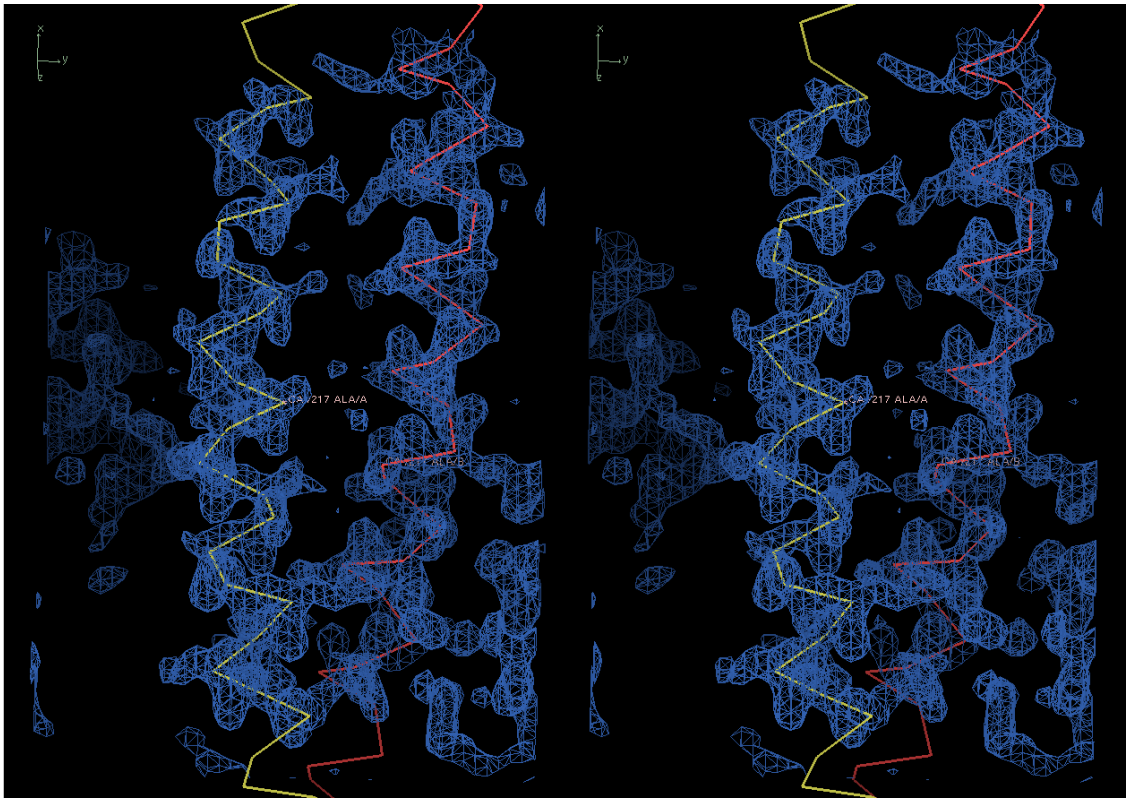
Supplementary Figures

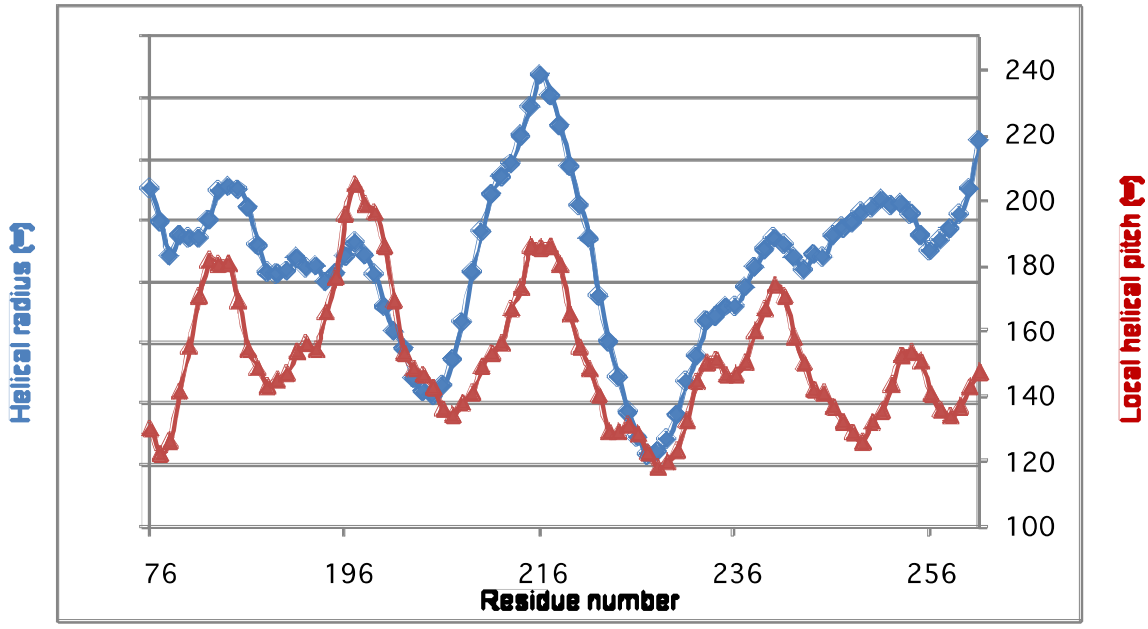
Supplementary Figure S1. The domain structure of Beclin 1.

Beclin 1 contains a BH3 domain, a coiled coil domain (CCD) and an evolutionarily conserved domain (ECD).



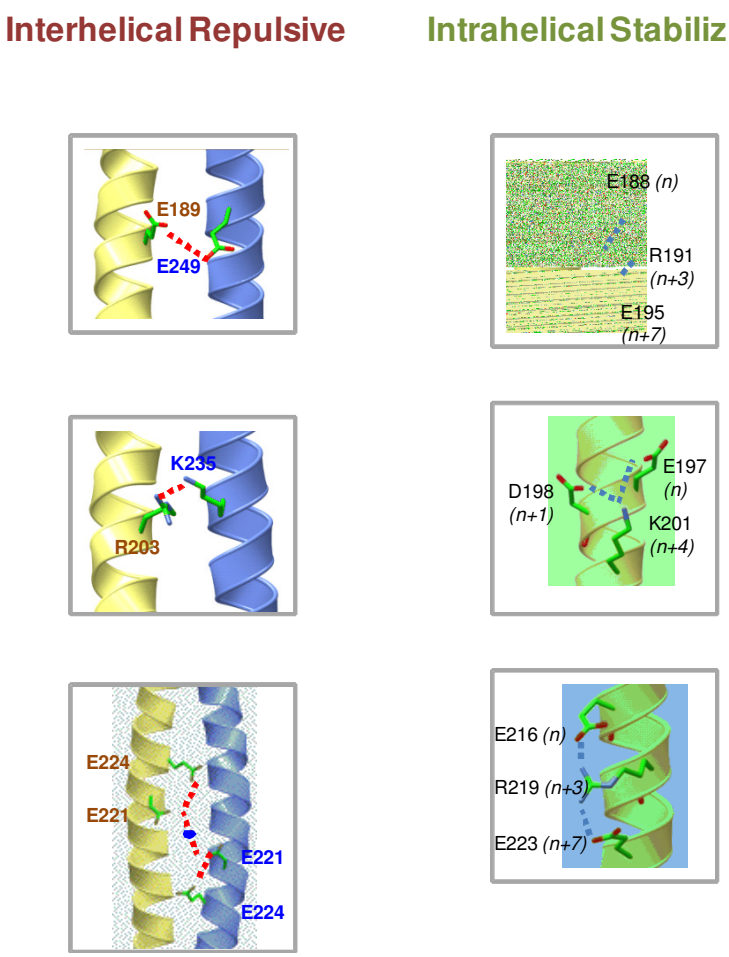
Supplementary Figure S2. Stereo representation of electron density map for the central region of Beclin 1 CC domain

2Fo-Fc electron density map, contoured at 2σ level, for the central region of the Beclin 1 CC domain near A217. The dimeric protein structure is shown in Cα trace in yellow and red color.



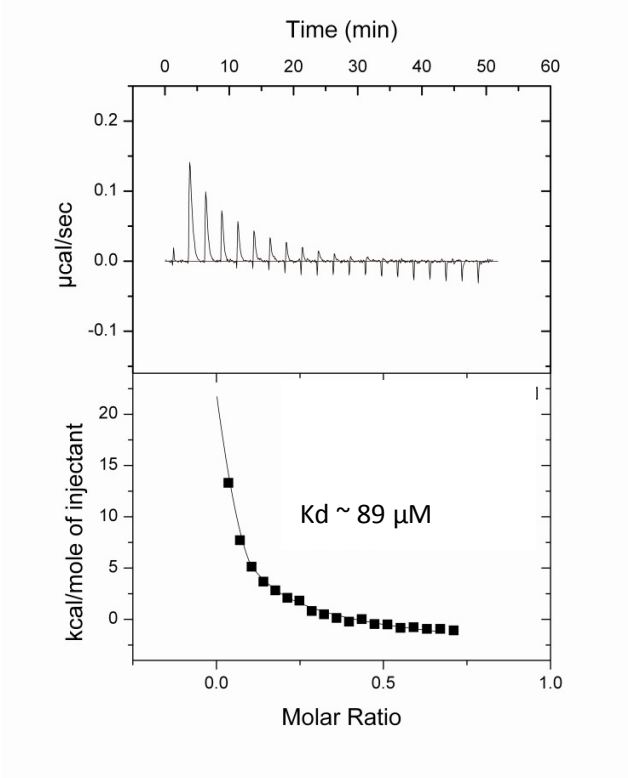
Supplementary Figure S3. Beclin 1 CC domain analyzed by TWISTER

The parameters for the coiled coil geometry of Beclin 1 CC domain, including the helical radius and the local helical pitch, were analyzed using TWISTER [42] and plotted by residue number.



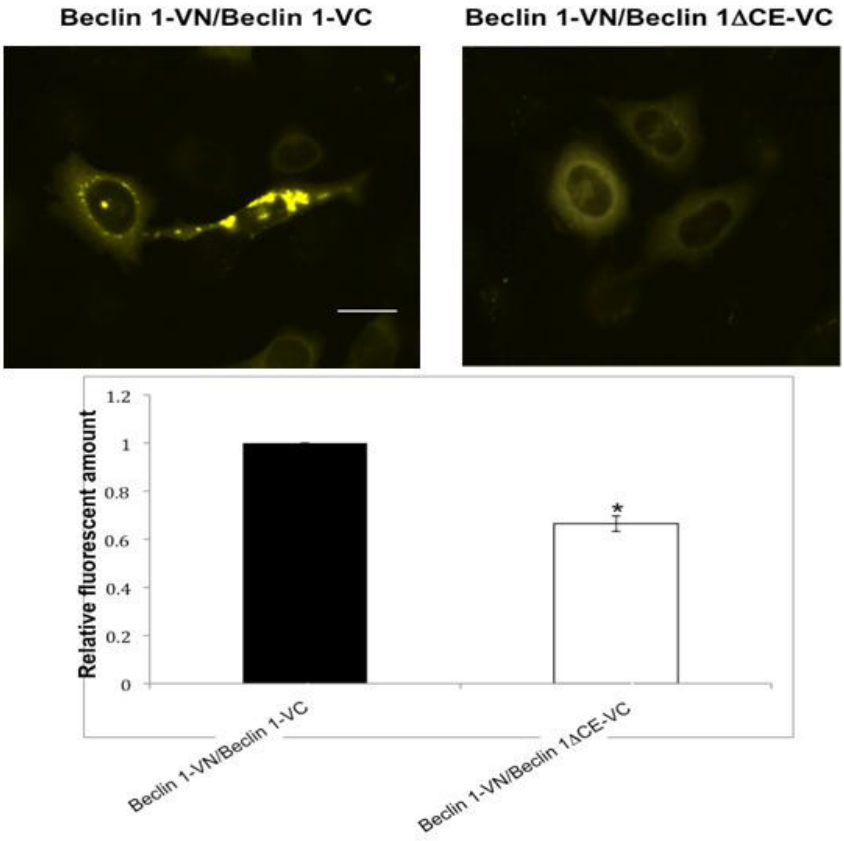
Supplementary Figure S4. The imperfect dimer interface of Beclin 1 CC domain

The notable electrostatic interactions at the dimer interface of Beclin 1 CC domain are plotted, including the interhelical repulsive interactions (left) and intrahelical stabilizing interactions (right). The two Beclin 1 CC domain molecules are shown in ribbons style and colored yellow or blue. The residues involved in the electrostatic interactions are shown in stick model and cpk coloring. The dashed lines indicate the electrostatic interactions. The oval dot indicates 2-fold symmetry.



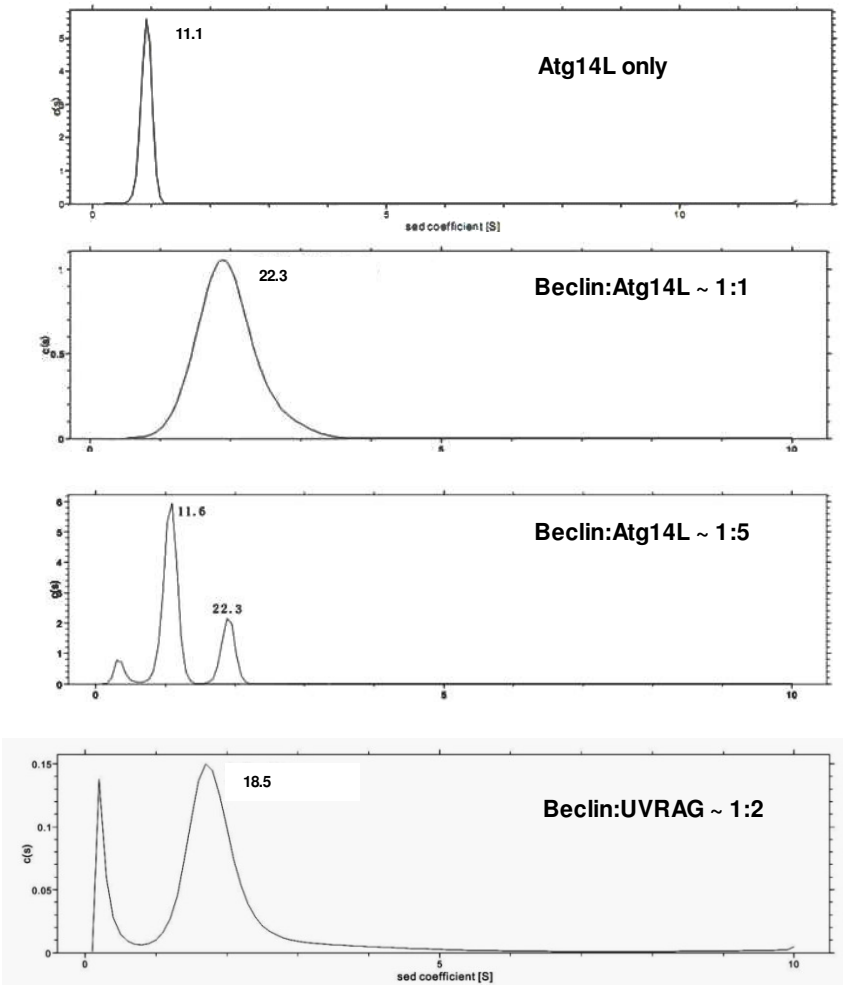
Supplementary Figure S5. Estimation of the self-dissociation constant (Kd) of Beclin 1 CC domain.

The ITC titration profile of Beclin 1 CC domain at buffer condition of pH 8.0, 150 mM NaCl. The background of this ITC profile has been corrected for the buffer-to-buffer effect.



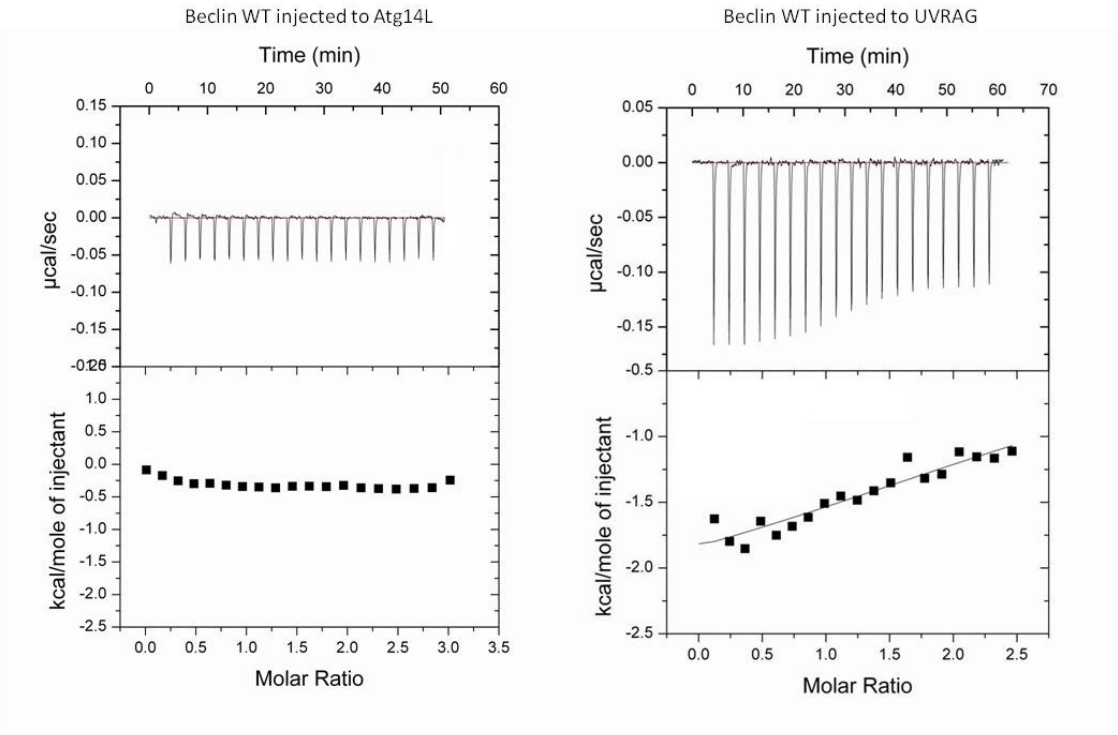
Supplementary Figure S6. Bimolecular fluorescence complementation (BiFC) assay to assess Beclin 1 self-association *in vivo*.

(top) Representative fluorescent images (YFP) acquired from HeLa cells co-expressing Beclin 1-VN and Beclin 1-VC or Beclin 1(ΔCE)-VC. Beclin 1(ΔCE) is a dimerization deficient mutant of Beclin 1 with CC domain deleted and used as a negative control. VN and VC refer to the N- and C-terminal constructs of YFP as designed by Hu *et. al*. Scale bar, 20 μM. (2) (bottom) fluorescence quantification was acquired from three independence experiments. * p=0.006. Student *t* test. Error bar, ± SEM, n=3.



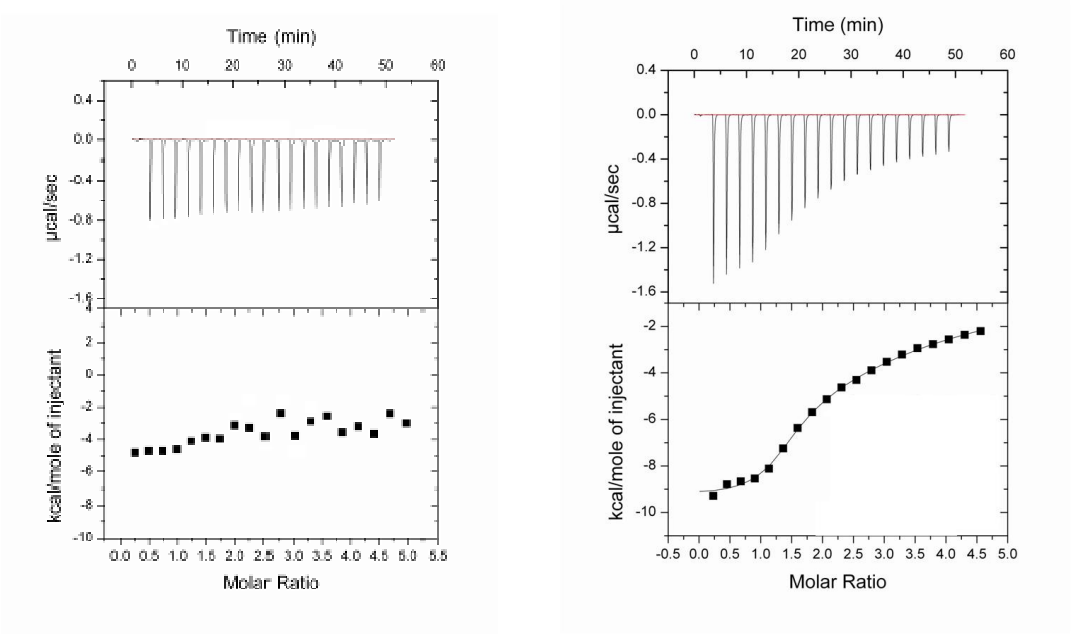
Supplementary Figure S7. Stoichiometry of Beclin 1 CC domain-Atg14L/UVRAG complex

The sedimentation velocity profile of Atg14L coiled coil region alone, and the complex of Beclin 1 CC domain with Atg14L and UVRAG coiled coil region respectively as measured by analytical ultracentrifugation. The numbers marked near the peaks of the curves indicate the estimated molecular weight of the corresponding protein samples. The molar ratio of the two proteins in the mixture for each experiment is indicated on the right of the profile panel.



Supplementary Figure S8. Interaction of Beclin 1 CC domain with Atg14L and UVRAG under high-salt condition

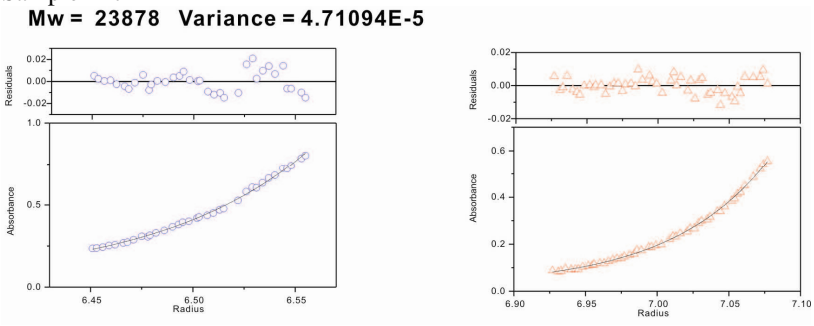
The ITC titration profile of Beclin 1 CC domain with the coiled coil region of Atg14L (left) and UVRAG (right), in buffer containing 1M NaCl representing high-salt condition.



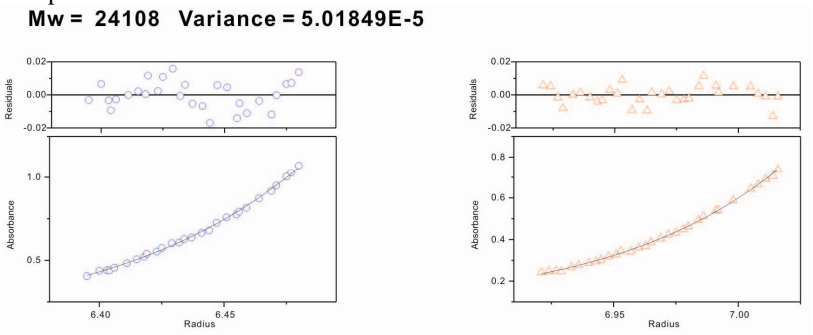
Supplementary Figure S9. Competitive binding of Atg14L and UVRAG to Beclin 1 CC domain

The ITC titration profile of Beclin 1 CC domain with the coiled coil region of Atg14L, in the presence of excessive amount of UVRAG (left); or with the coiled coil region of UVRAG, in the presence of excessive amount of Atg14L (right).

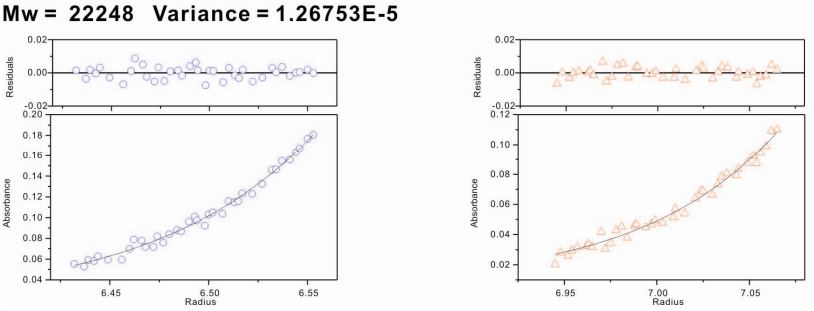
Sample 217-224



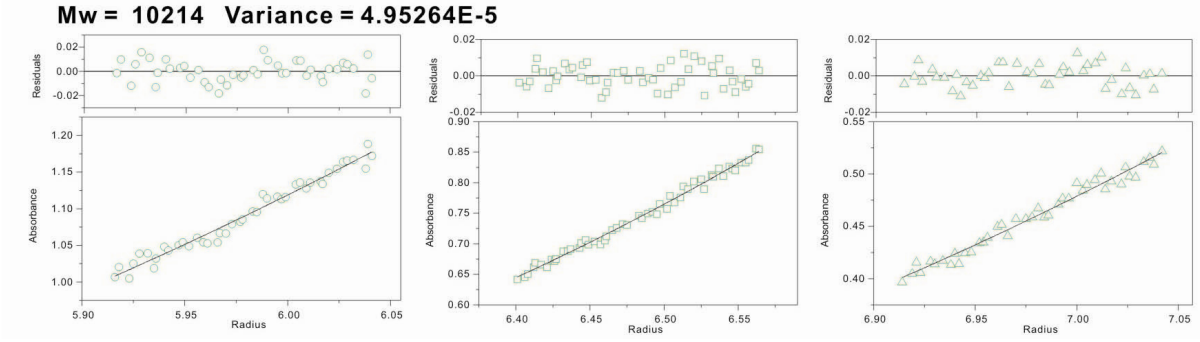
Sample 189-255



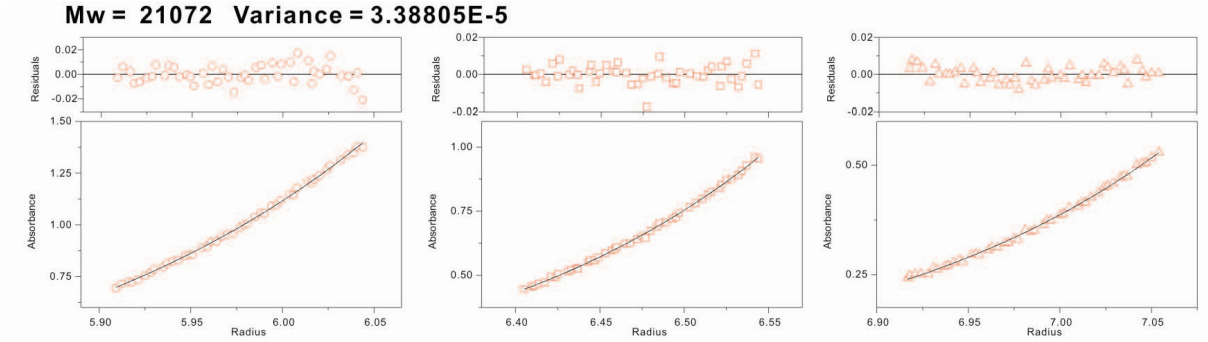
Sample 217-224-255



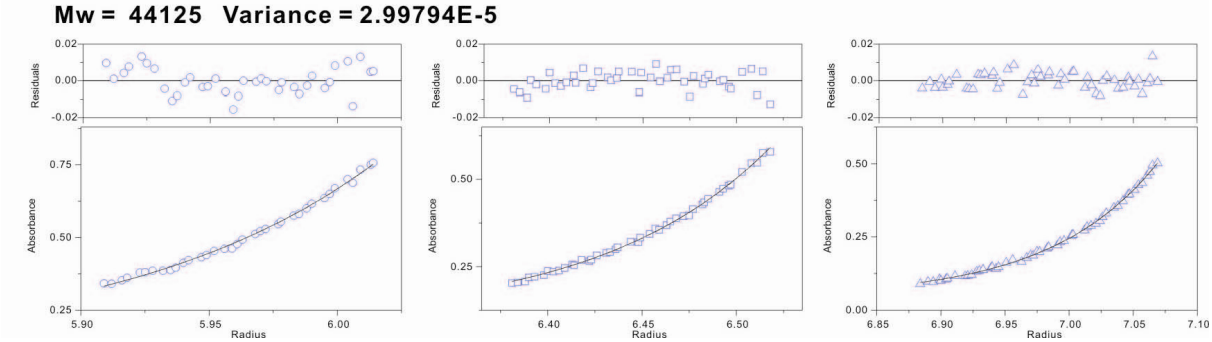
Sample 178-259



Sample wild-type

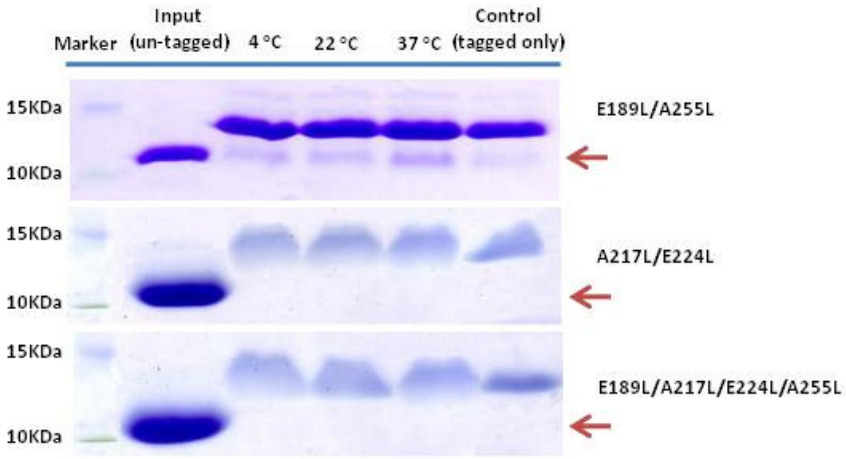


Sample 189-217-224-255



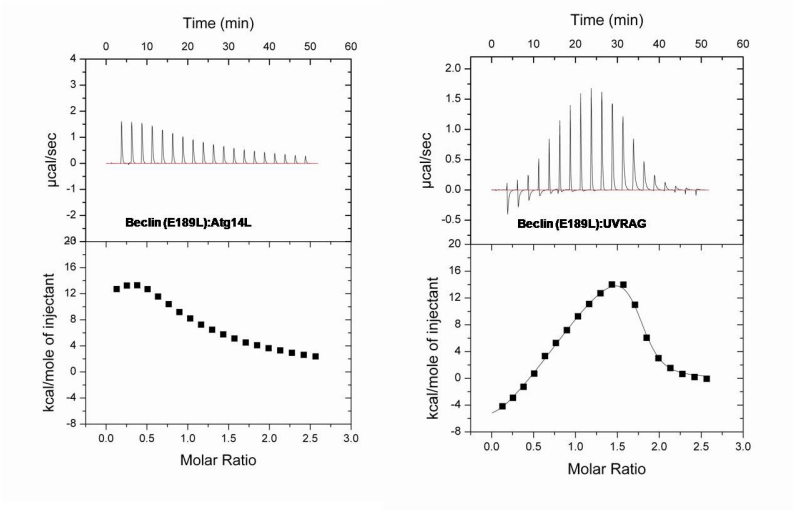
Supplementary Figure S10. The oligomeric state of MutM and MutStab mutants of Beclin 1 CC domain

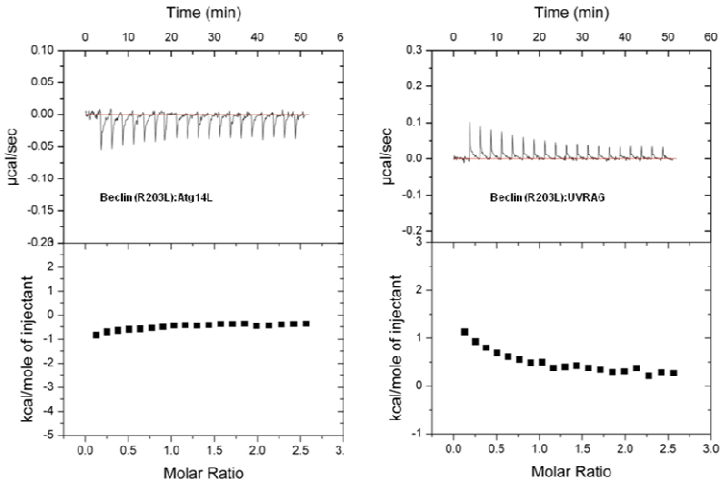
The sedimentation equilibrium profile of MutM and MutStab mutants of Beclin 1 CC domain as measured by analytical ultracentrifugation.

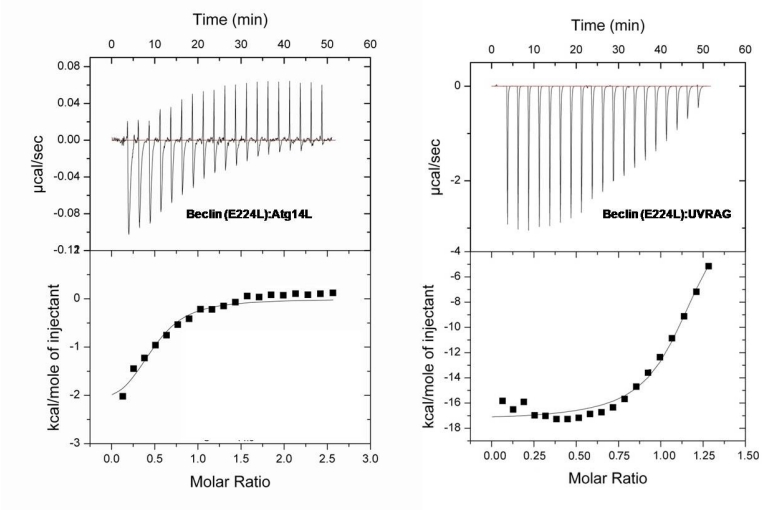


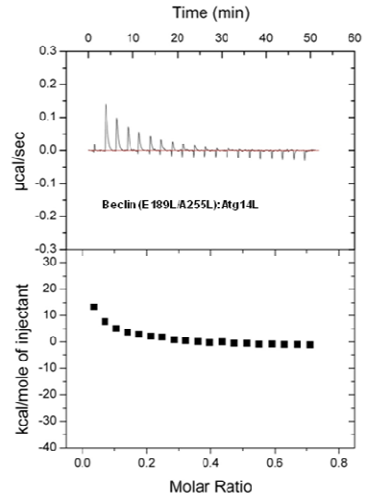
Supplementary Figure S11. The Dynamic exchange assay of MutStab constructs of Beclin 1

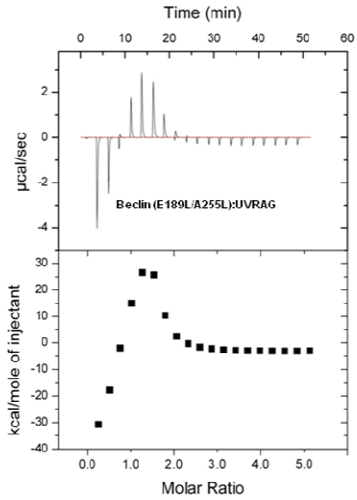
The assay was conducted as described in Fig. 2c. The expected heterodimeric Beclin 1 CC domain, consisting of one monomer with his-tag and the other monomer without tag, is indicated by the arrows.

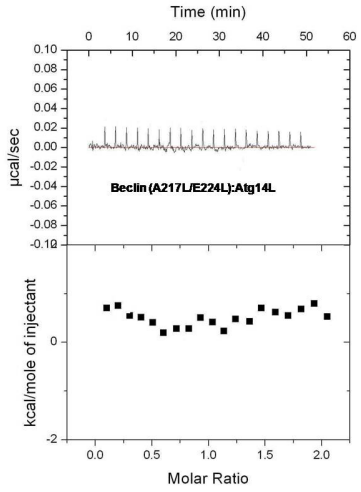

Time (min)
µcal/sec
Beclin (E189L):Atg14L
kcal/mole of injectant
Molar Ratio
Time (min)
µcal/sec
Beclin (E189L):UVRAG
kcal/mole of injectant
Molar Ratio

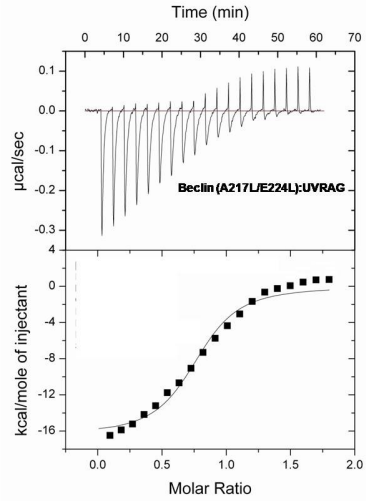

Time (min)
µcal/sec
Beclin (R203L):Atg14L
kcal/mole of injectant
Molar Ratio
Time (min)
µcal/sec
Beclin (R203L):UVRAG
kcal/mole of injectant
Molar Ratio

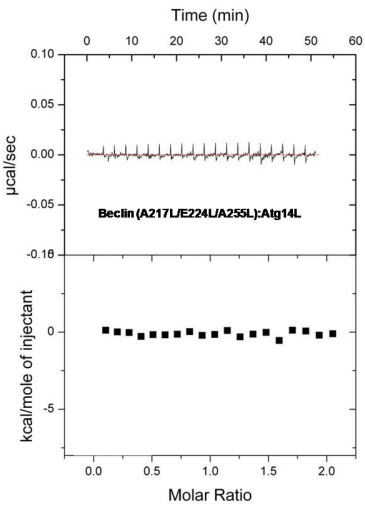

Time (min)
µcal/sec
Beclin (E224L):Atg14L
kcal/mole of injectant
Molar Ratio
Time (min)
µcal/sec
Beclin (E224L):UVRAG
kcal/mole of injectant
Molar Ratio

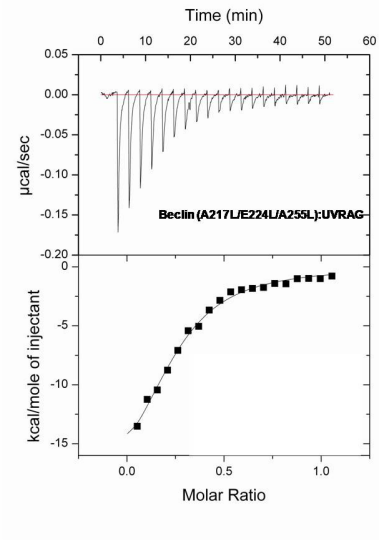

Time (min)
µcal/sec
Beclin (E189L/A255L):Atg14L
kcal/mole of injectant
Molar Ratio

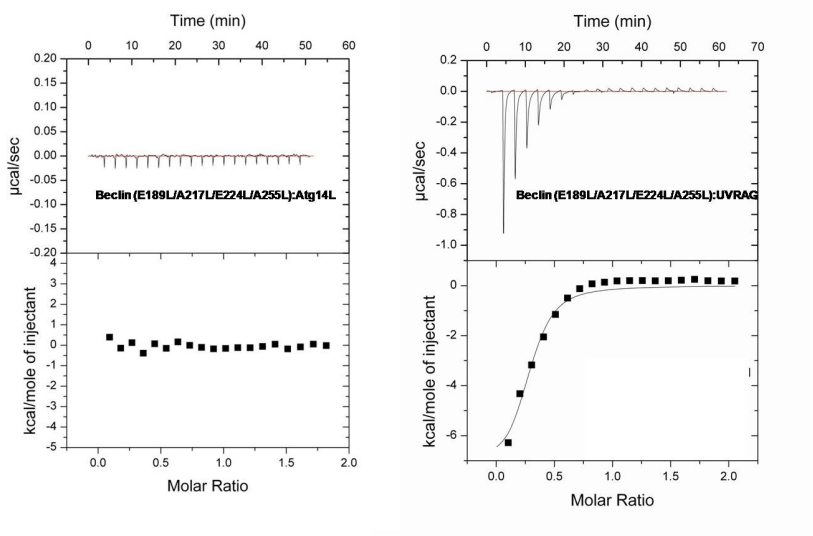

Time (min)
µcal/sec
Beclin (E189L/A255L):UVRAG
kcal/mole of injectant
Molar Ratio


Time (min)
µcal/sec
Beclin (A217L/E224L):Atg14L
kcal/mole of injectant
Molar Ratio


Time (min)
µcal/sec
Beclin (A217L/E224L):UVRAG
kcal/mole of injectant
Molar Ratio


Time (min)
µcal/sec
Beclin (A217L/E224L/A255L):Atg14L
kcal/mole of injectant
Molar Ratio


Time (min)
µcal/sec
Beclin (A217L/E224L/A255L):UVRAG
kcal/mole of injectant
Molar Ratio



Supplementary Figure S12. Interaction of MutM and MutStab mutants of Beclin 1 CC domain with Atg14L and UVRAG coiled coil region

The ITC titration profile of various MutM and MutStab mutants of Beclin 1 CC domain with the coiled coil region of Atg14L (left) and UBRAG (right) respectively.

Supplementary Table S1 Data collection, phasing and refinement statistics (MIR)

	Native	$AuCl_4$	NH_4I
Data collection			
Space group	C2	C2	C2
Cell dimensions			
a, *b*, *c* (Å)	103.78, 38.35, 65.75	103.77, 38.37, 62.82	103.50, 38.37, 65.43
α, β, γ (°)	90, 97.92, 90	90, 97.94, 90	90, 98.65, 90
Resolution (Å)[a]	37.88-1.86 (1.96-1.86)	37.90-1.94 (2.04-1.94)	37.48-1.86 (1.96-1.86)
R_{sym} or R_{merge} [b]	2.9% (11.0%)	2.3% (8.1%)	7.1% (17.4%)
$I / \sigma I$ [b]	32.5 (12.2)	34.5 (14.1)	23.1 (8.5)
Completeness (%)[b]	100 (100)	100 (100)	96.6 (94.3)
Redundancy [b]	4.0 (3.9)	3.9 (3.8)	7.3 (7.4)
Refinement			
Resolution (Å)	37.90-1.90		
No. reflections	19438		
R_{work} / R_{free}	20.88% / 23.63%		
No. atoms			
Protein	1592		
Ligand/ion	N/A		
Water	279		
B-factors			
Protein	23.68		
Ligand/ion	N/A		
Water	38.50		
R.m.s deviations			
Bond lengths (Å)	0.014		
Bond angles (°)	1.243		

[a] Numbers in parenthesis define the highest resolution shell of data.
[b] Numbers in parenthesis are the statistics for the highest resolution shell of data

Supplementary References:

42 Strelkov, S. V. & Burkhard, P. Analysis of alpha-helical coiled coils with the program TWISTER reveals a structural mechanism for stutter compensation. *J Struct Biol* **137**, 54-64 (2002).

43 Hu, C.D., Chinenov, Y., and Kerppola, T.K. Visualization of interactions among bZIP and Rel family proteins in living cells using bimolecular fluorescence complementation. *Mol Cell* **9**, 789-798 (2002).