Supplementary information

This supplementary information accompanies the article:

Molecular determinants of positive allosteric modulation of the human metabotropic glutamate receptor 2

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SUPPLEMENTARY MATERIAL 1: BUILDING THE $MGLU_2$ RECEPTOR HOMOLOGY MODEL.

An $mGlu_2$ receptor model was built from the recent $mGlu_1$ transmembrane structure 4OR2 (Wu *et al*, 2014). The $mGlu_1$ and $mGlu_2$ sequence alignment from Wu *et al* was applied. The 3D model was built using the homology model approaches in the Molecular Operating Environment (MOE) software. Default parameters were used except for the following modifications: the number of mainchain template models was increased from 10 to 100, intermediate and final model refinement was set to fine detail with RMS gradients of 0.1, and the ligand from 4OR2 was retained during model building to maintain an open binding site. The Amber 12HT forcefield was employed for energy minimisation and refinement. Dihedral angles were examined via Ramachandran plots and outlier amino acids were subjected to further optimisation.

SUPPLEMENTARY MATERIAL 2: PROTEIN LIGAND DOCKING.

Molecules were docked into the $mGlu_2$ receptor model using the software GOLD v5.2.2. Molecules were prepared with the MOE wash function, and their protonation state was also checked via pKa calculation with ACDLabs v12.0; this resulted in the deprotonation of the phenol in LY2607540. The GoldScore fitness function was used for docking with the so called 'most accurate approach', specifying 10 rounds of genetic algorithm optimization per ligand. A large 30 Å radius around CA of Leu732 defined the putative 7-TM binding site used for the docking calculations. This was sufficient to encompass almost the entire protein except for a small number of amino acids on the intracellular face. The top ten binding poses for each molecule were examined to identify plausible binding modes.

SUPPLEMENTARY MATERIAL 3: DATA CONFIRMING THE INTEGRITY OF THE ORTHOSTERIC RECEPTOR BINDING SITE.

Table S1: Summary of pIC_{50} (displacement of [^{3}H]LY341495 binding by glutamate) and pEC_{50} values (glutamate-induced [^{35}S]GTPγS binding) for stably and transiently transfected $mGlu_2$-wt (and $mGlu_2$ mutants. In each experiment, concentration-response measurements were performed in triplicate. SD values are provided when 2 or more experiments were performed.

Mutation	pIC_{50}	pEC_{50}	Response amplitude (%)
Stable mGlu2-wt	4.9 ± 0.10	5.0 ± 0.10	664 ± 174
Transient mGlu2-wt	4.9 ± 0.07	5.3 ± 0.15	250 ± 70
C616S	4.7	5.4	185
I622F	4.7	5.3	205
R635A	4.9	4.9 ± 0.22	205 ± 53
R636A	4.8	5.1 ± 0.12	185 ± 24
L639A	4.9	5.2 ± 0.14	261 ± 57
T641S	4.7	5.2	181
A642S	4.7	5.4	225
F643A	4.8 ± 0.06	5.0 ± 0.19	211 ± 55
S644A	4.7	5.2 ± 0.16	198 ± 67
A681F	5.2	5.4	237
S688L	4.7 ± 0.06	5.2 ± 0.12	259 ± 71
G689V	4.7	5.2 ± 0.17	225 ± 69
I693M	4.7	5.3	199
V695S	4.7	5.3 ± 0.09	180 ± 16
A696V	4.7	5.9 ± 0.54	184 ± 26
V700L	4.9	5.2 ± 0.14	243 ± 32
G706R	4.7	5.3 ± 0.07	212 ± 35
E708Y	4.6	5.1 ± 0.02	178 ± 5
A710L	4.7	5.2 ± 0.14	293 ± 58
P711A	4.7	5.4 ± 0.16	214 ± 28
V716T	4.7	5.2	217
T718I	4.7	5.2	176
H723V	4.6	5.1 ± 0.13	200 ± 61
D725A	5.0	5.3 ± 0.12	303 ± 11
A726S	4.6	5.3 ± 0.01	192 ± 8
M728A	4.9	5.1 ± 0.12	274 ± 132
G730I	4.7	5.3 ± 0.10	231 ± 19
S731A	5.0	5.3 ± 0.25	331 ± 61
L732A	4.9 ± 0.06	5.4 ± 0.18	336 ± 105

A733T	4.6	5.2 ± 0.14	174 ± 18
N735D	4.6	5.2 ± 0.15	225 ± 52
V736A	4.9	5.1 ± 0.07	248 ± 19
A740I	4.6	5.3 ± 0.13	165 ± 36
W773A	4.9 ± 0.12	5.0 ± 0.46	169 ± 29
F776A	4.8	5.3 ± 0.12	313 ± 87
F780A	4.8	5.2 ± 0.13	295 ± 46
S688L/G689V	4.7	5.1 ± 0.17	260 ± 59
S644A/V700L/H723V	4.8	5.2 ± 0.22	240 ± 60
S688L/G689V/N735D	4.7	5.0 ± 0.17	262 ± 52

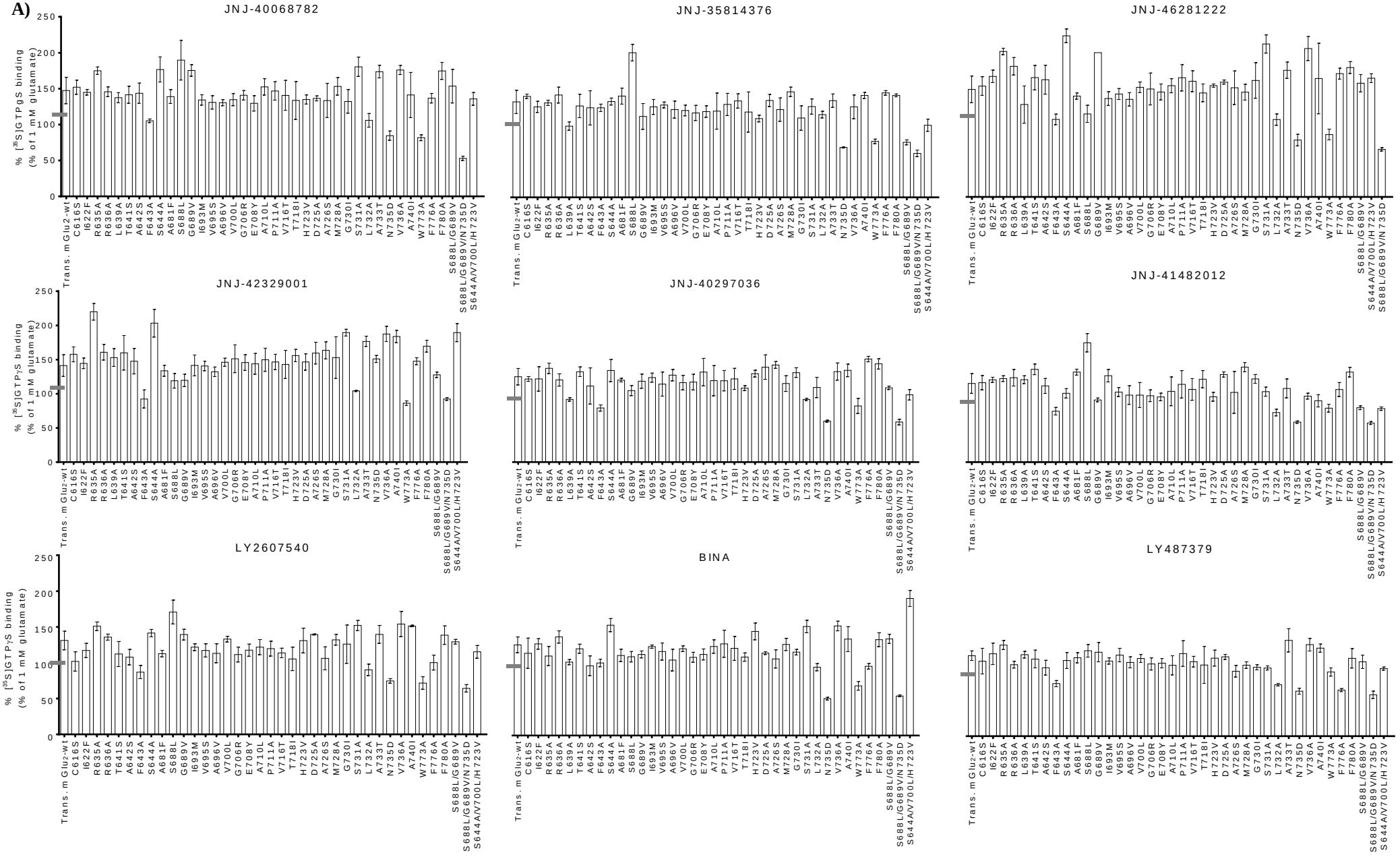
SUPPLEMENTARY MATERIAL 4: DETAILS ON THE INITIAL SCREENING OF EACH PAM ON ALL 39 MUTANT MGLU$_2$ RECEPTORS.

For the initial screening, two concentrations of PAM were tested: a concentration equivalent to the compound's EC_{50} and a concentration producing a maximal PAM response (typically 3 or 10 µM), both previously determined with the use of stably transfected cells. The resulting PAM effect at each concentration, calculated as a percentage of the response to 1 mM glutamate, was then compared between $mGlu_2$ mutants and the transiently transfected mGlu2-wt receptor. The response obtained in transient $mGlu_2$-wt was considered the baseline; mutations that elicited a similar response, i.e. these that produced a response within 25% variation of the response obtained in transient $mGlu_2$-wt, were considered to behave as the transient $mGlu_2$-wt. This 25% cut-off was chosen based on the overall variability of the PAM response in transient $mGlu_2$-wt, i.e. the standard deviation on the compound responses was not higher than 25% and hence this was considered a safe net to capture any changes from baseline. Results from the initial screening of each PAM on all mutant $mGlu_2$ receptors are shown in Figure S1 below.

To validate the screening approach, a set of 60 compound-mutant pairs with pre-screen data and twelve point concentration-response EC_{50} values was used. Data were ranked from high to low effect by both pre-screen and then full EC_{50}. A Spearman's correlation (r_s = 0.72) between the two ranks indicated that the two approaches were correlated and produce similar results.

To further assess the validity of the screening approach, 17 compound-mutant pairs for which no apparent changes in response to two compound concentrations were noted during initial testing, were still analyzed in greater detail: concentration-response analyses confirmed that EC_{50} values were unchanged (see for example follow-up work on S688L in Table S2).

Figure S1: Results from the initial screening of each PAM on all 39 mutant $mGlu_2$ receptors. For each transiently expressed $hmGlu_2$ receptor, the effect of the PAM was calculated as a percentage ± SD of the response to 1 mM glutamate. Results generally represent one or two experiments performed in quadruplicate,. The symbol ▬ indicates the cut-off that was chosen to select mutants for further evaluation. A) PAM effect at 3 or 10 µM of each compound, i.e. a concentration that should produce a maximal effect; B) PAM effect at a concentration of PAM equivalent to its EC_{50}.



B)

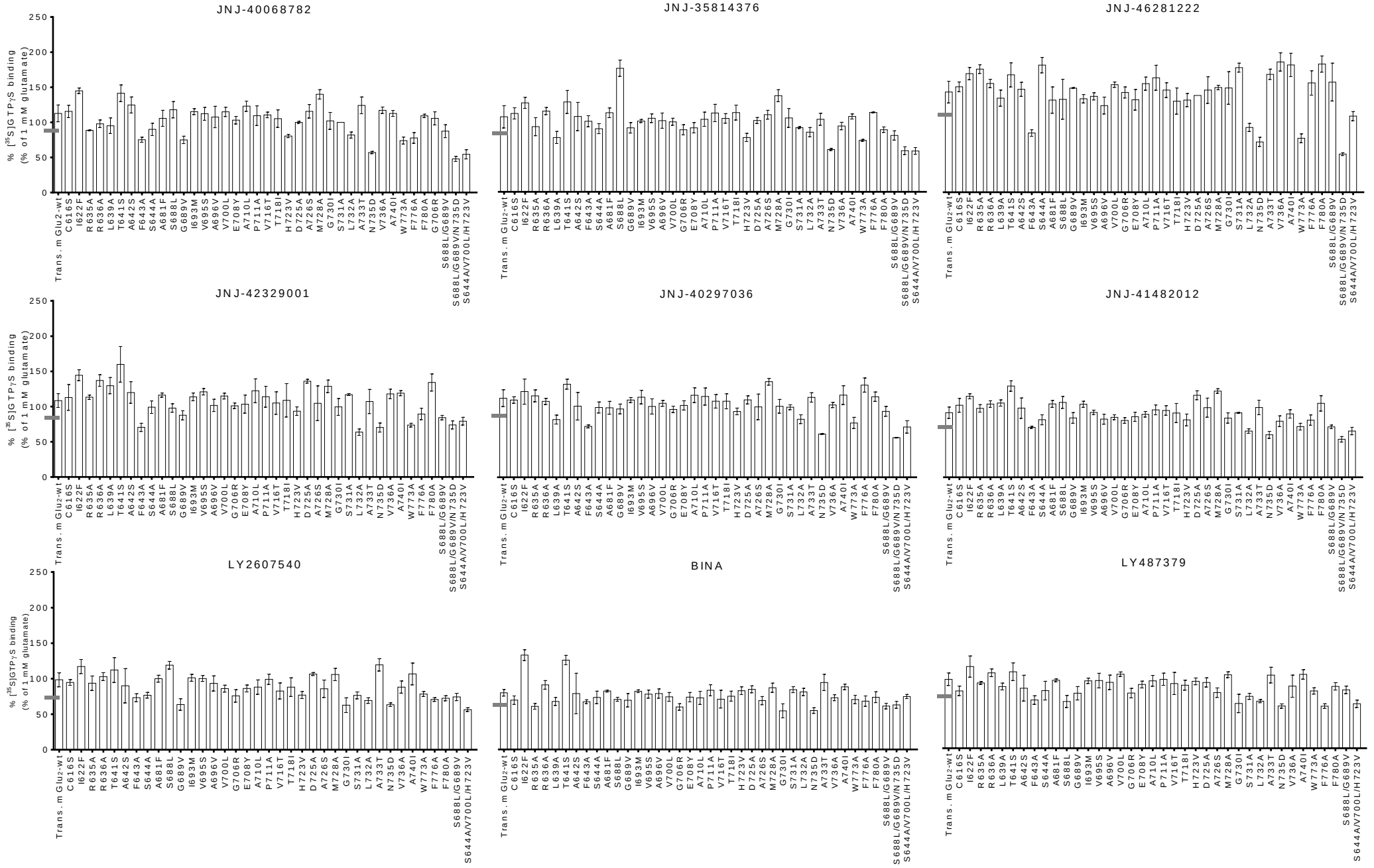

JNJ-40068782
JNJ-35814376
JNJ-46281222
JNJ-42329001
JNJ-40297036
JNJ-41482012
LY2607540
BINA
LY487379
% [35S]GTPγS binding
(% of 1 mM glutamate)
0
50
100
150
200
250
Trans. mGlu2-wt
C616S
I622F
R635A
R636A
L639A
T641S
A642S
F643A
S644A
A681F
S688L
G689V
I693M
V695S
A696V
V700L
G706R
E708Y
A710L
P711A
V716T
T718I
H723V
D725A
A726S
M728A
G730I
S731A
L732A
A733T
N735D
V736A
A740I
W773A
F776A
F780A
S688L/G689V
S688L/G689V/N735D
S644A/V700L/H723V

Table S2: pEC_{50} of each PAM studied on S688L compared to $mGlu_2$-wt. This mutation was found not to affect the response of each PAM tested during the initial screening round. Concentration-response analyses were nevertheless conducted to validate the screening approach. Data represent mean ± SD of at least 2-3 experiments, except for JNJ-46281222 (n=1).

	Transient $mGlu_2$-wt	**S688L**
JNJ-40068782	7.08 ± 0.13	6.92 ± 0.41
JNJ-35814376	6.36 ± 0.25	6.28 ± 0.06
JNJ-46281222	8.22 ± 0.29	7.69
JNJ-42329001	7.55 ± 0.2	7.50 ± 0.05
JNJ-40297036	6.75 ± 0.15	6.80 ± 0.17
JNJ-41482012	6.83 ± 0.15	6.89 ± 0.15
LY2607540 (THIIC)	6.97 ± 0.14	6.91 ± 0.35
BINA	7.11 ± 0.30	6.93 ± 0.33
LY487379	6.85 ± 0.20	6.92 ± 0.09

SUPPLEMENTARY MATERIAL 5: SEQUENCE ALIGNMENT.

Figure S2: Sequence alignment between human GRM1 to GRM8. Greyscale is based on column sequence similarity (BLOSUM62 matrix used, 100% conservation is black, ≥80% conservation is dark grey, ≥60% conservation is light grey). Secondary structural elements are based on 4OR2 structure assigned in MOE. Sheet segments are yellow, turn segments are blue, helix segments are pink. The start and end of the transmembrane alpha helices as defined from the $mGlu_1$ structure are highlighted. The sequence similarity follows the expected mGlu receptor subfamily denominations with human $mGlu_1$ and $mGlu_5$ receptors having 78% identity, $mGlu_2$ and $mGlu_3$ 75% identity, and $mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$ all having greater than 73% identity. Sequence identity between mGlu receptors of different subfamilies was typically in the range 45 to 52%, for instance, the sequence identity between human $mGlu_1$ and $mGlu_2$ receptors was 48%. Three amino acid positions differ in the transmembrane domain of human, rat and mouse $mGlu_2$ receptor sequences: I622 in human and mouse was V622 in rat; V695 in human was A695 in rat and mouse; and finally T705 in human and rat was I705 in mouse.