

Figure S1. Expression differences among the 14 predicted genes in the mapping region.

Relative expression profiles of the 14 predicted genes in the 5th instar wild-type ($+^{Bo}/+^{Bo}$, Dazao) and Bo mutant larvae. Data are mean \pm s.d. (n=3). *P<0.05, **P<0.01, paired Student's t-test. (n.s., not significant).

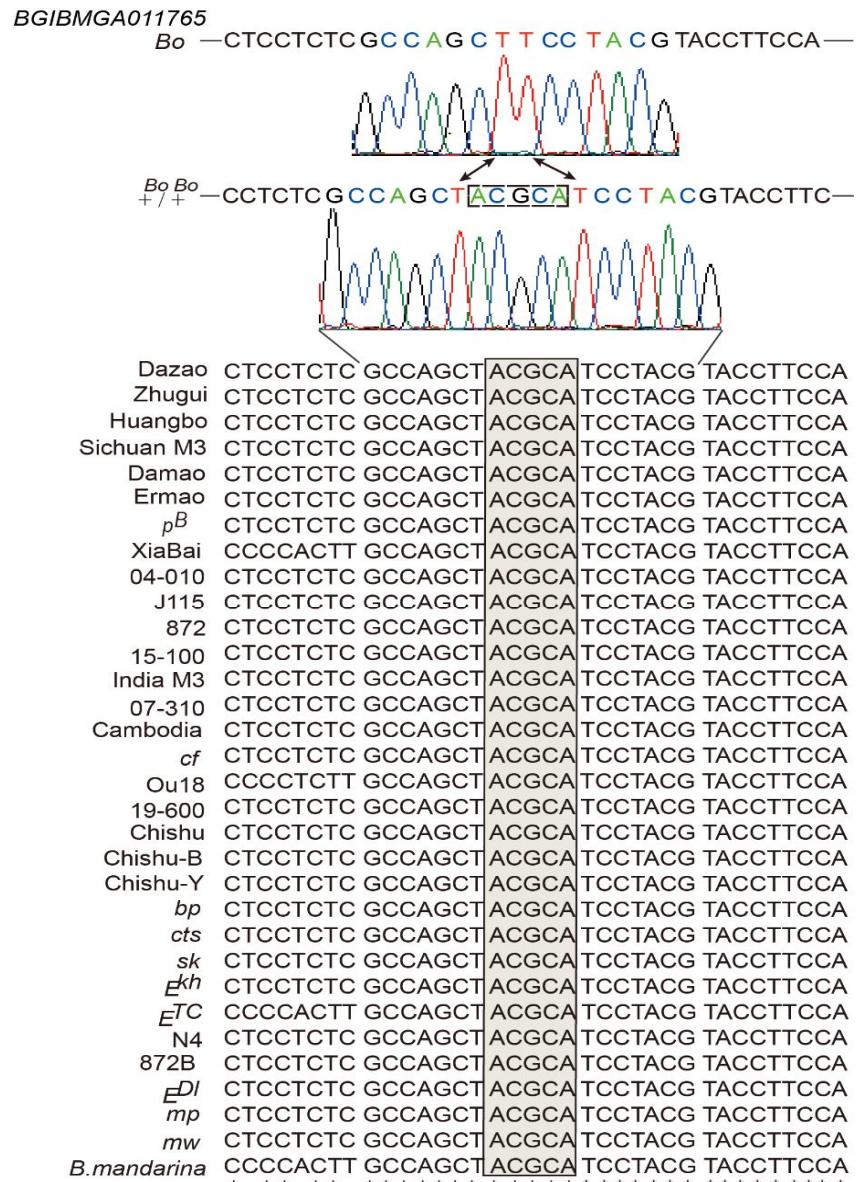


Figure S2. Multiple sequence alignment of *BGIBMGA011765* with homologs from 34 silkworm strains. PCR amplification and sequencing of amplified products from 34 silkworm strains (including *Bo*) confirmed the specificity of the 5bp deletion in *BmorCPH24* in the *Bo* mutant (grey frame). Asterisks identify identical nucleotides. Strains: *Bo*, *+Bo/+Bo*, Dazao, Zhugui, Huangbo, Sichuan M3, Damao, Ermao, *pB*, XiaBai, 04-010, J115, 872, 15-100, India M3, 07-310, Cambodia, crayfish (*cf*), Ou18, 19-600, Chishu, Chishu-B, Chishu-Y, black pupa (*bp*), cheek and tail spots (*cts*), Stick(*sk*), Kh-extra crescents (*E^{Kh}*), Triple crescents (*E^{Tc}*), N4, 872B, Extra-crescents and extra-legs (*E^{Dl}*), micropterous (*mp*), minute wing (*mw*), and *Bombyx mandarina* (*B. mandarina*).

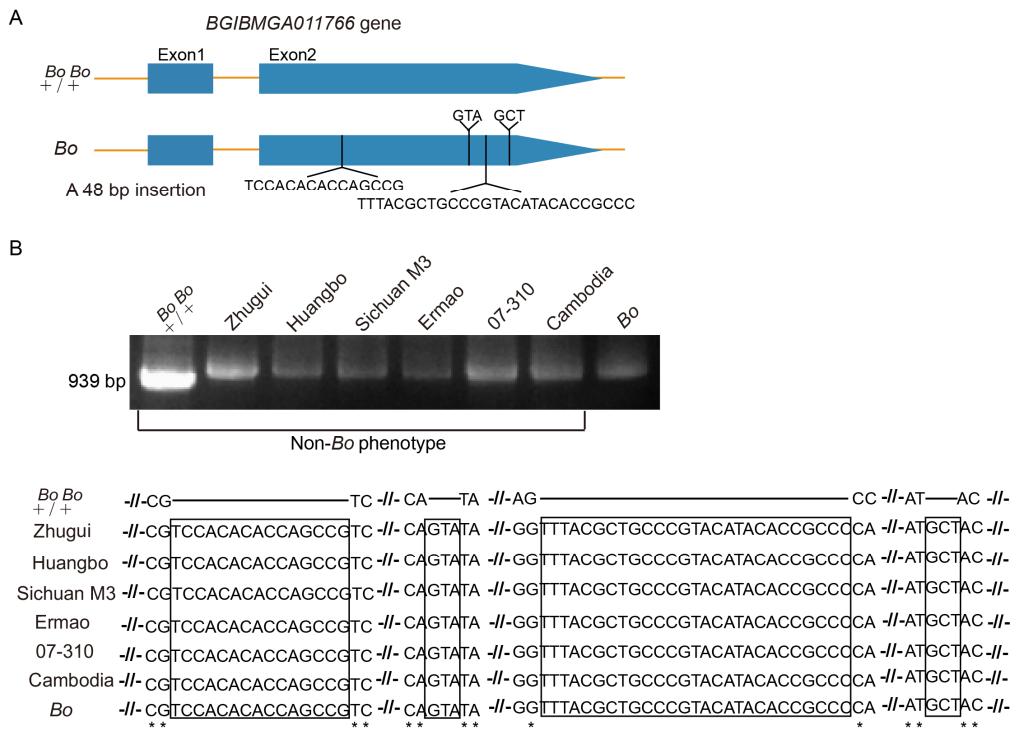


Figure S3. Sequence differences of *BGIBMGA011766* between wild-type and *Bo* mutant. (A) Structure of *BGIBMGA011766*. A 48 bp insertion in *Bo*. (B) Sequence analysis of *BGIBMGA011766* in multiple strains of silkworm. A sequence same to *Bo* was detected in 6 non-*Bo* phenotype silkworm strains (Zhugui, Huangbo, Sichuan M3, Ermao, 07-310, Cambodia). Asterisks and black frames indicate identical nucleotides and the 48 bp insertion in *Bo*, respectively.

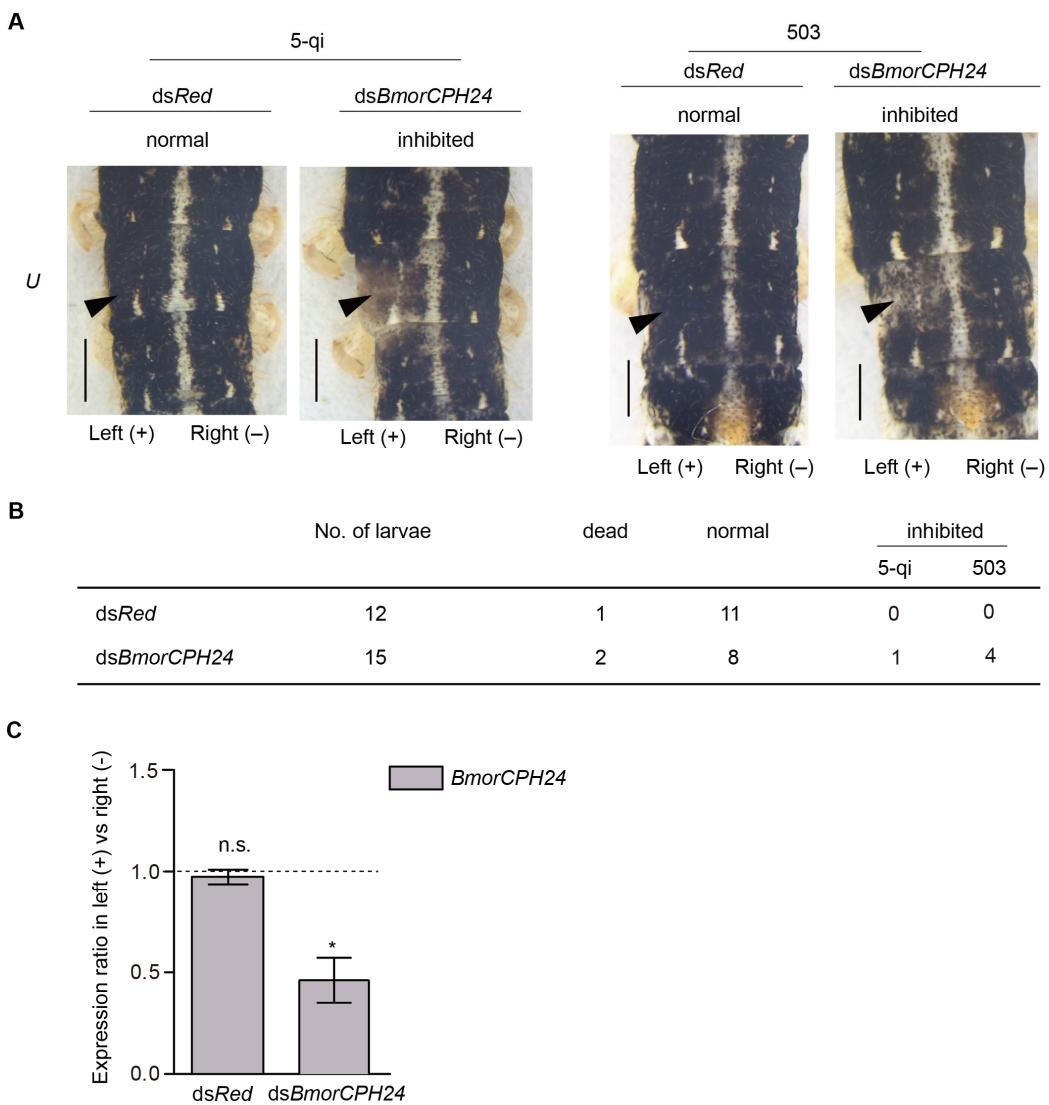


Figure S4. Knockdown of *BmorCPH24* in the *U* epidermis by RNAi. (A) Effect of RNAi on melanin in the *U* epidermis. Black arrows indicate the sites of injection and electric shock. Scale bars, 0.5cm. (B) Number of RNAi-treated individuals and results. (C) Analysis of *BmorCPH24* transcript levels in left versus right side of injected individuals. Data are mean \pm s.d. ($n=3-4$). * $P<0.05$, paired Student's *t*-test. (n.s., not significant). 5-qi and 503 indicate the start and day 3 of the 5th instar larvae, respectively.

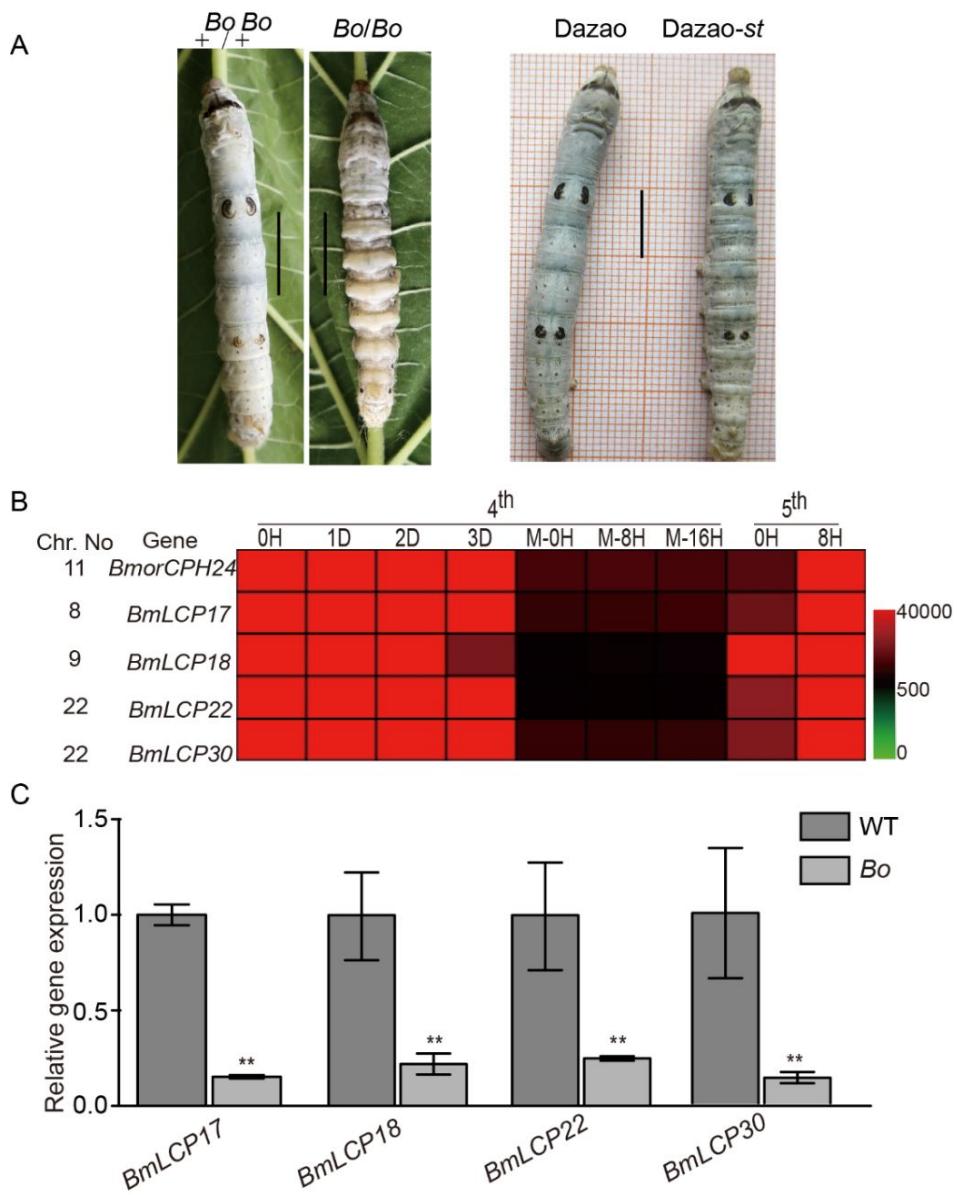


Figure S5. Analysis of the larval cuticle protein genes. (A) Phenotype of Bamboo (*Bo*) and stony (*st*) mutant larvae. Scale bars, 1cm. (B) Heat map of the relative expression level of 5 CP genes in *B. mori* by microarray. Red and green indicate higher and lower expression level, respectively. 4th fourth instar; 5th, fifth instar; H, hour; D, day; M, Molting. (C) Expression of the five cuticular protein genes (*BmLCP17*, *BmLCP18*, *BmLCP22*, *BmLCP30* and *BmCPT1*) in the epidermis of wild-type and *Bo* mutant. Data are mean \pm s.d. (n=3-4). **P<0.01, paired Student's *t*-test. (n.s., not significant).

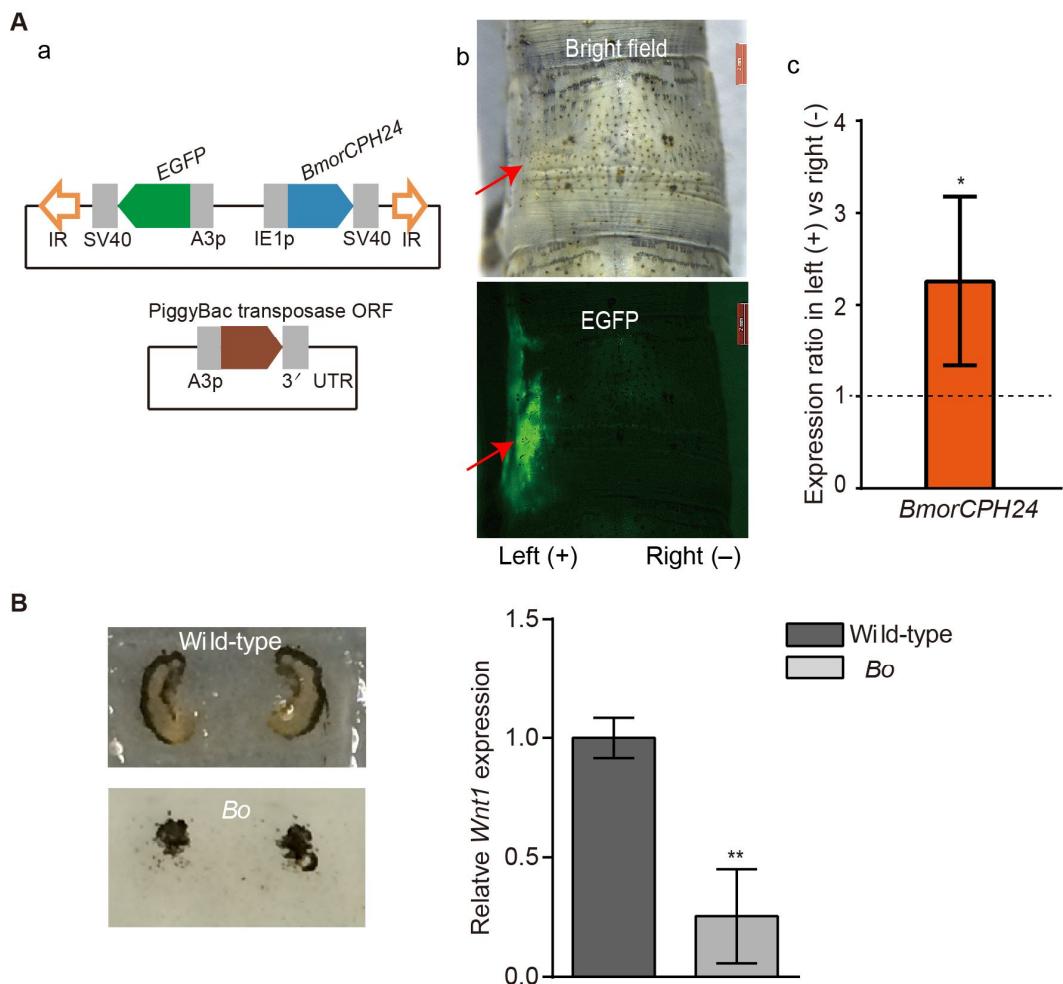


Figure S6. The relationship between *BmorCPH24* and pigmentation. (A) Overexpression of *BmorCPH24* in wild-type (+^P). (a) Schematic representation of the *BmorCPH24* overexpression system constructed in *piggyBac*. Grey boxes show promoters and terminators. IR represents recognition sequences of *piggyBac*. (b) *BmorCPH24* transgenic expression by electroporation into wild-type 5th instar larvae. '+' and '-' indicate the electrical current direction during electroporation. EGFP signal was visualized in the left (+) side. Scale bars, 2mm. (c) Expression of *BmorCPH24* in left (+) versus right (-) sides of the larval epidermis. Data are mean ± s.d. (n=5). *P<0.05, paired Student's t-test. (B) qRT-PCR analysis of *Wnt1* in wild-type and *Bo*. Data are mean ± s.d. (n=3). **P<0.01, paired Student's t-test.

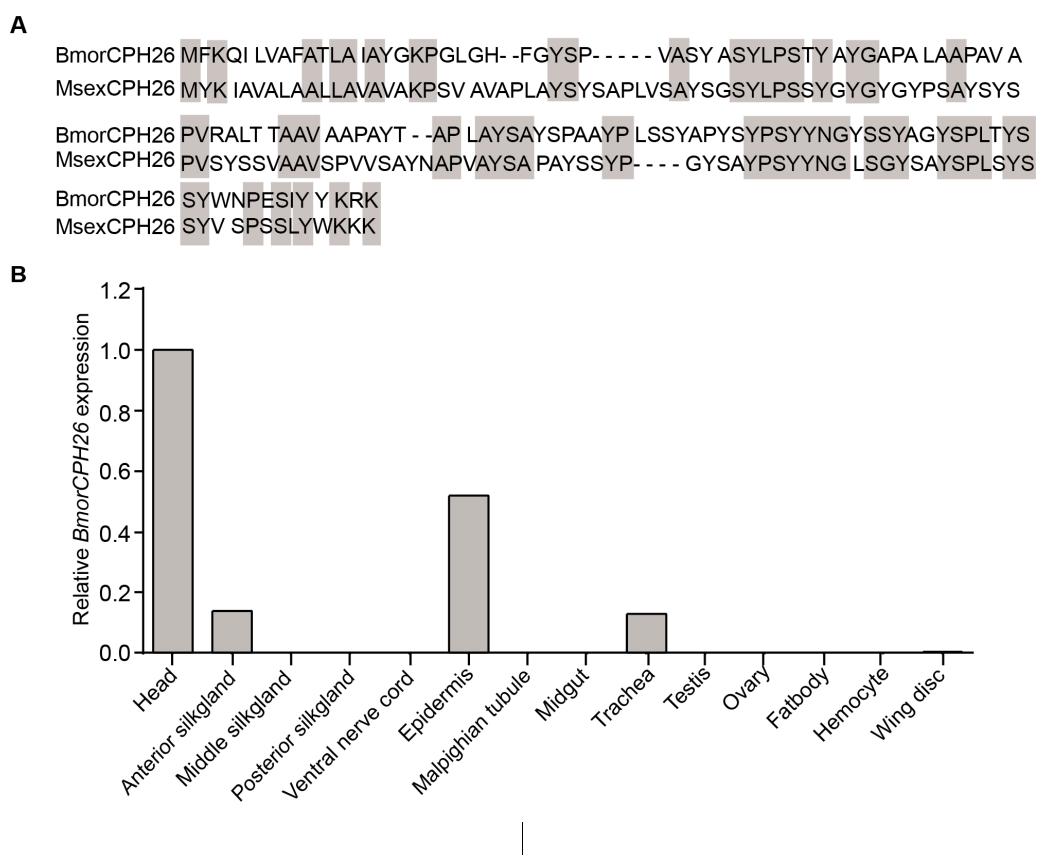


Figure S7. The sequence and expression characteristics of *BmorCPH26*, the paralog of *BmorCPH24*. (A) Protein sequence alignment between *BmorCPH26* and *MsexCPH26*. Grey represents conserved amino acids. (B) Expression pattern of *BmorCPH26* in different tissues of the wild-type larvae on day 3 of the 5th instar.

Table S1. Primers used in this study

Object	Name	Forward primer (5'-3')	Reverse primer (5'-3')
Genotyping of <i>Bo</i> Locus	a	GCTTTGCGAGTCTACTATCT	GTTCTGCTGGAGCGATTAA
	b	GGACTCCTTGACCTCGTAAC	AATTGTGACTTCGCCATCG
	c	GAAAATGTTGTCGTGGTTGT	GTGCTTGCCATCTGTGCT
	d	CTTCCTTGGTAAGAGGCTGAG	CGTGTAAACCAATGCCAGAG
	e	TGAGCAGCGGTCTTCAGTT	CTCCTTCTATGCCATCCA
	f	TATCTACTATCGTCCTGACACTAA	TTTATGAAACGGGTCAA
	g	TTTTTCTCGTAAAGGTGGAT	TCTATACAACGGTTATGGATT
	h	CACTATTCACTTGGCAACGAC	AACAATTGGGTGTCAGTT
	i	AACCTGTGAGCTGACCTAACCG	AACAAGATAGTCGCAACCCAG
Identifying the <i>Bo</i> mutation site	1765	CACACCACGATGTACACACTCAG	GTAGTAGACGGAGGCAGGAT
	1766	ACTTCGGCAGATTAGAGAG	AGAGACAACAGCTAACTGTT
<i>In situ</i> hybridization RNA probe (<i>BmorCPH24</i>)	sense	TAATACGACTCACTATAGGGAGA	GCGGTGTAGTCGTAGGTGGAAG
		TCAGAGTCCTTGTGCGATTG	
	antisense	TCAGAGTCCTTGTGCGATTG	TAATACGACTCACTATAGGGAGA
			GCGGTGTAGTCGTAGGTGGAAG
Primers for dsRNA synthesize	<i>dsBmorCP</i>	TAATACGACTCACTATAGGGAGA	TAATACGACTCACTATAGGGAGA
	<i>H24</i>	TCAGAGTCCTTGTGCGATTG	GCGGTGTAGTCGTAGGTGGAAG
	<i>dsRed</i>	TAATACGACTCACTATAGGGAGA	TAATACGACTCACTATAGGGAGA
		CTTCAAGGTGCGCATGGAG	TGTGGATCTGCCCTTCAG
Primers for plasmid construction		CGCGGATCCATGTACACACT	ATTTGCGGCCGCTCACTTCT
	<i>BmCPH24</i>	CAGAGTCCTT	TGTAGTAGACG
	(<i>Bam</i> H <i>I</i> , <i>Not</i> <i>I</i>)		

Table S2. Primers used for quantitative RT-PCR

Gene ID	Forward primer (5'-3')	Reverse primer (5'-3')
<i>BGIBMGA011719</i>	CCATCGCATCAACAAACGGA	ACAGGATTTTCGGTCTGGTATT
<i>BGIBMGA011718</i>	GGCATTGAGAATAACTTGGTC	GCTAAGAGGTGAGTAGGCAGGA
<i>BGIBMGA011762</i>	TGTTTATCTCACCTGTGTCCTTG	AATAGTAACGACGGAGGTAGTAAGC
<i>BGIBMGA011763</i>	ATTGTTCATCTTCGCTTGCTTC	GCGTAGGGTGATGCGTATGT
<i>BGIBMGA011717/16/15</i>	ACTGTTATCTCGCTTGCTTC	GCGTAGGGTGAGGCGTATGT
<i>BGIBMGA011764</i>	CAGTCAGTCTCGACGAACAAAC	CTAAGAGTATGGTGATGCGTATGAGT
<i>BGIBMGA011765</i>	TCAGAGTCCTTGTGCGATTG	GCGGTGAGTCGTAGGTGGAAG
<i>BGIBMGA011766</i>	GATTCTGTTACCCCTGCCACT	CAACGGAGGTGGTGAGAGC
<i>BGIBMGA011767</i>	TCTCGCCTCGCCACATT	AGCGTAGGACGACAGAGGGTAG
<i>BGIBMGA011768</i>	ATCCGTATGTGGTGCCTCTG	GCGTATGCGATTGTGGGT
<i>BGIBMGA011714</i>	ACCTGAGCCAGTGCTGACCT	GGCGTAATAAGAAGAGTAAGCGTAAG
<i>BGIBMGA011713</i>	GAACTTACGGTGGCTATGCTGC	TGTTGCCGAGGTTGTATGGAGA
<i>BGIBMGA011769</i>	CATTGTCGTTTCTCGCTC	ATAATCGTAGTCCAGGCTCGTGT
<i>BmLCP17</i>	CCGCCCCAGTGGTCAAAT	GTCGGCAGATGGCTTCCCT
<i>BmLCP18</i>	ACGGAATCAAGGCTCAGGAA	GCGTAGGTCACAGCGTAAGAAC
<i>BmLCP22</i>	ACTCCTACTCCGTTCTGCCTATC	CGTTACCGTTGGTTTCGT
<i>BmLCP30</i>	CTTCGTCGGAACCAAGGAGG	TGATGCCGTATTGTAGTCGTAGC
<i>BmWnt1</i>	GCGGTCTCACGGCAACCTC	TCTGCCGGCTCGTTGTTGTG
<i>BmICE</i>	AGTATTGCTGCCGACCAA	TAAGACGCCCTGCTTCAC
<i>BmICE2</i>	TCTGTTGACGGTTATCTTC	TATTGTTGGTCTCCTGACAT
<i>BmBuffy</i>	TCAGCTATGCTACGCTCAGACA	ATCCATGATCCAGGCTCCTC
<i>RpL3</i>	CGGTGTTGGATACATTGAG	GCTCATCCTGCCATTCTTACT