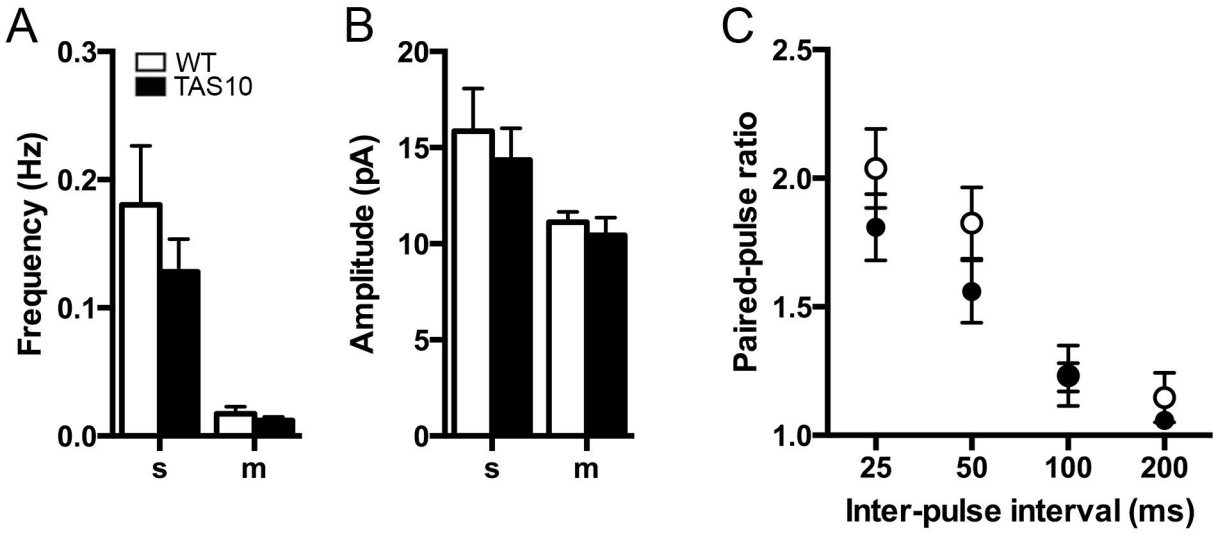

Supplementary Figure 3. No change in CA1 EPSCs in TAS10 mice compared to wild type littermate controls at 4 months. EPSCs were isolated in CA1 pyramidal neurones of the parent strain of the TASTPM mice (TAS10, transgenic for APP_{Swe}) and age-matched wild type littermate controls. *A & B*) No differences between genotypes were identified in frequencies (A) or amplitudes (B) of either spontaneous (s) or miniature (m) EPSCs. Wild type n=8; TAS10 n=7 animals. *C*) Paired-pulse ratios at CA3-CA1 synapses from TAS10 mice were not significantly different than wild type. Wild type n=7; TAS10 n=8 animals. Note: TAS10 express the APP_{Swe} transgene identical to TASTPM but have no detectable plaques or changes in gene expression until 18 months of age, reflecting lower levels of Aβ (Matarin *et al*. 2015).

Supplementary Methods

Animals

All experiments were performed in agreement with the Animals (Scientific Procedures) Act 1986, with local ethical approval and in agreement with the GlaxoSmithKline statement on use of animals. All mice in this study were bred at UCL in the same room and under identical conditions. To maximise the number of animals with genotypes of interest, male double heterozygous TASTPM mice were bred by crossing male homozygous TASTPM with female C57Bl/6j. Age-matched, non-littermate male C57Bl/6j mice were used as wild type controls. Founders were obtained from Charles River Laboratories (UK) and the colony subsequently maintained at UCL. In this study we avoided single housing by keeping mice in larger cages and enriching their environments. Under these conditions, while the aggressive nature of the TASTPM mouse is not completely avoided, it is less of a problem and allows group housing to be maintained over the lifetime of the mice. Large (20 × 35 × 45 cm) cages containing 2-10 mice were maintained on a 12-hour light/12-hour dark cycle with *ad libitum* supply of food and water. Environmental enrichment consisted of changes of food location, bedding type (e.g. tissue, shredded paper, paper roll, paper bags) and inanimate objects (e.g. running wheels, rodent balls, tubing, houses) within the cage at least once per week. Mice were used for experimentation at the ages stated (± 0.5 months where the age is stated in months) and were single-housed for no longer than 24 hours. Genotypes were tested post experimentation using standard PCR protocols on tails from all mice.

Acute hippocampal brain slice preparation

Mice were decapitated and the brain rapidly removed and placed in ice-cold dissection artificial cerebrospinal fluid (ACSF, containing (in mM): 125 NaCl, 2.4 KCl, 26 $NaHCO_3$, 1.4 NaH_2PO_4, 20 D-glucose, 3 $MgCl_2$, 0.5 $CaCl_2$, ~315 mOsm/l, pH 7.4). After approximately 1 minute in ice-cold dissection ACSF, the brain was prepared for slicing by removing the cerebellum, hemisection of the forebrain and a segment cut away from the dorsal aspect of each hemisphere at an angle of approximately 105° from the midline surface. Each hemisphere was then glued (cyanoacrylate, Henkel Loctite Limited, UK) on this surface onto the stage of a vibrating microtome (Integraslice model 7550 MM, Campden Instruments, Loughborough, UK) containing frozen dissection ACSF and 400 μm slices were cut, transverse to the long edge of the hippocampus and including the attached entorhinal cortex. From each slice, the hippocampus was dissected away retaining a portion of entorhinal cortex and the resulting slice was placed into a chamber containing 'Carbogenated' (95% O_2/5% CO_2; BOC Limited) dissection ACSF (pH 7.4) at room temperature (approximately 21 °C). In approximately 5 minutes intervals, slices were then transferred through a series of chambers held at 36 °C with fresh ACSF with varying Ca^{2+} and Mg^{2+} ion concentrations (in mM): i) 1 Mg^{2+}, 0.5 Ca^{2+}, ii) 1 Mg^{2+}, 1 Ca^{2+}; iii) 1 Mg^{2+}, 2 Ca^{2+}. Slices were subsequently allowed to return to room temperature and recover for at least a further 40 minutes prior to experimentation.

Patch-clamp recordings in brain slices

A single slice was transferred to a submerged chamber and superfused with recording ACSF at room temperature (~22 °C), described above and containing (in mM): 1 $MgCl_2$, 2 $CaCl_2$. Individual neurones were visualised using infrared-differential interference contrast microscopy (model BX50WI, Olympus, UK). Glass microelectrodes for patch-clamp were

pulled from borosilicate glass capillaries (model GC150F-7.5, 1.5 mm outer diameter × 0.86 mm inner diameter; Harvard Apparatus Ltd, Edenbridge, UK) on a vertical puller (model PP830, Narishige International Ltd, London UK). Electrodes (tip resistance approximately 5 MΩ) were filled with an internal solution containing (in mM): 140 CsCl, 5 HEPES, 10 EGTA, 2 Mg-ATP, ~290 mOsm/l, pH 7.4). Liquid junction potential, slow capacitance or series resistance were not compensated as errors introduced by these are calculated to be small when the membrane remains voltage-clamped at −70 mV. Patch-clamp recordings were performed using one of the following set ups: 1) EPC9/2 (HEKA Elektronik Dr Schulze GmbH, Lambrecht/Pfalz Germany) connected via the built-in ITC-16 digitizer board to a computer running Pulse software (version 8.80, HEKA); 2) An Axopatch 1D (Molecular Devices, Sunnyvale, CA, USA) connected via a digitizer (Digidata 1322A, Molecular Devices; or 1401plus, Cambridge Electronic Design, Limited, Cambridge, UK) to a computer running WinWCP (for isolated events; Strathclyde University, UK) and WinEDR (for continuous recordings; Strathclyde University, UK). Stimulation was applied *via* a patch electrode (4-6 MΩ) filled with ACSF, placed extracellularly in the appropriate axon path and using a square pulse constant-voltage stimulator (model SD9, Grass Technologies, Slough, UK; or DS2A-MkII (Digitimer Ltd, UK) triggered by the software used for data collection (Pulse or WinWCP).

Field potential recordings in brain slices

Following transfer of slices to recording ACSF (1 mM Mg^{2+} and 2 mM Ca^{2+}), slices were transferred as needed to a heated (30 ± 1 °C) submerged chamber and superfused with ACSF and allowed to recover for 1 hour in the recording chamber. A glass stimulating electrode (filled with ACSF, resistance 1-2 MΩ) was positioned as described in the text. A glass field recording electrode (filled with ACSF, resistance 1-2 MΩ) was positioned as described to record a dendritic field potential (fEPSP). Recordings were controlled by and recorded to WinWCP software (as above) and digitzed at 10 kHz via either an ITC16 interface (Digitimer Ltd, UK) or 1401plus interface (Cambridge Electrical Designs, UK). Stimuli (constant voltage, 100 μs; model Digitimer DS2A-MkII or Grass SD9) were applied at 0.1 Hz and resultant fEPSPs subsequently averaged over consecutive 1-minute intervals. Stimulation intensity was set at approximately 30% of the intensity required to evoke a population spike or the maximum fEPSP amplitude obtained and a >15 minute stable baseline was recorded. LTP conditioning consisted of 3 trains of tetani, each consisting of 20 pulses at 100 Hz (test-pulse intensity), 1.5 s inter-train interval. Following conditioning, fEPSPs were evoked at 0.1 Hz for 1 hour.

Hippocampal histology

Transcardial perfusion with phosphate buffered saline (PBS), followed by 4% paraformaldehyde (PFA) in PBS was performed under deep anaesthesia with Euthatal (200 μg/ml) and Lidocaine. Intact brains were extracted, post-fixed in 4% PFA and stored in 0.1% sodium azide in PBS. Brains were cryoprotected in 30% sucrose and serially sectioned (30 μm, ~720 μm apart) using a frozen microtome (Leica, Germany). For estimating plaque load, free-floating sections were permeabilized with 0.3% Triton-X100 in PBS and blocked in 8% horse serum. Primary Aβ-40 (1:300, Invitrogen, USA) antibody was diluted in 8% horse serum solution with 0.3% Triton-X100 and incubated overnight. Sections were then washed with PBS and incubated in goat anti-rabbit secondary antibody (1:600, Jackson ImmunoResearch, USA). For estimating neuronal loss, sections were incubated in 0.2% cresyl violet solution, destained in glacial acetic acid and 95% ethanol solution, and dehydrated in series of alcohols and xylene. Entire hippocampus was imaged in each section

using an EVOS FL Auto microscope (Life Technologies, USA). A 480 × 360 μm region was defined in CA1, CA3 and DG of each section. For plaque analysis, each region was converted to 8 bit, an equivalent threshold applied and each plaque was traced and measured in ImageJ (NIH, USA). For estimation of neuronal loss, neurones in each region were manually counted using ImageJ. Experimenter was blind to the age and genotype of the sample during quantification and analysis.

Immunoprecipitation-Mass Spectrometry

Brain tissue was snap frozen and then homogenized on ice in tris-buffered saline containing complete protease inhibitor (Roche Diagnostics GmBH, Mannheim, Germany). Briefly, formic acid (FA) was added (final concentration 70%) followed by further homogenization, sonication and centrifugation (30,000 x g, 1 hour, +4 °C). The supernatant was collected and dried in a vacuum centrifuge. The dried fraction was dissolved in 70% FA followed by centrifugation (30,000 x g, 1 hour, +4 °C) and the supernatant neutralised using 0.5 M Tris. Immunoprecipitation was performed using a KingFisher magnetic particle processor (Thermo Scientific, Waltman, MA, USA) using a modified protocol (Portelius *et al.* 2010). Briefly, 4 μg of the Aβ-specific antibodies 6E10 and 4G8 (epitope 4-9 and epitope 18-22, respectively, Signet Laboratories, Inc., Dedham, MA, USA) were separately added to 25 μl Dynabeads M-280 (Dynal) sheep anti-mouse according to the manufacturer's product description. The washed beads with bound antibody (25 μl 6E10 and 25 μl 4G8) were combined and used for immunoprecipitation of the neutralised FA fraction to which Tween-20 (Bio-Rad Laboratories Inc.; end concentration 0.025%) was added and incubated. The beads/FA fraction was transferred to a KingFisher magnetic particle processor (polypropylene tubes, Thermo Scientific) for automated washing and elution of the Aβ peptides. The collected supernatant was dried in a vacuum centrifuge and re-dissolved in 5 μl 0.1% FA in 20% acetonitrile. MALDI-TOF/TOFMS measurements were performed using a Bruker Daltonics UltraFleXtreme instrument (Bruker Daltonics, Bremen, Germany) operating in reflector mode.

RNA extraction, microarrays and network analysis

Hippocampal total RNA was prepared from 2 and 4 month old male mice using miRNeasy mini columns (Qiagen), using the manufacturer's protocol. The quality and concentration of the total RNA was determined using a NanoDrop spectrophotometer and Experion system (Bio-Rad). Microarray hybridisation was performed by AROS Applied Biotechnology. Briefly, 100 ng of total RNA was used for cDNA synthesis followed by *in vitro* transcription using the Ambion Illumina TotalPrep kit (Life Technologies). The *in vitro* transcription products were randomly hybridized to Illumina MouseRef8 v2 BeadArrays overnight at 48 °C and then scanned in an Illumina BeadStation Scanner. The raw data were quantile normalized using the Illumina Genome Studio software. Individual probes were excluded from analyses if P(detection)>0.05 in more than 50% of the samples in a given genotype at any age. After these quality control steps, data was available for 41 hippocampal samples, representing 16,465 gene probes.

Weighted Gene Co-expression Network Analyses (WGCNA) were performed using the WGCNA package in R. Genes with variable expression (coefficient of variation >5%) were selected for network analyses leaving 3,384 gene probes (Oldham *et al.* 2006). WGCNA were performed on these most variable genes as previously described (Zhang and Horvath 2005, Horvath *et al.* 2006, Oldham *et al.* 2006). For each set of genes a pair-wise correlation matrix and an adjacency matrix were calculated by raising the correlation matrix

to the power of 12 (chosen using the scale-free topology criterion). For each pair of genes, a robust measure of network interconnectedness (topological overlap measure; TOM (Yip and Horvath 2007)) was calculated based on the adjacency matrix. TOM-based dissimilarity was then used as input for average linkage hierarchical clustering: groups of genes based on TOM. Branches of the resulting clustering tree were defined as gene modules (Langfelder *et al.* 2008). To cut the branches, hybrid dynamic tree-cutting, a minimum module size of 30 genes and a minimum height for merging modules at 0.25 was used. Each module was summarised by the first principal component of the scaled (standardised) module expression profiles. For each module, the module membership measure (module eigengene-based connectivity kME) was defined as the correlation between gene expression values and the module eigengene. Genes were assigned to a module if they had a high module membership (kME >0.7). Each module was assigned a unique colour identifier and genes that did not fulfil these criteria for any of the modules, were assigned to the grey module. For functional analyses, the group of genes within each module of interest were analysed for common functional themes and similarity to previously generated molecular signatures using the DAVID database (http://david.abcc.ncifcrf.gov; Dennis *et al.* 2003).

Reverse-transcription and quantitative PCR (qPCR)

The first strand of cDNA was synthesized from total RNA (1 μg) and treated with 0.5 U of DNAase I (Invitrogen) containing 10 U of RNAase OUT (Invitrogen) at 37 °C for 15 minutes followed by enzyme inactivation at 75 °C for 15 minutes. The RNA was reverse-transcribed using the High Capacity cDNA synthesis protocol (Applied Biosystems) following the manufacturer's guidelines. First strand cDNA reactions were diluted four times with nuclease-free water. For qPCR, the primers were designed to span at least one intron using Primer3-BLAST software (NCBI; available online at:
http://www.ncbi.nlm.nih.gov/tools/primer-blast).

Primer sequences:

Primers for murine *Rps28* (product size = 121 bp):
Forward: 5' ATC AAG CTG GCT AGG GTA ACC 3'
Reverse: 5' GGC CTT TGA CAT TTC GGA TGA 3'

Primers for murine *Rps29* (product size = 263 bp):
Forward: 5' TCG TTC CTT TCT CCT CGT TGG 3'
Reverse: 5' AGA CTA GCA TGA TCG GTT CCA C 3'

Primers for murine *Rpl4* (product size = 178 bp):
Forward: 5' GCT GAA CCC TTA CGC CAA GAC 3'
Reverse: 5' CTT TCC TTT CTT GCC TAC CGC 3'

Primers for murine *Atf5* (product size = 293 bp):
Forward: 5' GCT GAA CAA TCT TCC ACG CTG 3'
Reverse: 5' CAC CCG CTC AGT CAT CCA ATC 3'

Primers for murine *Faim2* (product size = 233 bp):
Forward: 5' CGG TCT ATG CTG TAC TGG GAG 3'
Reverse: 5' GGA AGG GAC ATT CTC TGG AGG 3'
Primers for murine *Bdnf* (product size = 222 bp):

Forward: 5’ ATC CAC TGA GCA AAG CCG AAC 3’
Reverse: 5’ GGA CGT TTA CTT CTT TCA TGG GC 3’

Primers for murine *Rab4a* (product size = 198 bp):
Forward: 5’ ACA GGA GTG TGG CTG CTA GG 3’
Reverse: 5’ TAC TGT CCG CTG AGT TAG TTC G 3’

Primers for murine *Rims3* (product size = 253 bp):
Forward: 5’ TGG AAG TGA TTG AAG CTC GGG 3’
Reverse: 5’ GCC ATA CCC ATG AAG CAC TTG 3’

Prior to use, the primers were tested for specificity by performing a test PCR reaction and resolving the samples on an agarose gel with ethidium bromide. The cDNA samples were tested in triplicate in a 20 µl reaction volume in a 96 well plate format, containing 250 nM of each oligonucleotide primer and SYBR Green PCR Master Mix (BioRad). Negative control PCR reactions were also performed in parallel on total RNA that had not been processed with reverse-transcriptase to test for the presence of genomic DNA in each sample with each primer pair. Cycling parameters: 3 minutes at 95 °C, followed by 40 cycles (95 °C for 10 s, annealing at 58 °C for 30 s and extension at 72 °C for 30 s) and then a melt-curve analysis was performed by holding the samples at 95 °C for 1 minute followed by an increase in temperature from 60 °C to 95 °C in 0.5 °C increments every 5 s. The real-time PCR was performed in a CFX96 system with a C1000 thermal cycler (BioRad). We ensured that all qPCR reactions produced a single peak with the melt curve analysis. The results for the RT-qPCR experiments were expressed as:

$$2 - (\text{Gene of interest mean Ct value} - \text{Control gene mean Ct value})$$

In order to increase the accuracy of gene expression levels for normalization, we used the mean of three internal control reference genes: *Rps28*, *Rps29* and *Rpl4* (Vandesompele *et al*. 2002). Because the expression ratio of ideal control reference genes should be the same across all samples, we selected these reference genes among a set of twelve candidate reference genes that also included β-actin and *Rps18* in order to minimize the pair-wise variation in gene expression across hippocampal tissue from different mice. The calculations were performed as described on the geNorm website:
http://medgen.ugent.be/~jvdesomp/genorm.

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