Supplementary figure legends

Figure S1

Development of cell surface labeling with ACP fused to the N-terminus or inserted in the VFT domain of GB1. (A) Ribbon views of a GB1 VFT model where ACP was attached to the N-terminus (green) or inserted in lobe 1 of GB1 VFT (cyan). The residue (red) that is covalently labeled with fluorescent coenzyme A derivatives is indicated. (B, C) Amount at the cell surface of HA-tagged GB1 with ACP fused to the N-terminus (ACPGB1) or inserted in lobe 1 of GB1 VFT (GB1(ACP)) co-expressed with wild type GB2 (B), and the corresponding intracellular Ca\textsuperscript{2+} responses (C). (D, E) Specific CoA-DY647 labeling with different concentrations of substrate and enzyme, respectively. (F) Time-course of ACP labeling of the indicated constructs with 5 \textmu M CoA-DY647 and 1 \textmu M Sfp synthase. (G) CoA-DY647 labeling (open bars) of mock-transfected cells and cells expressing ACPGB1 alone or with GB2 and the corresponding amount of HA-tagged ACPGB1 at the cell surface as measured by ELISA (closed bars).

Figure S2

(A) Amount of HA-tagged GB1 at the cell surface as measured by ELISA when co-expressed with the indicated GB2 subunits. (B) Effect of CGP54626, a non-permeant antagonist of the GABA\textsubscript{B} receptor, on Ca\textsuperscript{2+} signals in cells expressing the indicated GABA\textsubscript{B} subunits. Cells were stimulated with concentrations of agonist equivalent to the EC\textsubscript{80} of GABA for the wild type (300 nM) and truncated (100 \textmu M) GABA\textsubscript{B} receptor.
Figure S3

**Similar amounts of HA-tagged GB1 at the cell surface in inositol phosphate measurements.** The amount of HA-tagged wild type GB1 at the cell surface as measured by ELISA when associated with the indicated GB2 subunits.

Figure S4

**Similar affinity of CGP35348 in both wild type and truncated receptor.** Displacement of \[^{125}\text{I}\]CGP64213 by GABA at the surface of cells expressing the wild type (A) or truncated (B) GABA\_B receptor.

Figure S5

Intracellular Ca\(^{2+}\) response mediated by the wild type GB1 subunit co-expressed with GB2 or the truncated mGlu1 subunit (ΔVmGlu1-C2), in response to increasing concentrations of Ro 01-6128, a positive allosteric modulator of mGlu1. As a control, cells were transfected with pRK5 empty vector (mock).

Figure S6

Similar amounts of HA-tagged GB1 at the cell surface when associated with truncated GB2 in which the SNAP-tag or ACP-tag was fused to the N-terminus of GB2 or in the second extracellular loop (e2), as measured by ELISA.

Figure S7
Direct interaction between GB1 and GB2 mutants. Amount of HA-tagged receptors at the cell surface, measured by ELISA (open bars), and TR-FRET signal (closed bars) in cells that express two SNAP-tagged subunits, labeled with a mix of DY647 and Lumi4-Tb™ dyes. As indicated, the SNAP-tag was fused to the N-terminus of all constructs: wild type or truncated GABA_B subunits compared to the GB1/2 chimera, in which GB1 VFT is associated to GB2 7TM (A), to the GB1-TM7 subunit in which GB1 VFT is anchored to the membrane through the seventh TM (B) or to the GB1-DCRC mutant in which two residues of the 7TM domain were mutated to cysteines (C).

Figure S8

Amount of HA-tagged receptors at the cell surface as measured by ELISA (open bars) and TR-FRET signal (closed bars) detected in cells transfected with SNAP-tagged GABA_B subunits labeled with DY647 and Flag-tagged subunits labeled with an antibody coupled to a cryptate fluorophore.
Figure S1

A. GB1 VFT

lobe 1

lobe 2

B. Emission signal at 682 nm (x10^3)

Time (s)

GB1(ACP) + GB2

GB1 + GB2

GB1(ACP)

GB2

C. Emission signal at 682 nm (x10^3)

Enzyme log(M)

HA-ACP

GB1

GB2

F. Calcium release (AU x 10^3)

GABA Log[M]

GB1(ACP) + GB2

GB1 + GB2

GB1(ACP)

GB2

mock

D. Emission signal at 682 nm (x10^3)

Substrate log(M)

GB1(ACP) + GB2

E. Emission signal at 682 nm (x10^3)

Enzyme log(M)

GB1(ACP) + GB2

H. HA epitope cell surface (% WT)

lobe 1

lobe 2

GB1 VFT
Figure S2

A

HA epitope
(cell surface)

GB1

GB2

AVGB2

AVGB2-L686P

B

Ca²⁺ release

CGP54626 Log[N]

[Graph showing HA epitope cell surface and Ca²⁺ release with different treatments.]

Figure S2
Figure S3

[Diagram showing HA-epitope cell surface (AU x 10^4) with data points for HA-GB1, GB2, ΔVGB2]
Figure S4

A

% Binding (%) of GABA vs. Log[M] for GABA and CGP35348.

B

% Binding (%) of GABA vs. Log[M] for GABA and CGP35348.
Figure S5

[Graph showing data points for GB1 + VmGlu1-C2, GB1 + GB2, and mock treatments with Ro 01-6128 Log(M) on the x-axis and Ca2+ release (AUX x 10^-7) on the y-axis.]
Figure S6
Figure S7

Cell surface expression FRET intensity

HA epitope cell surface (AU x 10^7)

FRET intensity (c.p.s x 10^3)

A

B

C

Table 1

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<th>HA epitope cell surface</th>
<th>FRET intensity</th>
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<td>HA^{m dip}GB1</td>
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HA^{m dip}GB1

HA^{m dip}GB1-TM7

HA^{m dip}GB1-DCRC

HA^{m dip}GB2

HA^{m dip}GB2

HA^{m dip}GB2

Table 2

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<td>HA^{m dip}GB2</td>
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</table>
Figure S8

Cell surface expression

FRET intensity

- HA
- Flag-VG2
- HA
- Flag-VG2

0.5 1.0 1.5 2.0

0 2 4 6 8

HA expression (AU x 10^7)

FRET intensity (c.p.s x 10^3)