Supplementary Material

Molecular basis of the scalp-ear-nipple syndrome unraveled by the characterization of disease-causing KCTD1 mutants.

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B

А

Figure S1: Secondary structure prediction of KCTD1 sequence computed by using Psipred server ⁴⁷ (A) and Vendruscolo Lab. Software ⁴⁸ (B).



Figure S2. Thioflavin T Fluorescence assay: emission spectra obtained by adding a ThT solution (50 μ M) to KCTD1^{P20S} (A), KCTD1^{H33P} (B), KCTD1^{G62D} (C), KCTD1^{D69E} (D), and KCTD1^{H74P} (E) at protein concentrations ranging from 0.37 μ M to 12 μ M.



Figure S3: Evaluation of the binding of KCTD1 SEN related mutants KCTD1^{P20S} (A), KCTD1^{H33P} (B), KCTD1^{G62D} (C), KCTD1^{D69E} (D), KCTD1^{H74P} (E) to AP- $2\alpha^{NTD}$ by Isothermal Titration Calorimetry. ITC experiments were performed by titrating KCTD1 variants (72 µM-expressed as pentamer) into a solution of AP- $2\alpha^{NTD}$ (7 µM). Top and bottom panels report raw and integrated data, respectively.

Α	KCTD1	<u>MSR</u> PLI <u>TRS</u> PASPLNN <u>OGI</u> ETPAQLTKSNAP VHIDV GG HMYTSS	44
	KCTD15	PPHRKERPSGSSLHTHGSTGTAEGGN <u>MSR</u> LSL <u>TRS</u> PV <u>SPL</u> AA <u>OGI</u> EL <u>FAQLTKSNAP</u> VHIDVGGHMYTSS	70
	KCTD19a		27
	KCTD4	MERKINRREKEKEYEGKHNSLEDTDQGKNCKSTLMTLNVGGYLYITQ	47
	KCTD19b	IKVYVGSHWYATT	393
	KCTD6	MDNGDWGYMMTDPVTLNVGGHLYTTS	26
В	KCTD21	MSDPITLNVGGKLYTTS	17
	KCTD11	ISPPFVPSSPPSFGGPVTLNVGGTLYSTT	29
	KCTD10	MEEMSGESVVSSAVPAAATRTTSFKGTSPSSK YVKLNV GG ALYYTT	46
С	TNFAIP1	MSGDTCLCPASGAKPKLSGFKGGGLGNKYVQLNVGGSLYYTT	42
	KCTD13	MSAEASGPAAAAAPSLEAPKPSGLEPGPAAYGLKPLTPNSK <mark>yvklnv</mark> gg <mark>slhytt</mark>	55
	KCTD7	MVVVTGREDSRQDGAMSSSDAEDDFLEPATPTATQAGHALPLLPQEFEVVPLNIGGAHFTTR	65
	KCTD14	MWQGCAVERPVGRMTSQTPLPQSPRPRRPTMSTVVELNVGGEFHTTT	47
_	KCTD3	MAGGHCGSFPAAAAGSGEIVQLNVGGTRFSTS	32
D	SHKBP1	MAAAATAAEGVPSRGPPGE <mark>vihlnv</mark> gg krFsts	37
	KCTD9	MRRVTLFLNGSEKNGKVVAVYGTLSDLLSVASSKLGIKATSVYNGKGGLIDDIALIRDDDVLFVCEGEEFIDEQTDSKEPEGLLGFHTDWLTLNVGGRYFTT	104
	KCTD2	MAELQLD PAMAGLGGGGGSGVGDGGG PVRG PPS PR PAG PT PRGHGR PAAAVAQ PLE PG PG P PERAGGGGAARWVRLNVGGTY FVTT	85
Ε	KCTD5		58
	KCTD17		45
	KCTD18	MEGHKAEEEVLDVLRLNVGGCIYTAR	26
	KCTD8	MALKDTGSGGSTILEISEMVSSSSSPGASAAAABGPCAESEFEEVVELNVGGQVYVTK	58
F	KCTD16		39
	KCTD12	MALADSTRGLPNGGGGGGGSGSSSSSAEPPLFPDIVELNVGGQVYVTR	48
	KCNRG		19
۸	KCTD1	LATLT-KYPESRIGRLFDGTEPIVLDSLKQHYFIDRDGQMFRYILNFLRTSKLLIPDDFKDYTLLYEEAKYFQLQPMLLEMERWKQDR	131
~	KCTD15	LATLT-KYPDSRISRLFNGTEPIVLDSLKOHYFIDRDEEIFRYVLSFLRTSKLLLPDDFKDFSLLYEEARYYOLOPMVRELERWOODO	157
	KCTD19a	RSKLS-OFPDSLLWKEASALTSSESORLFIDRDGSTFRHVHYYLYTSKLSFSS-CAELNLLYEOALGLOLMPLLOTLDNLKECKH	110
	KCTD4	KOTLT-KYPDTFL-EGIVNGKILCPFDADGHYFIDRDGLLFRHVLNFLRNGELLLPEGFRENOLLAGEAEFFOLKGLAEEVKSRWEKE	133
	KCTD19b	LOTLL-KYPELLSNPORVYWITYGOTLLIHGDGOMFRHILNFLRLGKLFLPSEFKEWPLFCOEVEEYHIPSLSEALAOCEAYK	492
	KCTD6	LTTLT-RYPDSMLGAMFGGDFPTARDPOGNYFIDRDGPLFRYVLNFLRTSELTLPLDFKEFDLLRKEADFYOIEPLIOCLNDPKPLY	112
R	KCTD21	LATLT-SFPDSMLGAMFSGKMPTKRDSGNCFTDRDGKVFRYTLNFLRTSHLDLPEDFOEMGLLRREADFYOVOPLIEALOEKEVEL	103
	KCTD11		110
	KCTD10	LETLT-RFPDSMLGAMFRAGTPMPPNLNSOGGGHYFIDRDGKAFRHILNFLRLGRLDLPRGYGETALLRAEADFYOIRPLLDALRELEASO	119
^		LETLT-RFPDSMLGAMFRAGTPMPPNLNSQGGGHYFIDRDGKAFRHILNFLRLGRLDLPRGYGETALLRAEADFYQIRPLLDALRELEASQ MOTLT-K-ODTMLKAMFSGRMEVLTDSEGWILIDRCGKHFGTILNYLRDGAVPLPESRREIEELLAEAKYYLVOGLVEECOAALONKDTY	134
L	TNFAIP1	LETLT-RFPDSMLGAMFRAGTPMPPNLNSQGGGHYFIDRDGKAFRHILNFLRLGRLDLPRGYGETALLRAEADFYQIRPLLDALRELEASQ MQTLT-K-QDTMLKAMFSGRMEVLTDSEGWILIDRCGKHFGTILNYLRDGAVPLPESRREIEELLAEAKYYLVQGLVEECQAALQNKDTY VRALT-R-HDTMLKAMFSGRMEVLTDKEGWILIDRCGKHFGTILNYLRDDTITLPONROEIKELMAEAKYYLIOGLVNMCOSALODKKDS	119 134 130
C	TNFAIP1 KCTD13	LETLT-RFPDSMLGAMFRAGTPMPPNLNSQGGGHYFIDRDGKAFRHILNFLRLGRLDLPRGYGETALLRAEADFYQIRPLLDALRELEASQ MQTLT-K-QDTMLKAMFSGRMEVLTDSEGWILIDRCGKHFGTILNYLRDGAVPLPESRREIEELLAEAKYYLVQGLVEECQAALQNKDTY VRALT-R-HDTMLKAMFSGRMEVLTDKEGWILIDRCGKHFGTILNYLRDDTITLPQNRQEIKELMAEAKYYLIQGLVNMCQSALQDKKDS LRTLT-G-ODTMLKAMFSGRVEVLTDAGGWYLIDRSGRHFGTILNYLRDGSVPLPESTRELGELLGEARYYLVOGLIEDCOLALOOKRETL-	119 134 130 144
C	TNFAIP1 KCTD13 KCTD7	LETLT-RFPDSMLGAMFRAGTPMPPNLNSQGGGHYFIDRDGKAFRHILNFLRLGRLDLPRGYGETALLRAEADFYQIRPLLDALRELEASQ MQTLT-K-QDTMLKAMFSGRMEVLTDSEGWILIDRCGKHFGTILNYLRDGAVPLPESRREIEELLAEAKYYLVQGLVEECQAALQNKDTY VRALT-R-HDTMLKAMFSGRMEVLTDKEGWILIDRCGKHFGTILNYLRDDTITLPQNRQEIKELMAEAKYYLIQGLVNMCQSALQDKKDS LRTLT-G-QDTMLKAMFSGRVEVLTDAGGWVLIDRSGRHFGTILNYLRDGSVPLPESTRELGELLGEARYYLVQGLIEDCQLALQQKRETL- LSTLR-CYEDTMLAAMFSGRHYIPTDSEGRYFIDRDGTHFGDVLNFLRSGDLPPRERVRAVYKEAOYYAIGPLLEOLENMOPLKG	119 134 130 144 149
U	TNFAIP1 KCTD13 KCTD7 KCTD14	LETLT-RFPDSMLGAMFRAGTPMPPNLNSQGGGHYFIDRDGKAFRHILNFLRLGRLDLPRGYGETALLRAEADFYQIRPLLDALRELEASQ MQTLT-K-QDTMLKAMFSGRMEVLTDSEGWILIDRCGKHFGTILNYLRDGAVPLPESRREIEELLAEAKYYLVQGLVEECQAALQNKDTY VRALT-R-HDTMLKAMFSGRMEVLTDKEGWILIDRCGKHFGTILNYLRDDTITLPQNRQEIKELMAEAKYYLIQGLVNMCQSALQDKKDS LRTLT-G-QDTMLKAMFSGRVEVLTDAGGWVLIDRSGRHFGTILNYLRDGSVPLPESTRELGELLGEARYYLVQGLIEDCQLALQQKRETL- LSTLR-CYEDTMLAAMFSGRHYIPTDSEGRYFIDRDGTHFGDVLNFLRSGDLPPRERVRAVYKEAQYYAIGPLLEQLENMQPLKG LGTLR-KFPGSKLAEMFSSLAKASTDAEGRFFIDRPSTYFRPILDYLRTGOVPTOHIPEVYREAOFYEIKPLVKLLEDMPOIF	119 134 130 144 149 129
U	TNFAIP1 KCTD13 KCTD7 KCTD14 KCTD3	LETLT-RFPDSMLGAMFRAGTPMPPNLNSQGGGHYFIDRDGKAFRHILNFLRLGRLDLPRGYGETALLRAEADFYQIRPLLDALRELEASQ MQTLT-K-QDTMLKAMFSGRMEVLTDSEGWILIDRCGKHFGTILNYLRDGAVPLPESRREIEELLAEAKYYLVQGLVEECQAALQNKDTY VRALT-R-HDTMLKAMFSGRMEVLTDKEGWILIDRCGKHFGTILNYLRDGSVPLPESTRELGELLGEAKYYLVQGLVECQAALQDKKDS LRTLT-G-QDTMLKAMFSGRVEVLTDAGGWVLIDRSGRHFGTILNYLRDGSVPLPESTRELGELLGEAXYYLVQGLVECQAALQDKKDS LSTLR-CYEDTMLAAMFSGRVEVLTDAGGWVLIDRSGRHFGTILNYLRDGSVPLPESTRELGELLGEAXYYLVQGLVECQAALQDKKDS LGTLR-KFPGSKLAEMFSGRVEVLTDAGGWFIDRDGTHFGDVLNFLRSGDLPPRERVRAVYKEAQYYAIGPLLEQLENMQPLKG LGTLR-KFPGSKLAEMFSGRISTLRDEGGFFIDRPSTYFRPILDYLRTGQVPTQHIPEVYREAQFYEIKPLVKLLEDMPQIF BOTLM-WIPDSFESSLLSGRISTLRDETGAIFTDRDPAAFAPILNFLRTKELDLRGVSINVLRHEAEFYGITPLVRRLLCEELER	119 134 130 144 149 129 117
D	TNFAIP1 KCTD13 KCTD7 KCTD14 KCTD3 SHKBP1	LETLT-RFPDSMLGAMFRAGTPMPPNLNSQGGGHYFIDRDGKAFRHILNFLRLGRLDLPRGYGETALLRAEADFYQIRPLLDALRELEASQ MQTLT-K-QDTMLKAMFSGRMEVLTDSEGWILIDRCGKHFGTILNYLRDGAVPLPESRREIEELLAEAKYYLVQGLVEECQAALQNKDTY VRALT-R-HDTMLKAMFSGRMEVLTDKEGWILIDRCGKHFGTILNYLRDGSVPLPESTRELGELLGEAKYYLVQGLUEOCQLALQQKKEDL- LRTLT-G-QDTMLKAMFSGRVEVLTDAGGWVLIDRSGRHFGTILNYLRDGSVPLPESTRELGELLGEAKYYLVQGLUEOCQLALQQKRETL- LSTLR-CYEDTMLAAMFSGRVEVLTDAGGWFIDRDGTHFGDVLNFLRSGDLPPRERVRAVYKEAQYYAIGPLLEQLENMQPLKG LGTLR-KFPGSKLAEMFSGRISTLRDEGAIFIDRDFSTYFRPILDYLRTGQVPTQHIPEVYREAQYYAIGPLLEQLENMQPLKG RQTLM-WIPDSFFSSLLSGRISTLRDETGAIFIDRDPAAFAPILNFLRTKELDL-RGVSINVLRHEAEFYGITPLVRRLLCREELRE ROTLMW-IPDSFFSSLLSGRISTLRDETGAIFIDRDPTVFAPILNFLRTKELDP-RGVSINVLRHEAEFYGITPLVRRLLCREELRE	119 134 130 144 149 129 117 121
D	TNFAIP1 KCTD13 KCTD7 KCTD14 KCTD3 SHKBP1 KCTD9	LETLT-RFPDSMLGAMFRAGTPMPPNLNSQGGGHYFIDRDGKAFRHILNFLRLQRLDLPRGYGETALLRAEADFYQIRPLLDALRELEASQ MQTLT-K-QDTMLKAMFSGRMEVLTDSEGWILIDRCGKHFGTILNYLRDGAVPLPESRREIEELLAEAKYYLVQGLVEECQAALQNKDTY VRALT-R-HDTMLKAMFSGRMEVLTDKEGWILIDRCGKHFGTILNYLRDGSVPLPESTRELGELLGEAKYYLVQGLIEDCQLALQQKRETL- LRTLT-G-QDTMLKAMFSGRVEVLTDAGGWVLIDRSGRHFGTILNYLRDGSVPLPESTRELGELLGEAKYYLVQGLIEDCQLALQQKRETL- LSTLR-CYEDTMLAAMFSGRVEVLTDAGGWVLIDRSGRHFGIDLNYLRDGSVPLPESTRELGELLGEAKYYLVQGLIEDCQLALQQKRETL- LSTLR-CYEDTMLAAMFSGRHYIPTDSEGRYFIDRDGTHFGDVLNFLRSGDLPPRERVRAVYKEAQYYAIGPLLEQLENMQPLKG LGTLR-KFPGSKLAEMFSGRHYIPTDSEGRYFIDRDGTHFDLDYLRTGQVPTQHIPEVYREAQFYEIKPLVKLLEDMPQIF RQTLM-WIPDSFFSSLLSGRISTLRDETGAIFIDRDPAAFAPILNFLRTKELDLRGVSINVLRHEAEFYGITPLVRRLLCEELER RQTLTW-IPDSFFSSLLSGRISTLKDETGAIFIDRDPTVFAPILNFLRTKELDPRGVHGSSLLHEAQFYGLTPLVRRLQLREELDRSSC- RSTLWKEPDSMLAHMF-KDSGRISTLKDETGAIFIDRDPTVFAPILNYLRHGOLUVN-DGINLLGVLERAFFGDSJLHEAQFYGLTPLVRRLQLREELDRSSC	119 134 130 144 149 129 117 121
D	TNFAIP1 KCTD13 KCTD7 KCTD14 KCTD3 SHKBP1 KCTD9	LETLT-RFPDSMLGAMFRAGTPMPPNLNSQGGGHYFIDRDGKAFRHILNFLRLGRLDLPRGYGETALLRAEADFYQIRPLLDALRELEASQ MQTLT-K-QDTMLKAMFSGRMEVLTDSEGWILIDRCGKHFGTILNYLRDGAVPLPESRREIEELLAEAKYYLVQGLVEECQAALQNKDTY VRALT-R-HDTMLKAMFSGRMEVLTDKEGWILIDRCGKHFGTILNYLRDGSVPLPESTRELGELLGEAKYYLVQGLUEDCQLALQQKRETL- LRTLT-G-QDTMLKAMFSGRVEVLTDAGGWVLIDRSGRHFGTILNYLRDGSVPLPESTRELGELLGEAKYYLVQGLUEDCQLALQQKRETL- LSTLR-CYEDTMLAAMFSGRVEVLTDAGGWVLIDRSGRHFGTILNYLRDGSVPLPESTRELGELLGEAKYYLVQGLUEDCQLALQQKRETL- LSTLR-KFPGSKLAEMFSGRHYIPTDSEGRYFIDRDGTHFGDVLNFLRSGDLPPRERVRAVYKEAQYYAIGPLLEQLENMQPLKG LGTLR-KFPGSKLAEMFSGRISTLRDETGAIFIDRDSTYFRPILDYLRTGQVPTQHIPEVYREAQFYEIKPLVKLLEDMPQIF RQTLM-WIPDSFFSSLLSGRISTLRDETGAIFIDRDPAFAPILNFLRTKELDLRGVSINVLRHEAEFYGITPLVRRLQLERELDRSSC- RQTLTW-IPDSFFSSLLSGRISTLKDETGAIFIDRDPTVFAPILNFLRTKELDPRGVHGSSLLHEAQFYGLTPLVRRLQLERELDRSSC- RSTLVNKEPDSMLAHMF-KDKGVWGNKQDHRGAFLIDRSPTYFGPILNYLRHGQLIVN-DGINLLGVLEEARFFGIDSLIEHLEVAIKNS	119 134 130 144 149 129 117 121 106
D	TNFAIP1 KCTD13 KCTD7 KCTD14 KCTD3 SHKBP1 KCTD9 KCTD2 KCTD5	LETLT-RFPDSMLGAMFRAGTPMPPNLNSQGGGHYFIDRDGKAFRHILNFLRLGRLDLPRGYGETALLRAEADFYQIRPLLDALRELEASQ MQTLT-K-QDTMLKAMFSGRMEVLTDSEGWILIDRCGKHFGTILNYLRDGAVPLPESRREIEELLAEAKYYLVQGLVEECQAALQNKDTY VRALT-R-HDTMLKAMFSGRMEVLTDKEGWILIDRCGKHFGTILNYLRDGSVPLPESTREIGELLGEAKYYLVQGLVECCQAALQNKDTS LRTLT-G-QDTMLKAMFSGRVEVLTDAGGWVLIDRSGRHFGTILNYLRDGSVPLPESTREIGELLGEAKYYLVQGLIEDCQLALQQKRETL- LSTLR-CYEDTMLAAMFSGRVEVLTDAGGWVLIDRSGRHFGTILNYLRDGSVPLPESTREIGELLGEAKYYLVQGLIEDCQLALQQKRETL- LSTLR-CYEDTMLAAMFSGRHYIPTDSEGRYFIDRDGTHFGDVLNFLRSGDLPPRERVRAVYKEAQYYAIGPLLEQLENMQPLKG LGTLR-KFPGSKLAEMFSGRISTLRDETGAIFIDRDGTHFGDVLNFLRSGDLPPRERVRAVYKEAQYYAIGPLLEQLENMQPLKG RQTLM-WIPDSFFSSLLSGRISTLRDETGAIFIDRDPAFAPILNFLRTKELDLRGVSINVLRHEAEFYGITPLVRLLCEELER RQTLTW-IPDSFFSSLLSGRISTLKDETGAIFIDRDPTVFAPILNFLRTKELDPRGVHGSSLLHEAQFYGLTPLVRLQLREELDRSSC- RSTLVNKEPDSMLAHMF-KDKGVWGNKQDHRGAFLIDRSPEYFEPILNYLRHGQLIVN-DGINLLGVLEEAFFGIDSLIEHLEVAIKNS RQTLG-REPKSFLCRLCCQEDPELDSDKDETGAYLIDRDPTYFGPULNYLRHGKLIIT-KELAEEGVLEEAEFYNIASLVRLVKERIRDNENRT- RQTLG-RDKSFLVRLC-0ADPLDSDKDETGAYLIDRDPTYFGPULNYLRHGKLIUN-KDIAERGVLEEAEFYNIASLVRLVKERIRDNENRT-	119 134 130 144 149 129 117 121 106 177 151
D	TNFAIP1 KCTD13 KCTD7 KCTD14 KCTD3 SHKBP1 KCTD9 KCTD2 KCTD5 KCTD17	LETLT-RFPDSMLGAMFRAGTPMPPNLNSQGGGHYFIDRDGKAFRHILNFLRLGRLDLPRGYGETALLRAEADFYQIRPLLDALRELEASQ MQTLT-K-QDTMLKAMFSGRMEVLTDSEGWILIDRCGKHFGTILNYLRDGAVPLPESRREIEELLAEAKYYLVQGLVEECQAALQNKDTY VRALT-R-HDTMLKAMFSGRMEVLTDKEGWILIDRCGKHFGTILNYLRDGSVPLPESRREIEELLAEAKYYLVQGLVEECQAALQNKDTS LRTLT-G-QDTMLKAMFSGRMEVLTDAGGWVLIDRSGRHFGTILNYLRDGSVPLPESTRELGELLGEARYYLVQGLIEDCQLALQQKRETL- LSTLR-CYEDTMLAAMFSGRHYIPTDSEGRYFIDRDGTHFGDVLNFLRSGDLPPRERVRAVYKEAQYYAIGPLLEQLENMQPLKG LGTLR-KFPGSKLAEMFSGRHYIPTDSEGRYFIDRDGTHFGDVLNFLRSGDLPPRERVRAVYKEAQYYAIGPLLEQLENMQPLKG RQTLM-WIPDSFFSSLLSGRISTLRDETGAIFIDRDFYFRPILDYLRTGQVPTQHIPEVYREAQFYEIKPLVKLLEDMPQIF RQTLTW-IPDSFFSSLLSGRISTLRDETGAIFIDRDPAFAPILNFLRTKELDLRGVSINVLRHEAEFYGITPLVRRLQLREELDRSSC- RSTLVNKEPDSMLAHMF-KDSGRISTLKDETGAIFIDRDPTVFAPILNFLRTKELDPRGVHGSSLLHEAQFYGLTPLVRRLQLREELDRSSC- RQTLG-REPKSFLCRLCCQEDPELDSDKDETGAYLIDRDPTYFGPILNYLRHGKLI'N-KELAEEGVLEEAEFYNIASLVRLVKERIRDNENRT- RQTLC-REPKSFLSRLCQDPLDSDKDETGAYLIDRDPTYFGPILNYLRHGKLVIN-KDLAEEGVLEEAEFYNIASLVRLVKERIRDNENRT- RQTLC-REPKSFLSRLCQGFUNGSNETGAYLIDRDPTYFGPILNYLRHGKLVIN-KDLAEEGVLEEAEFYNIASLVRLVKERIRDNENRT- RQTLC-REPKSFLSRLCQDPLDSDKDETGAYLIDRDPTYFGPILNYLRHGKLVIN-KDLAEEGVLEEAEFYNIASLVRLVKERIRDNENRT-	119 134 130 144 149 129 117 121 106 177 151
D	TNFAIP1 KCTD13 KCTD7 KCTD14 KCTD3 SHKBP1 KCTD9 KCTD2 KCTD5 KCTD17 KCTD18	LETLT-RFPDSMLGAMFRAGTPMPPNLNSQGGGHYFIDRDGKAFRHILNFLRLGRLDLPRGYGETALLRAEADFYQIRPLLDALRELEASQ MQTLT-K-QDTMLKAMFSGRMEVLTDSEGWILIDRCGKHFGTILNYLRDGAVPLPESRREIEELLAEAKYYLVQGLVEECQAALQNKDTY VRALT-R-HDTMLKAMFSGRMEVLTDKEGWILIDRCGKHFGTILNYLRDGSVPLPESRREIEELLAEAKYYLVQGLVEECQAALQNKDTY LRTLT-G-QDTMLKAMFSGRWEVLTDAGGWVLIDRSGRHFGTILNYLRDGSVPLPESRREIGELLGEARYYLVQGLIEDCQLALQQKRETL- LSTLR-CYEDTMLAAMFSGRHYIPTDSEGRYFIDRDGTHFGDVLNFLRSGDLPPRERVRAVYKEAQYYAIGPLLEQLENMQPLKG LGTLR-KFPGSKLAEMFSGRHYIPTDSEGRYFIDRDGTHFGDVLNFLRSGDLPPRERVRAVYKEAQYYAIGPLLEQLENMQPLKG RQTLM-WIPDSFFSSLLSGRISTLRDETGAIFIDRDPAFAPILNFLRTKELDLRGVSINVLRHEAEFYGITPLVRRLQLREELDRSSC- RQTLTW-IPDSFFSSLLSGRISTLKDETGAIFIDRDPTVFAPILNFLRTKELDLRGVSINVLRHEAEFYGITPLVRRLQLREELDRSSC- RSTLVNKEPDSMLAHMF-KDKGVWGNKQDHRGAFLIDRSPEYFEPILNYLRHGQLIVN-DGINLLGVLEEAAFFGIDSLIEHLEVAIKNS RQTLG-REPKSFLCRLCCQEDPELDSDKDETGAYLIDRDPTYFGPILNYLRHGKLIIT-KELAEEGVLEEAEFYNIASLVRLVKERIRDNENRT- RQTLC-RDPKSFLSRLCGEELQSDRDETGAYLIDRDPTYFGPILNYLRHGKLVIN-KDLAEEGVLEEAEFYNIASLVRLVKERIRDNENRT- RQTLC-REPKSFLCRLCQAGEELQSDRDETGAYLIDRDPTYFGPILNYLRHGKLVID-KDMAEEGVLEEAEFYNISLIKLVKDKIRERDSKTS RCC-REPKSFLSRLCQGEELQSDRDETGAYLIDRDPTYFGPILNYLRHGKLVID-KDMAEEGVLEEAEFYNISLIKLVKDKIRERDSKTS REFLC-REPKNLSMFSGEFPILKDPESCACVIDRDGELFKVILDVIDGINLLGVLEEAEFYNISLIKLVKDKIRERDSKTS	119 134 130 144 149 129 117 121 106 177 151 135
D	TNFAIP1 KCTD13 KCTD7 KCTD14 KCTD3 SHKBP1 KCTD9 KCTD2 KCTD5 KCTD17 KCTD18	LETLT-RFPDSMLGAMFRAGTPMPPNLNSQGGGHYFIDRDGKAFRHILNFLRLGRLDLPRGYGETALLRAEADFYQIRPLLDALRELEASQ MQTLT-K-QDTMLKAMFSGRMEVLTDSEGWILIDRCGKHFGTILNYLRDGAVPLPESRREIEELLAEAKYYLVQGLVEECQAALQNKDTY VRALT-R-HDTMLKAMFSGRMEVLTDKEGWILIDRCGKHFGTILNYLRDGSVPLPESRREIEELLAEAKYYLVQGLVEECQAALQNKDTS LRTLT-G-QDTMLKAMFSGRMEVLTDAGGWVLIDRSGRHFGTILNYLRDGSVPLPESTRELGELLGEARYYLVQGLIEDCQLALQQKRETL- LSTLR-CYEDTMLAAMFSGRHYIPTDSEGRYFIDRDGTHFGDVLNFLRSGDLPPRERVRAVYKEAQYYAIGPLLEQLENMQPLKG LGTLR-KFPGSKLAEMFSGRISTLRDETGAIFIDRDFYFRPILDYLRTGQVPTQHIPEVYREAQFYEIKPLVKLLEDMPQIF RQTLM-WIPDSFFSSLLSGRISTLRDETGAIFIDRDPAFAPILNFLRTKELDLRGVSINVLRHEAEFYGITPLVRRLQLREELDRSSC- RSTLVNKEPDSMLAHMF-KDSGRISTLKDETGAIFIDRDPTVFAPILNFLRTKELDPRGVHGSSLLHEAQFYGLTPLVRRLQLREELDRSSC- RSTLVNKEPDSMLAHMF-KDKGVWGNKQDHRGAFLIDRSPEYFEPILNYLRHGQLIVN-DGINLLGVLEEAFFGIDSLIEHLEVAIKNS RQTLG-REPKSFLCRLCCQEDPELDSDKDETGAYLIDRDPTYFGPILNYLRHGKLIIT-KELAEEGVLEEAEFYNISLKLVKKERIRDNENRT- RQTLC-RDPKSFLSRLC-QAGEELQSDRDETGAYLIDRDPTYFGPILNFLRHGKLVIN-KDLAEEGVLEEAEFYNISLKLVKDKIRERDSKTS RQTLC-REQKSFLSRLCQFLKTDESGACVIDRDGRLFKYLLDYLHGEVQIPTDEQTRIALQEEADFYGIPIPYSLSDHLANEM RESLC-RFKDSMLASMFSGRFPLKTDESGACVIDRDGRLFKYLLDYLHGEVQIPTDEQTRIALQEEADFYGIPPYSLSDHLANEM RESLC-RFKDSMLASMFSGRFPLKTDESGACVIDRDGRLFKYLLDYLHGEVQIPTDEQTRIALQEEADFYGIPPYSLSDHLANEM RESLC-RFKDSMLASMFSGRFPLKTDESGACVIDRDGRLFKYLLDYLHGEVQIPTDEQTRIALQEEADFYGIPPYSLSDHLANEM	119 134 130 144 149 129 117 121 106 177 151 135 111
C D E	TNFAIP1 KCTD13 KCTD7 KCTD14 KCTD3 SHKBP1 KCTD9 KCTD2 KCTD2 KCTD5 KCTD17 KCTD18 KCTD8 KCTD16	LETLT-RFPDSMLGAMFRAGTPMPPNLNSQGGGHYFIDRDGKAFRHILNFLRLGRLDLPRGYGETALLRAEADFYQIRPLLDALRELEASQ MQTLT-K-QDTMLKAMFS	119 134 130 144 149 129 117 121 106 177 151 135 111 153 129
D E F	TNFAIP1 KCTD13 KCTD7 KCTD14 KCTD3 SHKBP1 KCTD9 KCTD2 KCTD2 KCTD5 KCTD17 KCTD18 KCTD16 KCTD16 KCTD12	LETLT-RFPDSMLGAMFRAGTPMPPNLNSQGGGHYFIDRDGKAFRHILNFLRLGRLDLPRGYGETALLRAEADFYQIRPLLDALRELEASQ MQTLT-K-QDTMLKAMFSGRMEVLTDSEGWILIDRCGKHFGTILNYLRDGAVPLPESRREIEELLAEAKYYLVQGLVEECQAALQNKDTY VRALT-R-HDTMLKAMFSGRMEVLTDKEGWILIDRCGKHFGTILNYLRDGSVPLPESRREIEELLAEAKYYLVQGLVEECQAALQNKDTY LRTLT-G-QDTMLKAMFSGRMEVLTDAGGWVLIDRSGRHFGTILNYLRDGSVPLPESTRELGELLGEARYYLVQGLIEDCQLALQQKRETL- LSTLR-CYEDTMLAAMFSGRHYIPTDSEGRYFIDRDGTHFGDVLNFLRSGDLPPRERVRAVYKEAQYYAIGPLLEQLENMQPLKG LGTLR-KFPGSKLAEMFSGRISTLRDETGAIFIDRDFTYFRPILDYLRTGQVPTQHIPEVYREAQFYEIKPLVKLLEDMPQIF RQTLM-WIPDSFFSSLLSGRISTLRDETGAIFIDRDPAFAPILNFLRTKELDLRGVSINVLRHEAEFYGITPLVRRLQLREELDRSSC- RSTLVNKEPDSMLAHMF-KDSGRISTLKDETGAIFIDRDPTVFAPILNFLRTKELDPRGVHGSSLLHEAQFYGLTPLVRRLQLREELDRSSC- RSTLVNKEPDSMLAHMF-KDKGVWGNKQDHRGAFLIDRSPEYFEPILNYLRHGQLIVN-DGINLLGVLEEAFFGIDSLIEHLEVAIKNS RQTLG-REPKSFLCRLCCQEDPELDSDKDETGAYLIDRDPTYFGPILNYLRHGKLVIN-KDLAEEGVLEEAEFYNISLKLVKKERIRDNENRT- RQTLC-RDPKSFLSRLC_QADPLDSDKDETGAYLIDRDPTYFGPILNYLRHGKLVIN-KDLAEEGVLEEAEFYNISLKLVKKDKIRERDSKTS RQTLC-REQKSFLSRLCQGEELQSDRDETGAYLIDRDPTYFGPILNYLRHGKLVIN-KDLAEEGVLEEAEFYNIGPLIRIIKDRMEEKDYTV- RESLC-RFKDSMLASMFSPSSPRGGARRGELPRDSRARFFIDRDGFLFYVLDYLRDKQLALPEHFPEKERLLREAEYFQLTDLVKLLSPKUKQL HSTLL-SIPHSLLWKMFSPKRDTANDLAKDSKGRFFIDRDGFLFYVLDYLRDKQLALPEHFPEKERLLREAEYFQLTDLVKLLSPKVTKQL HSTLU-SVPDSTLASMFSPSFRDLAKDSKGRFFIDRDGFLFYVLDYLDVLVRDVLPDHFPEKGRLKREAEYFQLTDLVKLLSPKVTKQL HSTLD-SVPDSTLASMFSPKRDTANDLAKDSKGRFFIDRDGFLFYVLDYLDYLDVLVVPDHFPEKGRLKREAEYFQLTDLVKLLSPKVTKQL HSTLD-SVPDSTLASMFSPKRDTANDLAKDSKGRFFIDRDGFLFYVLDYLDYLDYLDVLDVFDGFLFPEKGRLKREAEYFQLTDLVKLLSPKVTKQL	119 134 130 144 149 129 117 121 106 177 151 135 111 153 129 136
D E F	TNFAIP1 KCTD13 KCTD7 KCTD14 KCTD3 SHKBP1 KCTD9 KCTD2 KCTD2 KCTD5 KCTD17 KCTD18 KCTD8 KCTD16 KCTD12 KCNPC	LETLT-RFPDSMLGAMFRAGTPMPPNLNSQGGGHYFIDRDGKAFRHILNFLRLGRLDLPRGYGETALLRAEADFYQIRPLLDALRELEASQ MQTLT-K-QDTMLKAMFSGRMEVLTDSEGWILIDRCGKHFGTILNYLRDGAVPLPESRREIEELLAEAKYYLVQGLVEECQAALQNKDTY VRALT-R-HDTMLKAMFSGRMEVLTDKEGWILIDRCGKHFGTILNYLRDGSVPLPESRREIEELLAEAKYYLVQGLVECQAALQNKDTS LRTLT-G-QDTMLKAMFSGRMEVLTDAGGWVLIDRSGRHFGTILNYLRDGSVPLPESTRELGELLGEARYYLVQGLIEDCQLALQQKRETL- LSTLR-CYEDTMLAAMFSGRHYIPTDSEGRYFIDRDGTHFGDVLNFLRSGDLPPRERVRAVYKEAQYYAIGPLLEQLENMQPLKG LGTLR-KFPGSKLAEMFSGRISTLRDETGAIFIDRDFTYFRPILDYLRTGQVPTQHIPEVYREAQFYEIKPLVKLLEDMPQIF RQTLM-WIPDSFFSSLLSGRISTLRDETGAIFIDRDPAAFAPILNFLRTKELDLRGVSINVLRHEAEFYGITPLVRRLQLREELDRSSC- RSTLVNKEPDSMLAHMF-KDKGVWGNKQDHRGAFLIDRSPEYFEPILNYLRHGQLIVN-DGINLLGVLEEAFFGIDSLIEHLEVAIKNS RQTLG-REPKSFLCRLCCQEDPELDSDKDETGAYLIDRDPTYFGPILNYLRHGKLVIT-KELAEEGVLEEAEFYNISLIKLVKKERIRDNENRT- RQTLC-RDPKSFLSRLC-QADPLDSDKDETGAYLIDRDPTYFGPILNYLRHGKLVIN-KDLAEEGVLEEAEFYNISLKLVKDKIRERDSKTS RQTLC-REQKSFLSRLCQGEELQSDRDETGAYLIDRDPTYFGPILNYLRHGKLVIN-KDLAEEGVLEEAEFYNIGPLIRIIKDRMEEKDYTV- RESLC-RFKDSMLASMFSGRFPLKTDESGACVIDRDGFLFYVLDYLRHGKLVIN-KDLAEEGVLEEAEFYNIGPLIRIIKDRMEEKDYTV- RSTL-SVPDSTLASMFSPSSPRGGARRRGELPRDSRARFFIDRDGFLFYVLDYLRDKQLALPEHFPEKERLLREAEYFQLDLVKLLSPKVTKQ HSTLL-SVPDSTLASMFSPSPSPRGGARRRGELPRDSRARFFIDRDGFLFYVLDYLRDQUVLPDHFPEKGRLKREAEYFQLDLVKLLSPKVTKQ RCTVV-SVPDSLLWRMFFQKRDTANDLAKDSKGRFFIDRDGFLFYVLDYLRDQUVLPDHFPEKSRLQREAEFYNLGPLDVKLLSPKVTKQ RCTVV-SVPDSLLWRMFFQKRDTANDLAKDSKGRFFIDRDGFLFYLLDYLRDLVLDFYFPERSRLQREAEFYGLPDLVKLLSPKVTKQ RCTVV-SVPDSLLWRMFFQKRDTANDLAKDSKGRFFIDRDGFLFYLLDYLRDQVVLPDHFPEKSRLQREAEFYGLPDLVKLLSPKVTKQ RCTVV-SVPDSLLWRMFFQQQPQELARDSKGRFFIDRDGFLFYLLDYLDFTWDUULUPTPERSRLQREAEFYGLPDLVKLLSPKVTKQ	119 134 130 144 149 129 117 121 106 177 151 135 111 153 129 136 106

Figure S4: Sequence alignment of members of the KCTD family grouped into the clades defined by Skoblov et al. ²⁴. The proline residues of the pre-BTB regions are highlighted in grey. Secondary structure elements of the KCTD proteins whose crystal structure has been reported are colored in blue (α -helices) and red (β -sheets). KCTD20 and BTBD10 are not reported as they present a rather low sequence identity with the other members of the family.

Protein	Retention Volume (mL)	Calculated Molecular weight (kDa)	Theoretical Molecular weight of the monomer (kDa)	Ratio
KCTD1	12.5	131.22	29.46	4.5
KCTD1 ^{BTB}	14.6	60.81	13.20	4.6
KCTD1 ^{CTD}	14.5	63.10	14.04	4.5

Table S1. SEC data of KCTD1, KCTD1^{BTB}, and KCTD1^{CTD}.

Table S2: Percentages of sequence identity, computed on the BTB domain and on the pre-BTB portion, between human KCTD1 and its orthologs isolated from five species: 4 different classes of the Chordata phylum (*Gallus gallus, Danio rerio, Mouse, Alligator mississippiensis*) and 1 of the Nematoda phylum (*Caenorhabditis elegans*).

KCTD1	Mouse	Caenorhabditis elegans	Danio rerio	Alligator mississippiensis	Gallus gallus
BTB	100	95.0	89.1	97.0	97.0
Pre-BTB	100	96.7	61.3	100	100

Table S3. Oligonucleotides used for the preparation of KCTD1 proteins. NcoI and XhoI restriction site used for KCTD1^{CTD} cloning are underlined.

Protein	Oligonucleotides
KCTD1 ^{CTD}	Fw: CATG <u>CCATGG</u> GGACCGGTCGTTTTAGCCGTC
	Rev: CATG <u>CTCGAG</u> TTAATCCAGCGGTTCTTGTT
KCTD1 ^{P20S}	Fw: CTGAATAATCAGGGTATTTCGACACCGGCACAGCTGAC
	Rev: GTCAGCTGTGCCGGTGTCGAAATACCCTGATTATTCAG
KCTD1 ^{H33P}	Fw: CAAAAGCAATGCACCGGTTCCTATTGATGTTGGTGGTCATATG
	Rev: CATATGACCACCAACATCAATAGGAACCGGTGCATTGCTTTTG
KCTD1 ^{G62D}	Fw: GGTCGTCTGTTTGATGACACCGAACCGATTGTTCTG
	Rev: CAGAACAATCGGTTCGGTGTCATCAAACAGACGACC
KCTD1 ^{D69E}	Fw: GAACCGATTGTTCTGGAAAGCCTGAAACAGCACTATTTC
	Rev: GAAATAGTGCTGTTTCAGGCTTTCCAGAACAATCGGTTC
KCTD1 ^{H74P}	Fw: CTGGATAGCCTGAAACAGCCCTATTTCATTGATCGTGATG
	Rev: CATCACGATCAATGAAATAGGGCTGTTTCAGGCTATCCAG