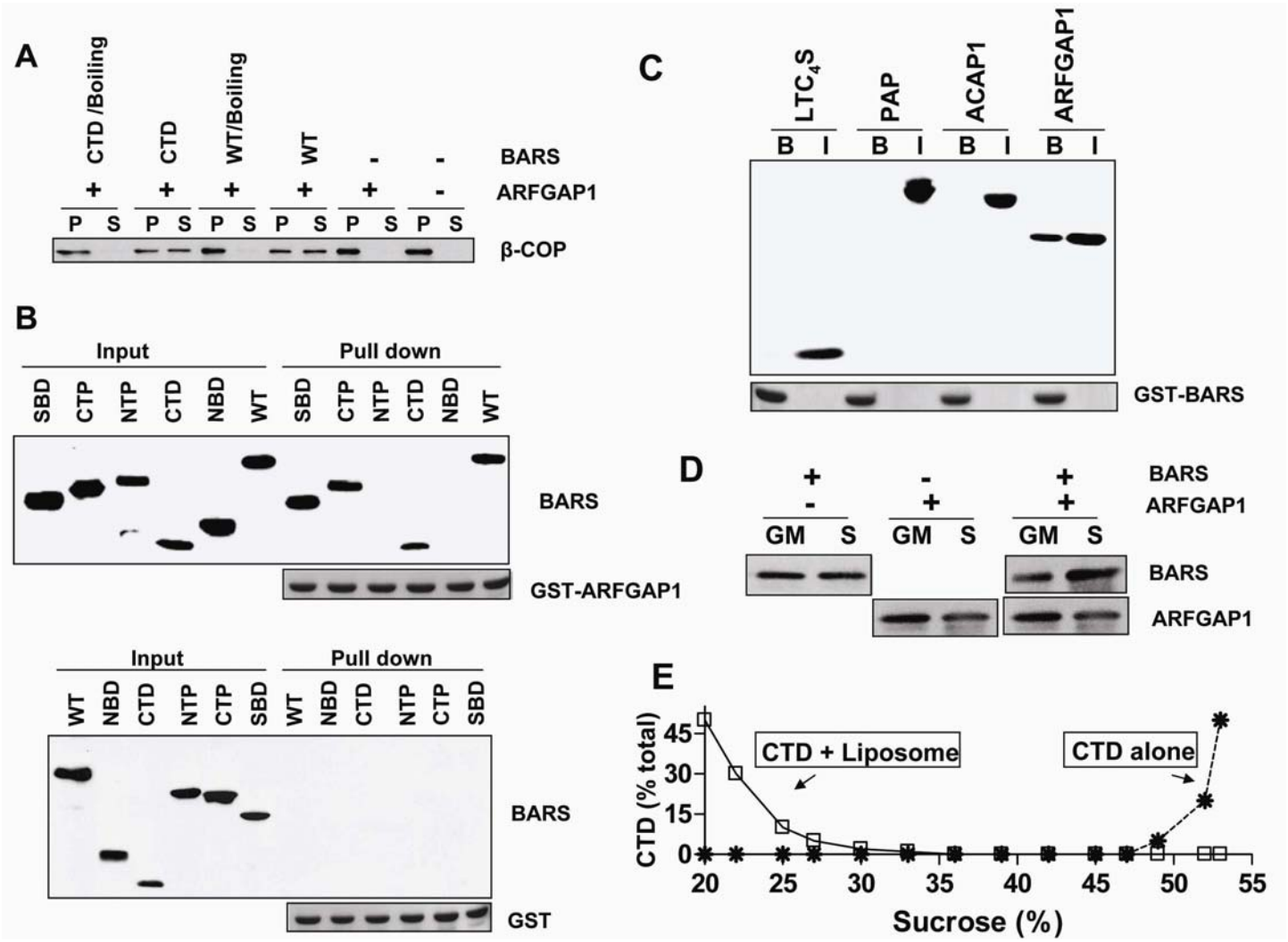

Supplementary Figure 3. Analysis of mutant BARS in vesicle formation and interaction with GAP. (A) Denaturation of the CTD mutant by boiling abolishes its ability to support vesicle formation. CHO Golgi membrane washed with 3M KCl was subjected to the two-stage incubation system, with the second stage incubation containing GAP and different forms of BARS as indicated, followed by centrifugation and then immunoblotting of both the pellet and supernatant fractions for β-COP. (B) Binding to GAP by different domains of BARS. [Top set] ARFGAP1 was fused to GST and then gathered onto glutathione beads for incubation with different forms of BARS in a pulldown assay. The beads were then immunoblotted for BARS and Coomassie-stained for GST fusion proteins. Input indicates the levels of different soluble BARS added for the incubation. [Bottom set] A similar pulldown was performed using GST on beads as control. (C) BARS does not interact with other proteins including other ARFGAPs. GST-BARS was bound to glutathione beads and then incubated with different purified proteins as indicated. In the upper panel, the bound fraction (B) and the input fraction (I) were then analyzed by immunoblotting using antibodies directed against the different input proteins. In the lower panel, the level of GST-BARS on beads was analyzed by Coomassie staining. Leukotriene C4 (LTC4) synthase, an intracellular enzyme involved in lipid metabolism, is used as a further control. (D) Binding of Golgi membrane by soluble BARS is not affected by the presence of GAP. Different soluble proteins as indicated were incubated with Golgi membrane and then the incubation was pelleted for Golgi membrane followed by immunoblotting of both the membrane and soluble fractions as indicated. (E) The CTD mutant binds to liposomal membrane. Liposomal membrane that mimics Golgi composition was generated and then incubated with CTD, followed by equilibrium centrifugation on a flotation sucrose gradient. Fractions were then analyzed for membrane lipids by fluorescence intensity (detecting fluorescence-labeled phosphatidylcholine incorporated into liposomal membrane) and CTD by western blotting. Note that soluble proteins remain at the bottom of this flotation gradient, while membrane-bound CTD floats to the top.