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Supplemental information

DIPA-CRISPR is a simple and accessible method for insect gene editing

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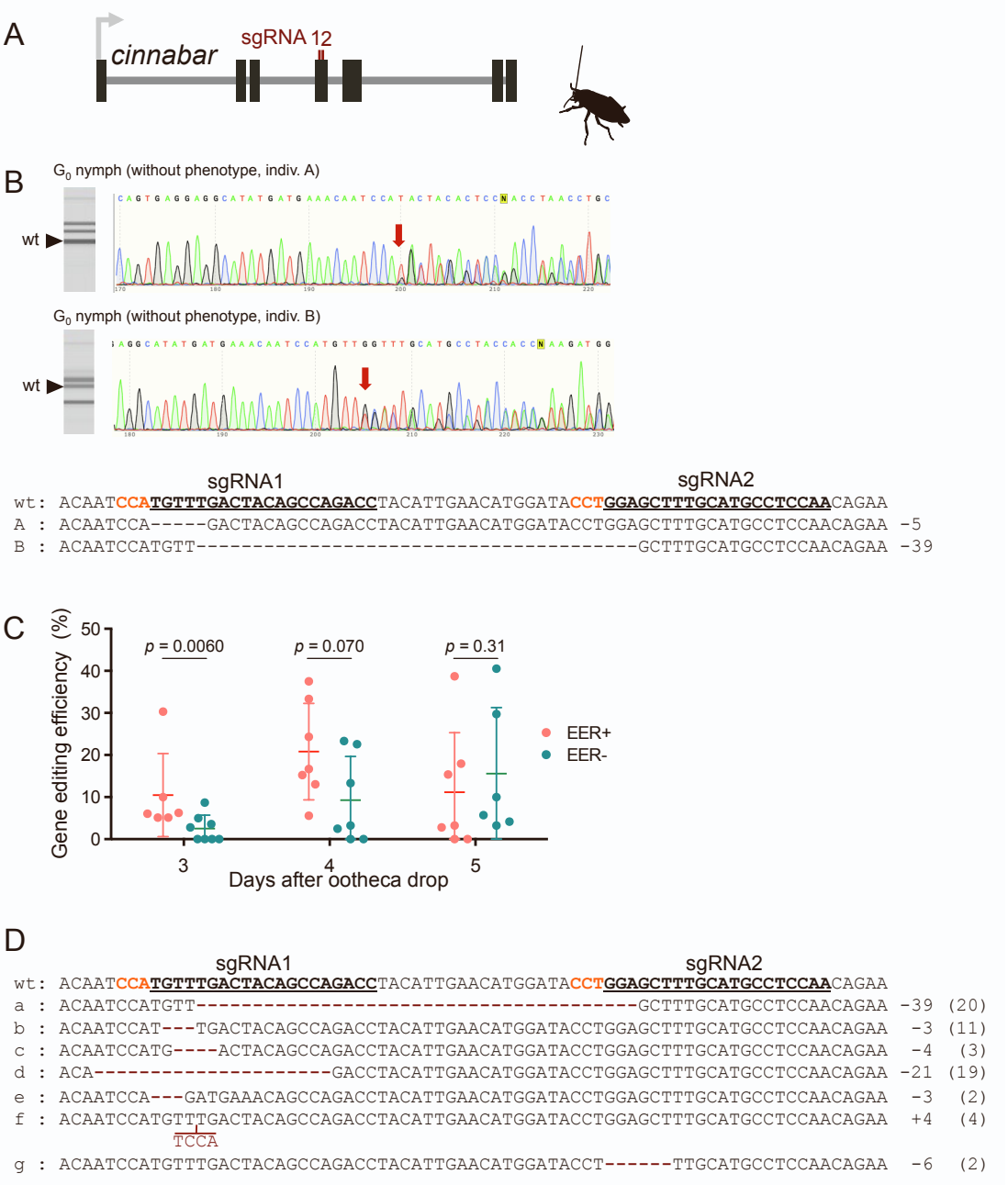

Figure S1. Disruption of *Blattella germanica cinnabar* by DIPA-CRISPR. Related to Figure 1.

(A) CRISPR target sites of *B. germanica cinnabar* (PSN36199).

(B) Representative results of genotyping of G_0 edited nymphs. Two G_0 nymphs that carried mutations (judged by heteroduplex mobility assay) but do not show eye color phenotypes were subjected to direct Sanger sequencing of genomic PCR products. Red arrows indicate the presence of double peaks caused by indel mutations. The DNA sequences of recovered mutant alleles are shown below the panel with sgRNA (underlined) and PAM (orange letters) sequences.

(C) Gene editing efficiency (GEF) in the G_0 progenies. Each point represents an individual adult female injected. Bars indicate mean ± SD (n = 6–8). The GEF values were analyzed with the Mann-Whitney nonparametric U test.

(D) The DNA sequences of *cinnabar* mutant alleles shown in Figure 2B (i.e., alleles a–g) with sgRNA (underlined) and PAM (orange letters) sequences. The length of indels and the number of G_1 insects (in parenthesis) are shown on the right.

A

B

Gene	Stage of injection	EER	Females injected	Survival rates	Screened G_0	G_0 edited animals		Gene editing efficiency (GEF)
						white	mosaic	
cardinal	3 days AE	+	84	100%	85			0%
		+	68	100%	17			0%
		-	65	100%	34			0%
		-	87	98.9%	91			0%
	4 days AE	+	84	98.8%	9	5		55.6% [a]
		+	76	100%	4	1	2	75.0% [a]
		-	73	100%	63	25	7	50.8%
		-	36	97.2%	54	12	5	31.5%
	5 days AE	-	76	98.7%	21	11	4	71.4%
		-	92	100%	132	31	15	34.9%
	10 days AE	-	106	98.1%	66	3	2	7.6%
		-	97	99.0%	119	5	2	5.9%
	30 days AE	+	94	100%	133	3	2	3.8%
		+	88	97.7%	132			0%
		-	79	94.9%	117	6	2	6.8%
		-	80	97.5%	230	4	1	2.2%

C

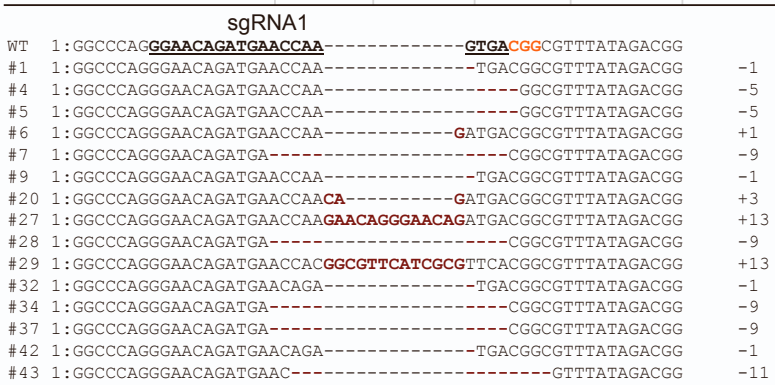

Figure S2. Disruption of *Tribolium castaneum cardinal* by DIPA-CRISPR. Related to Figures 3 and 4.

(A) The CRISPR target site of *T. castaneum cardinal* (XP_008200769). The sgRNA1 targeting the exon 3 of *cardinal* (Shirai and Daimon, 2020) was used.

(B) The detailed results of DIPA-CRISPR in *T. castaneum*. Cas9 ribonucleoprotein (RNP) solution containing 3.3 μg/μL Cas9 (IDT, Cat#1081059) and 1.3 μg/μL sgRNA were injected into adult females of selected days (i.e., 3, 4, 5, 10 or 30 days) after adult emergence (AE). The injected females were pooled, and the results are from the eggs laid during the first 24 h (females at 4 , 5, 10 or 30 days AE) or 48 h (females at 3 days AE, as

they were too young to lay eggs during the first 24 h) after injection. The results from two independent experiments are shown. EER, presence (+) or absence (-) of an endosomal escape reagent saponin (100 ng/μL) in injection solution. a: the gene-editing efficiency (GEF) values are very high, but these values may not be reliable as they are calculated based on very small numbers of G_0 insects hatched and/or survived.

(C) The DNA sequences of *cardinal* mutant alleles in G_0 edited insects. Hemizygous G_0 males with white eyes were randomly chosen and subjected to direct Sanger sequencing of genomic PCR products. Each row represents each G_0 mutant, and the length of indel is shown on the right.

A

Company	Stage of injection	EER	Females injected	Survival rates	Screened G_0	G_0 edited animals		Gene editing efficiency (GEF)
						white	mosaic	
IDT	4 days AE	-	73	100%	63	25	7	50.8%
		-	36	97.2%	54	12	5	31.5%
Sigma-Aldrich	4 days AE	-	59	100%	22	3	3	27.3%
		-	76	98.7%	55	14		25.5%
FUJIFILM Wako	4 days AE	-	73	98.6%	101	14	10	23.8%
		-	78	100%	29	6	1	24.1%
Fasmac	4 days AE	-	50	100%	22	6	1	31.8%

B

Conc. of Cas9 (IDT)	Stage of injection	EER	Females injected	Survival rates	Screened G_0	G_0 edited animals		Gene editing efficiency (GEF)
						white	mosaic	
3.3 μg/μL	4 days AE	-	73	100%	63	25	7	50.8%
		-	36	97.2%	54	12	5	31.5%
1.65 μg/μL	4 days AE	-	85	100%	37	1	7	21.6%
		-	59	100%	20	4	2	30.0%
0.83 μg/μL	4 days AE	-	65	100%	23	1	2	13.0%
		-	90	100%	43	2	3	11.6%
0.41 μg/μL	4 days AE	-	64	100%	38	3	5	21.1%
		-	80	100%	67	2	1	4.5%

C

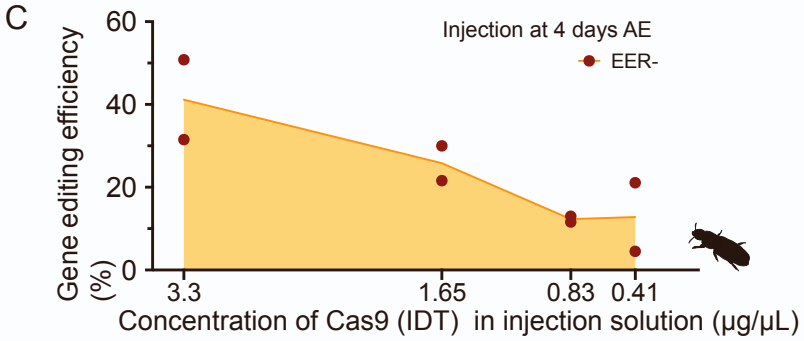

Figure S3. Performance of different Cas9 products and different doses of Cas9 in *Tribolium castaneum*. Related to Figures 3 and 4.

(A) Comparisons of Cas9 products from four vendors. Cas9 RNP solution containing 3.3 μg/μL Cas9 and 1.3 μg/μL sgRNA were injected into adult females of 4 days after adult eclosion (AE).

(B) A dilution series of Cas9 RNPs (diluted by water, the molar ratio of Cas9 and sgRNA was fixed to be 1 : 2) were injected into females.

(C) Gene editing efficiency shown in (B) was plotted against the concentration of Cas9 in injection solution. Each point represents the result of each replication.

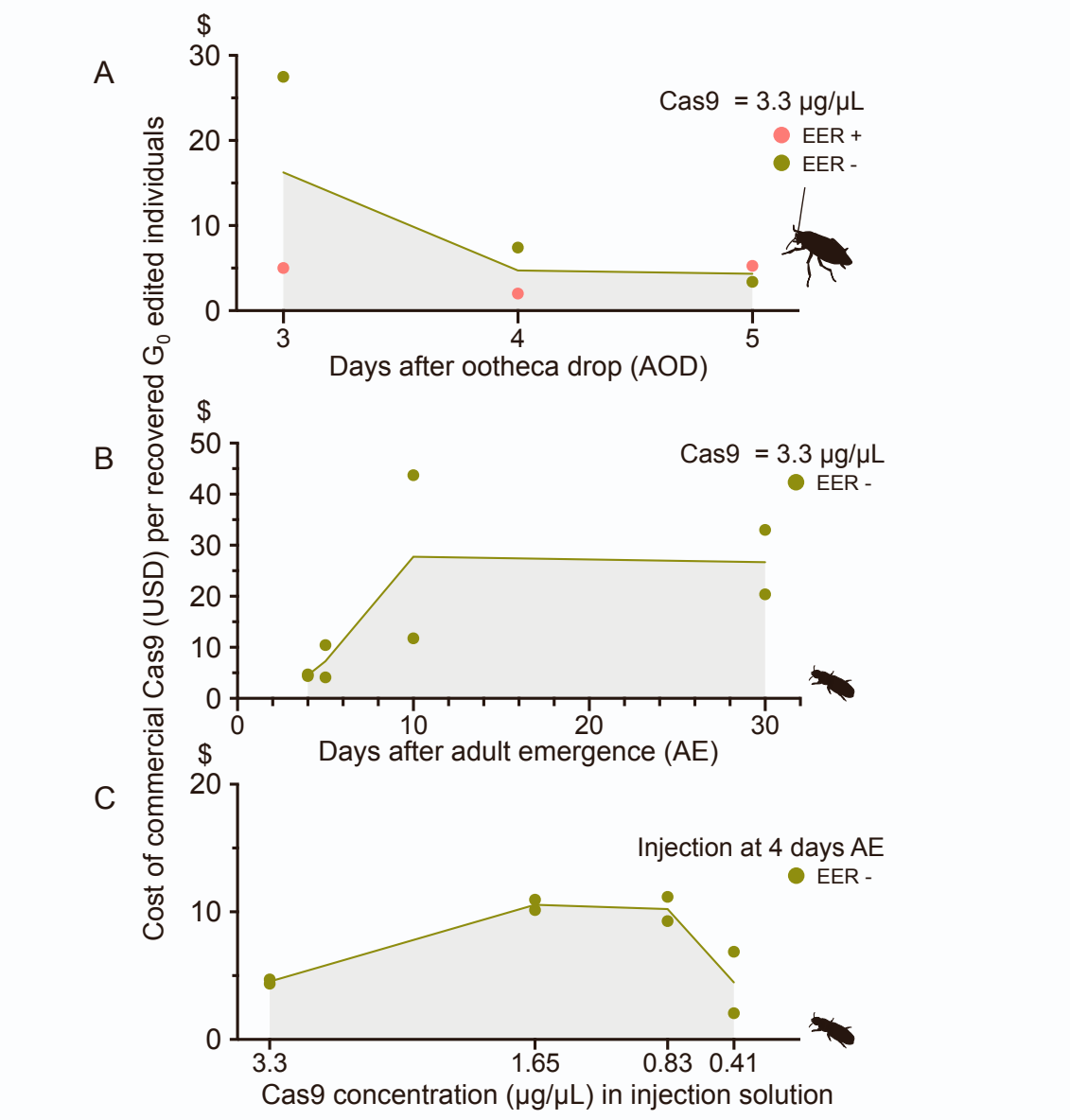

Figure S4. Cost of commercial Cas9 in DIPA-CRISPR. Related to Figures 1 and 3.

(A and B) Cost of commercial Cas9 (IDT, cat#10000735, 1.25 USD/µg) per recovered G_0 edited individuals was plotted against the time of injection for *B. germanica* (A) and *T. castaneum* (B). The results show that the cost can be significantly reduced by injecting at an appropriate timing in both species. Cost was calculated as follows: 1.25 (USD/µg) × amount (µg) of Cas9 injected per adult × total number of adults injected / total number of G_0 edited individuals recovered. Note that this calculation does not include the cost for sgRNA synthesis, as it greatly varies depending on the method (i.e., *in vitro* transcription or chemical synthesis).

(C) Relationship of the cost of Cas9 and the concentration of Cas9 in injection solution in *T. castaneum*. The results shown in Figure S3B were used for calculation.

Supplementary Table 1. DIPA-CRISPR experiments in *Drosophila melanogaster.* Related to Figure 4.

Gene	sgRNA	Stage of injection	EER	Females injected	Survival Rates	Screened G_0	G_0 edited animals		Gene editing efficiency (GEF)
							white	mosaic	
white	chemical synthesis	2 h AE	-	59	81.4%	212			0%
		2 h AE	-	29	96.6%	101			0%
		10 h AE	-	31	87.1%	2			0%
		10 h AE	-	26	96.2%	33			0%
		10 h AE	-	80	96.3%	270			0%
		24 h AE	-	61	85.2%	481			0%
		24 h AE	-	71	91.5%	426			0%
		2 d AE	-	47	53.2%	324			0%
		2 d AE	-	58	77.6%	437			0%
		4 d AE	-	53	60.4%	329			0%
		4 d AE	-	63	76.2%	421			0%
		7 d AE	-	62	66.1%	841			0%
		7 d AE	-	65	80.0%	573			0%
		Random	Chl 2 mM	113	93.1%	891			0%
	in vitro transcription	Random	-	71	91.5%	340			0%
		Random	Chl 0.5 mM	74	95.9%	417			0%
		Random	Chl 2 mM	70	88.6%	662			0%

Cas9 ribonucleoprotein (RNP) solution containing 3.3 µg/µL Cas9 (IDT, Cat#1081059) and 1.3 µg/µL sgRNA (a mixture of sgRNA1 and sgRNA2) were injected into adult females of selected hours or days after adult emergence (AE), or females that were randomly chosen from vials (Random). sgRNAs were purchased (chemical synthesis) or synthesized by in vitro transcription. The injected females were pooled, and the results are from the eggs laid during the first 48 h after injection. EER, presence or absence (-) of an endosomal escape reagent chloroquine (Chl) in injection solution.