

SUPPLEMENTARY INFORMATION

Supplemental Table 1: List of primers used in this study

Primer Name	Sequence
DhPPZ1 cloning	
DhPPZ1_ORF F	5'-CTAGCTAGCATGGGAACAATTCATCTAAG-3'
DhPPZ1_ORF R	5'-CCGCTCGAGTTACAGCTGTTGCAACTGAG-3'
DhPPZ1_Full F	5'-CGGGATCCTTATCCTACACGTCCAAC-3'
DhPPZ1_Full R	5'-CCGCTCGAGTAACAGATTCTAATAAGGGC-3'
DhPPZ1_ORF new RFP F	5'-TATACCATGGATGGGAACAATTCATCTAA-3'
DhPPZ1_ORF RFP R	5'-CCGCTCGAGCAGCTGTTGCAACTGAGC-3'
DhPPZ1 mutants	
DhPPZ1_seq1	5'-AGAAATGATTAACACTTGGAC-3'
DPPZ1_flanking F	5'-TTTCTACGAACGCTCACG-3'
DhPPZ1_DEL1 F	5'-CTCGGAAAGATGCTGAGGGATGATAATTCA-3'
DhPPZ1_DEL1 R	5'-CCCTCAGCATCTTGCCAGTTCCCG-3'
DhPPZ1_DEL2 F	5'-TTCACGCAAGATACCGATTAACGGGAATAAC-3'
DhPPZ1_DEL2 R	5'-TAATCGGTATCTGCGTGAAGTAGCCTTC-3'
DhPPZ1_DEL3 F	5'-GATTAACGGGAAAATCCATCAAATCAGCAT-3'
DhPPZ1_DEL3 R	5'-ATGGATTTTCCCCTTAATCGGTATGTTC-3'
DhPPZ1_DEL4F	5'-CTTACATAATCAATGATACAAGTGGAACCTAA-3'
DhPPZ1_DEL4R	5'-GTATCATTGATTATGTAAGCTTAGGTCGAAA-3'
DhPPZ1_DEL5F	5'-TCATTGGCACATTACACATCATTCTGGAAGTAT-3'
DhPPZ1_DEL5R	5'-GATGTGAATGTGCCAATGAGGTGAATTTC-3'
DhPPZ1_DEL6 F	5'-CCTACTGCATATTGATATTGAAAACCTGATTCAAG-3'
DhPPZ1_DEL6 R	5'-CAATATCAATATGCAGTAGGTTATTAAATCAGTACAT-3'
DhPPZ1_F NEW	5'-AAGGATCCTTATCCTACACGTCCAACATTA-3'
DhPPZ1_R NEW	5'-AATAGAGCTCTAACAGATTCTAACATAAGGGCTCG-3'
DhPPZ1_int R1	5'-TATACCATGGTCTCTAGTTAAACTACCAACG-3'
DhPPZ1_int F1	5'-AATACCATGGGAACAATTCTAACAG-3'
DhPPZ1_int R2	5'-CATATGTACATGCCAACCAACCTAACAG-3'
DhPPZ1_NterR	5'-TATCTCGAGTTAGTGTATGATGAGAGGCCGC TATCGTCG-3'
DhPPZ1_Cter F	5'-TTAACCATGGATGATTGATATTGAAAACCTGATTCA-3'
DhPPZ1_303R	5'-TATAAGCTTAGGTCAAAACGAA-3'
4SA for	5'-GCTCAAGAGCCATTAGAGCAAGGATAGCTATGCTGA GGGATGATAATTCA-3'
4SA rev	5'-AGCTATCCTGCTCTAATGGCTCTGAAGCCTTGCCGA GTTTCCCG-3'
3RE for	5'-TCTTCAGAACATTGAATCAGAGATATCTATGCTGAGG GATGATAATTCA-3'
3RE rev	5'-AGATATCTCTGATTCAATGGATTCTGAAGACTTGCCG AGTTTCCCG-3'
3RA for	5'-TCTTCAGCATCCATTGCATCAGCGATATCTATGCTGAGG GATGATAATTCA-3'
3RA rev	5'-AGATATCGCTGATGCAATGGATGCTGAAGACTTGCC GAGTTTCCCG-3'
SRAA for	5'-GCTTCAGCAGCCATTGCAGCAGCGATAGCTATGCTGAGG

	GATGATAATTCA-3'
SRAA rev	5'-AGCTATCGCTGCTGCAATGGCTGCTGAAGCCTTGCC GAGTTTCCCC-3'
SRAE for	5'-GCTTCAGAACCCATTGAAGCAGAGATAGCTATGCTGAGG GATGATAATTCA-3'
SRAE rev	5'-AGCTATCTCTGCTTCAATGGCTCTGAAGCCTTGCCGA GTTTCCCC-3'
S27A for	5'-AACTCGGCAAAGgCTTCAAGATCCATTAGATCAAGG-3'
S27A rev	5'-TGGATCTTGAAGCCTTGCCGAGTTCCCC-3'
S30A for	5'-AAGTCTCAAGAgCCATTAGATCAAGGATATCTATGCT-3'
S30A rev	5'-TTGATCTAATGGcTCTTGAAGACTTGCAGTT-3'
S33A for	5'-AGATCCATTAGAgCAAGGATATCTATGCTGAGGG-3'
S33A rev	5'-TAGATATCCTTGCCTAATGGATCTTGAAGACTTGC-3'
S36A for	5'-AGATCAAGGATAgCTATGCTGAGGGATGATAATTCA-3'
S36A rev	5'-CCCTCAGCATAGcTATCCTTGATCTAATGGATCTTGAA-3'
R29A for	5'-GCAAAGTCTTCAGcATCCATTAGATCAAGGATATCTATGC-3'
R29A rev	5'-GATCTAATGGATgcTGAAGACTTGCAGTTTC-3'
R32A for	5'-TCAAGATCCATTgcATCAAGGATATCTATGCTGAGGG-3'
R32A rev	5'-GATATCCTTGATgcAATGGATCTTGAAGACTTGC-3'
R34A for	5'-TCCATTAGATCAgCATACTATGCTGAGGGATGATAAT-3'
R34A rev	5'-AGCATAGATATCgcTGATCTAATGGATCTTGAAGACTT-3'

DhMPK1 cloning

DHMPK11_full f	5'-CGGGATCCAATATGTAGTCAAAGAGTGCG-3'
DHMPK11_full R	5'-CCGCTCGAGGCTAGTAATTGATTGTCGC-3'
DhMPK1_flankingF	5'-CGTATTGAAGCAATGGCTAG-3'

PPZ1 cloning

Scppz1 Full F	5'-AAATGCATGCGCCTCCAATTCAACAAACTA-3'
Scppz1 orf R	5'-AAGGTACCTTACTGTTGAGATTGTTATCA-3'
Scppz1 del F	5'-AAATCAAATAATCGTCCACTACGAATACTAATTC-3'
Scppz1del R	5'-TCGTAGTGGACGATTATTGATTTCAGACTTC-3'

RT-PCR Primers

DHTRK1_RTF	5'-TCAGAGGGAGGCACAGAGGTTG-3'
DHTRK1_RTR	5'-GCATTCCCATCATCCAAGCTATTG-3'
DHENA1_RTF	5'-TTTCCCAGTCGCTACATTCTGTAATC-3'
DHENA1_RTR	5'-TGGATCATTCTCTCTAAGTCGTAATCTGG-3'
DHNHA1_RTF	5'-GTGGATCTGAAGAGACTGATGAGGATG-3'
DHNHA1_RTR	5'-TCATCTTCTTCGCTATGAGTTGGC-3'
Dh_GPD F	5'-TTGTCTCCACCGATTCTTAG-3'
Dh_GPD R	5'-CAAGTCGACAACCTGGTAGAG-3'

Two hybrid cloning

DhHAL3 ORFf	5'-GGAATTCCATATGGTTCAGAAAATGGTG-3'
DhHAL3 ORFr	5'-CCGCTCGAGTTAAGTGTATTAGATTCTCCTC-3'
DhPPZ1 2HF	5'-ATCGGATCCATATGGGAACAATTCTAAG-3'
DhPPZ1_ORF R	5'-CCGCTCGAGTTACAGCTGTCAGACTGAG-3'

SUPPLEMENTAL FIGURES

Figure S1. DhPPZ1 protein comparison. Multiple alignment of DhPpz1p (XP_459586.2) with *S. cerevisiae* Ppz1p (NP_013696.1) and Ppz2p (NP_010724.1). N-terminal region is in black and C-terminal catalytic domain is in red. N- myristoylation site is colored and underlined.

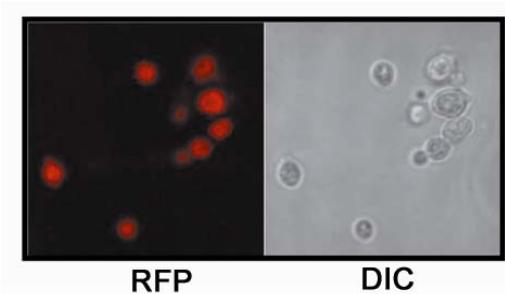
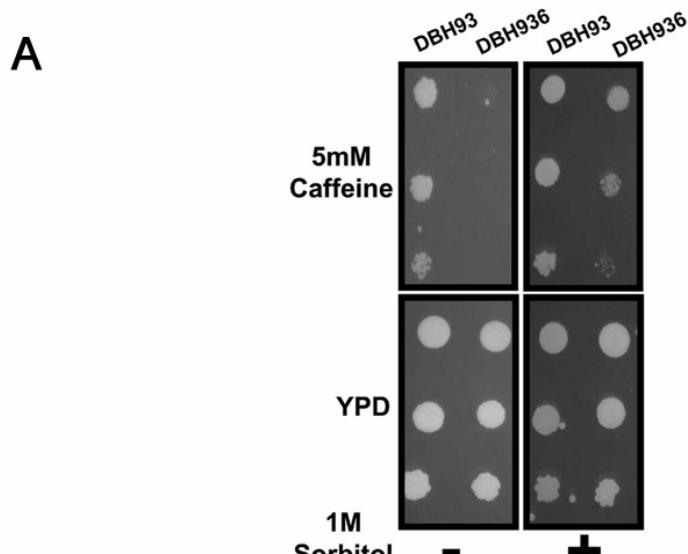


Figure S2. Localization of the DhPpz1p-RFP fusion protein. *D. hansenii* strain DBH93 harboring plasmid pAN4 (DhPPZ1-RFP) at logarithmic phase was observed under fluorescence microscope. RFP fluorescence is shown in red. Right panel shows the DIC image of the same cells.



B

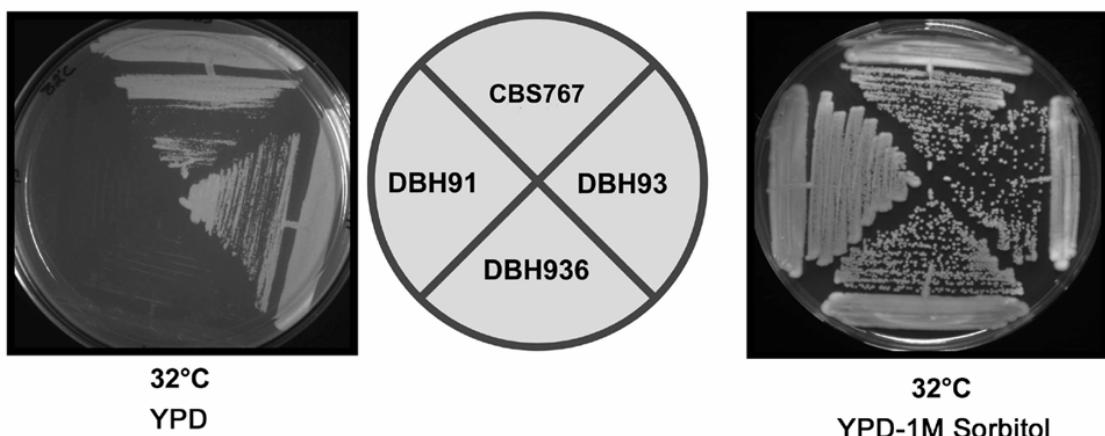


Figure S3. A. Sensitivity of *dhppz1* mutant towards cell wall destabilizing agent. Serial dilution of DBH936 (*dhppz1*) and DBH93 (parent) strains on YPD or YPD plus 1M sorbitol with 5mM caffeine incubated four days at 28°C **B. Temperature-dependent cell lysis defect of *dhppz1* mutants.** Growth of *dhppz1* mutants (DBH91 and DBH936) on YPD and YPD plate supplemented with 1M sorbitol at 32°C. CBS767 and DBH93 strains were used as control.



Figure S4. Temperature-dependent cell lysis defect of *dhmpk1* mutants. Growth of *dhmpk1* mutants (two independent transformants - DBH932 and DBH933) on YPD and YPD plate supplemented with 1M sorbitol at 32°C. CBS767 and DBH93 strains were used as control.

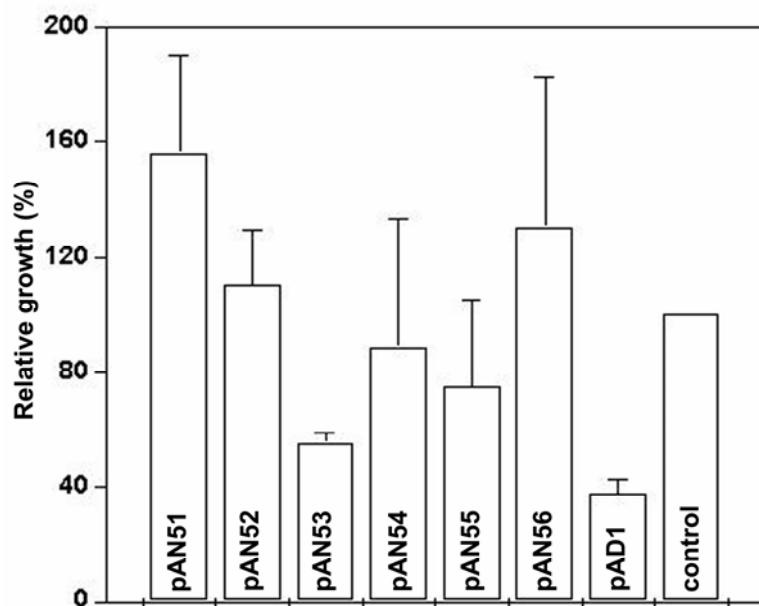


Figure S5. Effect of different N-terminal deletions on slow growth phenotype of *dhppz1* mutant. Saturated cultures of *dhppz1* mutant (DBH936) carrying different mutant plasmids pAN51, pAN52, pAN53, pAN54, pAN55, pAN56 or vector pDA1 were used to re-inoculate 25 ml YNB medium at initial OD₆₀₀ of 0.025. After 24 hr of growth at 28C, OD₆₀₀ of each culture was measured and expressed as relative growth as percentage of control strain (100%). DBH93 harboring plasmids pDA1 and pDH4 was used as control. Data presented as mean ± sd of three independent experiments.