Supplementary Data

Elevated MARK2-Dependent Phosphorylation of Tau in Alzheimer’s Disease

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Supplementary Figure 1. Evaluation of transfection efficiency in rhTau 3T3 cells. Green fluorescent protein (GFP) was co-expressed with MARK2 in rhTau 3T3 cells, stably expressing 4R tau protein. Transfection efficiency was calculated after 24h expression as the number of GFP expressing cells divided by the total number of observed cells (n>500). Images were acquired using 20 × magnification (A) or 10 × magnification (B).

Supplementary Figure 2. Expression of MARK1, MARK3, and MARK4 in transfected rhTau 3T3 cells. To confirm the expression of all MARK isoforms for validation of MARK-2 antibody, anti-MARK1 (AG06175; 1 μg/ml, AstraZeneca), anti-MARK3 (0.5 μg/ml, Cell Signaling), and anti-MARK4 (0.75 μg/ml, Cell Signaling) were used to detect expression of MARK1 (A), MARK3 (B), and MARK4 (C), respectively, using in situ proximity ligation assay (PLA). In situ PLA signals (red) were observed in cells co-expressing GFP (green). Cell nuclei were counterstained with DAPI (blue). Scale bars 20 μm.
Supplementary Figure 3. Detection of MARK2 in transfected rhTau 3T3 cells with or without staurosporine treatment. In situ PLA was performed to detect expression level of MARK2 (red, Cy3) with staurosporine treatment (staurosporine (+)) and without staurosporine treatment (staurosporine (-)) in transiently GFP (green) and MARK2-expressing cells (yellow, merged). Cell nuclei were counterstained with DAPI (blue). Scale bars 20 μm.
Supplementary Figure 4. Staurosporine treatment has no effect on expression level of tau. In situ PLA was performed to measure expression level of tau in staurosporine-treated cells (staurosporine (+)) and non-treated cells (staurosporine (-)). Signals from more than 30 transfected cells were recorded for each condition and digitally counted using the Duolink ImageTool. The data were analyzed in Microsoft Excel 2010.
Supplementary Figure 5. Control experiments for validation of in situ PLA signals in transfected rhTau 3T3 cells. A) In a technical control experiment for validation of the signals for interaction between MARK2 and tau (shown in Fig. 3), the primary antibody against MARK2 was omitted, which resulted in abolition of the in situ PLA signals. B) To confirm the specificity of the signals obtained for MARK2-dependent phosphorylation of tau (illustrated in Fig. 6), we omitted the primary antibody against phospho-Ser262. This resulted in a significant reduction of in situ PLA signals confirming that the signals indeed reflect the increased numbers of tau molecules phosphorylated at Ser262. Scale bars 20 μm.
Supplementary Figure 6. Expression levels of MARK2 in hippocampal neurons in post-mortem AD brains compared to controls. In situ PLA performed on human brain sections disclosed no difference in expression level of MARK2 in CA2-field of hippocampus in AD (A) compared to age-matched non-demented elderly controls (B). Quantification of in situ PLA signals in the cell bodies of neurons (n ≥ 100 per brain sample) are shown for AD cases (filled circles) and controls (open circles) (C). In situ PLA signals were digitally recorded using the Duolink ImageTool and analyzed in Microsoft Excel 2010. N.G.: Not significant.
Supplementary Figure 7. Technical control experiments for validation of PLA signals in human AD brain tissue. A) In situ PLA was performed in hippocampus of human AD brain tissues using anti-human MARK2 and anti-human tau antibodies. B) When the primary antibody against human MARK2 was omitted in a technical control experiment, the in situ PLA signals were significantly reduced in numbers. C) In another control experiment the MARK2-specific antibody was pre-blocked with an excess of immune peptide prior to use in in situ PLA. This pre-blocking of the antibody again resulted in significantly fewer in situ PLA signals, supporting the specificity of the signals for MARK2.