GIPC recruits GAIP (RGS 19) to attenuate dopamine D_2 receptor signaling

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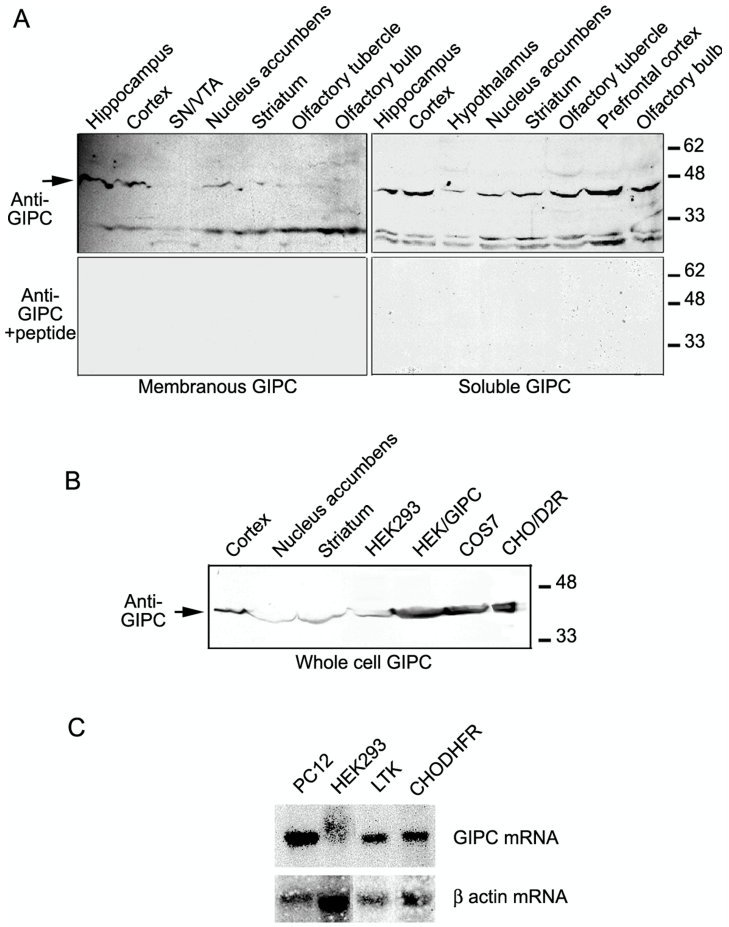
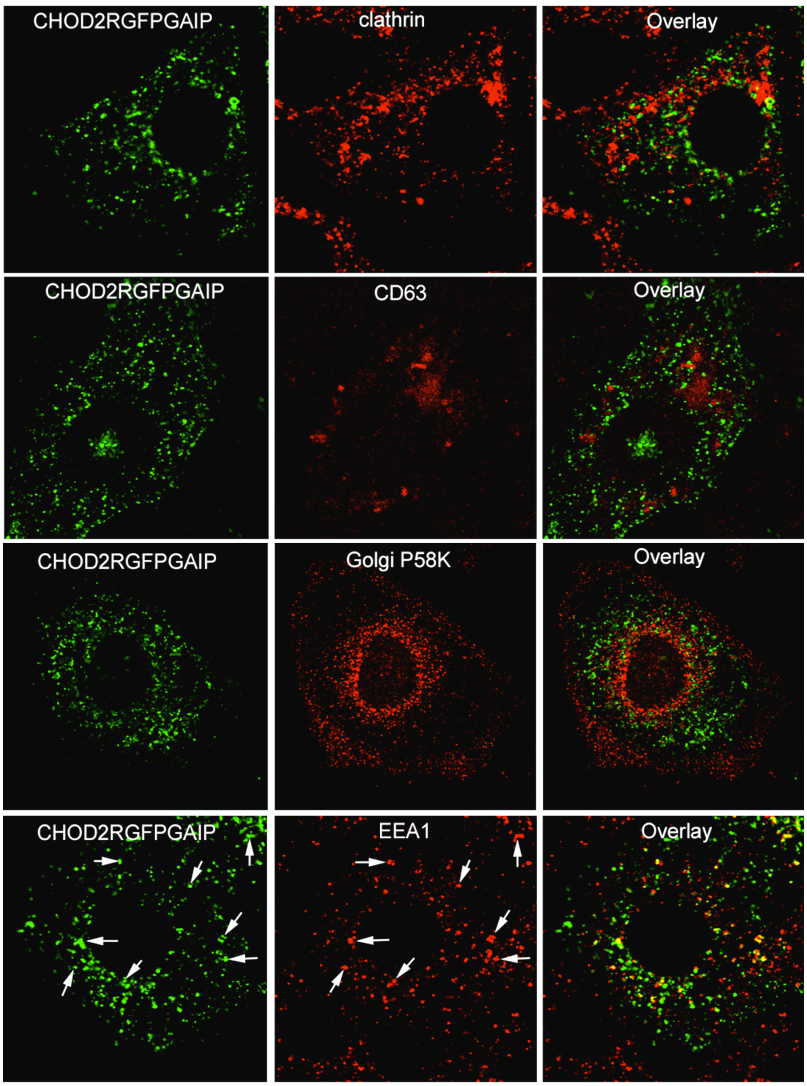
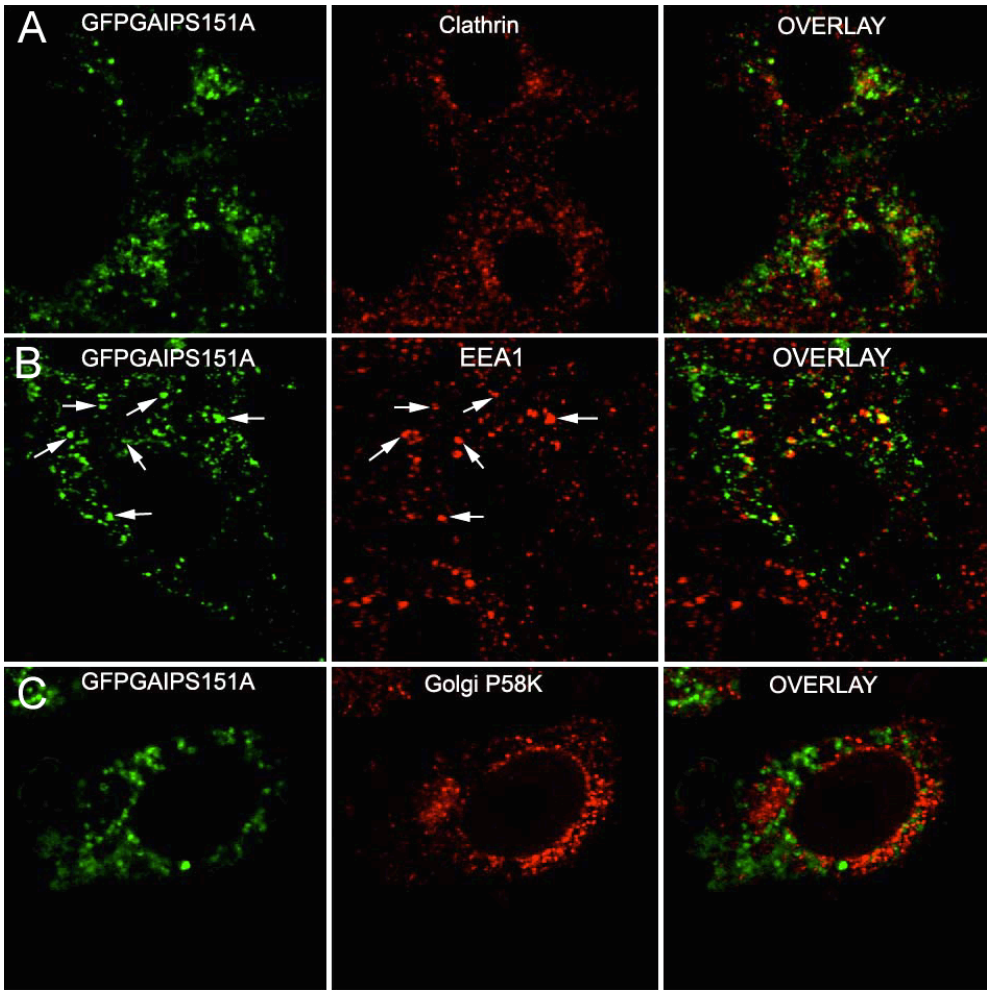
Supplementary Information Online



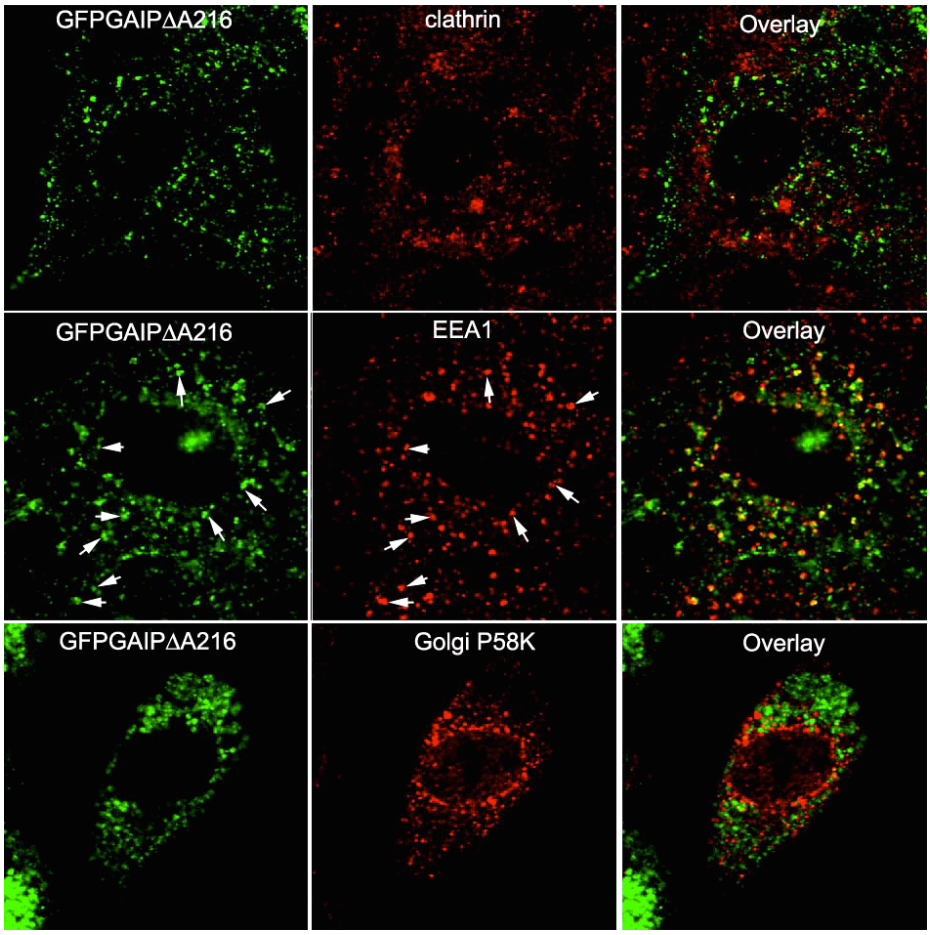
Supplementary information 1: Expression level of endogenous GIPC in various brain tissues and cell lines. (A) Membranes of dissected brain tissue lysates from three male Wistar rats were separated from soluble fraction by centrifugation and rinsed several times in sample buffer. Fifty micrograms of protein from each sample were resolved by 10 % SDS-PAGE and proteins were transferred to membranes. Blots were probed with the anti-GIPC N19 antibody in the presence or absence of the immunizing peptide N19-P (Santa Cruz Biotechnology, Santa Cruz, CA). Anti-GIPC immunoreactivity was completely circumvented by the addition of the immunizing peptide. The higher band of about 40 KDa coincides with the GIPC molecular weight reported in several previous studies. However, the lower apparent molecular weight species (about 30 KDa) of the GIPC-related immunosignals are unidentified (B) Analysis of GIPC expression level in whole cell lysate –(50 µg of protein) from various cell lines is indicated with brain tissue controls to allow comparison. (C) A comparison of GIPC mRNA expression level in total RNAs from various cell lines by Northern blot hybridized with GIPC (BC070505, nucleotides 98-475) and actin (BC002409, nucleotides 201-831) probes. GIPC was found expressed at a higher level in CHODHFR and CHO/D_2R cells than HEK293 cell line at the mRNA and the protein level.



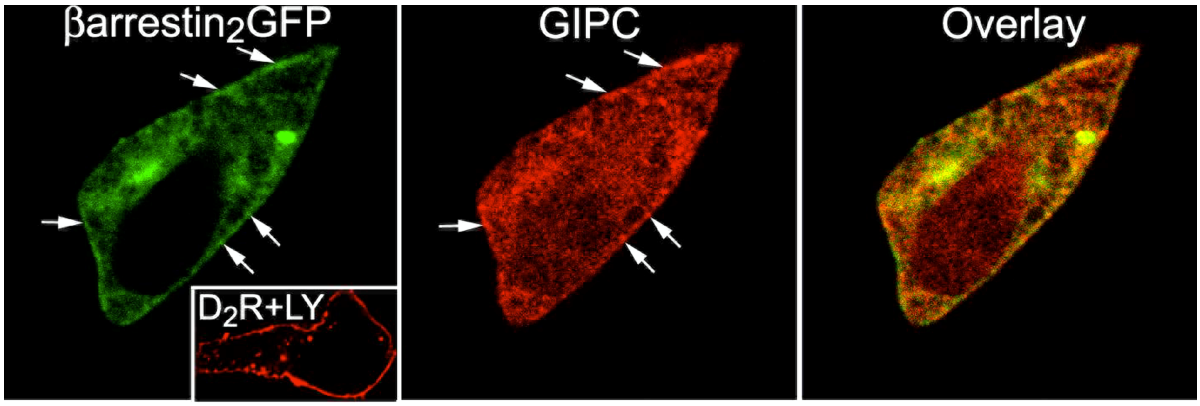
Supplementary information 2: Endosomal-based distribution of GFPGAIP in $CHOD_2R$ cells. GFPGAIP-stably transfected $CHOD_2R$ cells were fixed and permeated before subsequent detection of different subcellular markers. All markers were revealed with a CY3-conjugated anti-mouse antibody and GFP fluorescence visualized by confocal microscopy. Clathrin (A), CD63 (B), P58K (C), EEA1 (D), labeled the clathrin coats, lysosomes, Golgi apparatus, and early endosomes, respectively. Arrows indicate co-localization.



Supplementary information 3: Endosomal-based distribution of GFPGAIPS151A in $CHOD_2R$ cells. Clathrin (A), EEA1 (B) and P58K (C) markers were tested with their respective antibodies to characterize the subcellular distribution of GFPGAIPS151A in stably-transfected $CHOD_2R$ cells by confocal fluorescence microscopy. Arrows indicate co-localization.



Supplementary information 4: Endosomal-based distribution of GFPGAIP□A216 in CHO$_2$R cells. Clathrin (A), EEA1 (B) and P58K (C) markers were tested with their respective antibodies to characterize the subcellular distribution of GFPGAIP□A216 in stably-transfected CHO$_2$R cells by confocal fluorescence microscopy. Arrows indicate co-localization.



Supplementary information 5: β-arrestin2-GFP co-localized with GIPC upon D_2R activation. β-arrestin2-GFP and His_6GIPC were transiently co-transfected in HEK293 cells stably expressing mycD_2R. Cells were stimulated with 2 µM quinpirole (LY) for 5 min before detection of the His_6GIPC with anti-Xpress and CY3-conjugated secondary antibodies. Inset, detection of mycD_2R with anti-myc and CY3-conjugated secondary antibodies. β-arrestin2 was detected with the GFP fluorescence and visualized by confocal microscopy. His_6GIPC and β-arrestin2 co-localized at the PM as indicated by arrows, only when D_2R was activated.