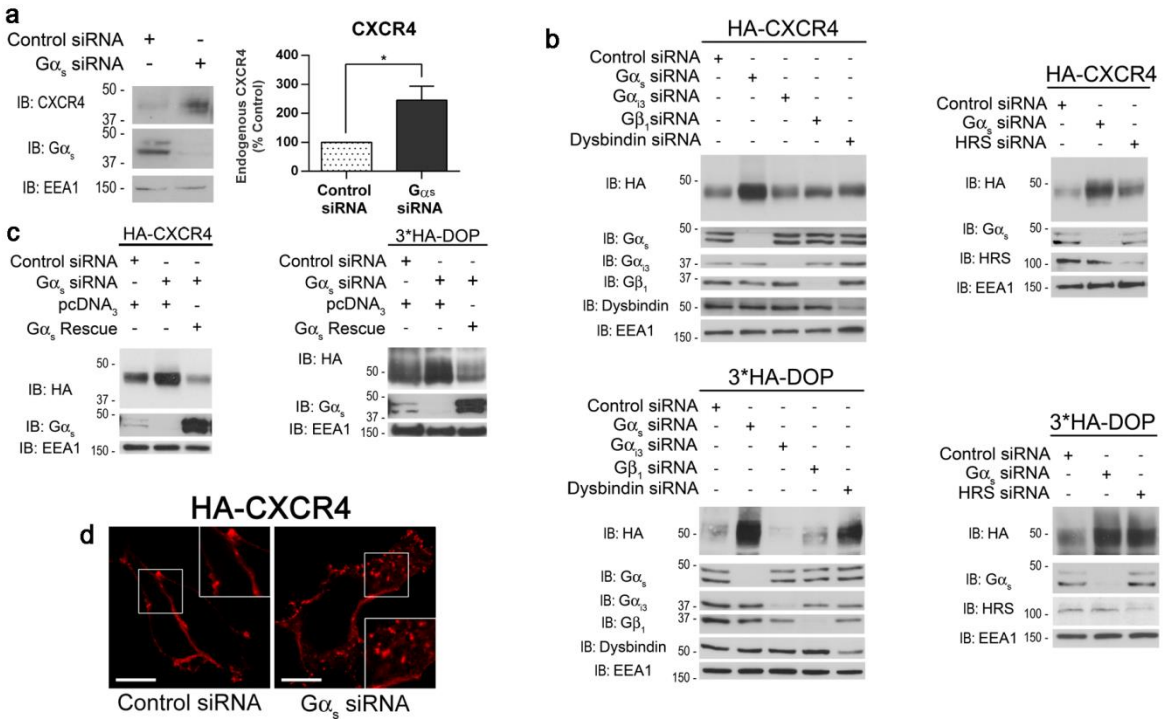
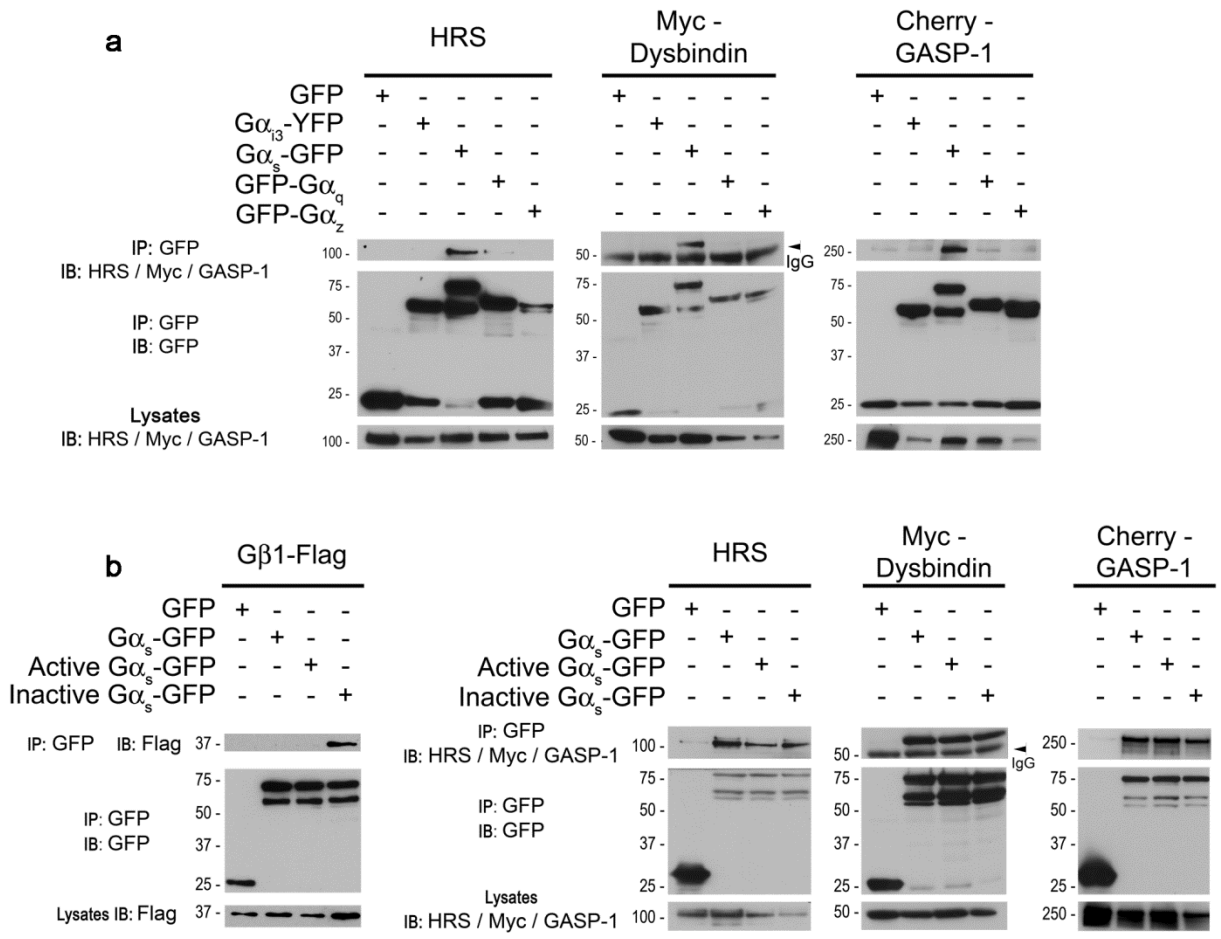
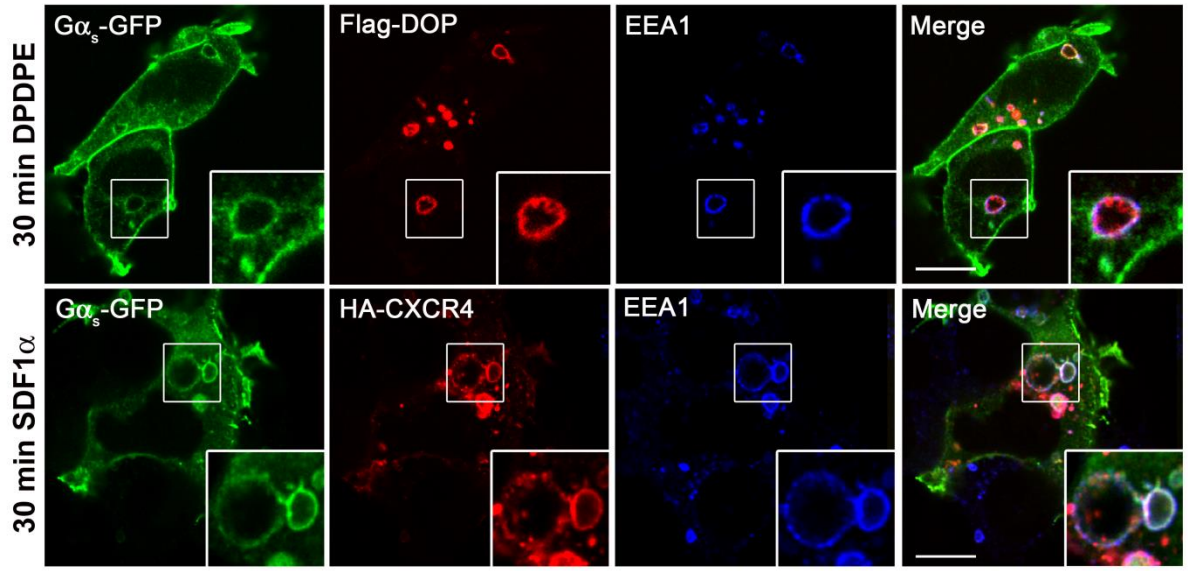
Supplementary Information



Supplementary Figure 1. Specificity of the effect of $G\alpha_s$ knockdown on the basal levels of CXCR4 and DOP. (a) Hela cells endogenously expressing CXCR4 receptor were transfected with control or $G\alpha_s$ siRNA for 72 h. Steady-state levels of these tagged-GPCRs were analyzed by immunoblotting using anti-CXCR4 antibody. EEA1, loading controls. The histogram depicts the quantification of CXCR4 endogenous level of expression presented as percentage of levels quantified in control cells. Values shown are means ± SEM of ≥3 separate experiments. * p≤0.05. (b) Effect of depletion of other heterotrimeric G proteins and components of the endosomal sorting machinery on the basal levels of HA-CXCR4 and Flag-DOP. HEK293 cells were transfected with control, $G\alpha_s$, $G\alpha_i3$, Gβ1, dysbindin or HRS siRNA. Twenty-four hours later, cells were transfected with HA-CXCR4 and HA-DOP and incubated for another 48 hours. Cells were lysed and analyzed by immunoblotting (IB) using the indicated antibody. EEA1 was used as a loading controls. (c) Expression of $G\alpha_s$ rescues the basal levels of HA-CXCR4 and HA-DOP. Control or $G\alpha_s$ siRNA-treated HEK293 cells expressing HA-CXCR4 or HA-DOP were transfected with pcDNA3 (control vector) or siRNA-resistant versions of the short and long forms of $G\alpha_s$ ($G\alpha_s$ Rescue) 10 h after the initial human $G\alpha_s$ siRNA transfection. Cells were lysed and analyzed by immunoblotting (IB) using the indicated antibody. EEA1 was used as loading controls. (d) Immunofluorescence analysis of the steady state distribution of HA-CXCR4 in control or $G\alpha_s$ siRNA-treated HEK293 cells. Cells were fixed, permeabilized, immunostained with anti-HA antibodies and processed for confocal microscopy analysis. In controls cells, HA-CXCR4 localized at cell surface whereas in $G\alpha_s$ siRNA treated cells, GPCR localized at cell surface and in intracellular compartments. Scale bars, 10 µm.



Supplementary Figure 2. Dysbindin and GASP-1 interact specifically with $G\alpha_s$-GFP independently of its activity state. (a) Dysbindin and GASP-1 do not interact with other Gα proteins. Lysate from HEK293 cells transiently transfected with GFP, $G\alpha_s$-GFP, $G\alpha_i3$-GFP, $G\alpha_q$-GFP or $G\alpha_z$-GFP together with HRS, Myc-dysbindin or Cherry-GASP1 were immunoprecipitated (IP) with anti-GFP and immunoblotted (IB) using the indicated antibody. Only $G\alpha_s$-GFP coimmunoprecipitated with all these proteins. (b) Constitutively active and inactive forms of $G\alpha_s$ similarly co-immunoprecipitated with dysbindin, HRS and GASP-1. HEK293 cells were transiently transfected with GFP, wild-type $G\alpha_s$-GFP, constitutively active $G\alpha_s$-GFP (Q227L) or inactive $G\alpha_s$-GFP (G226A; R280T; T284D; I285T and A366S) together with HRS, Myc-dysbindin or Cherry-GASP1. Cells were lysed, immunoprecipitated (IP) with anti-GFP and immunoblotted (IB) using the indicated antibody. Coimmunoprecipitation of endogenous Gβ1 was used as a control to confirm the activation status of $G\alpha_s$ mutants since Gβ1 only binds inactive $G\alpha_s$.



Supplementary Figure 3. $G\alpha_s$ colocalized with internalized GPCR on early endosomes. $G\alpha_s$-GFP and FLAG-DOP or HA-CXCR4 are together on early endosomes. HEK293 cells stably expressing Flag-DOP or transiently expressing HA-CXCR4 were transfected with $G\alpha_s$-GFP and Rab5Q79L, which created enlarged early endosomes. Cell-surface Flag-DOP or HA-CXCR4 was labeled with anti-Flag or HA at 4°C before incubation at 37°C in the presence of agonist for 30 min. Cells were fixed, permeabilized, stained with anti-GFP and anti-EEA1 (endogenous marker of early endosomes) and examined by confocal microscopy. The merged image indicates colocalization between $G\alpha_s$ and internalized Flag-DOP or HA-CXCR4 on EEA1-labeled enlarged endosomes. Scale bars, 10 μm.