Supplementary Materials for

**HSF1 physically neutralizes amyloid oligomers to empower overgrowth and bestow neuroprotection**

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Published 11 November 2020, *Sci. Adv.* 6, eabc6871 (2020)
DOI: 10.1126/sciadv.abc6871

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SUPPLEMENTAL MATERIALS AND METHODS

Cell lines
HEK293T cells were purchased from GE Dharmacon, and HeLa and A2058 cells were purchased from ATCC. They were all authenticated by ATCC. Immortalized Rosa26-CreERT²; Hsf1flo/flo MEFs (male) were described previously (21). Primary mouse astrocytes were prepared from the brains of P1 newborn mice as described previously (58) with a minor modification, wherein trypsin was replaced with Accumax Cell Dissociation Solution. All cell cultures were maintained in DMEM supplemented with 10% HyClone bovine growth serum. These cell lines have been routinely tested for mycoplasma contamination using MycoAlert Mycoplasm Detection kits.

Primary human neurons were cultured in complete neuronal medium. For the PLA, human neurons were plated on 8-well Nunc Lab-Tek II CC2™ Chamber Slides coated with both 20µg/ml laminin and 50µg/ml poly-L-Lysine. Half of the culture medium was changed every four days. After 12 days in culture, neurons were transduced with lentiviral particles overnight in the absence of polybrene and cultured for another four days, followed by transfection with Aβ1-42 peptides overnight.

Dual HSF1 reporter assay
Plasmids were co-transfected with the dual reporter system, comprising the heat shock element (HSE)-secreted embryonic alkaline phosphatase (SEAP) and CMV-Gaussia luciferase (GLuc) reporter plasmids, into HEK293T cells using TurboFect transfection reagents. After 48 hr, SEAP and luciferase activities in culture supernatants were quantitated using a NovaBright Phospha-Light EXP Assay Kit for SEAP and a Pierce Gaussia Luciferase Glow Assay Kit, respectively. Luminescence signals were measured by a CLARIOstar microplate reader (BMG LABTECH), and SEAP activities were normalized against GLuc activities.

In vitro kinase assays
The AKT kinase assays were performed in 30µl kinase buffer comprising 25mM MOPS pH 7.2, 12.5mM β-glycerol-phosphate, 25mM MgCl2, 5mM EGTA, 2mM EDTA, 0.25mM dithiothreitol, 250µM ATP. Reactions were incubated at 30°C for 30 min with 1,200 rpm mixing in an Eppendorf Thermomixer® C (Eppendorf North America). Reactions were stopped by adding 30µl of 2x SDS-PAGE sample buffer with 3% 2-mercaptoethanol.

Real-time quantitative RT-PCR
The extraction of total RNAs and qRT-PCR were described previously (21). Signals were detected by an Agilent Mx3000P qPCR System (Agilent Genomics). ACTB was used as the internal control. The sequences of individual primers for each gene are listed in Table S3.

Chromatin immunoprecipitation (ChIP)
ChIP experiments were performed according to the procedures described previously (5). Rabbit anti-HSF1 Abs H-311 or rabbit monoclonal anti-DYKDDDDK Tag Abs (D6W5B) were used for ChIP. Normal rabbit IgG served as the negative control. The sequences of individual primers for each gene are listed in Table S3.

Cytosolic and nuclear fractionation
Cytosolic and nuclear fractions were separated using a NE-PER™ Nuclear and Cytoplasmic Extraction Kit. Equal amounts of the same fractions were loaded for SDS-PAGE.
Measurement of cell size and quantitation of nuclei and DNA content
The sizes of cultured astrocytes were measured by a Scepter™ 2.0 Handheld Automated Cell Counter (Millipore) equipped with 60 µm sensors. Nuclei and DNAs were extracted from 30mg pulverized frozen mouse brain tissues. Nuclei were extracted using a Detergent-free Nuclei Isolation Kit and counted using the Scepter™ 2.0 Cell Counter. DNAs were extracted using a NucleoSpin® TriPrep Kit and quantitated by a NanoDrop™ 2000 Microvolume Spectrophotometer (Thermo Fisher Scientific).

Measurement of global protein translation rate
Cultured astrocytes were labeled with 50nM 6-FAM-dc-puromycin in vitro for 30 min and analyzed by flow cytometry.

Congo red staining
Following deparaffinization or air drying, paraffin-embedded brain or frozen liver sections were stained with 0.5% CR dissolved in PBS at RT for 20 min followed by differentiation in alkaline solutions (0.01% NaOH in 50% alcohol). Nuclei were counterstained with hematoxylin.

Peptide and antibody transfection
Aβ42-1 or Aβ1-42 peptides and A11 or OC antibodies were transfected into primary mouse astrocytes, immortalized MEFs, or primary human neurons using the Xfect™ Protein Transfection Reagent.

Mitochondria fractionation
1x10⁶ astrocytes were used to isolate the cytoplasmic and mitochondrial fractions using a Mitochondrial Isolation Kit according to the manufacturer’s instructions. Equal amounts of the same fractions were loaded for SDS-PAGE.

Quantitation of mitochondrial mass
After detaching cells with trypsin from culture plates, live cells were incubated with the culture medium containing 100nM MitoView™ Green dyes, which are not dependent on the mitochondrial membrane potential, for 15 min at 37°C. After washing once with PBS, stained cells were analyzed by a BD FACSCalibur™ flow cytometer (BD Biosciences) using the FL1 channel. The data were analyzed using the FlowJo™ v10 software (FlowJo LLC.).

siRNA and shRNA knockdown
siRNAs were transfected at 10nM final concentration using Mission® siRNA transfection reagent or jetPRIME® transfection reagent. HEK293T cells stably expressing lentiviral HSF1-targeting (hA6) shRNAs were described previously (21). The target sequences of siRNAs and shRNAs are listed in Table S3.

Separation of detergent-soluble and -insoluble cell/tissue fractions
All centrifugation was performed in Eppendorf Benchtop 5424 Microcentrifuges at 4°C. First, 1x10⁶ cells or 1mg pulverized snap-frozen tissues were incubated with the whole-cell lysis buffer (100 mM NaCl, 30 mM Tris-HCl pH 7.6, 1% Triton X-100, 1 mM EDTA, 1x Halt™ phosphatase inhibitor cocktail, and 1x Halt™ protease inhibitor cocktail) on ice for 20 min. Following a brief centrifugation at 500xg for 5 min at 4°C, the lysates were separated into pellets (P1) and supernatants (S1). The P1, which contains nuclei, membrane debris, and large aggregates, was then treated with 50µl DNA digestion buffer (three units DNase I in 40 mM Tris-HCl, pH 8.0, 10 mM NaCl, 6 mM MgCl₂, 10 mM CaCl₂ and 1% Triton X-100) at RT for 20 min to digest genomic DNAs, followed by membrane resolubilization with 2% SDS for 30 min at RT. The re-solubilized P1 was centrifugated at 16,813xg for 10 min at 4°C to obtain pellets (P2), which contain large aggregates and some SDS-resistant materials that cannot be resolubilized, and supernatants (S2), which mainly contain resolubilized membrane-
associated proteins and are therefore designated as the membrane-associated fractions. The combined S1 and P2 were further centrifuged at 16,813xg for 10 min at 4°C to obtain pellets (P3) and supernatants (S3). The P3, which contains both large aggregates from the P2 and small aggregates pelleted from the S1, is thus designated as the detergent-insoluble fractions. By contrast, the S3, which now contains all soluble proteins, is designated as the detergent-soluble fractions. For downstream SDS-PAGE and ELISA, the detergent-insoluble fractions were further re-solubilized by sonication for 10 min in PBS containing 2% SDS at high intensity using a Bioruptor® Sonication System (Diagenode Inc.) or a Q125 sonicator (Qsonica, LLC).

Apoptosis detection
Four independent approaches were applied to detect apoptosis in cultured cells and frozen tissues, including quantitation of caspase 3 activity using either a Caspase-3 Colorimetric Assay Kit or a Caspase 3 DEVD-R110 Fluorometric and Colorimetric Assay Kit, immunostaining with rabbit monoclonal anti-cleaved Caspase-3 (Asp175) (5A1E) Abs, detection of DNA fragmentation (TUNEL) in frozen sections using a NeuroTACS™ II In Situ Apoptosis Detection Kit, and measurement of mitochondrial membrane potential changes by FACS using a JC-1 Mitochondrial Membrane Potential Detection Kit. For the JC-1 staining, both floating and adherent cells were collected for analyses.

Immunoblotting and Immunoprecipitation
Whole cell lysates were extracted in lysis buffer, which comprises 100 mM NaCl, 30 mM Tris-HCl pH 7.6, 1% Triton X-100, 1 mM EDTA, 1x Halt™ phosphatase inhibitor cocktail, and 1x Halt™ protease inhibitor cocktail. Following incubation on ice for 20 min, lysates were centrifuged at 15,000 rpm for 10 min in an Eppendorf Benchtop 5424 Microcentrifuge at 4°C.

For immunoblotting, nitrocellulose membranes were incubated with primary antibodies (1:1,000 dilution in the blocking buffer) overnight at 4°C, followed by incubation with peroxidase-conjugated secondary antibodies (1: 2,500 dilution in the blocking buffer) at RT for 1 hr. Signals were generated using SuperSignal West Pico PLUS or Femto chemiluminescent substrates and captured by either X-ray films or an iBright™ FL1000 imaging system (Life Technologies Corporation). Uncropped blot images are provided as Fig. S8.

For IP, either 1mg whole cell and mouse tissue lysates or 500µg human AD brain lysates were incubated at 4°C overnight with primary antibodies, including: 10µl rabbit monoclonal anti-AKT (pan) (C67E7) Abs, 2µg rabbit polyclonal anti-amyloid oligomers (A11) or anti-amyloid fibrils (OC) Abs, 2µg mouse monoclonal anti-Aβ17-24 (4G8) Abs, and 2µg rabbit anti-HSF1 (H-311) Abs or 2µg mouse monoclonal anti-HSP60 Abs clone LK1. Either normal rabbit or mouse IgG or rabbit anti-PI3K p110α (C73F8) were used as the negative controls. Protein G MagBeads were used to precipitate primary Abs. After washing with the lysis buffer three times, beads were boiled in 1x sample loading buffer for 5 min before loading on SDS-PAGE.

To minimize the cross-reactivity between secondary Abs and reduced, denatured IP Abs during immunoblotting, EasyBlot® anti-Rabbit or anti-Mouse IgG Kits, which also include EasyBlocker to reduce the background caused by Protein G, were applied.

Dot blotting of Aβ1-42
1µM Aβ1-42 was mixed with GST or HSF1 proteins at different molar ratios in 100µl PBS and incubated at RT for 4 hr. After centrifugation at 15,000 rpm for 10 min at 4°C in an Eppendorf
Benchtop 5424 microcentrifuge, 50µl of supernatants were loaded on a 96-well Bio-Dot® Microfiltration Apparatus with Immobilon® PVDF membranes (0.45µm pore size) pre-soaked in PBS. A vacuum was used to drain the samples. Following blocking with 5% non-fat dry milk in PBS, the membranes were incubated with 4G8, ab2539, or D54D2 Abs (1:1000) at 4°C overnight, followed by incubation with secondary Abs-HRP conjugates (1:2500) at RT for 1 hr.

**Immunofluorescence**
Following fixation with 4% formaldehyde in 1xPBS for 15 minutes at RT, cells were blocked with 5% normal goat serum in PBS containing 0.3% Triton X-100 for 1 hr at RT. Primary antibodies 1:100 diluted in 5% normal goat serum were incubated at 4°C overnight, followed by incubation with donkey anti-rabbit or anti-mouse IgG (H+L) CF®594 or CF®488A conjugates (1:200) at RT for 1 hr. For immunofluorescence staining of HSP60 in mouse brains, frozen sections were first incubated with mouse monoclonal anti-HSP60 Abs clone LK1 (1:100) at 4°C overnight, followed by incubation with anti-mouse IgG (H+L) CF®594 conjugates (1:200) at RT for 1 hr. To co-stain neurons, sections were further incubated with mouse anti-βIII Tubulin Abs clone 2G10-TB3 Alexa Fluor® 488 conjugates (1:200) at RT for 4 hr. A set of brain sections incubated only with conjugated secondary Abs served as the negative controls. Nuclei were counterstained with Hoechst 33342, and fluorescent signals were documented by a Zeiss LSM780 confocal microscope.

**Nissl staining**
Following deparaffinization, brain sections were stained with 0.1% Cresyl violet solution (NovaUltra™ Nissl Stain Kit) for 5 min, followed by differentiation in 95% alcohol for 1 min.

**Lentiviral production and transduction**
Lentiviral particles were produced in HEK293T cells by co-transfection of pLKO vectors, pCMV-dR8.2 dvpr, and pCMV-VSV-G using the TurboFect™ Transfection Reagent. Culture supernatants containing lentiviral particles were collected and filtered through sterile 0.45µm syringe filters. Lentiviral titers were determined using the Lenti-X™ GoStix™ Plus. To transduce target cells, different amounts of viral supernatants, based on the MOIs, were diluted in the culture medium containing 10µg/ml polybrene and incubated with target cells overnight.
SUPPLEMENTARY FIGURE LEGENDS

Figure S1: AKT directly activates HSF1.
(A) Following heat shock at 43°C for 30 min, HEK293T cells were fixed and stained with phospho-AKT Thr308 and Ser473 antibodies. The fluorescence intensities were quantitated by flow cytometry (mean±SD, n=3 experiments, two-tailed Student’s t test). NHS: no heat shock. (B) Following pre-treatment with 20µM inhibitors for 3 hr, NIH3T3 cells were heat shocked at 43°C for 30 min and recovered at 37°C for 8 hr. The mRNAs of Hsp72 and Hsp25 were quantitated by qRT-PCR (mean±SD, n=3 experiments, One-way ANOVA). (C) Following pre-treatment with 20µM PI3K or AKT inhibitors for 3 hr, NIH3T3 cells were heat shocked at 43°C for 30 min and recovered at 37°C for 8 hr. HSP induction was detected by immunoblotting (images of a single experiment). (D) Following treatment with 20µM AKT inhibitors for 3 hr, the binding of HSF1 to the HSP72 promoter in HEK293T cells in the absence of heat shock was quantitated by chromatin immunoprecipitation (ChIP)-qPCR (mean±SD, n=3 experiments, One-way ANOVA). (E) and (F) Following transfection of HEK293T cells with LacZ or AKT1Myr plasmids for 48 hr, the binding of endogenous HSF1 to the HSP72 and HSP27 promoters were quantitated by ChIP-qPCR (E) and the transcripts of HSPs were quantitated by qRT-PCR (F) (mean±SD, n=3 experiments, One-way ANOVA). (G) Following co-transfection of plasmids encoding individual AKT isoforms along with the dual HSF1 reporter system in HEK293T cells for 48 hr, the reporter activities in culture media were measured, and SEAP activities were normalized against GLuc activities (mean±SD, n=3 experiments, One-way ANOVA). (H) HEK293T cells, stably expressing either scramble or PTEN-targeting shRNAs, were transfected with the dual HSF1 reporter system comprising the heat shock element (HSE)-secreted embryonic alkaline phosphatase (SEAP) and the CMV-Gaussia luciferase (GLuc) reporter plasmids. After 16 hr, transfected cells were treated with 20µM AKT inhibitors for 48 hr. The reporter activities in culture media were measured, and SEAP activities were normalized against GLuc activities (mean±SD, n=3 experiments, One-way ANOVA). (I) In HEK293T cells stably expressing either scramble or PTEN-targeting shRNAs, the binding of HSF1 to the HSP72 promoter was quantitated by ChIP-qPCR with and without 20µM MK2206 treatment overnight (mean±SD, n=3 experiments, One-way ANOVA). (J) Following heat shock at 43°C for 30 min, the endogenous AKT-HSF1 interactions were detected by co-IP with the EasyBlot™ reagents in HEK293T cells (representative images of three experiments). Rabbit monoclonal anti-PI3K p110α Abs served as the negative control. HC: heavy chain. WCL: whole cell lysate. (K) Following transfection of control or combined Akt1/2/3-targeting siRNAs for four days, immortalized Rosa26-CreERT2, Hsf1β/β MEFs without 4-OHT treatment were stained with rabbit monoclonal anti-pan AKT (C67E7) Abs (images of a single experiment). Following treatment with and without 1µM 4-OHT for seven days to delete Hsf1, these MEFs were stained with mouse monoclonal anti-HSF1 (E-4) Abs (images of a single experiment). The endogenous AKT-HSF1 interactions (green) were visualized by PLA using the anti-HSF1 (E-4) Ab and the anti-pan AKT (C67E7) Ab in these MEFs (representative images of three experiments performed by two individuals). Actin filaments and nuclei were labeled with phalloidin-Alexa Fluor® 594 conjugates (red) and Hoechst 33342 (blue), respectively. Scale bars: 10µm. (L) The consensus AKT phosphorylation sequence and corresponding Ser230 site on both human and mouse HSF1 proteins. (M) In vitro His-HSF1 Ser230 phosphorylation by recombinant active AKT isoforms (representative images of three experiments). Following co-incubation of 100ng recombinant His-HSF1 proteins with 100ng GST or AKT isoforms at 30°C for 30 min with and without 20µM AKT inhibitors, HSF1 Ser230 phosphorylation was detected by immunoblotting. (N) In vitro phosphorylation of His-HSF1 by recombinant active MEK1 and AKT1 proteins independently (representative images of three experiments). Phosphorylation was detected by immunoblotting. (O) Following heat shock at 43°C for
30 min, HSF1 and AKT phosphorylation in HEK293T cells pre-treated with 20μM PI3K or AKT inhibitors for 3 hr was detected by immunoblotting (images of a single experiment). (P) HSF1 and AKT phosphorylation in HEK293T cells stably expressing either scramble or PTEN-targeting shRNAs was detected by immunoblotting (images of a single experiment). (Q) In HEK293T cells stably expressing a shRNA (A6) that targets the 3’ UTR of HSF1, indicated plasmids were co-transfected along with the dual HSF1 reporter system. After 48 hr, the reporter activities in culture media were measured, and SEAP activities were normalized against GLuc activities (mean±SD, n=3 experiments, One-way ANOVA). The expression of HSF1WT and HSF1S230A was detected by immunoblotting. (R) HEK293T cells stably expressing HSF1-targeting shRNAs (A6) were co-transfected with indicated plasmids along with the dual reporter plasmids. After 16 hr, transfected cells were pre-treated with 20μM MK2206 for 3 hr, followed by heat shock at 43°C for 30 min. Forty-eight hours after heat shock, the reporter activities in culture media were measured, and SEAP activities were normalized against GLuc activities (mean±SD, n=3 experiments, One-way ANOVA). (S) Following transfection with FLAG-HSF1WT or -HSF1S230A plasmids and heat shock at 43°C for 30 min, the cytosolic and nuclear fractions of HEK293T cells were prepared, and FLAG-HSF1 was detected by immunoblotting (representative images of three experiments). LDH and Lamin A/C were used as the cytosolic and nuclear markers, respectively. C: cytosolic; N: nuclear. (T) HEK293T cells stably expressing HSF1-targeting shRNAs (A6) were transfected with either FLAG-HSF1WT or -HSF1S230A plasmids. After 48 hr, the binding of HSF1 to the HSP72 and HSP27 promoters was quantitated by ChIP-qPCR (mean±SD, n=3 experiments, One-way ANOVA). (U) Both heat stress and oncogenic stimuli converge on the AKT-mediated HSF1 activation, which manages to sustain the proteomic stability. (C), (O), and (P) were done once; all the others were repeated thrice.
Figure S2: HSF1 is required for megalencephaly driven by constitutively active PI3K.

(A) Measurement of global protein translation rate in cultured astrocytes by puromycin labeling. The labeling fluorescence intensity (FL1-H) was quantitated by FACS and represented as geometric means (mean±SD, n=3 lines of astrocytes each genotype, One-way ANOVA). The histogram represents a single line. (B) Quantitation of DNA content in frozen mouse brain tissues (mean±SD, n=5 mice per group, One-way ANOVA). (C) Immunoblotting of neuronal, astrocytic, and microglial markers in the lysates of whole mouse brains (three mice per group). (D) Microglial activation and neuronophagia, indicated by the arrows, in P*H* brains (representative images of three brains each genotype). Scale bars: 20µm. (E) Flowchart of the fractionation procedures and validation of the fractionation method by immunoblotting using mouse brains. Three individual p110* -expressing Hsf1+/+ brains were tested. β-Actin served as the marker for detergent-soluble fractions, E-cadherin and TOM20 served as the markers for membrane-associated fractions, and β-amyloid served as the marker for detergent-insoluble fractions. For the soluble and insoluble fractions, 40μg proteins were loaded, and only 20μg proteins were loaded for the membrane-associated fractions. Of note, some Histone remained insoluble. Detergent-insoluble fractions were re-solubilized by sonication before loading for SDS-PAGE. (F) Quantitation of PAOs in the membrane-associated fractions of brain lysates by ELISA (mean±SD, n=5 mice per group, One-way ANOVA). (G) Representative images of frozen brain sections stained with anti-Aβ1-42 oligomer Abs or TUNEL assays (from three brains of each genotype and the TUNEL staining was performed by two individuals). Scale bars: 100µm for main images; 10µm for insets. (H) Quantitation of endogenous mouse Aβ1-42 in the insoluble fractions of mouse brain lysates (mean±SD, n=5 mice per group, One-way ANOVA). (I) Validation of the specificity of anti-Aβ (ab2539) antibodies. Upper panel: immortalized Rosa26-CreERT<sup>2</sup>; Hsf1<sup>fl/fl</sup> MEFs, treated with 4-OHT to delete Hsf1, were transfected with either 10µM control Aβ12-1 or 10µM mouse Aβ1-42 overnight, followed by staining with anti-Aβ (ab2539) Abs (representative images of three experiments). Scale bars: 10µm. Lower panel: the paraffin sections of P*H* brains were stained with the mixture of ab2539 and the control Aβ12-1 or mouse Aβ1-42 peptides at a 1:20 molar ratio (representative images of three brains). Sale bars: 100µm. (J) Representative images of intracellular Aβ accumulation, plaque-like Aβ deposits, and amyloid angiopathy in P*H* brains (from three brains). Arrowheads denote amyloid angiopathy. Scale bars: 20µm for main images and 10µm for insets. (K) Neurodevelopmental defects and neuronal loss in p110* -expressing brains revealed by H&E and Nissl staining, respectively (images of a single experiment). Scale bars: 100µm. (L) and (M) Quantitation of soluble A11+ PAOs and caspase 3 activities in cultured astrocytes (mean±SD, n=3 lines of astrocytes each genotype, One-way ANOVA). The caspase 3 activities were measured using a SensoLyte<sup>®</sup> Homogeneous Rh110 Caspase-3/7 Assay Kit. (N) and (O) Quantitation of free soluble PAOs and insoluble AFs in p110* -expressing astrocytes treated with and without 10µM CR for two days, as described in Fig. 2A (mean±SD, n=3 lines of astrocytes each genotype, One-way ANOVA). (P) Measurement of the cell size of Pten-deficient astrocytes with and without Hsf1 deletion (mean±SD, n=3 lines of astrocytes each genotype, two-tailed Student’s t test). (Q) Measurement of the translation rate of Pten-deficient astrocytes with and without Hsf1 deletion by puromycin labeling (mean±SD, n=3 lines of astrocytes each genotype, two-tailed Student’s t test). (B), (C), (H), and (K) were done once; (F) was repeated twice; and all the others were repeated thrice with different sets of astrocytes or brains.
Figure S3: HSF1 suppresses amyloidogenesis in livers with constitutively active PI3K or PTEN deficiency.

(A) Quantitation of PAOs in the membrane-associated fractions of livers expressing p110* by ELISA (mean±SD, n=5 mice per group, One-way ANOVA). (B) Quantitation of endogenous Aβ1-42 levels in the soluble fractions of mouse liver lysates expressing p110* by ELISA (mean±SD, n=5 mice per group, One-way ANOVA). (C) Quantitation of PAOs in the membrane-associated fractions of livers deficient in Pten by ELISA (mean±SD, n=5 mice per group, One-way ANOVA). (D) Representative images of frozen mouse liver sections stained with anti-Aβ oligomer Ab, Congo red (CR), or anti-cleaved caspase 3 Abs (from three livers of each genotype). Scale bars: 50µm for main images; 10µm for insets.

(B) was done once; (A) and (C) were repeated twice; and (D) was repeated thrice with different sets of livers.
Figure S4: Loss of HSP60 function leads to mitochondrial damage, mitophagy, and apoptosis. 

(A) Measurement of global protein translation rate by puromycin labeling in p110*-expressing astrocytes treated with and without 50µM 4EGI-1 or 20µM LY2584702 overnight. The histogram represents a single experiment. (B) Quantitation of amyloid levels in Pten-deficient astrocytes with Hsf1 deletion treated with and without 50µM 4EGI-1 or 20µM LY2584702 overnight (mean±SD, n=3 lines of astrocytes of each genotype, One-way ANOVA). (C) Measurement of the mitochondrial membrane potentials of Pten-deficient astrocytes with Hsf1 deletion treated with and without 50µM 4EGI-1 or 20µM LY2584702 for four days (mean±SD, n=3 lines of astrocytes of each genotype, One-way ANOVA). (D) Detection of HSP60 aggregates in whole brain lysates by filter-trap assays (three mice per group). (E) Quantitation of mitochondrial mass in astrocytes treated with and without 10µM CR or 20µM CQ for six days by FACS using MitoView™ Green. The histogram depicts a single experiment, and three independent experiments are summarized in Fig. 5A. (F) JC-1 Red/Green (FL2-H/FL1-H) fluorescence ratios of p110*-expressing astrocytes transduced with lentiviral LacZ or HSP60 at a MOI=10 for six days (mean±SD, n=3 lines of astrocytes of each genotype, two-tailed Student’s t test). (G) JC-1 Red/Green fluorescence ratios of Pten-deficient astrocytes with Hsf1 deletion transduced with lentiviral LacZ or HSP60 at a MOI=10 for six days (mean±SD, n=3 lines of astrocytes of each genotype, two-tailed Student’s t test). (H) Measurement of the mitochondrial membrane potentials in hGFAP-Cre⁺; Hsf1⁺/⁻ astrocytes transfected with control or Hsp60-targeting siRNAs for four days (representative contour plot of three lines of astrocytes). (I) Detection of HSP60 and TOM20 in the detergent-soluble and detergent-insoluble fractions of Pten-deficient livers by immunoblotting (three mice of each genotype). (J) Detection of HSP60 aggregates in Pten-deficient livers by filter-trap assays (three mice of each genotype). (A), (D), (I), and (J) were done once; the others were repeated thrice with different sets of astrocytes. (G) was repeated by two individuals.
Figure S5

A. hGFAP-Cre: PI3K p110^−^\textsuperscript{;}\ Hsf1^+^/\textsuperscript{−}^; Hsp60 γ\textsuperscript{−}\textsuperscript{−}^/\textsuperscript{+}^; IP Abs: IgG, A11. β-amyloid (05403), Tau, IgG HC.

B. hGFAP-Cre: PI3K p110^−^\textsuperscript{;}\ Hsf1^+^/\textsuperscript{−}^; IP Abs: IgG, 4G8. β-amyloid, HSP60, HSF1, IgG HC.

C. hGFAP-Cre: PI3K p110^−^\textsuperscript{;}\ Hsf1^+^/\textsuperscript{−}^; IP Abs: IgG, HSF1. β-amyloid, HSP60, Tau, IgG HC.

D. Adα42\textsubscript{1}\textsuperscript{+}, Adα42\textsubscript{4}\textsuperscript{+}, HSF1^+^/\textsuperscript{−}^; 4G8, ab2539, D54D2.

E. Alb-Cre: Pten^+^/\textsuperscript{−}^; Hsf1^+^/\textsuperscript{−}^; IP Abs: IgG, A11. HSP60, HSF1, IgG HC.

F. Alb-Cre: Pten^+^/\textsuperscript{−}^; Hsf1^+^/\textsuperscript{−}^; IP Abs: IgG, OC. HSP60, HSF1, IgG HC.

G. siControl, siHsp60_A, siHsp60_B. HSP60, Nuclei.

H. HSF1^+^/\textsuperscript{−}^, HSF1^+^/\textsuperscript{−}^. Nuclei, HSP60 PLA.

I. GST: h–HSF1, αβ peptides: 42-1, 42-2, 1-42, 1-42. HSP60, β-amyloid (05403). His-HSF1, Ponceau red.

J. Aβ\textsubscript{42}\textsubscript{1}\textsuperscript{−}, Aβ\textsubscript{42}\textsubscript{4}. Supernatant, Pellets.

K. Soluble HSF1 inputs (ng/g total proteins). n.s., HGFAP-Cre: PI3K p110^−^\textsuperscript{;}\ Hsf1^+^/\textsuperscript{−}^.

L. Relative changes in soluble HSP60 inputs (%). n.s., HGFAP-Cre: PI3K p110^−^\textsuperscript{;}\ Hsf1^+^/\textsuperscript{−}^.

M. 4A4 Relative fluorescence ratio. n.s., n.s., n.s., HGFAP-Cre: PI3K p110^−^\textsuperscript{;}\ Hsf1^+^/\textsuperscript{−}^.

N. hGFAP-Cre: PI3K p110^−^\textsuperscript{;}\ Hsf1^+^/\textsuperscript{−}^; IP Abs: IgG, A11. HSP60, FLAG, IgG HC.

O. Adeno-Cre: shRNA: Hsf1^+^/\textsuperscript{−}^, LacZ^+^/\textsuperscript{−}^; 1-329, 1-323, 324-329.
Figure S5: HSF1 protects HSP60 from AO attack.

(A) Detection of PAOs of Aβ and Tau by IP with the EasyBlot® reagents in p110*-expressing mouse brains (representative images of two sets of brains). (B) and (C) Detection of physical Aβ-HSP60 and Aβ-HSF1 interactions in p110*-expressing mouse brains by co-IP with the EasyBlot® reagents (representative images of three sets of brains). (D) Detection of Aβ1-42 co-incubated with and without recombinant HSF1 proteins at increased molar ratios by dot blotting (representative images of three experiments). (E) and (F) Detection of physical AO-HSF1 and AO-HSP60 interactions in Pten-deficient livers by co-IP with the EasyBlot® reagents (representative images of three sets of livers). (G) Validation of the mouse monoclonal anti-HSP60 antibody (LK1) used for PLA by immunofluorescence in hGFAP-Cre+; Hsf1+/+ astrocytes transfected with control or Hsp60-targeting siRNAs for four days (images of a single experiment). Scale bars: 10µm. (H) Visualization of PAO-HSP60 interactions in cultured astrocytes by PLA using rabbit anti-PAOs (A11) Abs and mouse monoclonal anti-HSP60 (LK1) Abs (representative images of two lines of astrocytes of each genotype). Scale bars: 10µm. A similar result was also observed in Pten-deficient astrocytes following Hsf1 deletion. (I) Detection of HSP60 and HSF1 aggregation due to Aβ1-42 interactions in vitro, as described in Fig. 6C, by filter-trap assays (representative images of three experiments). (J) Detection of the insolubility of HSPs in the presence of Aβ1-42 in vitro. Recombinant HSP60, HSP90β, HSP72, HSP27, and HSP10 proteins were incubated with either Aβ42-1 or Aβ1-42 at a 1:1 molar ratio at RT for 4 hr (representative images of three experiments). (K) Quantitation of the absolute levels of HSF1, Aβ42, and HSP60 in 10µg of mouse brain lysates by commercial ELISA kits (mean±SD, n=5 mice per genotypic group, One-way ANOVA). (L) Quantitation of soluble AOs in P*H- mouse brain lysates before and after IP with Aβ (D54D2) Abs (n=5 mice, two-tailed paired Student’s t test). Normal rabbit IgG served as the control. (M) Measurement of the mitochondrial membrane potentials of Pten-deficient astrocytes with Hsf1 deletion transduced with lentiviral HSF1 at a MOI=10 for six days by JC-1 staining (mean±SD, n=3 lines of astrocytes of each genotype, One-way ANOVA). (N) Detection of physical PAO-HSF1 and PAO-HSP60 interactions by co-IP with the EasyBlot® reagents in astrocytes transduced with lentiviral LacZ, HSF11-529, HSF11-323, or HSF1324-529 at a MOI=10 for six days (images of a single experiment). (G), (L), and (N) were done once; (A) and (H) were repeated twice with different sets of brains or astrocytes; (K) was repeated twice; (B), (C), (E), (F), and (M) were repeated thrice with different sets of tissues or astrocytes; and (D), (I), and (J) were repeated thrice with the same reagents.
Figure S6

A

- GST+Aβ1-42
- HS1+Aβ1-42
- GST+Aβ2-1
- HS1+Aβ2-1

Fold changes in THT fluorescence

Hours

B

- Aβ1-42+GST
- Aβ1-42+HS1
- Aβ1-42+HSF1

***

n.s.

Fold changes in A11-positive PA0 levels

C

- Aβ1-42+GST
- Aβ1-42+HS1
- Aβ1-42+HSF1

***

n.s.

Fold changes in OC-positive insoluble AF levels

D

- Aβ1-42 + IgG (1:4)
- Aβ1-42 + OC (1:1)
- Aβ1-42 + OC (1:2)
- Aβ1-42 + OC (1:4)

Fold changes in THT fluorescence

Hours

E

- Aβ1-42: GST
- Aβ1-42: HSF1 (1:1)
- Aβ1-42: HSF1 (1:2)
- Aβ1-42: HSF1 (1:4)

***

F

- Aβ1-42+GST (1:32)
- Aβ1-42+HSF1 (1:32)

G

- hGFAP-Cre
- P300 p110
- Hsf1: +/+  fl/fl

Nephelometric turbidity units (NTUs)

H

- Aβ1-42+GST
- Aβ1-42+HSP60
- Aβ1-42+GST
- Aβ1-42+HSP60

Fold changes in THT fluorescence

I

- Aβ1-42+GST
- Aβ1-42+HSP60
- Aβ1-42+GST
- Aβ1-42+HSP60

Fold changes in A11-positive PA0 levels

J

- Aβ1-42+GST
- Aβ1-42+HSP60
- Aβ1-42+GST
- Aβ1-42+HSP60

Fold changes in OC-positive insoluble AF levels

K

- GST
- HSP60
- HSP90B
- HSP90
- HSP72
- HSP27
- HSP10

Fold changes in THT fluorescence

Hours
Figure S6: HSF1 impairs amyloidogenesis through physical interactions.

(A) Measurements of the fibrillation of 2µM Aβ1-42 incubated with recombinant GST or HSF1 proteins in vitro at a 1:1 molar ratio (mean±SD, n=3 experiments, Two-way ANOVA). Non-amyloidogenic Aβ42-1 peptides served as the negative control. The curves are fitted with the Boltzmann sigmoid equation. (B) and (C) Quantitation of PAOs and AFs formed by 2µM Aβ1-42 described in (A) by ELISA (mean±SD, n=3 experiments, One-way ANOVA). (D) Measurements of the fibrillation of 0.8µM Aβ1-42 incubated with OC Abs in vitro at increasing molar ratios (mean±SD, n=3 experiments, Two-way ANOVA). Normal rabbit IgG served as the control. The curves are fitted with the Boltzmann sigmoid equation. (E) Detection of protein aggregates in the experiments described in Fig. 8B by filter-trap assays (representative images of three experiments). The yellow color is due to ThT. Photo credit: Zijian Tang, NCI. (F) Dynamic measurements of the nephelometric turbidities of 0.2µM Aβ1-42 incubated with GST or HSF1 at a 1:32 molar ratio for 48 hr (mean±SD, n=2 experiments, Two-way ANOVA). (G) Measurements of the nephelometric turbidities of detergent-soluble brain lysates prior to incubation at 37°C (mean±SD, n=5 mice per group, One-way ANOVA). (H) In vitro fibrillation of Aβ1-42 incubated with either recombinant GST or HSP60 proteins at a 1:1 molar ratio (mean±SD, n=3 experiments, Two-way ANOVA). Non-amyloidogenic Aβ42-1 peptides served as the negative control. The curves are fitted with the Boltzmann sigmoid equation. (I) and (J) Quantitation of PAOs and AFs formed in (H) by ELISA (mean±SD, n=3 experiments, One-way ANOVA). (K) In vitro fibrillation of Aβ1-42 incubated with either recombinant GST or various HSP proteins at a 1:1 molar ratio (mean±SD, n=3 experiments, Two-way ANOVA). The curves are fitted with the Boltzmann sigmoid equation. (G) was done once; (F) was repeated twice; and all the others were repeated thrice with the same reagents.
Figure S7: Human AD brains display elevated AOs and apoptosis but diminished HSF1 and HSP60 proteins.

(A) Validations of the mouse anti-biotin and rabbit anti-HSP60 Abs in HeLa cells by immunofluorescence (images of a single experiment). Following transfection of 1µM non-biotinylated (NB) or biotinylated Aβ1-42 overnight, cells were stained with mouse monoclonal anti-biotin (BTN.4) Abs. Following transfection of 10nM HSP60-targetting siRNAs for four days, HeLa cells were stained with rabbit anti-HSP60 (D6F1) Abs. Scale bars: 10µm. (B) and (C) AD and normal control brain sections on tissue arrays were stained with A11 (B) and OC (C) Abs, respectively (images of a single experiment). Scale bars: 100µm for main images; 10µm for insets. (D) AD and normal control brains were stained with rabbit anti-cleaved caspase 3 (Asp175) (representative images of two experiments). Scale bars: 50µm for main images; 10µm for insets. (E) Validations of the mouse monoclonal anti-HSP60 (LK1) Ab in HeLa cells, transfected with 10nM HSP60-targetting siRNAs for four days, by immunofluorescence (images of a single experiment). Scale bars: 10µm. (F) AD and normal control brain sections on tissue arrays were stained with mouse monoclonal anti-Aβ17-24 (4G8) Abs (images of a single experiment). A similar result was observed for anti-Aβ1-14 (ab2539) Abs. The 4G8 antibody detected numerous amyloid plaques in AD QC control slides. Scale bars: 100µm. (G) Visualization of HSP60-AOs interactions (brown) in AD patients’ brains by brightfield PLA using a mouse monoclonal anti-HSP60 Ab (LK1) and the rabbit polyclonal anti-AOs (OC) Ab (representative images of two experiments). Scale bars: 20µm for low magnification; 10µm for high magnification. (H) AD and normal control brains on tissue arrays were stained with rabbit monoclonal anti-HSF1 (EP1710Y) Abs (representative images of three experiments). Scale bars: 100µm. (I) AD and normal control brains on tissue arrays were stained with anti-HSP60 (D6F1) Abs (representative images of four experiments). Scale bars: 100µm. (A)–(C), (E), and (F) were done once; (D) and (G) were repeated twice; and (H) and (I) were repeated three and four times, respectively.
Fig. 2B
Fig. 4F

Insoluble HSP60

Soluble β-Actin

Soluble HSP60

p-Tau S404

Insoluble Aβ

Insoluble Tau

Soluble TOM20

Soluble HSF1
Fig. 5

**K48 Polyub**

**TOM20**

**HSP60**

**HSF1**

**PARKIN**

**Cytochrome c**

**β-Actin**
Fig. 6A
Supernatant IgG HC

Pellet Aβ

Pellet HSP60

Supernatant HSP60

Fig. 6D
**Fig. 6E**

Soluble βActin

Insoluble HSF1

Soluble HSF1

Insoluble HSP60

Soluble HSP60

**Aβ**

- **Aβ**
  - **Aβ**
  - **Aβ**
  - **Aβ**

- **Aβ**
  - **Aβ**
  - **Aβ**

- **Aβ**
  - **Aβ**

- **Aβ**

- **Aβ**
  - **Aβ**

- **Aβ**

**72KD**

**55KD**

**95KD**

**72KD**

**55KD**

**43KD**
**Fig. 1A**

- **Iped HSP60**
- **IgG HC**
- **Iped HSF1**
- **HSP60**
- **HSP72**
- **HSP27**
- **βActin**
- **GST**
- **95KD**
- **72KD**
- **55KD**
- **43KD**
- **34KD**
- **26KD**
- **17KD**

**Whole brain**
**Figure 11**

**IgG HC**

**K48 PolyUb**

Hippocampus

**HSP60**

**HSF1**

**HSF1 Ab**

**IgG Ab**

**Western Blot**

**55KD**

**43KD**

**95KD**

**72KD**

**55KD**

**43KD**

**34KD**

**26KD**

**17KD**

**10KD**

**180kDa**

**250kDa**

**130kDa**

**95kDa**

**72kDa**

**55kDa**

**43kDa**

**34kDa**

**26kDa**

**17kDa**

**10kDa**
Fig. S1C

HSP25

HSP72

β-Actin

DMSO
LY294002
MK2206
RG7440

DMSO
LY294002
MK2206
RG7440

NHS
HS

DMSO
LY294002
MK2206
RG7440

DMSO
LY294002
MK2206
RG7440

NHS
HS

DMSO
LY294002
MK2206
RG7440

DMSO
LY294002
MK2206
RG7440

NHS
HS

55KD
43KD

72KD
55KD

26KD
17KD
**Fig. S1J**

HSF1

IP Abs:
PI3K  
AKT  
AKT+HS

IPed AKT

IP Abs:
PI3K  
AKT  
AKT+HS

IPed HSF1

IP Abs:
PI3K  
AKT  
AKT+HS

IgG HC

IP Abs:
PI3K  
AKT  
AKT+HS

βActin

55KD  43KD  72KD  55KD  43KD  72KD  95KD

NHS  72KD  55KD  43KD  72KD  95KD

P-AKT T308  
P-AKT S473  
AKT

NHS  HS

βActin

55KD  43KD  72KD  55KD  43KD  72KD  95KD

NHS  72KD  55KD  43KD  72KD  95KD

P-Akt

NHS  HS
Fig. S1N

p-HSF1 S326

His-HSF1

GST
MEK1
AKT1

GST
MEK1
AKT1

95KD
72KD

72KD
95KD
Figure S8: Uncropped immunoblot images.
Images highlighted in red fonts were captured by an iBright™ FL1000 imaging system. Photo credit: Zijian Tang, NCI.
Table S1: Information of human tissues used in this study.

| Tissue Types                                      | Disease State               | Sex | Age (years) |
|--------------------------------------------------|-----------------------------|-----|-------------|
| Brain (paraffin sections)                        | Alzheimer’s                 | M   | 73          |
| Brain (paraffin sections)                        | Alzheimer’s                 | M   | 72          |
| Brain (paraffin sections)                        | Alzheimer’s                 | M   | 88          |
| Brain (paraffin sections, positive control for amyloid plaques) | Alzheimer’s                 | N/A | N/A         |
| Brain (total lysates)                            | Alzheimer’s                 | M   | 65          |
| Brain (Hippocampus lysates)                      | Alzheimer’s                 | F   | 93          |
| Brain (paraffin sections)                        | Normal aged control         | M   | 54          |
| Brain (paraffin sections)                        | Normal aged control         | F   | 54          |
| Brain (paraffin sections)                        | Normal aged control         | M   | 73          |
| Brain (total lysates)                            | Normal aged control         | M   | 82          |
| Brain (Hippocampus lysates)                      | Normal aged control         | M   | 71          |
Table S2: Detailed information of all experimental materials.

| Antibodies | SOURCE | IDENTIFIER |
|------------|--------|------------|
| Anti-phospho-AKT Thr380 (D25E6) | Cell Signaling Technology | Cat#: 13038 |
| Anti-phospho-AKT Ser473 (D9E) | Cell Signaling Technology | Cat#: 4060 |
| Anti-HSF1 (H-311) | Santa Cruz Biotechnology | Cat#: sc-9144 |
| Anti-Pi3K p110α (C73F8) | Cell Signaling Technology | Cat#: 4249 |
| Anti-AKT (pan) (C67E7) | Cell Signaling Technology | Cat#: 4691 |
| Anti-HSF1 (E-4) | Santa Cruz Biotechnology | Cat#: sc-17757 |
| Anti-HSF1 (10H8) | Santa Cruz Biotechnology | Cat#: sc-30443-R |
| Anti-phospho-HSF1 Ser230 | Santa Cruz Biotechnology | Cat#: 9188 |
| Anti-βActin (GT5512) | GeneTex | Cat#: GTX629630 |
| Anti-PTEN (D4.3) | Cell Signaling Technology | Cat#: 14793 |
| Anti-DYKDDDDK Tag (FLAG) (D6W5B) | Cell Signaling Technology | Cat#: ADI-SPA-812 |
| Anti-HSP72 | Enzo Life Science | Cat#: ADI-SPA-801 |
| Anti-HSP25 | Enzo Life Science | Cat#: sc-13516 |
| Anti-phospho-HSF1 Ser326 (EP1713Y) | Abcam | Cat#: ab76076 |
| Anti-LDH (EP1563Y) | Abcam | Cat#: ab134187 |
| Anti-Lamin A/C (4C11) | Cell Signaling Technology | Cat#: 4777 |
| Anti-MCM2 (D7G11) | Cell Signaling Technology | Cat#: 3619 |
| Anti-PCNA (PC10) | Cell Signaling Technology | Cat#: 2586 |
| Anti-phospho-p70 S6K Thr389 (108D2) | Cell Signaling Technology | Cat#: 9234 |
| Anti-p70 S6K (49D7) | Cell Signaling Technology | Cat#: 2708 |
| Anti-β III tubulin (AA10) | STEMCELL Technologies | Cat#: 60100 |
| Anti-AMPA1/GluA1 (D4N9V) | Cell Signaling Technology | Cat#: 13185 |
| Anti-NMDAR1/GluN1 (D65B7) | Cell Signaling Technology | Cat#: 5704 |
| Anti-PSD95 (D27E11) | Cell Signaling Technology | Cat#: 3450 |
| Anti-GFAP (E4L7M) | Cell Signaling Technology | Cat#: 80788 |
| Anti-Synaptophysin (D8F6H) | Cell Signaling Technology | Cat#: 36406 |
| Anti-Iba-1/AIF1 | GeneTex | Cat#: GTX100042 |
| Anti-amyloid oligomer (A11) | StressMarq Biosciences | Cat#: SPC-506D |
| Biotin-conjugated Anti-amyloid oligomer (A11) | StressMarq Biosciences | Cat#: SPC-506D-BI |
| Anti-amyloid fibrils (OC) | StressMarq Biosciences | Cat#: SPC-507D |
| Biotin-conjugated Anti-amyloid fibrils (OC) | StressMarq Biosciences | Cat#: SPC-507D-BI |
| Anti-cleaved caspase 3 (Asp175) (5A1E) | Cell Signaling Technology | Cat#: 9664 |
| Anti-E-cadherin (24E10) | Cell Signaling Technology | Cat#: 3195 |
| Anti-TOM20 (D8T4N) | Cell Signaling Technology | Cat#: 42406 |
| Anti-Histone H3 (D1H2) | Cell Signaling Technology | Cat#: 4499 |
| Anti-β-amyloid, 17-24 (4G8) | BioLegend | Cat#: 800701 |
| Anti-β-amyloid, 1-14 | Abcam | Cat#: ab2539 |
| Antibody/Reagent Description                                           | Vendor                                    | Catalog Number |
|---------------------------------------------------------------------|-------------------------------------------|----------------|
| Anti-β-amyloid (D54D2)                                              | Cell Signaling Technology                 | Cat#: 8243     |
| Anti-Aβ1-42, oligomer specific                                      | GeneTex                                  | Cat#: GTX134510|
| Anti-HSP60 (D6F1)                                                   | Cell Signaling Technology                 | Cat#: 12165    |
| Anti-HSP60 (LK1)                                                    | EMD Millipore                            | Cat#: MAB3514   |
| Anti-Tau (Tau46)                                                    | Cell Signaling Technology                 | Cat#: 4019     |
| Anti-phospho-Tau (Ser404) (D2Z4G)                                   | Cell Signaling Technology                 | Cat#: 35834    |
| Anti-Lys48 polyubiquitin (Apu2)                                     | EMD Millipore                            | Cat#: 05-1307  |
| Anti-β III tubulin (2G10-TB3), Alexa Fluor 488                     | Thermo Fisher Scientific                  | Cat#: 53-4510-80|
| Anti-Parkin (Prk8)                                                  | Cell Signaling Technology                 | Cat#: 4211     |
| Anti-Cytochrome C (7H8.2C12)                                       | Thermo Fisher Scientific                  | Cat#: 33-8500  |
| Anti-HSP90α/β                                                       | Enzo Life Science                        | Cat#: ADI-SPA-846-D|
| Anti-GST (91G1)                                                     | Cell Signaling Technology                 | Cat#: 2625     |
| Anti-HSP27                                                          | Enzo Life Science                        | Cat#: ADI-SPA-803|
| Anti-HSP10                                                          | Enzo Life Science                        | Cat#: ADI-SPA-110-D|
| Anti-Biotin (BTN.4)                                                 | Thermo Fisher Scientific                  | Cat#: MA5-11251|
| Anti-DYKDDDDKD Tag, DyLight 680                                     | Thermo Fisher Scientific                  | Cat#: MA1-91878-D680|
| Anti-DYKDDDDKD Tag, Alexa Fluro 488                                 | Cell Signaling Technology                 | Cat#: 15008    |
| Anti-HSF1 (EP1710Y)                                                 | Abcam                                    | Cat#: ab52757  |
| Anti-Aβ1-42 (mOC98)                                                 | Abcam                                    | Cat#: ab201061 |
| Normal mouse and rabbit IgG                                         | Santa Cruz Biotechnology                  | Cat#: sc-2025 and sc-2027|
| Peroxidase AffiniPure Goat Anti-Rabbit IgG (H+L)                   | Jackson ImmunoResearch                    | Cat#: 111-035-144|
| Peroxidase AffiniPure Goat Anti-Mouse IgG (H+L)                    | Jackson ImmunoResearch                    | Cat#: 115-035-003|
| Peroxidase AffiniPure Goat Anti-Rat IgG (H+L)                      | Jackson ImmunoResearch                    | Cat#: 112-035-143|
| Duolink® In Situ PLA® anti-rabbit Plus probes                      | Sigma-Aldrich                            | Cat#: DUO92002 |
| Duolink® In Situ PLA® anti-mouse MINUS probes                      | Sigma-Aldrich                            | Cat#: DUO92004 |
| CF®594 Donkey anti-mouse IgG (H+L)                                  | Biotium                                  | Cat#: 201115   |
| CF®594 Donkey anti-rabbit IgG (H+L)                                 | Biotium                                  | Cat#: 20152    |
| CF®488A Donkey anti-mouse IgG (H+L)                                 | Biotium                                  | Cat#: 20014    |
| CF®488A Donkey anti-rabbit IgG (H+L)                                | Biotium                                  | Cat#: 20015    |

**Cell Culture Reagents, Chemicals, Peptides, and Recombinant Proteins**

| Reagent Description                                                | Vendor                                    | Catalog Number |
|---------------------------------------------------------------------|-------------------------------------------|----------------|
| Accumax Cell Dissociation Solution                                  | Innovative Cell Technologies              | Cat# AM105     |
| HyClone™ bovine growth serum                                         | HyClone Laboratories                      | Cat# SH30541.03IR|
| LY294002                                                            | Selleck Chemicals                        | Cat#: S1105    |
| MK2206                                                              | ApexBio                                  | Cat#: A3010    |
| RG7440 (Ipatasertib)                                                | ApexBio                                  | Cat#: A3006    |
| Product Name                                      | Supplier                              | Cat#          |
|--------------------------------------------------|---------------------------------------|---------------|
| Halt™ phosphatase inhibitor cocktail             | Thermo Fisher Scientific               | Cat#: 78420   |
| Halt™ protease inhibitor cocktail                | Thermo Fisher Scientific               | Cat#: 87785   |
| ActinRed™ 555 ReadyProbes™ Reagent              | Thermo Fisher Scientific               | Cat#: R37112  |
| Hoechst 33342                                    | Thermo Fisher Scientific               | Cat# H1399    |
| 2% uranyl acetate solution                       | Electron Microscopy Sciences           | Cat# 22400-2  |
| SuperSignal West Pico PLUS or Femto chemiluminescent substrates | Thermo Fisher Scientific               | Cat#34580 or 34095 |
| Protein G MagBeads                               | GenScript                             | Cat#L00274    |
| Recombinant active AKT1, AKT2, and AKT3 proteins | SignalChem                            | Cat#: A16-10G-10, A17-10G-10, A18-10G-10 |
| Recombinant active MEK1 proteins                 | SignalChem                            | Cat#: M02-10G-10 |
| TurboFect™ transfection reagents                 | Thermo Fisher Scientific               | Cat#R0531     |
| Mission® siRNA transfection reagent              | Sigma-Aldrich                         | Cat#: S1452    |
| jetPRIME® transfection reagent                   | Polyclon-transfection® SA             | Cat#: 114-15   |
| RNA STAT-60™ reagent                             | Tel-Test, Inc.                        | Cat#: CS-111   |
| Xfect™ Protein Transfection Reagent              | Takara Bio USA                        | Cat#: 631324   |
| 6-FAM-dc-puromycin                               | Jena Bioscience                       | Cat#: NU-925-6FM |
| 1-Step™ Ultra TMB-ELISA substrates               | Thermo Fisher Scientific               | Cat#: 34029    |
| Synthetic human Aβ1-42 peptides                  | GenScript                             | Cat#: RP10017  |
| Thioflavin T (ThT)                               | Thermo Fisher Scientific               | Cat#: AC211760050 |
| BLOXALL blocking solution                        | Vector Laboratories                   | Cat#: SP-6000  |
| Mouse on mouse (M.O.M) blocking reagents         | Vector Laboratories                   | Cat#MKB-2213  |
| Congo Red (CR)                                   | Thermo Fisher Scientific               | Cat#: C580-25  |
| 4EGI-1                                           | EMD Millipore                         | Cat#: 324517-10MG |
| LYT2584702                                       | BioVision                             | Cat#: 9445-25  |
| Pan-caspase inhibitor (CI), Q-VD-OPH             | APEX BIO                              | Cat#: A1901    |
| MitoView™ Green dyes                             | Biotium                               | Cat#: 70054    |
| Chloroquine diphosphate (CQ)                     | Axxora                                | Cat#: LKT-C2950-G025 |
| Synthetic Aβ42-1 peptides                        | AnaSpec                               | Cat#: AS-27275 |
| Recombinant GST proteins                         | SignalChem                            | Cat#: G52-30U-50 |
| Recombinant human HSF1 proteins                  | Enzo Life Science                     | Cat#: ADI-SPP-900-F |
| Recombinant human HSP60 proteins                 | R&D Systems                           | Cat#: AP-140-050 |
| Recombinant human HSP90β                         | Enzo Life Science                     | Cat#: ALX201147C025 |
| Recombinant human HSP72 proteins                 | Enzo Life Science                     | Cat#: ADI-SPP-715-D |
| Recombinant human HSP27 proteins                 | Enzo Life Science                     | Cat#: ADI-NSP-555-D |
| Recombinant human HSP10 proteins                 | Enzo Life Science                     | Cat#: ADI-SPP-110-D |
| HiLyte™ Fluor 488-labeled human Aβ1-42           | AnaSpec                               | Cat#: AS-60479-01 |
| DABCYL acid, SE                                  | AnaSpec                               | Cat#: AS-81801  |
| Synthetic human Biotin-Aβ1-42 peptides           | AnaSpec                               | Cat#: AS-23523-05 |
| Product Description | Supplier | Cat# |
|---------------------|----------|------|
| Synthetic human Biotin-Ab42-1 peptides | GenScript | Custom synthesis |
| Poly-L-Lysine | ScienCell Research Laboratories | Cat#: 0403 |
| Purified mouse laminin | EMD Millipore | Cat#: CC095 |
| **Commercial Kits** | | |
| EasyBlot anti-Rabbit or anti-Mouse IgG Kits | GeneTex | Cat# GTX225856-01, GTX225857-01 |
| MycoAlert™ Mycoplasma Detection kits normocin | Lonza | Cat# LT07-418 |
| Complete neuronal medium | ScienCell Research Laboratories | Cat# 1521 |
| Pierce™ BCA Protein Assay Kit | Thermo Fisher Scientific | Cat#: 23225 |
| NovaBright™ Phospha-Light™ EXP Assay Kit for SEAP | Thermo Fisher Scientific | Cat#: N10578 |
| Pierce™ Gaussia Luciferase Glow Assay Kit | Thermo Fisher Scientific | Cat#: 16160 |
| Duolink® In Situ Detection Reagents Red, Green, or Brightfield | Sigma-Aldrich | Cat#: DUO92008, DUO92014, DUO92012 |
| Verso cDNA Synthesis kit | Thermo Fisher Scientific | Cat#: AB1453B |
| DyNaMo HS SYBR Green qPCR kit | Thermo Fisher Scientific | Cat#: F410L |
| Q5® Site-Directed Mutagenesis Kit | New England Biolabs | Cat#: E0554S |
| NE-PER™ Nuclear and Cytoplasmic Extraction Kit | Thermo Fisher Scientific | Cat#: 78835 |
| NucleoSpin® TriPrep Kit | Takara Bio USA | Cat#: 740966.50 |
| Detergent-free Nuclei Isolation Kit | 101Bio, LLC | Cat#: P524-20 |
| Caspase-3 Colorimetric Assay Kit | R&D Systems | Cat#: K106-100 |
| Caspase 3 DEVD-R110 Fluorometric and Colorimetric Assay Kit | Biotium | Cat#: 30008-2 |
| NeuroTACS™ In Situ Apoptosis Detection Kit | R&D Systems | Cat#: 4823-30-K |
| JC-1 Mitochondrial Membrane Potential Detection Kit | Biotium | Cat#: 30001 |
| NovaUltra Nissl Stain Kit | IHCWORLD | Cat#: IW-3007 |
| ImmPRESS™ HRP horse anti-rabbit IgG Polymers Detection Kit | Vector Laboratories | Cat#: MP-7401-15 |
| ImmPRESS™-AP Anti-Mouse IgG (alkaline phosphatase) Polymer Detection Kit | Vector Laboratories | Cat#: MP-5402-15 |
| ImmPACT™ DAB Peroxidase (HRP) Substrate Kit | Vector Laboratories | Cat#: SK-4105 |
| ImmPACT™ NovaRED™ Peroxidase (HRP) Substrate Kit | Vector Laboratories | Cat#: SK-4805 |
| ImmPACT® Vector Red Alkaline Phosphatase (AP) substrate | Vector Laboratories | Cat#: SK-5105 |
| Vector Blue Alkaline Phosphatase (Blue AP) Substrate Kit | Vector Laboratories | Cat#: SK-5300 |
| ImmPRESS™ Excel Amplified HRP Polymer Staining Kit (Anti-Rabbit IgG) | Vector Laboratories | Cat#: MP-7601 |
| HSF1 ELISA Kit | Enzo Life Sciences | Cat# ADI-900-198 |
| Amyloid beta 42 Mouse ELISA Kit | Thermo Fisher Scientific | Cat# KMB3441 |
| Mouse HSP60 ELISA Kit | Abcam | Cat# Ab208344 |
| Mitochondrial Isolation Kit | Sigma-Aldrich | Cat#: MITOISO2-1KT |
| Molecular Probes Alexa Fluor™ 594 Microscale Protein Labeling Kit | Thermo Fisher Scientific | Cat#: A30008 |
| Lenti-X™ GoStix™ Plus | Takara Bio USA | Cat#: 631280 |
| CellTiter-Blue® Cell Viability Assay | Promega | Cat# G8080 |

**Cell Lines and Mouse Strains**

- HEK293T cells: GE Dharamcon, Cat#: HCL4517
- HeLa cells: ATCC, Cat#: CCL-2
- A2058 cells: ATCC, Cat#: CRL-11147
- NIH3T3 cells: Lab Collection, N/A
- HEK293T cells stably expressing HSF1-targeting lentiviral shRNAs (A6): Lab Collection, N/A
- Rosa26-CreER<sup>T2</sup>; Hsf1<sup>fl/fl</sup> MEFs (male): Lab Collection, N/A
- hGFAP-Cre<sup>+</sup>; PI3K<sup>p110</sup>* STOP<sup>fl</sup>; Hsf1<sup>+/−</sup> or <sup>fl/fl</sup> astrocytes: This study, N/A
- Hsf1<sup>fl/fl</sup> astrocytes stably expressing Scramble or Pten-targeting shRNAs: This study, N/A
- Primary human neurons: ScienCell Research Laboratories, Cat#: 1520
- Hsf1<sup>fl/fl</sup> mice: Lab Collection, N/A
- R26Stop<sup>p110</sup> mice: The Jackson Laboratory, Stock#: 012343
- hGFAP-Cre mice: The Jackson Laboratory, Stock#: 004600
- Alb-Cre mice: The Jackson Laboratory, Stock#: 016832
- Pten<sup>fl/fl</sup> mice: The Jackson Laboratory, Stock#: 006440

**Oligonucleotides**

- All listed in Table S3: Fisher Scientific and IDT, N/A

**Recombinant DNAs, shRNAs, and siRNAs**

- pHSE-SEAP: Clontech Laboratories, Cat#: 631910
- pCMV-Gaussia Luc: Thermo Fisher Scientific, Cat#: 16147
- pcDNA3-Myr-HA-AKT1: Addgene, Cat#: 9008
- pcDNA3-Myr-HA-AKT2: Addgene, Cat#: 9016
- pcDNA3-Myr-HA- AKT3: Addgene, Cat#: 9017
- pCMV-dR8.2 dvpr: Addgene, Cat#: 8455
- pCMV-VSV-G: Addgene, Cat#: 8454
- pLKO.1-shScramble: Addgene, Cat#: 1864
| Vector/Reagent | Source | Catalog Number |
|----------------|--------|----------------|
| pLKO.1-shPTEN_A | Addgene | Cat#: 25638 |
| pLKO.1-shPTEN_B | Addgene | Cat#: 25639 |
| pLKO-shPten_A | Sigma-Aldrich | Cat#: TRCN0000322421 |
| pLKO-shPten_B | Sigma-Aldrich | Cat#: TRCN0000322487 |
| pLenti6-LacZ | Lab Collection | N/A |
| pLenti6-FLAG-HSF1<sup>WT</sup> | Lab Collection | N/A |
| pLenti6-FLAG-HSF1<sup>S230A</sup> | This study | N/A |
| pLX304-HSP60 | DNASU repository | Cat#: HsCD00442045 |
| siControl | Thermo Fisher Scientific | Cat#: D-001810-01 |
| siAkt1 | Sigma-Aldrich | Cat#: SIHK0096 |
| siAkt2 | Sigma-Aldrich | Cat#: SIHK0099 |
| siAkt3 | Sigma-Aldrich | Cat#: SIHK0102 |
| siHsp60_A | Sigma-Aldrich | Cat#: SASI_Hs01_00136360 |
| siHsp60_B | Sigma-Aldrich | Cat#: SASI_Hs01_00136363 |
| siHSP60_A | Sigma-Aldrich | Cat#: SASI_Hs01_00136360 |
| siHSP60_B | Sigma-Aldrich | Cat#: SASI_Hs01_00136363 |
| pLenti6-FLAG-HSF1<sup>1-323</sup> | This study | N/A |
| pLenti6-FLAG-HSF1<sup>324-529</sup> | This study | N/A |

**Software and Algorithm**

| Software | Source | Catalog Number |
|----------|--------|----------------|
| Prism 8 | GraphPad Software | N/A |
| FlowJo v10 | FlowJo, LLC | N/A |
| Fiji v1.0 | NIH | N/A |

**Others**

| Item | Source | Catalog Number |
|------|--------|----------------|
| Ad5CMVhr-GFP and Ad5CMVCre viral particles | University of Iowa Gene Transfer Vector Core | Cat#: VVC-U of Iowa-2161 and -5 |
| Immobilon® PVDF membranes, 0.45µm pore size | EMD Millipore | Cat# IPVH07850 |
| 200-mesh carbon-coated nickel grid | Electron Microscopy Sciences | Cat# CF200-Ni |
| 8-well Nunc™ Lab-Tek™ II CC2™ Chamber Slides | Thermo Fisher Scientific | Cat# 154941 |
| Tissue arrays, Alzheimer’s Disease | US Biological | Cat#: T5595-6325 |
| Alzheimer QC control slides | StatLab Medical Products, LLC | Cat#: CSA0224P |
Table S3: Nucleotide sequences of primers and target sequences of siRNAs and shRNAs.

### qRT-PCR primers

| Primer ID                      | Primer sequences (5'→3')                  |
|-------------------------------|------------------------------------------|
| **Mouse**_Hspa1a/Hsp72__Forward | ATGGACAAGGCGCAGATCC                      |
| **Mouse**_Hspa1a/Hsp72__Reverse | CTCGGACTTGTCCCAT                         |
| **Mouse**_Hspb1/Hsp25__Forward  | ATCCCCCTGAGGGCACACTTA                    |
| **Mouse**_Hspb1/Hsp25__Reverse  | GGAATGTTGATCTCCGTCGAC                    |
| **Mouse**_Hsp90aa1/Hsp90α__Forward | AATTGCCAGTTAATGTCCTTGAs                |
| **Mouse**_Hsp90aa1/Hsp90α__Reverse | GTCCCGATGAATTGGAGATGAG                   |
| **Mouse**_Hspd1/Hsp60__Forward  | CACAGTCCTTCGACAGATGAG                    |
| **Mouse**_Hspd1/Hsp60__Reverse  | CTACACCTGGAAGCATTAAGGCT                  |
| **Mouse**_βActin_FWDOR          | GGCTGTATTCCTCCATCG                      |
| **Mouse**_βActin.Reverse        | CCAGTTGGAACATGCCCATGT                   |
| **Human**_HSPA1A/HSP72__Forward | CAAGATCACCATCACCAACG                    |
| **Human**_HSPA1A/HSP72__Reverse | TCGTCCTCGGCTTTGTACTT                    |
| **Human**_HSPB1/HSP27__Forward  | GGACGAGCTGACGTTCAAG                     |
| **Human**_HSPB1/HSP27__Reverse  | AGCGTGTATTTCCCGCTGTA                    |
| **Human**_βACTIN_FORWARD        | CATGTACGTTGCTATCCAGGC                   |
| **Human**_βACTIN.Reverse        | CTCCCTAATGTCACGCAGAT                   |

### ChIP qPCR primers

| Primer ID                  | Primer sequences (5'→3')                  |
|----------------------------|------------------------------------------|
| **Human**_HSP72__HSE Forward | GGCGAAAACCTGGAATATTCCCGA                 |
| **Human**_HSP72__HSE Reverse | AGCCTTGGGAACACGGGAG                     |
| **Human**_HSP27__HSE Forward | GTCGCGCTCTCGAATTCAT                    |
| **Human**_HSP27__HSE Reverse | CCTCCCATGCACTTCCT                     |

### Mutagenesis primers

| Primer ID                  | Primer sequences (5'→3')                  |
|----------------------------|------------------------------------------|
| **HSF1_1-323__Forward      | GACTACAAGGACGACGATGACAGTAG               |
| **HSF1_323__Reverse        | GGTTGACGACGACGATGACG                    |
| **HSF1_324-529__Forward    | CTCTTGTCCCAGAC                       |
| **HSF1_324-529__Reverse    | CATCTCGAACGAGA                       |
| **HSF1_S230A__Forward      | CGGCAGTTGCGCCCTGGAGCACGTC             |
| **HSF1_S230A__Reverse      | GCTATACCTGGAATGTC                      |

### siRNAs

| Gene ID | Sequences  | Vector | Vendor        | Cat#    |
|---------|------------|--------|---------------|---------|
| *Akt1*  | Proprietary| N/A    | Sigma-Aldrich | SIHK0096|
| Gene ID          | Sequences          | Vector | Vendor         | Cat#          |
|-----------------|--------------------|--------|----------------|---------------|
| Akt2            | Proprietary        | N/A    | Sigma-Aldrich  | SIHK0099      |
| Akt3            | Proprietary        | N/A    | Sigma-Aldrich  | SIHK0102      |
| HSPD1/HSP60_A   | Proprietary        | N/A    | Sigma-Aldrich  | SASI_Hs01_00136360 |
| HSPD1/HSP60_B   | Proprietary        | N/A    | Sigma-Aldrich  | SASI_Hs01_00136363 |
| Hspd1/Hsp60_A   | Proprietary        | N/A    | Sigma-Aldrich  | SASI_Mm01_00146427 |
| Hspd1/Hsp60_B   | Proprietary        | N/A    | Sigma-Aldrich  | SASI_Mm01_00146428 |
| Non-targeting control | Proprietary        | N/A    | Thermo Fisher Scientific | D-001810-01 |

**shRNAs**

| Gene ID          | Sequences          | Vector | Vendor         | Cat#          |
|-----------------|--------------------|--------|----------------|---------------|
| PTEN_A          | CCACAGCTAGAACCTTATCAA | pLKO   | Addgene        | #25638        |
| PTEN_B          | CCACAAATGAAGGGATATAAA | pLKO   | Addgene        | #25639        |
| Pten_A          | CGACTTAGACTTGACCTAT | pLKO   | Sigma-Aldrich  | TRCN0000322421 |
| Pten_B          | ACATTATGACACCGCACAATT | pLKO   | Sigma-Aldrich  | TRCN0000322487 |
| Scramble control| CCTAAGGGTTAAGTCGCCCTCG | pLKO   | Addgene        | #1864         |