Supporting Information

The human protein-L-isoaspartate *O*-methyltransferase domain-containing protein 1 (PCMTD1) associates with Cullin-RING ligase proteins

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Materials included:
Figures S1-S7

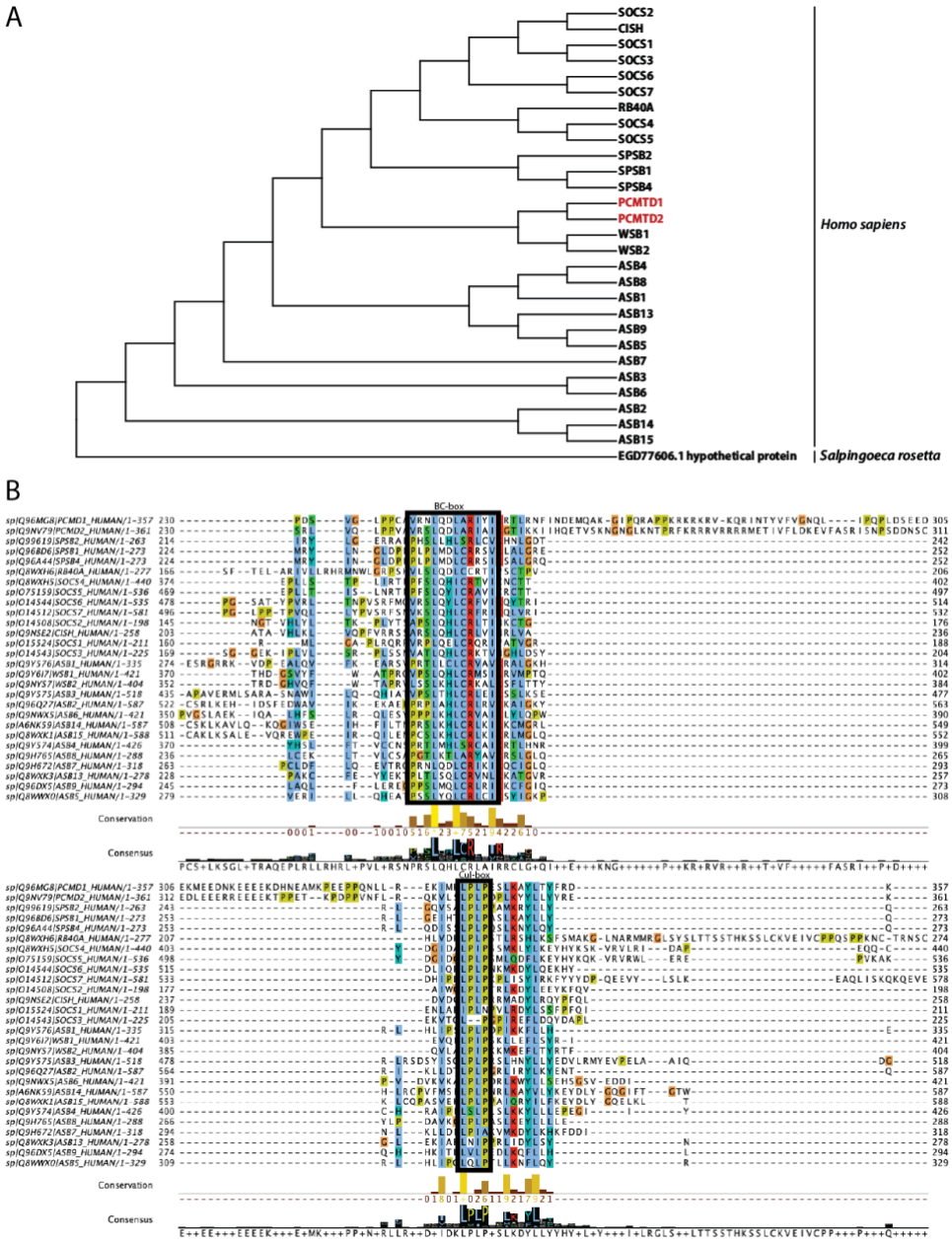

Figure S1. Sequence variation across different human SOCS box-containing proteins. **A.** A maximum likelihood tree comparing full sequences of human PCMTD proteins to full sequences of human SOCS box-containing proteins. The sequence of EGD77606.1 is a putative SOCS box protein from the choanoflagellate *S. rosetta* and acts as an outgroup to the human species. Accession numbers are indicated in bars, followed by protein name, and amino acid length of sequence. **B.** Sequences of the PCMTDs were aligned with SOCS box-containing proteins from the suppressor of cytokine signaling proteins (SOCS1-7), WD repeat and SOCS box-containing proteins (WSB1-2), Cytokine-inducible SH2-containing protein (CISH), Ras-related protein (RB40A), SPRY domain-containing SOCS box proteins (SPSB1-2, 4), and the Ankyrin repeat and SOCS box proteins. Only partial C-termini of the proteins are displayed to highlight the BC-box and the Cul-box (outlined). Unconserved residues are white. For conserved residues the color scheme is as follows: hydrophobic (blue), positive charge (red), negative charge (magenta), polar (green), cysteine (pink), glycine (orange), proline (yellow), aromatic (cyan).

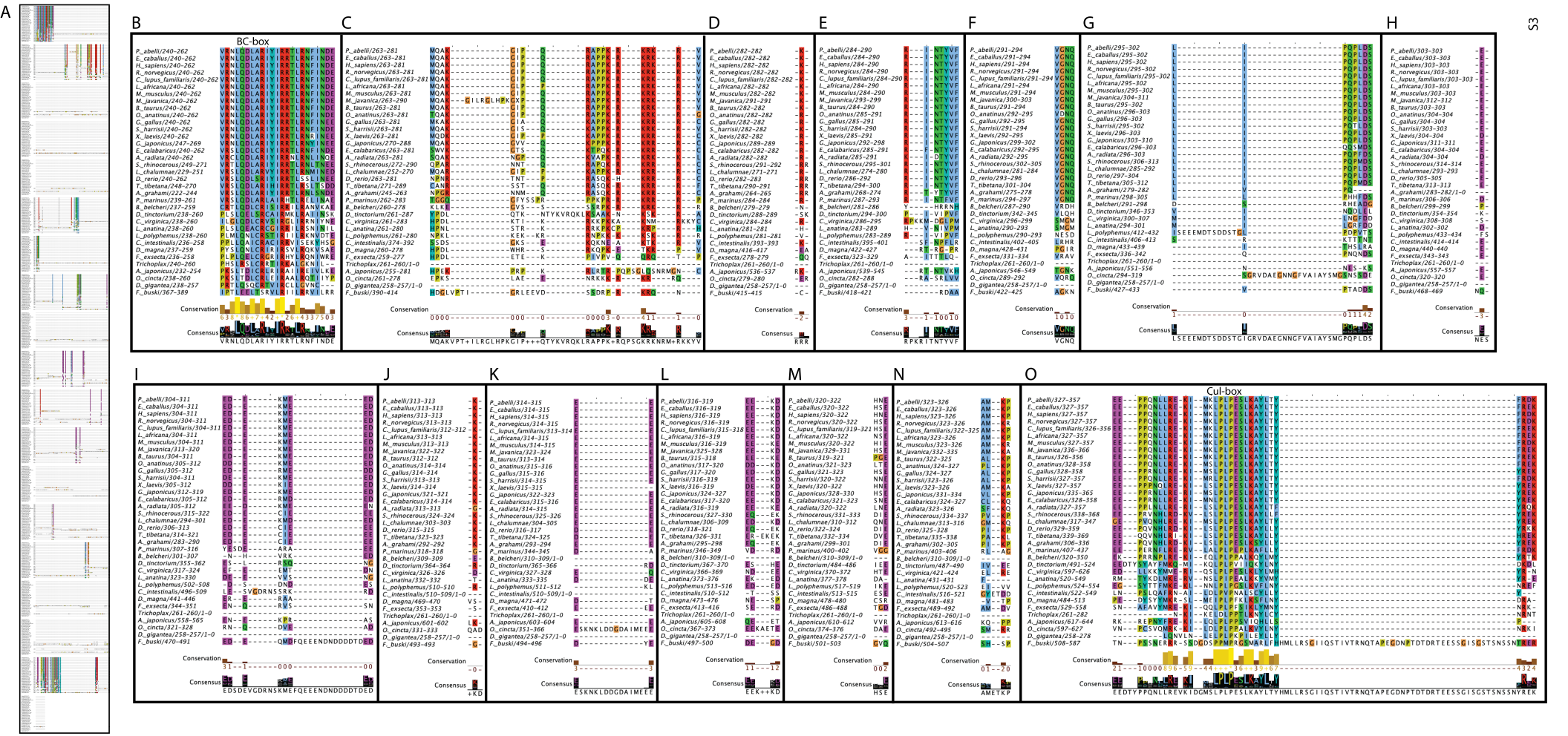
A
B
BC-box
C
D
E
F
G
H
S3
I
J
K
L
M
N
O
Cul-box
Conservation
Consensus

Figure S2. Conserved regions between the PCMTD1 BC-box and Cul-box across metazoan phyla. A protein BLAST search was performed against PCMTD1 isoform 1. Species were selected from each phylum that was identified and a multiple sequence alignment was performed. **A**. A T-Coffee multiple sequence alignment of PCMTD1 sequences was generated. **B-O**. Panels represent consecutive regions between the BC-box and the Cul-box of isoform 1 that have more than one or more conserved residues. Unconserved residues are white. For conserved residues the color scheme is as follows: hydrophobic (blue), positive charge (red), negative charge (magenta), polar (green), cysteine (pink), glycine (orange), proline (yellow), aromatic (cyan).

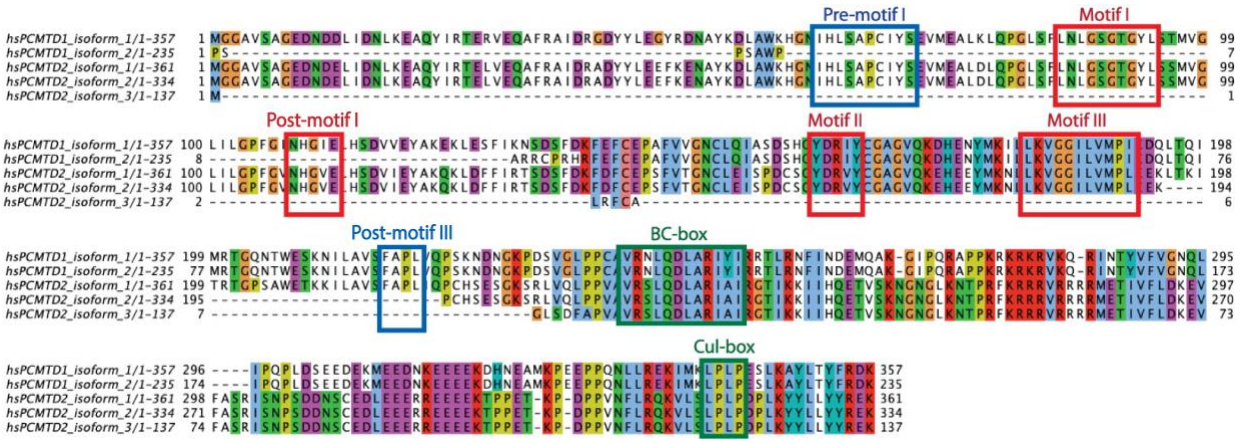

Figure S3. Multiple human isoforms of PCMTD result from alternative splicing. T-Coffee sequence alignment of the human PCMTD1 isoform 1, PCMTD1 isoform 2 (predicted), PCMTD2 isoform 1, PCMTD2 isoform 2 (predicted), and PCMTD2 isoform 3 (predicted; refs. 34-37). Isoform 1 for PCMTD1 and PCMTD2 represents the canonical sequence. In PCMTD1 isoform 2, residues 1-137 are replaced by the sequence PSPSAWPARRCPRHR. In PCMTD2 isoform 2 residues 195-221 are missing. In PCMTD2 isoform 3, residues 1-224 are missing and residues 225-236 are replaced with MLRFCAGLSDFA. Sequence outlined in blue corresponds to PCMT1 isoaspartyl-binding motifs, while sequence outlined in red represent PCMT1 AdoMet-binding motifs. Residues boxed in green in the PCMTD proteins comprise the BC-box and Cul-5 box binding motifs of the SOCS box domain. Unconserved residues are white. For conserved residues the color scheme is as follows: hydrophobic (blue), positive charge (red), negative charge (magenta), polar (green), cysteine (pink), glycine (orange), proline (yellow), aromatic (cyan).

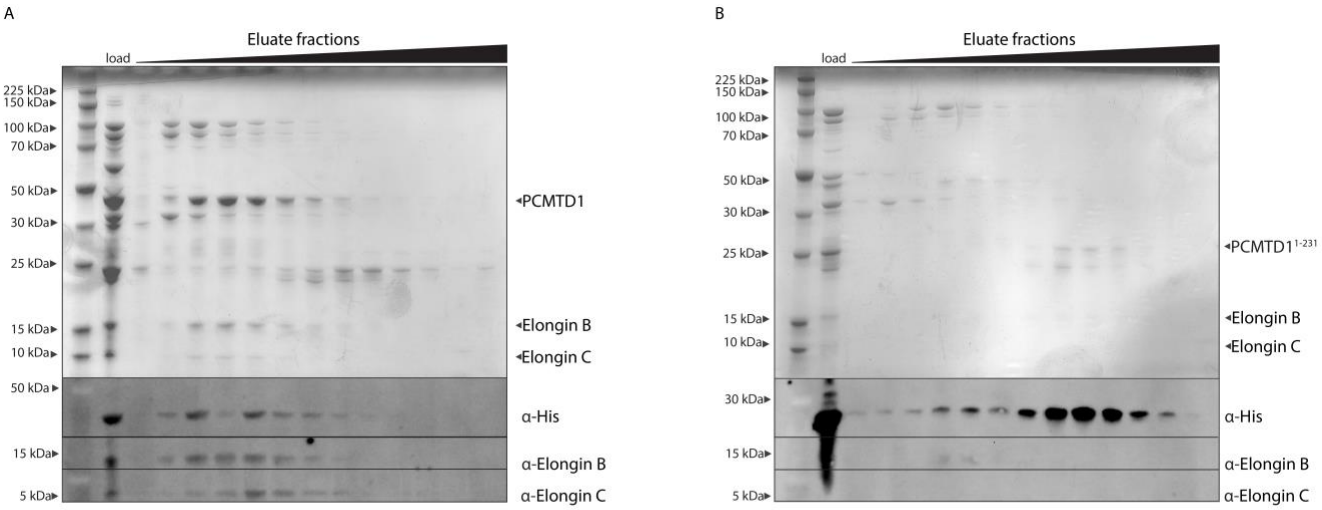

Figure S4. Elongins B and C co-purifies with full-length PCMTD1 but not with truncated PCMTD1$^{1-231}$. **A.** Following His-tag purification of a full-length N-terminal His-tagged PCMTD1 construct co-expressed with EloB and EloC, eluates containing protein were pooled and concentrated for size exclusion chromatography. Here, Elongins B and C were found to co-elute with the full-length PCMTD1 protein. The top gel represents a Coomassie stain of the size exclusion fractions and the immunoblots below represent immunostaining of a blot of a gel that was loaded equivalently. Load lane represents samples pooled after His-tag purification of recombinantly co-expressed 6xHis-PCMTD1 and Elongins B and C used for further purification via gel filtration. **B.** Following His-tag purification of a truncated N-terminal His-tagged PCMTD1$^{1-231}$ construct co-expressed with Elongins B and C, fractions were pooled and concentrated. Size exclusion chromatography indicated that the N-terminal His-tagged PCMTD1$^{1-231}$ does not co-elute with Elongins B and C. Load lane represents samples pooled after His-tag purification of recombinantly co-expressed 6xHis-PCMTD1$^{1-231}$ and Elongins B and C used for further purification via gel filtration.

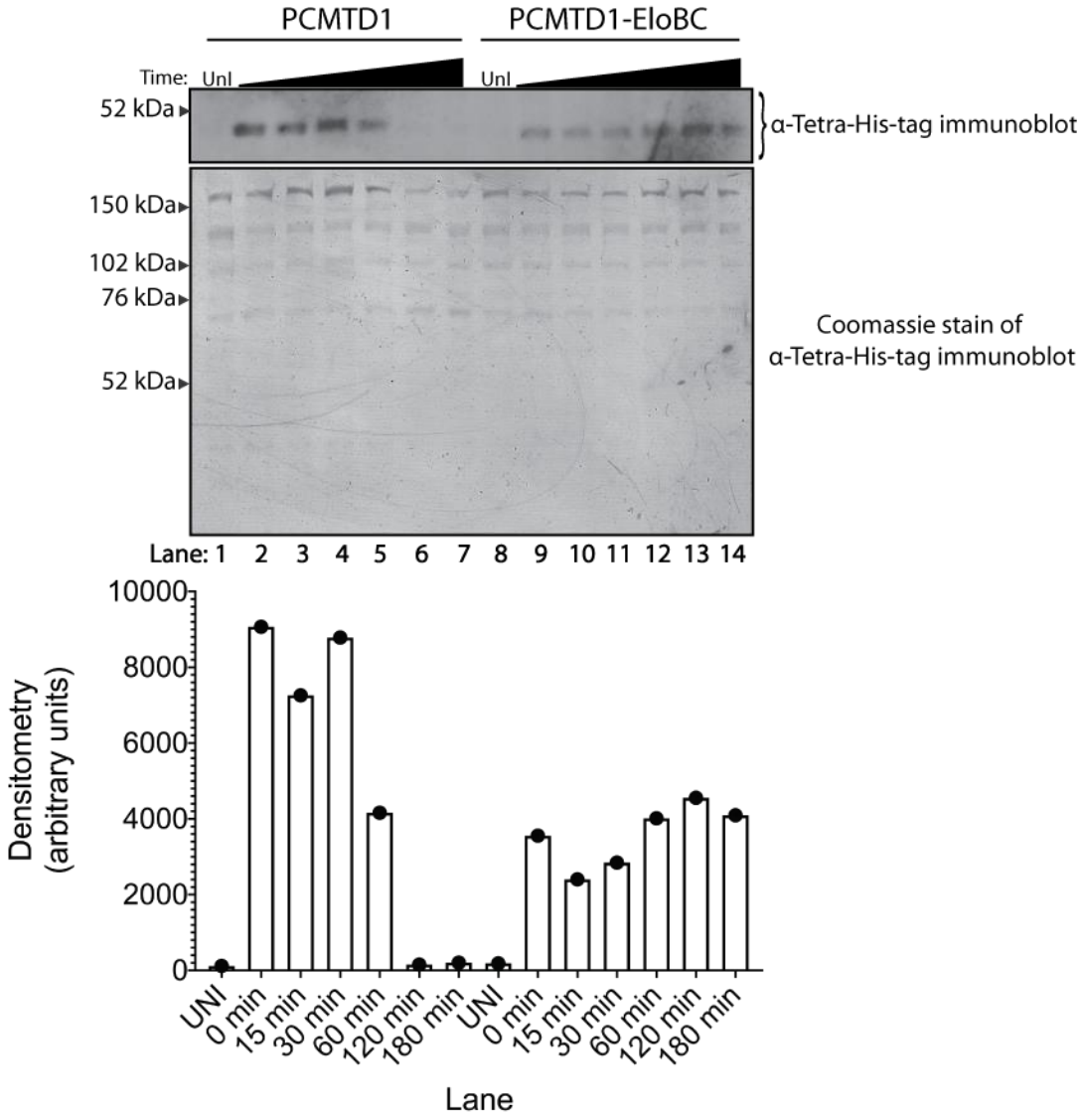

Figure S5. Elongins B and C help stabilize recombinant PCMTD1 replicate experiment. *E. coli* cells expressing PCMTD1 alone (lanes 1-7) or PCMTD1 with Elongins B and C (lanes 8-14) were treated with 25 μg/mL tetracycline as described in the Methods section. Lanes 1 and 8 represent uninduced cultures. Succeeding lanes show increasing time points after induced strains were treated with tetracycline. Lower panel represents densitometric quantification of the PCMTD1 band detected by anti-Tetra-His-tag antibody.

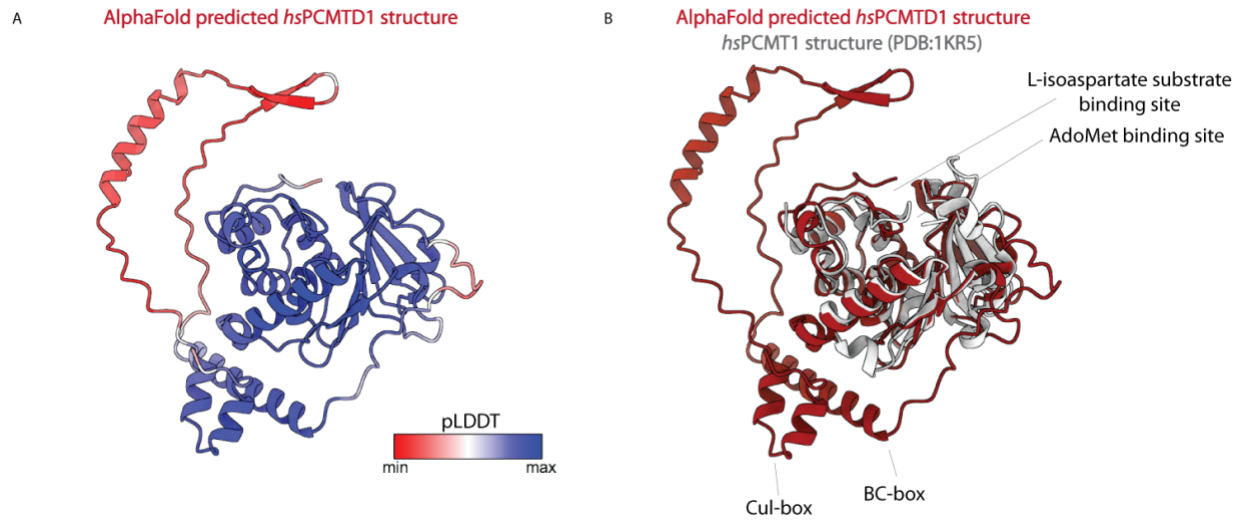

Figure S6. AlphaFold predicted structural model of human PCMTD1. **A.** Predicted structural model of human PCMTD1 from the AlphaFold Protein Structure Database (48). Backbone is colored by the per-residue confidence metric, predicted local distance difference test (pLDDT) with areas of higher confidence in blue and areas of lower confidence in red. **B.** Predicted structural model of human PCMTD1overlaid with the human PCMT1 crystal structure (PDB 1KR5; ref. 49).

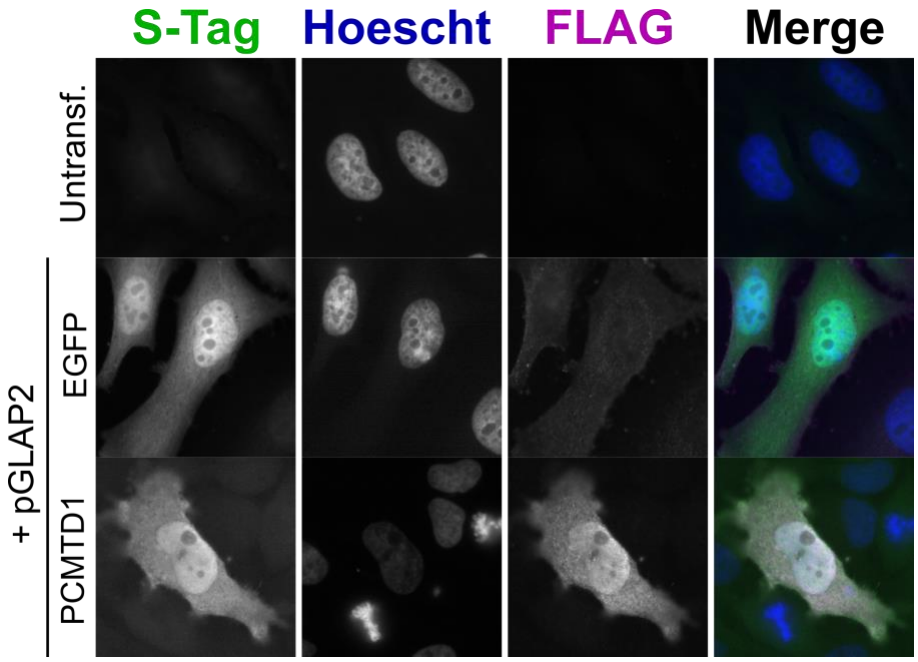

Figure S7. Microscopy of pGLAP2 EGFP and PCMTD1 constructs. 500 ng of pGLAP2 EGFP and PCMTD1 were transiently transfected into HeLa cells with 1.5 µL of Fugene 6. 48 hours after transfection, the cells were fixed with 4% PFA in PBS for 15 minutes at 37° C, permeabilized with 0.2% Triton X-100 in PBS for 1 minute, and stained with Hoescht 33342 (1 µg/mL) and antibodies (S-Tag: GeneTex, GTX19321, used at 1:100; FLAG: Cell Signaling Technology, #14793, used at 1:200; secondary antibodies from Jackson Immuno, used at 1:500). Images were captured with a Leica DMI6000 microscope (Leica DFC360 FX Camera; 63×/1.40-0.60 NA oil objective; Leica AF6000 software). Each image is 71.67 microns square. Untransf., untransfected HeLa cells.