Effect of G protein $\beta\gamma$ on PLC$\beta$ activation. CHO cells stably expressing M2 muscarinic receptor were transiently transfected with $\alpha_6$, $\beta_1$, YFP-$\gamma_9$ subunits and PH-mCh sensor. The cells were imaged as described in the materials and methods section. Briefly, they are mounted on imaging chamber and sequentially exposed to 100 $\mu$M of M2 receptor agonist (carbachol) followed by 100 $\mu$M of antagonist (atropine). Images for YFP-$\gamma_{11}$ and PH-mCh were captured at every 10 sec interval. Translocation of YFP-$\gamma_{11}$ in response to M2 receptor activation was observed indicating that receptor activation status. The translocating $\beta\gamma$ reverse translocated on plasma membrane on deactivation of the receptor. On the other hand no change in localization of PH-mCh was observed indicating towards failure of G$\beta\gamma$ to activate PLC$\beta$ which leads to PIP2 hydrolysis. Substitution of G$\gamma_{11}$ with other gamma subunits ($\gamma_2$ or $\gamma_3$) has no impact on the observations.

To ascertain that the cells are not mutated for PLC$\beta$ activity, transient introduction of a G$\alpha_q$ coupled receptor, M3 induced significant translocation of PH-mCh indicating that the cells used in the study were completely functionally proficient. These observations clearly indicated that no PLC$\beta$ activation through G$\beta\gamma$ in living cells is detected.