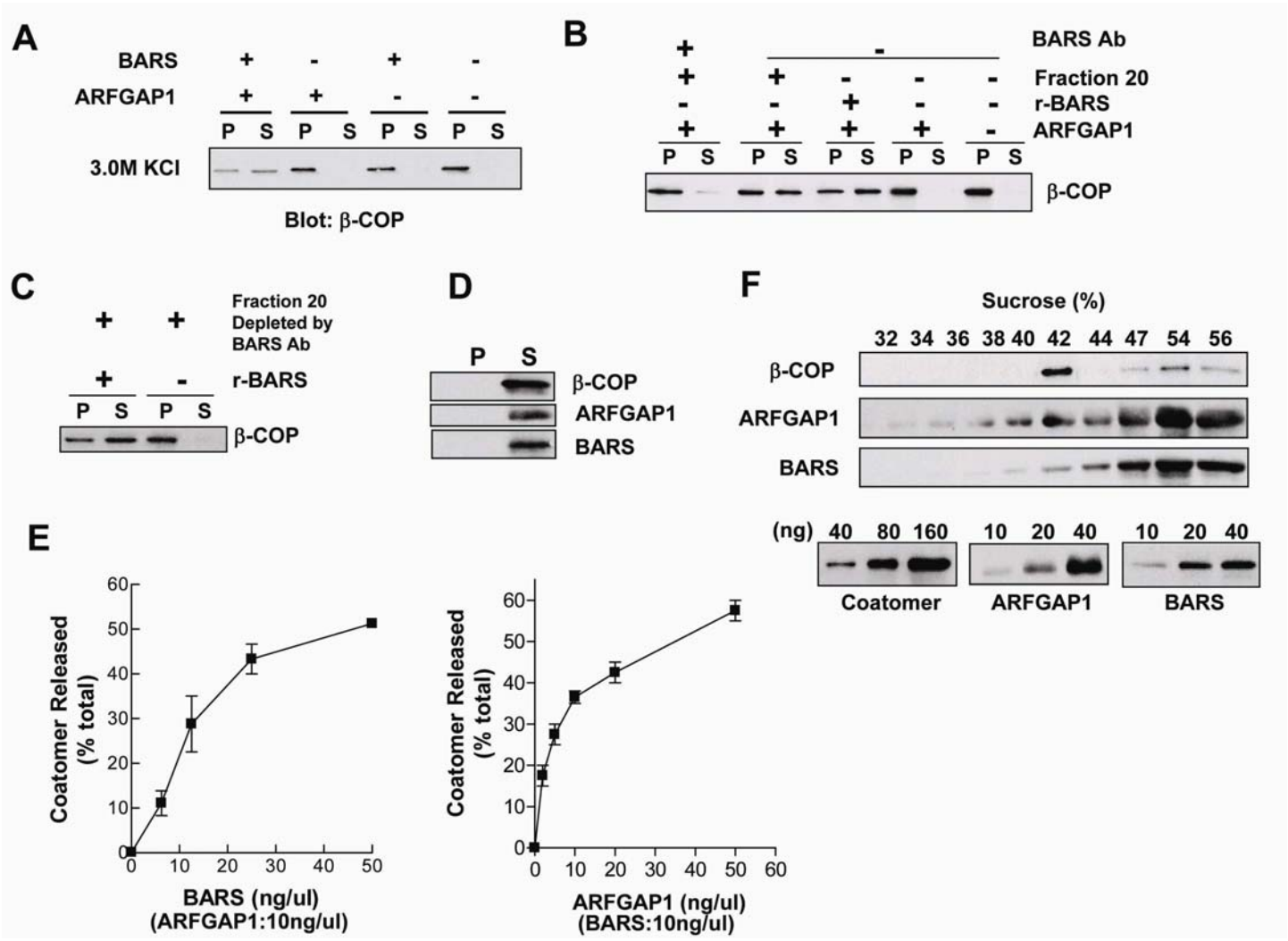

Supplementary Figure 1. Vesicle reconstitution using Golgi membrane washed with 3M KCl
(A) The addition of BARS restores the ability of GAP to complete the second-stage incubation using Golgi membrane washed with 3M KCl. Rat liver Golgi membrane washed with 3M KCl was used for the two-stage incubation system, followed by immunoblotting of pellet (P) and supernatant (S) for β-COP after the second-stage incubation. (B) The active fraction in gel filtration loses its activity upon the immunodepletion of BARS. CHO Golgi membrane was washed with 3M KCl, and then subjected to the two-stage incubation system. At the second stage, different components as indicated were added in conjunction with GAP, followed by centrifugation and then immunoblotting of both the pellet and supernatant fractions for β-COP. (C) Addition of recombinant BARS restores the activity of the active fraction that had been depleted of endogenous BARS. The experiment was performed as described in (B) with additional conditions as indicated. (D) Coatomer does not aggregate with GAP and BARS to form a complex that can be pelleted by ultracentrifugation. Soluble forms of coatomer, GAP and BARS were mixed together, and then subjected to ultracentrifugation at 200,000 x g for 1 hour to obtain pellet (P) and supernatant (S), followed by immunoblotting for β-COP. (E) Similar levels of GAP and BARS are needed in completing the second-stage incubation. CHO Golgi membrane washed with 3M KCl was used for the two-stage incubation system, with the second-stage incubation titrating in increasing levels of BARS or GAP. The level of β-COP released into the supernatant after this stage was quantified and then expressed as a percentage of the total. The mean of this calculated value derived from three independent experiments is shown with standard error. (F) Analysis of reconstituted COPI vesicles by equilibrium centrifugation reveals that BARS does not exhibit a similar peak at 42% sucrose density as coatomer and GAP. CHO Golgi membrane washed with 3M KCl was used in the two-stage incubation system, and then the supernatant from the second stage was subjected to equilibrium centrifugation followed by fractionation and immunoblotting for β-COP, GAP, and BARS. Bottom panels are used as quantitation references, showing immunoblotting of known levels of the different proteins in their purified forms.