SUPPLEMENTARY INFORMATION

Efficient population modification gene-drive rescue system in the malaria mosquito *Anopheles stephensi*

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This document contains:

- Supplementary Figures 1-6
- Supplementary Tables 1-13

Supplementary Figure 1. Confirmation of precise site-specific integration and *kh* recoding in the Rec*kh* gene drive line. a) Schematic representation of the integration locus in Rec*kh*, as in Fig. 1b, showing annealing sites of the primers used to verify the integration sites (black and green triangles) of the pRec*kh* recoding element into the nRec line. Recoded *kh* A.gam3': recoded portion of the *kh* cDNA sequence followed by the 3'-end regulatory sequence of the *An. gambiae kh* gene. attP: recombination site for *φC31*-mediated integration. 3xP3-GFP: fluorescent marker driven by an eye specific promoter. *vasa* promoter-Cas9: Cas9 driven by the germline-specific *vasa* promoter. U6A gRNA-kh2: guide RNA targeting *kh* driven by the ubiquitous promoter of the U6A gene. Internal primers Rec-kh-rv2 and eGFP-fw anneal within the pRec*kh* donor element while external primers Kh1-ext-fw and Vasa-rv anneal to sequences outside of the homology arms present in the donor plasmid. **b)** Gene amplification and sequencing of a diagnostic fragment spanning the 5'-end integration site following the cut mediated by gRNA-sw4 (PCR1), which is also the *kh* recoding site. **c)** Gene amplification and sequencing of the 3'-end integration site following the cut mediated by gRNA-sw3 (PCR2). Mosquitoes from the nRec and wild-type (WT) lines were included as controls. Ladder is GeneRuler 1 kb Plus. Source data are provided as a Source Data file.
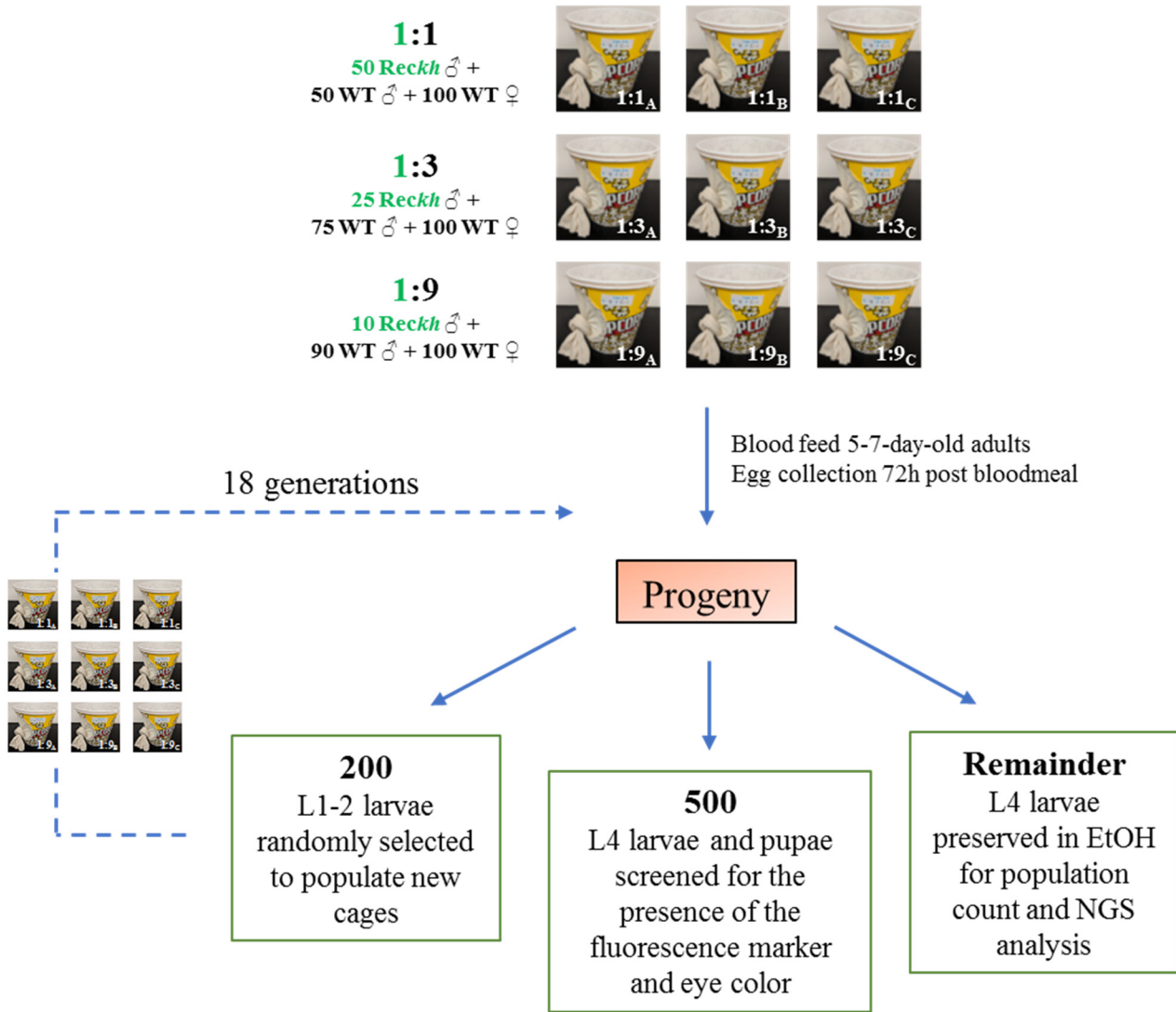
Supplementary Figure 2. Schematic representation of the small cage protocol. A total of nine 5,000 cm^3 cages were set up with triplicate (A, B and C) initial release ratios of 1:1, 1:3 and 1:9 Rec*kh* drive to wild-type (WT) males. WT females were added to reach a sex ratio of 1:1. A subset of 500 larvae was selected randomly from the progeny obtained from each cage immediately after hatching and screened as L4 larvae and pupae for the presence of the GFP fluorescent marker and the eye color, respectively. A subset of 200 larvae was selected randomly immediately after hatching and reared to adulthood to populate the following set of cages. All individuals from the first generation after release of cages 1:9 were screened and added to seed new cages in proportion to their GFP$^+$ phenotype. The remainder were reared to L4 and stored in ethanol for population counting and sequencing analyses. This protocol was repeated every three weeks for 18 consecutive generations.

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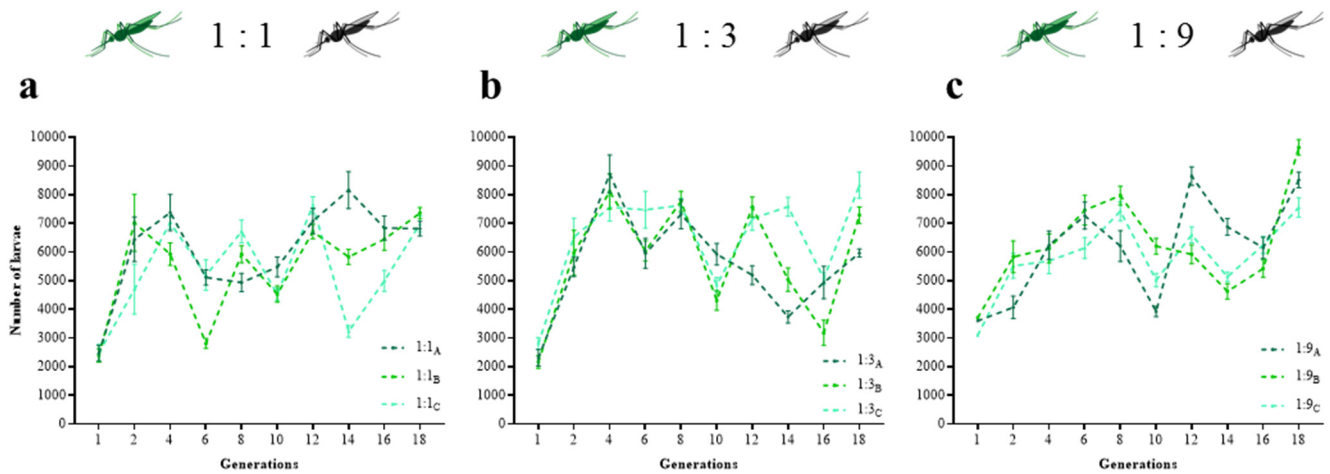
Supplementary Figure 3. Population size over time in caged populations seeded with three different release ratios of Rec*kh* to wild-type (WT) males. The total larval population was estimated every two generations in each of the triplicate cages (A, B, and C) seeded with 1:1 (a), 1:3 (b) and 1:9 (c) release ratios of Rec*kh* to WT males by taking 6-9 replicate measurements of randomly-selected L4 larvae. Bars represent the standard deviation from the mean. **a)** Each data point represents the mean obtained from nine replicate measurements (n = 9) for generations 1-2 and six replicate measurements (n = 6) for generations 4-18. **b)** Each data point represents the mean obtained from nine replicate measurements (n = 9) for generation 1 and six replicate measurements (n = 6) for generations 2-18. **c)** The entire larval population was counted in generation 1, while for the other generations each data point represents the mean obtained from 6 replicate measurements (n = 6). Source data are provided as a Source Data file.
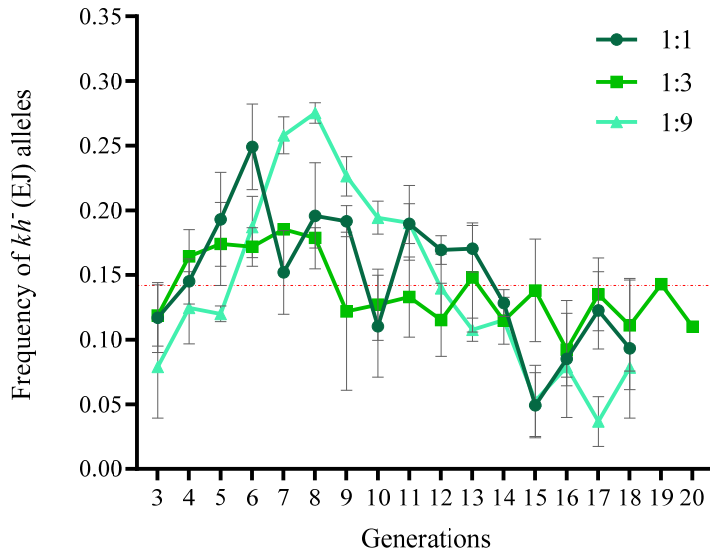
Supplementary Figure 4. EJ-induced kh^- (white) alleles. The frequency of EJ-induced kh^- alleles over time in all cages was inferred from the number of GFP$^-$/white individuals carrying two copies of non-functional mutated alleles using the formula √*N white-eyed individuals / N total mosquitoes* derived from the Hardy-Weinberg equation. Each data point represents the mean with SEM from three replicate cages. Dotted red line represents the average kh^- allele frequency in all cages during the entire experiment. Source data are provided as a Source Data file.
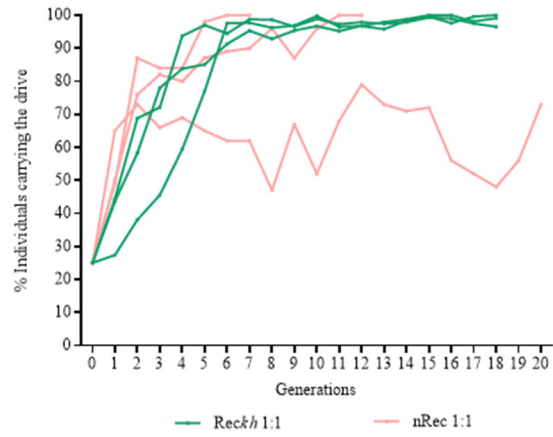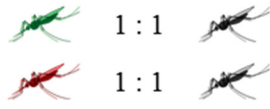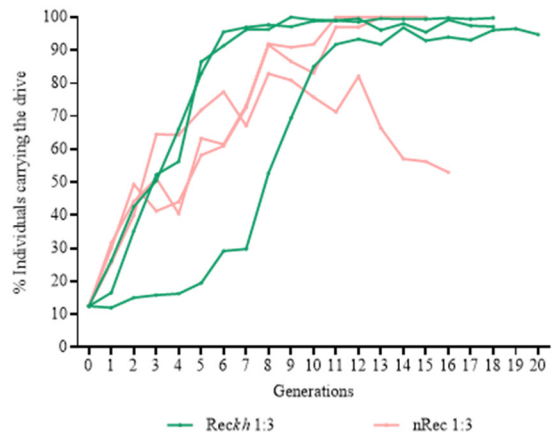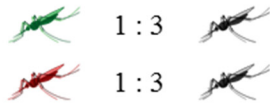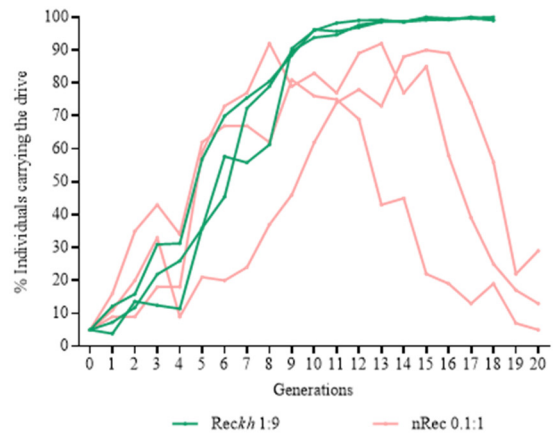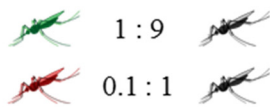
Supplementary Figure 5. Comparative long-time dynamics of the Rec*kh* and nRec gene drive lines in small cage laboratory trials. Rec*kh* carries a recoding system that maintains *kh* gene function after insertion, while the insertion of nRec determines the loss-of-function of the *kh* allele. Drive efficiency is measured as the accumulation of the fluorescence marker in three replicate populations of Rec*kh* (green) and nRec (red) *An. stephensi* mosquitoes with initial release ratios of drive to wild-type males of 1:1 **(a)**, 1:3 **(b)**, and 1:9 **(c)**. nRec data are from Pham *et al*.[1].
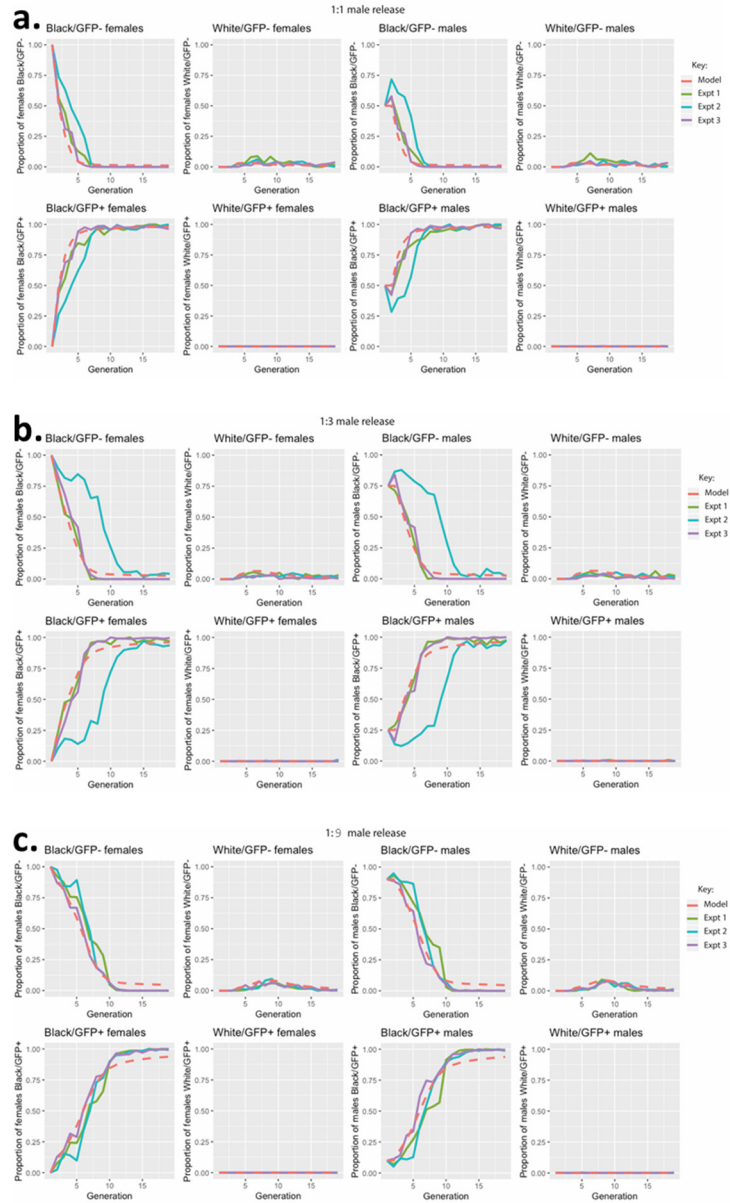
Supplementary Figure 6. Observed and model predicted Rec*kh* gene drive dynamics. Observed (Expts 1-3, solid lines) and predicted (Model, dashed lines) *GFP* and *kh* marker phenotype combinations for 1:1 **(a)**, 1:3 **(b)**, and 1:9 **(c)** Rec*kh*(HW):wild-type(WW) male releases in 18 non-overlapping generations. *Black*/*GFP*⁺ individuals have at least one copy of the H drive allele (i.e. genotypes HH, HW, HR and HB, where R represents an in-frame, cost-free resistant allele, and B represents an out-of-frame or otherwise-costly resistant allele). These individuals spread to near-fixation within ~7 generations (a), 7-12 generations (b), 12 generations (c) of introduction through the inheritance-biasing action of the H allele. *White*/*GFP*⁻ individuals lack both the gene drive construct and a copy of the W or R allele, and hence have genotype BB. B alleles are selected against when present in BB females, but their elimination is slowed due to their viability in heterozygotes and BB males. *Black*/*GFP*⁻ individuals are initially WW, but also include RR, RB, RW and BW genotypes. These genotypes are depleted through the action of the gene drive system but persist at low levels largely due to a small number of R alleles that are generated and persist in the population as a result of their cut-resistant phenotype.

AsMCRkh2 (nRec) embryos	G_0 larvae	G_0 adults	G_1 GFP$^+$ DsRed$^-$ larvae/tot	G_1 GFP$^+$ DsRed$^-$ adults	
				From ♂4 founder	From ♀4 founder
504	259	184 (85 ♂ + 99 ♀)	96/25,293	59 (33 ♂ + 26 ♀)	13 (7 ♂ + 6 ♀)

Supplementary Table 1. Swap microinjection data.

Embryos were obtained from DsRed$^+$ AsMCRkh2 (nRec) females mated to wild-type (WT) males.
G_0 individuals were grouped in 27 male founder pools of 2-4 males and 11 female founder pools of 7-10 females and crossed to WT.

Supplementary Table 2. Drive transmission through Rec*kh* males and females.

G₁	G₂	Rep	G₃ progeny GFP⁺ Black	GFP⁺ Mos	GFP⁻ Black	GFP⁻ Mos	White	Total (n)	Transmission (% GFP⁺)		HDR %	
Hh ♂	Hh ♂	A	149	0	0	0	0	149	100		100	
		B	248	0	0	0	0	248	100		100	
		C	313	0	0	0	0	313	100		100	
		tot	710	0	0	0	0	710	**100**	99.8	**100**	99.5
	Hh ♀	A	269	0	0	0	0	269	100		100	
		B	258	1	0	0	1	260	99.2		98.5	
		C	329	1	0	0	1	331	99.4		98.8	
		tot	856	2	0	0	2	860	**99.5**		**99.1**	
Hh ♀	Hh ♂	A	387	0	322	0	0	709	54.6		9.2	
		B	201	0	143	0	0	344	58.4		16.9	
		C	98	0	50	0	0	148	66.2		32.4	
		tot	686	0	515	0	0	1201	**57.1**	57	**14.2**	14
	Hh ♀	A	217	0	57	1	98	373	58.2		16.4	
		B	228	0	96	0	98	422	54		8.1	
		C	63	0	24	0	38	125	50.4		0.9	
		tot	508	0	177	1	234	920	**55.2**		**10.4**	

'H' is the kh^{Rec+} drive allele; 'h' is a non-drive allele.

G1 individuals derived from the cross between HH males and wild-type (hh) females.

All drive individuals were outcrossed to wild-type counterparts *en masse* in three replicate cages (Rep A, B, C).

Transmission % is the proportion of individuals inheriting the drive element (GFP⁺).

HDR % is the proportion of WT alleles converted into drive alleles by HDR and is calculated using the formula $2(X - 0.5n)/n$ ('X' is the number of GFP⁺ individuals and 'n' the total number of mosquito counted).

The transmission and HDR rates for the male and female lineages were calculated by averaging the percentages observed in a total of 6 cages (3 from the male parent and 3 from the female parent).

Supplementary Table 3. Reproductive parameters of Rec*kh* and *kh⁻/kh⁻* females.

	WT kh^+/kh^+	Rec*kh* homoz kh^{Rec+}/kh^{Rec+}	Rec*kh* heteroz kh^{Rec+}/kh^-	White kh^-/kh^-	One-Way ANOVA
Feeding success[a]	69/75 (92%)	78/87 (90%)	96/99 (97%)	82/94 (87%)	$p = 0.766$ $F(3, 6) = 0.388$
Survival after blood meal[b]	68/69 (99%)	75/78 (96%)	96/96 (100%)	24/82 (29%)	$p = 0.0013$** $F(3, 6) = 21.3$
Laying females[c]	44/59 (75%)	46/73 (63%)	48/65 (74%)	10/24 (42%)	$p = 0.9109$ $F(3, 6) = 0.173$
Mean No. eggs/female[d] (Fecundity)	91 (± 6 SEM) n=31	87 (± 5 SEM) n=30	89 (± 4 SEM) n=30	45 (± 13 SEM) n=10	$p = 0.0001$*** $F(3, 97) = 7.65$
Mean No. larvae/female[e] (Fertility)	73 (± 5 SEM) n=31	75 (± 5 SEM) n=30	79 (± 4 SEM) n=30	26 (± 8 SEM) n=10	$p < 0.0001$**** $F(3, 97) = 11.7$

[a] Females that fed/total number of females in the cage. 2-3 replicate experiments were performed for each line.
[b] Females that survived a blood meal/total number that fed. 2-3 replicate experiments were performed for each line.
[c] Females that laid eggs/total blood-fed female set up. 2-3 replicate experiments were performed for each line.
[d] Mean (±SEM) number of eggs per laying female.
[e] Mean (±SEM) number of larvae per laying female.
See Supplementary Table 4 for adjusted *p* values after multiple comparisons test.
Source data are provided as a Source Data file.

Supplementary Table 4. Adjusted *p* values for parameter in Supplementary Table 3 calculated by applying the Tukey's multiple comparisons test.

FEEDING	kh^+/kh^+	kh^{Rec+}/kh^{Rec+}	kh^{Rec+}/kh^-
kh^{Rec+}/kh^{Rec+}	0.9983		
kh^{Rec+}/kh^-	0.9482	0.8759	
kh^-/kh^-	0.9601	0.9827	0.7211
SURVIVAL	kh^+/kh^+	kh^{Rec+}/kh^{Rec+}	kh^{Rec+}/kh^-
kh^{Rec+}/kh^{Rec+}	0.9952		
kh^{Rec+}/kh^-	0.9996	0.9853	
kh^-/kh^-	0.0036**	0.0024**	0.0033**
LAYING	kh^+/kh^+	kh^{Rec+}/kh^{Rec+}	kh^{Rec+}/kh^-
kh^{Rec+}/kh^{Rec+}	0.9473		
kh^{Rec+}/kh^-	>0.9999	0.9414	
kh^-/kh^-	0.9665	0.9997	0.9619
EGGS	kh^+/kh^+	kh^{Rec+}/kh^{Rec+}	kh^{Rec+}/kh^-
kh^{Rec+}/kh^{Rec+}	0.9633		
kh^{Rec+}/kh^-	0.9949	0.9954	
kh^-/kh^-	<0.0001****	0.0004***	0.0002***
LARVAE	kh^+/kh^+	kh^{Rec+}/kh^{Rec+}	kh^{Rec+}/kh^-
kh^{Rec+}/kh^{Rec+}	0.9949		
kh^{Rec+}/kh^-	0.7869	0.9029	
kh^-/kh^-	<0.0001****	<0.0001****	<0.0001****

Supplementary Table 5. Rec*kh* male contribution to the following generation in the presence of an equal number of wild-type males.

Replicate	GFP$^+$	GFP$^-$	Tot	% GFP$^+$	% GFP$^-$	*p* value*
Cage A	657	511	1168	54.8%	45.2%	0.4167
Cage B	639	709	1348	46.7%	53.3%	0.6173
Cage C	705	769	1474	46%	54%	0.4841

Each cage was set up with 75 Rec*kh* homozygous males, 75 wild-type males, and 150 wild-type females.
*Two-tail Binomial Test.

Supplementary Table 6. Eye phenotypes scored in a subset of ~500 individuals isolated at each generation in the 1:1 cages.

Generations	1:1_A						1:1_B						1:1_C					
	GFP+			GFP−			GFP+			GFP−			GFP+			GFP−		
	kh^+ Black	kh^- White	kh^{mos} Mos	kh^+ Black	kh^- White	kh^{mos} Mos	kh^+ Black	kh^- White	kh^{mos} Mos	kh^+ Black	kh^- White	kh^{mos} Mos	kh^+ Black	kh^- White	kh^{mos} Mos	kh^+ Black	kh^- White	kh^{mos} Mos
G_1	200	0	0	259	0	0	137	0	0	363	0	0	217	0	0	272	0	0
G_2	288	0	0	206	0	0	181	0	0	294	0	0	324	0	0	147	0	0
G_3	368	0	0	87	17	6	203	0	0	235	8	0	351	0	0	134	2	0
G_4	410	0	0	63	16	1	271	0	0	179	6	0	448	0	0	19	11	1
G_5	388	0	0	35	33	2	378	0	0	97	16	0	517	0	0	5	11	1
G_6	452	0	0	1	50	0	455	0	0	16	24	0	445	0	0	0	21	0
G_7	450	0	0	0	22	0	472	0	0	3	8	0	479	0	0	0	6	0
G_8	468	0	0	0	36	0	481	0	0	0	19	0	500	1	0	0	7	0
G_9	456	0	0	0	22	0	471	0	0	0	15	0	457	0	0	0	16	0
G_{10}	469	0	0	0	16	0	481	0	0	0	1	0	464	0	0	0	5	0
G_{11}	459	0	0	0	23	0	475	0	0	0	17	0	466	0	0	0	13	0
G_{12}	486	0	0	0	16	0	466	0	0	0	16	0	501	0	0	0	11	0
G_{13}	474	0	0	0	10	0	477	0	0	0	21	0	482	0	0	0	13	0
G_{14}	491	0	0	0	6	0	476	0	0	0	8	0	462	0	0	0	10	0
G_{15}	486	0	0	0	0	0	472	0	0	0	2	0	436	0	0	0	3	0
G_{16}	464	0	0	0	0	0	495	0	0	0	12	0	489	0	0	0	5	0
G_{17}	463	0	0	0	9	0	473	0	0	0	2	0	466	1	0	0	12	0
G_{18}	459	0	0	0	4	0	475	0	0	0	0	0	467	0	0	0	17	0
TOT	**7731**	**0**	**0**	**651**	**280**	**9**	**7339**	**0**	**0**	**1187**	**175**	**0**	**7971**	**2**	**0**	**577**	**163**	**2**

Supplementary Table 7. Eye phenotypes scored in a subset of ~500 individuals isolated at each generation in the 1:3 cages.

Generations	1:3_A						1:3_B						1:3_C					
	GFP+			GFP−			GFP+			GFP−			GFP+			GFP−		
	kh^+ Black	kh^- White	kh^{mos} Mos	kh^+ Black	kh^- White	kh^{mos} Mos	kh^+ Black	kh^- White	kh^{mos} Mos	kh^+ Black	kh^- White	kh^{mos} Mos	kh^+ Black	kh^- White	kh^{mos} Mos	kh^+ Black	kh^- White	kh^{mos} Mos
G_1	111	0	0	312	0	0	63	0	0	461	0	0	80	0	0	403	0	0
G_2	207	0	0	279	0	0	73	0	0	413	0	0	168	0	0	309	0	0
G_3	259	1	0	247	8	4	75	0	0	377	22	9	250	1	0	224	5	2
G_4	296	1	0	131	21	3	78	0	0	392	10	0	247	0	0	180	12	3
G_5	409	0	0	56	28	1	101	0	0	402	15	1	449	0	0	62	8	0
G_6	452	0	0	0	19	0	144	0	0	315	14	3	456	0	0	24	13	0
G_7	503	2	0	1	15	0	145	2	0	330	16	1	456	1	0	2	16	0
G_8	470	2	0	0	11	0	257	0	0	214	16	0	476	0	0	0	18	0
G_9	473	1	0	0	14	0	342	0	0	132	18	0	495	0	0	0	0	0
G_{10}	496	0	0	0	6	0	390	0	0	54	15	0	481	0	0	0	4	0
G_{11}	477	0	0	0	5	0	435	0	0	21	18	0	478	0	0	0	5	0
G_{12}	492	0	0	0	2	0	426	0	0	18	12	0	479	0	0	0	7	0
G_{13}	466	0	0	0	19	0	445	0	0	24	16	0	482	0	0	0	2	0
G_{14}	463	0	0	0	9	0	479	0	0	8	8	0	479	0	0	0	3	0
G_{15}	465	0	0	0	22	0	431	0	0	26	7	0	484	0	0	0	3	0
G_{16}	493	0	0	0	4	0	468	0	0	20	10	0	489	0	0	0	1	0
G_{17}	453	2	0	0	12	0	443	0	0	22	11	0	467	0	0	0	3	0
G_{18}	488	0	0	0	14	0	464	3	0	15	4	0	476	0	0	0	1	0
G_{19}	-	-	-	-	-	-	471	0	0	7	10	0	-	-	-	-	-	-
G_{20}	-	-	-	-	-	-	473	0	0	20	6	0	-	-	-	-	-	-
TOT	7473	9	0	1026	209	8	6203	5	0	3271	228	14	7392	2	0	1204	101	5

Supplementary Table 8. Eye phenotypes scored in a subset of ~500 individuals isolated at each generation in the 1:9 cages.

Generations	1:9_A						1:9_B						1:9_C					
	GFP+			GFP−			GFP+			GFP−			GFP+			GFP−		
	kh^+ Black	kh^- White	kh^{mos} Mos	kh^+ Black	kh^- White	kh^{mos} Mos	kh^+ Black	kh^- White	kh^{mos} Mos	kh^+ Black	kh^- White	kh^{mos} Mos	kh^+ Black	kh^- White	kh^{mos} Mos	kh^+ Black	kh^- White	kh^{mos} Mos
G_1	36	0	0	457	0	0	19	0	0	479	0	0	62	0	0	447	0	0
G_2	56	0	0	419	0	0	69	0	0	437	0	0	79	0	0	418	0	0
G_3	97	0	0	347	0	0	65	0	0	449	10	2	145	0	0	315	9	3
G_4	118	1	0	334	6	2	56	0	0	428	6	2	145	0	0	300	19	4
G_5	170	0	0	297	8	0	171	0	0	297	8	1	287	0	0	210	8	2
G_6	266	0	1	214	18	1	242	0	0	210	11	1	373	0	0	128	32	4
G_7	275	0	0	182	36	2	342	0	0	92	39	2	354	0	0	86	29	4
G_8	297	0	0	154	34	0	372	0	0	59	40	0	376	0	0	57	34	0
G_9	455	0	0	19	29	0	445	0	0	31	20	1	436	0	0	27	30	1
G_{10}	430	0	0	1	17	0	441	0	0	7	22	0	453	0	0	4	14	0
G_{11}	487	0	0	0	9	0	471	0	0	0	27	0	489	0	0	1	21	0
G_{12}	506	0	0	1	4	0	481	0	0	0	12	0	473	0	0	0	15	0
G_{13}	494	0	0	0	4	0	452	0	0	0	6	0	489	0	0	0	7	0
G_{14}	461	0	0	1	6	0	460	0	0	0	6	0	478	0	0	0	7	0
G_{15}	472	0	0	0	0	0	466	0	0	0	2	0	462	0	0	0	4	0
G_{16}	486	0	0	0	2	0	481	0	0	0	4	0	445	0	0	0	3	0
G_{17}	476	0	0	0	2	0	483	0	0	0	1	0	506	0	0	0	0	0
G_{18}	489	0	0	0	4	0	485	1	0	0	0	0	491	0	0	0	5	0
TOT	6071	1	1	2426	179	5	6001	1	0	2489	214	9	6543	0	0	1993	237	18

Supplementary Table 9. Sequences of non-drive alleles in non-drive white-eyed (GFP⁻/kh⁻) individuals from all cages at generation G₃.

C: cage; I: individual mosquito; WT: wild-type; gRNA; PAM; mutation; F: frame; FS: frameshift; IF: in frame; HOM: homozygous.

C	I	Allele 1	Allele 2	F
	WT	CACGCGATGGTTCCGTTCTACGGGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACGGGCAGGGCATGAACGCGGG	
1:1ₐ	1	CACGCGATGGTTCCGTTCTACGCAGGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTTATGGAT--GGGCATGAACGCGGG	FS/FS
	2	CACGCGATGGTTCCGTTCTGCACGAAAAGGGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACAGGCGCTGGATCAAGGCAGGGCATGAACGCGGG	FS/FS
	3	CACGCGATGGTTCCGTTCTAC-GGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACAGGGCAGGGCATGAACGCGGG	FS/FS
	4	CACGCGATGGTTCCGTTCTACAGGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTAC----AGGGCATGAACGCGGG	IF/FS
	5	CACGCGATGGTTCCGTTCAT----CAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTA--GGCAGGGCATGAACGCGGG	FS/FS
	6	CACGCGATGGTTCCGTTCTACCGGGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTAAATCGCAGGCAGGGCATGAACGCGGG	FS/FS
	7	CACGCGATGGTTCCGTTCTACCAGGGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACAGTCGCACGGCAGGGCATGAACGCGGG	FS/FS
	8	CACGCGATGGTTCCGTTCTAC-GGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCGAT-GGCAGGGCATGAACGCGGG	FS/FS
	9	CACGCGATGGTTCCGT---ACGGGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCCCCGGCAGGGCATGAACGCGGG	IF/IF
1:1ᵦ	1	CACGCGATGGTTCCGTTCTACG--CAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACAGGCAGGGCATGAACGCGGG	FS/IF
	2	CACGCGATGGTTCCGTTCTACG--CAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACGATGGCAGGGCATGAACGCGGG	FS/FS
	3	CACGCGATGGTTCCGTTCT---GGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCCACG--CAGGGCATGAACGCGGG	IF/FS
	4	CACGCGATGGTTCCGTTCTACG--CAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACGGGACCAGGCAGGGCATGAACGCGGG	FS/IF
	5	CACGCGATGGTTCCG--------GCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCC---GGCAGGGCATGAACGCGGG	FS/IF
	6	CACGCGATGGTTCCGTTCTACGCG--GGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACAGGGAACCATCAAAGGCACGTCAAGAACGCGGG	FS/FS
	7	CACGCGATGGTTCCGTTCTACGCAGGGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCGCGGCAGGGCAGGGCATGAACGCGGG	IF/IF
	8	CACGCGATGGTTCCGTTCA---GGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTTGCACGCGTTGGCAGGGCATGAACGCGGG	FS/FS
1:1ᴄ	1	CACGCGATGGTTCCGTTCT---GGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCCACAGGGGCAGGGCATGAACGCGGG	IF/FS
	2	CACGCGATGGTTCCGTTCTACGGGCATGGCAGGGCATGAACGCGGG	CACGCGATGGTTCCG------GCGGGCATTGCATGAACGCGGG	FS/FS
1:3ₐ	1	CACGCGATGGTTCCGTT-----GGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACGCAGGGCAGGGCATGAACGCGGG	FS/FS
	2	CACGCGATGGTTCCGTTCTAC--GCAGGGCATGAACGCGGG	CACGCGATGGTTCCGAT-----GGCAGGGCATGAACGCGGG	FS/FS

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	#	Sequence (allele 1)	Sequence (allele 2)	Class
1:3ᴮ	1	CACGCGATGGTTC-----TACG--CAGGGCATGAACGCGGG	CACGCGATGGTTC-----TACG--CAGGGCATGAACGCGGG	**FS HOM**
	2	CACGCGATGGTTCCGTTCTAC--GCAGGGCATGAACGCGGG	------------------------AGGGCATGAACGCGGG	**FS/FS**
	3	CACGCGATGGTTCCGTTCTACAGGGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACGCGATGGTTCCGGCAGGGCATGAACGCGGG	**FS/FS**
	4	CACGCGATGGTTCCGTTCTAC--GCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACAGGGCAGGGCATGAACGCGGG	**FS/FS**
	5	CACGCGATGGTTCCGTTCTACG--CAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACGCGATGGCAGGGCATGAACGCGGG	**FS/FS**
	6	CACGCGATGGTTCCGTTCACGCGGGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACAGGCAGGGCATGAACGCGGG	**FS/IF**
	7	CACGCGATGGTTCCGTTCGCCAGGGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTAGTTCCGGCAGGGCATGAACGCGGG	**FS/FS**
	8	CACGCGATGGTTCCGTTCTACGCCGGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCCGTT-GCAGGGCATGAACGCGGG	**FS/FS**
	9	CACGCGATGGTTCCGTTCTA--GGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACCGCGCGATGGTTCCGTGGTTCCGGCAGGGCATGAACGCGGG	**FS/FS**
1:3ᶜ	1	CACGCGATGGTTCCGTTCTAC----AGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACATGAACGGCAGGGCATGAACGCGGG	**FS/FS**
1:9ᴮ	1	CACGCGATGGTTCCGTTCTACGGGCA-----TGAACGCGGG	CACGCGATGGTTCCGTTCTACAG-CAGGGCATGAACGCGGG	**FS/FS**
	2	CACGCGATGGTTCCGTTCTACTCCGGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACGGAACGTTCACGGCAGGGCATGAACGCGGG	**FS/FS**
	3	CACGCGATGGTTCCGTTCTACG-GCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACG-GCAGGGCATGAACGCGGG	**FS HOM**
1:9ᶜ	1	CACGCGATGGTTCCGTTCTACAAGGGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCCGCGTGAACGGCGGCCTGGTTCATGGTATGACATGAACGCGGG	**FS/FS**
	2	CACGCGATGGTTCCGTTCC---GGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCCGCGATGGCAGGGCATGAACGCGGG	**IF/FS**
	3	CACGCGATGGTTCCGAT-----GGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGAT-----GGCAGGGCATGAACGCGGG	**FS HOM**
	4	CACGCGATGGTTCCGTTCTAC-GGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACTCGAAATCACGCGATGGTTCCGTTCTAAGGCAGGGCATGAACGCGGG	**FS/FS**
	5	CACGCGATGGTTC-----TACG--CAGGGCATGAACGCGGG	CACGCGATGG------TTCTACGGCAGGGCATGAACGCGGG	**FS/FS**

Supplementary Table 10. Sequences of drive and non-drive alleles in drive white-eyed (GFP⁺/kh⁻) individuals.

C: Cage; G: generation; I: individual mosquito; WT: wild-type; gRNA; PAM; mutation; Recoded-*kh*.

C	G	I	Non-drive Allele	Rec*kh* Drive Allele
		WT	CACGCGATGGTTCCGTTCTACGGGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACGGACAAGGAATGAATGCAGGATTC
1:1$_C$	G$_8$	1	CACGCGATGGTTCCGTTCTACATGAACGCGGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCCGATGGTTCCGGACAAGGAATGAATGCAGGATTC
1:3$_A$	G$_7$	2	CACGCGATGGTTCCGTTCTACAGGGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCCGATGGTTCCGGACAAGGAATGAATGCAGGATTC
1:3$_A$	G$_7$	3	CACGCGATGGTTCCGTTCTACGC-CAGGGTATGAACGCGGG	CACGCGATGGTTCCGTTC----GACAAGGAATGAATGCAGGATTC
1:3$_A$	G$_8$	4	CACGCGATGGTTCCGTTCTACGC-CAGGGTATGAACGCGGG	CACGCGATGGTTCCGTTC----GACAAGGAATGAATGCAGGATTC
1:3$_A$	G$_8$	5	CACGCGATGGTTCCGTTCTACGG-CAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACAAGGAAGGGACAAGGAATGAATGCAGGATTC
1:9$_A$	G$_4$	6	CACGCGATGGTTCCGTTCC---GGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTGGACAAGGACAAGGAATGAATGCAGGATTC

Supplementary Table 11. Amplicon sequencing of non-drive alleles in pooled individuals from cage 1:3$_B$ at generations G$_0$, G$_8$, and G$_{14}$.		
gRNA and PAM in the wild-type allele; Mutation.		
Cage 1:3$_B$ Generation G$_0$		
Reads	**Sequence**	**Relative abundance (%)**
315911	GATGGTTCCGTTCTACGGGCAGGGCATGAACGCG	97.65
938	GATGGTTCCGTTCTACGGGCAGGACATGAACGCG	0.29
730	GATGGT-----------GGCAGGGCATGAACGCG	0.23
600	GATGGTTCCGTTCTACGGACAGGGCATGAACGCG	0.19
583	GATGG------------GGCAGGGCATGAACGCG	0.18
500	GATGGTTCCGTT-----GGCAGGGCATGAACGCG	0.15
391	GATGGTTCCGT------GGCAGGGCATGAACGCG	0.12
357	GATGGTT----------GGCAGGGCATGAACGCG	0.11
343	GATGGTTCC--------GGCAGGGCATGAACGCG	0.11
325	GATGGTTCCGTTCTCCGGGCAGGGCATGAACGCG	0.10
235	GATGGTTCCGTTCC---GGCAGGGCATGAACGCG	0.07
229	GATGGTTCCGTTCTA--GGCAGGGCATGAACGCG	0.07
194	GATG-------------GGCAGGGCATGAACGCG	0.06
190	GATGGTTCCG-------GGCAGGGCATGAACGCG	0.06
183	GATGGTTCCGTTCT---GGCAGGGCATGAACGCG	0.06
161	GATGGTTCCGTTCTACGAGCAGGGCATGAACGCG	0.05
158	GATGGTTCCGTTC----GGCAGGGCATGAACGCG	0.05
156	GATGG--------------CAGGGCATGAACGCG	0.05
139	GATGGTTCCGTTCTACG-GCAGGGCATGAACGCG	0.04
136	GATGGTTCCGTTC-------AGGGCATGAACGCG	0.04
129	GATGGTTC---------GGCAGGGCATGAACGCG	0.04
129	GATGGTTCCGTTCTATGGGCAGGGCATGAACGCG	0.04
126	GATGGTTCCGTTCTACGGGCAGGGCATGGACGCG	0.04
122	AATGGTTCCGTTCTACGGGCAGGGCATGAACGCG	0.04
115	GATGGTTCCGTTCTACGGGCAGTGCATGAACGCG	0.04

111	GATGGTTCCGTTCTACGGGCAGGGCATGAACACG	0.03
103	GATGGTTCCGTTCTAC----AGGGCATGAACGCG	0.03
103	GATGGTTCCGTTC--ATGGCAGGGCATGAACGCG	0.03
101	GATGGTTCCGTTCTACGGGCAGGGCATGAACGCT	0.03
323498		

Cage 1:3$_B$ Generation G$_8$		
Reads	**Sequence**	**Relative abundance (%)**
175157	GATGGTTCCGTTCTACGGGCAGGGCATGAACGCG	83.28
6438	GATGGTTCCGTTCTACAGGGCAGGGCATGAACGCG	3.06
4558	GATGGTTCCGTTCTACTAAACACGCGTTGCCATGAACGCGGTTCTACTAAACAGGCAGGGCATGAACGCG	2.17
4382	GATGGTTCCGTTCTACGCATGAACGCAGGGCATGAACGCG	2.08
4137	GATGGTTCCGTTCCGATGGCAGGGCATGAACGCG	1.97
4123	GATGGTTCCGTTCTACAACGCAACGTTCTACAACGGGGCAGGGCATGAACGCG	1.96
4042	GATGGTTCCGTTCC---GGCAGGGCATGAACGCG	1.92
2124	GATGGTTCCGTTCTACGGGGCAGGCATGAACGCG	1.01
2085	GATGGTTACGTTCTACATGGCAGGGCATGAACGCG	0.99
2083	GATGGTTCCGTTCTACGTTCGCGGCAGGGCATGAACGCG	0.99
478	GATGGTTCCGTTCTACGGACAGGGCATGAACGCG	0.23
473	GATGGTTCCGTTCTACGGGCAGGACATGAACGCG	0.22
241	GATGGTTCCGTTCTCCGGGCAGGGCATGAACGCG	0.11
210321		

Cage 1:3$_B$ Generation G$_{14}$		
Reads	**Sequence**	**Relative abundance (%)**
147513	GATGGTTCCGTTCTACAACGCAACGTTCTACAACGGGGCAGGGCATGAACGCG	48.16
77514	GATGGTTCCGTTCTACGT---------------	25.31
35534	GATGGTTCCGTTCTACGGGGCAGGCATGAACGCG	11.60
22641	GATGGTTCCGTTCTACGGAACCGGCAGGCATGAACGCG	7.39
14200	GATGGTTCCGTTCTACCAGCGCAGGGCAGGGCATGAACGCG	4.64
4036	GATGGTTCCGTTCATGT----GGGCATGAACGCG	1.32

1091	GATGGTTCCGTTCCCGCGTGGGCAGGCATGAACGCG	0.36
796	GATGG----------------CAGGCATGAACGCG	0.26
661	GATGGTTCCGTTCTACAACGCAACGTTCTACAACTGGGCAGGGCATGAACGCG	0.22
623	GATGGTTCCGTTCTACAACGCACCGTTCTACAACGGGGCAGGGCATGAACGCG	0.20
593	G----------------GGCAGGCATGAACGCG	0.19
455	GATGGTTCCGTTCTACAACGCAACGTTCTACACCGGGGCAGGGCATGAACGCG	0.15
236	GATGGTTCCGTTCTACGGGCAGGGCATGAACGCG	0.08
151	GATGGTTCCGTTCTACAACACAACGTTCTACAACGGGGCAGGGCATGAACGCG	0.05
134	GATGGTTCCGTTCTACAACGCAACGTTATACAACGGGGCAGGGCATGAACGCG	0.04
107	GATGGTTCCGTTCTACGGGGCAGACATGAACGCG	0.03
306285		

Supplementary Table 12. Sequences of non-drive alleles in single non-drive black-eyed (GFP⁻/*kh*⁺) individuals from cage 1:3_B at generation G₁₆.

I: individual mosquito; WT: wild-type; gRNA; PAM; mutation; F: frame; IF: in frame; FS: frameshift; HOM: homozygous; AAC: amino acid change in IF allele.

I	Allele 1	Allele 2	F	AAC
WT	CACGCGATGGTTCCGTTCTACGGGCAGGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACGGGCAGGGCATGAACGCGGGCTTTGAAGACTGTAGC		
1	CACGCGATGGTTCCGTTCTACGGGGCAGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACGGGGCAGGCATGAACGCGGGCTTTGAAGACTGTAGC	IF/IF HOM	Q330A
2	CACGCGATGGTTCCGTTCTACGGGGCAGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACGGGGCAGGCATGAACGCGGGCTTTGAAGACTGTAGC	IF/IF HOM	Q330A
3	CACGCGATGGTTCCGTTCTACGGGGCAGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACGGGGCAGGCATGAACGCGGGCTTTGAAGACTGTAGC	IF/IF HOM	Q330A
4	CACGCGATGGTTCCGTTCTACGGGGCAGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACGGGGCAGGCATGAACGCGGGCTTTGAAGACTGTAGC	IF/IF HOM	Q330A
5	CACGCGATGGTTCCGTTCTACGGGGCAGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACGGGGCAGGCATGAACGCGGGCTTTGAAGACTGTAGC	IF/IF HOM	Q330A
6	CACGCGATGGTTCCGTTCTACGGGGCAGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACGGGGCAGGCATGAACGCGGGCTTTGAAGACTGTAGC	IF/IF HOM	Q330A
7	CACGCGATGGTTCCGTTCTACGGGCCAGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACG------------------TTGTAAGACTGTAGC	IF/FS	Q330P
8	CACGCGATGGTTCCGTTCTACGGGCCAGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACG------------------TTGTAAGACTGTAGC	IF/FS	Q330P
9	CACGCGATGGTTCCGTTCTACGGGGCAGGCATGAACGCGGG	CACGCGATGGTTCCGTTCAACGGG----GCATGAACGCGGGCTTTGAAGACTGTAGC	IF/FS	Q330A
10	CACGCGATGGTTCCGTTCTACGGGGCAGGCATGAACGCGGG	CACGCGATGGTTCCGTTCAACGGG----GCATGAACGCGGGCTTTGAAGACTGTAGC	IF/FS	Q330A
11	CACGCGATGGTTCCGTTCTACGGGGCAGGCATGAACGCGGG	CACGCGATGGTTCCGTTCAACGGG----GCATGAACGCGGGCTTTGAAGACTGTAGC	IF/FS	Q330A
12	CACGCGATGGTTCCGTTCTACGGGGCAGGCATGAACGCGGG	CACGCGATGGTTCCGTTCATGT----GGGCATGAACGCGGGCTTTGAAGACTGTAGC	IF/FS	Q330A
13	CACGCGATGGTTCCGTTCTTGTGGGCAGGCATGAACGCGGG	CACGCGATGGTTCCGTTCAACGGG----GCATGAACGCGGGCTTTGAAGACTGTAGC	IF/FS	W328L; G329W; Q330A
14	CACGCGATGGTTCCGTTCTACGGGGCAGGCATGAACGCGGG	CACGCGATGGTTCCGTTCTACAACGCAAGGTTCTACGCGGGCTTTGAACATGAACGC	IF/FS	Q330A

Supplementary Table 13. Eye-phenotype proportions scored for kh^{Rec+} vs kh^{+R} allelic challenge.

Generations	Replicate Cages											
	A			B			C			D		
	GFP$^+$	GFP$^-$	Total	GFP$^+$	GFP$^-$	Total	GFP$^+$	GFP$^-$	Total	GFP$^+$	GFP$^-$	Total
G$_1$	79.3%	20.7%	300	81.2%	18.8%	394	77.9%	22.1%	653	81.4%	18.6%	420
G$_2$	80.7%	19.3%	373	85.9%	14.1%	396	82.3%	17.7%	362	87.9%	12.1%	390
G$_3$	84.2%	15.8%	310	89.2%	10.8%	305	84.0%	16.0%	357	89.0%	11.0%	308
G$_4$	86.8%	13.2%	318	90.0%	10.0%	359	85.7%	14.3%	357	89.6%	10.4%	326
G$_5$	88.3%	11.7%	315	91.0%	9.0%	310	87.5%	12.5%	327	89.9%	10.1%	338
G$_6$	89.4%	10.6%	320	95.7%	4.3%	304	88.9%	11.1%	341	90.8%	9.2%	336

Experiments were conducted in four replicate cages (A-D) each seeded with 200 individuals heterozygous for a copy of the Reckh drive allele and a copy of the kh functional resistant allele (kh^{Rec+}/kh^{+R}) with a 1:1 sex ratio.
All mosquitos displayed WT black eye color.

REFERENCES

1. Pham, T. B. et al. Experimental population modification of the malaria vector mosquito, Anopheles stephensi. *PloS Genet.* **15,** e1008440 (2019).