



HAL
open science

Probing the ionotropic activity of glutamate GluD2 receptor in HEK cells with genetically-engineered photopharmacology

Damien Lemoine, Sarah Mondoloni, Jérôme Tange, Bertrand Lambolez, Philippe Faure, Antoine Taly, Ludovic Tricoire, Alexandre Mourot

► To cite this version:

Damien Lemoine, Sarah Mondoloni, Jérôme Tange, Bertrand Lambolez, Philippe Faure, et al.. Probing the ionotropic activity of glutamate GluD2 receptor in HEK cells with genetically-engineered photopharmacology. *eLife*, 2020, 9, pp.e59026. 10.7554/eLife.59026 . hal-03087878

HAL Id: hal-03087878

<https://hal.science/hal-03087878v1>

Submitted on 15 Oct 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 | **Probing the ionotropic activity of glutamate GluD2 receptor in HEK cells with**
2 **genetically-engineered photopharmacology.**

3
4
5
6 Damien Lemoine¹, Sarah Mondoloni¹, Jérôme Tange¹, Bertrand Lambolez¹, Philippe
7 Faure¹, Antoine Taly^{2,3*}, Ludovic Tricoire^{1*} and Alexandre Mourot^{1*}.

8
9
10 ¹ Neuroscience Paris Seine - Institut de Biologie Paris Seine (NPS - IBPS), CNRS,
11 INSERM, Sorbonne Université, Paris, France

12 ² CNRS, Université de Paris, UPR 9080, Laboratoire de Biochimie Théorique, UPR
13 9080, 13 rue Pierre et Marie Curie, F-75005, Paris, France

14 ³ Institut de Biologie Physico-Chimique-Fondation Edmond de Rothschild, PSL
15 Research University, Paris, France

16 * Equal contribution

17
18
19 Correspondence to: alexandre.mourot@inserm.fr ; ludovic.tricoire@upmc.fr

20
21
22 Keywords: optogenetics, photopharmacology, Glutamate receptors, tethered ligands,
23 ion channels, azobenzene

26 **Abstract**

27 Glutamate delta (GluD) receptors belong to the ionotropic glutamate receptor family,
28 yet they don't bind glutamate and are considered orphan. Progress in defining the ion
29 channel function of GluDs in neurons has been hindered by a lack of
30 pharmacological tools. Here we used a chemo-genetic approach to engineer specific
31 and photo-reversible pharmacology in GluD2 receptor. We incorporated a cysteine
32 mutation in the cavity located above the putative ion channel pore, for site-specific
33 conjugation with a photoswitchable pore blocker. In the constitutively-open GluD2
34 Lurcher mutant, current could be rapidly and reversibly decreased with light. We then
35 transposed the cysteine mutation to the native receptor, to demonstrate with high
36 pharmacological specificity that metabotropic glutamate receptor signaling triggers
37 opening of GluD2. Our results assess the functional relevance of GluD2 ion channel
38 and introduce an optogenetic tool that will provide a novel and powerful means for
39 probing GluD2 ionotropic contribution to neuronal physiology.

Introduction

The delta glutamate receptors, GluD1 and GluD2, belong to the ionotropic glutamate receptor (iGluR) family, yet they don't bind glutamate (Yuzaki and Aricescu 2017). They are considered as glutamate receptors solely based on their strong sequence and structure homology with AMPA, NMDA and kainate receptors (Lomeli et al. 1993; Araki et al. 1993; Schmid and Hollmann 2008; Elegheert et al. 2016; Burada, Vinnakota and Kumar 2020a; Burada, Vinnakota and Kumar 2020b). GluD receptors are widely expressed throughout the brain, GluD1 predominantly in the forebrain, while GluD2 is highly enriched in cerebellar Purkinje cells (PCs) (Konno et al. 2014; Hepp et al. 2015; Nakamoto, Konno, et al. 2020). Both GluD1 and GluD2 play a role in the formation, stabilization, function and plasticity of synapses through their interaction with members of the cerebellin (Cbln) family (Fossati et al. 2019; Tao et al. 2018; Matsuda et al. 2010; Kakegawa et al. 2008). Cbln1 notably binds both the N-terminal domain of postsynaptic GluD2 and presynaptic neurexins, leading to a trans-synaptic bridge that promotes synaptogenesis and is essential for GluD2 signaling *in vivo* (Elegheert et al. 2016; Suzuki et al. 2020). Deletion of genes coding for GluD1 or GluD2 in mouse results in marked behavioral alterations (Yadav et al. 2012; Lalouette et al. 2001; Yadav et al. 2013; Nakamoto, Kawamura, et al. 2020), and mutations in these genes in humans have been associated with neurodevelopmental and psychiatric diseases (Griswold et al. 2012; Treutlein et al. 2009; Greenwood et al. 2011; Cristino et al. 2019), attesting to their functional importance in brain circuits.

Despite their structural similarity with other iGluRs, and notably the presence of a ligand binding domain (LBD), GluDs stand out because they are not activated by

65 glutamate (Araki et al. 1993; Lomeli et al. 1993). Nonetheless, recent studies
66 revealed that GluD pore opening could be triggered indirectly, through the activation
67 of Gq-coupled metabotropic receptors, and contributes to neurotransmission and
68 neuronal excitability (Ady et al. 2014; Dadak et al. 2017; Benamer et al. 2017; Gantz
69 et al. 2020). Indeed, the activation of GluD channels triggered by metabotropic
70 glutamate receptors (mGlu1/5) underlies the slow excitatory postsynaptic current in
71 cerebellar PCs (GluD2, Ady et al. 2014) and in midbrain dopaminergic neurons
72 (GluD1, Benamer et al. 2017). Moreover, the demonstration that GluD1 channels
73 carry the noradrenergic slow excitatory current in dorsal raphe neurons (Gantz et al.
74 2020), suggests that the contribution of GluD channels to neuronal excitability and
75 synaptic physiology may be widespread. The above studies relied largely on genetic
76 tools, such as dead-pore mutants or targeted deletions, to assess the ion channel
77 function of GluDs. Yet, due to the absence of specific pharmacological tools to block
78 their ion permeation, the role of GluD1/2 channels in the regulation of neural activity
79 remains largely elusive

80
81 Pore blockers for GluDs, such as pentamidine and 1-Naphthyl acetyl spermine
82 (NASPM), were previously identified using a point mutation (A654T) in GluD2 that
83 confers constitutive ion flow and causes the degeneration of cerebellar PCs in
84 *Lurcher* (GluD2^{L^c}) mice (Wollmuth et al. 2000; Zuo et al. 1997). These molecules are
85 however also pore blockers of NMDA and AMPA receptors, respectively. Other
86 ligands such as D-serine and glycine bind to the LBD and reduce spontaneous
87 currents in GluD2^{L^c}, which suggests a coupling between the LBD and the channel
88 (Naur et al. 2007; Hansen et al. 2009), but these molecules have broad spectrum
89 activity. Finally, 7-chlorokynurenic acid has been identified to modulate GluD2^{L^c}

90 current by binding to the D-serine site but it is also a GluN1 competitive antagonist
91 (Kristensen et al. 2016).

92
93 To fill this gap, we bestowed light-sensitivity to the GluD ion channel pore
94 using a photoswitchable tethered ligand (PTL) approach (Paoletti et al. 2019;
95 Mondoloni et al. 2019). Using structure-based design, we incorporated a cysteine
96 point mutation at the surface of GluD2, right above the hypothetical channel lumen,
97 onto which can be anchored a photoswitchable pore blocker. Different wavelengths
98 of light are then used to modify the geometry of the PTL, thereby
99 presenting/removing the blocker to/from the channel, resulting in optical control of
100 ionotropic activity. Here we demonstrate rapid and reversible, optical control of ion
101 current through a cysteine-substituted GluD2 receptor. This novel tool, called light-
102 controllable GluD2 (LiGluD2), allows rapid, reversible and pharmacologically-specific
103 control of ionic current through GluD2, and may help provide a mechanistic
104 understanding of how this receptor contributes to brain circuit function and behaviors.

105 106 Results

107 Designing a light-controllable GluD receptor

108 Our approach to probing the functionality of the ion channel in GluD is to install
109 a photo-isomerizable pore blocker at the extracellular entrance to the channel lumen
110 (Figure 1A). The tethered ligand is site-specifically attached to a cysteine-substituted
111 residue. In darkness or under green light (500-535 nm), the PTL adopts an elongated
112 shape and reaches the lumen, resulting in ion channel blockade, while under violet
113 light (380-390 nm), it switches to a twisted, shorter configuration, relieving blockade.
114 Our design of the PTL was based on the chemical structure of pentamidine (Figure

115 | 1B), a pore blocker that efficiently blocks current through GluD2^{Lc} receptors ([Williams](#)
116 | [et al. 2003](#)). The PTL, called MAGu, contains a thiol-reactive maleimide (M) moiety, a
117 | central photo-isomerizable azobenzene (A) chromophore, and a guanidinium (Gu)
118 | head group that resembles the amidinium groups of pentamidine (Figure 1C). MAGu
119 | was selected notably because its synthesis route has been described (referred to as
120 | PAG1c in the original article) [and because it was shown to have no adverse effect on](#)
121 | [native brain tissue](#) ([Lin et al. 2015](#)). In aqueous solution, MAGu could be converted
122 | to its *cis* form using 380 nm light, and converted back to *trans* either slowly in
123 | darkness ($t_{1/2} \sim 20$ min) or rapidly upon illumination with 525 nm light ([Figure 1-figure](#)
124 | [supplement 1A-B](#)), in agreement with previous reports ([Lin et al. 2018](#)). To find the
125 | best attachment site for MAGu on GluD, we developed a homology model of the
126 | GluD2 receptor, based on the structure of the recently crystallized GluA2 receptor
127 | ([Twomey et al. 2017](#)) (see methods). Using this model, we selected a series of 15
128 | residues, located on the peptide that links the LBD to the third transmembrane
129 | domain (M3) that lines the channel lumen, for mutation to cysteine (Figure 1D-E).

130

131 | [Cysteine Screening](#)

132 | Since no known ligand directly gates the ion channel of GluD2, we used a Lc
133 | mutant, L654T, which displays a constitutively open channel ([Wollmuth et al. 2000;](#)
134 | [Zuo et al. 1997](#)), for screening the 15 single-cysteine mutations. Accordingly, we
135 | found that heterologous expression of GluD2-L654T, but not of the wild-type (WT)
136 | protein, in HEK cells produces large currents that reverse at membrane potential
137 | close to 0 mV and are reduced by externally-applied pentamidine (Figure 2A).
138 | Subtracted Lc current showed clear rectification at positive potentials, as reported
139 | with the blockade by NASP, another GluD blocker ([Kohda et al. 2000](#)). Therefore, the

140 L654T Lc mutant was subsequently used as a screening platform to find the best
141 attachment site for MAGu on GluD2. Each of the 15 residues identified in [Figure 1D](#)
142 were mutated individually to cysteine on the L654T background, and tested using
143 patch-clamp electrophysiology. Cells were treated with MAGu (20 μ M, 20 min) and
144 Lc currents were measured in voltage-clamp mode (-60 mV) under different
145 illumination conditions to toggle MAGu between its *cis* and *trans* states. [As expected,](#)
146 [current](#) through L654T was not affected by light, indicating that [in the absence of a](#)
147 [properly positioned cysteine,](#) MAGu has no effect on this Lc channel. In contrast, we
148 found several cysteine mutants for which current was significantly larger under 380
149 than under 535 nm light, and one mutant (Q669C) for which there was a tendency for
150 “reverse photoswitching”, i.e. larger currents under 535 than under 380 nm light
151 (Figure 2B). We then quantified the degree of photoswitching by comparing the block
152 in darkness (*trans* state) to the block evoked by [a saturating concentration of](#)
153 [pentamidine \(100 \$\mu\$ M\).](#) We excluded from the analysis mutants that displayed no
154 pentamidine-decreased leak current (i.e. mutants for which pentamidine block was
155 significantly smaller than that observed on A654T, [Figure 2-figure supplement 1A-B](#)),
156 because they were likely either not expressed or not functional. Photoswitching was
157 significant for Q666C, Q669C, D670C, Q674C and I677C, suggesting that MAGu
158 covalently reacted with these cysteine mutants and that, once tethered, it could
159 modulate current in one of its conformer (Figure 2C). Importantly, photomodulation
160 was absent in the control A654T and the other cysteine mutants, indicating that the
161 effect of light is specific to the attachment of MAGu to the above-mentioned cysteine
162 mutants. From a structural point of view, the photocontrollable mutants are all located
163 at the very top of the linker, i.e. further away from the [membrane domain](#) compared
164 to other tested residues (Figure 2D).

165

166 **Photocontrol of GluD2-L654T-I677C tethered with MAGu**

167 We then selected the best mutant GluD2-L654T-I677C for further
168 characterization. Because pentamidine does not fully block GluD2, even at saturating
169 concentrations (Williams et al. 2003), we quantified the extent of photoswitching by
170 blocking leak current completely, using impermeant N-Methyl-D-glucamine (NMDG).
171 We found that MAGu blocked about 33% of the leak current in its *trans* form (Figure
172 3A). Photoregulation was fully reversible over many cycles of 380 and 535 nm light
173 (Figure 3B), in agreement with the fact that azobenzenes photobleach minimally
174 (Beharry and Woolley 2011). Under our illumination conditions, light pulses of 200 ms
175 were sufficient to fully unblock the current, while shorter illumination times could be
176 used to finely tune the degree of blockade (Figure 3C). Once in the *cis* configuration,
177 MAGu relaxes back to its thermodynamically stable *trans* state slowly, with a half-life
178 of about 20 min in solution (Figure S1B). Accordingly, relief of blockade persisted for
179 many seconds in darkness after a brief flash of 380 nm light (Figure 3D), eliminating
180 the need for constant illumination, an important feature for future neurophysiology
181 experiments.

182

183 From a pharmacological point of view, current blockade occurred in the *trans*
184 state (535 nm) and was relieved in the *cis* configuration (380 nm) for all membrane
185 potential tested, with very little voltage-dependence (Figure 4A), which contrasts with
186 the profound voltage-dependence of block observed with pentamidine. This
187 suggested to us that the positive charge of MAGu may not sense the electrical field
188 of the membrane as much as pentamidine does, and thus that the two molecules
189 may bind to different sites. To investigate whether MAGu and pentamidine compete

190 for the same binding site, we evaluated the dose-response relationship of
191 pentamidine block on GluD2-L654T-I677C conjugated with MAGu, under both 380
192 and 535 nm light (Figure 4B). We found the IC50s under both wavelengths to be
193 virtually indistinguishable, favoring the idea that MAGu and pentamidine have
194 distinct, non-overlapping binding sites. To get further molecular insight into *trans*
195 MAGu-induced reduction of current, we performed molecular modeling experiments.
196 After inserting the cysteine mutation, *trans* MAGu was docked by covalent docking,
197 i.e. the reactive maleimide moiety was forced to be in contact with the cysteine while
198 the rest of the molecule was free to move. We found that the guanidinium headgroup
199 of *trans* MAGu couldn't reach the membrane-embedded lumen of GluD2 (Figure 4C),
200 in agreement with our electrophysiology data. The effect of *trans* MAGu on the ion
201 current was tested with the MOLEonline webserver (Pravda et al. 2018), which
202 allowed to compute the geometry of the ion channel. We found that the photoswitch
203 has a direct steric effect on the size of the cavity above the channel, as shown by the
204 comparison of the computed channel in presence or absence of the photoswitch
205 (Figure 4D). In addition, the charge of the photoswitch could modify the electrostatic
206 potential in the cavity and thereby affect ion transfer.

207

208 Optical control of the native GluD2 channel.

209 We next sought to determine whether the non-Lc, native channel could be
210 photocontrolled after installation of MAGu on the cysteine-substituted GluD2-I677C
211 receptor. In heterologous expression system, activation of mGlu1 using the selective
212 agonist 3,5-Dihydroxyphenylglycine (DHPG) was reported to trigger opening of
213 GluD2 receptors (Ady et al. 2014; Dadak et al. 2017). Therefore, we co-expressed
214 the b isoform of mGlu1, which displays low basal activity (Prézeau et al. 1996),

215 together with GluD2 in HEK cells. Cells were labeled with MAGu and DHPG currents
216 were recorded while alternating between 380 and 535 nm light. We found that
217 DHPG-induced currents were reversibly reduced by about 23 % under 535 nm
218 compared to 380 nm light for I677C, indicating that optical blockade with MAGu could
219 be transposed to the native, non-Lc GluD2 (Figure 5A). Importantly, DHPG-induced
220 currents were identical in both wavelengths of light for the WT receptor (Figure 5B),
221 confirming that the effect of light is specific to the attachment of MAGu to I677C
222 (Figure 5C). In addition, we observed that the holding current increased when
223 switching from darkness to 380 nm light for I677C, and decreased when switching
224 back to 535 nm light, but remained constant in both wavelengths of light for WT
225 (Figure 5D). This suggests that a fraction GluD2 receptors are constitutively open
226 prior to DHPG application, likely due to some basal mGlu1 activity in these cells.
227 Altogether, these results show that the GluD2 I677C mutant labeled with MAGu
228 (a.k.a. LiGluD2) possesses a functional ion channel, which can be gated through the
229 mGlu signaling pathway, and which can be reversibly blocked and unblocked with
230 green and purple light, respectively.

231

232 In order to evaluate the usefulness of LiGluD2 in neurons, we verified that
233 MAGu treatment does not lead to photosensitization of native glutamate currents in
234 PCs of WT mice. Since GluD2 is enriched at the parallel fiber-PC synapse (Landsend
235 et al. 1997), we used local application of DHPG (200 μ M) to induce inward current in
236 both MAGu- and vehicle-treated slices. We found that the DHPG current amplitude
237 remained unchanged under 535 nm compared to 380 nm in the two treatment
238 conditions, resulting in a ratio of current amplitude I_{380}/I_{535} measured for each cell not
239 significantly different from 1 (Figure 5-figure supplement 1A). We then recorded

240 [AMPA-mediated excitatory post-synaptic currents \(EPSCs\) in both vehicle- and](#)
241 [MAGu-treated PCs. We found that the amplitude of electrically evoked EPSCs was](#)
242 [stable under 535 nm compared to 380 nm, and that the ratio of EPSC amplitudes](#)
243 [I₃₈₀/I₅₃₅ was not significantly different from 1 in both conditions \(Figure 5-figure](#)
244 [supplement 1B\). These control experiments demonstrate that wild-type GluD and](#)
245 [GluA receptors, which lack a properly-positioned cysteine residue near the pore](#)
246 [lumen, remain insensitive to light after MAGu treatment.](#)

247

248 **Discussion**

249 The PTL strategy has been successfully applied to several members of the
250 iGluR family, including kainate [\(Volgraf et al. 2005\)](#) and NMDA [\(Berlin et al. 2016\)](#)
251 receptors. In these former studies, the photoswitches were made with a glutamate
252 head group, and were tethered to the LBD in proximity to the glutamate binding
253 pocket, providing photocontrol of channel gating [\(Reiner et al. 2015\)](#). Because [their](#)
254 [activation mechanism is still unknown](#), we adopted a different strategy for
255 photocontrolling GluD receptors. We installed the photoswitchable ligand MAGu in
256 proximity to the pore lumen, in hope to alter ion conduction through non-competitive
257 antagonism. We found several cysteine mutants for which current was specifically
258 modulated by light after attachment of MAGu, notably I677C (a.k.a. LiGluD2). In
259 LiGluD2, *trans* MAGu likely does not reach the pore lumen as originally designed.
260 Nevertheless, it reversibly modulates current through the open GluD2 channel with
261 high temporal and pharmacological precision.

262

263 The compounds traditionally used to probe the ionic function of GluDs, such
264 as pentamidine and NASPM [\(Kohda et al. 2000; Williams et al. 2003\)](#), are not

265 specific of GluD and also block NMDA and AMPA receptors. As to D-serine and
266 glycine, they partially inhibit GluD2^{Lc} and mGlu1-gated GluD currents ([Naur et al.
267 2007; Ady et al. 2014; Benamer et al. 2017](#)), but they are also co-agonists of NMDA
268 receptors. Here, the pharmacological specificity of LiGluD2 is [exquisite](#): [after MAGu
269 treatment, only the I677C mutant, and not](#) the WT receptor, [became](#) sensitive to light.
270 Likewise, MAGu [did](#) not photosensitize [other WT glutamate receptors expressed in
271 native brain tissue, in agreement with previous report demonstrating that MAGu has
272 no off-target effects on WT GABA receptors, glutamate receptors and voltage-gated
273 ion channels \(Lin et al. 2015\). Indeed, even though the maleimide group of MAGu
274 reacts in principle with any extracellular cysteine freely accessible on the cell surface,
275 the ability of the tethered ligand \(here guanidinium\) to reach a particular site of action
276 on any given protein in one configuration, but not the other \(e.g. *trans* but not *cis*\), is
277 highly improbable.](#) In fact, the PTL approach has already demonstrated exquisite
278 pharmacological specificity for a large variety of cysteine-substituted ion channels
279 and receptors ([Paoletti et al. 2019; Mondoloni et al. 2019](#)), even in complex biological
280 settings such as brain slices ([Berlin et al. 2016](#)) or intact neuronal circuits *in vivo* ([Lin
281 et al. 2015; Durand-de Cuttoli et al. 2018](#)).

282
283 Attachment of MAGu to GluD2 requires a single amino acid substitution, which
284 is unlikely to disrupt the function of the receptor. In line with this, we found that the
285 functional coupling of GluD2 with mGlu1 signaling ([Ady et al. 2014; Dadak et al.
286 2017](#)) was intact in LiGluD2. This enabled us to [validate](#) that activation of mGlu1
287 triggers the opening of the GluD2 channel in heterologous expression system, in
288 support of earlier evidence that opening of the ion channel of GluD receptors can be
289 triggered in response to metabotropic signaling mechanisms (Ady et al. 2014; Dadak

290 et al. 2017; Benamer et al. 2017; Gantz et al. 2020). Even though light-induced
 291 blockade in LiGluD2 is partial, the rapid kinetics of block/unblock, coupled to the
 292 genetic specificity of the methodology, provide a unique opportunity to detect even
 293 small variations in GluD2 current, such as the tonic current we observed in
 294 heterologous expression system. LiGluD2 remains to be deployed in neuronal
 295 setting, yet we believe it will be a crucial tool for probing the ionotropic contribution of
 296 this orphan receptor to synaptic physiology.

297

298

299 **Materials and methods**

Key Resources Table				
Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
cell line (<i>H. sapiens</i>)	HEK tsa201	Sigma-Aldrich #96121229	RRID:CVCL_2737	
Chemical compound, drug	MAGu	10.1016/j.neuron.2015.10.026		Originally named PAG-1c. Custom-synthesized by Enamine, Ukraine
Chemical compound, drug	DMSO	Sigma-Aldrich	D2650	
Chemical compound, drug	Pentamidine	Sigma-Aldrich	1504900	

Chemical compound, drug	N-methyl-d-glucamine (NMDG)	Sigma-Aldrich	M2004	
Chemical compound, drug	(R,S)-3,5-DHPG	Hello-bio	HB0026	
Chemical compound, drug	CNQX	Hello-bio	HB0205	
Chemical compound, drug	D-APV	Hello-bio	HB0225	
Chemical compound, drug	SR 95531 (Gabazine)	Hello-bio	HB0901	
Chemical compound, drug	CGP 55845	Hello-bio	HB0960	
gene (<i>Mus musculus</i>)	Grid2 (glutamate receptor, ionotropic, delta 2)	Genbank	GeneID: 14804	
gene (<i>Rattus norvegicus</i>)	Grm1 (glutamate receptor, metabotropic 1)	Genbank	Gene ID: 24414	
transfected construct (<i>Mus musculus</i>)	pcDNA3-GluD2	https://doi.org/10.1002/embr.201337371	NM_008167.3	
transfected construct (<i>Mus musculus</i>)	pRK5-mGlu1b	Laurent Prezeau (IGF, Montpellier, France).	NM_001114330.1	

Software, algorithm	R Project for Statistical Computing	http://www.r-project.org/	RRID:SCR_001905	
Software, algorithm	Modeller 9.19	https://salilab.org/modeller/9.19/release.html	RRID:SCR_008395	
Software, algorithm	Smina	https://sourceforge.net/projects/smina/		

300

301

302 Chemicals

303 Bio-grade Chemicals products was provided by Sigma-Aldrich from Merck. MAGu
304 was synthesized as previously described ([Lin et al. 2015](#)) and provided by Enamine
305 Ltd., Kyiv, Ukraine (www.enamine.net). MAGu was stored at -80°C as stock solutions
306 in anhydrous DMSO.

307

308 Spectrophotometry

309 UV-visible spectra were recorded on a Nanodrop 2000 (Thermo Scientific, 1 mm
310 path) with 100 μM MAGu in PBS pH 7.4 (10% final DMSO). The sample was
311 illuminated for 1 minute using ultra high-power LEDs (Prizmatix) connected to an
312 optical fiber (URT, 1 mm core, Thorlabs), followed by an immediate measurement of
313 absorbance. Light intensity at the tip of the 1 mm fiber was 100 mW for the 390 nm
314 LED, and 150 mW for the 520 nm LED.

315

316 Molecular biology

317 The single-cysteine mutations of GluD2 were generated by site-directed mutagenesis
318 using the Quick Change II kit (Agilent technology) performed on pcDNA3-GluD2 ([Ady
319 et al. 2014](#)). All mutants were verified by sequencing.

320

321 Cell line

322 We used human Embryonic Kidney cells (HEK tsA201, Sigma-Aldrich # 96121229).
323 Cells were certified by Sigma-Aldrich. Mycoplasma contamination status were
324 negative.

325

326 Cell culture

327 Cells were cultured in 25 cm² tissue culture flask (Falcon, Vented Cap, 353109) with
328 a culture medium composed of Dulbecco's Modified Eagle Medium (Gibco life
329 technologies, 31966047) containing Glutamax and supplemented with Fetal Bovine
330 Serum (10%, Gibco life technologies, 10500064), Nonessential Amino-Acids (1%,
331 Life Technologies, 11140-035), ampicillin, streptomycin (50,000 U, Gibco, life
332 technologies, 15140-122) and mycoplasma prophylactic (2.5 mg, InvivoGen)
333 antibiotics.

334

335 Transfection

336 HEK tsA201 cells were freshly seeded and plated out in a 6-well plate, on coverslips
337 (10 mm) treated with poly-L-lysine hydrobromide (Sigma, P6282-5MG). Cells were
338 transiently transfected using calcium-phosphate precipitation, as described in
339 ([Lemoine et al. 2016](#)), using 1 µg of cDNA of GluD2 cysteine mutant per well. For co-
340 transfection experiments, we used mGlu1b/GluD2 ratio from 0.7 to 1, with a

341 maximum of 2 µg of total DNA. The plasmid pRK5-mGlu1b used in this study is a
342 generous gift of L. Prezeau (IGF, Montpellier).

343

344 In vitro electrophysiology

345 Electrophysiological currents were recorded on HEK tsA201 cells at room
346 temperature (21-25°C), 24-48h after transfection. Prior to whole cell patch-clamp
347 experiments, cells were incubated for 20 minutes with an extracellular solution
348 containing 20 µM MAGu, and then washed for at least 5 minutes with a fresh external
349 solution. Cells were perfused with an external solution containing (in mM): 140 NaCl,
350 2.8 KCl, 2 CaCl₂, 2 MgCl₂, 12 glucose, 10 HEPES and NaOH-buffered at pH 7.32.

351 The external NMDG solution contained (in mM): 140 NMDG, 2.8 KCl, 2 CaCl₂, 2
352 MgCl₂, 12 glucose, 10 HEPES and was KOH-buffered at pH 7.32. Cells were
353 patched with a borosilicate pipette (4-5 MΩ) containing an intracellular solution
354 containing (in mM): 140 KCl, 5 MgCl₂, 5 EGTA, 10 HEPES, and pH-adjusted to 7.32
355 with KOH. For recording metabotropic activation of GluD2 by mGlu1, the internal
356 solution contained (in mM): 140 K-gluconate, 6 KCl, 12.6 NaCl, 0.1 CaCl₂, 5 Mg-
357 ATP, 0.4 Na-GTP, 1 EGTA, 10 HEPES, and was adjusted to pH 7.32 with KOH.

358 Pentamidine and NMDG solutions were applied using a fast-step perfusion system
359 equipped with three square tubes (SF77B, warning instruments), as described in
360 (Lemoine et al. 2016). Illumination was carried out using a high-power LED system
361 (pE-2, Cooled) mounted directly on the epifluorescence port of a vertical microscope
362 (SliceScope Pro 6000, Scientifica). Light output at the focal plane was 5 and 11.7
363 mW/mm² for the 380 and 535 nm LEDs, respectively. Currents were recorded with
364 an axopatch 200B and digitized with a digidata 1440 (Molecular devices). Signals
365 were low-pass filtered (Bessel, 2 kHz) and collected at 10 kHz using the data

366 acquisition software pClamp 10.5 (Molecular Devices). Electrophysiological
367 recordings were extracted using Clampfit (Molecular Devices) and analyzed with R.

368

369 Slice electrophysiology

370 Animal breeding and euthanasia were performed in accordance to European
371 Commission guidelines and French legislation (2010/63/UE) and procedures were
372 approved by the French Ministry of Research (Agreement APAFIS#16198-
373 2018071921137716v3). Mice at age P30-40 were anesthetized with isoflurane and
374 decapitated. Cerebella were rapidly extracted and transferred into ice-cold ACSF
375 supplemented with 50 mM sucrose and 1mM kynurenic acid. The composition of
376 ACSF in mM was as follows: 126 NaCl, 26 NaHCO₃, 2.5 KCl, 1.25 NaH₂PO₄, 1
377 MgCl₂, 2 CaCl₂, 20 glucose. pH was adjusted to 7.4 by continuous gassing with
378 carbogen. Sagittal slices (250 μm) were sectioned from the vermis on a vibratome
379 (Leica VT 1200S) and transferred to oxygenated ACSF. Slices were incubated for 15
380 min at 30°C then transferred at room temperature before recording. Unless stated
381 otherwise, all the steps were performed at room temperature. Purkinje cells were
382 visually identified using infrared Dodt contrast imaging with a 60 × water immersion
383 objective. Whole-cell recordings from Purkinje cells in cerebellar lobules IV-VI
384 (voltage-clamped at -70 mV, liquid junction potentials not corrected) were performed
385 with borosilicate glass pipettes (WPI, 2–4 MΩ) pulled with a horizontal micropipette
386 puller (Sutter instruments). Internal pipette solutions contained (in mM): 140 Cs-
387 gluconate, 5 CsCl, 2 MgCl₂, 0.5 EGTA, 2 Na-ATP (pH 7.3, adjusted with CsOH).
388 Whole-cell currents were recorded at 20 kHz and filtered with a Bessel low pass filter
389 at 4 kHz using a patch-clamp amplifier (Multiclamp 700B, Molecular Devices)
390 connected to a Digidata 1440A interface board (Molecular Devices). Only Purkinje

391 cells with a series resistance <12 MΩ (not compensated; monitored during
392 experiments by applying 200 ms, -5 mV voltage pulses) were used for the analyses.
393 Slices were incubated for 20 min under continuous oxygenation with MAGu 20 μM or
394 vehicle (DMSO 0.4%) dissolved in 750 μl ACSF in a well of 24-well plate. The slices
395 were then washed in ACSF for at least 20 min, and transferred in the recording
396 chamber. Photoswitching was achieved by illuminating the slice as described above
397 alternatively at 380 nm and 535 nm for 1 s.
398 (RS)-DHPG (200 μM) was diluted in ACSF and locally pressure-applied using a
399 patch pipette placed in the dendrites of the recorded Purkinje-cell. A pneumatic
400 microinjector (Picopump, WPI) was used to deliver 0.1-0.2 ms air pressure pulses (4-
401 10 PSI) every minute. DHPG-mediated currents were recorded at room temperature
402 in presence of CNQX 10 μM, D-APV 25 μM, gabazine 10 μM and CGP 55845 0.5
403 μM. DHPG was applied immediately after the light stimulation.
404 Parallel fiber stimulation was achieved every 10 s with a glass pipette filled with
405 ACSF and placed in the outer half part of molecular layer. A constant voltage
406 isolation unit (DS3, Digitimer Ltd) was used to deliver 10 μs rectangular pulses (50-
407 200 μA) for extracellular stimulation. Parallel fibers inputs were identified by paired-
408 pulse facilitation at 50 ms inter stimulus interval. Amplitudes of evoked EPSCs were
409 averaged from 6 to 12 traces. EPSCs were recorded at 30°C in presence of gabazine
410 10μM. Electrical stimulations were performed immediately after the light stimulation.

411

412 Molecular modeling

413 The model of the GluD receptor has been obtained by homology modeling using the
414 software modeller version 9.19 ([Webb and Sali 2016](#)). The template was that of the
415 glutamate receptor GluA2 (PDB code 5weo) ([Twomey et al. 2017](#)). The automodel

416 class has been used with slow level of MD refinement and the optimisation has been
417 repeated 3 times for each model. 500 models were prepared and the best, as
418 assessed by the DOPE score, was retained for further studies.

419 The structure of the protein and ligand were converted to pdbqt files with the software
420 open babel 2.4.1. Covalent docking was then performed with the software smina
421 (Koes et al. 2013). The box of 25*25*25 angstrom was defined manually to
422 encompass the mutated residue and extend to axis of symmetry. Covalent docking
423 forced the maleimide to be in direct contact with the SG atom of the cysteine with
424 which it is shown experimentally to form a covalent bond. The geometry of the ion
425 channel has been computed with MOLEonline webserver, with the 'pore' mode. The
426 resulting ion channel was color-coded as a function of the diameter of the channel
427 allowing to illustrate the reduction of the diameter from a large (green) to a small
428 (red) diameter.

429

430 Data analysis

431 Data are plotted as mean \pm SEM. Total number (n) of cells in each group and
432 statistics used are indicated in figure and/or figure legend. Comparisons between
433 means were performed using parametric tests (two-sample t-test, Normality always
434 verified, Shapiro-Wilk test of normality). Homogeneity of variances was tested
435 preliminarily and the t-tests were Welch-corrected accordingly. For comparison with
436 theoretical values of 0 or 1, we performed either one-sample t-tests when Normality
437 was verified, or a non-parametric test (one-sample Wilcoxon tests) when Normality
438 was not verified. #p<0.1, *p<0.05, **p<0.01, ***p<0.001.

439

440 Time-course of current unblock and of thermal relaxation were fitted with the
441 following mono-exponential function:

$$442 \quad y = 100 \times (1 - e^{-kx}) \quad \text{Eq.1}$$

443 with k the [decay](#) constant, and $\ln 2/k$ the half-life.

444 Dose-response relationships were fitted with the following equation:

$$445 \quad y = MIN + \frac{MAX-MIN}{1+(\frac{x}{IC50})^{nH}} \quad \text{Eq.2}$$

446 with MAX the maximal current, MIN the minimal current, IC50 the pentamidine
447 concentration yielding half block, and n_H the Hill number.

448

449

450 **Acknowledgements**

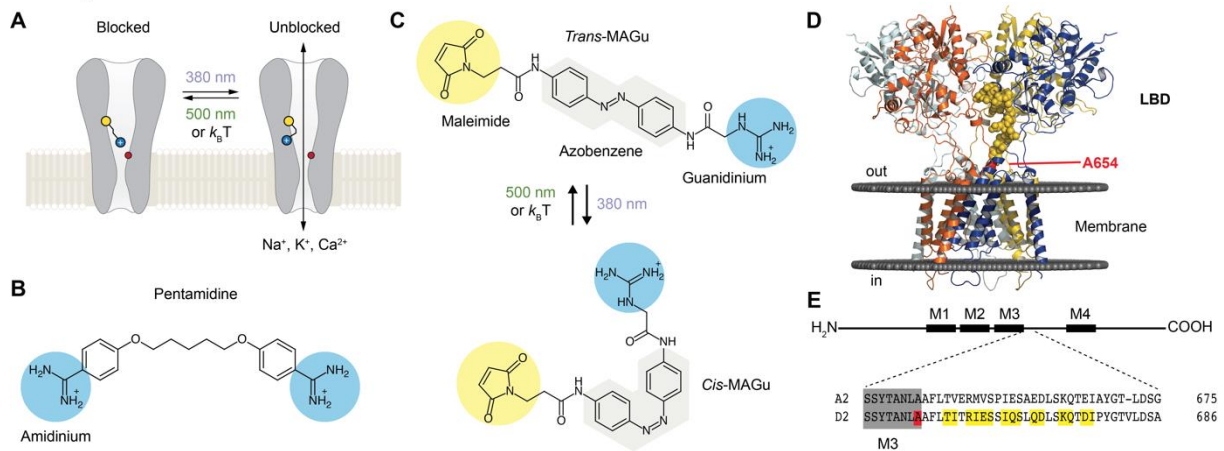
451 Authors would like to thank Nadine Mouttajagane and Manel Badsı for their help with
452 molecular biology work. This work was supported by funding provided by the French
453 Agency for Research (ANR-16-CE16-0014-01 to LT, ANR-11-LABX-0011 to AT), by
454 the "Initiative d'Excellence" (cluster of excellence LABEX Dynamo) to AT, by the
455 Foundation for Medical Research (FRM, Equipe FRM EQU201903007961 to P.F)
456 and by a post-doctoral fellowship from the Labex BioPsy to DL.

457

458

459 **Figure legends.**

Figure 1



460

461 **Figure 1: Optogenetic pharmacology strategy to probe the ionotropic activity of**

462 **GluD receptors.** A. GluD2 is genetically-modified to incorporate a cysteine residue

463 (yellow) at the entrance to the pore, which serves as a handle for the covalent

464 attachment of a synthetic, photoswitchable tethered ligand (PTL). Under green light

465 (500 nm), the PTL adopts an elongated state and places its cationic head group in

466 the lumen, resulting in ion channel blockade. Under violet light (380 nm), the PTL

467 switches to a twisted, shorter form and unblocks the channel. The position of the

468 Lurcher (Lc) mutation, which produces a permanently open channel, is depicted in

469 red. B. Chemical structure of pentamidine, a non-selective iGluR blocker with two

470 amidinium head groups. C. Chemical structures of the PTL MAGu in its *trans* (top)

471 and *cis* (bottom) configurations. MAGu is composed of a cysteine-reactive maleimide

472 group, a central azobenzene chromophore, and a guanidinium cationic head group.

473 D. Molecular model of GluD2, based on the structure of activated GluA2 (5weo).

474 Residues mutated to cysteine are depicted in yellow, while the Lc mutant is shown in

475 red. E. Top, schematic representation of one GluD subunit, with its ligand-binding

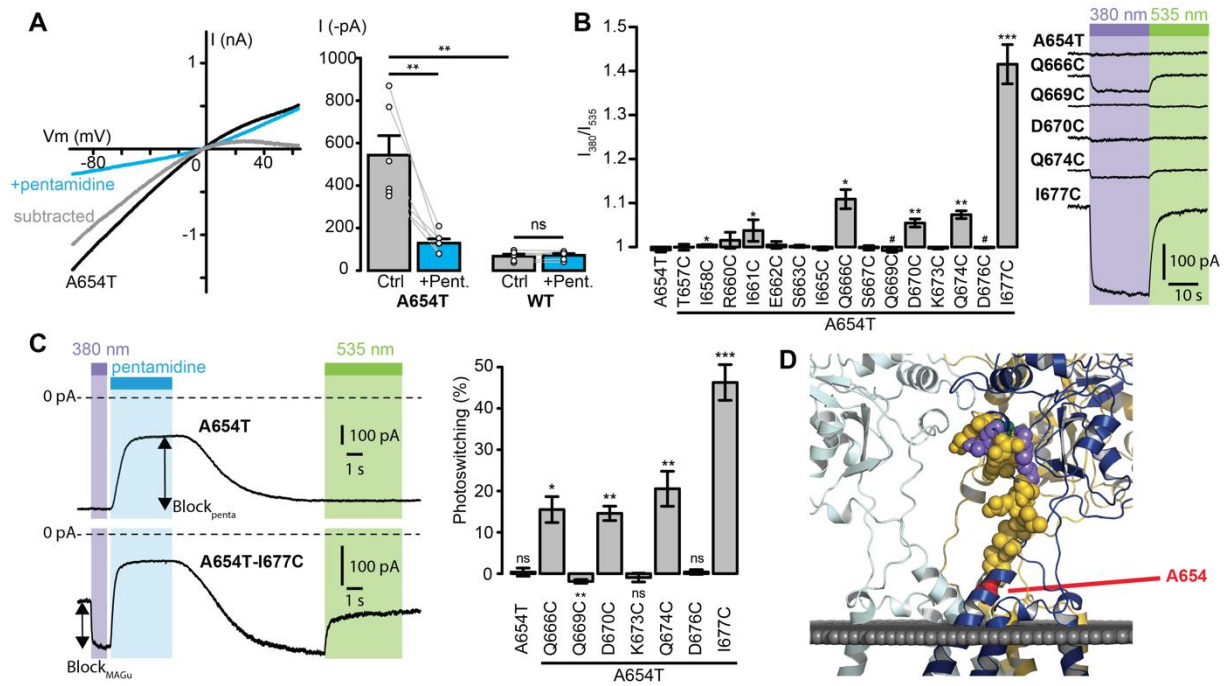
476 domain (LDB) and its four membrane segments (M1-4, M2 being a non-membrane

477 spanning pore loop). Bottom, sequence alignment between the mouse GluA2 and

478 GluD2 receptors around the engineered mutations. M3 is shown in grey, the 15
479 residues mutated to cysteine in yellow, and the position of the A654T Lc mutation in
480 red.

481

Figure 2



482

483 **Figure 2: Screening of the fifteen single-cysteine mutants engineered on the Lc**

484 **background.** A. Left, representative current-voltage relationship for GluD2-A654T

485 Lc, with (blue) and without (black) pentamidine (100 μ M). The subtracted current

486 (grey) shows clear inward rectification and block at positive voltages. Right, currents

487 recorded at -60 mV were larger for GluD2-A654T than for WT (n=6 cells, p = 0.0033,

488 two-sample t-test), and were strongly reduced with 100 μ M pentamidine for A654T

489 (n=6 cells, p = 0.0045, paired t-test) but not for the WT GluD2 (n = 6 cells, p = 0.34,

490 paired t-test). B. Left, ratio of the currents recorded at -60 mV under 380 and 535 nm

491 light, for A654T and the fifteen cysteine mutants engineered on the A654T

492 background (n = 3-8 cells, one-sample t-test, or Wilcoxon when normality is not

493 verified, compared with a theoretical mean value of 1). Right, representative change

494 in holding current when switching between dark, 380 and 535 nm light, for A654T

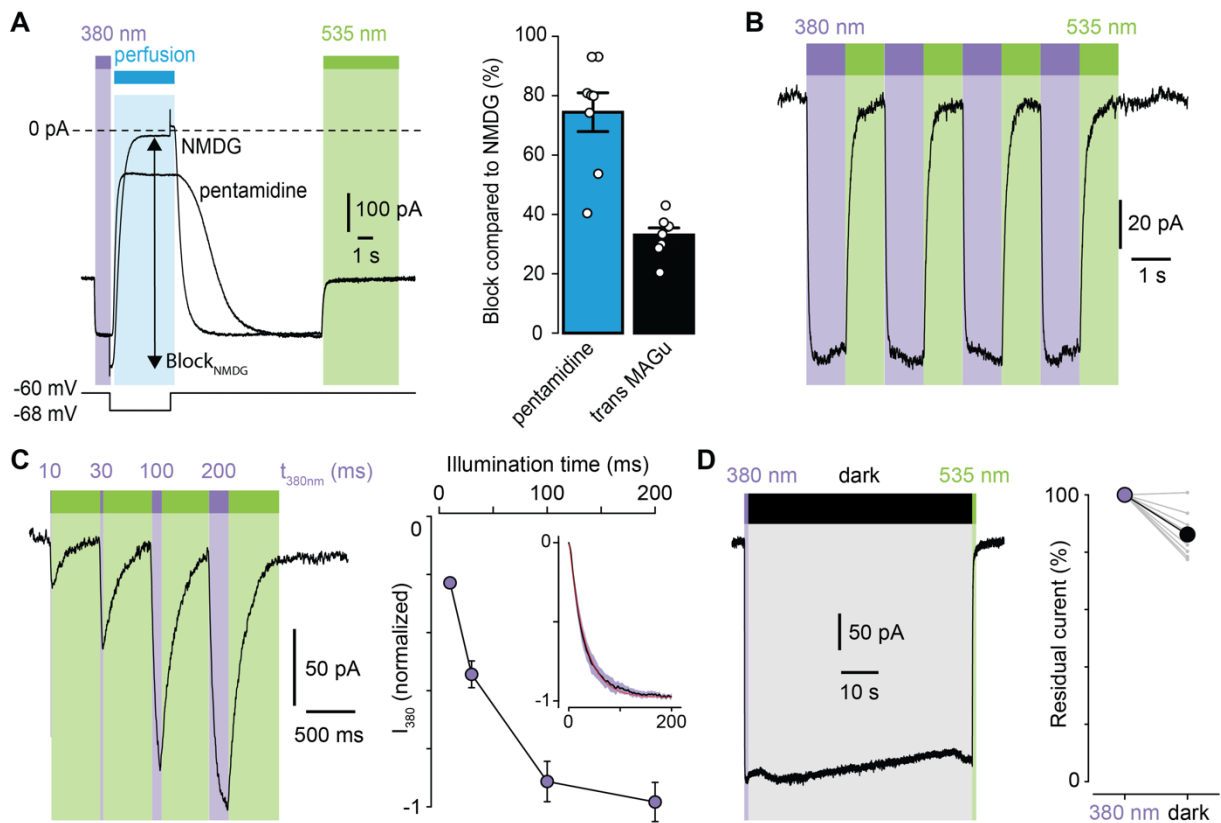
495 and for 5 mutants that show modulation of the holding current when switching

496 between 380 and 535 nm light (p< 0.05 except for Q669C where p = 0.09). C. Left,

497 representative current traces ($V_m = -60$ mV) recorded for A654T and A654T-I677C in

498 | [darkness, then under 380 nm light \(violet\), upon application of pentamidine \(blue,](#)
499 | [100 \$\mu\$ M\), and then under 535 nm after pentamidine washout \(green\).](#) Right, percent
500 | photoswitching (PS = $\text{Block}_{\text{photo}}/\text{Block}_{\text{penta}}$) for A654T and the cysteine mutants that
501 | show both clear leak current and pentamidine block. Photoswitching reached $15.5 \pm$
502 | 3.1 % for Q666C ($p=0.016$, $n = 4$), -1.9 ± 0.4 % for Q669C ($p=0.008$, $n =6$), $14.6 \pm$
503 | 1.7 % for D670C ($p=0.003$, $n=4$), 20.5 ± 4.2 % for Q674C ($p=0.008$, $n=5$) and $46.3 \pm$
504 | 4.3 % for I677C ($p = 1.34E-05$, $n= 8$, one-sample t-test, or Wilcoxon when normality
505 | is not verified, compared with a theoretical mean value of 0). Photoswitching was
506 | absent in the control A654T mutant (0.37 ± 0.9 %, $p = 0.72$, $n = 5$) and in the other
507 | two cysteine mutants K673C (-0.9 ± 1.06 %, $p = 0.43$) and D676C (0.39 ± 0.57 %, p
508 | $= 0.53$). D) Model of GluD2 showing the location of A654 (red), Q666, D670, Q674
509 | and I677C (violet), Q669 (green), and all the non-photocontrollable cysteine mutants
510 | (yellow). Data are presented as mean value \pm sem. [Source files of individual data](#)
511 | [points used for the quantitative analysis are available in the Figure 2 –source data 1.](#)

Figure 3



512

513 **Figure 3. Photoregulation of GluD2-A654T-I677C labeled with MAGu. A.**

514 representative current traces (Vm = -60 mV) recorded for A654T-I677C in darkness,

515 then under 380 light (violet), upon application of pentamidine or NMDG (blue, 100

516 μM), and then under 535 nm (green). Membrane potential is switched from -60 to -68

517 mV during NMDG application to correct for the change in junction potential, and

518 percent Block_{NMDG} is calculated for Vm = -68 mV. Right, percent block for

519 pentamidine (blue, 74.4 ± 6.5 %) and for transMAGu (green, 535 nm, 33.0 ± 2.4 %)

520 compared to NMDG block (n = 8 cells). B. Representative recording showing the

521 reversibility of block/unblock over multiple cycles of 380/535 nm light. C.

522 Left, representative recording showing the extent of current unblock when varying the

523 illumination time under violet light. Right, quantification of current unblock as a

524 function of illumination time (n = 8 cells). Inset, averaged time course of current

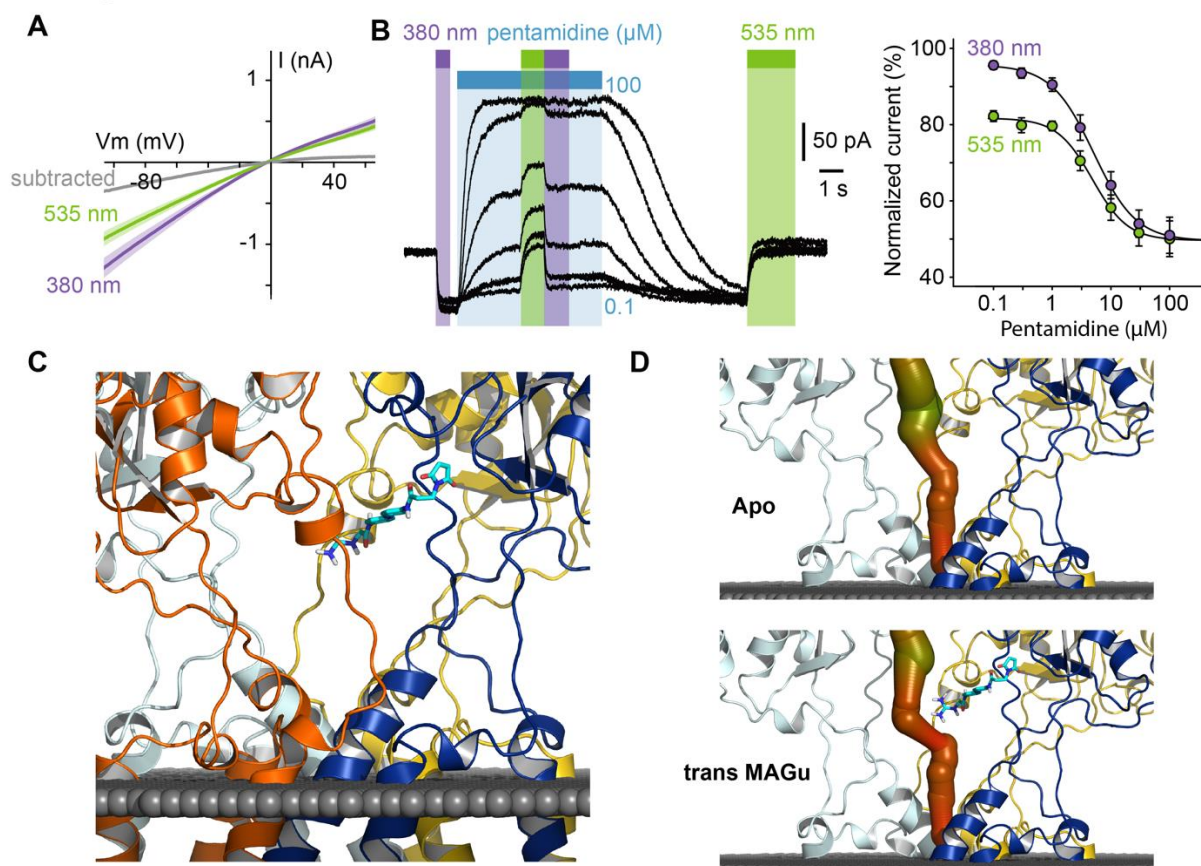
525 unblock when switching from dark to 380 nm light (mean value in black, ±SEM in

526 purple, n = 7 cells) and corresponding mono-exponential fit (red, $k = 0.0296 \pm 0.0002$
527 ms^{-1} ; $t_{1/2} = 23.4 \text{ ms}$). D. Left, representative current trace showing the thermal
528 stability of *cis* MAGu in darkness after a brief flash of 380 nm light. Right, 86.2 ± 2.5
529 % of the residual current remains after 1 min in darkness (n = 9 cells). Data are
530 presented as mean value \pm sem. [Source files of individual data points used for the](#)
531 [quantitative analysis are available in the Figure 3 –source data 1.](#)

532

533

Figure 4

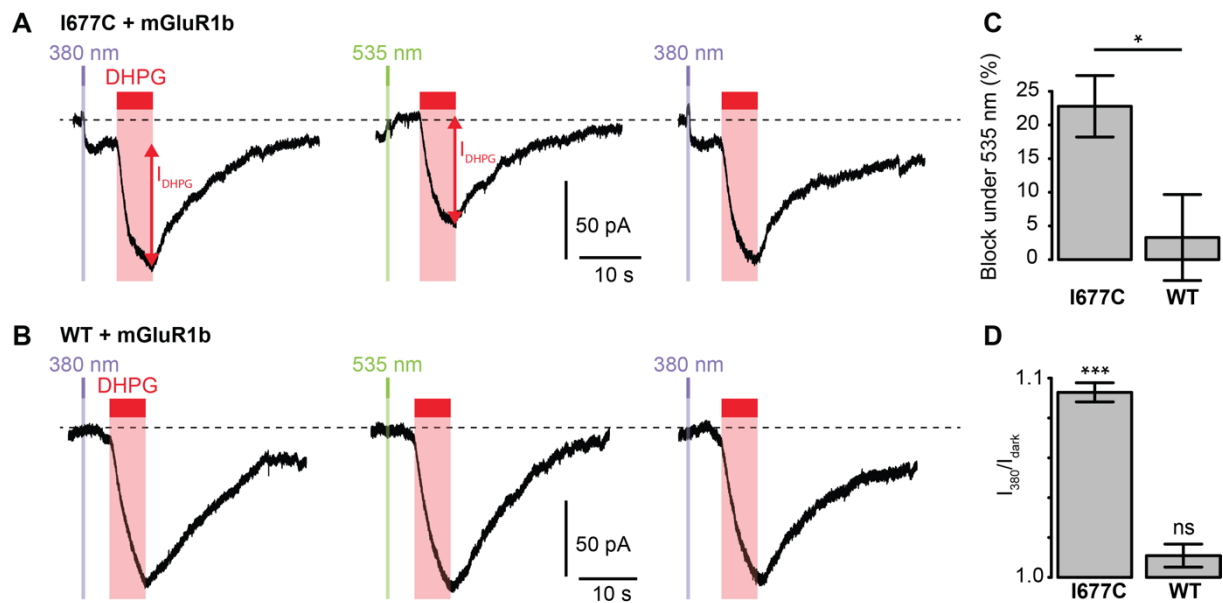


534

535 **Figure 4. Pharmacological action of MAGu at GluD2-A654T-I677C.** A. Average
536 current-versus-voltage relationship under 380 and 535 nm light ($n = 21$). S.E.M is
537 shown in shade, and subtracted current in grey. B. Left, representative dose-
538 dependent blockade of the current upon pentamidine (0.1-100 μ M) application,
539 under 380 and 535 nm light. Right, quantification of pentamidine blockade under both
540 wavelengths of light. $IC_{50_{380}} = 5.1 \pm 0.3 \mu$ M, $IC_{50_{535}} = 4.8 \pm 0.5 \mu$ M ($n = 6$ cells). C.
541 Molecular modeling showing *trans* MAGu tethered to I677C. D. Molecular modeling
542 showing the ion channel computed in the absence (top) and presence (bottom) of
543 *trans* MAGu. The channel is represented with a color coding of the diameter in order
544 to facilitate observation of the change induced by *trans* MAGu (from green, large, to
545 red, small). One subunit is omitted for clarity. Data are presented as mean value \pm

546 | sem. Source files of individual data points used for the quantitative analysis are
547 | available in the Figure 4 –source data 1.

Figure 5



548

549 **Figure 5. Photocontrol of GluD2-I677C (LiGluD2).** A. Representative DHPG-

550 induced current (measured as indicated by the red arrow) for a MAGu-treated (20

551 μM , 20_min) cell co-expressing mGlu1b and GluD2-I677C, under 380, 535 and

552 380nm light. Note the drop in holding current at the onset of the 380-nm illumination,

553 and the return to the baseline under 535 nm light. B. Representative DHPG-induced

554 current for a MAGu-treated cell co-expressing mGlu1b and WT GluD2, under 380,

555 535 and 380nm light. C. DHPG-induced currents were reduced under 535 compared

556 to 380 nm light for I677C ($22.8 \pm 4.6\%$, $n = 8$ cells) but not for WT GluD2 (3.3 ± 6.4

557 $\%$, $n = 6$ cells, $p = 0.02$, two-sample t-test). D. Ratio of the holding current recorded

558 under 380 nm light and in darkness is different from 1 for I677C ($1.09 \pm 0.004\%$, $p =$

559 $2.44 \text{ e-}7$, $n = 8$ cells) but not for WT GluD2 ($1.01 \pm 0.006\%$, $p = 0.10$, $n = 8$ cells,

560 one-sample t-test). Source files of individual data points used for the quantitative

561 analysis are available in the Figure 5 –source data 1.

562 **References**

563

564 Ady, V. et al. 2014. Type 1 metabotropic glutamate receptors (mGlu1) trigger the
565 gating of GluD2 delta glutamate receptors. *EMBO reports* 15(1), pp. 103–109.

566 Araki, K. et al. 1993. Selective expression of the glutamate receptor channel delta 2
567 subunit in cerebellar Purkinje cells. *Biochemical and Biophysical Research
568 Communications* 197(3), pp. 1267–1276.

569 Beharry, A.A. and Woolley, G.A. 2011. Azobenzene photoswitches for biomolecules.
570 *Chemical Society Reviews* 40(8), p. 4422.

571 Benamer, N. et al. 2017. GluD1, linked to schizophrenia, controls the burst ring of
572 dopamine neurons. *Molecular Psychiatry*, pp. 1–11.

573 Berlin, S. et al. 2016. A family of photoswitchable NMDA receptors. *eLife* 5.

574 Burada, A.P., Vinnakota, R. and Kumar, J. 2020a. Cryo-EM structures of the
575 ionotropic glutamate receptor GluD1 reveal a non-swapped architecture. *Nature
576 Structural & Molecular Biology* 27(1), pp. 1–22.

577 Burada, A.P., Vinnakota, R. and Kumar, J. 2020b. The architecture of GluD2
578 ionotropic delta glutamate receptor elucidated by cryo-EM. *Journal of Structural
579 Biology* 211(2), p. 107546.

580 Cristino, A.S. et al. 2019. Neurodevelopmental and neuropsychiatric disorders
581 represent an interconnected molecular system. *Molecular Psychiatry* 19(3), pp. 1–8.

582 Dadak, S. et al. 2017. mGlu1 receptor canonical signaling pathway contributes to the
583 opening of the orphan GluD2 receptor. *Neuropharmacology* 115, pp. 92–99.

584 Durand-de Cuttoli, R. et al. 2018. Manipulating midbrain dopamine neurons and
585 reward-related behaviors with light-controllable nicotinic acetylcholine receptors.
586 *eLife* 7, p. 15991.

587 Elegheert, J. et al. 2016. Structural basis for integration of GluD receptors within
588 synaptic organizer complexes. *Science* 353(6296), pp. 295–299.

589 Fossati, M. et al. 2019. Trans-Synaptic Signaling through the Glutamate Receptor
590 Delta-1 Mediates Inhibitory Synapse Formation in Cortical Pyramidal Neurons.
591 *Neuron* 104(6), pp. 1081–1094.e7.

592 Gantz, S.C. et al. 2020. Delta glutamate receptor conductance drives excitation of
593 mouse dorsal raphe neurons. *eLife* 9, p. 103.

594 Greenwood, T.A. et al. 2011. Analysis of 94 candidate genes and 12
595 endophenotypes for schizophrenia from the Consortium on the Genetics of
596 Schizophrenia. *The American journal of psychiatry* 168(9), pp. 930–946.

597 Griswold, A.J. et al. 2012. Evaluation of copy number variations reveals novel
598 candidate genes in autism spectrum disorder-associated pathways. *Human*
599 *molecular genetics* 21(15), pp. 3513–3523.

600 Hansen, K.B. et al. 2009. Modulation of the Dimer Interface at Ionotropic Glutamate-
601 Like Receptor 2 by D-Serine and Extracellular Calcium. *Journal of Neuroscience*
602 29(4), pp. 907–917.

603 Hepp, R. et al. 2015. Glutamate receptors of the delta family are widely expressed in
604 the adult brain. *Brain Structure and Function* 220(5), pp. 2797–2815.

605 Kakegawa, W. et al. 2008. Differential regulation of synaptic plasticity and cerebellar
606 motor learning by the C-terminal PDZ-binding motif of GluRdelta2. *Journal of*
607 *Neuroscience* 28(6), pp. 1460–1468.

608 Koes, D.R. et al. 2013. Lessons learned in empirical scoring with smina from the
609 CSAR 2011 benchmarking exercise. *Journal of Chemical Information and Modeling*
610 53(8), pp. 1893–1904.

611 Kohda, K. et al. 2000. Mutation of a glutamate receptor motif reveals its role in gating
612 and delta2 receptor channel properties. *Nature Neuroscience* 3(4), pp. 315–322.

613 Konno, K. et al. 2014. Enriched Expression of GluD1 in Higher Brain Regions and Its
614 Involvement in Parallel Fiber-Interneuron Synapse Formation in the Cerebellum.
615 *Journal of Neuroscience* 34(22), pp. 7412–7424.

616 Kristensen, A.S. et al. 2016. Pharmacology and Structural Analysis of Ligand Binding

617 to the Orthosteric Site of Glutamate-Like GluD2 Receptors. *Molecular Pharmacology*
618 89(2), pp. 253–262.

619 Lalouette, A. et al. 2001. Neurobiological effects of a null mutation depend on genetic
620 context: comparison between two hotfoot alleles of the delta-2 ionotropic glutamate
621 receptor. *Neuroscience* 105(2), pp. 443–455.

622 Landsend, A.S. et al. 1997. Differential localization of delta glutamate receptors in the
623 rat cerebellum: coexpression with AMPA receptors in parallel fiber-spine synapses
624 and absence from climbing fiber-spine synapses. *Journal of Neuroscience* 17(2), pp.
625 834–842.

626 Lemoine, D. et al. 2016. Optogenetic Control of Mammalian Ion Channels with
627 Chemical Photoswitches. *Methods in molecular biology (Clifton, N.J.)* 1408, pp. 177–
628 193.

629 Lin, W.-C. et al. 2015. A Comprehensive Optogenetic Pharmacology Toolkit for In
630 Vivo Control of GABA(A) Receptors and Synaptic Inhibition. *Neuron* 88(5), pp. 879–
631 891.

632 Lin, W.-C. et al. 2018. Design of a Highly Bistable Photoswitchable Tethered Ligand
633 for Rapid and Sustained Manipulation of Neurotransmission. *Journal of the American*
634 *Chemical Society* 140(24), pp. 7445–7448.

635 Lomeli, H. et al. 1993. The rat delta-1 and delta-2 subunits extend the excitatory
636 amino acid receptor family. *FEBS Letters* 315(3), pp. 318–322.

637 Matsuda, K. et al. 2010. Cbln1 is a ligand for an orphan glutamate receptor delta2, a
638 bidirectional synapse organizer. *Science* 328(5976), pp. 363–368.

639 Mondoloni, S. et al. 2019. Cell-Specific Neuropharmacology. *Trends in*
640 *Pharmacological Sciences* 40(9), pp. 696–710.

641 Nakamoto, C., Kawamura, M., et al. 2020. GluD1 knockout mice with a pure
642 C57BL/6N background show impaired fear memory, social interaction, and enhanced
643 depressive-like behavior. Ikeda, K. ed. *PLoS ONE* 15(2), p. e0229288.

644 Nakamoto, C., Konno, K., et al. 2020. Expression mapping, quantification, and

645 complex formation of GluD1 and GluD2 glutamate receptors in adult mouse brain.
646 *The Journal of Comparative Neurology* 528(6), pp. 1003–1027.

647 Naur, P. et al. 2007. Ionotropic glutamate-like receptor delta2 binds D-serine and
648 glycine. *PNAS* 104(35), pp. 14116–14121.

649 Paoletti, P. et al. 2019. Optical control of neuronal ion channels and receptors.
650 *Nature Reviews Neuroscience* 112, pp. 1–19.

651 Pravda, L. et al. 2018. MOLEonline: a web-based tool for analyzing channels, tunnels
652 and pores (2018 update). *Nucleic Acids Research* 46(W1), pp. W368–W373.

653 Prézeau, L. et al. 1996. Changes in the carboxyl-terminal domain of metabotropic
654 glutamate receptor 1 by alternative splicing generate receptors with differing agonist-
655 independent activity. *Molecular Pharmacology* 49(3), pp. 422–429.

656 Reiner, A. et al. 2015. ScienceDirectControlling ionotropic and metabotropic
657 glutamate receptors with light: principles and potential. *Current Opinion in*
658 *Pharmacology* 20, pp. 135–143.

659 Schmid, S.M. and Hollmann, M. 2008. To gate or not to gate: are the delta subunits
660 in the glutamate receptor family functional ion channels? *Molecular Neurobiology*
661 37(2-3), pp. 126–141.

662 Suzuki, K. et al. 2020. A synthetic synaptic organizer protein restores glutamatergic
663 neuronal circuits. *Science* 369(6507), p. eabb4853.

664 Tao, W. et al. 2018. Postsynaptic δ 1 glutamate receptor assembles and maintains
665 hippocampal synapses via Cbln2 and neurexin. *Proceedings of the National*
666 *Academy of Sciences* 115(23), pp. E5373–E5381.

667 Treutlein, J. et al. 2009. Dissection of phenotype reveals possible association
668 between schizophrenia and Glutamate Receptor Delta 1 (GRID1) gene promoter.
669 *Schizophrenia research* 111(1-3), pp. 123–130.

670 Twomey, E.C. et al. 2017. Channel opening and gating mechanism in AMPA-subtype
671 glutamate receptors. *Nature* 549(7670), pp. 60–65.

672 Volgraf, M. et al. 2005. Allosteric control of an ionotropic glutamate receptor with an
673 optical switch. *Nature Chemical Biology* 2(1), pp. 47–52.

674 Webb, B. and Sali, A. 2016. Comparative Protein Structure Modeling Using
675 MODELLER. *Current protocols in bioinformatics* 54(1), pp. 5.6.1–5.6.37.

676 Williams, K. et al. 2003. Pharmacology of delta2 glutamate receptors: effects of
677 pentamidine and protons. *Journal of Pharmacology and Experimental Therapeutics*
678 305(2), pp. 740–748.

679 Wollmuth, L.P. et al. 2000. The Lurcher mutation identifies delta 2 as an
680 AMPA/kainate receptor-like channel that is potentiated by Ca(2+). *Journal of*
681 *Neuroscience* 20(16), pp. 5973–5980.

682 Yadav, R. et al. 2012. Deletion of glutamate delta-1 receptor in mouse leads to
683 aberrant emotional and social behaviors. Christie, B. ed. *PLoS ONE* 7(3), pp.
684 e32969–13.

685 Yadav, R. et al. 2013. Deletion of Glutamate Delta-1 Receptor in Mouse Leads to
686 Enhanced Working Memory and Deficit in Fear Conditioning Christie, B. ed. *PLoS*
687 *ONE* 8(4), pp. e60785–12.

688 Yuzaki, M. and Aricescu, A.R. 2017. A GluD Coming-Of-Age Story. *Trends in*
689 *Neurosciences* 40(3), pp. 138–150.

690 Zuo, J. et al. 1997. Neurodegeneration in Lurcher mice caused by mutation in delta2
691 glutamate receptor gene. *Nature* 388(6644), pp. 769–773.

692

693

694 **Supplementary figures**

695

696 **Figure 1-figure supplement 1. Photochemical properties of MAGu.** A. UV-visible
697 spectra of MAGu under 520 nm light (green, mostly *trans*) or under 390 nm light
698 (pink, mostly *cis*). B. Thermal relaxation of *cis* MAGu in aqueous solution in
699 darkness. Absorbance at 362 nm is plotted as a function of time in darkness following
700 illumination with 390 nm light. Data points were fitted with the following
701 monoexponential decay equation: $y = A \exp(-x/k)$ and yielded: $A = 95.8 \pm 1.9$ and $k =$
702 $5.8e-04 \pm 0.2e-04 \text{ s}^{-1}$. The half-life of *cis* MAGu was $\ln(2)/k = 1195 \text{ s}$.

703

704 **Figure 2-figure supplement 1. Functional characterization of the cysteine**
705 **mutants.** A) Currents recorded at -60 mV for A654T and each of the fifteen cysteine
706 mutants (n = 3-8 cells). B. Pentamidine block for A654T and each of the fifteen
707 cysteine mutants (n = 3-8 cells). Data are presented as mean value \pm sem.

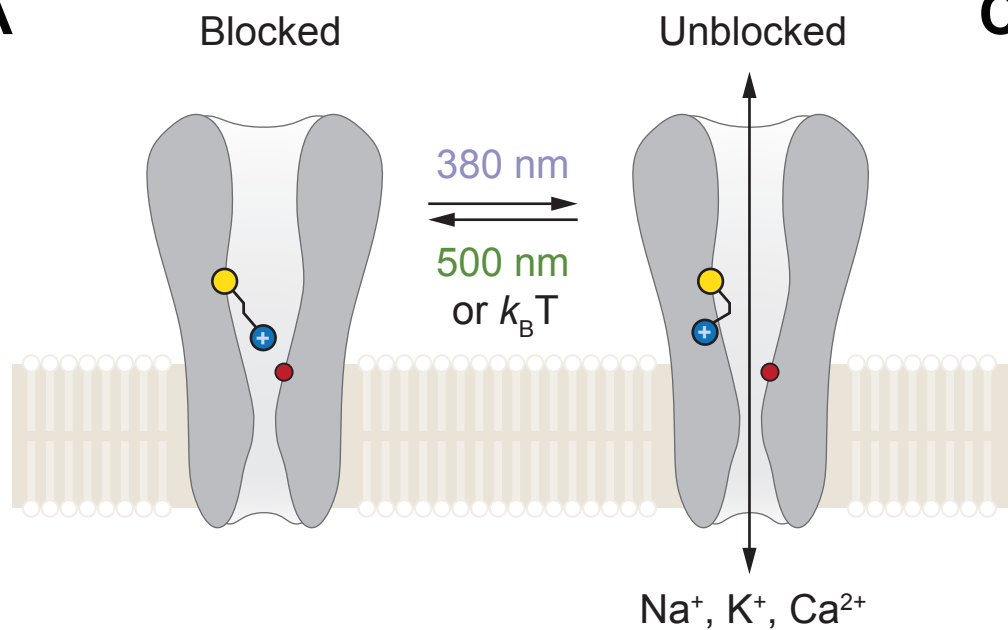
708

709 **Figure 5-figure supplement 1. MAGu does not photosensitize native GluD and**
710 **GluA currents in Purkinje cells.** A. Top, DHPG (200 μM)-induced current in
711 vehicle-treated cerebellar slices were of similar amplitude under 380 and 535 nm
712 light ($-800 \pm 57 \text{ pA}$ vs. $-799 \pm 57 \text{ pA}$, $p > 0.999$, $n = 10$). Middle, DHPG (200 μM)-
713 induced current in MAGu-treated cerebellar slices were of similar amplitude under
714 380 and 535 nm light ($-666 \pm 113 \text{ pA}$ vs. $-661 \pm 111 \text{ pA}$, $p = 0.625$, $n = 13$). Bottom,
715 ratio between the current measured under 380 and 535 nm light are not different
716 from 1 for both vehicle-treated (1.004 ± 0.008 , $p = 0.6475$) and MAGu-treated cells
717 (1.003 ± 0.01 , $p = 0.7755$). B. Top, Excitatory post-synaptic currents (EPSCs) in
718 vehicle-treated cerebellar slices were of similar amplitude under 380 and 535 nm
719 light ($-272 \pm 32 \text{ pA}$ vs $-273 \pm 34 \text{ pA}$, $p = 0.742$, $n = 8$). Middle, EPSCs in MAGu-

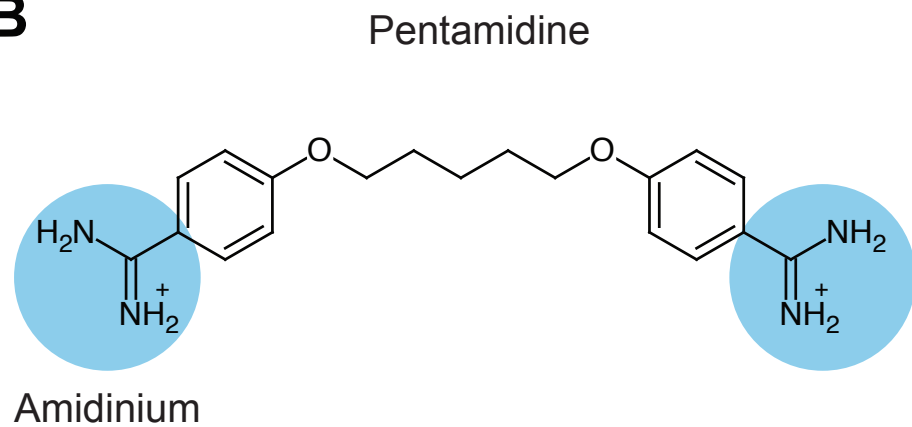
720 treated cerebellar slices were of similar amplitude under 380 and 535 nm light (-176
721 ± 20 pA vs. -180 ± 19 pA, $p= 0.320$, $n = 11$). Bottom, ratio between the current
722 measured under 380 and 535 nm light are not different from 1 for both vehicle-
723 treated (0.9976 ± 0.0185 , $p>0.999$) and MAGu-treated cells (1.035 ± 0.020 ,
724 $p=0.1748$). Data are presented as mean value \pm sem.
725

Figure 1

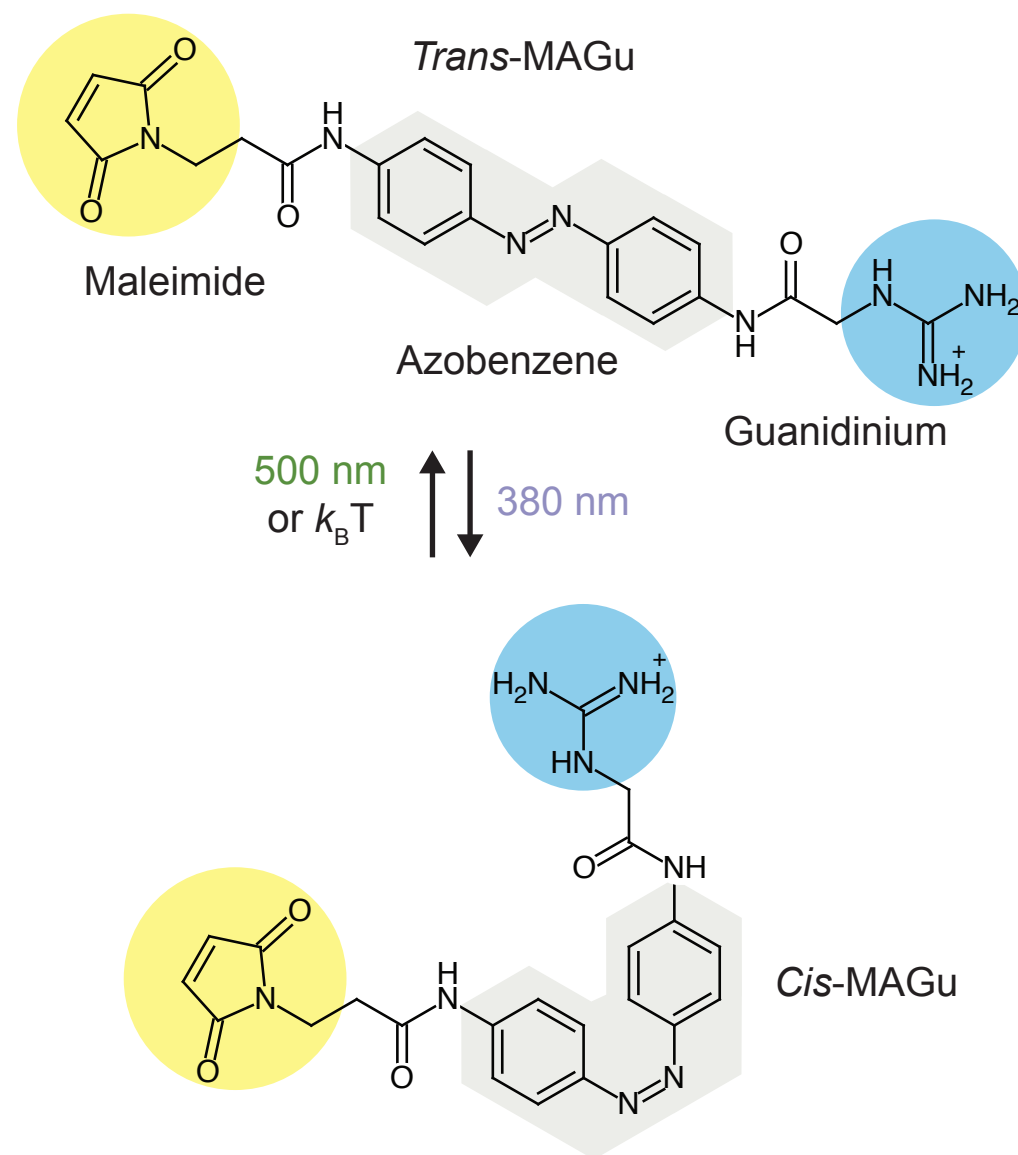
A



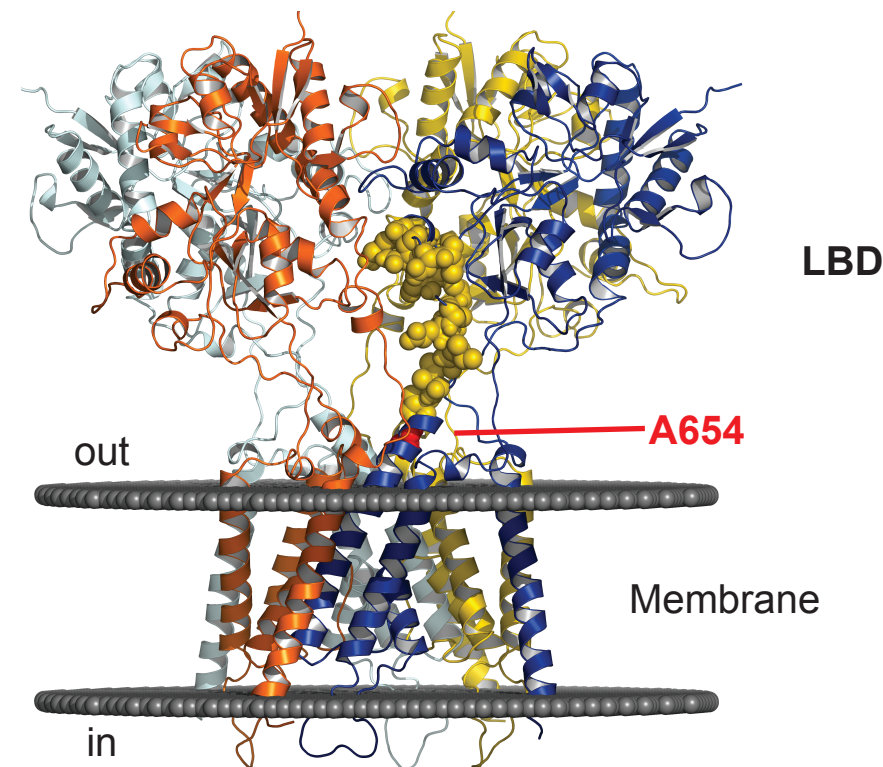
B



C



D



E

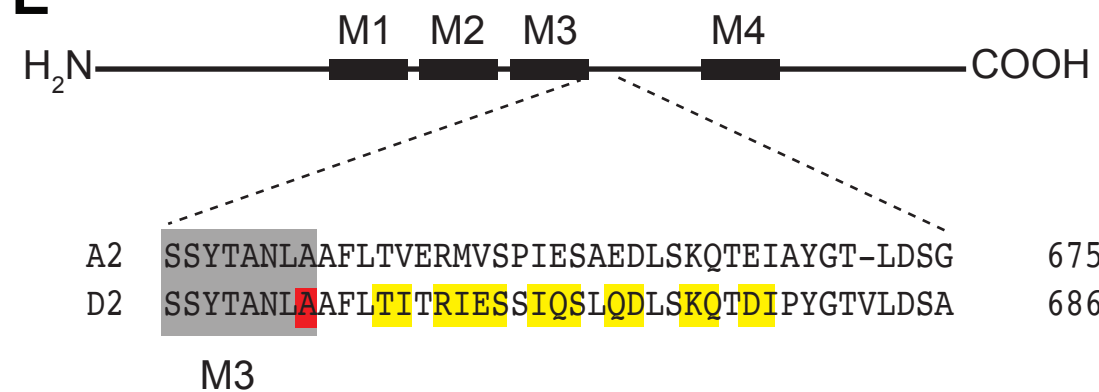


Figure 2

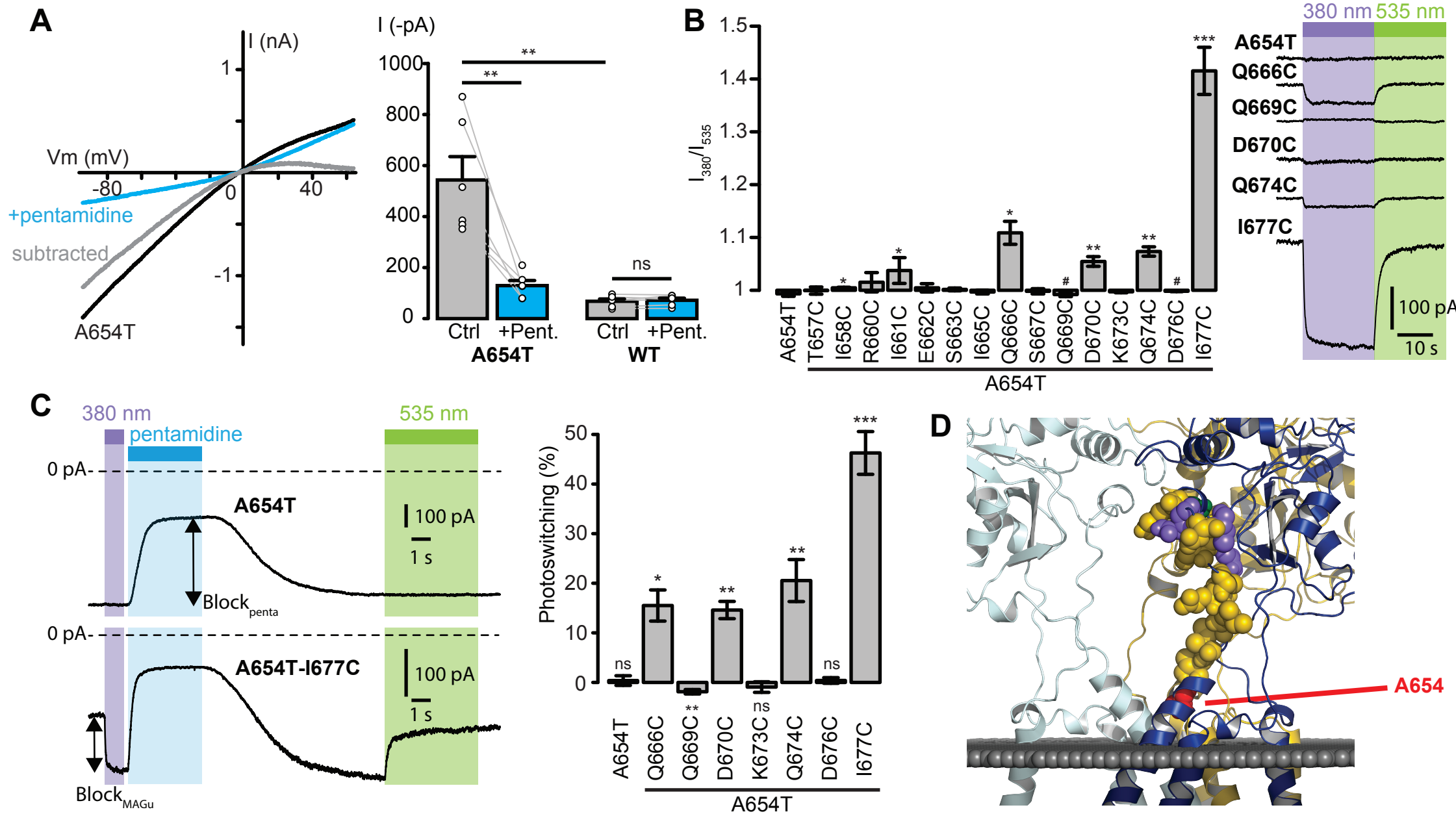


Figure 3

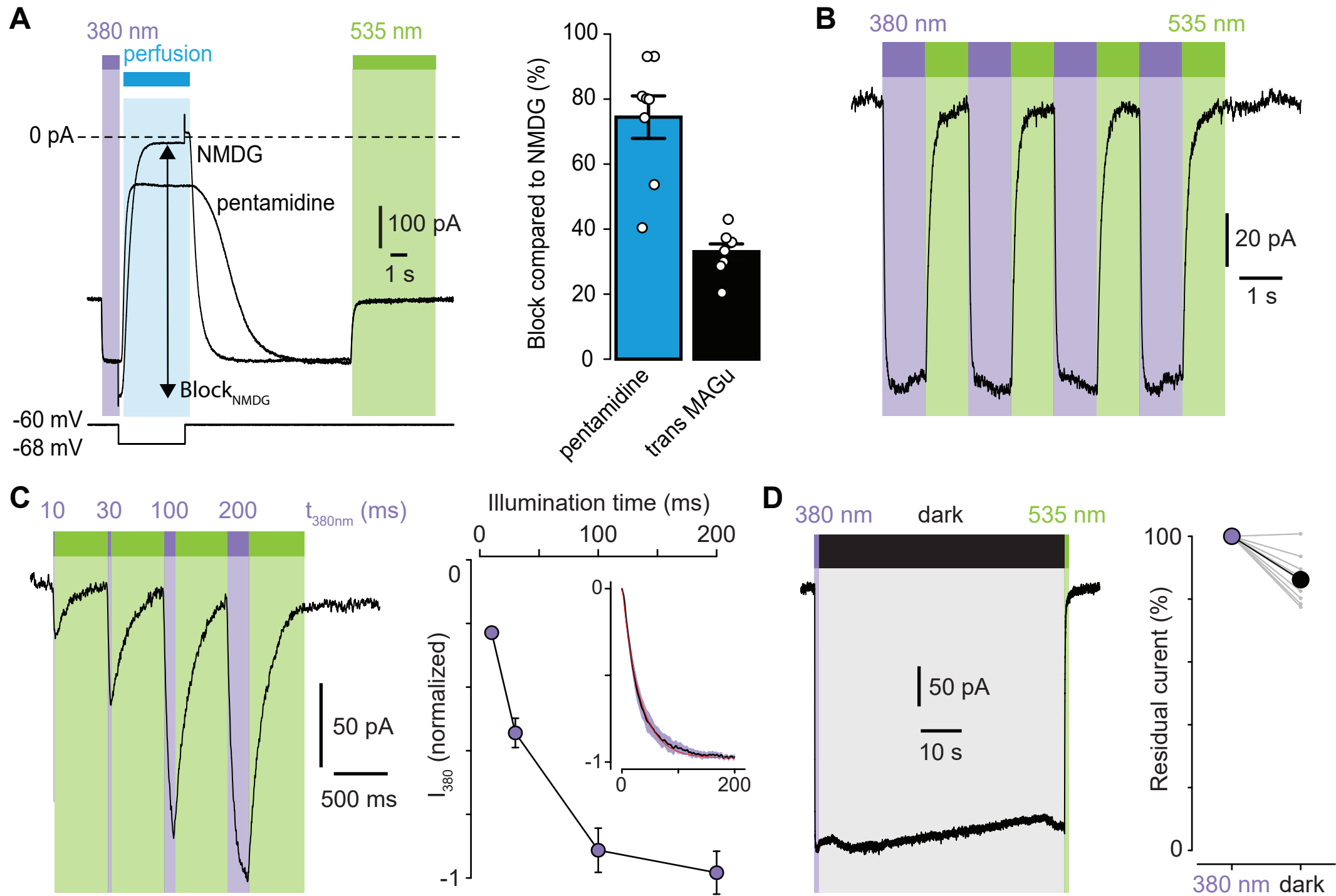
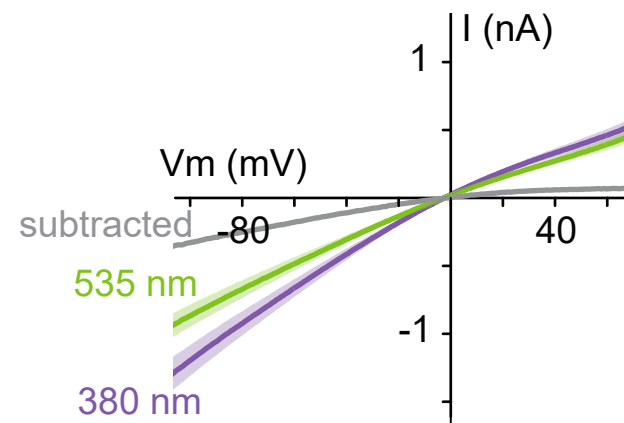
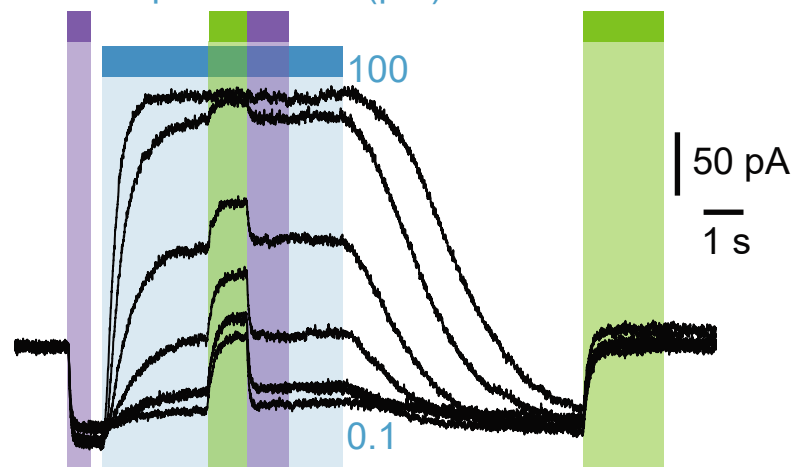


Figure 4

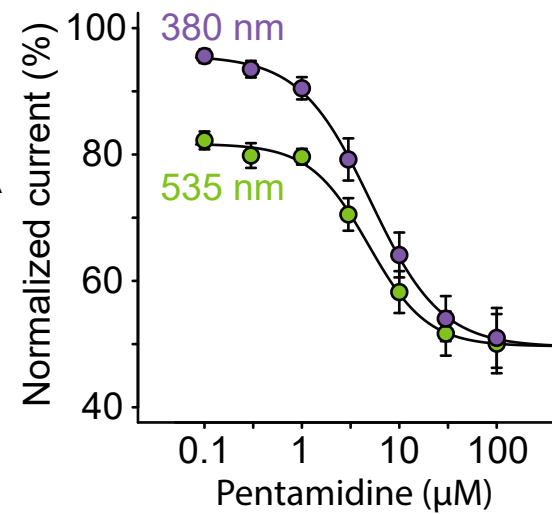
A



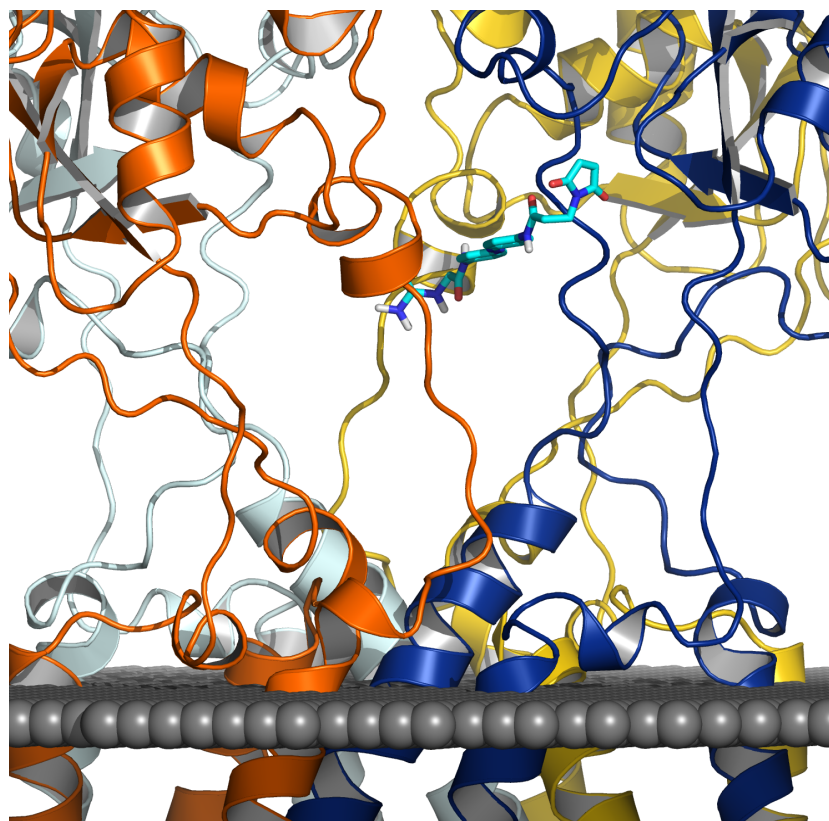
B 380 nm pentamidine (μM)



535 nm



C



D

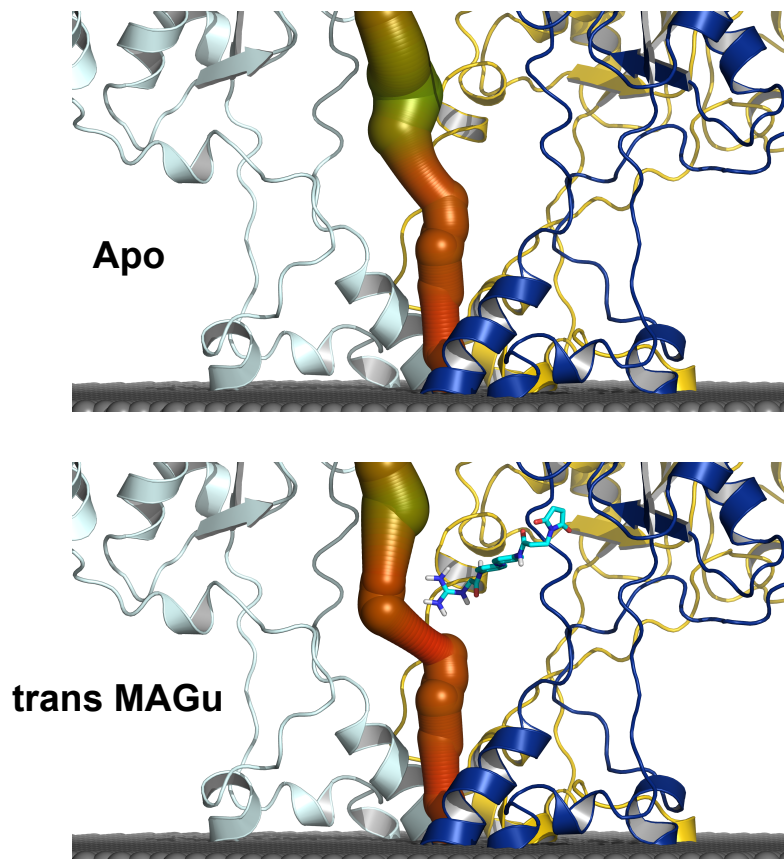
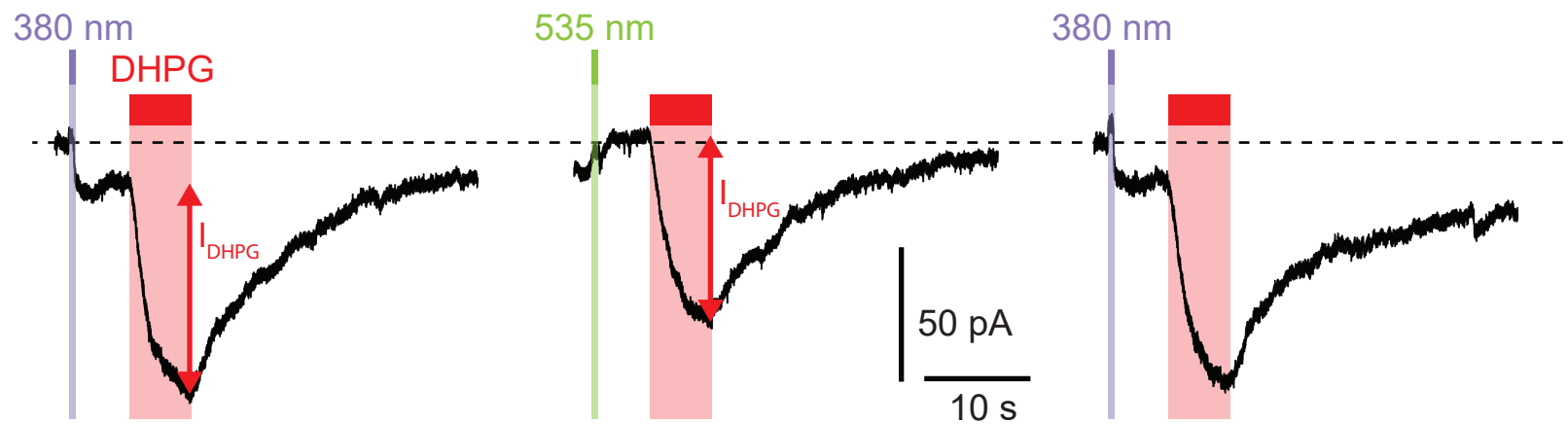
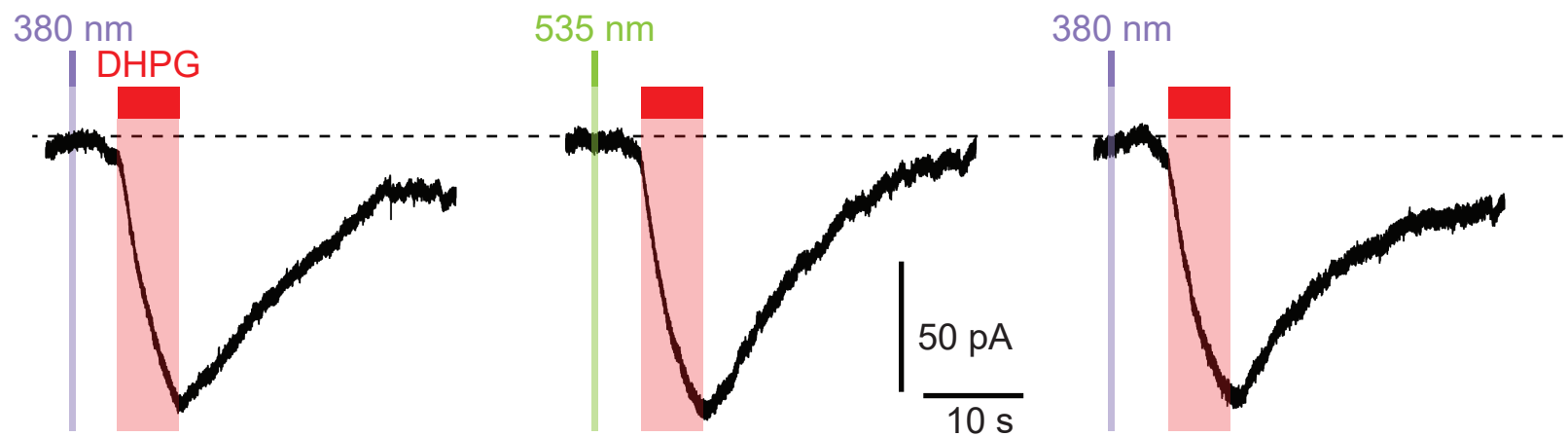


Figure 5

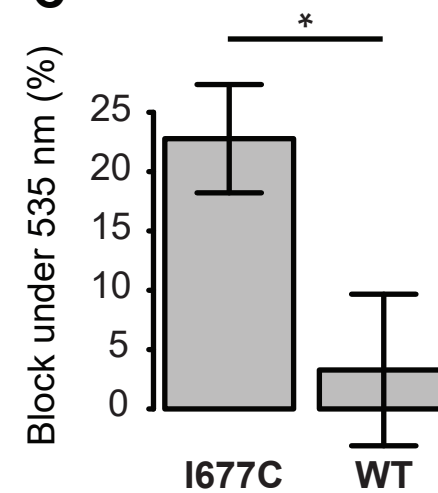
A I677C + mGluR1b



B WT + mGluR1b



C



D

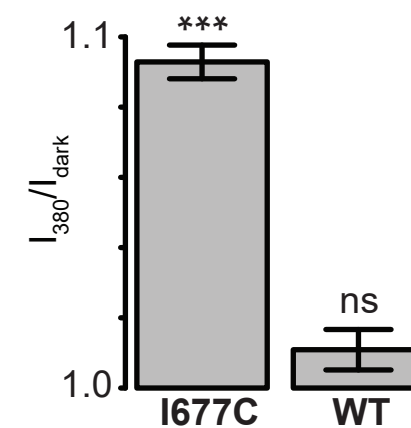


Figure 1-Figure Supp 1

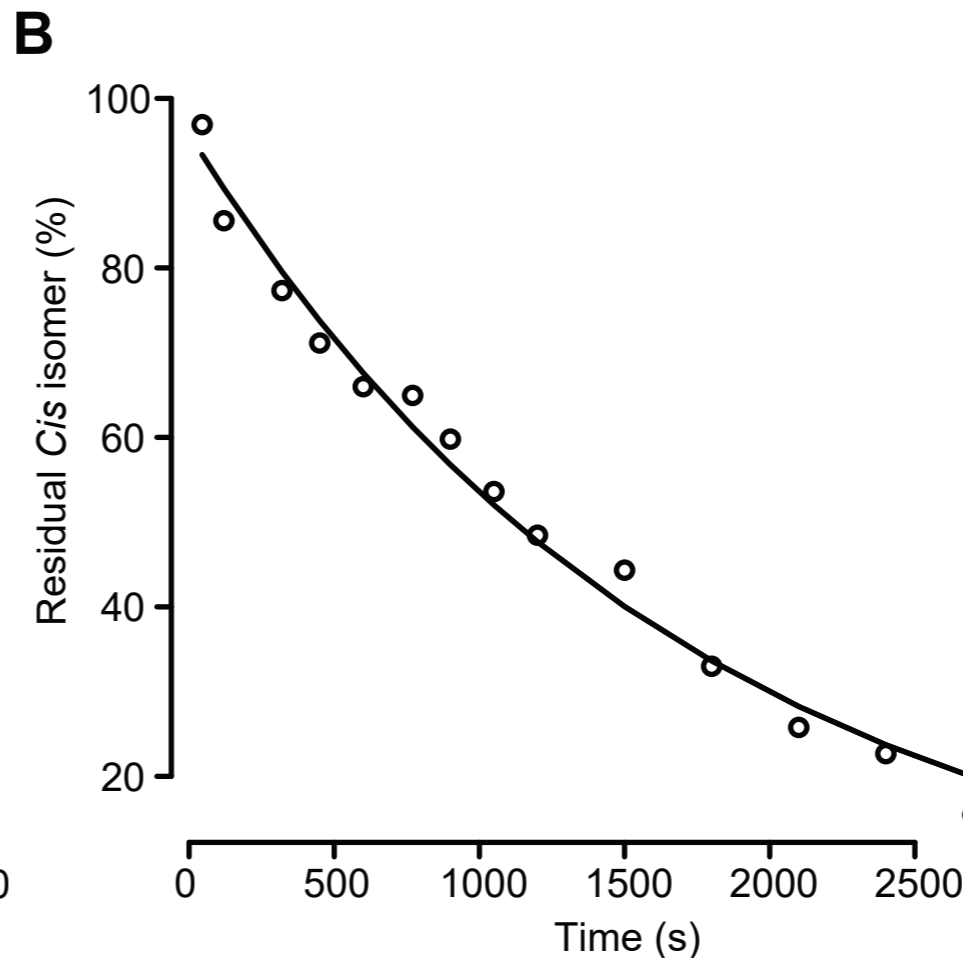
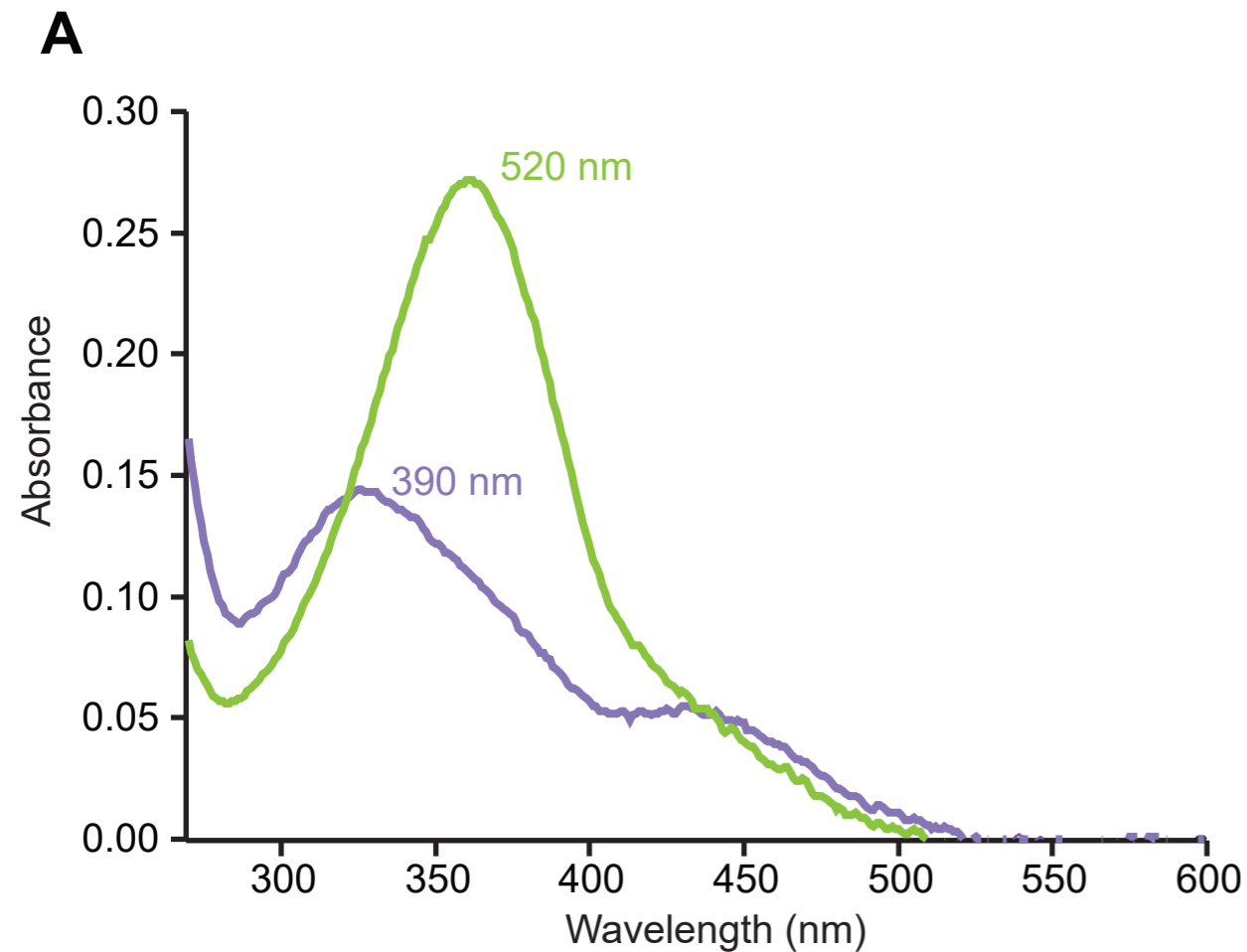


Figure 2-Figure supp1

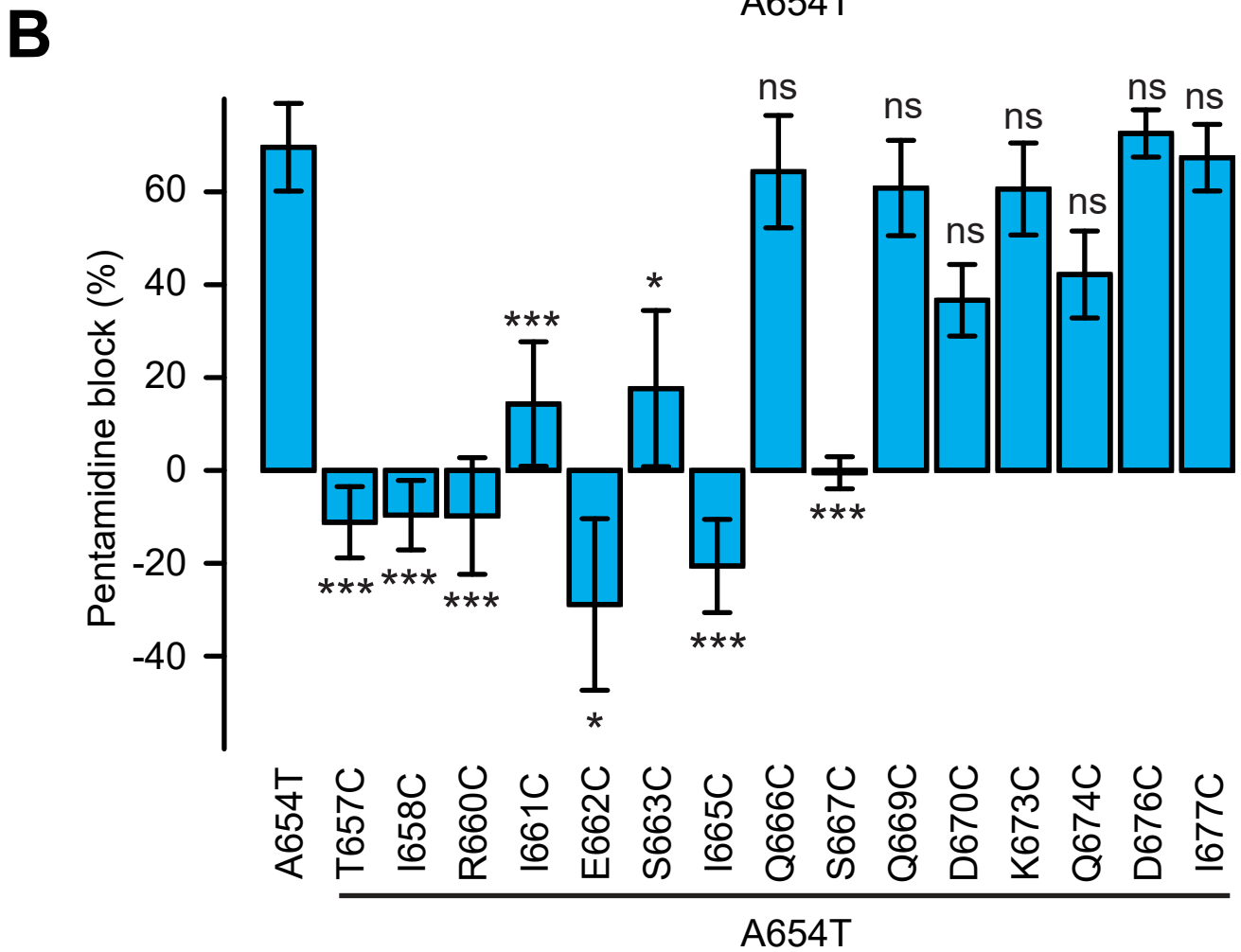
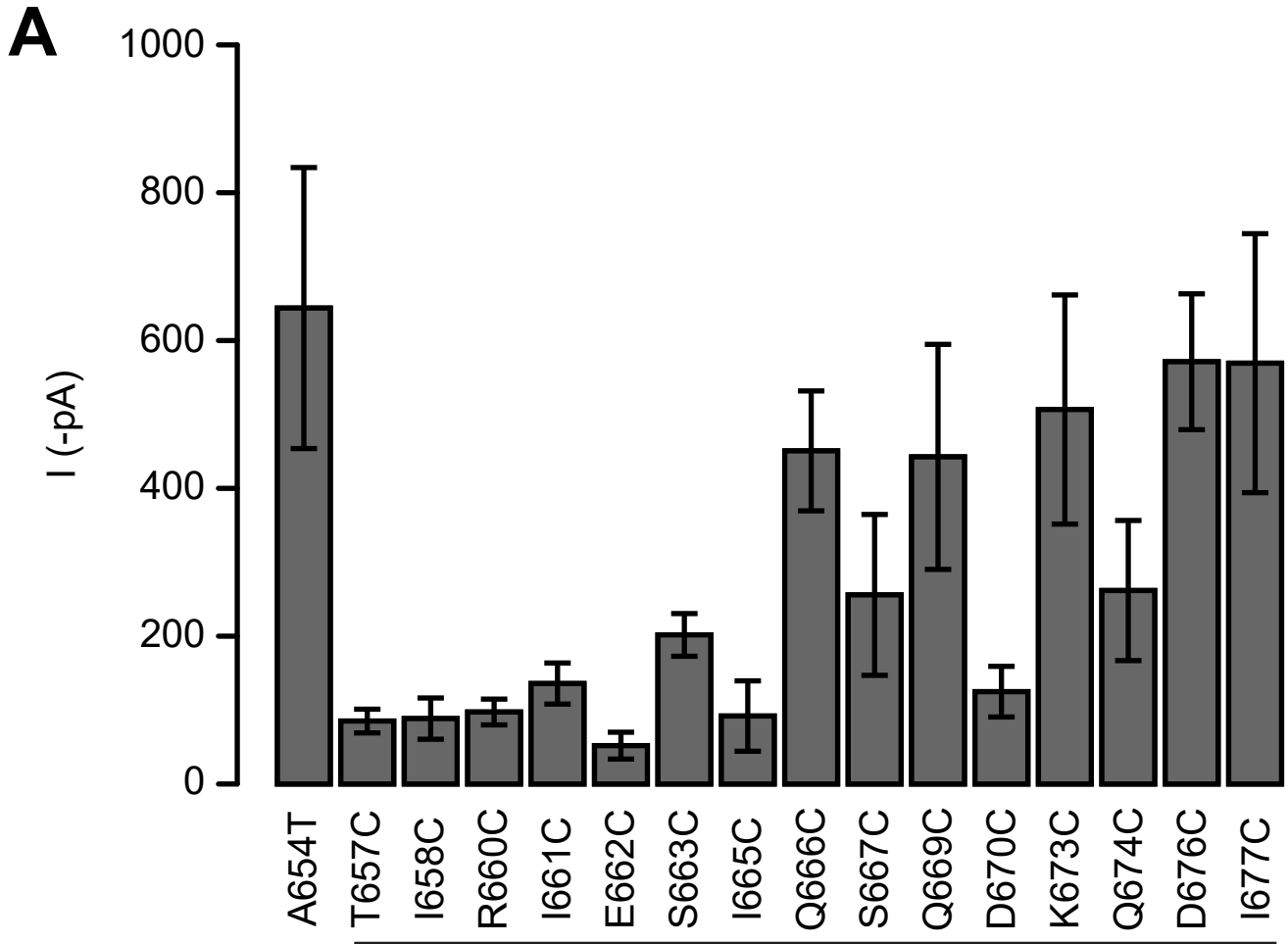


Figure 5-Figure Supp 1

