

Figure S1. The phylogenetic trees of 14 candidate genes from MAPK signaling pathway in *C. bowringi*. ExPASy translate tool was used to determine the protein sequences of candidate genes. The 14 rooted phylogenetic trees were built in MEGA 6 using the neighbor-joining method. Protein sequences used in phylogenetic analyses came from various insect orders, including Coleoptera, Diptera, Lepidoptera, Hemiptera, and Hymenoptera.

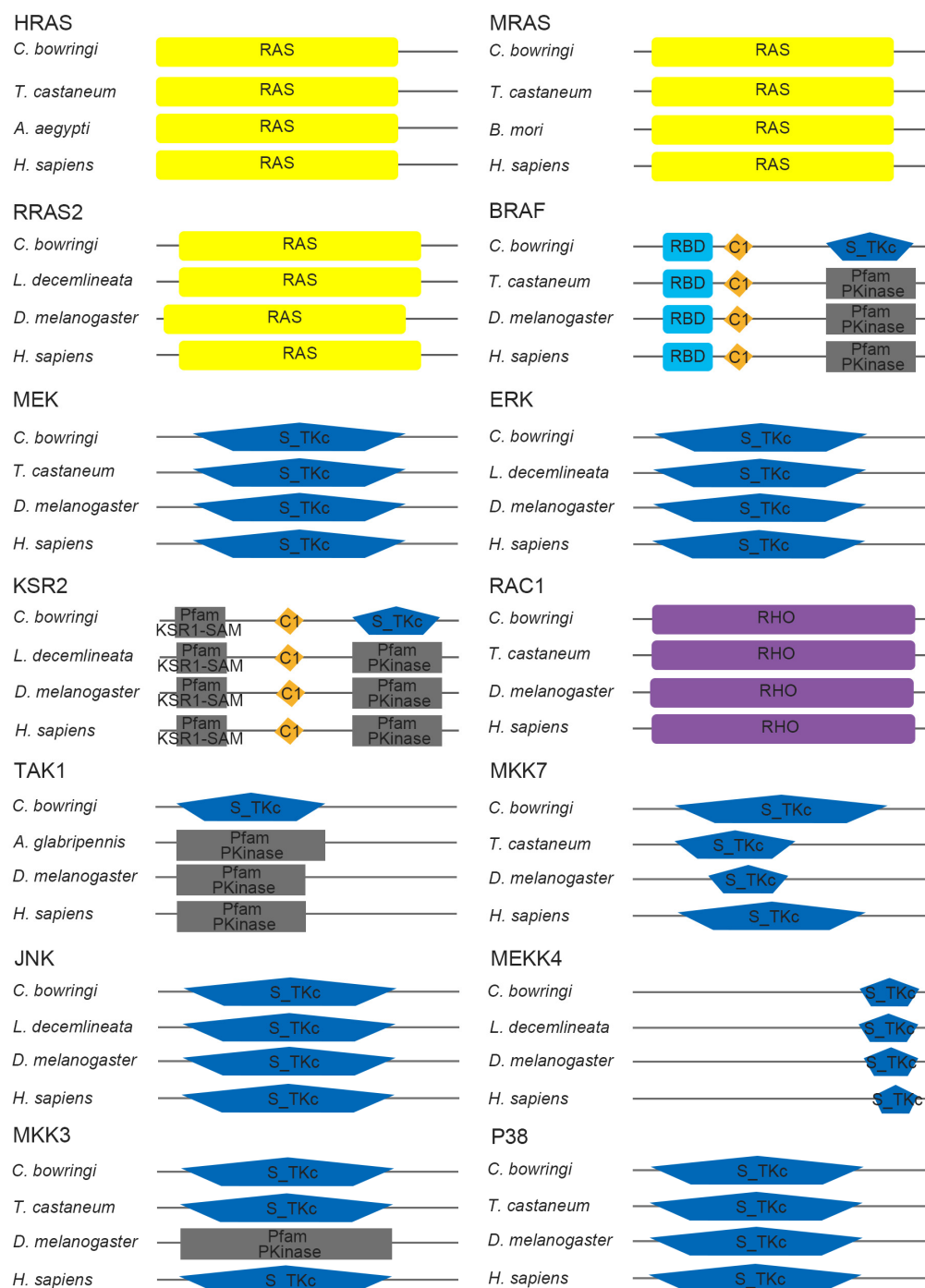


Figure S2. The protein domains of candidate genes from MAPK signaling pathway in *C. bowringi*. The ExPASy translate tool was used to deduce protein sequences of candidate genes, and SMART tool was used to predict protein domains. The protein sequences (the accession numbers were shown in Fig. S1) used for protein domains prediction came from a

variety of species, including *Colaphellus bowringi*, *Tribolium castaneum*, *Leptinotarsa decemlineata*, *Anoplophora glabripennis*, *Bombyx mori*, *Aedes aegypti*, *Drosophila melanogaster*, and *Homo sapiens*.

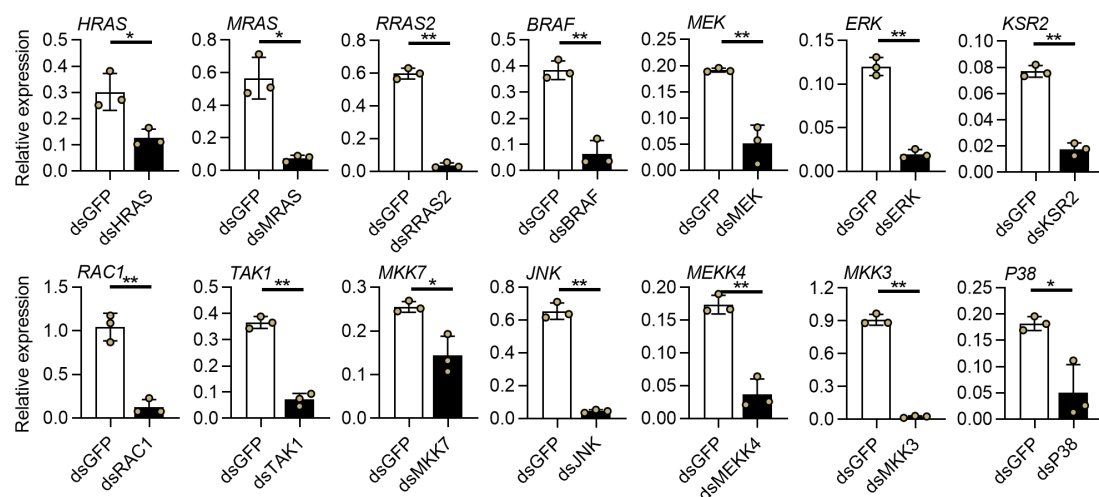


Figure S3. The efficacy of RNAi knockdown of candidate genes. After injecting dsRNAs into female pupae of *C. bowringi*, the expression levels of 14 candidate genes were detected in the ovaries. All data were collected at 4-day PE and presented as mean \pm SD, and the significance test was performed with an independent *t*-test, * $P < 0.05$, ** $P < 0.01$.

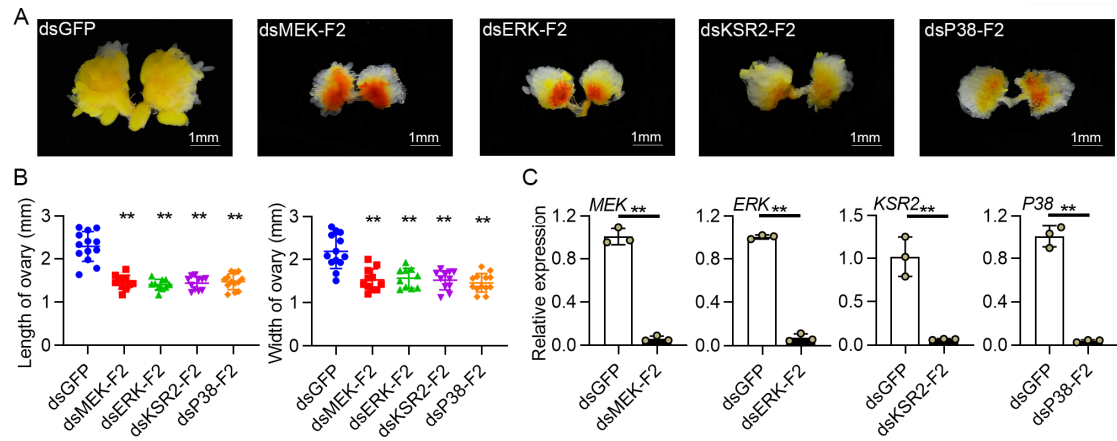


Figure S4. The effect of knocking down another non-overlapping region of key genes in *C. bowringi* ovarian development. (A) Representative ovarian samples following 2 μ g dsRNA microinjection into female pupae. (B) The size of ovaries as a result of silencing key genes $n = 10-13$. (C) The expression levels of key genes in the ovaries were measured. All data were collected at 4-day PE and presented as mean \pm SD, with the significance test using independent *t*-test, * $P < 0.05$, ** $P < 0.01$.

Table S1. The blastp analysis of candidate genes for RNAi

Gene (Abbreviation)	Gene ID	Closest species	Genbank number	E value
<i>GTPase HRas (HRAS)</i>	CL223.Contig3_All	<i>Leptinotarsa decemlineata</i>	XP_023017346.1	7E-122
<i>Ras-related protein M-Ras (MRAS)</i>	Unigene2066_All	<i>Anoplophora glabripennis</i>	XP_018563656.1	2E-141
<i>Ras-related protein R-Ras2 (RRAS2)</i>	Unigene1450_All	<i>Leptinotarsa decemlineata</i>	XP_023015173.1	1E-146
<i>B-Raf proto-oncogene serine/threonine-protein kinase (BRAF)</i>	CL3151.Contig3_All	<i>Leptinotarsa decemlineata</i>	XP_023012550.1	0
<i>Mitogen-activated protein kinase kinase 1 (MEK)</i>	Unigene14333_All	<i>Anoplophora glabripennis</i>	XP_018576061.1	0
<i>Mitogen-activated protein kinase 1 (ERK)</i>	CL5074.Contig3_All	<i>Leptinotarsa decemlineata</i>	XP_023027470.1	0
<i>Kinase suppressor of Ras 2 (KSR2)</i>	Unigene549_All	<i>Leptinotarsa decemlineata</i>	XP_023017147.1	0
<i>Ras-related C3 botulinum toxin substrate 1 (RAC1)</i>	CL2204.Contig2_All	<i>Anoplophora glabripennis</i>	XP_018569526.1	7E-139
<i>Mitogen-activated protein kinase kinase kinase 7 (TAK1)</i>	CL5016.Contig2_All	<i>Leptinotarsa decemlineata</i>	XP_023017563.1	0
<i>Mitogen-activated protein kinase kinase 7 (MKK7)</i>	CL3117.Contig2_All	<i>Leptinotarsa decemlineata</i>	ALE20541.1	0
<i>C-Jun N-terminal kinase (JNK)</i>	CL1663.Contig3_All	<i>Leptinotarsa decemlineata</i>	XP_008197492	0
<i>Mitogen-activated protein kinase kinase kinase 4 (MEKK4)</i>	Unigene5981_All	<i>Leptinotarsa decemlineata</i>	XP_023025197.1	0
<i>Mitogen-activated protein kinase kinase 3 (MKK3)</i>	CL3731.Contig2_All	<i>Leptinotarsa decemlineata</i>	XP_023017722.1	0
<i>P38 MAP kinase (P38)</i>	Unigene12015_All	<i>Anoplophora glabripennis</i>	XP_018573142.1	0

Table S2. Primers for qRT-PCR

Genes	Forward primers (5'-3')	Reverse primers (5'-3')	PCR efficiency (%)	Standard curve R ²
<i>RPL19</i>	gtaatgcgatgcggcaagaa	gagtgcaccgctacaggttt	102.2	0.998
<i>Actin1</i>	tcaagcgggtgtagctctgg	tatcgatcacgatgccgggtg	100.2	0.996
<i>HRAS</i>	ggagacatcagccaagac	agcacctcaacttgaatgg	100.4	0.999
<i>MRAS</i>	gctcacaattgggcattc	ggattattctcaccacctcat	107.6	0.999
<i>RRAS2</i>	agagtagtttggaagaaga	agcattatcgacgttcattc	112.2	1.000
<i>BRAF</i>	agcaattccaccaacctac	gcaactgaccagacaaca	99.2	0.999
<i>MEK</i>	ttgaagggttgagctatct	tctctggcgacataataactt	104.2	0.999
<i>ERK</i>	cgatactgagttggatgatt	tggttctatgtgatgcctt	101.3	0.998
<i>KSR2</i>	ggactcttcagtgtagcaa	gtagacttctcataacttctgg	96.2	0.997
<i>RAC1</i>	ccaatcacatatccacagg	gcatcacaggacatagtac	111.8	0.995
<i>TAK1</i>	accaacacaatgtcagagaa	agtgcgtgtccataattcctt	114.2	0.998
<i>MKK7</i>	accgaatctgaagtatggat	taaggcttctactgttgcta	106.2	0.996
<i>JNK</i>	cattgtatgtgctgctgtc	ggagaaggcggttcagtaa	96.9	0.998
<i>MEKK4</i>	ggaatgtaagcaagggtgtt	tatagcgtctgtaaggtagag	99.3	0.997
<i>MKK3</i>	cctcctcggttaccttca	tggctgtagttcgggtcta	109.6	0.998
<i>P38</i>	cattatcagcagttgggtca	agcagtttcagttccctata	96.9	0.999
<i>Vg1</i>	ggaatgcgcgtgagattgac	cgaagcagttttgtgggggtg	100.2	0.996
<i>Vg2</i>	tggaccgattcacaacctg	tcgaagatgaggaatgcggg	105.4	0.997
<i>JHAMT1</i>	gggttttcagtgtagattctgga	cgaaaatcagttgtccctctgt	91.5	0.998
<i>Kr-h1</i>	caactcaaatgcacacccg	ccgtaatgcgcaacctgatg	102.0	0.998
<i>Shade</i>	actgacgagcccaaaaacca	acgacgtttcgagacccaat	106.0	0.999
<i>E74</i>	agggattcgggcactgaaag	tgcctcgaaagtagctgggtg	100.7	0.999
<i>Nanos</i>	ctttccaacgggtgtcaaa	gaaacagaaacaactaagcc	108.5	0.994
<i>Vasa</i>	tgtatcaccaacacgagaat	gacagccactcaacacat	112.0	0.996

Table S3. Primers for RNAi

Genes	dsRNA region	Forward primers (5'-3')	Reverse primers (5'-3')
<i>GFP</i>	region 1	gcgtaatacgactcactatagggtgcccaattctcgtggaac	gcgtaatacgactcactataggcttgaagttgaccttgatgcc
<i>HRAS</i>	region 1	gcgtaatacgactcactataggcagcattgaccattcagttg	gcgtaatacgactcactataggctcatccacagcccagttt
<i>MRAS</i>	region 1	gcgtaatacgactcactataggtagtcgtagttggagatgga	gcgtaatacgactcactataggctcggataagtgctctgtct
<i>RRAS2</i>	region 1	gcgtaatacgactcactataggattagtctcgtgggtggag	gcgtaatacgactcactataggaaaccttattgccgaccataa
<i>BRAF</i>	region 1	gcgtaatacgactcactatagggtggaacaactgagtaatgga	gcgtaatacgactcactatagggttggtgacataagggaaggaa
<i>MEK</i>	region 1	gcgtaatacgactcactataggccaactgtaactccgacttc	gcgtaatacgactcactataggaaatcaagagaaccagcatcc
	region 2	gcgtaatacgactcactataggctcagtcagagtgacatat	gcgtaatacgactcactatagggcctgctatatccacatctt
<i>ERK</i>	region 1	gcgtaatacgactcactataggacgatcacaggggtctc	gcgtaatacgactcactataggctcatcagccggatcgtaat
	region 2	gcgtaatacgactcactataggcttacgatactgtgacgaa	gcgtaatacgactcactatagggtgagaagtagattgcttggt
<i>KSR2</i>	region 1	gcgtaatacgactcactataggaaacctcaggaagtgaattg	gcgtaatacgactcactataggataggatgagatggactacg
	region 2	gcgtaatacgactcactataggatgatgtcgtctgtgtggtta	gcgtaatacgactcactataggatgactgaatgtggatgtga
<i>RAC1</i>	region 1	gcgtaatacgactcactatagggtctctgataagctacact	gcgtaatacgactcactataggaaatagtcgctctatcgtctc
<i>TAK1</i>	region 1	gcgtaatacgactcactataggaggtgttcacatcatctcat	gcgtaatacgactcactatagggttctcaacttcgggtgaat
<i>MKK7</i>	region 1	gcgtaatacgactcactatagggtgcttatatggctccagaac	gcgtaatacgactcactataggcatccgattgttgaagaagt
<i>JNK</i>	region 1	gcgtaatacgactcactataggccgattgcattgaagata	gcgtaatacgactcactataggccaccgatattctactctgt
<i>MEKK4</i>	region 1	gcgtaatacgactcactataggacttgagttcgggaagagga	gcgtaatacgactcactatagggcaaacttgctaccaccat
<i>MKK3</i>	region 1	gcgtaatacgactcactataggccagtgggacaataatggct	gcgtaatacgactcactatagggttgcttggggatctatt
<i>P38</i>	region 1	gcgtaatacgactcactatagggtgggtgtatcatggcagaa	gcgtaatacgactcactataggctcatcgttgggatcagcata
	region 2	gcgtaatacgactcactataggccgagacacgataatagtca	gcgtaatacgactcactataggaaagattcaactggtggatgt

Note: T7 promoter sequence is “gcgtaatacgactcactatagg”.