Yan et al., http://www.jcb.org/cgi/content/full/jcb.201502078/DC1

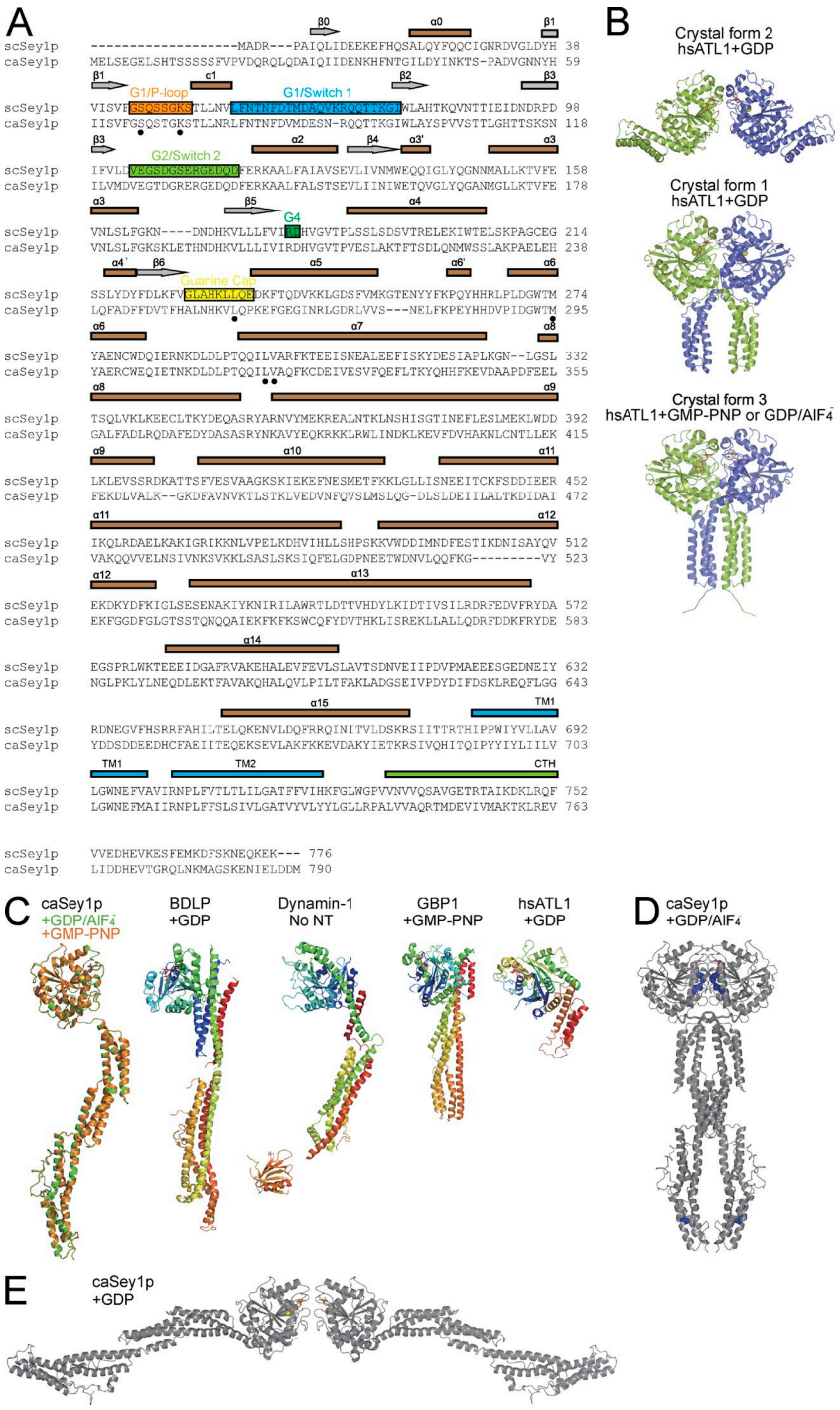

Figure S1. **Comparison of Sey1p and other dynamin-like proteins.** (A) *C. albicans* Sey1p (caSey1p) and *S. cerevisiae* Sey1p (scSey1p) were aligned. Predicted secondary structural elements, with α helices shown in bars and β strands given in arrows, and GTPase motifs of scSey1p are highlighted. Residues mutated in this paper are indicated by dots. TM, transmembrane domain; CTH, predicted C-terminal amphipathic helix. Residues important for hydrolysis and dimerization are indicated by black dots. (B) Human ATL1 dimers observed in crystal form 1 (PDB code: 3QNU, middle, GDP bound), form 2 (PDB code: 3QOF, top, GDP bound in the presence of inorganic phosphate), and form 3 (PDB code: 4IDP, bottom, GMP-PNP bound or GDP/AlF_4^- bound) are shown. The protomers in the dimer are shown in lime color and slate cartoon representation. Nucleotides are shown as orange sticks, and magnesium ions are shown as yellow spheres. (C) Structures of caSey1p in complex with GMP-PNP or GDP/AlF_4^- are compared with other dynamin-like proteins, including GDP-bound BDLP (PDB code: 2W6D), nucleotide-free dynamin-1 (PDB code: 3ZVR), GMP-PNP–bound GBP1 (PDB code: 1F5N), and GDP-bound ATL1 (PDP code: 3QOF). All structures of dynamin superfamily members are shown in rainbow-colored cartoon representation. (D) Mutations of RHD3 are mapped into caSey1p dimer structure (blue spheres). (E) Crystallographic dimer of GDP-bound caSey1p. GDP is shown as orange sticks, and magnesium ions are shown as yellow spheres.

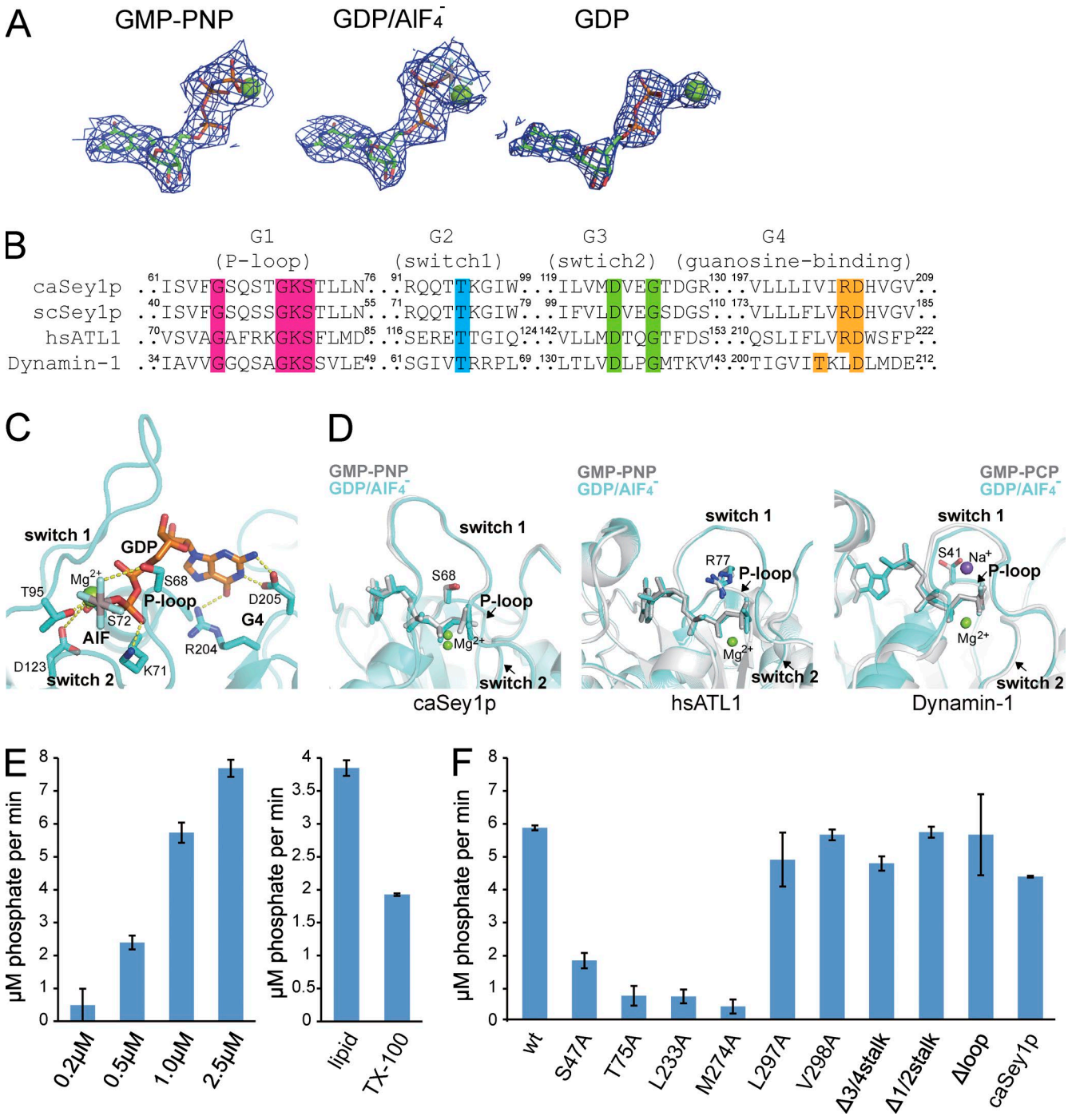

Figure S2. **The GTPase of Sey1p.** (A) The $2F_o - F_c$ electron density maps (1.6σ contour) are shown as wire mesh (blue) with the refined atomic model of the nucleotides superimposed. Magnesium ions are shown as green spheres. (B) Sequence alignment of the conserved GTPase motifs. Key residues are highlighted and numbered. (C) The catalytic center of caSey1p with GDP/AlF_4^- bound is shown with key side chains in cyan sticks, GDP is given in orange, and AlF_4^- is shown in gray and light blue. (D) Comparison of the catalytic centers in caSey1p, hsATL1, and dynamin-1. GMP-PNP or GMP-PCP states are shown in gray, and GDP/AlF_4^- is in cyan. The catalytic residue is shown in sticks. (E) GTPase activity of wild-type scSey1p. Left graph shows cytSey1p at different concentrations, and the right one shows full-length scSey1p (0.3 µM) in either proteoliposomes or detergents Triton X-100 (TX-100). (F) GTPase activity of Sey1p proteins. The activities were measured by phosphate release at saturating GTP concentrations (0.5 mM) using the cytosolic domains (1 µM). Each bar is the mean and SE of four measurements. All measurements are representative of at least three repetitions. wt, wild type.

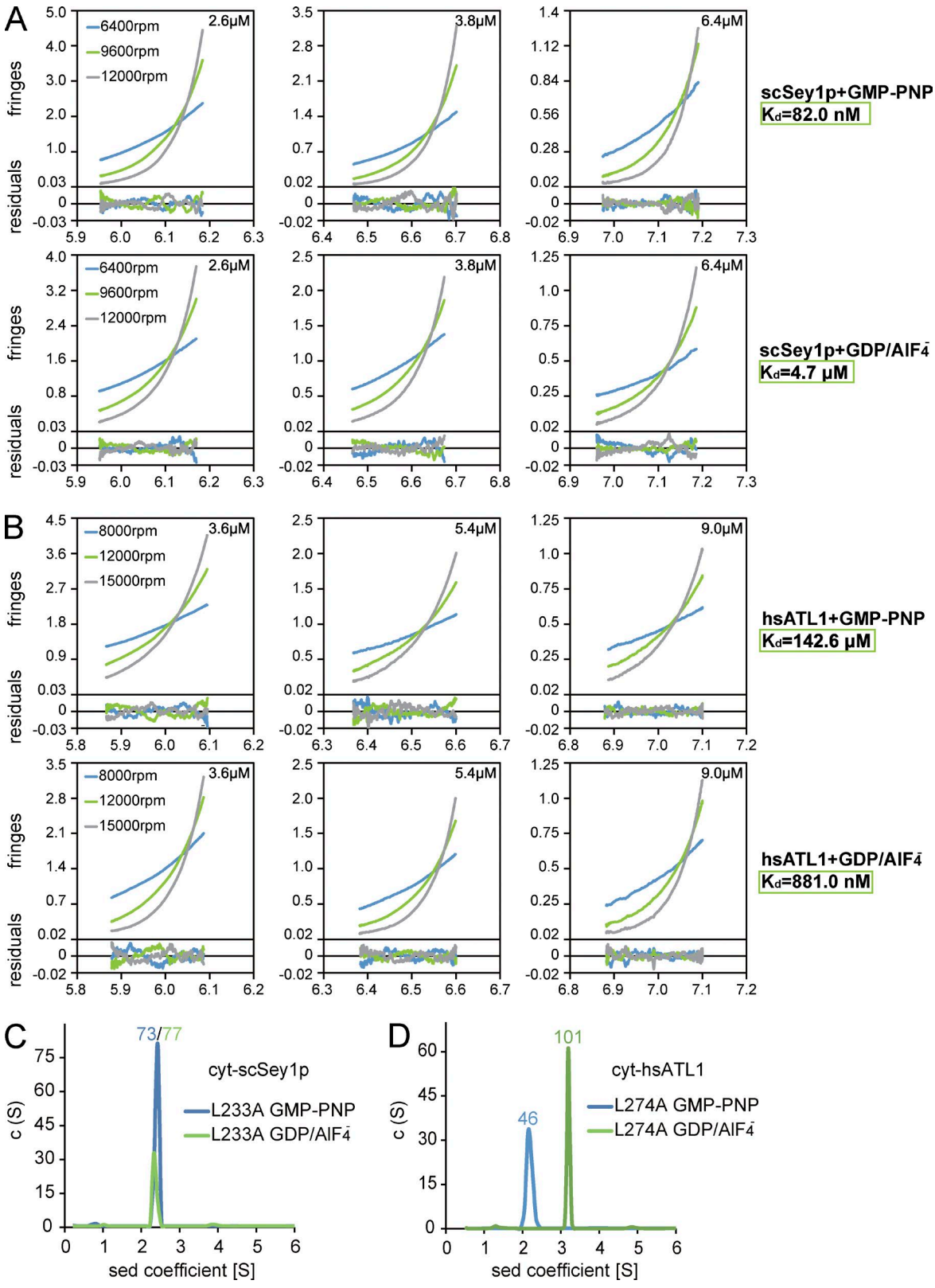

Figure S3. **AUC analysis of Sey1p and ATL1.** (A) AUC analysis of wild-type scSey1p was performed at three concentrations and three different speeds in the presence of two different nucleotides as indicated. The absorbance profiles at sedimentation equilibrium were recorded and used to calculate the dissociation constant (highlighted in green boxes) of the dimers. The corresponding residuals for the fit are shown in the lower portion of each graph. (B) As in A, but with wild-type hsATL1. Data shown in A and B are representative of three repeats. (C) The size of cyt-scSey1p L233A (40 μM, theoretical molecular mass 80.8 kD) was determined by AUC in the presence of the indicated nucleotides. The estimated molecular masses are given above the peaks in kilodaltons. (D) As in C, but with cyt-hsATL1 L274A (40 μM, theoretical molecular mass 51.7 kD). All measurements shown in C and D are representative of three repetitions.

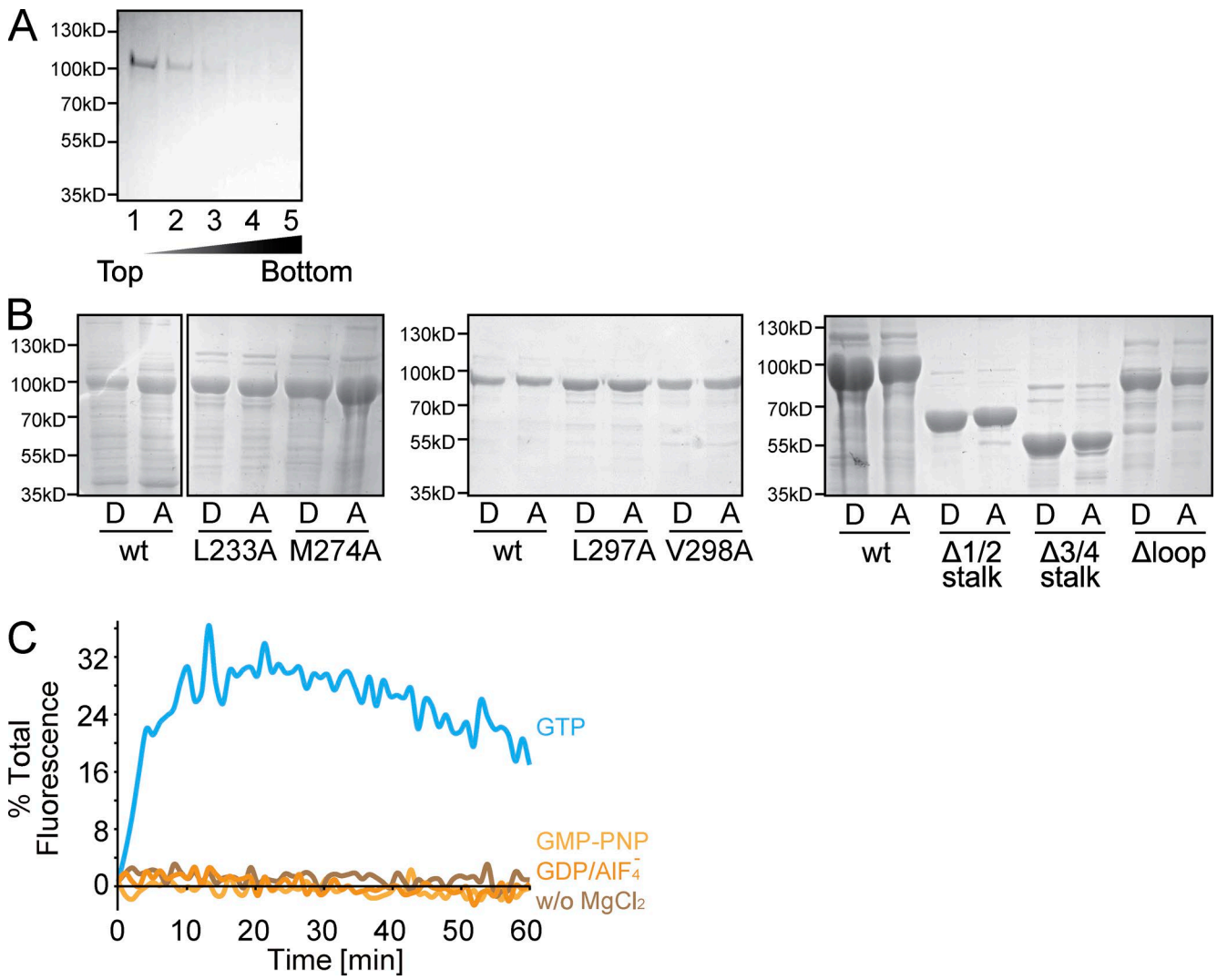

Figure S4. **Controls for fusion assay of Sey1p.** (A) Proteoliposomes containing wild-type (wt) full-length scSey1p were floated in a sucrose gradient and the fractions analyzed by SDS/PAGE and Coomassie staining. (B) Full-length wild-type scSey1p or the indicated mutants were purified and reconstituted into donor (D) and acceptor (A) vesicles. Aliquots of the proteoliposomes were floated in a discontinuous sucrose gradient and analyzed by SDS/PAGE and Coomassie blue staining. The same vesicles were used for the fusion assay in Figs. 2 (D and E) and 3 (G and H). (C) Fusion assay of the wild-type *Drosophila* ATL with indicated nucleotides. The data shown are representative of at three repetitions.

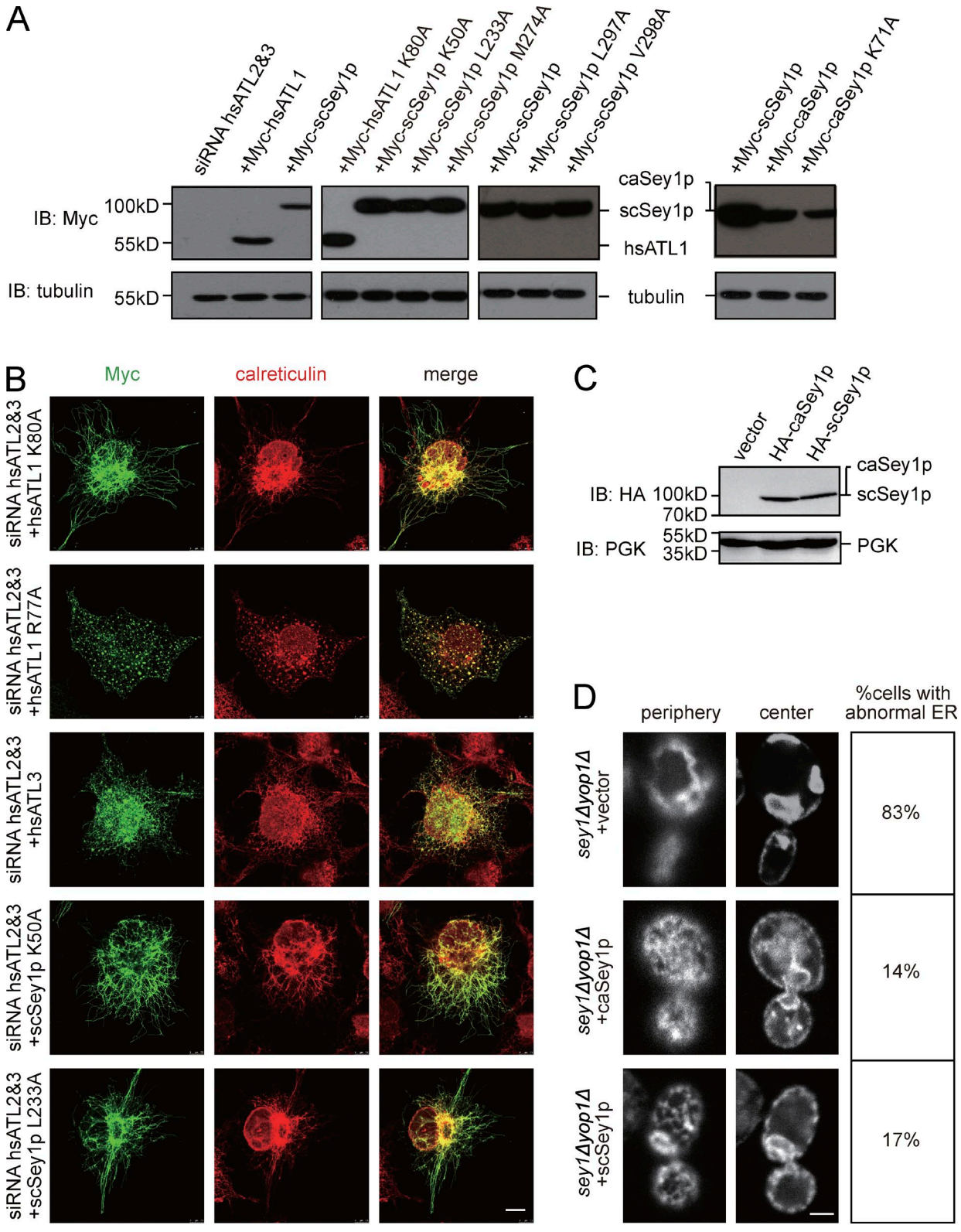

Figure S5. **Analysis of Sey1p in cells.** (A) COS-7 cells used in Fig. 4 were double depleted for hsATL2 and hsATL3 and then transfected with Myc-hsATL1, Myc-scSey1p, or Myc-caSey1p. The levels of Myc-tagged proteins were determined by immunoblotting. Tubulin was used as a loading control. (B) Myc-tagged hsATL1 or scSey1p were expressed in COS-7 cells depleted of ATLs. Localization was determined by anti-Myc antibodies and compared with calreticulin using indirect immunofluorescence and confocal microscopy. Bar, 10 µm. (C) Yeast cells lacking Sey1p and Yop1p were transformed with CEN plasmids expressing HA-tagged scSey1p and caSey1p under the endogenous promoter. The levels of scSey1p and caSey1p were determined by immunoblotting. Phosphoglycerate kinase was used as a loading control. (D) The ER morphology of the cells used in C was visualized by confocal microscopy using a GFP fusion of the ER protein Sec63p, with the microscope focused either at the center or the periphery of the cell. Bar, 2 µm. The percentage of cells with abnormal ER (loss of reticular network in the cortex as shown on the right) was determined from ≥200 cells per sample. IB, immunoblot.