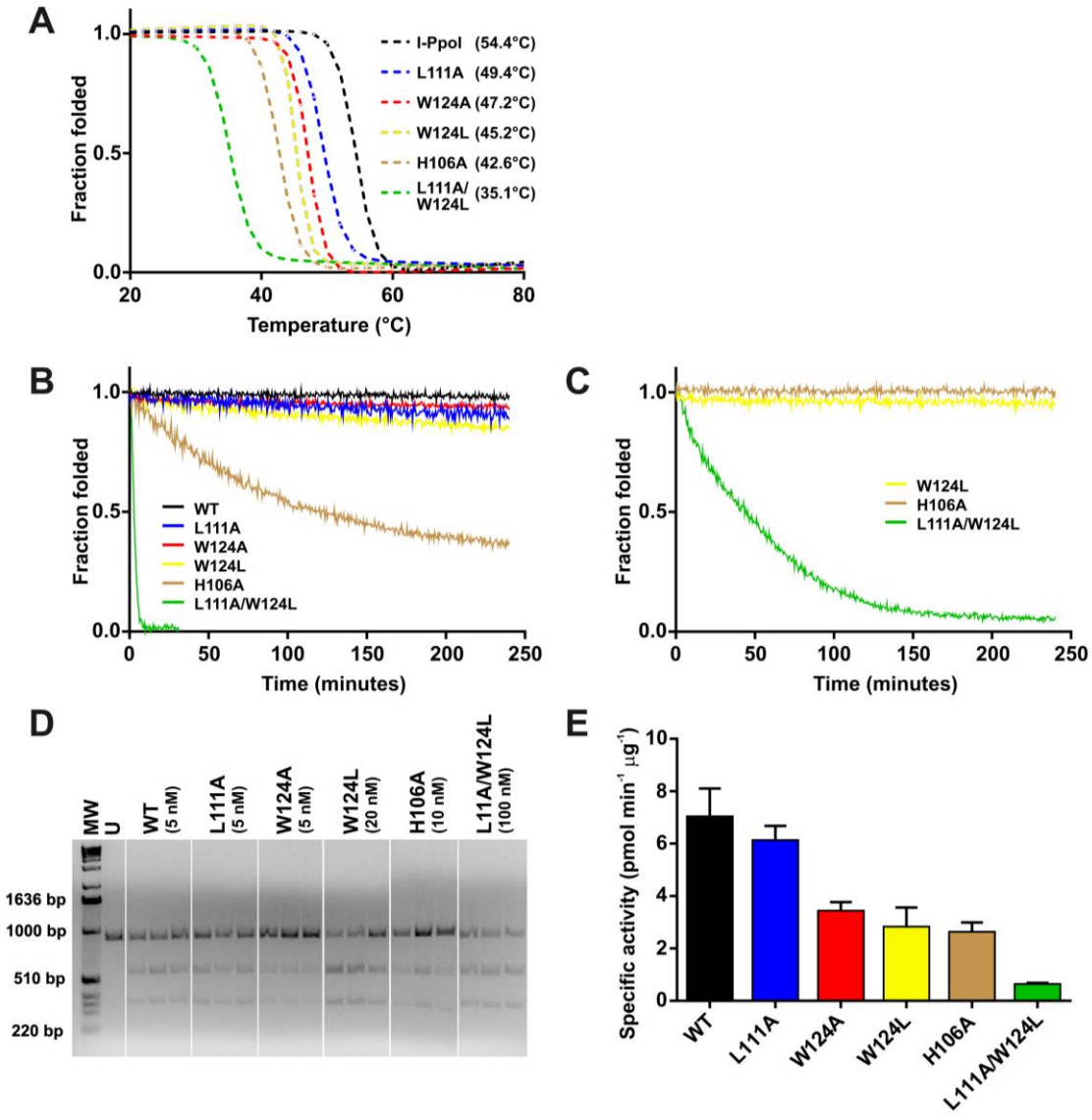
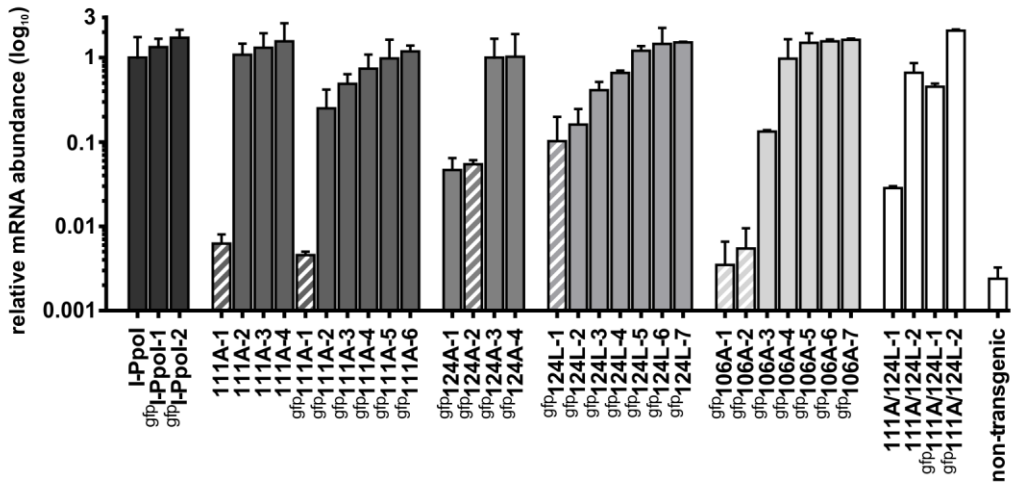
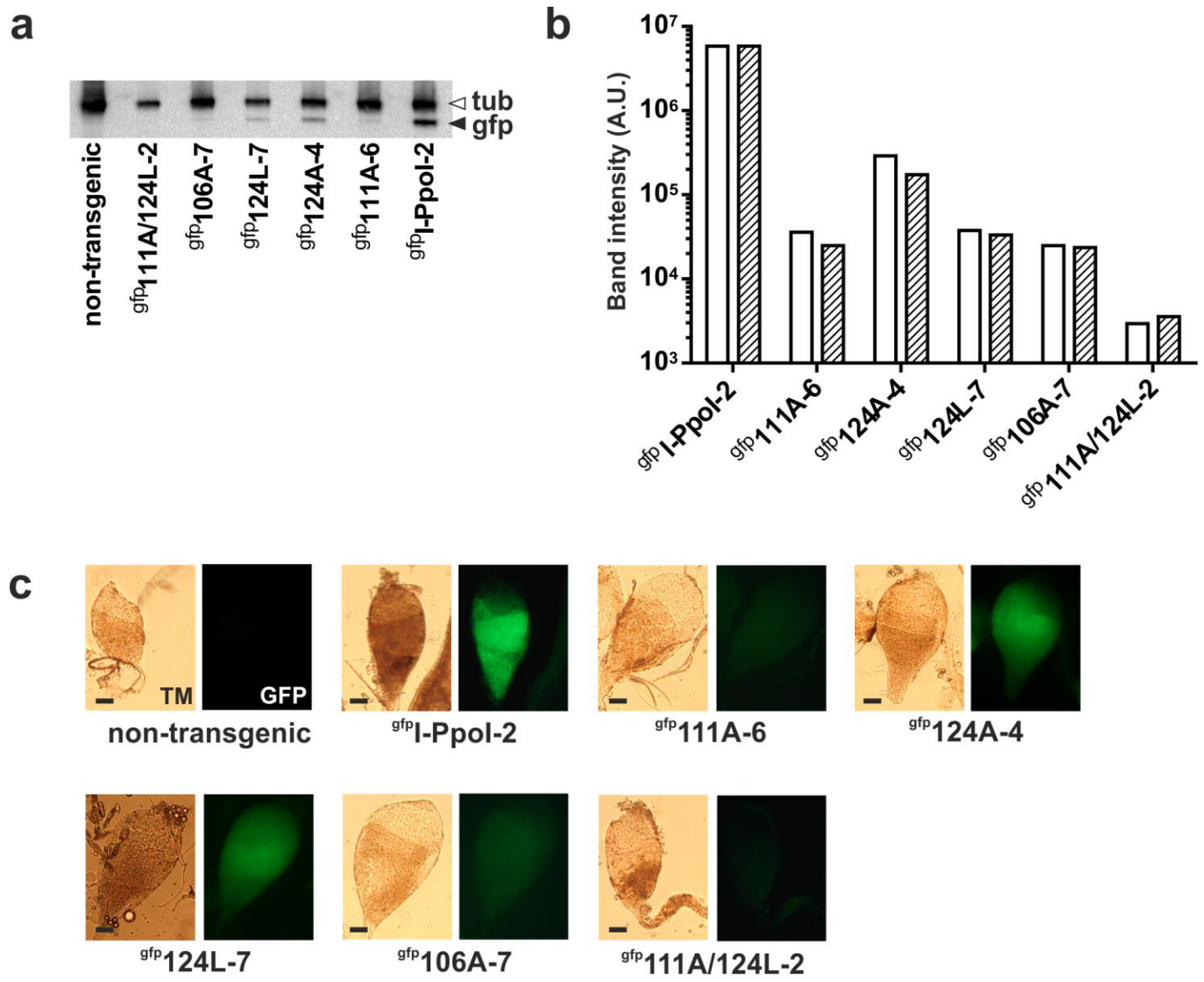


Supplementary Figure 1. ***In-vitro*** **characterization of I-PpoI variants. (A)** Temperature-dependent denaturation of wild-type and mutated I-PpoI structural variants, measured by circular dichroism (CD) spectroscopy. CD signals (corresponding to rotation of polarized incident UV light) were measured every 2° of increasing temperature at 206 nm (corresponding to maximum difference between folded and unfolded protein samples). All constructs display cooperative thermal denaturation profiles, and a range of thermal unfolding mid-points ("Tm values") ranging from 54.4°C to 35.1°C. **(B)** and **(C)** Kinetic denaturation profiles of wild-type and mutant I-PpoI enzymes. A sample of each purified construct was subjected to a kinetic analysis of thermal denaturation as described in the methods text, which was also monitored using CD spectroscopy while maintaining a constant temperature of 37°C **(B)** or 30°C **(C)**. The values and range of *in vitro* half-lives (Table 1.) displayed by the endonuclease constructs was calculated as described in the methods. **(D)** and **(E)** Determination of specific activities of I-PpoI constructs. Error bars represent the standard error of the mean from 3 replicate digests. **(D)** Typical DNA digest experiments in which a sample of each purified construct was incubated with a linear double stranded DNA substrate containing a

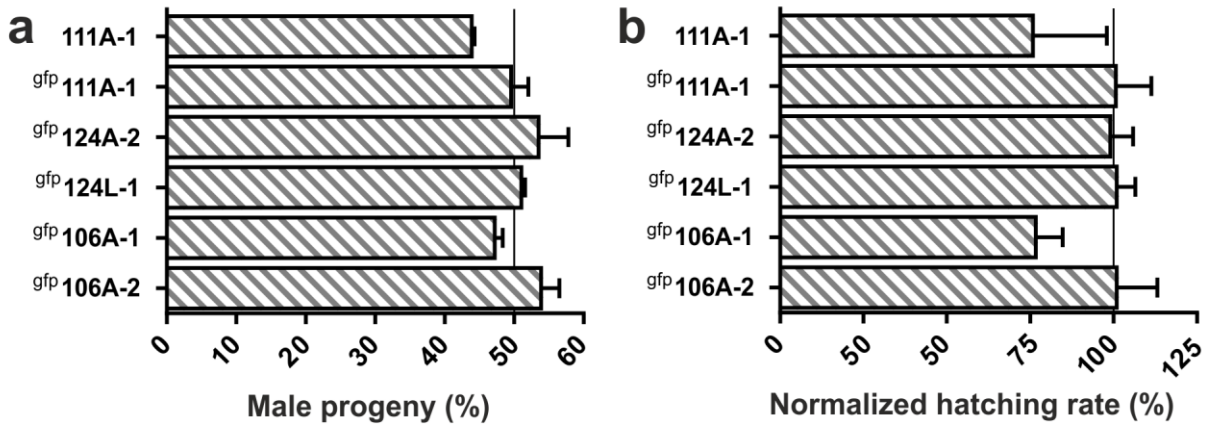
single copy of the I-PpoI target site as described in the methods text, and the specific activity of each enzyme was calculated and converted into consistent units (picomoles of DNA cleaved per minute per microgram of enzyme; pmol min^{-1} μg^{-1}) The concentration of the DNA substrate, temperature and incubation was the same for all constructs; the enzyme concentrations were adjusted to ensure partial digestion of substrate (and corresponding production of visible product) that could easily be quantitated. All digests for each construct were done in triplicate in the same experimental run. The resulting digest products were run on multiple gels, resulting in the composite image shown in **(D)**. **(E)** Relative specific activities of the constructs used in construction of transgenic mosquito lines.



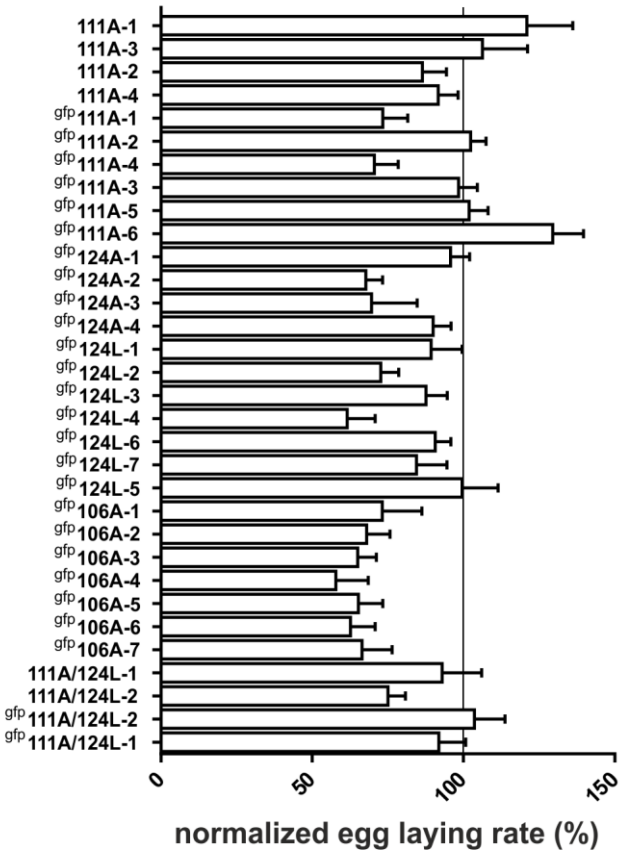
Supplementary Figure 2. Relative I-PpoI mRNA abundance in testes of transgenic mosquitoes lines. The mRNA levels were quantified by qRT-PCR and normalized against the ribosomal protein gene *Rpl19*. Shown is the range of the minimum and maximum fold changes detected (whiskers) and the mean (bars) relative to the wild-type I-PpoI reference. Dashed bars indicate strains carrying X-linked transgenes.



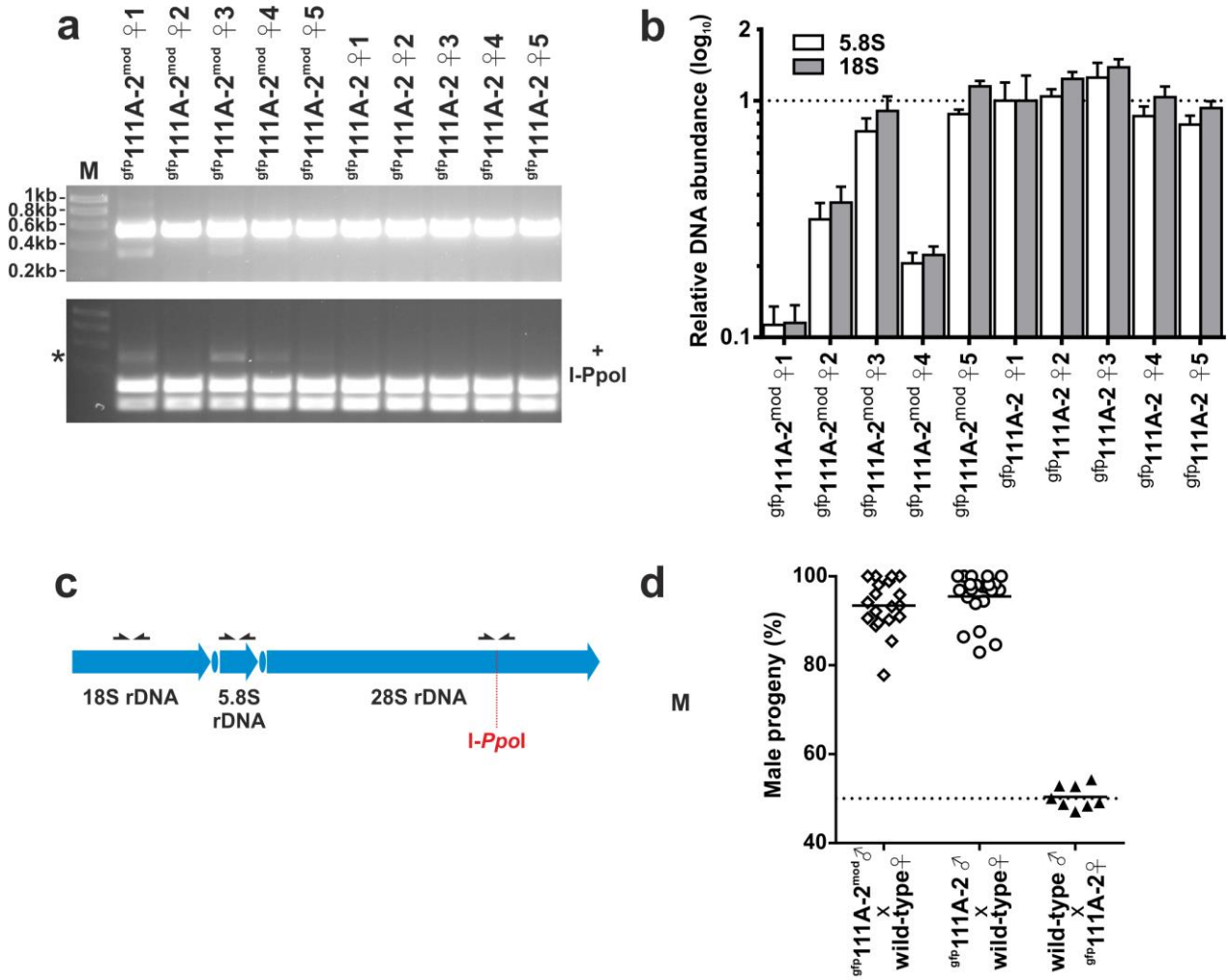
Supplementary Figure 3. Analysis of the protein levels of I-PpoI variants in the testes of transgenic mosquitoes. (A) Western blot analysis of wild-type and mutant I-PpoI protein (46.4kDa) in the transgenic lines with the highest mRNA expression levels of the corresponding I-PpoI variant compared to the α-tubulin control (50.9kDa). **(B)** Quantification of GFP::I-PpoI band intensities in (A) normalized for the levels of the α-tubulin control (solid bars) or for the α-tubulin control in addition to the relative mRNA expression levels of these lines (striped bars). **(C)** Transmission and fluorescent images of dissected adult testes of these transgenic lines. Scale bars 50μm.



Supplementary Figure 4. X-linked I-PpoI transgenes are inactive. (A) The adult sex ratio (±SEM) of the progeny of hemizygous transgenic males and wild-type females of all transgenic lines with single copy X-linked integrations. **(B)** The larval hatching rate (±SEM) of the progeny of hemizygous transgenic males and wild-type females of these X-linked transgenic lines normalized against the hatching rate of hemizygous transgenic females and wild-type males of the same lines.

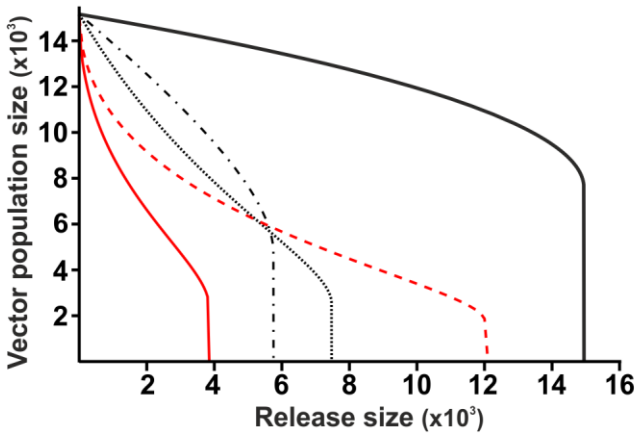


Supplementary Figure 5. Egg laying rates of of transgenic mosquitoes. Shown is the percentage of eggs laid by wild-type females crossed to hemizygous transgenic males of all lines normalized against the number of eggs laid by hemizygous transgenic females of the same strains when crossed to wild-type males. Error bars represent the standard error of the mean of experiments performed in at least two independent generations.



Supplementary Figure 6. Analysis of modified X-chromosomes. (A) I-PpoI re-cleavage assay. Daughters of ^{gfp}111A-2 males were backcrossed to ^{gfp}111A-2 males and their female progeny (5 different individuals) analysed for potential modifications to the ribosomal DNA on their X-chromosomes. Stock ^{gfp}111A-2 females (5 individuals) were used as a comparison. 28S rDNA was amplified by PCR (upper panel) and the PCR product digested by I-PpoI *in-vitro* (lower panel). The asterisk marks cleavage resistant rDNA indicating prior miss-repair *in-vivo*. **(B)** Genomic DNA of the same females was subjected to quantitiative PCR. Primers were used to amplified the 5.8S and 18S rDNA genes which were normalized against the single-copy autosomal gene *Rpl19*. Shown is the range of the minimum and maximum fold changes detected (whiskers) and the mean (bars) relative to the stock ^{gfp}111A-2 female #1 used as a reference. **(C)** Shown is the structure of the rDNA repeat unit including the 5.8S, 18S and 28S ribosomal genes, the location of the I-PpoI recognition site and the internal transcribed spacers (circles) as well as the relative position of the primers used for PCR. **(D)** The transgenic daughters of ^{gfp}111A-2 males were backcrossed to wild-type to generate transgenic males potentially carrying a cleavage resistant X-chromosome referred to as ^{gfp}111A-2^{mod}. These males were individually crossed to wild-type females and the adult sex ratio of the progeny of each

female was determined. As a positive control ^{gfp}111A-2 stock males were individually crossed to wild-type females and as a negative control wild-type males were crossed to stock ^{gfp}111A-2 females. The bars indicate the mean sex ratios of these groups.



Supplementary Figure 7. Comparison of vector control strategies. Equilibrium number of vectors as a function of the number of intervention males released at each generation according to a discrete generation model. The population is initially composed of $3x10^4$ individuals with a 50% sex ratio. The released males are assumed to be sterile (solid black line), carry a bisexual late-acting RIDL (Release of insects containing a dominant lethal) construct (dot-dashed black line), carry a female specific late acting RIDL construct (dotted black line) or carry an autosomal sex distorter inducing a bias towards males of 95%, either as hemizygotes (dashed red line) or as homozygotes (solid red line).

Supplementary Table 1. Characterization of transgenic lines originating from a single integration event.

Transformation construct	Strain	Chromosome band	Insertion site	% transgenic (G2 progeny)
pBac [3xP3-DsRed]β2-eGFP(F2A)I-PpoI	I-PpoI	2L-20C	TTTCACTTGATTAA-**PB**-TTAAAGATGGACTG	46.4 (28)
pBac [3xP3-DsRed]β2-eGFP(F2A)I-PpoI-111A	111A-1	X-5D>	GTTTCGTAGTTTAA-**PB**-TTAACGTTACCGCG	46.6 (88)
	111A-2	3L-44B	GATTAGAGAGTTAA-**PB**-TTAAAAGAGAATAC	N/A
	111A-3	2L-21F	AACCCTTTTGTTAA-**PB**-TTAATCAACGTGCCT	48.2 (112)
	111A-4	3R-35C	CATTTTTTTTTTAA-**PB**-TTAAGGTCGCTGTT	41.1 (112)
pBac [3xP3-DsRed]β2-eGFP::I-PpoI-111A	^{gfp}111A-1	X-5D	TCTTCATGGTTTAA-**PB**-TTAAGAACGATCCT	53.2 (77)
	^{gfp}111A-2	2R-19C	GCATTGTGTATTAA-**PB**-TTAATACGGTTACA	35.0 (40)
	^{gfp}111A-3	2R-<7B	TTACTTTTTATTAA-**PB**-TTAAGTAACAATCT	53.3 (30)
	^{gfp}111A-4	3L-44B	GCAGCGCTTTTTAA-**PB**-TTAATGAAACGTGA	52.9 (70)
	^{gfp}111A-5	3R-37C>	ATCCAGAACTTTAA-**PB**-TTAATGTAGATTCC	47.6 (21)
	^{gfp}111A-6	2R-13E	CTTTTGGCGTTTAA-**PB**-TTAATGAGAACCAT	40.7 (59)
pBac [3xP3-DsRed]β2-eGFP::I-PpoI-124A	^{gfp}124A-1	2L-20C	TGATGTGTGTTTAA-**PB**-TAATATTTACATTT	33.3 (96)
	^{gfp}124A-2	X-1C	ACCAACCTTCTTAA-**PB**-TTAACGCGATGCAC	45.5 (112)
	^{gfp}124A-3	2R-15D	ATTATAAGCCTTAA-**PB**-TTAACCTTGTACAA	53.6 (112)
	^{gfp}124A-4	2R-<7B	GCACGAATCGTTAA-**PB**-TTAAACAAAACTTC	42.2 (56)
pBac [3xP3-DsRed]β2-eGFP::I-PpoI-124L	^{gfp}124L-1	X-1B	GGTATCGAATTTAA-**PB**-TTAAAAGAGCACTG	49.1 (110)
	^{gfp}124L-2	3R-36D	TTTGTTTCTATTAA-**PB**-TTAATTATAGCAAG	52.7 (112)

	^{gfp}124L-3	3L-43A or 2R-14B	**PB**-TTAAAGCAAGTAAAA	50.0 (112)
	^{gfp}124L-4	2L-20C	CATTTTATACTTAA-**PB**-TTAAATAATACCCA	43.8 (112)
	^{gfp}124L-5	2R-8B	AGTATTTAGTTTAA-**PB**-TTAAAGCATTACAG	50.0 (112)
	^{gfp}124L-6	3L-45C	TCAGTGTCCTTTAA-**PB**-TTAAAGGGCTCAAT	51.8 (112)
	^{gfp}124L-7	2R-15D	TAGCTTAATCTTAA-**PB**-TTAATTAAGCGTAA	43.9 (98)
pBac [3xP3-DsRed]β2-eGFP::I-PpoI-106A	^{gfp}106A-1	X-5D>	CAGCATTGTTTTAA-**PB**-TTAATCGATTCGCA	46.9 (96)
	^{gfp}106A-2	X-1B	GTGTAATAAATTAA-**PB**-TTAAGCGGACAAAC	51.1 (88)
	^{gfp}106A-3	2L-20C	TCCACCTTGGTTAA-**PB**-TTAAGCTACGGCCC	38.5 (96)
	^{gfp}106A-4	3L-38B	AAAACGTCGTTTAA-**PB**-TTAATGGACCGTTC	42.0 (88)
	^{gfp}106A-5	2R-8A	TGGAGCTTTGTTAA-**PB**-TTAAGAAACATGAA	46.9 (96)
	^{gfp}106A-6	3R-32A	ATTGATGCCTTTAA-**PB**-TTAAAAGATGCACA	32.3 (96)
	^{gfp}106A-7	2L-28C>	TTGTCACGCTTTAA-**PB**-TTAAACAATCTGAA	43.8 (96)
pBac [3xP3-DsRed]β2-eGFP::I-PpoI-111A/124L	111A/124L-1	2R-10A	ACAGCAATATTTAA-**PB**-TTAATAGATAGGCT	48.4 (31)
	111A/124L-2	N/A	N/A	43.8 (64)
pBac [3xP3-DsRed]β2-eGFP(F2A)I-PpoI-111A/124L	^{gfp}111A/124L-1	3R-34C	CAAAGGAAGCTTAA-**PB**-TTAAGCGATGAAAT	53.3 (90)
	^{gfp}111A/124L-2	2L-27D	TACAATACTGTTAA-**PB**-TTAAAAAGTTTGCA	52.7 (93)

The 14 base pairs flanking the integration site of the transformation construct are shown. The final column shows the inheritance rate of the transgene scored in the G2 progeny. The total number of larvae counted is given in parentheses.

Supplementary Table 2. Outcome of crosses between transgenic and wild-type mosquitoes.

Transgenic line	Cross	# eggs / female[1]	% eggs hatching[2]	% normalized hatching rate[3]	% transgenics[4]	% male sex ratio[5]	*p* value
I-PpoI	I-PpoI ♂ x wild-type ♀	102.2 ±17.7 n=10 (1022)	0.0 n=10	0.0	-	-	-
	I-PpoI ♀ x wild-type ♂	147.9 ±8.9 n=11 (1627)	67.8 ±5.2 n=11		48.3 (211)	51.8 (1565)	
111A-1	I-PpoI ♂ x wild-type ♀	136.6 ±17.2 n=7 (956)	51.6 ±15.1 n=7	75.8	68.7 (163)	43.6 (477)	0.0003
	I-PpoI ♀ x wild-type ♂	112.9 ±13.7 n=10 (1129)	68.0 ±9.3 n=10		50.6 (239)	56.3 (384)	
111A-2	I-PpoI ♂ x wild-type ♀	118.9 ±11.0 n=19 (2260)	7.0 ±2.1 n=19	10.2	41.7 (103)	81.3 (139)	<0.0001
	I-PpoI ♀ x wild-type ♂	137.6 ±11.5 n=18 (2476)	68.5 ±7.9 n=18		53.8 (158)	47.4 (914)	
111A-3	I-PpoI ♂ x wild-type ♀	136.2 ±19.2 n=9 (1226)	4.2 ±1.2 n=9	9.2	51.0 (51)	83.3 (102)	<0.0001
	I-PpoI ♀ x wild-type ♂	128.2 ±19.6 n=9 (1154)	46.2 ±13.7 n=9		52.5 (238)	50.6 (433)	
111A-4	I-PpoI ♂ x wild-type ♀	133.7 ±9.5 n=20 (2674)	4.7 ±1.7 n=20	6.8	48.1 (52)	79.3 (82)	<0.0001
	I-PpoI ♀ x wild-type ♂	145.8 ±14.0 n=12 (1750)	69.2 ±7.7 n=12		44.8 (259)	50.2 (470)	
^{gfp}111A-1	I-PpoI ♂ x wild-type ♀	128.2 ±14.4 n=13 (1667)	61.6 ±6.5 n=13	100.6	49.7 (197)	49.9 (1250)	0.4267
	I-PpoI ♀ x wild-type ♂	174.9 ±11.6 n=10 (1749)	61.2 ±4.5 n=10		50.7 (300)	51.4 (1980)	
^{gfp}111A-2	I-PpoI ♂ x wild-type ♀	196.1 ±9.7 n=27 (5294)	57.1 ±4.0 n=27	88.5	50.6 (399)	95.0 (2232)	<0.0001
	I-PpoI ♀ x wild-type ♂	191.6 ±12.6 n=22 (4216)	64.4 ±2.8 n=22		48.7 (509)	51.9 (1834)	
^{gfp}111A-3	I-PpoI ♂ x wild-type ♀	189.1 ±12.0 n=13 (2458)	3.0 ±0.7 n=13	6.6	58.6 (58)	89.1 (55)	<0.0001
	I-PpoI ♀ x wild-type ♂	192.2 ±14.1 n=13 (2499)	46.3 ±6.4 n=13		50.3 (364)	55.7 (1383)	

^{gfp}111A-4	I-PpoI ♂ x wild-type ♀	128.2 ±14.4 n=13 (1667)	1.1 ±0.3 n=13	1.4	54.8 (42)	90.0 (40)	<0.0001
	I-PpoI ♀ x wild-type ♂	181.8 ±5.2 n=11 (2000)	77.5 ±8.5 n=11		51.7 (263)	44.5 (1293)	
^{gfp}111A-5	I-PpoI ♂ x wild-type ♀	171.8 ±10.7 n=10 (1718)	2.2 ±0.6 n=10	3.7	48.7 (230)	95.0 (119)	<0.0001
	I-PpoI ♀ x wild-type ♂	168.7 ±12.2 n=13 (2193)	58.9 ±7.7 n=13		54.1 (257)	50.2 (997)	
^{gfp}111A-6	I-PpoI ♂ x wild-type ♀	180.7 ±14.2 n=10 (1807)	1.6 ±0.4 n=10	3.8	51.9 (54)	91.8 (49)	<0.0001
	I-PpoI ♀ x wild-type ♂	139.5 ±13.8 n=10 (1395)	42.6 ±7.3 n=10		48.8 (240)	49.1 (1465)	
^{gfp}124A-1	I-PpoI ♂ x wild-type ♀	167.9 ±11.0 n=7 (1175)	20.5 ±6.6 n=7	24.1	43.9 (66)	85.1 (114)	<0.0001
	I-PpoI ♀ x wild-type ♂	175.3 ±21.9 n=7 (1227)	85.1 ±2.1 n=7		31.9 (144)	48.8 (504)	
^{gfp}124A-2	I-PpoI ♂ x wild-type ♀	117.5 ±9.6 n=8 (940)	81.0 ±5.6 n=8	99.0	38.5 (91)	53.9 (568)	0.0995
	I-PpoI ♀ x wild-type ♂	173.5 ±13.9 n=11 (1909)	81.8 ±3.7 n=11		50.5 (283)	48.8 (508)	
^{gfp}124A-3	I-PpoI ♂ x wild-type ♀	107.0 ±23.3 n=7 (749)	4.6 ±2.3 n=7	6.1	N/A	76.7 (60)	0.0003
	I-PpoI ♀ x wild-type ♂	153.8 ±11.4 n=18 (2768)	74.6 ±8.5 n=18		49.8 (301)	52.3 (938)	
^{gfp}124A-4	I-PpoI ♂ x wild-type ♀	154.1 ±10.1 n=7 (1079)	2.0 ±0.6 n=7	2.2	42.9 (7)	90.1 (111)	<0.0001
	I-PpoI ♀ x wild-type ♂	171.3 ±31.8 n=6 (1028)	88.4 ±1.9 n=6		51.6 (155)	50.1 (693)	
^{gfp}124L-1	I-PpoI ♂ x wild-type ♀	167.6 ±19.4 n=11 (1844)	76.4 ±4.3 n=11	100.9	46.7 (199)	51.0 (974)	0.2759
	I-PpoI ♀ x wild-type ♂	187.8 ±23.2 n=6 (1127)	75.7 ±7.0 n=6		44.7 (273)	48.3 (700)	
^{gfp}124L-2	I-PpoI ♂ x wild-type ♀	145.3 ±11.8 n=12 (1744)	75.2 ±7.6 n=12	100.4	48.9 (135)	95.7 (1104)	<0.0001
	I-PpoI ♀ x wild-type ♂	199.9 ±15.7 n=10 (1999)	74.8 ±8.5 n=10		45.6 (193)	50.1 (721)	
^{gfp}124L-3	I-PpoI ♂ x wild-type ♀	130.5 ±10.5 n=15 (1958)	74.4 ±7.9 n=15	104.3	40.7 (145)	96.6 (1096)	<0.0001

	I-PpoI ♀ x wild-type ♂	149.0 ±16.6 n=10 (1490)	71.3 ±9.6 n=10		49.8 (279)	47.5 (731)		
^{gfp}124L-4	I-PpoI ♂ x wild-type ♀	96.6 ±14.4 n=13 (1256)	63.0 ±8.4 n=13	80.3	50.8 (122)	97.4 (779)	]	<0.0001
	I-PpoI ♀ x wild-type ♂	156.9 ±17.3 n=10 (1569)	78.5 ±9.0 n=10		46.8 (267)	47.4 (593)		
^{gfp}124L-5	I-PpoI ♂ x wild-type ♀	147.9 ±17.6 n=11 (1627)	31.7 ±8.1 n=11	42.1	53.9 (102)	91.6 (333)	]	<0.0001
	I-PpoI ♀ x wild-type ♂	148.5 ±14.8 n=13 (1931)	75.3 ±6.6 n=13		53.0 (264)	51.9 (882)		
^{gfp}124L-6	I-PpoI ♂ x wild-type ♀	165.9 ±9.5 n=13 (2157)	36.9 ±10.4 n=13	56.3	50.0 (84)	89.9 (583)	]	<0.0001
	I-PpoI ♀ x wild-type ♂	183.1 ±22.0 n=7 (1282)	65.7 ±17.2 n=7		52.1 (280)	51.7 (775)		
^{gfp}124L-7	I-PpoI ♂ x wild-type ♀	104.4 ±12.4 n=14 (1461)	41.7 ±8.5 n=14	59.5	45.2 (135)	95.1 (473)	]	<0.0001
	I-PpoI ♀ x wild-type ♂	123.5 ±12.9 n=13 (1605)	70.1 ±6.4 n=13		45.5 (246)	54.2 (696)		

^{gfp}106A-1	I-PpoI ♂ x wild-type ♀	133.6 ±23.8 n=13 (1737)	62.3 ±6.6 n=13	76.6	46.8 (158)	47.0 (521)	]	0.5162
	I-PpoI ♀ x wild-type ♂	182.5 ±17.1 n=21 (3833)	81.4 ±2.7 n=21		52.2 (268)	48.8 (993)		
^{gfp}106A-2	I-PpoI ♂ x wild-type ♀	134.6 ±15.2 n=13 (1750)	64.7 ±7.8 n=13	101.0	39.0 (182)	53.6 (573)	]	0.0615
	I-PpoI ♀ x wild-type ♂	197.9 ±18.9 n=9 (1781)	64.1 ±11.4 n=9		52.6 (175)	48.1 (597)		
^{gfp}106A-3	I-PpoI ♂ x wild-type ♀	121.2 ±11.5 n=16 (1939)	55.0 ±8.5 n=16	69.9	54.1 (111)	93.1 (304)	]	<0.0001
	I-PpoI ♀ x wild-type ♂	186.4 ±17.2 n=18 (3356)	78.7 ±7.1 n=18		42.9 (259)	54.6 (621)		
^{gfp}106A-4	I-PpoI ♂ x wild-type ♀	118.8 ±22.0 n=12 (1426)	29.3 ±3.8 n=12	37.9	55.1 (69)	83.4 (265)	]	<0.0001
	I-PpoI ♀ x wild-type ♂	205.6 ±24.9 n=12 (2467)	77.4 ±2.4 n=12		45.6 (204)	48.5 (895)		
^{gfp}106A-5	I-PpoI ♂ x wild-type ♀	109.5 ±13.6 n=12 (1314)	9.4 ±3.2 n=12	12.5	37.9 (58)	93.9 (98)	]	<0.0001
	I-PpoI ♀ x wild-type ♂	167.8 ±12.3 n=13 (2182)	75.0 ±8.4 n=13		43.3 (215)	51.6 (601)		

^{gfp}106A-6	I-PpoI ♂ x wild-type ♀	97.1 ±12.6 n=27 (2622)	5.4 ±2.3 n=27	6.5	53.8 (26)	82.1 (67)	<0.0001
	I-PpoI ♀ x wild-type ♂	154.9 ±11.8 n=20 (3098)	82.3 ±5.1 n=20		39.9 (228)	52.7 (818)	
^{gfp}106A-7	I-PpoI ♂ x wild-type ♀	118.0 ±17.5 n=15 (1770)	9.7 ±2.6 n=15	13.2	48.0 (50)	84.8 (125)	<0.0001
	I-PpoI ♀ x wild-type ♂	177.5 ±15.2 n=14 (2485)	73.3 ±7.2 n=14		47.7 (239)	49.3 (764)	
111A/124L-1	I-PpoI ♂ x wild-type ♀	142.7 ±20.1 n=13 (1855)	75.1 ±4.1 n=13	89.4	50.0 (142)	47.1 (1164)	0.1191
	I-PpoI ♀ x wild-type ♂	153.5 ±25.4 n=14 (2149)	84.0 ±4.7 n=14		58.0 (162)	50.7 (805)	
111A/124L-2	I-PpoI ♂ x wild-type ♀	128.4 ±9.9 n=14 (1797)	79.3 ±6.4 n=14	92.9	51.6 (95)	54.6 (491)	0.0655
	I-PpoI ♀ x wild-type ♂	171.1 ±14.5 n=11 (1882)	85.4 ±1.8 n=11		45.2 (221)	49.2 (782)	
^{gfp}111A/124L-1	I-PpoI ♂ x wild-type ♀	175.8 ±17.0 n=10 (1758)	50.7 ±7.1 n=10	87.0	51.6 (206)	50.5 (877)	0.9648
	I-PpoI ♀ x wild-type ♂	191.4 ±9.1 n=9 (1723)	58.3 ±10.6 n=9		45.2 (194)	50.4 (1229)	
^{gfp}111A/124L-2	I-PpoI ♂ x wild-type ♀	151.4 ±14.7 n=11 (1665)	50.6 ±9.7 n=11	101.0	51.6 (194)	46.0 (678)	0.1067
	I-PpoI ♀ x wild-type ♂	146.0 ±15.1 n=11 (1606)	50.1 ±7.3 n=11		45.2 (189)	51.1 (421)	

Analysis of the progeny of 10 hemizygote males crossed to 10 wild-type females. As control 10 hemizygote females from the same line were crossed to 10 wild-type males. The experiments and the controls were repeated for at least 2 generations. (1) Average number of eggs laid by n number of females analysed (± represents the standard error of the mean; SEM). (2) Average percentage of larvae hatching from the eggs (±SEM), from n females analysed. (3) The hatching rate was normalized against the respective control. (4) Percentage of transgenic offspring in the pooled progeny of all females. (5) Percentage of males in the pooled progeny of all females progeny. Significance (Fisher's exact test, two-tailed, 2x2 contingency table) was tested comparing the total number of males and females observed versus the respective control. The total number of eggs or individuals counted in each experiment is given in parentheses.

Supplementary Table 3. Effect of rearing temperature on the phenotype of transgenic distorter strains.

Transgenic line	Rearing temperature (°C)	Cross	# eggs female[1]	% eggs hatching[2]	% normalized hatching rate[3]	% male sex ratio[4]	*p* value
^{gfp}111A-2	24	^{gfp}111A-2 ♂ x wild-type ♀	95.1 ±6.4 n=22 (2093)	19.0 ±4.1 n=22	29.0	90.1 ±1.8 (261) n=16	<0.0001
		^{gfp}111A-2 ♀ x wild-type ♂	148.0 ±13.3 n=20 (2959)	65.6 ±8.0 n=20		49.5 ±2.4 (1084) n=8	
	28	^{gfp}111A-2 ♂ x wild-type ♀	178.2 ±8.0 n=43 (7662)	59.6 ±4.5 n=43	75.0	95.7 ±0.7 (3190) n=36	<0.0001
		^{gfp}111A-2 ♀ x wild-type ♂	170.4 ±7.3 n=58 (9882)	79.4 ±2.2 n=58		51.6 ±0.7 (6894) n=58	
	32	^{gfp}111A-2 ♂ x wild-type ♀	103.4 ±17.7 n=7 (724)	75.7 ±4.7 n=7	104.7	91.7 ±1.7 (447) n=7	<0.0001
		^{gfp}111A-2 ♀ x wild-type ♂	107.0 ±19.0 n=6 (642)	72.3 ±5.3 n=6		48.0 ±3.6 (375) n=6	
^{gfp}124L-2	24	^{gfp}124L-2 ♂ x wild-type ♀	98.7 ±4.0 n=29 (2664)	66.8 ±5.3 n=29	99.4	94.2 ±1.3 (1085) n=17	<0.0001
		^{gfp}124L-2 ♀ x wild-type ♂	102.3 ±13.2 n=9 (921)	67.2 ±9.1 n=9		50.4 ±3.0 (592) n=8	
	28	^{gfp}124L-2 ♂ x wild-type ♀	161.7 ±14.1 n=12 (1940)	73.9 ±6.3 n=12	99.6	94.6 ±0.9 (1235) n=12	<0.0001
		^{gfp}124L-2 ♀ x wild-type ♂	166.1 ±13.9 n=10 (1661)	74.2 ±2.7 n=10		50.5 ±1.7 (1007) n=10	
	32	^{gfp}124L-2 ♂ x wild-type ♀	66.9 ±4.9 n=25 (1672)	74.6 ±5.1 n=25	107.8	87.9 ±1.5 (651) n=18	<0.0001
		^{gfp}124L-2 ♀ x wild-type ♂	68.6 ±7.4 n=17 (1167)	69.2 ±5.4 n=17		49.4 ±2.5 (437) n=13	

Outcome of crosses between ^{gfp}111A-2 and ^{gfp}124L-2 hemizygote males crossed to wild-type females. As a control hemizygote females were crossed to wild-type males. Mosquitoes were reared at 24, 28 or 32 °C. (1) Average number of eggs laid by n number of females analysed (±SEM). (2) Average percentage of larvae hatching from the eggs (±SEM), from n females analysed. (3) The hatching rate was normalized against the respective control. (4) Average percentage of males in the progeny (±SEM) established from n females. Significance (Welch's t-test, two-tailed, unpaired two-sample with unequal variance) was tested comparing *logit* transformed percentages observed from the progeny of n females versus the respective control values. The total number of eggs or individuals counted in each experiment is given in parentheses.

Supplementary Table 4. Heritability of the sex distortion phenotype through the male germline.

Generation	Cross	# eggs female[1]	% eggs hatching[2]	% normalized hatching rate[3]	% transgenics[4]	% male sex ratio[5]	*p* value
G2	^{gfp}111A-2 ♂ x wild-type ♀	N/A	N/A	N/A	55.8 (52)	95.2 (600)	<0.0001
	^{gfp}111A-2 ♀ x wild-type ♂	N/A	N/A		50.3 (157)	44.1 (145)	
G3	^{gfp}111A-2 ♂ x wild-type ♀	N/A	N/A	N/A	58.0 (112)	94.8 (940)	<0.0001
	^{gfp}111A-2 ♀ x wild-type ♂	N/A	N/A		56.3 (96)	52.2 (584)	
G4	^{gfp}111A-2 ♂ x wild-type ♀	227.4 ±30.2 n=5 (1137)	65.0 ±6.8 n=5	102.4	49.4 (326)	96.0 (683)	<0.0001
	^{gfp}111A-2 ♀ x wild-type ♂	156.8 ±32.0 n=6 (941)	63.5 ±5.7 n=6		46.4 (112)	51.4 (840)	
G5	^{gfp}111A-2 ♂ x wild-type ♀	189.3 ±15.0 n=9 (1704)	60.3 ±2.7 n=9	94.4	41.1 (56)	98.6 (568)	<0.0001
	^{gfp}111A-2 ♀ x wild-type ♂	203.7 ±16.9 n=9 (1833)	63.8 ±5.2 n=9		43.8 (144)	56.6 (265)	

Outcome of crosses between hemizygote ^{gfp}111A-2 males, originated from ^{gfp}111A-2 fathers, crossed to wild-type females for four consecutive generations (G2 to G5). As a control, hemizygote ^{gfp}111A-2 females, of mothers from the same line, were crossed to wild-type males. (1) Average number of eggs laid by n number of females analysed (±SEM). (2) Average percentage of larvae hatching from the eggs (±SEM), from n females analysed. (3) The hatching rate was normalized against the respective control. (4) Percentage of transgenic offspring. (5) Percentage of males in the progeny. Significance (Fisher's exact test, two-tailed, 2x2 contingency table) was tested comparing number of males and females observed versus the respective control. The total number of eggs or individuals counted in each experiment is given in parentheses.

Supplementary Table 5. Effect of transgene copy-number on the phenotype of transgenic distorter strains.

Transgenic line	Cross	# eggs female[1]	% eggs hatching[2]	% relative hatching rate[3]	% male sex ratio[4]	*p* value
^{gfp}111A-2	^{gfp}111A-2$^{+/+}$ ♂ x wild-type ♀	135.9 ±10.6 n=16 (2175)	28.8 ±8.4 n=16	48.3	88.9 ±2.5 (504) n=11	0.0080
	^{gfp}111A-2$^{+/-}$ ♂ x wild-type ♀	144.8 ±10.0 n=17 (2461)	59.5 ±7.7 n=17		95.3 ±1.3 (1193) n=17	
^{gfp}124L-2	^{gfp}124L-2$^{+/+}$ ♂ x wild-type ♀	179.6 ±8.5 n=17 (3053)	82.0 ±3.2 n=17	111.0	94.7 ±1.0 (2047) n=17	0.3434
	^{gfp}124L-2$^{+/-}$ ♂ x wild-type ♀	161.7 ±14.1 n=12 (1940)	73.9 ±6.3 n=12		94.6 ±0.9 (1235) n=12	
^{gfp}124L-3	^{gfp}124L-3$^{+/+}$ ♂ x wild-type ♀	187.8 ±19.3 n=8 (1502)	67.2 ±8.4 n=8	80.3	96.2 ±1.3 (846) n=8	0.2670
	^{gfp}124L-3$^{+/-}$ ♂ x wild-type ♀	178.1 ±12.1 n=8 (1425)	83.7 ±1.2 n=8		98.2 ±0.8 (983) n=8	

Outcome of crosses between homozygote males of ^{gfp}111A-2, ^{gfp}124L-2 and ^{gfp}124L-3 transgenic lines crossed to wild-type females. As a control hemizygote males were crossed to wild-type females. (1) Average number of eggs laid by n number of females analysed (±SEM). (2) Average percentage of larvae hatching from the eggs (±SEM), from n females analysed. (3) The hatching rate was normalized against the respective control. (4) Average percentage of males in the progeny (±SEM) established from n females. Significance (Welch's t-test, two-tailed, unpaired two-sample with unequal variance) was tested comparing *logit* transformed percentages observed from the progeny of n females versus the respective control values. The total number of eggs or individuals counted in each experiment is given in parentheses.

Supplementary Table 6. Analysis of the daughters of ^{gfp}111A-2 males and their progeny.

Transgenic line	Cross	# eggs female[1]	% eggs hatching[2]	*p* value	% normalized hatching rate[3]	% male sex ratio[4]	*p* value
^{gfp}111A-2	^{gfp}111A-2^{mod} ♀ x wild-type ♂	128.1 ±8.4 n=49	66.0 ±3.8 n=49 (6276)	0.0068	83.0	44.9 ±2.5 n=39 (2372)	0.0011
	^{gfp}111A-2 ♀ x wild-type ♂	170.4 ±7.3 n=58	79.4 ±2.2 n=58 (9882)			51.6 ±0.7 n=58 (6894)	

Outcome of crosses between ^{gfp}111A-2^{mod} females crossed to wild-type males. As a control stock ^{gfp}111A-2 females were crossed to wild-type males. (1) Average number of eggs laid by n number of females analysed (±SEM). (2) Average percentage of larvae hatching from the eggs (±SEM), from n females analysed. (3) The hatching rate was normalized against the respective control. (4) Average percentage of males in the progeny (±SEM) established from n females. Significance (Welch's t-test, two-tailed, unpaired two-sample with unequal variance) was tested comparing *logit* transformed percentages observed from the progeny of n females versus the respective control values. The total number of eggs or individuals counted in each experiment is given in parentheses.