

Supplementary Table 1: PCR conditions

	Final concentration	DNA-Polymerase	Hot-Start	Initial denaturation	Number of cycles	Temperature cycling		Final extension		PCR product
Standard PCR	1X <i>Taq</i> buffer 0.18 mM dNTPs 0.4 μM primer 1.5 mM MgCl ₂	<i>Taq</i> DNA Polymerase [3 U]	No	95 °C 4 min	30	95 °C 40 s 52 °C 40 s 72 °C 60 s		72 °C 5 min		~ 450 bp
RACE-PCR “Touch Down”	1X <i>Taq</i> buffer 0.18 mM dNTPs 0.4 μM primer 1.5 mM MgCl ₂	<i>Taq</i> DNA Polymerase [3 U]	No	95 °C 4 min	5	95 °C 40 s 62 °C 40 s 72 °C 60 s		72 °C 5 min		N-terminal ~ 1250 bp C-terminal ~ 800 bp
					10	95 °C 40 s 60 °C 40 s 72 °C 60 s				
					25	95 °C 40 s 58 °C 40 s 72 °C 60 s				
					5	95 °C 40 s 62 °C 40 s 72 °C 240 s				
Amplification of the <i>ScTPase</i> gene “Touch Down”	1X <i>Pfu</i> buffer with MgSO ₄ 0.18 mM dNTPs 0.4 μM primer	<i>Pfu</i> DNA Polymerase [3 U]	No	95 °C 4 min	10	95 °C 40 s 60 °C 40 s 72 °C 240 s		72 °C 5 min		2229 bp
					25	95 °C 40 s 58 °C 40 s 72 °C 60 s				
					4	95 °C 50 s 60 °C 50 s 72 °C 720 s				
					18	95 °C 50 s 60 °C 50 s 72 °C 720 s				
Two-Stage PCR	1X <i>Pfu</i> buffer with MgSO ₄ 0.2 mM dNTPs 0.3 μM primer	<i>Pfu</i> DNA Polymerase [3 U]	No	95 °C 1 min	4	95 °C 50 s 60 °C 50 s 72 °C 720 s		72 °C 7 min		5644 bp
				95 °C 1 min	18	95 °C 50 s 60 °C 50 s 72 °C 720 s		72 °C 7 min		

Supplementary Table 2: Detailed summary of the purification of recombinant *Sc*TPase.

All steps were carried out at 6 °C, enzyme activities and protein concentrations indicated were measured after concentrating steps.

Step	Total volume [ml]	Total activity [U]	Total protein [mg]	Specific activity [U/mg]	Yield [%]	Purification [-fold]
Crude cell extract	15	302.6	445.0	0.68	100	1.0
Anion-exchange	10	204.7	53.6	3.82	68	5.6
HiPrep 26/10 Desalting	10	132.7	26.9	4.93	44	7.3
Hydroxylapatite	5	94.9	15.1	6.3	32	9.3

```

GfTPase : -MAPPHQFOSKPSDVIRRRLLSSAVSSK--RP---NTPG--YTLSTPMWAGIAGAVVNNNTQFEVAISHDSVYNTDFASSVVFYSPNEPEAQAGITEKHVLETLRKFSTEHMCKFLGAGVTVLLREAQFNLCRLW : 128
PsTPase : MSTPHHQFESKSSSTAIRRRLLSSSVSSKQRP---NIMTTTTFASLTPMWAGVAGTLVNNNTQYEIAVTVHDGVYSTDFASVIEVTPGFTVKNKSDIEAQVLLNLRKFSAEHLCKFLGAGITLALLKEQFNLCRLW : 132
ScTPase : -MSPPHGFSVPESTAAARRRLSSKASLTNRPTFTKLTITQIYASLTPMWAGIAGAPINNGSQFELAISVHDSVYSTDFASFVIDHTPLDPEKAAAKEIEKHVLDALRKFSCQEHLCCKFLGAGITLALLRESFNICTRLW : 134

GfTPase : LDMDIVPIVFNPKPFHTDSITRPNVHRHRSSTTGSYVPSGAEETPTVYVYDPAQLQDFENKLSANVQTRLPVPTVDEQADSAARKCICYFGPCNNPRLQIGPRNOVAVDAGGKIHLIDDIIEYRKTIVGKCTWNSVTK : 263
PsTPase : LDMDIVPIVFNPKPFHTDSVTRPNIKHRISSTTGSYVPSGSEETPTVYVEASHLGDPSHLSFNAAQKLPVPTVDEQADSAARKCLMYFGPNNPRLSIGARNEVTVDAGGKIHLIDDIIEYRMTVCACTWNAVTK : 267
ScTPase : LDDLIVPIVFNPKPFHTDSLTRPNIKHRISSTTGSYVPSGAEETPTVYVEASHLGN--NLSAGTASKLPVPTVDEQADSAARKAIMYFGPNNPRLTIGPRNOVAVDAGGKIHLIDDIIEYRKTIVGKSTWNAVTK : 267

GfTPase : LADELREKRIKIGFFSSTPQGGVALMRHAIIRFETALDVAAWYVNPSPSVFRTTKNNHNILQGVADPSLRRLTKEADNFDSWILKNGLRWTAEGGPLAPGGVDIAFIDDPQMPGLIPLIKRIRPDLPIIYRS : 398
PsTPase : LADELREKVKIGFFSSTPQGGVALMRHALIRFETALDVDVAWYVNPSPQVFRRTTKNNHNILQGVAAAPDLRLTQEAKDADFDAWILKNGLRWTAEGGPLAPGGVDVVFIDDPQMPGLIPLIKKVRPEVPIVYRS : 402
ScTPase : LADELRERCIKIGFFSSTPQGGVALMRHALIRFETALDVAAWYVNPSPSVFRTTKNNHNILQGVASPDRLRLTQEAKDNFDAWITKNGLRWTAEGGPLAPGGVDIAFIDDPQMPGLIPLIKKVRPELPIIYRS : 402

GfTPase : HIEIRSDLVHVKGSFQEEVWNYLWNNIQHSDLFISHPVNKFVPSDVPLEKLALLCAATDWLDGLSKHLDAWDSOYYMGEFR-----NLCVKEKMNELGWPAREYTVQIARFDPKIGIPNVIDSYARFR : 521
PsTPase : HIEIRNDLVHVAWSFQEEVWNYLWNNIQLADLFISHPVSKFVPSDVPLEKLALLCAATDWLDGLNKDLDPWDSPFYMGFRPRGSHLNRGEFRSLCAKEKMHLELNWPARDYTVQVARFDPKIGIPNVVDSYKFR : 537
ScTPase : HIEIRSDLVHIAGSFQEEVWNYLWNNIQLADLFISHPVKAFVPEVPIERVALLPAAATDWLDGLNRELSDWDRQYYMGEFR-----ALCQKDKMNTLDWENREYCTQIARFDPKIGIPNVIDSYARFR : 525

GfTPase : KLCVDK---VMEDDTPQLLLCGHGAVDDPDASIIYDQVLLIHK--YKEYAPDIVVMRCPPSDQLLNTLMANAKFALQQLSTREGFEVKVSEALHAGKPVIACTRGGIPLQIEHGKSGYLCPEGDNAAVAQHMLDL : 652
PsTPase : NLLRTRSPMDSEHPQLLLCGHGAVDDPDASIIYDQIMLVNSDPYKEYAHDIVVMRIPPSDELLNAMMANSRIALQQLSTREGFEVKVSEALHTGKPVIACTRGGIPLQIQHGKSGYLTPGKDAVAGHEVDF : 672
ScTPase : RLLEAG-DVDADDQFQLLLCGHGAVDDPDASIIYDQVLLIHEK--YAEFAKDIVVMRIPPSDQLLCLMANARIALQQLSTREGFEVKVSEAVHAGKPIAATGGIPLQVEHGKSGFLTEPGDNAQVARHMYDL : 658

GfTPase : YTDEDLYDTMSEYARTHVSDEVGTVGNAAAWMLAVMYVSRGVKLRPHGAWINDLMRTEMGEPYRPGEPRLPRGELHVG----- : 732
PsTPase : YTDEALYRKMSEFARTHVSNEVGTVGNAAAWLYLAVMYSRGEKIKPNGAWINDFFREETGEPYKGETKLPRTKLDMQ----- : 751
ScTPase : YTDVALYDRMSQYARTHVSDEVGTVGNAAAWLYLAAVYTRGOKLAPKGAWLNDLIREETGTPYVEGEPRLPRGGIKVQD----- : 737

```

Supplementary Figure 1: Sequence comparison of fungal trehalose phosphorylases from *Grifola frondosa* (GfTPase, acc. no. BAA31350), *Pleurotus sajor-caju* (PsTPase, acc. no. AAF22230) and *Schizophyllum commune* (ScTPase, acc. no. ABC84380) was performed with Vector NTI program using blosum62mt2 scoring matrix and standard settings. Amino acids conserved are shaded in black. The primary structure of ScTPase is 76.7% and 73.0% identical to the sequences of GfTPase and PsTPase, respectively.

AtGS ----- : -
OtsA ----- : -
EcMalP ----- : -
ScTPase MSPPHGFSSVPSTAARRRLSSKASLTNRPTFTKITTQTYASLTPMWAGIAGAPINNGSQFELAISVHDSVYSTDFASFVIDHTPLDPEKAAKEIEKHVLDALRKFSQEHL : 110

AtGS ----- : -
OtsA ----- : -
EcMalP -----MSQPIFNDKQFQEALSQRQWQRYGLNSAAEMTPRQWWLAVSEALAEM : 46
ScTPase CKFLGAGITLALLRESPNICTRLWLDLDIVP IVFNKPFHTDSLTRPNIKHRISSTSGSYVPSGAETPTVYVEASHLGNNLSAGTASKLP IPRTLDEQADSAARKAIMYY : 220

AtGS -----MNVLVSSEIYPL---IKTGGLADVVGALPIAL-----E : 31
OtsA -----SRLVVVSNRIAPPDEHAASAAGGLAVGILGALKAAGGLWFGWSGETGNEDQPLKK : 54
EcMalP LRAQPFAPVANQRHVNYISMEFLIGRL-----TGNNLLNLGWYQDVQDSLKAYDINLTDLLEEIDPALGNCGGLGRLAACFLDSMATVQGSATGYGLNYQYGLFR : 147
ScTPase GPNNNPRLTIGPRNQVAVDAGGKIHLDDIDEYRKTVPSTWTAVNKLADLRERQIKIGFFSST-----PQGGVALMRHALIRFF-----S : 303

AtGS A-----HGVTRRTLIPGYPAVKA AVTDPVKCFEFTDLLGEKADLLEVQHERLDLLILDAPA-----YY--- : 89
OtsA V-----KKG NITWASFNLS-----EQDLDEYYNQFSNAVLWP--- : 86
EcMalP QSFVDGKQVEAPDDWHRSNYPWFRHNEALDVQVGIGGKVTKDGRWEPEFTITGQAWDL-----PVG YRNGVAQ-----PLRLW : 221
ScTPase A-----LDVDAAWYVPNPS-----PSVFRTTKNNH-----NI--- : 330

AtGS -----ERSGGPYLGQTGKDYDPDNWKRFAALS LAAARIGAGVLPGW-----RPDMVHAHDWQAAMTPVVMRYAETPEIP----- : 157
OtsA -----AFHYRLDLVQFORPAWDGYLRVNALLADKLLPLLQ-----DDDIWIWHDYHL-LPFAHELKRGVNNR----- : 149
EcMalP QATHAHPFDLTKFNDGDFLRAEQGINA EKLTKVLYPNDNHTAGKKLRLMQYFQCACSVADILRRHHLAGRKLHELADYEVIQINDTHP-TIAIPELLRVLIDEHQMSW : 330
ScTPase -----LQGVASPDRLRTQEAKDNFDAWITKNGLRRTAEGGPLAPG-----GVDIAFIIDDPQM-PGLIPLIKKVRPELP----- : 397

AtGS -----SLLTIHNI AFQQFGANIFSKLALP-----AHAFGMGIEIYNDVS-----FLKGGQTATALSTVSPS- : 216
OtsA -----IGFFLHIP-----FP-----TPEIFNALPTYDTLLE-----QLC-----DYDLLGFQTEN- : 189
EcMalP DDAWAITSKTFAYTNHTL-----MPEALERWDVKLVKGLLPRHQI INEINTRFKTLVEKTWP GDEKVVAKLAVVHDKQVHMANLCVVGGFVAVNGVAALH : 425
ScTPase -----IIYRSHIE-----IR-----SDLVHIAGSPQEEVWK-----YLWNNIQLADLFISHPVK- : 441

AtGS -YAE EILTA EFGMGLEGV-----IGSR-----AHVLHGIVNGIDADVWNPATDHLIHDNYSAANLK-----NRALNKK : 278
OtsA -DRLAFLDCLSNLTRVTT RSAKSH TAWGK-----AFRTEVYPIGIEP-----KEIAKQAAG-----PL--PPK : 244
EcMalP SDLVV-----KDLFPEYHQLWPNK FHNVTNGITPRRWIKQCNPALAALLDKSLQKEWANDLDQLINLEKFADDAKFRQQYREIKQANKVR--LAE : 513
ScTPase -AFVP-----EDVP-----IERVALLPAATDWDLDGLNKELSDWRQYYMGEFR-----AL--CQK : 488

AtGS	AVA EHFRI---DDD GSPLFCVLSRLTWQKGGIDLMAEAVDEIVSLGG-----RLVVLGAG--DVALE-GALLAAASRH-----HGR--V-----GVAIGY	: 354
OtsA	LAQLKAE L---K--NVQNI FSVRLDY SKG LPERFLAYEALLEKYPQHH-----GKIRY TQIAPT SRGDVQAYQDIRHQLENEAGRINGKYGLGWTP L-----YYLNQH	: 339
EcMalP	FVKV RTGIE INP--QAIFDIOIKRLEHEYK RQHLNLLLHILALYKEIRENPQ-ADRVPRVFLFGAKAAPGYLLA-KNII FAINKVA----DVINNDPL--VGDKLKV VFLPD	: 613
ScTPase	DKMNTLDW---P--NREYCIQ IARFDPAKGI PNVIDSYARFRRLLNEAGDVDADDQPLLICGHGAVDDPD A-SIIYDQVLT LI--HEKYAEFAKD--I-----VVMRLP	: 583
AtGS	-NEPLSHLMQAGCD AIIIPSR--FEP CGITQLYALRYGCIPVVARTG----GLAD--TVIDANHAALASKAATGVQFSPVTL DGLKQAI RRTVRY YHDPK LWTQM QKLG M	: 455
OtsA	FDRKLLMKIFRYSDVGLVTP L--RDGMNLVAK EYVAAQDPANPGVLV----LSQFAGAANELT-----SALIVN PYDRDEVAAALDRAL TMSLAERISRHAEMLDV	: 434
EcMalP	YCVSAAEK LIPAADISEQI STAGKEASGTGNM KKLALNGALT VGTLDGANVEIAEK--VGEENI-----FIFGH TVEVKAILAKGYDPVKRKKGKVLD AVLKELESQ	: 712
ScTPase	PSDQLLDCLMANARIALQLST--REGFEVKVSEAVHAGKPIIAATTG----GIPL--QVEHGK-----SGFLTEPGDNAQVARHMYDLYTDVALYDRMSQYARTHV	: 676
AtGS	KSDVSW EKSAGLYAALYSQLISKGH-----	: 480
OtsA	IVKNDINH WQECFISDLKQIVPRSAESQQRDKVATFPKLA-----	: 474
EcMalP	GKYSDGDKHAFDQMLHSIGKQGGDPYLVMADFAAYVEAQKQ--WVDVLYRDQEAWTRAAI LNTARCGMFS SDRSIRDYQARIWQAKR	: 797
ScTPase	SDEVGTVGNAAAWLYLAAVYTRGQKLAPKGAWLNDLLREETGTPYVEGEPRLPRGGIKVQD-----	: 737

Supplementary Figure 2: Full-length multiple sequence alignment of primary structures of *ScTPase* (acc. no. ABC84380, GT 4) and retaining glycosyltransferases of fold family GT-B including glycogen synthase from *Agrobacterium tumefaciens* (*AtGS*, acc. no. AAD034474, GT 5), trehalose 6-phosphate synthase from *E. coli* (*OtsA*, acc. no. BAA15717, GT 20) and maltodextrin phosphorylase from *E. coli* (*EcMalP*, acc. no. AAC76442, GT 35). Conserved regions surrounding catalytic centre residues are shaded. The predicted secondary structural elements for *ScTPase* are shown as black cylinders for α -helices and arrows for β -sheets. Secondary structure prediction was made with the public PSIPRED server (<http://bioinf.cs.ucl.ac.uk/psipred/>) using mGenTHREADER method.