Fig. S1. Bundle structures of the long form of Syn5 are similar to those of CLIMP-63. At 18 hours after transfection with the plasmid for CLIMP-63-FLAG (top two rows) or the long form of FLAG-Syn5 (bottom two rows), COS-7 cells were fixed and double stained for FLAG and HSP47 (top), α-tubulin (second), or CLIMP-63 (third and bottom). The panels in the bottom row show enlargement of the boxed areas in the third row. Scale bars, 10 μm.
Fig. S2. Interaction of the long form of Syn5 and its mutants with CLIMP-63. (A) Lysates of 293T cells expressing FLAG-Syn5 (long form), -Syn5 (R74A), or -Syn5 (K82A) were subjected to immunoprecipitation with anti-FLAG M2 beads and immunoblotted with the antibodies against CLIMP-63 (upper panel) and FLAG (lower panel). The experiment was repeated with similar results. (B) HeLa cells were transfected with FLAG-Syn5 (long form: WT), -Syn5 (R74A), or -Syn5 (K82A). At 24 hours after transfection, the cells were fixed and double stained for FLAG (upper row) and α-tubulin (lower row). Scale bar, 10 μm. The bottom panel shows the quantitative data. (C) Lysates of 293T cells transfected with plasmid for FLAG-Syn5 (long form) together with pcDNA vector, pcDNA-CLIMP-63 (full), or pcDNA-CLIMP-63 (Δ2-21) were subjected to immunoprecipitation with anti-FLAG M2 beads and immunoblotted with the antibodies against CLIMP-63 (upper panel) and FLAG (lower panel). Asterisks indicate non-specific bands. The experiment was repeated with similar results.
Fig. S3. Effect of Syn5 knockdown on the Golgi structure. (A) HeLa cells were transfected without (Control) or with one of the siRNAs targeting Syn5 (siRNA (432) or (390)). At 72 hours after transfection, the cells were lysed and equal amounts of total proteins were separated by SDS-PAGE and analyzed by immunoblotting with antibodies against calnexin (loading control) and Syn5. The expression level of Syn5 isoforms was estimated using ImageJ software after normalization to the loading control (bottom panel). The average values of the two independent experiments are shown. (B) At 72 hours after transfection, the cells were fixed and double stained for Syn5 and GM130. Scale bar, 10 μm.
Fig. S4. MTs are intact in Syn5-depleted cells. HeLa cells were transfected without (Mock) or with siRNA (390). At 72 hours after transfection, the cells were fixed and double stained for α-tubulin and acetylated tubulin. Scale bar, 10 μm.
Fig. S5. Morphology of the ER in COS-7 cells depleted of Syn5. COS-7 cells were transfected without (Control) or with siRNA (390). At 72 hours after transfection, the cells were fixed and double stained for Syn5 and CLIMP-63. Scale bar, 10 µm.
Fig. S6. Knockdown of Sec22b does not affect the ER morphology. (A) HeLa cells were transfected without (Control) or with siRNA targeting Sec22b. At 72 hours after transfection, the cells were fixed and double stained for GM130 and Sec61β. The boxed areas are enlarged in the insets. White lines indicate cell edges. Note that the Golgi apparatus was disassembled in Sec22b siRNA-treated cells, as seen in GM130 staining (lower left). Scale bars, 10 μm. (B) The distribution of Sec61β fluorescence was measured along a line from the nucleus to the cell periphery using ImageJ software. The average values of fluorescence brightness in five cells are shown.
Fig. S7. Knockdown of CLIMP-63 affects the ER structure. (A,B) HeLa cells were transfected without (Control) or with siRNA targeting CLIMP-63. At 72 hours after transfection, cell lysates (5 or 10 μg) were subjected to SDS-PAGE and analyzed by immunoblotting with antibodies against calnexin (upper panel) and CLIMP-63 (lower panel) (A), or cells were fixed and stained for Sec61β (B). The boxed areas are enlarged in the insets. White lines indicate cell edges. Scale bars, 10 μm. The bottom panel shows the distribution of Sec61β fluorescence along a line from the nucleus to the cell periphery. The average values of fluorescence brightness in five cells are shown. (C) HeLa cells were transfected without (Control) or with siRNA targeting CLIMP-63. At 72 hours after transfection, the cells were treated without (Vehicle) or with 5 μg/ml nocodazole for 90 minutes (Noc-treatment). The cells were fixed and double stained for CLIMP-63 and Sec61β. Scale bar, 10 μm.
**Movie S1.** Live cell imaging of mRFP-Sec61β-stably expressing cells. Control HeLa cells were incubated with 5 μg/ml nocodazole for 80 minutes, and images were obtained at 300 second intervals.

**Movie S2.** Live cell imaging of mRFP-Sec61β-stably expressing cells. Syn5-depleted HeLa cells were incubated with 5 μg/ml nocodazole for 80 minutes, and images were obtained at 300 second intervals.