Supporting Information

Pendin et al. 10.1073/pnas.1106421108

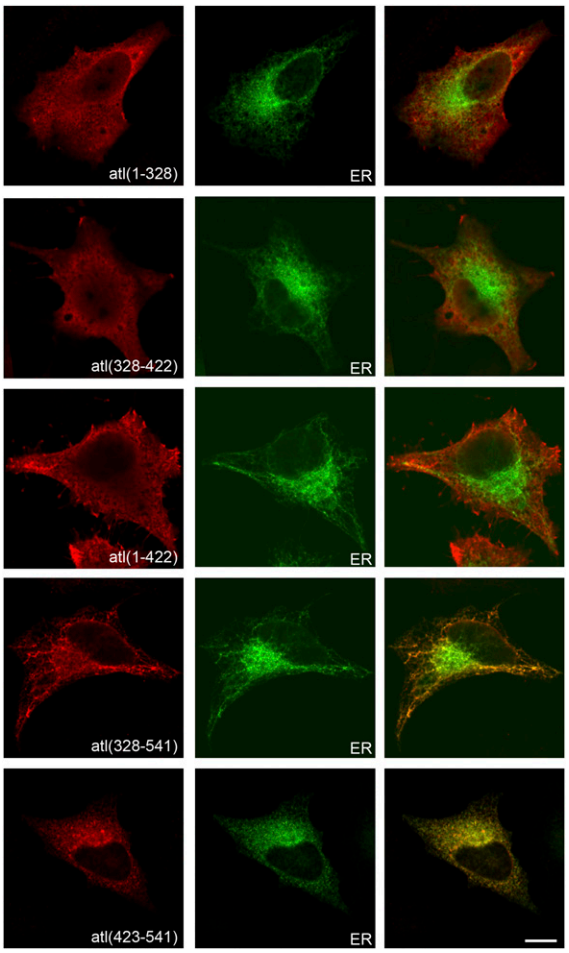

Fig. S1. Subcellular localization of atlastin fragments. HeLa cells were transfected with the indicated atlastin fragments. Only fragments containing the transmembrane domains display reticular localization. GFP-Sec61β was used as endoplasmic reticulum (ER) marker. (Scale bar, 10 μm.)

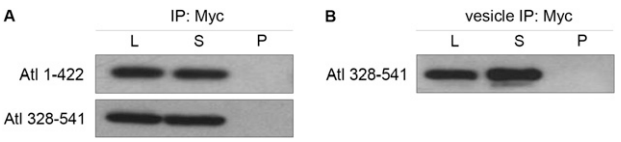

Fig. S2. HeLa cells were transfected with the indicated HA-tagged constructs. Lysates (*A*) or microsome fraction (*B*) were prepared and immunoprecipitated with anti-myc antibodies. Western blot analysis using anti-HA antibodies shows that these fragments were not immunoprecipitated aspecifically. L, lysate; S, supernatant; P, pellet.

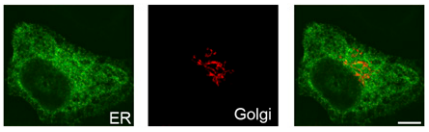

Fig. S3. Endoplasmic reticulum (ER) and Golgi morphology in untransfected control cells. PDI and GM130 were used as ER and Golgi markers, respectively. (Scale bar, 10 μm.)

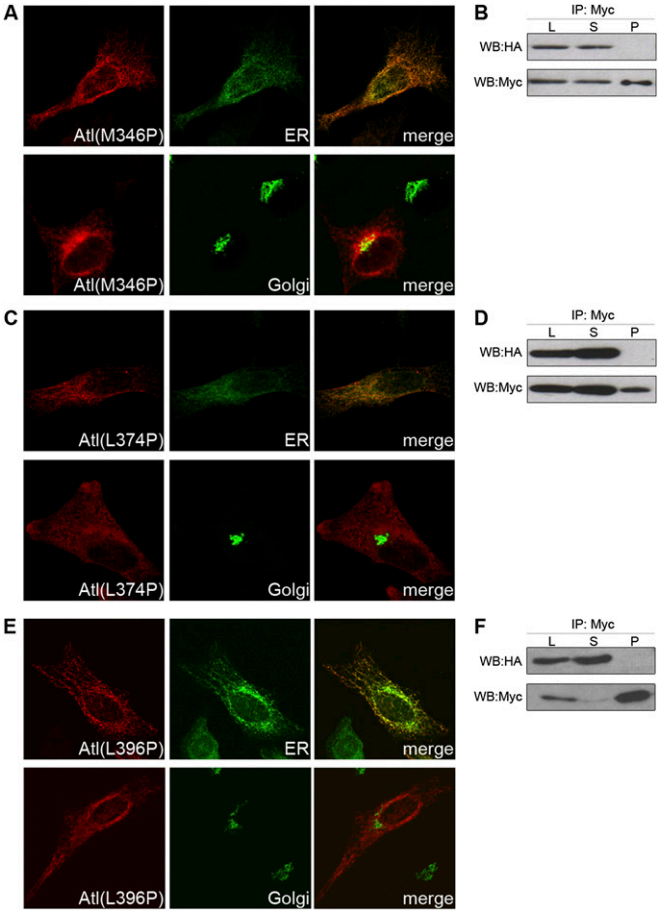

Fig. S4. Proline mutations within the α-helices of the middle domain prevent self-association and abolish atlastin function. (*A*, *C*, and *E*) HeLa cells transfected with atlastin(M346P) (*A*), atlastin(L374P) (*C*), or atlastin(L396P) (*E*) show normal endoplasmic reticulum (ER) and Golgi morphology; PDI and GM130 were used as ER and Golgi markers, respectively. (Scale bars, 10 μm.) (*B*, *D*, and *F*) Coimmunoprecipitation experiments using the single proline mutants M346P (*B*), L374P (*D*), or L396P (*F*) demonstrate the inability of these mutants to self-assemble. L, lysate; S, supernatant; P, pellet.

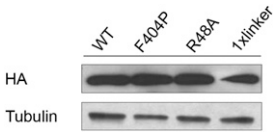

Fig. S5. Expression levels of wild-type, F404P, R48A, and 1xlinker atlastins upon transfection in HeLa cells. Western blot analysis shows that all atlastin variants are expressed at similar levels. Equal amounts of proteins were loaded in each lane.

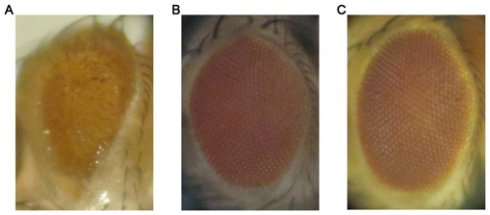

Fig. S6. (*A*) Overexpression of atlastin under the control of GMR-Gal4 causes a small eye phenotype. (*B* and *C*) GMR-Gal4–driven overexpression of atlastin (F404P) (*B*) and atlastin(3xlinker) (*C*) has no phenotypic consequences.

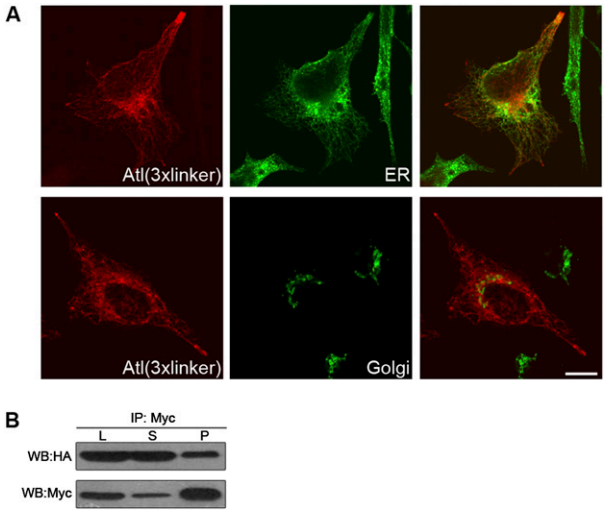

Fig. S7. Increasing the distance between the 3HB and the membrane anchor causes inactivation of atlastin without inhibiting its oligomerization properties. (*A*) Endoplasmic reticulum (ER) and Golgi morphology is unperturbed in HeLa cells transfected with atlastin(3xlinker); PDI and GM130 were used as ER and Golgi markers, respectively. (Scale bar, 10 μm.) (*B*) Coimmunoprecipitation and Western blot analysis of lysates from HeLa cells cotransfected with atlastin (3xlinker)-myc and atlastin(3xlinker)-HA demonstrate that atlastin(3xlinker) retains the ability to oligomerize. L, lysate; S, supernatant; P, pellet.

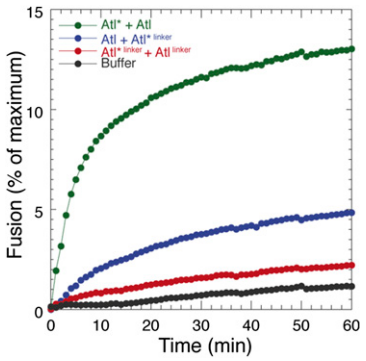

Fig. S8. Membrane fusion mediated by atlasin(1xlinker) is impaired. Fusion reactions performed as described in *Experimental Procedures* (main text), with wild-type atlastin proteoliposomes mixed together, atlastin(1xlinker) proteoliposomes together, or wild-type atlastin proteoliposomes mixed with atlastin(1xlinker) proteoliposomes. Fusion was reduced by ≈68% by having linker on one side and by ≈90% with linker on both sides. Asterisks indicate labeled proteoliposomes.