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Supplemental Data

A Class of Dynamin-like GTPases

Involved in the Generation

of the Tubular ER Network

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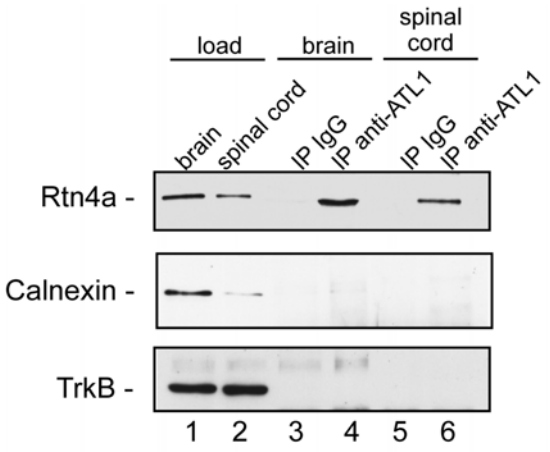

Figure S1. Endogenous ATL1 does not interact with control membrane proteins.
Detergent extracts from mouse brain or spinal cord were incubated with ATL1 antibodies or control IgG. Precipitated proteins were then analyzed by immunoblotting (IB) with Rtn4a, calnexin, or TrkB antibodies. Lanes 1 and 2 (loads) show 10% of the starting material used for immunoprecipitation.

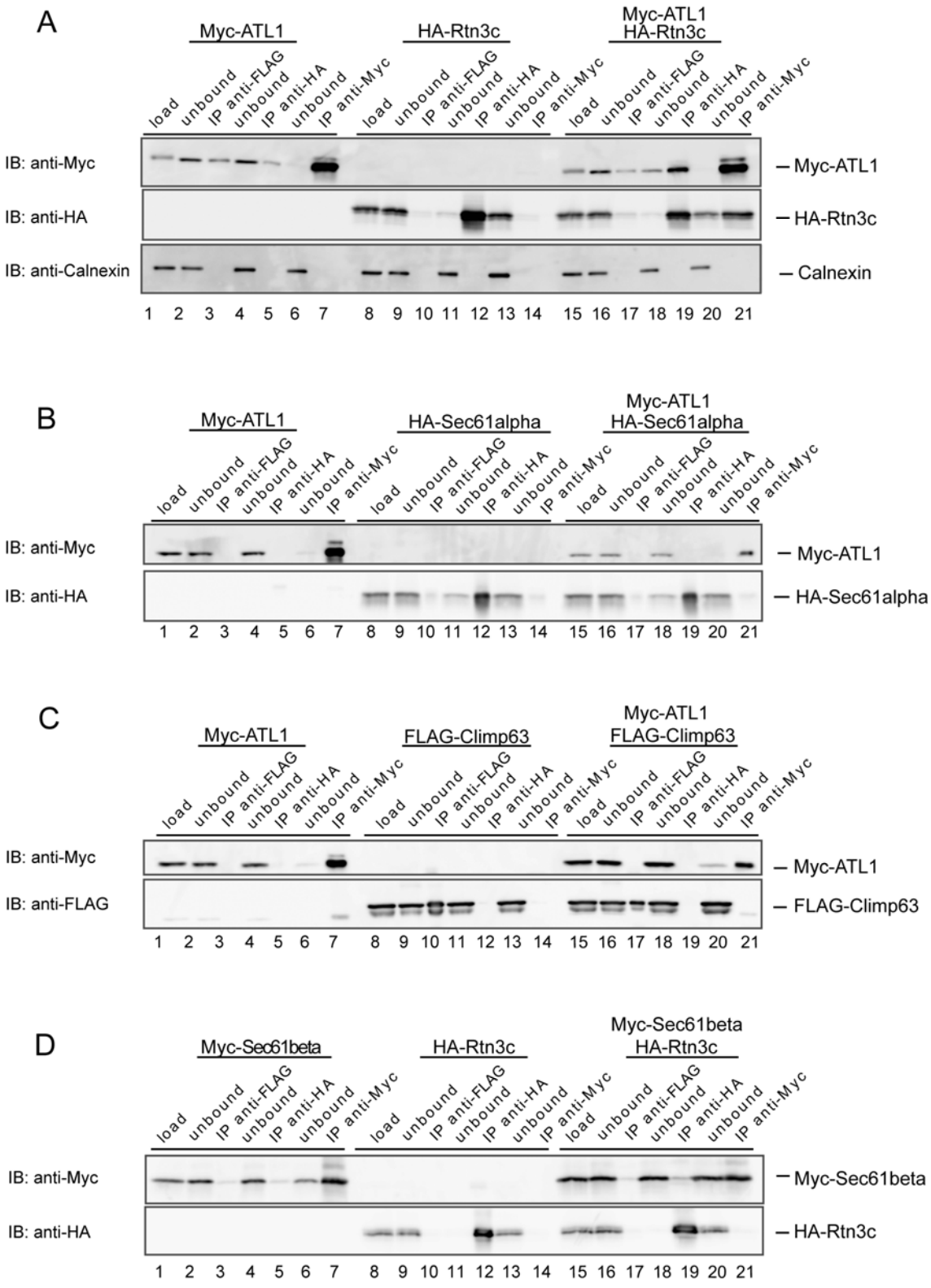

Figure S2. Overexpressed ATL1 and Rtn3c do not interact with control membrane proteins.
(A) Myc-ATL1 and HA-Rtn3c were expressed either individually or together in COS-7 cells. Digitonin-extracts were used for immunoprecipitation (IP) with anti-HA, anti-Myc, or anti-FLAG antibodies. Ten

percent of the starting material (load) and of the material not bound to the antibodies (unbound), as well as the precipitates were analyzed by SDS-PAGE and immunoblotting (IB) with anti-HA or anti-Myc antibodies. As a control, the blot was also probed with antibodies to the integral ER membrane protein calnexin.
(B) Co-immunoprecipitation as in (A), but with cells expressing Myc-ATL1 and the HA-tagged integral membrane protein Sec61alpha (HA-Sec61alpha).
(C) Co-immunoprecipitation as in (A), but with cells expressing Myc-ATL1 and the FLAG-tagged integral membrane protein CLIMP63 (FLAG-CLIMP63). The blot was probed with anti-FLAG, instead of HA-antibodies.
(D) Co-immunoprecipitation as in (A), but with cells expressing Myc-Sec61beta and HA-Rtn3c.

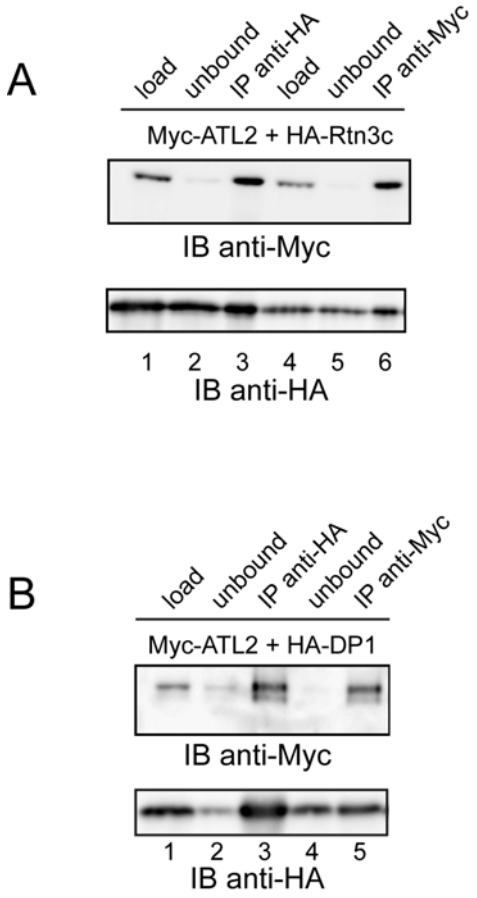

Figure S3. **ATL2 interacts with Rtn3c and DP1.**
(A) Myc-tagged ATL2 (Myc-ATL2) was expressed together with HA-tagged Rtn3c (HA-Rtn3c) in COS-7 cells. Digitonin-solubilized extracts were used for immunoprecipitation (IP) with anti-HA or anti-Myc antibodies. Ten percent of the starting material (load) and of the material not bound to the antibodies (unbound), as well as the material bound to the antibodies were analyzed by SDS-PAGE and immunoblotting (IB) with anti-HA or anti-Myc antibodies.
(B) As in (A), but with HA-tagged DP1 (HA-DP1) instead of HA-Rtn3c.

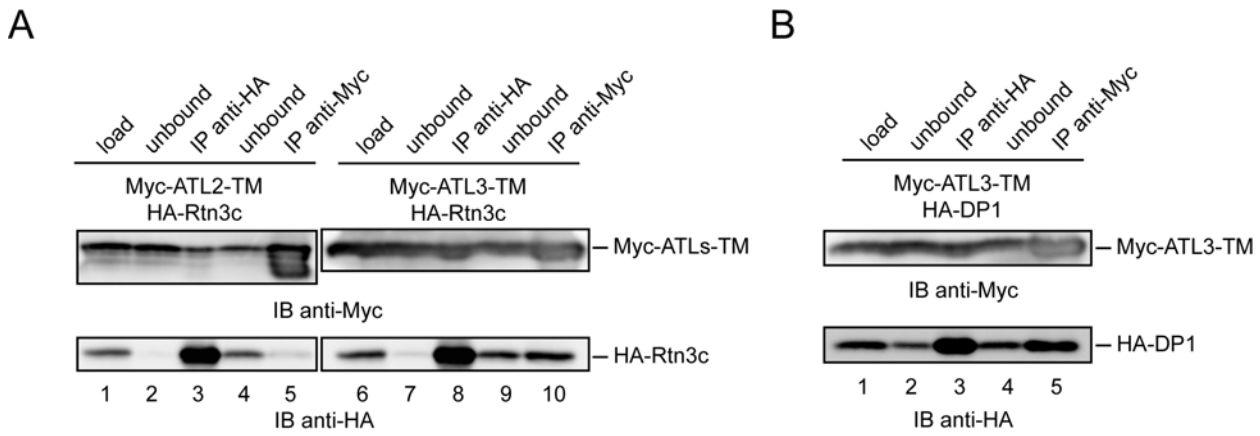

Figure S4. Interactions of the transmembrane segments of ATL2 and ATL3.
(A) The Myc-tagged C-terminal domains of ATL2 or ATL3 harboring the transmembrane domains (Myc-ATL2-TM or Myc-ATL3-TM) were expressed together with HA-tagged Rtn3c (HA-Rtn3c) in COS-7 cells. Digitonin-solubilized extracts were used for immunoprecipitation (IP) with anti-HA or anti-Myc antibodies. Ten percent of the starting material (load) and of the material not bound to the antibodies (unbound), as well as the material bound to the antibodies were analyzed by SDS-PAGE and immunoblotting (IB) with anti-HA or anti-Myc antibodies.
(B) As in (A), but with Myc-ATL3-TM and HA-DP1.

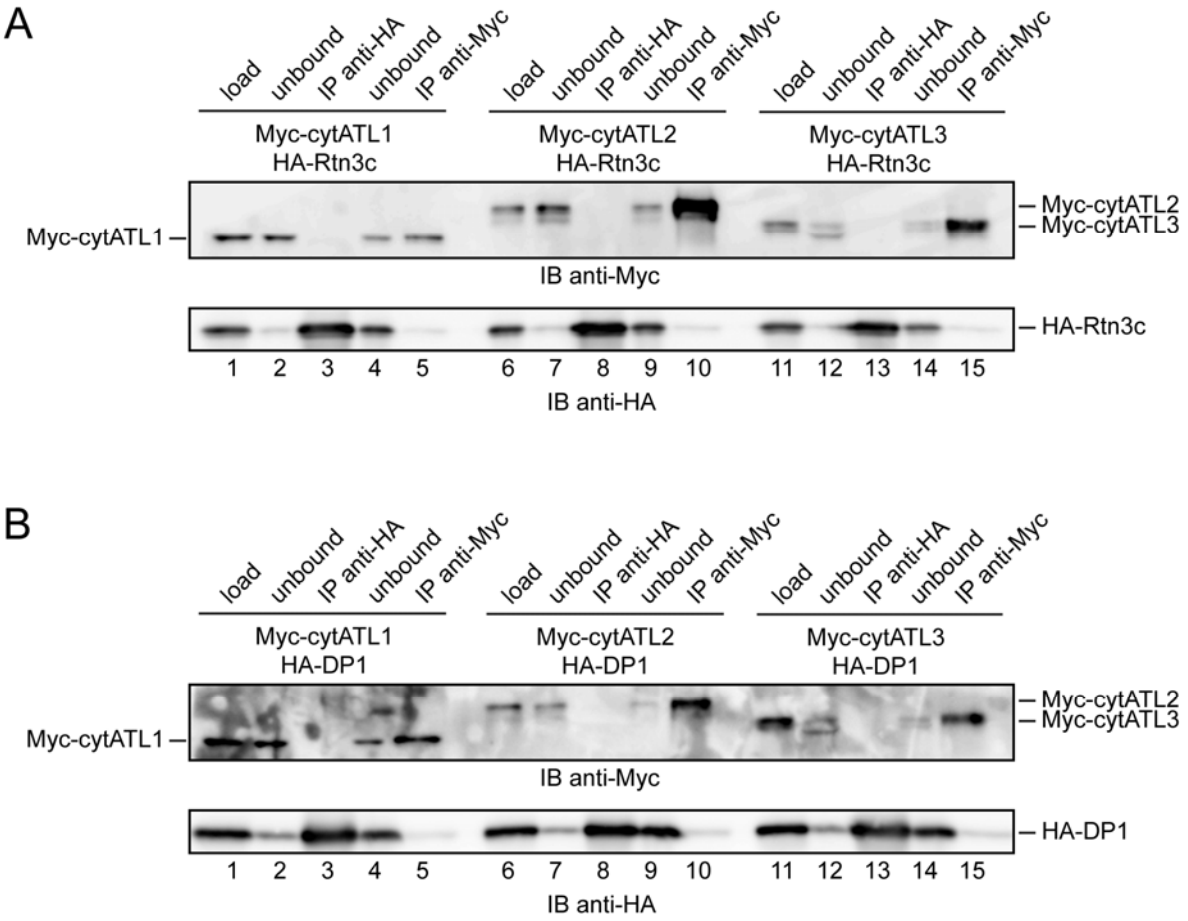

Figure S5. The cytoplasmic domains of the atlastins do not interact with Rtn3c or DP1.
(A) Myc-tagged versions of the large N-terminal cytoplasmic domains of ATL1, ATL2, or ATL3 (Myc-cytATL1, Myc-cytATL2, or Myc-cytATL3) and HA-tagged Rtn3c (HA-Rtn3c) were co-expressed in COS-7 cells. Digitonin-solubilized extracts were used for immunoprecipitation (IP) with anti-HA or anti-Myc antibodies. Ten percent of the starting material (load), the material not bound to the antibodies (unbound), and the material bound to the antibodies were analyzed by SDS-PAGE and immunoblotting (IB) with anti-HA or anti-Myc antibodies.
(B) As in (A), but with HA-tagged DP1 (HA-DP1) instead of HA-Rtn3c.

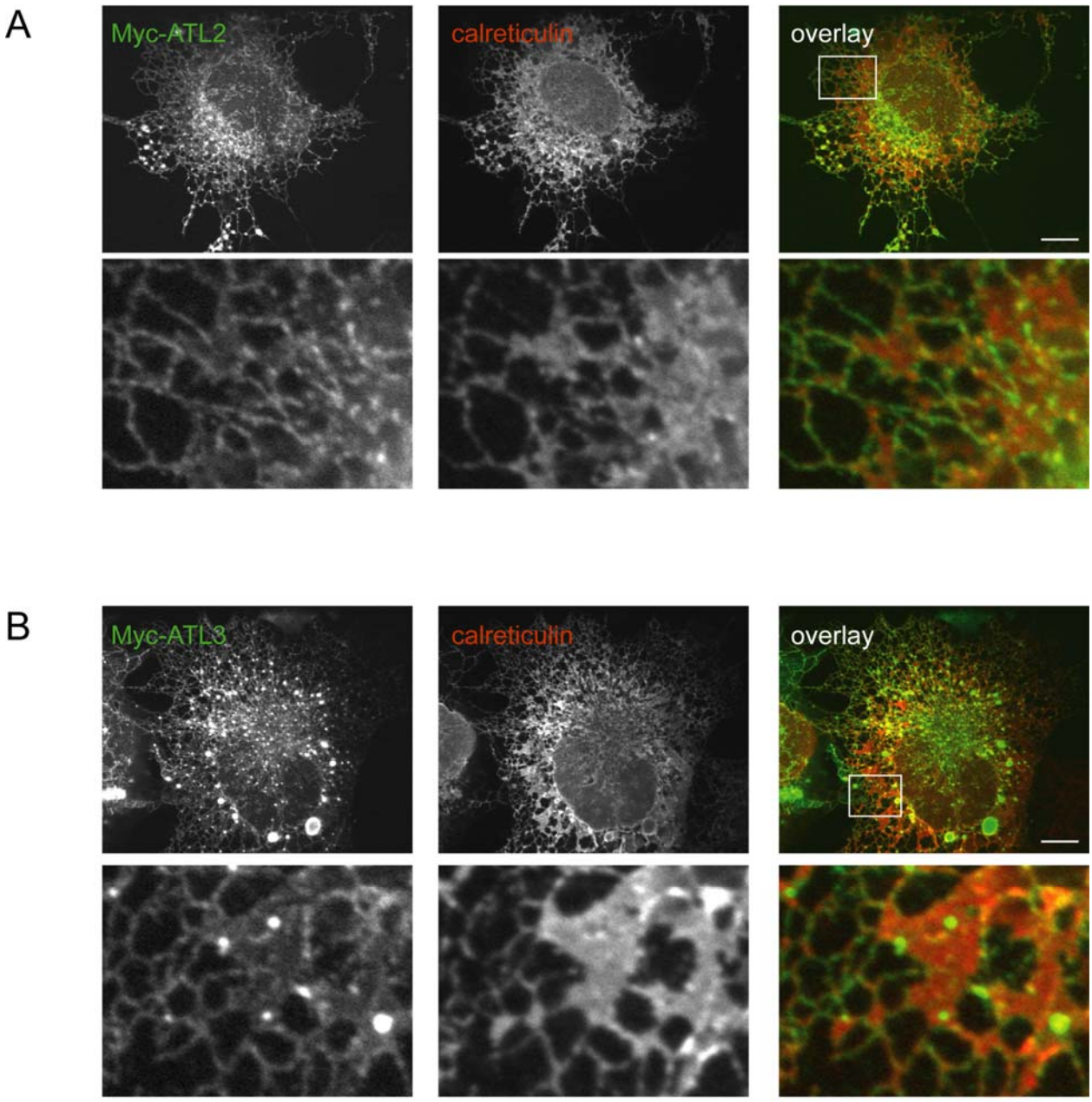

Figure S6. ATL2 and ATL3 localize to the tubular ER.
(A) Myc-ATL2 (green) was expressed in COS-7 cells. Its localization was identified with anti-Myc antibodies and compared to that of an endogenous luminal ER protein, calreticulin (red) using indirect immunofluorescence and confocal microscopy. The lower panels show an enlargement of the boxed region centered on ER sheets. Scale bar, 10 μm.
(B) As in (A), but with Myc-ATL3 instead of Myc-ATL2.

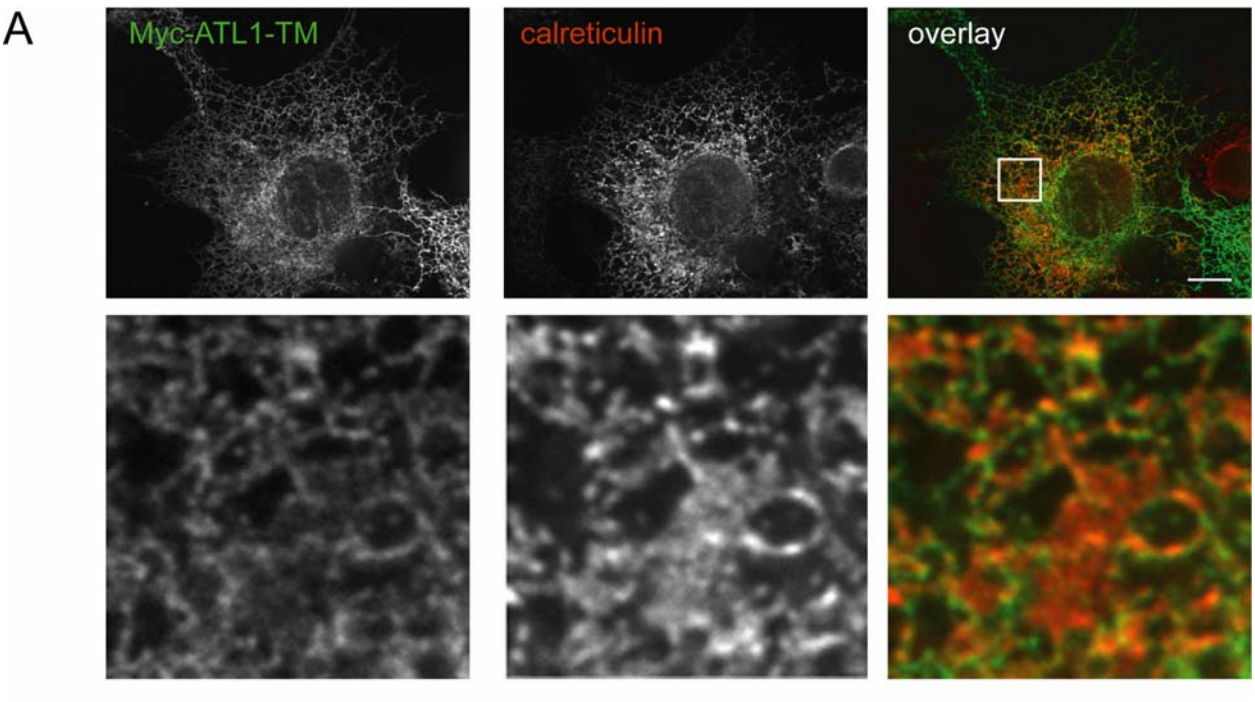

Figure S7. The membrane-anchoring domain of the atlastins localizes to the tubular ER network. Myc-tagged C-terminal region of ATL1 harboring the transmembrane domains (Myc-ATL1-TM) (green) was expressed in COS-7 cells. Its localization was revealed with anti-Myc antibodies and compared to that of an endogenous luminal ER protein, calreticulin (red), using indirect immunofluorescence and confocal microscopy. The lower panels show an enlargement of the boxed region centered on ER sheets. Scale bar, 10 μm.

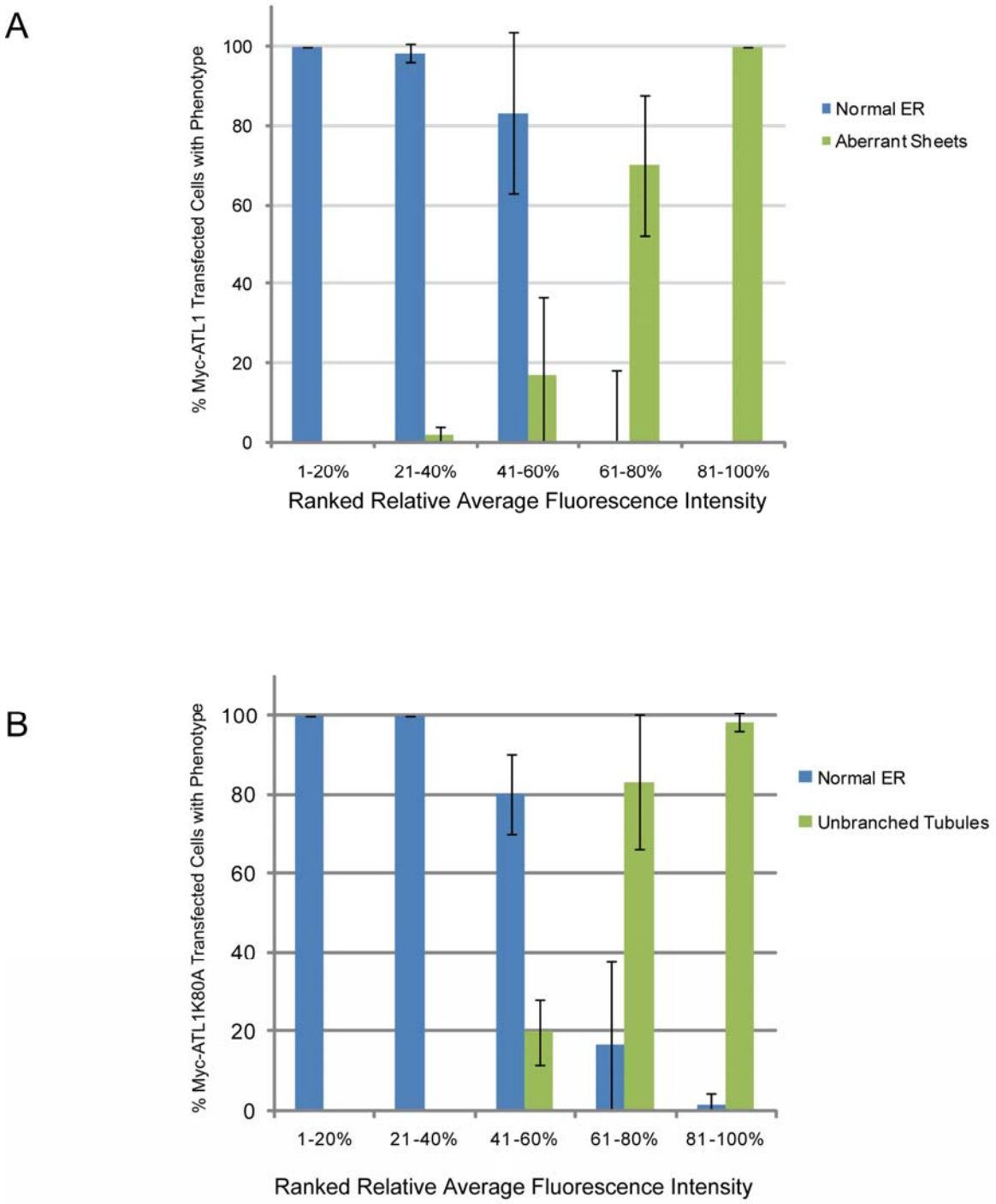

Figure S8. ER morphology defects correlate with the expression level of ATL1 and ATL1 K80A. Myc-ATL1 or Myc-ATL1 K80A was expressed in COS-7 cells. The cells were stained with Myc-antibodies and imaged by fluorescence microscopy. For each cell, the ER morphology was visually scored, and the average fluorescence intensity, defined as the total fluorescence in the ER divided by its area, was measured using MetaMorph software. The cells were divided into five groups according to their expression levels. Shown is the percentage of cells with normal and abnormal ER in each group (means ± standard errors from three different transfections; 100 cells per sample).

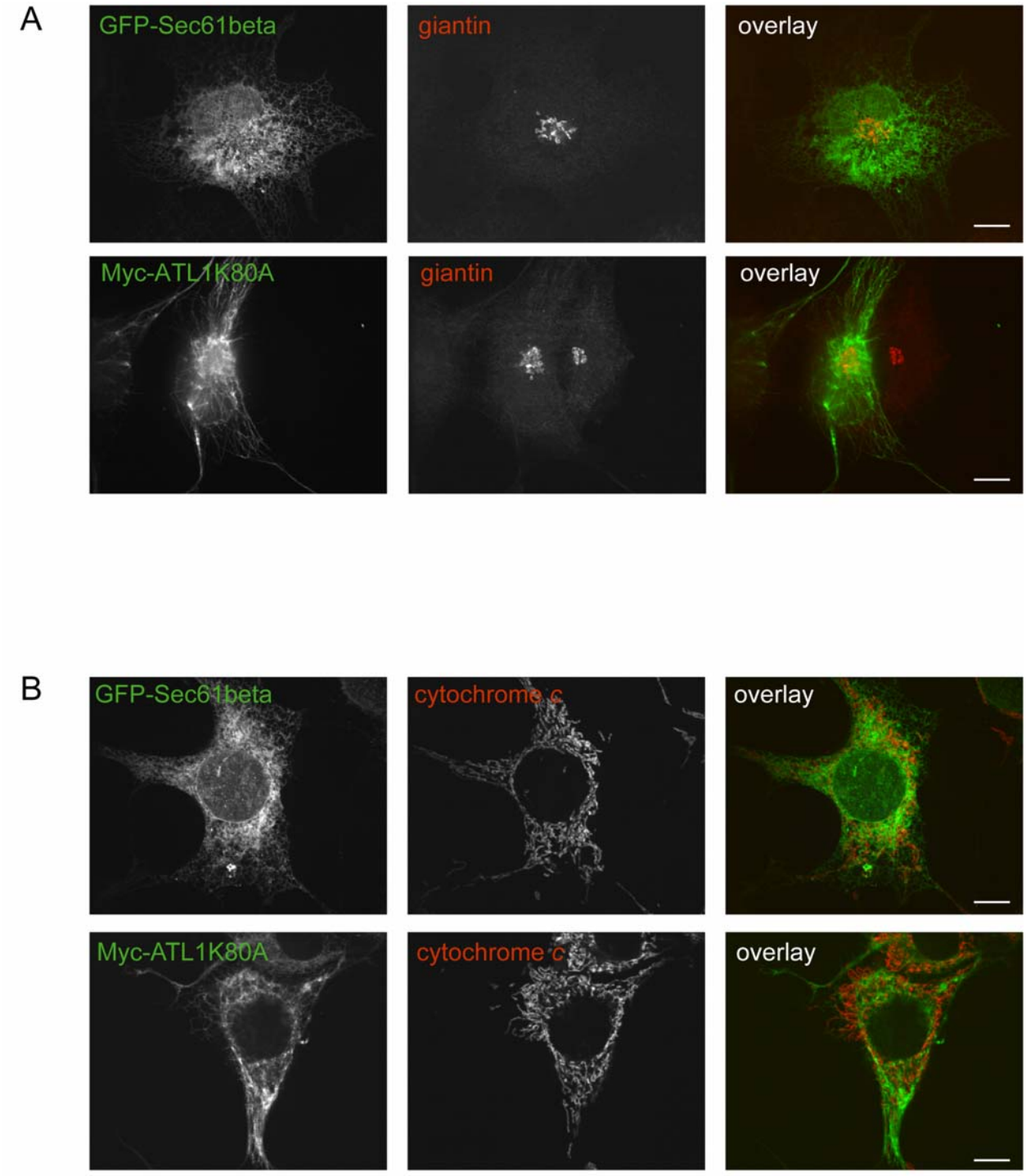

Figure S9. Expression of ATL1 K80A changes the morphology of the ER, but not that of other organelles.
(A) GFP-Sec61beta or Myc-ATL1 K80A was expressed in COS-7 cells. The localization of the Golgi marker giantin was analyzed using indirect immunofluorescence and confocal microscopy. Bar, 10 μm.
(B) As in (A), but the cells were stained with antibodies to the mitochondrial marker cytochrome *c*.

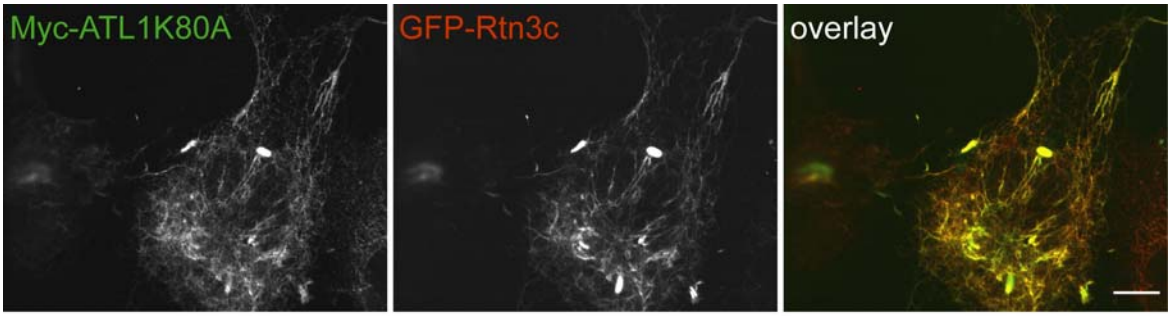

Figure S10. Co-localization of a GTP-binding mutant of ATL1 with Rtn3c.
Myc-ATL1 K80A was co-expressed with GFP-Rtn3c in COS-7 cells. The proteins were visualized by indirect immunofluorescence using Myc-antibodies and by GFP fluorescence. Bar, 10 μm.

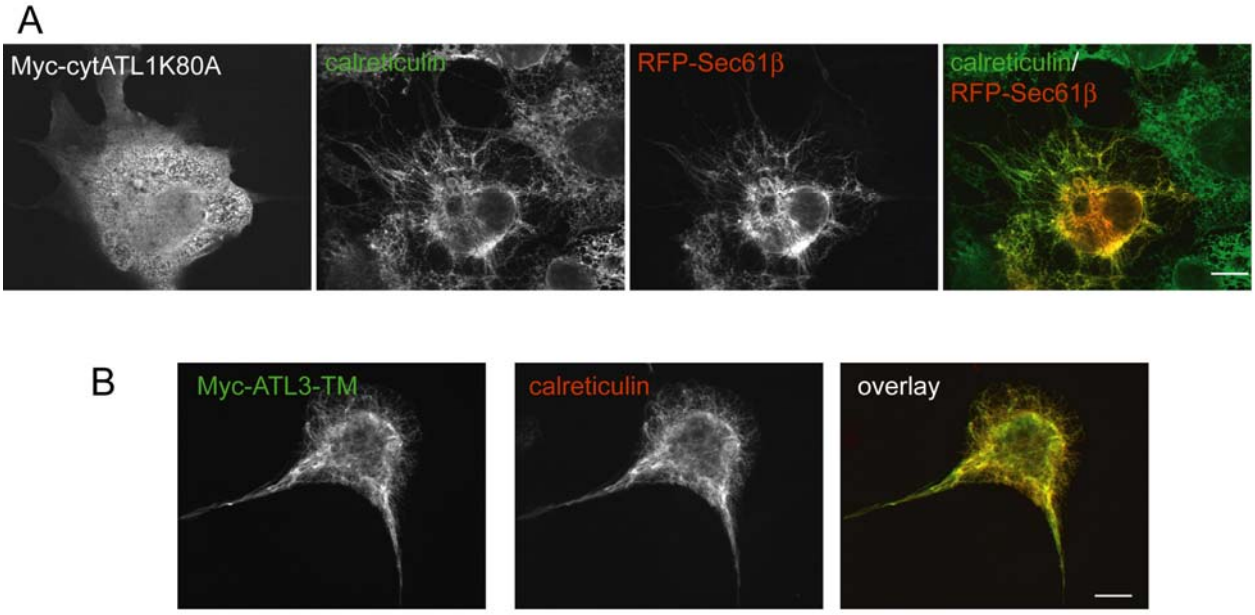

Figure S11. ER morphology changes caused by overexpression of atlastin mutants.
(A) A Myc-tagged version of the N-terminal, cytoplasmic domain of the GTP-binding mutant of ATL1 (Myc-cytATL1 K80A) was co-expressed in COS-7 cells with an RFP-tagged version of the ER protein Sec61β. The cells were stained with Myc-epitope and calreticulin antibodies and visualized by indirect immunofluorescence and confocal microscopy. Shown is a cell with an unbranched ER tubule phenotype. Bar, 10 μm.
(B) A Myc-tagged version of the membrane-anchoring domain of ATL3 (Myc-ATL3-TM) was expressed in COS-7 cells. The cells were stained with Myc-epitope and calreticulin antibodies and visualized by indirect immunofluorescence and confocal microscopy. Note the unbranched tubular ER morphology.

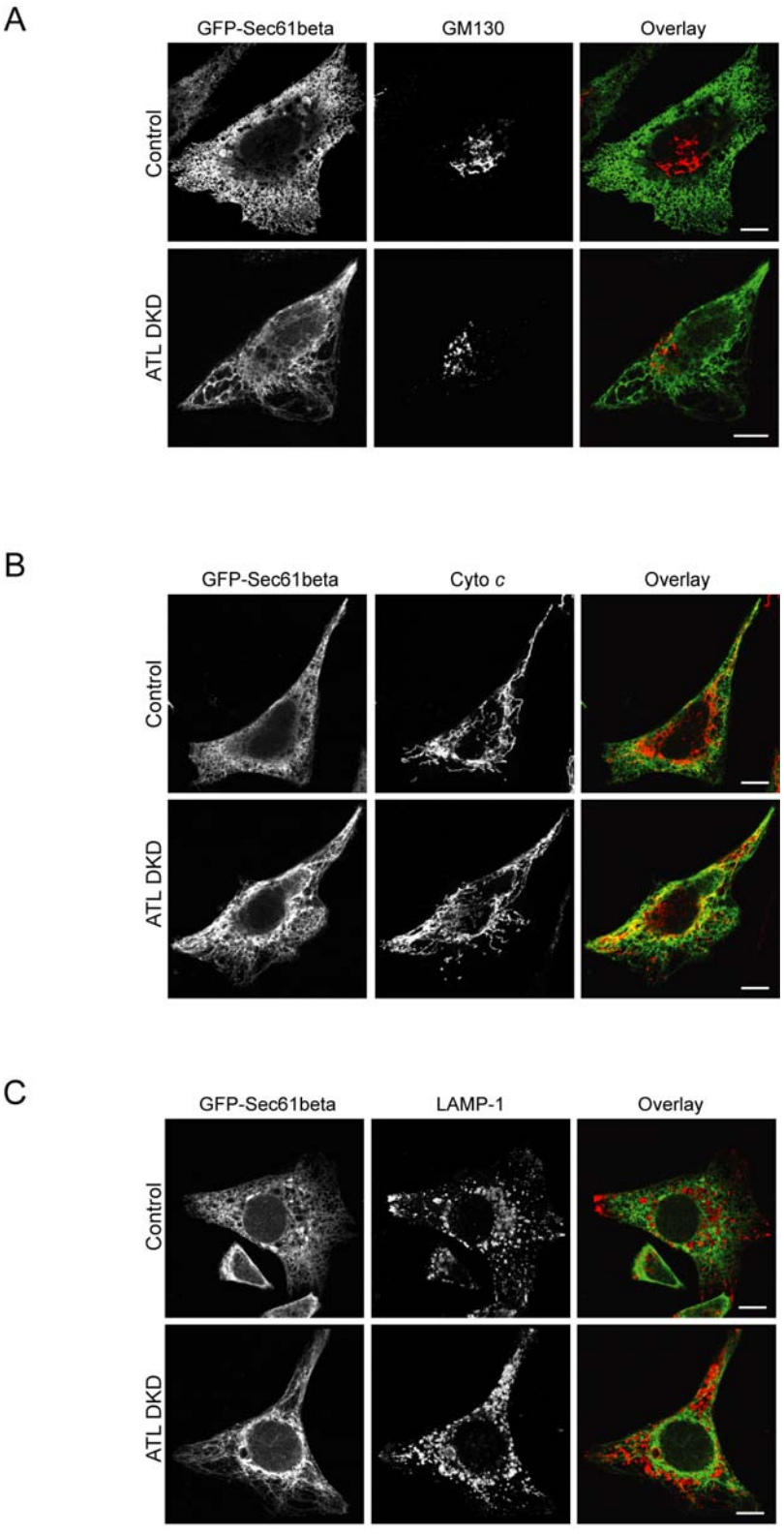

Figure S12. Effect of atlastin depletion on the morphology of organelles.
(A) HeLa cells were transfected with siRNA oligonucleotides directed against ATL2 and ATL3 (ATL DKD) or else with an equivalent concentration of control siRNA. The cells were transfected with GFP-Sec61β (green) and visualized by fluorescence confocal microscopy after staining for the Golgi marker GM130 (red). Merged images are to the right. Bar, 10 μm.
(B) As in (A), except cells were immunostained for the mitochondrial marker cytochrome *c* (Cyto *c*; red). Bar, 10 μm.
(C) As in (A), except cells were immunostained for the lysosomal marker LAMP-1 (red). Bar, 10 μm.

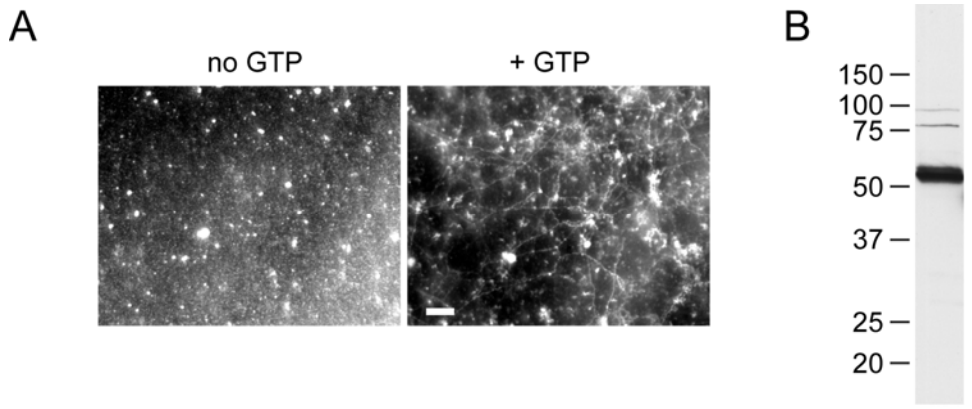

Figure S13. *Xenopus* egg membranes contain atlastins and form an ER network *in vitro*
(A) Membranes from *Xenopus* eggs were incubated with or without GTP for 60 min, stained with octadecyl rhodamine, and visualized by fluorescence microscopy. Bar, 20 μm.
(B) Membrane extracts from *Xenopus* eggs were separated by SDS-PAGE and analyzed by immunoblotting with anti-ATL antibodies that recognize all *Xenopus* atlastin isoforms. Molecular mass standards (in kDa) are on the left.

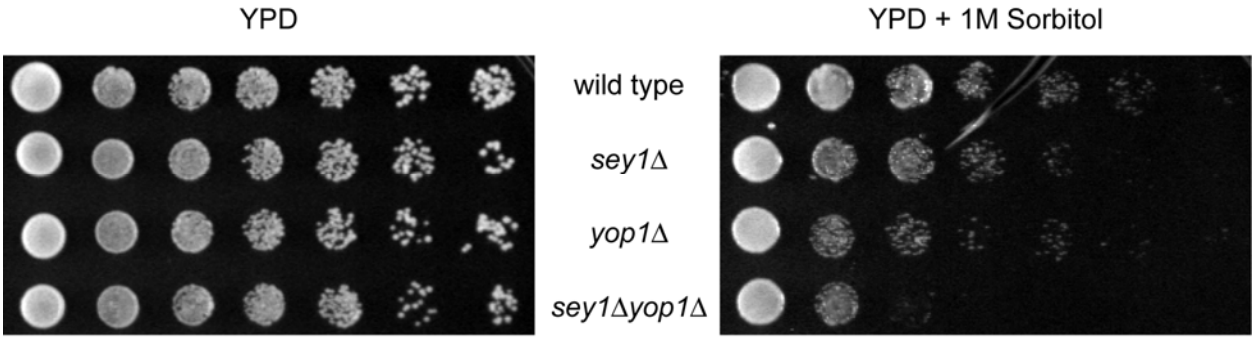

Figure S14. A *sey1Δyop1Δ* mutant shows a growth defect in high-osmolarity medium
The indicated yeast strains were grown to saturation in YPD media and diluted sequentially ten-fold. Three μl of each dilution were spotted onto YPD plates in the absence or presence of 1 M sorbitol, and the plates were incubated at 30°C for 2 days.

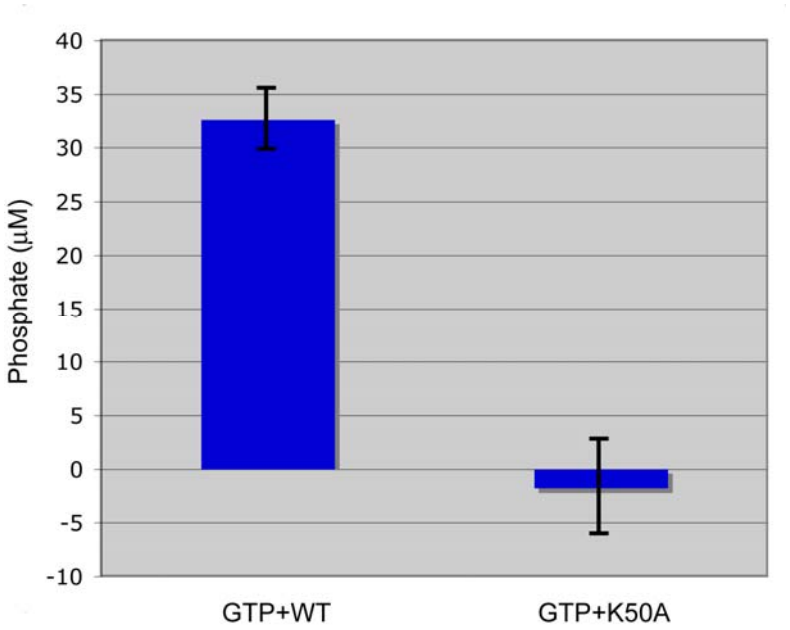

Figure S15. GTPase activity of the cytoplasmic domain of the Sey1p K50A mutant.
The Sey1p GTPase domain (residue 1-351) with an N-terminal 6xHis-tag and with or without the K50A mutation was expressed from the pET28a vector (Novagen) in *E. coli.* Cells were broken in 50 mM Tris pH 7.5, 150 mM NaCl and 5 mM $MgCl_2$ with protease inhibitors and 5 mM 2-mercaptoethanol. The protein was purified from cleared lysates by Ni-NTA chromatography (Qiagen) and gel filtration chromatography (Superdex 200, GE Healthcare). Purified proteins were mixed with 500 µM GTP and 2.5 mM $MgCl_2$, and assayed for generation of phosphate using the PiPer Phosphate Assay (Invitrogen) at 37ºC.