Supplementary materials

Supplementary Figures

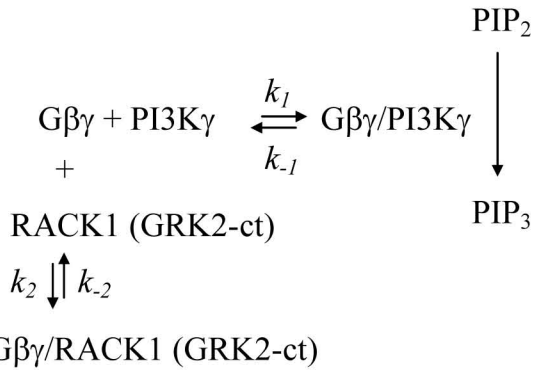
Supplementary Figure 1. Mathematical modeling of RACK1 and GRK2-ct dependent inhibition of Gβγ-mediated PI3Kγ activation. (A). Reactions that describe the quantitative relationship between the binding of RACK1 and GRK2-ct to Gβγ and Gβγ-dependent PI3Kγ activation. (B). Equations describing the affinities of PI3Kγ for Gβγ ($Kd1_{\_PI3K\gamma}$), RACK1 for Gβγ ($Kd2_{\_RACK1}$) and GRK2-ct for Gβγ ($Kd2_{\_GRK2\text{-}ct}$). (C). Equations describing the initial concentration of Gβ1γ2 ($[G\beta1\gamma2]_0$), PI3Kγ ($[PI3K\gamma]_0$), RACK1 ($[RACK1]_0$) and GRK2-ct ($[GRK2\text{-}ct]_0$).

Supplementary Figure 2. Comparison between empirical results and theoretical simulations of RACK1 and GRK2-ct mediated inhibition of PI3Kγ activation. The empirical results were taken from Figure 8A in the paper. Mathematical simulations were performed by Matlab using the following parameters, $[G\beta1\gamma2]_0$ = 100 nM, $[PI3K\gamma]_0$ = 5.6nM, [RACK1] = 10 nM-20 μM, [GRK2-ct] = 10 nM-20 μM, (A) $Kd1_{\_PI3K\gamma}$ = 5.6 nM, $Kd2_{\_RACK1}$ = 520 nM, $Kd2_{\_GRK2\text{-}ct}$ = 100nM; (B), $Kd1_{\_PI3K\gamma}$ = 5.6 nM, $Kd2_{\_RACK1}$ = 1040 nM, $Kd2_{\_GRK2\text{-}ct}$ = 200 nM; (C), $Kd1_{\_PI3K\gamma}$ = 2.8 nM, $Kd2_{\_RACK1}$ = 520 nM, $Kd2_{\_GRK2\text{-}ct}$ = 100nM. Dash lines, empirical data; Solid lines, theoretical simulations.

Supplementary videos 1-5. The chemotactic response and translocation of PH-AKT-GFP in transiently transfected dHL60 cells to a point source of fMLP (10 μM) delivered by a micropipette. Video 1, cells expressing PH-AKT-GFP alone; Video 2, cells transfected with PH-AKT-GFP together with RACK1 siRNA; Video 3-1 and 3-2, cells transfected with PH-AKT-GFP together with RACK1; Video 4, cells transfected with PH-AKT-GFP together with BD1-2; Video 5, cells transfected with PH-AKT-GFP together with BD5-7. Videos 1, 2, 3-1, 4 and 5 are related to Figure 10A-E. The first frame of each video shows the cells prior to the exposure to fMLP. The position of the

micropipette filled with fMLP is indicated by an asterisk in the second frame of each video. Videos are played at 6 frames per second and can be opened by QuickTime. Note that the cell in video 3-2 failed to migrate toward the chemoattractant probably due to higher levels of RACK1 overexpression.

A



B

$$Kd1_{\_PI3K\gamma} = \frac{k_{-1}}{k_1} = \frac{[G\beta\gamma][PI3K\gamma]}{[G\beta\gamma/PI3K\gamma]}$$

$$Kd2_{\_RACK1} = \frac{k_{-2}}{k_2} = \frac{[G\beta\gamma][RACK1]}{[G\beta\gamma/RACK1]}$$

$$Kd2_{\_GRK2\text{-}ct} = \frac{k_{-2}}{k_2} = \frac{[G\beta\gamma][GRK2\text{-}ct]}{[G\beta\gamma/GRK2\text{-}ct]}$$

C

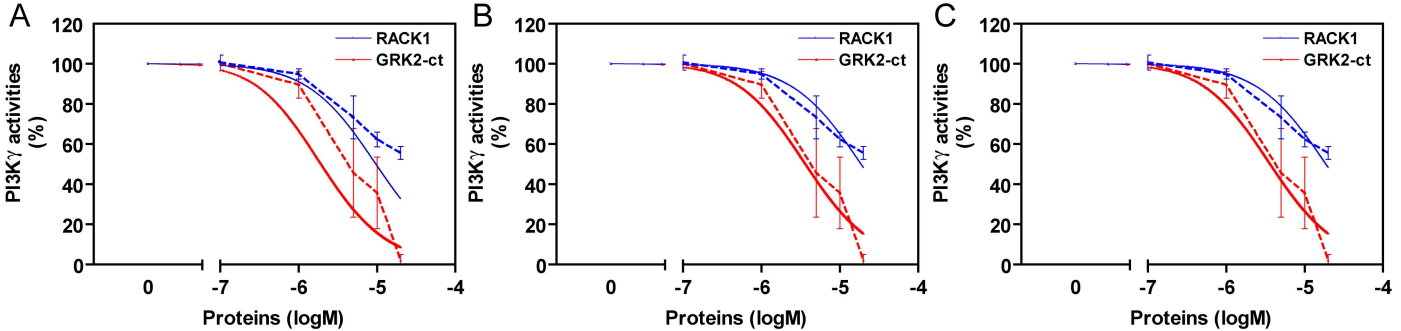
$$\mathbf{[G\beta1\gamma2]_0=[G\beta1\gamma2]+[\ G\beta1\gamma2/PI3K\gamma]+[\ G\beta1\gamma2/RACK1]}$$

$$\mathbf{[PI3K\gamma]_0=[\ PI3K\gamma]+[G\beta1\gamma2/PI3K\gamma]}$$

$$\mathbf{[RACK1]_0=[RACK1]+[G\beta1\gamma2/RACK1]}$$

$$\mathbf{[GRK2\text{-}ct]_0=[GRK2\text{-}ct]+\ [G\beta1\gamma2/GRK2\text{-}ct]}$$

Supplementary Figure 1



Supplementary Figure 2