

Supplementary Information.

Table S1: Residues at the interaction interface (a, d positions of heptad repeats) in the various BECN1/VPS30-containing dimers.

Dimer:	BECN1 homodimer (anti-parallel)		Pairing	BECN1:ATG14 heterodimer (parallel)		Pairing	VPS30:VPS38 heterodimer (parallel)	
Monomers:	BECN1			BECN1	ATG14		VPS30	VPS38
Heptad positions:	a	d'		a	a'		a	a'
Interface residues:	S177	L264	NI	S177	F92	NI		
	L184	A257	OK	L184	A99	OK		
	E191	V250	NI	E191	T106	P	E244	R224
	L198	L243	I	L198	I113	I	L251	E231
	R205	F236	NI	R205	I120	NI	D258	L238
	L212	Y229	NI	L212	I127	I	L265	K245
	A219	L222	OK	A219	M134	OK	K272	L252
	E226	V215	NI	E226	L141	NI	K279	N259
	Y233	V208	NI	Y233	N148	NI		
	Q240	V201	NI	Q240	A155	NI	N293	E272
	L247	L194	I	L247	K162	NI	L300	G279
	M254	L187	OK	M254	N169	NI	F307	V286
L261	L180	I	L261	V176	I	L314	D293	
Heptad positions:	d	a'		d	d'		d	d'
Interface residues:	L180	L261	I	L180	E95	NI		
	L187	M254	OK	L187	G102	NI		
	L194	L247	I	L194	L109	I	L247	Q227
	V201	Q240	NI	V201	C116	NI	L254	E234
	V208	Y233	NI	V208	L123	I	L261	K241
	V215	E226	NI	V215	G130	NI	L268	E248
	L222	A219	OK	L222	N137	NI	L275	N255
	Y229	L212	NI	Y229	T144	P	K282	T263
	F236	R205	NI	F236	L151	OK		
	L243	L198	I	L243	H158	NI	F296	N275
	V250	E191	NI	V250	I165	I	L303	Y282
	A257	L184	OK	A257	L172	OK	S310	K289
L264	S177	NI						

Pairing Key: I: Ideal; NI: Non-Ideal; OK: Acceptable hydrophobic; P: paired polar residues

Figure S1. ATG14 sequence alignment. Sequence alignment of predicted CCDs from five diverse eukaryotic ATG14 proteins. Yellow, orange and red represent increasing sequence conservation, with red corresponding to invariant residues. Predicted secondary structures are shown below the sequence alignment. The predicted CCD region used in this study is boxed. Conserved residues involved in hydrophobic pairing in the heterodimer, that were selected for mutagenesis are indicated by red arrowheads.

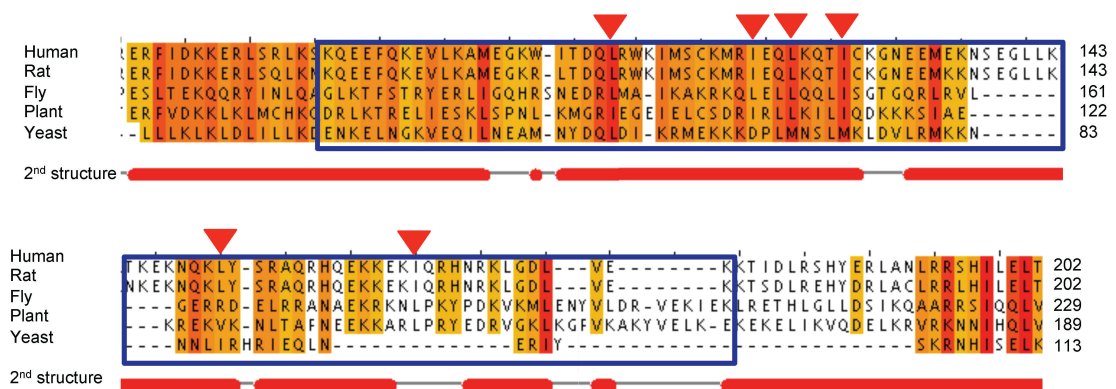


Figure S2. SEC purification of the BECN1:ATG14 CCD heterodimer complex.

SEC curves shown correspond to BECN1 CCD (green) and BECN1:ATG14 CCD (blue). Elution positions for different molecular weight markers are indicated. The molecular weight calculated from SEC is indicated. SDS-PAGE analysis of the peak fractions is shown.

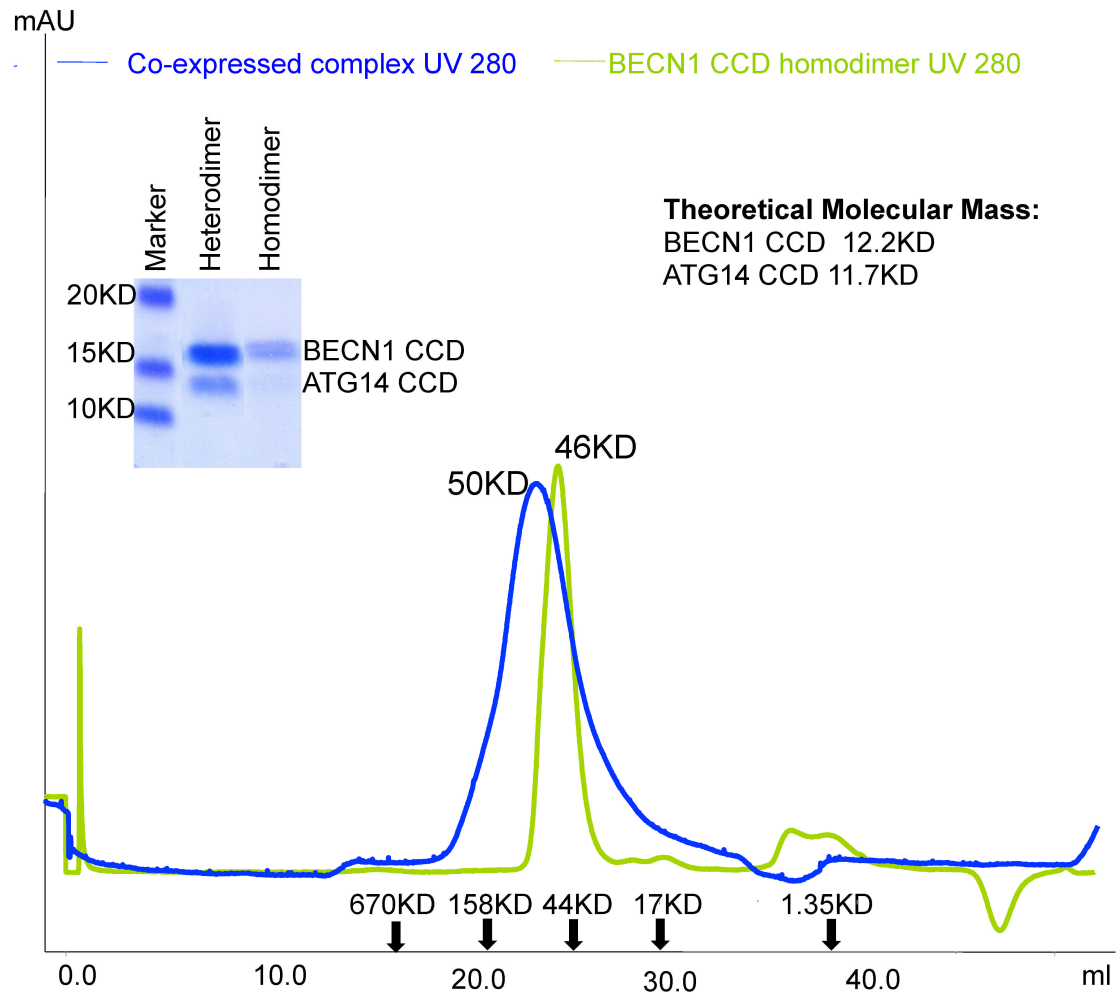


Figure S3. CD spectra of different CCDs. The spectra for the MBP-ATG14 CCD fusion protein, the BECN1:ATG14 CCD complex, the BECN1 CCD, and MBP are color coded as indicated in the legend.

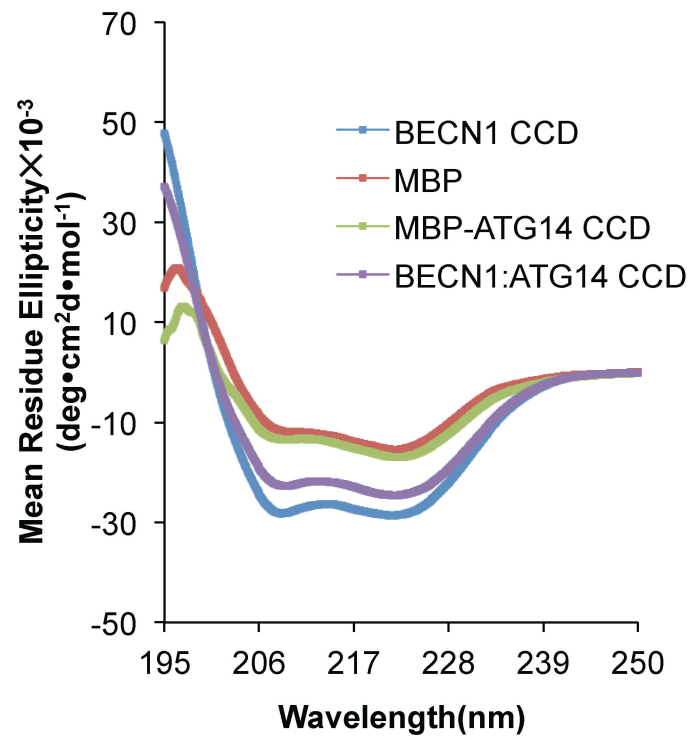


Figure S4. Comparison of BECN1 CCD containing dimers. All proteins are rendered in ribbon, colored as follows: BECN1, magenta; ATG14, wheat; VPS30, yellow and VPS38, green. The residues involved in interface interactions are shown in stick with atoms color-coded by atom type: O, red; N, blue; S, yellow; and C, colored according to main chain ribbon for that molecule. Superposition of (A) BECN1:ATG14 CCD complex and the BECN1 CCD homodimer. (B) VPS30:VPS38 complex and the BECN1 CCD homodimer. (C) BECN1:ATG14 CCD complex and the VPS30:VPS38 complex.

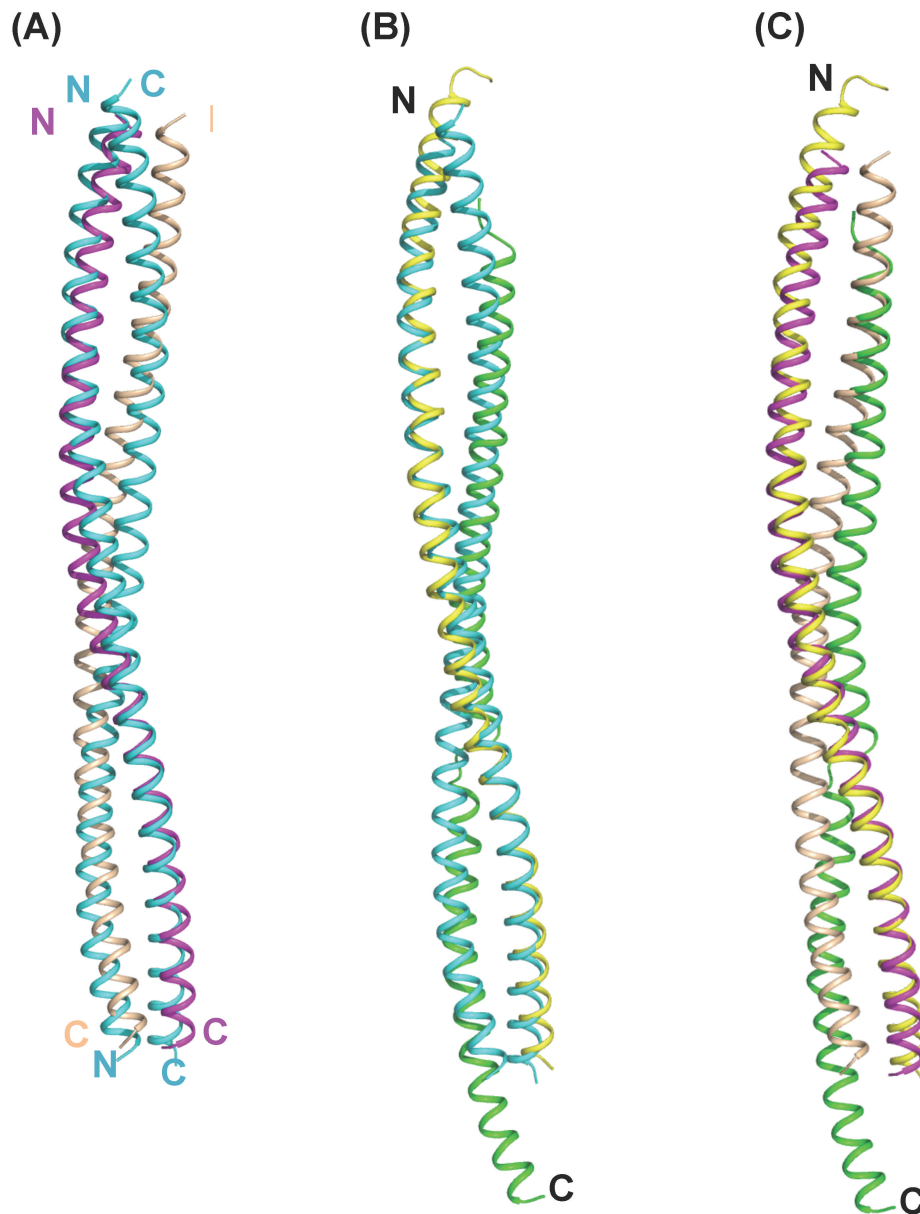


Figure S5. Displacement of the BECN1 BARAD domain due to the curved BECN1:ATG14 quaternary structure. All proteins are shown in ribbon, colored as follows: BECN1, magenta; ATG14, wheat; VPS30, yellow; VPS38, green; VPS15, grey, and VPS34, blue. Protein domains implicated in membrane interaction, the BECN1 BARAD, VPS38 BARAD and PI3KC3 catalytic domain are labeled. Arrows indicate altered positions of equivalent BECN1/VPS30 residues in a complex containing ATG14.

