

SUPPLEMENTAL DATA

EXPERIMENTAL PROCEDURES

Electrophysiology-

To confirm whether or not GB1a-i1Cer, -i2Cer or -i3Cer construct paired with GB2 wild-type (wt) is functional, the inward current through G-protein coupled inwardly rectifying potassium type 2 (GIRK2) channel upon receptor activation was recorded. The membrane currents of cells expressing Cerulean were measured using whole cell patch-clamp procedures with Axopatch 200B amplifier and pClamp 9 software (Axon Instruments). Macroscopic whole cell K⁺ currents were recorded at a holding potential

of -80 mV, using the following solutions. The normal internal solution contained 120 KCl, 5 K₂-ATP, 10 NaCl, 3 EGTA, 10 HEPES, 0.1 CaCl₂, 4 MgCl₂ (in mM) (pH 7.4, adjusted with KOH). Right before the experiments, the internal solutions were supplemented with GTP (final 0.3 μM) to fully activate the GIRK channel. The external solution contained 140 NaCl, 1 CaCl₂, 4 KCl, 0.3 MgCl₂, 10 HEPES (in mM) (pH 7.4, adjusted with NaOH). One minute prior to the 100 μM GABA application, bath solution was switched to 140 mM KCl solution to increase the driving force for K⁺ ions.

FIGURE LEGENDS

Supplemental data 1. Omitting the subtraction of bleed-through fraction of EYFP does not cause qualitative alteration in FRET changes. *A*, Left: FRET decreases by 100 μM GABA from the GB1a-i2EYFP & GB2-i2Cer pair, acquired by the protocol used throughout the present study (no EYFP excitation). Right: plots of mean ± se, n=12. *B*, Left: FRET decreases from the same set of cells as in *A* acquired by a protocol that also excites EYFP. For the calculation, EYFP bleed-through fraction into the FRET channel by 442-nm excitation was experimentally determined as 0.18, and was incorporated into a formula as follows:

$$nF/Cerulean = [I_{FRET} - (I_{Cerulean} \times 0.37 + I_{EYFP} \times 0.18)] / I_{Cerulean}.$$

Right: plots of mean ± se, n=12. Although exhibiting lower baseline and enhanced change, the direction of FRET change is kept the same as in *A*.

Supplemental data 2. GB1a intracellular loop constructs are functional when paired with GB2 wild-type (wt). GIRK channel currents evoked by applying 100 μM GABA for 30 s (black bars) are shown. Holding potential was -80 mV. *A*, GB1a wt & GB2 wt. *B*, GB1a-i1Cer & GB2 wt. *C*, GB1a-i2Cer & GB2 wt. *D*, GB1a-i3Cer & GB2 wt.

Supplemental data 3. Counteractions in the GB1a-i2 & GB2-i2 and the GB1a-i2 & GB2-i1 pairs. The FRET pairs exhibited synchronized positive-going Cerulean and negative-going EYFP fluorescence intensities when 100 μM Baclofen was applied for 60 s. All intensities are shown in arbitrary units. *A*, Representative traces from the GB1a-i2EYFP & GB2-i2Cer pair. *B*, Representative traces from the GB1a-i2Cer & GB2-i1EYFP pair.

Supplemental data 4. When a saturated concentration of GABA was used, CGP7930 still potentiated the FRET decrease from the GB1a-i2 & GB2-i2 pair, but not the one from the GB1a-i2 & GB2-i1 pair. *A*, Left: individual traces from the GB1a-i2EYFP & GB2-i2Cer pair. 100 μM GABA was applied for 300 s (long black bar). Within this period, DMSO was added as a vehicle for 60 s (short black bar), followed by 100 μM CGP7930 for 60 s (short black bar). Note the FRET decreases first evoked by GABA were further enhanced by CGP7930. Right: plots of mean ± se, n=10. *B*, Left: individual traces from the GB1a-i2EYFP & GB2-i1Cer pair. Application profiles are the same as those in *A*. Right: plots of mean ± se, n=9. *C*, Summary of

FRET changes shown in *A* and *B*. The Y-axis represents changes in $\Delta(nF/Cerulean)$. Bars represent the normalized FRET decreases by 100 μ M GABA only, + DMSO, and + 100 μ M CGP7930 applications.

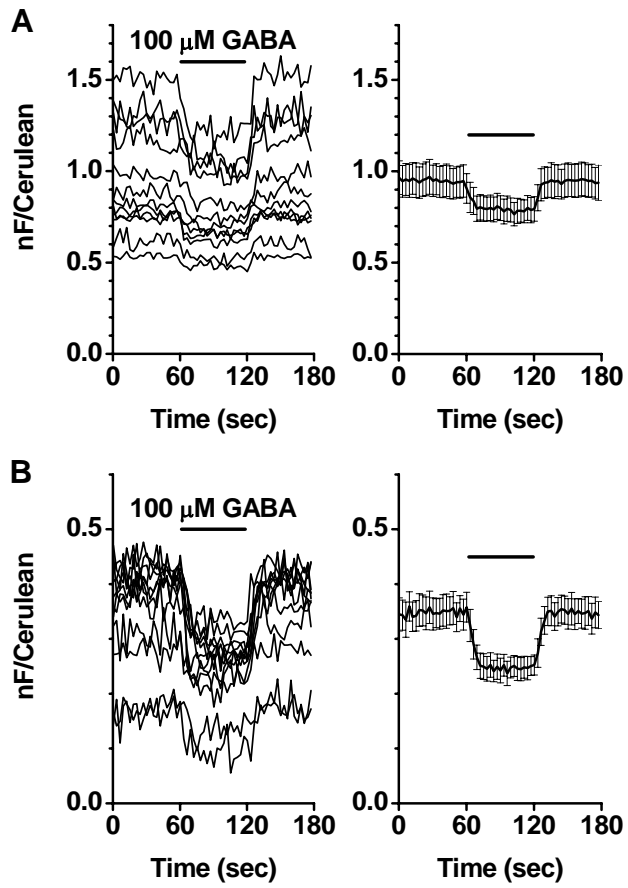
Supplemental data 5. Characterization of other CGP7930 properties. *A*, CGP7930 itself did not evoke FRET changes from both the GB1a-i2 & GB2-i2 and the GB1a-i2 & GB2-i1 pairs. Two traces are plots of mean \pm se of individual recordings from the GB1a-i2EYFP & GB2-i2Cer pair (n=5) and the GB1a-i2EYFP & GB2-i1Cer pair (n=5). DMSO added as a vehicle at first for 60 s (black bar) and then 100 μ M CGP7930 applied for 60 s (black bar). The ligand was washed for 60 s. *B*, The action of CGP7930 was specific to the GABA_BR, as there was no effect on the mGluR1 α . Plots of mean \pm se of individual recordings from the GB1a-i2EYFP & GB2-i2Cer pair (n=3) and the mGluR1 α -i2EYFP & mGluR1 α -i2Cer pair (n=2). 100 μ M Glutamate was applied for 60 s (black bar) and washed for 60 s. 100 μ M GABA was applied for 240 s (black bar). Within this period, DMSO was added as a vehicle for 60 s (black bar), followed by 100 μ M CGP7930 for 60 s (black bar). The ligands were washed for 60 s.

Supplemental data 6. GB1a/GB2-i2 & GB2/GB1a-2 chimeric pair demonstrated a FRET decrease by applying GABA. Left: plots of mean \pm se of individual traces from GB1a/GB2-i2Cer & GB2/GB1a-2EYFP (n=10). 100 μ M GABA was applied for 60 s and washed out for 60 s. The same procedure was repeated again to confirm the reproducibility. Right: schematic drawing of the chimeric heterodimer.

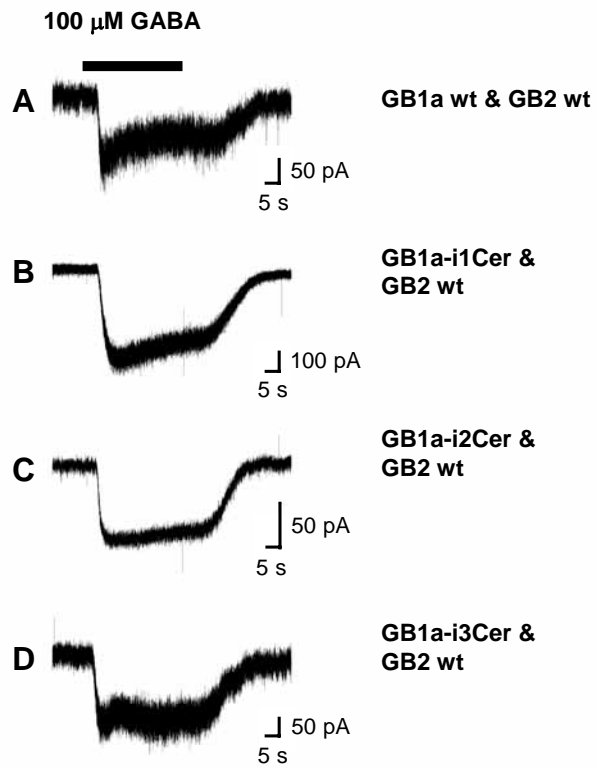
Supplemental data 7. GB1a intra-subunit FRET. *A*, Three types of GB1a, possessing Cerulean fixed at T872 and EYFP at one of different intracellular loops, were paired with GB2 wt. No sign of response from GABA or when the agonist coapplied with CGP7930. Plots of mean \pm se of individual recordings from GB2 wt & GB1a-i1EYFP-T872Cer (n=6) or GB1a-i2EYFP-T872Cer (n=10) or GB1a-i3EYFP-T872Cer (n=6) are shown. Application profile was the same as in Fig. 6. On the right hand side, schematic drawing of GB2 wt & GB1a-i3EYFP-T872Cer is shown as a representative. *B*, Three types of GB1a, the same as shown in *A*, were paired with GB2 T749 stop mutant. No sign of response from GABA or when the agonist coapplied with CGP7930. Plots of mean \pm se of individual recordings from GB2 T749 stop & GB1a-i1EYFP-T872Cer (n=3), GB1a-i2EYFP-T872Cer (n=6) and GB1a-i3EYFP-T872Cer (n=5) are shown. Application profile was the same as in Fig. 6. On the right, schematic drawing of GB2 T749 stop & GB1a-i3EYFP-T872Cer is shown as a representative.

Supplemental data 8. mAChR M₁ construct for detection of intra-subunit structural change exhibited agonist-induced FRET decrease. *A*, Left: plots of mean \pm se of individual FRET traces from M₁-i3Cer-EYFP (n=8). 10 μ M Oxotremorine M was applied for 60 s, washed for 60 s and repeated again for checking the reproducibility. Right: schematic drawing of the M₁ construct. *B*, Averaged normalized FRET decreases from M₁-i3Cer-EYFP and M₁-i3EYFP-Cer intra-subunit FRET pairs.

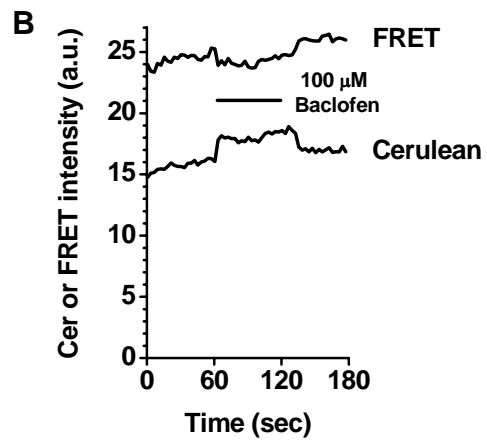
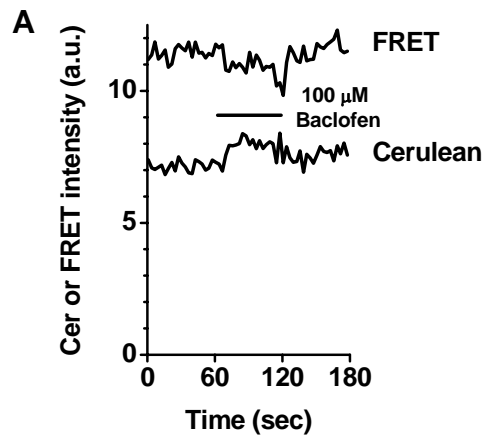
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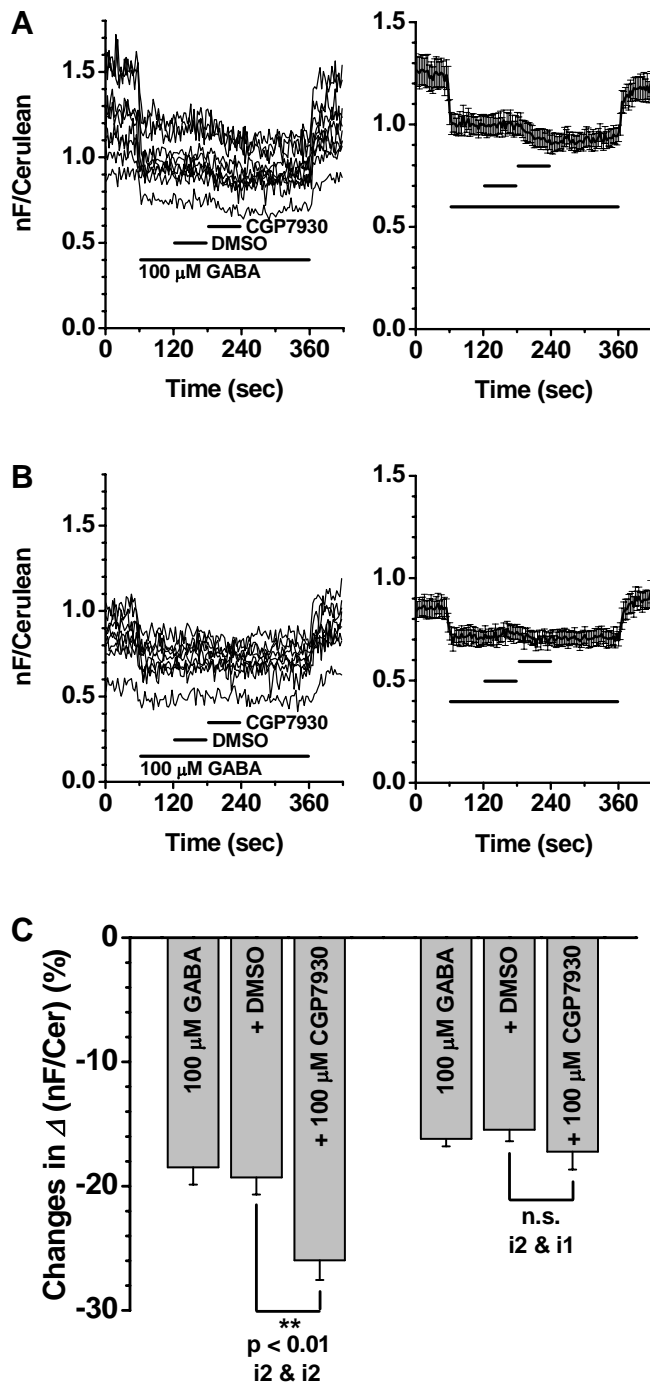
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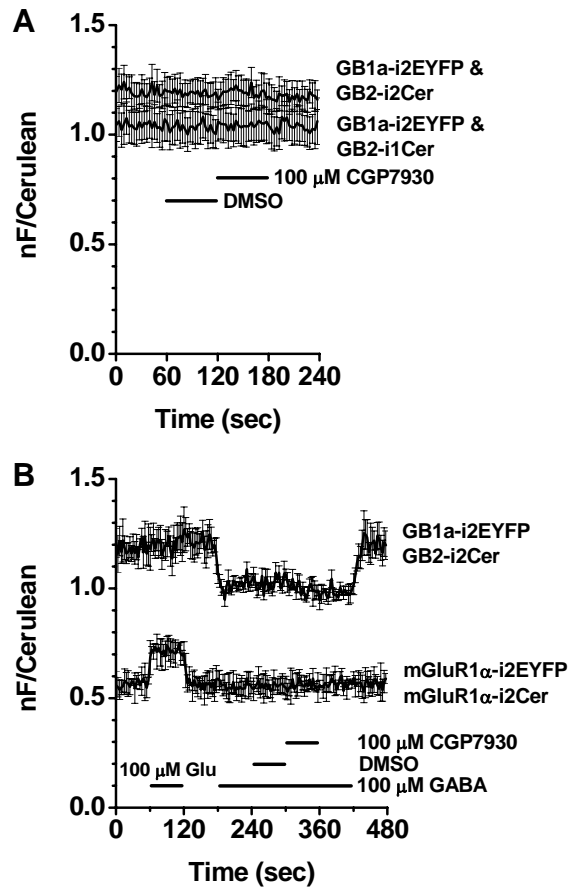
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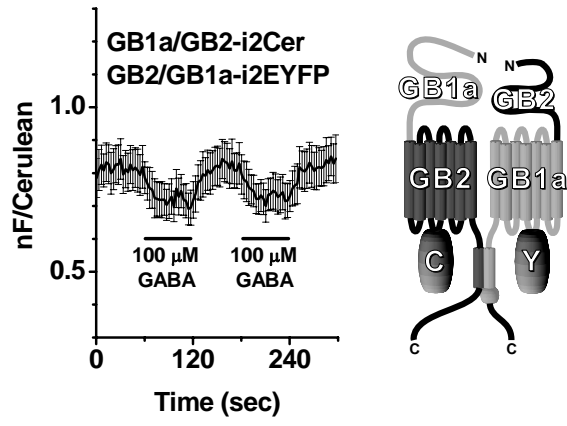
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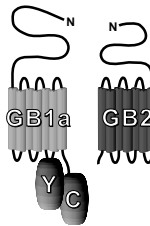
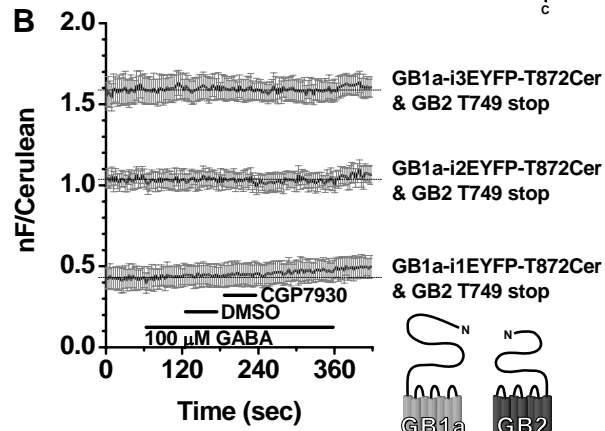
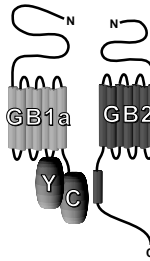
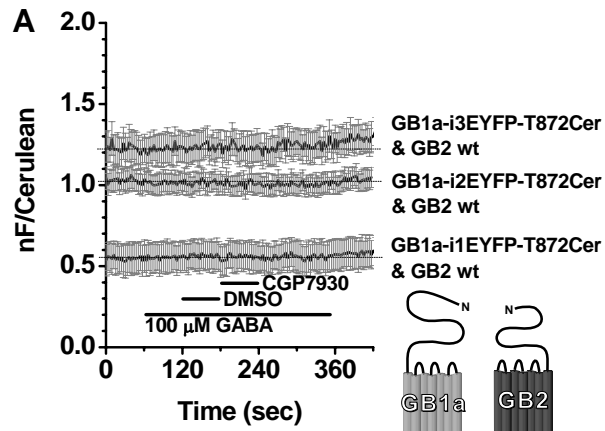
Supplemental data 5



Supplemental data 6



Supplemental data 7



Supplemental data 8

