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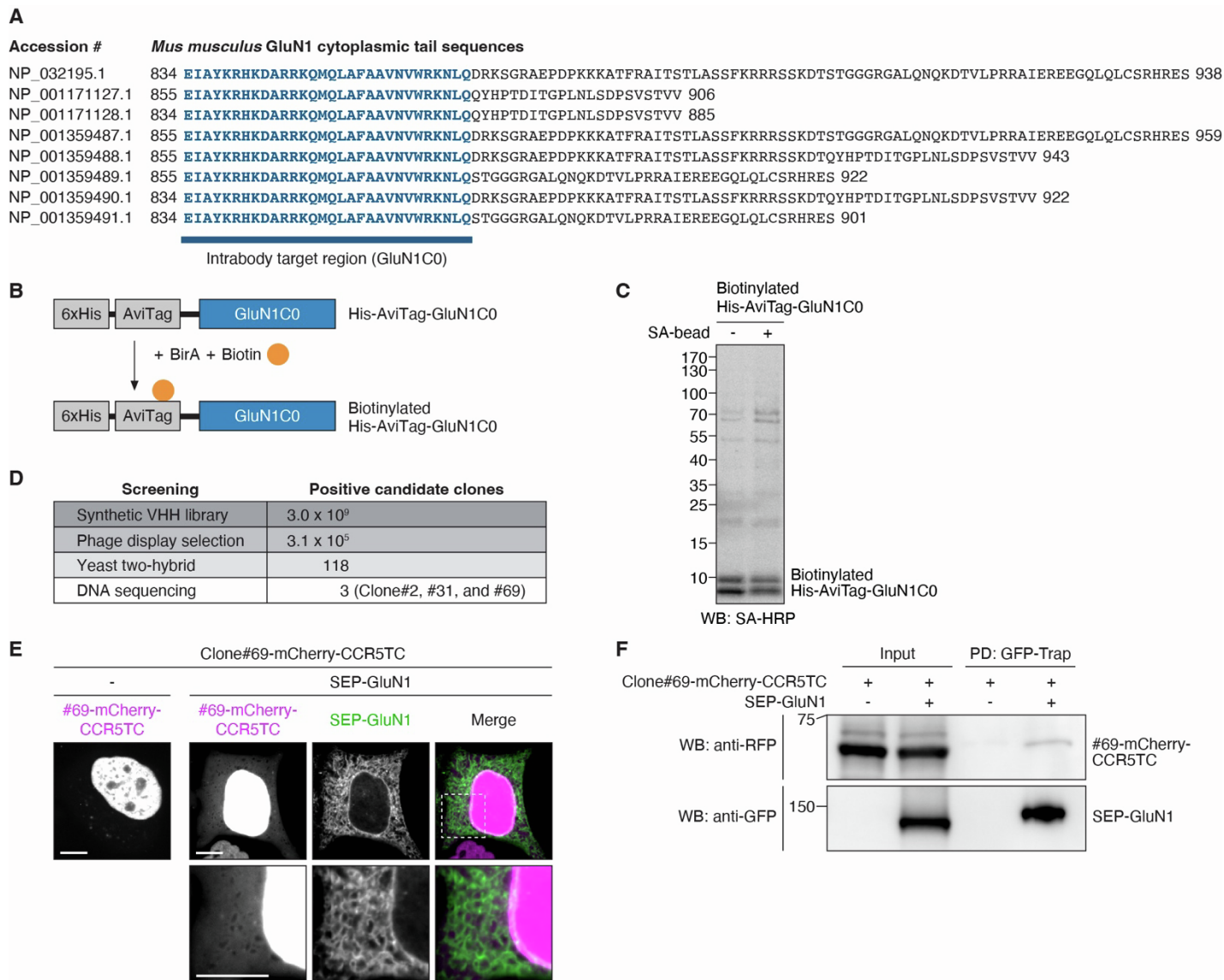
## **Supplemental information**

**NMDA receptor-targeted**

**enrichment of CaMKII $\alpha$**

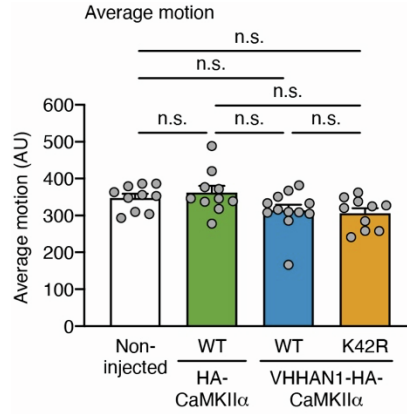
**improves fear memory**

**Anthony Chifor, Jeongyoon Choi, and Joongkyu Park**



**Figure S1. Screening and validation of anti-GluN1 intrabody candidates, Related to Figure 1 (A)**

Comparison of eight splice variants of mouse GluN1 cytoplasmic tail sequences. The conserved region (834–863 amino acids of NP\_032195.1; C0 cassette) was chosen as the intrabody target (indicated in blue). (B and C) The bacterially purified His-AviTag-tagged GluN1C0 fragment (~11 kDa) was biotinylated by a BirA enzyme *in vitro* (B) and immobilized on streptavidin beads (SA-bead) (C). *In vitro* biotinylation of His-AviTag-GluN1C0 fragment (C, left lane) and immobilization on SA-beads (C, right lane) were confirmed by Western blot using streptavidin-horseradish peroxidase conjugates (SA-HRP). (D) Summary of positive clone numbers of anti-GluN1 intrabody candidates after each screening. (E and F) Colocalization and interaction tests of Clone#69 with GluN1 in heterologous cells. Single transfection of DNA constructs encoding mCherry- and CCR5TC-fused Clone#69 in HeLa cells showed its nuclear localization due to the CCR5TC (E, left panel). Co-expression of SEP-GluN1 (green) showed minimal colocalization of Clone#69 (magenta; E, right panel). GFP-Trap pull-down (PD) showed that Clone#69-mCherry-CCR5TC can bind to SEP-GluN1 in HeLa cells (F). However, Clone#69 was not selected as the anti-GluN1 intrabody because it did not show robust colocalization with SEP-GluN1 (E). Scale bars, 10  $\mu$ m.



**Figure S2. General locomotor activity, Related to Figure 4** There were no significant differences in average motion (AU, arbitrary unit) measured during the habituation session. Data are shown as mean  $\pm$  SEM;  $n = 10-12$  mice per group; n.s., not significant ( $P = 0.4759$  for non-injected vs. VHHAN1-HA-CaMKII $\alpha$  [wild-type, WT];  $P = 0.2069$  for non-injected vs. VHHAN1-HA-CaMKII $\alpha$  [K42R];  $P = 0.4028$  for HA-CaMKII $\alpha$  vs. VHHAN1-HA-CaMKII $\alpha$  [WT];  $P = 0.1723$  for HA-CaMKII $\alpha$  vs. VHHAN1-HA-CaMKII $\alpha$  [K42R];  $P > 0.9999$  for the rest); Kruskal-Wallis tests with multiple comparisons.

**Table S1.** Synthetic DNAs used in this work, Related to STAR Methods.

<p><b>AviTag-GluN1(834-863):</b> 5'-GCACAC<b>ATATGGGT</b>CTGAACGACATCTTCGAGGCTCAGAA AATCGAATGGCACGAAGGTGGAAGTGGATCTGGTGAGATCGCCTACAAGCGACACAAGGATGCC CGTAGGAAGCAGATGCAGCTGGCTTTTGCAGCCGTGAACGTGTGGAGGAAGAACCTGCAGTGAC <b>TCGAGT</b>TGTGC-3'</p> <p>(NdeI and XhoI are in bold)</p>
<p><b>mCherry-IRES-Cre:</b> 5'-CGCG<b>GATCC</b>GTGAGCAAGGGCGAGGAGGATAACATGGCCATC ATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGA TCGAGGGCGAGGGCGAGGGCCGCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCA AGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCTCAGTTCATGTACGGCTCCAAGGC CTACGTGAAGCACCCCGCGACATCCCCGACTACTTGAAGCTGTCTTCCCGAGGGGTTCAAGT GGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGC AGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCTCCGACGGCCCCGT AATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGC CCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGT CAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCAAG TTGGACATCACCTCCACAACGAGGACTACCCATCGTGGAACAGTACGAACGCGCCGAGGGCC GCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTAAGGCGCGCCCCCCTAACGTTACTGG CCGAAGCCGCTTGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCGT TTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTCTAGGGGTCTTT CCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCT TCTTGAAGACAAACAACGTCTGTAGCGACCCTTTCAGGCAGCGGAACCCCCACCTGGCGACA GGTGCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCAGTG CCACGTTGTGAGTTGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGG GGCTGAAGGATGCCAGAAGGTACCCATTGTATGGGATCTGATCTGGGGCTCGGTGCACATG CTTTACATGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTT TCTTTGAAAAACACGATGATAATATGGCCACAACCATGCCAAGAAGAAGAGGAAGGTGTCCAA TCTCCTGACTGTTCAACCAGAACCTCCCTGCGCTGCCAGTAGATGCCACTAGCGATGAGGTCAGGA AAAATCTCATGGATATGTTTAGGGATAGACAGGCGTTTTCTGAACACACCTGAAAATGCTGCTTA GCGTGTGCCGATCCTGGGCAGCCTGGTGTAAAGCTGAACAATCGAAATGGTTCCCCGCCGAGCC GGAGGACGTGCGGATTACCTGCTGTATCTCCAGGCAAGAGGGCTGGCTGTCAAGACTATCCAG CAGCACTTGGGCCAACTGAATATGCTGCATCGACGCAGCGGGCTCCCCGGCCTAGCGATTCAA ACGCAGTCTCCCTTGTATGAGGAGAATTAGAAAGGAAAACGTAGATGCGGGTGAGAGGGCTAAG CAGGCTCTCGCTTTTGTAGCGGACTGATTTGACCCAGGTCAGATCCCTGATGGAGAACAGCGATCG GTGCCAGGACATCAGGAACCTCGCATTCTGGGAATTGCATATAACACACTTCTGCGCATAGCTG AGATCGCCCGGATCAGAGTGAAAGACATCAGTCCAACGGACGGCGGCCGGATGCTTATTCATATT GGACGCACAAAGACATTGGTCAACCCGCTGGCGTTGAAAAGGCCTTGTCCCTGGGCGTAACGA AGCTGGTGAAAAGATGGATCTCAGTGTCCGGCGTGGCTGACGACCCTAATAATTACTTGTCTGT CGAGTGAGAAAAAACGGAGTCGCCGCGCCCTCTGCCACCAGCCAATTGAGTACACGGGCCCTTG AAGGGATCTTTGAGGCAACCCACCGACTCATATACGGAGCCAAGGATGACAGTGGCCAGAGGTA TCTCGCCTGGTCAAGTCACTTCTGCTAGGGTGGGGGCCGACGAGACATGGCGCGGGCAGGAGT CTCCATAACAGAGATTATGCAAGCTGGAGGTTGGACAAATGTGAACATCGTTATGAACTATATCCG CAATCTTACTCTGAAACCGGGGCCATGGTGAGACTGCTCGAAGATGGTACT<b>GAATTC</b>CGG-3'</p> <p>(BamHI and EcoRI are in bold)</p>
<p><b>HA-CaMKII<math>\alpha</math> (wild-type):</b> 5'- GCATAG<b>GATCC</b>GGTGGTACCCTTATGATGTGCCTGATTATGCTG GCGGCGGTAGCATGGCTACCATCACCTGCACCCGATTCACGGAAGAGTACCAGCTCTTCGAGGA ACTGGGAAAGGGAGCCTTCTCCGTGGTGCGCAGGTGTGTGAAGGTGCTGGCTGGCCAGGAGTAT GCTGCCAAGATTATCAACACCAAGAAGCTCTCAGCCAGAGATCACCAGAAGTTGGAACGCGAGGC CCGATCTGCCGCTTGTGAAGCACCCCAATATCGTCCGACTCCATGACAGCATCTCCGAGGAGG GGCACCACCTTATCTTCGATCTGGTCACTGGTGGGGAGCTGTTTGAAGACATTGTGGCCCGG GAGTATTACAGTGAGGCTGATGCCAGCCACTGTATCCAGCAGATCCTGGAGGCTGTGCTACACTG</p>

TCACCAGATGGGGGTGGTGCATCGCGACCTGAAGCCTGAGAATCTGTTGCTGGCTTCGAAGCTC  
AAGGGTGTGCGGTGAAGCTGGCAGACTTTGGCCTGGCCATAGAGGTTGAGGGAGAGCAGCAG  
GCATGGTTTGGTTTCGCAGGGACACCTGGATACCTCTCCCCAGAAGTGCTGCGGAAGGACCCAT  
ACGGGAAGCCTGTGGACCTGTGGGCCTGTGGCGTCATCCTGTATATCTTGCTGGTTGGGTATCCC  
CCATTCTGGGATGAGGACCAGCACCGCCTGTACCAGCAGATCAAAGCTGGTGCCTACGATTTCCC  
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TGTGGCCTCCTGCATGCACAGACAGGAGACCGTGGACTGCCTGAAGAAGTTCAATGCCAGGAGG  
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GAGGAAACAAGAAGAATGATGGCGTGAAGGAATCCTCTGAGAGCACCAACACCACCATCGAGGA  
TGAAGACACCAAAGTGCGCAAACAGGAAATTATCAAAGTGACAGAGCAGCTGATCGAAGCCATAA  
GCAATGGAGACTTTGAATCCTACACGAAGATGTGCGACCCTGGAATGACAGCCTTTGAACCGGAG  
GCCCTGGGGAACCTGGTCGAGGGCCTGGACTTTTCATCGATTCTATTTTGA AACCTGTGGTCCCG  
GAACAGCAAGCCCGTGCACACCACCATCCTGAACCCTCACATCCACCTGATGGGTGACGAGTCA  
GCCTGCATCGCCTACATCCGCATCACTCAGTACCTGGATGCGGGTGGCATCCCCCGCACGGCCC  
AGTCAGAGGAGACCCGTGTCTGGCACCGCAGGGATGGAAAATGGCAGATCGTCCACTTCCACAG  
ATCTGGGGCGCCCTCCGTCTGCCCCATTGAG**GGCGCGCC**TAGTC-3'

(BamHI and Ascl are in bold)

**HA-CaMKII $\alpha$  (K42R):** 5'-

GCATAG**GGATCC**GGTGGTGGCTACCCTTATGATGTGCCTGATTATGCTGGCGGGCGGTAGCATGGC  
TACCATCACCTGCACCCGATTCACGGAAGAGTACCAGCTCTTCGAGGAACTGGGAAAGGGAGCCT  
TCTCCGTGGTGCAGGTGTGTGAAGGTGCTGGCTGGCCAGGAGTATGCTGCCCGGATTATCAA  
CACCAAGAAGCTCTCAGCCAGAGATCACCAGAAGTTGGAACGCGAGGCCCGCATCTGCCGCTTG  
TTGAAGCACCCCAATATCGTCCGACTCCATGACAGCATCTCCGAGGAGGGGGCACCCTACCTTAT  
CTTCGATCTGGTCACTGGTGGGGAGCTGTTCAAGACATTGTGGCCCCGGGAGTATTACAGTGAG  
GCTGATGCCAGCCACTGTATCCAGCAGATCCTGGAGGCTGTGCTACACTGTCACCAGATGGGGG  
TGGTGCATCGCGACCTGAAGCCTGAGAATCTGTTGCTGGCTTCAAGCTCAAGGGTGTGCGGT  
GAAGCTGGCAGACTTTGGCCTGGCCATAGAGGTTGAGGGAGAGCAGCAGGCATGGTTTGGGTTT  
GCAGGGACACCTGGATACCTCTCCCCAGAAGTGCTGCGGAAGGACCCATACGGGAAGCCTGTGG  
ACCTGTGGGCCTGTGGCGTCATCCTGTATATCTTGCTGGTTGGGTATCCCCATTCTGGGATGAG  
GACCAGCACCGCCTGTACCAGCAGATCAAAGCTGGTGCCTACGATTTCCCATCACCAGAATGGGA  
CACCGTCACCCCGGAAGCCAAGGATCTGATCAATAAGATGCTGACCATCAACCCGTCCAAACGCA  
TCACGGCCGCTGAGGCTCTCAAGCACCCCTGGATCTCGCACCGCTCCACTGTGGCCTCCTGCAT  
GCACAGACAGGAGACCGTGGACTGCCTGAAGAAGTTCAATGCCAGGAGGAAACTGAAGGGAGCC  
ATCCTCACCCTATGCTGGCCACCAGGAACCTTCTCCGGAGGGAAGAGTGGAGGAAACAAGAAGA  
ATGATGGCGTGAAGGAATCCTCTGAGAGCACCAACACCACCATCGAGGATGAAGACACCAAAGTG  
CGAAACAGGAAATTATCAAAGTGACAGAGCAGCTGATCGAAGCCATAAGCAATGGAGACTTTGA  
ATCCTACACGAAGATGTGCGACCCTGGAATGACAGCCTTTGAACCGGAGGCCCTGGGGAACCTG  
GTCGAGGGCCTGGACTTTTCATCGATTCTATTTTGA AACCTGTGGTCCCGGAACAGCAAGCCCGT  
GCACACCACCATCCTGAACCCTCACATCCACCTGATGGGTGACGAGTCAGCCTGCATCGCCTACA  
TCCGCATCACTCAGTACCTGGATGCGGGTGGCATCCCCCGCACGGCCAGTCAGAGGAGACCCG  
TGTCTGGCACCGCAGGGATGGAAAATGGCAGATCGTCCACTTCCACAGATCTGGGGCGCCCTCC  
GTCCTGCCCCATTGAG**GGCGCGCC**TAGTC-3'

(BamHI and Ascl are in bold; CGG encoding R42 is underlined)

**Kozak consensus sequence:** 5'-CTAGCGGTACCGCCACCATGGGATC-3'

(Kozak is in bold; overhangs are underlined)

**Table S2.** Primers used for plasmid construction, Related to STAR Methods.

<b>Construct</b>	<b>Primer</b>	
pCAG-Clone#2/#31/#69-mCherry-CCR5TC	Fwd#1	5'-GCACAG <b>GTACCGCC</b> ACCATGGCGGAAGT-3' (KpnI in bold)
	Rev#1	5'-GCCAGCTCCCCTAGACTTGTACAGCTCGTCC-3'
	Fwd#2	5'-GGACGAGCTGTACAAGTCTAGGGGAGCTGGC-3'
	Rev#2	5'-GCACA <b>ACGCGT</b> TTAAGCCATAGAAGCAAGATTAG AATAATTTTCAAGC-3' (MluI in bold)
pENTR-VHHAN1-EGFP-CCR5TC	Fwd	5'-GCACA <b>AAGCTT</b> GCCACCATGGCGGAAGTGCAGCTG-3' (HindIII in bold)
	Rev	5'-GCACAG <b>GATCCG</b> CTACTCACAGTTACCTGCGTC-3' (BamHI in bold)