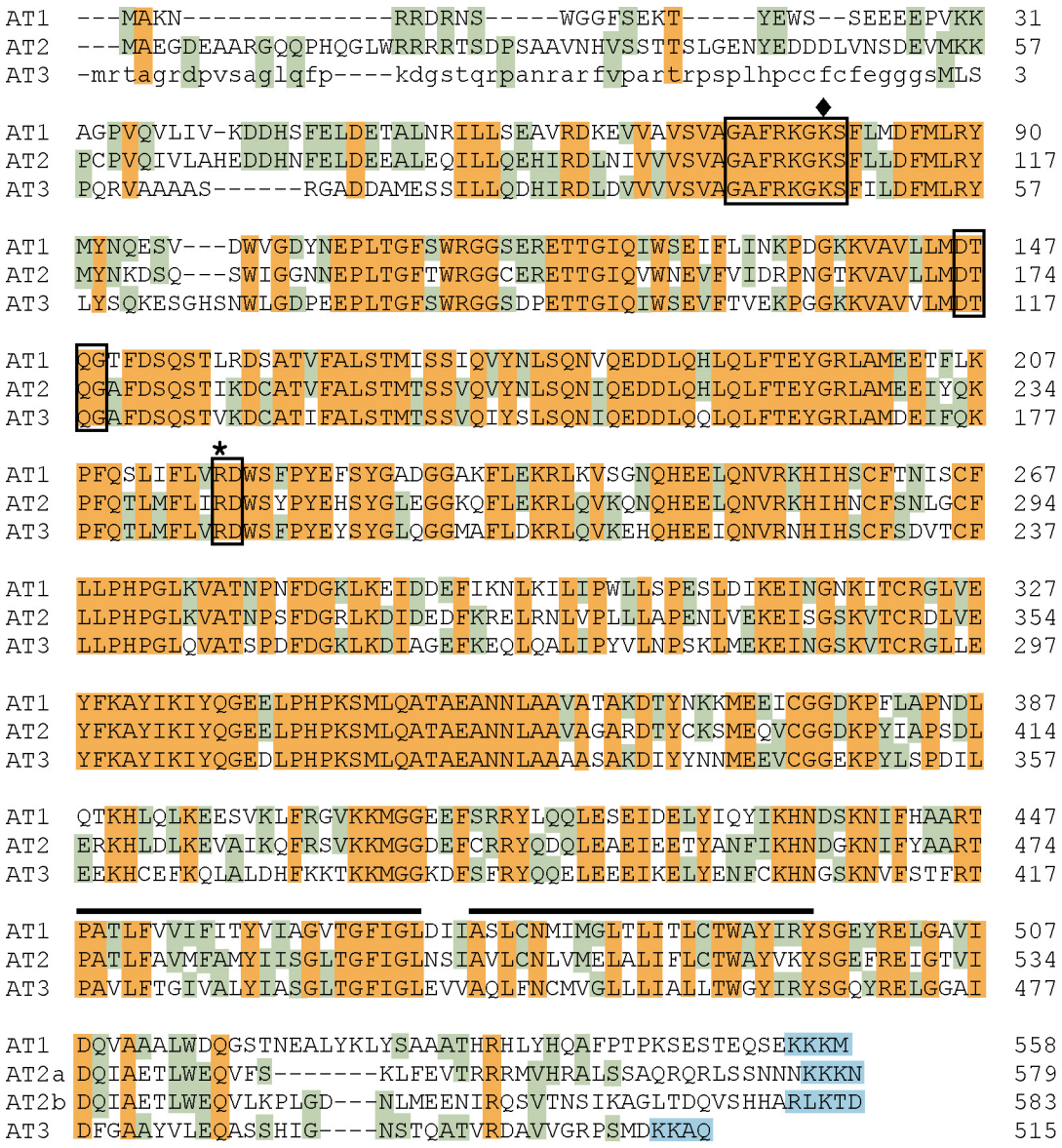
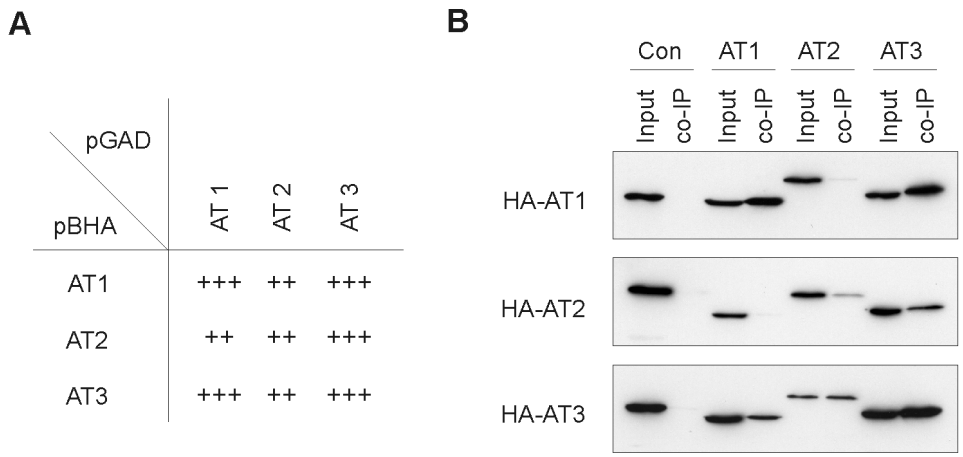


Supplementary Material, Figure S1. Atlastin family of proteins in humans. Residues identical in all three atlastins are highlighted in orange, while those residues identical in at least two of the atlastins (exclusive of different splice variants of the same atlastin) are shaded green. Bold lines are above predicted transmembrane domains. A variant of atlastin-3 with a 26-residue insertion following Ser^{22} (GenBank accession number CAB56010) and a variant of atlastin-1 lacking exon 13 (b isoform; coding for residues 518-522), both generated by alternative splicing, are also known. Numbering for the atlastin-3 start site is based on results shown in Fig. 1C (Trans-S), but additional residues at the N-terminus of atlastin-3 predicted if an alternate start site (Trans-L) were utilized are shown in lower case. GTP-binding motifs are boxed. A filled diamond (♦) denotes the site of the dominant-negative, GTP-binding deficient mutation and an asterisk (*) identifies the site of a *SPG3A* mutation investigated in this study. Potential C-terminal ER retrieval motifs are shaded in blue.



Supplementary Material, Figure S2. Hetero-oligomerization of atlastins upon overexpression. (A) Matrix of yeast two-hybrid tests showing interactions of atlastin-1, -2, and -3 with one another. Strength of interaction was assayed by *HIS3* and *β-galactosidase* induction and semi-quantitated as described previously (35). In all cases, *HIS3* and *β-galactosidase* induction correlated. (B) Co-immunoprecipitation (co-IP) of overexpressed atlastin proteins with one another. HeLa cells were co-transfected with Myc-tagged atlastins (across top) and HA-tagged atlastins (left) in all nine possible combinations and immunoprecipitated with anti-Myc antibodies. Immune complexes were resolved by SDS-PAGE and immunoblotted with anti-HA antibodies. In control experiments (Con), immuno-precipitations were performed on extracts from cells expressing only the HA-tagged constructs. On longer exposures, co-precipitations of HA-AT1 and Myc-AT2 as well as HA-AT1 and Myc-AT2 are apparent.