The Autophagy-related Beclin-1 Protein requires both the Coiled-coil and BARA Domains to form a Homodimer with Sub-micromolar affinity

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Supporting information

Figure S1. Sedimentation velocity analytical ultracentrifugation of full-length BECN1 at 10.3 μM.

Figure S2. SEC-MALS of full-length BECN1.

Figure S3. CD thermal melts of full-length BECN1 with varying salt concentration.

Figure S4. Biophysical analysis of the $BECN^{1-265}$ and BARA constructs.

Figure S5. Line width and integration data for select 2D sfHMQC peaks of ^{15}N-labeled BARA spectra.

Figure S6. Sedimentation equilibrium analytical ultracentrifugation of the V250A/M254A/L261A mutant of full-length BECN1

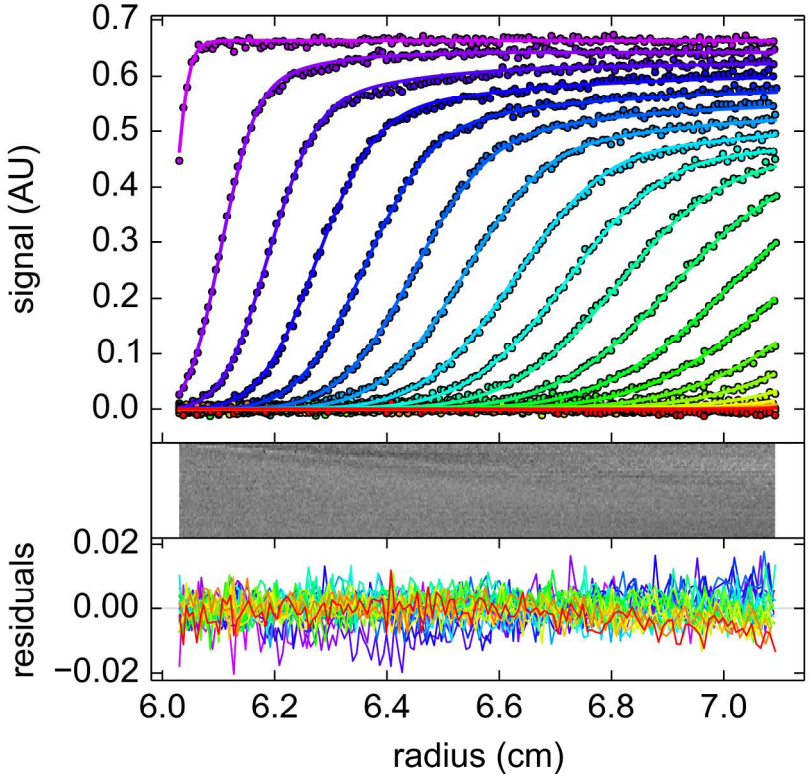

Figure S1. Sedimentation velocity analytical ultracentrifugation data for full-length BECN1 (10.3 μM) in 25 mM HEPES (pH 7.5), 150 mM NaCl, 0.5 mM TCEP at 20˚C. The data were fit using a *continuous c(s)* model in SedFIT.

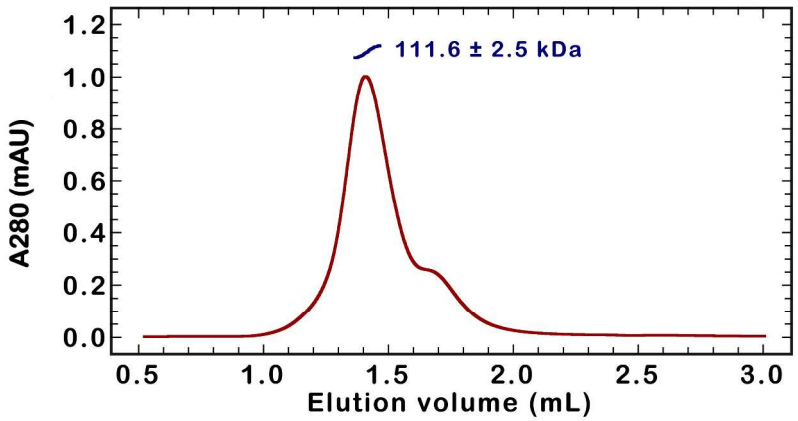

Figure S2. SEC-MALS of full-length BECN1 (9.6 μM) on a Superdex 200 5/15 GL Increase column at 0.4 mL/min in 25 mM Tris (pH 8), 500 mM NaCl, 0.5 mM TCEP. An average mass of 111.6 kDa was observed.

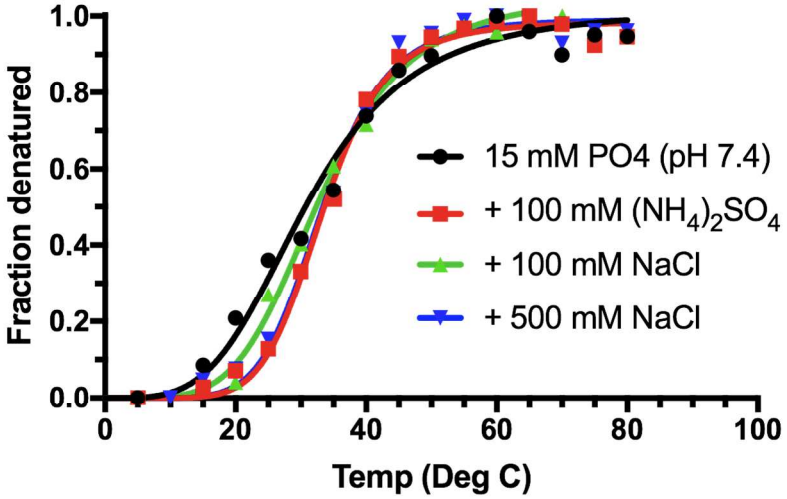

Figure S3. CD thermal melt of full-length BECN1 in phosphate buffer alone (*black;* T_M = 31˚C), phosphate with 100 mM $(NH_4)_2SO_4$ (*red;* T_M = 33˚C), phosphate with 100 mM NaCl (*green;* T_M = 33˚C), or phosphate with 500 mM NaCl (*blue;* T_M = 33˚C). Experiments were conducted as described in the methods for Figure 1G.

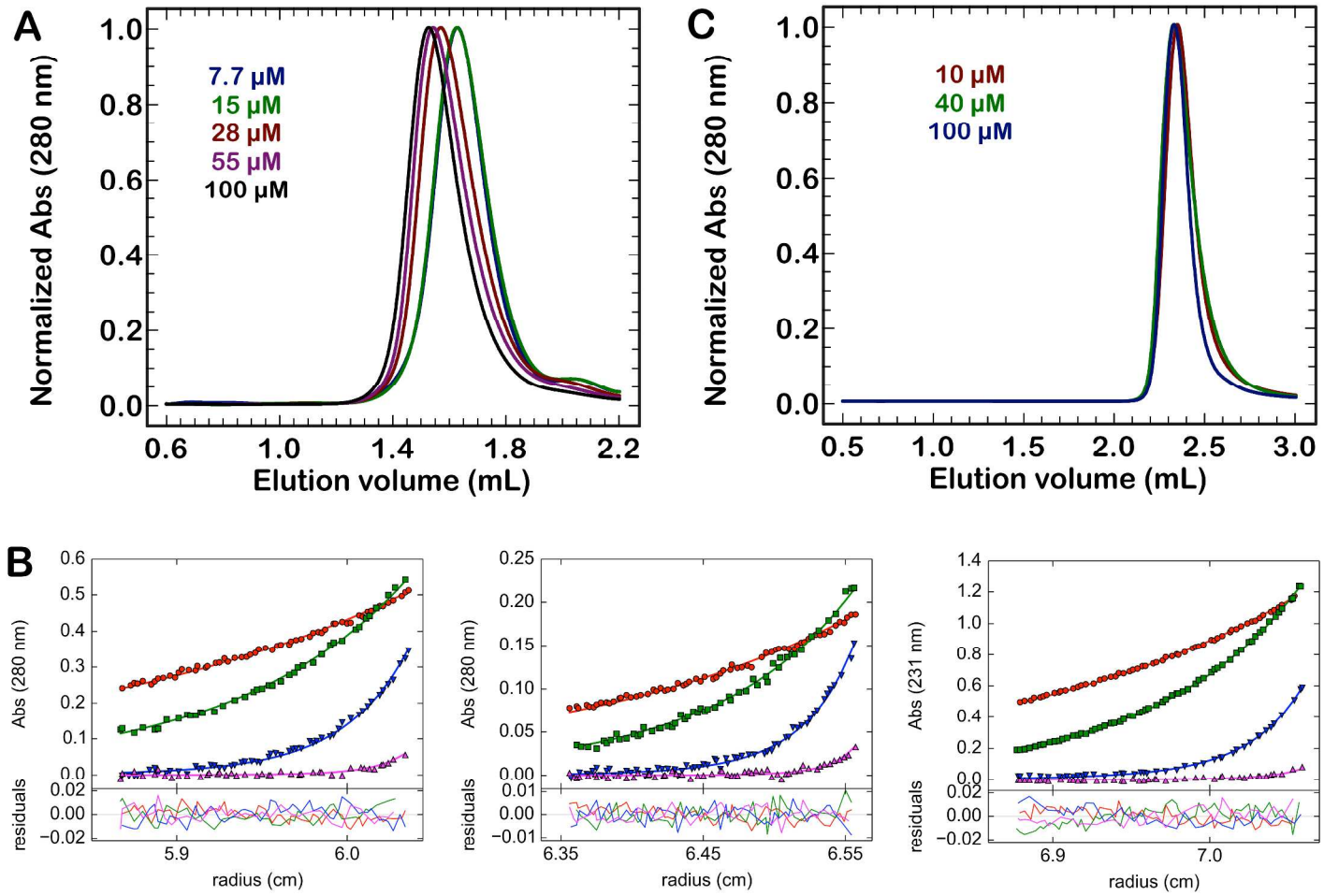

Figure S4. Biophysical analysis of the BECN$^{1-265}$ and BARA constructs. (A) SEC-MALS of the BECN$^{1-265}$ construct at various concentrations to determine whether a concentration-sensitive oligomerization state was present up to 100 μM. Samples were run over a Superdex 200 5/15 GL Increase column at 0.4 mL/min in 25 mM HEPES (pH 7.5), 150 mM NaCl, 0.5 mM TCEP. (B) Sedimentation equilibrium analytical ultracentrifugation of the truncated BECN$^{1-265}$ construct. The samples were run at 9,500 rpm (red *circles*), 14,500 rpm (*green squares*), 24,000 rpm (*blue inverted triangles*), or 35,000 (*magenta triangles*). (C) SEC-MALS of the BARA construct as described in S4*A*.

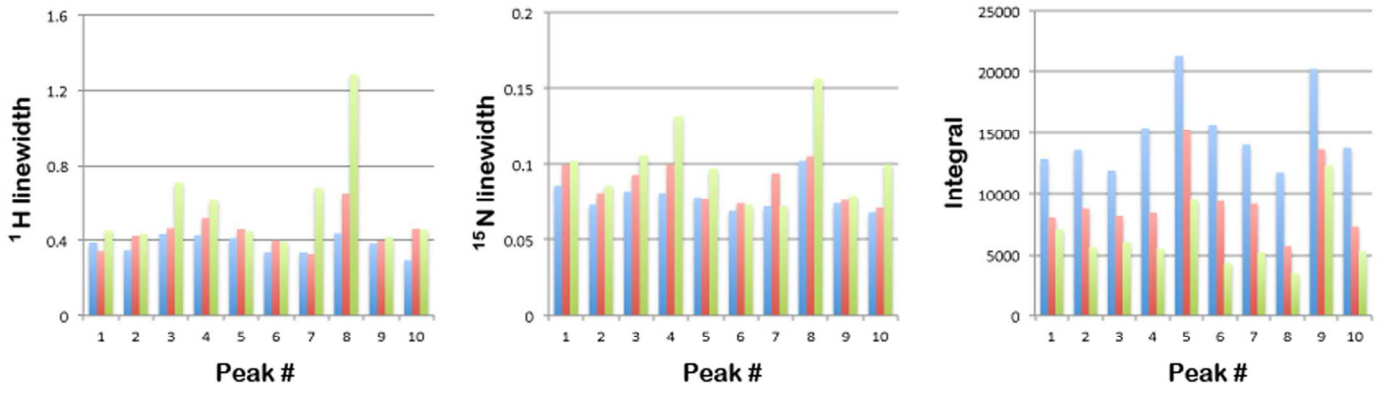

Figure S5. Line width and integration data for select 2D sfHMQC peaks of ^{15}N-labeled BARA spectra. Spectra for the ^{15}N-labeled BARA (30-25 μM) were collected at 298 K with 0 (*blue bars*), 0.5 (*red bars*), or 0.9 (*green bars*) molar equivalents of the unlabeled BECN$^{1-265}$ construct.

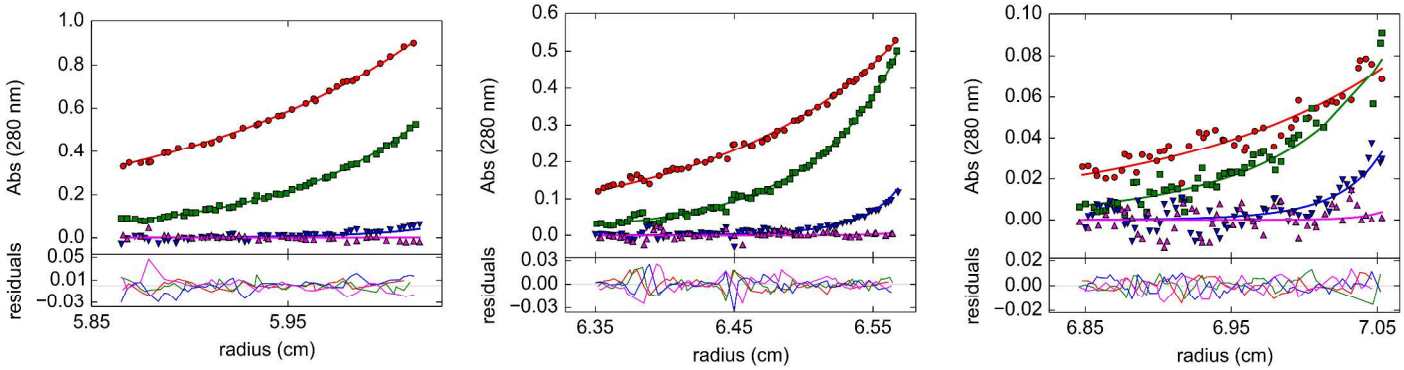

Figure S6. Sedimentation equilibrium analytical ultracentrifugation of the V250A/M254A/L261A mutant of full-length BECN1 at 15.4 μM (*left*), 5.7 μM (*center*), or 0.96 μM (*right*). The samples were run at 9,500 rpm (red *circles*), 14,500 rpm (*green squares*), 24,000 rpm (*blue inverted triangles*), or 35,000 (*magenta triangles*).