iScience, Volume *26*

Supplemental information

Activity-dependent tau cleavage by caspase-3 promotes neuronal dysfunction and synaptotoxicity

Carli K. Opland, Miles R. Bryan, Braxton Harris, Jake McGillion-Moore, Xu Tian, Youjun Chen, Michelle S. Itano, Graham H. Diering, Rick B. Meeker, and Todd J. Cohen

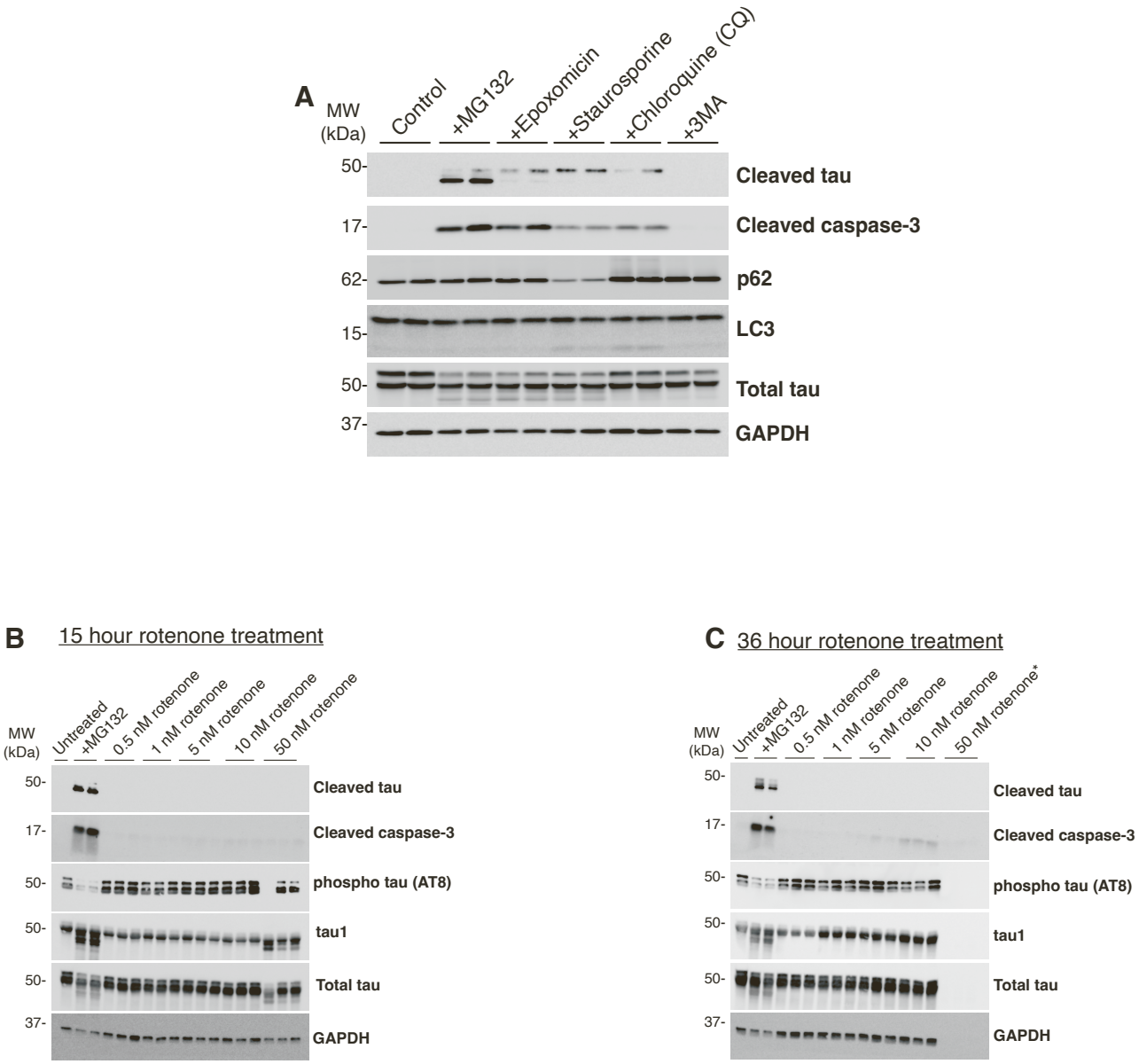
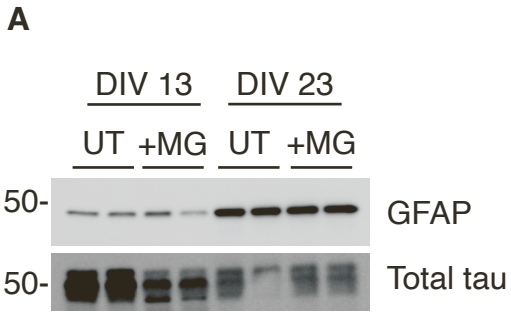


Figure S1. Cleaved tau is primarily generated in response to proteasome impairment, Related to Figure 1

A Primary mouse cortical neurons (E16) from C57BL/6 WT mice cultured for 10 days in vitro (DIV 10) and treated with either MG132 (overnight), epoxomicin (overnight), staurosporine (1 μM, 5 hours), chloroquine diphosphate (CQ, 10 μM, overnight) or 3MA (10 μM, overnight) and analyzed by immunoblotting. **B** Primary WT neurons cultured to DIV 11 were either treated with MG132 or different concentrations of rotenone (0.5 nM, 1nM, 5 nM, 10 nM or 50 nM) overnight for 15 hours and analyzed by immunoblotting. **C** Primary WT neurons cultured to DIV 11 were either treated with MG132 (overnight) or different concentrations of rotenone (0.5 nM, 1 nM, 5 nM, 10 nM or 50 nM) for 36 hours and analyzed by immunoblotting. Neurons treated with 50 nM rotenone for 36 hours (denoted by the asterisk) were not viable and did not generate any detectable protein yields.



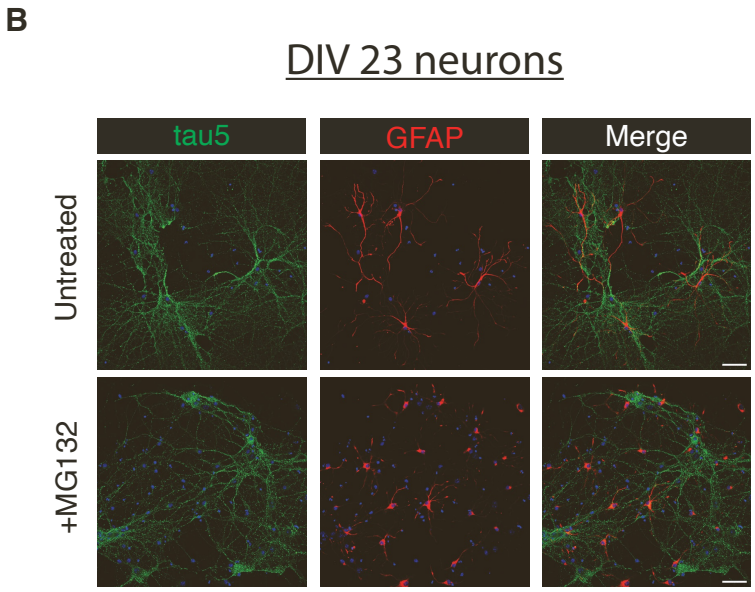


Figure S2. Astrocytes accumulate in aged neuronal cultures beyond DIV 12, Related to Figure 4
A Primary WT neurons were left untreated or treated with MG132 overnight at DIV 13 or DIV 23 and analyzed by immunoblotting. **B** Primary WT neurons were left untreated or treated with MG132 overnight at DIV 23 and analyzed by immunostaining to detect total tau levels (tau5, green) and astrocytes (GFAP, red), while nuclei were marked by DAPI (blue). Scale bar = 50 μm. Representative images are shown from n = 3 independent experiments.

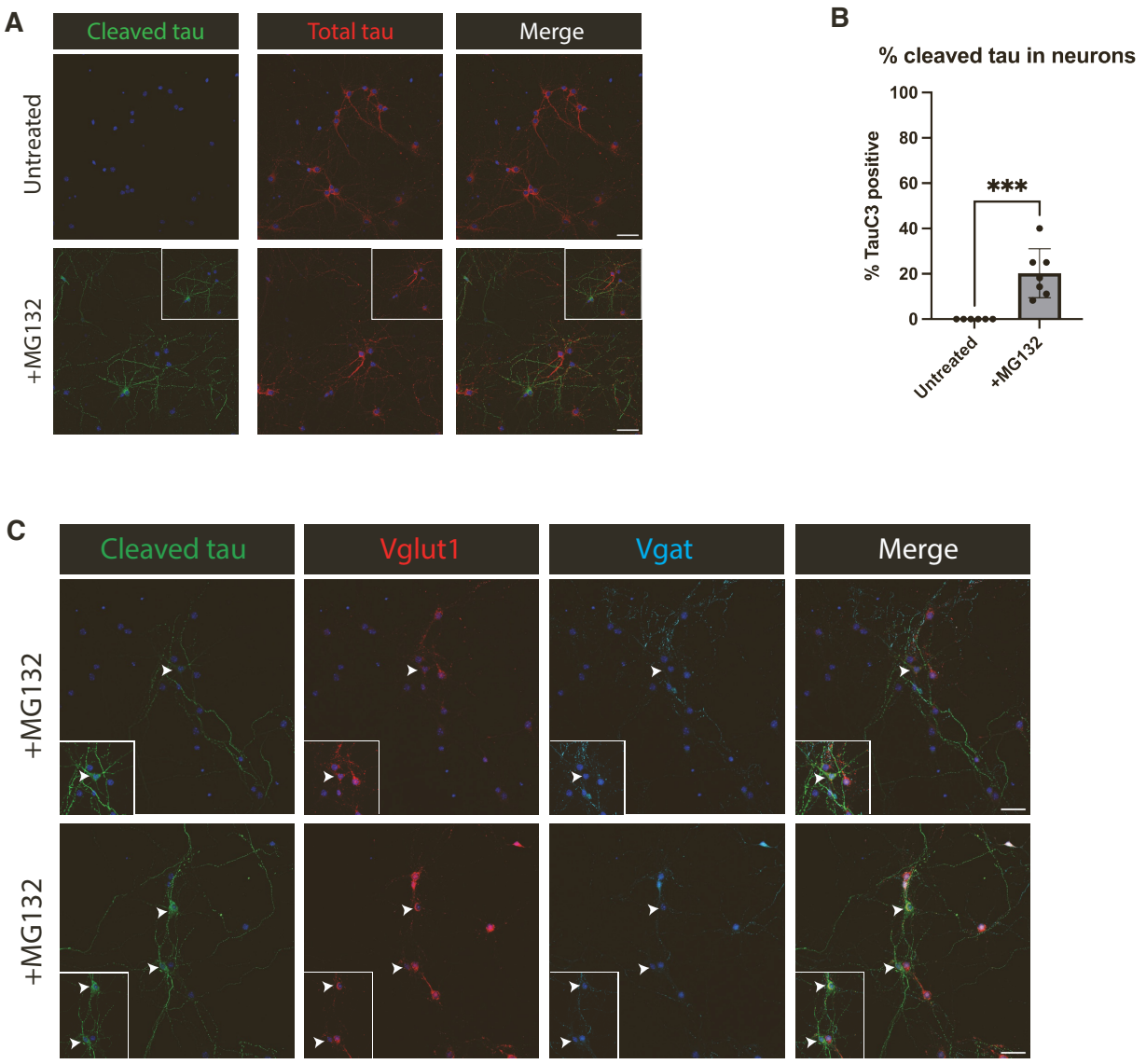


Figure S3. Cleaved tau accumulates preferentially in excitatory neurons, Related to Figure 4
A-B Primary WT neurons were left untreated or treated with MG132 overnight at DIV 10 and analyzed by IF to detect cleaved tau (green) and total tau (red), while nuclei were marked by DAPI (blue) followed by the quantification of percentage of cleaved tau neurons compared to total tau. The white inset highlights a region of neurons where total tau (K9JA) and cleaved tau (tauC3) signals are non-overlapping, indicating cleaved tau selectivity among specific neuronal subpopulations. Scale bar = 50 µm. **C** Primary WT neurons were treated with MG132 overnight and analyzed by confocal imaging to detect cleaved tau (green), Vglut1 (excitatory, red), and Vgat (inhibitory, cyan), while nuclei were marked by DAPI (blue). Arrowheads highlight cleaved tau-positive neurons that are preferentially localized to excitatory neurons (Vglut1 positive). The white inset highlights cleaved tau positive neurons that preferentially label excitatory neurons. Scale bar = 50 µm. Representative neurons are shown from n = 3 **(A)** and n = 2 **(C)** biologically independent experiments. Statistical tests: p value determined by two-sided unpaired t-test **(B)** from n = 6. Error bars represent means ± SEM **(B)**. *** $p < 0.001$

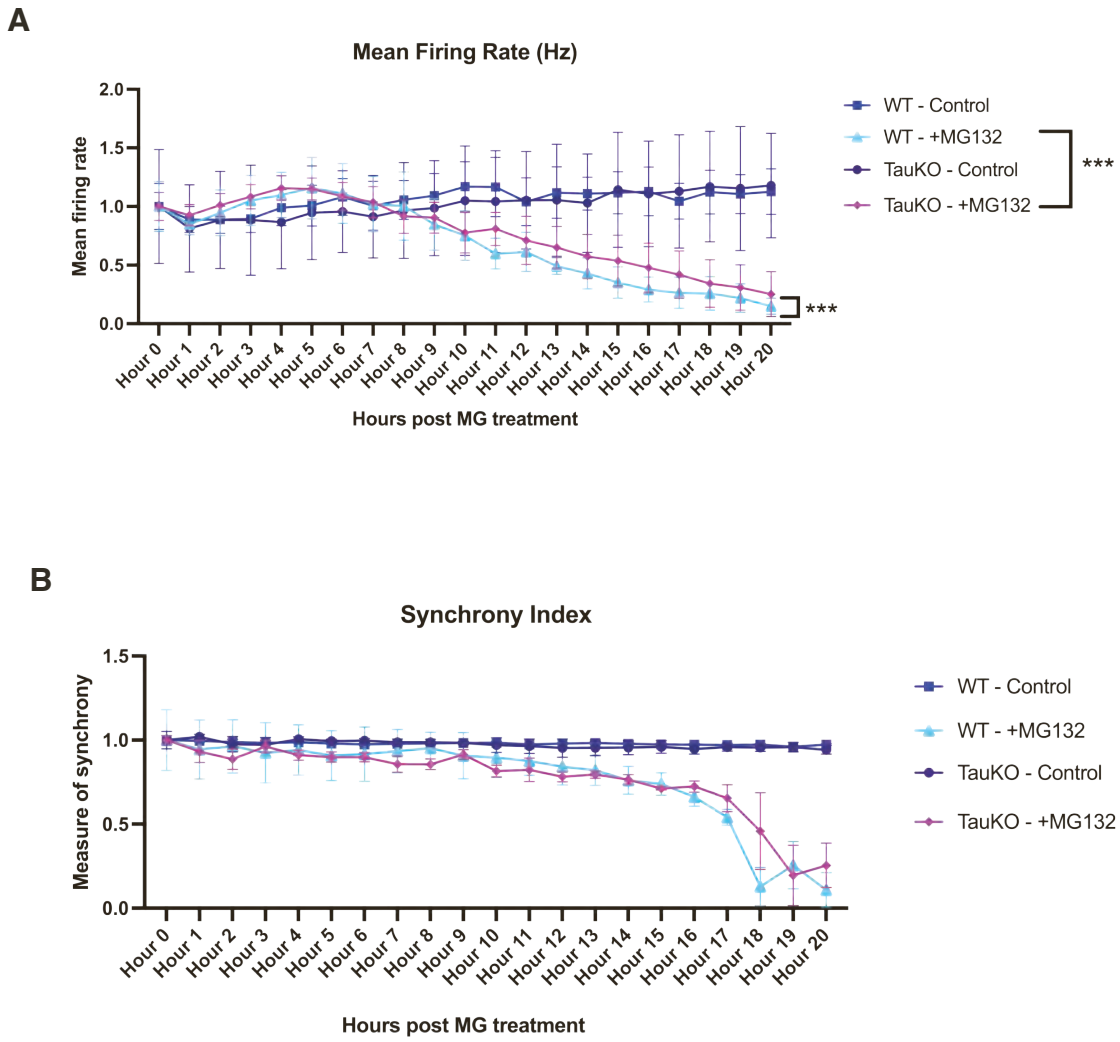


Figure S4. Tau depletion partially prevents the reduction in neuronal firing in response to proteasome inhibition, Related to Figure 7

A-B Primary WT and tau KO neurons were cultured to DIV 18, treated with MG132 or left untreated, and recorded on Axion Biosystems for 10 minutes every hour over a 21-hour time period, and then analyzed for mean firing rate and synchrony index. Hour 0 represents the initial recording post MG132 exposure. For both mean firing rate and synchrony index, all data was normalized by setting hr 0 equal to 1 on the y-axis. Statistical tests: p value determined by one-way ANOVA with Tukey's post hoc test from n = 3 biologically independent experiments. Error bars represent means ± SEM. ***$p < 0.001$.

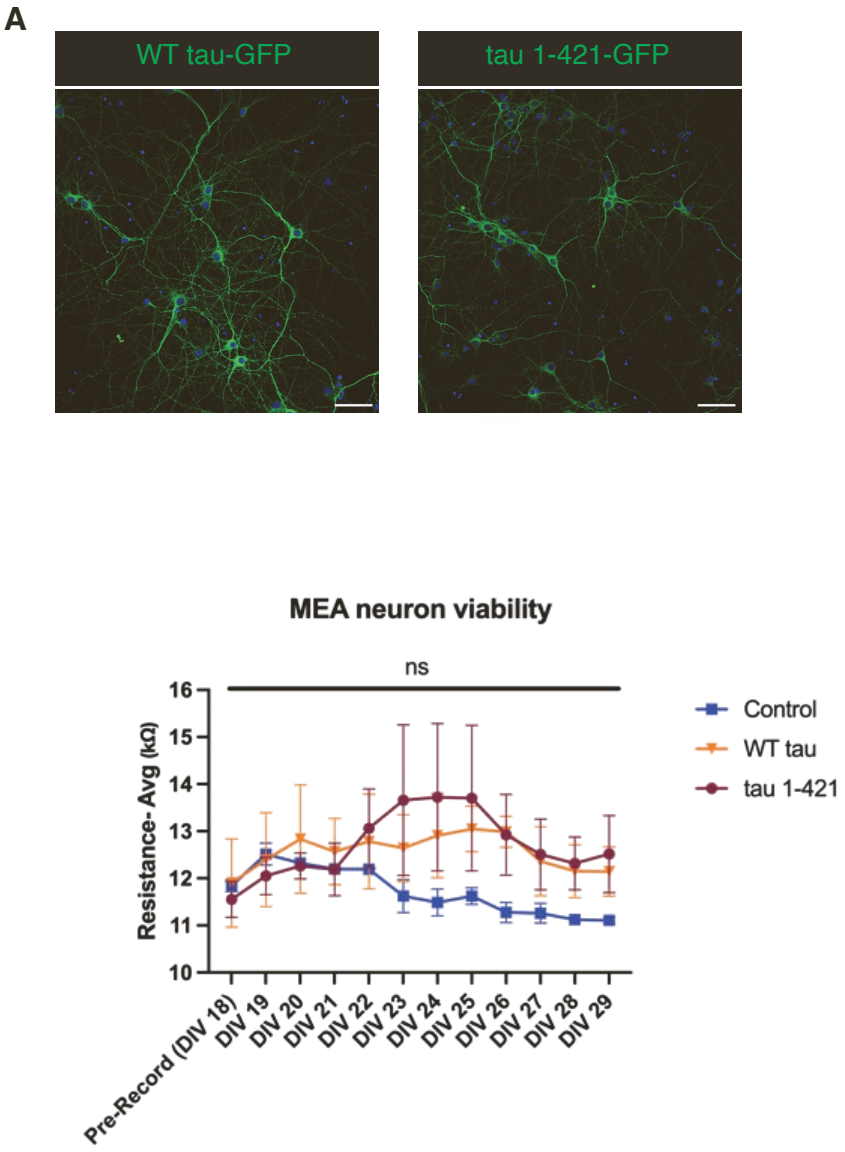


Figure S5. Transduction efficiency and viability of WT-tau and 1-421 expressing neurons, Related to Figure 7

A Primary WT neurons were transduced with either WT tau-GFP or tau 1-421-GFP and analyzed by confocal imaging to detect ectopically expressed tau (green), while nuclei were marked by DAPI (blue). Scale bar = 50 µm. Representative neurons are shown from n = 3 biologically independent experiments. Transduction efficiencies using lentiviral delivery was > 90%. **B** Primary WT neurons cultured at DIV 18 were analyzed for viability using the Axion Biosystems viability module. After baseline analysis at DIV 18, neurons were transduced with control GFP, WT tau, or tau 1-421, and viability was analyzed daily up until DIV 29. Statistical tests: p value determined by two-way ANOVA with Tukey's post hoc test from n = 2 biologically independent experiments. Error bars represent means ± SEM **(B)**. ns = not significant.

Table S1: Human patient demographics used in this study, Related to STAR Methods

Case no.	Clinical diagnosis	Ages	Developmental stage
1-5 (IB)	Normal	73-92	N/A
6-12 (IB & IHC)	Alzheimer's disease	56-90	Braak stage V-V1

Table S2: Primary antibodies used in this study, Related to STAR Methods

Primary Antibodies				
Antibody	**Vendor**	**Catalog number**	**Western Blot dilution**	**IF dilution**
TauC3 (Cleaved Asp421,Asp422)	Invitrogen	#AHB0061	1:500	1:500
Cleaved caspase-3 (Asp175)	Cell Signaling	#9661S	1:1000	1:400
Total tau K9JA	DAKO	#A0024	1:5000	1:2000
GAPDH (clone 6C5)	MilliporeSigma	#MAB374	1:1000	
Anti-Tau (3-repeat isoform RD3) clone 8E6/C11	Millipore	#05-803	1:500	
Anti-Tau (4-repeat isoform RD4) clone 1E1/A6	Millipore	#05-804	1:500	
Anti-Tau-1 Ab, clone PC1C6	Millipore	#MAB3420	1:1000	
Total tau Tau12	Millipore	#MAB2241	1:1000	
anti-p62	Progen	#GP62-C	1:1000	
LC3A/B	Cell Signaling	#4108S	1:500	
Tau Monoclonal Antibody (Tau5)	Invitrogen	#AHB0042	1:1000	1:1000
Phospho-Tau (Ser202, Thr205) Monoclonal Antibody (AT8)	Invitrogen	#MN1020	1:1000	
VGLUT1	Synaptic Systems	#135304		1:500
VGAT	Synaptic Systems	#131008		1:500
Anti-PSD95	Abcam	#Ab18258		1:1000
Anti-PSD-95 Antibody (K28/43)	NeuroMab/Antibodies Inc	#75-028	1:1000	1:1000
Rabbit anti-GFAP	DAKO	#Z0334	1:2000	1:1000
Homer1	Synaptic Systems	#160002	1:25,000	
Anti-GluA1/GluR1 (N355/1)	NeuroMab/Antibodies Inc	#75-327	1:1000	
Anti-Glutamate Receptor 1 Ab, phosphoSer845	Millipore	#AB5849	1:1000	

Table S3: Secondary antibodies used in this study, Related to STAR Methods

Fluorescent Secondary Antibodies		
Antibody	**Vendor**	**Catalog number**
Goat anti-Mouse IgG (H+L)-Highly crossed absorbed (HA) Secondary Antibody, Alexa Fluor™ 488	Invitrogen	#A32723
Goat anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor™ 488	Invitrogen	#A11001
Donkey anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor™ 488	Life technologies	#A21202
Goat anti-Guinea pig IgG (H+L) Secondary Antibody, Alexa Fluor™ 568	Invitrogen	#A11075
Donkey anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor™ 568	Invitrogen	#A10037
Donkey anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor™ 594	Invitrogen	#A21207
Goat anti-Rabbit IgG (H+L)-HA Secondary Antibody, Alexa Fluor™ 594	Invitrogen	#A32740
Goat anti-Rabbit IgG (H+L)-HA Secondary Antibody, Alexa Fluor™ 647	Invitrogen	#A32733
Donkey anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor™ 647	Invitrogen	#A31573
Goat anti-Mouse IgG (H+L) Secondary Antibody, HRP	Invitrogen	#32430
Goat anti-Rabbit IgG (H+L) Secondary Antibody, HRP	Invitrogen	#32460