

Additional file 1

Table S1 Primers used in this study.

Primer	Sequence (5' to 3')	Applications
20aaC-VP3-VP1-F	<u>GGATCC</u> ATG GT TTTGGCATCTGC	Cloning 20C-VP3-VP1
<i>Sal</i> I-TAA-Co1-VP1-R	<u>GTCGACT</u> TTAT GGTAGTGGACT TCTAGCTGGTAGTTGATGGA AAGATTGTTCCACTGGTG	Cloning 20C-VP3-VP1
<i>Bam</i> HI-Ramyl-F	<u>GGATCC</u> ATG TCTCAGGTGCTG	Cloning surface display
Co1-20C-VP1-OL-F	AGTCCACTACCAGTTTTGGCA TCT	Cloning surface display
Co1-20C-VP1-OL-R	AGATGCCAAAAGTGGTAGTGG ACT	Cloning surface display
VP1-AGA1-OL-F	GGAACAAGGAGGGGGAGGCA GCGGTGGAGGGGGAAGTGGT GGAGGTGGGAGCGCAAGTGC CAAAAGCTC	Cloning surface display
VP1-AGA1-OL-R	GAGCTTTTGGCACTTGCGCTC CCACCTCCACCACTTCCCCCT CCACCGCTGCCTCCCCCTCCT TGTTCC	Cloning surface display
<i>Sal</i> I-TAA-AGA1-R	<u>GTCGACT</u> AAGA ATAGCAGGTA CG	Cloning surface display
GPD probe-F	GTTTCCCATGACGACAAGCAC A	Probe for Northern blot analysis
GPD probe-R	CAACTTGACGAACTTTGGAGA CAAT	Probe for Northern blot analysis

Underlines indicate recognizing sequences for corresponding restriction enzymes.
Bold sequences indicate start and stop codons.

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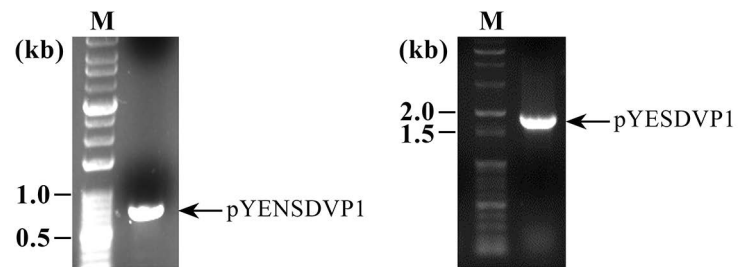


Fig. S1 The PCR analysis for the presence of 20aaC VP3-VP1-Co1 and Ramy1A-Co1-20aaC VP3-VP1-AGA1.

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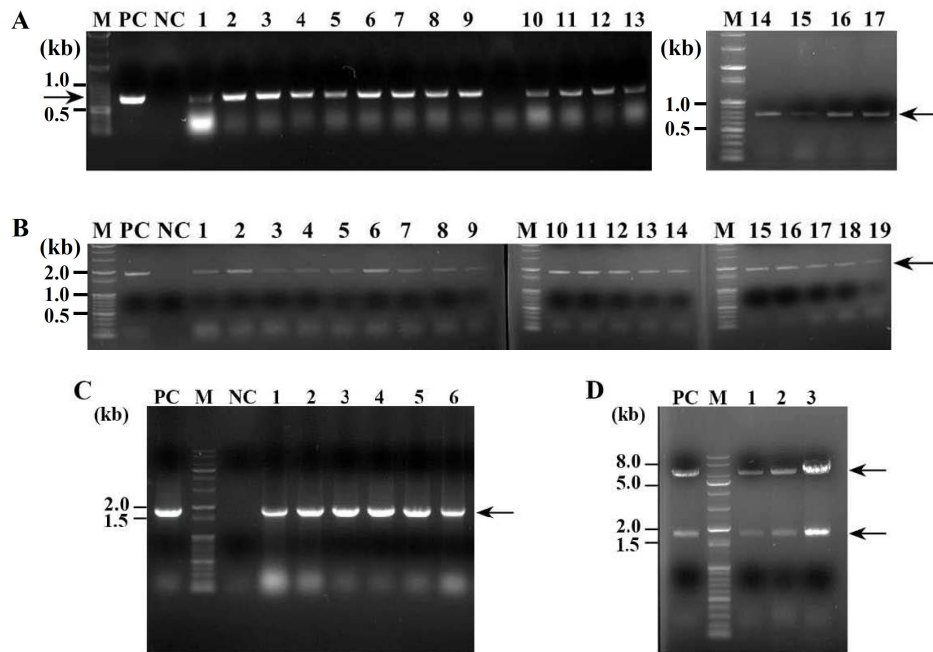


Fig. S2 The colony PCR products from yeast transformants harboring the non-surface construct pYENS DVP1(720bp) (A) and surface construct pYES DVP1 (1.8kb) (B). Products from the PCR of *E. coli* back-transformant colonies (1.8 kb) (C) and *Bam*HI and *Sal*I digests of three randomly selected plasmids (1.8 kb) (D). PC: original plasmid used for yeast transformation.

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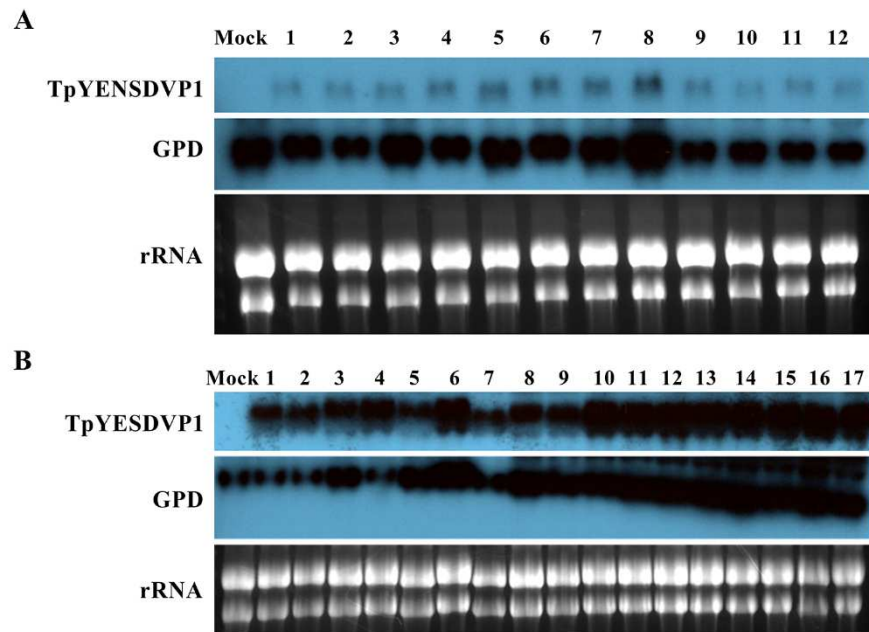


Fig. S3 Northern blot analysis of the non -surface construct pYENS DVP 1 (**A**) and the surface construct pYES DVP 1 (**B**). RNA preparations from cells harvested three days after cultivation at 20 °C. *GPD* was used as the internal control, and rRNAs indicate equal amounts of RNA loaded on each lane.

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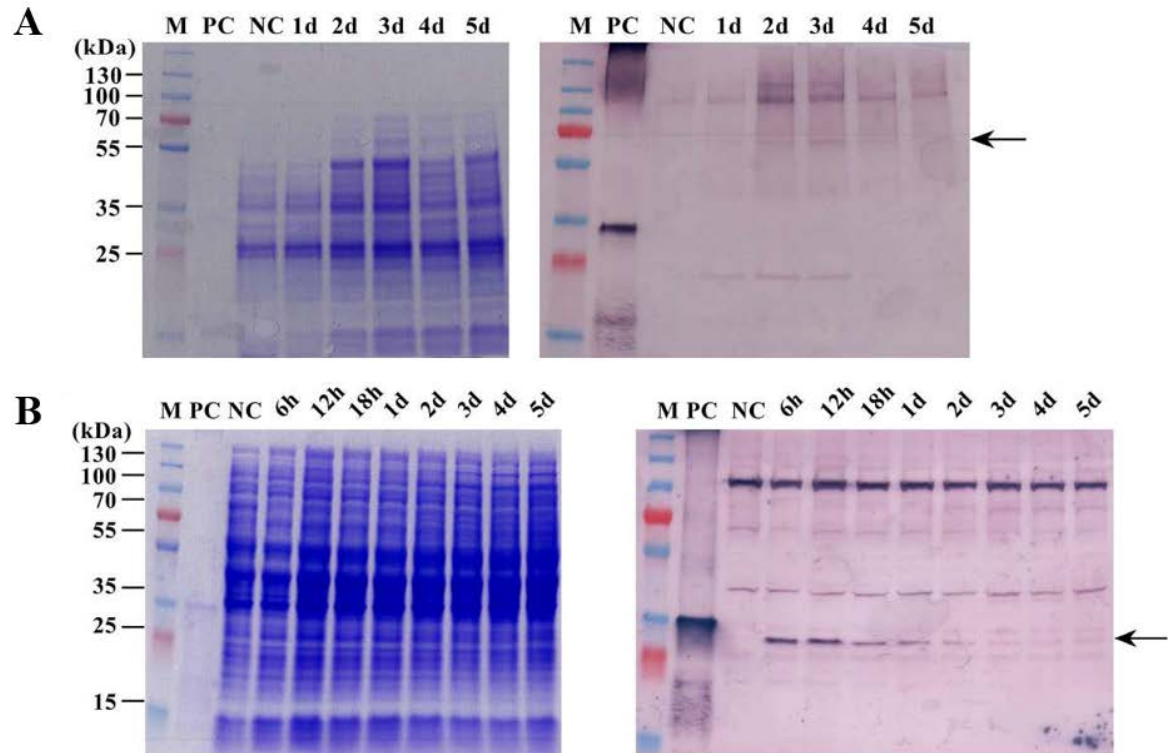


Fig. S4 Western blot analysis of TpYESDVP 1-6 (A) and TpYENSVDVP 1-8 (B) yeast transformants harvested after 1, 2, 3, 4, and 5 days of incubation at 20 °C using commercial antibodies against VP1. The corresponding *E. coli*-expressed VP1 protein was fused with a His tag and used as the PC. The NC contained the protein preparation from the mock transformant (vector only). Arrows indicate the target protein.

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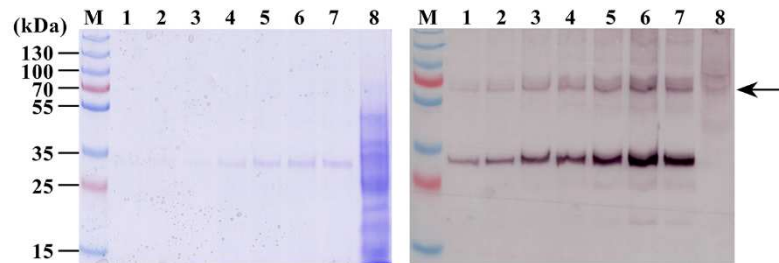


Fig. S5 Western blot analysis of yeast-expressed TpYESDVP 1-6 to quantify the expression of the fused target protein. Western blot analysis of serially diluted-known amounts of *E. coli*-expressed VP1, which were subsequently compared with those of yeast-expressing TpYESDVP1-6. Lanes 1-7 contain 0.15, 0.2, 0.3, 0.4, 0.5, 0.75, and 1.0 μg of *E. coli*-expressed VP1, respectively. Lane 8 contains 50 μg of total cell wall protein extracted from yeast-expressed TpYESDVP1-6. A representative twin SDS-PAGE gel stained with Coomassie Brilliant Blue is shown for comparison. Densitometry of cross-reacting band intensity was performed to estimate the protein expression levels. An arrow indicates the target protein.

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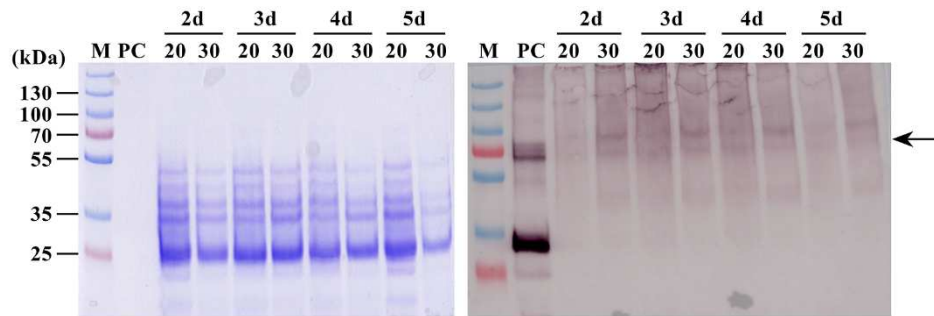


Fig. S6 Western blot analysis of the TpYESDVP1-6 selected using commercial antibodies against VP1. The corresponding *E. coli*-expressed VP1 protein was fused with His-tag and used as the PC. 20 and 30 indicate TpYESDVP 1-6 total proteins at 20 °C and 30 °C, which were extracted after cultivation at 2, 3, 4, and 5 days (2d, 3d, 4d, and 5d). Representative twin SDS-PAGE gels stained with Coomassie Brilliant Blue are shown for comparison. An arrow indicates the target protein.