Brain region-specific spread of Lewy body pathology in synucleinopathies is governed by α-synuclein conformations.

Laura de Boni[1,2], Aurelia Hays Watson[1], Ludovica Zaccagnini[1], Nora Kim[3], John Sanderson[4], Haiyang Jiang[5], Elodie Martin[1], Adam Cantlon[5], Matteo Rovere[7], Lei Liu[5], Marc Sylvester[7], Tammaryn Lashley[8], Ulf Dettmer[5], Zane Jaunmuktane[8,9,10], Tim Bartels[1*]

Correspondence to: t.bartels@ucl.ac.uk

This file includes:

Materials and Methods
Figs. S1 to S25
Table S1 and S2

Materials and Methods

Brain samples. The experimental use of human brain samples was approved under the protocol number 1999P001180 ('Aging In The Brain: Role Of Fibrous Proteins') by the Partners Human Research Committee, the Institutional Review Board of Partners Research Management and the REC reference 18/LO/0721 ('Queen Square Brain Bank for Neurological Disorders').

cDNA cloning. Single-mutation αS expression plasmids were generated from the pcDNA4 or pcDNA3/aS plasmid9 using the QuikChange II site-directed mutagenesis kit and appropriate primers.

Cell lines and transfection. Cells were cultured at 37 °C in 5 % CO_2. Human BE (2)-M17 neuroblastoma cells (called M17D, ATCC number CRL-2267) were cultured in DMEM/F12 GlutaMAX (Thermo Fisher Scientific), 10 % fetal bovine serum (Sigma) and 1 % Penicillin/Streptomycin. Prior to the transfection, cells were seeded at a density of $1.5x10^6$ cells per 6 cm dish. HEK cells were cultured in DMEM with 10% fetal bovine serum (Thermo Fisher Scientific), 1 % Penicillin/Streptomycin and L-glutamine (2 mM, Gibco). Transfections with αS wild type and fPD mutants (A30P, E46K, G51D, A53T, H50Q) were carried out using Lipofectamine 2000 according to the manufacturer's instructions. Cells were harvested 48 h after transfection, pelleted, snap frozen and further processed by crosslinking. HEK293 αS C-term Strep II-tagged lysates were purchased from GeneScript.

Protein extraction. Frozen brain or cell pellet samples were manually homogenized in 1x PBS / protease inhibitor / phosphatase inhibitor (Sigma, Thermo Fisher Scientific). Tissue suspensions were lysed by sonication (Sonic Dismembrator model 300, setting 40 for 15 seconds at 4 °C or

Fisher Scientific Model 705 Sonic Dismembrator, sonication of amplitude 5 % for 15 seconds at 4 °C). Samples were ultracentrifuged at 100,000*g* for 1 hour at 4 °C. The resultant supernatant contained soluble proteins. To separate membrane proteins from insoluble proteins, the pellet from the homogenized brain tissue was resuspended in 1 ml OG-RIPA buffer (0.5 % Nonidet P-40 substitute (NP-40, Pan Reac), 0.5 % sodium deoxycholate (Sigma), 0.1 % sodium dodecyl sulfate (SDS, Sigma), 10 mM calcium chloride dihydrate (Sigma) with 2 % n-octyl-ß-D-glucoside (abcam) and centrifuged at 175,000*g* for 30 min at 4 °C. The supernatant containing membrane-associated proteins was collected. The remaining pellet containing insoluble proteins was re-suspended in 200 µl 8 M urea (Sigma) / 5 % SDS (Sigma) / PBS (Sigma), sonicated for 30 s (5 s on, 5 s off setting, amplitude 20 %, room temperature (RT), Fisher Scientific Model 705 Sonic Dismembrator), and boiled for 10 min at 100 °C. Protein concentrations were measured with the Pierce BCA protein assay kit (Thermo Fisher Scientific) according to the manufacturer's instructions.

Purification of αS from erythrocytes: Freshly collected and washed erythrocytes were resuspended in 3-fold volume of ACK lysing buffer (Lonza, Walkersville MD, USA). (NH4)2SO4 to a final concentration of 25 % was added and incubated at 4 °C for 30 min. The lysate was centrifuged (20,000*g*, 20 min), and the supernatant brought up to 50 % (NH4)2SO4. The pellet was washed several time in 55 % (NH4)2SO4 to remove excess hemoglobin. The sample was centrifuged at 20,000*g* for 20 min and the pellet resolubilized in 50-fold volume of 50 mM phosphate buffer, pH 7.0, 1 M (NH4)2SO4. 5 ml of the resultant solution were injected onto a 5 ml HiTrap phenyl hydrophobic interaction column (GE Healthcare) equilibrated with 50

mM phosphate buffer, pH 7.0, 1 M (NH4)2SO4. αS was eluted with a 1 M to 0 M (NH4)2SO4 gradient in 50 mM phosphate buffer, pH 7.0 (αS eluted at ~0.75 M (NH4)2SO4).

Crosslinking of lysate. 20 µg of total protein in a total volume of 25 µl were incubated with 10 µl 5 mM DSG (Thermo Fisher Scientific, final concentration 1.43 mM, DSG was first dissolved in 50 µl of anhydrous DMSO and further in PBS pH 7.4 to a final volume of 1 ml) for 30 min at 37 °C shaking or rotating. The reaction was quenched with 3.5 µl 1 M Tris-HCl pH 7.6 (Sigma) for 5 min shaking at RT. Samples were analysed in biological duplicates and technical triplicates. The crosslinker Disuccinimidyl suberate (DSS, Thermo Scientific Pierce) was used for validation.

Immunoblotting (Western blot, WB). Samples were boiled at 90 °C in 10 µl 4x NuPage LDS sample buffer (Novex)/1:10 β-Mercaptoethanol (Sigma) for 5 min. Samples were electrophoresed at maximum 200 V on NuPAGE Bis Tris Midi gels (Invitrogen) with NuPage MES-SDS running buffer (Invitrogen) and the SeeBlue Plus2 pre-stained molecular weight marker (Invitrogen). The total volume of each sample containing 20 µg total protein was loaded on each lane. After electrophoresis, gels were incubated in 20 % ethanol (Decon Laboratories) for 5 min at RT and electroblotted onto iBlot 2 NC Regular Stacks (Invitrogen) using the iBlot Dry Blotting preset 7 min blotting program. The membrane was briefly rinsed in ultrapure water and incubated in 4 % paraformaldehyde/PBS (Alfa Aesar) for 30 min at RT. Some membranes were stained with 0.1 % Ponceau (Biotium) and rinsed with ultrapure water. Membranes were blocked in Odyssey blocking buffer (PBS)/PBS buffer 1:1 (LI-COR) or Casein buffer 0.5 % (BioRad) for 30 min at RT. After blocking, membranes were incubated with primary antibodies

overnight at 4 °C. Membranes were briefly rinsed in PBS-Tween 0.1 % and then washed 3 x 10 min in PBS-Tween 0.1 %. Membranes were incubated with the corresponding secondary LI-COR antibodies (1:20,000 in Odyssey blocking buffer (PBS)/PBS 1:1/Tween 0.1 % at RT for 1 h in the dark. Membranes were briefly rinsed in PBS-Tween 0.1 % and then washed 3 x 10 min in PBS-Tween 0.1 % in the dark. Membranes were imaged on a LI-COR Odyssey CLx imaging system (settings: custom, western, quality low, resolution 169 µm, focus offset 0.0, auto intensity).

Antibodies. Antibodies used were Syn1 to αS (clone 42, 610786, Becton-Dickinson, WB 1:500), 2F12 to αS(*1*) (MABN1817, Merck, WB 1:5,000), SOY1 to αS (MABN1818, Merck), 15G7 to αS(*2*) (hybridoma supernatants, generously contributed by U.D., WB 1:50), C20 to αS (sc-7011-R, Santa Cruz, WB 1:5,000), H3C to αS (Developmental Studies Hybridoma Bank, WB 1:50), 4B12 to αS (MA1-90346, Thermo Fisher Scientific, WB 1:5,000), 211 to αS (32-8100, Thermo Fisher Scientific, WB 1:1,000), MJFR1 to αS (ab138501, abcam, WB 1:1,000), 202 to αS (836601, Biolegend, WB 1:1,000), anti-DJ-1(*3*) (or GeneTex, WB 1:2,000). Anti-Tau (EP2456Y, abcam, WB 1:5,000), anti-β Tubulin III (T2200, Sigma-Aldrich, WB 1:5,000), anti-Heat Shock Protein 70 (HSP70, ab181606, abcam, WB 1:1,000), anti-14-3-3 beta (ab15260, abcam, WB 1:500), anti-β Actin (ab8227, abcam, WB 1:5,000), anti-HSP90 (HEK: ab clone 16F1, ADI-SPA-83, Enzo, WB 1:1,000, hubrain: ab PA3-013, Invitrogen, WB 1:500).

αS-specific ELISA. Multi-Array 96 HB plates (MSD) were coated with the capture antibody anti-αS 2F12 (200 ng 2F12(*1*) in PBS, 30 µl per well) and incubated over night at 4 °C. The next day, the remaining liquid was removed and the plates were blocked with 5 % Blocker A (MSD)

in PBS-Tween 0.1 % (150 µl per well, Sigma) shaking for 2 hours at RT. Plates were washed 5x with PBS-Tween 0.1 % (150 µl per well). Standards (recombinant αS, highest concentration 1 ng, ratio for serial dilution 1:4, 30 µl per well) and protein (30 µl per well, soluble protein fraction 1:500, membrane (OG-RIPA) and insoluble (UREA/SDS fraction 1:100) were diluted in 1 % Blocker A in PBS-Tween 0.1 % and the plates were incubated shaking for 2 hours at RT. The remaining liquid was removed and the plates were washed 5x with PBS-Tween 0.1 % (150 µl per well). The sulfo-tagged detection antibody SOY1 (50 ng SOY1 in 1 % Blocker A (MSD)/ PBS-Tween 0.1 %, 30 µl per well) was added and the plate incubated shaking for 1 hour at RT in the dark. Remaining liquid was removed and plates were washed 5x with PBS-Tween 0.1 % (150 µl per well). 2x read buffer (MSD)/MilliQ water was added (150 µl per well) and the plates were immediately measured using a MSD Sector 2400 imager or MSD Model No. 1300, QuickPlex SQ 120 according to the manufacturer's instructions. All samples and standards were analyzed in technical duplicates.

Immunoprecipitation of αS. αS from brain tissue was immunoprecipitated using the Pierce Direct IP Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. The 2F12 to αS(*1*) was used as a capture antibody (300 µg per reaction). At least, 800 µg total protein were used for each crosslinking of lysate reaction using 5 mM DSG (final concentration in total volume 1.43 mM). Volumes of wash and incubation buffers were adapted to the total volume input of the sample. Elution fractions were further concentrated using Amicon concentration columns (Millipore) according to the manufacturer's instructions. The fractions were checked for the presence of αS monomer and multimer using immunoblotting and Coomassie staining. Negative controls included the incubation and clearance of samples using the Pierce Control

Agarose Resin (non-amine reactive) and the incubation of samples on the AminoLink Plus™ Coupling Resin column without any antibody. Normal mouse IgG was used as a positive control (200 µg per AminoLink Plus™ Coupling Resin column, sc-2025, Santa Cruz Biotechnology). αS from HEK293 αS C-term Strep II-tagged lysates was immunoprecipitated using StrepTrap 5ml columns. Briefly, cells were lysed, ultracentrifuged (100,000*g* at 4 °C for one hour and the protein content measured in the supernatant. Crosslinking was performed according the protocol above and the crosslinked lysate loaded onto equilibrated Strep Tag 5 ml columns. Unbound samples was washed out with PBS buffer pH 7.4 and the sample eluted with elution buffer (2.5 mM desthiobiotin in PBS pH 7.4). For mock controls, HEK without αS tag were subjected to immunoprecipitation.

Size exclusion chromatography (SEC). Immunoprecipitated αS fractions were injected on a Superdex 200 (10/300 GL) column (GE Healthcare) at room temperature and eluted with 50 mM NH4Ac (pH 7.4) while measuring (in-line) the conductivity and the 280-nm absorption of the eluate at 0.7 mL/min. For size estimation, a gel filtration standard (Bio-Rad) was run on the column, and the calibration curve was obtained by semilogarithmic plotting of molecular weight versus the elution volume divided by the void volume. Immunoblotting was performed to identify the fractions containing αS multimers and monomers.

Circular Dichroism Spectroscopy (CDS). After SEC separation, samples containing αS multimers or monomers, were exchanged into 10 mM ammonium acetate using Zeba spin desalting columns (Thermo Fisher), lyophilized, and resuspended in 10 mM ammonium acetate at a concentration of approximately 10 µM. αS samples were added to a 1 mm path length quartz

cuvette for far-UV CD and analyzed using a J-1500 CD spectrometer (Jasco) at 25 °C. Temperature control with an accuracy of 0.1 °C was achieved with a heating/cooling accessory equipped with a Peltier element (PFD-425S) connected to a water thermostatic bath. Buffer spectra were recorded and subtracted.

Multi-angled light scattering (MALS). Immunoprecipitated and SEC-separated αS multimers and monomers were loaded at a flow rate of 0.15 mL/min onto a Superdex 200 3.2/300 GL Increase column (GE Healthcare) previously equilibrated in 50 mM ammonium acetate pH 7.4. The column was connected in line to a Dawn 8+ MALS detector (Wyatt Technology) using a laser emitting at 690 nm and by refractive index measurement using an Optilab T-rex (Wyatt Technology Corp.).

PFF generation. 5 mg/ml recombinant αS in PBS was aggregated for 3 days at 37 °C with nutation to form Thioflavin T positive fibrils. To generate soluble PFFs, αS fibrils were diluted to 1 mg/ml and sonicated at power level 50 for 3 x 10 s using a Sonic Dismembrator model 300 (Fisher). Aliquots of the resultant material were flash-frozen in liquid nitrogen and stored at -80 °C.

Preformed-fibril (PFF) transfection. All reagents were purchased from Thermo Fisher Scientific unless otherwise noted. Transfected M17D were incubated with 0.5 µg/ml αS PFF. After 48h, the medium was aspirated, cells were harvested by scraping, and washed 2 x in cold PBS (500*g* spin for 5 min). Cell pellets were resuspended in 1x PBS / protease inhibitor and sonicated with a Sonic Dismembrator model 300 (microtip setting 40; 2 x 15 s). Cells were spun

at 20,000*g* for 10 min. The supernatant was kept ("cytosol") and the pellet was incubated in 1 % triton X-100 at 4 °C for 30 min under nutation. After a 20,000*g* spin for 10 min, the supernatant was kept ("membrane fraction") and the pellet dissolved in 5 % SDS at 100 °C for 10 min to give "insoluble αS" fraction. Relative αS content in each fraction was analyzed by SDS-PAGE Western blot and ELISA.

Thioflavin T (ThT) binding. To detect amyloid fibril growth, a discontinuous assay was used. Aliquots (10 µL) were removed from each purified αS sample (lyophilized from 50 mM ammonium acetate, pH 7.4, and agitated at 37 °C at a concentration of 75 µM in 20 mM Bis-Tris propane, 100 mM LiCl, pH 7.4) and added to 2 mL of a 10 µM Thioflavin T (ThT) solution in 10 mM glycine buffer, pH 9. Fluorescence was directly quantified on a Varian Eclipse fluorescence spectrophotometer at 20 °C by exciting at 444 nm and scanning the emission wavelengths from 460 to 550 nm with slit widths set at 5 nm (PMT at 750 V).

Preparation of paraffin sections. Frozen tissue blocks were embedded in paraffin by the Specialized Histopathology Services Core at the Brigham and Women's Hospital, Boston, Ma, USA. The core also provided 6 µm sections. In total, 5 controls (control #4, control #5, control #6, control #18, control #19), 5 DLB (DLB #15, DLB, #16, DLB #19, DLB #21, DLB #22) and 4 sporadic PD (PD #10, PD #7, PD #11, PD #12) samples were processed.

Immunostaining. 6 µm paraffin sections were dewaxed in xylenes and rehydrated through an ethanol series into water. Antigen retrieval was performed by boiling the slides in citrate buffer, pH 6.0, for 15 minutes. Slides were blocked in 2 % milk in PBS with 0.3 % triton X-100 for 1

hour, and were then incubated with the 5G4 antibody to αS (Sigma) at 1:5,000 at RT overnight. Slides were then incubated in biotin-conjugated secondary antibodies in 2 % milk in PBS with 0.3 % triton X-100 for 2 hours (1:200, Southern Biotech) followed by avidin-biotin-peroxidase complex (Vectastain Elite) in PBS for 2 hours. Histochemical detection was performed with diaminobenzidine (ImmPACT DAB, Vector). Stainings were performed by our collaborators Prof. M. Feany and her team from the Department of Pathology, Brigham and Women's Hospital, Boston, MA, USA.

Mass spectrometry: HEK293 αS C-term Strep II-tagged lysates were crosslinked and immunoprecipitated as described above and the αS^{CH} and αS^{CU} were separated via SEC and run on a SDS-gel. Gel pieces containing the bands of interest were cut out and the gel pieces were lypholized prior Mass spectrometry analysis. Gel slices were subjected to gel digestion. In brief, slices were washed consecutively with water, 50 % acetonitrile (ACN), and 100 % ACN. Proteins were reduced with 20 mM DTT in 50 mM ammonium bicarbonate and alkylated with 40 mM acrylamide (in 50 mM bicarbonate). The slices were washed again and dehydrated with ACN. Proteolysis was performed with 330 ng chymotrypsin or trypsin (sequencing grade Promega, Mannheim, Germany) at 37 °C overnight. The peptide extracts were dried in a vacuum concentrator and stored at -20 °C. Dried peptides were dissolved in 10 µl 0.1 % formic acid (solvent A). 2 µl were injected onto a C18 analytical column (self-packed 300 mm length, 100 µm inner diameter, ReproSil-Pur 120 C18-AQ, 3 µm, Dr. Maisch GmbH, Ammerbuch-Entringen, Germany). Peptides were separated during a linear gradient from 2 % to 35 % solvent B (90 % acetonitrile, 0.1 % formic acid) within 90 min at a flow rate of 300 nl/min. The nanoHPLC was coupled online to an Orbitrap Lumos mass spectrometer (Thermo Fisher

Scientific, Bremen, Germany). Peptide ions between 330 and 1600 m/z were scanned in the Orbitrap detector with a resolution of 60,000 (maximum injection time 50 ms, AGC target 400,000). Precursor ions (threshold 25,000) were subjected to higher energy collision induced dissociation within a 2.5 s cycle and fragments were analyzed in the Orbitrap detector (maximum injection time 22 ms, resolution = 15,000). Fragmented peptide ions were excluded from repeat analysis for 15 s.

Statistics. Western Blot: Ratio of protein multimers to monomers was analysed using the Image Studio software western analysis according to the manufacturer instructions (background substraction: median, border with 3, top/bottom). Data analysis (except for correlation and regression analysis) was performed using GraphPad Prism 7 (GraphPad Software, La Jolla, CA, USA). Statistical significance was determined by Mann Whitney test ($p<0.05$, two-tailed). Samples are displayed as mean ± SD.

Correlation analysis was performed using the computing environment R (RStudio, Version 3.5.2) and Pearson correlation test. Additional software packages included "devtools" (https://github.com/r-lib/devtools), "easyGgplot2" (https://github.com/kassambara/easyGgplot2) and „Hmisc" (https://github.com/harrelfe/Hmisc). Samples were coded as follows: Gender male = 1, female = 2. McKeith: neocortical = 3, limbic = 2, brainstem = 1, controls (no McKeith staging) were set to 0. CERAD: none = 0, sparse = 1, mild = 2, mderate = 3, frequent = 4. Braak LB staging: controls were set to 0, PD and DLB patients were coded according to evaluated Braak LB staging.

ELISA: Raw data from the MSD Discovery Workbench was imported into Excel 2010 (Microsoft) for background calculation and subtraction. Values from excel were imported into

GraphPad Prism 7 (GraphPad Software, La Jolla, CA, USA) for interpolation together with the known standard concentrations (standard curve: sigmoidal, 4PL, X is log (concentration, no special handling for outliers). Final concentration of each analyzed sample was calculated in Excel according to the dilution factor.

Mass spectrometry: Raw data processing and analysis of database searches were performed with Proteome Discoverer software 2.4.0.305 (Thermo Fisher Scientific). The protein-specific peptide identification was done with an in house Mascot server version 2.6.1 (Matrix Science Ltd, London, UK) from Proteome Discoverer. MS2 data were searched against a database of common contaminants (cRAP of the Global Proteome Machine), and human SwissProt sequences. Precursor Ion m/z tolerance was 10 ppm, fragment ion tolerance 0.02 Da. Tryptic peptides with up to two missed cleavages were searched, propionamide set as a static modification of cysteines, oxidation as a dynamic modification of methionine. Mascot results from searches against SwissProt were sent to the Percolator algorithm[3] version 3.02 as implemented in Proteome Discoverer 2.4. If the data did not permit use of Percolator, PSMs were evaluated by a target-decoy procedure. Spectra without high confident (FDR 1 %) matches were sent to a second round Mascot search with semi-specific enzyme cleavage. The false discovery rate of proteins in these samples is 0.6 %.

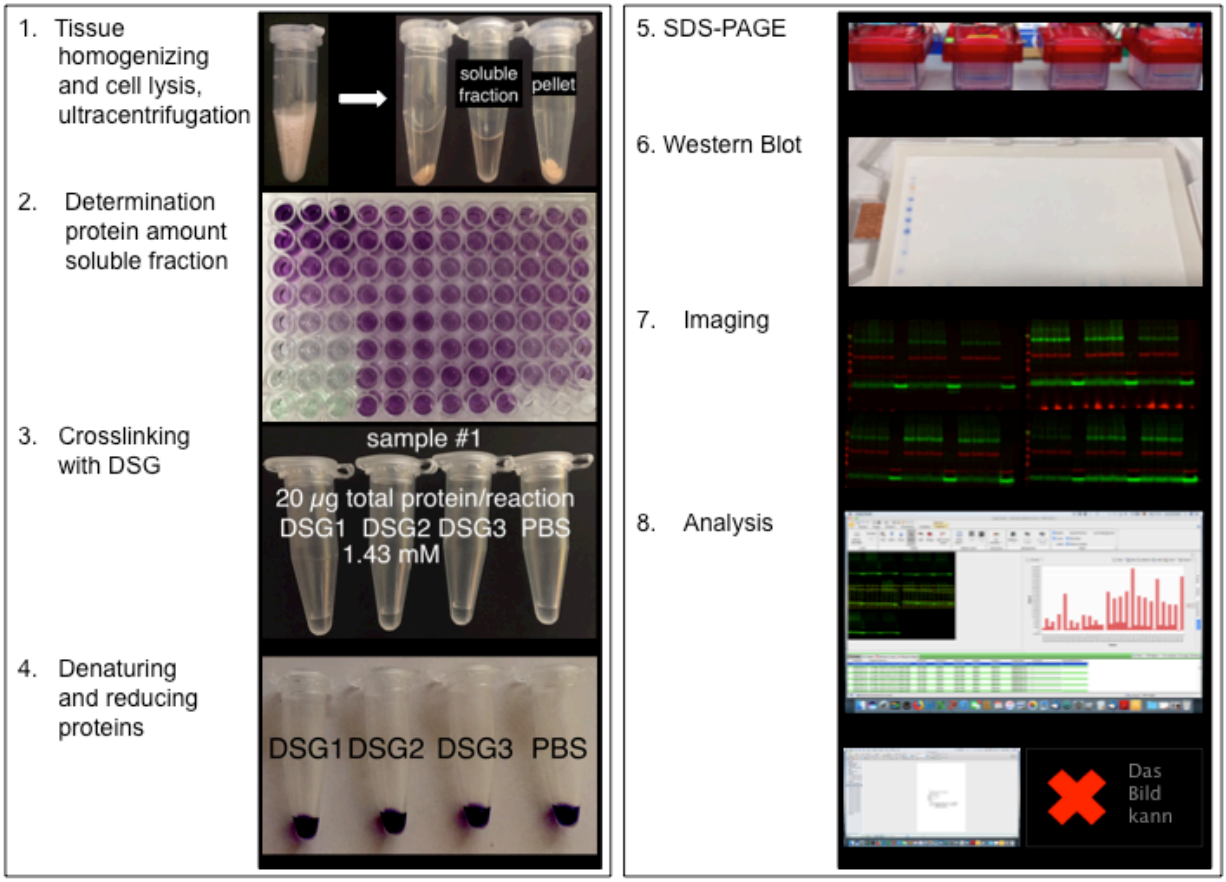
References:

1. U. Dettmer, A. J. Newman, E. S. Luth, T. Bartels, D. Selkoe, In vivo cross-linking reveals principally oligomeric forms of α-synuclein and β-synuclein in neurons and non-neural cells. *J. Biol. Chem.* **288**, 6371–6385 (2013).
2. P. J. Kahle, M. Neumann, L. Ozmen, V. Muller, H. Jacobsen, A. Schindzielorz, M.

Okochi, U. Leimer, H. van Der Putten, A. Probst, E. Kremmer, H. A. Kretzschmar, C. Haass, Subcellular localization of wild-type and Parkinson's disease-associated mutant alpha -synuclein in human and transgenic mouse brain. *J. Neurosci.* **20**, 6365–6373 (2000).

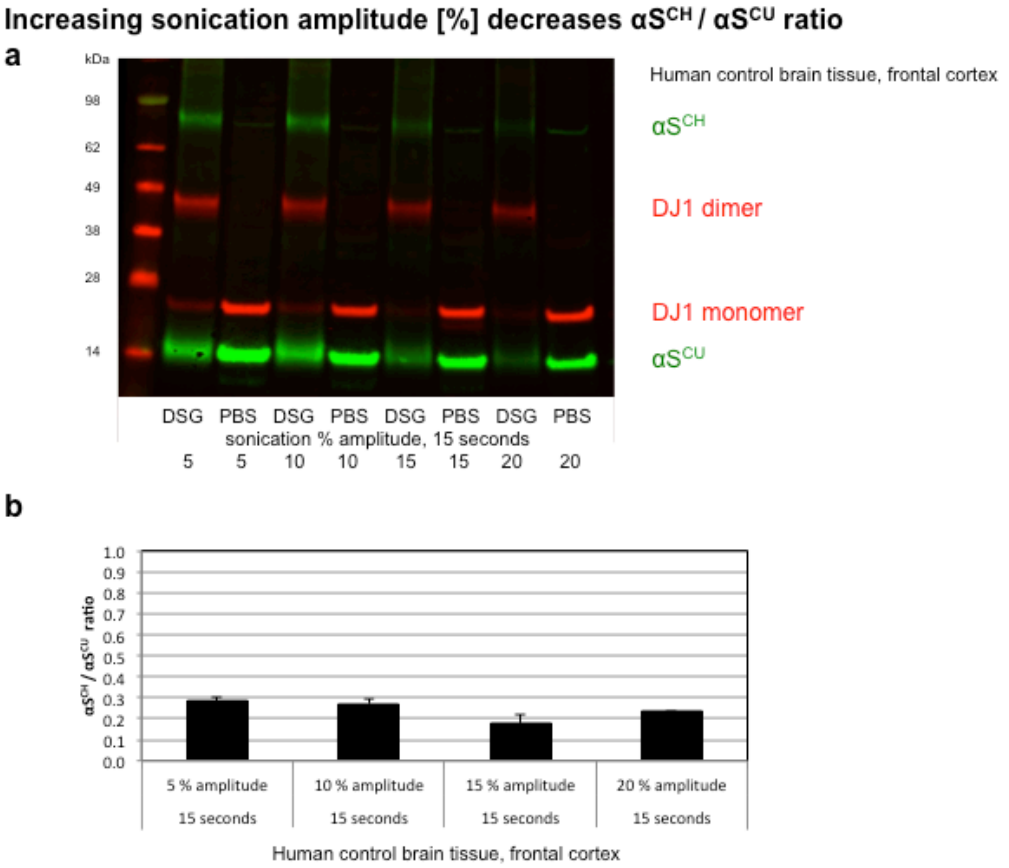
3. S. Baulac, M. J. LaVoie, J. Strahle, M. G. Schlossmacher, W. Xia, Dimerization of Parkinson's disease-causing DJ-1 and formation of high molecular weight complexes in human brain. *Mol. Cell. Neurosci.* **27**, 236–246 (2004).

Fig. S1 Crosslinking protocol for tissue and cell lysates



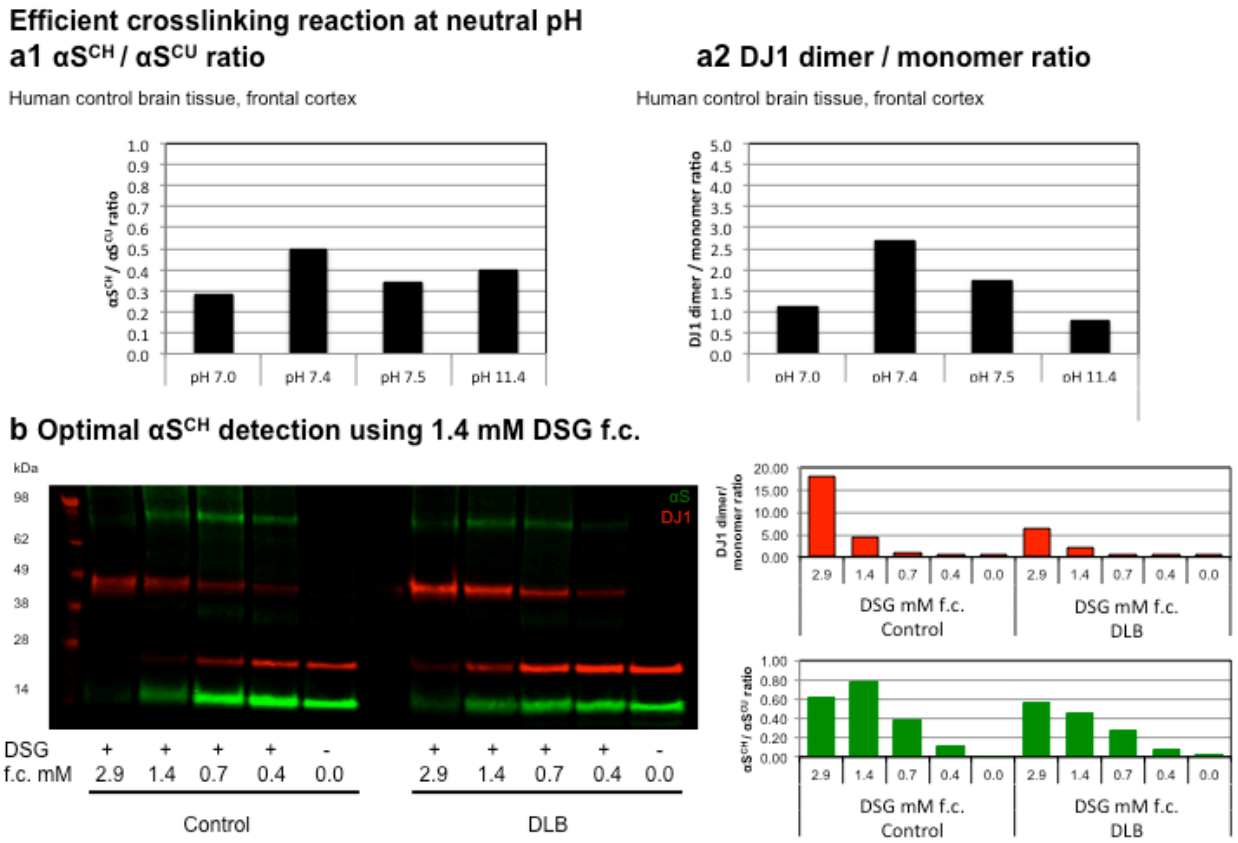
Supplementary Fig. 1 Protocol and workflow of the crosslinking protocol using tissue and cell lysates. The procedure is described in the Methods section.

Fig. S2 Validation of the crosslinking protocol



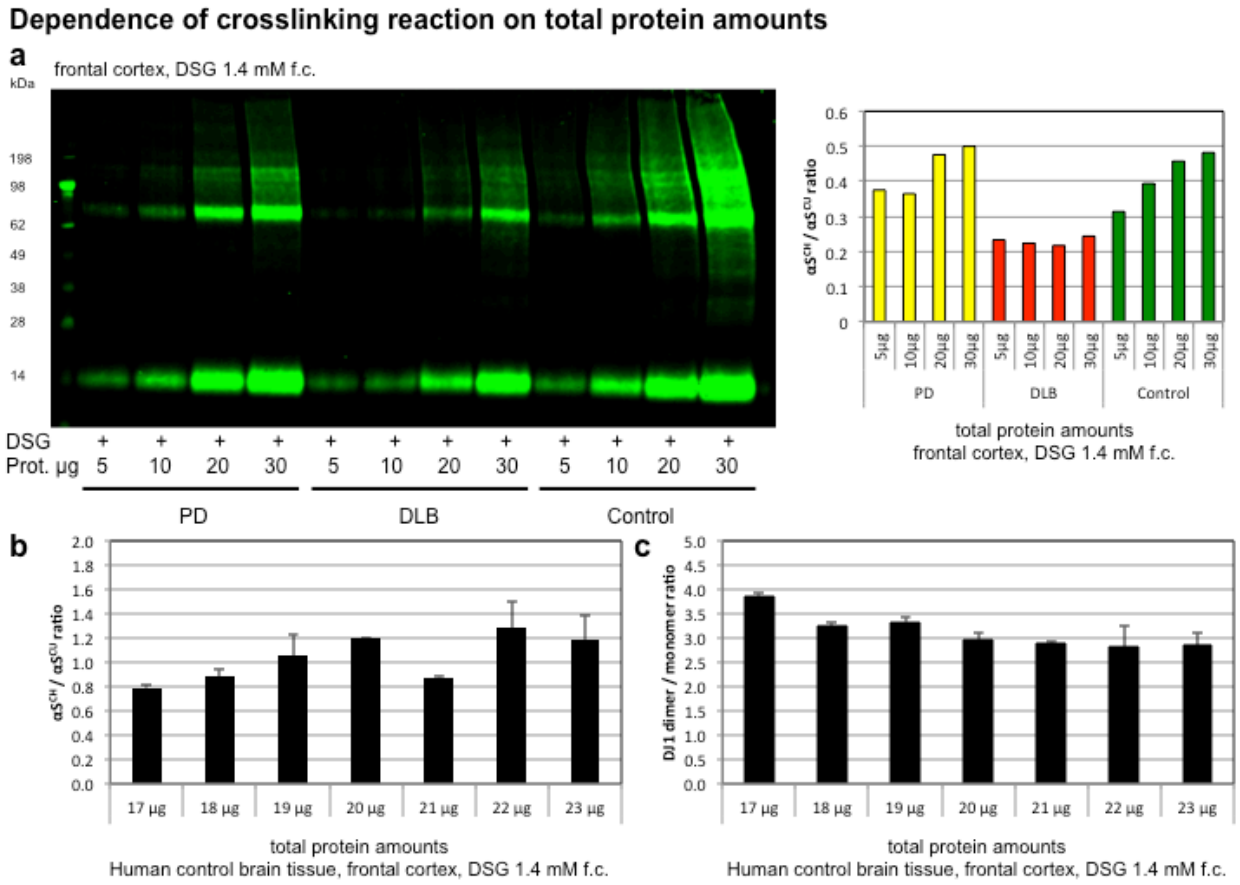
Supplementary Fig. 2 Validation of the crosslinking protocol. The procedure is described in the Methods section. **a**, Western blot and **b**, quantification of the Western blot of crosslinked (DSG) human control brain tissue (frontal cortex, n=1) subjected to different sonication amplitudes (Fisher Scientific Model 705 Sonic Dismembrator 5 %, 10 %, 15 %, 20 %). Non-crosslinked samples (PBS) are displayed in A. αS^{CH} are sensitive to sonication as described previously[1] and sonication settings were choosen accordingly (Sonic Dismembrator model 300 settings 40, 15 seconds, Fisher Scientific Model 705 Sonic Dismembrator 5 % of amplitude 15 seconds). Green = αS, red = DJ1

Fig. S3 Validation of the crosslinking protocol



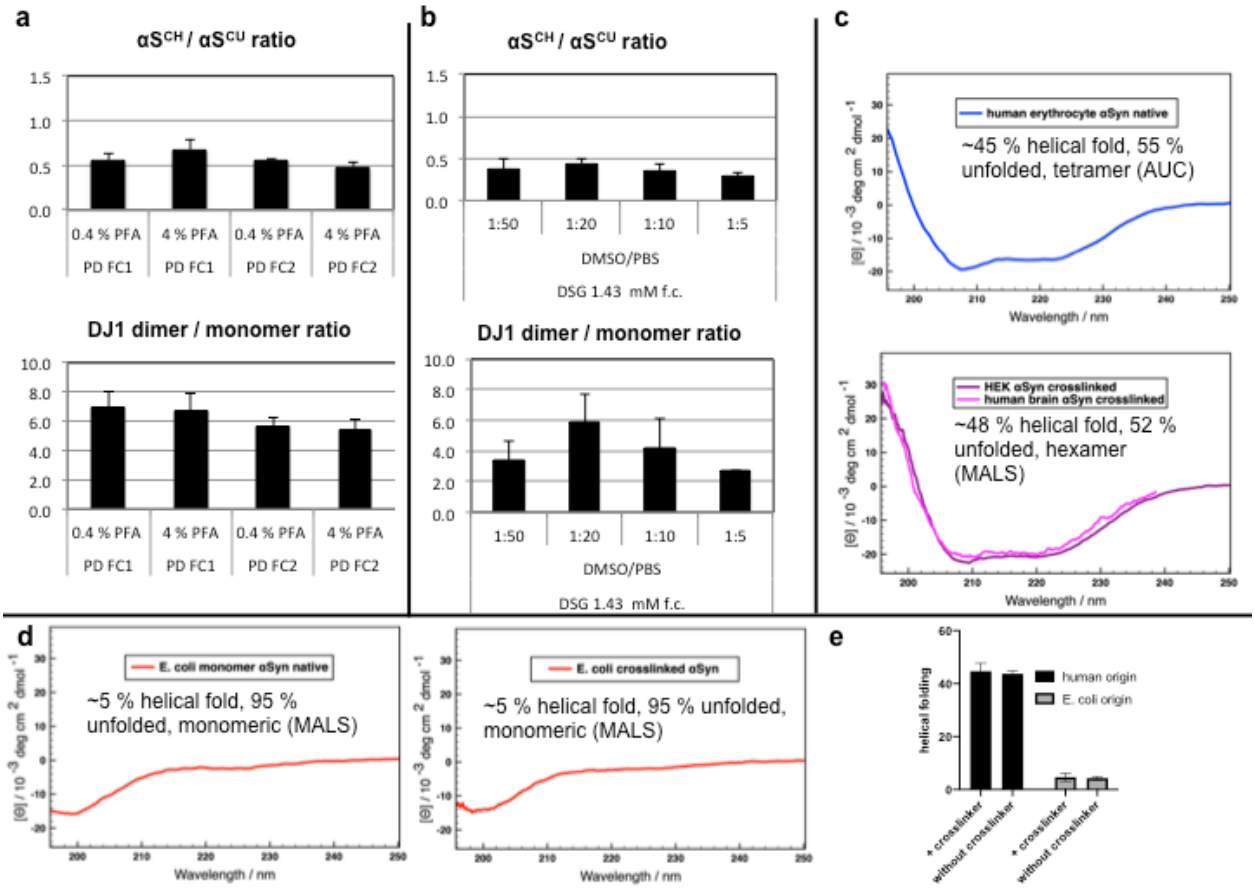
Supplementary Fig. 3 Validation of the crosslinking protocol. The procedure is described in the Methods section. **a1**, Quantification of αS^{CH} / αS^{CU} ratios and **a2**, DJ1 dimer / monomer ratios in human control brain tissue (frontal cortex, n=1) depending on different pH concentrations. 1x PBS has been used at different pH for solubilization of DSG and for filling up the sample volume to 25 µl total volume. Efficient crosslinking (controlled by DJ1) is carried out at pH 7.4. **b**, Western blot with quantifications of crosslinked human control and DLB brain tissue (frontal cortex, n=1 each). The crosslinking reaction is depending on the amount and final concentration of the crosslinker DSG as described previously[1]. Thus, for the crosslinking protocol used for frozen brain tissue and cell pellets, a final concentration (f.c.) of 1.4 mM DSG was chosen in accordance with the described optimal 1-2 mM working solution for intact cell crosslinking[1]. DSG "+" = crosslinked sample, DSG "-" = non-crosslinked (control) sample. Green = αS, red = DJ1

Fig. S4 Validation of the crosslinking protocol



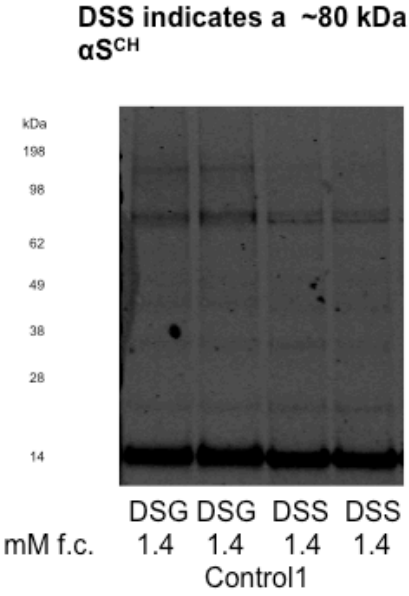
Supplementary Fig. 4 Validation of the crosslinking protocol. The procedure is described in the Methods section. **a**, Western blot and quantification demonstrating the efficiency of the crosslinking reaction (human brain tissue: control, DLB, PD (n=1 each), frontal cortex) depending on the total amount of protein input (5 µg, 10 µg, 20 µg, 30 µg) into the reaction and the failure to detect higher amounts of αS^{CH} in DLB despite a higher total protein input. DSG "+" = crosslinked sample. **b, c**, Minor changes in total protein input (17 µg, 18 µg, 19 µg, 20 µg, 21 µg, 22 µg, 23 µg) exhibit very similar crosslinking results and efficiency (controlled by DJ1). Human brain tissue, frontal cortex (n=1). F.c. = final concentration.

Fig. S5 Validation of the crosslinking protocol and CD spectra of purified αS



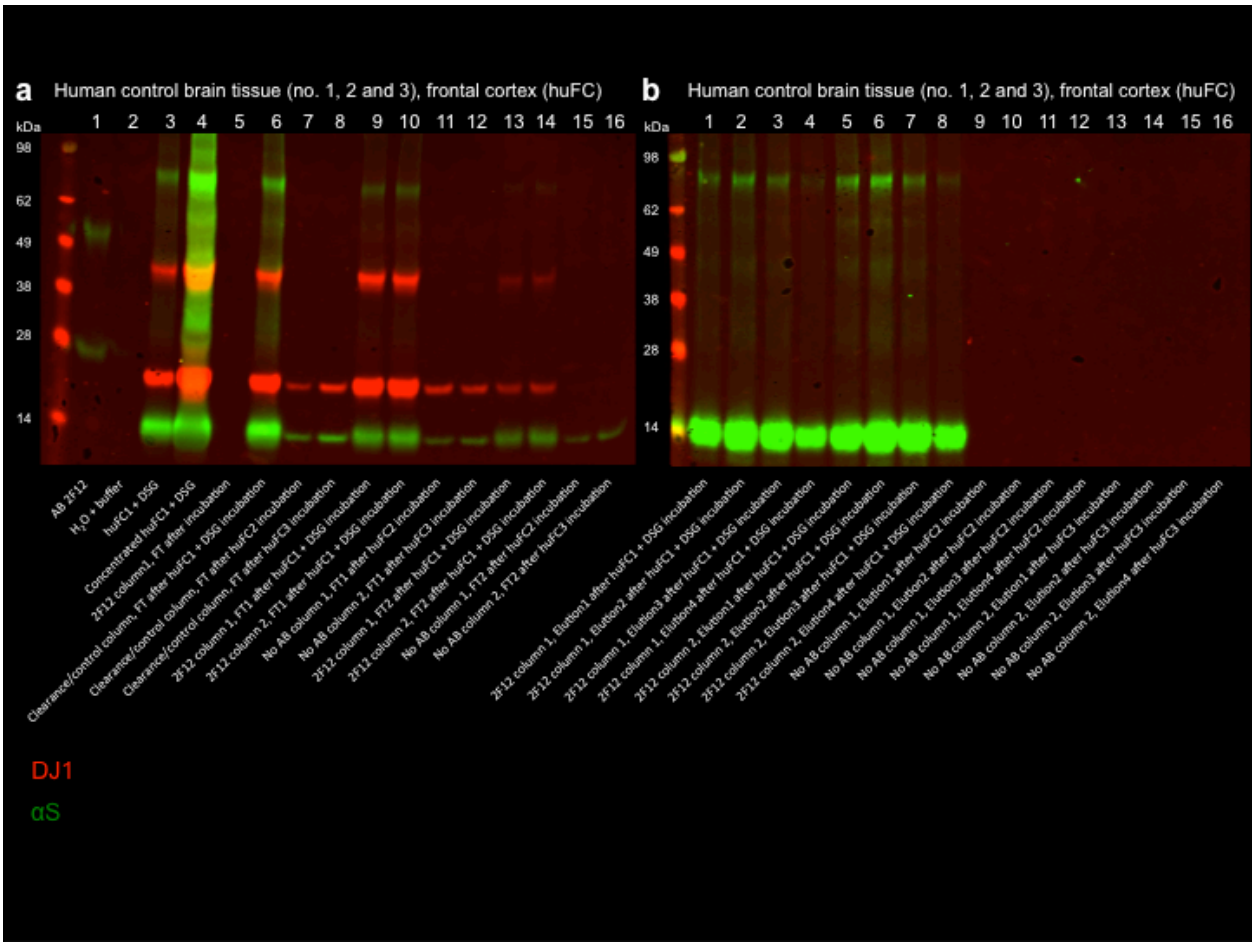
Supplementary Fig. 5 Validation of the crosslinking protocol and CD spectra of purified αS. The procedure is described in the Methods section. **a**, Comparison of different PFA concentrations for the fixation of αS. Similar $αS^{CH}$ / $αS^{CU}$ ratios (A) and DJ1 dimer / monomer ratios upon fixation with PFA 0.4 % or PFA 4 %. Brain samples (frontal cortex, FC) have been analyzed in biological duplicates (FC 1 and FC2). **b**, DMSO does not lead to an artificial oligomer formation. DSG was solubilized in different amounts of DMSO and added to a protein lysate from frontal cortex control brain tissue. The crosslinking reaction seems to be most efficient at a DSG PBS/DMSO ratio of 1:20. This ratio is used in the crosslinking protocol. PD = Parkinson's disease, FC = frontal cortex. **c**, Purified $αS^{CH}$ from red blood cells[2] (RBC) exhibits an α-helical secondary structure of approximately 45 %. The $αS^{CH}$ from human brain exhibits an α-helical secondary structure of approximately 48 %. **d**, Recombinant αS from E. coli is unfolded. The unfolded structure of recombinant αS from E. coli remains after crosslinking. **e**, Folded structure of αS without and with crosslinking demonstrating the difference from human and recombinant origin.

Fig. S6 αS^{CH} appearance after crosslinking with DSG and DSS



Supplementary Fig. 6 The crosslinker DSS detects αS^{CH}. The procedure for the crosslinking is described in the Methods section. Western blot displaying control (n=1) human brain tissue, frontal cortex, crosslinked (technical duplicates) with either DSG or disuccinimidyl suberate (DSS). DSS detects αS^{CH} migrating at ~80 kDa in accordance with DSG. All crosslinkers have been used at a final concentration (f.c.) of 1.4 mM in human brain tissue.

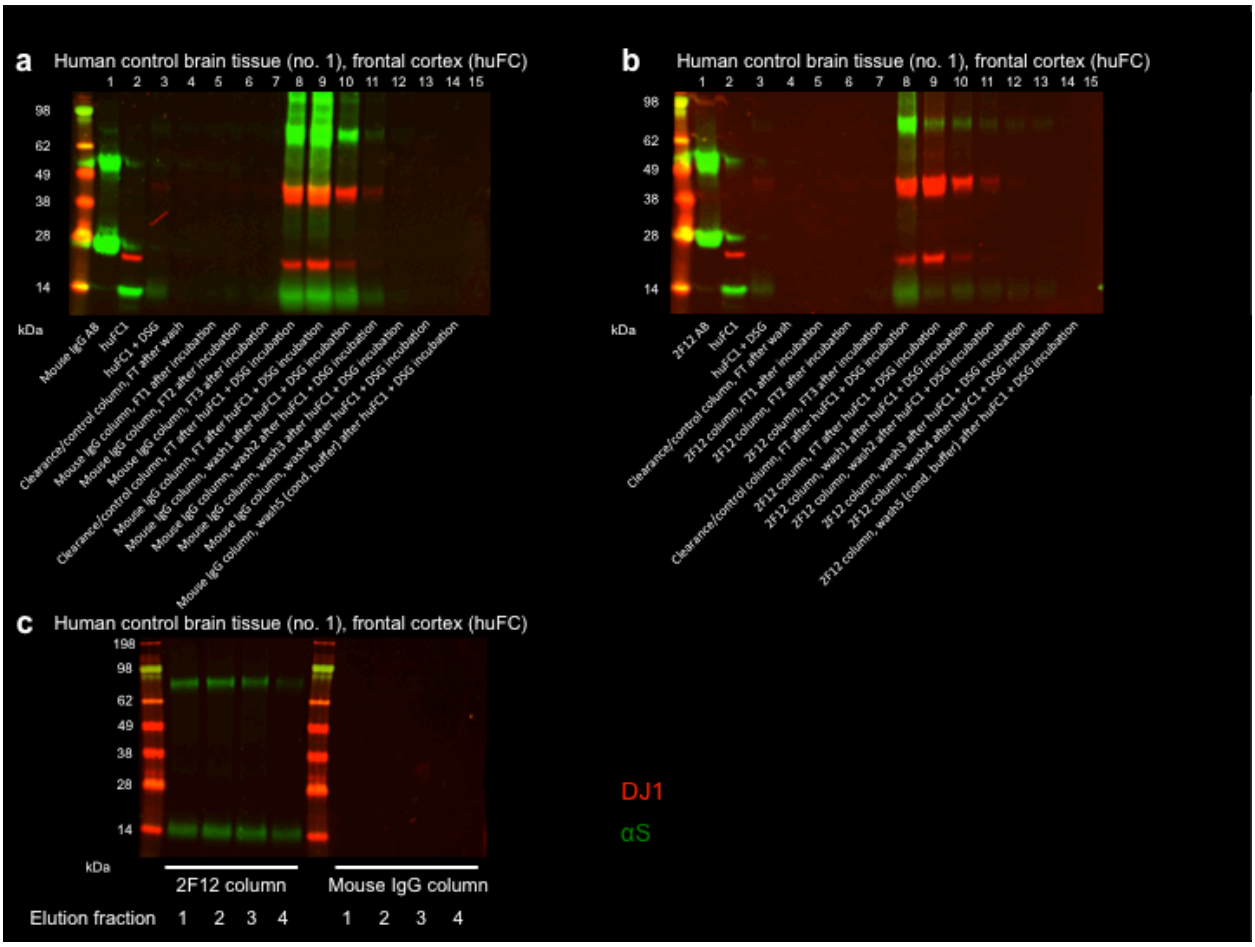
Fig. S7 Immunoprecipitation of αS from human post-mortem native brain tissue



Supplementary Fig. 7 Immunoprecipitation and purification of αS from human brain tissue. The procedure is described in the Methods section. Samples: 3 different human control brain tissues (no. 1, 2, 3), frontal cortex. The crosslinking procedure is performed as described in the Methods section. **a**, Lane 1: Displaying the applied anti-αS 2F12 antibody (25 kDa light and 50 kDa heavy chain). Lane 2: Used 1x conditioning buffer without artificial protein detection. Lane 3: Crosslinked human brain lysate (no. 1) displaying $αS^{CH}$ and $αS^{CU}$ (green) and DJ1 dimer and monomer (red). Lane 4: Concentrated crosslinked human brain lysate (no. 1). Lane 5: Flow through (FT) after clearing of the column (AminoLink Plus™ Coupling Resin) incubated with the 2F12 antibody demonstrating complete binding of the 2F12 antibody. Lane 6: FT after incubation of the crosslinked sample no. 1 on the control/clearance column (Pierce Control Agarose Resin, non-amine reactive). Lane 7: FT after incubation of the non-crosslinked sample no. 2 on the control/clearance column. Lane 8: FT after incubation of the non-crosslinked sample no. 3 on the control/clearance column. Lane 9: FT after incubation of the crosslinked sample no. 1 on the 2F12 antibody column. Column is saturated and some protein lost during the washing step. Lane 10: FT after incubation of the crosslinked sample no. 1 on another 2F12 antibody column. Lane 11: FT of column incubated without any antibody and sample no. 2. Lane 12: FT of column incubated without any antibody and sample no. 3. Lane 13: Second FT after incubation of the crosslinked sample no. 1 on the 2F12 antibody column. Column is saturated and some protein lost during the washing step. Lane 14: Second FT after incubation of the

crosslinked sample no. 1 on another 2F12 antibody column. Lane 15: Second FT of column incubated without any antibody and sample no. 2. Lane 16: Second FT of column incubated without any antibody and sample no. 3. **b**, Lane 1-4: Elution fractions 1-4 of 2F12 column no. 1 incubated with the crosslinked brain sample no 1 showing the purification of the αS protein (absence of DJ1 protein). Lane 5-8: Elution fractions 1-4 of 2F12 column no. 2 incubated with the crosslinked brain sample no. 1 showing the purification of the αS protein (absence of DJ1 protein). Lane 9-12: Elution fractions 1-4 of one column without antibody incubated with the non-crosslinked brain sample no 2. No protein binding to the column, no elution of protein in the final elution steps. Lane 13-16: Elution fractions 1-4 of a second column without antibody incubated with the non-crosslinked brain sample no 3. No protein binding to the column, no elution of protein in the final elution steps. Green = αS, red = DJ1

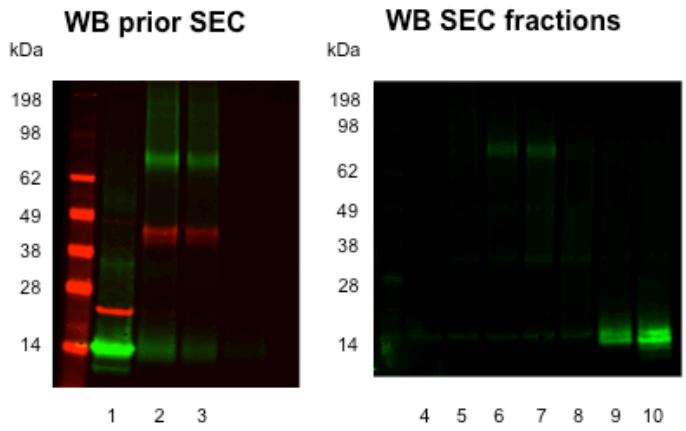
Fig. S8 Immunoprecipitation of αS from human post-mortem native brain tissue



Supplementary Fig. 8 Immunoprecipitation and purification of αSYN from human brain tissue. The procedure is described in the Methods section. Sample: human control brain tissues (no. 1), frontal cortex. The crosslinking is performed according to the procedure described in the Methods section. **a**, Lane 1: Displaying the applied mouse IgG (25 kDa light and 50 kDa heavy chain) used as a control antibody. Lane 2: non-crosslinked lysate human brain tissue no. 1. Slight overflow from Lane 1. Lane 3: Crosslinked lysate human brain tissue no. 1. Lane 4: Flow Through (FT) of the washed clearance/control column (Pierce Control Agarose Resin, non-amine reactive); no protein detection. Lane 5-8: FTs after incubation of the antibody column (AminoLink Plus™ Coupling Resin) with the mouse IgG demonstrating complete binding of the mouse IgG. Lane 8: FT after incubation of the clearance/control column with the crosslinked human brain lysate no. 1. Lane 9: FT after incubation of the mouse IgG antibody column with the crosslinked human brain lysate no. 1. Lane 10-15: FT after the washing steps of the mouse IgG antibody column incubated with the crosslinked human brain lysate no. 1. **b**, Lane 1: Displaying the applied anti-αS antibody (25 kDa light and 50 kDa heavy chain). Lane 2: Non-crosslinked lysate human brain tissue no. 1. Slight overflow from lane 1. Lane 3: Crosslinked lysate human brain tissue no. 1. Lane 4: Flow Through (FT) of the washed clearance/control column Pierce Control Agarose Resin, non-amine reactive); no protein detection. Lane 5-8: FTs after incubation of the antibody column (AminoLink Plus™ Coupling Resin) with the 2F12

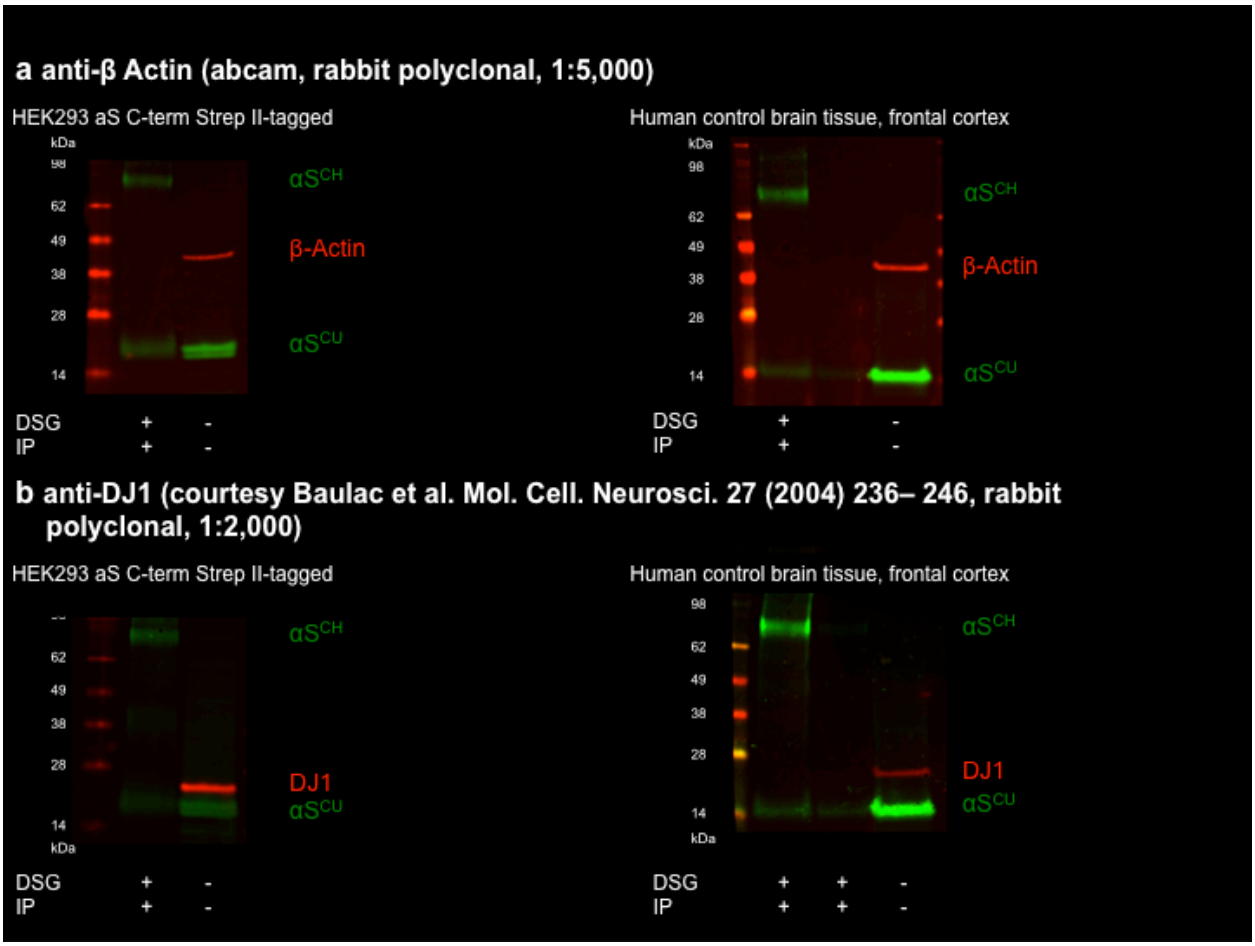
antibody demonstrating complete binding of the 2F12. Lane 8: FT after incubation of the clearance/control column with the crosslinked human brain lysate no. 1. Lane 9: FT after incubation of the 2F12 antibody column with the crosslinked human brain lysate no. 1. Column is saturated, no complete binding of the crosslinked lysate Lane 10-15: FT after the washing steps of the 2F12 antibody column incubated with the crosslinked human brain lysate no. 1. **c**, Lane 1-4 2F12 column: Elution fractions 1-4 of 2F12 column incubated with the crosslinked brain sample no 1 showing the purification of the αS protein (absence of DJ1 protein). Lane 1-4 mouse IgG column: Elution fractions 1-4 of mouse IgG column incubated with the crosslinked brain sample no 1 showing no binding and accordingly no elution of αSYN protein or DJ1. Green = αS, red = DJ1

Fig. S9 Separation of αS^{CH} / αS^{CU} from human control brain tissue



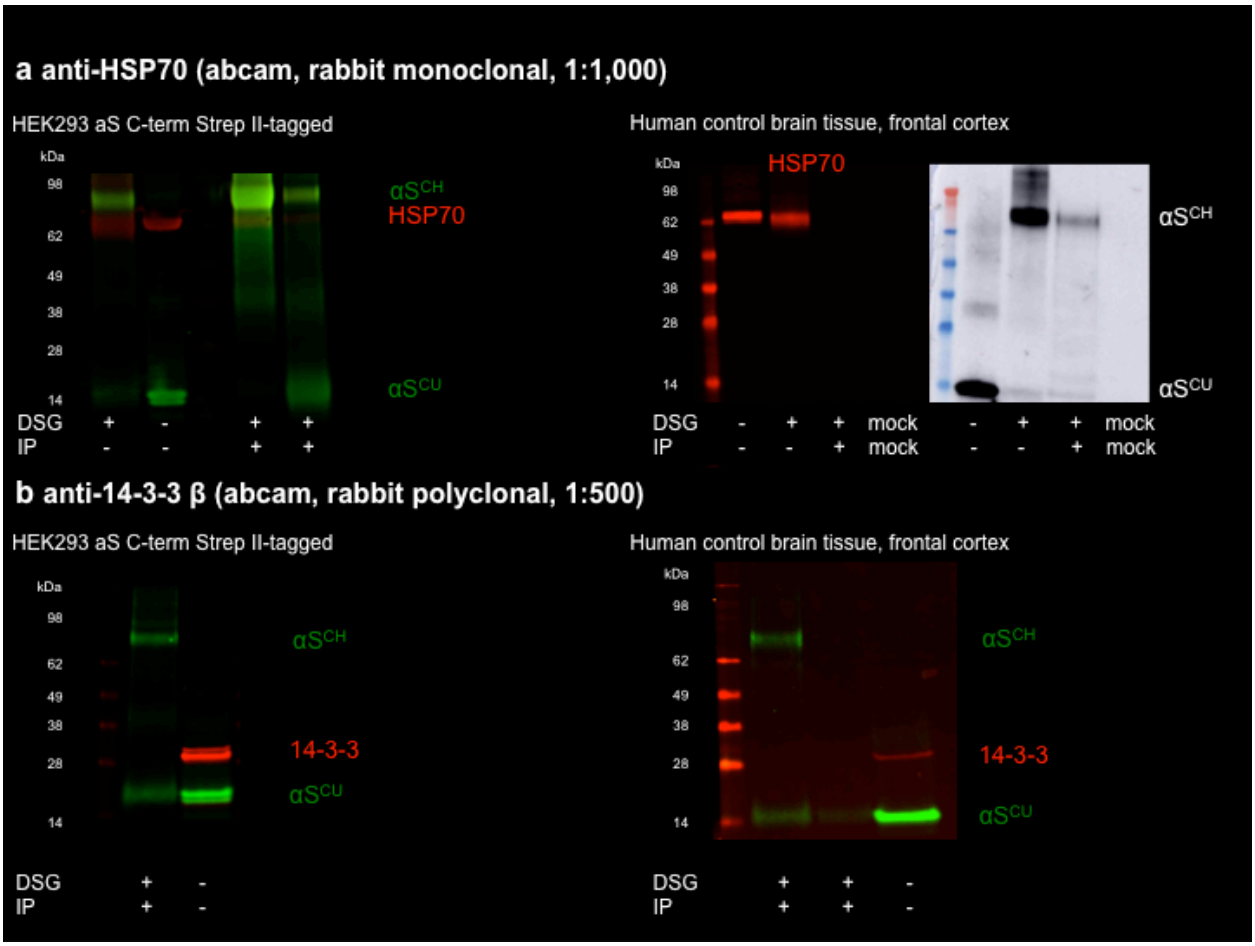
Supplementary Fig. 9 Separation of immunoprecipitated αS via SEC. The procedure is described in the Methods section. Western blot of human control brain tissue, frontal cortex. Lane 1: Non-crosslinked lysate. Lane 2: Crosslinked lysate. Lane 3: Concentrated sample after immunoprecipitation. Lane 4-10: SEC fractions 4-10 displaying separated αS^{CH} in fraction 6 + 7 and αS^{CU} in fraction 9 + 10

Fig. S10 Absent co-purification of β-Actin or DJ1 and αS^{CH}



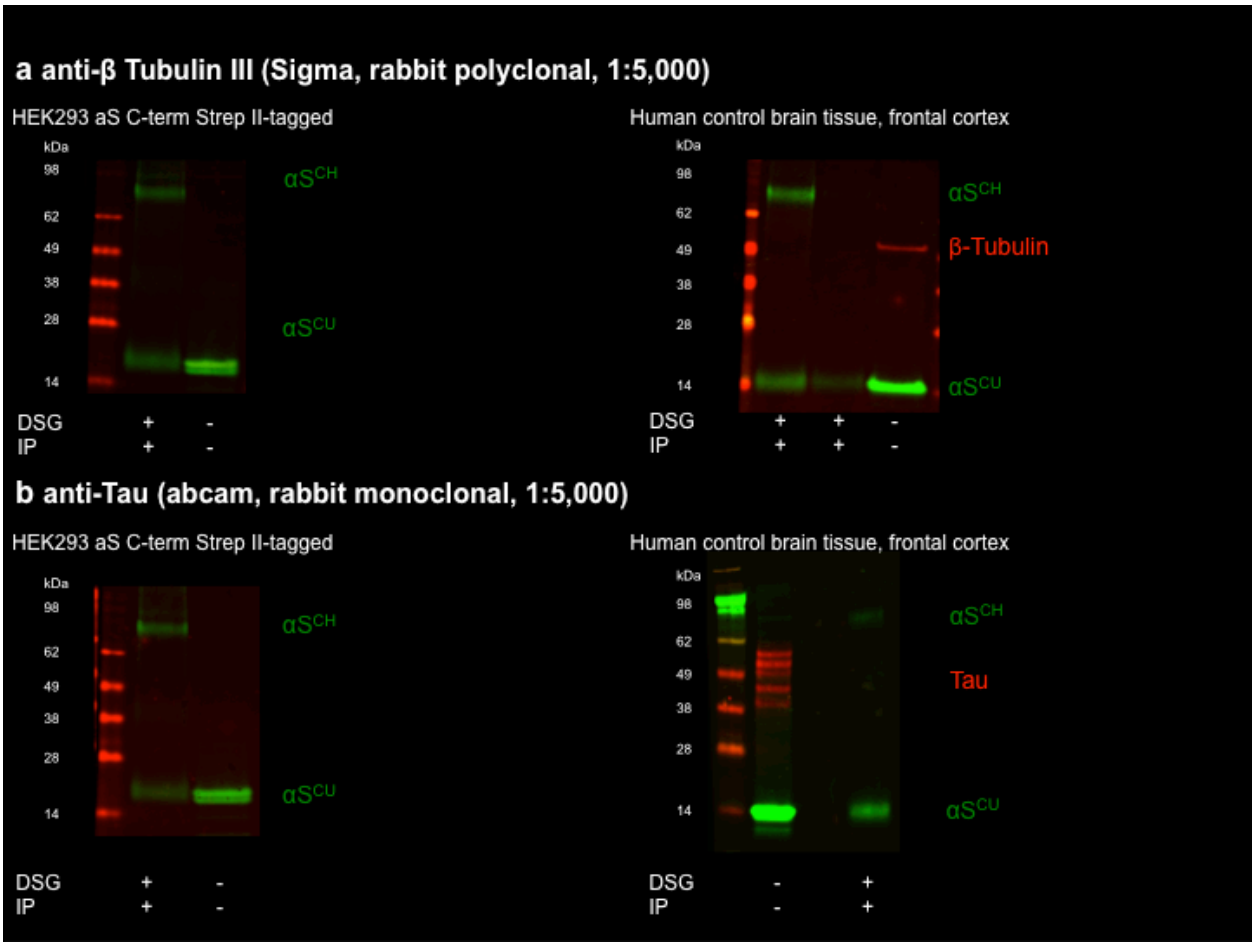
Supplementary Fig. 10 No co-purification of β-Actin or DJ1 and αS^{CH}. The crosslinking and immunoprecipitation procedures are described in the Methods section. Samples: HEK cells and human control brain tissue, frontal cortex. HEK293 αS C-term Strep II-tagged lysates were crosslinked and used to purify αS^{CH} and αS^{CU} using StrepTrap 5ml columns. The αS^{CH} and αS^{CU} from human brain lysate was immunoprecipitated after crosslinking using the Pierce™ Direct IP Kit. **a,** Western blot of immunoprecipitated and crosslinked protein lysate from HEK cells and human brain in comparison to the original lysates demonstrating no co-immunoprecipitation of the β-Actin protein and αS^{CH}. **b,** Western blot of immunoprecipitated and crosslinked protein lysate from HEK cells and human brain in comparison to the original lysates demonstrating no co-immunoprecipitation of the DJ1 protein and αS^{CH}. The immunoprecipitated human brain sample has been concentrated using an Amicon ultra 50 kDa filter unit and the first lane depicts the concentrated sample, second lane depicts the flow through. DSG "+" = crosslinked sample. DSG "-" = non-crosslinked (control) sample. IP "+" = immunoprecipitated sample, IP "-" = no immunoprecipitation performed.

Fig. S11 Absent co-purification of HSP70 or 14-3-3 and αS^{CH}



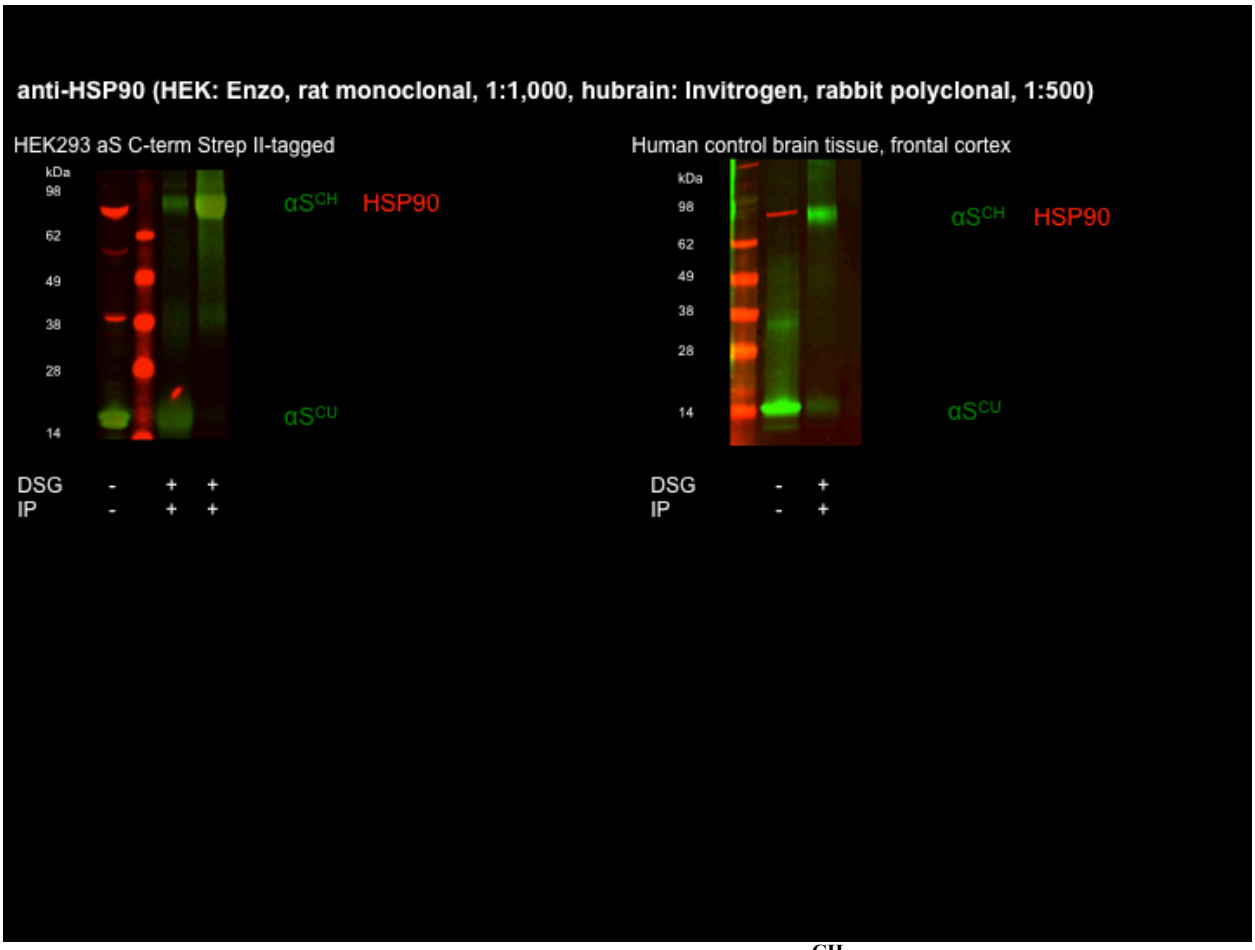
Supplementary Fig. 11 No co-purification of HSP70 or 14-3-3 and αS^{CH}. The crosslinking and immunoprecipitation procedures are described in the Methods section. Samples: HEK cells and human control brain tissue, frontal cortex. HEK293 αS C-term Strep II-tagged lysates were crosslinked and used to purify αS^{CH} and αS^{CU} using StrepTrap 5ml columns. The αS^{CH} and αS^{CU} from human brain lysate was immunoprecipitated after crosslinking using the Pierce™ Direct IP Kit. **a**, Western blot of immunoprecipitated and crosslinked protein lysate from HEK cells and human brain in comparison to the original lysates and one mock IP sample (human brain, no antibody for capturing used). HEK: First lane depicts the crosslinked original lysate, second lane depicts the non-crosslinked original lysate, third and fourth lane depict the αS after crosslinking, immunoprecipitation and SEC to separate αS^{CH} and αS^{CU}. IP human brain samples have been concentrated using Amicon ultra 50 kDa filter units. The Western blots demonstrate no co-immunoprecipitation of HSP70 protein (left) and αS^{CH} (right). **b**, Western blot of immunoprecipitated and crosslinked protein lysate from HEK cells and human brain in comparison to the original lysates demonstrating no co-immunoprecipitation of 14-3-3 protein and αS^{CH}. The immunoprecipitated human brain sample has been concentrated using an Amicon ultra 50 kDa filter unit and the first lane depicts the concentrated sample, second lane depicts the flow through. DSG "+" = crosslinked sample. DSG "-" = non-crosslinked (control) sample. IP "+" = immunoprecipitated sample, IP "-" = no immunoprecipitation performed.

Fig. S12 Absent co-purification of β-Tubulin III or Tau and αS^{CH}



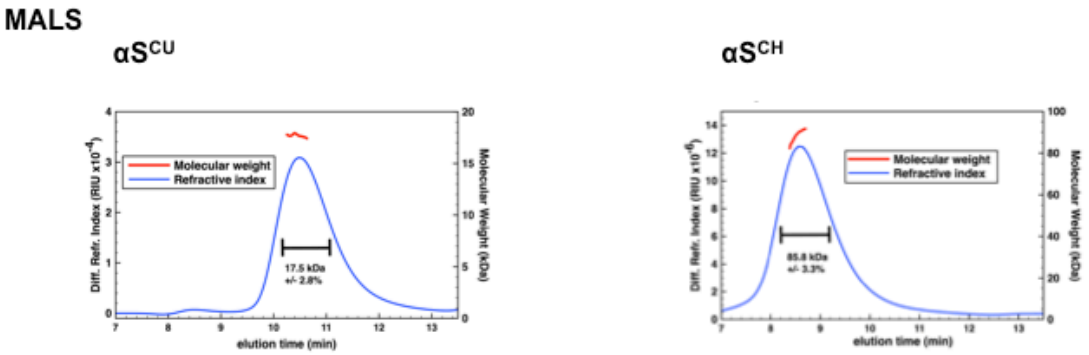
Supplementary Fig. 12 No co-purification of β-Tubulin or Tau and αS^{CH}. The crosslinking and immunoprecipitation procedures are described in the Methods section. Samples: HEK cells and human control brain tissue, frontal cortex. HEK293 αS C-term Strep II-tagged lysates were crosslinked and used to purify αS^{CH} and αS^{CU} using StrepTrap 5ml columns. The αS^{CH} and αS^{CU} from human brain lysate was immunoprecipitated after crosslinking using the Pierce™ Direct IP Kit. **a,** Western blot of immunoprecipitated and crosslinked protein lysate from HEK cells and human brain in comparison to the original lysates demonstrating no co-immunoprecipitation of β-Tubulin protein and αS^{CH} in the brain sample. The immunoprecipitated human brain sample has been concentrated using an Amicon ultra 50 kDa filter unit and the first lane depicts the concentrated sample, second lane depicts the flow through. β Tubulin could not be demonstrated in the original lysate and immunoprecipitated sample of HEK cells. **b**, Western blot of immunoprecipitated and crosslinked protein lysate from HEK cells and human brain in comparison to the original lysates demonstrating no co-immunoprecipitation of tau protein and αS^{CH} in the human brain sample. Tau could not be demonstrated in the original lysate and immunoprecipitated sample of HEK cells. DSG "+" = crosslinked sample. DSG "-" = non-crosslinked (control) sample. IP "+" = immunoprecipitated sample, IP "-" = no immunoprecipitation performed.

Fig. S13 Absent co-purification of HSP90 and αS^{CH}



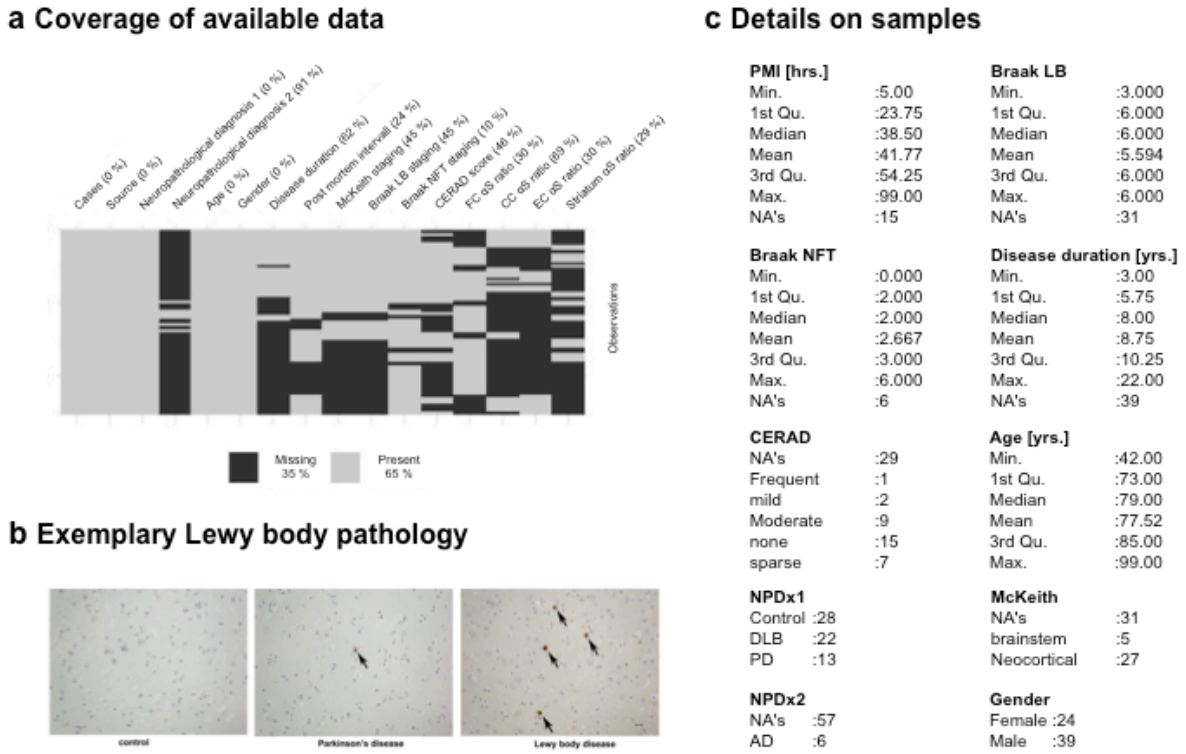
Supplementary Fig. 13 No co-purification of HSP90 and αS^{CH}. The crosslinking and immunoprecipitation procedures are described in the Methods section. Samples: HEK cells and human control brain tissue, frontal cortex. HEK293 αS C-term Strep II-tagged lysates were crosslinked and used to purify αS^{CH} and αS^{CU} using StrepTrap 5ml columns. The αS^{CH} and αS^{CU} from human brain lysate was immunoprecipitated after crosslinking using the Pierce Pierce™ Direct IP Kit. Western blot of immunoprecipitated and crosslinked protein lysate from HEK cells and human brain in comparison to the original lysates demonstrating no co-immunoprecipitation of HSP90 protein and αS^{CH}. HEK: First lane depicts the non-crosslinked original lysate, second lane and third lane depict the αS after crosslinking, immunoprecipitation and SEC to separate αS^{CH} and αS^{CU}. DSG "+" = crosslinked sample. DSG "-" = non-crosslinked (control) sample. IP "+" = immunoprecipitated sample, IP "-" = no immunoprecipitation performed.

Fig. S14 αS^{CH} exhibits a mass of 86 kDa



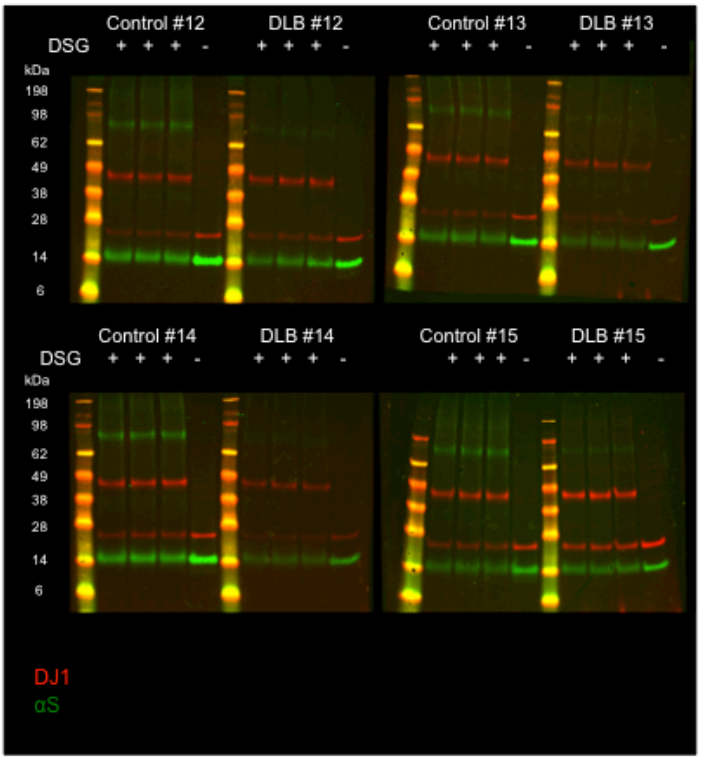
Supplementary Fig. 14 αS^{CH} exhibits a mass of 86 kDa. Immunoprecipitated αS^{CH} from frontal cortex tissue of a control was separated from αS^{CU} by size-exclusion chromatography and analyzed via multi-angle light scattering demonstrating a mass of approximately 86 kDa.

Fig. S15 Compendium of sample information



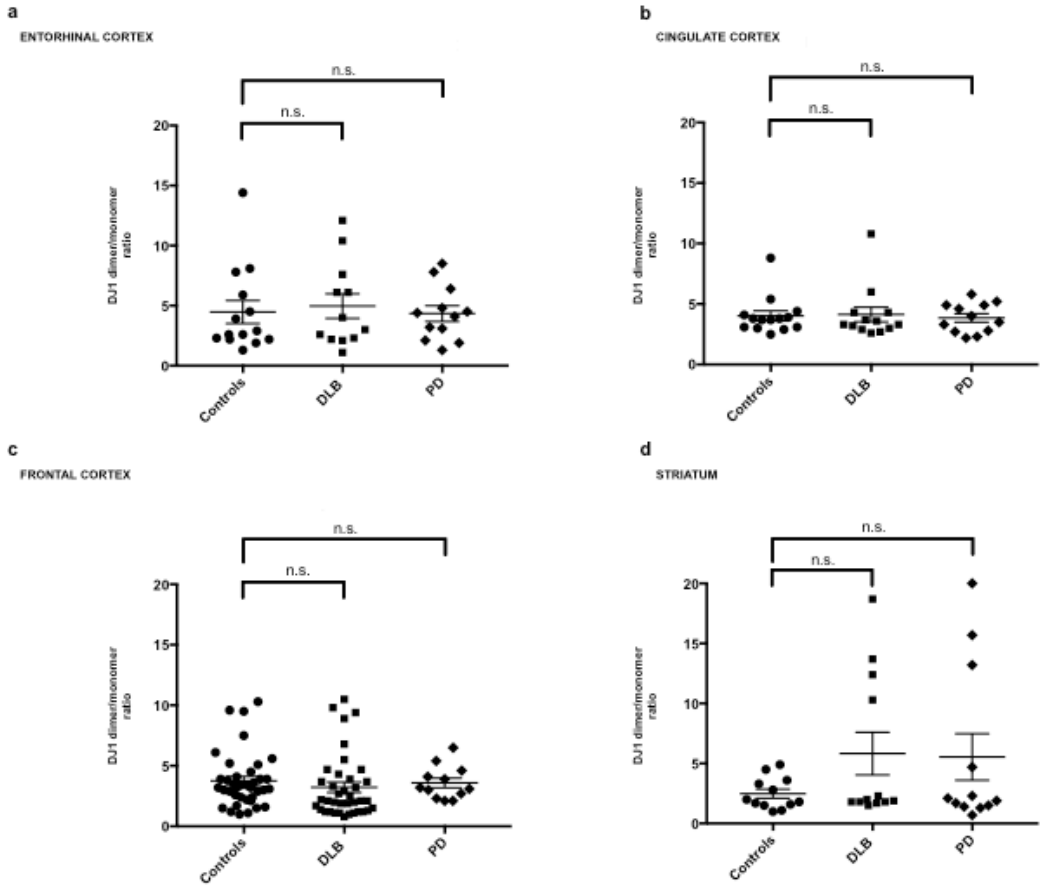
Supplementary Fig. 15 Summary of the clinical and neuropathological data of the analyzed human brain samples. a, Coverage (availability) of the clinical and neuropathological data. LB = Lewy body, NFT = neurofibrillary tangles, CERAD = Consortium to Establish a Registry for Alzheimer's Disease, FC = frontal cortex, CC = cingulate cortex, EC = entorhinal cortex. **b**, Details of clinical and neuropathological features. **c**, Representative immunohistochemial pictures of Lewy body pathology of one control, PD and DLB case.

Fig. S16 Exemplary Western blot of analyzed samples



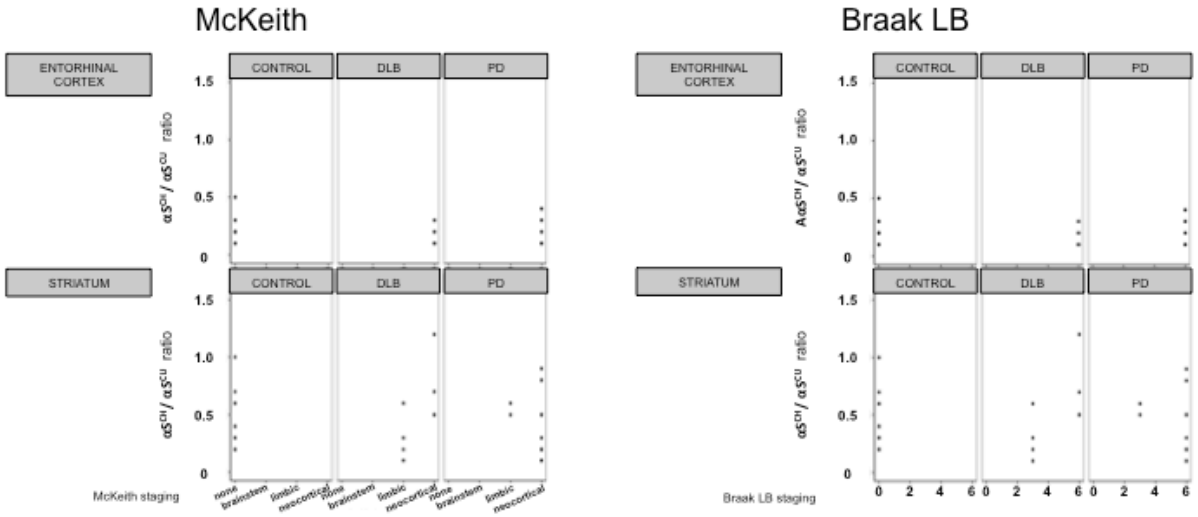
Supplementary Fig. 16 Western blot example of reduced αS^{CH} in 4 DLB patients. Western blot of crosslinked frontal cortex (FC) lysate (control vs. DLB, frontal cortex, n=4 each). The crosslinking reaction was performed in technical triplicates alongside with one non-crosslinked (PBS) sample. The Western blot demonstrates reduced αS^{CH} / αS^{CU} ratios in DLB patients compared to controls. DSG "+" = crosslinked sample. DSG "-" = non-crosslinked sample. Green = αS, red = DJ1

Fig. S17 Similar DJ1 dimer to monomer ratios across all brain regions



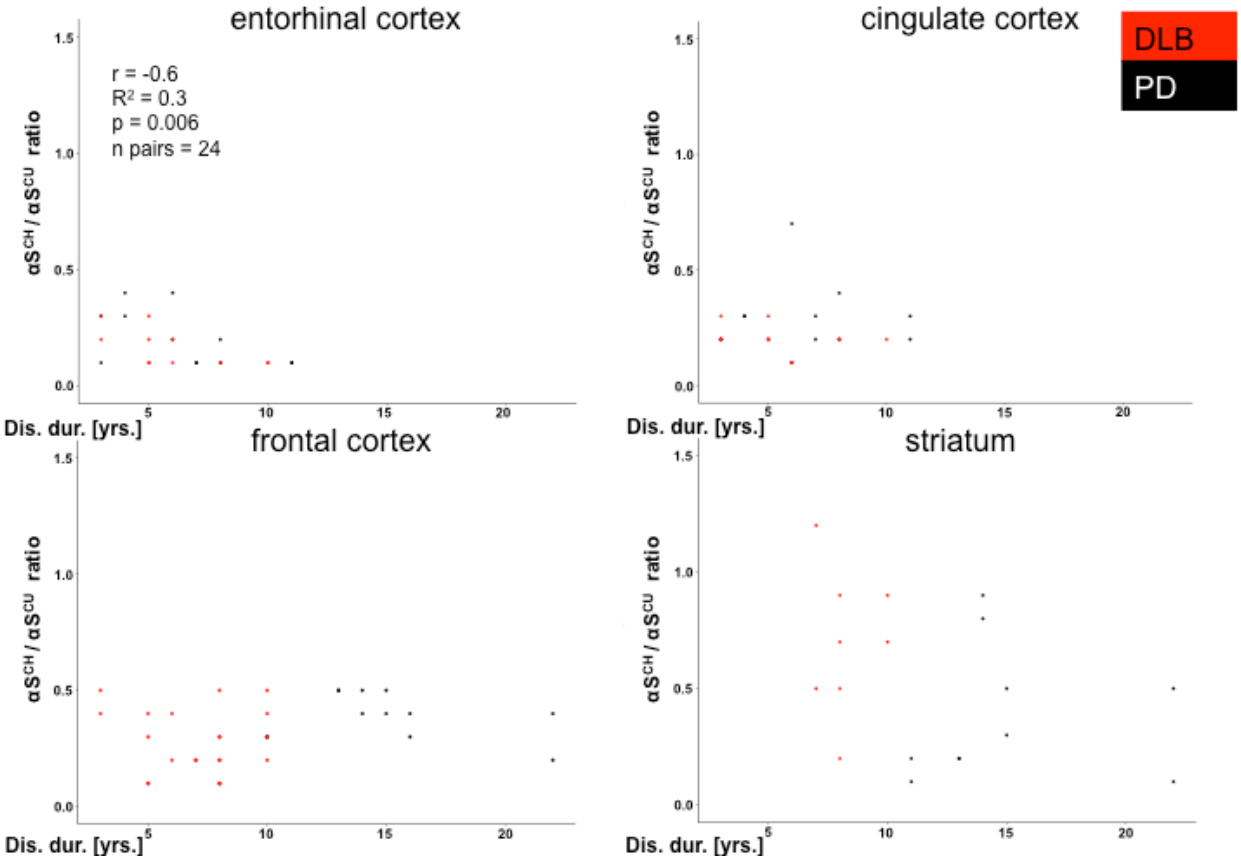
Supplementary Fig. 17 Similar DJ1 dimer to monomer ratios across all brain regions. The DJ1 serves as an internal control for the crosslinking procedure. Across different brain regions (**a**, entorhinal cortex, **b**, cingulate cortex, **c**, frontal cortex, **d**, striatum) no differences in DJ1 dimer to monomer ratios were detected comparing controls, DLB patients and PD patients. Entorhinal cortex controls n=7 individuals, DLB n=6 individuals, PD n=6 individuals. cingulate cortex controls n=5 individuals, DLB n=7 individuals, PD n=6 individuals. Frontal cortex controls n=19 individuals, DLB n=19 individuals, PD n=6 individuals. Striatum controls n=6 individuals, DLB n=6 individuals, PD n=6 individuals. Samples have been analyzed in biological duplicates and technical triplicates except for one cingulate cortex sample of one DLB individual (low amount of tissue). N.s.=not significant.

Fig. S18 No significant correlation of αS^{CH} / αS^{CU} ratios and Lewy body (LB) pathology in the entorhinal cortex or striatum



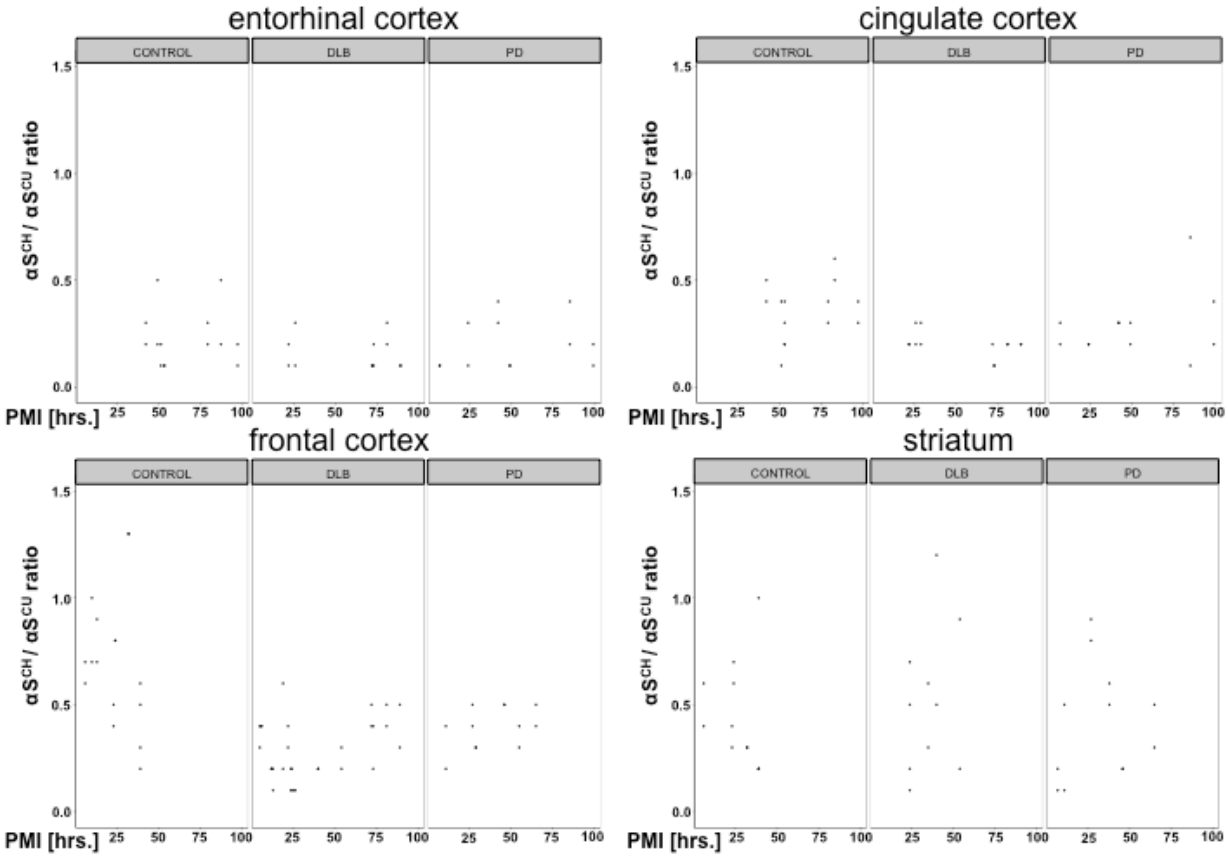
Supplementary Fig. 18 No significant correlation of αS^{CH} / αS^{CU} ratios and McKeith or Braak Lewy body (LB) pathology in the entorhinal cortex or striatum. Across the entorhinal cortex (EC) and striatum no significant correlation of αS^{CH} / αS^{CU} ratios and McKeith (none, brainstem, limbic, neocortical) or Braak LB staging (0, 1, 2, 3, 4, 5, 6) was detected comparing controls to DLB patients and PD patients. EC αS^{CH} / αS^{CU} ratios vs. McKeith staging r = -0.2, R^2 = 0.04, p = 0.2, n pairs = 38. EC αS^{CH} / αS^{CU} vs. Braak LB staging r = -0.2, R^2 = 0.05, p = 0.2, n pairs = 38. Striatum αS^{CH} / αS^{CU} vs. McKeith staging r = 0.09, R^2 = 0.009, p = 0.6, n pairs = 32. Striatum αS^{CH} / αS^{CU} ratios vs. Braak LB staging r = 0.09, R^2 = 0.009, p = 0.6, n pairs = 32.

Fig. S19 Significant correlation of αS^{CH} / αS^{CU} ratios and disease duration of the entorhinal cortex but not frontal cortex, cingulate cortex or striatum



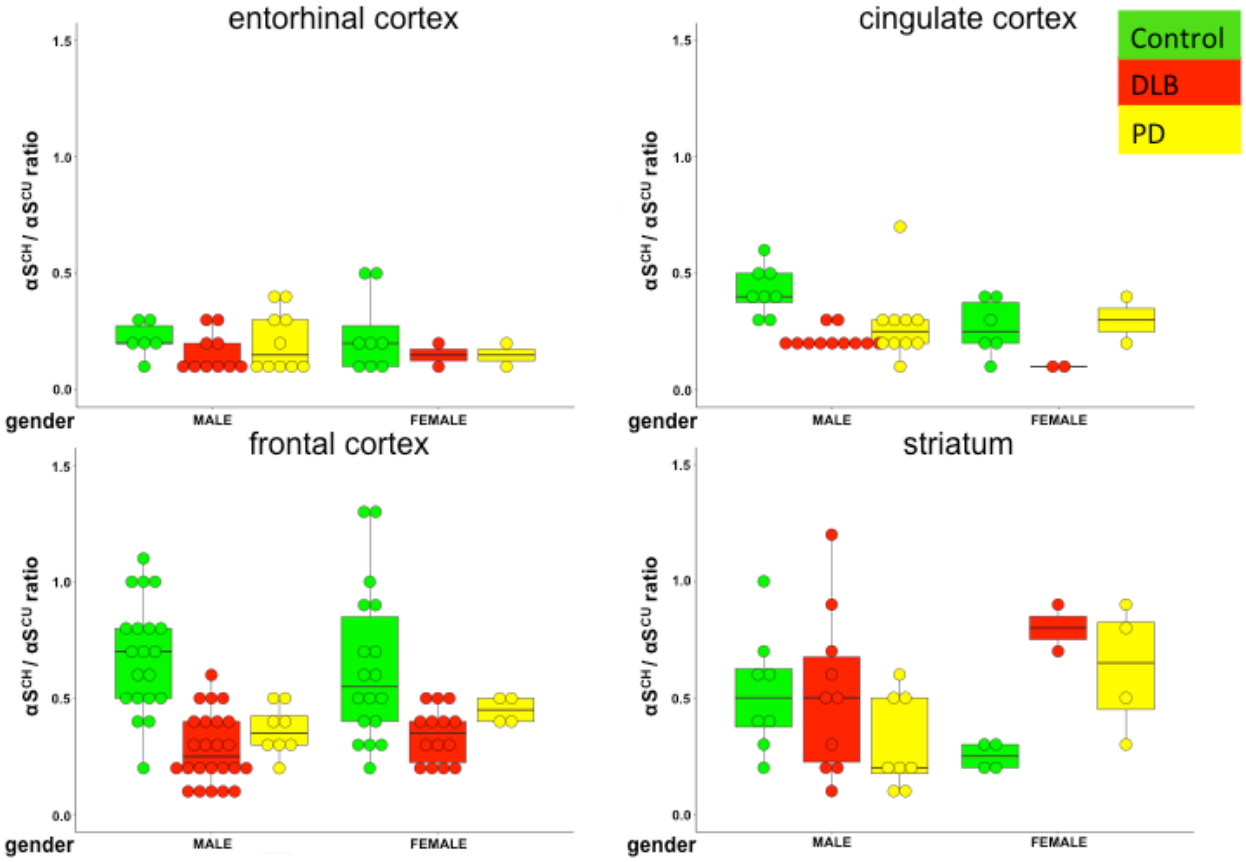
Supplementary Fig. 19 Significant negative correlation of αS^{CH} / αS^{CU} ratios and disease duration in the entorhinal cortex. In the entorhinal cortex (EC) significant negative correlation of αS^{CH} / αS^{CU} ratios and disease duration was detected comparing controls to DLB patients and PD patients. EC αS^{CH} / αS^{CU} ratios vs. disease duration r = -0.6, R^2 = 0.3, p = 0.006, n pairs = 24. No correlation could be found in the cingulate cortex (CC), frontal cortex (FC) or striatum between αS^{CH} / αS^{CU} ratios and disease duration comparing controls to DLB patients and PD patients. CC αS^{CH} / αS^{CU} ratios vs. disease duration r = 0.04, R^2 = 0.001, p = 0.9, n pairs = 25. FC αS^{CH} / αS^{CU} ratios vs. disease duration r = 0.3, R^2 = 0.07, p = 0.1, n pairs = 34. Striatum αS^{CH} / αS^{CU} ratios vs. disease duration r = -0.4, R^2 = 0.1, p = 0.2, n pairs = 18. Dis. dur. = Disease duration.

Fig. S20 No significant correlation of αS^{CH} / αS^{CU} ratios and PMI interval



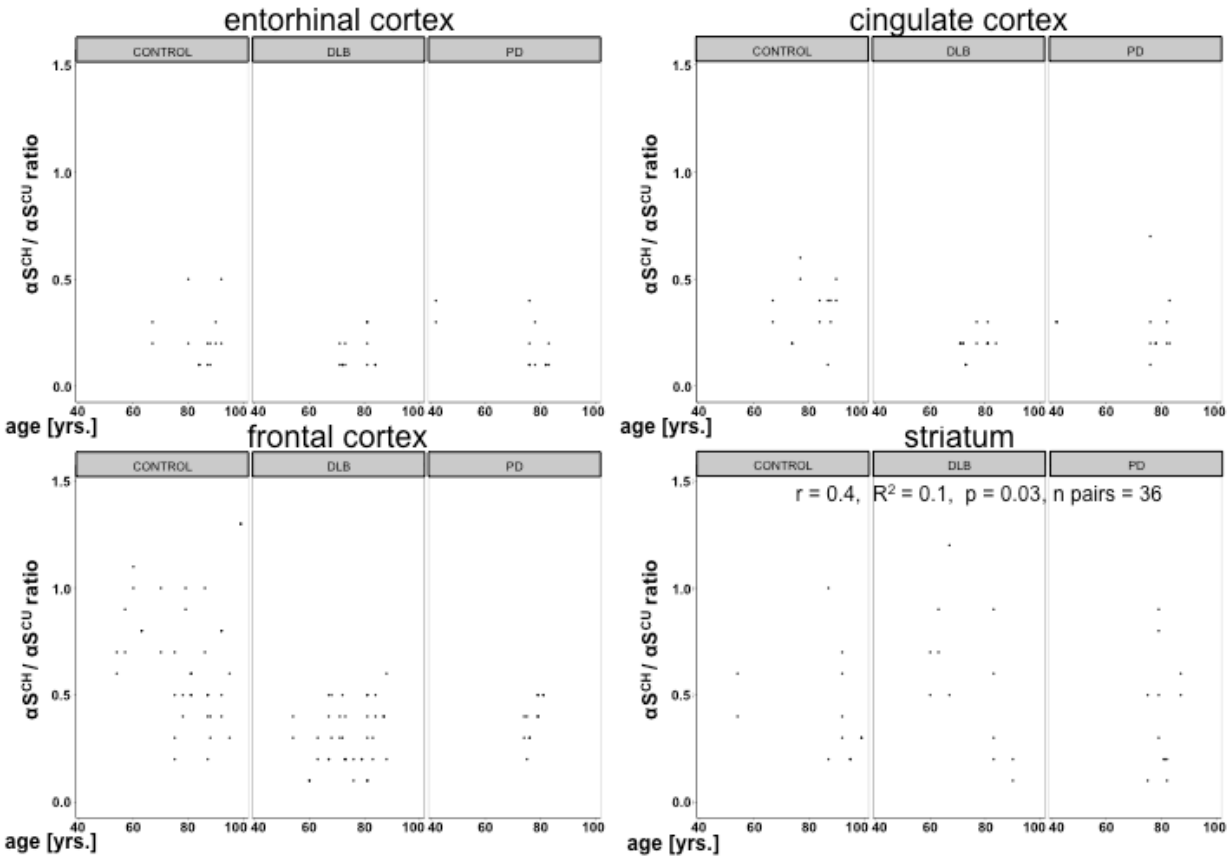
Supplementary Fig. 20 No significant correlation of αS^{CH} / αS^{CU} ratios and post mortem interval (PMI). Across different brain regions (entorhinal cortex (EC), cingulate cortex (CC), frontal cortex (FC) and striatum) no correlation of αS^{CH} / αS^{CU} ratios and PMI interval (hrs. = hours) was detected comparing controls to DLB patients and PD patients. EC αSYN αS^{CH} / αS^{CU} vs. PMI r = 0.07, R^2 = 0.005, p = 0.7, n pairs = 38. CC αS^{CH} / αS^{CU} ratios vs. PMI r = 0.2, R^2 = 0.04, p = 0.2, n pairs = 39. FC αS^{CH} / αS^{CU} vs. PMI r = -0.07, R^2 = 0.005, p = 0.6, n pairs = 58. Striatum αS^{CH} / αS^{CU} ratios vs. PMI r = 0.1, R^2 = 0.01, p = 0.5, n pairs = 34.

Fig. S21 No significant correlation of αS^{CH} / αS^{CU} ratios and gender



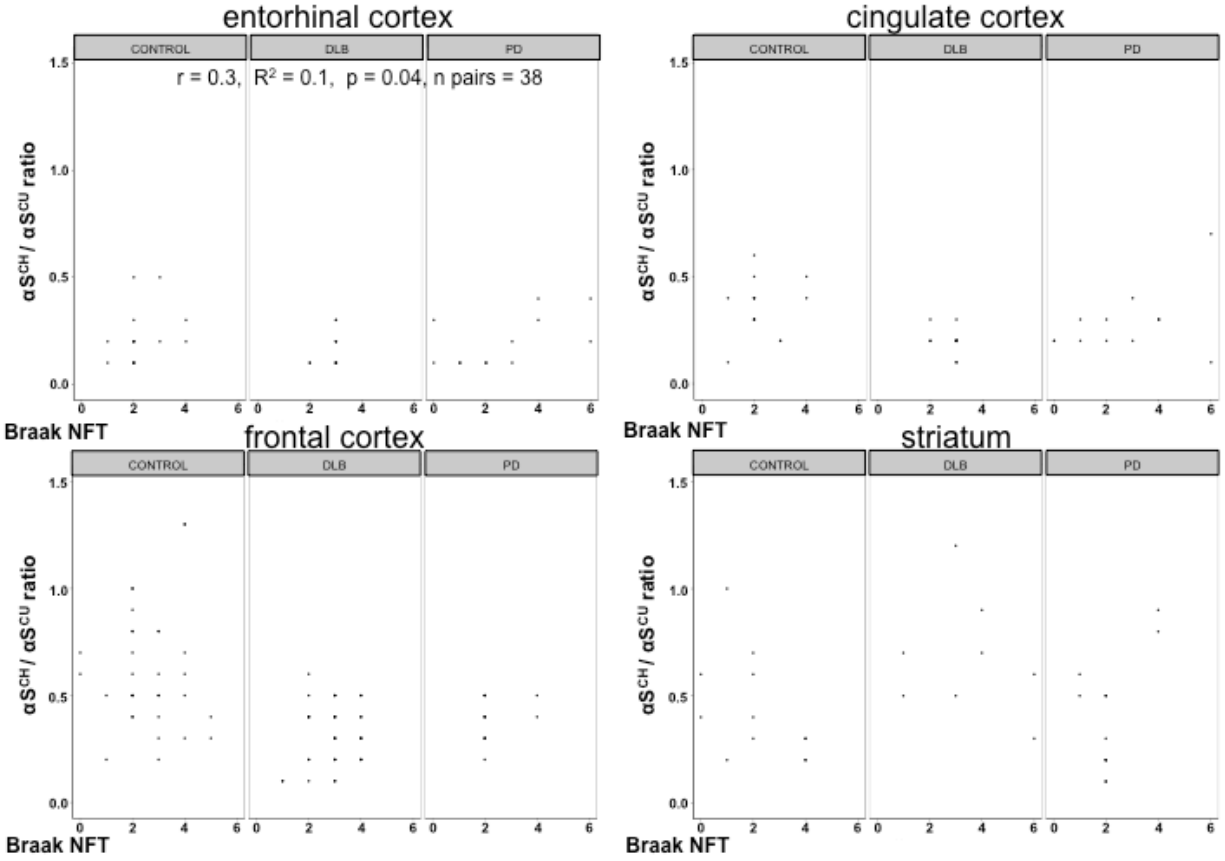
Supplementary Fig. 21 No significant correlation of αS^{CH} / αS^{CU} ratios and gender. Across different brain regions (entorhinal cortex (EC), cingulate cortex (CC), frontal cortex (FC) and striatum) no correlation of αS^{CH} / αS^{CU} ratios and gender was detected comparing controls to DLB patients and PD patients. EC αS^{CH} / αS^{CU} vs. gender r = 0.07, R^2 = 0.004, p = 0.7, n pairs = 38. CC αS^{CH} / αS^{CU} ratios vs. gender r = -0.2, R^2 = 0.03, p = 0.3, n pairs = 39. FC αS^{CH} / αS^{CU} ratios vs. gender r = 0.09, R^2 = 0.009, p = 0.4, n pairs = 88. Striatum αS^{CH} / αS^{CU} ratios vs. gender r = 0.09, R^2 = 0.008, p = 0.6, n pairs = 36.

Fig. S22 Significant correlation of αS^{CH} / αS^{CU} ratios and age of the striatum but not entorhinal cortex, frontal cortex or cingulate cortex



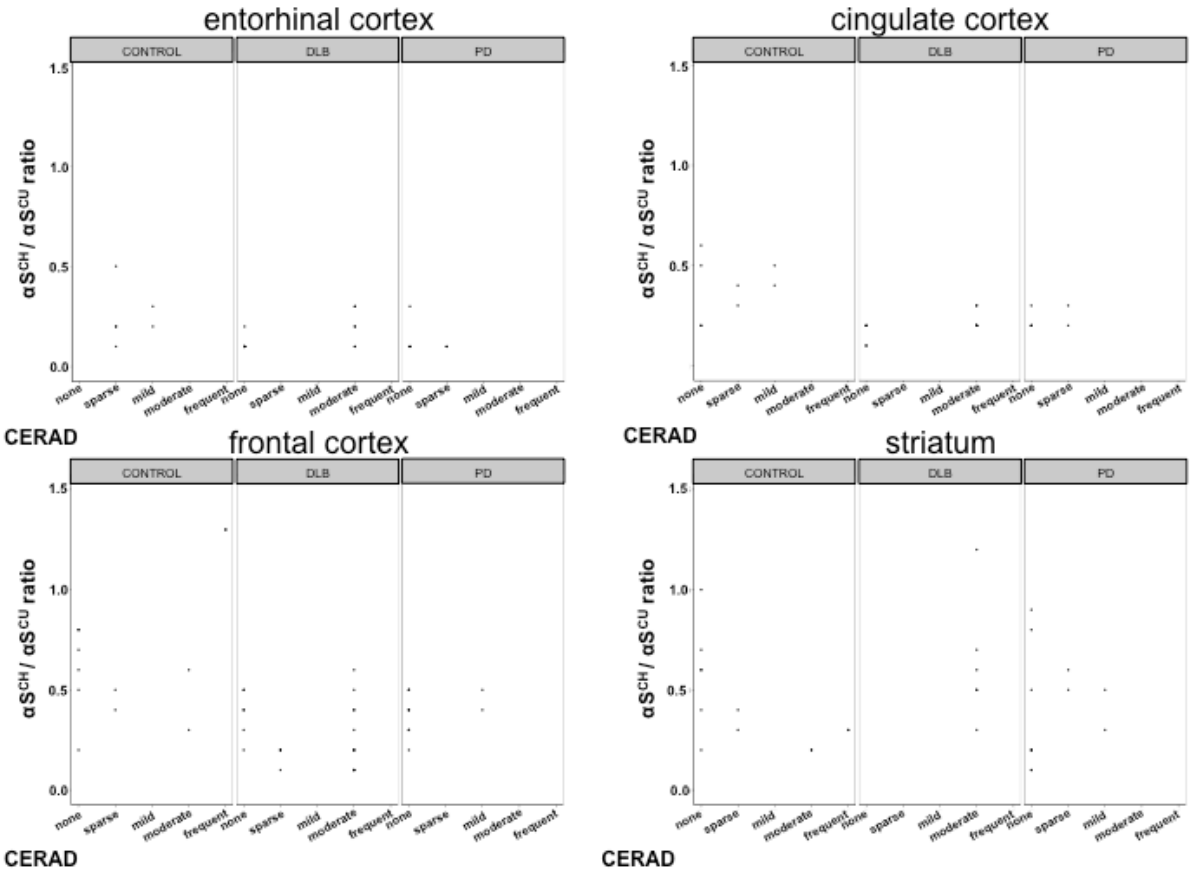
Supplementary Fig. 22 Significant negative correlation of αS^{CH} / αS^{CU} ratios and age in the striatum. In the striatum a significant negative correlation of αS^{CH} / αS^{CU} ratios and age (yrs. = years) was detected comparing controls to DLB patients and PD patients. Striatum αS^{CH} / αS^{CU} ratios vs. age r = -0.4, R^2 = 0.1, p = 0.02, n pairs = 36. No correlation could be found in the entorhinal cortex (EC), cingulate cortex (CC) or frontal cortex (FC) between αS^{CH} / αS^{CU} and age comparing controls to DLB patients and PD patients. EC αS^{CH} / αS^{CU} ratios vs. age r = -0.2, R^2 = 0.04, p = 0.3, n pairs = 38. αS^{CH} / αS^{CU} vs. age r = 0.1, R^2 = 0.01, p = 0.5, n pairs = 39. FC αS^{CH} / αS^{CU} vs. age r = 0.09, R^2 = 0.009, p = 0.4, n pairs = 88.

Fig. S23 Significant positive correlation of αS^{CH} / αS^{CU} ratios and Braak neurofibrillary tangle (NFT) staging in the entorhinal cortex.



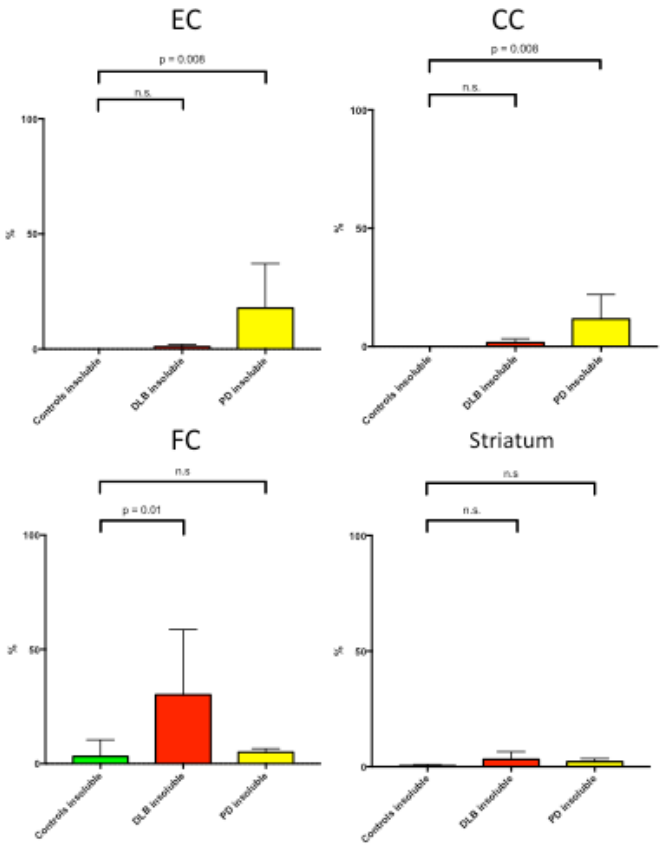
Supplementary Fig. 23 Significant positive correlation of αS^{CH} / αS^{CU} ratios and Braak neurofibrillary tangle (NFT) staging in the entorhinal cortex. In the entorhinal cortex (EC) a significant positive correlation of αS^{CH} / αS^{CU} ratios and Braak NFT staging (0, 1, 2, 3, 4, 5, 6) was detected comparing controls to DLB patients and PD patients. EC αS^{CH} / αS^{CU} ratios vs. Braak NFT staging r = 0.3, R^2 = 0.1, p = 0.04, n pairs = 38. No correlation could be found in the cingulate cortex (CC), frontal cortex (FC) or striatum between αS^{CH} / αS^{CU} ratios and Braak NFT staging comparing controls to DLB patients and PD patients. CC αS^{CH} / αS^{CU} ratios vs. Braak neurofibrillary tangle staging r = 0.2, R^2 = 0.03, p = 0.3, n pairs = 39. FC αS^{CH} / αS^{CU} ratios vs. Braak neurofibrillary tangle staging r = -0.007, R^2 = 0.00005, p = 1.0, n pairs = 78. Striatum αS^{CH} / αS^{CU} vs. Braak neurofibrillary tangle staging r = 0.05, R^2 = 0.003, p = 0.8, n pairs = 32.

Fig. S24 No significant correlation of αS^{CH} / αS^{CU} ratios and CERAD score



Supplementary Fig. 24 No significant correlation of αS^{CH} / αS^{CU} ratios and CERAD (Consortium to Establish a Registry for Alzheimer's Disease) score. Across different brain regions (entorhinal cortex (EC), cingulate cortex (CC), frontal cortex (FC) and striatum) no correlation of αS^{CH} / αS^{CU} ratios and CERAD score (none, sparse, mild, moderate and frequent) was detected comparing controls to DLB patients and PD patients. EC αS^{CH} / αS^{CU} ratios vs. CERAD r = 0.3, R^2 = 0.09, p = 0.2, n pairs = 24. CC αS^{CH} / αS^{CU} ratios vs. CERAD r = -0.004, R^2 = 0.00001, p = 1.0, n pairs = 27. FC αS^{CH} / αS^{CU} ratios vs. CERAD r = 0.09, R^2 = 0.007, p = 0.6, n pairs = 46. Striatum αS^{CH} / αS^{CU} ratios vs. CERAD r = -0.01, R^2 = 0.0001, p = 1.0, n pairs = 30

Fig. S25 DLB and sPD patients exhibit higher levels of pathological, insoluble αS compared to controls



Supplementary Fig. 25 PD and DLB patients exhibit higher levels of pathological, insoluble αS compared to controls. The procedure for the protein fractionation and the ELISA is described in the Methods section. The amount of each fraction was analyzed by ELISA and calculated as percentage for the overall αS levels in each fraction (cytosol, membrane, insoluble). PD patients exhibit higher levels of pathological, insoluble αS in early affected brain regions (entorhinal cortex (EC) and cingulate cortex (CC)) according to the classical caudal-rostral progression. DLB patients exhibiting early cortical involvement display higher levels of pathological, insoluble αS in the frontal cortex (FC).

References:

1 Dettmer U, Newman AJ, Luth ES, Bartels T, Selkoe D. In vivo cross-linking reveals principally oligomeric forms of α-synuclein and β-synuclein in neurons and non-neural cells. *J Biol Chem*. 2013;288(9):6371-6385. doi:10.1074/jbc.M112.403311

2 Bartels T, Choi JG, Selkoe DJ. α-Synuclein occurs physiologically as a helically folded tetramer that resists aggregation. Nature. 2011 Aug 14;477(7362):107-10. doi: 10.1038/nature10324. PMID: 21841800; PMCID: PMC3166366.

Table S1. Mass Spectrometry Results of purified αS from multiple sources

αS sample (gel piece)	Acce-ssion	Description	Protein Group IDs	Coverage [%]	Unique Peptides	Protein Unique Peptides	AAs	MW [kDa]	calc. pI	Abun-dances	Gene Symbol
mock 14 kDa	P81605	Dermicidin	11	20	3	3	110	11.3	6.54	7.3E+06	DCD
mock 14 kDa	Q86YZ3	Hornerin	4	1	2	2	2850	282.2	10.04	5.7E+06	HRNR
mock 14 kDa	P37840	α-Synuclein	12	21	4	2	140	14.5	4.7	2.4E+06	SNCA
αS cytosolic unfolded, 14 kDa	P37840	α-Synuclein	19	40	9	5	140	14.5	4.7	2.8E+07	SNCA
αS cytosolic unfolded, 14 kDa	Q86YZ3	Hornerin	5	3	3	3	2850	282.2	10.04	2.0E+07	HRNR
αS cytosolic unfolded, 14 kDa	P14923	Junction plakoglobin	16	4	3	3	745	81.7	6.14	6.9E+06	JUP
αS cytosolic unfolded, 14 kDa	P15924	Desmoplakin	13	1	5	5	2871	331.6	6.81	3.0E+06	DSP
αS cytosolic unfolded, 14 kDa	P81605	Dermicidin	18	12	3	3	110	11.3	6.54	1.7E+06	DCD
mock 80 kDa	P37840	α-Synuclein	24	52	12	9	140	14.5	4.7	1.3E+08	SNCA
mock 80 kDa	Q86YZ3	Hornerin	8	3	2	2	2850	282.2	10.04	1.7E+07	HRNR
mock 80 kDa	P0DMV8	Heat shock 70 kDa protein	21	14	5	1	641	70	5.66	7.2E+06	HSPA1B; HSPA1A
mock 80 kDa	P15924	Desmoplakin	17	1	3	3	2871	331.6	6.81	1.5E+06	DSP
mock 80 kDa	P81605	Dermicidin	23	21	2	2	110	11.3	6.54	4.9E+05	DCD
αS cytosolic helical, 80 kDa	P0DMV8	Heat shock 70 kDa protein 1A	172	89	43	43	641	70	5.66	1.6E+11	HSPA1B; HSPA1A
αS cytosolic helical, 80 kDa	P11021	Endoplasmic reticulum chaperone BiP	151	57	40	40	654	72.3	5.16	1.9E+10	HSPA5
αS cytosolic helical, 80 kDa	P37840	α-Synuclein	200	81	11	9	140	14.5	4.7	1.1E+10	SNCA
αS cytosolic helical, 80 kDa	P11142	Heat shock cognate 71 kDa protein	128	59	25	25	646	70.9	5.52	6.7E+09	HSPA8
αS cytosolic helical, 80 kDa	P38646	Stress-70 protein, mitochondrial	205	44	25	25	679	73.6	6.16	2.2E+09	HSPA9
αS cytosolic helical, 80 kDa	P14136	Glial fibrillary acidic protein	32	6	1	1	432	49.9	5.52	1.4E+09	GFAP
αS cytosolic helical, 80 kDa	P04406	Glyceraldehyde-3-phosphate dehydrogenase	147	59	14	13	335	36	8.46	5.2E+08	GAPDH
αS cytosolic helical, 80 kDa	P40926	Malate dehydrogenase, mitochondrial	99	30	7	7	338	35.5	8.68	3.3E+08	MDH2
αS cytosolic helical, 80 kDa	P13667	Protein disulfide-isomerase A4	34	25	13	13	645	72.9	5.07	3.0E+08	PDIA4
αS cytosolic helical, 80 kDa	P15924	Desmoplakin	113	8	23	23	2871	331.6	6.81	1.9E+08	DSP
αS cytosolic helical, 80 kDa	P49915	GMP synthase	70	15	7	7	693	76.7	6.87	1.6E+08	GMPS
αS cytosolic helical, 80 kDa	Q5D862	Filaggrin-2	106	2	5	5	2391	247.9	8.31	1.6E+08	FLG2
αS cytosolic helical, 80 kDa	P31944	Caspase-14	5	30	7	7	242	27.7	5.58	1.5E+08	CASP14
αS cytosolic helical, 80 kDa	Q12931	Heat shock protein 75 kDa, mitochondrial	112	17	8	8	704	80.1	8.21	1.5E+08	TRAP1

αS cytosolic helical, 80 kDa	Q02413	Desmoglein-1	108	17	12	12	1049	113.7	5.03	1.4E+08	DSG1
αS cytosolic helical, 80 kDa	P07237	Protein disulfide-isomerase	96	31	11	11	508	57.1	4.87	1.0E+08	P4HB
αS cytosolic helical, 80 kDa	P10809	60 kDa heat shock protein, mitochondrial	207	21	7	7	573	61	5.87	8.6E+07	HSPD1
αS cytosolic helical, 80 kDa	Q5T750	Skin-specific protein 32	211	4	2	2	250	26.2	7.97	7.5E+07	C1orf68
αS cytosolic helical, 80 kDa	P17066	Heat shock 70 kDa protein 6	75	22	3	3	643	71	6.14	7.1E+07	HSPA6
αS cytosolic helical, 80 kDa	P08133	Annexin A6	11	27	15	15	673	75.8	5.6	7.1E+07	ANXA6
αS cytosolic helical, 80 kDa	P07355	Annexin A2	145	37	12	4	339	38.6	7.75	6.5E+07	ANXA2
αS cytosolic helical, 80 kDa	Q08554	Desmocollin-1	246	7	5	5	894	99.9	5.43	5.2E+07	DSC1
αS cytosolic helical, 80 kDa	P07195	L-lactate dehydrogenase B chain	137	22	7	7	334	36.6	6.05	5.1E+07	LDHB
αS cytosolic helical, 80 kDa	P50395	Rab GDP dissociation inhibitor beta	226	28	5	5	445	50.6	6.47	5.1E+07	GDI2
αS cytosolic helical, 80 kDa	Q15181	Inorganic pyrophosphatase	63	21	4	4	289	32.6	5.86	5.0E+07	PPA1
αS cytosolic helical, 80 kDa	Q15084	Protein disulfide-isomerase A6	210	9	2	2	440	48.1	5.08	4.7E+07	PDIA6
αS cytosolic helical, 80 kDa	P14923	Junction plakoglobin	153	12	8	7	745	81.7	6.14	4.5E+07	JUP
αS cytosolic helical, 80 kDa	P07900	Heat shock protein HSP 90-alpha	43	15	5	4	732	84.6	5.02	4.3E+07	HSP90AA1
αS cytosolic helical, 80 kDa	P17812	CTP synthase 1	248	17	8	8	591	66.6	6.46	4.1E+07	CTPS1
αS cytosolic helical, 80 kDa	Q92945	Far upstream element-binding protein 2	146	3	2	2	711	73.1	7.3	4.0E+07	KHSRP
αS cytosolic helical, 80 kDa	P14618	Pyruvate kinase PKM	222	17	6	5	531	57.9	7.84	3.5E+07	PKM
αS cytosolic helical, 80 kDa	P23526	Adenosylhomocysteinase	184	9	4	4	432	47.7	6.34	3.4E+07	AHCY
αS cytosolic helical, 80 kDa	P00338	L-lactate dehydrogenase A chain	171	14	4	4	332	36.7	8.27	3.3E+07	LDHA
αS cytosolic helical, 80 kDa	P29401	Transketolase	102	12	5	5	623	67.8	7.66	2.9E+07	TKT
αS cytosolic helical, 80 kDa	P19623	Spermidine synthase	141	8	3	3	302	33.8	5.49	2.6E+07	SRM
αS cytosolic helical, 80 kDa	P13797	Plastin-3	203	17	9	6	630	70.8	5.6	2.6E+07	PLS3
αS cytosolic helical, 80 kDa	P40925	Malate dehydrogenase, cytoplasmic	216	13	3	3	334	36.4	7.36	2.6E+07	MDH1
αS cytosolic helical, 80 kDa	Q13867	Bleomycin hydrolase	193	5	2	2	455	52.5	6.27	2.6E+07	BLMH
αS cytosolic helical, 80 kDa	P00491	Purine nucleoside phosphorylase	185	34	7	7	289	32.1	6.95	2.5E+07	PNP
αS cytosolic helical, 80 kDa	P05089	Arginase-1	158	22	5	5	322	34.7	7.21	2.2E+07	ARG1
αS cytosolic helical, 80 kDa	P06733	Alpha-enolase	206	14	4	3	434	47.1	7.39	2.1E+07	ENO1
αS cytosolic helical, 80 kDa	P14625	Endoplasmin	15	11	6	4	803	92.4	4.84	2.1E+07	HSP90B1
αS cytosolic helical, 80 kDa	P54652	Heat shock-related 70 kDa protein 2	92	38	8	8	639	70	5.74	2.0E+07	HSPA2

αS cytosolic helical, 80 kDa	P78371	T-complex protein 1 subunit beta	174	13	4	4	535	57.5	6.46	1.9E+07	CCT2
αS cytosolic helical, 80 kDa	Q96QA5	Gasdermin-A	230	9	3	3	445	49.3	5.29	1.8E+07	GSDMA
αS cytosolic helical, 80 kDa	Q96AE4	Far upstream element-binding protein 1	16	7	3	3	644	67.5	7.61	1.8E+07	FUBP1
αS cytosolic helical, 80 kDa	P36551	Oxygen-dependent coproporphyrinogen -III oxidase, mitochondrial	215	11	4	4	454	50.1	8.25	1.7E+07	CPOX
αS cytosolic helical, 80 kDa	P48147	Prolyl endopeptidase OS=Homo sapiens	67	4	2	2	710	80.6	5.86	1.5E+07	PREP
αS cytosolic helical, 80 kDa	P22234	Multifunctional protein ADE2 OS=Homo sapiens	115	8	2	2	425	47	7.23	1.5E+07	PAICS
αS cytosolic helical, 80 kDa	P38117	Electron transfer flavoprotein subunit beta	170	7	2	2	255	27.8	8.1	1.4E+07	ETFB
αS cytosolic helical, 80 kDa	P13639	Elongation factor 2	179	10	6	6	858	95.3	6.83	1.3E+07	EEF2
αS cytosolic helical, 80 kDa	P07384	Calpain-1 catalytic subunit	238	6	3	3	714	81.8	5.67	1.3E+07	CAPN1
αS cytosolic helical, 80 kDa	P26038	Moesin	116	14	4	4	577	67.8	6.4	1.3E+07	MSN
αS cytosolic helical, 80 kDa	P08238	Heat shock protein HSP 90-beta	101	15	4	2	724	83.2	5.03	1.2E+07	HSP90AB1
αS cytosolic helical, 80 kDa	P62258	14-3-3 protein epsilon	247	13	3	2	255	29.2	4.74	1.2E+07	YWHAE
αS cytosolic helical, 80 kDa	O95302	Peptidyl-prolyl cis-trans isomerase FKBP9	103	3	2	2	570	63	5.08	1.2E+07	FKBP9
αS cytosolic helical, 80 kDa	P06576	ATP synthase subunit beta, mitochondrial	167	11	4	4	529	56.5	5.4	1.1E+07	ATP5B
αS cytosolic helical, 80 kDa	P32119	Peroxiredoxin-2 OS=Homo sapiens	244	27	3	3	198	21.9	5.97	1.1E+07	PRDX2
αS cytosolic helical, 80 kDa	P15311	Ezrin	127	11	3	3	586	69.4	6.27	1.1E+07	EZR
αS cytosolic helical, 80 kDa	Q04837	Single-stranded DNA-binding protein, mitochondrial	39	16	2	2	148	17.2	9.6	1.1E+07	SSBP1
αS cytosolic helical, 80 kDa	Q9BWD1	Acetyl-CoA acetyltransferase, cytosolic	132	14	3	3	397	41.3	6.92	1.0E+07	ACAT2
αS cytosolic helical, 80 kDa	P00505	Aspartate aminotransferase, mitochondrial	220	8	3	3	430	47.5	9.01	1.0E+07	GOT2
αS cytosolic helical, 80 kDa	P41250	Glycine--tRNA ligase	240	9	4	4	739	83.1	7.03	9.4E+06	GARS
αS cytosolic helical, 80 kDa	P19338	Nucleolin	178	11	6	6	710	76.6	4.7	8.2E+06	NCL
αS cytosolic helical, 80 kDa	Q00839	Heterogeneous nuclear ribonucleoprotein U	136	4	2	2	825	90.5	6	7.8E+06	HNRNPU
αS cytosolic helical, 80 kDa	Q99798	Aconitate hydratase, mitochondrial	225	4	2	2	780	85.4	7.61	7.8E+06	ACO2
αS cytosolic helical, 80 kDa	P34932	Heat shock 70 kDa protein 4	129	6	4	4	840	94.3	5.19	7.8E+06	HSPA4
αS cytosolic helical, 80 kDa	P26639	Threonine--tRNA ligase, cytoplasmic	148	3	2	2	723	83.4	6.67	7.8E+06	TARS
αS cytosolic helical, 80 kDa	Q96P63	Serpin B12 OS=Homo sapiens	152	11	4	4	405	46.2	5.53	7.8E+06	SERPINB12

αS cytosolic helical, 80 kDa	P30101	Protein disulfide-isomerase A3	159	4	2	2	505	56.7	6.35	7.4E+06	PDIA3
αS cytosolic helical, 80 kDa	P61604	10 kDa heat shock protein, mitochondrial	114	29	3	3	102	10.9	8.92	7.3E+06	HSPE1
αS cytosolic helical, 80 kDa	Q01813	ATP-dependent 6-phosphofructokinase, platelet type	104	6	2	1	784	85.5	7.55	5.1E+06	PFKP
αS cytosolic helical, 80 kDa	P26641	Elongation factor 1-gamma	4	5	2	2	437	50.1	6.67	5.0E+06	EEF1G
αS cytosolic helical, 80 kDa	O00154	Cytosolic acyl coenzyme A thioester hydrolase	224	6	2	2	380	41.8	8.54	4.9E+06	ACOT7
αS cytosolic helical, 80 kDa	Q96P16	Regulation of nuclear pre-mRNA domain-containing protein 1A	27	5	2	2	312	35.7	7.55	4.8E+06	RPRD1A
αS cytosolic helical, 80 kDa	P31150	Rab GDP dissociation inhibitor alpha	62	14	1	1	447	50.6	5.14	4.8E+06	GDI1
αS cytosolic helical, 80 kDa	Q9UI42	Carboxypeptidase A4	9	7	2	2	421	47.3	6.7	4.7E+06	CPA4
αS cytosolic helical, 80 kDa	P50991	T-complex protein 1 subunit delta	38	5	2	2	539	57.9	7.83	4.2E+06	CCT4
αS cytosolic helical, 80 kDa	P22735	Protein-glutamine gamma-glutamyltransferase K	30	5	3	3	817	89.7	6.04	4.0E+06	TGM1
αS cytosolic helical, 80 kDa	Q08188	Protein-glutamine gamma-glutamyltransferase E	23	13	8	8	693	76.6	5.86	3.9E+06	TGM3
αS cytosolic helical, 80 kDa	P37837	Transaldolase OS=Homo sapiens	202	7	2	2	337	37.5	6.81	3.8E+06	TALDO1
αS cytosolic helical, 80 kDa	P08670	Vimentin	41	5	1	1	466	53.6	5.12	3.8E+06	VIM
αS cytosolic helical, 80 kDa	Q8NBF2	NHL repeat-containing protein 2	255	7	4	4	726	79.4	5.55	3.5E+06	NHLRC2
αS cytosolic helical, 80 kDa	P25786	Proteasome subunit alpha type-1	60	10	2	2	263	29.5	6.61	3.3E+06	PSMA1
αS cytosolic helical, 80 kDa	P09960	Leukotriene A-4 hydrolase	236	6	2	2	611	69.2	6.18	2.8E+06	LTA4H
αS cytosolic helical, 80 kDa	O43175	D-3-phosphoglycerate dehydrogenase	66	6	3	3	533	56.6	6.71	2.5E+06	PHGDH
αS cytosolic helical, 80 kDa	P16278	Beta-galactosidase	76	5	3	3	677	76	6.57	2.2E+06	GLB1
αS cytosolic helical, 80 kDa	O95757	Heat shock 70 kDa protein 4L	74	3	2	2	839	94.5	5.88	1.6E+06	HSPA4L
E. coli recombinant αS	P0DMV8	Heat shock 70 kDa protein 1A	23	49	34	17	641	70	5.66	2.6E+09	HSPA1B; HSPA1A
E. coli recombinant αS	P37840	Alpha-synuclein	26	69	16	12	140	14.5	4.7	1.3E+09	SNCA
E. coli recombinant αS	P11021	Endoplasmic reticulum chaperone BiP	20	19	9	9	654	72.3	5.16	2.0E+08	HSPA5
E. coli recombinant αS	P11142	Heat shock cognate 71 kDa protein	18	22	7	5	646	70.9	5.52	6.1E+07	HSPA8
E. coli recombinant αS	P38646	Stress-70 protein, mitochondrial	27	4	3	3	679	73.6	6.16	2.8E+07	HSPA9
E. coli recombinant αS	P0ACF8	DNA-binding protein H-NS	29	15	3	3	137	15.5	5.47	1.6E+07	hns
E. coli recombinant αS	P02452	Collagen alpha-1(I) chain	15	1	2	2	1464	138.9	5.8	9.1E+06	COL1A1

E. coli recombinant αS	Q86YZ3	Hornerin	8	3	2	2	2850	282.2	10.04	4.1E+06	HRNR
E. coli recombinant αS	P08123	Collagen alpha-2(I) chain	22	1	2	2	1366	129.2	8.95	3.0E+06	COL1A2

Table S1

Mass spectrometry data of αS from HEK. The detailed procedure is described in the methods section. HEK293 αS C-term Strep II-tagged lysates were crosslinked and αS^{CH} and αS^{CU} were immunoprecipitated using StrepTrap 5ml columns. αS^{CH} and αS^{CU} were separated via size exclusion chromatography, run on a SDS-gel and gel pieces were lyophilized prior Mass spectrometry analysis. Lyophilized gel pieces were digested with trypsin. The quantification analysis was based on protein-specific peptides. For the mock sample, regular HEK293 were used. To further exclude false positive hits, recombinant αS^{CU} was analyzed as well. The mock αS from regular HEK has a low abundance of αS, depicting αS^{CU} remnants from HEK trapped in the StrepTap column. The αS from the 80 kDa gel piece exhibits 100 times more αS than the mock 80 kDa sample and the false discovery rate of proteins in these samples is 0.6 %. The E. coli samples shows several false positive hits including HSP70. The data show that the most abundant protein in the αS^{CH} sample is indeed αS.

Table S2. Information on samples, crosslinking spread analysis (9 brain regions)

Individual	PMI [hours]	Dementia	Age at death [years]	Disease duration [years]	Gender	Thal score	B&B tau staging	CERAD score	Braak Lewy Body staging	McKeith staging
Control 1	79	No	84	n.a.	Male	0	0	Absent	0	None
Control 2	120	No	86	n.a.	Female	0	2	Absent	0	None
Control 3	105	No	79	n.a.	Male	0	2	Absent	0	None
PD 1	24	No	63	21	Male	0	1	Absent	6	Diffuse neocortical
PD 2	25	No	70	27	Female	1	1	Absent	6	Diffuse neocortical
PD 3	87	No	76	15	Female	0	1	Absent	6	Diffuse neocortical
DLB 1	55	Yes	79	10	Male	4	2	Sparse	6	Diffuse Neocortical
DLB 2	56	Yes	79	10	Male	4	2	Sparse	6	Diffuse Neocortical
DLB 3	24	Yes	60	8	Male	1	1	Moderate	6	Diffuse Neocortical

PD = Parkinson's disease, DLB = Dementia with Lewy bodies, B&B = Braak and Braak, CERAD = Consortium to Establish a Registry for Alzheimer's Disease