Supporting Information

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SI Materials and Methods

Generation of *LRRTM1, 2* floxed mouse line. Targeting vectors for generation of *LRRTM1*$^{flox/flox}$ and *LRRTM2*$^{flox/flox}$ mouse lines were generated by the recombineering method (1) from 129S7 bMQ BAC clones, specifically bMQ315C13 (*LRRTM1*) and bMQ453P2 (*LRRTM2*) obtained from the Sanger Institute (2). The targeting vectors contained two loxP sites flanking exon 2 followed by a Neomycin resistance gene (Neo) cassette flanked by Frt sites and a herpes simplex virus thymidine kinase expression cassette for negative selection. Targeting vector linearized by NotI was transfected to 129/Ola embryonic stem (ES) cells for homologous recombination (3, 4). Positive selection of ES cells was in the presence of G418 and negative selection against random integration by Gancyclovir. Homologous recombination was verified by Southern blot analysis and PCR. Positive ES cells were further expanded and a single Neo insertion was verified by Southern blotting against a probe for Neo. Positive clones were injected into C57BL/6J blastocysts which were implanted into surrogate female mice. Founder chimeras were backcrossed 9-10 times with C57BL/6J mice. The conditional *LRRTM1*$^{flox/+}$ and *LRRTM2*$^{flox/+}$ line were crossed with a FLP1-expressing deleter line [B6;SJL-Tg(ACTFLPe) 9205Dym/J ; Jackson Laboratory] to remove the Neo cassette. *LRRTM1*$^{flox/+}$ and *LRRTM2*$^{flox/+}$ with deleted Neo and with floxed exon 2 for the respective genes were crossed with each other to generate the *LRRTM1*$^{flox/flox}$ *LRRTM2*$^{flox/flox}$ line (*LRRTM1, 2* floxed). Both wild type and *LRRTM1, 2* floxed mice were maintained on the C57BL/6J background (Jackson Laboratory, Strain 000664). Mice were maintained in groups of 5 or less with 12 hour on/off light cycles. All experiments were performed in accordance with US National Institutes of Health guidelines and approved by the Stanford University Administrative Panel on Laboratory Animal Care.

Plasmid Construction. The lentiviral vector used for all *in vivo* experiments was pHUGW, a vector based on FUGW (5). The vector includes the HIV-1 flap sequence, an H1 promoter for expressing shRNA (which was not used in these experiments), the human polyubiquitin promoter-C, and WPRE element. The ubiquitin promoter was used to express nuclear localization signal (NLS) EGFP Cre or a truncated variant, NLS EGFP ΔCre, lacking recombinase activity (6). For rescue experiments, the stop codon was removed and the auto cleavage peptide was added before a signal peptide from influenza hemagglutinin (HA) (7) and an HA epitope tag. LRRTM cDNA (LRRTM2: NM_178005.4**,** LRRTM4: NM_001282928.2), omitting endogenous signal peptides, was then inserted between AgeI and PspXI sites after the HA epitope. For PDZ motif swap experiments, LRRTM2 PDZ motif was replaced with that of

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beta-2 adrenergic receptor using the following primer (5'-caagtgtcagcagctgccaAGTACAAATGACTCACTGCTGTAA), where lowercase sequence is from LRRTM2 and uppercase is from B2AR. For GPI anchored constructs, GPI attachment site and signal peptide of mouse Thy1 (NM_009382.3) were amplified using the following PCR primers (5'- acaatgccatctttactcagagaGCTAGCTATCCATATG, 5'-TCTCTGAGTAAAGATGGCATTGT), where the lowercase region is from LRRTM2, and added by PCR tiling to the truncated c-terminus of the LRRTM2 constructs. For experiments utilizing mRuby p2a paGFP-GluA1, the lentiviral backbone vector used was FSW, which is derivative of FUGW (8), which includes the HIV-1 flap sequence, synapsin promoter, and WPRE element. Expressed off the synapsin promoter, the paGFP-GluA1 construct was modified from a previously published GFP p2a SEP-GluA1 construct, where GFP was excised and replaced with mRuby and SEP was excised and replaced with paGFP (9, 10).

Lentiviral Production. Lentiviruses were produced by transfection of HEK293T cells with four plasmids, the lentiviral shuttle vector, pVSVG, pRRE and pREV, using Lipofectamine 2000 (Thermofisher). The HEK293T culture media was collected 40-44 h after transfection, and spun at 900 x g to remove cellular debris followed by centrifugation at 50,000 x g to concentrate the virus. Concentrated virus was dissolved in a small volume of medium, aliquoted, and stored frozen at -80°C.

Stereotaxic Injection. Briefly, animals were anesthetized with a mixture of ketamine (60 mg/kg body weight) and dexmedetomidine (0.6 mg/kg body weight) by intraperitoneal injection. Mice were immobilized on a Kopf stereotaxic apparatus and bilateral small holes were made in the skull at -1.6 mm posterior and -1.6 mm lateral to bregma for injection in the CA1 region of the hippocampus. Glass cannula filled with viral solution was lowered to a depth of 1.35-1.40 mm (from the dura) and the viral medium (0.5 µl) was injected using a microinjection pump (Harvard Apparatus) at a flow rate of 0.1 µl/min on each hemisphere sequentially. The scalp was then sealed and Atipamezole (0.6 mg/kg body weight) was injected by intraperitoneal injection to reverse the effect of dexmedetomidine.

Electrophysiology in Acute Slices. 14 to 21 days following the *in vivo* injection of virus, animals were anesthetized with isofluorane and the brains rapidly removed and placed in ice-cold, high sucrose cutting solution containing (in mM): 228 sucrose, 26 $NaHCO_3$, 11 glucose, 2.5 KCl, 1 NaH_2PO_4, 7 $MgSO_4$, and 0.5 $CaCl_2$. Slices were cut on a vibratome (Leica Biosystems) in high sucrose cutting solution, and immediately transferred to an incubation chamber containing artificial cerebrospinal fluid (ACSF) containing (in mM) 119 NaCl, 26 $NaHCO_3$, 10 glucose, 2.5 KCl, 1 NaH_2PO_4, 1.3 $MgSO_4$, and 2.5 $CaCl_2$. The slices were allowed to recover at 32°C for 30 min before being allowed to equilibrate at room temperature for a

further 1 h. During recordings, the slices were placed in a recording chamber constantly perfused with heated ACSF (28-30°C) and gassed continuously with 95% O_2 / 5% CO_2. All recordings were made with the GABA-A receptor antagonist picrotoxin (50 µM) added to the ACSF. Whole-cell recording pipettes (3-5 MΩ) were filled with a solution containing (in mM) 135 $CsMeSO_4$, 8 NaCl, 10 HEPES, 0.25 EGTA, 2 Mg_2ATP, 0.3 Na_3GTP, 0.1 spermine, and 7 phosphocreatine (pH 7.35-7.4; osmolarity 297-299).

Data were collected with a MultiClamp 700A amplifier (Axon Instruments) and digitized at 10 kHz with a 2 kHz low pass filter using the A/D converter ITC-18 (Instrutech Corporation). All electrophysiological data were acquired and analyzed using the Recording Artist package (Dr. Rick Gerkin) written in Igor Pro (Wavemetrics). All experiments examining LTP were performed and analyzed blindly without knowledge of the specific virus that had infected that cell. CA1 pyramidal cells were visualized by infrared differential interference contrast and GFP positive neurons were identified by epifluorescence on an upright microscope (Eclipse E600FN, Nikon). A double-barreled glass pipette filled with ACSF was used as a bipolar stimulation electrode and was placed in stratum radiatum to evoke EPSCs in CA1 pyramidal cells. Cells were held at -70 mV to record AMPAR EPSCs while stimulating afferent inputs at 0.1 Hz. Slices were only used when infections were limited in the CA1 hippocampal region, ensuring that effects from LRRTM1,2 cKO are isolated to the postsynaptic cell.

LTP was induced by 2 trains of high frequency stimulation (100 Hz, 1 s) separated by 20 s, while cells were depolarized to -10 mV. This induction protocol was always applied within 15 minutes of achieving whole-cell configuration, to avoid “wash-out” of LTP. Voltage pulse LTP was induced under constant perfusion of D-AP5 (50 µM) by voltage stepping the cells to +10 mV for 1 s with an inter-stimulus interval of 7 s for a total of twenty times. To induce LTD, cells were held at -45 mV and given a train of 600 stimuli at 1 Hz. To generate summary graphs, individual experiments were normalized to the baseline and were averaged to generate 1-minute bins. These were then averaged together to generate the final summary graphs. The magnitude of LTP and LTD was calculated based on the EPSC values in minutes 55-60 after the end of the induction protocols for LTP or 45-50 for LTD. mEPSCs were recorded in ACSF with 4 mM $CaCl_2$, as opposed to 2.5 mM, to increase mEPSC frequency. mEPSCs were recorded in the presence of TTX (0.5 µM) while holding the cells at -70 mV. Analysis of mEPSCs was performed using a mEPSC analysis program (Synaptosoft).

Measurement of AMPAR EPSCs and NMDAR EPSCs was conducted by simultaneous dual cell recording of neighboring infected and uninfected cells, within 4 cell bodies of each other. The AMPAR/NMDAR ratio was calculated as the peak averaged AMPAR EPSC (20-30

consecutive events) at -70 mV divided by the averaged NMDAR EPSCs (20-30 consecutive events) measured at 50 ms after the onset of the dual component EPSC at +40 mV.

Dissociated Neuronal Cultures. Dissociated hippocampal cultures were prepared from newborn mice as previously described (11). Sex of pups was not determined. Briefly, hippocampi were isolated and incubated with a digestion solution containing papain for 30 min at 37°C. A papain inactivation solution containing fetal bovine serum and bovine serum albumin was then applied. Tissue was triturated and cells were plated on poly-D-lysine coated glass coverslips placed in 12 mm wells at a density of 80,000 cells per well. Wells contained Neurobasal A media (Invitrogen) supplemented with B-27 (Invitrogen) and Glutamax (Invitrogen). Glial growth was inhibited by FUDR at DIV 3 or 4 and fresh medium was provided every 3-4 days. The cells were infected with lentiviruses at DIV 10 and processed for experiments 10-12 days later.

qPCR. Cultures were harvested using the Qiashredder kit (Qiagen) and RNA was isolated using the RNAeasy kit (Qiagen). VeriQuest SYBR Green One-Step qRT-PCR Master Mix (Affymetrix) was used and samples run on a 7900HT Fast Real-Time PCR system (Applied Biosystems). Previously published primers for ActB, LRRTM1, and LRRTM2 were used (12). LRRTM levels were referenced within samples against ActB and normalized to LRRTM1,2 levels in untreated WT culture.

Live Cell Imaging. For photoactivation experiments, hippocampal cultures were thoroughly washed with extracellular solution (ECS) containing (in mM): 150 NaCl, 2 $CaCl_2$, 5 KCl, 10 HEPES, 3 Glucose (pH 7.4). After washing, coverslips were mounted on a live imaging chamber immersed in extracellular solution (ECS) at 37°C. Initially, a Z-stack image of a region containing secondary dendrites of a transfected neuron was obtained with a 40X 1.3 NA oil-immersion objective, mounted on a Nikon A1 laser-scanning confocal microscope. A 5 µm thick linear ROI was then activated with 15 sequential scanning runs of the 405 nm laser at 5% laser power. Immediately after photoactivation, another Z-stack image was taken and then at 5-minute intervals for the duration of the experiment. Z-projections were obtained from each time point using Nikon's summed intensity projection method. Images from multiple time points were then stacked as time-series in ImageJ (13). This time-series was then precisely aligned using 'StackReg' plugin in ImageJ. Fluorescent puncta juxtaposing secondary dendrites that appeared after photoactivation and assumed to be spines were selected into multiple ROI's and integrated intensity was measured across time. Intensity values were background subtracted and normalized against photobleaching in the image. Activation in the spines was measured as specific signal in the GFP channel after activation over signal prior to activation. Each intensity value was then normalized with respect to the initial intensity for temporal quantification of signal

loss. Activated regions were only analyzed if the activating ROI was approximately perpendicular to the dendrite.

Immunocytochemistry and Fixed Cell Imaging. Neuronal cultures expressing n-terminally FLAG tagged paGFP-GluA1 were fixed with 4% paraformaldehyde and 4% sucrose in PBS for 15 min on ice. Coverslips were incubated with M1 anti-FLAG (Sigma, F3040, 1:100) for two hours and then secondary antibody for 1 hr, both under saturating conditions. Cells were then permeabilized using 0.05% Triton X-100 for 30 min at room temperature. Post permeabilization, cells were incubated with saturating amounts of M1 and another secondary antibody. Fixed cells were imaged on a Nikon A1 laser scanning confocal using a 40x 1.4 NA oil objective. All images were acquired with parameters maintaining Nyquist criteria for digital microscopy. Z-stacks of primary dendrites were turned into summed projections and the background corrected intensities of the non-permeabilized (surface) and permeabilized (internal) channels was used to form a ratio. The ratio was then normalized to the values found in cells not expressing Cre virus.

Generation of custom antibodies against LRRTM1 and LRRTM2. Anti-LRRTM1 antibody (BC267) was raised in rabbit against mouse LRRTM1 ectodomain fused to His tag (6His-WKSLTSITLAGNLWDCGRNVCALASWLSNFQGRYDANLQCASPEYAQGEDVLDAVYAFHLCEDGAEPTSGHLLSVAVTNRSDLTPPESSATTLVDGGEGHDGTFEPITVALPGGEHAENAVQIHK). Anti-LRRTM2 antibody (512) was raised in rabbit against peptide NH2-CQQLPYKECEV-COOH conjugated to Keyhole Limpet Hemocyanin (KLH). Both anti-sera were affinity purified and eluted in 3M KSCN.

Immunoblotting. Whole brain homogenates from wild-type and embryonic-deleted *LRRTM1* and *LRRTM2* mice were prepared as described (14). For all samples, protein concentrations were normalized and run on 10% polyacrylamide gels. Gels were transferred onto Immobilon P membranes (Millipore) which were blocked in 5% skim milk in Tris-buffered saline/0.05% Tween-20 or 5% BSA in PBS and incubated with primary antibodies against LRRTM1 (BC2567) or LRRTM2 (512) at a dilution of 1:500 followed by secondary antibody (goat anti-rabbit HRP conjugate from Millipore). Immunoblot signals were detected using the SuperSignal Chemiluminescent kit (Thermo Scientific) and a Bio-Rad gel documentation system.

Surface Staining of Rescue Constructs. Neuronal cultures from *LRRTM1,2* cKO pups were sparsely infected with lentivirus expressing Cre recombinase and n-terminally HA-tagged LRRTM rescue constructs at DIV 10. On DIV 17 cultures were fixed with 4% paraformaldehyde and 4% sucrose in PBS for 15 min on ice. Coverslips were not permeabilized prior to incubation with 3F10 anti-HA antibody (Sigma, 11867423001, 1:1000) to stain only surface LRRTMs.

Quantification and Statistical analysis. All graphs show individual data points (no error bars) or means ± SEMs. Experimental “n” values are given in all figure panels and described in the

relevant figure legend. Statistical significance was calculated between interleaved control cells and test cells using Graphpad (Prism). For comparisons between two groups, unpaired t-tests were used. For data sets with two experimental variables (virus and time) two-way Anova were performed with Bonferroni post-test. Statistical tests were performed as two-tailed variants. Approximate sample set sizes were determined in advance using power analysis performed on historical data from the lab. This analysis suggested n values of 6 to 8 were needed to detect effects on LTP. Sufficient numbers of mice were injected to obtain roughly double that set size in order to accommodate for experimental loss due to off target injections and experimental variability.

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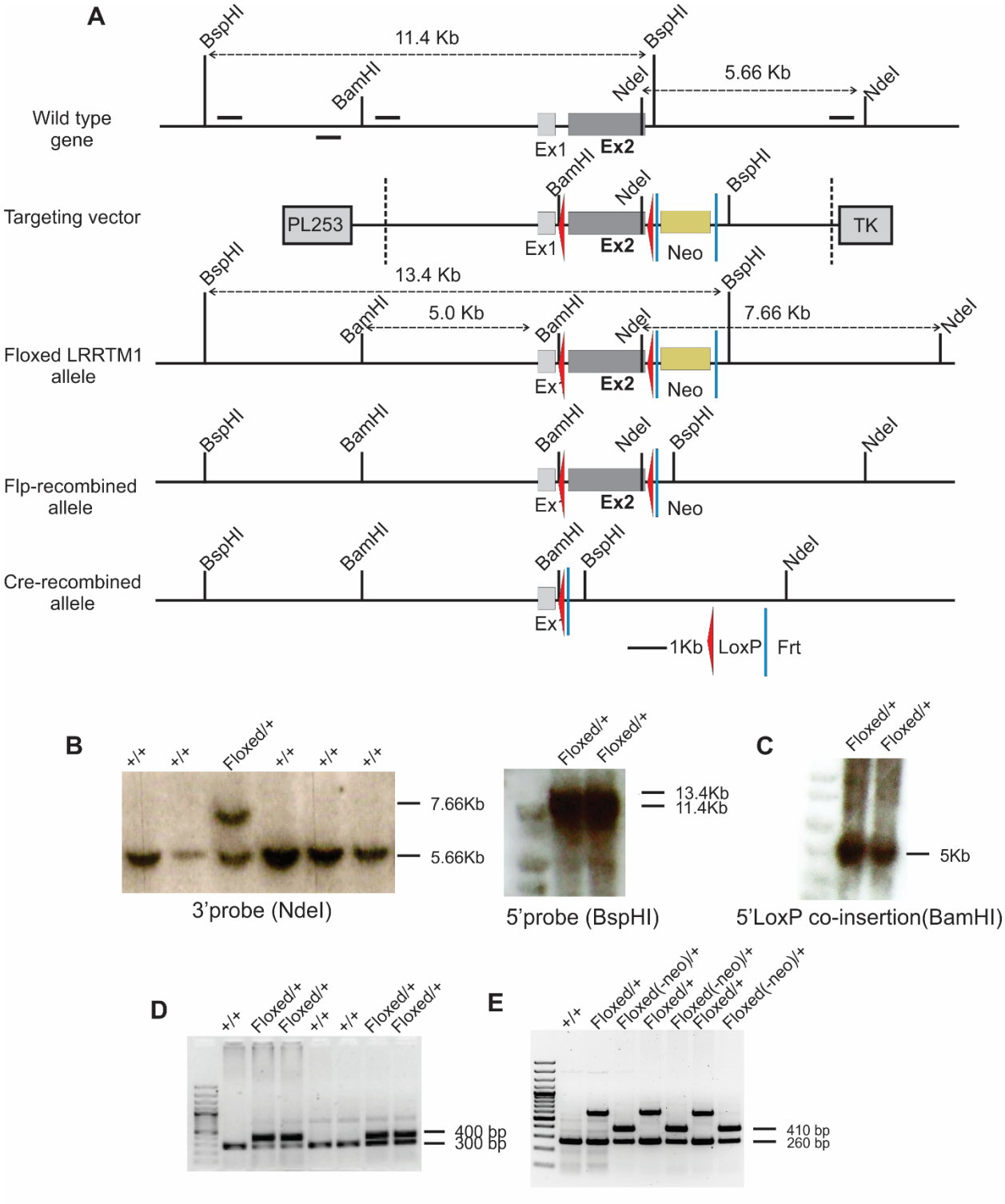

Figure S1. Generation of *LRRTM1*$^{flox/flox}$ mice. (A) Targeting strategy for *LRRTM1* gene. Exon 2 (Ex2), the major coding exon, was floxed. The restriction enzyme sites for BspHI and NdeI used

for Southern blot analysis are shown. Black bars represent outside southern blot probes to monitor homologous recombination. The structure of the final targeting vector introduced into the mouse ES cells contains 5’ and 3’ homologous regions flanking the targeted region. Thymidine kinase (TK) was used for negative selection and neomycin resistance cassette (Neo) was used for positive selection. The floxed allele without the neomycin coding sequence was generated by crossing with B6;LTJ-Tg(ACTFLPe)9205Dym/J mice. (B) Confirmation of homologous recombination in ES cells by Southern blot analysis. The 3’ probe was used for screening and the 5’ probe was used for confirmation of targeted insertion. (C) A separate probe was used to determine 5’LoxP site co-insertion into the homologous targeting region. (D) PCR test to identify floxed and wild-type alleles. (E) PCR analysis showing wild-type allele, allele with LoxP sites and neomycin (floxed) and allele with only LoxP sites after neomycin excision (floxed (-neo)).

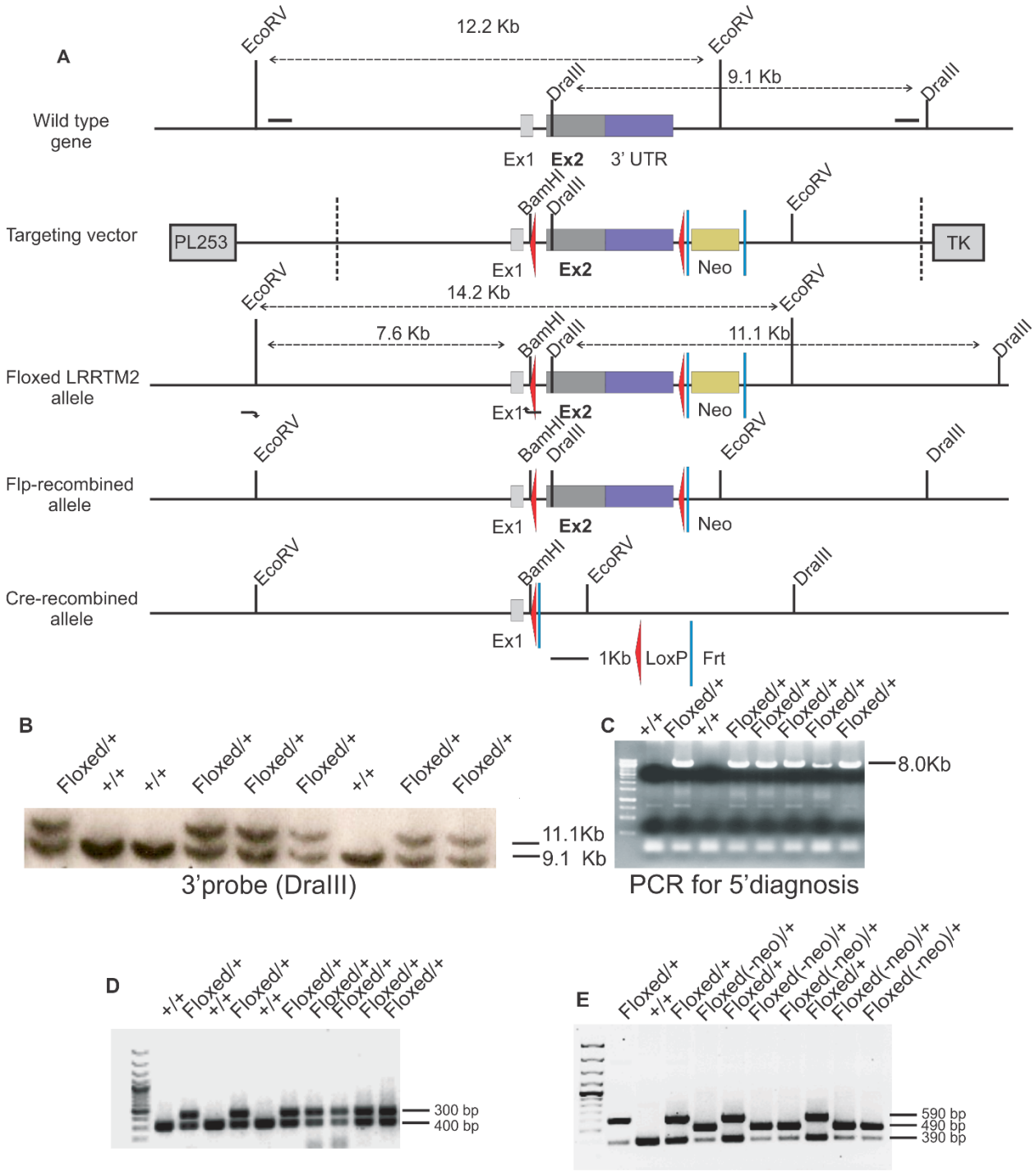

Figure S2. Generation of *LRRTM2*$^{flox/flox}$ mice. (A) Targeting strategy for LRRTM2 gene. Exon 2, the major coding exon with associated 3' untranslated region, was excised. The restriction enzyme sites for DraIII used for Southern blot analysis are shown. PCR primers used for 5' diagnosis are shown by curved arrows in the *LRRTM2* targeted allele. The black bar represents the outside southern blot probe to monitor homologous recombination. The structure of the final

targeting vector introduced into the mouse ES cells contains 5’ and 3’ homologous regions flanking the targeted region. Thymidine kinase (TK) was used for negative selection and neomycin resistance cassette (Neo) was used for positive selection. The floxed allele without the neomycin coding sequence was generated by crossing with B6;LTJ-Tg(ACTFLPe)9205Dym/J mice. (B) Confirmation of homologous recombination in ES cells by Southern blot analysis. The 3’ probe was used for screening for targeted insertion. (C) Confirmation of homologous recombination in ES cells by PCR at the 5’ end. (D) PCR test to identify floxed and wild-type alleles. (E) PCR analysis showing wild-type allele, allele with LoxP sites and neomycin (floxed) and allele with only LoxP sites after neomycin excision (floxed (-neo)).

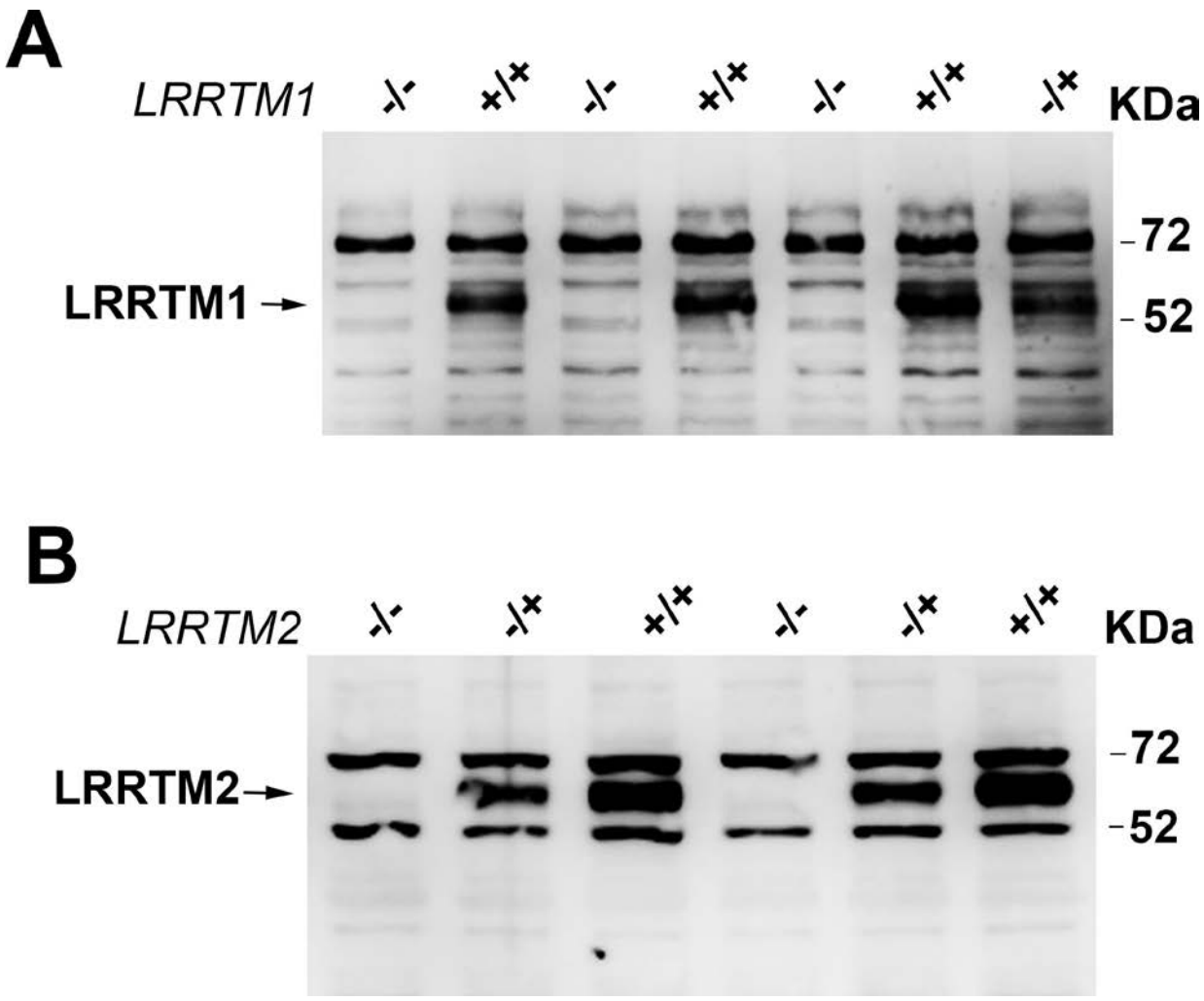

Figure S3. Immunoblot of LRRTM1 and LRRTM2 from *LRRTM1*$^{flox/flox}$ and *LRRTM2*$^{flox/flox}$ mice crossed to germ line Cre mice. The null alleles were generated by crossing *Lrrtm* mice floxed with LoxP and Frt sites with B6;LTJ-Tg(ACTFLPe)9205Dym/J and 6.FVB-Tg(EIIa-cre)C5379Lmgd/J mice at the germ line stage. (A) Confirmation of loss of LRRTM1 protein in brain homogenate from *LRRTM1-/-* mouse. (B) Confirmation of loss of LRRTM2 protein in brain homogenate from *LRRTM2-/-* mouse.

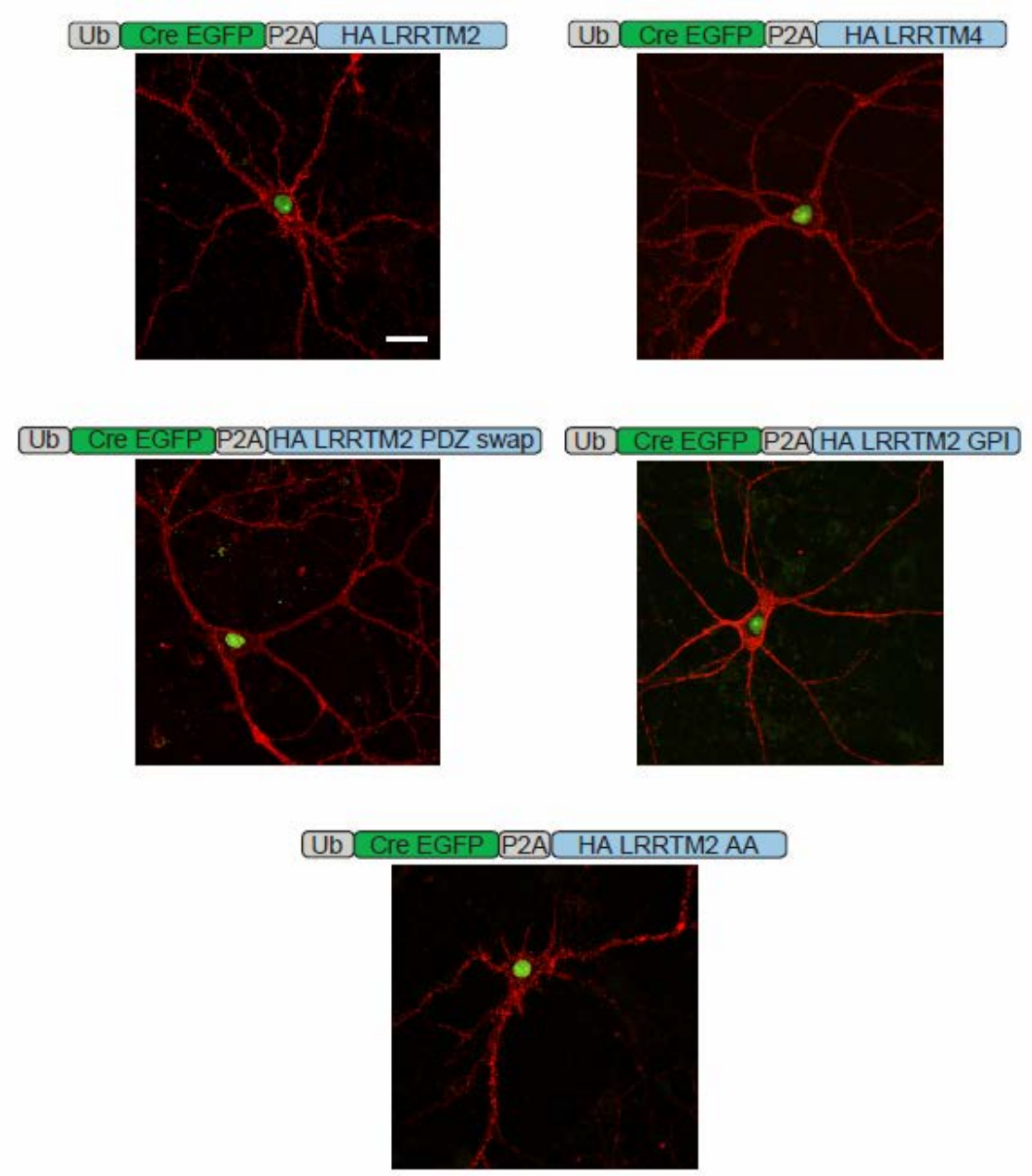

Figure S4. HA-tagged LRRTM2 and LRRTM4 rescue constructs traffic to the plasma membrane in the absence of endogenous LRRTM1, 2. Representative images from *LRRTM1, 2* cKO hippocampal primary culture neurons sparsely infected with lentivirus for rescue constructs used in this paper. Schematics showing construct design are shown above the images. Cre-GFP is shown in green and non-permeabilized HA staining is shown in red. Scale bar: 25 µm.