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# Structures of human mGlu2 and mGlu7 homo- and heterodimers

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34 **Summary**

35 **The metabotropic glutamate receptors (mGlu) are involved in modulation of synaptic**  
36 **transmission and neuronal excitability in the central nervous system<sup>1</sup>. These receptors**  
37 **likely exist as both homo- and heterodimers with unique pharmacological and**  
38 **functional properties<sup>2-4</sup>. Here we report four cryo-electron microscopy structures of the**  
39 **human mGlu, including inactive mGlu2 and mGlu7 homodimers, agonist/PAM-bound**  
40 **mGlu2 homodimer, and inactive mGlu2-7 heterodimer. A subtype-dependent**  
41 **dimerization mode of mGlu was observed, as a unique dimer interface mediated by**  
42 **helix IV important to limit receptor activity exists in the inactive mGlu2 structure only.**  
43 **The structures provide molecular details of inter- and intrasubunit conformational**  
44 **changes that are required for receptor activation, which distinguish class C G-protein-**  
45 **coupled receptors (GPCRs) from classes A and B receptors. Furthermore, the mGlu2-7**  
46 **heterodimer structure and functional studies suggest that the mGlu7 subunit plays a**  
47 **dominant role in controlling dimeric association and G protein activation in the**  
48 **heterodimer. These insights into mGlu homo- and heterodimers highlight the complex**  
49 **landscape of mGlu dimerization and activation.**

50 The mGlu contain a large extracellular domain (ECD) composed of the Venus flytrap (VFT)  
51 domain that binds agonist and a cysteine-rich domain (CRD) connected to a seven-helical  
52 transmembrane domain (TMD) responsible for G protein coupling<sup>4</sup>. It has been  
53 acknowledged that homodimerization is mandatory for the function of the mGlu, making  
54 them complex allosteric proteins with two subunits influencing each other<sup>3</sup>. In addition, there  
55 is increasing evidence suggesting that different mGlu subunits can associate to form multiple  
56 types of heterodimers, adding complexity of function modulation in this receptor family<sup>2,4</sup>.  
57 However, conformational difference between the mGlu homo- and heterodimerization and  
58 how these dimers control mGlu function remain unclear. Thus, we performed single-particle

59 cryo-electron microscopy (cryo-EM), X-ray crystallography, and functional studies to solve  
60 structures of mGlu2 and mGlu7 in both homo- and heterodimeric organizations and provide  
61 valuable insights into mGlu activity modulation.

## 62 **Structure determination of mGlu2 and mGlu7**

63 For structure determination of inactive mGlu2 and mGlu7 homodimers, a modified mGlu2 or  
64 mGlu7 was purified in presence of its selective negative allosteric modulator (NAM)  
65 [NAM563 (ref. 5) for mGlu2; MMPIP<sup>6</sup> for mGlu7] (see Methods for construct design and  
66 function characterization; Extended Data Fig. 1a-l). The stable agonist-bound mGlu2 was  
67 obtained by co-purifying another modified receptor with the agonist LY354740 (ref. 7), the  
68 positive allosteric modulator (PAM) JNJ-40411813 (ref. 8), and the nanobody DN13 that acts  
69 as an mGlu2 PAM<sup>9</sup> (Extended Data Fig. 1e, i, n-q). To solve the mGlu2-7 heterodimer  
70 structure, an FK506 binding protein (FKBP)-tagged mGlu2 and a rapamycin binding  
71 fragment (FRB)-tagged mGlu7 were co-purified in the presence of NAM563 and MMPIP  
72 (Extended Data Fig. 1r and Supplementary Fig. 1). The inactive structures of mGlu2, mGlu7,  
73 and mGlu2-7 and the agonist/PAM-bound mGlu2 structure were determined by cryo-EM  
74 with overall resolutions of 3.6 Å, 4.0 Å, 3.9 Å, and 3.1 Å, respectively, and the resolutions in  
75 the ECDs of mGlu7 and mGlu2-7 were further improved to 3.6 Å and 3.5 Å, respectively  
76 (Extended Data Figs. 2 and 3; Extended Data Table 1). To provide high-resolution molecular  
77 details of NAM binding mode, we also solved two crystal structures of the mGlu2 TMD  
78 bound to two NAMs, NAM563 and NAM597 (ref. 10), at 2.5 Å and 2.7 Å resolution,  
79 respectively (Extended Data Table 2).

80 The inter-VFT disulfide bonds (mGlu2, C121-C121; mGlu7, C136-C136; mGlu2-7,  
81 C121-C136) were resolved in all the structures, but exhibit distinct conformations (Extended  
82 Data Fig. 3). Compared to the inactive mGlu2 structure, the junction loop between the

83 disulfide bond and B helix in the VFT adopts a more extended conformation in the inactive  
84 structures of mGlu7 and mGlu2-7, resulting in a higher position of this disulfide bond  
85 (Extended Data Fig. 4a). Associated with the conformational rearrangement of the VFTs  
86 upon agonist binding, the disulfide bond undergoes an upward movement in the  
87 agonist/PAM-bound mGlu2 structure relative to the inactive state (Extended Data Fig. 4b),  
88 which may be consistent with previous observation that the intersubunit disulfide bond  
89 stabilizes the receptor active state<sup>2</sup>.

### 90 **Multiple dimerization modes of inactive mGlu**

91 Similar to the previously published inactive structures of mGlu5<sup>11</sup> and mGlu2-VFT<sup>12</sup>, the  
92 inactive mGlu2 and mGlu7 structures reveal an open conformation of the VFTs and a  
93 common homodimer interface at the apex of the VFTs (Extended Data Fig. 4c, d). In contrast,  
94 the CRDs adopt distinct relative positioning in the inactive structures of mGlu2, mGlu5, and  
95 mGlu7, with the CRDs in mGlu2 exhibiting the closest positions (Extended Data Fig. 4e).  
96 This difference is associated with the distinct dimerization modes of the receptor TMDs,  
97 where close contact is observed in mGlu2 but is absent in mGlu5 and mGlu7 (Fig. 2a). The  
98 TMDs of mGlu5 and mGlu7 are completely separated with helices V in the two subunits  
99 facing each other, while the TMDs in the inactive mGlu2 form extensive interactions along  
100 the whole length of helix IV (Figs. 1a, b and 2a). This dimerization mode is supported by  
101 previous crosslinking studies, showing that helices IV and V of mGlu2 are involved in  
102 intersubunit interaction in basal or inactive state<sup>13</sup>. The additional involvement of helix V in  
103 dimerization suggests a dynamic nature of the dimer interface.

104 The different dimerization modes of the inactive mGlu agree with previous investigation  
105 of inter-TMD interaction, in which the mGlu2 TMD showed a higher proportion of dimers  
106 compared to that of mGlu1, mGlu3, or mGlu5<sup>14</sup>, suggesting subtype-specific inter-TMD

107 interaction strength of mGlu2. In the inactive mGlu2, the TMD dimer interface is mainly  
108 mediated by helix IV and the extracellular tip of helix III through hydrophobic contacts (Fig.  
109 2b, c). Mutations of most of the key residues in the dimer interface resulted in an elevated  
110 constitutive activity (Fig. 2d and Extended Data Table 3), and the full dose-response curves  
111 excluded the possible effects of increased glutamate potency and maximal activation  
112 potentially induced by the environmental glutamate (Extended Data Fig. 1s and Extended  
113 Data Table 3). Furthermore, a substantially impaired receptor activity was observed when the  
114 inactive dimer interface is locked through a disulfide bridge by introducing a cysteine  
115 substitution, L698<sup>4.51</sup>C or V699<sup>4.52</sup>C (Extended Data Fig. 1u, v and Supplementary Fig. 1).  
116 These data imply that the helix IV-mediated dimerization plays a role in stabilizing the  
117 receptor inactive conformation.

118 The mGlu2 residues that participate in dimerization are poorly conserved between mGlu2  
119 (Extended Data Fig. 5), suggesting that the mGlu2 dimer interface along helix IV may be  
120 subtype-dependent. Some of the key residues such as L684<sup>4.37</sup>, L692<sup>4.45</sup>, L698<sup>4.51</sup>, and  
121 V699<sup>4.52</sup> are substituted by phenylalanine or residues with small side chains in other mGlu2,  
122 which may disturb the dimer interface by introducing a spatial hindrance or disrupting the  
123 hydrophobic interaction. Replacing these residues with their counterparts in other mGlu2  
124 increased the receptor basal activity (Fig. 2d, Extended Data Fig. 1t, and Extended Data  
125 Table 3), further reflecting the disturbance of the inactive state. In contrast, a wild-type level  
126 of basal activity was observed for the mGlu7 mutants in helices III and IV (Fig. 2e). These  
127 data support that the dimer interface along helix IV in the inactive mGlu2 homodimer is  
128 subtype-specific. However, this does not rule out the possibilities of specific inactive  
129 conformation that is required for facilitating conformational change upon receptor activation  
130 and structural constraints coming from the specific conformation of the VFTs and CRDs in  
131 different mGlu2.

132 **Intersubunit interactions upon activation**

133 Comparison of the inactive and agonist/PAM-bound structures of mGlu2 revealed a closure  
134 of the VFTs and CRDs upon receptor activation (Fig. 1b, c; Extended Data Fig. 4f, g),  
135 leading to a dimer interface mediated by a hydrophobic interaction core at the apex of the  
136 CRDs (Extended Data Fig. 4h). Locking this interface by introducing a disulfide bond  
137 resulted in constitutive activation of mGlu2 and mGlu7 (Extended Data Fig. 1w, x),  
138 consistent with previous studies showing that a precise association of the two CRDs in an  
139 mGlu dimer is required for full receptor activation<sup>15</sup>.

140 Similar to what was observed for mGlu5<sup>11</sup>, in the agonist/PAM-bound mGlu2 structure  
141 the CRD and ECL2 are in proximity (Extended Data Fig. 6a and Extended Data Fig. 3c).  
142 Disturbing the interaction interface by introducing alanine mutation(s) or removing the ECL2  
143 residues R714 and E715 substantially reduced the maximal response ( $E_{\max}$ ) of the receptor in  
144 the agonist-induced inositol phosphate (IP) accumulation assay but had no effect on the  
145 PAM-induced receptor signalling (Extended Data Fig. 1y, z; Extended Data Table 3). A  
146 similar detrimental effect was also shown when residues at the apex of ECL2 in mGlu7  
147 (T737-E741) were removed (Extended Data Fig. 1x and Extended Data Table 3). These data  
148 suggest that the CRD and ECL2 may play a similar role in transmitting signal from the VFT  
149 to the TMD in different mGlus.

150 Upon receptor activation, the helix IV-mediated dimeric association of mGlu2 switches to  
151 an active state mainly contributed by helix VI (Fig. 1b, c), a hallmark of class C GPCR  
152 activation<sup>11,13</sup>. An increased constitutive activity of mGlu7 mutant A812<sup>6.61</sup>C was observed  
153 (Extended Data Fig. 1x), suggesting that helix VI is most likely also involved in mGlu7  
154 dimerization in the active state. Despite a similar dimer interface along helix VI in mGlu2  
155 and mGlu5, the agonist/PAM-bound mGlu2 structure reveals a slight movement of the TMDs

156 relative to the mGlu5 structure, leading to additional contacts between residues V782<sup>6.59</sup> and  
157 V789<sup>7.27</sup> at the extracellular tips of helices VI and VII (Extended Data Fig. 6b). Our cysteine  
158 crosslinking and signalling studies showed that in contrast to mutant C121A, additional  
159 introduction of mutations V782<sup>6.59</sup>C and V789<sup>7.27</sup>C in the two subunits, respectively,  
160 displayed an increased dimer proportion in presence of agonist and PAM (Extended Data Fig.  
161 1u and Supplementary Fig. 1) and an elevated constitutive activity (Extended Data Table 3),  
162 which reflect the close contact between helices VI and VII in the active state and importance  
163 of such contact in stabilizing the receptor active conformation.

#### 164 **Intrasubunit conformational change of mGlu2**

165 Following the relative movement of the two subunits, further conformational change within  
166 the TMD of one subunit occurs, resulting in G protein activation<sup>16</sup>. The crystal structures of  
167 the mGlu2-TMD bound to NAM563 and NAM597 (Extended Data Fig. 6c) and our recently  
168 determined structure of the fully active mGlu2–G<sub>i</sub> complex<sup>17</sup> allowed us to obtain molecular  
169 details of the conformational changes required for receptor activation within the receptor  
170 TMD.

171 In mGlu2, NAM563 and NAM597 bind to a cavity mainly formed by helices III and V-  
172 VII, which is similar to the NAM-binding pockets previously observed in mGlu1<sup>18</sup> and  
173 mGlu5<sup>19</sup> and the ligand-binding sites in some class A GPCRs (Extended Data Fig. 6d). These  
174 two NAMs exhibit similar binding patterns that share many common interactions with the  
175 receptor but are distinct on the extracellular side, with the trifluoromethyl-morpholin group of  
176 NAM563 squeezing into a gap between helices VI and VII while the methyl-pyrazol group in  
177 NAM597 extending in an opposite direction to enable extra interactions with helix II and  
178 ECL2 (Extended Data Fig. 6c). This may account for the slightly higher activity of NAM597  
179 in inhibiting receptor signalling (Extended Data Fig. 1m and Extended Data Table 3). The

180 binding modes of these two NAMs are supported by a 5–1,000-fold reduction of their  
181 inhibitory activity associated with mutations within the binding pocket, which had a weak  
182 effect on agonist-induced receptor signalling (Extended Data Table 3).

183 Compared to the inactive structures of mGlu2 and mGlu2-TMD–NAMs, the pronounced  
184 outward movement of helix VI, a hallmark of receptor activation for classes A and B GPCRs,  
185 was not observed in both the  $G_i$ -bound and -free structures of agonist-bound mGlu2  
186 (Extended Data Fig. 6e). Instead, helix VI in the  $G_i$ -bound subunit of the mGlu2– $G_i$  complex  
187 undergoes a downward shift towards the intracellular surface by half a helical turn (Extended  
188 Data Fig. 6f). However, neither the G protein-free agonist/PAM-bound structure nor the  $G_i$ -  
189 free subunit in the mGlu2– $G_i$  structure displays such conformational change, suggesting that  
190 this movement is associated with G protein binding. Close inspection of the mGlu2-TMD–  
191 NAM and mGlu2– $G_i$  structures revealed a rotamer conformational change of the highly  
192 conserved class C GPCR residue W<sup>6.50</sup> with its side chain rotating downwards by  
193 approximate 90° in the  $G_i$ -bound structure, which may release the transmembrane helical  
194 bundle from its inactive state by disrupting an interaction core involving helices III and V–  
195 VII and potentially leads to the downward shift of helix VI (Extended Data Fig. 6g). In the  
196 structures of mGlu2-TMD–NAMs, the NAMs form a close contact with W773<sup>6.50</sup>,  
197 constraining the conformational change of the side chain to stabilize the receptor in the  
198 inactive state (Extended Data Fig. 6c).

199 Although not directly involved in G protein binding of mGlu2<sup>17</sup>, helix VI may play a role  
200 in modulating receptor activation by altering the interaction network on the intracellular  
201 surface of the receptor to facilitate G protein recognition. A salt bridge between the highly  
202 conserved residues K<sup>3.50</sup> and E<sup>6.35</sup>, which is known as the ‘ionic lock’ of class C GPCRs<sup>18,19</sup>,  
203 and another ionic interaction formed by the less well conserved residue R656<sup>3.53</sup> in helix III  
204 and E754 in ICL3 are intact in both mGlu2-TMD–NAM structures but broken in the  $G_i$ -

205 bound subunit of the mGlu2-G<sub>i</sub> complex likely due to the downward shift of helix VI and  
206 rearrangement of ICL3 (Extended Data Fig. 6h). Our signalling assay showed an increased  
207 constitutive activity resulted from mutation E754A (Extended Data Table 3), implying that  
208 the salt bridge between R656<sup>3,53</sup> and E754 may act as a secondary ‘ionic lock’ to stabilize the  
209 inactive conformation of mGlu2.

## 210 **Heterodimerization of mGlu2 and mGlu7**

211 It was recently found that mGlu2 can associate with mGlu7 in hippocampus, suggesting a  
212 physiological relevance of such a heterodimer<sup>20</sup>. The mGlu2-7 heterodimerization was further  
213 confirmed by our tr-fluorescence resonance energy transfer (tr-FRET) assay (Extended Data  
214 Fig. 7a, b). Like the homodimers, the VFTs in the mGlu2-7 heterodimer adopt a similar open  
215 conformation forming contacts mainly through the B and C helices (Extended Data Fig. 4i, j),  
216 suggesting a conserved VFT dimerization mode shared by the mGlu homo- and heterodimers.  
217 In addition to this common dimer interface, extra contacts between the mGlu7 residue R191  
218 and the mGlu2 residues D174 and S176 were observed in the heterodimer (Fig. 3a). Similar  
219 interactions also exist in the inactive mGlu2 homodimer, but not in the inactive mGlu7  
220 homodimer due to the substitution of S176 (mGlu2) with R190 (mGlu7), which repels the  
221 residue R191 in the opposite subunit (Fig. 3a). Compared to the heterodimer mutant that  
222 lacks the inter-VFT disulfide bond, further introduction of mutation D174C or S176C in  
223 mGlu2 and mutation R191C in mGlu7 greatly increased the dimer proportion in our  
224 crosslinking studies (Extended Data Fig. 1aa and Supplementary Fig. 1), supporting the close  
225 contacts between these residues. A substantially reduced potency of both the mGlu2 and  
226 mGlu7 agonists was observed for the crosslinked heterodimer mutants and mutants with the  
227 mGlu2 residues D174 and S176 replaced with arginines or alanine mutation(s) introduced to  
228 disrupt the inter-VFT interactions (Extended Data Fig. 7c-g), suggesting that this heterodimer  
229 interface may play a role in modulating receptor activation.

230 The interaction difference in the top lobes of the VFTs brings the rest of the heterodimer  
231 in closer distance compared to that in the mGlu7 homodimer (Fig. 3a). While the TMDs in  
232 the mGlu2 and mGlu7 homodimers adopt distinct dimerization modes, the TMDs in the  
233 mGlu2-7 heterodimer assemble in a similar manner to that in the mGlu7 homodimer with  
234 helices V facing each other (Fig. 1d), implying that mGlu7 may be more dominant in  
235 controlling heterodimer assembly. Previous investigations of mGlu homo- and  
236 heterodimerization suggested that mGlu2 dimerized with mGlu7 with a lower propensity  
237 compared to that of mGlu2 homodimerization<sup>2</sup>. This is likely due to the distinct dimerization  
238 modes of the TMDs, which form extensive contacts in the mGlu2 homodimer but not in the  
239 mGlu2-7 heterodimer.

240 Asymmetric activation has been observed for some mGlu heterodimers<sup>2,21</sup>. The mGlu2-7  
241 heterodimer displayed a 3-fold higher  $E_{\max}$  than the mGlu7 homodimer when activated by the  
242 mGlu7 agonist L-AP4 (ref. 22) (Fig. 3c), which indicates that the heterodimerization boosts  
243 the G protein activation even when one of the subunits is not occupied with agonist,  
244 demonstrating a clear cooperativity between the two subunits. Further investigation of  
245 heterodimer signal transduction showed an agonist-induced response comparable to the  
246 controls when the mGlu2 subunit carried mutation F756S in ICL3 that blocks the G protein  
247 coupling<sup>23</sup>, while no response was observed if the mutation was introduced in the mGlu7  
248 subunit (Fig. 3b, c). Such data imply that the mGlu7 subunit is mainly responsible for G  
249 protein activation within the mGlu2-7 heterodimer, consistent with previous observation that  
250 the group III mGlu subunit plays a predominant role in mediating signal transduction in the  
251 heterodimers between groups II and III mGlu<sup>21</sup>. Furthermore, a recovery of agonist-induced  
252 response was observed in presence of the mGlu2 PAM JNJ-40411813 with the heterodimer  
253 containing the blocked mGlu7 subunit (Fig. 3d, e), demonstrating that the mGlu2 subunit  
254 within the mGlu2-7 heterodimer is capable of G protein coupling when its TMD activation is

255 facilitated by a PAM. However, the signal transduction could not be redirected to mGlu2 if  
256 the mGlu7 blocked subunit was locked in its inactive state with a selective NAM (Fig. 3d and  
257 Extended Data Fig. 7h). This is in contrast to the previous finding on the mGlu2-4  
258 heterodimer, in which mGlu2 took over for G protein activation when an mGlu4 NAM was  
259 used<sup>21</sup>. These observations suggest subtype-specific manners of signal transduction within  
260 different mGlu heterodimers.

## 261 **References**

- 262 1 Niswender, C. M. & Conn, P. J. Metabotropic glutamate receptors: physiology, pharmacology,  
263 and disease. *Annu. Rev. Pharmacol. Toxicol.* **50**, 295-322 (2010).
- 264 2 Levitz, J. *et al.* Mechanism of assembly and cooperativity of homomeric and heteromeric  
265 metabotropic glutamate receptors. *Neuron* **92**, 143-159 (2016).
- 266 3 Kniazeff, J., Prezeau, L., Rondard, P., Pin, J. P. & Goudet, C. Dimers and beyond: The  
267 functional puzzles of class C GPCRs. *Pharmacol. Ther.* **130**, 9-25 (2011).
- 268 4 Pin, J. P. & Bettler, B. Organization and functions of mGlu and GABA<sub>B</sub> receptor complexes.  
269 *Nature* **540**, 60-68 (2016).
- 270 5 Bungard, C. J. *et al.* Quinoline carboxamide and quinoline carbonitrile derivatives as  
271 mGluR2-negative allosteric modulators, compositions, and their use. *PCT Int. Pat. Appl* **WO**  
272 **2013/066736 A1** (2013).
- 273 6 Suzuki, G. *et al.* In vitro pharmacological characterization of novel isoxazopyridone  
274 derivatives as allosteric metabotropic glutamate receptor 7 antagonists. *J. Pharmacol. Exp.*  
275 *Ther.* **323**, 147-156 (2007).
- 276 7 Monn, J. A. *et al.* Design, synthesis, and pharmacological characterization of (+)-2-  
277 aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY354740): a potent, selective, and orally  
278 active group 2 metabotropic glutamate receptor agonist possessing anticonvulsant and  
279 anxiolytic properties. *J. Med. Chem.* **40**, 528-537 (1997).
- 280 8 Cid, J. M. *et al.* Discovery of 1-butyl-3-chloro-4-(4-phenyl-1-piperidinyl)-(1H)-pyridone  
281 (JNJ-40411813): a novel positive allosteric modulator of the metabotropic glutamate 2  
282 receptor. *J. Med. Chem.* **57**, 6495-6512 (2014).
- 283 9 Scholler, P. *et al.* Allosteric nanobodies uncover a role of hippocampal mGlu2 receptor  
284 homodimers in contextual fear consolidation. *Nat. Commun.* **8**, 1967 (2017).
- 285 10 Arasappan, A. *et al.* Tetrahydronaphthyridine derivatives as mGluR2-negative allosteric  
286 modulators, compositions, and their use. *PCT Int. Pat. Appl* **WO 2016/029454 A1** (2016).
- 287 11 Koehl, A. *et al.* Structural insights into the activation of metabotropic glutamate receptors.  
288 *Nature* **566**, 79-84 (2019).
- 289 12 Chappell, M. D. *et al.* Discovery of (1*S*,2*R*,3*S*,4*S*,5*R*,6*R*)-2-Amino-3-[(3,4-  
290 difluorophenyl)sulfanylmethyl]-4-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic Acid  
291 Hydrochloride (LY3020371.HCl): A Potent, Metabotropic Glutamate 2/3 Receptor  
292 Antagonist with Antidepressant-Like Activity. *J. Med. Chem.* **59**, 10974-10993 (2016).
- 293 13 Xue, L. *et al.* Major ligand-induced rearrangement of the heptahelical domain interface in a  
294 GPCR dimer. *Nat. Chem. Biol.* **11**, 134-140 (2015).
- 295 14 Gutzeit, V. A. *et al.* Conformational dynamics between transmembrane domains and  
296 allosteric modulation of a metabotropic glutamate receptor. *Elife* **8** (2019).
- 297 15 Huang, S. *et al.* Interdomain movements in metabotropic glutamate receptor activation. *Proc.*  
298 *Natl. Acad. Sci. U S A* **108**, 15480-15485 (2011).

299 16 Hlavackova, V. *et al.* Sequential inter- and intrasubunit rearrangements during activation of  
300 dimeric metabotropic glutamate receptor 1. *Sci. Signal.* **5**, ra59 (2012).  
301 17 Lin, S. *et al.* Structures of G<sub>i</sub>-bound metabotropic glutamate receptors mGlu2 and mGlu4.  
302 *Nature*, accepted (2021).  
303 18 Wu, H. *et al.* Structure of a class C GPCR metabotropic glutamate receptor 1 bound to an  
304 allosteric modulator. *Science* **344**, 58-64 (2014).  
305 19 Dore, A. S. *et al.* Structure of class C GPCR metabotropic glutamate receptor 5  
306 transmembrane domain. *Nature* **511**, 557-562 (2014).  
307 20 Habrian, C. H. *et al.* Conformational pathway provides unique sensitivity to a synaptic  
308 mGluR. *Nat. Commun.* **10**, 5572 (2019).  
309 21 Liu, J. *et al.* Allosteric control of an asymmetric transduction in a G protein-coupled receptor  
310 heterodimer. *Elife* **6** (2017).  
311 22 Saugstad, J. A., Kinzie, J. M., Mulvihill, E. R., Segerson, T. P. & Westbrook, G. L. Cloning  
312 and expression of a new member of the L-2-amino-4-phosphonobutyric acid-sensitive class of  
313 metabotropic glutamate receptors. *Mol. Pharmacol.* **45**, 367-372 (1994).  
314 23 Francesconi, A. & Duvoisin, R. M. Role of the second and third intracellular loops of  
315 metabotropic glutamate receptors in mediating dual signal transduction activation. *J. Biol.*  
316 *Chem.* **273**, 5615-5624 (1998).  
317

318 **Figure legends**

319 **Fig. 1 | Maps and structures of mGlu2 and mGlu7.** The maps and structures are colored  
320 according to chains. The structures are shown in both side and intracellular views. **a**, Inactive  
321 mGlu7 homodimer. The red arrow indicates the most proximal transmembrane helix pair,  
322 with the distance between the intracellular tips labelled. The disulfide bonds are shown as  
323 yellow sticks. **b**, Inactive mGlu2 homodimer. **c**, Agonist/PAM-bound mGlu2 homodimer. **d**,  
324 Inactive mGlu2-7 heterodimer.

325 **Fig. 2 | Inactive mGlu structures.** **a**, Comparison of the TMDs in the inactive structures of  
326 mGlu2, mGlu5 (PDB ID: 6N52), and mGlu7. The distance between the C $\alpha$  atoms of the  
327 residues at position 5.41 in each structure is indicated by a dashed line. The red arrow  
328 indicates the movement of the TMDs in mGlu5 and mGlu7 relative to that in mGlu2. **b**, **c**,  
329 The TMD dimer interface in the inactive mGlu2 structure. The residues that are involved in  
330 dimerization are shown as sticks. **d**, Basal activity of the wild-type (WT) mGlu2 and mutants  
331 measured by IP accumulation assay. The basal activity of the WT was substantially reduced  
332 by the orthosteric antagonist LY341495 (50  $\mu$ M) or the NAM NAM563 (25  $\mu$ M). **e**, Basal  
333 activity of the WT mGlu7 and mutants. The basal activity of the WT was substantially  
334 reduced by the orthosteric antagonist XAP044 (25  $\mu$ M) or the NAM MMPIP (25  $\mu$ M). Data  
335 are presented as percent of WT activity and displayed as mean  $\pm$  SEM (bars) from at least  
336 five independent experiments performed in technical triplicate with individual data points  
337 shown (dots). \*\*\* $P$ <0.001 by one-way ANOVA followed by Dunnett's post-test compared to  
338 the response of WT. See Extended Data Table 3 for detailed independent experiment  
339 numbers (n),  $P$  values, and expression level.

340 **Fig. 3 | Structure and activation of mGlu2-7 heterodimer.** **a**, Structural comparison of  
341 inactive mGlu2-7 heterodimer and mGlu7 homodimer. The distances between the conserved

342 cysteine residues (C540 in mGlu2, C563 in mGlu7) in the two structures are shown as dashed  
343 lines (right middle panel). The red arrows indicate the movement of each transmembrane  
344 helix in mGlu2-7 relative to mGlu7 (right bottom panel). **b-e**, Calcium release assays of  
345 mGlu2 and mGlu7 homo- and heterodimers. Data are shown as mean  $\pm$  SEM from at least  
346 three independent experiments performed in technical triplicate and correspond to the peak  
347 response. The numbers of independent experiments (n) are shown in the parentheses.  
348 Schemes illustrating the activation of the mGlu2-7 heterodimer upon binding to agonist alone  
349 or agonist together with mGlu2 PAM (or mGlu7 NAM) are shown on the right of each panel.  
350 **b**, LY354740-induced calcium release of mGlu2 and mGlu2-7. 'x' indicates the F<sup>ICL3</sup>S  
351 mutation introduced in the mGlu2 or mGlu7 subunit. **c**, L-AP4-induced calcium release of  
352 mGlu7 and mGlu2-7. **d**, Calcium release of mGlu2-7<sup>x</sup> in presence of LY354740 or  
353 LY354740+mGlu2 PAM (JNJ-40411813)/mGlu7 NAM (MMPiP, ADX71743 or MDiP). **e**,  
354 Calcium release of mGlu2-7<sup>x</sup> in presence of L-AP4 or L-AP4+JNJ-40411813.

## 355 **Methods**

### 356 **Construct design and expression of mGlu2, mGlu7, and mGlu2-7**

357 To solve the inactive mGlu2 structure, the human mGlu2 gene was cloned into a modified  
358 **pTT5** vector with a hemagglutinin (HA) signal peptide and a Flag-tag at the N terminus and  
359 a PreScission protease (PPase) site and a 10 × His-tag at the C terminus. For the inactive  
360 mGlu7, the human mGlu7 gene was cloned into the same vector with the HA signal peptide  
361 at the N terminus and the PPase site, 2 × Strep-tag, and Flag-tag at the C terminus. The  
362 flexible C termini of the two receptors were truncated with different lengths, among which  
363 removing 47 residues (V826-L872) in mGlu2 and 56 residues (K860-I915) in mGlu7  
364 produced the most optimal protein yield and homogeneity (Extended Data Fig. 1a, b). To  
365 further improve stability of the inactive receptors, mutation N<sup>3.52</sup>Y was introduced in both  
366 receptors as suggested by previous structural studies of mGlu5-TMD<sup>19</sup>. Furthermore,  
367 mutation H815<sup>7.53</sup>Y in mGlu2 and mutations G722<sup>4.52</sup>I, I775<sup>5.59</sup>F, and P789<sup>6.38</sup>Y in mGlu7  
368 were designed by replacing these residues with their counterparts in other mGlu5 to  
369 potentially increase stability of the transmembrane helical bundle by introducing extra intra-  
370 and/or inter-helical contacts through larger side chains (Extended Data Fig. 5). With these  
371 mutations, the protein yield and homogeneity were greatly improved (Extended Data Fig. 1c,  
372 d). To assess effects of the truncations and mutations on receptor function, we performed IP  
373 accumulation assay and a bioluminescence resonance energy transfer (BRET) assay using  
374 TRUPATH biosensors<sup>24</sup>, which measures the proximal interaction between the  $\alpha$  and  $\gamma$   
375 subunits upon G<sub>i</sub> activation. The results confirmed that these modifications had little effect on  
376 receptor signalling and NAM activity (Extended Data Fig. 1e-j).

377 For structural studies of agonist-bound mGlu2, the wild-type receptor was modified by  
378 introducing a mutation S601A, which, together with the C-terminal truncation (residues

379 V826-L872), greatly improved protein yield (Extended Data Fig. 1n) but did not alter  
380 receptor activation and PAM activity (Extended Data Fig. 1e, i, o, p). In addition, the C-  
381 terminal His-tag was replaced by a 2 × Strep-tag to facilitate protein purification.

382 To aid formation of mGlu2-7 heterodimer, two fusion proteins FKBP and FRB, which  
383 form an interaction pair to stabilize the heterodimerization<sup>25</sup>, were linked to the C termini of  
384 mGlu2 (E19-V825) and mGlu7 (Q35-R859), respectively. FRET analysis indicates that these  
385 fusions do not affect heterodimerization between these two mGlu (Extended Data Fig. 7a, b).  
386 To allow tandem affinity-chromatography, 10 × His-tag and 2 × Strep-tag were added to the  
387 C terminus of mGlu2-FKBP and the N terminus of mGlu7-FRB, respectively. The mGlu2  
388 mutations N655<sup>3.52</sup>Y and H815<sup>7.53</sup>Y and the mGlu7 mutations G722<sup>4.52</sup>I, I775<sup>5.59</sup>F, and  
389 P789<sup>6.38</sup>Y were introduced to facilitate expression and purification of the heterodimer.

390 All the constructs were expressed in HEK293F cells (Invitrogen) with the starting density  
391 at  $1.2 \times 10^6$  cells ml<sup>-1</sup> in 5% CO<sub>2</sub> at 37 °C. For the mGlu2-7 heterodimer, the mGlu2 and  
392 mGlu7 constructs were co-expressed with a plasmid ratio of 1:1 and 100 nM rapamycin was  
393 added during expression. The cells were harvested by centrifugation (1,000g for 15 min) after  
394 48 h transfection and stored at -80 °C until use.

### 395 **Purification of mGlu2 and mGlu7 homo- and heterodimers**

396 The cells expressing mGlu2, mGlu7, or mGlu2-7 were lysed in a hypotonic buffer containing  
397 10 mM HEPES, pH 7.5, 10 mM MgCl<sub>2</sub>, 20 mM KCl, and EDTA-free protease inhibitor  
398 cocktail tablets (Roche). Extensive washes of membranes were performed by repeated  
399 dounce homogenization and centrifugation (160,000g for 30 min) in the hypotonic buffer and  
400 a high-osmotic buffer (hypotonic buffer supplemented with 1 M NaCl). The purified  
401 membranes were suspended in 10 mM HEPES, pH 7.5, 10 mM MgCl<sub>2</sub>, 20 mM KCl, and 30%  
402 glycerol and stored at -80 °C until use.

403 The membranes were thawed and incubated with different ligands [50  $\mu$ M NAM563 for  
404 inactive mGlu2; 50  $\mu$ M MMPIP for inactive mGlu7; 50  $\mu$ M LY354740, 50  $\mu$ M JNJ-  
405 40411813, and DN13 (prepared as below) (DN13 : mGlu2 = 3:1) for agonist/PAM-bound  
406 mGlu2; 50  $\mu$ M NAM563 and 25  $\mu$ M MMPIP for mGlu2-7 heterodimer], 1mg ml<sup>-1</sup>  
407 iodoacetamide (Sigma), and EDTA-free protease inhibitor cocktail tablets at 4 °C for 1 h.  
408 After that, equal volume of solubilization buffer containing 100 mM HEPES, pH 7.5, 300  
409 mM NaCl, 1% (w/v) n-dodecyl- $\beta$ -D-maltopyranoside (DDM, Anatrace) and 0.2% (w/v)  
410 cholesteryl hemisuccinate (CHS, Sigma) was added to the membranes and incubated at 4 °C  
411 for 3 h. The supernatant was then isolated by centrifugation at 160,000g for 30 min, and  
412 incubated with TALON resin (Clontech) (for the inactive mGlu2 and mGlu2-7) or STREP  
413 resin (IBA) (for the agonist/PAM-bound mGlu2 and inactive mGlu7) at 4 °C overnight.

414 To purify the inactive mGlu2 homodimer, the TALON resin was washed with 10 column  
415 volumes of 0.05% (w/v) DDM, 0.01% (w/v) CHS, 25 mM HEPES, pH 7.5, 150 mM NaCl,  
416 30 mM imidazole, and 50  $\mu$ M NAM563. Then the detergent was exchanged to glyco-  
417 diosgenin (GDN, Anatrace) by incubating the resin with a buffer containing 50 mM HEPES,  
418 pH 7.5, 0.25% (w/v) GDN, 150 mM NaCl, 30 mM imidazole, and 50  $\mu$ M NAM563 at 4 °C  
419 for 2 h. The resin was then washed by another 10 column volumes of wash buffer containing  
420 25 mM HEPES, pH 7.5, 0.01% (w/v) GDN, 150 mM NaCl, 30 mM imidazole, and 50  $\mu$ M  
421 NAM563. The protein was then eluted by the wash buffer supplemented with 300 mM  
422 imidazole. The imidazole was removed using a PD MiniTrap G-25 column (GE healthcare),  
423 and the protein was treated with His-tagged PPase (custom-made) at 4 °C overnight. Ni-NTA  
424 Superflow resin (Qiagen) was used to remove the cleaved His-tag and PPase. The final  
425 sample was concentrated to about 5 mg ml<sup>-1</sup>.

426 For the inactive mGlu7 homodimer, the STREP resin was washed with 10 column volumes  
427 of wash buffer containing 25 mM HEPES, pH 7.5, 0.05% (w/v) DDM, 0.01% (w/v) CHS,  
428 150 mM NaCl, and 50  $\mu$ M MMPIP. The detergent was exchanged to GDN following the  
429 same protocol mentioned above. The protein was eluted by 200 mM Tris, pH 8.0, 0.01% (w/v)  
430 GDN, 150 mM NaCl, 50 mM biotin, and 50  $\mu$ M MMPIP, and concentrated to about 5 mg ml<sup>-1</sup>  
431 <sup>1</sup>.

432 The agonist/PAM-bound mGlu2 homodimer was purified following the same protocol of  
433 inactive mGlu7 except that the ligands 50  $\mu$ M LY354740 and 50  $\mu$ M JNJ-40411813 were  
434 used throughout the whole process.

435 Tandem affinity-chromatography was performed to prepare the mGlu2-7 heterodimer  
436 sample. In brief, the TALON resin was washed with 10 column volumes of 25 mM HEPES,  
437 pH 7.5, 0.05% (w/v) DDM, 0.01% (w/v) CHS, 150 mM NaCl, 30 mM imidazole, 50  $\mu$ M  
438 NAM563, and 25  $\mu$ M MMPIP. Then, the detergent was exchanged to GDN and the protein  
439 was eluted by 25 mM HEPES, pH 7.5, 0.01% (w/v) GDN, 150 mM NaCl, 300 mM  
440 imidazole, 50  $\mu$ M NAM563, and 25  $\mu$ M MMPIP. The elution was then incubated with the  
441 STREP resin to remove mGlu2 homodimer. The final concentration of mGlu2-7 heterodimer  
442 was about 7 mg ml<sup>-1</sup>.

#### 443 **Expression and purification of DN13**

444 The nanobody DN13 was prepared as previously described<sup>9</sup>. In brief, DN13 was cloned into  
445 the **pET32a** vector and transformed into *E. coli* BL21DE3 strain for expression. The  
446 transformed cells were cultured in LB media supplemented with 100  $\mu$ g ml<sup>-1</sup> ampicillin,  
447 0.1% (w/v) glucose and 1 mM MgCl<sub>2</sub> at 37 °C until the OD<sub>600</sub> reached 0.7. Then the cells  
448 were induced with 1 mM IPTG and grown at 28 °C overnight. The cells were collected by

449 centrifugation at 4,000g for 15 min and resuspended in ice-cold TES buffer containing 200  
450 mM Tris, pH 8.0, 0.5 mM EDTA, and 0.5 M sucrose. After incubation at 4 °C for 2 h, same  
451 volume of a diluted TES buffer (TES buffer : double distilled water = 1:3) was added for  
452 further incubation for 2 h. The periplasmic extract was collected by centrifugation at 50,000g  
453 for 30 min and incubated with the TALON resin at 4 °C overnight. The resin was then  
454 washed with 20 mM Tris, pH 8.0, 100 mM NaCl, and 30 mM imidazole and eluted by the  
455 same buffer supplemented with 300 mM imidazole. The protein was concentrated to about 10  
456 mg ml<sup>-1</sup> and stored at -80 °C with addition of 20% glycerol until further use.

#### 457 **Cryo-EM sample preparation, data acquisition and processing**

458 Using Vitrobot Mark IV (ThermoFisher), 3 µl of protein sample were applied to glow-  
459 discharged 300 mesh amorphous NiTi foil 1.2/1.3 (Au)<sup>26</sup> (mGlu2 homodimers), Quantifoil  
460 R1.2/1.3 (Au) (mGlu7 homodimer), or CryoMatrix R0.6/1 (Au) (mGlu2-7 heterodimer) grids.  
461 The grids were blotted at 4 °C and 100% humidity for 4 s, 6 s and 1 s, respectively, and  
462 rapidly plunged into liquid ethane for vitrification.

463 For mGlu2, all datasets were collected on a 300-kV Titan Krios G2 electron microscope  
464 (FEI) equipped with K2 Bioquantum direct electron detector (Gatan) and GIF Quantum  
465 energy filter. The defocus range varied from -1.2 µm to -2.2 µm and the dose was 1.75  
466 electrons per frame. 10,137 and 2,425 movies of inactive mGlu2 were collected by  
467 SerialEM<sup>27</sup> with a pixel size of 0.82 Å and 1.04 Å, respectively. A total of 6,989 movies of  
468 the agonist/PAM-bound mGlu2 were collected with a pixel size of 1.04 Å. All movies were  
469 subjected to beam-induced motion correction and dose-weighting using RELION's  
470 implementation<sup>28</sup>. Contrast transfer function (CTF) parameters for each micrograph were  
471 estimated by Gctf<sup>29</sup>. Gautomatch (developed by K. Zhang, MRC Laboratory of Molecular  
472 Biology, Cambridge, UK; <http://www.mrc-lmb.cam.ac.uk/kzhang/Gautomatch/>) was used to

473 pick particles for topaz training<sup>30</sup> and CryoSPARC<sup>31</sup> was used to generate initial models.  
474 Total 1,711,322 particles and 1,076,522 particles were picked for inactive and agonist/PAM-  
475 bound mGlu2, respectively. 801,134 particles of inactive mGlu2 were subjected to three-  
476 dimensional (3D) auto-refinement after 3D classification, followed by Bayesian polishing  
477 and magnification CTF refinement to yield a 3.4-Å resolution map. Four rounds of TMD-  
478 masked 3D classification were performed to improve the map quality of the TMDs and the  
479 best class was selected to run 3D auto-refinement with C2 symmetry and Sidesplitter  
480 refinement<sup>32</sup>. A 3.6-Å map was obtained. For the agonist/PAM-bound mGlu2, total 440,306  
481 particles were selected after 3D classification. A similar data processing procedure was  
482 followed as above. Final 365,101 particles were selected and subjected to several refinements  
483 and yielded a 3.1-Å resolution map. Both maps were post-processed with LocSpiral<sup>33</sup> and  
484 further locally filtered according to the local resolution values for the following model  
485 building and refinement.

486 The datasets of mGlu7 were collected on a 200-kV Talos Arctica electron microscope  
487 (FEI) equipped with K2 Bioquantum direct electron detector (Gatan) and GIF Quantum  
488 energy filter. The defocus range varied from  $-0.5\ \mu\text{m}$  to  $-2.0\ \mu\text{m}$  and the dose was 1.47  
489 electrons per frame. A total of 13,282 movies were collected by SerialEM<sup>27</sup> with a physical  
490 pixel size of 0.8 Å. All movies were subjected to beam-induced motion correction and dose-  
491 weighting using RELION's implementation<sup>28</sup>. CTF parameters for each micrograph were  
492 estimated by Gctf<sup>29</sup>. Particle selection was performed using template-free auto-picking in  
493 RELION-3.0. A total of 3,051,969 particles were extracted and then subjected to three rounds  
494 of reference-free two-dimensional classification and four rounds of 3D classification. The  
495 best-looking classes with 1,011,214 particles were selected for 3D auto-refinement and  
496 Bayesian polishing with C2 symmetry imposed to yield a 4.0-Å resolution map. Focused  
497 refinement of the ECDs was performed, which further improved the ECD map resolution to

498 3.6 Å. A composite map was generated in UCSF Chimera<sup>34</sup> using ‘vop maximum’ command  
499 and used for model building and refinement.

500 For the mGlu2-7 heterodimer, the data were collected on a 300-kV Titan Krios G3 electron  
501 microscope (FEI) equipped with K3 Summit direct electron detector (Gatan) and GIF  
502 Quantum energy filter. The final magnified pixel size was 1.045 Å in the super-resolution  
503 counting mode. Each movie stack was dose-fractionated over 32 frames with the dose of 2.2  
504 electrons per frame. The defocus values of all movie stacks varied from  $-1.3\ \mu\text{m}$  to  $-2.3\ \mu\text{m}$ .  
505 A total of 18,547 movies were collected by SerialEM<sup>27</sup>. Beam-induced motion and drift  
506 correction were performed using MotionCor2<sup>35</sup>. The CTF parameters for each micrograph  
507 were determined by Gctf<sup>29</sup>. Particle selection was performed using template-free auto-picking  
508 in RELION 3.0 (ref. 28). A total of 6,587,999 particles were extracted and then subjected to  
509 three rounds of reference-free 2D classification and six rounds of 3D classification. The best  
510 classes with 1,113,538 particles were selected and subjected to 3D auto-refinement and  
511 Bayesian polishing to get a 3.9-Å resolution map. Focused refinement of the ECDs was  
512 performed, which further improved the ECD map resolution to 3.5 Å. A composite map was  
513 generated in UCSF Chimera<sup>34</sup> using ‘vop maximum’ command and used for subsequent  
514 model building and analysis. The maps of mGlu2 and mGlu7 homo- and heterodimers exhibit  
515 a higher resolution in the VFT region compared to that in the CRDs and TMDs, indicating  
516 that these regions are more structurally dynamic.

### 517 **Cryo-EM model building and refinement**

518 The structures of inactive mGlu2 and mGlu7 were built using the inactive mGlu5 structure  
519 (PDB ID: 6N52) as an initial model. The structure of agonist/PAM-bound mGlu2 was built  
520 using the agonist/PAM-bound mGlu5 structure (PDB ID: 6N51) as an initial model. The  
521 model of the inactive mGlu2-7 heterodimer was built using the solved structures of inactive

522 mGlu2 and mGlu7. All the models were docked into the cryo-EM density maps using  
523 Chimera<sup>34</sup>, followed by iterative manual adjustment in Coot<sup>36</sup> and real space refinement in  
524 Phenix<sup>37</sup>. The crystal structures of mGlu2-TMD–NAM complexes (see below for materials  
525 and methods) were used to aid refinement of the TMDs in the inactive mGlu2 structure due  
526 to a relatively low resolution of the cryo-EM map in this region. The model quality was  
527 validated using MolProbity<sup>38</sup>. The final refinement statistics are provided in Extended Data  
528 Table 1. The final models include most of residues in the receptors and the agonist LY354740  
529 and nanobody DN13 in the agonist/PAM-bound mGlu2 structure. The mGlu2 NAM  
530 NAM563 (inactive mGlu2 and mGlu2-7), mGlu7 NAM MMPIP (inactive mGlu7 and  
531 mGlu2-7), mGlu2 PAM JNJ-40411813 (agonist/PAM-bound mGlu2), and the FKBP and  
532 FRB fusion proteins in the C termini of the mGlu2-7 heterodimer were not modeled due to  
533 the relatively low resolution in the TMD region.

#### 534 **Expression, purification, and crystallization of mGlu2-TMD–NAM complexes**

535 The gene of mGlu2-TMD (residues 556-845) was modified by introducing the mutations  
536 N655<sup>3.52</sup>Y and H815<sup>7.53</sup>Y and replacing residues 663-670 at ICL2 with a fusion protein  
537 flavodoxin<sup>39</sup>, and cloned into a modified **pFastBac1** vector with the HA signal sequence and  
538 Flag-tag at the N terminus and the PPase site followed by a 10 × His-tag at the C terminus.  
539 The optimized mGlu2-TMD construct was expressed in *Spodoptera frugiperda* (*Sf9*)  
540 (Invitrogen) insect cells using the Bac-to-Bac Baculovirus Expression System (Invitrogen).  
541 *Sf9* cells at a cell density of  $2 \times 10^6$  cells per ml were infected with high-titer virus at a MOI  
542 (multiplicity of infection) of 5. Cells were harvest 48 h post infection by centrifugation.

543 The cells expressing mGlu2-TMD were lysed in the hypotonic buffer containing 10 mM  
544 HEPES, pH 7.5, 10 mM MgCl<sub>2</sub>, 20 mM KCl, and EDTA-free protease inhibitor cocktail  
545 tablets (Roche). Extensive washing of the membranes was performed by repeated dounce

546 homogenization and centrifugation in the hypotonic and high-osmotic buffers. Purified  
547 membranes were suspended in the hypotonic buffer with addition of 30% glycerol, 20  $\mu$ M  
548 NAM563 (ref. 5) or NAM597 (ref. 10) (provided by Merck), and EDTA-free protease  
549 inhibitor cocktail. After incubation at 4 °C for 1 h, the complex was extracted by adding equal  
550 volume of solubilization buffer containing 100 mM HEPES, pH 7.5, 1 M NaCl, 1% (w/v)  
551 DDM, and 0.2% (w/v) CHS. After incubation at 4 °C for 3 h, the supernatant was isolated by  
552 centrifugation at 160,000g for 30 min and incubated with TALON resin (Clontech) at 4 °C  
553 overnight. The resin was washed with 25 column volumes of wash buffer containing 25 mM  
554 HEPES, pH 7.5, 500 mM NaCl, 0.05% (w/v) DDM, 0.01% (w/v) CHS, 10% glycerol, 50  $\mu$ M  
555 NAM563 (or NAM597), and 30 mM imidazole, and eluted by increasing the concentration of  
556 imidazole to 300 mM. PD MiniTrap G-25 column (GE Healthcare) was then used to remove  
557 imidazole. The sample was supplemented with 100  $\mu$ M NAM563 (or NAM597) and  
558 incubated with His-tagged PPase (custom-made) at 4 °C overnight to cut off His-tag. The Ni-  
559 NTA resin (Qiagen) was added to remove the cleaved His-tag and PPase. The purified  
560 sample was concentrated to 20–30 mg ml<sup>-1</sup> with a 100-kDa molecular weight cut-off  
561 concentrator (Millipore).

562 The mGlu2-TMD–NAM complexes were crystallized using the lipidic cubic phase (LCP)  
563 method<sup>40</sup>. The proteins were mixed with lipid (monoolein/cholesterol 10:1 by mass) at a ratio  
564 of 1:1.5 (w/w) using a syringe lipid mixer. The LCP mixture was dispensed onto 96-well  
565 glass sandwich plates (Shanghai FAlstal BioTech) in 35 nl drop and overlaid with 800 nl  
566 precipitant solution using a Gryphon robot (Art-Robbins). The crystals of mGlu2-TMD–  
567 NAM563 appeared in 100 mM HEPES, pH 7.0, 100–200 mM NaCl, 100–200 mM MgCl<sub>2</sub>,  
568 and 20–30% (v/v) PEG 400 after 1 day and reached full size in 4–8 days. The crystals of  
569 mGlu2-TMD–NAM597 grew in 100 mM MES, pH 6.0–6.5, 50–200 mM NaCl, 50–150 mM  
570 MgCl<sub>2</sub>, 20%–35% (v/v) PEG 400 and reached full size in 7 days. Crystals were harvested

571 using 50–75  $\mu\text{m}$  micro mounts (M2-L19-50/150, MiTeGen) and immediately flash-frozen in  
572 liquid nitrogen.

### 573 **X-ray data collection, processing, and structure determination of mGlu2-TMD–NAMs**

574 Crystal diffraction data collection was performed at the SPring-8 beam line 41XU, Hyogo,  
575 Japan, using a Pilatus3 6M detector (X-ray wavelength 1.0000 Å). The crystals were exposed  
576 with a 10  $\mu\text{m}$   $\times$  8  $\mu\text{m}$  mini-beam for 0.2 s and 0.2° oscillation per frame. Due to radiation  
577 damage, data collection was limited to 10–15° per crystal. HKL2000<sup>41</sup> was used for  
578 integrating and scaling data from 21 mGlu2-TMD–NAM563 crystals and 26 mGlu2-TMD–  
579 NAM597 crystals. Both structures were solved by molecular replacement with Phaser<sup>42</sup> using  
580 mGlu1-TMD (PDB: 4OR2) and flavodoxin (PDB: 1I1O) structures as searching models. The  
581 correct molecular replacement solutions contained one mGlu2-flavodoxin molecule in the  
582 asymmetric unit. Refinements were performed with Refmac<sup>543</sup>, Buster<sup>44</sup>, and Phenix<sup>37</sup>  
583 followed by manual examination into  $|2F_o| - |F_c|$  and  $|F_o| - |F_c|$  maps with Coot<sup>36</sup>. The final  
584 models of the mGlu2-TMD–NAM563 and mGlu2-TMD–NAM597 complexes contain 262  
585 residues of mGlu2 (G564–V825) and 147 residues of flavodoxin (A1–I147). In both crystal  
586 structures, the receptor TMDs form dimers through a dimer interface mediated by helices I,  
587 VI, and VII, which is likely resulted from crystal packing.

### 588 **IP accumulation assay**

589 Flag-tagged wild-type and mutant mGlu2 and mGlu7 receptors were cloned into the **pTT5**  
590 vector and expressed in HEK293F cells. Cells were co-transfected with the plasmids of  
591 receptor, glutamate transporter EAAC1, and a chimera G protein  $G\alpha_{qi9}$ , which allows  
592 efficient signalling through the phospholipase C pathway<sup>13</sup>, at a ratio of 2:1:2. Cells were  
593 harvested 48 h post transfection. The surface expression of receptors was measured with the  
594 Monoclonal Anti-Flag M2-FITC antibody (Sigma; 1:100 diluted in TBS+4% BSA) using a

595 flow cytometry reader (Millipore). IP1 accumulation was measured using IP-One G<sub>q</sub> assay kit  
596 (Cisbio Bioassays) following the manufacturer's instructions.

597 For LY354740-induced IP accumulation of mGlu2, the cells expressing the wild-type  
598 mGlu2 or mutants (18,000 cells per well) were incubated with increasing concentrations of  
599 LY354740 (1.58 pM – 25 μM) at 37 °C for 45 min. For glutamate-induced IP accumulation of  
600 mGlu7, the cells expressing the wild-type mGlu7 or mutants (12,000 cells per well) were  
601 incubated with 2.5 μM VU0422288 (mGlu7 PAM)<sup>45</sup> and increasing concentrations of L-  
602 glutamate (1.58 μM – 100 mM) at 37 °C for 1 h. To measure the basal activity of mGlu2 and  
603 mGlu7, cells were incubated with stimulation buffer at 37 °C for 90 min. Inhibition of IP  
604 accumulation by the NAMs of mGlu2 and mGlu7 was measured by incubating the cells with  
605 the agonist at EC<sub>80</sub> concentration (100 nM LY354740 for mGlu2; 10 mM glutamate for  
606 mGlu7) and increasing concentrations of NAMs (0.13 nM – 50 μM NAM563; 0.76 nM – 50  
607 μM MMPiP) for 90 min. Cells were then incubated with cryptate-labelled anti-IP1  
608 monoclonal antibody (1:20 diluted in lysis and detection buffer) and IP1-d2 antibody (1:20  
609 diluted in lysis and detection buffer) for 1 h at room temperature. Fluorescence signal was  
610 measured using a Synergy II (Bio-Tek) plate reader with excitation at 330 nm and emission at  
611 620 nm and 665 nm. The accumulation of IP1 was calculated according to a standard dose-  
612 response curve using GraphPad Prism 8.0 (GraphPad software). The basal activity was  
613 calculated by subtracting the IP production measured in the control (Gα<sub>q19</sub> alone) for the WT  
614 receptor and all the mutants.

#### 615 **BRET assay using TRUPATH biosensors**

616 To measure G<sub>i</sub> protein activation, a BRET assay using TRUPATH biosensors<sup>24</sup>, which  
617 measures the proximal interaction between the α and γ subunits, was performed. In brief,  
618 HEK293T cells (Invitrogen) were plated in 6-well plates at a density of 6–7 × 10<sup>5</sup> cells per

619 well one day before transfection. Then, the cells were co-transfected with plasmids of  
620 receptor, G $\alpha$ -RLuc8, G $\beta$ , and G $\gamma$ -GFP2 [TRUPATH was a gift from Bryan Roth (Addgene  
621 kit # 1000000163)] at a ratio of 1:1:1:1 (500 ng per plasmid). After 24 h post transfection,  
622 cells were digested by 0.25% Trypsin-EDTA (Thermo Fisher Scientific) and plated in poly-  
623 D-lysine-coated white 96-well assay plates (Corning) at a density of  $2\text{--}5 \times 10^4$  cells per well.  
624 After 24 h, the culture medium was replaced with 60  $\mu$ l of assay buffer [ $1 \times$  Hank's balanced  
625 salt solution (HBSS), 20 mM HEPES, pH 7.4, and 0.1% BSA), followed by adding 10  $\mu$ l  
626 freshly prepared 50  $\mu$ M coelenterazine 400a (Nanolight Technologies) and equilibrating for 5  
627 min. Then, plates were measured by Synergy II (Bio-Tek) plate reader with 410 nm (RLuc8-  
628 coelenterazine 400a) and 515 nm (GFP2) emission filters for 5 min. The cells were then  
629 treated with 30  $\mu$ l ligand for 5 min and continued to read serially 8 times. The last  
630 measurements were used in all analyses. The BRET ratios were calculated as the ratio of the  
631 GFP2 emission to RLuc8 emission.

### 632 **Intracellular calcium release measurement**

633 The Intracellular calcium release was measured as describe before<sup>21</sup>. In brief, the HEK293  
634 cells (ATCC, CRL-1573) were transfected with the chimeric G protein G $\alpha_{qi9}$  and the  
635 glutamate transporter EAAC1 together with the mGlu constructs. The transfected cells were  
636 loaded in 96-well plates with 1  $\mu$ M Fluo4-AM in fresh buffer (20 mM HEPES, pH 7.4, 130  
637 mM NaCl, 5.1 mM KCl, 0.42 mM KH<sub>2</sub>PO<sub>4</sub>, 0.32 mM Na<sub>2</sub>HPO<sub>4</sub>, 5.27 mM glucose, 3.3 mM  
638 Na<sub>2</sub>CO<sub>3</sub>, 0.1% BSA, and 2.5 mM probenecid) for 1 h at 37 °C. The fluorescence (excitation  
639 485 nm, emission 525 nm) was measured for 60 s by a FlexStation 3 microplate reader  
640 (Molecular Devices, Sunnyvale, CA, USA). The ligand was injected at 20 s during recording.

### 641 **tr-FRET measurement and ELISA**

642 HEK293 cells were transiently transfected with indicated plasmids and plated in 96-well  
643 plates at  $10^5$  cells per well. After 24 h transfection, the cells were washed with PBS and  
644 incubated with fluorophore-conjugated antibodies HA-Tb cryptate antibody (Pekin Elmer;  
645 1:160 diluted in Tris KREBS buffer) and Flag-d2 antibody (Pekin Elmer; 1:267 diluted in  
646 Tris KREBS buffer) overnight at 4 °C. The fluorescence of the cryptate (620 nm) and d2 (665  
647 nm) was measured 40  $\mu$ s after excitation at 337 nm using PheraStar instrument (BMG  
648 Labtechnologies). tr-FRET signals were expressed as  $\Delta F\% = [(665/620)_{\text{sample}} - (665/620)_{\text{mock}}]$   
649  $\times 100 / (665/620)_{\text{mock}}$ .

650 The expression levels of different proteins were evaluated by ELISA. The transfected cells  
651 were fixed for 5 min with paraformaldehyde (4%), then blocked with PBS containing 1%  
652 fetal bovine serum (FBS) and incubated for 30 min with monoclonal anti-HA horseradish  
653 peroxidase (Roche; 1:2,000 diluted in PBS+1% FBS) or anti-Flag horseradish peroxidase  
654 (Sigma; 1:20,000 diluted in PBS+1% FBS). Bound antibody was detected using a  
655 SuperSignal substrate (Pierce) and a Tecan infinite 200Pro plate reader.

### 656 **Cysteine crosslinking and fluorescent-labelled blot experiments**

657 Crosslinking of mGlu2 and mGlu2-7 mutants were measured by fluorescent-labelled blot as  
658 previously described<sup>13</sup>. After 48 h transfection, the HEK293 cells plated in 12-well plates  
659 were incubated in GlutaMAX medium (Gibco) overnight. Then, the cells were labelled with  
660 100 nM SNAP-Surface 647 (NEW ENGLAND BioLabs Inc.) in culture medium at 37 °C for  
661 1 h. Cells were then washed once with PBS and crosslinked with 1.5 mM CuP in crosslinking  
662 buffer (16.7 mM Tris, pH 8.0, 100 mM NaCl, 1 mM CaCl<sub>2</sub>, and 5 mM MgCl<sub>2</sub>) at room  
663 temperature for 20 min. After incubation with 10 mM N-ethylmaleimide at 4 °C for 15 min to  
664 stop the crosslinking reaction, cells were lysed with lysis buffer (50 mM Tris, pH 7.4, 150  
665 mM NaCl, 1% Nonidet P-40, and 0.5% sodium deoxycholate) at 4 °C for 1 h. After

666 centrifugation at 12,000g for 30 min at 4 °C, supernatants were mixed with loading buffer at  
667 37 °C for 10 min. Equal amounts of proteins were resolved by 6% SDS-PAGE. Proteins were  
668 transferred to nitrocellulose membranes (Millipore). Membranes were imaged by Odyssey  
669 CLx imager (LI-COR Bioscience, Lincoln, NE, USA).

## 670 **Data availability**

671 Atomic coordinates and cryo-EM density maps for the structures of inactive mGlu2,  
672 agonist/PAM-bound mGlu2, inactive mGlu7, and inactive mGlu2-7 have been deposited in  
673 the RCSB Protein Data Bank (PDB) under accession codes 7EPA, 7EPB, 7EPC, and 7EPD,  
674 and the Electron Microscopy Data Bank (EMDB) under accession codes EMD-31235, EMD-  
675 31236, EMD-31237, and EMD-31238. Atomic coordinates for the structures of mGlu2-  
676 TMD–NAM563 and mGlu2-TMD–NAM597 have been deposited in the RCSB PDB under  
677 accession codes 7EPE and 7EPF. The uncropped gels shown in Extended Data Fig. 1r, u, aa  
678 are displayed in Supplementary Figure 1.

## 679 **References**

- 680 24 Olsen, R. H. J. *et al.* TRUPATH, an open-source biosensor platform for interrogating the  
681 GPCR transducerome. *Nat. Chem. Biol.* **16**, 841-849 (2020).  
682 25 Fegan, A., White, B., Carlson, J. C. & Wagner, C. R. Chemically controlled protein assembly:  
683 techniques and applications. *Chem. Rev.* **110**, 3315-3336 (2010).  
684 26 Huang, X. *et al.* Amorphous nickel titanium alloy film: A new choice for cryo electron  
685 microscopy sample preparation. *Prog. Biophys. Mol. Biol.* **156**, 3-13 (2020).  
686 27 Mastronarde, D. N. Automated electron microscope tomography using robust prediction of  
687 specimen movements. *J. Struct. Biol.* **152**, 36-51 (2005).  
688 28 Scheres, S. H. RELION: implementation of a Bayesian approach to cryo-EM structure  
689 determination. *J. Struct. Biol.* **180**, 519-530 (2012).  
690 29 Zhang, K. Gctf: Real-time CTF determination and correction. *J. Struct. Biol.* **193**, 1-12 (2016).  
691 30 Bepler, T. *et al.* Positive-unlabeled convolutional neural networks for particle picking in cryo-  
692 electron micrographs. *Nat. Methods* **16**, 1153-1160 (2019).  
693 31 Punjani, A., Rubinstein, J. L., Fleet, D. J. & Brubaker, M. A. cryoSPARC: algorithms for  
694 rapid unsupervised cryo-EM structure determination. *Nat. Methods* **14**, 290-296 (2017).  
695 32 Ramlal, K., Palmer, C. M., Nakane, T. & Aylett, C. H. S. Mitigating local over-fitting during  
696 single particle reconstruction with SIDESPLITTER. *J. Struct. Biol.* **211**, 107545 (2020).  
697 33 Kaur, S. *et al.* Local computational methods to improve the interpretability and analysis of  
698 cryo-EM maps. *Nat. Commun.* **12**, 1240 (2021).  
699 34 Pettersen, E. F. *et al.* UCSF Chimera--A Visualization System for Exploratory Research and  
700 Analysis. *J. Comput. Chem.* **25**, 1605-1612 (2004).

- 701 35 Zheng, S. Q. *et al.* MotionCor2: anisotropic correction of beam-induced motion for improved  
702 cryo-electron microscopy. *Nat. Methods* **14**, 331-332 (2017).
- 703 36 Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. Features and development of *Coot*.  
704 *Acta Crystallogr. D Biol. Crystallogr.* **66**, 486-501 (2010).
- 705 37 Adams, P. D. *et al.* PHENIX: a comprehensive Python-based system for macromolecular  
706 structure solution. *Acta Crystallogr. D Biol. Crystallogr.* **66**, 213-221 (2010).
- 707 38 Chen, V. B. *et al.* MolProbity: all-atom structure validation for macromolecular  
708 crystallography. *Acta Crystallogr. D Biol. Crystallogr.* **66**, 12-21 (2010).
- 709 39 Chun, E. *et al.* Fusion Partner Toolchest for the Stabilization and Crystallization of G Protein-  
710 Coupled Receptors. *Structure* **20**, 967-976 (2012).
- 711 40 Caffrey, M. & Cherezov, V. Crystallizing Membrane Proteins Using Lipidic Mesophases. *Nat.*  
712 *Protoc.* **4**, 706-731 (2009).
- 713 41 Otwinowski, Z. & Minor, W. Processing of X-ray diffraction data collected in oscillation  
714 mode. *Methods Enzymol.* **276**, 307-326 (1997).
- 715 42 McCoy, A. J. *et al.* Phaser crystallographic software. *J. Appl. Crystallogr.* **40**, 658-674  
716 (2007).
- 717 43 Vagin, A. A. *et al.* REFMAC5 dictionary: organization of prior chemical knowledge and  
718 guidelines for its use. *Acta Crystallogr. D Biol. Crystallogr.* **60**, 2184-2195 (2004).
- 719 44 Smart, O. S. *et al.* Exploiting structure similarity in refinement: automated NCS and target-  
720 structure restraints in BUSTER. *Acta Crystallogr. D Biol. Crystallogr.* **68**, 368-380 (2012).
- 721 45 Jalan-Sakrikar, N. *et al.* Identification of positive allosteric modulators VU0155094 (ML397)  
722 and VU0422288 (ML396) reveals new insights into the biology of metabotropic glutamate  
723 receptor 7. *ACS Chem. Neurosci.* **5**, 1221-1237 (2014).

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738 **Author contributions** J.D. developed the protein expression and purification procedures of  
739 mGlu2, prepared the mGlu2 protein samples for cryo-EM and crystallization, solved the  
740 crystal structures of mGlu2-TMD–NAMs, performed IP accumulation assay, and helped with  
741 manuscript preparation. D.W. developed the protein expression and purification procedures  
742 of mGlu7, prepared the mGlu7 protein sample for cryo-EM, performed model building and  
743 structure refinement of the homodimers, and helped with the IP accumulation assay and  
744 manuscript preparation. H.F. performed the cryo-EM studies of the mGlu2 homodimers. C.X.  
745 performed crosslinking, calcium release, and FRET assays. L.T. performed the cryo-EM  
746 studies of the mGlu7 homodimer. S.L. developed the protein expression and purification  
747 procedures of the mGlu2-7 heterodimer, prepared the mGlu2-7 protein sample for cryo-EM,  
748 and performed model building and structure refinement of the heterodimer. S.H. performed  
749 the cryo-EM studies of the mGlu2-7 heterodimer. Q.T. performed BRET assay. X.W. and  
750 T.X. helped with protein preparation and functional assays. H.Z. collected the crystal  
751 diffraction data. X.C. and C.Y. expressed the proteins. P.L. and Xi.W. helped with  
752 crosslinking and calcium release assays. J.-P.P. and P.R. helped with data  
753 analysis/interpretation and edited the manuscript. Y.Z. and H.L. provided mGlu2 ligands for  
754 structural and functional studies. J.L. oversaw crosslinking, calcium release, and FRET  
755 assays, helped with data analysis/interpretation, and edited the manuscript. F.S. oversaw the  
756 cryo-EM studies of mGlu2 and mGlu7 homodimers, helped with data analysis/interpretation,  
757 and edited the manuscript. B.W. and Q.Z. initiated the project, planned and analyzed  
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763 or Q.Z.

764 **Extended Data legends**

765 **Extended Data Fig. 1 | Optimization and characterization of mGlu2 and mGlu7 homo-**  
766 **and heterodimers. a,** Comparison of the mGlu2 homodimers with different C-terminal  
767 truncations. The curves of size-exclusion chromatography (SEC) of purified protein samples  
768 show higher yield and better homogeneity for the protein with the C-terminal residues V826-  
769 L872 truncated. **b,** Comparison of the mGlu7 homodimers with different C-terminal  
770 truncations. The SEC curves of purified protein samples show higher yield and better  
771 homogeneity for the protein with the C-terminal residues K860-I915 truncated. **c,**  
772 Comparison of the mGlu2 homodimers with different mutations. The SEC curves of purified  
773 protein samples show higher yield for the mutant N655<sup>3.52</sup>Y/H815<sup>7.53</sup>Y (construct 1, used to  
774 determine the inactive mGlu2 structure). **d,** Comparison of the mGlu7 homodimers with  
775 different mutations. The SEC results of purified protein samples show higher yield for the  
776 mutant N678<sup>3.52</sup>Y/G722<sup>4.52</sup>I/I775<sup>5.59</sup>F/P789<sup>6.38</sup>Y (construct 2, used to determine the inactive  
777 mGlu7 structure). **e,** LY354740-induced IP accumulation assay of wild-type (WT) mGlu2  
778 and mutants. The IP accumulation data are shown as mean  $\pm$  SEM from at least three  
779 independent experiments performed in technical triplicate (same for below). The numbers of  
780 independent experiments (n) are shown in the parentheses (same for below). Surface  
781 expression levels of the mutants are reported as percent compared to the WT from at least  
782 three independent measurements performed in duplicate (% of WT): N655<sup>3.52</sup>Y, 112  $\pm$  29;  
783 H815<sup>7.53</sup>Y, 56  $\pm$  10; S601A, 88  $\pm$  13; C-truncation (V826-L872), 53  $\pm$  3. **f,** Inhibition of  
784 LY354740-induced IP accumulation of WT mGlu2 and mutants by NAM563. **g,** Glutamate-  
785 induced IP accumulation assay of WT mGlu7 and mutants in presence of VU0422288.  
786 Surface expression levels of the mutants (% of WT): N678<sup>3.52</sup>Y, 200  $\pm$  25; G722<sup>4.52</sup>I, 144  $\pm$   
787 14; I775<sup>5.59</sup>F, 136  $\pm$  13; P789<sup>6.38</sup>Y, 129  $\pm$  12; C-truncation (K860-I915), 110  $\pm$  10. **h,**  
788 Inhibition of glutamate-induced IP accumulation of WT mGlu7 and mutants by MMPiP. **i,**

789 LY354740-induced  $G_i$  activation of WT mGlu2 and mutants measured by the BRET assay.  
790 The BRET data are shown as mean  $\pm$  SEM from at least three independent experiments  
791 performed in technical duplicate (same for below). **j**, Glutamate-induced  $G_i$  activation of WT  
792 mGlu7 and mutants in presence of VU0422288 measured by the BRET assay. **k**, Ligand  
793 screening for the inactive mGlu2 homodimer. The SEC curves show that the NAM563-bound  
794 mGlu2 has higher yield and better homogeneity compared to the apo receptor and  
795 LY341495-bound mGlu2. **l**, Ligand screening for the inactive mGlu7 homodimer. The SEC  
796 curves show that the NAM MMP-IP-bound mGlu7 has higher yield and better homogeneity  
797 compared to the apo receptor and the receptor bound to other ligands. **m**, Inhibition of  
798 LY354740-induced IP accumulation of WT mGlu2 by NAM563 or NAM597. **n**, Comparison  
799 of WT mGlu2 and construct 3 (used to determine the agonist/PAM-bound mGlu2 structure).  
800 The SEC curves of purified protein samples show higher yield and better homogeneity for  
801 construct 3. **o**, JNJ-40411813-induced IP accumulation assay of WT mGlu2 and mutants. **p**,  
802 JNJ-40411813-induced  $G_i$  activation of WT mGlu2 and mutants measured by the BRET  
803 assay. **q**, Ligand screening for the agonist/PAM-bound mGlu2 homodimer. The SEC curves  
804 show higher yield for the agonist- or PAM-bound receptor than the apo protein. **r**, Nu-PAGE  
805 and western-blot results of the mGlu2-7 heterodimer. Double staining (anti-His for his-tagged  
806 mGlu2 and anti-Strep for Strep-tagged mGlu7) confirms the existence of mGlu2-7  
807 heterodimer. Three independent experiments were performed with similar results. Results  
808 from a representative experiment are shown. For gel source data, see Supplementary Figure 1.  
809 **s**, **t**, Glutamate-induced IP accumulation of WT mGlu2 and mutants. See Extended Data  
810 Table 3 for detailed independent experiment numbers (n), statistical evaluation, and  
811 expression level. **u**, Crosslinking of the mGlu2 mutants C121A/L698<sup>4.51</sup>C, C121A/V699<sup>4.52</sup>C,  
812 and C121A/V782<sup>6.59</sup>C–C121A/V789<sup>7.27</sup>C with (+) or without (-) preincubation with the  
813 agonist LY354740 and PAM JNJ-40411813 or the antagonist LY341495 and NAM Ro64-

814 5299. The mutant C121A/V782<sup>6.59</sup>C–C121A/V789<sup>7.27</sup>C was tested using an optimized  
815 GABA<sub>B</sub> quality control system, in which the C-terminal tails of the two mGlu2 subunits were  
816 replaced by the modified C1 and C2 domains of GABA<sub>B1</sub> and GABA<sub>B2</sub>, respectively, to only  
817 allow the mGlu2 dimers containing the C1-C2 dimer reach the cell surface<sup>15</sup>. The WT and  
818 mutant C121A were tested in parallel as controls. Three independent experiments were  
819 performed with similar results. Results from a representative experiment are shown. For gel  
820 source data, see Supplementary Figure 1. **v**, LY354740-induced IP accumulation assay of  
821 mGlu2 in presence of the oxidant CuP. Surface expression levels of the mGlu2 mutants are  
822 reported as percent compared to the WT from at least three independent measurements  
823 performed in duplicate (% of WT): C121A, 100 ± 6; C121A/L698<sup>4.51</sup>C, 84 ± 11;  
824 C121A/V699<sup>4.52</sup>C, 92 ± 9. **w**, LY354740-induced IP accumulation assay of the mGlu2  
825 mutants in the CRD. See Extended Data Table 3 for detailed statistical evaluation and  
826 expression level. **x**, Glutamate-induced IP accumulation assay of WT mGlu7 and mutants in  
827 presence of VU0422288. See Extended Data Table 3 for detailed statistical evaluation and  
828 expression level. **y**, LY354740-induced IP accumulation assay of mGlu2 mutants in the CRD  
829 and ECL2. See Extended Data Table 3 for detailed independent experiment numbers (n),  
830 statistical evaluation, and expression level. **z**, JNJ-40411813-induced IP accumulation assay  
831 of WT mGlu2 and the mutant in ECL2. **aa**, Crosslinking of the mGlu2-7 mutants  
832 C121A/D174C (mGlu2)-C136A/R191C (mGlu7) and C121A/S176C (mGlu2)-  
833 C136A/R191C (mGlu7). The crosslinking studies were carried out using the GABA<sub>B</sub> C1-C2  
834 quality control system that allows cell surface targeting of the heterodimer only<sup>21</sup>. The WT  
835 heterodimer and mutant C121A (mGlu2)-C136A (mGlu7) were tested in parallel as controls.  
836 Four independent experiments were performed with similar results. Results from a  
837 representative experiment are shown. For gel source data, see Supplementary Figure 1.

838 **Extended Data Fig. 2 | Cryo-EM processing and 3D reconstruction workflow. a-f,**  
839 Results of the inactive mGlu2 homodimer. **a**, Processing workflow. **b**, Representative cryo-  
840 EM image from four independent experiments with similar results. **c**, 2D averages. **d**, Cryo-  
841 EM map colored according to local resolution (Å). **e**, Gold-standard FSC curve showing an  
842 overall resolution at 3.6 Å. **f**, Cross-validation of model to cryo-EM density map. FSC curves  
843 for the final model versus the final map and half maps are shown in black, red, and green,  
844 respectively. **g-l**, Results of the inactive mGlu7 homodimer. **g**, Processing workflow. **h**,  
845 Representative cryo-EM image from five independent experiments with similar results. **i**, 2D  
846 averages. **j**, Cryo-EM map colored according to local resolution (Å). **k**, Gold-standard FSC  
847 curves showing an overall resolution at 4.0 Å and a resolution at 3.6 Å for the ECDs. **l**,  
848 Cross-validation of model to cryo-EM density map. **m-r**, Results of the agonist/PAM-bound  
849 mGlu2 homodimer. **m**, Processing workflow. **n**, Representative cryo-EM image from three  
850 independent experiments with similar results. **o**, 2D averages. **p**, Cryo-EM map colored  
851 according to local resolution (Å). **q**, Gold-standard FSC curve showing an overall resolution  
852 at 3.1 Å. **r**, Cross-validation of model to cryo-EM density map. **s-x**, Results of the inactive  
853 mGlu2-7 heterodimer. **s**, Processing workflow. **t**, Representative cryo-EM image from four  
854 independent experiments with similar results. **u**, 2D averages. **v**, Cryo-EM map colored  
855 according to local resolution (Å). **w**, Gold-standard FSC curves showing an overall resolution  
856 at 3.9 Å and a resolution at 3.5 Å for the ECDs. **x**, Cross-validation of model to cryo-EM  
857 density map.

858 **Extended Data Fig. 3 | Cryo-EM density maps of mGlu2 and mGlu7 homo- and**  
859 **heterodimers.** The models are shown as cartoon and sticks. The density maps are colored  
860 gray. **a**, Map and model of the inactive mGlu2 structure shown for all transmembrane helices,  
861 B and C helices in VFTs, CRDs, and intersubunit disulfide bond. The model is colored blue  
862 (molecule A) and gray (molecule B). **b**, Map and model of the inactive mGlu7 structure

863 shown for all transmembrane helices, B and C helices in VFTs, CRDs, and intersubunit  
864 disulfide bond. The model is colored orange (molecule A) and yellow (molecule B). **c**, Map  
865 and model of the agonist/PAM-bound mGlu2 structure shown for all transmembrane helices,  
866 B and C helices in VFTs, LY354740, DN13, CRDs, and intersubunit disulfide bond. The  
867 model is colored blue (molecule A) and gray (molecule B). **d**, Map and model of the inactive  
868 mGlu2-7 structure shown for all transmembrane helices, B and C helices in VFTs, CRDs,  
869 intersubunit disulfide bond, and some non-conserved residues in the two subunits that aided  
870 modelling. The model is colored blue (mGlu2) and orange (mGlu7).

871 **Extended Data Fig. 4 | VFT and CRD conformations in mGlu.** **a**, Comparison of  
872 intersubunit disulfide bonds in the inactive mGlu2 and mGlu7 homo- and heterodimers. The  
873 structures of inactive mGlu2 homodimer, mGlu7 homodimer, and mGlu2-7 heterodimer are  
874 shown in cartoon representation. The disulfide bonds are shown as yellow sticks. The  
875 intersubunit disulfide bonds are highlighted by a red dashed box. **b**, Comparison of  
876 intersubunit disulfide bonds in the inactive mGlu2 and agonist/PAM-bound mGlu2. The  
877 structures of inactive mGlu2 homodimer and agonist/PAM-bound mGlu2 homodimer are  
878 shown in cartoon representation. **c**, Comparison of VFT conformations in inactive mGlu  
879 structures. The structures of inactive mGlu2, inactive mGlu5 (PDB ID: 6N52), inactive  
880 mGlu7, apo mGlu2-VFT (PDB ID: 5KZN), and LY3020371 (antagonist)-bound mGlu2-VFT  
881 (PDB ID: 5KZQ) are shown in cartoon representation. **d**, Comparison of the VFTs in the  
882 inactive structures of mGlu2, mGlu5, and mGlu7. The structures are shown in a side view.  
883 The C $\alpha$  atoms of the mGlu2 residue C234 and its counterparts in mGlu5 and mGlu7 are  
884 shown as spheres. The green arrow indicates the shift of the VFT in the mGlu5 structure  
885 compared to that in the mGlu2 and mGlu7 structures. **e**, Comparison of the CRDs in the  
886 inactive structures of mGlu2, mGlu5, and mGlu7. The structures are shown in both side and  
887 extracellular views. The C $\alpha$  atoms of the mGlu2 residue C540 and its counterparts in mGlu5

888 and mGlu7 are shown as spheres. The distance between the C $\alpha$  atoms of the cysteine residues  
889 in each structure is indicated by a dashed line and colored blue (mGlu2), magenta (mGlu5),  
890 or orange (mGlu7). The red arrow indicates the movement of the CRDs in mGlu5 and mGlu7  
891 relative to that in mGlu2. **f**, Comparison of VFT conformations in agonist-bound mGlu  
892 structures. The structures of LY354740/JNJ-40411813-bound mGlu2, L-quisqualate/CDPPB-  
893 bound mGlu5 (PDB ID: 6N51), LY354740-bound mGlu2-VFT (PDB ID: 4XAQ), and  
894 glutamate-bound mGlu2-VFT (PDB ID: 5CNI) are shown in cartoon representation and  
895 colored blue, magenta, red, and light gold, respectively. **g**, Comparison of the CRDs in the  
896 agonist/PAM-bound mGlu2 and mGlu5 structures. The structures are shown in both side and  
897 extracellular views. **h**, Interactions between the CRDs in the agonist/PAM-bound mGlu2  
898 structure. The residues that are involved in dimerization are shown as blue and gray sticks,  
899 respectively. The disulfide bonds are shown as yellow sticks. **i**, Comparison of VFT  
900 conformations in the inactive mGlu2 and mGlu7 homo- and heterodimers. **j**, VFT dimer  
901 interface in the inactive mGlu2-7 heterodimer structure. The residues that are involved in  
902 dimerization are shown as sticks.

903 **Extended Data Fig. 5 | Sequence alignment of the TMDs of human mGlu.** Colors  
904 represent the similarity of residues: red background, identical; red text, strongly similar. The  
905 red arrows indicate the positions with mutations in the inactive mGlu2 and mGlu7 structures  
906 (2, mGlu2; 7, mGlu7). The mGlu2 residues that are involved in TMD dimerization in the  
907 inactive mGlu2 structure are indicated by green arrows. The conserved residues with the  
908 modified Ballesteros-Weinstein numbers for class C GPCRs, 1.50, 2.50, 3.50, 4.50, 5.50,  
909 6.50, and 7.50, are indicated by black arrows. The alignment was generated using UniProt  
910 (<http://www.uniprot.org/align/>) and the graphic was prepared on the ESPript 3.0 server  
911 (<http://esprict.ibcp.fr/ESPript/cgi-bin/ESPript.cgi>).

912 **Extended Data Fig. 6 | Inter- and intrasubunit interactions upon mGlu2 activation and**  
913 **NAM-binding sites in mGlu2.** **a**, Interactions between the CRD and ECL2 in the  
914 agonist/PAM-bound mGlu2 and mGlu5 structures. The structures of agonist/PAM-bound  
915 mGlu2 and mGlu5 (PDB ID: 6N51) are shown in cartoon representation and colored blue and  
916 pink, respectively. The residues that potentially form interactions between the CRD and  
917 ECL2 are shown as sticks. **b**, Comparison of the helical bundles in the agonist/PAM-bound  
918 structures of mGlu2 and mGlu5. The mGlu2 residues V782<sup>6.59</sup> and V789<sup>7.27</sup> that are involved  
919 in dimerization are shown as sticks. The red arrows indicate the movement of each helix in  
920 the mGlu2 structure relative to the mGlu5 structure. **c**, Crystal structures of mGlu2-TMD–  
921 NAMs. The receptor is colored cyan. The NAMs NAM563 and NAM597 are shown as sticks  
922 and colored yellow and magenta, respectively. The mGlu2 residues that form interactions  
923 with the NAMs are shown as cyan sticks (middle panels). The ligand electron densities are  
924 contoured at  $1.0\sigma$  from an  $|2F_o| - |F_c|$  map, and colored gray (bottom panels). **d**, Comparison  
925 of ligand-binding sites in mGlu2 and class A GPCRs. The ligands in the crystal structures of  
926 mGlu2-TMD–NAM563, mGlu1-TMD–FITM (PDB ID: 4OR2), mGlu5-TMD–mavoglurant  
927 (PDB ID: 4OO9),  $\beta_2$ AR–carazalol (PDB ID: 2RH1), M2–AF-DX384 (PDB ID: 5ZKB), and  
928 D3R–eticlopride (PDB ID: 3PBL) are shown as sticks. Only the receptor in the mGlu2-  
929 TMD–NAM563 structure is shown in cyan cartoon representation for clarity. **e**, Comparison  
930 of the helical bundles in the inactive and active structures of mGlu2. The helical bundles in  
931 the structures of mGlu2-TMD–NAM563, inactive mGlu2, agonist/PAM-bound mGlu2, and  
932 the  $G_i$ -free (free) and  $G_i$ -bound subunits (G) in the mGlu2– $G_i$  complex are shown in an  
933 intracellular view. **f**, Comparison of helix VI in the mGlu2-TMD–NAM563 and mGlu2– $G_i$   
934 structures. The red arrow indicates the downward shift of helix VI in the  $G_i$ -bound subunit of  
935 the mGlu2– $G_i$  complex relative to the mGlu2-TMD–NAM563 structure and the  $G_i$ -free  
936 subunit in the  $G_i$ -bound structure. **g**, Comparison of the W<sup>6.50</sup> conformation in the inactive

937 and active mGlu2 structures. The mGlu2-TMD–NAM563 structure and the G<sub>i</sub>-bound subunit  
938 in the mGlu2–G<sub>i</sub> structure are colored cyan and dark red, respectively. The residue W773<sup>6,50</sup>  
939 in the two structures and the residues that form interactions with W773<sup>6,50</sup> in the mGlu2-  
940 TMD–NAM563 structure are shown as sticks. The red arrow indicates the rotamer  
941 conformational change of W773<sup>6,50</sup> in the active structure relative to the inactive structure. **h**,  
942 Comparison of the ionic locks in the inactive and active mGlu2 structures. The mGlu2-  
943 TMD–NAM563 structure is colored cyan. The G<sub>i</sub>-bound subunit and G<sub>i</sub> protein in the  
944 mGlu2–G<sub>i</sub> structure are colored dark red and green, respectively. The residues K653<sup>3,50</sup>,  
945 R656<sup>3,53</sup>, E754, and E758<sup>6,35</sup> that form ionic interactions in the inactive structure are shown  
946 as sticks in both structures. The ionic interactions in the inactive structure are shown as red  
947 dashed lines and the interaction between R656<sup>3,53</sup> and G $\alpha_i$  in the active structure is shown as  
948 a blue dashed line.

949 **Extended Data Fig. 7 | tr-FRET and functional assays of mGlu2 and mGlu7 homo- and**  
950 **heterodimers. a**, Validation of mGlu2-7 heterodimer and construct used for structural  
951 determination by tr-FRET assay. The signals were measured using the N-terminal HA- and  
952 Flag-tags in each subunit. In each panel, cartoons illustrating the dimer compositions are  
953 shown at the bottom. Data are displayed as mean  $\pm$  SEM (bars) from three independent  
954 experiments performed in technical triplicate with individual data points shown (dots). **b**,  
955 Cell surface expression of homo- and heterodimers quantified by ELISA. Data were  
956 calculated as the fold of mock. Data are displayed as mean  $\pm$  SEM (bars) from three  
957 independent experiments performed in technical triplicate with individual data points shown  
958 (dots). **c-g**, Calcium release assays of mGlu2-7 heterodimer. Data are shown as mean  $\pm$  SEM  
959 from at least three independent experiments performed in technical triplicate and correspond  
960 to the peak response. The numbers of independent experiments (n) are shown in the  
961 parentheses. **c**, **e**, and **f**, LY354740-induced calcium release of wild-type (WT) mGlu2-7

962 heterodimer and mutants. **d, g**, L-AP4-induced calcium release of WT mGlu2-7 heterodimer  
963 and mutants. **h**, LY354740-induced  $G_i$  activation of mGlu2-7<sup>x</sup> in presence of LY354740,  
964 LY354740+mGlu2 PAM (JNJ-40411813), or LY354740+mGlu7 NAM (MMPiP,  
965 ADX71743, or MDIP) measured by the BRET assay. Data are shown as mean  $\pm$  SEM from  
966 at least three independent experiments performed in technical duplicate. The numbers of  
967 independent experiments (n) are shown in the parentheses.

968 **Extended Data Table 1 | Cryo-EM data collection, refinement and validation statistics**

969 \*For inactive mGlu2, different datasets were collected with different magnification of  
970 165,000 and 130,000, corresponding to different pixel sizes of 0.82 Å and 1.04 Å,  
971 respectively.

972 †Particles picked from different datasets with different pixel sizes. The particles were then  
973 combined using RELION 3.1.

974 ‡n.a., not applicable. The LocSpiral performs the local map enhancement based on  
975 thresholding of amplitudes, which are obtained by using the spiral phase transformation.  
976 The algorithm does not estimate or use the *B* factor.

977 **Extended Data Table 2 | Data collection and structure refinement statistics of mGlu2-**  
978 **TMD–NAM563 and mGlu2-TMD–NAM597 complexes**

979 \*Numbers in parentheses refer to the highest-resolution shell.

980 **Extended Data Table 3 | IP accumulation assays of wild-type (WT) and mutant mGlu2**  
981 **using a chimeric  $G\alpha$  protein  $G\alpha_{qi9}$**

982 †The basal activity was calculated by subtracting the IP production measured in the control  
983 ( $G\alpha_{qi9}$ ) for the WT receptor and all the mutants, and is presented as percent of WT activity.

984 ‡Data are shown as mean  $\pm$  SEM from at least three independent experiments. nd (not  
985 determined) refers to data where a robust concentration response curve could not be

986 established within the concentration range tested. \*\*\* $P < 0.001$  by one-way ANOVA followed  
987 by Dunnett's post-test compared to the response of WT.

988 §Sample size, the number of independent experiments performed in technical triplicate.

989 ¶Protein expression levels of mGlu2 and mGlu7 constructs at the cell surface were  
990 determined in parallel by flow cytometry with an anti-Flag antibody (Sigma) and reported as  
991 percent compared to the WT from at least three independent measurements performed in  
992 duplicate.

993 ¶The mutations were introduced in the wild-type receptor.

994 #The span is defined as the window between the maximal agonist response ( $E_{max}$ ) and the  
995 vehicle (no agonist/PAM).

996 ††The  $IC_{50}$  ratio ( $IC_{50(mutant)}/IC_{50(WT)}$ ) characterizes the effect of mutations on inhibitory  
997 activity of NAMs.

998 ††n=29.





