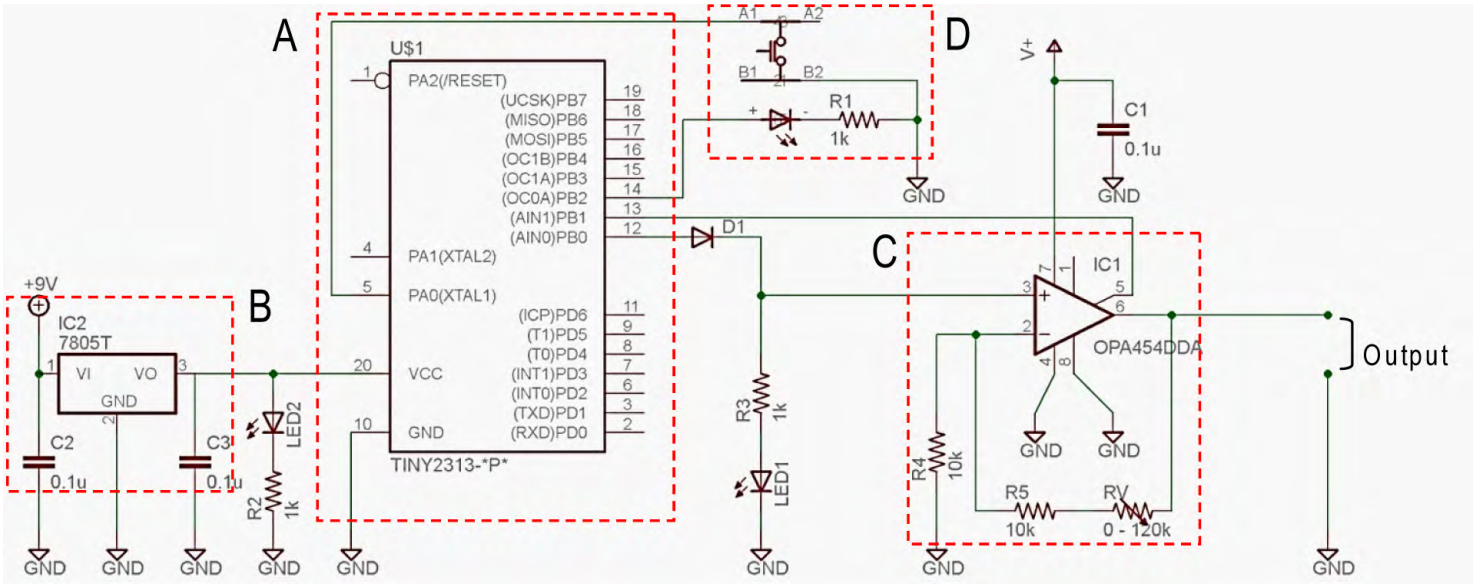

Fig. S1. Electronic circuit of the electroporator. (**A**) AVR microcontroller. (**B**) Power supply unit for AVR. (**C**) Operational amplifier unit. (**D**) LED-containing push switch unit. C1-C3, capacitor; R1-R5 and RV, resistor; D1, diode; GND, ground.

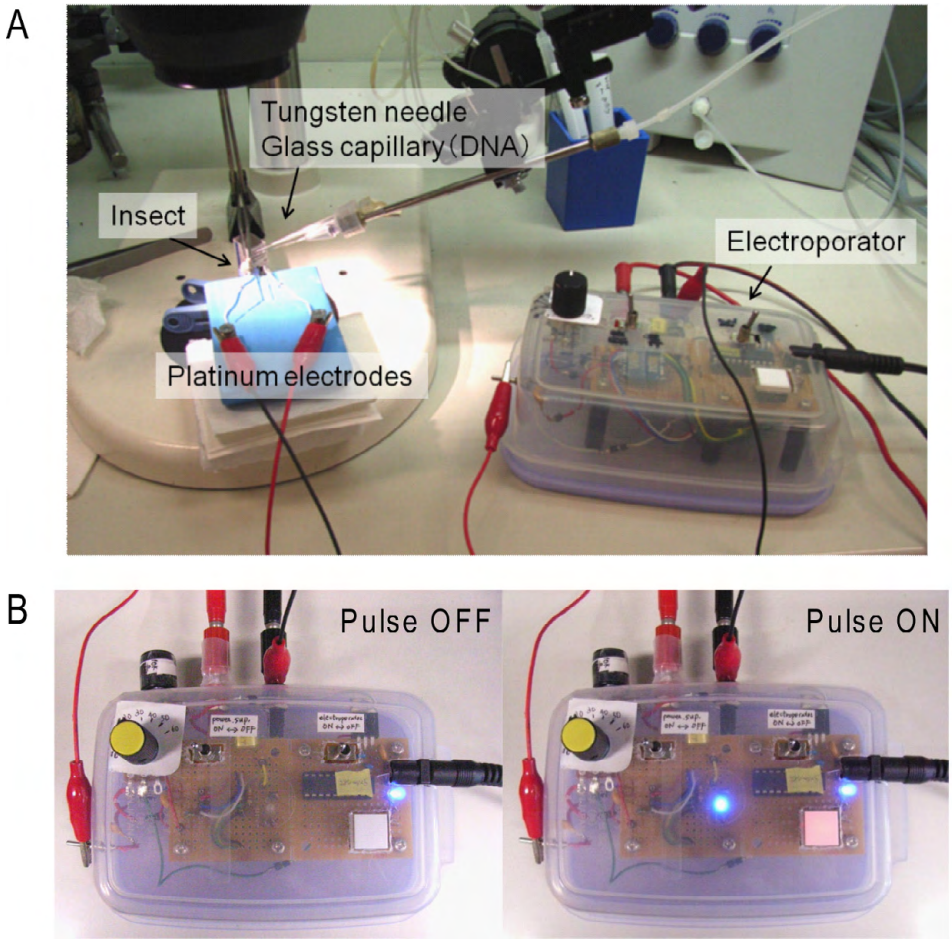

Fig. S2. Overview of the *in vivo* electroporator. (**A**) A set of *in vivo* electroporation apparatuses. (**B**) Overview of the electroporator while applying voltage pulses. Voltage status can be monitored with lighting of the LED.

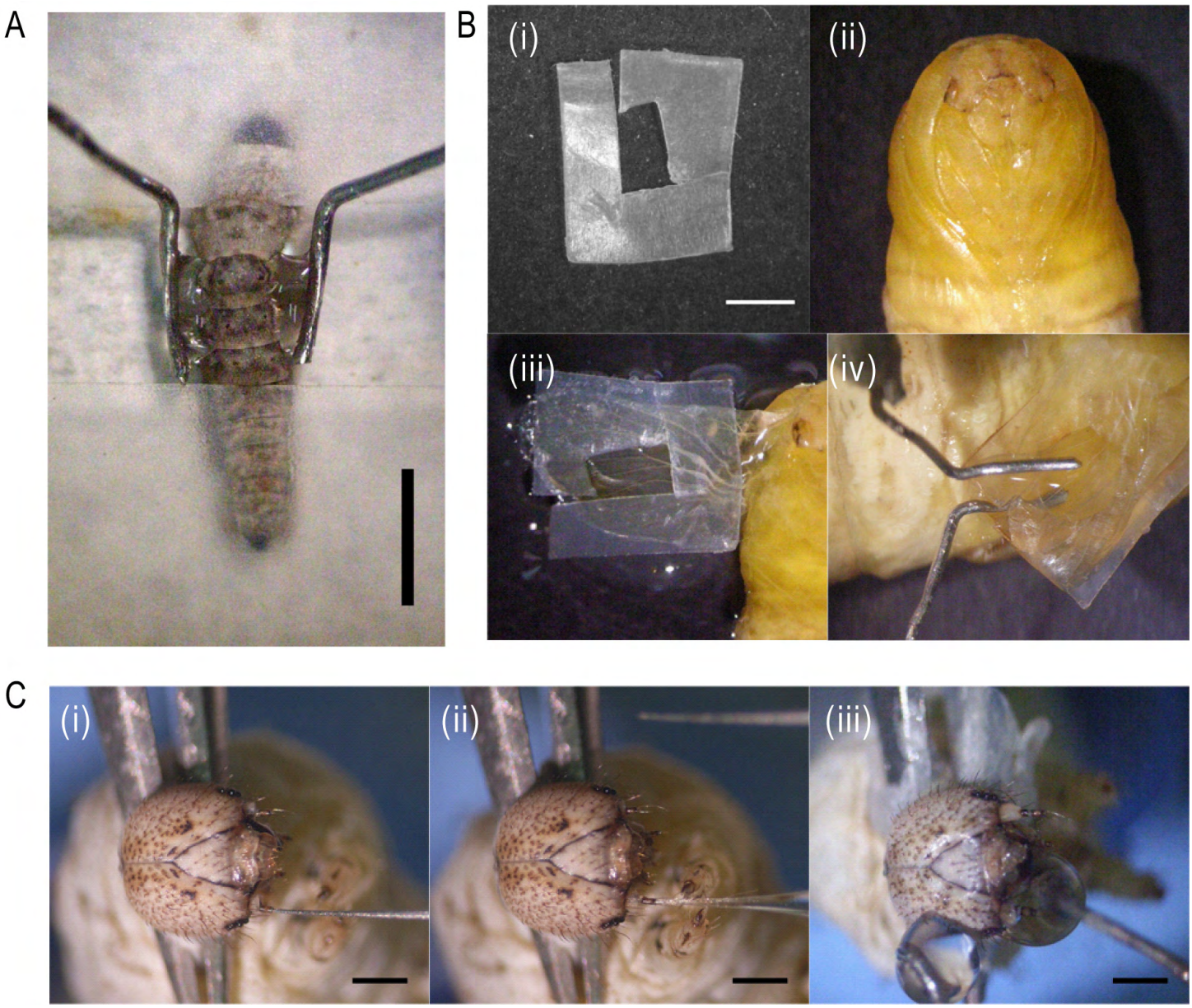

Fig. S3. Procedures of *in vivo* electroporation for each tissue. (**A**) Gene transfer to larval epidermis. After injecting DNA solution, platinum electrodes and droplets of PBS were placed near the injection site, and voltage pulses were applied. Scale bar: 2 mm. (**B**) Gene transfer to pupal wing. (i) A Parafilm gasket was prepared to be inserted between a fore wing and a hind wing. (ii) A pupa that had just pupated was selected. (iii) The fore wing was floated on water and the gasket was placed on the ventral side of the wing. (iv) After wiping water droplets from the body surface, DNA solution was injected into the hemocoel of the forewing. The platinum electrodes and droplets of PBS were placed on both the ventral and dorsal sides of the wing and voltage pulses were applied. Scale bar: 1 mm. (**C**) Gene transfer to antennal primordium. (i) A larval head was grasped with forceps and the tip of the larval antenna was pierced with a sharp tungsten needle. The tungsten needle was prepared using electrolytic grinding (ii) DNA solution was injected through the hole. (iii) Platinum electrodes and droplets of PBS were placed as above and voltage pulses were applied. Scale bars: 1 mm.

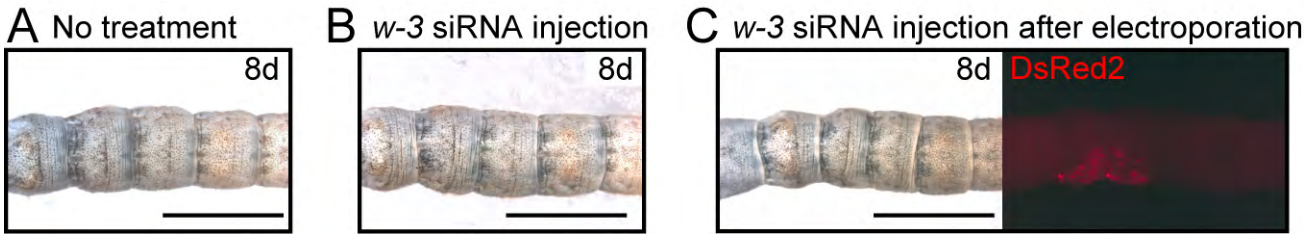

Fig. S4. Negative control experiments for RNAi against *w-3*. (**A**) No treatment (*n*=10). (**B**) *w-3* siRNA injection (300 μM, 0.5 μl) (*n*=10). (**C**) *w-3* siRNA injection (300 μM, 0.5 μl) 1 hour after electroporation of pPIG-A3GR (*n*=6). Left, bright field; right, DsRed2 signal. Images were collected 8 days after each treatment. No attenuation of white pigmentation was observed under each condition. Scale bar: 5 mm.

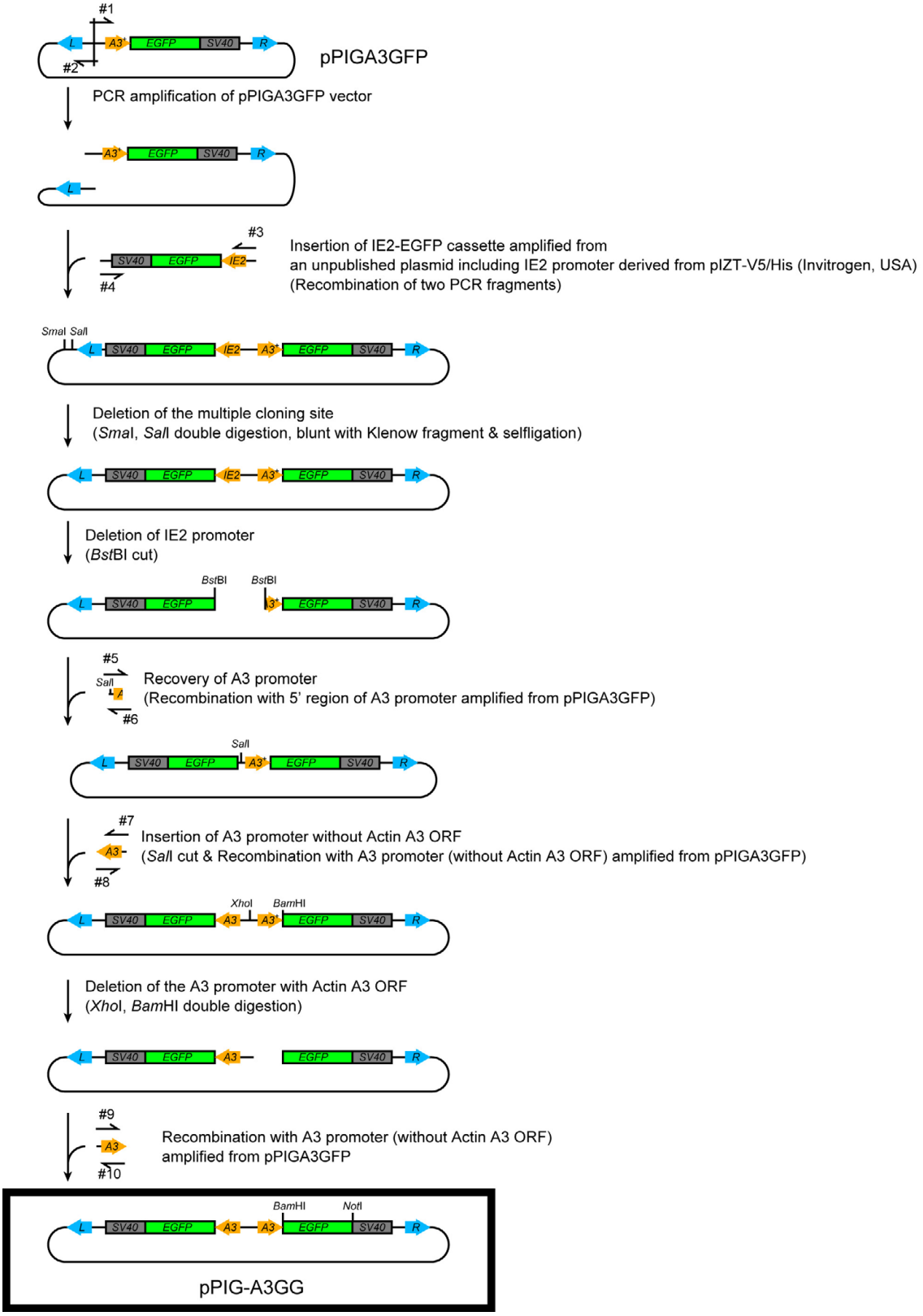

Fig. S5. Construction of the pPIG-A3GG transitional vector. pPIG-A3GG was constructed based on pPIGA3GFP (Tamura et al., 2000). Each step is indicated next to each arrow. When a PCR fragment was added, the structure of the amplified fragment and primer number are shown together. Primer sequences (#1-#10) are listed in supplementary material Table S2.

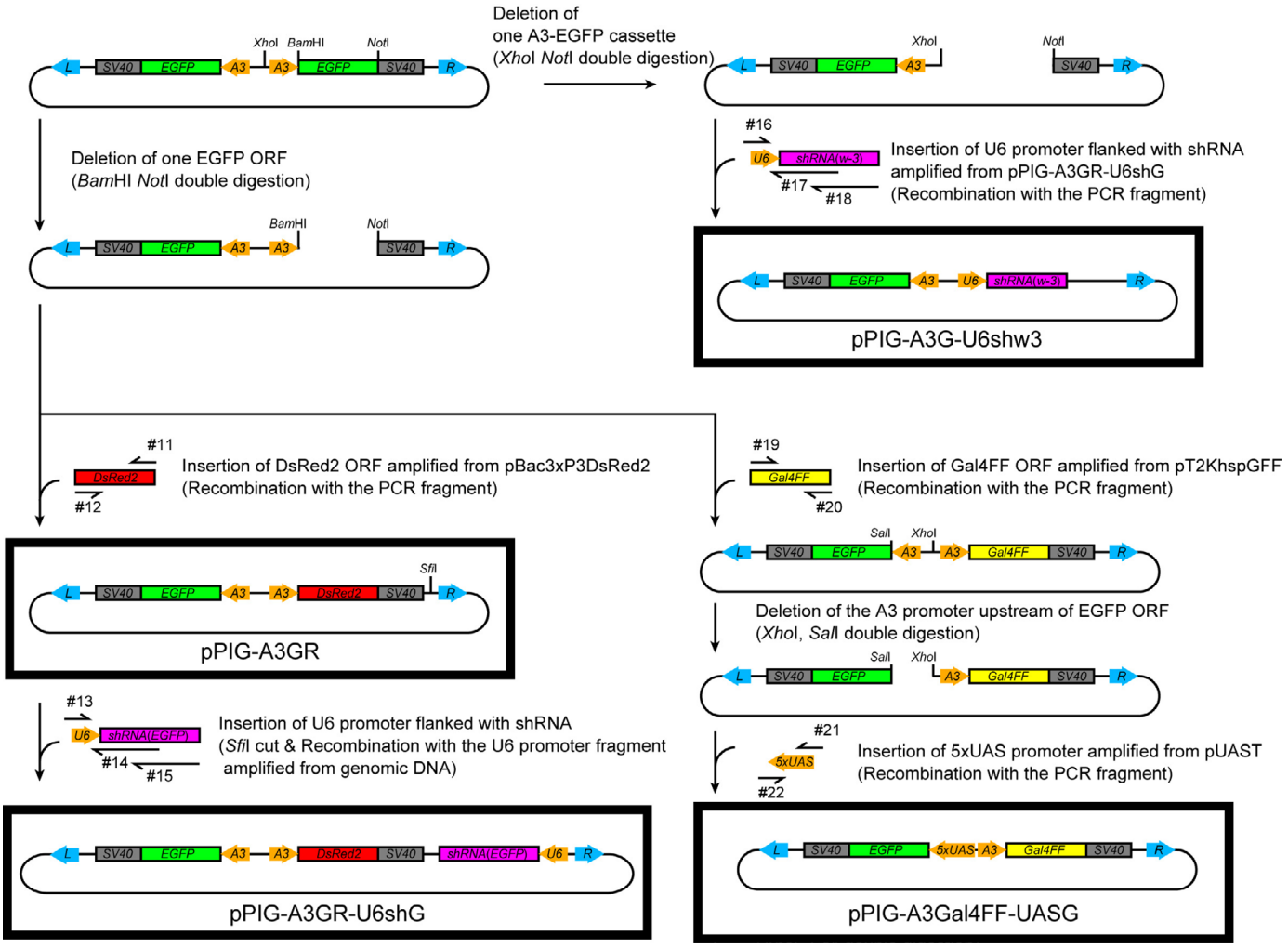

Fig. S6. Construction of donor vectors. pPIG-A3GR, pPIG-A3GR-U6shG, pPIG-A3G-U6shw3 and pPIG-A3GFF-UASG were based on the transitional vector pPIG-A3GG (supplementary material Fig. S4). Each step is indicated next to each arrow. When a PCR fragment was added, the structure of the amplified fragment and primer number are shown together. Primer sequences (#11-#22) are listed in supplementary material Table S2.

Table. S1. Electroporation conditions, survival rate and gene transfer efficiency

Insect species	Target tissue	Electroporation condition	Survival rate (5 days after electroporation)	Fluorescence signal in the survived (5 days after electroporation)	Notes
Bombyx mori	Larval epidermis	45V, 280ms/1s, 5 pulses (2nd instar)	0/15 (0%)	–	The larvae in the experiments for Fig. 2A (+ *piggyBac*) were counted.
		45V, 280ms/1s, 5 pulses (3rd instar)	2/4 (50%)	2/2 (100%)	The larvae in the experiments for Fig. 2A (+ *piggyBac*) were counted.
		20V, 280ms/1s, 5 pulses (2nd instar)	11/21 (52%)	11/11 (100%)	The larvae in the experiments for Fig. 2A (+ *piggyBac*) were counted.
	Pupal wing	45V, 280ms/1s, 5 pulses	2/2 (100%)	0/4 (0%)*	The larvae in the experiments for Fig. 2B were counted.
		30V, 280ms/1s, 5 pulses	4/4 (100%)	0/4 (0%)*	The larvae in the experiments for Fig. 2B were counted.
		20V, 280ms/1s, 5 pulses	4/4 (100%)	2/4 (50%)*	The larvae in the experiments for Fig. 2B were counted.
	Antennal primordium	45V, 280ms/1s, 10 pulses	13/20 (65%)	11/13 (85%)	The larvae in the experiments for Fig. 2C were counted.
		20V, 280ms/1s, 5 pulses	4/4 (100%)	0/4 (0%)	The larvae in the experiments for Fig. 2C were counted.
Papilio xuthus	Larval epidermis	20V, 280ms/1s, 5 pulses (2nd instar)	1/3 (33%)	1/1 (100%)	The larvae in the experiments for Fig. 4A were counted.
		30V, 280ms/1s, 5 pulses (3rd instar)	3/5 (60%)	3/3 (100%)	Both larvae treated with and without siRNA (n = 2 and 3, respectively) in the experiments for Fig. 4B were counted together.
Tribolium castaneum	Larval abdomen	20V, 280ms/1s, 5 pulses	7/11 (64%)	7/7 (100%)	The larvae in the experiments for Fig. 4C were counted.

*Insects with fluorescent scale cells were counted

Table. S2. The PCR primers used for vector constructions

Primer	Sequence (5'-3')
#1	cagaggactattagaggtaagaataaac
#2	tggcaaggtcaagattctgttagaag
#3	tctaatagtcctctggatcatgatgataaacaatgtatggtgc
#4	atcttgaccttgccatttatttgtaaccattataagctgcaat
#5	gctcaccatggttcggtcgaccagaggactattagaggtaa
#6	gacggagaaccttcgaaattc
#7	tagtcctctggtcgatcgcgttaccatatatggtg
#8	ccatggttcggtcgaccttgaattagtctgcaagaaaag
#9	attgtcagatctcgagctca
#10	ggcgaccggtggatccttgaattagtctgcaagaaaag
#11	tctagagtcgcggccgct
#12	actcgtacggggatccaccggtcgccaccatggcctcctccgagaacgt
#13	atagctcagaggccgagaatttttcaaatcggaccag
#14	gaggacagcacactcttgaaattcaccttaatacacttgataatctttttattttgga
#15	cagaggccgaggccgaaaaagcatcaaggtgaacttcaagaggacagcacactcttgaaattca
#16	attgtcagatctcgaagaatttttcaaatcggaccag
#17	ggggacagcacacctttcaatacctatataacaacttgataatctttttattttgga
#18	tctagagtcgcggccgcaaaaacgttatacaggtactgaaaggggacagcacacctttcaatacc
#19	actaattcaaggatctccgcggcgccaccatgaagctactgtcttctatcgaac
#20	tctagagtcgcggccaggcctttagttacccgggagcatat
#21	caagcttgagctcgactcgcgagcatgcctgcaggtcggagt
#22	gacctgcaggcatgcatttaaattccgatccagacatgataagatacat

Table. S3. siRNA sequences

Target gene	Direction	Sequence (5'-3')
EGFP	Sense	GCAUCAAGGUGAACUUCAAGA
	Antisense	UUGAAGUUCACCUUGAUGCCG
w-3	Sense	CAUUUAUGGCCCAAAACGUUA
	Antisense	ACGUUUUGGGCCAUAAAUGAA