- Rutz W, Lüscher M. 1974. The occurrence of vitellogenin in workers and queens of Apis mellifica and the possibility of its transmission to the queen. J Insect. Physiol. 20 (5): 897-909.
- Seehuus SC, Norberg K, Gimsa U, Krekling T, Amdam GV. 2006. Reproductive protein protects functionally sterile honey bee workers from oxidative stress. Proc. Natl. Acad. Sci. USA 103: 962-967.
- Seehuus SC, Norberg K, Krekling T, Fondrk K, Amdam GV. 2007. Immunogold localization of vitellogenin in the ovaries, hypopharyngeal glands and head fat bodies of honeybee workers, *Apis mellifera*. J. Insect. Sci. 7: 1-14.
- Shevchenko A, Tomas H, Havli J, Olsen JV. Mann M. 2006. In-gel digestion for mass spectrometric characterization of proteins and proteomes. Nature. protocols, 1(6): 2856.
- Spieth J, Nettleton M, Zuckeraprison E, Lea K, Blumenthal, T. 1991. Vitellogenin motifs conserved in nematodes and vertebrates. J.Mol. Evol. 32: 429–438.
- Snodgrass RE. 1956. Anatomy of the Honey Bee. Cornell. University. Press.
- Tufail M, Takeda M. 2005. Molecular cloning, characterisation and regulation of the cockroach vitellogenin receptor during oogenesis. Insect. Molecul. Biol. 14 (4): 389-401.
- Tufail M, Takeda M. 2007. Molecular cloning and developmental expression pattern of the vitellogenin receptor from the cockroach *Leucophaea maderae*, Insect. Biochem. Molecul. Biol. 37: 235-245.
- Wheeler DE, Kawooya JK. 1990. Purification and characterization of honey bee vitellogenin.

  Arch. Insect. Biochem. Physiol. 14 (4): 253-267.
- Winston ML, Fergusson LA. 1985. The effect of worker loss on temporal caste structure in colonies of the honeybee (*Apis mellifera L.*). Canadian. J. Zoology. 63 (4): 777-780. Winston ML. 1987. The biology of the honey bee. Cambridge: Harvard University Press.

**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  $\mu$ 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 μg) 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 μg) were loaded in each lane. Numbers to the left give molecular weights in kilodaltons as given by the protein ladder M (L2020 UP-BBio). 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

This article is protected by copyright. All rights reserved