

Supplementary File 1

MudPIT and mass spectrometry analyses

The protein digest was pressure-loaded into a 250 μm i.d. capillary packed with 2.5 cm of 5 μm Partisphere strong cation exchanger (SCX) (Whatman, USA), and 2 cm of 3 μm Aqua C18 reversed phase (RP) (Phenomenex, USA), attached a packed column with 11 cm of 3 μm Aqua C18. The set was placed in line with an Agilent 1100 HPLC and analyzed using a modified 7-step MudPIT separation as previously described [14], ranging from 0 to 100% buffer C. The following buffers were used: buffer A (5% acetonitrile; 0.1% formic acid), buffer B (80% acetonitrile; 0.1% formic acid) and buffer C (500 mM ammonium acetate; 5% acetonitrile; 0.1% formic acid).

Peptides eluting from the microcapillary column were electrosprayed directly into an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, San Jose, CA) with the application of a distal 2.5 kV spray voltage. A cycle consisted of one full scan of the mass range (MS) (400–2000 m/z, resolution of 60 000) followed by five data-dependent collision-induced dissociation (CID) MS/MS spectra in the LTQ. Dynamic exclusion was enabled with a repeat count of 1, a repeat duration of 30 s, an exclusion list size of 150, and exclusion duration of 180 s. Also, the mass window for precursor ion selection was set to 400–1600, unassigned, and charge 1 was rejected, and the normalized collision energy for CID was 35. Mass spectrometer scan functions and HPLC solvent gradients were controlled through the XCalibur data system. Protein identification and quantification analyses were done using the Integrated Proteomics Pipeline (IP2, Integrated Proteomics Applications, Inc., www.integratedproteomics.com). The search was made using ProLuCID algorithm [15] against human and Zika reviewed proteins list from UniProt (downloaded in August 22nd, 2017). ProLuCID results were assembled and filtered using the DTASelect program [16] resulting in a data set with a false discovery rate of 1% for protein. Also, the search was made using the following parameters: the protein must contain at least 1 peptide (minimum of 6 amino acids) and 1 tryptic end per peptide, the subset proteins were included, and the precursor delta mass cutoff in ppm was set to 10.