Supplementary Material

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

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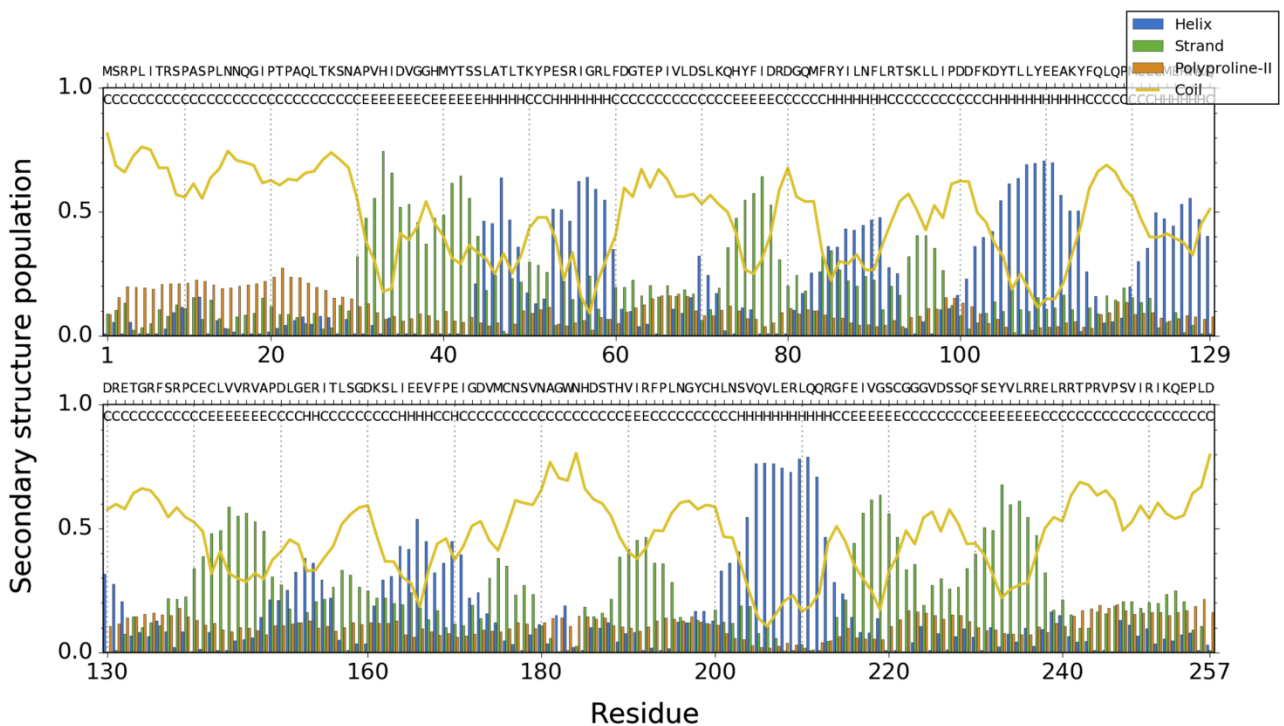
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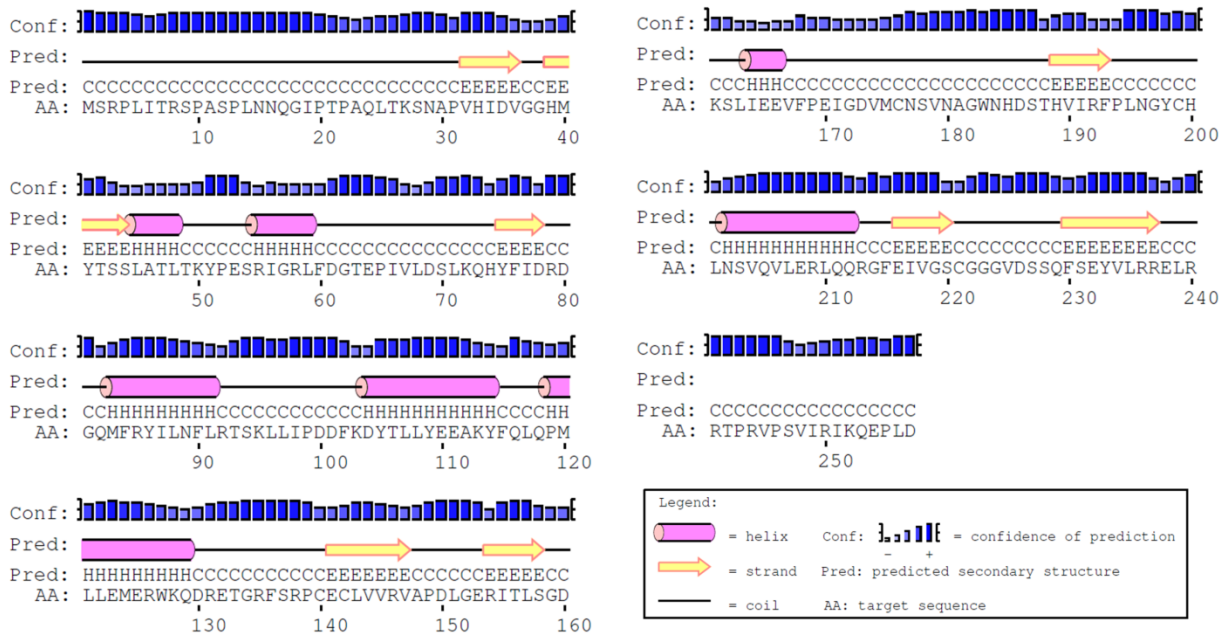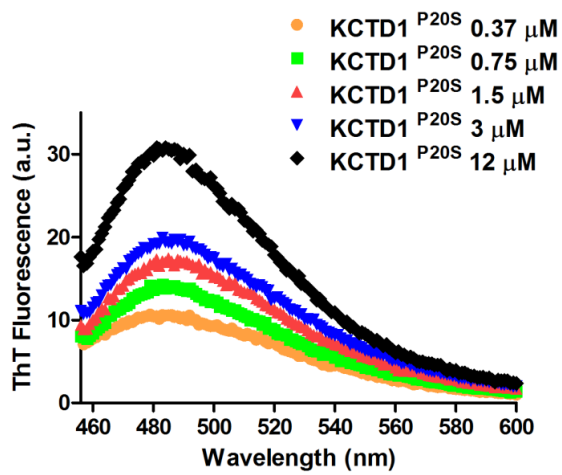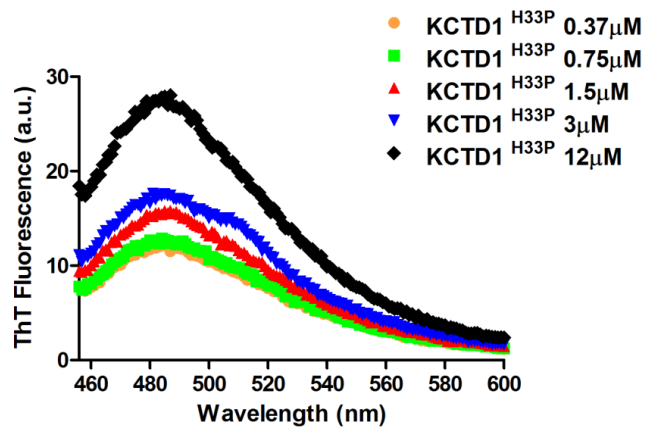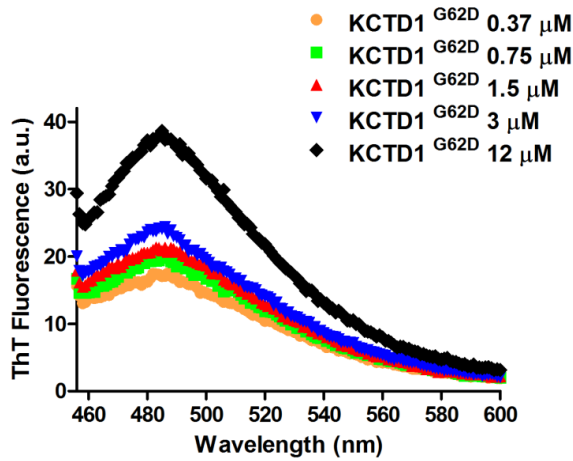
Figure S1: Secondary structure prediction of KCTD1 sequence computed by using Psipred server [47] (A) and Vendruscolo Lab. Software [48] (B).
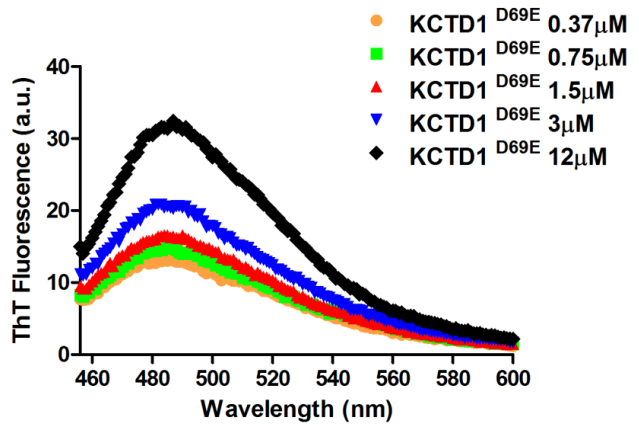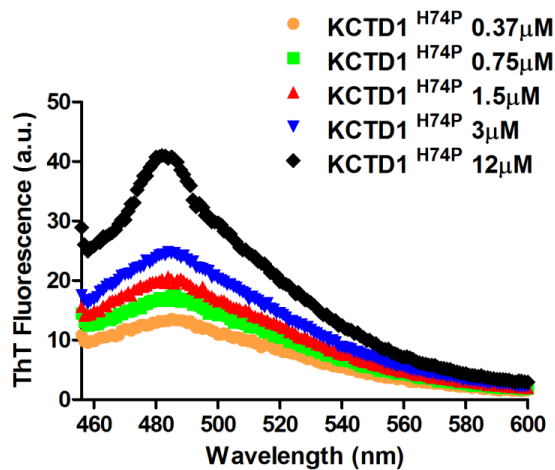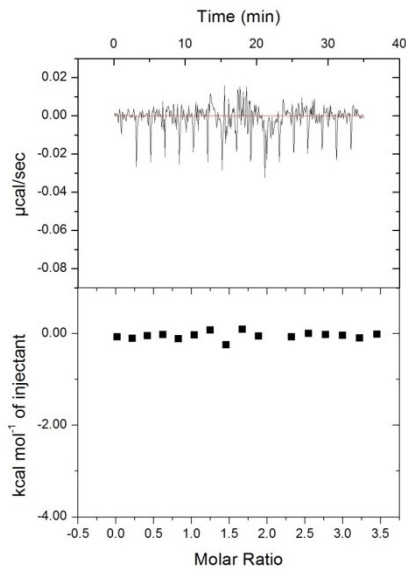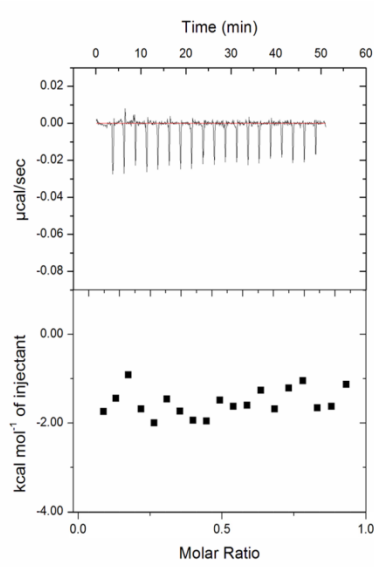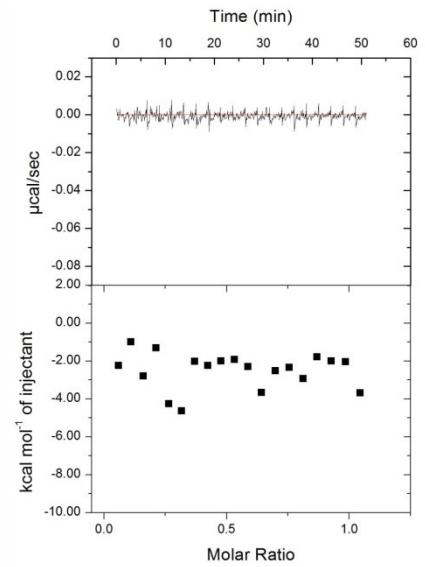
Figure S2. Thioflavin T Fluorescence assay: emission spectra obtained by adding a ThT solution (50μM) to KCTD1^{P20S} (A), KCTD1^{H33P} (B), KCTD1^{G62D} (C), KCTD1^{D69E} (D), and KCTD1^{H74P} (E) at protein concentrations ranging from 0.37 μM to 12 μM.
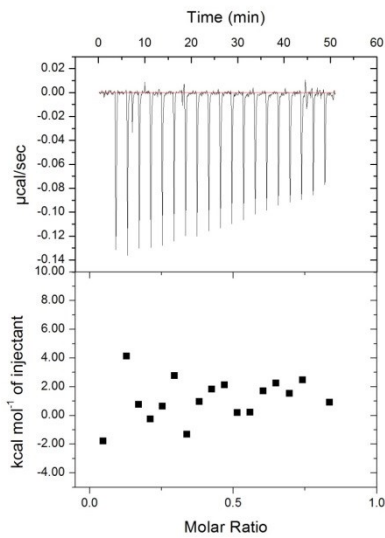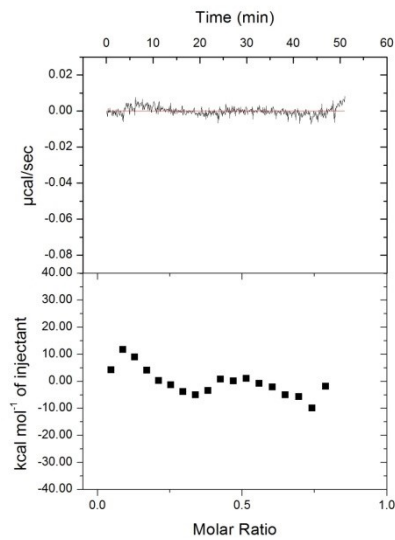
Figure S3: Evaluation of the binding of KCTD1 SEN related mutants KCTD1^{P20S} (A), KCTD1^{H33P} (B), KCTD1^{G62D} (C), KCTD1^{D69E} (D), KCTD1^{H74P} (E) to AP-2α^{NTD} by Isothermal Titration Calorimetry. ITC experiments were performed by titrating KCTD1 variants (72 μM-expressed as pentamer) into a solution of AP-2α^{NTD} (7 μM). Top and bottom panels report raw and integrated data, respectively.

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	*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: CATGCCATGGGGACCGGTCGTTTTAGCCGTC
	Rev: CATGCTCGAGTTAATCCAGCGGTTCTTGTT
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