



### Supplementary Figure 5. Effects of inhibiting BARS activity in vivo

(A) Knocking down BARS by siRNA. Cells were treated with different siRNA conditions as indicated, and then analyzed by immunoblotting for different protein levels as indicated. (B) Anterograde ER-to-Golgi transport is not significantly affected by knocking down BARS. COS cells transfected with GFP-tagged VSVG(ts045) were either treated with siRNA for BARS or mock treated as control. Cells were then examined for the distribution of VSVG by confocal microscopy upon release from ER for 20 minutes. Representative images are shown (bar, 10  $\mu\text{m}$ ). (C) The CTP mutant inhibits the retrograde transport of a chimeric KDEL. COS cells were transfected with VSVG-ts045-KDEL-myc and also vector alone, wild-type BARS, or the CTP mutant BARS. Cells were then examined at the permissive temperature and also upon shift to the non-permissive temperature for the distribution of the chimeric KDEL by immunofluorescence microscopy. Representative images are shown (bar, 10  $\mu\text{m}$ ). (D) The CTP mutant increases the level of Golgi coated buds. COS cells were treated either with siRNA for BARS or with a scrambled sequence as control, followed by EM examination, using the correlative light-electron microscopy technique to identify cells with reduced BARS. Coated buds on Golgi cisternae were confirmed by EM tomography performed on 200 nm sections, and then quantified by counting the number of buds and vesicles, 50-80 nm in diameter, on 30 micrographs. The number was divided by the area (in  $\mu\text{m}^2$ ). The mean with standard error from three independent experiments is shown.