ESM1: RNA extraction and qPCR analysis

RNA was extracted from approximately 20 mg samples of wing tissue or 5-10 mg samples of lymph node. Samples were homogenized, using a MagNA Lyser (Roche, Basel, Switzerland) for 2.5 min in 30-s intervals. RNA was extracted from wing tissues using Qiagen Allprep RNA/DNA/miRNA Universal kit (Qiagen, Redwood CA, USA) with the following modifications to the manufacturer's protocol: After wing tissue was homogenized in 600 µL buffer RLT Plus with β-mercaptoethanol, samples were spun 3 min at 16,000 x g. Additionally, instead of washing with buffer FRN the samples were washed with buffer RPE, and two additional washes with ethanol were performed before eluting RNA in molecular grade water. RNA from lymph nodes was extracted using the Qiagen RNeasy Mini kit (Qiagen, Redwood CA, USA) with the following modifications in protocol. After homogenization of the samples in 600 µL buffer RLT with β-mercaptoethanol, the samples were spun for 3 min at 16,000 x g. The supernatant was mixed with 150 µL chloroform and centrifuged 3 min at 16,000 x g at 4ºC. The separated RNA phase was mixed with an equal volume of 70% ethanol and transferred to RNeasy Mini spin columns. The extraction was finished following the manufacturer's protocol. RNA extractions from both wing and lymph node included an on-column DNase I treatment. RNA concentration was measured using Qubit broad range RNA assay kit (ThermoFisher Scientific) and RNA purity using Nanodrop 1000 instrument (Thermo Scientific). All samples had $OD_{260/280} > 1.8$. Samples with $OD_{260/230} < 1.5$ were precipitated using 3M Na-acetate, pH 5.5, and washed with 75% ethanol o/n at –20ºC. RNA integrity was evaluated using agarose gel electrophoresis with ethidium bromide. Two distinct bands of 18S rRNA and 28s rRNA on the gel were observed indicating high quality RNA. RNA samples were stored at –80ºC until cDNA synthesis.

Complementary DNA (cDNA) was synthesized from 700 ng RNA from the wing tissue and 1000 ng RNA from the lymph node tissue using High Capacity RNA-to-cDNA kit (Life Technologies), which uses a mixture of random hexamers and oligo-d(T) primers, following the manufacturer's protocol. cDNA samples were stored at –20ºC until qPCR analysis.

Reaction volume was 20 μL and included 500 nM forward- and reverse-primers and the equivalent of 17.5 ng and 25 ng cDNA from wing or lymph node tissues, respectively. PCR programs consisted of 10 min pre-incubation at 95ºC, followed by 40 cycles of 10 s at 95ºC and 30 s at 50ºC for *Il6*, *Il1b*, *Ifng* and *aup1* or 58ºC for *Il17a* and *Il4*. Each program was followed by a melting curve analysis that consisted of the following incubations: 10 s at 95ºC, 60 s at 65ºC and 1 s at 97ºC. Standard curves were generated for each gene from a three-fold dilution series of pooled cDNA from lymph node and wing samples separately. All samples and the standard curve for a given gene in each tissue were run on one plate. Reactions with atypical melting curves were excluded from analysis. Further, no-template controls, RNA samples and reverse-transcription controls were analyzed using the reference gene to verify that significant amounts of genomic DNA or contamination were not present in the samples. Target gene expression was calculated with efficiency-corrected ddCt-method, as described in Yuan et al [1] (Eq. 7), where the normalization was performed against the mean of low-titer, uninfected group.

1. Yuan, J. S., Reed, A., Chen, F. & Stewart, C. N. 2006 Statistical analysis of real-time PCR data. *BMC Bioinformatics* **7**, 85. (doi:10.1186/1471-2105-7-85)