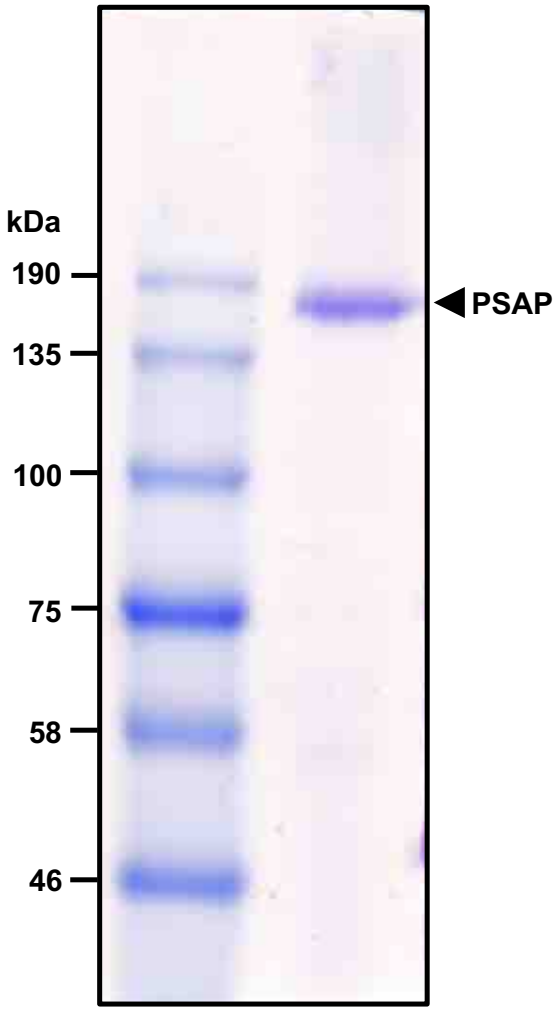
A



B

Migration distance of dye front (mm)
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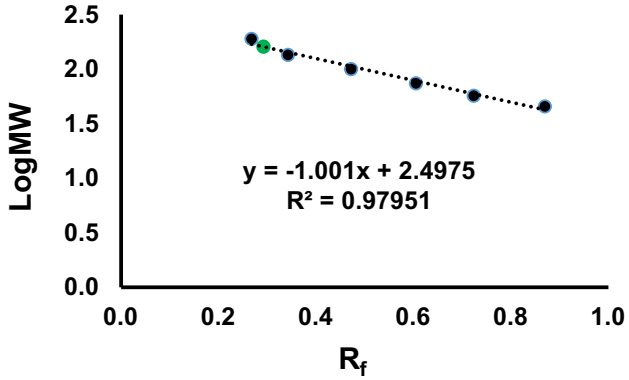
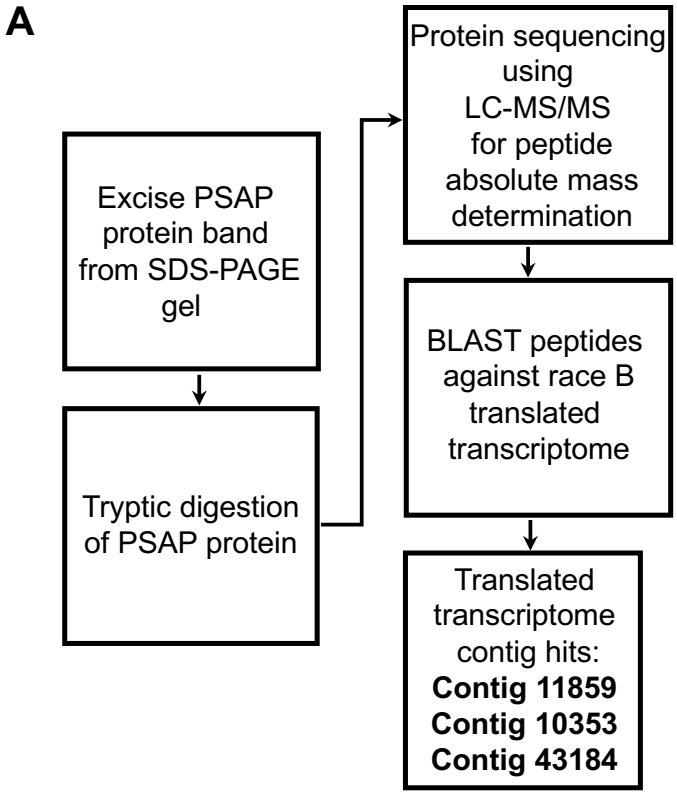
Migration distance (mm)	R_f values	Log MW	kDa
Protein Markers			
32	0.268	2.28	190
41	0.343	2.13	135
56.4	0.472	2.00	100
72.3	0.605	1.87	75
86.5	0.724	1.76	58
104	0.870	1.66	46
PSAP			
35	0.293	2.20	162.2



Supplementary Fig. 1. Analysis of PSAP molecular mass by SDS-PAGE. (A) The PSAP protein was extracted from shell material using SDS-PAGE sample buffer and separated by 12% SDS-PAGE followed by staining with Coomassie blue. (B) Calculation of PSAP molecular mass based on SDS-PAGE migration in comparison to molecular weight markers.



B

Contig 11859

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STASIFDANDVIQIFDTGDGRATVSVTLGGVTQPPFVITTASPPQFLYGKQCIF
LWASTVAITFSRNDLGVAFNPNNINTFLTILGLNTVQVISGAVTVQVTHTPNPI
PAGISPRFFTALQQAQALTFQECQNCLVNTNVAATVPRLVSLPGFALYVKSWNF
AARSGAQTSIFIKNTLFSNIGLTLASIACSPGNLQITGNTALLSLAGLTNMQTT
IVPGPTVFITNNQLLFGAPEVFQLQRLASCVGAATSPLFSAILIQSAACTSTCW
NQYCSFVNGFGCNACSPPPPPPQSPPP

Contig 10353

PSPPPPLPACNTPISPTTAPTCGSLYGFLSIADNGDGTCSAFRIEQTSATGTPT
TVALNCNQLAFPGIASCASIQSAVKITIRRTRSVAYIQPDINKWLTNMGLNNVI
VIANALTVEVDHSPFPIPQFINPVFFTLLRQVEAVVAQECANCATSTNVGPALP
ALASLAGLRAIQQFRGFKGGVSLGSLIIKGTALLDLTSFSGISCTPGFIQITNN
ARLGSFNGLEGARTFFNPGPTVFAQGNPLLNSQSVAPIRQLAQCPSGNTSPLNS
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PPPSPPSPPPPPPDATCFDPMPLPADICGDGITWTLQVQYIGNGTCFARLFFNG
TQ

Contig 43184

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ASIQTGTPPNTGIRNIPCNQLAFGQQCGSLNGSLTLTSFRASNGTAYRGPFFNS
WLSLMGLQNINALNGNLIVLHNQLINSIPPVTPADFLPRLNEVSGGLEANELRR
PGQTPSLLSVPGFKSVVFAQSAISVNGTAFPDFRNTFNGLICP

Supplementary Fig. 2. Identification of *B. braunii* race B transcriptome contigs matching PSAP MS peptides. (A) Step-by-step outline of PSAP MS analysis and identification of matching contigs. (B) Translation of the three *B. braunii* race B contigs that matched MS peptides from PSAP. PSAP peptides identified by MS that match the contigs are shown in alternating dark orange and light orange lettering. Methionine start site in contig 11859 is shown in black bold lettering.

A

Contig 11859

GACTGCGCTAAGGCCCAGTTGTCAAGCAGTTCTCCCGAAATATAAGAGCTTCGGTGAAAGGTAAAGGAGCAAAGA
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B

Contig 10353

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CCAACATGGGCCTTAACAACGTGATTGTGATTGCAAACGCCCTGACTGTTGAAGTCGACCACTCCCCTTTCCCGA
TTCCCCAATTCATTAATCCAGTTTTCTTCACTCTTCTGAGA**CAGGTGGAAGCAGTTGTGGCTCAAGAGTGTGCCA**
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AGTTCCGAGGCTTCAAG**GGAGGGGTTTCTCTTGGTTCCCTCATCATCAAG**GGCACAGCGTTATTGGACTTGACAT
CCTTCTCAGGCATCAGTTGCACGCCAGGCTTCATCCAGATCACCAACAATGCCCGT**CTGGGCAGCTTCAATGGGT**
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TCTTTAACGCACAG

C

Contig 43184

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GTTGAAGTCCTCGACTACGGTGATGCAACTTGCACTGCCTCTATTCAGACAGGAACCCCCCCCAACACTGGTATC
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GATTTCCTACCAAGACTGAATGAAGTCTCTGGTGGTCTGGAAGCAAACGAGCTTCGTAGGCCTGGCCAGACTCCG
TCACTTCTTTCAGTTCCTGGATTCAAGTCAGTTGTATTTGCACAGTCAGCCATTTCTGTTAATGGCACAGCTTTC
CCTGATTTCAGAAATACCTTCAACGGCCTGATCTGCCCACC

Supplementary Fig. 3. Nucleotide sequence of the three *B. braunii* race B contigs that matched MS peptides from PSAP. (A) Contig 11859, translation start codon shown in red lettering. (B) Contig 1053. (C) Contig 43184. Bases in alternating dark orange and light orange lettering correspond to the peptides found by MS analysis.

A **PSAP ORF sequence, 3,798 bp**

ATGGCGAAGGTGCCGCTGGCACTACTGCTGCTGAGTTTGACCTTTGCCGCCCACGGTCTCGAGGCCCCAGCTCTCGCTCCCGACTCTCAGGCCGCGGCGGA
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CCTCACCATCCTGGGATTGAATACAGTGCAGGTCATCTCAGGTGCCGTTACAGTGCAGGTCACCCACACTCCAAACCCTATTCCTGCTGGCATCAGCCCCC
GGTTCTTCACTGCTCTACAGCAGGCACAAGCACTCACATTCCAGGAGTGCCAGAACTGTTTGGTGAACACCAATGTTGCTGCAACAGTCCCCAGGCTTGTG
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TGGTCTCACCTTGGCCTCAATTGCTTGCTCGCCTGGTAACCTCCAGATTACTGGTAACACAGCTCTGTTAAGCCTGGCTGGGTTGACCAATATGCAAACTA
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GCAACCAGTCCCCTGTTTTCAGCAATCTTGATTCAGTCAGCTGCTTGCACATCAACGTGCTGGAATCAATACTGCTCCTTCGTCAATGGATTTGGCTGCAA
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GGATGTCCCACCCCCTGTACCCCGACTGGTAGGCCTTCCGGGCCTGACGGGCGTGAGGAGGATCATTGCCCCACCAGGCACTGGCTTCGGTGACTTCACCT
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CCAGCAGATTTCCTACCAAGACTGAATGAAGTCTCTGGTGGTCTGGAAGCAAACGAGCTTCGTAGGCCTGGCCAGACTCCGTCACTTCTTTCAGTTCCTGG
ATTCAAGTCAGTTGTATTTGCACAGTCAGCCATTTCTGTTAATGGCACAGCTTTCCCTGATTTCAGAAATACCTTCAACGGCCTGATCTGCCCACCCTACC
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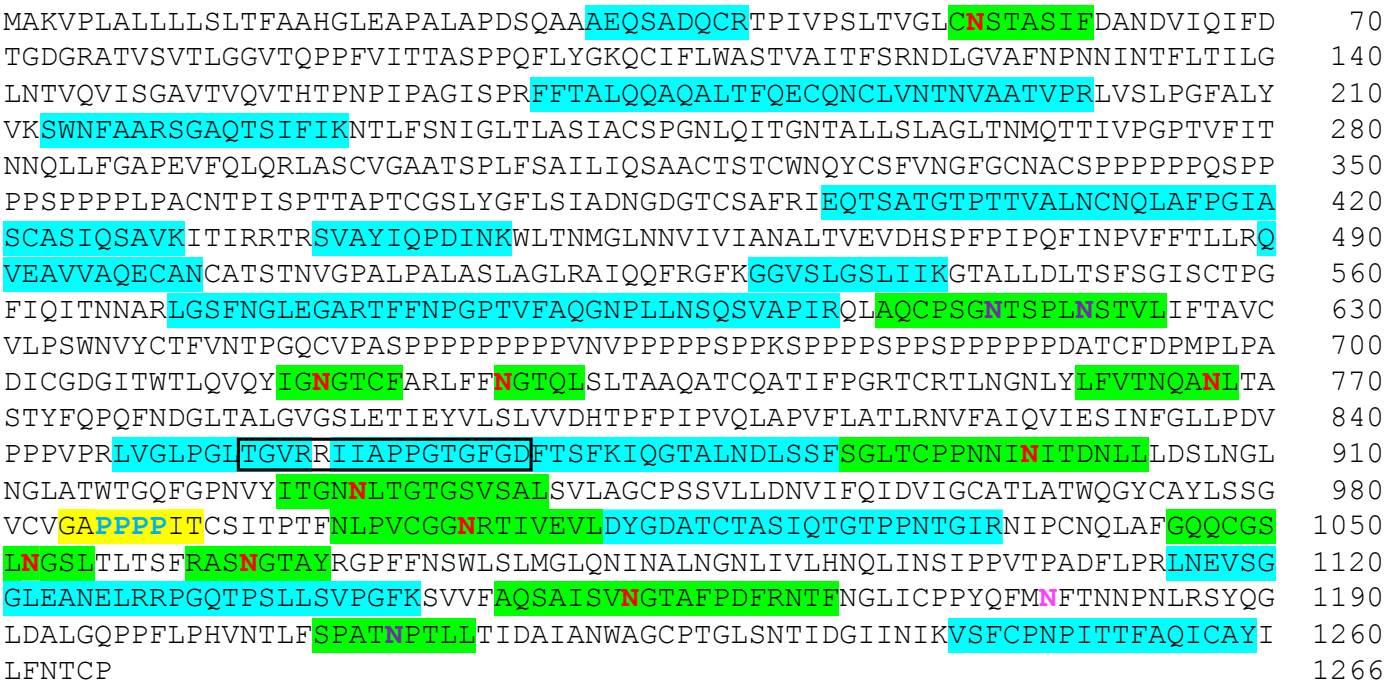
CCTGCCACCAATCCTACACTCCTCACCATTGATGCAATTGCAAACTGGGCAGGCTGCCCTACGGGCCTTAGTAACACAATCGACGGTATCATTAATATTAA
AGTTTCATTCTGCCCTAATCCCATCACCACTTTCGCACAAATCTGCGCTTATATTTTATTCAACACGTGCCCATAA

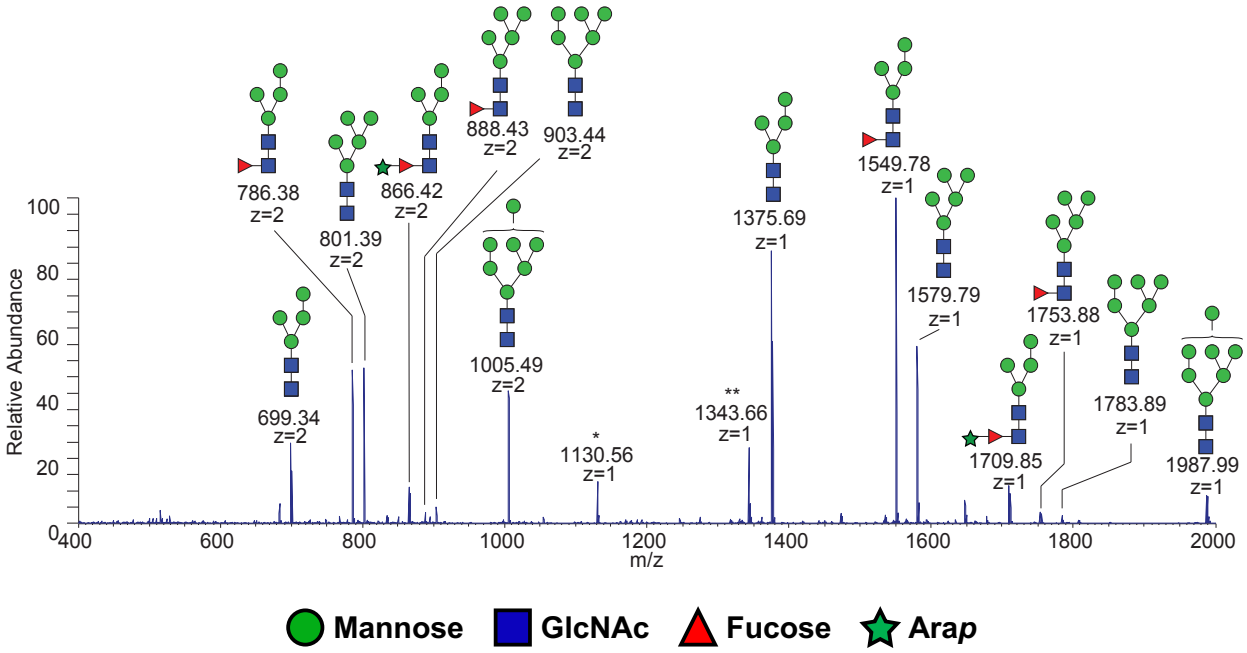
B **PSAP protein sequence, 1,266 amino acids, 132.8 kDa**

MAKVPLALLLLSLTFAAHGLEAPALAPDSQAAAEQSADQCRTPIVPSLTVGLCNSTASIFDANDVIQIFDTGDGRATVSVTLGGVTQPPFVITTASPPQFL
YGKQCIFLWASTVAITFSRNDLGVAFNPNNINTFLTILGLNTVQVISGAVTVQVTHTPNPIPAGISPRFFTALQQAQALTFQECQNCLVNTNVAATVPRLV
SLPGFALYVKSWNFAARSGAQTSIFIKNTLFSNIGLTLASIACSPGNLQITGNTALLSLAGLTNMQTTIVPGPTVFITNNQLLFGAPEVFQLQRLASCVGA
ATSPLFSAILIQSAACTSTCWNQYCSFVNGFGCNACSPPPPPPQSPPPPSPPPPLPACNTPISPTTAPTCGSLYGFLSIADNGDGTCSAFRIEQTSATGTP
TTVALNCNQLAFPGIASCASIQSAVKITIRRTRSVAYIQPDINKWLTNMGLNNVIVIANALTVEVDHSPFPIPQFINPVFFTLLRQVEAVVAQECANCATS
TNVGPALPALASLAGLRAIQQFRGFKGGVSLGSLIIKGTALLDLTSFSGISCTPGFIQITNNARLGSFNGLEGARTFFNPGPTVFAQGNPLLNSQSVAPIR
QLAQCPSGNTSPLNSTVLIFTAVCVLPSWNVYCTFVNTPGQCVPASPPPPPPPPVNVPPPPPSPPKSPPPPSPPSPPPPPPDATCFDPMPLPADICGDGI
TWTLQVQYIGNGTCFARLFFNGTQLSLTAAQATCQATIFPGRTCRTLNGNLYLFVTNQANLTASTYFQPQFNDGLTALGVGSLETIEYVLSLVVDHTPFPI
PVQLAPVFLATLRNVFAIQVIESINFGLLPDVPPPVPRLVGLPGLTGVRRIIAPPGTGFGDFTSFKIQGTALNDLSSFSGLTCPPNNINITDNLLLDSLNG
LNGLATWTGQFGPNVYITGNNLTGTGSVSALSVLAGCPSSVLLDNVIFQIDVIGCATLATWQGYCAYLSSGVCVGAPPPPITCSITPTFNLPVCGGNRTIV
EVLDYGDATCTASIQTGTPPNTGIRNIPCNQLAFGQQCGSLNGSLTLTSFRASNGTAYRGPFFNSWLSLMGLQNINALNGNLIVLHNQLINSIPPVTPADF
LPRLNEVSGGLEANELRRPGQTPSLLSVPGFKSVVFAQSAISVNGTAFPDFRNTFNGLICPPYQFMNFTNNPNLRSYQGLDALGQPPFLPHVNTLFSPATN
PTLLTIDAIANWAGCPTGLSNTIDGIINIKVSFCPNPITTFAQICAYILFNTCP

Supplementary Fig. 4. Nucleotide and protein sequence of PSAP. (A) PSAP ORF nucleotide sequence. Bases shown in alternating dark orange and light orange lettering correspond to the peptides found by MS analysis. Contig locations are identified by a black box for contig 11859, a red box for contig 10353, and a purple box for contig 43184. (B) PSAP protein sequence. The PSAP signal peptide is highlighted in green. The PSAP peptides identified by MS that match the contigs are shown in alternating dark orange and light orange lettering. The Pro-rich motifs are shown in magenta lettering.

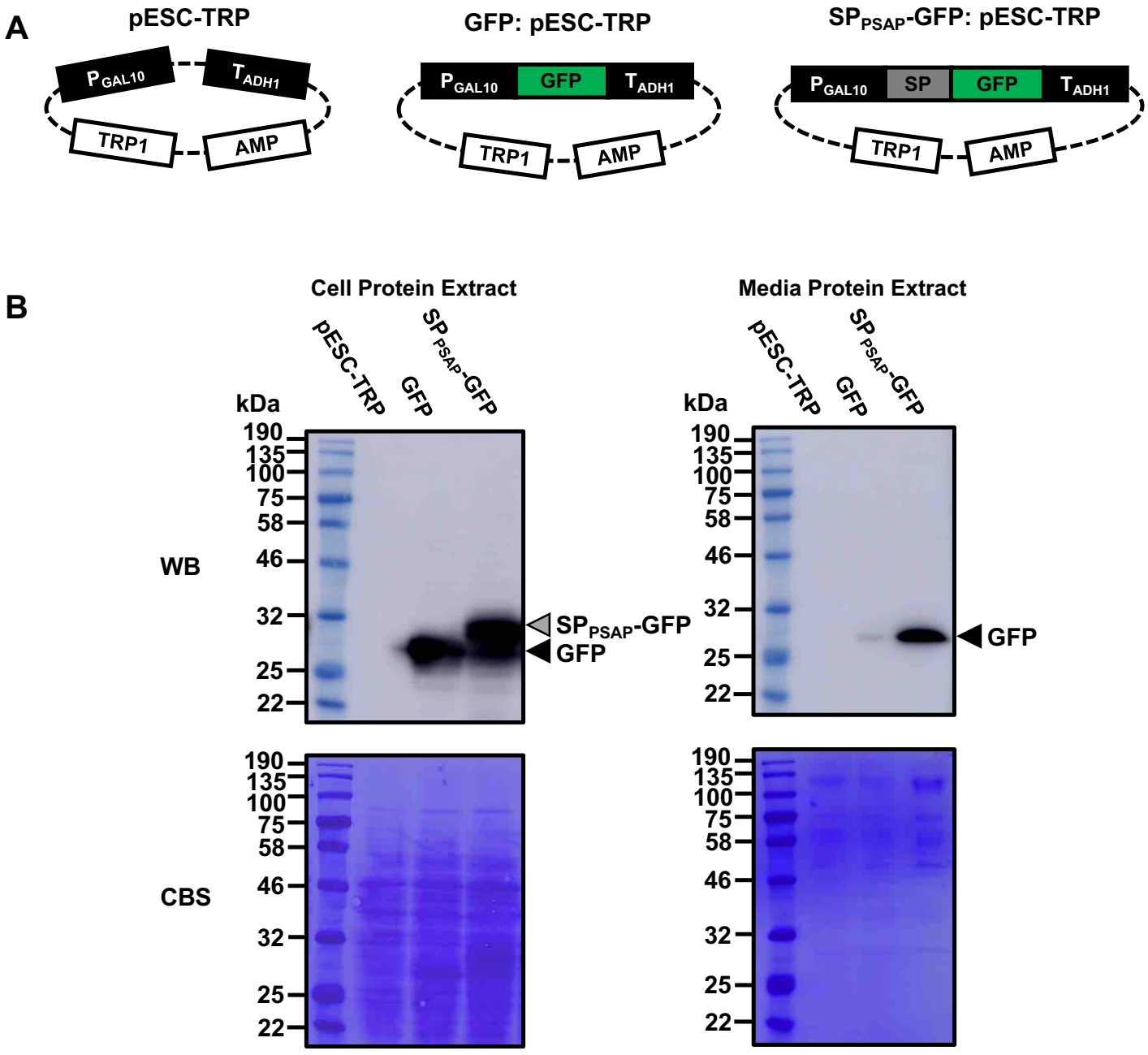


Supplementary Fig. 5. PSAP *N*-linked and *O*-linked glycan analysis. Peptides highlighted in cyan and green represent the peptides identified by LC-MS/MS in *N*-linked glycan analysis. Cyan peptides do not contain predicted *N*-linked glycosylation sites. Green peptides contain predicted *N*-linked glycosylation sites. Asn (N) residues highlighted in red were confirmed to be an *N*-linked glycosylation site; N54, N718, N728, N767, N897, N930, N1006, N1052, N1064, N1155. Asn (N) residues highlighted in purple were confirmed to not be an *N*-linked glycosylation site; N615, N620, and N1212 . Potential *N*-linked glycan site N1178, highlighted in magenta, was not found in any peptide identified in this study. The peptide highlighted in yellow represents the peptide identified to contain Hyp *O*-linked glycans; Hyp986, Hyp987, Hyp988, Hyp989 are shown in blue lettering. The protein sequence in the box identifies the peptide used for PSAP antibody development.

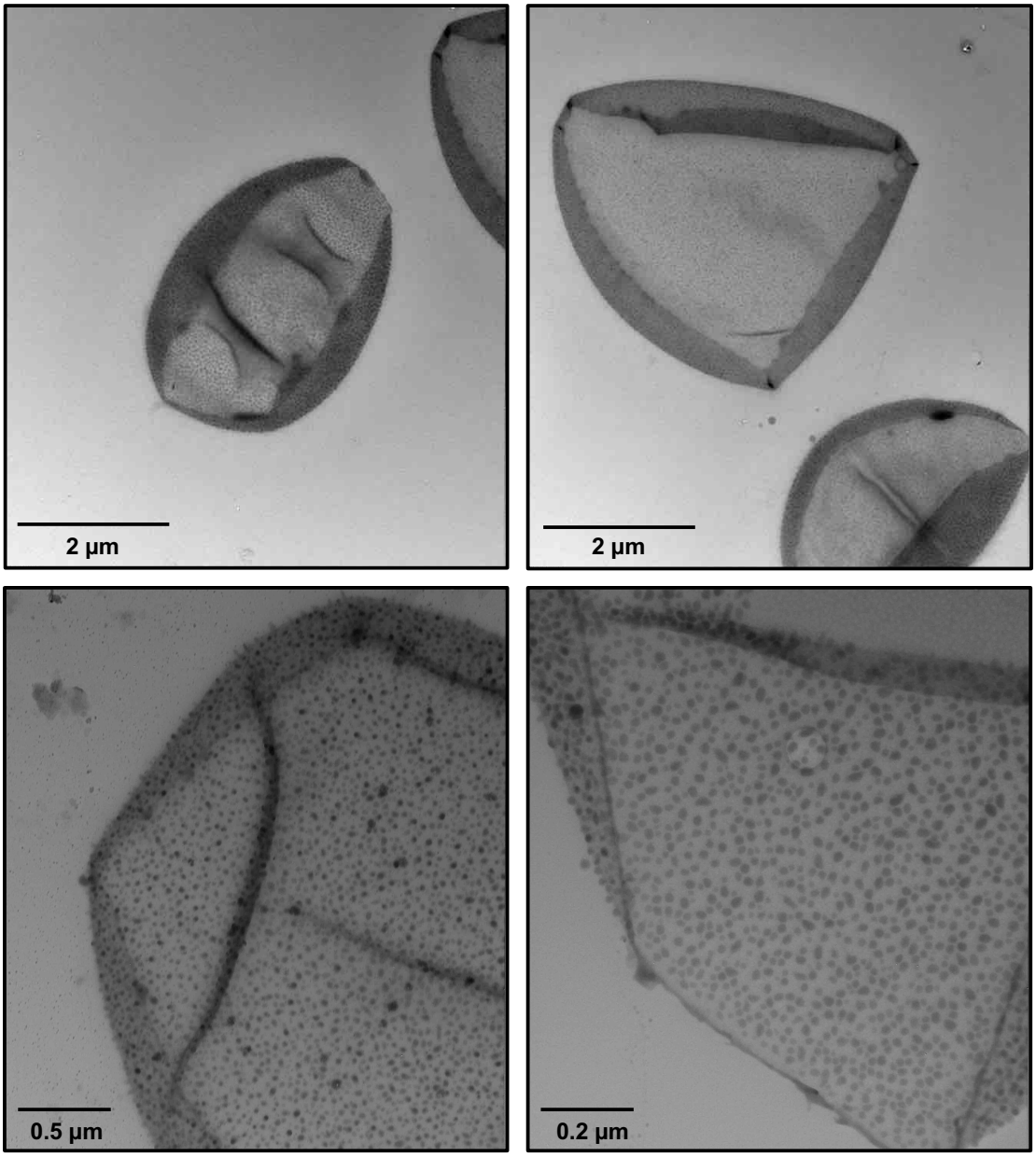


Supplementary Fig. 6. PSAP *N*-linked glycan analysis by NSI-MSn**.** *N*-linked glycans were released enzymatically from PSAP with PNGAse A, permethylated, and profiled by NSI-MSn. Full MS profile of the released permethylated glycans showing a series of high-mannose type *N*-glycans and fucosylated *N*-glycans as the major components. In addition, a unique *N*-glycan carrying fucose and arabinose was detected at *m/z* = 1709 (1+) and *m/z* = 866 (2+). A single asterisk indicates an *N*-glycan fragment with $Hex_4HexNAc_1$. Two asterisks indicates the in-source fragment ion, presumably a $Z_{1\beta}$ fragment ion from the precursor at *m/z* = 1709 ($Hex_4HexNAc_2Pent_1Deoxyhex_1$) due to a labile *O*-glycosyl linkage of the internal fucose.

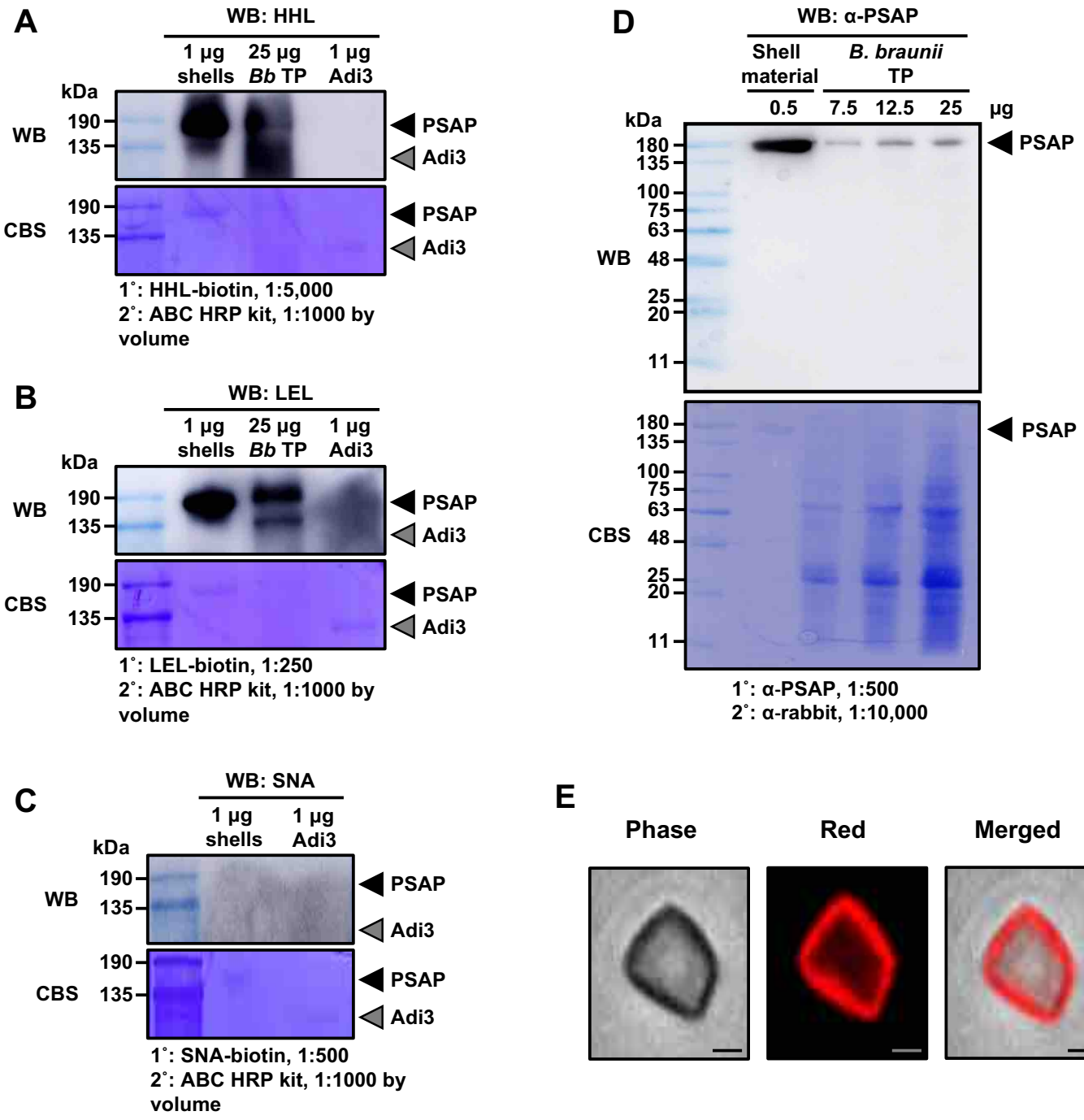
Figure 5



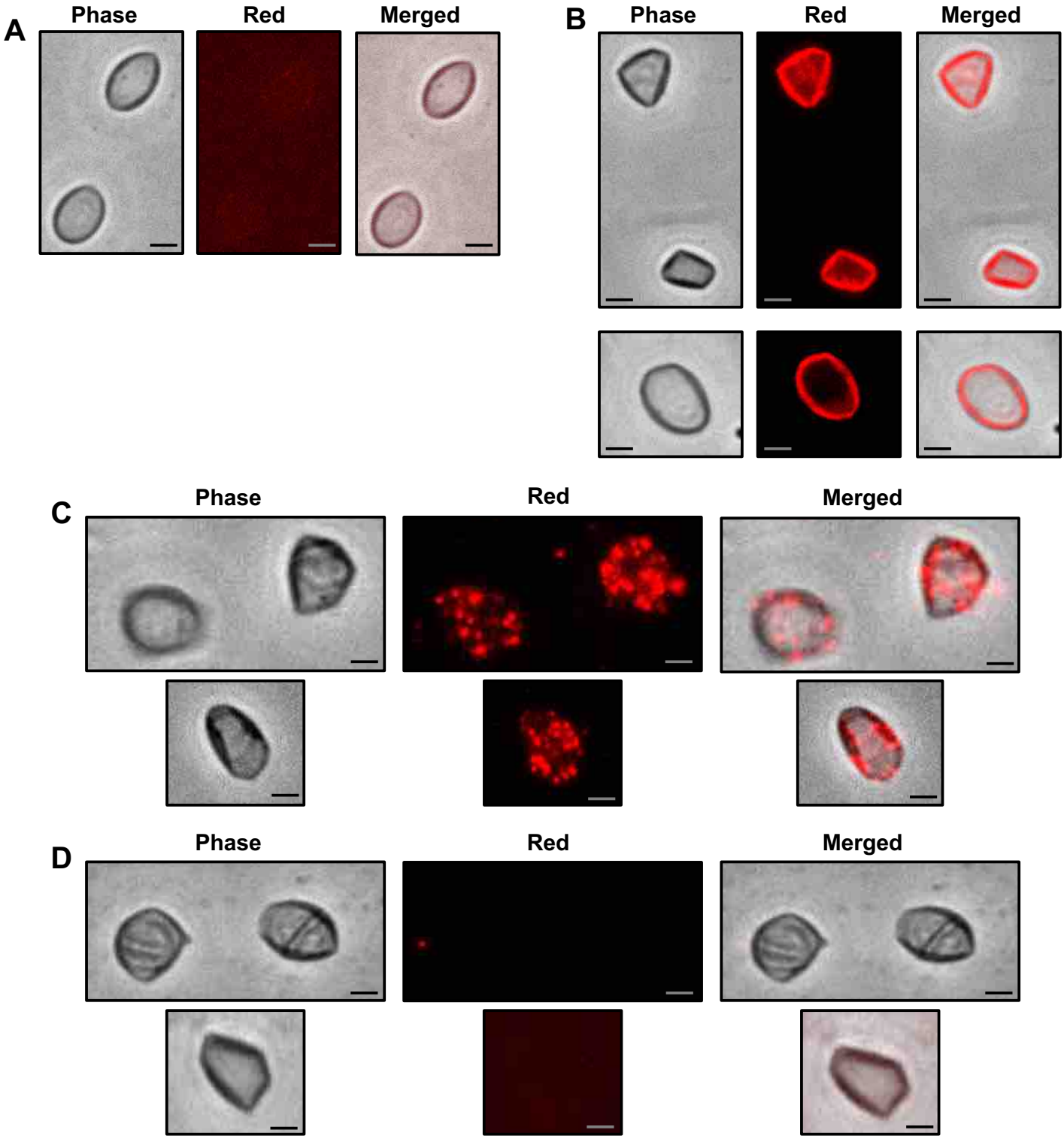
Supplementary Fig. 7. Functional analysis of the PSAP signal peptide. (A) The three constructs used for GFP secretion analysis in *S. cerevisiae*; pESC-TRP, GFP: pESC-TRP, and SP_{PSAP}-GFP: pESC-TRP. SP_{PSAP}, PSAP signal peptide. (B) Western blot detection of GFP from *S. cerevisiae* cell protein extracts and media protein extracts using the α-GFP-HRP antibody (1:3,000 for cell protein extracts and 1:1,500 for media protein extracts). WB, western blot; CBS, Coomassie blue stain of WB.



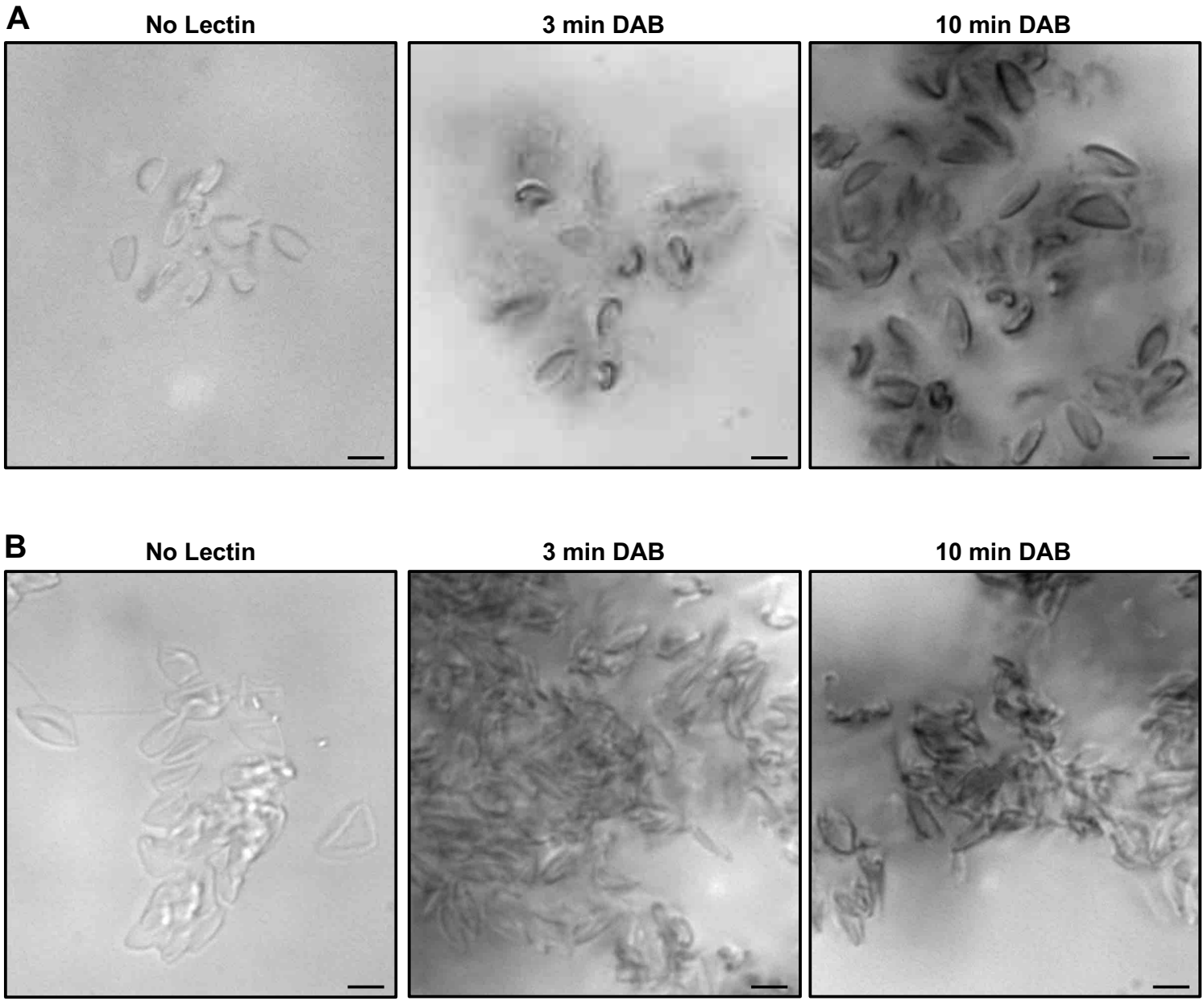
Supplementary Fig. 8. Electron micrographs of *B. braunii* race B shell material. Purified shells were resuspended in water and placed on an EM copper grid with carbon support film and allowed to dry. The shells were then visualized by TEM.



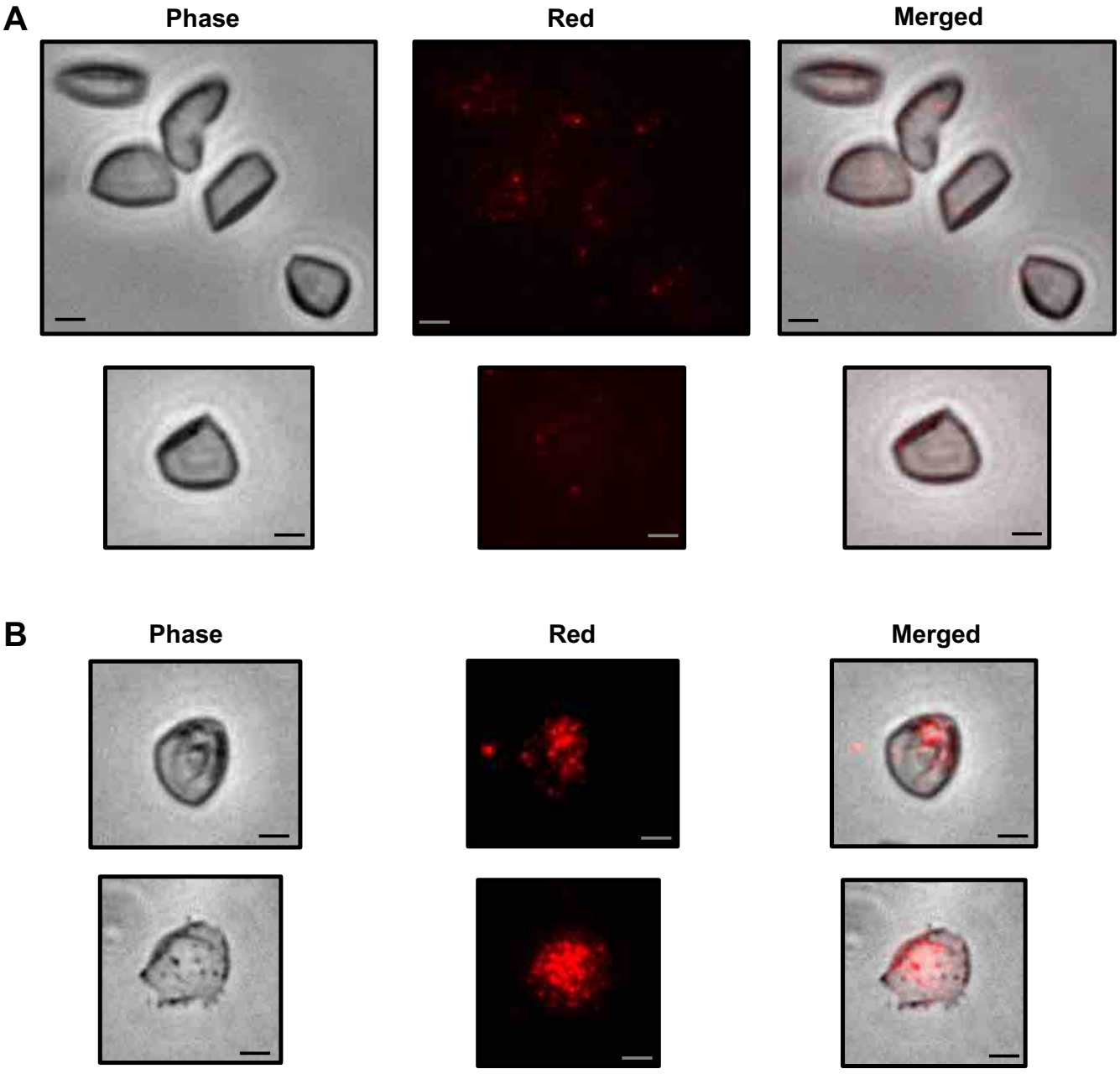
Supplementary Fig. 9. Lectin blot and western blot detection of PSAP from shell material. (A) HHL lectin blot (B) LEL lectin blot. (C) SNA lectin blot. (D) Western blot detection of PSAP from shell material and a *B. braunii* race B total protein extract using the α-PSAP antibody. (E) PSAP detection in shell material using the HHL lectin. Shell material was incubated with HHL (1:5,000) followed by incubation with streptavidin-Texas red (1:1,000) and fluorescence microscopy; Scale bar, 2 µm. WB, western blot; CBS, Coomassie blue stain of WB; TP, total protein.



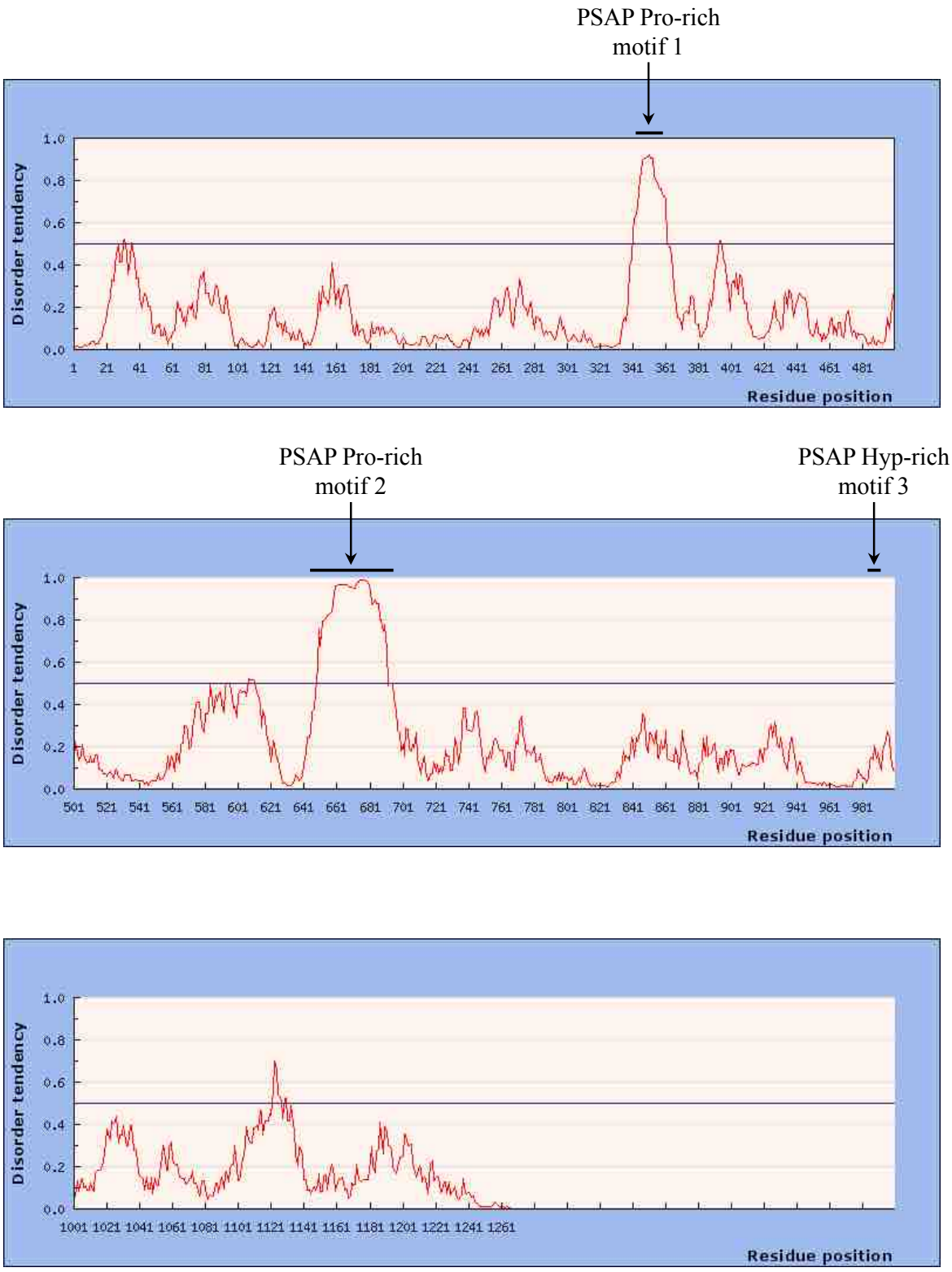
Supplementary Fig. 10. Detection of PSAP in shell material by fluorescence microscopy using lectins specific for PSAP glycans. (A) Negative control; no lectin used. (B) HHL lectin, 1:5,000; streptavidin-Texas red, 1:1,000. (C) LEL lectin, 1:250; streptavidin-Texas red, 1:1,000. (D) Negative control; SNA lectin, 1:500; streptavidin-Texas red, 1:1,000. Scale bars, 2 μm.



Supplementary Fig. 11. Light microscopy detection of PSAP in shell material using lectins specific for PSAP glycans and DAB. (A) PSAP localization in shells using the HHL lectin. Left panel, control with no lectin; middle panel, 3 min DAB exposure; right panel, 10 min DAB exposure. (B) PSAP localization in shells using the LEL lectin. Left panel, control with no lectin; middle panel, 3 min DAB exposure; right panel, 10 min DAB exposure. Scale bare, 5 μm



Supplementary Fig. 12. Detection of PSAP in shell material by fluorescence microscopy using the PSAP specific antibody (α-PSAP). (A) Negative control, no α-PSAP, anti-rabbit Texas red 1:1,000. (B) α-PSAP 1:100, anti-rabbit Texas red 1:1,000. Scale bar, 2 μm.



Supplementary Fig. 13. Identification of disordered regions in the PSAP proteins sequence. The PSAP protein sequence was analyzed by the IUPred web server (http://iupred.enzim.hu/) for the prediction of intrinsically unstructured regions in proteins. A value above the 0.5 line indicates disordered regions within the protein.