Supplemental Data

Statins perturb Gβγ signaling and cell behavior in a Gγ subtype dependent manner

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Molecular Pharmacology

Supplemental Figure Legends

Supplemental Figure 1

A) **Comparison of cellular distribution of WT mCh-Gγ3 with and without +Flu and mCh-Gγ3 (C72A) mutant.** While WT mCh-Gγ3 expressing control cells showed a strong distribution of Gγ3 on both PM and IMs, +Flu cells having lost their ability to bind to IM exhibited Gγ3 only on the PM (Yellow arrow). Comparatively mCh-Gγ3(C72A) mutant expressing cells showed Gγ3 distribution all over the cytosol, but not bound to either PM or IMs (scale bar: 5 μm).

B) **Comparison of the extents of Gγ membrane binding inhibition by fluvastatin.** As the numerical value indicates, while farnesylated Gγ1 exhibited the highest inhibition, the other geranylgeranylated Gγs showed comparatively low inhibition. One-way ANOVA was performed, and the data were statistically different F (4, 77) = 79.19, P<0.05 (error bars: SD, n > 10, p < 0.05).

C) **Gγ-type independent inhibition of IM binding by fluvastatin.** HeLa cells expressing CFP-KDEL and either mCh-Gγ9 or Gγ3. Compared to control cells of both Gγ9 and Gg3 with clear distribution of Gγ bound to IMs (yellow arrow), regardless of the Gγ subtype, +Flu cells exhibited absence of Gγ on the IMs. CFP-KDEL shows the intact IMs even after +Flu treatment to cells (scale bar: 5 μm)

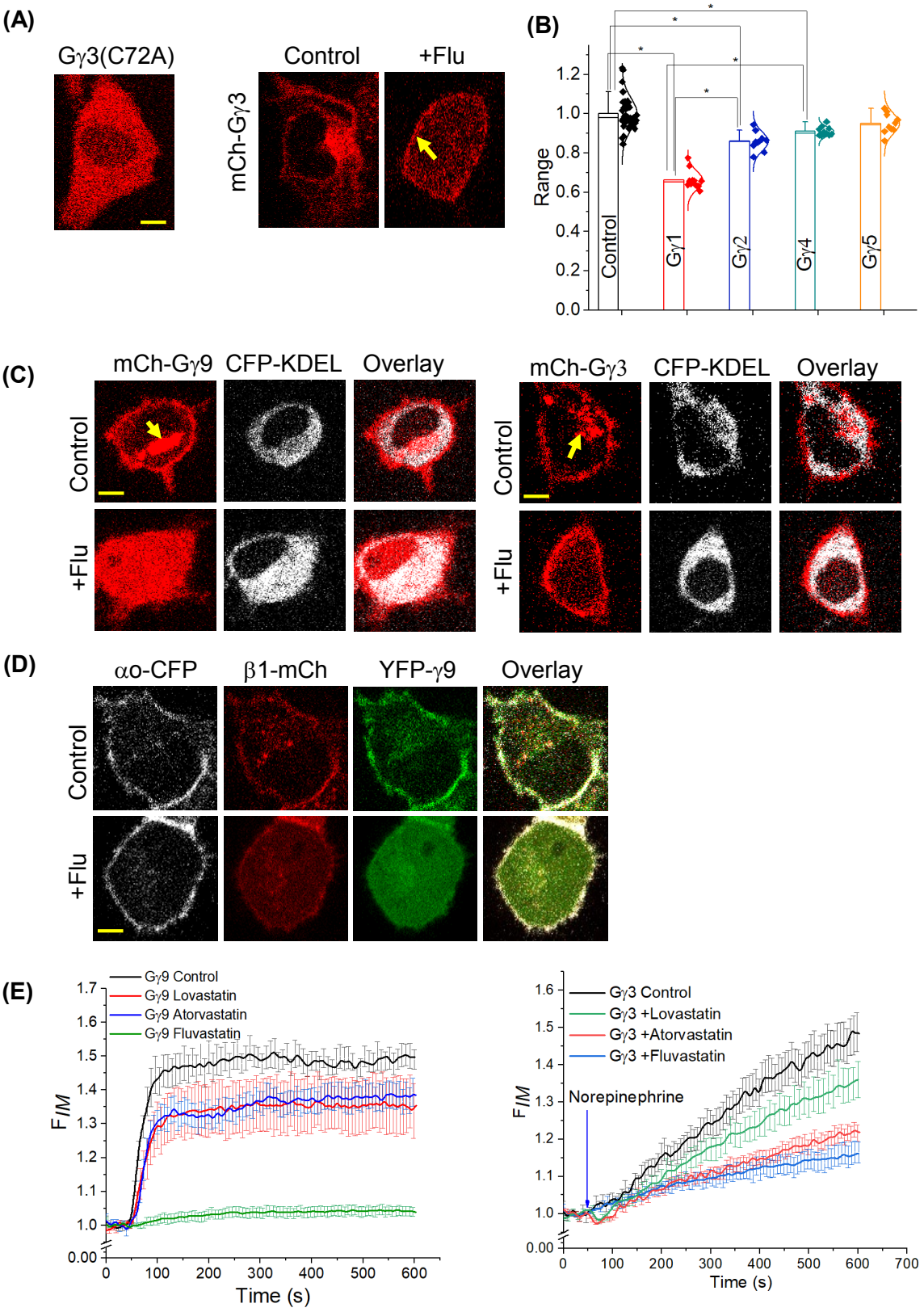
D) **Effects of fluvastatin treatment on subcellular distribution of heterotrimeric G protein subunits.** HeLa cells expressing Gαo-CFP, Gβ1-mCh, and Gg9-YFP. Compared to control cells, PM localization of Gαo subunit of many +Flu cells remained intact. However, both Gβ1 and Gγ9 showed primarily a cytosolic distribution (scale bar: 5 μm).

E) **The varying effect of Gγ3 and Gγ9 translocation from PM to IM by different drug.** Plots shows the extent of translocation of Gγ9 (left) and Gγ3 (right) with different drug treatment. Compared to the effect on +Flu cells, in the Gγ9 and Gg3 expressing cells the effect by +Ator and +Lov is moderate. (error bars: SEM, n > 12, p < 0.05)

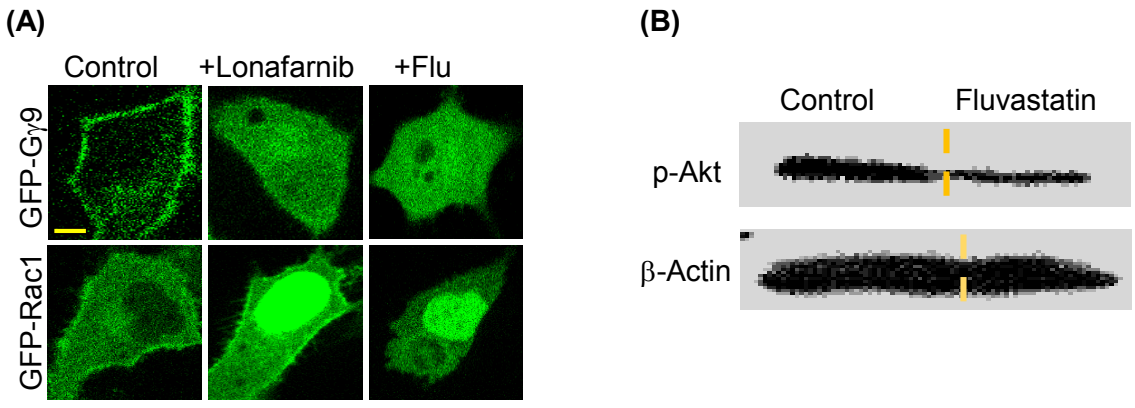
Supplemental Figure 2

A) **Comparison of prenylation-inhibition by fluvastatin and farnesyl transferase inhibitor, Lonafarnib.** Hela cells expressing GFP-Gγ9 and GFP-Rac1 exhibited identical and complete inhibitions of PM-binding when exposed to fluvastatin and lonafarnib, indicating that both inhibit farnesylation (scale bar: 5 μm).

B) The original images of Western blot analysis showing the expression of p-Akt and β-Actin with and without Fluvastatin. The yellow dashed line indicates the spliced-border where the band splicing was done in the main figure, due to merging of bands.



Supplemental Figure 1



Supplemental Figure 2