

Figure 1: A: Protein sequence of vitellogenin

(https://www.ncbi.nlm.nih.gov/nuccore/NM_001011578, accession number:

NM_001011578). Position of the two peptides used for immunization is marked in violet. B:

Dot blot analysis: hemolymph, fat body and brain of adult honey bees were processed as described and 1 μ L was spotted on a nitrocellulose membrane, following by incubation with vitellogenin antibody. Proteins were detected by IR-fluorescence.

Figure 2: Expression of vitellogenin in different body tissues of honey bees processed as described. (A) Vitellogenin in fat body (FS), honey stomach (HS), hemolymph (HL). Shown are all cross-reactive bands. (B) Vitellogenin in brain samples. Equal amounts of lysates (20 μ g) were loaded in each lane. Numbers to the left give molecular weights in kilodaltons as given by the protein ladder M (L2020 UPBBio). Full-length vitellogenin is represented by the band below 180 kDa, lighter vitellogenin is presented by the band below 140 kDa (both marked with arrows), possible degradation product of vitellogenin visible at 75 kDa, * marks uncharacterized *Apis mellifera* protein, which is detected by the anti-vitellogenin antibody and used as loading control. Proteins were detected by IR-fluorescence. C: Analysis of protein bands in hemolymph by mass spectroscopy. Data shown of the proteins, which were detected by the vitellogenin antibody.

Figure 3: Expression of vitellogenin in different body tissues of control bees and bees exposed to clothianidin. Hemolymph (A, n=4), brain (B, n=3) and fat body (D, n=4) of control honey bees (solvent control) and honey bees exposed to 3 ng/bee clothianidin for 24 h were processed as described. Equal amounts of lysates (20 μ g) were loaded in each lane. Numbers to the left give molecular weights in kilodaltons as given by the protein ladder M (L2020 UPBBio). Full-length vitellogenin is the band below 180 kDa, processed vitellogenin is the band below 135 kDa (both marked with arrows), * marks uncharacterized *Apis mellifera* protein, which is detected by the anti-vitellogenin antibody and used as loading control. Proteins were

detected by IR-fluorescence. The intensity of the fluorescent signals was quantified using Image J software of full length and lighter vitellogenin in brain sample (C) and of full length vitellogenin in hemolymph sample (E).