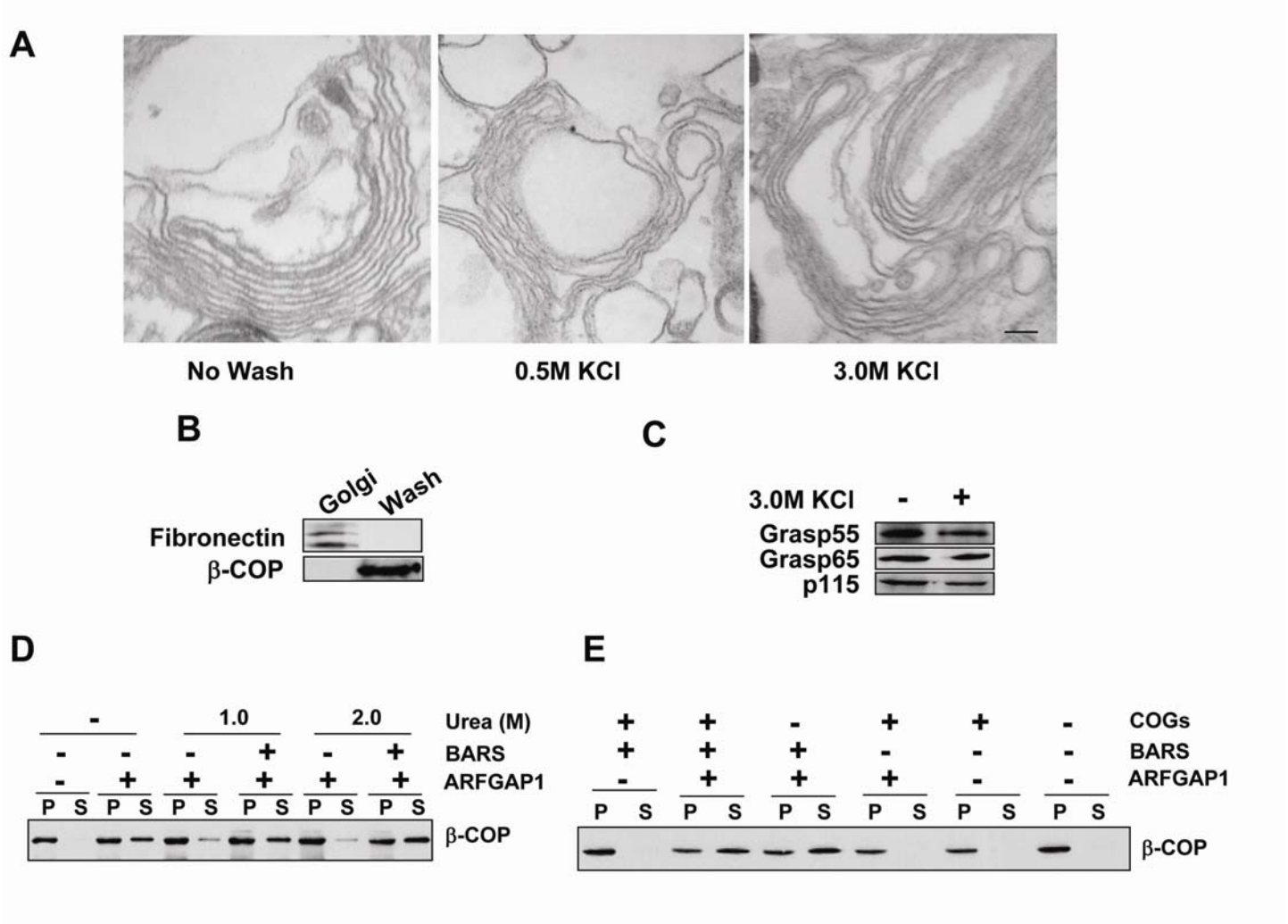

Supplementary Figure 2. Characterization of the Golgi membrane washed with 3M KCl
(A) Golgi membrane washed with 3M KCl appears similar as unwashed membrane. Golgi membrane from rat liver was washed with 0.5M KCl, 3M KCl, or not washed, and then embedded in epon for serial sections and examination by EM; bar, 100 nm. (B) Luminal Golgi proteins are not released by washing with 3M KCl. CHO Golgi membrane was washed with 3M KCl, and then subjected to centrifugation, followed by immunoblotting of both the pelleted membrane fraction and the supernatant fraction containing the wash for proteins as indicated. Note that fibronectin exists in multiple forms recognized by the antibody. (C) Proteins that stabilize the Golgi stacks are not significantly released by the 3M KCl wash. Rat liver Golgi membrane was washed with either transport buffer that contained 50 mM KCl or 3M KCl. Washed membranes were then subjected to centrifugation, followed by immunoblotting for proteins as indicated. (D) BARS is required for the release of coatomer from Golgi membrane washed with 2M urea during the second-stage incubation. CHO Golgi membrane, washed with different levels of urea as indicated, was used for the two-stage incubation system, followed by centrifugation to obtain pellet (P) and supernatant (S), and then immunoblotting for β-COP. (E) The addition of the COG complex does not induce the release of coatomer from Golgi membrane during the second-stage incubation. CHO Golgi membrane washed with 3M KCl was used for the two-stage incubation system, with GAP and the COG complex added in the second stage, followed by centrifugation to obtain pellet (P) and supernatant (S), and then immunoblotting for β-COP.