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

Matthew J. Ranaghan,[1] Michael A. Durney,[1] Michael F. Mesleh,[1] Patrick R. McCarren,[1] Colin W. Garvie,[1] Douglas S. Daniels,[2] Kimberly L. Carey[1], Adam P. Skepner,[1] Beth Levine,[3] and Jose R. Perez[1]*

[1]Center for the Development of Therapeutics, Broad Institute of MIT and Harvard, 415 Main Street, Cambridge, MA 02142

[2]Department of Chemistry, University of Dayton, 300 College Park, Dayton, OH 45469

[3]Department of Internal Medicine and Howard Hughes Medical Institute, UT Southwestern Medical Center, Dallas, TX 75390, USA

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

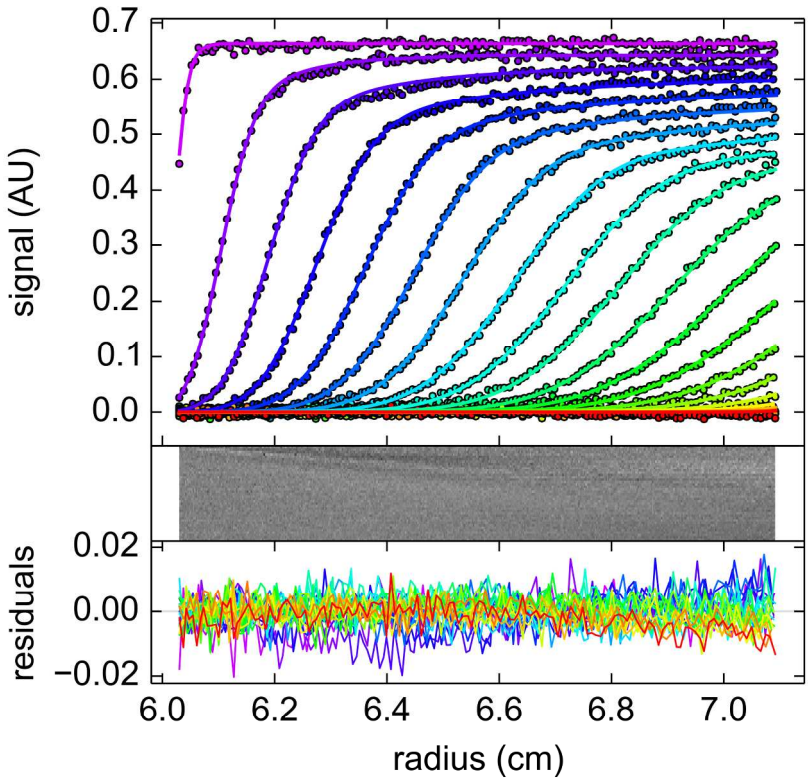

Figure S1. Sedimentation velocity analytical ultracentrifugation data for full-length BECN1 (10.3 μM) in 25 mM HEPES (pH 7.5), 150 mM NaCl, 0.5 mM TCEP at 20˚C. The data were fit using a *continuous c(s)* model in SedFIT.

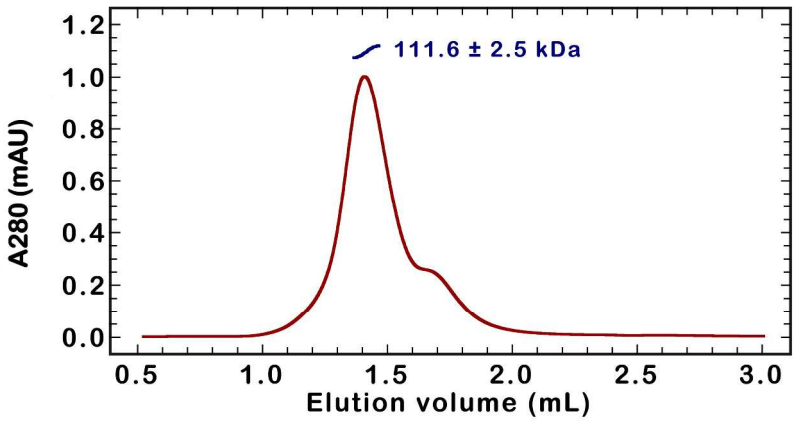

Figure S2. SEC-MALS of full-length BECN1 (9.6 μM) on a Superdex 200 5/15 GL Increase column at 0.4 mL/min in 25 mM Tris (pH 8), 500 mM NaCl, 0.5 mM TCEP. An average mass of 111.6 kDa was observed.

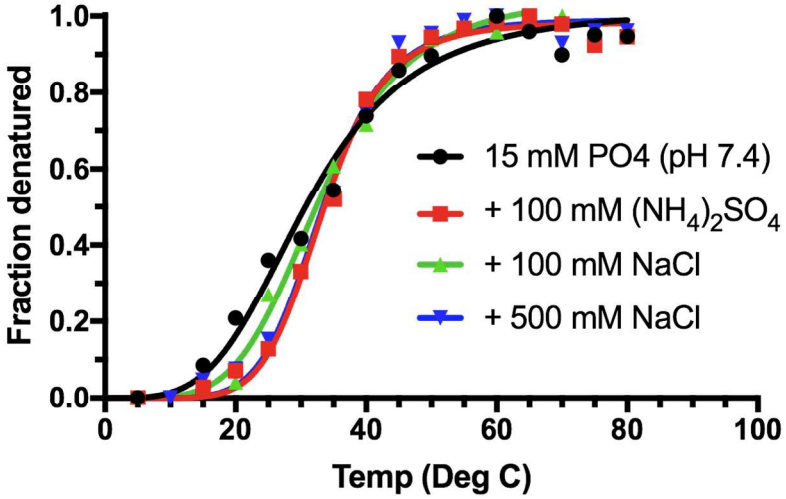

Figure S3. CD thermal melt of full-length BECN1 in phosphate buffer alone (*black;* T_M = 31˚C), phosphate with 100 mM $(NH_4)_2SO_4$ (*red;* T_M = 33˚C), phosphate with 100 mM NaCl (*green;* T_M = 33˚C), or phosphate with 500 mM NaCl (*blue;* T_M = 33˚C). Experiments were conducted as described in the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

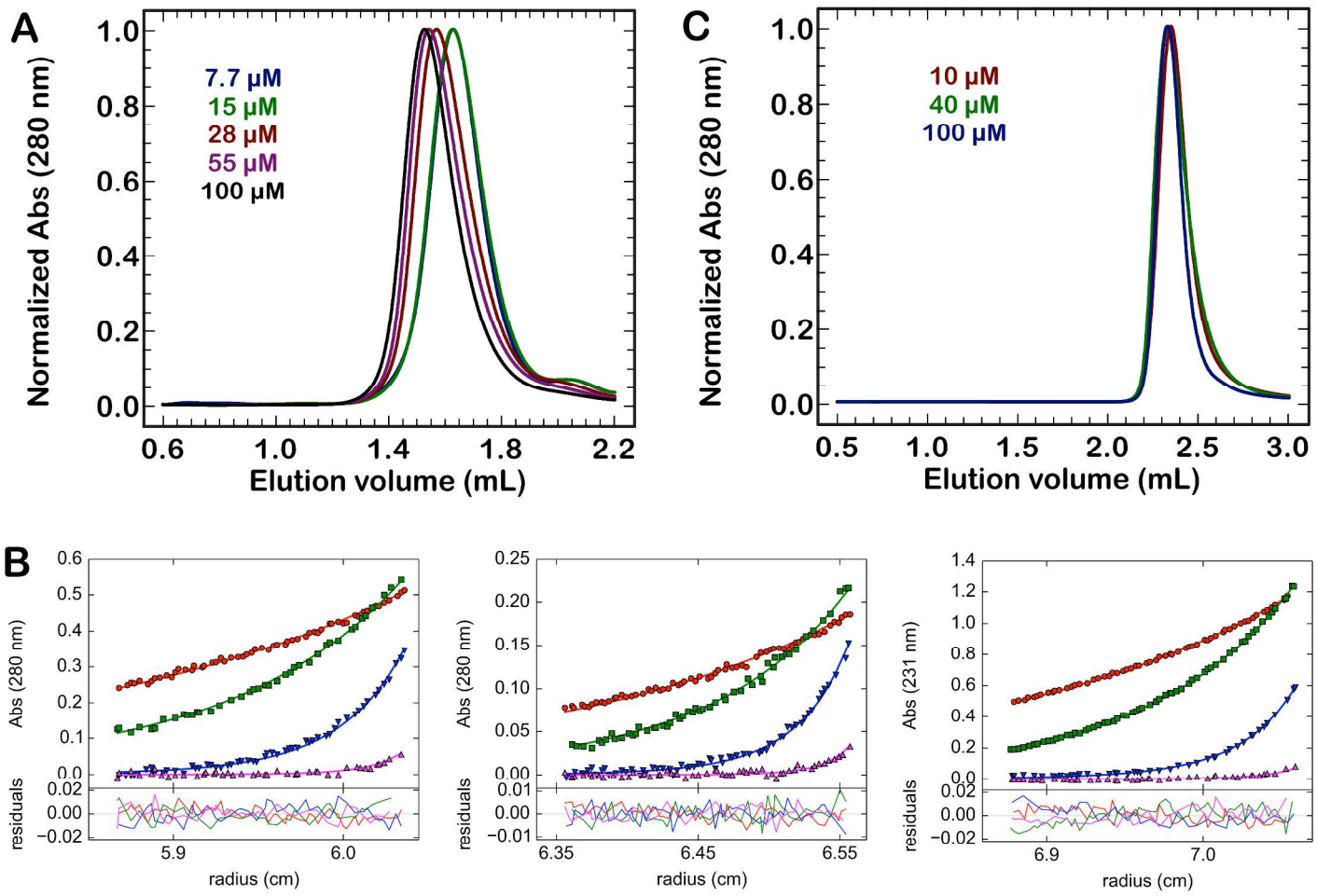

Figure S4. Biophysical analysis of the BECN$^{1\text{-}265}$ and BARA constructs. (A) SEC-MALS of the BECN$^{1\text{-}265}$ construct at various concentrations to determine whether a concentration-sensitive oligomerization state was present up to 100 μM. Samples were run over a Superdex 200 5/15 GL Increase 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$^{1\text{-}265}$ 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*.

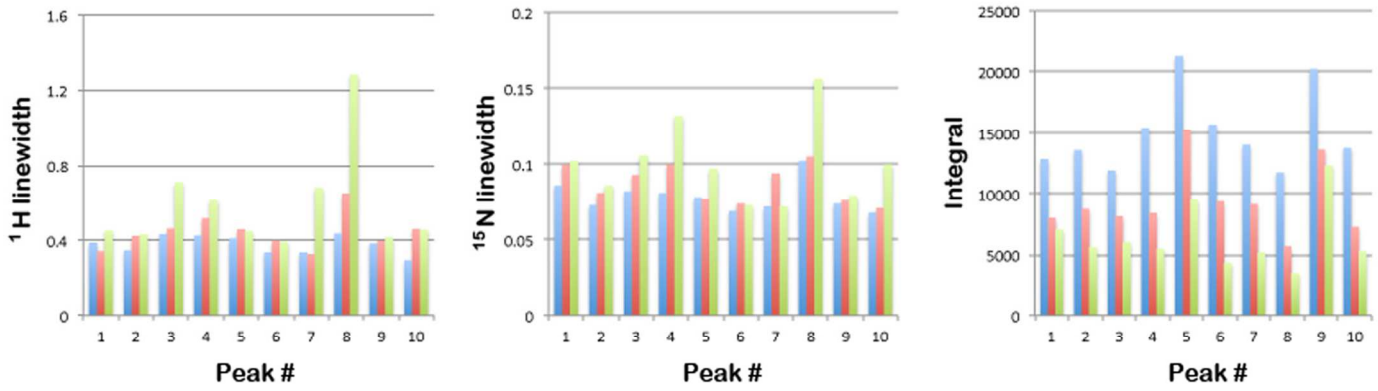

Figure S5. Line width and integration data for select 2D sfHMQC peaks of ^{15}N-labeled BARA spectra. Spectra for the ^{15}N-labeled BARA (30-25 μM) were collected at 298 K with 0 (*blue bars*), 0.5 (*red bars*), or 0.9 (*green bars*) molar equivalents of the unlabeled BECN$^{1\text{-}265}$ construct.

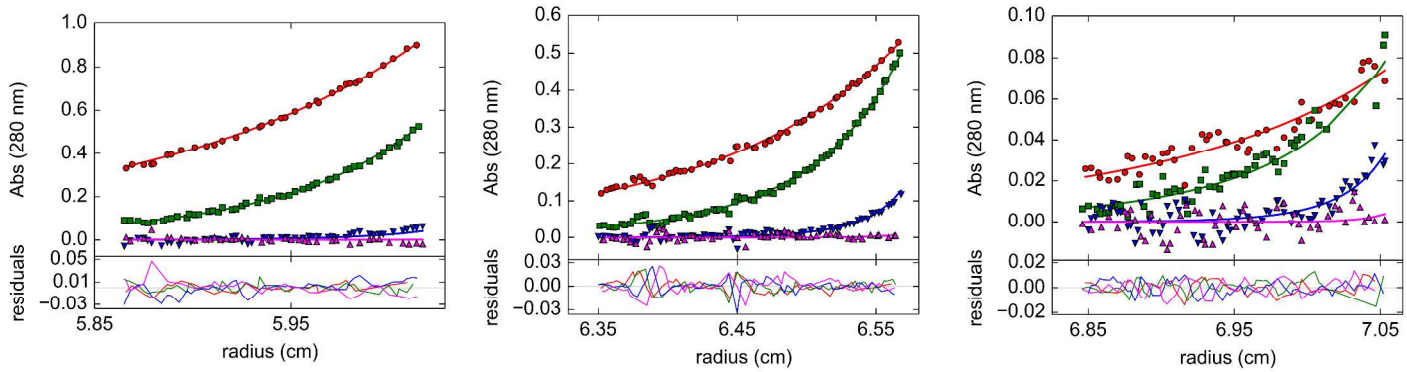

Figure S6. Sedimentation equilibrium analytical ultracentrifugation of the V250A/M254A/L261A mutant of full-length BECN1 at 15.4 μM (*left*), 5.7 μM (*center*), or 0.96 μM (*right*). The samples were run at 9,500 rpm (red *circles*), 14,500 rpm (*green squares*), 24,000 rpm (*blue inverted triangles*),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