SUPPLEMENTARY MATERIAL

Appendix 1: Data S1

Peptides resulting from in-solution digestion were loaded first onto an Acclaim PepMap 100 Trapping column (Thermo Scientific, 20mm x 75 µm ID, 3 µm C18 resin with 100 Å pore size) using buffer A (2% acetonitrile, 0.1% formic acid) and then were separated and eluted on a C18 resin NTCC analytical column (NikkyoTechnos Co., Ltd., 150mmx75µm ID, 3 µm C18 resin with 100 Å pore size) using a 150 minute gradient of 5% to 35% Buffer B (100% acetonitrile, 0.1% formic acid) in Buffer A at constant flow rate of 250 nl/min.

Data acquisition was performed with a Q-Exactive HF. Data were acquired using an ion spray voltage 1.8 Kv and ion transfer temperature of 250ºC. All data were acquired using data-dependent acquisition (DDA) and in positive mode with Xcalibur 4.0 software. For the MS2 scan, top 17 most abundant precursors with charges of 2–6 in MS1 scans were selected for higher-energy collisional dissociation (HCD) fragmentation with a dynamic exclusion of 20 s. The MS1 scans were acquired at a m/z range of 350–2000Da with a mass resolution of 60,000 and an automatic gain control (AGC) target of 3E6 at a maximum Ion Time (ITmax) of 60 ms. The threshold to trigger MS2 scans was 2E3; the normalized collision energy (NCE) was 27%; the resolved fragments were scanned at a mass resolution of 30,000 and an AGC target value of 1E5 with anITmax of 80ms.

Appendix 2: Table S1. Identification by LC-MS of the raw venom of *A. mellifera*

IUIS	Accession number	Description	Score Mascot	Coverage [%]	PSM/MW	% Total PSM/MW	Grouping % Total PSM/MW
Api m 1	NP_001011614.1	Phospholipase A2 precursor	5294	70	28.579	26.26	30.00
	XP_001120293.1	Phospholipase A2-like	384	48	2.634	2.42	
	XP_012142828.1	Phospholipase A2-like	468	5	1.371	1.26	
	XP_006616412.1	Phospholipase A2-like	0	8	0.065	0.06	
Api m 2	XP_016771467.1	Hyaluronidase isoform X1	538	29	1.496	1.37	1.37
Api m 3	XP_006569959.1	Venom acid phosphatase Acph-1-like	39	7	0.047	0.04	0.17
	XP_017792604.1	Venom acid phosphatase Acph-1-like isoform X1	42	3	0.134	0.12	
Api m 4	XP_016916903.1	Melittin, partial	1705	15	35.364	32.49	35.34
	AMP81999.1	Prepromelittin, partial	60	81	3.103	2.85	
Api m 5	XP_006614167.1	Venom dipeptidyl peptidase 4-like, partial	353	12	0.664	0.61	1.47
	NP_001119715.1	Venom dipeptidyl peptidase 4 precursor	363	41	0.933	0.86	
Api m 6	P83563.1	Allergen Api m 6	372	62	4.211	3.87	3.87
Api m 7	NP_001011584.1	Venom serine protease 34 precursor	362	50	2.374	2.18	2.41
	XP_392669.1	Venom serine protease 34	49	21	0.247	0.23	
Api m 8	NP_001119716.1	venom carboxylesterase-6 precursor	110	25	0.252	0.23	0.23
Api m 9	XP_016771167.1	Venom serine carboxypeptidase isoform X1	153	37	0.372	0.34	0.63
	NP_001152775.1	Venom serine carboxypeptidase precursor	143	27	0.317	0.29	
Api m 10	NP_001012431.1	Icarapin-like precursor	237	22	1.290	1.19	1.26
	XP_003704678.2	Icarapin-like	0	5	0.078	0.07	
Api m 11	XP_006558987.1	Major royal jelly protein 8 isoform X1	243	38	0.821	0.75	1.50
	ACD84799.1	Major royal jelly protein 8	243	39	0.810	0.74	
Other proteins					23.685	21.76	21:76

IUIS: International Union of Immunological Societies; PSM/MW: peptide-spectrum match

normalized by molecular weight of the protein