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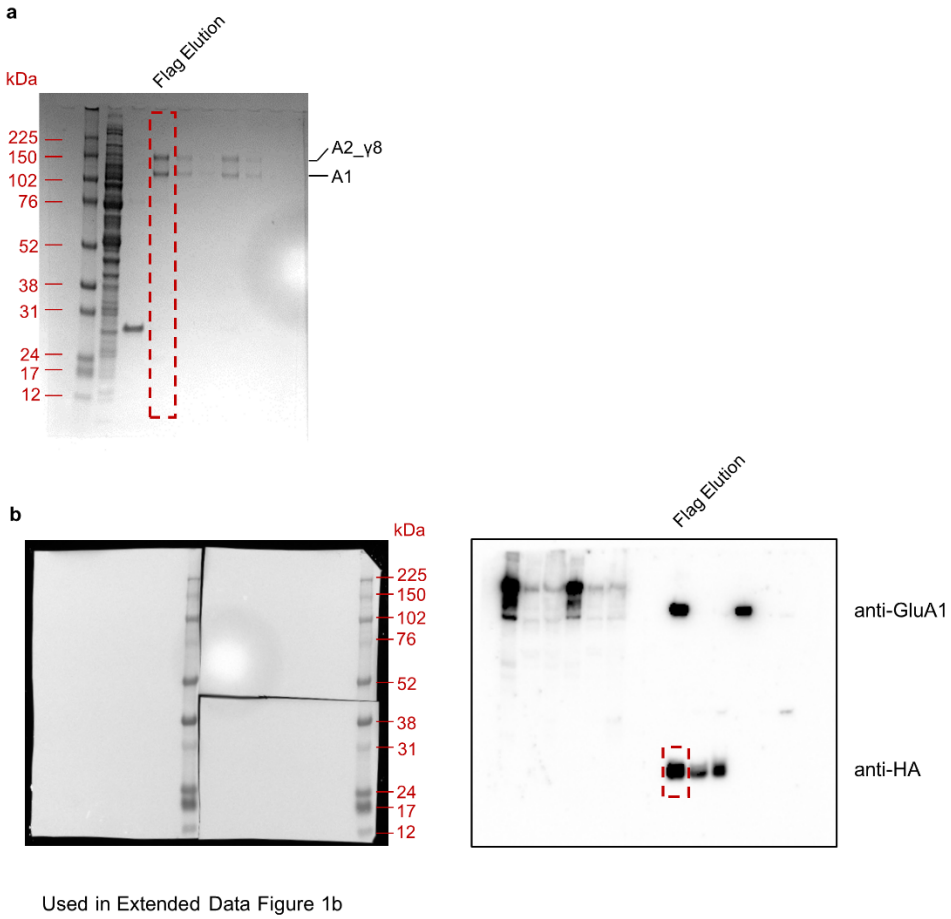
Supplementary information

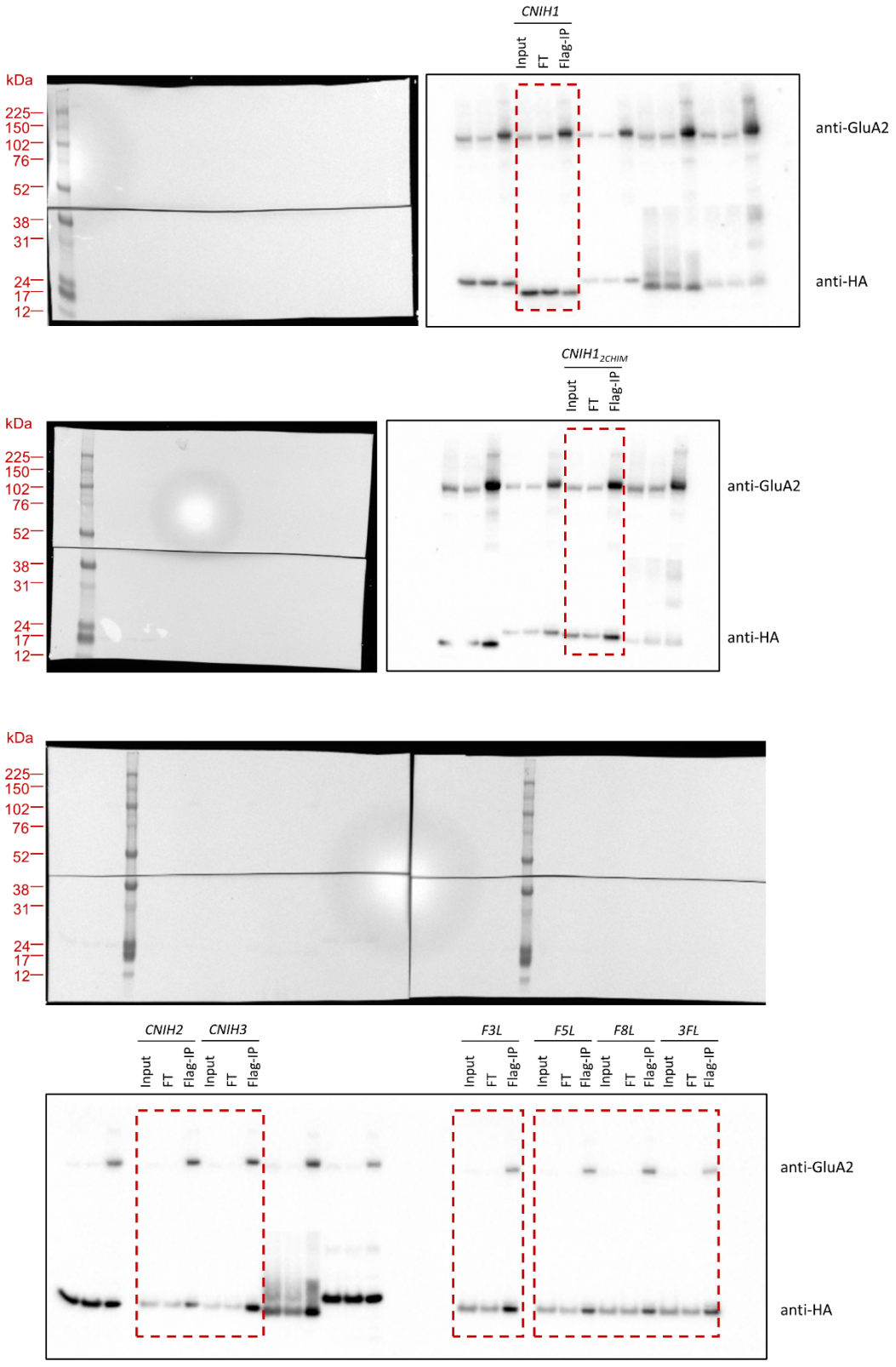
Gating and modulation of a hetero-octameric AMPA glutamate receptor

In the format provided by the authors and unedited

Supplementary Figure



Uncropped gel and immunoblots from Extended Data Fig1. a, 4-12% Bis-Tris gel stained with coomassie blue, indicating elution of A1/2_γ8/C2 purification. Image is used in Extended Data Fig 1b left panel. **b,** Immunoblots of CNIH2 by probing for the C-terminal HA tag. Image is used in Extended Data Fig 1b left panel.



Used in Extended Data Figure 10a

Uncropped immunoblots from Extended Data Fig10.

Immunoblots from Flag IP of CNIH homologues and CNIH2 mutants in complex with Flag tagged GluA2 homomers. Images are used in Extended Data Fig 10a.

Supplementary Table 1. Electrophysiology P values, Figure 1 and Extended Data Figure 1

Figure 1b & Extended Data Figure 1a: GluA1/2_γ8+CNIH2 Recombinant receptors Welch's ANOVA test; Dunnett's multiple comparisons – adjusted p values				
Comparison	*Desensitisation*	*Rise time*	*RI*	*Eq'm Current*
GluA1/2 vs GluA1/2_γ8	0.0002	0.0941	0.1120	0.0723
GluA1/2 vs GluA1/2_γ8 + CNIH2	<0.0001	0.0527	0.2759	0.0083
GluA1/2_γ8 vs GluA1/2_γ8 + CNIH2	0.0003	0.7232	0.6548	0.0567

Figure 1b & Extended Data Figure 1a: Neuronal receptors Welch's ANOVA test; Dunnett's multiple comparisons – adjusted p values				
Comparison	*Desensitisation*	*Rise time*	*RI*	*Eq'm Current*
CA1 - PC vs. CA1 - IN	0.3687	0.9993	0.3687	0.0002
CA1 - PC vs. DG - GC	0.9908	0.0247	0.9908	0.0003
CA1 - PC vs. CA3 - PC	0.8330	0.7584	0.8330	0.9634
CA1 - IN vs. DG - GC	0.5602	0.1790	0.5602	0.9977
CA1 - IN vs. CA3 - PC	0.4393	0.6645	0.4393	0.0190
DG - GC vs. CA3 - PC	>0.9999	0.1098	>0.9999	0.0334

Supplementary Table 2. Electrophysiology P values, Figure 3 and Extended Data Figure 10

Figure 3a & Extended Data Figure 10d: CNIH2 FL mutations Welch's ANOVA test with Dunnett's multiple comparisons test – adjusted p values		
	Desensitisation (Figure 3)	*Eq'm Current (Figure ED 9)*
GluA2Q + CNIH2 vs. GluA2Q	0.0002	0.0038
GluA2Q + CNIH2 vs. F3L	0.0295	0.0414
GluA2Q + CNIH2 vs. F5L	0.0107	0.0375
GluA2Q + CNIH2 vs. F8L	0.0065	0.0267
GluA2Q + CNIH2 vs. 3FL	0.0003	0.0049

Figure 3c & Extended Data Figure 10e: CNIH1/2 chimeric recordings Welch's ANOVA test with Dunnett's multiple comparisons test – adjusted p values		
	Desensitisation (Figure 3)	*Eq'm Current (Figure ED 9)*
GluA2Q vs GluA2Q + CNIH1	0.0016	0.057
GluA2Q vs GluA2Q + CNIH1/2	<0.0001	<0.0001
GluA2Q vs GluA2Q + CNIH2	0.0011	0.0002
GluA2Q + CNIH1 vs GluA2Q CNIH1/2	<0.0001	0.0003
GluA2Q + CNIH1 vs GluA2Q CNIH2	0.0021	0.0005
GluA2Q + CNIH1/2 vs GluA2Q CNIH2	0.0362	0.016

Supplementary Table 3. Immunology P values, Extended Data Figure 10b

Extended Data Figure 10b: CNIH immunocytochemistry Kruskal-Wallis test, Comparisons – adjusted p values		
	Surface Fluorescence	*Total Fluorescence*
No CNIH vs CNIH1	<0.0001	<0.0001
No CNIH vs CNIH2	<0.0001	<0.0001
No CNIH vs CNIH3	<0.0001	<0.0001
No CNIH vs CNIH1/2chimera	<0.0001	<0.0001
No CNIH vs CNIH2 F3L	0.0001	0.0009
No CNIH vs CNIH2 F5L	<0.0001	<0.0001
No CNIH vs CNIH2 F8L	<0.0001	<0.0001
No CNIH vs CNIH2 3FL	0.1497	<0.0001

Extended Data Figure 10b: CNIH immunocytochemistry One Sample Wilcoxon test vs Median = 0	
	Total Fluorescence
CNIH1	<0.0001
CNIH2	<0.0001
CNIH3	<0.0001
CNIH1/2chimera	<0.0001
CNIH2 F3L	<0.0001
CNIH2 F5L	<0.0001
CNIH2 F8L	<0.0001
CNIH2 3FL	<0.0001