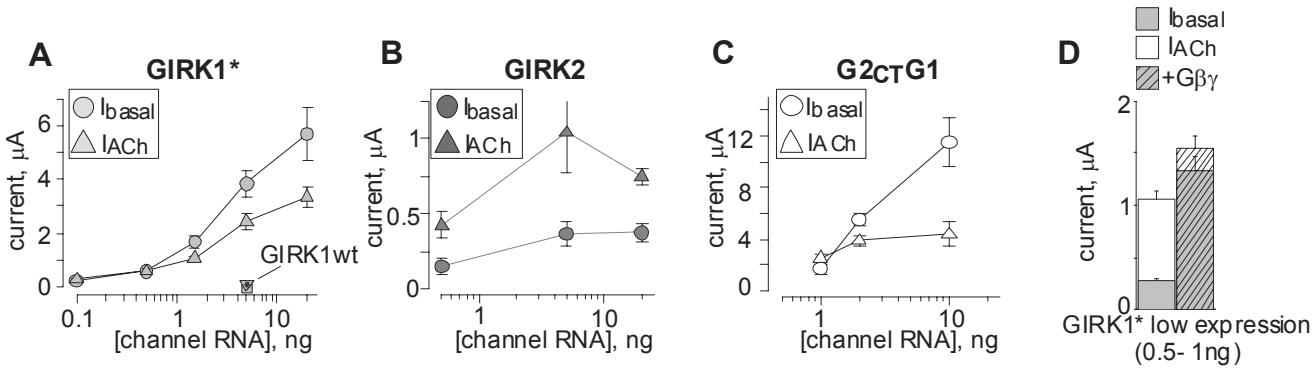
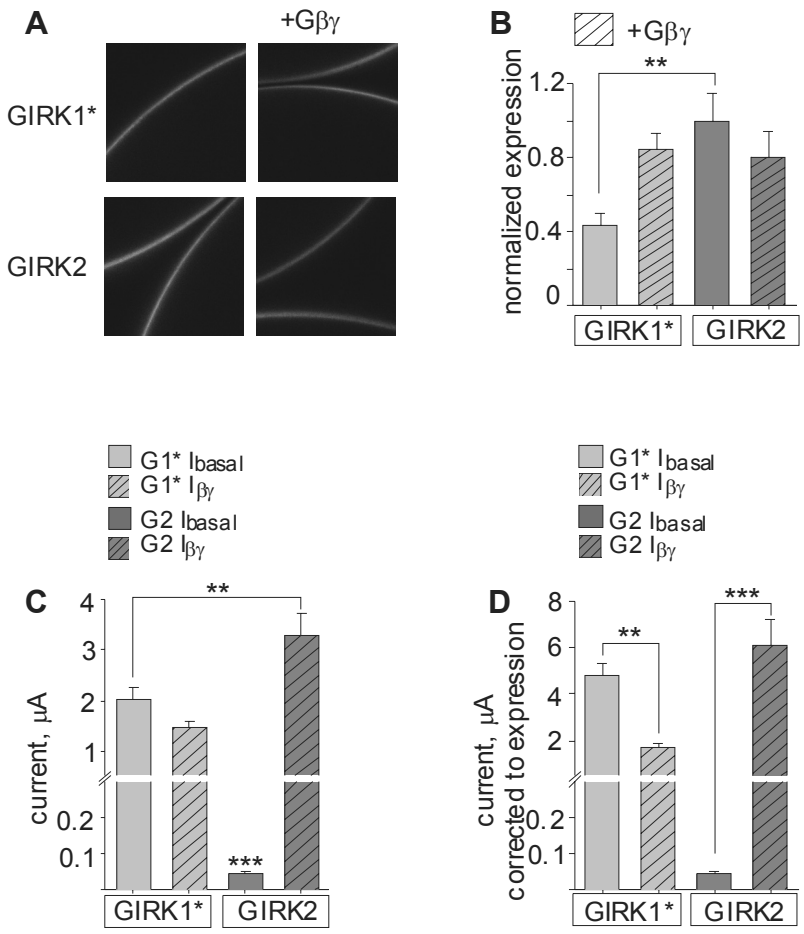
Supplemental material.

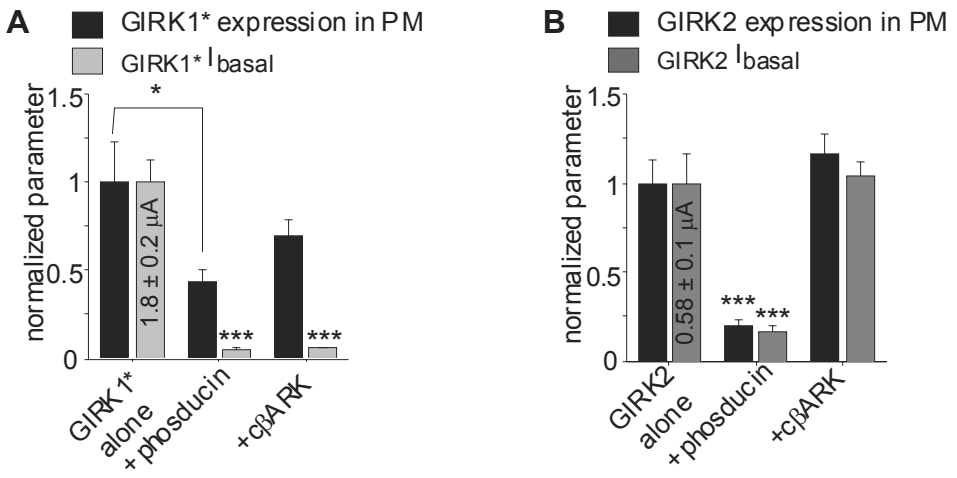
Supplemental Figures



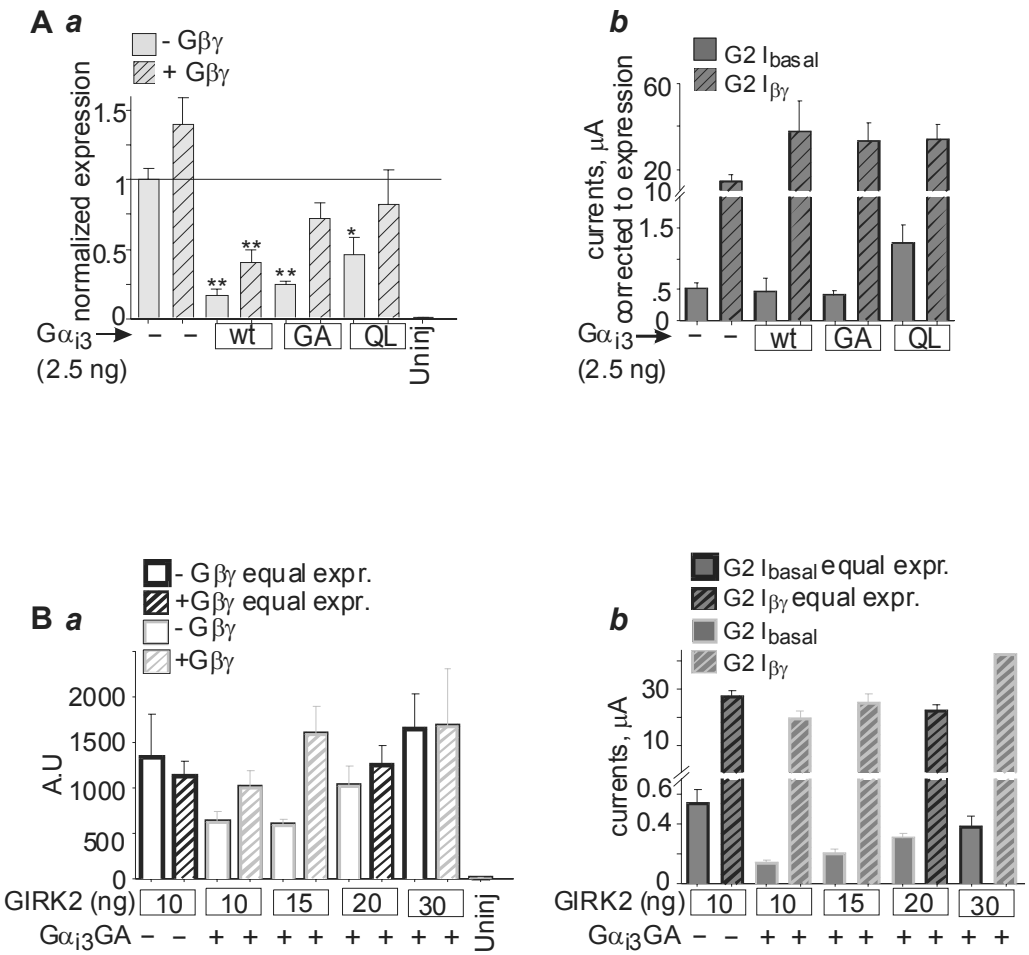
Supplemental Fig. 1. Titrated expression of GIRK1*, GIRK2 and $G2_{CT}G1$ chimera. (A-C), titrated expression of GIRK1* (A), GIRK2 (B) and $G2_{CT}G1$ chimera (C). 0.5 ng RNA of m2R was coinjected with the indicated amounts of RNA of the channel. Average I_{basal} (circles) and I_{ACh} (triangles) measured in 24 mM K^+ are shown (for all the channels); n=4-5 for each point. Only very small I_{basal} and I_{ACh} were produced by injection of 5 ng wt GIRK1 alone, without the GIRK5 antisense (A, arrow). (D) GIRK1* was activated by Gβγ when expressed at low expression levels (0.5-1 ng RNA/oocyte). The bottom bar shows I_{basal}, the top empty bar shows I_{ACh}, the total height of each bar presents I_{total}. Diagonal strips indicate coexpression of Gβγ. n=20-21.



Supplemental Fig. 2. GIRK1* and GIRK2 homomers express similarly in the plasma membrane. Injection of equal doses of RNA (10 ng each) of GIRK1* and GIRK2 results in similar levels of expression of these channels in the PM. Confocal images (A) and summary of PM levels (B) of whole oocytes expressing YFP-labeled GIRK1* or YFP-labeled GIRK2 in the same experiment, with or without Gβγ coexpression, are shown. (C, D) Summary of the basal and Gβγ-evoked currents in the same experiments as in A, B. GIRK1* is depicted in light grey and GIRK2 in dark grey. C summarizes the recorded currents and D shows the same but with a correction to changes in PM expression measured in each experiment (as shown in B). 2 batches of oocytes, n=16-19. G1* stands for GIRK1*, G2 for GIRK2. **, $p < 0.01$; ***, $p < 0.001$.



Supplemental Fig. 3. The effect of coexpression of Gβγ scavengers, m-phosducin and m-cβARK on GIRK1* and GIRK2 homomeric channels. (A) PM expression level of GIRK1* (black, examples in Fig. 5A) and I_{basal} (grey), each normalized to the same parameter in control (GIRK1* only) group. The actual average amplitude of I_{basal} (in μA) in the control group is shown within the corresponding bar. GIRK1* currents were measured in 24 mM external K^+, GIRK2 - in 48 mM external K^+ . (B) PM expression of GIRK2 (black, examples in Fig. 5A) and I_{basal} (dark grey), each normalized to the same parameter in control (GIRK2 only) group. n= 20-21. *, $p<0.05$; ***, $p < 0.001$.



Supplemental Fig. 4. GIRK2 is not regulated by $G\alpha_{i3}$. (A*a*) Summary of changes in the amount of GIRK2 channels in PM caused by $G\alpha_{i3}$ (wt and GA, QL mutants) and Gβγ. Data obtained by measurements in giant excised patches using anti GIRK2 antibody, or in whole oocytes with the external HA tag (from 4 different experiments) were pooled. Statistical comparisons were done using one way ANOVA followed by Dunnett's test (against the control group, GIRK2 alone). n=13-26. (A*b*) The effect of coexpression of $G\alpha_{i3}$ (wt, GA or QL, 2.5 ng each) on GIRK2 I_{basal} and $I_{\beta\gamma}$. The recorded currents of each experiment were corrected to changes in PM expression, as measured in the same experiment. The corresponding $R_{\beta\gamma}$ is shown in Fig. 8B. n=12-28. (B) In one experiment, $GIRK2_{HA}$ PM expression was titrated by injecting varying amounts of $GIRK2_{HA}$ RNA, in the presence of $G\alpha_{i3}GA$ (2.5 ng RNA), with or without Gβγ coexpression (5 and 1 ng RNA, respectively). Representative currents and confocal images from oocytes with similar PM expression are shown in Fig. 8C*a*. (*a*) Increasing amount of $GIRK2_{HA}$ RNA were injected, as indicated below the bars, to produce similar PM expression. Bars framed with thick black lines indicate the groups of oocytes selected as having equal expression, for the summary shown in Fig. 8C. (*b*) Summary of the currents; bars framed with thick black lines indicate equal PM expression, strips denote coexpression of Gβγ. $R_{\beta\gamma}$ is shown in Fig. 8C*c*. n=8. *, $p < 0.05$; **, $p < 0.01$.