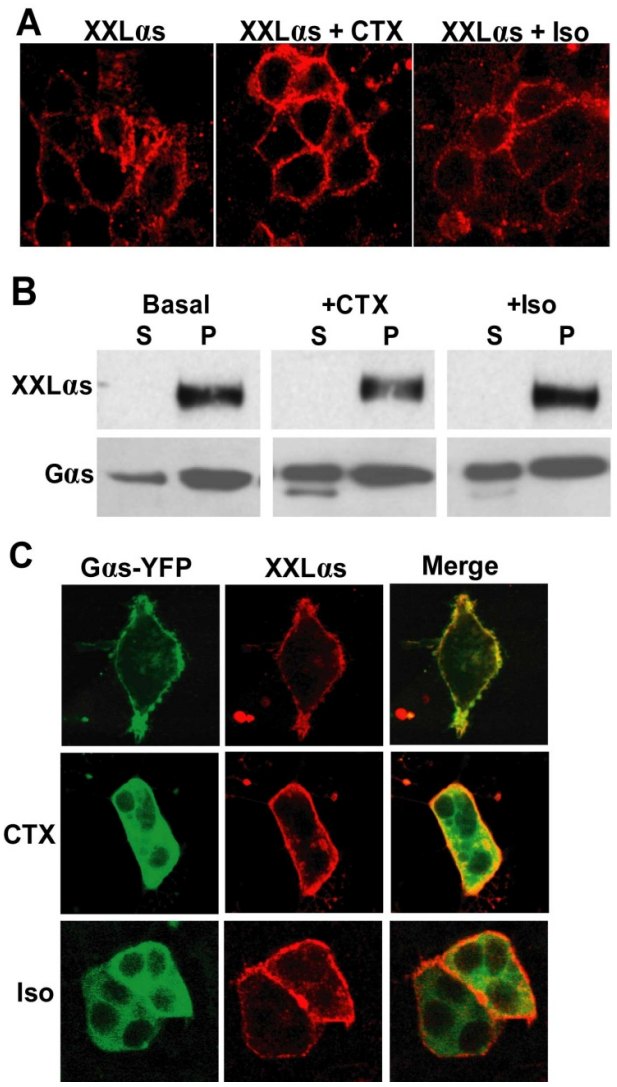

Fig- S1.

Supplemental Figure 1. XXLαs are targeted differently from wild-type Gsα upon activation. A-B: HEK293 cells were transiently transfected with cDNA encoding either HA-tagged Gsα or XXLαs. Forty-eight hours after transfection, cells were treated with 10^{-5} M isoproterenol for 20 min, or with 1μg/ml Cholera toxin (CTX) for four hours, and subcelluar localization of Gsα and XXLαs were examined by immunocytochemical (IC) analysis by using the anti-HA antibody (A), and Western blot analysis by using the anti-HA antibody (B). C: HEK293 cells were transiently co-transfected with cDNA encoding HA-tagged XXLαs and Gsα-YFP. Forty-eight hours after transfection, cells were treated with 10^{-5} M isoproterenol (iso) for 20 min or with 1μg/ml Cholera toxin (CTX) for four hours, and subcellular localization of XXLαs were examined by immunocytochemical (IC) analysis by using the anti-HA antibody (Red), and Gsα-YFP was visualized by green fluorescence directly through a confocal microscope.

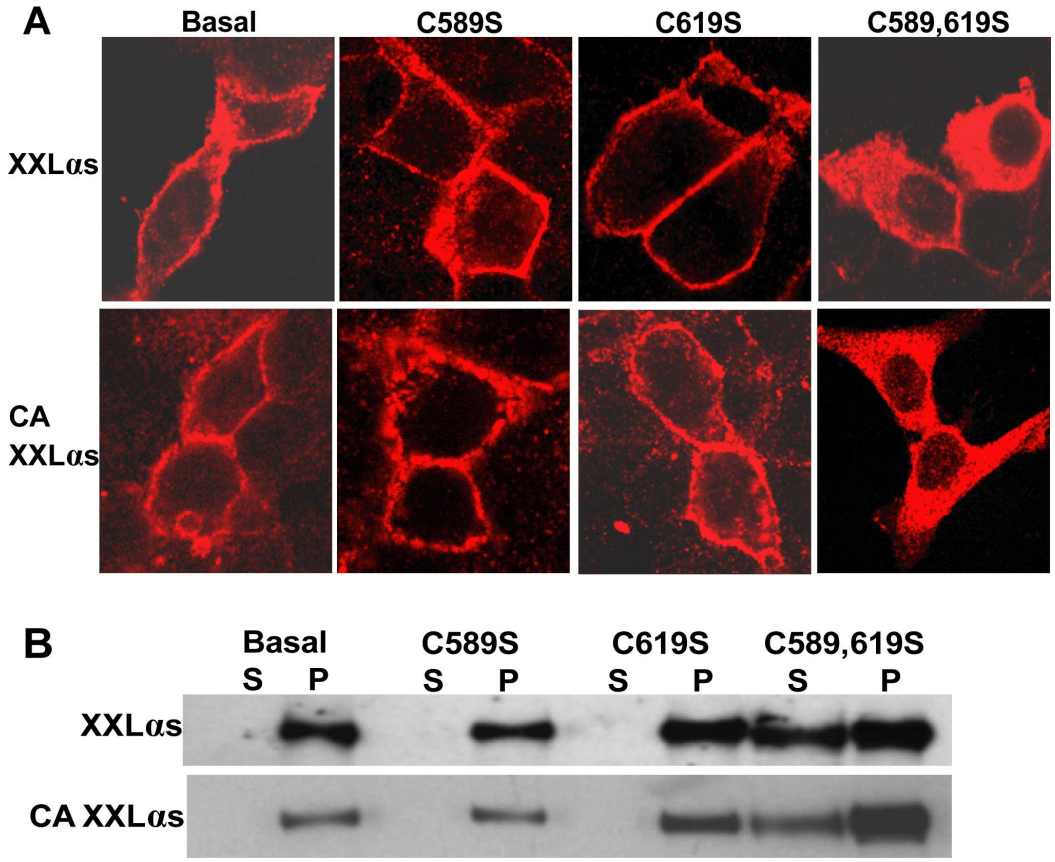

Fig- S2.

Supplemental Figure 2. Substitution of conserved cysteine residues in the XL domain and inhibition of protein palmitoylation disrupts plasma membrane targeting at the steady state. A: Immunocytochemical analysis of subcellular distribution for wild-type and Cys-to-Ser mutants of XXLαs in HEK293 cells by using the anti-HA antibody. Expression constructs encoding the wild-type or Cys-to-Ser mutants of XXLαs (C589, C619) were transiently transfected into HEK cells. Forty eight hours after transfection, the subcellular localization of these HA-tagged recombinant XXLαs were investigated. CA, constitutively active form carrying GTPase inhibiting mutation analogous to R201H. B: Western blots using the anti-Gsα C-terminal antibody performed for determining the subcellular localizations of Cys-to-Ser mutants of XXLαs in HEK293 cells. S, soluble fraction; P, particular fraction.