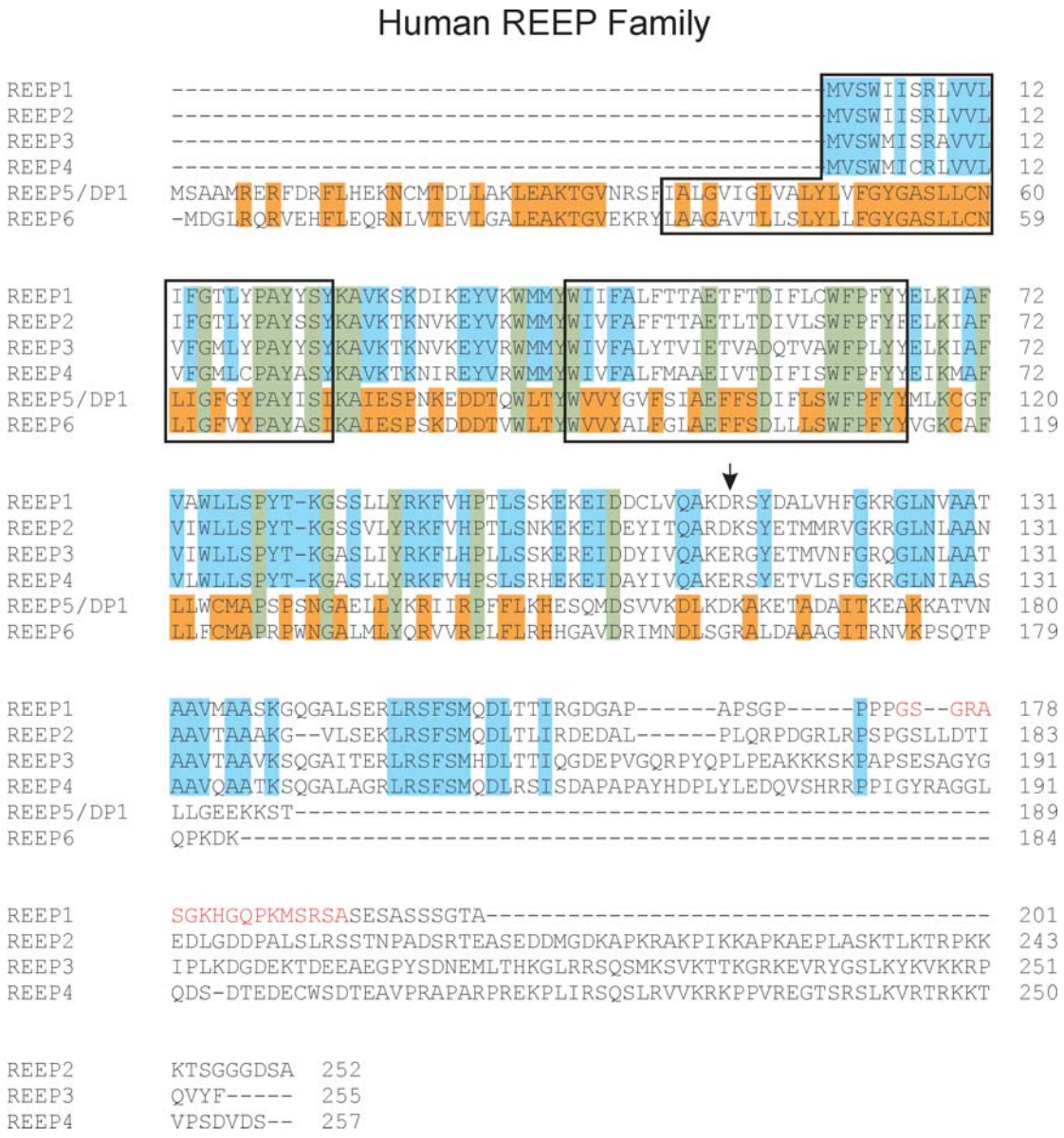
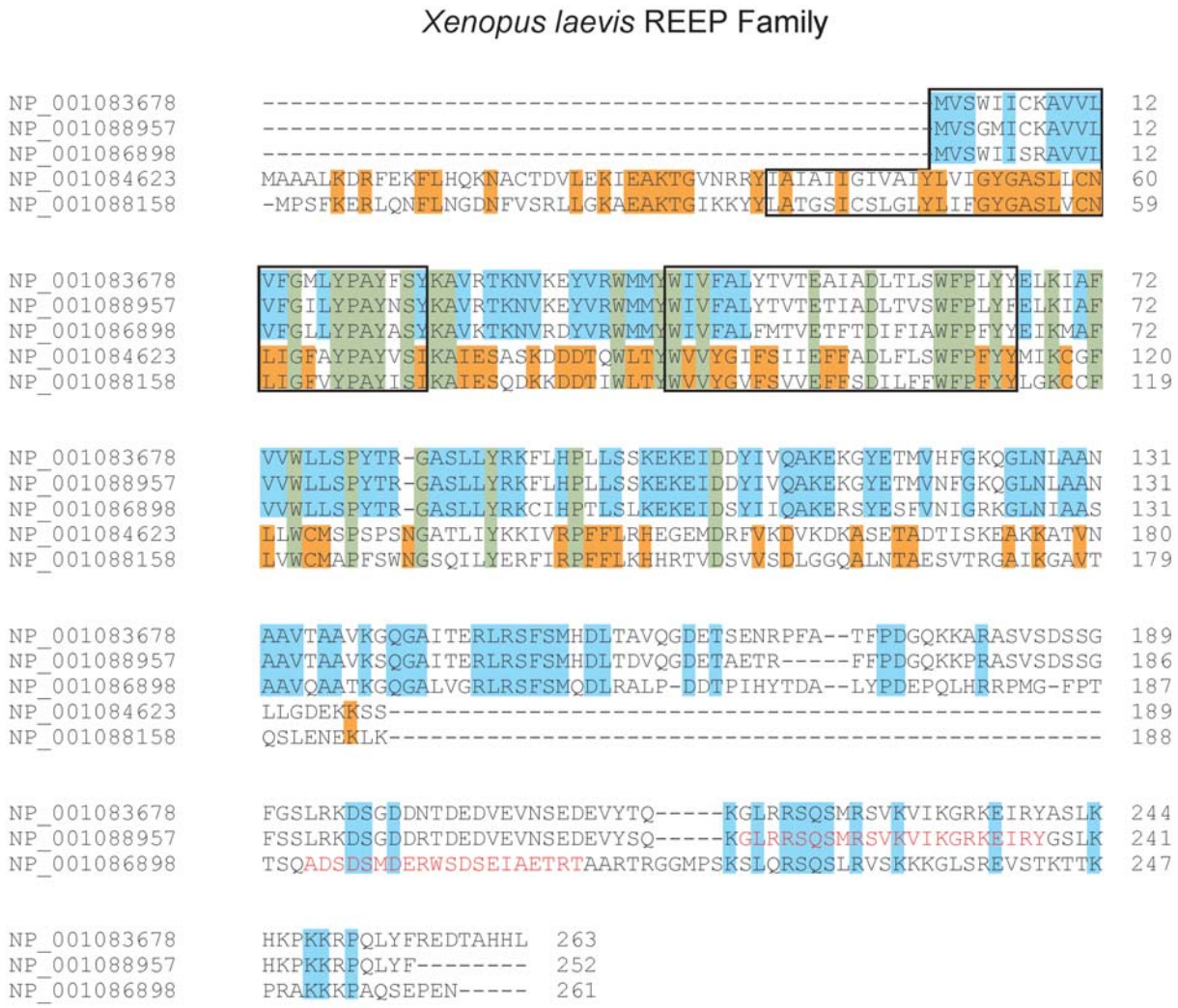
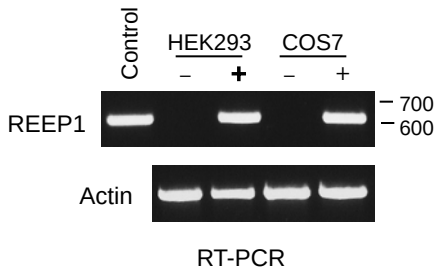


Supplemental Figure 1

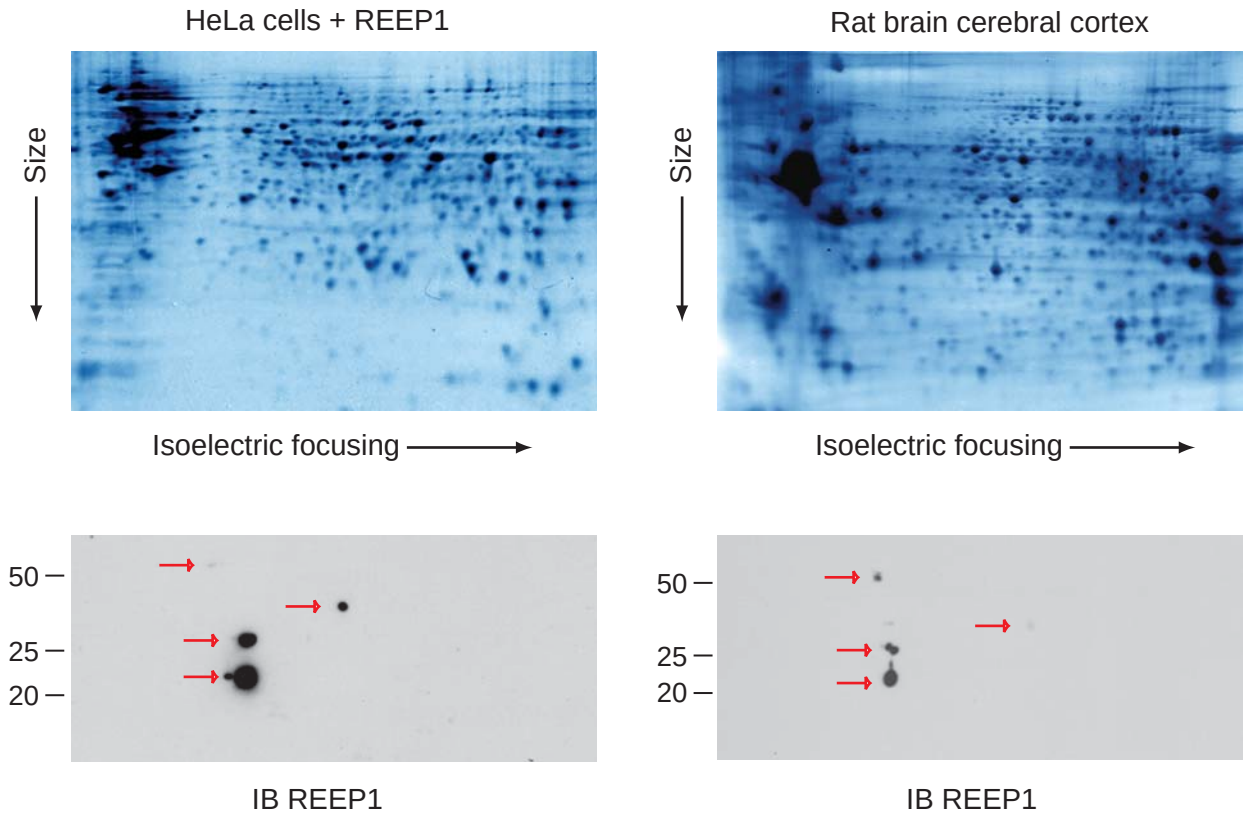
Human REEP family of proteins can be divided into two distinct subfamilies. Residues (single letter amino acid code) identical in all six REEPs are highlighted in green. Additional residues identical in REEP1-4 are in blue, and residues identical in REEP5-6 are in orange. Hydrophobic segments are boxed. An arrow identifies the point of truncation of the SPG31 nonsense mutant REEP1 p.Arg113X investigated in this study. The sequence selected for generation of REEP1-specific anti-peptide antibodies is shown in red. Amino acid numbers are indicated at the right. GenBank protein accession numbers are shown in Figure 1A.



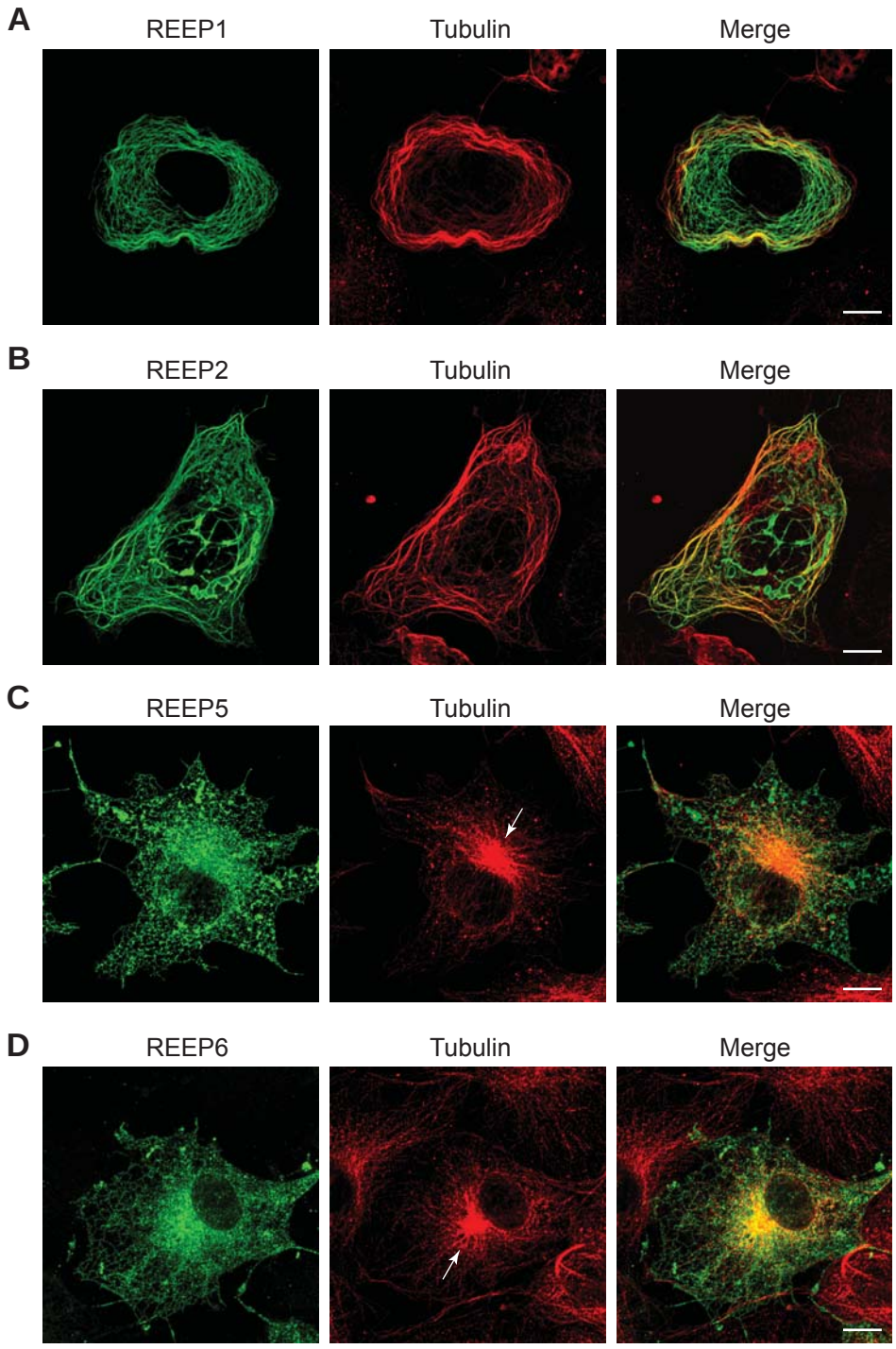
Supplemental Figure 2
REEP family of proteins in *Xenopus* can be divided into two subfamilies. Residues (single letter amino acid code) identical in all five REEPs shown are highlighted in green. Additional residues identical in *Xenopus* proteins related to human REEP1-4 are in blue, and residues identical in *Xenopus* proteins related to REEP5-6 are in orange. Hydrophobic domains are boxed. The sequences selected for generation of anti-REEP antibodies used for in vitro ER network formation assays are shown in red. Amino acid numbers are indicated at the right. GenBank accession numbers are at the left and are also shown in Figure 1A.



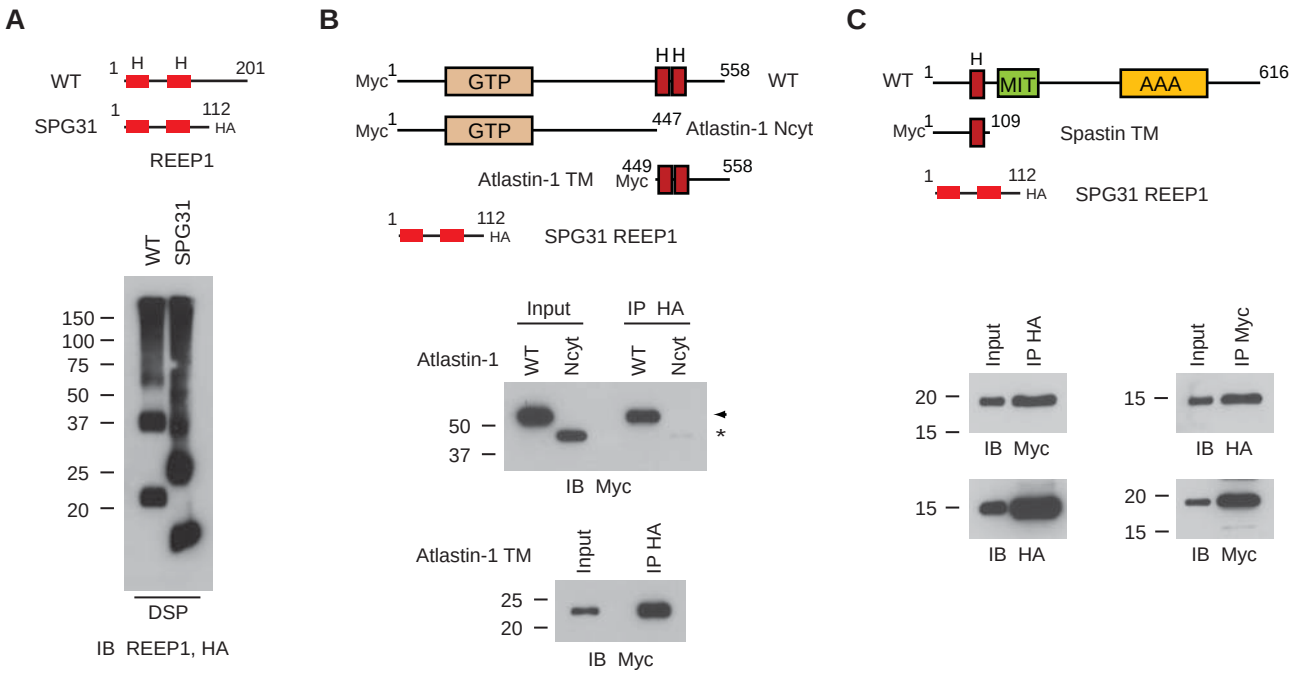
Supplemental Figure 3
RT-PCR analysis of REEP1 mRNA expression in cell lines. Total RNA was extracted using the TRI Reagent (Invitrogen) from both untransfected and REEP1-transfected COS7 and HEK293 cells. RNA was reverse transcribed with the ThermoScript RT-PCR System (Invitrogen) using an Oligo (dT)20 primer. Gene-specific PCR was carried out using primers specific for REEP1 (Forward: 5'-ATGGTGTCATGGATCATCTCC-3' Reverse: 5'-CTAGGCGGTGCCTGAGCTGCTAG-3') or actin (Forward: 5'- GCTCACCATGGATGATGATATCGC-3' Reverse: 5'-GGAGGAGCAATGATCTTGATCTTC -3'). Reactions were subjected to an initial denaturing step of 60 sec at 94°C, followed by 30 cycles of 45 s at 94°C, 60 s at 55°C, and 60 s at 72°C. Full-length cDNA of REEP1 served as a positive control. There is no detectable REEP1 mRNA in untransfected COS7 or HEK293 cells. Size markers (in bp) are to the right.



Supplemental Figure 4
Two-dimensional gel analysis of endogenous rat brain and recombinant human REEP1 expressed in HeLa cells. Top, representative gel staining with 0.1% Coomassie Brilliant Blue G-250 colloidal stain. Bottom, REEP1 immunoblots (IB), with red arrows identifying common immunoreactive protein spots. MW standards (in kDa) are indicated. An immunoblot of a two-dimensional gel resolving untransfected HeLa cell extracts showed no immunoreactive spots (data not shown).

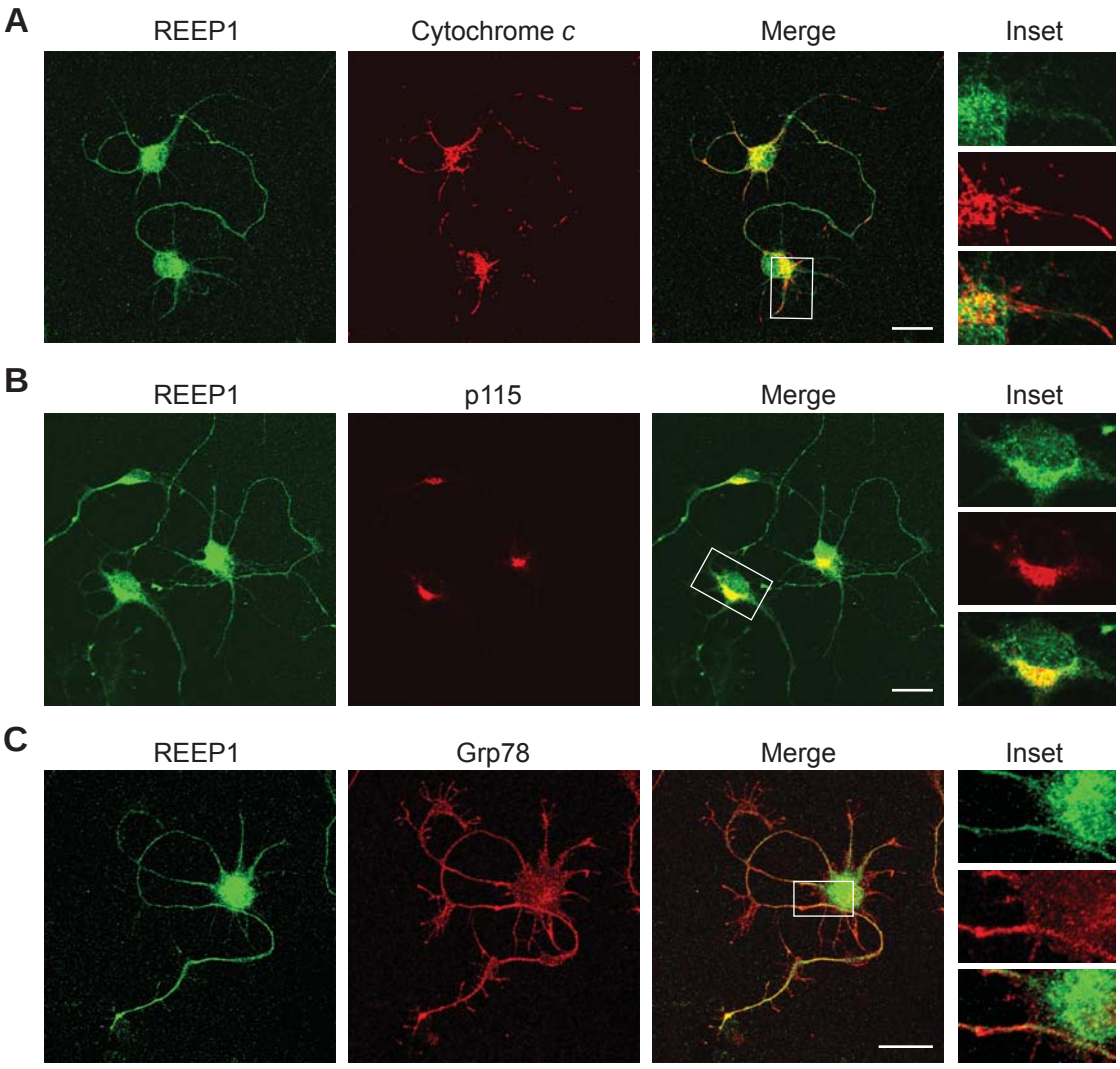


Supplemental Figure 5
REEP1 and REEP2, but not REEP5 and REEP6, are within the tubular ER aligned along bundled microtubules. The indicated REEP proteins tagged with HA-epitope at the C-terminus were expressed in COS7 cells and costained for HA-epitope (green) and β-tubulin (red). Merged images are to the right. Note the formation of thickened and bundled microtubules aligned with the ER in REEP1 (A) and REEP2 (B) expressing cells, but not in cells expressing REEP5 (C) and REEP6 (D). The MTOC is only clearly visible in cells expressing REEP5 and REEP6 (arrows in C and D). Scale bars: 10 μm.

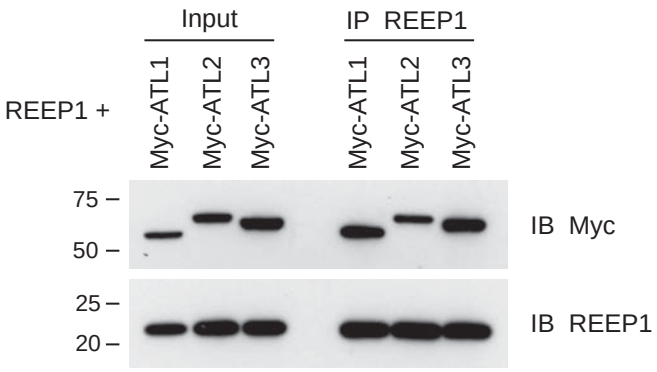


Supplemental Figure 6

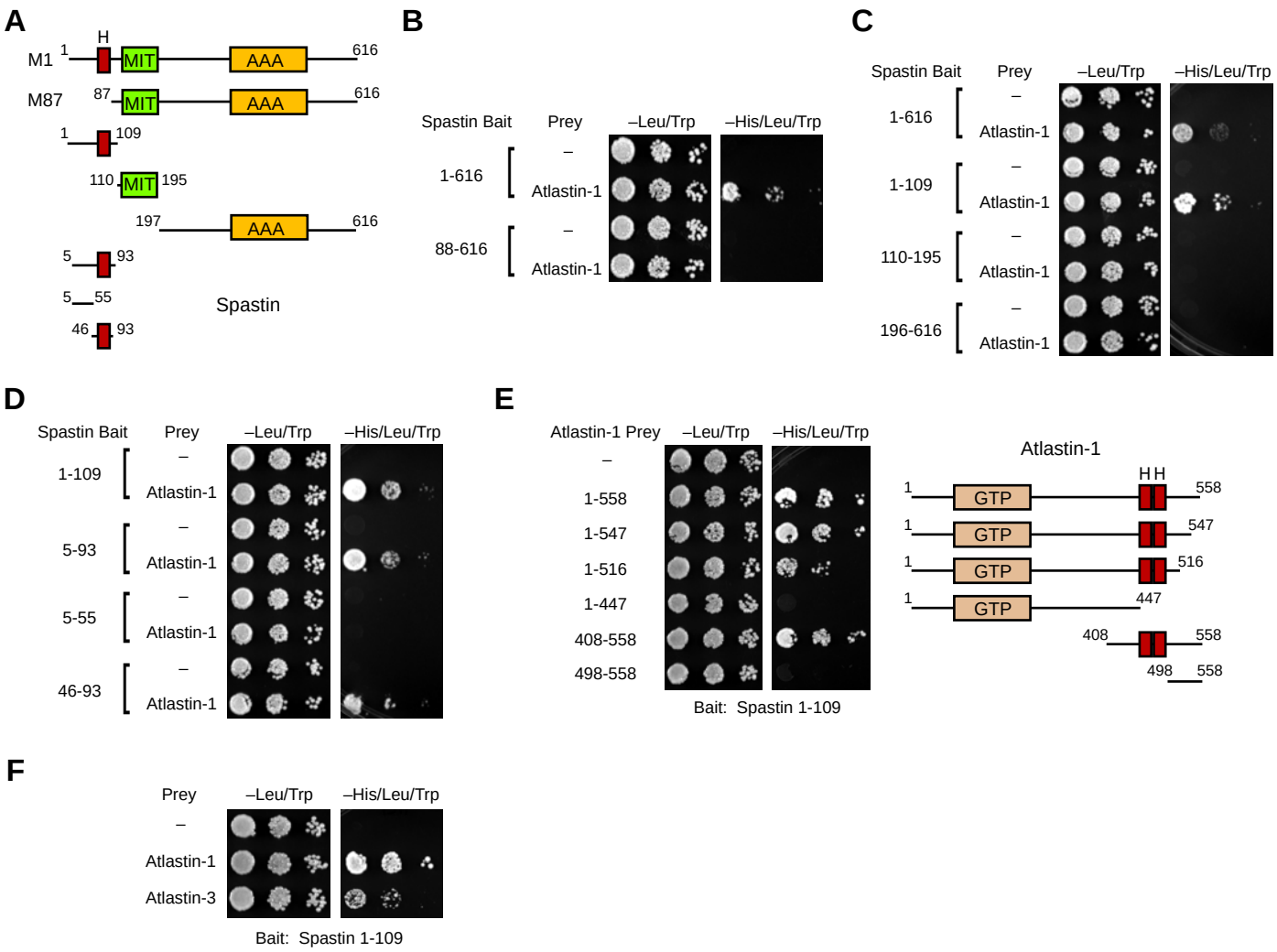
SPG31 mutant REEP1 is oligomeric and interacts with both M1 spastin and atlastin-1. (A) Similar oligomerization of WT and SPG31 mutant (Mut) REEP1, with immunodetection using anti-REEP1 and HA-epitope antibodies, respectively. Schematic diagrams of the constructs are shown at the top. (B) Domain mapping of atlastin-1 interaction with mutant REEP1. The indicated Myc-atlastin-1 constructs, with boundary amino acid residues shown (top), were co-expressed with REEP1, and cell extracts were immunoprecipitated (IP) with anti-HA antibodies. Immunoprecipitates were immunoblotted (IB) for Myc-epitope. Arrowheads identify co-immunoprecipitated WT atlastin-1, and an asterisk (*) shows lack of co-precipitation of mutant REEP1 with atlastin-1 Ncyt which lacks the hydrophobic domains. SPG31 mutant REEP1 co-precipitates with atlastin-1 TM that harbors the membrane-spanning segments (bottom).
(C) Mutant REEP1 co-immunoprecipitates with spastin TM that harbors the hydrophobic membrane-spanning segment. Schematic diagrams of the constructs are shown at the top. Myc-spastin TM was co-expressed with SPG31 mutant REEP1 and immunoprecipitated (IP) with anti-HA-epitope or anti-Myc-epitope antibodies. Immunoprecipitates were immunoblotted for Myc-epitope and HA-epitope. MW standards (in kDa) are to the left in all panels.



Supplemental Figure 7
Endogenous REEP1 localizes to ER in cultured rat cerebral cortical neurons. (A-C) REEP1 (green) colocalizes with the ER-marker Grp78 (A) and is enriched in the vicinity of the vesicular tubular complexes and *cis*-Golgi apparatus within the neuronal soma as identified by co-staining for p115 (B; red). REEP1 (green) does not localize to mitochondria, which are labeled with antibodies to cytochrome c (C; red). Boxed areas are enlarged to the right. An area of Grp78 enrichment in the soma is identified with an arrow. Scale bars: 20 μm.

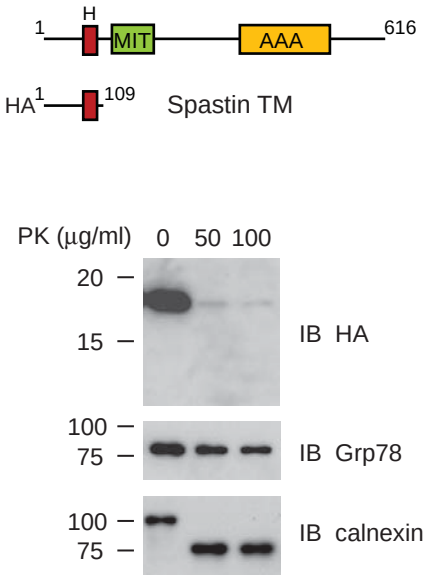


Supplemental Figure 8
REEP1 interacts with all known human atlastin proteins. REEP1 was coexpressed with the indicated Myc-tagged atlastin proteins (ATL1-3), and cell extracts were immunoprecipitated (IP) with anti-REEP1 antibodies. Immunoprecipitates were immunoblotted (IB) for Myc-epitope and REEP1 as indicated. MW standards (in kDa) are shown.

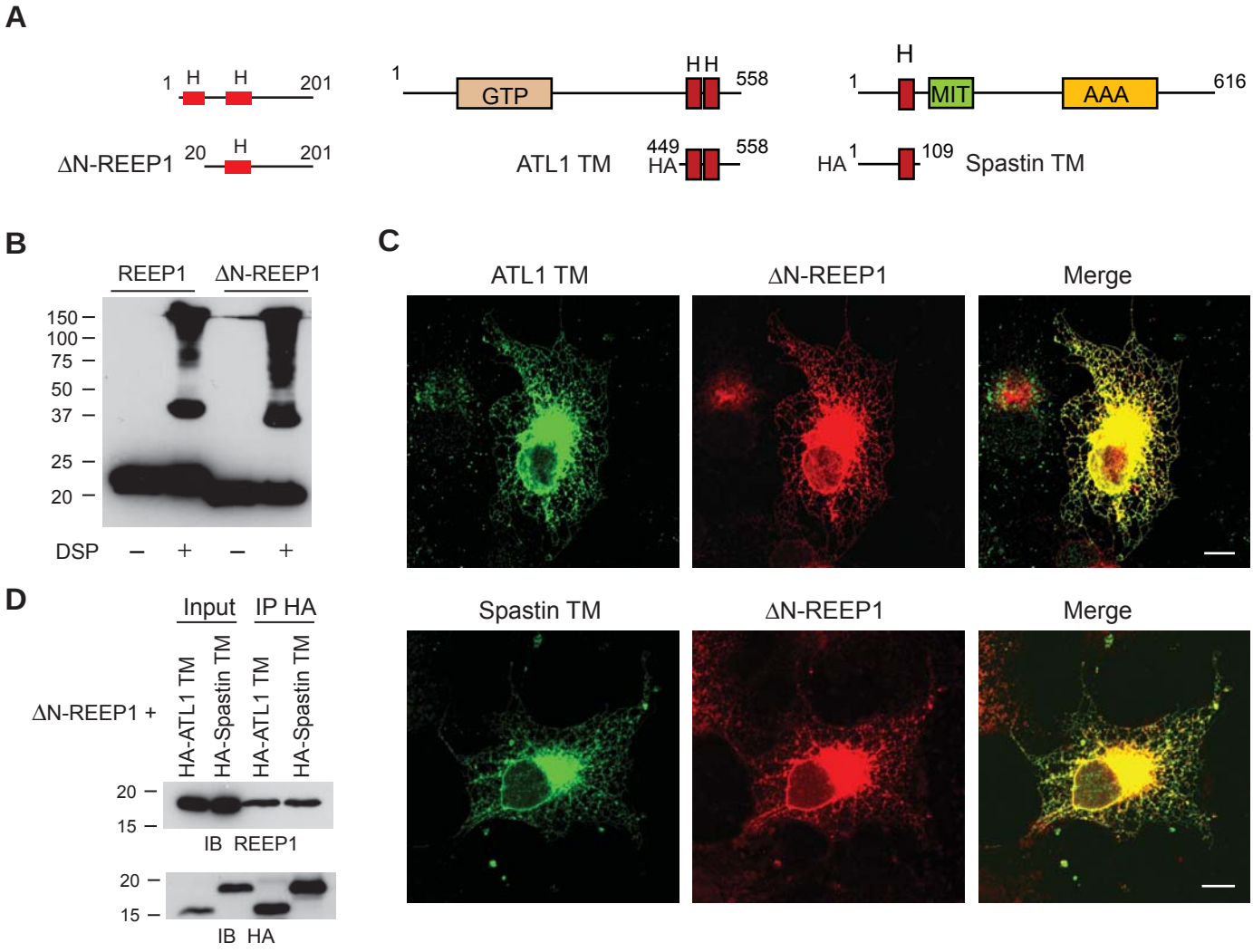


Supplemental Figure 9

Atlastin-1 binds to the hydrophobic segment of M1 spastin. (A) Schematic diagram of spastin constructs used, with boundary amino acid residues indicated. H, hydrophobic segment; MIT, present in microtubule-interacting and trafficking proteins; AAA, ATPases associated with diverse cellular activities domain. (B) Yeast two-hybrid interactions of M1 (aa 1-616) and M87 (aa 88-616) spastin baits with full-length atlastin-1 or control (empty pGAD10 vector) prey were assayed using the *HIS3* reporter (–His/Leu/Trp), with ten-fold sequential yeast dilutions shown. The –Leu/Trp panel demonstrates equal transformation efficiencies for the constructs. (C and D) Yeast two-hybrid tests of the indicated spastin baits with atlastin-1 or control prey. (E) Yeast two-hybrid tests of the spastin (1-109) bait with the indicated control or atlastin-1 deletion prey, with schematic representations of each construct directly to the right. Hydrophobic segments (H) and GTP-binding domain (GTP) as well as boundary amino acid residues are indicated. (F) Yeast two-hybrid tests of the spastin (aa 1-109) bait with control, atlastin-1, or atlastin-3 prey. We attempted to use various REEP1 constructs as both baits and prey for similar studies, but some REEP1 baits exhibited self-activation with control prey, and the others did not exhibit specific interactions with spastin, atlastin-1, or REEP1 itself (data not shown).



Supplemental Figure 10
Membrane topology of spastin TM. The indicated concentrations of proteinase K (PK) were added to intact microsomes from COS7 cells expressing M1 spastin residues 1-109 tagged with HA-epitope at the N-terminus (spastin TM). Aliquots were immunoblotted (IB) for HA-epitope as well as the endogenous ER luminal protein Grp78 and the endogenous ER membrane-spanning protein calnexin. The HA-epitope is proteolyzed, indicating that it is cytoplasmic. MW standards (in kDa) are indicated. A schematic diagram of the spastin TM construct is shown at the top. H, hydrophobic segment; MIT, present in microtubule-interacting and trafficking proteins; AAA, ATPases associated with diverse cellular activities domain.



Supplemental Figure 11

First hydrophobic segment of REEP1 is required for microtubule interaction in cells but not for interaction with atlastin-1 or M1 spastin. (A) Schematic diagrams of the constructs used. H, hydrophobic segment; MIT, present in microtubule-interacting and trafficking proteins; AAA, ATPases associated with diverse cellular activities domain. (B) Oligomerization of ΔN-REEP1. Membranes from WT and ΔN-REEP1-expressing cells were cross-linked with DSP and resolved by SDS-PAGE on non-reducing gels. MW standards (in kDa) are indicated. (C) ΔN-REEP1 (red) was co-expressed with HA-atlastin-1 TM or HA-spastin TM (green) and identified in cells using confocal immunofluorescence microscopy. ΔN-REEP1 colocalizes with both proteins, as shown in the merged images. Scale bars: 10 μm. (D) ΔN-REEP1 interacts with spastin TM and atlastin-1 TM. ΔN-REEP1 was co-expressed with HA-spastin TM or HA-atlastin-1 TM , and cell extracts were immunoprecipitated (IP) with anti-HA-epitope antibodies. Immunoprecipitates were immunoblotted (IB) for REEP1 and HA-epitope. MW standards (in kDa) are indicated.