Supplementary Methods

Immunohistochemistry and confocal imaging

Hippocampal slices (300-μm-thick) prepared from transgenic mice expressing yellow fluorescence protein (YFP) in a subset of neurons (YFP-2J line, Jackson Laboratory) were placed in 4% paraformaldehyde overnight (4°C) and transferred to 30% sucrose [in 0.1 M sodium phosphate (SP) buffer] overnight before re-sectioning to 20 μm thickness using a freezing sliding microtome (Leica). The sections were collected in cryoprotectant (20% sucrose, 30% ethylene glycol in pH 7.4 SP buffer) and kept at -20°C. The day before primary antibody incubation, the sections were frozen at -80°C in 30% sucrose (in 0.1 M SP buffer). The following day, sections were defrosted and rinsed in PBS (4×5 min) and permeabilized [2% Triton X-100 (TX-100) in SP buffer, 1 hour]. They were then incubated in -20°C methanol (10 min), rinsed in PBS (4×5 min), and placed in 1% TX-100 blocking solution [10% normal donkey serum (NDS), 4% BSA in PBS] for 1 h at room temperature. Sections were then incubated in 0.2% TX-100 blocking solution with α7-nAChR antibody (1:500, rabbit polyclonal antibody, Abcam, Cambridge, MA) and synaptophysin antibody (1:20, mouse monoclonal antibody, Millipore, Billerica, MA) for 7 days at 4°C. After washing (PBS, 4×5 min), sections were incubated for 2 h at room temperature in secondary antibodies [Alexa633 goat anti-rabbit IgG (1:500, Molecular Probes, Eugene, OR) and Alexa555 goat anti-mouse IgG (1:200, Molecular Probes, Eugene, OR)] in 1.5% NDS in PBS. Sections were rinsed in PBS, mounted on glass slides, and air-dried. The slides were coverslipped with Prolong™ mounting solution (Molecular Probes, Eugene, OR) and sealed with nail polish. The stained sections were imaged using a Leica SP5X confocal microscope with a 63×oil immersion objective lens. The CA3 subfield of the hippocampus was imaged through the z-axis at 0.5 μm steps with x/y/z resolution of 0.24/0.24/0.50 μm/pixel.

Steady-state surface biotinylation

Hippocampal slices (400 μm thick) were prepared as described above, and the CA3 region was isolated. After 30 min recovery at room temperature, the isolated CA3 slices were transferred to 30°C for additional 30 min recovery. The slices were then transferred to ice-cold ACSF for 10 min, and subsequently to ice-cold ACSF containing 2

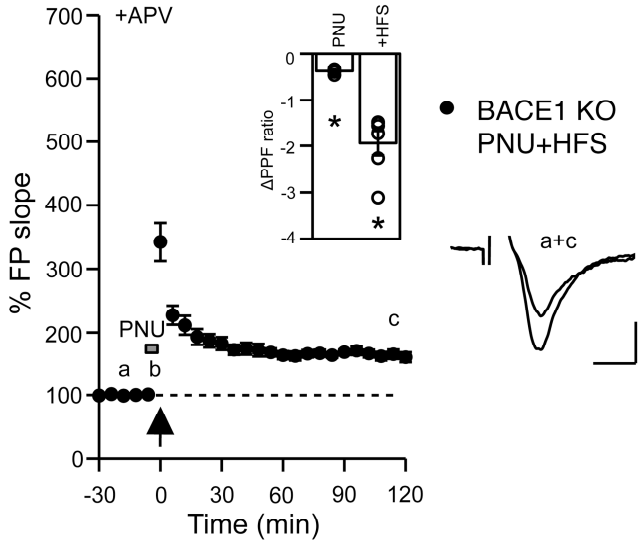
mg/ml biotin (EZ-Link Sulfo-NHS-Biotin, Pierce) saturated with 5% CO_2/95% O_2 for 15 min. The slices were then washed in tris-buffered saline (TBS: 50 mM Tris, 0.9% NaCl, pH 7.4) containing 100 mM glycine (5 times, 1 min each) before homogenized in ice-cold 0.2% SDS/1% Triton X-100 IPB (20 mM Na_3PO_4, 150 mM NaCl, 10 mM EDTA, 10 mM EGTA, 10 mM $Na_4P_2O_7$, 50 mM NaF, and 1 mM Na_3VO_4, pH 7.4; with 1 μM okadaic acid and 10 KIU/ml aprotinin) by ~30 gentle strokes using glass-teflon tissue homogenizers (Pyrex). The homogenates were centrifuged for 10 min at 13,200 × g, 4°C. Protein concentration of the supernatant was measured and normalized to 2 mg/ml. Some of the supernatants were saved as inputs by adding gel sampling buffer and boiled for 5 min. 300 μg of each supernatant was mixed with neutravidin slurry [1:1 in 1% Triton X-100 IPB (TX-IPB)] and rotated overnight at 4°C. The neutravidin beads were isolated by brief centrifugation at 1,000 × g. Some of the supernatants were saved by adding gel sample buffer and boiled for 5 min. The neutravidin beads were washed 3 times with 1% TX-IPB, 3 times with 1% TX-IPB containing 500 mM NaCl, followed by 2 washes in 1% TX-IPB. The biotinylated surface proteins were then eluded from the neutravidin beads by boiling in gel sampling buffer for 5 min. The input (I, total homogenate), supernatant (S, intracellular fraction), and biotinylated samples (B, surface fraction) were run on the same gel, and processed for immunoblot analysis using α7-nAchR and actin antibodies. The band intensity in the input lanes and biotin lanes, which fell within the linear range, was quantified to calculate the % of total α7-nAchR on the surface for each sample.

Immunoblot analysis

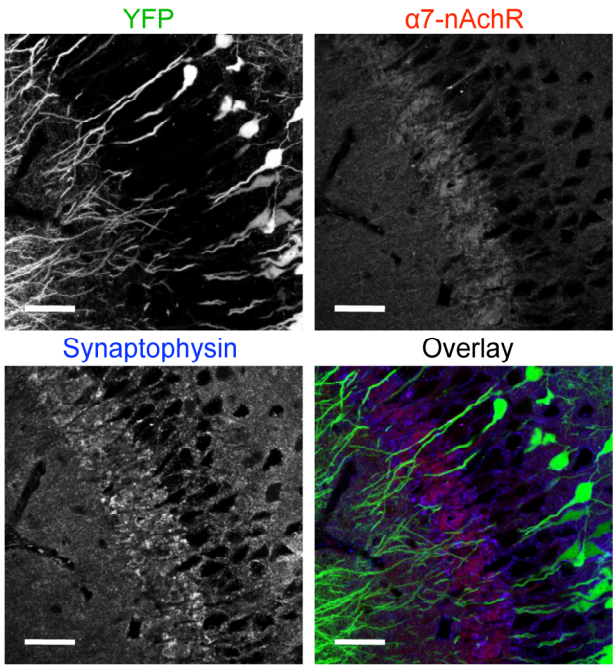
SDS-PAGE gels were transferred to polyvinyl difluoride (PVDF) membranes (Immobilon^{TM}, Millipore). The PVDF membrane blots were blocked for ~1 hr in blocking buffer (1% bovine serum albumin and 0.1% Tween-20 in phosphate buffered saline (PBS), pH 7.4), and subsequently incubated for 1-2 hrs in primary antibodies (Ab's) diluted in blocking buffer. After 5 x 5 min washes in blocking buffer, the blots were incubated for 1 hr in 2nd Ab linked to Cy3 and Cy5. After washes, blots were scanned using Typhoon Trio (GE Health), and signals were quantified using Image Quant TL software (GE Health). The fluorescence intensity values for each band was then

normalized to the average value of WT samples on the same blot to obtain the % of average WT values, which were compared across different experimental groups using unpaired Student's t-test. The biotinylation blots were also probed simultaneously with α7-nAchR and actin antibodies using the ECL plex system. The band intensities of biotinylated samples were normalized to that of the input lanes (total protein) to obtain the level of surface α7-nAchR as a % of total value.

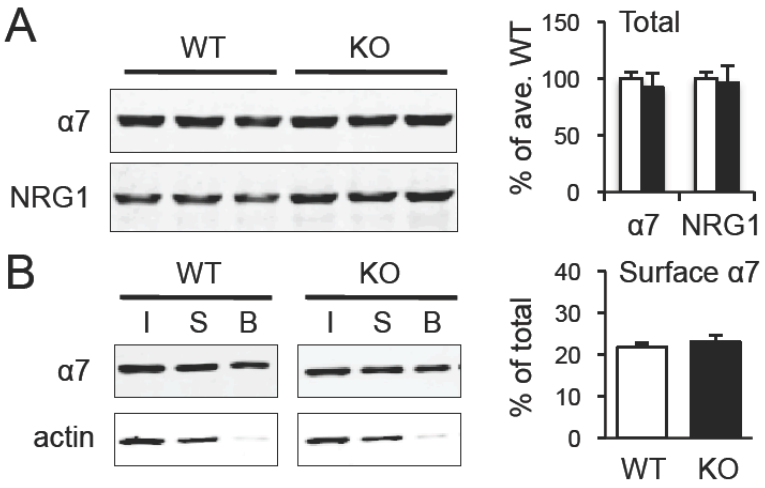
Supplementary Figures



Supplementary Figure 1. Transient bath application of PNU282987 (PNU: 500 nM, 10 min; gray bar) restored mfLTP in BACE1 KOs for up to 2 hours (black circles). 2 hour post-HFS: 163 ± 7%, n = 6 slices/3 mice; paired t-test: $P < 0.001$. Inset: ΔPPF ratio in BACE1 KO PNU+HFS experiments. ΔPPF ratio with PNU282987 application [= (PPF at time point b) – (PPF at time point a)]; ΔPPF ratio with HFS [= (PPF at time point c) – (PPF at time point a)], *paired t-test: $P < 0.001$. Bars: average ± sem. Black circles: individual data points. Arrow: HFS (100 Hz, 1s × 3). Right: Superimposed FP traces. Scale: 0.5 mV, 5 ms.



Supplementary Figure 2. Immunohistochemical labeling of α7-nAChRs in the CA3 region of a YFP-2J mouse. Top left: YFP signal in a subset of CA3 neurons. Top right: α7-nAChR immunoreactivity (Alexa633-linked secondary antibody). Note a higher signal in the stratum lucidum. Bottom left: Synaptophysin immunoreactivity (Alexa555-linked secondary antibody). Bottom right: Overlay of YFP (green), α7-nAChR (red), and synaptophysin (blue). Note purple signal in stratum lucidum, which indicates overlap of α7-nAChR and synaptophysin immunoreactivity. Scale: 40 µm.



Supplementary Figure 3. Normal expression of α7-nAChRs in the CA3 region of BACE1 KOs.

(A) Comparison of α7-nAChR and neuregulin-1 (NRG1) levels in the total homogenates of isolated CA3 regions of WT and KOs. Left: Example immunoblots probed with α7-nAChR or NRG1 antibody. Right: Quantification of the immunoblots for total α7-nAChR (WT = 100 ± 5.7% of average WT value; KO = 92 ± 12.9% of average WT value; n = 10 mice each group; t-test: $P > 0.58$) and total NRG1 (WT = 100 ± 5.2% of average WT value; KO = 96 ± 14.6% of average WT value; n = 10 mice each group; t-test: $P > 0.82$).

(B) Comparison of cell surface α7-nAChR levels in isolated CA3 slices between WT and KO. Cell surface α7-nAChRs were isolated using steady-state surface biotinylation. The total protein (input, I), the resulting supernatant (S: intracellular protein fraction), and biotin pull-down (B: cell surface fraction) were loaded onto the same gel, transferred to blots, and simultaneously probed with α7-nAChR and actin antibodies using the ECLplex system (GE Health). The intensity of signals in the input and the biotin lanes was used to calculate the fraction of surface α7-nAChRs as a % of total α7-nAChR. Actin signal was used to assess the success of the biotinylation assay. Only the blots showing less than 5% actin signal in the biotin lanes were used for quantification. Left: An example immunoblot from WT and KO. Right: Quantification of biotinylated α7-nAChR signals (Surface α7-nAChR: WT = 21 ± 1.0% of total, n = 10 mice; KO = 23 ± 1.7% of total, n = 8 mice; t-test: $P = 0.51$).