



Supplementary Materials

cpPstDNV Sinnuengnong	(1)	1	80
cpPstDNV-dspro	(1)	ATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCTGGTGCCGCGCGGCAGCCATATGTGCGCCGATTCAACAAG	
FJ848675 YHV protease	(1)	ATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCTGGTGCCGCGCGGCAGCCATATGTGCGCCGATTCAACAAG	
YHV anti protease	(1)	-----	
cpPstDNV Sinnuengnong	(81)	81	160
cpPstDNV-dspro	(81)	AGCAAGCCCAAGGAAAAGATCCAGGAGGGATGCACATAATGAAGACGAAGAACACGCCGAAGGATCAAGTGGACCAGACC	
FJ848675 YHV protease	(1)	AGCAAGCCCAAGGAAAAGATCCAGGAGGGATGCACATAATGAAGACGAAGAACACGCCGAAGGATCAAGTGGACCAGACC	
YHV anti protease	(1)	-----	
cpPstDNV Sinnuengnong	(161)	161	240
cpPstDNV-dspro	(161)	CACACAGATGTCTACAATTCAATACTGGAGACTCAATATATATTACTTTCCAAACAAGAAGATACTTCGAATTCGACGCT	
FJ848675 YHV protease	(1)	CACACAGATGTCTACAATTCAATACTGGAGACTCAATATATATTACTTTCCAAACAAGAAGATACTTCGAATTCGACGCT	
YHV anti protease	(1)	-----	
cpPstDNV Sinnuengnong	(241)	241	320
cpPstDNV-dspro	(241)	GCCAATGATGGAACTTCGACGGAAAAATTTATACTGCCTCCCACTACATTGGATGAACCTTATCTCTATGGCTTAAA	
FJ848675 YHV protease	(1)	GCCAATGATGGAACTTCGACGGAAAAATTTATACTGCCTCCCACTACATTGGATGAACCTTATCTCTATGGCTTAAA	
YHV anti protease	(1)	-----	
cpPstDNV Sinnuengnong	(321)	321	400
cpPstDNV-dspro	(321)	GAGCAGCGACAGTTTCAGCAACAGAAACACAACGATATAAGATGGTAAATCAATGATGAAGACCTACGGATGGAAAGTAC	
FJ848675 YHV protease	(1)	GAGCAGCGACAGTTTCAGCAACAGAAACACAACGATATAAGATGGTAAATCAATGATGAAGACCTACGGATGGAAAGTAC	
YHV anti protease	(1)	-----	
cpPstDNV Sinnuengnong	(401)	401	480
cpPstDNV-dspro	(401)	ATAAAGCAGGCGTAGTGATGCATCGATGGTACCCTTATGAAAGACTTAAAGTATCAGGAGGCACATCATTTGAGACT	
FJ848675 YHV protease	(1)	ATAAAGCAGGCGTAGTGATGCATCGATGGTACCCTTATGAAAGACTTAAAGTATCAGGAGGCACATCATTTGAGACT	
YHV anti protease	(1)	-----	
cpPstDNV Sinnuengnong	(481)	481	560
cpPstDNV-dspro	(481)	CTCACATTTACAGACCCCCATATTTAGAAATATTTAAGGATACTACTGGACTACATAATCAACTATCAACTAAGGAAGC	
FJ848675 YHV protease	(1)	CTCACATTTACAGACCCCCATATTTAGAAATATTTAAGGATACTACTGGACTACATAATCAACTATCAACTAAGGAAGC	
YHV anti protease	(1)	-----	
cpPstDNV Sinnuengnong	(561)	561	640
cpPstDNV-dspro	(561)	CGACGTAACATTGGCAAAATGGATACAAAATCCCCAACTTGTGACCGTACAATCAACAGCAGCAAACTATGAAGACCCAA	
FJ848675 YHV protease	(1)	CGACGTAACATTGGCAAAATGGATACAAAATCCCCAACTTGTGACCGTACAATCAACAGCAGCAAACTATGAAGACCCAA	
YHV anti protease	(1)	-----	
cpPstDNV Sinnuengnong	(641)	641	720
cpPstDNV-dspro	(641)	TCCAACAATTTGGATTTCATGGAACAAATGCGAACCAGGTGACAGAAAAGCCTATACAATCCATGGTGACACTAGAAATTGG	
FJ848675 YHV protease	(1)	TCCAACAATTTGGATTTCATGGAACAAATGCGAACCAGGTGACAGAAAAGCCTATACAATCCATGGTGACACTAGAAATTGG	
YHV anti protease	(1)	-----	
cpPstDNV Sinnuengnong	(721)	721	800
cpPstDNV-dspro	(721)	TATGGCGGAGAAATACCAACAACCGGACCCACCTTCATCCCAAAATGGGGTGGTCAATTAATAATGGGACAAACCATCCCT	
FJ848675 YHV protease	(1)	TATGGCGGAGAAATACCAACAACCGGACCCACCTTCATCCCAAAATGGGGTGGTCAATTAATAATGGGACAAACCATCCCT	
YHV anti protease	(1)	-----	
cpPstDNV Sinnuengnong	(801)	801	880
cpPstDNV-dspro	(801)	TGGAAACCTAGTCTACCCAGCAGACCACCATACAAACGACTGGCAACAGATCTTCATGAGAATGTCACCAATCAAAGGAC	
FJ848675 YHV protease	(1)	TGGAAACCTAGTCTACCCAGCAGACCACCATACAAACGACTGGCAACAGATCTTCATGAGAATGTCACCAATCAAAGGAC	
YHV anti protease	(1)	-----	
cpPstDNV Sinnuengnong	(881)	881	960
cpPstDNV-dspro	(881)	CAAATGGAGACGAACCTAAACTTGGCTGCAGAGTACAAGCCGACTTCTTCTACATCTAGAAGTACGACTCCCACCACAA	
FJ848675 YHV protease	(1)	CAAATGGAGACGAACCTAAACTTGGCTGCAGAGTACAAGCCGACTTCTTCTACATCTAGAAGTACGACTCCCACCACAA	
YHV anti protease	(1)	-----	
cpPstDNV Sinnuengnong	(961)	961	1040
cpPstDNV-dspro	(961)	GGATGTGTCGCAAGTTTGGGGATGTTACAATATCTTCACGCACCATGTACTGGACAACCTTAACAAATGTTATATTATGCA	
FJ848675 YHV protease	(1)	GGATGTGTCGCAAGTTTGGGGATGTTACAATATCTTCACGCACCATGTACTGGACAACCTTAACAAATGTTATATTATGCA	
YHV anti protease	(1)	-----	
cpPstDNV Sinnuengnong	(1041)	1041	1120
cpPstDNV-dspro	(1041)	TACTAACTAA-----XhoI	
FJ848675 YHV protease	(1)	TACTAACTAACTCGAGTCAGCGGCAAAATTCCTCTACTTTCCTCGTCACATCTTCGACTCCTGTACTGACAACACTCT	
YHV anti protease	(1)	-----CTCGAGTCAGCGGCAAAATTCCTCTACTTTCCTCGTCACATCTTCGACTCCTGTACTGACAACACTCT	
cpPstDNV Sinnuengnong	(990)	1121	1200
cpPstDNV-dspro	(1121)	CACACGACACATCCGCGTCACAAAAGGTGAAGAACTCATGACATCGAATTGTTGAGCGAAGAATATGACGCCACTCCTT	
FJ848675 YHV protease	(68)	CACACGACACATCCGCGTCACAAAAGGTGAAGAACTCATGACATCGAATTGTTGAGCGAAGAATATGACGCCACTCCTT	
YHV anti protease	(1)	-----	

		1201		1280
cpPstDNV Sinnuengnong	(990)	-----		-----
cpPstDNV-dspro	(1201)	TCATCAAACCGACAGTCCATTTCGCAGAAGCAACTGTACTCAAATTCGGTAAACTCCAACGCACTCAGTACGCATACTTC		
FJ848675 YHV protease	(148)	TCATCAAACCGACAGTCCATTTCGCAGAAGCAACTGTACTCAAATTCGGTAAACTCCAACGCACTCAGTACGCATACTTC		
YHV anti protease	(1)	-----		-----
		1281		1360
cpPstDNV Sinnuengnong	(990)	-----		-----
cpPstDNV-dspro	(1281)	GTCACGTGCTGATGACATCAGGGTTGGTTCAATGTCCGCCGACGGCTACCACAACATTTCTACCAAGGATGGTGACTGCGG		
FJ848675 YHV protease	(228)	GTCACGTGCTGATGACATCAGGGTTGGTTCAATGTCCGCCGACGGCTACCACAACATTTCTACCAAGGATGGTGACTGCGG		
YHV anti protease	(1)	-----		-----
		1361		1440
cpPstDNV Sinnuengnong	(990)	-----		-----
cpPstDNV-dspro	(1361)	TTCACCTCCTCTTTGACCACCTTCACAATGTTGTTGGAGCTCACATCGTCGGCATTGCTAGCATCCCTCCTGTTAACGGTG		
FJ848675 YHV protease	(308)	TTCACCTCCTCTTTGACCACCTTCACAATGTTGTTGGAGCTCACATCGTCGGCATTGCTAGCATCCCTCCTGTTAACGGTG		
YHV anti protease	(1)	-----		-----
		1441		1520
cpPstDNV Sinnuengnong	(990)	-----		-----
cpPstDNV-dspro	(1441)	CCCTGACCTGGAATGCAGAAAAGGAAATGCTCTGCGGACCAAAATGATGACTACGATTACGATCCAGAAAAAGTCGGTCCA		
FJ848675 YHV protease	(388)	CCCTGACCTGGAATGCAGAAAAGGAAATGCTCTGCGGACCAAAATGATGACTACGATTACGATCCAGAAAAAGTCGGTCCA		
YHV anti protease	(1)	-----		-----
		1521	loop	1600
cpPstDNV Sinnuengnong	(990)	-----		-----
cpPstDNV-dspro	(1521)	CCCAAGGTATGGCCTGTAGAATCAATCACTGCTCTCAGCACGATCCTCAATCAGCTCAAGTATGTCACCGGAGATGGCTT		
FJ848675 YHV protease	(468)	CCCAAGGTATGGC-----		
YHV anti protease	(1)	-----		-----
		1601	loop	1680
cpPstDNV Sinnuengnong	(990)	-----		-----
cpPstDNV-dspro	(1601)	CACCTACACCCAACTCCTTACAACTATCAGCTAATTGGTTGCGAGACACTGGACCAAGCTTGCATACCTTGGGTGGAC		
FJ848675 YHV protease	(480)	-----		
YHV anti protease	(1)	-----GCCATACCTTGGGTGGAC		
		1681		1760
cpPstDNV Sinnuengnong	(990)	-----		-----
cpPstDNV-dspro	(1681)	CGACTTTTCTGGATCGTAATCGTAGTCATCATTTGGTCCGCAGAGCATTTCCTTTTCTGCATTCCAGGTCAGGGCACCGT		
FJ848675 YHV protease	(480)	-----		
YHV anti protease	(18)	CGACTTTTCTGGATCGTAATCGTAGTCATCATTTGGTCCGCAGAGCATTTCCTTTTCTGCATTCCAGGTCAGGGCACCGT		
		1761		1840
cpPstDNV Sinnuengnong	(990)	-----		-----
cpPstDNV-dspro	(1761)	TAACAGGAGGGATGCTAGCAATGCCGACGATGTGAGCTCCAACAACATTGTGAAGGTGGTCAAAGAGGAGTGAACCGCAG		
FJ848675 YHV protease	(480)	-----		
YHV anti protease	(98)	TAACAGGAGGGATGCTAGCAATGCCGACGATGTGAGCTCCAACAACATTGTGAAGGTGGTCAAAGAGGAGTGAACCGCAG		
		1841		1920
cpPstDNV Sinnuengnong	(990)	-----		-----
cpPstDNV-dspro	(1841)	TCACCATCCTTGGTAGAAATGTTGTGGTAGCCGTCGGCGGACATTGAACCAACCTGATGTCATCAGCAGTGACGAAGTA		
FJ848675 YHV protease	(480)	-----		
YHV anti protease	(178)	TCACCATCCTTGGTAGAAATGTTGTGGTAGCCGTCGGCGGACATTGAACCAACCTGATGTCATCAGCAGTGACGAAGTA		
		1921		2000
cpPstDNV Sinnuengnong	(990)	-----		-----
cpPstDNV-dspro	(1921)	TGCGTACTGAGTGCGTTGGAGTTTACCGAATTGAGTACAGTTGCTTCTGCGAATGGACTGTCGGTTTTGATGAAAGGAG		
FJ848675 YHV protease	(480)	-----		
YHV anti protease	(258)	TGCGTACTGAGTGCGTTGGAGTTTACCGAATTGAGTACAGTTGCTTCTGCGAATGGACTGTCGGTTTTGATGAAAGGAG		
		2001		2080
cpPstDNV Sinnuengnong	(990)	-----		-----
cpPstDNV-dspro	(2001)	TGGCGTCATATTCTTCGCTCAACAATTCGATGTCATGAGTTTCTTCACCTTTTGTGACGCGGATGTGTCGTGTGAGAGTG		
FJ848675 YHV protease	(480)	-----		
YHV anti protease	(338)	TGGCGTCATATTCTTCGCTCAACAATTCGATGTCATGAGTTTCTTCACCTTTTGTGACGCGGATGTGTCGTGTGAGAGTG		
		2081	XhoI	
cpPstDNV Sinnuengnong	(990)	-----		2140
cpPstDNV-dspro	(2081)	TTGTCAGTACAGGAGTCGAAGATGTGACGAGGAAAGTAGAGGAATTGCCGCTGATCGAG		2140
FJ848675 YHV protease	(480)	-----		2140
YHV anti protease	(418)	TTGTCAGTACAGGAGTCGAAGATGTGACGAGGAAAGTAGAGGAATTGCCGCTGATCGAG		2140

Figure S1. Nucleotide sequence alignment of pET28a-Linked cpPstDNV-dspro with other relevant plasmid and gene.

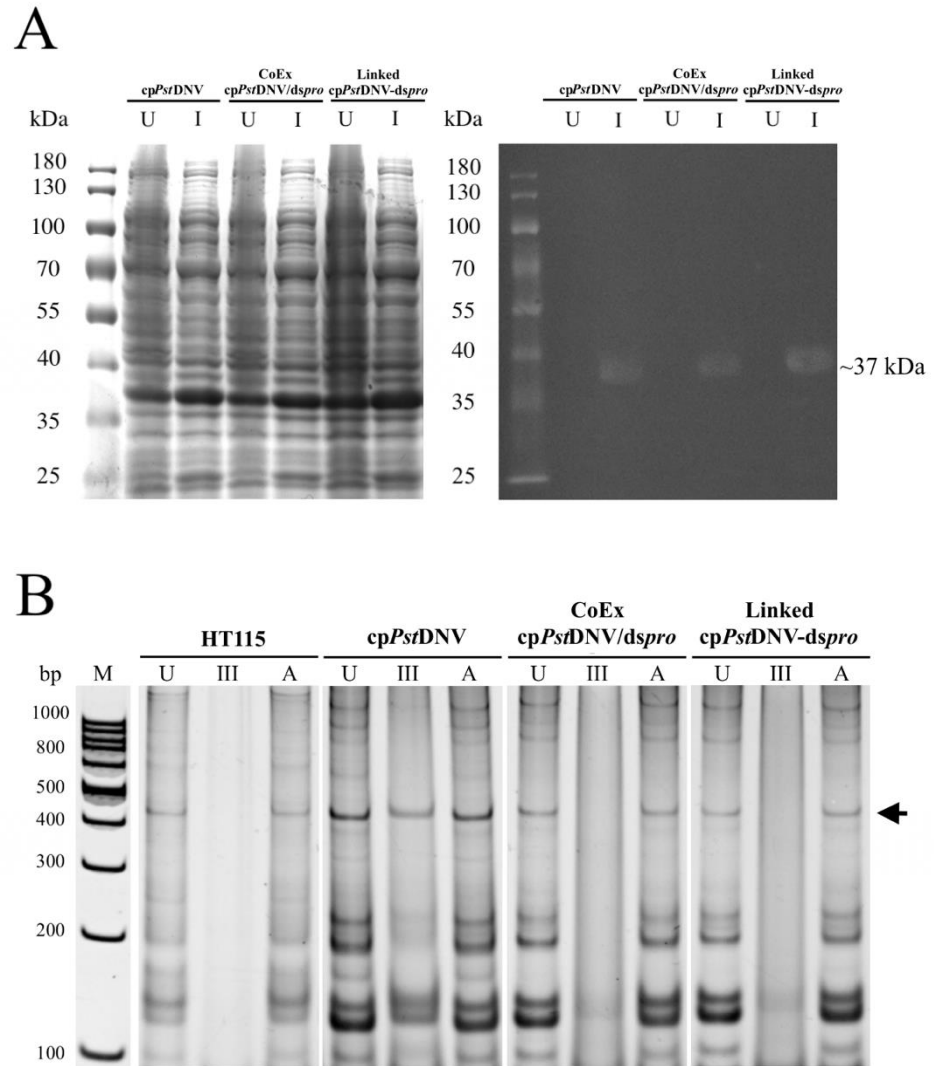


Figure S2. Expression of recombinant Linked *cpPstDNV-dspro*, *cpPstDNV* and CoEx *cpPstDNV/dspro* in *E. coli* strain Rosetta-gami 2(DE3) pLysS. (A) SDS-PAGE and western blot analysis from the total cell lysate of induced (lane I) and uninduced (U) recombinant bacteria. Lane M, PageRuler™ Prestained Protein Ladder (Thermo Fisher Scientific, Waltham, MA, USA) (B) The dsRNA-YHV *protease* gene expression and its properties were analyzed on 12% TAE buffered-polyacrylamide gel after total RNAs isolated from induced recombinant *E. coli* strain Rosetta-gami 2(DE3) pLysS and HT115 (lane U) were treated with RNase III (lane III) or RNase A (lane A). Lane M, GeneRuler 100 bp DNA Ladder (Thermo Fisher Scientific, Waltham, MA, USA). Arrow indicates *dspro*.

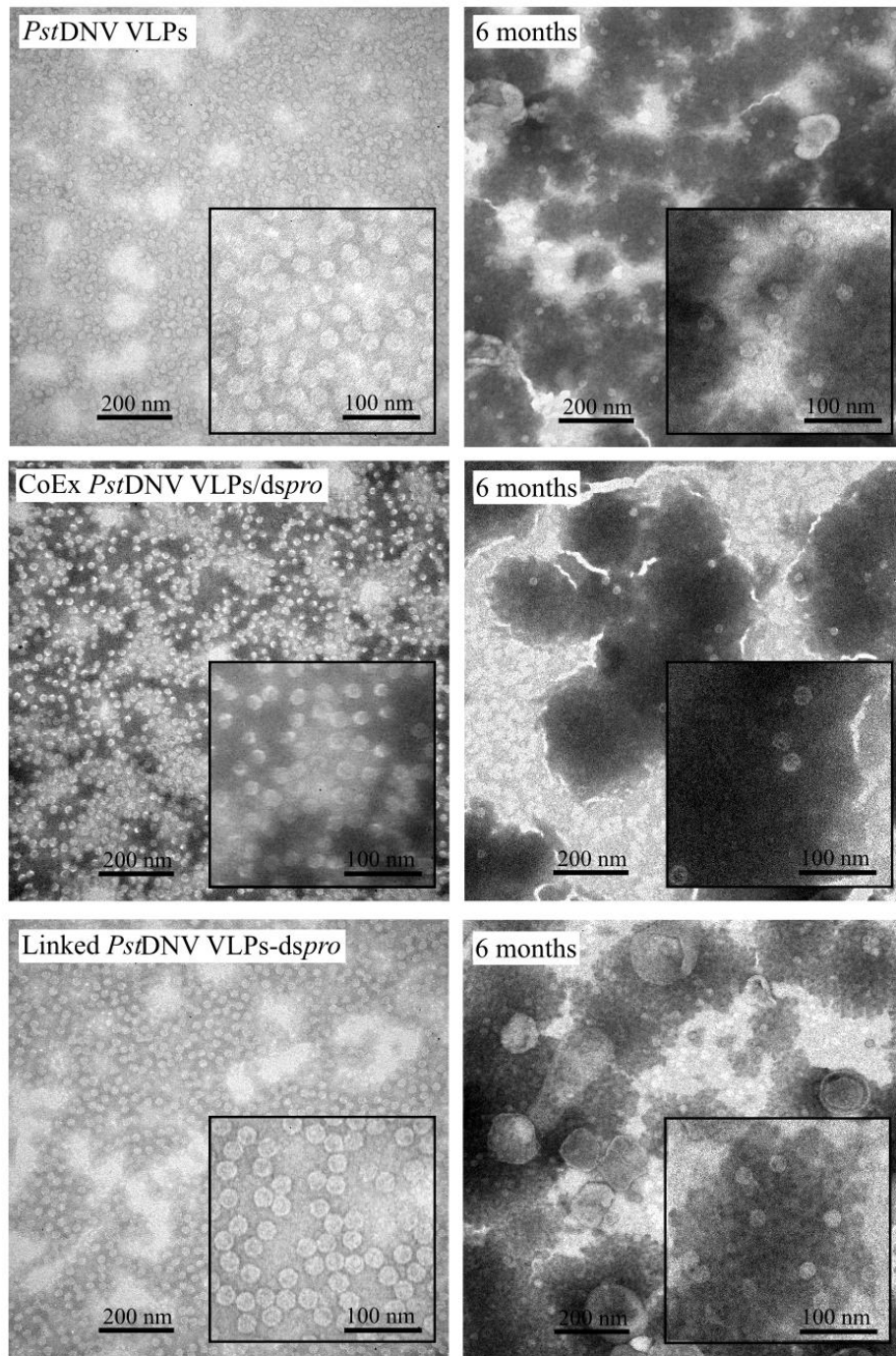


Figure S3. Transmission electron micrograph of *PstDNV* VLPs, CoEx *PstDNV* VLPs/*dspro* and Linked *PstDNV* VLPs-*dspro* and their stability after being kept at 4 °C for 6 months. Bars = 200 and 100 nm.

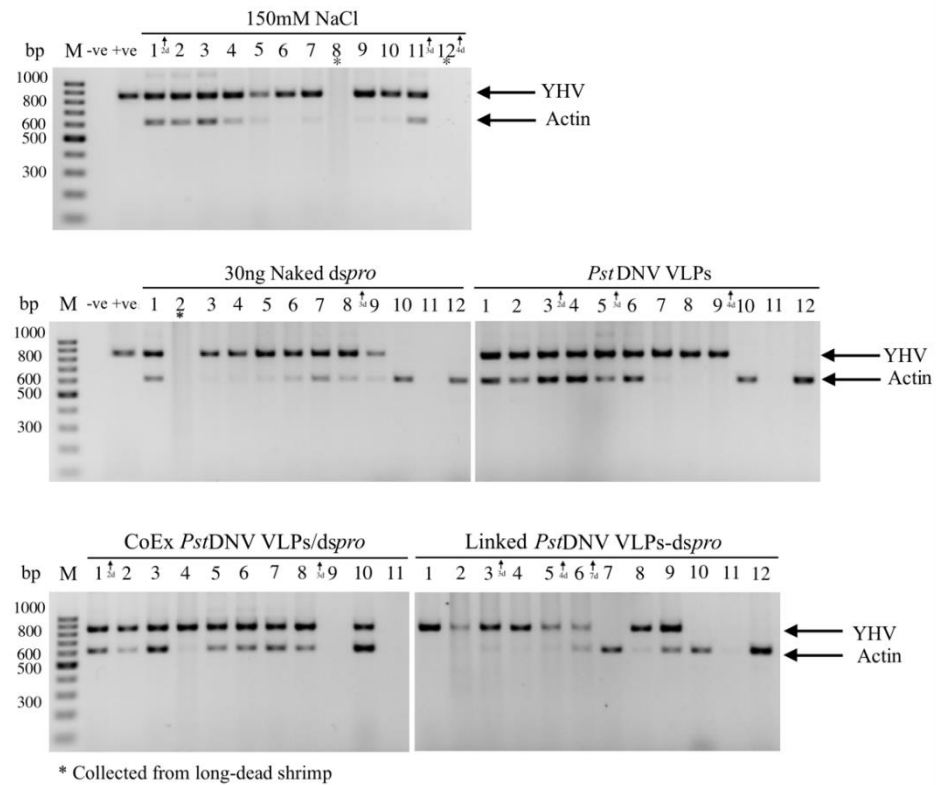


Figure S4. Confirmation of shrimp in all treatments infected with YHV. Agarose gel electrophoresis of RT-PCR products specific for YHV *helicase* from challenged shrimps. Host's *actin* gene was used as an internal control. Small arrows indicate the date on which each animal found dead following a virus challenge. Asterisk indicates sample collected from a long-dead shrimp. Lane M; GeneRuler 100 bp DNA Ladder (Thermo Fisher Scientific, Waltham, MA, USA), Lane +ve; positive control PCR, Lane -ve; negative control PCR.

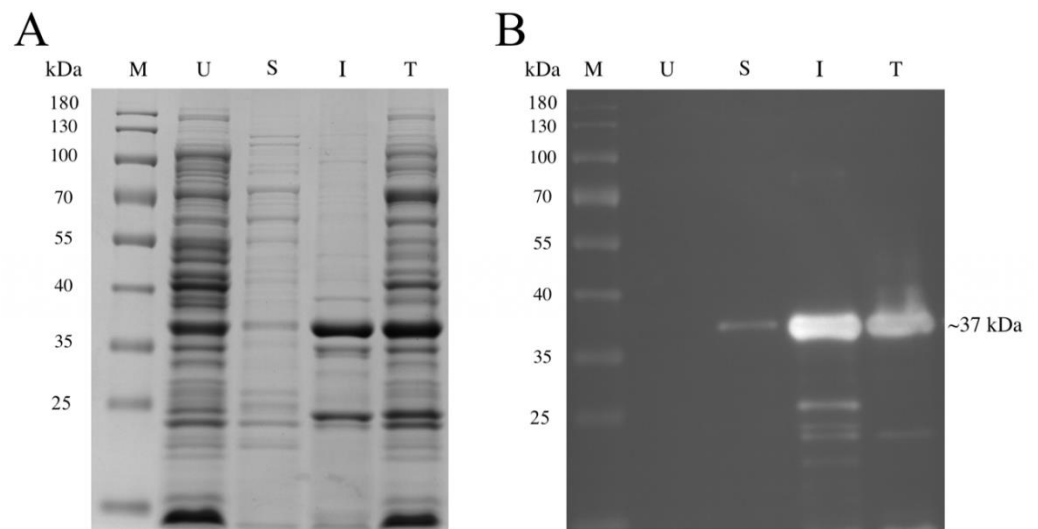


Figure S5. The solubility of recombinant Linked cpPstDNV-dspro in ENZhance lysis buffer. Induced bacteria (1 OD_{600nm}) were resuspended in ENZhance lysis buffer and incubated on ice for 30 min. After the bacterial suspension was centrifuged at 17,000 xg at 4 °C for 15 min, supernatant was taken (lane S) and cell debris was washed twice with sterile water (lane I). Both samples were then analyzed by SDS-PAGE (A) and western blotting (B). Lane M, PageRuler™ Prestained Protein Ladder (Thermo Fisher Scientific, Waltham, MA, USA), Lane U; total cell lysate of uninduced cell, Lane T, total cell lysate of induced cell.