Supplementary Materials for

Gene drive that brings about addiction to a temperature sensitive version of an essential gene triggers a population collapse in *Drosophila*

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This PDF file includes:

Fig. S1 to S3

Table S1 to S9

Other Supplementary Materials for this manuscript include the following:

Data S1: Gene drive counts, Control drive counts, Escaper crosses, Escaper target site sequencing results, primers, synthetic constructs genbank files

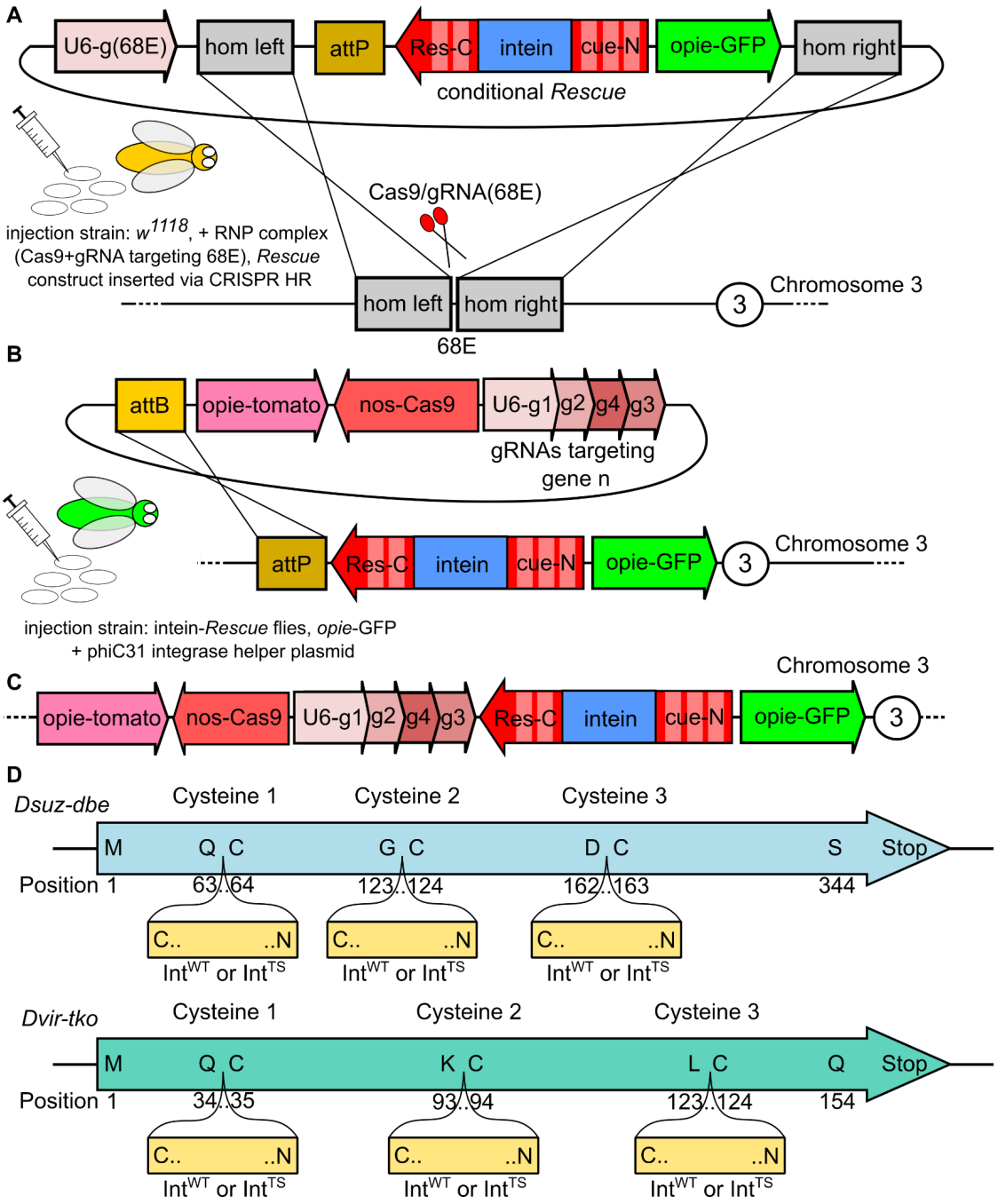
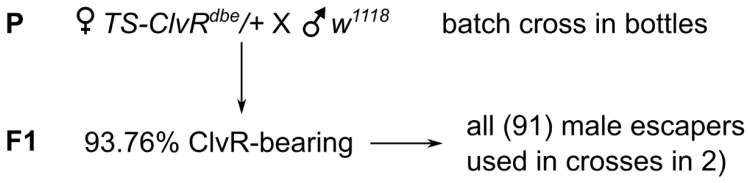
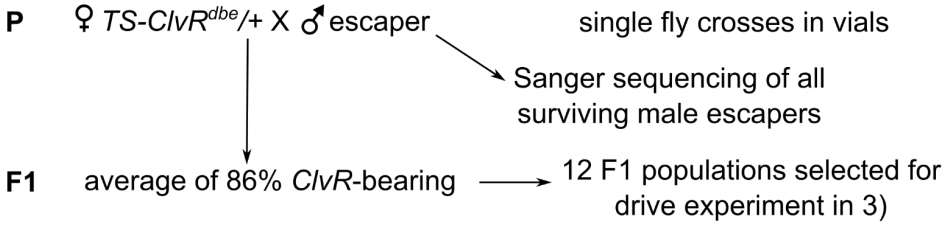


Fig. S1: (A) Genomic insertion of the *Rescue*-INT constructs. We assembled plasmids that had TS and WT versions of the VMA intein inserted into the coding regions of *dbe* and *tko*. The constructs also had a ubiquitous *OpIE*-GFP marker, and an attP landing site for subsequent modifications of the locus. These were flanked by homology arms to facilitate CRISPR-HR mediated insertion into the genome. The construct was injected into w^{1118} flies along with a Cas9 RNP complex that targeted the genomic region at 68E on the third chromosome. **(B) Genomic integration of Cas9/gRNAs.** The second part of the *ClvR* drive mechanism, Cas9 and the gRNAs, were integrated into the genomic site of the TS-*Rescue* to yield complete TS-$ClvR^{dbe}$ flies. This second step was performed only with flies carrying the INT^{TS}(*dbe*)Cys2. **(C) Schematic of the final TS-$ClvR^{dbe}$ drive element. (D) Intein insertion positions in *dbe* and *tko* coding regions**

1) Cross of heterozygous TS-*ClvR*^{dbe} (or *ClvR*^{dbe} controls) females to *w*^{1118} males. Results in Table S3.



2) Backcross of all male escapers from 1) to *TS-ClvR*^{dbe} (or *ClvR*^{dbe} controls). After mating, the male escaper fly was removed and we extracted genomic DNA, PCR amplified over the target gene, and Sanger sequenced. Results in Data S1 "Escaper Analysis" sheet.



3) All progeny from the crosses in 2) were again scored to determine cleavage rates. We selected 12 crosses from 2) and introduced all progeny from each cross into a food bottle to start a drive. Bottles were transferred and monitored until *TS-ClvR*^{dbe} (or *ClvR*^{dbe} controls) reached genotype fixation (3-5 generations). Populations were then shifted to bottles at the restrictive temperature and characterized for survival and fertility as described in text and Data S1 "Escaper Analysis" sheet.

Fig. S2: Identification and characterization of "escapers", progeny of *ClvR*-bearing females that survive and lack *ClvR*.

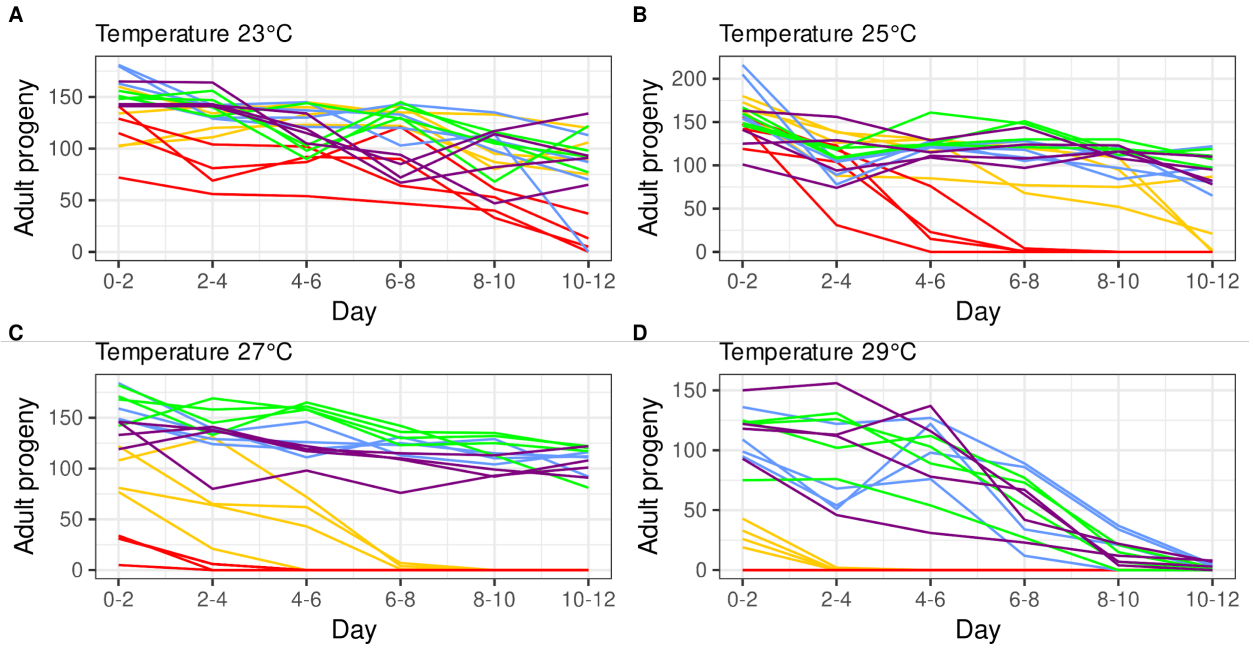


Fig. S3: Adult fly output at different temperatures. Shown are the numbers of adult flies in four replicates that eclosed from different crosses incubated at 23° C **(A)**, 25° C **(B)**, 27° C **(C)**, and 29° C **(D)** over 12 days of egg-laying. Crosses were ♀TS-*ClvR*^{dbe}/+ X ♂*w*^{1118} in red, ♀TS-*ClvR*^{dbe}/TS-*ClvR*^{dbe} X ♂TS-*ClvR*^{dbe}/TS-*ClvR*^{dbe} in yellow, ♀*w*^{1118} *X* ♂TS-*ClvR*^{dbe}/TS-*ClvR*^{dbe} in violet, ♀*ClvR*^{dbe} X ♂*ClvR*^{dbe} (control) in green, and ♀*w*^{1118} *X* ♂*w*^{1118} (control) in blue. Cumulative sums of adult progeny are shown in Fig. 3 in the main text.

Supplementary Tables

Table S1: Screening of *Rescue*-INT function for *tko*. Shown are the numbers of offspring from single fly crosses of heterozygous female $ClvR^{tko}$/+ to males that carry a copy of different versions of the *Rescue*-INT for *tko*. Crosses were kept at 23º C or 27º C. WT (+/+) offspring were dying from maternal carryover activity of $ClvR^{tko}$ at both temperatures. Offspring that carry the *Rescue* within $ClvR^{tko}$ were not affected by temperature. Neither INT^{WT} nor INT^{TS} versions of the *tko Rescue* were able to rescue the LOF phenotypes induced by the $ClvR^{tko}$ element. Note: We did not obtain transformants for the INT^{TS} version inserted after cysteine 2 of *tko*. Since the INT^{WT}version of that *Rescue* did not provide gene function we reasoned that the TS-version will not work either. Thus, the construct was not pursued further.

Rescue	Temperature	Replicate	***Rescue*/+**	*Rescue/ClvR*	*ClvR/+*	+/+	notes
INT^{TS}(tko)Cys1	23	A	**0**	41	46	0	
	23	B	**0**	42	53	0	
	23	C	**0**	50	55	0	
	27	A	**0**	49	49	0	
	27	B	**0**	51	59	0	
	27	C	**0**	44	41	0	
INT^{TS}(tko)Cys3	23	A	**0**	45	43	0	
	23	B	**0**	53	58	0	
	23	C	**0**	41	39	0	
	27	A	**0**	44	46	0	
	27	B	**0**	60	53	0	
	27	C	**0**	41	36	0	
INT^{WT}(tko)Cys1	23	A	**0**	58	48	0	
	23	B	**0**	54	52	0	
	23	C	**0**	55	48	0	
	27	A	**0**	44	48	0	
	27	B	**0**	41	40	0	
	27	C	**-**	-	-	-	sterile
INT^{WT}(tko)Cys2	23	A	**0**	48	44	0	
	23	B	**1**	42	45	0	
	23	C	**0**	53	60	0	
	27	A	**0**	52	57	0	
	27	B	**0**	40	45	0	
	27	C	**0**	62	60	0	
INT^{WT}(tko)Cys3	23	A	**1**	40	42	0	
	23	B	**0**	50	46	0	
	23	C	**0**	45	44	0	
	27	A	**0**	47	47	0	
	27	B	**0**	36	40	0	
	27	C	**0**	63	62	0	

Table S2. Screening of intein-*Rescue* function for *dbe*. Shown are the numbers of adult offspring output from single fly crosses of heterozygous female *ClvR*^{dbe}/+ to males that carry a copy of different versions of the *Rescue*-INT for *dbe*. Crosses were kept at 23° C or 27° C. WT (+/+) offspring of *ClvR*^{dbe} mothers die due to LOF allele creation in the female germline and zygote at both temperatures. Offspring that carry the *Rescue* within *ClvR*^{dbe} were not affected by temperature. Versions with a INT^{WT} inserted after cysteine 2 or 3 of *dbe* were functional at both temperatures. Versions with a INT^{TS} inserted after cysteine and 1 and 3 did not provide *Rescue* function at either temperature. However, a INT^{TS} inserted after cysteine 2 showed promising behavior, having *Rescue* activity at 23° C, whereas at 27° C most of the flies that carried it did not develop into adults (highlighted in red). We chose this *Rescue*-INT^{TS} to build a full TS-*ClvR* element by inserting Cas9 and gRNAs from *ClvR*^{dbe}. Note: For the INT^{WT} inserted after cysteine 1 we did not obtain transformants after a first round of injections. Since the INT^{TS} version inserted after cysteine 1 did not show any *Rescue* activity we did not pursue this construct further.

Rescue	Temperature	Replicate	***Rescue*/+**	*Rescue/ClvR*	*ClvR/+*	+/+
INT^{TS}(dbe)Cys1	23	A	**0**	35	39	0
	23	B	**0**	35	32	0
	23	C	**0**	31	36	0
	27	A	**0**	40	45	0
	27	B	**0**	39	32	0
	27	C	**0**	47	50	0
INT^{TS}(dbe)Cys2	**23**	**A**	**20**	**26**	**23**	**0**
	23	**B**	**31**	**24**	**24**	**2**
	23	**C**	**30**	**32**	**36**	**0**
	27	**A**	**3**	**25**	**23**	**0**
	27	**B**	**4**	**37**	**39**	**0**
	27	**C**	**3**	**28**	**24**	**0**
INT^{TS}(dbe)Cys3	23	A	**0**	49	49	0
	23	B	**0**	33	35	0
	23	C	**0**	36	32	0
	27	A	**0**	44	47	0
	27	B	**0**	38	37	0
	27	C	**0**	32	30	0
INT^{WT}(dbe)Cys2	23	A	**47**	45	54	0
	23	B	**41**	30	25	0
	23	C	**48**	46	43	0
	27	A	**41**	33	26	0
	27	B	**29**	23	31	0
	27	C	**26**	41	43	0
INT^{WT}(dbe)Cys3	23	A	**47**	43	52	0
	23	B	**20**	52	40	0
	23	C	**6**	51	36	0
	27	A	**41**	33	26	0
	27	B	**29**	23	31	0
	27	C	**26**	41	43	0

Table S3: Cleavage to LOF of *ClvR^{dbe}* and TS-*ClvR^{dbe}* at 22°C. We assayed the cleavage activity of TS-*ClvR^{dbe}* at the permissive temperature of 22° C by crossing heterozygous TS-*ClvR^{dbe}* females to *w^{1118}* males and scoring the offspring for the dominant *td-tomato* marker. The observed frequency of TS-*ClvR*-bearing flies in the offspring was lower than what we previously observed with *ClvR^{dbe}* (>99%, (27)). That experiment was performed at a higher temperature of 26° C. Since the cleaving components (Cas9/gRNAs) of TS-*ClvR^{dbe}* are exactly the same as for *ClvR^{dbe}* we reasoned that the lower cleavage activity might be due to the lower incubation temperature. To confirm this, we set up the same crosses with the original *ClvR^{dbe}* stock incubated at 22° C and found a lower rate of cleavage to LOF in that stock as well.

Control crosses ♀*ClvR^{dbe}*/+ X ♂*w^{1118}*						
Bottle	*ClvR*-bearing	♂*w^{1118}*	♀*w^{1118}*	sum	*ClvR*-freq (%)	cleavage to LOF (%)
A	817	17	14	848	96.34	92.69
B	800	21	19	840	95.24	90.48
C	831	16	13	860	96.63	93.26
D	597	18	14	629	94.91	89.83
total	**3045**	**72**	**60**	**3177**	**95.85**	**91.69**

Crosses with TS-*ClvR^{dbe}*/+ X *w^{1118}*						
Bottle	*ClvR*-bearing	♂*w^{1118}*	♀*w^{1118}*	sum	*ClvR*-freq (%)	cleavage to LOF (%)
A	832	22	24	878	94.76	89.52
B	975	31	37	1043	93.48	86.96
C	385	14	10	409	94.13	88.26
D	575	24	22	621	92.59	85.19
total	**2767**	**91**	**93**	**2951**	**93.76**	**87.53**

Table S4: Incubations at a restrictive temperature of 29ºC. In a first test we crossed homozygous ♀TS-*ClvR*^{dbe} to ♂TS-*ClvR*^{dbe} and incubated them at a potentially restrictive temperature of 29º C. We also set up controls with *w*^{1118} X *w*^{1118} and homozygous *ClvR*^{dbe} X *ClvR*^{dbe}. All flies were reared at 22ºC, crossed to each other in a fresh food vial and transferred to a 29º C incubator. All the crosses were fertile and gave progeny in the F1 generation. We transferred all the F1 flies to a fresh vial and kept them at 29º C. F1 progeny of TS-*ClvR*^{dbe} X TS-*ClvR*^{dbe} were completely sterile, whereas F1 progeny from the two control crosses remained fertile. F1 progeny of all crosses were monitored for 1 week at 29º C. Afterwards we took two male TS-*ClvR*^{dbe} flies and crossed them to *w*^{1118} virgins. We also took two females and crossed them to *w*^{1118} males. Both crosses did not yield offspring. The remaining F1 flies of the TS-*ClvR*^{dbe} cross were put back at 22º C and monitored for another week after which most of them had died. All of the flies remained sterile.

Cross	Vial	F1	F2
w^{1118} X *w*^{1118}	1	66	fertile
	2	85	fertile
	3	102	fertile
	4	110	fertile
	5	95	fertile
ClvR^{dbe} X *ClvR*^{dbe}	1	95	fertile
	2	99	fertile
	3	98	fertile
	4	103	fertile
	5	106	fertile
TS-*ClvR*^{dbe} X TS-*ClvR*^{dbe}	1	32	sterile
	2	60	sterile
	3	63	sterile
	4	25	sterile
	5	18	sterile
	6	64	sterile
	7	69	sterile
	8	67	sterile
	9	63	sterile
	10	83	sterile

Table S5: Male *TS-ClvR^{dbe}* and *w^{1118}* (control) fertility at 23ºC. TS-*ClvR^{dbe}* (*w^{1118}* as controls) adult males raised at 23ºC were crossed to four *w^{1118}* virgins in a vial and incubated at a restrictive temperature of 29ºC and a permissive temperature of 23ºC. Every two days we transferred the males to a fresh food vial and added new virgins.

♂*TS-ClvR^{dbe}* X ♀ *w^{1118}* @ 23ºC			♂*w^{1118}* X ♀ *w^{1118}* @ 23ºC		
Time (days)	Replicate	Fertility	Time (days)	Replicate	Fertility
0-2	A	fertile	0-2	A	fertile
0-2	B	fertile	0-2	B	fertile
0-2	C	fertile	0-2	C	fertile
0-2	D	fertile	0-2	D	fertile
0-2	E	fertile	0-2	E	fertile
2-4	A	fertile	2-4	A	fertile
2-4	B	fertile	2-4	B	fertile
2-4	C	fertile	2-4	C	fertile
2-4	D	fertile	2-4	D	fertile
2-4	E	fertile	2-4	E	fertile
4-6	A	fertile	4-6	A	fertile
4-6	B	fertile	4-6	B	fertile
4-6	C	fertile	4-6	C	fertile
4-6	D	fertile	4-6	D	fertile
4-6	E	fertile	4-6	E	fertile
6-8	A	fertile	6-8	A	fertile
6-8	B	fertile	6-8	B	fertile
6-8	C	fertile	6-8	C	fertile
6-8	D	fertile	6-8	D	fertile
6-8	E	fertile	6-8	E	fertile
8-10	A	fertile	8-10	A	fertile
8-10	B	fertile	8-10	B	fertile
8-10	C	fertile	8-10	C	fertile
8-10	D	fertile	8-10	D	fertile
8-10	E	fertile	8-10	E	fertile

Table S6: Male *TS-ClvR^{dbe}* and *w^{1118}* (control) fertility at 29°C

♂*TS-ClvR^{dbe}* X ♀ *w^{1118}* @ 29°C			♂*w^{1118}* X ♀ *w^{1118}* @ 29°C		
Time (days)	Replicate	Fertility	Time (days)	Replicate	Fertility
0-2	A	fertile	0-2	A	fertile
0-2	B	fertile	0-2	B	fertile
0-2	C	fertile	0-2	C	fertile
0-2	D	fertile	0-2	D	fertile
0-2	E	fertile	0-2	E	fertile
2-4	A	fertile	2-4	A	fertile
2-4	B	fertile	2-4	B	fertile
2-4	C	fertile	2-4	C	fertile
2-4	D	fertile	2-4	D	fertile
2-4	E	fertile	2-4	E	fertile
4-6	A	fertile	4-6	A	fertile
4-6	B	fertile	4-6	B	fertile
4-6	C	fertile	4-6	C	fertile
4-6	D	fertile	4-6	D	fertile
4-6	E	fertile	4-6	E	fertile
6-8	A	**sterile**	6-8	A	fertile
6-8	B	fertile	6-8	B	**sterile**
6-8	C	**sterile**	6-8	C	**sterile**
6-8	D	**sterile**	6-8	D	fertile
6-8	E	fertile	6-8	E	**sterile**
8-10	A	**sterile**	8-10	A	**sterile**
8-10	B	**sterile**	8-10	B	**sterile**
8-10	C	**sterile**	8-10	C	**sterile**
8-10	D	**sterile**	8-10	D	**sterile**
8-10	E	**sterile**	8-10	E	**sterile**

Table S7. Allele frequencies of TS-*ClvR*^{dbe} in the drive experiment at generation 18. Allele frequencies were measured by individually outcrossing 100 males from the drive populations to w^{1118} females. Males that produced 100% TS-*ClvR* bearing offspring were considered to be homozygous. Males that produced 50% TS-*ClvR* bearing offspring were considered to be heterozygous.

Drive replicate	homozygous	heterozygous	total alleles	allele freq (%) TS-ClvR
A	71	4	150	97.33
B	70	11	162	93.21
C	61	6	134	95.52
D	78	4	164	97.56

Table S8: Incubation of gene drive populations at a restrictive temperature of 29° C. Flies from the gene drive experiment described in Fig. 4 were transferred to a fresh food bottle and incubated at 29° C. They produced offspring for one more generation. That next generation was sterile resulting in a complete population collapse.

Replicate	Generation drive	output in Generation n+1	output in Generation n+2
A	10	486	**0**
B	10	575	**0**
C	10	433	**0**
D	10	188	**0**
A	12	513	**0**
B	12	477	**0**
C	12	430	**0**
D	12	367	**0**
A	13	476	**0**
B	13	481	**0**
C	13	588	**0**
D	13	456	**0**
A	16	321	**0**
B	16	272	**0**
C	16	251	**0**
D	16	369	**0**
A	17	245	**0**
B	17	295	**0**
C	17	214	**0**
D	17	229	**0**

Table S9: *ClvR^{dbe}* drive populations at 29^{o} C. As a control we took flies carrying *ClvR^{dbe}* (non-TS) from a previously performed gene drive experiment (27) and transferred them to an incubator at 29^{o} C. Flies were handled as with the other gene drive experiments. Every generation was transferred to a fresh food bottle and always kept at 29^{o} C. This cycle was repeated for a total of 6 generations. Population size remained constant around the carrying capacity of the food bottles with no obvious fitness effects.

Replicate	Generation drive (n)	Fly output generation (n+1)	Fly output generation (n+2)	Fly output generation (n+3)	Fly output generation (n+4)	Fly output generation (n+5)	Fly output generation (n+6)
A	44	581	430	642	508	416	488
B	44	575	547	611	610	535	530
C	44	540	514	678	636	516	606
D	44	381	445	709	657	403	484