

Supplementary Material

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

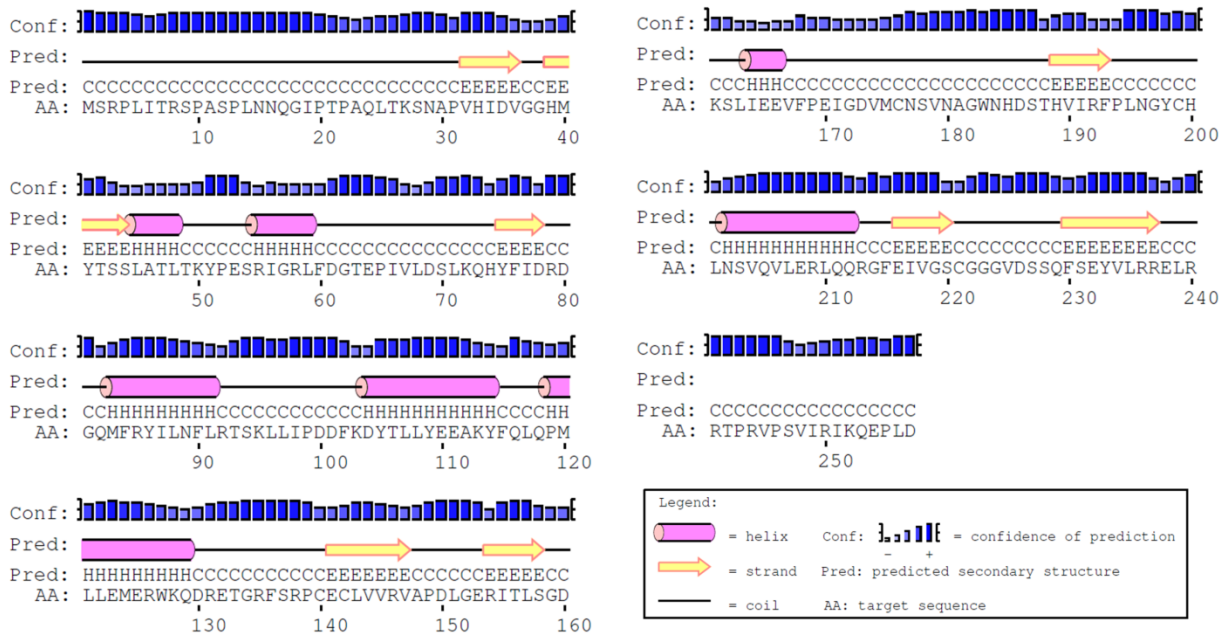
Giovanni Smaldone^a, Nicole Balasco^b, Luciano Pirone^b, Daniela Caruso^{b,c}, Sonia Di Gaetano^b, Emilia Maria Pedone^b, and Luigi Vitagliano^{b,*}

^aIRCCS SDN, Via Gianturco 113, 80143 Napoli, Italy

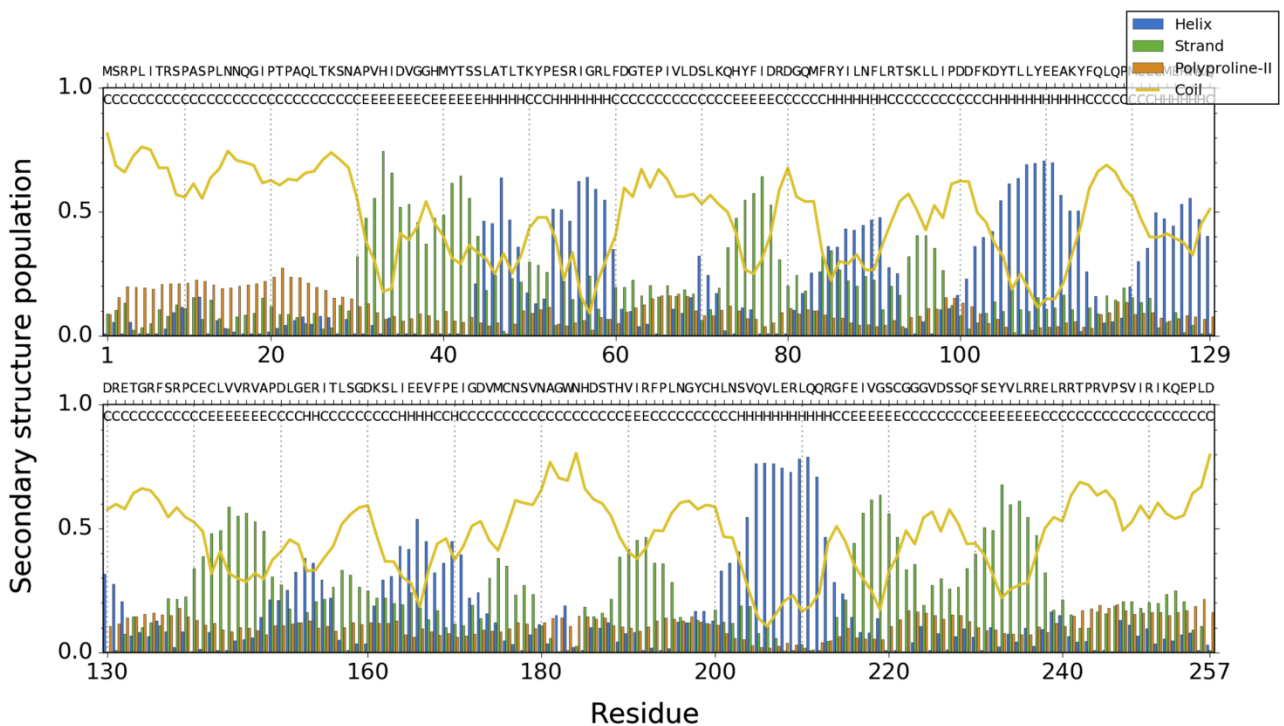
^bInstitute of Biostructures and Bioimaging, CNR, Via Mezzocannone 16, 80134 Napoli, Italy

^cUniversità degli Studi della Campania "Luigi Vanvitelli", Viale Abramo Lincoln 5, 81100 Caserta, Italy

*corresponding author: Luigi Vitagliano, luigi.vitagliano@unina.it, 0039 081 2534506

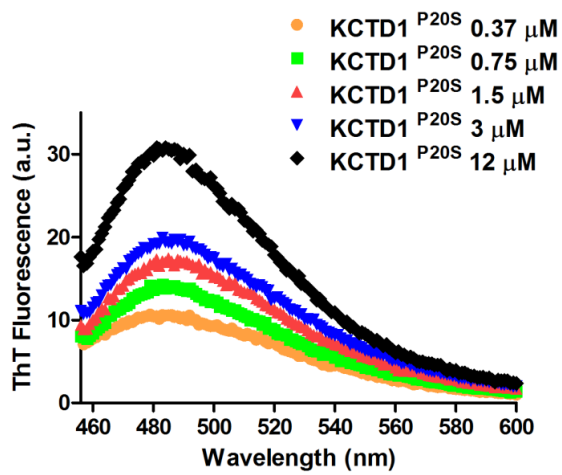


A

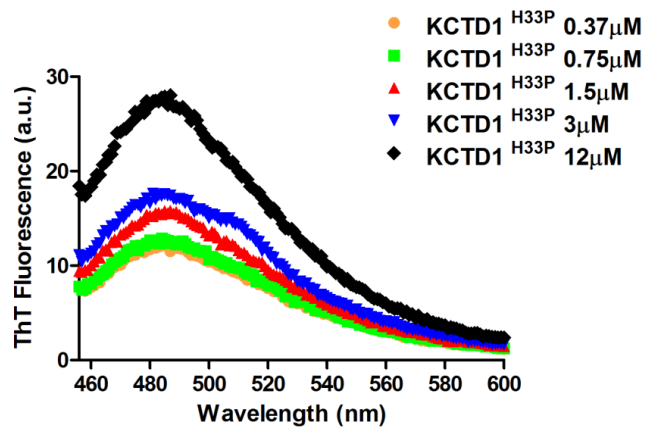


B

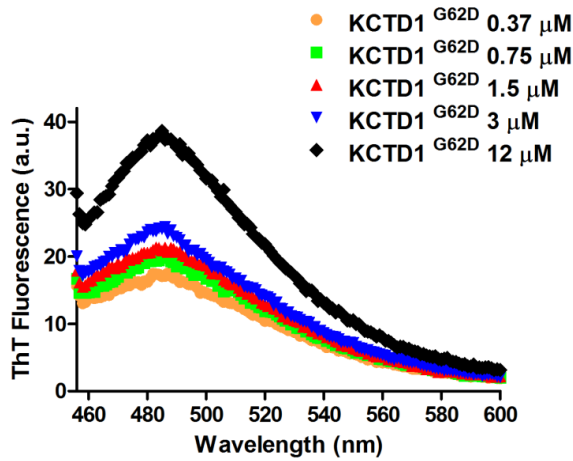
Figure S1: Secondary structure prediction of KCTD1 sequence computed by using Psipred server⁴⁷ (A) and Vendruscolo Lab. Software⁴⁸ (B).



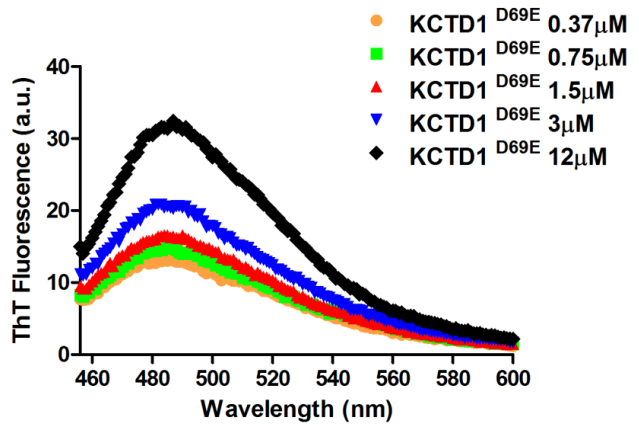
A



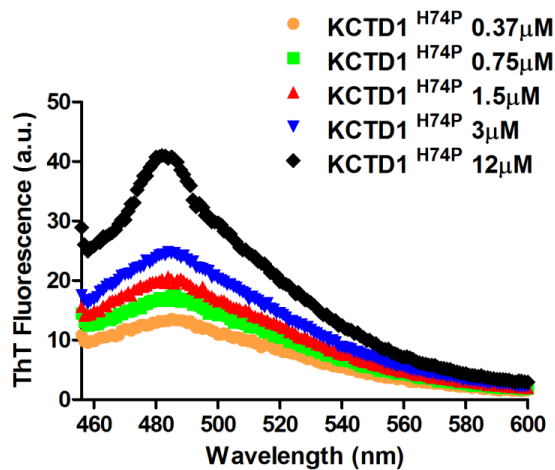
B



C

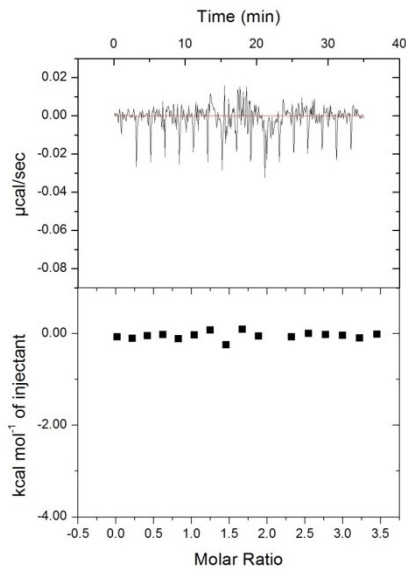


D

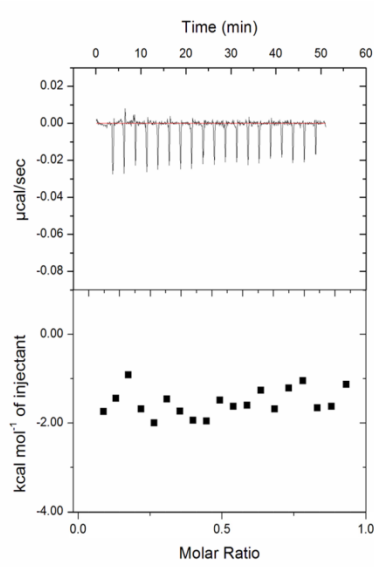


E

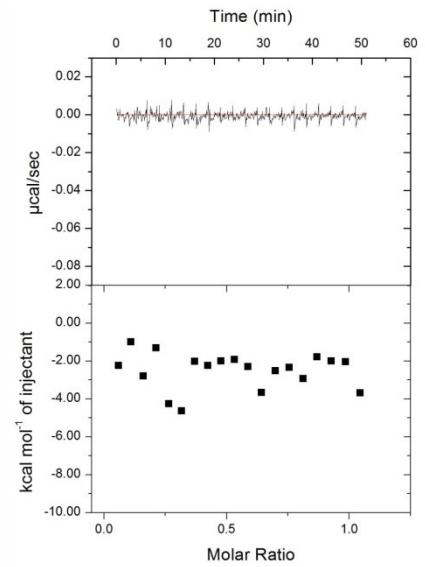
Figure S2. Thioflavin T Fluorescence assay: emission spectra obtained by adding a ThT solution (50 μ M) to KCTD1^{P20S} (A), KCTD1^{H33P} (B), KCTD1^{G62D} (C), KCTD1^{D69E} (D), and KCTD1^{H74P} (E) at protein concentrations ranging from 0.37 μ M to 12 μ M.



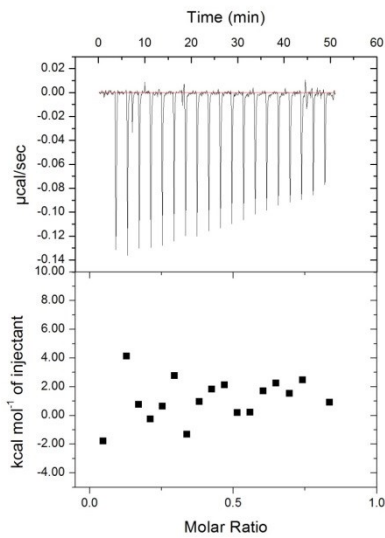
A



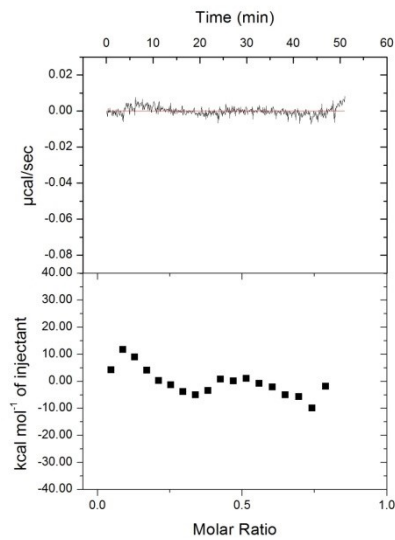
B



C



D



E

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 α ^{NTD} by Isothermal Titration Calorimetry. ITC experiments were performed by titrating KCTD1 variants (72 μ M-expressed as pentamer) into a solution of AP-2 α ^{NTD} (7 μ M). Top and bottom panels report raw and integrated data, respectively.

A	KCTD1	-----MSRFLITRSASFLNNQGITPAQLTKSNAE VHIDVGGHMYTSS	44
	KCTD15	-----MPHRKERESGSSSLHTGSGTAEAGNMSRSLSTRSEVSEFLAAQGITPAQLTKSNAE VHIDVGGHMYTSS	70
	KCTD19a	-----MEESGMAHESAEDLFPHNVGGWHFVSVP	27
	KCTD4	-----MERKINRREKEKEYEGKHNLEDTDQGNCKSTLMTLNVGGYLYITQ	47
	KCTD19b	-----IKVYVGS SHWYATT	393
B	KCTD6	-----MDNGDWGYMMTDVTLNVGGHLYTTS	26
	KCTD21	-----MSDITLNVGGKLYTTS	17
	KCTD11	-----ISPPPVSESSPSPFGGVTLNVGGTLYSTT	29
C	KCTD10	-----MEEMSGESVVSASVAAAATRTTSFKGTS SSKVVKLVNNGGALYTTT	46
	TNFAIP1	-----MSGDTCLCPASGAKPKLSGFKGGGLNKYVQLNVGGSLYTTT	42
	KCTD13	-----MSAEASGFAAAAAE SLEAPKESGLEPGPAAYGLKELTNSKYVVLNNGGSLHYTT	55
	KCTD7	-----MVVVTGREEDSRRQDGAMSSSDAEDDFLEATPTATQAGHALLELQEFEEVVPLNIGGAHFTTR	65
	KCTD14	-----MWQCAVERE VGRMTSQTLEQSPRRRTMSTVVELNVGGEFHTTT	47
D	KCTD3	-----MAGGHCGSFAAAAGSGEIVQLNVGGTRFSTS	32
	SHKBP1	-----MAAATAAEGV ESRGPGEVHILNVGGKRFSTT	37
	KCTD9	MRRVTLFLNGSE KNGKVVAVYGTLSDLLSVASSKLGIKATSVYNGKGLIDDIALLRDDDLVFCVCEGEFFIDPQTDSEKPEGLLGFHTDWTLNVGGGRYFTTT	104
E	KCTD2	-----MAELQLDEAMAGLGGGGSGVGDGGG EVRGPESEPRAGETPRGHGRFAAAVAQPLEPCGPPERAGGGGAARWVRLNVGGTYFVTT	85
	KCTD5	-----MAENHCELLSE PARGGIGAGLGGGLCRRCSAGLGALAQREGSVSKWVRLNVGGTYFLTT	58
	KCTD17	-----MQT PREPAMRMEAGEAAPAGAGGRAAGGWGKWVRLNVGGTVFLTT	45
	KCTD18	-----MEGHKAEVEEVLVRLNVGGCIYAR	26
F	KCTD8	-----MALKDTGSGGSTILP ISEMVSSSSSPGASAAAAPGCAESPFEVVELNVGGQVYVTK	58
	KCTD16	-----MALSGNCSRY PREQGSAVNSPEVVELNVGGQVYFTR	39
	KCTD12	-----MALADSTRGLE NGGGGGGGSGSSSSSAEPPLFDIVELNVGGQVYVTR	48
	KCNRG	-----MSSQELVTLNVGGKIFTRR	19
A	KCTD1	LATLT -KYPES SRIGRLF DGT----EPI VLD SLKQ-- HYFI DRDQ MFRIYLNFLR TSKLLIPDDFKDY TLLYEAKYQLQPLM LEMER WKQDR ----	131
	KCTD15	LATLT -KYPDSRIS RFLNGT ----EPIVLD SLKQH ---YFIDR DGEIFRYVLSFLR TSKLLLPDDFKDFSLLYE EARYQLQPMVRELER WQQEQ----	157
	KCTD19a	RSKLS -QFPDS----LL WKE -----ASALT SSESQR ---LFIDR DGSTRFHVHYLYTSKLS FSS-CAELNLL YEQALGLQ LMPLL QTL DLN KEGK H----	110
	KCTD4	KQTLT -KYPDT FL -EGIVNG----KIL CFD ADGH---YFIDR DGLFRHVLNFLR NGEL LLPEGFRENQ LLA EQAEF Q LKGLAE EVKS RWEKE ----	133
	KCTD19b	LQTLT -KYP----EL LSNP ----Q RVYWI TYG QT ---LLI HGDQ MF RHILN FL R L LGK FL PSE FK EWPL FC QEV EY HI PS LSE AL AQ CE AYK ----	492
B	KCTD6	LTTLT -RYPDS MLGAM FGG-----DF PTAR DP Q G--NYFIDR DGFL RY VLN FL R TSE L TP LD FD KE FD LLR KE AD FY QIE PL I Q CL ND PK PLY----	112
	KCTD21	LATLT -SFPDS MLGAM FSG-----K MPT KRDS Q G--N CFID R DGK V F RY ILN FL R TS H LD LP ED FQ EM GLLR RE AD FY Q VP LI E AL Q KE VE L ----	103
	KCTD11	LETLT -RFPDS MLGAM FRAG----T PMP PN LS Q GG GHY FID R DG K A FR HLN FL R L GR LD L PR GY GE TAL L R AE AD FY Q IR PL LD AL RE LE AS Q ----	119
C	KCTD10	MQTLT -K-QD TMLKAM F S -----G R MEV LT D SE GV ILID R CG K H FG TIL NY L R DG AV PL PE S R REI E ELLA E AKY YL VQ GL VEE C Q AAL Q N K DTY--	134
	TNFAIP1	VRALT -R-HD TMLKAM F-----S G RMEV LT D KE GW ILID R CG K H FG TIL NY L R DD TI T LP Q NR Q E I K EL MA E AKY YL I Q GL V N M C S AL Q D K K D S--	130
	KCTD13	LRTL T-G-QD TMLKAM F-----S G RVEV LT D AG GW LI DR S GR H FG TIL NY L R DG SV LP EST REL G ELL GE ARY YL VQ GL I ED Q L AL Q Q K R ETL-	144
	KCTD7	LSTLR -CYED TMLA AMF-----S G RHY I PT D SE GR Y FID R DG TF H GD V LN FL RS GD LP--R ER V AV Y KEA Q Y AI G PL LE Q LEN M Q PL K G----	149
	KCTD14	LGTLR -K FP GS KLA EMF-----S S LAK AST D A E GR FF ID RP ST Y FR PI LD Y L R T G Q VP---T Q HI PE VY REA Q F Y E IK PL V K LE D MP Q IF----	129
D	KCTD3	RQTL M-WIPDS FF SS LL -----S G R I ST L R DE T GA IF ID R D PA AF AP IL N FL R T KE LD L--R G V S I N V L R H E A E F Y G IT PL V R RL LL C E E L E R ----	117
	SHKBP1	RQTL TW-IPDS FF SS LL -----S G R I ST L K DE T GA I FID R D PT VF AP IL N FL R T KE LD P--R G V H G S SS LL H EA Q F Y GL T PL V R RL Q L R E E L D R S C-	121
	KCTD9	RSTL VN KE PD SML A H MF-KD----K G V W G N Q D H R GA FLI DR S PE Y F EP IL NY L R H G Q L I V N-D G I N LL G V LE E AR FF G D S L E H LE V A I K N S ----	106
E	KCTD2	RQTL G-REP K S FL CR LCC QE-----D P EL D SD K DE T GA YLID R D PT Y FG PI LN Y L R H G K L I T -K E L A E E GV LE E A E F Y N I A S L V R L V K E R I DN EN RT-	177
	KCTD5	RQTL C-RDP K S FL Y R LC-QA-----D P DL D SD K DE T GA YLI DR D PT Y FG PI LN Y L R H G K L V I N-K D L A E E GV LE E A E F Y N I T S L I K L V K D K I R ER D S K T S	151
	KCTD17	RQTL C-REQ K S FL S R LC Q -----G E EL Q S D R DE T GA YLI DR D PT Y FG PI LN Y L R H G K L V L D-K D M A E E GV LE E A E F Y N I G PL R I I K D R M E E K D Y T V-	135
	KCTD18	RESL C-R F K D S ML AS M FS G R-----F P LT D ES G AC VID R D GR L FK Y LL D Y L H G -E V Q I PT D E Q TR I AL Q E E A D Y F G I P Y P S Y S L D H L AN E M-----	111
F	KCTD8	HSTL L-SVPDS TL AS M FS P SS PR GGARR R G EL PR D SR AR FF ID R D GF L FR Y LD Y L R D K Q L AL P EH F PE K ER LL RE A E F Y Q L T D L V K L S PK V T R Q----	153
	KCTD16	HSTL I-S I PH S LL W K M F S PK--R D TAN---D L AK D SK GR FFI DR D GF L FR Y I L D Y L R D R Q V VP D HP PE K GR L K R E A E F Y Q L P D L V K L L T P D E IK Q ----	129
	KCTD12	RCTV V-SVPDS LL WR M FT Q Q---Q P Q---E L AR D SK GR FF L DR D GF L FR Y I L D Y L R D L Q V LP D Y F P E RS R L Q RE A E F Y EL PE L V R RL G AP Q Q P G----	136
	KCNRG	FSTI K-Q F PA S R L AR L MD G R---D Q ---E F K M V G -G Q I F V D R D GD L FS F IL D FL R TH Q LL P TE F S D Y L RL Q RE A L F Y E L R S L V D L N P Y LL Q PR----	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.

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

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 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	<i>Mouse</i>	<i>Caenorhabditis elegans</i>	<i>Danio rerio</i>	<i>Alligator mississippiensis</i>	<i>Gallus gallus</i>
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: CATGCC <u>ATGGG</u> ACCGGTCGTTTTAGCCGTC Rev: CATG <u>CTCGAG</u> TTAATCCAGCGGTTCTTGTT
KCTD1 ^{P20S}	Fw: CTGAATAATCAGGGTATTTTCGACACCGGCACAGCTGAC Rev: GTCAGCTGTGCCGGTGTCGAAATACCCTGATTATTCAG
KCTD1 ^{H33P}	Fw: CAAAAGCAATGCACCGGTTCTATTGATGTTGGTGGTCATATG Rev: CATATGACCACCAACATCAATAGGAACCGGTGCATTGCTTTTG
KCTD1 ^{G62D}	Fw: GGTCGTCTGTTTGATGACACCGAACCGATTGTTCTG Rev: CAGAACAATCGGTTCCGGTGTCATCAAACAGACGACC
KCTD1 ^{D69E}	Fw: GAACCGATTGTTCTGGAAAGCCTGAAACAGCACTATTTTC Rev: GAAATAGTGCTGTTTCAGGCTTTCCAGAACAATCGGTTTC
KCTD1 ^{H74P}	Fw: CTGGATAGCCTGAAACAGCCCTATTTTCATTGATCGTGATG Rev: CATCACGATCAATGAAATAGGGCTGTTTCAGGCTATCCAG