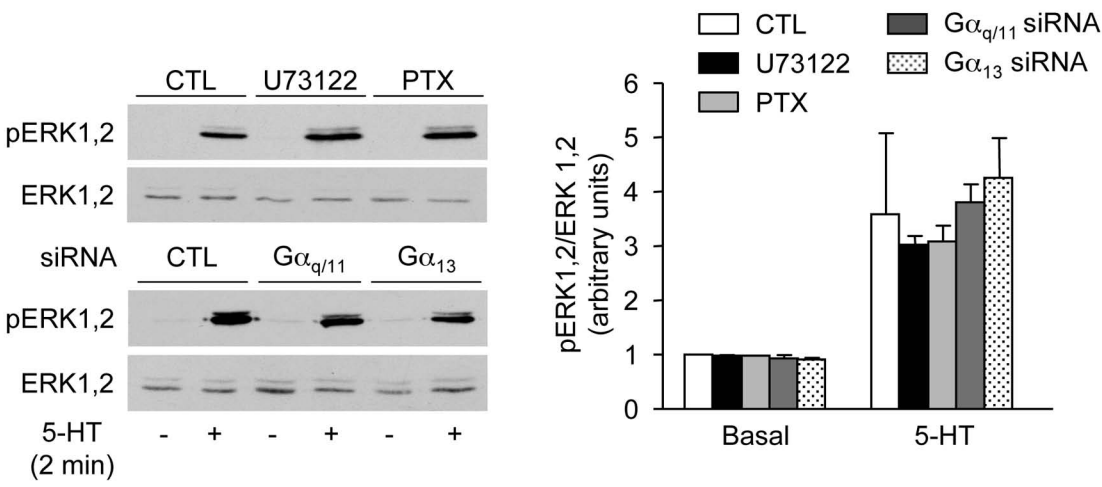
E08-04-0422 Marin

Supplemental Figure 1. 5-HT_{2C} receptor-mediated ERK1,2 signaling does not involve G proteins even after 2-min 5-HT exposure. HEK-293 cells, transfected with the 5-HT_{2C} receptor, were pretreated or not (CTL) with either U73122 (5 μM, 10 min) or Pertussis toxin (PTX, 1 μg/ml, 18 hr). For experiments using siRNAs, cells were transfected with either control (CTL siRNA), $G\alpha_{q/11}$ or $G\alpha_{13}$ siRNA as indicated in Figure 4. They were then exposed to 1 μM 5-HT for 2 min and ERK1,2 phosphorylation was analyzed by immunoblotting as indicated in the legend to Figure 2. Data are means ± SEM of values obtained in three independent experiments.

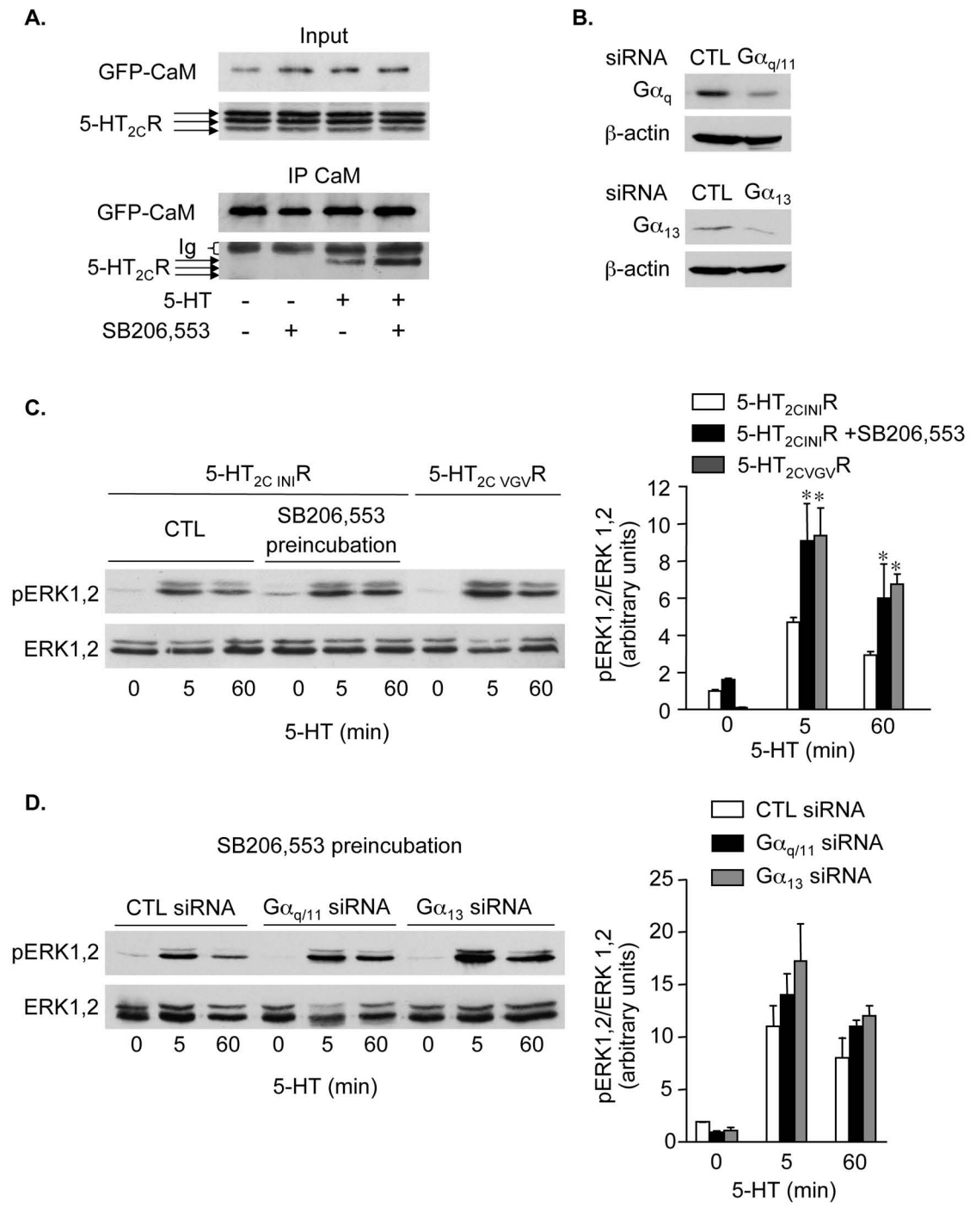
Supplemental Figure 2. G protein-independent ERK1,2 signaling mediated by 5-HT_{2C} receptors exhibiting enhanced plasma membrane localization. (A) HEK-293 cells co-transfected with GFP-CaM and wild-type 5-HT_{2C} receptor were pretreated for 18 hr with vehicle or SB206,553 (0.1 μM). After washing, they were exposed to either 5-HT (1 μM) or vehicle for 5 min. Solubilized protein extracts were immunoprecipitated with the polyclonal anti-GFP antibody. Immunoprecipitated proteins were analyzed by Western blotting using the monoclonal anti-GFP antibody and the polyclonal anti-5-HT_{2C} receptor 522 antibody. Inputs represent 5% of the total protein amount used in immunoprecipitations. The data illustrated are representative of three independent experiments. (B) $G\alpha_q$ and $G\alpha_{13}$ expression in HEK-293 cells transfected with the 5-HT_{2CINI} receptor and pretreated for 18 hr with SB106,553 (0.1 μM). The data are representative of three independent experiments performed on different cultures. (C,D) HEK-293 cells transfected with either 5-HT_{2CINI} or 5-HT_{2CVGV} receptor were exposed to 1 μM 5-HT for 5 and 60 min. When indicated, they were co-transfected with either control (CTL), $G\alpha_{q/11}$ or $G\alpha_{13}$ siRNA, or pretreated with SB206,553 (0.1 μM, 18 hr). ERK1,2 phosphorylation was analyzed by immunoblotting as indicated in the legend to Figure 2. Data are means ± SEM of values obtained in three independent experiments. * $p<0.05$ *vs.* corresponding value measured in cells expressing 5-HT_{2CINI} receptor and not pretreated with SB206,553.

Supplemental Figure 3. 5-HT_{2C} receptor-mediated ERK1,2 signaling does not involve Ras activation. HEK-293 cells, transfected with 5-HT_{2C} receptor or co-transfected with the receptor and a dominant-negative Ras mutant (RasN17), were deprived of serum for 18 hr and exposed to 1 μM 5-HT for 5 min. Ras expression, phosphorylated ERK1,2 and total ERK1,2 were analyzed by immunoblotting as indicated in the legend to Figure 2. The data illustrated are representative of three independent experiments performed on different cultures.

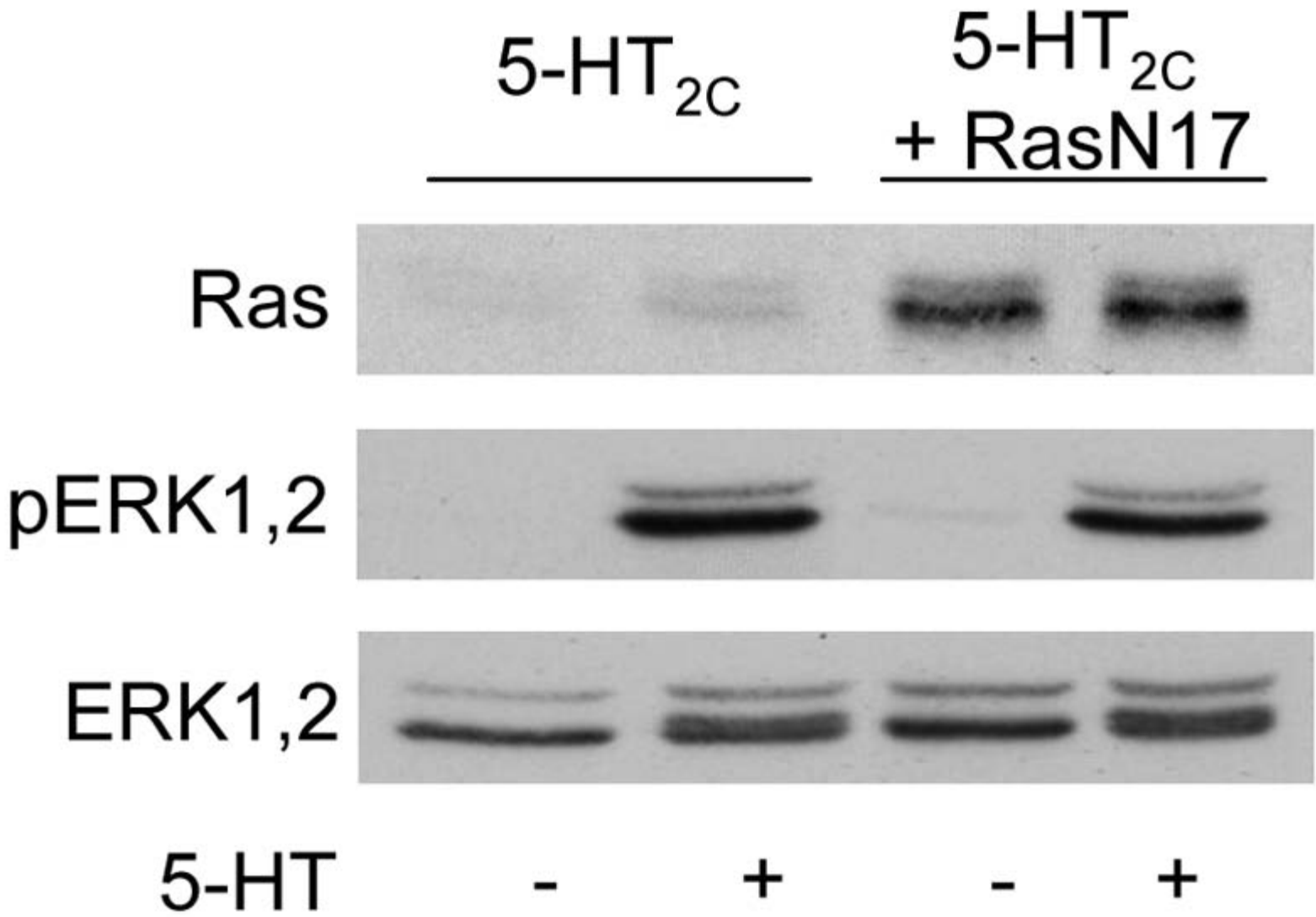
Supplemental Figure 4. Model of assembly of liganded 5-HT_{2C} receptor with calmodulin and β-arrestin. In the presence of 5-HT, β-arrestin binds to 5-HT_{2C} receptor, probably *via* a recognition motif located in the i2 loop and common to the rhodopsin family GPCRs. β-arrestin is also connected to the receptor C-terminus by a Ca^{2+}-CaM dimer, that binds to the receptor upon agonist stimulation. This CaM-dependent scaffold might function to stabilize 5-HT_{2C} receptor/β-arrestin complex.



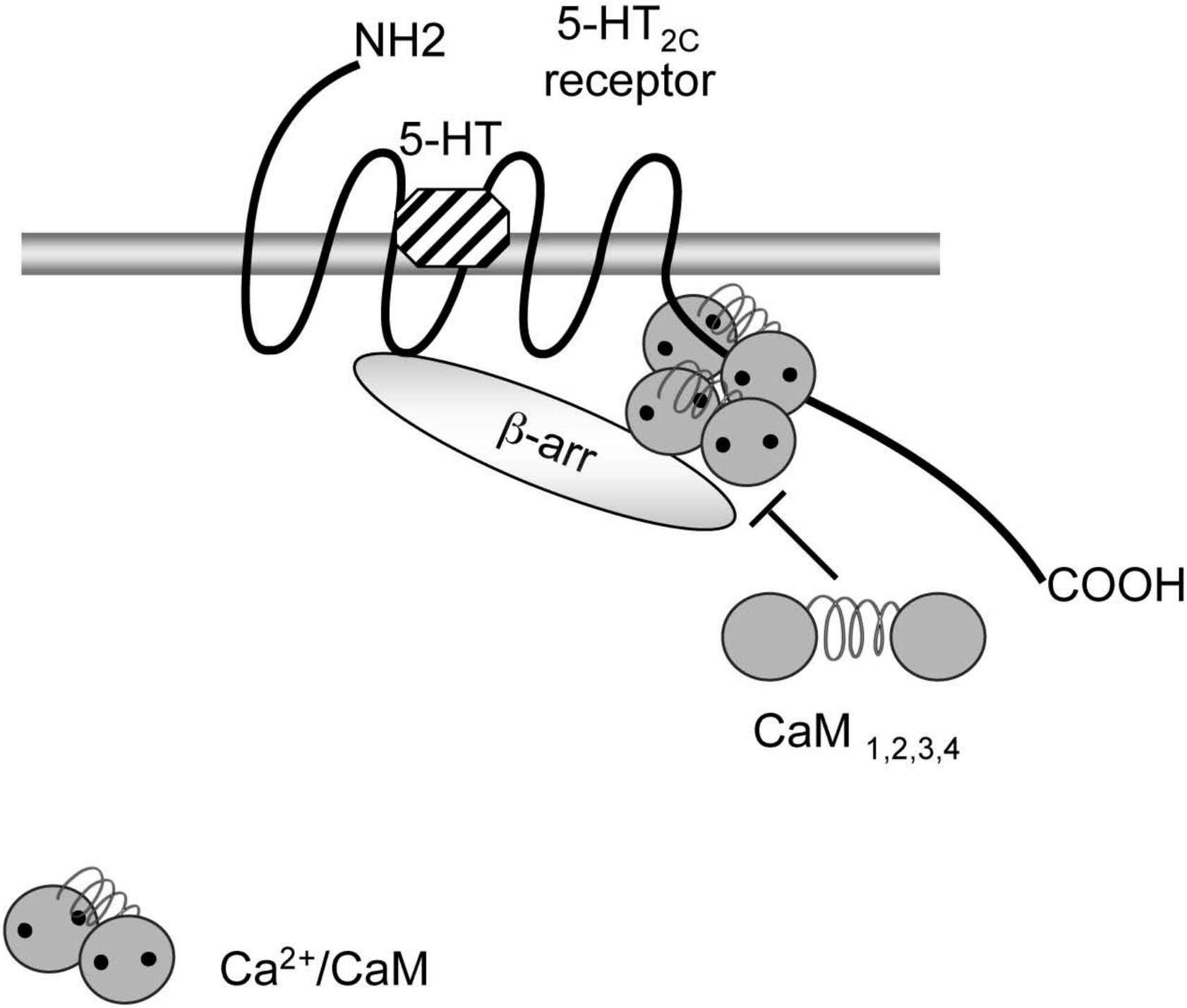
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4