Supporting Information

Piggott *et al*. 10.1073/pnas.0712100105

SI Materials and Methods

To peform the immunoprecipitation-adenylyl cyclase assay, HEK293 cells were rinsed with PBS, resuspended in lysis buffer, and homogenized using a 23-gauge syringe. Competing proteins were added during homogenization. Cellular debris was removed by centrifugation, and the appropriate antibody was added. A 30 μl of aliquot was saved to measure AC activity in the starting material. Samples were rotated at 4°C for 1 h, and then Protein A Sepharose (30 μl of 50% slurry) was added for 1 h. Samples were washed two times with wash buffer (50 mM Hepes pH 7.4, 1 mM EDTA, 1 mM $MgCl_2$, 150 mM NaCl, 0.05% $C_{12}E_9$, and protease inhibitors), resuspended in buffer lacking NaCl with 0.04% $C_{12}E_9$, and analyzed by Western blotting and AC activity assays. For the latter, an AC mix was added to the IP containing the indicated stimulators and assay components as previously described (1).

1. Dessauer CW (2002) Kinetic analysis of the action of P-site analogs. *Methods Enzymol* 345:112–126.

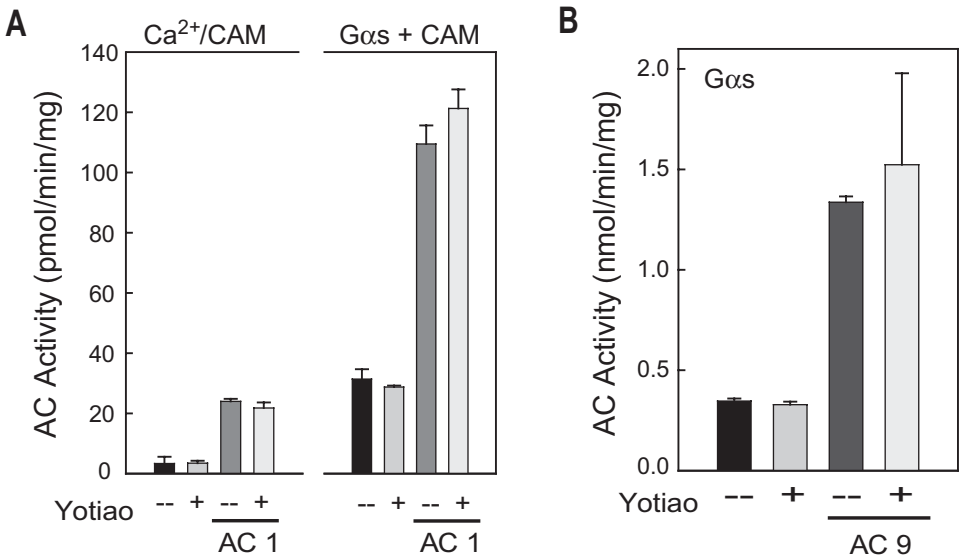

Fig. S1. Yotiao does not decrease the activity of AC 1 or 9. Membranes from HEK293 cells transfected with vector, AC 1 or 9 +/− Yotiao were assayed for AC activity. (*A*) Membranes containing AC1 were stimulated with 100 μM Ca^{2+} plus 300 nM calmodulin, or 50 nM activated Gαs plus Ca^{2+}/calmodulin (AC1). (*B*) Membranes containing AC9 were stimulated with 400 nM Gαs (AC9).