Caption of figures and table

Table 1. Details of the binding results obtained from different binding and activating domain fusions. Results are indicated as (+) when the interaction occurs (blue colony on selective medium [SD/-Trp/-Leu/-His/-Ade containing 5-bromo-4-chloro-3-indolyl-α-D-galactopyranoside (X-α-Gal)] and (–) when there was no interaction (no growth on SD medium). Diagrams illustrate the structure of Pm-syntenin and α_2M deduced from their amino acid sequences The distinct domains within each protein are shown by different annotations as follows:

Full length DNA of Pm-Syntenin

N-terminal of Pm-Syntenin

PDZ 1

PDZ2

C-terminal of shrimp α_2M

Fig. 1 (A) Yeast two-hybrid assay. *S. cerevisiae* AH109 cells were cotransformed with full length syntenin in the pGBKT7 vector, and the C-terminal 181 amino acids residues of α_2M in pGADT7 (SA1) and 286 amino acids residues of α_2M in pGADT7 (SA2). Transformed cells were selected on SD medium. The positive control is yeast cells that were transformed with pGBKT7-53 and pGADT7-T (CLONTECH) and the negative control for α-galactosidase activity is yeast cell that did not activate the *MEL1*.

(B) The filter containing the selected lysed yeast cells and liquid 5-bromo-4-chloro-3-indolyl-β-D-galactopyransoside (X-Gal) was used to verify the activation of *lacZ* by interaction between two known proteins. *S. cerevisiae* AH109 cells were cotransformed with 1) pGBKT7-53 and pGADT7-T (positive control), 2) BD-syntenin and AD-α_2M, 3) N-terminal of syntenin in pGBKT7 (BD-NS) and AD-α_2M, 4) PDZ1-PDZ2 in pGBKT7 (BD-PDZ1,2) and AD-α_2M.

Transformed cells were selected on medium (SD/-Trp/-Leu/-His/-Ade) except for BD-PDZ1,2 was obtained from SD/-Leu/-Trp).

Fig. 2 ***In vitro*** **binding assay I.** Purified GST-α_2M and GST proteins were detected by specific antibody in the presence of 6xHis-syntenin. A glutathione sepharose bead pull-down was performed on the combined proteins. The eluted material was loaded on SDS-PAGE gels, transferred and detected using specific antibodies. When not pulled down, there was no band detected with anti-His Tag antibody (lane 1) but a GST band was detected with anti-GST antibody (lane 3). In the case where the combined proteins were pulled down (lane 2, 4), syntenin was detected with anti-His Tag antibody and α_2M was detected with anti-GST antibody.

Fig. 3 ***In vitro*** **binding assay II.** α_2M protein was obtained from an *in vitro* transcription/translation of the plasmid pGADT7-SA1 in the presence of ^{35}S-Met and the ^{35}S – Met labeled protein was combined with GST-syntenin. A glutathione sepharose bead pull-down was performed on the combined proteins. The material was loaded on SDS-PAGE gels, transferred to a nitrocellulose membrane and detected by using fluorography.

Fig. 4 (A) Reverse transcription PCR experiments performed with total RNA isolated from haemocytes of 5 uninfected individuals of *P. monodon* and 5 individuals infected with WSSV. Amplification with β-actin was performed in parallel (bottom 2 panels) as a control. Images are ethidium bromide staining of the RT-PCR product after 25 cycles of amplification, N.1-N.5: normal (uninfected samples); I.1-I.5: infected samples (48 hours WSSV post-injection samples).

(B) The normalized α_2M was calculated from the images using Scion Image software. The data represent the average results obtained from individuals of normal and infected shrimps.

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[i] Revised 08/05/2548

Table 1

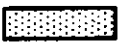
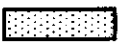
Binding Domain Fusion	Activating Domain Fusion	SD+ X-α-Gal
1.  BD-Syntenin	-	(-)
2. -	 AD-α_2M	(-)
3.  BD-Syntenin	 AD-α_2M	(+)
4.  BD-NS	 AD-α_2M	(+)
5.  BD-PDZ1,2	 AD-α_2M	(-)
6.  BD-PDZ1	 AD-α_2M	(-)

Fig. 1A

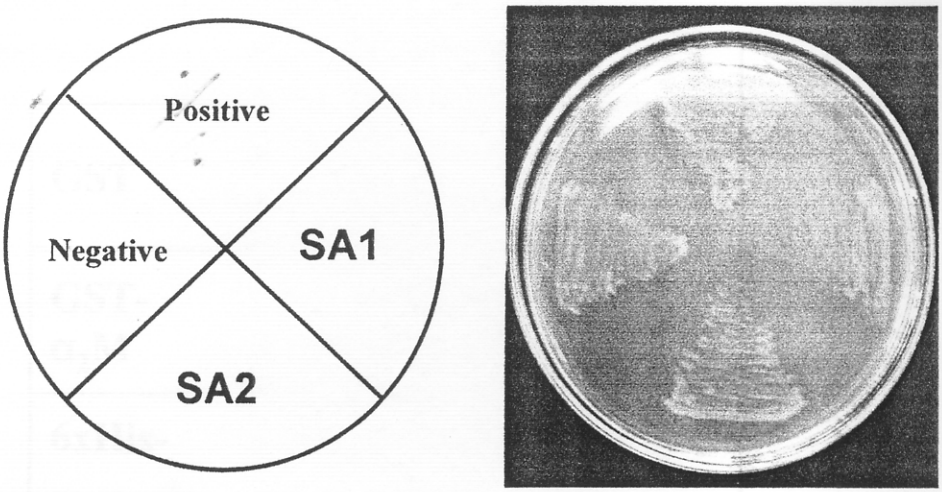

Positive
Negative
SA1
SA2

Fig. 1B

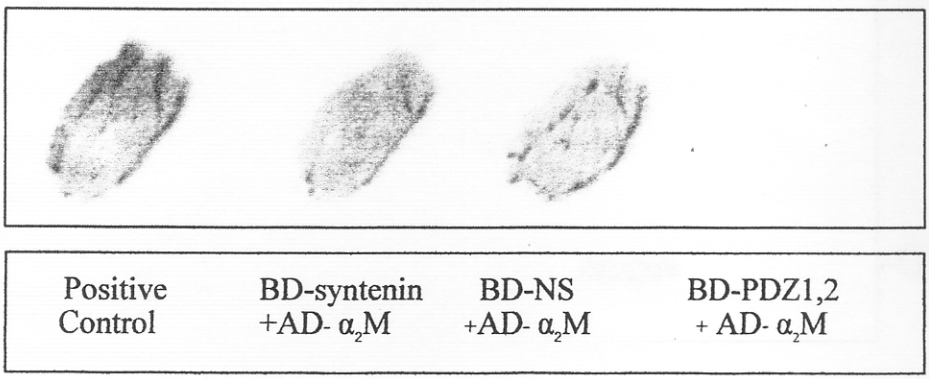

Positive Control
BD-syntenin +AD- α2M
BD-NS +AD- α2M
BD-PDZ1,2 + AD- α2M

Fig. 2

	1	2	3	4
GST	+	-	+	-
GST-α_2M	-	+	-	+
6xHis-syntenin	+	+	+	+

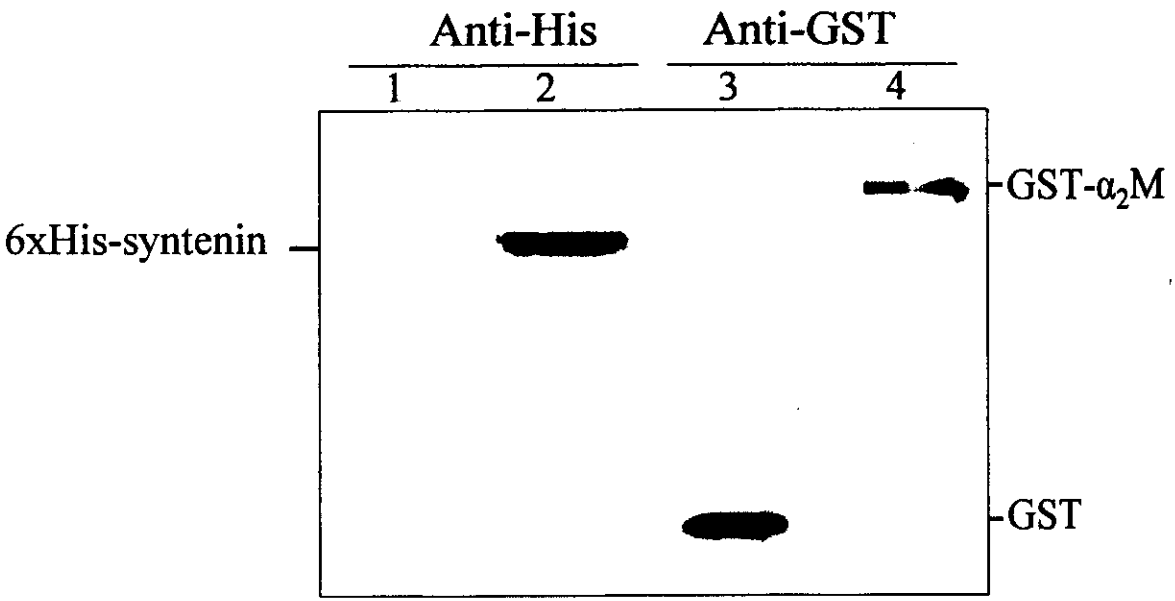


Fig. 3

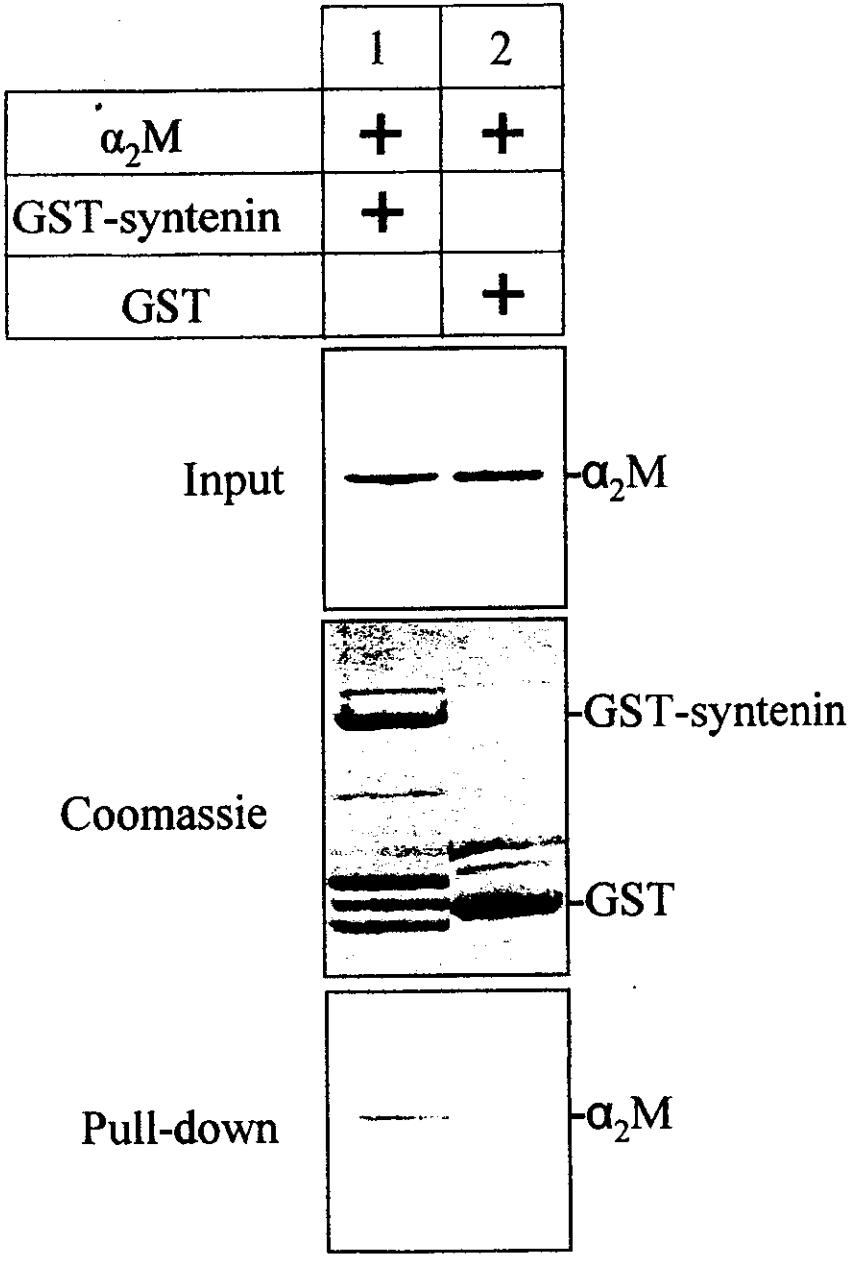

1
2
α2M
+
+
GST-syntenin
+
GST
+
Input
α2M
Coomassie
GST-syntenin
GST
Pull-down
α2M

Fig 4A

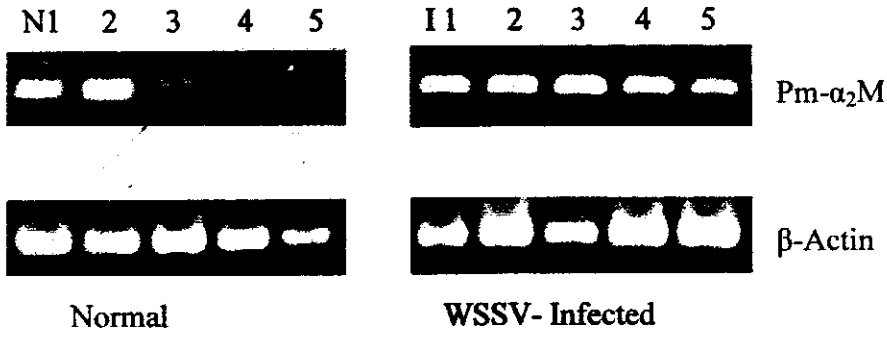
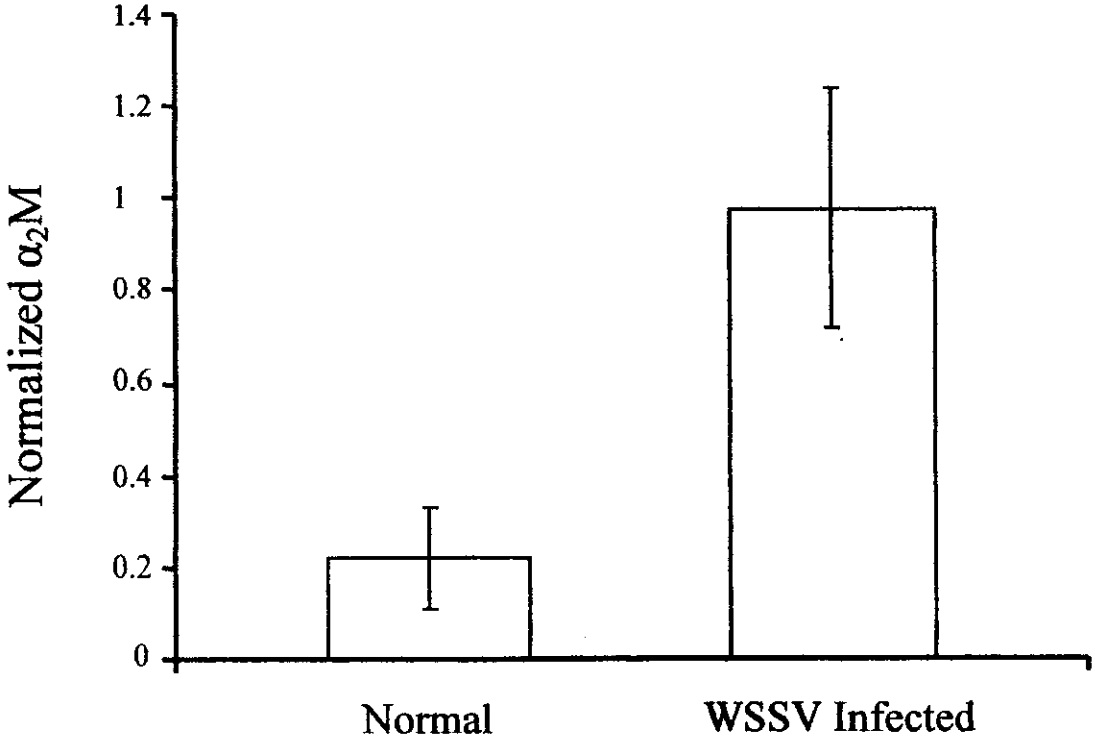

N1 2 3 4 5
I 1 2 3 4 5
Pm-α2M
β-Actin
Normal
WSSV- Infected

Fig 4B


Normalized α2M
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Normal
WSSV Infected

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Bielefeld, 01. Juni 2005

Notice of Acceptance
MS No. 05-01-035

Dear Doctor Phongdara,

The following manuscript has been accepted for publication in **Journal of Biotechnology** and has been forwarded to the publisher from whom you will receive further information soon.

Author(s): Tonganunt et al.

Title: Identification and characterization of syntenin binding protein in the black tiger shrimp *Penaeus monodon*

Date of submission: January 17, 2005
Receipt of revised version: May 17, 2005
Date of acceptance: June 1, 2005

Sincerely yours

(A. Pühler)