

Expanded View Figures

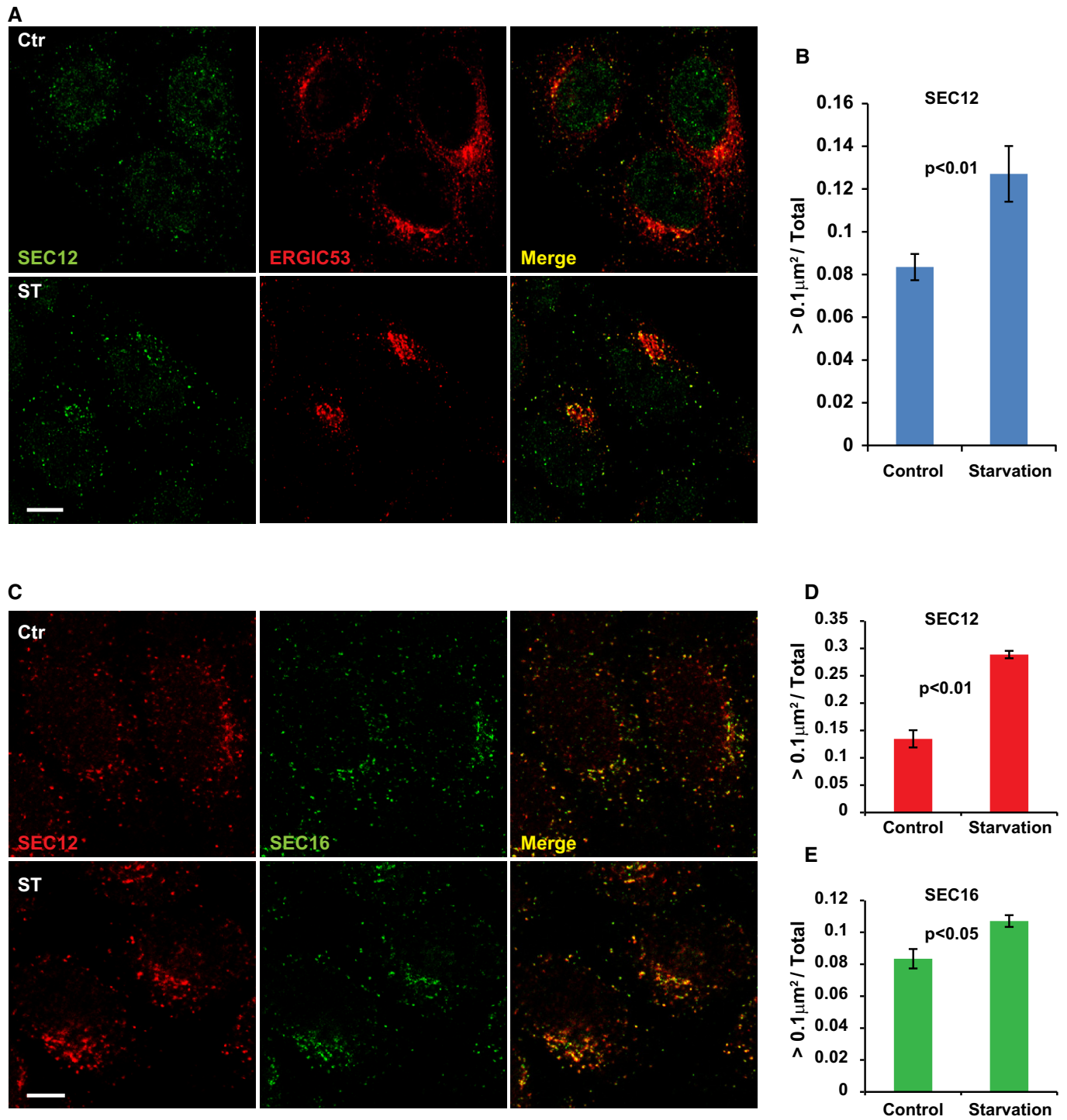


Figure EV1.

Figure EV1. Enlargement of SEC12-positive compartment upon starvation.

- A HeLa cells were incubated in nutrient-rich medium (Ctr) or starved in EBSS (ST) for 1 h. Immunofluorescence and confocal microscopy were performed to visualize SEC12 and ERGIC53. Scale bar: 10 μm
- B Quantification of the fraction of SEC12 compartments larger than 0.1 μm^2 in area analyzed in (A). Error bars represent standard deviations. *P*-value was obtained from two-tailed *t*-test. Five experiments (50–100 cells/experiment) were performed for the statistics.
- C HeLa cells were incubated in nutrient-rich medium (Ctr) or starved in EBSS (ST) for 1 h. Immunofluorescence and confocal microscopy were performed to visualize SEC12 and SEC16 compartments. Scale bar: 10 μm
- D Quantification of the percentage of SEC12 compartments larger than 0.1 μm^2 in area analyzed in (C). Error bars represent standard deviations. *P*-value was obtained from two-tailed *t*-test. Five experiments (50–100 cells/experiment) were performed for the statistics.
- E Quantification of the percentage of SEC16 compartments larger than 0.1 μm^2 in area analyzed in (C). Error bars represent standard deviations. *P*-value was obtained from two-tailed *t*-test. Five experiments (50–100 cells/experiment) were performed for the statistics.

Figure EV2. PI3K is not required for the starvation-induced enlargement of SEC12 compartment.

- A HeLa cells were incubated in nutrient-rich medium (Ctr) or starved in EBSS (ST) with control, wortmannin (10 nM) or 3-methyladenine (3-MA, 10 mM) for 1 h. Immunofluorescence and confocal microscopy were performed to visualize SEC12 compartments. Scale bar: 10 μm
- B Quantification of the fraction of SEC12 compartments larger than 0.1 μm^2 in area analyzed in (A). Error bars represent standard deviations. *P*-value was obtained from two-tailed *t*-test. Five experiments (50–100 cells/experiment) were performed for the statistics.
- C HeLa cells were incubated in nutrient-rich medium (Ctr) or starved in EBSS (ST) with control, PIK-III, SAR405, or VPS34-IN1 (20 μM each) for 1 h. Immunofluorescence and confocal microscopy were performed to visualize SEC12 compartments. Scale bar: 10 μm
- D Quantification of the fraction of SEC12 compartments larger than 0.1 μm^2 in area analyzed in (C). Error bars represent standard deviations. *P*-value was obtained from two-tailed *t*-test. Five experiments (50–100 cells/experiment) were performed for the statistics.

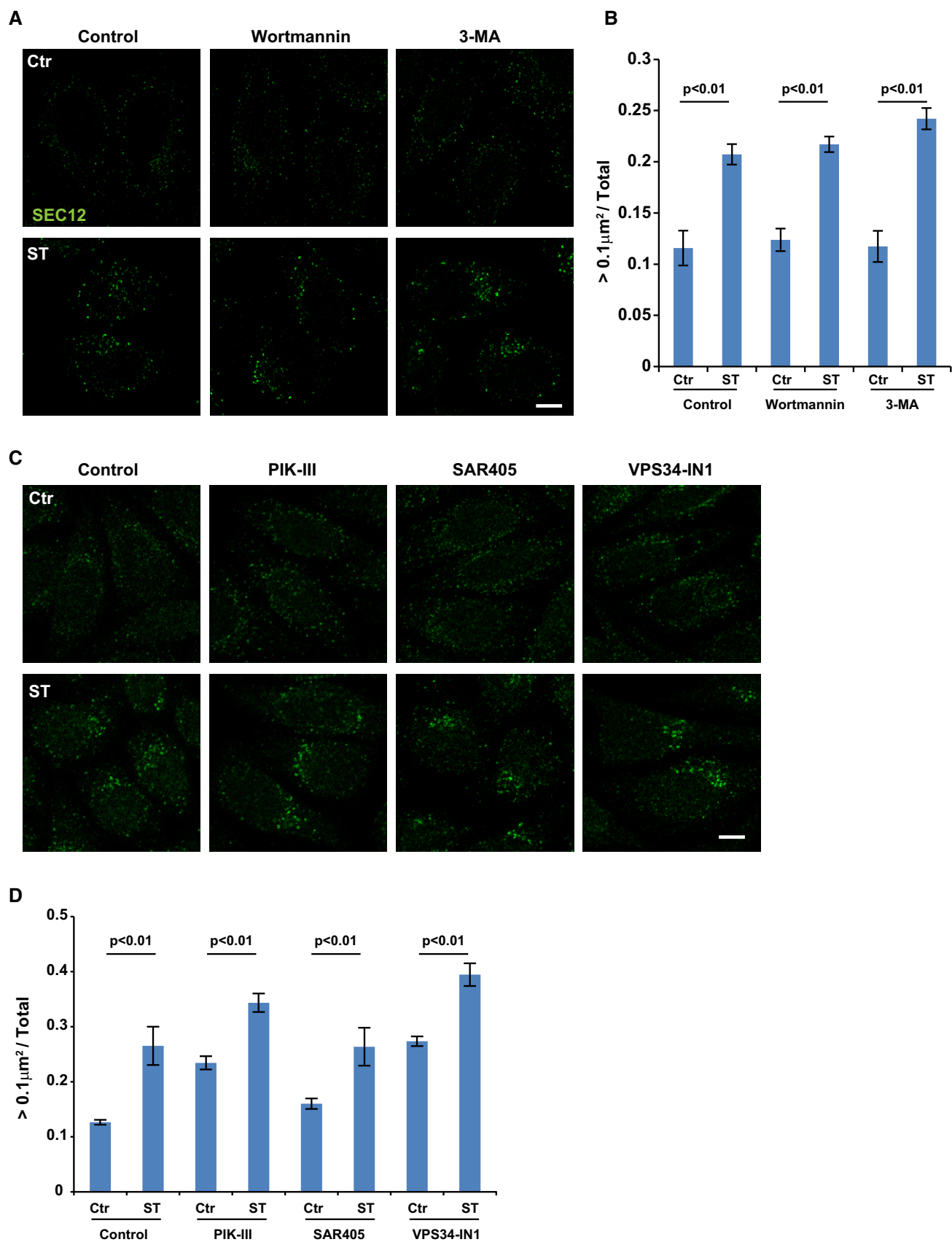


Figure EV2.

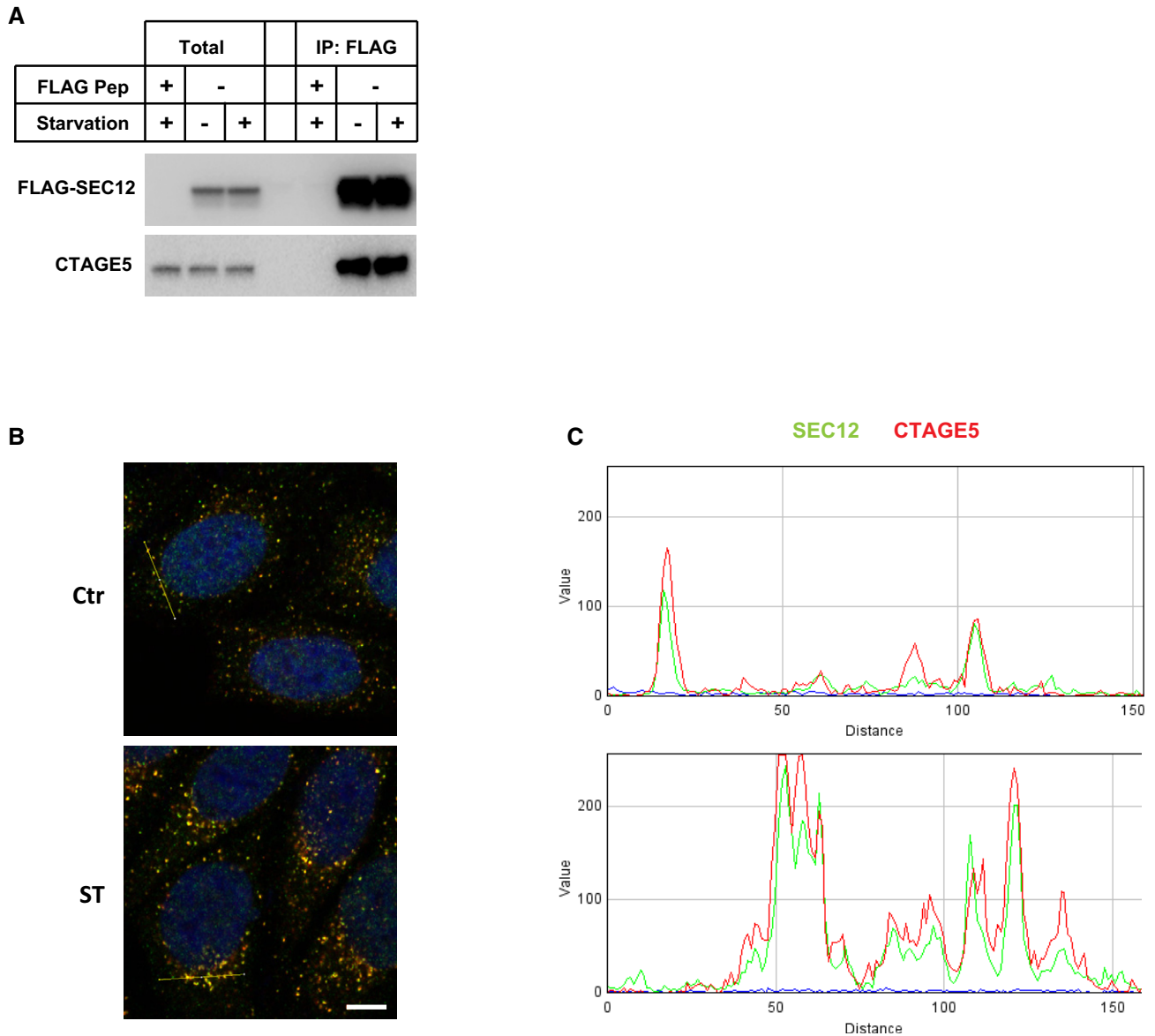


Figure EV3. Analysis of SEC12 and CTAGE5 colocalization and requirement of CTAGE5 for LC3 lipidation.

- A HEK293T cells were transfected with control plasmids or plasmids encoding FLAG-SEC12. After 24 h, the cells were incubated in nutrient-rich medium or starved in EBSS for 1 h. Immunoprecipitation of FLAG-SEC12 was performed, and the levels of indicated proteins from indicated fractions were determined by immunoblot.
- B, C RGB profile plots showing the colocalization of SEC12 (green) and CTAGE5 (red) through the lines drawn on the merged images shown in Fig 2A (yellow lines in panel B).

Figure EV4. Blue Native-PAGE analysis of the TANGO1-CTAGE5-SEC12 complex.

HeLa cells were incubated in nutrient-rich medium or starved in EBSS for 1 h in the absence or presence of 20 nM wortmannin. Cells were lysed, and Blue Native-PAGE was performed to separate protein complexes. SDS-PAGE and immunoblot were followed to determine the migration of the indicated proteins in the Blue Native-PAGE gel. Triangles point to indicate the respective protein bands.

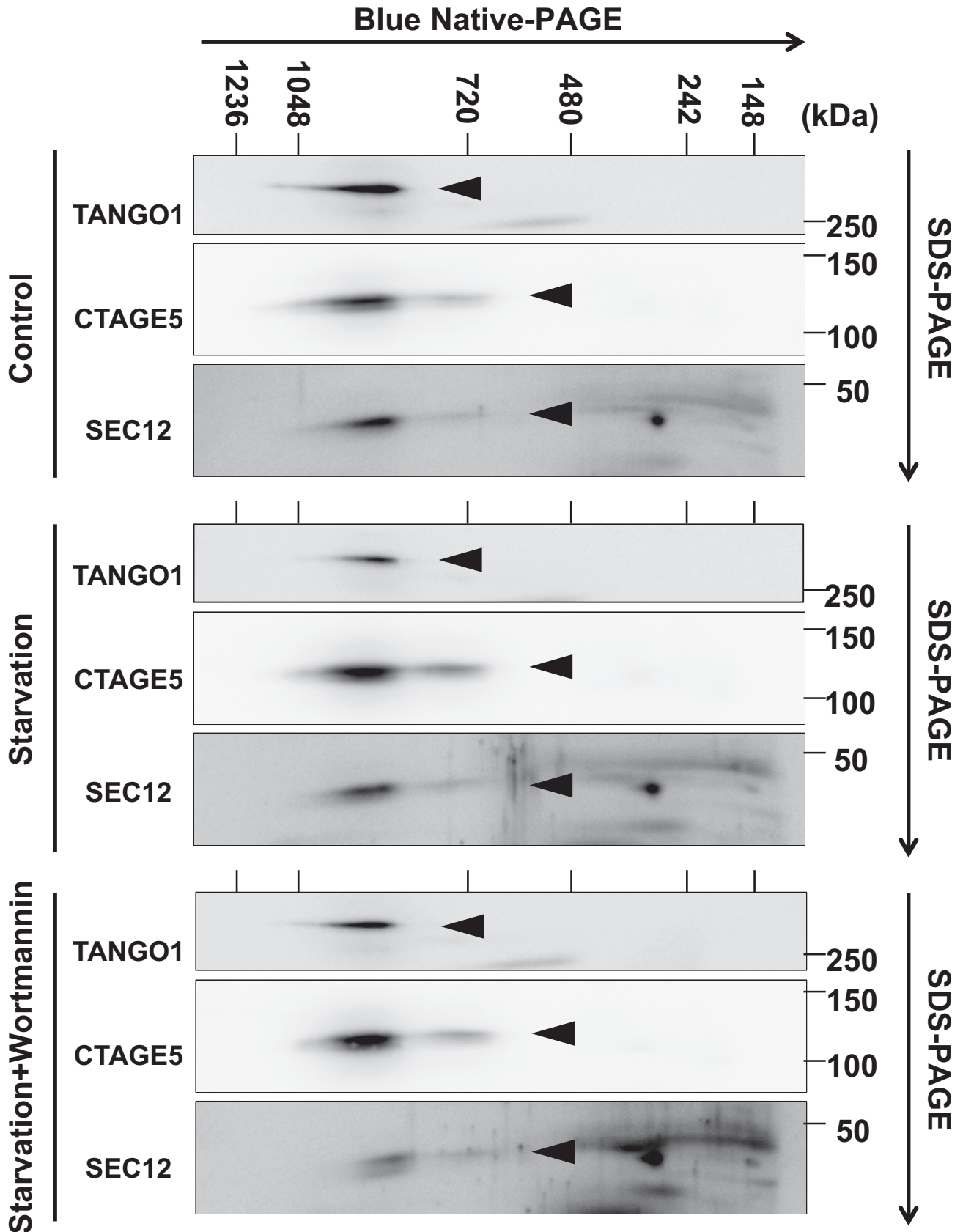


Figure EV4.

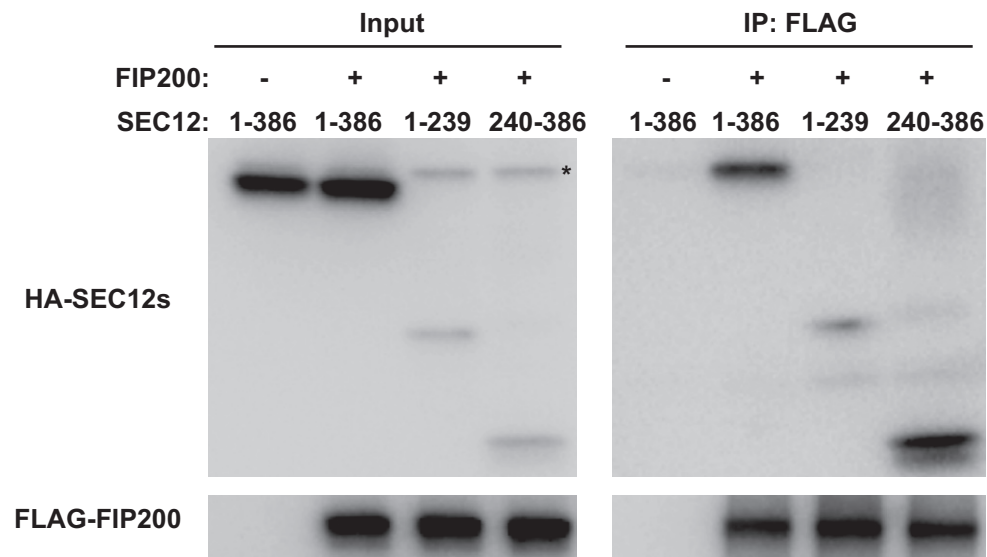


Figure EV5. The association between SEC12 cytoplasmic domains and FIP200.

HEK293T cells were transfected with plasmids encoding SEC12 cytoplasmic domain variants together with FLAG-FIP200. After 24 h, the cells were starved in EBSS for 1 h. Immunoprecipitation of HA-FIP200 was performed, and the levels of indicated proteins from indicated fractions were determined by immunoblot. Asterisk indicates a non-specific band.