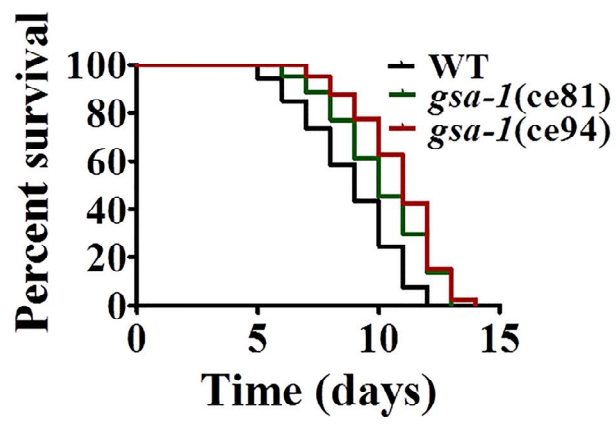
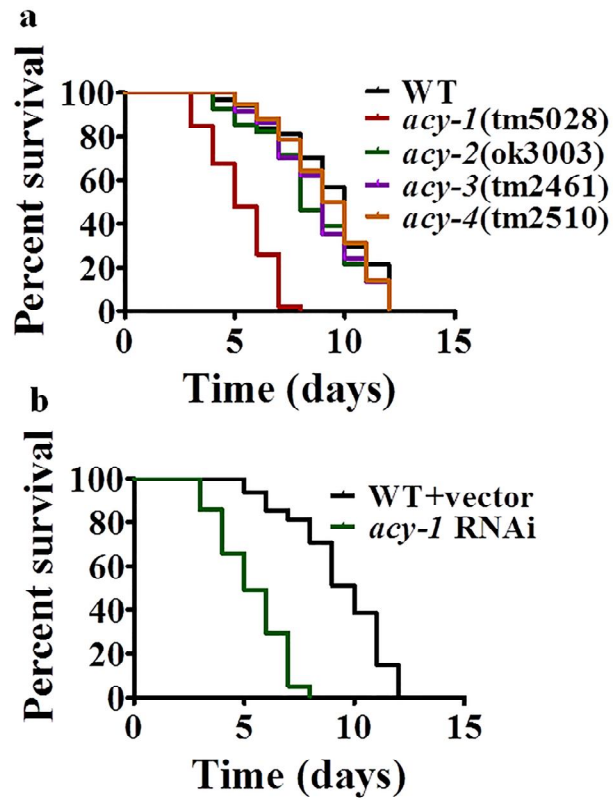


The cAMP-PKA pathway-mediated fat mobilization is required for cold tolerance in *C. elegans*

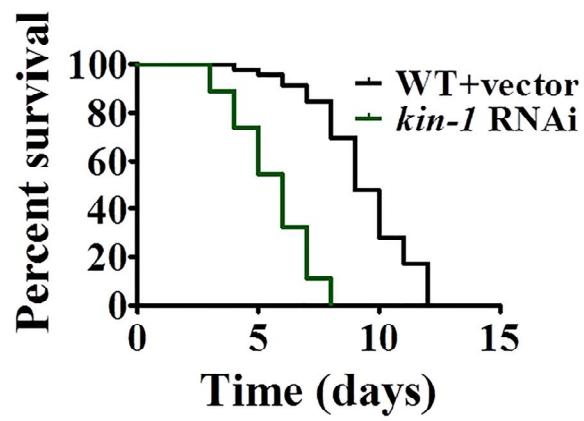
Fang Liu, Yi Xiao, Xing-Lai Ji, Ke-Qin Zhang, Cheng-Gang Zou



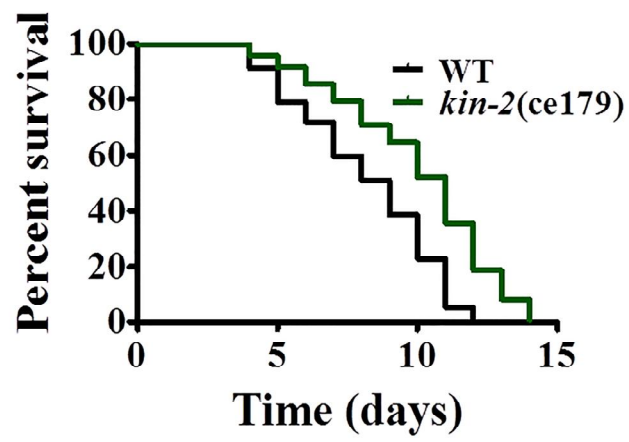
Supplementary Figure 1. Mutations in *gsa-1(ce94)* and *gsa-1(ce81)* promote survival of worms under cold conditions. $P < 0.01$ relative to wild type (WT) worms.



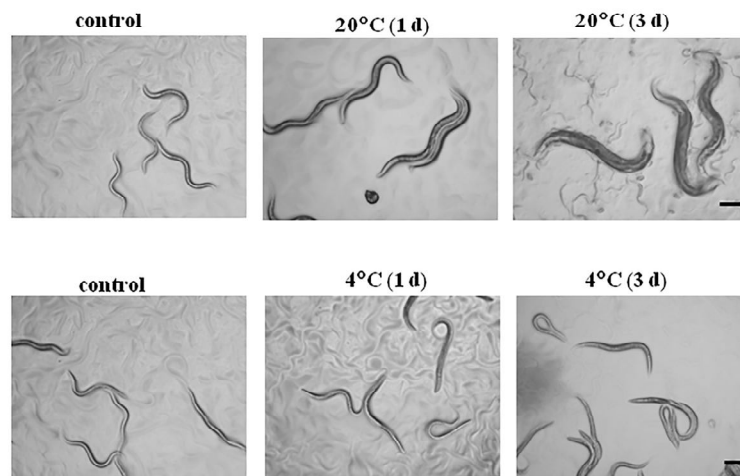
Supplementary Figure 2. Mutation or knockdown of *acy-1* reduces survival of worms under cold conditions. (a) $P < 0.001$ relative to wild type (WT) animals. (b) $P < 0.01$ relative to WT with empty vector.



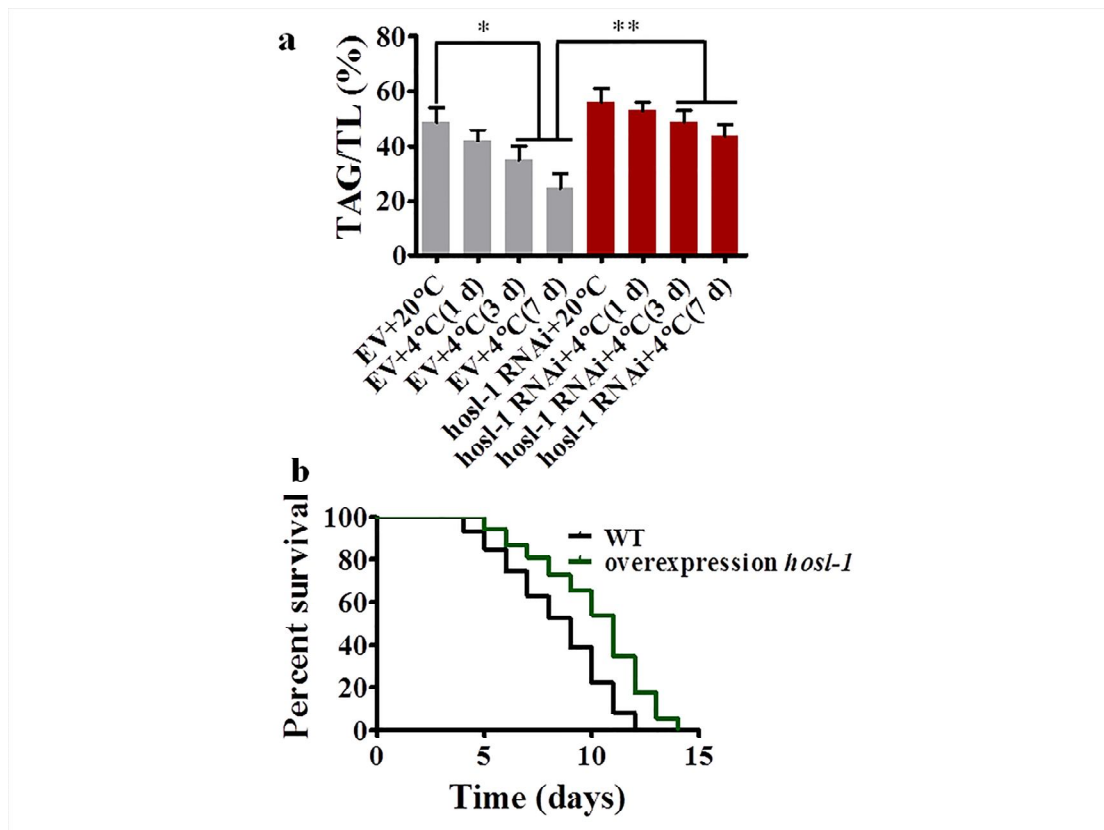
Supplementary Figure 3. Knockdown of *kin-1* by RNAi reduces survival of worms under cold conditions. $P < 0.01$ relative to wild type (WT) animals with empty vector.



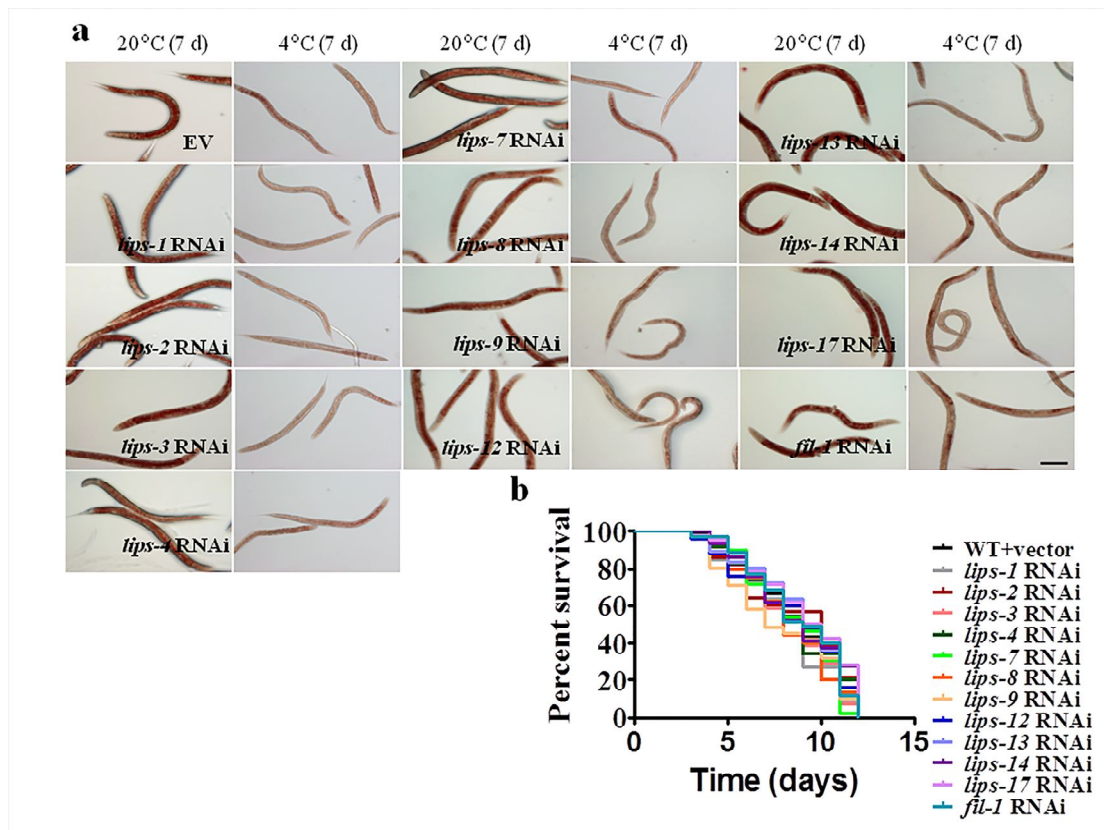
Supplementary Figure 4. Mutation in *kin-2(ce179)* promotes survival of worms under cold conditions. $P < 0.01$ relative to wild type (WT) worms.



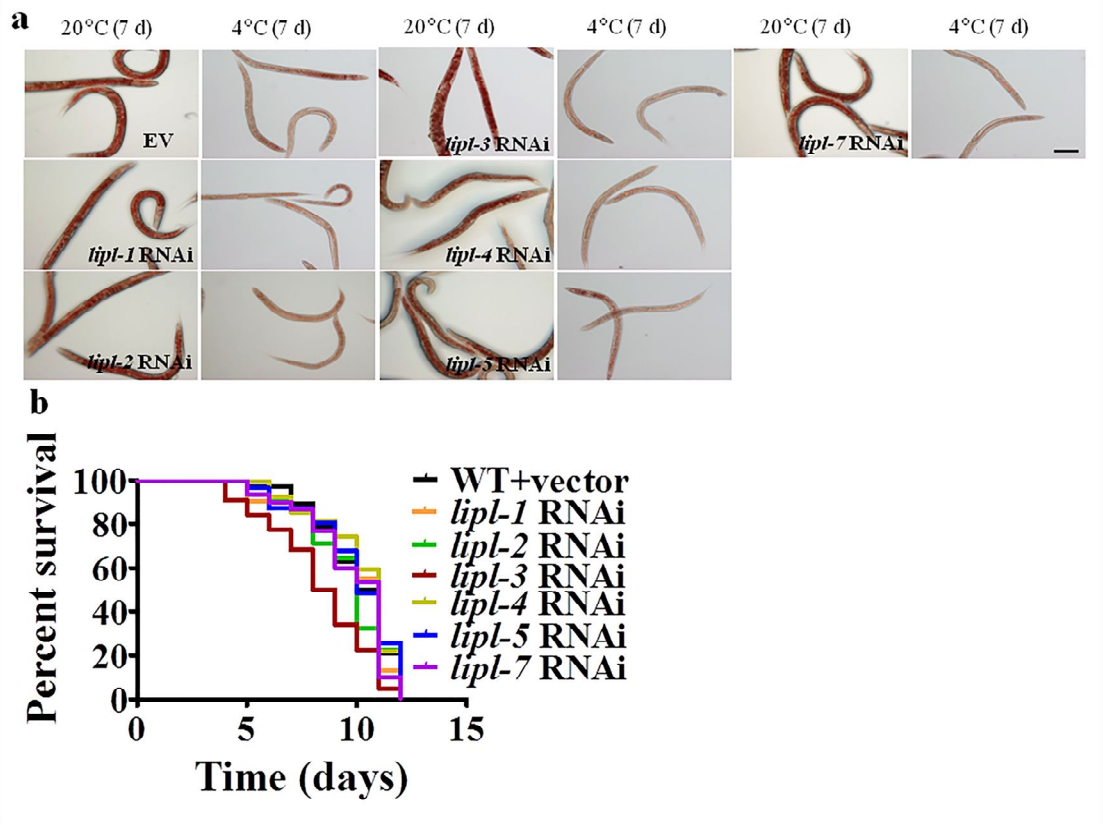
Supplementary Figure 5. Exposure to chill (4 °C) resulted the defects in egg production. Scale bar, 100 μ m.



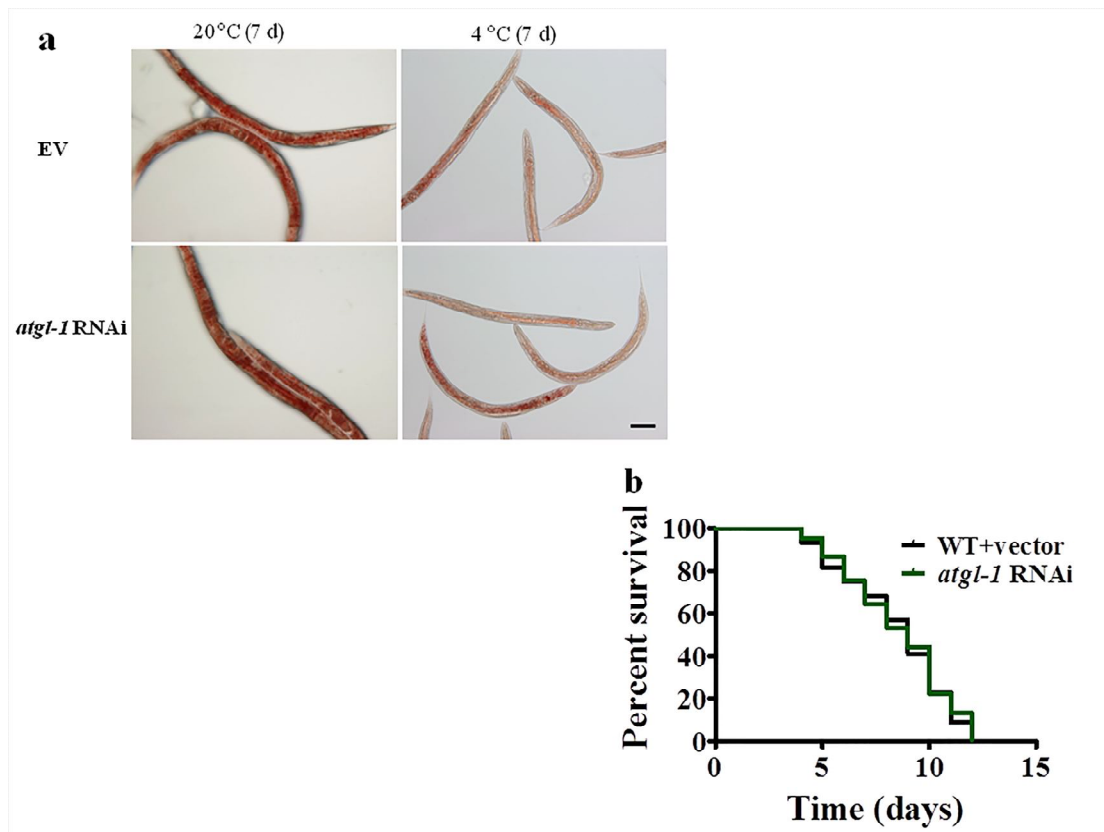
Supplementary Figure 6. Knockdown of *hosl-1* by RNAi inhibits cold-induced lipid hydrolysis. (a) The relative triacylglycerol contents were determined by GC-MS. * $P < 0.05$; ** $P < 0.01$. **Overexpression *hosl-1* in worms exhibited enhanced resistance to cold stress.** (b) $P < 0.01$ relative to wild type (WT) worms.



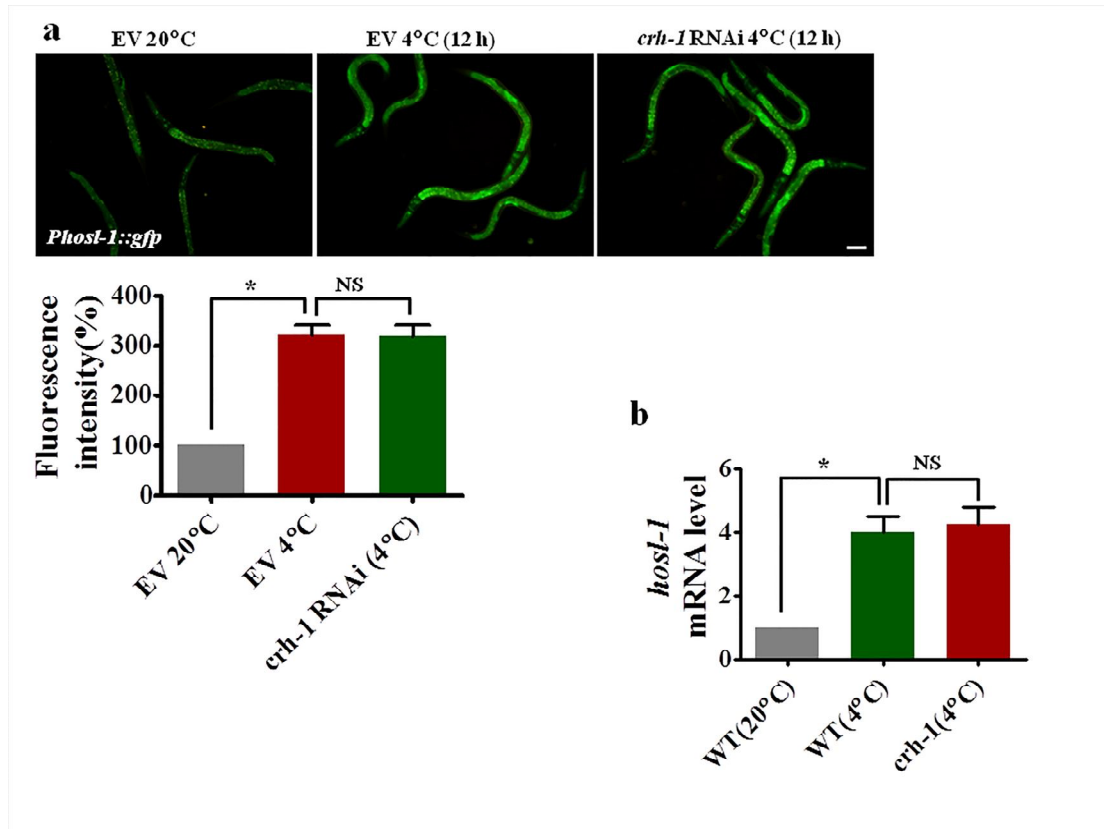
Supplementary Figure 7. Twelve class II lipase genes are not involved in lipid hydrolysis and cold tolerance. (a) Lipid content was determined by staining with Oil Red O. Scale bar, 150 μ m. (b) Knockdown of these genes by RNAi did not influence survival of worms at 4°C.



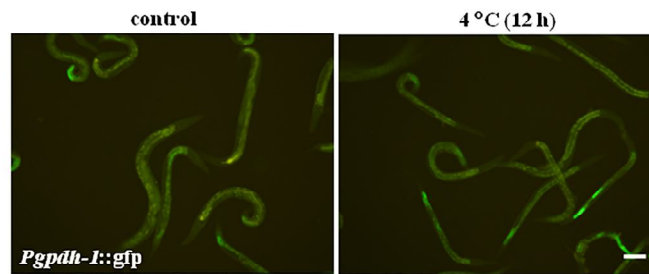
Supplementary Figure 8. Six lipase-related genes are not involved in lipid hydrolysis and cold tolerance. (a) Lipid content was determined by staining with Oil Red O. Scale bar, 150 μ m. (b) Knockdown of these genes by RNAi did not influence survival of worms at 4°C.



Supplementary Figure 9. *atgl-1* is not involved in lipid hydrolysis and cold tolerance. (a) Lipid content was determined by staining with Oil Red O. Scale bar, 100 μm . (b) Knockdown of *atgl-1* by RNAi did not influence survival of worms at 4°C.



Supplementary Figure 10. CRH-1 is not involved in up-regulation of *hosl-1* expression mediated by KIN-1. (a) Knockdown of *crh-1* by RNAi did not influence the expression of *Phosl-1::gfp* at 4°C. Scale bar, 100 μ m. (b) The mutation in *crh-1*(*tz2*) did not influence the mRNA levels of *hosl-1* at 4°C. * $P < 0.05$; NS, not significant.



Supplementary Figure 11. Cold stress does not influence the expression of *gpdh-1*.

The expression of *Pgdh-1::gfp* was not altered in worms exposed to cold (4 °C).

Scale bar, 100 μ m.

Supplementary Table 2. The efficiency of RNAi is detected by quantitative RT-PC.

Gene	The efficiency of RNAi
<i>gsa-1</i>	85%
<i>acy-1</i>	80%
<i>kin-1</i>	88%
<i>hosl-1</i>	81.5%
<i>atgl-1</i>	90%
<i>lipl-1</i>	75%
<i>lipl-2</i>	78%
<i>lipl-3</i>	90%
<i>lipl-4</i>	82%
<i>lipl-5</i>	78%
<i>lipl-7</i>	70%
<i>lips-1</i>	85%
<i>lips-2</i>	80%
<i>lips-3</i>	90%
<i>lips-4</i>	89%
<i>lips-7</i>	77%
<i>lips-8</i>	87%
<i>lips-9</i>	92%
<i>lips-12</i>	72%
<i>lips-13</i>	79%
<i>lips-14</i>	84%
<i>lips-17</i>	91%
<i>fil-1</i>	80%
<i>crh-1</i>	75%

Supplementary Table 3. Primer sequences used for quantitative RT-PC.

Gene	Sequences (5'→3')	Product length (bp)
<i>gsa-1-F</i> <i>gsa-1-R</i>	TGCTGGAAAGACAAGGGAGT ATCGTAGTTGTTCTGTGCGCAC	117
<i>acy-1-F</i> <i>acy-1-R</i>	GCCGCTCAATACCAAAGGTT GCAACTGGAGTCATAAACGC	119
<i>kin-1-F</i> <i>kin-1-R</i>	GAAGGACAACAAGAACTCGGC GAATGATCCGGTTCCAAGG	145
<i>hosl-1-F</i> <i>hosl-1-R</i>	GGCTCGCTCATCAACACTGG CACCATTTCTCCACTCTTCC	231
<i>atgl-1-F</i> <i>atgl-1-R</i>	CGGGTTGTGGATTCTTTGTG TGAGACCACAGGCAACGAT	126
<i>lipl-1-F</i> <i>lipl-1-R</i>	TCCTGGAGCACGGTTATGTT TTTGTGGCGTGGTCACTTC	94
<i>lipl-2-F</i> <i>lipl-2-R</i>	ACGGACAAGATACACCACCA TCTCCAAGCCAATCATCGTCA	91
<i>lipl-3-F</i> <i>lipl-3-R</i>	CTGTGACAATGTGTGCTTCCT TCCGTGCCGAACCATCTGAA	142
<i>lipl-4-F</i> <i>lipl-4-R</i>	GATGTGAATAATTCCCCTCC GCCCAACCACACATCAAACC	135
<i>lipl-5-F</i> <i>lipl-5-R</i>	GAGTGCTGGTTTCTGTTCG TCCCAACTCCAGTCCCAGAA	129
<i>lipl-7-F</i> <i>lipl-7-R</i>	CAAATAGCCGTGGAACCTCCTG CCAAATCCACACTCGCTGTC	123
<i>lips-1-F</i> <i>lips-1-R</i>	TCGGACCCAACCTATCGCAT AGTCATTTCTGGGCAGGTGC	146
<i>lips-2-F</i> <i>lips-2-R</i>	TCCCAGGCAGCAACATTTG TTGGTCCGCTGCTGTTTCA	77
<i>lips-3-F</i> <i>lips-3-R</i>	AAACTGTTCCCCTTGGTGC AGCGACCGTTCATTCCTGGA	258
<i>lips-4-F</i> <i>lips-4-R</i>	GTGGCAAAGTTGATGTGTTTCG ATAGCCTCCACACGAATCTCC	137
<i>lips-7-F</i> <i>lips-7-R</i>	CCTTCCTGTCAGTTGCTGGT CCATCACGCTTCACAAATCCA	253
<i>lips-8-F</i> <i>lips-8-R</i>	TTTGTTGGTGTGCTGGGC CAATCCGATAACCCTGCTGC	120
<i>lips-9-F</i> <i>lips-9-R</i>	TCGGTTTCTCGCTTGGTGT TGTCAGCGGTTCTCCAAGGT	92
<i>lips-12-F</i> <i>lips-12-R</i>	GAAACCGTCTACGCAACATCA CCCTTCTCCATACGCCACAAT	156
<i>lips-13-F</i> <i>lips-13-R</i>	GGGCAATACAACCGACACTTC GTGCCACAATATCAACCTGA	122
<i>lips-14-F</i> <i>lips-14-R</i>	CATACAGCACAACCTCACACCT TGAACCTTCGTCCGGTAGGCAA	109

<i>lips-17-F</i> <i>lips-17-R</i>	TGTATCTGTTGCTGGAGCC CTTTATCGTCTCCTGGTCCGT	176
<i>fil-1-F</i> <i>fil-1-R</i>	GGAGTTACCCTTGCCAGAAAG AGACCCAAATCACAGGAACC	74
<i>crh-1-F</i> <i>crh-1-R</i>	CGCCGTTGATGATGCTCTTG TCACCTTATCCGCCGTTTCT	141