Takac et al., 2010, SUPPLEMENTAL DATA:

Protein extraction for two-dimensional electrophoresis and 2-D LC MS/MS

The homogenate was resuspended in 0.5 ml of extraction buffer (0.15 M Tris HCl pH 8.8; 0.4 M EDTA; 0.2% (v/v) 2-mercaptoethanol; 0.9 M sucrose; 25 mM KCl), thoroughly vortexed and supplemented with 0.5 ml Tris-buffered phenol (pH 8.8). After 30 min of incubation at 4°C, the extract was centrifuged at 8000g at 4°C for 5 min. The upper phenol phase was collected, and the residual proteins from aqueous phase were re-extracted using extraction buffer and Tris-buffered phenol. Following centrifugation, the phenol phases were combined, and additional extraction buffer was added to maximize the removal of the non-protein compounds. Next, the mixture was intensely mixed, centrifuged, and the phenol phase was collected for protein precipitation. The proteins were precipitated by addition of 5 volumes of 0.1 M ammonium acetate (in 100% methanol) to the phenol phase, and leaving at -20°C overnight. Subsequently, the precipitate was centrifuged at 16,000g at 4°C for 20 min. The supernatant was discarded and the pellet was washed twice with 0.1 M ammonium acetate (in 100% methanol), twice in 80% acetone, and twice in 75% ethanol, consequently. Between washing steps pellet was thoroughly resuspended and incubated at -20°C for 15 min followed by centrifugation at 12,000g at 4°C for 10 min. Finally, the pellet was air-dried for 10 min.

2-D electrophoresis

The protein (50 µg) were focused on 7 cm long IPG strips (pH range 5-8) using Protean IEF Cell (Bio-Rad) with the following conditions: 150 V for 150 VH, 500 V for 500 VH and 4000 V for 15000 VH including initial active rehydration for 12 hours at 50 V. Next, the protein strips were equilibrated in 1% (w/v) dithiothreitol (DTT) containing equilibration solution (6 M urea; 30% (v/v) glycerin; 70 mM sodium dodecyl sulfate; 0.006% (w/v) bromphenol blue) for 15 min, and subsequently 15 min in equilibration solution containing 2.5% (w/v) iodacetamide. The strips were applied onto the SDS-gel and sealed with

agarose solution (0.5% (w/v) agarose; 0.002% bromphenol blue in 1x SDS running buffer), and separated on 10% SDS-PAGE gels at 80 V.

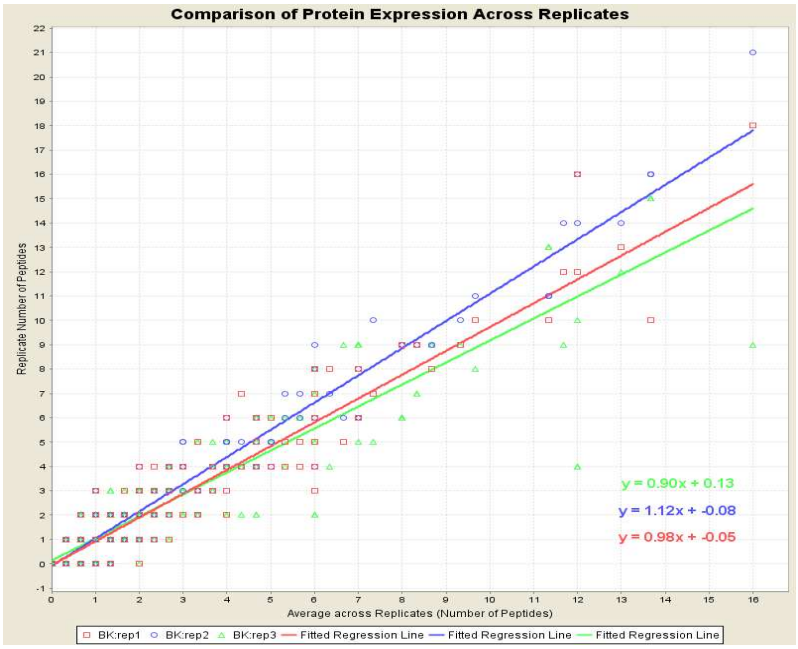
LCQ Deca Xp Plus mass spectrometer set up

Global setting: Dynamic exclusion "on", repeat count: 2, exclusion mass width low = 1.00, high = 2.50; Segment setting: Reject masses: 317.0 and 444.0, Normalized collision energy: 35%, Minimum MS signal: 10 x 10^4, Minimum MS/MS: 1 x 10^4, Isolation width (m/z): 3.0. Details of instrument setting: Spray voltage 2.7 kV, Capillary temperature 170^{o}C, AGC (automatic gain control) "on" with MSn Target 4 x 10^4. Collected spectra (RAW files) were processed by Bioworks 3.1 SR1 (ThermoFinnigan, San Jose; CA) software. The subprogram TurboSequest carried out the protein identification by matching the experimental data (masses of parent and fragmented ions/peptides) to those in the database. Search parameters as follows were employed:

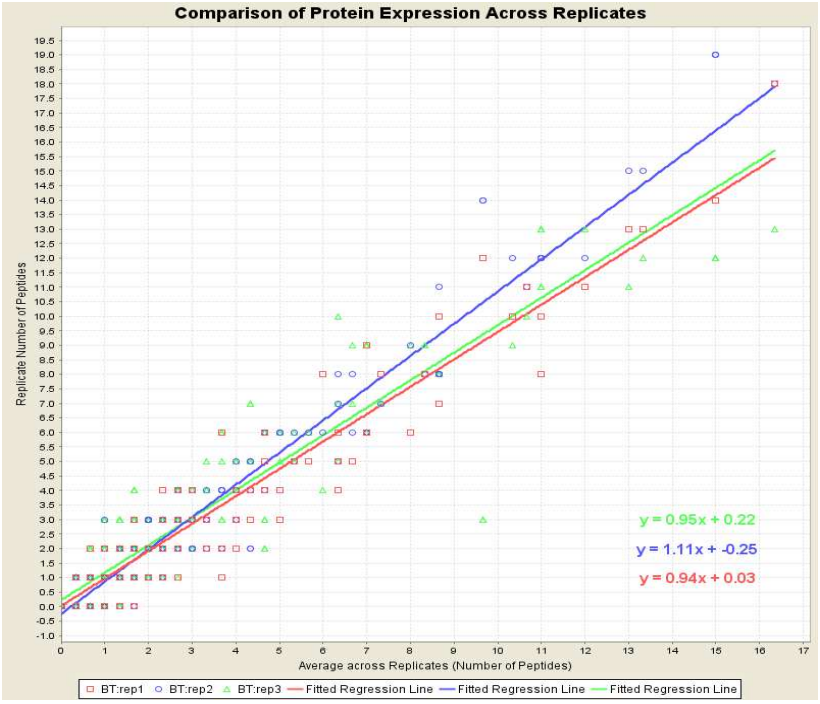
DTA Search: Trypsin with two internal cleavage sites, Precursor (□) Mass: 1.40 amu, Group Scan: 7, Minimum Group Count: 1, Minimum Ion Count: 15, Charge State: Auto, MSn level: Auto, Peptide: 2.50, Fragment Ions: 0.00, Ion Series: B and Y;

Modifications: C=57.05 (differential) for carbamidomethylation of cysteins by iodoacetamide, M= 32.0 (differential) for oxidation of methionines;Charge State Analysis: "off ".

Protein samples replicas reproducibility validations



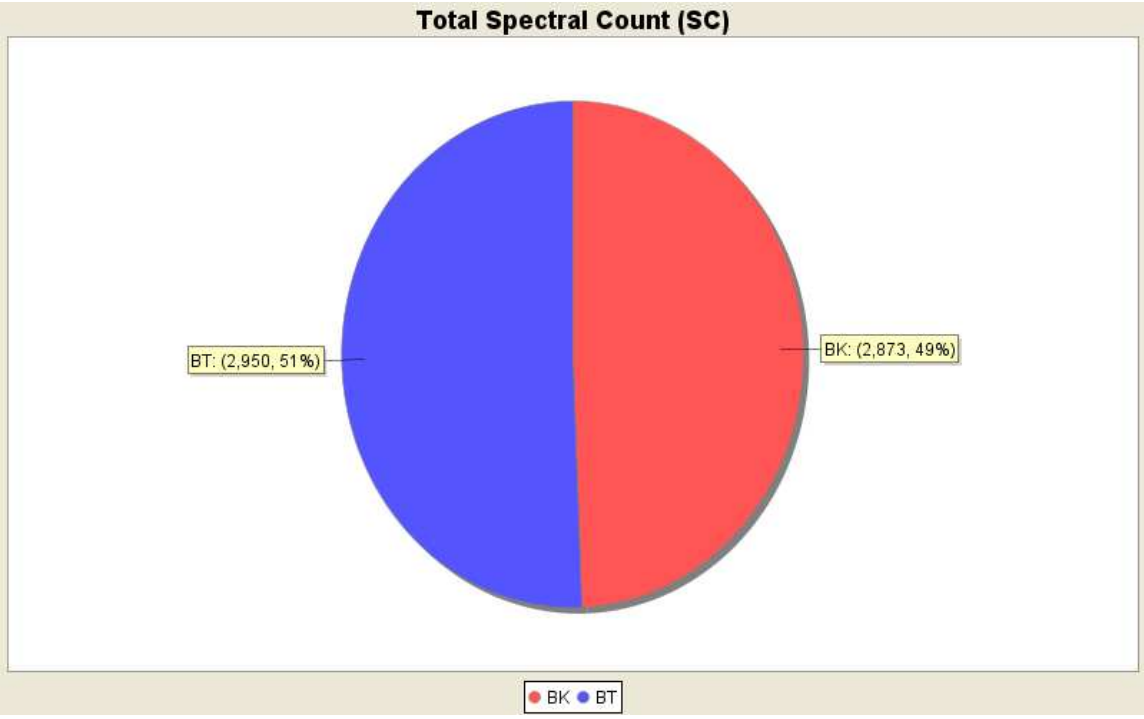
A



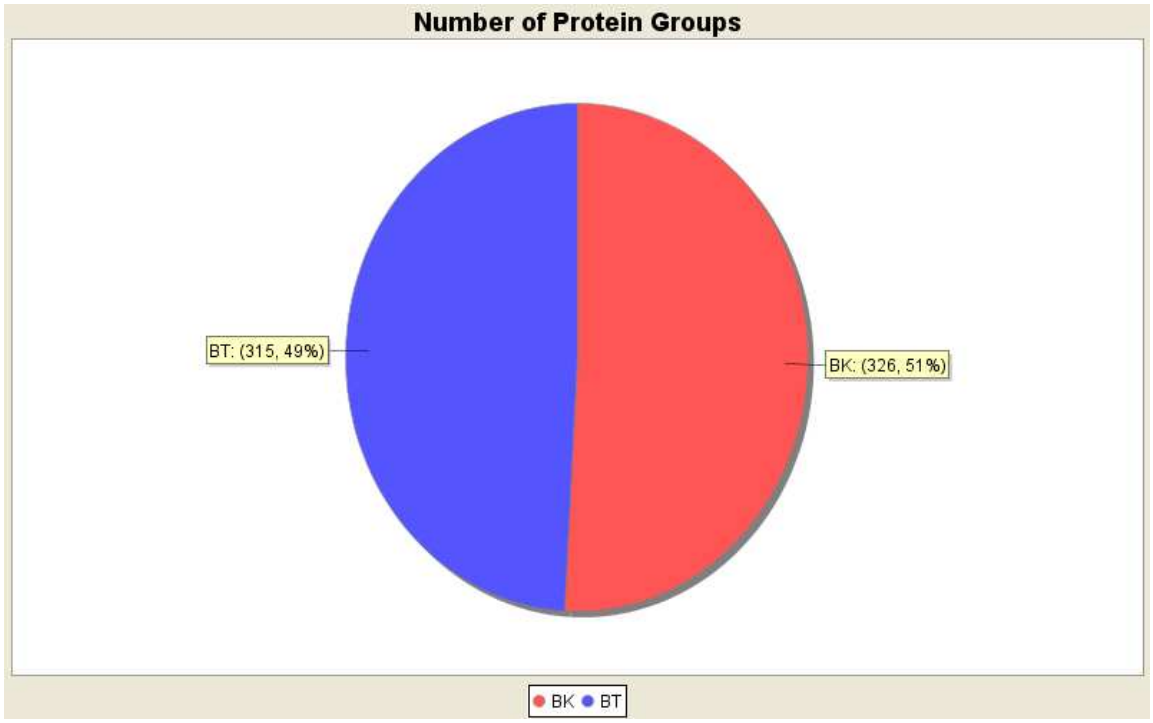
B

Figure 1/SD. Number of peptides identified in particular replicas *vs* averaged number of peptides in all replicas. **A**- control samples, **B** – BFA treatead samples.

Protein samples biological samples reproducibility validation



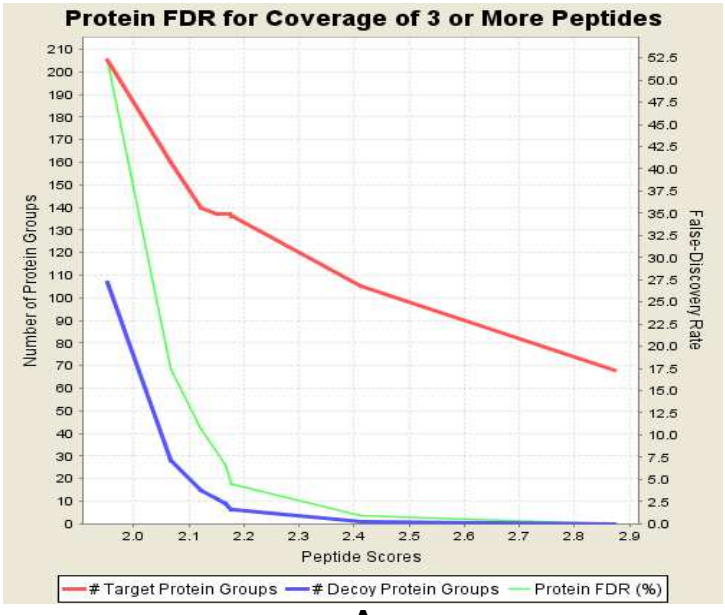
A



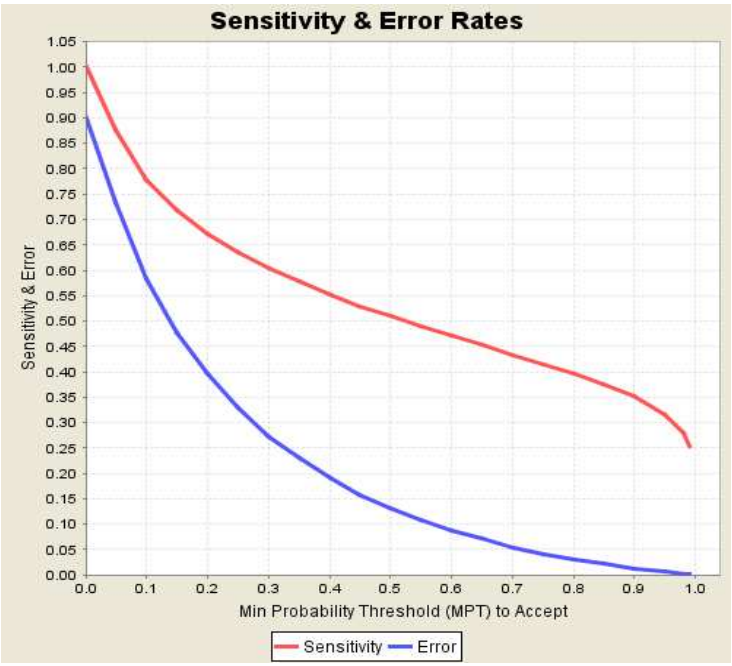
B

Figure 2/SD. Total spectral count (**A**) and number of protein groups (**B**) detected in control (BK) and BFA treated (BT) samples.

Validation of selection of false discovery rate (FDR) and probability values

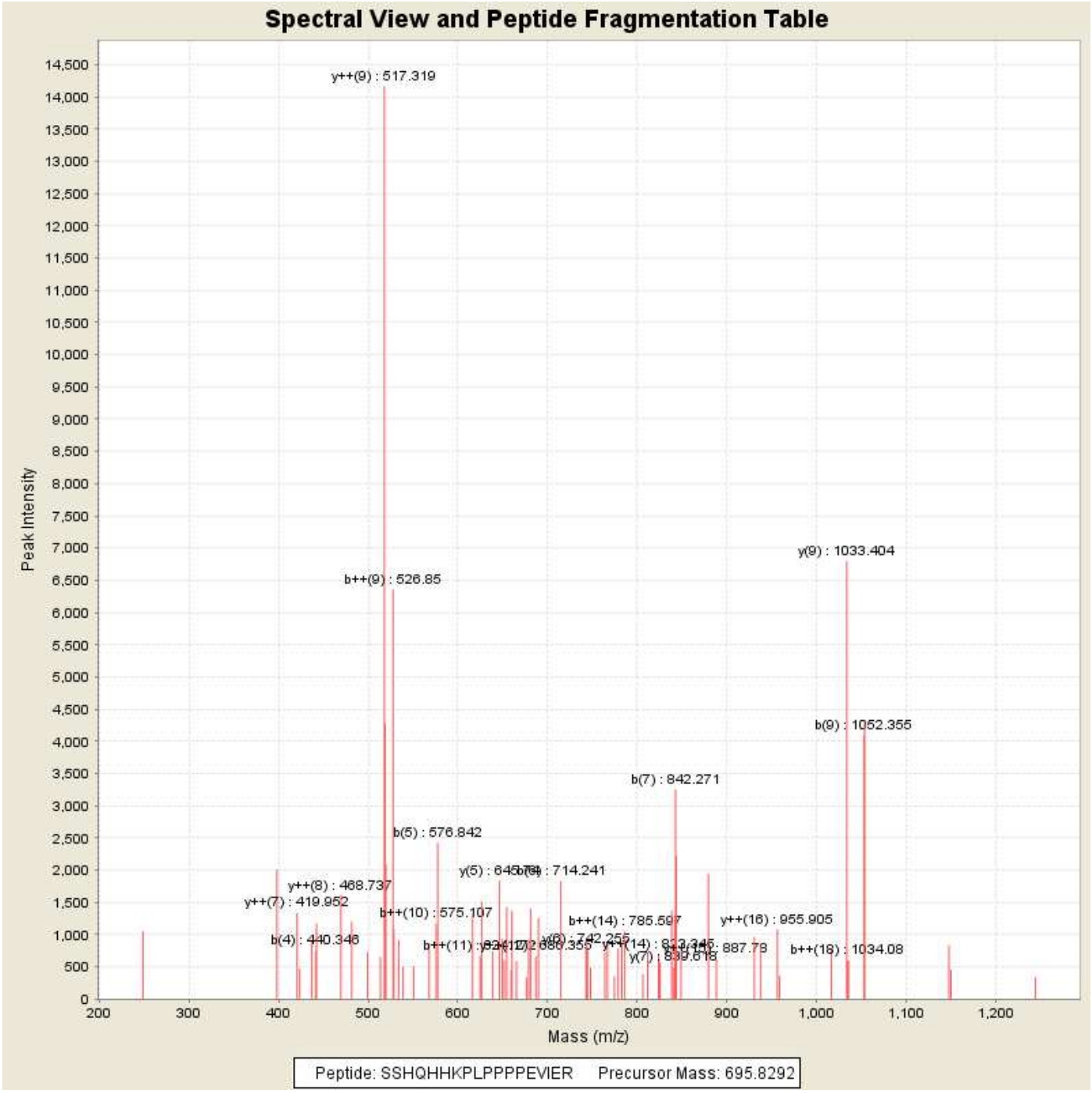


A

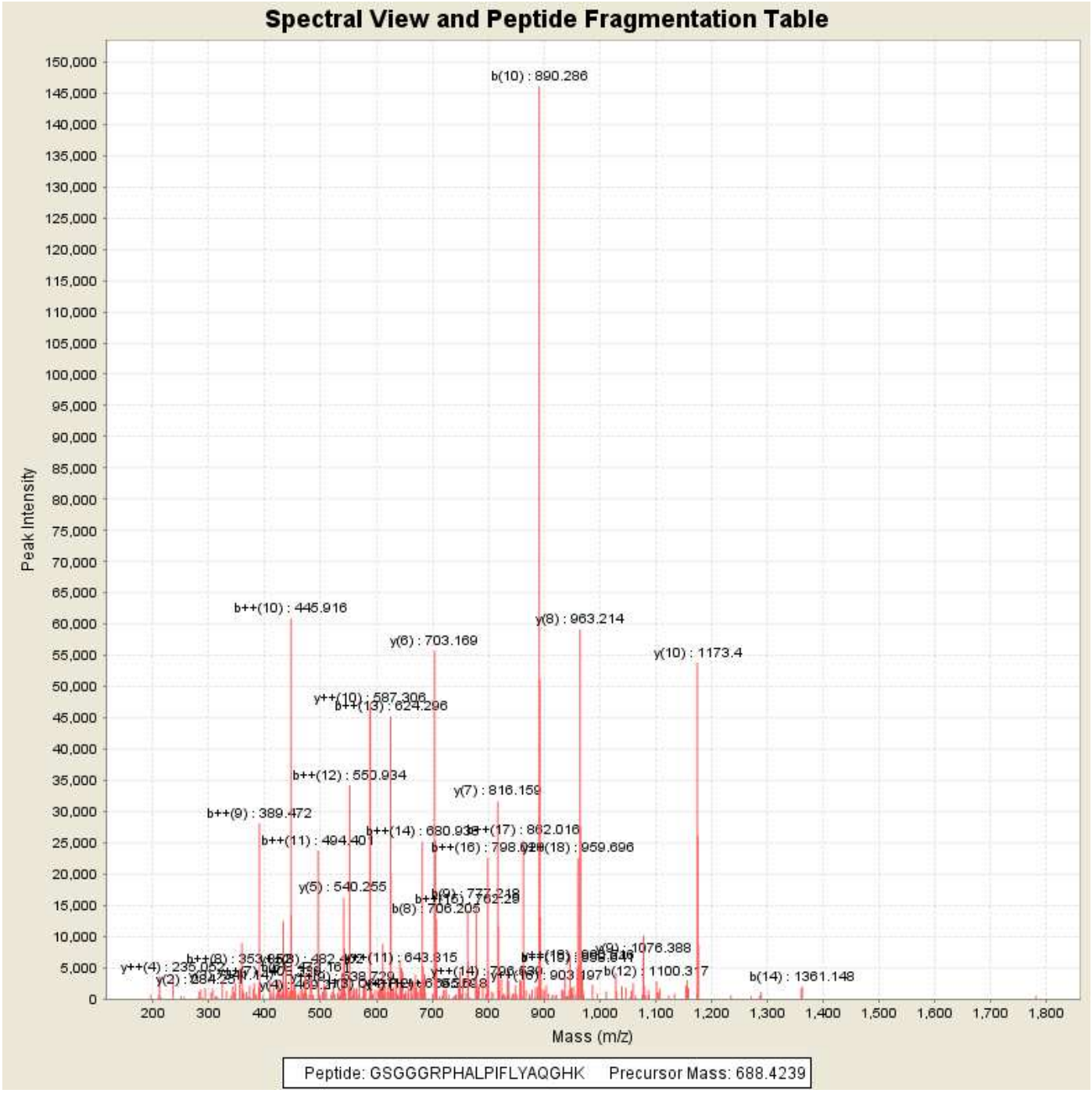


B

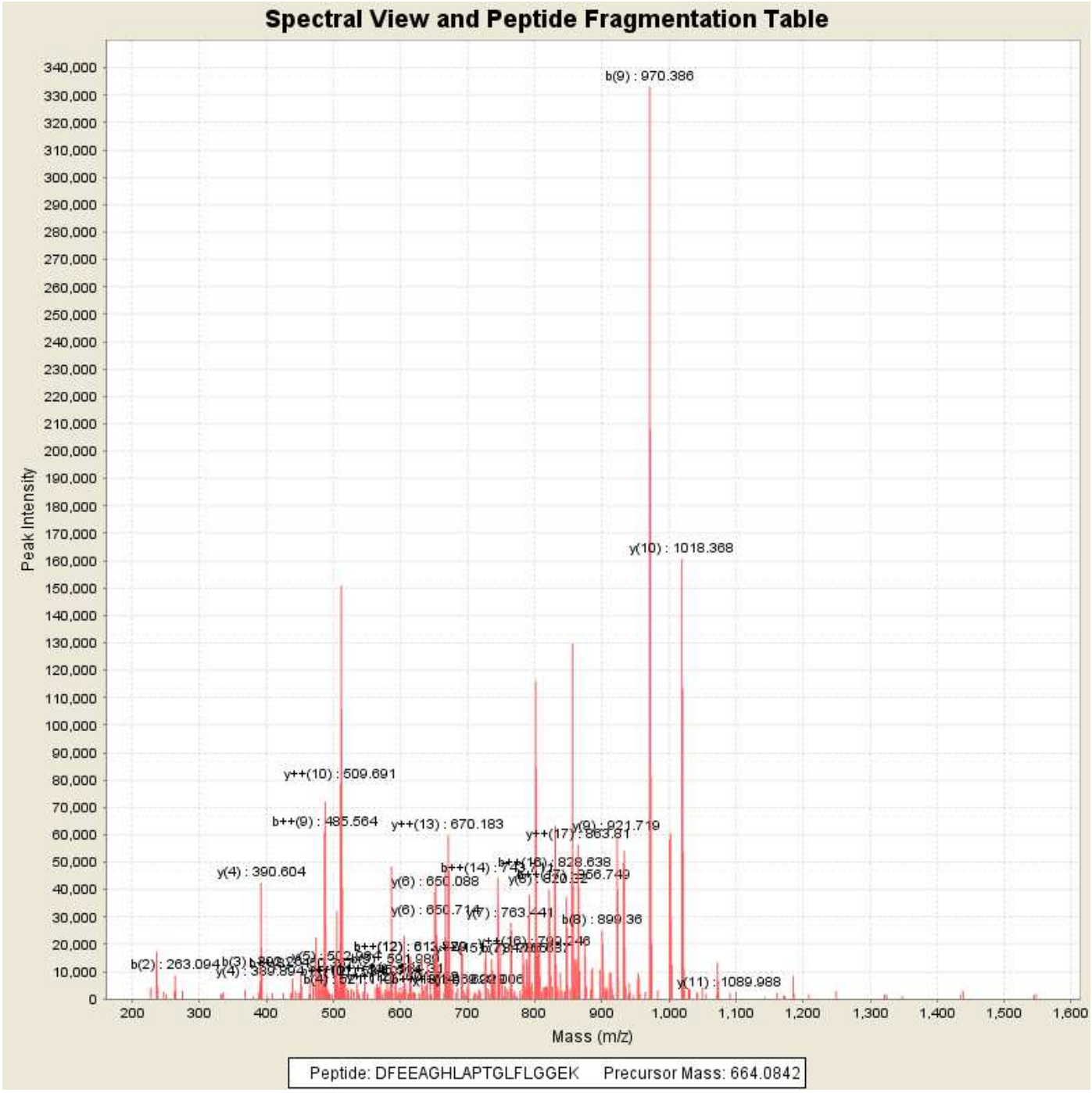
Figure 3/SD. FDR vs peptide scores (**A**), and protein probability *vs* sensitivity & error (**B**) for total sample set analyzed by ProteoIQ software (Bioinquire).


Spectral View and Peptide Fragmentation Table
Peak Intensity
Mass (m/z)
y++(9) : 517.319
y(9) : 1033.404
b++(9) : 526.85
b(9) : 1052.355
b(7) : 842.271
b(5) : 576.842
y++(8) : 468.737
y++(7) : 419.952
b++(10) : 575.107
b++(14) : 785.597
y++(16) : 955.905
b(4) : 440.346
b++(18) : 1034.08
Peptide: SSHQHHKPLPPPPEVIER Precursor Mass: 695.8292

A


Spectral View and Peptide Fragmentation Table
Peak Intensity
Mass (m/z)
b(10) : 890.286
b++(10) : 445.916
y(8) : 963.214
y(6) : 703.169
y(10) : 1173.4
y++(10) : 587.306
b++(13) : 624.296
b++(12) : 550.934
y(7) : 816.159
b++(9) : 389.472
b++(14) : 680.938
b++(17) : 862.016
b++(11) : 494.401
b++(16) : 798.021
b++(18) : 959.696
y(5) : 540.255
b(8) : 706.205
b(14) : 1361.148
Peptide: GSGGGRPHALPIFLYAQGHK Precursor Mass: 688.4239

B



C

Figure 4/SD. Annotated MSMS spectrum of peptide that were used to identify A) copine-related protein, B) meprin and TRAF homology domain-containing protein, C) PFN2 protein.