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(54) Title: PHOSPHOSPECIFIC ANTIBODIES RECOGNISING TAU

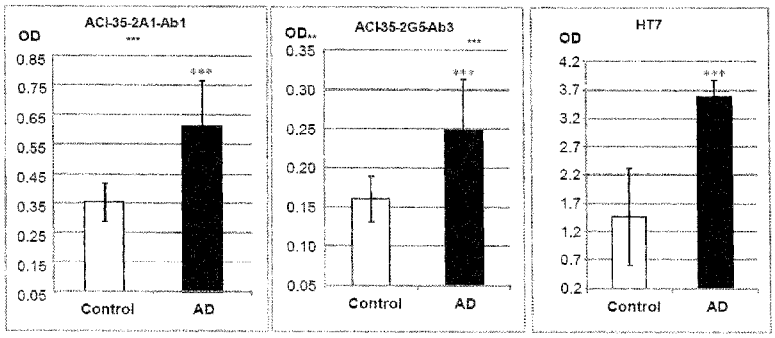

Figure 1.

(57) Abstract: The present invention relates to methods and compositions for the therapeutic and diagnostic use in the treatment of diseases and disorders which are caused by or associated with neurofibrillary tangles, in particular, the invention relates to antibodies, which specifically recognize and bind to phosphorylated pathological protein tau-conformers and to methods and compositions involving said antibodies for the therapeutic and diagnostic use in the treatment of tauopathies including Alzheimer's Disease (AD).

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PHOSPHOSPECIFIC ANTIBODIES RECOGNISING TAU

This application claims the benefit of European Patent Application No. EP12163319.2 filed on April 5, 2012 and International Patent Application No. PCT/EP2011/067604 filed on October 7, 2011, the contents of each of which is hereby incorporated by reference it its entirety.

The present invention is related to methods and compositions for the therapeutic and diagnostic use in the treatment of diseases and disorders which are caused by or associated with neurofibrillary tangles. In particular, the invention relates to antibodies, which specifically recognize and bind to phosphorylated pathological protein tau-conformers and to methods and compositions involving said antibodies for the therapeutic and diagnostic use in the treatment of tauopathies including Alzheimer's Disease (AD).

Neurofibrillary tangles and neuropil threads (NTs) are the major neuropathological hallmarks of Alzheimer's Disease (AD). They are composed of the microtubule-associated protein tau that has undergone posttranslational modifications, including phosphorylation, deamidation and isomerization on asparaginyl or aspartyl residues. They originate by the aggregation of hyper-phosphorylated protein tau and its conformers. AD shares this pathology with many neurodegenerative tauopathies, in particularly with specified types of frontotemporal dementia (FTD).

Protein Tau is a freely soluble, "naturally unfolded" protein that binds avidly to microtubules (MTs) to promote their assembly and stability. MTs are of major importance for the cytoskeletal integrity of neurons - and thereby for the proper formation and functioning of neuronal circuits, hence for learning and memory. The binding of tau to MT is controlled by dynamic phosphorylation and de-phosphorylation, as demonstrated mainly *in vitro* and in non-neuronal cells. Due to the large number of possible phosphorylation sites (>80), the exact contribution of each and the identity of the responsible kinases remain largely undefined *in vivo*.

In AD brain, tau pathology develops later than, and therefore probably in response to amyloid pathology, which constitutes the essence of the amyloid cascade hypothesis. This is based on and indicated by studies in AD and Down syndrome patients, and is corroborated by studies in transgenic mice with combined amyloid and tau pathology (Lewis et al., 2001; Oddo et al., 2004; Ribe et al., 2005; Muyllaert et al, 2006; 2008; Terwel et al, 2008).

The exact timing of both pathologies in human AD patients as well as mechanisms that link amyloid to tau pathology remain largely unknown, but are proposed to involve activation of neuronal signaling pathways that act on or by GSK3 and cdk5 as the major "tau-kinases" (reviewed by Muyllaert et al, 2006, 2008).

The hypothesis that tauopathy is not an innocent side-effect but a major pathological executor in AD is based on sound genetic, pathological and experimental observations that corroborate each other fully:

- in early-onset familial AD cases that are due to mutations in amyloid protein precursor (APP) or presenilin, the obligate pathogenic cause is amyloid accumulation, but invariably the pathology comprises collateral tauopathy, identical to that in the late-onset sporadic AD cases;
- severity of cognitive dysfunction and dementia correlates with tauopathy, not with amyloid pathology, exemplified most recently by several clinical phase-1&2 studies that include PIB-PET imaging for amyloid and identify many "false positives": cognitively normal individuals with high brain amyloid load;
- in familial FTD, the tauopathy is provoked by mutant tau and causes neurodegeneration directly, without amyloid pathology;
- in experimental mouse models the cognitive defects caused by amyloid pathology are nearly completely alleviated by the absence of protein tau (Roberson et al, 2007).

The combined arguments support the hypothesis that protein tau is a major player in the cognitive demise in AD and related neurodegenerative tauopathies.

A prominent emerging treatment of AD is by passive immunotherapy with specific mAbs, to clear amyloid peptides and their aggregates that are presumed to be neuro-toxic or synapto-toxic.

Immunotherapy targeting tau pathology, as proposed here, is anticipated to counteract the pathological protein tau-conformers that are known or postulated to cause synaptic dysfunction and neurodegeneration.

Other therapeutic approaches that target protein tau are scarce and comprise mainly:

- inhibitors of the kinases that are thought to increase the phosphorylation of tau to pathological levels
- compounds that block the cytoplasmic aggregation of hyper-phosphorylated protein tau.

These approaches suffer various draw-backs of specificity and efficacy, a problem they share with attempts to modify the metabolism of APP and amyloid, all emphasizing the importance of a continuous search for additional treatment options, including immunotherapy against tau. Indeed, immunotherapy targeting amyloid in a preclinical mouse model with combined AD-like pathology demonstrated also an effect on tau pathology although tau aggregates persisted (Oddo et al., 2004).

Some doubts have been cast on the feasibility of approaching intra-cellular protein tau by immunotherapy. These have been countered by the most recent experimental study in a tauopathy mouse model (Asuni et al., 2007). They showed reduction in tangle pathology and functional improvements by vaccination with a protein tau derived phospho-peptide. These data corroborate previous reports of immunotherapy targeting α-synuclein in Parkinson's Disease (PD) and Lewy body disease models (Masliah et al., 2005, 2011) and of superoxide dismutase in an amyotrophic lateral sclerosis (ALS) model (Urushitiani et al., 2007). These diseases are examples wherein intra-cellular proteins lead to synaptic defects and neurodegeneration by as yet not fully understood mechanisms.

There is an unmet need for passive and/or active immunotherapies that work to counteract the pathological protein conformers that are known - or presumed - to cause neurodegenerative disorders, such as amyloid pathology in AD caused, for example, by intra-neuronal aggregates of hyper-phosphorylated protein tau that are as typical for AD as amyloid.

This unmet need is met by the present invention which provides for binding proteins recognizing and binding to major pathological phospho-epitopes of the tau protein. In particular, the present invention provides specific antibodies against linear and conformational, simple and complex phospho-epitopes on protein tau, particularly on aggregated tau protein that are believed to be responsible for synapto- and neuro-toxicity in tauopathies, including AD.

Accordingly, the present invention relates in one embodiment to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, which binding peptide or protein or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a phospho-epitope on aggregated Tau protein, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes, wherein said binding peptide or antibody has a high binding affinity to soluble, oligomeric and insoluble phosphorylated Tau protein and is capable of detecting and/or modulating levels of

soluble, oligomeric and insoluble phosphorylated Tau protein *in vivo*, particularly in the brain, particularly with a dissociation constant of at least 10 nM, particularly of at least 8 nM, particularly of at least 5 nM, particularly of at least 2 nM, particularly of at least 1 nM, particularly of at least 500 pM, particularly of at least 400 pM, particularly of at least 300 pM, particularly of at least 200 pM, particularly of at least 100 pM, particularly of at least 50 pM.

In particular, the dissociation constant is in a range of between 2 nM and 80 nM, particularly between 2 nM and 40 nM , particularly between 2 nM and 10 nM.

In a certain aspect, provided herein is an antibody or a functional fragment thereof, wherein said antibody or antibody fragment binds to a phospho-epitope having, or within, the amino acid sequence VYKSPVVSGDTSPRHL (SEQ ID NO: 62) (Tau aa 393-408 of SEQ ID NO: 67, *e.g.*, as set forth in Table 1) comprising a phosphorylated Ser at position 396 (pS396) and at position 404 (pS404).

In a second embodiment, the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes, wherein said binding peptide or antibody has a high binding affinity to soluble, oligomeric and insoluble phosphorylated Tau protein and is capable of detecting and/or modulating levels of soluble, oligomeric and insoluble phosphorylated Tau protein *in vivo* and has an association rate constant of 10^4 $M^{-1}s^{-1}$ or greater, particularly of between 3 - 5 x 10^4 $M^{-1}s^{-1}$ or greater, particularly of 10^5 $M^{-1}s^{-1}$ or greater; particularly of 2 – 9 x 10^5 $M^{-1}s^{-1}$ or greater; particularly of 10^6 $M^{-1}s^{-1}$ or greater, particularly of 1 – 4 x 10^6 $M^{-1}s^{-1}$ or greater, particularly of 10^7 $M^{-1}s^{-1}$ or greater.

In particular, the association rate constant is in a range of between 1.6 x 10^3 and 5 x 10^5, particularly between 2.4 x 10^4 and 5 x 10^5, between 3 x 10^3 and 5 x 10^5.

In a third embodiment, the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes, wherein said binding peptide or antibody has a high binding affinity to soluble, oligomeric and

insoluble phosphorylated Tau protein and is capable of detecting and/or modulating levels of soluble, oligomeric and insoluble phosphorylated Tau protein *in vivo* and has a high binding affinity with a dissociation constant of at least 4 nM and an association rate constant of 10^5 $M^{-1}s^{-1}$ or greater, particularly a dissociation constant of at least 3 nM and an association rate constant of 10^6 $M^{-1}s^{-1}$ or greater, particularly a dissociation constant of at least 2 nM and an association rate constant of 10^4 $M^{-1}s^{-1}$ or greater, particularly a dissociation constant of at least 1 nM and an association rate constant of 10^5 $M^{-1}s^{-1}$ or greater, particularly a dissociation constant of at least 200 pM and an association rate constant of 10^5 $M^{-1}s^{-1}$ or greater, particularly a dissociation constant of at least 100 pM and an association rate constant of 10^6 $M^{-1}s^{-1}$ or greater.

In particular, the dissociation constant is in a range of between 2 nM and 80 nM and the association rate constant is in a range of between 1.6×10^3 and 5×10^5, particularly the dissociation constant is in a range of between 2 nM and 40 nM and the association rate constant is in a range of between 2.4×10^4 and 5×10^5, particularly the dissociation constant is in a range of between 2 nM and 10 nM and the association rate constant is in a range of between 3×10^3 and 5×10^5.

One embodiment (4) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes, wherein said binding peptide or antibody has a high binding affinity to soluble, oligomeric and insoluble phosphorylated Tau protein and is capable of detecting and/or modulating levels of soluble, oligomeric and insoluble phosphorylated Tau protein *in vivo* and wherein said binding peptide or antibody binds to an epitope on a mammalian, particularly on the human Tau protein as shown in SEQ ID NO: 67, selected from the group consisting of Tau aa 393-401, comprising a phosphorylated Ser at position 396 (pS396), Tau aa 396-401 comprising a phosphorylated Ser at position 396 (pS396), Tau aa 394-400 comprising a phosphorylated Ser at position 396 (pS396), Tau aa 402-406 comprising a phosphorylated Ser at position 404 (pS404) and Tau aa 393-400 comprising a phosphorylated Ser at position 396 (pS396).

One embodiment (5) relates to the binding peptide or antibody of any of the preceding embodiments, wherein said peptide binds to an epitope on a mammalian, particularly on the human Tau protein, but especially the human Tau protein as shown in SEQ ID NO: 67, comprising Tau aa 393-401 comprising a phosphorylated Ser at position 396 (pS396).

One embodiment (6) relates to the binding peptide or antibody of any o the preceding embodiments, wherein said peptide binds to an epitope on a mammalian, particularly on the human Tau protein, but especially the human Tau protein as shown in SEQ ID NO: 67, comprising Tau aa 396-401 comprising a phosphorylated Ser at position 396 (pS396).

One embodiment (7) relates to the binding peptide or antibody of any of the preceding embodiments, wherein said peptide binds to an epitope on a mammalian, particularly on the human Tau protein, but especially the human Tau protein as shown in SEQ ID NO: 67, comprising Tau aa 394-400 comprising a phosphorylated Ser at position 396 (pS396).

One embodiment (8) relates to the binding peptide or antibody of any of the preceding embodiments, wherein said peptide binds to an epitope on a mammalian, particularly on the human Tau protein, but especially the human Tau protein as shown in SEQ ID NO: 67, comprising Tau aa 402-406 comprising a phosphorylated Ser at position 404 (pS404).

One embodiment (9) relates to the binding peptide or antibody of any of the preceding embodiments, wherein said peptide binds to an epitope on a mammalian, particularly on the human Tau protein, but especially the human Tau protein as shown in SEQ ID NO: 67, comprising Tau aa 393-400 comprising a phosphorylated Ser at position 396 (pS396).

One embodiment (10) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody has a high binding affinity to soluble, oligomeric and insoluble phosphorylated Tau protein and is capable of detecting and/or modulating levels of soluble, oligomeric and insoluble phosphorylated Tau protein *in vivo* and wherein said binding peptide or antibody comprises a first binding domain comprising in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 73, a CDR2 with the amino acid sequence shown in SEQ ID NO: 74, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 75, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto and/or a second binding domain comprising in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 70, or an amino acid sequence at least 95%, particularly 98%, particularly 99% identical thereto, a CDR2 with the amino acid sequence shown in SEQ ID NO: 71, or an

amino acid sequence at least 94%, 95%, 96%, 97%, 98%, or 99% identical thereto, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 72, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto.

In one aspect, provided herein is an antibody or a functional fragment thereof, which antibody or fragment thereof recognizes and specifically binds to a phospho-epitope on a mammalian Tau protein or on a fragment thereof, wherein said antibody or fragment thereof comprises:

(a) a first binding domain comprising a CDR1 comprising the amino acid sequence shown in SEQ ID NO: 73, a CDR2 comprising the amino acid sequence shown in SEQ ID NO: 74, and a CDR3 comprising the amino acid sequence shown in SEQ ID NO: 75; and/or

(b) a second binding domain comprising an amino acid sequence comprising a CDR1 comprising the amino acid sequence shown in SEQ ID NO: 70, a CDR2 comprising the amino acid sequence shown in SEQ ID NO: 71, and a CDR3 comprising the amino acid sequence shown in SEQ ID NO: 72.

One embodiment (11) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody has a high binding affinity to soluble, oligomeric and insoluble phosphorylated Tau protein and is capable of detecting and/or modulating levels of soluble, oligomeric and insoluble phosphorylated Tau protein *in vivo* and wherein said binding peptide or antibody comprises a first binding domain comprising in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 81, a CDR2 with the amino acid sequence shown in SEQ ID NO: 82, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 83, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto and/or a second binding domain comprising in sequence a CDR1 with the amino acid sequence shown in

SEQ ID NO: 78, a CDR2 with the amino acid sequence shown in SEQ ID NO: 79, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 80, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto.

In one aspect, provided herein is an antibody or a functional fragment thereof, which antibody or fragment thereof recognizes and specifically binds to a phospho-epitope on a mammalian Tau protein or on a fragment thereof, wherein said antibody or fragment thereof comprises:

(a) a first binding domain comprising a CDR1 comprising the amino acid sequence shown in SEQ ID NO: 81, a CDR2 comprising the amino acid sequence shown in SEQ ID NO: 82, and a CDR3 comprising the amino acid sequence shown in SEQ ID NO: 83; and/or

(b) a second binding domain comprising an amino acid sequence comprising a CDR1 comprising the amino acid sequence shown in SEQ ID NO: 78, a CDR2 comprising the amino acid sequence shown in SEQ ID NO: 79, and a CDR3 comprising the amino acid sequence shown in SEQ ID NO: 80.

One embodiment (12) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody has a high binding affinity to soluble, oligomeric and insoluble phosphorylated Tau protein and is capable of detecting and/or modulating levels of soluble, oligomeric and insoluble phosphorylated Tau protein *in vivo* and wherein said binding peptide or antibody comprises a first binding domain comprising in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 93, a CDR2 with the amino acid sequence shown in SEQ ID NO: 94, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 95, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs and/or a second binding domain comprising in sequence a CDR1 with the amino acid

sequence shown in SEQ ID NO: 89, a CDR2 with the amino acid sequence shown in SEQ ID NO: 90, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 91, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs.

In a certain aspect, provided herein is an antibody or a functional part thereof, which antibody or fragment thereof recognizes and specifically binds to a phospho-epitope on a mammalian Tau protein or on a fragment thereof, wherein said antibody or fragment thereof comprises:

(a) a first binding domain comprising a CDR1 comprising the amino acid sequence shown in SEQ ID NO: 93, a CDR2 comprising the amino acid sequence shown in SEQ ID NO: 94, and a CDR3 comprising the amino acid sequence shown in SEQ ID NO: 95; and/or

(b) a second binding domain comprising an amino acid sequence comprising a CDR1 comprising the amino acid sequence shown in SEQ ID NO: 89, a CDR2 comprising the amino acid sequence shown in SEQ ID NO: 90, and a CDR3 comprising the amino acid sequence shown in SEQ ID NO: 91.

One embodiment (13) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody has a high binding affinity to soluble, oligomeric and insoluble phosphorylated Tau protein and is capable of detecting and/or modulating levels of soluble, oligomeric and insoluble phosphorylated Tau protein *in vivo* and wherein said binding peptide or antibody comprises a first binding domain comprising in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 101, a CDR2 with the amino acid sequence shown in SEQ ID NO: 102, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 103, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs and/or a second binding domain comprising in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 98, a CDR2 with the amino acid sequence shown in SEQ ID

NO: 99, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 100, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs.

In one aspect, provided herein is an antibody or a functional fragment thereof, which antibody or fragment thereof recognizes and specifically binds to a phospho-epitope on a mammalian Tau protein or on a fragment thereof, wherein said antibody or fragment thereof comprises:

(a) a first binding domain comprising a CDR1 comprising the amino acid sequence shown in SEQ ID NO: 101, a CDR2 comprising the amino acid sequence shown in SEQ ID NO: 102, and a CDR3 comprising the amino acid sequence shown in SEQ ID NO: 103; and/or

(b) a second binding domain comprising an amino acid sequence comprising a CDR1 comprising the amino acid sequence shown in SEQ ID NO: 98, a CDR2 comprising the amino acid sequence shown in SEQ ID NO: 99, and a CDR3 comprising the amino acid sequence shown in SEQ ID NO: 100.

One embodiment (14) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody has a high binding affinity to soluble, oligomeric and insoluble phosphorylated Tau protein and is capable of detecting and/or modulating levels of soluble, oligomeric and insoluble phosphorylated Tau protein *in vivo* and wherein said binding peptide or antibody comprises a first binding domain comprising in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 106, a CDR2 with the amino acid sequence shown in SEQ ID NO: 107, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 108, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs and/or a second binding domain comprising in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 89, a CDR2 with the amino acid sequence shown in SEQ ID

NO: 115, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 91, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs.

In a particular aspect, provided herein is an antibody or a functional fragment thereof, which antibody or fragment thereof recognizes and specifically binds to a phospho-epitope on a mammalian Tau protein or on a fragment thereof, wherein said antibody or fragment thereof comprises:

(a) a first binding domain comprising a CDR1 comprising the amino acid sequence shown in SEQ ID NO: 106, a CDR2 comprising the amino acid sequence shown in SEQ ID NO: 107, and a CDR3 comprising the amino acid sequence shown in SEQ ID NO: 108; and/or

(b) a second binding domain comprising an amino acid sequence comprising a CDR1 comprising the amino acid sequence shown in SEQ ID NO: 89, a CDR2 comprising the amino acid sequence shown in SEQ ID NO: 115, and a CDR3 comprising the amino acid sequence shown in SEQ ID NO: 91.

In a certain embodiment, the first binding domain of an antibody or antibody fragment thereof described herein is a light chain variable region, and the second binding domain of an antibody or antibody fragment thereof described herein is a heavy chain variable region.

In another embodiment (15), the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes, wherein said binding peptide or antibody has a high binding affinity to soluble, oligomeric and insoluble phosphorylated Tau protein and is capable of detecting and/or modulating levels of soluble, oligomeric and insoluble phosphorylated Tau protein *in vivo* and wherein said binding peptide or antibody comprises a first binding domain comprising the amino acid sequence shown in SEQ ID NO: 69, 77, 116/92, 118, 97, 105, or an amino acid sequence particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto, and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 68, 76, 88, 96,

104, or an amino acid sequence at least 80%, particularly at least 85%, particularly at least 86%, particularly at least 87%, particularly at least 88%, particularly at least 89%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto.

One embodiment (16) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody has a high binding affinity to soluble, oligomeric and insoluble phosphorylated Tau protein and is capable of detecting and/or modulating levels of soluble, oligomeric and insoluble phosphorylated Tau protein *in vivo* and wherein said binding peptide or antibody comprises a first binding domain comprising the amino acid sequence shown in SEQ ID NO: 69, or an amino acid sequence at least 98% or 99% identical thereto; and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 68, or an amino acid sequence at least 90%, 91%, 92% or 93% identical thereto.

One embodiment (17) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody has a high binding affinity to soluble, oligomeric and insoluble phosphorylated Tau protein and is capable of detecting and/or modulating levels of soluble, oligomeric and insoluble phosphorylated Tau protein *in vivo* and wherein said binding peptide or antibody comprises a first binding domain comprising the amino acid sequence shown in SEQ ID NO: 77, or an amino acid sequence at least 93%, 94% or 95% identical thereto; and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 76, or an amino acid sequence at least 88%, 89%, or 90% identical thereto.

One embodiment (18) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody has a high binding affinity to soluble, oligomeric and insoluble phosphorylated Tau protein and is capable of detecting and/or modulating levels of soluble, oligomeric and insoluble phosphorylated Tau protein *in vivo* and wherein said binding peptide or antibody comprises a first binding domain comprising the amino acid sequence shown in SEQ ID NO: 116, 92, or 118, or an amino acid sequence at least 93%, 94% or 95% identical thereto; and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 88, or an amino acid sequence at least 90%, 91%, 92% or 93% identical thereto.

One embodiment (19) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody has a high binding affinity to soluble, oligomeric and insoluble phosphorylated Tau protein and is capable of detecting and/or modulating levels of soluble, oligomeric and insoluble phosphorylated Tau protein *in vivo* and wherein said binding peptide or antibody comprises a first binding domain comprising the amino acid sequence shown in SEQ ID NO: 97, or an amino acid sequence at least 99% identical thereto; and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 96, or an amino acid sequence at least 86%, 87%, 88% or 90% identical thereto.

One embodiment (20) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody has a high binding affinity to soluble, oligomeric and

insoluble phosphorylated Tau protein and is capable of detecting and/or modulating levels of soluble, oligomeric and insoluble phosphorylated Tau protein *in vivo* and wherein said binding peptide or antibody comprises a first binding domain comprising the amino acid sequence shown in SEQ ID NO: 105, or an amino acid sequence at least 98%, or 99% identical thereto; and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 104, or an amino acid sequence at least 88%, 89%, or 90% identical thereto.

One embodiment (21) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, wherein said binding peptide or antibody comprises a first binding domain the amino acid sequence shown in SEQ ID NO: 69, or an amino acid sequence at least 99% identical thereto; and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 68, or an amino acid sequence at least 93% identical thereto.

One embodiment (22) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, wherein said binding peptide or antibody comprises a first binding domain comprising the amino acid sequence shown in SEQ ID NO: 77, or an amino acid sequence at least 95% identical thereto and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 76, or an amino acid sequence at least 90% identical thereto.

One embodiment (23) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, wherein said binding peptide or antibody comprises a first binding domain comprising the amino acid sequence shown in SEQ ID NO: 116, 92, or 118, or an amino acid sequence at least 93 95% identical thereto; and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 88, or an amino acid sequence at least 93% identical thereto.

One embodiment (24) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, wherein said binding peptide or antibody comprises a first binding domain comprising the amino acid sequence shown in SEQ ID NO: 97, or an amino acid sequence at least 99% identical thereto; and/or a second binding domain comprising the amino acid

sequence shown in SEQ ID NO: 96, or an amino acid sequence at least 90% identical thereto.

One embodiment (25) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, wherein said binding peptide or antibody comprises a first binding domain comprising the amino acid sequence shown in SEQ ID NO: 105, or an amino acid sequence at least 98%, or 99% identical thereto; and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 104, or an amino acid sequence at least 90% identical thereto.

One embodiment (26) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, according to embodiment (16), wherein said first binding domain contains the CDRs as shown in SEQ ID NOs: 73-75, and said second binding domain contains the CDRs as shown in SEQ ID NOs: 70-72.

One embodiment (27) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, according to embodiment (17), wherein said first binding domain contains the CDRs as shown in SEQ ID NOs: 81-83, and said second binding domain contains the CDRs as shown in SEQ ID NOs: 78-80.

One embodiment (28) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, according to embodiment (18), wherein said first binding domain contains the CDRs as shown in SEQ ID NOs: 93-95, and said second binding domain contains the CDRs as shown in SEQ ID NOs: 89-91.

One embodiment (29) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, according to embodiment (19), wherein said first binding domain contains the CDRs as shown in SEQ ID NOs: 101-103, and said second binding domain contains the CDRs as shown in SEQ ID NOs: 98-100.

One embodiment (30) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, according to embodiment (18), wherein said first binding domain contains the CDRs as shown in SEQ ID NOs: 89, 115, and 91, and said second binding domain contains the CDRs as shown in SEQ ID NOs: 106-108.

In still another embodiment (31), the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody has a high binding affinity to soluble, oligomeric and insoluble phosphorylated Tau protein and is capable of detecting and/or modulating levels of soluble, oligomeric and insoluble phosphorylated Tau protein *in vivo* and wherein said binding peptide or antibody comprises a

a. first binding domain comprising the amino acid sequence shown in SEQ ID NO: 69 and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 68; or a

b. first binding domain comprising the amino acid sequence shown in SEQ ID NO: 77 and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 76; or a

c. first binding domain comprising the amino acid sequence shown in SEQ ID NO: 116 and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 88; or a;

d. first binding domain comprising the amino acid sequence shown in SEQ ID NO: 92 and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 88; or a

e. first binding domain comprising the amino acid sequence shown in SEQ ID NO: 97 and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 96; or a

f. first binding domain comprising the amino acid sequence shown in SEQ ID NO: 105 and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 104.

g. first binding domain comprising the amino acid sequence shown in SEQ ID NO: 118 and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 88

In one embodiment (32) of the invention, the binding peptide of any of the preceding embodiments is an antibody, particularly an antibody of the IgG2a, IgG2b or the IgG3

isotype, particularly a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a humanized antibody or a fully human antibody.

One embodiment (33) of the invention relates to a polynucleotide encoding the binding peptide of any one of the preceding embodiments.

In one embodiment (34), said polynucleotide comprises a nucleic acid molecule selected from the group consisting of

a. a nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide comprising the amino acid sequence as depicted in SEQ ID NOs: 84-87, SEQ ID NO: 109-114, 117 and 119;

b. a nucleic acid molecule comprising a nucleotide sequence that has at least 85% sequence identity to the sequence shown in SEQ ID NOs: 84-87, SEQ ID NO: 109-114, 117 and 119;

c. a nucleic acid molecule comprising a nucleotide sequence that has at least 90% sequence identity to the sequence shown in SEQ ID NOs: 84-87, SEQ ID NO: 109-114, 117 and 119;

d. a nucleic acid molecule comprising a nucleotide sequence that has at least 95% sequence identity to the sequence shown in SEQ ID NOs: 84-87, SEQ ID NO: 109-114, 117 and 119;

e. a nucleic acid molecule comprising a nucleotide sequence that has at least 98% sequence identity to the sequence shown in SEQ ID NOs: 84-87, SEQ ID NO: 109-114, 117 and 119;

f. a nucleic acid molecule comprising a nucleotide sequence that has at least 99% sequence identity to the sequence shown in SEQ ID NOs: 84-87, SEQ ID NO: 109-114, 117 and 119;

g. a nucleic acid molecule comprising a nucleotide sequence the complementary strand of which hybridizes to the nucleic acid molecule of any of a) – f);

h. a nucleic acid molecule comprising a nucleotide sequence that deviates from the nucleotide sequence defined in any of a) – g) by the degeneracy of the genetic code,

wherein said nucleic acid molecule as defined in any of a) – h) recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly on the human Tau protein as shown in SEQ ID NO: 67, selected from the group consisting of Tau aa 393-401, comprising a phosphorylated Ser at position 396 (pS396), Tau aa 396-401 comprising a phosphorylated Ser at position 396 (pS396), Tau aa 394-400 comprising a phosphorylated Ser at position 396 (pS396), Tau aa

402-406 comprising a phosphorylated Ser at position 404 (pS404), and Tau aa 393-400 comprising a phosphorylated Ser at position 396 (pS396), wherein, in one embodiment, said binding peptide has a high binding affinity with a dissociation constant of at least 10 nM, particularly of at least 8 nM, particularly of at least 5 nM, particularly of at least 2 nM, particularly of at least 1 nM, particularly of at least 500 pM, particularly of at least 400 pM, particularly of at least 300 pM, particularly of at least 200 pM, particularly of at least 100 pM, particularly of at least 50 pM and/or has an association rate constant of 10^4 $M^{-1}s^{-1}$ or greater, particularly of between 3 - 5 x 10^4 $M^{-1}s^{-1}$ or greater, particularly of 10^5 $M^{-1}s^{-1}$ or greater; particularly of 6 – 9 x 10^5 $M^{-1}s^{-1}$ or greater; particularly of 10^6 $M^{-1}s^{-1}$ or greater, particularly of 1 – 4 x 10^6 $M^{-1}s^{-1}$ or greater, particularly of 10^7 $M^{-1}s^{-1}$ or greater, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes.

In various embodiments (35) of the invention, a binding peptide is provided or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide, according to any one of the preceding embodiments, or a combination thereof, which is capable of specifically recognizing and binding to a phospho-epitope on a mammalian, particularly on the human Tau protein, particularly a microtubule-associated protein tau, particularly an aggregated microtubule-associated and hyperphosphorylated protein tau such as that present in paired helical filaments (PHF), which are the predominant structures in neurofibrillary tangles, neuropil threads and dystrophic neurites, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes.

In a specific embodiment (36) of the invention, the human tau protein is the human Tau protein as shown in SEQ ID NO: 67.

The binding peptides and antibodies according to any one of the preceding embodiments can thus be used (37) for reducing the levels of total soluble tau protein, particularly of soluble phosphorylated tau protein, in the brain, particularly in the brain cortex and/or hippocampus, of a mammal or a human containing increased levels of soluble tau protein and/or soluble phosphorylated tau protein.

The binding peptides and antibodies according to any one of the preceding embodiments can also be used (38) for reducing the levels of paired helical filaments containing hyperphosphorylated tau protein (pTau PHF) in the brain, particularly in the brain cortex and/or hippocampus, of a mammal or a human containing increased levels of said pTau paired helical filaments.

Reduction of the level of total soluble tau protein and/or soluble phosphorylated tau protein and/or pTau paired helical filaments in the brain, particularly in the brain cortex and/or hippocampus, of a mammal or a human containing increased levels of said tau protein

variants, which contribute to tau-protein-associated diseases, disorders or conditions in said mammal or human, may lead to an improvement and/or alleviation of the symptoms associated with such tau-protein-associated diseases, disorders or conditions (39).

The binding peptides and antibodies according to any one of the preceding embodiments can therefore be used (40) in therapy, particularly in human therapy, for slowing or halting the progression of a tau-protein-associated disease, disorder or condition.

The binding peptides and antibodies according to any one of the preceding embodiments can further be used (41) in therapy, particularly in human therapy, for improving or alleviating the symptoms associated with tau-protein-associated diseases, disorders or conditions such as, for example, impairment or loss of cognitive functions including reasoning, situational judgement, memory capacity, learning, special navigation, etc.

In one embodiment (42), the invention relates to the binding peptides and antibodies according to any one of the preceding embodiments for use in therapy, particularly for use in the treatment of tauopathies, a group of tau-protein-associated diseases and disorders, or for alleviating the symptoms associated with tauopathies.

In one embodiment (43), the invention relates to the binding peptides and antibodies according to any one of the preceding embodiments for retaining or increasing cognitive memory capacity in a mammal suffering from a tauopathy.

In another specific embodiment (44) of the invention, binding peptides and antibodies comprising at least one or all of the light chain CDRs of antibodies ACI-35-2A1-Ab1; ACI-35-2A1-Ab2; ACI-35-4A6-Ab1; ACI-35-4A6-Ab2; ACI-35-1D2-Ab1; ACI-35-2G5-Ab1; ACI-35-2G5-Ab2; ACI-35-2G5-Ab3; as given in SEQ ID NOs: 73-75, 81-83, 93-95, 101-103, 106-108 and/or at least one or all of the heavy chain CDRs of antibodies ACI-35-2A1-Ab1; ACI-35-2A1-Ab2; ACI-35-4A6-Ab1; ACI-35-4A6-Ab2; ACI-35-1D2-Ab1; ACI-35-2G5-Ab1; ACI-35-2G5-Ab2; ACI-35-2G5-Ab3; as given in SEQ ID NOs: 70-72, 78-80, 89-91, 98-100, (89, 115, 91) are used in therapy, particularly in human therapy, for improving or alleviating the symptoms associated with tau-protein-associated diseases, disorders or conditions such as, for example, impairment or loss of cognitive functions including reasoning, situational judgement, memory, learning, special navigation, etc.

In another specific embodiment (45) of the invention, binding peptides and antibodies comprising at least one or all of the light chain CDRs of antibodies ACI-35-2G5-Ab2; ACI-35-2G5-Ab3 as given in SEQ ID NOs: 106-108 and/or at least one or all of the heavy chain CDRs of antibodies ACI-35-2G5-Ab2; ACI-35-2G5-Ab3; as given in SEQ ID NOs: 89, 115 and 91, are used in therapy, particularly in human therapy, for improving or alleviating the symptoms associated with tau-protein-associated diseases, disorders or conditions such as,

for example, impairment or loss of cognitive functions including reasoning, situational judgement, memory, learning, special navigation, etc.

Binding of the peptides or antibodies according to the preceding embodiments to tau tangles and pTau on brains may be determined by applying protein immuno-reactivity testing of selected brain sections and by Western blotting of brain homogenates, respectively, as described in the Examples.

In another embodiment (46), the present invention provides a pharmaceutical composition comprising a binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide, according to any one of the preceding embodiments, or a combination thereof, in a therapeutically effective amount together with a pharmaceutically acceptable carrier.

In one embodiment (47), the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide, or a pharmaceutical composition, according to any one of the preceding embodiments, or a combination thereof, is used in therapy, particularly in human therapy for the treatment or alleviation of the symptoms of tau-protein-associated diseases or disorders including neurodegenerative disorders such as tauopathies.

The binding peptides, antibodies and/or pharmaceutical compositions according to any one of the preceding embodiments may thus be used (48) for slowing or halting the progression of a tau-protein-associated disease, disorder or condition, upon administration of said binding peptides, antibodies and/or pharmaceutical compositions to an animal, particularly a mammal, particularly a human, suffering from such a disease or condition.

The binding peptides, antibodies and/or pharmaceutical compositions according to any one of the preceding embodiments may further be used (49) for improving or alleviating the symptoms associated with tau-protein-associated diseases, disorders or conditions such as, for example, impairment or loss of cognitive functions including reasoning, situational judgement, memory capacity, learning, special navigation, etc, upon administration of said binding peptides, antibodies and/or pharmaceutical compositions to an animal, particularly a mammal, particularly a human, suffering from such a disease or condition.

In one embodiment (50), the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide or a pharmaceutical composition, according to any one of the preceding embodiments, or a combination thereof, is used in the treatment of diseases and disorders which are caused by or associated with the formation of neurofibrillary lesions, the predominant brain pathology in tauopathy comprising a heterogeneous group of neurodegenerative diseases or disorders including diseases or disorders which manifest both tau and amyloid pathologies including,

but not limited to, Alzheimer's Disease, Creutzfeldt-Jacob disease, Dementia pugilistica, Down's Syndrome, Gerstmann-Sträussler-Scheinker disease, inclusion-body myositis, and prion protein cerebral amyloid angiopathy, traumatic brain injury and further diseases or disorders which do not show a distinct amyloid pathology including, but not limited to, amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam, Non-Guamanian motor neuron disease with neurofibrillary tangles, argyrophilic grain dementia, corticobasal degeneration, diffuse neurofibrillary tangles with calcification, frontotemporal dementia with parkinsonism linked to chromosome 17, Hallevorden-Spatz disease, multiple system atrophy, Niemann-Pick disease, type C, Pallido-ponto-nigral degeneration, Pick's disease, progressive subcortical gliosis, progressive supranuclear palsy, Subacute sclerosing panencephalitis, Tangle-only dementia, Postencephalitic Parkinsonism, Myotonic dystrophy.

In one embodiment (51), the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide or a pharmaceutical composition, according to any one of the preceding embodiments, or a combination thereof, is used in the treatment of Alzheimer's Disease.

In one embodiment (52) of the invention, a method is provided for detecting and/or modulating levels of soluble and/or, oligomeric and/or insoluble phosphorylated Tau protein, particularly *in vivo*, particularly in the brain, particularly in the brain cortex and/or hippocampus, of an animal, particularly a mammal or a human, comprising administering to said animal, particularly to said mammal or human, the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide or a pharmaceutical composition, according to any one of the preceding embodiments, or a combination thereof.

In one aspect, modulation relates to reducing the levels of soluble tau protein, particularly of soluble phosphorylated tau protein, in the brain, particularly in the brain cortex and/or hippocampus, of an animal, particularly a mammal or a human containing increased levels of soluble tau protein and/or soluble phosphorylated tau protein.

In one embodiment (53) of the invention, a method is provided for reducing the levels of insoluble tau protein, particularly of paired helical filaments containing hyperphosphorylated tau protein (pTau PHF) in the brain, particularly in the brain cortex and/or hippocampus, of an animal, particularly a mammal or a human, containing increased levels of insoluble tau protein, particularly of pTau paired helical filaments (pTau PHF) comprising administering to said animal, particularly to said mammal or human, the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide or a pharmaceutical composition, according to any one of the preceding embodiments, or a combination thereof.

In one embodiment (54), the present invention relates to a method for slowing or halting the progression of a tau-protein-associated disease, disorder or condition in an animal, particularly a mammal or human comprising administering to said animal, particularly said mammal or human, suffering from such a disease or condition, the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide or a pharmaceutical composition, according to any one of the preceding embodiments, or a combination thereof.

In one embodiment (55), the present invention relates to a method for improving or alleviating the symptoms associated with tau-protein-associated diseases, disorders or conditions such as, for example, impairment or loss of cognitive functions including reasoning, situational judgement, memory capacity, learning, special navigation, etc., in an animal, particularly a mammal or a human, comprising administering to said animal, particularly to said mammal or human, suffering from such a disease or condition, the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide or a pharmaceutical composition, according to any one of the preceding embodiments, or a combination thereof.

In one embodiment (56), the present invention relates to a method for retaining or increasing cognitive memory capacity in a mammal suffering from a tauopathy.

In still another embodiment (57) of the invention, a method is provided for the treatment of a tau-protein-associated disease or disorder including a neurodegenerative disease or disorder such as a tauopathy comprising administering to an animal, particularly to a mammal, but especially to human, suffering from such a disease or disorder, the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide or a pharmaceutical composition, according to any one of the preceding embodiments, or a combination thereof.

In one embodiment (58) of the invention, a method is provided for the treatment of diseases and disorders which are caused by or associated with the formation of neurofibrillary lesions, the predominant brain pathology in tauopathy comprising a heterogeneous group of neurodegenerative diseases or disorders including diseases or disorders which manifest both tau and amyloid pathologies including, but not limited to, Alzheimer's Disease, Creutzfeldt-Jacob disease, Dementia pugilistica, Down's Syndrome, Gerstmann-Sträussler-Scheinker disease, inclusion-body myositis, and prion protein cerebral amyloid angiopathy, traumatic brain injury and further diseases or disorders which do not show a distinct amyloid pathology including, but not limited to, amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam, Non-Guamanian motor neuron disease with neurofibrillary tangles, argyrophilic grain dementia, corticobasal degeneration, diffuse neurofibrillary tangles with

calcification, frontotemporal dementia with parkinsonism linked to chromosome 17, Hallevorden-Spatz disease, multiple system atrophy, Niemann-Pick disease, type C, Pallido-ponto-nigral degeneration, Pick's disease, progressive subcortical gliosis, progressive supranuclear palsy, Subacute sclerosing panencephalitis Tangle-only dementia, Postencephalitic Parkinsonism, Myotonic dystrophy, which method comprises administering to an animal, particularly to a mammal, but especially to human, suffering from such a disease or disorder, the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide or a pharmaceutical composition according to any one of the preceding embodiments, or a combination thereof.

In another embodiment (59) of the invention, a method is provided for inducing a passive immune response in an animal, particularly a mammal or a human, suffering from a neurodegenerative disorder such as tauopathy by administering to said animal or human the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide, or a pharmaceutical composition, according to any one of the preceding embodiments, or a combination thereof.

In still another embodiment (60) of the invention, a method of diagnosing a tau-protein-associated disease, disorder or condition in a patient is provided comprising detecting the immunospecific binding of a binding peptide or an active fragment thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, according to any one of the preceding embodiments, to an epitope of the tau protein in a sample or *in situ* which includes the steps of

a. bringing the sample or a specific body part or body area suspected to contain the tau protein into contact with a binding peptide or a fragment thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, according to any one of the preceding claims, wherein said binding peptide or antibody or fragment thereof binds an epitope of the tau protein;
b. allowing said binding peptide, particularly said antibody, particularly said monoclonal antibody or a functional part thereof, to bind to the tau protein to form an immunological complex;
c. detecting the formation of the immunological complex; and
d. correlating the presence or absence of the immunological complex with the presence or absence of tau protein in the sample or specific body part or area.

In still another embodiment (61) of the invention, a method for diagnosing a predisposition to tau-protein-associated disease, disorder or condition in a patient is provided comprising detecting the immunospecific binding of a binding peptide or an active fragment thereof,

particularly an antibody, particularly a monoclonal antibody or a functional part thereof, according to any one of the preceding embodiments, to an epitope of the tau protein in a sample or *in situ,* which includes the steps of

a. bringing the sample or a specific body part or body area suspected to contain the tau antigen into contact with a binding peptide or an active fragment thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, according to any one of the preceding embodiments, which peptide or fragment thereof binds an epitope of the tau protein;
b. allowing said binding peptide, particularly said antibody, particularly said monoclonal antibody or a functional part thereof, to bind to the tau antigen to form an immunological complex;
c. detecting the formation of the immunological complex; and
d. correlating the presence or absence of the immunological complex with the presence or absence of tau antigen in the sample or specific body part or area;
e. comparing the amount of said immunological complex to a normal control value;

wherein an increase in the amount of said aggregate compared to a normal control value indicates that said patient is suffering from or is at risk of developing an tau- protein-associated disease or condition.

In one embodiment (62) of the invention, a method is provided for monitoring minimal residual disease in a patient following treatment with the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide, or a pharmaceutical composition, according to any one of the preceding embodiments, wherein said method comprises:

a. bringing the sample or a specific body part or body area suspected to contain the tau antigen into contact with the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, according to any one of the preceding embodiments, which peptide or fragment thereof binds to an epitope of the tau protein;
b. allowing said binding peptide, particularly said antibody, particularly said monoclonal antibody or a functional part thereof, to bind to the tau antigen to form an immunological complex;
c. detecting the formation of the immunological complex; and
d. correlating the presence or absence of the immunological complex with the presence or absence of tau antigen in the sample or specific body part or area,
e. comparing the amount of said immunological complex to a normal control value,

wherein an increase in the amount of said aggregate compared to a normal control value indicates that said patient still suffers from a minimal residual disease.

In one embodiment (63), a method is provided for predicting responsiveness of a patient being treated with the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide, or a pharmaceutical composition, according to any one of the preceding embodiments, comprising

a. bringing the sample or a specific body part or body area suspected to contain the tau antigen into contact with a binding peptide or an active fragment thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof according to any one of the preceding embodiments, which peptide or fragment thereof binds to an epitope of the tau protein;
b. allowing said binding peptide, particularly said antibody, particularly said monoclonal antibody or a functional part thereof, to bind to the tau antigen to form an immunological complex;
c. detecting the formation of the immunological complex; and
d. correlating the presence or absence of the immunological complex with the presence or absence of tau antigen in the sample or specific body part or area,
e. comparing the amount of said immunological complex before and after onset of the treatment,

wherein a decrease in the amount of said aggregate indicates that said patient has a high potential of being responsive to the treatment.

Anti-Tau antibodies and fragments thereof may be used in the above methods of the invention. In the above methods the sample containing the antibody or fragment thereof may be immune-enriched to increase the concentration of Tau protein in the sample by contacting the sample with an anti-Tau antibody or a fragment thereof attached to a solid support.

Prior to the step step (a), the sample is immune-enriched to increase the concentration of Tau protein in the sample by contacting the sample with an anti-Tau antibody or a fragment thereof attached to a solid support

In another embodiment (64), the invention relates to a test kit for detection and diagnosis of tau-protein-associated diseases, disorders or conditions comprising a binding peptide or an active fragment thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, according to any one of the preceding embodiments.

In one embodiment (65) said test kit comprises a container holding one or more binding peptides or active fragments thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, according to any one of the preceding embodiments

and instructions for using the binding peptides or antibodies for the purpose of binding to tau antigen to form an immunological complex and detecting the formation of the immunological complex such that presence or absence of the immunological complex correlates with presence or absence of tau antigen.

In still another embodiment (66), the present invention relates to an epitope on a mammalian, particularly on the human Tau protein as shown in SEQ ID NO: 67, selected from the group consisting of Tau aa 393-401, comprising a phosphorylated Ser at position 396 (pS396), Tau aa 396-401 comprising a phosphorylated Ser at position 396 (pS396), Tau aa 394-400 comprising a phosphorylated Ser at position 396 (pS396), Tau aa 402-406 comprising a phosphorylated Ser at position 404 (pS404) and Tau aa 393-400 comprising a phosphorylated Ser at position 396 (pS396).

In one embodiment (67), said epitope consists of Tau aa 393-401, comprising a phosphorylated Ser at position 396 (pS396).

In one embodiment (68), said epitope consists of Tau aa 396-401 comprising a phosphorylated Ser at position 396 (pS396).

In one embodiment (69), said epitope consists of Tau aa 394-400 comprising a phosphorylated Ser at position 396 (pS396).

In one embodiment (70), said epitope consists of Tau aa 402-406 comprising a phosphorylated Ser at position 404 (pS404).

In one embodiment (71), said epitope consists of Tau aa 393-400 comprising a phosphorylated Ser at position 396 (pS396).

In another embodiment (72), the invention relates to a cell line producing a binding peptide or an active fragment thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof according to any one of the preceding embodiments.

In one embodiment (73), the invention relates to a cell line, which is hybridoma cell line A4-4A6-48 deposited on August 30, 2011 as DSM ACC3136.

In one embodiment (74), the invention relates to a cell line, which is hybridoma cell line A6-2G5-30 deposited on August 30, 2011 as DSM ACC3137.

In one embodiment (75), the invention relates to a cell line, which is hybridoma cell line A6-2G5-41 deposited on August 30, 2011 as DSM ACC3138.

In one embodiment (76), the invention relates to a cell line, which is hybridoma cell line A4-2A1-18 deposited on August 30, 2011 as DSM ACC3139.

In one embodiment (77), the invention relates to a cell line, which is hybridoma cell line A4-2A1-40 deposited on August 30, 2011 as DSM ACC3140.

In one embodiment (78), the invention relates to a cell line, which is hybridoma cell line A6-1D2-12 deposited on September 6, 2011 as DSM ACC3141.

In one embodiment (79), the invention relates to a monoclonal antibody or a functional part thereof comprising a light chain (VL) and/or a heavy chain (VH) domain, which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line A4-2A1-18 deposited on August 30, 2011 as DSM ACC3139 using

a. a primer pair comprising a 5'-primer of SEQ ID NO: 149 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 120, 123, 124, 136, 137, 138, 139, and 140 and a 3'-primer selected from the group consisting of SEQ ID NOs: 131, 134, and 141-148, for amplification of a second binding domain.

In one embodiment (80), the invention relates to a monoclonal antibody or a functional part thereof comprising a light chain (VL) and/or a heavy chain (VH) domain, which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line A6-2G5-30 deposited on August 30, 2011 as DSM ACC3137 using

a. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 51 and 169-174 and a 3'-primer of SEQ ID NO: 51, for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 124, 127, and 150-158 and a 3'-primer selected from the group consisting of SEQ ID NOs: 130, and 159-168, for amplification of a second binding domain.

In one embodiment (81), the invention relates to a monoclonal antibody or a functional part thereof comprising a light chain (VL) and/or a heavy chain (VH) domain, which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line A4-2A1-40 deposited on August 30, 2011 as DSM ACC3140 using

a. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 178, 179 and 180 and a 3'-primer of SEQ ID NO: 51, for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 121, 127, 139, 154, 155, and 175 and a 3'-primer selected from the group

consisting of SEQ ID NOs: 128, 129, 147, 176, and 177, for amplification of a second binding domain.

In one embodiment (82), the invention relates to a monoclonal antibody or a functional part thereof comprising a light chain (VL) and/or a heavy chain (VH) domain, which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line A6-2G5-41 deposited on August 30, 2011 as DSM ACC3138 using

a. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 51 and 188-192 and a 3'-primer of SEQ ID NO: 51, for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 120, 124, 126, 181, 182 and 183 and a 3'-primer selected from the group consisting of SEQ ID NOs: 144, 145 and 184-187, for amplification of a second binding domain.

In one embodiment (83), the invention relates to a monoclonal antibody or a functional part thereof comprising a light chain (VL) and/or a heavy chain (VH) domain, which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line A4-4A6-48 deposited on August 30, 2011 as DSM ACC3136 using

a. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 50 and 201-204 and a 3'-primer of SEQ ID NO: 51, for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 121, 137, 151 and 193-197 and a 3'-primer selected from the group consisting of SEQ ID NOs: 131, 141, 144, 166, 198, 199 and 200, for amplification of a second binding domain.

In one embodiment (84), the invention relates to a monoclonal antibody or a functional part thereof comprising a light chain (VL) and/or a heavy chain (VH) domain, which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line A6-1D2-12 deposited on September 6, 2011 as DSM ACC3141 using

a. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 209-214, and 219-221 a 3'-primer of SEQ ID NO: 215, for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 216, 217 and 218 and a 3'-primer of SEQ ID NOs: 208, for amplification of a second binding domain.

In one embodiment (85), the antibody according to any one of the preceding embodiments may be a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a humanized antibody, a human antibody, a camelid antibody, a diabody, or a modified or engineered antibody.

In one embodiment (86), the invention provides a method for producing the binding peptides or antibodies of any one of the preceding embodiments, comprising the step of culturing the cell line of any of the preceding embodiments in a suitable cultivation medium and, optionally, purifying the binding peptides or antibody from the cell line or cultivation medium.

In another embodiment (87), the present invention provides a method of detecting phosphoTau (pTau) multimers in a brain sample comprising

a. bringing the sample into contact with an antibody or a fragment thereof according to any one of the preceding claims, which peptide or fragment thereof binds an epitope of the phosphoTau protein;
b. allowing the antibody to bind to the tau protein to form an immunological complex;
c. detecting the formation of the immunological complex, particularly by applying an ELISA assay.

In particular, the invention relates in a specific embodiment (88) to a method of *post mortem* detection of phosphoTau (pTau) multimers in brain homogenates from a subject suspected to suffer from a tau-associated disease or disorder and from a healthy control subject comprising

a. bringing a sample of brain homogenates from both subjects into contact with an antibody or a fragment thereof according to any one of the preceding claims, which peptide or fragment thereof binds an epitope of the phosphoTau protein;
b. allowing the antibody to bind to the tau protein to form an immunological complex;
c. detecting the formation of the immunological complex, particularly by applying an ELISA assay and
d. comparing the amount or intensity of the immunological complex in the sample obtained from the subject suspected to suffer from a tau-associated disease or to that of the control sample,

wherein an increase in the amount or intensity of said immunological complex compared to the control value indicates that said patient had suffered from a minimal residual disease.

In one embodiment (89), the increase observed in the test sample compared to the control sample is between 30% and 50%, particularly between 35% and 45%.

In one embodiment (90) the invention provides an a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, according to any one of the preceding embodiment, which antibody or fragment shows a favourable pK profile. In particular, said antibody or fragment has a hight serum concentration up to 10-day post administration, which indicates a pharmacokinetic (PK) profile that favorably supports the use of said antibodies as a therapeutic antibody. (Deng et al., Expert Opin Drug Metab Toxicol, 2012, 8(2) 141-60; Putman et al., Trends Biotech, 2010 (28) 509-516 ; Bai, S. Clin Pharmacokinet 2012; 51 (2): 119-135.

Brief Description of Figures and Sequences

FIGURES

Figure 1 shows results of detection of phosphorylated Tau multimers by ACI-35-2A1-Ab1 (left panel), ACI-35-2G5-Ab3 (middle panel), and a control antibody (HT7; right panel) in human brain homogenates from control and AD subjects.

Figure 2 (2A and 2 B) shows results detection of total and p-Tau by commercial antibodies in human brain homogenates.

Figure 3 (3A, 3B, 3C) shows Detection of phospho-Tau by ACI-35-2A1-Ab1 (A), ACI-35-1D2-Ab1 (B), and ACI-35-2G5-Ab3 (C) in human brain homogenates.

Figure 4 (4A, 4B, 4C) shows results of detection of Tau-pS396 in human AD and control (Ctrl) brain by ACI-35-2A1-Ab1 (A), ACI-35-1D2-Ab1 (B), and ACI-35-2G5-Ab3 (C) antibodies using AlphaLISA. ****$p<0.0001$, **$p<0.01$ by Mann-Whitney test.

Figure 5 (5A and 5B) shows results of Tau-pT231 (AT180) IHC staining in the amygdala (A) and hippocampus (B), following treatment of Tau transgenic mice with ACI-35-2G5-Ab3.

Figure 6 (6A and 6B) shows results of total Tau (HT7) IHC staining in the amygdala (A) and hippocampus (B) , following treatment of Tau transgenic mice with ACI-35-2G5-Ab3.

Figure 7 shows an SDS-PAGE for Tau-pS396 generated using different GSK3β conditions, and the membrane blotted using the ACI-35-2G5-Ab3 antibody.

Figure 8 shows specific AlphaLISA assay setup using ACI-35-2G5-Ab3-BT and Tau-13 antibodies.

Figure 9 shows detection of Tau-pS396 in human S1 brain fraction from one AD donor; comparison of signal obtained from samples enriched by IP and non-IP samples.

Figure 10 shows results of detection of Tau-pS396 in human AD and control (Ctrl) CSF by ACI-35-2G5-Ab3 antibody using IP followed by AlphaLISA. *** p=0.0003 by Mann-Whitney test.

SEQUENCES

SEQ ID NO: 46 – 57 depicts the nucleotide sequences of VH/VK forward and reverse primers.

SEQ ID NO: 62 depicts the amino acid sequence of Tau antigen, peptide T3 (see Table 1).

SEQ ID NO: 67 depicts the amino acid sequence of longest isoform of human tau (441 aa) also called Tau40.

SEQ ID NO: 68 depicts the amino acid sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-35-4A6-Ab1 produced by hybridoma cell line A4-4A6-18.

SEQ ID NO: 69 depicts the amino acid sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-4A6-Ab1 produced by hybridoma cell line A4-4A6-18.

SEQ ID NO: 70 depicts the amino acid sequence of the CDR1 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-4A6-Ab1.

SEQ ID NO: 71 depicts the amino acid sequence of the CDR2 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-4A6-Ab1.

SEQ ID NO: 72 depicts the amino acid sequence of the CDR3 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-4A6-Ab1.

SEQ ID NO: 73 depicts the amino acid sequence of the CDR1 of the light chain variable region (VK) of monoclonal antibody ACI-35-4A6-Ab1.

SEQ ID NO: 74 depicts the amino acid sequence of the CDR2 of the light chain variable region (VK) of monoclonal antibody ACI-35-4A6-Ab1.

SEQ ID NO: 75 depicts the amino acid sequence of the CDR3 of the light chain variable region (VK) of monoclonal antibody ACI-35-4A6-Ab1.

SEQ ID NO: 76 depicts the amino acid sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-35-1D2-Ab1 produced by hybridoma cell line A6-1D2-12.

SEQ ID NO: 77 depicts the amino acid sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-1D2-Ab1 produced by hybridoma cell line A6-1D2-12.

SEQ ID NO: 78 depicts the amino acid sequence of the CDR1 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-1D2-Ab1.

SEQ ID NO: 79 depicts the amino acid sequence of the CDR2 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-1D2-Ab1.

SEQ ID NO: 80 depicts the amino acid sequence of the CDR3 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-1D2-Ab1.

SEQ ID NO: 81 depicts the amino acid sequence of the CDR1 of the light chain variable region (VK) of monoclonal antibody ACI-35-1D2-Ab1.

SEQ ID NO: 82 depicts the amino acid sequence of the CDR2 of the light chain variable region (VK) of monoclonal antibody ACI-35-1D2-Ab1.

SEQ ID NO: 83 depicts the amino acid sequence of the CDR3 of the light chain variable region (VK) of monoclonal antibody ACI-35-1D2-Ab1.

SEQ ID NO: 84 depicts the nucleotide sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-35-4A6-Ab1 produced by hybridoma cell line A4-4A6-18.

SEQ ID NO: 85 depicts the nucleotide sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-4A6-Ab1 produced by hybridoma cell line A4-4A6-18.

SEQ ID NO: 86 depicts the nucleotide sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-35-1D2-Ab1 produced by hybridoma cell line A6-1D2-12.

SEQ ID NO: 87 depicts the nucleotide sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-1D2-Ab1 produced by hybridoma cell line A6-1D2-12.

SEQ ID NO: 88 depicts the amino acid sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2A1-Ab1, ACI-35-2A1-Ab2, and ACI-35-4A6-Ab2, respectively, produced by hybridoma cell line A4-2A1-18, A4-2A1-40 and A4-4A6-48, respectively.

SEQ ID NO: 89 depicts the amino acid sequence of the CDR1 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2A1-Ab1, ACI-35-2A1-Ab2, ACI-35-4A6-Ab2, ACI-35-2G5-AB2 and ACI-35-2G5-AB3, respectively.

SEQ ID NO: 90 depicts the amino acid sequence of the CDR2 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2A1-Ab1, ACI-35-2A1-Ab2, and ACI-35-4A6-Ab2, respectively.

SEQ ID NO: 91 depicts the amino acid sequence of the CDR3 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2A1-Ab1, ACI-35-2A1-Ab2, ACI-35-4A6-Ab2, ACI-35-2G5-AB2 and ACI-35-2G5-AB3, respectively.

SEQ ID NO: 92 depicts the amino acid sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-2A1-Ab2 produced by hybridoma cell line A4-2A1-40

SEQ ID NO: 93 depicts the amino acid sequence of the CDR1 of the light chain variable region (VK) of monoclonal antibody ACI-35-2A1-Ab2.

SEQ ID NO: 94 depicts the amino acid sequence of the CDR2 of the light chain variable region (VK) of monoclonal antibody ACI-35-2A1-Ab2.

SEQ ID NO: 95 depicts the amino acid sequence of the CDR3 of the light chain variable region (VK) of monoclonal antibody ACI-35-2A1-Ab2.

SEQ ID NO: 96 depicts the amino acid sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2G5-Ab1 produced by hybridoma cell line A6-2G5-08.

SEQ ID NO: 97 depicts the amino acid sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-2G5-Ab1 produced by hybridoma cell line A6-2G5-08.

SEQ ID NO: 98 depicts the amino acid sequence of the CDR1 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2G5-Ab1.

SEQ ID NO: 99 depicts the amino acid sequence of the CDR2 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2G5-Ab1.

SEQ ID NO: 100 depicts the amino acid sequence of the CDR3 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2G5-Ab1.

SEQ ID NO: 101 depicts the amino acid sequence of the CDR1 of the light chain variable region (VK) of monoclonal antibody ACI-35-2G5-Ab1.

SEQ ID NO: 102 depicts the amino acid sequence of the CDR2 of the light chain variable region (VK) of monoclonal antibody ACI-35-2G5-Ab1.

SEQ ID NO: 103 depicts the amino acid sequence of the CDR3 of the light chain variable region (VK) of monoclonal antibody ACI-35-2G5-Ab1.

SEQ ID NO: 104 depicts the amino acid sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2G5-AB2 and ACI-35-2G5-AB3, respectively, produced by hybridoma cell line A6-2G5-30 and A6-2G5-41, respectively.

SEQ ID NO: 105 depicts the amino acid sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-2G5-AB2 and ACI-35-2G5-AB3, respectively, produced by hybridoma cell line A6-2G5-30 and A6-2G5-41, respectively.

SEQ ID NO: 106 depicts the amino acid sequence of the CDR1 of the light chain variable region (VK) of monoclonal antibody ACI-35-2G5-AB2 and ACI-35-2G5-AB3, respectively.

SEQ ID NO: 107 depicts the amino acid sequence of the CDR2 of the light chain variable region (VK) of monoclonal antibody ACI-35-2G5-AB2 and ACI-35-2G5-AB3, respectively.

SEQ ID NO: 108 depicts the amino acid sequence of the CDR3 of the light chain variable region (VK) of monoclonal antibody ACI-35-2G5-AB2 and ACI-35-2G5-AB3, respectively.

SEQ ID NO: 109 depicts the nucleotide sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2A1-Ab1, ACI-35-2A1-Ab2, and ACI-35-4A6-Ab2, respectively, produced by hybridoma cell line A4-2A1-18, A4-2A1-40 and A4-4A6-48, respectively.

SEQ ID NO: 110 depicts the nucleotide sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-2A1-Ab2 produced by hybridoma cell line A4-2A1-40.

SEQ ID NO: 111 depicts the nucleotide sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2G5-AB1 produced by hybridoma cell line A6-2G5-08.

SEQ ID NO: 112 depicts the nucleotide sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-2G5-AB1 produced by hybridoma cell line A6-2G5-08.

SEQ ID NO: 113 depicts the nucleotide sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2G5-AB2 and ACI-35-2G5-AB3, respectively, produced by hybridoma cell line A6-2G5-30 and A6-2G5-41, respectively.

SEQ ID NO: 114 depicts the nucleotide sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-2G5-AB2 and ACI-35-2G5-AB3, respectively, produced by hybridoma cell line A6-2G5-30 and A6-2G5-41, respectively.

SEQ ID NO: 115 depicts the amino acid sequence of the CDR2 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2G5-AB2 and ACI-35-2G5-AB3.

SEQ ID NO: 116 depicts the amino acid sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-2A1-Ab1produced by hybridoma cell line A4-2A1-18.

SEQ ID NO: 117 depicts the nucleotide sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-2A1-Ab1 produced by hybridoma cell line A4-2A1-18.

SEQ ID NO: 118 depicts the amino acid sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-4A6-Ab2 produced by hybridoma cell line A4-4A6-48.

SEQ ID NO: 119 depicts the nucleotide sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-4A6-Ab2 produced by hybridoma cell line A4-4A6-48.

SEQ ID NO: 120 – 221 depicts the nucleotide sequences of VH/VK forward and reverse primers.

Definition of Terms

The terms "polypeptide", "peptide", and "protein", as used herein, are interchangeably and are defined to mean a biomolecule composed of amino acids linked by a peptide bond.

The term "peptides," or "binding peptide" are used herein interchangeably and refer to chains of amino acids (typically L-amino acids) whose alpha carbons are linked through peptide bonds formed by a condensation reaction between the carboxyl group of the alpha carbon of one amino acid and the amino group of the alpha carbon of another amino acid. The terminal amino acid at one end of the chain (i.e., the amino terminal) has a free amino group, while the terminal amino acid at the other end of the chain (i.e., the carboxy terminal) has a free carboxyl group. As such, the term "amino terminus" (abbreviated N-terminus) refers to the free alpha-amino group on the amino acid at the amino terminal of the peptide, or to the alpha-amino group (imino group when participating in a peptide bond) of an amino acid at any other location within the peptide. Similarly, the term "carboxy terminus" (abbreviated C-terminus) refers to the free carboxyl group on the amino acid at the carboxy terminus of a peptide, or to the carboxyl group of an amino acid at any other location within the peptide. A binding peptide may constitutes antibodies such as polyclonal or monoclonal antibodies, human or humanized antibodies, diabodies, camelid antibodies, etc, or functional parts thereof as defined herein.

The terms "fragment thereof" or "fragment" as used herein in the context of a peptide refer to a functional peptide fragment which has essentially the same (biological) activity as an intact peptide defined herein. The terms when used herein in the context of an antibody refers to an antibody fragment comprising a portion of an intact antibody that contains an antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; single-chain antibody molecules, including single-chain Fv (scFv) molecules; and bispecific and multispecific antibodies and/or antibody fragments.

Typically, the amino acids making up a peptide are numbered in order, starting at the amino terminal and increasing in the direction toward the carboxy terminal of the peptide. Thus, when one amino acid is said to "follow" another, that amino acid is positioned closer to the carboxy terminal of the peptide than the preceding amino acid.

The term "residue" is used herein to refer to an amino acid that is incorporated into a peptide by an amide bond. As such, the amino acid may be a naturally occurring amino acid or, unless otherwise limited, may encompass known analogs of natural amino acids that function in a manner similar to the naturally occurring amino acids (i.e., amino acid mimetics). Moreover, an amide bond mimetic includes peptide backbone modifications well known to those skilled in the art.

The phrase "consisting essentially of" is used herein to exclude any elements that would substantially alter the essential properties of the peptides to which the phrase refers. Thus, the description of a peptide "consisting essentially of . . ." excludes any amino acid

substitutions, additions, or deletions that would substantially alter the biological activity of that peptide.

Furthermore, one of skill will recognize that, as mentioned above, individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids (typically less than 5%, more typically less than 1%) in an encoded sequence are conservatively modified variations where the alterations result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. The following six groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Serine (S), Threonine (T);
2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

The phrases "isolated" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany it as found in its native state. Thus, the peptides described herein do not contain materials normally associated with their in situ environment. Typically, the isolated, immunogenic peptides described herein are at least about 80% pure, usually at least about 90%, and preferably at least about 95% as measured by band intensity on a silver stained gel.

Protein purity or homogeneity may be indicated by a number of methods well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualization upon staining. For certain purposes high resolution will be needed and HPLC or a similar means for purification utilized.

When the immunogenic peptides are relatively short in length (i.e., less than about 50 amino acids), they are often synthesized using standard chemical peptide synthesis techniques.

Solid phase synthesis in which the C-terminal amino acid of the sequence is attached to an insoluble support followed by sequential addition of the remaining amino acids in the sequence is a preferred method for the chemical synthesis of the immunogenic peptides described herein. Techniques for solid phase synthesis are known to those skilled in the art.

Alternatively, the immunogenic peptides described herein are synthesized using recombinant nucleic acid methodology. Generally, this involves creating a nucleic acid sequence that encodes the peptide, placing the nucleic acid in an expression cassette under the control of a particular promoter, expressing the peptide in a host, isolating the expressed peptide or

polypeptide and, if required, renaturing the peptide. Techniques sufficient to guide one of skill through such procedures are found in the literature.

Once expressed, recombinant peptides can be purified according to standard procedures, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like. Substantially pure compositions of about 50 % to 95 % homogeneity are preferred, and 80 % to 95 % or greater homogeneity is most preferred for use as therapeutic agents.

One of skill in the art will recognize that after chemical synthesis, biological expression or purification, the immunogenic peptides may possess a conformation substantially different than the native conformations of the constituent peptides. In this case, it is often necessary to denature and reduce the antiproliferative peptide and then to cause the peptide to re-fold into the preferred conformation. Methods of reducing and denaturing proteins and inducing re-folding are well known to those of skill in the art.

Antigenicity of the purified protein may be confirmed, for example, by demonstrating reaction with immune serum, or with antisera produced against the protein itself.

The terms "a", "an" and "the" as used herein are defined to mean "one or more" and include the plural unless the context is inappropriate.

The terms "detecting" or "detected" as used herein mean using known techniques for detection of biologic molecules such as immunochemical or histological methods and refer to qualitatively or quantitatively determining the presence or concentration of the biomolecule under investigation.

By “isolated” is meant a biological molecule free from at least some of the components with which it naturally occurs.

The terms "antibody" or “antibodies" or “functional parts thereof" as used herein is an art recognized term and is understood to refer to molecules or active fragments of molecules that bind to known antigens, particularly to immunoglobulin molecules and to immunologically active portions of immunoglobulin molecules, i.e molecules that contain a binding site that immunospecifically binds an antigen. The immunoglobulin according to the invention can be of any type (IgG, IgM, IgD, IgE, IgA and IgY) or class (IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclasses of immunoglobulin molecule.

“Antibodies" are intended within the scope of the present invention to include monoclonal antibodies, polyclonal, chimeric, single chain, bispecific, simianized, human and humanized antibodies, camelid antibodies, diabodies, as well as functional parts or active fragments thereof. Examples of active fragments of molecules that bind to known antigens include Fab and $F(ab')_2$ fragments, including the products of a Fab immunoglobulin expression library and epitope-binding fragments of any of the antibodies and fragments mentioned above.

These active fragments can be derived from an antibody of the present invention by a number of techniques. For example, purified monoclonal antibodies can be cleaved with an enzyme, such as pepsin, and subjected to HPLC gel filtration. The appropriate fraction containing Fab fragments can then be collected and concentrated by membrane filtration and the like. For further description of general techniques for the isolation of active fragments of antibodies, see for example, Khaw, B. A. et al. J. Nucl. Med. 23:1011-1019 (1982); Rousseaux et al. Methods Enzymology, 121:663-69, Academic Press, (1986).

A "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one (or more) human immunoglobulin(s).

A humanized antibody may further refer to an antibody having a variable region where one or more of its framework regions have human or primate amino acids. In addition, framework support residues may be altered to preserve binding affinity. Methods to obtain "humanized antibodies" are well known to those skilled in the art. (see, e.g., Queen et al., Proc. Natl Acad Sci USA, 86:10029-10032 (1989), Hodgson et al., Bio/Technoloy, 9:421 (1991)).

A "humanized antibody" may also be obtained by a novel genetic engineering approach that enables production of affinity-matured humanlike polyclonal antibodies in large animals such as, for example, rabbits (http://www.rctech.com/bioventures/therapeutic.php).

The term "fully human antibody" or "human" antibody is meant to refer to an antibody derived from transgenic mice carrying human antibody genes or from human cells. To the human immune system, however, the difference between "fully human", "human", and "humanized" antibodies may be negligible or nonexistent and as such all three may be of equal efficacy and safety.

The term "monoclonal antibody" is also well recognized in the art and refers to an antibody that is mass produced in the laboratory from a single clone and that recognizes only one antigen. Monoclonal antibodies are typically made by fusing a normally short-lived, antibody-producing B cell to a fast-growing cell, such as a cancer cell (sometimes referred to as an "immortal" cell). The resulting hybrid cell, or hybridoma, multiplies rapidly, creating a clone that produces large quantities of the antibody.

The term "antigen" refers to an entity or fragment thereof which can induce an immune response in an organism, particularly an animal, more particularly a mammal including a human. The term includes immunogens and regions responsible for antigenicity or antigenic determinants.

As used herein, the term "soluble" means partially or completely dissolved in an aqueous solution.

Also as used herein, the term "immunogenic" refers to substances which elicit or enhance the production of antibodies, T-cells and other reactive immune cells directed against an immunogenic agent and contribute to an immune response in humans or animals.

An immune response occurs when an individual produces sufficient antibodies, T-cells and other reactive immune cells against administered immunogenic compositions of the present invention to moderate or alleviate the disorder to be treated.

The term "hybridoma" is art recognized and is understood by those of ordinary skill in the art to refer to a cell produced by the fusion of an antibody-producing cell and an immortal cell, e.g. a multiple myeloma cell. This hybrid cell is capable of producing a continuous supply of antibody. See the definition of "monoclonal antibody" above and the Examples below for a more detailed description of the method of fusion.

The term "carrier" as used herein means a structure in which antigenic peptide or supramolecular construct can be incorporated into or can be associated with, thereby presenting or exposing antigenic peptides or part of the peptide to the immune system of a human or animal. Any particle that can be suitably used in animal or human therapy such as, for example, a vesicle, a particle or a particulate body may be used as a carrier within the context of the present invention.

The term "carrier" further comprises methods of delivery wherein supramolecular antigenic construct compositions comprising the antigenic peptide may be transported to desired sites by delivery mechanisms. One example of such a delivery system utilizes colloidal metals such as colloidal gold.

Carrier proteins that can be used in the supramolecular antigenic construct compositions of the present invention include, but are not limited to, maltose binding peptide "MBP"; bovine serum albumin "BSA"; keyhole lympet hemocyanin "KLH"; ovalbumin; flagellin; thyroglobulin; serum albumin of any species; gamma globulin of any species; syngeneic cells; syngeneic cells bearing Ia antigens; and polymers of D- and/or L- amino acids.

Further, the term "therapeutically effective amount" or "pharmaceutically effective amount" refers to the amount of binding peptide which, when administered to a human or animal, is sufficient to result in a therapeutic effect in said human or animal. The effective amount is readily determined by one of ordinary skill in the art following routine procedures.

"pTau PHF", "PHF", and "paired helical filaments" are used herein synonymously and refer to pairs of approximately 10 nm filaments wound into helices with a periodicity of 160 nm visible on electron microscopy. Width varies between 10 and 22 nm. PHF are the predominant structures in neurofibrillary tangles of Alzheimer's Disease (AD) and neuropil threads. PHF may also be seen in some but not all dystrophic neurites associated with neuritic plaques. The major component of PHF is a hyperphosphorylated form of microtubule-associated

protein tau. PHF are composed of disulfide-linked antiparallel hyper-phosphorylated tau proteins. PHF tau may be truncated of its C-terminal 20 amino acid residues. The mechanisms underlying PHF formation are uncertain but hyper- phosphorylation of tau may disengage it from microtubules, increasing the soluble pool of tau.

Within the scope of the present invention, it was demonstrated that the antibody induced response to the antigenic composition according to the invention is largely T-cell independent. A nude mouse model was used in this respect and nude mice were vaccinated and antibody responses measured to evaluate the Aβ-specific antibody response induced by the antigenic composition according to the invention in the immunized nude mice. The nude mice carry the Foxn1nu mutation and as a consequence, have reduced T-cell function due to the lack of a proper thymus.

A "pharmaceutically effective amount" as used herein refers to a dose of the active ingredient in a pharmaceutical composition adequate to cure, or at least partially arrest, the symptoms of the disease, disorder or condition to be treated or any complications associated therewith.

The present invention provides binding peptides recognizing and binding to major pathological phospho-epitopes of the tau protein. In particular, the present invention provides specific antibodies against linear and conformational, simple and complex phospho-epitopes on protein tau that are believed to be responsible for synapto- and neuro-toxicity in tauopathies, including AD.

Accordingly, the present invention relates in one embodiment to a binding peptide or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes, wherein said binding peptide or antibody has a high binding affinity with a dissociation constant of at least 10 nM, particularly of at least 8 nM, particularly of at least 5 nM, particularly of at least 2 nM, particularly of at least 1 nM, particularly of at least 500 pM, particularly of at least 400 pM particularly of at least 300 pM, particularly of at least 200 pM, particularly of at least 100 pM, particularly of at least 50 pM.

"Soluble Tau" protein as used herein refers to proteins consisting of both completely solubilized Tau protein/peptide monomers or of Tau-like peptides/proteins, or of modified or truncated Tau peptides/proteins or of other derivates of Tau peptides/proteins monomers, and of Tau protein oligomers. "Soluble Tau" excludes particularly neurofibrillary tangles (NFT).

"Insoluble Tau" as used herein refers to multiple aggregated monomers of Tau peptides or proteins, or of Tau-like peptides/proteins, or of modified or truncated Tau peptides/proteins or of other derivates of Tau peptides/proteins forming oligomeric or polymeric structures which are insoluble both *in vitro* in aqueous medium and *in vivo* in the mammalian or human body more particularly in the brain, but particularly to multiple aggregated monomers of Tau or of modified or truncated Tau peptides/proteins or of derivatives thereof, which are insoluble in the mammalian or human body more particularly in the brain, respectively. "Insoluble Tau" particularly includes neurofibrillary tangles (NFT).

""Monomeric Tau" or "Tau monomer" as used herein refers to completely solubilized Tau proteins without aggregated complexes in aqueous medium.

"Aggregated Tau", "oligomeric Tau" and "Tau oligomer" refer to multiple aggregated monomers of Tau peptides or proteins , or of Tau-like peptides/proteins, or of modified or truncated Tau peptides/proteins or of other derivates of Tau peptides/proteins forming oligomeric or polymeric structures which are insoluble or soluble both in vitro in aqueous medium and in vivo in the mammalian or human body more particularly in the brain, but particularly to multiple aggregated monomers of Tau or of modified or truncated Tau peptides/proteins or of derivatives thereof, which are insoluble or soluble in the mammalian or human body more particularly in the brain, respectively."

A "modulating antibody " refers to an antibody or a functional fragment thereof as described herein in the various embodiments, which may either up-regulate (e.g., activate or stimulate), down-regulate (e.g., inhibit or suppress) or otherwise change a functional property, biological activity or level of soluble and/or insoluble and/or oligomeric Tau protein, particularly of soluble phosphorylated tau protein, *in vivo,* particularly in the brain, particularly in the brain cortex and/or hippocampus, of an animal, particularly a mammal or a human containing increased levels of soluble tau protein and/or soluble phosphorylated tau protein. A modulating antibody or functional fragment thereof may act to modulate a tau protein or a polypeptide encoding said tau protein either directly or indirectly. In certain embodiments, a modulating antibody or functional fragment thereof reduces the levels of soluble and/or insoluble and/or oligomeric, particularly soluble and insoluble tau protein, particularly soluble and insoluble and oligomeric tau protein. In one aspect, the soluble and/or insoluble and/or oligomeric tau protein is phosphorylated tau protein, particularly soluble phosphorylated tau protein, in the brain, particularly in the brain cortex and/or hippocampus, of an animal, particularly a mammal or a human containing increased levels of tau protein and/or phosphorylated tau protein, particularly of soluble tau protein and/or soluble phosphorylated tau protein."

In one embodiment, the present invention provides a pharmaceutical composition comprising a binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide comprising a nucleic acid sequence encoding said binding peptide or antibody, according to any one of the embodiments described and claimed herein, or a combination thereof, in a therapeutically effective amount together with a pharmaceutically acceptable carrier.

Suitable pharmaceutical carriers, diluents and/or excipients are well known in the art and include, for example, phosphate buffered saline solutions, water, emulsions such as oil/water emulsions, various types of wetting agents, sterile solutions, etc.

The binding peptides according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, can be prepared in a physiologically acceptable formulation and may comprise a pharmaceutically acceptable carrier, diluent and/or excipient using known techniques. For example, the binding peptides according to the invention and as described herein including any functionally equivalent binding peptides or functional parts thereof, in particular, the monoclonal antibodies of the invention including any functionally equivalent antibodies or functional parts thereof, are combined with a pharmaceutically acceptable carrier, diluent and/or excipient to form a therapeutic composition. Suitable pharmaceutical carriers, diluents and/or excipients are well known in the art and include, for example, phosphate buffered saline solutions, water, emulsions such as oil/water emulsions, various types of wetting agents, sterile solutions, etc.

Formulation of the pharmaceutical composition according to the invention can be accomplished according to standard methodology know to those of ordinary skill in the art.

The compositions of the present invention may be administered to a subject in the form of a solid, liquid or aerosol at a suitable, pharmaceutically effective dose. Examples of solid compositions include pills, creams, and implantable dosage units. Pills may be administered orally. Therapeutic creams may be administered topically. Implantable dosage units may be administered locally, for example, at a tumor site, or may be implanted for systematic release of the therapeutic composition, for example, subcutaneously. Examples of liquid compositions include formulations adapted for injection intramuscularly, subcutaneously, intravenously, intra-arterially, and formulations for topical and intraocular administration. Examples of aerosol formulations include inhaler formulations for administration to the lungs.

The compositions may be administered by standard routes of administration. In general, the composition may be administered by topical, oral, rectal, nasal, interdermal, intraperitoneal, or parenteral (for example, intravenous, subcutaneous, or intramuscular) routes.

In addition, the composition may be incorporated into sustained release matrices such as biodegradable polymers, the polymers being implanted in the vicinity of where delivery is

desired, for example, at the site of a tumor. The method includes administration of a single dose, administration of repeated doses at predetermined time intervals, and sustained administration for a predetermined period of time.

A sustained release matrix, as used herein, is a matrix made of materials, usually polymers which are degradable by enzymatic or acid/base hydrolysis or by dissolution. Once inserted into the body, the matrix is acted upon by enzymes and body fluids. The sustained release matrix desirably is chosen by biocompatible materials such as liposomes, polylactides (polylactide acid), polyglycolide (polymer of glycolic acid), polylactide co-glycolide (copolymers of lactic acid and glycolic acid), polyanhydrides, poly(ortho)esters, polypeptides, hyaluronic acid, collagen, chondroitin sulfate, carboxylic acids, fatty acids, phospholipids, polysaccharides, nucleic acids, polyamino acids, amino acids such phenylalanine, tyrosine, isoleucine, polynucleotides, polyvinyl propylene, polyvinylpyrrolidone and silicone. A preferred biodegradable matrix is a matrix of one of either polylactide, polyglycolide, or polylactide co-glycolide (co-polymers of lactic acid and glycolic acid).

It is well known to those of ordinary skill in the pertinent art that the dosage of the composition will depend on various factors such as, for example, the condition of being treated, the particular composition used, and other clinical factors such as weight, size, sex and general health condition of the patient, body surface area, the particular compound or composition to be administered, other drugs being administered concurrently, and the route of administration.

The composition according to the invention may be administered in combination with other compositions comprising an biologically active substance or compound such as, for example, a known compound used in the medication of tauopathies and/or of amyloidoses, a group of diseases and disorders associated with amyloid or amyloid-like protein such as the amyloid β protein involved in Alzheimer's Disease.

The other biologically active substance or compound may exert its biological effect by the same or a similar mechanism as the therapeutic vaccine according to the invention or by an unrelated mechanism of action or by a multiplicity of related and/or unrelated mechanisms of action.

Generally, the other biologically active compound may include neutron-transmission enhancers, psychotherapeutic drugs, acetylcholine esterase inhibitors, calcium-channel blockers, biogenic amines, benzodiazepine tranquillizers, acetylcholine synthesis, storage or release enhancers, acetylcholine postsynaptic receptor agonists, monoamine oxidase-A or –B inhibitors, N-methyl-D-aspartate glutamate receptor antagonists, non-steroidal anti-inflammatory drugs, antioxidants, and serotonergic receptor antagonists.

In particular, the biologically active agent or compound may comprise at least one compound selected from the group consisting of compounds against oxidative stress, anti-apoptotic compounds, metal chelators, inhibitors of DNA repair such as pirenzepin and metabolites, 3-amino-1-propanesulfonic acid (3APS), 1,3-propanedisulfonate (1,3PDS), secretase activators, [beta]- and 7-secretase inhibitors, tau proteins, neurotransmitter, /3-sheet breakers, antiinflammatory molecules, "atypical antipsychotics" such as, for example clozapine, ziprasidone, risperidone, aripiprazole or olanzapine or cholinesterase inhibitors (ChEIs) such as tacrine, rivastigmine, donepezil, and/or galantamine and other drugs and nutritive supplements such as, for example, vitamin B 12, cysteine, a precursor of acetylcholine, lecithin, choline, Ginkgo biloba, acyetyl-L-carnitine, idebenone, propentofylline, or a xanthine derivative, together with a binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, and, optionally, a pharmaceutically acceptable carrier and/or a diluent and/or an excipient and instructions for the treatment of diseases.

In a further embodiment, the composition according to the invention may comprise niacin or memantine together with a binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, and, optionally, a pharmaceutically acceptable carrier and/or a diluent and/or an excipient.

In still another embodiment of the invention compositions are provided that comprise “atypical antipsychotics” such as, for example clozapine, ziprasidone, risperidone, aripiprazole or olanzapine for the treatment of positive and negative psychotic symptoms including hallucinations, delusions, thought disorders (manifested by marked incoherence, derailment, tangentiality), and bizarre or disorganized behavior, as well as anhedonia, flattened affect, apathy, and social withdrawal, together with the binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, and, optionally, a pharmaceutically acceptable carrier and/or a diluent and/or an excipient.

Other compounds that can be suitably used in compositions in addition to the binding peptide according to the invention, are those disclosed, for example, in WO 2004/058258 (see especially pages 16 and 17) including therapeutic drug targets (page 36-39), alkanesulfonic acids and alkanolsulfuric acid (pages 39-51), cholinesterase inhibitors (pages 51-56), NMDA receptor antagonists (pages 56-58), estrogens (pages 58-59), non-steroidal anti-inflammatory drugs (pages 60-61), antioxidants (pages 61-62), peroxisome proliferators-activated receptors (PPAR) agonists (pages 63-67), cholesterol–lowering agents (pages 68-75); amyloid inhibitors (pages 75-77), amyloid formation inhibitors (pages 77-78), metal chelators (pages 78-79), anti-psychotics and anti-depressants (pages 80-82), nutritional

supplements (pages 83-89) and compounds increasing the availability of biologically active substances in the brain (see pages 89-93) and prodrugs (pages 93 and 94), which document is incorporated herein by reference, but especially the compounds mentioned on the pages indicated above.

Proteinaceous pharmaceutically active matter may be present in amounts between 1 ng and 10 mg per dose. Generally, the regime of administration should be in the range of between 0.1 µg and 10 mg of the antibody according to the invention, particularly in a range 1.0 µg to 1.0 mg, and more particularly in a range of between 1.0 µg and 100 µg, with all individual numbers falling within these ranges also being part of the invention. If the administration occurs through continuous infusion a more proper dosage may be in the range of between 0.01 µg and 10 mg units per kilogram of body weight per hour with all individual numbers falling within these ranges also being part of the invention.

Administration will generally be parenterally, e.g. intravenously or subcutaneously. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions and emulsions. Non-aqueous solvents include, without being limited to, propylene glycol, polyethylene glycol, vegetable oil such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous solvents may be chosen from the group consisting of water, alcohol/aqueous solutions, emulsions or suspensions including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose) and others. Preservatives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, inert gases, etc.

The pharmaceutical composition may further comprise proteinaceous carriers such as, for example, serum albumin or immunoglobulin, particularly of human origin. Further biologically active agents may be present in the pharmaceutical composition of the invention dependent on its the intended use.

When the binding target is located in the brain, certain embodiments of the invention provide for the binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, to traverse the blood-brain barrier. Certain neurodegenerative diseases are associated with an increase in permeability of the blood-brain barrier, such that the binding peptide according to the invention including antibodies, particularly monoclonal antibodies or active fragment thereof can be readily introduced to the brain. When the blood-brain barrier remains intact, several art-known approaches exist for transporting molecules across it, including, but not limited to, physical methods, lipid-based methods, and receptor and channel-based methods.

Physical methods of transporting the binding peptide according to the invention including antibodies, particularly monoclonal antibodies, or active fragment thereof across the blood-brain barrier include, but are not limited to, circumventing the blood-brain barrier entirely, or by creating openings in the blood-brain barrier. Circumvention methods include, but are not limited to, direct injection into the brain (see, e.g., Papanastassiou et al., Gene Therapy 9: 398-406 (2002)) and implanting a delivery device in the brain (see, e.g., Gill et al., Nature Med. 9: 589-595 (2003); and Gliadel Wafers(TM), Guildford Pharmaceutical). Methods of creating openings in the barrier include, but are not limited to, ultrasound (see, e.g., U.S. Patent Publication No. 2002/0038086), osmotic pressure (e.g., by administration of hypertonic mannitol (Neuwelt, E. A., Implication of the Blood-Brain Barrier and its Manipulation, Vols 1 & 2, Plenum Press, N. Y. (1989)), permeabilization by, e.g., bradykinin or permeabilizer A-7 (see, e.g., U.S. Patent Nos. 5,112,596, 5,268,164, 5,506,206, and 5,686,416), and transfection of neurons that straddle the blood-brain barrier with vectors containing genes encoding the binding peptide or antigen-binding fragment (see, e.g., U.S. Patent Publication No. 2003/0083299).

Lipid-based methods of transporting the binding peptide according to the invention including antibodies, particularly monoclonal antibodies, or an active fragment thereof across the blood-brain barrier include, but are not limited to, encapsulating the binding peptide according to the invention including antibodies, particularly monoclonal antibodies, or active fragment thereof in liposomes that are coupled to active fragments thereof that bind to receptors on the vascular endothelium of the blood-brain barrier (see, e.g., U.S. Patent Application Publication No. 20020025313), and coating the binding peptide according to the invention including antibodies, particularly monoclonal antibodies, or active fragment thereof in low-density lipoprotein particles (see, e.g., U.S. Patent Application Publication No. 20040204354) or apolipoprotein E (see, e.g., U.S. Patent Application Publication No. 20040131692).

Receptor and channel-based methods of transporting the binding peptide according to the invention including antibodies, particularly monoclonal antibodies, or active fragment thereof across the blood-brain barrier include, but are not limited to, using glucocorticoid blockers to increase permeability of the blood-brain barrier (see, e.g., U.S. Patent Application Publication Nos. 2002/0065259, 2003/0162695, and 2005/0124533); activating potassium channels (see, e.g., U.S. Patent Application Publication No. 2005/0089473), inhibiting ABC drug transporters (see, e.g., U.S. Patent Application Publication No. 2003/0073713); coating antibodies with a transferrin and modulating activity of the one or more transferrin receptors (see, e.g., U.S. Patent Application Publication No. 2003/0129186), and cationizing the antibodies (see, e.g., U.S. Patent No. 5,004,697).

Single or repeated administrations of the binding peptide according to the invention including antibodies, particularly monoclonal antibodies, or an active fragment thereof, or of a pharmaceutical composition according to the invention may be provided to a subject over an extended period of time. The duration of administration may be between 1 week and up to 12 month or more. During this time the binding peptide, antibody or pharmaceutical composition may be administerd once a week, once every two weeks, three weeks, four weeks, etc, or at a higher or lower frequency depending on the needs of the subject to be treated.

In a further embodiment the present invention provides methods and kits for the detection and diagnosis of tau-protein-associated diseases, disorders or conditions, including neurodegenerative diseases or disorders such as tauopathies comprising a heterogeneous group of neurodegenerative diseases or disorders including diseases or disorders which manifest both tau and amyloid pathologies including, but not limited to, Alzheimer's Disease, Creutzfeldt-Jacob disease, Dementia pugilistica, Down's Syndrome, Gerstmann-Sträussler-Scheinker disease, inclusion-body myositis, and prion protein cerebral amyloid angiopathy, traumatic brain injury and further of diseases or disorders which do not show a distinct amyloid pathology including, but not limited to, amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam, Non-Guamanian motor neuron disease with neurofibrillary tangles, argyrophilic grain dementia, corticobasal degeneration, diffuse neurofibrillary tangles with calcification, frontotemporal dementia with parkinsonism linked to chromosome 17, Hallevorden-Spatz disease, multiple system atrophy, Niemann-Pick disease, type C, Pallido-ponto-nigral degeneration, Pick's disease, progressive subcortical gliosis, progressive supranuclear palsy, Subacute sclerosing panencephalitis Tangle-only dementia, Postencephalitic Parkinsonism, Myotonic dystrophy. The pathological abnormalities may be caused by or associated with the formation of neurofibrillary lesions, the predominant brain pathology in tauopathy.

Further, the present invention provides methods and kits for diagnosing a predisposition to tau-protein-associated diseases, disorders or conditions, including neurodegenerative diseases or disorders such as tauopathies comprising a heterogeneous group of neurodegenerative diseases or disorders including diseases or disorders which show co-manifest both tau and amyloid pathologies, or for monitoring minimal residual disease in a patient or for predicting responsiveness of a patient to a treatment with a binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, or a composition according to the invention and as described herein. These methods include known immunological methods commonly used for detecting or quantifying substances in biological samples or in an in situ condition.

Diagnosis of a tau-protein-associated disease or condition or of a predisposition to an tau-protein-associated disease or condition in a subject in need thereof, particularly a mammal, more particularly a human, including neurodegenerative diseases or disorders such as tauopathies comprising a heterogeneous group of neurodegenerative diseases or disorders including diseases or disorders which manifest both of tau and amyloid pathologies, may be achieved by detecting the immunospecific binding of a binding peptide of the invention, particularly of an antibody, particularly of a monoclonal antibody or an active fragment thereof, to an epitope of the tau protein in a sample or in situ, which includes bringing the sample or a specific body part or body area suspected to contain the tau protein into contact with an antibody which binds an epitope of the tau protein, allowing the antibody to bind to the tau protein to form an immunologic complex, detecting the formation of the immunologic complex and correlating the presence or absence of the immunologic complex with the presence or absence of tau protein in the sample or specific body part or area, optionally comparing the amount of the immunologic complex to a normal control value, wherein an increase in the amount of the immunologic complex compared to a normal control value indicates that the subject is suffering from or is at risk of developing an tau protein-associated disease or condition.

Monitoring minimal residual disease in a subject, particularly a mammal, more particularly a human, following treatment with a binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, or a composition according to the invention may be achieved by detecting the immunospecific binding of a binding peptide of the invention, particularly of an antibody, particularly a monoclonal antibody or an active fragment thereof to an epitope of the tau protein in a sample or *in situ*, which includes bringing the sample or a specific body part or body area suspected to contain the tau protein into contact with a binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, which binds an epitope of the tau protein, allowing the binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, to bind to the tau protein to form an immunologic complex, detecting the formation of the immunologic complex and correlating the presence or absence of the immunologic complex with the presence or absence of tau protein in the sample or specific body part or area, optionally comparing the amount of said immunologic complex to a normal control value, wherein an increase in the amount of said immunologic complex compared to a normal control value indicates that the subject may still suffer from a minimal residual disease.

Predicting responsiveness of a subject, particularly a mammal, more particularly a human, to a treatment with a binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, or a composition according to the

invention may be achieved by detecting the immunospecific binding of a binding peptide, particularly of a monoclonal antibody or an active fragment thereof to an epitope of the tau protein in a sample or *in situ*, which includes bringing the sample or a specific body part or body area suspected to contain the tau protein into contact with a binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, which binds an epitope of the tau protein, allowing the binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, to bind to the tau protein to form an immunologic complex, detecting the formation of the immunologic complex and correlating the presence or absence of the immunologic complex with the presence or absence of tau protein in the sample or specific body part or area, optionally comparing the amount of said immunologic complex before and after onset of the treatment, wherein an decrease in the amount of said immunologic complex indicates that said patient has a high potential of being responsive to the treatment.

Biological samples that may be used in the diagnosis of a tau protein-associated disease or condition, for diagnosing a predisposition to a tau protein-associated disease or condition, including neurodegenerative diseases or disorders such as tauopathies comprising a heterogeneous group of neurodegenerative diseases or disorders including diseases or disorders which manifest both tau and amyloid pathologies, or for monitoring minimal residual disease in a patient or for predicting responsiveness of a patient to a treatment with a binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, or a composition according to the invention and as described herein are, for example, fluids such as serum, plasma, saliva, gastric secretions, mucus, cerebrospinal fluid, lymphatic fluid and the like or tissue or cell samples obtained from an organism such as neural, brain, cardiac or vascular tissue. For determining the presence or absence of the tau protein in a sample, any immunoassay known to those of ordinary skill in the art may be used such as, for example, assays which utilize indirect detection methods using secondary reagents for detection, ELISA's and immunoprecipitation and agglutination assays. A detailed description of these assays is, for example, given in Harlow and Lane, Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory, New York 1988 555-612, WO96/13590 to Maertens and Stuyver, Zrein et al. (1998) and WO96/29605.

For *in situ* diagnosis, the binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, of the invention or any active and functional part thereof may be administered to the organism to be diagnosed by methods known in the art such as, for example, intravenous, subcutaneous, intranasal, intraperitoneal, intracerebral, intraarterial injection such that a specific binding between an antibody according to the invention with an eptitopic region on the amyloid protein may

occur. The binding peptide/antigen complex may conveniently be detected through a label attached to the binding peptide according to the invention including antibodies, particularly monoclonal antibodies, or a functional fragment thereof or any other art- known method of detection.

The immunoassays used in diagnostic applications or in applications for diagnosing a predisposition to a tau protein-associated disease or condition, including neurodegenerative diseases or disorders such as tauopathies comprising a heterogeneous group of neurodegenerative diseases or disorders including diseases or disorders which manifest both tau and amyloid pathologies, or for monitoring minimal residual disease in a patient or for predicting responsiveness of a patient to a treatment with a binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, or a composition according to the invention and as described herein typically rely on labelled antigens, binding peptides, or secondary reagents for detection. These proteins or reagents can be labelled with compounds generally known to those of ordinary skill in the art including enzymes, radioisotopes, and fluorescent, luminescent and chromogenic substances including, but not limited to colored particles, such as colloidal gold and latex beads. Of these, radioactive labelling can be used for almost all types of assays and with most variations. Enzyme-conjugated labels are particularly useful when radioactivity must be avoided or when quick results are needed. Fluorochromes, although requiring expensive equipment for their use, provide a very sensitive method of detection. Binding peptides useful in these assays are those disclosed claimed herein including antibodies, particularly monoclonal antibodies, polyclonal antibodies, and affinity purified polyclonal antibodies.

Alternatively, the binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, may be labelled indirectly by reaction with labelled substances that have an affinity for immunoglobulin, such as protein A or G or second antibodies. The binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, may be conjugated with a second substance and detected with a labelled third substance having an affinity for the second substance conjugated to the antibody. For example, the binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, may be conjugated to biotin and the binding peptide/biotin conjugate detected using labelled avidin or streptavidin. Similarly, the binding peptide may be conjugated to a hapten and the binding peptide/hapten conjugate detected using labelled anti-hapten binding peptide.

Those of ordinary skill in the art will know of these and other suitable labels which may be employed in accordance with the present invention. The binding of these labels to binding

peptides or fragments thereof can be accomplished using standard techniques commonly known to those of ordinary skill in the art. Typical techniques are described by Kennedy, J. H., et al., 1976 (Clin. Chim. Acta 70:1-31), and Schurs, A. H. W. M., et al. 1977 (Clin. Chim Acta 57:1-40). Coupling techniques mentioned in the latter are the glutaraldehyde method, the periodate method, the dimaleimide method, and others, all of which are incorporated by reference herein.

Current immunoassays utilize a double antibody method for detecting the presence of an analyte, wherein, the antibody is labeled indirectly by reactivity with a second antibody that has been labeled with a detectable label. The second antibody is preferably one that binds to antibodies of the animal from which the monoclonal antibody is derived. In other words, if the monoclonal antibody is a mouse antibody, then the labeled, second antibody is an anti-mouse antibody. For the antibody to be used in the assay described herein, this label is preferably an antibody-coated bead, particularly a magnetic bead. For the antibody to be employed in the immunoassay described herein, the label is preferably a detectable molecule such as a radioactive, fluorescent or an electrochemiluminescent substance.

An alternative double antibody system, often referred to as fast format systems because they are adapted to rapid determinations of the presence of an analyte, may also be employed within the scope of the present invention. The system requires high affinity between the antibody and the analyte. According to one embodiment of the present invention, the presence of the amyloid protein is determined using a pair of antibodies, each specific for amyloid protein. One of said pairs of antibodies is referred to herein as a "detector antibody" and the other of said pair of antibodies is referred to herein as a "capture antibody". The monoclonal antibody of the present invention can be used as either a capture antibody or a detector antibody. The monoclonal antibody of the present invention can also be used as both capture and detector antibody, together in a single assay. One embodiment of the present invention thus uses the double antibody sandwich method for detecting amyloid protein in a sample of biological fluid. In this method, the analyte (amyloid protein) is sandwiched between the detector antibody and the capture antibody, the capture antibody being irreversibly immobilized onto a solid support. The detector antibody would contain a detectable label, in order to identify the presence of the antibody-analyte sandwich and thus the presence of the analyte.

Exemplary solid phase substances include, but are not limited to, microtiter plates, test tubes of polystyrene, magnetic, plastic or glass beads and slides which are well known in the field of radioimmunoassay and enzyme immunoassay. Methods for coupling antibodies to solid phases are also well known to those of ordinary skill in the art. More recently, a number of

porous material such as nylon, nitrocellulose, cellulose acetate, glass fibers and other porous polymers have been employed as solid supports.

The present invention also relates to a diagnostic kit for detecting tau protein in a biological sample comprising a composition as defined above. Moreover, the present invention relates to the latter diagnostic kit which, in addition to a composition as defined above, also comprises a detection reagent as defined above. The term "diagnostic kit" refers in general to any diagnostic kit known in the art. More specifically, the latter term refers to a diagnostic kit as described in Zrein et al. (1998).

It is still another object of the present invention to provide novel immunoprobes and test kits for detection and diagnosis of tau protein-associated diseases and conditions, comprising binding peptides according to the present invention. For immunoprobes, the binding peptides are directly or indirectly attached to a suitable reporter molecule, e.g., an enzyme or a radionuclide. The test kit includes a container holding one or more binding peptides according to the present invention and instructions for using the binding peptides for the purpose of binding to tau antigen to form an immunologic complex and detecting the formation of the immunologic complex such that presence or absence of the immunologic complex correlates with presence or absence of tau protein.

EXAMPLES

EXAMPLE 1: Generation and screening of hybridomas and antibodies

The objective of this study was to generate and screen anti-Tau mAbs (monoclonal antibodies). Hybridomas were generated by fusion of tau vaccine immunized mouse spleen cells with a myeloma cell line. The hybridomas were assessed for reactivity against both phosphorylated and non-phosphorylated full-length Tau protein, as well as the phosphorylated and non-phosphorylated Tau antigenic peptides used in the vaccine preparation. Hybridoma screening was also performed for reactivity of hybridomas supernatant for tau tangles using immunochemistry on Tau transgenic mouse brain slices.

1.1 Methods

1.1.1 Fusion

A wild type C57BL/6 mouse vaccinated with ACI-35 (Tau393-408 [pS396, pS404]) was used for hybridoma production. The mouse was boosted with ACI-35 vaccine on day 0 then again on day 4 and the fusion was performed on day 7.

6 $x10^7$ (ACI-35), splenocytes from the immunized mouse were fused with 2 x 10^7 SP2-O-Ag14 myeloma cells at a ratio of 3 splenocytes / 1 myeloma cell.

The fusions resulted in 8x96 well plates and the clones were named according to the plate (1-8) then the row (A-G) and finally the column (1-12).

1.1.2 Screening method to select clones

The 8x96 well plates were first screened twice for IgG expression. Positive expressing clones were then transferred in 24 well plates and cell supernatants (=clones) of growing cells were tested in a Tau ELISA screen and a immunohistochemistry TAUPIR screen. Positive supernatants in ELISA and/or TAUPIR were transferred to T25 flasks and clones were screened again for IgG expression in a Tau ELISA screen and TAUPIR screen.

1.1.3 IgG screen

ELISA plates (Costar; Sigma) were coated with 50 µl/well of anti-mouse IgG antibody (AbD Serotec, Düsseldorf, Germany) in coating buffer for 16 hr at 4°C. After washing plates with PBS/Tween, wells were blocked with 100 µl/well of blocking solution for 1 hr at ambient temperature. Undiluted hybridoma supernatants (50 µl per well) were incubated for 1 hr at ambient temperature. After a washing, a mix of horseradish peroxidase (HRP)-conjugated anti-mouse IgG1, IgG2a, IgG2b, IgG3, or IgM (AbD Serotec) was applied for 1 hr at ambient temperature. After a final wash, detection was performed with HRP substrate (TMB; 3-3',5,5'-tetramethylbenzidine), and plates were read at 405 nm using a microplate reader. Results are expressed as optical density (O.D.).

1.1.4 Hybridomas Tau ELISA screen

Hybridomas ELISA screen was performed on pTau peptide (ACI-35, T3.5: Tau393-408[pS396/pS404; PolyPeptide Laboratories, Hillerød, Denmark), the corresponding non-phosphorylated Tau peptide (T3.6: Tau393-408, PolyPeptide Laboratories), phosphorylated full-length (441aa) Tau protein (pTau protein, Vandebroek et al., 2005) and full-length (441aa) Tau protein (Tau protein, SignalChem, Richmond, Canada). Finally Bovine Serum Albumin (BSA) was used as negative control.

Plates were coated with 10 µg/ml of corresponding Tau peptide and 1 µg/ml of corresponding Tau protein overnight at 4°C. After washing each well with PBS-0.05% Tween 20 and blocking with 1% BSA in PBS-0.05% Tween 20, undiluted hybridoma supernatant or medium negative control were added to the plates and incubated at 37°C for 2 hours. After washing plates were incubated with an alkaline phosphatase (AP)-conjugated anti-mouse IgG total antibody (Jackson Laboratories, Baltimore, PA, USA) for 2 hours at 37°C. After washing plates were incubated with pNPP (para-nitro-phenyl-phosphate), the phosphatase substrate for AP, and read at 405 nm using an ELISA plate reader. Results are expressed as O.D. (Optical Density).

1.1.5 Hybridomas IHC screen: Binding of anti-Tau antibodies to tangles in brain sections from transgenic mice (TAUPIR)

TAUPIR experiments were done according to protocol from EXAMPLE 3.1.2.

1.1.6 T25 flasks IgG screen

ELISA plates were coated with 5ug/ml of anti-mouse IgG F(ab')2 fragment specific antibody (Jackson Laboratories, Baltimore, PA, USA) in carbonate-bicarbonate coating buffer pH 9.6 (Sigma, Buchs, Switzerland) overnight at 4°C. After washing plates, undiluted hybridoma supernatant, positive control IgG1 antibody (6E10 at 1ug/ml: Covance, Emeryville, CA, USA) or negative control (culture medium alone) were incubated for 1 hr at RT. After a washing step, the secondary AP-conjugated goat anti-mouse IgG (subclasses 1+2a+2b+3) Fcγ fragment specific antibody (Jackson Laboratories, Baltimore, PA, USA) was incubated on the plates for 2 hrs at 37°C. After a final washing, detection was performed with pNPP (para-nitro-phenyl-phosphate), the phosphatase substrate for AP, and plates were read at 405 nm using an ELISA plate reader. Results are expressed as O.D. (Optical Density).

1.2 Results

The cell supernatants from the 8x96 well plates resulting from the fusion were screened for production of IgG. Out of 768 wells (8x96 wells) tested, 48 wells positive for IgG production were selected based on the best binding to the vaccine phospho-peptide, and to full-length phospho-Tau. Selection was based on binding to the peptide and full-length phospho-Tau protein by ELISA, and also to selectivity when comparing to non-phospho-peptide and non-phospho full-length Tau protein. 24 selected hybridomas were subcloned by seeding 2 plates per hybridoma at 1 cell/well and 1 plate at 0.5 cell/well. Supernatants were tested again for binding to phospho-peptide and phospho-protein to verify binding profile, after which stability was evaluated in a 6-week culture. Eight stable clones were then selected and tested for isotyping, and binding using ELISA and TAUPIR as described in Methods.

1.3. Conclusion

The antibodies generated have shown high specificity to pTau peptides with only marginal binding to non-phosphorylated peptides.

A total of 8 clones were selected for further subcloning and were sequenced (see Table 6 and Table 7) and 6 clones were deposited at DSMZ (see Table 10).

The positive mother clones mentioned above were further cultivated in 96 well plates, then 24 well plates and finally T25 flasks. At each stage, the supernatants of the hybridoma clones were screened by ELISA, Taupir and Western Blot.

EXAMPLE 2: Cloning of Antibody Light Chain and Heavy Chain Variable Regions

Antibody heavy and light variable region genes from the hybridoma cells are cloned and the DNA sequences and location of the complementarity determining regions (CDRs) determined as well as the antibodies binding features.

Total RNA was prepared from 3 x 10^6 hybridoma cells (1 vial) using the Qiagen RNeasy mini kit (Cat No: 74104). RNA was eluted in 50uL water and checked on a 1.2% agarose gel.

V_H and V_K cDNAs were prepared using reverse transcriptase with IgG and kappa constant region primers. The first strand cDNAs were amplified by PCR using a large set of signal sequence primers. The amplified DNAs were gel-purified and cloned into the vector pGem® T Easy (Promega). The V_H and V_K clones obtained were screened for inserts of the expected size. The DNA sequence of selected clones was determined in both directions by automated DNA sequencing. The locations of the complementarity determining regions (CDRs) in the sequences were determined with reference to other antibody sequences (Kabat EA *et al.*, 1991).

EXAMPLE 3: Binding Studies I

The objective was to measure the phospho-Tau (pTau) binding of the antibodies generated from subcloned hybridomas derived from mice immunized with the tau liposomal vaccines. To test this, an enzyme-linked immunosorbant assay (ELISA) was used to measure the binding of the purified antibodies to both phosphorylated and non-phosphorylated full-length Tau protein, as well as the phosphorylated and non-phosphorylated Tau antigenic peptides used for the liposomal vaccine preparation.

The screening was completed by two other methods. Immunohistochemistry (IHC) on brain sections from a Tau transgenic animal (TAUPIR) using an anti-tau antibody as the primary antibody was done. Additionally, a western blot (WB) on brain protein homogenates from Tau transgenic mice was performed, using an anti-tau antibody as the blotting antibody.

3.1 Methods

3.1.1. *ELISAs: Phospho-Tau binding assay*

To test the binding of the purified antibodies to Tau and pTau, an ELISA assay was used. Briefly, Nunc MaxiSorp 96-well plates (Nunc, Roskilde, Denmark) were coated with 1 µg/mL of full-length (441 aa) Tau protein (SignalChem, Richmond, Canada) or phosphorylated full-length (441 aa) Tau protein (Vandebroek et al., 2005). Additionally, plates were coated with 10 µg/mL of the Tau-derived vaccine peptide, Tau393-408 (phosphorylated or not on S396 and S404). To test for cross-reactivity to Tau and pTau sequences of different pTau epitopes that were not used in the vaccine preparation, plates were coated with 10 µg/mL of the

following peptides:, Tau393-408 (phosphorylated or not on S396 and S404), Coating was done overnight in phosphate-buffered saline (PBS) at 4°C. Plates were washed thoroughly with 0.05% Tween20/PBS and then blocked with 1% bovine serum albumin (BSA) in 0.05% Tween20/PBS for 1 hr at 37°C. The antibody being tested was then added in an 8 or 16 two-fold dilution series between 20 and 0 µg/mL, and allowed to incubate for 2 hr at 37°C. Plates were then washed as described previously, and AP-conjugated anti-mouse IgG secondary antibody (Jackson ImmunoResearch Laboratories, Suffolk, England) was added at 1/6000 dilution in 0.05% Tween20/PBS for 2 hr at 37°C. After washing, plates were incubated with *p*-nitrophenyl phosphate disodium hexahydrate (pNPP; Sigma-Aldrich, Buchs, Switzerland) phosphatase substrate solution, and read at 405 nm following 30 min, 1, 2 or 16 hr incubation times using an ELISA plate reader.

3.1.2. TAUPIR and Western-blots: Binding of anti-Tau antibody to Tau tangles in brain sections from a Tau transgenic animal (TAUPIR)

For TAUPIR staining, brain sections were from TPLH mice (transgenic mice expressing the longest isoform (441aa) of $hTau^{P301L}$), old (>18 months old) double transgenic biGT (GSK-3β transgenic mice crossed with TPLH) mice, and double transgenic biAT ($hAPP^{V717I}$ transgenic mice crossed with TPLH) mice. As a negative control, sections from Tau knock-out mice (TKO; 6 months old) were used. Brain sections were washed for 5 min in PBS then incubated for 15 min at RT in 1.5% H_2O_2 in PBS:MeOH (1:1) to block endogenous peroxidase activity. After washing the sections 3 times in PBST (PBS/0.1% TritonX100) they were incubated for 30 min at RT in PBST+10% FCS (fetal calf serum) blocking solution. The incubation with the anti-Tau antibody being tested was done overnight at 4°C using the following antibody concentrations: ACI-35-2A1-Ab1 at 0.0053 µg/mL, ACI-35-2A1-Ab2 at 0.0048 µg/mL, ACI-35-4A6-Ab1 at 0.015 µg/mL, ACI-35-1D2-Ab1 at 0.0047 µg/mL, ACI-35-2G5-Ab1 at 0.0055 µg/mL, and ACI-35-2G5-Ab2 and ACI-35-2G5-Ab3 at 0.01 µg/mL in PBST/10% FCS. Sections were next washed 3 times in PBST before incubation with an HRP-conjugated goat anti-mouse (purchased from Dako, Glostrup, Denmark) secondary antibody in PBST/10% FCS for 1 hour at RT. Prior to detection, sections were washed 3 times with PBST and incubated in 50 mM Tris/HCl pH7.6 for 5 min. Detection was done by incubating the sections for 3 min in Diaminobenzidine (DAB: 1 tablet in 10 ml of 50 mM Tris.HCl + 3 µl H_2O_2 30%; MP Biomedicals, Solon, OH, USA). The reaction was stopped by washing the sections 3 times in PBST. Sections were then transferred onto silanized glass-plates and air-dried on warm-plate at 50°C for 2 hours. Counterstaining was done using incubation with Mayers hematoxylin (Fluka Chemie, Buchs, Switzerland) for 1 min, followed by a washing step for 4 min in running tap-water. Sections were dehydrated by passing in 50%, 70%, 90% and twice in 100% ethanol bath then in Xylol 2 times for 1 min. Finally

sections were mounted with DePeX (BDH Chemicals Ltd., Poole, England) under glass cover-slips for imaging.

Additional staining (Western-blotting) was done on SDS-PAGE (10%) separated brain homogenate proteins from wild-type mice (FVB) Tau transgenic mice (TPLH and biGT), or Tau knock-out mice (TKO). For Western-blotting, antibodies were used at the following concentrations: ACI-35-2A1-Ab1 at 0.53 µg/mL, ACI-35-2A1-Ab2 at 0.48 µg/mL, ACI-35-4A6-Ab1 at 0.5 µg/mL, ACI-35-1D2-Ab1 at 0.47 µg/mL, ACI-35-2G5-Ab1 at 0.55 µg/mL, ACI-35-2G5-Ab2 at 0.33 µg/mL, and ACI-35-2G5-Ab3 at 0.5 µg/mL.

3.2 Results

Antibody ACI-35-2A1-Ab1, ACI-35-2A1-Ab2, ACI-35-1D2-Ab1, ACI-35-2G5-Ab2 and ACI-35-2G5-Ab3 demonstrated high binding activity and specificity to phosphorylated human Tau protein (Table 2), more specifically to the antigenic phospho-Tau peptide used in the corresponding vaccine. No cross-reactivity to non-phosphorylated Tau was observed, or to other phosphorylated and non-phosphorylated Tau-derived peptides tested. Antibody ACI-35-4A6-Ab1, as per its selection, displayed high binding activity only to the antigenic phospho-Tau peptide used in the vaccine preparation. Low cross-reactivity was found to the non-phospho counterpart of the antigenic peptide used in the vaccine preparation which was expected based on the clone selection. Antibody ACI-35-2G5-Ab1, displayed high binding activity only to the antigenic phospho-Tau peptide used in the vaccine preparation. Small cross-reactivity was observed to the T4.5 phospho-peptide, comprising part of the antigenic peptide sequence used in the vaccine.

TAUPIR and WBs were used to look at binding to Tau tangles in brains of mice with advanced tauopathy (biGT > 18 months), and to full-length Tau in denatured homogenates derived from these mice. Different brain regions were analyzed: cortex and CA1, CA3 and dentate gyrus (DG) part of the hippocampus. Antibodies ACI-35-2A1-Ab1 and ACI-35-2A1-Ab2 displayed the best TAUPIR results with a dense cytoplasmic staining and clear neuropil threads, especially in the CA1 and CA3 regions of the hippocampus. Antibody ACI-35-4A6-Ab1 was negative in TAUPIR with only faint sporadic tangle like structures lightly stained. Antibody ACI-35-1D2-Ab1 showed a good cytoplasmic TAUPIR staining with neuropil threads in the CA1 region. Antibody ACI-35-2G5-Ab1 was negative in TAUPIR with nuclear staining and only some tangle staining. Finally, ACI-35-2G5-Ab2 and ACI-35-2G5-Ab3 antibodies displayed similar good cytoplasmic TAUPIR staining with neuropil threads observed in the CA1 and CA3 of the hippocampus. The rating of staining quality using + or - signs is shown in Table 2. Brain homogenates from Tau transgenic mice were blotted, showing that all antibodies bound well to expected Tau bands (Table 2, rated as +), with ACI-35-1D2-Ab1 and ACI-35-2G5-Ab1 also showing additional non-specific binding (-/+).

EXAMPLE 4: Binding Studies II

4.1 Methods

4.1.1 SPR binding assay

All SPR experiments were carried out on a Biacore X instrument (GE Healthcare). Sensor chip SA (Streptavidin derivatized carboxymethyl dextran) was purchased from GE Healthcare. Running buffer was PBS (Dulbecco's PBS, Sigma D8537). Non-covalently bound Streptavidin was firstly removed from the sensor surface by injecting 8 pulses (each ~1 µL) of 16 mM NaOH (aq). Phospho-tau peptide was then solubilized in PBS to give a final peptide concentration of 1 µM and then injected (35 µL) over flow cell (fc) 2 of the sensor chip at 5 µl/min. After coupling, a final immobilization level of 130 RUs was obtained. To study the binding of the antibodies to the chip surface, several concentrations of antibodies were prepared by serial 2-fold dilutions with running buffer. The injections were performed over both fc 1 and 2 at a flow rate of 50 µL/min for 120 s. Flow cell 1 was not derivatized and responses of fc 1 were subtracted from fc 2 to correct for instrument noise and bulk refractive changes. After each injection, the surfaces were washed immediately with running buffer for 100 s. To remove any remaining bound antibody from the chip, surface regeneration was performed by injecting 1 µL of 10 mM Glycine-HCl pH 1.7. Kinetic analyses were performed using algorithms for numerical integration and global analysis using BIAevaluation 3.0. The sensograms obtained for injections of antibody at different concentrations were overlaid and the baselines adjusted to zero. For curve fitting, all data were fit simultaneously to a 1:1 homogeneous (Langmuir) model.

Peptides used

T3.30	Biotin-LC linker-GVYKS[PO3H2]PVVSGDTS[PO3H2]PRHL-NH2	lot MI89P9-P12-2 (64 % pure)
		lot MI89P9-P12-3 (87 % pure)

4.2 Results

The binding of the anti-tau antibodies to the phosphorylated Tau peptide was monitored in real-time using SPR. Analyses of the association and dissociation phases of antibody binding could be used to determine the association rate constant (k_a), dissociation rate constant (k_d) as well as dissociation constant K_D.

All antibodies were found to bind specifically to peptide T3.30 over the non-derivatized carboxymethyl dextran surface in the range 46 → 734 nM of antibody analyzed (or 11.5 → 184 nM for ACI-35-4A6-Ab1). Kinetic analyses of the sensograms revealed the dissociation constant K_D for the binding interaction between the different antibodies and T3.30 to be

between 2 and 82 nM. This therefore demonstrates that the antibodies recognize the phosphopeptide T3.30 with very high affinity (Table 3).

EXAMPLE 5: Binding Studies III ELISA on human brain samples (ELISA for detection of multimers of phosphorylated Tau)

5.1 Methods

5.1.1. Human samples: Preparation of human brain samples used for the assays described here

Temporal post-mortem cortex for ten Alzheimer's disease (AD) and ten age-matched controls were obtained from the Brain Endowment Bank of the University of Miami. The mean age at death for the AD patients (seven females, three males) was 81.1 ± 7.3 years and for the controls (free of neurological symptoms; nine females, one male) was 87.0 ± 5.8 (not significantly different from the AD patients by student t-test). All samples were of Caucasian origin. The AD samples were characterized for Braak disease stage (Braak and Braak (1991) Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 82:239-259) as shown in Table 4.

Temporal post-mortem cortex for ten AD and ten age-matched controls were homogenized according to the following protocol. Brain fragments were weighted and homogenized in 9 volumes of 25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1mM EDTA, 1 mM EGTA containing phosphatase inhibitors (30 mM NaF, 0.2 mM Na3VO4, 1 nM okadaic acid, 1 mM PMSF, 5 mM Na4P2O7) and protease inhibitors (Complete Mini; Roche, Switzerland). Homogenization was done on ice using a glass potter. This constitutes the Total Homogenate fraction (TH). Protein concentrations were measured using Bradford reagent (Sigma).

5.1.2. Setup 1 ELISA: Setup 1 ELISA assay to detect the presence of multimers of phosphorylated Tau in human post-mortem cortical brain homogenates from AD-affected individuals and age-matched controls

Multititer 96-well plates were coated with antibodies overnight at 4 °C at 5 µg/ml in carbonate/bicarbonate buffer. After 4 washes in PBS-Tween, plates were saturated with PBS-Tween 10% BSA for 1 hr at 37°C. Brain homogenates were then added to the wells at a concentration of 100 ng/µL in 50 µL PBS, and incubated for 2 hr at 37°C. After washing the plates, the same antibody as used for coating, but biotinylated, was incubated for 1 hr at 37°C at a final concentration of 5 µg/mL. Plates were washed and after addition of avidin-peroxydase (Vectastain ABC kit, Vector Laboratories) and its substrate (ABTS, Roche 10881420) the plates were read at different time points. Values are expressed as mean OD ±SD for 10 AD and 10 control subjects.

5.2 Results

Antibodies ACI-35-2A1-Ab1 and ACI-35-2G5-Ab3 were tested for their ability to detect phosphoTau (pTau) multimers in brain homogenates from AD and control subjects, using a phospho- and multimer-specific Setup 1 ELISA. We observed a highly significant ($p<0.001$) difference between AD and age-matched controls (n=10) in this assay for both antibodies (Figure 1). Using human post-mortem cortical homogenates from AD and age-matched control brain, we demonstrated the ability of ACI-35-2A1-Ab1 and ACI-35-2G5-Ab3 anti-pTau antibodies to detect multimers of Tau-pS396 in post-mortem human brain samples.

EXAMPLE 6: Binding Studies IV - Western-blots on human brain samples.

6.1 Methods

6.1.1. Human samples: The same method for preparation of human samples as described in Method 5.1.1.

6.1.2. Western-blots: Western-blot assay to detect the presence of phosphorylated Tau in human post-mortem cortical brain homogenates from AD-affected individuals and age-matched controls

The anti-human Tau antibodies used in this study were the mouse ACI-35-2A1-Ab1, ACI-35-1D2-Ab1, and ACI-35-2G5-Ab3, all directed against Tau-pS396. The mouse monoclonal TAU-13 antibody (Abcam ab24636) directed against total human Tau, and the rabbit monoclonal antibody E178 directed against Tau-pS396 (Abcam ab32057) were used as controls. 20 µg of each total homogenate was loaded per lane of on a 10% polyacrylamide Bis-TRIS precast gel (Nupage Novex 10% Bis-TRIS Midi Gel, Invitrogen). Proteins were resolved as recommended by the manufacturer in NuPAGE MOPS SDS running buffer (Invitrogen NP0001). Protein blotting was done for 3 hr in 25 mM TRIS pH 8.6, 190 mM Glycin buffer, 20% methanol, on ice on PVDF membranes (Immobilon-FL, Millipore IPFL00010). Membranes were blocked for 1 hr in Licor blocking buffer (Odyssey) diluted 1/3 in PBS. Membranes were incubated overnight with primary antibodies at the following concentrations: TAU-13 at 0.6 µg/mL, E178 diluted 1/5000, ACI-35-2A1-Ab1 at 0.53 µg/mL, ACI-35-1D2-Ab1 at 0.47 µg/mL, and ACI-35-2G5-Ab3 at 0.5 µg/mL, diluted 1/3 in Licor buffer and 2/3 PBS with 0.1% Tween-20 (PBS-T). After 4 washes in PBS-T, membranes were incubated with a goat anti-mouse antibody coupled with the LICOR 800 dye (Goat anti-mouse IRDye 800 CW, Odyssay) for 1 hr at room temperature, washed again 4 times with PBS-T, and scanned for image reproduction using the LICOR system.

6.2 Results

Antibodies ACI-35-2A1-Ab1, ACI-35-1D2-Ab1, and ACI-35-2G5-Ab3 were tested for their ability to detect phosphoTau (pTau) in brain homogenates from AD and control subjects, using a phospho-specific Western-blots. All post-mortem human cortex samples were first characterized using commercial antibodies against human Tau: anti-total Tau (TAU-13) and anti-pS396 Tau (E178) antibodies. As shown in Figure 2A, using the TAU-13 antibody, we detected in all samples the characteristic Tau ladder corresponding to different Tau isoforms in the range of 50-70 kDa. Interestingly, in AD brain homogenates we also observed a relative shift in the migration pattern of Tau as expected for the presence of hyper-phosphorylated Tau in AD brains. Confirming this hypothesis, the commercial anti-pS396 Tau antibody discriminated very well controls and AD (Figure 2B). Indeed, the anti-pS396 Tau antibody revealed three main immunoreactive bands corresponding to (hyper)-phosphorylated isoforms of Tau in all AD brain homogenates and with a very weak intensity, or absent in the healthy controls. In addition, the AD samples displayed a high molecular weight TAU-13 immunoreactive smear likely reflecting the presence of aggregated Tau (Figure 2A).

Western-blotting with ACI-35-2A1-Ab1 revealed the presence of two immunoreactive protein bands of the expected size for phospho Tau in the AD brain homogenates but not in controls (Figure 3A). Weak immunoreactions by western blot using ACI-35-2A1-Ab1 may be explained by the presence of two major nonspecific bands at ~35 and ~40 kDa. Western-blotting with ACI-35-1D2-Ab1 revealed the presence of two immunoreactive protein bands of the expected size for phospho Tau in the AD brain homogenates but not in controls (Figure 3B). Weak immunoreactions by western blot using ACI-35-1D2-Ab1 may be explained by the presence of nonspecific bands at ~40 and ~50 kDa, as well as 4 nonspecific bands between 80 kDa and 150 kDa. Western-blotting with ACI-2G5-Ab3 revealed the presence of three main immunoreactive bands corresponding to (hyper)-phosphorylated isoforms of Tau in all AD brain homogenates and absent in the healthy controls, except for one control subject (C22), who has a family history of AD (Figure 3C). This report demonstrated that ACI-35-2A1-Ab1, ACI-35-1D2-Ab1, and ACI-35-2G5-Ab3, can discriminate between AD and age-matched controls for the presence of pS396 Tau in human post-mortem cortex, and thus these monoclonal antibodies recognize AD-associated pathological Tau isoforms.

EXAMPLE 7: Binding Studies V - Setup 1 (ELISA on human brain samples)

7.1 Methods

7.1.1. Human samples. The same method for preparation of human samples as described in Method 5.1.1., except for the last part, S1 fraction preparation.

7.1.2. S1 Tau protein fraction: Subfractionation of total homogenate fractions to obtain soluble Tau and phospho-Tau proteins.

To prepare the soluble Tau (S1) fraction used for the AlphaLISA assay, half volume of TH fraction was aliquoted and stored at -80°C. The remainder of the TH fraction was further processed by adding Triton X-100 to a final concentration of 0.4%. Samples were mixed well and vortexed several times before being centrifuged at 5'000 rpm for 5 min at 4°C. The supernatant constitutes the S1 fraction. The samples were aliquoted and stored at -80°C. Protein concentrations were measured using Bradford reagent.

7.1.3. AlphaLISA: AlphaLISA assay to detect the presence phosphorylated Tau in human post-mortem cortical brain homogenates from AD-affected individuals and age-matched controls.

Antibodies ACI-35-2A1-Ab1, ACI-35-1D2-Ab1, and ACI-35-2G5-Ab3, all directed against Tau-pS396, were biotinylated using EZ-Link Micro Sulfo-NHS-LC-Biotinylation Kit (Thermo Scientific), according to manufacturer's instructions. Twentyfive-fold molar excess of Biotin over antibody was used in the labeling reaction. After biotinylation, the excess of free biotin was removed by dialysis against PBS using the Slide-A-Lyzer MINI Dialysis Devices, 10K MWCO (Thermo Scientific). The biotinylated antibodies are designated as ACI-35-2A1-Ab1-BT, ACI-35-1D2-Ab1-BT, and ACI-35-2G5-Ab3-BT. Antibody Tau-13 was conjugated to the activated Alpha Acceptor beads (Perkin Elmer) using the following protocol: 0.1 mg of Tau-13 antibody solution (purified on Protein A column) was mixed with 1 mg of AlphaLISA Acceptor Bead pellets and complemented with 0.13 M phosphate buffer (pH 8.0) to a final reaction volume of 200 µL. Next, 1.25 µL of 10% Tween-20 and 10 µL of a 25 mg/mL solution of $NaBH_3CN$ was added and the tube was incubated for 48 h at 37°C with a mild rotation (7 rpm). After the conjugation reaction, the active sites on beads were blocked by adding 10 µL of a Carboxy-methoxylamine solution and further incubated at 37°C for 1 h. Finally, the beads were washed two times with 200 µL of 0.1 M Tris-HCl pH 8.0 and stored at 4°C in 200 µL storage buffer (PBS with 0.05% Proclin-300) that resulted in a final AlphaLISA Acceptor beads concentration of 5 mg/ml.

AlphaLISA is a homogenous assay based on bead proximity chemiluminescence. If Alpha Donor and Acceptor beads are in close proximity, upon laser excitation, a cascade of chemical reactions produces an amplified signal. Upon excitation at 680 nm, the photosensitizer contained in Donor beads converts ambient oxygen into more reactive singlet oxygen species. These singlets diffuse (up to 200 nm, within 4 µsec of a half-life) and produce a chemiluminescent reaction in the Acceptor beads, leading to light emission. The assay setting was as follows:

S1 samples were pre-diluted in Alpha Assay buffer (PerkinElmer AL000C) to obtain a 20 µg/mL stock concentration. The following reagents were added to a 384-well white OptiPlate

(PerkinElmer) to a final volume of 50 µL: S1 brain homogenate (5 µL), 10 µL of ACI-35-2A1-Ab1-BT, ACI-35-1D2-Ab1-BT, or ACI-35-2G5-Ab3-BT for a final antibody concentration of 0.2 nM, 0.5 nM, or 0.5 nM, respectively, and 10 µL of Tau13-Acceptor beads conjugate for a final bead concentration of 2.5 µg/mL. The reaction mix was incubated for 1 h at room-temperature, and 25 µL of Streptavidin Donor beads was added and further incubated for 2 h at room-temperature in the dark. Readout was done using EnSpire Alpha instrument and analysis using EnSpire Workstation version 3.00. Statistical analysis of data was performed using the GraphPad Prism software. Results are presented as Alpha units ±SD.

7.2 Results

An AlphaLISA assay was used to test antibodies ACI-35-2A1-Ab1, ACI-35-1D2-Ab1, and ACI-35-2G5-Ab3 for the ability to detect Tau-pS396 in post-mortem human brain homogenates, and to discriminate AD from age-matched controls. All antibodies detected Tau-pS396 (Figure 4A, 4B, 4C). The difference in signal detection between AD and controls (n=10) was also highly significant for all antibodies, showing increased signal in brains of AD subjects; ACI-35-2A1-Ab1 ($p<0.0001$), ACI-35-1D2-Ab1 ($p<0.0001$), and ACI-35-2G5-Ab3 ($p=0.002$). In conclusion, AlphaLISA technology was used to demonstrate the capability of ACI-35-2A1-Ab1, ACI-35-1D2-Ab1, and ACI-35-2G5-Ab3 to detect pS396-Tau in brains of AD subject, and to differentiate between AD and control donors.

EXAMPLE 8: In vivo efficacy of ACI-35-2G5-Ab3 antibody

8.1 Methods

8.1.1. Study setup: In vivo treatment effects of 2 administrations of anti-pTau antibody ACI-35-2G5-Ab3 in Tau transgenic mice

Female and male Tau transgenic mice (TMHT) with a C57BL/6xDBA background, at an age of 6-7 months, were administered by i.p injection 3 or 10 mg/kg of ACI-35-2G5-Ab3, or vehicle control (PBS) two times, one week apart. On day 14, animals were euthanized, brains were harvested and processed for immunohistochemistry (IHC). For the determination of Tau pathology in the hippocampus and the amygdala 5 slices (1 from each level) per brain were labeled using AT180 (for Tau-pT231) and HT7 (for total human Tau) antibodies and subsequently immunoreactive area were evaluated using Image Pro Plus (v6.2) software. Immunoreactive objects were measured above a size restriction (30 μm^2 in the amygdala, 7 μm^2 in the hippocampus) and above a dynamic intensity threshold. Total area and intensity of objects and the individual threshold were automatically filed. If used, a dynamic threshold was defined as mean intensity within area of intesity (AOI) plus a factor times the standard deviation of pixel intensities within the AOI. The region size was measured by manual

delineation of the hippocampus and amygdala. AT180 and HT7 IR area data were normalized to the region (in hippocampus) or AOI size (in amygdala).

8.2 Results

The AT180 pTau antibody detects the endogenous and human pTau (doubly phosphorylated at Thr231 and Ser235). For the Tau transgenic mice used in this study, AT180 histological measurements concentrated on hippocampal and amygdaloideic neurons. Mice treated with ACI-35-2G5-Ab3 had a significant reduction for AT180 mean and normalized sum intensity of somal labeling, in both amygdala and hippocampus (Figure 5A and 5B), showing reduction of overall somal AT180-positive pTau in treated mice.

For total human (transgenic) Tau, the HT7 antibody was used. HT7 recognizes normal human Tau between residue 159 and 163. Histological measurements concentrated on immunoreactive somata of hippocampal and amygdaloideic neurons. Mice treated with ACI-35-2G5-Ab3 had reduced HT7 immunoreactive area, as well as sum and mean HT7 intensity of immunoreactivity in the amygdala (Figure 6A). In the hippocampus, the same was observed for mean intensity (Figure 6B). However, an increase in HT7 labeling was observed for immunoreactive area, and sum intensity in the hippocampus in mice treated at 10 mg/kg. This increase observed in the hippocampus was mainly due to three mice out of the total eight mice investigated.

ACI-35-2G5-Ab3 treatment significantly decreased AT180 immunoreactive pTau levels in both investigated regions, thus in somata of hippocampal and amygdaloideic neurons. In the amygdala, the sum intensity of labeling was decreased for both AT180 immunoreactive pTau and HT7 immunoreactive total human Tau. Treatment with a dose of 3 mg/kg also significantly decreased mean HT7 intensity in both regions. However, at 10 mg/kg the average HT7 immunoreactive area and sum intensity in the hippocampus were increased over that of control treated mice, suggesting that a ACI-35-2G5-Ab3 treatment leads to shift from pathological pTau.

EXAMPLE 9: Epitope mapping of anti pTau antibodies

9.1 Methods

Epitope mapping of anti-phospho Tau mouse monoclonal antibodies was performed by ELISA using different phospho and non-phospho peptide libraries. The amino acid sequences of peptide library T3 used are shown in Table 11A. Each library consisted of short biotinylated peptides spanning phospho and non-phospho sequences present in the peptide vaccine. Additionally, a peptide library was generated substituting each residue of a peptide sequence that binds to the antibody with Alanine (Ala), as shown in Tables 11B and 11C.

Each library consisted of short biotinylated peptides spanning phospho and non-phospho sequences present in the peptide vaccine. Peptide libraries were purchased from ANAWA Trading SA. Peptide libraries were purchased from ANAWA Trading SA. Epitope mapping was done according to the manufacturer's (Mimotopes) instructions. Briefly, streptavidin coated plates (NUNC) were blocked with 0.1% BSA in phosphate-buffered saline (PBS) overnight at 4°C. After washing with PBS-0.05% Tween 20, plates were coated for 1 hr at RT with the different peptides from each library, diluted in 0.1% BSA, 0.1% sodium azide in PBS to a final concentration of 10 µM. After washing, plates were incubated for 1 hr at RT with the antibody to be tested diluted to 40 ng/ml in 2% BSA, and 0.1% sodium azide in PBS. Plates were washed again and incubated with AP-conjugated anti-mouse IgG secondary antibody (Jackson ImmunoResearch Laboratories, Suffolk, England) at 1/6000 dilution for 1 hr at RT. After a final wash, plates were incubated with *p*-nitrophenyl phosphate disodium hexahydrate (pNPP; Sigma-Aldrich, Buchs, Switzerland) phosphatase substrate solution, and read at 405 nm following 2 hr incubation using an ELISA plate reader. Binding was considered positive if the optical density (O.D.) was at least 2-times over background O.D.

9.2 Results

As a result of the epitope mapping experiments, epitopes could be identified including the required phosphorylated amino acid residue (see table 5) to which the antibodies disclosed herein specifically bind.

- Tau aa 393-401, with requirement for pS396 (ACI-35-2A1-Ab1; ACI-35-2A1-Ab2)
- Tau aa 396-401, with requirement for pS396 (ACI-35-4A6-Ab1)
- Tau aa 394-400, with requirement for pS396 (ACI-35-1D2-Ab1)
- Tau aa 402-406, with requirement for pS404 (ACI-35-2G5-Ab1)
- Tau aa 393-400, with requirement for p396 (ACI-35-2G5-Ab2; ACI-35-2G5-Ab3)

EXAMPLE 10: *Phosphorylation of Tau at serine 396 (pS396) using GSK3β kinase, and SDS-PAGE / Western-blot analysis*

10.1 Methods

The longest isoform of human full-length Tau (TAU441; SignalChem) at a final concentration of 16 µM (20 µg Tau/25 µL reaction) was incubated with 0.018 U GSK3β/pmol of Tau in phosphorylation buffer containing HEPES pH 7.64 (40 mM), EGTA (5 mM), $MgCl_2$ (3 mM), and ATP (2 mM) for 1, 6, or 20 h at 4, 30, or 37°C. One unit of GSK3β is defined by the manufacturer (New England BioLabs) as the amount of enzyme that will transfer 1 pmol phosphate from ATP to CREB phosphopeptide (KRREILSRRPpSYR) in 1 minute at 30°C. Tau phosphorylated with GSK3β (pTau-GSK3β) was probed with antibodies directed against

Tau phosphorylated at serine 202, 396, 404, 409, threonine 181, 205, and 231, and total Tau, run on direct ELISAs and Western-blots (WBs), to optimize and verify kinase activity and specificity (not shown). Additionally, blots were probed for the presence of GSK3β using an anti-GSK3α/β antibody (BioSource Invitrogen). For all WBs, pTau-GSK3β was diluted by adding an equal volume of sample buffer A (125 mM Tris-HCl pH 6.8, 4% [w/v] sodium dodecyl sulfate [SDS], 20% glycerol, 0.01% bromophenol blue, 5% β-mercaptoethanol), and the samples were heated to 95°C for 10 min. 30 µg of sample was loaded onto a 4-12% Bis-Tris gel (Invitrogen) and run in MOPS SDS buffer (Invitrogen). Proteins were transferred to a 0.45 µm PVDF membrane in transfer buffer (25 mM Tris pH 8.6, 190 mM glycine, 20% methanol). To verify protein transfer, membranes were stained with Ponceau S for 5 min. Membranes were then washed and then blocked for 1 hour in blocking buffer (5% BSA in TBS [50 mM Tris-HCl, pH 7.6, 150 mM NaCl]). Membranes were blotted over-night at 4°C with the primary antibodies in blocking buffer and 0.1% Tween. Blotting with the ACI-35-2G5-Ab3 was done at 0.5 µg/mL antibody dilution.

10.2 Results

Tau treated with GSK3β resulted in high presence of phosphorylation at Tau serine 396 (Tau-pS396), as verified using antibodies specific to different Tau phospho-serine and -threonine residues (not shown). Figure 7 shows an SDS-PAGE for Tau-pS396 generated using different GSK3β conditions, and the membrane blotted using the ACI-35-2G5-Ab3 antibody. The ACI-35-2G5-Ab3 antibody, specific for Tau-pS396, demonstrated a good signal for Tau-pS396, with bands also observed suggesting that it binds to Tau-pS396 dimers (Figure 7, lanes 11 and 13). No bands were observed in the absence of GSK3β treatment (lanes 6-8 and 14-15).

EXAMPLE 11: *Detection of phosphorylation of Tau (pSer396) in human Cerebrospinal fluid (CSF) samples*

11.1 Methods

11.1.1 Human samples – post-mortem brain samples

Temporal post-mortem cortex of one Alzheimer's disease (AD) donor AD19 was obtained from the Brain Endowment Bank of the University of Miami. We kindly acknowledge the University of Miami Brain Endowment Bank for providing samples for this study. The demographic information about the donor is shown in Table 12 below, where the Braak disease stage (Braak and Braak (1991) Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 82:239-259) is also indicated.

Sample ID	Gender	Age at death	Age at diagnosis	Disease duration	disease stage
AD 19	F	81	77	4	Braak V

Table 12. Description of the brain sample AD19 used in this study

11.1.2. Preparation of homogenate fraction S1 from post-mortem brain

Temporal post-mortem cortex of the AD19 donor was homogenized according to the following protocol. Brain fragment was weighted and homogenized in 9 volumes of 25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1mM EDTA, 1 mM EGTA containing phosphatase inhibitors (30 mM NaF, 0.2 mM Na_3VO_4, 1 nM okadaic acid, 1 mM PMSF, 5 mM $Na_4P_2O_7$) and protease inhibitors (Complete Mini, Roche 04 693 124 001). Homogenization was done on ice using a glass potter. This constitutes the Total Homogenate fraction (TH). Half volume of TH fraction was aliquoted and stored at -80°C. The remainder of the TH fraction was further processed by adding Triton x-100 to a final concentration of 0.4%. The sample was mixed well and vortexed several times before being centrifuged at 5'000 rpm for 5 min at 4°C. The supernatant constitutes the S1 fraction. The sample was aliquoted and stored at -80°C. Protein concentration was measured using Bradford reagent (Sigma B6916-500).

11.1.3 Human CSF samples

Cerebrospinal fluid (CSF) samples from clinically confirmed mild-to-moderate Alzheimer's disease (AD) patients and healthy volunteer control donors (Ctrls) were provided by the Charité School of Medicine Berlin. We kindly acknowledge the Charité School of Medicine Berlin for providing samples for this study. The samples were aliquoted, stored at -80°C and used without further processing. Demographic and clinical information on CSF sample donors is shown in Table 13 below.

diagnosis	n	mean age (StDev)	age range	% females	MMSE score (StDev)	MMSE range
AD	17	72.5 (8)	57-85	35	21.2 (4)	13-27
Ctrl	16	65 (7)	53-77	69	29 (1)	27-30

Table 13. Demographic and clinical information on CSF sample donors

11.1.4 Immuno-enrichment of CSF Tau

11.1.4.1 Antibody coupling

For immuno-enrichment of CSF Tau, a commercial human Tau antibody (clone HT7, Thermo Scientific MN 1000) was used. In order to couple HT7 to Protein G Dynabeads (Life Technologies 10004D), for each sample 1.5 mg (50 µL) Protein G Dynabeads were resuspended by vortexing and transferred to a 1.7 mL Maximum Recovery tube (Axygen MCT-175-L-C). The tubes were placed on a magnetic support (DynaMag, Life Technologies

123.21D) in order to concentrate the beads on the side of the tube and to remove the buffer. Binding of 1 µg HT7 in 200 µL of PBS to the Protein G Dynabeads was performed using a Hula Mixer (Life Technologies) at 10/20 rpm, 25°/10 tilt, 5°/2 vibro for 10 min, after which tubes were placed on the magnet, the buffer was removed and tubes were washed once by gentle pipetting with 200 µL PBS/0.02% Tween 20 and two times with 200 µL conjugation buffer (20 mM Na Phosphate, 150 mM NaCl, prepared freshly). The washing buffers were always removed using the magnet. For crosslinking HT7 to the Protein G Dynabeads, the HT7-beads were resuspended in 250 µL of 5 mM BS3 solution (Sigma-Aldrich S5799) dissolved in conjugation buffer and incubated with rotation (same settings as above) for 30 min at room temperature (RT), the reaction was terminated by adding 12.5 µL of quenching buffer (1M Tris-HCl pH 7.5) for 15 min followed by three washes with 200 µL PBS/0.02% Tween 20.

11.1.4.2 CSF Tau immuno-enrichment

CSF was used undiluted and 1 mL of CSF for each donor was transferred to the tube containing the HT7 cross-linked beads and incubated for 1 hr 4°C under continuous rotation (10 rpm). After removing the unbound material on the magnet, the beads were washed with 200 µL PBS/0.02% Tween 20 and Tau was eluted in 20 µL 1% sodium dodecyl sulphate (SDS) in PBS at 70°C for 10 min. In order to avoid sedimentation of beads, tubes were mixed shortly (300 rpm in the heated horizontal mixer, for 5 seconds, every minute). After this incubation, the eluted samples were collected by placing the tubes on the magnet.

As a positive control, Tau was also enriched from human brain homogenates. For this serial dilutions of human brain S1 fraction from AD19 donor were prepared in PBS (0.5 µg/mL, 0.17 µg/mL, 0.056 µg/mL, 0.019 µg/mL, 0.006 µg/mL, 0.002 µg/mL, 0.0007 µg/mL). Each sample (1 mL) was then treated as described above and eluted in 25 µL 1% SDS.

11.1.5 AlphaLISA.

11.1.5.1 AlphaLISA Assay description

AlphaLISA is a homogenous assay that utilizes the bead-based Alpha technology. AlphaLISA was selected as a technology platform based on sensitivity and minimal number of steps. Briefly, the assay is based on bead proximity. Upon excitation at 680 nm, the photosensitizer containing Donor beads converts ambient oxygen into singlet oxygen species, these diffuse (up to 200 nm, within 4 µsec of a half-life) and produce a chemiluminescent reaction in the Acceptor beads, leading to light emission.

The assay setting used in our experiments was the following (see also Figure 8):

- Pan-Tau antibody Tau-13 (Abcam ab24636), coupled to Alpha Acceptor beads binds human Tau present in the sample and forms the "Tau protein-Tau-13 antibody-Acceptor beads" complex
- Detection antibody ACI-35-2G5-Ab3-BT binds to the pS396 of human Tau and allows binding the Streptavidin-coated (SAv) Alpha Donor beads to the complex.

After bringing all reagents into the reaction, the chemiluminescent signal is read using EnSpire Alpha 2390 Reader.

11.1.5.2 Biotinylation of ACI-35-2G5-Ab3 antibody

In order to be used in the AlphaLISA assay, the antibody ACI-35-2G5-Ab3 was biotinylated using EZ-Link Micro Sulfo-NHS-LC-Biotinylation Kit (Thermo Scientific 21935), according to manufacturer's instructions. Twenty five-fold molar excess of Biotin over the antibody was used in the labeling reaction. After the biotinylation, the excess of free biotin was removed by washing the antibody four time in PBS using 50'000 MWCO Spin-X UF 500 Concentrator (Corning 431480). The biotinylated ACI-35-2G5-Ab3 antibody is indicated as ACI-35-2G5-Ab3-BT.

11.1.5.3 Coupling of the Tau-13 antibody to AlphaLISA Acceptor beads.

In order to be used in the AlphaLISA assay, the antibody Tau-13 was conjugated to the activated Alpha Acceptor beads (Perkin Elmer 6772001). The following conjugation protocol was used: 0.1 mg of Tau-13 antibody solution (purified on Protein A column) was mixed with 1 mg of AlphaLISA Acceptor Beads pellet and complemented with 0.13 M phosphate buffer (pH 8.0) to a final reaction volume of 200 µL. Next, 1.25 µL of 10% Tween-20 and 10 µL of a 25 mg/mL solution of NaBH3CN were added and the tube was incubated for 48 h at 37°C with a mild rotation (7 rpm). After the conjugation reaction, the active sites on beads were blocked by adding 10 µL of a Carboxy-methoxylamine solution and further incubated at 37°C for 1 h. Finally, the beads were washed two times with 200 µL of 0.1 M Tris-HCl pH 8.0 and stored at 4°C in 200 µL storage buffer (PBS with 0.05% Proclin-300) that resulted in a final AlphaLISA Acceptor beads concentration of 5 mg/ml.

11.1.5.4 Limit of detection determination using brain pS396-Tau

Immuno-enriched Tau brain samples, S1 brain fraction samples and buffer blanks were used for this experiment. Each sample was analyzed in 50 µL final volume using a 384-well white OptiPlate (PerkinElmer 6007291). Dilutions of all reagents were done with Alpha Assay buffer (PerkinElmer AL000C).

- 5 µL sample (1/10 of the final volume, therefore the final protein concentration in the assay corresponds to the 1/10 of the sample concentration).

- 10 µL 0.5% SDS for S1 brain fraction samples or 10 µL plain buffer for the immuno-enriched Tau brain samples was added
- 15 µL of ACI-35-2G5-Ab3-BT antibody (final concentration: 5 nM) mixed with Tau13-Acceptor beads conjugate (final bead concentration: 2.5 µg/mL)
- Incubation at room temperature for 1 hr
- 20 µL of Streptavidin Donor beads (final bead concentration: 25 µg/mL)
- Incubation at room temperature for 30 min (protected from light)
- Readout using EnSpire Alpha instrument and analysis using EnSpire Workstation version 3.00

11.1.5.5 Determination of immuno-enriched pS396-Tau in CSF

Each sample was analyzed in 50 µL final volume using a 384-well white OptiPlate (PerkinElmer 6007291). Dilutions of all reagents were done with Alpha Assay buffer (PerkinElmer AL000C).

- 5 µL of immunoprecipitated eluate from each donor
- 20 µL of ACI-35-2G5-Ab3-BT antibody (final concentration: 5 nM) mixed with Tau13-Acceptor beads conjugate (final bead concentration: 2.5 µg/mL)
- Incubation at RT for 1 h
- 25 µL of Streptavidin Donor beads (final bead concentration: 25 µg/mL)
- Incubation at RT for 30 min (protected from light)
- Readout using EnSpire Alpha instrument and analysis using EnSpire Workstation version 3.00

11.1.6 Statistical analysis

Statistical analysis of data was performed using the GraphPad Prism software.

11.2 Results

Preliminary experiments indicated that the amount of pS396 present in human CSF was too low for detection. For this reason, an immuno-enrichment protocol coupled to high-sensitivity immuno-detection was developed. The immuno-enrichment protocol was first validated using human AD post-mortem brain material. Side-by-side comparison of untreated brain homogenate samples with Tau immuno-enriched samples revealed that at corresponding concentrations, the limit of detection of the Tau13/ACI-35-2G5-Ab3 AlphaLISA assay was reached at 0.5 µg/mL for the untreated samples and at between 0.002-0.006 µg/mL for the immuno-enriched samples, indicating a 100-fold enrichment (Figure 9).

Next, the immuno-enrichment protocol was applied on the live-donor CSF samples (n=17 for mild-to-moderate AD patients and n=16 for age-matched healthy volunteers). The data obtained (Figure 10) demonstrate that: a) following the immuno-enrichment protocol the Tau13/ACI-35-2G5-Ab3 AlphaLISA detected pS396-Tau in all human CSF samples; and b) more importantly, a significant increased in the amount of pS396-Tau in AD CSF was observed when compared to the control (p=0.0003, Mann-Whitney test).

In conclusion, an immuno-enrichment / immuno-detection protocol was developed, allowing for the detection of pS396-Tau in human CSF. Increase of pS396-Tau in CSF of mild-to-moderate AD suggests that this method could be successfully used in clinical biomarker studies to assess disease progression, patient stratification and therapy efficacy. ACI-35-2G5-Ab3 antibody detected pS396-Tau in all human CSF samples, and more importantly, the antibody was able to discriminate AD CSF when compared to the control.

Table 1. Tau sequence, vaccine and antibody description

Description	Vaccine	Sequence*, length (n), sequence ID number	Hybridoma	Antibodies
T3: Tau 393-408 [pS396, pS404]	ACI-35	VYKS(p)PVVSGDTS(p)PRHL (n = 16) (SEQ ID NO: 62)	A4-4A6-48	ACI-35-4A6-Ab2
			A6-2G5-30	ACI-35-2G5-Ab2
			A6-2G5-41	ACI-35-2G5-Ab3
			A4-2A1-18	ACI-35-2A1-Ab1
			A4-2A1-40	ACI-35-2A1-Ab2
			A6-1D2-12	ACI-35-1D2-Ab1
			A4-4A6-18	ACI-35-4A6-Ab1
			A6-2G5-08	ACI-35-2G5-Ab1

*Based on the longest isoform of human Tau (Tau441). p indicates phosphorylated residue.

Table 2. Screening of hybridomas for binding to target

Hybridoma	Antibodies	ELISA				TAUPIR	Western Blot
		Tau p-peptide	Tau peptide	Full-length pTau	Full-length Tau		
A4-2A1-18	ACI-35-2A1-Ab1	+	-	+	-	+++	+
A4-2A1-40	ACI-35-2A1-Ab2	+	-	+	-	+++	+
A4-4A6-18	ACI-35-4A6-Ab1	+	-	-	+	-	+
A6-1D2-12	ACI-35-1D2-Ab1	+	-	+	-	++	-/+
A6-2G5-08	ACI-35-2G5-Ab1	+	-	-	-	-	-/+
A6-2G5-30	ACI-35-2G5-Ab2	+	-	+	-	++	+
A6-2G5-41	ACI-35-2G5-Ab3	+	-	+	-	++	+

The intensity of binding can be compared only within the same column, within the same assay (ELISA, or TAUPIR, or WB).
- Not good binding or absent; + Good binding; ++ Very good binding; +++ Great binding (better than very good binding)

Table 3. Binding affinity of anti-tau antibodies

Hybridoma	Antibodies	Association rate constant (k_d) (1/Ms)	Dissociation rate constant (k_a) (1/s)	Dissociation constant (K_D) (nM)
A4-4A6-18	ACI-35-4A6-Ab1	2.00×10^5 1.10×10^5	3.10×10^{-3} 1.70×10^{-3}	16 [a] 15 [b]
A6-1D2-12	ACI-35-1D2-Ab1	1.60×10^3 2.20×10^4	9.30×10^{-6} 1.80×10^{-3}	≤ 6 [a] 82 [b]
A6-2G5-08	ACI-35-2G5-Ab1	4.80×10^5 3.20×10^4	5.30×10^{-3} 2.20×10^{-3}	10 [a] 70 [b]
A6-2G5-30	ACI-35-2G5-Ab2	2.40×10^4	2.30×10^{-4}	10 [b]
A6-2G5-41	ACI-35-2G5-Ab3	1.70×10^4	3.80×10^{-5}	2 [b]
A4-2A1-18	ACI-35-2A1-Ab1	2.70×10^4	1.00×10^{-3}	38 [b]
A4-2A1-40	ACI-35-2A1-Ab2	3.00×10^4	9.00×10^{-4}	30 [b]

[a] Analyses performed with a Phospho-peptide purity of 64 % by HPLC.
[b] Analyses performed with a Phospho-peptide purity of 87 % by HPLC.

Table 4. Description of the AD subjects used for this study

AD subject ID	Gender	Age at death	Age at diagnosis	Disease duration	AD Braak stage
AD 18	F	82	66	16	Braak V
AD 19	F	81	77	4	Braak V
AD 20	M	88	82	6	Braak V
AD 21	F	82	77	5	Braak VI
AD 22	M	62	49	13	Braak V
AD 23	F	76	65	11	Braak VI
AD 24	F	86	84	2	Braak V
AD 25	M	81	78	3	Braak V
AD 26	F	88	83	5	Braak V
AD 27	F	85	82	3	Braak V

Table 5. Tau amino acids and phospho-residues required for antibody binding.

Hybridoma	Antibody	Epitope*
A4-2A1-18	ACI-35-2A1-Ab1	Tau aa 393-401, with requirement for pS396
A4-2A1-40	ACI-35-2A1-Ab2	Tau aa 393-401, with requirement for pS396
A4-4A6-18	ACI-35-4A6-Ab1	Tau aa 396-401, with requirement for pS396
A6-1D2-12	ACI-35-1D2-Ab1	Tau aa 394-400, with requirement for pS396
A6-2G5-08	ACI-35-2G5-Ab1	Tau aa 402-406, with requirement for pS404
A6-2G5-30	ACI-35-2G5-Ab2	Tau aa 393-400, with requirement for pS396
A6-2G5-41	ACI-35-2G5-Ab3	Tau aa 393-400, with requirement for pS396

*Based on the longest isoform of human Tau (Tau441)

Table 6. Amino Acid Sequence of the heavy chain and light chain variable regions (VH and VK) and the CDRs

Antibody	Hybridoma	VH	VK	VH CDR1	VH CDR2	VH CDR3	VK CDR1	VK CDR2	VK CDR3
ACI-35-4A6-Ab1	A4-4A6-18	QVQLQQPGAELLKPGASVKLSCKASGYTFTSYWMHWVKQRPGRGLEWIGRIDPNSDRTKYNEKFKRKATLTVDKSSSTAYMQLSSLTSEDSAVYYCARDDYAWFAYWGQGTLVTVSA (SEQ ID NO: 68)	DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSNGNTYLEWYLQKPGQSPKLLIYKLSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPPTFGGGTKLEIK (SEQ ID NO: 69)	GYTFTSYWMH (SEQ ID NO: 70)	RIDPNSDRTKYNEKFKR (SEQ ID NO: 71)	DDYAWFAY (SEQ ID NO: 72)	RSSQSIVHSNGNTYLE (SEQ ID NO: 73)	KLSNRFS (SEQ ID NO: 74)	FQGSHVPPT (SEQ ID NO: 75)
ACI-35-1D2-Ab1	A6-1D2-12	QVTLKESGPGILQSSQTLSLTCSFSGFSLSTSGMGVSWIRQPSGKGLEWLAHIYWDDDKRYNASLKSRLTISKDTSRNQVFLKITCVDTADTATYYCARLLRPYALDYWGQGTSVTVSS (SEQ ID NO: 76)	NILMTQSPSSLAVSAGEKVTMSCKSSQSVLYSSNQKNYLAWYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCLQYLSSLTFGAGTKLELK (SEQ ID NO: 77)	GFSLSTSGMGVS (SEQ ID NO: 78)	HIYWDDDKRYNASLKS (SEQ ID NO: 79)	LLRPYALDY (SEQ ID NO: 80)	KSSQSVLYSSNQKNYLA (SEQ ID NO: 81)	WASTRES (SEQ ID NO: 82)	LQYLSSLT (SEQ ID NO: 83)
ACI-35-2A1-Ab1	A4-2A1-18	EVQLQQSGPELVKPGASVKISCKASGYTFTDYYMNWVKQSHGKSLEWIGDINPNNGGTSYNQKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCVREGRFAYWGHGTLVTVSA (SEQ ID NO: 88)	DIVMTQAAPSVPVTPGESVSISCRSSKSLLHSNGNTYLYWFLQRPGQSPQLLIHRMSNLASGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLKSPYTFGGGTKLEIK (SEQ ID NO: 116)	GYTFTDYYMN (SEQ ID NO: 89)	DINPNNGGTSYNQKFKG (SEQ ID NO: 90)	EGRFAY (SEQ ID NO: 91)	RSSKSLLHSNGNTYLY (SEQ ID NO: 93)	RMSNLAS (SEQ ID NO: 94)	MQHLKSPYT (SEQ ID NO: 95)
ACI-35-2A1-Ab2	A4-2A1-40	EVQLQQSGPELVKPGASVKISCKASGYTFTDYYMNWVKQSHGKSLEWIGDINPNNGGTSYNQKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCVREGRFAYWGHGTLVTVSA (SEQ ID NO: 88)	DIX*MTQAAPSVPVTPGESVSISCRSSKSLLHSNGNTYLYWFLQRPGQSPQLLIYRMSNLASGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLKSPYTFGGGTKLEIK (SEQ ID NO: 92) *X = M or V	GYTFTDYYMN (SEQ ID NO: 89)	DINPNNGGTSYNQKFKG (SEQ ID NO: 90)	EGRFAY (SEQ ID NO: 91)	RSSKSLLHSNGNTYLY (SEQ ID NO: 93)	RMSNLAS (SEQ ID NO: 94)	MQHLKSPYT (SEQ ID NO: 95)

Antibody	Hybridoma	VH	VK	VH CDR1	VH CDR2	VH CDR3	VK CDR1	VK CDR2	VK CDR3
ACI-35-A46-Ab2	A4-4A6-48	EVQLQQSGPELVKPGA SVKISCKASGYTFTDYY MNWVKQSHGKSLEWIG DINPNNGGTSYNQKFK GKATLTVDKSSSTAYM ELRSLTSEDSAVYYCVR EGRFAYWGHGTLVTVS A (SEQ ID NO: 88)	DIVMTQAAPSVPVTPGE SVSISCRSSKSLLHSNG NTYLYWFLQRPGQSPQ LLIYRMSNLASGVPDRF SGSGSGTAFTLRISRVE AEDVGVYYCMQHLKSP YTFGGGTKLEIK (SEQ ID NO: 118)	GYTFTD YYMN (SEQ ID NO: 89)	DINPNN GGTSYN QKFKG (SEQ ID NO: 90)	EGRFA Y (SEQ ID NO: 91)	RSSKSLL HSNGNT YLY (SEQ ID NO: 93)	RMSNLA S (SEQ ID NO: 94)	MQHLKS PYT (SEQ ID NO: 95)
ACI-35-2G5-Ab1	A6-2G5-08	QVQLKQSGAELVRPGA SVKLSCKASGYTFTDYY INWVKQRPGQGLEWIA RIYPGRGNIYYNEKFKG KATLTAEKSSSTAYMQL SSLTSEDSAVYFCARF WDVTYWGQGTLVTVSA (SEQ ID NO: 96)	DVLMTQTPLSLPVSLGD QASISCRSSQSIVHSNG NTYLEWFLQKPGQSPK LLIYKVSNRFSGVPDRF SGSGSGTDFTLKISRVE AEDLGVYYCFQGSHVP YTFGGGTKLEIK (SEQ ID NO: 97)	GYTFTD YYIN (SEQ ID NO: 98)	RIYPGR GNIYYN EKFKG (SEQ ID NO: 99)	FWDVT Y (SEQ ID NO: 100)	RSSQSIV HSNGNT YLE (SEQ ID NO: 101)	KVSNRF S (SEQ ID NO: 102)	FQGSHV PYT (SEQ ID NO: 103)
ACI-35-2G5-Ab2	A6-2G5-30	EVQLQQSGPELVKPGA SVKISCKASGFTFTDYY MNWVKQSHGKSLEWIG DINPNNGGTSYHQKFK GKATLTVDKSSSTAYM ELRSLTSEDSAVYYCVR EGRFAYWGQGTLVTVS A (SEQ ID NO: 104)	DIVMTQSQKFMSTSVG DRVSVTCKASQNVGTN VAWYQQKPGQSPKALI YSASYRYSGVPDRFTG SGSGTDFTLTISNVQSE DLAEYFCQQYNSYPYT FGGGTKLEIK (SEQ ID NO: 105)	GFTFTD YYMN (SEQ ID NO: 89)	DINPNN GGTSYH QKFKG (SEQ ID NO: 115)	EGRFA Y (SEQ ID NO: 91)	KASQNV GTNVA (SEQ ID NO: 106)	SASYRY S (SEQ ID NO: 107)	QQYNSY PYT (SEQ ID NO: 108)
ACI-35-2G5-Ab3	A6-2G5-41	EVQLQQSGPELVKPGA SVKISCKASGFTFTDYY MNWVKQSHGKSLEWIG DINPNNGGTSYHQKFK GKATLTVDKSSSTAYM ELRSLTSEDSAVYYCVR EGRFAYWGQGTLVTVS A (SEQ ID NO: 104)	DIVMTQSQKFMSTSVG DRVSVTCKASQNVGTN VAWYQQKPGQSPKALI YSASYRYSGVPDRFTG SGSGTDFTLTISNVQSE DLAEYFCQQYNSYPYT FGGGTKLEIK (SEQ ID NO: 105)	GFTFTD YYMN (SEQ ID NO: 89)	DINPNN GGTSYH QKFKG (SEQ ID NO: 115)	EGRFA Y (SEQ ID NO: 91)	KASQNV GTNVA (SEQ ID NO: 106)	SASYRY S (SEQ ID NO: 107)	QQYNSY PYT (SEQ ID NO: 108)

Table 7. Nucleotide Sequence of the heavy chain and light chain variable regions (VH and VK)

Antibody	Hybridoma	VH	VK
ACI-35-4A6-Ab1	A4-4A6-18	CAGGTCCAACTGCAGCAGCCTGGGGCTGAGCTTCT GAAGCCTGGGGCTTCAGTGAAACTGTCCTGCAAGG CTTCTGGCTACACCTTCACCAGCTACTGGATGCACT GGGTGAAGCAGAGGCCTGGACGAGGCCTTGAGTG GATTGGAAGGATTGATCCTAATAGTGATCGTACTAA GTACAATGAGAAGTTCAAGCGCAAGGCCACACTGA CTGTAGACAAATCCTCCAGCACAGCCTACATGCAGC TCAGCAGCCTGACATCTGAGGACTCTGCGGTCTATT ATTGTGCAAGGGATGATTACGCCTGGTTTGCTTACT GGGGCCAAGGGACTCTGGTCACTGTCTCTGCA (SEQ ID NO: 84)	GATGTTTTGATGACCCAAACTCCACTCTCCCTGCCTGTC AGTCTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGT CAGAGCATTGTACATAGTAATGGAAACACCTATTTAGAAT GGTACCTGCAGAAACCAGGCCAGTCTCCAAAGCTCCTG ATCTACAAACTTTCCAACCGATTTTCTGGGGTCCCAGAC AGGTTCAGTGGCAGTGGATCAGGGACAGATTTCACACT CAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTT ATTACTGCTTTCAAGGTTCACATGTTCCTCCGACGTTCG GTGGAGGCACCAAGCTGGAAATCAAA (SEQ ID NO: 85)
ACI-35-1D2-Ab1	A6-1D2-12	CAGGTTACTCTGAAAGAGTCTGGCCCTGGGATATTG CAGTCCTCCCAGACCCTCAGTCTGACTTGTTCTTTC TCTGGGTTTTCACTGAGCACTTCTGGTATGGGTGTG AGCTGGATTCGTCAGCCTTCAGGAAAGGGTCTGGA GTGGCTGGCACACATTTACTGGGATGATGACAAGC GCTATAACGCATCCCTGAAGAGCCGGCTCACAATCT CCAAGGATACCTCCAGAAACCAGGTATTCCTCAAGA TCACCTGTGTGGACACTGCAGATACTGCCACATACT ACTGTGCTCGGTTACTGCGTCCTTATGCTTTGGACT ACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCA (SEQ ID NO: 86)	AACATTTTGATGACACAGTCGCCATCATCTCTGGCTGTG TCTGCAGGAGAAAAGGTCACTATGAGCTGTAAGTCCAGT CAAAGTGTTTTATACAGTTCAAATCAGAAGAACTACTTGG CCTGGTACCAGCAGAAACCAGGGCAGTCTCCTAAACTG CTGATCTACTGGGCATCCACTAGGGAATCTGGTGTCCCT GATCGCTTCACAGGCAGTGGATCTGGGACAGATTTTACT CTTACCATCAGCAGTGTACAAGCTGAAGACCTGGCAGTT TATTACTGTCTTCAATACCTCTCCTCGCTCACGTTCGGTG CTGGGACCAAGCTGGAGCTGAAA (SEQ ID NO: 87)
ACI-35-2A1-Ab1	A4-2A1-18	GAGGTCCAGCTGCAACAATCTGGACCTGAGCTGGT GAAGCCTGGGGCTTCAGTGAAGATATCCTGTAAGG CTTCTGGATACACGTTCACTGACTACTACATGAACT GGGTGAAGCAGAGCCATGGAAAGAGCCTTGAGTGG ATTGGAGATATTAATCCTAACAATGGTGGTACTAGC TACAACCAGAAGTTCAAGGGCAAGGCCACATTGACT GTAGACAAGTCCTCCAGCACAGCCTACATGGAGCT CCGCAGTCTGACATCTGAGGACTCTGCAGTCTATTA TTGTGTAAGAGAGGGGCGGTTTGCTTACTGGGGTC ATGGGACTCTGGTCACTGTCTCTGCA (SEQ ID NO: 109)	GATATTGTGATGACTCAGGCTGCACCCTCTGTACCTGTC ACTCCTGGAGAGTCAGTATCCATCTCCTGCAGGTCTAGT AAGAGTCTCCTGCATAGTAATGGCAACACTTACTTGTATT GGTTCCTGCAGAGGCCAGGCCAGTCTCCTCAGCTCCTG ATACATCGGATGTCCAACCTTGCCTCAGGAGTCCCAGAC AGGTTCAGTGGCAGTGGGTCAGGAACTGCTTTCACACT GAGAATCAGTAGAGTGGAGGCTGAGGATGTGGGTGTTT ATTACTGTATGCAACATCTAAAATCTCCGTACACGTTCGG AGGGGGGGACCAAGCTGGAAATAAAA (SEQ ID NO: 117)

Antibody	Hybridoma	VH	VK
ACI-35-2A1-Ab2	A4-2A1-40	GAGGTCCAGCTGCAACAATCTGGACCTGAGCTGGT GAAGCCTGGGGCTTCAGTGAAGATATCCTGTAAGG CTTCTGGATACACGTTCACTGACTACTACATGAACT GGGTGAAGCAGAGCCATGGAAAGAGCCTTGAGTGG ATTGGAGATATTAATCCTAACAATGGTGGTACTAGC TACAACCAGAAGTTCAAGGGCAAGGCCACACATTGACT GTAGACAAGTCCTCCAGCACAGCCTACATGGAGCT CCGCAGTCTGACATCTGAGGACTCTGCAGTCTATTA TTGTGTAAGAGAGGGGCGGTTTGCTTACTGGGGTC ATGGGACTCTGGTCACTGTCTCTGCA (SEQ ID NO: 109)	GATATTR*TGATGACTCAGGCTGCACCCTCTGTACCTGT CACTCCTGGAGAGTCAGTATCCATCTCCTGCAGGTCTAG TAAGAGTCTCCTGCATAGTAATGGCAACACTTACTTGTAT TGGTTCCTGCAGAGGCCAGGCCAGTCTCCTCAGCTCCT GATATATCGGATGTCCAACCTTGCCTCAGGAGTCCCAGA CAGGTTCAGTGGCAGTGGGTCAGGAACTGCTTTCACAC TGAGAATCAGTAGAGTGGAGGCTGAGGATGTGGGTGTT TATTACTGTATGCAACATCTAAAATCTCCGTACACGTTCG GAGGGGGGACCAAGCTGGAAATAAAA (SEQ ID NO: 110) R* = A or G
ACI-35-4A6-Ab2	A4-4A6-48	GAGGTCCAGCTGCAACAATCTGGACCTGAGCTGGT GAAGCCTGGGGCTTCAGTGAAGATATCCTGTAAGG CTTCTGGATACACGTTCACTGACTACTACATGAACT GGGTGAAGCAGAGCCATGGAAAGAGCCTTGAGTGG ATTGGAGATATTAATCCTAACAATGGTGGTACTAGC TACAACCAGAAGTTCAAGGGCAAGGCCACACATTGACT GTAGACAAGTCCTCCAGCACAGCCTACATGGAGCT CCGCAGTCTGACATCTGAGGACTCTGCAGTCTATTA TTGTGTAAGAGAGGGGCGGTTTGCTTACTGGGGTC ATGGGACTCTGGTCACTGTCTCTGCA (SEQ ID NO: 109)	GATATTGTGATGACTCAGGCTGCACCCTCTGTACCTGTC ACTCCTGGAGAGTCAGTATCCATCTCCTGCAGGTCTAGT AAGAGTCTCCCTGCATAGTAATGGCAACACTTACTTGTATT GGTTCCTGCAGAGGCCAGGCCAGTCTCCTCAGCTCCTG ATATATCGGATGTCCAACCTTGCCTCAGGAGTCCCAGAC AGGTTCAGTGGCAGTGGGTCAGGAACTGCTTTCACACT GAGAATCAGTAGAGTGGAGGCTGAGGATGTGGGTGTTT ATTACTGTATGCAACATCTAAAATCTCCGTACACGTTCGG AGGGGGGGACCAAGCTGGAAATAAAA (SEQ ID NO: 119)
ACI-35-2G5-Ab1	A6-2G5-08	CAGGTCCAGCTGAAGCAGTCTGGGGCTGAGCTGGT GAGGCCTGGGGCTTCAGTGAAACTGTCCTGCAAGG CTTCTGGCTACACTTTCACTGACTACTATATAAACTG GGTGAAGCAGAGGCCTGGACAGGGACTTGAGTGGA TTGCAAGGATTTATCCTGGAAGAGGTAATATTTACTA CAATGAGAAGTTCAAGGGCAAGGCCACACTGACTG CAGAAAAATCCTCCAGCACTGCCTACATGCAGCTCA GCAGCCTGACATCTGAGGACTCTGCTGTCTATTTCT GTGCAAGATTCTGGGACGTGACTTACTGGGGCCAA GGGACTCTGGTCACTGTCTCTGCA (SEQ ID NO: 111)	GATGTTTTGATGACCCAAACTCCACTCTCCCTGCCTGTC AGTCTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGT CAGAGCATTGTACATAGTAATGGAAACACCTATTTAGAAT GGTTCCTGCAGAAACCAGGCCAGTCTCCAAAGCTCCTG ATCTACAAAGTTTCCAACCGATTTTCTGGGGTCCCAGAC AGGTTCAGTGGCAGTGGATCAGGGACAGATTTCACACT CAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTT ATTACTGCTTTCAAGGTTCACATGTTCCGTACACGTTCG GAGGGGGGACCAAGCTGGAAATAAAA (SEQ ID NO: 112)

Antibody	Hybridoma	VH	VK
ACI-35-2G5-Ab2	A6-2G5-30	GAGGTCCAGCTGCAACAATCTGGACCTGAGCTGGT GAAGCCTGGGGCTTCAGTGAAGATATCCTGTAAGG CTTCTGGATTCACGTTCACTGACTACTACATGAACT GGGTGAAGCAGAGCCATGGAAAGAGCCTTGAGTGG ATTGGAGATATTAATCCTAACAATGGTGGTACTAGC TACCACCAGAAGTTCAAGGGCAAGGCCACATTGACT GTAGACAAGTCCTCCAGCACAGCCTACATGGAGCT CCGCAGCCTGACATCTGAGGACTCTGCAGTCTATTA CTGTGTAAGAGAGGGAAGATTTGCTTACTGGGGCC AAGGGACTCTGGTCACTGTCTCTGCA (SEQ ID NO: 113)	GACATTGTGATGACCCAGTCTCAAAAATTCATGTCCACA TCAGTAGGAGACAGGGTCAGCGTCACCTGCAAGGCCAG TCAGAATGTGGGTACTAATGTAGCCTGGTATCAACAGAA ACCAGGGCAATCTCCTAAAGCACTGATTTACTCGGCATC CTACCGGTACAGTGGAGTCCCTGATCGCTTCACAGGCA GTGGATCTGGGACAGATTTCACTCTCACCATCAGCAATG TGCAGTCTGAAGACTTGGCAGAGTATTTCTGTCAGCAAT ATAACAGCTATCCGTACACGTTCGGAGGGGGGGACCAAG CTGGAAATAAAA (SEQ ID NO: 114)
ACI-35-2G5-Ab3	A6-2G5-41	GAGGTCCAGCTGCAACAATCTGGACCTGAGCTGGT GAAGCCTGGGGCTTCAGTGAAGATATCCTGTAAGG CTTCTGGATTCACGTTCACTGACTACTACATGAACT GGGTGAAGCAGAGCCATGGAAAGAGCCTTGAGTGG ATTGGAGATATTAATCCTAACAATGGTGGTACTAGC TACCACCAGAAGTTCAAGGGCAAGGCCACATTGACT GTAGACAAGTCCTCCAGCACAGCCTACATGGAGCT CCGCAGCCTGACATCTGAGGACTCTGCAGTCTATTA CTGTGTAAGAGAGGGAAGATTTGCTTACTGGGGCC AAGGGACTCTGGTCACTGTCTCTGCA (SEQ ID NO: 113)	GACATTGTGATGACCCAGTCTCAAAAATTCATGTCCACA TCAGTAGGAGACAGGGTCAGCGTCACCTGCAAGGCCAG TCAGAATGTGGGTACTAATGTAGCCTGGTATCAACAGAA ACCAGGGCAATCTCCTAAAGCACTGATTTACTCGGCATC CTACCGGTACAGTGGAGTCCCTGATCGCTTCACAGGCA GTGGATCTGGGACAGATTTCACTCTCACCATCAGCAATG TGCAGTCTGAAGACTTGGCAGAGTATTTCTGTCAGCAAT ATAACAGCTATCCGTACACGTTCGGAGGGGGGGACCAAG CTGGAAATAAAA (SEQ ID NO: 114)

Table 8. Primers used for CDR sequencing of antibody variable regions

Subclone	Ab isotype	Primer sequences			SEQ ID NO
A4-4A6-48	IgG2b	VH primers	5'	ACTAGTCGACATGGGATGGAGCTTATCATGTTCTT	193
				ACTAGTCGACATGGGATGGAGCTTATCATGCTCTT	194
				GGGAATTCATGGAATGCACCTGGGTTTTCCTCTT	137
				GGGAATTCATGGAATGGACCTGGGTTTTCCTCTT	195
				GGGAATTCATGGAATGGACCTGGGTCTTTCTCTT	196
				GGGAATTCATGAAATGGAGCTGGGTTATTCTCTT	197
				GGGAATTCATGGAATGCAGCTGGGTTATTCTCTT	151
				GGGAATTCATGGAATGGAGCTGGGTCTTTCTCTT	121
			3'	CCCAAGCTTCCAGGGGCCAATGGATAGACGATGG	198
				CCCAAGCTTCCAGGGACCAAGGGATAGACGGATGG	199
				CCCAAGCTTCCAGGGACCAAGGGATAGACGATGG	200
				CCCAAGCTTCCAGGGGCCAATGGATAACGGTGG	141
				CCCAAGCTTCCAGGGACCAGTGGATAAACGATGG	166
				CCCAAGCTTCCAGGGACCAATGGATAAACGGATGG	131
				CCCAAGCTTCCAGGGACCAAGGGATAAACGGATGG	144
		VK primers	5'	ACTAGTCGACATGATGTACCCGGCTCAGTTTCTGGG	201
				ACTAGTCGACATGAGGACTTCGATTCAGTTCTTGGG	202
				ACTAGTCTACATGAAGTTGCCTGTTAGGCTGTTGGTGCT	203
				ACTAGTCGACATGAAGTTGTCTGTTAGGCTGTTGGTGCT	204
				ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT	50
			3'	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
A4-4A6-18	IgG2b	VH primers	5'	ATGGGATGGAGCTRTATCATSYTCTT	205
				ATGAAGWTGTGGBTRAACTGGRT	206
				ATGGRATGGASCKKIRTCTTTMTCT	207
			3'	CCAGGGRCCARKGGATARACIGRTGG	208

Subclone	Ab isotype	Primer sequences			SEQ ID NO
		VK primers	5'	ATGGAGACAGACACACTCCTGCTAT	209
				ATGGAGWCAGACACACTSCTGYTATGGGT	210
				ATGAAGTTGCCTGTTAGGCTGTTGGTGCT	211
				ATGGATTTWCARGTGCAGATTWTCAGCTT	212
				ATGGTYCTYATVTCCTTGCTGTTCTGG	213
				ATGGTYCTYATVTTRCTGCTGCTATGG	214
			3'	ACTGGATGGTGGGAAGATGGA	215
A6-1D2-12	IgG2a	VH primers	5'	ATGAAATGCAGCTGGRTYATSTTCTT	216
				ATGGRCAGRCTTACWTYYTCATTCCT	217
				ATGATGGTGTTAAGTCTTCTGTACCT	218
			3'	CCAGGGRCCARKGGATARACIGRTGG	208
		VK primers	5'	ATGRAGWCACAKWCYCAGGTCTTT	219
				ATGGAGACAGACACACTCCTGCTAT	209
				ATGGAGWCAGACACACTSCTGYTATGGGT	210
				ATGAGGRCCCCTGCTCAGWTTYTTGGIWTCTT	220
				ATGGGCWTCAAGATGRAGTCACAKWYYCWGG	221
				ATGAAGTTGCCTGTTAGGCTGTTGGTGCT	211
				ATGGATTTWCARGTGCAGATTWTCAGCTT	212
				ATGGTYCTYATVTCCTTGCTGTTCTGG	213
				ATGGTYCTYATVTTRCTGCTGCTATGG	214
			3'	ACTGGATGGTGGGAAGATGGA	215
A4-2A1-18	IgG2b	VH primers	5'	GGGAATTCATGGAATGGAGCTGGGTCATTCTCTT	136
				GGGAATTCATGGAATGCAGCTGGGTTTTTCTCTT	120
				GGGAATTCATGGAATGGAGCTGGGTTTTTCTCTT	123
				GGGAATTCATGGAATGCACCTGGGTTTTCCTCTT	137
				GGGAATTCATGGAATGGAGCTGGGTCTTCCTCTT	138
				GGGAATTCATGGAATGGAGCTGGGTCATCCTCTT	139
				GGGAATTCATGGAATGGAGCTGGGTTATTCTCTT	124
				ACTAGTCGACATGGGATGAGCTTATCATCCTCTT	140
			3'	CCCAAGCTTCCAGGGGCCAATGGATAACGGTGG	141
				CCCAAGCTTCCAGGGACCAGTGGGATAAACGGGTGG	142

Subclone	Ab isotype	Primer sequences			SEQ ID NO
				CCCAAGCTTCCAGGGGCCAATGGATAAACGGGTGG	134
				CCCAAGCTTCCAGGGACCAAGGGATAGACGGGTGG	143
				CCCAAGCTTCCAGGGACCAAGGGATAAACGGATGG	144
				CCCAAGCTTCCAGGGACCAGGGGATAAACGGATGG	145
				CCCAAGCTTCCAGGGACCAATGGATAAACGGATGG	131
				CCCAAGCTTCCAGGGGCCAGGGATAAACGGGTGG	146
				CCCAAGCTTCCAGGGGCCAATGGATAAACCGGTGG	147
				CCCAAGCTTCCAGGGACCAGTGGATAAACGGTGG	148
		VK primers	5'	ACTAGTCGACATGGTGTCCACAGCTCAGTTCCTTG	149
			3'	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
A4-2A1-40	IgG2b	VH primers	5'	GGGAATTCATGGAATGGAGCTGGGTCATCCTCTT	139
				GGGAATTCATGGAATGCAGCTGGGTTTTCCTCTT	154
				GGGAATTCATGGAATGCAGCTGGGTCTTTCTCTT	155
				GGGAATTCATGGAATGGAGCTGGGTTTTCCTCTT	127
				GGGAATTCATGGAATGGAGCTGGGTCTTTCTCTT	121
				ACTAGTCGACATGGATGGAGCTTATCATCCTCTT	175
			3'	CCCAAGCTTCCAGGGACCAAGGGATAAACGGTGG	176
				CCCAAGCTTCCAGGGGCCAATGGATAAACCGGTGG	147
				CCCAAGCTTCCAGGGACCAATGGATAAACGATGG	129
				CCCAAGCTTCCAGGGGCCAGTGGATAAACGGGTGG	177
				CCCAAGCTTCCAGGGACCAATGGATAACGGGTGG	128
		VK primers	5'	ACTAGTCGACATGAGGTACTCGGCTCAGTTCCTGGG	178
				ACTAGTCGACATGAGGTCCCCGGCTCAGTTCCTGGG	179
				ACTAGTCGACATGAGGACGTCGATTCAGTTCTTGGG	180
			3'	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
A6-2G5-08	IgG2a	VH primers	5'	GGGAATTCATGGAATGCAGCTGGGTTTTTCTCTT	120
				GGGAATTCATGGAATGGAGCTGGGTCTTTCTCTT	121
				GGGAATTCATGGAATGCAGCTGGGTCATTCTCTT	122
				GGGAATTCATGGAATGGAGCTGGGTTTTTCTCTT	123
				GGGAATTCATGGAATGGAGCTGGGTTATTCTCTT	124
				GGGAATTCATGAAATGGAGCTGGGTCTTTTCTT	125
				GGGAATTCATGGAATGCAGCTGGGTCTTCCTCTT	126

Subclone	Ab isotype	Primer sequences			SEQ ID NO
				GGGAATTCATGGAATGGAGCTGGGTTTTCCTCTTC	127
			3'	CCCAAGCTTCCAGGGACCAATGGATAACGGGTGG	128
				CCCAAGCTTCCAGGGACCAATGGATAAACGATGG	129
				CCCAAGCTTCCAGGGACCAATGGATAAACGGTGG	130
				CCCAAGCTTCCAGGGACCAATGGATAAACGGATGG	131
				CCCAAGCTTCCAGGGACCAGTGGATAGACGGGTGG	132
				CCCAAGCTTCCAGGGACCAAGGGATAGATGATGG	133
				CCCAAGCTTCCAGGGGCCAATGGATAAACGGGTGG	134
				CCCAAGCTTCCAGGGGCCAATGGATAAACGATGG	135
		VK primers	5'	ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT	50
			3'	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
A6-2G5-30	IgG2b	VH primers	5'	GGGAATTCATGAAATGGAGCTGGGTCTTCCTCTT	150
				GGGAATTCATGGAATGCAGCTGGGTTATTCTCTT	151
				GGGAATTTATGGAATGGAGCTGGGTCTTCCTCTT	152
				GGGAATTCATGGAATGGAGCTGGGTTTTCCTCTT	127
				GGGAATTCATGGAATGCAGCTGGGTCATCCTCTT	153
				GGGAATTCATGGAATGGAGCTGGGTTATTCTCTT	124
				GGGAATTCATGGAATGCAGCTGGGTTTTCCTCTT	154
				GGGAATTCATGGAATGCAGCTGGGTCTTTCTCTT	155
				ACTAGTCGACATGGGATGGAGCTATATCATCCTCTT	156
				ACTAGTCGACATGGGATGGAGCTTATCATCTTCTT	157
				ACTAGTCGACATGTAGATGTGGTTAAACTGGGT	158
			3'	CCCAAGCTTCCAGGGGCCAGGGGATAAACGGATGG	159
				CCCAAGCTTCCAGGGGCCAAGGGATAGACGGATGG	160
				CCCAAGCTTCCAGGGACCAGGGGATAGACGGGTGG	161
				CCCAAGCTTCCAGGGACCAGGGGATAGACGGATGG	162
				CCCAAGCTTCCAGGGGCCAGTGGATAAACGGATGG	163
				CCCAAGCTTCCAGGGGCCAATGGATAACGATGG	164
				CCCAAGCTTCCAGGGGCCAGTGGATAAACGATGG	165
				CCCAAGCTTCCAGGGACCAATGGATAAACGGTGG	130
				CCCAAGCTTCCAGGGACCAGTGGATAAACGATGG	166
				CCCAAGCTTCCAGGGACCAATGGATAACGATGG	167

Subclone	Ab isotype	Primer sequences			SEQ ID NO
		VK primers		CCCAAGCTTCCAGGGACCATGGATAAACGGGTGG	168
			5'	ACTAGTCGACATGGGCATCAAGATGAAGTCACATACTCTGG	169
				ACTAGTCGACATGGGCATCAAGATGAGTCACATACTCTGG	170
				ACTAGTCGACTGGGCATCAGATGAGTCACATACTCTGG	171
				ACTAGTCGACATGGGCATCAAGATGAAGTCACAGACCCAGG	172
				ACTAGTCGACATGGGCTTCAAGATGAAGTCACATTCTCTGG	173
				ACTAGTCGACATGGGCTTCAAGATGAAGTCACATATTCAGG	174
				CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
			3'	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
A6-2G5-41	IgG2b	VH primers	5'	GGGAATTCATGGAATGGACCTGGGTCATCCTCTT	181
				GGGAATTCATGGAATGCAGCTGGGTTTTTCTCTT	120
				GGGAATTCATGGAATGCAGCTGGGTTATCCTCTT	182
				GGGAATTCATGGAATGGAGCTGGGTTATTCTCTT	124
				GGGAATTCATGGAATGCAGCTGGGTCTTCCTCTT	126
				GGGAATTCATGAATGGATCTGGGTTATTCTCTT	183
			3'	CCCAAGCTTCCAGGGACCAGGGGATAAACGGGTGG	184
				CCCAAGCTTCCAGGGACCAAGGGACGGGTGG	185
				CCCAAGCTTCCAGGGACCAATGGATAAACAGATGG	186
				CCCAAGCTTCCAGGGACCAAGGGATAAACGGGATGG	144
				CCCAAGCTTCCAGGGACCAGGGGATAAACGGGATGG	145
				CCCAAGCTTCCAGGGACCAAGGGATAAACGGGTGG	187
		VK primers	5'	GGGAATTCATGGAGACACATTCCCAGGTCTTT	188
				GGGAATTCATGGAGTCACAGTCTCAGGTCTTT	189
				ACTAGTCGACATGGGCTTCAAGATGGAGTCACATTTTCAGG	190
				ACTAGTCGACATGGGCATCAAGATGAAGTCACATATTCAGG	191
				ACTAGTCGACATGGGCTTCAAGATGAAGTCACATTCTCAGG	192
				CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
			3'	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51

Degenerate Codons:

R	= A or G	S	= C or G	D	= A or G or T	B	= C or G or T
Y	= C or T	M	= A or C	H	= A or C or T		
K	= G or T	W	= A or T	V	= A or G or C		

Table 9. Longest isoform of human Tau (441aa), also called Tau40

Longest isoform of human Tau (441aa), also called Tau40 (SEQ ID NO: 67) Microtubule-associated protein tau isoform 2 [Homo sapiens] NCBI Reference Sequence: NP_005901.2	MAEPRQEFEV MEDHAGTYGL GDRKDQGGYT MHQDQEGDTD AGLKESPLQT PTEDGSEEPG SETSDAKSTP TAEDVTAPLV DEGAPGKQAA AQPHTEIPEG TTAEEAGIGD TPSLEDEAAG HVTQARMVSK SKDGTGSDDK KAKGADGKTK IATPRGAAPP GQKGQANATR IPAKTPPAPK TPPSSGEPPK SGDRSGYSSP GSPGTPGSRS RTPSLPTPPT REPKKVAVVR TPPKSPSSAK SRLQTAPVPM PDLKNVKSKI GSTENLKHQP GGGKVQIINK KLDLSNVQSK CGSKDNIKHV PGGGSVQIVY KPVDLSKVTS KCGSLGNIHH KPGGGQVEVK SEKLDFKDRV QSKIGSLDNI THVPGGGNKK IETHKLTFRE NAKAKTDHGA EIVYKSPVVS GDTSPRHLSN VSSTGSIDMV DSPQLATLAD EVSASLAKQG L (SEQ ID NO: 67)

Deposits:

Table 10. The following hybridoma cell lines were deposited in the name of AC Immune SA, PSE-EPFL Building B, 1015 Lausanne/Switzerland and Katholieke Universiteit Leuven, Waaistraat 6 - Box 5105, 3000 Leuven/Belgium with the "Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in Braunschweig, Inhoffenstrasse 7 B, 38124 Braunschweig, under the provisions of the Budapest Treaty:

Hybridoma name	Deposit number	Date of deposit
A4-4A6-48	DSM ACC3136	August 30, 2011
A6-2G5-30	DSM ACC3137	August 30, 2011
A6-2G5-41	DSM ACC3138	August 30, 2011
A4-2A1-18	DSM ACC3139	August 30, 2011
A4-2A1-40	DSM ACC3140	August 30, 2011
A6-1D2-12	DSM ACC3141	September 6th, 2011

Table 11A. Peptide library used for epitope mapping

Peptide library for T3

	Tau(441) amino acid number	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408
	Amino acid	V	Y	K	S(p)	P	V	V	S	G	D	T	S(p)	P	R	H	L
Phospho peptides	Peptide no.																
	T3.9	V	Y	K	S(p)	P	V	V	S								
	T3.10		Y	K	S(p)	P	V	V	S	G							
	T3.11			K	S(p)	P	V	V	S	G	D						
	T3.12				S(p)	P	V	V	S	G	D	T					
	T3.13					P	V	V	S	G	D	T	S(p)				
	T3.14						V	V	S	G	D	T	S(p)	P			
	T3.15							V	S	G	D	T	S(p)	P	R		
	T3.16								S	G	D	T	S(p)	P	R	H	
	T3.17									G	D	T	S(p)	P	R	H	L

	Amino acid	V	Y	K	S	P	V	V	S	G	D	T	S	P	R	H	L
Non-phospho peptides	Peptide no.																
	T3.18	V	Y	K	S	P	V	V	S								
	T3.19		Y	K	S	P	V	V	S	G							
	T3.20			K	S	P	V	V	S	G	D						
	T3.21				S	P	V	V	S	G	D	T					
	T3.22					P	V	V	S	G	D	T	S				
	T3.23						V	V	S	G	D	T	S	P			
	T3.24							V	S	G	D	T	S	P	R		
	T3.25								S	G	D	T	S	P	R	H	
	T3.26									G	D	T	S	P	R	H	L

Table 11B. Alanine (Ala) substitution peptide library used for epitope mapping of pS396-specific antibodies

Peptide No.	393	394	395	396	397	398	399	400
T3-Ala.A1	V	Y	K	S	P	V	V	S
T3-Ala.A2	V	Y	K	S(p)	P	V	V	S
T3-Ala.A3	A	Y	K	S(p)	P	V	V	S
T3-Ala.A4	V	A	K	S(p)	P	V	V	S
T3-Ala.A5	V	Y	A	S(p)	P	V	V	S
T3-Ala.A6	V	Y	K	A	P	V	V	S
T3-Ala.A7	V	Y	K	S(p)	A	V	V	S
T3-Ala.A8	V	Y	K	S(p)	P	A	V	S
T3-Ala.A9	V	Y	K	S(p)	P	V	A	S
T3-Ala.A10	V	Y	K	S(p)	P	V	V	A

Table 11C. Alanine (Ala) substitution peptide library used for epitope mapping of pS404-specific antibodies

Peptide No.	400	401	402	403	404	405	406	407
T3-Ala.B1	S	G	D	T	S	P	R	H
T3-Ala.B2	S	G	D	T	S(p)	P	R	H
T3-Ala.B3	A	G	D	T	S(p)	P	R	H
T3-Ala.B4	S	A	D	T	S(p)	P	R	H
T3-Ala.B5	S	G	A	T	S(p)	P	R	H
T3-Ala.B6	S	G	D	A	S(p)	P	R	H
T3-Ala.B7	S	G	D	T	A	P	R	H
T3-Ala.B8	S	G	D	T	S(p)	A	R	H
T3-Ala.B9	S	G	D	T	S(p)	P	A	H
T3-Ala.B10	S	G	D	T	S(p)	P	R	A

REFERENCE LIST

Alonso A.D., et al. (1997), Proc.Natl.Acad.Sci.U.S.A., 94, 298-303

Alving et al.,(1992) Infect. Immun. 60:2438-2444

Asuni et al., (2007) J Neurosc. 27 (34), 9115-29

Braak and Braak (1991) Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 82:239-259)

Braak H., et al. (1993), Eur.Neurol., 33, 403-408

Gill et al., Nature Med. 9: 589-595 (2003)

Greenberg S.G., et al. (1992), J Biol.Chem., 267, 564-569.

Harlow and Lane, Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory, New York 1988 555-612

Hodgson et al.,(1991) Bio/Technoloy, 9:421

Hoffmann R., et al (1997), Biochemistry, 36, 8114-8124.

Kabat EA, Wu TT, Perry HM, Gottesman KS, Foeller C. *Sequences of proteins of Immunological Interest,* US Department of Health and Human Services, 1991

Kennedy, J. H., et al., 1976 (Clin. Chim. Acta 70:1-31)

Khaw, B. A. et al. (1982) J. Nucl. Med. 23:1011-1019

Lewis et al., (2000) Nature Genetics, 25 :402-405

Masliah et al., (2005) Neuron, 46(6), 857-68

Masliah et al., (2011) PLoS ONE, Volume 6(4), e19338, pp- 1-17

Muhs et al., (2007) Proc Natl Acad Sci USA, 104(23), 9810-5

Muyllaert et al, (2006) Rev Neurol, 162(10), 903-907

Muyllaert et al, (2008) Genes Brain Behav., Suppl. 1, 57-66

Neuwelt, E. A., Implication of the Blood-Brain Barrier and its Manipulation, Vols 1 & 2, Plenum Press, N. Y. (1989))

Nicolau et. al. (2002) Proc Natl. Acad. Sci USA 99, 2332-2337

Nicoll et al., (2003) Nature Med, 9, 448-452

Oddo et al., (2004) Neuron, 43, 321-332

Queen et al.,(1989) Proc. Natl Acad Sci USA, 86:10029-10032

Papanastassiou et al., Gene Therapy 9: 398-406 (2002)

Reig S., et al. (1995), Acta Neuropathol., 90, 441-447

Ribe et al., (2005) Neurobiol Dis, 20(3), 814-22

Roberson et al, (2007) Science, 316 (5825), 750-4

Rosenmann et al., (2006) Arch Neurol, 63(10), 1459-67

Rousseaux et al. Methods Enzymology, (1986) , Academic Press 121:663-69

Schurs, A. H. W. M., et al. 1977 {Clin. Chim Acta 57:1-40

Terwel et al., (2006) J Biol Chem, 280, 3963-3973

Terwel et al, (2008) Am J pathol., 172(3), 786-98

Urushitiani et al., (2007) Proc. Natl Acad Sci USA, 104(79, 2495-500

Vandebroek et al., "*Phosphorylation and Aggregation of Protein Tau in Humanized Yeast Cells and in Transgenic Mouse Brain*"; 7th International Conference on Alzheimer's and Parkinson's Disease, Sorrento, Italy, March 9-13, 2005, pp 15-19

Wagner et al (2002) Journal of Liposome Research Vol 12(3), pp 259 – 270

WO 2004/058258

WO 96/13590

WO 96/29605

U.S. Patent Publication No. 2002/0038086

U.S. Patent Publication No. 2003/0083299

U.S. Patent Publication No. 2002/0025313

U.S. Patent Publication No 2004/0204354

U.S. Patent Publication No 2004/0131692

U.S. Patent Publication No 2002/0065259

U.S. Patent Publication No 2003/0162695

U.S. Patent Publication No 2005/0124533

U.S. Patent Publication No 2005/0089473

U.S. Patent Publication No 2003/0073713

U.S. Patent Publication No 2003/0129186

U.S. Patent No. 5,112,596,

U.S. Patent No. 5,268,164,

U.S. Patent No. 5,506,206,

U.S. Patent No. 5,686,416

U.S. Patent No. 5,004,697

CLAIMS

1. An antibody, or a functional fragment thereof, which recognizes and specifically binds to a phospho-epitope on the mammalian Tau protein or on a fragment thereof, wherein said antibody or antibody fragment has a high binding affinity to soluble, oligomeric and insoluble phosphorylated Tau protein and is capable of detecting and/or modulating levels of soluble, oligomeric and insoluble phosphorylated Tau protein *in vivo*.

2. An antibody or a functional fragment thereof, wherein said antibody or antibody fragment binds to a phospho-epitope having, or within, the amino acid sequence VYKSPVVSGDTSPRHL (SEQ ID NO: 62) (Tau aa 393-408 of SEQ ID NO: 67) comprising a phosphorylated Ser at position 396 (pS396) and at position 404 (pS404).

3. The antibody of claim 2 or a functional fragment thereof, wherein said antibody or antibody fragment is a modulating antibody which modulates soluble and/or insoluble Tau levels in the brain of a mammal.

4. The antibody of claim 3 or a functional fragment thereof, wherein said antibody or antibody fragment modulates soluble and/or insoluble Tau levels in brain cortex and/or hippocampus.

5. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment reduces the levels of total soluble and/or insoluble tau protein.

6. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment reduces the levels of soluble and/or insoluble phosphorylated tau protein.

7. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment reduces the levels of paired helical filaments containing hyperphosphorylated tau protein.

8. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment reduces the levels of total soluble tau protein, soluble phosphorylated tau protein and pTau paired helical filaments.

9. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said mammal is a human.

10. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has a dissociation constant of at least 10 nM.

11. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has a dissociation constant of at least 82 nM, 5 nM, at least 2 nM; at least 1 nM, at least 500 pM, at least 300 pM, at least 200 pM, at least 100 pM.

12. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has an association rate constant of 10^4 $M^{-1}s^{-1}$ or greater; of 10^5 $M^{-1}s^{-1}$ or greater, of 10^6 $M^{-1}s^{-1}$ or greater.

13. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has a high binding affinity with a dissociation constant of at least 4 nM and an association rate constant of 10^5 $M^{-1}s^{-1}$ or greater.

14. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has a high binding affinity with a dissociation constant of at least 3 nM and an association rate constant of 10^6 $M^{-1}s^{-1}$ or greater.

15. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has a high binding affinity with a dissociation constant of at least 2 nM and an association rate constant of 10^4 $M^{-1}s^{-1}$ or greater.

16. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has a high binding affinity with a dissociation constant of at least 1 nM and an association rate constant of 10^5 $M^{-1}s^{-1}$ or greater.

17. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has a high binding affinity with a dissociation constant of at least 200 pM and an association rate constant of 10^5 $M^{-1}s^{-1}$ or greater.

18. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has a high binding affinity with a dissociation constant of at least 100 pM and an association rate constant of 10^6 $M^{-1}s^{-1}$ or greater.

19. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment binds to an epitope selected from the group consisting of:

a. VYKSPVVSG (Tau aa 393-401 of SEQ ID NO: 67) comprising a phosphorylated Ser at position 396 (pS396);

b. SPVVSG (Tau aa 396-401 of SEQ ID NO: 67) comprising a phosphorylated Ser at position 396 (pS396);

c. YKSPVVS (Tau aa 394-400 of SEQ ID NO: 67) comprising a phosphorylated Ser at position 396 (pS396);

d. DTSPR (Tau aa 402-406 of SEQ ID NO: 67) comprising a phosphorylated Ser at position 404 (pS404); and

e. VYKSPVVS (Tau aa 393-400 of SEQ ID NO: 67) comprising a phosphorylated Ser at position 396 (pS396).

20. The antibody of any of the preceding claims or a functional fragment thereof, wherein said antibody or fragment thereof binds to an epitope on a mammalian Tau protein, particularly on the human Tau protein, but especially the human Tau protein as shown in SEQ ID NO: 67, comprising Tau aa 393-401 (VYKSPVVSG) comprising a phosphorylated Ser at position 396 (pS396).

21. The antibody of any of the preceding claims or a functional fragment thereof, wherein said antibody or fragment thereof binds to an epitope on a mammalian Tau protein, particularly on the human Tau protein, but especially the human Tau protein as shown in SEQ ID NO: 67, comprising Tau aa 396-401 (SPVVSG) comprising a phosphorylated Ser at position 396 (pS396).

22. The antibody of any of the preceding claims or a functional fragment thereof, wherein said antibody or fragment thereof binds to an epitope on a mammalian Tau protein, particularly on the human Tau protein, but especially the human Tau protein as shown in SEQ ID NO: 67, comprising Tau aa 394-400 (YKSPVVS) comprising a phosphorylated Ser at position 396 (pS396).

23. The antibody of any of the preceding claims or a functional fragment thereof, wherein said antibody or fragment thereof binds to an epitope on a mammalian Tau protein, particularly on the human Tau protein, but especially the human Tau protein as shown in SEQ ID NO: 67, comprising Tau aa 402-406 (DTSPR) comprising a phosphorylated Ser at position 404 (pS404).

24. The antibody of any of the preceding claims or a functional fragment thereof, wherein said antibody or fragment thereof binds to an epitope on a mammalian Tau protein, particularly on the human Tau protein, but especially the human Tau protein as shown in SEQ ID NO: 67, comprising Tau aa 393-400 (VYKSPVVS) comprising a phosphorylated Ser at position 396 (pS396).

25. The antibody of any of the preceding claims or a functional fragment thereof, which antibody or fragment thereof recognizes and specifically binds to a phospho-epitope on a mammalian Tau protein or on a fragment thereof, wherein said antibody or fragment thereof comprises a first binding domain comprising a CDR1 comprising the amino acid sequence shown in SEQ ID NO: 73, a CDR2 comprising the amino acid sequence shown in SEQ ID NO: 74, and a CDR3 comprising the amino acid sequence shown in SEQ ID NO: 75, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto and/or a second binding domain comprising a CDR1 comprising the amino acid sequence shown in SEQ ID NO: 70, or an amino acid sequence at least 95%, particularly 98%, particularly 99% identical thereto, a CDR2 comprising the amino acid sequence shown in SEQ ID NO: 71, or an amino acid sequence at least 94%, 95%, 96%, 97%, 98%, or 99% identical thereto, and a CDR3 comprising the amino acid sequence shown in SEQ ID NO: 72, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto.

26. The antibody of any of the preceding claims or a functional fragment thereof, which antibody or fragment thereof recognizes and specifically binds to a phospho-epitope on a mammalian Tau protein or on a fragment thereof, wherein said antibody or fragment thereof comprises:

    a. a first binding domain comprising a CDR1 comprising the amino acid sequence shown in SEQ ID NO: 73, a CDR2 comprising the amino acid sequence shown in SEQ ID NO: 74, and a CDR3 comprising the amino acid sequence shown in SEQ ID NO: 75; and/or

    b. a second binding domain comprising an amino acid sequence comprising a CDR1 comprising the amino acid sequence shown in SEQ ID NO: 70, a CDR2 comprising the amino acid sequence shown in SEQ ID NO: 71, and a CDR3 comprising the amino acid sequence shown in SEQ ID NO: 72.

27. The antibody of any of the preceding claims or a functional fragment thereof, which antibody or fragment thereof recognizes and specifically binds to a phospho-epitope on a mammalian Tau protein or on a fragment thereof, wherein said antibody or fragment thereof comprises a first binding domain comprising a CDR1 with the amino acid sequence shown in SEQ ID NO: 81, a CDR2 with the amino acid sequence shown in

SEQ ID NO: 82, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 83, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto and/or a second binding domain comprising a CDR1 with the amino acid sequence shown in SEQ ID NO: 78, a CDR2 with the amino acid sequence shown in SEQ ID NO: 79, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 80, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto.

28. The antibody of any of the preceding claims or a functional fragment thereof, which antibody or fragment thereof recognizes and specifically binds to a phospho-epitope on a mammalian Tau protein or on a fragment thereof, wherein said antibody or fragment thereof comprises:

    a. a first binding domain comprising a CDR1 comprising the amino acid sequence shown in SEQ ID NO: 81, a CDR2 comprising the amino acid sequence shown in SEQ ID NO: 82, and a CDR3 comprising the amino acid sequence shown in SEQ ID NO: 83; and/or

    b. a second binding domain comprising an amino acid sequence comprising a CDR1 comprising the amino acid sequence shown in SEQ ID NO: 78, a CDR2 comprising the amino acid sequence shown in SEQ ID NO: 79, and a CDR3 comprising the amino acid sequence shown in SEQ ID NO: 80.

29. The antibody of any of the preceding claims or a functional fragment thereof, which antibody or fragment thereof recognizes and specifically binds to a phospho-epitope on a mammalian Tau protein or on a fragment thereof, wherein said antibody or fragment thereof comprises a first binding domain comprising a CDR1 with the amino acid sequence shown in SEQ ID NO: 93, a CDR2 with the amino acid sequence shown in SEQ ID NO: 94, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 95, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs and/or a second binding domain comprising a CDR1 with the amino acid sequence shown in SEQ ID NO: 89, a CDR2 with the amino acid sequence shown in

SEQ ID NO: 90, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 91, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs.

30. The antibody of any of the preceding claims or a functional fragment thereof, which antibody or fragment thereof recognizes and specifically binds to a phospho-epitope on a mammalian Tau protein or on a fragment thereof, wherein said antibody or fragment thereof comprises:

    a. a first binding domain comprising a CDR1 comprising the amino acid sequence shown in SEQ ID NO: 93, a CDR2 comprising the amino acid sequence shown in SEQ ID NO: 94, and a CDR3 comprising the amino acid sequence shown in SEQ ID NO: 95; and/or

    b. a second binding domain comprising an amino acid sequence comprising a CDR1 comprising the amino acid sequence shown in SEQ ID NO: 89, a CDR2 comprising the amino acid sequence shown in SEQ ID NO: 90, and a CDR3 comprising the amino acid sequence shown in SEQ ID NO: 91.

31. The antibody of any of the preceding claims or a functional fragment thereof, which antibody or fragment thereof recognizes and specifically binds to a phospho-epitope on a mammalian Tau protein or on a fragment thereof, wherein said antibody or fragment thereof comprises a first binding domain comprising a CDR1 with the amino acid sequence shown in SEQ ID NO: 101, a CDR2 with the amino acid sequence shown in SEQ ID NO: 102, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 103, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs and/or a second binding domain comprising in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 98, a CDR2 with the amino acid sequence shown in SEQ ID NO: 99, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 100, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs.

32. The antibody of any of the preceding claims or a functional fragment thereof, which antibody or fragment thereof recognizes and specifically binds to a phospho-epitope on a mammalian Tau protein or on a fragment thereof, wherein said antibody or fragment thereof comprises:

    a. a first binding domain comprising a CDR1 comprising the amino acid sequence shown in SEQ ID NO: 101, a CDR2 comprising the amino acid sequence shown in SEQ ID NO: 102, and a CDR3 comprising the amino acid sequence shown in SEQ ID NO: 103; and/or

    b. a second binding domain comprising an amino acid sequence comprising a CDR1 comprising the amino acid sequence shown in SEQ ID NO: 98, a CDR2 comprising the amino acid sequence shown in SEQ ID NO: 99, and a CDR3 comprising the amino acid sequence shown in SEQ ID NO: 100.

33. The antibody of any of the preceding claims or a functional fragment thereof, which antibody or fragment thereof recognizes and specifically binds to a phospho-epitope on a mammalian Tau protein or on a fragment thereof, wherein said antibody or fragment thereof comprises a first binding domain comprising a CDR1 with the amino acid sequence shown in SEQ ID NO: 106, a CDR2 with the amino acid sequence shown in SEQ ID NO: 107, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 108, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs and/or a second binding domain comprising a CDR1 with the amino acid sequence shown in SEQ ID NO: 89, a CDR2 with the amino acid sequence shown in SEQ ID NO: 115, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 91, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs.

34. The antibody of any of the preceding claims or a functional fragment thereof, which antibody or fragment thereof recognizes and specifically binds to a phospho-epitope on a mammalian Tau protein or on a fragment thereof, wherein said antibody or fragment thereof comprises:

    a. a first binding domain comprising a CDR1 comprising the amino acid sequence shown in SEQ ID NO: 106, a CDR2 comprising the amino acid

sequence shown in SEQ ID NO: 107, and a CDR3 comprising the amino acid sequence shown in SEQ ID NO: 108; and/or

b. a second binding domain comprising an amino acid sequence comprising a CDR1 comprising the amino acid sequence shown in SEQ ID NO: 89, a CDR2 comprising the amino acid sequence shown in SEQ ID NO: 115, and a CDR3 comprising the amino acid sequence shown in SEQ ID NO: 91.

35. The antibody of any one of the preceding claims or a functional fragment thereof comprising a first binding domain comprising the amino acid sequence shown selected from SEQ ID NO: 69, 77, 116, 92, 118, 97, 105, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto, and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 68, 76, 88, 96, 104, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto.

36. The antibody of any one of the preceding claims or a functional fragment thereof, which recognizes and specifically binds to a phospho-epitope on a mammalian Tau protein or on a fragment thereof, wherein said antibody or functional fragment thereof comprises a first binding domain comprising the amino acid sequence shown in SEQ ID NO: 69, or an amino acid sequence at least 98% or 99% identical thereto; and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 68, or an amino acid sequence at least 90%, 91%, 92% or 93% identical thereto.

37. The antibody of any one of the preceding claims or a functional fragment thereof, which recognizes and specifically binds to a phospho-epitope on a mammalian Tau protein or on a fragment thereof, wherein said antibody or functional fragment thereof comprises a first binding domain comprising the amino acid sequence shown in SEQ ID NO: 77, or an amino acid sequence at least 93%, 94% or 95% identical thereto; and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 76, or an amino acid sequence at least 88%, 89%, or 90% identical thereto.

38. The antibody of any one of the preceding claims or a functional fragment thereof, which recognizes and specifically binds to a phospho-epitope on a mammalian Tau protein or on a fragment thereof, wherein said antibody or functional fragment thereof comprises a first binding domain comprising the amino acid sequence shown in SEQ ID NO: 116, 92,

or 118, or an amino acid sequence at least 93%, 94% or 95% identical thereto; and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 88, or an amino acid sequence at least 90%, 91%, 92% or 93% identical thereto.

39. The antibody of any one of the preceding claims or a functional fragment thereof, which recognizes and specifically binds to a phospho-epitope on a mammalian Tau protein or on a fragment thereof, wherein said antibody or functional fragment thereof comprises a first binding domain comprising the amino acid sequence shown in SEQ ID NO: 97, or an amino acid sequence at least 99% identical thereto; and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 96, or an amino acid sequence at least 86%, 87%, 88% or 90% identical thereto.

40. The antibody of any one of the preceding claims or a functional fragment thereof, which recognizes and specifically binds to a phospho-epitope on a mammalian Tau protein or on a fragment thereof, wherein said antibody or functional fragment thereof comprises a first binding domain comprising the amino acid sequence shown in SEQ ID NO: 105, or an amino acid sequence at least 98%, or 99% identical thereto; and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 104, or an amino acid sequence at least 88%, 89%, or 90% identical thereto.

41. The antibody of any one of the preceding claims or a functional fragment thereof comprising:

a. a first binding domain comprising the amino acid sequence shown in SEQ ID NO: 69 and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 68; or

b. a first binding domain comprising the amino acid sequence shown in SEQ ID NO: 77 and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 76; or

c. a first binding domain comprising the amino acid sequence shown in SEQ ID NO: 116 and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 88; or

d. a first binding domain comprising the amino acid sequence shown in SEQ ID NO: 92 and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 88; or

e. a first binding domain comprising the amino acid sequence shown in SEQ ID NO: 97 and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 96; or

f. a first binding domain comprising the amino acid sequence shown in SEQ ID NO: 105 and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 104, or

g. a first binding domain comprising the amino acid sequence shown in SEQ ID NO: 118 and/or a second binding domain comprising the amino acid sequence shown in SEQ ID NO: 88.

42. The antibody of any one of the preceding claims or a functional fragment thereof, which is polyclonal antibody, a monoclonal antibody, a chimeric antibody, a humanized antibody, or a fully human antibody or a functional fragment thereof.

43. The antibody of any one of the preceding claims or a functional fragment thereof which is of the IgG2b, IgG2a or the IgG3 isotype.

44. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said mammalian Tau protein is a human Tau protein.

45. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or fragment thereof binds to a pathological protein Tau conformer, but does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes.

46. A polynucleotide encoding the antibody of any one of the preceding claims or a functional fragment thereof.

47. The polynucleotide of claim 46 comprising a nucleic acid molecule selected from the group consisting of:

a. a nucleic acid molecule comprising a nucleotide sequence that has at least 85% sequence identity to the sequence shown in SEQ ID NOs: 35-45; SEQ ID NOs: 84-87, SEQ ID NO: 109-112 and 117-119;

b. a nucleic acid molecule comprising a nucleotide sequence that has at least 90% sequence identity to the sequence shown in SEQ ID NOs: 35-45; SEQ ID NOs: 84-87, SEQ ID NO: 109-112 and 117;

c. a nucleic acid molecule comprising a nucleotide sequence that has at least 95% sequence identity to the sequence shown in SEQ ID NOs: 35-45; SEQ ID NOs: 84-87, SEQ ID NO: 109-112 and 117-119;

d. a nucleic acid molecule comprising a nucleotide sequence that has at least 98% sequence identity to the sequence shown in SEQ ID NOs: 35-45; SEQ ID NOs: 84-87, SEQ ID NO: 109-112 and 117-119;

e. a nucleic acid molecule comprising a nucleotide sequence that has 100% sequence identity to the sequence shown in SEQ ID NOs: 35-45; SEQ ID NOs: 84-87, SEQ ID NO: 109-112 and 117-119;

f. a nucleic acid molecule comprising a nucleotide sequence the complementary strand of which hybridizes to the nucleic acid molecule of any of a) – d); or

g. a nucleic acid molecule comprising a nucleotide sequence that deviates from the nucleotide sequence defined in any of a) – e) by the degeneracy of the genetic code:

wherein said nucleic acid molecule as defined in any of a) – g) encodes an antibody, or a functional fragment thereof which recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes, wherein said antibody or antibody fragment has a high binding affinity to soluble, oligomeric and insoluble phosphorylated Tau protein and is capable of detecting and/or modulating levels of soluble, oligomeric and insoluble phosphorylated Tau protein *in vivo*.

48. A pharmaceutical composition comprising an antibody or a functional fragment thereof according to any one of the preceding claims, or a combination thereof, in a therapeutically effective amount together with a pharmaceutically acceptable carrier.

49. The pharmaceutical composition of claim 48, which comprises at least two antibodies or functional fragments thereof according to any one of the preceding claims, which recognize and bind to two different phospho-epitopes on the mammalian Tau protein.

50. The pharmaceutical composition of claim 48 or 49, which further comprises an additional antibody or a functional fragment thereof which recognizes and binds to an amyloidogenic protein or peptide.

51. The antibody or a functional fragment thereof, polynucleotide or a pharmaceutical composition according to any one of the preceding claims, or a combination thereof, for use in therapy, particularly for use in the treatment of a neurodegenerative disease or disorder such as a tauopathy in a mammal, particularly a human in need of such a treatment.

52. The antibody or functional fragment thereof, polynucleotide, pharmaceutical composition, or combination thereof according to claim 51 for use in the treatment of a neurodegenerative disease or disorder in a mammal, wherein the neurodegenerative disease or disorder is a tauopathy.

53. The antibody or functional fragment thereof, polynucleotide, pharmaceutical composition, or combination thereof according to claim 51 or 52, for use in the treatment of a neurodegenerative disease or disorder such as a tauopathy in a mammal, wherein the mammal is a human.

54. The antibody or a functional fragment thereof, polynucleotide, or pharmaceutical composition according to any one of the preceding claims for use in treatment or alleviation of cognitive deficits in a mammal, particularly a human suffering from such a deficit.

55. The antibody or a functional fragment thereof, polynucleotide, or pharmaceutical composition according to claim 54, wherein the treatment or alleviation of cognitive deficits in a mammal, particularly a human, leads to an arrest in the progression of the cognitive deficits.

56. The antibody or a functional fragment thereof, polynucleotide, or pharmaceutical composition according to claim 54, wherein the treatment or alleviation of cognitive deficits in a mammal, particularly a human, leads to an increase in the retention, particularly a complete restoration of cognitive memory capacity in the treated subject.

57. The antibody or a functional fragment thereof, polynucleotide, or the pharmaceutical composition according to claim 52 wherein the tauopathy is selected from the group consisting of Alzheimer's Disease, Creutzfeldt-Jacob disease, Dementia pugilistica, Down's Syndrome, Gerstmann-Sträussler-Scheinker disease, amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam, Non-Guamanian motor neuron disease with neurofibrillary tangles, argyrophilic grain dementia, corticobasal degeneration, diffuse neurofibrillary tangles with calcification, frontotemporal dementia with parkinsonism linked to chromosome 17, frontotemporal dementia, Hallevorden-Spatz disease, multiple system atrophy, Niemann-Pick disease, type C, Pallido-ponto-nigral degeneration, Pick's disease, progressive subcortical gliosis, progressive supranuclear palsy, Subacute sclerosing panencephalitis, Tangle-only dementia, Postencephalitic Parkinsonism, and Myotonic dystrophy.

58. The antibody or a functional fragment thereof, polynucleotide, or pharmaceutical composition according to any one of the preceding claim for use in the treatment of Alzheimer's Disease or frontotemporal dementia.

59. The antibody or a functional fragment thereof, polynucleotide, or pharmaceutical composition according to claim 51, wherein the neurodegenerative disease or disorder shows the presence of both tau and amyloid pathologies.

60. The antibody or a functional fragment thereof, polynucleotide, or the pharmaceutical composition according to any one of the preceding claims for use in the treatment of diseases and disorders which are caused by or associated with the formation of neurofibrillary lesions, the predominant brain pathology in tauopathy comprising a heterogeneous group of neurodegenerative diseases or disorders including diseases or disorders which show the presence of both tau and amyloid pathologies including, but not limited to, Alzheimer's Disease, Creutzfeldt-Jacob disease, Dementia pugilistica, Down's Syndrome, Gerstmann-Sträussler-Scheinker disease, inclusion-body myositis, and prion protein cerebral amyloid angiopathy, traumatic brain injury and further diseases or disorders which do not show a distinct amyloid pathology including, but not limited to, amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam, Non-Guamanian motor neuron disease with neurofibrillary tangles, argyrophilic grain dementia, corticobasal degeneration, diffuse neurofibrillary tangles with calcification, frontotemporal dementia with parkinsonism linked to chromosome 17, frontotemporal dementia, Hallevorden-Spatz disease, multiple system atrophy, Niemann-Pick disease, type C, Pallido-ponto-nigral degeneration, Pick's disease, progressive subcortical gliosis, progressive supranuclear palsy, Subacute sclerosing panencephalitis, Tangle only dementia, Postencephalitic Parkinsonism, and Myotonic dystrophy.

61. The antibody or a functional fragment thereof, polynucleotide, or pharmaceutical composition according to claim 54, 55, 56, or 58, wherein the mammal is a human.

62. A method for the treating, alleviating, or protecting against a neurodegenerative disease or disorder such as a tauopathy comprising administering to a mammal, suffering from such a disease or disorder, an antibody or a functional fragment thereof, a polynucleotide or a pharmaceutical composition according to any one of the preceding claims, or a combination thereof.

63. The method according to claim 62, wherein the neurodegenerative disease or disorder is tauopathy.

64. The method according to claim 63 wherein the tauopathy is selected from the group consisting of Alzheimer's Disease, Creutzfeldt-Jacob disease, Dementia pugilistica, Down's Syndrome, Gerstmann-Sträussler-Scheinker disease, inclusion-body myositis, and prion protein cerebral amyloid angiopathy, traumatic brain injury, amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam, Non-Guamanian motor neuron disease with neurofibrillary tangles, argyrophilic grain dementia, corticobasal degeneration, diffuse neurofibrillary tangles with calcification, frontotemporal dementia with parkinsonism linked to chromosome 17, frontotemporal dementia, Hallevorden-Spatz disease, multiple system atrophy, Niemann-Pick disease, type C, Pallido-ponto-nigral

degeneration, Pick's disease, progressive subcortical gliosis, progressive supranuclear palsy, Subacute sclerosing panencephalitis Tangle-only dementia, Postencephalitic Parkinsonism, Myotonic dystrophy.

65. The method of claim 62, 63, or 64, wherein the mammal is a human.

66. A method for inducing a passive immune response in an animal, particularly a mammal or a human, suffering from a neurodegenerative disorder such as tauopathy by administering to said animal or human an antibody or a functional fragment thereof, or a pharmaceutical composition according to any one of the preceding claims, or a combination thereof.

67. A method for diagnosing a tau-protein-associated disease, disorder or condition or a predisposition to tau-protein-associated disease, disorder or condition in a patient comprising detecting the immunospecific binding of an antibody or an active fragment thereof to an epitope of the tau protein in a sample or in situ which comprising the steps of:

a. bringing the sample or a specific body part or body area suspected to contain the tau antigen into contact with an antibody or a fragment thereof according to any one of the preceding claims, which antibody or fragment thereof binds an epitope of the tau protein;
b. allowing the antibody to bind to the tau antigen to form an immunological complex;
c. detecting the formation of the immunological complex; and
d. correlating the presence or absence of the immunological complex with the presence or absence of tau antigen in the sample or specific body part or area,

wherein an increase in the amount of said aggregate compared to a normal control value indicates that said patient is suffering from or is at risk of developing an tau protein-associated disease or condition.

68. A method for monitoring minimal residual disease in a patient following treatment with an antibody or a pharmaceutical composition according to any one of the preceding claims, wherein said method comprises:

a. bringing the sample or a specific body part or body area suspected to contain the tau antigen into contact with an antibody or a fragment thereof according to any one of the preceding claims, which peptide or fragment thereof binds an epitope of the tau protein;

b. allowing the antibody to bind to the tau antigen to form an immunological complex;

c. detecting the formation of the immunological complex; and

d. correlating the presence or absence of the immunological complex with the presence or absence of tau antigen in the sample or specific body part or area,

wherein an increase in the amount of said immunological complex compared to a normal control value indicates that said patient still suffers from a minimal residual disease.

69. A method for predicting responsiveness of a patient being treated with an antibody or a pharmaceutical composition according to any one of the preceding claims comprising

a. bringing the sample or a specific body part or body area suspected to contain tau antigen into contact with an antibody or a fragment thereof according to any one of claims 1-45, which peptide or fragment thereof binds an epitope of the tau protein;

b. allowing the antibody to bind to the tau antigen to form an immunological complex;

c. detecting the formation of the immunological complex; and

d. correlating the presence or absence of the immunological complex with the presence or absence of tau antigen in the sample or specific body part or area,

e. comparing the amount of said immunological complex before and after onset of the treatment,

wherein a decrease in the amount of said immunological complex indicates that said patient has a high potential of being responsive to the treatment.

70. The method of any one of claim 67-69, wherein the step of correlating the presence or absence of the immunological complex with the presence or absence of tau antigen in the sample or specific body part or area involves a step of comparing the amount of said immunological complex to a normal control value.

71. The method of any one of claims 67-70, wherein the sample is a brain homogenate or cerebral spinal fluid.

72. A method of post-mortem detection of phosphoTau (pTau) multimers in a brain sample from a subject suspected to suffer from a tau-associated disease or disorder comprising:

a. bringing a brain sample of the subject into contact with an antibody or a fragment thereof according to any one of the preceding claims, which antibody or fragment thereof binds an epitope of the phosphoTau protein;

b. allowing the antibody to bind to the tau protein to form an immunological complex;

c. detecting the formation of the immunological complex, and

d. comparing the amount or intensity of the immunological complex in the sample obtained from the subject to the amount or intensity of the immunological complex obtained from a healthy control subject using the same conditions,

wherein an increase in the amount or intensity of said immunological complex compared to the control value indicates that said patient had suffered from a tau-associated disease or disorder.

73. The method of claim 72, wherein the increase observed in the test sample compared to the control sample is between 30% and 100%.

74. The method for diagnosing a tau-protein-associated disease of any one of claims 67-73, wherein, prior to step (a), the sample is immune-enriched to increase the concentration of Tau protein in the sample by contacting the sample with an anti-Tau antibody or a fragment thereof attached to a solid support.

75. Test kits for detection and diagnosis of tau protein-associated diseases, disorders or conditions comprising an antibody according to claims 1-45.

76. A test kit according to the preceding claim comprising a container holding one or more antibodies or functional fragments thereof according to any one of claims 1-45 and instructions for using the antibodies for the purpose of binding to tau antigen to form an immunological complex and detecting the formation of the immunological complex such that presence or absence of the immunological complex correlates with presence or absence of tau antigen.

77. An epitope selected from the group consisting of VYKSPVVSG (Tau aa 393-401 of SEQ ID NO: 67) comprising a phosphorylated Ser at position 396 (pS396), SPVVSG (Tau aa 396-401 of SEQ ID NO: 67) comprising a phosphorylated Ser at position 396 (pS396), YKSPVVS (Tau aa 394-400 of SEQ ID NO: 67) comprising a phosphorylated Ser at position 396 (pS396), DTSPR (Tau aa 402-406 of SEQ ID NO: 67) comprising a phosphorylated Ser at position 404 (pS404), and VYKSPVVS (Tau aa 393-400 of SEQ ID NO: 67) comprising a phosphorylated Ser at position 396 (pS396).

78. A cell line producing an antibody according to any of the preceding claims.

79. The cell line of claim 78, which is hybridoma cell line A4-4A6-48 deposited on August 30, 2011 as DSM ACC3136.

80. The cell line of claim 78, which is hybridoma cell line A6-2G5-30 deposited on August 30, 2011 as DSM ACC3137.

81. The cell line of claim 78, which is hybridoma cell line A6-2G5-41 deposited on August 30, 2011 as DSM ACC3138.

82. The cell line of claim 78, which is hybridoma cell line A4-2A1-18 deposited on August 30, 2011 as DSM ACC3139.

83. The cell line of claim 78, which is hybridoma cell line A4-2A1-40 deposited on August 30, 2011 as DSM ACC3140.

84. The cell line of claim 78, which is hybridoma cell line A6-1D2-12 deposited on September 6, 2011 as DSM ACC3141.

85. A vector comprising the polynucleotide of claims 46 or 47, wherein said polynucleotide is operably linked.

86. A host cell comprising the vector of claim 85.

87. The host cell of claim 86 producing the antibody according to any one of claims 1-45, or a functional fragment thereof.

88. The host cell of claim 86 or claim 87, which is mammalian cell.

89. The host cell of claim 86 or claim 87, which is a Chinese Hamster Ovary cell

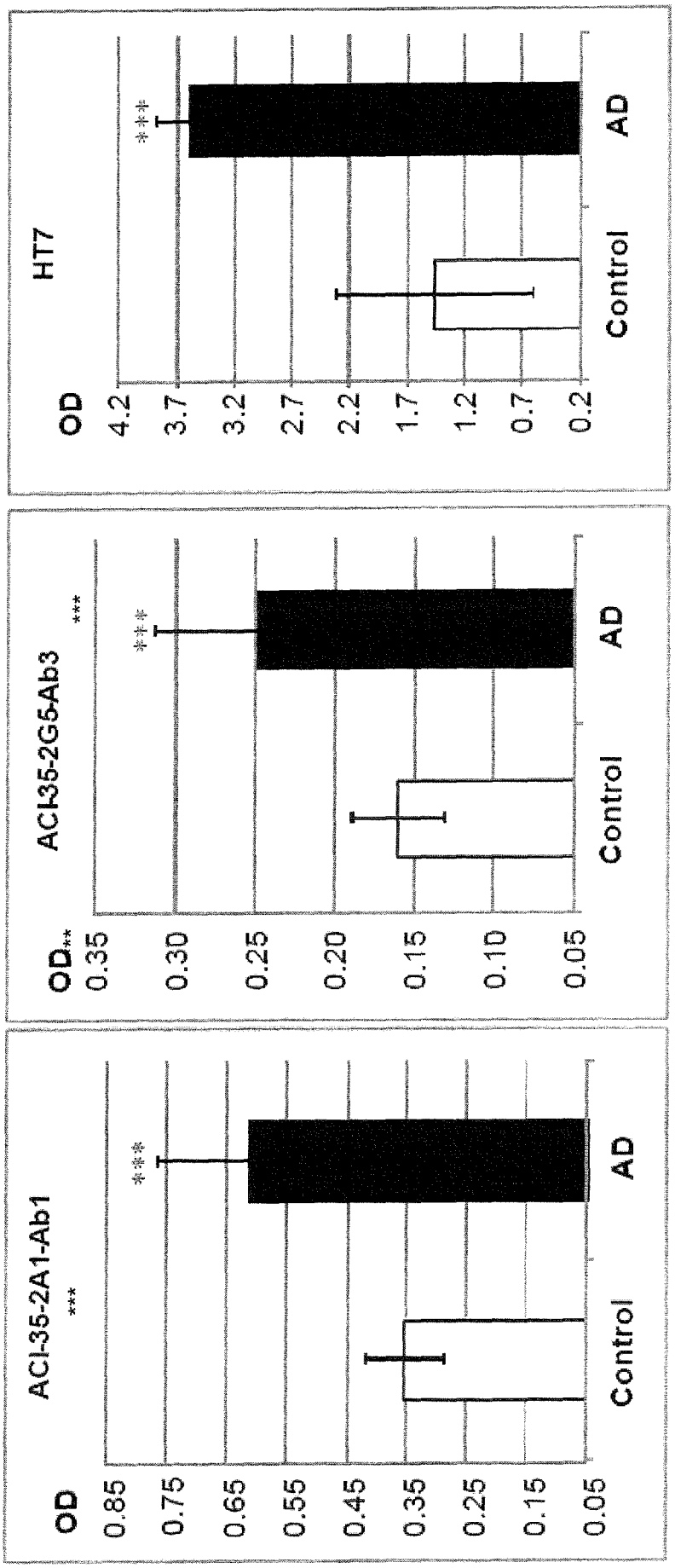
90. A process for producing the antibody of any of the preceding claims, or a functional fragment thereof comprising culturing the host cell of any one of claims 86-89 under conditions suitable for expression of the antibody or fragment, and recovering the antibody or fragment.

91. A method of detecting phosphoTau (pTau) multimers in a brain sample comprising

a. bringing the sample into contact with an antibody or a fragment thereof according to any one of claims 1-45, which antibody or fragment thereof binds an epitope of the phosphoTau protein;

b. allowing the antibody or fragment thereof to bind to the tau protein to form an immunological complex; and

c. detecting the formation of the immunological complex.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0.75
0.65
0.55
0.45
0.35
0.25
0.15
0.05
***
Control
AD
ACI35-2G5-Ab3
***
OD**
0.35
0.30
0.25
0.20
0.15
0.10
0.05
***
Control
AD
HT7
OD
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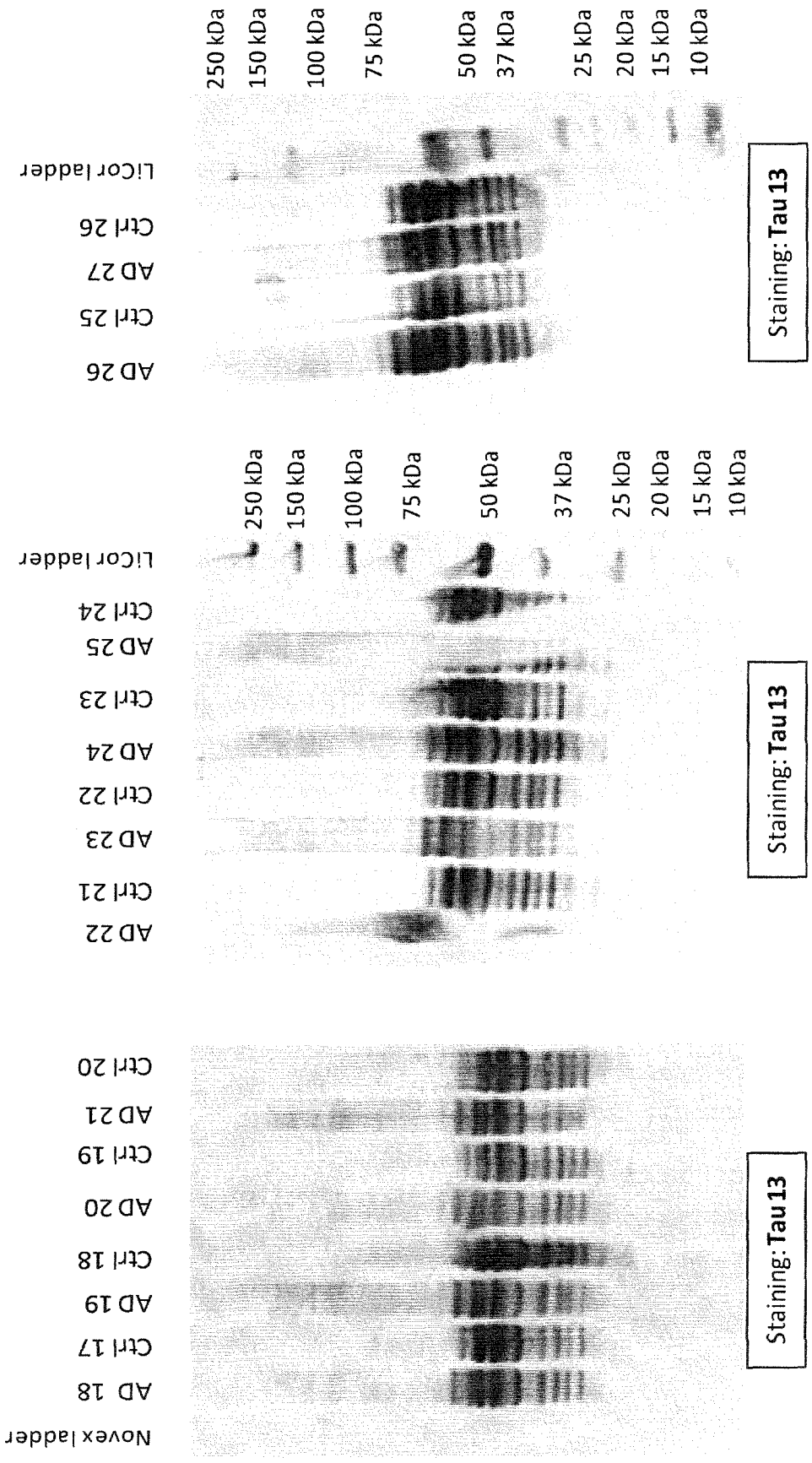
3.7
3.2
2.7
2.2
1.7
1.2
0.7
0.2
***
Control
AD

Figure 1.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Novex ladder
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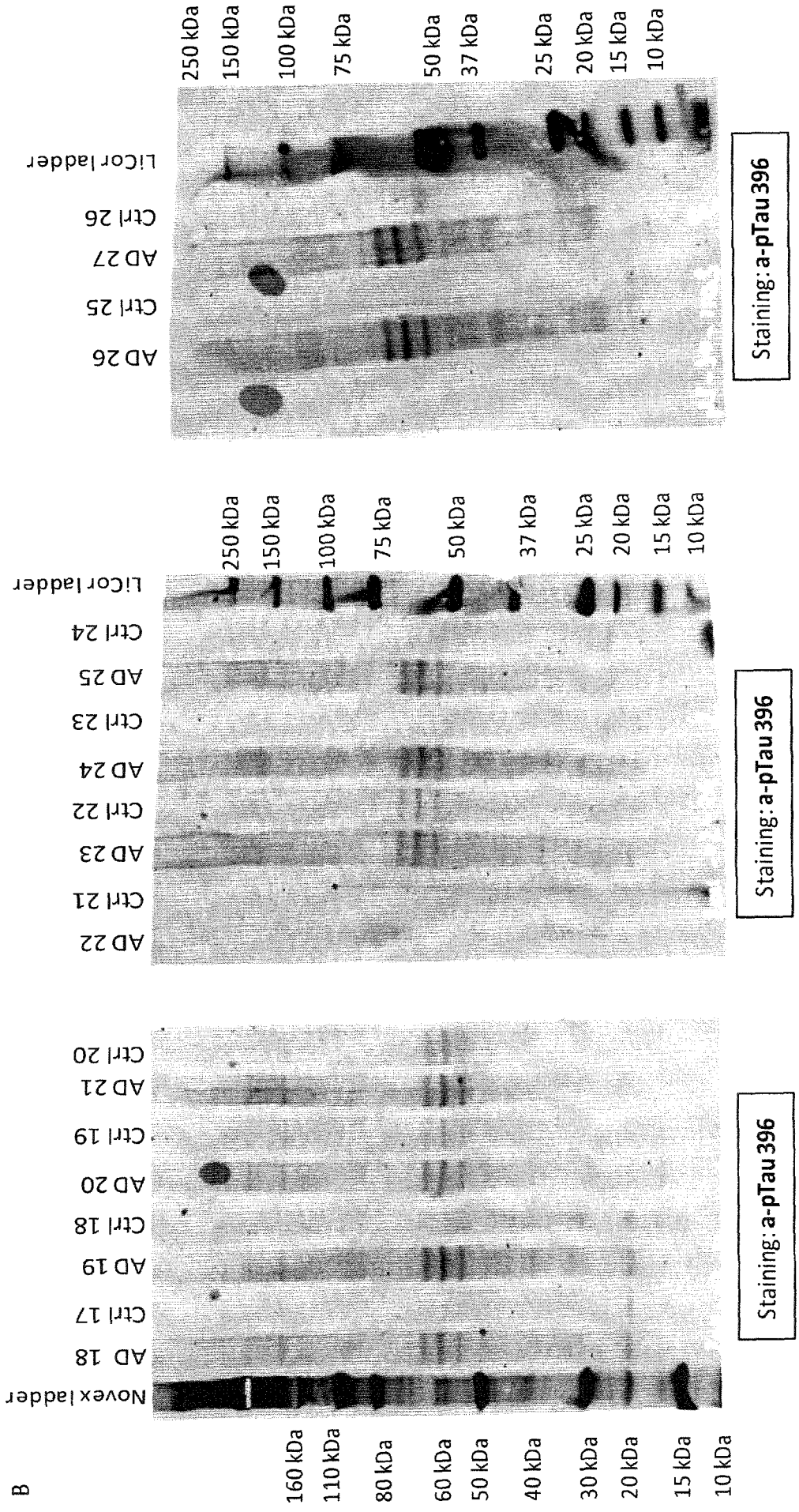
Ctrl 17
AD 19
Ctrl 18
AD 20
Ctrl 19
AD 21
Ctrl 20
160 kDa
110 kDa
80 kDa
60 kDa
50 kDa
40 kDa
30 kDa
20 kDa
15 kDa
Staining: Tau 13
AD 22
Ctrl 21
AD 23
Ctrl 22
AD 24
Ctrl 23
AD 25
Ctrl 24
LiCor ladder
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa
Staining: Tau 13
AD 26
Ctrl 25
AD 27
Ctrl 26
LiCor ladder
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa
Staining: Tau 13

Figure 2A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Ctrl 17
AD 19
Ctrl 18
AD 20
Ctrl 19
AD 21
Ctrl 20
160 kDa
110 kDa
80 kDa
60 kDa
50 kDa
40 kDa
30 kDa
20 kDa
15 kDa
10 kDa
Staining: a-pTau 396
AD 22
Ctrl 21
AD 23
Ctrl 22
AD 24
Ctrl 23
AD 25
Ctrl 24
LiCor ladder
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa
Staining: a-pTau 396
AD 26
Ctrl 25
AD 27
Ctrl 26
LiCor ladder
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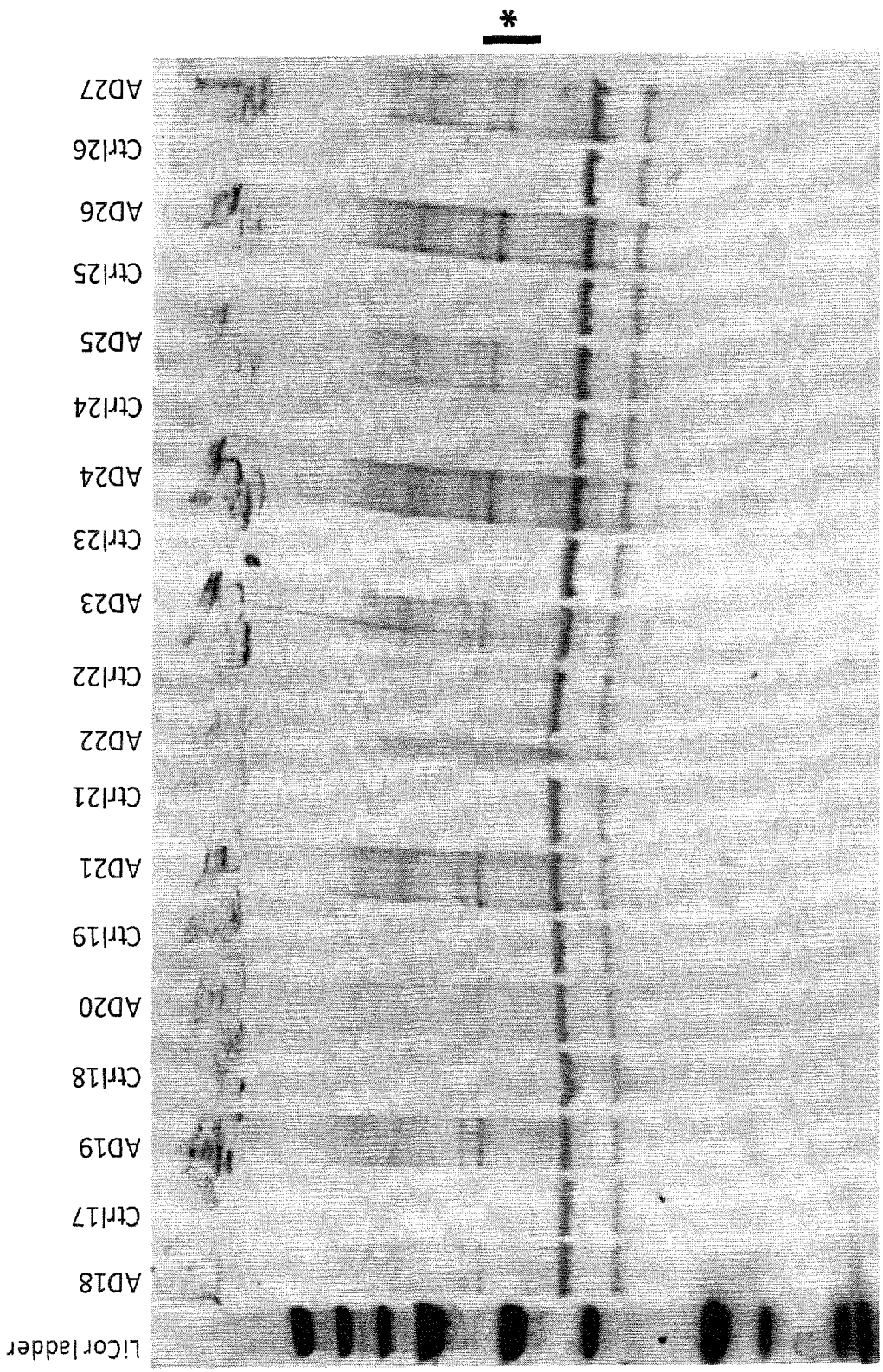
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa
Staining: a-pTau 396

Figure 2B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AD20
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Ctrl24
AD25
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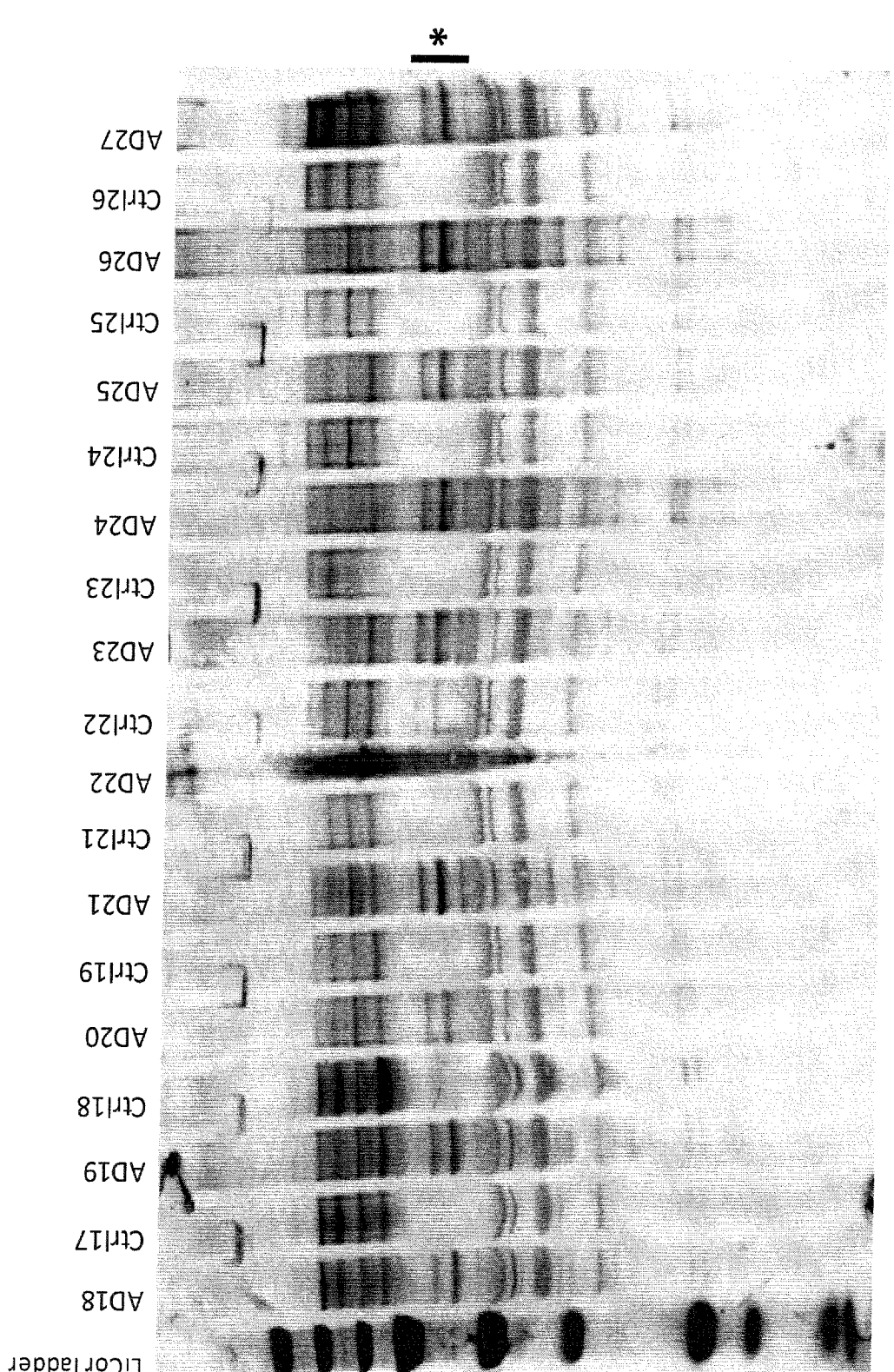
AD26
Ctrl26
AD27
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa

Figure 3A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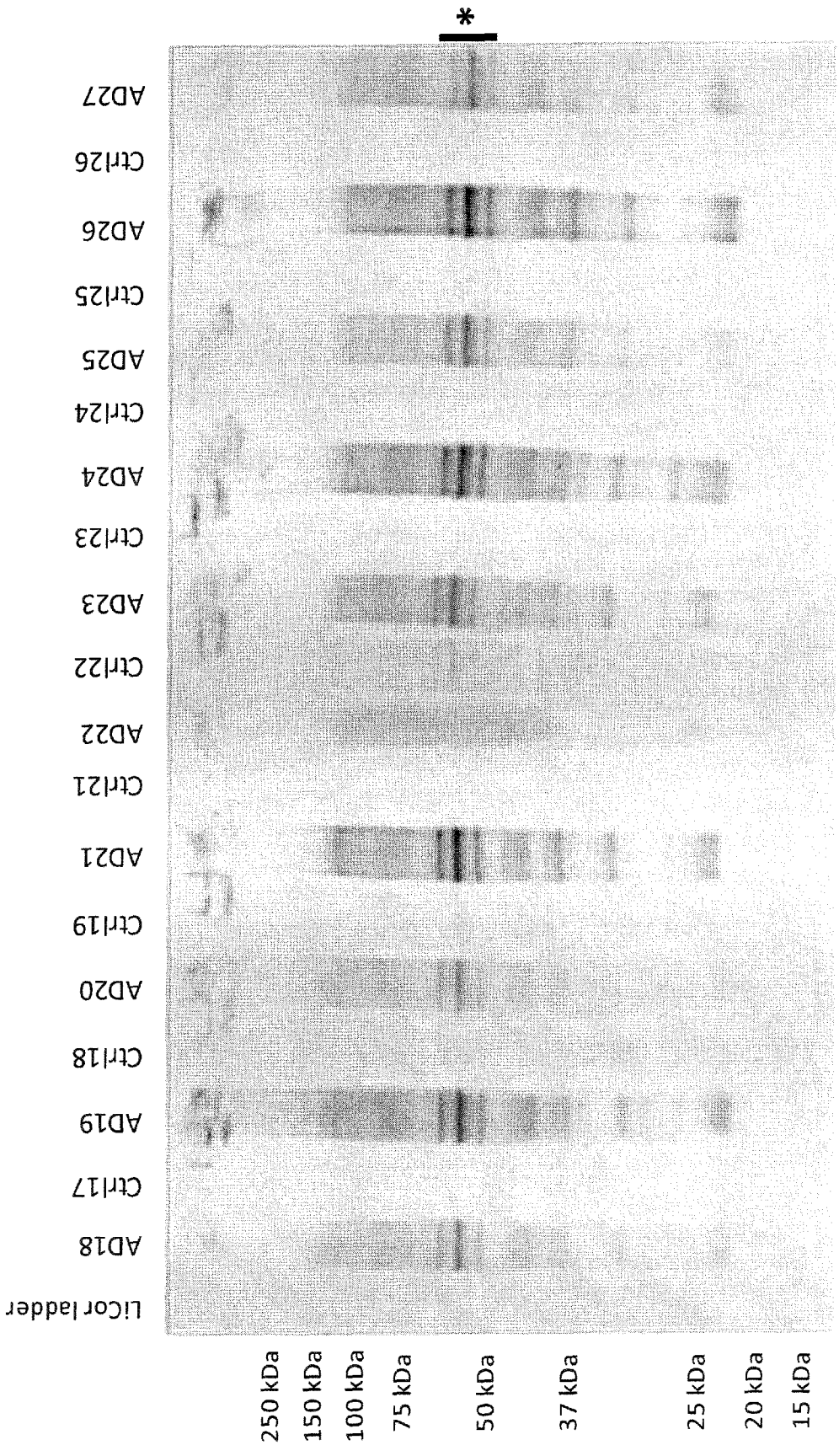
20 kDa
15 kDa

Figure 3B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AD20
Ctrl18
AD19
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LiCor ladder
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150 kDa
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50 kDa
37 kDa
25 kDa
20 kDa
15 kDa

Figure 3C

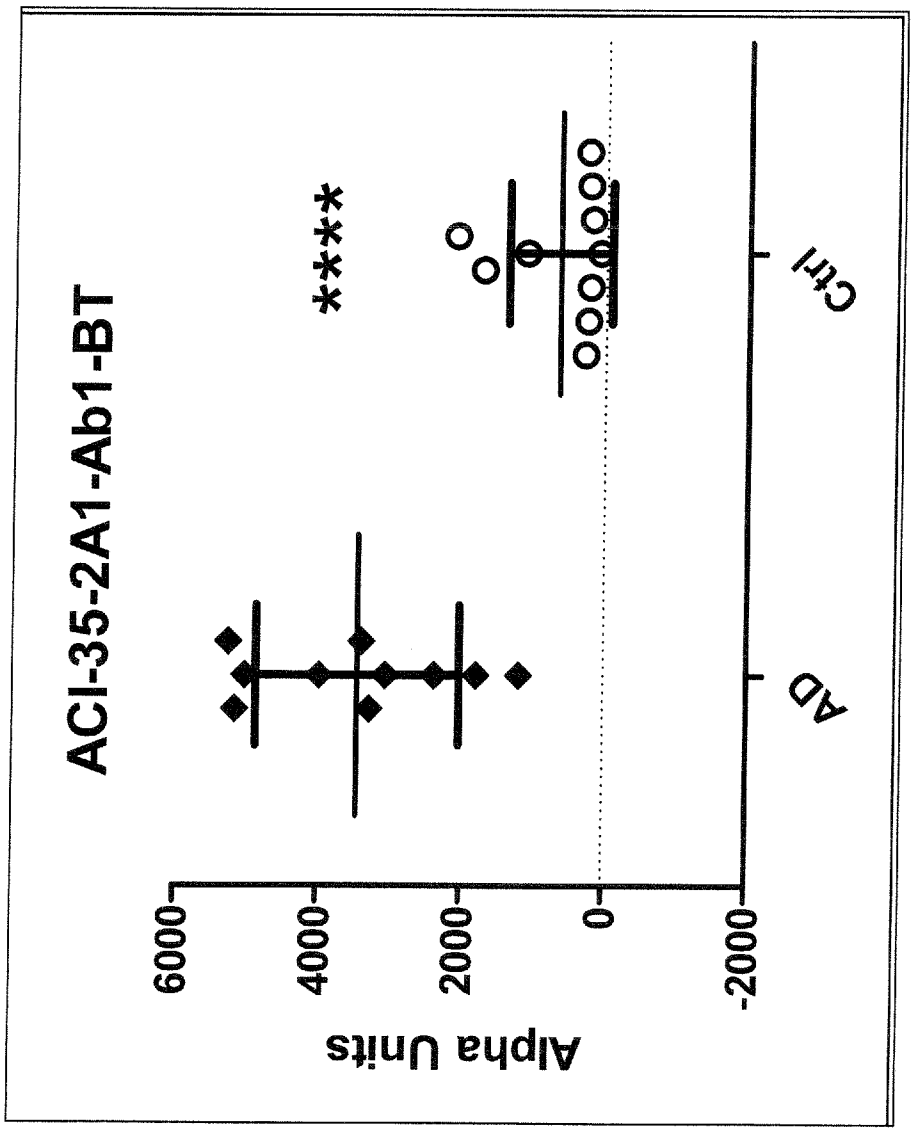


Figure 4A

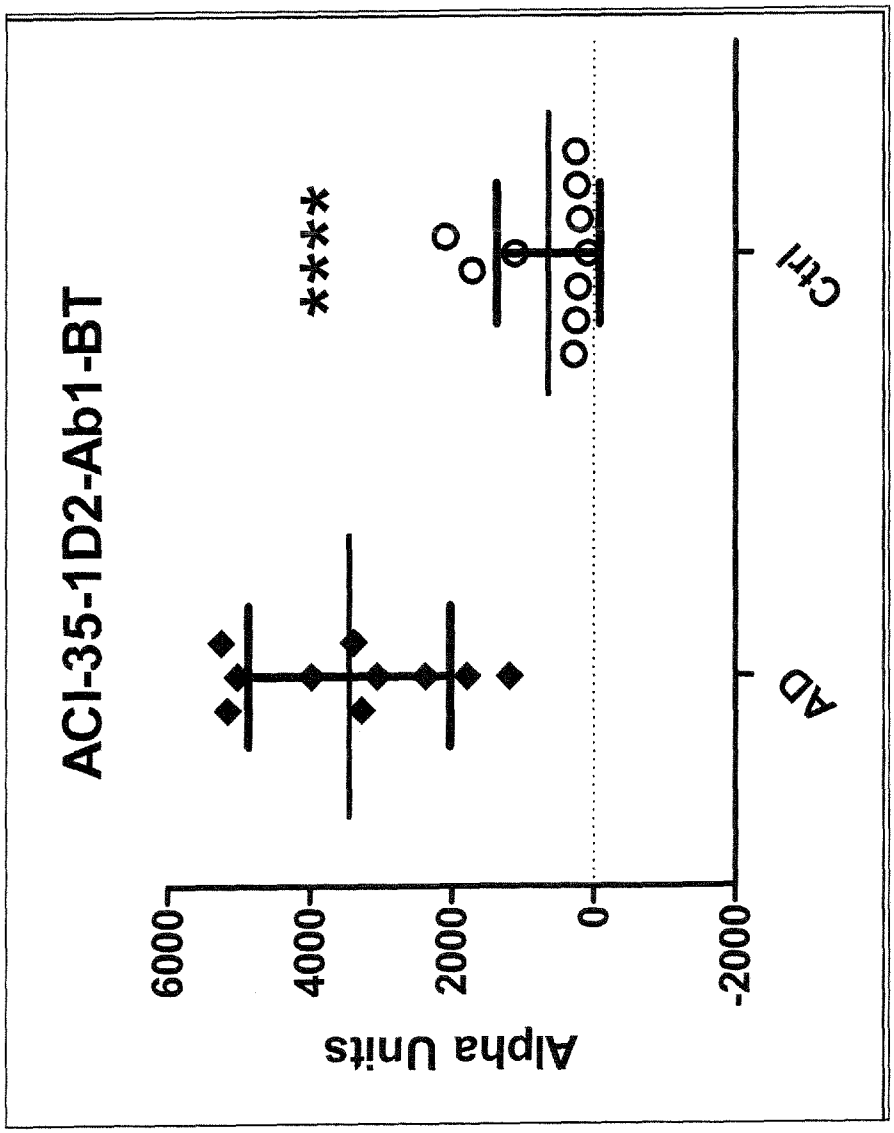
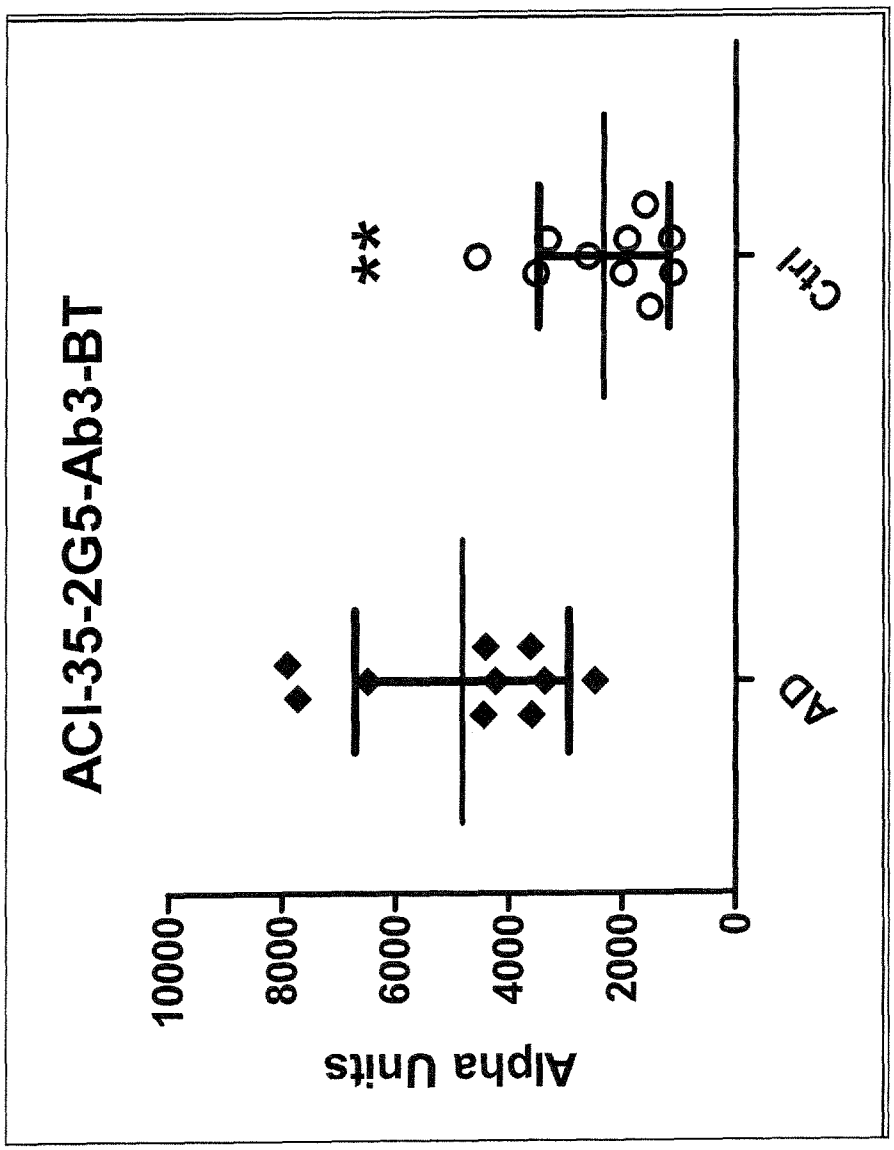


Figure 4B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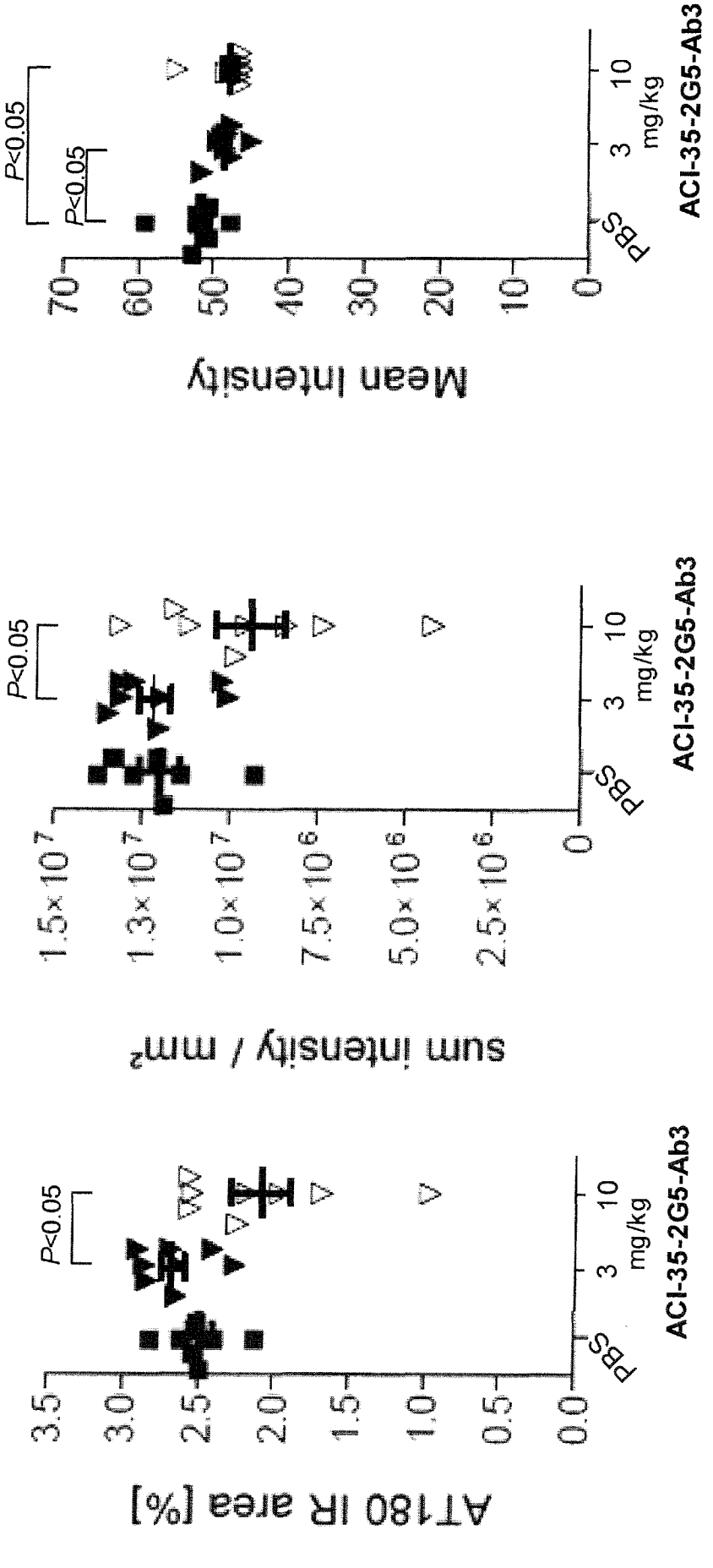
8000
6000
4000
2000
0
**
AD
Ctrl

Figure 4C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sum intensity / mm²
Mean intensity
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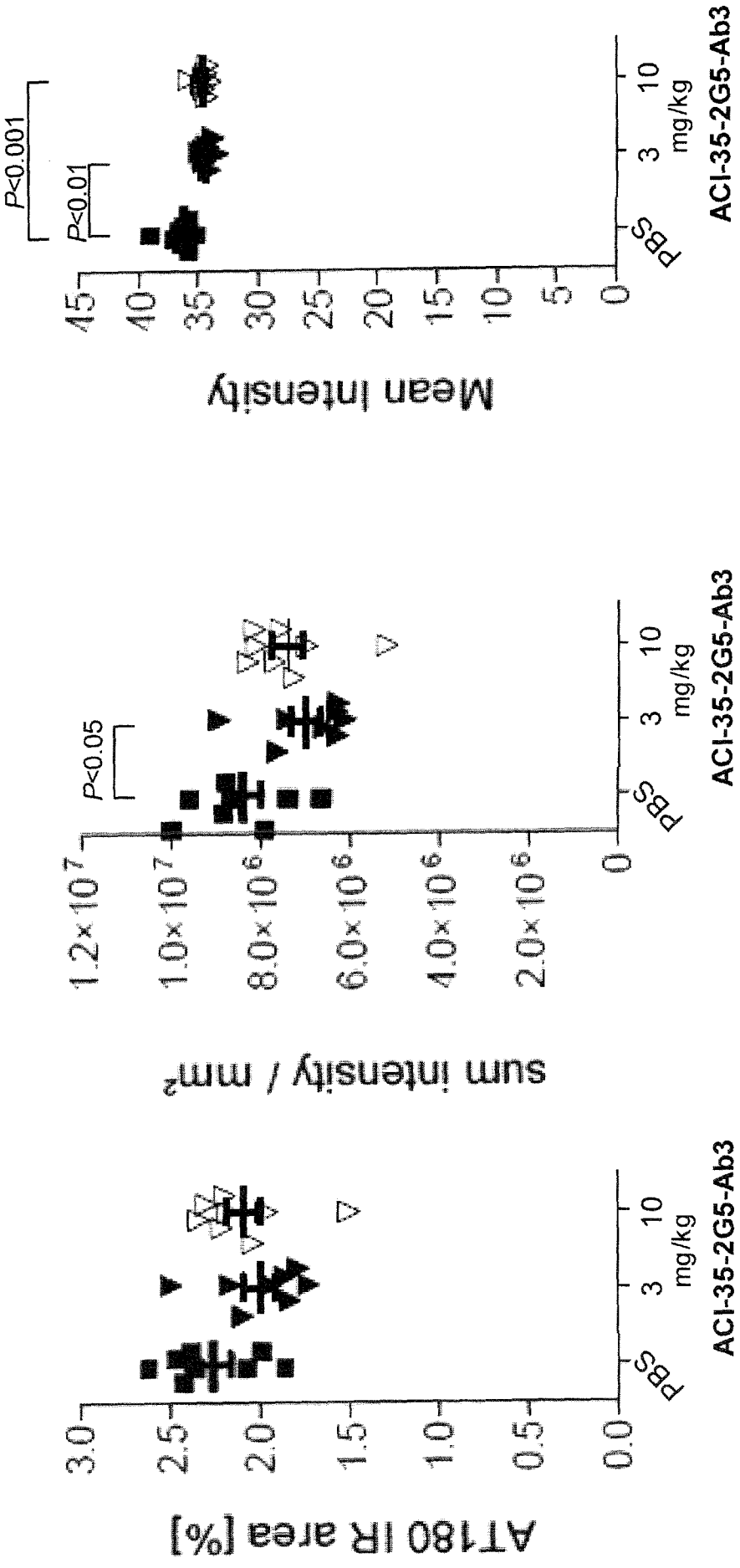
10
mg/kg
ACI-35-2G5-Ab3

Figure 5A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0.5
0.0
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mg/kg
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sum intensity / mm²
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1.0×10⁷
8.0×10⁶
6.0×10⁶
4.0×10⁶
2.0×10⁶
0
P<0.05
PBS
3
10
mg/kg
ACI-35-2G5-Ab3
Mean intensity
15
10
35
30
25
20
15
10
5
0
P<0.001
P<0.01
PBS
3
10
mg/kg
ACI-35-2G5-Ab3

Figure 5B

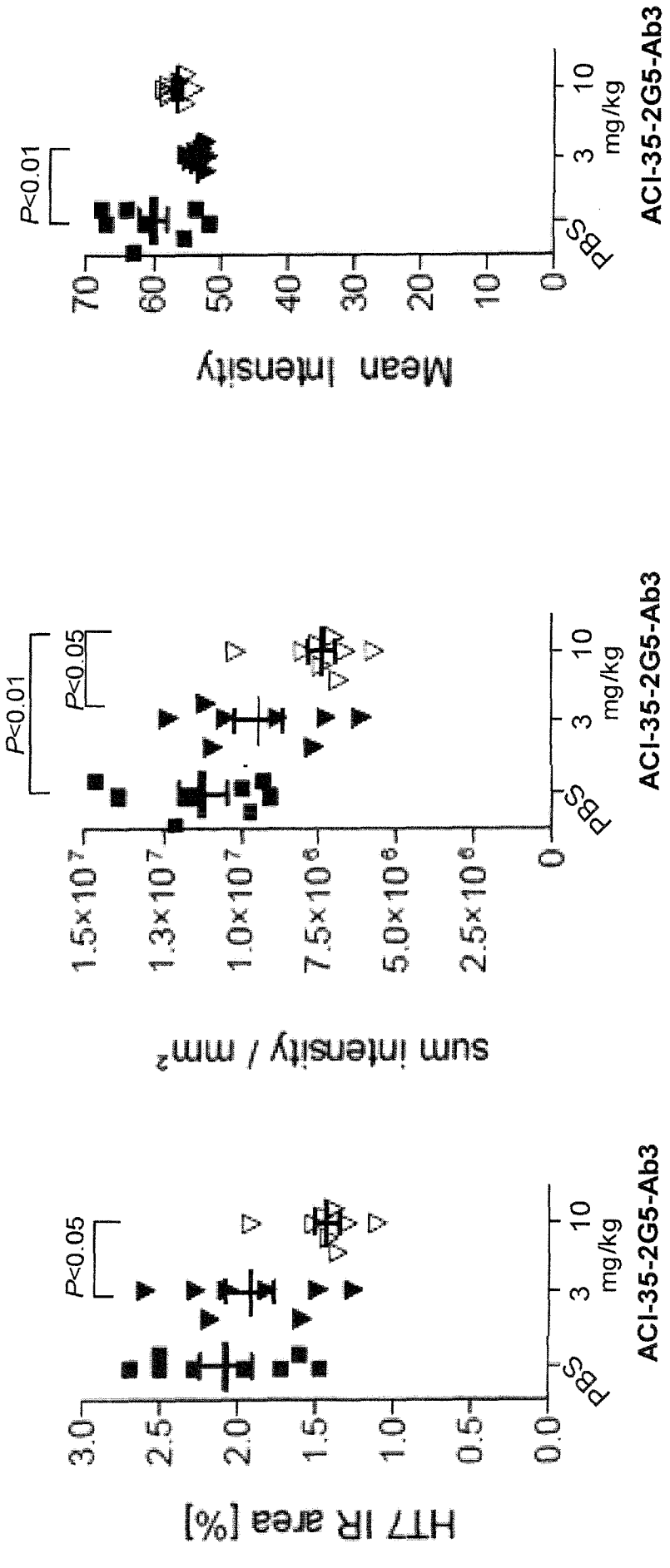

HT7 IR area [%]
3.0
2.5
2.0
1.5
1.0
0.5
0.0
P<0.05
PBS
3
10
mg/kg
ACI-35-2G5-Ab3
sum intensity / mm²
1.5×10⁷
1.3×10⁷
1.0×10⁷
7.5×10⁶
5.0×10⁶
2.5×10⁶
0
P<0.01
P<0.05
PBS
3
10
mg/kg
ACI-35-2G5-Ab3
Mean Intensity
70
60
50
40
30
20
10
0
P<0.01
PBS
3
10
mg/kg
ACI-35-2G5-Ab3

Figure 6A

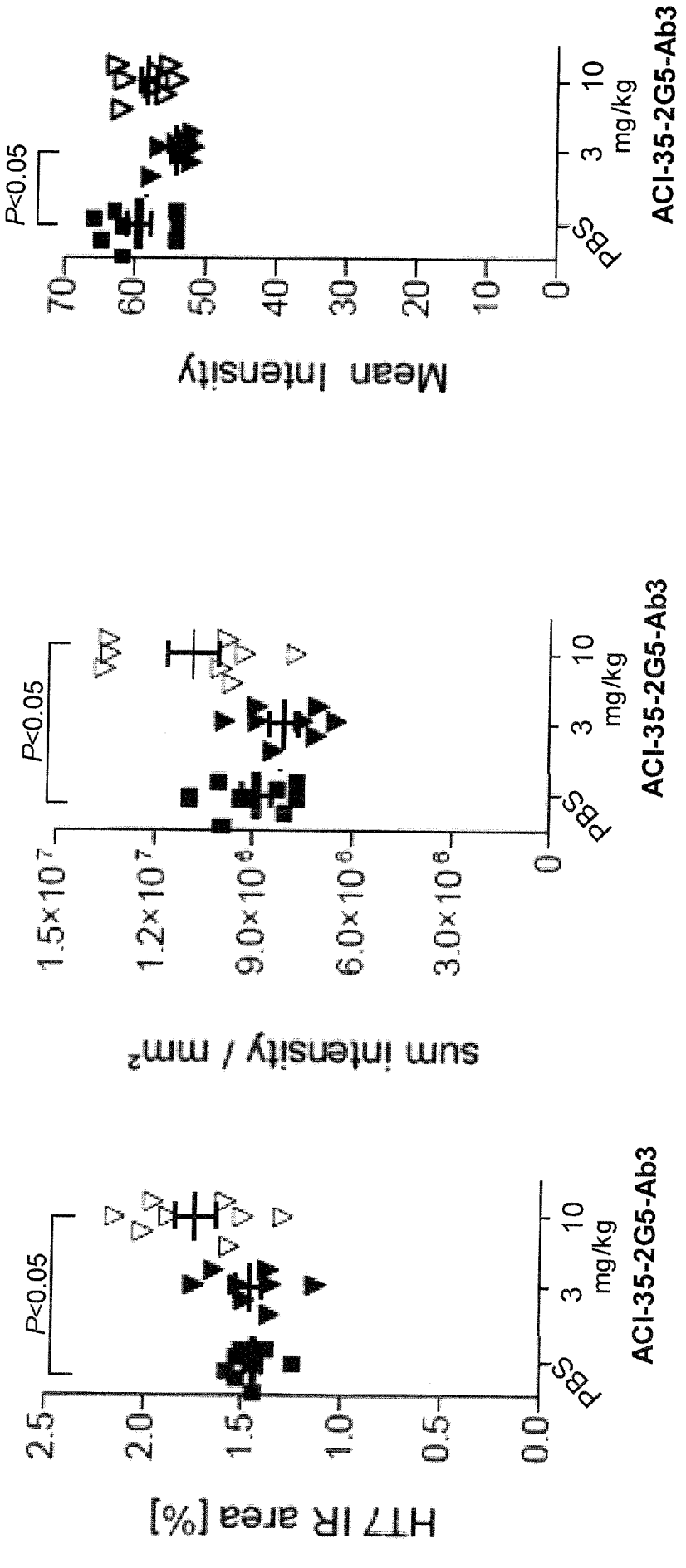
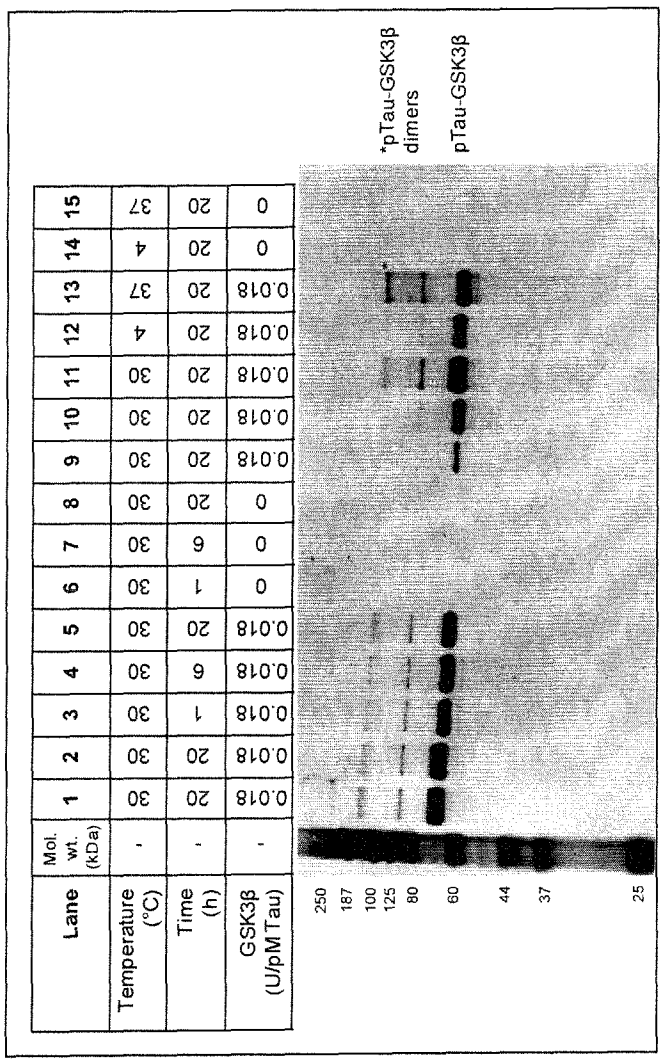

HT7 IR area [%]
2.5
2.0
1.5
1.0
0.5
0.0
P<0.05
PBS
3
10
mg/kg
ACI-35-2G5-Ab3
sum intensity / mm²
1.5×10⁷
1.2×10⁷
9.0×10⁶
6.0×10⁶
3.0×10⁶
0
P<0.05
PBS
3
10
mg/kg
ACI-35-2G5-Ab3
Mean Intensity
70
60
50
40
30
20
10
0
P<0.05
PBS
3
10
mg/kg
ACI-35-2G5-Ab3

Figure 6B



Lane	Mol. wt. (kDa)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Temperature (°C)	-	30	30	30	30	30	30	30	30	30	30	30	4	37	4	37
Time (h)	-	20	20	1	6	20	1	6	20	20	20	20	20	20	20	20
GSK3β (U/pM Tau)	-	0.018	0.018	0.018	0.018	0.018	0	0	0	0.018	0.018	0.018	0.018	0.018	0	0

Figure 7

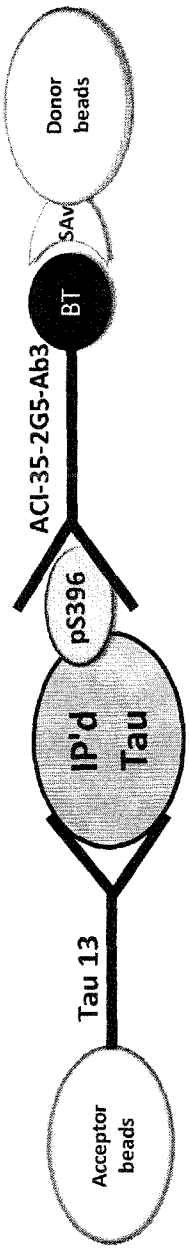

Donor beads
SAv
BT
ACI-35-2G5-Ab3
pS396
IP'd Tau
Tau 13
Acceptor beads

FIGURE 8

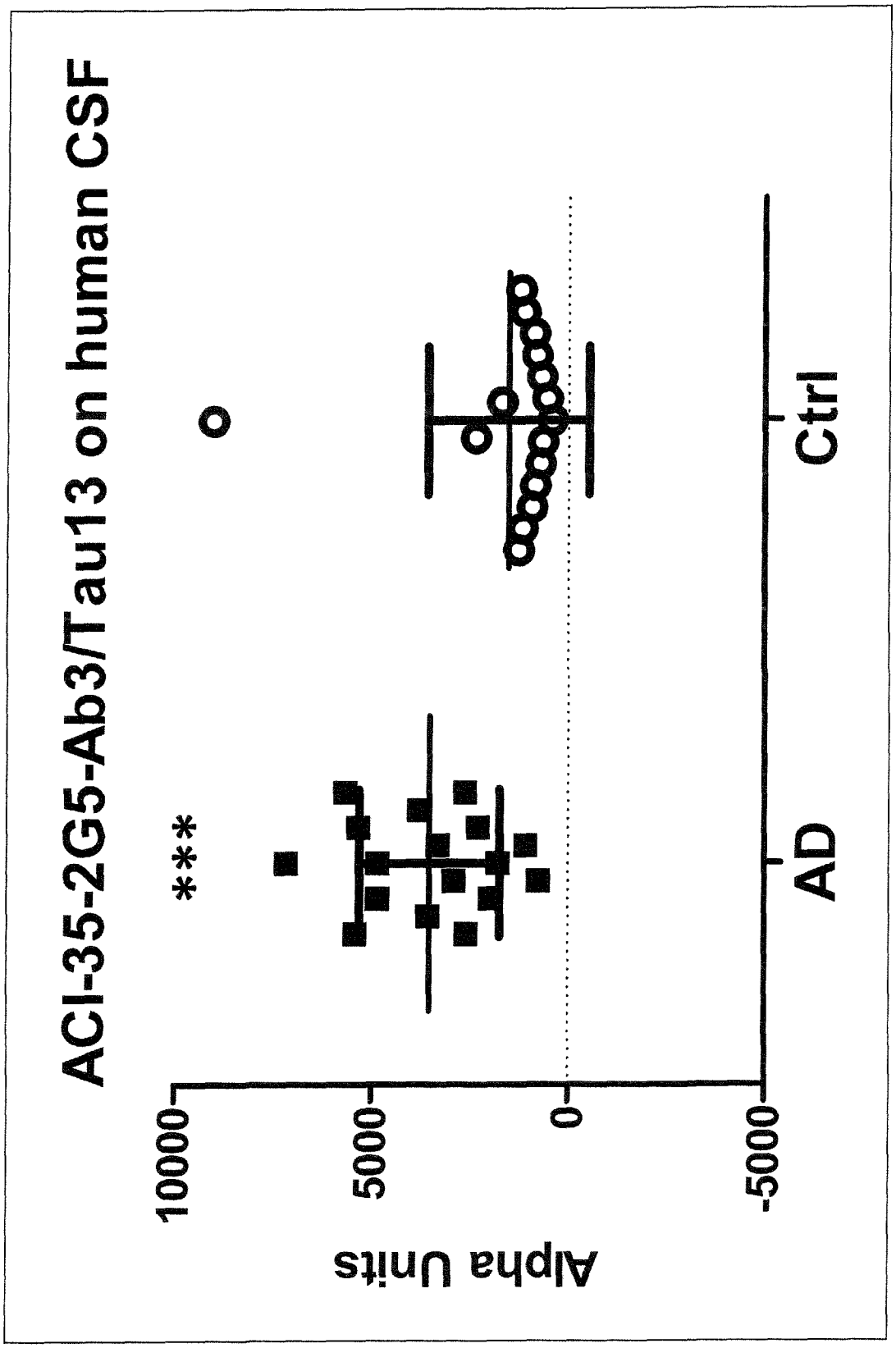

ACI-35-2G5-Ab3/Tau13 on human CSF
***
Alpha Units
10000
5000
0
-5000
AD
Ctrl

FIGURE 9

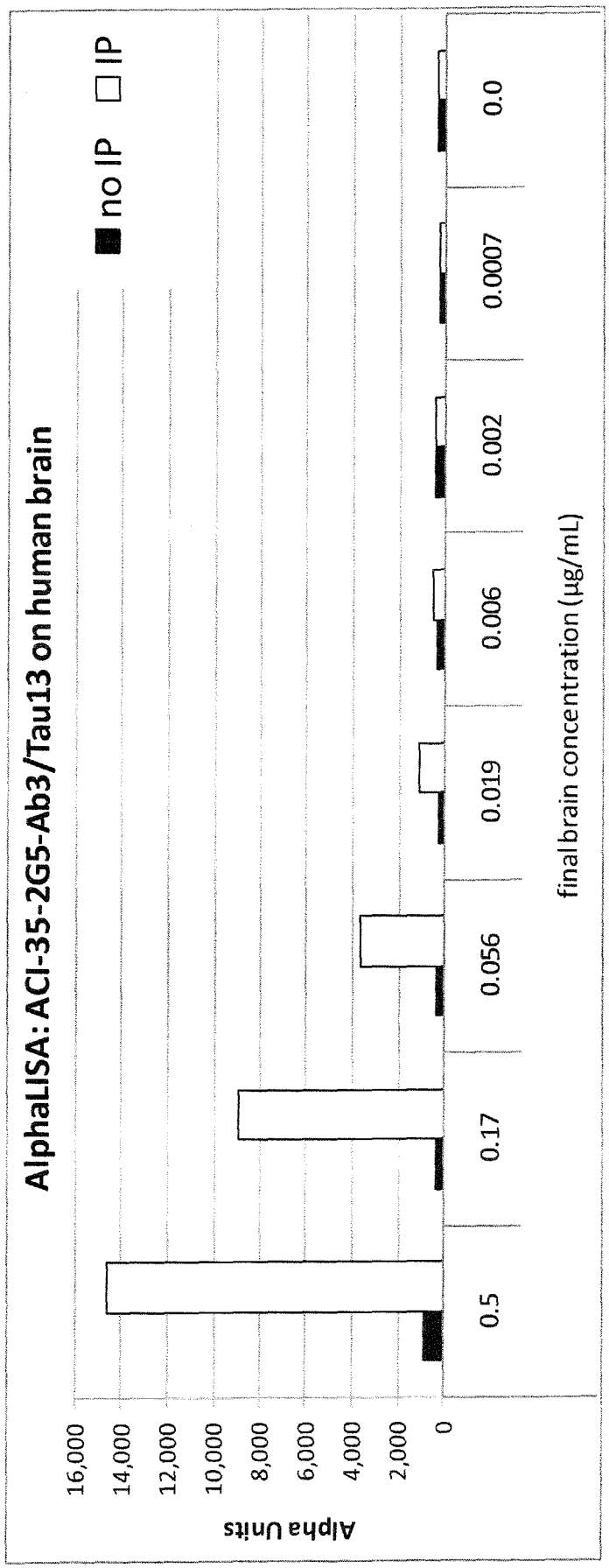

AlphaLISA: ACI-35-2G5-Ab3/Tau13 on human brain
no IP
IP
Alpha Units
16,000
14,000
12,000
10,000
8,000
6,000
4,000
2,000
0
0.5
0.17
0.056
0.019
0.006
0.002
0.0007
0.0
final brain concentration (µg/mL)

FIGURE 10

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/069783

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/18 G01N33/68 A61K39/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P L	WO 2012/045882 A2 (AC IMMUNE SA [CH]; LEUVEN K U RES & DEV [BE]; PFEIFER ANDREA [CH]; MUH) 12 April 2012 (2012-04-12) the whole document claims 1,2,18 tables 6,9 -----	1-91
X	US 2008/050383 A1 (SIGURDSSON EINAR [US] ET AL) 28 February 2008 (2008-02-28) the whole document claims 1,6,31,37,38 page 11; sequence 6 -----	1-91
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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Date of the actual completion of the international search	Date of mailing of the international search report
3 January 2013	25/01/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Jenkins, Gareth

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/106127 A2 (AC IMMUNE SA [CH]; PFEIFER ANDREA [CH]; MUHS ANDREAS [CH]; PIHLGREN MA) 23 September 2010 (2010-09-23) the whole document claims 1-62 pages 44,67 sequence 2	1-91
X	WO 2010/115843 A2 (AC IMMUNE SA [CH]; LEUVEN K U RES & DEV [BE]; PFEIFER ANDREA [CH]; MUH) 14 October 2010 (2010-10-14) the whole document page 52; table 1 page 77; table 10 claims 1-97	1-91
X	WO 98/22120 A1 (WISTAR INST [US]; UNIV PENNSYLVANIA [US]; OTVOS LASZLO [US]; HOFFMANN) 28 May 1998 (1998-05-28) the whole document claims 1,7,8,18,26	1-91
X	EP 2 210 901 A1 (IMMUNAS PHARMA INC [JP]; NAT CT FOR GERIATRICS AND GERO [JP]) 28 July 2010 (2010-07-28) the whole document claims 2,9 sequences 3,15,17,19	1-91
X	WO 2010/144711 A2 (UNIV NEW YORK [US]; SIGURDSSON EINAR M [US]) 16 December 2010 (2010-12-16) the whole document claim 1 table 1	1-91
X	HOFFMANN R ET AL: "Unique Alzheimer's disease paired helical filament specific epitopes involve double phosphorylation at specific sites", BIOCHEMISTRY, AMERICAN CHEMICAL SOCIETY, US, vol. 36, no. 26, 1 July 1997 (1997-07-01), pages 8114-8124, XP002625812, ISSN: 0006-2960, DOI: 10.1021/BI970380+ the whole document abstract tables 1,2	1-91

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LICHTENBERG-KRAAG B ET AL: "'Phosphorylation-dependent epitopes of neurofilament antibodies on tau protein and relationship with Alzheimer tau", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE, WASHINGTON, DC; US, vol. 89, no. 12, 1 June 1992 (1992-06-01), pages 5384-5388, XP002226003, ISSN: 0027-8424, DOI: 10.1073/PNAS.89.12.5384 the whole document figure 7	1-91
X	FRANCOIS TORREILLES ET AL: "Binding specificity of monoclonal antibody AD2: influence of the phosphorylation state of tau", MOLECULAR BRAIN RESEARCH, vol. 78, 1 January 2000 (2000-01-01), pages 181-185, XP055022594, the whole document figure 2B	1-91
X	L OTVOS ET AL: "Monoclonal Antibody PHF4 Recognizes Tau Protein Phosphorylated at Serine Residues 396 and 404", JOURNAL OF NEUROSCIENCE RESEARCH, vol. 39, 1 January 1994 (1994-01-01), pages 669-673, XP055022593, the whole document table 1 figure 1	1-91
X	HIRATA-FUKAE C ET AL: "Levels of soluble and insoluble tau reflect overall status of tau phosphorylation in vivo", NEUROSCIENCE LETTERS, LIMERICK, IE, vol. 450, no. 1, 23 January 2009 (2009-01-23), pages 51-55, XP025800599, ISSN: 0304-3940, DOI: 10.1016/J.NEULET.2008.11.023 [retrieved on 2008-11-13] the whole document figure 1	1-91

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TOM VANDEBROEK ET AL: "Identification and Isolation of a Hyperphosphorylated, Conformationally Changed Intermediate of Human Protein Tau Expressed in Yeast +", BIOCHEMISTRY, vol. 44, no. 34, 1 August 2005 (2005-08-01), pages 11466-11475, XP055044797, ISSN: 0006-2960, DOI: 10.1021/bi0506775 the whole document figure 5E	1-91
X	US 2008/220449 A1 (VASAN SARA [US] ET AL) 11 September 2008 (2008-09-11) the whole document paragraph [0071] examples 4-6	1-91
X	S. ODDO ET AL: "Reduction of Soluble Abeta and Tau, but Not Soluble Abeta Alone, Ameliorates Cognitive Decline in Transgenic Mice with Plaques and Tangles", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 281, no. 51, 1 January 2006 (2006-01-01), pages 39413-39423, XP055022863, ISSN: 0021-9258, DOI: 10.1074/jbc.M608485200 the whole document page 39417	1-91

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2012/069783

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012045882 A2	12-04-2012	TW 201216985 A	01-05-2012
		US 2012276009 A1	01-11-2012
		WO 2012045882 A2	12-04-2012
US 2008050383 A1	28-02-2008	US 2008050383 A1	28-02-2008
		US 2011318358 A1	29-12-2011
WO 2010106127 A2	23-09-2010	AU 2010224824 A1	22-09-2011
		CA 2755683 A1	23-09-2010
		CN 102428101 A	25-04-2012
		EP 2408807 A2	25-01-2012
		JP 2012520852 A	10-09-2012
		KR 20120004994 A	13-01-2012
		SG 174871 A1	28-11-2011
		US 2010285108 A1	11-11-2010
		WO 2010106127 A2	23-09-2010
WO 2010115843 A2	14-10-2010	AU 2010233856 A1	27-10-2011
		CA 2757345 A1	14-10-2010
		CO 6390113 A2	29-02-2012
		CR 20110509 A	09-02-2012
		EP 2413957 A2	08-02-2012
		JP 2012522754 A	27-09-2012
		KR 20120034609 A	12-04-2012
		SG 175037 A1	28-11-2011
		US 2012183599 A1	19-07-2012
		WO 2010115843 A2	14-10-2010
WO 9822120 A1	28-05-1998	AU 5508798 A	10-06-1998
		WO 9822120 A1	28-05-1998
EP 2210901 A1	28-07-2010	AU 2008312802 A1	23-04-2009
		CA 2702880 A1	23-04-2009
		CN 101965365 A	02-02-2011
		EP 2210901 A1	28-07-2010
		KR 20100115340 A	27-10-2010
		US 2010260783 A1	14-10-2010
		WO 2009051220 A1	23-04-2009
WO 2010144711 A2	16-12-2010	CA 2765099 A1	16-12-2010
		CN 102596221 A	18-07-2012
		EA 201171397 A1	30-05-2012
		EP 2440234 A2	18-04-2012
		US 2010316564 A1	16-12-2010
		WO 2010144711 A2	16-12-2010
US 2008220449 A1	11-09-2008	US 2008220449 A1	11-09-2008
		WO 2008140639 A2	20-11-2008