Figure legends

Figure S1. Characterization of GBR2 species expressed in CHO cells

CHO cells stably expressing HA-GBR2 were used. We defined the nature of carbohydrate modifications of each of GBR2 species detected in these cells by performing some deglycosylation treatments. Taking advantage of the fact that the lower molecular weight species (~100kDa) is preferentially recognized by the by anti-HA antibodies, endoglycosidase H (EndoH) treatments were performed on this species following its immunoprecipitation. The sensitivity to the EndoH treatment indicated the presence of unprocessed high mannose oligosaccharides, characteristic of immature glycoproteins, suggesting that the ~100kDa species represent an ER-localized precursor form of the receptor. In contrast, the higher molecular weight specie (~120kDa) of GBR2, purified by streptavidin pull down after cell surface biotinylation, was found to be resistant to EndoH treatment but sensitive to PNGase F (an enzyme removing all kinds of N-linked oligosaccharides from glycoproteins) indicating that it corresponds to the fully processed glycosylated form of the receptor. Protocol used to perform these experiments may be found in supplemental materials and methods.

Figure S2. GBR does not induce a massive change in the NSF cellular distribution

CHO cells expressing or not GBR1a and GBR2 were transfected with a GFP-GRK5 plasmid. The co-localization between endogenous NSF (red) detected with appropriate antibodies and the GFP-GRK5 (green) protein used as a marker of the plasma membrane was assessed by confocal fluorescence microscopy as described in material and methods. Overlay panels at different Z position are presented and the regions where co-localization was detected are enlarged in inset.

Figure S3. Plasma membrane expression of GBR1 and GBR2 following GABA treatment

CHO cells stably expressing myc-GBR1b/HA-GBR2 were treated with 1mM GABA for the indicated period. The level of surface expression of each of the receptor was measure using ELISA assays (performed following SIGMA's instructions). Black and white bars correspond to the quantification of GBR1 and GBR2, respectively. These results represent the mean ± SEM of three independent experiments performed in triplicates.

Figure S1

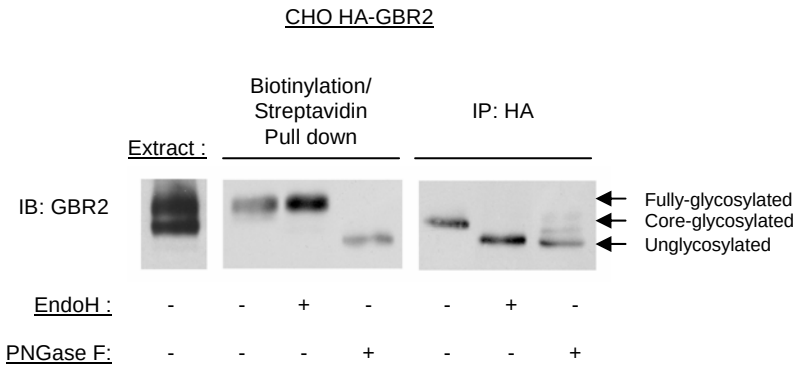

CHO HA-GBR2
Biotinylation/
Streptavidin
Pull down
IP: HA
Extract :
IB: GBR2
Fully-glycosylated
Core-glycosylated
Unglycosylated
EndoH : - - + - - + -
PNGase F: - - - + - - +

Figure S2

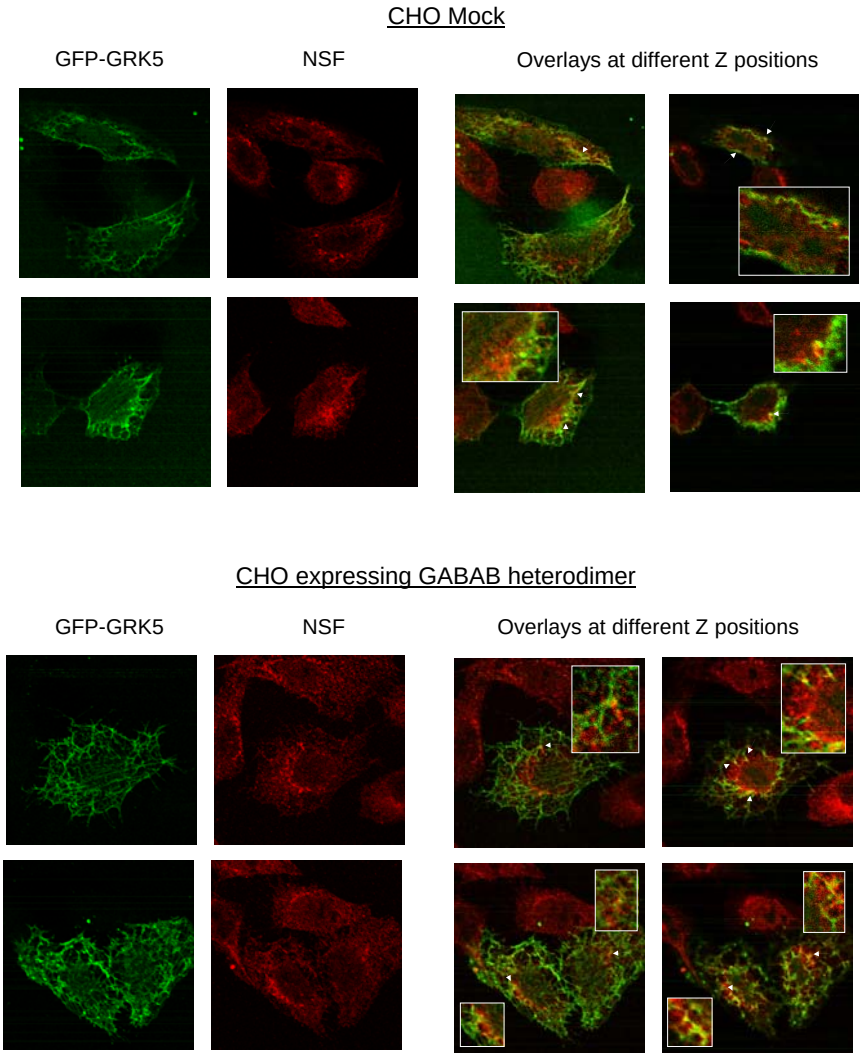
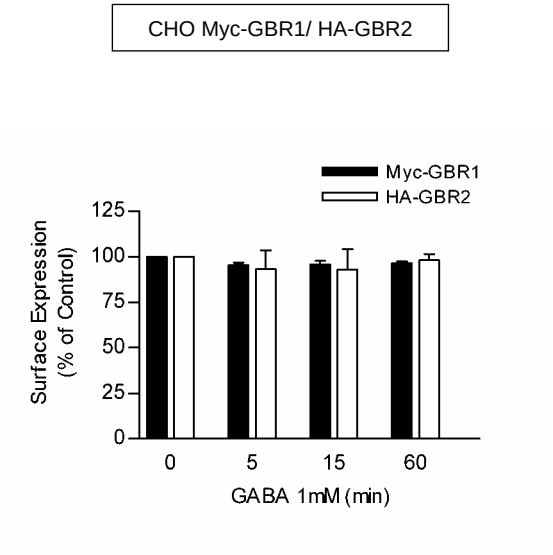

CHO Mock
GFP-GRK5
NSF
Overlays at different Z positions
CHO expressing GABAB heterodimer
GFP-GRK5
NSF
Overlays at different Z positions

Figure S3


CHO Myc-GBR1/ HA-GBR2
Myc-GBR1
HA-GBR2
Surface Expression
(% of Control)
125
100
75
50
25
0
0
5
15
60
GABA 1mM (min)