

Figure S1. Adulticidal activity of the first-generation laboratory Sh.463 yeast strain. The Sh.463 laboratory strain induced significant adult morbidity in *A. aegypti* (left) and *A. gambiae* (right) when delivered as an ATSB in 5% sucrose. *** = $P < 0.001$ vs 5% sucrose (ASB) or vs. the control yeast treatment (Control). ~5 μ l of 0.4 μ g/ μ l yeast in 5% sucrose was delivered to each of 25 adult females in replicate trials. Mean mortalities observed in six replicate trials are shown, and error bars refer to SEM.

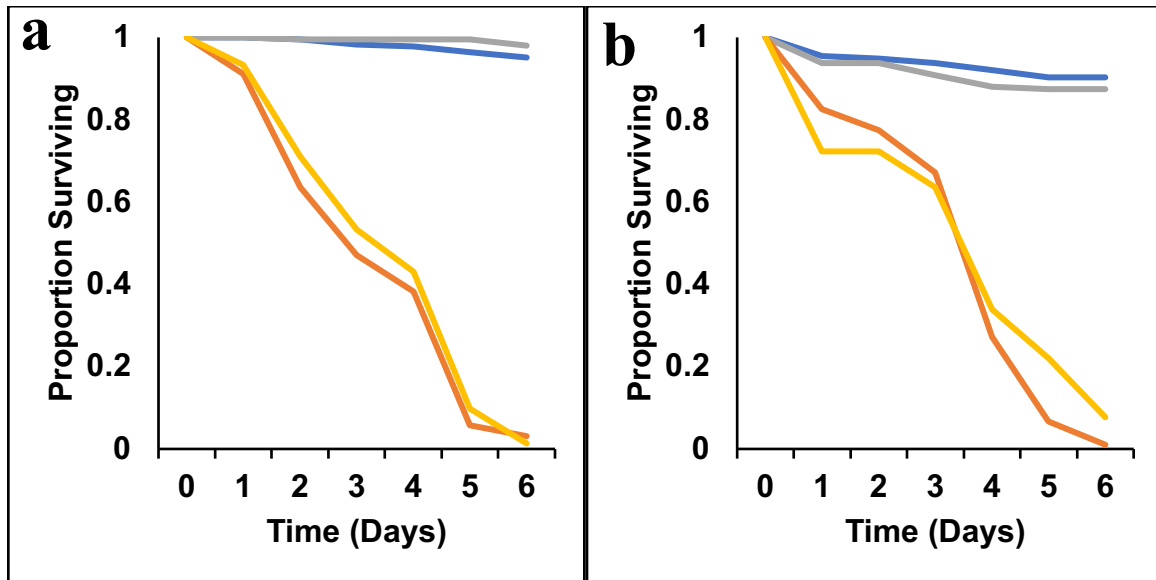


Figure S2. Ingestion of Sh.463 Cas-CLOVER/piggyBac-synthesized yeast strains results in adult mosquito mortality. *A. aegypti* (a) and *A. gambiae* (b) adult females were fed sugar bait alone (blue), or sugar bait with insecticidal DMT9-52.2R#3 yeast (yellow), insecticidal DMT9-56.10R#3 yeast (orange), or control yeast (grey). Although mosquitoes that fed on sugar bait alone or sugar bait with control yeast survived, ingestion of either of the insecticidal yeasts resulted in mosquito death within six days in simulated field trials performed in the insectary. Data were prepared from six replicate trials in which each of 25 adult females fed on $\sim 5 \mu\text{l}$ of $0.2 \mu\text{g}/\mu\text{l}$ yeast in sugar bait.

Table S1

Description	Sequence
shRNA of siRNA.463	gttaaattatctaggcattcgattcaagagatcgaatgcctagataatttaa
Target sequence <i>Shaker</i> (AAEL000242)	auuuuuuuuaucuaggcauucgaaa
Truncated (defective) <i>leu2</i> nutritional promoter	atatatatattcaaggatataccattcta
3' piggyBac Inverted Terminal Repeat transposable element	ttaaccctagaaagataatcatattgtgacgtacgttaaagataatcatgcgtaaaattgacgcatgtgtttatcggtctgtatcgcaggtttatttatta attgaatagatattaagttttattatatttacacttacatactaataataaattcaacaacaattttattatgtttatttattataaaaaaaacaaaaactc aaaatttctctataaagtaacaaaactttta
5' piggyBac Inverted Terminal Repeat transposable element	gatatctataacaagaaaatatatatataataagttatcacgtaagtagaacatgaataacaataataattatcgtatgagttaaactctaaaagtcacgt aaaagataatcatgcgtcattttgactcacgcggtcgttatagttcaaaatcagtgacacttaccgcattgacaagcacgcctcacgggagctccaa gcggcgactgagatgtcctaataatgcacagcgacggattcgcgctatttagaaagagagagcaatattcaagaatgcatgcgtcaattttacgcag actatctttctagggttaa
piggyBac Core Insulators	gaggggacagccccccccaaagccccagggatgtaattacgtccctccccgctaggggggcagcagcgagccgccccggggctccgctccg gtccggcgctcccccgcatccccgagccggcagcgtgcggggacagccggggcacggggaagggtggcacgggatcgcttcctctgaacg cttcctcgtcgtctttgagcctgcagacacctgggggggatacggggaaaa

<p>piggyBac Transposon</p> <p>carrying Sh.463 expression</p> <p>cassette for DMT9-52.2R #3</p>	<p> ttaaccctagaaagataatcatattgtgacgtacgttaaagataatcatgcgtaaaattgacgcatgtgtttatcggtctgtatcgcaggtttatttatta atttgaatagatattaagttttattatatttacacttacataactaataataaattcaacaaacaattttattatgtttatttatttataaaaaaaacaaaaactc aaaattttcttataaagtaacaaaacttttatagaattctatctagagggacagccccccccaaagccccaggatgtaattacgtccctcccc gctagggggcagcagcgagccgccccggggtccgctccgggtccggcgctccccccgcacccccgagccggcagcgtgcggggacagccc gggcacggggaaggtggcacgggatcgcttctctgaacgcttctcgctgctctttgagcctgcagacacctggggggatcggggaaaaggc ctccaaggccagcttccacaataagttgggtgaattttggctcattctcttctataggattgaggtcagagctttgtatgggaattctgtggaat gtgtgtcagttagggtgtggaagtcccgcgacgctcattatcaatactgccatttcaaagaatacgtaaataattaatagtagtgatttcttaactt tatttagtcaaaaaattagccttttaattctgctgtaacccgtacatgccccaaatagggggcggttacacagaatatataacatcgtaggtgtctgg gtgaacagtttattcctggcatccactaaatataatggagcccgttttaagctggcatccagaaaaaaaagaatccagcaccaaaattgtttt cttcaccaaccatcagttcataggtccattctcttagcgcaactacagagaacagggggcacaacaggcaaaaaacgggcacaacctcaatgga gtgatgcaacctgcctggagtaaattgatgacacaaggcaattgaccacgcgatgtatctatctcttcttacaccttctattaccttctgctctctga tttgaaaaagctgaaaaaaaaggttgaaccagttccctgaaattattcccctacttgactaataagtataaaagacggtaggtattgattgtaattct gtaaacttatttctaaactttaaattctacttttatagttagcttttttttagttttaaaacaccagaacttagtttcgacggattctagaactagtgatcc gttaaattatctaggcattcgattcaagagatcgaaatgcctagataatttaatttttctcagtcagtaattagttatgtcacgcttacattcacgcctc cccccacatccgctctaaccgaaaagggaaggttagacaacctgaagtctaggtccctatttttttatagttatgttagtattaagaacgttattta tattcaaatttttcttttttctgtacagacgctgtacgcatgtaacattatactgaaaacctgttgagaagggtttgggacgctcgaaggctttaatt tgccggccggcctgcataatataattcaaggatataaccattctaattgctgcccctaagaagatcgctgtttgccagggtgaccacgttggtcaagaa atcacagccgaagccattaaggttctaaagctatttctgatgttcgttccaatgtcaagttcgatttcgaaaatcatttaattggtggtgctgctatcgat gctacaggtgttccactccagatgaggcgctggaagcctccaagaaggctgatccgtttgttaggtgctgtgggtgtcctaaatggggtacc ggtagtgttagacctgaacaaggtttactaaaaatccgtaagaacttcaattgtacgccaacttaagaccatgtaactttgcatccgactctcttttag acttatctccaatcaagccacaatttgctaagggtactgacttcgtgtgtgcagagaattagtgagggtatttactttggaagagaaggaagacg atggtgatggtgtcgttgggatagtgacaataacaccgttcagaagtgcaagaatcacaagaatggccgctttcatggccctacaacatgagc caccattgcctatttggctcctggataaagctaattgtttggcctctcaagattatggagaaaaactgtggaggaaaccatcaagaacgaattcccta cattgaaggttcaacatcaattgattgattctgccgccatgatctagttaagaaccaacccacctaaatgggtattataatcaccagcaacatgtttgg tgatatcatctccgatgaagcctccgttatccagggttccttgggtttgttgcctatctgcgtccttggcctctttgccagacaagaacaccgcatttgggt tgtacgaacctgccacgggtctgtccagatttgcaaagaataaggtaaccttatgccactatctgtctgctgcaatgatgttgaaattgtcatt gaacttgctgaagaaggtaaggccattgaagatgcagttaaaaagggttttgatgcaggtatcagaactgggtgatttaggtggttccaacagtacc accgaagtcggtgatgctgcgccgaagaagttaaaaaatcctgtctaaaaagattctctttttatgatattgtacataaaactttataaatgaaattc ataatagaaacgacacgaaattacaaaatggaatatgttcatagggtgagcgaactatatacgcaatctacatacatttatcaagaaggagaaaaa ggaggatgtaaaggaatacaggttaagcaattgataactaatggctcaacgtgataaggaaaaagaattgcatttaacattaatattgacaaggag gagggcaccacacaaaaagttagggtgaacagaaaaatcatgaaactatgattcctaatttatatttgaggattttcttaaaaaaaaaaaaaatacaa caaataaaaaaactcaatgacctgaccatttgatggagttaagtcaataccttcctgcaggtgtcctcacaggaacgaagtccctaaagaaaca gtggcagccagggttagccccgaattgactggattccttttttagggccattgggtatggcttttccccgtatccccccagggtgtctgcaggctcaa </p>
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Native leucine promoter	aactgtgggaataactcaggtatcgtaagatgcaagagttcgaatctcttagcaaccattatTTTTTctcaacataacgagaacacacaggggcgct atcgcacagaatcaaattcgatgactggaaatTTTTgttaattcagaggtcgcctgacgcatatacctTTTcaactgaaaaattgggagaaaaagga aagggtgagaggccggaaccggctTTTcatatagaatagagaagcgttcatgactaaatgctgcatcacaatacttgaaagtgacaatattttaag gacctattgTTTTccaataggtggttagcaatcgtcttacttctaactTTTcttacTTTTacatttcagcaatatatatatatatttcaaggatataccattct a
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Table S1. Sequences of the indicated features are provided.