Supplementary material

Protein preparation and quality control

To express CDIN1 and Codanin1$_{Cterm}$, we used a codon-optimized bacterial system developed to produce mammalian proteins efficiently. We purified both CDIN1 and Codanin1$_{Cterm}$ using a three-step purification. At first, we applied ultra-affinity chromatography involving CL7/Im7, followed by His-trap affinity chromatography. In the final polishing step, we used size-exclusion chromatography (SEC) to obtain CDIN1 and Codanin1$_{Cterm}$ with a purity above 95% (Fig. S1A, B). Subsequently, MALDI-TOF MS confirmed the identity of both proteins (Fig. S1C, D).

To evaluate protein quality and stability, we used a combination of biophysical methods. We recorded circular dichroism (CD) spectra to indicate that proteins are appropriately folded (Fig. S2A). Comparing secondary structure composition based on experimental CD data evaluated by BeStSel software[28] and the composition calculated from predicted PDB structure using PDBMD2CD[29] suggested that structured parts are preferentially alpha-helical for both CDIN1 and Codanin1$_{Cterm}$ (Fig. S3A, B). Additionally, we employed dynamic light scattering (DLS) to measure the size distribution of purified proteins. DLS plots (Fig. S2B) showed single peaks, implying that our proteins are homogenous without aggregations. Finally, we used differential scanning fluorimetry (nanoDSF) to determine the thermal stability of CDIN1 (48 °C) and Codanin1$_{Cterm}$ (55 °C) (Fig. S2C), suggesting that both proteins are stable at room temperature. Taken together, we prepared pure proteins that were adequately folded, homogenous, stable, and well-suitable for subsequent studies.

Supplementary table

Table S1: SAXS-derived structural parameters for CDIN1 and Codanin1$_{Cterm}$.
I(0), R_g, and D_{max} values were calculated from SAXS data.

	Frame	Guinier analysis		Distance distribution analysis		
		I(0)	R_g (nm)	I(0)	R_g (nm)	D_{max} (nm)
CDIN1	504-615	0.059	2.95 ± 0.06	0.0571	2.84 ± 0.06	8.18
Codanin1$_{Cterm}$ second peak	762-817	0.041	2.66 ± 0.07	0.0396	2.59 ± 0.03	7.51
Codanin1$_{Cterm}$ first peak	605-643	0.080	4.11 ± 0.14	0.0765	3.94 ± 0.03	10.69

Supplementary figures and legends

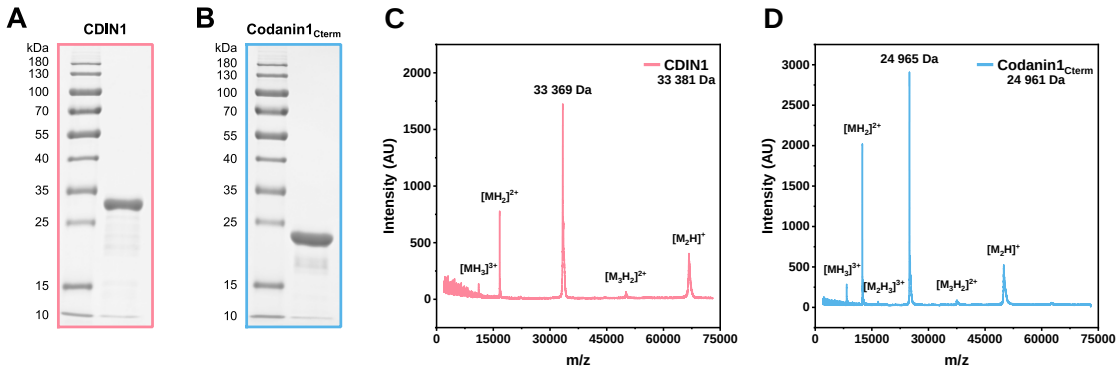

Figure S1: Protein purity and identity analyses.
(A, B) Coomassie-stained 12% SDS-PAGE gels show the purity of recombinant proteins CDIN1 and Codanin1$_{Cterm}$. CDIN1 (4 µg) and Codanin1$_{Cterm}$ (4 µg) were loaded in corresponding wells after three

purification steps. Molecular weight marker PageRuler™ Prestained Protein Ladder (Thermo Fisher Scientific) and band sizes are depicted on the left. **(C, D)** MALDI-TOF MS analysis of CDIN1 and Codanin1$_{Cterm}$ confirms the identity of both proteins. The highest observed peak with experimental molecular weight corresponds with the expected molecular weight shown under the protein name. The differences between calculated and experimental molecular weights were smaller than the size of a single amino acid, suggesting that our proteins were prepared as intended and did not contain any extra residues.

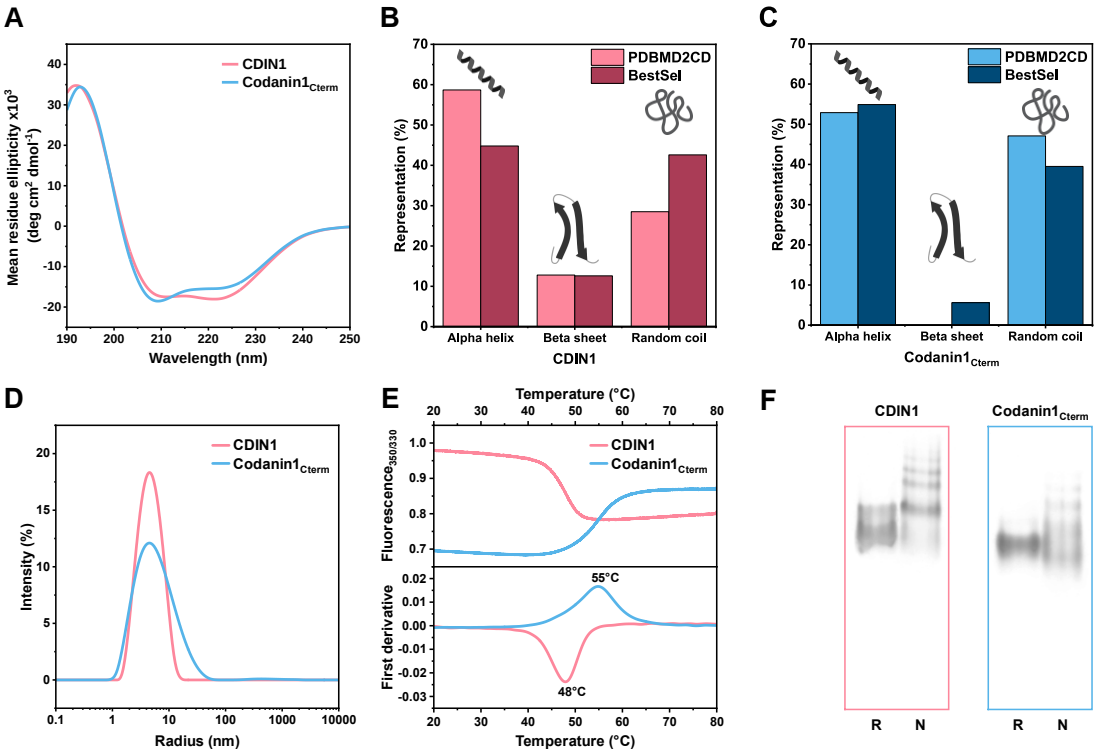

Figure S2: Secondary structure, homogeneity, and thermal stability of CDIN1 and Codanin1$_{Cterm}$. **(A)** Far-UV Circular dichroism (CD) spectra of CDIN1 and Codanin1$_{Cterm}$ display secondary structure profiles of folded proteins. **(B, C)** Bar graphs showing the difference between calculated secondary structure composition from AlphaFold predicted structures using PDBMD2CD software in light colors and experimentally obtained data analyzed by BeStSel software in dark colors. **(D)** The dynamic light scattering (DLS) graph demonstrates the homogeneity of both proteins without aggregation. **(E)** Differential scanning fluorimetry (nanoDSF) shows thermal stability with inflection points corresponding to 48 °C and 55 °C for CDIN1 and Codanin1$_{Cterm}$, respectively. **(F)** 12% native acrylamide gel shows the presence of oligomers in non-denaturing conditions. R and N stand for reducing and non-reducing conditions.

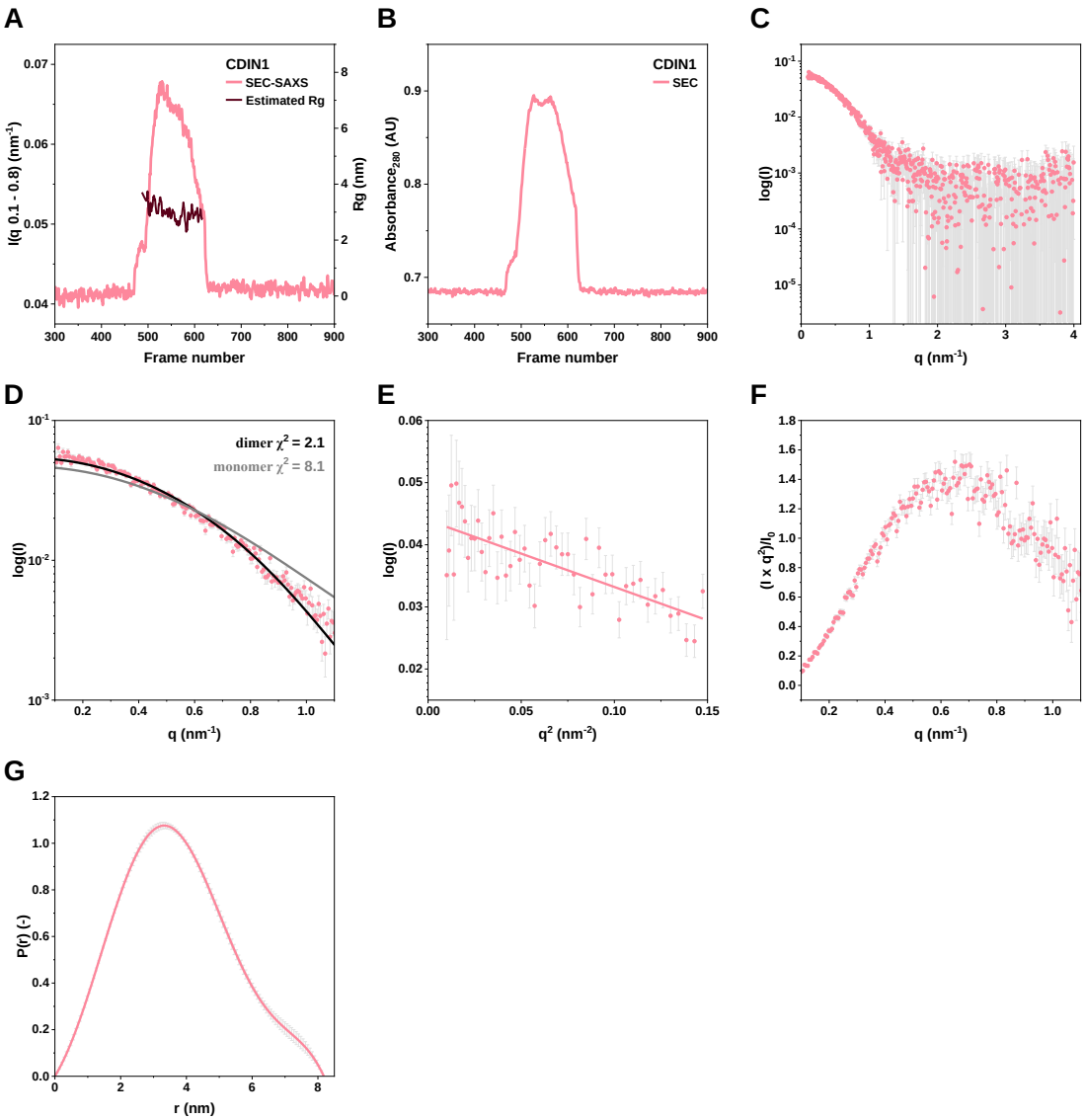

Figure S3: SEC peak of CDIN1 analyzed by SAXS.

(A) Estimated R_g (dark) and I(q 0.1-0.8) (light) plots for CDIN1 SEC-SAXS. **(B)** SEC absorbance profile of CDIN1 obtained using UV_{280} detector. **(C)** Solution SAXS intensity profile of CDIN1 with subtracted background plotted as log(I) versus q. **(D)** Fit of the scattering curve calculated from CDIN1 dimer AlphaFold model (black line) and CDIN1 monomer AlphaFold model (gray line) with SAXS data (red dots) generated by CRYSOL. **(E)** Linear Guinier plot of the initial part of the scattering curve showing the quality of the measured protein sample. **(F)** Dimensionless Kratky plot indicating folded structures. **(G)** Pairwise distance distribution function P(r) indicating maximum dimensions D_{max}.

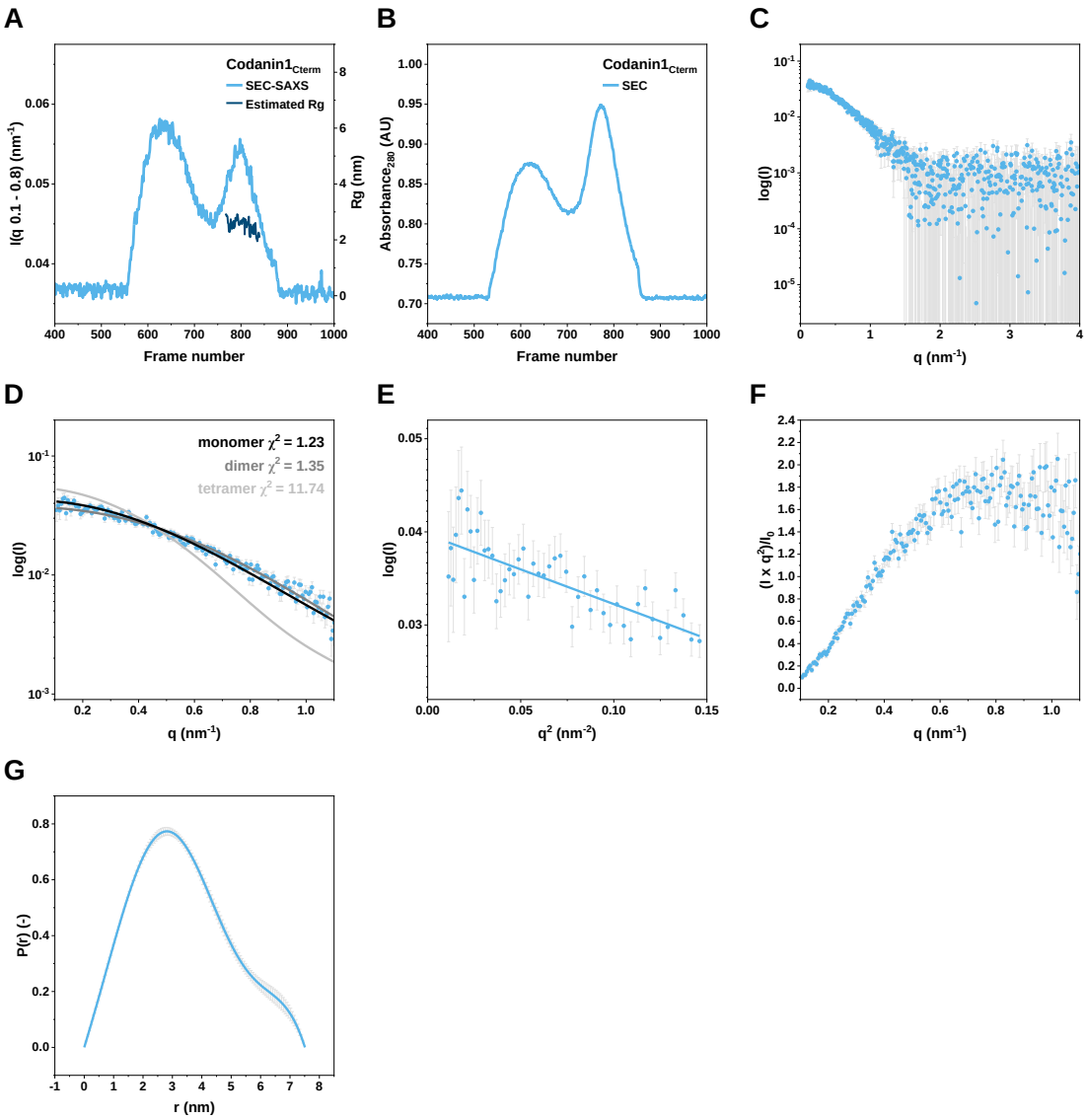

Figure S4: SEC second peak of Codanin1$_{Cterm}$ analyzed by SAXS.

(A) Estimated R_g (dark) and I(q 0.1-0.8) (light) plots for Codanin1$_{Cterm}$ SEC-SAXS. **(B)** SEC absorbance profile of Codanin1$_{Cterm}$ obtained using UV_{280} detector. **(C)** Solution SAXS intensity profile of Codanin1$_{Cterm}$ with subtracted background plotted as log(I) versus q. **(D)** Fit of the scattering curve calculated from the Codanin1$_{Cterm}$ monomer AlphaFold model (black line), Codanin1$_{Cterm}$ dimer AlphaFold model (dark gray line), and Codanin1$_{Cterm}$ tetramer AlphaFold model (light gray line) with SAXS data (blue dots) generated by CRYSOL. **(E)** Linear Guinier plot of the initial part of the scattering curve showing the quality of the measured protein sample. **(F)** Dimensionless Kratky plot indicating folded structures. **(G)** Pairwise distance distribution function P(r) indicating maximum dimensions D_{max}.

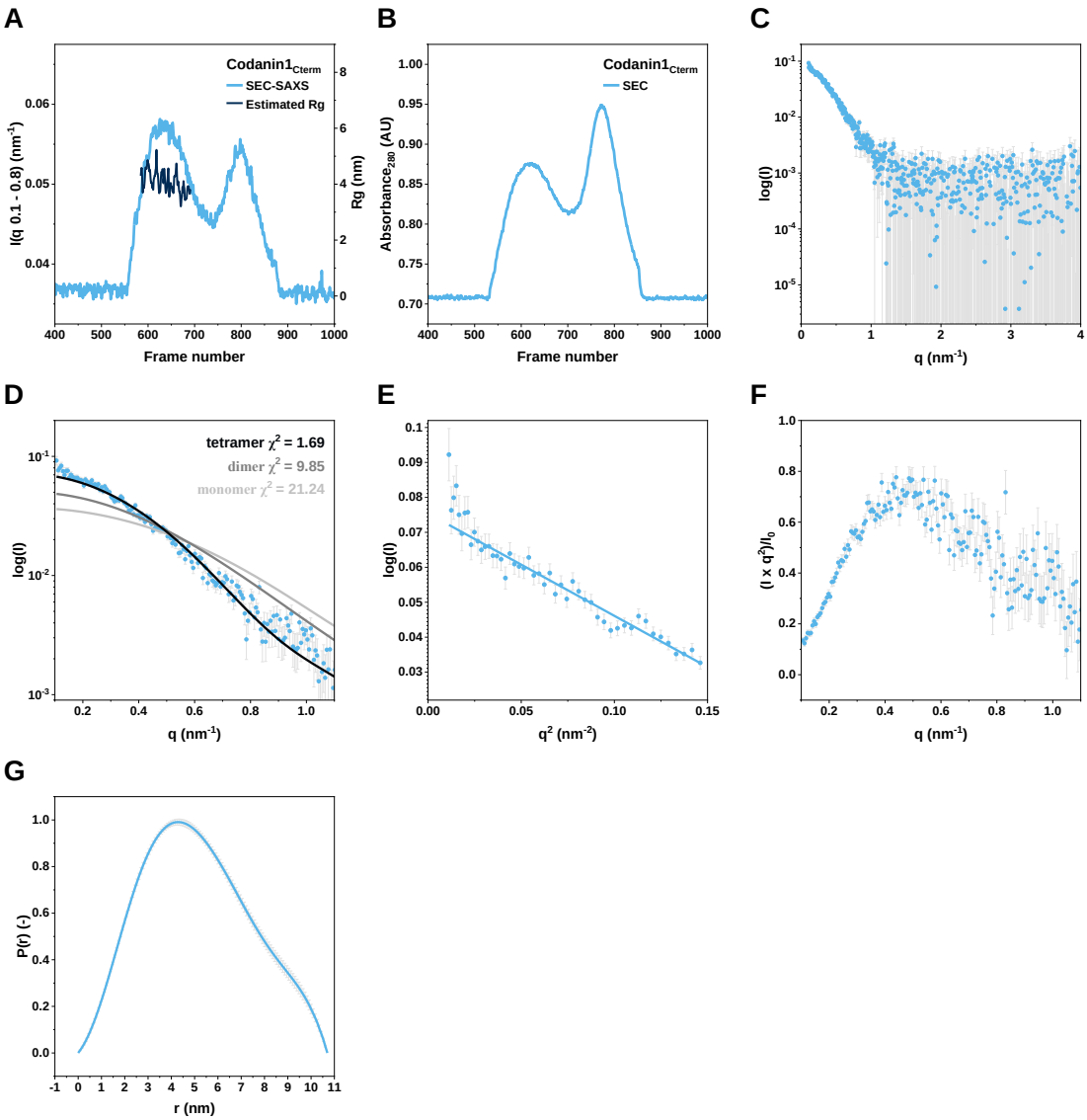

Figure S5: SEC first peak of Codanin1$_{Cterm}$ analyzed by SAXS.

(A) Estimated R_g (dark) and I(q 0.1-0.8) (light) plots for Codanin1$_{Cterm}$ SEC-SAXS. **(B)** SEC absorbance profile of Codanin1$_{Cterm}$ obtained using UV_{280} detector. **(C)** Solution SAXS intensity profile of Codanin1$_{Cterm}$ with subtracted background plotted as log(I) versus q. **(D)** Fit of the scattering curve calculated from the Codanin1$_{Cterm}$ tetramer AlphaFold model (black line), Codanin1$_{Cterm}$ dimer AlphaFold model (dark gray line), and Codanin1$_{Cterm}$ monomer AlphaFold model (light gray line) with SAXS data (blue dots) generated by CRYSOL. **(E)** Linear Guinier plot of the initial part of the scattering curve showing the quality of the measured protein sample. **(F)** Dimensionless Kratky plot indicating folded structures. **(G)** Pairwise distance distribution function P(r) indicating maximum dimensions D_{max}.

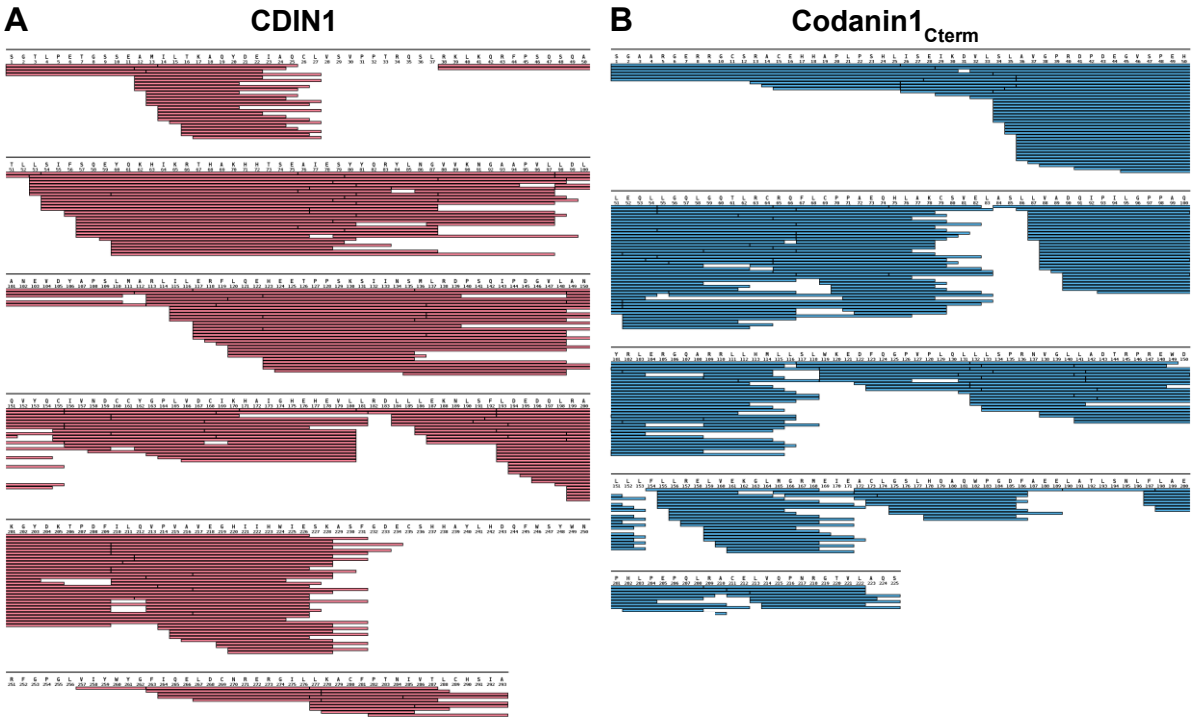

Figure S6: Proteolytic coverage of CDIN1 and Codanin1$_{Cterm}$ proteins.
Graphical representation of CDIN1 and Codanin1$_{Cterm}$ peptide coverage after proteolytic digestion identified by LC-MS/MS under conditions used for hydrogen-deuterium exchange mass spectrometry. (A, B) The red boxes present 242 unique peptides of CDIN1, covering 89% of the sequence, whereas the blue boxes present 232 unique peptides of Codanin1, covering the whole sequence.

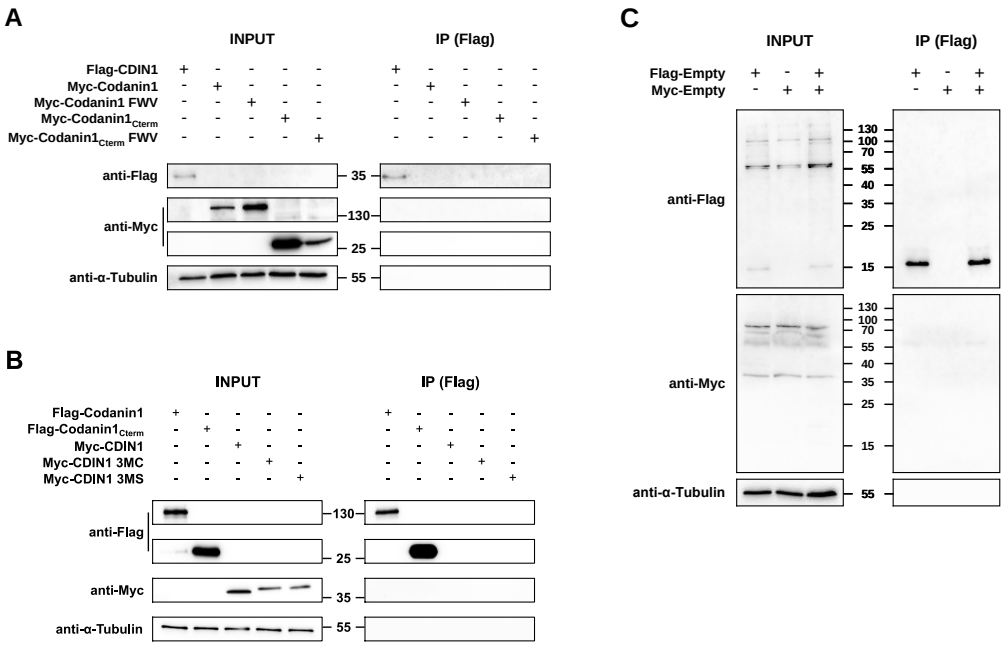

Figure S7: Co-IP controls confirmed specificity of antibodies.
(A, B) Western blot analysis of Co-IP samples obtained from HEK293T transfected by each of CDIN1 and Codanin1 plasmids separately shows the specificity of M2 Flag magnetic beads. **(C)** Western blot analysis of Co-IP samples obtained from HEK293T transfected by empty vectors shows no non-specific signal at the molecular level of proteins of interest.