SUPPORTING INFORMATION

A. SUPPORTING FIGURES

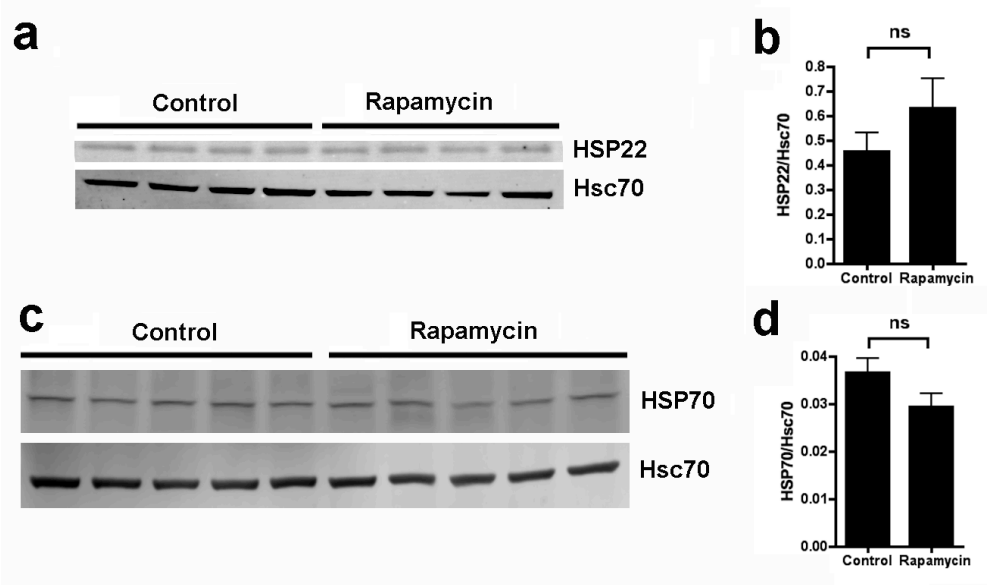

Figure S1. Levels of HSP22 and HSP70 in brains of PDAPP mice are not affected by rapamycin treatment. a. Representative immunoblots of the indicated HSPs in whole-brain extracts. **b,** Densitometric quantitation of immunoblots normalized by Hsc70. Bars represent the mean of 4 (HSP22) and 5 (HSP70) mice/group +/- SEM. * indicates $P<0.05$.

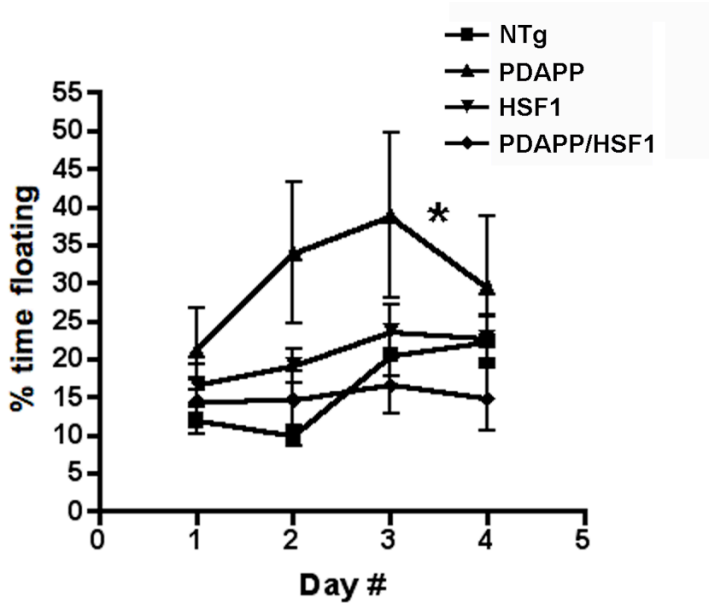

Figure S2. Increased floating in PDAPP mice. Percent time spent making no attempt to escape ("floating") was significantly increased for PDAPP mice with respect to all other experimental groups [*P<0.01, Bonferroni's post hoc test applied to a significant effect of genotype, $F(3,111)=3.82$, P<0.01, two-way repeated measures ANOVA]. No significant interaction between day of training and percent time spent floating for different genotypes was observed ($F(9,111)=1.08$, P=0.38), thus genotype had the same effect at all times during training. Data are means ± SEM.

B. SUPPORTING METHODS

Rapamycin treatment. Rapamycin was used at 14 mg per kg food (verified by HPLC). On the assumption that the average mouse weighs 30 g and consumes 5 g of food/day, this dose supplied 2.24 mg rapamycin per kg body weight/day{Harrison, 2009 #107}. All mice were given *ad libitum* access to rapamycin or control food and water for the duration of the experiment. Body weights and food intake were measured weekly. Food consumption remained constant for both control- and rapamycin-fed groups during treatment (no significant effect of week number on food consumption by two-way ANOVA). As previously reported, food consumption was higher for rapamycin-fed animals. This may be a result of the inhibition of the mTOR pathway, which is expected to mimic the unfed state. Littermates (transgenic and non-transgenic mice) were housed together, thus we could not

distinguish effects of genotype on food consumption. In spite of the differences in food consumption, overall body weight of control- and rapamycin-fed groups was not significantly different.

2D Gel Electrophoresis: Tissues were homogenized with a teflon probe on ice in 50mM Tris 10mM $MgSO_4$ pH7.4 with a protease inhibitor cocktail (Roche) and processed for 2D gel electrophoresis as described{Pierce, 2008 #121}. Homogenates were centrifuged at 16,000*g* for 30 min at 4°C. The supernatant was then precipitated in 10% trichloroacetic acid at 4°C, then protein pellets were washed with 1:1 ice cold ethanol:ethyl acetate. Protein pellets were dissolved in 8M urea with 4% 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPS). Protein concentration was determined by BCA assay. Two-hundred µg of protein was mixed with 0.4% ampholytes (Biorad), and 4 µl of destreak reagent (GE Healthcare), diluted to a volume of 300 µl and loaded on 4-7 immobilized pharmalyte gradient (IPG) strips for isoelectric focusing. Focused strips were separated on 12% PAGE gels following reduction with 10 mg/ml dithiothreitol and blocking with 25 mg/ml iodoacetimide in equilibration buffer (50mM Tris, pH 8.8, 6M urea, 30%(v/v) glycerol, 2%(w/v) SDS). Spots were visualized by staining gels with Sypro Ruby, and quantitated using PDQuest Advanced (Biorad).

Behavioral testing. The novel object recognition task was used to test recognition memory{Antunes, #76}. Mice tend to spend more time interacting with a new object rather than one they have previously encountered. Experimental animals were habituated to the testing arena, a clean cage, 24 h before training. On day 1, mice were presented with two different objects and allowed to explore them. On day 2, one of the objects was replaced with a new one. The amount of time mice spent exploring the novel and the previously encountered objects was recorded. The Morris water maze (MWM){Morris, 1984 #21; Galvan, 2006 #18; Galvan, 2008 #19; Zhang, #20} was used to test spatial memory. All animals showed no deficiencies in swimming abilities, directional swimming or climbing onto a cued platform during pre-training and had no sensorimotor deficits as determined with a battery of neurobehavioral tasks performed 2 weeks prior to testing. The procedure described by Morris et al.{Morris, 1984 #21} was followed as described{Galvan, 2006 #18; Spilman, #22}. Briefly,

transgenic and non-transgenic PDAPP mice were given a series of 6 trials, 1 hour apart in a light-colored tank filled with opaque water whitened by the addition of non-toxic paint at a temperature of 24.0±1.0°C. Animals were trained to find a 12x12-cm submerged platform (1 cm below water surface) placed in one quadrant of a water tank surrounded by opaque dark panels 30 cm away from the tank, serving as distal cues. The animals were released at different locations in each 60' trial. If mice did not find the platform in 60 seconds, they were gently guided to it. After remaining on the platform for 20 seconds, the animals were removed and placed in a dry cage under a warm heating lamp. Twenty minutes later, each animal was given a second trial using a different release position. This process was repeated a total of 6 times for each mouse, with each trial approximately 20 minutes apart, for a total of 4 days. At the end of training, a 45-second probe trial was administered in which the platform was removed from the pool. The time spent in the quadrant where the platform had been located and the Gallagher proximity measure{Gallagher, 1993 #78} was determined as a measure of platform location retention. Animals that showed high anxiety ("thigmotaxers or wall huggers" spending more than 85% of trial time within 10 cm of the tank wall) were taken out of the study. During the course of testing, animals were monitored daily, and their weights were recorded weekly. Performance in all tasks was recorded by a computer-based video tracking system (Water2020, HVS Image, U.K). Data were analyzed offline by using HVS Image and processed with Microsoft Excel.

Immunoblots: For the experiments of **Figure 1**, membranes were immunoblotted with a multi-plexed cocktail of antibodies containing HSC70 (rat, SPA815 1:10000), HSP70 (mouse, SPA810 1:10000), HSP105 (rabbit, abcam24503 1:5000), HSP90 (rabbit, SPA846 1:10000), HSP60 (rabbit, cell signaling #4870 1:1000), HSP25 (rabbit, SPA801 1:5000), CryAB (rabbit, abcam ab13497 1:2000), GRP94 (rat, santacruz sc-56399 1:10000), and α–Synuclein (mouse, abcam ab78541 1:2000) overnight at 4º C. Primary antibodies were detected using anti-rat Alexa 647 (Invitrogen), anti-mouse IRDye 680LT, or anti-rabbit IR Dye800 CW (Li-Cor) conjugated antibodies. Secondary antibodies were incubated at 1:3000 (rat) or 1:10000 (mouse and rabbit) for 2 hours at room temperature and images were captured using a Typhoon 9410 variable mode imager (GE Healthcare) for Alexa 647 or

Odyssey Imaging System (Li-Cor, Lincoln) for IRDye 680LT, IR Dye800. In all other experiments, proteins from soluble fractions of brain LN_2 homogenates were resuspended in 1 mM α-glycerophosphate, 5 mM sodium orthovanadate, 1 mM EDTA, 1 mM EGTA, 2 mM dithiotreitol and protease inhibitors cocktail (Roche) in phosphate-buffered saline. Homogenates were then sonicated and centrifuged at 15,000 g for 15 min at 4°C. The protein concentration in supernatants was determined by the BCA assay and resolved by SDS/PAGE (Invitrogen, Temecula, CA) under reducing conditions and transferred to a PVDF membrane, which was incubated in a 5% solution of non-fat milk or in 5% BSA for 1 hour at 20°C. After overnight incubation at 4°C with the following primary antibodies, at the indicated dilutions: anti-HSF1 (Stressgen #SPA-901, rabbit 1:1000); anti-HSF1 PSer326 (Abcam, ab76076 1:5000), anti-CryAB as described above, anti-Aβ (6E10, Covance #SIG 39340, 1:1000), anti-GAPDH (Open Biosystems # TAB1001, 1:2000); anti-4E-BP1 (Cell Signaling #9452, 1:1000); anti-(P)4E-BP1(Thr37/46) (Cell Signaling # 9459, 1:1000). Membranes were washed in TBS-Tween 20 (TBS-T) (0.02% Tween 20, 100mM Tris pH 7.5; 150 nM NaCl) for 20 minutes and incubated at room temperature with appropiate secondary antibodies. The blots were then washed 3 times for 20 minutes each in TBS-T and then incubated for 5 min with Super Signal (Pierce, Rockford, IL), washed again and exposed to film or imaged with a Typhoon 9200 variable mode imager (GE Healthcare, NJ).

Real-time quantitative PCR (RT-PCR): Whole mouse brain tissues (corresponding to one complete hemisphere) were snap frozen on dry ice and were later homogenized in liquid nitrogen. Total RNA was extracted from each sample using the mirVana isolation Kit (Ambion #AM1560) and subsequently treated with DNAse (Invitrogen #18068-015). Thirty ng of RNA were reverse transcribed into cDNA using a commercially available kit (Invitrogen #18080-051). qRT-PCR reactions were performed in triplicates using PowerSYBR Green master mix (Promega #4367659).

Expression levels for control and rapamycin treated experimental groups (n = 6 per group) were determined by normalization against the gene that showed the least variation among samples

(GRP94) using the comparative C(T) method [Schmittgen and Livak (2008) Nat Protoc 3:1101] with cycle threshold ($2^{-\Delta Ct}$). Primers used were:

HSP60 FWD 5-OH-TGTAGACCTTTTAGCAGATGCTG-OH-3; HSP60 rev 5-OH-CGTCCTGAACAAGTTTAGCTCCA-OH-3;HSP105 FWD 5-OH-CAGGTACAAACTGATGGTCAACA-OH-3; HSP105rev 5-OH-TGAGTTAAGTTCAGGTGAAGGG-OH-3; GRP78FWD 5-OH-ACTTGGGGACCACCTATTCCT-OH-3; GRP78 rev 5-OH-ATCGCCAATCAGACGCTCC-OH-3; GRP94 FWD 5-OHTCGTCAGAGCTGATGATGAAGT-OH-3; GRP94 rev 5-OH-GCGTTTAACCCATCCAACTGAAT-OH-3; HSP16 FWD 5-OH-GTTCTTCGGAGAGCACCTGTT-OH-3; HSP16 rev 5-OHGAGAGTCCGGTGTCAATCCAG-OH-3; HSP22 FWD 5-OH-TCCCGTGCTCCTACCCAAG-OH-3; HSP22 rev 5-OH-GCTGTCAAGTCGTCTGGAAAAG -OH-3.

Immunohistochemistry. Ten-micrometer coronal cryosections from snap-frozen brains were post-fixed in ice-cold methanol and stained with CryAB-specific antibodies (Abcam ab13497) and with antibodies that recognize the Aß portion of the APP protein (6E10) followed by AlexaFluor488-conjugated donkey anti-rabbit IgG (1:500, Molecular Probes, Invitrogen, CA) and AlexaFluor594-conjugated donkey anti-mouse IgG (1:500, Molecular Probes, Invitrogen, CA), and imaged with a laser scanning confocal microscope (Zeiss LSM 510) using a 488 Argon laser and a 505 long-pass filter. Images were obtained using a 60X objective. Z-stacks of confocal images were processed using Image J. Colocalization analyses were performed using the Colocalization Indices plugin in ImageJ (Kouichi Nakamura, Kyoto University, Japan). All images were collected in the molecular layer of the cornu ammonis region 3 of the hippocampus at Bregma ~-2.18. The MBL Mouse Brain Atlas was used for reference.