Journal of Alzheimer's Disease 33 (2013) 1–7
IOS Press

Supplementary Data

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

Gucci Jijuan Gu[a], Di Wu[a], Harald Lund[b,1], Dan Sunnemark[b], Alexander J. Kvist[c], Roy Milner[c], Sonia Eckersley[c], Lars N.G. Nilsson[d,2], Karin Agerman[b], Ulf Landegren[a] and Masood Kamali-Moghaddam[a,*]

[a]*Department of Immunology, Genetics and Pathology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden*

[b]*Department of Neuroscience, iMed, CNS and Pain Södertälje, AstraZeneca Research and Development, Södertälje, Sweden*

[c]*Antibody Generation Group, Discovery Sciences, iMed, AstraZeneca Research and Development, Mereside, Alderley Park, Macclesfield, Cheshire, UK*

[d]*Department of Public Health and Caring Sciences, Molecular Geriatrics, Uppsala University, Uppsala, Sweden*

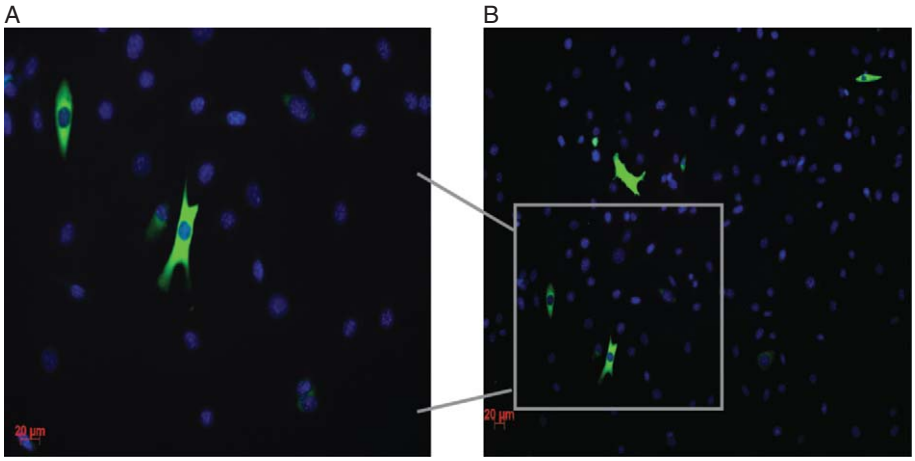
Accepted 16 August 2012

[1]Present address: Department of Clinical Neuroscience, Centre for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden.

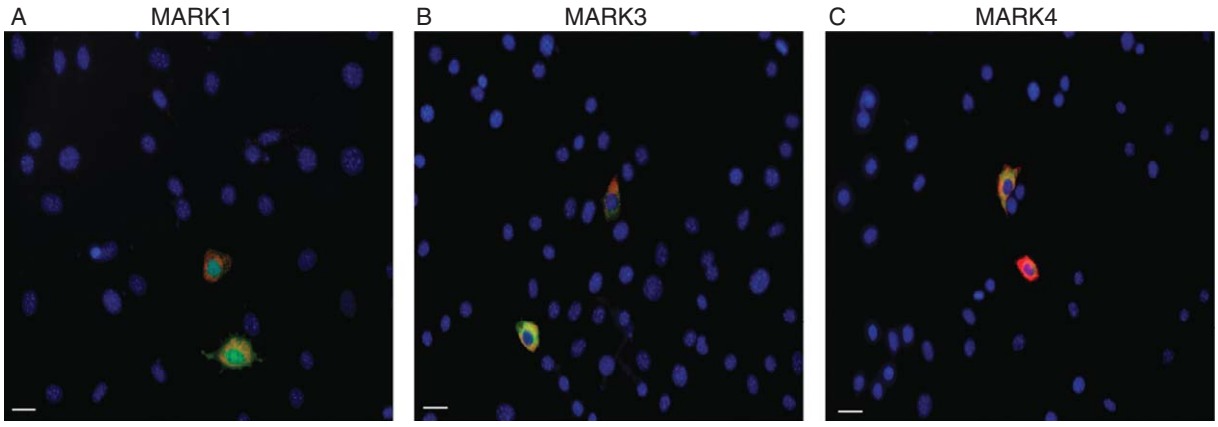
[2]Present address: Department of Pharmacology, Oslo University, Oslo, Norway.

*Correspondence to: Masood Kamali-Moghaddam, Department of Immunology, Genetics and Pathology, Rudbeck Laboratory, SE-751 85 Uppsala, Sweden. Tel.: +46 18 471 4454; Fax: +46 18 471 4808; E-mail: Masood.Kamali@igp.uu.se.

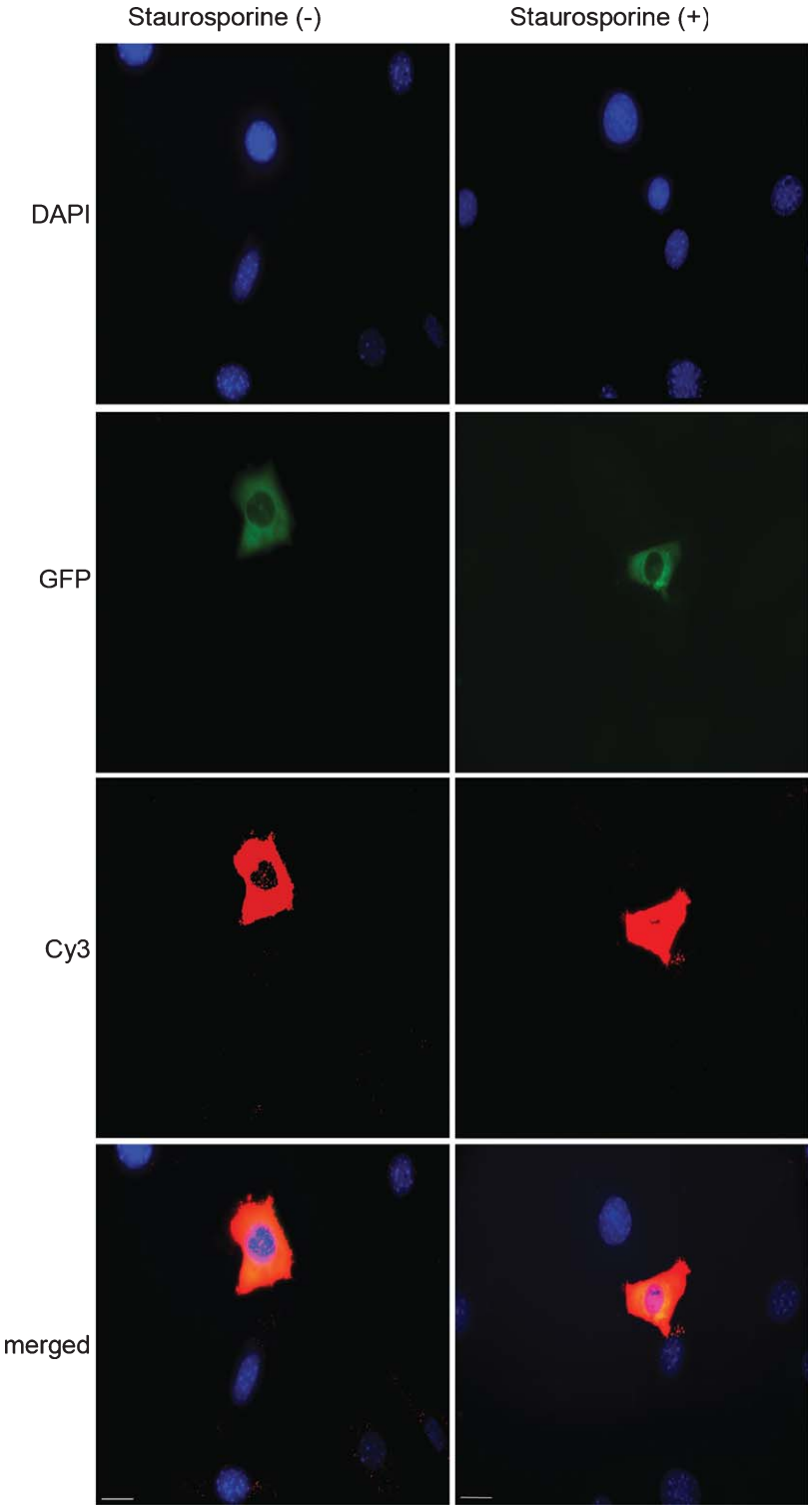
ISSN 1387-2877/13/$27.50



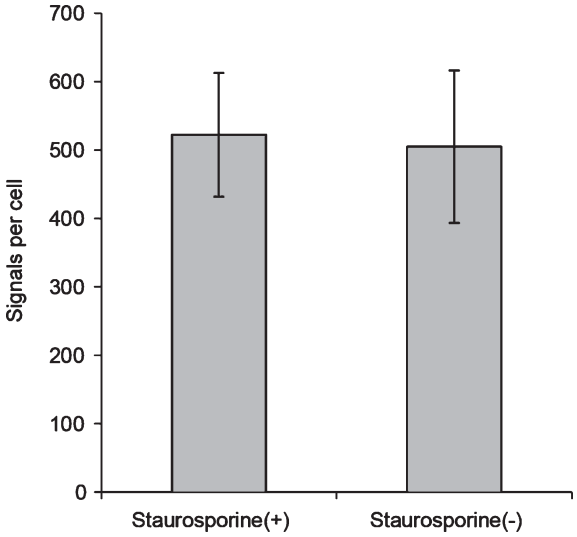
Supplementary Figure 1. Evaluation of transfection efficiency in rhTau 3T3 cells. Green fluorescent protein (GFP) was co-expressed with MARK2 in rhTau 3T3 cells, stably expressing 4R tau protein. Transfection efficiency was calculated after 24 h expression as the number of GFP expressing cells divided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



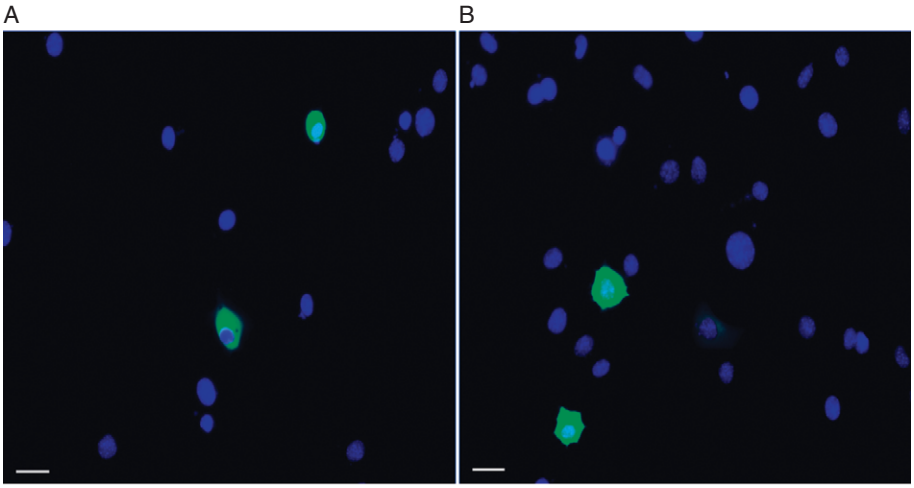
Supplementary Figure 2. Expression of MARK1, MARK3, and MARK4 in transfected rhTau 3T3 cells. To confirm the expression of all MARK isoforms for validation of MARK-2 antibody, anti-MARK1, AGG6175 (1 µg/ml, AstraZeneca), anti-MARK3 (0.5 µg/ml, Cell Signaling), and anti-MARK4 (0.75 µg/ml, cell signaling) 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.



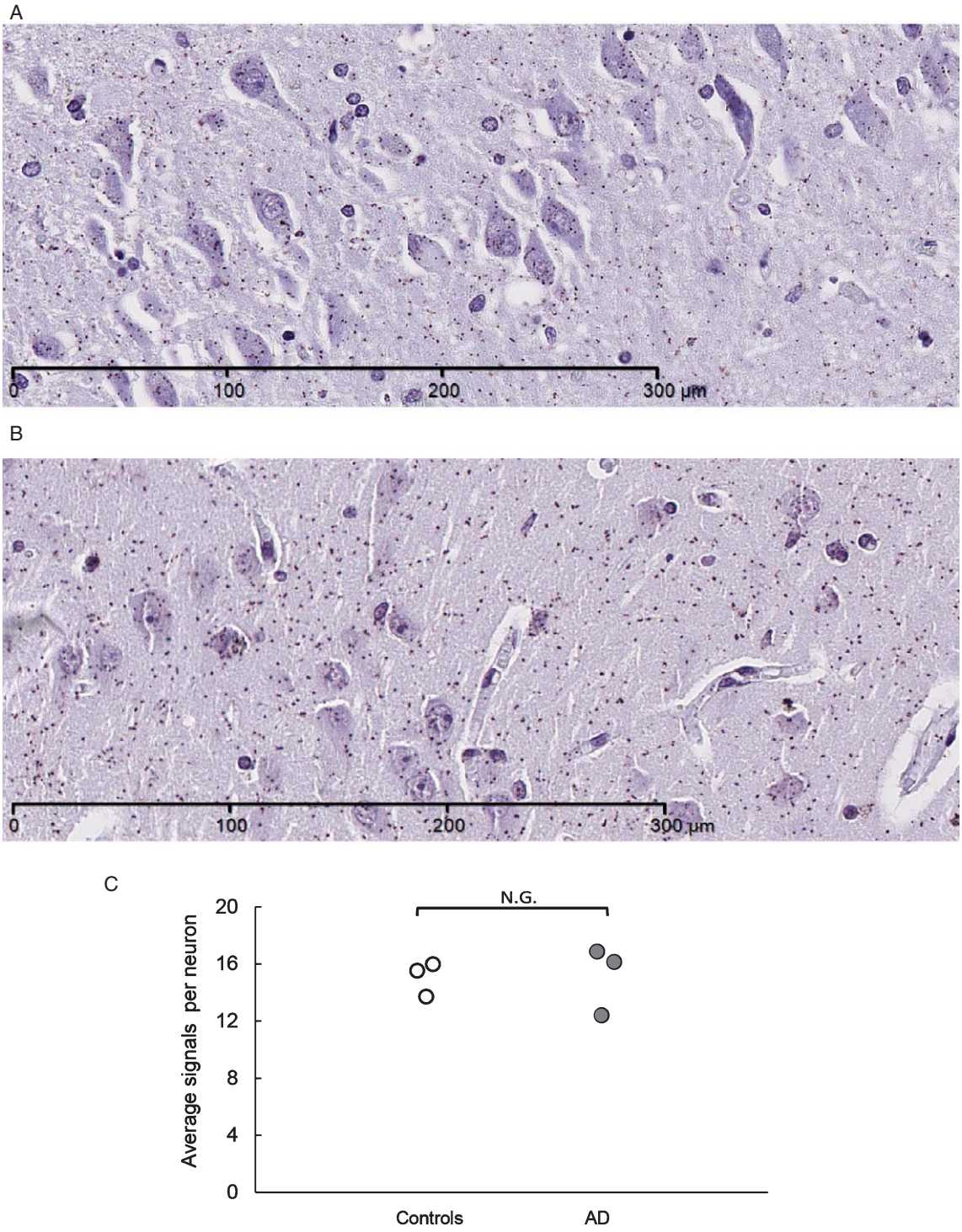
Supplementary Figure 3. Detection of MARK2 in transfected rhTau 3T3 cells with or without staurosporine treatment. *In situ* PLA was performed to detect expression level of MARK2 (red, Cy3) with staurosporine treatment (staurosporine (+)) and without staurosporine treatment (staurosporine (-)) in transiently GFP (green) and MARK2-expressing cells (yellow, emerged). Cell nuclei were counterstained with DAPI (blue). Scale bars 20 μm.



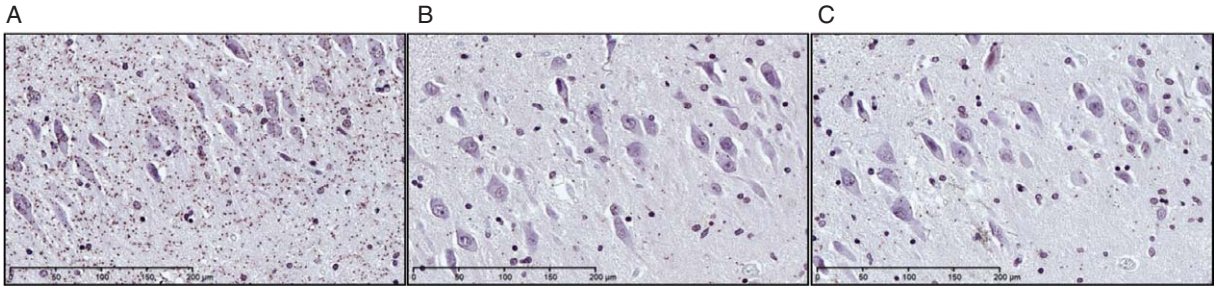
Supplementary Figure 4. Staurosporine treatment has no effect on expression level of tau. *In situ* PLA was performed to measure expression level of tau in staurosporine-treated cells (staurosporine (+)) and non-treated cells (staurosporine (-)). Signals from more than 30 transfected cells were recorded 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-Ser^{262}. 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 Ser^{262}. 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 on 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 ($n \geq 100$ 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.