Supporting Information

Zheng *et al*. 10.1073/pnas.0802253105

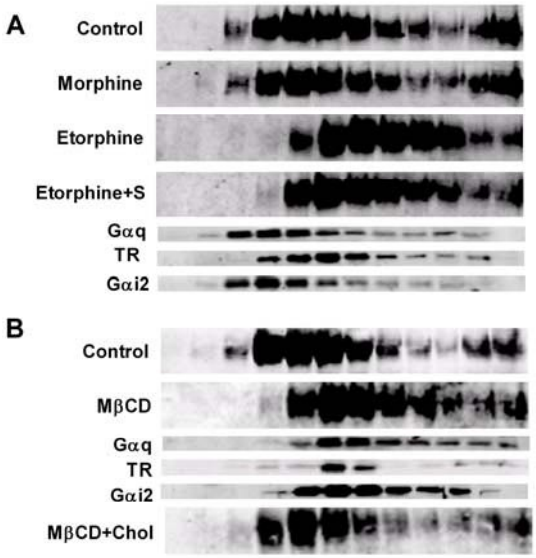

Fig. S1. Immunobloting image of tMOR localization. (*A*) Sucrose gradient fractionation of the cell homogenates. Immunoblotting image for Fig. 1*A*. HEK293 cells were treated with 1 μM morphine, 10 nM etorphine, or 10 nM etorphine with 0.4 M sucrose pretreatment for 10 min (Etorphine+S). Then MOR location on the cell membrane was determined, as described in *Materials and Methods*. Gαq represented the location of lipid raft domains, while transferin receptor (TR) showed nonraft domains. (*B*) Distribution of MOR after MβCD and cholesterol treatment. Immunoblotting image for Fig. 2. HEK293 were treated with 1 mM MβCD for 1 h (MβCD) or 1 mM MβCD for 1 h and then 10 μg/ml cholesterol for 3 h (MβCD+Chol). Immunoreactivities of Gαi2, Gαq, and TR were also detected.

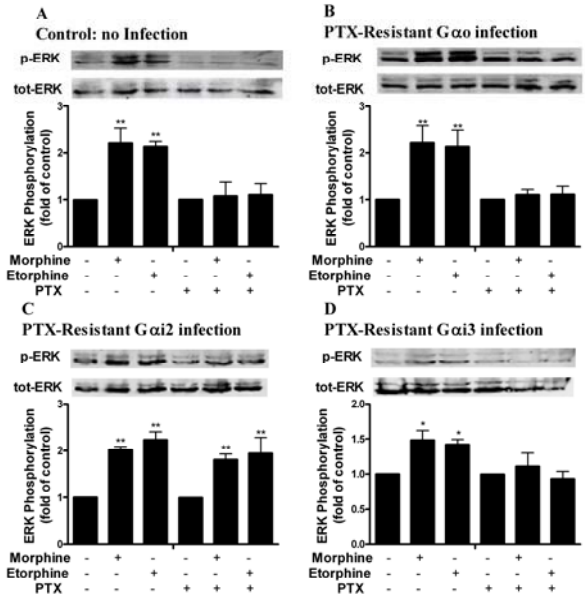

Fig. S2. MOR used Gαi2 to mediate ERK1/2 phosphorylation. N2A cells with MOR-HA stably expressed were infected with different PTX-insensitive Gα, control (*A*), Gαo (*B*), Gαi2 (*C*), and Gαi3 (*D*). Before experiments, 100 ng/ml PTX was used to treat the cells overnight. Then the cells were exposed to 1 μM morphine or 10 nM etorphine for 10 min. The bar graphs represent the averages of ERK1/2 phosphorylation increases from the basal level determined in experiments repeated at least three times. *, $P \leq 0.05$ and **, $P \leq 0.005$ in two-tail *t* test.

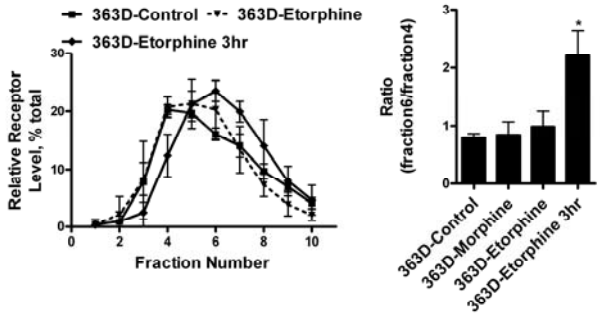

Fig. S3. MOR translocation in a mutant lacking GRK phosphorylation sites. HEK293 cells transfected with MOR363D were treated either with 1 μM morphine (10 min) or with 10 nM etorphine (10 min or 3 h). The locations of MOR in the sucrose gradient fractions were then determined. All of the experiments were repeated at least three times. *, $P \leq 0.05$ and **, $P \leq 0.005$ in two-tail *t* test.

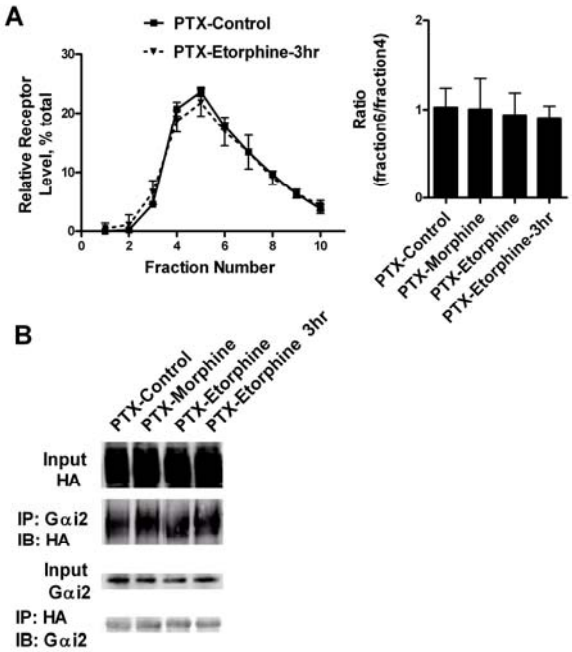

Fig. S4. PTX block the dissociation of Gαi2 from MOR and etorphine-induced translocation. (*A*) PTX pretreatment blocked the etorphine-induced MOR translocation. HEK293 cells were treated with 100 ng/ml PTX overnight followed by 10 nM etorphine (10 min or 3 h) treatment. Then the location of MOR on cell membrane was determined by the sucrose gradient fractionations. (*B*) PTX pretreatment blocked the agonist-induced decrease in MOR-Gαi2 interaction. HEK293 was treated as described in *A* and HA or Gαi2 antibodies were used for immunoprecipitation after the cells were exposed to either 1 μM morphine or 10 nM etorphine for 10 min or 3 h. All of the experiments were repeated at least three times. *, $P \leq 0.05$ and **, $P \leq 0.005$ in two-tail *t* test.