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Supplementary Information

Figures S1-S9

Table S2

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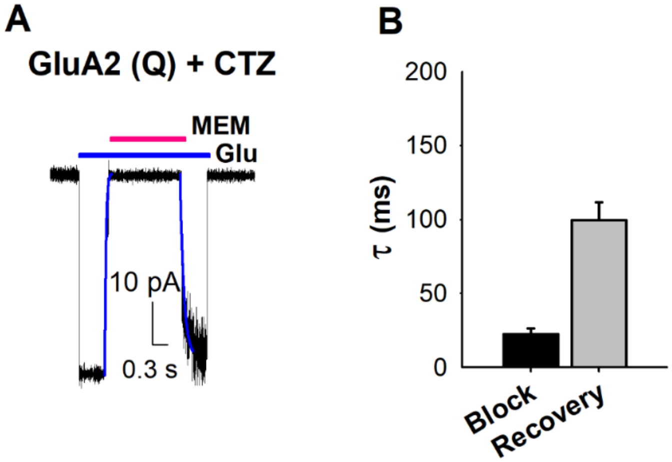

Fig. S1. Time course of memantine inhibition. (A) Time course of the inhibition and recovery by 500 µM of memantine (MEM) in the presence of 10 mM glutamate and 100 µM CTZ. The inhibition and recovery phases were fitted to a single exponential function. (B) Bar graph showing the fits for the inhibition and recovery of memantine inhibition (n=6).

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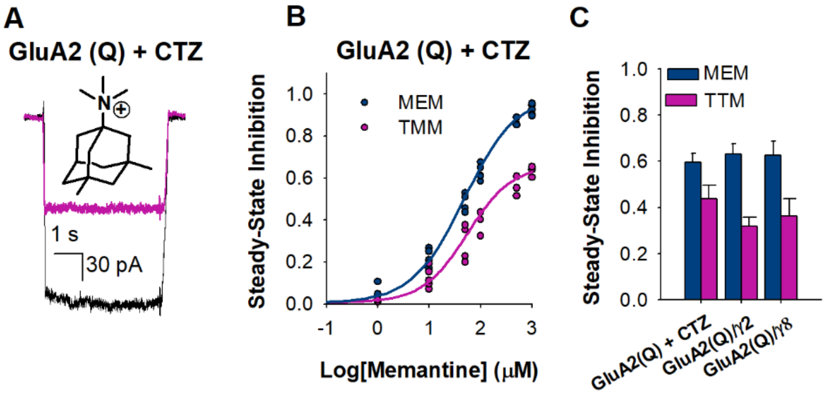

Fig. S2. Memantine versus TMM inhibition of CP-AMPARs. (A) Chemical structure of trimethylmemantine (TMM), and representative GluA2 (Q) + CTZ current traces due to 10 mM glutamate in the absence (black) and presence of 500 µM TMM (pink). (B) The dose-dependent inhibitory effects of memantine (MEM) (•) and TMM (•) on GluA2 (Q) in the presence of CTZ, with IC$_{50}$ 48 ± 3 µM and 384 ± 8 µM, respectively. Each dot represents data from a different cell. (C) Comparison of inhibition by 100 µM of Memantine and TMM inhibition for GluA2(Q)+CTZ, GluA2(Q)/γ2, and GluA2(Q)/γ8, (n ≥ 4).

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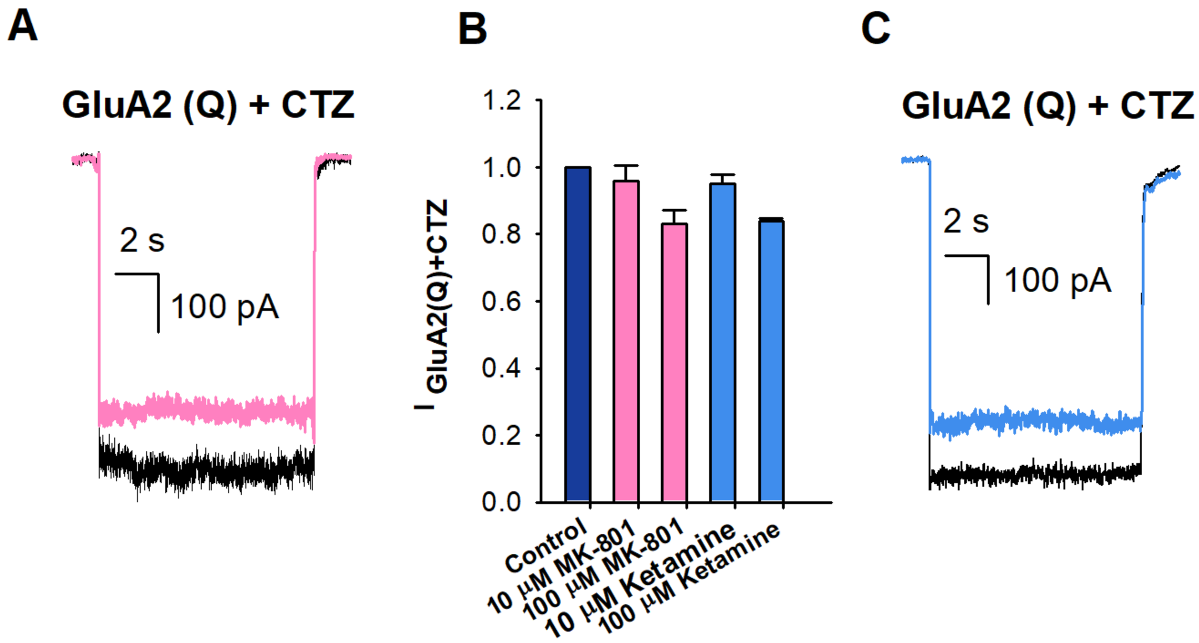

Figure S3. MK-801 and Ketamine responses to GluA2 (Q). (A) Representative whole-cell recordings in response to 10 mM glutamate alone (black) or in the presence of 100µM of MK-801 (pink). (B) Comparison of inhibition of 10 mM glutamate (dark blue) by 10 and 100 µM of MK-801 (pink); and 10 and 100 µM of Ketamine (blue). (C) Representative whole-cell recordings in response to 10 mM glutamate alone (black) or in the presence of 100µM of Ketamine (blue). (n ≥ 4)

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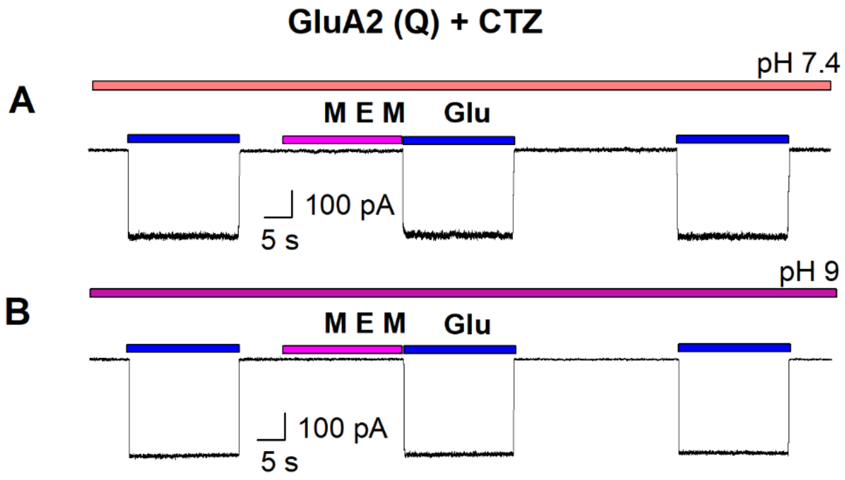

Fig. S4. Representative current traces of memantine (MEM) inhibition showing rapid on and off rates. Current traces of memantine inhibition showing rapid on and off rates at pH 7.4 (A) and at pH 9.0 (B).

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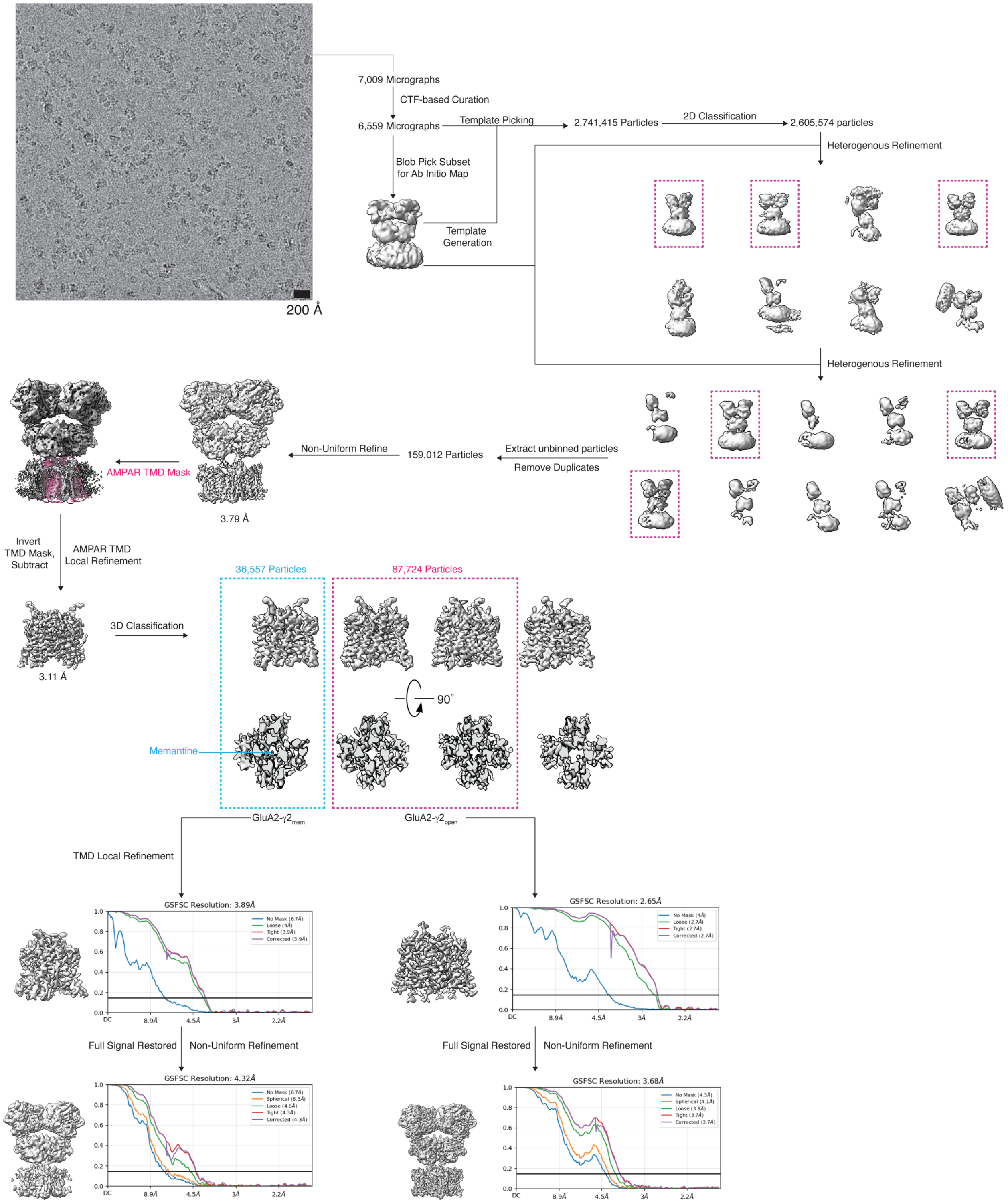

Fig. S5. Cryo-EM processing workflow in Cryosparc.

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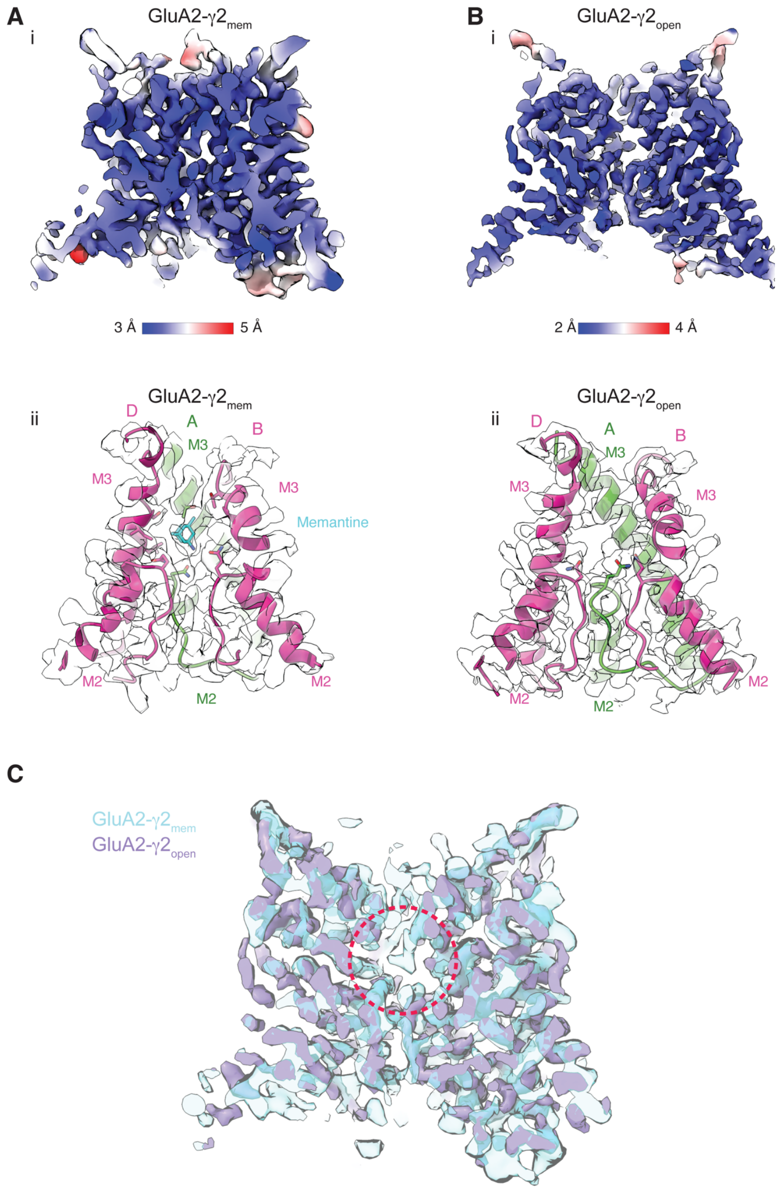

Figure S6. Local refinement maps and details for GluA2-γ2$_{\text{mem}}$ and GluA2-γ2$_{\text{open}}$. (A) Inset i, GluA2-γ2$_{\text{mem}}$ local resolution map, colored blue (3.0 Å) to red (5.0 Å). Inset ii, GluA2-γ2$_{\text{mem}}$ pore model fit into locally refined map. Subunit C omitted for clarity. (B) Inset i, GluA2-γ2$_{\text{open}}$ local resolution map, colored blue (2.0 Å) to red (4.0 Å). Inset ii, GluA2-γ2$_{\text{open}}$ pore model fit into locally refined map. Subunit C is omitted for clarity. (C) Overlay of GluA2-γ2$_{\text{mem}}$ (cyan, transparent) and GluA2-γ2$_{\text{open}}$ (purple) local maps. Red dashed circle indicates the memantine binding site.

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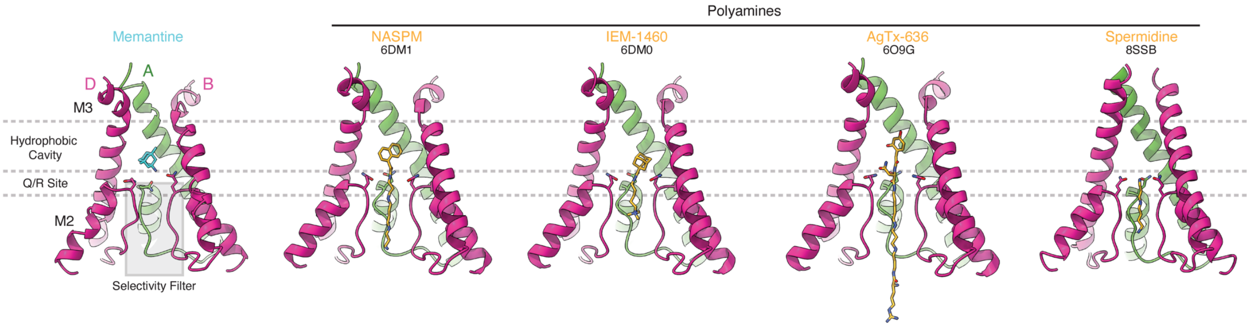

Figure S7. Comparison of memantine block to polyamine block. Polyamines are shown in yellow, memantine in cyan. Both are shown as sticks. Carbon molecules are colored the same as the molecule, nitrogen atoms blue, oxygen atoms red. Nitrogens in polyamine tails are directly coordinated by the selectivity filter and Q/R site, and polyamine derivates or toxins (e.g., NASPM – pdb 6DM1, IEM-1460 – pdb 6DM0, AgTx-636 – pdb 6O9G) have a hydrophobic head above the polyamine tail that sits in the hydrophobic cavity. Spermidine (pdb 8SSB) sits directly at the Q/R site and within the selecitivty filter below.

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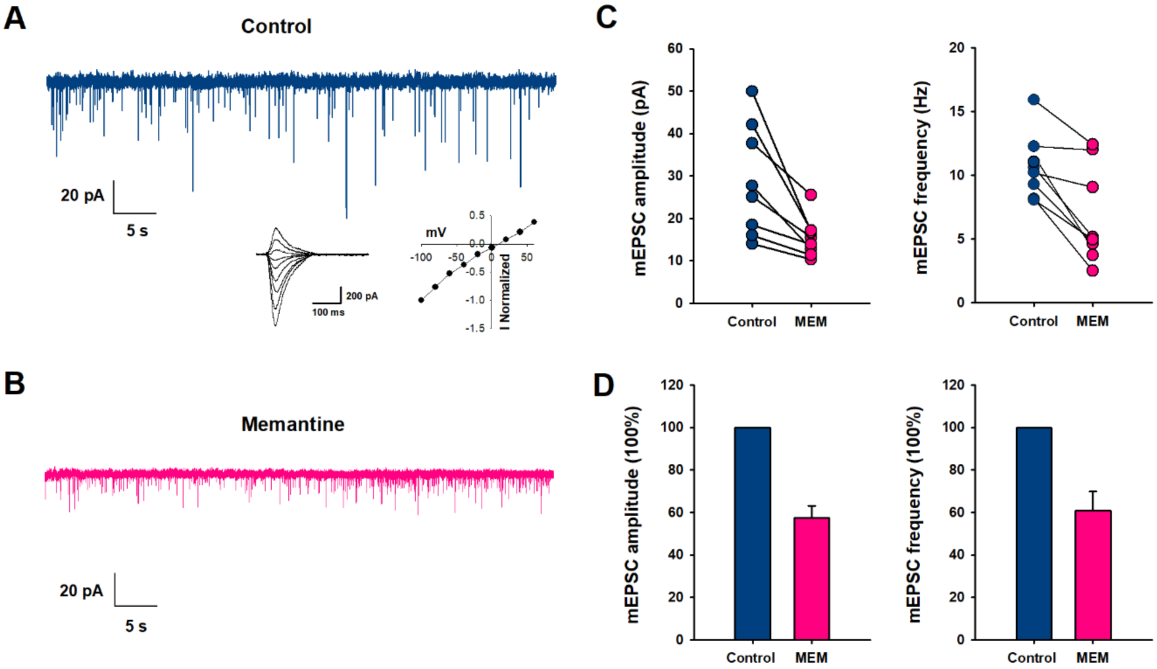

Fig. S8. mEPSCs inhibition by 500 µM of Memantine (MEM). (A) Representative spontaneous mEPSCs, in control (blue) and (B) in the presence of 500 µM of Memantine (pink). Inset: Representative currents activated by fast application of 10 mM of glutamate (from -100 to +60 mV) from hippocampus neurons. (C) mEPSC amplitude and frequency were measured from individual neurons. Paired data from each experiment are connected by a line. (D) Bar graphs of the average values of the normalized mEPSC amplitude and frequency, in control (blue) and in the presence of 500 µM of Memantine (pink).

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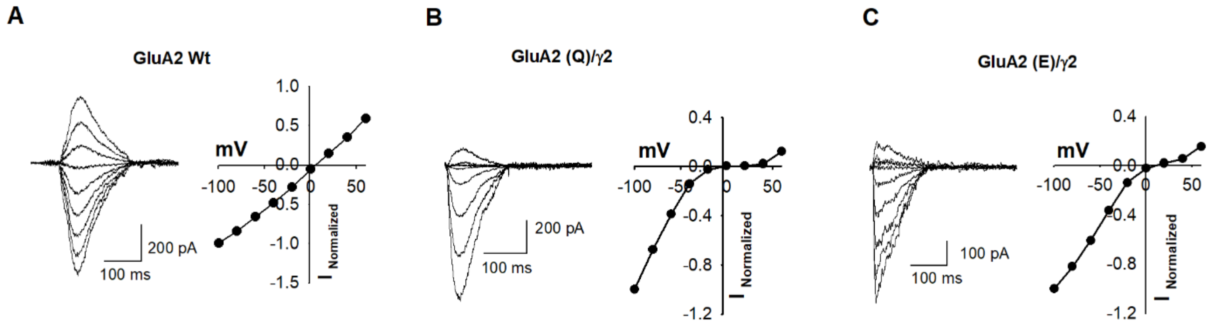

Figure S9. Rectification of synaptic AMPA receptors. Representative currents activated by fast application of 10 mM of glutamate (from -100 to +60 mV) from hippocampus neurons in native conditions (A), and hippocampus neurons transfected with GluA2 (Q)/γ2 (B) and GluA2 (E)/γ2 (C).

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Table S1

Calibrated pixel size (Å)	0.93			
Total Exposure (e/Å^2)	40			
Micrographs (#)	7,009			
Starting particle images	2,741,415 Particles			
Image analysis software	*cryoSPARC 4.5.1*			
Cryo-EM maps	GluA2-γ2$_{mem}$		GluA2-γ2$_{open}$	
	AMPAR TMD	Full	AMPAR TMD	Full
EMDB ID	EMD-XXXX		EMD-XXXX	
Particle images contributing to maps	36,557	36,557	87,724	87,724
Global resolution (FSC = 0.143, Å)	3.9	4.3	2.7	3.7
Resolution range (Å)	5.0 – 3.0	8.4 – 2.6	4.0 – 2.0	7.1 – 2.2
Model Building				
Associated PDB ID	XXXX		XXXX	
Software	*Phenix 1.21.1, Coot 0.9.8.92, Isold 1.7e, ChimeraX 1.6.1*		*Phenix 1.21.1, Coot 0.9.8.92, Isold 1.7e, ChimeraX 1.6.1*	
Protein residues	3954		3954	
Ligand	4 Glu		4 Glu	
	4 CTZ		4 CTZ	
	1 Mem		0 Mem	
RMSD bond length (Å)	0.015		0.015	
RMSD bond angle (º)	0.76		0.69	
Ramachandaran outliers (%)	0.18%		0.08%	
Ramachandran favored (%)	93.01%		92.46	
Rotamer outliers (%)	0		0.03%	
Clashscore	6.63		6.86	
MolProbity score	1.81		1.85	