Supplementary methods

1. Competition assay

Antibodies (1 ng/ml, 6.7 pM) were incubated at room temperature for 1 h with increasing concentrations of tau peptide (386-408)-pS396/pS404 (0-10000 nM). The amount of non-bound fraction of antibody was measured in MSD plates coated with 100 ng/ml tau (386-408)-pS396/pS404. IC_{50} values were estimated from one-site fitted inhibition curves using GraphPad Prism software.

2. Western blot

Samples were boiled in 1x LDS loading buffer and 100 mM DTT and loaded on a 4-12% Bis-Tris NuPAGE Gel (LifeTech Novex). After electrophoresis, the proteins were blotted over to a Immobilon–FL PVDF membrane (0.45 µm, IPFL10100, Millipore) blocked with SEA blocking buffer (Product #37527, Thermo Fisher) and probed with 1 µg/ml antibody overnight at 4°C. A secondary fluorophore conjugated IgG antibody was used (IRDye 680 Goat anti-mouse, IRDye 800CW Goat anti-rabbit, LICOR biosciences) and the signal was quantified using Odyssey CLx and Image studio software (LI-COR biosciences).

3. *In vitro* seeding assay in HEK293 cells

<u>rTg4510 extracts for HEK293 seeding experiments</u>: rTg4510 and tTa brain extracts were prepared from 48 weeks old mice as described by Holmes et al.

<u>Transient HEK293 Seeding assay:</u> 600,000 HEK293 cells were plated per well in a 6-well plate in DMEM supplemented with 10% heat-inactivated FCS and 1% penicillin/streptomycin. The following day, cells were transfected with 4 µg plasmid DNA (pcDNA3.1 encoding human tau 0N4R-P301L) using 10 µl lipofectamine2000™ per manufacturer's protocol and the medium was exchanged 4 hrs post-transfection. On day two, cells were seeded with immunodepleted extracts

containing 40 µg total protein per well. Six hrs post-seeding, medium was changed to low serum medium. Twenty-four hrs post-seeding, cells were trypsinized for 3 min to degrade extracellular seeds. The cells were re-plated at 800,000 cells per well in 6-well plates for fractionation experiments; and 20,000 cells per well in 96-well plates for the Cisbio tau aggregation assay.

Cellular fractionation: 48 hrs post-seeding, cells were harvested in cold PBS using a cell scraper, pelleted and lysed (TBS, 1% Triton X-100, phosphatase and protease inhibitors) and finally sonicated for 5 s at 15%, 0 cycles. Following ultra-centrifugation (100,000g for 30 min at 4 °C), pellets were re-suspended in 1% SDS, sonicated and ultra-centrifuged once more. The Triton X-100-insoluble/SDS-soluble fractions were analyzed by Western blotting for total tau (E1 antibody) and phosphorylation of tau on pS396 (D1.2). The D1.2-positive tau signal was quantified using Odyssey CLx and Image studio software.

Cisbio™ tau aggregation assay: 48 hrs post-seeding, cells were washed in ice-cold PBS and plates were frozen dry at -20 °C, covered with a plate sealer. Cells were lysed in 100 µl/well ice-cold TBS supplemented with 1% Triton X-100 with phosphatase/protease inhibitors (Phos-stop/Complete) and 0.125 U/ml Benzonase and incubated on an orbital shaker, at 650 rpm for 40 min at 4 °C. Total cell lysates were analyzed for tau aggregates by the Cisbio tau aggregation assay, according to manufacturer's protocol using a Pherastar FS microplate reader. Total protein was determined for all samples by BCA and the HTRF signal-to-noise ratio of the sample was normalized to protein and plotted as relative tau aggregation using 8 technical replicates.

Stable HEK293 cell line generation, seeding, antibody treatment and imaging: pcDNA3.1 constructs, for generation of stable HEK293 cell lines expressing the microtubule binding region (MTBR) of tau with P301L/V337M double mutation fused to GFP, was designed according to Kfoury et al. Clone JFP2A was selected for a high seeding ability and normal cell morphology. Clone JFP2A was plated at 8000 cells/well in collagen-coated black clear-bottom plates (CellCarrier, PerkinElmer) and rTg4510 brain extract (3.3 µg total protein per well) pre-incubated with different concentrations of antibodies was added. Four days later, Hoechst 33342 (2 µg/ml) was added and after a 30-min incubation at 37 °C the cells were fixed in 4% paraformaldehyde supplemented with 4% sucrose (W/V). Upon incubation with seeds the MTBR(LM)-GFP construct was recruited from a diffuse distribution in the cytoplasm into dense inclusion bodies

with a high fluorescent contrast that can be quantified by a spot-detection algorithm. We used a Cellomics Arrayscan VTI HCS reader using spot algorithm from Cellomics Scan software version 6.5.0 to quantify aggregates per cell. For the peptide competition experiment, 133 nM antibody were pre-incubated with a 100-fold exes (13.3 µM) of non-phos (386-408), pS396 and pS404 peptide for 30 min prior to adding seeds.

References:

Holmes, B.B., et al., *Proteopathic tau seeding predicts tauopathy in vivo.* Proc Natl Acad Sci U S A, 2014. **111**(41): p. E4376-85.

Kfoury, N., *Holmes, BB., Jiang, H., Holtzman, DM., Diamond, MI., Trans-cellular propagation of Tau aggregation by fibrillar species.* J. Biol. Chem, 2012. **287**(23): p. 19440-19451.

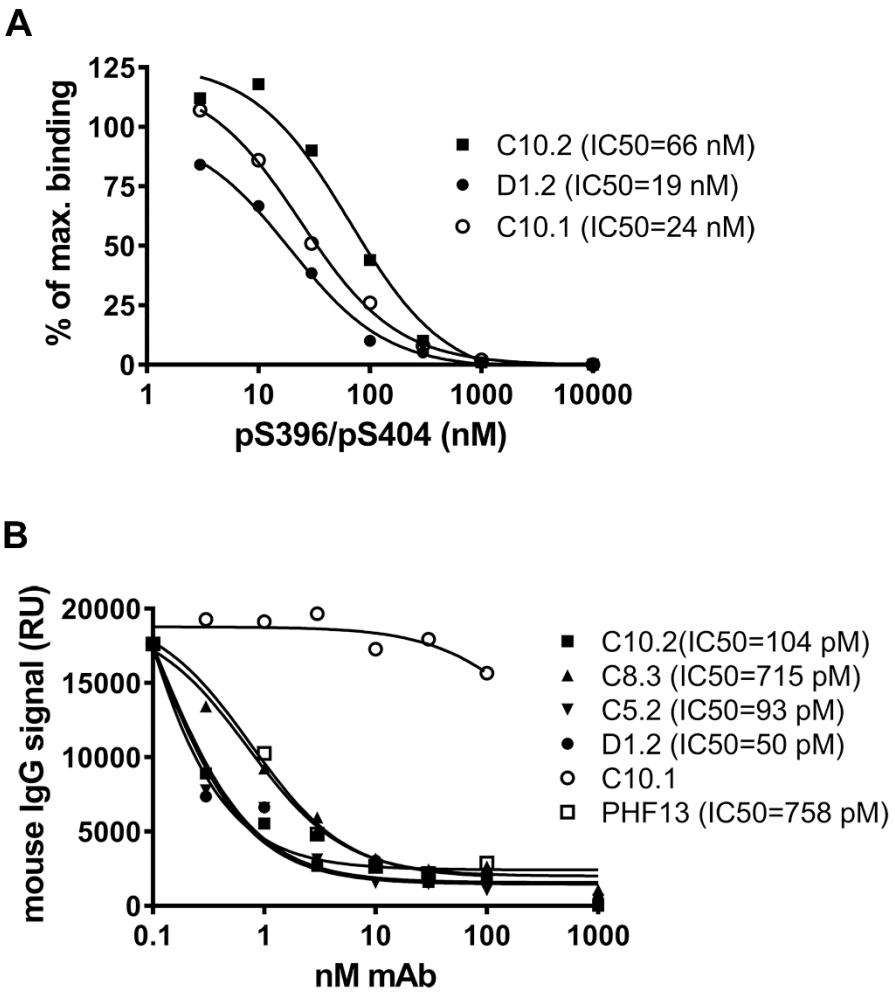
Table S1

Demographics of frontal cortex specimens obtained from Tissue Solutions

Fixed paraffin-embedded frontal cortex			
Subject	Gender	Age (y)	Autolysis (h)
AD – Braak VI	F	70	2
AD – Braak V	F	65	3.5
AD – Braak V	M	63	3
HC	F	96	4.5
HC	M	82	7.5
HC	F	82	5.5
Frozen samples of frontal cortex			
Subject	Gender	Age (y)	Autolysis (h)
AD – Braak VI *	F	70	2
AD – Braak VI *	F	82	2
AD – Braak V	M	63	3
AD – Braak V	F	63	3.5
HC	F	80	3
HC	F	57	4
HC	M	77	5
HC	M	70	3

*Specimens used in seeding assays. AD, Alzheimer's disease; HC, healthy control

Figure S1



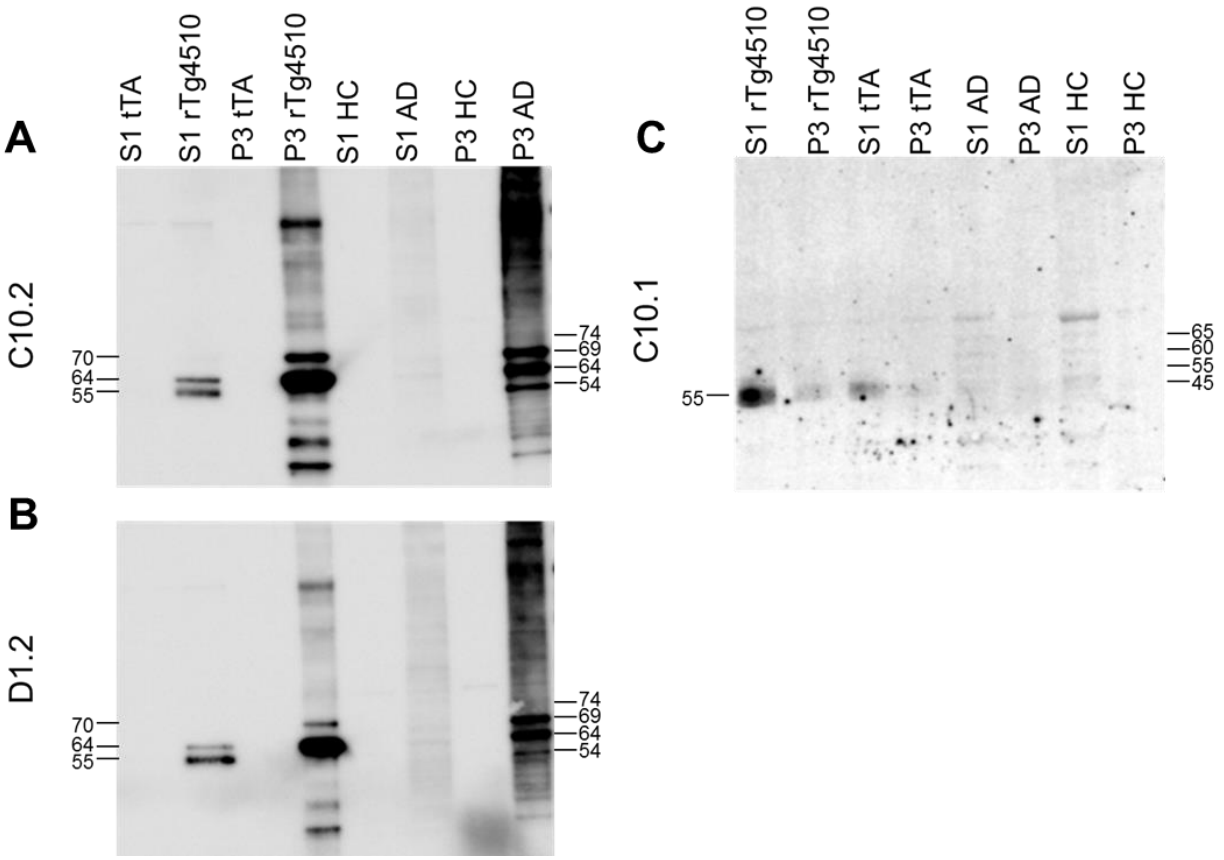
A: tau (386-408)-pS396/pS404 peptide binding of C10.2, D1.2 and C10.1

Direct competition assay. Antibodies (1 ng/ml) were pre-incubated 60 min at room temperature with graded concentration (0-10000 nM, indicated at X axis) of tau (386-408)-pS396/pS404 peptide and subsequently analyzed for presence of free antibody binding in MSD plates coated with 100 ng/ml tau (386-408)-pS396/pS404. The apparent affinities of the antibody binding to tau (386-408)-pS396/pS404 were determined for D1.2 (IC50 19 nM), C10.2 (IC50 66 nM) and C10.1 (IC50 24 nM).

B: In-solution antibody binding to phosphorylated tau-441

Recombinant phosphorylated tau-441 were incubated 60 min at room temperature with graded concentrations (final conc: 0-1000nM as indicated on X-axis) of individual antibodies; C10.2, C8.3, C5.2, D1.2, PHF13 and C10.1 and subsequently the amount of free antibodies were analyzed in C10.2 coated MSD plates. Binding activity in presence of recombinant phosphorylated tau was reported as IC50 (indicated in parenthesis) for the different antibodies.

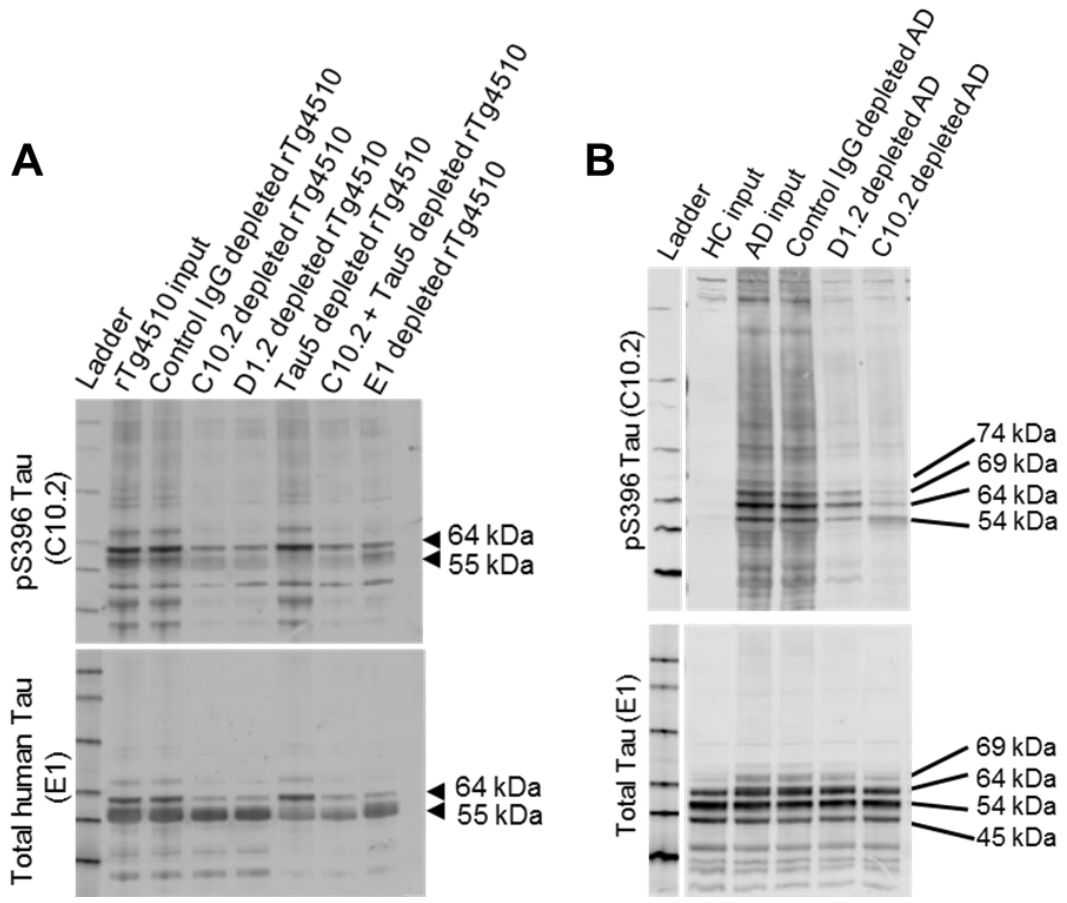
Figure S2



Detection of pathological tau with C10.2 and D1.2 by western blot.

Forebrain pooled from three rTg4510 mice and single-transgenic control (tTA) littermates euthanized at 32 weeks of age and pooled cortical specimen from four Alzheimer's disease (AD) and four healthy control (HC) donors, respectively, were fractionated into soluble (S1) and sarkosyl-insoluble (P3) fractions. The fractions were analyzed by Western blot for tau phosphorylated at the S396 epitope with 1 µg/ml C10.2 or D1.2 and at the S404 epitope with 1 µg/ml C10.1. In rTg4510, normal human 4R0N tau is displayed at 55 kDa in S1 fraction, while hyperphosphorylated tau species are displayed at 64 and 70 kDa in S1 and P3 fractions. In HC and AD samples normal human tau is displayed as four bands of 45, 55, 60, and 65 kDa in S1 fractions. In AD samples, hyperphosphorylated tau species are displayed as four bands of 54, 64, 69 and 74 kDa, with a variable amount of AD typical smear in the S1 and P3 fractions.

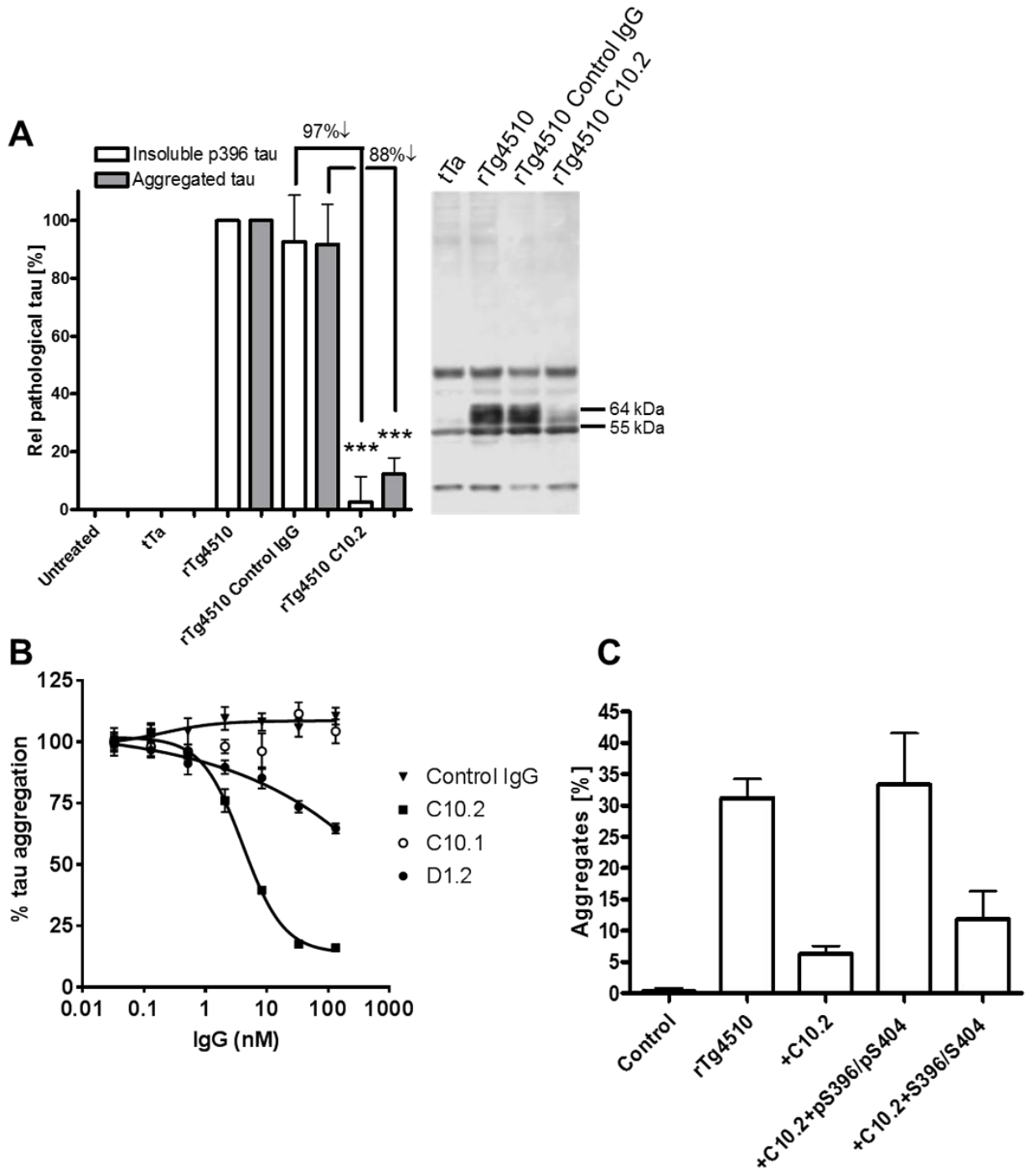
Figure S3



C10.2 and D1.2 remove hyperphosphorylated tau from brain extracts

D1.2, C10.2 or control antibodies, E1 (rabbit antibody raised against tau 19-33) and Tau5 (AHB0042, Thermo Fisher) were conjugated to magnetic beads and incubated with brain extracts. The amount of residual tau after the immunodepletion was analysed by western blot. A: C10.2 and D1.2 had removed the majority of pS396 phosphorylated 64kDa tau from the rTg4510 extracts while leaving the 55kDa tau, detected by E1. B: Immunodepletion using C10.2 and D1.2 removed the majority of pS396 phosphorylated tau from AD brain extracts, but did not affect the normal tau bands, as demonstrated in the E1 western blot.

FigureS4



A: Seeding potential of C10.2 immunodepleted rTg4510 brain extracts

Depleted and non-depleted extracts were used to seed HEK293 cells transiently expressing human tau (0N4R-P301L). Seeding was assessed by a tau aggregation assay (Cisbio) on total cell lysate

or by western blots quantitation of Triton X-100-insoluble tau (p396). Representative tau-pS396 (D1.2) western blot of insoluble tau from cells seeded with depleted or non-depleted rTg4510 extracts is shown. Seeding resulted in a strong increase in insoluble D1.2-positive 64 kDa hyperphosphorylated tau, which was prevented by C10.2 depletion. Data presented as mean +/- SEM, normalized to seeding with non-depleted material. One way ANOVA with Tukey post-test, ***P<0.001, *n*=3.

B: Seeding prevention of C10.2 in cell seeding assay

HEK293 cells stably expressing Tau-GFP were incubated with rTg4510 brain extracts alone or pre-incubated with increasing amounts of C10.1, C10.2 or D1.2. Antibody-mediated prevention of intracellular Tau-GFP aggregation was quantified by a spot-detection algorithm using high-content imaging, Cellomics. C10.2 and D1.2 resulted in a concentration-dependent inhibition of seeding whereas C10.1 did not. Data presented as mean +/- SEM, *n*=6.

C: Peptide competition in HEK293 seeding

Specificity of C10.2 was tested by pre-incubating antibody with tau (386-408)-pS396/pS404 or non-phosphorylated peptide tau (386-408) prior to incubation with rTg4510 brain extracts and addition to cells. Four days post-seeding, MTBR(LM)-GFP positive inclusions were quantified using high-content imaging, Cellomics. Only the phosphorylated peptide competed out the C10.2 mediated effect on seeding. Data presented as mean +/-SEM, *n*=6.