Supporting information for manuscript:

A novel insertion mutation in Atlastin 1 is associated with spastic quadriplegia, increased membrane tethering, and aberrant conformational switching

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List of materials included:

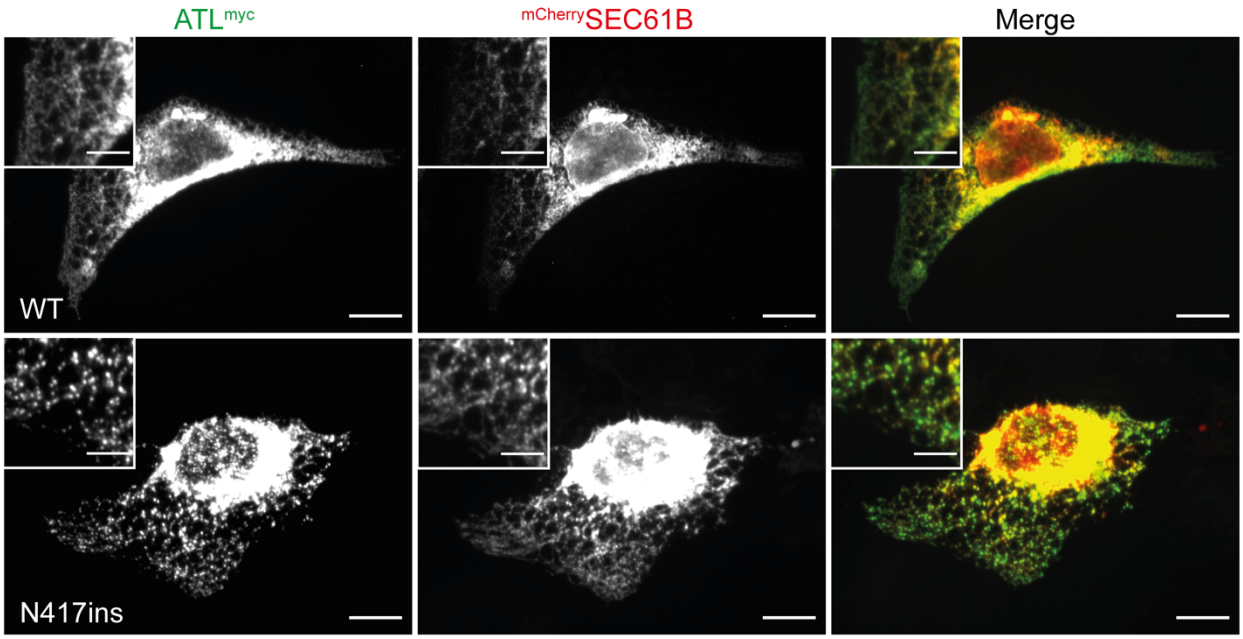

Figure S1. Colocalization of ATL1 with an ER marker in U2OS cells. Immunofluorescence was carried out on U2OS cells transiently co-transfected with ^{mCherry}SEC61B and either ATL1 WT^{myc} (top) or ATL1 N417ins^{myc} (bottom). The first column shows ATL1 signal (green), the second column shows SEC61B (red), and the third column overlays the signals. Scale bars = 10 μm; inset scale bar = 5 μm.

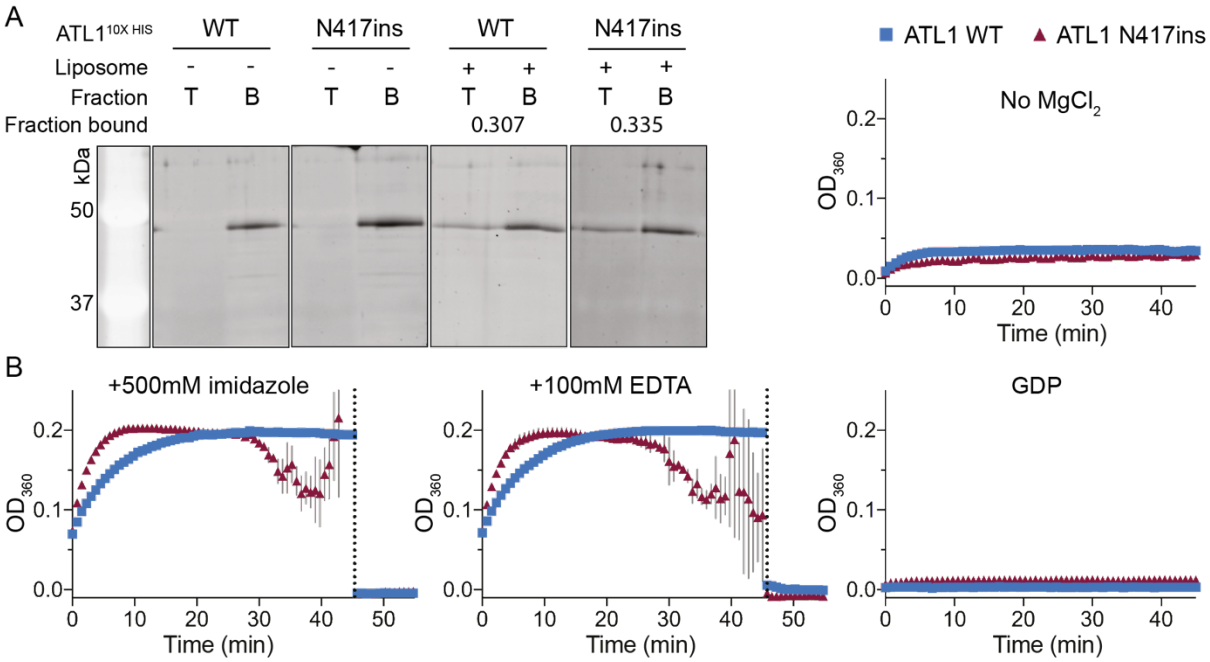

Figure S2. Vesicle tethering controls. (A) Flotation assay with 1 μM ATL1 WT or N417ins catalytic core proteins with a C-terminal His_{10}-tag in the presence (right two panes) or absence (left two panes) or lipids containing 1% molar ratio of Ni-NTA-modified lipids. Top fraction (indicated by "T") contains vesicles and bottom fraction ("B") contains unbound protein. For conditions with lipids, the fraction of protein bound to lipids was calculated using ImageJ (77). (B) Tethering reactions with 1 μM protein including addition of 10 mM EDTA or 500 mM imidazole at 45 minutes, or in the absence of $MgCl_2$ or presence of 2 mM GDP.

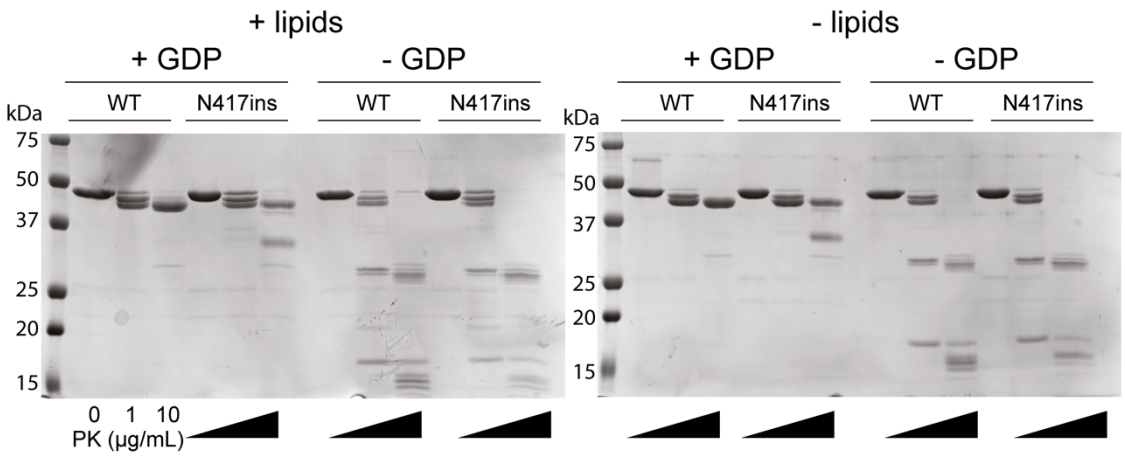

Figure S3. Limited proteolysis controls. SDS-PAGE gels show limited proteolysis reactions as described in Figure 6, but in the presence or absence of liposomes as prepared for vesicle tethering assays (see Figure 5). Reactions were carried out with increasing concentrations of PK (0 to 10 µg/mL) as indicated at the bottom of the gel and by the black triangles.

References cited in supporting information

77. Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012) NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **9**, 671-675.