The colony was infested with *Varroa destructor* and hence treated with formic acid in summer 2016 and oxalic acid in winter 2016. The experimental procedure was identical as described previously (Christen et al. 2016). Ten bees were placed in one PET bottle. Bees were fed either with sucrose solution containing 0.1% DMSO (solvent control, four bottles) or with 3 ng/bee clothianidin (four bottles) for 24 h. The selection of clothianidin concentration and exposure time is based on our previous study where the strongest induction of *vitellogenin* transcript was found after 24 h exposure to 3 ng/bee clothianidin (Christen et al. 2016). Per bottle, 3 bees were pooled to one hemolymph, brain, and fat body sample.

Hemolymph collection

Hemolymph collection was done according to Rutz and Lüscher (1974) and Randolt et al., (2008) with some slide modifications. In brief, hemolymph from frozen unexposed and exposed bees was collected from the dorsal part of the bees by using a sterile 10 µL micro tip. Individual bees were fixated between a pair of tweezers and the intersegmental membrane was slit slightly with the micro tip between the fourth and fifth tergite of the honeybee abdomen. Emerging clear hemolymph was collected with the micro tip which had been pre-wetted with PTU (N-Phenylthiourea, Sigma P7629) and protease inhibitor cocktail solution (Roche complete 04693124001) to prevent immediate melanisation and protein degradation. Turbid hemolymph was discarded. The hemolymph (5–10 µL per bee) was transferred into an Eppendorf tube containing an ice-cold mixture of 10% PTU and protease inhibitor cocktail. The samples were stored on ice during collection. The volume was adjusted to 60 - 80 µL with ice- cold PBS pH 7.4 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.

Fat body collection

To obtain abdominal fat body tissue, the frozen bee was briefly adjusted to room temperature. The thorax was cut off and the abdomen was fixated with needles to styrofoam. By gripping

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