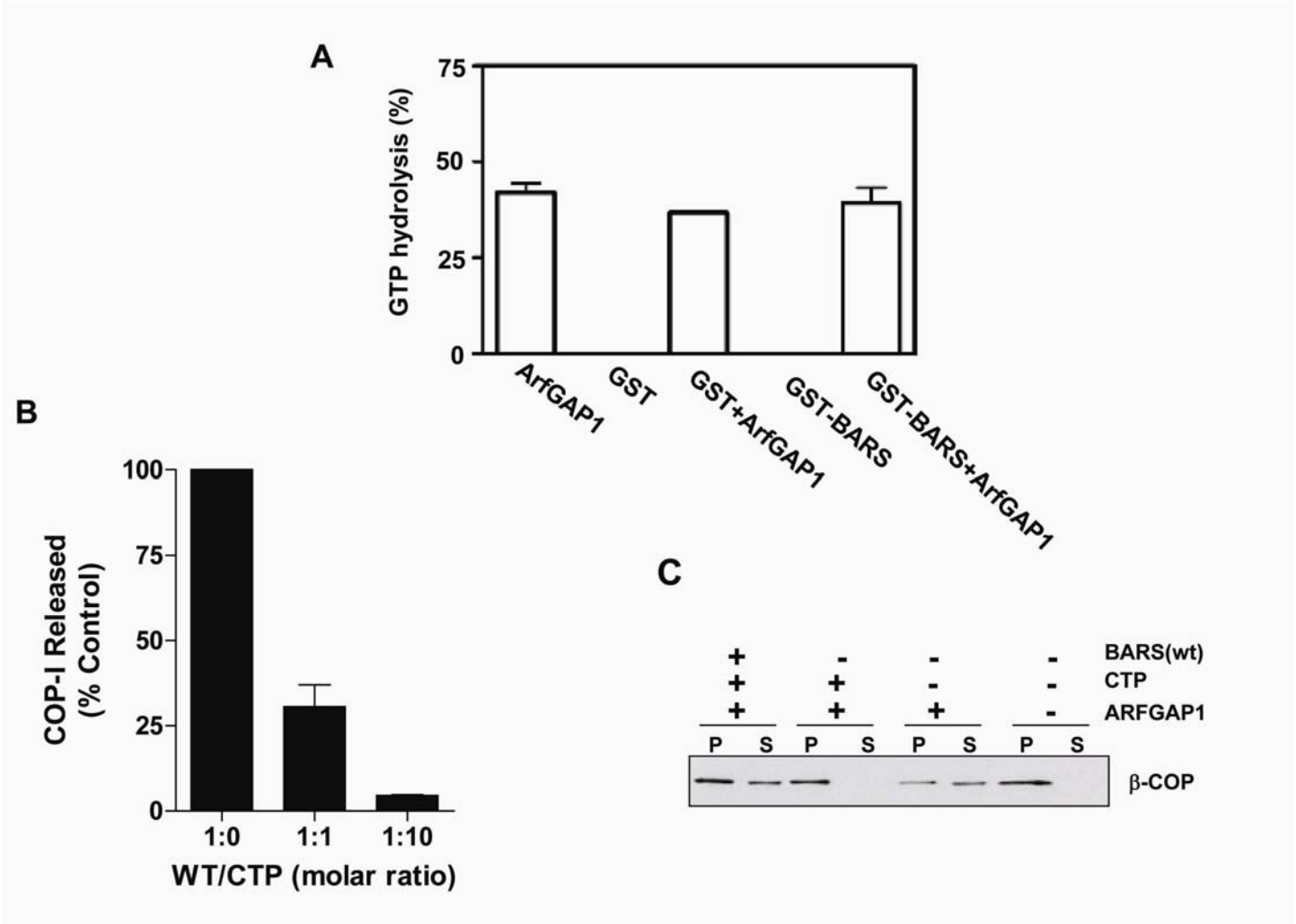

Supplementary Figure 4. Insights into how the interaction between GAP and BARS is critical for vesicle formation

(A) The GAP catalytic activity is not affected by the presence of BARS. GAP was incubated with GST-BARS (or GST as control), and then incubated with ARF1 (previously loaded with GTP) in the presence of large unilamellar vesicles. GTP hydrolysis was then quantified. The mean from three independent experiments is shown with standard error. (B) Relative level of the CTP mutant needed to inhibit the ability of wild-type BARS in promoting the completion of the second-stage incubation. CHO Golgi membrane washed with 3M KCl was used for the two-stage incubation system. After the second-stage incubation that contained GAP with varying levels of wild-type and the CTP mutant BARS (as indicated by their molar ratio), the level of β-COP released into the supernatant was quantified and expressed as a percentage of total (represented by β-COP level in both pellet and supernatant). This value was then normalized to control, which was derived from the condition that did not have the CTP mutant in the incubation. The mean of this normalized value derived from three independent experiments is shown with standard error. (C) The inhibitory effect of CTP at the second-stage incubation is reversed by adding excess wild-type BARS. CHO Golgi membrane washed with 0.5M KCl was used for the two-stage incubation system, with different components added at the second stage as indicated.