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.

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_blots/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,

0.45 µm). After drying, the membrane was incubated in Licor Blocking buffer (927-40100) for 1h at room temperature with agitation. After addition of 0.2% Tween-20, the membrane was incubated with the polyclonal antibody against vitellogenin in a 1:7000 dilution (Davids Biotechnologie Regensburg) for 1 h at room temperature with agitation. After three washes with PBS-T (0.2% Tween 20) the membrane was incubated for 45 min at room temperature in secondary antibody solution (IRDye® 800CW Goat anti-Rabbit IgG (H + L),1:40.000 in Licor blocking buffer diluted 1:1 with PBS-T). After 3 washes with PBS-T (0.2% Tween 20) the vitellogenin specific signals were acquired at 800 nm using the Licor Odyssey® CLx Imaging System.

SDS Polyacrylamide gel electrophoresis

SDS polyacrylamide gel electrophoresis was run according to the protocols on the Licor home page (https://www.licor.com/bio/applications/quantitative_western_blots/protocol.htm) with slight modifications. In brief, one-dimensional gel electrophoresis was carried out in vertical polyacrylamide gels (10.1 x 7.3 x 0.1 cm) containing 0.1% SDS with a 4% stacking gel on top of the separating gel. Samples were diluted with 4x concentrated Orange G sample buffer for Licor blots (250 mM Tris-HCl, pH 6.8, 12% SDS, 50% Glycerol, 6% 2-mercaptoethanol, 0.2% Orange G), heated for 5 min at 95°C and subjected to electrophoresis at constant voltage (140 V) for 20 min and 190 V until the dye front has run out. Two types of one-dimensional gels were run (10% and 7.5% polyacrylamide/ 0.1% SDS gels) for the separation of proteins in the range of 30–200 kDa, with the later found to be superior for separation of the two vitellogenin forms.

Protein extraction and digestion

The extracted proteins were resolved on one‐dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using a pre-cast gradient gel (4 - 20% polyacrylamide,