

A

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Ce_TWK-1      1  MPTNDGD-NDRIGKEEEHGLIENQIISOKTKKQLPSWLDREYHVWRDQFQODKCCPKKM
Hs_TREK-2     1  MKKPIETPRKQVNWDPKVAVPAAPVCPKKSATNGQPPAPAPPTPTPLSISSRATVVARM

Ce_TWK-1     60  VKR-----ELMFASTYVLLILNVALILVVLFCQVFDLRHS-----
Hs_TREK-2     61  EGTSQGGQLQTVMKWKTVAIAFVVVVVVLVTCGLVFRALQPFESSQKNTIALEKAEFLRD

                                     M1
Ce_TWK-1     95  -----STNNEASFLDR---ALFCITITISTIGYGNIAF
Hs_TREK-2    121  HVCVSPQELETLIQHALDADNAGVSPIGNSSNNSSHWDLGSAFFFACTVITITIGYGNIAF

                                     P1
Ce_TWK-1    124  FDDRGGVICILYCVAGIPLFFMTVATN---SVLVVDICNIVHRSYSSQNVENSGRWYV
Hs_TREK-2    181  STEGGKIFCILYALFGIPLFGFLLAGIGDQLGTIFGKSIARVEKVFRRKQVSOTKIRVIS
                                     ▼tn1397(W177stop)

                                     M2
Ce_TWK-1    180  SAILLAHHCFFGALFFSLWIDQLD---FDADFYSFISITTICTVCDYSPTPEG-----
Hs_TREK-2    241  TILFILAGCIVFVTIPAVIFKYIEGTALLESIVFVVVITITVCFGDFVAGGNAGINREW

                                     M3                                     P2
Ce_TWK-1    230  ---LFOYIIVTVYLCTGVATML---LFFASLQKGMWIIHYGRKVDSEEAEIFWGG
Hs_TREK-2    301  YKPLVWFVILVGLAYFAAVLSMIGDWLRVLSKKTKEEVGEIKAHAAEWKANVTAEFRETR
                                     ▼tn1403(E272stop)

ΔC →                                     M4                                     ▼tn1398(W330stop)
Ce_TWK-1    281  QMMIVKDLVTVAEKFGSTPEKLRVVDHLDKIIEVACKQAE---EDDDEEDWRSVNNSE
Hs_TREK-2    361  RRLSVEIHDKIQRAATIRSMERRRLGIDQRAHSIDMLSPKRSVFAALDTGRFKASSQES

Ce_TWK-1    338  ALSPPAPR--KKTVFISGSSDGDCCQATTHSFTSNQRSIPKDEFAIALALGTIQHHLRRL
Hs_TREK-2    421  INNRNNLRLKGPQGNKHGOGASEDNIINKFGSISRLTKRKNKDLKKTLPEDVOKIYKT

Ce_TWK-1    396  PSVRGIHGHHHPKIPFSKPSADSN-----IESKEAITSLDLRKKAHRVHSDGRLLEQ
Hs_TREK-2    481  FRNYSLDEEKEEETEKMCSNDNSSTAMLTDCIQQHAELNGMIPTDTKDREPNNSLIE

Ce_TWK-1    449  QDV
Hs_TREK-2    541  DRN

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B

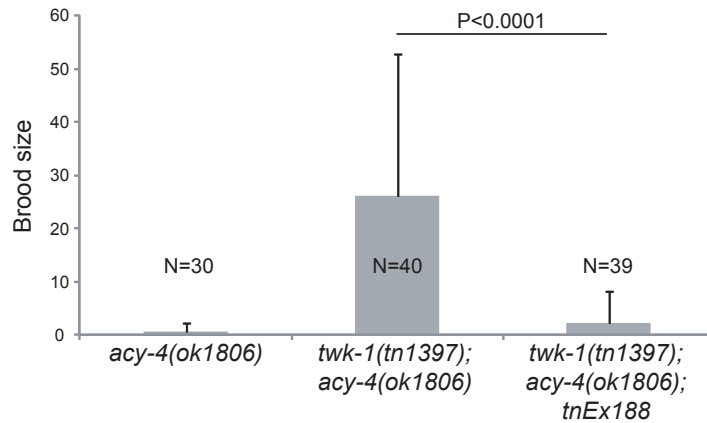


Figure S12 The TWK-1 C-terminus is not essential for function. (A) ClustalW alignment of *C. elegans* TWK-1 and human TREK-2 (NP_612190). Using RT-PCR, we found that the TWK-1 prediction (NP_492054.2) was incorrect in that a small predicted intron was found to be coding, resulting in an additional 14 amino acids. The RT-PCR data is in agreement with the gene prediction based on the FN890018 cDNA. We thank Bernard Lakowski for pointing out the discrepancy in the *twk-1* gene prediction. The four transmembrane domains (M1-M4) and two pore domains (P1 and P2) are underlined. The three *twk-1* nonsense mutations and the position of the $\Delta C284$ deletion are indicated. TWK-1 exhibits less similarity to TREK-1 (not shown) in the C-terminal region because of the latter's smaller size. (B) The TWK-1DC284::GFP fusion (*tnEx188*) rescues *twk-1(tn1397)*. Error bars represent one standard deviation. Student's *t*-test was used for statistical analysis.