**Figure S1:** Mutagenesis of glutamic acids at positions 52 and 53 of Cog4 impairs the binding to Sly1. HEK293 cells were transiently transfected with Sly1-HA or cotransfected with Sly1-HA and Myc-tagged Cog4(1-231) containing the indicated point mutations. The interaction between Sly1-HA and Cog4(1-231)-Myc was examined by immunoprecipitation (IP) with anti-Myc antibody and immunoblotting (IB) with anti-HA antibody. The expression levels of the indicated proteins are shown in the lower panels. As shown, the interaction of Sly1 with the Cog4(E52/53A) double mutant was very similar to its interaction with the Cog4(E53A) mutant.

**Figure S2:** The E53/71A Cog4 mutant interacts with other COG subunits. A. The E53/71A mutant of Cog4 interacts with the Cog2 subunit. Cog2 was expressed as GST-fusion protein in HEK293 cells together with either Myc-tagged wild-type or the E53/71A mutant of Cog4. The cell lysates were subjected to glutathione-agarose beads (GB) pull-down. The interaction between mGST-Cog2 and Cog4 was determined by immunoblotting with anti-Myc antibody. Mammalian GST (mGST) was used as a control, whereas the expression level of the wild-type and mutant Cog4 was determined by immunoblotting with anti-Myc antibody. B. The E53/71A Cog4 double mutant is incorporated into the COG complex. The wild-type or the E53/71A Myc-tagged Cog4 mutant were transiently expressed in HeLa cells, and immunoprecipitated with anti-Myc antibody. Their ability to be incorporated into the endogenous COG complex was assessed by Western blotting using antibodies against either Cog3 or Cog8. As seen, the wild-type and the E53/71A Cog4 mutant similarly interact with Cog2 and are incorporated into the COG complex.

**Figure S3:** Depletion of Cog4 disrupts the colocalization between Syntaxin 5 and GS15. Control and Cog4 knocked-down (KD) HeLa cells were fixed and double immunostained with monoclonal antibody against GS15 (green) and polyclonal antibody against Syntaxin 5 (red). Shown are representative confocal images demonstrating the localization of each protein along with their colocalization, which appears in yellow in the merged image. Scale bar, 10 μm.

**Figure S4:** The wild-type Cog4 restores the colocalization of Syntaxin 5 and GS15 in Cog4-knocked down cells. Cog4-knocked down HeLa cells were transiently transfected with either Myc-tagged wild-type or the E53/71A double mutant of Cog4 containing the silent mutations within the RNAi targeting sequence. Two days later, the cells were fixed and coimmunostained with anti-Myc (green), anti-GS15 (red), and anti-Syntaxin 5 (light blue) antibodies. Colocalization is shown in the merged images. Transfected cells are indicated by arrowheads. Scale bar, 10 μm.
Figure S5: Depletion of Cog4 attenuates Golgi-to-ER retrograde transport in response to BFA treatment, but has no effect on ER-to-Golgi anterograde transport following BFA wash-out.  A. Control and Cog4-depleted HeLa cells were treated with BFA (5 µg/ml) for the indicated time points, fixed and immunostained with anti-mannosidase II antibody and analyzed by confocal microscope. Shown are representative confocal images of each time point. Scale bar, 10 µm. B. Control and Cog4-depleted HeLa cells were treated with 0.25 µg/ml BFA for 1 h, extensively washed in PBS, and allowed to recover in regular medium for the indicated time points. The cells were then fixed and immunostained as described above. Scale bar, 10 µm.

Figure S6: Depletion of Cog4 has no effect on VSV-G anterograde transport. Control and Cog4-depleted HeLa cells were infected with ts045 strain of VSV at 37°C for 30 min, washed, and incubated at 40°C for 3 h. The cells were then shifted to 32°C in the presence of cyclohexamide (100 µg/ml) for the indicated time periods, fixed, and double-immunostained with anti-VSV-G (red) and anti-GRASP-65 (green) antibodies. Scale bar, 10 µm.

Figure S7: Cog4 can interact simultaneously with Sly1 and Syntaxin 5. The T7/F10A double mutant of Syntaxin 5 failed to interact with Sly1 (A), though it did interact with Cog4 (B). HEK293 cells were transiently cotransfected with expression vectors encoding the indicated HA-tagged Syntaxin 5 proteins together with either Sly1-Myc (A) or GST-Cog4 (B). The interaction between the wild-type or the T7/F10A double mutant of Syntaxin 5 and Sly1 was determined by immunoprecipitation with anti-Myc antibody and immunoblotting with anti-HA antibody. The interaction between the indicated Syntaxin 5 proteins and Cog4 was determined by pull-down experiments using glutathione-agarose beads (GB), followed by immunoblotting with anti-HA antibody. C. HEK293 cells were transiently cotransfected with expression vectors encoding the indicated HA-tagged Syntaxin 5 proteins, Myc-tagged Cog4 proteins, and Myc-tagged Sly1. The cells were lysed and subjected to immunoprecipitations with anti-Sly1 antibody followed by immunoblotting with the indicated antibodies. The expression level of the transfected proteins was assessed by immunoblotting of total cell lysates with the indicated antibodies (A,B,C). As seen, Sly1 interacts with the T7/F10A mutant of Syntaxin 5 only in the presence of wild-type Cog4, suggesting that Cog4 can interact simultaneously with Sly1 and Syntaxin 5.