

## **SUPPORTING ONLINE MATERIAL**

### **Materials and methods**

#### **Mosquito stocks**

*Aedes aegypti* strains kindly donated by the O'Neill laboratory, University of Queensland, were PGYP1 that had been transfected with *w*MelPop, and the uninfected PGYP1.tet created by tetracycline curing PGYP1, with the genetic backgrounds of the two having been homogenized by introgression (*SI*). The filaria-susceptible strain Ref<sup>m</sup> was kindly donated by E. Devaney, University of Glasgow. All strains were maintained under standard mosquito insectary conditions.

#### **Microarray analysis of gene expression.**

A custom gene expression oligo microarray was developed using Agilent Technologies' eArray website (<https://earray.chem.agilent.com/earray/>) to design a minimum of two unique 60-mer probes per *Ae. aegypti* gene transcript (Vectorbase genome build 1.1). The 44 000 feature array (4 x 44k format) contained Agilent's standard control features and was filled with the custom *Ae. aegypti* probe set.

Total RNA was extracted from pools of 2 and 15 day old adult females *w*MelPop-infected and uninfected *Aedes aegypti* using Trizol reagent (Invitrogen). Gene expression array experiments were conducted according to Agilent Technologies' Two-colour Microarray-based Gene Expression Analysis protocol (Version 5.5). Briefly, 1.5 $\mu$ g of total RNA was amplified and labelled using the Low RNA Input Linear Amplification Kit PLUS, Two-colour (Agilent Technologies). Labelled cRNA was prepared and hybridized to arrays with the Gene Expression Hybridization Kit (Agilent Technologies) following the manufacturer's protocol. Hybridized arrays were washed and then

protected against ozone degradation with a stabilization and drying solution (Agilent Technologies). Arrays were scanned at  $5\mu\text{m}$  resolution using the extended dynamic range feature (PMT 100% and 10%) of the Agilent Microarray Scanner. Feature Extraction software (Agilent Technologies, version 9.5.3) was used to quantify fluorescence data from the scanned array images following Agilent's standard two-colour array protocol (GE2-v5\_95\_Feb07). Array analysis was conducted with Genespring GX software (Agilent Technologies, version 4.0). Processed Cy5 and Cy3 signal data for replicate probes were averaged, normalized using the LOWESS normalization strategy, thresholded to 1.0, log (base 2) transformed and then Cy5/Cy3 ratios calculated. The array experiment consisted of four biological replicates and Cy5/Cy3 signal ratios were further averaged across these replicates. Transcripts with signal data below the 20<sup>th</sup> percentile in more than one of the four replicate arrays were removed (2331 transcripts), as were data without positive and significant signal as determined during feature extraction (111 transcripts). Signal ratios from all remaining transcripts were subjected to a *t*-test against zero at  $p \leq 0.05$ .

### **qRT-PCR**

Gene expression levels were monitored using quantitative RT-PCR (qRT-PCR). Total RNA was extracted with Trizol reagent (Invitrogen) from pools of ten *Ae. aegypti* PGYP1 or PGYP.tet females that were either 2 or 15 days post eclosion. Infected mosquitoes were approximately 40 generations post transinfection. cDNAs were synthesized from 1  $\mu\text{g}$  of total RNA using SuperScript II enzyme (Invitrogen). qRT-PCR was performed on a 1 to 20 dilution of the cDNAs using dsDNA dye SYBR Green I. Reactions were run on a DNA Engine thermocycler (MJ Research) with Chromo4 real-

time PCR detection system (Bio-Rad) using the following cycling conditions: 95°C for 15 minutes, then 45 cycles of 95°C for 10s, 59°C for 10s, 72°C for 20s, with fluorescence acquisition at the end of each cycle, then a melting curve analysis after the final one. The cycle threshold (C<sub>t</sub>) values were determined and background fluorescence was subtracted. Gene expression levels of target genes were calculated, relative to the internal reference gene *RpS17* (*ribosomal protein S17*; AAEL004175). Primer pairs used to detect target gene transcripts are listed in table S1.

### **Bacterial infection and survival experiments**

Infections with *Erwinia carotovora* and *Micrococcus luteus* were performed by pricking mosquitoes in the thorax with a thin needle previously dipped into a concentrated pellet of bacterial culture (OD = 200). Mosquitoes were counted at different time points to monitor survival. Each experiment was performed with 15 or 25 individuals and the results shown are representative of at least three independent tests.

### **⚠MelPop introgression and filarial worm challenge**

The Ref<sup>m</sup> strain has a red eye phenotype closely associated (less than 0.1 crossover) with the sensitivity locus to filarial nematodes (*S2*). This locus was backcrossed into the *Ae. aegypti* PGYP1 and control PGYP1.tet backgrounds using the red eye color marker for selection. Sixty virgin PGYP1 (or control PGYP1.tet) females were crossed to Ref<sup>m</sup> males, then approximately 200 G1 females (PGYP1/Ref<sup>m</sup> or PGYP1.tet/Ref<sup>m</sup>) were backcrossed to Ref<sup>m</sup> males. Only the resulting first backcross (BC1) individuals with red eyes were self-crossed to establish infected and control introgressed lines and used to perform worm challenge experiments. Challenge experiments A, C and D were

performed using BC1 red eye mosquitoes, while experiment B was done on the next generation after intercrossing. Separately reared cages of mosquitoes were used in each case. The mosquitoes were fed on sheep blood containing 11 (experiments A and B), 13 (experiment C) or 23 (experiment D) *B. pahangi* microfilaria per  $\mu\text{L}$ . The females that failed to feed or partially fed were removed from the cages. Dissections to determine the infection status were done 10 to 13 days after the infective blood meal under a dissecting stereomicroscope. Results were analyzed with a Mann-Whitney *U* Test.

**Table S1:** Quantitative RT-PCR primers used for validation of microarray results. All primers have an annealing temperature of 60°C.

| <b>Primer name</b> | <b>Sequence (5' - 3')</b> |
|--------------------|---------------------------|
| AAEL000611-F1      | TTGCACTCGTTCTGCTCATC      |
| AAEL000611-R1      | ACACGTTTTCCGACTCCTTC      |
| AAEL000598-F1      | GCTAGGTCAAACCGAAGCAG      |
| AAEL000598-R1      | TCCTACAACAACCGGGAGAG      |
| AAEL015458-F1      | AGCGAACGATGGTTTGAGTT      |
| AAEL015458-R1      | TATGGCATGCCTTGTACCAC      |
| AAEL005641-F1      | GTCTCCGGGTGCAATACACT      |
| AAEL005641-R1      | CCCTATCGTTCCAATTCCAA      |
| AAEL015458-F1      | GTTCCGGTACAACCTGGAGA      |
| AAEL015458-R1      | TTCAGCTCGATCAGGGAAGT      |
| AAEL005431-F1      | TCTATTGCGGAGGAGTGCTT      |
| AAEL005431-R1      | TGTCCCGGTACAATCCAAAT      |
| AAEL005093-F1      | TTGGGGGAAAACAGAAACAG      |
| AAEL005093-R1      | GATCTGCTTCCCAGAGAACG      |
| AAEL009474-F1      | TGGAGCGACATTGGTTACAA      |
| AAEL009474-R1      | GCGATGCCAATCGACTTACT      |
| AAEL001794-F1      | ATTTTTGACGGCTTTTGTGG      |
| AAEL001794-R1      | TGGATTACTTGCCCCACTTC      |
| AAEL003467-F1      | AATTCCGCAAATGTTTGGAC      |
| AAEL003467-R1      | CATGCAAACGTGGTAGTTGG      |
| AAEL011455-R1      | CGGTAGAGTTCTGCACCACA      |
| AAEL011455-F1      | TTGGCGAATTTATCCGACTC      |
| RpS17-F            | CAGGTCCGTGGTATCTCCAT      |
| RpS17-R            | CAGGACATCATCGAAGTCGA      |

**Table S2:** *Aedes aegypti* gene transcripts showing significant differential expression between females infected with *wMelPop* versus uninfected females. Shaded rows indicate gene transcripts with putative immune function. Transcripts are ranked according to mean  $\log_2$  signal ratio ( $\log_2$ ), which corresponds to the absolute fold change (FC) in gene expression. Gene names, putative gene products and transcript identifiers were compiled from Vectorbase, the Insect Immune-related Gene Database (S3) and relevant literature (S4).

**Table S2**

| Gene name | Putative gene product                                     | Transcript ID | Absolute FC | Regulation | log2  | p-value |
|-----------|---|---------------|-------------|------------|-------|---------|
| CECE      | Cecropin  | AAEL000611-RA | 56.390      | up         | 5.817 | 0.002   |
|           | hypothetical protein                                      | AAEL003843-RA | 55.448      | up         | 5.793 | 0.000   |
| FREP38    | Fibrinogen and fibronectin                                | AAEL015428-RA | 41.577      | up         | 5.378 | 0.000   |
| FREP28    | Fibrinogen and fibronectin                                | AAEL003156-RA | 37.839      | up         | 5.242 | 0.000   |
| CECA      | Cecropin  | AAEL000627-RA | 37.438      | up         | 5.226 | 0.017   |
| CECD      | Cecropin  | AAEL000598-RA | 37.326      | up         | 5.222 | 0.006   |
| CECG      | Cecropin  | AAEL015515-RA | 34.306      | up         | 5.100 | 0.015   |
| CECN      | Cecropin  | AAEL000621-RA | 30.937      | up         | 4.951 | 0.001   |
| CECF      | Cecropin  | AAEL000625-RA | 27.087      | up         | 4.760 | 0.010   |
|           | Galactose-specific C-type lectin                          | AAEL005641-RA | 25.496      | up         | 4.672 | 0.001   |
| DEFC      | Defensin  | AAEL003832-RA | 20.672      | up         | 4.370 | 0.002   |
| DEFD      | Defensin  | AAEL003857-RA | 19.362      | up         | 4.275 | 0.002   |
| DEFA      | Defensin  | AAEL003841-RA | 19.322      | up         | 4.272 | 0.001   |
|           | hypothetical protein                                      | AAEL003816-RB | 15.442      | up         | 3.949 | 0.001   |
|           | hypothetical protein                                      | AAEL003816-RA | 15.043      | up         | 3.911 | 0.002   |
| FREP10    | Fibrinogen and fibronectin                                | AAEL008646-RA | 14.186      | up         | 3.826 | 0.009   |
|           | hypothetical protein                                      | AAEL009537-RA | 13.677      | up         | 3.774 | 0.028   |
|           | Transferrin   | AAEL015458-RA | 13.648      | up         | 3.771 | 0.001   |
|           | hypothetical protein                                      | AAEL001392-RA | 12.122      | up         | 3.600 | 0.000   |
|           | Transferrin   | AAEL015639-RA | 11.755      | up         | 3.555 | 0.001   |
|           | Cubulin   | AAEL010965-RA | 10.563      | up         | 3.401 | 0.009   |
| LYSC11    | Lysozyme P  | AAEL003723-RA | 9.921       | up         | 3.310 | 0.002   |
| CLIPB27   | CLIP-domain serine protease (Lumbrokinase 1 T4 precursor) | AAEL007993-RA | 9.781       | up         | 3.290 | 0.001   |
|           | Cubulin   | AAEL014312-RA | 9.562       | up         | 3.257 | 0.009   |
|           | conserved hypothetical protein                            | AAEL003699-RA | 9.450       | up         | 3.240 | 0.001   |
| CLIPB37   | CLIP-domain serine protease                               | AAEL005431-RA | 9.224       | up         | 3.205 | 0.002   |
| CLIPB21   | CLIP-domain serine protease                               | AAEL001084-RA | 8.865       | up         | 3.148 | 0.001   |
|           | Galactose-specific C-type lectin                          | AAEL014390-RA | 6.866       | up         | 2.780 | 0.000   |
| CLIPC15   | CLIP-domain serine protease                               | AAEL010270-RA | 6.866       | up         | 2.779 | 0.030   |
|           | conserved hypothetical protein                            | AAEL006971-RA | 6.790       | up         | 2.763 | 0.036   |
| CLIPB46   | CLIP-domain serine protease                               | AAEL005093-RA | 6.346       | up         | 2.666 | 0.002   |
| PGRPS1    | Peptidoglycan recognition protein                         | AAEL009474-RA | 5.833       | up         | 2.544 | 0.003   |
|           | CLIP-domain serine protease                               | AAEL001098-RA | 5.536       | up         | 2.469 | 0.002   |
| TEP20     | Thio-ester containing protein (Macroglobulin/complement)  | AAEL001794-RB | 5.463       | up         | 2.450 | 0.001   |
|           | Galactose-specific C-type lectin                          | AAEL011619-RA | 5.442       | up         | 2.444 | 0.002   |
|           | Sugar transporter   | AAEL011411-RA | 5.375       | up         | 2.426 | 0.007   |
|           | Antifreeze protein  | AAEL012353-RA | 5.369       | up         | 2.425 | 0.008   |
|           | Serine-type endopeptidase                                 | AAEL013284-RA | 5.316       | up         | 2.410 | 0.012   |
|           | Juvenile hormone esterase                                 | AAEL008757-RA | 5.298       | up         | 2.406 | 0.021   |
|           | Cysteine-rich venom protein                               | AAEL000374-RA | 5.279       | up         | 2.400 | 0.030   |
|           | conserved hypothetical protein                            | AAEL007703-RB | 5.209       | up         | 2.381 | 0.009   |
|           | Cysteine-rich venom protein                               | AAEL012194-RA | 5.144       | up         | 2.363 | 0.025   |
|           | conserved hypothetical protein                            | AAEL001862-RA | 5.139       | up         | 2.362 | 0.004   |
|           | Juvenile hormone esterase                                 | AAEL008757-RB | 4.948       | up         | 2.307 | 0.013   |
|           | conserved hypothetical protein                            | AAEL002524-RA | 4.914       | up         | 2.297 | 0.019   |
|           | conserved hypothetical protein                            | AAEL010875-RA | 4.906       | up         | 2.295 | 0.014   |
|           | Carboxylesterase  | AAEL004022-RA | 4.881       | up         | 2.287 | 0.024   |
|           | conserved hypothetical protein                            | AAEL008173-RA | 4.860       | up         | 2.281 | 0.032   |
| CLIPA16   | CLIP-domain serine protease                               | AAEL008404-RA | 4.536       | up         | 2.181 | 0.001   |
|           | hypothetical protein                                      | AAEL012346-RA | 4.488       | up         | 2.166 | 0.048   |
|           | Serine protease   | AAEL011622-RA | 4.488       | up         | 2.166 | 0.000   |
| CLIPB28   | CLIP-domain serine protease (Proacrosin)                  | AAEL013245-RA | 4.336       | up         | 2.116 | 0.009   |
|           | conserved hypothetical protein                            | AAEL003442-RA | 4.207       | up         | 2.073 | 0.009   |
| FREP12    | Fibrinogen and fibronectin                                | AAEL011634-RA | 4.204       | up         | 2.072 | 0.009   |
| TEP20     | Thio-ester containing protein (Macroglobulin/complement)  | AAEL001794-RA | 4.141       | up         | 2.050 | 0.013   |
|           | conserved hypothetical protein                            | AAEL010874-RA | 4.108       | up         | 2.038 | 0.006   |
| TEP23     | Thio-ester containing protein (Macroglobulin/complement)  | AAEL001163-RA | 4.080       | up         | 2.029 | 0.030   |
|           | conserved hypothetical protein                            | AAEL001271-RA | 4.075       | up         | 2.027 | 0.007   |
|           | conserved hypothetical protein                            | AAEL001508-RA | 4.023       | up         | 2.008 | 0.006   |
|           | conserved hypothetical protein                            | AAEL014384-RA | 4.019       | up         | 2.007 | 0.049   |
|           | conserved hypothetical protein                            | AAEL011621-RA | 4.018       | up         | 2.006 | 0.000   |
|           | Trypsin   | AAEL012842-RA | 4.008       | up         | 2.003 | 0.001   |
|           | conserved hypothetical protein                            | AAEL013775-RA | 4.004       | up         | 2.001 | 0.003   |
|           | conserved hypothetical protein                            | AAEL004667-RA | 3.965       | up         | 1.987 | 0.010   |
|           | hypothetical protein                                      | AAEL010439-RA | 3.922       | up         | 1.971 | 0.022   |
| LYSC10    | Lysozyme P  | AAEL003712-RA | 3.903       | up         | 1.965 | 0.002   |
|           | predicted protein   | AAEL002580-RA | 3.893       | up         | 1.961 | 0.004   |
|           | hypothetical protein                                      | AAEL008935-RA | 3.892       | up         | 1.961 | 0.002   |
|           | hypothetical protein                                      | AAEL003656-RA | 3.834       | up         | 1.939 | 0.003   |
|           | Serine protease   | AAEL010139-RA | 3.821       | up         | 1.934 | 0.009   |
|           | conserved hypothetical protein                            | AAEL015078-RA | 3.806       | up         | 1.928 | 0.037   |
|           | Galactose-specific C-type lectin                          | AAEL011610-RA | 3.799       | up         | 1.926 | 0.003   |
|           | hypothetical protein                                      | AAEL008050-RA | 3.789       | up         | 1.922 | 0.024   |
|           | conserved hypothetical protein                            | AAEL013774-RA | 3.752       | up         | 1.908 | 0.001   |
|           | Elastase  | AAEL012956-RA | 3.733       | up         | 1.900 | 0.002   |
|           | conserved hypothetical protein                            | AAEL015200-RA | 3.723       | up         | 1.896 | 0.003   |
| TEP21     | Thio-ester containing protein (Macroglobulin/complement)  | AAEL001802-RA | 3.617       | up         | 1.855 | 0.032   |
| FREP26    | Fibrinogen and fibronectin                                | AAEL005194-RA | 3.551       | up         | 1.828 | 0.021   |
|           | conserved hypothetical protein                            | AAEL002242-RA | 3.488       | up         | 1.803 | 0.000   |
|           | conserved hypothetical protein                            | AAEL007999-RA | 3.399       | up         | 1.765 | 0.007   |
| CLIPA1    | CLIP-domain serine protease                               | AAEL002601-RA | 3.378       | up         | 1.756 | 0.006   |

|         |  |               |       |    |       |       |
|---------|--|---------------|-------|----|-------|-------|
|         | Serine protease  | AAEL003642-RA | 3.339 | up | 1.739 | 0.025 |
|         | Trypsin  | AAEL007992-RA | 3.289 | up | 1.718 | 0.003 |
| GNBP4   | Beta glucan binding protein (Gram-negative bacteria binding protein) | AAEL009178-RA | 3.230 | up | 1.692 | 0.041 |
|         | hypothetical protein   | AAEL007262-RA | 3.128 | up | 1.645 | 0.016 |
|         | hypothetical protein   | AAEL004591-RB | 3.125 | up | 1.644 | 0.011 |
|         | hypothetical protein   | AAEL008936-RA | 3.124 | up | 1.644 | 0.005 |
| CLIPB35 | CLIP-domain serine protease  | AAEL000037-RA | 3.053 | up | 1.610 | 0.014 |
|         | Nitric oxide synthase  | AAEL009745-RA | 3.044 | up | 1.606 | 0.001 |
|         | hypothetical protein   | AAEL007109-RA | 3.001 | up | 1.585 | 0.007 |
|         | conserved hypothetical protein                                       | AAEL013885-RA | 2.999 | up | 1.585 | 0.002 |
|         | Galactose-specific C-type lectin                                     | AAEL014382-RA | 2.992 | up | 1.581 | 0.034 |
|         | conserved hypothetical protein                                       | AAEL009985-RA | 2.977 | up | 1.574 | 0.002 |
| CLIPB15 | CLIP-domain serine protease  | AAEL014349-RA | 2.974 | up | 1.572 | 0.008 |
|         | Galactose-specific C-type lectin                                     | AAEL011607-RA | 2.944 | up | 1.558 | 0.033 |
|         | hypothetical protein   | AAEL012534-RA | 2.935 | up | 1.553 | 0.012 |
|         | Peritrophin  | AAEL002467-RA | 2.916 | up | 1.544 | 0.017 |
|         | Serine protease  | AAEL012775-RA | 2.880 | up | 1.526 | 0.007 |
| CLIPB26 | CLIP-domain serine protease  | AAEL003280-RA | 2.873 | up | 1.522 | 0.003 |
|         | hypothetical protein   | AAEL004591-RC | 2.857 | up | 1.514 | 0.007 |
|         | conserved hypothetical protein                                       | AAEL004672-RA | 2.852 | up | 1.512 | 0.018 |
|         | Serine protease  | AAEL002585-RA | 2.821 | up | 1.496 | 0.000 |
|         | conserved hypothetical protein                                       | AAEL000496-RA | 2.813 | up | 1.492 | 0.020 |
| LRIM1   | Luicine-rich repeat-containing protein                               | AAEL012086-RA | 2.769 | up | 1.470 | 0.025 |
|         | Coagulation factor X   | AAEL002582-RA | 2.739 | up | 1.453 | 0.004 |
|         | hypothetical protein   | AAEL000859-RA | 2.726 | up | 1.447 | 0.048 |
|         | Carboxylesterase   | AAEL004724-RA | 2.719 | up | 1.443 | 0.007 |
|         | Stearoyl-coa desaturase  | AAEL003645-RA | 2.715 | up | 1.441 | 0.033 |
|         | conserved hypothetical protein                                       | AAEL008259-RA | 2.709 | up | 1.438 | 0.012 |
|         | hypothetical protein   | AAEL003821-RA | 2.707 | up | 1.437 | 0.017 |
| CLIPB32 | CLIP-domain serine protease  | AAEL000086-RA | 2.696 | up | 1.431 | 0.034 |
|         | Coagulation factor X   | AAEL002582-RB | 2.684 | up | 1.425 | 0.007 |
|         | Serine protease  | AAEL007107-RA | 2.676 | up | 1.420 | 0.005 |
|         | Ornithine decarboxylase  | AAEL000044-RA | 2.658 | up | 1.410 | 0.020 |
|         | conserved hypothetical protein                                       | AAEL012953-RA | 2.649 | up | 1.405 | 0.003 |
|         | hypothetical protein   | AAEL004591-RA | 2.639 | up | 1.400 | 0.011 |
|         | conserved hypothetical protein                                       | AAEL002631-RA | 2.622 | up | 1.391 | 0.022 |
|         | Trypsin-eta  | AAEL008080-RA | 2.615 | up | 1.387 | 0.006 |
|         | Ornithine decarboxylase  | AAEL000044-RB | 2.612 | up | 1.385 | 0.019 |
| GNBPA1  | Beta glucan binding protein (Gram-negative bacteria binding protein) | AAEL007626-RA | 2.610 | up | 1.384 | 0.001 |
|         | hypothetical protein   | AAEL014166-RA | 2.610 | up | 1.384 | 0.002 |
| FREP9   | Fibrinogen and fibronectin   | AAEL004156-RA | 2.604 | up | 1.381 | 0.045 |
|         | conserved hypothetical protein                                       | AAEL002589-RA | 2.601 | up | 1.379 | 0.023 |
|         | hypothetical protein   | AAEL003842-RA | 2.599 | up | 1.378 | 0.001 |
|         | conserved hypothetical protein                                       | AAEL001571-RA | 2.590 | up | 1.373 | 0.006 |
| CLIPB22 | CLIP-domain serine protease (MASP-2 protein)                         | AAEL008668-RA | 2.580 | up | 1.367 | 0.045 |
|         | conserved hypothetical protein                                       | AAEL003567-RA | 2.575 | up | 1.365 | 0.029 |
|         | hypothetical protein   | AAEL012805-RA | 2.572 | up | 1.363 | 0.035 |
|         | conserved hypothetical protein                                       | AAEL004863-RA | 2.567 | up | 1.360 | 0.019 |
|         | Calmodulin   | AAEL006921-RA | 2.559 | up | 1.356 | 0.018 |
|         | hypothetical protein   | AAEL014565-RA | 2.556 | up | 1.354 | 0.026 |
|         | Glucose dehydrogenase  | AAEL004021-RA | 2.547 | up | 1.349 | 0.001 |
|         | conserved hypothetical protein                                       | AAEL000507-RA | 2.533 | up | 1.341 | 0.018 |
|         | Galactose-specific C-type lectin                                     | AAEL011404-RA | 2.531 | up | 1.339 | 0.002 |
|         | conserved hypothetical protein                                       | AAEL015527-RA | 2.530 | up | 1.339 | 0.005 |
|         | Atrial natriuretic peptide receptor                                  | AAEL005330-RA | 2.530 | up | 1.339 | 0.015 |
| PPO10   | Prophenoloxidase   | AAEL011764-RA | 2.520 | up | 1.333 | 0.049 |
|         | Serine protease  | AAEL002301-RA | 2.514 | up | 1.330 | 0.021 |
| FREP37  | Fibrinogen and fibronectin   | AAEL011007-RA | 2.512 | up | 1.329 | 0.038 |
|         | conserved hypothetical protein                                       | AAEL012577-RA | 2.493 | up | 1.318 | 0.014 |
|         | Prophenoloxidase   | AAEL006874-RA | 2.489 | up | 1.316 | 0.048 |
|         | Tyrosine-protein kinase  | AAEL007742-RA | 2.486 | up | 1.314 | 0.000 |
|         | hypothetical protein   | AAEL010634-RA | 2.480 | up | 1.310 | 0.037 |
|         | conserved hypothetical protein                                       | AAEL004332-RB | 2.480 | up | 1.310 | 0.006 |
|         | hypothetical protein   | AAEL010686-RA | 2.478 | up | 1.309 | 0.009 |
|         | hypothetical protein   | AAEL009329-RA | 2.466 | up | 1.302 | 0.014 |
|         | conserved hypothetical protein                                       | AAEL014914-RA | 2.461 | up | 1.299 | 0.018 |
|         | Cysteine-rich secretory protein-2                                    | AAEL008488-RA | 2.440 | up | 1.287 | 0.004 |
|         | A-kinase anchoring protein AKAP120                                   | AAEL010185-RA | 2.428 | up | 1.280 | 0.014 |
|         | Serine protease  | AAEL011611-RA | 2.413 | up | 1.271 | 0.038 |
|         | hypothetical protein   | AAEL005253-RA | 2.407 | up | 1.267 | 0.025 |
|         | hypothetical protein   | AAEL003848-RA | 2.396 | up | 1.261 | 0.021 |
|         | conserved hypothetical protein                                       | AAEL006109-RA | 2.395 | up | 1.260 | 0.045 |
|         | Type II transmembrane receptor OtB7                                  | AAEL011407-RA | 2.376 | up | 1.248 | 0.020 |
| CLIPB45 | CLIP-domain serine protease  | AAEL001077-RA | 2.374 | up | 1.247 | 0.020 |
|         | conserved hypothetical protein                                       | AAEL003114-RA | 2.367 | up | 1.243 | 0.001 |
|         | Metalloproteinase  | AAEL011557-RA | 2.365 | up | 1.242 | 0.016 |
|         | conserved hypothetical protein                                       | AAEL012866-RA | 2.357 | up | 1.237 | 0.003 |
|         | conserved hypothetical protein                                       | AAEL014385-RA | 2.346 | up | 1.230 | 0.018 |
|         | Latisemin  | AAEL008487-RA | 2.340 | up | 1.226 | 0.007 |
|         | conserved hypothetical protein                                       | AAEL002767-RA | 2.336 | up | 1.224 | 0.001 |
| CLIPB8  | CLIP-domain serine protease  | AAEL005792-RA | 2.314 | up | 1.210 | 0.017 |
|         | conserved hypothetical protein                                       | AAEL009737-RA | 2.311 | up | 1.208 | 0.047 |
|         | conserved hypothetical protein                                       | AAEL013004-RA | 2.301 | up | 1.202 | 0.009 |
|         | Maltose phosphorylase  | AAEL014541-RA | 2.277 | up | 1.187 | 0.015 |

|        |   |               |       |      |        |       |
|--------|---|---------------|-------|------|--------|-------|
|        | Apolipoprotein D                                  | AAEL009561-RA | 2.251 | up   | 1.171  | 0.025 |
|        | Testisin precursor                                | AAEL002686-RA | 2.251 | up   | 1.171  | 0.005 |
|        | hypothetical protein                              | AAEL014363-RA | 2.248 | up   | 1.168  | 0.036 |
|        | conserved hypothetical protein                    | AAEL007344-RA | 2.239 | up   | 1.163  | 0.009 |
|        | conserved hypothetical protein                    | AAEL001491-RA | 2.226 | up   | 1.154  | 0.003 |
|        | conserved hypothetical protein                    | AAEL002549-RA | 2.199 | up   | 1.137  | 0.043 |
|        | Serine protease                                   | AAEL006136-RA | 2.194 | up   | 1.133  | 0.010 |
| GALE6B | Galactoside-binding lectins (Keratinocyte lectin) | AAEL012003-RA | 2.178 | up   | 1.123  | 0.017 |
|        | conserved hypothetical protein                    | AAEL008283-RA | 2.166 | up   | 1.115  | 0.016 |
|        | Serine protease                                   | AAEL002593-RB | 2.155 | up   | 1.108  | 0.008 |
| REL2   | Relish-like protein                               | AAEL007624-RA | 2.144 | up   | 1.101  | 0.004 |
|        | hypothetical protein                              | AAEL004115-RA | 2.141 | up   | 1.098  | 0.014 |
|        | Nucleoprotein                                     | AAEL002517-RB | 2.141 | up   | 1.098  | 0.008 |
|        | conserved hypothetical protein                    | AAEL010601-RA | 2.140 | up   | 1.097  | 0.037 |
| PPO2   | Prophenoloxidase                                  | AAEL013499-RA | 2.122 | up   | 1.085  | 0.041 |
|        | Cytochrome P450                                   | AAEL009121-RA | 2.115 | up   | 1.081  | 0.003 |
| FREP21 | Fibrinogen and fibronectin                        | AAEL006691-RA | 2.111 | up   | 1.078  | 0.021 |
|        | AMP dependent ligase                              | AAEL005793-RA | 2.106 | up   | 1.075  | 0.015 |
|        | conserved hypothetical protein                    | AAEL006826-RA | 2.100 | up   | 1.070  | 0.032 |
|        | Lysosomal alpha-mannosidase                       | AAEL005752-RA | 2.097 | up   | 1.068  | 0.037 |
|        | Maltose phosphorylase                             | AAEL006276-RA | 2.092 | up   | 1.065  | 0.044 |
|        | Gustatory receptor 64a                            | AAEL015071-RA | 2.092 | up   | 1.065  | 0.013 |
|        | conserved hypothetical protein                    | AAEL005543-RA | 2.085 | up   | 1.060  | 0.015 |
|        | hypothetical protein                              | AAEL011305-RA | 2.079 | up   | 1.056  | 0.023 |
|        | Nucleoprotein                                     | AAEL002535-RA | 2.075 | up   | 1.053  | 0.009 |
| FREP18 | Fibrinogen and fibronectin                        | AAEL006704-RA | 2.074 | up   | 1.052  | 0.033 |
|        | conserved hypothetical protein                    | AAEL005428-RA | 2.058 | up   | 1.041  | 0.001 |
|        | hypothetical protein                              | AAEL007371-RA | 2.057 | up   | 1.041  | 0.028 |
|        | Galactose-specific C-type lectin                  | AAEL011453-RA | 2.055 | up   | 1.039  | 0.022 |
| CLIPB1 | CLIP-domain serine protease                       | AAEL000074-RA | 2.052 | up   | 1.037  | 0.045 |
|        | conserved hypothetical protein                    | AAEL000139-RA | 2.047 | up   | 1.034  | 0.013 |
|        | conserved hypothetical protein                    | AAEL014945-RB | 2.027 | up   | 1.020  | 0.038 |
|        | Gamma-glutamyl hydrolase                          | AAEL000271-RA | 2.006 | up   | 1.005  | 0.011 |
| PGRPLB | Peptidoglycan recognition protein sb2             | AAEL010171-RA | 2.004 | up   | 1.003  | 0.023 |
|        | hypothetical protein                              | AAEL014300-RA | 2.005 | down | -1.003 | 0.019 |
|        | conserved hypothetical protein                    | AAEL000083-RA | 2.032 | down | -1.023 | 0.040 |
|        | hypothetical protein                              | AAEL000521-RA | 2.051 | down | -1.036 | 0.020 |
|        | 2-amino-3-ketobutyrate coenzyme a ligase          | AAEL012318-RA | 2.055 | down | -1.039 | 0.003 |
|        | hypothetical protein                              | AAEL009683-RA | 2.071 | down | -1.050 | 0.006 |
|        | hypothetical protein                              | AAEL009409-RA | 2.089 | down | -1.063 | 0.036 |
|        | conserved hypothetical protein                    | AAEL006332-RA | 2.091 | down | -1.064 | 0.008 |
|        | conserved hypothetical protein                    | AAEL000151-RA | 2.112 | down | -1.078 | 0.002 |
|        | Cytochrome P450                                   | AAEL000325-RA | 2.165 | down | -1.114 | 0.034 |
|        | Toll  | AAEL001771-RA | 2.180 | down | -1.124 | 0.032 |
|        | hypothetical protein                              | AAEL001138-RA | 2.206 | down | -1.141 | 0.043 |
|        | hypothetical protein                              | AAEL012022-RA | 2.218 | down | -1.149 | 0.008 |
|        | hypothetical protein                              | AAEL015019-RA | 2.229 | down | -1.156 | 0.026 |
|        | conserved hypothetical protein                    | AAEL010726-RA | 2.258 | down | -1.175 | 0.017 |
|        | Carboxylesterase                                  | AAEL002391-RA | 2.326 | down | -1.218 | 0.031 |
|        | Cytochrome P450                                   | AAEL013554-RA | 2.339 | down | -1.226 | 0.003 |
|        | conserved hypothetical protein                    | AAEL002449-RA | 2.356 | down | -1.237 | 0.011 |
|        | Threonine dehydrogenase                           | AAEL003443-RA | 2.387 | down | -1.255 | 0.015 |
|        | Serine collagenase 1 precursor                    | AAEL007432-RA | 2.389 | down | -1.257 | 0.008 |
|        | Oxidoreductase                                    | AAEL010075-RA | 2.393 | down | -1.259 | 0.024 |
|        | Alcohol dehydrogenase                             | AAEL011450-RA | 2.409 | down | -1.268 | 0.035 |
|        | hypothetical protein                              | AAEL014937-RA | 2.486 | down | -1.314 | 0.028 |
|        | hypothetical protein                              | AAEL008754-RA | 2.491 | down | -1.317 | 0.003 |
|        | Splicing factor pTSR1                             | AAEL001889-RA | 2.511 | down | -1.328 | 0.025 |
|        | CLIP-domain serine protease                       | AAEL006576-RA | 2.533 | down | -1.341 | 0.014 |
|        | CLIP-domain serine protease                       | AAEL006576-RB | 2.553 | down | -1.352 | 0.011 |
|        | conserved hypothetical protein                    | AAEL005542-RA | 2.648 | down | -1.405 | 0.028 |
|        | conserved hypothetical protein                    | AAEL014647-RA | 2.798 | down | -1.484 | 0.034 |
|        | Cathepsin b                                       | AAEL007599-RA | 2.811 | down | -1.491 | 0.018 |
|        | Argininosuccinate synthase                        | AAEL004701-RA | 2.831 | down | -1.502 | 0.005 |
|        | Galactose-specific C-type lectin                  | AAEL011455-RA | 3.041 | down | -1.605 | 0.024 |
|        | Matrix metalloproteinase                          | AAEL002655-RA | 3.065 | down | -1.616 | 0.033 |
|        | Short-chain dehydrogenase                         | AAEL009625-RA | 3.307 | down | -1.726 | 0.002 |
|        | hypothetical protein                              | AAEL001274-RA | 3.314 | down | -1.729 | 0.022 |
|        | conserved hypothetical protein                    | AAEL010775-RA | 3.486 | down | -1.802 | 0.025 |
|        | conserved hypothetical protein                    | AAEL010093-RA | 3.497 | down | -1.806 | 0.048 |
|        | Glucosyl/glucuronosyl transferases                | AAEL014244-RA | 3.718 | down | -1.894 | 0.004 |
|        | hypothetical protein                              | AAEL003663-RA | 3.825 | down | -1.935 | 0.009 |
|        | conserved hypothetical protein                    | AAEL004342-RA | 4.024 | down | -2.009 | 0.003 |
|        | hypothetical protein                              | AAEL003482-RA | 4.167 | down | -2.059 | 0.026 |
|        | hypothetical protein                              | AAEL009095-RA | 4.206 | down | -2.072 | 0.031 |
|        | Trypsin   | AAEL006903-RA | 4.429 | down | -2.147 | 0.028 |
|        | hypothetical protein                              | AAEL005199-RA | 4.534 | down | -2.181 | 0.004 |
|        | Juvenile hormone esterase                         | AAEL005178-RA | 4.799 | down | -2.263 | 0.003 |
|        | conserved hypothetical protein                    | AAEL003467-RA | 8.250 | down | -3.044 | 0.018 |

## REFERENCES

- S1. C. J. McMeniman *et al.*, *Science* **323**, 141 (2009).
- S2. W. W. Macdonald, P. M. Sheppard, *Ann. Trop. Med. Parasitol.* **59**, 74 (1965).
- S3. R. M. Waterhouse *et al.* *Science* **16**, 1738 (2007).
- S4. M. Povelones, R. M. Waterhouse, F. C. Kafatos, G.K. Christophides. *Science* **324**, 258-261 (2009).