Table S2 IP accumulation and G_i activation assays of wild-type and mutant mGlu hetero- and homodimers

Basal activity of mGlu2–mGlu3 measured by IP accumulation assay				
WT/mutants	Basal activity[a] % of WT[b]	n[c]	Expression[d] % of WT[b]	
WT	100	23	100	
WT+LY341495	38 ± 3***	7	/	
mGlu2	$E604^{2.37}W$	115 ± 7	7	99 ± 13
	$G611^{2.44}W$	135 ± 6*	7	94 ± 8
	$L615^{2.48}W$	130 ± 5	8	88 ± 10
	$A630^{3.27}W$	159 ± 11***	7	100 ± 14
	$T633^{3.30}W$	162 ± 15***	6	69 ± 11
	$L637^{3.34}W$	135 ± 7*	7	58 ± 6
	$S678^{4.31}W$	143 ± 7*	7	87 ± 18
	$A681^{4.34}W$	135 ± 8*	7	89 ± 20
	$L684^{4.37}W$	144 ± 7**	8	82 ± 8
	$S688^{4.41}W$	150 ± 15**	6	83 ± 17
	$V695^{4.48}W$	109 ± 13	7	78 ± 12
	$V699^{4.52}W$	153 ± 8***	9	82 ± 8
mGlu3	$F598^{1.61}A$	141 ± 16*	7	73 ± 9
	$Y616^{2.40}A$	115 ± 7	7	124 ± 9
	$F620^{2.44}A$	133 ± 10	6	127 ± 19
	$L624^{2.48}W$	142 ± 9*	7	126 ± 11
	$C627^{2.51}W$	149 ± 10**	6	122 ± 14
	$F631^{2.55}A$	139 ± 11*	7	142 ± 11*
	$V639^{3.27}W$	150 ± 13**	5	118 ± 14
	$L643^{3.31}W$	144 ± 11**	7	127 ± 13
	$L646^{3.34}W$	155 ± 11***	8	130 ± 8
	$S650^{3.38}W$	138 ± 4*	7	106 ± 12
	$V701^{4.45}W$	128 ± 7	8	103 ± 15
	$S686^{4.30}W$	131 ± 13	5	114 ± 24
	$F690^{4.34}A$	170 ± 7***	5	147 ± 16
	$G694^{4.38}W$	163 ± 9***	7	100 ± 11

Glutamate-induced G_i activation of mGlu2–mGlu3						
WT/mutants[g]	EC_{50} (μM)	EC_{50} ratio[d]	pEC_{50} mean ± s.e.m.[b]	E_{max}[f] % of WT[b]	n[c]	Expression[d] % of WT[b]
WT	4.4	1	5.36 ± 0.06	100 ± 3	20	100
$mGlu2^{X}$–mGlu3	17	4	4.76 ± 0.51	19 ± 4***	10	56 ± 5*
mGlu2–$mGlu3^{X}$	4.5	1	5.34 ± 0.02	97 ± 8	8	97 ± 12
WT+NAM563	19	4	4.72 ± 0.21	90 ± 9	4	100 ± 0
$mGlu2^{X}$–mGlu3+NAM563	78	18	4.11 ± 0.53	51 ± 15***	4	58 ± 12
$mGlu2^{C121A}$–$mGlu3^{C127A}$	2.5	1	5.61 ± 0.27	81 ± 9	8	125 ± 13
$mGlu2^{C121A/I693C}$–$mGlu3^{C127A/Y743C}$	2.8	1	5.56 ± 0.34	44 ± 6***	10	77 ± 22
$mGlu2^{C121A/A726C}$–$mGlu3^{C127A/L709C}$	8.7	2	5.06 ± 0.48	36 ± 8***	5	106 ± 11
$mGlu2^{C121A/V700C}$–$mGlu3^{C127A/S735C}$	6.2	1	5.21 ± 0.44	42 ± 8***	6	112 ± 17
$mGlu2^{C121A/A630C}$–$mGlu3^{C127A/I708C}$	72	16	4.14 ± 1.04	21 ± 12***	3	65 ± 10
WT	13	1	4.90 ± 0.07	100 ± 3	16	100
$mGlu2^{YADA}$–mGlu3	27	2	4.57 ± 0.20	105 ± 9	5	83 ± 7
mGlu2–$mGlu3^{YADA}$	122	10	3.91 ± 0.31**	41 ± 6***	4	90 ± 8
$mGlu2^{YADA}$–$mGlu3^{X}$	26	2	4.59 ± 0.26	70 ± 8*	4	67 ± 10**

mGlu2^{X}–mGlu3^{YADA}	nd	nd	nd	nd	4	31 ± 4***
mGlu2^{X}–mGlu3	nd	nd	nd	nd	6	78 ± 7*
mGlu2^{X}–mGlu3^{D744N}	22	2	4.66 ± 0.24	84 ± 8	4	76 ± 7*
mGlu2^{X}–mGlu3^{D671G}	224	17	3.65 ± 0.20***	85 ± 8	4	94 ± 8

Glutamate-induced G_i activation of mGlu2–mGlu4

WT/mutants[g]	EC_{50} (μM)	EC_{50} ratio[d]	pEC_{50} mean ± s.e.m.[b]	E_{max}[f] % of WT[b]	n[c]	Expression[d] % of WT[b]
WT	6.3	1	5.20 ± 0.11	100 ± 5	10	100
mGlu2^{X}–mGlu4	11	2	4.96 ± 0.18	102 ± 9	5	41 ± 6**
mGlu2–mGlu4^{X}	nd	nd	nd	nd	5	97 ± 19
WT	13	1	4.88 ± 0.55	100 ± 2	17	100
mGlu2–mGlu4^{X}	42	3	4.38 ± 0.63	13 ± 4***	7	75 ± 9
mGlu2^{G663Q}–mGlu4^{X}	138	10	3.86 ± 0.26*	38 ± 5***	7	82 ± 7
mGlu2^{N735S}–mGlu4^{X}	361	28	3.44 ± 0.47**	33 ± 7***	6	72 ± 8

Glutamate/ADX88178-induecd G_i activation of mGlu2–mGlu4

WT/mutants		ADX88178 EC_{50} (μM)	ADX88178 EC_{50} ratio[d]	ADX88178 pEC_{50} mean ± s.e.m.[b]	ADX88178 E_{max}[f] % of WT[b]	ADX88178 n[c]	Glutamate EC_{50} (μM)	Glutamate EC_{50} ratio[d]	Glutamate pEC_{50} mean ± s.e.m.[b]	Glutamate E_{max}[f] % of WT[b]	Glutamate n[c]	Expression[d] % of WT[b]
WT		2.0	1	5.70 ± 0.07	100 ± 3	18	13	1	4.88 ± 0.55	100 ± 2	17	100
mGlu2	W567$^{1.39}$A	nd	nd	nd	nd	5	31	2	4.51 ± 0.44	55 ± 10***	5	27 ± 5***
	G570$^{1.42}$W	4.7	2	5.33 ± 0.46	45 ± 10***	5	10	1	5.00 ± 0.12	105 ± 5	5	81 ± 7
	I772$^{6.49}$A	nd	nd	nd	nd	5	13	1	4.89 ± 0.12	88 ± 5	5	39 ± 4*
	T792$^{7.30}$W	nd	nd	nd	nd	5	10	1	5.00 ± 0.12	88 ± 5	5	71 ± 8
	V796$^{7.34}$W	16	8	4.79 ± 0.74	38 ± 15***	5	19	1	4.73 ± 0.17	104 ± 8	5	84 ± 8
	S797$^{7.35}$W	0.99	0.5	6.01 ± 0.19	113 ± 9	5	24	2	4.63 ± 0.13	109 ± 6	5	110 ± 14
	L800$^{7.38}$A	nd	nd	nd	nd	4	25	2	4.61 ± 0.20	99 ± 8	4	87 ± 16
	I771$^{6.48}$A	17	8	4.78 ± 0.38	64 ± 14	5	14	1	4.85 ± 0.23	110 ± 11	3	132 ± 34
	L774$^{6.51}$A	18	9	4.75 ± 0.28	83 ± 13	5	17	1	4.76 ± 0.20	135 ± 12*	3	157 ± 5*
	A775$^{6.52}$W	2.4	1	5.63 ± 0.56	47 ± 12	5	22	2	4.66 ± 0.22	101 ± 9	4	106 ± 23
	P778$^{6.55}$W	2.6	1	5.59 ± 0.24	70 ± 8	5	32	2	4.50 ± 0.36	90 ± 13	4	64 ± 6
mGlu4	W587$^{1.39}$A	2.2	1	5.66 ± 0.48	44 ± 10*	5	27	2	4.57 ± 0.24	82 ± 8	3	82 ± 40
	L590$^{1.42}$A	4.4	2	5.35 ± 0.35	84 ± 14	6	16	1	4.79 ± 0.19	115 ± 9	5	119 ± 21
	V797$^{6.49}$A	4.0	2	5.39 ± 0.54	62 ± 16	3	26	2	4.59 ± 0.50	74 ± 15	4	57 ± 18
	T820$^{7.30}$W	2.3	1	5.64 ± 0.59	49 ± 13*	4	14	1	4.85 ± 0.25	108 ± 12	4	76 ± 4
	V824$^{7.34}$W	6.2	3	5.21 ± 0.67	38 ± 12***	4	20	1	4.71 ± 0.29	91 ± 11	4	74 ± 15
	S825$^{7.35}$W	7.8	4	5.11 ± 0.47	62 ± 14*	6	28	2	4.55 ± 0.11	94 ± 4	4	88 ± 17
	L828$^{7.38}$A	2.9	1	5.53 ± 0.28	59 ± 8*	5	17	1	4.78 ± 0.21	122 ± 11	4	103 ± 19
	I796$^{6.48}$A	4.0	2	5.39 ± 0.54	37 ± 9***	5	29	2	4.54 ± 0.17	91 ± 7	5	113 ± 20
	L799$^{6.51}$A	5.5	3	5.26 ± 0.27	78 ± 10	5	16	1	4.78 ± 0.14	102 ± 6	5	107 ± 20
	A800$^{6.52}$W	nd	nd	nd	nd	4	48	4	4.32 ± 0.25	84 ± 9	3	87 ± 21
	I802$^{6.54}$W	nd	nd	nd	nd	5	32	2	4.49 ± 0.13	112 ± 6	5	170 ± 6**
	P803$^{6.55}$W	nd	nd	nd	nd	5	21	2	4.68 ± 0.12	92 ± 5	5	101 ± 13

PAM-induced G_i activation of mGlu2–mGlu4

WT/ Mutants[g]	ADX88178 EC_{50} (μM)	ADX88178 EC_{50} ratio[d]	ADX88178 pEC_{50} mean ± s.e.m.[b]	ADX88178 E_{max}[f] % of WT[b]	ADX88178 n[c]	JNJ-40411813 EC_{50} (μM)	JNJ-40411813 EC_{50} ratio[d]	JNJ-40411813 pEC_{50} mean ± s.e.m.[b]	JNJ-40411813 E_{max}[f] % of WT[b]	JNJ-40411813 n[c]	Expression[d] % of WT[b]
WT	2.0	1	5.70 ± 0.07	100 ± 3	18	6.1	1	5.22 ± 0.16	100 ± 9	9	100
mGlu2^{X}–mGlu4	2.1	1	5.67 ± 0.19	109 ± 10	7	nd	nd	nd	nd	9	54 ± 15*
mGlu2–mGlu4^{X}	nd	nd	nd	nd	7	5.7	1	5.24 ± 0.25	110 ± 17	9	84 ± 12

Glutamate/PAM-induced G_i activation of mGlu4

WT/ mutants	Glutamate EC_{50} (μM)	Glutamate EC_{50} ratio[d]	Glutamate pEC_{50} mean ± s.e.m.[b]	Glutamate E_{max}[f] % of WT[b]	ADX88178 EC_{50} (μM)	ADX88178 EC_{50} ratio[d]	ADX88178 pEC_{50} mean ± s.e.m.[b]	ADX88178 E_{max}[f] % of WT[b]	VU0364770 EC_{50} (μM)	VU0364770 EC_{50} ratio[d]	VU0364770 pEC_{50} mean ± s.e.m.[b]	VU0364770 E_{max}[f] % of WT[b]	n[c]	Expression[d] % of WT[b]

WT	24	1	4.62 ± 0.11	100 ± 4	0.65	1	6.19 ± 0.08	100 ± 3	27	1	4.57 ± 0.13	100 ± 7	7	100
I796$^{6.48}$A	166	7	3.78 ± 0.19*	80 ± 7	nd	nd	nd	nd	nd	nd	nd	nd	5	115 ± 32
A800$^{6.52}$W	57	2	4.24 ± 0.21	76 ± 7	nd	nd	nd	nd	nd	nd	nd	nd	7	29 ± 6*
S825$^{7.35}$W	83	3	4.08 ± 0.19	86 ± 8	nd	nd	nd	nd	nd	nd	nd	nd	6	44 ± 4*

[a]The basal activity was calculated by subtracting the IP production measured in the control ($G\alpha_{qi9}$, EAAT1, and empty PTT5 vector) for the wild-type (WT) heterodimer and all the mutants, and is presented as percent of WT activity.

[b]Data are shown as mean ± s.e.m. from at least three independent experiments. nd (not determined) refers to data where a robust concentration response curve could not be established within the concentration range tested. *P<0.05, **P<0.001, ***P<0.0001 by one-way ANOVA followed by Dunnett's post-test compared to the response of WT.

[c]Sample size, the number of independent experiments performed in technical duplicate.

[d]Protein expression levels of the constructs at the cell surface were determined in parallel by flow cytometry with an anti-Flag antibody (Sigma) and reported as percent compared to the WT from at least three independent measurements performed in duplicate.

[e]The EC_{50} ratio (EC_{50}(mutant)/EC_{50}(WT)) represents the shift between the WT and mutant curves, and characterizes the effect of the mutations on G_i activation.

[f]The maximal response is reported as a percentage of the maximal effect at the WT.

[g]The 'X' indicates that the G protein coupling of the subunit was blocked by introducing a mutation in ICL3 (mGlu2, F756S; mGlu3, F765S; mGlu4, F781S).