

SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIG. 1.

(A) Schematic diagram of AtTPPD showing the site of the T-DNA insertion in *tppd*. Black boxes indicate coding and white boxes intron regions.

(B) qRT-PCR analysis of wild type Col-0, *tppd* and AtTPPD overexpressor lines AtTPPD OE20 and AtTPPD OE22 showing the absence of a full-length *AtTPPD* transcript in *tppd* plants and enhanced *AtTPPD* transcript levels in AtTPPD overexpressor lines. Data are means \pm relative SD of three independent experiments.

(C) Western-blot analysis of total protein extract from wild type Col-0, *tppd* and AtTPPD-CFP overexpressor lines AtTPPD OE20 and AtTPPD OE22 using an anti-GFP antibody. Upper panel: AtTPPD-CFP protein levels. Lower panel: protein levels (CBB, Coomassie Brilliant Blue).

(D, E) Fresh weight (D) and chlorophyll content (E) of four-week old soil-grown *A. thaliana* wild type Col-0, *tppd* and AtTPPD overexpressor lines AtTPPD OE20 and AtTPPD OE22. Data are means \pm relative SD of twenty plants per genotype.

(F) Three-week old soil-grown *A. thaliana* wild type Col-0, *tppd* and AtTPPD overexpressor lines AtTPPD OE20 and AtTPPD OE22.

(G, H) Enhanced salt stress tolerance of plants overexpressing AtTPPD. Two-week old soil-grown *A. thaliana* wild type Col-0, *tppd* and AtTPPD overexpressor lines AtTPPD OE20 and AtTPPD OE22 were watered for 3 weeks with 200 mM NaCl. Image of single representative individuals (G) and chlorophyll content (H) of the phenotypes' mean of 20 plants. Asterisks indicate a significant difference (***) $P \leq 0.001$ using Student's *t*-test for pairwise comparison to Col-0 within the same condition.

SUPPLEMENTAL FIG. 2.

Redox sensitivity of AtTPPD activity. The activity of recombinant, purified AtTPPD was analyzed in the presence of DTT (A) or GSH (B) or increasing concentrations of H₂O₂ (A) or GSSG (B). To test for AtTPPD reactivation, AtTPPD was oxidized using H₂O₂ or GSSG, followed by addition of DTT (A) or GSH (B).

SUPPLEMENTAL FIG. 3.

Protein sequence alignment of AtTPPD and TaTTP using ClustalW. The bottom line represents the degree of homology: (*) identical amino acid; (:) conserved substitution; (.) semi-conserved

substitution. The cap domain is underlined. The three motifs characteristic for HAD superfamily of phosphatases are boxed. The four cysteine residues in AtTPPD are indicated by arrows.

SUPPLEMENTAL FIG. 4.

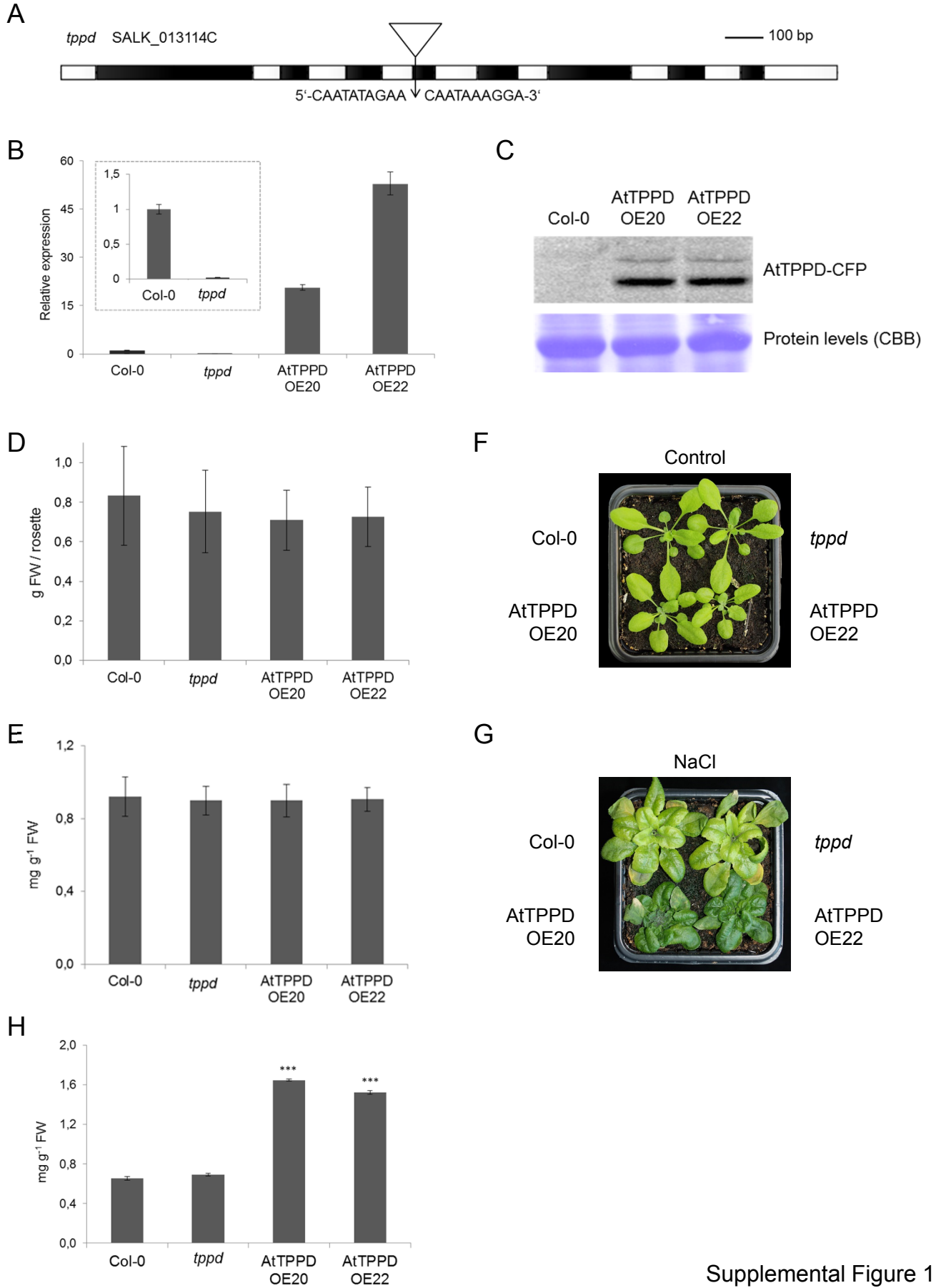
Amino acid sequence alignment of Arabidopsis TPPs using CLC Main workbench. Cysteine residues are highlighted in boxes, and the positions of C159, C216, C235 and C242 from AtTPPD are indicated by arrows.

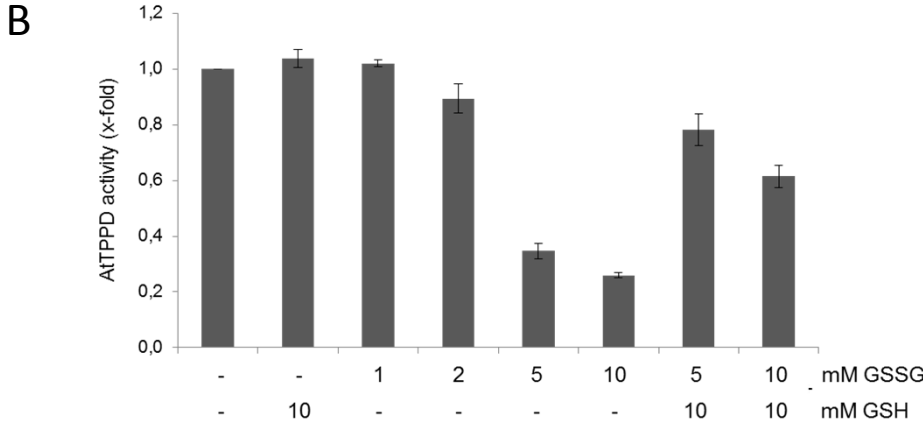
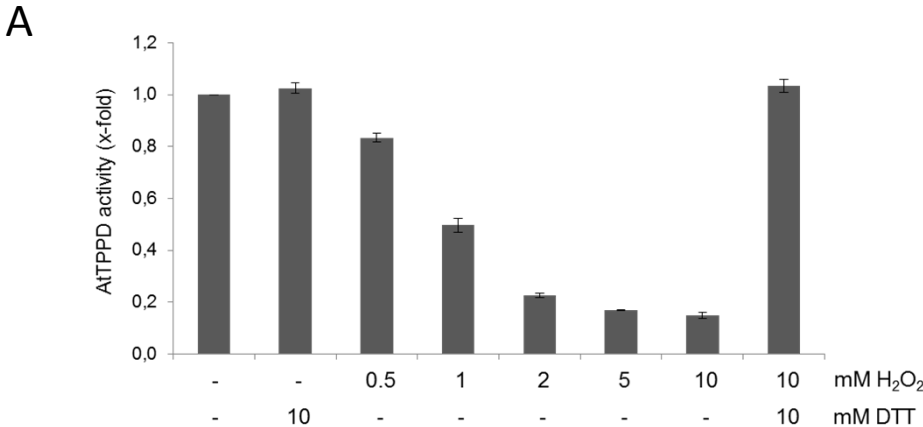
SUPPLEMENTAL TABLE 1

Specific activities of AtTPP enzyme purifications. Specific activity is expressed as unit per milligram protein. One unit is the amount of enzyme that catalyzes the release of 1 μ mol inorganic phosphate from trehalose-6-phosphate in one minute at 37°C.

SUPPLEMENTAL TABLE 2

List of primers.





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AtTPPD      -MTNHNALISDAKGSIGVAVRVPNQSLFSPGGGRYISIPRKKLVQKLEADPSQTRIHTWI
TaTPP      -M-----

AtTPPD      EAMRASSPTRTRPGNISPLPESDEEDEYSSWMAQHPSALTMFEEIAEASKGKQIVMFIDY
TaTPP      -----SLIFIDY
                        : : ****

AtTPPD      DGTLSPIVENPDRAYMSEEMREAVKGVARYFPPTAIVTGRCDKVRRFVKLPGLYYAGSHG
TaTPP      DGLVPIIXNPEESYADAGLLSLISDLKERFDTYIVTGRSPEEISRFLPL-----
          **** ** : ** : : * . : . : . : . * * * * * . : : : ** : *

AtTPPD      MDIKGPSKRKHNKNNKGVLFQ-AANEF↓LPIDK↓VS↓SKCLVEKMRDIEGANVENNKFCVSV
TaTPP      -DINXICYHGACSKINGQIVYNNGSDRFLGVFDRIYEDTRSWVSDFPGLRIYRKNLAVLY
          ** : . : . . * * : : : . : . * * : * : : : . : * : * . : : : . *

AtTPPD      HYRCVDQKDWGLVAEHVTSILSEYPKLRLTQGRK↓VLEIR-PTIKWDK↓GKALEFLLES↓LG↓F
TaTPP      HGLXGADXKPKLRSRIEEIARIFG-VETYYGKXIIELRVP↓GV--NKGSAIRSV-----
          * . . : : : . * : : . * : : * : * * : : * * . * . :

AtTPPD      ANSNDVLP↓PIYIGDDRTDEDAFKVLRNKGQGF↓GILVSKI↓PK-ETSATYSLQ---EPSEVGE
TaTPP      --RGERPAIIAGDDATDEAAFEANDDA-----LTIKVGEGETHAKFHVADYIEXRKILK
          . : . * * * * * * * : . : * . * : : * * * . : : * : : :

AtTPPD      FLQRL-VEWKQMSLRGR
TaTPP      FIEXLGVQKK-----
          * : : * * : *
    
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		20		40		60	
AITPPA	MDMKSGHSSP	VMTD-SPPIS	NSRLTIRQNR	LPYSSAAATA	ISQNNLLLT	VPRKKTGILD	DV----- 61
AITPPB	M---TNQNV	VSDRKPILGL	KTITVSVSNS	PLFS-----	NSFP--TYFN	FPRRKLKLL	EAADKNNLVV 59
AITPPC	M---K-----	ITD-----					-----I 6
AITPPD	M---TNHNL	ISDAK--GS	IGVAVRVPNQ	SLFSPGGGR	-----YIS	IPRKKLV--Q	KLEADPS-- 51
AITPPE	MVRFIEENI	--TK--ML	E--TKAISNS	EVLYVGGDDG	DTSP-----	--TTKVL--H	DFQINSGGGL 51
AITPPF	MDLNSNHKSS	VLKDPSPSVN	QSRL-----	-----G	VS--SRFMM	SQWKKPAKLD	DV----- 44
AITPPG	MDLNIKTTTP	VLSDPTTPVS	KTRL-----	-----G	SSFPSGRFMM	NSRKKIPKLD	DV----- 47
AITPPH	MVRFIEEN--	--TK--LV	EKETGNKSN	DV-----	-TTT-----	--KKKAL--Q	DIINNGVGL 43
AITPPI	MS--ASQNV	VSETT--MS	SIIPNNNNN	N--NSSSQKL	P--P-GLIS	ISKKKLL--K	NIDIINGG-- 55
AITPPJ	M--VSQNVV	VSDAK--TG	IITVSTVSN	SVFTPTAQKP	PTAP--GYIS	VSKKKLL--K	NLEI-NGADQ 59
	80	100	120	140			
AITPPA	--KSNGLDA	MKSSSPPTI	LNKD-NLS-N	DATDMTYREW	MLKYPSALT	SFEKIMFAK	GKRIALFLDY 127
AITPPB	APKITSMIDS	MRDSSPTRLR	-SSSYDS-VS	-DNDD-KTSW	I-VRFPSALN	MFDEIVNAAK	GKQIVMFLDY 124
AITPPC	SGKIETLVDS	LRDMSPTRVR	-SSFSDHEVS	-ENDDESSW	I-ALHPSALD	MFEQIMRDAE	GKQIMFLDY 73
AITPPD	QTRIHWTIEA	MRASSPTRTR	-PGNISPLPE	SDEEDEYSSW	M-AQHPSALT	MFEELAEASK	GKQIVMFLDY 119
AITPPE	--IRSWVDS	MRACSPTRPK	-----SFNSQ	-----SCW	I-KEHPSALN	MFEELHKSSE	GKQIVMFLDY 105
AITPPF	--RSGWLDA	MISSPPRRK	LVKDFNVEVA	PEDDFAQRAW	M-VKYPSAIS	SFAHIAAQAK	KKKIAVFLDY 111
AITPPG	--RSGWLDA	MISSPPRRK	LVKDFNIEIA	PEDDFSQRAW	M-LKYPSAIT	SFAHIAAQAK	NKKIAVFLDY 114
AITPPH	--INSWVDS	MRACSPTHLK	-----SLLKQ	-----SSW	L-TEHPSALD	MFEELHLSE	GKQIVMFLDY 97
AITPPI	GQRINAWVDS	MRASSPTHLK	-----SLPSS	ISTQQQLNSW	I-MQHPSALE	KFEQIMEASR	GKQIVMFLDY 119
AITPPJ	SQRLNSWVDS	MRASSPTHLK	-----SL-SS	FSSEEEHNSW	I-KRHPSALN	MFERIEEAR	GKQIVMFLDY 122
	160	180	200				
AITPPA	DGTLSPIVEE	PDCAYMSSAM	RSAVQNVAKY	FPTAII SGRS	RDKVYEFVNL	SELYYAGSHG	MDIMSPAGES 197
AITPPB	DGTLSPIVED	PDKAFITHEM	REVVKDVASN	FPTAIVTGRS	IEKVRSFVQV	NEIYYAGSHG	MDIEGP---- 190
AITPPC	DGTLSLITED	HDRAIITDEM	REVVKEVATY	FKTAII SGRS	TDKVQSFVKL	TGIHYAGSHG	MDIKGP---- 139
AITPPD	DGTLSPIVEN	PDRAYMSEEM	REAVKGVARY	FPTAIVTGRS	RDKVRRFVKL	PGLIYAGSHG	MDIKGP---- 185
AITPPE	DGTLSPIVDD	PDRAFMSKKM	RNTVTKLAKC	FPTAIVSGRS	REKVSSFVKL	TELYYAGSHG	MDIKGP---- 171
AITPPF	DGTLSPIVDD	PDRAIMSDAM	RSAVKDVASY	FPTAII SGRS	RDKVYQLVGL	TELYYAGSHG	MDIMT---- 176
AITPPG	DGTLSPIVDD	PDRAIMSDAM	RAAVKDVASY	FPTAII SGRS	RDKVYQLVGL	TELYYAGSHG	MDIMTPVNP 184
AITPPH	DGTLSPIVDD	PDRAFMSRKM	RRTVTKLANC	FPTAIVSGRS	IEKVYNFVKL	TELYYAGSHG	MDIKGP---- 163
AITPPI	DGTLSPIVDD	PDKAFMSSKM	RRTVTKLANC	FPTAIVTGRS	IDKVYNFVKL	AELYAGSHG	MDIKGP---- 185
AITPPJ	DGTLSPIVDD	PDRAFMTSKM	RRTVTKMKAC	FPTSIVTGRS	IDKVYSFVKL	AELYAGSHG	MDIKGP---- 188
	220	240	260	280			
AITPPA	LNHEHSRTV-	SVYEQGDVDN	LFQPASEFLP	MIDKVLCSLI	ESTDKIKGVK	VEDNKFCLSV	HYRNVEEKNW 266
AITPPB	-----TNE	SNGQSNERV	LFQPAREFLP	MIEKVVNILE	EKTKWIPGAM	VENNKFCLSV	HFRRVDEKRW 253
AITPPC	-----TND	QSNQ--EEV	MFQPASDYL P	MIDEVVNLK	EKTKSIPGAT	VEHNKFCLTV	HFRRVDEGW 200
AITPPD	-----SKRN	KHNKNNKGV-	LFQAAANFLP	MIDKVSCLLV	EKMRDIEGAN	VENNKFCLSV	HFRVVDQKDW 248
AITPPE	--EQG-SKYK	K--ENQSL	LFQPAEFLLP	VINEVYKCLV	ENTQSI PGAK	VENNKFCLSV	HFRVVEENKW 234
AITPPF	--SSDGNCFK	STDQQGKEVN	LFQPAKEFIP	VIDEVFRTL V	EKMKD IKGAK	VENHKFCLSV	HYRNVDEKDW 245
AITPPG	GSPEDPNCIK	TTDQQGEEVN	LFQPAKEFIP	VIEEVYNNLV	EITDKIKGAK	VENHKFCLSV	HYRNVDEKDW 254
AITPPH	--EQG-SKYE	QILQDSKSL-	LFQPAEFLLP	MIDEVYHKL V	EKTKSTPGAQ	VENNKFCLSV	HFRRVDENNW 229
AITPPI	--AGFSRHK	R--VKQSL-	LYQPANDYLP	MIDEVYRQLL	EKTKSTPGAQ	VENNKFCLSV	HFRRVDEKDW 249
AITPPJ	--TKGFSRYN	K--DKPSV-	LYQPAGDFLP	MIDEVYKQLV	EKTKSTPGAQ	VENNKFCLSV	HFRRVDEKDW 252
	300	320	340				
AITPPA	TLVAQCVDV	IRTYPKLRLT	HGRKVL EIRP	VIDWDK GKAV	TFLLESGLGN	NCEDVLP IYV	GDDRTDEDAF 336
AITPPB	PALAEVVKSV	LIDYPKLKL T	QGRKVL EIRP	TIKWDK GKAL	NFLLKSLGYE	NSDDVVPVYI	GDDRTDEDAF 323
AITPPC	AALAEQVRLV	LIDYPKLRLT	QGRKVL EIRP	SIKWDK GKAL	EFLNLSLGI A	ESKDVLPVYI	GDDRTDEDAF 270
AITPPD	GLVAEHVRLV	LSEYPKLRLT	QGRKVL EIRP	TIKWDK GKAL	EFLLESGLGFA	NSNDVLP IYI	GDDRTDEDAF 318
AITPPE	SDLAHQVRSV	LKNYPKLM L T	QGRKVL EIRP	IIKWDK GKAL	EFLLESGLYD	NCIDVFP IYI	GDDRTDEDAF 304
AITPPF	PIIAQRVHDH	LKQYPR LRLT	HGRKVL EIRP	VIDWNKGRAV	EFLLESGLS	NKDDL P IYI	GDDTTDEDAF 315
AITPPG	PLVAQRVHDH	LKRYPR LRLT	HGRKVL EIRP	VIEWNKGRAV	EFLLESGLS	NNDFLP IFI	GDDTTDEDAF 324
AITPPH	SDLANQVRSV	MKDYPK LRLT	QGRKVL EIRP	IIKWDK GKAL	EFLLESGLYA	NCIDVFP IYI	GDDRTDEDAF 299
AITPPI	SELVLQVRSV	LKKFPTLQLT	QGRKVL EIRP	MI EWDK GKAL	EFLLESGLFG	NTNNVFPVYI	GDDRTDEDAF 319
AITPPJ	SELASKVRSV	VKNYPTL KLS	QGRKVL EIRP	IIKWNK GKAL	EFLLESGLFE	NCIDVFP IYI	GDDKTDEDAF 322
	360	380	400				
AITPPA	KVLRDGNH G	YGILVSAV PK	DSNAFYSLRD	PSEVME-FLK	S---LVTW-	---KRSMG-	385
AITPPB	KVLR E-RGQ G	FGILVSKV PK	DTNASYSLQD	PSQVNK-FLE	---RLVEWK	R--KTVGEE	374
AITPPC	KVLC E-RGQ G	FGIIVSKT IK	ETYSATYSLQD	PSQVKE-FLE	---RLVWK	K--QLTGE	320
AITPPD	KVLRN-KGQ G	FGILVSKIPK	ETSATYSLQE	PSEVGE-FLQ	---RLVEWK	Q--MSLRGR	369
AITPPE	KILRD-KKQ G	LGILVSKYAK	ETNASYSLQE	PDEV MV-FLE	---RLVEWK	Q--SRC-GA	354
AITPPF	KVLRD-NRG	FGILVSSIPK	ESNAFYSLRD	PSEVKK-FLK	T---LVKWA	KLEKNSTGF	368
AITPPG	KVLR E-NRG	FGILVSSV PK	ESNAFYSLRD	PSEVKK-FLK	T---LVKWA	KMSSKTSF	377
AITPPH	KVLR E-RRQ G	LGILVSKF PK	ETSATYSLQE	PDEV ME-FLQ	---RLVEWK	Q--LRS-GA	349
AITPPI	KMLRD-RGEG	FGILVSKF PK	DTNASYSLQD	PSEASDGFLT	TIGGMETNAA	K--NV--G	372
AITPPJ	KLLRG-RGQ G	FGILVSKF PK	DTNASYSLQD	PPEVMN-FL-	---GRLVEWK	Q--MQ--Q	370

	Specific activity
AtTPPA	0,073
AtTPPE	0,037
AtTPPH	0,058
AtTPPD	0,057
AtTPPD_C159S	0,069
AtTPPD_C216S	0,062
AtTPPD_C235S	0,040
AtTPPD_C242S	0,031
AtTPPD_C159/235S	0,051

Gene	Primer Name	Primer Sequence	Purpose
AtTPPA	AtTPPA cDNA_Inf	(F) 5'-ggatctgctggtgcgGACATGAAATCTGGTCACTC-3'	Cloning
		(R) 5'-ccagcgctaccagcgccACCCATTGATCTCTTCCATG-3'	Cloning
AtTPPB	AtTPPB cDNA_Inf	(F) 5'-ggatctgctggtgcgACTAACCAGAATGTCATCGT-3'	Cloning
		(R) 5'-ccagcgctaccagcgccCTCTTCTCCCACTGTCTTCC-3'	Cloning
AtTPPC	AtTPPC cDNA_Inf	(F) 5'-ggatctgctggtgcgAAGATTACGGATATTTCCGG-3'	Cloning
		(R) 5'-ccagcgctaccagcgccTTCTCCAAGTGTGGTTTCT-3'	Cloning
AtTPPD	AtTPPD cDNA_Inf	(F) 5'-ggatctgctggtgcgACAAACCATAATGCCTTAAT-3'	Cloning
		(R) 5'-ccagcgctaccagcgccTCTTCCTCTTAGTGACATTT-3'	Cloning
	qRT_AtTPPD_ex5/6	(F) 5'-GTTGATCAAAAAGGACTGGGG-3'	qRT-PCR
		(R) 5'-CCCATTTGATGGTTGGTCGA-3'	qRT-PCR
	AtTPPD_ex1	(F) 5'-TCAAAAAGGGAAACAAATCGTG-3'	Genotyping
	AtTPPD_ex6	(R) 5'-GAGATTCGACCAACCATCAAA-3'	Genotyping
AtTPPE	AtTPPE cDNA_Inf	(F) 5'-ggatctgctggtgcgGTTAGATTCATCGAAGAAAA-3'	Cloning
		(R) 5'-ccagcgctaccagcgccTGCCCCACACCTTGACTGTT-3'	Cloning
	qRT_AtTPPE_ex9/10	(F) 5'-AGATGATCGTACCGACGAAGACG-3'	qRT-PCR
		(R) 5'-TACGCAAAGGAGACTAATGC-3'	qRT-PCR
AtTPPF	AtTPPF cDNA_Inf	(F) 5'-ggatctgctggtgcgGATTTAACTCAAACCACAA-3'	Cloning
		(R) 5'-ccagcgctaccagcgccAAAACCAGTAGAATTCTTCT-3'	Cloning
AtTPPG	AtTPPG cDNA_Inf	(F) 5'-ggatctgctggtgcgGATTTGAATATAACAAGAC-3'	Cloning
		(R) 5'-ccagcgctaccagcgccAAAACCTGTTTTGAACTTT-3'	Cloning
AtTPPH	AtTPPH cDNA_Inf	(F) 5'-ggatctgctggtgcgGTTAGATTCATAGAAGAAAA-3'	Cloning
		(R) 5'-ccagcgctaccagcgccTGCTCCAGATCTCAATTGTT-3'	Cloning
AtTPPI	AtTPPI cDNA_Inf	(F) 5'-ggatctgctggtgcgTCAGCTAGTCAAAACATTGT-3'	Cloning
		(R) 5'-ccagcgctaccagcgccCATTCTTGGCTGCATTTGTT-3'	Cloning
AtTPPJ	AtTPPJ cDNA_Inf	(F) 5'-ggatctgctggtgcgGTGAGCCAAAACGTCGTCGT-3'	Cloning
		(R) 5'-ccagcgctaccagcgccTTGCTGCATCTGTTTCCACT-3'	Cloning
AtPP2A	qRT_AtPP2A	(F) 5'-TAACGTGGCCAAAATGATGC-3'	qRT-PCR
		(R) 5'-GTTCTCCACAACCGCTTGGT-3'	qRT-PCR
Ubiquitin1	qRT_Ubiquitin1	(F) 5'-ATGCAGATCTTCGTGAAAACC-3'	qRT-PCR
		(R) 5'-CTACTTGATCTTCTTCTTAGGCCTC-3'	qRT-PCR
Salk T-DNA LB	SK_LB	(R) 5'-GTTACGCTAGTGGGCCATCG-3'	Genotyping