

the tip of the last body segment, the bee was slightly stretched and cut open on one side of the abdomen, using fine-tipped scissors. The sternum was again fixated with needles, all unwanted body tissue was carefully removed before collecting fat body tissue layer, lining the tergum wall. The tissue was collected into ice-cold 2x concentrated lysis buffer (100 mM Tris pH 7.4, 300 mM NaCl, 30 mM EDTA, 0.2% Triton, Duong et al. (2004)) with addition of protease inhibitor cocktail (Roche cOmplete 04693124001). The samples were stored on ice during collection. For tissue disruption the collected fat bodies were ground using a small glass bar as pestle before lysing on ice for 30 min with two intermediate vortexing steps. Lysis was finally enhanced by sonication on ice by 1-3 cycles with 10 second bursts with 30 second cooling intervals (amplitude 100%, cycle 1 in a Branson Sonifier 450 W). Cellular debris was removed by centrifugation at 13000 rpm at 4°C for 10 min. The supernatant was carefully collected and stored at -20°C until further analyses. Protein concentrations were determined using a bicinchoninic acid (BCA) protein assay kit (Pierce™ BCA Protein Assay Kit, Thermo Fisher) according to the manufacturer's protocol.

Honey stomach collection

To obtain honey stomach, the frozen bee was briefly thawed at room temperature. The thorax was cut off and the abdomen fixated between thumb and forefinger. Carefully, the honey stomach was removed from the front part of the abdomen using forceps. The tissue was collected into ice-cold 2x concentrated lysis buffer (100 mM Tris pH 7.4, 300 mM NaCl, 30 mM EDTA, 0.2% Triton, Duong et al. (2004)) with addition of protease inhibitor cocktail (Roche cOmplete 04693124001). The samples were stored on ice during collection. For tissue disruption, the collected honey stomach were ground using a small glass bar as pestle before lysing on ice for 30 min with two intermediate vortexing steps. Lysis was finally enhanced by sonication on ice by 1-3 cycles with 10 second bursts with 30 second cooling intervals (amplitude 100%, cycle 1 in a Branson Sonifier 450 W). Cellular debris was removed by centrifugation at

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Brain collection

The brain of frozen bees was removed in total by opening the cranium using a scalpel and forceps, as described before (Christen et al. 2016). The tissue was collected into ice-cold 2x concentrated lysis buffer (100 mM Tris pH 7.4, 300 mM NaCl, 30 mM EDTA, 0.2% Triton; Duong et al. 2004)) with addition of protease inhibitor cocktail (Roche cOmplete 04693124001). The samples were stored on ice during collection. For tissue disruption the collected brains were ground using a small glass bar as pestle before lysing on ice for 30 min with two intermediate vortexing steps. Lysis was finally enhanced by sonication on ice by 1-3 cycles with 10 second bursts with 30 second cooling intervals (amplitude 100%, cycle 1 in a Branson Sonifier 450 W). Cellular debris was removed by centrifugation at 13000 rpm at 4°C for 10 min. The supernatant was carefully collected and stored at –20°C until further analyses. Protein concentrations were determined using a bicinchoninic acid (BCA) protein assay kit (Pierce™ BCA Protein Assay Kit, Thermo Fisher) according to the manufacturer's protocol.

Analysis of antibody binding by dot blot

Dot blot analysis was run according to the protocols on the Licor home page (https://www.licor.com/bio/applications/quantitative_western_blot/protocol.htm) with slight modifications. BSA (1 µL of 2 mg/mL BSA in PBS) as negative control, secondary antibody solution (IRDye® 800CW Goat anti-Rabbit IgG, 1:500 in Licor blocking buffer diluted 1:1 with PBS-T) as positive control, hemolymph, fat body and brain lysate were spotted separately on a nitrocellulose membrane (Amersham™ Protran® Western blotting membranes, This article is protected by copyright. All rights reserved