Maeda et al., https://doi.org/10.1083/jcb.201703084

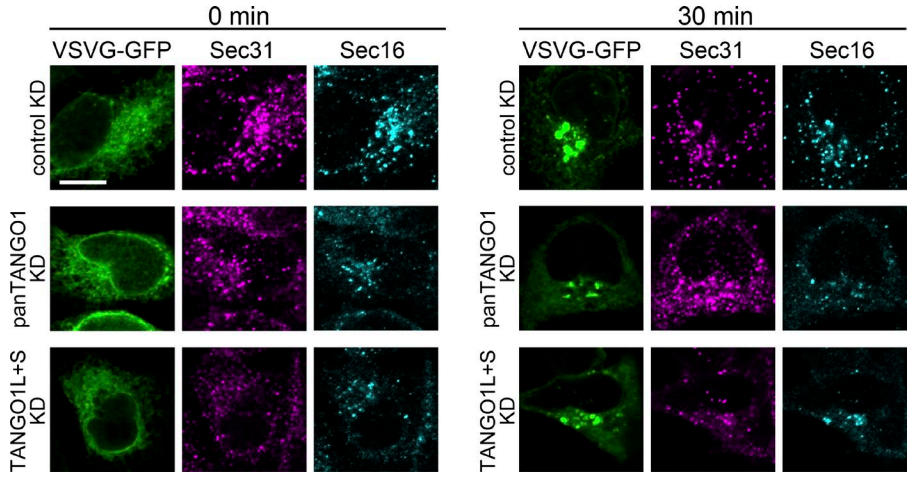

Figure S1. **VSVG transport assay in TANGO1-depleted cells.** HeLa cells twice transfected with the indicated siRNAs were transfected with VSVG-ts045-GFP. The cells were cultured at 39.5°C to accumulate the protein in the ER, then incubated for the indicated times at 37°C before fixation. Fixed cells were stained with anti–Sec16-C and anti-Sec31 antibodies. Bars, 10 µm.

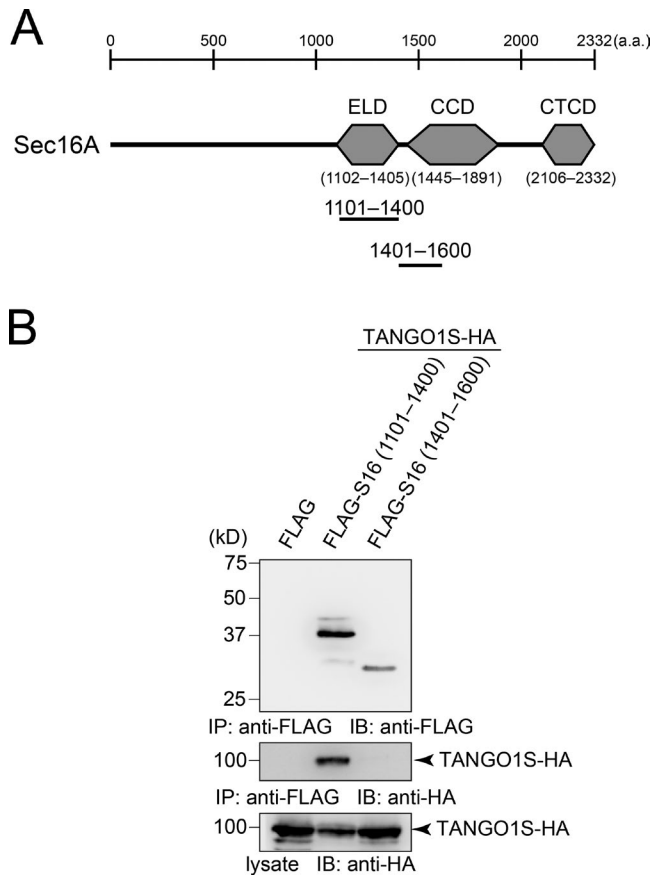

Figure S2. **Sec16 ELD is responsible for interaction with TANGO1.** (A) Schematic representation of human Sec16 domain organization. (B) 293T cells were transfected with FLAG-Sec16 (1101–1400 aa) or FLAG-Sec16 (1401–1600 aa) and TANGO1S-HA constructs as indicated. Cell lysates were immunoprecipitated with anti-FLAG antibody and eluted with a FLAG peptide. Eluates were then subjected to SDS-PAGE followed by Western blotting with anti-FLAG and anti-HA antibodies.

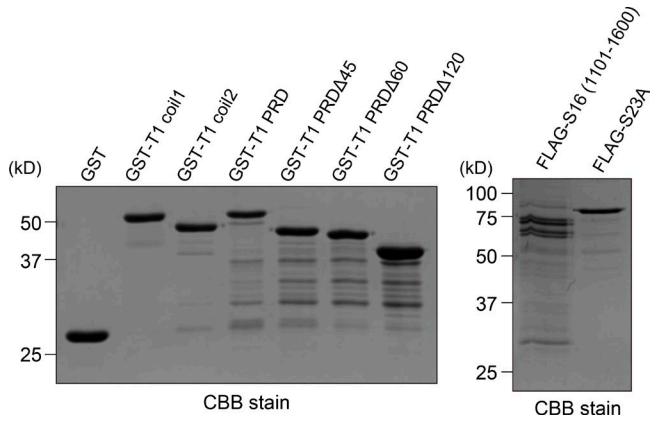

Figure S3. **Purified recombinant proteins used in the in vitro binding assay.** GST or GST-tagged TANGO1-coil1 (1211–1440 aa), GST-tagged TANGO1-coil2 (1441–1650 aa), GST-tagged TANGO1-PRD (1651–1907 aa), GST-tagged TANGO1-PRDΔ45 (1651–1862 aa), GST-tagged TANGO1-PRDΔ60 (1651–1847 aa), or GST-tagged TANGO1-PRDΔ120 (1651–1787 aa) were expressed in *E. coli* and purified with glutathione Sepharose resin. FLAG-Sec16 (1101–1600 aa) and FLAG-Sec23A expressed in 293T cells were purified with FLAG-M2 agarose beads. Purified proteins were analyzed by SDS-PAGE followed by Coomassie brilliant blue (CBB) staining.

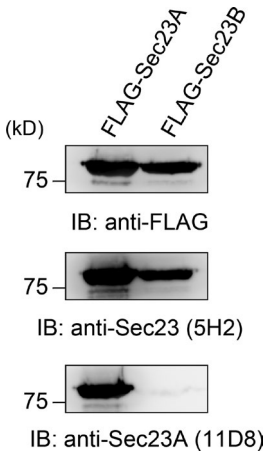

Figure S4. **Specificities and properties of rat monoclonal antibodies for Sec23.** FLAG-Sec23A and FLAG-Sec23B expressed in 293T cells were purified with FLAG-M2 agarose beads. Purified proteins were subjected to SDS-PAGE, followed by Western blotting with anti-FLAG, anti-Sec23 (5H2), and anti-Sec23A (11D8) antibodies.

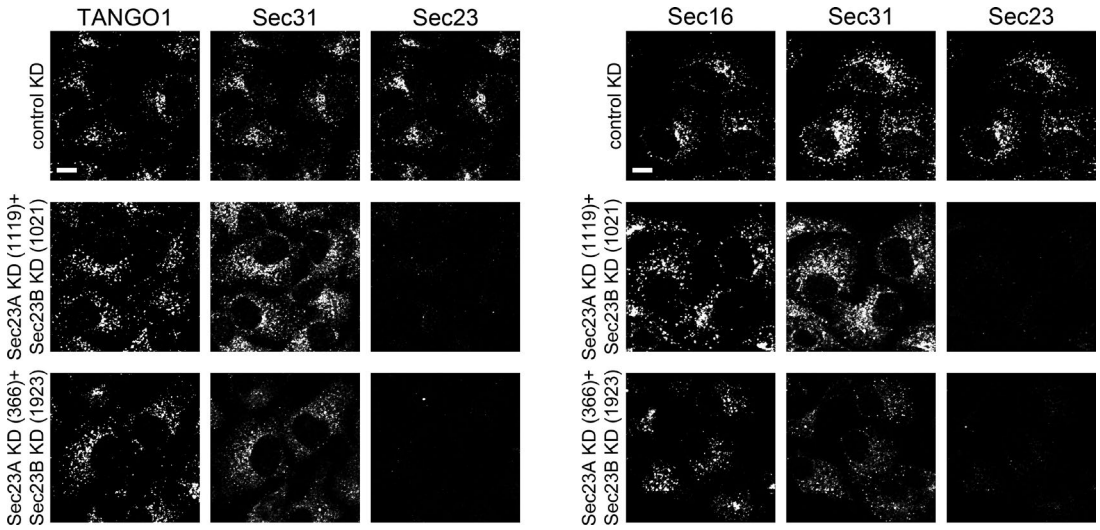

Figure S5. **Sec23 depletion does not affect the localization of endogenous TANGO1.** HeLa cells were transfected with the indicated siRNAs. After 48 h, cells were fixed and costained with anti–TANGO1-CT, anti–Sec16-C, anti-Sec31, and anti-Sec23 (5H2) antibodies. Bars, 10 µm.