

Supporting Information

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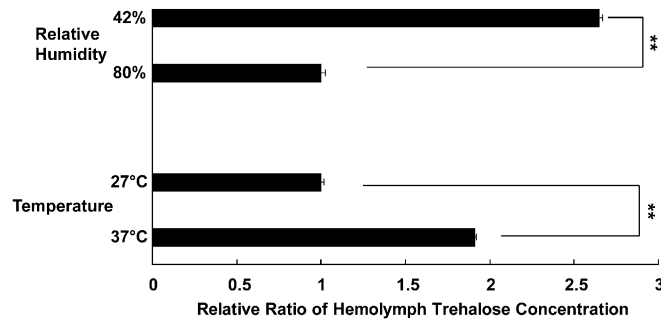


Fig. S1. Normalized hemolymph trehalose concentrations after stress treatments. Mosquito hemolymph was extracted after desiccation and heat for 2 d, and trehalose concentrations were measured. Using trehalose levels under control conditions (80% relative humidity and 27 °C) as references, the relative trehalose concentration in mosquitoes at 42% relative humidity is 2.6 ± 0.02 times of that in control mosquitoes, and 1.9 ± 0.01 times of control at 37 °C. Both are statistically significant with $P \leq 0.01$ by Student's *t* test.

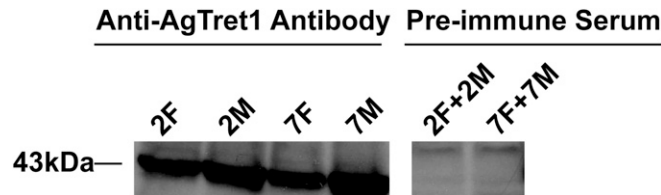


Fig. S2. Western blot of pooled whole adult *Anopheles gambiae* mosquitoes. (Left) Western blot using AgTret1 antibody; sample in each lane is 2F (2-d-old females), 2M (2-d-old males), 7F (7-d-old females), and 7M (7-d-old males). (Right) Western blot using preimmune serum, sample in each lane is 2F+2M (2-d-old adults including same numbers of females and males) and 7F+7M (7-d-old adults including same numbers of females and males). Sample loading is normalized with mosquito numbers.

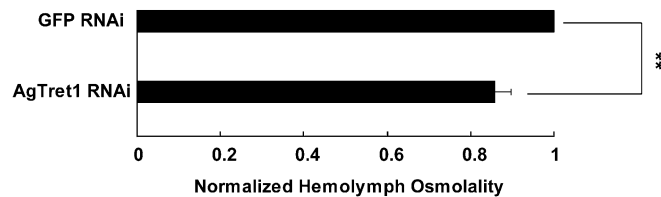


Fig. S3. Normalized hemolymph osmolality in RNAi mosquitoes. Using the hemolymph osmolality of GFP dsRNA-injected mosquitoes as reference, the normalized hemolymph osmolality in AgTret1-silenced mosquitoes is reduced by 14% compared with controls. $**P \leq 0.01$ by Student's *t* test.

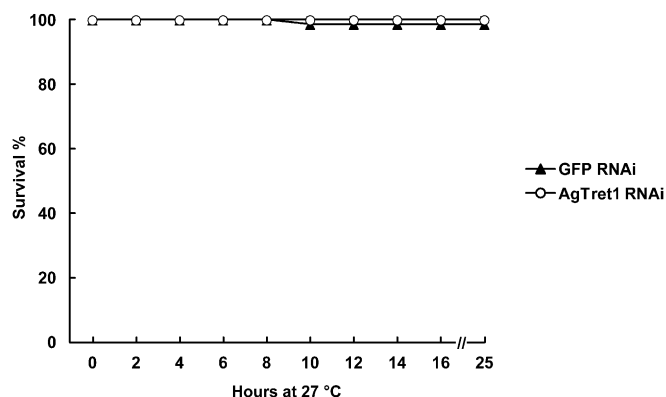


Fig. S4. Survival rate of dsRNA-injected mosquitoes under control condition (27 °C and 80% relative humidity). In three repeats with 22–25 mosquitoes injected with GFP- or AgTret1-dsRNA each time, the survival rate is $98.7 \pm 2.3\%$ or $100 \pm 0\%$. There is no significantly different death rate at the 27 °C control condition. x axis, hours at 27 °C; y axis, mosquito survival rate in %.

Table S1. Summary of desiccation and heat assays

Exp. no.	<i>n</i> (GFP RNAi)	<i>n</i> (AgTret1 RNAi)	<i>P</i> value of survival analysis
Antidesiccation assay			
1*	31	38	$P \leq 0.05$
2	35	34	$P \leq 0.01$
3	36	34	$P \leq 0.05$
Antiheat assay			
1*	39	39	$P \leq 0.05$
2	39	25	$P \leq 0.01$
3	27	32	$P \leq 0.05$

Total mosquito numbers in control (GFP RNAi) or AgTret1 RNAi group and the corresponding *P* values of survival analysis in three experiments are shown. The experiments with * are the ones chosen as the representative plots in Fig. 3 A and B.

Table S2. Summary of oocyst-counting results

Exp. no.	Mean oocysts and <i>n</i> (GFP RNAi)	Mean oocysts and <i>n</i> (AgTret1 RNAi)	<i>P</i> of Mann–Whitney <i>U</i> test
1*	Mean = 16.5, <i>n</i> = 59	Mean = 4.6, <i>n</i> = 60	$P \leq 0.01$
2	Mean = 3.0, <i>n</i> = 78	Mean = 1.0, <i>n</i> = 71	$P \leq 0.05$
3	Mean = 18.9, <i>n</i> = 43	Mean = 7.0, <i>n</i> = 46	$P \leq 0.05$

Mean oocysts per midgut, mosquito numbers in control (GFP RNAi) or AgTret1 RNAi group, and the corresponding *P* values of nonparametric Mann–Whitney *U* test in three biological repeat experiments are shown. The experiment with * is the one chosen as the representative plot in Fig. 4.

Table S3. Primers used for AgTreT1 cloning, RT-qPCR, and RNAi

For AgTreT1 Cloning	
TreT1_F	ACAGAATTC CATGAAATGGGCACGAAAGAG
TreT1_R	TCTAGCTAGCTTACATGTTGAACGACAGC
For AgTreT1 RNAi	
TreT1_RNAiF2b	TAATACGACTCACTATAGGGAGAAGAAGCTGTGCACGATCATCGTCG
TreT1_Rb	TAATACGACTCACTATAGGGAGATTACATGTTGAACGACAG C
EGFP-RNAi-F2	TAATACGACTCACTATAGGGAGACTCGTGACCACCTGACCTACG
EGFP-RNAi-R2	TAATACGACTCACTATAGGGAGAGATCTTGAAGTTCACCTTGATGCC
For AgTreT1 qPCR	
AgTreT1_qF	TTCGGTAACATCGGCATCC
AgTreT1_qR	CTCGGAATGAGGAACATCAG
For S7 qPCR control	
S7A	TCCTGGAGCTGGAGATGAAC
S7B	GACGGTCTGTACCTTCTGG