Cell Reports, Volume *34*

Supplemental information

The AD tau core spontaneously self-assembles and recruits full-length tau to filaments

Yari Carlomagno, Sireesha Manne, Michael DeTure, Mercedes Prudencio, Yong-Jie Zhang, Rana Hanna Al-Shaikh, Judith A. Dunmore, Lillian M. Daughrity, Yuping Song, Monica Castanedes-Casey, Laura J. Lewis-Tuffin, Katharine A. Nicholson, Zbigniew K. Wszolek, Dennis W. Dickson, Anthony W.P. Fitzpatrick, Leonard Petrucelli, and Casey N. Cook

Supplementary figure 1

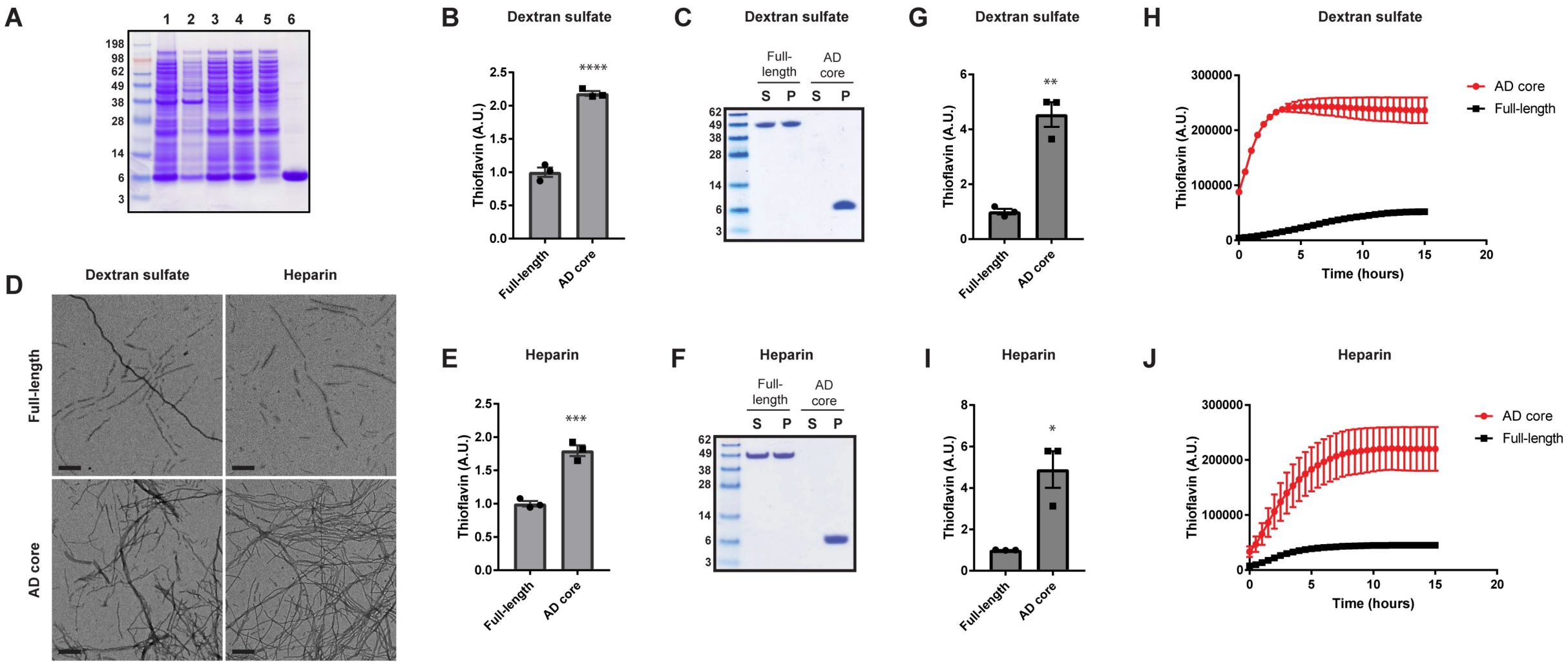

Figure S1. Assembly of the AD tau core
Related to Figure 1

(**A**) The recombinant AD tau core was expressed in E.coli and purified from the soluble fraction. The expression level and purity at various steps of the purification protocol were evaluated by Coomassie blue staining of an SDS-PAGE gel. The cell homogenate (lane 1) was separated into the inclusion body (lane 2) and soluble fraction (lane 3). The soluble fraction was then injected into a monoS column (lane 4), and the unbound (lane 5) and bound fractions (lane 6) confirming elimination of contaminants in the bound, highly pure recombinant AD tau core (6kDa).

(**B-F**) Full-length tau or the AD tau core (0.4ug/uL) were incubated with either dextran sulfate (**B-D**) or heparin (**D-F**) to induce aggregation, and polymerization assessed by thioflavin (**B, E**), ultracentrifugation analysis of supernatant (S) and pellet (P) fractions by SDS-PAGE followed by Coomassie blue staining (**C, F**), and electron microscopy (**D**). Experiment performed in triplicate, with data represented as mean±SEM. Scale bar represents 0.2 microns.

(**G-J**) Full-length tau or the AD tau core (10mM) were incubated with either dextran sulfate (**G-H**) or heparin (**I-J**) to induce aggregation, and polymerization assessed by thioflavin. Experiment performed in triplicate, with data represented as mean±SEM. *p=0.01, **p<0.01, ***p<0.001, ****p<0.0001

Supplementary figure 2

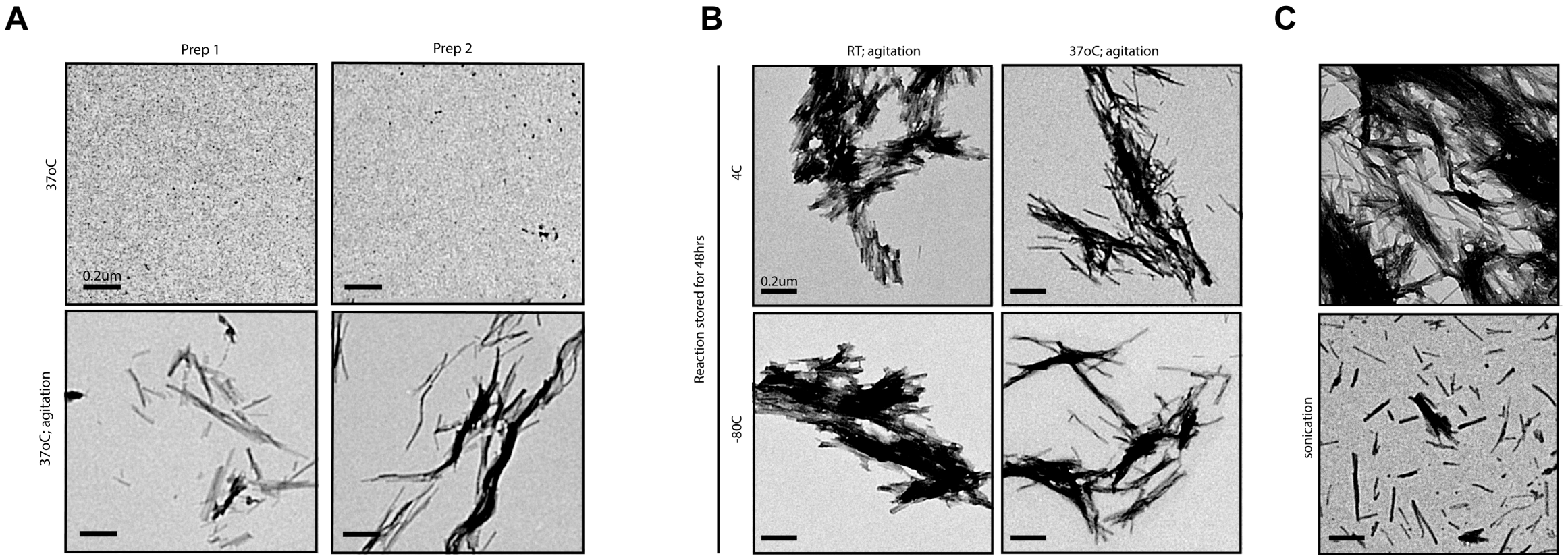

Figure S2. Filament formation of AD tau core without inducer
Related to Figure 1

(**A**) Electron microscopy (EM) was used to evaluate filament assembly in two different preps of the recombinant AD tau core following incubation at 37°C for 16 hours either stationary (top panels) or with constant agitation (bottom panels). Scale bar represents 0.2 microns.

(**B**) EM was used to evaluate filament assembly of the recombinant AD tau core upon constant agitation at either room temperature (RT; left panels) or 37°C (right panels) for 24 hours, followed by storage of the reaction at 4°C (top panels) or -80°C (bottom panels) for 48 hours. Scale bar represents 0.2 microns.

(**C**) The recombinant AD tau core was polymerized by shaking at 220RPM at 37°C for 24 hours, and EM used to evaluate filament assembly prior to (top panel) or following sonication (bottom panel). Scale bar represents 0.2 microns.

Supplementary figure 3

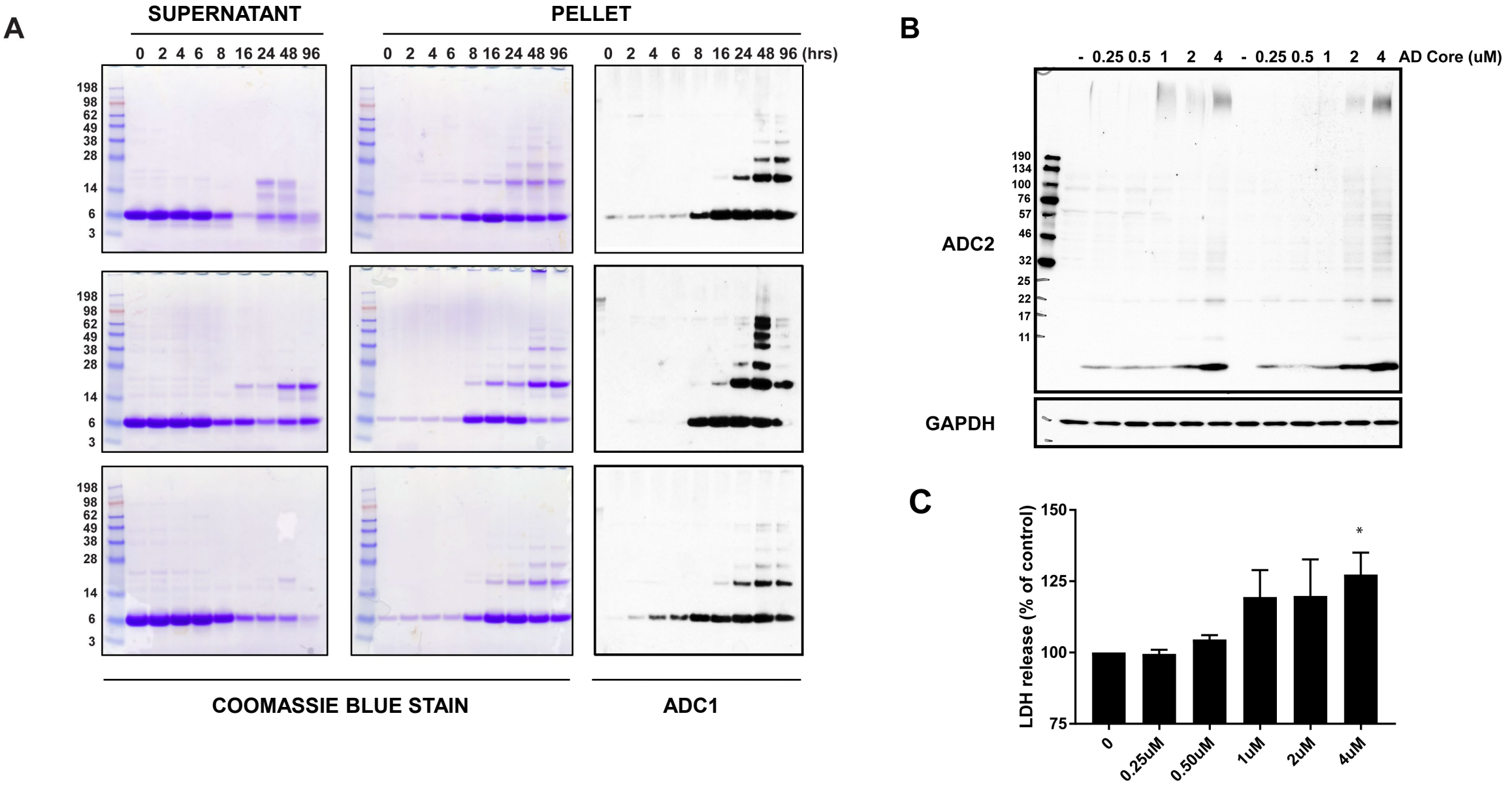

Figure S3. Aggregation and toxicity of AD tau core filaments
Related to Figure 1

(**A**) Three separate preparations of recombinant AD tau core were incubated shaking at 37°C for the indicated time points. Assembly reactions were pelleted, and the supernatant and pellet fractions evaluated by SDS-PAGE followed by Coomassie blue staining. The pellet fraction was also evaluated by immunoblotting with ADC1, an antibody that detects the AD tau core.

(**B**) M17 cell lysates treated for 72 hours with increasing concentrations of recombinant AD tau core filaments were evaluated by Western blotting with ADC2 and GAPDH to control for protein loading.

(**C**) An LDH assay was performed on cell culture media collected from M17 cells treated for 72 hours with recombinant AD tau core filaments. Data represented as mean±SEM from 6 biological replicates. Statistical significance evaluated by 1-way ANOVA with Dunnett's multiple comparisons postdoc test. *$p<0.05$

Supplementary figure 4

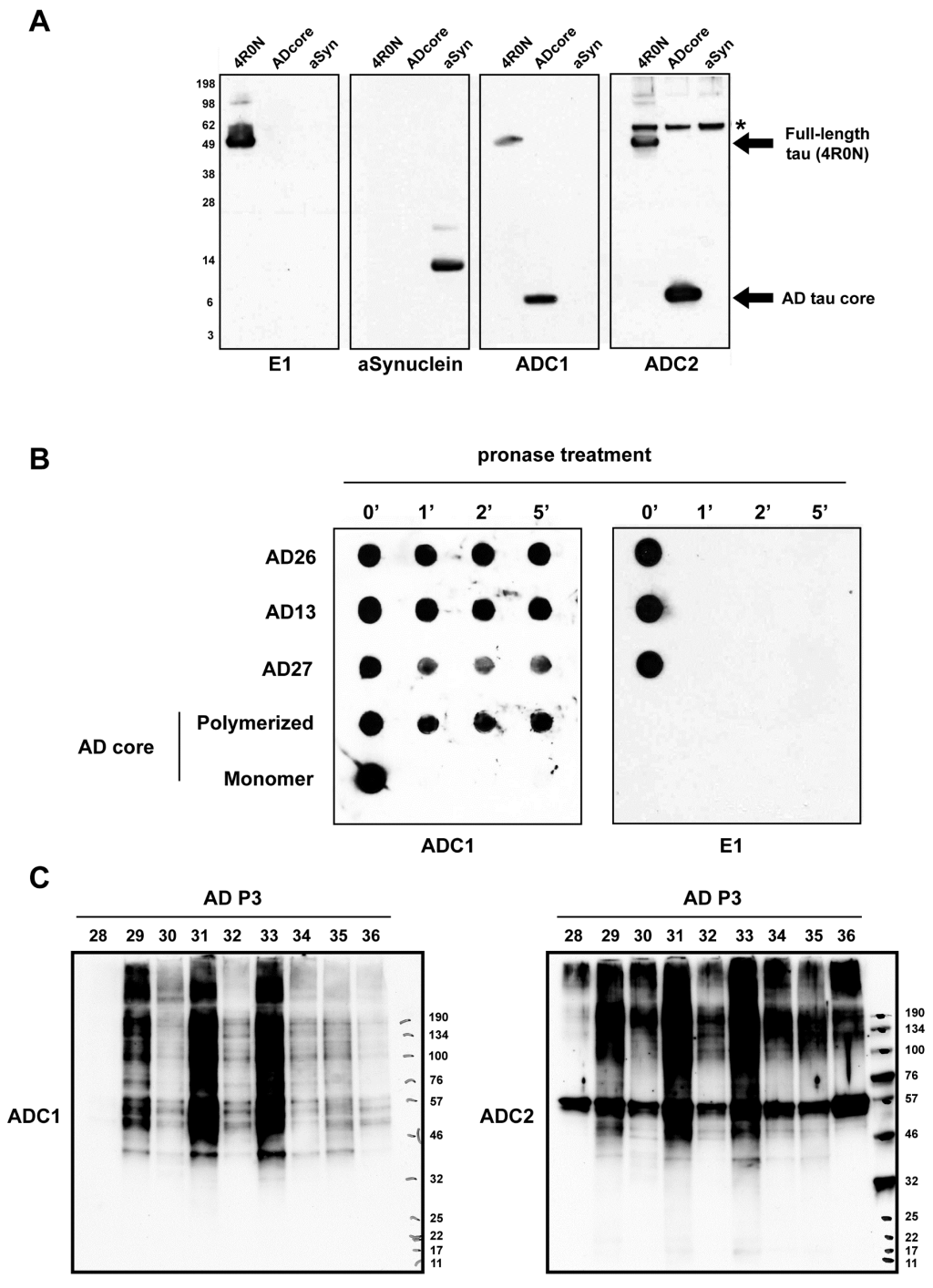

Figure S4. Characterization of AD tau core antibodies
Related to Figure 2

(**A**) Recombinant full-length tau (4R0N isoform), AD tau core, and alpha-synuclein (aSyn) were separated by SDS-PAGE, and immunoblotting performed to evaluate specificity of the AD tau core antibodies ADC1 and ADC2. E1 is an N-terminal tau antibody that detects full-length tau, but not the AD tau core. Asterisk denotes non-specific band in ADC2 immunoblot.

(**B**) AD P3 fractions or recombinant AD tau core (polymerized or monomeric) were treated for the indicated amount of time with pronase, and reactions evaluated by dot blot. Dot blots were immunoblotted with either ADC1 or E1, with the N-terminal epitope of E1 providing a postive control for pronase treatment.

(**C**) AD P3 fractions were separated by SDS-PAGE and immunoblotting performed to assess tau species detected by ADC1 and ADC2 tau core antibodies.

Supplementary figure 5

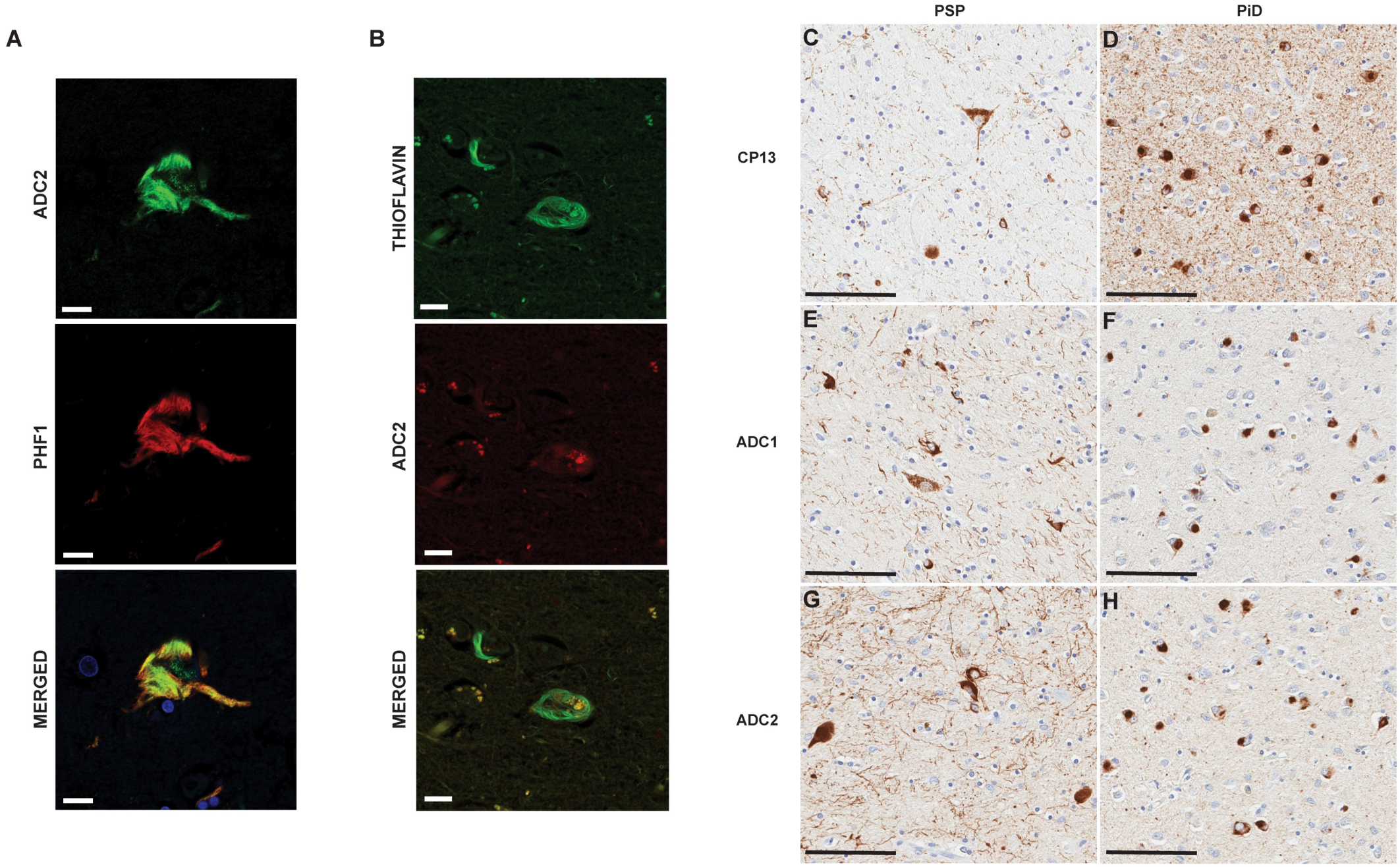

Figure S5. AD tau core antibody colocalizes with classic markers of tau pathology
Related to Figure 3

(**A**) Double-immunofluorescence staining for ADC2 (green) and PHF1 (red) in the hippocampus of a patient with AD. Nuclei are labeled with Hoescht 33258. Scale bars, 10 μm.

(**B**) Thioflavin S staining and ADC2 immunofluorescence (red) in paraffin-embedded tissue sections from the hippocampus of an AD patient. Scale bars, 10 μm.

(**C-H**) Immunohistochemical analysis with CP13 (**C-D**), ADC1 (**E-F**), and ADC2 (**G-H**) antibodies in paraffin-embedded tissue sections from the basal ganglia of a PSP patient (**C, E, G**) and the frontal cortex of a PiD patient (**D, F, H**). Nuclei are counterstained with hematoxylin. Scale bar represents 100 microns.

Supplementary figure 6

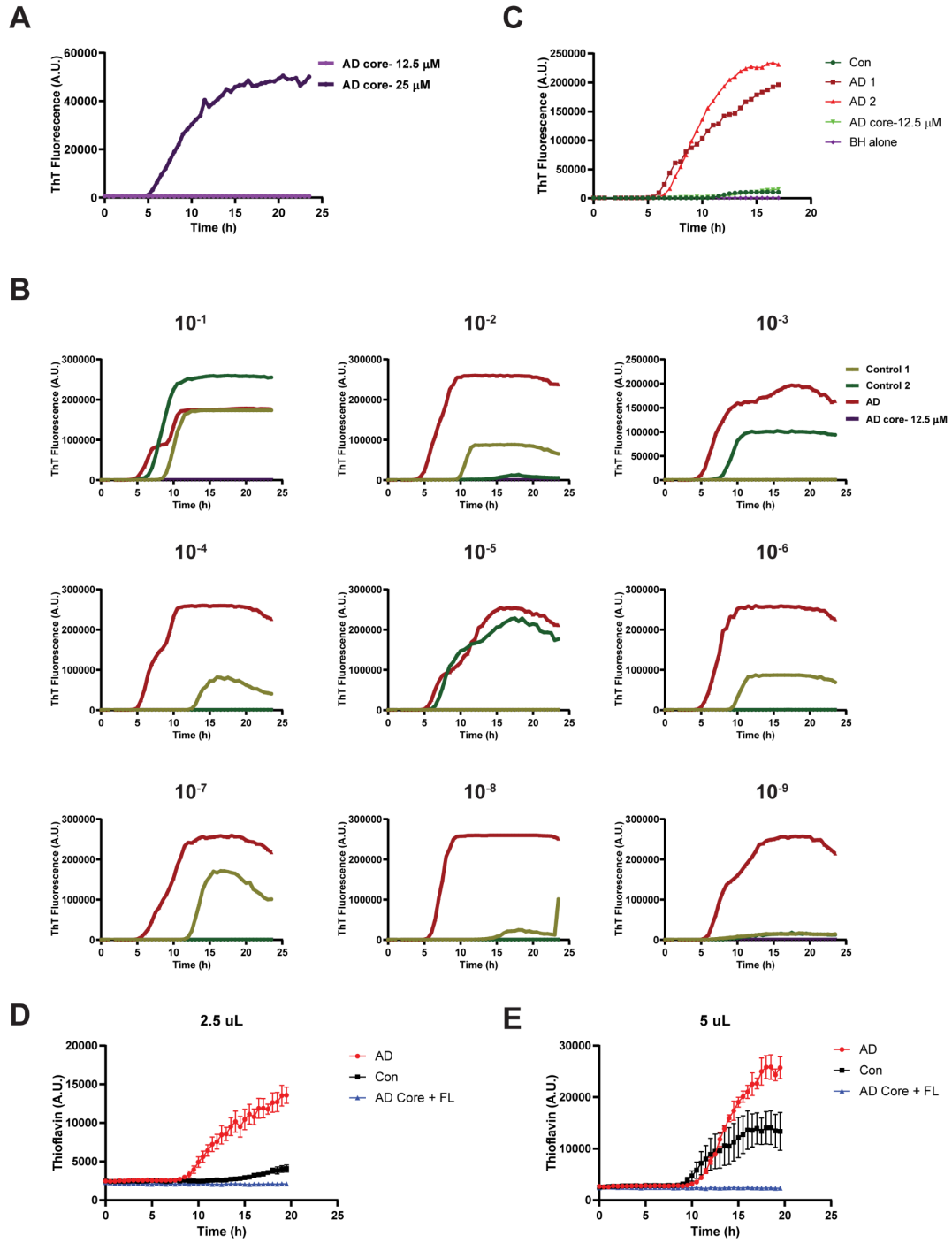

Figure S6. Optimization of the RT-QuIC assay
Related to Figure 5

(**A**) Two different concentrations of the recombinant AD tau core (12.5mM and 25mM) was tested in the assay. Enhanced thioflavin T (ThT) fluorescence was observed with 25mM of AD tau core.

(**B**) Dilutions of control and AD brain homogenates (10^{-1} to 10^{-9}) were tested, with maximum lag-phase separation between the AD and control cases achieved at 10^{-9}. Each trace represents the average of three technical replicates.

(**C**) Control and AD brain homogenates were tested at the optimal 10^{-9} dilution in the presence and absence (BH alone) of recombinant AD tau core. Only AD samples in the presence of recombinant AD tau core exhibited an increase in ThT fluorescence.

(**D-E**) Control and AD CSF was tested at 2.5uL (**D**) or 5uL (**E**) in the RT-QuIC reaction, with recombinant AD tau core and full-length tau alone (AD Core + FL) included as a negative control.

Case #	Neuropathological Diagnosis	Sex	Age at death (years)	Braak stage	Use
C1	VaD/PA	F	94	2.5	RT-QuIC
C2	PA	F	94	1.5	RT-QuIC
C3	PA	M	89	3	RT-QuIC
C4	VaD/PA	F	92	3.5	RT-QuIC
C5	PA	M	80	2.5	RT-QuIC
C6	PA	M	74	3	RT-QuIC
C7	SC	M	93	3	RT-QuIC
C8	VaD	M	83	3	RT-QuIC
C9	VaD	F	79	2.5	RT-QuIC
C10	VaD	M	69	2	RT-QuIC
C11	Normal	M	82	2	RT-QuIC
C12	Normal	M	84	3	RT-QuIC
C13	Normal	F	58	0	IHC
C14	PA	F	97	2	IHC
C15	PA	M	81	3	IHC
C16	PA	M	81	3	IHC/IF
AD1	AD	F	56	6	RT-QuIC
AD2	AD	M	81	6	RT-QuIC
AD3	AD	M	73	6	RT-QuIC
AD4	AD	M	85	6	RT-QuIC
AD5	AD	F	63	6	RT-QuIC
AD6	AD	M	83	6	RT-QuIC
AD7	AD	M	78	6	RT-QuIC
AD8	AD	M	60	6	RT-QuIC
AD9	AD	M	81	6	RT-QuIC
AD10	AD	F	72	6	RT-QuIC
AD11	AD	M	92	6	RT-QuIC
AD12	AD	M	83	6	RT-QuIC
AD13	AD	F	59	6	RT-QuIC/pronase
AD14	AD	F	91	6	IHC
AD15	AD	F	94	5	IHC
AD16	AD	M	89	5	IHC
AD17	AD	M	89	5	IHC
AD18	AD	M	89	5	IHC
AD19	AD	F	78	4	IHC
AD20	AD	M	94	4	IHC/IF
AD21	AD	F	95	3	IHC
AD22	AD	F	91	4	IHC
AD23	AD	M	91	3	IHC
AD24	AD	M	98	5	IHC
AD25	AD	F	58	6	IHC
AD26	AD	F	60	6	pronase
AD27	AD	M	70	6	pronase

AD28	AD	M	82	6	WB
AD29	AD	F	65	6	WB
AD30	AD	F	74	6	WB
AD31	AD	F	73	6	WB
AD32	AD	M	85	6	WB
AD33	AD	M	56	6	WB
AD34	AD	M	73	6	WB
AD35	AD	M	70	6	WB
AD36	AD	F	91	6	WB
CBD1	CBD	M	75	2	RT-QuIC
CBD2	CBD	M	65	1	RT-QuIC
CBD3	CBD	F	69	2	RT-QuIC
CBD4	CBD	M	70	2	RT-QuIC
CTE1	CTE/MSA	M	53	5	RT-QuIC
CTE2	CTE/LBD	M	78	4	RT-QuIC
CTE3	CTE/ALS	M	38	4	RT-QuIC
PSP1	PSP	M	59	2	RT-QuIC
PSP2	PSP	M	55	0	RT-QuIC
PSP3	PSP	M	82	2	RT-QuIC
PSP4	PSP	M	66	2	RT-QuIC
PSP5	PSP	F	77	2	IHC
PSP6	PSP	M	68	0	IHC
PiD1	PiD	F	59	2	IHC

Table S1. Information about human postmortem tissue samples. Abbreviations: VaD, Vascular dementia; PA, pathological aging; SC, senile changes; AD, Alzheimer's disease; CBD, corticobasal degeneration; CTE, chronic traumatic encephalopathy; MSA, multiple system atrophy; LBD, lewy body dementia; ALS, amyotrophic lateral sclerosis; PSP, progressive supranuclear palsy; PiD, Pick's disease; F, female; M, male; RT-QuIC, real-time quaking-induced conversion; IHC, immunohistochemistry; IF, immunofluorescence; WB, western blotting.

Related to Figure 2-3, 5

Case #	Clinical Diagnosis	Sex	Age at draw (years)	MMSE
C17	Normal	F	64	-
C18	Normal	M	61	-
C19	Normal	F	68	-
C20	Normal	M	68	-
C21	Normal	F	55	-
C22	Normal	F	55	-
C23	Normal	M	54	-
C24	Normal	M	69	-
C25	Normal	M	73	-
C26	Normal	F	62	-
AD32	AD	M	79	16
AD33	AD	F	79	21
AD34	AD	M	63	20
AD35	AD	F	58	20
AD36	AD	M	64	19
AD37	AD	F	65	14
AD38	AD	F	66	25
AD39	AD	M	82	18
AD40	AD	M	58	15
AD41	AD	F	81	20

Table S2. Information about human antemortem CSF samples. Abbreviations: AD, Alzheimer's disease; F, female; M, male; MMSE, Mini Mental State Exam.

Related to Figure 5