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Electronic Supplementary Material

3H-31, A Non-structural Protein of Heliothis virescens ascovirus 3h, Inhibits the Host Larval Cathepsin and Chitinase Activities

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Supplementary Data

Preparation of 3H-31 specific antiserum

The specific primer pairs of *3h-31* (31-F: 3'- GGATCCATGAACGGACACGTCGAG -5' and 31-R: 3'- CTCGAGTTATTCGATTACACAAGT -5', *Bam*HI and *Xho*I site were underlined) were used to amplify the CDs region of *3h-31*. The resulting PCR products purified using EasyPure PCR Purification Kit (TRANSGEN, CHN) and then cloned into pGEM-T easy vector (Promega) to generate vector 31-T. White clones were picked and sent to TsingKe Biological Technology, Limited, Co. to confirm the clone by sequencing. The constructed 31-T vector were then extracted and digestion with *Bam*HI (NEB) and *Xho*I (NEB), followed by ligating with *Bam*HI and *Xho*I digested pET-28a(+) (Novagen, GER) vector to generate prokaryotic expression vector 28a-31.

The constructed expression vectors were then transformed into *Escherichia coli* BL21 (DE3) and induced with 1 mmol/L isopropyl-β-d-thiogalactoside (IPTG) (Sigma, USA) at 28 °C by centrifuging at 180 rpm for 20 hrs. The cultured bacteria were collected and destroyed by ultrasonication. The resulting products were centrifuged for 20 min at 12000 g. The pellets were then macerated with 8 mol/L urea to dissolute the His-tag fused 3h-31 (Pr3H-31) inclusion bodies. After centrifugation (14000 g for 30 min), Pr3H-31 was purified with His-Tag Purification Resin (Roche, SUI) according to the manufacturer's instructions.

As can be seen from Supplementary Figure S1, the His-tag fused 3H-31 started to be eluted from pH 5.6 buffer, and was most abundant with the elution of the pH 5.2 buffer. But the content and the purity was not qualified for antigen used in the following immunology. The N-terminal 200 amino acid residues were intercepted (31-F: 3'- GGATCCATGAACGGACACGTCGAG -5' and 31N-R: 3'- CTCGAGAACTTTATGTTATTGTCAGC -5', *Bam*HI and *Xho*I site were underlined), expressed, and purified according to the above description.

The yield of the intercepted N-terminal 200 amino acid residues of 3H-31 (Pr3H-31-N200) was obviously higher than that of Pr3H-31 (Supplementary Figure S2). The flow through with elution buffer pH 5.2 was further used as antigen to produce specific rabbit antiserum. The purified protein in complete Freund's adjuvant (Sigma, USA) was injected subcutaneously to immunize New Zealand white rabbits. Two booster injections in incomplete Freund's adjuvant at two-week intervals were followed before exsanguination. The polyclonal rabbit antiserum against 3h-31 were prepared for the following immunoassays.

Purification of virions by sucrose density gradient centrifugation

After centrifugation, the interfaces between the sonic disrupted hemolymph-15% sucrose, 15%-35% sucrose, and 35%-55% sucrose was collected and observed by TEM (Supplementary Figure S3). Seldom virions were found from the 35%-55% sucrose interface (Bottom), and a lot of impurities were found in the disrupted hemolymph-15% sucrose interface (Top). Clean and abundant virions were found from the 15%-35% sucrose interface (Middle), which was used to extract virion structural protein for analysis.

Construction of bidirectional expression donor plasmid

To facilitate the *polyhedrin* reparation and foreign gene expression, a bidirectional expression donor plasmid was constructed based on the commercial obtained pFast Bac HTb vector. A 1865-bp ph-Pp10 fragment was synthesized (Supplementary Figure S4), in which a *p10* promoter was employed to express foreign genes and the AcMNPV *polyhedrin* and its native promoter was in the opposite direction of *p10* promoter to express polyhedrin.

Synthetise of dsRNA

By using T7 Ribomax Express RNAi System (Promega), the dsRNA of *egfp* and the dsRNA of *3h-31* was synthesized and confirmed by agarose gel electrophoresis (Supplementary Figure S5).

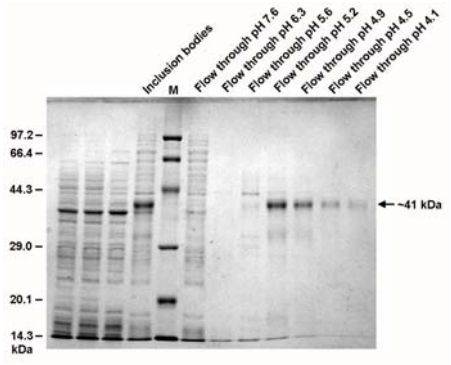

Fig. S1 Purification of Pr3H-31. Elution buffers (300 mmol/L NaCl, 50 mmol/L NaH_2PO_4 8 mol/L Urea) with different pH value were used to elute out target protein.

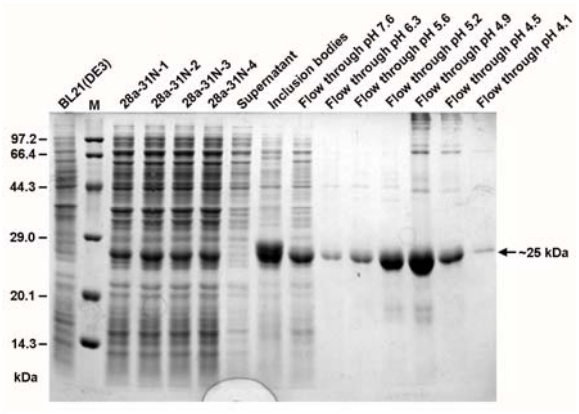

Fig. S2 Purification of Pr3H-31-N200. Elution buffers (300 mmol/L NaCl, 50 mmol/L NaH_2PO_4 8 mol/L Urea) with different pH value were used to elute out target protein.

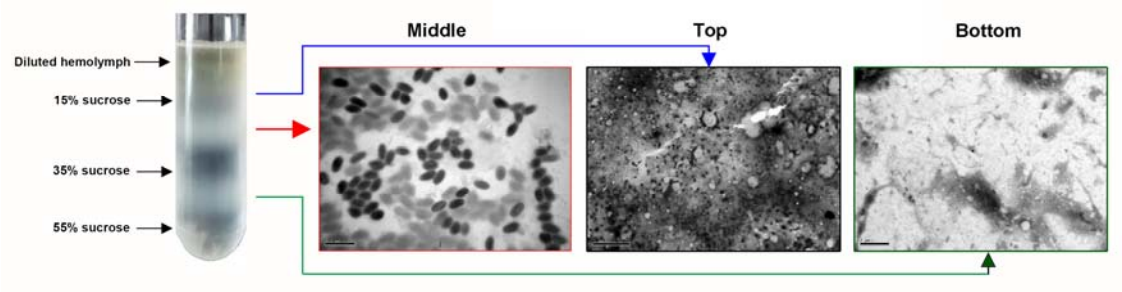

Fig. S3 Purification of virions from HvAV-3h infected *Mythimna separata*. The interfaces between different sucrose solutions were monitored separately.

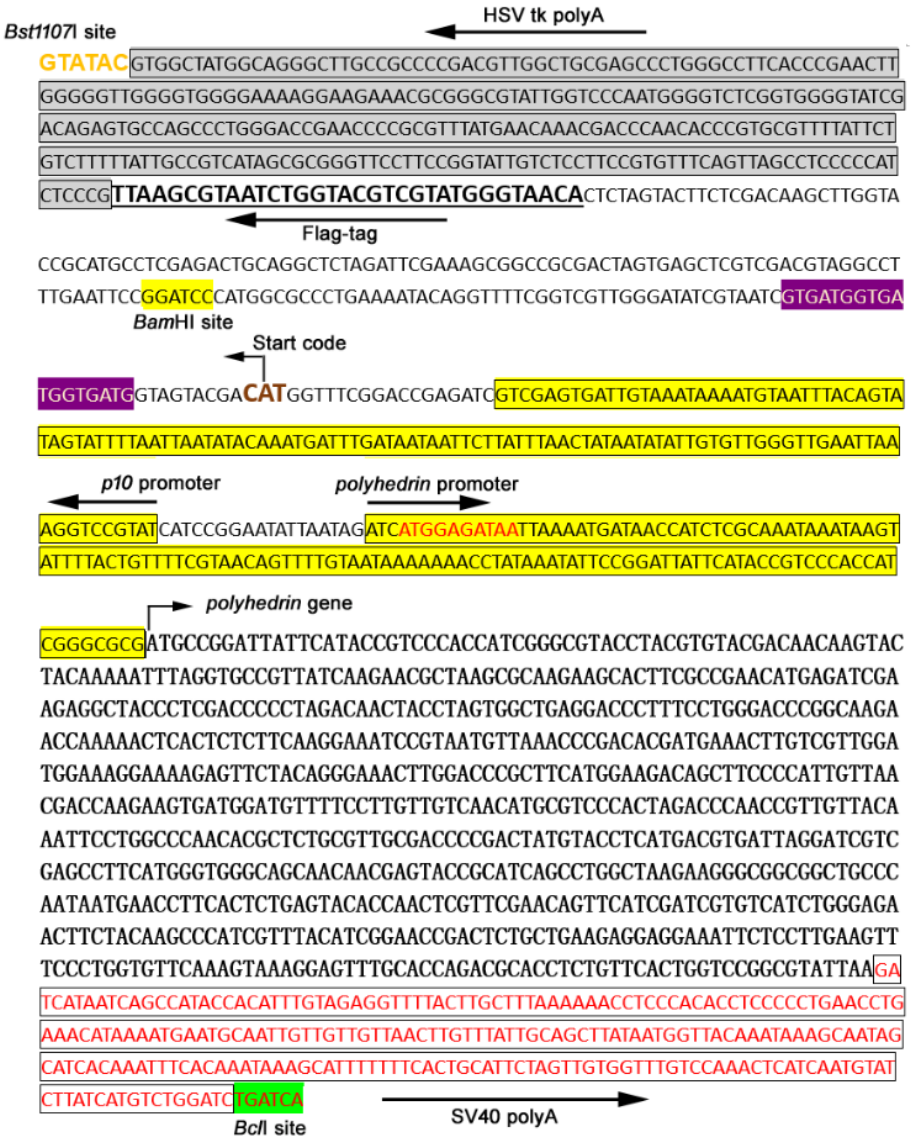

Fig. S4 The sequence of synthesized 1865-bp ph-Pp10 fragment. AcMNPV *polyhedrin* gene under its native promoter and an opposite directed *p10* promoter was contained, and the *polyhedrin* was modified to silence the *Hind*III and *Eco*RI site in the nature sequence for the further application.

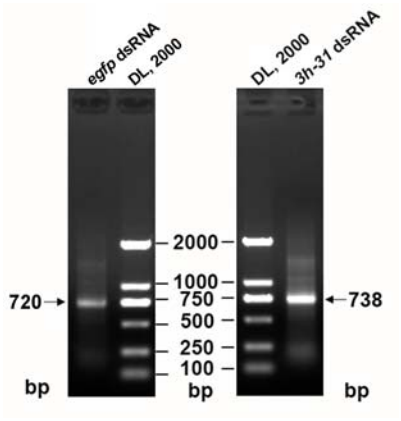

Fig. S5 Agarose gel electrophoresis detection of the synthetized dsRNA.