Kienzle et al., http://www.jcb.org/cgi/content/full/jcb.201311052/DC1

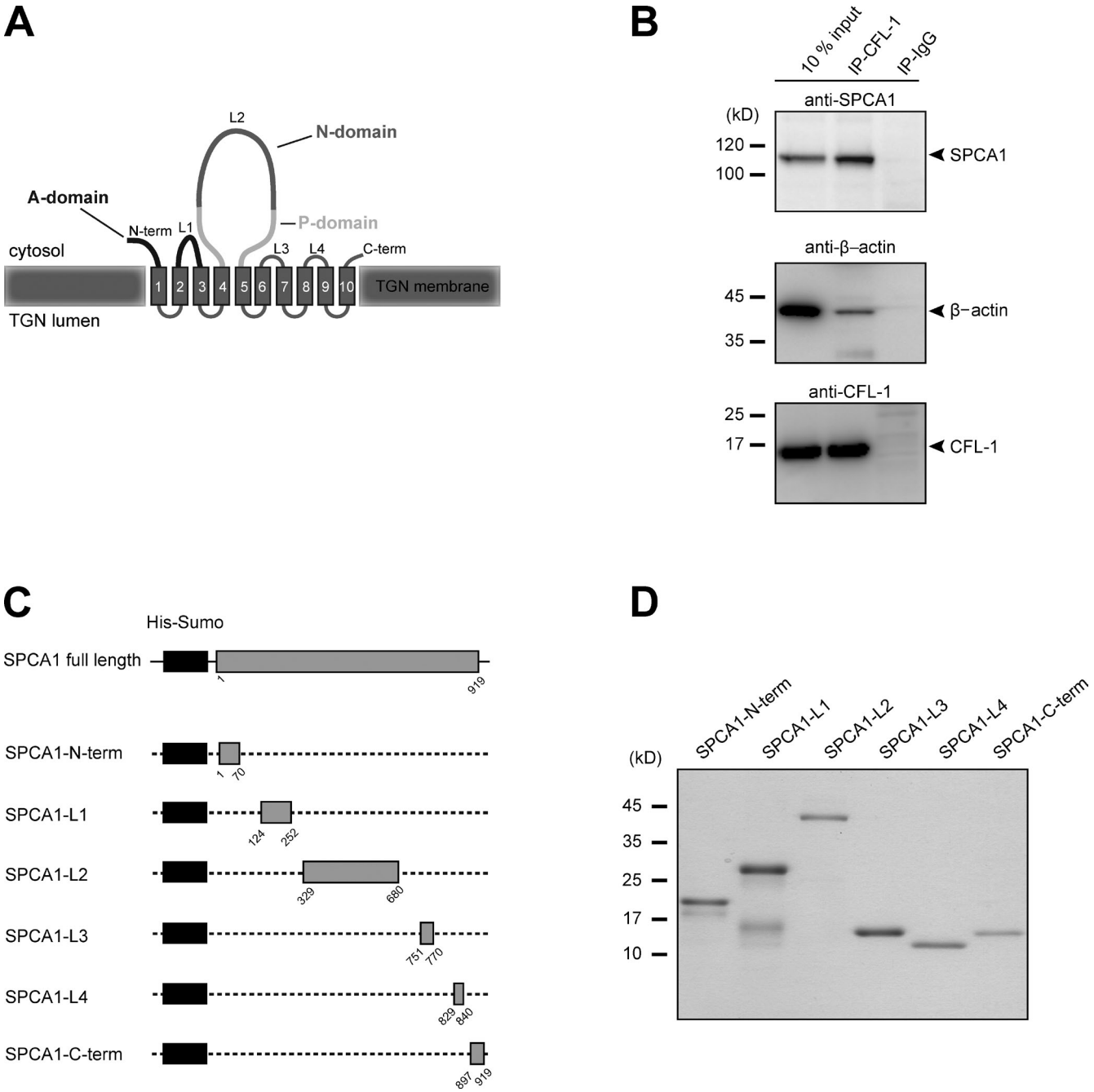

Figure S1. **Expression and purification of the putative actin–CFL-1 interaction domains of SPCA1.** (A) Illustration of SPCA1 structure: SPCA1 contains 10 hydrophobic transmembrane helices. N and C termini (N-term and C-term) are exposed to the cytosolic side as well as four intertransmembrane domains, labeled Loop1 through Loop4 (L1–L4, Fig. 1 A). The N-term and L1 of SPCA1 fold to an A-domain. The largest cytosolic domain (L2) contains an N-domain and a P-domain. (B) CFL-1 was immunoprecipitated from HeLa cell lysates. The lysate was also incubated with a rabbit IgG antibody as a negative control. 10% total input and the CFL-1 and IgG immunoprecipitates were separated by SDS-PAGE and detected by Western blotting with SPCA1 and CFL-1 antibodies. (C) Schematic representation of the His-Sumo expression constructs of SPCA1 cytosolic domains (N-term, L1, L2, L3, L4, and C-term). (D) The cytosolic domains were expressed and purified from *E. coli*. Proteins were separated by SDS-PAGE and visualized by Coomassie staining.

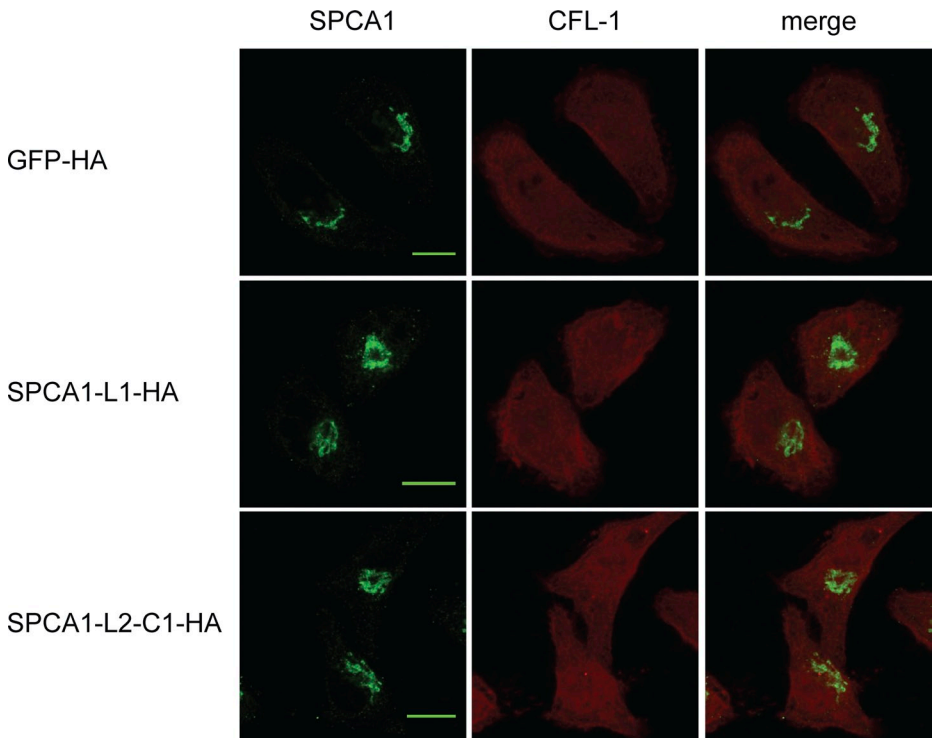

Figure S2. **CFL-1 is diffusely distributed in the cytosol in nonpermeabilized cells.** HeLa cells stably expressing GFP-HA, SPCA1-L1-HA, and SPCA1-L2-C1-HA were fixed with formaldehyde, then permeabilized with 0.2% Triton X-100 and 0.5% SDS before incubation with anti-SPCA1 (green) or anti–CFL-1 (red) antibodies. Bars, 10 µm.

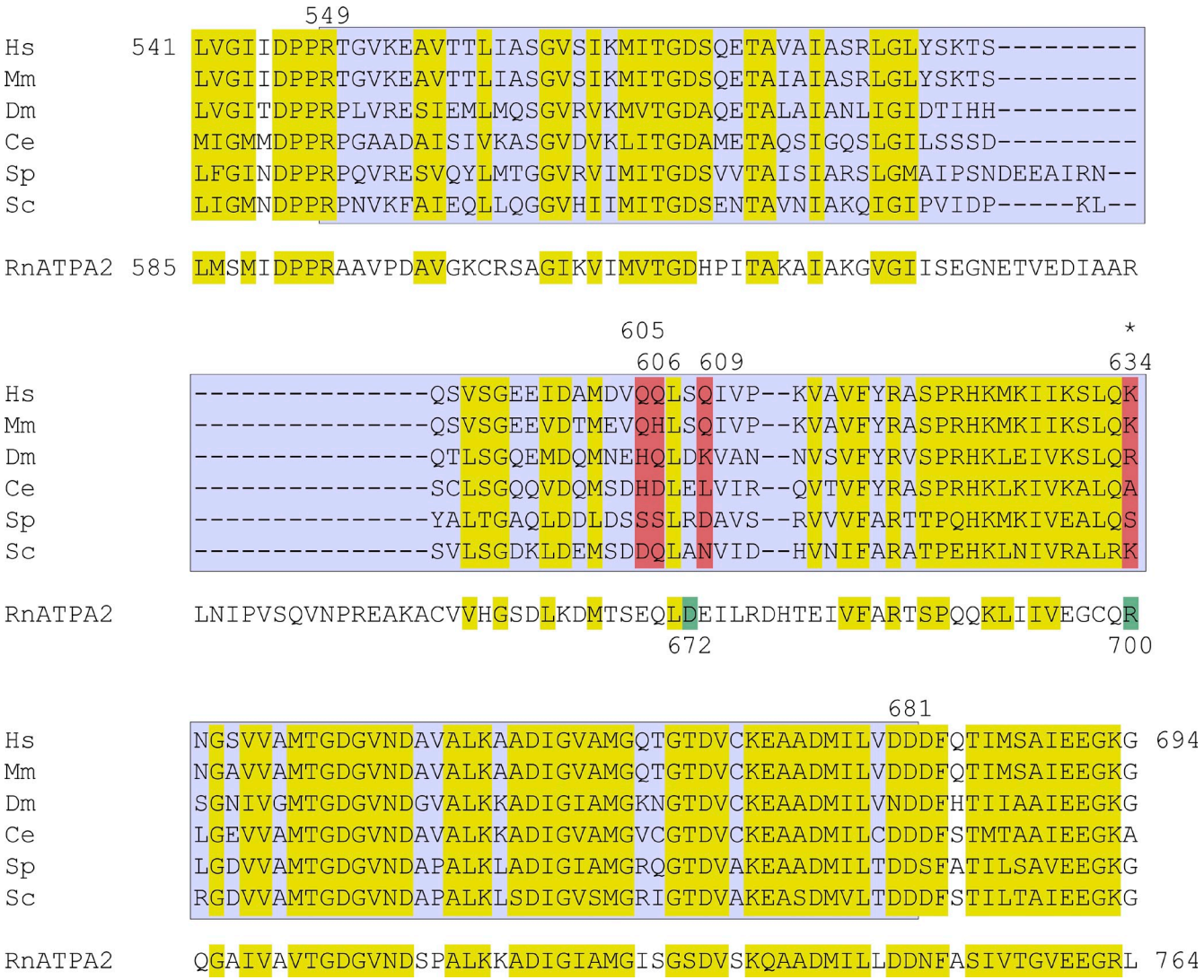

Figure S3. **Multiple sequence alignment of SPCA1 orthologues.** Conserved residues are highlighted in yellow, and the CFL-1–interacting region is indicated by light-blue shading. Residues that influence CFL binding are highlighted in red in SPCA1 sequences and green in Rat ATPA2 (Na^+/K^+-ATPase).

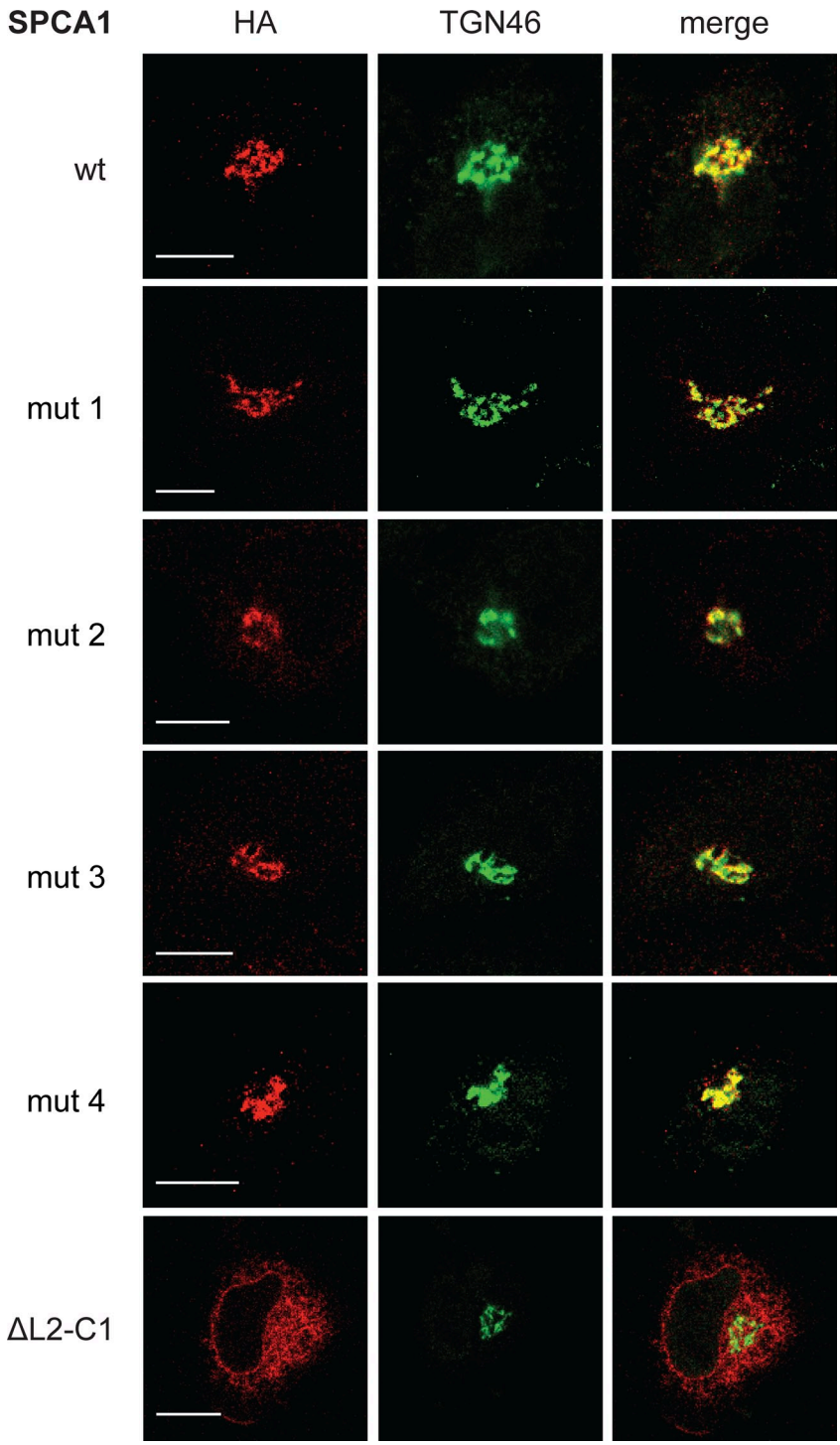

Figure S4. **Localization of HA-tagged SPCA1 mutants.** HeLa cells stably expressing siRNA-resistant SPCA1-wt, mut1 ($Q^{605}A/S^{608A}/K^{634}A$), mut2 ($R^{623}A/K^{627}A/K^{630}A/K^{634}A$), mut3 ($K^{589}A/K^{613}A$,), mut4 ($Q^{605}A/Q^{606}A/Q^{609}A/K^{634}A$), and SPCA1-ΔL2-C1 were fixed with formaldehyde and permeabilized using 0.2% Triton X-100 and 0.5% SDS before incubation with anti-SPCA1 (green) or anti–CFL-1 (red) antibodies. Bars, 10 μm.

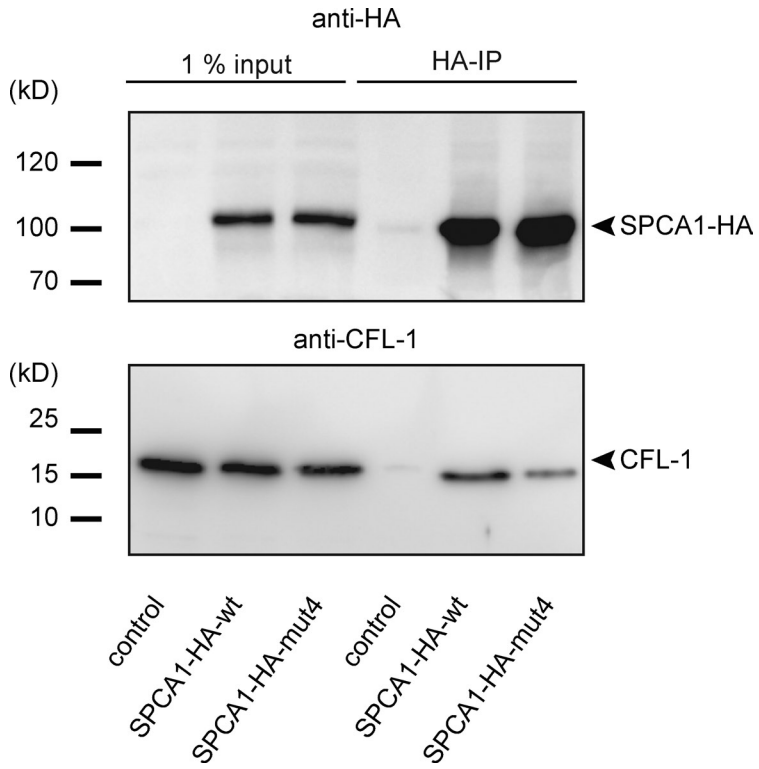

Figure S5. **SPCA1-mut4 binding to CFL-1 is impaired.** HeLa cells transduced with a control plasmid, SPCA1-HA-wt, or -mut4 were lysed, and lysates were incubated with μMACS anti-HA magnetic microbeads. Associated proteins were eluted, separated by SDS-PAGE, and analyzed by Western blotting with anti-HA and anti-CFL-1 antibodies.

Table S1. **Accession numbers and species abbreviations of human *ATP2C1***

Abbreviation	Accession number	Species
Hs	NP_055197	*Homo sapiens*
Mm	NP_778190	*Mus musculus*
Dm	NP_730744	*Drosophila melanogaster*
Ce	NP_001021862	*Caenorhabditis elegans*
Sp	NP_595098	*Schizosaccharomyces pombe*
Sc	NP_011348	*Saccharomyces cerevisiae*
RnATPA2	NP_036637	*Rattus norvegicus*