Supporting Information:

Figure S1. Characterization of transgenic mice. **A**. Schematic depiction of transgenic cassettes for generation of transgenic mice expressing PreP specifically in neurons under thy-1 promoter. **B**. PCR for identification of transgenic PreP mice. Tg PreP or PE mice were identified as bearing the transgene from analysis of tail DNA based on PCR amplification. **C.** Synaptic mitochondria were prepared from the indicated Tg mice and subjected to immunoblotting for PreP (upper panel). Hsp60, a mitochondrial matrix protein, serves as the mitochondria protein loading control (the lower panel). **D.** Cytosol fractions from the indicated transgenic mice were subjected to immunoblotting to detect PreP (upper panel) and β-actin (lower panel), which is not normally present in mitochondria, was used as a marker of the absence of mitochondria. Mitochondrial faction was loaded as a positive control for PreP immunoreaction. No PreP immunoreactive bands were found in cytosolic fractions from the indicated Tg mice (upper panel). **E**. Brain slices from single Tg mice (nonTg, PreP and PE) were subjected to confocal microscopy with double immunofluorescent staining of PreP (Green) and the mitochondrial marker, CCO (Red), to show the mitochondrial localization of PreP. Scale bar=20 μm. **F**. Immunogold electron microscopy showed PreP immunogold particles (18 nm) in neuronal mitochondria of the indicated Tg mice. Black arrows point to mitochondria and * denotes synaptic cleft. Scale bar = 200 nm. **G-H**. Mitochondrial PreP activity in the indicated Tg mice was measured by changes in fluorescence quenching for substrate V degradation. The Substrate V degradation rate is shown as Relative Fluorescence Units (RFU, **G**). RFUs were increased in PreP neuronal mitochondria. Mitochondrial matrix PreP proteins (15 μg) from nonTg (lanes 1-2), PreP (lane 3), and PE (lane 4) were incubated with Biotin-Aβ (1-42, 10 ng) at 37ºC for 2.5 hours followed by immunobloting with ExtraAvidin Peroxidase Conjugate to detect Aβ immunoreactive bands. Lane 5 denotes vehicle-treated biotin-Aβ as a positive control.

Figure S2. Localization of PreP in cortical neurons of Tg mAPP, mAPP/PreP, and mAPP/PE mice, **A**. Brain sections were subjected to confocal microscopy with double immunofluorescent staining of PreP (Green) and CCO (Red), the mitochondrial marker, to show the localization of PreP in mitochondria. The scale bar is 20μm. **B**. Immunogold electron microscopy showed PreP immunogold particles (18 nm) in neuronal mitochondria of the indicated Tg mice. Black arrows point to mitochondria and * denotes synaptic cleft. Scale bar = 200 nm. **C-D**. Quantification of Aβ-immunoreactive plaques in cerebral cortex including hippocampus in the indicated Tg mice (**C**). Representative sections stained with 3D6 from the indicated Tg mice at 12 months of age (**D**). N= 5-8 mice per group. No Aβ plaque was found in nonTg mice (data not shown). Scar bar = 30μm.

Figure S3. Representative images for MitoSox (red) staining with nuclear staining (blue) in the indicated Tg mice. Scale bar = 50 μm. There were no detectable MitoSox-positive cells (red) in both hippocampus and cortex of NonTg, PreP and PE mice, indicating no ROS accumulation in the mitochondria of these mice. N = 3-5 mice per group.

Figure S4. Effect of PreP overexpression and activity on basal synaptic transmission and hippocampal LTP. The fEPSPs (field-excitatory post-synaptic potential) were recorded in the CA1 region of the indicated transgenic mice at 12–13 months of age. (**A-B**) Tg PreP or PE mice have no significant alterations on either the basal synaptic transmission (amplitude of fEPSPs plotted with the corresponding stimulus (**A**) or hippocampal LTP (**B**) compared to that of in nonTg controls. (**C**) Slices from 12-13 months old mAPP mice showed a reduction of basal synaptic transmission compared to nonTg slices. However, overexpression of PreP in mAPP mice (mAPP/PreP) restored the impaired basal synaptic transmission (*$P < 0.05$ compared to nonTg or mAPP/PreP slices). Data are presented as mean ± SE. N = 7-12 slices from -5 mice per group.

Figure S5. Effect of PreP overexpression on spatial learning and memory. The radial arm water maze demonstrated no significant difference of learning capacity among the indicated groups of control mice (**A**). Furthermore, Morris Water Maze test showed the average latency to locate the hidden platform during each day of training sessions (**B**) and swimming speed of the indicated Tg mice (**C** and **D**). N = 6-13 mice per group. There was no significant difference of latency among PreP, PE, and nonTg mice. The transgenic mice among these groups had similar swimming speed by the visual swimming speed test.

Figure S1

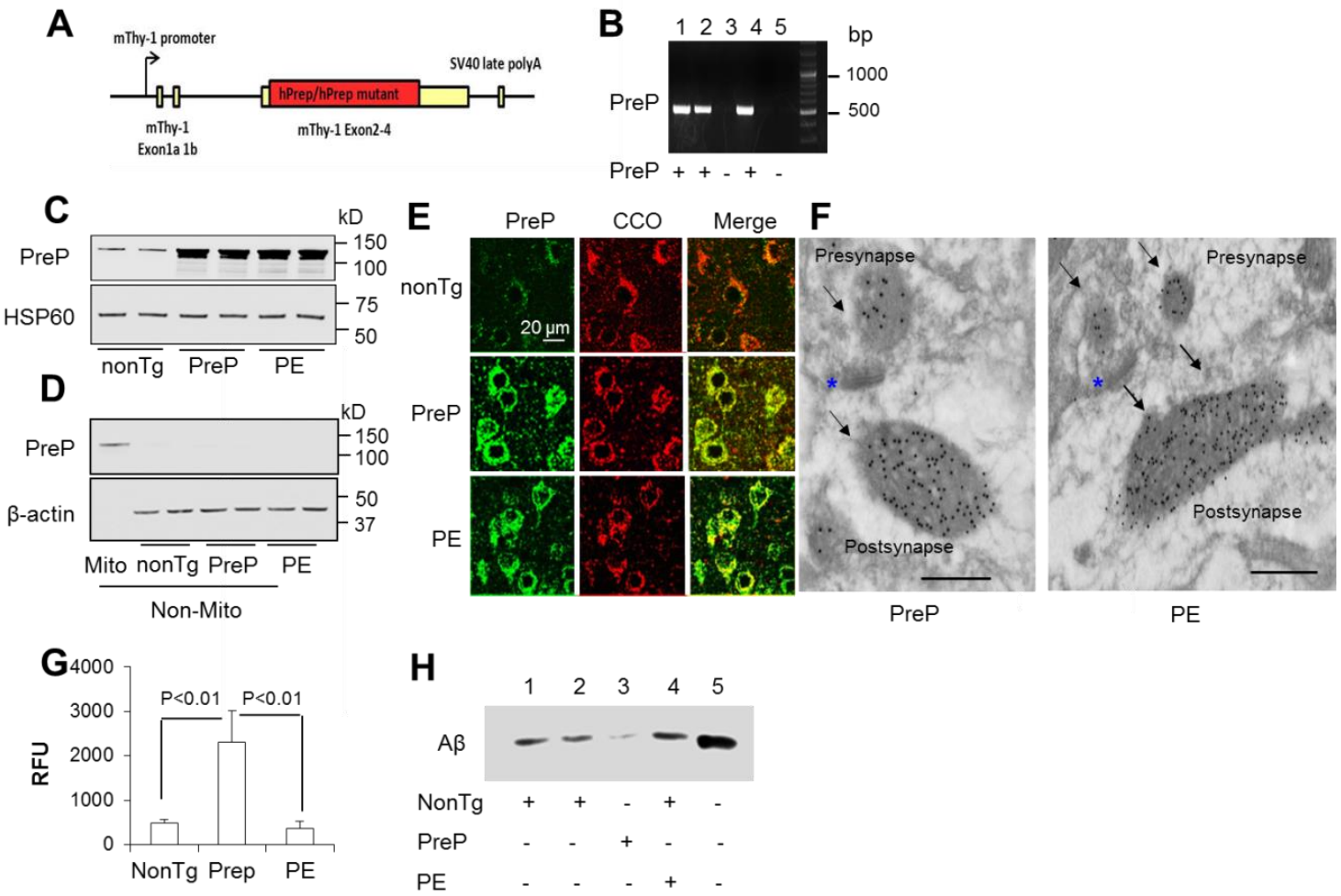

A
mThy-1 promoter
SV40 late polyA
hPrep/hPrep mutant
mThy-1 Exon1a 1b
mThy-1 Exon2-4
B
1 2 3 4 5
bp
1000
500
PreP
PreP + + - + -
C
kD
PreP
HSP60
150
100
75
50
nonTg PreP PE
D
kD
PreP
β-actin
150
100
50
37
Mito nonTg PreP PE
Non-Mito
E
PreP
CCO
Merge
nonTg
PreP
PE
20 μm
F
Presynapse
Postsynapse
PreP
PE
G
RFU
4000
3000
2000
1000
0
P<0.01
P<0.01
NonTg Prep PE
H
1 2 3 4 5
Aβ
NonTg + + - + -
PreP - - + - -
PE - - - + -

Figure S2

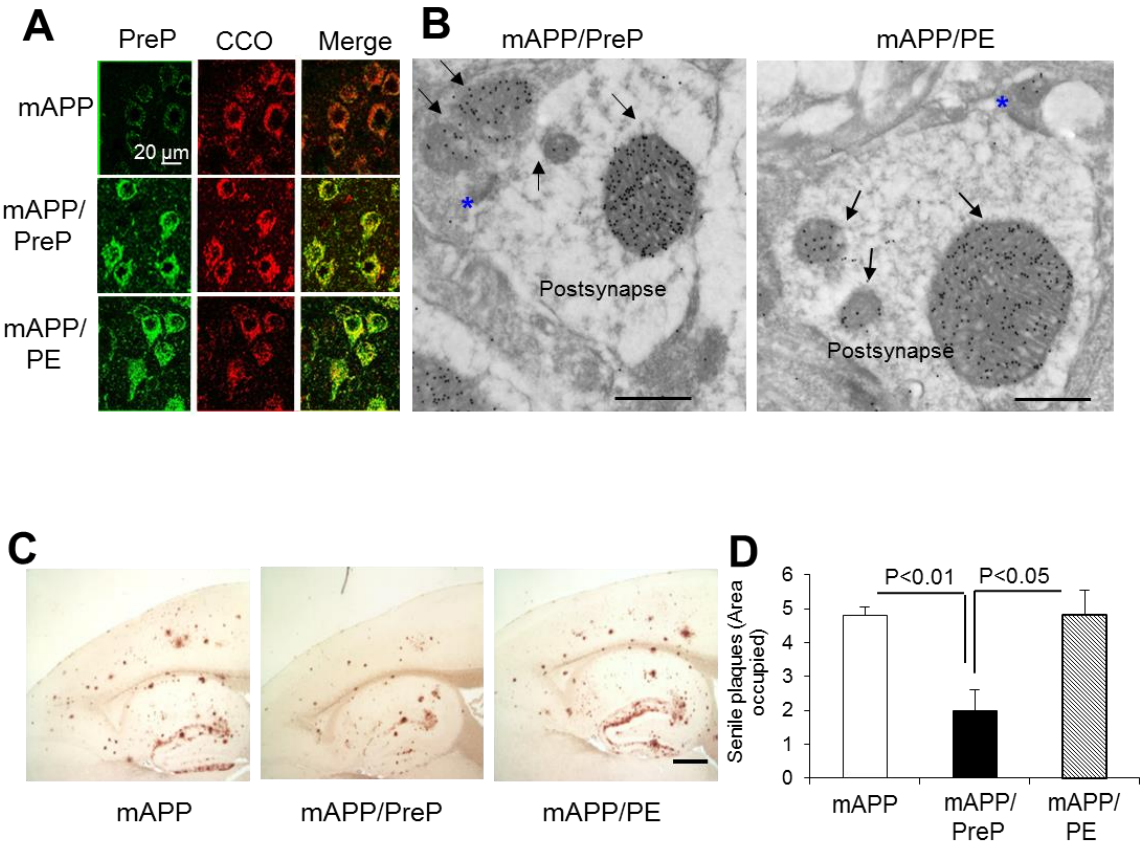

A
PreP
CCO
Merge
mAPP
mAPP/
PreP
mAPP/
PE
20 μm
B
mAPP/PreP
mAPP/PE
Postsynapse
Postsynapse
C
mAPP
mAPP/PreP
mAPP/PE
D
P<0.01
P<0.05
Senile plaques (Area occupied)
0
1
2
3
4
5
6
mAPP
mAPP/
PreP
mAPP/
PE

Figure S3

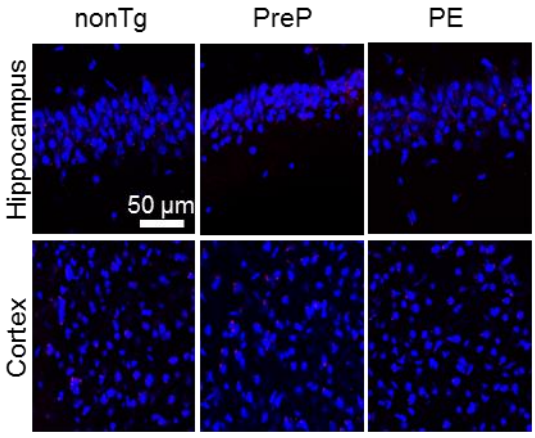


Figure S4

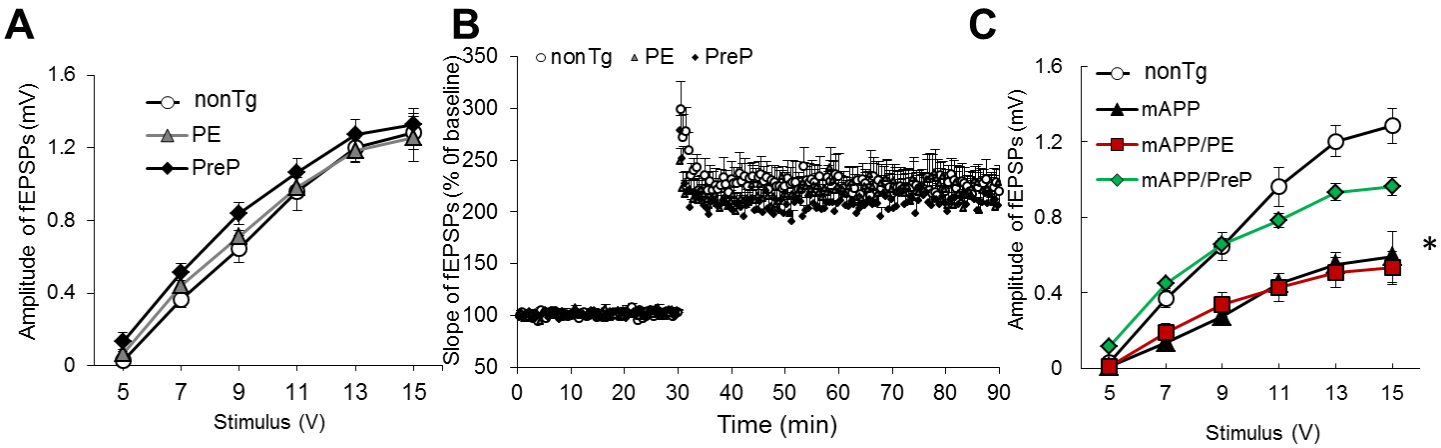
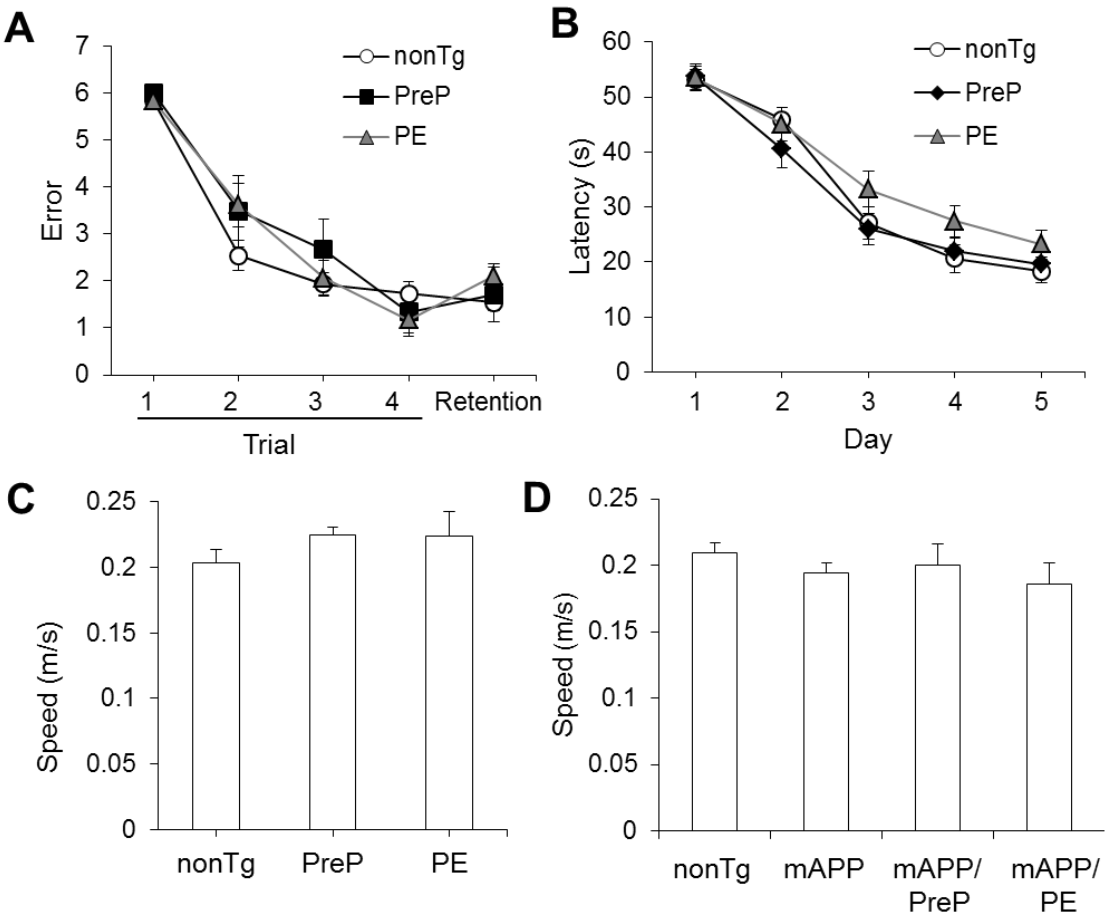

A
Amplitude of fEPSPs (mV)
1.6
1.2
0.8
0.4
0
nonTg
PE
PreP
5
7
9
11
13
15
Stimulus (V)
B
Slope of fEPSPs (% 0f baseline)
350
300
250
200
150
100
50
nonTg
PE
PreP
0
10
20
30
40
50
60
70
80
90
Time (min)
C
Amplitude of fEPSPs (mV)
1.6
1.2
0.8
0.4
0
nonTg
mAPP
mAPP/PE
mAPP/PreP
*
5
7
9
11
13
15
Stimulus (V)

Figure S5


A
Error
Trial
Retention
nonTg
PreP
PE
B
Latency (s)
Day
C
Speed (m/s)
nonTg
PreP
PE
D
Speed (m/s)
nonTg
mAPP
mAPP/ PreP
mAPP/ PE