The amino acid sequence for wild type TchPAM:

MGFAVESRSHVKDILGLINAFNEVKKITVDGTTPITVAHVAALARRHDVKVALEAEQCRARVETC
SSWVQRKAEDGADIYGVTTGFGACSSRRTNRLSELQESLIRCLLAGVFTKGCAPSVDELPATATR
SAMLLRLNSFTYGCSGIRWEVMEALEKLLNSNVSPKVPLRGSVSASGDLIPLAYIAGLLIGKPSVIA
RIGDDVEVPAPEALSRVGLRPFKLQAKEGLALVNGTSFATAVASTVMYDANVLLLLVETLCGMF
CEVIFGREEFAHPLIHKVKPHPGQIESAELLEWLLRSSPFQELSREYYSIDKLKKPKQDRYALRSSPQ
WLAPLVQTIRDATTTVETEVNSANDNPIIDHANDRALHGANFQGSAVGFYMDYVRIAVAGLGK
LLFAQFTELMIEYYSNGLPGNLSLGPDLSVDYGLKGLDIAMAAYSSELQYLANPVTTHVHSAEQH
NQDINSLALISARKTEEALDILKLMIASHLTAMCQAVDLRQLEEALVKVVENVVSTLADECGLPN
DTKARLLYVAKAVPVYTYLESPCDPTLPLLLGLKQSCFDTILALHKKDGIETDTLVDRLAEFEKRLSD
RLENEMTAVRVLYEKKGHKTADNNDALVRIQGSKFLPFYRFVREELDTGVMSARREQTPQEDV
QKVFDAIADGRITVPLLHCLQGFLGQPNGCANGV

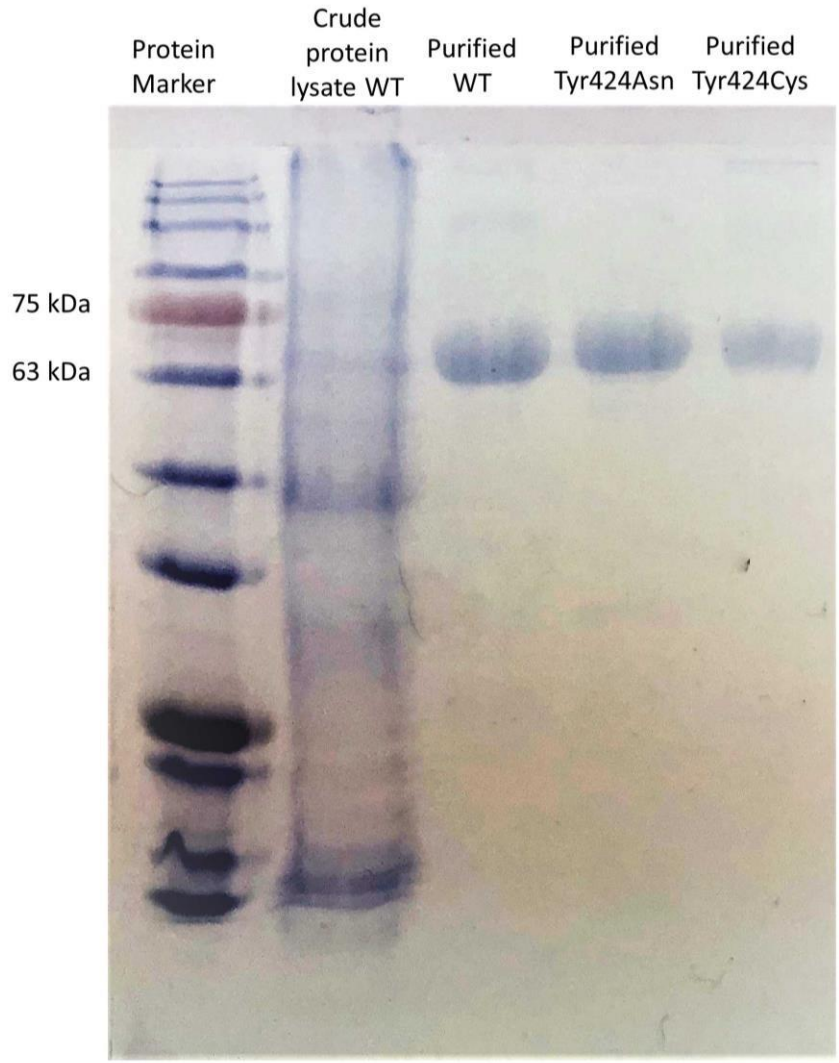
Supplementary Table S1: The list of primers for site-directed mutagenesis through touch down method

MUTANT	PRIMERS	
F86Y	fw	TGT GAC CAC CGG TTA TGG TG
	rw	ACC GGT GGT CAC ACC ATA G
L104S	fw	CTG CAG GAA AGT AGC ATT CGC TGT CT
	rw	ACT TTC CTG CAG TTC GCT CAG G
L104T	fw	CTG CAG GAA AGT ACC ATT CGC TGT CT
	rw	ACT TTC CTG CAG TTC GCT CAG G
L108Q	fw	CTG ATT CGC TGT CAA CTG GCA G
	rw	ACA GCG AAT CAG ACT TTC CTG CA
L108S	fw	CTG ATT CGC TGT AGC CTG GCA G
	rw	ACA GCG AAT CAG ACT TTC CTG CA
K427C	fw	GAT TAT GGC CTG TGC GGT CTG GAT ATT
	rw	CAG GCC ATA ATC AAC GCT CAG A

K427E	fw	GAT TAT GGC CTG GAA GGT CTG GA
	rw	CAG GCC ATA ATC AAC GCT CAG A
K427M	fw	GAT TAT GGC CTG ATG GGT CTG GA
	rw	CAG GCC ATA ATC AAC GCT CAG A
K427N	fw	ATC TGA GCG TTG ATA ATG GCC TG
	rw	CAG GCC ATA ATC AAC GCT CAG A
K427Q	fw	GAT TAT GGC CTG CAG GGT CTG GA
	rw	CAG GCC ATA ATC AAC GCT CAG A
I431E	fw	TGA AAG GTC TGG ATG AAG CAA TGG C
	rw	ATC CAG ACC TTT CAG GCC ATA ATC
I431N	fw	TGA AAG GTC TGG ATA ATG CAA TGG C
	rw	ATC CAG ACC TTT CAG GCC ATA ATC
E455D	fw	TGCA TAG TGC AGA TCA GCA TAA TCA GGA
	rw	GCA CTA TGC ACA TGG GTG GTC
E455H	fw	TGC ATA GTG CAC ATC AGC ATA ATC AGG A
	rw	GCA CTA TGC ACA TGG GTG GTC
E455N	fw	TGT GCA TAG TGC AAA TCA GCA TAA TCA GGA
	rw	GCA CTA TGC ACA TGG GTG GTC

Supplementary Table S2: The list of primers for site-directed mutagenesis through KLD (Kinase, Ligase and DpnI enzymes) method

MUTANT	PRIMERS	
Y424C	fw	GAGCGTTGATtgcGGCCTGAAAGG
	rw	AGATCCGGACCCAGACTC
Y424D	fw	GAGCGTTGATgatGGCCTGAAAG
	rw	AGATCCGGACCCAGACTC
Y424N	fw	GAGCGTTGATaatGGCCTGAAAG
	rw	AGATCCGGACCCAGACTC
Y424Q	fw	GAGCGTTGATcaaGGCCTGAAAGG
	rw	AGATCCGGACCCAGACTC



Supplementary Figure S1: The representative SDS-PAGE gel of the crude protein lysate and purified enzymes. The band of TchPAM was observed between 63 kDa and 75 kDa. WT: wild type.