

Supplemental Data

Differential Activity of Orthosteric Agonists and Allosteric Modulators at Metabotropic Glutamate Receptor 7

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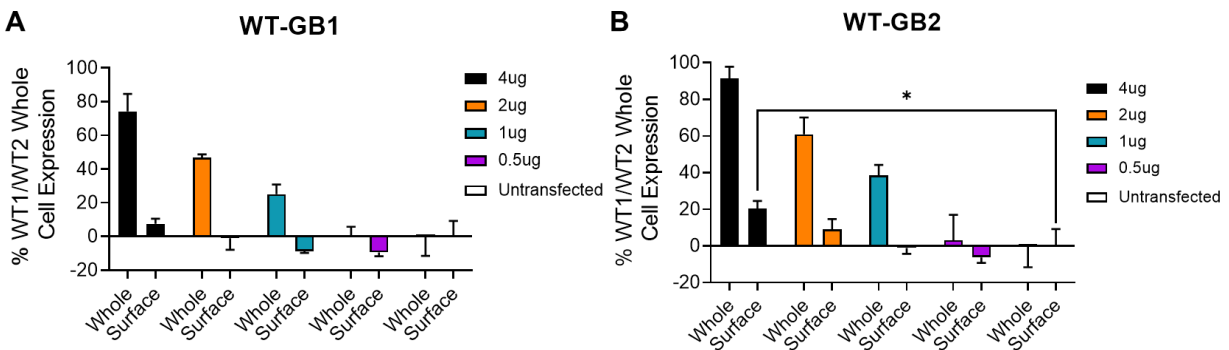


Figure S1. Cell surface expression is optimal with 2 μ g plasmid for WT mGlu7-GB1/GB2. Total and surface expression levels measured after transfecting different amounts of plasmid encoding (A) WT-GB1 or (B) WT-GB2. HEK293A/G_{a15} cells were transfected with 4 μ g (black), 2 μ g (orange), 1 μ g (turquoise), 0.5 μ g (purple) of DNA or untransfected controls (white). pcDNA3.1(+) was added to create a total transfection amount of 4 μ g for each condition. The expression level is shown as % of WT-GB1/WT-GB2 total expression. Expression levels were obtained using an on-cell ELISA that records the intensity of secondary antibody and normalized to cell number indicated by DRAQ5 intensity. Data are Mean \pm SD, n=4 independent experiments with 4 technical

replicates each. 2-way ANOVA with Turkey's multiple comparisons. * $p < 0.05$ between surface expression of the 4 μg transfection of WT-GB2 and untransfected cells.

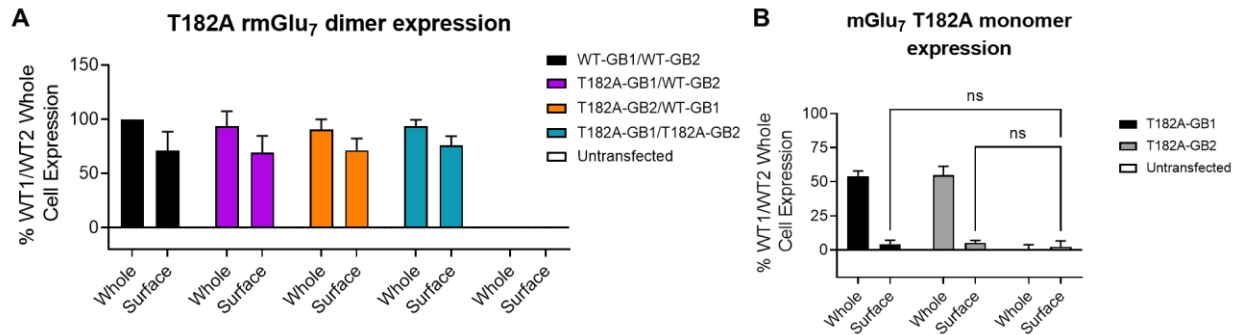


Figure S2. Surface and total expression level of mGlu₇ receptors with or without T182A mutations. (A) Expression levels of paired GB1/GB2 plasmids with T182A mutation(s) after transfection into HEK293A/G_{a15} cells. Cells were transfected with plasmids encoding the following mGlu₇ receptors: WT-GB1/WT-GB2 (black), T182-GB1/WT-GB2 (purple), T182A-GB2/WT-GB1 (orange), mutations in both subunits T182A-GB1/T182A-GB2 (turquoise), or no DNA (untransfected, white) and total and surface expression was measured using on-cell ELISA. (B) Total and surface expression levels of HEK 293A/G_{a15} cells transfected with T182A-GB1 alone (black), T182A-GB2 alone (grey), or untransfected (white). Data are Mean \pm SD, n=3 independent experiments with 4 technical replicates each. 2-way ANOVA with Turkey's multiple comparisons. ns, not significant.



Figure S3. mGlu₇ also requires 2 agonist binding sites for GIRK channel activation.

Concentration-response curves from HEK293A/GIRK cells co-expressing WT-GB1/WT-GB2 with or without mutations in the agonist binding site(s). Cells were transfected with WT-GB1/WT-GB2 (black), T182A-GB1/WT-GB2 (orange), T182A-GB2/WT-GB1 (turquoise), or mutations in both subunits, T182A-GB1/T182A-GB2 (white). Cells were pretreated with vehicle (DMSO) and thallium flux was measured as described in Materials and Methods. Data are Mean±SD, n=3 independent experiments with 3 technical replicates each.

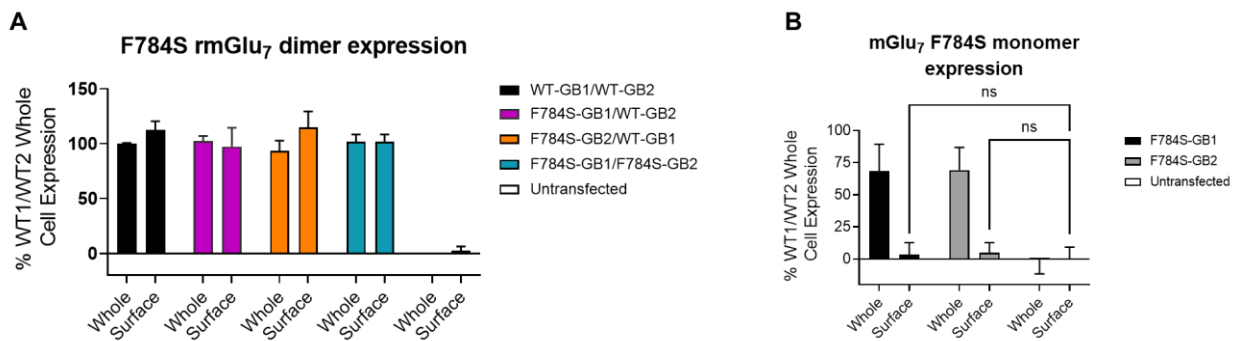


Figure S4. Total and surface expression levels of HEK 293A/G_{a15} cells transfected

with paired GB1/GB2 plasmids with F784S mutation(s). (A) Cells were transfected with WT-GB1/WT-GB2 (black), F784S-GB1/WT-GB2 (purple), F784S-GB2/WT-GB1 (orange), mutations in each subunit, F784S-GB1/F784S-GB2 (turquoise), or untransfected (white) and total and surface expression was measured. (B) Total and surface expression levels of HEK 293A/G_{a15} cells transfected with F784SA-GB1 alone (black), F784SA-GB2 alone (grey), or untransfected (white). Data are Mean±SD, n=3 independent experiments with 4 technical replicates each. 2-way ANOVA with Turkey's multiple comparisons. ns, not significant.

Supplemental Table 1. Statistical comparison of data in Figure 6. Data represent Student's t-tests (n=7 independent replicates performed in duplicate or triplicate) for pEC₅₀ and % Max values between various conditions for WT/WT receptors.

Agonist	PAM	Receptor Combination	pEC ₅₀ ± SD	P value
Glutamate	None	WT/WT	2.89±0.32	****<0.0001
L-AP4	None	WT/WT	3.74±0.10	
Glutamate	None	WT/WT	2.89±0.32	***0.0007
Glutamate	VU288	WT/WT	3.46±0.10	
Glutamate	None	WT/WT	2.89±0.32	****<0.0001
Glutamate	VU094	WT/WT	3.95±0.13	
Glutamate	None	WT/WT	2.89±0.32	****<0.0001

Glutamate	VU649	WT/WT	3.89±0.10	
L-AP4	None	WT/WT	3.74±0.10	****<0.0001
L-AP4	VU288	WT/WT	4.11±0.13	
L-AP4	None	WT/WT	3.74±0.10	****<0.0001
L-AP4	VU094	WT/WT	4.59±0.08	
L-AP4	None	WT/WT	3.74±0.10	****<0.0001
L-AP4	VU649	WT/WT	4.54±0.09	
Agonist	PAM	Receptor Combination	Max ± SD	P value
Glutamate	None	WT/WT	22.5±4.3	****<0.0001
L-AP4	None	WT/WT	101.0±1.7	
Glutamate	None	WT/WT	22.5±4.3	****<0.0001
Glutamate	VU288	WT/WT	85.1±18.4	
Glutamate	None	WT/WT	22.5±4.3	****<0.0001
Glutamate	VU094	WT/WT	166.2±19.3	
Glutamate	None	WT/WT	22.5±4.3	****<0.0001
Glutamate	VU649	WT/WT	123.2±16.6	
L-AP4	None	WT/WT	101.0±1.7	****<0.0001
L-AP4	VU288	WT/WT	163.5±17.6	
L-AP4	None	WT/WT	101.0±1.7	****<0.0001
L-AP4	VU094	WT/WT	205.6±21.1	
L-AP4	None	WT/WT	101.0±1.7	****<0.0001
L-AP4	VU649	WT/WT	156.3±23.7	

Supplemental Table 2. Statistical comparison of data in Figure 7. Data represent unpaired Student's t-tests (n=7 independent replicates performed in duplicate or triplicate for WT/WT and n=3 for T182A/WT) for pEC₅₀ and % Max values between various conditions for WT/WT and T182A/WT combinations.

Agonist	PAM	Receptor Combination	pEC₅₀ ± SD	P value
Glutamate	VU094	WT/WT	3.95±0.13	****<0.0001
Glutamate	VU094	T182A/WT	3.28±0.10	
Glutamate	VU649	WT/WT	3.89±0.10	***0.0001
Glutamate	VU649	T182A/WT	3.37±0.13	
L-AP4	VU094	WT/WT	4.59±0.08	****<0.0001
L-AP4	VU094	T182A/WT	3.86±0.06	
L-AP4	VU649	WT/WT	4.54±0.09	****<0.0001
L-AP4	VU649	T182A/WT	3.89±0.07	
Agonist	PAM	Receptor Combination	Max ± SD	P value
Glutamate	None	WT/WT	22.5±4.3	****<0.0001
Glutamate	None	T182A/WT	-2.1±5.7	
Glutamate	VU288	WT/WT	85.1±18.4	****<0.0001
Glutamate	VU288	T182A/WT	-0.5±1.3	
Glutamate	VU094	WT/WT	166.2±19.3	****<0.0001
Glutamate	VU094	T182A/WT	50.6±19.5	

Glutamate	VU649	WT/WT	123.2±16.6	****<0.0001
Glutamate	VU649	T182A/WT	28.1±9.1	
L-AP4	None	WT/WT	101.0±1.7	****<0.0001
L-AP4	None	T182A/WT	0.5±1.9	
L-AP4	VU288	WT/WT	163.5±17.6	****<0.0001
L-AP4	VU288	T182A/WT	7.9±4.2	
L-AP4	VU094	WT/WT	205.6±21.1	****<0.0001
L-AP4	VU094	T182A/WT	87.2±18.2	
L-AP4	VU649	WT/WT	156.3±23.7	***0.0001
L-AP4	VU649	T182A/WT	54.9±3.6	

Supplemental Table 3. Statistical comparison of data in Figures 8 and 9. Data represent unpaired Student's t-tests (n=7 independent replicates performed in duplicate or triplicate for WT/WT and n=4 for F784S/WT combinations) for pEC₅₀ and % Max values between various conditions for WT/WT and F784S/WT combinations.

Agonist	PAM	Receptor Combination	pEC₅₀ ± SD	P value
Glutamate	VU288	WT/WT	3.46±0.10	0.2273
Glutamate	VU288	F784S/WT	3.37±0.15	
Glutamate	VU094	WT/WT	3.95±0.12	*0.0488
Glutamate	VU094	F784S/WT	3.76±0.13	
Glutamate	VU649	WT/WT	3.89±0.10	0.2832
Glutamate	VU649	F784S/WT	3.79±0.15	
L-AP4	None	WT/WT	3.74±0.10	*0.0131
L-AP4	None	F784S/WT	3.53±0.12	
L-AP4	VU288	WT/WT	4.11±0.13	0.0572
L-AP4	VU288	F784S/WT	3.93±0.13	
L-AP4	VU094	WT/WT	4.59±0.03	**0.0063
L-AP4	VU094	F784S/WT	4.38±0.12	
L-AP4	VU649	WT/WT	4.54±0.09	0.0804
L-AP4	VU649	F784S/WT	4.40±0.16	
L-AP4	None	F784S/WT	3.53±0.12	**0.0051
L-AP4	VU288	F784S/WT	3.93±0.13	

L-AP4	None	F784S/WT	3.53±0.12	****<0.0001
L-AP4	VU094	F784S/WT	4.38±0.12	
L-AP4	None	F784S/WT	3.53±0.12	***0.0007
L-AP4	VU649	F784S/WT	4.40±0.16	
Agonist	PAM	Receptor Combination	Max ± SD	P value
Glutamate	None	WT/WT	22.5±4.3	****<0.0001
Glutamate	None	F784S/WT	2.1±1.2	
Glutamate	VU288	WT/WT	85.1±18.4	**0.0069
Glutamate	VU288	F784S/WT	52.1±3.2	
Glutamate	VU094	WT/WT	166.2±19.3	*0.0480
Glutamate	VU094	F784S/WT	141.6±11.7	
Glutamate	VU649	WT/WT	123.2±16.6	0.6381
Glutamate	VU649	F784S/WT	118.4±13.9	
L-AP4	None	WT/WT	101.0±1.7	****<0.0001
L-AP4	None	F784S/WT	58.4±8.0	
L-AP4	VU288	WT/WT	163.5±17.6	*0.0166
L-AP4	VU288	F784S/WT	131.6±16.8	
L-AP4	VU094	WT/WT	205.6±21.1	0.1168
L-AP4	VU094	F784S/WT	184.8±14.2	
L-AP4	VU649	WT/WT	156.3±23.7	0.8465
L-AP4	VU649	F784S/WT	159.1±19.9	

Glutamate	None	F784S/WT	2.1±1.2	****<0.0001
Glutamate	VU288	F784S/WT	52.1±3.2	
Glutamate	None	F784S/WT	2.1±1.2	****<0.0001
Glutamate	VU094	F784S/WT	141.6±11.7	
Glutamate	None	F784S/WT	2.1±1.2	****<0.0001
Glutamate	VU649	F784S/WT	118.4±13.9	
L-AP4	None	F784S/WT	58.4±8.0	**0.0022
L-AP4	VU288	F784S/WT	131.6±16.8	
L-AP4	None	F784S/WT	58.4±8.0	****<0.0001
L-AP4	VU094	F784S/WT	184.8±7.1	
L-AP4	None	F784S/WT	58.4±8.0	****<0.0001
L-AP4	VU649	F784S/WT	159.1±19.9	