G protein-coupled glutamate and GABA receptors form complexes and mutually modulate their signals

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Figure S1. Establishment of stable HEK293 cell lines with inducible expression of mGluR1, GBR1, and GBR2.

To examine the expression of GPCRs, cell lysates were electrophoresed and immunoblotted with antibodies against mGluR1, GBR1, GBR2, and beta-tubulin. HEKmg12 (clone #04) cells were treated with 2 μM Dox for 0 to 24 h.

Figure S2. Measurement of intracellular cAMP using the LANCE cAMP assay system.

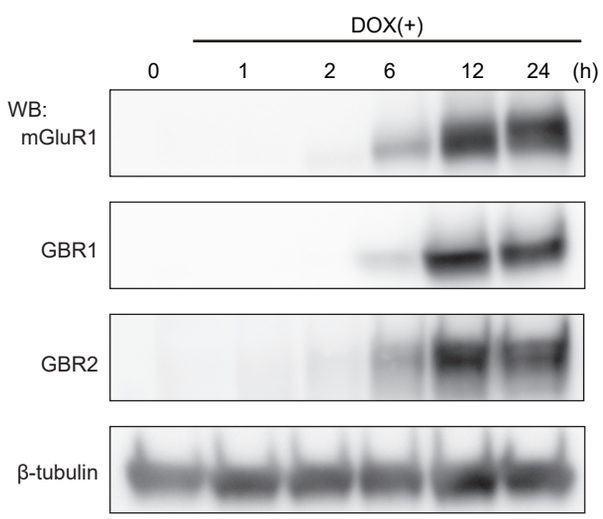
(A-C) Densitometry of cAMP was performed using the LANCE cAMP assay system. Reactions were assembled with the indicated cAMP concentrations in a 384-well plate. The fluorescence intensity of 665 nm (A) and 615 nm (B) and ratio of 665 nm to 615 nm (C, TR-FRET signal). The TR-FRET signal showed negative correlation to the cAMP concentrations. N=4. (D) HEKmg12 cells were treated with FK or vehicle (control). Administration of FK decreased the TR-FRET signal in a dose-dependent manner. N=4. Error bars are ± SD.

Figure S3. Bioluminescence-based cAMP assay for GBR signaling in HEKmg12 cells.

(A) Densitometry of cAMP was performed using the cAMP-Glo^{TM} assay system. Reactions were assembled with the indicated concentrations of purified cAMP in a low-volume 96-well plate. Data were collected using a plate-reading luminometer. N=4. (B) Cells were treated with the indicated concentrations of FK. FK decreased cAMP-Glo luminescence in a dose-dependent manner. N=3. (C, D) DHPG by itself does not affect basal $[cAMP]_i$ (C, N=3–4), and FK (1 μM)-induced increase in $[cAMP]_i$ (D, N=6). (E) Cells were treated with the indicate concentrations of baclofen in the presence of 1 uM FK. Baclofen inhibited the increase in $[cAMP]_i$ induced by FK. N=4. One-way ANOVA was used to test for statistical significance, and differences between pairs were analyzed using Dunnett's multiple comparison test vs. cells treated with 1 μM FK in the absence of baclofen. (F) Cells were treated with the indicated concentrations of DHPG in the presence of 1 μM FK and/or 0.3 μM baclofen. DHPG reversed the effect of 0.3 μM baclofen in a dose-dependent manner, starting

at 3 μM. N=7. One-way ANOVA was used to test for statistical significance, and differences between pairs were analyzed using Dunnett's multiple comparison test vs. cells treated with 1 μM FK and 0.3 μM baclofen in the absence of DHPG. Error bars are ± SD. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Table S1. List of antibodies used for experiments.



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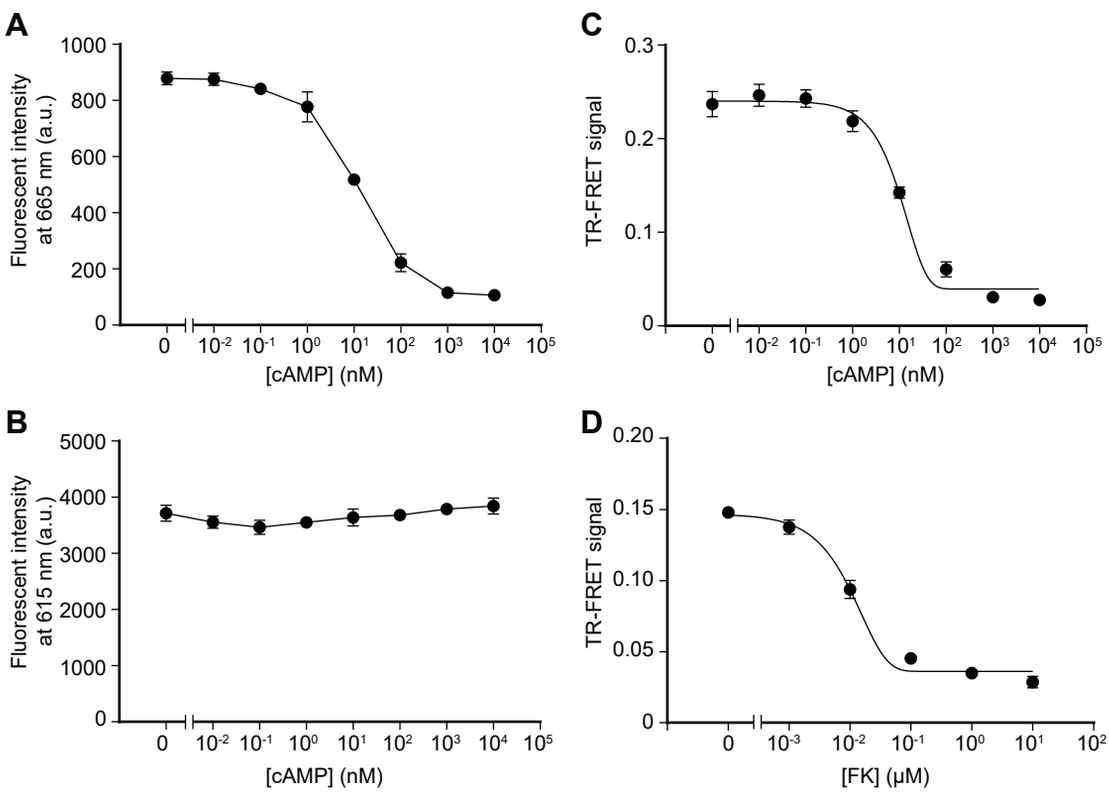

Figure S2. Measurement of intracellular cAMP using the LANCE cAMP assay system.

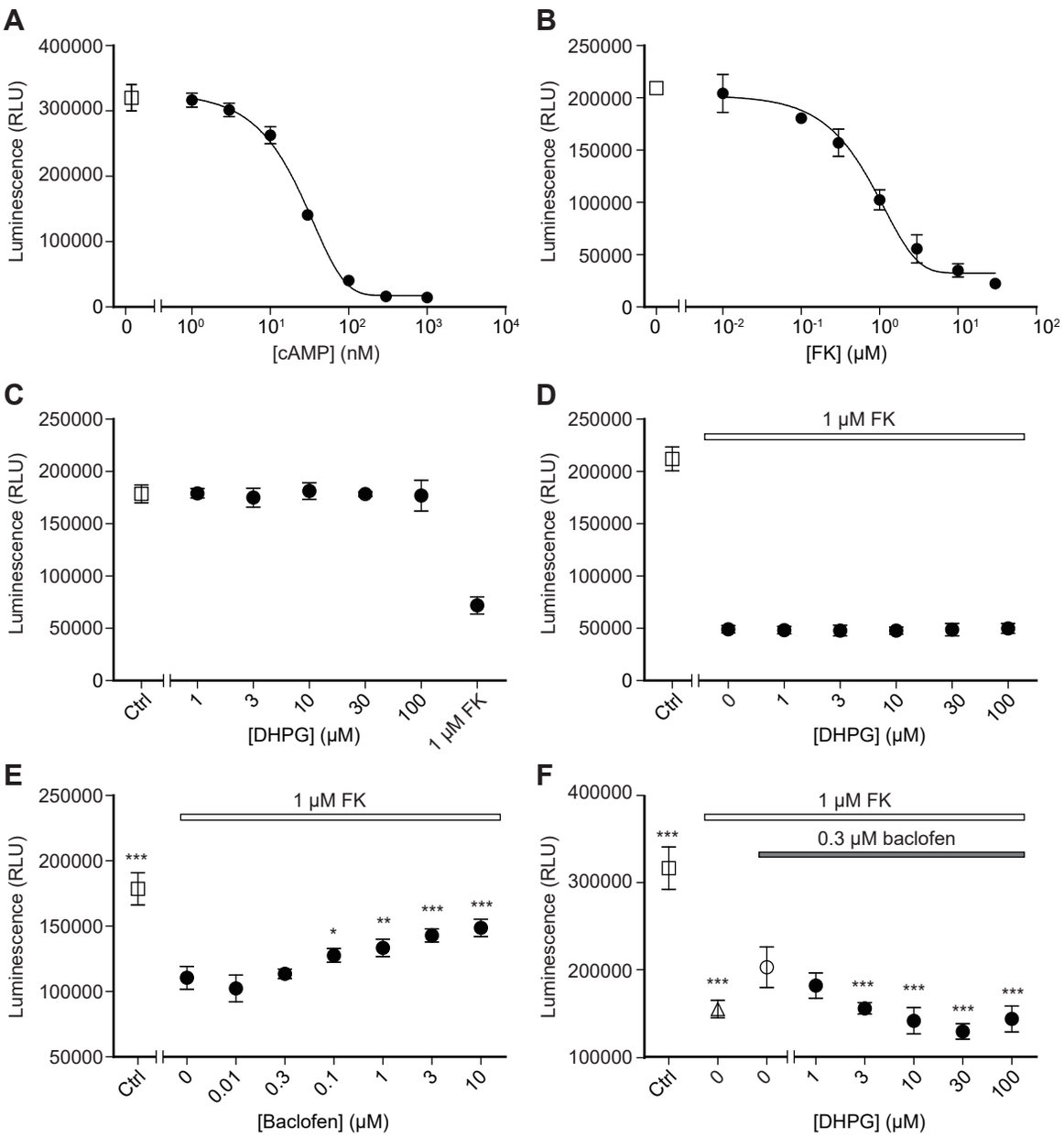

Figure S3. Bioluminescence-based cAMP assay for GBR signaling in HEKmg12 cells.

Table S1 List of antibodies used for experiments

	Antibody	Species	Supplier	Code
Immunoprecipitation	anti-GBR1	Rabbit	Invitrogen	PA5-17075
	anti-GBR2	Rabbit	Invitrogen	PA5-17035
	anti-mGluR1	Rabbit	Details are described in Kamikubo *et al*., 2013	
	anti-GFP	Mouse	Life Technologies	A11120
Immunoblot	anti-GBR1	Sheep	R&D Systems	AF7000
	anti-GBR1	Rabbit	Details are described in Kulik *et al*., 2002	
	anti-GBR2	Goat	R&D Systems	AF1188
	anti-mGluR1	Mouse	BD Biosciences	610965
	anti-mGluR1	Sheep	R&D Systems	AF4836
	anti-mGluR1	Rabbit	Details are described in Kamikubo *et al*., 2013	
	anti-HA	Rabbit	Promega	G928A
	anti-VSVG	Rabbit	Sigma-Aldrich	V4888
TIRF imaging	anti-GBR1	Rabbit	Invitrogen	PA5-17075
	anti-GBR2	Rabbit	Invitrogen	PA5-17035
	anti-mGluR1	Mouse	BD Biosciences	610965
	anti-rabbit (AF488)	Donkey	Invitrogen	A21206
	anti-mouse (AF594)	Donkey	Molecular Probes	A21203
	anti-HA (AF594)	Mouse	MBL	M180-A59
	anti-VSVG (DL649)	Rabbit	Rockland	600-443-386
Antibody patching	anti-GBR1	Sheep	R&D Systems	AF7000
	anti-GBR2	Rabbit	Invitrogen	PA5-17035
	anti-mGluR1	Rabbit	Details are described in Kamikubo *et al*., 2013	
	anti-NAKA	Rabbit	Abcam	ab76020
	anti-sheep (AF488)	Donkey	Invitrogen	A11015
	anti-rabbit (AF594)	Donkey	Life Technologies	A21207