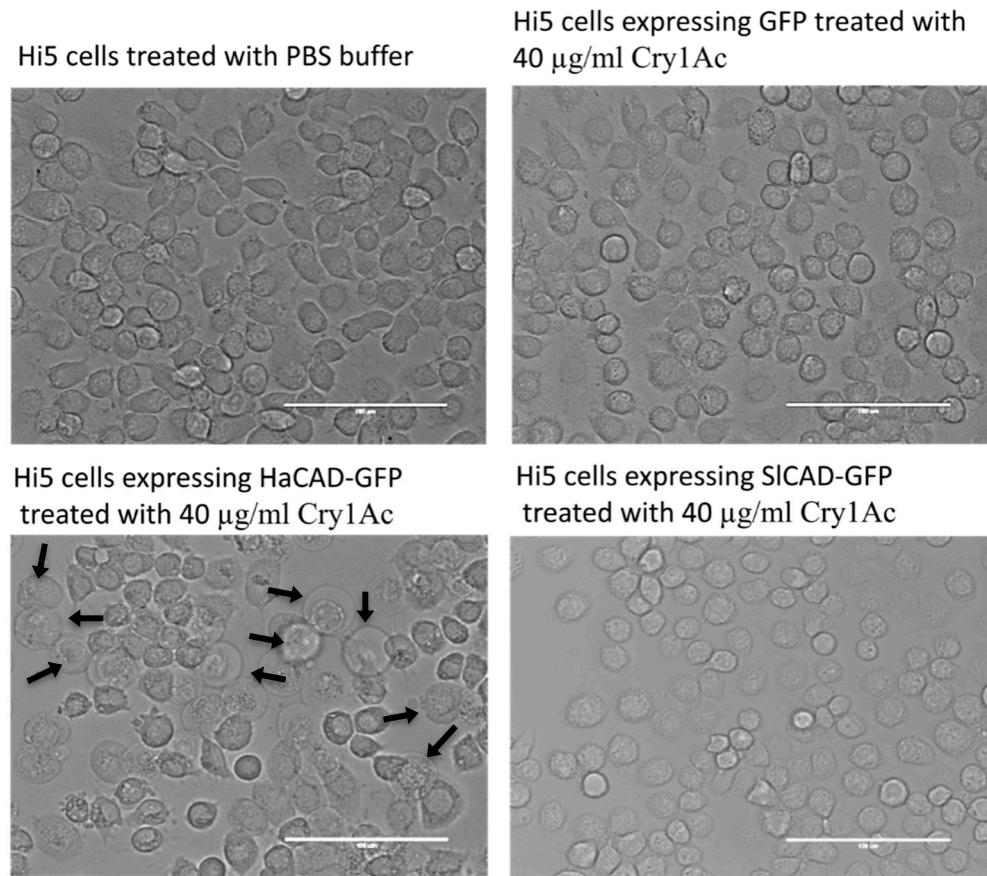
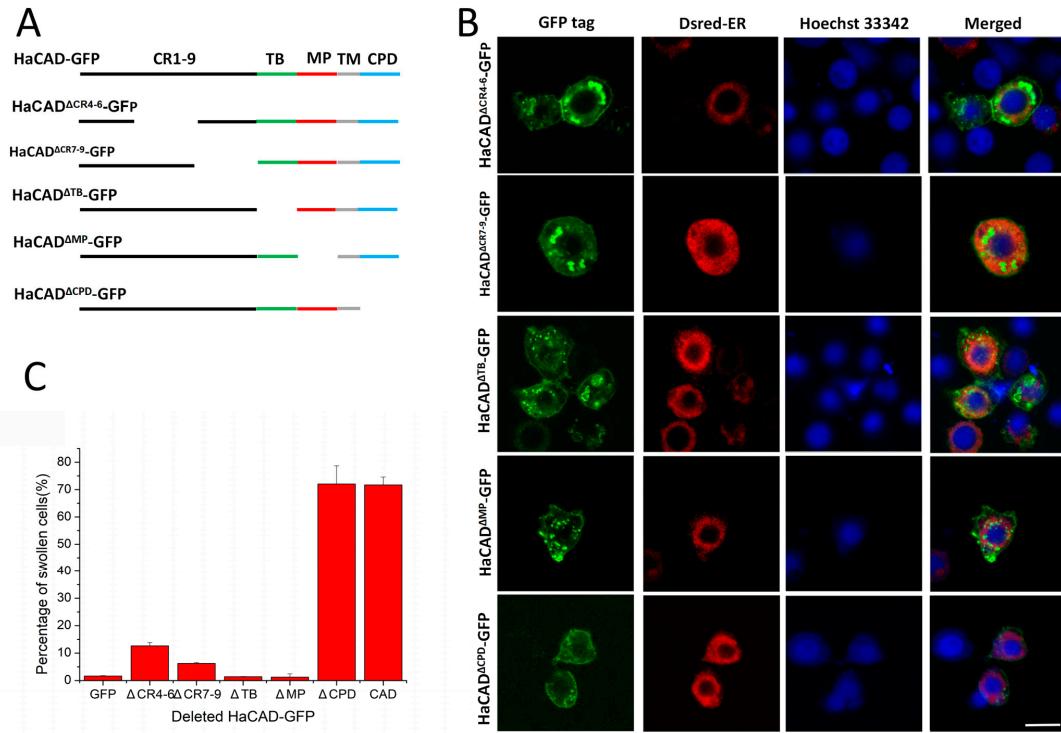


## Supplementary data: The cadherin Cry1Ac binding-region is necessary for the cooperative effect with ABCC2 transporter enhancing insecticidal activity of Cry1Ac toxin from *Bacillus thuringiensis*

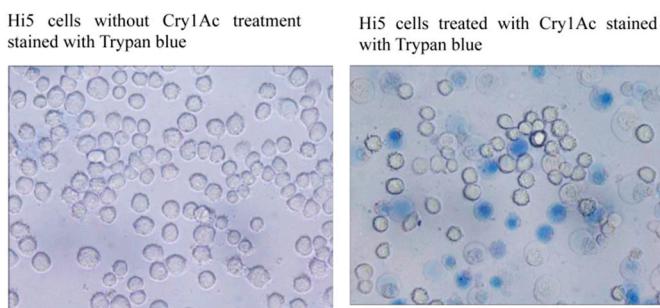
Yuemin Ma, Jianfeng Zhang, Yutao Xiao, Yanchao Yang, Chenxi Liu, Rong Peng, Yongbo Yang, Alejandra Bravo, Mario Soberón and Kaiyu Liu



**Figure S1.** Images of Hi5 cells expressing GFP or GFP tagged CAD with Cry1Ac treatment. The Hi5 cells were treated with or without Cry1Ac at the indicated concentration for 1 h after they had been transfected for 24 h. Only Hi5 cells expressing HaCAD-GFP treated with a 40 µg/mL concentration of Cry1Ac showed cell swelling (arrows). Bar, 100 µm.



**Figure S2.** Construction of CAD proteins deleted of some regions. **A**, shows a diagram explaining the different deleted proteins that were constructed. **B**, shows the localization of these constructions observing GFP fluorescence. Only HaCAD-GFP<sup>ΔCPD</sup> localized in the membrane. The rest of the deleted CAD proteins were localized mainly in cytoplasm. The endoplasmic reticulum was labeled with Dsred-ER and nuclei were stained with Hoechst 33342 (1 µg/mL). **C**, shows percentage of swollen cells after treatment with the different deletion CAD constructions with 40 µg/mL Cry1Ac for 1 h. The number of the cells emitting green fluorescence was about 300 to 800 in each group. Bar, 20 µm.



**Figure S3.** Staining with Trypan blue showing that the swollen cells were dead. Hi5 cells expressing HaCAD-GFP were cultured into 24-well plates and then treated with PBS buffer or Cry1Ac at 40 µg/mL for 1 h, the solution containing toxin was removed and the Trypan blue solution (0.4%, w/v) was added into wells and incubated for 5 min. The cells were observed and photographed under optical microscope.

**Table S1.** Cytotoxicity of Cry1Ac mediated by Cry1Ac receptors with different tags.

|                         | Percentage of swollen cells at 40 µg/mL (%) | Slope              | EC <sub>50</sub> (µg/mL) | 95% CI      | x <sup>2</sup> | df |
|-------------------------|---|--------------------|--------------------------|-------------|----------------|----|
| HaCAD-GFP               | N <sup>a</sup>                              | 1.484(1.189–1.778) | 7.223                    | 5.887–8.686 | 1.766          | 3  |
| HaABCC2-GFP             | N   | 1.749(1.442–2.055) | 0.127                    | 0.108–0.149 | 3.996          | 3  |
| HaCAD-GFP+HaABCC2-GFP   | N   | 2.219(1.860–2.578) | 0.010                    | 0.006–0.014 | 7.126          | 3  |
| HaCAD-Flag              | 29.02 ± 10.7                                | N                  | N                        | N           | N              | N  |
| HaABCC2-Flag            | N   | 1.698(1.395–2.001) | 0.267                    | 0.234–0.326 | 1.906          | 3  |
| HaCAD-Flag+HaABCC2-Flag | N   | 2.439(2.076–2.802) | 0.013                    | 0.012–0.015 | 1.971          | 3  |

<sup>a</sup> N, not tested. When the GFP tag was replaced with Flag tag, the expression levels of the fusion HaCAD-Flag decreased. The EC<sub>50</sub> of Cry1Ac in cells transfected with HaCAD-Flag can not be calculated since the percentage of cell swelling treated with maximum Cry1Ac concentration of 40 µg/mL for 1 h was 29%.

**Table S2.** Influences of the deletion of domains of HaCAD on cytotoxicity of Cry1Ac on Hi5 cells.

| Protein                    | EC <sub>50</sub> (µg/mL) | 95% of CI (µg/mL) | Slope | x <sup>2</sup> | df |
|----------------------------|--------------------------|-------------------|-------|----------------|----|
| GFP                        | >40 <sup>a</sup>         | N                 | N     | N              | N  |
| HaCAD-GFP                  | 7.35                     | 6.23–8.59         | 3.11  | 2.90           | 3  |
| HaCAD <sup>ΔCPD</sup> -GFP | 8.35                     | 7.11–9.75         | 3.11  | 3.16           | 3  |

<sup>a</sup> The EC<sub>50</sub> of Cry1Ac in cells transfected with GFP can not be calculated since the percentage of the aberrant Hi5 cells treated with Cry1Ac at 40 µg/mL for 1 h were less than 5%. N, not determined. The number of the cells emitting green fluorescence was about 300 to 800 in each group.

**Table S3.** The potentiation effect on Cry1Ac toxicity when the deleted HaCAD-GFP proteins were co-expressed with HaABCC2-GFP.

| Protein                                  | EC <sub>50</sub> (µg/mL) | 95% CI (µg/mL) | Slope | x <sup>2</sup> | df | Synergism |
|--|--------------------------|----------------|-------|----------------|----|-----------|
| GFP                                      | >40 <sup>a</sup>         | N              | N     | N              | N  | N         |
| GFP+HaABCC2-GFP                          | 0.28                     | 0.23–0.34      | 3.00  | 0.63           | 3  | —         |
| HaCAD-GFP+ HaABCC2-GFP                   | 0.01                     | 0.01–0.02      | 3.33  | 11.17          | 3  | +         |
| HaCAD <sup>ΔCR4-6-</sup> GFP+HaABCC2-GFP | 0.06                     | 0.05–0.06      | 3.95  | 1.80           | 3  | +         |
| HaCAD <sup>ΔCR7-9-</sup> GFP+HaABCC2-GFP | 0.06                     | 0.04–0.09      | 3.35  | 6.00           | 3  | +         |
| HaCAD <sup>ΔTB-</sup> GFP+HaABCC2-GFP    | 0.27                     | 0.21–0.36      | 5.25  | 6.02           | 3  | —         |

|   |      |           |      |       |   |   |
|---|------|-----------|------|-------|---|---|
| HaCAD <sup>ΔAMP-</sup><br>GFP+HaABCC2-GFP | 0.22 | 0.15–0.32 | 3.96 | 7.04  | 3 | — |
| HaCAD <sup>ΔCPD-</sup><br>GFP+HaABCC2-GFP | 0.02 | 0.01–0.05 | 2.85 | 12.81 | 3 | + |

<sup>a</sup> The EC<sub>50</sub> of Cry1Ac in cells transfected with GFP can not be calculated since the percentage of the aberrant Hi5 cells treated with Cry1Ac at 40 µg/mL for 1 h was less than 5%. N, not determined; −, no synergism; +, synergism. The number of the cells emitting green fluorescence was 300 to 800 in each group.

**Table S4.** Transfection efficiency of Hi5 and Sf9 cells.

| plasmid      | Hi5 (M ± SD)%           | Sf9 (M ± SD)%           |
|--------------|-------------------------|-------------------------|
| pHaCAD-GFP   | 63.1 ± 3.4 <sup>a</sup> | 33.7 ± 5.1 <sup>b</sup> |
| pSiCAD-GFP   | 60.8 ± 1.9 <sup>a</sup> | 32.6 ± 5.0 <sup>b</sup> |
| pHaABCC2-GFP | 54.5 ± 7.9 <sup>a</sup> | 31.6 ± 2.0 <sup>b</sup> |

N = 250–280 cells each group. The positive transfected cells emitted green fluorescence under fluorescence microscope. The different upper-letters showed significant differences in the same row. M ± SD, mean ± standard deviation.

**Table S5.** Analysis of the CAD and ABCC2 expression in Hi5 cells by RT-qPCR.

| Gene        | Expression level (M±SD)                     |
|-------------|---|
| TnCAD       | (6.6 ± 1.2) × 10 <sup>-5</sup> <sup>a</sup> |
| TnABCC2     | 0 <sup>b</sup>                              |
| HaCAD-GFP   | 5.4 ± 2.1 <sup>c</sup>                      |
| HaABCC2-GFP | 3.4 ± 0.7 <sup>c</sup>                      |

HaCAD-GFP and HaABCC2-GFP were co-expressed in Hi5 cells through co-transfection using the two plasmids. The expression levels of four genes were quantified by real time RT- qPCR using the method of 2<sup>-ΔCT</sup> at 36 h post co-transfection. *T. ni rps3A* gene was used as an internal gene and its expression level was set as 1. The primers were listed in table S15. The different upper-letters showed significant differences in the same row. M ± SD, mean ± standard deviation.

**Table S6.** Sequence of primers used for cloning CAD proteins genes from different lepidopteran.

| Primer  | Sequence (5'-3')  | Expressed protein |
|---------|---|-------------------|
| SiCAD-F | AGATCTCGAGCTCAAGCTTGGGCCACCATGGCGCTTGATGTGCGAT          | SiCAD-GFP         |
| SiCAD-R | GGTGGCGACCGGTGGATCACCTCCGCCACCGCCTCACTTTAAACTGGGAGTTCAC |                   |
| HaCAD-F | AGATCTCGAGCTCAAGCTTGGGCCACCATGGCAGTCGACGTGAGAA          | HaCAD-GFP         |
| HaCAD-R | GGTGGCGACCGGTGGATCACCTCCGCCACCGCCTCTCTGAAGTGGTTCGC      |                   |
| SeCAD-F | AGATCTCGAGCTCAAGCTTGGGCCACCATGGCGCTGGACGTGCGAATACT      | SeCAD-GFP         |
| SeCAD-R | GGTGGCGACCGGTGGATCACCTCCGCCACCGCCTCTCTAAACTGGGAGTTCGCG  |                   |

**Table S7.** Sequence of primers used for PCR amplification of the deleted HaCAD fragments.

| Primer                          | Sequence (5'-3')                                 | Expressed protein            |
|---------------------------------|--|------------------------------|
| HaCAD-up-F                      | CCCTCGAGGCCACCATGGCAGTCGACGTGAGAA                | Used for overlapping PCR     |
| HaCAD-down-R                    | TCCCCGGGACCTCCGCCACCGCCTCTTCTGAAGTCGCT<br>GTTCGC | Used for overlapping PCR     |
| HaCAD <sup>ACR4-6</sup> -down-F | GGGATGTTGGTGTATAAGTCGTCACTGAGTGTCTCC             | HaCAD <sup>ACR4-6</sup> -GFP |
| HaCAD <sup>ACR4-6</sup> -up-R   | GACTTATCACCAACATCCGGTCTCGCCAC                    |                              |
| HaCAD <sup>ACR7-9</sup> -down-F | GTGTGACCTCTCACTACGATTCTGCTACGG                   | HaCAD <sup>ACR7-9</sup> -GFP |
| HaCAD <sup>ACR7-9</sup> -up-R   | AGT GAGGAGGT CAC ACT TGT CACT AGCT ATG           |                              |
| HaCAD <sup>ATB</sup> -down-F    | CGAGAGTTCCAGGTGATATCGGACCGCAACCG                 | HaCAD <sup>ATB</sup> -GFP    |

|                              |   |                           |
|------------------------------|---|---------------------------|
| HaCAD <sup>ATB</sup> -up-R   | CACCTGGAACTCTCGGAAGTCTTCAGGGAAAG                  |                           |
| HaCAD <sup>AMP</sup> -down-F | GAAGTGAAAGGTGTACCGAGCTCTGTACCGCGCTGG              | HaCAD <sup>AMP</sup> -GFP |
| HaCAD <sup>AMP</sup> -up-R   | GTACACCTTCACTTCCGTGCCGGCAGTTTC                    |                           |
| HaCAD <sup>ACP</sup> -down-F | CCCTCGAGGCCACCATGGCAGTCGACGTGAGAA                 | HaCAD <sup>ACP</sup> -GFP |
| HaCAD <sup>ACP</sup> -up-R   | GGACCGCGGTGCCCTCCACCGCCAGTCCTAACAAAGAAC<br>ACAATG |                           |

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Note: The two fragments (up-fragment and down-fragment) were amplified. Then the overlapping PCR was carried out using the mixture of up-fragment and down-fragment as described previously [1].

**Table S8.** Sequence of primers used for amplification of short inserted fragments of *SICAD*.

| Primer                     | Sequence (5'-3')                             | Expressed protein            |
|----------------------------|--|------------------------------|
| HaCAD <sup>SICR</sup> -F   | ACAGTTGAGTTGACGATTCTGTCTACGGACG              | HaCAD <sup>SICR</sup> -GFP   |
| HaCAD <sup>SICR</sup> -R   | CCGCCACCGCCTCCTCTGAACCTGCGTTCGC              |                              |
| HaCAD <sup>SITB</sup> -F   | AGAGTTCCAGGTGGTATTCGTGCCACGGATG              | HaCAD <sup>SITB</sup> -GFP   |
| HaCAD <sup>SITB</sup> -R   | TGCGGTCCGATATCATGTAGATTATAACTTT              |                              |
| HaCAD <sup>SIMP</sup> -F   | GAAGGTGTACCTGATATCGACACGTGTCAGAG             | HaCAD <sup>SIMP</sup> -GFP   |
| HaCAD <sup>SIMP</sup> -R   | CCGCCAGCGCGTAAACGCCAGGGCGTAATCT              |                              |
| HaCAD <sup>SITM</sup> -F   | GGCGCGAGCTCTGTATATCCTCGCCGGTATCG             | HaCAD <sup>SITM</sup> -GFP   |
| HaCAD <sup>SITM</sup> -R   | TTAGTGTCTAGTCTGATGAAGAAAGCGATG               |                              |
| HaCAD <sup>SICPD</sup> -F  | GTTCTTGTTAGGAACCGAACGTTGAATGCC               | HaCAD <sup>SICPD</sup> -GFP  |
| HaCAD <sup>SICPD</sup> -R  | CCGCCACCGCCTCCACTTTAAACTGGGAGTTCAC           |                              |
| HaCAD <sup>SICR9</sup> -F  | ACTGGCTACGGTAGACGGAGAGTCCTCGACCGACTCTCGCTA   | HaCAD <sup>SICR9</sup> -GFP  |
| HaCAD <sup>SICR9</sup> -R  | ACATGATGTTCTCGGGTCCCTCGGCTTGAGGCAGCTCGAACCTC |                              |
| HaCAD <sup>SICR10</sup> -F | GGAACCTACCTCGCCCTCAGACCCAAGAACTACAGGTGTACGG  | HaCAD <sup>SICR10</sup> -GFP |
| HaCAD <sup>SICR10</sup> -R | GATTCTTCGATGAAATCTGTGTAAAGATGCCAGCTGTAA      |                              |
| HaCAD <sup>SICR11</sup> -F | AGCTGGCATCTGCTGGCGATAACGTACACAGGGATCTCATTT   | HaCAD <sup>SICR11</sup> -GFP |
| HaCAD <sup>SICR11</sup> -R | CTCGGTTGCGGTCCGATATCATGTAGATTATAACTTTGCTCGA  |                              |

Note: The short inserted fragment and the following long vector fragment were used for gene fusion [2].

**Table S9.** Sequence of primers used for amplification of long vector fragments of *HaCAD*.

| Primer                     | Sequence (5'-3')                            | Expressed protein            |
|----------------------------|---|------------------------------|
| HaCAD <sup>SICR</sup> -F   | GCGAACACCGCAGTTCAGAAGAGGGAGGCGGTGGCG        | HaCAD <sup>SICR</sup> -GFP   |
| HaCAD <sup>SICR</sup> -R   | TAGCACGAATCGTCAACTCAAACCTGTTGGACA           |                              |
| HaCAD <sup>SITB</sup> -F   | TATAATCTACATGATATCGGACCGAACCGAG             | HaCAD <sup>SITB</sup> -GFP   |
| HaCAD <sup>SITB</sup> -R   | TGGCACGAATAACCACCTGAACTCTCGGAAG             |                              |
| HaCAD <sup>SIMP</sup> -F   | GTGTAACATAGACCAGACGTGGTGGGCCAGCG            | HaCAD <sup>SIMP</sup> -GFP   |
| HaCAD <sup>SIMP</sup> -R   | CACGTGTCGATATCAGGTACACCTTCACTTCC            |                              |
| HaCAD <sup>SITM</sup> -F   | TTTCTTCATCAGGACTAGGACACTAACCGCT             | HaCAD <sup>SITM</sup> -GFP   |
| HaCAD <sup>SITM</sup> -R   | CGGCGAGGATATACTAGAGCTCGCCCTGCGCG            |                              |
| HaCAD <sup>SICPD</sup> -F  | GTGAACTCCCAGTTAAAGTGGAGGCCGTGGCG            | HaCAD <sup>SICPD</sup> -GFP  |
| HaCAD <sup>SICPD</sup> -R  | TCAACGTTCGGTTCTAACAAAGAACACAATG             |                              |
| HaCAD <sup>SICR9</sup> -F  | CGAGCTGCCCAAGCCGAGGACCCGAGGAACATCATGTGTGAAG | HaCAD <sup>SICR9</sup> -GFP  |
| HaCAD <sup>SICR9</sup> -R  | AGAGTCGGTCGAGGAACCTCCGTCTACCGTAGCCAGTACCG   |                              |
| HaCAD <sup>SICR10</sup> -F | TGCTGGCATCTTACACAGATTCTACGAAAGAAATCTGTGA    | HaCAD <sup>SICR10</sup> -GFP |
| HaCAD <sup>SICR10</sup> -R | ACCTGTAGTTCTGGGGTCTGAGGCCGAGGTAGTCCGCCCTC   |                              |
| HaCAD <sup>SICR11</sup> -F | AAAAGTTATAATCTACATGATATCGGACCGAACCGAGTGTCT  | HaCAD <sup>SICR11</sup> -GFP |
| HaCAD <sup>SICR11</sup> -R | GATCCCTGTGTACGTTATGCCACCGAGAGATGCCAGCTGTAG  |                              |

Note: The short inserted fragment and the following long vector fragment were used for gene fusion [2].

**Table S10.** Sequence of primers used primers for amplification of short inserted fragments of *HaCAD*.

| Primer  | Sequence (5'-3')                           | Expressed protein              |
|---|--|--------------------------------|
| SICAD <sup>HaCR</sup> -F  | AGAGTTCCAGGTGGTTATCGTGCCACGGATG            | SICAD <sup>HaCR</sup> -GFP     |
| SICAD <sup>HaCR</sup> -R  | CCGCCACCGCCTCCACTTTAAACTGGGAGTTCAC         |                                |
| SICAD <sup>HaTB</sup> -F  | ACAGTTGAGTTGACGATTCTGCTACGGACG             | SICAD <sup>HaTB</sup> -GFP     |
| SICAD <sup>HaTB</sup> -R  | CACGTGTCGATATCAGGTACACCTTCACTTCC           |                                |
| SICAD <sup>HaMP</sup> -F  | TATAATCTACATGATATCGGACCGCAACCGAG           | SICAD <sup>HaMP</sup> -GFP     |
| SICAD <sup>HaMP</sup> -R  | CGGGGAGGATATACAGAGCTCGGCCTGCGCG            |                                |
| SICAD <sup>HaTM</sup> -F  | AGATTACGCCCTGGCGGTTACGGCTGGCGGGTAGCTGCGG   | SICAD <sup>HaTM</sup> -GFP     |
| SICAD <sup>HaTM</sup> -R  | GGCGATTCAACGTTGGTTCTAACAAAGAACACAATGAGCAGC |                                |
| SICAD <sup>HaCPD</sup> -F   | CATCGCTTCTCATCAGGACTAGGACACTAAACCGTCGCTTGC | SICAD <sup>HaCPD</sup> -GFP    |
| SICAD <sup>HaCPD</sup> -R   | CCGCCACCGCCTCCTCTGAACACTCGCTGTTCGC         |                                |
| SICAD <sup>HaCR-TB</sup> -F   | GAAGGTGTACCTGATATCGACACGTGTCAGAG           | SICAD <sup>HaCR-TB</sup> -GFP  |
| SICAD <sup>HaCR-TB</sup> -R   | CCGCCACCGCCTCCACTTTAAACTGGGAGTTCAC         |                                |
| SICAD <sup>HaTB-MP</sup> -F   | ACAGTTGAGTTGACGATTCTGCTACGGACG             | SICAD <sup>HaTB-MP</sup> -GFP  |
| SICAD <sup>HaTB-MP</sup> -R   | CGGGGAGGATATACAGAGCTCGGCCTGCGCG            |                                |
| SICAD <sup>HaTB-TM</sup> -F(SICAD <sup>HaTM</sup> was used as a background)   | ACAGTTGAGTTGACGATTCTGCTACGGACG             | SICAD <sup>HaTB-TM</sup> -GFP  |
| SICAD <sup>HaTB-TM</sup> -R   | CACGTGTCGATATCAGGTACACCTTCACTTCC           |                                |
| SICAD <sup>HaTB-CPD</sup> -F(SICAD <sup>HaCPD</sup> was used as a background) | ACAGTTGAGTTGACGATTCTGCTACGGACG             | SICAD <sup>HaTB-CPD</sup> -GFP |
| SICAD <sup>HaTB-CPD</sup> -R  | CACGTGTCGATATCAGGTACACCTTCACTTCC           |                                |

**Table S11.** Sequence of primers used for amplification of long vector fragments of *SICAD*.

| Primer   | Sequence (5'-3')                            | Expressed protein              |
|--|---|--------------------------------|
| SICAD <sup>HaCR</sup> -F   | GTGAACCTCCAGTTAAAAGTGGAGGCCGTGGCGG          | SICAD <sup>HaCR</sup> -GFP     |
| SICAD <sup>HaCR</sup> -R   | TGGCACATAACCACCTGAACTCTCGGAAG               |                                |
| SICAD <sup>HaTB</sup> -F   | GAAGGTGTACCTGATATCGACACCGTGTAGAG            | SICAD <sup>HaTB</sup> -GFP     |
| SICAD <sup>HaTB</sup> -R   | TAGCACGAATCGTCAACTCAAACGTGGACA              |                                |
| SICAD <sup>HaMP</sup> -F   | GGCCGAGCTCTGTATATCCTCGCCGTATGC              | SICAD <sup>HaMP</sup> -GFP     |
| SICAD <sup>HaMP</sup> -R   | TGCGTCCGATATCATGTAGATTATAACTTT              |                                |
| SICAD <sup>HaTM</sup> -F   | CATTGTGTTCTTGTAGAACCGAACGTTGAATCGCCGATCG    | SICAD <sup>HaTM</sup> -GFP     |
| SICAD <sup>HaTM</sup> -R   | CTACCGCCGCCAGCGCGTAAACCGCCAGGGCGTAATCTCCGCG |                                |
| SICAD <sup>HaCPD</sup> -F  | GCGAACACCGCAGTCAGAAGAGGAGGCCGTGGCGG         | SICAD <sup>HaCPD</sup> -GFP    |
| SICAD <sup>HaCPD</sup> -R  | TTAGTGTCCCTAGTCCTGATGAAGAAAGCGATG           |                                |
| SICAD <sup>HaCR-TB</sup> -F  | GTGAACCTCCAGTTAAAAGTGGAGGCCGTGGCGG          | SICAD <sup>HaCR-TB</sup> -GFP  |
| SICAD <sup>HaCR-TB</sup> -R  | CTCTGACACGTGTCGATATCAGGTACACCTCACTCCGTGCGG  |                                |
| SICAD <sup>HaTB-MP</sup> -F (SICAD <sup>HaTM</sup> was used as a background)   | GAAGGTGTACCTGATATCGACACCGTGTAGAG            | SICAD <sup>HaTB-MP</sup> -GFP  |
| SICAD <sup>HaTB-TM</sup> -R  | TAGCACGAATCGTCAACTCAAACGTGGACA              |                                |
| SICAD <sup>HaTB-CPD</sup> -F (SICAD <sup>HaCPD</sup> was used as a background) | GAAGGTGTACCTGATATCGACACCGTGTAGAG            | SICAD <sup>HaTB-CPD</sup> -GFP |
| SICAD <sup>HaTB-CPD</sup> -R   | TAGCACGAATCGTCAACTCAAACGTGGACA              |                                |

**Table S12.** Sequence of primers used for amplification of short inserted fragments of *HevCAD*.

| Primer                              | Sequence (5'-3')                           | Expressed protein                     |
|-------------------------------------|--|---------------------------------------|
| SICAD <sup>HevTB-MP-TM-CPD</sup> -F | CCAACAGTTGAGTTGGTTATCGTGTACGGATGGTGGTACGG  | SICAD <sup>HevTB-MP-TM-CPD</sup> -GFP |
| SICAD <sup>HevTB-MP-TM-CPD</sup> -R | CACCTCCGCCACCGCCTCCTCCTGAGCTGCGAGTCGCGAAC  |                                       |
| SICAD <sup>HevTB</sup> -F           | CCAACAGTTGAGTTGGTTATCGTGTACGGATGGTGGTACGG  | SICAD <sup>HevTB</sup> -GFP           |
| SICAD <sup>HevTB</sup> -R           | CTCTGACACGTGTCGATATCAGGTACACCTCACTCCGTGCGC |                                       |

**Table S13.** Sequence of primers used for amplification of long vector fragments of *SICAD*.

| Primer                              | Sequence (5'-3')                             | Expressed protein                     |
|-------------------------------------|--|---------------------------------------|
| SICAD <sup>HevTB-MP-TM-CPD</sup> -F | GAACTCGCAGCTCAGGAGAGGAGGCCGTGGCGGAGGTATCCAC  | SICAD <sup>HevTB-MP-TM-CPD</sup> -GFP |
| SICAD <sup>HevTB-MP-TM-CPD</sup> -R | CACCATCCGTAGCACGAATAACCAACTCAAACGTGGACACCA   |                                       |
| SICAD <sup>HevTB</sup> -F           | CGAAGTGAAGGTGTACCTGATATCGACACCGTGTAGAGTAGCCT | SICAD <sup>HevTB</sup> -GFP           |
| SICAD <sup>HevTB</sup> -R           | CACCATCCGTAGCACGAATAACCAACTCAAACGTGGACACCA   |                                       |

Note: The short inserted fragment and the following long vector fragment were used for gene fusion [2].

**Table S14.** Sequence of primers used for expression of GST tagged CAD fragments in *E.coli* bacteria.

| Primer                        | Sequence (5'-3')                          | Expressed protein          |
|-------------------------------|---|----------------------------|
| GST-TB-MP <sup>HaCAD</sup> -F | CCCGAATTCTAGGAGGCCGTGGAGGCCGTACGGTACGGACG | GST-TB-MP <sup>HaCAD</sup> |
| GST-TB-MP <sup>HaCAD</sup> -R | CCCAAGCTTCTACAGAGCTCGCCCTGCCG             |                            |

|                               |  |                             |
|-------------------------------|--|-----------------------------|
| GST-TB-MP <sup>HaCAD</sup> -F | CCGGAATTCTAGGAGGCCGTGGAGGCCGTATTCTGCCACGGATGGT     | GST-TB-MP <sup>HaCAD</sup>  |
| GST-TB-MP <sup>HaCAD</sup> -R | CCCAAGCTTCAAACGCCAGGCCGTAAATCT                     |                             |
| GST-CR7-9 <sup>HaCAD</sup> -F | TGCTCTAGACGGAGGCCGTGGAGGCCGTAAAGTCGAGGCACGGTGT     | GST-CR-7-9 <sup>HaCAD</sup> |
| GST-CR7-9 <sup>HaCAD</sup> -R | CCGCTCGAGCTACACCTGAACTCTCGAAGTCCT                  |                             |
| GST-TB <sup>HaCAD</sup> -F    | CCCAAGCTTAATTCTCGTACTGACTGACGAT                    | GST-TB <sup>HaCAD</sup>     |
| GST-TB <sup>HaCAD</sup> -R    | CCCAAGCTTCACAGGTACACCTTCACCTCCGT                   |                             |
| GST-MP <sup>HaCAD</sup> -F    | CCGGAATTCTAGGAGGCCGTGGAGGCCGTATATCGGACCGAACCGAGTGT | GST-MP <sup>HaCAD</sup>     |
| GST-MP <sup>HaCAD</sup> -R    | CCCAAGCTTCTACAGAGCTCGCCCTGCGCG                     |                             |

**Table S15.** Sequences of the primers used for real time qPCR analyzes.

| primers   | sequence (5'-3')          |
|-----------|---------------------------|
| HaCAD-F   | CTACGCCATCACAGGTCTTAC     |
| HaCAD-R   | CCCCAATAGCCTCTCAAATCC     |
| HaABCC2-F | TTTGGGGCACTTGGTGAT        |
| HaABCC2-R | TTCCGTGGGTAGTTGGTGT       |
| TnCAD-F   | TGCTGGACAGAGACGGCGACTAT   |
| TnCAD-R   | GTCGTTCACATCTAACAGAACCCAG |
| TnABCC2-F | CACTACACTAGCGACACGGTGCT   |
| TnABCC2-R | GTGCTGACCATAAGGACGGATCTC  |
| Tnrps3A-F | GCGAAAATTCGTCGGAGACGGAG   |
| Tnrps3A-R | ATAATGATGATCTCCGAGCGAGTC  |

## References

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