File S1

Expression levels of miRNAs chosen for investigation – from a preliminary screen of responses of miRNAs to SP receipt (T. Rathjen, H. Pais, C.J. Pennington, S. Moxon, T.D. & T.C., unpublished data).

(A) Small RNA sequencing: Fly samples were prepared and Total RNA was extracted as in (Gioti *et al*. 2012). miRNA expression data were generated from 2 replicates each (from the total of 4 described in Gioti *et al*. (2012)) for Head+Thorax (HT) and Abdomen (Abd) samples from wild type females, at 3 hr post mating to either SP^0 null or control SP^+ males (Liu and Kubli 2003). Total RNA was isolated, using the mirVana™ kit (Ambion), following the manufacturer's protocol for total RNA (TRNA) isolation. Quality and quantity of the isolated RNA were verified using a spectrophotometer and by gel electrophoresis. miRNA assays were performed using a service provider (BaseClear). cDNA libraries of the short RNA fractions were generated as described in Szittya *et al.* (2008) and sequenced on the Illumina GA2 platform. All sRNA FASTQ files were first converted to FASTA format and adapter sequences were removed by trimming sequences with exact matches to the first eight bases of the 3' adapter (only this 3' adapter sequence is read). Any sequences without adapter matches or shorter than 16 nucleotides after processing were excluded. Processed sRNA reads were mapped to the *D. melanogaster* genome (release 5.9; Drysdale *et al*. 2008). This mapping was performed using PatMaN (Pruefer *et al*. 2008) allowing only perfect ungapped alignment of sRNAs to the reference genome. After pre-preprocessing, reads were mapped to the sequences of known miRNAs (miRbase v.13.0, Griffiths-Jones *et al*. 2008) using miRProf (Moxon *et al*. 2008). For each sample the number of reads assigned to each miRNA was normalized to the total number of reads mapping to at least one miRNA in that sample. Mapping of Illumina deep sequencing data against the database of known miRNAs (miRbase, v13.0) identified 75 expressed miRNAs. 28 Abd and 29 HT miRNAs showed evidence of signal:noise >1. Of these, the top 15 provided some basis for further investigation, with a final estimate of 5-10 miRNAs of interest in each body part. Expression levels for the four candidate miRNA loci chosen for investigation in this study are shown in Table S1.

(B) Validation of small RNA sequencing by quantitative RT-PCR: qRT-PCR was used to validate the differences in miRNA expression detected in the small RNA sequencing screen described above (C.J. Pennington & T.C. unpublished data). We used Applied Biosystems (Warrington, UK) probesets to confirm differential expression in response to SP in two of the miRNAs chosen for investigation: miR-184 and miR-279. Probes were used according to the manufacturer's instructions given in the TaqMan small RNA Assays Protocol (Applied Biosytems). 10 ng RNA was used per reaction and tests were done on all four biological replicate samples of (Gioti *et al*. 2012). Two of these replicates were subjected to the small RNA sequencing described above. Expression values for the miRNAs were determined using a standard curve method and normalized to the expression level of miR-2s. The probe target sequences used were: miR-184 (FBgn0262391, uggacggagaacugauaagggc); miR-279 (FBgn0262448, ugacuagauccacacucauuaa); miR-2S (tgcttggactacatatggttgagggttgta).