## Supplementary Data

## A NH<sub>2</sub> Tau Fragment Targets Neuronal Mitochondria at AD Synapses: Possible Implications for Neurodegeneration

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Supplementary Fig. 1. Characterization of affinity and specificity of the polyclonal NH<sub>2</sub> 4268 tau antibody. A-E) NH<sub>2</sub> 4268 tau antiserum (SG-4268) was generated in Sigma-Genosys (UK) laboratories against an HPLC-purified synthetic peptide, which corresponds to residues 26-36 of the longest isoform of human tau protein (project 18966-1). KLH-conjugated synthetic NH<sub>2</sub> 26–36 tau peptide was used for immunization of New Zealand white rabbit and test bleeds were further assessed for epitope mapping by enzyme linked immunosorbant assay (ELISA). Affinity-purified NH<sub>2</sub> 4268 tau antibody (0.5 mg/ml) reacted with a synthetic peptide corresponding to residues 26-36 in human tau, but not with a panel of other peptides corresponding within the amino-terminal half of the protein, including peptide 1-25. The specificity of purified polyclonal antibody was next analyzed by comparing to its relative pre-immune serum, by SDS-PAGE of human protein extratcs (A). A NH2 tau fragment of 20-22 kDa molecular weight was detected only by immune serum (A left) in total protein extracts from differentiated SH-SY5Y induced to apoptosis upon 6 h staurosporine treatment, in agreement with our previous report [1]. Finally, Western blotting of AD synaptosomes compared the relative immunoreactivity of NH2 4268 tau and CCP-NH2 tau antibodies. Proteins samples from AD synaptosomes were applied on the gel in two different lanes and after transfer the membrane was cut in two equal parts and analyzed in parallel with NH2 4268 tau and CCP-NH2 tau antibodies (B). Notice also that the NH2 tau fragment of 20-22 kDa molecular weight was only detected in AD synaptosomes, while it is indetectable in equivalent protein amounts from AD total extract or ND synaptosomes. alpha-tubulin was used as control loading. The identity of the NH<sub>2</sub> 20-22 kDa tau truncated fragment probed with NH<sub>2</sub> 4268 tau was finally confirmed by Western blotting analysis of equal amounts of human synaptosomes fractions with several commercial and non-commercial phosphorylation-independent tau antibodies, i.e., Tau12-27 (aa NH<sub>2</sub> 12-27), DC39N1 (aa NH<sub>2</sub> 45-73), HT7 (aa NH<sub>2</sub> 159-163), which are directed against the proximal/middle NH<sub>2</sub>-end of full length human tau protein, respectively. Although with different intensity, immunological affinity epitopes-mapping identified the NH2 20-22 kDa tau fragment as positive for DC39N1 (C) and HT7 (D), while only the upper band of the doublet was positive for Tau 12-27 (E), likely because of a partial destruction of epitope that could remove crucial interactions between antibody and antigen and reduce the antibody affinity. Furthermore, in agreement with our previous data in cellular and animal AD models [1], Tau13 (aa NH<sub>2</sub> 1-13), an antibody reacting with the extreme NH<sub>2</sub> end of full length human tau protein, did not visualize the NH<sub>2</sub> 20-22 kDa tau truncated fragment, which were instead detectable at long exposition with Tau21 (aa NH<sub>2</sub> 21-36) (not shown), suggesting that a removal of near part not involved in antibody binding could disrupt the conformation of adiacent molecule region necessary for the in vivo epitope presentation.



Supplementary Fig. 2. Biochemical characterization of neuron-derived conditioned media from differentiated human SY5Y incubated with 10  $\mu$ M synthetic human A $\beta_{1-42}$  and oligomers-enriched AD soluble brain extracts (SBH). A, B) Differentiated human SY5Y neurons were incubated for 12 h with vehicle alone, with 10  $\mu$ M of soluble human A $\beta_{1-42}$  [2], with 1 mg/ml AD-SBH [3]. Equal amounts of unheated and DTT-free conditioned melieu were loaded for lane and subjected to Western blotting analysis. After resolving on SDS-PAGE (4-12% Tris-glycine) and Ponceau staining to verify the homogeneous proteins loading, the filter was probed with 6E10 antibody (anti-A $\beta_{4-9}$ ). Notice that the conditioned medium from  $A\beta$  peptide-exposed cultures contained a mixture of SDS-stable soluble dimers, trimer/tetramers while a faint immunoreactivity of oligometric A $\beta$  species was present in melieu from AD-SBH-treated neurons, which in contrast contained sA $\beta$ PP $\alpha$ and full length A $\beta$ PP (A). However, several 6E10-immunoreactive, SDS-stable, A $\beta$  aggregates, which theoretically corresponded to trimeric (14-kDa), hexameric (27 kDa), nonameric (40 kDa), and dodecameric (56 kDa) and HMW multiples of  $A\beta_{1-42}$  assemblies (50-75 kDa), were contextually detected in oligomeric-enriched AD-SBH preparation (S2B, left) suggesting that, upon 12 h treatment, such aggregates were in vitro largely membrane-bound or internalized [4,5]. Finally when denaturated, by mild boiling or by low concentration of reducing agents, i.e., DTT, the protein-smear in the HMW region disappeared concomitant with a parallel increase in levels of examers and, to a lesser extent, of tetrameric/trimeric A $\beta$  species (S2B, right). All together, these results suggest that (i) the 6E10-positive A $\beta$  complexes are held together by SDS-resistant hydrogen bonds or S-S covalent bonds. In addition, as previously reported [6], the great resistance to trimers/tetramers to denaturating conditions confirmed that these A $\beta$  aggregates are the primary A $\beta$  assembly unit *in vivo*. Arrows indicate respective migration positions of monomers (1-mer), dimers (2-mer), trimers (3-mer), tetramers (4-mer), hexamers (6-mer), nonamers (9-mer), dodecamers (12-mer) and  $sA\beta PP\alpha$  (secreted form of  $A\beta PP$  that has been cleaved by  $\alpha$ -secretase). Asterisk indicates the protein-smear in high molecular region around 50-75 kDa.