Supporting Online Material

Materials and Methods

Mosquito rearing and parasite infections

Anopheles gambiae L3-5, 4Arr and G3 strains were reared as described (*1*). Mosquitoes were infected with *PbGFP$_{CON}$* (*2*) and live and melanised parasites were counted on midguts dissected 7–9 days post infection (*3, 4*).

Reciprocal genetic crosses

For genetic mapping, the refractory L3-5 and susceptible 4Arr strains were crossed. Virgin males and females of each strain were separated at the pupal stage, and allowed to mate as follows: (i) cross 1, 50 L3-5 females with 50 4Arr males; and (ii) cross 2, 88 L3-5 males and 66 4Arr females. A total of 18 F1 females were isolated singly, and each placed together with four males and fed on human blood to enhance egg production. Four out of eight and six out of ten isolated F1 females laid eggs for crosses 1 and 2, respectively, giving rise to a total of 10 independent F2 families. All F2 females were infected with *PbGFPcon* (*2*). In addition, females of the parental L3-5 and 4Arr strains, and the remaining F1 females that were not used to give rise to F2 families were infected with *PbGFPcon*. The midguts of blood fed mosquitoes were dissected 7–9 days post infection, and the remaining carcasses were frozen for genomic DNA extraction.

For reciprocal allele-specific RNAi, virgin males and females of the L3-5 and G3 strains were separated at the pupal stage and allowed to mate. The F1 progeny was injected with different dsRNA probes and analyzed further.

Mendelian inheritance of the resistance and melanising traits

Simple Mendelian inheritance was assessed for the two binary traits "resistant" (no live parasite) and "melanising" (at least one melanised parasite) using the counts of mosquitoes falling into each category in the two F0 parental populations, the F1 and the F2 populations. Let k_{AA}, k_{aa}, k_{Aa} and k_{F2} out of n_{AA}, n_{aa}, n_{Aa} and n_{F2} mosquitoes show the phenotype in the two parental, the F1 and the F2 populations, respectively. Assuming a single causative locus with two alleles (A and a) and a genotype distribution in the F2 population of ¼ AA, ½ Aa and ¼ aa, the probability of an F2 individual to show the phenotype is $p_{F2} = 0.25 p_{AA} + 0.5 p_{Aa} + 0.25 p_{aa}$, where p_{xy} is the penetrance of genotype *xy*. The three genotype penetrances p_{AA}, p_{Aa} and p_{aa} were estimated under this hypothesis by numerically maximizing the overall likelihood of the observed F0, F1 and F2 counts assuming binomial distributions and independence of the counts given the genotype. Significance levels of the deviation of the observed counts to the expected ones were estimated using the chi-squared statistic defined as $X^2 = \sum_{xy \in \{AA, Aa, aa, F2\}} \frac{\left(k_{xy} - n_{xy} p_{xy}\right)^2}{n_{xy} p_{xy} (1 - p_{xy})}$ which approximately follows a chi-squared distribution with 1 degree of freedom (4 variables minus 3 model parameters).

Genetic mapping

Genomic DNA was extracted from mosquitoes and microsatellites were genotyped as described (*5, 6*). SNP genotyping was performed using fluorescence polarization (FP SNP) (*7*). For this, PCR primers and one base extension SNP primers were designed as described (*7*), and PCR conditions (annealing temperature, number of cycles) were optimized for each locus. PCR was performed as follows: 125 pM of each primer, 0.10mM of each dNTP, 0.025 U/µl of Taq polymerase, and 0.3-0.9 ng/µl of genomic DNA from a single mosquito. Cycling conditions were: denaturation at 94ºC for 2 min, followed by 28-34 cycles of 94ºC for 15 s, 56-62ºC for 30s, and 72ºC for 30 s, and a final 72ºC extension of 10 min. Degradation of primers and dNTPs was performed as follows: 3 µl of water and 2 µl PCR of Clean-Up Reagent (provided in the AcycloPrimer[TM]-FP SNP Detection Kit, PerkinElmer) was added into 2 µl of PCR product and the mixture was incubated at 37ºC for 1 hour, followed by heating at 80ºC for 15 minutes. Then the necessary components from the kit, together with 250 pM of SNP primer, were added for another 25-cycle PCR reaction. FP SNP detection was performed on Victor 3 (PerkinElmer) according to manufacturer instructions. The markers used in this study are listed in Table S1.

Two mapping strategies were employed: (i) composite interval mapping (CIM) on binary traits, and (ii) non-parametric single interval mapping (SIM) for parasite counts. (i) Two binary traits were defined: "resistant" (no live parasites) vs. non-resistant, and "melanising" (bearing at least one melanised parasite) vs. non-melanising. For each trait, comparison of the genotypes of both groups was performed using CIM from the software suite QTL Cartographer (*8, 9*) in "model 6 mode" with a walking speed of 0.5 cM, a window size of 12 cM and 8 control markers identified by the forward stepwise regression method. We performed CIM in its default mode (linear regression) because in practice little gain is obtained when using models for binary traits (*10*). QTL boundaries were determined by taking the interval 2-LOD scores below the point of greatest LOD score. (ii) SIM was performed with the actual numbers of live or melanised parasites as traits using the R/qtl package version 1.11-12 in its non-parametric mode (*11*). Confidence intervals for the genomic position with maximum LOD score over each chromosome were derived by bootstrapping of the cases (1,000 times with replacement) (*12*). For both CIM and SIM, LOD score cut-offs were set at the 5% experiment-wise significance level, and were estimated from 1,000 permutations of the trait values (*13*).

Sequencing and genotyping of the *TEP1* locus

Preliminary genotyping of the parental 4Arr and L3-5 mosquitoes at the *TEP1* locus using previously published primer pairs for *TEP1s* and *TEP1r* (*3*) indicated that the L3-5 strain bore a single *TEP1r* allele while the 4Arr strain gave positive PCR signals for both *TEP1s* and *TEP1r*. Genotyping of the G3 strain suggested it was homozygous for *TEP1s*. Thus, for each strain and each allele, we cloned and sequenced the entire *TEP1* ORF from 2-3 homozygous individuals.

Using specific primers (see below) and high-fidelity DNA polymerases (Phusion from Finnzymes, or iProof from BioRad), TEP1 was amplified by PCR from genomic

DNA (4Arr and G3) or from cDNA (L3-5) obtained from individual mosquitoes yielding 4.8 kb and 4 kb fragments, respectively. PCR conditions were: 2 min at 98°C; followed by 35 cycles of 10 sec at 98°C, 2 min 30 sec at 72°C and a final extension of 7 min at 72°C. The PCR products were cloned into the pCR®-XL-TOPO® vector (Invitrogen) and at least three clones were fully sequenced per homozygous individual using M13 universal primers (mapping in the vector) and six additional TEP1-specific primers (see below).

For each clone, the reads were assembled, and a consensus for each allele (*TEP1*R1, *R2, *S2* and **S3*) was derived from 6–9 sequences (2–3 homozygous individuals, three sequenced clones each) using DNAstar Seqman. The different allelic sequences obtained above, and the previously described *TEP1r* (renamed *TEP1*R1*) and *TEP1s* (renamed *TEP1*S1*) were aligned using Muscle (*14, 15*). Intronic sequences were trimmed from genomic DNA sequences in the aligned set using Seaview (*16*) and the cDNA sequences of *TEP1*S1* and **R1* as templates. The corresponding amino acid sequences were aligned using Muscle and displayed in Jalview (*17*). A phylogenetic tree was drawn using Phyml (*18*). The JTT amino acid substitution model was applied with 4 substitution rate categories, estimating the proportion of invariable sites and the gamma distribution, and optimising the tree topology and branch length. The robustness of the tree was estimated by performing a bootstrap analysis with 1000 samples. Average divergence between alleles (cDNAs) at non synonymous sites (Ka) was calculated with a sliding window of 50 and a step of 10 non-synonymous sites using DnaSP 4.5 (*19, 20*).

Four pairs of primers were used to genotype F2 mosquitoes from the mapping crosses at the *TEP1* locus (Table S2).

Primer sequences (5'-3'):
Cloning:
EL152_TEP1F: CCG CTA GCA CCA TGT GGC AGT TCA TAA GGT CAC G
EL157_TEP1R: GCA CTC TGC AGG ACA GTC TTC TTC GTC
Sequencing:
EL314-TEP1seq1: TACGGTGAACTTCCGTGTGA
EL315-TEP1seq2: CGTACGGTGGTCAAACAGTCA
EL316-TEP1seq3: ATTGATGCGTTTCATGGTGA
EL317-TEP1seq4: GGTGTCGTATCGGACGAACT
EL318-TEP1seq5: GATAATGCTGGGACACGAAAC
EL319-TEP1seq6: GCTACGAATTTGTTGCGTCA

Allele-specific RNAi
Three pairs (*dsR/dsS*, a-c) of fragments (75–139bp) were amplified from L3-5 (*dsR*) and G3 (*dsS*) cDNA in polymorphic regions of *TEP1*, and cloned into pLL10 to produce dsRNA (*21*) (Table S3). Mosquitoes were injected with these probes, *dsLacZ* or *dsTEP1* (69 nL, 3 μg/μL) (*3, 21*). Hemolymph and total RNA were extracted from 10 mosquitoes for each sample 3-4 days after dsRNA-treatment. Hemolymph extracts were analyzed by immunoblotting using TEP1 and PPO antisera (*4*). Total RNA samples were reverse transcribed and expression levels of *TEP1*R1* and **S3* were measured by quantitative Real-

Time PCR using allele-specific primers and probes, and normalized to their levels in the *dsLacZ* control. The ribosomal protein transcript *RpL19* (AGAP004422) was used as an internal control in all experiments.

qRT-PCR reactions were run on a 7500 Real-Time PCR instrument (Applied Biosystems) using the TaqMan Universal PCR Master Mix (Applied Biosystems). Primers and probes (see below) were designed with the PrimerExpress software (Applied Biosystems). Data were analyzed with the 7500 Software v2.0.1 (Applied Biosystems).

TEP1*R1: AG484 forward primer 5'-ATACGGATCTCAGCTACACCAAATC-3'
 AG485 reverse primer 5'-GCTTGCGGGCCTTGATG-3'
 TaqMan probe 5'-TGAGCGTTCCTCCAAAA-3'
TEP1*S3: AG486 forward primer 5'-ATACGGATCTCAGCTATACCAAATCG-3'
 AG487 reverse primer 5'-TGCGGGCCTTTATGAGAAAA-3'
 TaqMan probe 5'-TCCGAAGGTTGGTGTTC-3'
Rpl19: AG490 forward primer 5'-CCAACTCGCGACAAAACATTC-3'
 AG491 reverse primer 5'-ACCGGCTTCTTGATGATCAGA-3'
 TaqMan probe 5'-*VIC*-CAAACTGATCAAGGATG-*MGB*-3'

Correlation between *TEP1* genotype and phenotype upon infection in F2

Pair-wise comparisons between selected genotypes were performed for the proportions of resistant and melanising mosquitoes. In order to take potential F2-family effects into account, we modeled the data using generalized linear models (*22*) with family as a covariate. Specifically, we performed logistic regression, i.e. we modeled the observed number of mosquitoes showing the phenotype out of the n_{ij} mosquitoes in the *j*-th family with the *i*-th genotype as a binomial variable $Y_{ij} \sim Bin(\pi_{ij}, n_{ij})$ with $\log(\frac{\pi_{ij}}{1 - \pi_{ij}}) = \mu + \alpha_i + \beta_j$. The more complex model that includes genotype-family interactions did not appear to significantly improve the fit while the model without family effect was significantly worse (chi-squared test, p-value <0.01). Statistical tests for contrasts between all pairs of genotypes (Tukey's null hypotheses) were performed using the multcomp package version 1.1-0 (*23*) of the R software. Significance for differences is indicated for key comparisons for which we could verify that the difference between the two groups was consistent over the different F2 families where they appeared simultaneously. In particular, we did not include the comparison between **R2/R2* and **S2/S2* as they were never found in the same F2 family.

Supporting Text

Reciprocal crosses of resistant L3-5 and susceptible 4Arr mosquitoes
We noted that the distribution of the number of live parasites per midgut was wider in the F2 generation than in F0 4Arr mosquitoes (Fig. 1A). This might be due to several reasons: (i) a higher number of mosquitoes were analyzed in the two F2 generations (271 and 131) compared to 43 F0 4Arr, which may thus sample more cases with extreme phenotypes; (ii) by necessity, mosquitoes from different generations were infected on different mice (parasitemia vary between mice), and (iii) recombination between certain alleles inherited from the susceptible and refractory strains that would make mosquitoes more susceptible.

Identification of informative markers for genetic mapping
To map loci that control mosquito resistance to malaria parasites and clearance of dead parasites, we first screened a set of microsatellites (*24, 25,* and newly designed microsatellites) and SNPs to identify informative markers. A marker is defined as "informative" when the parental strains have two different homozygous genotypes and the F1 progeny is heterozygous bearing one allele from each parent. 132 microsatellite markers (Fig. S1) were tested on three mosquitoes of each parental strain, as well as on all ten F1 mothers. We identified 34 informative markers and selected 32 of them to genotype mosquitoes from the crosses (Fig. S1 and Table S1). Seven additional informative SNPs were selected in regions devoid of informative microsatellites, or to increase microsatellite coverage on chromosome 3L (Fig. S1 and Table S1).

Genome-wide mapping of loci controlling resistance to malaria parasites and the mode of clearance of dead parasites
Among the 402 F2 females, 206 individuals, mostly with extreme phenotypes, were selected for genotyping: 50 mosquitoes bore exclusively melanised parasites, 49 only live parasites, 5 mosquitoes mostly melanised but also a few live parasites, 11 with mostly live but also a few melanised parasites, and 91 were devoid of parasites. The latter mosquitoes are likely to have killed all parasites and cleared them through lysis, although we cannot rule out the possibility that some of them were not resistant, but were not infected although they had taken blood (or where parasites died before midgut invasion). All selected mosquitoes, except those devoid of parasites, carried more than 5 parasites in total. Mosquitoes where no live parasites were detected were considered "resistant"; mosquitoes with at least one melanised parasite were classified as "melanising".

To identify genomic loci that control resistance to *P. berghei* and the mode of clearance of dead parasites, we employed 2 strategies: (i) we compared the genotypes of resistant vs. non-resistant mosquitoes, and of melanising vs. non-melanising mosquitoes using composite interval mapping (Fig. 1C); and (ii) we performed non-parametric simple interval mapping (SIM) with the number of live and the number of melanised parasites as traits (Fig. S2B). Both methods identified QTLs at the same locations (Fig. S2B): a single resistance locus on chromosome 3L, *Pbres1*; and 2 loci controlling the mode of clearance

of dead parasites on chromosome 2R, *Pbmel1*, and on chromosome 3, *Pbmel2*, encompassing the centromere and partially overlapping *Pbres1*.

The resistance QTL *Pbres1* contains ~975 genes, spanning from AGAP010342 to AGAP011319, among which at least 35 can be classified as "immune-related" (*26*): 4 Lectins, 10 thioester-containing proteins (TEPs), 3 CLIP domain serine proteases, 4 Toll receptors, Frizzled 2, the transcription factor STAT1, 4 caspases, 1 antimicrobial peptide and 7 regulators of redox homeostasis and iron binding molecules.

Allele-specific RNAi

We designed 3 pairs of short dsRNAs (*dsR / dsS*, 75–139 bp) targeting specifically **R1* and **S3*, and tested their efficiency and specificity in the parental G3 and L3-5 strains by immunoblotting of hemolymph extracts 3–4 days after dsRNA-treatment (Fig. S4A). Polyclonal antibodies recognizing both the full-length and cleaved form of TEP1 were used (*27*). For each pair, we expected to deplete TEP1 from L3-5 mosquitoes when injecting *dsR* but not *dsS*, and reciprocally in G3 mosquitoes, with *dsS* but not *dsR*. We used *dsLacZ* as a negative control, and *dsTEP1* that targets both alleles as a positive control (*3*). All *dsS* probes efficiently silenced *TEP1*S3* in G3 mosquitoes, to a level comparable with that of *dsTEP1*. However, we observed substantial cross-silencing of *TEP1*S3* upon treatment with *dsRb* and *dsRc*, but not with *dsRa*, suggesting pair a was the most specific in the G3 strain. In L3-5 mosquitoes, pair a also behaved as expected although we detected slight cross-silencing of *TEP1*R1* by the *dsSa* probe. These results were further confirmed at the mRNA level: *TEP1* expression was measured relative to the *dsLacZ* control by quantitative RT-PCR on whole mosquitoes 3–4 days post dsRNA injection (Fig. S4B). Injection of *dsRa* or *dsTEP1* decreased *TEP1*R1* expression level to ~35% compared to *dsLacZ* in L3-5 mosquitoes, and injection of *dsSa* or *dsTEP1* decreased *TEP1*S3* expression level to ~25% compared to *dsLacZ* in G3 mosquitoes. Again, we detected a slight cross-silencing of *TEP1*R1* by the *dsSa* probe (**R1* expression level was decreased to 84% upon injection of *dsSa* in L3-5).

Three to four days after dsRNA-treatment, parental strains were allowed to feed on an infected mouse carrying *PbGFPcon*. As expected, L3-5 mosquitoes treated with *dsTEP1* or *dsRa* were rendered susceptible and unable to melanise parasites, while G3 mosquitoes depleted from TEP1 by treatment with *dsSa* or *dsTEP1* carried 3–4 times more oocysts than control *dsLacZ* mosquitoes.

To evaluate the efficiency and specificity of allele-specific RNAi in the F1 progeny of reciprocal crosses between resistant L3-5 and susceptible G3 mosquitoes, we quantified the expression of each *TEP1* allele by qRT-PCR using allele-specific primers and probes (Figs. 3B and S4B). Similar efficiencies were observed for both probes on their respective targets (the expression of **R1* and **S3* was reduced to ~40% in L3-5xG3 mosquitoes upon treatment with *dsRa* and *dsSa* respectively, and to ~25% in G3xL3-5 mosquitoes), and these efficiencies were similar to those obtained with *dsTEP1*. In L3-5xG3 mosquitoes, injection of *dsRa*, but not of *dsSa*, triggered the upregulation of the non-targeted allele (expression of **S3* was 1,5 fold higher in *dsRa*- than in *dsLacZ*-treated mosquitoes, Fig. 3C), although this was not observed in G3 x L3-5 (expression of **S3* was similar in *dsRa*- and *dsLac*-treated mosquitoes, Fig. S4B). Still, even if *dsRa*-treated mosquitoes produce

more TEP1 than *dsSa*-treated mosquitoes in the L3-5 x G3 cross, they were more susceptible than those injected with *dsSa*, which is the reverse of what would be expected if the efficiency of TEP1 was strictly quantity-dependent. Indeed *dsRa*-treated mosquitoes displayed more live parasites than *dsSa*-treated ones, and were completely devoid of melanised parasites. These results demonstrate that *R1 is more efficient than *S3 to kill parasites, and that *S3 alone is not able to promote melanisation of dead parasites in F1 mosquitoes.

Association between phenotypes and *TEP1* genotypes in the F2 generation
Out of the 402 individuals, 17 were excluded due to incoherent (3) or no genotype (14).

For each genotype, we calculated the percentages of resistant and of melanising mosquitoes (Fig. 4A). We note that because not all genotypes were present simultaneously in one family, some comparisons (especially *R2/R2* vs. *S2/S2*) would need to be repeated in new crosses designed differently (for instance in the F3 generation). Still, several patterns emerge from this analysis. In particular, *R1/R1* mosquitoes were more prone to melanise dead parasites than *S2/S2* mosquitoes: the percentage of melanising mosquitoes and the number of melanised parasites per midgut were higher in *R1/R1* than in *S2/S2* mosquitoes (Fig. 4). This and the fact that depletion of *R1 in the F1 progeny of reciprocal crosses between resistant L3-5 and susceptible G3 mosquitoes completely inhibits melanisation of dead parasites, suggest that polymorphisms in *TEP1* not only affect the efficiency of parasite killing, but may also determine the mode of clearance of dead parasites. As TEP1 binds to the surface of invading parasites, it is possible that polymorphims in TEP1 affect the recruitment of key enzymes of the melanisation cascade. In line with this, we have previously observed parasites surrounded by concentric layers of TEP1, PPO (prophenoloxidases which are enzymes involved in melanisation in insects) and TEP1 in resistant L3-5 mosquitoes infected with *P. berghei*, although no colocalisation of the TEP1 and PPO signals was detected (*3*). Further studies are required to confirm the role of TEP1 in controlling the mode of clearance of dead parasites.

X
4 — XH145, XH293
XH503, XH484, XH36
3 — XH53, XH19
2 — XH454
1 — XH711w
5 — XH766
C XH678
6

2R
7 — 2H46, 2H682, 2H417
8 — 2EG15, 2EA1, 2H290, 2EB4, 2EB6
2EC1, 2EC2, 2H175, 2EC4, 2EE2, 2EF1
2EF2, 2ED2, 2EG1, 2ED8, 2EG6, 2ED7
9 — 2ED11, 2EG8, 2EG9, 2EG10, 2EG11
2H157, 2EG12
10 — 2H197, 2H801, SCRASP3
11 — 2H187, 2H85, CLIPB7
12 — 2H799, 2H769
2H815, 2H757
E122, 2H26
13 — 2E123, GNBPB3
2H590
14 — 2E141
15 — 2H135
2H770
16 — 2H147
17 — 2H125
2H786
18 — CLIPB5
2H1
19 —
C —
20 —
21 —
2H161
22 — 2H796
23 — 2H1012
24 — 2H637
2H802
25 — 2H787, 2H215
2Hr7, 2R007
26 — 2H523, 2H778
2H603, 3B20-B1, 2H772
27 —
28 — 2H675, 2H117
2L

3R
29 — 3H93
3H776
3H746, 3H59
30 — 6B10-1
3H249
31 — 3H525, 3A13-12
3H119
32 —
33 — 3H158CD
3H555, 3E33B
3E33C, 9D17-42, 3E33D
3A6-17, 3E34B
34 — 3A23-100, 3E34B2
35 — 3E35B
36 — CLIPB15
3E36D, 3E37A
37 — 3E37B, 3E37B1
C 3E37C1, 3E37C2, 3E37D
38 — 3E38B1, 3E38B2, 3E38B3
3E38B4, 3E38C1, 3E38C2
39 — 3L09-C1, 3E39C, CTL2
40 — 3E40A1, TEP4, 3E40B1
3E40B2, 3E40B3, 3E40C1
41 — 3A2-29, 3H811
3H127, 3H750, 3O10-C2
42 — 3H577, 3H83, 3H544EG, 3H544EB
3H154, 3H758
43 — 3A14-47
44 — 3A19-32
3H817
45 — 3A5-25
3H242
46 — 19A18-54
3L 19C20-B1, 3H88

Fig. S1. Informative markers for QTL mapping. 132 microsatellite markers have been genotyped in parental L3-5 and 4Arr mosquitoes and in the F1 progeny of reciprocal crosses of these two strains to identify markers that are informative, i.e. for which parental L3-5 and 4Arr mosquitoes have two different homozygous genotypes and the F1 progeny is heterozygous bearing one allele from each parent. 34 microsatellites were found informative (orange). An additional seven informative SNPs (cyan) were used for QTL mapping in regions devoid of informative microsatellites, and to increase coverage on chromosome 3L. Pictures of the polytene chromosomes were downloaded from Anobase (http://www.anobase.org/graphic_map/), and microsatellite markers and SNPs were positioned according to their position in the *A. gambiae* genome sequence.
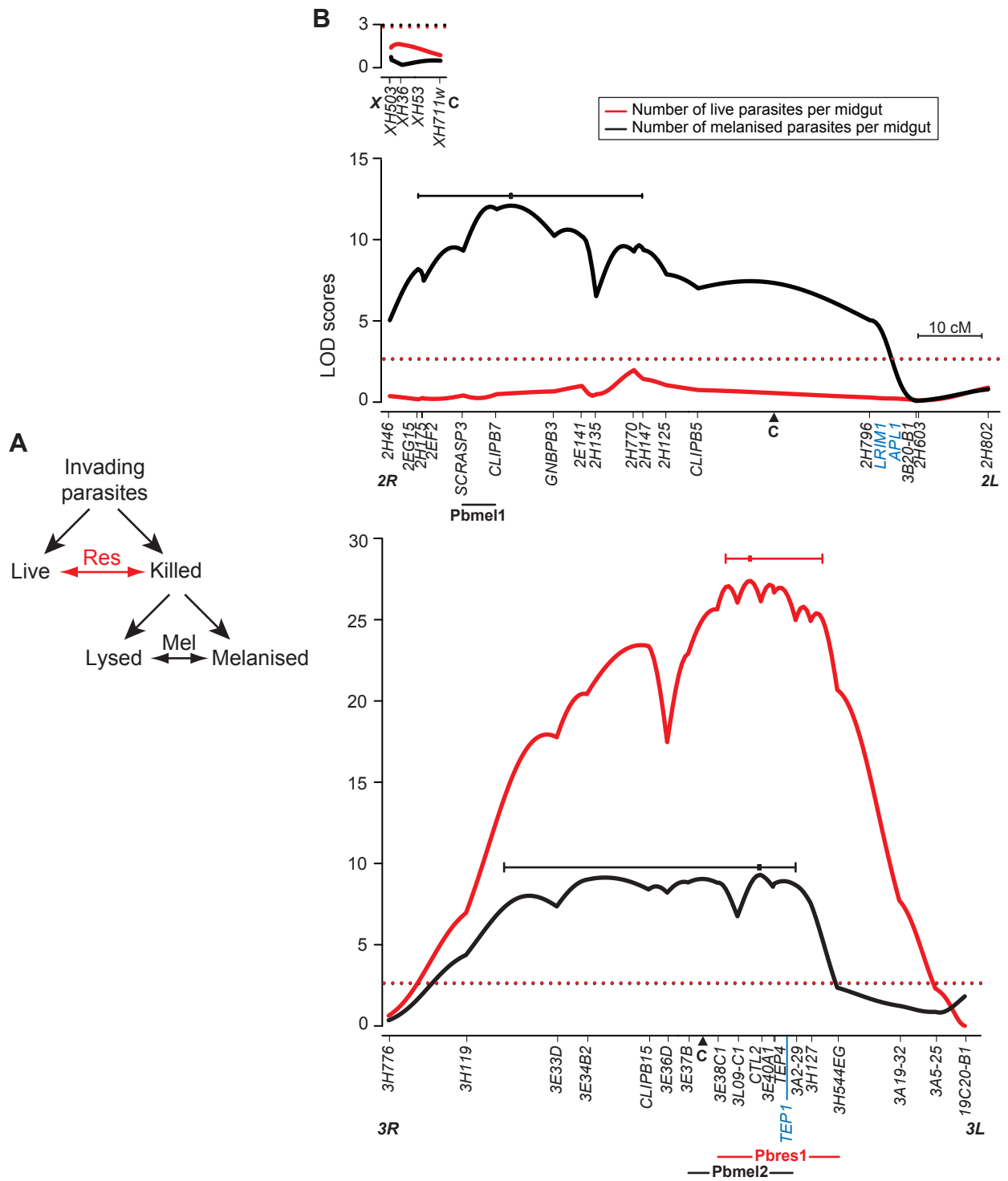
Fig. S2. Mapping of loci controlling resistance and the mode of clearance of dead parasites using counts as traits. (**A**) Parasite elimination in *A. gambiae*. Some of the invading parasites are killed, and dead parasites are disposed of either by melanisation or by lysis. Resistance loci (Res) determine the efficiency of parasite killing, while melanisation loci (Mel) control the choice of mechanism(s) for the clearance of dead parasites. (**B**) Non-parametric simple interval mapping was used to detect association between the number of live (red) or the number of melanised (black) parasites and markers. Estimated LOD scores thresholds are indicated as dotted lines (2.67 for both traits). Confidence intervals (horizontal bars) for the genomic position with maximum LOD score were derived by bootstrapping of the cases. This method maps QTLs at the same location as those identified by CIM using the binary traits "resistant" and "melanising" (*Pbres1*, *Pbmel1* and *Pbmel2*, positioned below chromosomes for comparison). Genetic markers, centromere positions (C), chromosome arms and the *TEP1, LRIM1* and *APL1* loci (in cyan) are indicated below axes.

9

A

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TEP1*R1              βA   βA'     βB          βC          βC'   T    βE              βF
          10        20        30        40        50        60        70        80        90

*S1  MWQFIRSRILTVIIFIGAAHGLLVVGPKFIRANQEYTLVISNFNSQLSKVDLLLKLEGETDNGLSVLNVTKMVDVRRNMNRMINFNMPEDLTAGNYKITI
*S3  MWQFIRSRILTVIIFIGAAHGLLVVGPKFIRANQEYTLVISNFNSQLSKVDLLLKLEGETDNGLSVLNVTKMVDVRRNMNRMINFNMPEDLTAGNYKITI
*S2  MWQFIRSRILTVIIFIGAAHGLLVVGPKFIRANQEYTLVISNFNSQLSKVDLLLKLEGETDNGLSVLNVTKMVDVRRNMNRMINFNMPEDLTAGNYKITI
*R2  MWQFIRSRILTVIIFIGAAHGLLVVGPKFIRANQEYTLVISNFNSQLSKVDLLLKLEGETDNGLSVLNVTKMVDVRRNMNRMINFNMPEDLTAGNYKITI
*R1  MWQFIRSRILTVIIFIGAAHGLLVVGPKFIRANQEYTLVISNFNSQLSKVDLLLKLEGETDNGLSVLNVTKMVDVRRNMNRMINFNMPEDLTAGNYKITI


TEP1*R1  →       βG        βA    βA' TT    βB    TT        βC    TT    βC'    TT  βE              βF
         110       120       130       140       150       160       170       180       190

*S1  DGQRGFSFHKEAELVYLSKSISGLIQVDKPVFKPGDTVNFRVILLDTELKPPARVKSVYYVTIRDPQRNVIRKWSTAKLYAGVFESDLQIVPTPMLGVWNI
*S3  DGQRGFSFHKEAELVYLSKSISGLIQVDKPVFKPGDTVNFRVIVLDTELKPPARVKSVYVTIRDPQRNVIRKWSTAKLYAGVFESDLQIAPTPMLGVWNI
*S2  DGQRGFSFHKEAELVYLSKSISGLIQVDKPVFKPGDTVNFRVIVLDTELKPPARVKSVYVTIRDPQRNVIRKWSTAKLYAGVFESDLQIAPTPMLGVWNI
*R2  DGQRGFSFHKEAELVYLSKSISGLIQVDKPVFKPGDTVNFRVIVLDTELKPPARVKSVYVTIRDPQRNVIRKWSTAKLYAGVFESDLQIAPTPMLGVWNI
*R1  DGQRGFSFHKEAELVYLSKSISGLIQVDKPVFKPGDTVNFRVIVLDTELKPPARVKSVYVTIRDPQRNVIRKWSTAKLYAGVFESDLQIAPTPMLGVWNI


TEP1*R1  → TT    βG          βA      ηA'    βB    TT     βC          βC'          βE              βF
         210       220       230       240       250       260       270       280       290

*S1  SVEVEGEELVSKTFEVKEYVLSTFDVQVMPSVIPLEEHQAVNLTIRANYHFGKPVQGVAKVELYLDDDKLNQKKELTVYGKGQVELRFDNFAMDADQQDV
*S3  SVEVEGEELVSKTFEVKEYVLSTFDVQVMPSVIPLEEHQAVNLTIRANYHFGKPVQGVAKVELYLDDDKLNQKKELTVYGKGQVELRFDNFAMDADQQDV
*S2  SVEVEGEELVSKTFEVKEYVLSTFDVQVMPSVIPLEEHQAVNLTIRANYHFGKPVQGVAKVELYLDDDKLNQKKELTVYGKGQVELRFDNFAMDADQQDV
*R2  SVEVEGEELVSKTFEVKEYVLSTFDVQVMPSVIPLEEHQAVNLTIDANYHFGKPVQGVAKVELYLDDDKLNQKKELTVYGKGQVELRFDNFAMDADQQDV
*R1  SVEVEGEELVSKTFEVKEYVLSTFDVQVMPSVIPLEEHQAVNLTIRANYHFGKPVQGVAKVELYLDDDKLKLKKELTVYGKGQVELRFDNFAMDADQQDV


TEP1*R1  →  TTT     βG          βA      T  T    βB    TT    TT    βC    ηC  βC'    TT   βE        TT   βF
         310       320       330       340       350       360       370       380       390

*S1  RVKVSFIEQYTNRTVVKQSQITVYRYAYRVELIKESPQFRPGLPFKCALQFTHHDGTPAKGITGKVEVSDVGFETTTTSDNDGLIKLELQPSEGTEQLGI
*S3  RVKVSFIEQYTNRTVVKQSQITVYRYAYRVELIKESPQFRPGLPFKCALQFTHHDGTPAKGITGKVEVSDVGFETTKTSDNDGLIKLELQPSEGSEQLGI
*S2  RVKVSFIEQYTNRTVVKQSQITVYRYAYRVELIKESPQFRPGLPFKCALQFTHHDGTPAKGITGKVEVSDVGFETTTTSDNDGLIKLELQPSEGTEQLGI
*R2  RVKVSFIEQHTNRTVVKQSQITVYRYAYRVELIKESPQFRPGLPFKCALQFTHHDGTPAKGITGKVEVSDVGFETTTTSDNDGLIKLELQPSEGTEQLGI
*R1  PVKVSFVEQYTNRTVVKQSQITVYRYAYRVELIKESPQFRPGLPFKCALQFTHHDGTPAKGISGKVEVSDVRFETTTTSDNDGLIKLELQPSEGTEQLSI


TEP1*R1  →   TT    βG    αG     βA      TT      βB          βC    TT     βC'          βE    ηE    βF      TT
         410       420       430       440       450       460       470       480       490

*S1  NFNAVDGFFFYEDVNKVETVTDAYIKLELKSPIKRNKLMRFMVTCTERMTFFVYYVMSKGNIIDAGFMRPNKQTKYLLQLNATEKMIPKAKILIATVAGR
*S3  NFNAVDGFFFYEDVNKVETVTDAYIKLELKSPIKRNKLMRFMVTCTERMTFFVYYVMSKGNIIDAGFMRPNKQTKYLLQLNATEKMIPKAKILIATVAGR
*S2  NFNAVDGFFFYEDVNKVETVTDAYIKLELKSPIKRNKLMRFMVTCTERMTFFVYYVMSKGNIIDAGFMRPNKQTKYLLQLNATEKMIPKAKILIATVAGR
*R2  NFNAVDGFFFYEDVNKVETVTDAYIKLELKSPIKRNKLMRFMVTCTERMTFFVYYVMSKGNIIDAGFMRPNKQTKYLLQLNATEKMIPKAKILIATVAGR
*R1  HFNAVDGFFFYEDVNKVETVTDAYIKLELKSPIKRNKLMRFMVTCTERMTFFVYYVMSKGNIIDAGFMRPNKQPKYLLQLNATEKMIPRAKILIATVAGR


TEP1*R1  βG      →     βA        TT    βB    TT    βC      α1          α2                  α3      β1    β1'
         510       520       530       540       550       560       570       580       590

*S1  TVVYDYADLDFQELRNNFDLSIDEQEIKPGRQIELSMSGRPGAYVGLAAYDKALLLFNKNHDLFWEDIGQVFDGFHAINENEFDIFHSLGLFARTLDDIL
*S3  TVVYDYADLDFQELRNNFDLSIDEQEIKPGRQIELSMSGRPGAYVGLAAYDKALLLFNKNHDLFWEDIGQVFDGFHAINENEFDIFHSLGLFARTLDDIL
*S2  TVVYDYADLDFQELRNNFDLSIDEQEIKPGRQIELSMSGRPGAYVGLAAYDKALLLFNKNHDLFWEDIGQVFDGFHAINENEFDIFHSLGLFARTLDDIL
*R2  TVVYDFADLDFQELRNNFDLSIDEQEIKPGRQIELSMFGRPGAYVGLAAYDKALLLFNKNHDLFWEDIGQVFDGFHAINENEFDIFHSLGLFARTLDDIL
*R1  TVVYDFADLDFQELRNNFDLSIDEQEIKPGRQIELSMSGRPGAYVGLAAYDKALLLFNKNHDLFWEDIGQVFDGFHAINENEFDIFHSLGLFARTLDDIL


TEP*R1   ►                        βC'    TT     βE          βF      TTT   βG    βG'       βA      βA'
         610       620       630       640       650       660       670       680       690

*S1  FDSANEKTGRNALQSGKPIGKLVSYRTNFQESWLWKNVSIGRSGSRKLIEVVPDTTTSWYLTGFSIDPVYGLGIIKKPIQFTTVQPFYIVENLPYSIKRG
*S3  FDSANEKTGRNALQSGKPIGKLVSYRTNFQESWLWKNVSIGRSGSRKLIEVVPDTTTSWYLTGFSIDPVYGLGIIKKPIQFTTVQPFYIVENLPYSIKRG
*S2  FDSANEKTGRNALQSGKPIGKLVSYRTNFQESWLWKNVSIGRSGSRKLIEVVPDTTTSWYLTGFSIDPVYGLGIIKKPIQFTTVQPFYIVENLPYSIKRG
*R2  FDSANEKTGRNALQSGKPIGKLVSYRTNFQESWLWKNVSIGRSGSRKLIEVVPDTTTSWYLTGFSIDPVYGLGIIKKPIQFTTVQPFYIVENLPYSIKRG
*R1  FDSANEKTGRNALQSGKPIGKLVSYRTNFQESWLWKNVSIGRSGSRKLIEVVPDTTTSWYLTGFSIDPVYGLGIIKKPIQFTTVQPFYIVENLPYSIKRG


TEP1*R1  βB      →   βC        βC''  T   TT     βC'    TT    βE          βF    ηF    βG              ■
         710       720       730       740       750       760       770       780       790

*S1  EAVVLQFTLFNNLGAEYIADVTLYNVANQTEFVGRPDTDLSYTKSVSVPPKVGVPISFLIKARKLGEMAVRVKASIMLGHETDALEKVIRVMPESLAQPK
*S3  EAVVLQFTLFNNLGAEYIADVTLYNVANQTEFVGRPDTDLSYTKSVSVPPKVGVPISFLIKARKLGEMAVRVKASIMLGHETDALEKVIRVMPESLAQPK
*S2  EAVVLQFTLFNNLGAEYIADVTLYNVANQTEFVGRPDTDLSYTKSVSVPPKVGVPISFLIKARKLGEMAVRVKASIMLGHETDALEKVIRVMPESLAQPK
*R2  EAVVLQFTLFNNLGAEYIADVTLYNVANQTEFVGRPNTDLSYTKSVSVPPKVGVPISFLIKARKLGEMAVRVKASIMLGHETDALEKVIRVMPESLVQPR
*R1  EAVVLQFTLFNNLGAEYIADVTLYNVANQTEFVGRPNTDLSYTKSVSVPPKVGVPISFLIKARKLGEMAVRVKASIMLGHETDALEKVIRVMPESLVQPR


TEP1*R1  β3      β4        TT   β5       α0       ★      α2                          α3
         810       820       830       840       850       860       870       880       890

*S1  MDTSFFCFDDYKNQTFPFNLDINKKADNGSKKIEFRLNPNLLTMVIKNLDNLLAVPTGCGEQNMVKFVPNILVLDYLYATGSKEQHLIDKATNLLRQGYQ
*S3  MDTSFFCFDDYKNQTFPFNLDINKKADNGSKKIEFRLNPNLLTMVIKNLDNLLAVPTGCGEQNMVKFVPNILVLDYLYATGSKEQHLIDKATNLLRQGYQ
*S2  MDTSFFCFDDYKNQTFPFNLDINKKADNGSKKIEFRLNPNLLTMVIKNLDNLLAVPTGCGEQNMVKFVPNILVLDYLYATGSKEQHLIDKATNLLRQGYQ
*R2  MDTRFFCFDDYKNQTFPINLDINKKADNGSTKIEFRLNPNLLTTVIKNLDHLLGVPTGCGEQNMVKFVPNILVLDYLHAIGSKEQHLIDKATNLLRQGYQ
*R1  MDTRFFCFDDHKNQTFPINLDINKKADSGSTKIEFRLNPNLLTTVIKNLDHLLGVPTGCGEQNMVKFVPNILVLDYLHAIGSKEQHLIDKATNLLRQGYQ


TEP1*R1            TT        α4                  α5             TT              α6
         910       920       930       940       950       960       970       980       990

*S1  NQMRYRQTDGSFGVWEKSGSSVFLTAFVATSMQTASKYMNDIDAAMVEKALDWLASKQHSSGRFDETGKVWHKDMQGGLRNGVALTSYVLTALLENDIAK
*S3  NQMRYRQTDGSFGVWEKSGSSVFLTAFVATSMQTASKYMNDIDAAMVEKALDWLASKQHSSGRFDETGKVWHKDMQGGLRNGVALTSYVLTALLENDIAK
*S2  NQMRYRQTDGSFGVWEKSGSSVFLTAFVATSMQTASKYMNDIDAAMVEKALDWLASKQHSSGRFDETGKVWHKDMQGGLRNGVALTSYVLTALLENDIAK
*R2  NQMRYRQTDGSFGLWETTGGSVFLTAFVGTSMQTAAKYISDIDAAMVEKALDWLASKQHFSGRFDKAGAEVHKEMQGGLRNGVALTSYVLMALLENDIAK
*R1  NQMRYRQTDGSFGLWETTNGSVFLTAFVGTSMQTAVKYISDIDAAMVEKALDWLASKQHFSGRFDKAGAEVHKEMQGGLRNGVALTSYVLMALLENDIAK
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Fig. S3. Allelic variation in *TEP1*. (**A**) TEP1 amino-acid sequence alignment with secondary structure assignment as in (*28*). TEP1*S1 from PEST, TEP1*R1 from L3-5, TEP1*R2 and *S2 from 4Arr and TEP1*S3 from G3. Amino acid differences were colored according to the following groups: AVLICM (gray), GP (yellow), FYW (purple), HNQ (green), ST (cyan), DE (blue), KR (red). (**B**) Crystal structure and domain arrangement of TEP1*R1 from (*28*), where domains are colored as in (A). MG, macroglobulin domain; LNK, linker; TED, thioester domain; ANK, anchor; the red star indicates the position of the thioester bond. (**C**) Divergence at non synonymous sites (Ka) between each 4Arr allele of *TEP1* (*S2* and *R2*) and *S1* (red) and *R1* (violet), and between the two 4Arr alleles (green). The 3'-half is the most polymorphic region. *S2* is most similar to *S1* apart from a short stretch of ~60 bp where it resembles *R1* and *R2* (arrows). *R2* is most similar to *S1* in the 5'-half and to *R1* in the 3'-half.
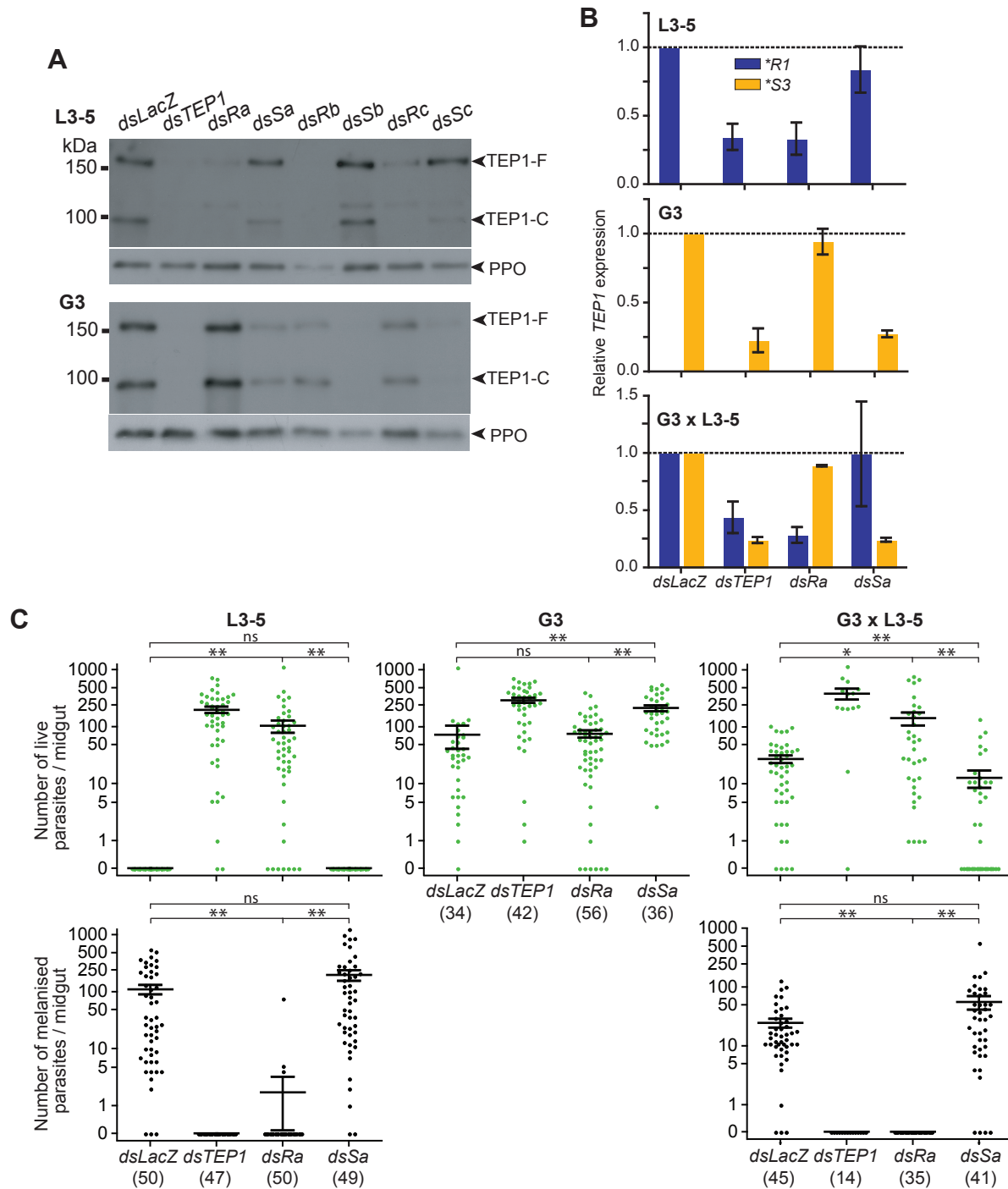
Fig. S4. Reciprocal allele-specific RNAi. (**A**) Hemolymph was collected from L3-5 (top panel) and G3 (bottom panel) mosquitoes four days after dsRNA treatment (10 individuals per dsRNA), and analyzed by immunoblotting. For each pair of short dsRNA (*dsR/dsS*, a-c), *dsR* targets the L3-5 allele (*TEP1*R1*) and *dsS*, the G3 allele (*TEP1*S3*). Controls include *dsLacZ* specific for *LacZ*, and *dsTEP1* which silences both alleles. In G3 and L3-5 mosquitoes, polyclonal TEP1 antibodies recognize a full length (TEP1-F) and a cleaved (TEP1-C) forms. An antibody against the hemocyte-specific prophenoloxidase (PPO) was used as a loading control. (**B**) *TEP1* expression in L3-5, G3 and in the F1 progeny of G3 x L3-5 was measured by quantitative real-time PCR three days after dsRNA-treatment using allele-specific primers and probes. Expression levels of *TEP1*R1* and *S3* were normalised to their levels in the *dsLacZ* control. Ribosomal protein gene *RpL19* was used as an internal control. Mean ± SEM of 3-4 independent experiments. (**C**) Parasite counts in L3-5, G3 and in the F1 progeny of G3 x L3-5 after dsRNA treatment. Results of three independent experiments were pooled, sample sizes are shown in brackets. Mean ± SEM. Significance for differences between groups are indicated (Mann-Whitney on key comparisons): **, $p<0.001$; *, $p<0.05$; ns, not significant.

Tables

Table S1. Informative markers used for genotyping of selected F2 individuals.

Chromosome	Marker	Cytogenetic Position	Genome Position (AgamP3)	Mapping Distance (Mb)	Forward primer (5'-3') [a]	Reverse primer (5'-3') [a]	Repeats / SNP primer (5'-3') [b]	Allele size (L3-5/4Arr) / SNP [c]
X	XH503	4B	1825845-928	0	AGGTTAGAGTGAGCAACCC	GCACTGCATCTCTCCAATAC	(GT)30	82/84
	XH36	4B	1915346-533	0.09	CGTATGTTTGCTAGGGGTGG	GTCAAGAAATGGGCCACAGG	(GT)14	189/193
	XH53	4A	3630267-360	1.8	GTTTCGGGGCTTGAGAAGTG	CTTCACGTGGCTTTGCTGTG	(GT)7	91/95
	XH711w	2B	9884944-5074	8.06	CCCACAGCAAAACGAGAAG	GACAACTTGCATTTCACTATG	(GA)9+4	125/129
2R	2H46	7A	1247911-8052	0	CGCCCATAGACAACGAAAGG	TGTACAGCTGCAGAACGAGC	(GT)8	147/143
	2EG15	8B	5798929-9171	4.55	CCTGTTTCCTTTCGCTTCTG	GCAATCTATCATGCGGAGGT	(GT)12	235/245
	2H175	8D	6554063-159	5.31	AGGAGCTGCATAATTCACGC	AGAAGCATTGCCCGCATTCC	(CA)8	97/95
	2EF2	8D	6688476-738	5.44	CAGACACATTTTCGCTCACG	CACACAATGGCACTGGTTTT	(GT)8G	261/264
	SCRASP3	9C	13048252-271	11.8	CATCCGGCCATCTACAATTC	AACCGTTACGGAGGTGGTAA	CGCAACACCGTCGGGAATGA	TC
	CLIPB7	11A	18410803-25	17.16	CTTCCTGCTCACAGCTTCCT	ACACAGGTTCCCGGCTCT	GTAGTGTGTGGTACAAACAAAGC	TC

	GNBPB3	13B	27646565-84	26.39	ATCAACCCCATCAAGAGTGC	AGAGCCAGTCACCGGTAGG	AAGTACGGACGGATGGAGGT	GA
	2E141	14A	32093046-65	30.85	GCAAAAGCGGCAAGGACTCG	GCGTCCCCGTCTCCTTATC	(GT)10	134/137
	2H135	14D	34371317-96	33.12	TCATGCACTGTTTGCTCGGC	CTGCCCCATTCAATTGCAGC	(GT)7	104/81
	2H770	15D	40443556-721	39.19	CAAGATGGAGGCGCATGATC	GCGTTCCATCGAAATCAGAC	(GT)8+4	172/156
	2H147	16D	41979597-768	40.73	CTGCTGTTGCTGCCAAAATG	AGCTTCACGGAAAGCAAAGG	(GT)8	179/175
	2H125	17A	45660190-281	44.41	AGGAGCATAACACATCGCCC	CGCTCGTCAAAGAAACTGGC	(GT)11	91/93
	CLIPB5	18B	50743688-707	49.49	CACTGCGTCAACGGTAAAGA	GGAGCAGTCAACGTCATCG	GGCGAATGGGACACCTCTAC	GT
C	2H796	22C	16729412-98	77.03	CTTTGCCATTGCACGGTCCC	TTCGGCTCCGCTCACTCAAC	(GT)10	85/97
	20-B1	26C	42424297-418	84.48	GAGACCAAAACATGTCAACCG	GTATGTCTGCGCATGAACGAT	(CA)17	105/121
	2H603	26C	42014745-850	84.89	TGCACCGTTGATGCACATGC	GTGGACGATGTGAAAGATAAGG	(GT)7	153/107
2L	2H802	24C	30876135-263	96.03	TTTGGTGAGGGGTTTGTTCC	GGCAATTCATTCGTCACACC	(TG)31+24	136/134
3R	3H776	29C	2439524-617	0	TGCGGATCATAATCGAGTCC	TCACAAACACGCAACGAGTC	(GT)7	98/96

	3H119	31A	14828037-231	12.39	GGTTGAT GCTGAAG AGTGGG	ATGCCAG CGGATAC GATTCG	(GT)6	185/195
	3E33D	33D	29371886-991	26.93	CCAAGAG AGGGAGG CACATA	CCAGCCT CTCAGCTT TGGTA	(GT)14	96/98
	3E34B2	34B	34193977-4151	31.75	TCGGTTG GGTACAA GAAAGG	GGTTTGCC ATTCTGCT TTGT	(GT)14	162/175
	CLIPB15	35D	44101708-28	41.66	CTTTTCGC ACTACGA CACGA	CGCACGT AGGTGAA TTGCTA	GGTGCC TATGCG CAAAAA GCC	CA
	3E36D	36B	47071241-524	44.63	TCTGTTAG CCGATCA TGTGC	GGTTAGG GCAGTGT GGGTATT	(GT)10	279/275
	3E37B	37B	50439481-608	48	TGGAATTT GGACTCG TGTGA	AACGTGT CTCGCTAC TGCAC	(CA)9	120/126
C	3E38C1	38A	1878876-9144	52.64	TCGCCGT ATATATTA AAGCTTG TG	GGCACAA CAACAAT GAATGTG	(CA)17	263/261
	3L09-C1	38B	5123942-4110	55.89	GAAACTT CCCTGAA TTTCCGC	ATGGAGG CGACTAA ACGTTGC	(CA)10+8	167/169
	CTL2	39B	8879556-75	59.64	CAAACGG CCTTTCAA AATTC	CCACTCAT CCTTCCAA CAGG	GCCAAG CTGCGTT CCCGAA A	GC
	3E40A1	39C	10802338-543	61.56	CGCATCA AAAGTGA ACATGC	GAATTATT CCCGCCA GTGTG	(GT)10	206/194
	TEP4	39C	11004857-76	61.77	TGTTTGCA CGAACAA TGGAG	CACAGCC AAGATTC GAGAAA	AGACGG CTCGAG ATGGAT CG	GT
	3A2-29	40C	14448042-230	65.21	GGCTGCT CTAGGAG GATGTG	AATCGAG GGTGGTG TGAATG	(CT)16	173/179

	3H127	41A	16858897-976	67.62	CCTCTAAC TCGATTAC CGTG	GTCAGGG AATTGGA AAGAGC	(GT)12	80/72
	3H544E G	42A	21119886-904	71.88	GCTCGTC GATCCTG ATCGAA	GCTCTTTA ATTTCTCG CCC	(GT)9	174/176
	3A19-32	43D	31065213-350	81.83	CGTGCTG GCATTAA AAAGTG	CTACGCA TGCATCTT TCTCC	(CA)15	125/119
	3A5-25	45A	36841460-584	87.6	CCTTGGG CGCTATA AAACAC	TATTAGC AGCAGGG GAAACC	(TG)10	126/132
3L	19C20-B1	46C	41558503-660	92.32	ACCAAGT GCTCTTTG GGTTG	ACGAATG CTGGACA ATGTGA	(TG)15	159/161

32 microsatellites: black, and 7 SNPs: blue.

[a] Forward and reverse primers used to amplify microsatellites or SNPs.

[b] Microsatellites: repeats and number of repeats; SNPs: one base extension SNP primer.

[c] Microsatellites: allele sizes in the L3-5 and 4Arr parental strains; SNPs: SNP type.

Table S2. Genotyping of F2 mosquitoes at the *TEP1* locus.

PCR[a]			Signal per Genotype					
Forward Primer	Reverse Primer	Ta (ºC)	*R1/ R1	*R1/ R2	*R1/ S2	*R2/ R2	*R2/ S2	*S2/ S2
AAAGCTACGAA TTTGTTGCGTCA (EL101)	CCTCTGCGTGCT TTGCTT (EL119)	60	X	X	X	X	X	
AAAGCTACGAA TTTGTTGCGTCA (EL101)	ATAGTTCATTCC GTTTTGGATTAC CA (EL117)	60			X		X	X
GCTGTACCTAG ACGACGATAAG CTAAAACT (EL295)	CCGTGCACGTC ACCATGAAA (EL296)	67	X	X	X			
GCTGTACCTAG AMGACGATAAG CTAAATCA (EL307)	CCGTGCACGTC ACCATGAAG (EL309)	63		X	X	X	X	X

[a] PCR conditions: 2 min at 94°C; followed by 35 cycles of 10 sec at 94°C, 15 sec at Ta, 15-30 sec at 72°C and a final extension of 5 min at 72°C. PCRs were performed on genomic DNAs extracted from F2 mosquitoes.

Table S3. Primers and restriction enzymes used to prepare allele-specific dsRNA probes.

	PCR [a]			plasmid linearization [b]	
	Forward Primer (5'-3')	**Reverse Primer (5'-3')**	**Size (bp)**	**RE1**	**RE2**
dsRa	TTAGCCTCGAAGCAGCATTT	CCCTCCTTGCATTTCTTTGT	75	Hind III	Pst I or Bam HI
dsSa	TTGGCCTCGAAGCAGCACAG	CCCTCCTTGCATATCTTTGT	75	Hind III	Pst I or Bam HI
dsRb	GGAGCAAATTGATGTGGAAAA	TCTCAAAGTTGACGAGATTCAAA	134	Nde I or Hind III	Pst I or Bam HI
dsSb	CGGAGCAAATTGATTTCCAA	CGATGCTCAAAGTTGACGAG	139	Nde I or Xho I	Pst I or Bam HI
dsRc	ATGTGTTGATGGCATTGCTG	GGATCCAAACTGATTGCTCA	98	Hind III	Pst I or Xma I
dsSc	TCGTATGTGTTGACGGCATT	GAATGCAAGCTGATTGCTCA	102	Hind III	Pst I or Bam HI

[a] PCR conditions: 2 min at 95°C; then 30 sec at 95°C; 30 sec at 60°C; 30 sec at 72°C for 35 cycles and 10 min at 72°C. PCR were performed on cDNA obtained from L3-5 and G3 mosquitoes.
[b] Plasmids were linearized separately with two restriction enzymes produce templates for ssRNA synthesis.

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