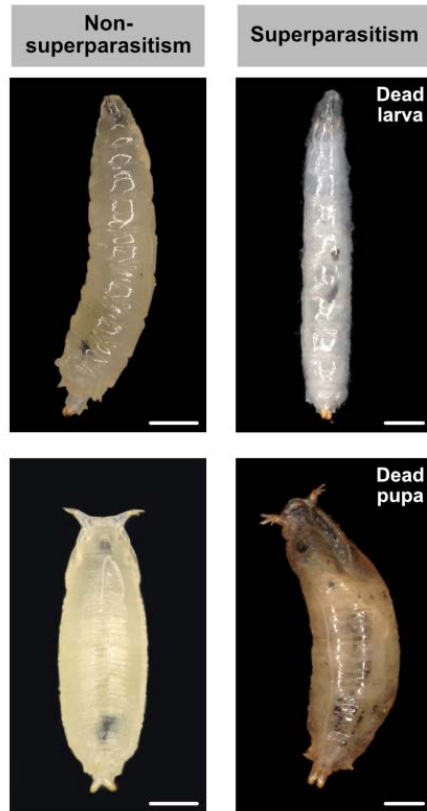


Supplementary Information for

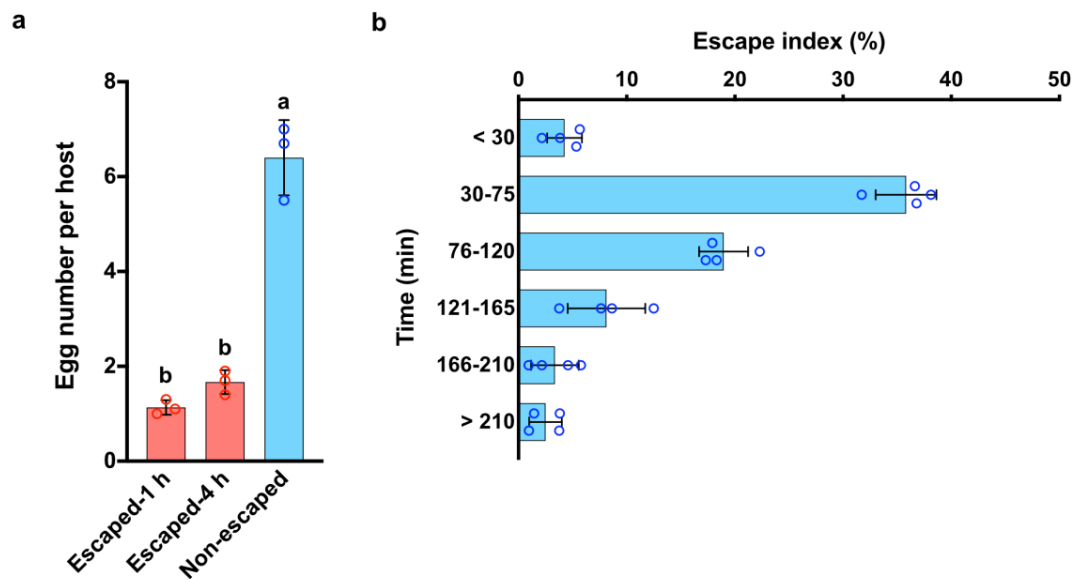
**Neofunctionalization of an ancient domain allows parasites to avoid intraspecific competition by manipulating host behaviour**

Supplementary Figure 1	-----	2
Supplementary Figure 2	-----	3
Supplementary Figure 3	-----	4
Supplementary Figure 4	-----	5
Supplementary Figure 5	-----	6
Supplementary Figure 6	-----	7
Supplementary Figure 7	-----	8
Supplementary Figure 8	-----	9
Supplementary Figure 9	-----	10
Supplementary Figure 10	-----	11
Supplementary Figure 11	-----	12
Supplementary Table 1	-----	13
Supplementary Table 2	-----	14
Supplementary Table 3	-----	15
Supplementary Table 4	-----	16
Supplementary Table 5	-----	17



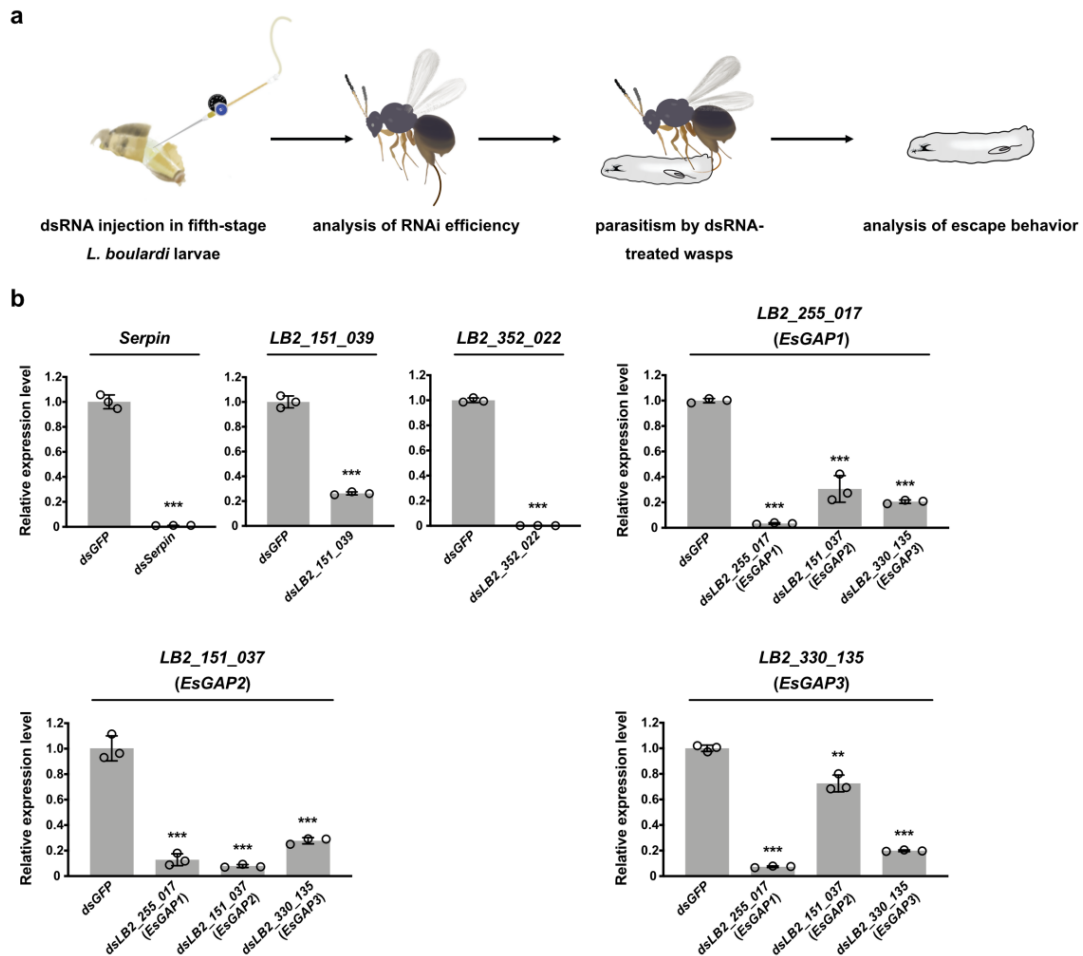
**Supplementary Figure 1 Representative images of premature death in superparasitized hosts**

The development of escaped (superparasitism avoidance) and non-escaped hosts (superparasitism) was observed in the 4 h parasitization assay. Bar: 1 mm. The photos were taken by Jia'ni Chen using a KEYENCE VHX-2000C digital microscope system.



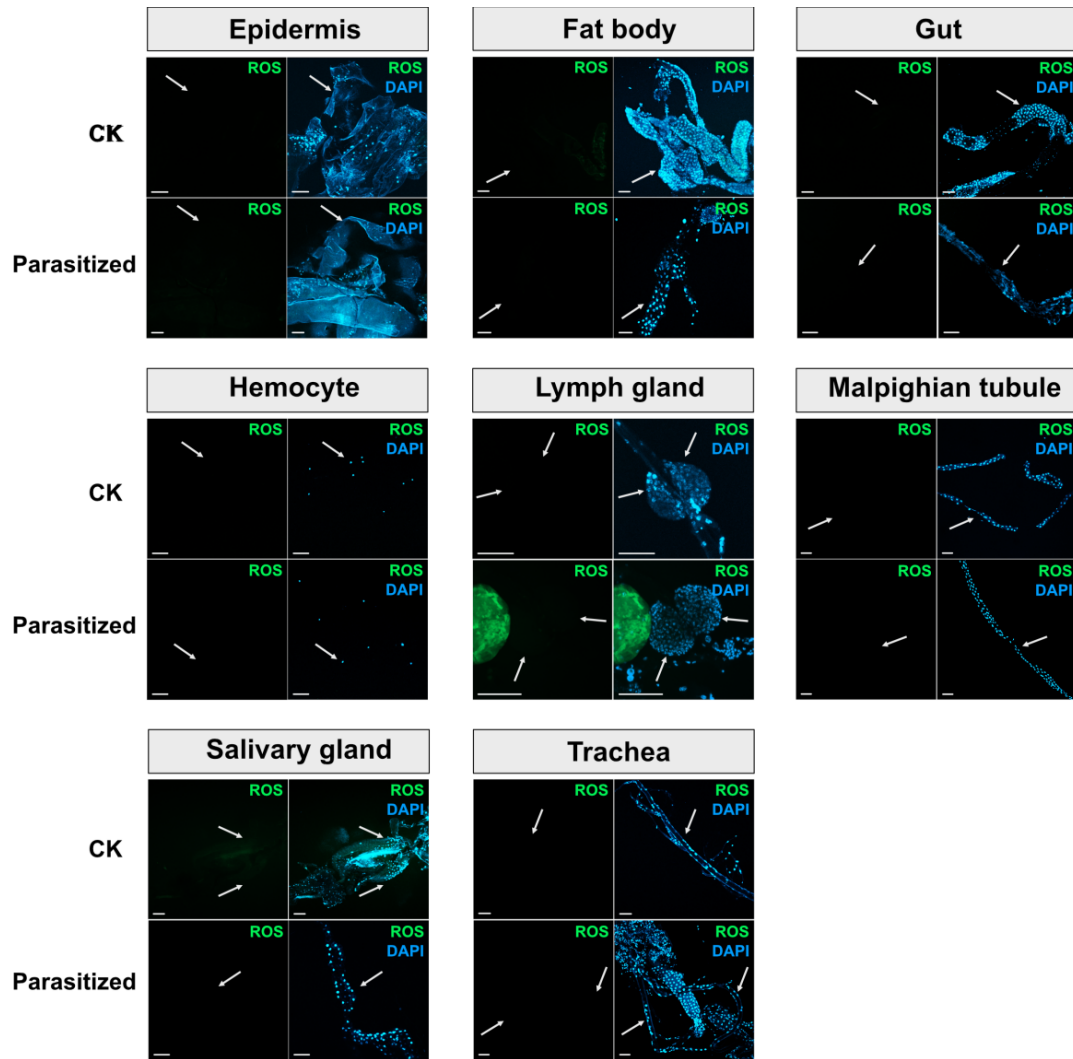
**Supplementary Figure 2 Host escape statistics across different time points after exposure to Lb parasitoids**

**a** Average number of parasitoid eggs found in escaped (red column, n=30) and non-escaped (blue column, n=30) hosts of *Drosophila* larvae after exposure to Lb at 1:10. The hosts were randomly selected from non-escaped and escaped groups after 4h exposure to Lb to check parasitoid eggs. The escaped hosts were additionally assayed at an early time point (1 h), which is the peak time of escape (see **b**). Three biological replicates were performed for each assay. Data are presented as mean values ± SD. Significance was analyzed by one-way ANOVA along with Fisher's least significant difference test (Escaped-1 h vs Escaped-4 h:  $P=0.4279$ ; Escaped-1 h vs Non-escaped:  $P=2.9 \times 10^{-5}$ ; Escaped-4 h vs Non-escaped:  $P=5.4 \times 10^{-5}$ ). The differences between treatments were considered significant when  $P < 0.05$ . **b** Escape preference of hosts across different time points after exposure to Lb at 1:10. Four biological replicates were performed for each assay. Data are presented as mean values ± SD. Source data are provided as a Source data file.



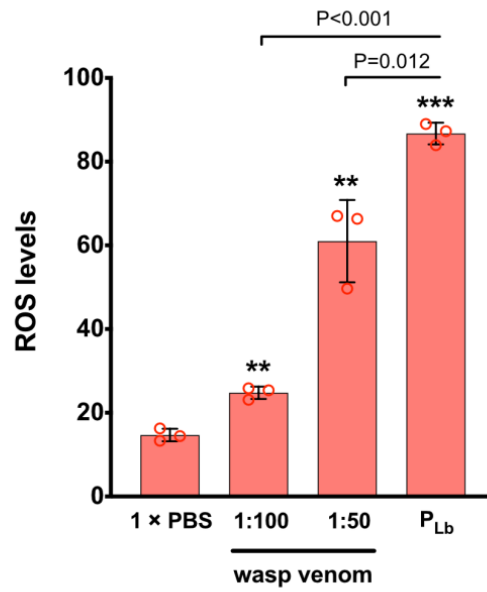
### Supplementary Figure 3 RNAi effects of significantly expressed VP genes on reducing gene expression

**a** Schematic diagram of analyzing the role of candidate genes in host escape behaviour by inhibiting gene expression. The images were crafted using Affinity Designer v1.8.6 and Procreate v4.2.2. **b** Relative mRNA levels of all significantly highly expressed VP genes, including *Serpin*, *LB2\_151\_039*, *LB2\_352\_022*, *LB2\_255\_017 (EsGAP1)*, *LB2\_151\_037 (EsGAP2)*, and *LB2\_330\_135 (EsGAP3)*, in *Lb* venom apparatus after RNAi treatments. *dsGFP* was used as control. Three biological replicates were performed. Data are presented as mean values  $\pm$  SD; significance was determined by two-sided unpaired Student's t-test (*dsSerpin*:  $P=6.5 \times 10^{-6}$ ; *dsLB2\_151\_039*:  $P=1.4 \times 10^{-5}$ ; *dsLB2\_352\_022*:  $P=7.7 \times 10^{-8}$ ; *dsEsGAP1*:  $P=8.2 \times 10^{-8}$  for *EsGAP1*,  $P=0.0003$  for *EsGAP2*,  $P=3.9 \times 10^{-7}$  for *EsGAP3*; *dsEsGAP2*:  $P=0.0002$  for *EsGAP1*,  $P=8.4 \times 10^{-5}$  for *EsGAP2*,  $P=0.0002$  for *EsGAP3*; *dsEsGAP3*:  $P=3.8 \times 10^{-7}$  for *EsGAP1*,  $P=0.0024$  for *EsGAP2*,  $P=6.4 \times 10^{-7}$  for *EsGAP3*; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ). Source data are provided as a Source data file.



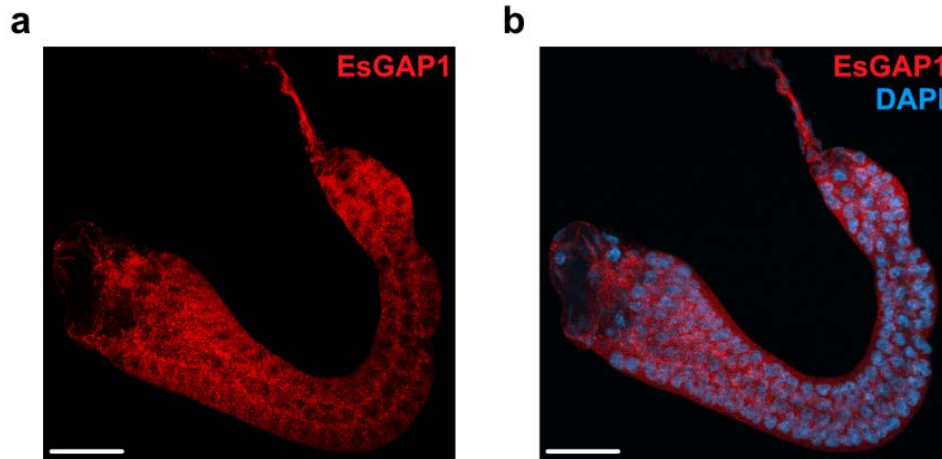
#### Supplementary Figure 4 ROS levels in non-CNS host tissues after Lb parasitism

Representative fluorescent images of epidermis, fat body, gut, hemocyte, lymph gland, malpighian tubule, salivary gland and trachea from a nonparasitized larva (CK) and from a parasitized host that exhibited escape behavior. Tissue was harvested immediately after 1 h post infection by Lb females. ROS levels were detected by DCFH-DA (green) and tissue nuclei were labeled by DAPI (blue). At least 20 *Drosophila* larvae were examined for each individual case. Arrows indicate the particular tissue. Scale bars: 50 μm.

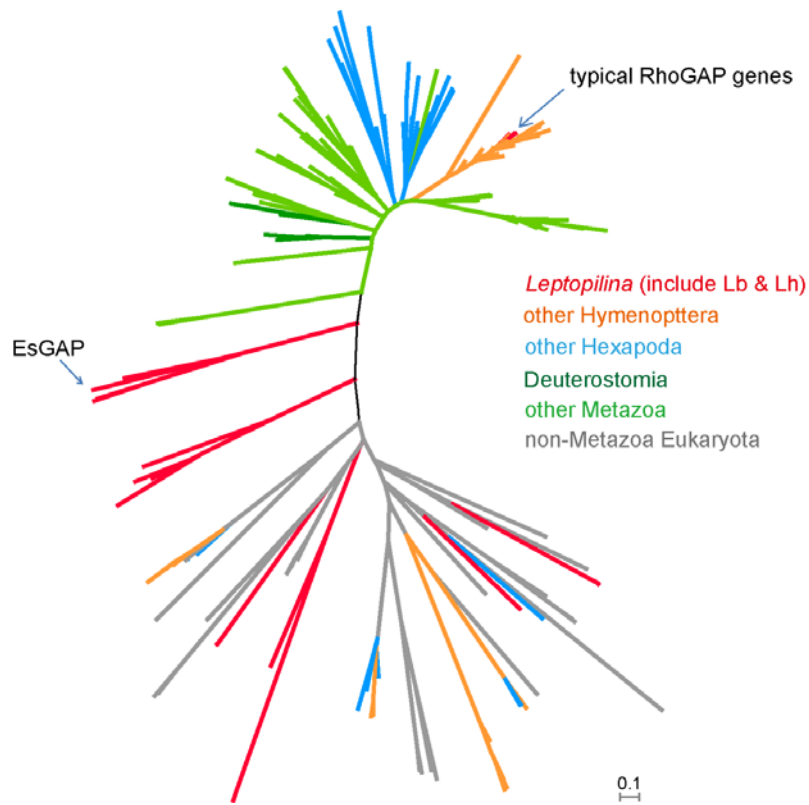


### Supplementary Figure 5 Lb venom triggers ROS induction in host CNS

Quantification of ROS levels in CNS of larvae injected with different venom dilutions from a single Lb female wasp ( $n \geq 10$  in each case). Elevation of ROS levels upon injection of 1:50 dilution of venom was much similar to the level in Lb parasitized host larvae ( $P=0.012$ ). Data are presented as mean values  $\pm$  SD. Significance was determined by two-sided unpaired Student's t-test (1:100 venom:  $P=0.0011$ ; 1:50 venom:  $P=0.0013$ ; P<sub>Lb</sub>:  $P=1.9 \times 10^{-6}$ ; 1:100 venom vs. P<sub>Lb</sub>:  $P=3.5 \times 10^{-6}$ ; 1:50 venom vs. P<sub>Lb</sub>:  $P=0.012$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ). Source data are provided as a Source data file.



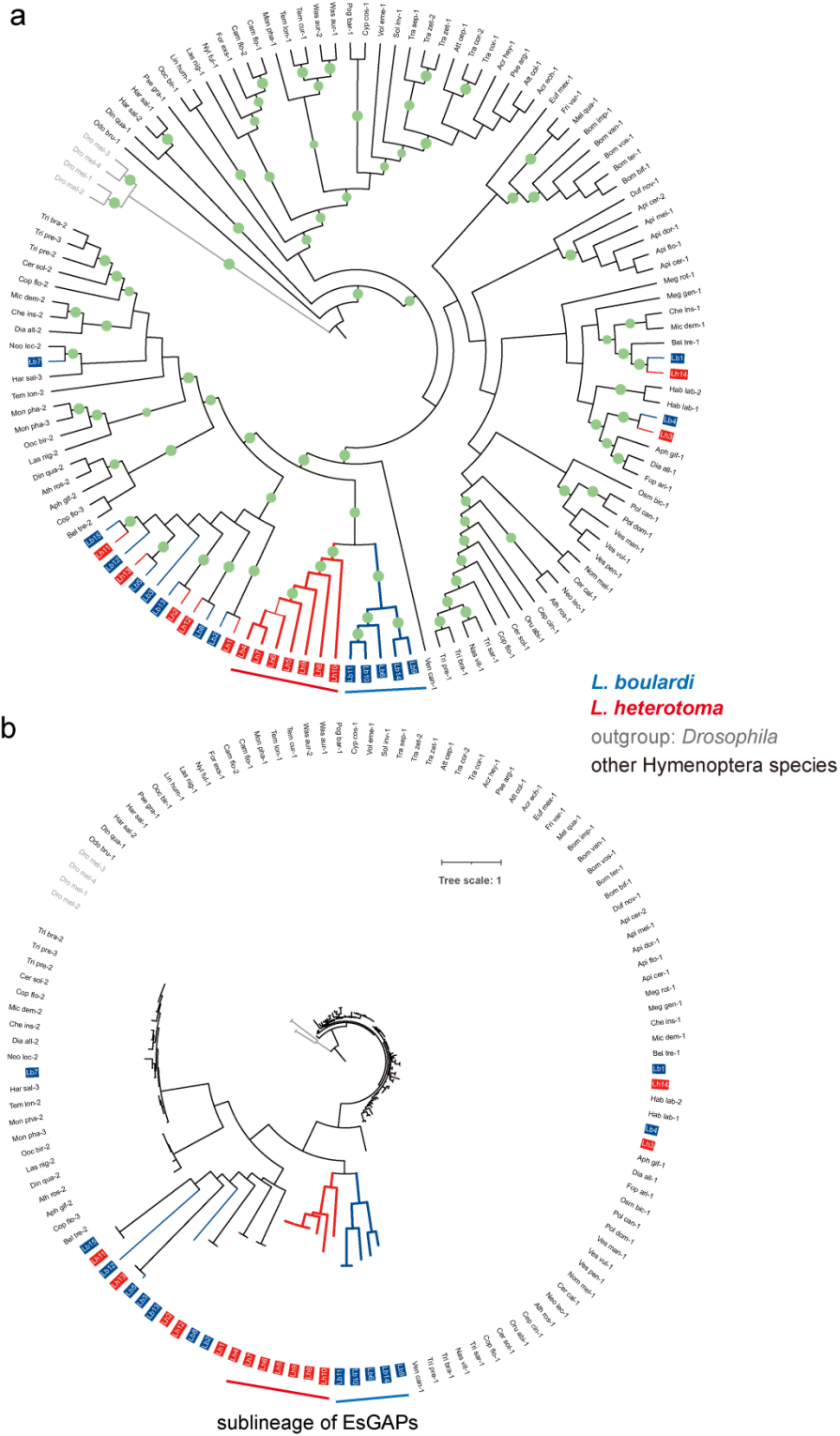
**Supplementary Figure 6 Immunolocalization assay of EsGAP1 expression in Lb**  
**a** Confocal immunolocalization of EsGAP1 (red) in Lb venom gland. Venom glands of at least 20 parasitoids were examined. **b** Merged image of EsGAP1 staining (red) and nuclei stained with DAPI (blue). Scale bars: 50  $\mu$ m.



**Supplementary Figure 7 Phylogenetic analyses of RhoGAP genes across eukaryotes**

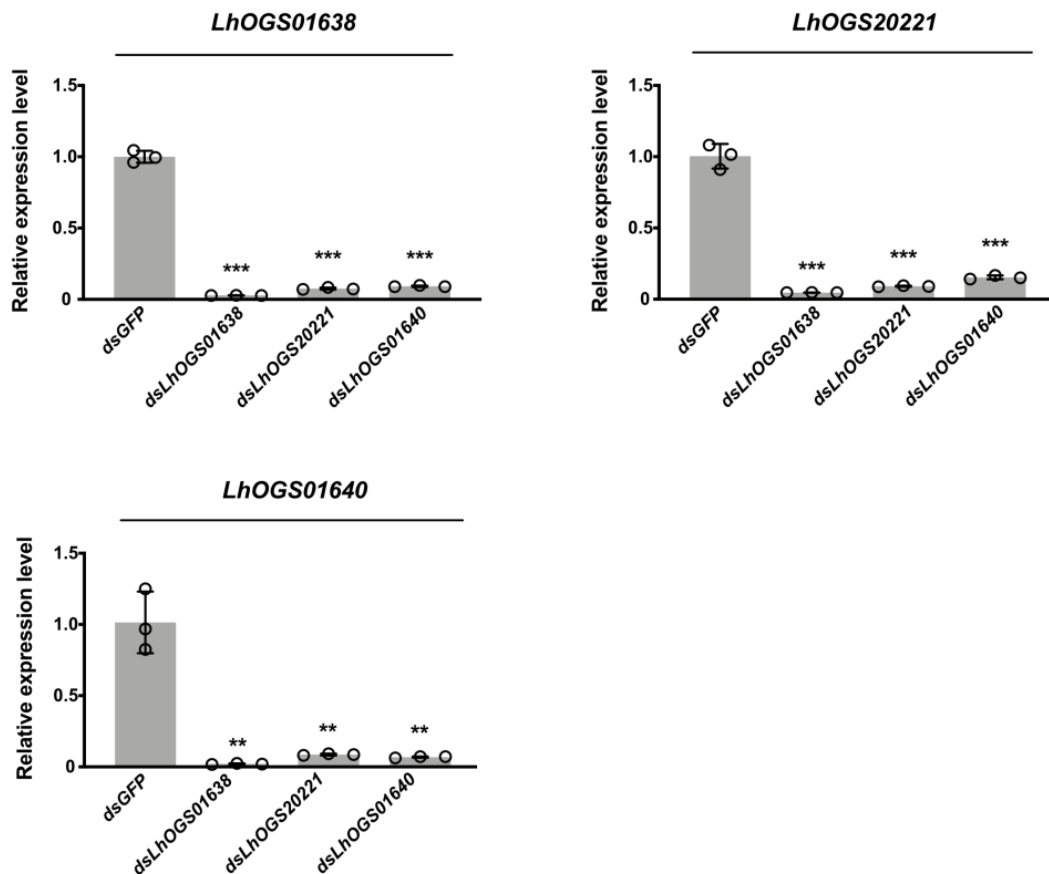
A total of 177 selective sequences from the representative nodes during animal evolution were used in this analysis (see Methods). Given the lower conservation out of the domain region, we retrieved the sequence of the RhoGAP domain (~170 AAs) from each full sequence prior to multiple alignment. The maximum-likelihood phylogenetic tree was inferred under the JTT model.





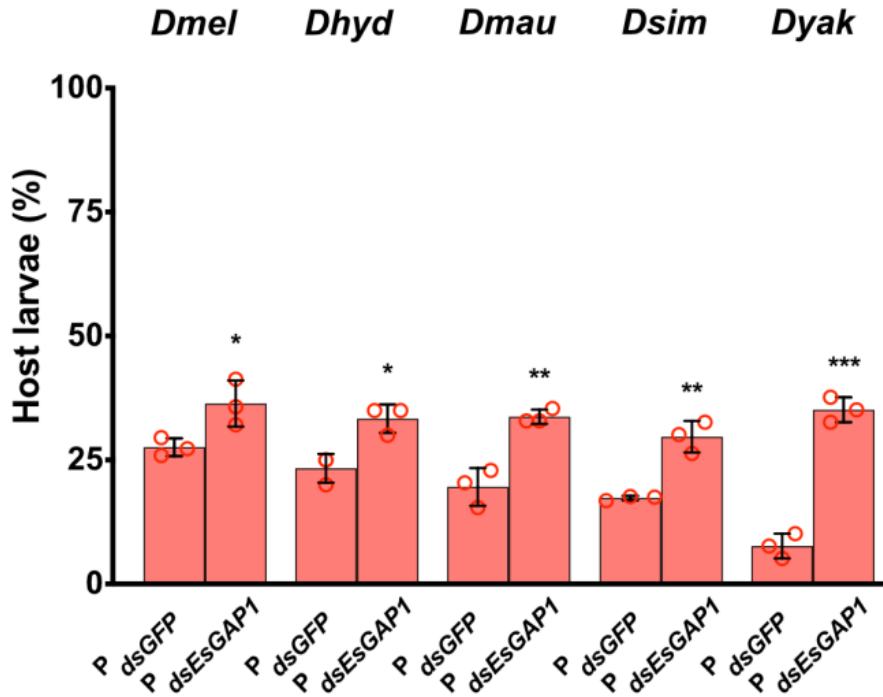
**Supplementary Figure 8 Phylogenetic analyses of RhoGAP genes across Hymenoptera**

All 127 identified genes, from 70 hymenopteran species, were used in this analysis (see Methods). Only the sequences around the RhoGAP domain (165 AAs) from each full sequence were retained for analysis. The maximum-likelihood phylogenetic tree was inferred under the JTT model. **a** The tree in cladogram with labelled bootstraps. Nodes with green circles indicate being with >75% bootstrap supports. **b** The tree in phylogram with branch length. The sublineages of EsGAPs in Lb and Lh are underlined.



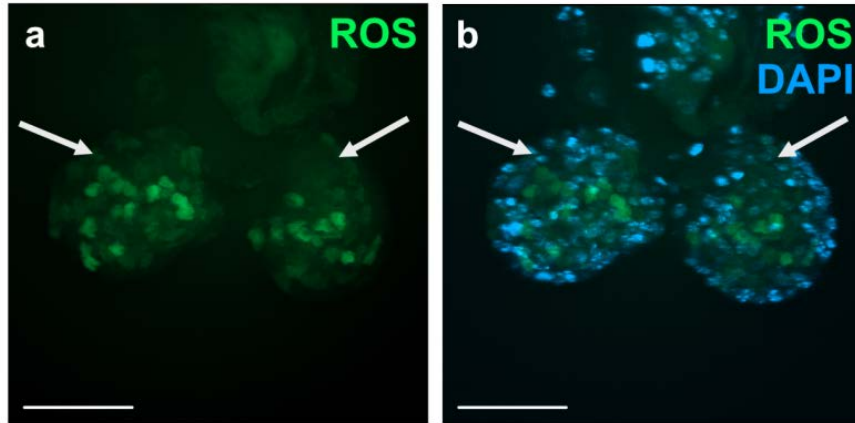
**Supplementary Figure 9 RNAi effects of three EsGAP genes on reducing gene expression in Lh**

Relative mRNA levels of *LhOGS01638* (LhEsGAP1), *LhOGS20221* (LhEsGAP2) and *LhOGS01640* (LhEsGAP3) in Lh venom apparatus after RNAi treatments. *dsGFP* was used as control. Three biological replicates were performed. Data are presented as mean values  $\pm$  SD; significance was determined by two-sided unpaired Student's t-test (*dsLhOGS01638*:  $P=2.4 \times 10^{-6}$  for *dsLhOGS01638*,  $P=3.1 \times 10^{-6}$  for *dsLhOGS20221*,  $P=3.2 \times 10^{-6}$  for *dsLhOGS01640*; *dsLhOGS20221*:  $P=4.4 \times 10^{-5}$  for *dsLhOGS01638*,  $P=5.1 \times 10^{-5}$  for *dsLhOGS20221*,  $P=7.2 \times 10^{-5}$  for *dsLhOGS01640*; *dsLhOGS01640*:  $P=0.0014$  *dsLhOGS01638*,  $P=0.0017$  for *dsLhOGS20221*,  $P=0.0016$  for *dsLhOGS01640*; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ). Source data are provided as a Source data file.



**Supplementary Figure 10 RNAi of EsGAP1 leads to more nonparasitized hosts remained under the condition of superparasitism**

Dissected hosts of without parasitoid eggs inside are considered as nonparasitized hosts. See the ratios of other fates (being parasitized only once and superparasitized) in Fig. 4a. Three biological replicates were performed. Data are presented as mean values  $\pm$  SD. Significance was analysed by two-sided unpaired Student's t-test ( $P_{EsGAP1}$  with *Dmel*:  $P=0.0376$ ;  $P_{EsGAP1}$  with *Dhyd*:  $P=0.0132$ ;  $P_{EsGAP1}$  with *Dmau*:  $P=0.0039$ ;  $P_{EsGAP1}$  with *Dsim*:  $P=0.0026$ ;  $P_{EsGAP1}$  with *Dyak*:  $P=0.0002$ ; \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ). *Dmel*, *D. melanogaster*; *Dhyd*, *D. hydei*; *Dmau*, *D. mauritiana*; *Dsim*, *D. simulans*; *Dyak*, *D. yakuba*. Source data are provided as a Source data file.



**Supplementary Figure 11 ROS levels in host lymph glands 12 hours after exposure to Lb**  
**a** Representative fluorescent images of *Drosophila* lymph glands stained for ROS with DCFH-DA (green). **b** Merged image of tissue stained with DCFH-DA (green) and with DAPI (blue) to label nuclei. Larval lymph glands of at least 20 *Drosophila* were examined for each group. Lymph gland is indicated by arrows. Scale bars: 50  $\mu$ m.

**Supplementary Table 1 Basic features of Lb genome assembly**

Genome assembly	
Contig size (bp)	354,804,354
#Contigs	409
Contig N <sub>50</sub> (bp)	2,668,382
Genome annotation	
GC (%)	28.0
Repeats (simple repeats) (%)	44.5 (3.6)
#Protein-coding genes	12,613
Coding (%)	8.9
Quality control	
BUSCO (insecta) partial (%)	98.3
BUSCO (insecta) complete (%)	97.8
BUSCO (hymenoptera) partial (%)	95.4
BUSCO (hymenoptera) complete (%)	91.8
CEGMA partial (%)	97.2
CEGMA complete (%)	93.2

**Supplementary Table 2 Venom protein genes of Lb**

Gene ID	#SP	TPM	Annotation*	Gene ID	#SP	TPM	Annotation*
LB2_216_018	25	100297	Venom serpin	LB2_302_012	11	203	Hypothetical protein
LB2_151_039s	6	46364	n/a	LB2_258_003	12	192	Mme11_3 protein
LB2_330_135	7	24835	RhoGAP	LB2_112_062	6	189	Trehalase
LB2_352_022	5	23249	n/a	LB2_038_141	8	184	Endoplasmic reticulum ATPase
LB2_255_017	19	16501	RhoGAP	LB2_210_047	8	183	Leukotriene A(4) hydrolase
LB2_213_059	8	14031	Superoxide dismutase	LB2_399_112	7	181	Ionotropic glutamate receptor
LB2_151_037	8	9248	RhoGAP	LB2_300_065	7	152	Hypothetical protein
LB2_248_134	17	9072	Lipase	LB2_038_115	4	152	Heat shock
LB2_310_056s	15	7161	n/a	LB2_055_006s	5	140	n/a
LB2_362_187s	5	5641	n/a	LB2_080_098	3	130	Nucleobindin-2
LB2_090_011	3	4134	Calreticulin	LB2_318_034	17	112	Lysosomal alpha-mannosidase
LB2_273_020	8	3163	Hypothetical protein	LB2_126_037	5	108	Hypothetical protein
LB2_325_090	5	2915	n/a	LB2_384_006	6	101	Catalase
LB2_259_013s	5	2859	n/a	LB2_384_064	3	97	Protein toll
LB2_405_013	3	2794	Hypothetical protein	LB2_342_106	3	95	Hypothetical protein
LB2_223_093	20	2597	Hypothetical protein	LB2_123_060	7	90	GTP-binding protein 2
LB2_229_038	4	2438	Elongation factor 1-alpha	LB2_195_053s	47	89	n/a
LB2_055_066s	20	1910	Ubiquitin	LB2_202_122	3	88	Importin subunit alpha
LB2_064_044	15	1849	Hypothetical protein	LB2_248_035	29	85	n/a
LB2_328_010	12	1558	n/a	LB2_032_029	3	52	Cytochrome P450 6a2
LB2_392_037	16	1524	Hypothetical protein	LB2_342_009	8	49	Hypothetical protein
LB2_269_001	8	1364	Lysozyme	LB2_041_025s	6	40	n/a
LB2_223_100	10	1317	Hypothetical protein	LB2_362_044	3	40	n/a
LB2_032_074	5	1125	Heat shock 70 kDa protein	LB2_055_010	4	39	n/a
LB2_368_216	5	996	Peptidylprolyl isomerase	LB2_384_060	4	34	Hypothetical protein
LB2_351_090	17	941	Aminopeptidase	LB2_055_008	4	30	n/a
LB2_202_072	4	897	ribosomal protein L40	LB2_055_131	3	25	CLIP-associating protein
LB2_362_047	16	852	Serine protease easter	LB2_165_094	3	23	Oligomeric Golgi complex
LB2_195_052s	64	761	n/a	LB2_055_040	4	22	n/a
LB2_195_039	57	733	n/a	LB2_193_063	3	21	Hypothetical protein
LB2_169_090	39	710	n/a	LB2_210_050	3	20	Hypothetical protein
LB2_273_025s	5	608	n/a	LB2_216_153	3	18	Arginine kinase
LB2_351_102s	16	562	n/a	LB2_362_055	5	17	Transgelin
LB2_302_125	6	549	Hypothetical protein	LB2_055_009	7	14	n/a
LB2_040_036	16	481	Hypothetical protein	LB2_316_055	3	14	Hypothetical protein
LB2_202_067	6	474	Hypothetical protein	LB2_055_007	3	13	n/a
LB2_121_078	21	376	Hypothetical protein	LB2_273_001	5	12	Ankyrin-2
LB2_278_199	11	331	Actin-5C	LB2_310_087	3	12	Spectrin beta chain
LB2_351_089	7	324	Aminopeptidase	LB2_179_055	4	11	Hypothetical protein
LB2_259_015	7	317	n/a	LB2_151_033	3	11	Hypothetical protein
LB2_249_032	8	316	Hypothetical protein	LB2_392_160	4	11	retinitis pigmentosa GTPase
LB2_399_119	25	303	Hypothetical protein	LB2_112_177	3	10	Hypothetical protein
LB2_377_061	3	297	CALM_0 protein	LB2_195_051	40	9	n/a
LB2_202_100	3	272	Hypothetical protein	LB2_123_202	3	8	Hypothetical protein
LB2_310_078	3	268	n/a	LB2_112_123	3	8	Hypothetical protein
LB2_248_036	23	208	n/a				

\*SP, the count of peptide detected by proteome with 100% sequence identity; TPM, transcripts per million, normalized expression value of RNAseq; Annotation, the best BLASTP hit against the UniProt database. Genes in blue background indicate of significantly high expression level in the venoms (Z-test,  $P < 0.01$ ).

**Supplementary Table 3 Homologous regions encoding the RhoGAP domain in Lb and Lh genomes**

Contig ID <sup>a</sup>	Strand	Locus position	Corresponding OGS ID <sup>b</sup>
LbouV2_1	+	868,856-872,281	LB2_001_063
Lhet_53	+	395,566-399,842	LhOGS03178
LbouV2_150	-	1,303,091-1,309,834	LB2_150_055
Lhet_18	-	1,465,913-1,480,789	LhOGS01184
LbouV2_169	+	2,154,376-2,164,465	LB2_169_103
LbouV2_278	+	4,265,093-4,278,742	LB2_278_172
Lhet_405	+	195,397-219,634	LhOGS11401
LbouV2_371	+	3,353,377-3,392,780	LB2_371_076
Lhet_108	+	2,221,126-2,327,245	LhOGS06035
LbouV2_1	-	1,587,155-1,587,806	LB2_001_148
LbouV2_179	-	957,879-979,728	LB2_179_004
Lhet_42	-	3,480,592-3,513,911	LhOGS02298
Lhet_166	+	1,359,807-1,364,726	LhOGS07661
LbouV2_302	+	1,597,777-1,610,241	LB2_302_118
LbouV2_112	+	3,034,506-3,043,583	LB2_112_162
LbouV2_151	-	64,210-70,326	LB2_151_005
Lhet_52	+	5,133,557-5,148,895	LhOGS03040
Lhet_26	+	4,080,189-4,080,907	n/a
Lhet_26	+	863,738-8,644,42	Lh-EsGAP3 <sup>c</sup>
Lhet_26	+	845,813-852,290	Lh-EsGAP2
Lhet_26	+	741,969-742,519	Lh-EsGAP1' <sup>d</sup>
Lhet_26	+	770,024-770,574	Lh-EsGAP1' <sup>d</sup>
Lhet_26	+	759,013-759,563	Lh-EsGAP1' <sup>d</sup>
Lhet_26	+	730,957-731,507	Lh-EsGAP1
LbouV2_216	-	4,232,052-4,235,053	LB2_216_152
LbouV2_330	+	2,748,394-2,753,571	EsGAP3
LbouV2_151	-	822,831-823,968	EsGAP2 <sup>e</sup>
LbouV2_255	+	620,159-620,919	EsGAP1' <sup>f</sup>
LbouV2_255	+	601,067-601,827	EsGAP1

The order of loci listed here corresponds to those shown in Fig. 5a (from top to bottom).

<sup>a</sup> Contig IDs from Lb (initiated with Lb) and Lh genomes (initiated with Lh), respectively; <sup>b</sup> the predicted OGS gene that encompasses the locus encoding the domain; <sup>c</sup> the length ratio of domain to the entire length of OGS; <sup>d</sup> this gene model concatenates four neighboring homologous loci, indicative of very recently duplication; <sup>e</sup> this gene is near-identical to a previously described LbGAP gene GU300066.1, but short by 39- and 126-bp of non-domain sequences on 5'- and 3'- ends, respectively; <sup>f</sup> this locus has no gene being predicted but shows high identity to its neighboring locus that encompassing LB2\_255\_017.

**Supplementary Table 4 Detailed statistics analysis of Fig. 1d**

Time (min)	CK	Female wasps	Male wasps	<i>P</i> value		
				CK vs Female wasps	CK vs Male wasps	Female wasps vs Male wasps
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	—	—	—
15	0.00 ± 0.00 <sup>b</sup>	1.91 ± 0.84 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	0.0070	0.9999	0.0070
30	0.00 ± 0.00 <sup>b</sup>	5.31 ± 2.08 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	0.0040	0.9999	0.0040
45	0.00 ± 0.00 <sup>b</sup>	20.36 ± 5.40 <sup>a</sup>	0.61 ± 0.53 <sup>b</sup>	0.0005	0.9689	0.0006
60	0.24 ± 0.41 <sup>b</sup>	32.67 ± 6.30 <sup>a</sup>	1.18 ± 0.67 <sup>b</sup>	9.0e-5	0.9479	0.0001
75	0.24 ± 0.41 <sup>b</sup>	42.41 ± 6.26 <sup>a</sup>	2.03 ± 0.78 <sup>b</sup>	1.8e-5	0.8248	2.4e-5
90	0.24 ± 0.41 <sup>b</sup>	50.42 ± 7.07 <sup>a</sup>	2.43 ± 0.87 <sup>b</sup>	1.4e-5	0.7985	1.8e-5
105	0.52 ± 0.45 <sup>b</sup>	56.80 ± 6.17 <sup>a</sup>	2.43 ± 0.87 <sup>b</sup>	3.0e-5	0.7992	3.7e-6
120	0.52 ± 0.45 <sup>b</sup>	60.53 ± 7.01 <sup>a</sup>	2.43 ± 0.87 <sup>b</sup>	4.3e-6	0.8390	5.3e-6
135	0.75 ± 0.72 <sup>b</sup>	63.53 ± 7.87 <sup>a</sup>	2.95 ± 0.40 <sup>b</sup>	6.5e-6	0.8299	8.1e-6
150	0.75 ± 0.72 <sup>b</sup>	66.03 ± 7.40 <sup>a</sup>	3.48 ± 0.09 <sup>b</sup>	3.5e-6	0.7283	4.5e-6
165	1.03 ± 0.90 <sup>b</sup>	69.46 ± 6.19 <sup>a</sup>	3.64 ± 0.21 <sup>b</sup>	1.2e-6	0.6691	1.4e-6
180	1.03 ± 0.90 <sup>b</sup>	71.44 ± 5.30 <sup>a</sup>	3.81 ± 0.49 <sup>b</sup>	5.5e-7	0.5537	6.6e-7
195	1.55 ± 0.66 <sup>b</sup>	73.94 ± 4.92 <sup>a</sup>	3.97 ± 0.77 <sup>b</sup>	3.4e-7	0.5907	4.0e-7
210	1.83 ± 0.88 <sup>b</sup>	75.04 ± 5.03 <sup>a</sup>	4.21 ± 0.69 <sup>b</sup>	3.7e-7	0.6141	4.3e-7
225	1.83 ± 0.88 <sup>b</sup>	76.45 ± 4.61 <sup>a</sup>	4.21 ± 0.69 <sup>b</sup>	2.0e-7	0.5670	2.5e-7
240	1.83 ± 0.88 <sup>b</sup>	77.78 ± 5.04 <sup>a</sup>	4.21 ± 0.69 <sup>b</sup>	3.0e-7	0.6146	3.6e-7

One-way analysis of variance (ANOVA) along with Fisher's least significant difference tests was used. Data are presented as the mean ± standard deviation (mean ± SD) and differences between treatments are considered significant when  $P < 0.05$ . All statistical analyses were conducted using SPSS 26.0 Statistics and EXCEL.



**Supplementary Table 5 Detailed statistics analysis of Fig. 4c (*D. hydei*)**

Time (min)	P <sub>Lb</sub>	CK	P <sub>dsEsGAP1</sub>	P value		
				P <sub>Lb</sub> vs CK	P <sub>Lb</sub> vs P <sub>dsEsGAP1</sub>	CK vs P <sub>dsEsGAP1</sub>
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	—	—	—
15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	—	—	—
30	3.98 ± 2.24 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	1.00 ± 1.00 <sup>b</sup>	0.0317	0.0923	0.6791
45	22.20 ± 12.66 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	3.33 ± 1.15 <sup>b</sup>	0.0234	0.0453	0.8472
60	37.43 ± 15.05 <sup>a</sup>	0.24 ± 0.41 <sup>b</sup>	6.00 ± 1.00 <sup>b</sup>	0.0047	0.0106	0.7108
75	47.94 ± 12.64 <sup>a</sup>	0.24 ± 0.41 <sup>b</sup>	7.67 ± 1.53 <sup>b</sup>	0.0005	0.0013	0.4763
90	55.56 ± 12.15 <sup>a</sup>	0.24 ± 0.41 <sup>b</sup>	9.67 ± 0.58 <sup>b</sup>	0.0002	0.0005	0.2997
105	59.67 ± 11.43 <sup>a</sup>	0.52 ± 0.45 <sup>b</sup>	11.67 ± 2.08 <sup>b</sup>	9.2e-5	0.0003	0.1845
120	66.59 ± 8.39 <sup>a</sup>	0.52 ± 0.45 <sup>c</sup>	13.00 ± 1.73 <sup>b</sup>	7.8e-6	2.8e-5	0.0487
135	70.23 ± 7.83 <sup>a</sup>	0.75 ± 0.72 <sup>c</sup>	14.67 ± 2.08 <sup>b</sup>	4.1e-6	1.6e-5	0.0256
150	72.79 ± 5.62 <sup>a</sup>	0.75 ± 0.72 <sup>c</sup>	15.67 ± 2.89 <sup>b</sup>	1.0e-6	3.0e-6	0.0060
165	73.74 ± 4.78 <sup>a</sup>	1.03 ± 0.90 <sup>c</sup>	17.33 ± 1.53 <sup>b</sup>	3.6e-7	1.1e-6	0.0012
180	75.30 ± 3.57 <sup>a</sup>	1.03 ± 0.90 <sup>c</sup>	18.00 ± 2.00 <sup>b</sup>	8.6e-8	4.4e-7	0.0003
195	76.77 ± 2.14 <sup>a</sup>	1.55 ± 0.66 <sup>c</sup>	19.00 ± 2.00 <sup>b</sup>	1.7e-9	4.3e-8	4.3e-5
210	77.25 ± 1.73 <sup>a</sup>	1.83 ± 0.88 <sup>c</sup>	20.00 ± 3.00 <sup>b</sup>	1.6e-8	1.8e-7	9.3e-5
225	78.43 ± 2.21 <sup>a</sup>	1.83 ± 0.88 <sup>c</sup>	20.67 ± 2.52 <sup>b</sup>	9.3e-9	1.4e-7	6.3e-5
240	78.43 ± 2.21 <sup>a</sup>	1.83 ± 0.88 <sup>c</sup>	20.67 ± 2.52 <sup>b</sup>	9.3e-9	1.4e-7	6.3e-5

One-way analysis of variance (ANOVA) along with Fisher's least significant difference tests was used. Data are presented as the mean ± standard deviation (mean ± SD) and differences between treatments are considered significant when  $P < 0.05$ . All statistical analyses were conducted using SPSS 26.0 Statistics and EXCEL.

**Supplementary Table 5 Detailed statistics analysis of Fig. 4c (*D. mauritiana*)**

Time (min)	$P_{Lb}$	CK	$P_{dsEsGAP1}$	<i>P</i> value		
				$P_{Lb}$ vs CK	$P_{Lb}$ vs $P_{dsEsGAP1}$	CK vs $P_{dsEsGAP1}$
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	—	—	—
15	3.13 ± 0.20 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	1.8e-7	1.8e-7	0.9999
30	11.19 ± 3.14 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	1.83 ± 0.76 <sup>b</sup>	0.0008	0.0021	0.4932
45	19.06 ± 2.63 <sup>a</sup>	0.55 ± 0.95 <sup>b</sup>	3.92 ± 1.01 <sup>b</sup>	2.9e-5	9.2e-5	0.1154
60	27.15 ± 3.37 <sup>a</sup>	0.55 ± 0.95 <sup>c</sup>	6.42 ± 1.51 <sup>b</sup>	1.4e-5	6.3e-5	0.0391
75	34.87 ± 3.55 <sup>a</sup>	0.55 ± 0.95 <sup>c</sup>	8.75 ± 3.70 <sup>b</sup>	2.1e-5	0.0001	0.0360
90	40.94 ± 3.02 <sup>a</sup>	0.97 ± 0.86 <sup>c</sup>	9.83 ± 3.62 <sup>b</sup>	4.7e-6	2.2e-5	0.0182
105	46.05 ± 3.80 <sup>a</sup>	0.97 ± 0.86 <sup>c</sup>	10.83 ± 3.82 <sup>b</sup>	5.0e-6	2.3e-5	0.0202
120	52.18 ± 3.96 <sup>a</sup>	0.97 ± 0.86 <sup>c</sup>	11.17 ± 3.75 <sup>b</sup>	2.6e-6	9.9e-6	0.0185
135	56.85 ± 3.14 <sup>a</sup>	0.97 ± 0.86 <sup>c</sup>	11.83 ± 4.25 <sup>b</sup>	1.4e-6	4.5e-6	0.0121
150	60.03 ± 3.66 <sup>a</sup>	1.40 ± 1.30 <sup>c</sup>	12.25 ± 3.63 <sup>b</sup>	1.1e-6	3.0e-6	0.0117
165	62.74 ± 4.72 <sup>a</sup>	1.40 ± 1.30 <sup>c</sup>	12.58 ± 3.64 <sup>b</sup>	1.7e-6	5.2e-6	0.0190
180	64.52 ± 5.72 <sup>a</sup>	1.78 ± 0.72 <sup>c</sup>	12.58 ± 3.64 <sup>b</sup>	2.7e-6	8.4e-6	0.0351
195	66.01 ± 5.38 <sup>a</sup>	1.78 ± 0.72 <sup>c</sup>	13.33 ± 3.06 <sup>b</sup>	1.5e-6	4.4e-6	0.0180
210	66.86 ± 5.87 <sup>a</sup>	1.78 ± 0.72 <sup>c</sup>	14.08 ± 2.50 <sup>b</sup>	1.6e-6	5.2e-6	0.0156
225	67.18 ± 6.35 <sup>a</sup>	1.78 ± 0.72 <sup>c</sup>	14.08 ± 2.50 <sup>b</sup>	2.2e-6	7.6e-6	0.0209
240	67.18 ± 6.35 <sup>a</sup>	1.78 ± 0.72 <sup>c</sup>	14.08 ± 2.50 <sup>b</sup>	2.2e-6	7.6e-6	0.0209

One-way analysis of variance (ANOVA) along with Fisher's least significant difference tests was used. Data are presented as the mean ± standard deviation (mean ± SD) and differences between treatments are considered significant when  $P < 0.05$ . All statistical analyses were conducted using SPSS 26.0 Statistics and EXCEL.

**Supplementary Table 5 Detailed statistics analysis of Fig. 4c (*D. simulans*)**

Time (min)	$P_{Lb}$	CK	$P_{dsEsGAP1}$	P value		
				$P_{Lb}$ vs CK	$P_{Lb}$ vs $P_{dsEsGAP1}$	CK vs $P_{dsEsGAP1}$
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	—	—	—
15	1.11 ± 1.92 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.4827	0.4827	0.9999
30	10.70 ± 5.35 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	2.92 ± 0.72 <sup>b</sup>	0.0133	0.0504	0.5231
45	23.67 ± 8.88 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	5.42 ± 1.91 <sup>b</sup>	0.0035	0.0125	0.4622
60	33.16 ± 6.04 <sup>a</sup>	0.41 ± 0.70 <sup>c</sup>	8.75 ± 3.31 <sup>b</sup>	0.0001	0.0007	0.0949
75	38.71 ± 6.89 <sup>a</sup>	0.41 ± 0.70 <sup>c</sup>	10.42 ± 4.39 <sup>b</sup>	0.0002	0.0008	0.0912
90	43.23 ± 5.87 <sup>a</sup>	0.41 ± 0.70 <sup>c</sup>	12.50 ± 5.00 <sup>b</sup>	5.7e-5	0.0004	0.0371
105	49.96 ± 4.37 <sup>a</sup>	0.89 ± 0.78 <sup>c</sup>	14.17 ± 5.64 <sup>b</sup>	1.6e-5	0.0001	0.0182
120	51.66 ± 4.34 <sup>a</sup>	0.89 ± 0.78 <sup>c</sup>	14.17 ± 5.64 <sup>b</sup>	1.3e-5	7.8e-5	0.0180
135	53.90 ± 5.28 <sup>a</sup>	1.30 ± 1.23 <sup>c</sup>	15.00 ± 5.00 <sup>b</sup>	1.3e-5	7.5e-5	0.0179
150	55.52 ± 5.54 <sup>a</sup>	1.30 ± 1.23 <sup>c</sup>	15.83 ± 5.20 <sup>b</sup>	1.4e-5	8.6e-5	0.0167
165	56.60 ± 6.27 <sup>a</sup>	1.78 ± 1.56 <sup>c</sup>	16.25 ± 4.51 <sup>b</sup>	1.5e-5	8.9e-5	0.0188
180	58.21 ± 7.06 <sup>a</sup>	1.78 ± 1.56 <sup>c</sup>	16.67 ± 3.82 <sup>b</sup>	1.5e-5	9.3e-5	0.0195
195	58.77 ± 7.88 <sup>a</sup>	2.93 ± 0.72 <sup>c</sup>	17.08 ± 4.02 <sup>b</sup>	2.7e-5	0.0001	0.0341
210	58.77 ± 7.88 <sup>a</sup>	3.41 ± 1.09 <sup>c</sup>	17.92 ± 3.61 <sup>b</sup>	2.6e-5	0.0001	0.0289
225	59.32 ± 8.73 <sup>a</sup>	3.41 ± 1.09 <sup>c</sup>	17.92 ± 3.61 <sup>b</sup>	4.0e-5	0.0002	0.0406
240	59.32 ± 8.73 <sup>a</sup>	3.41 ± 1.09 <sup>c</sup>	18.33 ± 2.89 <sup>b</sup>	3.4e-5	0.0002	0.0326

One-way analysis of variance (ANOVA) along with Fisher's least significant difference tests was used. Data are presented as the mean ± standard deviation (mean ± SD) and differences between treatments are considered significant when  $P < 0.05$ . All statistical analyses were conducted using SPSS 26.0 Statistics and EXCEL.

**Supplementary Table 5 Detailed statistics analysis of Fig. 4c (*D. yakuba*)**

Time (min)	$P_{Lb}$	CK	$P_{dsEsGAP1}$	P value		
				$P_{Lb}$ vs CK	$P_{Lb}$ vs $P_{dsEsGAP1}$	CK vs $P_{dsEsGAP1}$
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	—	—	—
15	2.24 ± 1.94 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.1096	0.1096	0.9999
30	6.38 ± 2.98 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	0.33 ± 0.58 <sup>b</sup>	0.0102	0.0131	0.9707
45	14.22 ± 2.60 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	2.33 ± 1.15 <sup>b</sup>	0.0001	0.0003	0.2668
60	20.59 ± 2.75 <sup>a</sup>	0.24 ± 0.41 <sup>c</sup>	5.00 ± 1.73 <sup>b</sup>	2.9e-5	0.0001	0.0491
75	26.24 ± 6.30 <sup>a</sup>	0.24 ± 0.41 <sup>b</sup>	5.33 ± 2.08 <sup>b</sup>	0.0004	0.0013	0.3060
90	29.82 ± 4.75 <sup>a</sup>	0.47 ± 0.82 <sup>b</sup>	5.67 ± 2.52 <sup>b</sup>	6.6e-5	0.0002	0.1869
105	37.35 ± 5.93 <sup>a</sup>	1.13 ± 1.07 <sup>b</sup>	8.00 ± 1.73 <sup>b</sup>	4.4e-5	0.0001	0.1276
120	41.76 ± 6.29 <sup>a</sup>	1.13 ± 1.07 <sup>c</sup>	9.00 ± 2.00 <sup>b</sup>	3.3e-5	0.0001	0.1023
135	44.06 ± 6.87 <sup>a</sup>	1.13 ± 1.07 <sup>c</sup>	10.67 ± 2.52 <sup>b</sup>	4.3e-5	0.0002	0.0755
150	47.55 ± 6.38 <sup>a</sup>	1.53 ± 0.52 <sup>c</sup>	11.33 ± 2.89 <sup>b</sup>	2.1e-5	8.5e-5	0.0569
165	50.56 ± 5.64 <sup>a</sup>	1.53 ± 0.52 <sup>c</sup>	11.67 ± 3.21 <sup>b</sup>	9.0e-6	3.7e-5	0.0377
180	53.33 ± 4.79 <sup>a</sup>	1.53 ± 0.52 <sup>c</sup>	12.00 ± 3.46 <sup>b</sup>	3.6e-6	1.5e-5	0.0224
195	55.62 ± 3.79 <sup>a</sup>	1.94 ± 0.67 <sup>c</sup>	12.00 ± 3.46 <sup>b</sup>	1.5e-6	4.5e-6	0.0146
210	56.22 ± 4.01 <sup>a</sup>	2.35 ± 0.19 <sup>c</sup>	12.33 ± 3.79 <sup>b</sup>	1.9e-6	6.4e-6	0.0201
225	59.59 ± 1.91 <sup>a</sup>	2.35 ± 0.19 <sup>c</sup>	12.33 ± 3.79 <sup>b</sup>	4.8e-7	1.1e-6	0.0059
240	59.59 ± 1.91 <sup>a</sup>	2.35 ± 0.19 <sup>c</sup>	12.67 ± 4.16 <sup>b</sup>	6.7e-7	1.6e-6	0.0074

One-way analysis of variance (ANOVA) along with Fisher's least significant difference tests was used. Data are presented as the mean ± standard deviation (mean ± SD) and differences between treatments are considered significant when  $P < 0.05$ . All statistical analyses were conducted using SPSS 26.0 Statistics and EXCEL.