Supplementary material

Appendix S1. Sample preparation for LC-MS/MS analysis, setup of the LC-MS/MS analysis, and the list of identified proteins in four samples of two *S. multipunctatus* individuals, with protein of known toxic function listed as subsample.

Sample preparation for LC-MS/MS analysis

The samples of AXG secretion were mixed with 8M urea in 100 mM Tris-HCl and 0.1M DTT. This mixture was then loaded onto a Microcon device equipped with a 10 kDa cut-off filter (Merck Millipore) and centrifuged at 7,000 × g for 30 minutes at 20 °C. The retained proteins were washed (with all subsequent centrifugation steps conducted at 14,000 × g) using 200 μL of 8M urea in 100 mM Tris-HCl and 0.1M DTT. The final protein concentrates were mixed with 100 μL of 8M urea in 100 mM Tris-HCl containing 50 mM iodoacetamide and incubated in the dark for 20 minutes. Following this incubation, the samples were washed three times with 100 μL of 8M urea in 100 mM Tris-HCl buffer and three times with 100 μL of 50 mM NH_4HCO_3. Next, 500 ng of trypsin (SOLu-Trypsin, Sigma-Aldrich) was added to the filter, and the mixture was incubated at 37 °C for 18 hours. The tryptic peptides were then eluted by centrifugation, followed by two additional elutions with 50 μL of 50 mM NH_4HCO_3. Finally, the peptides were extracted into LC-MS vials using 2.5% formic acid (FA) in 50% acetonitrile (ACN) and then by 100% ACN, with the addition of polyethylene glycol (20,000, final concentration of 0.001%). The samples were then concentrated using a SpeedVac concentrator (Thermo Fisher Scientific) prior to LC-MS analysis.

LC-MS/MS analysis setup

The peptides were separated using an Aurora C18 column (3rd generation, 25 cm long, 75 μm inner diameter, 1.7 μm particles) from IonOpticks, following a 60-minute gradient program with a flow rate of 150 nL/min using an UltiMate 3000 RSLCnano system (Thermo Fisher Scientific). The mobile phase B was linearly increasing from 3% to 42%, while mobile phase A consisted of 0.1% formic acid (FA) in water, and mobile phase B was composed of 0.1% FA in 80% acetonitrile. After the separation, a wash step with 80% mobile phase B was performed. The analytical column was maintained inside a Column Toaster at 50 °C (Bruker), and the emitter side was connected to the CaptiveSpray ion source (Bruker) of a timsTOF Pro spectrometer (Bruker Daltomics). MS/MS data were acquired in *m*/*z* range of 100-1,700 and 1/k0 range of 0.6-1.4 V × s × cm^{-2} using DDA-PASEF method acquiring 10 PASEF scans with scheduled target intensity of 20,000 and intensity threshold of 2,500. Active exclusion was set for 0.4 min with precursor reconsideration for 4× more intense precursors.