Supporting Information

Chang et al. 10.1073/pnas.1307391110

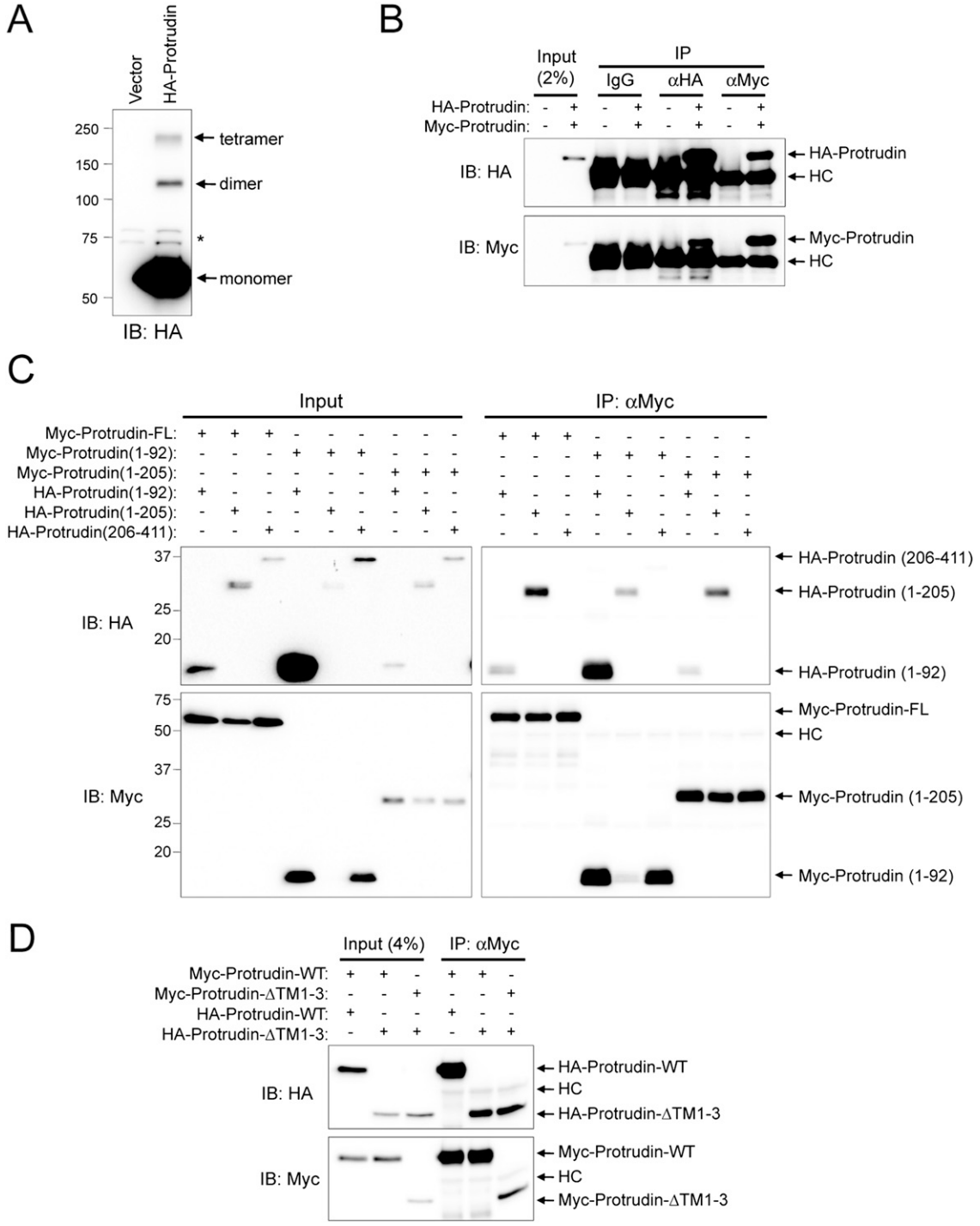

Fig. S1. Protrudin is an oligomeric protein. (*A*) HEK293T cells were transfected with vector and HA-protrudin constructs, and cell lysates were immunoblotted for HA-epitope. An asterisk (*) identifies probable cross reacting bands. (*B*) HA- and Myc-tagged protrudin were coexpressed in HEK293T cells. Lysates were immunoprecipitated (IP) and immunoblotted (IB) with the indicated antibodies. (*C*) HA- and Myc-tagged full-length or truncated protrudin proteins were coexpressed in cells, and lysates were immunoprecipitated and immunoblotted. (*D*) HA- and Myc-tagged full-length or membrane domain (TM)-lacking protrudin proteins were coexpressed, and lysates were immunoprecipitated and immunoblotted as shown. HC, IgG heavy chain. Migrations of molecular-mass standards (in kilodaltons) are to the left in *A* and *C*.

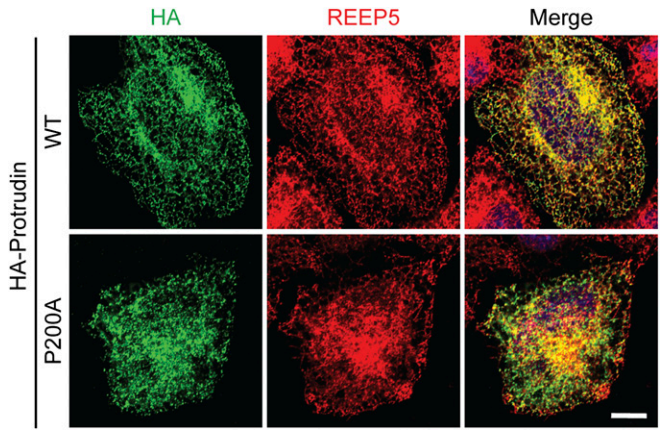

Fig. S2. Pro200 is important for the reticular endoplasmic reticulum (ER) localization of protrudin. HA-tagged wild-type (WT) and P200A mutant protrudin were expressed in HeLa cells and immunostained for HA (green) and REEP5 (red). Merged images are to the right. (Scale bar: 10 μm.)

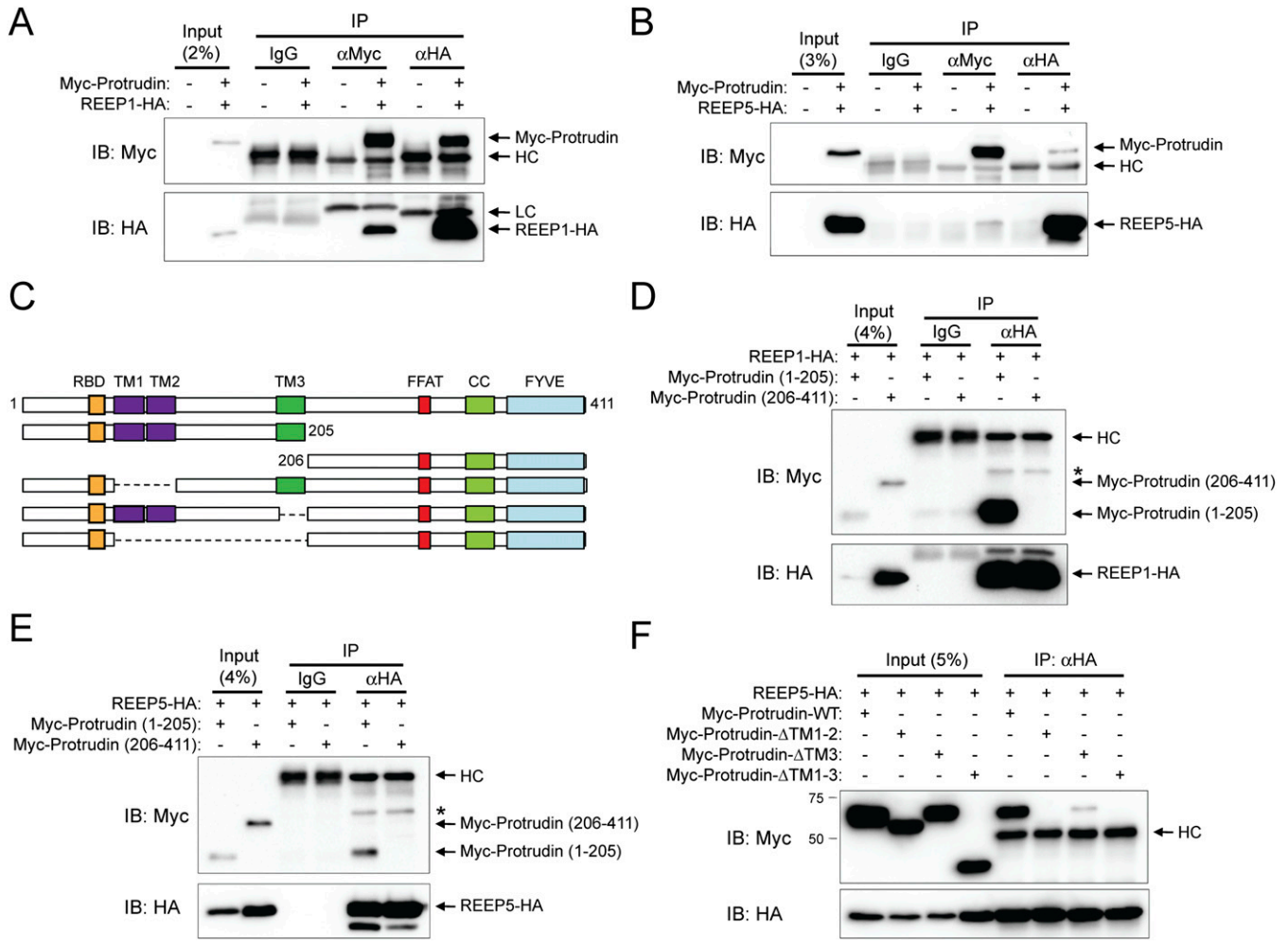

Fig. S3. Protrudin associates with ER-shaping receptor expression-enhancing proteins (REEPs). (*A* and *B*) Myc-tagged protrudin was coexpressed with REEP1-HA (*A*) or REEP5-HA (*B*) in HEK293T cells, and lysates were immunoprecipitated (IP) and immunoblotted (IB) with the indicated antibodies. (*C*–*E*) N terminus of protrudin containing all membrane domains (TM) or C terminus lacking the TM domains (*C*) were coexpressed with REEP1-HA (*D*) or REEP5-HA (*E*), and lysates were immunoprecipitated and immunoblotted as shown. (*F*) Wild-type (WT) protrudin or deletion mutants lacking the indicated TM domains (*C*) were coexpressed with REEP5-HA, and lysates were immunoprecipitated and immunoblotted. HC, IgG heavy chain; LC, IgG light chain. Asterisks (*) denote cross-reacting bands.

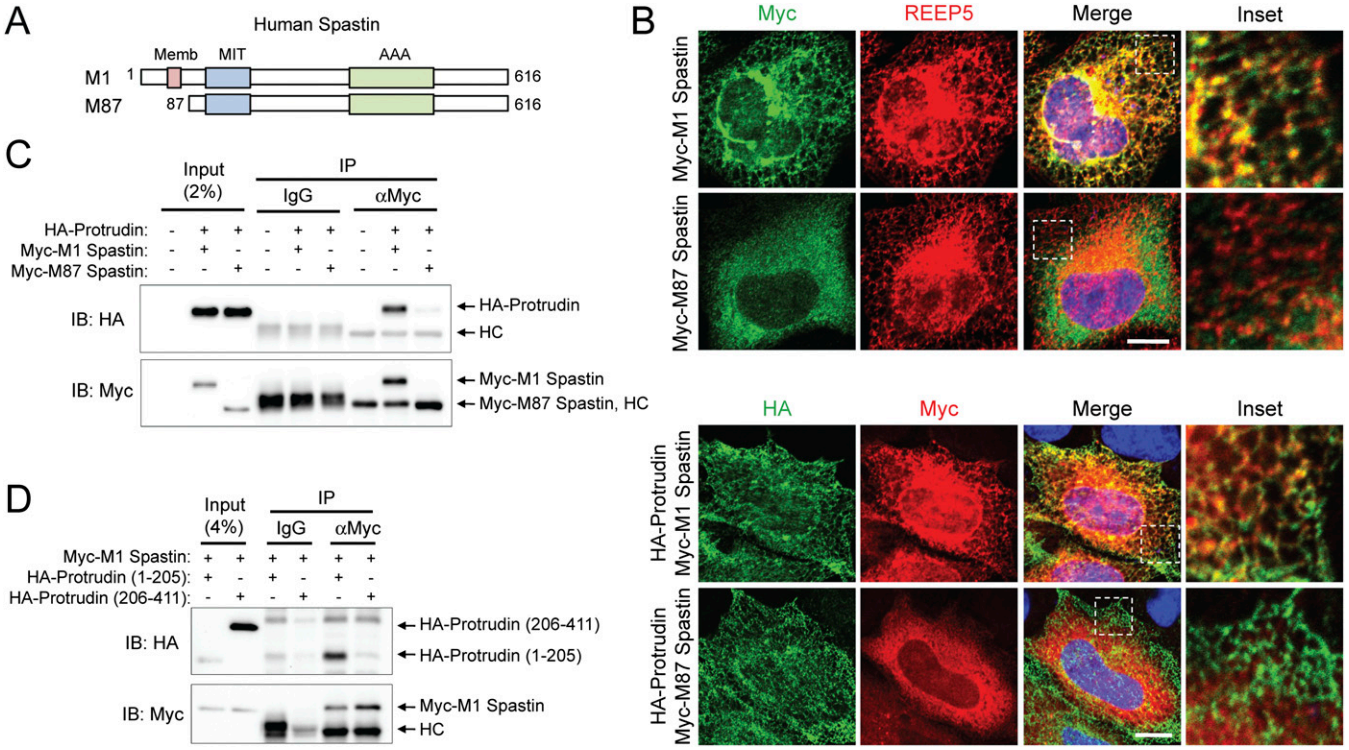

Fig. S4. Protrudin associates selectively with the M1 spastin isoform. (*A*) Schematic diagram showing two major forms of human spastin protein. (*B*, *Upper*) Myc-tagged M1 or M87 spastin-expressing HeLa cells were immunostained for Myc-epitope (green) and endogenous REEP5 (red). (*B*, *Lower*) HA-protrudin was coexpressed with Myc-tagged M1 or M87 spastin, and cells were immunostained for HA- (green) and Myc-epitopes (red). *Insets* in the merged images (with DAPI nuclear staining) are enlarged to the far right. (*C*) HA-protrudin was coexpressed with Myc-tagged M1 or M87 spastin in HEK293T cells, and lysates were immunoprecipitated (IP) and immunoblotted (IB) with the indicated antibodies. (*D*) Myc-tagged M1 spastin was coexpressed with N-terminal (1–205) or C-terminal (206–411) fragments of protrudin, tagged with HA-epitope; lysates were immunoprecipitated and immunoblotted as shown. AAA, ATPases associated with a variety of cellular activities; HC, IgG heavy chain; MIT, present in microtubule-interacting and trafficking molecules. (Scale bars: 10 μm.)

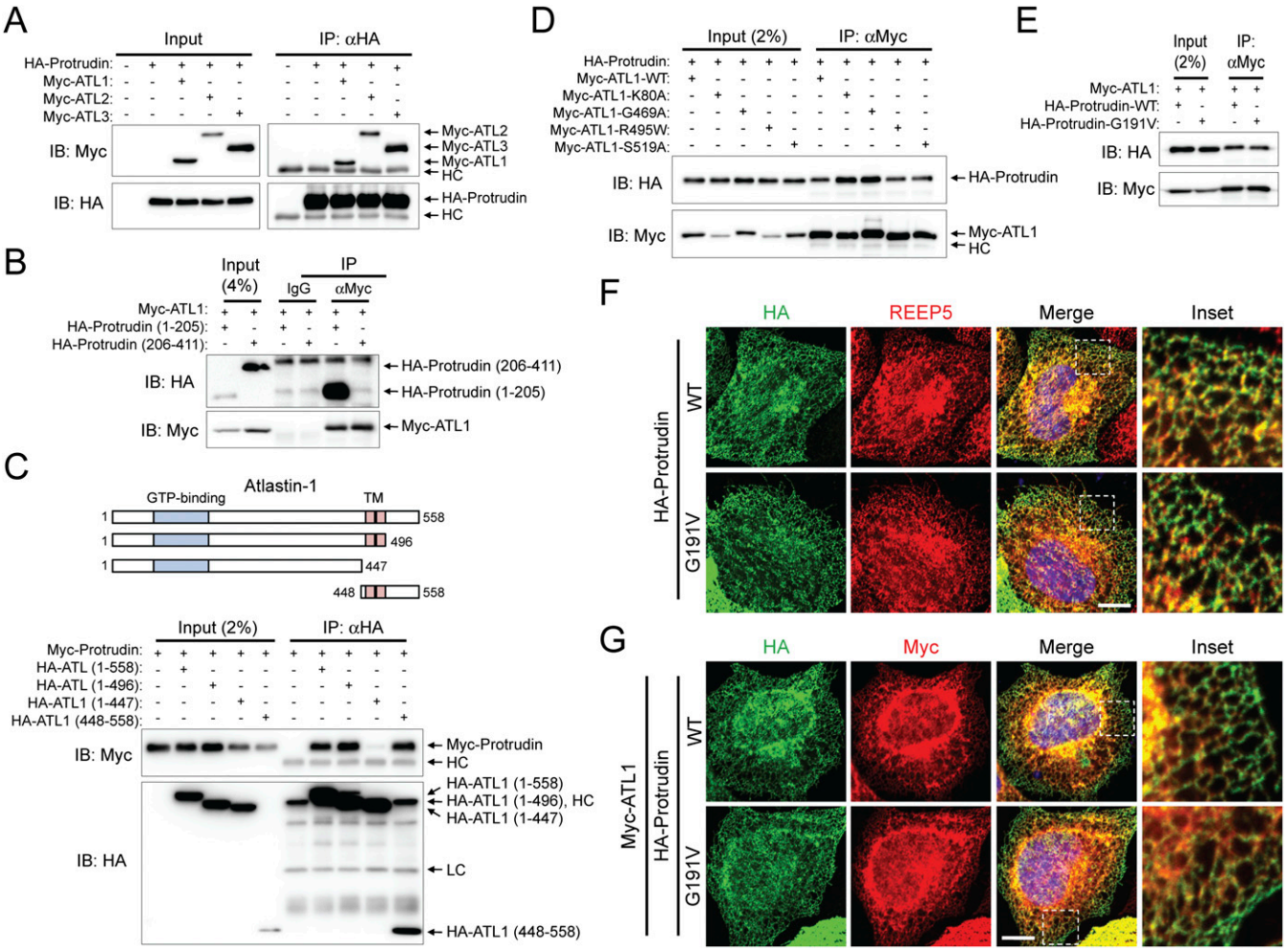

Fig. S5. Protrudin associates with atlastins. (*A*) HA-protrudin was coexpressed with Myc-tagged atlastin-1, atlastin-2, or atlastin-3 in HEK293T cells, and cell lysates were immunoprecipitated (IP) and immunoblotted (IB) with indicated antibodies. (*B*) Myc–atlastin-1 was coexpressed with either the N-terminal (1–205) or C-terminal (206–411) portion of protrudin, and lysates were immunoprecipitated and immunoblotted. (*C* and *D*) HA-protrudin was coexpressed with the indicated Myc-tagged truncation (*C*) or missense mutants (*D*) of atlastin-1, and lysates were immunoprecipitated and immunoblotted as shown. (*E*) Myc–atlastin-1 was coexpressed with HA-tagged wild-type (WT) or SPG33 mutant (G191V) protrudin, and lysates were immunoprecipitated and immunoblotted. (*F*) HeLa cells expressing HA-tagged wild-type or G191V mutant protrudin were immunostained for HA-epitope (green) and endogenous REEP5 (red). (*G*) Myc–atlastin-1 was coexpressed with HA-tagged wild-type or G191V mutant protrudin, and cells were immunostained for HA- (green) and Myc- (red) epitopes. *Insets* in the merged images (with DAPI staining of nuclei) in *F* and *G* are enlarged to the right. ATL, atlastin; HC, IgG heavy chain; LC, IgG light chain. (Scale bars: 10 μm.)

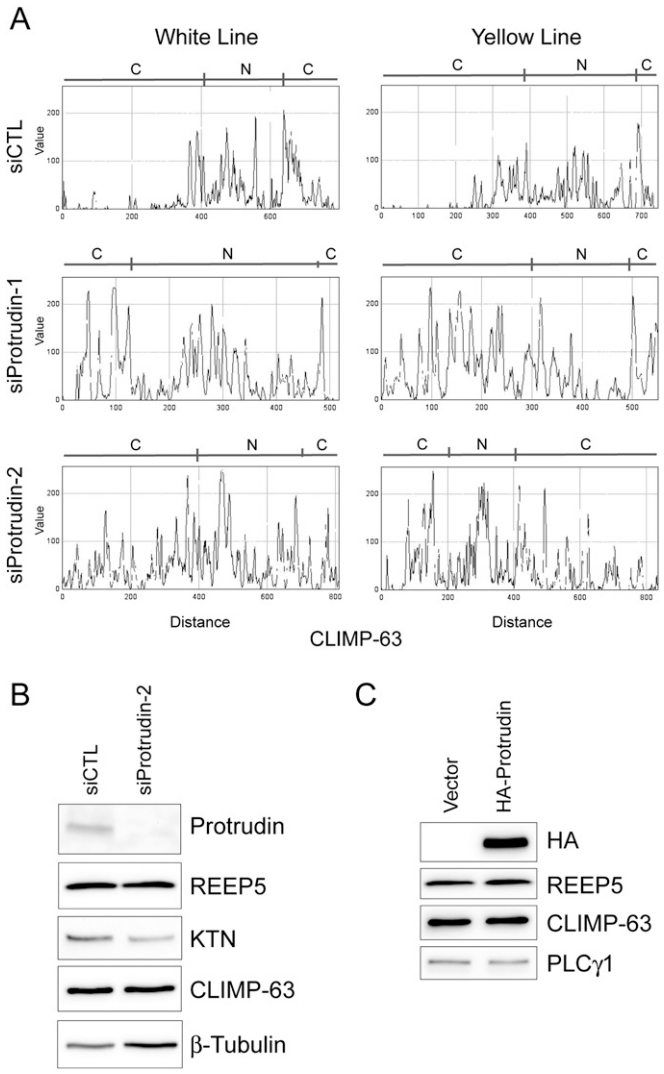

Fig. S6. Protrudin modulates the ER sheet-to-tubule balance without changing levels of ER sheet and tubule proteins. (*A*) Line-scan plots associated with Fig. 3*B*. Line-intensity measurement tools (ImageJ plug in) were used to measure the distributions CLIMP-63 signals in the representative images. C, cytoplasm; N, nucleus. (*B*) HeLa cells were transfected with control (siCTL) or protrudin-2 siRNAs for 72 h, and cell lysates (10 μg protein per lane) were immunoblotted with the indicated antibodies. (*C*) HeLa cells were transfected with vector and HA-protrudin, and cell lysates were immunoblotted with the indicated antibodies (10 μg protein per lane). KTN, kinectin.

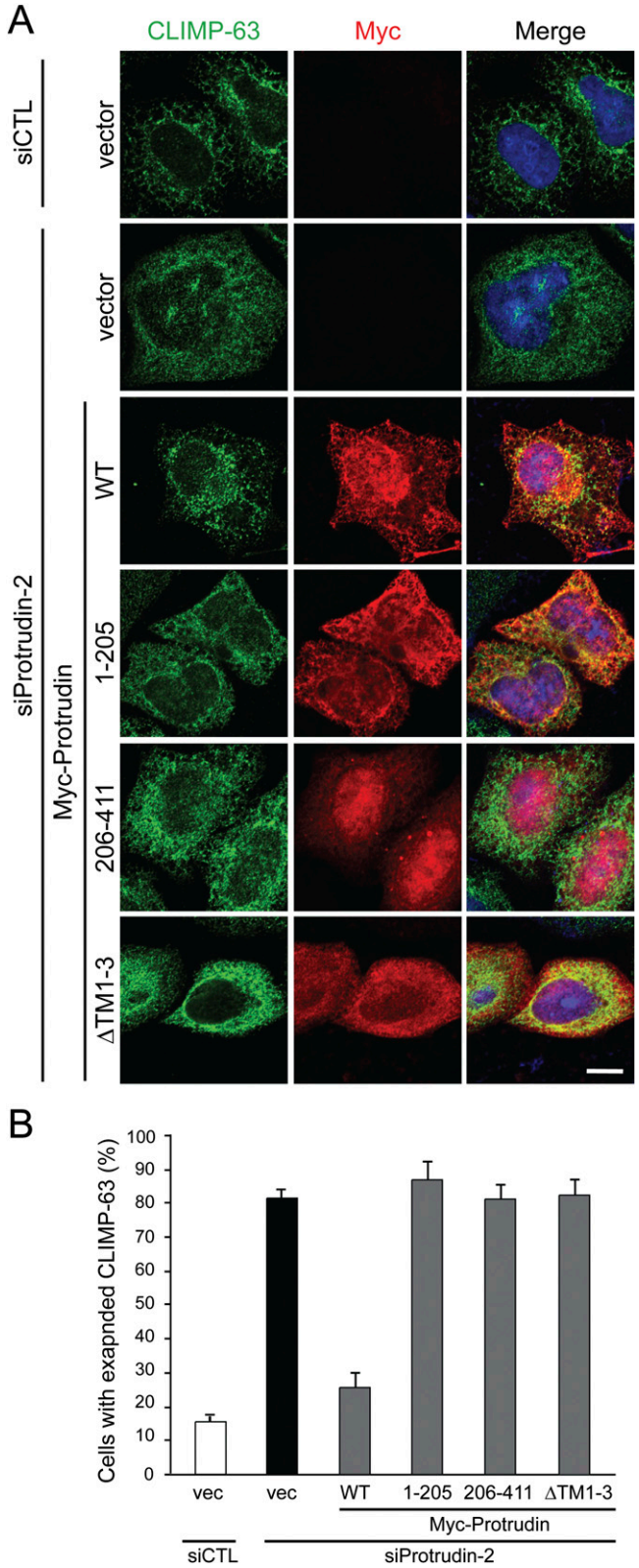

Fig. S7. Suppression of ER-morphology phenotype in protrudin-depleted cells requires multiple protrudin domains. HeLa cells were transfected with control (siCTL) or protrudin-specific siRNAs and subsequently with the indicated Myc-protrudin constructs. Cells were immunostained for CLIMP-63 (green) and Myc-epitope (red) (*A*), with merged images with DAPI nuclear staining to the right, and numbers of cells with expanded CLIMP-63 signals were quantified ($n = 3$; >200 cells/experiment) (*B*). Means ± SD are shown. (Scale bar: 10 μm.) WT, wild-type.

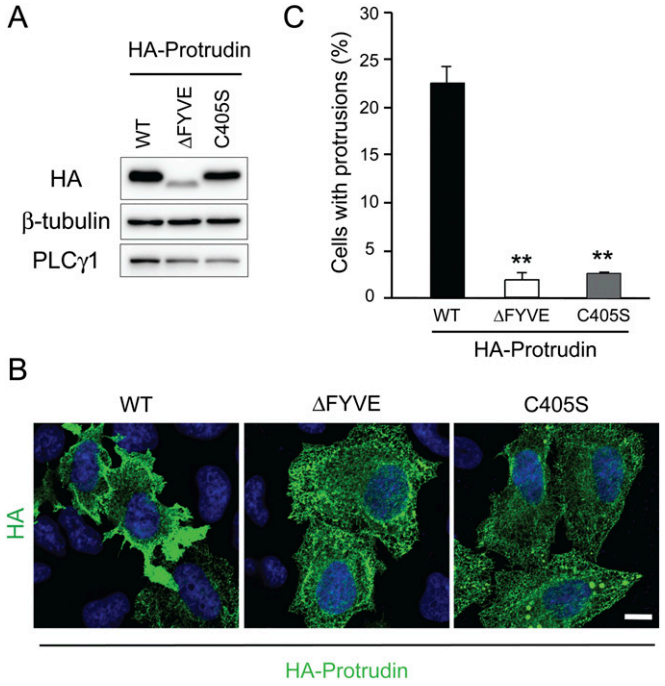

Fig. S8. FYVE domain mutations impair protrudin induction of cellular protrusions. (*A*) HeLa cells were transfected with wild-type (WT) or else ΔFYVE and C405S mutant protrudin constructs, and cell lysates were subjected to immunoblotting with the indicated antibodies. (*B* and *C*) Cells expressing protrudin proteins as in *A* were immunostained for HA-epitope (green), and the number of cells with protrusions was quantified. DAPI stained the nucleus (blue). Means ± SD are shown. Paired Student *t* test: $^{**}P < 0.01$. (Scale bar: 10 μm.)

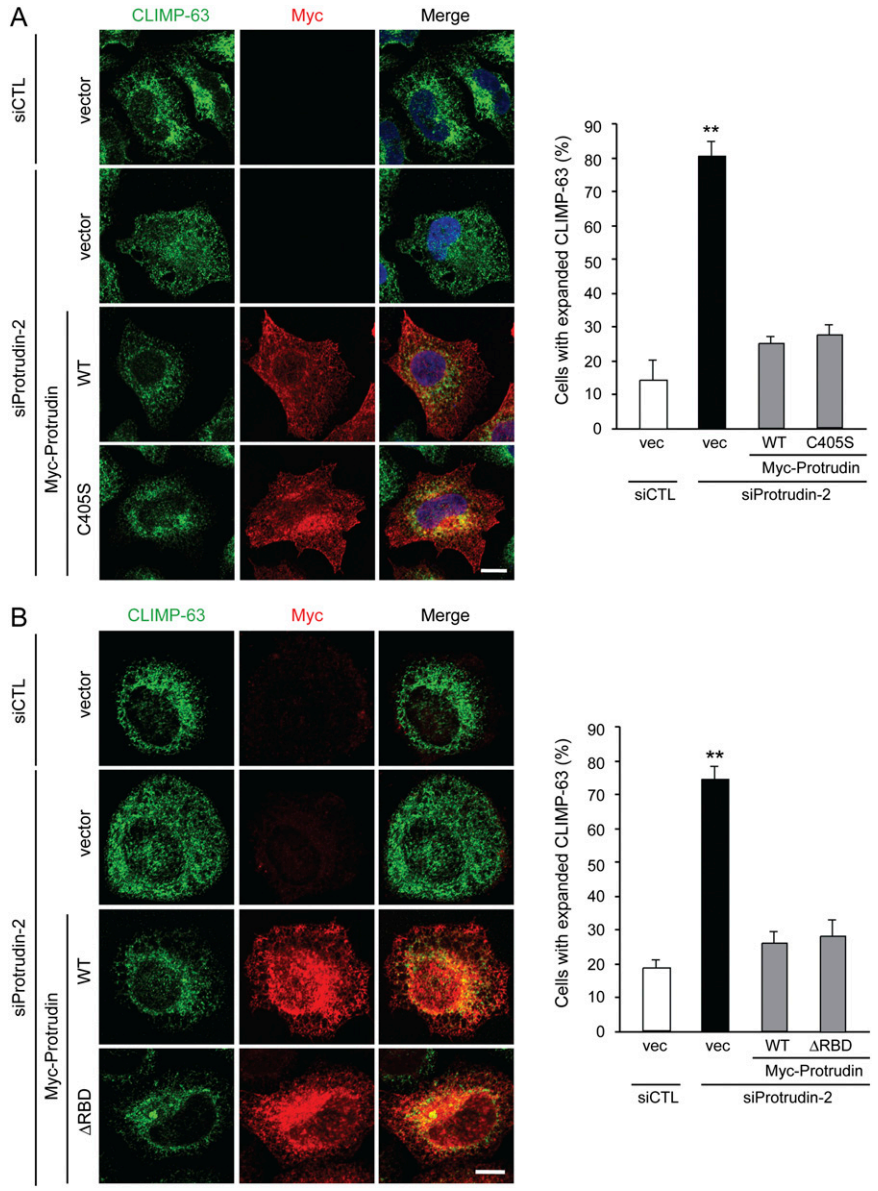

Fig. S9. Protrudin harboring either a mutation in the FYVE domain or lacking the Rab-binding domain (ΔRBD; Δ51–71) can rescue the protrudin siRNA-induced ER phenotype. (*A* and *B*, *Left*) HeLa cells were transfected with control (siCTL) or protrudin-specific siRNA and subsequently with the indicated Myc-protrudin constructs or empty vector (vec), and then immunostained for CLIMP-63 (green) and Myc-epitope (red). Merged images also show DAPI nuclear staining (blue) in *A*. (*A* and *B*, *Right*) Percentages of cells with expanded CLIMP-63 signal area were quantified ($n = 3$; >200 cells per experiment) and presented graphically. Means ± SD are shown. Paired Student *t* test: $^{**}P < 0.01$. (Scale bar: 10 μm.) RBD, Rab-binding domain; WT, wild-type.