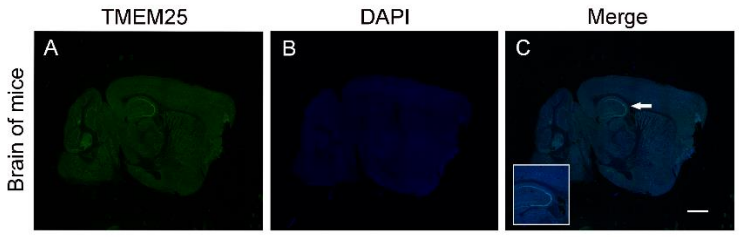
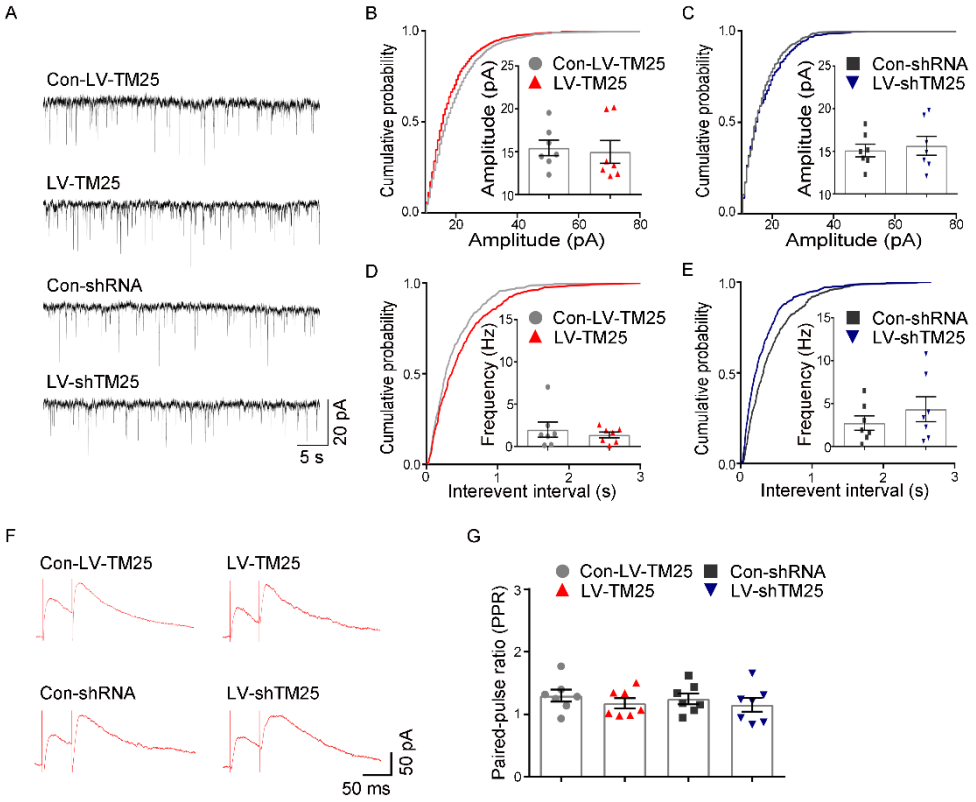
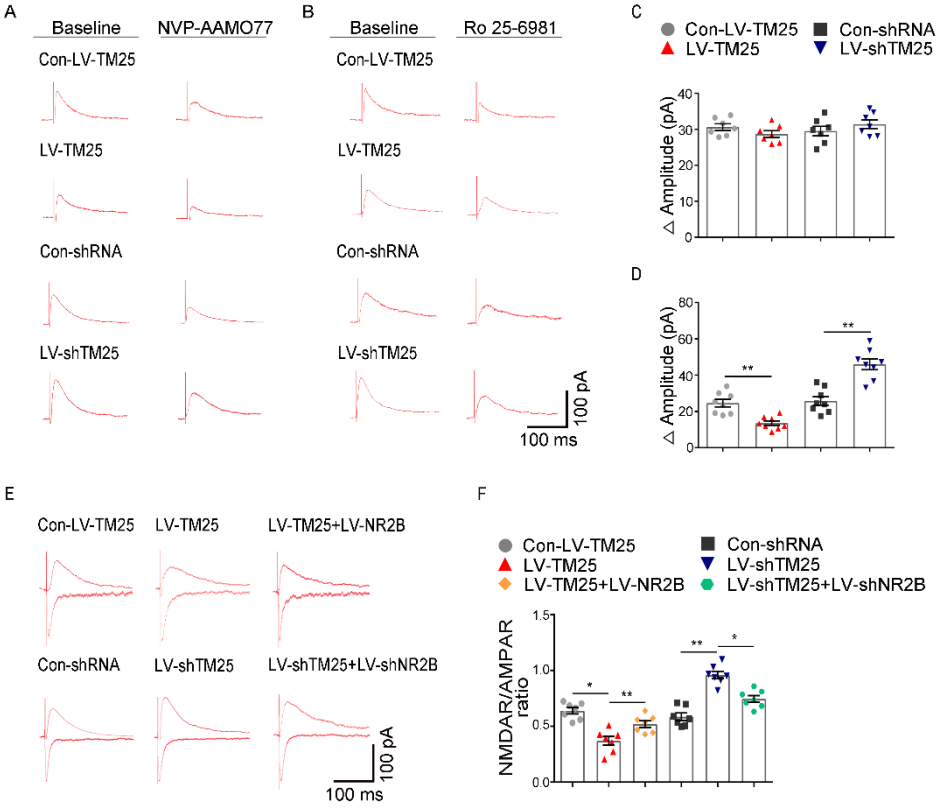


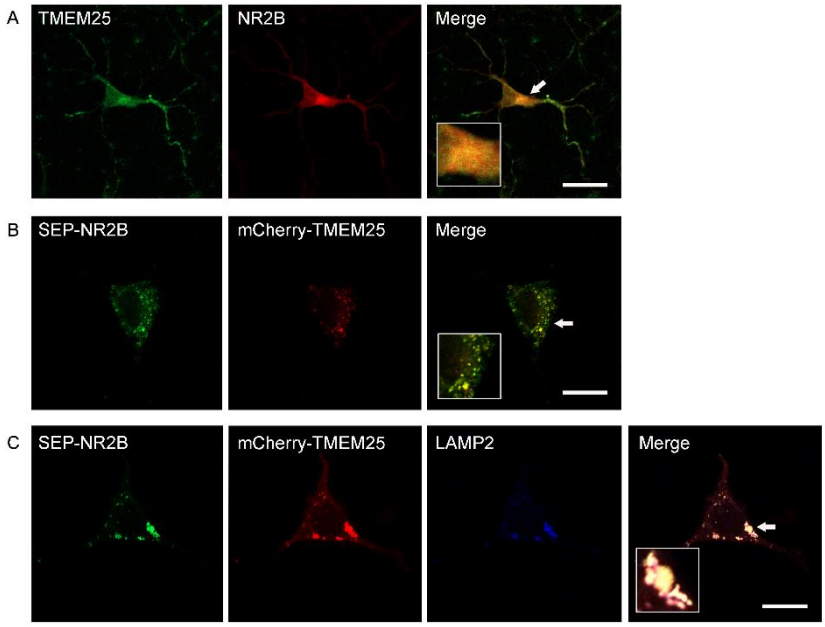
Supplemental Figure 1. Distribution of TMEM25 throughout the brain of normal mice. (A-C) TMEM25 localization in the brains of adult C57BL/6 mice. Arrow, TMEM25 expression in hippocampal regions. Scale bar: 1 mm.



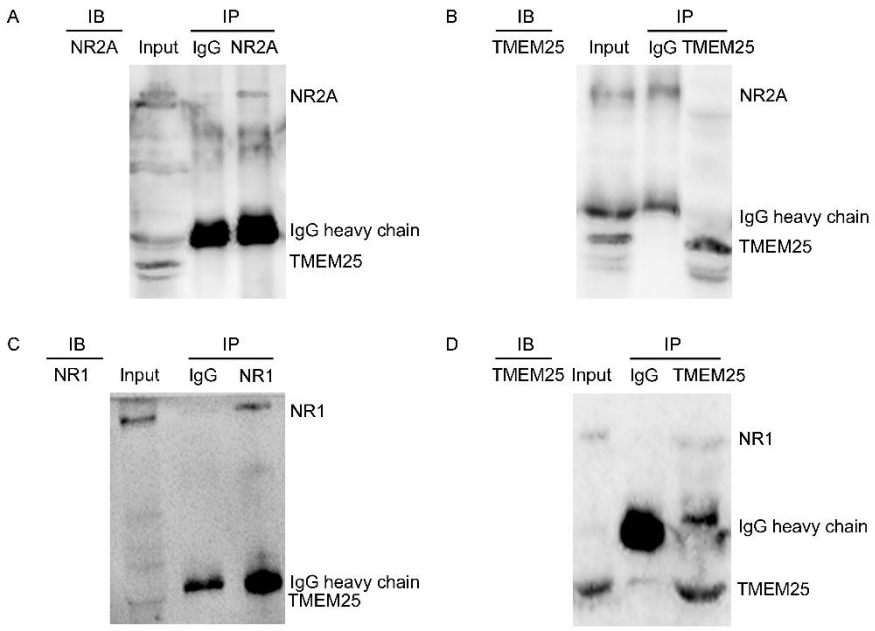
Supplemental Figure 2. TMEM25 has no effect on mIPSCs or PPRs in the hippocampal CA1 region. (A-E) Representative traces of mIPSCs recorded from the examined groups and summary of the frequency and amplitude of mIPSCs. **(F** and **G)** Representative traces of PPRs for NMDAR-mediated EPSCs and summary of PPRs among the groups. Data are presented as means ± SEM, *n* = 7 per group. Student's *t*-test **(B**, **C** and **E)**, or Mann-Whitney *U*-test **(D)**, or one-way ANOVA followed by LSD-*t* test **(G)**.



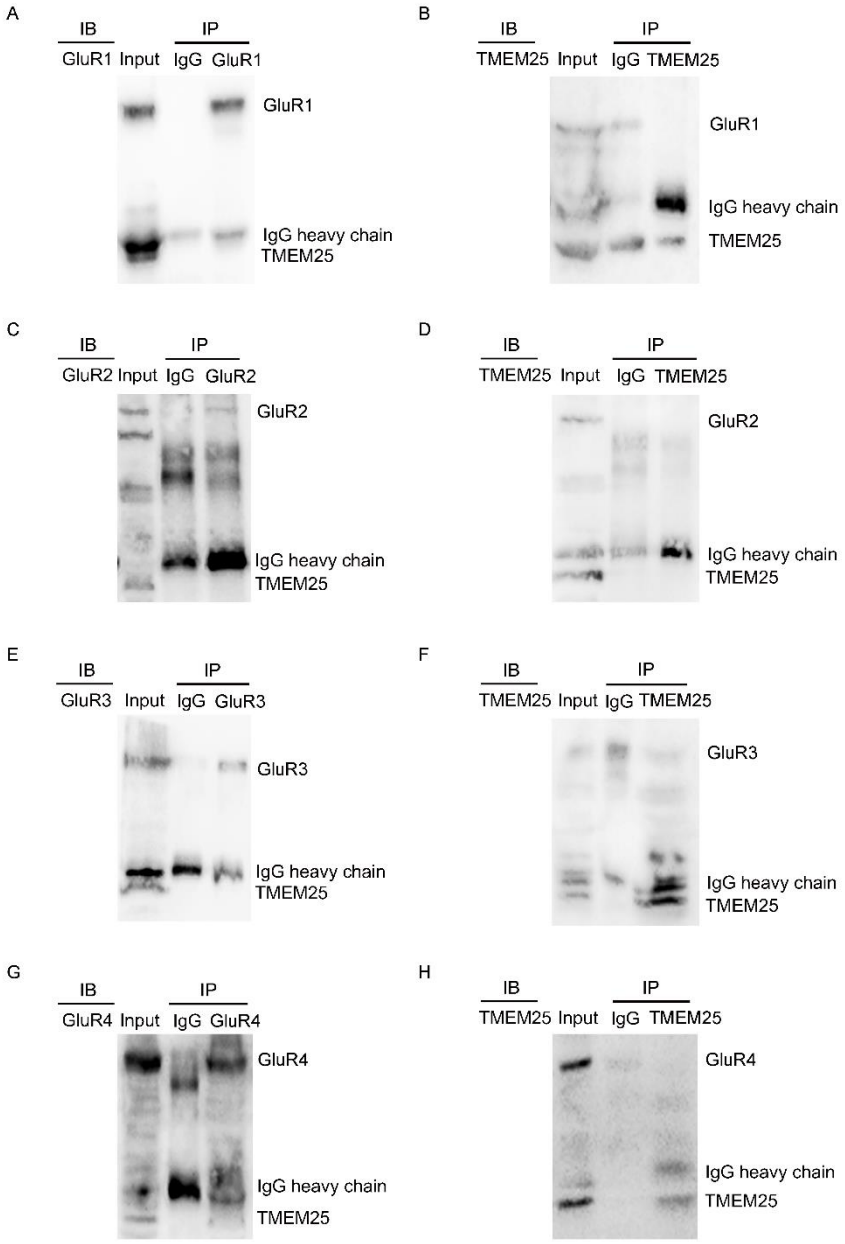
Supplemental Figure 3. Effect of TMEM25 on NMDAR subunit currents in CA1 pyramidal neurons. (A-D) Representative traces of NMDAR-mediated EPSCs on the same neuron recorded from different groups before and after 10 minutes of application of NVP-AAM077 (50 nM) or Ro 25-6981 (0.5 μM) and summary of the EPSC Δ amplitude. **(E** and **F)** Representative traces of NMDAR- and AMPAR-mediated EPSCs and summary of the NMDAR/AMPAR ratio in each group. Data are presented as means ± SEM, n = 7 per group. *P < 0.05; **P < 0.01, by one-way ANOVA followed by LSD-t test.



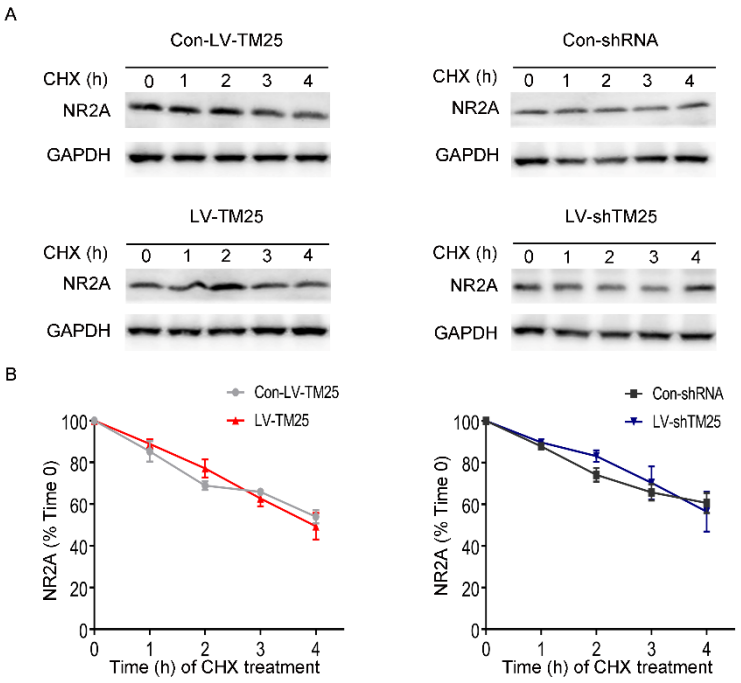
Supplemental Figure 4. TMEM25 colocalizes with NR2B in late endosomes/lysosomes. (A) Neurons were stained with anti-TMEM25 and anti-NR2B antibodies on DIV 14. Arrow, cells positive for TMEM25 and NR2B. **(B)** HEK293 cells were transfected with mCherry-TMEM25 and SEP-NR2B and stained with anti-GFP antibody. Arrow, cells positive for exogenous TMEM25 and NR2B. **(C)** HEK293 cells were transfected with mCherry-TMEM25 and SEP-NR2B and stained with anti-LAMP2 and anti-GFP antibodies. Arrow, cells positive for exogenous TMEM25 and NR2B in LAMP2-positive late endosome/lysosome compartments. Scale bars: 10 μm.



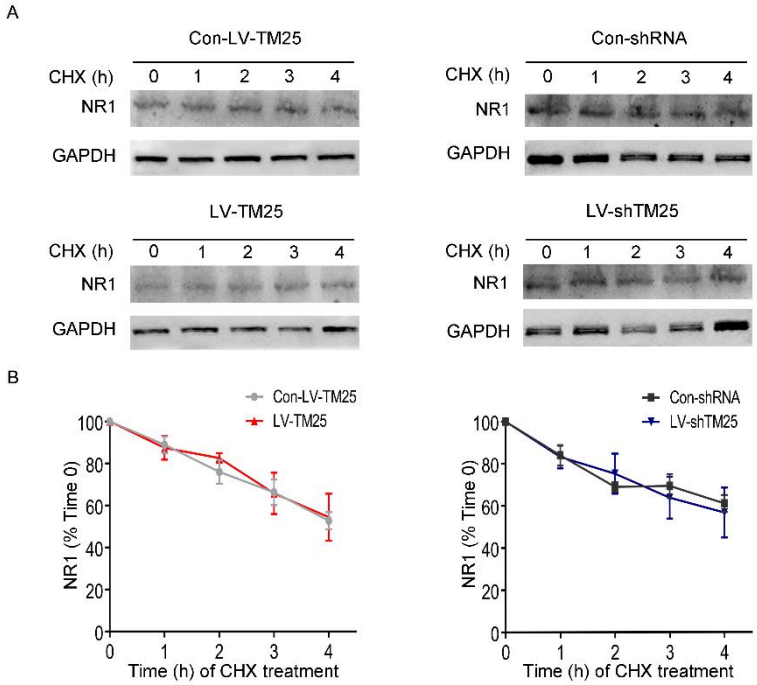
Supplemental Figure 5. Coimmunoprecipitation of TMEM25 with NMDAR NR2A subunit and NR1 subunit. (A and **B)** No interaction was observed between TMEM25 and NR2A. **(C** and **D)** A weak interaction was observed between TMEM25 and NR1.



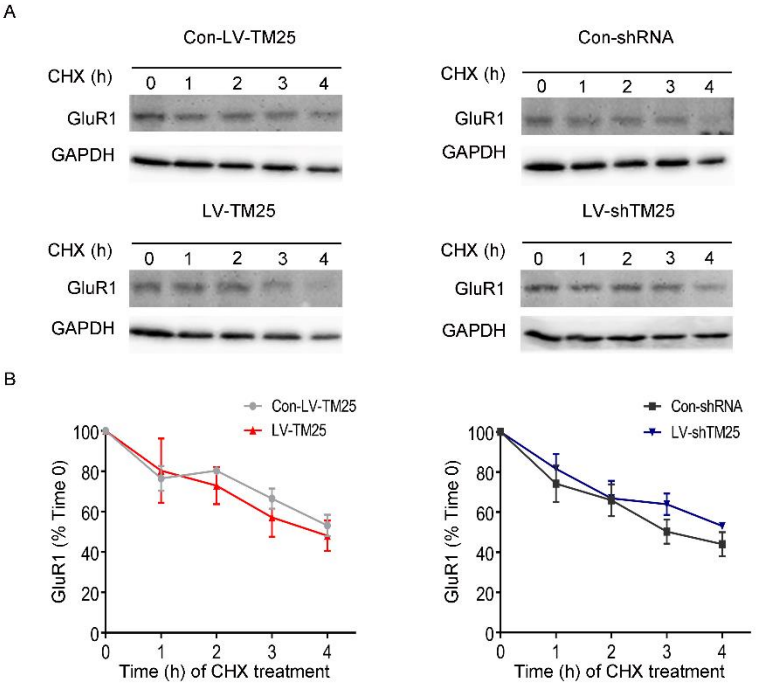
Supplemental Figure 6. Coimmunoprecipitation of TMEM25 with AMPAR GluR1-4 subunits. (A and **B)** No interaction was observed between TMEM25 and GluR1. **(C** and **D)** No interaction was observed between TMEM25 and GluR2. **(E** and **F)** No interaction was observed between TMEM25 and GluR3. **(G** and **H)** No interaction was observed between TMEM25 and GluR4.



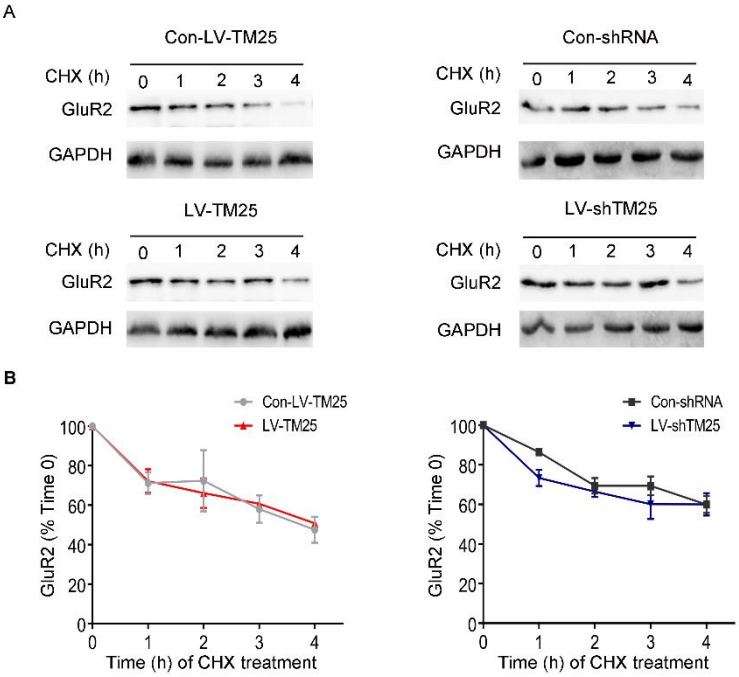
Supplemental Figure 7. Effect of TMEM25 on the rate of NMDAR NR2A subunit degradation. (A and **B)** Representative images of the expression of total NR2A in hippocampal neurons transfected with lentiviral vector and cultured with CHX and quantification of immunoblots. Data are presented as means ± SEM and are representative of at least 3 independent repeats, *n* = 3 per group, by two-way ANOVA followed by post-hoc test.



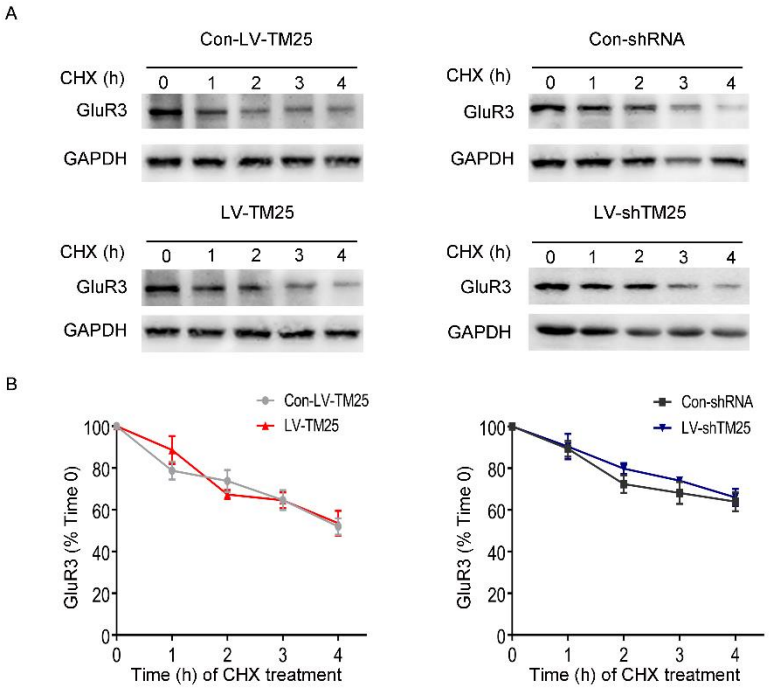
Supplemental Figure 8. Effect of TMEM25 on the rate of NMDAR NR1 subunit degradation. (A and **B)** Representative images of the expression of total NR1 in hippocampal neurons transfected with lentiviral vector and cultured with CHX and quantification of immunoblots. Data are presented as means ± SEM and are representative of at least 3 independent repeats, *n* = 3 per group, by two-way ANOVA followed by post-hoc test.



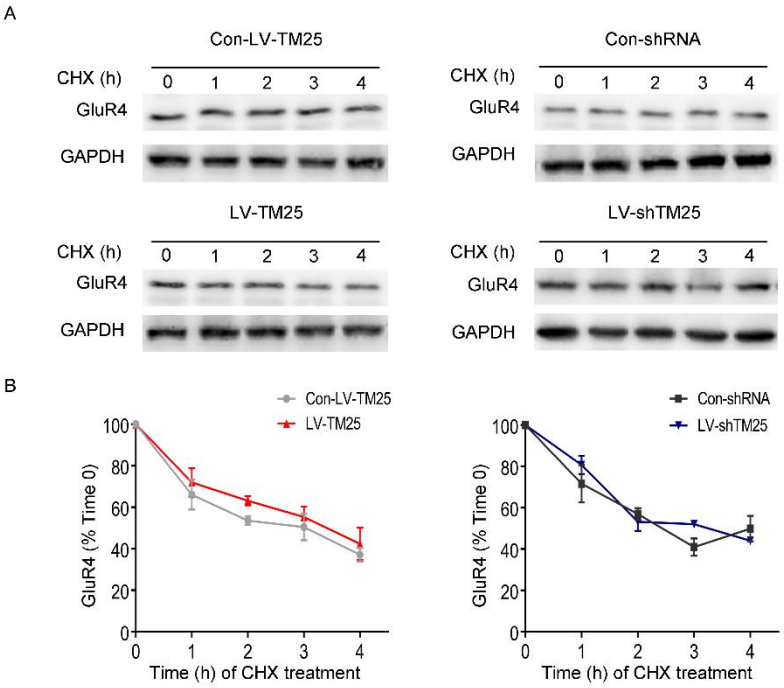
Supplemental Figure 9. Effect of TMEM25 on the rate of AMPAR GluR1 subunit degradation. (A and **B)** Representative images of the expression of total GluR1 in hippocampal neurons transfected with lentiviral vector and cultured with CHX and quantification of immunoblots. Data are presented as means ± SEM and are representative of at least 3 independent repeats, *n* = 3 per group, by two-way ANOVA followed by post-hoc test.



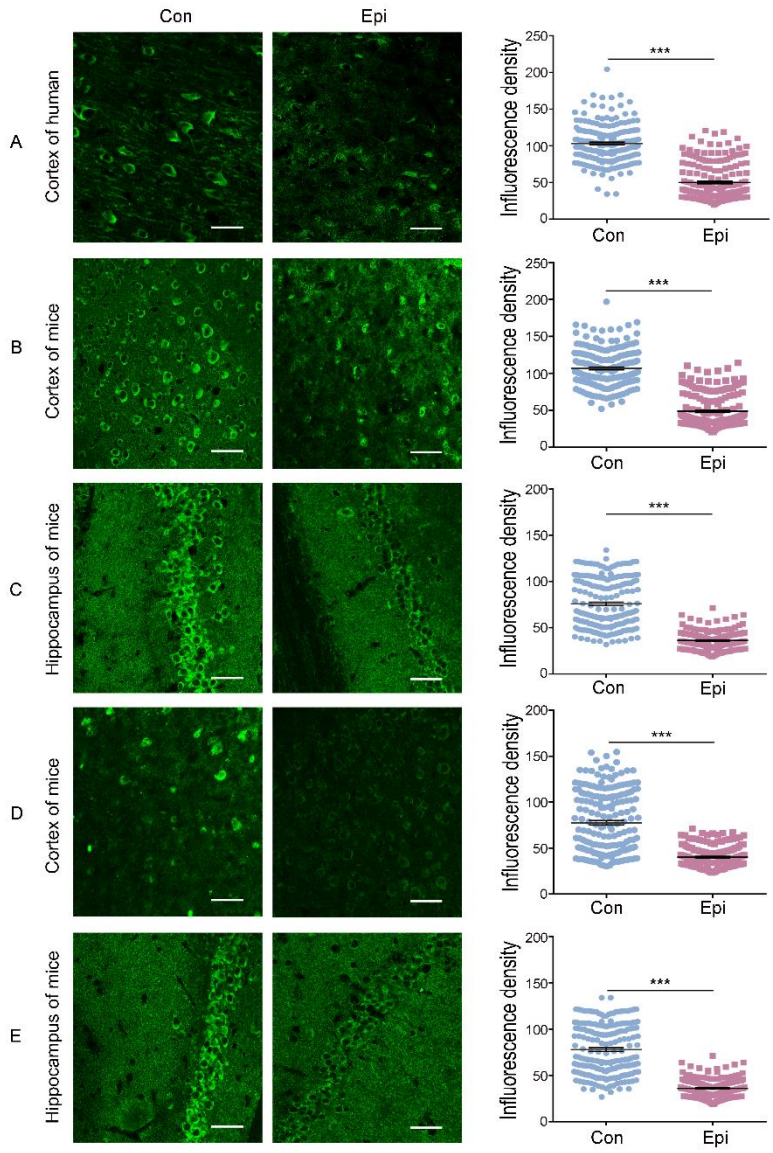
Supplemental Figure 10. Effect of TMEM25 on the rate of AMPAR GluR2 subunit degradation. (A and **B)** Representative images of the expression of total GluR2 in hippocampal neurons transfected with lentiviral vector and cultured with CHX and quantification of immunoblots. Data are presented as means ± SEM and are representative of at least 3 independent repeats, *n* = 3 per group, by two-way ANOVA followed by post-hoc test.



Supplemental Figure 11. Effect of TMEM25 on the rate of AMPAR GluR3 subunit degradation. (A and **B)** Representative images of the expression of total GluR3 in hippocampal neurons transfected with lentiviral vector and cultured with CHX and quantification of immunoblots. Data are presented as means ± SEM and are representative of at least 3 independent repeats, *n* = 3 per group, by two-way ANOVA followed by post-hoc test.

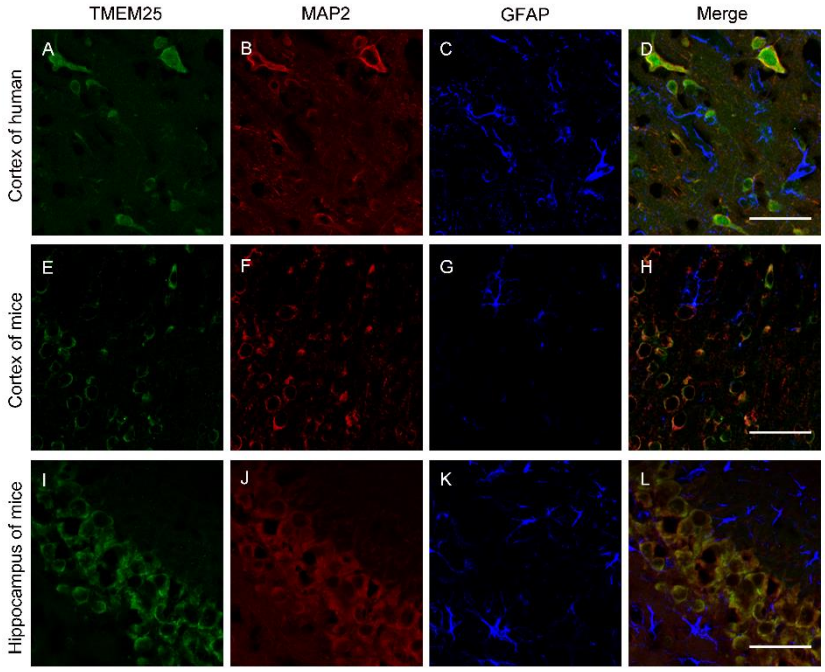


Supplemental Figure 12. Effect of TMEM25 on the rate of AMPAR GluR4 subunit degradation. (A and **B)** Representative images of the expression of total GluR4 in hippocampal neurons transfected with lentiviral vector and cultured with CHX and quantification of immunoblots. Data are presented as means ± SEM and are representative of at least 3 independent repeats, *n* = 3 per group, by two-way ANOVA followed by post-hoc test.

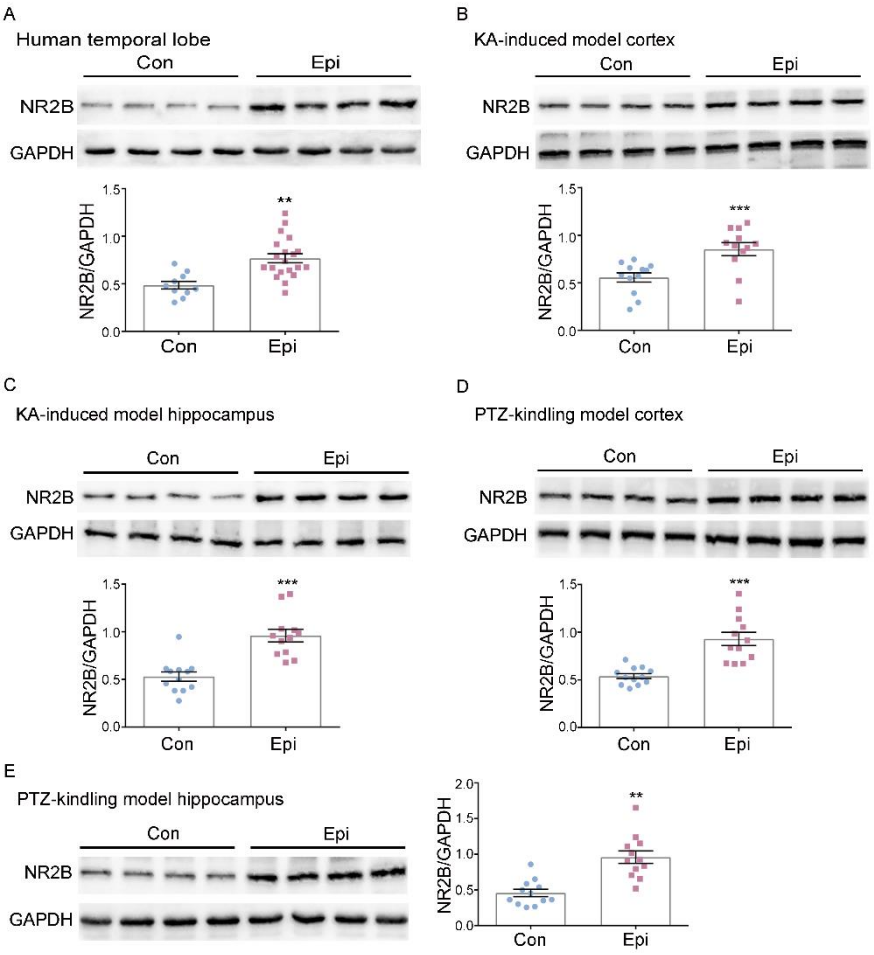


Supplemental Figure 13. Quantification of immunofluorescence intensity for TMEM25 in epileptic and nonepileptic brain tissues. (A) Representative images of immunofluorescence staining for TMEM25 in the brain tissues of patients with TLE or nonepileptic patients and quantification of fluorescence intensity. **(B-E)** Representative images of immunofluorescence staining for TMEM25 in the cortex and hippocampus from chronic epileptic mice or normal mice and quantification of fluorescence intensity. Scale bars: 50 μm. Data are presented as means ± SEM and are

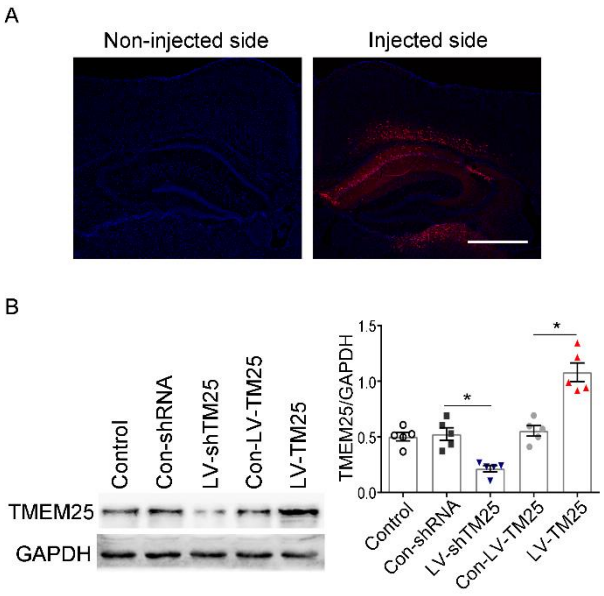
representative of at least 3 independent repeats, n = 200 sites per group. ***$P < 0.001$, by Mann-Whitney U-test.



Supplemental Figure 14. Localization of TMEM25 in epileptic brain tissues. (A-D) In the temporal cortical tissue from TLE patients, TMEM25 colocalized with MAP2. **(E-L)** In the cortex and hippocampus from a KA-induced epileptic mouse model, TMEM25 colocalized with MAP2. Scale bars: 50 μm.



Supplemental Figure 15. Expression of NR2B in the brain tissues of epileptic patients and mice. (A) Representative images of the expression of NR2B in brain tissues from pharmacoresistant TLE patients (n = 20) and control individuals with head trauma (n = 10) and quantification of immunoblots. **(B-E)** Representative images of the expression of NR2B in the cortex and hippocampus from chronic epileptic mice (n = 12) and normal mice (n = 12) and quantification of immunoblots. Data are presented as means ± SEM and are representative of at least 3 independent repeats. $^{**}P < 0.01$; $^{***}P < 0.001$, by independent Student's t-test **(A, B, C** and **E)** or Mann-Whitney U-test **(D)**.



Supplemental Figure 16. Expression of mCherry and TMEM25 after the injection of lentiviral vectors. (A) Representative images showing mCherry fluorescence in the hippocampus of mice at the end of behavioral observations. Bar: 500 μm. **(B)** Western blot analysis of TMEM25 levels in the hippocampus of mice injected with or without lentiviral vectors at the end of behavioral observations. Data are presented as means ± SEM and are representative of at least 3 independent repeats, $n = 5$ per group. $^*P < 0.05$, by one-way ANOVA followed by LSD-*t* test.

Supplemental Table 1. Primer sequences used for quantitative real-time PCR.

Gene	Forward and reverse primers	Amplified fragment (bp)
Grin2b	F: 5'-CATCGGCATCGCTGTCATC-3' R: 5'-CTACTGTGGCTGGTTCTTCG-3'	219
Gapdh	F: 5'-TGTTGCCATCAATGACCCCTT-3' R: 5'-GCGACTCATGCAGCACCTC-3'	202

Supplemental Table 2. The clinical characterization of TLE patients and controls.

Cases	Sex	Age (years)	Duration (years)	Preoperative AED consumption	Side of resected temporal lobe	Pathological diagnosis
E1	F	26	7	CBZ, VPA, TPM, OXC	L	G
E2	M	22	5	CBZ, TPM, CZP	L	NL, G
E3	F	25	10	CBZ, PB, LTG, LEV	R	NL, G
E4	M	24	8	VPA, CBZ, TPM, PB	L	NL, G
E5	M	18	7	PHT, PB, CBZ, VPA	L	G
E6	F	28	13	CBZ, PHT, VPA, PB	R	G
E7	F	20	4	CBZ, VPA, LTG	R	NL
E8	M	29	14	CBZ, PB, LTG, LEV	R	NL, G
E9	F	22	9	CBZ, TPM, CZP	L	NL, G
E10	F	35	10	CBZ, VPA, TPM, OXC	L	G
E11	M	31	15	PB, CBZ, TPM, LTG	L	G
E12	M	30	18	VPA, PB, CBZ, LEV	R	NL, G
E13	M	27	13	VPA, CBZ, TPM, LTG	R	NL, G
E14	F	36	26	PHT, PB, CBZ, VPA	R	NL, G
E15	F	28	18	VPA, CBZ, TPM, PB	L	G
E16	M	36	27	VPA, CBZ, CZP	L	NL, G
E17	M	25	15	VPA, PHT, CBZ	L	NL, G
E18	F	16	14	CBZ, TPM, CZP	R	NL, G
E19	F	38	18	VPA, PB, CBZ, LTG	L	G
E20	F	36	19	VPA, TPM, GBP	R	NL, G
C1	F	30	0	None	L	N
C2	F	31	0	None	L	N
C3	M	34	0	None	L	N
C4	M	26	0	None	R	N
C5	F	22	0	None	L	N
C6	M	29	0	None	R	N
C7	F	27	0	None	L	N
C8	M	30	0	None	R	N
C9	M	36	0	None	R	N
C10	F	34	0	None	L	N

AEDs, antiepileptic drugs; E, epilepsy; C, control; F, female; M, male; CBZ, carbamazepine; CZP, clonazepam; GBP, gabapentin; LEV, levetiracetam; LTG, lamotrigine; OXC, oxcarbazepine; PB, phenobarbital; PHT, phenytoin; TPM, topiramate; VPA, valproate; L, left; R, right; NL, neuron loss; G, gliosis; N, normal.