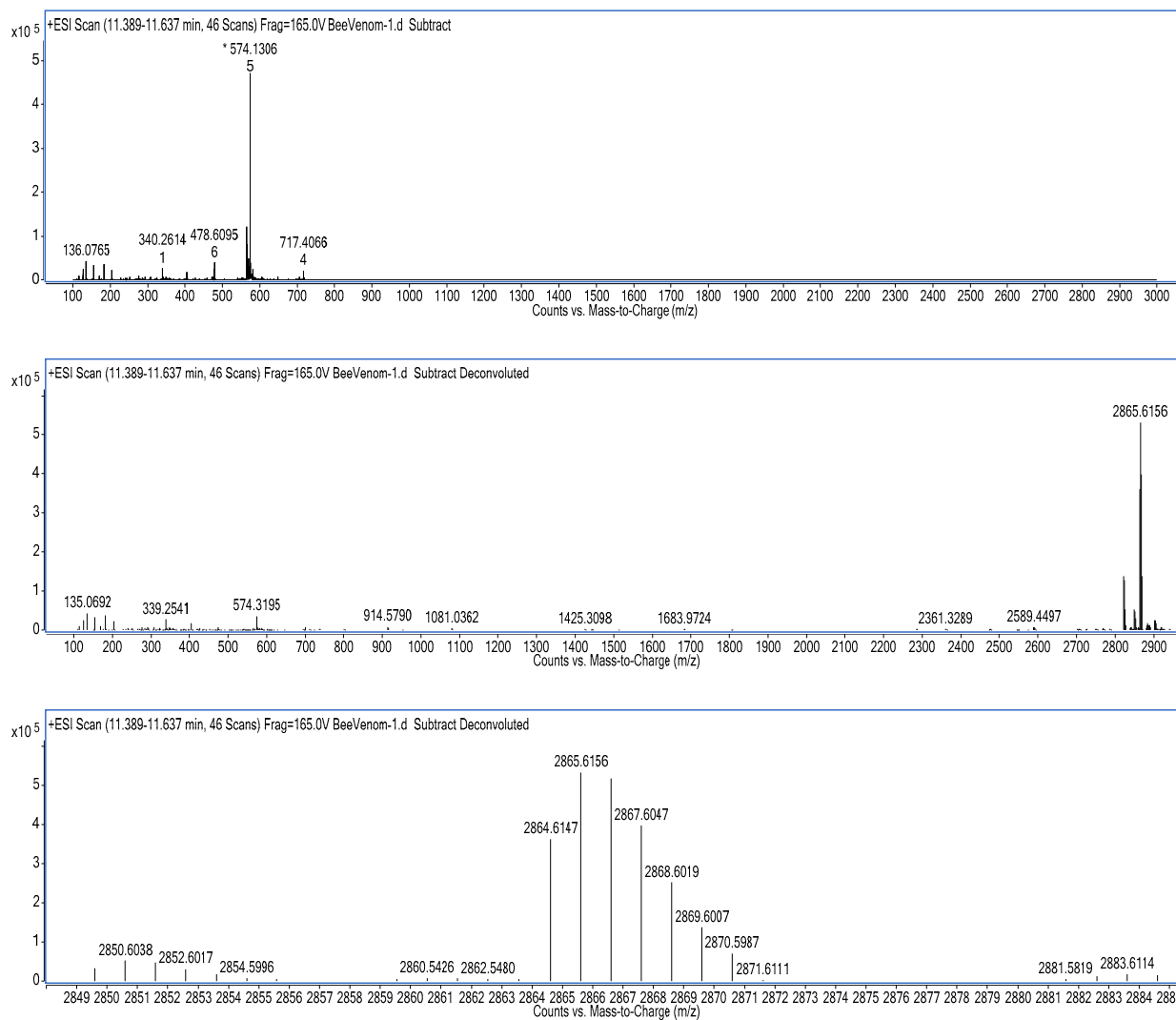


Fig. S5 LC-MS spectrum and deconvoluted spectrum of Secapin.

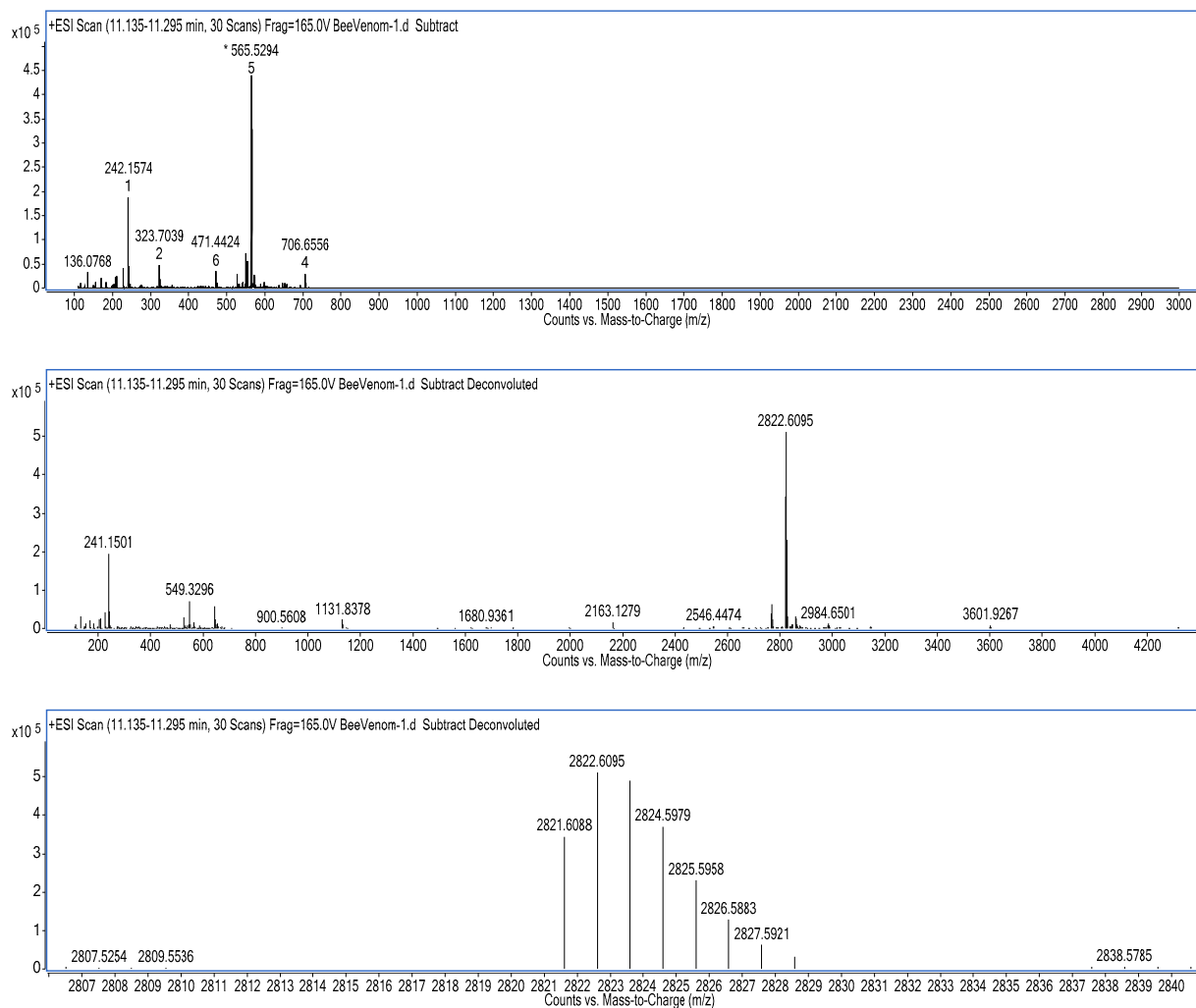


Fig. S6 LC-MS spectrum and deconvoluted spectrum of Secapin-1.

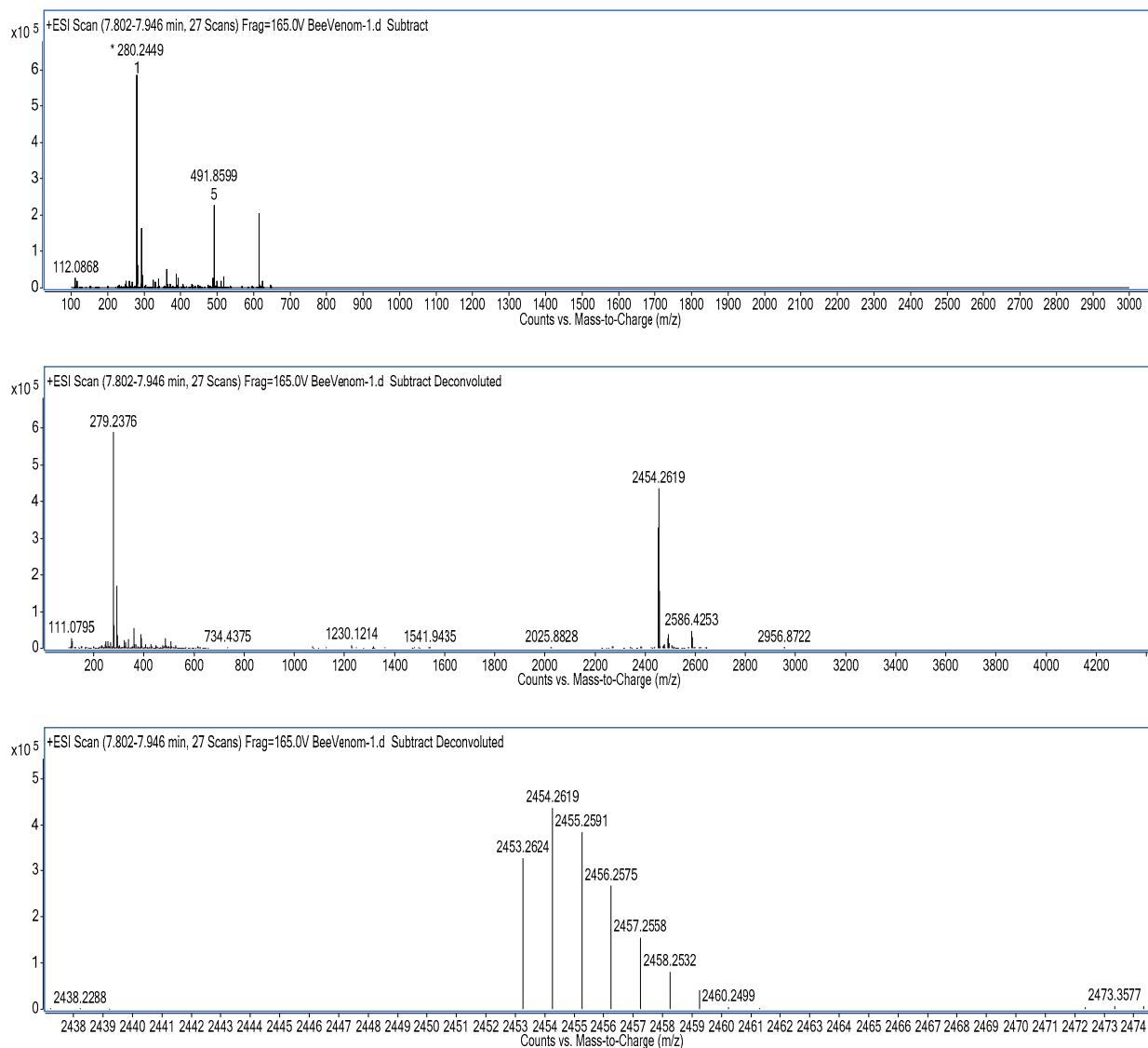


Fig. S7 LC-MS spectrum and deconvoluted spectrum of Tertiapin.

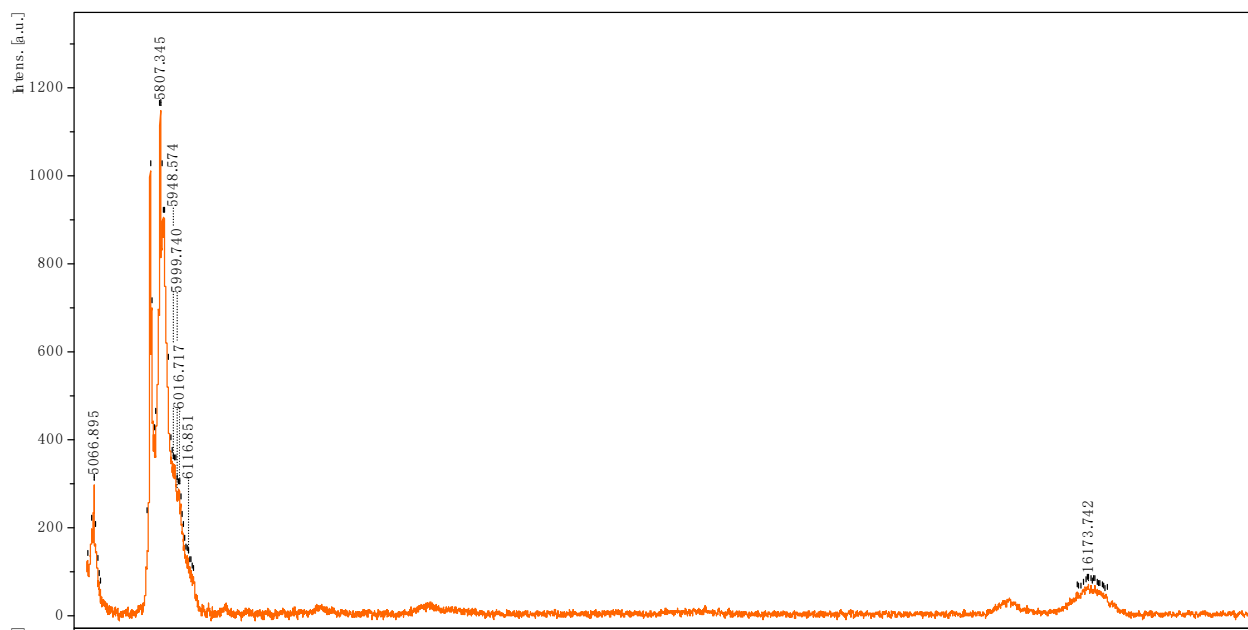


Fig. S8 MALDI-TOF spectrum of bee venom showing PLA2 enzyme mass around 16 kDa.

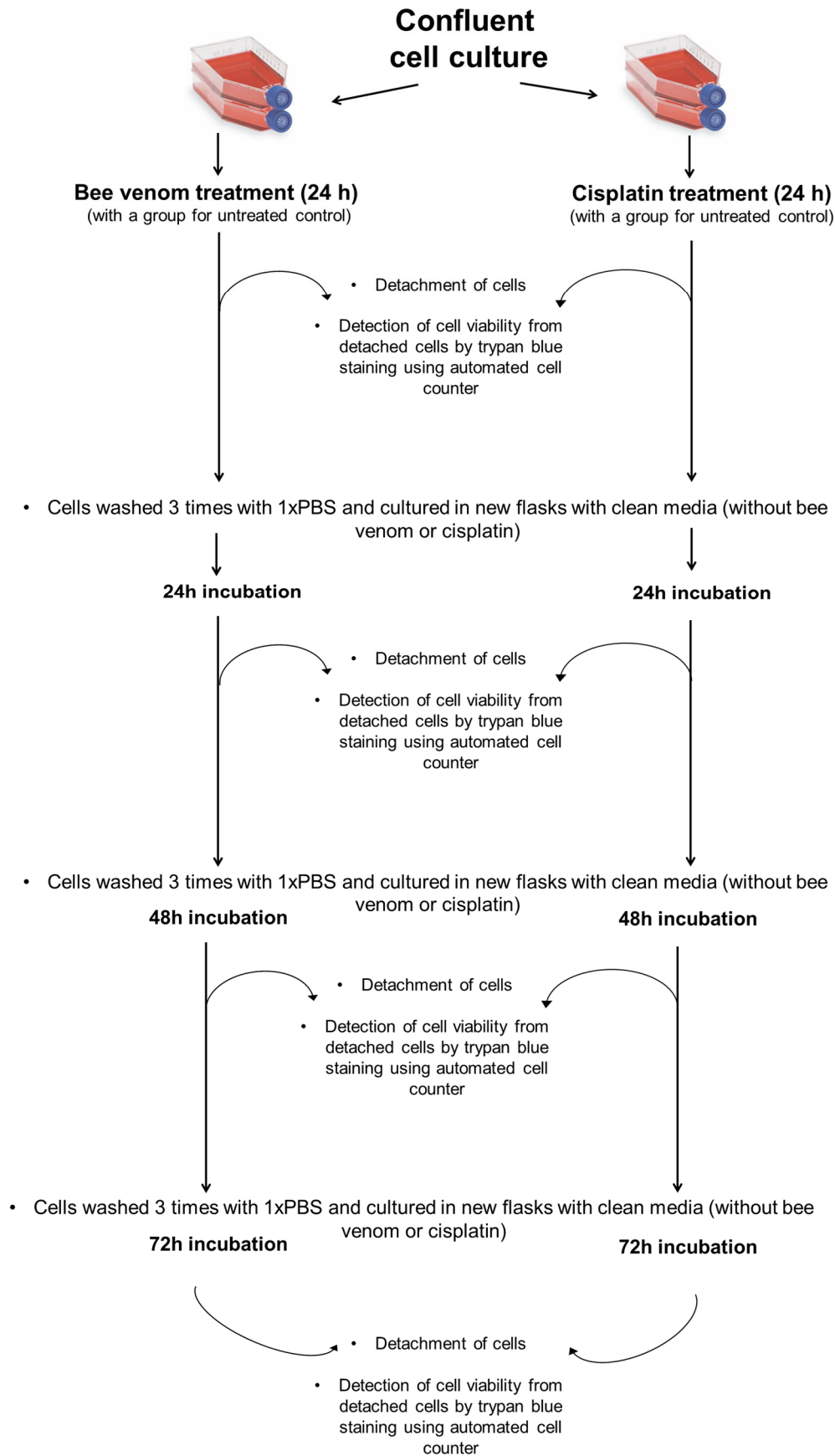


Fig. S9 Experimental plan for anastasis.