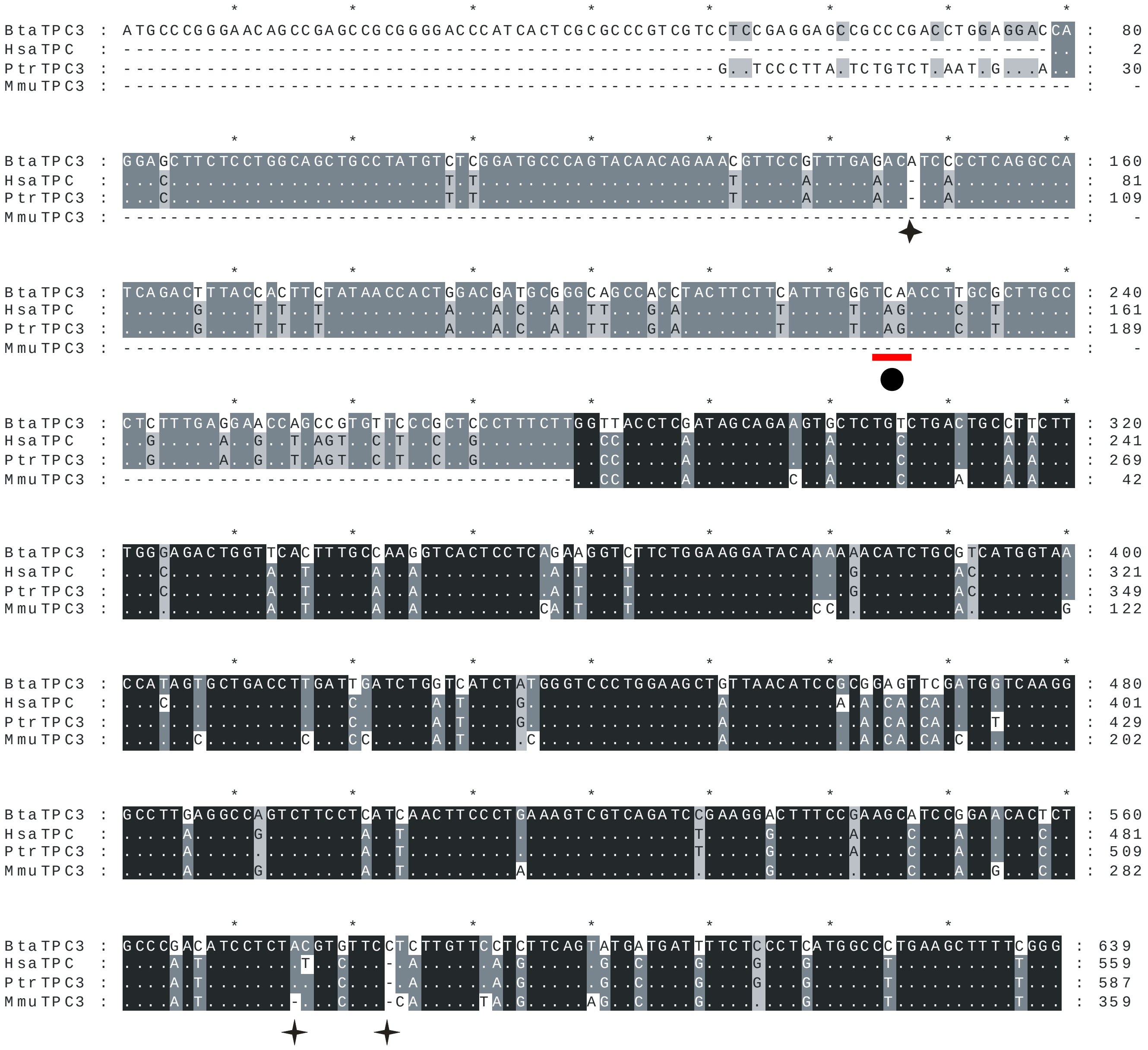
Supplementary Figure 1. TPC3 pseudogene sequences in primates. Multiple sequence alignment of coding nucleotide sequences of cow *TPC3* (BtaTPC3) and genomic sequences from human (*Hsa*), chimpanzee (*Ptr*) and monkey (*Mmu*). Nucleotide deletions in primate sequences are marked with asterisks and stop codons are indicated with a filled circle. The genomic sequences were derived from the following unique trace identifiers: TI1571518716, TI232317170, TI222576964 and TI1008067773 (human); TI368718204, TI235691386, TI441856022 and TI229241163 (chimpanzee); TI541405745, TI541924217 and TI460077731 (monkey).

Supplementary Figure 2. Sea urchin TPCs. Multiple sequence alignment of the deduced amino acid sequences of *TPC* genes from the sea urchin (SpTPC1-3) and human (HsTPC1 and HsTPC2). Position of the two putative pores is highlighted in red.

Supplemental Fig. S1



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                   *         *         *         *         *         *         *         *
BtaTPC3 : ATGCCCGGGAACAGCCGAGCCGCGGGGACCCATCACTCGCGCCCGTCGTCCTCCGAGGAGCCGCCCGACCTGGAGGACCA : 80
HsaTPC  : ------------------------------------------------------------------------------.. : 2
PtrTPC3 : --------------------------------------------------G..TCCCTTA.TCTGTCT.AAT.G...A.. : 30
MmuTPC3 : -------------------------------------------------------------------------------- : -

                   *         *         *         *         *         *         *         *
BtaTPC3 : GGAGCTTCTCCTGGCAGCTGCCTATGTCTCGGATGCCCAGTACAACAGAAACGTTCCGTTTGAGACATCCCCTCAGGCCA : 160
HsaTPC  : ...C.......................T.T.....................T.....A.....A..-..A.......... : 81
PtrTPC3 : ...C.......................T.T.....................T.....A.....A..-..A.......... : 109
MmuTPC3 : -------------------------------------------------------------------------------- : -
                                                                            ✦

                   *         *         *         *         *         *         *         *
BtaTPC3 : TCAGACTTTACCACTTCTATAACCACTGGACGATGCGGGCAGCCACCTACTTCTTCATTTGGGTCAACCTTGCGCTTGCC : 240
HsaTPC  : ......G....T.T..T..........A...A.C..A..TT...G.A........T.....T..AG....C..T...... : 161
PtrTPC3 : ......G....T.T..T..........A...A.C..A..TT...G.A........T.....T..AG....C..T...... : 189
MmuTPC3 : -------------------------------------------------------------------------------- : -
                                                                         ▬▬▬
                                                                          ●

                   *         *         *         *         *         *         *         *
BtaTPC3 : CTCTTTGAGGAACCAGCCGTGTTCCCGCTCCCTTTCTTGGTTACCTCGATAGCAGAAGTGCTCTGTCTGACTGCCTTCTT : 320
HsaTPC  : ..G.....A..G..T.AGT..C.T..C..G..........CC.....A...........A.....C........A.A... : 241
PtrTPC3 : ..G.....A..G..T.AGT..C.T..C..G..........CC.....A...........A.....C........A.A... : 269
MmuTPC3 : --------------------------------------..CC.....A........C..A.....C....A...A.A... : 42

                   *         *         *         *         *         *         *         *
BtaTPC3 : TGGGAGACTGGTTCACTTTGCCAAGGTCACTCCTCAGAAGGTCTTCTGGAAGGATACAAAAAACATCTGCGTCATGGTAA : 400
HsaTPC  : ...C........A..T.....A..A...........A.T...T..................G........AC........ : 321
PtrTPC3 : ...C........A..T.....A..A...........A.T...T..................G........AC........ : 349
MmuTPC3 : ............A..T.....A..A..........CA.T...T...............CC..........A........G : 122

                   *         *         *         *         *         *         *         *
BtaTPC3 : CCATAGTGCTGACCTTGATTGATCTGGTCATCTATGGGTCCCTGGAAGCTGTTAACATCCGCGGAGTTCGATGGTCAAGG : 480
HsaTPC  : ...C...............C......A.T....G................A.........A.A.CA.CA........... : 401
PtrTPC3 : ...................C......A.T....G................A...........A.CA.CA....T...... : 429
MmuTPC3 : ......C........C...CC.....A.T.....C...............A...........A.CA.CA.C......... : 202

                   *         *         *         *         *         *         *         *
BtaTPC3 : GCCTTGAGGCCAGTCTTCCTCATCAACTTCCCTGAAAGTCGTCAGATCCGAAGGACTTTCCGAAGCATCCGGAACACTCT : 560
HsaTPC  : .....A.....G........A..T........................T.....G......A....C...A......C.. : 481
PtrTPC3 : .....A..............A..T........................T.....G......A....C...A......C.. : 509
MmuTPC3 : .....A.....G........A..T.........A....................G...........C...A..G...C.. : 282

                   *         *         *         *         *         *         *
BtaTPC3 : GCCCGACATCCTCTACGTGTTCCTCTTGTTCCTCTTCAGTATGATGATTTTCTCCCTCATGGCCCTGAAGCTTTTCGGG : 639
HsaTPC  : ....A.T........T..C...-.A......A.G......G..C....G....G...G......T..........T... : 559
PtrTPC3 : ....A.T...........C...-.A......A.G......G..C....G....G...G......T..........T... : 587
MmuTPC3 : ....A.T.......-...C...-CA.....TA.G.....AG..C....G........G......T..........T... : 359
                        ✦       ✦
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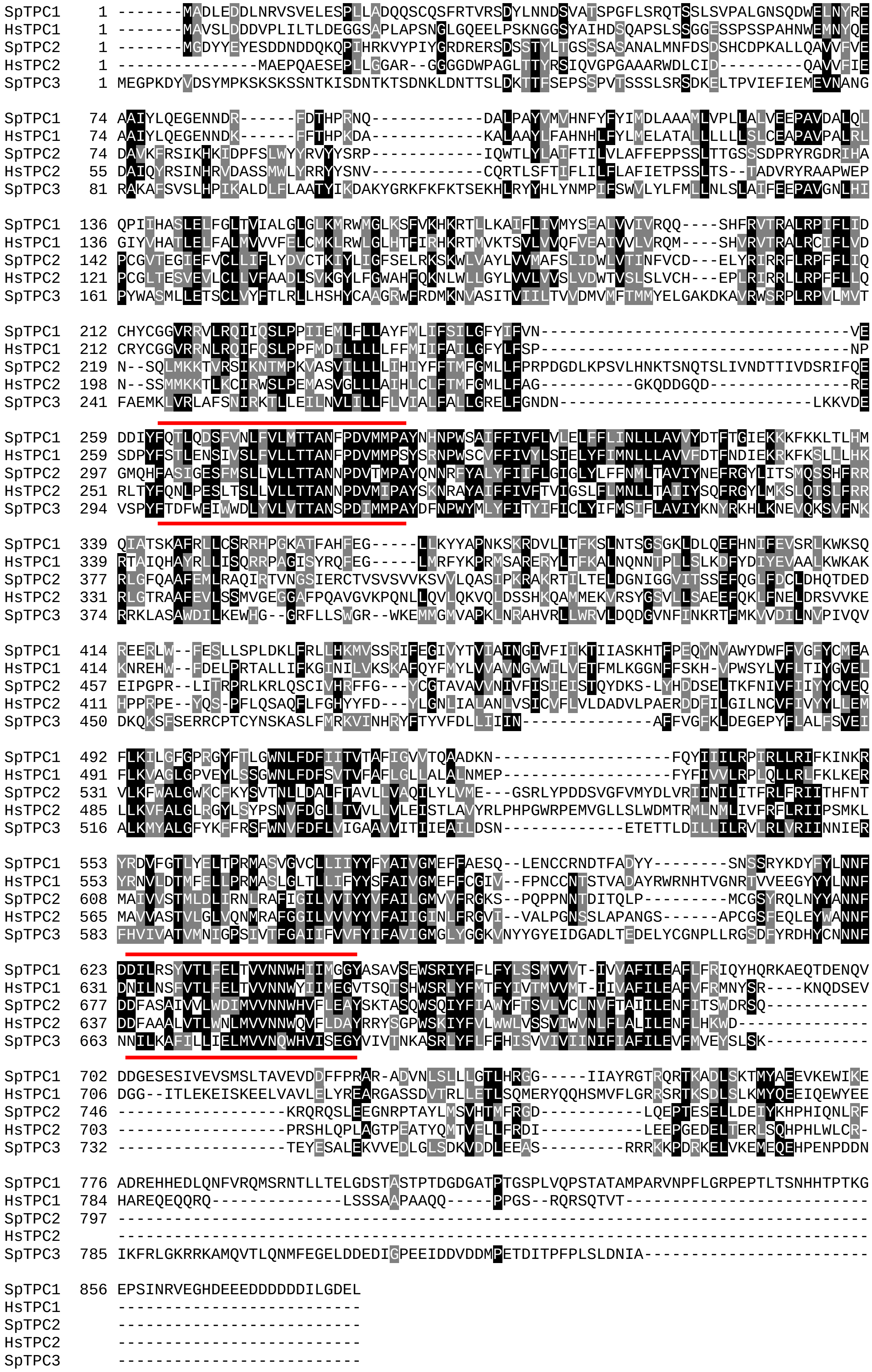
Supplemental Fig. S2



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SpTPC1     1 -------MADLEDDLNRVSVELESPLLADQQSCQSFRTVRSDYLNNDSVATSPGFLSRQTSSLSVPALGNSQDWELNYRE
HsTPC1     1 -------MAVSLDDDVPLILTLDEGGSAPLAPSNGLGQEELPSKNGGSYAIHDSQAPSLSSGGESSPSSPAHNWEMNYQE
SpTPC2     1 -------MGDYYEYESDDNDDQKQPIHRKVYPIYGRDRERSDSSTYLTGSSSASANALMNFDSDSHCDPKALLQAVVFVE
HsTPC2     1 ---------------MAEPQAESEPLLGGAR--GGGGDWPAGLTTYRSIQVGPGAAARWDLCID---------QAVVFIE
SpTPC3     1 MEGPKDYVDSYMPKSKSKSSNTKISDNTKTSDNKLDNTTSLDKTTFSEPSSPVTSSSLSRSDKELTPVIEFIEMEVNANG

SpTPC1    74 AAIYLQEGENNDR------FDTHPRNQ------------DALPAYVMVHNFYFYIMDLAAAMLVPLLALVEEPAVDALQL
HsTPC1    74 AAIYLQEGENNDK------FFTHPKDA------------KALAAYLFAHNHLFYLMELATALLLLLLSLCEAPAVPALRL
SpTPC2    74 DAVKFRSIKHKIDPFSLWYYRVYYSRP------------IQWTLYLAIFTILVLAFFEPPSSLTTGSSSDPRYRGDRIHA
HsTPC2    55 DAIQYRSINHRVDASSMWLYRRYYSNV------------CQRTLSFTIFLILFLAFIETPSSLTS--TADVRYRAAPWEP
SpTPC3    81 RAKAFSVSLHPIKALDLFLAATYIKDAKYGRKFKFKTSEKHLRYYHLYNMPIFSWVLYLFMLLNLSLAIFEEPAVGNLHI

SpTPC1   136 QPIIHASLELFGLTVIALGLGLKMRWMGLKSFVKHKRTLLKAIFLIVMYSEALVVIVRQQ----SHFRVTRALRPIFLID
HsTPC1   136 GIYVHATLELFALMVVVFELCMKLRWLGLHTFIRHKRTMVKTSVLVVQFVEAIVVLVRQM----SHVRVTRALRCIFLVD
SpTPC2   142 PCGVTEGIEFVCLLIFLYDVCTKIYLIGFSELRKSKWLVAYLVVMAFSLIDWLVTINFVCD---ELYRIRRFLRPFFLIQ
HsTPC2   121 PCGLTESVEVLCLLVFAADLSVKGYLFGWAHFQKNLWLLGYLVVLVVSLVDWTVSLSLVCH---EPLRIRRLLRPFFLLQ
SpTPC3   161 PYWASMLLETSCLVYFTLRLLHSHYCAAGRWFRDMKNVASITVIILTVVDMVMFTMMYELGAKDKAVRWSRPLRPVLMVT

SpTPC1   212 CHYCGGVRRVLRQIIQSLPPIIEMLFLLAYFMLIFSILGFYIFVN---------------------------------VE
HsTPC1   212 CRYCGGVRRNLRQIFQSLPPFMDILLLLLFFMIIFAILGFYLFSP---------------------------------NP
SpTPC2   219 N--SQLMKKTVRSIKNTMPKVASVILLLLIHIYFFTMFGMLLFPRPDGDLKPSVLHNKTSNQTSLIVNDTTIVDSRIFQE
HsTPC2   198 N--SSMMKKTLKCIRWSLPEMASVGLLLAIHLCLFTMFGMLLFAG----------GKQDDGQD---------------RE
SpTPC3   241 FAEMKLVRLAFSNIRKTLLEILNVLILLFLVIALFALLGRELFGNDN---------------------------LKKVDE

SpTPC1   259 DDIYFQTLQDSFVNLFVLMTTANFPDVMMPAYNHNPWSAIFFIVFLVLELFFLINLLAVVYDTFTGIEKKKFKKLTLHM
HsTPC1   259 SDPYFSTLENSIVSLFVLLTTANFPDVMMPSYSRNPWSCVFFIVYLSIELYFIMNLLAVVFDTFNDIEKRKFKSLLLHK
SpTPC2   297 GMQHFASIGESFMSLLVLLTTANNPDVTMPAYQNNRFYALYFIIFLGIGLYLFFNMLTAVIYNEFRGYLITSMQSSHFRR
HsTPC2   251 RLTYFQNLPESLTSLLVLLTTANNPDVMIPAYSKNRAYAIFFIVFTVIGSLFLMNLLTAIIYSQFRGYLMKSLQTSLFRR
SpTPC3   294 VSPYFTDFWEIWWDLYVLVTTANSPDIMMPAYDFNPWYMLYFITYIFICLYIFMSIFLAVIYKNYRKHLKNEVQKSVFNK

SpTPC1   339 QIATSKAFRLLCSRRHPGKATFAHFEG-----LLKYYAPNKSKRDVLLTFKSLNTSGSGKLDLQEFHNIFEVSRLKWKSQ
HsTPC1   339 RTAIQHAYRLLISQRRPAGISYRQFEG-----LMRFYKPRMSARERYLTFKALNQNNTPLLSLKDFYDIYEVAALKWKAK
SpTPC2   377 RLGFQAAFEMLRAQIRTVNGSIERCTVSVSVVKSVVLQASIPKRAKRTILTELDGNIGGVITSSEFQGLFDCLDHQTDED
HsTPC2   331 RLGTRAAFEVLSSMVGEGGAFPQAVGVKPQNLLQVLQKVQLDSSHKQAMMEKVRSYGSVLLSAEEFQKLFNELDRSVVKE
SpTPC3   374 RRKLASAWDILKEWHG--GRFLLSWGR--WKEMMGMVAPKLNRAHVRLLWRVLDQDGVNFINKRTFMKVVDILNVPIVQV

SpTPC1   414 REERLW--FESLLSPLDKLFRLLHKMVSSRIFEGIVYTVIAINGIVFIIKTIIASKHTFPEQYNVAWYDWFFVGFYCMEA
HsTPC1   414 KNREHW--FDELPRTALLIFKGINILVKSKAFQYFMYLVVAVNGVWILVETFMLKGGNFFSKH-VPWSYLVFLTIYGVEL
SpTPC2   457 EIPGPR--LITRPRLKRLQSCIVHRFFG---YCGTAVAVVNIVFISIEISTQYDKS-LYHDDSELTKFNIVFIIYYCVEQ
HsTPC2   411 HPPRPE--YQS-PFLQSAQFLFGHYYFD---YLGNLIALANLVSICVFLVLDADVLPAERDDFILGILNCVFIVYYLLEM
SpTPC3   450 DKQKSFSERRCPTCYNSKASLFMRKVINHRYFTYVFDLLIIIN--------------AFFVGFKLDEGEPYFLALFSVEI

SpTPC1   492 FLKILGFGPRGYFTLGWNLFDFIITVTAFIGVVTQAADKN-------------------FQYIIILRPIRLLRIFKINKR
HsTPC1   491 FLKVAGLGPVEYLSSGWNLFDFSVTVFAFLGLLALALNMEP------------------FYFIVVLRPLQLLRLFKLKER
SpTPC2   531 VLKFWALGWKCFKYSVTNLLDALFTAVLLVAQILYLVME---GSRLYPDDSVGFVMYDLVRIINILITFRLFRIITHFNT
HsTPC2   485 LLKVFALGLRGYLSYPSNVFDGLLTVVLLVLEISTLAVYRLPHPGWRPEMVGLLSLWDMTRMLNMLIVFRFLRIIPSMKL
SpTPC3   516 ALKMYALGFYKFFRSFWNVFDFLVIGAAVVITIIEAILDSN-------------ETETTLDILLILRVLRLVRIINNIER

SpTPC1   553 YRDVFGTLYELTPRMASVGVCLLIIYYFYAIVGMEFFAESQ--LENCCRNDTFADYY--------SNSSRYKDYFYLNNF
HsTPC1   553 YRNVLDTMFELLPRMASLGLTLLIFYYSFAIVGMEFFCGIV--FPNCCNTSTVADAYRWRNHTVGNRTVVEEGYYYLNNF
SpTPC2   608 MAIVVSTMLDLIRNLRAFIGILVVIYYVFAILGMVVFRGKS--PQPPNNTDITQLP---------MCGSYRQLNYYANNF
HsTPC2   565 MAVVASTVLGLVQNMRAFGGILVVVYYVFAIIGINLFRGVI--VALPGNSSLAPANGS------APCGSFEQLEYWANNF
SpTPC3   583 FHVIVATVMNIGPSIVTFGAIIFVVFYIFAVIGMGLYGGKVNYYGYEIDGADLTEDELYCGNPLLRGSDFYRDHYCNNNF

SpTPC1   623 DDILRSYVTLFELTVVNNWHIIMGGYASAVSEWSRIYFFLFYLSSMVVVT-IVVAFILEAFLFRIQYHQRKAEQTDENQV
HsTPC1   631 DNILNSFVTLFELTVVNNWYIIMEGVTSQTSHWSRLYFMTFYIVTMVVMT-IIVAFILEAFVFRMNYSR----KNQDSEV
SpTPC2   677 DDFASAIVVLWDIMVVNNWHVFLEAYSKTASQWSQIYFIAWYFTSVLVCLNVFTAIILENFITSWDRSQ-----------
HsTPC2   637 DDFAAALVTLWNLMVVNNWQVFLDAYRRYSGPWSKIYFVLWWLVSSVIWVNLFLALILENFLHKWD--------------
SpTPC3   663 NNILKAFILLIELMVVNQWHVISEGYVIVTNKASRLYFLFFHISVVIVIINIFIAFILEVFMVEYSLSK-----------

SpTPC1   702 DDGESESIVEVSMSLTAVEVDDFFPRAR-ADVNLSLLLGTLHRGG-----IIAYRGTRQRTKADLSKTMYAEEVKEWIKE
HsTPC1   706 DGG--ITLEKEISKEELVAVLELYREARGASSDVTRLLETLSQMERYQQHSMVFLGRRSRTKSDLSLKMYQEEIQEWYEE
SpTPC2   746 ------------------KRQRQSLEEGNRPTAYLMSVHTMFRGD-----------LQEPTESELLDEIYKHPHIQNLRF
HsTPC2   703 ------------------PRSHLQPLAGTPEATYQMTVELLFRDI-----------LEEPGEDELTERLSQHPHLWLCR-
SpTPC3   732 ------------------TEYESALEKVVEDLGLSDKVDDLEEAS---------RRRKKPDRKELVKEMEQEHPENPDDN

SpTPC1   776 ADREHHEDLQNFVRQMSRNTLLTELGDSTASTPTDGDGATPTGSPLVQPSTATAMPARVNPFLGRPEPTLTSNHHTPTKG
HsTPC1   784 HAREQEQQRQ--------------LSSSAAPAAQQ-----PPGS--RQRSQTVT--------------------------
SpTPC2   797 --------------------------------------------------------------------------------
HsTPC2       --------------------------------------------------------------------------------
SpTPC3   785 IKFRLGKRRKAMQVTLQNMFEGELDDEDIGPEEIDDVDDMPETDITPFPLSLDNIA------------------------

SpTPC1   856 EPSINRVEGHDEEEDDDDDDILGDEL
HsTPC1       --------------------------
SpTPC2       --------------------------
HsTPC2       --------------------------
SpTPC3       --------------------------
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