

Figure S1. A schematic representation of the pAehsp-pBac helper plasmid. Schematic representation of the pAehsp-pBac helper construct. This helper consists of the 0.66-kb *Aehsp70* promoter upstream of the *piggyBac* transposase coding region and polyadenylation.

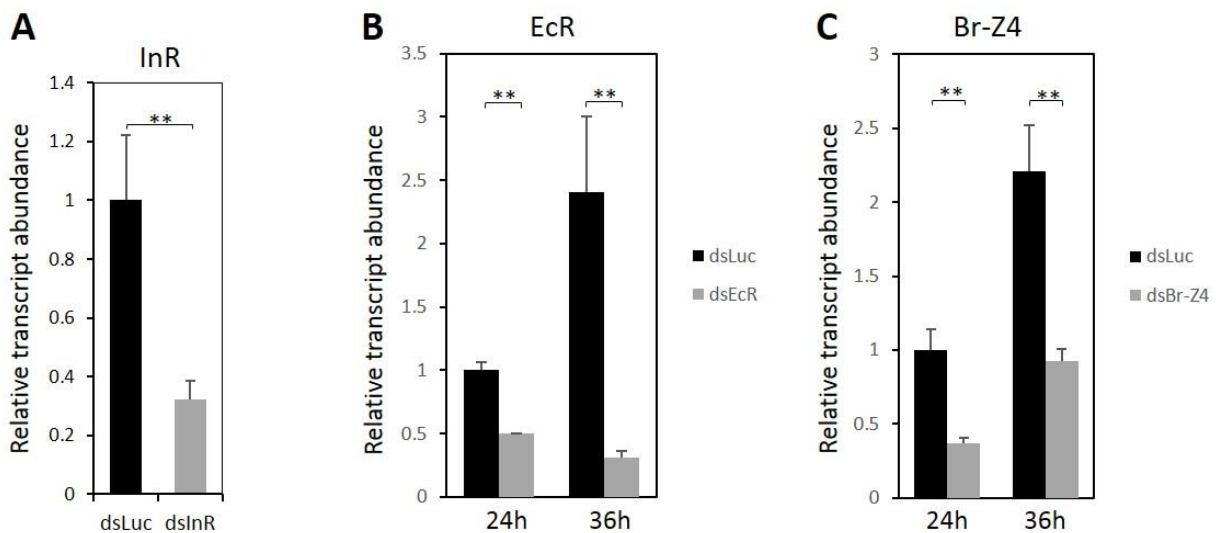


Figure S2. RNAi depletion of *InR*, *EcR* and *Br-Z4* genes. Transcript levels of *InR* (dsInR), *EcR* (dsEcR) and *Br-Z4* (dsBr-Z4) in the CP-Gal4>UAS-EGFP female mosquitoes after their respective dsRNA knockdown. Transcript levels of these genes were expressed relative to the Luciferase RNAi (dsLuc) control (standardized to 1). Times indicate hours after blood feeding.

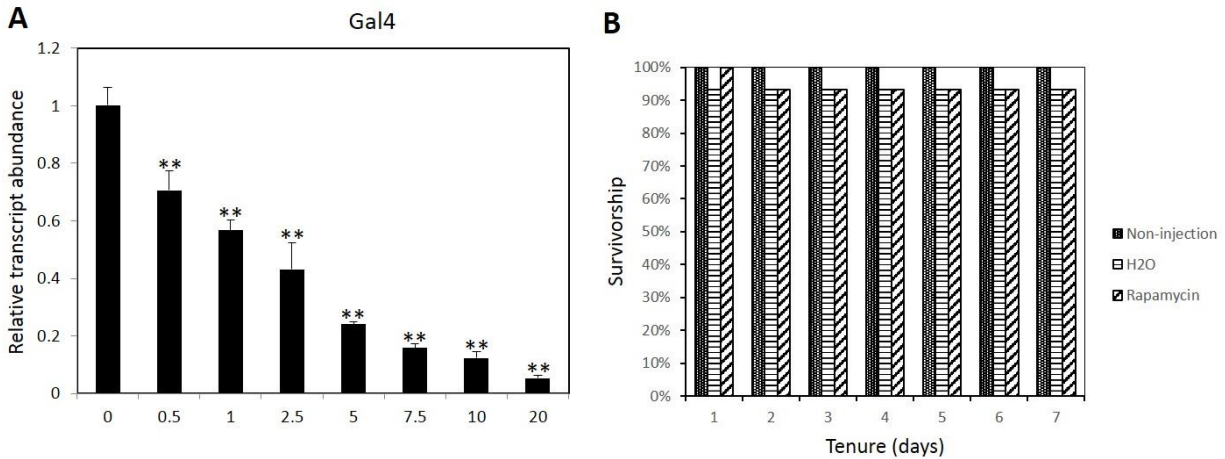


Figure S3. Effect of rapamycin on transgene expression and survival in the hybrid female mosquitoes. (A) A dose–response effect of the TOR inhibitor rapamycin on expression of the *CP-Gal4>UAS-EGFP* transgene. Three-day-old hybrid female mosquitoes were injected with rapamycin (0–20 pmol) and blood fed 1 day later. The transcript level of the *CP-Gal4>UAS-EGFP* transgene was measured in midguts 24 h PBM by means of qRT-PCR analysis using the Gal4 primer. The transcript level of control mosquitoes injected with dH<sub>2</sub>O was set at 1, while transcript levels for all other treatments were expressed relative to the control. Times designate injection amount (pmol). (B) Survivorship of female mosquitoes injected with 20 pmol of rapamycin. Non-injected mosquitoes served as a control. For each experimental set, the initial population size is 15 *CP-Gal4>UAS-EGFP* female mosquitoes. No difference was observed in survival between mosquitoes injected with H<sub>2</sub>O and those treated with rapamycin. Times indicate days after injection.

No. of embryos injected	No. of G <sub>0</sub> survived to adulthood	No. of transgenic lines	Transformation efficiency
1084	90	2	2/90=2.2%

Table S1. Transformation data from *Ae. aegypti* lines using *piggyBac* transposable elements as insertion vectors.

Line	Location	Sequence
M16-1	39 kb from 5' end of AAEL010518	AATAACGTTATAAAAAAAAAACTTAA- <i>piggyBac</i> - TTAAACTAAAGGCTCAACC
F4-4	8 kb from 5' end of AAEL004671	TGCAGTAACAGCTCTCTATTAA- <i>piggyBac</i> - TTAAACTTTATGTGTATCAGATAC

Table S2. Genomic integration sites in *Ae. aegypti* transgenic lines M16-1 and F4-4.

Gene (PCR and RT-PCR)	Sequence 5'-3'
<u><i>Actin</i></u>	
Forward	AAGGCCAACCGTGAGAAGATGACT
Reverse	GCTCGTTGCCAATGGTGATGAC
<u><i>CP</i></u>	
Forward	GGGATTTGCCTATGTTTCGGAGT
Reverse	GCACCTCTGGCACGCTTCGA
<u><i>CPT</i></u>	
Forward (CPT-F)	TGGATCCGCGAGCTTGAAG
Reverse (CPT-R)	CGTTGCTACTGTTAGTGAAAGTGAA
<u><i>UAS-EGFP</i></u>	
Forward (UAS)	CAAGAAGAGAACTCTGAATAGGG
Reverse (158)	TGAAGTCGATGCCCTTCAGCT
<u><i>piggyBac (Left Arm)</i></u>	
Forward (L1)	TGTCAATGCCGGTAAGTGTC

Reverse (L2)	CCTCTGTGGCAAGGTCAAGA
<u><i>piggyBac</i> (Right Arm)</u>	
Forward (R1)	TGATGACCTGCAGTAGGAAGACG
Reverse (R2)	AGAAACAACCTTTGGCACATATCA
<u><i>Inverse PCR</i> (Left Arm)</u>	
574F	TCTTGACCTTGCCACAGAGG
157R	TGACACTTACCGCATTGACA
<u><i>Inverse PCR</i> (Right Arm)</u>	
2388F	GTCAGTCCAGAAACAACCTTTGGC
2123R	CCTCGATATACAGACCGATAAAAACACATG
<u><i>Aahsp70</i></u>	
Forward	CTCGAGTCCAGTCTTTTTGAAGTCGCGAA
Reverse	AAGCTTCTTTAATTAGTGTTGTTTTGACGAGA

Gene (qRT-PCR)	Sequence 5'-3'
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*Actin*

Forward	GACTACCTGATGAAGATCCTGAC
Reverse	GCACAGCTTCTCCTTAATGTCAC

*CP*

Forward	CTGGAGCAGTCGAGTGATAGTTTG
Reverse	CAATCTGACACGGACACCTTCG

*Gal4*

Forward	TTCATCTTTCAGGAGGCTTGCT
Reverse	GAACTATAAATGGCACCTGATTGC

*EGFP*

Forward	CAGGGTGGTCACGAGGGTGG
Reverse	GTGCCCATCCTGGTCGAGCT

*InR*

Forward	GATAAACTGCGGGACATCGTG
Reverse	TGTCGGTGACGTCGATGGTA

*EcR*

Forward	AAGCGAGGTTATGATGTTGCG
Reverse	CAGCAGGTCCTCTATCGTGTCC

*Br-Z4*

Forward TTCACCACAAGGTATGAGCACAG  
Reverse GCAGTCAGCGGAAGCGGT

Gene (dsRNA) Sequence 5'-3'

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*dsLuc*

Forward TAATACGACTCACTATAGGGCTCTGCCTCATAGAACTGCCTG  
Reverse TAATACGACTCACTATAGGGAACCTTCGCTTCAAAAATGGA

*dsInR*

Forward TAATACGACTCACTATAGGGGAGATCACCGAGTACCTGCTGC  
Reverse TAATACGACTCACTATAGGGGCAACAGACCCCGGTCTTG

*dsEcR*

Forward TAATACGACTCACTATAGGGAAGCGAGGTTATGATGTTGCGAATG  
Reverse TAATACGACTCACTATAGGGTGAGGACGAGGACTGGGTGCC

*dsBr-Z4*

Forward TAATACGACTCACTATAGGGATCGGATTTAAACCACTCCAGCG  
Reverse TAATACGACTCACTATAGGGTGCGCAGTCTTTGTTGAACATCTAT

Table S3. List of primers.