Supplemental Data

Molecular Pharmacology

Non-classical ligand-independent regulation of Go protein by an orphan Class C GPCR

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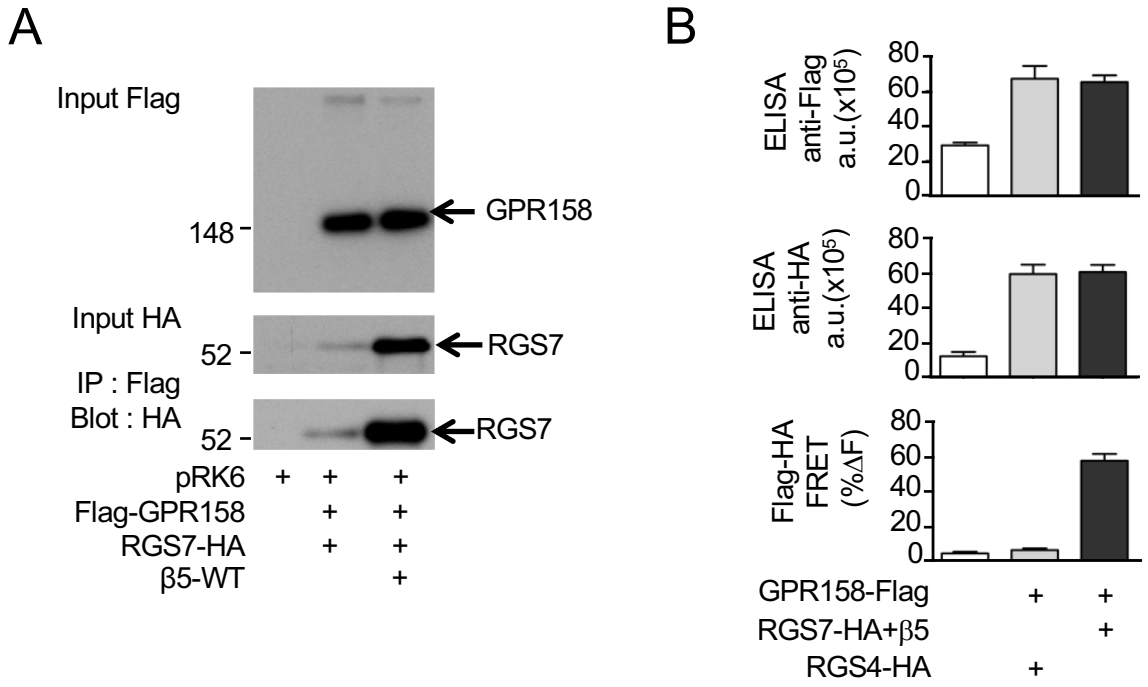


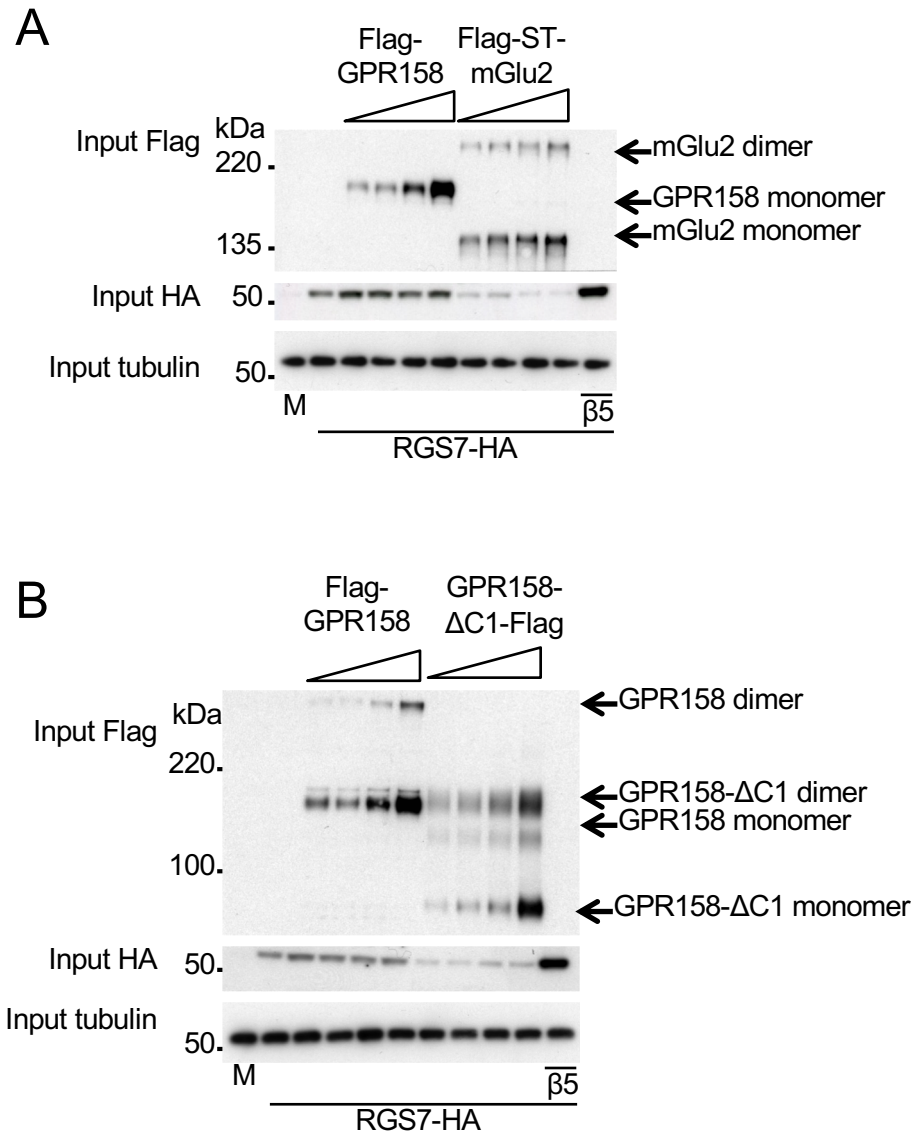
Supplemental Figure 1: **RGS7 interaction with GPR158 measured by TR-FRET. A.** β5 was necessary for RGS7 expression, as showed using Western blot analysis (Middle panel) from cells transfected with 150 ng of RGS7 coding plasmid, with or without 30 ng of plasmid coding for β5 G protein subunit. RGS7-HA was co-immunoprecipitated by GPR158-Flag when co-expressed in HEK293 cells, even at very low concentration of RGS7 in absence of co-transfection of its partner β5 proteins. This experiment is representative of three independent experiments. **B.** TR-FRET (HTRF®) experiments showed a significant FRET signal (Lower panel) in cells expressing GPR158-Flag and RGS7-HA but not in cells expressing GPR158-Flag and RGS4-HA. RGS7 and RGS4 were expressed at similar level (middle panel) and GPR158 expression was similar in both conditions (upper panel). This experiment is representative of 3 independent experiments.

Supplemental Figure 1

	V1006 E1010	V1071 E1075	V1171 E1175
H sap	FDIGE **VCPWE** VYDLT	IDKAE **VCLWE** SQGQS	TSRAE **VCPWE** FETPA
P tro	FDIGE **VCPWE** VYDLT	IDKAE **VCLWE** SQGQS	TSRAE **VCPWE** FETPA
N leu	FDIGE **VCPWE** VYDLT	IDKAE **VCLWE** SQGQS	TSRAE **VCPWE** FETPA
M mul	FDIGE **VCPWE** VYDLT	TDKAE **VCLWG** IQGQS	MSRAE **VCPWE** FETPA
E cab	FDIGE **VCPWE** IYDLT	TDKAE **VCPWE** SQGQS	ISRAE **VCPWE** FETPD
C fam	FDIGE **VCPWE** IYDLA	IDKAE **VCPWK** SQGQP	TSRAE **VCPWE** FETPN
R nor	FDIGE **VCPWE** VYDLT	IDKTE **VCPWE** SHGQS	TSRAE **VCPWE** FEPLE
M mus	FDIGE **VCPWE** VYDLT	IDKTE **VCPWE** IHSQS	TSRAE **VCPWE** FEPLE
M dom	FDIGE **VCPWE** VYDLT	IDKTE **VCPWE** SPEQC	TSRAE **VCPWE** YEAPS
G gal	FDIGE **VCPWE** IYDQT	PQKLE **AGTRE** VQEQH	ASRAE **VCPWE** FDTPD
T gut	FNIGE **VCPWE** IYDQV	SENVE **AATQE** TQEQQ	ASRAE **VCPWE** YDTAD
D rer	CDLSE **VCPWE** VEDL-	ADRAD **ICPWE**/SQQQA	-SKAD **VCPWD** FETMS

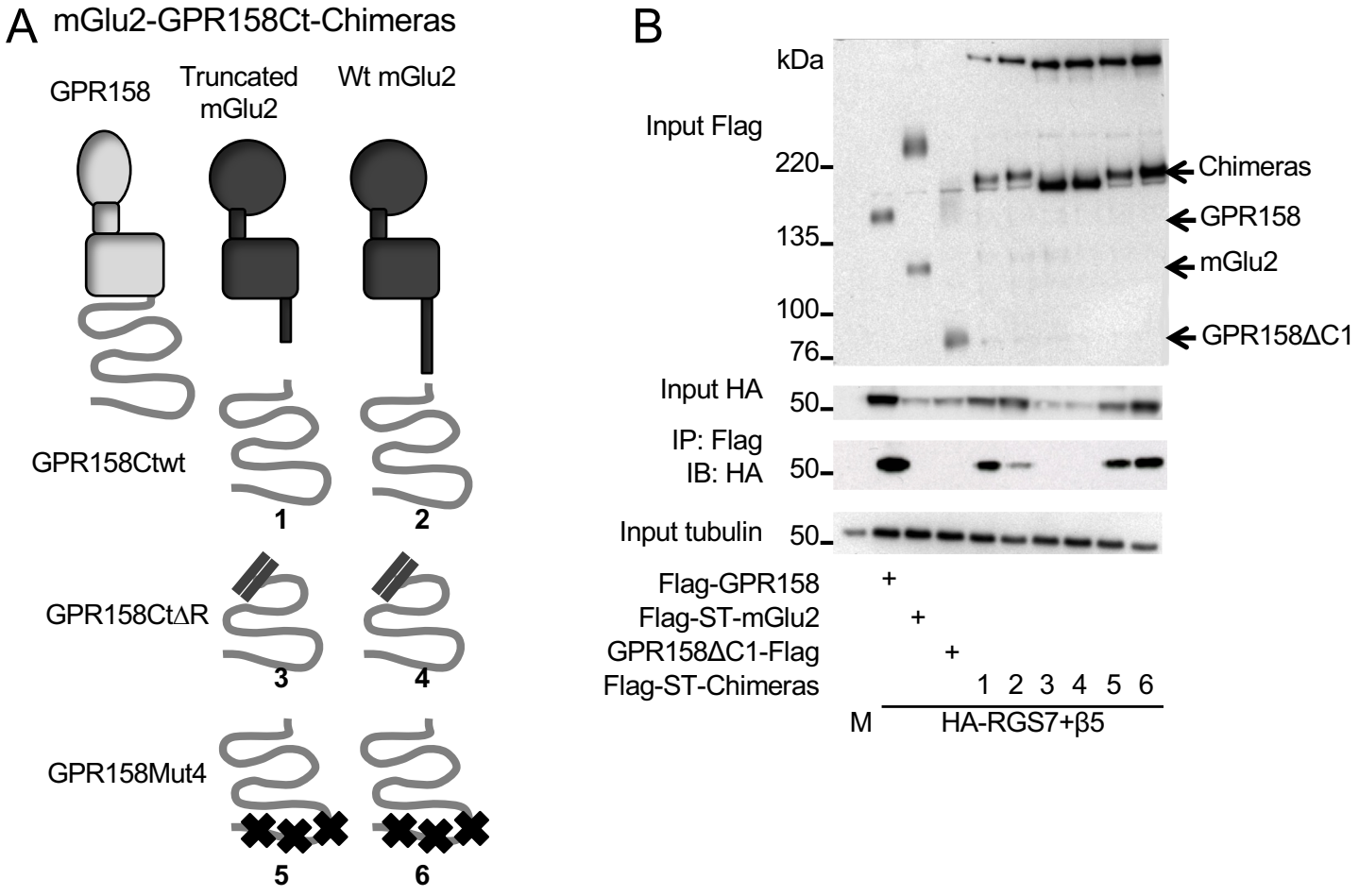
Supplemental figure 2: **Conserved VCPWE motifs in the C-terminal domain of GPR158**. The sequences of each motif in the sequence of GPR158 from different species are aligned. Indicated are the residues numbers of the human sequence. H sap stands for, Homo sapiens, P tro for Pan troglodits, N leu for Nomascus leucogenys, M mul for Macaca mulatta, E cab for Equus caballus, C fam for Canis familiaris, R nor for Ratus norvegicus, M mus for mus musculus, M dom for Monodelphis domestica, G gal for Gallus gallus, T gut for Taeniopygia guttata, D rer for Danio rerio.

Supplemental Figure 2



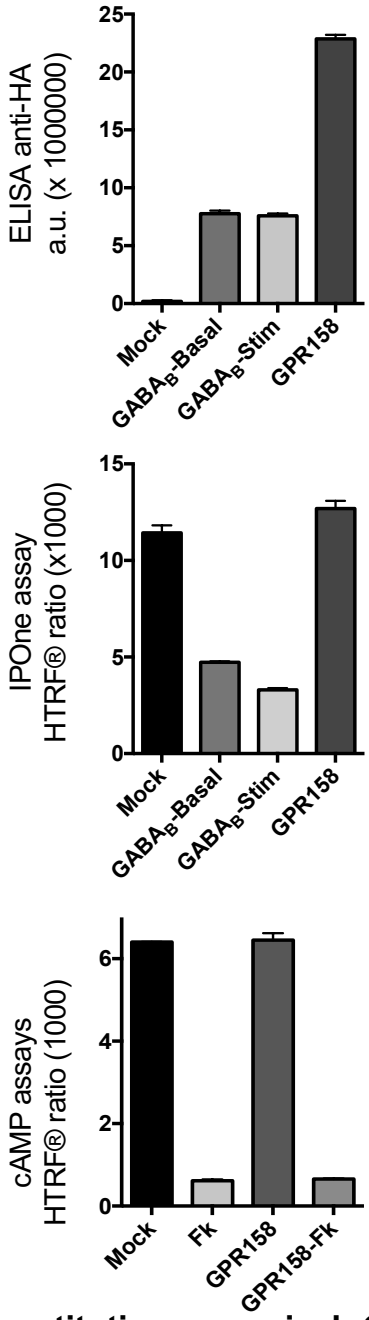
Supplemental Figure 3: **RGS7 expression stabilized when co-expressed with GPR158 even in absence of β5**. HEK293 cells were transfected with constant amounts of plasmids coding for RGS7-HA (150ng) and increasing amounts of plasmids coding for Flag-GPR158 or Flag-mGlu2 receptors (1 to 50ng) (**A**), or Flag-GPR158 or Flag-GPR158-DC1 (1 to 50ng) (**B**). The expression of HA-RGS7 was analyzed using Western blot analysis against the HA epitope. The tubulin expression was used as a Western blot loading control. β5 was not co-expressed with RGS7. Each experiment is representative of three independent experiments.

Supplemental Figure 3



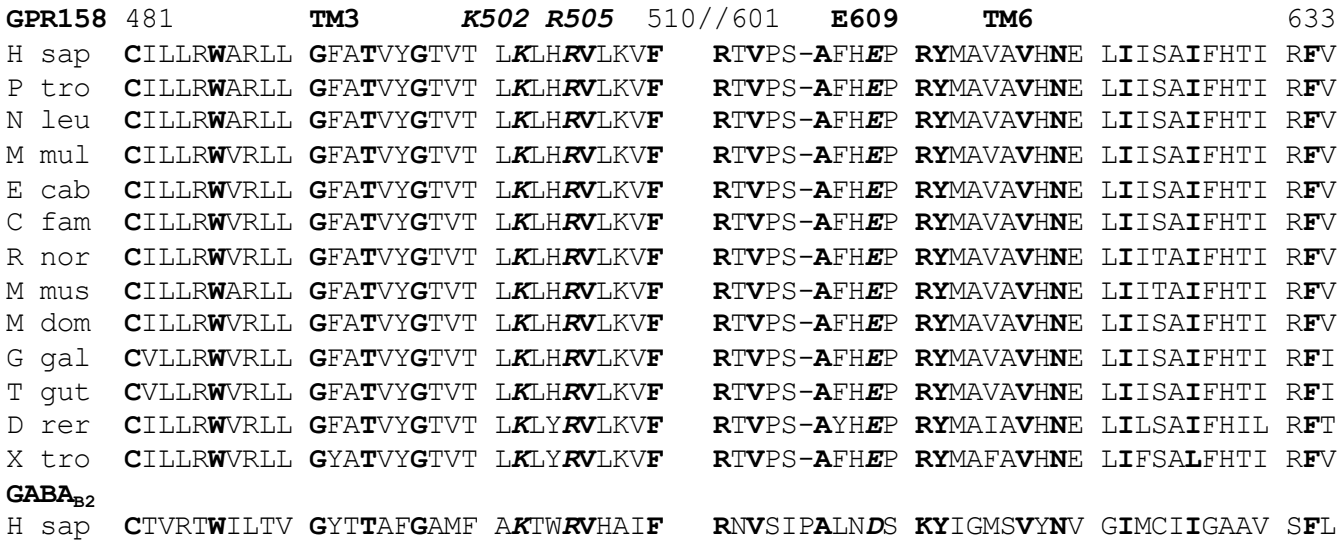
Supplemental Figure 4: **The C-terminal domain of GPR158 was sufficient and the region 714-764 necessary for RGS7 interaction and stabilization**. **A.** Schematic representation of the chimeric mGlu2 receptors fused to the C-terminal domain of GPR158, either WT, with the three motifs mutated (stars) or deleted of the Ile714-Thr764 region (double oblique sticks) used for co-immunoprecipitation (**B**) from cells co-expressing RGS7 and β5. The truncated mGlu2 has been deleted of its last 32 residues. This experiment is representative of three independent experiments.

Supplemental Figure 4



Supplemental Figure 5: **No constitutive canonical G protein coupling detected for GPR158.** HEK293 cells were transfected with plasmids coding for Flag-GPR158, or the subunits Flag-GB1 and HA-GB2 of the Gi/o-coupled $GABA_B$ receptor, with or without the chimeric G protein GqTop. GqTop allows Gi/o-coupled receptors to couple to Gq and its IP1-3 second messenger pathway. The IP1-3 and cAMP second messenger production was quantified to assess the constitutive (Basal) and ligand-induced activity (Stim) of Gi/o-coupled receptors, as illustrated with $GABA_B$ receptor in basal conditions or stimulated with 1 mM of GABA, or with Forskoline (Fk). **Upper panel** shows the expression level of $GABA_B$, wild type and mutated GPR158 using an ELISA against the N-terminal epitope tag. No constitutive basal GPR158 activity was detected using the IP1-3 production measurement IPOne (**Middle panel**) or the cAMP production measurement HTRF® assays (Cisbio Bioassays) (**Lower panel**). GABA (1 mM) was used to stimulate $GABA_B$ receptor, and 0.1 µM forskoline (Fk) was added for pre-activating the adenylyl cyclase (**Lower panel**). Of note, these experimental conditions allowed the analysis of the coupling to both endogenous Gq and chimeric GqTop-mediated Gi/o. This experiment is representative of several independent experiments.

Supplemental Figure 5



Supplemental Figure 6: Region of TM3 and TM6 of GPR158 from various species. Bold letters indicate conserved residues among the species. Bold italic letters indicated the residues involved in coupling activity in $GABA_B$ class C receptor and conserved in GPR158.

Supplemental Figure 6