

## E07-10-0997 McNiven

**Supplementary Figure S1.** The perinuclear localization of Eps15 is disrupted by brefeldin A treatment. (A–C’’) Brefeldin A (BFA) treatment disrupts the perinuclear co-localization of Eps15 and AP-1. HeLa cells were treated with methanol carrier (A–A’’) or 5 µg/ml of BFA for 5 min (B–B’’) or 10 min (C–C’’) and then immunostained with antibodies against AP-1 (A, B, and C) and Eps15 (A’, B’, and C’). Eps15 and AP-1 co-localize in a perinuclear region in control cells (A–A’’). However, both proteins become dispersed and no longer co-localize in the Golgi region in BFA-treated cells (B–C’’), consistent with a Golgi association for Eps15. Scale bars, 20 µm.

**Supplementary Figure S2.** Expression of the appendage domain of  $\gamma$ -adaptin, a subunit of the AP-1 complex, alters VSVG-ts-GFP transport from the trans-Golgi network to the plasma membrane. Trafficking of VSVG-ts-GFP in BHK-21 cells was assayed and quantitated essentially as described in the legend to Figure 6. Here, cells expressing Myc-tagged versions of either wild-type (wt)  $\gamma$ -adaptin appendage domain (A–A’’) or a  $\gamma$ -adaptin appendage domain mutant (A716D) defective in Eps15 binding (B–B’’) were analyzed. It was predicted that the wild-type  $\gamma$ -adaptin appendage domain would bind the AP-1-binding motif of Eps15 and act as a competitive inhibitor, thereby reducing Golgi-to-plasma membrane transport of VSVG-ts-GFP. In contrast, VSVG-ts-GFP trafficking would be normal in cells expressing the  $\gamma$ -adaptin appendage domain A716D mutant that does not interact with Eps15. VSVG-ts-GFP was retained in the endoplasmic reticulum at the restrictive temperature (40°C) and transported to the Golgi following a 15 min incubation at the permissive temperature (32°C) in cells expressing either wild-type (A and A’) or mutant (B and B’)  $\gamma$ -adaptin appendage domain. Upon longer incubation at the permissive temperature (1 h and 2 h), VSVG-ts-GFP was subsequently transported to the plasma membrane in cells expressing the  $\gamma$ -adaptin appendage domain A716D mutant (B’’ and B’’’), whereas VSVG-ts-GFP was retained in a perinuclear region in cells expressing wild-type  $\gamma$ -adaptin

appendage domain (A'' and A'''). (C) Graph representing quantitation of the average fluorescence intensity of VSVG-ts-GFP in a standardized area covering the Golgi compartment at the indicated time points following release from the restrictive temperature in cells expressing either  $\gamma$ -adaptin appendage domain wt or  $\gamma$ -adaptin appendage domain A716D. Cells expressing wild-type  $\gamma$ -adaptin appendage domain retained ~2.5-fold more VSVG-ts-GFP in the Golgi area than did cells expressing the  $\gamma$ -adaptin appendage domain A716D mutant. Forty cells were measured for each condition. Error bars indicate the standard error. Scale bars, 10  $\mu$ m (A–B''').

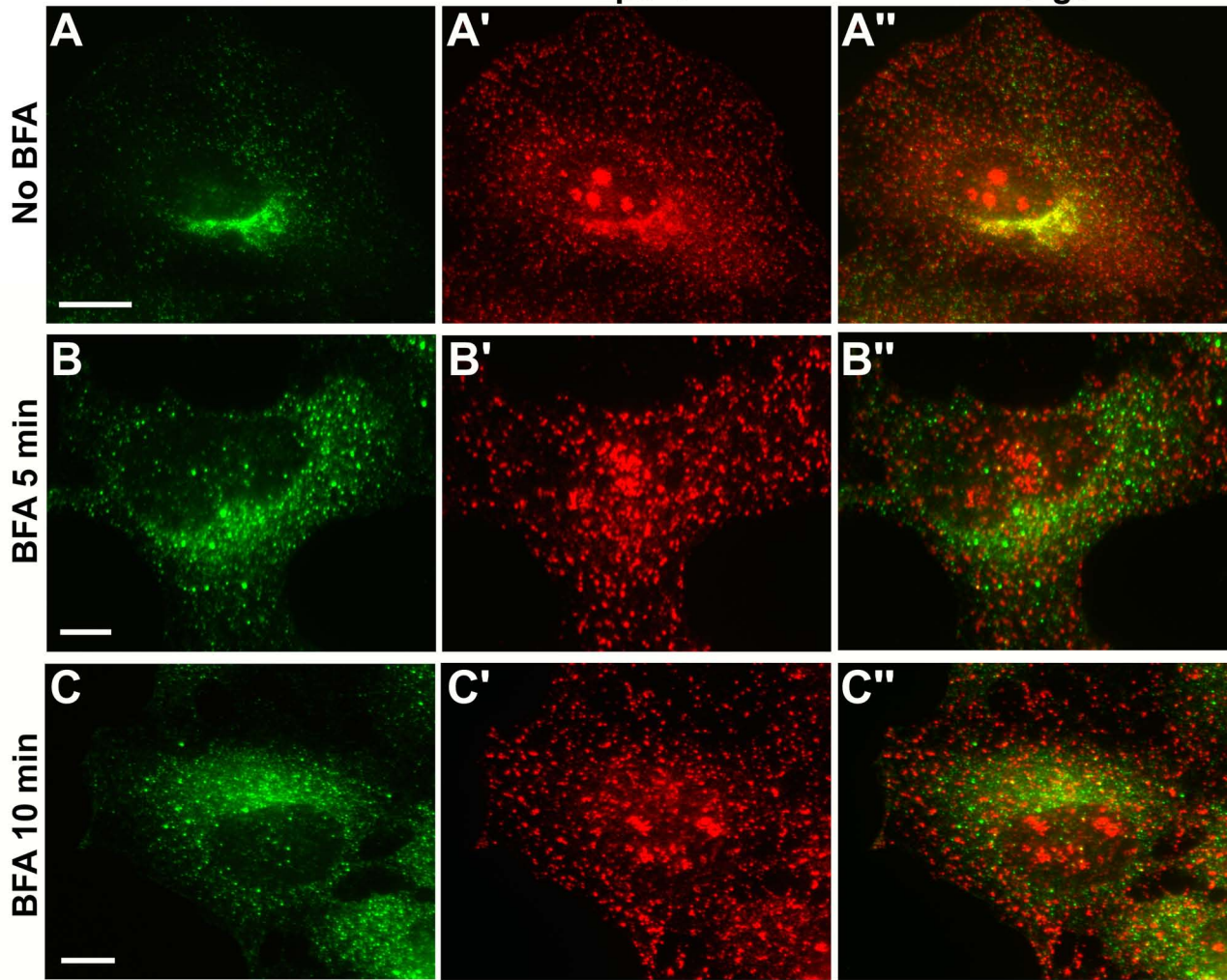
**Supplementary Figure S3.** VSVG-ts-GFP is retained at the trans-Golgi network by inhibition of Eps15-AP-1 interaction. Trafficking of VSVG-ts-GFP in BHK-21 cells was assayed as described in Figure 6. The localization of retained VSVG-ts-GFP in cells expressing Myc-tagged-Eps15  $\Delta$ 14aa was examined after 2 h at permissive temperature (32°C) using the TGN marker, p230 (A-A''), or Alexa 594-transferrin (B-B'', recycling endosomes). Note that retained VSVG-ts-GFP shows a marked co-localization with the TGN marker, p230 (arrows in A''), but not with Alexa 594-transferrin (B-B''). Expression of Eps15  $\Delta$ 14aa was confirmed by immunostaining with anti-Myc antibody (data not shown). Scale bars, 10  $\mu$ m.

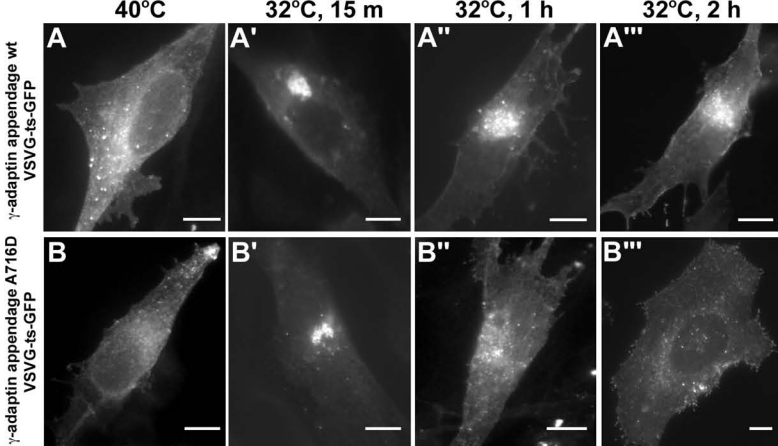
**Supplementary Figure S4.** Inhibition of Eps15-AP-1 interaction does not disrupt endocytosis. HeLa cells were transfected with Myc-tagged-Eps15  $\Delta$ 14aa (asterisk) and transferrin uptake was performed for 20 mins using Alexa 594-transferrin (A and B). (C) Graph representing % of cells internalizing transferrin showing that expression of Eps15  $\Delta$ 14aa does not block endocytosis. Scale bars, 20  $\mu$ m.

AP-1

Eps15

Merge





**C**

